Transcriptional profiling and Functional studies of Zinc cluster transcription factors in *Candida albicans*

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A Thesis

in

The Department

of

Biology

Presented in Partial Fulfillment of the Requirements

For the Degree of Doctorate of Philosophy at

Concordia University,

Montreal, Quebec, Canada

December 15th, 2021

CONCORDIA UNIVERSITY

School of Graduate Studies

This is to certify that the thesis prepared

By: Raha Parvizi Omran

Entitled: Transcriptional regulatory and functional studies of Zinc cluster transcription factors in *Candida albicans*

and submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (Biology)

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Abstract

Transcriptional regulatory and functional studies of Zinc cluster transcription factors in *Candida albicans*

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Concordia University, 2021

The yeast *Candida albicans* is a commensal member of the gastrointestinal and urogenital tracts of most healthy humans. However, its capacity to function as an opportunistic pathogen allows it to cause systematic infections of immunocompromised individuals. Over the past two decades, the *C. albicans* zinc cluster transcription factor family (ZCFs) has been a fascinating subject of research – with studies identifying their roles in virulence, morphogenesis, biofilm formation, drug resistance and many other cellular processes. An understanding of these ZCFs may reveal new targets for therapeutic strategies.

My work focused on generating genome-wide transcriptional profiling for a large subset of 35 ZCF gain-of-function mutants (GOF) to elucidate the transcriptional profiles among the ZCFs, and on investigating in depth the function of some specific ZCFs in the fungal pathogen.

Transcriptional profiling revealed the target genes that are activated by the ZCF-GOF mutants and provided insight into the underlying roles of the factors. My study focused on establishing the transcriptional regulatory relationship among the ZCFs and understanding the function of some uncharacterized ZCFs. In chapter 2, I selected a set of 35 mostly uncharacterized ZCF, or little is known about them to explore their function using RNA-based transcriptional profiling in collaboration with professor M. Hallett lab. The network approach often shows a specific ZCF-GOF caused activation of expression of other ZCFs, which highlights the extensive interactions among ZCFs. We suggest that most expression changes can be the result of downstream longer-term adaptive responses that induce the expression of intermediate transcription factors.

In chapter 3, I characterized a new element involved in hyphal development regulation as a previously unstudied *Candida*-specific ZCF encoded by *CaORF19.1604* that I named Rha1 (Regulator of Hyphal Activity). I identified Rha1 through screening a ZCF-GOF library and noting the Rha1-GOF strain was in a filamentous form under yeast growth conditions. I have characterized Rha1 inactivation mutants and GOF alleles, and I explored the Rha1 regulatory network involving

Brg1 and Ume6, which are upregulated hyphal activators that appeared in the Rha1-GOF profile to show that Rha1 affects hyphal gene expression and upregulates Brg1/Ume6 and downregulates Nrg1.

In chapter 4, I investigated the role of *ZCF4* in cell wall biogenesis, filamentation, biofilm formation, and drug resistance. I explored the *ZCF4* function after noting its upregulation in most of the activated ZCF profiles like Rha1-GOF. Zcf4-GOF showed a severe filamentation defect on serum-based medium but exhibited normal filamentation under other cues. I have shown that *ZCF4*-influenced filamentation is nutrient dependent.

In chapter 5, I showed the robust ability of *C. albicans* to use proline as a carbon and nitrogen source by describing *Ca*Put3 as a proline catabolism regulator. The functional studies demonstrated Put3 has a conserved role in regulating proline catabolism in *C. albicans* and *Saccharomyces cerevisiae*, but *Ca*Put3 initiates the degradation of proline even in the presence of a rich nitrogen source such as ammonium sulphate.

Collectively, this study established a framework of functional study TFs and generated robust transcriptional data from an activated set of 35 ZCFs to help understand the biology of *C. albicans*, an important human pathogen.

KEYWORDS: Transcription factors, Transcriptional regulators, *Candida albicans*, Zinc cluster factors (ZCFs)

Acknowledgements

I would like to first express my deepest gratitude to my supervisor Dr. Malcolm Whiteway for his support, and intellectual guidance throughout my degree. You have been the role model and mentor I needed as a young scientist. I thank you for your trust, and for the words of encouragement that have given me the confidence I lacked and for your enthusiasm for science which has been a continuous motivation. The knowledge I have gained from his expertise is invaluable. Thank you for your support, both intellectually and emotionally, and I am forever grateful for your guidance.

I would like to thank the members of my committee, Dr. Patrick Gulick and Dr. David Walsh, for their valuable suggestions during my graduate research. There are no words to express my gratitude to Dr. Vanessa Dumeaux and the Mike Hallett lab for all they have contributed to my studies. I would also like to extend my thanks to former and current lab mates Walters Aji Tebung, Yuan Sun, Tuana Mesquita, Anna Carolina Borges Pereira da Costa, Adithi Sundarakrishnan, Amjad Islam, Samira Masshai and Golnaz Rastghalam for their encouragement and scientific help. I would like to thank Hoai Anh Nguyen, Abir akoum and Yana Moscovitz for their contributions during my studies.

I am grateful for my family, especially my parents, for their love, encouragement, and sacrifice. I could not have accomplished this work without their generous support in particular my mother, who has always prayed for my success. I would like to dedicate my thesis to my brother Mohammad Javad, whom I lost at his early age of childhood. Mohammad Javad, I dedicate this and all my future pursuits of knowledge to you! I will love you and miss you always!

Contribution of Authors

Chapter 1: Introduction

Throughout this chapter, you will find an overview of the background information that will be discussed in subsequent chapters. Raha Parvizi Omran compiled the writing and Dr. Malcolm Whiteway edited it. All figures were created by Raha Parvizi Omran, except for Figure 1.1, which was taken from Shapiro *et al*. (2011), Figure 1.2 (Mayer *et al.* 2013), Figure 1.3 (Rodriguez *et al.* 2020) , Figure 1.4 (Shapiro *et al.* 2011), Figure 1.5 (MacPherson *et al.* 2006). (copyright permission was not required).

Chapter 2: Transcriptional profiling of the zinc cluster transcription factors.

In this chapter, we explored transcription-based RNA sequencing (RNA-seq) profiles of ZCFs. By using Illumina miSeq technology, 35 of the 82 Zinc cluster transcription factors with gain-of-function mutants were assessed for transcriptional profiling with two biological replicates. Raha Parvizi Omran compiled the writing. The RNA-seq profiles were processed by Dr. V Dumeaux. As part of Dr. Michael Hallett's lab, Anaika Van kooten wrote the method section and prepared differential expression profiles. Anh Nguyen constructed *HAL9* heterozygous mutants and performed phenotypic studies.

Chapter 3 (published)**:** Raha Parvizi Omran, Bernardo Ramírez-Zavala, Walters Aji Tebung, Shuangyan Yao, Jinrong Feng, Chris Law, Vanessa Dumeaux, Joachim Morschhäuser, Malcolm Whiteway. **The zinc cluster transcription factor Rha1 is a positive filamentation regulator in *Candida albicans*.**

This chapter explored a new player of hyphal development in *Candida albicans* named Rha1 (Regulator of Hyphal Activity) that is a member of the zinc cluster family of transcription factors. Raha Parvizi Omran designed and performed the experimental studies and wrote the paper. Bernardo Ramírez-Zavala contributed to the experimental work and edited the paper. Walters Aji Tebung performed the Chip-chip assays. Shuangyan Yao and Jinrong Feng contributed to the virulence assay. Chris Law assisted with microscopy and the elongation factor assay. Vanessa Dumeaux provided bioinformatic support for the RNA-seq data postprocessing. Joachim Morschhäuser provided strains and edited the paper. Malcolm Whiteway designed experiments and edited the paper.

Chapter 4: Raha Parvizi Omran, Yana Moscovitz, Vanessa Dumeaux, Joachim Morschhäuser, Malcolm Whiteway. **Regulation of Hyphal Development by Zcf4 in *Candida albicans***.

This chapter explored the function of zinc cluster transcription factors Zcf4 using transcriptional profiling, bioinformatic analysis and phenotypic study. Raha Parvizi Omran designed and performed the experimental studies and wrote the chapter. Yana Moscovitz did construction of *ZCF4* null and heterozygous mutant strains and performed phenotypic studies. Joachim Morschhäuser provided activated transcription factor reagents Vanessa Dumeaux provided bioinformatic support for the RNA-seq data postprocessing. Malcolm Whiteway edited the paper.

Chapter 5 (published)**:** Walters Aji Tebung, Raha Parvizi Omran, Debra L Fulton, Joachim Morschhäuser, Malcolm Whiteway. mSphere 2017. **Put3 Positively Regulates Proline Utilization in *Candida albicans*.**

This chapter explored the function of zinc cluster transcription factors Put3 using transcriptional profiling, ChIP-chip, and phenotypic study. Walters Aji Tebung performed experiments and wrote the paper. Raha Parvizi Omran did construction of associated mutants' strains and performed phenotypic studies and edited the paper. Joachim Morschhäuser provided activated transcription factor reagents and edited the paper. Debra Fulton provided bioinformatic support for the RNA-seq data postprocessing. Malcolm Whiteway designed experiments and edited the paper.

Chapter 6: Conclusions and future work

Chapter six summarizes the main findings of the thesis and discusses the implications for the work and future directions.

Other contributions not included in this thesis:

Anna Carolina Borges Pereira Casta, **Raha Parvizi Omran**, Chris Law, Vanessa Dumeaux, and Malcolm Whiteway. "Signal-mediated localization of *Candida albicans* pheromone response pathway components." G3 11, no. 3 (2021): jkaa033.

Bettauer, Van, Anna CBP Costa, **Raha Parvizi Omran**, Samira Massahi, Eftyhios Kirbizakis, Shawn Simpson, Vanessa Dumeaux, Chris Law, Malcolm Whiteway, and Michael T. Hallett. "A deep learning approach to capture the essence of *Candida albicans* morphologies." *bioRxiv* (2021).

Bettauer, V., S. Massahi, S. Khurdia, A. C. B. P. Costa, **R. P. Omran**, N. Khosravi, S. Simpson et al. "*Candida albicans* exhibits distinct cytoprotective responses to anti-fungal drugs that facilitate the evolution of drug resistance." bioRxiv (2020).

Feng, Jinrong, Amjad Islam, Bjorn Bean, Jia Feng, Samantha Sparapani, Manjari Shrivastava, Aashima Goyal, **Raha Parvizi Omran**, Jaideep Mallick, and Malcolm Whiteway. "Hof1 plays a checkpoint-related role in MMS-induced DNA damage response in *Candida albicans*." *Molecular biology of the cell* 31, no. 5 (2020): 348-359.

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and Malcolm Whiteway. "MAP kinase regulation of the *Candida albicans* pheromone pathway." Msphere 4, no. 1 (2019): e00598-18.

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List of abbreviations

aa: Amino Acid

AD: Activation Domain

ALS: Agglutinin-Like Sequence

arg: Arginine

bHLH: basic helix-loop-helix

BLAST: Basic Local Alignment Search Tool

bp: Base Pairs

cAMP: Cyclic Adenosine Monophosphate

Cas9: CRISPR associated protein 9

$CaCl_2$: Calcium Chloride

CFW: Calcofluor White

ChIP: Chromatin immunoprecipitation

CGD: Candida Genome Database

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

CO_2: Carbon Dioxide

CSRE: Carbon Source-Responsive Element

$CuSO_4$: Copper(II) Sulfate

DBD: DNA-Binding Domain

DIC: Differential Interference Contrast

FAD: Flavin Adenine Dinucleotide

GABA: Gamma-AminoButyrate

GI: GastroIntestinal

GlcNAc: N-acetylglucosamine

GO: Gene Ontology

GOF: Gain-Of-Function

GPI: Glycosylphosphatidylinositol

GTFs: General Transcription Factors

GTP: Guanosine Triphosphate

h: Hour

HAGs: Hyphae Associated Genes

HAT: Histone Acyltransferase

HIV: Human Immunodeficiency Virus

Hsps: Heat shock proteins

$FeCl_3$: Iron(III) Chloride

$FeSO_4$: Iron(II) Sulfate

KGDH: α-Ketoglutarate Dehydrogenase

MAPK: Mitogen-Activated Protein Kinase

MMS: Methyl Methane Sulfate

LiCl: Lithium Chloride

MES: 2-(N-morpholino)ethanesulfonic acid

ml: Milliliters

mM: Millimolar

MMS: Methyl Methane Sulfonate

mRNA: Messenger RNA

MTL: Mating Type Like

NaCl: Sodium chloride

NAT: Nourseothricin

NCBI: National Centre for Biotechnology Information

nm: Nanometers

OD: Optical Density

PBS: Phosphate-Buffered Saline

PCR: Polymerase Chain Reaction

RNA-seq: RNA Sequencing

s: Second

SGD: Saccharomyces Genome Database

SAGA : (Spt-Ada-Gcn5-acetyltransferase)

Saps: Secreted Aspartyl Proteinase

SC: Synthetic Complete

TBP: TATA-box binding protein

TCA: Tricarboxylic Acid

TF: Transcription Factor

TFs: Transcription factors

TOR: Target of Rapamycin

IHW: Independent Hypothesis Weighting

vs: Versus

WT: Wild Type

WGD: Whole genome duplication

YCB: Yeast Carbon Base

YNB: Yeast Nitrogen Base

YPD: Yeast-extract-peptone-dextrose

Succinyl-CoA: Succinyl coenzyme A

ZCF: Zinc cluster transcription factor

ZCFs: Zinc cluster transcription factors

°C: Degree Centigrade

%: Percent

µg: Micrograms

µl: Microliters

µm: Micrometers

Chapter 1: Introduction

1.1 *Candida albicans* as a human fungal pathogen

Candida albicans is a commensal fungal pathogen that inhabits the skin, gastrointestinal (GI) and genitourinary tracts of warm-blooded animals such as humans. As an opportunistic fungus, it causes a range of infections from discomforting vaginal yeast infections and oral-pharyngeal candidiasis to life-threatening bloodstream infections in immunocompromised human immunodeficiency virus (HIV) patients, organ transplant recipients, and preterm infants (Kim and Sudbery 2011). In the U.S, *C. albicans* is the fourth-most common source of hospital-acquired infections and has a mortality rate associated with systematic infections as high as 50% even in the presence of antifungal drug treatments (Pfaller and Diekema 2010; Kullberg and Arendrup 2015). Invasive fungal infections kill annually over 1.5 million people, which is a mortality rate comparable with that of tuberculosis or malaria (Brown *et al.* 2012). Nearly 90% of human mortality associated with fungal infections is caused by opportunistic species of *Candida*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Pneumocystis jirrovecii* (Pfaller and Diekema 2010). The incidence of fungal infections is ever-expanding because of increasing numbers of immunocompromised patients; such as cancer patients undergoing chemotherapy, growing numbers of HIV patients and expanding cases of individuals undergoing organ transplant surgery (Pfaller and Diekema 2010).

To date, there are three major classes of antifungal drugs to treat *C. albicans* infections: azoles, polyenes, and echinocandins (Morschhäuser 2010). Four azole antifungal drugs are clinically available - fluconazole, itraconazole, voriconazole, and posaconazole; all of which target a key enzyme in ergosterol biosynthesis - the fungal cytochrome P450 lanosterol 14α-demethylase, encoded by *ERG11* in *C. albicans* (Shapiro *et al.* 2011; Greenblatt and Greenblatt 2014). In other fungal species like *A. fumigatus*, cytochrome P450 is encoded by *cyp51A* and *cyp51B* (Shapiro *et al.* 2011). Inhibiting cytochrome P450 leads to the accumulation of toxic methylated sterols generated by Erg3, which are then incorporated into the plasma membrane and replace ergosterol. By depleting ergosterol and accumulating a toxic sterol intermediate, azoles cause severe membrane stress and alter activity of membrane-bound enzymes like chitin synthase, leading to growth arrest in fungi (White *et al.* 1998).

The polyene amphotericin B kills the fungal cells at two different levels. One consists of pore formation in the cellular membrane through binding to ergosterol, the predominant sterol in the fungal membrane component. The other is through creating oxidative and reactive oxygen species (Shapiro *et al.* 2011; Gray *et al.* 2012) (Figure 1.1). Amphotericin B possesses strong antifungal activity, but has high host toxicity, with side effects like renal dysfunction. Moreover, *C. albicans* can generate resistance to amphotericin B through reduction and alteration in ergosterol production or changes in the fungal cell wall (Shapiro *et al.* 2011; Gray *et al.* 2012).

Caspofungin, micafungin, and anidulafungin are in the echinocandin class of antifungals which target the fungal cell wall by inhibiting 1,3 β-D-glucan synthases. In the absence of 1,3 β-D-glucan, fungal cell walls are damaged, generating severe stress on the cell (Shapiro *et al.* 2011). Despite this, fungi have a mechanism for protecting themselves from echinocandin drugs by mutations in the genes encoding the two major subunits of glucan synthetase *FKS1* and *FKS2*. These mutations can reduce the sensitivity to drugs by several thousand-fold, allowing continued synthesis in the presence of the drugs (Perlin 2011). Overall, a low level of side effects has been reported for this class of agents because it affects a component of fungi that is not conserved in mammalian cells. (Shapiro *et al.* 2011). A rising tide of drug resistance in fungal pathogens compromises all the antifungal drugs currently used in clinical practice. For instance, the Centers for Disease Control and Prevention has classified azole-resistant Candida as one of the serious threats to human health, given the 46000 infections reported in the USA alone each year and the fact that around 30% of patients with those infections die during hospitalization (CDC, 2013). So, the identification of novel approaches to treat life-threatening fungal infectious diseases and combat fungal drug resistance is urgently necessary. Moreover, a comprehensive understanding of *C. albicans* biology including factors that regulate growth and virulence are urgently needed for the development of new antifungal agents to treat life-threatening fungal infectious disease and combat the growing instances of fungal drug resistance.

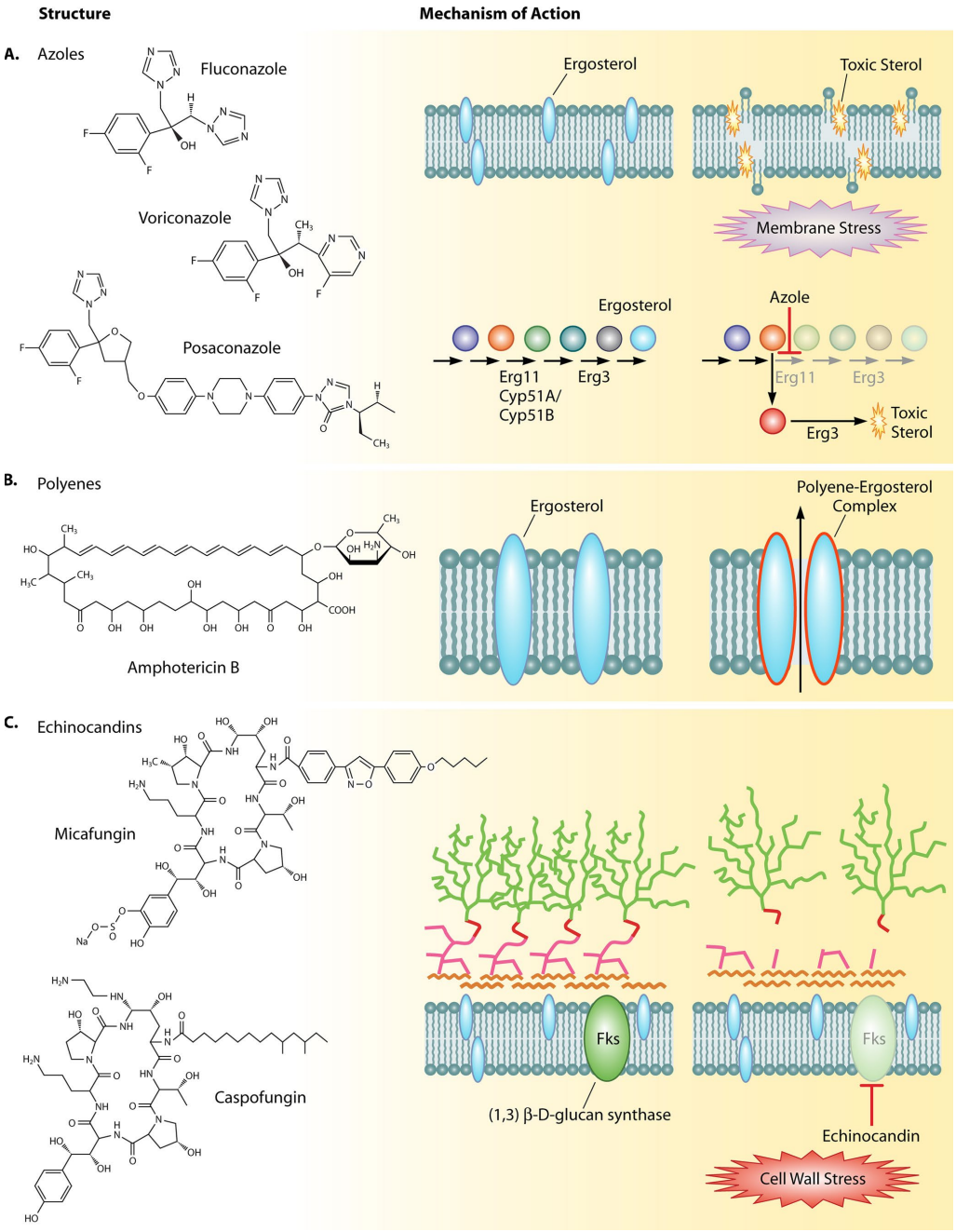


Figure 1.1. A diagram illustrating the target of antifungal drugs (Shapiro *et al.* 2011). **A.** Azoles prevent the activity of the ergosterol biosynthesis enzyme lanosterol demethylase, encoded by *ERG11* in *C. albicans* or by *cyp51A* and *cyp51B* in *A. fumigatus*. Azoles block ergosterol production and result in accumulation of a toxic sterol by *ERG3 activity*. **B.** Amphotericin B creates a drug-lipid complex through binding to ergosterol which results in membrane pore formation and oxidative stress. **C.** The Echinocandins target a specific component of the fungal cell wall, 1,3 β-D-glucan, and cause dysfunction in cell wall integrity and cell wall stress response.

1.2 *Candida albicans* virulence factors

The ability of *C. albicans,* as an effective pathogen, to both grow as a commensal and to switch to a pathogenic state, relies on several virulence traits. These traits include characteristics such as the production of adhesion factors, the potential to switch morphologies, the ability to form biofilms and a capacity for metabolic adaptation (Calderone and Clancy 2011; Polke *et al.* 2015) (Figure 1.2).

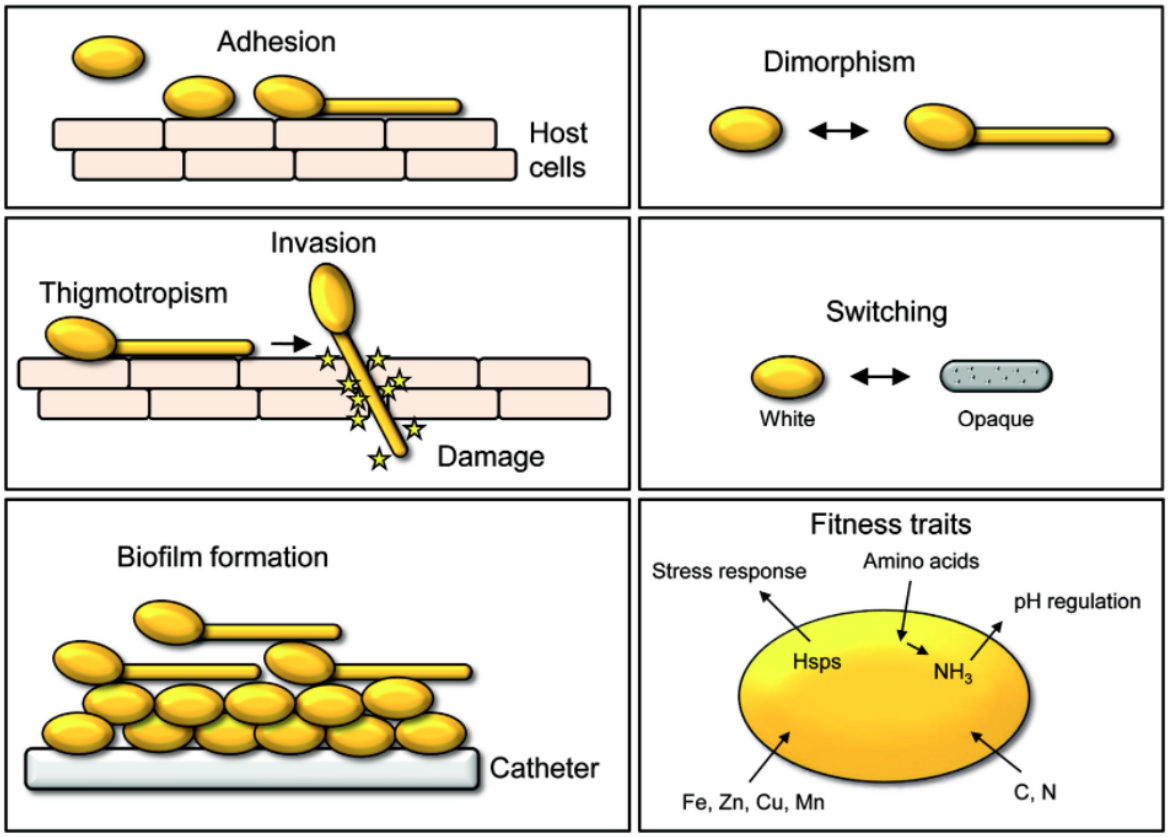


Figure 1.2. Diagram of selected *C. albicans* pathogenicity mechanisms (Mayer *et al.* 2013). The growth transition between yeast and hyphae, the expression of adhesins and invasions on the surface of the cells, thigmotropism, the formation of biofilms and phenotypic switching are all considered virulence factors. Additional fitness attributes include rapid adaptation to pH fluctuations, metabolic flexibility, powerful nutrient acquisition systems, and robust stress response mechanisms.

Adhesion of *C. albicans* to host cells is an early sign of disease development and an important virulence factor. *C. albicans* has a variety of adhesion proteins that facilitate binding to abiotic

surfaces, to other *C. albicans* cells and other microorganisms, or the surface of the host cells. The best-studied adherence proteins of *C. albicans* are ALS (agglutinin-like sequence) proteins comprised eight members (Als1-7 and Als9) which are glycosylphosphatidylinositol (GPI)-linked cell surface glycoproteins. Among the eight members of the ALS family, the hyphal-state-associated protein Als3 has a critical adhesion role in oral and vaginal infections (Liu and Filler 2011). Hwp1 is another GPI-linked protein characteristically expressed in hyphae that acts as substrate for mammalian transglutaminase (Mayer *et al.* 2013). Studies showed *C. albicans* cells deficient in Als3 and Hwp1 displayed notable defects in host cell interactions assay, in biofilm formation, and in iron acquisition *in vitro* (Liu and Filler 2011; Mayer *et al.* 2013). Other adhesion and invasion related genes, commonly known as hyphae associated genes (HAGs) include *ECE1*, *HGC1*, *RBT1*, *SAP4*, *SOD5*, which are correlated with the yeast to hyphae transition and contribute to host infection by *C. albicans*. Several transcription factors, such as Efg1, Tec1, and Bcr1 are known to activate HAGs, while Nrg1 and Tup1 inhibit HAGs expression (Fan *et al.* 2013). Overall, adherence factors serve as critical components needed for *C. albicans* to invade endothelial cells and to degrade host proteins using hydrolytic enzymes such as secreted aspartyl proteinases (Saps), phospholipases and lipases (Naglik *et al.* 2004; Zhu and Filler 2010).

Another key virulence factor in *C. albicans* is its ability to transition among morphologies, such as the switch from white cells to mating competent opaque cells, or from yeast cells to chlamydospores in response to specific environmental conditions (Kim and Sudbery 2011; Shapiro *et al.* 2011; Noble *et al.* 2017). *C. albicans* cells that are homozygous at the Mating Type Like (*MTL*) locus are capable of switching from yeast morphology (white) to elongated mating competent opaque cells (Miller and Johnson 2002). The opaque formation is repressed by heterozygosity at the *MTL* locus, as the **a** and **α** alleles encode proteins that associate as a heterodimer and bind to the promoter of the master regulator of the switch, *WOR1* (Miller and Johnson 2002). However, in *MTL* homozygous cells, the white-opaque transitions can be induced by environmental cues such as elevated CO_2 levels, and lower temperatures compared to yeast growth conditions. These two cell types differ dramatically in terms of transcriptional profile, mating efficacy and virulence and have different impacts on the host niches (Miller and Johnson 2002). *C. albicans* can also switch from growth as a budding yeast to forming a large, spherical, thick-walled cell termed a chlamydospore at the tips of filamentous cells under nutrient poor

environments and changes in temperature and pH (Staib and Morschhäuser 2007). *Candida* chlamydospores are high in lipid droplets for energy, but they are not more resistant to heat or starvation compared to yeast cells (Böttcher *et al.* 2016). There are six genes required for chlamydospore formation including *ISW2*, *MDS3*, *RIM13*, *RIM101*, *SCH9* and *SUV3*. A forward genetic analysis showed that cells lacking *ISW2*, *MDS3*, and *SCH9* were completely incapable of chlamydospore formation, but that *RIM13*, *RIM101*, and *MSD3* were necessary for correct timing of their production (Nobile *et al.* 2003). It remains to be determined whether chlamydospore functions as a spore, what structure it takes on, and why it is unique to the close relatives *C. albicans* and *C. dubliniensis* within the *Candida* clade (Staib and Morschhäuser 2007).

Perhaps the most clinically relevant morphological transition is the interchange between yeast, pseudohyphal and hyphae in response to specific environmental cues. The yeast growth form has a round to oval shape morphology, is fundamentally unicellular and reproduces by budding, while the hyphal or filamentous forms are multicellular, and have an elongated tubular shape in which the daughter cells do not detach from the mother cells (Sudbery *et al.* 2004; Noble *et al.* 2017). Pseudohyphal cells are ellipsoid cells that share traits of both yeast and hyphal cells, but in comparison to hyphae they are wider and have constrictions at the septation sites (Sudbery 2011). Both yeast and filamentous forms are observed during *ex vivo* experiments in different tissues and organs with the distinctions that yeast forms are mainly found in skin infections while filamentous forms are associated with infected kidneys, and with oral and vaginal mucosa (Raz-Pasteur *et al.* 2011; Böhm *et al.* 2017). *C. albicans* can undergo the filamentation transitions *in vitro* when triggered by external stimuli such as serum, nutrient starvation, temperature above 37 °C, alkaline pH and CO_2 concentrations that mimic the host environments (Sudbery 2011; Shapiro *et al.* 2011). Signal transduction pathways can be activated by this environmental cue, thus favoring *Candida* pathogenicity. Numerous studies suggested this *C. albicans* filamentous transition to be an important virulence trait which in turn depends on the primary control of gene expression by transcriptional regulators (Transcription factors) that act as activators or repressors in response to a specific environmental signal to direct appropriate cellular behavior (Maicas *et al.* 2005; Homann *et al.* 2009). It has been found that there is not necessarily a correlation between filamentation and the virulence of *Candida* cells. As an example, single mutants of the hyphal activators Efg1 or Cph1 are still virulent despite the fact that the cells cannot form hyphae, but

cells after deletion of both hyphal activators (*cph1/cph1 efg1/efg1)* are locked in yeast form and are avirulent (Lo *et al.* 1997). As such, it is possible that *Candida* virulence traits are most closely related to its transcription factors, which control gene expression, rather than its morphological cell type.

The ability of *C. albicans* to form biofilms on abiotic (catheters) or biotic (host cell) surfaces is another critical virulence factor. In biofilms, there are different types of cells, including round yeast cells, pseudohyphal and elongated hyphae covered by extracellular matrix (Chandra *et al.* 2001). There are multiple stages involved in biofilm formation, from yeast adhesion to growth as cell communities encased in extracellular matrix, to dispersal, where some yeast cells disperse from the biofilm to cause infections. A major medical issue is biofilms attached to implanted medical devices such as indwelling catheters (Blankenship and Mitchell 2006; Gulati and Nobile 2016) (Figure 1.3). Biofilm-based infections are a significant clinical challenge because biofilms show resistance to both antifungal drugs and the host immune system (Nobile and Johnson 2015). There are six "master" transcription factors proposed to control biofilms - Tec1, Bcr1, Efg1, Brg1, Ndt80, Rob1 – functioning as a transcriptional network (Nobile *et al.* 2012; Fanning and Mitchell 2012) . Studies showed these transcription factors (TFs) directly bind in total to nearly 1,000 target genes, which results in a complex interconnected network of genes involved in biofilm development. Moreover, mutants of 44 additional transcription factors showed some defect in *C. albicans* biofilm formation (Nobile and Johnson 2015). Therefore, screening libraries of activated or deleted transcription factors can still be a powerful genetic tool for identifying new regulatory genes that are involved in Candida biofilm formation.

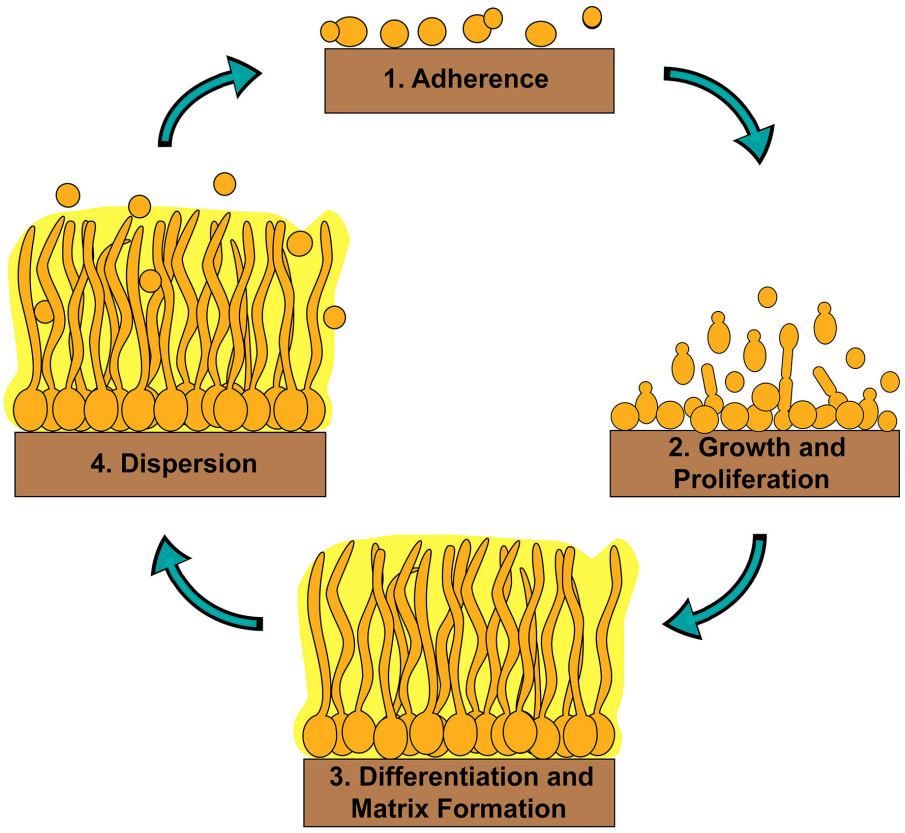


Figure 1.3. *C. albicans* stages of biofilm formation (Rodriguez *et al.* 2020)**. A**. Surface adhesion of yeast cells. **B**. Formation of biofilms, where cells proliferate to create a basal layer of adherent cells. **C.** Biofilm maturation occurs when complex layers of polymorphic cells are enclosed by an extracellular matrix. **D.** Dispersion occurs when yeast cells leave the mature biofilm to seed new sites.

In addition to these more classical virulence factors, a variety of fitness traits also play a role in fungal pathogenicity. There is a robust stress response mediated by heat shock proteins (Hsps). This response includes the auto-induction of hyphal formation through amino acid uptake, excretion of ammonia NH_3 and concomitant extracellular alkalinization; uptake of different compounds as carbon (C) and nitrogen (N) sources; and uptake of essential trace metals like iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) and metabolic flexibility among these fitness traits. For instance, nutrient availability changes considerably through the *C. albicans* transition from commensality to pathogenicity and during dissemination and colonization of different host niches (Whittington *et al.* 2014; Miramón and Lorenz 2017). For example, *C. albicans* utilizes glucose, its favored carbon source during systematic infection, but switches to alternative carbon sources including amino acids and organic acids when exposed to nutrient starvation within the

phagosome (Whittington *et al.* 2014; Miramón and Lorenz 2017). Therefore, the fungal pathogen has developed metabolic plasticity to use diverse sources of carbon, nitrogen, oxygen, phosphorus, sulfur, and micronutrients to survive under nutrient limitation and adapt to various host-imposed stresses (Brown *et al.* 2014). Studies show a complex network of regulators, which have undergone major transcriptional rewiring during yeast evolution, control metabolic adaptation in *C. albicans* (Askew *et al.* 2009). In the sugar metabolic circuitry for example (Sabina and Brown 2009), the glycolysis pathways responsible for carbon metabolism in the model organism *Saccharomyces cerevisiae* are regulated by Gcr1, not Gal4 and Tye7 in as in *C. albicans* (Askew *et al.* 2009; Lavoie *et al.* 2010). As well, the fungal pathogen can use a varied range of primary nitrogen sources like ammonia, glutamine, asparagine and glutamate, but when confronted with a limitation in primary nitrogen sources, *C. albicans* is capable of switching to secondary nitrogen sources like amino acids and polyamines or can start to hydrolyze proteins (Marzluf 1997). Thus, knowledge about the regulators of virulence factors could lead to the identification of new drug targets.

1.3 Transcriptional regulation of gene expression

Regulation of gene expression plays a central role in all living organisms to ensure proper development, normal cellular functions, response to environmental changes and the capability to cope with disease as well as to maintain phenotypic variety both within and across species (de Souza *et al.* 2018). In eukaryotes, transcription is regulated by TFs that coordinate the phase, location, and efficiency of RNA polymerase in eukaryotes. TFs are divided into two classes: general transcription factors (GTFs) and sequence-specific transcription factors. GTFs including TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH form transcription initiation complexes that enable basal expression by interacting with core promoters near transcription sites (Müller 2001; Sikorski and Buratowski 2009). The transcriptional initiation complexes can also work in other contexts, including stimulation by a multi-subunit protein complex named Mediator that bridges the interaction between regulatory information from enhancers and other control elements to the basal RNA polymerase II transcription machinery. The transcriptional initiation complexes can also be brought to promoters by a coactivator multiprotein SAGA (Spt-Ada-Gcn5-acetyltransferase) complex which recruits the TATA-box binding protein (TBP) to modulate gene expression (Boonyasiri *et al.* 2013).

Overall, sequence-specific transcription factors that bind to specific DNA sequences

located upstream of a gene's transcription start site can be classified as positive or negative regulators for a particular gene, and these factors allow the cell to co-ordinate control of its gene expression (Müller 2001). The DNA-binding domain (DBD) and transcription activation domain (AD) are the two main components of such sequence-specific TFs. TFs are mostly categorized based on their DBDs, which selectively bind to 4-12 base pairs (bp) of *cis*-acting elements in gene regulatory sequences. To modulate transcriptional output, AD can bind to co-activators, such as mediator, or contribute to the remodeling or modification of chromatin. AD can be classified as acidic, proline-rich, serine/threonine-rich, or glutamine-rich based on their amino acid profile (Boija *et al.* 2018).

Overall, the yeast *Saccharomyces cerevisiae* has emerged as an important model organism in the study of transcriptional regulation, in part through the discovery of transcription factor families. As *C. albicans* both possesses pathogenic properties and shares the same common ancestor with *S. cerevisiae*, it would be a prime candidate to allow us to gain general insights into TF roles in signaling pathways controlling distinct biological processes such as cell growth, development, and stress responses.

1.4 Transcriptional regulation of filamentation in *C. albicans*

C. albicans can sense filament-inducing cues and transduce the information by signal transduction cascades to the transcription factors controlling morphogenesis transitions. *C. albicans* has two major filamentation-regulating signal pathways - cyclic AMP- protein kinase A (cAMP-PKA) and Cek1-mitogen-activated protein kinase (MAPK) - for controlling morphogenesis (Figure 1.4). A guanine nucleotide binding protein, Ras1, is essential in the regulation of the cyclic/AMP- protein kinase (PKA) and Cek1-MAPK pathways (Leberer *et al.* 2001; Shapiro *et al.* 2011). Active guanosine triphosphate (GTP)-bound Ras1 binds to the adenylyl cyclase Cyr1 protein and increases cAMP production from ATP in response to hyphal signals (Leberer *et al.* 2001; Biswas and Morschhäuser 2005; Shapiro *et al.* 2011). At high cAMP levels, cAMP binds to the PKA regulatory subunit Bcy1 leading to conformational changes and release of the two catalytic subunits Tpk1 and Tpk2 from the PKA complex. These catalytic subunits phosphorylate downstream transcription factors including Efg1 (Bockmühl and Ernst 2001; Cassola *et al.* 2004). Efg1 is a member of the basic helix-loop-helix (bHLH) transcription factor family and is a central morphogenesis regulator; deletion of *EFG1* inhibits filamentation under a

variety of hyphae-triggering conditions (Stoldt *et al.* 1997; Braun and Johnson 2000). Efg1 then interacts with the histone acyltransferase (HAT) NuA4 during the yeast to hyphae transition, allowing an increase in histone H4 acetylation at the promoters of HAGs (Lu *et al.* 2008). As mentioned earlier, these HAGs consist of elements such as adhesion proteins from the agglutinin-like sequence Als family, *HWP1* a gene encoding a cell wall mannoprotein, and hyphal controllers such as Ume6 which is important in *C. albicans* pathogenicity.

The *C. albicans* Cek1-MAPK pathway responds to nutrient limitation, embedding matrix, cell wall damage and invasive growth on solid medium (Leberer *et al.* 1996). Ras1 transduces the signal to the MAPK cascade through the small GTPase Cdc42 and the kinase Cst20. This leads to sequential phosphorylation of the MAPKKK Ste11, the MAPKK Hst7 and the MAPK Cek1, ending in the activation of the downstream transcription factor Cph1 (Csank *et al.* 1998; Leberer *et al.* 2001) (Figure 1.4).

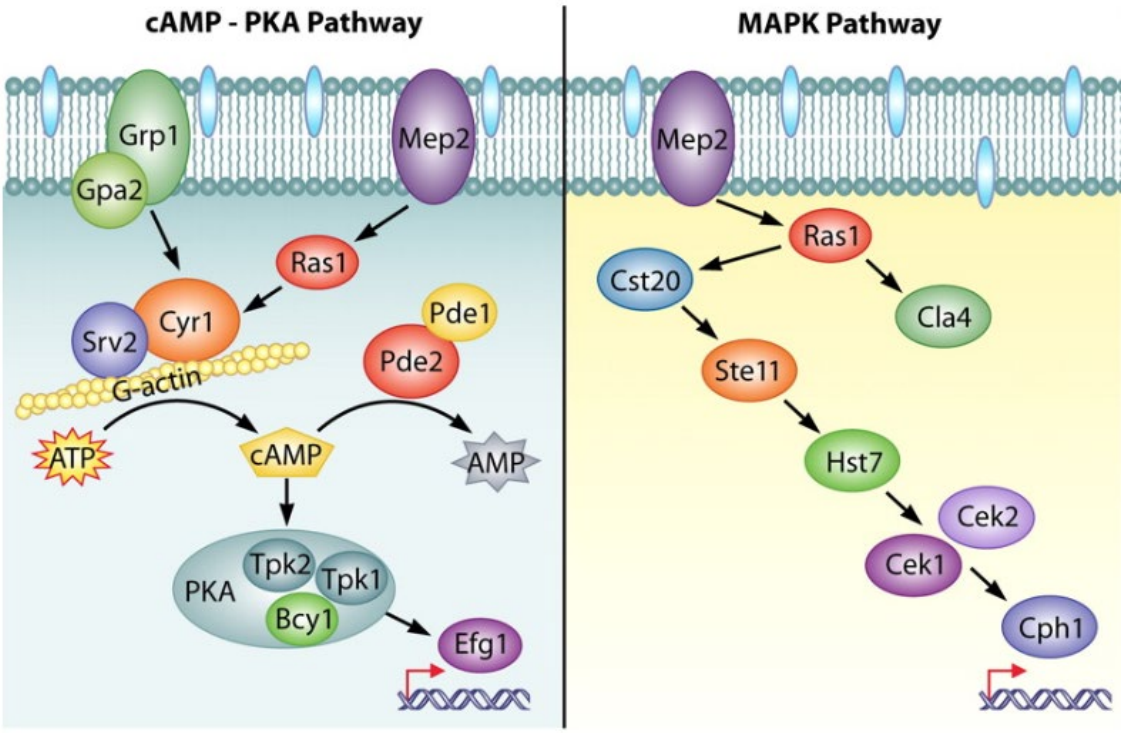


Figure 1.4. Diagrams of the cAMP-PKA and MAPK pathways (Shapiro *et al.* 2011). Key cellular signaling cascades regulating morphogenesis in *C. albicans*. Two of the most well-characterized pathways are shown, along with several key proteins involved in each pathway.

1.5 Zinc cluster proteins in *C. albicans*

Gene expression regulation is crucial for the ability of cells to adjust to changes in their environment (Latchman 1997). Transcription factors that activate or repress gene expression in response to specific signals play a central role in coordinating appropriate cellular behaviors (Latchman 1997). The target genes that are controlled by transcription factors and the signals to which they react can change over evolutionary timescales. Such changes in transcriptional networks alter the properties of organisms and are an intrinsic part of species development (Dowell 2010).

TFs are categorized into several classes based on their DNA binding domains: these include the basic helix-loop-helix (bHLH) class, the zinc finger class, the homeobox family, and the leucine zipper TFs. The largest family of transcriptional regulators in eukaryotes is the zinc finger protein family (MacPherson *et al.* 2006). The zinc-finger transcription factors contain a common zinc finger motif, consisting of one α helix and a pair of antiparallel β strands. This family also has one or more zinc atoms bound by cysteines or histidines, stabilizing the domain and contributing to the structure and function of the protein (MacPherson *et al.* 2006). Most zinc finger proteins bind DNA as transcription factors, but some act as mediators for protein/protein interactions, chromatin remodeling, protein chaperoning, lipid binding, and zinc sensing (Laity *et al.* 2001; MacPherson *et al.* 2006) There are fundamentally three classes of zinc finger proteins. The classic zinc finger protein is the Cys2His2 class that binds DNA as monomers with the zinc finger structure CysX2-4-Cys-X12-His-X3-5-His. The second class of zinc finger proteins is the Cys4 (Cys-X2-Cys-XnCys-X2-Cys-Xn-Cys-X2-Cys-Xn-Cys-X2-Cys) class of proteins. These zinc finger proteins bind DNA as homodimers or heterodimers (MacPherson *et al.* 2006). The homodimers typically bind inverted repeats while the heterodimers bind direct repeats (MacPherson *et al.* 2006). Cys6, the third class of zinc finger proteins, has six conserved cysteine residues that bind to two zinc atoms named zinc cluster transcription factors (ZCFs), and appears exclusive to fungi and amoebae. ZCFs bind DNA as homodimers utilizing their zinc finger motif at the N-terminus to hydrogen bond with triplets of CGG nucleotides that are oriented as everted, inverted, or direct repeats. These proteins can bind DNA as monomers, homodimers, and heterodimers (MacPherson *et al.* 2006) (Figure 1.5). The genome of *C. albicans* encodes 82 ZCFs (zinc binuclear cluster TFs or (Zn (II)2 Cys6) family members with the conserved Cys6 DNA-binding motif ($CX_2CX_6CX_{5–12}CX_2CX_{6–8}C$) usually located at the N-terminus (MacPherson *et al.*

2005; Schillig and Morschhäuser 2013). These ZCFs regulate diverse processes including metabolism of sugars and amino acids, switching to mating competent opaque form filamentation, drug resistance and virulence-related traits (MacPherson *et al.* 2005; Banerjee *et al.* 2008; Schillig and Morschhäuser 2013; Issi *et al.* 2017)

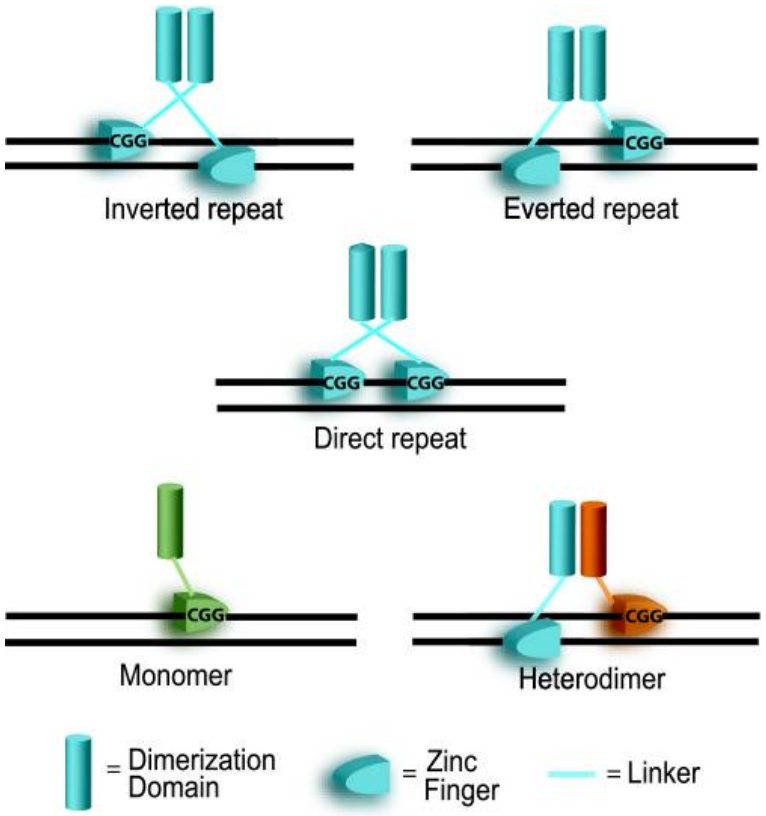


Figure 1.5. Zinc cluster protein DNA recognition model (MacPherson *et al.* 2006). Zinc cluster proteins preferentially bind to CGG triplets of three orientations: inverted, everted, and direct repetitions.

For the past decade, several studies have been conducted to characterize the functions among previously uncharacterized ZCFs. Schillig and Morschhäuser generated a library of artificially activated ZCFs in *C. albicans* by fusing the heterologous Gal4 activation domain from *Saccharomyces cerevisiae* to the C terminus of complete ZCF proteins. Screening this ZCF- gain-of-function (GOF) library identified new regulators in filamentation and new mediators of fluconazole resistance such as Mrr2 (multidrug resistance regulator 2) that controls expression of *CDR1* encoding the multidrug efflux pump (Schillig and Morschhäuser 2013). Studies by Kakade *et al*. (2016) characterized Zcf32 as a negative regulator of biofilm gene circuitry. Zcf32 represses

C. albicans biofilms by downregulating cell wall proteins, chitins, and GPI anchored proteins (Kakade *et al.* 2016). Tebung *et al*. (2016) have identified a rewiring case of allantoin degrading enzymes from Prp1 in *C. albicans* to Dal82 in *S. cerevisiae*. This conserved Prp1 zinc cluster TF protein underwent a switch in function from regulating the breakdown allantoin into ammonia and carbon dioxide in *C. albicans* to the control of *URA* genes in *S. cerevisiae* (Tebung *et al.* 2016). Despite the many currently characterized ZCFs in *C. albicans*, many non-orthologous ZCFs still have unknown biological roles, and the functions of many structurally orthologous *Ca*ZCFs still require experimental verification. Therefore, the functional study of currently uncharacterized ZCF family members is central to both advancing our understanding of *C. albicans* biology and potentially in directing the development of new antifungal strategies.

Chapter 2: Transcriptional profiling of under-characterized zinc cluster transcription factors

2.1 Introduction

Cells respond and adapt to environmental signals in part by turning on or off the expression of various genes. The regulation of gene function can occur at many stages, such as chromatin accessibility, transcription control, modulation of RNA processing, or even post-translational modification of expressed proteins. In the transcriptional stage, TFs, which bind DNA, function as central decision makers in a cell's response to environmental and developmental cues (Grove and Walhout 2008). Research into these proteins has made significant progress over the past several decades, establishing a better understanding of their role in regulating gene expression and the impact of evolution on their function among related species (Grove and Walhout 2008; Spitz and Furlong 2012).

TFs can be classified into several categories: including the basic helix-loop-helix (bHLH) class, zinc finger class, homeobox family, and leucine zipper family based on their DNA-binding domains (MacPherson *et al.* 2006). In fungi, their roles include regulating major cellular processes including sugar metabolism, amino acid synthesis, meiosis and mitosis, nitrate utilization and response to stimuli and environmental conditions. We are interested in a subclass of class III zinc finger proteins in the fungus *C. albicans*: the zinc cluster proteins or zinc binuclear cluster proteins (Zn(II)2Cys6). Large-scale studies of inactivation mutants uncovered several ZCFs, including Lys144, Zcf8, Zfu2, Zcf22, and Lys14 which control the expression of genes related to virulence development in *C. albicans* (Vandeputte *et al.* 2011; Perez *et al.* 2013). This latter study also reported a transcriptional regulatory network among TFs that orchestrates the commensal and pathogenicity lifestyles of the fungal pathogen (Perez *et al.* 2013). These studies focused on libraries of TF deletions to find regulators involved in *C. albicans* environmental adaptation, antifungal resistance, or host-pathogen interaction *in vivo* and served to identify *C. albicans* virulence factors (Homann *et al.* 2009; Vandeputte *et al.* 2011). However, such inactivation mutant analyses are not an optimal approach in the case of essential genes, where inactivation leads to inviability, and in the case of genes which exhibit functional redundancy, where inactivation of one gene is covered by the remaining redundant member.

As an alternative to deletion studies, a heterologous activation strategy has been applied to

allow functional analyses of ZCFs. A GOF library of zinc cluster TFs generated by the Morschhäuser group has uncovered novel fluconazole resistance regulators, including the multidrug resistance modulator, Mrr1, which controls the multidrug efflux pump Cdr1 (Schillig and Morschhäuser 2013). Screening these libraries can involve phenotypic assessments using collections of challenge conditions. However, it may be possible to get an assessment of the potential function of any TF under analysis by determining the pattern of gene expression in the activated strain. Genes that are up-regulated in expression may represent direct or indirect targets of the TF, and patterns of commonly up-regulated genes may provide insight into the normal regulatory function of the TF.

We therefore monitored the expression profiles of members of this extensive set of activated zinc cluster proteins created by adding a constitutive activation domain from the Gal4 zinc cluster TFs of *S. cerevisiae* to each of the 82 *C. albicans* ZCFs. We initially selected a set of relatively uncharacterized *C. albicans* ZCFs from this previously constructed gain-of-function library of *C. albicans* strains (Schillig and Morschhäuser 2013). Three categories were created from 35 ZCFs. For the first two categories, we included ZCFs that had a best hit candidate in their protein sequence alignments or had an ortholog in the well-characterized model organism *S. cerevisiae*. We used the known or suggested functions of the orthologous and best-hit categories as a reference to investigate the potential functions for the *C. albicans* ZCFs by examining the expression profiles. For the last group, there were no orthologs in the model organism *S. cerevisiae*; therefore, any biological processes linked to the transcriptional profile could not be suggested from ortholog analysis. We were interested to identify genes, pathways, and processes that are unique or nearly unique to each ZCF. The study can also identify genes, pathways, and processes across subgroups of ZCFs. The selected ZCFs have been analyzed by transcription-based RNA sequencing (RNA-seq). The top significantly expressed genes identified by differential analysis are anticipated to be genes regulated by the specific Zn (II)2Cys6 protein under investigation. Gene ontology (GO) analyses were performed on gene lists obtained by RNA-seq to identify putative enriched biological functions, processes, or cell compartments: (GO Term Finder; http://www.candidagenome.org/cgi-bin/GO/goTermFinder) to assign differentially regulated genes to specific biological processes.

2.2 Methods

2.2.1 Construction of the TF-GOF strains

We utilized a subset of the Schillig and Morschhäuser GOF collection for the ZCFs (Schillig and Morschhäuser 2013). For 35 of these TFs-GOF strains, two independent biological replicates obtained from two independent colonies grown on the same plate derived from the same initial transformation were harvested for downstream profiling. The colonies were grown in YPD medium, at 30 °C, with shaking at 220 rpm. Total RNA was extracted using the RNeasy minikit protocol (Qiagen Inc.), and RNA quality and quantity were determined using a BioAnalyzer (Agilent Inc.). Paired-end sequencing (2x150bp) of the RNA samples was carried out at the McGill University and Quebec Genome Innovation Center using the Illumina miSEQ sequencing platform. The TFs were profiled in five batches with two biological replicates for each target ZCF. Controls profiles (n=2) were included for all but batch 3

2.2.2 Bioinformatics

The data were used for Gene Ontology analyses was performed by the CGD tool "Go Term Finder" (http://www.candidagenome.org/cgi-bin/GO/goTermFinder) (Inglis *et al.* 2012). The protein sequences were aligned using SIM Alignment (http://web.expasy.org/sim/) (Huang and Miller 1991), and a graphical representation was created using the LALNVIEW program. (Duret *et al.* 1996).

2.2.3 Basic pipeline for processing RNA-seq files

Raw reads were examined and pre-processed with the tools from the Trim Galore package (REF). FASTQC was used to assess quality of the reads and cutadapt (version 0.4.1) was used for trimming and filtering (`--phred33 --length 36 -q 5 --stringency 1 -e 0.1`). Paired-end reads were then mapped against the *C. albicans* SC5314 haplotype A, version A22 downloaded from the Candida Genome Database (www.candidagenome.org) using HISAT2 (version 2.0.4). SAMtools was then used to sort and convert SAM files (Kim *et al.* 2019). The read alignments and *C. albicans* SC5314 genome annotation were provided as input to StringTie (version 1.3.3) (Pertea *et al.* 2015), a tool which estimates abundances of each gene in each sample.

2.2.4 Basic statistics, informatics and visualization

Analysis was conducted using the R programming language (version 4.0.3) (R Core Team, 2020). Fisher's exact test was conducted using the newGOM function in the R package GeneOverlap (Shen, 2013). Pairwise overlap was visualised using the drawHeatmap function with a cutoff value of 0.01 for adjusted p-value, using the Benjamin-Hochberg method. Cytoscape (version 3.6.1) (Shannon *et al.* 2003) was used to visualize the TF networks; Cytoscape was accessed using the R package Rcy3 (version 3.7) (Gustavsen *et al.* 2019). Edges were set at log2 fold change of 2 for upregulated and log2 fold change of -2 for downregulated genes.

2.2.5 Normalization

We opted to use the so-called median of ratios approach from DESeq2 to normalize data, since this approach is particularly suited for cross-sample comparisons. The approach does not normalize for gene length but does normalize across the total number of reads generated per sample. The approach also normalizes for bias in the RNA composition of transcripts, a property that may affect the propensity for a transcript to be sequenced. First, the row-wise (gene-wise) geometric mean is calculated for each gene, referred to as a pseudo- reference as follows

$$X'[g,i] = X[g,i] / (\Pi_k X[g, k])^{1/m}$$

where X is the gene n x m gene by sample count matrix. Second, normalization between differences in counts across samples is addressed by dividing by the column medians:

$$X''[g,i] = X'[g,i] / \text{median}(X'[,i]),$$

followed by a log2 transformation.

2.2.6 Data access

A detailed list of differentially expressed genes for 35 ZCFs is available at the following address: https://github.com/Rahaomran/Raha-Omran.git

2.3 Results and discussion

2.3.1 Transcriptional profiling of ZCFs gain-of-function in *C. albicans*

The data used in this study were generated by RNA-sequencing, using the Illumina MiSeq RNA-sequencing method. The ZCFs gain-of-function mutants were obtained from Morschhäuser's lab (Schillig and Morschhäuser 2013). The mutants contain a conserved DNA binding domain with the motif CX2CX6CX5–12CX2CX6–8C for each of the ZCFs, fused to a constitutively active activation domain obtained from the *S. cerevisiae* Gal4 protein. The activated version of each ZCF was placed under the control of the strongly expressed *ADH1* promoter in the wild type strains of *C. albicans* SC5314; therefore, the target TF should be both hyperactive and overexpressed in the modified strain when compared to the Wild-type. We selected strains expressing 35 of the 82 Zinc cluster transcription factors with GOF mutants that were viable and used them for transcriptional profiling with Illumina MiSeq technology with two biological replicates. The 35 ZCFs were selected based on three criteria; the first set consists of ZCFs that lack an ortholog in *S. cerevisiae* and whose function is unknown. The second group has an ortholog in *S. cerevisiae*, however, their function has not been confirmed in *C. albicans*. Our third set involves genes (with or without orthologs in *S. cerevisiae*) with verified functions in *C. albicans* that can be used to validate our approach for elucidating the ZCFs' function based primarily on its transcriptional profile. Dr. V. Dumeaux processed the RNA-seq profiles by performing basic quality control and trimming of the reads, and then aligning them to the *C. albicans* SC5314 haplotype A/version A22. Anika Vankooten from Dr. Michael Hallett's lab prepared the differential expression profiles of individual ZCFs.

2.3.2 Genes differentially expressed by each ZCF

We identified genes that are differentially expressed for each ZCF. For each ZCF, we applied a linear model from the DESeq2 (Love *et al.* 2014) R package to identify all genes whose expression differs significantly from the control, with log2 fold ≥ 1.5 and adjusted P-value < 0.05. We investigated possible functional patterns within the up-regulated genes using the GO term finder for processes or functions for each ZCFs using a P-value cut-off of ≤ 0.00001. We also suggest hypothetical functions based on an enrichment of genes related to a common biological process when there is no indication from GO term analysis.

CTF1 encodes a protein with a Zn(2)-Cys(6)-type zinc finger motif in the N-terminal domain. There is no highly similar protein in *S. cerevisiae*. The best hit for aa sequence similarity in *S. cerevisiae* is *ASG1* with 21% identity over the zinc cluster region (Figure 2.1); *ASG1* encodes a protein that regulates the stress response and fatty acid utilization in the model yeast (Jansuriyakul *et al.* 2016). Ramirez and Lorenz determined that in *C. albicans CTF1* function is to control β-oxidation of fatty acids and peroxisome biogenesis. Mutants lacking *CTF1* exhibited no growth on fatty acids as sole carbon sources and reduced expression of β-oxidation enzymes like *POT1*, *POX1*, *ECI1* and the peroxisomal import receptor *PEX5.* In addition, the *CaCTF1* mutant slightly reduced virulence (Ramírez and Lorenz 2009). Our transcriptional profiling of Ctf1-GOF showed positive expression of genes involved in fatty acid degradation like *FOX2*, *POT1*, *POX1-3*, *PEX5* (Appendix 1). It also showed upregulation of core genes required for alternative carbon metabolism like isocitrate lyase (*ICL1*) (Appendix 1). The GO-term analysis identified enrichment in fatty acid β-oxidation processes. These findings connecting *CTF1* to a role in β-oxidation of fatty acids and peroxisome biogenesis contrasted with a previous study by Coste and coworkers, who identified three *C. albicans* zinc finger transcription factors, Ctf1 as well as Cta4 and Asg1, using *S. cerevisiae* screens to identify suppressors of mutants in *PDR1* and *PDR3*. Because Pdr1 and Pdr3 transcriptionally control drug pumps in *S. cerevisiae*, and when defective lead to multidrug sensitivity, it was anticipated that Cta4, Asg1 and Ctf1 would be regulators of drug pumps and drug response in *C. albicans*. However, fluconazole susceptibility was not altered after deletion of *CTA4*, *ASG1*, and *CTF1* in *C. albicans* and there were no expression changes in the ABC-transporter genes *CDR1* and *CDR2* (for *Candida* drug resistance) or the major multidrug transporter gene *MDR1* (Coste *et al.* 2008) in these strains. They also found no phenotype for carbon utilization in a *CTF1* mutant, but suggested a possible role for *CaASG1* as a regulator of glyoxylate cycle genes (Coste *et al.* 2008). Overall, our Ctf1-GOF profiling phenotype agrees with the proposed role of *CTF1* in β-oxidation of fatty acids and peroxisomal biogenesis suggested by others (Ramírez and Lorenz 2009).

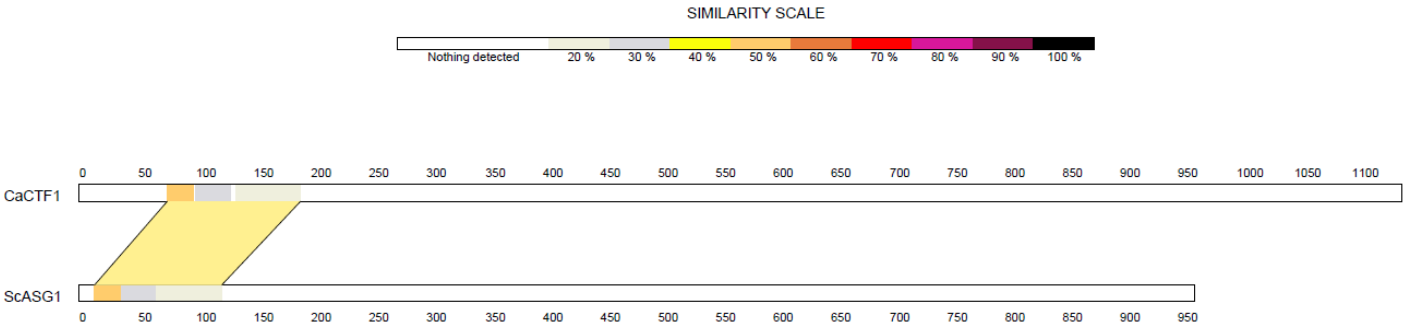


Figure 2.1: *C. albicans* Ctf1p aligned with *S. cerevisiae* Asg1. The shaded alignment area has 21% identity. This shows that although the *S. cerevisiae ASG1* gene is the closest match to *CTF1* in *C. albicans*, the genes are not orthologs; the similarity is limited to the zinc cluster domain.

In *C. albicans*, ***ASG1*** the actual ortholog of *ScASG1*; the name stands for activator of stress response genes, and the transcription factor directly regulates the utilization of fatty acids and accumulation of lipids in *S. cerevisiae*. *Sc*Asg1 stimulates genes in β-oxidation (*POX1*, *FOX2*, and *POT1*), gluconeogenesis (*PCK1*), the glyoxylate cycle (*ICL1*), triacylglycerol breakdown (*TGL3*), and peroxisomal transport (*PXA1*) (Jansuriyakul *et al.* 2016), and thus provides essentially the same function in *S. cerevisiae* that *CTF1* provides in *C. albicans*. *CaASG1* is 40% identical to the *S. cerevisiae* protein (Figure 2.2). As noted, a previous study showed *CaASG1* complements the pleiotropic drug sensitivity caused by loss of the multidrug-transporter-regulating transcription factors Pdr1/Pdr3 in *S. cerevisiae* (Coste *et al.* 2008). While deletion of *ASG1* in *C. albicans* did not affect fluconazole susceptibility and did not alter the expression of the ABC-transporter genes *CDR1* and *CDR2* or the major facilitator gene *MDR1* (Coste *et al.* 2008), we found upregulation of genes involved in fluconazole resistance like *CDR1*, *MRR1*, *PDR16* and *SNQ2* in the activated Asg1 strain (Appendix 2). As well, we observed upregulation of fluconazole repressed genes such as *FCR1,* a negative regulator of drug resistance (Talibi and Raymond 1999). Phenotypic screening showed that *CaASG1* is required to sustain growth on non-fermentative carbon sources (sodium acetate, acetic acid, ethanol) (Coste *et al.* 2008). However, our Asg1-GOF transcriptional profile revealed no upregulation of genes related to β-oxidation, gluconeogenesis and the glyoxylate cycle; as noted, this circuit appears controlled by Ctf1 in the pathogen. An *in vivo* study of *C. albicans* ZCFs revealed that the *ASG1* homozygous deletion results in decreased hyphal growth

(Vandeputte *et al.* 2011). Our transcriptional profile supports this link as it shows upregulation of either characterized or putative genes involved in hyphal growth like *GPR1*, *ORF19.3088*, *ORF19.4634* and *CLG1*. Asg1-GOF significantly induced many genes (279) above log2 fold ≥ 1.5, padj ≤ 0.05. Due to the presence of 279 positively expressed genes associated with *ASG1,* including several transcription factors, the identification of ontology terms may be challenging. In particular, a major fraction (35 out of 105) of the genes that encode the proteins acting in the ribosomal large subunit assembly process are upregulated.

There were also several transcription factors among the upregulated genes in the Asg1-GOF strain such as *ZCF35* (profiled here in a later section), *CTA8*; an essential transcription factor that mediates heat shock transcription induction (Nicholls *et al.* 2009), *SUT1* with an established role in *C. albicans* virulence (Xu *et al.* 2015), *SEF1* which regulates iron uptake (Chen *et al.* 2011), *CRZ1 a* calcineurin-regulated C2H2 transcription factor (Santos and de Larrinoa 2005), *ADR1*, *PDC2*, a regulator of pyruvate decarboxylase (Kaiser *et al.* 1999), *HAP42* and *HAP41* (Johnson *et al.* 2005), *GAL4* (Askew *et al.* 2009), and *MRR1* a regulator of the plasma membrane multidrug efflux pump *MDR1* (Dunkel *et al.* 2008). In the Asg1-GOF profile, for example, one of the upregulated ZCFs, Zcf35, itself activates 330 expressed genes. *ASG1* appears to perform multiple functions, and it looks to cooperate with other transcription factors. Combined double knockout mutants of *ASG1* and the most prominent transcription factors it apparently regulates may provide insight into its function in *C. albicans*. Compared to previous findings, the *ASG1* revealed both positive and negative roles in fluconazole resistance and no involvement in utilization of non-fermentable carbon sources.

The transcriptomic profiles of activated *ASG1* and *CTF1* revealed more details about their roles in *C. albicans*. We are not surprised that *C. albicans CTF1* did not exhibit the phenotype of multidrug resistance because *Sc*Asg1, its closest TF by sequence comparison, plays a role in fatty acid catabolism, and the Ctf1-GOF profile along with Lorenz's studies support its role into β-oxidation of fatty acids. The orthologs *Ca*Asg1 and *Sc*Asg1 appear to have different functions between the two closely related organisms. This could be an example of 'transcriptional rewiring' in which transcription factors of two closely related organisms that are orthologs by sequence similarity show functional assays identifying divergent functions (Martchenko *et al.* 2007).

Overall, The *Ca*Asg1-GOF transcriptional profile suggests its role as an activator and repressor for multidrug drug resistance which will require further investigation.

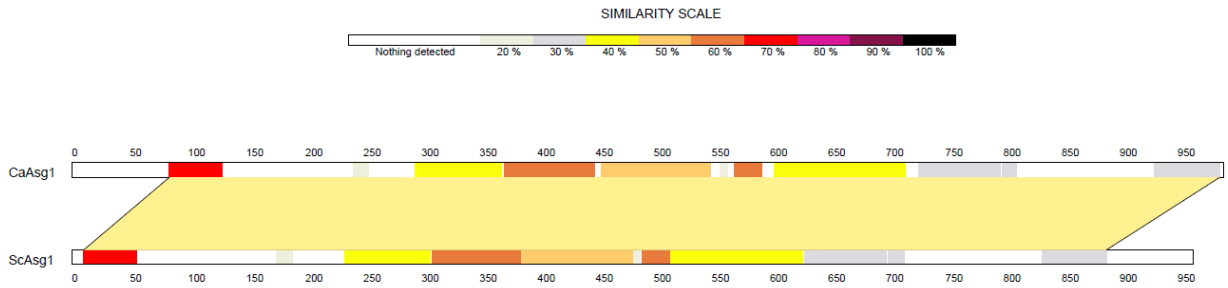


Figure 2.2: *Ca*Asg1 aligned with *Sc*Asg1. The result of sequence alignment displays 40% sequence identity spanning 925 amino acids.

C. albicans ***ARO80*** encodes a Zn(2)-Cys(6) finger TF with a DNA-binding motif with 40% similarity with its *S. cerevisiae* homolog over a stretch of 587 amino acids (Figure 2.3). *C. albicans* produces aromatic alcohols such as phenethyl alcohol, tyrosol, and tryptophol by using the same pathways that, in *S. cerevisiae,* are under Aro80 and pH signaling pathway regulation (Figure 2.4). *C. albicans* Aro80 positively controlled expression of genes for transamination (*ARO8*, *ARO9*), decarboxylation (*ARO10*), and then reduction by alcohol dehydrogenase, depending on pH and availability of oxygen (Iraqui *et al.* 1999; Ghosh *et al.* 2008) (Figure 2.4). The Aro80-GOF transcriptional profile agrees with its suggested role in controlling aromatic alcohol production as it showed upregulation of *ARO8*, *ARO9, ARO10* and *ADH5* (Figure 2.4, highlighted in yellow, Appendix 3). Interestingly, the Aro80-GOF transcriptional profile is also enriched in genes encoding enzymes required for biosynthesis of amino acids such as arginine and glutamine, upregulating the *ARG1*, *CPA1/2*, *ARG5, 6* and *8*, *CAR2*, *PUT1, GAD1, ECM42* genes as well as the amino acid permease encoding gene *CAN2* (Appendix 3). The cluster frequency of GO term supported the transcriptional profile for biosynthesis of cellular amino acids and not exclusively the aromatic alcohol process. Overall, the profile suggests a new role of the transcription factor

Aro80 in coordinating expression of genes involved in aromatic alcohol production and biosynthesis of amino acids as opposed to its close relative *S. cerevisiae.*

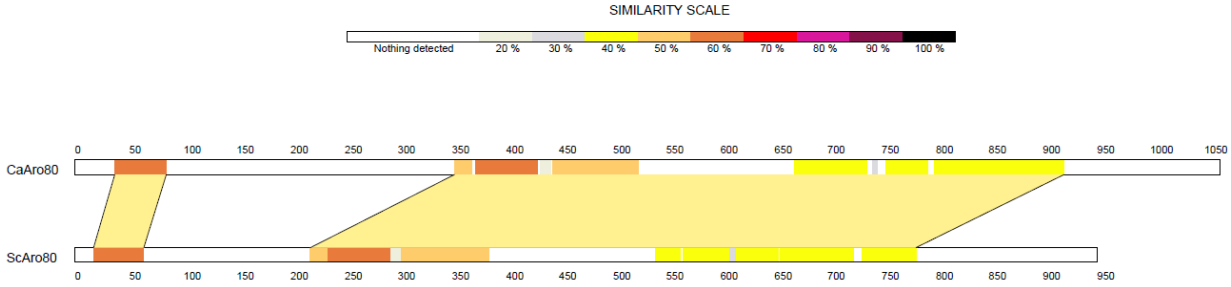


Figure 2.3: *C. albicans* Aro80 aligned with *S. cerevisiae* Aro80. The shaded alignment area has average 40% identity and includes the zinc finger domain at the amino terminus around aa 50.

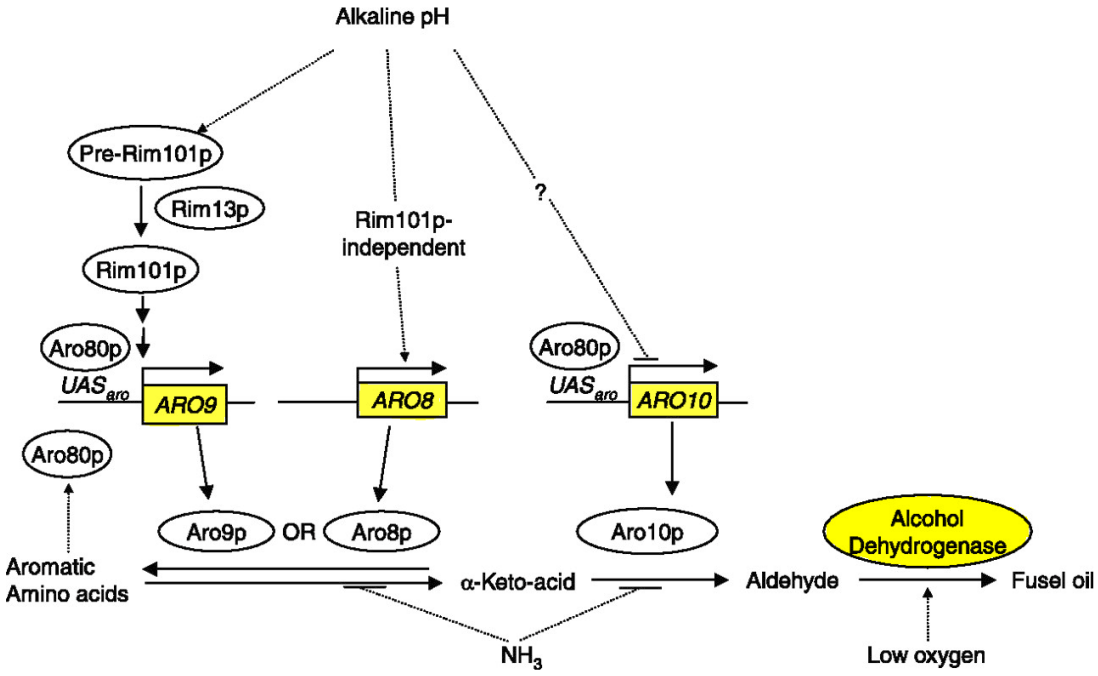


Figure 2.4: A diagram illustrating regulation of aromatic alcohol production from aromatic amino acids in *C. albicans* (Ghosh *et al.* 2009). Genes highlighted in yellow show upregulation in the Aro80-GOF transcriptional profiles.

CTA7 is a member of the ZCF family having *STB4* as an ortholog in *S. cerevisiae*; these two proteins have 31% sequence identity spanning 567 amino acids (Figure 2.5). *Sc*Stb4 is predicted to regulate transporters in a study by Ward and Bussemaker. To determine the TF's

functional regulatory target, the authors analyzed nucleosome occupancy, ChIP-chip, and *S. cerevisiae* expression data. A model was built by calculating the total promoter affinity of functional target genes of those TFs and comparing it across orthologous promoters (Ward and Bussemaker 2008).

A two-hybrid screen assay identified *ScSTB4* as encoding one of the five yeast proteins, Stb1-Stb5, that interact with Sin3. Sin3 is part of Rpd3S and Rpd3L histone deacetylase complexes which does not bind DNA directly and is involved in repression and activation of diverse processes, including mating-type switching and meiosis; the complex is also involved in the maintenance of chromosomal integrity (Kasten and Stillman 1997). It has been suggested that Stb1 and Stb2 are required in part for the Sin3 transcriptional repression (Kasten and Stillman 1997). A large-scale phenotypic study showed a reduction in invasive growth for the *cta7* heterozygous mutant in *C. albicans* (Oh *et al.* 2010). Our data from the activated construct demonstrated increases in genes encoding hyphal cell wall and GPI-anchored proteins, including *ALS2*, *ALS4*, *ALS9*, *IHD1*, *FLO9*, *GPI14*, *PGA45* and *PGA57*, consistent with Cta7 having a positive effect on hyphal development, which is linked to invasiveness (Appendix 4). Interestingly, we observed both upregulation and downregulation of genes involved in drug, glucose, and phosphate transporters. Cta7-GOF revealed significant downregulation of drug and glucose transporters including *CDR1*, *FLU1, ROA1, ORF19.304*, *ORF19.6578*; *PXA1*, *RGT1*; a transcriptional repressor of glucose transporter genes, *HNM1*; Putative choline/ethanolamine transporter, *ORF19.4384.1*, *PHO84*, *ORF19.3782*, *HGT1, HGT16*, *HGT2*, *HXT5*, *NAG3* and *HGT1.* We also observed upregulation of either putative or characterized transporter genes like *ORF19.2350*, *ORF19.6209*, *ORF19.3395*, *QDR2*, *BIO5*, *ORF19.7554*. As for the other ZCF members in the Cta7-GOF profile, *ZCF20* (profiled here in a later file) and hyphal regulator *ZCF1* are upregulated, whereas *ZCF2* and *ZCF23* are downregulated, but their functions remain unknown. We can predict the role played by *CTA7* in *C. albicans* filamentation and transcriptional activator and repressor for the transporter genes, which is unlike the role of its ortholog gene in *S. cerevisiae*.

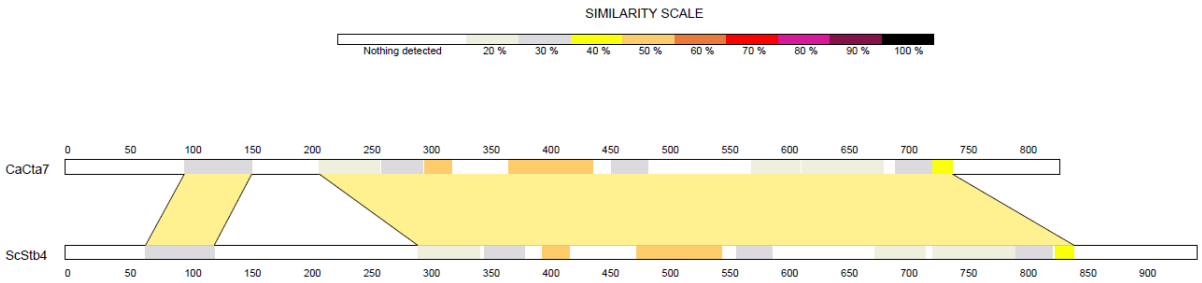


Figure 2.5: *C. albicans* Cta7p aligned with *S. cerevisiae* Stb4p. The result of sequence alignment with 31 % sequence identity over 567 amino acids.

Leu3 is the *S. cerevisiae* ortholog of *C. albicans* Leu3 (45% identity); the yeast transcription factor regulates branched-chain amino acid synthesis and ammonia assimilation (Figure 2.6). In particular, *LEU1*, *LEU2*, *LEU4* and *ILV2* are leucine biosynthesis genes whose expression is controlled by the transcription factor *ScLEU3* (Friden and Schimmel 1988). This transcriptional regulator (*Sc*Leu3) represses transcription in the absence of *LEU4* (α-isopropylmalate) and becomes a strong transcriptional activator in its presence (Kohlhaw 2003) (Figure 2.7). There are four genes specific for leucine biosynthesis; *LEU4* and *LEU9* (encoding the α-isopropylmalate synthase isoenzymes), *LEU1* (isopropylmalate isomerase), and *LEU2* (β-isopropylmalate dehydrogenase) (Kohlhaw 2003) (Figure 2.7). Although *C. albicans* and *S. cerevisiae* share the leucine pathway, this pathway is poorly characterized in *C. albicans*. (Figure 2.8). In *C. albicans*, there is an ortholog of the *S. cerevisiae LEU4*/*ORF19.6086* as well as another related gene, *LEU42*/*ORF19.1375*, but there is no designated ortholog of *S. cerevisiae LEU9* in *C. albicans* (Remm *et al.* 2001). *LEU4/LEU9* paralogs in *S. cerevisiae* seemed to be result of the whole genome duplication (WGD) proceeding from the pre-whole-genome duplication status of the precursor leading to *C. albicans*. The *LEU4/LEU42* paralogs in the *C. albicans* branch would have generated 4 paralogs of *LEU4/LEU42* during the WGD, then a pair of these duplicated genes need be lost to get to the 2 paralogs in *S. cerevisiae.* The sequence similarity and phylogeny tree of these genes suggest that the two paralogs that were conserved in *S. cerevisiae* were both derived from the *LEU42* branch (Figure 2.9 and 2.10).

While we observed upregulation of the leucine biosynthesis enzymes *LEU4* and *LEU42*, there was no evidence of upregulation of *LEU1*, *LEU2* and *ILV2* in the Leu3-GOF profile (Appendix 5). According to our findings, the amino acid transmembrane transporter and permease genes *CAN3* and *GAP6* respectively are upregulated. Using GO term analysis, we also observed upregulated genes involved in biotin biosynthesis. Leu3-GOF induces *GLN3*, which is implicated in nitrogen starvation-induced filamentous growth (Dabas and Morschhäuser 2007), *HMX1*, which is heme oxygenase (Santos *et al.* 2003), the membrane transporter involved in biotin import *VHT1* (Sprenger *et al.* 2020) as well as amino acid permease *CAN3* (Lan *et al.* 2002). The transcriptional data suggest that *CaLEU3* participates in leucine biosynthesis by just controlling *LEU4*, distinct from that of its ortholog *ScLEU3;* this supports our approach to identifying unknown functions of transcription factors. *LEU3* may play a role in the initial stages of leucine biosynthesis, which could still allow efficient control of the pathway. This could suggest *C. albicans* has a distinct regulation pattern of branched-chain amino acids.

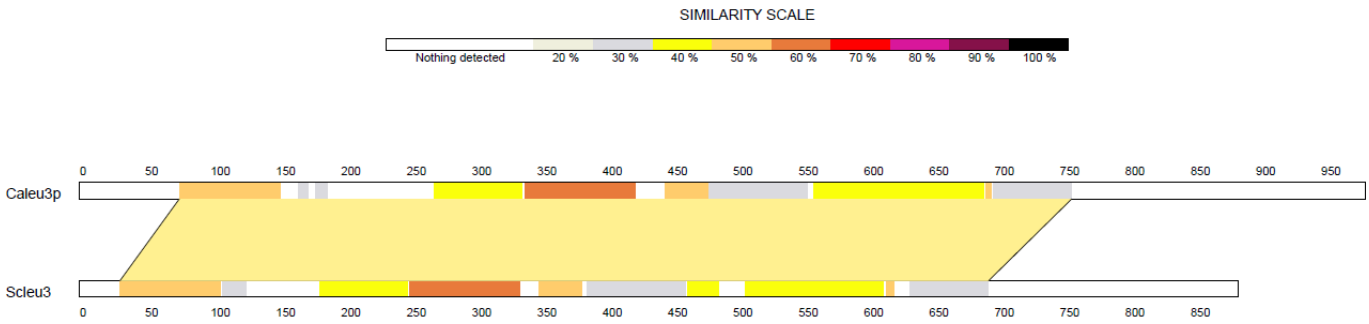


Figure 2.6: *C. albicans* Leu3 aligned with *S. cerevisiae* Leu3p. The result of sequence alignment with 45% sequence identity covering 720 amino acids.

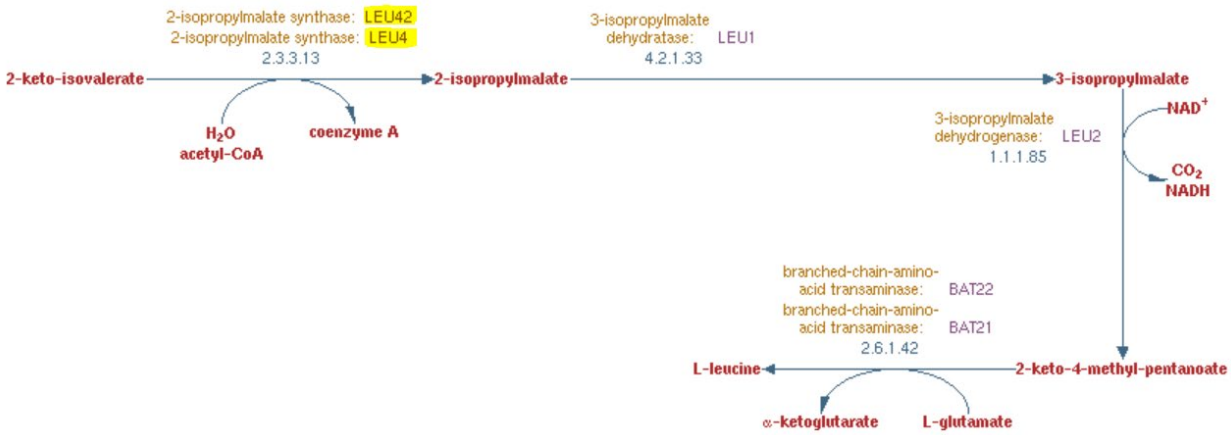


Figure 2.7: *Saccharomyces cerevisiae* L-leucine biosynthesis pathway.

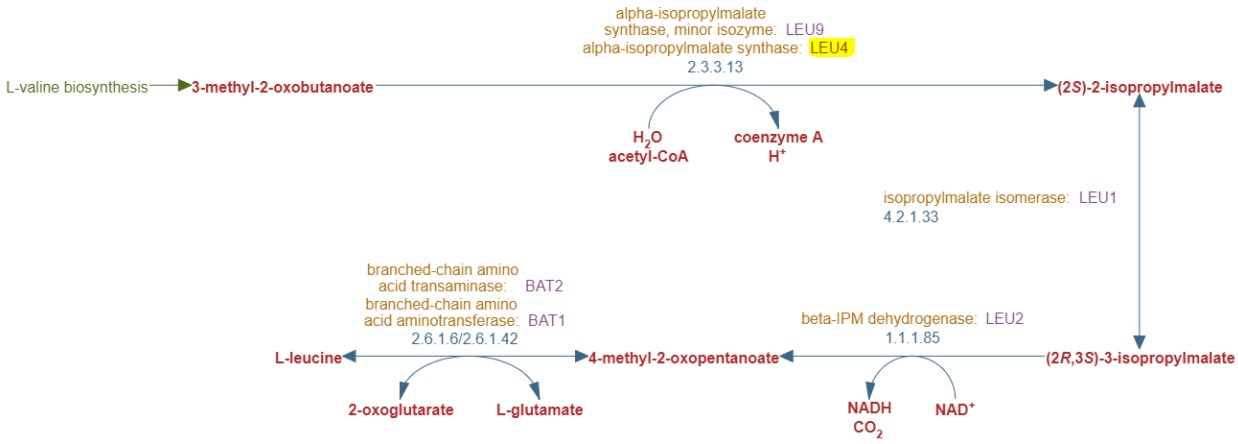
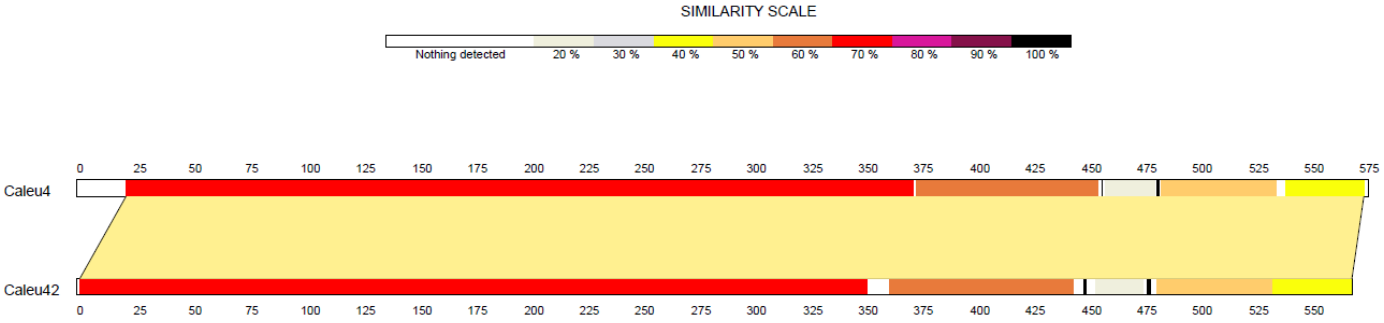


Figure 2.8: *Candida albicans* L-leucine biosynthesis pathway.

A.


SIMILARITY SCALE
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30 %
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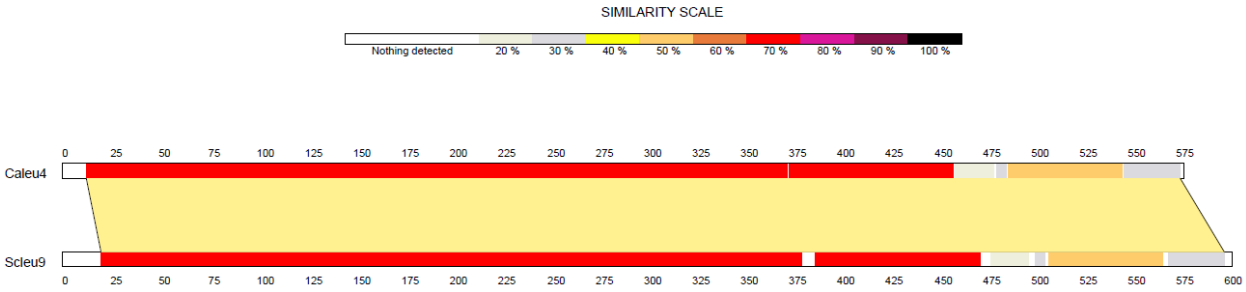
Caleu4
Caleu42

B.


SIMILARITY SCALE
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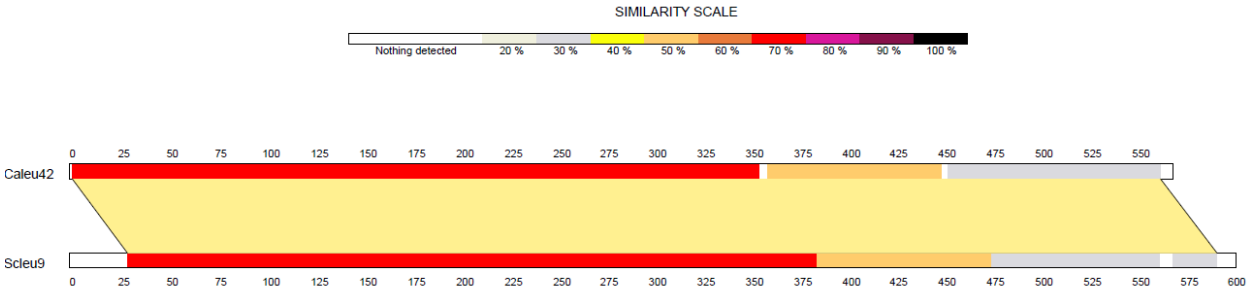
Caleu4
Scleu9

C.


SIMILARITY SCALE
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Caleu42
Scleu9

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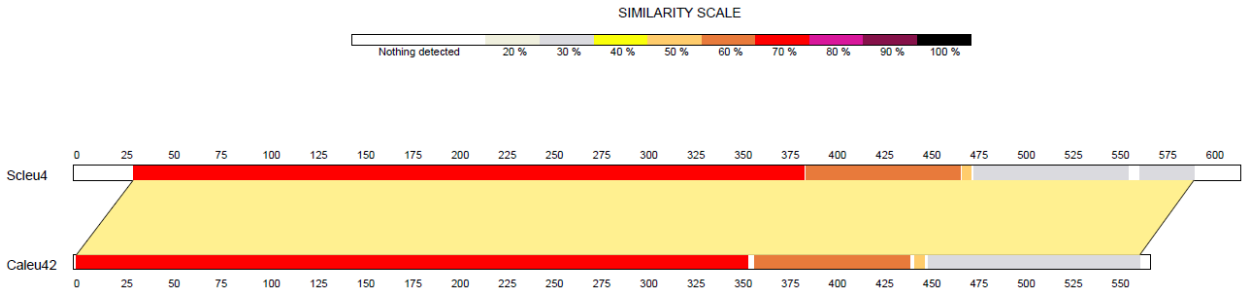


Figure 2.9: Sequence alignment comparison between *C. albicans Leu4/Leu42 and S. cerevisiae Leu4/Leu9* **A**. *C. albicans* Leu4 aligned with *C. albicans* Leu42. The result of sequence alignment with 65% sequence identity covering 575 amino acids. **B**. *C. albicans* Leu4 aligned with *S. cerevisiae* Leu9. The result of sequence alignment with 65% sequence identity for 581 amino acids length. C. *C. albicans* Leu42 aligned with *S. cerevisiae* Leu9. The result of sequence alignment with 61% sequence identity crossing 571 amino acids. **D**. *S. cerevisiae* Leu4 aligned with *C. albicans* Leu42. The result of sequence alignment with 70% sequence identity crossing 581 amino acids.

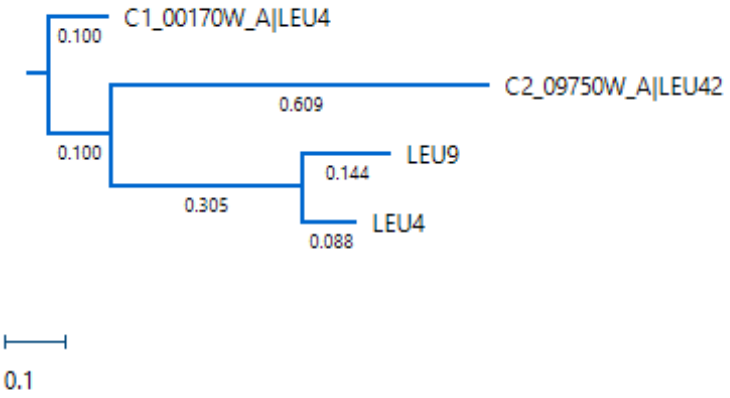


Figure 2.10: Phylogeny tree of *C. albicans* C1_00170W_A|Leu4, C2_09750W_A|Leu42 and *S. cerevisiae* Leu4 and Leu9.

Zcf16 is the ZCF family member related to *Sc*Sip4 (57% sequence identity limited to the zinc cluster domain), with the yeast gene playing a role in binding to the carbon source-responsive element (CSRE) of gluconeogenic genes in *S. cerevisiae*. *Sc*Sip4 regulates the glyoxylate cycle genes *ICL1* (generating succinate and glyoxylate from isocitrate) and *MLS1* (encoding malate synthesis) and glucogenesis genes *PCK1* (encoding phosphoenolpyruvate carboxykinase) and

FBP1 (encoding fructose 1,6- bisphosphatase) (Valdés-Hevia *et al.* 1989; Fernández *et al.* 1992; Hernández-Cervantes *et al.* 2020). *ICL1*, *MLS1*, *FBP1*, *PCK1* have CSREs in their promoter regions and are expressed when glucose is substituted for ethanol or acetate, while being repressed when glucose is present (Vincent and Carlson 1998).

CaZCF16 encodes a 1078 amino acid protein with a zinc cluster type DNA-binding domain at the N-terminus that shows limited sequence similarity to that of *Sc*Sip4; Zcf16 is unrelated to *Sc*Sip4 in the rest of the encoded protein (Figure 2.11). A *ZCF16* homozygous deletion increased colony wrinkling in *C. albicans* (Vandeputte *et al.* 2011); wrinkling is a common characteristic of colonies with a high proportion of hyphal cells. As well, Zcf16-GOF significantly upregulates all the four genes in *C. albicans ICL1*, *MLS1*, *FBP1*, *PCK1* that make up the Sip4 regulon in *S. cerevisiae* (Appendix 6). Zcf16-GOF also downregulated the glucose transporter encoding genes *HGT2* and *HGT12*. Upregulated genes are associated with carbon utilization processes; the gene enrichment of Zcf16 upregulated genes was quite specific for carbohydrate transporter genes. Overall, the profile suggests *CaZCF16* may function in the *C. albicans* gluconeogenesis pathway, and may be functionally equivalent to Sip4 in *S. cerevisiae*. Thus, while sometimes clearly orthologous transcription factors may have distinct functions in *S. cerevisiae* and *C. albicans*, there also appear to be cases where non-orthologous TFs with similar DNA binding domains can regulate the same circuits in the two fungi.

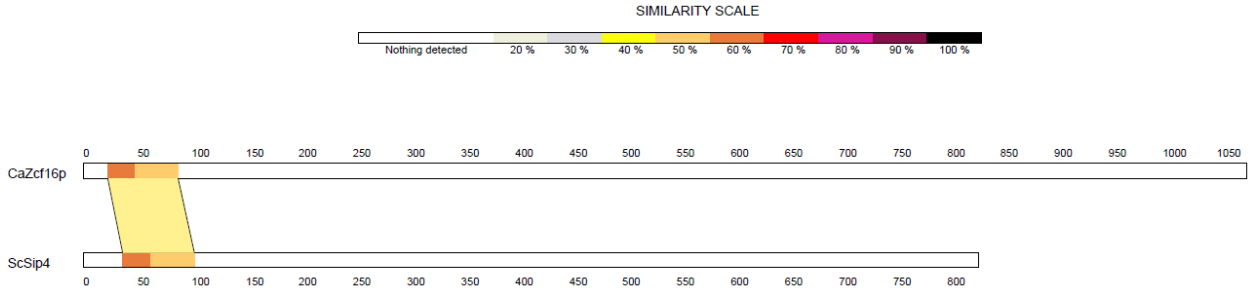


Figure 2.11: *C. albicans* Zcf16 aligned with *S. cerevisiae* Sip4. The result of sequence alignment with 57% sequence identity covering 68 amino acids length.

Zcf20 has limited similarity to Hap1 in *S. cerevisiae* with 45% sequence identity in the zinc cluster type DNA-binding domain at the N-terminus covering 67 amino acids (Figure 2.12) *ScHAP1* is involved in response to the level of oxygen and heme (Pfeifer *et al.* 1989), as Hap1 is a heme-responsive activator of genes induced in aerobic conditions. Our transcriptional profile of activated Zcf20 showed upregulation of the ferric reductase genes *FRP1* and heme oxygenase *HMX1* that use hemin iron which suggest its role in *C. albicans* could be related to that of *Sc*Hap1. Zcf20-GOF also highly induced expression of the hyphal regulator *EFH1* and GPI-linked cell wall component-encoding gene *RBT5* (Braun and Johnson 2000; Doedt *et al.* 2004) (Appendix 7). The *ZCF20* heterozygous mutant causes cell size increases in *C. albicans* (Chaillot *et al.* 2017). In the regulation of iron acquisition, *Ca*Sef1 is a transcriptional activator of iron uptake genes and promotes virulence in a mouse model of bloodstream infection (Chen *et al.* 2011). Chen *et.al*. 2011 demonstrated, by RNA expression profiling of *sef1Δ/Δ* and wild type *C. albicans* under iron-limiting conditions, the downregulation of genes including *ZCF20* affecting *C. albicans* iron homeostasis. Zcf20-GOF upregulated 37 genes significantly enriched in processes like amino acid transport and cellular iron ion homeostasis (Appendix 7). The transcriptional profile suggests *CaZCF20* has functions in iron hemostasis, expression of amino acid transporters and plays a positive role in *C. albicans* filamentation.

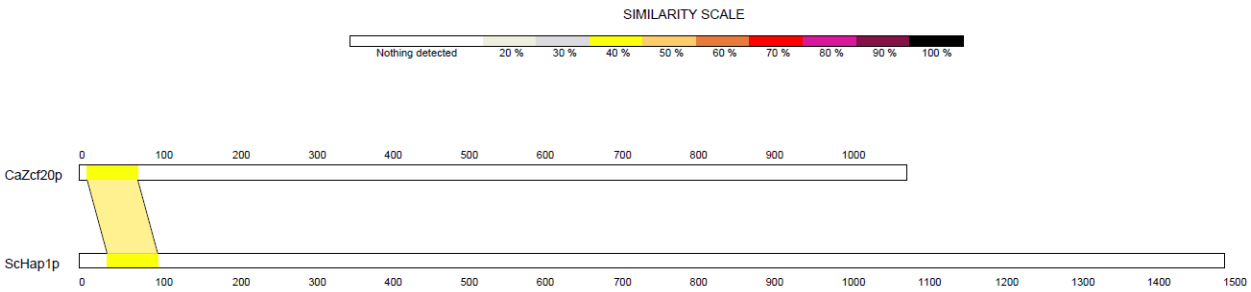


Figure 2.12: *C. albicans* Zcf20 aligned with *S. cerevisiae* Hap1. The result of sequence alignment with 45% sequence identity spanning 67 amino acids.

Tea1 is a putative ZCF member with similarity in *S. cerevisiae* Tea1 with 46% sequence identity (Figure 2.13). *TEA1* is present in other *Candida* species. *ScTEA1* is involved in Ty retrotransposon enhancer-mediated transcription (Gray and Fassler 1996). Based on the large-scale studies, lack of *CaTEA1* caused abnormal morphology, an increase in hyphal growth, an invasion decrease and flucytosine drug sensitivity (Homann *et al.* 2009; Vandeputte *et al.* 2011). Hyphal regulator *BRG1*, hyphal cell wall protein encoding *ALS1*, and *RHB1*, encoding a small G protein from the Ras family involved in cell wall integrity and filamentous growth, are noticeable filament-related downregulated genes (Sheppard *et al.* 2004; Tsao *et al.* 2009; Du *et al.* 2012) (Appendix 8). This is consistent with the increases of hyphal growth for the deleted *TEA1* strain. In addition, Tea1-GOF profiles showed upregulation of the genes for the GPI-anchored protein Rbt5 and filament regulators, such as Cph1, which could be involved in decreased invasion (Appendix 8). In addition, Tea1-GOF upregulated other ZCFs members like *ZCF22* (profiled here with an implied role in controlling sterol synthesis) and *ZCF10* (profiled here with possible role in filamentation), which might indicate a regulatory network among these three ZCFs. This finding suggests *TEA1* plays both a positive and negative role in regulating *C. albicans* filamentation.

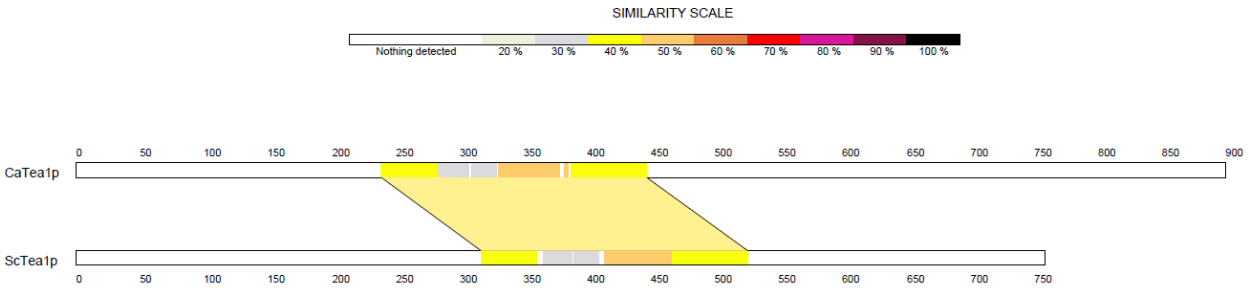


Figure 2.13: *C. albicans* Tea1 aligned with *S. cerevisiae* Tea1. The result of sequence alignment with 46 % sequence identity covering 217 amino acids.

Zcf23 has an ortholog in *S. cerevisiae*, Gsm1, which is proposed to be involved in regulation of energy metabolism (Ho *et al.* 2006). Zcf23 aligned with *S. cerevisiae* Gsm1 showed 30% sequence identity spanning 541 amino acids (Figure 2.14). Activated Zcf23 expressed a large number of genes (n=633), and while many of the over-expressed ones are poorly characterized, there is an enrichment for carbohydrate and hexose metabolic processes. Zcf23-GOF up-regulated zinc finger and zinc cluster TFs including *TRY5* and *SUC1,* respectively, which are both implicated in cell adherence, *RME1* involved in fluconazole resistance, *ZCF39*, *ZCF27* (profiled here with possible role in filamentation), *ZCF9* (profiled here with suggested role in copper transport) and *ZCF29* a hypocolonizer in host organs (Vandeputte *et al.* 2011) (Appendix 9). *ZCF5* with unknown function and *ZCF17,* a negative regulator of hyphal development, are among the downregulated genes (Appendix 9). Our transcriptional profile suggests that *ZCF23* is positively involved in *C. albicans* filamentation and metabolism that requires further investigation. It could be an example of a "rewiring event" where Zcf23 function diverged from controlling metabolism and filaments in *C. albicans* to only controlling energy metabolism in *S. cerevisiae*.

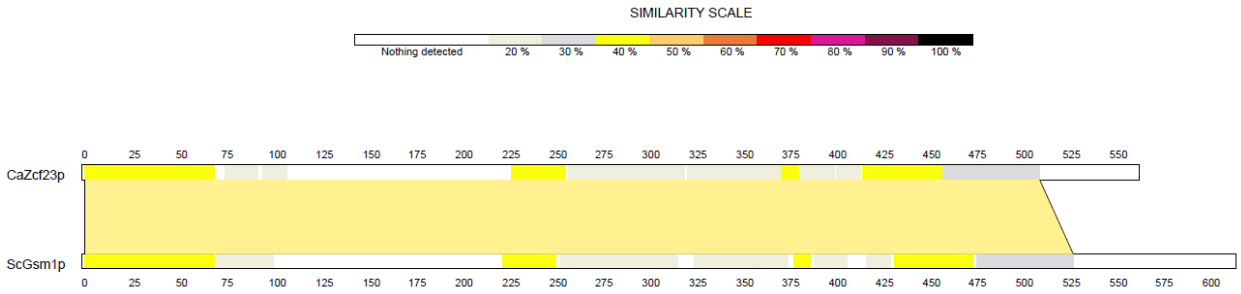


Figure 2.14: *C. albicans* Zcf23 aligned with *S. cerevisiae* Gsm1. The result of sequence alignment with 30% sequence identity involving 541 amino acids.

ORF19.2230 encodes a ZCF member homologous to *Sc*Rds3. *ScRDS3* encodes a Zinc cluster protein with 57% sequence identity covering 110 amino acids. It regulates pre mRNA splicing and cycloheximide resistance (Akache and Turcotte 2002; Vincent *et al.* 2003) (Figure 2.15). Interestingly its transcriptional profile has many common genes with Lys142-GOF and Zcf23-GOF (n=255). The GO term analysis showed enrichment in carbohydrate catabolic and nitrogen utilization processes. Unlike its ortholog, the *Ca*Orf19.2230-GOF profile showed genes related to mRNA splicing being downregulated including *ORF19.6356*, *ORF19.674*, *DBR1*, *ORF19.4205.1*, *ISY1*, *CWC22*, *PRP5*, *PRP3*, *PRP39*, *ORF19.1687* (Appendix 10). This could suggest Orf19.2230 diverged its function compared to its ortholog *Sc*Rds3; this will require further investigation.

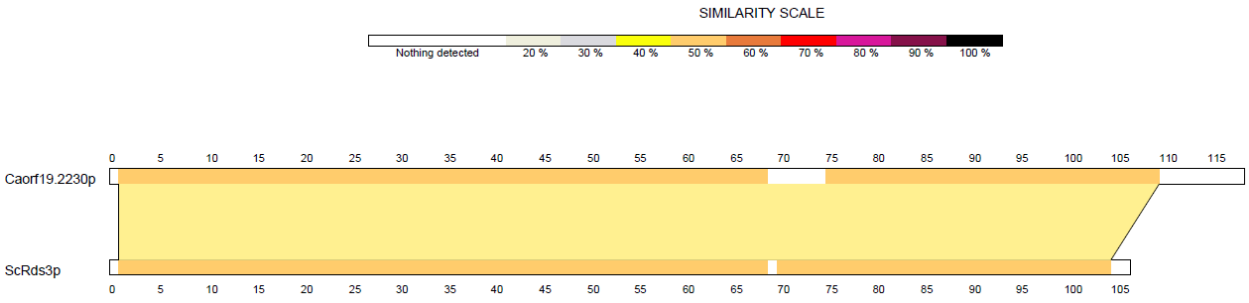


Figure 2.15: *C. albicans* Orf19.2230 aligned with *S. cerevisiae* Rds3. The result of sequence alignment with 57% sequence identity and include the zinc cluster domain.

FGR17 encodes a putative ZCF member with no clear *S. cerevisiae* ortholog. The best hit of *FGR17* in *S. cerevisiae* is *CHA4* that mediates serine/threonine activation of the catabolic L-serine (L-threonine) deaminase (CHA1). large-scale studies showed its negative influence in hyphal growth as the *fgr17* mutant causes filamentation increases and its heterozygous state reduces hyphal development (Uhl *et al.* 2003; Vandeputte *et al.* 2011). A significant number of upregulated genes are involved in biotin processes. Among the upregulated genes, we observed the adhesion genes *ALS2* and *ALS4,* although deletion of both genes did not affect the cell wall adhesion significantly (Zhao *et al.* 2005) (Appendix 11). In addition, Fgr17-GOF significantly downregulated the expression of a hyphal gene regulator, *TEC1,* which agrees with its reduction in hyphal growth. A number of the downregulated genes are involved in the catabolism of galactose. Fgr17 may act as a repression factor for *C. albicans* filamentation.

FGR27 is another member of the ZCF family with no ortholog in *S. cerevisiae*. Large-scale studies showed that the absence of *FGR27* causes decreases in adhesion and filamentous growth with a suggested function in the cell adherence of *C. albicans* to a silicone substrate, and thus contributes to the biofilm formation (Uhl *et al.* 2003; Vandeputte *et al.* 2011; Finkel *et al.* 2012). Fgr27-GOF upregulated genes involved in hypha formation, such as *HGC1*, *HWP1*, and *ALS1* as well as hyphal regulators such as *BRG1*and *TEC1*. Additionally, *RAS1*, which controls cAMP and MAP kinase pathways for hyphal induction, is induced (Feng *et al.* 1999) (Appendix 12). GO term analysis of upregulated genes indicated enrichment in carbohydrate transport, redox homeostasis, and oxidative stress response. In summary, looking at individual upregulated genes, our data indicate that *FGR27* plays a critical role in the development of *C. albicans* hyphae, along with other functions such as carbohydrate transport and oxidative stress-response.

Lys142 in *C. albicans* is similar in protein sequence to *Sc*Lys14 with 28% sequence identity covering 309 amino acids including the zinc cluster domain; in *S. cerevisiae,* Lys14 controls lysine biosynthesis genes (Feller *et al.* 1994) (Figure 2.16). The protein sequence alignment of *C. albicans* Lys142 and Lys144 against *Sc*Lys14 revealed high similarity of *Ca*Lys142 to *Sc*Lys14 outside the Zn(II)2Cys6 binuclear cluster DNA binding motif (Figures 2.16 and 2.17). There are four *LYS14*-like genes, *LYS141*, *LYS142*, *LYS143*, and *LYS144* in *C. albicans* but they play no apparent role in the regulation of *LYS* gene expression (Priyadarshini and Natarajan 2016). This study suggested Gcn1 as an essential regulator for *C. albicans* growth under

lysine deprivation, as well as the essential direct activator of multiple lysine biosynthesis genes (Priyadarshini and Natarajan 2016). However, three out of nine lysine biosynthesis genes identified were upregulated in Lys142-GOF, which could suggest *LYS142* may be involved in *C. albicans* lysine biosynthesis. However, the Lys142-GOF transcriptional profile revealed upregulation of 416 genes in total along with ZCFs members like *ZCF19* (profiled here with positive role in filamentation). *ZCF17* and *ZCF35* (profiled here with a suggested role in arginine biosynthesis) are downregulated (Appendix 13). The GO term analysis was enriched in alpha-amino acid biosynthesis processes. We suggest that *Ca*Lys142 plays a role in amino acid processes directly/indirectly requiring further phenotypic studies.

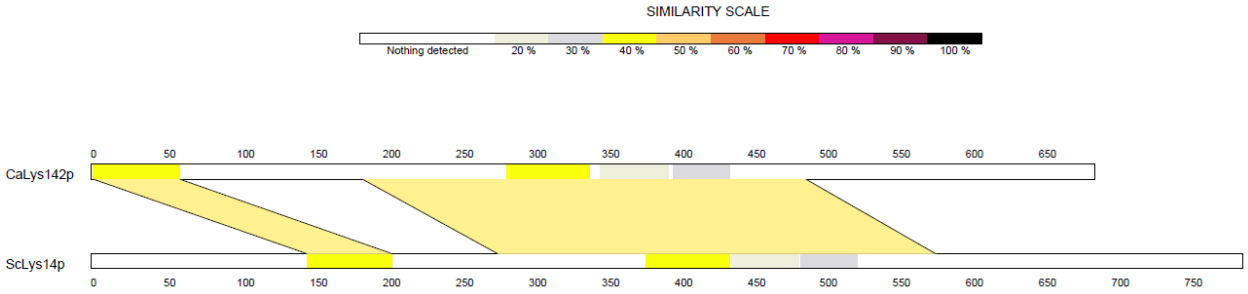


Figure 2.16: *C. albicans* Lys142 aligned with *S. cerevisiae* Lys14. The result of sequence alignment with 28 % sequence identity and include the zinc cluster domain.

Lys144 belongs to ZCF family with sequence similarity of 33% to *Sc*Lys14 (Figure 2.19). A study has shown that cells lacking *LYS144* do not show changes in the expression of genes involved in lysine biosynthesis, which fits well with our Lys144-GOF profile as we did not observe upregulation of lysine biosynthesis genes (Priyadarshini and Natarajan 2016). The screening of a library of transcription regulator deletion strains using mouse models of intestinal colonization and systemic infection demonstrated that cells lacking *LYS144* are seriously impaired in GI tract colonization but do not exhibit abnormalities in a systemic infection model (Perez *et al.* 2013). The Lys144-GOF construct causes upregulation of 134 genes with significant functional enrichment in biotin metabolic proceLys144-GOF upregulated uncharacterized ZCFs such as *ZCF1*, *ZCF2*, *ZCF9* (profiled here with suggested role in in copper transporter), and *ZCF22*

(profiled here with possible role in regulation of sterol synthesis) (Appendix 14). *ZCF22*, *ZCF9* are the target genes based on ChIP-chip data for Lys144 which agrees with our Lys144-GOG profile. The results of our studies confirmed that Lys144 controls *C. albicans* colonization and that the regulatory network with *ZCF22*, *ZCF9* should be further investigated.

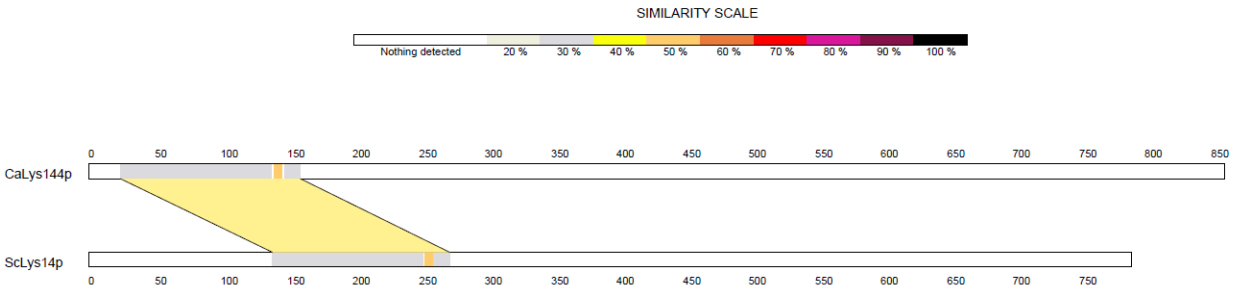


Figure 2.17: *C. albicans* Lys144 aligned with *S. cerevisiae* Lys14. The result of Blastp sequence alignment with 42% sequence identity covering 137 amino acids.

Uga32 is related to the regulator of gamma-aminobutyrate (GABA) metabolism gene *UGA3* in *S. cerevisiae* with 23% sequence identity spanning 254 amino acids (Figure 2.18). A *C. albicans uga32* mutant showed increases in hyphal growth with normal colony appearance on solid Spider medium (Homann *et al.* 2009; Vandeputte *et al.* 2011). There are 59 regulated genes in the Uga32-GOF cells; 20 genes are upregulated and 39 are negatively regulated by Uga32-GOF. Two GPI-anchored protein encoding genes *ESM33* and *PGA37* were among the upregulated and downregulated genes, respectively. There was no sign of GABA related gene upregulation, and the profile showed downregulation of GABA transporter *GPT1* (Appendix 15). We noticed downregulation of two ZCFs members, Zcf2 involved in adaptation to reactive sulfur species and Zcf17 with unknown function. We suggest *UGA32* may have a different role in *C. albicans* than that implied by its assigned name which require further studies.

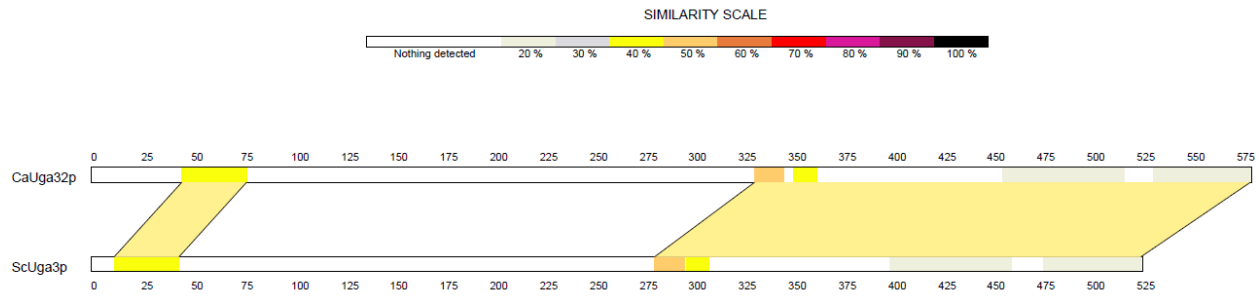


Figure 2.18: *C. albicans* Uga32 aligned with *S. cerevisiae* Uga3. The colored alignment area has 23% sequence identity.

Zcf10 is a ZCF member that up-regulates zinc finger TF encoding genes including *ZCF20* (profiled here), cell wall protein genes *RBT1*, *RBE1* and GPI-linked cell wall protein *RBT5* and GPI-linked hyphal surface antigen *PGA7, SAP10* with roles in adhesion (Appendix 16). It has no ortholog in *S. cerevisiae*. A noticeable gene among the downregulated genes is *RAS2* that has an opposing effect of *RAS1* (Zhu *et al.* 2009), controller of hyphal growth. However, there was no significant ontology term from the upregulated or downregulated genes. The heterozygous mutant of *ZCF10* reduced invasive growth and decreased colony wrinkling based on the large-scale studies (Oh *et al.* 2010; Vandeputte *et al.* 2011). Thus, the transcriptional profile of activated Zcf10 suggest its potential positive impact as a regulator of hyphal growth.

Zcf13 has some similarity to, but is not an ortholog of, *HAP1* in *S. cerevisiae* with 57% sequence identity in the DNA binding region (Figure 2.19). There are no paralogs of *ZCF13* in *C. albicans*, and the gene is present in some *Candida* species but not in *S. cerevisiae.* The Zcf13-GOF significantly upregulates 17 genes associated with lipid catabolic and fatty acid beta oxidation (Appendix 17). According to Vandeputte's large-scale study, lack of *ZCF13* is associated with abnormal colony morphology, enhanced hyphal growth, and decreased heat sensitivity. Within the Zcf13-GOF GO, no significant enrichment was found for processes or functions. This may be due to the large number of uncharacterized genes in the Zcf13-GOF profile. Despite this, *RBT1* encoding a hyphal cell wall protein, and *PGA17*, encoding a putative GPI-anchored protein, are among the genes that were upregulated and downregulated respectively (Appendix 17). Hence, the Zcf13-GOF profile suggests it may have a role in *C. albicans* hyphal growth.

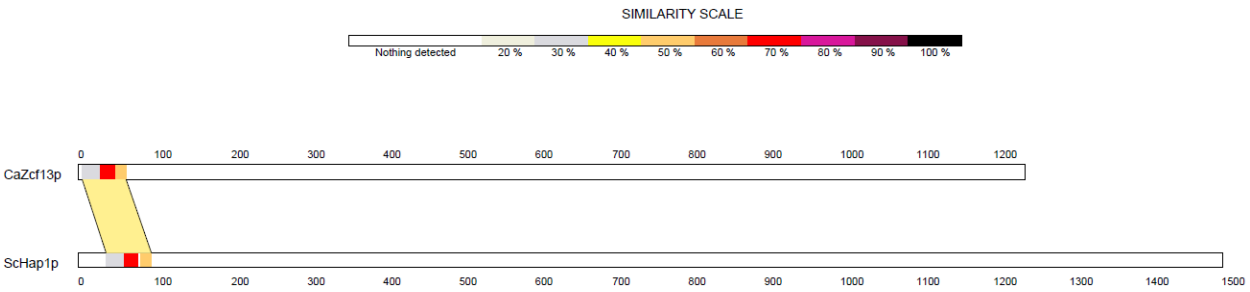


Figure 2.19: *C. albicans* Zcf13 aligned with *S. cerevisiae* Hap1. The result of sequence alignment with 57% sequence identity covering 61 amin acids length.

Zcf24 has a similar domain to *ScASG1* with 41% sequence identity covering 54 amino acids (Figure 2.20). *CaZCF24* is present in other *Candida* species like *C. dubliniensis*, *C. guilliermondii* and *C. tropicalis* but not in *S. cerevisiae*. There was no significant phenotypic hint from *zcf24* null mutants in the large-scale studies (Noble *et al.* 2010; Segal *et al.* 2018). The profile showed upregulation of genes regulated by Tup1 such as *QDR1*, *RHR2, ADH5, GAP2, PRD1, ORF19.1691, GST2, GDB1* (Appendix 18). It also upregulated genes with positive impact on *C. albicans* filamentation like *DEF1*, *UCF1*, *ORF19.1381*, *ORF19.2350* (Appendix 18). We also noticed downregulation of *ZCF24* across many of our ZCFs profiles. *ZCF27* (profiled here) is the only ZCF member among the genes upregulated by the Zcf24-GOF. There was a large-scale study that demonstrated hyphal production of *zcf27* (Vandeputte *et al.* 2011) which may be directly or indirectly connected to *ZCF24*. These two ZCFs appear to be linked in a direct or indirect manner in functions related to *C. albicans* filamentation that needs to be explored further.

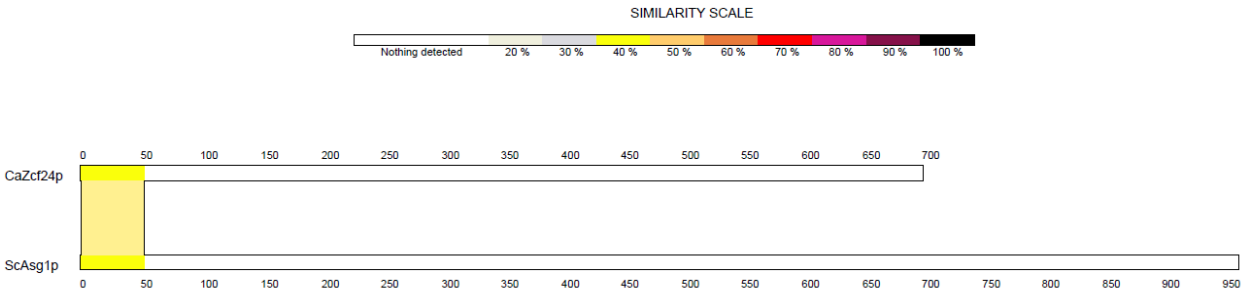


Figure 2.20: *C. albicans* Zcf24 aligned with *S. cerevisiae* Asg1. The result of sequence alignment with 41% sequence identity limited to the zinc cluster domain.

Zcf15 is ZCF member with *PDR1* as its similar protein with 54% sequence identity spanning 37 amino acids in the zinc cluster region in *S. cerevisiae* (Figure 2.21). *ZCF15* is present in the pathogenic *Candida* species *C. tropicalis* and *C. dubliniensis* but not in *S. cerevisiae*. *ZCF15* has two paralogs *ZCF25* and *ZCF26* (profiled here with possible role in DNA repair). There were no significant phenotypes with a *ZCF15* deletion as shown in large-scale studies (Homann *et al.* 2009; Vandeputte *et al.* 2011). It significantly upregulated 140 genes with 80.0% enrichment in ribosome biogenesis processing. Zcf24 (profiled here) is one of the downregulated genes with potential positive effects on *C. albicans* filamentation and DNA repair (Appendix 19). This could suggest a regulatory network between *ZCF15* and *ZCF24* will require further studies by constructing double mutants of *ZCF24* and *ZCF15* and performing phenotypic studies. Overall, it seems *Ca*Zcf15 has a function related to ribosomal biogenesis.

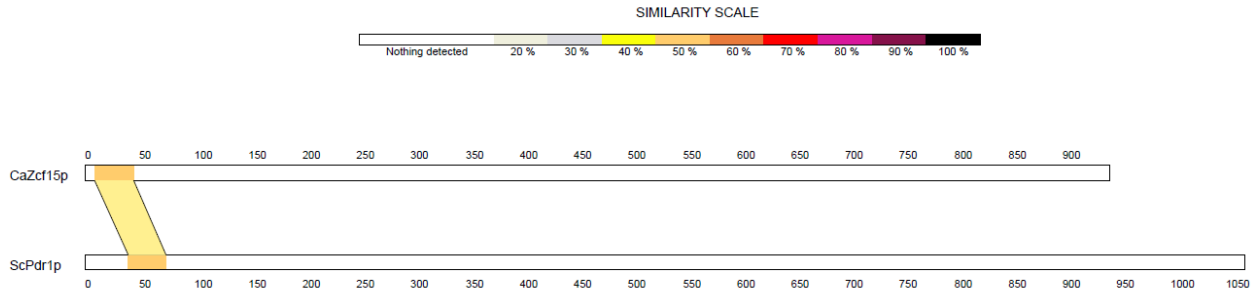


Figure 2.21: *C. albicans* Zcf15 aligned with *S. cerevisiae* Pdr1. The result of sequence alignment with 54% sequence identity spanning 37 amino acids length.

The **Zcf18** related protein in *S. cerevisiae* is Lys14 with 32% sequence identity covering 84 amino acids (Figure 2.22). As within Zcf15-GOF, genes related to ribosomal biogenesis are enriched among Zcf18-GOF upregulated genes. Deletion of *zcf18* resulted in hyphal growth development (Vandeputte *et al.* 2011) which could suggest its role as negative regulator of *C. albicans* hyphal growth. We noticed downregulation of a number of hyphal related genes such as *ALS9*, *DUR1,2* and adhesin genes *IFR1, FAV2* in the Zcf18-GOF profile which agrees with its suggested role as negative controller in *C. albicans* hyphal growth (Appendix 20).

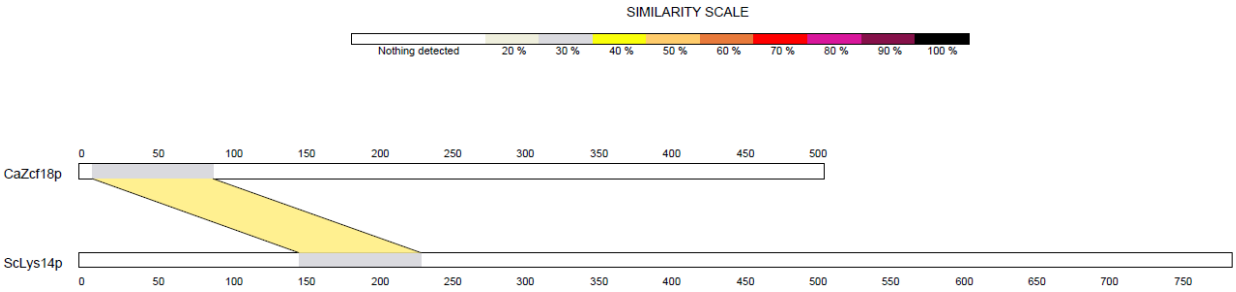


Figure 2.22: *C. albicans* Zcf18 aligned with *S. cerevisiae* Lys14. The result of sequence alignment with 32% sequence identity.

Zcf19 is another ZCF member with no ortholog in *S. cerevisiae.* Zcf19-GOF profile showed similar processes enriched as Zcf15-GOF and Zcf18-GOF for its upregulated genes. Zcf19-GOF induced a number of noticeable genes like *HCA4*; putative role in regulation of cell

wall biogenesis, *TEC1*; hyphal regulator, *ALS7* from *ALS* family protein, *ROA1* with putative role in PDR-subfamily ABC transporter. Basic amino acid permease *CAN2*, putative oxidoreductase gene *CIP1* and GABA/polyamine transporter *GTP1* are downregulated by Zcf19-GOF (Appendix 21).

Zcf21 is a ZCF member with *ScLys14* as its similar protein with 26% sequence identity covering 149 amino acids (Figure 2.23). The absence of *ZCF21* resulted in sensitivity to chemical stresses like calcofluor white and caffeine and showed a weak defect in GI tract colonization (Homann *et al.* 2009; Perez *et al.* 2013). GO term enrichment of upregulated genes is the same as for activated *ZCF15*, *ZCF18*, and *ZCF19* with no significant ontology function term. Overall, there was no enrichment in cell wall related genes for Zcf21-GOF (Appendix 22).

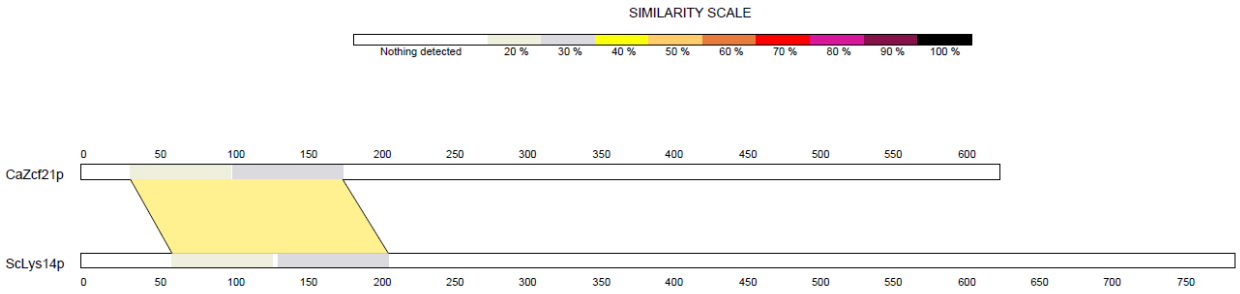


Figure 2.23: *C. albicans* Zcf21 aligned with *S. cerevisiae* Lys14. The result of sequence alignment with 26% sequence identity.

Zcf22 has a similar domain as *S. cerevisiae UPC2,* which is a regulator of sterol biosynthesis. Sequence alignment of *Ca*Zcf22 with *Sc*Upc2 showed 49% sequence identity covering 41 amino acids length limited in the zinc cluster domain (Figure 2.24). *UPC2* overexpression in *S. cerevisiae* causes filamentation and cells lacking *UPC2* do not form filaments (Vik A and Rine 2001; Woods and Höfken 2016). A large-scale phenotypic screen in *C. albicans* showed that a heterozygous mutant of *zcf22* has an invasion defect (Oh *et al.* 2010). There was no hyphal-associated-gene enrichment in the upregulated genes of Zcf22-GOF. Overall, Zcf22-GOF upregulated *CDR4*, a putative ABC transporter superfamily, as well as genes that are induced under fluconazole like *PLB1*, *ADH5*, *OPT3*, *DAK2*, and *orf19.1691* (Appendix 23). Our data suggest that *ZCF22* may play a role similar to *ScUPC2*. However, *ZCF22* does not play a role in filamentation.

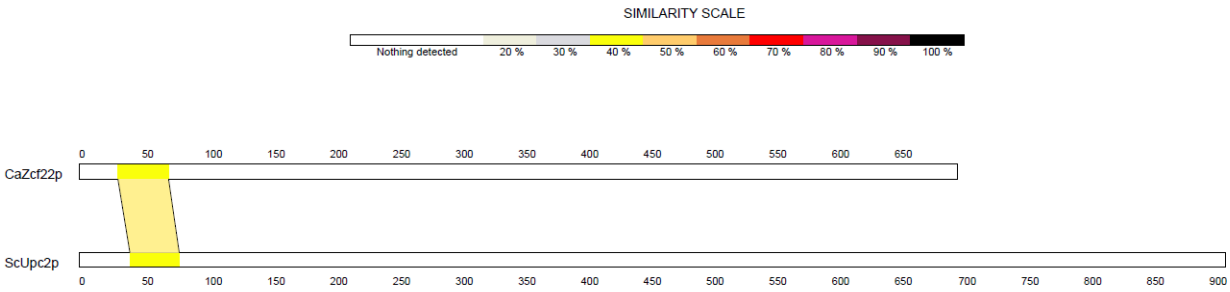


Figure 2.24: *C. albicans* Zcf22 aligned with *S. cerevisiae* Upc2. The result of sequence alignment with 49% sequence identity.

Zcf26 has no ortholog in *S. cerevisiae* but exhibits some similarity to *ScGal4* DBD domain. Zcf26-GOF induced many genes (n = 380). The GO is highly enriched in terms associated with ribosomal processes. In addition, activated Zcf26 induced genes involved in DNA repair including *orf19.6907*, *MMS22* and *GIN1, SMC5, orf19.4161*, *orf19.2673, orf19.50* (Appendix 24). It also upregulates genes in filamentous growth, including *GIR2*, *CLA4* and *BRE1*. Our data suggest ZCF26 may play a role in *C. albicans* DNA repair.

Zcf27 has a similar DBD domain to that of *S. cerevisiae OAF1* with 48% identity that is involved in beta-oxidation of fatty acids, peroxisome organization and biogenesis (Smith *et al.* 2011) (Figure 2.25). A large-scale study showed that *ZCF27* caused hyphal growth development and decreased agar invasion (Vandeputte *et al.* 2011). There were no significant GO terms for the downregulated genes. But, the Zcf27-GOF profiles showed upregulation of genes related to the hyphal cell wall like *ALS1*, *RBT1*, *RBT5*, *RHD3* and the hyphal regulator *BRG1*, and known/putative heat shock genes *HSP12*, *C5_02110W_A*. It also activated genes related to oxidative stress, such as *YCP4*, *GSY1*, *PST3*, *orf19.7085* (Appendix 25). The observed upregulation of HAGs and TFs contrasts with *zcf27* hyphal induction, but it agrees with the reduction in agar invasion. It's possible that *ZCF27* has a role in filamentation triggered on agar medium, as a recent study showed *C. albicans* has a distinct transcriptional program in hyphal development induced by liquid or solid. There was no upregulation of β-oxidation of fatty acids genes like *FOX2*, *POT1*, *PEX1-3*, *PEX5* (Ramírez and Lorenz 2009). Overall, the Zcf27-GOF profile reveals a potential role in *C. albicans* filamentation and oxidative stress response.

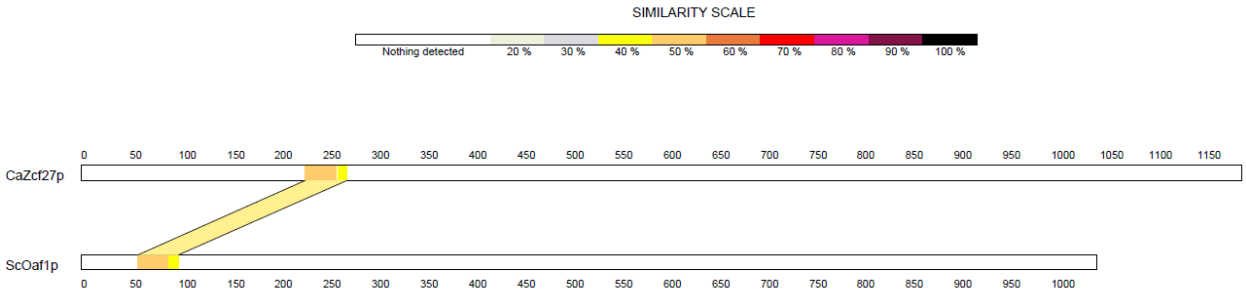


Figure 2.25: *C. albicans* Zcf27 aligned with *S. cerevisiae* Oaf1. The result of sequence alignment with 48% sequence identity covering 43 amino acids.

Zcf31 is a *C. albicans* specific ZCF member with no *S. cerevisiae* orthologs. It is also found in other pathogenic *Candida* species *C. tropicalis* and *C. dubliniensis*. Based on the large-scale studies, *zcf31* null mutants showed strong sensitivity to copper and SDS, and weak sensitivity to fluphenazine, fenpropimorph, caffeine, and low pH, and strong resistance to calcofluor white; and was suggested to be required for yeast cell adherence to silicone substrate GPI anchor protein Ihd1 (Homann *et al.* 2009; Finkel *et al.* 2012). The Zcf31-GOF transcriptional profile revealed an upregulation of GPI-anchored protein Ihd1, consistent with this proposed function (Appendix 26).

Zcf35 has no clear ortholog in *S. cerevisiae* but its best hit is *Oaf3*, a putative transcriptional repressor negatively regulating transcription in response to oleate levels (Smith *et al.* 2007). Sequence alignment of Zcf35 with Oaf3 from *S. cerevisiae* showed 33% sequence identity covering 86 amino acids (Figure 2.26). Zcf35-GOF upregulated a large number of genes (n=330). Genes that have been upregulated are significantly enriched in processes such as amino acid metabolism and nitrogen utilization. In the GO term component ontology, it was found that 21% of genes are significantly associated with the periphery of the cell, 9% with the external encapsulating structure, and 7% with the cell wall. We noticed upregulation number of hyphal cell wall genes like *ALS2*, *RBT1*, *ORF19.5267*, *ATC1*, *PIR1*, *RBE1*, *orf19.675* and GPI-anchored protein *FLO9*, *RBR1*, *RHD3*, *PGA10*, *PGA13*, *PGA14* (Appendix 27). Zcf35-GOF revealed upregulation of *GAT1* and *RME1* that regulate nitrogen utilization and chlamydosporulating genes in response to starvation, respectively (Ramachandra *et al.* 2014; Hernández-Cervantes *et al.* 2020). Zcf35-GOF induced other ZCF members including *ZCF27* (profiled here), *ZCF1* with role in hyphal formation, *ZCF10* (profiled here), *LYS143* (profiled here). It also downregulated *LEU3* (profile here), *ZCF22* (profile here) and *ZCF17* whose deletion shows increased hyphae formation

(Uppuluri and Chaffin 2007; Vandeputte *et al.* 2011) (Appendix 27). Zcf35 seems to be a multifunctional ZCF member that has an interconnected network with a number of ZCF family members with possible roles in carbohydrate metabolism and nitrogen utilization. As a result, it may be useful to examine the function of Zcf35 in combination with double mutants of upregulated ZCFs that are found in its profile.

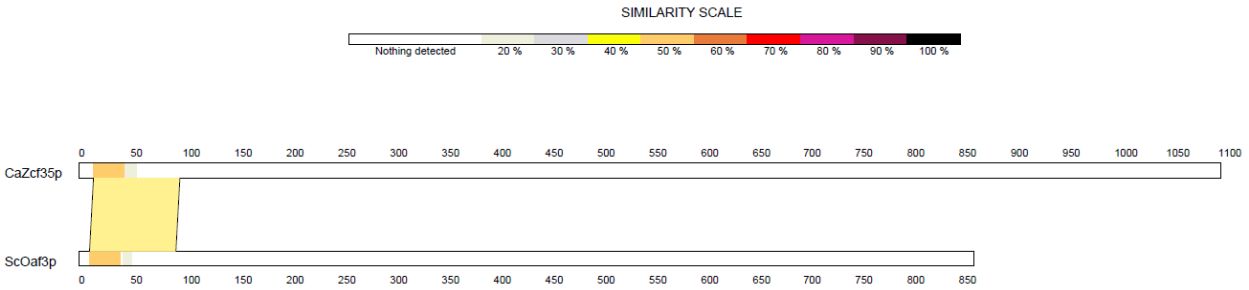


Figure 2.26: *C. albicans* Zcf35 aligned with *S. cerevisiae* Oaf1. The result of sequence alignment with 33% sequence identity limited to the zinc cluster domain.

Zcf38 is ZCF member with no ortholog in *S. cerevisiae. ZCF38* heterozygous deletion caused a decrease in *C. albicans* invasive growth but showed normal biofilm and virulence phenotypes for the *zcf38* deleted strain (Nobile and Mitchell 2005; Chamilos *et al.* 2009; Oh *et al.* 2010). The GO term ontology associated with upregulated genes is enriched for biotin biosynthesis and metabolism. Zcf38 induces a number of genes involved in cell wall adhesions (*ALS4*, *ALS9*, *ALS2, SAP9*) and GPI-anchored protein encoding genes (*PGA13*, *PGA7*, *GPI14*). There are a number of genes related to oxidative stress among the upregulated genes including *GSY1*, *MLS1*, *IFR1*, *TPS3*, *GPI14* (Appendix 28). Our data suggest that *CaZCF38* may play a positive role in filamentous growth and oxidative stress response.

Zcf5 has no ortholog in *S. cerevisiae* but its most similar protein is *ScHAP1* with 31% sequence identity over 131 amino acids (Figure 2.27). It strongly upregulated ALS family genes like *ALS2*, *ALS9* and *ALS4*, but here was no significant functional enrichment seen for the top 34-upregulated genes (Appendix 29). Studies showed the homozygous deletion of *zcf5* decreased colony wrinkling but cells formed normal biofilms in large-scale screening studies (Nobile and Mitchell 2005; Vandeputte *et al.* 2011). Upregulation of hyphal cell wall proteins of the ALS

family in Zcf5-GOF may relate its function to *C. albicans* morphology regulation; this requires further investigation.

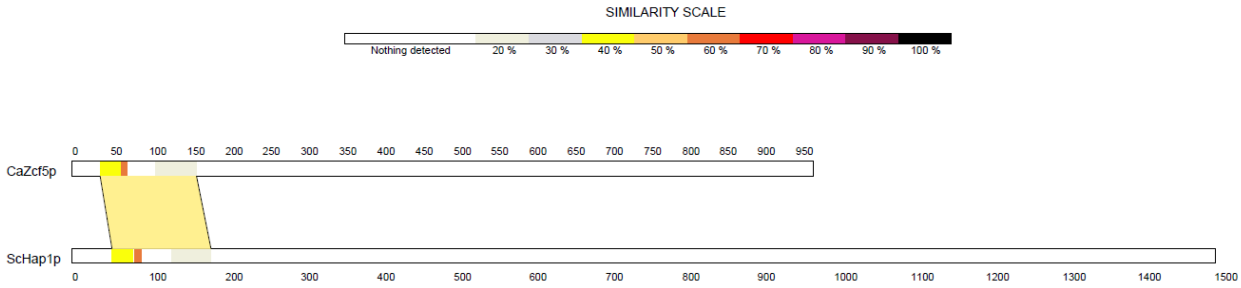


Figure 2.27: *C. albicans* Zcf5 aligned with *S. cerevisiae* Hap1. The result of sequence alignment with 31% sequence identity.

Zcf6 is related to *Sc*Asg1 with 47% sequence identity limited mainly to the DNA-binding domain covering 47 amino acids **(**Figure 2.28). The *ZCF6* gene does not have orthologs in other *Candida* species, and thus potentially regulates pathways or processes that differentiate *C. albicans* from these other species. Zcf6-GOF triggered the upregulation of genes related to the glycolytic process, while most of Zcf6-GOF downregulated genes are poorly characterized. *zcf6* mutant phenotypic studies showed increased colony wrinkling, normal biofilm with avirulence in mouse intravenous infection studies (Nobile and Mitchell 2005; Vandeputte *et al.* 2011; Amorim-Vaz *et al.* 2015). Zcf6-GOF induced hyphal-related adhesion genes *ALS2*, *ALS4*, *SAP10* and GPI anchored protein encoding genes *PGA10* and *RHD3* (Appendix.30). This is same case as Zcf27-GOF transcriptional profile that has positive impact in *C. albicans* filamentation, but the large-scale study is suggesting the negative impact of *ZCF6* in filamentation. Interestingly, Zcf6-GOF expressed *ZCF27* (profiled here), and *ZCF1* that have potential roles in *C. albicans* filamentation and biofilm formation. Overall, Zcf6 has a potential role in glycolytic process and filamentation with possible interconnected networks with *ZCF27* and *ZCF1* that require further studies.

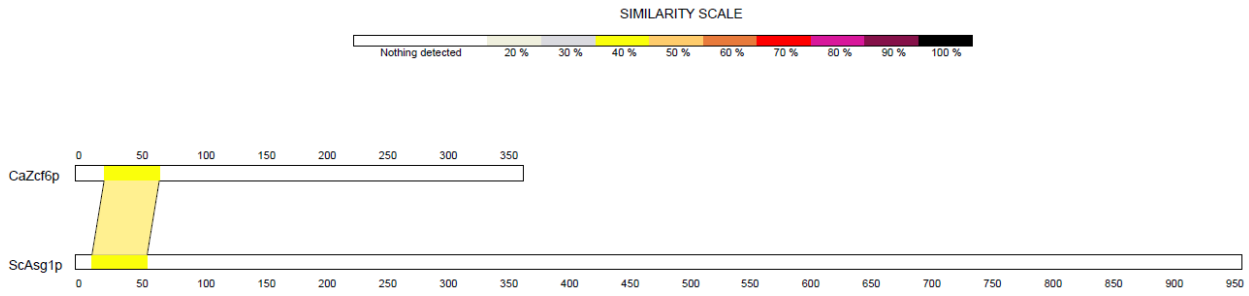


Figure 2.28: *C. albicans* Zcf6 aligned with *S. cerevisiae* Asg1. The sequence alignment with 47% sequence identity and includes the zinc cluster domain.

Zcf8 lacks a clear ortholog in the model yeast *S. cerevisiae*. Zcf8-GOF strongly upregulates *DAL52*, encoding an allantoate transmembrane and dipeptide transporter, as well as genes for other membrane transporters including *ORF19.1308*, *GDT1*, *HGT20*, *OPT8, FRP3*, *YOR1*, *ORF19.6976* and *CTP1* (Appendix 31). An *in vitro* genetic screening for cell-substrate adhesion using silicone polydimethyl siloxane as a substrate identified the *zcf8* mutant (Finkel *et al.* 2012). In the context of the gastrointestinal tract, Zcf8 functions to promote adhesion to silicone and contribute to attachment to surfaces (Böhm *et al.* 2017). The upregulated genes did not exhibit a significant enrichment in cell wall related genes. Among the upregulated genes were transcription factors such as *ADR1*, which contributes to ergosterol metabolism (M. Shrivastava, personal communication), and *RLM1*, which promotes cell wall remodeling during carbon adaptation (Oliveira-Pacheco *et al.* 2018), and the downregulated gene *ORF19.6888*, a poorly studied ZCF family member. In agreement with upregulation of *ADR1*, there are genes related to fluconazole repression in the *ZCF8* profile like *ATO1* and *FPR3*. Overall, the induced genes suggest *ZCF8* may function in regulation of *C. albicans* membrane transport.

Zcf9 is a ZCF member that lacks an ortholog in *S. cerevisiae*. Zcf9-GOF expressed genes are implicated in copper ion import and transport, and in cellular detoxification of aldehyde (Appendix 32). Its activation downregulates several genes involved in generic stress responses and oxidative reduction. *ZCF9* is significantly induced in a *zcf29* mutant, which plays a positive role in *C. albicans*' response to oxidative stress (Issi *et al.* 2017). *ZCF9* has a negative effect on oxidative stress, as the Zcf9-GOF profile supports this notion. A large-scale screening found that the homozygous null mutant of *zcf9* was not recovered, suggesting that it may be an essential gene (Nobile and Mitchell 2005). The *ZCF9* profile suggests it may function in copper transport.

Hal9 was named because of its similarity (22% identity) to Hal9 in *S. cerevisiae*. *ScHAL9* encodes a TF involved in salt tolerance (Mendizabal *et al.* 1998) (Figure 2.29). Sequence alignments demonstrated that *Ca*Tac1 was in fact the closest match to *Sc*Hal9 in *C. albicans* with 42% sequence similarity but there existed two other proteins, named Hal9 and Znc1, that also showed similarity with *Sc*Hal9 40% and 41% respectively. Blastp results showed Hal9, Tac1 and Znc1 were related to each other, and intriguingly, synteny alignments showed the 3 genes were clustered adjacent to each other on chromosome five. In two large-scale studies, the *CaHAL9* deletion increased agar invasiveness and decreased colony wrinkles (Nobile *et al.* 2003; Vandeputte *et al.* 2011). Our results suggest that the homozygous null mutant of *HAL9* could not be recovered, as well as two other reports suggesting that it is an essential gene (Coste *et al.* 2004; Chaillot *et al.* 2017).

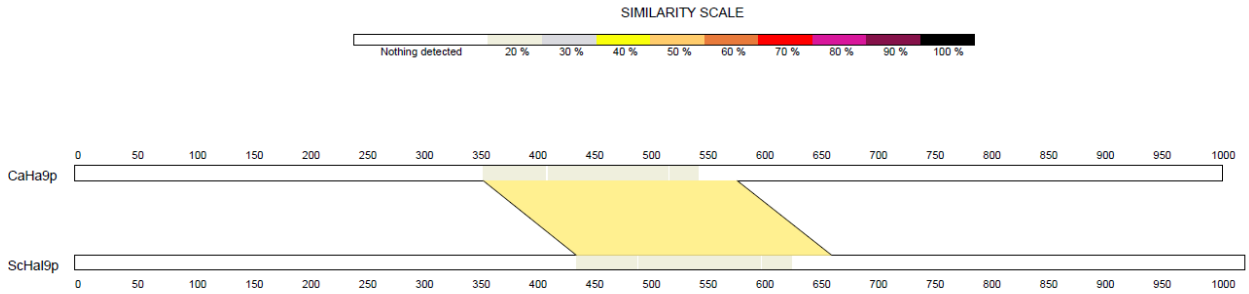
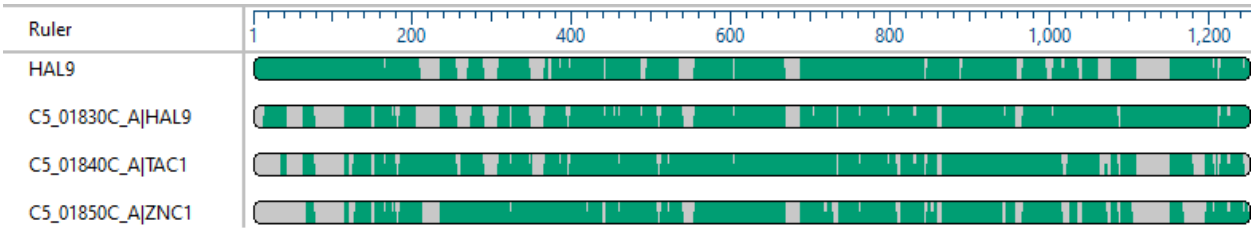


Figure 2.29: *C. albicans* Hal9 aligned with *S. cerevisiae* Hal9. The result of sequence alignment with 22% sequence identity containing 227 amino acids.

A.



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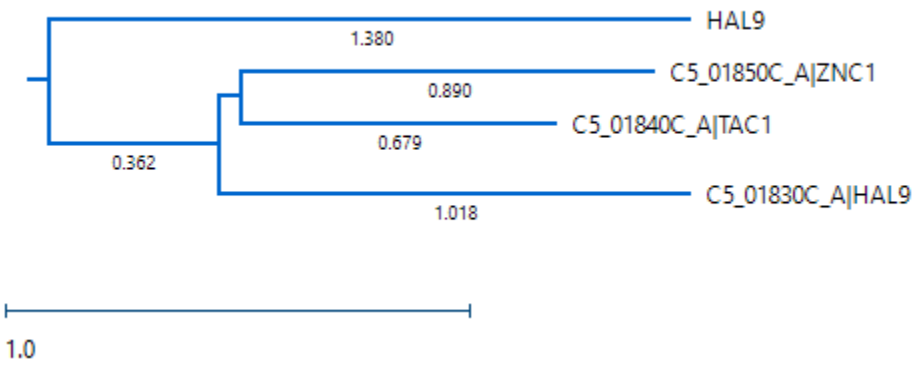


Figure 2.30: A. The multiple sequence alignment of *Sc*Hal9 and *C. albicans* C5_01830C_A|Hal9, C5_01840C_A|Tac1 and C5_01850C_A|Znc1. **B**. The phylogeny tree after *S. cerevisiae* Hal9 alignment with *C. albicans* C5_01830C_A|Hal9, C5_01840C_A|Tac1 and C5_01850C_A|Znc1.

*Sc*Hal9 is involved in salt tolerance through the expression of the Ena1p Na+/Li+ extrusion pump where the overexpression of *Sc*Hal9 increased sodium and lithium tolerance (Mendizabal *et al.* 1998). Whereas the overexpression of *Ca*Hal9 showed more sensitivity to salt than the wild type in *C. albicans* and the *HAL9* heterozygous mutants showed similar sensitivity as the wild type. The salt stress assays also showed that *Ca*Tac1's function was not similar as *Sc*Hal9 as the overexpression of the gene resulted in more sensitivity to salt compared to wild type SC5314. Growth trends of Hal9-GOF and Tac1-GOF looked similar in YPD containing NaCl, LiCl and $CaCl_2$ (Figure 2.31), which illustrated the similarity of the two proteins in sensitivity to salt if compared to the wild-type. The halotolerance assays further confirmed that *CaHAL9* did not function the same way as *ScHAL9* beside the difference in protein sequence of those two transcription factors. *Ca*Hal9 was shown to function differently from *Sc*Hal9 through the halotolerance assays, which was also consistent with the low identity value between the two

proteins using BLAST (22%). However, BLASTp results suggested *Ca*Hal9 and *Ca*Tac1 are related to each other based on the sequence alignment and the best hit of both proteins in *S. cerevisiae* being *Sc*Hal9; thus Hal9 might function similarly as Tac1. Together with BLASTp results, it was suggested that CaHal9's function might be like CaTac1's instead of *Sc*Hal9's. Tac1 is a zinc-cluster transcription factor involved in the regulation of genes mediating resistance to antifungal drugs such as fluconazole (Coste *et al.* 2004). The fluconazole test was conducted to determine whether Hal9 was involved in fluconazole resistance like Tac1. Thus, Tac1-GOF was used in the test as a control for comparison of the drug resistance. Indeed, Tac1-GOF showed more resistance to fluconazole than the wild type particularly in agar YPD media containing 2.5 and 5 μg/ml fluconazole. At those two fluconazole concentrations, Hal9-GOF was also more resistant than the wild type although its resistivity looked relatively weaker than the Tac1-GOF's (Figure 2.32). The results of the test illustrated that Hal9 functioned in fluconazole resistance like Tac1. However, at higher fluconazole concentrations, Tac1-GOF no longer showed its higher resistance compared to the wild-type whereas Hal9-GOF still remained its resistance to fluconazole. Tac1 had been shown to be the activator of *CDR1* and *CDR2*, which encoded ABC transporters that mediating azole resistance (Coste *et al.* 2004). The consistent resistance to fluconazole of Hal9-GOF suggested the involvement of Hal9 in fluconazole resistance through a different mechanism other than through the regulation of *CDR1* and *CDR2*. Interestingly, Hal9-GOF upregulated genes and transcription factors related to *C. albicans* fluconazole resistance like *PDR16* and *RME1* but there was no sign of well-known ABC transporters *CDR1* and *CDR2* expressions. The *hal9* heterozygous cells showed sensitivity to fluconazole than the Hal9-GOF but its sensitivity was similar as the wild type's. The results also demonstrated the involvement of Tac1 in fluconazole resistance through the strong growth of Tac1-GOF in YPD + 2.5 or 5.0 ug/ml fluconazole compared to that of other strains. However, in higher fluconazole concentrations, the growth of Tac1-GOF became similar as that of SC5314 and *HAL9/hal9Δ* whereas Hal9-GOF still showed the resistance consistently (Figure 2.32). From the fluconazole test, Hal9 also showed fluconazole resistance but with a different mechanism, since Tac1-GOF only showed resistance to fluconazole at low concentrations while Hal9-GOF maintained its resistance even at high concentrations.

In addition, Znc1 is also emerging as a novel drug resistance regulator in *C. albicans* and its activation causes the cells to be more resistant to fluconazole by upregulating the expression of *CDR1* (Schillig and Morschhäuser 2013) . That is consistent with the result that Znc1 was the best

hit of Tac1 in *C. albicans* with 30% similarity (Coste *et al.* 2004) thus Znc1's function might be more like Tac1's than Hal9's. Overall, *HAL9*, *TAC1* and *ZNC1* that are not only close in protein sequence but also in functions in fluconazole resistance. We further constructed a phylogeny tree using the NCBI's BLAST tool to determine if the proteins have orthologs in other organisms and therefore to acknowledge their evolution (Figure 2.33). The genomes of organisms in the CTG (or CUG) clade have most of the Hal9, Tac1, and Znc1 orthologs, but only Tac1 orthologs in the Protoploids and WGD clades except for Lachancea thermotolerance. There might have been a triplication event to produce those three genes, and they may have remained in the evolution of the CTG clade, but some of them have been lost in the Protoploids and WGDs.

Our Hal9-GOF transcriptional profile showed upregulation of 143 genes above log2 fold $\geq$ 1.5, padj $\leq$ 0.05 genes. The GO analysis of Hal9-GOF did not identify genes enriched significantly in specific processes or functions. However, Hal9-GOF upregulated genes implicated in the white-opaque switch (*PTH2*, *CZF1* and *RPD3*), and genes involved in the oxidative stress response-like *SOD3*, *YCP4*, *PSD3*, *RIM2*, *PST3* and *OYE32*. It positively expressed GPI-anchored proteins and cell wall proteins like *PGA13*, *WSC2*, *CRH11* and repressed hyphal associated cell wall gene *RBT1* (Appendix 33). This may suggest that Hal9 is involved in *C. albicans* filamentation in different ways. It also caused upregulation of ZCF members like *ZCF31* and downregulation of *ZCF23* that have been profiled here. Overall, *Sc*Hal9 is involved in salt tolerance whereas the three genes in *C. albicans* functioned in drug resistance. This further illustrates the divergence of transcription factors for the adaptation of a species to different environments especially *C. albicans* in its human host. Our study suggest that Hal9 plays multiple critical roles with variable mechanisms in *C. albicans*.

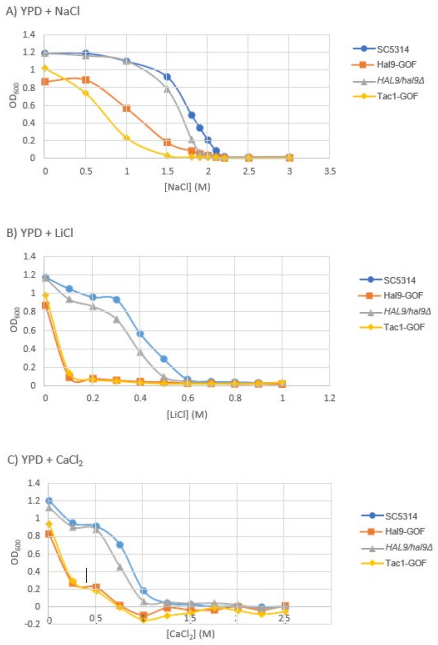


Figure 2.31: *C. albicans* Hal9 halotolerance assay**.** Graphs showed the growth of SC5314 (WT), Hal9-GOF*, HAL9/hal9*Δ, and Tac1-GOF in YPD containing different salts with different concentrations. The overnight cultures were diluted to OD600 of 0.1 in YPD + salt media prepared in 96-well plates and the growth of cells was monitored after 24 hours by measuring OD600. A) Growth of cells in YPD + different [3M NaCl]; B) Growth of cells in YPD + different [2M LiCl]; C) Growth of cells in YPD + different [2.5M CaCl2].

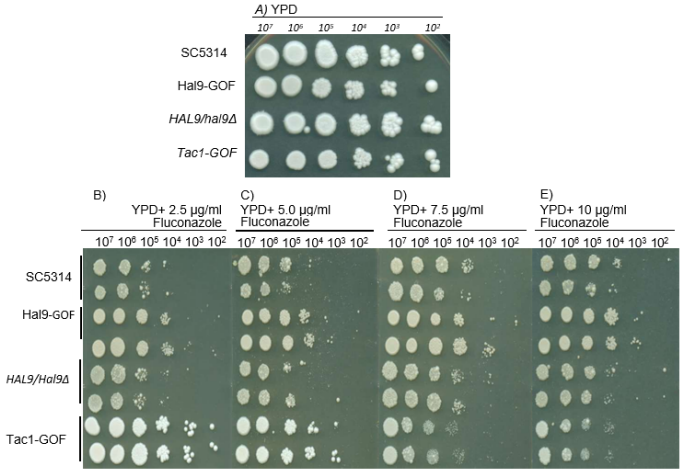
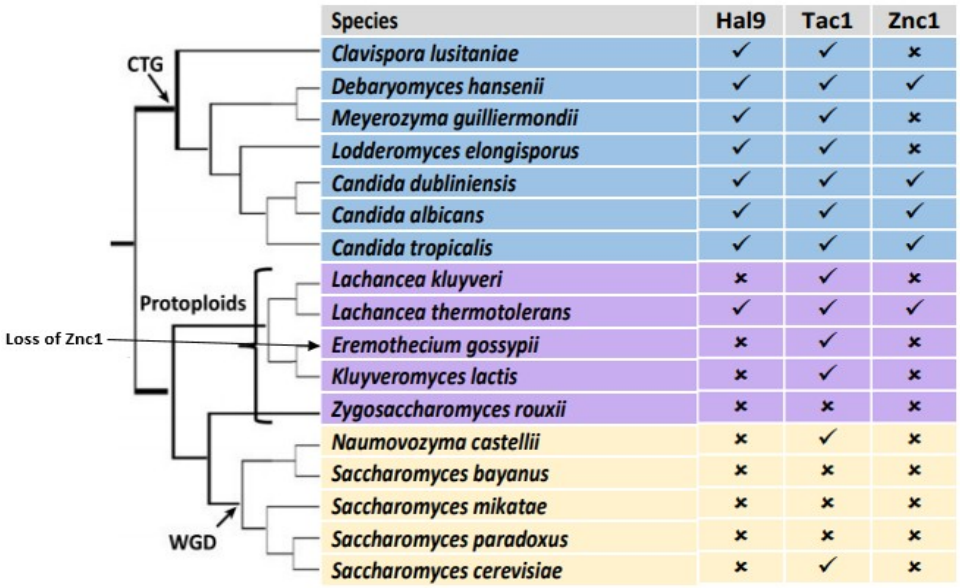


Figure 2.32: Growth of cells on YPD + Fluconazole (with different concentrations) plates. The overnight cultures of Sc5314, Hal9-GOF, *HAL9/hal9Δ* and Tac1-GOF were washed twice with PBS and diluted to OD600 = 1 and further serial 10-fold diluted in PBS before being spotted.



Species	Hal9	Tac1	Znc1
Clavispora lusitaniae	✓	✓	×
Debaryomyces hansenii	✓	✓	✓
Meyerozyma guilliermondii	✓	✓	×
Lodderomyces elongisporus	✓	✓	×
Candida dubliniensis	✓	✓	✓
Candida albicans	✓	✓	✓
Candida tropicalis	✓	✓	✓
Lachancea kluyveri	×	✓	×
Lachancea thermotolerans	✓	✓	✓
Eremothecium gossypii	×	✓	×
Kluyveromyces lactis	×	✓	×
Zygosaccharomyces rouxii	×	×	×
Naumovozyma castellii	×	✓	×
Saccharomyces bayanus	×	×	×
Saccharomyces mikatae	×	×	×
Saccharomyces paradoxus	×	×	×
Saccharomyces cerevisiae	×	✓	×

Figure 2.33: Phylogeny tree of yeast for Hal9, Tac1 and Znc1 in *C. albicans*. The phylogeny was constructed based on the order of HAL9, TAC1 and ZNC1 in *C. albicans* to determine whether their orthologs exist in other yeast species using NCBI Blastp tool. It is noted that the *S. cerevisiae* Hal9 was the closest in sequence to *Ca*Tac1, so the presence of this gene was checked in the Tac1 column.

Orf19.1604 is the only member of the ZCF panel where the gain-of-function mutant induces the hyphae transition in *C. albicans.* The differential expression genes for Orf19.1604 include *ECE1* (a well-established signal of hyphal induction; log fold change 6.49 log2, *NGR1* (a known repressor of hyphal induction; log fold change (-1.2 log2) , and *BCR1* encoding an established inducer of the hyphal transition (1.9 log2) (Kumamoto and Vinces 2005). This small sample of findings suggest the possible role of *ORF19.1604* in *C. albicans* filamentation; we follow this up in Chapter 3.

Zcf4 upregulates the zinc cluster TF encoding gene *ZCF32* that is not amongst our 34 profiled ZCFs, but which has been identified as a negative regulator of biofilm formation (Kakade *et al.* 2016). We observe strong down-regulation of *ZRT2* (-4 log2 fold change) which is essential for zinc uptake. Interestingly, we observe upregulation of several genes involved in proline and arginine metabolism incl. *CAR1* and *CAR2*, *PRO3* and *PUT1*. The Zcf4-GOF GO term ontology analysis implicates - proline metabolic and process to proline via ornithine. In Chapter 4, we will examine this further.

2.4 Conclusions

DNA-binding proteins known as transcription factors control gene expression by binding to a gene promoter or upstream activation domain enhancer, and play pivotal roles in cellular processes and organismal phenotypes. The regulation of a gene can occur on several levels, including the binding of transcription factors to the promoter region, post-translational modifications of TFs, or binding of transcription factors to cofactors. Zinc cluster transcription factors (ZCFs) are a subfamily of zinc finger transcription factors found in fungi and amoebae, and they may offer potential targets for medications against pathogens such as *C. albicans* since they are not found in mammals. To get insight into the biological processes controlled by ZCFs we need studies to decipher the individual functions of ZCFs. Phenotypic screening of ZCF knockdown libraries on different cellular stresses is one of the ways to study ZCFs. This could be challenging because transcriptionally regulated proteins work cooperatively, and transcriptional regulation is not an isolated process. It is possible that other TFs compensate for the absence of deleted ZCF and result in an unchanged phenotype. On the other hand, one of the promising strategies to identify ZCFs function is artificial activation of ZCFs *in vivo* without knowing the natural condition which TFs act. We combined this strategy with genome wide expression profile of individual ZCFs to suggest potential functions for a set of mostly uncharacterized or poorly characterized proteins.

RNA-seq profiles from 35 ZCFs were subjected to comprehensive quality control and normalization process that produced robust profiles that provided insight into the biology of several members. In summary, we identified hypothetical functions using GO term enrichment attuned to a common function or observing gene enrichment associated to the same functions and compared it to a known function when the candidate has an orthologous or best hit in *S. cerevisiae*. As an example, we were able to decode the role of two ZCFs member, Ctf1 and Asg1, with their closest protein sequence and orthologous member which was *Sc*Asg1. Our study suggested *Sc*Asg1 rewired its function from a role in *C. albicans* drug resistance to be a *S. cerevisiae* activator of stress response and utilization of β-oxidation of fatty acids, and it seems *Ca*Ctf1 oversees β-oxidation of fatty acids in *C. albicans*. The profiles revealed hypothetical functions for many uncharacterized ZCFs member and identified orthologous transcription factors from the ZCF

family that appeared to be functionally divergent between two closely related species, *C. albicans* and *S. cerevisiae.*

Our study provided a possibility to find the genes regulated by two ZCFs in order to identify the TF pairs that work as a complex. Accordingly, we propose that much of the expression changes observed in this example were not direct responses to the gain-of-function ZCF, but downstream, longer-term adaptive responses that may have engaged several intermediate transcription factors. We observed quite frequently in our data that these gain-of-function ZCFs influenced the expression levels of other ZCFs and transcription factors that were not profiled here. Our understanding of the regulatory relationships between ZCFs could be improved by making available a complete catalog of gain-of-function mutants for all ZCFs or all TFs. We identified many novel potential functions and cooperative TFs that could lead directly to new hypotheses for future experiments.

C. albicans ZCFs could be studied by using their orthologs or similarities in DNA-binding domains with their closest relative, *S. cerevisiae*. This would facilitate functional studies of ZCFs and provide insight into the regulatory process. As far as orthologous ZCFs are concerned, many of them had rewired functions compared to their orthologs in *S. cerevisiae*, such as Asg1, Aro80, Cta7, and Leu3. In contrast, *Ca*ZCFs that are not orthologs may have potential similar functions to their *S. cerevisiae*-related proteins, for example Ctf1, Zcf16, Zcf20, and Zcf22. It is interesting to note that non-ortholog ZCFs were mainly similar in the N-terminus DNA-binding domain with its *S. cerevisiae* best protein hits. The functional similarity could be due to the specificity of DNA-binding domain to the target genes. Previously published studies on protein engineering which compared affinity and specificity of zinc finger DNA-binding domains with their performance as zinc finger nucleases in human cells concluded that both DNA-binding affinity and specificity of the zinc finger domain influenced the activity (Cornu *et al.* 2008). Thus, non-orthologous transcription factors that have a similar DNA-binding domain tend to have similar DNA-binding specificities, leading to the possibility of revealing hypothetical functions that could be elucidated.

In the following chapters, we will explore the functional characterization of ZCF proteins Orf19.1604 and Zcf4. We selected these two members mainly due to the data of screening a library of *C. albicans* strains overexpressing activated zinc cluster transcription factors and finding Orf19.1604 activation generated wrinkled colonies containing filamentous cells on solid media

under non-hyphal-inducing conditions. This observation further agreed with RNA-Seq analysis that revealed up-regulation of genes required for filamentation and cell-wall-adhesion-related proteins in the Orf19.1604 hyperactivation strain compared with the wild type. The up-regulated genes include the hyphal-inducing transcription factors Brg1 and Ume6, while the hyphal repressor Nrg1 is downregulated in its transcriptional profile. In chapter 4, I investigate an unknown ZCF that is upregulated in Orf19.1604-GOF named *ZCF4*. I decipher the function of the zinc cluster transcription factor (ZCF) Zcf4 through transcription profiling, bioinformatic analysis and phenotypic investigation of null mutants. The potential role of *ZCF4* could differ from what has been previously established for *C. albicans* filamentation control.

Chapter 3: The zinc cluster transcription factor Rha1 is a positive filamentation regulator in *Candida albicans*

Abstract

Zinc cluster transcription factors (TFs) are essential fungal regulators of gene expression. In the pathogen *Candida albicans*, the gene orf19.1604 encodes a zinc cluster TF regulating filament development. Hyperactivation of orf19.1604, which we have named *RHA1* for Regulator of Hyphal Activity, generates wrinkled colony morphology under nonhyphal growth conditions, triggers filament formation, invasiveness, and enhanced biofilm formation and causes reduced virulence in the mouse model of systemic infection. The strain expressing activated Rha1 shows up-regulation of genes required for filamentation and cell-wall-adhesion-related proteins. Increased expression is also seen for the hyphal-inducing TFs Brg1 and Ume6, while the hyphal repressor Nrg1 is downregulated. Inactivation of *RHA1* reduces filamentation under a variety of filament-inducing conditions. In contrast to the partial effect of either single mutant, the double *rha1 ume6* mutant strain is highly defective in both serum- and Spider-medium-stimulated hyphal development. While the loss of Brg1 function blocks serum-stimulated hyphal development, this block can be significantly bypassed by Rha1 hyperactivity, and the combination of Rha1 hyperactivity and serum addition can generate significant polarization even in *brg1 ume6* double mutants. Thus, in response to external signals, Rha1 functions with other morphogenesis regulators including Brg1 and Ume6, to mediate filamentation.

Keywords: ZCFs; filamentation; *Candida albicans*; biofilms

3.1 Introduction

Transcriptional control of cellular processes is critical for normal functioning of the medically important opportunistic fungal pathogen, *Candida albicans*. Central to this regulation is the DNA- binding transcription factors (TFs) that typically associate with target sequences in the promoters of regulated genes. The bound factors serve to activate or repress transcription in response to signals that generally represent either internal cellular states or external conditions (Tong and Young 2000; Kornitzer 2019). One of the main classes of *C. albicans* TFs is the zinc cluster proteins, named because of a cysteine-rich region that coordinates two zinc ions as part of

the DNA binding domain of the protein (MacPherson *et al.* 2006). Zinc cluster transcription factors (ZCFs) are found only in fungi and amoebae, but related zinc finger proteins serve as TFs throughout eukaryotes (MacPherson *et al.* 2006; Clarke *et al.* 2013). Many studies have been conducted to characterize ZCF function; a powerful tool in these studies has been the use of activated versions of the ZCFs. For example, screening by Schillig and Morschhäuser (2013) of a comprehensive collection of Zn(II)2 Cys6 gain-of-function mutants identified regulatory genes controlling fluconazole resistance, including Mrr2 (multidrug resistance regulator) that regulates the multi-drug efflux pump Cdr1. Tebung *et al.* (2016) used ZCF activation during the characterization of an example of rewiring between purine and pyrimidine metabolism in the ascomycetes, and recently in establishing a conserved role for Put3 in regulating proline catabolism in both *C. albicans* and *Saccharomyces cerevisiae* (Tebung *et al.* 2017). These studies led us to investigate ZCFs further to uncover the transcriptional regulatory circuits involved in pathogenicity-critical processes such as hyphal growth.

An essential characteristic of *C. albicans* critical for its success as a commensal and opportunistic pathogen is its ability to grow in different morphological forms. The two most common cellular forms are the yeast form, where cells grow by budding as individual, rounded cells, and the hyphal form, where cells grow as extended, branching filaments, with individual cells delineated by septa (Whiteway and Bachewich 2007). *C. albicans* cells use the filament form to escape from human macrophages and to invade into deeper tissues during infection (Rooney and Klein 2002; Kumamoto and Vinces 2005; Mayer *et al.* 2013). Transcriptional control is essential for the ability of cells to make the switch between the yeast and filamentous forms. Several TFs have been identified with functions in this switch. These include Efg1, a target of the Ras1/cAMP pathway (Stoldt *et al.* 1997), and Cph1, a target of the MAP kinase pathway (Liu *et al.* 1994), both being essential elements of the specific signal transduction pathways. They also include transcriptional repressors such as Tup1 (Braun and Johnson 1997) and Nrg1 (Braun *et al.* 2001).

The molecular mechanism of hyphal initiation suggests that environmental stimuli such as serum, Spider medium, and GlcNAc, serve to trigger a switch from a yeast state, where the Nrg1 repressor blocks activity of hyphal-associated genes (HAGs), to a hyphal state, where the Brg1 TFs and the associating Hda1 histone deacetylase remodel the chromatin state of the HAG promoters. This remodeling leads to the activation of the hyphal program (Lu *et al.* 2011). Thus, hyphal development involves downregulation of TF Nrg1 by the cAMP-dependent protein kinase

A (PKA) pathway, together with upregulation of Brg1 and Ume6 (Braun *et al.* 2001), and it has been proposed that the chromatin state of the promoters of crucial genes represents a central regulator of transcriptional control of the transition between the yeast and hyphal states (Lu *et al.* 2012). This circuit regulating chromatin structure required for hyphal initiation and extension involves the GATA family member Brg1 (Lu *et al.* 2011) and Ume6, a zinc cluster family member (Banerjee *et al.* 2008; Zeidler *et al.* 2009). Brg1 expression is repressed by Nrg1 (Cleary *et al.* 2012), and Brg1 and Nrg1 compete to control the chromatin state.

Our work identifies a new TF involved in the hyphal transition. The zinc cluster TF Rha1 interacts with the Nrg1/Brg1 switch; activated Rha1 can trigger filamentous growth in the absence of external signals, and in the presence of external signals like serum, Rha1 activation can bypass the need for Brg1. Loss of Rha1 function leads to a reduced ability to generate hyphal growth in the presence of external signals, and when coupled with loss of Ume6 function, creates completely non-hyphal cells. This establishes Rha1 as a critical ZCF functioning in the hyphal control circuitry of the human pathogen *C. albicans*.

3.2 Materials and Methods

3.2.1 Strains, media and growth conditions

All *C. albicans* strains, oligonucleotides, used in this study are listed in tables S3.1 and S3.2. For long term storage, cells were kept at -80 °C in 25% glycerol supplemented YPD. The strains were routinely cultured in liquid YPD (10 g yeast extract, 20 g peptone, 20 g glucose per liter, 50 µg/ml uridine (2% w/v Bacto-agar for solid medium) at 30 °C in a shaking incubator. The *C. albicans* transformations were plated on YPD agar plates containing 200 µg/ml nourseothricin (Werner Bioagents, Jena, Germany) or 500-600 µg/ml Hygromycin B (HygB) (Bioshop Inc, Canada), for 48 h at 30 °C.

3.2.2 *C. albicans* mutant strains

All *C. albicans* mutants except ChIP-chip strains were constructed in the wild-type (WT) strain SC5314 (Gillum *et al.* 1984) using the lithium acetate method of transformation (Gietz *et al.* 1995) or electroporation (Köhler *et al.* 1997). Two Rha1 mutants were created using different approaches in strain SC5314. The *rha1-/-* strain was constructed by using the CRISPR/Cas9 method to insert tandem stop codons into both *RHA1* alleles. The *rha1Δ/Δ* mutant is the result of

the deletion of both *RHA1* alleles, giving rise to the *rha1Δ/Δ* mutant SC1604M4B. A CRISPR/Cas9 system (Vyas *et al.* 2015) was used to construct the *RHA1* mutation strain. The sgRNA of *RHA1* was formed by annealing primers *RHA1*-sg-F and *RHA1*-sg-R and cloning the fragment into the BsmBI site of pV1093 to make plasmid pV1093-Rha1-sgRNA. A repair DNA was generated using Rha1-Rep-F, and Rha1-Rep-R primers to PCR amplify a fragment containing in-frame stop codons with a disrupted PAM region and directing introduction of an XhoI site for the confirmation of transformants with the correct insertions. The repair DNA template and linearized plasmid pV1093-Rha1-sgRNA were transformed, then transformants with the correct mutations were verified by PCR. We used a transient CRISPR/Cas9 system (Min *et al.* 2016) to delete *C. albicans BRG1* and *UME6* in the Rha1-GOF background strains. A Cas9 gene was amplified using a pV1093 plasmid and P7 and P8 standard primers. The final sgRNA fragment was amplified using P5 and P6 primers from the product of two separate PCR reactions, including the sgRNA sequence. The Hygromycin B repair template was amplified from pYM70 plasmid (Basso Jr *et al.* 2010) as a selection marker to create homozygous null mutants *brg1Δ/Δ* and *ume6Δ/Δ*. Correct deletions were confirmed using primers internal to the HygB markers in combination with primers for the upstream regions of *UME6* or *BRG1* and by using primers internal to the *BRG1* and *UME6* ORFs. The double mutant strains *rha1-/- ume6Δ/Δ* and *rha1-/- brg1Δ/Δ* were constructed in the *rha1-/-* Nat^R strain background. An *rha1* deletion mutant of strain SC5314 was also generated by the *SAT1*-flipping method (Reuß *et al.* 2004) . For this, *RHA1* upstream and downstream sequences were amplified with the primer pairs orf19.1604-5‘FW/orf19.1604-5’RV and orf19.1604-3‘FW/orf19.1604-3’RV, respectively, and cloned on both sides of the *SAT1* flipper cassette in plasmid pSFS5 (Sasse *et al.* 2011) The insert from the resulting plasmid p1604M1 was used to sequentially delete the two *RHA1* alleles of strain SC5314, generating the *rha1Δ/Δ* mutant SC1604M4B. For reinsertion of an intact *RHA1* copy, the *RHA1* coding and flanking sequences were amplified with primers 1604-5‘FW-Kompl and 1604-3‘RV-Kompl and substituted for the *RHA1* upstream flanking sequence in p1604M1 to yield p1604K2. The insert from this plasmid was used to reintegrate *RHA1* into its endogenous locus in the *rha1Δ/Δ* mutant SC1604M4B, followed by recycling of the *SAT1* flipper cassette to generate the complemented strain SC1604MK2B. A *ume6Δ/Δ* mutant of strain SC5314 was constructed in an analogous fashion. *UME6* upstream and downstream sequences were amplified with the primer pairs UME6-ko1/UME6-ko2 and UME6-ko3/UME6-ko4, respectively, and cloned on both sides

of the *SAT1* flipper cassette in plasmid pSFS5. The insert from the resulting plasmid pUME6M2 was used to sequentially delete the two *UME6* alleles of strain SC5314, generating the *ume6Δ/Δ* mutant SCUME6M4A. We constructed the *RHA1*-TAP strain from the strain SN148 (Noble and Johnson 2005; Lavoie *et al.* 2008) by transformation using a TAP-URA3 PCR product containing 99 bp of homologous sequence immediately upstream and downstream of the *RHA1* stop codon. The TAP-URA3 portion of the oligomer used for transformation was amplified from the pFA-TAP-URA3 plasmid.

3.2.3 Filamentation assays

The environmental filament induction assays were performed by growing the selected strains overnight in liquid YPD at 30 °C, 220-rpm. The next day, the cells were washed twice with 1× PBS and resuspended at OD_{600} = 0.1 into fresh liquid Spider (1.35% agar for solid medium ,1% nutrient broth, 0.4% potassium phosphate, and 2% mannitol, pH 7.2), YPD, or YPD plus 20% serum (Fetal Bovine serum; Sigma) then incubated at 37 °C for 4 hours. For solid medium, 5 µl from an OD_{600} = 0.1 culture was spotted onto YPD plus 20% serum plates and incubated at 37 °C for 3 days.

3.2.4 Biofilm assays

Strains were grown overnight in 5 ml liquid YPD at 30 °C on a shaker at 220 rpm. In the morning, after washing the cells twice with 1× PBS, the cell densities were adjusted to an OD_{600} = 0.5 in 200 µl Spider medium and then were added to a 96 well plate at 37 °C for 1.5 h in static condition to allow cell adherence to the surface of the wells. Next, non-adherent cells were removed by washing with PBS and 200 µl of fresh Spider was added to each of the washed wells. The plate was incubated for 24 h at 37 °C in a shaker at 75 rpm. Each well was washed twice with 200 µl of PBS, and the level of biofilm formation was determined by using the crystal violet assay, as reported previously (Daniels *et al.* 2013).

3.2.5 Stress assays

For stress assays, single colonies from WT and Rha1-GOF strains were grown overnight in YPD at 30 °C and resuspended in 1× PBS to a cell density of 1.5×10^{7} cells/ml at 600 nm, then were diluted in 10-fold stages from 10^{6} to 10^{2} in 1× PBS. These ten-fold serial dilutions of each strain were spotted onto YPD plates containing 5 mM hydrogen peroxide, 0.02% MMS, 10 mM $FeSO_4$, 20 mM $FeCl_3$, and 5 mM $CuSO_4$, 0.4 M $CaCl_2$, 1 M sorbitol, 0.15 mM menadione, 38 mM

hydroxyurea, pH 10, 250 mM Glycerol, or 100 µg/ml Hygromycin B. Plates were incubated for 3 days at 30 °C. The chemical concentrations were selected based on recent literature values used in large scale phenotypic screenings.

3.2.6 Microscopy and imaging

Single colonies of *C. albicans* strains were inoculated in 5 ml of liquid YPD and incubated overnight at 30 °C with shaking at 220 rpm. The cells were then washed twice with 1× PBS and imaged using differential interference contrast (DIC). For the hyphal induction images, cells were washed twice with 1× PBS, followed by the addition of Calcofluor white stain (2 µg/ml; Sigma, USA) for 20 min and imaged as described below. For quantification of elongation factor, cells were stained with Calcofluor white stain, then imaged using a Leica DM6000 microscope with both DIC optics and a DAPI filter cube (377/50ex, 447/60em), using a 100x (N.A. 1.3), 60x (N.A. 1.4) or 40x (N.A. 0.75) objective lens and a Hamamatsu Orca R2 camera. After capture, Calcofluor white stain images were presented to a Region-based Convolutional Neural Net (R-CNN, He et al. 2017) trained to recognize yeast cells, resulting in a binary mask that represents the outline of most cells in the image; these masks were verified by a trained human observer, who could discard inappropriate masks that did not correlate well with merged DIC and Calcofluor white stain images. The remaining masks were measured in FIJI (NIH, Bethesda), using the Shape Descriptors option to extract the aspect ratio of each cell, being the ratio of the width of the cell to its height (Lu *et al.* 2019). The aspect ratio of filament cells was calculated for each compartment of the filament. Of note, this R-CNN was not trained on elongated cells and failed to find the most elongated hyphal cells, resulting in an underestimation of aspect ratio in samples containing extremely elongated cells. Kruskal-Wallis and Dunn's multiple comparisons tests were conducted on the data to determine statistical significance. GraphPad Prism 6.00 was used for analysis.

3.2.7 Invasion assays

Single colonies of the selected strains were grown overnight in 5 ml YPD at 30 °C, 220 rpm. Then the cells were washed twice with 1× PBS and diluted to an optical density of OD_{600} = 0.1. Five µL from the adjusted cell density were spotted on YPD, incubated for 1 day at 30 °C or when spotted on Spider agar, incubated at 37 °C, 5 days. The spots on the Spider plates were then washed with sterile water for 15 seconds. Plates were scanned before and after washing using an Epson Perfection V500 Photo color scanner.

3.2.8 RNA isolation and RNA-seq experiment

Two biological replicates of both Rha1-GOF and WT strains were diluted to OD_{600} = 0.1 in YPD and inoculated to reach OD_{600} = 0.8-1. Total RNA was extracted using QIAGEN RNA extraction kit, then RNA quality and quantity were determined using an Agilent bioanalyzer. Paired end 150bp illumina misEQ sequencing was carried out at the Quebec Genome Innovation center. Raw reads were pre-processed as described previously (Costa *et al.* 2019). Briefly, the sequence-grooming tool cutadapt version 0.4.1 (Martin 2011) with the following quality trimming and filtering parameters (`--phred33 --length 36 -q 5 --stringency 1 -e 0.1`) was used. Each set of paired-end reads was mapped against the *C. albicans* SC5314 haplotype A version A22 downloaded from the *Candida* Genome Database (CGD) (http://www.candidagenome.org/) using HISAT2 version 2.0.4 (Kim *et al.* 2015). SAMtools (Li *et al.* 2009) was then used to sort and convert SAM files. The read alignments and SC5314 genome annotation were provided as input into StringTie v1.3.3 (Pertea *et al.* 2015), which returned gene abundances for each sample. Raw and processed data have been deposited in NCBI's Gene Expression Omnibus under accession number GSE 143825 (Edgar *et al.* 2002). We imported gene abundances into R using the tximport R package and conducted differential analysis of transcript count data using the DESeq2 R package (Soneson *et al.* 2016). We use the independent hypothesis weighting (IHW) Bioconductor package (Ignatiadis *et al.* 2016) to weight p-values and adjust for multiple testing using the procedure of Benjamini Hochberg (BH) (Benjamini and Hochberg 1995).

Expression heatmaps of selected genes were plotted using the pheatmap R package (https://cran.r-project.org/web/packages/pheatmap/index.html). Hierarchical clustering based on Euclidean distance and average linkage was applied to both samples (in columns) and genes (in rows). Gene ontology (GO) analyses were achieved on gene lists obtained by RNA-seq to identify putative enriched biological functions, processes, or cell compartments (*Candida* Genome Database GO Term Finder; (http://www.candidagenome.org/cgi-bin/GO/goTermFinder) and to assign differentially regulated genes to specific biological processes (*Candida* Genome Database GO Slim Mapper; http://www.candidagenome.org/cgi-bin/GO/goTermMapper).

3.2.9 ChIP-chip

ChIP-chip experiments were performed as described previously (Tebung *et al.* 2016) with minor changes. Briefly, the strain containing the chromosomally integrated Rha1-TAP fusion, as well as the background strain SN148 (untagged), were grown to an OD_{600} of 0.6 in 50 ml of YPD medium and then used for ChIP. Cross-linking for each 50 ml culture was carried out in 1.5 ml of 37% formaldehyde for 30 min, and then ChIP was performed as described previously (Tebung *et al.* 2016). ChIP DNA extracted from tagged strains was labeled with Cy5 dye, ChIP DNA from untagged strain SN148 was labeled with Cy3 dye, and the samples were then cohybridized to Agilent 8X15K whole-genome arrays containing 14490 60-mer intergenic and intragenic oligonucleotide probes. Microarray hybridization, washing, scanning, and normalization were performed as described previously (Nantel *et al.* 2006) with the following modifications: The Axon GenePix 4000B microarray scanner was used to perform scanning, and GenePix data analysis software and Multiexperiment Viewer (MeV) software were used to analyze and normalize data; a 0.05 P value cut-off was used for MeV analyses. The scanning settings used were 635 nm for Cy5 and 532 nm for Cy3. The log of ratios of Cy5 to Cy3 (635 nm/532 nm) with a cut-off of at least 1.5 for each spot was considered to be an indicator of significant orf19.1604 binding.

3.2.10 Virulence studies

Male ICR mice of 6 weeks age (n = 12) were used for virulence assay as previous described (Feng *et al.* 2020). Briefly, 200 µl of 5×10^6 cells ml^{-1} were injected intravenously into the tail vein of mice. Survival rates were checked daily and survival curves were generated according to the Kaplan–Meier method using the PRISM program (version 5.0; GraphPad Software) and compared using the log-rank test. Moribund mice were sacrificed and sections were prepared from the kidneys and stained with periodic acid-Schiff's (PAS) stain for histological examination (Feng *et al.* 2020). Mouse studies were carried out under the guidelines established by the Ethics Committee of Nantong University, China.

3.2.11 Data access

ChIP-chip, transcriptional profiling, and GO term analysis for the Rha1-GOF are available at https://github.com/Rahaomran/Raha-Omran.git.

3.3 Results

3.3.1 Activation of Rha1 triggers *C. albicans* filamentation

We screened a library of *C. albicans* strains containing overexpressed and activated zinc cluster transcription factors (Schillig and Morschhäuser 2013) and identified orf19.1604 as generating an abnormal colony morphology; colonies of cells over-expressing orf19.1604 fused to a mutant Gal4 activation domain were wrinkled and crenulated whereas the control strain SC5314 colonies were smooth. The strains expressing the activated orf19.1604 were also invasive and resistant to washing from the surface of YPD medium plates after growth at 30 °C. By contrast, cells of the control strain SC5314 were washed easily from the surface of the agar (Figure 3.1A). When grown in liquid YPD medium at 30 °C, strains containing activated orf19.1604 were highly flocculant (Figure 3.1A), and assessment of the cellular morphology showed a high frequency of filamentous cells exhibiting primarily pseudohyphal characteristics (Figure 3.1B). As a consequence of this role in filamentation, we named the gene encoding orf19.1604 as *RHA1*, for Regulator of Hyphal Activity. Furthermore, we observed enhanced biofilm formation in the *RHA1* activated strain compared to strain SC5314 (Figure 3.1C).

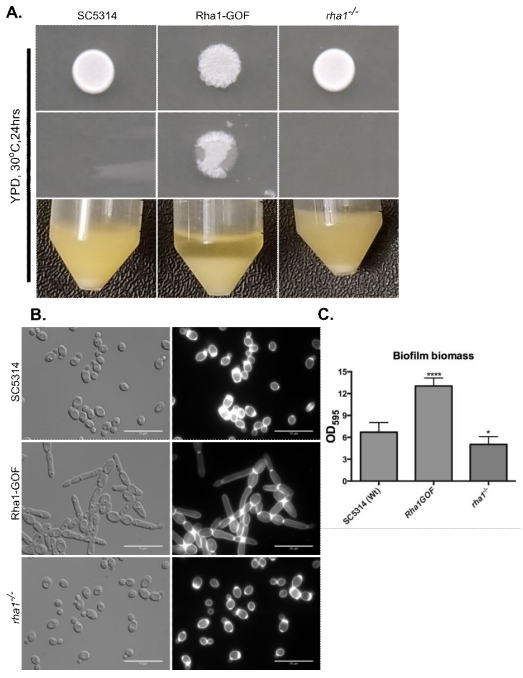


Figure 3.1: Morphological and biofilm results of the WT, Rha1-GOF, and *rha1* mutant. **A**. WT, Rha1-GOF, and *rha1-/-* strains were spotted on the solid nonfilament-inducing medium YPD and grown for 24 h before washing with a stream of water for 15 s. The Rha1-GOF strain was invasive and resistant to washing.

The indicated strains were grown in liquid YPD medium at 30 C. Rha1-GOF cells are flocculent in YPD medium **B**. The cellular morphology of the indicated strains is displayed after growth overnight at 30 C in liquid YPD medium. Cells were washed twice with 1x PBS, stained with CFW, and visualized by DIC optics (Bar, 10 lm). **C**. A biofilm assay was run in biological triplicate in Spider medium at 37 C after 48 h. $*P < 0.05$, $***P < 0.001$ relative to the WT strain (one-way ANOVA with Dunnet test multiple comparison test).

3.3.2 Rha1 orthologs are limited to the CTG clade

In *C. albicans*, *RHA1* (C2_09460C_A) encodes a 989 amino acid protein with a zinc cluster type DNA-binding domain at the N-terminus that shows limited sequence similarity to that of Lys14 in *Saccharomyces cerevisiae*, although Rha1 is unlike ScLys14 in the rest of the protein. In order to identify orthologs of *C. albicans* Rha1, the protein sequence from the *Candida* Genome Database (CGD) (http://www.candidagenome.org/) was compared across the ascomycetes using Blastp searches of the multiple fungal genome database (https://www.yeastgenome.org/blast-fungal). Convincing orthologs with sequence similarity outside of the DNA binding region of Rha1 are limited to the CTG clade-specific species that use CTG to encode serine rather than leucine. They are not present in several phylogenetically related non-pathogenic species, including *Saccharomyces cerevisiae*, *S. paradoxus*, *S. mikatae*, *S. bayanus*, *S. castelli, and K. lactis*.

3.3.3 Activated Rha1 does not influence general stress phenotypes

We examined the sensitivity of strains expressing activated Rha1 to a variety of stresses. Hyperactivity of Rha1 (Rha1-GOF) did not enhance sensitivity to the DNA damaging agent MMS at 0.02%, the oxidative stress-inducing agent hydrogen peroxide (H_2O_2) at 5 mM, the metal stressors 10 mM $FeSO_4$, 20 mM $FeCl_3$, and 5 mM $CuSO_4$, and the osmotic stressor 1 M sorbitol. As well, cells were not sensitive to 0.4 M $CaCl_2$, 0.15 mM menadione, 38 mM hydroxyurea, pH 10, glycerol at 250 mM, and Hygromycin B at 100 µg/ml (Supplemental Material, Figure S3.1).

3.3.4 Activated Rha1 modulates gene expression

We performed RNA sequencing to determine the transcriptional profile of cells carrying the activated *RHA1* allele (n = 2) compared to the WT cultured under the non-hyphae-inducing conditions of YPD medium at 30 °C (n = 2). Three notable classes of genes were up-regulated above 4-fold with adjusted p-value < 0.001, compared to the reference strain SC5314 (Table 3.1; Figure S3.2A). The first class of genes encodes classic cell wall adhesins such as Als1, Als3, and

hypha-related proteins including Hgc1, Hwp1, Ece1, and Hgt1, consistent with the observed phenotype of filamentous cells (Martin *et al.* 2013). A second class consists of genes encoding transcription factors that themselves regulate hyphal development, specifically Brg1 and Ume6. The third notable collection of significantly upregulated genes encodes arginine metabolism enzymes such as Arg1, Arg3, Arg4, and the arginine permease Can2. Among the top 35 downregulated genes, there is the well-studied hyphal repressor Nrg1, which is repressed 2.5-fold in Rha1-GOF cells. Interestingly, a number of transcription factors of the ZCF class including ZCF1, ZCF3, and FCR1, appear among the downregulated genes (Figure S3.2B). Overall, the downregulated genes in Rha1-GOF compared to WT were enriched for biological function in transcription regulator activity. As well, Rha1-GOF down-regulated the arginine catabolism gene, *CAR1*, consistent with the high proportion of arginine biosynthesis genes upregulated in Rha1-GOF cells. A gene ontology (GO) analysis indicated that the upregulated genes in Rha1-GOF were involved significantly in carbohydrate transport (35.8%), biological process involved in interspecies interaction between organisms (20%), regulation of growth (16.8%), biological adhesion (14.7%), filamentous growth (29.4%) and biofilm formation (27.4%) (https://github.com/Rahaomran/Raha-Omran.git). These groupings were not mutually exclusive – many genes fell into more than one class.

To further investigate the targets of Rha1, we investigated the binding site of Rha1::TAP using ChIP-chip. This analysis identified a set of candidate target filament or biofilm related genes like *CEK1*, *BRG1*, *DEF1* (*EED1*) as well as other genes of unknown function (Table 3.2; https://github.com/Rahaomran/Raha-Omran.git). Overall, the overlap in these candidate targets with transcriptionally up-regulated genes in the Rha1-GOF strain shows strong enrichment in genes induced during biofilm formation (Table 3.2).

Table 3.1: RNAseq result of Rha1-GOF. Duplicated cultures of WT and Rha1-GOF strains were grown overnight and processed for RNA seq. Upregulated classes of genes identified include genes involved in *Candida albicans* filament formation, hyphal-specific TFs, and arginine biosynthesis. To be classified as up-regulated or down-regulated, RNAs must show a fourfold change in abundance with *P*-values <0.001.

Gene	Name	log2FC	Adjusted P value	Description
orf19.3981	*MAL31*	10	3.29E-09	Putative high-affinity maltose transporter
orf19.4936.1	-	9.13	6.32E-07	Putative adhesin-like protein; Spider biofilm induced
orf19.4527	*HGT1*	9.03	5.85E-07	High-affinity MFS glucose transporter
orf19.3374	*ECE1*	9.02	1.18E-11	Candidalysin; hypha-specific protein
orf19.113	*CIP1*	8.55	1.57E-04	Possible oxidoreductase
orf19.6028	*HGC1*	7.8	2.02E-04	Hypha-specific G1 cyclin-related protein
orf19.1822	*UME6*	7.73	3.32E-04	Zn(II)2Cys6 transcription factor; role in hyphal extension
orf19.1227	*ZCF4*	7.63	3.10E-04	Putative Zn(II)2Cys6 transcription factor
orf19.7469	*ARG1*	7.21	1.07E-152	Argininosuccinate synthase; arginine synthesis
orf19.1321	*HWP1*	7.03	5.92E-11	Hyphal cell wall protein
orf19.5610	*ARG3*	5.56	9.49E-69	Putative ornithine carbamoyltransferase
orf19.5741	*ALS1*	5.38	2.23E-40	Cell-surface adhesin; adhesion
orf19.4630	*CPA1*	5.26	1.12E-82	Putative carbamoyl-phosphate synthase subunit
orf19.6689	*ARG4*	5.11	8.33E-84	Argininosuccinate lyase
orf19.4377	*KRE1*	5	6.96E-24	Cell wall glycoprotein; beta glucan synthesis
orf19.7610	*PTP3*	4.95	5.97E-82	Putative protein tyrosine phosphatase; hypha induced
orf19.4056	*BRG1*	4.82	8.10E-33	Transcription factor; recruits Hda1 to hypha-specific promoters
orf19.3770	*ARG8*	4.8	1.09E-60	Putative acetylornithine aminotransferase
orf19.3221	*CPA2*	4.74	7.76E-97	Putative arginine-specific carbamoylphosphate synthetase
orf19.4788	*ARG5,6*	4.62	3.21E-91	Arginine biosynthetic enzyme
orf19.3282	*BMT3*	4.48	5.13E-13	Beta-mannosyltransferase
orf19.1816	*ALS3*	4.42	2.62E-59	Cell wall adhesin

Table 3.2: Overlap summary between ChIP-chip and RNA-seq data. 502 of Rha1-GOF ChIP-chip > 1.5 log of ratio bound site and 106 Rha1-GOF RNAseq upregulated genes with log2FoldChange > 1.5 identified.

Feature	Orf	Gene	Description
C1_08170C_A	orf19.5094	*BUL1*	Protein similar but not orthologous to *S. cerevisiae* Bul1
C1_08940C_A	orf19.4752	*MSN4*	Zinc finger transcription factor; similar to *S. cerevisiae* Msn4, but not a significant stress response regulator in *C. albicans*
C2_08920W_A	orf19.215	-	Component of a complex containing the Tor2p kinase; possible a role in regulation of cell growth; Spider biofilm induced
C3_01800C_A	orf19.1666	-	Ortholog of Dig2, a MAP kinase-responsive inhibitor of Ste12
C4_02990C_A	orf19.2693	*GST2*	Glutathione S transferase; induced by benomyl and in populations of cells exposed to fluconazole over multiple generations
C4_06480C_A	orf19.2886	*CEK1*	ERK-family protein kinase; required for wild-type yeast-hypha switch, mating efficiency, virulence in mice
C5_03930C_A	orf19.3219	-	Ortholog of *S. cerevisiae* Sia1
C7_01390W_A	orf19.6920	-	Protein of unknown function; induced during chlamydospore formation in both *C. albicans* and C. dubliniensis
C7_01680C_A	orf19.6556	-	Protein of unknown function; rat catheter, flow model and Spider biofilm induced
CR_00290W_A	orf19.7504	-	Ortholog of *S. cerevisiae* Rts3; a component of the protein phosphatase type 2A complex
CR_03890W_A	orf19.467	*WOR3*	Transcription factor; modulator of white-opaque switch; induced in opaque cells; promoter bound by Wor1
CR_06790C_A	orf19.1855	-	Predicted membrane transporter, member of the anion:cation symporter (ACS) family, major facilitator superfamily (MFS); Gcn4p-regulated
CR_09880W_A	orf19.7561	*DEF1*	RNA polymerase II regulator; role in filamentation, epithelial cell escape, dissemination in RHE model
C1_05140W_A	orf19.4056	*BRG1*	Transcription factor; recruits Hda1 to hypha-specific promoters; Tn mutation affects filamentation; Hap43-repressed

3.3.5 Loss of Rha1 function modulates hyphal development

Activation of Rha1 leads to filamentation, and ChIP-chip and RNA-seq analysis showed a link to biofilm formation. We therefore asked if Rha1 function is required for normal hyphal development in response to hyphal inducing conditions. We inactivated both alleles of *RHA1* in the WT background and assessed filamentation and biofilm formation. In comparison to the WT strain, the *rha1-/-* strain showed a 33% reduction in biofilm formation (Figure 3.1C). We assessed hyphal development in response to both serum medium and growth on Spider medium at 37 °C. Colony morphology appeared unchanged on solid serum media (Figure 3.2A) and showed indistinguishable invasion on Spider medium (Figure 3.3A). However, the *rha1-/-* strain formed less inner colony wrinkling compared to the WT and *ume6Δ/Δ* (Figure 3.3A). Cell morphology was changed in response to growth in liquid serum and Spider medium. Under serum inducing conditions at 37 °C at time 4 h, the mutants *rha1-/-*, *brg1Δ/Δ* and *ume6Δ/Δ* displayed a filamentation defect compared with the WT (Figure 3.2, B and C). As is shown in Figure 3.2C, the WT cells can filament as expected, with median aspect ratio (AR, a length to width ratio serving as a measure of cell polarity) of 2.1. Cells lacking Rha1 showed reduced filamentation (median AR = 1.3), as did mutant strains lacking the classic hyphal regulators Ume6 (slightly, median AR = 1.4) and Brg1 (dramatically, median AR = 1.2). The filamentation defect was more extreme for Spider induction conditions as shown in Figure 3.3B; here, the *rha1* mutant strain was strongly compromised in hyphal development after 4 hours at 37 °C. The WT strain filamented with median AR of 2.4, but cells lacking Rha1 (AR median of 1.3), Brg1 (AR median of 1.3) and Ume6 (AR median of 1.5) showed significantly reduced filamentation compared to the WT (Figure 3.3C). Taken together, these results suggest that Rha1 plays a key role in filamentation in liquid serum and Spider medium.

We also assessed whether the *rha1* inactivated strain was auxotrophic for arginine, because activation of Rha1 also leads to the up-regulation of genes involved in arginine metabolism (Table 3.1). We grew the strain in liquid SC-arg medium for 72 h at 30 °C. In the absence of arginine supplementation, the mutant strain grew identically to the control strain (Figure S3.3). Finally, we tested complementation to further confirm Rha1 function by adding a single copy of *RHA1* back to a *rha1Δ/Δ* mutant. The complemented strain rescued the *rha1Δ/Δ* mutant filamentation defects, confirming that the initial observed phenotype was due to loss of Rha1 function (Figure S3.4).

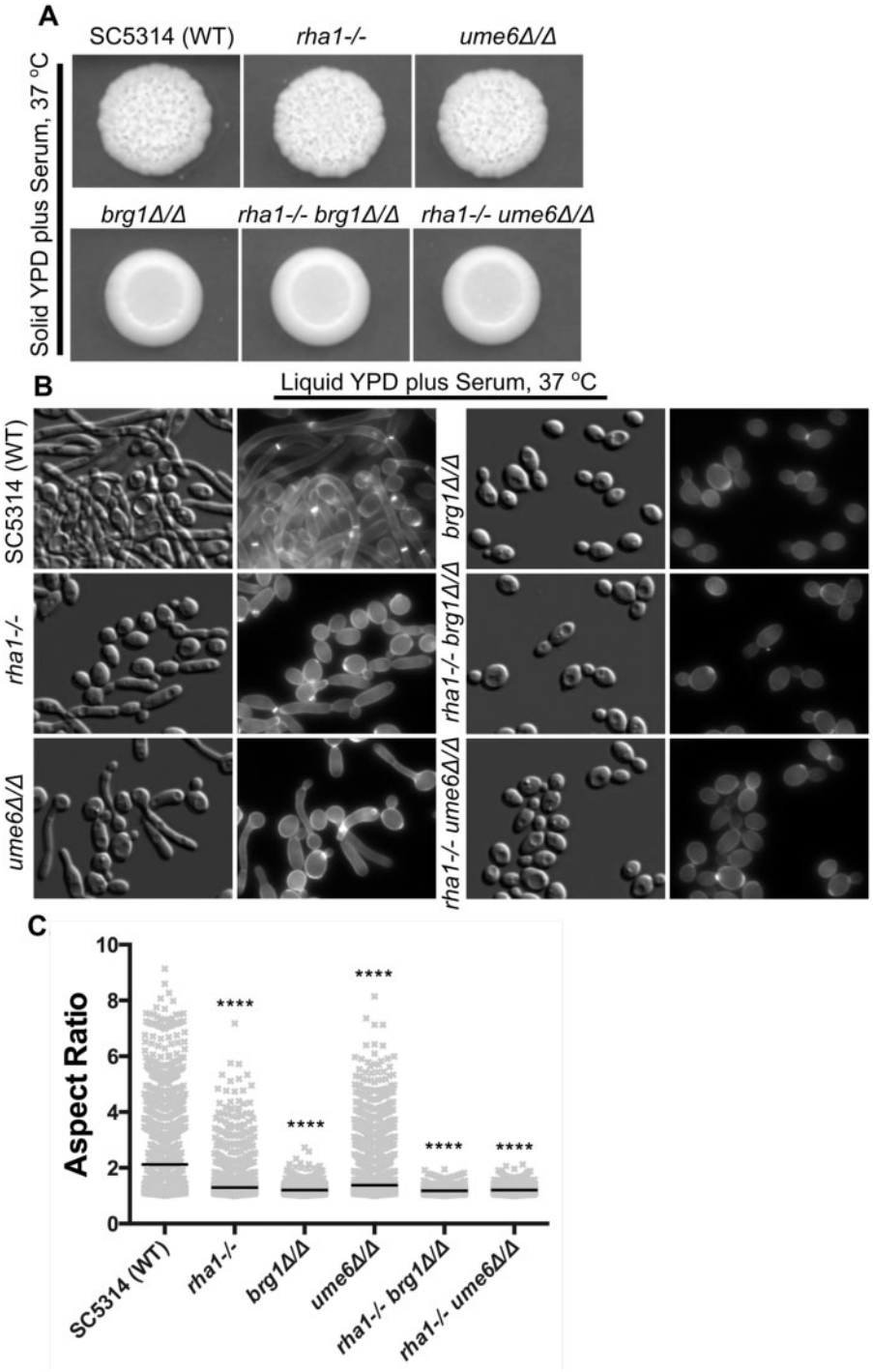


Figure 3.2. Deletion of *RHA1* and *UME6* causes defects in filamentation under serum stimuli. (A) The wrinkled colony morphology of the *rha1*, *ume6* and *brg1* single mutants, and *rha1*-/- *ume6Δ/Δ* and *rha1*-/- *brg1Δ/Δ* double mutants, together with the WT strain SC5314 on 20% solid serum medium are shown after three days of growth at 37 °C. (B) Strains were grown in liquid YPD supplemented with 20% serum for 4 h, 37 °C, stained with CFW, and examined at 63x magnification by DIC optics and a DAPI filter cube (Bar,10 μm). (C). Aspect ratio analysis of the cellular morphology of strains for each experimental group,

N ≥ 1314 cells derived from at least 3 different experimental repeats. The horizontal lines indicate the median. **** P < 0.0001; Kruskal–Wallis test with Dunn's multiple comparison test.

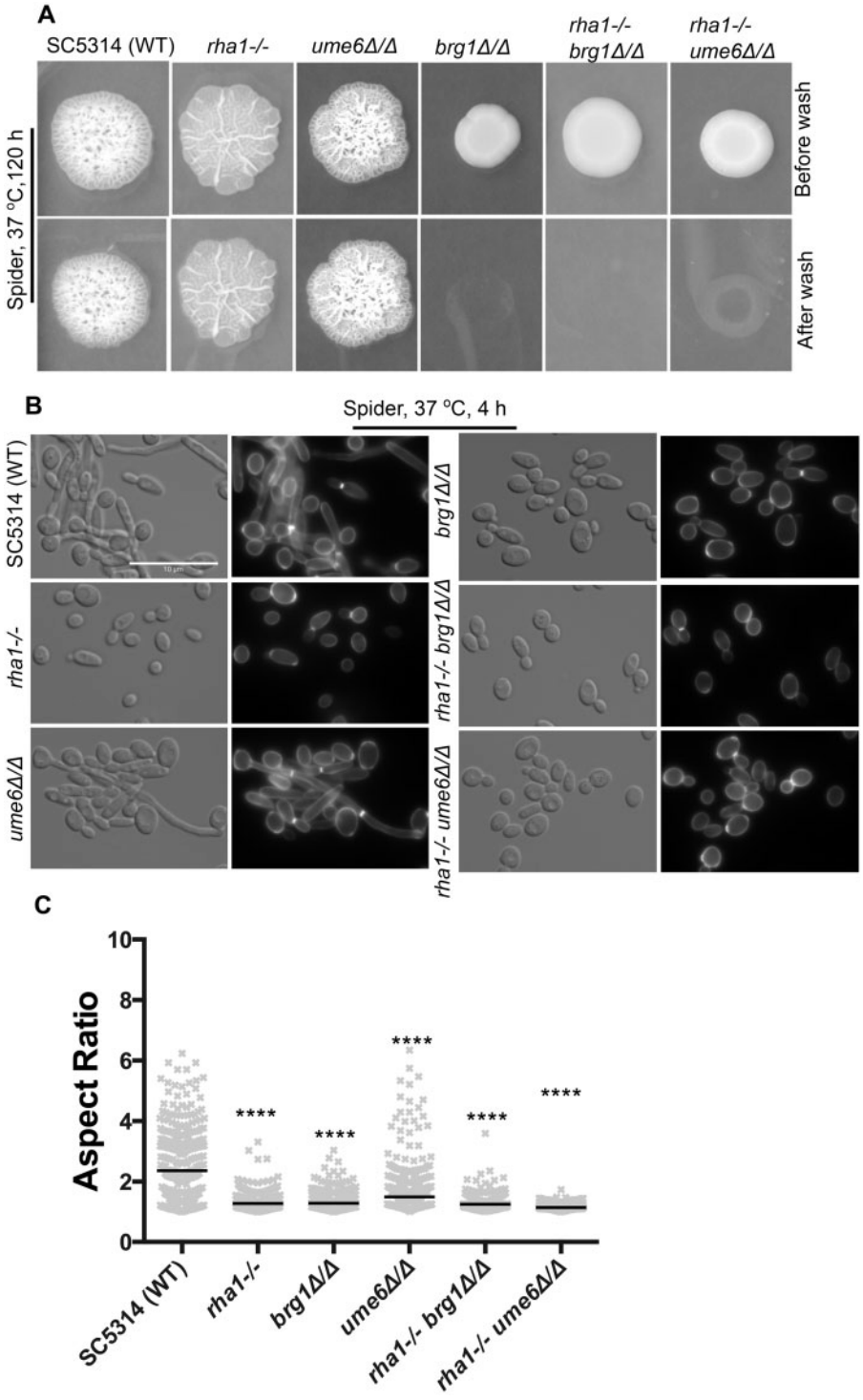


Figure 3.3. Inactivation of *RHA1* causes defects in hyphal formation on Spider medium. (A) The invasiveness of strains after growth on Spider medium for 120 h was tested after washing colonies with a stream of water. (B) The cellular morphology of the indicated strains grown in the liquid Spider medium,

4 h at 37 °C. Scale bar is 10 μm. The *rha1*-/- *ume6Δ/Δ* double mutants were highly defective in hyphal formation when grown in Spider medium. (C) Aspect ratio analysis of Spider-treated strains is shown to quantify the reduction in filamentation. Horizontal lines show the median AR. n ≥ 315. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001; Kruskal–Wallis test with Dunn's multiple comparison test.

3.3.6 Gain of function of Rha1 impairs virulence in a mouse model

Given the role of Rha1 in hyphae-formation regulation, we checked the function of the transcription factor in virulence by injecting the Rha1 gain-of-function strain and the *rha1* deletion strain separately into mice. The mice infected with the WT cells all died by day 8, showing an average survival of 6 days, while the mice infected with the *rha1* deletion cells and complemented strain both showed an average survival of 5 days, with no significant difference with the WT group (Figure 3.4A). However, the Rha1 gain-of-function strain showed an impaired virulence; only 4 mice infected with this strain died by day 18, a significant difference from the WT group. Consistently, in the kidneys of mice, WT cells, the *rha1* deletion, the *RHA1* complemented, and the Rha1 gain-of-function cells all showed filamentous forms (Figure 3.4B), although the null mutant infections appeared to have fewer of these forms. Overall, it appears that the gain-of-function allele of *RHA1*, but not the deletion, impairs virulence significantly compared to the WT.

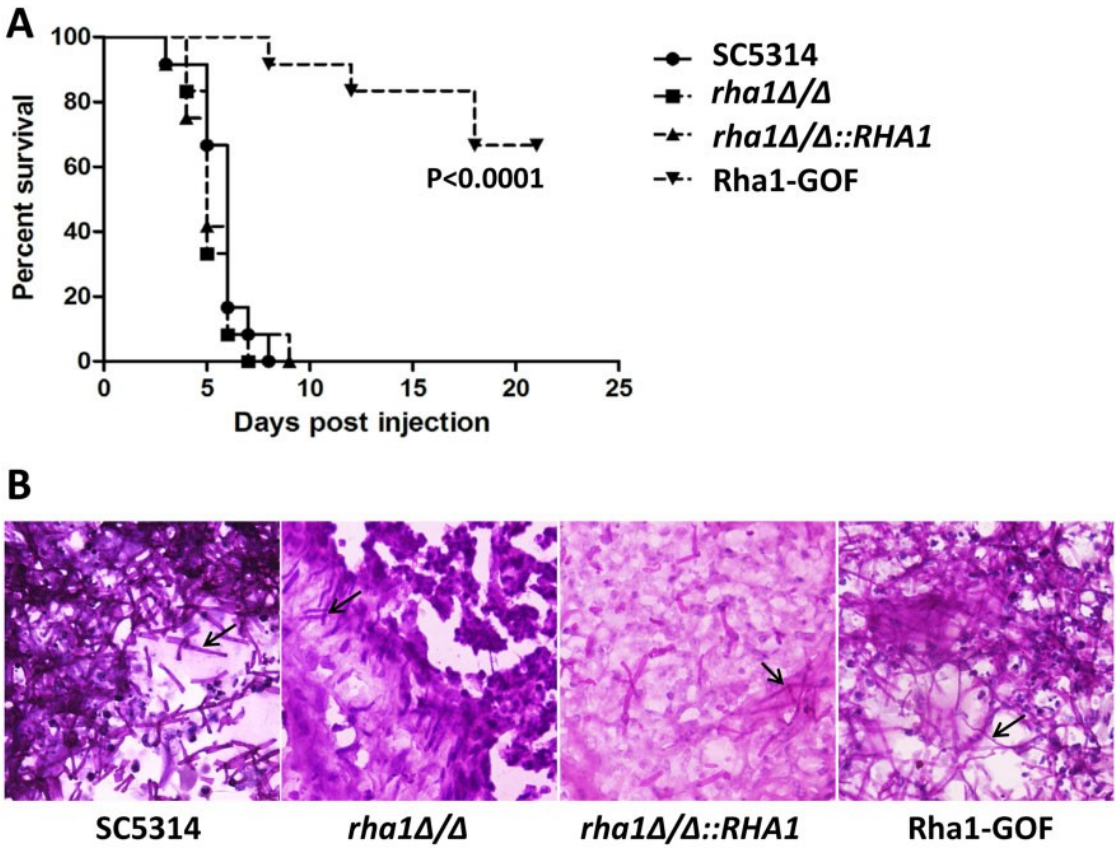


Figure 3.4: Hyperactivity of Rha1 reduces virulence in mice. **A.** Survival curves of mice intravenously infected with the indicated *C. albicans* strains. Male ICR mice (6 weeks old, 12 mice for each group) were injected with 1×10^6 stationary-phase cells. Mice were checked daily for morbidity. **B.** Histopathological examination of kidney tissues of moribund mice was performed after sacrifice. The infected kidney tissues were stained with periodic acid-Schiff's reagent. The hyphae cells were indicated by black arrows.

3.3.7 Brg1 and Ume6 are required for the Rha1 hyperactive phenotype

The expression profiling results showed that the key hyphal-regulator-encoding genes *BRG1* and *UME6* were overexpressed in strains with hyperactive Rha1. We investigated whether the function of either gene was required for the filamentous phenotype generated by Rha1 hyperactivation under normal yeast growth conditions (Figure 3.5A). The strain with the activated Rha1 construct shows an aspect ratio (median AR of 2.1). We deleted *UME6* and *BRG1* in this strain and observed a significant loss of induced filamentation in the Rha1-GOF *brg1Δ/Δ* strain, with the median of the aspect ratio dropping to 1.4, supporting a role of Brg1 in activated Rha1-triggered filamentation. There was much less reduction in filamentation in the Rha1-GOF *ume6Δ/Δ* strain (median AR= 1.9) (Figure 3.5B). As expected, the single mutants of Brg1 and Ume6 alone

displayed normal yeast morphology with median ARs of 1.3 and 1.1, respectively. These results indicate that *BRG1* contributes to proper filamentation in the Rha1 activated strain. In contrast, the *UME6* deletion only reduces the hyphal extension in the Rha1 hyperactive strain, which is consistent with its defined role in the normal extension process (Banerjee *et al.* 2008).

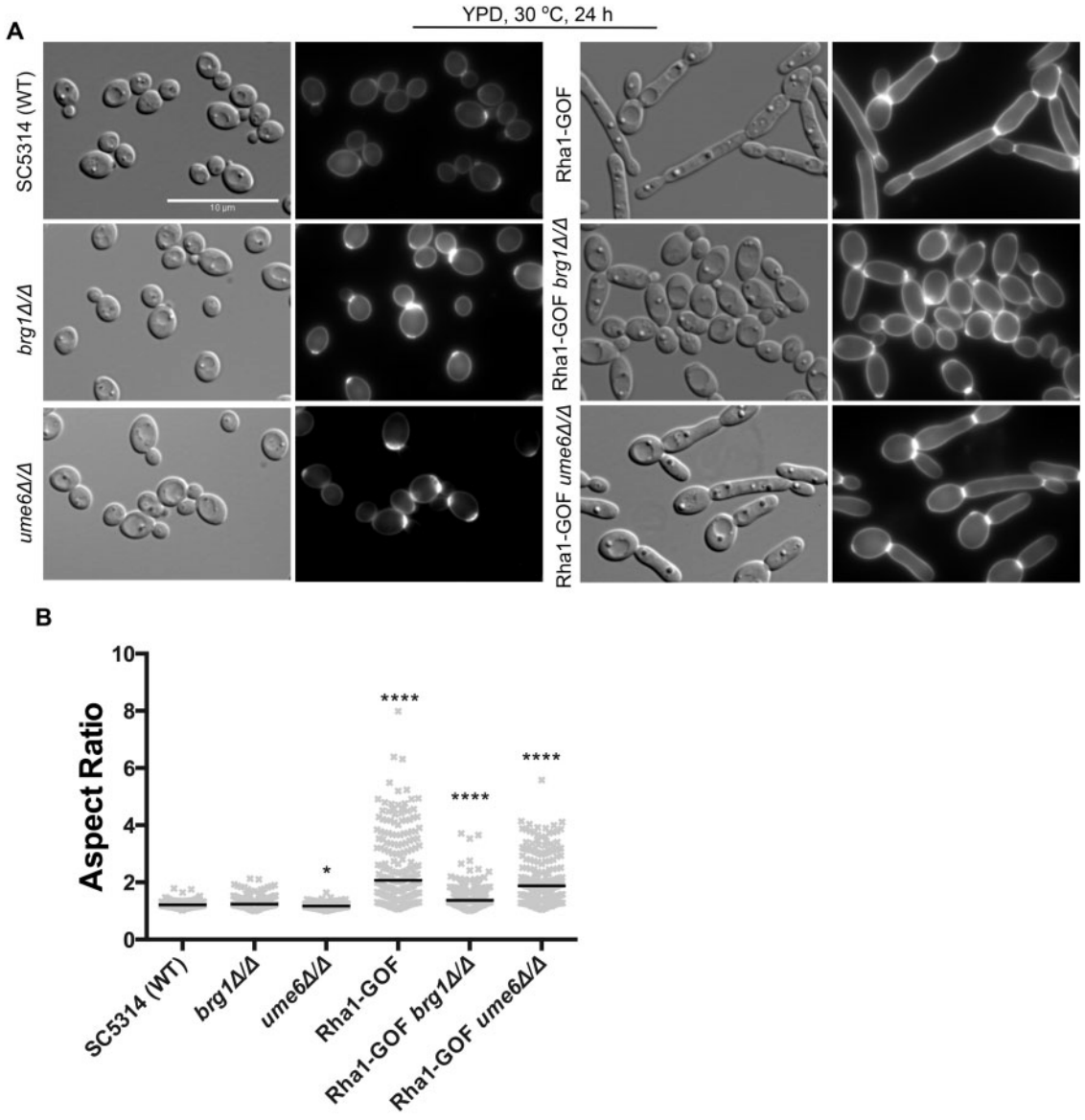


Figure 3.5: Deletion of *BRG1* and *UME6* causes defects in Rha1-GOF-induced morphology. **A.** Cellular morphology of the indicated strains under yeast growth conditions and imaged with DIC optics and DAPI filter cube **B.** Horizontal lines represent the median AR. n ≥ 200. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001; Kruskal–Wallis test with Dunn's multiple comparison test. Scale bar is 10µm.

3.3.8 Rha1 and TFs controlling *C. albicans* filamentation

We further investigated the relationship between Rha1 and the hyphal-controlled transcription factors identified in our profiling experiment. We first constructed double mutant combinations of the *RHA1* with *UME6* and *BRG1* deletions to extend our examination of the effect of *RHA1* on hyphal development in response to environmental stimuli. As shown in Figure 3.2B and Figure 3.3B, the *rha1 brg1* double mutant was not significantly different from the *brg1* single mutant, which was by itself essentially non-hyphal in response to either serum or Spider inducing conditions (median ARs of 1.2 and 1.3, respectively). By contrast, however, the *rha1 ume6* double mutant was significantly compromised in hyphal formation in response to either serum (median AR = 1.2) or Spider (median AR = 1.1) stimulation compared to the WT, even though the single mutants still showed significant morphological response to either hyphal inducing condition (Figure 3.2C and 3.3C). As previously noted, filamentation was reduced in *ume6* single mutants under serum (median AR =1.4) and not significantly under Spider (median AR = 1.5) stimuli, and cells lacking Rha1 showed aspect ratio median of 1.3 under serum and Spider induced conditions.

We also examined the effect of the Rha1 activated allele on the serum and Spider medium stimulation of hyphal development in the *ume6* and *brg1* null mutants. As can be seen in Figure 3.6, cells that were defective in hyphal formation in response to serum due to deletion of *UME6* or *BRG1* formed long filaments when the environmental stimulus was combined with the hyperactivated Rha1 construct. Serum treatment in the presence of the hyperactive Rha1 construct (median AR = 3.4) was able to significantly stimulate some cellular elongation even in cells deleted for both *BRG1* and *UME6* compared to the parental strain in yeast growth condition (Figure 3.6, A and B). Serum stimulation caused the Rha1-GOF *ume6Δ/Δ* strain to form filaments with median AR of 2.5, and even the Rha1-GOF *brg1Δ/Δ* strain to filament with a mean AR of 1.5 and Rha1-GOF *ume6Δ/Δ brg1Δ/Δ* strain (median AR = 2) (Figure 3.6B). As well, strains with activated Rha1 and *BRG1* or *UME6* deleted showed filaments on Spider medium but generated no wrinkled morphology on solid plates (Figure 3.7A and B). As is shown in Figure 3.7C, the Rha1-GOF *brg1Δ/Δ* strain incubated in Spider medium demonstrated cells lacking yeast morphologies (median AR = 1.7) compared to the Rha1-GOF (median AR = 2.4). However, Rha1-GOF *ume6Δ/Δ* formed elongated cells (median AR = 2.4), notably better than Rha1-GOF incubated in Spider medium (median AR = 2.4). Furthermore, Rha1-GOF cells lacking *UME6* and *BRG1* incubated in

the same medium were not triggered to form filaments (median AR = 1.6) (Figure 3.7C). This suggests that while hyperactivation of Rha1 was capable of bypassing the requirement of Brg1 and Ume6 in serum-rich media to trigger filamentation, this response was less effective in Spider medium.

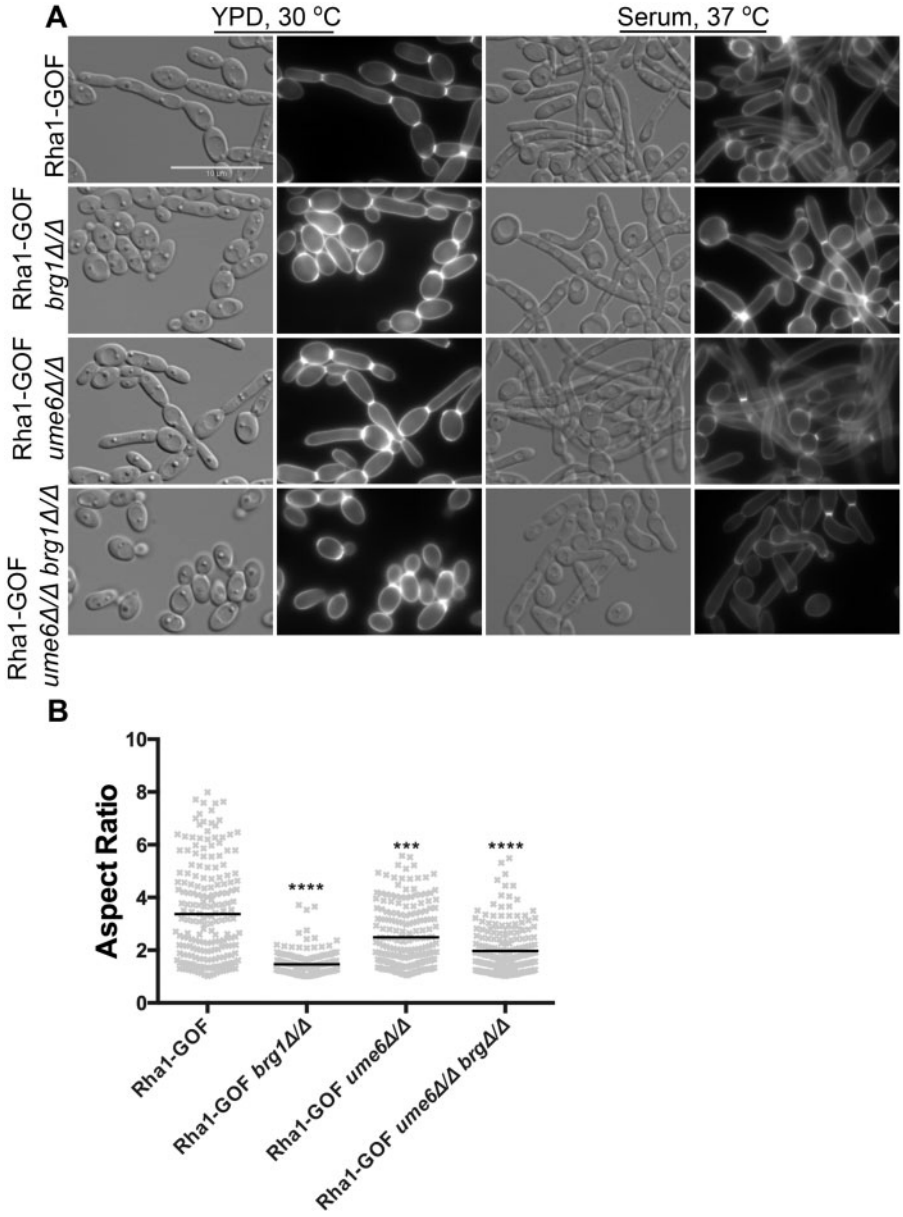


Figure 3.6: Rha1-GOF rescues filament development in *brg1* and *ume6* single mutants in the presence of 20% serum. **A**. Overnight cultures of strains grown in YPD plus 20% serum **B**. The aspect ratio of cells after 4 h in 20% serum and overexpression of Rha1. When supplemented with serum the Rha1-GOF could bypass the effect of *brg1* and *ume6* single mutants. The cells imaged by DIC and a DAPI filter cube. N ≥ 200; Horizontal lines represent the median AR. n ≥ 200. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001; Kruskal–Wallis test with Dunn's multiple comparison test.

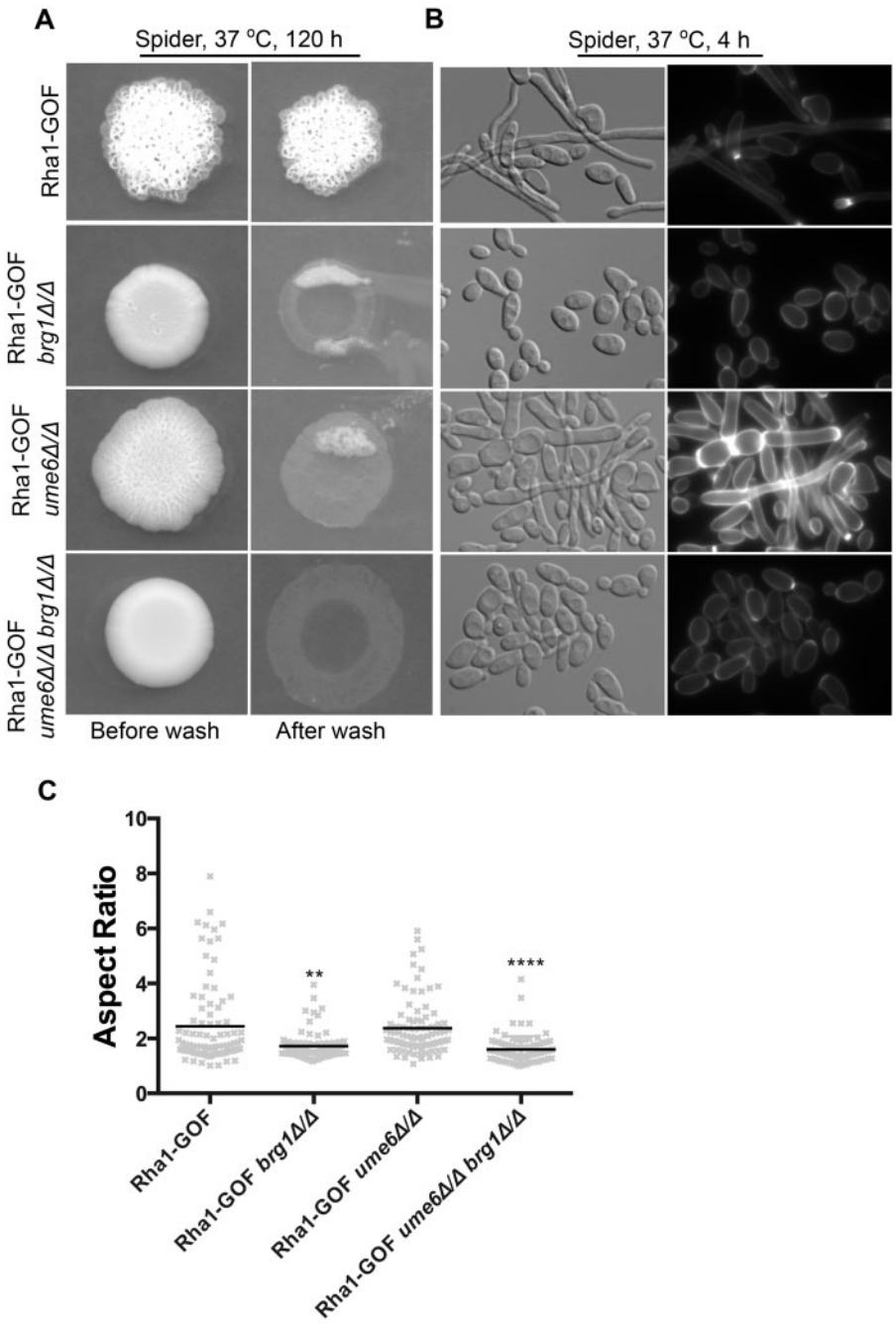


Figure 3.7: The media specificity of Rha1 for bypass of the filamentation defects of single *brg1* and *ume6* mutants. (A) Strains were spotted on Spider medium, and invasiveness assayed after being washed twice with 1× PBS and incubated 5 days. (B) DIC and fluorescence images represented cellular morphology of strains grown in liquid Spider medium. Rha1 overexpression coupled with Spider medium showed some morphological effects in the *ume6Δ/Δ* mutant but failed to bypass the phenotypes of *brg1Δ/Δ* or *brg1Δ/Δ ume6Δ/Δ* mutants. Horizontal lines represent the median AR. n ≥ 91. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; Kruskal–Wallis test with Dunn's multiple comparison test. Scale bar represents 10 μm.

3.4 Discussion

One of the critical characteristics that allows *C. albicans* to be a successful fungal pathogen is its capability of transitioning from a budding yeast to a filamentous form. *C. albicans* cells use this morphological plasticity to escape from human macrophages and to invade into deeper tissues during infection (Lo *et al.* 1997; Rooney and Klein 2002; Mayer *et al.* 2013). Numerous studies have identified a variety of signaling pathways, with a complex interconnected network of transcription factors (TFs), required for regulation of hyphae-associated genes (HAGs) under different external stimuli (Biswas *et al.* 2007). These signaling pathways include the mitogen-activated protein kinase (MAPK), the target of rapamycin (TOR), the regulation of Ace2 morphogenesis (RAM), and the RAS/cAMP pathways (Basso *et al.* 2019).

The use of gene deletions to investigate the roles of individual members of such complex transcriptional regulatory circuits can be problematic in cases of functional redundancy (Kafri *et al.* 2006). As an alternative, artificial gene activation can be a powerful strategy to uncover specific gene functions and to relate a phenotype to genotype for functional discovery in such interlinked systems (Prelich 2012). In this study, we used a variety of approaches, including genetic activation, to explore the molecular role of a new member of the hyphal control circuit, a zinc cluster family member we have termed Rha1 for regulator of hyphal activity. The Rha1 protein is a classic zinc cluster transcription factor; it is 989 amino acids long, with the zinc cluster DNA binding domain found from aa 16 to aa 47. Protein sequence alignments of CaRha1p revealed putative orthologs exclusively in the CTG clade of Saccharomycotina, whereas searches directed outside of the *Candida* clade show sequence similarity limited to the generally conserved zinc cluster DNA binding domain. The CTG group contains a large number of closely related pathogenic yeast, including *C. dubliniensis*, *C. parapsilosis*, *C. tropicalis*, *C. lusitaniae*, and *C. guilliermondii*, and filamentation has been connected with pathogenesis (Turner and Butler 2014). Activation of Rha1 established that it functions in the hyphal development circuit; transcriptional profiling and analysis of genetic interactions suggest that its role in this circuit connects it to chromatin structure through the Nrg1 repressor and the Brg1 and Ume6 activators.

Regulation of transcription factors such as Brg1 and Ume6 has been connected to a central signaling pathway controlling hyphal development that requires the cAMP-dependent PKA. PKA is activated by a cAMP signal created through Ras1 and adenylyl cyclase (Cdc35/Cyr1). The action

of this pathway ultimately results in the relief of Nrg1-mediated repression of hyphal associated genes (HAGs) (Lu *et al.* 2011, 2014). Overall, PKA regulates chromatin conformation by changing Nrg1-mediated repression into Brg1-mediated activation at the promoters of HAGs This transition also involves a subunit of the histone chaperone complex (Hir1), which has been shown to be involved in the downregulation of Nrg1 and initiation of hyphal development (Jenull *et al.* 2017), and the hyphal elongation transcription factor Ume6, which functions during the hyphal maintenance phase (Lu *et al.* 2011).

Strains defective in Brg1 are unable to initiate hyphal development and remained locked in the yeast state in response to activating signals like serum (Homann *et al.* 2009; Cleary *et al.* 2012). Strains defective in Ume6 can initiate but not maintain induced hyphal development, and thus generate short germ tubes under inducing conditions (Banerjee 2008; Zeidler *et al.* 2009). However, loss of Brg1 or Ume6 were bypassed in serum-stimulated cells that contained a hyperactive allele of *RHA1*; hyphal development in response to serum proceeded efficiently, suggesting Rha1 hyperactivity can compensate for the loss of either Brg1 or Ume6. However, in the presence of hyperactivated Rha1, serum treatment of the *brg1 ume6* double mutant triggered polarized growth but not extensive filaments, showing that Rha1 activation cannot altogether bypass the Brg1/Ume6 circuit. Intriguingly, on solid medium hyperactive Rha1 did not overcome the filamentation defect of the *brg1* mutant in the presence of serum. Previous studies have established different genetic and transcriptional programs during hyphal development in cells responding to growth in either liquid or solid media (Azadmanesh *et al.* 2017).

In addition to bypassing blocks in serum-stimulated hyphal development in *brg1* and *ume6* mutants, we found that hyperactive Rha1 directs constitutive hyphal growth, creates an invasive phenotype in the absence of external hyphal inducing signals, and stimulates biofilm formation. Consistent with the phenotypic observations, transcriptional profiling of the hyperactivated Rha1 strain in the absence of external signals for hyphal development revealed induction of a variety of hyphal-specific genes like *ECE1* and *HWP1*, as well as genes for the transcription factors Brg1 and Ume6, and also showed downregulation of the gene for the hyphal repressor Nrg1. Aside from *NRG1* downregulation, we noted repression for several ZCF members such as *ZCF1*, *ZCF3*, and *FCR1*, which are in some way related to filament and biofilm formation. In particular, Vandeputte *et al*. (2011) reported that deletion of *ZCF3* significantly enhances hyphal formation. Thus, it will

be useful to further investigate the effects of Rha1 on *ZCF3* and the other transcriptionally influenced zinc cluster TF genes.

The induction of filamentation caused by Rha1 activation in the absence of external signals depended entirely on Brg1, while loss of Ume6 reduced, but did not eliminate, Rha1-hyperactivity-induced filamentation. The deletion of both *BRG1 and UME6* together completely blocked the filamentation caused by hyperactive Rha1 under yeast growth conditions. Recent analysis of gene expression during growth in both solid and liquid conditions showed that a series of hyphal induction conditions (10% fetal bovine serum, Lee's media, RPMI media, and Spider media) did not significantly induce or repress *RHA1* expression (Azadmanesh *et al.* 2017). *RHA1* expression has however been found to increase in cells lacking Tup1 or Nrg1 when compared with the WT, and in these strains is primarily expressed within the first two hours after serum treatment at 37 °C (Kadosh and Johnson 2005; Banerjee *et al.* 2008). Overall then, hyperactivation of Rha1 in the presence or absence of serum stimulation establishes an active link between Rha1 function and the Nrg1-Brg1 switch regulating chromatin structure at HAG promoters and the initiation of the hyphal developmental program.

The inactivation of *RHA1* also provides evidence for a link between Rha1 and the Nrg1/Brg1-Ume6 circuit. The inactivation mutants showed limited defects in hyphal development, as filamentation was moderately delayed in serum-stimulated cells, and more significantly delayed in Spider medium induced cells. However, the *rha1 ume6* double mutant was utterly defective in serum or Spider-induced hyphal development, although either mutant alone was capable of initiating hyphal formation. This suggests some redundancy in the roles of Rha1 and Ume6 in the hyphal developmental program.

Interestingly, Rha1 may function in more than just the yeast to hyphal transition. Genes involved in arginine biosynthesis were also up-regulated in cells with a hyperactivated Rha1. Previous work has suggested a linkage between hyphal development and arginine biosynthesis (Jiménez-López *et al.* 2013); Rha1 may thus play a role in connecting these two cellular processes. Hyperactivity of Rha1 resulted in attenuated virulence in the mouse model, with a significant change in survival curves. This may result from driving hyphal growth through Rha1 hyperactivity affecting dissemination, as evidence suggests that yeast form cells are vital for escape from the bloodstream, and adhere better than filament cells to endothelial cells (Saville *et al.* 2003). The

result is consistent with previous reports where *BRG1* overexpression or the inactivation of *NRG1* attenuated virulence in systemic infections (Murad *et al.* 2001; Cleary *et al.* 2012). By contrast, the *rha1Δ/Δ* single mutants showed normal virulence in the mouse tail-vein injection assay even though they exhibited a minor defect in establishing abundant hyphae.

In Figure 3.8, we propose a genetic interaction model to highlight the relationships among Rha1, Ume6 and Brg1 in response to hyphal cues. This model proposes that Brg1/Hda1 can act through Rha1 and Ume6 after being exposed to hyphal-inducing stimuli like serum. A prior study demonstrated that constitutively expressed Ume6 corrected the hyphal growth defect in *hda1* mutant cells (Lu *et al.* 2012), and we found that Rha1 hyperactivation bypassed the inactivation of Brg1 in serum-stimulated cells. According to this model, Rha1 and Ume6 can contribute cooperatively to hyphal formation, as we found single *rha1* or *ume6* mutants permit filamentation, but the double mutant fails to form hyphal cells in response to inducing cues. We suggest Rha1 and Ume6 play primarily distinct roles in filamentation; Rha1 in initiation and Ume6 in elongation. Rha1 activation uniquely induced arginine biosynthesis genes that are known to be critical for the initiation step in *C. albicans* filamentation (Ghosh *et al.* 2009), while it did not induce Ume6-triggered genes such as *CDC10*, *RBT4*, *CBP1*, *FAV2*, *CDC10*, *PHR1* and *RDI1*, which may be important in elongation (Carlisle and Kadosh 2013). Key hyphal-associated genes like *ALS3*, *ECE1*, *HWP1*, and *HGC1* can be up-regulated by activation of either Rha1 or Ume6. In addition, Rha1 may also influence upstream elements, as the overlap of the transcriptional profiles and the ChIP-chip profiles suggest Brg1, Cek1 and Def1 (Eed1) are potential Rha1 target genes. Because the Rha1-GOF expression profile also contains uncharacterized transcription factors from the ZCF family, including ZCF4 that may play a role in Rha1-responsive filamentation, it appears there is considerable complexity in the network that still needs to be resolved.

Overall, our study provides potential insight into the mechanism of hyphal development controlled by the Nrg1/Brg1 switch. Under hyphal-inducing conditions, Rha1 functions to facilitate both downregulation of the Nrg1 repressor and upregulation (directly or indirectly) of the Brg1 and Ume6 transcription factors required for initiation and maintenance of HAGs. This switches the cell from a repressed chromatin configuration at the HAGs controlled by Nrg1 to the activated state controlled by Brg1, and thus Rha1 represents an important new regulator of the yeast to hyphal transition critical for *C. albicans* pathogenicity.

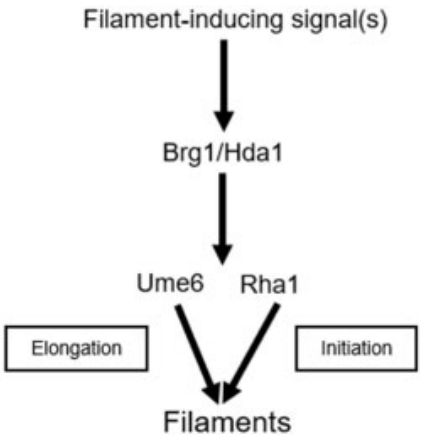


Figure 3.8: Schematic representation of Rha1 genetic interactions during the yeast to hyphal transition. Filament generating signal(s) like serum induce the expression of Brg1 and stimulate the downstream pathways. We propose that Brg1/Hda1 directs filamentation via both Rha1, which primarily acts in initiation, and Ume6, which primarily acts in hyphal elongation. Ume6 has previously been implicated in elongation and, when activated, has been shown to bypass the requirement of Brg1/Hda1 (Lu *et al.* 2012). We show here that activated Rha1 in the presence of serum stimulus can similarly bypass the deletion of Brg1. We also find that although either single mutant is able to facilitate filamentation, the double *rha1 ume6* mutant is unable to form hyphae. Furthermore, activation of Rha1 in a *ume6* mutant generates frequent short hyphae that are inefficiently extended, consistent with a role of Ume6 in extension, while activation of Ume6 in a *rha1* mutant background allows extension of all initiated filaments.

3.5 Acknowledgments

We thank all members of the M. Hallett lab for helpful discussions, and M.W lab members for valuable contribution during this study. We want to acknowledge funding support from the NSERC discovery RGPIN/4799, and NSERC Canada Research Chair 950-228957 awards. Work in J.M.s lab was funded by the Deutsche Forschungsgemeinschaft (DFG grant MO 846/7). Work in J.F.s lab was supported by a NSFC funding (No 82072261).This paper is dedicated to Rebecca Schillig (deceased), who originally discovered orf19.1604 (*RHA1*) as a regulator of filamentous growth of *C. albicans* during her PhD studies in J.M.s lab.

3.6 Supplementary material

Table S3.1: *Candida albicans* strains used in this study

Strain	Parent	Description	Source
SC5314	Clinical Isolate	Wild type	(Gillum *et al.* 1984)
SN148	SN76	*arg4/arg4 leu2/leu2 his1/his1 ura3/ura3*	(Noble and Johnson 2005)
SC1604GAD1A	SC5314	*ADH1/adh1*::P_{ADH1}*-RHA1-GAL4AD-HA-caSAT1*	(Schillig and Morschhäuser 2013)
SC1604M4B	SC5314	*rha1Δ::FRT/rha1Δ::FRT*	This study
SC1604MK2B	SC1604M4B	*rha1Δ::FRT/RHA1::FRT*	This study
RHA1-TAP	SN148	*RHA1/RHA1-TAP-URA3*	This study
rha1-/-	SC5314	*rha1/rha1 ENO1/eno1:: natMX4*	This study
brg1Δ/Δ	SC5314	*brg1::HYGB/brg1::HYGB*	This study
um6Δ/Δ	SC5314	*ume6::HYGB/ume6::HYGB*	This study
rha1 -/- brg1Δ/Δ	*rha1-/-*	*rha1/rha1 ENO1/eno1:: natMX4brg1::HYGB/brg1::HYGB*	This study
rha1 -/- ume6Δ/Δ	*rha1-/-*	*rha1/rha1 ENO1/eno1:: natMX4ume6::HYGB/ume6::HYGB*	This study
Rha1-GOF *brg1Δ/Δ*	Rha1-GOF	*brg1::HYGB/brg1::HYGB ADH1/adh1*::P_{ADH1}*-RHA1-GAL4AD-HA-caSAT1*	This study
Rha1-GOF *ume6Δ/Δ*	Rha1-GOF	*ume6::HYGB/ume6::HYGB ADH1/adh1*::P_{ADH1}*-RHA1-GAL4AD-HA-caSAT1*	This study
SCUME6M4A	SC5314	*ume6Δ::FRT/ume6Δ::FRT*	This study
SC*ume6*Δ1604GAD1A	SCUME6M4A	*ume6Δ::FRT/ume6Δ::FRT ADH1/adh1*::P_{ADH1}*-RHA1-GAL4AD-HA-caSAT1*	This study
Rha1GOF *brg1 Δ/Δ ume6 Δ/Δ*	*SCume6Δ1604GAD1A*	*brg1::HYGB/brg1::HYGB ume6Δ::FRT/ume6Δ::FRT ADH1/adh1*::P_{ADH1}*-RHA1-GAL4AD-HA-caSAT1*	This study

Table S3.2: Oligonucleotides used in this study

Oligo	**Sequence (5'-3')**	**Description**
P7	ATCTCATTAGATTTGGAACTTGTGGGTT	5' CAS9
P8	TTCGAGCGTCCCAAAACCTTCT	3' CAS9
P1	AAGAAAGAAAGAAAACCAGGAGTGAA	5' sgRNA
P4	ACAAATATTTAAACTCGGGACCTGG	3' sgRNA
P5	GCGGCCGCAAGTGATTAGACT	5' sgRNA
P6	GCAGCTCAGTGATTAAGAGTAAAGATGG	3' sgRNA
orf19.1604-5'FW	TATAGGGCGAATTGGAGCTCGACGTTGATAATTTCATCACC	*RHA1* deletion
orf19.1604-5'RV	AGAGCGGCCGCCACCGCGGTGGTATAAGTATAATTTAGGGG	*RHA1* deletion
orf19.1604-3'FW	AGTATAGGAACTTCCTCGAGCAAAATGAAATAGGTAAGCGA	*RHA1* deletion
orf19.1604-3'RV	AAAAGCTGGGTACCGGGCCCAATCAGGGAACCAAAGGTGA	*RHA1* deletion
1604-5'FW-Kompl	ATATGAGCTCGGGCCCGACGTTGATAATTTCATCACC	*RHA1* reinsertion

1604-3‘RV-Kompl	AAAAGCTGGGTACCCCGCGGAATCAGGGAACCAAAGGTGA	*RHA1* reinsertion
UME6-ko1	CATTAGAGCTCAAATTCTTGGAGAAAGATCAAAG	*UME6* deletion
UME6-ko2	AAACCCGCGGAACTAATTGGAAGTAAATTGAGG	*UME6* deletion
UME6-ko3	GTTAATCTCGAGGCTAAGTTAAGAATTAAGAATTAACAGG	*UME6* deletion
UME6-ko4	CATTAGGGCCCAGACTTTTCCGTATGATGAAACA	*UME6* deletion
RHA1-sg-F	ATTTGCAGCTTATAGAAAATAACACG	sgRNA for *RHA1*
RHA1-sg-R	AAAACGTGTTATTTTCTATAAGCTGC	sgRNA for *RHA1*
RHA1-Rep-F	CAGTCATACTCCTGTACCTTTGCATTTGAACGACCAGGCTCAGCTTATAGAATAATAACACTCGAG	repair DNA for *RHA1*
RHA1-Rep-R	ATATTGAGGACCCAGTGTCGCCGCTGGCATTGAAAAACTCGAGTGTTATTATTCTATAAGCTGAGC	repair DNA for *RHA1*
RHA1-In-F	AGTGCACAGAAGAGCTACCTTC	5’ *RHA1* Internal checking
RHA1-In-R	GTTGGTGCTGATGGTTGTGGTT	3’ *RHA1* Internal checking
RHA1-F	TCTCAAGCAGCGAAAATCTCCA	5’ *RHA1* checking primer
RHA1-R	CGGGGTACCATCTGGTTCAATAAAGGC	3’ *RHA1* checking primer
BRG1-sg-F	TCACGATCAACCATTAGTGGGTTTTAGAGCTAGAAATAGCAAGTTAAA	sgRNA for *BRG1* promoter
BRG1-sg-R	CCACTAATGGTTGATCGTGACAAATTAAAAATAGTTTACGCAAGTC	sgRNA for *BRG1* promoter
BRG1-HygBRep-F	ATTTGATATTTCAACGTTATTTCTCCATCCATACTTGTTACATTATAAATATTCACTACTATTCCAGAAATTCAAATTGCAAGTATATCTGGCAAACCTG	repair DNA for *BRG1* promoter
BRG1-HygBRep-R	TGTGCAAAAAATTAAATTAAAACTATTTTAATGACGAATTAAAGGAATTTGGGTTGGGTAAGCAACAGGAATACCGCCAGTTTATGATGGAATGAATGGG	repair DNA for *BRG1* promoter
BRG1-In-F	GCAACAACAACCAGTGCACGTA	5’ *BRG1* Internal checking
BRG1-In-R	TGTGGTTGTTGTGGTGGGGTAT	3’ *BRG1* Internal checking
BRG1-F	ACCCGTTTAACATTTGCAGCTT	5’ *BRG1* checking primer
BRG1-R	TTCGGTTTGATCCAGCTCAGGA	3’ *BRG1* checking primer
UME6-sg-F	ATGAGATCTTTGGCACCAGTGTTTTAGAGCTAGAAATAGCAAGTTAAA	sgRNA for *UME6* promoter
UME6-sg-R	ACTGGTGCCAAAGATCTCATCAAATTAAAAATAGTTTACGCAAGTC	sgRNA for *UME6* promoter
UME6-HygBRep-F	ATATTCTATATCTTCACTCCAAACATCAATATCCTCAATTTACTTCCAATTAGTTTTATTTTTTAATCCACTGTATAAGCAAGTATATCTGGCAAACCTG	5’ UME6 Repair DNA
UME6-HygBRep-R	GCAACAACAACAACAACAATAACCACCGTCAACCGTCAACCTGTTAATTCTTAATTCTTAACTTAGCCTAATTTTTATGATGGAATGAATGGG	3’ *UME6* Repair DNA
UME6-In-F	TCTTCAGCACCAAATTCGCCTT	5’ *UME6* Internal checking
UME6-In-R	GCAGCACTAACACTGACACCAC	3’ *UME6* Internal checking
UME6-F	TGGCTCATTATTGCTTTGCTTT	5’ *UME6* checking primer
UME6-R	CGGGAAAAGTTGCAAGAGTTGGT	3’ *UME6* checking primer
*RHA1*_TAP_URA3_F	ATATTTGGACGTGAGGGTAAAGTCTTTGATGATATTGGAACCATAAAACAGGGACTTGAAAACTTTGAGTTGGCACAAAAGGAAATAGCAAAATGTGTTGGTCGACGGATCCCCGGG TT	5’ *RHA1*_TAP_URA3
*RHA1*_TAP_URA3_R	TGCTACATTCACTAAAAACTGTATCTACATAATGGTTTTATTGATAGCCAGTACTAAAATTTCAATACAATCGCTTAC	3’ *RHA1*_TAP_URA3

	CTATTTCATTTTGTTAACACATCGATGAATTCGAGCTCG TT	
HygB-F-5’	TGCTTCTGCTGCTTTGCCAATTCCAG	5’ HYGB checking primer
Hygb-R-3’	ACAACCATCAGTCCAAACAGCAGCTGA	3’ HYGB checking primer
Nat-in-Fwd	TGATTGATCTGTCGGCAGTGGT	5’ NAT Internal checking
Nat-in-Rvs	GTCAATGCCGCCGAGAGTAAAG	3’ NAT Internal checking

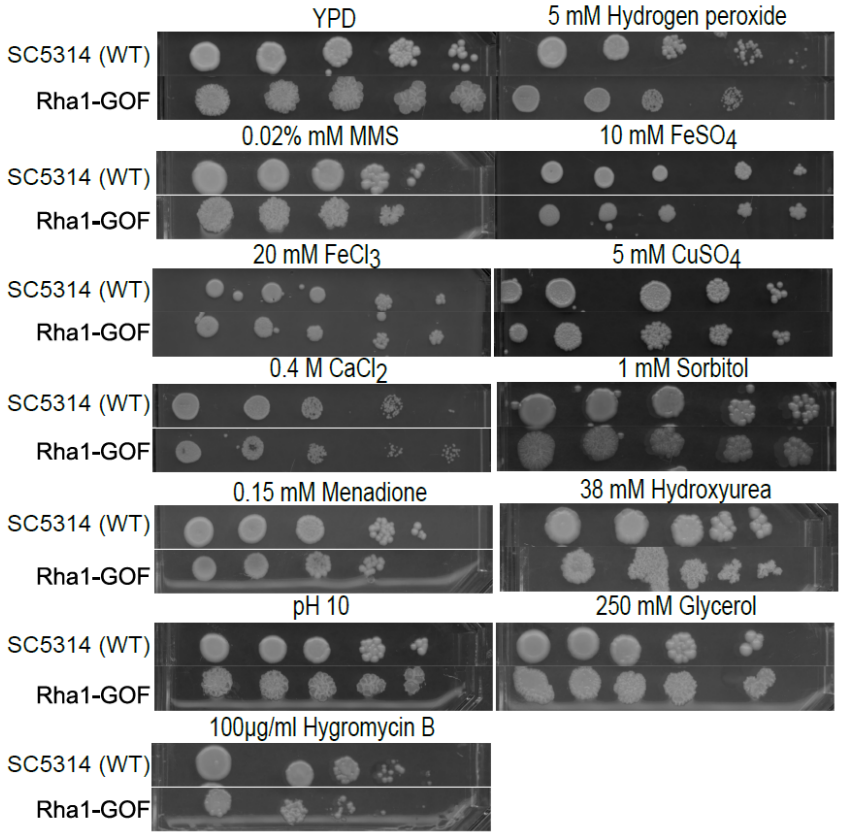


Figure S3.1: The effect of different stresses on strains expressing Rha1-GOF. Overnight cultures of SC5314 and Rha1-GOF were spotted under the indicated stress conditions. Rha1-GOF cultures grow normally under the different stress conditions including, the metal stressors, 100 mM $FeSO_4$,20 mM $FeCl_3$, and 5 mM $CuSO_4$, the oxidative stress-inducing agent hydrogen peroxide at 5 mM and the osmotic stressor sorbitol at 1 M, 0.4 M $CaCl_2$, 0.15 mM menadione, 38 mM hydroxyurea, pH 10, glycerol 250 mM, hygromycin B 100 µg/ml and 0.02% MMS.

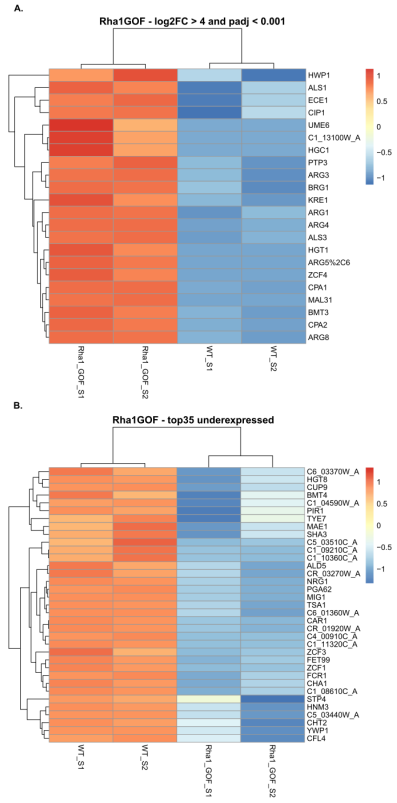


Figure S3.2: RNAseq analysis of Rha1-GOF. (A) Heatmap of up-regulated genes (log2 fold >4, Padj <0.001) versus the wild-type under non hyphal conditions. (B) Top 35 down- regulated genes P-values <0.03 in the Rha1-GOF compared with the wild-type under non-hyphal inducing conditions.

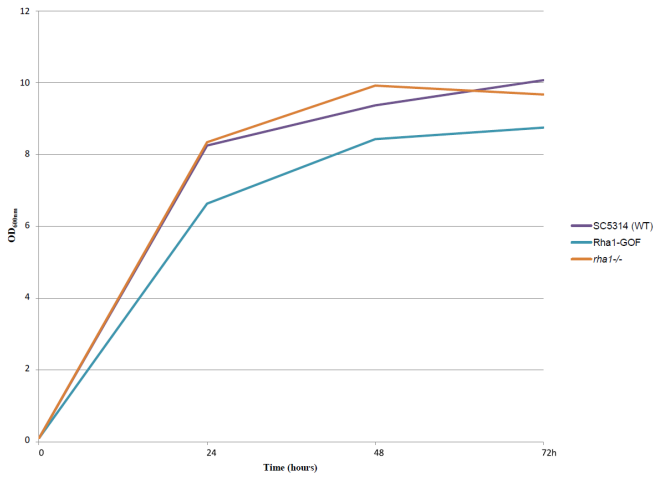


Figure S3.3. Rha1 strains grow normally on media without arginine. Growth curves (OD_{600} of *C. albicans* SC5314(wild-type), Rha1-GOF and *rha1-/-* to determine influence of Rha1 function in arginine biosynthesis. The overnight strains were cultured in liquid SC without arginine and incubated at 30 °C for 72 h.

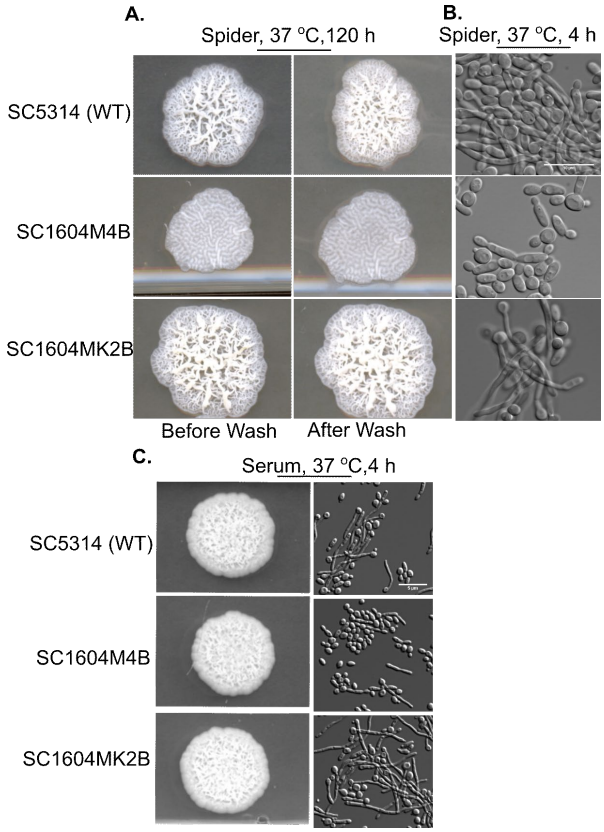


Figure S3.4: One copy of Rha1 restores the filamentation morphology in *rha1Δ/Δ*. **A**. The WT, SC1604M4B, (*rha1Δ/Δ*), SC1604MK2B (*rha1Δ/Δ::RHA1*) were assessed with an invasion plate assay on Spider medium for 5 days to test invasiveness. **B**. The DIC images of the indicated strains grown in the liquid Spider medium, 4 h at 37 °C. The complemented strain SC1604MK2B induced hyphal formation in Spider medium. Scale bar represents 10 µm. **C**. Serum treated cells after 4 h at 37 °C. Cells which represent one copy of Rha1 add back the filamentation function. Scale bar is 5 µm.

Chapter 4: Regulation of Hyphal Development by *ZCF4* in *Candida albicans*

Abstract

Candida albicans is an opportunistic fungal pathogen of humans. The morphogenetic switch from the unicellular budding yeast to the hyphal form has been linked to the fungi's pathogenesis, but at present our understanding of the regulatory circuit inducing filamentation is incomplete. Here, we have investigated *ZCF4*, a zinc cluster transcription factor, for involvement in the regulation of filamentation development. Although deletion of *ZCF4* generated no dramatic phenotype, we found that activation of *ZCF4* caused enhanced filamentation in response to a starvation-like cue, while reducing hyphal development triggered by serum treatment. We performed RNA-sequencing analysis to determine the transcriptional profile of the activated *ZCF4* and identified a cluster of genes implicated in arginine catabolism. Arginine metabolism has previously been associated with hyphal switching through the arginase Car1. In this study, we identified *ZCF4* as a potential new member of the filamentation regulatory circuit and established that in an activated state it plays distinct roles in hyphae formation in response to environmental conditions. Our results from phenotypic studies and transcription profiling propose Zcf4 as a new regulator in the establishment of filamentation, with effects that are different from previously identified regulators.

4.1 Introduction

The opportunistic pathogen *Candida albicans* is an important component of the human microbiome, and a significant medical issue. It can cause lethal infections in immunocompromised individuals (Mayer *et al.* 2013). *C. albicans* is a dimorphic yeast that forms hyphal filaments in response to a variety of environmental cues like elevated CO_2 and temperature (Klengel *et al.* 2005; Shapiro *et al.* 2009), serum (Taschdjian *et al.* 1960), nutrient limitation (Lu *et al.* 2011), N-acetylglucosamine (GlcNAc) (Simonetti *et al.* 1974), and pH (Buffo *et al.* 1984). The morphogenetic switch from the unicellular budding yeast to the hyphal form where cells grow as branching filaments is seen as one of the attributes linked to its pathogenesis. This yeast-to-hypha transition is a critical virulence determinant in *C. albicans* infections as it enables the opportunistic yeast to evade immune cells and penetrate endothelial tissues (Lorenz *et al.* 2004; Ghosh *et al.* 2009; Mayer *et al.* 2013). Thus, understanding the mechanism of morphogenesis is critical in

determining aspects of its virulence.

A complex network of transcription factors (TFs) has been identified that function to regulate morphogenesis and play key roles in the regulatory mechanisms for hyphal associated genes. These TFs regulate gene expression of their target genes as activators or repressors by binding to their promoters. A main family of TFs in *C. albicans* are the zinc cluster transcription factors (ZCFs), which contain the conserved motif $CX_2CX_6CX_{5–24}CX_2CX_{6–9}C$ in the DNA binding domain and interact with CGG nucleotide triplets (MacPherson *et al.* 2006). These ZCFs are known to regulate various cellular processes such as invasive filamentous growth, metabolism, meiosis, and drug resistance (Vandeputte *et al.* 2011; Schillig and Morschhäuser 2013).

As a major component of fungal-specific regulatory circuits, the activity of ZCFs is modulated by several environmental cues such as temperature, nutrient starvation, pH, as well as serum and certain amino acids as nutritional signals (Taschdjian *et al.* 1960; Buffo *et al.* 1984; Lorenz *et al.* 2004; Shapiro *et al.* 2009; Lu *et al.* 2011). The transition to different cellular forms allows *C. albicans* to exploit numerous niches within the human body, helping it to be a successful pathogen (Barelle *et al.* 2006). *C. albicans,* as both a successful commensal and opportunistic fungal pathogen, is required to respond to variable pH in host niches. pH, as a morphological signal, prompts the transition from round, budding cells to the filamentous hyphal form (Saville *et al.* 2003; Biswas *et al.* 2007). The ability to acquire carbon and nitrogen nutrients from various sources increases the fungi's success within its host (Barelle *et al.* 2006). Barelle *et al.* have shown that mutations in *C. albicans'* metabolic pathways reduce its pathogenicity. Previous studies examining the amino acid arginine report that upon ingestion by the host macrophages, the induction of arginase and the triggering of filamentous growth allows the yeast to escape macrophage killing (Ghosh *et al.* 2009; Silao *et al.* 2019).

An ongoing area of intense research interest in *C. albicans* involves the regulatory systems controlling morphological switching. *RHA1*, a regulator of filament development, was found to strongly up-regulate another zinc cluster transcription factor *ZCF4* in cells with a hyperactivated Rha1 (Omran *et al.* 2021). Recently, gene expression studies conducted under liquid and solid growth conditions shows that *ZCF4* is significantly induced under liquid RPMI media conditions (Azadmanesh *et al.* 2017). Here, we investigated *ZCF4* in *C. albicans*, as the function of the encoded TF is unknown. Despite being located between *CaORF19.2778* and *HAP2*, which are orthologous to *Saccharomyces cerevisiae*'s *URB1* and *HAP2* and syntenic in their respective

genomes, *ZCF4* has not been identified outside the Candida clade, and thus appears to have been lost from the branch leading to *S. cerevisiae*, or uniquely picked up in the branch leading to the CUG clade. Here, we use an artificial gene activation of *ZCF4*, generating a gain-of-function (GOF) mutant (Schillig and Morschhäuser 2013). RNA-sequencing-based transcriptional profiling data from the Zcf4-GOF has pointed to the possibility of it being involved in filament formation, as its activation is linked to the up-regulation of another TF gene, *ZCF32,* implicated in filamentation and biofilm formation (Kakade *et al.* 2016). The goal of our study was to investigate the involvement of Zcf4 in the yeast-to-hyphal transition. Up-regulation in the Zcf4-GOF strain was also observed for the genes encoding enzymes playing key roles in arginine catabolism. Therefore, we investigated the metabolism of arginine by using growth assays involving arginine as sole carbon and/or nitrogen source.

4.2 Materials and methods

4.2.1 Strains and culture conditions

C. albicans strains routinely were cultivated in yeast-extract-peptone-dextrose (YPD) medium (10 g yeast extract, 10 g peptone, 2% glucose, 50 mg uridine and 20 g agar). Spider medium (10 g D-mannitol, 10 g nutrient broth, 2 g K_2HPO_4, 50 mg uridine, and 20 g agar in 1 L of water) at pH 7.2 (Liu *et al.* 1994), Lee's media (12.5 g glucose, 5 g $(NH_4)2SO_4$, 0.2 g $MgSO_4x7H_20$, 3.28 g K_2HPO_4, 5 g NaCl, 0.5 g l-proline, 0.001 g biotin and 20 g agar in 1 L of water) (Lee *et al.* 1975), 10% serum media (YPD media and 10% fetal bovine serum), RPMI-MOPS media (10.4 g RPMI, 0.165 M MOPS buffer, pH 7.0, and 20 g agar in 1 L of water), minimal medium YNB (Yeast Nitrogen Base) with ammonium sulfate and/or arginine (0.11 mM), and YCB (Yeast Carbon Base) with ammonium sulfate and/or arginine were used as indicated. Cells were grown at 30 °C for yeast-like growth, and at 37 °C for filamentous growth. Strains included gain-of-function *ZCF4* (Zcf4-GOF) artificially activated by addition of a constitutive *S. cerevisiae* Gal4 activation domain (Schillig and Morschhäuser 2013) in background xxx. Wild-type (WT) *C. albicans* strain *SC5314* was used to generate *ZCF4*/*Zcf4*Δ and *Zcf4*Δ/Δ (C1_07670W_A) by using the CRISPR-Cas9 gene editing. SC5314 is representative of a significant fraction of clinical isolates (Odds *et al.* 2004). The solo system plasmid pV1093 was used in the CRISPR editing (Vyas *et al.* 2015). Transformants were selected on solid YPD with

Hygromycin B (600 µg/ml) and screened by colony PCR. All primers and guide RNA sequences are listed in Table S4.1 in the supplemental material.

4.2.2 RNA isolation and RNA-seq experiment

Two biological replicates of both Zcf4-GOF and the WT SC5314 strains were diluted to OD600=0.1 in YPD and inoculated to reach OD600 =0.8-1. Total RNA was extracted using QIAGEN RNA extraction kit, then RNA quality and quantity were determined using an Agilent bioanalyzer. Paired end 150bp illumina misSEQ sequencing. was carried out at the Quebec Genome Innovation center. Raw reads were pre-processed as described previously (Costa *et al.* 2019). Briefly, the sequence-grooming tool cutadapt version 0.4.1 (Martin 2011) with the following quality trimming and filtering parameters (`--phred33 --length 36 -q 5 --stringency 1 -e 0.1`) was used. Each set of paired-end reads was mapped against the *C. albicans* SC5314 haplotype A version A22 downloaded from the Candida Genome Database (CGD) (http://www.candidagenome.org/) using HISAT2 version 2.0.4 (Kim *et al.* 2015). SAMtools (Li *et al.* 2009) was then used to sort and convert SAM files. The read alignments and SC5314 genome annotation were provided as input into StringTie v1.3.3 (Pertea *et al.* 2015), which returned gene abundances for each sample. We imported gene abundances into R using the tximport R package and conducted differential analysis of transcript count data using the DESeq2 R package (Soneson *et al.* 2016) . We use the independent hypothesis weighting (IHW) Bioconductor package (Ignatiadis *et al.* 2016) to weight p-values and adjust for multiple testing using the procedure of Benjamini Hochberg (BH) (Benjamini and Hochberg 1995). Expression heatmaps of selected genes were plotted using the pheatmap R package (https://cran.r-project.org/web/packages/pheatmap/index.html). Hierarchical clustering based on Euclidean distance and average linkage was applied to both samples (in columns) and genes (in rows).

The complete list of Zcf4-GOF differentially expressed genes can be found at https://github.com/Rahaomran/Raha-Omran.git. Gene ontology (GO) analyses were achieved on gene lists obtained by RNAseq to identify putative enriched biological functions, processes, or cell compartments (*Candid* Genome Database GO Term Finder; (http://www.candidagenome.org/cgi-bin/GO/goTermFinder) and to assign differentially regulated genes to specific biological processes (*Candid* Genome Database GO Slim Mapper; http://www.candidagenome.org/cgi-bin/GO/goTermMapper).

4.2.3 Filamentation assays

A single colony of each of WT, *ZCF4*/*Zcf4*Δ, *Zcf4*Δ/Δ, Zcf4-GOF strains were picked from a YPD agar plate struck from a glycerol frozen stock and incubated overnight in 5ml of liquid YPD media at 30 °C with shaking at 220 RPM. Aliquots of the overnight culture were spun down at high speed in a microcentrifuge and washed twice with 1 ml of phosphate-buffered saline (PBS). The washed cell pellets were resuspended in 1 ml PBS at an OD600 of 0.1. For the solid spotting assays, cells were spotted onto YPD, Spider, 10% serum, Lee's, and RPMI-MOPS agar plates and incubated at 37 °C for all except the yeast control on YPD at 30 °C. Colony morphology was analyzed after incubating for five days and photographs were taken using an Epson scanner. For the liquid assays, cells were grown in Spider, 10% serum media, and RPMI-MOPS for four hours. For quantification of cell elongation, cells were stained with Calcofluor white (1 μg/mL), then imaged using a Leica DM6000 microscope with both DIC optics and appropriate fluorescent filters, using a 40x (N.A. 0.75) objective lens. After capture, Calcofluor white images were presented to a Region Convolutional Neural Net (He *et al.* 2017; Lu *et al.* 2019) trained to recognize yeast cells, resulting in a binary mask that represents the outline of most yeast cells in the image; these masks were verified by a trained human observer, who could discard inappropriate masks that did not correlate well with merged DIC and Calcofluor white images. The remaining masks were measured in FIJI (NIH, Bethesda), The results were analyzed using the Kruskal-Wallis test, Dunn's multiple comparison test, and the Shape Descriptors option test. Analyses were conducted using GraphPad Prism version 6.00.

4.2.4 Growth assays

The WT, Zcf4-GOF, *ZCF4*/*Zcf4*Δ and *zcf4*Δ/Δ were each cultured overnight in 5 mL YPD. Cells were washed three times with PBS before diluting to an OD600 of 0.1. In a 96-well plate, four replicates of all strains were grown in YCB, YCB with ammonium sulfate, YCB with arginine, YNB, YNB with ammonium sulfate, and YNB with arginine for 2 days at 30 ℃ in a Sunrise plate reader (TECAN) with a 220-rpm shaker. The plate reader was set to collect OD600 data every 10 min throughout the incubation period.

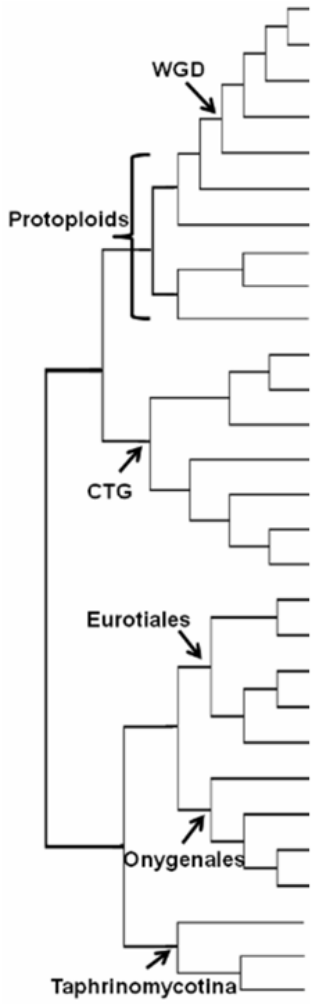
4.2.5 Specific arginase activity assay

Specific arginase activity was determined with QuantiChromTM Arginase Assay Kit following the manufacturer's instructions (BioAssay Systems, CA, USA). Cells were grown in 5 ml YPD media at 30 °C and harvested by centrifugation, and then washed three times with PBS buffer. Cells were lysed with beads using 500 µL of lysis buffer as recommended by the manufacturer and the FastPrep-24 homogenizer. The lysate was clarified by centrifugation at 10,000 ×g at 4 °C for 15 min. The supernatant was used to perform the assay. Optical density was detected in a multimode plate reader (BMG Labtech CLARIOstar®Plus) at 43.

4.3 Results

4.3.1 Identification of ZCF4 by transcription profiling

In chapter 2, we determined the function of the previously uncharacterized ORF, *ORF19.1604*, which we named *RHA1* for Regulator of Hyphal Activity, as encoding a positive regulator of hyphal development in *C. albicans*. As well, analysis of the up-regulated transcripts in the Rha1-GOF strain identified another uncharacterized zinc cluster transcription factor named *ZCF4*. *ZCF4* displayed significant upregulation (7.63 log2 fold ≥ 1.5, padj ≤ 0 in the expression profile of Rha1-GOF (Chapter 3, Table 3.1)). *ZCF4* encodes a protein of 197 amino acids with the N-terminal region from 15-56 aa on containing the binuclear zinc cluster DNA binding domain. The gene is located between *CaORF19.2778* and *HAP2* on chromosome one. The Synteny analysis based on *HAP2* arrangement showed *CaORF19.2778 and ZCF4* arrived next to each other in CTG clade (Figure 4.1). *ZCF4* (*ORF19.1227*) orthologs within the ascomycete with similarity outside the ZCFs DNA biding motif is absent from ancestral clades such as Protoplolds , Eurotiales, Onygenales, Taphrinomycotina and is variably present in the CTG clade. Overall, *ZCF4* appears to arise in the CTG clade between *CaORF19.2778* and *HAP2*.



Species	Clade	*ORF19.2778*	*ZCF4*	*HAP2*
S. cerevisiae	WGD	--	Δ	*HAP2* (YGL237C)
S. paradoxus	WGD	--	Δ	*HAP2* (SPAR_G00240)
S. mikatae	WGD	Δ	Δ	Δ
S. bayanus	WGD	Δ	Δ	Δ
S. castellii	WGD	--	Δ	NCAS_0E00300
Z. rouxii	Protoploids	--	Δ	ZYRO0E09372g
A. gossypii	Protoploids	--	Δ	AFR257Wp
K. thermotolerans	Protoploids	KLTH0C00836g	Δ	KLTH0G00880g
K. lactis	Protoploids	--	Δ	KLLA0_A00891g
S. kluyveri	Protoploids	Δ	Δ	Δ
D. hansenii	CTG	--	DEHA2F26466g	DEHA2F264688g
P. guilliermondii	CTG	--	--	Δ
C. lusitaniae	CTG	--	CLUG_05887	CLUG_05888
L. elongisporus	CTG	--	Δ	LELG_00868
C. tropicalis	CTG	--	--*	CTRG1_03974
C. dubliniensis	CTG	CD36_07130	CD36_07140	CD36_07150
C. albicans	CTG	*ORF19.2778*	*ZCF4* (*ORF9.1227*)	*HAP2* (*ORF19.1228*)
A. oryzae	Eurotiales	Δ	Δ	BAE60208.1
A. niger	Eurotiales	Δ	Δ	ASPNIDRAFT_35358
A. clavatus	Eurotiales	Δ	Δ	EAW14243.1
A. fumigatus	Eurotiales	Δ	Δ	Afu2g14720
A. nidulans	Eurotiales	Δ	Δ	AN7545
P. brasiliensis	Onygenales	Δ	Δ	EST07563.1
U. reesii	Onygenales	Δ	Δ	EEP76530.1
C. posadasii	Onygenales	Δ	Δ	EER28756.1
C. immitis	Onygenales	Δ	Δ	EAS37031.2
S. japonicus	Taphrinomycotina	Δ	Δ	RAH81897.1
S. octosporus	Taphrinomycotina	Δ	Δ	EPX74212.1
S. pombe	Taphrinomycotina	Δ	Δ	SPBC725.11c

Figure 4.1: *ZCF4* bioinformatics analysis. (A) Orthologous cluster for *ZCF4* (*ORF19.1227*). *ZCF4* has no orthologs in *S. cerevisiae* and the close relatives of budding yeast. On the left, there is a dendrogram illustrating the phylogenetic tree of a selected group of ascomycetes. The genes shown on the right establish the synteny between *ZCF4* and its orthologs in these species. The symbol "Δ" indicates that the gene is not present in the genome, and "--" indicates that it is present elsewhere in the genome. The Phylogenetic tree is based on (Wang *et al.* 2009; Shen *et al.* 2016) and the Fungal Orthogroups Repository at the Broad Institute (Wapinski *et al.* 2007) (not in scale). *: There is a flipped arrangement of Hap2 in *C. tropicalis*. In *C. albicans* the order is *ZCF4-HAP2*, while in tropicalis it is *Hap2-ZCF4*.Abbreviation: WGD, whole-genome duplication. Species name: *S*: *Saccharomyces*, *N*: *Naumovozyma, Z:* Zygosaccharomyces, *A*: *Ashbya*, *K*: *Kluyveromyces*, *L*: *Lachancea*, *D*: *Debaryomyces*, *P*: *Pichia*/*Meyerozymai*, *C*: *Candida*, *L*: *Lodderomyces*, *Candida A*: *Aspergillus*, *P*: *Paracoccidioides*, *U*: *Uncinocarpus*, *C*: *Coccidioides, S: Schizosaccharomyces.*

4.3.2 Zcf4-GOF filamentation phenotype differs depending on the inducing cue

Since *ZCF4* showed upregulation in cells with an activated Rha1-GOF, we investigated a possible role of Zcf4 in *C. albicans* filamentation. There are three morphologies of cells on the most solid media of *C. albicans* - round budding yeast form cells, pseudohyphal form cells that are defined by a string of elongated cells that remain attached after cell division, and hyphae, which are highly elongated tubular cells separated by septa following cell division (Sudbery *et al.* 2004) The latter two filamentous morphologies can be triggered by environmental stimuli such as elevated temperature and CO_2, serum, nutrient limitation, and alkaline pH. We examined wild type SC5314, the strain containing Zcf4-GOF, and the homozygous *zcf4Δ/Δ* deletion strain for their colony morphologies on known hyphal-inducing media including serum, Spider, Lee's media RPMI-MOPS, along with the control non-inducing media YPD (Lee *et al.* 1975; Sudbery *et al.* 2004). As shown in Figure 4.2, colony morphology of Zcf4-GOF on Spider media, which generates a starvation-like signal, showed an enhanced wrinkled colony morphology toward the center in comparison to the wild type; this could represent increased filamentous growth of the Zcf4-GOF strain. In dramatic contrast, on serum media, where the inducing signal represents rich growth filamentation condition, the Zcf4-GOF strain generated a smooth colony morphology, quite different from the wrinkled morphology of the wild type. In Lee's and RPMI medium, the Zcf4-GOF colonies look much like WT. In contrast to the wild type and Zcf4-GOF strains, the *zcf4Δ/Δ* homozygous null strain produced an expanded colony size on Lee's media, and a marginally reduced colony size on RPMI agar (Figure 4.2). Thus, activated *ZCF4* may repress *C. albicans* filamentation triggered by serum agar, and possibly increased filamentation on Spider solid medium when activated, while its absence may play a positive role in filament formation or growth on Lee's agar medium.

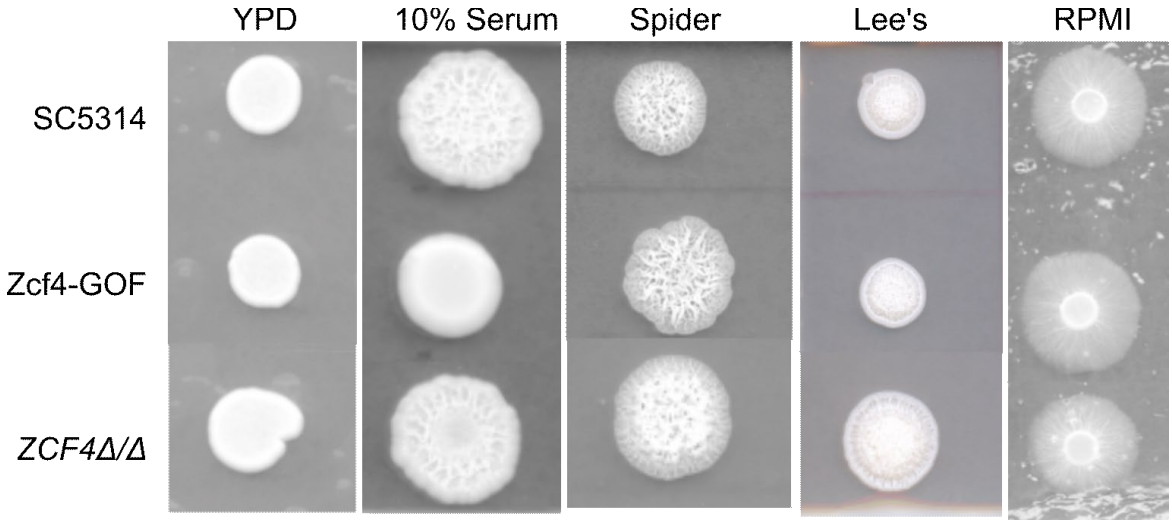


Figure 4.2: Morphology of *C. albicans* strains grown on solid media. The wrinkled colony morphology of the SC5314, Zcf4-GOF and *zcf4Δ/Δ*, on 10% solid Serum medium, Spider, Lee's and RPMI are shown after three days of growth at 37 °C. Zcf4-GOF results in invasive growth on Spider media while having an impaired agar invasion on serum media and decreased colony wrinkled on lee's media. The spot assay results shown are representative of two independent experiments. All strains grew normally on YPD at 30 °C. Photographs were taken using an Epson scanner.

As the ability of *C. albicans* to switch from yeast to hyphae is of crucial importance for its virulence, we further investigated hyphae formation on liquid Spider and serum media. We noted that the Zcf4-GOF strain exhibits enhanced elongation in Spider media in comparison to the wild type strain (Figure 4.3A), while having impaired filamentation on serum media (Figure 4.3B), consistent with the spot assay on solid media. Cells are presented in differential interference contrast (DIC) as well as stained with calcofluor white (CFW) to detect cell wall chitin. The microscopy images were used for image analysis where the aspect ratio of length to width of the cell captured was plotted against the relative bud length. The elongation of the cells in the images was quantified by a scatter plot (Figure 4.3C and D). This allowed us to distinguish the hyphal elongation present in different strains on Spider and serum media, as the aspect ratio analysis allows quantification of the filamentation. The GOF allele inhibited elongation generated by serum and enhanced filamentation directed by Spider medium.

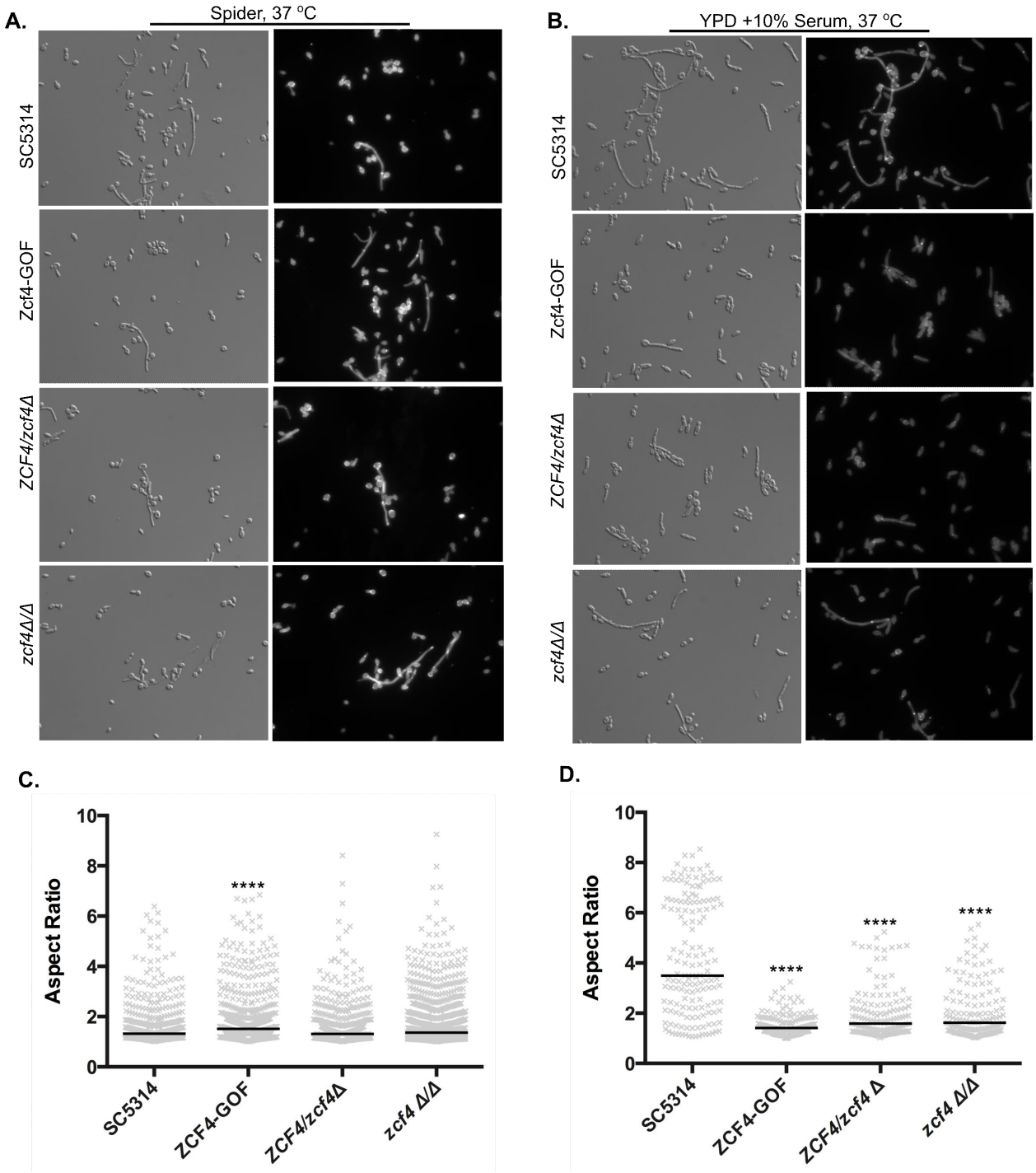


Figure 4.3: Morphology of *C. albicans* strains grown in liquid media. **A.** Cell morphology test of the ZCF4-GOF strain shows considerably more hyphal cells relative to the wild-type strain in Spider media. **B**. Cell morphology of the ZCF4-GOF strain shows impaired hyphal formation in serum media. **C.** The aspect ratio of Spider-induced filamentation shows a greater relative elongation of the cells of ZCF4-GOF. **D.** The aspect ratio of serum-induced filamentation shows a reduced relative elongation of the cells of the ZCF4-GOF strain compared to the WT. Strains were stained with CFW and imaged by Leica Dm 600 microscope.

Cells are shown at 40x magnification. Scale bar = 10 μm. The horizontal lines indicate the median. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, strains that differ from the wild-type (Kruskal–Wallis test with Dunn's multiple comparison test). Pictures and aspect ratios shown are representative of results from two independent experiments.

4.3.3 *Ca*Zcf4-GOF transcriptional profile undergoing serum and elevated temperature

We carried out RNAseq with Zcf4-GOF versus wild type in serum at 37 ˚C because the Zcf4-GOF strain was compromised in forming filaments under serum induction conditions. In general, we did not see a large decrease in the genes characterizing the standard hyphal induction signal *ALS3*, *HWP1*, *ECE1*, *HGC1* and the common serum responsive genes (Martin *et al.* 2013) like *CSA1*, *DDRR48*, *GNP1*, *HYR1*, *LYS12*, *SAP6*, *SKS1*, *TDH3* were not differentially downregulated, suggesting that although hyphal growth was compromised, the hyphal gene induction signal was not dramatically suppressed.

Approximately 30 genes were found to be upregulated (≥ 1.5 log2 fold change in expression level and padj ≤ 0.05) in Zcf4-GOF as compared to the wild type (Table 4.1). There were genes related to arginine catabolism, cell wall biogenesis, and the heat shock response. GO term analysis of upregulated genes established they were significantly enriched for genes in proline metabolic process, and arginine catabolic process (Table S1). Among the approximately 29 downregulated genes (Log2 fold ≤ 1, padj ≤ 0.05) an enrichment for genes encoding ribosomal proteins was observed, along with a set, (*MNN45*, *MNN42*, *MNN1*) of mannosyltransferase genes, and repressed adherence regulator *CRZ2* compared to the wild type cells (Table 4.2). Thus, *ZCF4* may contribute inversely to *C. albicans* filamentation which causes a defect in forming filaments under serum condition.

Table 4.1: RNAseq result of Zcf4-GOF under serum. RNA-seq data showing 30 *C. albicans* Up-regulated genes by Zcf4-GOF versus SC5314 under serum stimuli, Log2 fold > 1.5, padj < 0.05.

Feature	log2FoldChange	pvalue	padj	Description
ZCF4	11.03	7.5E-14	0	Putative Zn(II)2Cys6 transcription factor
C7_02920W_A	4.45	7.2E-14	0	Has domain(s) with predicted carbon-nitrogen ligase activity
CIS2	3.76	1.7E-38	0	Putative role in regulation of biogenesis of the cell wall; upregulated in biofilm; Gcn4p-regulated
PRO3	3.46	6.2E-36	0	Delta 1-pyrroline-5-carboxylate reductase; protein induced during the mating process
UGA1	3.22	2.2E-04	0.01	Putative GABA transaminase; transcription regulated by Mig1 and Tup1; stationary phase enriched protein; rat catheter and Spider biofilm induced
CAR1	3.16	9.5E-42	0	Arginase; arginine catabolism; transcript regulated by Nrg1, Mig1, Tup1
CAR2	3.16	6.6E-11	0	Ornithine aminotransferase
C7_03580C_A	3.1	2.1E-09	0	Protein of unknown function; Hap43-repressed gene
PHO89	2.93	5.8E-11	0	Putative phosphate permease
C5_02110W_A	2.61	3.4E-09	0	Putative heat shock protein; decreased expression in hyphae
CR_04870C_A	2.59	5.3E-17	0	Trimethylaminobutyraldehyde dehydrogenase, the third enzyme of the carnitine biosynthesis pathway
C1_01930W_A	2.59	2.6E-06	0	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
C4_01220C_A	2.57	5.9E-08	0	Protein with a glycoside hydrolase domain; mutants are viable
HSP12	2.56	4.9E-09	0	Heat-shock protein
CR_00130C_A	2.34	6.6E-09	0	Protein with a pyridoxal phosphate-dependent transferase domain
C4_03050C_A	2.2	4.4E-10	0	Ortholog(s) have carboxypeptidase activity, role in nitrogen compound metabolic process
AMO1	2.2	4.6E-08	0	Putative peroxisomal copper amine oxidase
APR1	2.15	4.9E-17	0	Vacuolar aspartic proteinase; transcript equivalent in yeast-form and mycelial cells but is elevated at lower growth temperatures
OSM1	2.15	4.5E-14	0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae; caspofungin repressed
C2_06890C_A	2.12	1.2E-05	0	Similar to oxidoreductases and to *S. cerevisiae* Yjr096wp; Sfu1 repressed; induced by benomyl treatment, Ssr1; Hap43-repressed; flow model biofilm repressed
ADH5	2.06	7.4E-10	0	Putative alcohol dehydrogenase; regulated by white-opaque switch; fluconazole-induced
C1_08350C_A	2.02	2.9E-12	0	-
OPT3	1.79	7.2E-07	0	Oligopeptide transporter: transcript induced by macrophage phagocytosis, BSA or peptides
C1_04590W_A	1.78	1.1E-12	0	Protein of unknown function; Spider biofilm induced
SOD5	1.76	3.2E-03	0.05	Cu-containing superoxide dismutase; protects against oxidative stress
C4_04230W_A	1.73	1.1E-04	0	Putative transporter; fungal-specific; Spider biofilm induced
AOX2	1.63	4.0E-04	0.01	Alternative oxidase; cyanide-resistant respiration; induced by antimycin A, oxidants
C3_01190C_A	1.62	4.9E-06	0	-
CR_09530C_A	1.5	8.9E-05	0	-

Table 4.2: RNAseq result of Zcf4-GOF under serum. RNA-seq data showing 29 *C. albicans* genes down-regulated by Zcf4-GOF under serum stimuli versus SC5314, Log2 fold ≤ -1, padj ≤ 0.05.

Feature	log2FoldChange	pvalue	padj	Description
ICL1	-2.82	0	0	Isocitrate lyase; glyoxylate cycle enzyme
C7_04090C_A	-2.54	0	0	Predicted mitochondrial cardiolipin-specific phospholipase
MNN1	-2.21	0	0	Putative alpha-1,3-mannosyltransferase
CRZ2	-1.89	0	0	C2H2 transcription factor
CR_06550C_A	-1.82	0	0	Protein of unknown function; induced by nitric oxide
C1_11270W_A	-1.72	0	0	Cell wall protein
HSP104	-1.7	0	0.01	Heat-shock protein; roles in biofilm and virulence
TPO4	-1.64	0	0	Putative sperimidine transporter
MNN45	-1.63	0	0	Mannosyltransferase
MNN42	-1.4	0	0.03	Protein of unknown function; repressed by Rim101
MET1	-1.38	0	0.03	Putative uroporphyrin-3 C-methyltransferase, methionine biosynthesis enzyme
CPA2	-1.32	0	0.04	Putative arginine-specific carbamoylphosphate synthetase
PDE2	-1.31	0	0	High affinity cyclic nucleotide phosphodiesterase
C4_00490W_A	-1.27	0	0.01	Has domain(s) with predicted role in peptidyl-diphthamide biosynthetic
C1_11280W_A	-1.24	0	0.03	Ortholog(s) have U6 snRNA binding activity
C2_10160W_A	-1.23	0	0.02	Secreted protein; fluconazole-induced
RRP9	-1.13	0	0	Ribosomal protein
C2_09880C_A	-1.12	0	0.02	Putative protein of unknown function
C6_01040C_A	-1.12	0	0	DEAH-box ATP-dependent RNA helicase, required for 18S rRNA synthesis
IML1	-1.1	0	0.03	Putative protein with a role in autophagy
CCP1	-1.09	0	0	Cytochrome-c peroxidase N terminus
AAH1	-1.08	0	0	Adenine deaminase
NCE103	-1.06	0	0.01	Carbonic anhydrase; converts of CO2 to bicarbonate
GAP1	-1.06	0	0	Amino acid permease; antigenic in human/mouse
CR_07480W_A	-1.06	0	0.03	S. pombe ortholog SPAC5D6.04 is a predicted auxin family transmembrane transporter
NAN1	-1.05	0	0	Putative U3 snoRNP protein; Hap43p-induced gene
C1_10950C_A	-1.02	0	0.02	Putative serine/threonine-protein kinase
C3_00030C_A	-1.01	0	0.01	Protein with a predicted DEAD-like DNA/RNA helicase domain
C3_01430W_A	-1	0	0.01	Protein of unknown function

4.3.4 Transcriptional profile of Zcf4-GOF under normal yeast growth conditions

To help in elucidating the role of *ZCF4*, we next investigated the gene expression profile of the Zcf4-GOF against the wild type under yeast growth conditions. RNA-seq of Zcf4-GOF indicated log2 fold ≥ 1.5, padj < 0.05 induction genes involved in arginine catabolism with log2foldchange of *CAR1* 2.55, *CAR2* 2.48, *PRO3* 3.15, *PUT1* 2.89, cell wall proteins (*IHD1* 2.44) and cell wall biogenesis *CIS2* 4.72, GABA transaminase (*UGA1* 3.89) (Table 4.3). Zcf4-GOF also induced *ZCF32*, encoding a negative regulator of biofilm formation from the ZCF gene family, that blocks adhesion and the yeast to hyphae transition (Kakade *et al.* 2016). GO ontology terms revealed enriched genes related to proline metabolic process, and the arginine catabolic process (Table S4.2). We observed log2 fold change downregulation of *TRY6*, regulator of adherence (Oh *et al.* 2010; Finkel *et al.* 2012), zinc transporter gene *ZRT2* 1.81 (Crawford *et al.* 2018) (Table 4.4). Downregulation of the yeast adhesion regulator by Zcf4-GOF may correlate with our phenotypic observations of the Zcf4-GOF strain under hyphal growth conditions. Overall, we hypothesize that Zcf4 may act in arginine catabolism and adherence.

Table 4.3: RNAseq result of Zcf4-GOF under yeast growth conditions. RNA-seq data showing top 34 *C. albicans* genes Up-regulated by ZCF4-GOF versus SC5314 under yeast growth conditions, Log2 fold ≥ 1.5, padj ≤ 0.05.

Gene_name	baseMean	log2FoldChange	pvalue	padj	Description
orf19.5169	63.03	6.90	1.7E-35	3.4E-32	Has domain(s) with predicted carbon-nitrogen ligase activity
orf19.1307	46.52	4.91	1.6E-09	5.4E-07	Predicted membrane protein
orf19.6690	83.01	4.84	3.6E-14	2.4E-11	Protein of unknown function
CIS2	110.00	4.72	3.9E-50	1.2E-46	Putative role in regulation of biogenesis of the cell wall
OPT3	214.52	4.03	2.4E-11	8.7E-09	Oligopeptide transporter
UGA1	94.41	3.89	4.9E-35	7.2E-32	Putative GABA transaminase
orf19.2049	33.73	3.60	1.6E-05	1.8E-03	Plasma membrane-associated protein;
AMO1	594.85	3.49	5.7E-09	1.7E-06	Putative peroxisomal copper amine oxidase
PRO3	373.27	3.15	2.0E-50	1.2E-46	Delta 1-pyrroline-5-carboxylate reductase
OPT2	27.32	3.13	1.7E-04	1.3E-02	Oligopeptide transporter
BIO3	281.78	2.94	4.3E-04	2.5E-02	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis
PUT1	70.45	2.89	1.7E-06	2.8E-04	Putative proline oxidase
orf19.1369	33.00	2.59	1.5E-06	2.5E-04	Protein with predicted peptidase domains
CAR1	295.76	2.55	6.7E-20	6.6E-17	Arginase; arginine catabolism
orf19.6306	95.20	2.54	8.7E-14	4.6E-11	Trimethylaminobutyraldehyde dehydrogenase
RBT1	17.02	2.54	6.2E-05	5.4E-03	Cell wall protein with similarity to Hwp1
CAR2	1253.45	2.48	1.0E-22	1.2E-19	Ornithine aminotransferase; arginine metabolism; alkaline induced
IFG3	115.31	2.47	1.3E-16	1.1E-13	Putative D-amino acid oxidase
IHD1	146.63	2.44	3.8E-08	8.9E-06	GPI-anchored protein
CAN2	236.88	2.36	7.3E-08	1.7E-05	Basic amino acid permease; arginine metabolism
orf19.6605	28.54	2.14	2.0E-07	4.1E-05	Has domain(s) with predicted integral component of membrane localization
orf19.1368	80.96	2.12	3.1E-04	2.0E-02	Protein of unknown function
orf19.2244	97.49	2.00	2.1E-06	3.2E-04	Similar to oxidoreductases and to *S. cerevisiae* Yjr096wp
orf19.1427	91.90	1.95	2.7E-09	8.9E-07	Putative transporter; fungal-specific
orf19.1169	33.20	1.91	4.5E-05	4.3E-03	Putative DnaJ-like molecular chaperone; Spider biofilm induced
orf19.22	57.61	1.82	2.1E-04	1.5E-02	Protein with homology to peroxisomal membrane proteins
orf19.2686	374.59	1.73	9.3E-14	4.6E-11	Ortholog(s) have carboxypeptidase activity
ZCF32	39.35	1.71	4.8E-06	6.4E-04	Zn(II)2Cys6 transcription factor involved in regulation of biofilm formation
VPS70	168.91	1.71	6.8E-05	5.8E-03	Has domain(s) with predicted peptidase activity and role in proteolysis
orf19.6852.1	439.37	1.67	2.3E-08	5.8E-06	Protein of unknown function
PRN1	27.74	1.66	4.1E-05	4.0E-03	Protein with similarity to pirins
GDE1	122.43	1.61	1.7E-12	6.9E-10	Glycerophosphocholine phosphodiesterase
orf19.3318	55.25	1.58	3.5E-06	5.1E-04	Ortholog(s) have lipid droplet, peroxisome localization
DUO1	19.72	1.54	5.3E-04	2.9E-02	Subunit of the Dam1 (DASH) complex

Table 4.4: Top Downregulated genes Zcf4-GOF under yeast growth conditions. RNA-seq data showing top 13 *C. albicans* genes down-regulated by Zcf4-GOF versus wild type under normal yeast growth condition Log2 fold ≤ -1.5, Padj ≤ 0.05.

Feature	baseMean	log2FoldChange	pvalue	padj	Description
TRY6	2.30	-29.60	0.00	0.00	Helix-loop-helix transcription factor; regulator of yeast form adherence; required for yeast cell adherence to silicone substrate; Spider and F-12/CO2 biofilm induced; repressed by alpha pheromone in Spider medium
orf19.750	2.01	-15.36	0.00	0.00	Protein of unknown function; exogenously expressed protein is a substrate for Kex2p processing in vitro
orf19.7385	3.01	-13.97	0.00	0.03	CCCH zinc finger protein; Spider biofilm induced
orf19.2515	21.72	-5.13	0.00	0.00	ZZ-type zinc finger protein; rat catheter and Spider biofilm induced
orf19.2812	7.97	-3.80	0.00	0.02	Protein of unknown function; Spider biofilm induced
orf19.3364	42.34	-2.82	0.00	0.00	Ortholog of C. parapsilosis CDC317
orf19.5701	27.57	-2.49	0.00	0.01	Ortholog(s) have role in DNA replication initiation
BMT4	246.89	-2.18	0.00	0.00	Beta-mannosyltransferase
orf19.3448	80.75	-2.04	0.00	0.04	Protein of unknown function; ketoconazole-repressed
OYE32	74.56	-1.87	0.00	0.00	NAD(P)H oxidoreductase family protein; induced by nitric oxide, amphotericin B
ZRT2	1631.38	-1.81	0.00	0.02	Zinc transporter, essential for zinc uptake and acidic conditions tolerance
orf19.1606	30.13	-1.69	0.00	0.02	Protein of unknown function; Plc1-regulated
orf19.1534	108.30	-1.66	0.00	0.00	Ortholog of *S. cerevisiae* Zrt3, vacuolar membrane zinc transporter

Then, we investigated amino acid catabolism and the connection to *CaZCF4* regulation of filament formation. Arginine impacts filamentation morphology via two branches of a pathway converting arginine to urea and L-ornithine through the Car1p enzyme arginase (Middelhoven 1964; Sumrada and Cooper 1987). Urea is converted via enzyme urea aminolyase Dur1,2 to CO2 and ammonia (Cooper *et al.* 1980) causing cAMP pathway activation to induce germ tube formation and yeast to hyphae transition. Studies by Lorenz (Lorenz *et al.* 2004) showed *C. albicans* cells were unable to escape from macrophage after mutation in argenine biosynthesis genes such as *arg1* and *arg3,* thus induction of arginine helps *C. albicans* escape from macrophage. On the other hand, ornithine subsequently converted to glutamate semialdehyde by ornithine aminotransferase CAR2, that converts to pyrroline-5-carboxylate (P5C). Cytoplasmic P5C is transformed to proline by Pro3 then requires transporting into mitochondria to switch back to P5C through *PUT1.* Then it converted to α-ketoglutarate by Gdh2. We assessed the ability of *Ca*Zcf4-GOF and deleted *zcf4* to induce filamentous growth in proline, arginine and ornithine that promote *C. albicans* filamentation in a mitochondria-dependent manner (Silao *et al.* 2019). Wild type, Zcf4-GOF and *zcf4Δ/Δ* were grown on synthetic dextrose (2% glucose) containing 10mM of selected amino acids as sole nitrogen source on MES buffered (pH=6). The result indicates strains can undergo filamentous growth on selected amino acids (Figure 4.4). These results suggest that Zcf4 filamentation formation is pH and media dependent.

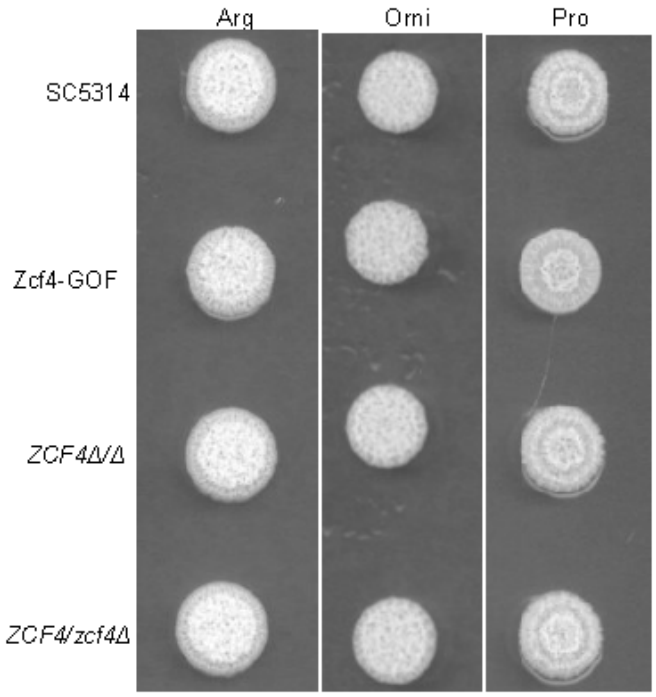


Figure 4.4: Morphology of *C. albicans* strains grown on synthetic complete media with amino acid hyphae inducer. Colonies of *C. albicans* wild type (SC5314), Zcf4-GOF and *zcf4 Δ/Δ, ZCF4/zcf4Δ* grown on SXD medium containing 10 mM of the indicated amino acids (X = Proline, Arginine, Ornithine) and 2% glucose after 48h of growth at 37 ˚C adjusted PH=6.

4.3.5 Determination of *ZCF4* in arginine utilization

We questioned whether *ZCF4* encodes a main player in the *C. albicans* arginine degradation processes since we significantly observed upregulation of arginine metabolism genes in the Zcf4-GOF profile. Arginine is converted to urea and l-ornithine by the enzyme arginase (Car1p). Ornithine is metabolized by ornithine aminotransferase (Car2p) to form glutamate γ-semialdehyde, which is digested to pyrroline-5-carboxylate (P5C) and subsequently to proline by 1-pyrroline-5-carboxylate reductase (Pro3p). This proline located in the cytosol is transported into the mitochondria where it is converted back to P5C by proline oxidase (Put1p). Catabolism of arginine can supply nitrogen and carbon for energy in *C. albicans*. These enzymes were found to be up-regulated in the RNA-seq data of Zcf4-GOF (Table 4.3), which led us to hypothesize that Zcf4-GOF would utilize arginine more efficiently than its wild-type strain and *ZCF4* mutants would be unable to grow on medium supplied with arginine as sole carbon and/or nitrogen source.

To confirm whether the *ZCF4* is a regulator of arginine catabolism as the RNA-seq of Zcf4-GOF suggests, we cultured the wild-type, Zcf4-GOF, *ZCF4*/*Zcf4*Δ and zcf4Δ/Δ, strains on Yeast Nitrogen Base (YNB) medium containing arginine as the sole carbon source (Figure 4.5E), as the sole carbon and nitrogen source (Figure 4.5C), on Yeast Carbon Base (YCB) medium containing arginine as the sole nitrogen source (Figure 4.5F) as well as on YCB with ammonium sulfate as a positive control (Figure 4.5D), and solely YNB (Figure 4.5A) and YCB (Figure 4.5B) as negative controls. The growth curves show the *ZCF4* mutants retained the ability to grow using arginine as a sole nitrogen source and/or sole carbon source (Figure 4.5D, E, F) and Zcf4-GOF does not seem to exhibit a superior ability to utilize arginine. This is not surprising because previous studies suggested the existence of an arginase independent pathway in *C. albicans* (Silao *et al.* 2019). The strains also grew normally on solid media with 2% glucose using lysine, tryptophan, glutamine, ornithine as the sole nitrogen source (Figure 4.6). Therefore, our result suggest *ZCF4* is not an essential player in arginine catabolism in *C. albicans.*

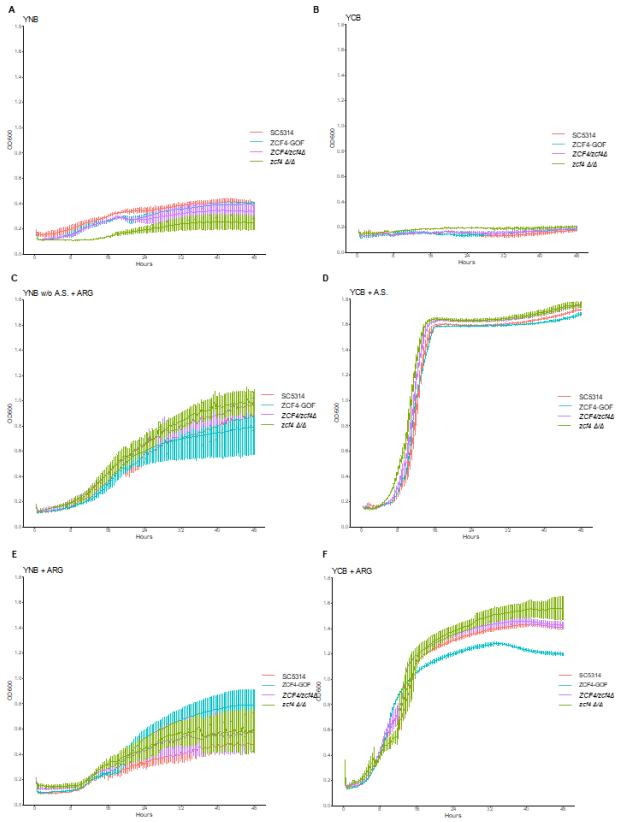


Figure 4.5: Arginine utilization as a carbon and nitrogen source in liquid media. Growth curves are the results SC5314, *ZCF4*/*Zcf4*Δ, *zcf4* Δ/Δ, and Zcf4-GOF strains grown on various media. **A.** YNB. (B) YCB. (C) YNB without ammonium sulfate with arginine as the sole carbon and nitrogen source. **D.** YCB with ammonium sulfate. **E** YNB with arginine as the sole carbon source. **F.** YCB with arginine as the sole nitrogen source. Error bars are based on the standard deviation from four replicates of each data point reading taken every 10 min for 48h using a Sunrise plate reader.

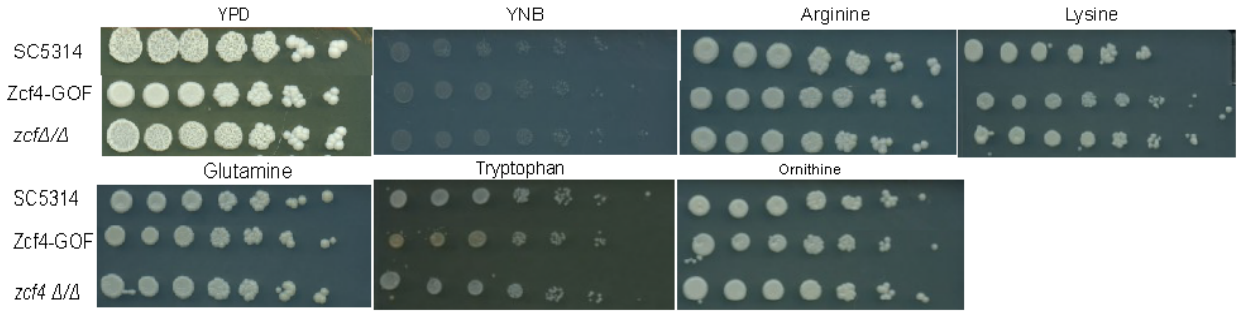


Figure 4.6: Morphology of *C. albicans* strains grown on different nitrogen source. Colonies of *C. albicans* wildtype (SC5314), Zcf4-GOF and *zcf4Δ/Δ, ZCF4/zcf4Δ* grown on solid media with 2% glucose using lysine, tryptophan, glutamine, ornithine as the sole nitrogen source and incubated at 30℃ for 2 days. Photographs were taken using an Epson scanner.

To further confirm that *ZCF4* mutants have maintained the ability to grow using arginine as a sole nitrogen source and/or sole carbon source, cells were spotted onto solid YNB medium containing arginine as the sole carbon source (Figure 4.7A) with YNB and glucose as a positive control (Figure 4.7B), as well as on YCB medium containing arginine as the sole nitrogen source (Figure 4.7C) and YCB with ammonium sulfate as a positive control (Figure 4.7D). Again, this is consistent with the data obtained previously where all the strains were able to grow properly, and no significant difference was observed.

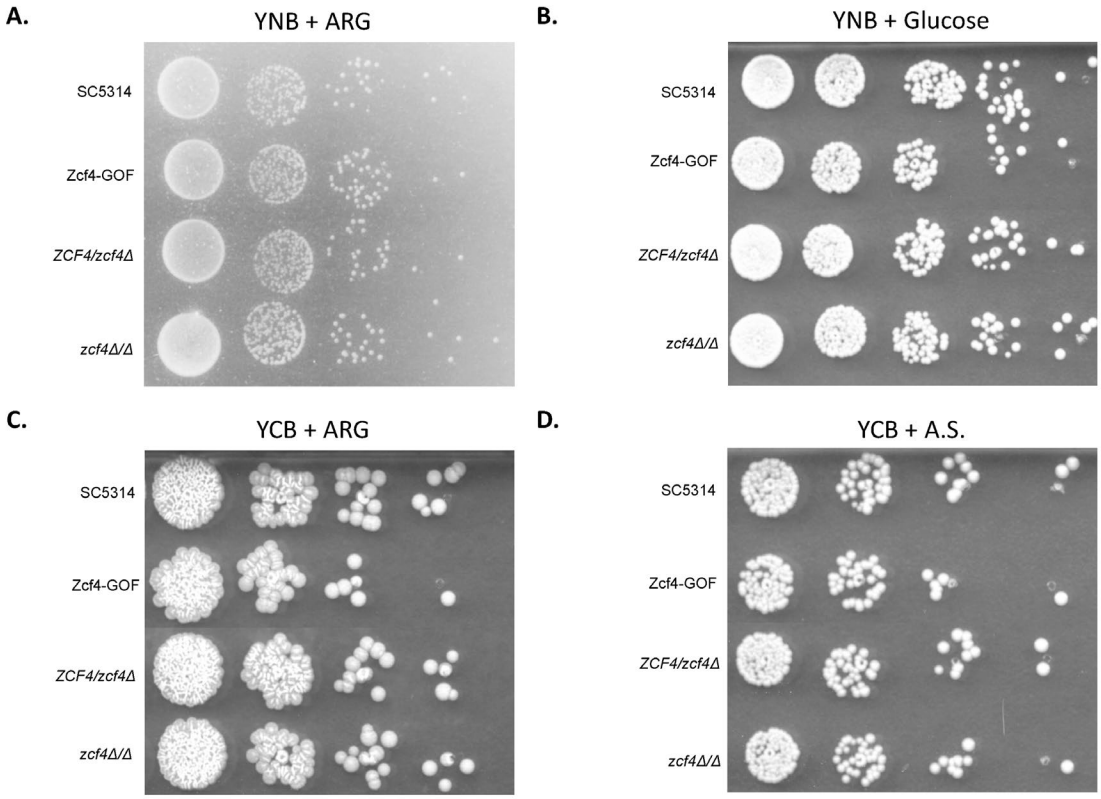


Figure 4.7: Arginine utilization as a carbon and nitrogen source on solid media. (A) YNB with arginine as the sole carbon source. (B) YNB with 2% glucose. (C) YCB with arginine as the sole nitrogen source. (D) YCB with ammonium sulfate. 5 µl of 10-fold serially diluted cells were spotted onto the surface and incubated at 30℃ for 2–3 days. Photographs were taken using an Epson scanner.

4.3.6 Assay of arginase activity

As the arginase activity (CAR1) is up-regulated in the Zcf4-GOF RNA-seq data, we decided to assess its activity in wild type, *ZCF4*/*Zcf4*Δ, *zcf4Δ/Δ*, and Zcf4-GOF strains, as well as using *Ca*Orf19.6888-GOF (C2_05770W_A) as supplementary strains (Figure 4.8). Both are zinc-cluster transcription factors that are up-regulated in the Zcf4-GOF data and possibly play a role in the mechanism of Zcf4. Using the QuantiChromTM Arginase Assay Kit (BioAssay Systems, CA, USA), the arginase activity can be determined with chromogen that forms a urea-specific coloured complex with the urea produced in the arginase reaction as it catalyzes the conversion of arginine to ornithine and urea. The intensity of the colour is directly proportional to the arginase activity in the sample. Following the manufacturer instructions, the specific arginase activities of the strains were determined, and *zcf4Δ/Δ* seems to exhibit the greatest arginase activity. However, the differences were not statistically significant. Consistent with our arginine growth and spotting assays, the Zcf4-GOF does not appear to have higher activity of arginase (Car1p).

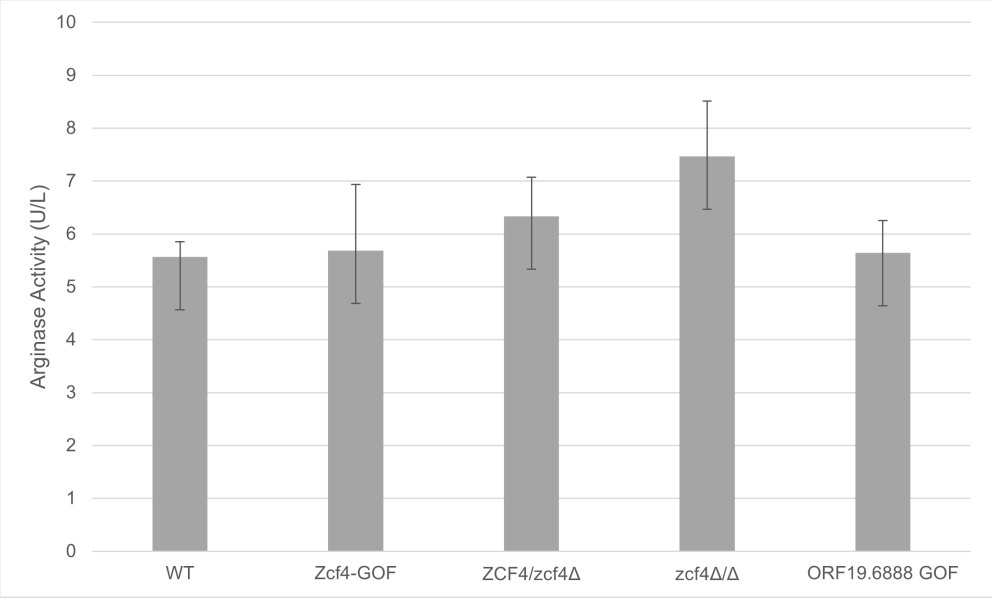


Figure 4.8: Arginase activity measure in *C. albicans* cells lysate. The 1 unit per litre of sample (U/L) represents 1 unit of arginase converting 1 μmole of L-arginine to ornithine and urea per minute. Error bars are based on the standard deviation from four replicates of each strain.

4.4 Discussion

The process of hyphal formation is crucial for *C. albicans* to cope with the requirements of its environment and plays a vital role in its virulence. Several decades of scientific research have led to a reasonable understanding of the wide range of nutritional and environmental cues inducing the morphological switch from yeast to hyphae. However, the mechanisms by which this yeast can mediate the response to these signals and modulate morphogenesis is still poorly understood. In this study, we identified Zcf4 as a potential new member of the filamentation regulatory circuit and demonstrated that it plays a role in the establishment of hyphae in different environmental conditions. Our work has shown that Zcf4-GOF exhibits enhanced growth on Spider media and impaired growth on serum induced conditions when spotted on solid plates (Figure 4.2). Consistent with these results, we observed similar morphologies in liquid media (Figure 3A and B). This was confirmed to be significant by the aspect ratio (Figure 4.3C and D). This suggests that some of the genetic requirements for filamentation vary between distinct inducing conditions. It is not yet clear how Zcf4 functions in the regulation of the morphological switching, and whether these differences are restricted to it being condition-specific or if larger differences exist between these induction conditions. One hypothesis is that distinct inducing conditions have different genetic requirements to filament as the regulatory circuit controlling filamentation in *C. albicans* is highly interconnected and complex.

We expect that the genetic requirements for filamentation and transcriptional responses to filamentation would be similar in liquid and solid versions of media with the same components. However, transcriptional profiles of the Zcf4-GOF strain growing under serum conditions showed no significant differences in expression levels of hyphae associated genes, which is in contrast with severe filamentation defect on the solid serum. This could be due to the ability of *C. albicans* to distinguish between filaments that are formed in various human niches, such as the gastrointestinal and urinary tracts. Our data strongly suggest that filamentation programs are distinct in solid and liquid media, both in the genetic requirements for filamentation and in the transcriptional response to filamentation. Based on these observations, we hypothesize that *C. albicans* must differentiate between filamentation in a free-floating state and on surfaces in the gastrointestinal and genitourinary tracts of the human body and that its ability to respond appropriately to these distinct conditions is important for its survival *in vivo*. Our data propose that we need to treat solid and liquid filamentation as two distinct phenotypes and that defects in each process will have different impacts on pathogenesis. Our results suggest that the *ZCF4* transcriptional response and genetic requirement are distinct in solid and liquid serum medium support the previous study inferred from (Azadmanesh *et al.* 2017).

To sustain the energy demands of hyphal growth, the energy status of the cell is crucial in the mechanisms underlying yeast-to-hyphal transition. To survive in sugar-limiting niches, the fungi must be able to utilize amino acids (Martínez and Ljungdahl 2004). Based on RNA-sequencing-based transcriptional profiling data, the hyperactivated Zcf4 up-regulates essential enzymes involved in arginine catabolism *CAR1*, *CAR2*, *PRO3*, *PUT1*, *AFP99* and *CaORF*19.5159. We then hypothesized that Zcf4-GOF would utilize arginine more efficiently than the WT strain and *ZCF4* mutants would be unable to grow on medium supplied with arginine as sole carbon and/or nitrogen source. Our data, however, does not seem to indicate this is the case when grown in liquid or solid media. The arginase activity was also assessed to not have be significant. Earlier reports by Han *et al.* proposed that an arginase-independent pathway exists in *C. albicans* as arginine could enter the TCA cycle via the urea cycle (Han *et al.* 2019). It seems likely that other regulators are involved in arginine degradation. Further studies are required to understand the role Zcf4 plays in the catabolism of arginine and the underlying mechanisms through which it influences filamentation remains to be defined.

Elucidating the role of TFs in the filamentation process of *C. albicans* would provide valuable insight into the environmentally responsive regulatory systems involved in morphological switching. A better understanding of how regulatory TFs integrate and respond to these environmental signals could lead to the exploration of novel therapeutic approaches in the future.

4.5 Supplementary material

Table S4.1: Oligonucleotides used in this study.

Name	Sequence (5' - 3')
SNR52/R-*ZCF4*-T2	TGGGTTTAGATATTCTTTTACAAATTAAAAATAGTTTACGCAAGTC
sgRNA/F-*ZCF4* -T3	TAAAAGAATATCTAAACCCAGTTTTAGAGCTAGAAATAGCAAGTTAAA
F-*ZCF4*-Check	ACGAATAGAAGAATGATGCTCTC
R-*ZCF4*-Check	ACAACACTGTTCCTTGTTCCCA
R-*ZCF4*_Inner-Check	TCCACACAATCTTGTTGCATAGC
R-Hyg_B_repair-Check	TGCTTCTGCTGCTTTGCCAATTCCAG
F-Hyg_B_repair-Check	ACAACCATCAGTCCAAACAGCAGCTGA

Table S4.2: GO term analysis for Zcf4-GOF upregulated genes. The Go term finder with process ontology is applied to Zcf4-GOF *versus* wild type under serum Log2 fold ≥ 1.5, Padj ≤ 0.05. Only significant hits with a p-value ≤ 0. 1 are shown.

GOID	GO_term	Cluster frequency	Background frequency	Corrected P-value	False discovery rate	Gene(s) annotated to the term
6560	proline metabolic process	3 out of 29 genes, 10.3%	8 out of 6473 background genes, 0.1%	0.00074	0.00%	CAR2:PRO3:CAR1
9064	glutamine family amino acid metabolic process	4 out of 29 genes, 13.8%	28 out of 6473 background genes, 0.4%	0.00102	0.00%	UGA1:CAR2:PRO 3:CAR1
10121	arginine catabolic process to proline via ornithine	2 out of 29 genes, 6.9%	2 out of 6473 background genes, 0.0%	0.00321	0.67%	CAR2:CAR1
190361 3	regulation of protein tyrosine phosphatase activity	2 out of 29 genes, 6.9%	2 out of 6473 background genes, 0.0%	0.00321	0.50%	HSP12:C5_02110 W_A
190361 4	negative regulation of protein tyrosine phosphatase activity	2 out of 29 genes, 6.9%	2 out of 6473 background genes, 0.0%	0.00321	0.40%	HSP12:C5_02110 W_A
19493	arginine catabolic process to proline	2 out of 29 genes, 6.9%	2 out of 6473 background genes, 0.0%	0.00321	0.33%	CAR2:CAR1
6527	arginine catabolic process	2 out of 29 genes, 6.9%	2 out of 6473 background genes, 0.0%	0.00321	0.29%	CAR2:CAR1
10923	negative regulation of phosphatase activity	2 out of 29 genes, 6.9%	7 out of 6473 background genes, 0.1%	0.06663	6.75%	HSP12:C5_02110 W_A
32515	negative regulation of phosphoprotein phosphatase activity	2 out of 29 genes, 6.9%	7 out of 6473 background genes, 0.1%	0.06663	6.00%	HSP12:C5_02110 W_A
6591	ornithine metabolic process	2 out of 29 genes, 6.9%	7 out of 6473 background genes, 0.1%	0.06663	5.40%	CAR2:CAR1
35305	negative regulation of dephosphorylation	2 out of 29 genes, 6.9%	8 out of 6473 background genes, 0.1%	0.08859	6.36%	HSP12:C5_02110 W_A
35308	negative regulation of protein dephosphorylation	2 out of 29 genes, 6.9%	8 out of 6473 background genes, 0.1%	0.08859	5.83%	HSP12:C5_02110 W_A

Chapter 5: Put3 Positively Regulates Proline Utilization in *Candida albicans*

Abstract

The zinc cluster transcription factor Put3 was initially characterized in *Saccharomyces cerevisiae* as the transcriptional activator of *PUT1* and *PUT2*, two genes acting early in the proline assimilation pathway. We have used phenotypic studies, transcription profiling, and chromatin immunoprecipitation with microarray technology (ChIP-chip) to establish that unlike *S. cerevisiae*, which uses proline as a nitrogen source and not carbon source, *Candida albicans* can use proline as a nitrogen source, a carbon source, or a source of both nitrogen and carbon. However, a *C. albicans put3* null mutant cannot grow on proline, suggesting that as in *S. cerevisiae*, *C. albicans* Put3 (*Ca*Put3) is required for proline catabolism, and because the *C. albicans put3* null mutant grew efficiently on glutamate as the sole carbon or nitrogen source. *Ca*Put3 showed direct binding to the *CaPUT1* promoter, and both *PUT1* and *PUT2* were upregulated in response to proline addition in a Put3-dependent manner, as well as in a *C. albicans* strain expressing a hyperactive Put3. *Ca*Put3 directs proline degradation even in the presence of a good nitrogen source such as ammonia, which contrasts with *S. cerevisiae* Put3 (*Sc*Put3)-regulated proline catabolism, which only occurs in the absence of a rich nitrogen source. Thus, while overall proline regulatory circuitry differs between *S. cerevisiae* and *C. albicans*, the specific role of Put3 appears fundamentally conserved.

IMPORTANCE

Candida albicans poses a significant threat to the lives of immunocompromised people. Historically, knowledge has been drawn from studies on *Saccharomyces cerevisiae* to understand the pathogen, and many *Candida albicans* genes are named after their *S. cerevisiae* orthologs. Direct studies on the pathogen have, however, revealed differences in the roles of some orthologous proteins in the two yeasts. We show that the Put3 transcription factor allows the pathogen to completely degrade proline to usable nitrogen and carbon by evading regulatory restrictions imposed on its *S. cerevisiae* ortholog, which mandates conditional use of proline only as a nitrogen source. The ability of *Candida albicans* to freely obtain nutrients from multiple sources may help it thrive as a commensal and opportunistic pathogen.

Keywords: *Candida albicans*; Put3; Put3 regulation; *Saccharomyces cerevisiae*; carbon source; nitrogen source; proline catabolism.

5.1 Introduction

Every organism requires carbon and nitrogen for survival, but nutrient choices and nutrient assimilation mechanisms vary among species. For example, *Candida albicans* uses available galactose as a carbon source even in the presence of glucose, in contrast to *Saccharomyces cerevisiae*, which shuts down the galactose catabolic pathway and preferentially uses glucose as a carbon source when both galactose and glucose are available (Sellick *et al.* 2008). Differences in this regulatory circuitry are correlated with an exchange in key transcription factors; Gal4 controls galactose metabolism in *S. cerevisiae* through regulation of the Leloir pathway genes (Martchenko *et al.* 2007; Sellick *et al.* 2008), while Rtg1 and Rtg3 regulate expression of the orthologous genes in *C. albicans* (Dalal *et al.* 2016). *S. cerevisiae* has the ability to acquire carbon and nitrogen from various sources, but preferentially utilizes nutrients from available sources (Huang and Brandriss 2000; Sellick *et al.* 2008). Such examples of Gal4 transcriptional rewiring and altered metabolic dynamics in *C. albicans* and *S. cerevisiae* highlight a need to be cautious when relying solely on orthologous gene identification to infer protein function and metabolic pathways.

We have studied the role of Put3 in *C. albicans*, and our findings suggest that Put3 function is largely conserved between *C. albicans* and *S. cerevisiae*. However, *C. albicans* can use proline as both a carbon source and nitrogen source, unlike *S. cerevisiae*, where Put3 only activates proline catabolism in the absence of a rich nitrogen source. The presence of a rich nitrogen source does not prevent *C. albicans* Put3 (*Ca*Put3) from directing the breakdown of proline to acquire nitrogen and carbon for cell growth. Nitrogen catabolism has been shown to be hierarchical in *S. cerevisiae*; proline is not used as a nitrogen source in the presence of a more readily assimilated source such as ammonium sulfate (Huang and Brandriss 2000). Furthermore, proline only serves as a nitrogen source in *S. cerevisiae*: it does not provide both carbon and nitrogen availability (Brandriss and Magasanik 1979). In *S. cerevisiae*, proline functions as an inducer of Put3, and available nitrogen sources dictate the phosphorylation status of Put3 and fine-tune its activation of Put1 and Put2 (Huang and Brandriss 2000), an alteration that appears to be bypassed in *C. albicans* since the pathogen can catabolize proline in medium containing ammonium sulfate. Our findings suggest that Put3 function is fundamentally preserved between *C. albicans* (*Ca*Put3) and *S. cerevisiae*

(*Sc*Put3), although their proline catabolism circuits show significant differences.

5.2 Materials and methods

5.2.1 Strains, media, plasmids, and transformation

(i) *Candida albicans* The *ScPUT3*GAD1A strain was constructed as described previously (Schillig and Morschhäuser 2013). To obtain the *put3* null mutant in the SN95 background strain, one *PUT3* allele was replaced by transformation with the *HIS1* marker and the other allele with the *ARG4* marker. Oligonucleotides used for the *put3* null mutant construction were amplified from plasmids pFA-HIS1 for the first allele knockout and pFA-ARG4 for the second allele knockout (Lavoie *et al.* 2008) using the respective forward and reverse primers PUT3_Marker_KO_F, which contains 97 nucleotides corresponding to the sequence just before the *PUT3* start codon, and PUT3_Marker_KO_R, which contains 95 nucleotides corresponding to the sequence just after the *PUT3* stop codon. Deletion of the first allele was confirmed by PCR using the forward primer PUT3_KO_Check_F, which binds upstream of the *PUT3* gene, and the reverse primer PUT3_KO_Check_R, which binds downstream of the gene. Deletion of the second allele was confirmed by PCR using two primer pairs: (i) forward primer PUT3_KO_Check_F, which binds upstream of the *PUT3* gene, and reverse primer FT-H2, which binds upstream of the *HIS1* gene (*HIS1* promoter region), and (ii) forward primer FT-U3, which binds inside the *HIS1* gene, and reverse primer PUT3_KO_Check_R, which binds downstream of *PUT3*. The *put3* null mutants were confirmed using the forward primer PUT3_KO_Check_Internal_F and the reverse primer PUT3_KO_Check_Internal_R, which both bind inside the *PUT3* gene; no band is expected for *put3* null mutants for this primer pair. Oligonucleotides used for transformation in this study are presented in Table S1. Standard procedures for *C. albicans* cell growth and transformation (Gola *et al.* 2003) were followed. *C. albicans* strains for transformation, ChIP-chip analyses, and transcriptional profiling experiments were cultured in YPD (1% [wt/vol] yeast extract, 2% [wt/vol] peptone, 2% [wt/vol] dextrose). Yeast nitrogen base medium (YNB) at 6.8 mg/ml supplemented with glucose (20 mg/ml) or proline (15.3 mg/ml) was used for phenotypic studies testing for the ability of the put3 null mutant to utilize proline as a carbon source. YNB without ammonium sulfate at 6.8 mg/ml supplemented with proline (15.3 mg/ml) was used for phenotypic studies testing for the ability of *C. albicans* (as well as *S. cerevisiae*) wild-type and *put3* null mutant strains to utilize proline as the sole source of carbon and nitrogen. Yeast carbon base (YCB) medium at

11.7 mg/ml supplemented with ammonium sulfate (5 mg/ml) or proline (8.7 mg/ml) was used for phenotypic studies testing for the ability of the put3 null mutant to utilize proline as a nitrogen source. The *put3* null mutant strains were also cultured in control media (YNB at 6.7 mg/ml without a carbon supplement, YCB at 11.7 mg/ml without a nitrogen source, and YPD).

Saccharomyces cerevisiae. *S. cerevisiae* strains were routinely cultured in YPD medium with 40 mg/liter uridine at 30 °C. Standard genetic procedures were used for mating of *S. cerevisiae* strains, selection of diploids, induction of meiosis, and tetrad dissection (Rose *et al.* 1990) . *S. cerevisiae* transformation was carried out by the lithium acetate procedure (Bartel 1991). *PUT3* was replaced by the one-step gene disruption procedure. The KANMAX4 marker was amplified by PCR of the Put3 null mutant from the *S. cerevisiae* knockout (YKO) deletion collection using the forward primer *PUT3-F* and reverse primer *PUT3-R*, which provided homology to the flanking regions of the relevant KANMX4 insert (Giaever *et al.* 2002) . The KANMAX4 marker was transformed into the prototrophic strain RO-1A (WT-Sacc), and transformants were selected on YPD using Geneticin (G418). Successful deletion of *PUT3* was further confirmed by PCR using two primer pairs: (i) forward primer *PUT3*-F, which binds upstream of the *PUT3* gene, and reverse primer kanB-R, which binds inside the KANMAX4 gene, and (ii) forward and reverse internal primers PUT3-In-F and PUT3-In-R, respectively, which bind inside the *PUT3* gene. Oligonucleotides used for transformation in this study are presented in Table S5.1.

5.2.2 Proline utilization assays

(i) *Candida albicans*. The wild-type (WT) strain SC5314, the *put3* null mutant strain *ΔΔput3*, the Put3 gain-of-function (Put3-GOF) strain *ScPUT3*GAD1A, and the ppr1 null mutant strain *ΔΔppr1* were each cultured in 10 ml of each of the following media in 50-ml Falcon tubes for 3 days at 30 °C with shaking at 220 rpm: 6.8 mg/ml YNB, YNB plus proline (15.3 mg/ml), YNB plus glucose (20 mg/ml), YPD, 11.7 mg/ml YCB (Sigma-Aldrich), YCB plus proline (8.7 mg/ml), and YCB plus ammonium sulfate (5 mg/ml). The put3 null mutant strain and SC5314 (wild-type strain) were each also cultured for 6 days as described above in the following media: 6.8 mg/ml YNB without ammonium sulfate and YNB plus proline (15.3 mg/ml). Optical density at 600 nm (OD600) data were collected every 24 h throughout each incubation period.

(ii) *Saccharomyces cerevisiae*. Overnight cultures of the *S. cerevisiae* prototroph RO-1A

and the *S. cerevisiae put3* null mutant grown in YPD at 30 °C were washed twice in water, adjusted to an OD600 of 0.1, and then incubated at 30 °C in 10 ml of each of the following media: 6.8 mg/ml YNB, YNB plus proline (15.3 mg/ml), YNB plus glucose (20 mg/ml), YPD, 11.7 mg/ml YCB (Sigma-Aldrich), YCB plus proline (8.7 mg/ml), YCB plus ammonium sulfate (5 mg/ml), 6.8 mg/ml yeast nitrogen base (YNB) without ammonium sulfate, and YNB without ammonium sulfate plus proline (15.3 mg/ml). OD600 data were collected every 24 h for up to 3 days or 4 days for cultures in YNB without ammonium sulfate containing proline or no proline.

5.2.3 Transcriptional profiling experiments

Transcriptional profiling experiments were carried out as described previously (Tebung *et al.* 2016), with a few modifications. Briefly, experiments were performed for strain *SCPUT3*GAD1A (Put3 gain-of-function mutant) compared with the background wild-type strain SC5314. Single colonies of each strain were each inoculated into 10 ml YPD and incubated overnight at 30 °C on a 220-rpm shaker. The overnight cultures were diluted to an OD600 of 0.1 in 50 ml YPD and grown to an OD600 of 0.8. Total RNA was extracted using the Qiagen RNeasy minikit protocol, and RNA quantity was determined using a NanoQuant machine. For direct dye incorporation, 20 µg of total RNA from each sample was reverse transcribed using oligo(dT)23VN and Superscript III reverse transcriptase (Invitrogen) in the presence of Cy3 or Cy5; dye swaps were employed for each sample. Template RNA was eliminated from the synthesized cDNA by simultaneously adding RNase A (Sigma) to a final concentration of 0.05 mg/ml and 0.05 U/µl RNase H (New England Biolabs) to each sample and then incubating the mixture for 30 min at 37 °C before purifying the labeled cDNA with a QIAquick PCR purification kit (Qiagen). Microarray hybridization, washing, scanning, and normalization were performed as described previously (Nantel *et al.* 2006), with the following exceptions. Scanning was carried out using an Axon GenePix 4000B microarray scanner, and data analyses and normalizations were done using GenePix data analysis software. The scanning settings were 635 nm for Cy5 and 532 nm for Cy3. The median of ratios of mutant Cy5-tagged to nontagged Cy3 or mutant Cy3-tagged to nontagged Cy5 values were statistically analyzed in the MultiExperiment Viewer (MeV) software using a P value cutoff at 0.05. Positive significant genes (upregulated genes) were candidates for Put3 regulation.

5.2.4 ChIP-chip

ChIP-chip experiments were performed as described previously (Tebung *et al.* 2016) with minor changes. Briefly, the *ScPUT3*GAD1A strain containing the chromosomally inserted Put3-HA fusion and the background strain SC5314 (untagged) were cultured to an OD600 of 0.6 in 50 ml of YPD. Cross-linking of protein bound to DNA for each 50-ml culture was carried out in 1.5 ml of 37% formaldehyde for 30 min, and then ChIP was performed as described previously (Tebung *et al.* 2016). ChIP DNA extracted from tagged strains was labeled with Cy5 dye, ChIP DNA from untagged strain SC5314 was labeled with Cy3 dye, and the samples were then cohybridized to Agilent 8X15K whole-genome arrays containing 14490 60-mer intergenic and intragenic oligonucleotide probes. Microarray hybridization, washing, scanning, and normalization were performed as described previously (Nantel *et al.* 2006) with the following modifications: the Axon GenePix 4000B microarray scanner was used to perform scanning, and GenePix data analysis software and Multiexperiment Viewer (MeV) software were used to analyze and normalize data; a 0.05 P value cutoff was used for MeV analyses. The scanning settings used were 635 nm for Cy5 and 532 nm for Cy3. The log of ratios of Cy5 to Cy3 (635 nm/532 nm) with a cutoff of at least 1.5 for each spot was considered to be an indicator of significant Put3 binding.

5.2.5 RNA-seq

Single colonies of the *C. albicans* wild-type strain SC5314 and the *put3* null mutant strain were each inoculated into 10 ml YPD and incubated overnight at 30 °C on a 220-rpm shaker. The overnight cultures were diluted to OD600 of 0.1 in 10 ml and grown to an OD600 of between 0.8 and 1.3 in various media at 30 °C with shaking at 220 rpm. SC5314 was cultured in YNB plus ammonium sulfate, glucose, and proline (YNGP_SC), YNB plus ammonium sulfate and glucose (YNG_SC), YNB plus ammonium sulfate and proline (YNP_SC), YNB plus glucose and proline (YGP_SC), and YNB plus proline (YP_SC). The *put3* null mutant strain was cultured in YNB plus ammonium sulfate, glucose, and proline (YNGP_ΔΔPut3) and YNB plus ammonium sulfate and glucose (YNG_ΔΔPut3). YNB (6.8 mg/ml), proline (15.3 mg/ml), glucose (20 mg/ml), and ammonium sulfate (5 mg/ml) were used when required. Total RNA was extracted using the Qiagen RNeasy minikit protocol, and RNA quality and quantity were determined using an Agilent bioanalyzer. Sequencing of extracted RNA samples was carried out at the Quebec Genome Innovation Center located at McGill University using an Illumina miSEQ sequencing platform.

Each RNA-seq data file was postprocessed to correct read sequences (Song and Florea 2015), trim adapters (Jiang *et al.* 2014), and remove rRNA reads (Kopylova *et al.* 2012) . The *C. albicans*_SC5314_Assembly22 ORF/gene coding sequences (SC5314_V22) were downloaded from the CGD website (Skrzypek *et al.* 2017). A PERL script was written to create a gene/open reading frame ID and description file. The reads were then mapped to the SC5314_V22 sequences (Patro *et al.* 2017) to produce raw counts and TPM (transcripts per million) values. Raw counts and TPM values were annotated with gene descriptions using a PERL script and imported to an Excel spreadsheet for further analysis. Expression ratios of mutants versus controls in each experiment were calculated to identify proline-dependent changes in gene expression. To minimize false positives, gene expression values with log2 1.5 threshold were considered in the calculation of the ratios of experiments versus controls.

5.2.6 Bioinformatics

The CGD tool "Go Term Finder" (http://www.candidagenome.org/cgi-bin/GO/goTermFinder) (Inglis *et al.* 2012) was used for Gene Ontology analyses. Fungal BLAST analysis of *C. albicans* Put3 was performed using the Saccharomyces Genome Database (SGD) fungal BLAST tool (http://yeastgenome.org/blast-fungal) (Cherry *et al.* 2012) . Protein sequences were aligned using the SIM Alignment tool (http://web.expasy.org/sim/) (Huang and Miller 1991), and graphical representation of protein alignment was generated using the LALNVIEW program (Duret *et al.* 1996) . Put3 alignments throughout the ascomycete lineage were carried out using Fungal Orthogroups Repository (https://portals.broadinstitute.org/regev/orthogroups/) (Wapinski *et al.* 2007). Color coding of amino acid classes in the Put3 and ortholog sequences was done using an online tool, the Sequence Manipulation Suite (Stothard 2000). A Newick file for Put3 yeast phylogeny was generated using Fungal Orthogroups Repository (Wapinski *et al.* 2007) and then modified and used to generate the Put3 phylogenetic tree using an online Newick Viewer, T-REX (Boc *et al.* 2012).

5.2.7 Data access

The complete data obtained from ChIP-chip and transcription profiling (microarray) data, and RNA-seq data can be found on https://github.com/Rahaomran/Raha-Omran.git.

5.3 Results

5.3.1 The Put3 ortholog in *C. albicans*.

In the pathogen *C. albicans*, the orf19.6203 (*PUT3*) gene encodes the proposed ortholog of *S. cerevisiae* Put3. The *S. cerevisiae* and *C. albicans* proteins have about 42% sequence identity spanning 821 amino acids (over 83% of each protein) at the N-terminal part of each ortholog (Figure 5.1). The next closest *S. cerevisiae* protein homolog to *Ca*Put3 is Asg1, which has a stretch of 214 amino acids with just 24.8% identity and does not include the zinc cluster domain. These findings suggest that the *C. albicans* orf19.6203 gene may be orthologous to the yeast put3 gene.

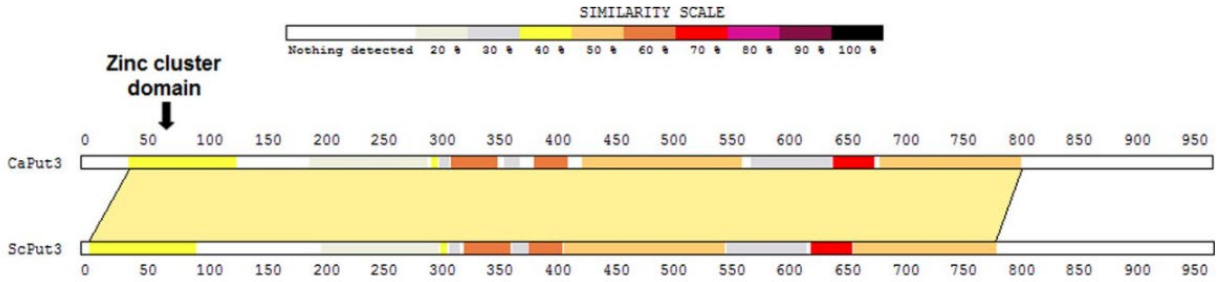


Figure 5.1 *C. albicans* Put3 aligned with *S. cerevisiae* Put3. The shaded alignment area has 41.7% identity and includes the zinc cluster domain.

5.3.2 Proline utilization.

Phenotypic studies showed that the *C. albicans* WT strain SC5314 and the disruption *ppr1* null mutant strain grow in yeast nitrogen base medium with ammonium sulfate (YNB plus NH_4^+) that contains 15.3 mg/ml proline as the sole carbon source (Figure 5.2A). These findings revealed that *C. albicans* is able to metabolize and use proline as the sole carbon source even in the presence of ammonium sulfate (Figure 5.2B), an observation that contrasts with previous findings, further confirmed in this study (Figure 5.2B), that *S. cerevisiae* cannot use proline as the sole carbon source (Brandriss and Magasanik 1979). This suggests that the pathogen is able to make more liberal use of proline as a carbon source (Figure. 5.2A).

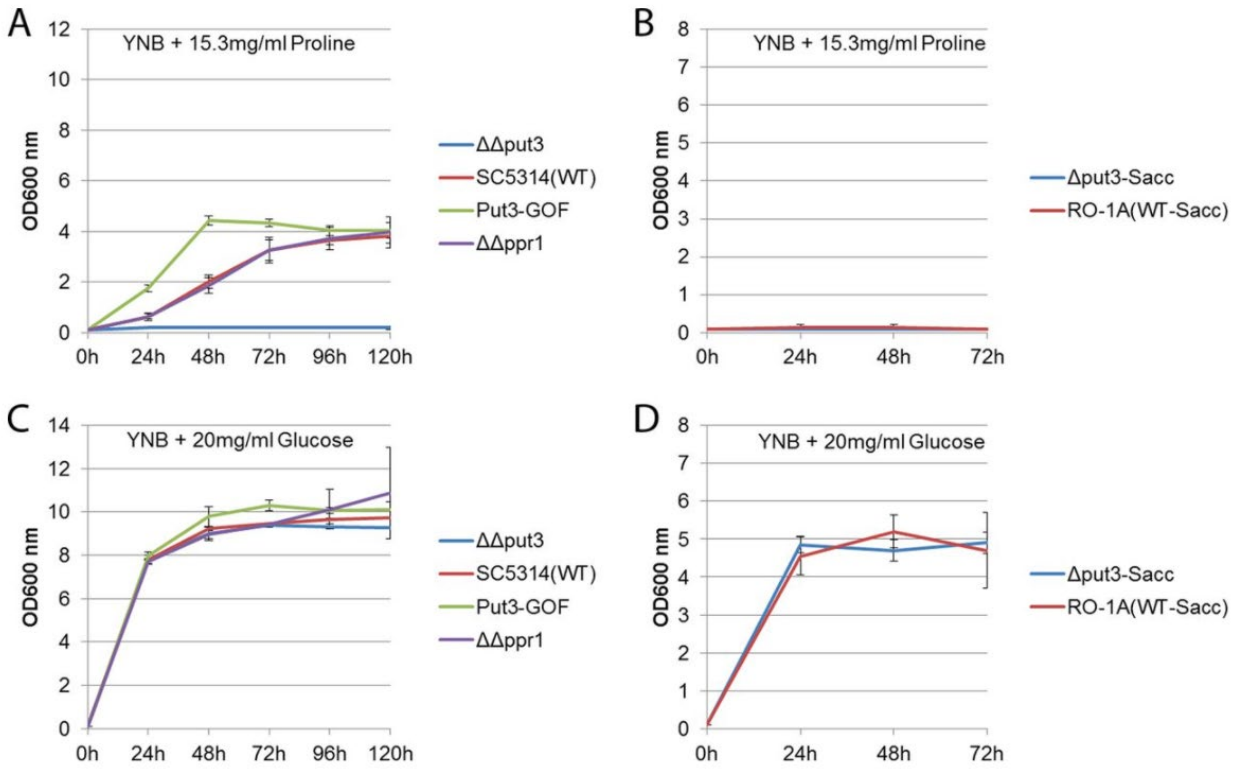


Figure 5.2:. Proline utilization as a carbon source. Shown are the results from an assay of a *C. albicans put3* null mutant (*ΔΔput3*), the *C. albicans* wild-type (WT) strain, SC5314, a *C. albicans* Put3 gain-of-function mutant (Put3-GOF), the *C. albicansppr1* null mutant (*ΔΔppr1*), the *S. cerevisiaeput3* null mutant (*Δput3*-Sacc), and the *S. cerevisiae* wild-type strain, RO-1A (WT-Sacc), at 30 °C. YNB plus glucose was used as a control at 30 °C. **A.** Growth curve of the *C. albicans ΔΔput3* mutant, SC5314, the Put3-GOF mutant, and the *ΔΔppr1* mutant in YNB with proline as the sole carbon source at 30 °C. **B**. Growth curve of the *S. cerevisiae* Δput3-Sacc mutant and RO-1A in YNB with proline as the sole carbon source at 30 °C. (C) Growth curve of the *C. albicans* ΔΔput3 mutant, SC5314, the Put3-GOF mutant, and the ΔΔppr1 mutant in YNB with glucose as the sole carbon source at 30 °C. **D.** Growth curve of the *S. cerevisiae* Δput3-Sacc mutant and RO-1A in YNB with glucose as the sole carbon source at 30 °C. Error bars are based on the standard deviation from two biological replicates of each data point reading taken every 24 h

To confirm whether the *C. albicans* Put3 ortholog is a regulator of proline catabolism, we cultured the wild type as well as the *put3* null mutant and Put3 gain-of-function (Put-GOF) mutant strains for 5 days at 30 °C in YNB medium containing 15.3 mg/ml proline as the sole carbon source. The *put3* null mutant strain did not grow in YNB with proline as the sole carbon source even after 5 days at 30 °C, suggesting an inability of the strain to metabolize proline (Figure 5.2A), while the Put3-GOF strain showed better growth than either the wild-type strain, SC5314, or the *ppr1* null mutant strain used as a control. Ppr1 is a different zinc cluster transcription factor functioning in purine catabolism (Tebung *et al.* 2016); it serves as a control for the *put3* null mutant

strain since it was constructed similarly (Figure 5.2A). These findings suggest that Put3 regulates proline catabolism in *C. albicans*. All *C. albicans* strains had normal and similar growth patterns in YNB with 20 mg/ml glucose as the sole carbon source (Figure 5.2C), as well as in YPD (yeast extract, peptone, dextrose), and all *C. albicans* strains showed no growth in YNB without any carbon source (data not shown). As expected, during growth under the same experimental conditions for 3 days, both the *S. cerevisiae* wild-type strain, RO-1A, and the *S. cerevisiae put3* null mutant strain had similar growth outcomes in YNB with 20 mg/ml glucose as the sole carbon source (Figure. 5.2D) and no growth in YNB with 15.3 mg/ml proline as the sole carbon source (Figure. 5.2B).

We also used 8.7 mg/ml proline as the sole nitrogen source in yeast carbon base medium (YCB) to culture wild-type *C. albicans* strain SC5314, the control *ppr1* null mutant strain, the *put3* null mutant strain, and the Put3 gain-of-function strain. All strains were able to grow in the medium except the *put3* null mutant strain (Figure 5.3A), suggesting that Put3 regulates the use of proline as both a carbon source and a nitrogen source in *C. albicans*. An *S. cerevisiae* prototrophic strain, RO-1A, was able to grow in 8.7 mg/ml proline as the sole nitrogen source, but the *S. cerevisiae put3* null mutant strain failed to grow in the same culture medium (Figure 5.3B). All *C. albicans* strains showed similar growth in YCB medium with 5 mg/ml ammonium sulfate as the sole nitrogen source (Figure 5.3C), and both the *S. cerevisiae* prototrophic strain RO-1A and the *S. cerevisiaeput3* null mutant strain had similar growth patterns with 5 mg/ml ammonium sulfate as the sole nitrogen source (Figure 5.3D). We further confirmed that the wild type *C. albicans* strain SC5314 grows in YNB medium containing proline as the sole source of both carbon and nitrogen, but as previously noted (Brandriss and Magasanik 1979), *S. cerevisiae* prototrophic strains such as RO-1A cannot use proline as a combined carbon and nitrogen source (Figure 5.4) and can only use proline as a nitrogen source in the absence of a rich nitrogen source such as ammonium sulfate. The inability of both *S. cerevisiae* and *C. albicans put3* mutants to utilize proline as the sole nitrogen source confirms that Put3 is essential for proline catabolism in both species. Both the *S. cerevisiae* wild type strain and the *S. cerevisiae put3* mutant strain also failed to use proline as the sole carbon source. This could be due to the inhibitory effect of ammonium sulfate present in the medium; however, both strains failed to utilize proline as the sole source of both nitrogen and carbon, even when other nitrogen sources were absent from the growth medium. The inability of

S. cerevisiae to degrade proline for carbon use even in the absence of a nitrogen source agrees with a previous report that *S. cerevisiae* can not utilize associated amino acids as a carbon source (Nishida *et al.* 2016) .

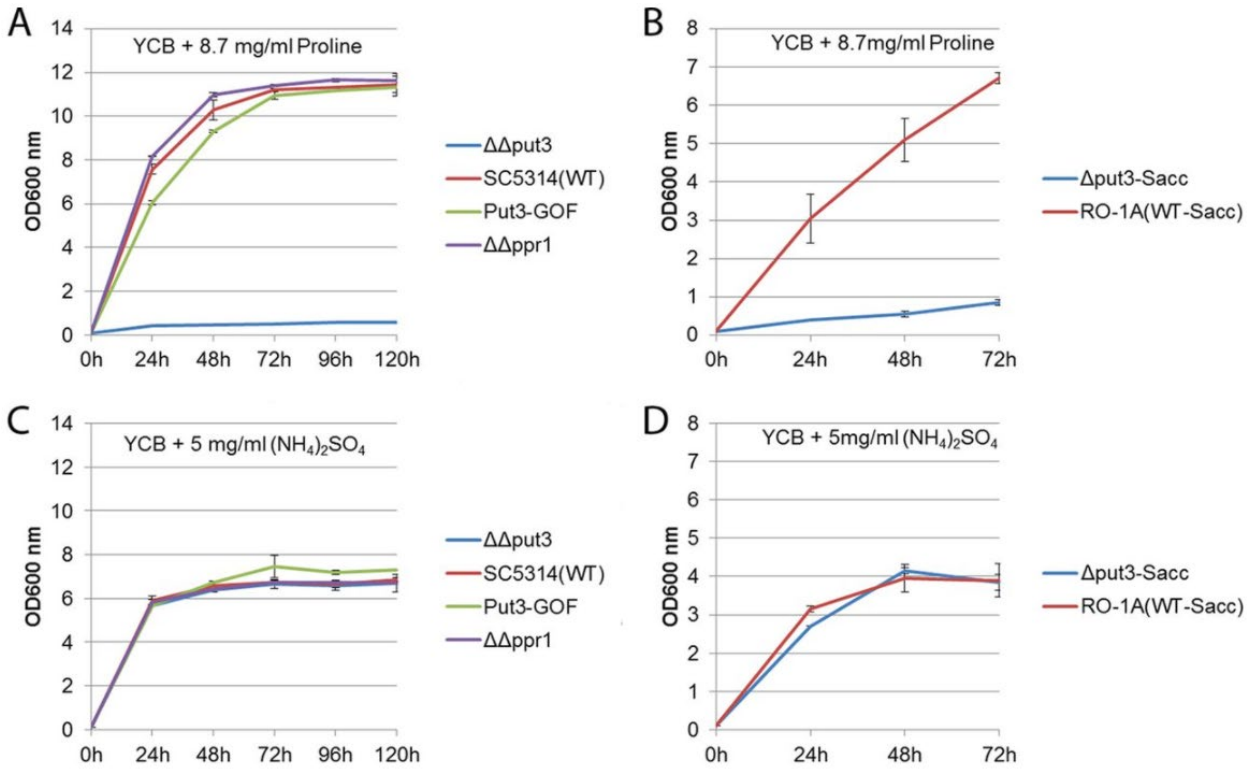


Figure 5.3: Proline utilization as a nitrogen source. Shown are the results from an assay of a *C. albicansput3* null mutant (*ΔΔput3*), the *C. albicans* wild-type (WT) strain, SC5314, a *C. albicans* Put3 gain-of-function mutant (Put3-GOF), the *C. albicansppr1* null mutant (*ΔΔppr1*), the *S. cerevisiaeput3* null mutant (*Δput3*-Sacc), and the *S. cerevisiae* wild-type strain, RO-1A (WT-Sacc), at 30 °C. YCB plus ammonium sulfate was used as a control at 30 °C. **A**. Growth curve of the *C. albicans* ΔΔput3 mutant, SC5314, the Put3-GOF mutant, and the *ΔΔppr1* mutant in yeast carbon base (YCB) with proline as the sole nitrogen source at 30 °C. **B.** Growth curve of the *S. cerevisiae* Δput3-Sacc mutant and RO-1A in YCB with proline as the sole nitrogen source at 30 °C. **C.** Growth curve of the *C. albicans ΔΔput3* mutant, SC5314, the Put3-GOF mutant, and the ΔΔppr1 mutant in YCB with ammonium sulfate as the sole nitrogen source at 30 °C. **D**. Growth curve of the *S. cerevisiae Δput3*-Sacc mutant and RO-1A in YCB with ammonium sulfate as the sole nitrogen source at 30 °C. Error bars are based on the standard deviation from two biological replicates of each data point reading taken every 24 h.

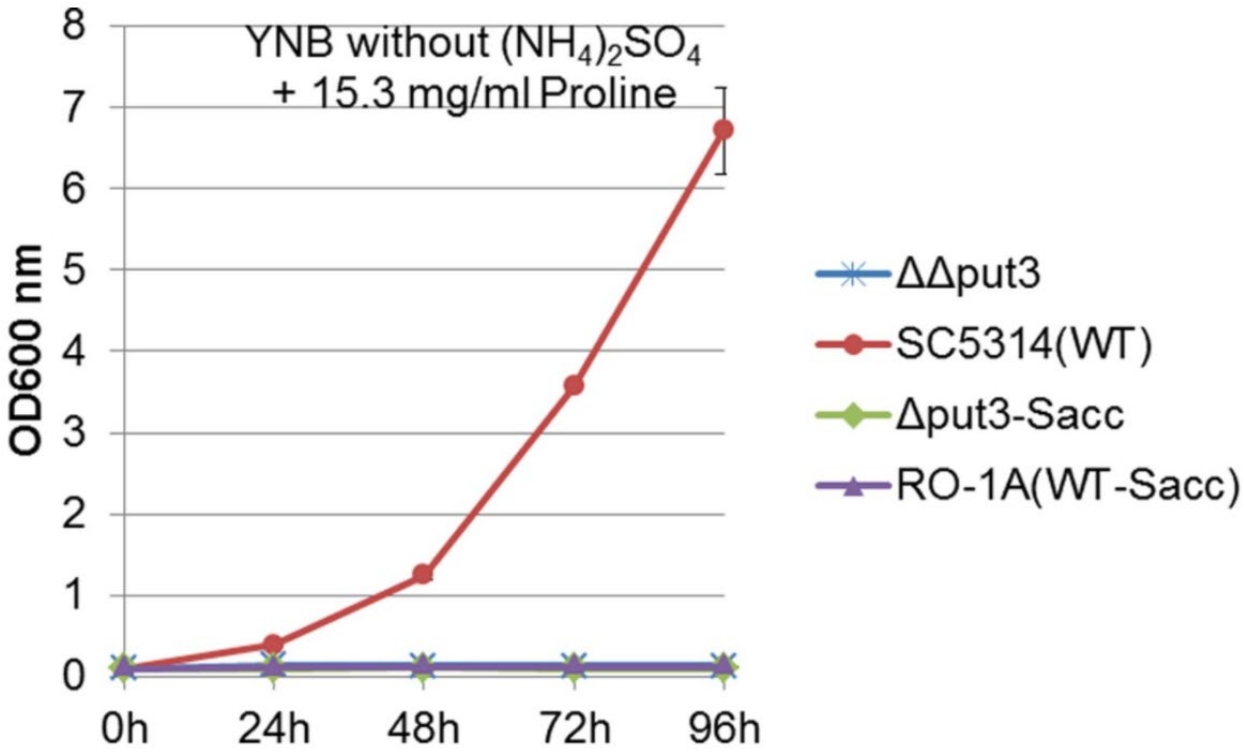


Figure 5.4: Proline utilization as a source of both carbon and nitrogen. Shown are the results from an assay of the *C. albicansput3* null mutant (*ΔΔput3*), the *C. albicans* wild-type (WT) strain, SC5314, the *S. cerevisiae put3* null mutant (*Δput3*-Sacc), and the *S. cerevisiae* wild-type strain, RO-1A (WT-Sacc), at 30 °C. The growth curves of the *C. albicans ΔΔput3* mutant and SC5314 and the *S. cerevisiae Δput3*-Sacc mutant and RO-1A in yeast nitrogen base (YNB) without ammonium sulfate but with proline as the sole carbon and nitrogen source at 30 °C were determined. Error bars are based on the standard deviation from two biological replicates of each data point reading taken every 24 h.

5.3.3 Identification of proline-regulated genes

To establish whether proline impacted *C. albicans* gene expression, we performed transcriptome sequencing (RNA-seq) analyses of *C. albicans* cells growing in the presence or absence of proline. As shown in Table 1, the addition of proline to cultures containing a readily accessible carbon (glucose) and nitrogen (ammonium) source dramatically activated the expression of *PUT1* and *PUT2*. We observed an even higher induction of *PUT1* and *PUT2* expression by proline when both ammonium sulfate and glucose were absent and when either ammonium sulfate or glucose was absent (Table 5.1). Expression of the Put3 transcription factor itself was not induced by proline, and its deletion eliminated the proline-induced expression of *PUT1* and *PUT2* (see https://github.com/Rahaomran/Raha-Omran.git). These findings suggest that proline induces the expression of *PUT1* and *PUT2* by interacting with Put3 at a posttranscriptional level. Ribosome biogenesis genes were also induced by proline addition when

ammonium sulfate, glucose, or both were missing from the media (Table 5.1). When both ammonium sulfate and glucose were present, the induction of genes involved in ribosome biogenesis was not observed, and induction was lower when ammonium sulfate was missing and glucose was present in the media (Table 5.1). This could indicate that glucose represses, to a certain degree, the Put3-dependent induction of ribosome biogenesis by proline. Proline also induced genes encoding carboxylic acid catabolism and glyoxylate enzymes, such as *ICL1*, *FOX2*, and *ADH2*, when glucose, ammonium sulfate, or both were missing in the media. These genes are required by the pathogen to obtain carbon from nonfermentable carbon sources.

Table 5.1. Proline-induced genes in *C. albicans* SC5314.

Gene or allele[a]	Fold change in expression[b]:			
	YNGP_SC/YNG_SC	YP_SC/YNG_SC	YNP_SC/YNG_SC	YGP_SC/YNG_SC
PUT1	47.05	202.58	238.72	215.67
PUT2	18.59	42.76	43.92	50.47
C2_02580W_A	2.65	14.21	10.61	12.57
ICL1	2.45	11.92	15.20	12.39
FOX2	1.97	8.21	9.86	7.97
ADH2	0.83	13.13	31.08	17.90
BUD23	1.02	15.52	13.79	8.68
C1_06760C_A,B	1.66	13.13	12.79	6.19
C1_10970W_A,B	0.84	7.77	9.25	3.18
C2_04570W_A,B	1.09	9.60	8.76	4.25
C3_02020W_A,B	1.53	11.84	9.94	6.35
C3_06760W_A,B	1.29	14.19	15.81	7.71
C5_04840C_A,B	1.78	11.16	13.42	5.90
DRS1	1.34	14.59	10.49	8.70
HIT1	0.00	6.85	12.08	4.79
SDA1	1.20	10.38	10.68	4.73
SPB4	1.15	8.80	10.61	4.87
UTP21	1.09	10.68	10.96	4.61
C1_02450C_A,B	1.07	5.73	7.97	3.33
C1_04040C_A,B	0.93	8.22	9.36	4.48
C2_05750W_A,B	0.54	5.86	8.67	3.71
CHR1	1.07	6.68	8.23	3.41
CR_03360W_A,B	0.92	6.50	8.23	3.24
CR_09800C_A,B	0.83	5.06	10.06	4.77
REI1	0.71	6.96	9.14	3.04
RPP1	0.43	7.12	10.72	4.52
RRP8	0.81	6.14	7.69	3.12
CR_01780W_A,B	1.73	9.20	3.60	4.62
CR_10410C_A,B	1.21	10.31	7.43	5.48
FYV5	0.71	8.15	5.67	4.11
LTV1	0.67	14.55	9.33	8.94
NOP14	0.82	10.75	8.97	5.70
YVH1	0.72	7.57	5.67	3.83

RNA-seq data showing *C. albicans* SC5314 genes induced by proline are presented (Table 5.1). **a.** The genes shown have at least one allele induced 10-fold or more under at least one of the conditions listed

and are involved in either proline degradation, the glyoxylate cycle, or ribosome biogenesis. When the allele name is used instead of the gene name, "A, B" is added at the end of the name if the data presented are for both alleles of the gene.

b. The average of the expression fold change for the two alleles of each gene is presented, except for C2_02580W_A, which is just one allele of the gene. The YNPG_SC/YNG_SC column shows the fold change in gene expression for the *C. albicans* SC5314 wild-type strain cultured in YNB containing ammonium sulfate, glucose, and proline compared against the same strain grown in YNB containing ammonium sulfate and glucose. The YP_SC/YNG_SC column shows the fold change in gene expression for the *C. albicans* SC5314 wild-type strain cultured in YNB containing proline only compared against the same strain grown in YNB containing ammonium sulfate and glucose. The YNP_SC/YNG_SC column shows the fold change in gene expression for the *C. albicans* SC5314 wild-type strain cultured in YNB containing ammonium sulfate and proline compared against the same strain grown in YNB containing ammonium sulfate and glucose. The YGP_SC/YNG_SC column shows the fold change in gene expression for the *C. albicans* SC5314 wild-type strain cultured in YNB containing glucose and proline compared against the same strain grown in YNB containing ammonium sulfate and glucose.

5.3.4 Identification of Put3-regulated genes using transcriptional profiling.

To further confirm the pathway through which Put3 regulates proline catabolism in *C. albicans*, we performed transcriptional profiling experiments using the Put3 gain-of-function (Put3-GOF) mutant strain *ScPUT3*GAD1A generated by addition of a Gal4 activation domain to Put3 (Schillig and Morschhäuser 2013). Such GOF mutant strains can allow for gene network upregulation in the absence of any stimulatory condition. Transcription profiling using hyperactive proteins is an efficient way to identify the regulatory pathway of transcription factors (Devaux *et al.* 2001; Hikkel *et al.* 2003; Onda *et al.* 2004). The key proline catabolism enzyme-encoding genes *PUT1* and *PUT2* (Table 5.2) were among the genes upregulated in strains expressing the hyperactive Put3. As well, the *C. albicans* gene orf19.1584, which is similar to the gene *MCH5* encoding a riboflavin transporter required for Put1 function in proline degradation in *S. cerevisiae* (Spitzner *et al.* 2008), was also upregulated. This suggests that Put3 has preserved its proline catabolism regulation role in *C. albicans* and *S. cerevisiae*, and it regulates this pathway by transcriptionally activating *PUT1* and *PUT2*, as well as the Put1 cofactor precursor importer gene, orf19.1584. Other upregulated genes observed in the transcription profiling data using the Put3 gain-of-function mutant strain include genes involved in ribosome biogenesis, carboxylic acid metabolic processes, filamentous growth, response to stress, RNA metabolic processes, and cellular protein modification (Table 5.2; see https://github.com/Rahaomran/Raha-Omran.git.).

Table 5.2 *C. albicans* Put3-regulated genes and potential target genes. Both ChIP-chip and transcriptional profiling data were analyzed using GenePix and MeV, and Put3 motif identification was carried out using the online motif scanning tool FIMO. *C. albicans* genes appearing in any two of the following four categories are presented: (i) gene is upregulated in transcription profiling data for SCPUTGAD1A, (ii) gene shows ChIP-chip binding by Put3, (iii) promoter has *Sc*Put3 binding motif, and (iv) ortholog is regulated by *Sc*Put3. Check marks indicate when a gene falls into a category. Transcription profiling median of ratio values above 1.94 and ChIP-chip log of ratio values above 1.4 are considered significant.

Gene	ORF	Gene categorization by[a]:			
		Transcription profiling	ChIP-chip	ScPut3 motif	ScPut3 regulates ortholog
	orf19.1584	✓	✓	✓	✓
CYB2	orf19.5000	✓	✓	✓	
	orf19.670.2	✓	✓	✓	
	orf19.3406	✓	✓	✓	
YHB1	orf19.3707	✓	✓	✓	
	orf19.1109	✓	✓		
CAS1	orf19.1135	✓	✓		
FAD2	orf19.118	✓	✓		
	orf19.1486	✓	✓		
	orf19.1549	✓	✓		
UTP4	orf19.1633	✓	✓		
	orf19.1789	✓	✓		
RGT1	orf19.2747	✓	✓		
	orf19.2782	✓	✓		
AIP2	orf19.300	✓	✓		
	orf19.3254	✓	✓		
	orf19.3585	✓	✓		
IDP2	orf19.3733	✓	✓		
	orf19.4273	✓	✓		
PUT1	orf19.4274	✓	✓		✓
PPS1	orf19.4405	✓	✓		
PTC8	orf19.4698	✓	✓		
	orf19.5026	✓	✓		
RPL35	orf19.5964.2	✓	✓		
GLN1	orf19.646	✓	✓		
GIN4	orf19.663	✓	✓		
	orf19.6828	✓	✓		
	orf19.6853	✓	✓		
ATP1	orf19.6854	✓	✓		
CAN3	orf19.84	✓	✓		
PET9	orf19.930	✓	✓		
	orf19.1054		✓	✓	
PEX11	orf19.1089		✓	✓	
	orf19.1490.1		✓	✓	
HEM3	orf19.1742		✓	✓	
	orf19.2105		✓	✓	
TIF5	orf19.4261		✓	✓	
RAD9	orf19.4275		✓	✓	
	orf19.6188		✓	✓	
	orf19.7266		✓	✓	
MIS12	orf19.7534		✓	✓	
PUT2	orf19.3974	✓			✓
TEC1	orf19.5908	✓			✓
AXL1	orf19.7342	✓			✓
STE3	orf19.2492		✓		✓
PCL1	orf19.2649		✓		✓
KIP4	orf19.5265		✓		✓
BEM2	orf19.6573		✓		✓
	orf19.68.2		✓		✓
MODF	orf19.5029			✓	✓
	orf19.5720			✓	✓

5.3.5 Direct identification of genes bound by *Candida albicans* Put3 using ChIP-chip.

Our phenotypic studies demonstrated that Put3 regulates proline use in *C. albicans*, and transcription profiling experiments highlighted that *C. albicans* Put3 regulates the proline catabolism pathway genes *PUT1*, *PUT2*, and orf19.1584, as well as genes involved in ribosome biogenesis and other cellular pathways. Chromatin immunoprecipitation followed by microarray analysis (ChIP-chip) can identify the direct binding targets of specific transcription factors. This analysis was carried out using the *C. albicans* strain *ScPUT3*GAD1A (Schillig and Morschhäuser 2013), which was constructed from SC5314 and contains a hemagglutinin (HA) epitope sequence fused to the Put3 protein. After chromatin cross-linking, target binding sequences were identified by amplifying and labeling immunoprecipitated DNA sequences and hybridizing these labeled sequences to Agilent 8X15K whole-genome tiling arrays containing a representative probe set for the *C. albicans* genome. Put3 target genes were ranked based on their log of ratio (PUT3-HA-Cy5 versus nontagged Cy3) values (the data is available at: https://github.com/Rahaomran/Raha-Omran.git.), and then target genes with log of ratio values of at least 1.4 that were also upregulated in the *ScPUT3*GAD1A strain expressing a hyperactive Put3 (Schillig and Morschhäuser 2013) (Table 2) were further annotated for function using the Candida Genome Database Gene Ontology tool. Consistent with transcription profiling data for the *ScPUT3*GAD1A strain, Put3 targets identified by ChIP-chip include the promoters of *PUT1* and orf19.1584, as well as genes involved in ribosome biogenesis, carboxylic acid metabolic processes, filamentous growth, response to stress, RNA metabolic processes, and cellular protein modification (Table 5.2). *PUT2*, however, was not significantly bound by *C. albicans* Put3 in our ChIP-chip data; this suggests that Put3 may regulate *PUT2* indirectly. Binding of *C. albicans* Put3 to the promoter of *PUT1* and orf19.1584 provides further support for Put3's regulatory role in proline catabolism in *C. albicans*, which aligns with the role played by its ortholog in *S. cerevisiae*. No classic Put3 DNA binding motif as defined in *S. cerevisiae* can be identified at the *C. albicans PUT1* promoter. The orf19.1584 promoter, however, has a sequence (P = 6.89E−6) identical to the predicted *S. cerevisiae* Put3 (*Sc*Put3) binding motif (Swaminathan *et al.* 1997) located at positions −42 to −57. Other genes with minimum log ratios of 1.4 identified as both upregulated in the gain-of-function strain and with demonstrated ChIP-chip binding were also assessed for the *S. cerevisiae* Put3 binding motif. In addition to orf19.1584, four other genes (orf19.5000, orf19.670.2, orf19.3406, and orf19.3707) in this set of 31 genes had a predicted *S. cerevisiae* Put3 motif. Overall, however, this motif is

relatively common in the *C. albicans* intergenic regions, with 668 sequences identified in the 6,206 *C. albicans* promoter sequences used by the FIMO motif scan tool (Grant *et al.* 2011). Thus, the predicted *S. cerevisiae* Put3 motif is not significantly enriched at the promoters of genes present in both our ChIP-chip and transcription profiling hits. However, no alternative putative motif was detected within the sequences of the Put3-bound and transcriptionally activated genes in *C. albicans*.

5.3.6 Conservation of Put3 phosphorylation sites.

Studies in *S. cerevisiae* have shown that Put3 regulation depends on various factors, including proline and available nitrogen sources. The presence of proline alters the conformation of Put3 to a more active form (Des Etages *et al.* 2001): meanwhile, different nitrogen sources induce specific Put3 phosphorylation states that range from an active state to a less active state as the quality of the nitrogen source increases (Huang and Brandriss 2000; Leverentz and Reece 2006). Phosphorylation of Y788 activates Put3, and phosphorylation of S969 inhibits Put3 (Leverentz *et al.* 2009). Put3 alignment among fungal species shows that the phosphorylatable Y788 and S969 both appear in the lineage leading to *Saccharomyces bayanus* and are conserved through *S. cerevisiae*. Y788 and S969 are not present in species appearing earlier than *S. bayanus* in the phylogeny, including *C. albicans* (Figure 5.5). These results suggest that Put3 may have acquired its potential to be regulated by these phosphorylations at the lineage leading to *S. bayanus* and the potential has been conserved through *S. cerevisiae*.

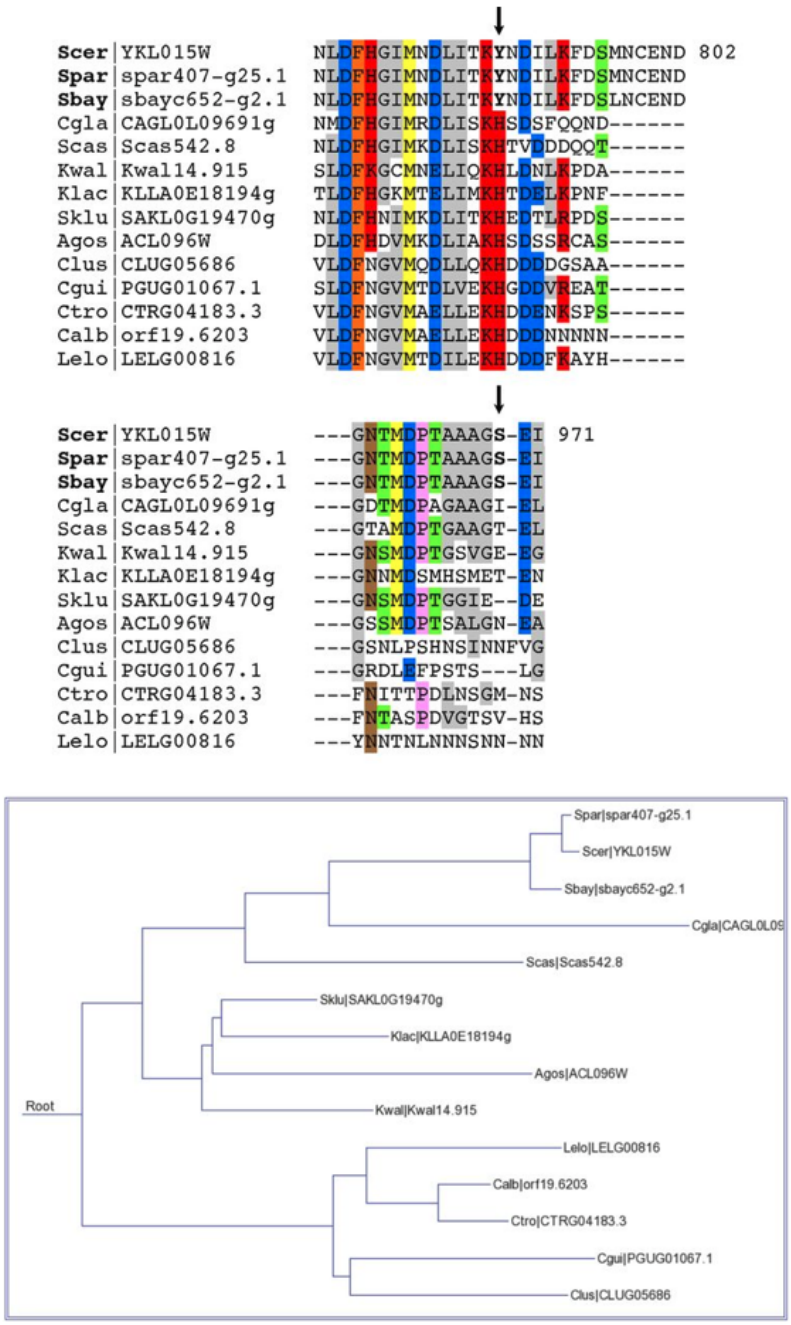


Figure 5.5: Alignment of Put3 through yeast phylogeny. Shown is alignment of Put3 and orthologs through yeast phylogeny. A conserved tyrosine essential for Put3 activation and conserved serine essential for Put3 inhibition in *S. cerevisiae* are in boldface, and boldface arrows indicate their positions in the aligned sequences. Species name abbreviations with the conserved activating tyrosine and inhibiting serine are in boldface: Scer, *Saccharomyces cerevisiae*; Spar, *Saccharomyces paradoxus*; Sbay, *Saccharomyces bayanus*. Amino acids of the same class that are 50% or more conserved are highlighted in specific colors based on amino acid class. A phylogenic tree shows Put3 phylogeny in yeasts.

5.4 Discussion

Put3 transcriptionally regulates enzymes of the proline catabolism pathway in *S. cerevisiae*. The *C. albicans* gene orf19.6203 has been named *PUT3* based on sequence similarity to *S. cerevisiae* Put3, but zinc cluster transcription factor-rewiring events such as those for Gal4 and Ppr1 (Martchenko *et al.* 2007; Tebung *et al.* 2016) highlight the need to study each transcription factor beyond sequence and structural alignments in order to accurately establish the role of each ortholog in other species. We performed phenotypic studies using a *C. albicans put3* null mutant and found that this mutant failed to grow in YNB medium containing proline as the sole carbon source (Figure 5.2A) or in YCB medium containing proline as the sole nitrogen source (Figure 5.3A), suggesting Put3 does play a role in proline catabolism in this opportunistic pathogen. In contrast, the wild-type strain SC5314, the Put3-GOF strain, and the *ppr1* null mutant strain used as controls all showed significant growth in both YNB medium containing proline as the sole carbon source (Figure 5.2A) and YCB medium containing proline as the sole nitrogen source (Figure 5.3A). Intriguingly, the Put3-GOF strain had a shorter lag phase in YNB medium with proline as the sole carbon source, which further suggests the importance of Put3 in proline catabolism (Figure 5.2A). All strains showed similar growth in YNB medium containing glucose as the sole carbon source (Figure 5.2C), in YCB medium containing ammonium sulfate as the sole nitrogen source (Figure 5.3C), and in YPD medium.

Put3 regulates proline use as a nitrogen source in *S. cerevisiae* by controlling expression of *PUT1* and *PUT2*, which encode the factors required to process proline to glutamate. We found that *C. albicans put3* null mutant strain utilizes glutamate for both its carbon and nitrogen needs, which led us to hypothesize that Put3 could direct expression of the *PUT1* and *PUT2* genes for proline utilization. This prediction was confirmed using RNA-seq; in *C. albicans*, proline induces *PUT1* and *PUT2* transcription in a Put3-dependent manner (Table 1) (Huang and Brandriss 2000). Furthermore, transcription profiling of an activated Put3 protein and ChIP-chip data support the role of Put3 in the positive regulation of *PUT1* and *PUT2*. This suggests that the zinc cluster transcription factor Put3 has conserved its role in proline catabolism between *C. albicans* and *S. cerevisiae*. Our data suggest that proline induces *PUT1* and *PUT2* by acting on Put3 at a posttranscriptional level since proline did not induce *PUT1* and *PUT2* in *put3* null mutant strains, and only *PUT1* (approximately 50-fold) and *PUT2* (approximately 20-fold), but not *PUT3*, were

transcriptionally induced when both ammonium sulfate and glucose were present. In fact, *PUT1* ranked first and *PUT2* ranked second in our list of most upregulated genes upon proline induction in the presence of ammonium sulfate and glucose (Table 5.1). The inducing effect of proline was significantly increased in the absence of both ammonium sulfate and glucose, with *PUT1* induced approximately 200-fold and *PUT2* induced approximately 40-fold (Table 5.1). These findings suggest that *C. albicans* simultaneously uses proline, ammonium sulfate, and glucose when all are available in the media, but further induces Put3 upregulation of *PUT1* and *PUT2* expression when proline becomes the only source of carbon, nitrogen, or both carbon and nitrogen, as this may allow more efficient breakdown of proline to usable carbon and nitrogen for cellular needs. Although *PUT1* and *PUT2* have greater expression when proline is the sole source of carbon, nitrogen, or both, sufficient expression is still attained in the presence of glucose, ammonium sulfate, or both to degrade proline for cellular growth. Proline also induced the expression of genes involved in ribosome biogenesis when ammonium sulfate, glucose, or both were missing in the media. Carboxylic acid catabolism and glyoxylate pathway enzymes such as *ICL1*, *FOX2*, and *ADH2* were also upregulated by proline, but only when glucose, ammonium sulfate, or both were missing in the media. The induction of these enzymes could act to allow *C. albicans* to efficiently utilize proline as a carbon source. It is possible that the presence of ammonium sulfate and glucose reduces the need for the cells to break down available proline, which circumvents upregulation of carboxylic acid catabolism and glyoxylate enzymes. Overall, *C. albicans* is able to use proline as the sole source of carbon (Figure 5.2A), nitrogen (Figure 5.3A), and both carbon and nitrogen (Figure 5.4). This is in clear contrast to *S. cerevisiae*, which can use proline only as a nitrogen source and does so only in the absence of a more readily assimilated source (Brandriss and Magasanik 1979) (Figure 5.2B, 3B, and 4). Notably, *C. albicans* can metabolize proline even in the presence of ammonium sulfate (Figure 5.2A).

This nitrogen-sensing regulation of *S. cerevisiae PUT1* and *PUT* by Put3 is achieved at least in part by fine-tuning Put3 activity through phosphorylation events that depend on the quality of available nitrogen in the growth medium (Huang and Brandriss 2000; Leverentz and Reece 2006). Rich nitrogen sources induce phosphorylations that block the ability of Put3 to induce genes involved in proline catabolism. Sequence alignment of *S. cerevisiae* and *C. albicans* Put3 ortholog sequences revealed that the amino acids Y788 and S969, which are essential phosphorylation targets for *S. cerevisiae* Put3 activation and inhibition, respectively, are not conserved in *C.*

albicans Put3 (Leverentz *et al.* 2009) . This finding suggests that in *C. albicans*, Put3 (*Ca*Put3) may not be equivalently regulated by phosphorylation in response to the quality of available nitrogen sources and may be compensated by the capacity of the pathogen to utilize proline as a carbon source in the presence of ammonium sulfate. Similar to *C. albicans*, other species appearing prior to *S. bayanus* in the yeast phylogeny lack these key amino acids implicated in Put3 regulation by phosphorylation.

While one might anticipate that differing phosphorylation states could reduce the capacity of *S. cerevisiae* to utilize proline as a nitrogen source in the presence of richer nitrogen sources, our study highlighted its complete inability to utilize proline when richer nitrogen sources are absent. This inability may be due in part to the role of the Fmp12 protein. Recent studies (Nishida *et al.* 2016) have shown that the Fmp12 protein in *S. cerevisiae* has sequence similarity to α-ketoglutarate-dependent dioxygenases of *Candida* species and in humans plays a role in the decarboxylation of α-ketoglutarate, an intermediate of proline catabolism. The Fmp12-dependent decarboxylation of α-ketoglutarate might enable the bypass of the tricarboxylic acid (TCA) cycle reactions mediated by α-ketoglutarate dehydrogenase (KGDH) and succinyl coenzyme A (succinyl-CoA) ligase. As such, in *S. cerevisiae* α-ketoglutarate could be metabolized through Fmp12 rather than KGDH, allowing the bypass of NADH production via KGDH and ATP production via succinyl-CoA ligase (Nishida *et al.* 2016) . Overall, this would result in the inability of *S. cerevisiae* to generate sustainable energy for cell growth using proline as the sole source of carbon or both carbon and nitrogen. Intriguingly, deletion of *FMP12* promotes the use of proline as the sole source of both nitrogen and carbon by *S. cerevisiae*, and this enhanced-growth phenotype is eliminated by deletion of *PUT1* or *PUT2* (Nishida *et al.* 2016). On the other hand, overexpression of *FMP12* negatively affected *S. cerevisiae* growth in media containing proline as the sole source of both nitrogen and carbon (Nishida *et al.* 2016). If the α-ketoglutarate-dependent dioxygenases in *C. albicans*, such as Bbh1 (the ortholog of Fmp12), have a lower affinity for α-ketoglutarate compared to KGDH, this may explain the differences observed in the use of proline as a carbon and nitrogen source in the two species.

We further confirmed the role of Put3 in *C. albicans* using transcriptional profiling and ChIP-chip experiments. Consistent with our observations from phenotypic studies and RNA-seq data, both *PUT1* and *PUT2* in *C. albicans* are under the regulation of Put3, as shown by our

transcriptional profiling data, and our ChIP-chip data suggest that Put3 binds the *PUT1* promoter (Table 5.2), while *PUT2* does not appear to be bound by Put3 in *C. albicans*. *MCH5* is also a direct target of Put3 in *S. cerevisiae* and encodes a riboflavin transporter (Spitzner *et al.* 2008). Riboflavin is required for the generation of flavin adenine dinucleotide (FAD), the catalytic cofactor required for Put1 activity (Spitzner *et al.* 2008). We discovered in *C. albicans* that one of the four genes with similarity to *MCH5*, orf19.1584, has the *S. cerevisiae* Put3 binding site CGG(N_{10})CCG (Harbison *et al.* 2004) at its promoter, demonstrated direct binding by Put3, and is transcriptionally activated by Put3 (Table 5.2), suggesting that orf19.1584 is the functional ortholog of *MCH5*. orf19.1584, like its *S. cerevisiae* ortholog, could therefore play a role in the proline degradation pathway by importing riboflavin in *C. albicans* under the regulation of Put3. As shown in our RNA-seq data (Table 5.1), orf19.1584 (C2_02580W_A) was also upregulated by proline when ammonium sulfate, glucose, or both were missing in the culture media. Some *S. cerevisiae* Put3 targets (including Put1 and Put2) that have the Put3 binding site CGG(N_{10})CCG (18) at their promoters have clear orthologs in *C. albicans*. We, however, did not identify a predicted *S. cerevisiae* Put3 motif at the promoter of these *C. albicans* orthologs, consistent with previous bioinformatics analysis (Whiteway *et al.* 2015), although some of these orthologs are upregulated by activated Put3 in *C. albicans* in our expression data. X-ray crystallography studies of *S. cerevisiae* Put3 DNA binding as well as substitution mutation studies of its binding site reveal that unlike other zinc cluster transcription factors, such as Gal4 and Ppr1, that predominantly bind the CGG DNA half-sites, Put3 binds to the spacer DNA sequence between the half-sites as well (Swaminathan *et al.* 1997). Extensive interaction of Put3 with DNA during such binding could render the CGG half-sites dispensable for this transcription factor, facilitating alternative interaction sites in *C. albicans* gene targets beyond its characterized zinc cluster DNA binding motif.

Our findings suggest that *PUT1* and *PUT2* may be under direct and indirect regulation of Put3, respectively, in *C. albicans*, unlike in *S. cerevisiae*, where Put3 regulates both genes through direct DNA interactions (Huang and Brandriss 2000). As revealed by our RNA-seq, transcription profiling, and ChIP-chip data, Put3 activation may also upregulate ribosome biogenesis genes and carboxylic acid metabolic process genes, as well as genes that are implicated in filamentous growth, the response to stress, RNA metabolic processes, and cellular protein modification (see Table S5.1 in the supplemental material). Put3 appears to exert a regulatory role in ribosome

biogenesis, as over 18% of the transcriptionally upregulated genes observed in our microarray transcription profiling data are annotated with ribosome biogenesis function, compared to just over 4% of *C. albicans* genes that are implicated in ribosome biogenesis. We also noted a very strong signal in our *C. albicans* RNA-seq data, with many ribosome biogenesis genes showing moderate upregulation by proline in the absence of glucose, ammonium sulfate, or both (Table 5.1). Ribosome biogenesis genes were not upregulated by proline when both glucose and ammonium sulfate were present in the media (Table 5.1). Such a role has not been previously reported for *S. cerevisiae* Put3. It will be necessary to carry out phenotypic studies to further clarify the role of *C. albicans* Put3 in these pathways and to investigate whether *S. cerevisiae* Put3 plays a similar role. Such investigations may further strengthen a proposed model of functional conservation for Put3 function between the two species and/or could illuminate cases of rewiring in Put3 function.

Overall our findings show that *C. albicans* can utilize proline as a carbon source, a nitrogen source, and both a carbon and nitrogen source. We also demonstrated that *C. albicans* Put3 regulates proline catabolism, even in the presence of ammonium sulfate, to provide the cells with carbon and nitrogen. These functions are in contrast to its role in *S. cerevisiae*, which can only use proline as a nitrogen source. Moreover, Put3 does not possess the ability to effectively activate the proline catabolic pathway in the presence of a rich nitrogen source such as ammonium sulfate in *S. cerevisiae*. Notably, Put3 proline catabolism molecular function is generally conserved between *C. albicans* and *S. cerevisiae*, but their specific catabolic pathways have diverged.

5.5 Acknowledgments

We acknowledge funding support from the Tier I Canada Research Chair program and from CIHR grant MOP42516 to M.W., a Concordia University Faculty of Arts and Science Graduate Fellowship and Concordia Accelerator Award to W.A.T., and DFG grant MO846/7 to J.M. We thank all members of the laboratory of M.W. for valuable input during this research. Walters Aji Tebung performed experiments and bioinformatic analyses and wrote the paper. Raha Parvizi Omran contributed to phenotypic experiments. Joachim Morschhäuser provided activated transcription factor reagents and edited the paper. Debra Fulton provided bioinformatic support for the RNA-seq data postprocessing. Malcolm Whiteway designed experiments and wrote the paper.

5.6 Supplementary material

Table S5.1: Oligonucleotides used for DNA amplification in this study.

Name	Sequence (5' - 3')
PUT3_Marker_KO_F	CATTCCGAACAAGGTATTAGAAATAATTATTTTCTATCAACC AACACCCACATAAATCATTCCTTCATTTACTTATATATAATC CGATTCTTGTACAGAAGCTTCGTACGCTGCAGGTC
PUT3_Marker_KO_R	GTTTTTGTAATATATTGTATTATATAGAAAATTTTATTACCAT CACAGAATAAATGTACAGACATAAATATATATTTGCCTCAC TCCCGCACAATTCTGATATCATCGATGAATTCGAG
PUT3_KO_Check_F	CGTTGTGCGAAACTACCAAA
PUT3_KO_Check_R	ACAGGCCAATGAGAATACGC
PUT3_KO_Check_Internal_F	AAGAGCCTTCGGAGGAAAAG
PUT3_KO_Check_Internal_R	TCGGGACTTTTTGTTTGGAG
FT-U3	TATAGGTCTTAGTGTTGACTGT
FT-H2	CAACGAAATGGCCTCCCCTACCACAG
ScPUT3-F	ACATCCACCAGGTGCTTGATGA
*Sc*PUT3-R	TGGACAGAAGGTGATAGCGACA
ScPUT3-In-F	GGCAACATTCCAGCAGGCTTAA
ScPUT3-In-R	TCAAATATAGGAGCCGCCGGAG
kanC-F	TGATTTTGATGACGAGCGTAAT
kanB-R	CTGCAGCGAGGAGCCGTAAT

Chapter 6: Conclusions and future work

This study aims to understand the function of the zinc (II)2 Cys6 transcription factors in the pathogenic fungus *C. albicans*. This organism encodes 82 ZCF proteins as compared to 55 in the nonpathogenic yeast *S. cerevisiae*. *Ca*ZCFs proteins may ensure survival and success of *C. albicans* under diverse conditions because they regulate crucial cellular processes characteristic of the organism. Overall, this transcription factor class is one of the most abundant protein classes in both fungi. The purpose of this study was to provide a framework to monitor the expression profiles of the comprehensive set of activated zinc cluster proteins created by adding a constitutive activation domain from the Gal4 zinc cluster TF of *S. cerevisiae* to each of the 82 *C. albicans* ZCFs. We deciphered the functional significance of orthologous and non-orthologous transcription factors by revealing that ZCFs can be studied individually or in combination with other TFs.

Based on their orthologies with the model fungus *S. cerevisiae*, the 82 ZCFs in *C. albicans* can be divided into two classes: ZCFs with strong orthologs in *S. cerevisiae* and those without. This provides two basic frameworks for the investigation of the proteins; open-ended for assessment of proteins with no *S. cerevisiae* orthology, and directed for proteins with a clear ortholog. While the concept of orthologous proteins having similar functions is a defining theme in biology, transcription factors are somewhat an exception to this general rule. In particular, the process of rewiring has been seen to link orthologous TFs to distinct cellular processes. One example is the rewiring of the galactose metabolic genes in fungi that have been switched from helix-loop helix proteins Rgt1-Rtg3 to a zinc finger protein Gal4. Rgt1 and Rgt3 control *GAL* gene expression in *C. albicans* and other ancestral fungi, whereas Gal4 plays this role in *S. cerevisiae* (Dalal *et al.* 2016). Several other cases of rewiring have been reported between these species (Tebung *et al.* 2016; Dalal and Johnson 2017). Thus, even factors with a strong ortholog in *S. cerevisiae* need to be assessed carefully to establish the extent of potential divergence in cellular function between the pathogenic fungus and the benign, industrially important yeast.

In Chapter 2, I examined the expression profiles generated from a large set of activated zinc cluster proteins selected based on their relative lack of characterization and their potential to be associated with unexplored aspects of cellular function in the pathogen. In addition to providing insights into the underlying biology of these 35 ZCFs, our work identified genes, pathways, and processes that are specific to each of the ZCF members, providing insights into their specific

functions. Overall, establishing the transcriptional profiles of individual ZCFs represent a powerful approach in deciphering the function of uncharacterized TFs. We presented hypothetical function for numbers of ZCF including Asg1, Aro80, Cta7, Zcf20, Tea1, Zcf23, Orf19.2230, Fgr17, Fgr27, Lys142, Zcf10, Zcf13, Zcf24, Zcf15, Zcf22, Zcf26, Zcf27, Zcf35, Zcf38, Zcf5, Zcf6, Zcf8, Zcf9, Hal9. We also showed whether expression of activated ZCFs for characterized *S. cerevisiae* orthologs agreed with their established functions. It is, however, difficult to ascribe hypothetical functions to differentially expressed genes solely based on gene ontology (GO) term analysis. This may arise from the fact that GO terms are built based on the non-pathogenic *S. cerevisiae*, which by far contains a greater number of characterized genes relative to *C. albicans*. As well, we frequently observed the expression of other ZCFs, and in fact other TFs of other classes, in each of the profiles, suggesting an interaction among transcription factors. Thus investigating the phenotypic consequences of multiple genetic interventions (activations and inactivations) may be needed to elucidate the function of many ZCFs. In general, this expression profile information will prove valuable to those interested in studying ZCF individually or as part of a network to test hypotheses quickly.

Non-orthologous transcription factors with similarity only in the DNA-binding domains are expected to play different roles among organisms due to the lack of structural similarity in the rest of the proteins. My study has challenged this view as the transcriptional profiles of several non-orthologous ZCFs; Ctf1, Zcf16, Zcf20, Zcf22 seemed to processes similar processes to ZCFs in *S. cerevisiae* whose sequence similarity was limited to the DNA binding region. We found that Ctf1's transcriptional profile in *C. albicans* was consistent with ScAsg1 regulated genes in *S. cerevisiae*; both proteins controlled the expression of genes that work in the process of beta-oxidation of fatty acids. Intriguingly, the true ortholog of *Sc*Asg1, *Ca*Asg1, showed both activator and repressor functions for multidrug resistance, and thus Asg1 represents an example of rewiring of the orthologous genes. This observation opens many questions for future research. For example, how do two closely related organisms accomplish the same function with non-orthologous TFs whose similarities are limited to their DNA-binding domains? Do these TFs possess the same DNA-binding motif in both organisms, and do they recognize the same DNA motifs in the promoters of their target genes? In their DNA-binding domains, is there a specific region which is crucial for their performance like in the case of ZCFs, which contains zinc finger, linker region,

dimerization domain, or is the entire domain equally important for the TF's performance? This could be due to the interaction between DNA-binding domains (DBDs) of transcription factors and their target sequences which are highly influenced by their structural features. Different DBDs appear to have developed their own DNA–protein recognition codes which affect their structure and could be critical for their functions, which could potentially be used to predict the function of an uncharacterized TFs. Hence, by examining the structural, functional, and evolutionary diversity of ZCFs we might be able to identify new drug targets by inhibiting their binding to target genes and thus combat the pathogenicity of *C. albicans*.

In Chapter 3, by combining the transcriptional profile of an uncharacterized ZCF, Orf19.1604, with phenotypic screens of the activated ZCF collection, I was able to determine the unknown function of this ZCF. This gene has been named *RHA1*, which stands for Regulator of Hyphal Activation. The loss of *RHA1* alters hyphal development in response to a variety of environmental conditions, including serum, Spider media, and growth at 37 °C. Rha1 exerts its effects through changes in cellular and colony morphology that are influenced by Ume6 and Brg1 as double mutants are unable to activate hyphal growth. Accordingly, Rha1, a newly characterized morphogenesis regulator, mediates filamentation by interacting with Brg1 and Ume6 in response to an external signal. Merging the Rha1 transcriptional profile and ChIP-chip data suggested common genes that have direct interaction with Rha1. We provided a scheme to decode an unknown TF by combining innovative genetic screening methods along with global expression profile. We need to investigate how filamentation signaling pathways can be activated in absence of chromatin remodeler regulator Brg1 and known filament extension regulator Ume6 by basically activating Rha1 and adding strong inducer signal like serum, and then decipher the mechanism behind it.

In Chapter 4, we explored the function of an uncharacterized ZCF which displayed positive expression in the Rha1 study. Despite the *ZCF4* deletion not producing significant phenotypic changes induced by starvation-like stimuli, activation of Zcf4 caused a reduction in hyphal development triggered by serum treatment. Using RNA sequencing, we determined the transcription profile of activated Zcf4 and identified genes involved in arginine metabolism. In the current study, we identified Zcf4 as a possible new member of the filamentation regulatory circuit and established that in an activated state, it plays distinct roles in the formation of hyphae.

According to our phenotypic studies and transcription profiling, Zcf4 is a new regulator in filamentation, with effects that differ from previously recognized regulators. This is interesting as we think the filamentation signal transduction pathway as interconnected scheme which the downstream process would lead to the same path to activate filamentation. This showed how *C. albicans* TFs can be selective in turning a pathway on or off based on the environmental conditions. By determining the set of regulated genes for each condition, we could learn how each condition favors *C. albicans* pathogenic traits.

In Chapter 5, we investigated if the sequence similarity of transcription factors can provide insight into the TF function. Rewiring can occur, shifting the regulatory function of a TF between species as was observed for Ppr1, which rewired from purine catabolism in *C. albicans* to pyrimidine biosynthesis in *S. cerevisiae* (Tebung *et al.* 2016). Differences in ribosomal protein gene expression observed between *C. albicans* and *S. cerevisiae* highlight how metabolic pathways could be flexible through evolution. Tbf1 is the primary regulator of ribosomal protein gene expression in *C. albicans*, a process that is centrally regulated by Rap1 in *S. cerevisiae* (Hogues *et al.* 2008). However, structural orthology is typically useful in establishing protein function, so we also investigated *C. albicans* TFs with substantial sequence similarity to functionally characterized TFs from the model yeast *S. cerevisiae*. We discovered that both species display transcriptional regulation of proline catabolism by Put3. Despite the presence of ammonium sulfate, *C. albicans* utilize proline as a carbon and nitrogen source, which contrasts with *S. cerevisiae*, which does not use proline as a sole carbon source, nor as a sole carbon and nitrogen source but only as a sole nitrogen source. Additionally, the *S. cerevisiae* Put3 ortholog only degrades proline if there is no rich nitrogen source available, contrary to the *C. albicans* Put3 ortholog. In both species, the transcriptional arrangement is similar, suggesting that the differences may be due to post-transcriptional regulation.

Our studies have several implications such as to enhance our understanding of the functional diversities in transcriptional factors and understand how the different TFs might regulate each other. The combined use of high throughput expression profile of ZCFs with ChIP-chip, bioinformatics, and phenotypic data can facilitate study of *Ca*ZCFs and cast light on the multi-level transcriptional regulatory networks in eukaryotes. Then, they can compare the transcriptional regulatory circuits between organisms like *C. albicans* and *S. cerevisiae* to discover

rewiring cases to understand the evolutionary development of regulatory mechanisms. Using the ZCFs expression data, researchers could identify pathogen-specific transcriptional regulatory circuits involved in *Candida*-specific processes such as virulence, the white–opaque transition, and hyphal growth. Future work will involve confirming the suggested function in-depth molecular and genetic analysis of the ZCFs that will provide new insight into developing novel classes of antifungal agents that could resolve the problems associated with the currently available drugs.

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Appendix 1: RNA-seq result of Ctf1-GOF. Top differentially expressed Up-regulated genes in *Candida albicans* Ctf1-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
PGA28	17.1	0.00	Protein similar to S. cerevisiae Wsc2p, which has roles in stress- and cell-wall-related processes;
orf19.7596	7.8	0.00	Protein with a phosphoglycerate mutase family domain
CTF1	6.4	0.00	Putative zinc-finger transcription factor, similar to A. nidulans FarA and FarB
ICL1	6.3	0.00	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice
orf19.3684	4.9	0.00	Putative oxidoreductase
orf19.3627	4.8	0.00	Ortholog of C. dubliniensis CD36 : Cd36_22640
TES1	4.5	0.00	Putative acyl-CoA thioesterase
orf19.4142	4.4	0.00	Putative transporter; decreased transcription is observed upon fluphenazine treatment
ANT1	4.4	0.00	Peroxisomal adenine nucleotide transporter; role in beta-oxidation of medium-chain fatty acid and peroxisome proliferation; rat catheter biofilm induced
orf19.2962	4.3	0.00	Protein of unknown function; Spider biofilm induced
orf19.7567	4.3	0.00	Protein of unknown function; induced by alpha pheromone in SpiderM medium
MEP2	4.0	0.01	Ammonium permease and regulator of nitrogen starvation-induced filamentation
orf19.3461	4.0	0.00	Protein of unknown function; oxidative stress-induced via Cap1; induced by alpha pheromone in SpiderM medium
LYP1	3.9	0.00	Putative permease; amphotericin B induced; flucytosine repressed; possibly an essential gene, disruptants not obtained by UAU1 method
CRC1	3.6	0.00	Mitochondrial carnitine carrier protein
POT1	3.6	0.00	Putative peroxisomal 3-oxoacyl CoA thiolase; transcript regulated by Nrg1 and Mig1; farnesol regulated; Hap43-repressed
orf19.6830	3.5	0.00	Putative enoyl-CoA hydratase; Spider biofilm induced
orf19.4287	3.4	0.00	Putative oxidoreductase; Hap43-repressed gene; clade-associated gene expression
MLS1	3.4	0.00	Malate synthase; glyoxylate cycle enzyme; no mammalian homolog
BIO5	3.4	0.00	Putative transporter; Hap43, flucytosine repressed
orf19.692	3.4	0.00	Protein of unknown function; Hap43-repressed gene; rat catheter and Spider biofilm induced
orf19.5205	3.2	0.01	Protein of unknown function; Hap43-repressed gene
CAT2	3.2	0.00	Major carnitine acetyl transferase
FOX3	3.1	0.00	Putative peroxisomal 3-oxoacyl CoA thiolase; transcript regulated by white-opaque switch; Spider biofilm induced
EHD3	3.1	0.00	Predicted 3-hydroxyisobutyryl-CoA hydrolase; mitochondrially localized; Spider biofilm induced
RCH1	3.1	0.00	Plasma membrane protein; involved in regulation of cytosolic calcium homeostasis
POX1-3	3.1	0.00	Predicted acyl-CoA oxidase; farnesol regulated; stationary phase enriched protein
SET6	3.1	0.00	Ortholog of S. cerevisiae Set6, a SET domain protein of unknown function
CSO99	3.0	0.00	Protein of unknown function; Hap43-repressed gene; protein not conserved in S. cerevisiae
FOX2	3.0	0.00	3-hydroxyacyl-CoA epimerase; fatty acid beta-oxidation
PEX4	3.0	0.00	Putative peroxisomal ubiquitin conjugating enzyme; regulated by Sef1, Sfu1, and Hap43; rat catheter biofilm induced; Spider biofilm induced
orf19.3610	2.9	0.01	Protein of unknown function; upregulation correlates with clinical development of fluconazole resistance; regulated by Sef1, Sfu1, and Hap43
orf19.2789	2.9	0.00	Ortholog of C. dubliniensis CD36 : Cd36_07040, C. parapsilosis CDC317 : CPAR2_208700, C. auris B8441 : B9J08_003149 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_108836
orf19.6020	2.8	0.00	Ortholog(s) have Atg8 ligase activity
orf19.3395	2.8	0.00	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family
orf19.1608	2.7	0.05	Has domain(s) with predicted catalytic activity, sulfuric ester hydrolase activity and role in metabolic process
orf19.1592	2.6	0.00	Protein of unknown function; Spider biofilm induced
FAA21	2.6	0.00	Predicted acyl CoA synthetase; upregulated upon phagocytosis; transcript regulated by Nrg1 and Mig1
EBP1	2.6	0.00	NADPH oxidoreductase; interacts with phenolic substrates (17beta-estradiol); possible role in estrogen response; induced by oxidative, weak acid stress, NO, benomyl, GlcNAc; Cap1, Mnl1 induced; Hap43-repressed; rat catheter biofilm induced
GAP2	2.6	0.00	General broad specificity amino acid permease; ketoconazole, flucytosine repressed; Ssy1-dependent histidine induction; regulated by Nrg1, Tup1; colony morphology-related gene regulation by Ssn6; Spider and flow model biofilm induced

orf19.5576	2.5	0.00	Putative pantothenate kinase; ortholog of S. cerevisiae Cab1; transposon mutation affects filamentous growth; repressed in core stress response
FAA2-3	2.5	0.00	Predicted acyl CoA synthetase
PDC2	2.5	0.00	Homeodomain-like transcription factor; regulator of pyruvate decarboxylase; contains a putative C-terminal activation domain, Glu- and Pro-rich; complements glucose utilization defect of *S. cerevisiae* pdc2 mutant
SFL2	2.4	0.00	Transcription factor involved in regulation of morphogenesis
SFC1	2.4	0.03	Putative succinate-fumarate transporter; involved in repression of growth on sorbose
orf19.7595	2.3	0.00	Has domain(s) with predicted dynactin complex localization
orf19.5578	2.3	0.00	Ortholog of C. dubliniensis CD36 : Cd36_63050 and *Candida albicans* WO-1 : CAWG_05094
orf19.7648	2.3	0.00	Has domain(s) with predicted antiporter activity, xenobiotic transmembrane transporter activity, role in drug transmembrane transport and membrane localization
orf19.4807	2.2	0.00	Ortholog(s) have inorganic diphosphatase activity, role in aerobic respiration and mitochondrion localization

Appendix 2: RNA-seq result of Asg1-GOF. Top differentially expressed Up-regulated genes in *Candida albicans* Asg1-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.2247	17.1	0.00	Protein similar to S. cerevisiae Wsc2p, which has roles in stress- and cell-wall-related processes;
orf19.5611	7.8	0.00	Protein with a phosphoglycerate mutase family domain
orf19.2310	6.4	0.00	Putative zinc-finger transcription factor, similar to A. nidulans FarA and FarB
orf19.1774	6.3	0.00	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice
HSP31	4.9	0.00	Putative oxidoreductase
HPD1	4.8	0.00	Ortholog of C. dubliniensis CD36 : Cd36_22640
orf19.5785	4.5	0.00	Putative acyl-CoA thioesterase
orf19.7279.1	4.4	0.00	Putative transporter; decreased transcription is observed upon fluphenazine treatment
orf19.6586	4.4	0.00	Peroxisomal adenine nucleotide transporter; role in beta-oxidation of medium-chain fatty acid and peroxisome proliferation; rat catheter biofilm induced
orf19.3088	4.3	0.00	Protein of unknown function; Spider biofilm induced
orf19.4921.1	4.3	0.00	Protein of unknown function; induced by alpha pheromone in SpiderM medium
orf19.6311	4.0	0.01	Ammonium permease and regulator of nitrogen starvation-induced filamentation
CFL4	4.0	0.00	Protein of unknown function; oxidative stress-induced via Cap1
orf19.5020	3.9	0.00	Putative permease; amphotericin B induced; flucytosine repressed
orf19.1117	3.6	0.00	Mitochondrial carnitine carrier protein
orf19.6899	3.6	0.00	Putative peroxisomal 3-oxoacyl CoA thiolase
orf19.344	3.5	0.00	Putative enoyl-CoA hydratase; Spider biofilm induced
orf19.4634	3.4	0.00	Putative oxidoreductase; Hap43-repressed gene; clade-associated gene expression
REI1	3.4	0.00	Malate synthase; glyoxylate cycle enzyme; no mammalian homolog
ASG1	3.4	0.00	Putative transporter; Hap43, flucytosine repressed
NOG2	3.4	0.00	Protein of unknown function; Hap43-repressed gene; rat catheter and Spider biofilm induced
orf19.4273	3.2	0.01	Protein of unknown function; Hap43-repressed gene
FDH1	3.2	0.00	Major carnitine acetyl transferase; intracellular acetyl-CoA transport
snR76	3.1	0.00	Putative peroxisomal 3-oxoacyl CoA thiolase; transcript regulated by white-opaque switch; Spider biofilm induced
AOX2	3.1	0.00	Predicted 3-hydroxyisobutyryl-CoA hydrolase; mitochondrially localized; Spider biofilm induced

DIM1	3.1	0.00	Plasma membrane protein; involved in regulation of cytosolic calcium homeostasis
orf19.5216	3.1	0.00	Predicted acyl-CoA oxidase; farnesol regulated; stationary phase enriched protein; Spider biofilm induced
ENP2	3.1	0.00	Ortholog of S. cerevisiae Set6, a SET domain protein of unknown function
AOX1	3.0	0.00	Protein of unknown function; Hap43-repressed gene; protein not conserved in S. cerevisiae
orf19.7088	3.0	0.00	3-hydroxyacyl-CoA epimerase; fatty acid beta-oxidation
ALD6	3.0	0.00	Putative peroxisomal ubiquitin conjugating enzyme
orf19.7042	2.9	0.01	Protein of unknown function
NOP14	2.9	0.00	Ortholog of C. dubliniensis CD36 : Cd36_07040, C. parapsilosis
RSM22	2.8	0.00	Ortholog(s) have Atg8 ligase activity
MAK16	2.8	0.00	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family
orf19.7091	2.7	0.05	Has domain(s) with predicted catalytic activity, sulfuric ester hydrolase activity and role in metabolic process
RTA3	2.6	0.00	Protein of unknown function; Spider biofilm induced
orf19.5207	2.6	0.00	Predicted acyl CoA synthetase; upregulated upon phagocytosis; transcript regulated by Nrg1 and Mig1
SET6	2.6	0.00	NADPH oxidoreductase; interacts with phenolic substrates (17beta-estradiol
RRN3	2.6	0.00	General broad specificity amino acid permease
STP4	2.5	0.00	Putative pantothenate kinase; ortholog of S. cerevisiae Cab1
orf19.4895	2.5	0.00	Predicted acyl CoA synthetase
ELP3	2.5	0.00	Homeodomain-like transcription factor
SMM1	2.4	0.00	Transcription factor involved in regulation of morphogenesis
orf19.5905	2.4	0.03	Putative succinate-fumarate transporter
orf19.2090	2.3	0.00	Has domain(s) with predicted dynactin complex localization
orf19.3470	2.3	0.00	Ortholog of C. dubliniensis CD36 : Cd36_63050 and *Candida albicans* WO-1 : CAWG_05094
RCH1	2.3	0.00	Has domain(s) with predicted antiporter activity

orf19.3406	2.2	0.00	Ortholog(s) have inorganic diphosphatase activity
POP3	17.1	0.00	Protein similar to S. cerevisiae Wsc2p, which has roles in stress- and cell-wall-related processes;
orf19.6675	7.8	0.00	Protein with a phosphoglycerate mutase family domain
orf19.2330	6.4	0.00	Putative zinc-finger transcription factor, similar to A. nidulans FarA and FarB
TRM2	6.3	0.00	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice
ENP1	4.9	0.00	Putative oxidoreductase
orf19.2320	4.8	0.00	Ortholog of C. dubliniensis CD36 : Cd36_22640
orf19.4160	4.5	0.00	Putative acyl-CoA thioesterase
orf19.6175	4.4	0.00	Putative transporter; decreased transcription is observed upon fluphenazine treatment
NOP8	4.4	0.00	Peroxisomal adenine nucleotide transporter
CTA8	4.3	0.00	Protein of unknown function; Spider biofilm induced
HBR3	4.3	0.00	Protein of unknown function; induced by alpha pheromone in SpiderM medium
orf19.341	4.0	0.01	Ammonium permease and regulator of nitrogen starvation-induced filamentation
orf19.2726	4.0	0.00	Protein of unknown function; oxidative stress-induced via Cap1; induced by alpha pheromone in SpiderM medium
RRP15	3.9	0.00	Putative permease; amphotericin B induced
SAS10	3.6	0.00	Mitochondrial carnitine carrier protein
BUD22	3.6	0.00	Putative peroxisomal 3-oxoacyl CoA thiolase; transcript regulated by Nrg1 and Mig1
SUT1	3.5	0.00	Putative enoyl-CoA hydratase; Spider biofilm induced
orf19.7601	3.4	0.00	Putative oxidoreductase; Hap43-repressed gene; clade-associated gene expression
orf19.3627	3.4	0.00	Malate synthase; glyoxylate cycle enzyme; no mammalian homolog
UTP18	3.4	0.00	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method
FCR1	3.4	0.00	Protein of unknown function; Hap43-repressed gene; rat catheter and Spider biofilm induced
orf19.494	3.2	0.01	Protein of unknown function; Hap43-repressed gene
orf19.7546	3.2	0.00	Major carnitine acetyl transferase; intracellular acetyl-CoA transport
orf19.1708	3.1	0.00	Putative peroxisomal 3-oxoacyl CoA thiolase; transcript regulated by white-opaque switch
UTP21	3.1	0.00	Predicted 3-hydroxyisobutyryl-CoA hydrolase; mitochondrially localized; Spider biofilm induced
CFL2	3.1	0.00	Plasma membrane protein; involved in regulation of cytosolic calcium homeostasis
orf6.5536	3.1	0.00	Predicted acyl-CoA oxidase; farnesol regulated; stationary phase enriched protein; Spider biofilm induced
MTG1	3.1	0.00	Ortholog of S. cerevisiae Set6, a SET domain protein of unknown function
orf19.5038	3.0	0.00	Protein of unknown function; Hap43-repressed gene; protein not conserved in S. cerevisiae
FYV5	3.0	0.00	3-hydroxyacyl-CoA epimerase; fatty acid beta-oxidation
CNS1	3.0	0.00	Putative peroxisomal ubiquitin conjugating enzyme
TAZ1	2.9	0.01	Protein of unknown function; upregulation correlates with clinical development of fluconazole resistance
orf19.107	2.9	0.00	Ortholog of C. dubliniensis CD36 : Cd36_07040, C. parapsilosis CDC317 : CPAR2_208700, C. auris B8441
orf19.2386	2.8	0.00	Ortholog(s) have Atg8 ligase activity
orf19.3831	2.8	0.00	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family
orf19.2167	2.7	0.05	Has domain(s) with predicted catalytic activity, sulfuric ester hydrolase activity and role in metabolic process
orf19.2934	2.6	0.00	Protein of unknown function; Spider biofilm induced
orf19.2018	2.6	0.00	Predicted acyl CoA synthetase; upregulated upon phagocytosis; transcript regulated by Nrg1 and Mig1
orf19.6662	2.6	0.00	NADPH oxidoreductase; interacts with phenolic substrates (17beta-estradiol); possible role in estrogen response
orf19.3448	2.6	0.00	General broad specificity amino acid permease
orf19.6234	2.5	0.00	Putative pantothenate kinase
KRR1	2.5	0.00	Predicted acyl CoA synthetase
DIP2	2.5	0.00	Homeodomain-like transcription factor; regulator of pyruvate decarboxylase
NSA1	2.4	0.00	Transcription factor involved in regulation of morphogenesis
TES1	2.4	0.03	Putative succinate-fumarate transporter
NAN1	2.3	0.00	Has domain(s) with predicted dynactin complex localization
orf19.3626	2.3	0.00	Ortholog of C. dubliniensis CD36 : Cd36_63050 and *Candida albicans* WO-1 : CAWG_05094
orf19.3483	2.3	0.00	Has domain(s) with predicted antiporter activity
IMP4	2.2	0.00	Ortholog(s) have inorganic diphosphatase activity, role in aerobic respiration and mitochondrion localization

HNM1	17.1	0.00	Protein similar to S. cerevisiae Wsc2p, which has roles in stress- and cell-wall-related processes;
RPL7	7.8	0.00	Protein with a phosphoglycerate mutase family domain
orf19.7011	6.4	0.00	Putative zinc-finger transcription factor, similar to A. nidulans FarA and FarB
TEM1	6.3	0.00	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice
orf19.7204	4.9	0.00	Putative oxidoreductase
orf19.3704	4.8	0.00	Ortholog of C. dubliniensis CD36 : Cd36_22640
orf19.6907	4.5	0.00	Putative acyl-CoA thioesterase
UTP5	4.4	0.00	Putative transporter; decreased transcription is observed upon fluphenazine treatment
orf19.1697	4.4	0.00	Peroxisomal adenine nucleotide transporter; role in beta-oxidation of medium-chain fatty acid and peroxisome proliferation; rat catheter biofilm induced
CDR1	4.3	0.00	Protein of unknown function; Spider biofilm induced
orf19.1791	4.3	0.00	Protein of unknown function; induced by alpha pheromone in SpiderM medium
orf19.2214	4.0	0.01	Ammonium permease and regulator of nitrogen starvation-induced filamentation
MRR1	4.0	0.00	Protein of unknown function; oxidative stress-induced via Cap1; induced by alpha pheromone in SpiderM medium
orf19.1887	3.9	0.00	Putative permease; amphotericin B induced
UTP13	3.6	0.00	Mitochondrial carnitine carrier protein
orf19.3556	3.6	0.00	Putative peroxisomal 3-oxoacyl CoA thiolase; transcript regulated by Nrg1 and Mig1; farnesol regulated; Hap43-repressed
PAM18	3.5	0.00	Putative enoyl-CoA hydratase; Spider biofilm induced
orf19.6156	3.4	0.00	Putative oxidoreductase; Hap43-repressed gene; clade-associated gene expression
orf19.3170	3.4	0.00	Malate synthase; glyoxylate cycle enzyme; no mammalian homolog
RRS1	3.4	0.00	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method
orf19.6418	3.4	0.00	Protein of unknown function; Hap43-repressed gene; rat catheter and Spider biofilm induced
IPT1	3.2	0.01	Protein of unknown function; Hap43-repressed gene
HEM1	3.2	0.00	Major carnitine acetyl transferase; intracellular acetyl-CoA transport; localized in peroxisomes and mitochondria
orf19.1687	3.1	0.00	Putative peroxisomal 3-oxoacyl CoA thiolase; transcript regulated by white-opaque switch; Spider biofilm induced
MAK5	3.1	0.00	Predicted 3-hydroxyisobutyryl-CoA hydrolase; mitochondrially localized; Spider biofilm induced
orf19.3357	3.1	0.00	Plasma membrane protein; involved in regulation of cytosolic calcium homeostasis
SDA1	3.1	0.00	Predicted acyl-CoA oxidase; farnesol regulated; stationary phase enriched protein; Spider biofilm induced
orf19.2847	3.1	0.00	Ortholog of S. cerevisiae Set6, a SET domain protein of unknown function
orf19.3755	3.0	0.00	Protein of unknown function; Hap43-repressed gene; protein not conserved in S. cerevisiae
orf19.4176	3.0	0.00	3-hydroxyacyl-CoA epimerase; fatty acid beta-oxidation
SMF12	3.0	0.00	Putative peroxisomal ubiquitin conjugating enzyme; regulated by Sef1, Sfu1, and Hap43
RPA190	2.9	0.01	Protein of unknown function; upregulation correlates with clinical development of fluconazole resistance; regulated by Sef1, Sfu1, and Hap43
JIP5	2.9	0.00	Ortholog of C. dubliniensis CD36 : Cd36_07040, C. parapsilosis CDC317 : CPAR2_208700, C. auris B8441 : B9J08_003149 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_108836
orf19.6818	2.8	0.00	Ortholog(s) have Atg8 ligase activity
GPR1	2.8	0.00	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family
NOP4	2.7	0.05	Has domain(s) with predicted catalytic activity, sulfuric ester hydrolase activity and role in metabolic process
DRS1	2.6	0.00	Protein of unknown function; Spider biofilm induced
orf19.5391	2.6	0.00	Predicted acyl CoA synthetase; upregulated upon phagocytosis; transcript regulated by Nrg1 and Mig1
orf19.1609	2.6	0.00	NADPH oxidoreductase; interacts with phenolic substrates (17beta-estradiol)
orf19.5433	2.6	0.00	General broad specificity amino acid permease
orf19.6227	2.5	0.00	Putative pantothenate kinase
CLG1	2.5	0.00	Predicted acyl CoA synthetase
NRP1	2.5	0.00	Homeodomain-like transcription factor; regulator of pyruvate decarboxylase
orf19.962	2.4	0.00	Transcription factor involved in regulation of morphogenesis

Appendix 3: RNA-seq result of Aro80-GOF. Top differentially expressed Up-regulated genes in *Candida albicans* Aro80-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
EFH1	17.4	0.0	APSES transcription factor; homodimer
orf19.5648	13.4	0.0	Ortholog of C. dubliniensis CD36 : Cd36_40300, C. parapsilosis CDC317 : CPAR2_402190, C. auris B8441 : B9J08_003511 and Candida tenuis NRRL Y-1498 : cten_CGOB_00197
orf19.4612	8.5	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
orf19.1473	7.7	0.0	2-hydroxyacid dehydrogenase domain-containing protein; Hap43-repressed gene; induced by alpha pheromone in SpiderM medium
ARO10	7.6	0.0	Aromatic decarboxylase; Ehrlich fusel oil pathway of aromatic alcohol biosynthesis; alkaline repressed; protein abundance affected by URA3 expression in CAI-4 strain; Spider biofilm induced
ALD6	7.6	0.0	Putative aldehyde dehydrogenase; stationary phase enriched protein
orf19.341	7.3	0.0	Putative spermidine export pump; fungal-specific (no human or murine homolog)
orf19.1438	7.2	0.0	Protein with homology to NADH dehydrogenase; regulated by Sef1p-, Sfu1p-, and Hap43p
ATO6	6.2	0.0	Putative fungal-specific transmembrane protein
RME1	6.2	0.0	Zinc finger protein, controls asexual sporulation
orf19.5952	5.8	0.0	Protein of unknown function; induced by nitric oxide independent of Yhb1
orf19.7495	5.5	0.0	Protein with NADPH oxidoreductase containing flavin mononucleotide (FMN) domain; induced by nitric oxide
orf19.3633	5.5	0.0	Ortholog(s) have role in purine nucleobase catabolic process
orf19.3461	5.0	0.0	Protein of unknown function; oxidative stress-induced via Cap1; induced by alpha pheromone in SpiderM medium
YDC1	4.9	0.0	Alkaline dihydroceramidase; involved in sphingolipid metabolism; Mob2-dependent hyphal regulation; transcript is regulated by Nrg1 and Mig1; Hap43-repressed
orf19.2905	4.7	0.0	Ortholog of *Candida albicans* WO-1 : CAWG_03194
ASR1	4.6	0.0	Heat shock protein; transcript regulated by cAMP, osmotic stress, ciclopirox olamine, ketoconazole; repressed by Cyr1, Ras1; colony morphology-related regulated by Ssn6; stationary phase enriched; Hap43-induced; Spider biofilm induced
orf19.4970	4.5	0.0	Protein of unknown function; Spider biofilm induced
MEP2	4.5	0.0	Ammonium permease and regulator of nitrogen starvation-induced filamentation; 11 predicted transmembrane regions; in low nitrogen cytoplasmic C-terminus activates Ras/cAMP and MAPK signal transduction pathways to induce filamentation
ARG1	4.5	0.0	Argininosuccinate synthase; arginine synthesis; Gcn4, Rim101 regulated; induced by amino acid starvation (3-AT), benomyl treatment; stationary phase enriched protein; repressed in alkalinizing medium; rat catheter, Spider biofilm induced
orf19.2846	4.4	0.0	Protein of unknown function; Hap43-repressed; induced in core caspofungin response; regulated by yeast-hypha switch; Spider biofilm repressed
PUT1	4.4	0.0	Putative proline oxidase; alkaline upregulated by Rim101; flow model biofilm induced; Spider biofilm induced
orf19.5514	4.0	0.0	Ortholog of S. pombe SPCC550.08, an N-acetyltransferase; transcript induced during growth in the mouse cecum
orf19.5785	4.0	0.0	Protein of unknown function; upregulated in a cyr1 or ras1 null mutant; induced by nitric oxide
TES1	3.9	0.0	Putative acyl-CoA thioesterase
MRF1	3.9	0.0	Putative mitochondrial respiratory protein; induced by farnesol, benomyl, nitric oxide, core stress response; oxidative stress-induced via Cap1; stationary-phase enriched protein; Spider biofilm induced
ARO80	3.9	0.0	Zn(II)2Cys6 transcription factor; transcriptional activator of aromatic amino acid catabolism; regulator of aromatic alcohol biosynthesis via the Ehrlich pathway; mutant is viable
LEU42	3.9	0.0	Putative alpha-isopropylmalate synthase; fungal-specific; induced by human blood or polymorphonuclear cells; regulated by Gcn2 and Gcn4; stationary phase enriched protein; Spider biofilm induced
GCS1	3.8	0.0	Gamma-glutamylcysteine synthetase; glutathione synthesis, required for virulence;induced in low iron, H2O2, Cd, or presence of human neutrophils; possibly adherence-induced; Spider and F-12/CO2 biofilm induced
CIP1	3.8	0.0	Possible oxidoreductase; transcript induced by cadmium but not other heavy metals, heat shock, yeast-hypha switch, oxidative stress (via Cap1), or macrophage interaction; stationary phase enriched protein; Spider biofilm induced
SNZ1	3.8	0.0	Stationary phase protein; vitamin B synthesis; induced byyeast-hypha switch, 3-AT or in azole-resistant strain overexpressing MDR1; soluble in hyphae; regulated by Gcn4, macrophage; Spider biofilm induced; rat catheter biofilm repressed
BAT22	3.7	0.0	Putative branched chain amino acid aminotransferase; regulated by Gcn4p; induced by farnesol treatment, GlcNAc, amino acid starvation (3-aminotriazole treatment); present in exponential and stationary growth phase yeast cultures

orf19.4287	3.7	0.0	Putative oxidoreductase; Hap43-repressed gene; clade-associated gene expression
orf19.3544	3.7	0.0	Putative protein of unknown function; Hap43p-repressed gene
orf19.7227	3.7	0.0	Protein phosphatase inhibitor; Hap43-repressed; homozygous Tn insertion decreases colony wrinkling but does not block hyphal growth in liquid media; mutation confers hypersensitivity to toxic ergosterol analog; Spider biofilm induced
orf19.1440.2	3.6	0.0	Ortholog of C. dubliniensis CD36 : Cd36_62310 and Candida tropicalis NEW ASSEMBLY : CTRG1_CGOB_00040
orf19.1307	3.6	0.0	Predicted membrane protein; rat catheter biofilm induced
orf19.2244	3.5	0.0	Similar to oxidoreductases and to S. cerevisiae Yjr096wp; Sfu1 repressed; induced by benomyl treatment, Ssr1; Hap43-repressed; flow model biofilm repressed
LAP3	3.5	0.0	Putative aminopeptidase; positively regulated by Sfu1; clade-associated gene expression; virulence-group-correlated expression; induced by alpha pheromone in SpiderM medium; Hap43-induced; Spider and flow model biofilm induced
CDR11	3.5	0.0	Putative transporter of PDR subfamily of ABC family; Gcn4-regulated; induced by Rim101 at pH 8; Spider biofilm induced
PEX5	3.4	0.0	Pex5p family protein; required for PTS1-mediated peroxisomal protein import, fatty acid beta-oxidation; similar to S. cerevisiae Pas10p peroxisomal targeting receptor; macrophage/pseudohyphal-repressed; Hap43p-repressed
AAT22	3.4	0.0	Aspartate aminotransferase; nitrogen metabolism; similar but not orthologous to S. cerevisiae Aat2; clade-associated gene expression; protein levels decrease in stationary phase yeast; mutant is viable; flow model biofilm repressed
orf19.1395	3.3	0.0	Ortholog(s) have copper ion transmembrane transporter activity, inorganic phosphate transmembrane transporter activity and role in cellular copper ion homeostasis, copper ion transmembrane transport, phosphate ion transmembrane transport
PSA2	3.3	0.0	Mannose-1-phosphate guanyltransferase; Hap43, macrophage-repressed; stationary phase enriched protein; Spider biofilm induced; rat catheter biofilm repressed
orf19.3610	3.3	0.0	Protein of unknown function; upregulation correlates with clinical development of fluconazole resistance; regulated by Sef1, Sfu1, and Hap43
DLD1	3.3	0.0	Putative D-lactate dehydrogenase; white cell-specific trancript; colony morphology-related gene regulation by Ssn6; Hap43-repressed; rat catheter biofilm induced; Spider biofilm repressed
orf19.2350	3.2	0.0	Protein similar to S. cerevisiae Yor378w; MFS family transporter; transposon mutation affects filamentous growth; null mutants are viable; fungal-specific (no human or murine homolog)
orf19.36.1	3.2	0.0	Ortholog of C. parapsilosis CDC317 : CPAR2_104640, Candida orthopsilosis Co 90-125 : CORT_0B05695 and Candida orthopsilosis NEW ASSEMBLY : CORT1B05695
EBP1	3.1	0.0	NADPH oxidoreductase; interacts with phenolic substrates (17beta-estradiol); possible role in estrogen response; induced by oxidative, weak acid stress, NO, benomyl, GlcNAc; Cap1, Mnl1 induced; Hap43-repressed; rat catheter biofilm induced

Appendix 4: RNA-seq result of Cta7-GOF. Top 50 differentially expressed Up-regulated genes in *Candida albicans* Cta7-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.1438	13.3	0.00	Protein with homology to NADH dehydrogenase; regulated by Sef1p-, Sfu1p-, and Hap43p
orf19.7495	9.4	0.00	Protein with NADPH oxidoreductase containing flavin mononucleotide (FMN) domain; induced by nitric oxide
orf19.2285	6.7	0.02	Protein of unknown function; transcription induced by benomyl treatment
orf19.2350	6.3	0.00	Protein similar to S. cerevisiae Yor378w; MFS family transporter; transposon mutation affects filamentous growth; null mutants are viable; fungal-specific (no human or murine homolog)
CTA7	6.1	0.00	Zn(II)2Cys6 transcription factor; activates transcription in 1-hybrid assay in S. cerevisiae; has similarity to S. cerevisiae Stb4
orf19.6209	5.2	0.00	Predicted membrane transporter; monocarboxylate porter (MCP) family, major facilitator superfamily (MFS); possibly essential, disruptants not obtained by UAU1 method; rat catheter and Spider biofilm induced
RCH1	5.0	0.00	Plasma membrane protein; involved in regulation of cytosolic calcium homeostasis; null mutation confers sensitivity to calcium and resistance to azoles and terbinafine; rat catheter biofilm induced
orf19.3395	4.4	0.00	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family; induced by nitric oxide, oxidative stress, alpha pheromone; fungal-specific; Hap43-repressed; Spider biofilm induced
orf19.308	4.1	0.00	Ortholog(s) have role in nucleobase-containing compound transport, regulation of fungal-type cell wall organization, regulation of phospholipid translocation and plasma membrane localization
PGA57	4.0	0.00	Putative GPI-anchored protein; Hap43p-induced gene
orf19.711	3.9	0.00	Protein of unknown function; induced by nitric oxide; predicted ORF from Assembly 19; removed from Assembly 20; restored based on transcription data
OYE23	3.9	0.00	Putative NAPDH dehydrogenase; induced by nitric oxide, benomyl; oxidative stress-induced via Cap1; Hap43p-repressed; rat catheter biofilm induced

orf19.5785	3.8	0.01	Protein of unknown function; upregulated in a cyr1 or ras1 null mutant; induced by nitric oxide
KRE1	3.8	0.00	Cell wall glycoprotein; beta glucan synthesis; increases glucan content in S. cerevisiae kre1, complements killer toxin sensitivity; caspofungin induced; Spider/rat catheter/flow model biofilm induced; Bcr1-repressed in RPMI a/a biofilms
PLB1	3.8	0.01	Phospholipase B; host cell penetration and virulence in mouse systemic infection; Hog1-induced; signal sequence, N-glycosylation, and Tyr phosphorylation site; induced in fluconazole-resistant strains; rat catheter biofilm repressed
MNN45	3.7	0.01	Mannosyltransferase; transcript upregulated in Ssk1 response regulator mutant or in nik1 null mutant, but not in chk1 or sln1 null mutants; pheromone induced; Spider biofilm induced
MODF	3.6	0.00	Has domain(s) with predicted ATP binding, ATPase activity, nucleoside-triphosphatase activity
IHD1	3.3	0.00	GPI-anchored protein; alkaline, hypha-induced; regulated by Nrg1, Rfg1, Tup1 and Tsa1, Tsa1B in minimal media at 37; oralpharyngeal candidasis induced ; Spider biofilm induced; regulated in Spider biofilms by Tec1, Efg1, Ndt80, Rob1, Brg1
SSP96	3.2	0.00	Putative flavin-containing monooxygenase; F-12/CO2 early biofilm induced
ALS2	3.0	0.00	ALS family protein; role in adhesion, biofilm formation, germ tube induction; expressed at infection of human buccal epithelial cells; putative GPI-anchor; induced by ketoconazole, low iron and at cell wall regeneration; regulated by Sfu1p
QDR2	3.0	0.00	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family; Spider biofilm induced
ATO2	2.9	0.00	Putative fungal-specific transmembrane protein; fluconazole repressed, Hap43-repressed; flow model biofilm induced; Spider biofilm induced
MLS1	2.8	0.00	Malate synthase; glyoxylate cycle enzyme; no mammalian homolog; regulated upon white-opaque switch; phagocytosis, strong oxidative stress induced; stationary phase enriched; flow model biofilm repressed; rat catheter, Spider biofilm induced
orf19.6222.1	2.8	0.00	Ortholog of C. parapsilosis CDC317 : CPAR2_208910, Candida tenuis NRRL Y-1498 : CANTEDRAFT_114047, Debaryomyces hansenii CBS767 : DEHA2D14388g and Pichia stipitis Pignal : PICST_37629
orf19.5525	2.7	0.00	Putative oxidoreductase; protein levels affected by URA3 expression in CAI-4 strain background; Efg1, Efh1 regulated; Rgt1-repressed; protein present in exponential and stationary growth phase yeast; rat catheter biofilm repressed
orf19.732	2.7	0.01	Possible dehydrogenase; flow model biofilm induced; rat catheter biofilm induced; Spider biofilm induced
orf19.4612	2.7	0.02	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
orf19.3483	2.6	0.03	Putative phosphatidyl glycerol phospholipase C; Plc1-regulated; flow model biofilm induced; Spider biofilm induced
YDC1	2.4	0.00	Alkaline dihydroceramidase; involved in sphingolipid metabolism; Mob2-dependent hyphal regulation; transcript is regulated by Nrg1 and Mig1; Hap43-repressed
ITS1	2.4	0.00	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58; in S. cerevisiae it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
orf19.7567	2.4	0.01	Protein of unknown function; induced by alpha pheromone in SpiderM medium
LCB4	2.3	0.00	Putative sphingosine kinase; Tac1p-regulated expression; rat catheter biofilm induced
SRR1	2.3	0.00	Two-component system response regulator; involved in stress response; Plc1-regulated; upregulated in cyr1 null mutant; flow model biofilm induced; Spider biofilm induced
GIG1	2.2	0.04	Protein induced by N-acetylglucosamine (GlcNAc); localized in cytoplasm; mutation causes increased resistance to nikkomycin Z
BIO2	2.2	0.01	Putative biotin synthase; induced by high iron; repressed by ciclopirox olamine; upregulated in clinical isolates from HIV+ patients with oral candidiasis; Spider biofilm induced; biotin-dependent transcription regulated by Vhr1p
BIO5	2.2	0.00	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method; Spider biofilm induced; transcription regulated by biotin and Vhr1p
ITS2	2.2	0.00	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25; in S. cerevisiae it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
orf19.1116	2.1	0.01	Protein of unknown function; planktonic growth-induced gene
orf19.3051	2.1	0.00	Protein of unknown function; S. pombe ortholog SPAC17A2.02c plays a role in resistance to cadmium; colony morphology-related gene regulation by Ssn6; Spider biofilm repressed
orf19.5070	2.1	0.00	Similar to cell-wall mannoproteins; induced in low iron; induced in cyr1 homozygous null; regulated by osmotic and oxidative stress via Hog1; Spider biofilm induced
ZCF20	2.1	0.00	Zn(II)2Cys6 transcription factor orthologous to S. cerevisiae Hap1; regulated by Sef1, Sfu1; Hap43-induced; Spider biofilm induced
RAD16	2.1	0.00	Ortholog of S. cerevisiae Rad16; a protein that recognizes and binds damaged DNA; flucytosine induced; rat catheter and Spider biofilm induced
ATC1	2.1	0.01	Cell wall acid trehalase; catalyzes hydrolysis of the disaccharide trehalose; similar to S. cerevisiae vacuolar acid trehalase (Ath1p); Hap43p-repressed gene

FLO9	2.1	0.00	Putative adhesin-like cell wall mannoprotein; repressed during the mating process; mutation confers hypersensitivity to toxic ergosterol analog; decreased transcription is observed upon fluphenazine treatment
UGA6	2.1	0.01	Putative GABA-specific permease; decreased transcription is observed upon benomyl treatment or in an azole-resistant strain that overexpresses MDR1
orf19.4843	2.1	0.00	Putative iron/copper reductas; involved in iron homeostasis; rat catheter and Spider biofilm induced
ARE2	2.0	0.01	Acyl CoA:sterol acyltransferase; uses cholesterol and oleoyl-CoA substrates; protoberberine derivative drug inhibits enzyme activity; ketoconazole-induced; Hap43-repressed; flow model biofilm induced; Spider biofilm induced
FGR13	2.0	0.03	Protein encoded in retrotransposon Zorro3 with a potential zinc finger; lacks an ortholog in S. cerevisiae
orf19.4835	2.0	0.00	Ortholog(s) have role in endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more

Appendix 5: RNA-seq result of Leu3-GOF. 19 differentially expressed Up-regulated genes in *Candida albicans* Leu3-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
LEU3	4.5	0.00	Zn(II)2Cys6 transcription factor; predicted regulator branched-c ofhain amino acid biosynthesis genes; alkaline induced; induced by Mnl1 under weak acid stress; required for yeast cell adherence to silicone substrate; Spider biofilm induced
BIO5	3.0	0.00	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method; Spider biofilm induced; transcription regulated by biotin and Vhr1p
VHT1	2.6	0.00	Predicted membrane transporter, involved in biotin import; member of the anion:cation symporter (ACS) family, major facilitator superfamily (MFS); biotin-dependent transcription regulated by Vhr1p; amphotericin B, caspofungin repressed
GPX2	2.5	0.00	Similar to glutathione peroxidase; induced in high iron; alkaline induced by Rim101; induced by alpha factor or interaction with macrophage; regulated by Efg1; caspofungin repressed; Spider biofilm induced
SEO1	2.1	0.02	Protein with similarity to permeases; Sfu1-repressed
LEU4	2.0	0.00	Putative 2-isopropylmalate synthase; regulated by Nrg1, Mig1, Tup1, Gcn4; induced by human whole blood or PMNs; macrophage/pseudohyphal-repressed after 16h; Spider biofilm repressed
BAS1	1.9	0.00	Putative Myb-like transcription factor; ortholog S. cerevisiae Bas1, a regulator of purine biosynthetic genes; mutant exhibits adenine auxotrophy and abnormal colony morphology
HMX1	1.9	0.02	Heme oxygenase; utilization of hemin iron; transcript induced by heat, low iron, or hemin; repressed by Efg1; induced by low iron; upregulated by Rim101 at pH 8; Hap43-induced; Spider and flow model biofilm induced
BIO3	1.9	0.00	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis; transcription regulated by biotin availability and Vhr1p
orf19.2372	1.8	0.01	Pol protein of retrotransposon Tca2; separated by a stop codon from Gag protein orf19.2371; likely translated as single polyprotein with Gag, reverse transcriptase, protease, and integrase; rat catheter biofilm repressed
GLN3	1.7	0.00	GATA transcription factor, involved in regulation of nitrogen starvation-induced filamentous growth; regulates transcription of Mep2 ammonium permease; regulated by Gcn2 and Gcn4; mRNA binds She3; Spider biofilm induced
CTP1	1.7	0.00	Putative citrate transport protein; flucytosine induced; amphotericin B repressed, caspofungin repressed; Hap43p-induced gene
orf19.2371	1.7	0.03	Putative Gag protein of retrotransposon Tca2; separated by a stop codon from Pol protein orf19.2372; likely translated as single polyprotein that includes Gag, reverse transcriptase, protease, and integrase; rat catheter biofilm repressed
CAN3	1.7	0.00	Predicted amino acid transmembrane transporter; transcript regulated by white-opaque switch; Hap43-repressed gene
GAP6	1.7	0.00	Broad-specificity amino acid permease; Plc1, Gcn4 regulated; rat catheter biofilm induced
BIO4	1.6	0.00	Putative dethiobiotin synthetase; transcript upregulated in clinical isolates from HIV+ patients with oral candidiasis; Hap43-repressed; GlcNAc-induced protein; Spider biofilm induced; biotin-dependent transcription regulated by Vhr1p
SHM2	1.6	0.00	Cytoplasmic serine hydroxymethyltransferase
HOM3	1.5	0.00	Putative L-aspartate 4-P-transferase; fungal-specific (no human or murine homolog); regulated by Gcn2 and Gcn4; early-stage flow model biofilm induced
LEU42	1.5	0.00	Putative alpha-isopropylmalate synthase

Appendix 6: RNA-seq result of Zcf16-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf16-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.5648	14.4	0.00	Ortholog of C. dubliniensis CD36 : Cd36_40300, C. parapsilosis CDC317 : CPAR2_402190, C. auris B8441 : B9J08_003511 and Candida tenuis NRRL Y-1498 : cten_CGOB_00197
ZCF16	7.0	0.00	Predicted Zn(II)2Cys6 transcription factor; mutants are viable; rat catheter biofilm induced
PEX11	4.4	0.00	Putative peroxisomal membrane protein; role in fatty acid oxidation; expression is Tac1-regulated; Hms1p-dependent induction by geldamycin; Spider biofilm induced
AOX2	4.1	0.04	Alternative oxidase; cyanide-resistant respiration; induced by antimycin A, oxidants; growth; Hap43, chlamydospore formation repressed; rat catheter, Spider biofilm induced; regulated in Spider biofilms by Bcr1, Tec1, Ndt80, Brg1
ICL1	3.7	0.00	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice; induced upon phagocytosis by macrophage; farnesol regulated; Pex5-dependent peroxisomal localization; stationary phase enriched; rat catheter, Spider biofilm induced
PCK1	3.5	0.02	Phosphoenolpyruvate carboxykinase; glucose, C-source, yeast-hypha, Hap43 regulated; fluconazole, phagocytosis, H2O2, oral candidasis, Spider/rat catheter/flow model biofilm induced; repressed in biofilm by Bcr1, Tec1, Ndt80, Rob1, Brg1
orf19.21	3.2	0.00	Ortholog(s) have role in ethanol metabolic process and mitochondrial inner membrane localization
orf19.4921.1	3.1	0.00	Protein of unknown function; Spider biofilm repressed
orf19.449	3.0	0.00	Putative phosphatidyl synthase; stationary phase enriched protein; transcript repressed by yeast-hypha switch; Hap43-repressed; rat catheter, Spider and flow model biofilm induced
FBP1	3.0	0.00	Fructose-1,6-bisphosphatase; key gluconeogenesis enzyme; regulated by Efg1, Ssn6; induced by phagocytosis; effects switch from glycolysis to gluconeogenesis in macrophage; rat flow model biofilm induced; overlaps orf19.6179
PRC3	2.9	0.00	Putative carboxypeptidase Y precursor; transcript regulated by Nrg1 and Mig1; regulated by Gcn2 and Gcn4
AAT21	2.9	0.00	Putative aspartate aminotransferase; stationary phase enriched protein; Gcn4-regulated; Spider biofilm induced
SFC1	2.7	0.02	Putative succinate-fumarate transporter; involved in repression of growth on sorbose; alkaline induced; rat catheter biofilm induced; Spider biofilm induced
CSH1	2.6	0.05	Aldo-keto reductase; role in fibronectin adhesion, cell surface hydrophobicity; regulated by temperature, growth phase, benomyl, macrophage interaction; azole resistance associated; Spider biofilm induced; rat catheter biofilm repressed
SNZ1	2.5	0.00	Stationary phase protein; vitamin B synthesis; induced byyeast-hypha switch, 3-AT or in azole-resistant strain overexpressing MDR1; soluble in hyphae; regulated by Gcn4, macrophage; Spider biofilm induced; rat catheter biofilm repressed
MLS1	2.5	0.00	Malate synthase; glyoxylate cycle enzyme; no mammalian homolog; regulated upon white-opaque switch; phagocytosis, strong oxidative stress induced; stationary phase enriched; flow model biofilm repressed; rat catheter, Spider biofilm induced
SYG1	2.4	0.00	Ortholog(s) have role in signal transduction and plasma membrane localization
OPT1	2.3	0.04	Oligopeptide transporter; transports 3-to-5-residue peptides; alleles are distinct, one has intron; suppresses S. cerevisiae ptr2-2 mutant defects; induced by BSA or peptides; Stp3p, Hog1p regulated; flow model biofilm induced
HBR2	2.3	0.00	Putative alanine glyoxylate aminotransferase; regulated by Gcn4p and hemoglobin; stationary phase enriched protein
orf19.2848	2.2	0.00	Predicted regulatory subunit of the Atg1 signaling complex; required for vesicle formation during autophagy and the cytoplasm-to-vacuole targeting (Cvt) pathway; Spider biofilm induced
PDK2	2.2	0.00	Putative pyruvate dehydrogenase kinase; mutation confers hypersensitivity to amphotericin B
ZWF1	2.2	0.00	Glucose-6-phosphate dehydrogenase; antigenic in mice; activity induced by O2 or oxidizing agents H2O2, menadione, macrophage; caspofungin repressed; induced in core stress response; regulated by Gcn2, Gcn4; rat catheter biofilm repressed
GAT1	2.0	0.03	GATA-type transcription factor; regulator of nitrogen utilization; required for nitrogen catabolite repression and utilization of isoleucine, tyrosine and tryptophan N sources; required for virulence in a mouse systemic infection model
orf19.6747	2.0	0.00	Ortholog(s) have acid phosphatase activity and role in dephosphorylation, intracellular sterol transport
orf19.2244	2.0	0.02	Similar to oxidoreductases and to S. cerevisiae Yjr096wp; Sfu1 repressed; induced by benomyl treatment, Ssr1; Hap43-repressed; flow model biofilm repressed
GPD1	2.0	0.00	Glycerol-3-phosphate dehydrogenase; glycerol biosynthesis; regulated by Efg1; regulated by Tsa1, Tsa1B under H2O2 stress conditions; Sflow model and Spider biofilm induced
NDE1	2.0	0.00	Putative NADH dehydrogenase; may act alternatively to complex I in respiration; caspofungin repressed; rat catheter biofilm induced; Spider biofilm repressed
orf19.94	1.9	0.02	Protein of unknown function; Spider biofilm induced
orf19.1796	1.9	0.00	Putative glyoxylate reductase; acts on glyoxylate and hydroxypyruvate substrates; Spider biofilm repressed

orf19.787.1	1.8	0.03	Protein of unknown function; ORF added to Assembly 21 based on comparative genome analysis; protein detected by mass spec in stationary phase cultures
orf19.7341.1	1.8	0.01	Protein of unknown function; Spider biofilm induced
orf19.3649	1.7	0.00	Ortholog(s) have adenyl-nucleotide exchange factor activity, role in cytoplasm protein quality control by the ubiquitin-proteasome system and cytosol localization
DEF1	1.6	0.02	RNA polymerase II regulator; role in filamentation, epithelial cell escape, dissemination in RHE model; induced by fluconazole, high cell density; Efg1/hyphal regulated; role in adhesion, hyphal growth on solid media; Spider biofilm induced
GRP2	1.6	0.02	NAD(H)-linked methylglyoxal oxidoreductase involved in regulation of methylglyoxal and pyruvate levels; regulation associated with azole resistance; induced in core stress response or by oxidative stress via Cap1, fluphenazine, benomyl
AAT1	1.6	0.00	Aspartate aminotransferase; soluble protein in hyphae; macrophage-induced protein; alkaline upregulated; amphotericin B repressed; gene used for strain identification by multilocus sequence typing; farnesol-, Hap43p-induced; GlcNAc-induced
AGC1	1.6	0.00	Putative mitochondrial carrier protein; transcript is alkaline upregulated rat catheter biofilm induced
orf19.1318	1.5	0.05	Ortholog of C. dubliniensis CD36 : Cd36_43350, C. parapsilosis CDC317 : CPAR2_403560, Debaryomyces hansenii CBS767 : DEHA2A08250g and Pichia stipitis Pignal : PICST_29838
orf19.904	1.5	0.04	Ortholog(s) have cytoplasm, nucleus localization
YUH2	1.5	0.00	Putative ubiquitin C-terminal hydrolase; sumoylation target
orf19.5648	14.4	0.00	Ortholog of C. dubliniensis CD36 : Cd36_40300, C. parapsilosis CDC317 : CPAR2_402190, C. auris B8441 : B9J08_003511 and Candida tenuis NRRL Y-1498 : cten_CGOB_00197
ZCF16	7.0	0.00	Predicted Zn(II)2Cys6 transcription factor; mutants are viable; rat catheter biofilm induced
PEX11	4.4	0.00	Putative peroxisomal membrane protein; role in fatty acid oxidation; expression is Tac1-regulated; Hms1p-dependent induction by geldamycin; Spider biofilm induced
AOX2	4.1	0.04	Alternative oxidase; cyanide-resistant respiration; induced by antimycin A, oxidants; growth; Hap43, chlamydospore formation repressed; rat catheter, Spider biofilm induced; regulated in Spider biofilms by Bcr1, Tec1, Ndt80, Brg1
ICL1	3.7	0.00	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice; induced upon phagocytosis by macrophage; farnesol regulated; Pex5-dependent peroxisomal localization; stationary phase enriched; rat catheter, Spider biofilm induced
PCK1	3.5	0.02	Phosphoenolpyruvate carboxykinase; glucose, C-source, yeast-hypha, Hap43 regulated; fluconazole, phagocytosis, H2O2, oral candidasis, Spider/rat catheter/flow model biofilm induced; repressed in biofilm by Bcr1, Tec1, Ndt80, Rob1, Brg1
orf19.21	3.2	0.00	Ortholog(s) have role in ethanol metabolic process and mitochondrial inner membrane localization
orf19.4921.1	3.1	0.00	Protein of unknown function; Spider biofilm repressed
orf19.449	3.0	0.00	Putative phosphatidyl synthase; stationary phase enriched protein; transcript repressed by yeast-hypha switch; Hap43-repressed; rat catheter, Spider and flow model biofilm induced
FBP1	3.0	0.00	Fructose-1,6-bisphosphatase; key gluconeogenesis enzyme; regulated by Efg1, Ssn6; induced by phagocytosis; effects switch from glycolysis to gluconeogenesis in macrophage; rat flow model biofilm induced; overlaps orf19.6179

Appendix 7: RNA-seq result of Zcf20-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf20-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
EFH1	30.0	0.00	APSES transcription factor; homodimer; minor role in transcriptional regulation vs Efg1; regulates filamentous growth, phenotypic switch; EFG1 and EFH1 genetically interact; expression interferes with mouse intestinal tract colonization
BMT9	7.5	0.05	Beta-mannosyltransferase, 9-gene family that includes characterized genes BMT1, BMT2, BMT3, and BMT4 with roles in beta-1,2-mannosylation of cell wall phosphopeptidomannan; regulated by Sef1, Sfu1, Hap43; rat catheter biofilm repressed
PGA7	5.2	0.00	GPI-linked hyphal surface antigen; induced by ciclopirox olamine, ketoconazole, Rim101 at pH 8; Hap43, fluconazole; flow model biofilm induced; Spider biofilm induced; required for RPMI biofilm; Bcr1-induced in a/a biofilm
TPO2	5.1	0.00	Putative polyamine transport protein; fungal-specific (no human or murine homolog)
PLB1	5.0	0.01	Phospholipase B; host cell penetration and virulence in mouse systemic infection; Hog1-induced; signal sequence, N-glycosylation, and Tyr phosphorylation site; induced in fluconazole-resistant strains; rat catheter biofilm repressed
FRP1	4.4	0.02	Ferric reductase; alkaline-induced by Rim101; iron-chelation-induced by CCAAT-binding factor; fluconazole-repressed; ciclopirox-, hypoxia-, Hap43-induced; colony morphology-related regulation by Ssn6; Spider and flow model biofilm induced
GAP2	3.9	0.00	General broad specificity amino acid permease; ketoconazole, flucytosine repressed; Ssy1-dependent histidine induction; regulated by Nrg1, Tup1; colony morphology-related gene regulation by Ssn6; Spider and flow model biofilm induced
ZCF20	3.5	0.00	Zn(II)2Cys6 transcription factor orthologous to S. cerevisiae Hap1; regulated by Sef1, Sfu1; Hap43-induced; Spider biofilm induced
RME1	3.5	0.02	Zinc finger protein, controls asexual sporulation; white-specific transcript; upregulation correlates with clinical development of fluconazole resistance; Upc2-regulated in hypoxia; flow model biofilm induced; Spider biofil
RBT5	3.3	0.03	GPI-linked cell wall protein; hemoglobin utilization; Rfg1, Rim101, Tbf1, Fe regulated; Sfu1, Hog1, Tup1, serum, alkaline pH, antifungal drugs, geldamycin repressed; Hap43 induced; required for RPMI biofilms; Spider biofilm induced
OPT3	3.3	0.01	Oligopeptide transporter; transcript induced by macrophage phagocytosis, BSA or peptides; fluconazole-induced; induced by Rim101 at pH 8; virulence-group-correlated expression; Hap43-repressed; Spider biofilm induced
IFE2	3.2	0.04	Putative alcohol dehydrogenase; yeast-enriched transcript; Efg1-regulated; induced by prostaglandins, Hog1, fluconazole; rat catheter biofilm induced
DUR1,2	3.2	0.00	Urea amidolyase; hydrolyzes urea to CO2; use of urea as N source and for hyphal switch in macrophage; regulated by Nrg1/Hap43; required for virulence; promotes mouse kidney and brain colonization; rat catheter and flow model biofilm induced
NUP	3.2	0.00	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not; similar to a nucleoside permease of S. pombe; possibly processed by Kex2p
HMX1	3.0	0.00	Heme oxygenase; utilization of hemin iron; transcript induced by heat, low iron, or hemin; repressed by Efg1; induced by low iron; upregulated by Rim101 at pH 8; Hap43-induced; Spider and flow model biofilm induced
RHR2	3.0	0.03	Glycerol 3-phosphatase; roles in osmotic tolerance, glycerol accumulation in response to salt; Spider/flow model biofilm induced; regulated by macrophage, stress, yeast-hyphal switch, pheromone, Gcn4, Hog1, Nrg1, Tup1
CAN2	2.9	0.00	Basic amino acid permease; arginine metabolism; regulated by Nrg1/Tup1; caspofungin, flucytosine induced; colony morphology-related regulation by Ssn6; Hap43-repressed; rat catheter and Spider biofilm induced; promoter bound by Efg1
OPT1	2.9	0.01	Oligopeptide transporter; transports 3-to-5-residue peptides; alleles are distinct, one has intron; suppresses S. cerevisiae ptr2-2 mutant defects; induced by BSA or peptides; Stp3p, Hog1p regulated; flow model biofilm induced
MNN14	2.9	0.00	Predicted alpha-1,3-mannosyltransferase activity with a role in protein glycosylation; Hap43-repressed; Spider biofilm induced
CSA1	2.9	0.00	Surface antigen on elongating hyphae and buds; strain variation in repeat number; ciclopirox, filament induced, alkaline induced by Rim101; Efg1-, Cph1, Hap43-regulated; required for WT RPMI biofilm formation; Bcr1-induced in a/a biofilms
orf19.5169	2.8	0.01	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
CAN1	2.4	0.01	Basic amino acid permease; complements lysine transport mutation; 10 predicted transmembrane regions, 3 predicted N-glycosylation sites; phagocytosis by macrophages induces transcript; rat catheter, Spider and flow model biofilm induced
FCY2	2.3	0.00	Purine-cytosine permease of pyrimidine salvage; mutation associated with resistance to flucytosine in clinical isolates; transposon mutation affects filamentation; farnesol-upregulated in biofilm

MNN22	2.2	0.03	Alpha-1,2-mannosyltransferase; required for normal cell wall mannan; regulated by Tsa1, Tsa1B at 37 deg; repressed in core stress response; NO, Hog1 induced; confers sensitivity to cell wall perturbing agents; Spider biofilm repressed
YOR1	2.1	0.00	Protein similar to S. cerevisiae Yor1; ABC-type plasma membrane transporter involved in resistance to aureobasidin A; white cell type-specific transcript; Spider biofilm induced
orf19.7445	2.0	0.02	Ortholog of S.c. Vid24; a peripheral membrane protein located at Vid (vacuole import and degradation) vesicles; regulated by Sef1, Sfu1, and Hap43; Spider biofilm induced
RBE1	2.0	0.00	Pry family cell wall protein; Rim101, Efg1, Ssn6, alkaline repressed; O-glycosylation; no GPI anchor predicted; ketoconazol induced; regulated by Sef1, Sfu1, Hap4; flow model biofilm induced; rat catheter and Spider biofilm repressed
FRP2	2.0	0.05	Putative ferric reductase; alkaline induced by Rim101; fluconazole-downregulated; upregulated in the presence of human neutrophils; possibly adherence-induced; regulated by Sef1, Sfu1, and Hap43
AUT7	1.9	0.02	Putative autophagosome protein; acts synergistically with Ysy6p to regulate unfolded protein response and mitochondrial function under ER stress; macrophage/pseudohyphal-repressed; alternatively spliced intron in 5' UTR
MEP1	1.9	0.02	Ammonium permease; Mep1 more efficient permease than Mep2, Mep2 has additional regulatory role; 11 predicted transmembrane regions; low mRNA abundance; hyphal downregulated; flow model biofilm induced
GDH3	1.7	0.01	NADP-glutamate dehydrogenase; Nrg1, Plc1 regulated; hypha, hypoxia, Efg1-repressed; Rim101-induced at pH 8; GlcNAc, ciclopirox, ketoconazole induced; exp and stationary phase protein; Spider biofilm repressed; rat catheter biofilm induced
IFM3	1.7	0.03	Protein with a 2-hydroxyacid dehydrogenase catalytic domain; Hap43-repressed; Plc1-regulated; overlaps orf19.2177
ADE13	1.7	0.01	Adenylosuccinate lyase; enzyme of adenine biosynthesis; soluble protein in hyphae; not induced during GCN response, in contrast to the S. cerevisiae ortholog; repressed by nitric oxide
GCV2	1.6	0.01	Glycine decarboxylase P subunit; protein of glycine catabolism; repressed by Efg1; Hog1-induced; induced by Rim101 at acid pH; transcript induced in elevated CO2; stationary phase enriched protein
RCH1	1.6	0.03	Plasma membrane protein; involved in regulation of cytosolic calcium homeostasis; null mutation confers sensitivity to calcium and resistance to azoles and terbinafine; rat catheter biofilm induced
orf19.6983	1.5	0.03	Protein of unknown function; Hap43-repressed gene; repressed by nitric oxide; Spider biofilm induced
orf19.993	1.5	0.04	Protein of unknown function; rat catheter biofilm repressed

Appendix 8: RNA-seq result of Tea1-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Tea1-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.5648	13.6	0.0	Ortholog of C. dubliniensis CD36 : Cd36_40300, C. parapsilosis CDC317 : CPAR2_402190, C. auris B8441 : B9J08_003511 and Candida tenuis NRRL Y-1498 : cten_CGOB_00197
orf19.5952	8.8	0.0	Protein of unknown function; induced by nitric oxide independent of Yhb1; Sef1, Sfu1, and Hap43-induced; rat catheter and Spider biofilm induced
MEP2	6.6	0.0	Ammonium permease and regulator of nitrogen starvation-induced filamentation; 11 predicted transmembrane regions; in low nitrogen cytoplasmic C-terminus activates Ras/cAMP and MAPK signal transduction pathways to induce filamentation
CIP1	6.3	0.0	Possible oxidoreductase; transcript induced by cadmium but not other heavy metals, heat shock, yeast-hypha switch, oxidative stress (via Cap1), or macrophage interaction; stationary phase enriched protein; Spider biofilm induced
orf19.3982	5.6	0.0	Maltase; induced during growth on sucrose; induced by alpha pheromone in SpiderM medium; early-stage flow model biofilm indced
ARO9	5.4	0.0	Aromatic transaminase; Ehrlich fusel oil pathway of aromatic alcohol biosynthesis; Rim101-dependent pH-regulation (alkaline induced); Hap43-induced gene
PAD1	5.1	0.0	Putative phenylacrylic acid decarboxylase; repressed by Rgt1p
orf19.5730	5.0	0.0	Putative phenylacrylic acid decarboxylase; clade-associated gene expression
orf19.4445	4.9	0.0	Protein of unknown function; Plc1p-regulated; expression induced early upon infection of reconstituted human epithelium (RHE), while expression of the C. dubliniensis ortholog is not; mutant is viable; Spider biofilm induced
orf19.6840	4.8	0.0	Protein of unknown function; transcript detected in high-resolution tiling arrays; transcription induced by alpha pheromone in SpiderM medium; Spider and early-stage flow model biofilm induced
ICL1	4.4	0.0	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice; induced upon phagocytosis by macrophage; farnesol regulated; Pex5-dependent peroxisomal localization; stationary phase enriched; rat catheter, Spider biofilm induced
RSN1	4.3	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; induced during the mating process; Hap43-repressed
orf19.419	4.3	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
orf19.1608	4.0	0.0	Has domain(s) with predicted catalytic activity, sulfuric ester hydrolase activity and role in metabolic process

HGT20	3.9	0.0	Putative glucose transporter of the major facilitator superfamily; the *C. albicans* glucose transporter family comprises 20 members; 12 probable membrane-spanning segments; regulated by Nrg1
RBT5	3.8	0.0	GPI-linked cell wall protein; hemoglobin utilization; Rfg1, Rim101, Tbf1, Fe regulated; Sfu1, Hog1, Tup1, serum, alkaline pH, antifungal drugs, geldamycin repressed; Hap43 induced; required for RPMI biofilms; Spider biofilm induced
CAN2	3.8	0.0	Basic amino acid permease; arginine metabolism; regulated by Nrg1/Tup1; caspofungin, flucytosine induced; colony morphology-related regulation by Ssn6; Hap43-repressed; rat catheter and Spider biofilm induced; promoter bound by Efg1
orf19.5785	3.8	0.0	Protein of unknown function; upregulated in a cyr1 or ras1 null mutant; induced by nitric oxide
orf19.7566	3.6	0.0	Predicted amino acid transport domain; transcript upregulated in clinical strains from HIV+ patients with oral candidiasis; alkaline upregulated by Rim101; rat catheter, Spider and flow model biofilm induced
orf19.4530.1	3.5	0.0	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
orf19.6888	3.5	0.0	Zn(II)2Cys6 domain transcription factor; regulated by Mig1 and Tup1; rat catheter and Spider biofilm induced
orf19.6311	3.5	0.0	Protein of unknown function; Hap43-induced; rat catheter and Spider biofilm induced
orf19.449	3.5	0.0	Putative phosphatidyl synthase; stationary phase enriched protein; transcript repressed by yeast-hypha switch; Hap43-repressed; rat catheter, Spider and flow model biofilm induced
AMO1	3.4	0.0	Putative peroxisomal copper amine oxidase
ZCF22	3.4	0.0	Predicted Zn(II)2Cys6 transcription factor
orf19.3460	3.3	0.0	Protein of unknown function; mRNA binds She3; transcript regulated upon yeast-hypha switch; induced in oralpharyngeal candidasis
orf19.1277	3.1	0.0	Protein of unknown function; Rgt1, Hap43-repressed; flow model biofilm induced; Spider biofilm induced
TEA1	3.1	0.0	Putative transcription factor with zinc cluster DNA-binding motif; has similarity to S. cerevisiae Tea1p; Hap43p-repressed gene
FUR4	3.0	0.0	Putative uracil permease
DOG1	3.0	0.0	Putative 2-deoxyglucose-6-phosphatase; haloacid dehalogenase hydrolase/phosphatase superfamily; similar to S. cerevisiae Dog1, Dog2, Hor1, Rhr2; regulated by Nrg1, Tup1; Spider biofilm repressed
DAL4	3.0	0.0	Putative allantoin permease; fungal-specific (no human or murine homolog)
OYE32	2.9	0.0	NAD(P)H oxidoreductase family protein; induced by nitric oxide, amphotericin B, oxidative stress via Cap1; upregulation associated with MDR1 overexpression or benomyl treatment; macrophage-downregulated protein; Spider biofilm induced
GAT1	2.9	0.0	GATA-type transcription factor; regulator of nitrogen utilization; required for nitrogen catabolite repression and utilization of isoleucine, tyrosine and tryptophan N sources; required for virulence in a mouse systemic infection model
PSA2	2.9	0.0	Mannose-1-phosphate guanyltransferase; Hap43, macrophage-repressed; stationary phase enriched protein; Spider biofilm induced; rat catheter biofilm repressed
SKN2	2.9	0.0	Protein with a potential role in beta-1,6 glucan biosynthesis; similarity to Kre6 and Skn1; possibly essential, disruptants not obtained by UAU1 method; Hap43-induced; flow model biofilm induced; rat catheter biofilm repressed
orf19.1656	2.8	0.0	Protein with a predicted FYVE/PHD zinc finger domain; Hap43-repressed; Spider biofilm induced
NUP	2.8	0.0	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not; similar to a nucleoside permease of S. pombe; possibly processed by Kex2p
orf19.1107	2.8	0.0	Protein of unknown function; Spider biofilm induced
orf19.413	2.7	0.0	Protein of unknown function; induced by Sfu1; Spider biofilm induced
TFS1	2.7	0.0	Putative carboxypeptidase y inhibitor; transcript regulated upon yeast-hypha switch; colony morphology-related gene regulation by Ssn6
CPH1	2.7	0.0	Transcription factor; for mating, filamentation on solid media, pheromone-stimulated biofilms; in pathway with Ess1, Czf1; required with Efg1 for host cytokine response; regulates galactose metabolism genes; rat cathether biofilm repressed
orf19.5719	2.6	0.0	Predicted ORF from Assembly 19; removed from Assembly 20; subsequently reinstated in Assembly 21 and merged with orf19.1020 based on comparative genome analysis
orf19.7091	2.6	0.0	Protein of unknown function; induced by nitric oxide; Spider biofilm repressed
orf19.3639	2.5	0.0	Ortholog(s) have DNA-3-methyladenine glycosylase activity, alkylbase DNA N-glycosylase activity, damaged DNA binding activity
RCT1	2.5	0.0	Fluconazole-induced protein; Ras1, Cyr1 repressed and Efg1 induced; regulated by Nrg1, Tup1, Tbf1, Ssn6; induced in oralpharyngeal candidasis; rat catheter biofilm repressed
ALT1	2.4	0.0	Putative alanine transaminase; mutation confers hypersensitivity to 5-fluorocytosine (5-FC); rat catheter and flow model biofilm induced
RPS27A	2.4	0.0	Ribosomal protein S27; rat catheter and Spider biofilm induced
PTC6	2.4	0.0	Mitochondrial protein phosphatase of the Type 2C-related family (serine/threonine-specific), functional homolog of S. cerevisiae Ptc6p; mutant shows virulence defect

orf19.1258	2.4	0.0	Adhesin-like protein; regulated by Tsa1, Tsa1B in minimal media at 37 deg; clade-associated gene expression; induced by alpha pheromone in SpiderM medium; Hap43-induced; Spider biofilm repressed
ADH5	2.3	0.0	Putative alcohol dehydrogenase; regulated by white-opaque switch; fluconazole-induced; antigenic in murine infection; regulated by Nrg1, Tup1; Hap43, macrophage repressed, flow model biofilm induced; Spider biofilm induced
orf19.6637	2.3	0.0	Predicted glycosyl hydrolase; hypoxia induced; flow model biofilm induced
MET15	2.3	0.0	O-acetylhomoserine O-acetylserine sulfhydrylase; sulfur amino acid synthesis; immunogenic; Hog1, adherence-induced; brown color of mutant in Pb(2+) medium a visual selection; chlamydospore formation induced, F-12/CO2 biofilm induced
orf19.29	2.3	0.0	Ortholog of S. cerevisiae Tah11, a DNA replication licensing factor required for pre-replication complex assembly; rat catheter, flow model and Spider biofilm induced
ANT1	2.2	0.0	Peroxisomal adenine nucleotide transporter; role in beta-oxidation of medium-chain fatty acid and peroxisome proliferation; rat catheter biofilm induced
orf19.5720	2.2	0.0	Predicted membrane transporter, member of the monocarboxylate porter (MCP) family, major facilitator superfamily (MFS); ketoconazole or caspofungin repressed; Spider biofilm induced
ROD1	2.2	0.0	Protein similar to S. cerevisiae Rod1;a membrane protein with a role in drug tolerance; repressed by Rgt1; mutant is viable
orf19.28	2.2	0.0	Putative thiamine transmembrane transporter; Spider biofilm induced
ZCF10	2.2	0.0	Putative transcription factor with zinc cluster DNA-binding motif
SUC1	2.2	0.0	Zinc-finger transcription factor; regulates alpha-glucosidase exression; complements S. cerevisiae suc2 for sucrose utilization and mal13 maltase defect; required for yeast cell adherence to silicone substrate; rat catheter biofilm induced
GAP2	2.2	0.0	General broad specificity amino acid permease; ketoconazole, flucytosine repressed; Ssy1-dependent histidine induction; regulated by Nrg1, Tup1; colony morphology-related gene regulation by Ssn6; Spider and flow model biofilm induced
MET4	2.1	0.0	Putative transcription coactivator; predicted role in sulfur amino acid metabolism; required for yeast cell adherence to silicone substrate; Spider biofilm induced
orf19.6753	2.0	0.0	Protein with a predicted RING-type zinc finger; possibly an essential gene, disruptants not obtained by UAU1 method
CAC2	2.0	0.0	Component of the chromatin assembly factor I (CAF-1); involved in regulation of white-opaque switching frequency; macrophage-induced
AIF1	1.9	0.0	Has domain(s) with predicted flavin adenine dinucleotide binding, oxidoreductase activity
ALD5	1.9	0.0	NAD-aldehyde dehydrogenase; decreased expression in fluconazole-resistant isolate, or in hyphae; biofilm induced; fluconazole-downregulated; protein abundance is affected by URA3 expression in the CAI-4 strain; stationary phase enriched
orf19.308	1.9	0.0	Ortholog(s) have role in nucleobase-containing compound transport, regulation of fungal-type cell wall organization, regulation of phospholipid translocation and plasma membrane localization
QDR2	1.9	0.0	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family; Spider biofilm induced
orf19.5525	1.9	0.0	Putative oxidoreductase; protein levels affected by URA3 expression in CAI-4 strain background; Efg1, Efh1 regulated; Rgt1-repressed; protein present in exponential and stationary growth phase yeast; rat catheter biofilm repressed
GAC1	1.9	0.0	Putative regulatory subunit of ser/thr phosphoprotein phosphatase 1; fluconazole-induced; caspofungin repressed; transcript induced by Mnl1 under weak acid stress; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
orf19.4795	1.9	0.0	Protein of unknown function; Sef1-, Sfu1-, and Hap43 regulated; Spider biofilm induced
NPR1	1.9	0.0	Predicted serine/threonine protein kinase, involved in regulation of ammonium transport; induced in core stress response; Hap43p-repressed gene
GST2	1.9	0.0	Glutathione S transferase; induced by benomyl and in populations of cells exposed to fluconazole over multiple generations; regulated by Nrg1, Tup1; induced by nitric oxide; stationary phase enriched; Spider biofilm induced
CPA2	1.9	0.0	Putative arginine-specific carbamoylphosphate synthetase; protein enriched in stationary phase yeast cultures; rat catheter biofilm induced; Spider biofilm induced
DPP1	1.9	0.0	Putative diacylglycerol pyrophosphate phosphatase of diacylglycerol production for phospholipid biosynthesis; downregulation correlates with clinical development of fluconazole resistance
LEU4	1.8	0.0	Putative 2-isopropylmalate synthase; regulated by Nrg1, Mig1, Tup1, Gcn4; induced by human whole blood or PMNs; macrophage/pseudohyphal-repressed after 16h; Spider biofilm repressed
orf19.1933	1.8	0.0	Ortholog(s) have role in ER-dependent peroxisome organization, peroxisome organization and endoplasmic reticulum, peroxisomal membrane, peroxisome localization
orf19.4735	1.8	0.0	Ornithine cyclodeaminase family protein; Sef1, Sfu1, and Hap43-regulated; ortholog of S. cerevisiae YGL159W and S. pombe SPAP11E10.01; rat catheter biofilm induced
MUM2	1.8	0.0	Protein similar to S. cerevisiae Mum2, a protein essential for meiotic DNA replication and sporulation; induced by alpha pheromone in SpiderM medium; transcript regulated by Tup1

PDX1	1.8	0.0	Pyruvate dehydrogenase complex protein X; essential component of the mitochondrial pyruvate dehydrogenase complex; role in the respiratory pathway; protein present in exponential and stationary growth phase yeast; Spider biofilm repressed
ARA1	1.8	0.0	D-Arabinose dehydrogenase; dehydro-D-arabinono-1,4-lactone synthesis; active on D-arabinose, L-fucose, L-xylose, L-galactose; inhibited by metal ions, thiol group-specific reagents; induced on polystyrene adherence; Spider biofilm induced
OSM2	1.7	0.0	Putative mitochondrial fumarate reductase; regulated by Ssn6p, Gcn2p, and Gcn4p; Hog1p-downregulated; stationary phase enriched protein; Hap43p-repressed gene
SIP5	1.7	0.0	Protein of unknown function; flow model, rat catheter and Spider biofilm induced
ADE13	1.7	0.0	Adenylosuccinate lyase; enzyme of adenine biosynthesis; soluble protein in hyphae; not induced during GCN response, in contrast to the S. cerevisiae ortholog; repressed by nitric oxide
PMR1	1.7	0.0	Secretory pathway P-type Ca2+/Mn2+-ATPase; calcium pump involved in control of calcium homeostasis; required for protein glycosylation and cell wall maintenance; required for hyphal tip oscillation in semisolid substrate
orf19.3810	1.7	0.0	Ortholog(s) have methylenetetrahydrofolate dehydrogenase (NAD+) activity, role in folic acid-containing compound biosynthetic process, one-carbon metabolic process, purine nucleobase biosynthetic process and cytosol localization
orf19.4736	1.7	0.0	Ortholog(s) have alkaline phosphatase activity, zinc ion sensor activity, role in nicotinamide nucleotide metabolic process, protein dephosphorylation and extracellular region, fungal-type vacuole, fungal-type vacuole membrane localization
LEU2	1.7	0.0	Isopropyl malate dehydrogenase; leucine biosynthesis; induced by human whole blood or PMNs; protein level decreases in stationary phase; GlcNAc-induced protein; flow model biofilm repressed
OXR1	1.7	0.0	Ortholog(s) have role in cellular response to oxidative stress and mitochondrion localization
orf19.95	1.7	0.0	Ortholog of S. cerevisiae : PRM5, C. dubliniensis CD36 : Cd36_60980, C. parapsilosis CDC317 : CPAR2_603060, C. auris B8441 : B9J08_003420 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_113703
FAA2-1	1.7	0.0	Predicted long chain fatty acid CoA ligase; upregulated upon phagocytosis; induced by nitric oxide independent of Yhb1
ARG5,6	1.7	0.0	Arginine biosynthetic enzyme; processed in S. cerevisiae into 2 polypeptides with acetylglutamate kinase (Arg6) activity and acetylglutamate-phosphate reductase (Arg5) activity; Gcn4 regulated; alkaline repressed; Spider biofilm induced
PYC2	1.7	0.0	Putative pyruvate carboxylase; binds biotin cofactor; repressed by Ssk1 response regulator, by benomyl treatment, or in an azole-resistant strain overexpressing MDR1; stationary phase enriched protein; flow model biofilm repressed
PHO113	1.7	0.0	Putative constitutive acid phosphatase; Rim101-repressed; DTT-extractable; N-glycosylated; possibly an essential gene, disruptants not obtained by UAU1 method
YPS7	1.7	0.0	Putative aspartic-type endopeptidase with limited ability to degrade alpha pheromone; mutants show increased sensitivity to alpha pheromone
MEP1	1.6	0.0	Ammonium permease; Mep1 more efficient permease than Mep2, Mep2 has additional regulatory role; 11 predicted transmembrane regions; low mRNA abundance; hyphal downregulated; flow model biofilm induced
IDP1	1.6	0.0	Putative isocitrate dehydrogenase; transcriptionally induced by interaction with macrophage; alkaline induced; Spider biofilm repressed
LAT1	1.6	0.0	Putative dihydrolipoamide acetyltransferase component (E2) of pyruvate dehydrogenase complex; sumoylation target; Spider biofilm repressed
orf19.4368	1.6	0.0	Has domain(s) with predicted hydrolase activity and role in cellular process
orf19.338	1.6	0.0	Putative glycoside hydrolase; stationary phase enriched protein; Hog1p-downregulated; shows colony morphology-related gene regulation by Ssn6p
orf19.6754	1.6	0.0	Protein of unknown function; Spider biofilm induced
RIB5	1.6	0.0	Putative riboflavin synthase; fungal-specific (no human or murine homolog); farnesol-downregulated; protein present in exponential and stationary growth phase yeast cultures
GDH3	1.6	0.0	NADP-glutamate dehydrogenase; Nrg1, Plc1 regulated; hypha, hypoxia, Efg1-repressed; Rim101-induced at pH 8; GlcNAc, ciclopirox, ketoconazole induced; exp and stationary phase protein; Spider biofilm repressed; rat catheter biofilm induced
BNA31	1.6	0.0	Putative arylformamidase, enzyme of the NAD biosynthesis pathway; Gcn4p-regulated
PDX3	1.5	0.0	Pyridoxamine-phosphate oxidase; transcript regulated by yeast-hypha switch and by Nrg1, Mig1, Tup1; Hap43, caspofungin repressed; present in exponential and stationary phase yeast cultures
orf19.7556	1.5	0.0	Ortholog of C. dubliniensis CD36 : Cd36_34965, C. parapsilosis CDC317 : CPAR2_200910, C. auris B8441 : B9J08_005167 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_95984
LEU1	1.5	0.0	3-isopropylmalate dehydratase; antigenic in humans
SHM2	1.5	0.0	Cytoplasmic serine hydroxymethyltransferase
orf19.1386	1.5	0.0	Ortholog(s) have SNAP receptor activity and role in endoplasmic reticulum to Golgi vesicle-mediated transport, retrograde vesicle-mediated transport, Golgi to endoplasmic reticulum, vesicle fusion
orf19.7196	1.5	0.0	Putative vacuolar protease; upregulated in the presence of human neutrophils; Spider biofilm induced

Appendix 9: RNA-seq result of Zcf23-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf23-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
TRY6	28.6	0.0	Helix-loop-helix transcription factor; regulator of yeast form adherence
LIP3	28.6	0.0	Secreted lipase; gene family member whose members are expressed differentially in response to carbon source and infection
orf19.5735.3	28.3	0.0	Protein of unknown function; Spider biofilm induced
orf19.3210	17.8	0.0	Predicted protein of unknown function; Plc1-regulated
orf19.3378	16.8	0.0	Protein of unknown function; regulated by Tsa1, Tsa1B in minimal media at 37 degrees C
OPT9	13.7	0.0	Probable pseudogene similar to fragments of OPT1 oligopeptide transporter gene; decreased expression in hyphae compared to yeast-form cells; transcriptionally induced upon phagocytosis by macrophage
DUR3	11.6	0.0	High affinity spermidine transporter; expression is induced by urea; fungal-specific (no human or murine homolog); not required for virulence in a mouse intravenous model
MEP2	11.1	0.0	Ammonium permease and regulator of nitrogen starvation-induced filamentation; 11 predicted transmembrane regions; in low nitrogen cytoplasmic C-terminus activates Ras/cAMP and MAPK signal transduction pathways to induce filamentation
OPT3	10.9	0.0	Oligopeptide transporter; transcript induced by macrophage phagocytosis, BSA or peptides
orf19.2583.2	9.8	0.0	Pseudogene; formerly an ORF Predicted by Annotation Working Group
OPT2	9.8	0.0	Oligopeptide transporter; induced upon phagocytosis by macrophage
orf19.1438	9.2	0.0	Protein with homology to NADH dehydrogenase; regulated by Sef1p-, Sfu1p-, and Hap43p
OPT1	8.8	0.0	Oligopeptide transporter; transports 3-to-5-residue peptides
BLP1	8.2	0.0	Protein of unknown function, serum-induced
AMO1	8.2	0.0	Putative peroxisomal copper amine oxidase
RME1	8.1	0.0	Zinc finger protein, controls asexual sporulation; white-specific transcript; upregulation correlates with clinical development of fluconazole resistance; Upc2-regulated in hypoxia; flow model biofilm induced; Spider biofil
HSP21	8.1	0.0	Small heat shock protein; role in stress response and virulence; fluconazole-downregulated; induced in cyr1 or ras1 mutant
GPT1	8.0	0.0	GABA/polyamine transporter; 9 to 11 membrane spanning segments; complements GABA uptake defect of an S. cerevisiae uga4 put4 gap1 triple mutant; complements growth of an S. cerevisiae spe1 mutant under polyamine limitation
MRV5	7.8	0.0	Planktonic growth-induced gene
ZCF23	7.7	0.0	Predicted Zn(II)2Cys6 transcription factor; ortholog of S. cerevisiae Gsm1; flow model biofilm induced
orf19.6398	7.3	0.0	S. pombe ortholog SPBC460.04c is a predicted sulfonate/alpha-ketoglutare dioxygenase; induced by nitric oxide; Spider biofilm induced
SAP99	7.2	0.0	Putative secreted aspartyl protease
GAP2	7.2	0.0	General broad specificity amino acid permease
orf19.1641	6.9	0.0	Ortholog(s) have extracellular region localization
PCK1	6.9	0.0	Phosphoenolpyruvate carboxykinase; glucose, C-source, yeast-hypha, Hap43 regulated
DAL4	6.8	0.0	Putative allantoin permease; fungal-specific (no human or murine homolog)
OPT4	6.7	0.0	Oligopeptide transporter; detected at germ tube plasma membrane
orf19.1691	6.7	0.0	Plasma-membrane-localized protein; filament induced; Hog1, ketoconazole, fluconazole and hypoxia-induced; regulated by Nrg1, Tup1, Upc2; induced by prostaglandins; flow model biofilm induced; rat catheter and Spider biofilm repressed
IFE2	6.5	0.0	Putative alcohol dehydrogenase; yeast-enriched transcript; Efg1-regulated; induced by prostaglandins, Hog1, fluconazole; rat catheter biofilm induced
tR(UCU)4	6.5	0.0	tRNA-Arg, predicted by tRNAscan-SE; UCU anticodon
ICL1	6.4	0.0	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice; induced upon phagocytosis by macrophage; farnesol regulated; Pex5-dependent peroxisomal localization; stationary phase enriched; rat catheter, Spider biofilm induced
DUR4	6.3	0.0	Putative urea permease; fungal-specific (no human or murine homolog); possibly an essential gene, disruptants not obtained by UAU1 method
FUR4	6.3	0.0	Putative uracil permease
orf19.1421	6.3	0.0	Ortholog(s) have ureidoglycolate lyase activity
orf19.5169	6.3	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
SOD6	6.2	0.0	Copper-containing superoxide dismutase; gene family includes SOD1, SOD4, SOD5, and SOD6; gene may contain an intron; Hap43-repressed; flow model and rat catheter biofilm induced
orf19.4873	6.2	0.0	Protein of unknown function; transcript regulated by white-opaque switch; flow model biofilm induced; Spider biofilm induced
FET99	6.2	0.0	Multicopper oxidase family protein
orf19.3910	6.2	0.0	Has domain(s) with predicted RNA binding, ribonuclease T2 activity
ADH5	6.1	0.0	Putative alcohol dehydrogenase

CIP1	6.1	0.0	Possible oxidoreductase
orf19.4450.1	6.1	0.0	Protein conserved among the CTG-clade; 2 adjacent upstream SRE-1 elements
orf19.1307	6.1	0.0	Predicted membrane protein; rat catheter biofilm induced
orf19.6501	6.0	0.0	Ortholog of C. dubliniensis CD36
RNR22	5.9	0.0	Putative ribonucleoside diphosphate reductase;colony morphology-related gene regulation by Ssn6
MOH1	5.8	0.0	Ortholog of S. cerevisiae Moh1, essential for staionary phase growth
LAP3	5.8	0.0	Putative aminopeptidase; positively regulated by Sfu1
STF2	5.7	0.0	Protein involved in ATP biosynthesis; repressed in hyphae
orf19.3544	5.7	0.0	Putative protein of unknown function; Hap43p-repressed gene
GAL10	5.7	0.0	UDP-glucose 4-epimerase; galactose utilization
CRZ2	5.6	0.0	C2H2 transcription factor, involved in regulation of early adaptation to murine GI tract
THI4	5.6	0.0	Thiamine biosynthetic enzyme precursor
orf19.7495	5.5	0.0	Protein with NADPH oxidoreductase containing flavin mononucleotide (FMN) domain
PGA23	5.5	0.0	Putative GPI-anchored protein of unknown function
orf19.5451	5.5	0.0	Ortholog of Candida guilliermondii ATCC 6260
TRY5	5.4	0.0	Zn(II)2Cys6 transcription factor; regulator of yeast form adherence
DUR1,2	5.4	0.0	Urea amidolyase; hydrolyzes urea to CO2
FDH1	5.4	0.0	Formate dehydrogenase; oxidizes formate to CO2; Mig1 regulated; induced by macrophages
orf19.7279.1	5.3	0.0	Protein of unknown function; Spider biofilm induced
HAK1	5.3	0.0	Putative potassium transporter; similar to Schwanniomyces occidentalis Hak1p
ATO6	5.3	0.0	Putative fungal-specific transmembrane protein
GAT1	5.2	0.0	GATA-type transcription factor; regulator of nitrogen utilization
orf19.3879	5.2	0.0	Predicted protein of unknown funtion; overlaps orf19.3879.1
RHD3	5.2	0.0	GPI-anchored yeast-associated cell wall protein
IFG3	5.2	0.0	Putative D-amino acid oxidase; Spider biofilm induced
SUC1	5.2	0.0	Zinc-finger transcription factor; regulates alpha-glucosidase exression
TPO3	5.2	0.0	Putative polyamine transporter; MFS-MDR family
GAL1	5.2	0.0	Galactokinase; galactose, Mig1, Tup1, Hap43 regulated
CDR4	5.1	0.0	Putative ABC transporter superfamily
orf19.1867	5.1	0.0	Putative malate permease
UCF1	5.1	0.0	Upregulated by cAMP in filamentous growth
orf19.670.2	5.1	0.0	Protein of unknown function; hypoxia, Hap43-repressed
NUP	5.0	0.0	Nucleoside permease
orf19.5282	4.9	0.0	Protein of unknown function
orf19.7204	4.9	0.0	Has domain(s) with predicted catalytic activity, nitronate monooxygenase activity
orf19.541	4.9	0.0	Has domain(s) with predicted 2-oxoglutarate-dependent dioxygenase activity
orf19.419	4.9	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
HNM4	4.8	0.0	Putative choline permease; fungal-specific (no human or murine homolog)
PRM1	4.8	0.0	Putative membrane protein with a predicted role in membrane fusion during mating
FBP1	4.7	0.0	Fructose-1,6-bisphosphatase; key gluconeogenesis enzyme
GCA1	4.7	0.0	Extracellular/plasma membrane-associated glucoamylase
GST2	4.7	0.0	Glutathione S transferase
orf19.6793	4.7	0.0	Protein of unknown function; Sef1, Sfu1, and Hap43 regulated
ARG8	4.7	0.0	Putative acetylornithine aminotransferase
MNN22	4.7	0.0	Alpha-1,2-mannosyltransferase; required for normal cell wall mannan
orf19.7158	4.7	0.0	Protein of allantoate permease family
ARG1	4.6	0.0	Argininosuccinate synthase; arginine synthesis
BIO32	4.6	0.0	Putative class III aminotransferase with a predicted role in biotin biosynthesis
orf19.1353	4.6	0.0	Protein of unknown function; repressed by yeast-hypha switch
orf19.2691	4.6	0.0	Planktonic growth-induced gene
orf19.6690	4.6	0.0	Protein of unknown function; Hap43-repressed gene
orf19.6950	4.5	0.0	Putative vacuolar membrane transporter for cationic amino acids; Spider biofilm induced
CRG1	4.5	0.0	Methyltransferase involved in sphingolipid homeostasis, methylates a drug cantharidin
OSM1	4.5	0.0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae
PGA10	4.5	0.0	GPI anchored membrane protein; utilization of hemin and hemoglobin for Fe in host
RSN1	4.5	0.0	Protein of unknown function
CSO99	4.5	0.0	Protein of unknown function; Hap43-repressed gene; protein not conserved in S. cerevisiae
orf19.1433	4.5	0.0	Protein of unknown function; Hap43-repressed
GCA2	4.5	0.0	Predicted extracellular glucoamylase
orf19.5270	4.4	0.0	Protein of unknown function; rat catheter biofilm induced
orf19.1608	4.4	0.0	Has domain(s) with predicted catalytic activity
GIT4	4.4	0.0	Glycerophosphocholine transporter; fungal-specific (no human or murine homolog)
orf19.7227	4.3	0.0	Protein phosphatase inhibitor; Hap43-repressed
SAP1	4.3	0.0	Secreted aspartyl proteinase

orf19.6316	4.3	0.0	Predicted membrane transporter, member of the L-amino acid transporter-3 (LAT3) family, major facilitator superfamily (MFS)
orf19.4612	4.3	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
orf19.6899	4.3	0.0	Putative oxidoreductase; mutation confers hypersensitivity to toxic ergosterol analog
orf19.6816	4.2	0.0	Putative xylose and arabinose reductase; flow model biofilm induced; Spider biofilm repressed
XYL2	4.2	0.0	D-xylulose reductase; immunogenic in mice

Appendix 10: RNA-seq result of Orf19.2230-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Orf19.2230-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
TRY6	28.5	0.0	Helix-loop-helix transcription factor; regulator of yeast form adherence
OPT9	9.1	0.0	Probable pseudogene similar to fragments of OPT1 oligopeptide transporter gene
ARG1	6.9	0.0	Argininosuccinate synthase; arginine synthesis
orf19.1691	6.8	0.0	Plasma-membrane-localized protein
RNR22	6.6	0.0	Putative ribonucleoside diphosphate reductase;colony morphology-related gene regulation by Ssn6
orf19.2583.2	6.5	0.0	Pseudogene; formerly an ORF Predicted by Annotation Working Group
OPT4	6.4	0.0	Oligopeptide transporter
PGA10	6.3	0.0	GPI anchored membrane protein
CIP1	6.1	0.0	Possible oxidoreductase
BLP1	5.8	0.0	Protein of unknown function, serum-induced
FDH1	5.7	0.0	Formate dehydrogenase
GAP2	5.6	0.0	General broad specificity amino acid permease
OPT3	5.6	0.0	Oligopeptide transporter; transcript induced by macrophage phagocytosis, BSA or peptides
ARG8	5.6	0.0	Putative acetylornithine aminotransferase; Gcn2, Gcn4 regulated; rat catheter biofilm induced
ARG3	5.5	0.0	Putative ornithine carbamoyltransferase
TPO3	5.4	0.0	Putative polyamine transporter; MFS-MDR family; induced by Sfu1, regulated upon white-opaque
orf19.1307	5.3	0.0	Predicted membrane protein; rat catheter biofilm induced
ATO6	5.2	0.0	Putative fungal-specific transmembrane protein
orf19.2048	5.2	0.0	Proten of unknown function; transcript positively regulated by Sfu1
IFE2	5.2	0.0	Putative alcohol dehydrogenase; yeast-enriched transcript; Efg1-regulated
SOD6	5.1	0.0	Copper-containing superoxide dismutase; gene family includes SOD1, SOD4, SOD5, and SOD6
OPT2	5.1	0.0	Oligopeptide transporter; induced upon phagocytosis by macrophage
PLB1	5.1	0.0	Phospholipase B; host cell penetration and virulence in mouse systemic infection
orf19.3910	5.0	0.0	Has domain(s) with predicted RNA binding, ribonuclease T2 activity
CRG1	5.0	0.0	Methyltransferase involved in sphingolipid homeostasis, methylates a drug cantharidin
AQY1	5.0	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance
orf19.1421	5.0	0.0	Ortholog(s) have ureidoglycolate lyase activity
STF2	4.9	0.0	Protein involved in ATP biosynthesis; repressed in hyphae
ADH5	4.9	0.0	Putative alcohol dehydrogenase; regulated by white-opaque switch
CAN2	4.9	0.0	Basic amino acid permease; arginine metabolism; regulated by Nrg1/Tup1
UCF1	4.9	0.0	Upregulated by cAMP in filamentous growth; induced in high iron, decreased upon yeast-hypha switch
CPA2	4.8	0.0	Putative arginine-specific carbamoylphosphate synthetase
ARG5,6	4.8	0.0	Arginine biosynthetic enzyme
HSP21	4.8	0.0	Small heat shock protein; role in stress response and virulence
HEM13	4.7	0.0	Coproporphyrinogen III oxidase; antigenic; on yeast cell surface, not hyphae
orf19.4450.1	4.7	0.0	Protein conserved among the CTG-clade; 2 adjacent upstream SRE-1 elements
RHR2	4.6	0.0	Glycerol 3-phosphatase; roles in osmotic tolerance, glycerol accumulation in response to salt
orf19.4607	4.5	0.0	Possible Golgi membrane protein; Hap43-repressed; hypha induced

orf19.1353	4.5	0.0	Protein of unknown function; repressed by yeast-hypha switch; Ras1-regulated; oral infection induced
orf19.5205	4.5	0.0	Protein of unknown function; Hap43-repressed gene
OPT1	4.4	0.0	Oligopeptide transporter; transports 3-to-5-residue peptides
OSM1	4.4	0.0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae
orf19.5169	4.4	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
RSN1	4.4	0.0	Protein of unknown function
orf19.1862	4.4	0.0	Possible stress protein; increased transcription associated with CDR1 and CDR2 overexpression or fluphenazine treatment; regulated by Sfu1, Nrg1, Tup1
FGR23	4.3	0.0	Protein of unknown function
orf19.4287	4.3	0.0	Putative oxidoreductase; Hap43-repressed gene; clade-associated gene expression
orf19.2230	4.3	0.0	Putative pre-mRNA-splicing factor; decreased transcription is observed upon benomyl treatment
MEP2	4.3	0.0	Ammonium permease and regulator of nitrogen starvation-induced filamentation
SAP1	4.3	0.0	Secreted aspartyl proteinase; acts in utilization of protein as nitrogen source
AMO1	4.3	0.0	Putative peroxisomal copper amine oxidase
orf19.1368	4.2	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
orf19.6950	4.2	0.0	Putative vacuolar membrane transporter for cationic amino acids; Spider biofilm induced
CRP1	4.1	0.0	Copper transporter; CPx P1-type ATPase; mediates Cu resistance
CSP2	4.1	0.0	Putative cell wall associated protein
CPA1	4.1	0.0	Putative carbamoyl-phosphate synthase subunit; alkaline repressed
DAK2	4.1	0.0	Putative dihydroxyacetone kinase; repressed by yeast-hypha switch
MNN14	4.1	0.0	Predicted alpha-1,3-mannosyltransferase activity with a role in protein glycosylation
GAT1	4.0	0.0	GATA-type transcription factor; regulator of nitrogen utilization
ARG4	4.0	0.0	Argininosuccinate lyase, catalyzes the final step in the arginine biosynthesis pathway
orf19.1867	4.0	0.0	Putative malate permease; induced during macrophage infection
XYL2	3.9	0.0	D-xylulose reductase; immunogenic in mice; soluble protein in hyphae
GPM2	3.9	0.0	Putative phosphoglycerate mutase; repressed in hyphae
orf19.2691	3.8	0.0	Planktonic growth-induced gene
orf19.6690	3.8	0.0	Protein of unknown function; Hap43-repressed gene
OPT7	3.8	0.0	Putative oligopeptide transporter; possibly transports GSH or related compounds
CDR4	3.8	0.0	Putative ABC transporter superfamily; fluconazole, Sfu1, Hog1, core stress response induced
orf19.1433	3.8	0.0	Protein of unknown function; Hap43-repressed; colony morphology-related gene regulation by Ssn6; Spider biofilm induced
orf19.951	3.7	0.0	Protein of unknown function; transcript repressed upon yeast-hyphal switch; fluconazole-induced; Hap43-repressed; flow model biofilm induced
orf19.787.1	3.7	0.0	Protein of unknown function; ORF added to Assembly 21 based on comparative genome analysis
orf19.3544	3.7	0.0	Putative protein of unknown function; Hap43p-repressed gene
orf19.5282	3.7	0.0	Protein of unknown function; Hap43-repressed gene; mRNA binds to She3
orf19.6660	3.7	0.0	Protein of unknown function; mRNA binds to She3; Hap43-repressed; rat catheter and flow model biofilm induced
orf19.6222.1	3.7	0.0	Ortholog of *C. parapsilosis* CDC317
MOH1	3.7	0.0	Ortholog of S. cerevisiae Moh1, essential for staionary phase growth
RTA4	3.7	0.0	Protein similar to S. cerevisiae Rsb1p, involved in fatty acid transport
QDR1	3.6	0.0	Putative antibiotic resistance transporter
orf19.4612	3.6	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
orf19.5223	3.6	0.0	Ortholog of *Candida albicans* WO-1 : CAWG_00199
orf19.7227	3.6	0.0	Protein phosphatase inhibitor
RHD3	3.5	0.0	GPI-anchored yeast-associated cell wall protein; induced in high iron
RME1	3.5	0.0	Zinc finger protein, controls asexual sporulation; white-specific transcript
NUP	3.5	0.0	Nucleoside permease;
LAP3	3.5	0.0	Putative aminopeptidase; positively regulated by Sfu1

ECM42	3.5	0.0	Ornithine acetyltransferase; Gcn2, Gcn4-regulated
orf19.5785	3.4	0.0	Protein of unknown function; upregulated in a cyr1 or ras1 null mutant; induced by nitric oxide
orf19.3021	3.4	0.0	Putative protein of unknown function; Hap43-repressed gene; Spider biofilm induced
orf19.7027	3.4	0.0	Protein of unknown function; Spider biofilm induced
orf19.1381	3.4	0.0	Ortholog of S. cerevisiae/S. pombe Lsb5
orf19.4791	3.3	0.0	Protein of unknown function; Spider biofilm induced
orf19.411	3.3	0.0	Protein similar to GTPase regulators; induced in low iron
DAL4	3.3	0.0	Putative allantoin permease; fungal-specific (no human or murine homolog)
CAN1	3.3	0.0	Basic amino acid permease; complements lysine transport mutation
YHB1	3.3	0.0	Nitric oxide dioxygenase; acts in nitric oxide scavenging/detoxification
ECM21	3.3	0.0	Predicted regulator of endcytosis of plasma membrane proteins
PFK1	3.2	0.0	Phosphofructokinase alpha subinit; activated by fructose 2,6-bisphosphate, AMP, ATP inhibited
ITS1	3.2	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58
orf19.4530.1	3.2	0.0	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
orf19.6983	3.2	0.0	Protein of unknown function; Hap43-repressed gene; repressed by nitric oxide; Spider biofilm induced
orf19.7396	3.1	0.0	Protein of unknown function; Spider biofilm induced; Hap43-repressed
HGT6	3.1	0.0	Putative high-affinity MFS glucose transporter; 20 family members; induced in core stress response
SKN2	3.1	0.0	Protein with a potential role in beta-1,6 glucan biosynthesis
orf19.4229	3.1	0.0	Putative polyphosphate phosphatase
PCK1	3.1	0.0	Phosphoenolpyruvate carboxykinase; glucose, C-source, yeast-hypha, Hap43 regulated
orf19.5438	3.0	0.0	Putative ubiquitin-protein ligase; role in protein sumoylation, protein ubiquitination
orf19.5114.1	3.0	0.0	Ortholog of C. dubliniensis CD36
PRD1	3.0	0.0	Putative proteinase; transcript regulated by Nrg1, Mig1, and Tup1
DUR1,2	3.0	0.0	Urea amidolyase; hydrolyzes urea to CO2
orf19.1314	3.0	0.0	Protein of unknown function; planktonic growth-induced gene
GCY1	3.0	0.0	Aldo/keto reductase; mutation confers hypersensitivity to toxic ergosterol analog
IFF4	3.0	0.0	Adhesin-like cell surface protein; putative GPI-anchor
orf19.419	3.0	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
BUB3	3.0	0.0	Protein similar to S. cerevisiae Bub3; a kinetochore checkpoint component
ITS2	2.9	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25
ERG1	2.9	0.0	Squalene epoxidase, epoxidation of squalene to 2,3(S)-oxidosqualene; ergosterol biosynthesis
AMS1	2.9	0.0	Putative alpha-mannosidase; transcript regulated by Nrg1; induced during cell wall regeneration
GST2	2.9	0.0	Glutathione S transferase
HGT20	2.9	0.0	Putative glucose transporter of the major facilitator superfamily
OSM2	2.9	0.0	Putative mitochondrial fumarate reductase; regulated by Ssn6p, Gcn2p, and Gcn4p
orf19.4550	2.8	0.0	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family; flow model biofilm induced
GPD1	2.8	0.0	Glycerol-3-phosphate dehydrogenase; glycerol biosynthesis
ALS2	2.8	0.0	ALS family protein; role in adhesion, biofilm formation, germ tube induction
MEP1	2.8	0.0	Ammonium permease; Mep1 more efficient permease than Mep2, Mep2 has additional regulatory role
GLK1	2.8	0.0	Putative glucokinase; transcript regulated upon yeast-hyphal switch
PHO15	2.8	0.0	HAD-family 2-phosphoglycolate phosphatase, likely involved in a metabolic repair system,
AGT1	2.8	0.0	Agmatinase, involved in metabolism of agmatine; downregulated upon adherence to polystyrene
UGA6	2.8	0.0	Putative GABA-specific permease
GSY1	2.7	0.0	Glycogen synthase (UDP glucose/starch glucosyltransferase)
MAF1	2.7	0.0	Putative negative regulator of RNA polymerase III; decreased expression in hyphae vs yeast cells
DAL9	2.7	0.0	Putative allantoate permease; fungal-specific (no human or murine homolog)
FUR4	2.7	0.0	Putative uracil permease
CAF16	2.7	0.0	ABC family protein, predicted not to be a transporter; Hap43, caspofungin repressed

MNN22	2.7	0.0	Alpha-1,2-mannosyltransferase; required for normal cell wall mannan
VPS70	2.7	0.0	Has domain(s) with predicted peptidase activity and role in proteolysis
orf19.1365	2.7	0.0	Putative monooxygenase; mutation confers hypersensitivity to toxic ergosterol analog
DLD2	2.7	0.0	Ortholog(s) have D-lactate dehydrogenase (cytochrome) activity
orf19.5103	2.7	0.0	Protein with a predicted phosphoglycerate mutase family domain
orf19.6816	2.7	0.0	Putative xylose and arabinose reductase; flow model biofilm induced; Spider biofilm repressed
PFK2	2.6	0.0	Phosphofructokinase beta subunit; fructose 2,6-bisphosphate, AMP activated; ATP inhibited
SYG1	2.6	0.0	Ortholog(s) have role in signal transduction and plasma membrane localization
DAP1	2.6	0.0	Similar to mammalian membrane-associated progesterone receptors involved in DNA damage response
TPS3	2.6	0.0	Predicted trehalose-phosphate synthase regulatory subunit; regulated by Efg1
orf19.6637	2.6	0.0	Predicted glycosyl hydrolase; hypoxia induced; flow model biofilm induced
orf19.915	2.6	0.0	Protein of unknown function; Spider biofilm induced
orf19.1034	2.6	0.0	Protein with a predicted cytochrome b5-like Heme/Steroid binding domain; Hap43, caspofungin repressed; flow model biofilm induced
orf19.1654	2.6	0.0	Predicted membrane protein; induced by prostaglandins
TPS2	2.6	0.0	Trehalose-6-phosphate (Tre6P) phosphatase
orf19.3820	2.5	0.0	Protein with DNA binding domain, an endonuclease domain
PTP2	2.5	0.0	Predicted protein tyrosine phosphatase; involved in regulation of MAP kinase Hog1 activity
orf19.7196	2.5	0.0	Putative vacuolar protease; upregulated in the presence of human neutrophils; Spider biofilm induced
orf19.4013	2.5	0.0	Putative protein of unknown function; Hap43p-repressed gene; S. cerevisiae ortholog YHR045W localizes to the endoplasmic reticulum
PSA2	2.5	0.0	Mannose-1-phosphate guanyltransferase
GIG1	2.5	0.0	Protein induced by N-acetylglucosamine (GlcNAc)
FCY2	2.5	0.0	Purine-cytosine permease of pyrimidine salvage
NPR1	2.5	0.0	Predicted serine/threonine protein kinase, involved in regulation of ammonium transport; induced in core stress response; Hap43p-repressed gene
SAP10	2.4	0.0	Secreted aspartyl protease; roles in adhesion, virulence (RHE model), cell surface integrity; distinct specificity from Sap9; at cell membrane and wall; GPI-anchored; induced in low iron; Tbf1-activated; Spider biofilm induced
RBR2	2.4	0.0	Cell wall protein; expression repressed by Rim101
CIRT4B	2.4	0.0	Cirt family transposase
CMK1	2.4	0.0	Putative calcium/calmodulin-dependent protein kinase II
orf19.2866	2.4	0.0	Has domain(s) with predicted DNA binding, nucleic acid binding activity
HXK2	2.4	0.0	Hexokinase II; antigenic in humans; repressed by human neutrophils
orf19.5525	2.4	0.0	Putative oxidoreductase; protein levels affected by URA3 expression in CAI-4 strain background
orf19.3004	2.4	0.0	Ortholog of S. cerevisiae
GLK4	2.4	0.0	Putative glucokinase; decreased expression in hyphae compared to yeast-form cells
orf19.5114	2.4	0.0	Sorting nexin; role in maintaining late-Golgi resident enzymes in their proper location by recycling molecules from the prevacuolar compartment; Spider biofilm induced
HGT7	2.4	0.0	Putative MFS glucose transporter
orf19.5686	2.4	0.0	Protein of unknown function; Spider biofilm induced
orf19.2838	2.4	0.0	Protein of unknown function; mutation confers hypersensitivity to amphotericin B; flow model biofilm induced
GDH3	2.4	0.0	NADP-glutamate dehydrogenase; Nrg1, Plc1 regulatedexp and stationary phase protein; Spider biofilm repressed; rat catheter biofilm induced
HEM14	2.4	0.0	Putative protoporphyrinogen oxidase; involved in heme biosynthesis; predicted Kex2p substrate; iron regulated transcript; Yfh1-induced; Hap43-repressed; rat catheter biofilm repressed
orf19.1395	2.4	0.0	Ortholog(s) have copper ion transmembrane transporter activity
snR42a	2.4	0.0	H/ACA box small nucleolar RNA (snoRNA)
ATC1	2.3	0.0	Cell wall acid trehalase; catalyzes hydrolysis of the disaccharide trehalose
MDH1-3	2.3	0.0	Predicted malate dehydrogenase; farnesol regulated
orf19.3325	2.3	0.0	Putative glycogen synthesis initiator
POS5	2.3	0.0	Protein similar to *S. cerevisiae* Pos5p, a mitochondrial NADH kinase involved in the oxidative stress response

RDN25	2.3	0.0	25S ribosomal RNA
orf19.4589	2.3	0.0	Ortholog(s) have polyamine oxidase activity and role in pantothenate biosynthetic process, spermine catabolic process
RDN58	2.3	0.0	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit
AUT7	2.3	0.0	Putative autophagosome protein
orf19.6559	2.3	0.0	RNA polymerase III transcription initiation factor complex (TFIIIC) subunit
CSO99	2.3	0.0	Protein of unknown function; Hap43-repressed gene; protein not conserved in S. cerevisiae
GDB1	2.3	0.0	Putative glycogen debranching enzyme; expression is regulated upon white-opaque switch
TPS1	2.3	0.0	Trehalose-6-phosphate synthase; role in hyphal growth and virulence in mouse systemic infection
HRK1	2.3	0.0	Putative serine/threonine kinase; predicted role in cellular ion homeostasis; Spider biofilm repressed
GPI14	2.3	0.0	Catalytic subunit of glycosylphosphatidylinositol-alpha 1,4 mannosyltransferase I, involved in GPI anchor biosynthesis
UGA2	2.3	0.0	Predicted succinate semialdehyde dehydrogenase; predicted role in glutamate catabolism; transcription regulated by Mig1, Tup1, Gcn4; mutants are viable
orf19.7531	2.3	0.0	Protein of unknown function; stationary phase enriched protein
GLT1	2.2	0.0	Putative glutamate synthase; regulated by Sef1, Sfu1, and Hap43; rat catheter biofilm repressed
orf19.5136	2.2	0.0	Putative pyridoxamine 5'-phosphate oxidase; planktonic growth and early-stage flow model biofilm induced
PIR1	2.2	0.0	1,3-beta-glucan-linked cell wall protein; N-mannosylated, O-glycosylated by Pmt1; cell wall defect in het mutant; Hog1/fluconazole/hypoxia induced; iron/Efg1/Plc1/temp regulated
RIB3	2.2	0.0	3,4-Dihydroxy-2-butanone 4-phosphate synthase; homodimeric enzyme of riboflavin biosynthesis; converts ribulose 5-phosphate to L-3,4-dihydroxy-2-butanone 4-phosphate; transcription regulated o
GPH1	2.2	0.0	Putative glycogen phosphorylase; role in glycogen metabolism; regulated by Ssk1, Mig1, Tup1, Hap43
orf19.4523	2.2	0.0	Ortholog(s) have 5-formyltetrahydrofolate cyclo-ligase activity and role in folic acid-containing compound biosynthetic process
UPC2	2.2	0.0	Zn2-Cys6 transcript factor; regulator of ergosterol biosynthetic genes and sterol uptake
orf19.4617	2.2	0.0	Predicted peptide alpha-N-acetyltransferase; flow model biofilm induced
URA1	2.2	0.0	Dihydroorotate dehydrogenase; de novo pyrimidine biosynthesis
orf19.1557	2.2	0.0	Ortholog(s) have S-adenosylmethionine-dependent methyltransferase activity and role in protein methylation
orf19.6316	2.2	0.0	Predicted membrane transporter, member of the L-amino acid transporter-3 (LAT3) family, major facilitator superfamily (MFS)
orf19.6117	2.2	0.0	S. pombe ortholog SPAC5D6.04 is a predicted auxin family transmembrane transporter; ketoconazole and hypoxia induced
orf19.6440	2.2	0.0	Ortholog(s) have ubiquitin-protein transferase activity and role in cellular response to amino acid stimulus, transcription factor catabolic process, ubiquitin-dependent protein catabolic process
orf19.3053	2.2	0.0	Protein of unknown function; present in exponential and stationary phase yeast; identified in extracts from biofilm and planktonic cells; flow model biofilm induced gene; GlcNAc-induced protein
XKS1	2.1	0.0	Putative xylulokinase; Hap43-repressed; induced by prostaglandins; rat catheter biofilm repressed
orf19.7459	2.1	0.0	Putative mitochondrial protein with a predicted role in respiratory growth
RAD16	2.1	0.0	Ortholog of S. cerevisiae Rad16; a protein that recognizes and binds damaged DNA
GLC3	2.1	0.0	Putative 1,4-glucan branching enzyme; fluconazole-induced
VTC4	2.1	0.0	Putative polyphosphate synthetase; decreased expression in hyphae compared to yeast-form cells
orf19.7567	2.1	0.0	Protein of unknown function; induced by alpha pheromone in SpiderM medium
orf19.5841	2.1	0.0	Ortholog of C. dubliniensis CD36 : Cd36_17700, C. parapsilosis CDC317 : CPAR2_212340, C. auris
orf19.1461	2.1	0.0	S. pombe ortholog SPCC576.01c is a predicted sulfonate dioxygenase; possibly transcriptionally regulated upon hyphal formation; Spider biofilm induced
orf19.7204	2.1	0.0	Has domain(s) with predicted catalytic activity, nitronate monooxygenase activity
ADE13	2.1	0.0	Adenylosuccinate lyase; enzyme of adenine biosynthesis; soluble protein in hyphae; not induced during GCN response, in contrast to the S. cerevisiae ortholog; repressed by nitric oxide
YTH1	2.1	0.0	Putative mRNA cleavage and polyadenylation specificity factor; transcription is regulated upon yeast-hyphal switch
ISN1	2.1	0.0	Putative inosine 5'-monophosphate 5'-nucleotidase; fungal-specific (no human or murine homolog)
PHO113	2.1	0.0	Putative constitutive acid phosphatase; Rim101-repressed; DTT-extractable; N-glycosylated; possibly an essential gene, disruptants not obtained by UAU1 method
orf19.4595	2.1	0.0	Ortholog of C. dubliniensis CD36 : Cd36_41860, C. parapsilosis CDC317 : CPAR2_400440, C. auris B8441 : B9J08_002205 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_103033
orf19.2371	2.1	0.0	Putative Gag protein of retrotransposon Tca2

orf19.2401	2.0	0.0	Ortholog(s) have Atg8-specific protease activity
ECI1	2.0	0.0	Protein similar to S. cerevisiae Eci1p, which is involved in fatty acid oxidation
DAC1	2.0	0.0	N-acetylglucosamine-6-phosphate (GlcNAcP) deacetylase
BNA31	2.0	0.0	Putative arylformamidase, enzyme of the NAD biosynthesis pathway; Gcn4p-regulated
PLB4.5	2.0	0.0	Phospholipase B; Hog1-induced; regulated by Ssn6
orf19.3411	2.0	0.0	Ortholog of *S. cerevisiae*
MRF1	2.0	0.0	Putative mitochondrial respiratory protein
orf19.2047	2.0	0.0	Putative protein of unknown function
orf19.3881	2.0	0.0	Ortholog of C. dubliniensis CD36
FBA1	2.0	0.0	Fructose-bisphosphate aldolase
orf19.2350	2.0	0.0	Protein similar to *S. cerevisiae* Yor378w
orf19.7437	2.0	0.0	Putative protein of unknown function; Hap43p-repressed gene; ortholog of S. cerevisiae YJL218W
orf19.4795	2.0	0.0	Protein of unknown function; Sef1-, Sfu1-, and Hap43 regulated; Spider biofilm induced
LYS12	2.0	0.0	Homoisocitrate dehydrogenase
orf19.3395	2.0	0.0	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family; induced by nitric oxide, oxidative stress, alpha pheromone
orf19.993	2.0	0.0	Protein of unknown function; rat catheter biofilm repressed
ARE2	2.0	0.0	Acyl CoA:sterol acyltransferase; uses cholesterol and oleoyl-CoA substrates; protoberberine derivative drug inhibits enzyme activity; ketoconazole-induced
TGL99	2.0	0.0	Has domain(s) with predicted role in lipid metabolic process
ARA1	2.0	0.0	D-Arabinose dehydrogenase; dehydro-D-arabinono-1,4-lactone synthesis
orf19.4580	2.0	0.0	Protein of unknown function; Hap43-repressed gene
orf19.5626	2.0	0.0	Protein of unknown function; Plc1-regulated; induced by Mnl1 under weak acid stress; flow model biofilm induced
PDX3	2.0	0.0	Pyridoxamine-phosphate oxidase; transcript regulated by yeast-hypha switch and by Nrg1, Mig1, Tup1; Hap43, caspofungin repressed; present in exponential and stationary phase yeast cultures
PRB1	2.0	0.0	Endoprotease B; regulated by heat, carbon source (GlcNAc-induced), nitrogen, macrophage response
MLS1	2.0	0.0	Malate synthase; glyoxylate cycle enzyme
PCL7	2.0	0.0	Putative cyclin-like protein; possible Pho85 cyclin; hyphal repressed; induced by Mnl1 under weak acid stress
CDC34	2.0	0.0	Putative ubiquitin-protein ligase; transcript regulated by Nrg1 and Tup1, and by Gcn2 and Gcn4; rat catheter biofilm induced
PDC11	2.0	0.0	Pyruvate decarboxylase; antigenic; on hyphal not yeast cell surface
MSO1	2.0	0.0	Putative secretory protein involved in S. cerevisiae sporulation
CUP1	2.0	0.0	Metallothionein; involved in copper resistance; copper induced; Spider biofilm induced; flow model biofilm repressed
IFE1	2.0	0.0	Putative medium-chain alcohol dehydrogenase; rat catheter and Spider biofilm repressed
orf19.5727	1.9	0.0	Ortholog of C. dubliniensis CD36 : Cd36_64130, C. parapsilosis CDC317 : CPAR2_601230, C. auris B8441 : B9J08_003898 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_115908
DES1	1.9	0.0	Putative delta-4 sphingolipid desaturase; planktonic growth-induced gene
TPI1	1.9	0.0	Triose-phosphate isomerase; antigenic in mouse/human; mutation affects filamentation; macrophage-repressed; protein in exponential and stationary growth phase yeast
orf19.1406	1.9	0.0	Ortholog(s) have DNA-directed DNA polymerase activity, role in error-free translesion synthesis, error-prone translesion synthesis and mitochondrion, zeta DNA polymerase complex localization
orf19.5814.1	1.9	0.0	Protein of unknown function
GRP2	1.9	0.0	NAD(H)-linked methylglyoxal oxidoreductase involved in regulation of methylglyoxal and pyruvate levels; regulation associated with azole resistance
orf19.7457	1.9	0.0	Protein with Mob2p-dependent hyphal regulation
SMF3	1.9	0.0	Putative vacuolar iron transporter; alkaline upregulated; caspofungin repressed; induced by Mnl1 under weak acid stress; Hap43-repressed
BPH1	1.9	0.0	Ortholog of S. cerevisiae Bph1; a putative ortholog of human Chediak-Higashi syndrome protein and murine beige gene implicated in disease syndromes involving defective lysosomal trafficking; mutant is viable
orf19.7445	1.9	0.0	Ortholog of S.c. Vid24; a peripheral membrane protein located at Vid (vacuole import and degradation) vesicles; regulated by Sef1, Sfu1, and Hap43; Spider biofilm induced
UBC8	1.9	0.0	Predicted ubiquitin-conjugating enzyme that negatively regulates gluconeogenesis
FMP27	1.9	0.0	Putative mitochondrial protein; mRNA binds She3

orf19.5527	1.9	0.0	Protein with a predicted role in 5.8S rRNA processing; flow model biofilm induced
orf19.6498	1.9	0.0	Ortholog(s) have role in nucleocytoplasmic transport, protein import into nucleus and cytoplasm, nucleus localization
PEX5	1.9	0.0	Pex5p family protein; required for PTS1-mediated peroxisomal protein import, fatty acid beta-oxidation
orf19.4828	1.9	0.0	WD repeat domain protein; Hap43-repressed gene; flow model biofilm induced
orf19.6527	1.9	0.0	Pheromone-regulated protein (Prm10) of S. cerevisiae; colony morphology-related gene regulation by Ssn6; induced by Mnl1 under weak acid stress
BNA32	1.9	0.0	Has domain(s) with predicted catalytic activity, pyridoxal phosphate binding activity and role in biosynthetic process
LPG20	1.9	0.0	Aldo-keto reductase family protein; similar to aryl alcohol dehydrogenases
ECM38	1.9	0.0	Putative gamma-glutamyltransferase; alkaline upregulated; Spider biofilm induced
CDC19	1.9	0.0	Pyruvate kinase at yeast cell surface; Gcn4/Hog1/GlcNAc regulated
DOT5	1.9	0.0	Putative nuclear thiol peroxidase; alkaline downregulated; sumoylation target
orf19.321	1.9	0.0	Ortholog(s) have L-methionine transmembrane transporter activity and role in methionine import across plasma membrane
orf19.4699	1.9	0.0	Putative phospholipase of patatin family; similar to S. cerevisiae Tgl3p; predicted Kex2p substrate
ZCF24	1.9	0.0	Predicted Zn(II)2Cys6 transcription factor; caspofungin induced; Hap43-repressed
ERG3	1.9	0.0	C-5 sterol desaturase; introduces C-5(6) double bond into episterol
orf19.7405	1.9	0.0	Ortholog of Rad33; involved in nucleotide excision repair in S. cerevisiae
HGT3	1.9	0.0	Putative glucose transporter of the major facilitator superfamily
orf19.5773	1.9	0.0	Putative dipeptidyl-peptidase III
PGK1	1.9	0.0	Phosphoglycerate kinase; localizes to cell wall and cytoplasm; antigenic in murine/human infection; flow model biofilm, Hog1-, Hap43-, GCN-induced
GYP7	1.8	0.0	Protein similar to S. cerevisiae Gyp7p (GTPase-activating protein for Ypt1p); caspofungin-induced
orf19.6396	1.8	0.0	Putative patatin-like phospholipase; similar to S. cerevisiae Nte1p, which is predicted to be a membrane protein; antigenic during human oral infection; Hap43p-repressed gene
PST2	1.8	0.0	Flavodoxin-like protein involved in oxidative stress protection and virulence; putative NADH:quinone oxidoreductase; similar to 1,4-benzoquinone reductase; induced by benomyl, oxidative stress via Cap1; fungal-specific
orf19.5572	1.8	0.0	Protein of unknown function; Spider biofilm repressed
PGM2	1.8	0.0	Ortholog of S. cerevisiae Pgm2; induced in planktonic culture; Tye7p-regulated; flow model biofilm induced; rat catheter biofilm repressed
orf19.6065	1.8	0.0	RNA polymerase II holoenzyme/mediator subunit; regulated by Mig1, Tup1; amphotericin B, caspofungin repressed; protein present in exponential and stationary growth phase yeast; Hap43-repressed; Spider biofilm repressed
HGT18	1.8	0.0	Putative glucose transporter of the major facilitator superfamily; the *C. albicans* glucose transporter family comprises 20 members; 12 probable membrane-spanning segments; expressed in rich medium with 2% glucose
orf19.1107	1.8	0.0	Protein of unknown function; Spider biofilm induced
PRC3	1.8	0.0	Putative carboxypeptidase Y precursor; transcript regulated by Nrg1 and Mig1; regulated by Gcn2 and Gcn4
orf19.1369	1.8	0.0	Protein with predicted peptidase domains; Hap43-repressed gene
orf19.3810	1.8	0.0	Ortholog(s) have methylenetetrahydrofolate dehydrogenase (NAD+) activity, role in folic acid-containing compound biosynthetic process, one-carbon metabolic process, purine nucleobase biosynthetic process and cytosol localization

Appendix 11: RNA-seq result of Fgr17-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Fgr17-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.5730	7.6	0.0	Putative phenylacrylic acid decarboxylase; clade-associated gene expression
PAD1	6.9	0.0	Putative phenylacrylic acid decarboxylase; repressed by Rgt1p
HMX1	3.0	0.0	Heme oxygenase; utilization of hemin iron; transcript induced by heat, low iron, or hemin
BIO5	2.9	0.0	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method
ALS2	2.7	0.0	ALS family protein; role in adhesion, biofilm formation, germ tube induction; expressed at infection of human buccal epithelial cells
GPX2	2.6	0.0	Similar to glutathione peroxidase; induced in high iron; alkaline induced by Rim101
orf19.1258	2.5	0.0	Adhesin-like protein; regulated by Tsa1, Tsa1B in minimal media at 37 deg; clade-associated gene expression
NUP	2.4	0.0	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not
SNZ1	2.0	0.0	Stationary phase protein; vitamin B synthesis; induced byyeast-hypha switch, 3-AT or in azole-resistant strain overexpressing MDR1
CAN3	1.9	0.0	Predicted amino acid transmembrane transporter; transcript regulated by white-opaque switch; Hap43-repressed gene
BIO3	1.9	0.0	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis
VHT1	1.8	0.0	Predicted membrane transporter, involved in biotin import
ALS4	1.8	0.0	GPI-anchored adhesin; role in adhesion, germ tube induction; growth, temperature regulated; expressed during infection of human buccal epithelial cells; repressed by vaginal contact; biofilm induced; repressed during chlamydospore formation
ADE13	1.8	0.0	Adenylosuccinate lyase; enzyme of adenine biosynthesis; soluble protein in hyphae; not induced during GCN response, in contrast to the S. cerevisiae ortholog; repressed by nitric oxide
MLS1	1.6	0.0	Malate synthase; glyoxylate cycle enzyme; no mammalian homolog; regulated upon white-opaque switch; phagocytosis, strong oxidative stress induced; stationary phase enriched; flow model biofilm repressed; rat catheter, Spider biofilm induced
BIO4	1.5	0.0	Putative dethiobiotin synthetase; transcript upregulated in clinical isolates from HIV+ patients with oral candidiasis; Hap43-repressed; GlcNAc-induced protein; Spider biofilm induced; biotin-dependent transcription regulated by Vhr1p
ITS2	1.5	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25; in S. cerevisiae it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species

Appendix 12: RNA-seq result of Fgr27 -GOF. Differentially expressed Up-regulated genes in *Candida albicans* Fgr27 -GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
HGC1	17.9	0.00	Hypha-specific G1 cyclin-related protein involved in regulation of morphogenesis, biofilm formation
orf19.2247	15.5	0.00	Ortholog of C. dubliniensis CD36 : Cd36_21220, C. parapsilosis CDC317 : CPAR2_406700, Candida tenuis NRRL Y-1498
orf19.1539	11.8	0.05	Protein of unknown function; F-12/CO2 early biofilm induced
HWP1	10.3	0.01	Hyphal cell wall protein; host transglutaminase substrate; opaque-, a-specific, alpha-factor induced; at MTLa side of conjugation tube
MDR1	8.6	0.00	Plasma membrane MDR/MFS multidrug efflux pump
RMS1	7.4	0.00	Putative lysine methyltransferase; Hap43-induced
orf19.4476	7.3	0.00	Protein with a NADP-dependent oxidoreductase domain; transcript induced by ketoconazole; rat catheter and Spider biofilm induced
CTN1	7.3	0.03	Carnitine acetyl transferase; required for growth on nonfermentable carbon sources, not for hyphal growth or virulence in mice
orf19.6592	7.2	0.00	Predicted membrane transporter, member of the aromatic acid:proton symporter (AAHS) family, major facilitator superfamily (MFS)
HGT1	7.1	0.00	High-affinity MFS glucose transporter; induced by progesterone, chloramphenicol, benomyl
orf19.2281	7.0	0.00	Has domain(s) with predicted CoA-transferase activity and role in metabolic process
orf19.1438	6.8	0.01	Protein with homology to NADH dehydrogenase; regulated by Sef1p-, Sfu1p-, and Hap43p
orf19.7279.1	6.3	0.01	Protein of unknown function; Spider biofilm induced

orf19.3337	6.1	0.00	Protein of unknown function; merged with orf19.3338; rat catheter, flow and Spider model biofilm induced
TNA1	5.9	0.00	Putative nicotinic acid transporter; detected at germ tube plasma membrane by mass spectrometry
orf19.7495	5.8	0.00	Protein with NADPH oxidoreductase containing flavin mononucleotide (FMN) domain; induced by nitric oxide
WOR3	5.7	0.00	Transcription factor; modulator of white-opaque switch; induced in opaque cells; promoter bound by Wor1; overexpression at 25 degr shifts cells to opaque state; deletion stabilizes opaque cells at higher temperatures; Spider biofilm induced
GRE2	5.6	0.00	Putative reductase; Nrg1 and Tup1-regulated; benomyl- and hyphal-induced; macrophage/pseudohyphal-repressed
BRG1	5.5	0.00	Transcription factor; recruits Hda1 to hypha-specific promoters; Tn mutation affects filamentation
RBR1	5.5	0.04	Glycosylphosphatidylinositol (GPI)-anchored cell wall protein; required for filamentous growth at acidic pH; expression repressed by Rim101 and activated by Nrg1; Hap43-induced
MAL31	5.4	0.03	Putative high-affinity maltose transporter; transcript is upregulated in clinical isolates from HIV+ patients with oral candidiasis; alkaline induced; Spider biofilm induced
ALS1	5.2	0.00	Cell-surface adhesin; adhesion, virulence, immunoprotective roles; band at hyphal base; Rfg1, Ssk1, Spider biofilm induced; flow model biofilm repressed
orf19.5290	4.9	0.03	Protein of unknown function; repressed by Sfu1; Hap43-induced gene
WH11	4.8	0.00	White-phase yeast transcript; expression in opaques increases virulence/switching; mutant switches as WT; Hap43, hypoxia, ketoconazol induced; required for RPMI biofilm
orf19.7306	4.8	0.00	Aldo-keto reductase; increased transcript associated with MDR1 overexpression, benomyl or long-term fluconazole treatment; overexpression does not affect drug or oxidative stress sensitivity
RBR3	4.5	0.00	Cell wall adhesin-like protein; repressed by Rim101; possibly an essential gene, disruptants not obtained by UAU1 method
OYE22	4.4	0.01	Putative NADPH dehydrogenase; rat catheter biofilm induced
ICL1	4.4	0.00	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice; induced upon phagocytosis by macrophage; farnesol regulated; Pex5-dependent peroxisomal localization
orf19.2059	4.2	0.00	Protein with homology to magnesium-dependent endonucleases and phosphatases; regulated by Sef1, Sfu1, and Hap43; Spider biofilm induced
FGR27	4.1	0.00	Zn(II)2Cys6 transcription factor; transposon mutation affects filamentous growth; required for yeast cell adherence to silicone substrate; Spider biofilm induced
ASM3	4.1	0.00	Putative secreted acid sphingomyelin phosphodiesterase; possible Kex2 substrate; transcript increased in an azole-resistant strain that overexpresses MDR1
ARO9	4.1	0.00	Aromatic transaminase; Ehrlich fusel oil pathway of aromatic alcohol biosynthesis; Rim101-dependent pH-regulation (alkaline induced); Hap43-induced gene
orf19.1409.2	4.0	0.00	Protein of unknown function; Spider biofilm induced
orf19.3639	4.0	0.00	Ortholog(s) have DNA-3-methyladenine glycosylase activity, alkylbase DNA N-glycosylase activity, damaged DNA binding activity
orf19.3439	3.8	0.01	Protein of unknown function; Cyr1-repressed; rat catheter and Spider biofilm induced
ASR2	3.7	0.00	Adenylyl cyclase and stress responsive protein; induced in cyr1 or ras1 mutant; stationary phase enriched protein; Spider biofilm induced
ECM331	3.7	0.00	GPI-anchored protein; mainly at plasma membrane, also at cell wall; Hap43, caspofungin-induced; Plc1-regulated; Hog1, Rim101-repressed; colony morphology-related regulated by Ssn6
orf19.1277	3.6	0.00	Protein of unknown function; Rgt1, Hap43-repressed; flow model biofilm induced
orf19.2607	3.5	0.00	Protein of unknown function; Spider biofilm induced
FAA2-1	3.5	0.00	Predicted long chain fatty acid CoA ligase; upregulated upon phagocytosis; induced by nitric oxide independent of Yhb1
GCA2	3.4	0.01	Predicted extracellular glucoamylase; induced by ketoconazole; possibly essential, disruptants not obtained by UAU1 method; promotes biofilm matrix formation
GCA1	3.3	0.03	Extracellular/plasma membrane-associated glucoamylase; expressed in rat oral infection; regulated by carbohydrates, pH, galactose; promotes biofilm matrix formation
GAC1	3.3	0.00	Putative regulatory subunit of ser/thr phosphoprotein phosphatase 1; fluconazole-induced; caspofungin repressed; transcript induced by Mnl1 under weak acid stress
orf19.7380	3.2	0.00	Has domain(s) with predicted nucleic acid binding, nucleotide binding activity
SFC1	3.1	0.00	Putative succinate-fumarate transporter; involved in repression of growth on sorbose
CPA1	3.1	0.01	Putative carbamoyl-phosphate synthase subunit; alkaline repressed
LYS143	3.1	0.00	Zn(II)2Cys6 transcription factor; ortholog of S. cerevisiae Lys14 involved in the regulation of lysine biosynthesis genes
CHK1	3.1	0.00	Histidine kinase; 2-component signaling, cell wall synthesis; hyphal growth defect
ALS3	3.1	0.00	Cell wall adhesin; epithelial adhesion, endothelial invasion; alleles vary in adhesiveness
HRQ2	3.1	0.01	Protein of unknown function; mutants are viable; rat catheter and Spider biofilm induced
GST2	3.0	0.00	Glutathione S transferase

orf19.5817	3.0	0.00	Ortholog(s) have role in cytoplasm to vacuole transport by the Cvt pathway, endoplasmic reticulum to Golgi vesicle-mediated transport, intra-Golgi vesicle-mediated transport, macroautophagy
orf19.1887	3.0	0.00	Ortholog(s) have sterol esterase activity, role in sterol metabolic process and integral component of membrane, lipid droplet localization
orf19.6705	2.9	0.00	Putative guanyl nucleotide exchange factor with Sec7 domainÀ
orf19.1344	2.9	0.01	Protein of unknown function; fluconazole-induced; Spider biofilm induced
orf19.1608	2.9	0.03	Has domain(s) with predicted catalytic activity, sulfuric ester hydrolase activity and role in metabolic process
DDR48	2.9	0.00	Immunogenic stress-associated protein; filamentation regulated; induced by benomyl/caspofungin/ketoconazole or in azole-resistant strain
OYE32	2.8	0.00	NAD(P)H oxidoreductase family protein; induced by nitric oxide, amphotericin B, oxidative stress via Cap1
orf19.1496	2.8	0.00	Putative transcription factor with zinc finger DNA-binding motif; Hap43p-repressed gene
orf19.1340	2.8	0.00	Putative aldose reductase; protein level decreases in stationary phase cultures; Spider biofilm repressed
IFR2	2.8	0.00	Zinc-binding dehydrogenase; induced by benomyl, ciclopirox olamine, alpha pheromone, Hap43; regulated by oxidative stress via Cap1, osmotic stress via Hog1
TOK1	2.7	0.00	Outwardly rectifying, noisily gated potassium channel; modulates sensitivity to human salivary histatin (Hst5); very similar to S. cerevisiae Tok1p; Bcr1-repressed in RPMI a/a biofilms
orf19.4583	2.7	0.01	Protein with a mitochondrial carrier protein domain; possibly an essential gene, disruptants not obtained by UAU1 method; Spider biofilm repressed
RFX2	2.6	0.01	Transcriptional repressor; regulator of filamentation, response to DNA damage, adhesion, virulence in murine mucosal, systemic infections; RFX domain; regulated by Nrg1, UV-induced; partially complements S. cerevisiae rfx1 mutant defects
FRP3	2.6	0.00	Putative ammonium transporter; upregulated in the presence of human neutrophils; fluconazole-downregulated; repressed by nitric oxide; Spider biofilm induced; rat catheter biofilm repressed
orf19.4216	2.6	0.01	Putative heat shock protein; decreased expression in hyphae; transcription is increased in populations of cells exposed to fluconazole over multiple generations; overexpression increases resistance to farnesol and azoles
HSP12	2.6	0.01	Heat-shock protein; induced by osmotic/oxidative/cadmium stress, fluphenazine treatment, low iron, CDR1 and CDR2 overexpression, or ssn6 or ssk1 null mutation; overexpression increases resistance to farnesol and azoles
orf19.5210	2.6	0.01	Putative Xbp1 transcriptional repressor; binds to cyclin gene promoters in S. cerevisiae; Hap43-repressed; possibly essential, disruptants not obtained by UAU1 method
orf19.4530.1	2.6	0.00	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
PGA55	2.5	0.00	GPI-anchored adhesin-like protein; filament induced; regulated by Nrg1, Tup1; regulated upon hyphal formation; mRNA binds to She3 and is localized to yeast-form buds and hyphal tips; induced during chlamydospore formation
HMX1	2.5	0.00	Heme oxygenase; utilization of hemin iron; transcript induced by heat, low iron, or hemin; repressed by Efg1; induced by low iron; upregulated by Rim101 at pH 8; Hap43-induced; Spider and flow model biofilm induced
ARE2	2.5	0.00	Acyl CoA:sterol acyltransferase; uses cholesterol and oleoyl-CoA substrates; protoberberine derivative drug inhibits enzyme activity; ketoconazole-induced; Hap43-repressed; flow model biofilm induced; Spider biofilm induced
orf19.1933	2.5	0.00	Ortholog(s) have role in ER-dependent peroxisome organization, peroxisome organization and endoplasmic reticulum, peroxisomal membrane, peroxisome localization
GPX2	2.5	0.00	Similar to glutathione peroxidase; induced in high iron; alkaline induced by Rim101; induced by alpha factor or interaction with macrophage; regulated by Efg1; caspofungin repressed; Spider biofilm induced
ZCF6	2.5	0.01	Putative transcription factor with zinc cluster DNA-binding motif; involved in virulence
BIO5	2.5	0.00	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method; Spider biofilm induced; transcription regulated by biotin and Vhr1p
orf19.4423	2.5	0.00	Putative glucosyltransferase; localized to the mitochondrial membrane
NTH1	2.4	0.00	Neutral trehalase; hyphal induction in mutant delayed but not reduced overall; not required for virulence in mice; possible regulatory cAMP-dependent phosphorylation at S10,S213; Hap43-repressed gene; Spider biofilm induced
DCK1	2.4	0.00	Putative guanine nucleotide exchange factor; required for embedded filamentous growth; activates Rac1; has a DOCKER domain; similar to adjacent DCK2 and to S. cerevisiae Ylr422wp; regulated by Nrg1; Spider biofilm induced
ENA21	2.4	0.00	Predicted P-type ATPase sodium pump; Gcn4p-regulated; flucytosine, amphotericin B, or ketoconazole-induced; osmotic stress-induced; overlaps orf19.5170.1, which is annotated as a blocked reading frame; Spider biofilm induced
ATS1	2.4	0.00	Protein required for modification of wobble nucleosides in tRNA; induced upon adherence to polystyrene; regulated by Sef1, Sfu1, and Hap43

GLX3	2.4	0.01	Glutathione-independent glyoxalase; binds human immunoglobulin E; alkaline, fluconazole, Hog1 repressed; hypoxia, oxidative stress via Cap1, Hap43 induced; stationary-phase enriched; rat catheter, Spider biofilm induced
CRH11	2.4	0.00	GPI-anchored cell wall transglycosylase, putative ortholog of S. cerevisiae Crh1p; predicted glycosyl hydrolase domain; similar to Csf4p and to antigenic A. fumigatus Aspf9; predicted Kex2p substrate; caspofungin-induced
AVT7	2.4	0.00	Ortholog of S. cerevisiae Avt7 transporter; repressed upon adherence to polystyrene; constitutive expression independent of MTL or white-opaque status; Spider biofilm induced
orf19.4150	2.4	0.00	Putative glutaredoxin; induced by nitric oxide; Spider biofilm induced
orf19.5620	2.4	0.00	Stationary phase enriched protein; Gcn4-regulated; induced by amino acid starvation (3-AT), benomyl or in azole-resistant strain that overexpresses MDR1; flow model biofilm induced; rat catheter biofilm repressed; overlaps orf19.5621
TEC1	2.4	0.00	TEA/ATTS transcription factor; white cell pheromone response, hyphal gene regulation; required for Spider and RPMI biofilm formation; regulates BCR1; Cph2 regulated transcript; alkaline, rat catheter, Spider, flow model biofilm induced
orf19.7648	2.4	0.00	Has domain(s) with predicted antiporter activity, xenobiotic transmembrane transporter activity, role in drug transmembrane transport and membrane localization
orf19.7235	2.4	0.00	Putative protein of unknown function; mutation confers hypersensitivity to amphotericin B
orf19.215	2.3	0.00	Component of a complex containing the Tor2p kinase; possible a role in regulation of cell growth; Spider biofilm induced
orf19.7499.1	2.3	0.02	Ortholog of C. parapsilosis CDC317 : CPAR2_800380, Candida tenuis NRRL Y-1498 : CANTEDRAFT_114079, Pichia stipitis Pignal : psti_CGOB_00127 and Candida tropicalis MYA-3404 : CTRG_05490
RIB1	2.3	0.00	GTP cyclohydrolase II, rate-limiting enzyme of riboflavin biosynthesis; fungal-specific (no human or murine homolog); regulated by Sef1p, Sfu1p, and Hap43p
orf19.310	2.3	0.00	Putative mitochondrial protein with a predicted role in cell wall biogenesis; possibly an essential gene, disruptants not obtained by UAU1 method
orf19.4901	2.3	0.01	Predicted methyltransferase; Spider biofilm induced
VHT1	2.3	0.00	Predicted membrane transporter, involved in biotin import; member of the anion:cation symporter (ACS) family, major facilitator superfamily (MFS); biotin-dependent transcription regulated by Vhr1p; amphotericin B, caspofungin repressed
SEO1	2.3	0.01	Protein with similarity to permeases; Sfu1-repressed; flucytosine induced; induced by Mnl1 under weak acid stress; flow model biofilm repressed
PST3	2.3	0.00	Flavodoxin-like protein involved in oxidative stress protection and virulence; YNB biofilm induced; stationary phase enriched protein; rat catheter and Spider biofilm repressed
PRN2	2.3	0.00	Protein similar to pirin; Hap43p-repressed gene
ECM18	2.3	0.00	Ortholog of S. cerevisiae : ECM18, C. glabrata CBS138 : CAGL0B01969g, C. parapsilosis CDC317 : CPAR2_103190, C. auris B8441 : B9J08_000758 and Debaryomyces hansenii CBS767 : DEHA2G08448g
GOR1	2.2	0.00	Ortholog(s) have glyoxylate reductase (NAD+) activity, role in glyoxylate catabolic process and extracellular region localization
POT1-2	2.2	0.00	Putative peroxisomal 3-ketoacyl CoA thiolase; Hap43-repressed
NUP	2.2	0.01	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not; similar to a nucleoside permease of S. pombe; possibly processed by Kex2p
PST1	2.2	0.00	Flavodoxin-like protein involved in oxidative stress protection and virulence; putative 1,4-benzoquinone reductase; hyphal-induced; regulated by Cyr1, Ras1, Efg1, Nrg1, Rfg1, Tup1; Hap43-induced; Spider biofilm induced
YMX6	2.2	0.00	Putative NADH dehydrogenase; macrophage-downregulated gene; induced by nitric oxide; rat catheter biofilm induced
orf19.1368	2.1	0.03	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
orf19.1486	2.1	0.00	Protein with a life-span regulatory factor domain; regulated by Sef1, Sfu1, and Hap43; flow model biofilm induced; Spider biofilm induced
NCE103	2.1	0.01	Carbonic anhydrase; converts of CO2 to bicarbonate; essential for virulence in host niches with limited CO2, normal white-opaque switch; Mnl1-induced in weak acid stress; Hap43-induced gene; F-12/CO2, rat catheter, Spider biofilm induced
MNN42	2.1	0.00	Protein of unknown function; repressed by Rim101; negatively modulates intracellular ATP levels during the development of azole resistance; induced by Ca(2+) in a calcineurin-dependent manner; Spider biofilm induced
ARO8	2.1	0.00	Aromatic transaminase of the Ehrlich fusel oil pathway of aromatic alcohol biosynthesis; Rim101 independent alkaline induction; protein abundance affected by URA3 expression in CAI-4 strain; Gcn4-regulated; stationary phase enriched
ZFU2	2.1	0.00	Zn(II)2Cys6 transcription factor
PRC2	2.1	0.02	Putative carboxypeptidase; induced by human neutrophils; Spider biofilm induced

orf19.850	2.0	0.00	Ortholog(s) have protein-N-terminal asparagine amidohydrolase activity, protein-N-terminal glutamine amidohydrolase activity and role in N-terminal protein amino acid modification, protein catabolic process
FAD3	2.0	0.01	Omega-3 fatty acid desaturase; production of alpha-linolenic acid, a major component of membranes
orf19.2165	2.0	0.00	Predicted hydrolase; induced by nitric oxide
orf19.1440.2	2.0	0.02	Ortholog of C. dubliniensis CD36 : Cd36_62310 and Candida tropicalis NEW ASSEMBLY : CTRG1_CGOB_00040
orf19.3219	2.0	0.00	Ortholog of S. cerevisiae Sia1
orf19.6941	2.0	0.00	Putative diacylglycerol acyltransferase; catalyzes the terminal step of triacylglycerol formation
orf19.5730	2.0	0.00	Putative phenylacrylic acid decarboxylase; clade-associated gene expression
orf19.2959.1	1.9	0.01	Gene induced by hypoxia and ketoconazole; oral infection upregulated; mutants have reduced capacity to damage oral epithelial cells
orf19.7615	1.9	0.01	Protein involved in endoplasmic reticulum (ER) to Golgi vesicle-mediated transport
orf19.5491	1.9	0.02	Ortholog of C. dubliniensis CD36 : Cd36_20670, C. parapsilosis CDC317 : CPAR2_104720, C. auris B8441 : B9J08_000085 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_103989
orf19.2988	1.9	0.00	Predicted aminotransferase based on S. pombe ortholog SPBC660.12c; flow model biofilm induced
HGT4	1.9	0.01	Glucose and galactose sensor; fermentation, filamentation, virulence roles; 20-member glucose transporter family
orf19.4756	1.9	0.01	Ortholog of S. cerevisiae : YTP1, C. dubliniensis CD36 : Cd36_08490, C. parapsilosis CDC317 : CPAR2_801590, C. auris B8441
BIO3	1.9	0.00	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis; transcription regulated by biotin availability and Vhr1p
DLD1	1.9	0.00	Putative D-lactate dehydrogenase; white cell-specific trancript; colony morphology-related gene regulation by Ssn6
RAS1	1.9	0.00	RAS signal transduction GTPase; regulates cAMP and MAP kinase pathways
ARG5,6	1.9	0.01	Arginine biosynthetic enzyme; processed in S. cerevisiae into 2 polypeptides with acetylglutamate kinase (Arg6) activity and acetylglutamate-phosphate reductase (Arg5) activity; Gcn4 regulated; alkaline repressed; Spider biofilm induced
SAC7	1.9	0.00	Putative GTPase activating protein (GAP) for Rho1; repressed upon adherence to polystyrene
RHO2	1.8	0.00	Ortholog(s) have GTPase activity and role in establishment or maintenance of actin cytoskeleton polarity, regulation of fungal-type cell wall (1->3)-alpha-glucan biosynthetic process
orf19.4142	1.8	0.04	Putative transporter; decreased transcription is observed upon fluphenazine treatment
CPA2	1.8	0.02	Putative arginine-specific carbamoylphosphate synthetase; protein enriched in stationary phase yeast cultures; rat catheter biofilm induced; Spider biofilm induced
orf19.4384.1	1.8	0.00	Putative MFS transporter; predicted ORF in Assembly 20; removed from Assembly 21; restored based on conservation among several Candida species
PUT3	1.8	0.00	Zn(II)2Cys6 transcription factor; has similarity to S. cerevisiae Put3, a transcription factor involved in the regulation of proline utilization genes
GRF10	1.8	0.01	Putative homeodomain transcription factor, involved in control of filamentous growth; null mutant is an adenine auxotroph; Spider biofilm induced; promoter bound by Bcr1, Tec1, Efg1, Ndt80 and Brg1
orf19.4864	1.8	0.00	Ortholog(s) have acylglycerol lipase activity, role in triglyceride metabolic process and lipid droplet, membrane localization
orf19.3473	1.8	0.00	Ortholog(s) have enzyme activator activity, histone acetyltransferase activity, structural molecule activity
BMT4	1.8	0.01	Beta-mannosyltransferase; for elongation of beta-mannose chains on the acid-labile fraction of cell wall phosphopeptidomannan
CTA8	1.8	0.00	Essential transcription factor, mediates heat shock transcriptional induction
PAD1	1.8	0.04	Putative phenylacrylic acid decarboxylase; repressed by Rgt1p
RBT4	1.8	0.00	Pry family protein; required for virulence in mouse systemic/rabbit corneal infections; not filamentation
orf19.3644	1.8	0.00	Protein of unknown function; Cyr1-repressed; rat catheter and Spider biofilm induced
GPH1	1.8	0.05	Putative glycogen phosphorylase; role in glycogen metabolism; regulated by Ssk1, Mig1, Tup1, Hap43; fluconazole-induced
orf19.2726	1.8	0.02	Putative plasma membrane protein; Plc1-regulated; Spider biofilm induced
orf19.2987	1.8	0.00	Ortholog(s) have role in cellular ion homeostasis, mitochondrion inheritance, mitochondrion organization, regulation of cardiolipin metabolic process and mitochondrial inner membrane, mitochondrion localization
orf19.511	1.7	0.00	Ortholog(s) have ribosylnicotinamide kinase activity and role in NAD biosynthesis via nicotinamide riboside salvage pathway, NAD biosynthetic process, nicotinamide riboside metabolic process
orf19.7504	1.7	0.00	Ortholog of S. cerevisiae Rts3; a component of the protein phosphatase type 2A complex; Plc1-regulated; induced in core caspofungin response; Spider biofilm induced

SCS7	1.7	0.00	Putative ceramide hydroxylase; regulated by Nrg1; induced in high iron
LEU42	1.7	0.00	Putative alpha-isopropylmalate synthase; fungal-specific; induced by human blood or polymorphonuclear cells
orf19.4807	1.7	0.00	Ortholog(s) have inorganic diphosphatase activity, role in aerobic respiration and mitochondrion localization
PRN1	1.7	0.02	Protein with similarity to pirins; induced by benomyl and in response to alpha pheromone in SpiderM medium
orf19.1855	1.7	0.01	Predicted membrane transporter, member of the anion:cation symporter (ACS) family, major facilitator superfamily (MFS)
orf19.7297	1.7	0.00	Putative cystathionine gamma-synthase; decreased levels in stationary phase cultures; Hog1p-induced; Gcn4p-regulated
orf19.6869	1.7	0.00	Putative lipid raft associated protein; Spider biofilm induced
ECM4	1.7	0.00	Cytoplasmic glutathione S-transferase; regulated by Nrg1, Tup1
UGT51C1	1.7	0.00	UDP-glucose:sterol glucosyltransferase; enzyme of sterol glucoside (membrane-bound lipid) biosynthesis
orf19.3142	1.7	0.01	Ortholog of C. dubliniensis CD36 : Cd36_46140, C. parapsilosis CDC317 : CPAR2_501210, C. auris B8441 : B9J08_001807 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_97195
orf19.2124	1.7	0.01	Predicted alcohol dehydrogenase; Spider biofilm induced
orf19.2244	1.6	0.02	Similar to oxidoreductases and to *S. cerevisiae* Yjr096wp; Sfu1 repressed; induced by benomyl treatment, Ssr1; Hap43-repressed; flow model biofilm repressed
orf19.5777	1.6	0.00	Protein of unknown function; F-12/CO2 early biofilm induced
orf19.7566	1.6	0.04	Predicted amino acid transport domain; transcript upregulated in clinical strains from HIV+ patients with oral candidiasis; alkaline upregulated by Rim101; rat catheter, Spider and flow model biofilm induced
orf19.4132	1.6	0.01	Protein of unknown function; UPF0057 protein family member; localizes to the plasma membrane; Spider biofilm induced
GAD1	1.6	0.01	Putative glutamate decarboxylase; alkaline, macrophage-downregulated gene; amphotericin B induced; induced by Mnl1 under weak acid stress; stationary phase enriched protein; rat catheter biofilm repressed
VHR1	1.6	0.01	Transcriptional activator of genes involved in biotin metabolism; required for survival and proliferation in macrophages; expression upregulated during growth in the mouse cecum; Spider biofilm induced
LYS22	1.6	0.02	Homocitrate synthase, minor isoform; repressed by nitric oxide and by hypoxia; protein level decreases in stationary phase cultures; induced by ketoconazole, Spider biofilm induced; flow model biofilm repressed
orf19.4898	1.6	0.01	Putative protein of unknown function; induced by prostaglandins
orf19.36.1	1.6	0.02	Ortholog of C. parapsilosis CDC317 : CPAR2_104640, Candida orthopsilosis Co 90-125 : CORT_0B05695 and Candida orthopsilosis NEW ASSEMBLY : CORT1B05695
orf19.4172	1.6	0.00	Has domain(s) with predicted hydrolase activity and role in metabolic process
orf19.1562	1.6	0.03	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; repressed by alpha pheromone in SpiderM medium
EVP1	1.6	0.01	Putative plasma membrane protein; predicted role in cell wall integrity; regulated by Nrg1, Tup1; induced during chlamydospore formation in both *C. albicans* and C. dubliniensis
WSC1	1.6	0.00	Putative cell wall component; transcript upregulated in cyr1 mutant (yeast or hyphae); Spider and flow model biofilm induced
orf19.1765	1.6	0.01	Secreted protein; fluconazole-induced
orf19.3007.2	1.6	0.02	Ortholog of C. dubliniensis CD36 : Cd36_02950, C. parapsilosis CDC317 : CPAR2_108520, C. auris B8441 : B9J08_002035 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_100285
orf19.7214	1.6	0.02	Glucan 1,3-beta-glucosidase; regulated by Nrg1, Tup1 and possibly Tac1; induced by NO and during cell wall regeneration; stationary phase enriched; possibly essential (UAU1 method); F-12/CO2 early biofilm induced; flow biofilm repressed
BIO4	1.6	0.01	Putative dethiobiotin synthetase; transcript upregulated in clinical isolates from HIV+ patients with oral candidiasis; Hap43-repressed; GlcNAc-induced protein; Spider biofilm induced; biotin-dependent transcription regulated by Vhr1p
orf19.6973	1.5	0.01	ATP-dependent LON protease family member; Hap43-repressed gene; regulated by Gcn2 and Gcn4; Spider biofilm induced
PEX8	1.5	0.01	Putative peroxisomal biogenesis factor; expression regulated during planktonic growth
GDB1	1.5	0.01	Putative glycogen debranching enzyme
PRN4	1.5	0.00	Protein with similarity to pirins; induced by benomyl treatment; flow model biofilm repressed
BMT8	1.5	0.02	Putative beta-mannosyltransferase, member of a 9-gene family including characterized BMT genes with roles in beta-1,2-mannosylation of cell wall phosphopeptidomannan; transposon insertion in promoter region causes decreased colony wrinkling
APG7	1.5	0.03	Ortholog(s) have Atg12 activating enzyme activity, Atg8 activating enzyme activity

Appendix 13: RNA-seq result of Lys142 -GOF. Differentially expressed Up-regulated genes in *Candida albicans* Lys142 -GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
HGC1	17.9	0.00	Hypha-specific G1 cyclin-related protein involved in regulation of morphogenesis, biofilm formation
orf19.2247	15.5	0.00	Ortholog of C. dubliniensis CD36 : Cd36_21220, C. parapsilosis CDC317 : CPAR2_406700, Candida tenuis NRRL Y-1498 : CANTEDRAFT_127772 and Candida tropicalis MYA-3404 : CTRG_01766
orf19.1539	11.8	0.05	Protein of unknown function; F-12/CO2 early biofilm induced
HWP1	10.3	0.01	Hyphal cell wall protein; host transglutaminase substrate; opaque-, a-specific, alpha-factor induced
MDR1	8.6	0.00	Plasma membrane MDR/MFS multidrug efflux pump
RMS1	7.4	0.00	Putative lysine methyltransferase; Hap43-induced
orf19.4476	7.3	0.00	Protein with a NADP-dependent oxidoreductase domain; transcript induced by ketoconazole; rat catheter and Spider biofilm induced
CTN1	7.3	0.03	Carnitine acetyl transferase; required for growth on nonfermentable carbon sources, not for hyphal growth or virulence in mice; induced in macrophage; macrophage/pseudohyphal-repressed after 16 hr; rat catheter, Spider biofilm induced
orf19.6592	7.2	0.00	Predicted membrane transporter, member of the aromatic acid:proton symporter (AAHS) family, major facilitator superfamily (MFS)
HGT1	7.1	0.00	High-affinity MFS glucose transporter; induced by progesterone, chloramphenicol, benomyl; likely essential for growth; protein newly produced during adaptation to the serum; rat catheter and Spider biofilm induced
orf19.2281	7.0	0.00	Has domain(s) with predicted CoA-transferase activity and role in metabolic process
orf19.1438	6.8	0.01	Protein with homology to NADH dehydrogenase; regulated by Sef1p-, Sfu1p-, and Hap43p
orf19.7279.1	6.3	0.01	Protein of unknown function; Spider biofilm induced
orf19.3337	6.1	0.00	Protein of unknown function; merged with orf19.3338; rat catheter, flow and Spider model biofilm induced; promoter bound by Bcr1, Efg1, Ndt80, and Rob1; orf19.3338 Bcr1-repressed in RPMI a/a biofilms
TNA1	5.9	0.00	Putative nicotinic acid transporter; detected at germ tube plasma membrane by mass spectrometry; transcript induced upon phagocytosis by macrophage; rat catheter biofilm induced
orf19.7495	5.8	0.00	Protein with NADPH oxidoreductase containing flavin mononucleotide (FMN) domain; induced by nitric oxide
WOR3	5.7	0.00	Transcription factor; modulator of white-opaque switch; induced in opaque cells; promoter bound by Wor1; overexpression at 25 degr shifts cells to opaque state; deletion stabilizes opaque cells at higher temperatures; Spider biofilm induced
GRE2	5.6	0.00	Putative reductase; Nrg1 and Tup1-regulated; benomyl- and hyphal-induced; macrophage/pseudohyphal-repressed; repressed by low iron; possibly involved in osmotic stress response; stationary phase enriched protein; Spider biofilm induced
BRG1	5.5	0.00	Transcription factor; recruits Hda1 to hypha-specific promoters; Tn mutation affects filamentation; Hap43-repressed; Spider and flow model biofilm induced; required for Spider biofilm formation; Bcr1-repressed in RPMI a/a biofilms
RBR1	5.5	0.04	Glycosylphosphatidylinositol (GPI)-anchored cell wall protein; required for filamentous growth at acidic pH; expression repressed by Rim101 and activated by Nrg1; Hap43-induced
MAL31	5.4	0.03	Putative high-affinity maltose transporter; transcript is upregulated in clinical isolates from HIV+ patients with oral candidiasis; alkaline induced; Spider biofilm induced
ALS1	5.2	0.00	Cell-surface adhesin; adhesion, virulence, immunoprotective roles; band at hyphal base; Rfg1, Ssk1, Spider biofilm induced; flow model biofilm repressed; CAI-4 strain background effects; promoter bound Bcr1, Tec1, Efg1, Ndt80, and Brg1
orf19.5290	4.9	0.03	Protein of unknown function; repressed by Sfu1; Hap43-induced gene
WH11	4.8	0.00	White-phase yeast transcript; expression in opaques increases virulence/switching; mutant switches as WT; Hap43, hypoxia, ketoconazol induced; required for RPMI biofilm; Bcr1-induced in RPMI a/a biofilm; rat catheter, Spider biofilm induced
orf19.7306	4.8	0.00	Aldo-keto reductase; increased transcript associated with MDR1 overexpression, benomyl or long-term fluconazole treatment; overexpression does not affect drug or oxidative stress sensitivity; stationary phase enriched; flow biofim repressed
RBR3	4.5	0.00	Cell wall adhesin-like protein; repressed by Rim101; possibly an essential gene, disruptants not obtained by UAU1 method
OYE22	4.4	0.01	Putative NADPH dehydrogenase; rat catheter biofilm induced
ICL1	4.4	0.00	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice; induced upon phagocytosis by macrophage; farnesol regulated; Pex5-dependent peroxisomal localization; stationary phase enriched; rat catheter, Spider biofilm induced

orf19.2059	4.2	0.00	Protein with homology to magnesium-dependent endonucleases and phosphatases; regulated by Sef1, Sfu1, and Hap43; Spider biofilm induced
FGR27	4.1	0.00	Zn(II)2Cys6 transcription factor; transposon mutation affects filamentous growth; required for yeast cell adherence to silicone substrate; Spider biofilm induced
ASM3	4.1	0.00	Putative secreted acid sphingomyelin phosphodiesterase; possible Kex2 substrate; transcript increased in an azole-resistant strain that overexpresses MDR1; flow model biofilm induced; F-12/CO2 early biofilm induced
ARO9	4.1	0.00	Aromatic transaminase; Ehrlich fusel oil pathway of aromatic alcohol biosynthesis; Rim101-dependent pH-regulation (alkaline induced); Hap43-induced gene
orf19.1409.2	4.0	0.00	Protein of unknown function; Spider biofilm induced
orf19.3639	4.0	0.00	Ortholog(s) have DNA-3-methyladenine glycosylase activity, alkylbase DNA N-glycosylase activity, damaged DNA binding activity
orf19.3439	3.8	0.01	Protein of unknown function; Cyr1-repressed; rat catheter and Spider biofilm induced
ASR2	3.7	0.00	Adenylyl cyclase and stress responsive protein; induced in cyr1 or ras1 mutant; stationary phase enriched protein; Spider biofilm induced
ECM331	3.7	0.00	GPI-anchored protein; mainly at plasma membrane, also at cell wall; Hap43, caspofungin-induced; Plc1-regulated; Hog1, Rim101-repressed; colony morphology-related regulated by Ssn6; induced by ketoconazole and hypoxia
orf19.1277	3.6	0.00	Protein of unknown function; Rgt1, Hap43-repressed; flow model biofilm induced; Spider biofilm induced
orf19.2607	3.5	0.00	Protein of unknown function; Spider biofilm induced
FAA2-1	3.5	0.00	Predicted long chain fatty acid CoA ligase; upregulated upon phagocytosis; induced by nitric oxide independent of Yhb1
GCA2	3.4	0.01	Predicted extracellular glucoamylase; induced by ketoconazole; possibly essential, disruptants not obtained by UAU1 method; promotes biofilm matrix formation; Spider biofilm induced; Bcr1-induced in RPMI a/a biofilms
GCA1	3.3	0.03	Extracellular/plasma membrane-associated glucoamylase; expressed in rat oral infection; regulated by carbohydrates, pH, galactose; promotes biofilm matrix formation; flow model biofilm induced; Bcr1 repressed in RPMI a/a biofilms
GAC1	3.3	0.00	Putative regulatory subunit of ser/thr phosphoprotein phosphatase 1; fluconazole-induced; caspofungin repressed; transcript induced by Mnl1 under weak acid stress; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
orf19.7380	3.2	0.00	Has domain(s) with predicted nucleic acid binding, nucleotide binding activity
SFC1	3.1	0.00	Putative succinate-fumarate transporter; involved in repression of growth on sorbose; alkaline induced; rat catheter biofilm induced; Spider biofilm induced
CPA1	3.1	0.01	Putative carbamoyl-phosphate synthase subunit; alkaline repressed; rat catheter, Spider and flow model biofilm induced
LYS143	3.1	0.00	Zn(II)2Cys6 transcription factor; ortholog of S. cerevisiae Lys14 involved in the regulation of lysine biosynthesis genes
CHK1	3.1	0.00	Histidine kinase; 2-component signaling, cell wall synthesis; hyphal growth defect; avirulent in mouse, not rat vaginal infection; phagocytosis rate increased; Spider biofilm induced
ALS3	3.1	0.00	Cell wall adhesin; epithelial adhesion, endothelial invasion; alleles vary in adhesiveness; immunoprotective in mice; binds SspB adhesin of S. gordonii in mixed biofilm
HRQ2	3.1	0.01	Protein of unknown function; mutants are viable; rat catheter and Spider biofilm induced
GST2	3.0	0.00	Glutathione S transferase; induced by benomyl and in populations of cells exposed to fluconazole over multiple generations
orf19.5817	3.0	0.00	Ortholog(s) have role in cytoplasm to vacuole transport by the Cvt pathway, endoplasmic reticulum to Golgi vesicle-mediated transport, intra-Golgi vesicle-mediated transport, macroautophagy
orf19.1887	3.0	0.00	Ortholog(s) have sterol esterase activity, role in sterol metabolic process and integral component of membrane, lipid droplet localization
orf19.6705	2.9	0.00	Putative guanyl nucleotide exchange factor with Sec7 domain; required for normal filamentous growth; regulated by yeast-hyphal switch; filament induced; regulated by Nrg1, Tup1, Mob2, Hap43
orf19.1344	2.9	0.01	Protein of unknown function; fluconazole-induced; Spider biofilm induced
orf19.1608	2.9	0.03	Has domain(s) with predicted catalytic activity, sulfuric ester hydrolase activity and role in metabolic process
DDR48	2.9	0.00	Immunogenic stress-associated protein; filamentation regulated; induced by benomyl/caspofungin/ketoconazole or in azole-resistant strain; Hog1, farnesol, alkaline repressed
OYE32	2.8	0.00	NAD(P)H oxidoreductase family protein; induced by nitric oxide, amphotericin B, oxidative stress via Cap1; upregulation associated with MDR1 overexpression or benomyl treatment
orf19.1496	2.8	0.00	Putative transcription factor with zinc finger DNA-binding motif; Hap43p-repressed gene
orf19.1340	2.8	0.00	Putative aldose reductase; protein level decreases in stationary phase cultures
IFR2	2.8	0.00	Zinc-binding dehydrogenase; induced by benomyl, ciclopirox olamine, alpha pheromone, Hap43;
TOK1	2.7	0.00	Outwardly rectifying, noisily gated potassium channel; modulates sensitivity to human salivary histatin (Hst5); very similar to S. cerevisiae Tok1p; Bcr1-repressed in RPMI a/a biofilms

orf19.4583	2.7	0.01	Protein with a mitochondrial carrier protein domain; possibly an essential gene, disruptants not obtained by UAU1 method; Spider biofilm repressed
RFX2	2.6	0.01	Transcriptional repressor; regulator of filamentation, response to DNA damage, adhesion, virulence in murine mucosal, systemic infections; RFX domain; regulated by Nrg1, UV-induced
FRP3	2.6	0.00	Putative ammonium transporter; upregulated in the presence of human neutrophils
orf19.4216	2.6	0.01	Putative heat shock protein; decreased expression in hyphae; transcription is increased in populations of cells exposed to fluconazole over multiple generations
HSP12	2.6	0.01	Heat-shock protein; induced by osmotic/oxidative/cadmium stress, fluphenazine treatment, low iron, CDR1 and CDR2 overexpression, or ssn6 or ssk1 null mutation
orf19.5210	2.6	0.01	Putative Xbp1 transcriptional repressor; binds to cyclin gene promoters in S. cerevisiae; Hap43-repressed; possibly essential, disruptants not obtained by UAU1 method
orf19.4530.1	2.6	0.00	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
PGA55	2.5	0.00	GPI-anchored adhesin-like protein; filament induced; regulated by Nrg1, Tup1
HMX1	2.5	0.00	Heme oxygenase; utilization of hemin iron; transcript induced by heat, low iron, or hemin
ARE2	2.5	0.00	Acyl CoA:sterol acyltransferase; uses cholesterol and oleoyl-CoA substrates; protoberberine derivative drug inhibits enzyme activity; ketoconazole-induced; Hap43-repressed; flow model biofilm induced; Spider biofilm induced
orf19.1933	2.5	0.00	Ortholog(s) have role in ER-dependent peroxisome organization, peroxisome organization and endoplasmic reticulum, peroxisomal membrane, peroxisome localization
GPX2	2.5	0.00	Similar to glutathione peroxidase; induced in high iron; alkaline induced by Rim101; induced by alpha factor or interaction with macrophage; regulated by Efg1; caspofungin repressed; Spider biofilm induced
ZCF6	2.5	0.01	Putative transcription factor with zinc cluster DNA-binding motif; involved in virulence
BIO5	2.5	0.00	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method; Spider biofilm induced; transcription regulated by biotin and Vhr1p
orf19.4423	2.5	0.00	Putative glucosyltransferase; localized to the mitochondrial membrane
NTH1	2.4	0.00	Neutral trehalase; hyphal induction in mutant delayed but not reduced overall; not required for virulence in mice; possible regulatory cAMP-dependent phosphorylation at S10,S213
DCK1	2.4	0.00	Putative guanine nucleotide exchange factor; required for embedded filamentous growth; activates Rac1; has a DOCKER domain; similar to adjacent DCK2 and to S. cerevisiae Ylr422wp
ENA21	2.4	0.00	Predicted P-type ATPase sodium pump; Gcn4p-regulated; flucytosine, amphotericin B, or ketoconazole-induced; osmotic stress-induced
ATS1	2.4	0.00	Protein required for modification of wobble nucleosides in tRNA
GLX3	2.4	0.01	Glutathione-independent glyoxalase; binds human immunoglobulin E; alkaline, fluconazole, Hog1 repressed;
CRH11	2.4	0.00	GPI-anchored cell wall transglycosylase, putative ortholog of S. cerevisiae Crh1p
AVT7	2.4	0.00	Ortholog of S. cerevisiae Avt7 transporter; repressed upon adherence to polystyrene; constitutive expression independent of MTL or white-opaque status; Spider biofilm induced
orf19.4150	2.4	0.00	Putative glutaredoxin; induced by nitric oxide; Spider biofilm induced
orf19.5620	2.4	0.00	Stationary phase enriched protein; Gcn4-regulated; induced by amino acid starvation (3-AT), benomyl or in azole-resistant strain that overexpresses MDR1
TEC1	2.4	0.00	TEA/ATTS transcription factor; white cell pheromone response, hyphal gene regulation; required for Spider and RPMI biofilm formation; regulates BCR1; Cph2 regulated transcript
orf19.7648	2.4	0.00	Has domain(s) with predicted antiporter activity, xenobiotic transmembrane transporter activity, role in drug transmembrane transport and membrane localization
orf19.7235	2.4	0.00	Putative protein of unknown function; mutation confers hypersensitivity to amphotericin B
orf19.215	2.3	0.00	Component of a complex containing the Tor2p kinase; possible a role in regulation of cell growth; Spider biofilm induced
orf19.7499.1	2.3	0.02	Ortholog of C. parapsilosis CDC317 : CPAR2_800380, Candida tenuis NRRL Y-1498
RIB1	2.3	0.00	GTP cyclohydrolase II, rate-limiting enzyme of riboflavin biosynthesis; fungal-specific (no human or murine homolog); regulated by Sef1p, Sfu1p, and Hap43p
orf19.310	2.3	0.00	Putative mitochondrial protein with a predicted role in cell wall biogenesis
orf19.4901	2.3	0.01	Predicted methyltransferase; Spider biofilm induced
VHT1	2.3	0.00	Predicted membrane transporter, involved in biotin import
SEO1	2.3	0.01	Protein with similarity to permeases
PST3	2.3	0.00	Flavodoxin-like protein involved in oxidative stress protection and virulence
PRN2	2.3	0.00	Protein similar to pirin; Hap43p-repressed gene
ECM18	2.3	0.00	Ortholog of S. cerevisiae : ECM18, C. glabrata CBS138 : CAGL0B01969g, C. parapsilosis CDC317 : CPAR2_103190, C. auris B8441
GOR1	2.2	0.00	Ortholog(s) have glyoxylate reductase (NAD+) activity, role in glyoxylate catabolic process and extracellular region localization

POT1-2	2.2	0.00	Putative peroxisomal 3-ketoacyl CoA thiolase; Hap43-repressed
NUP	2.2	0.01	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not; similar to a nucleoside permease of S. pombe; possibly processed by Kex2p
PST1	2.2	0.00	Flavodoxin-like protein involved in oxidative stress protection and virulence; putative 1,4-benzoquinone reductase; hyphal-induced
YMX6	2.2	0.00	Putative NADH dehydrogenase; macrophage-downregulated gene; induced by nitric oxide; rat catheter biofilm induced
orf19.1368	2.1	0.03	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
orf19.1486	2.1	0.00	Protein with a life-span regulatory factor domain; regulated by Sef1, Sfu1, and Hap43; flow model biofilm induced; Spider biofilm induced
NCE103	2.1	0.01	Carbonic anhydrase; converts of CO2 to bicarbonate; essential for virulence in host niches with limited CO2, normal white-opaque switch; Mnl1-induced in weak acid stress; Hap43-induced gene; F-12/CO2, rat catheter, Spider biofilm induced
MNN42	2.1	0.00	Protein of unknown function; repressed by Rim101; negatively modulates intracellular ATP levels during the development of azole resistance
ARO8	2.1	0.00	Aromatic transaminase of the Ehrlich fusel oil pathway of aromatic alcohol biosynthesis
ZFU2	2.1	0.00	Zn(II)2Cys6 transcription factor; regulator of yeast form adherence; mutants display increased colonization of mouse kidneys; required for yeast cell adherence to silicone substrate
PRC2	2.1	0.02	Putative carboxypeptidase; induced by human neutrophils; Spider biofilm induced
orf19.850	2.0	0.00	Ortholog(s) have protein-N-terminal asparagine amidohydrolase activity, protein-N-terminal glutamine amidohydrolase activity and role in N-terminal protein amino acid modification, protein catabolic process
FAD3	2.0	0.01	Omega-3 fatty acid desaturase; production of alpha-linolenic acid, a major component of membranes; caspofungin induced; Plc1-regulated
orf19.2165	2.0	0.00	Predicted hydrolase; induced by nitric oxide
orf19.1440.2	2.0	0.02	Ortholog of C. dubliniensis CD36 : Cd36_62310 and *Candida tropicalis* NEW ASSEMBLY : CTRG1_CGOB_00040
orf19.3219	2.0	0.00	Ortholog of S. cerevisiae Sia1; involved in activation of the Pma1 plasma membrane H+-ATPase by glucose in S. cerevisiae; Spider biofilm induced
orf19.6941	2.0	0.00	Putative diacylglycerol acyltransferase; catalyzes the terminal step of triacylglycerol formation
orf19.5730	2.0	0.00	Putative phenylacrylic acid decarboxylase; clade-associated gene expression
orf19.2959.1	1.9	0.01	Gene induced by hypoxia and ketoconazole; oral infection upregulated; mutants have reduced capacity to damage oral epithelial cells; Spider biofilm induced
orf19.7615	1.9	0.01	Protein involved in endoplasmic reticulum (ER) to Golgi vesicle-mediated transport; putative subunit of the transport protein particle (TRAPP) complex of the cis-Golgi; Spider biofilm induced
orf19.5491	1.9	0.02	Ortholog of C. dubliniensis CD36 : Cd36_20670, C. parapsilosis CDC317 : CPAR2_104720, C. auris B8441 : B9J08_000085 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_103989
orf19.2988	1.9	0.00	Predicted aminotransferase based on S. pombe ortholog SPBC660.12c; flow model biofilm induced
HGT4	1.9	0.01	Glucose and galactose sensor; fermentation, filamentation, virulence roles
orf19.4756	1.9	0.01	Ortholog of *S. cerevisiae*
BIO3	1.9	0.00	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis; transcription regulated by biotin availability and Vhr1p
DLD1	1.9	0.00	Putative D-lactate dehydrogenase; white cell-specific trancript; colony morphology-related gene regulation by Ssn6; Hap43-repressed; rat catheter biofilm induced; Spider biofilm repressed
RAS1	1.9	0.00	RAS signal transduction GTPase; regulates cAMP and MAP kinase pathways
ARG5,6	1.9	0.01	Arginine biosynthetic enzyme; processed in S. cerevisiae into 2 polypeptides with acetylglutamate kinase (Arg6) activity and acetylglutamate-phosphate reductase (Arg5) activity
SAC7	1.9	0.00	Putative GTPase activating protein (GAP) for Rho1; repressed upon adherence to polystyrene
RHO2	1.8	0.00	Ortholog(s) have GTPase activity and role in establishment or maintenance of actin cytoskeleton polarity, regulation of fungal-type cell wall (1->3)-alpha-glucan biosynthetic process
orf19.4142	1.8	0.04	Putative transporter; decreased transcription is observed upon fluphenazine treatment
CPA2	1.8	0.02	Putative arginine-specific carbamoylphosphate synthetase
orf19.4384.1	1.8	0.00	Putative MFS transporter; predicted ORF in Assembly 20
PUT3	1.8	0.00	Zn(II)2Cys6 transcription factor; has similarity to S. cerevisiae Put3, a transcription factor involved in the regulation of proline utilization genes
GRF10	1.8	0.01	Putative homeodomain transcription factor, involved in control of filamentous growth
orf19.4864	1.8	0.00	Ortholog(s) have acylglycerol lipase activity, role in triglyceride metabolic process and lipid droplet, membrane localization
orf19.3473	1.8	0.00	Ortholog(s) have enzyme activator activity, histone acetyltransferase activity, structural molecule activity
BMT4	1.8	0.01	Beta-mannosyltransferase

CTA8	1.8	0.00	Essential transcription factor, mediates heat shock transcriptional induction
PAD1	1.8	0.04	Putative phenylacrylic acid decarboxylase; repressed by Rgt1p
RBT4	1.8	0.00	Pry family protein; required for virulence in mouse systemic/rabbit corneal infections
orf19.3644	1.8	0.00	Protein of unknown function; Cyr1-repressed; rat catheter and Spider biofilm induced
GPH1	1.8	0.05	Putative glycogen phosphorylase; role in glycogen metabolism
orf19.2726	1.8	0.02	Putative plasma membrane protein; Plc1-regulated; Spider biofilm induced
orf19.2987	1.8	0.00	Ortholog(s) have role in cellular ion homeostasis, mitochondrion inheritance, mitochondrion organization, regulation of cardiolipin metabolic process and mitochondrial inner membrane, mitochondrion localization
orf19.511	1.7	0.00	Ortholog(s) have ribosylnicotinamide kinase activity and role in NAD biosynthesis via nicotinamide riboside salvage pathway, NAD biosynthetic process, nicotinamide riboside metabolic process
orf19.7504	1.7	0.00	Ortholog of S. cerevisiae Rts3; a component of the protein phosphatase type 2A complex
SCS7	1.7	0.00	Putative ceramide hydroxylase; regulated by Nrg1
LEU42	1.7	0.00	Putative alpha-isopropylmalate synthase; fungal-specific
orf19.4807	1.7	0.00	Ortholog(s) have inorganic diphosphatase activity
PRN1	1.7	0.02	Protein with similarity to pirins
orf19.1855	1.7	0.01	Predicted membrane transporter, member of the anion:cation symporter (ACS) family, major facilitator superfamily (MFS)
orf19.7297	1.7	0.00	Putative cystathionine gamma-synthase; decreased levels in stationary phase cultures; Hog1p-induced; Gcn4p-regulated
orf19.6869	1.7	0.00	Putative lipid raft associated protein; Spider biofilm induced
ECM4	1.7	0.00	Cytoplasmic glutathione S-transferase; regulated by Nrg1, Tup1
UGT51C1	1.7	0.00	UDP-glucose:sterol glucosyltransferase; enzyme of sterol glucoside (membrane-bound lipid) biosynthesis; has UDP-sugar binding domain
orf19.3142	1.7	0.01	Ortholog of C. dubliniensis CD36 : Cd36_46140, C. parapsilosis CDC317 : CPAR2_501210, C. auris B8441 : B9J08_001807 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_97195
orf19.2124	1.7	0.01	Predicted alcohol dehydrogenase; Spider biofilm induced
orf19.2244	1.6	0.02	Similar to oxidoreductases and to S. cerevisiae Yjr096wp; Sfu1 repressed
orf19.5777	1.6	0.00	Protein of unknown function; F-12/CO2 early biofilm induced
orf19.7566	1.6	0.04	Predicted amino acid transport domain; transcript upregulated in clinical strains from HIV+ patients with oral candidiasis
orf19.4132	1.6	0.01	Protein of unknown function; UPF0057 protein family member; localizes to the plasma membrane; Spider biofilm induced
GAD1	1.6	0.01	Putative glutamate decarboxylase
VHR1	1.6	0.01	Transcriptional activator of genes involved in biotin metabolism
LYS22	1.6	0.02	Homocitrate synthase, minor isoform; repressed by nitric oxide and by hypoxiae
orf19.4898	1.6	0.01	Putative protein of unknown function; induced by prostaglandins
orf19.36.1	1.6	0.02	Ortholog of C. parapsilosis CDC317 : CPAR2_104640, Candida orthopsilosis Co 90-125 : CORT_0B05695 and Candida orthopsilosis NEW ASSEMBLY : CORT1B05695
orf19.4172	1.6	0.00	Has domain(s) with predicted hydrolase activity and role in metabolic process
orf19.1562	1.6	0.03	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; repressed by alpha pheromone in SpiderM medium
EVP1	1.6	0.01	Putative plasma membrane protein; predicted role in cell wall integrity; regulated by Nrg1, Tup1
WSC1	1.6	0.00	Putative cell wall component; transcript upregulated in cyr1 mutant (yeast or hyphae); Spider and flow model biofilm induced
orf19.1765	1.6	0.01	Secreted protein; fluconazole-induced
orf19.3007.2	1.6	0.02	Ortholog of C. dubliniensis CD36 : Cd36_02950, C. parapsilosis CDC317 : CPAR2_108520, C. auris B8441 : B9J08_002035 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_100285
orf19.7214	1.6	0.02	Glucan 1,3-beta-glucosidase
BIO4	1.6	0.01	Putative dethiobiotin synthetase
orf19.6973	1.5	0.01	ATP-dependent LON protease family member; Hap43-repressed gene
PEX8	1.5	0.01	Putative peroxisomal biogenesis factor; expression regulated during planktonic growth
GDB1	1.5	0.01	Putative glycogen debranching enzyme
PRN4	1.5	0.00	Protein with similarity to pirins; induced by benomyl treatment; flow model biofilm repressed
BMT8	1.5	0.02	Putative beta-mannosyltransferase
APG7	1.5	0.03	Ortholog(s) have Atg12 activating enzyme activity, Atg8 activating enzyme activity

Appendix 14: RNA-seq result of Lys144 -GOF. Differentially expressed Up-regulated genes in *Candida albicans* Lys144 -GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
CEK2	29.3	0.0	MAP kinase required for wild-type efficiency of mating; component of the signal transduction pathway that regulates mating
orf19.4654	22.8	0.0	Protein of unknown function; Spider biofilm induced
orf19.1539	12.5	0.0	Protein of unknown function; F-12/CO2 early biofilm induced
orf19.3105	9.1	0.0	Putative cytochrome P450 protein; possibly an essential gene, disruptants not obtained by UAU1 method
orf19.7279.1	7.7	0.0	Protein of unknown function; Spider biofilm induced
GBU1	7.6	0.0	Guanidinobutyrase (Gbase), enzyme involved in metabolism of guanidinobutyrate
AGT1	7.2	0.0	Agmatinase, involved in metabolism of agmatine; downregulated upon adherence to polystyrene; regulated by Gcn2p and Gcn4p
orf19.6398	7.0	0.0	S. pombe ortholog SPBC460.04c is a predicted sulfonate/alpha-ketoglutare dioxygenase; induced by nitric oxide; Spider biofilm induced
orf19.5573	6.6	0.0	Protein of unknown function; expression downregulated in an ssr1 null mutant
LYS144	6.3	0.0	Zn(II)2Cys6 transcription factor; has similarity to S. cerevisiae Lys14, involved in the regulation of lysine biosynthesis genes
ZCF22	5.7	0.0	Predicted Zn(II)2Cys6 transcription factor
orf19.7648	5.4	0.0	Has domain(s) with predicted antiporter activity, xenobiotic transmembrane transporter activity, role in drug transmembrane transport and membrane localization
orf19.6679	5.4	0.0	Has domain(s) with predicted metal ion binding activity
orf19.6557	4.7	0.0	Protein with a predicted fatty acid amide hydrolase I domain; induced by Mnl1 under weak acid stress
orf19.1449	4.6	0.0	Protein of unknown function; induced in azole-resistant strain that overexpresses MDR1; protein present in exponential and stationary growth phase yeast cultures; Spider biofilm induced
AMO2	4.6	0.0	Protein similar to A. niger predicted peroxisomal copper amino oxidase; mutation confers hypersensitivity to toxic ergosterol analog; F-12/CO2 early biofilm induced
ECM38	4.4	0.0	Putative gamma-glutamyltransferase; alkaline upregulated; Spider biofilm induced; possibly an essential gene, disruptants not obtained by UAU1 method
orf19.6788	4.3	0.0	Protein with a predicted role in cotranslational protein targeting to membrane; induced during chlamydospore formation in both *C. albicans* and C. dubliniensis
AMO1	4.2	0.0	Putative peroxisomal copper amine oxidase
orf19.4316	4.2	0.0	Trimethyllysine dioxygenase, the first enzyme in the carnitine biosynthesis pathway; hypha-induced expression, regulated by Cyr1, Ras1, Efg1; rat catheter biofilm repressed
orf19.6976	4.2	0.0	Predicted MFS membrane transporter; member of the proton coupled folate transporter/heme carrier protein family; virulence-group-correlated expression; Spider biofilm induced
RTA2	4.0	0.0	Flippase involved in sphingolipid long chain base release; mediates calcineurin-dependent ER stress response and resistance to azoles; Plc1p, Ca2+, calcineurin-regulated;
orf19.6023	4.0	0.0	Protein with a predicted multidrug transporter domain; Hap43-repressed gene
DAL9	3.9	0.0	Putative allantoate permease; fungal-specific (no human or murine homolog)
ARG1	3.8	0.0	Argininosuccinate synthase; arginine synthesis; Gcn4, Rim101 regulated; induced by amino acid starvation (3-AT), benomyl treatment; stationary phase enriched protein; repressed in alkalinizing medium; rat catheter, Spider biofilm induced
HRQ2	3.8	0.0	Protein of unknown function; mutants are viable; rat catheter and Spider biofilm induced
OPT8	3.6	0.0	Oligopeptide transporter; similar to Opt1 and to S. cerevisiae Ygl114wp, but not other OPTs; induced by nitric oxide, amphotericin B; expression of OPT6, 7, 8 does not complement mutants lacking Opt1, Opt2, and Opt3; Spider biofilm induced
FAD3	3.6	0.0	Omega-3 fatty acid desaturase; production of alpha-linolenic acid, a major component of membranes; caspofungin induced; Plc1-regulated; colony morphology-related gene regulation by Ssn6; Spider biofilm induced, flow model biofilm repressed
ROA1	3.5	0.0	Putative PDR-subfamily ABC transporter involved in sensitivity to azoles; Spider biofilm induced
BUL1	3.5	0.0	Protein similar but not orthologous to S. cerevisiae Bul1; a protein involved in selection of substrates for ubiquitination; mutants are viable; macrophage/pseudohyphal-induced; rat catheter biofilm induced
orf19.164	3.4	0.0	Ortholog(s) have triglyceride lipase activity, role in triglyceride catabolic process and peroxisomal matrix localization
orf19.1340	3.4	0.0	Putative aldose reductase; protein level decreases in stationary phase cultures; Spider biofilm repressed
orf19.4735	3.4	0.0	Ornithine cyclodeaminase family protein; Sef1, Sfu1, and Hap43-regulated; ortholog of S. cerevisiae YGL159W and S. pombe SPAP11E10.01; rat catheter biofilm induced

TES1	3.4	0.0	Putative acyl-CoA thioesterase
orf19.1608	3.4	0.0	Has domain(s) with predicted catalytic activity, sulfuric ester hydrolase activity and role in metabolic process
orf19.6017	3.3	0.0	Protein of unknown function; Spider biofilm induced
FCA1	3.2	0.0	Cytosine deaminase; enzyme of pyrimidine salvage; functional homolog of S. cerevisiae Fcy1p; mutation is associated with resistance to flucytosine (5-FC) in a clinical isolate; hyphal downregulated; gene has intron
GAC1	3.2	0.0	Putative regulatory subunit of ser/thr phosphoprotein phosphatase 1; fluconazole-induced; caspofungin repressed; transcript induced by Mnl1 under weak acid stress; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
SCH9	3.2	0.0	Protein kinase; involved in growth control, ribosomal protein synthesis, cell size, resistance to rapamycin,, chlamydospore formation, filamentous growth, and virulence; prevents hyphal growth in hypoxia at high CO2
orf19.2065	3.2	0.0	Ortholog(s) have allantoicase activity and role in allantoin catabolic process
orf19.1306	3.2	0.0	Has domain(s) with predicted 2-oxoglutarate-dependent dioxygenase activity
ICL1	3.1	0.0	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice; induced upon phagocytosis by macrophage; farnesol regulated; Pex5-dependent peroxisomal localization; stationary phase enriched; rat catheter, Spider biofilm induced
orf19.1307	3.1	0.0	Predicted membrane protein; rat catheter biofilm induced
orf19.5840	3.1	0.0	Ortholog(s) have actin filament binding activity, role in negative regulation of actin filament polymerization and Aim21-Tda2 complex, actin cortical patch localization
orf19.5169	3.1	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
GPX2	3.0	0.0	Similar to glutathione peroxidase; induced in high iron; alkaline induced by Rim101; induced by alpha factor or interaction with macrophage; regulated by Efg1; caspofungin repressed; Spider biofilm induced
CIP1	3.0	0.0	Possible oxidoreductase; transcript induced by cadmium but not other heavy metals, heat shock, yeast-hypha switch, oxidative stress (via Cap1), or macrophage interaction; stationary phase enriched protein; Spider biofilm induced
orf19.2686	3.0	0.0	Ortholog(s) have carboxypeptidase activity, role in nitrogen compound metabolic process, proteolysis involved in cellular protein catabolic process and fungal-type vacuole lumen localization
orf19.1369	3.0	0.0	Protein with predicted peptidase domains; Hap43-repressed gene
orf19.2231	3.0	0.0	Protein of unknown function; Spider biofilm induced
orf19.7554	2.8	0.0	Transporter; similar to the Sit1 siderophore transporter; induced by nitric oxide independent of Yhb1; repressed during chlamydospore formation in *C. albicans* and C. dubliniensis; rat catheter biofilm repressed
BIO5	2.8	0.0	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method; Spider biofilm induced; transcription regulated by biotin and Vhr1p
orf19.4629	2.8	0.0	Ortholog(s) have ubiquitin-ubiquitin ligase activity, role in ATP export, mitochondrion inheritance, protein monoubiquitination, protein polyubiquitination, ubiquitin-dependent endocytosis and ubiquitin ligase complex localization
orf19.6306	2.7	0.0	Trimethylaminobutyraldehyde dehydrogenase, the third enzyme of the carnitine biosynthesis pathway
orf19.1365	2.7	0.0	Putative monooxygenase; mutation confers hypersensitivity to toxic ergosterol analog; constitutive expression independent of MTL or white-opaque status
DAL5	2.7	0.0	Allantoate permease; nitrogen catabolite repressed, induced in absence of preferred N sources; nitrogen source regulation requires Gat1; possibly essential gene (by UAU1 method); Hap43-repressed
SRB8	2.7	0.0	Putative RNA polymerase II mediator complex subunit; early-stage flow model biofilm induced
orf19.36.1	2.7	0.0	Ortholog of C. parapsilosis CDC317 : CPAR2_104640, Candida orthopsilosis Co 90-125 : CORT_0B05695 and Candida orthopsilosis NEW ASSEMBLY : CORT1B05695
orf19.3851	2.7	0.0	Protein of unknown function; induced by alpha pheromone in SpiderM medium
XOG1	2.6	0.0	Exo-1,3-beta-glucanase; 5 glycosyl hydrolase family member; affects sensitivity to chitin and glucan synthesis inhibitors; not required for yeast-to-hypha transition or for virulence in mice; Hap43-induced; Spider biofilm induced
VPS22	2.6	0.0	ESCRT-II complex protein with a role in multivesicular body (MVB) trafficking; required for processing of Rim8p; Hap43p-repressed gene
orf19.1301	2.6	0.0	Ortholog(s) have protein-macromolecule adaptor activity and role in proteasome regulatory particle assembly
orf19.5210	2.6	0.0	Putative Xbp1 transcriptional repressor; binds to cyclin gene promoters in S. cerevisiae; Hap43-repressed; possibly essential, disruptants not obtained by UAU1 method
orf19.2446	2.6	0.0	Has domain(s) with predicted 2-dehydropantoate 2-reductase activity, NADP binding, oxidoreductase activity, oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor activity

MLT1	2.6	0.0	Vacuolar membrane transporter; MRP subfamily of ABC family; may transport organic anions conjugated to glutathione, glucuronate, or sulfate; needed for virulence in mouse peritonitis; Spider biofilm induced; flow model biofilm repressed
HGT20	2.6	0.0	Putative glucose transporter of the major facilitator superfamily; the *C. albicans* glucose transporter family comprises 20 members; 12 probable membrane-spanning segments; regulated by Nrg1
ACF2	2.5	0.0	Putative endo-1,3-beta-glucanase; fungal-specific (no human or murine homolog)
MNN42	2.5	0.0	Protein of unknown function; repressed by Rim101; negatively modulates intracellular ATP levels during the development of azole resistance; induced by Ca(2+) in a calcineurin-dependent manner; Spider biofilm induced
orf19.6558	2.5	0.0	Ortholog(s) have GTPase activator activity and cytosol localization
orf19.1364	2.5	0.0	Ortholog of S. pombe Stm1 G-protein coupled receptor; PQ-loop domains; constitutive expression independent of MTL or white-opaque status; Hap43-repressed
orf19.6596	2.4	0.0	Putative esterase; possibly transcriptionally regulated by Tac1; induced by Mnl1 under weak acid stress; protein present in exponential and stationary growth phase yeast cultures; Spider biofilm repressed
HMX1	2.4	0.0	Heme oxygenase; utilization of hemin iron; transcript induced by heat, low iron, or hemin; repressed by Efg1; induced by low iron; upregulated by Rim101 at pH 8; Hap43-induced; Spider and flow model biofilm induced
orf19.7014	2.4	0.0	Putative protein of unknown function; transcription is positively regulated by Tbf1p; overlaps orf19.7015
orf19.4293	2.4	0.0	Ortholog(s) have role in protein maturation by iron-sulfur cluster transfer, tRNA wobble uridine modification and CIA complex, cytosol, nucleus localization
VHT1	2.4	0.0	Predicted membrane transporter, involved in biotin import; member of the anion:cation symporter (ACS) family, major facilitator superfamily (MFS); biotin-dependent transcription regulated by Vhr1p; amphotericin B, caspofungin repressed
orf19.752	2.4	0.0	Has domain(s) with predicted methyltransferase activity and role in metabolic process
ECM331	2.4	0.0	GPI-anchored protein; mainly at plasma membrane, also at cell wall; Hap43, caspofungin-induced; Plc1-regulated; Hog1, Rim101-repressed; colony morphology-related regulated by Ssn6; induced by ketoconazole and hypoxia
ARO8	2.4	0.0	Aromatic transaminase of the Ehrlich fusel oil pathway of aromatic alcohol biosynthesis; Rim101 independent alkaline induction; protein abundance affected by URA3 expression in CAI-4 strain; Gcn4-regulated; stationary phase enriched
SEO1	2.3	0.0	Protein with similarity to permeases; Sfu1-repressed; flucytosine induced; induced by Mnl1 under weak acid stress; flow model biofilm repressed
orf19.4172	2.3	0.0	Has domain(s) with predicted hydrolase activity and role in metabolic process
orf19.4280	2.3	0.0	Protein of unknown function; Spider biofilm induced; rat catheter biofilm repressed
CTP1	2.3	0.0	Putative citrate transport protein; flucytosine induced; amphotericin B repressed, caspofungin repressed; Hap43p-induced gene
LAP41	2.3	0.0	Putative aminopeptidase yscI precursor; mutant is viable; protein present in exponential and stationary growth phase yeast cultures; Spider biofilm repressed
ZCF9	2.3	0.0	Putative Zn(II)2Cys6 transcription factor; hypersensitive to toxic ergosterol analog ECC69 and/or ECC1384
orf19.3661	2.2	0.0	Putative deubiquitinating enzyme; induced by Mnl1 under weak acid stress
orf19.4376	2.2	0.0	Protein of unknown function; Spider biofilm induced
orf19.95	2.2	0.0	Ortholog of S. cerevisiae : PRM5, C. dubliniensis CD36 : Cd36_60980, C. parapsilosis CDC317 : CPAR2_603060, C. auris B8441 : B9J08_003420 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_113703
YOR1	2.2	0.0	Protein similar to S. cerevisiae Yor1; ABC-type plasma membrane transporter involved in resistance to aureobasidin A; white cell type-specific transcript; Spider biofilm induced
orf19.4128	2.2	0.0	Ortholog of C. dubliniensis CD36 : Cd36_19390, C. parapsilosis CDC317 : CPAR2_209600, C. auris B8441 : B9J08_004606 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_134010
orf19.1121	2.1	0.0	Ortholog of C. dubliniensis CD36 : Cd36_53470, Candida tenuis NRRL Y-1498 : CANTEDRAFT_93324, Candida tropicalis NEW ASSEMBLY : CTRG1_05538 and Spathaspora passalidarum NRRL Y-27907 : SPAPADRAFT_55715
CRD2	2.1	0.0	Metallothionein; for adaptation to growth in high copper; basal transcription is cadmium-repressed; Ssn6 regulated; complements copper sensitivity of an S. cerevisiae cup1 mutant; regulated by Sef1, Sfu1, and Hap43; Spider biofilm induced
orf19.4294	2.1	0.0	Ortholog(s) have oxidoreductase activity, role in cytochrome c-heme linkage, mitochondrial membrane organization and extrinsic component of mitochondrial inner membrane localization
BIO2	2.0	0.0	Putative biotin synthase; induced by high iron; repressed by ciclopirox olamine; upregulated in clinical isolates from HIV+ patients with oral candidiasis; Spider biofilm induced; biotin-dependent transcription regulated by Vhr1p
CAN3	2.0	0.0	Predicted amino acid transmembrane transporter; transcript regulated by white-opaque switch; Hap43-repressed gene

BIO3	2.0	0.0	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis; transcription regulated by biotin availability and Vhr1p
KEX1	2.0	0.0	Carboxypeptidase involved in maturation of candidalysin Ece1p
ATX1	2.0	0.0	Putative cytosolic copper metallochaperone; flucytosine induced; Ssr1-repressed; rat catheter biofilm induced
orf19.1143	2.0	0.0	Ortholog of Pichia stipitis Pignal : PICST_30878, Candida tropicalis NEW ASSEMBLY : CTRG1_01052, Spathaspora passalidarum NRRL Y-27907 : SPAPADRAFT_60017 and Candida guilliermondii ATCC 6260 : PGUG_05223
GLO1	2.0	0.0	Putative monomeric glyoxalase I; oxidative stress-induced via Cap1; flow model and rat catheter biofilm repressed
APE3	2.0	0.0	Putative vacuolar aminopeptidase Y,; regulated by Gcn2 and Gcn4; rat catheter and Spider biofilm repressed
DAO2	1.9	0.0	Putative D-amino acid oxidase; rat catheter biofilm induced
AGP2	1.9	0.0	Amino acid permease; hyphal repressed; white-opaque switch regulated; induced in core caspofungin response, during cell wall regeneration, by flucytosine; regulated by Sef1, Sfu1, and Hap43; rat catheter and Spider biofilm induced
AVT4	1.9	0.0	Putative vacuolar transporter of large neutral amino acids; induced by alpha pheromone in SpiderM medium
orf19.6003	1.9	0.0	Protein of unknown function; role in intracellular signal transduction; Spider biofilm induced
orf19.2669	1.9	0.0	ORF in retrotransposon Tca4; similar to Pol region of retrotransposons encoding reverse transcriptase, protease, integrase; downstream of RHD2, similar to the Gag region encoding nucleocapsid-like protein; rat catheter biofilm induced
ECM14	1.8	0.0	Has domain(s) with predicted metallocarboxypeptidase activity, zinc ion binding activity and role in proteolysis
WOR4	1.8	0.0	Predicted C2H2 zinc finger protein, involved in transcriptional regulation of white-opaque phenotypic switching; activator of the opaque cell type
PRN1	1.8	0.0	Protein with similarity to pirins; induced by benomyl and in response to alpha pheromone in SpiderM medium; transcript induced by Mnl1 in weak acid stress; rat catheter and Spider biofilm induced
ADE13	1.7	0.0	Adenylosuccinate lyase; enzyme of adenine biosynthesis; soluble protein in hyphae; not induced during GCN response, in contrast to the S. cerevisiae ortholog; repressed by nitric oxide
orf19.6263	1.7	0.0	Predicted MFS membrane transporter; member of the monocarboxylate porter (MCP) family; Spider biofilm induced
BIO4	1.7	0.0	Putative dethiobiotin synthetase; transcript upregulated in clinical isolates from HIV+ patients with oral candidiasis; Hap43-repressed; GlcNAc-induced protein; Spider biofilm induced; biotin-dependent transcription regulated by Vhr1p
orf19.1336.2	1.7	0.0	Ortholog(s) have role in mitochondrial respiratory chain complex assembly and mitochondrial intermembrane space localization
orf19.6982	1.7	0.0	Ortholog(s) have mitochondrial ribosome binding activity and role in mitochondrial translational initiation
BAS1	1.7	0.0	Putative Myb-like transcription factor; ortholog S. cerevisiae Bas1, a regulator of purine biosynthetic genes; mutant exhibits adenine auxotrophy and abnormal colony morphology
orf19.5574	1.7	0.0	Ortholog of S. cerevisiae : MCY1, C. glabrata CBS138 : CAGL0F08789g, C. dubliniensis CD36 : Cd36_63080, C. parapsilosis CDC317 : CPAR2_602080 and C. auris B8441 : B9J08_004864
BUD6	1.7	0.0	Protein required for Spitzenkorper formation in hyphal cells (wild-type localization of Mlc1p to the Spitzenkorper); localizes to polarisome
orf19.511	1.6	0.0	Ortholog(s) have ribosylnicotinamide kinase activity and role in NAD biosynthesis via nicotinamide riboside salvage pathway, NAD biosynthetic process, nicotinamide riboside metabolic process
CDC28	1.6	0.0	Cyclin-dependent protein kinase; interacts with regulatory subunit Cyb1; determination of cell morphology during the cell cycle; phosphorylated mostly by Swe1 and phosphorylation is regulated by Hsl1; 5'-UTR intron; Spider biofilm repressed
orf19.1855	1.6	0.0	Predicted membrane transporter, member of the anion:cation symporter (ACS) family, major facilitator superfamily (MFS); Gcn4p-regulated; flucytosine induced; ketoconazole-repressed; oxidative stress-induced via Cap1p
orf19.254	1.6	0.0	Protein of unknown function; Hog1p-repressed; Spider biofilm induced
GPD1	1.6	0.0	Glycerol-3-phosphate dehydrogenase; glycerol biosynthesis; regulated by Efg1; regulated by Tsa1, Tsa1B under H2O2 stress conditions; Sflow model and Spider biofilm induced
orf19.5495	1.6	0.0	Putative RNA-binding protein; induced by alpha pheromone in SpiderM medium
orf19.1486	1.6	0.0	Protein with a life-span regulatory factor domain; regulated by Sef1, Sfu1, and Hap43; flow model biofilm induced; Spider biofilm induced
orf19.5085	1.6	0.0	Ortholog(s) have nuclear import signal receptor activity, nuclear localization sequence binding activity
ADE5,7	1.6	0.0	Phosphoribosylamine-glycine ligase and phosphoribosylformylglycinamidine cyclo-ligase; interacts with Vps34p; required for hyphal growth and virulence; flucytosine induced; not induced in GCN response, in contrast to S. cerevisiae ortholog

orf19.2372	1.6	0.0	Pol protein of retrotransposon Tca2; separated by a stop codon from Gag protein orf19.2371; likely translated as single polyprotein with Gag, reverse transcriptase, protease, and integrase; rat catheter biofilm repressed
MET14	1.6	0.0	Putative adenylylsulfate kinase; predicted role in sulfur metabolism; possibly adherence-induced; protein present in exponential and stationary growth phase yeast; F-12/CO2 biofilm induced
NRP1	1.5	0.0	Ortholog(s) have cytoplasmic stress granule localization
ZCF1	1.5	0.0	Zn(II)2Cys6 transcription factor; transcript regulated during hypha formation; 5'-UTR intron; mutants show decreased colonization of mouse kidneys; flow model biofilm induced; Spider biofilm induced
RHD2	1.5	0.0	Predicted ORF in retrotransposon Tca4 with similarity to the Gag region encoding nucleocapsid-like protein; overlaps blocked reading frame orf19.2668.1; yeast-enriched transcript; rat catheter biofilm induced
OCH1	1.5	0.0	Alpha-1,6-mannosyltransferase; initiates N-glycan outer chain branch addition; similar to S. cerevisiae Och1p; required for wild-type virulence in mouse intravenous infection; fungal-specific (no human or murine homolog)
SNZ1	1.5	0.0	Stationary phase protein; vitamin B synthesis; induced byyeast-hypha switch, 3-AT or in azole-resistant strain overexpressing MDR1; soluble in hyphae; regulated by Gcn4, macrophage; Spider biofilm induced; rat catheter biofilm repressed
SWI4	1.5	0.0	Putative component of the SBF transcription complex involved in G1/S cell-cycle progression; periodic mRNA expression, peak at cell-cycle G1/S phase; predicted, conserved MBF binding sites upstream of G1/S-regulated genes
orf19.2961	1.5	0.0	Putative transcription factor with zinc finger DNA-binding motif
orf19.5842	1.5	0.0	Protein with predicted siroheme synthase middle domains NAD(P)-binding; Hap43-repressed gene

Appendix 15: RNA-seq result of Uga32-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Uga32-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	**log2FC**	**Adjusted P value**	**Description**
orf19.7279.1	7.4	0.0	Protein of unknown function; Spider biofilm induced
UGA32	6.5	0.0	Predicted Zn(II)2Cys6 transcription factor; has similarity to S. cerevisiae Uga3, a transcription factor involved in the regulation of gamma-aminobutyrate metabolism genes; rat catheter biofilm induced
orf19.6679	4.1	0.0	Has domain(s) with predicted metal ion binding activity
BIO5	2.8	0.0	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method; Spider biofilm induced; transcription regulated by biotin and Vhr1p
GPX2	2.6	0.0	Similar to glutathione peroxidase; induced in high iron; alkaline induced by Rim101; induced by alpha factor or interaction with macrophage; regulated by Efg1; caspofungin repressed; Spider biofilm induced
HMX1	2.5	0.0	Heme oxygenase; utilization of hemin iron; transcript induced by heat, low iron, or hemin; repressed by Efg1; induced by low iron; upregulated by Rim101 at pH 8; Hap43-induced; Spider and flow model biofilm induced
HGT20	2.2	0.0	Putative glucose transporter of the major facilitator superfamily; the *C. albicans* glucose transporter family comprises 20 members; 12 probable membrane-spanning segments; regulated by Nrg1
orf19.1272	2.2	0.0	Protein of unknown function; may play a role in regulation of cell size; rat catheter biofilm repressed
VHT1	2.1	0.0	Predicted membrane transporter, involved in biotin import; member of the anion:cation symporter (ACS) family, major facilitator superfamily (MFS); biotin-dependent transcription regulated by Vhr1p; amphotericin B, caspofungin repressed
ECM331	1.9	0.0	GPI-anchored protein; mainly at plasma membrane, also at cell wall; Hap43, caspofungin-induced; Plc1-regulated; Hog1, Rim101-repressed; colony morphology-related regulated by Ssn6; induced by ketoconazole and hypoxia
ITS1	1.9	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58; in S. cerevisiae it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
CAN3	1.8	0.0	Predicted amino acid transmembrane transporter; transcript regulated by white-opaque switch; Hap43-repressed gene
SNZ1	1.7	0.0	Stationary phase protein; vitamin B synthesis; induced byyeast-hypha switch, 3-AT or in azole-resistant strain overexpressing MDR1; soluble in hyphae; regulated by Gcn4, macrophage; Spider biofilm induced; rat catheter biofilm repressed
BIO3	1.7	0.0	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis; transcription regulated by biotin availability and Vhr1p
orf19.3810	1.6	0.0	Ortholog(s) have methylenetetrahydrofolate dehydrogenase (NAD+) activity, role in folic acid-containing compound biosynthetic process, one-carbon metabolic process, purine nucleobase biosynthetic process and cytosol localization
ADE13	1.6	0.0	Adenylosuccinate lyase; enzyme of adenine biosynthesis; soluble protein in hyphae; not induced during GCN response, in contrast to the S. cerevisiae ortholog; repressed by nitric oxide

RHD2	1.5	0.0	Predicted ORF in retrotransposon Tca4 with similarity to the Gag region encoding nucleocapsid-like protein; overlaps blocked reading frame orf19.2668.1; yeast-enriched transcript; rat catheter biofilm induced
ITS2	1.5	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25; in S. cerevisiae it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
RDN58	1.5	0.0	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
orf19.3222	1.5	0.0	Predicted vacuolar protein; rat catheter biofilm repressed; flow model biofilm repressed

Appendix 16: RNA-seq result of Zcf10-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf10-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.2285	10.1	0.0	Protein of unknown function; transcription induced by benomyl treatment
orf19.4459	5.1	0.0	Predicted heme-binding stress-related protein; Tn mutation affects filamentous growth
ZCF10	4.9	0.0	Putative transcription factor with zinc cluster DNA-binding motif
PGA7	4.7	0.0	GPI-linked hyphal surface antigen; induced by ciclopirox olamine, ketoconazole, Rim101 at pH 8
AQY1	4.4	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance
RBT1	4.2	0.0	Cell wall protein with similarity to Hwp1; required for virulence
orf19.5169	3.9	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
RBT5	3.5	0.0	GPI-linked cell wall protein; hemoglobin utilization; Rfg1, Rim101, Tbf1, Fe regulated; Sfu1, Hog1, Tup1, serum, alkaline pH, antifungal drugs, geldamycin repressed; Hap43 induced; required for RPMI biofilms; Spider biofilm induced
orf19.4337	3.1	0.0	Predicted membrane transporter; monocarboxylate porter (MCP) family, major facilitator superfamily (MFS); Spider biofilm induced; rat catheter biofilm repressed
SAP10	3.1	0.0	Secreted aspartyl protease
orf19.5817	2.6	0.0	Ortholog(s) have role in cytoplasm to vacuole transport by the Cvt pathway, endoplasmic reticulum to Golgi vesicle-mediated transport, intra-Golgi vesicle-mediated transport, macroautophagy
ROA1	2.6	0.0	Putative PDR-subfamily ABC transporter involved in sensitivity to azoles; Spider biofilm induced
orf19.263.1	2.6	0.0	Protein of unknown function; gene has intron; Spider biofilm induced
orf19.6920	2.4	0.0	Protein of unknown function; induced during chlamydospore formation in both *C. albicans* and C. dubliniensis; Hap43-induced gene; Spider biofilm induced; F-12/CO2 early biofilm induced
ZCF20	2.1	0.0	Zn(II)2Cys6 transcription factor orthologous to S. cerevisiae Hap1; regulated by Sef1, Sfu1; Hap43-induced; Spider biofilm induced
CLN3	1.9	0.0	G1 cyclin; depletion abolishes budding and causes hyphal growth defects
orf19.35	1.8	0.0	Predicted kinase; rat catheter, flow model, Spider biofilm induced
RBE1	1.6	0.0	Pry family cell wall protein; Rim101, Efg1, Ssn6, alkaline repressed; O-glycosylation; no GPI anchor predicted; ketoconazol induced
orf19.2285	10.1	0.0	Protein of unknown function; transcription induced by benomyl treatment
orf19.4459	5.1	0.0	Predicted heme-binding stress-related protein
ZCF10	4.9	0.0	Putative transcription factor with zinc cluster DNA-binding motif
PGA7	4.7	0.0	GPI-linked hyphal surface antigen; induced by ciclopirox olamine, ketoconazole, Rim101 at pH 8
AQY1	4.4	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance
RBT1	4.2	0.0	Cell wall protein with similarity to Hwp1; required for virulence; predicted glycosylation
orf19.5169	3.9	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
RBT5	3.5	0.0	GPI-linked cell wall protein; hemoglobin utilization; Rfg1, Rim101, Tbf1, Fe regulated; Sfu1, Hog1, Tup1, serum, alkaline pH, antifungal drugs, geldamycin repressed
orf19.4337	3.1	0.0	Predicted membrane transporter
SAP10	3.1	0.0	Secreted aspartyl protease; roles in adhesion, virulence (RHE model), cell surface integrity; distinct specificity from Sap9; at cell membrane and wall; GPI-anchored
orf19.5817	2.6	0.0	Ortholog(s) have role in cytoplasm to vacuole transport by the Cvt pathway, endoplasmic reticulum to Golgi vesicle-mediated transport, intra-Golgi vesicle-mediated transport, macroautophagy
ROA1	2.6	0.0	Putative PDR-subfamily ABC transporter involved in sensitivity to azoles; Spider biofilm induced
orf19.263.1	2.6	0.0	Protein of unknown function; gene has intron; Spider biofilm induced

orf19.6920	2.4	0.0	Protein of unknown function; induced during chlamydospore formation in both *C. albicans* and C. dubliniensis; Hap43-induced gene; Spider biofilm induced; F-12/CO2 early biofilm induced
ZCF20	2.1	0.0	Zn(II)2Cys6 transcription factor orthologous to S. cerevisiae Hap1
CLN3	1.9	0.0	G1 cyclin; depletion abolishes budding and causes hyphal growth defects; farnesol regulated, functional in S. cerevisiae; other biofilm induced; Spider biofilm induced
orf19.35	1.8	0.0	Predicted kinase; rat catheter, flow model, Spider biofilm induced
RBE1	1.6	0.0	Pry family cell wall protein; Rim101, Efg1, Ssn6, alkaline repressed

Appendix 17: RNA-seq result of Zcf13-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf13-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.3210	13.9	0.0	Predicted protein of unknown function; Plc1-regulated
LIP3	12.4	0.0	Secreted lipase; gene family member whose members are expressed differentially in response to carbon source and infection; possible role in nutrition and/or in creating an acidic microenvironment; flow model biofilm induced
INO2	5.5	0.0	Transcriptional activator that forms a heterodimer with Ino4p; likely regulates genes involved in phosphatidylcholine and phosphatidylinositol biosynthesis, fatty acid beta-oxidation, and peroxisome biogenesis
ZCF13	4.5	0.0	Predicted Zn(II)2Cys6 transcription factor; similar to but not the true ortholog of S. cerevisiae Hap1; mutants display decreased colonization of mouse kidneys
IFK2	4.2	0.0	Putative thiol-specific monooxygenase; mutant is viable; flow model biofilm induced
orf19.4128	3.9	0.0	Ortholog of C. dubliniensis CD36 : Cd36_19390, C. parapsilosis CDC317 : CPAR2_209600, C. auris B8441 : B9J08_004606 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_134010
orf19.3139	3.4	0.0	Putative NADP-dependent oxidoreductase; Hap43-repressed
ALK2	3.1	0.0	N-Alkane inducible cytochrome P450
POX1	2.8	0.0	Predicted acyl-CoA oxidase; regulated upon white-opaque switch; upregulated upon phagocytosis; Spider biofilm induced
orf19.899	2.1	0.0	Ortholog of C. dubliniensis CD36 : Cd36_18040, C. parapsilosis CDC317 : CPAR2_211790, C. auris B8441 : B9J08_004373 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_109928
RBT1	2.1	0.0	Cell wall protein with similarity to Hwp1; required for virulence; predicted glycosylation; fluconazole, Tup1 repressed; farnesol, alpha factor, serum, hyphal and alkaline induced; Rfg1, Rim101-regulated
orf19.1709	1.9	0.0	Sterol carrier domain protein; alkaline downregulated; colony morphology-related gene regulation by Ssn6; Spider biofilm induced
ANT1	1.9	0.0	Peroxisomal adenine nucleotide transporter; role in beta-oxidation of medium-chain fatty acid and peroxisome proliferation; rat catheter biofilm induced
orf19.4066	1.7	0.0	Putative glycerol-3-phosphate acyltransferase; Hog1-repressed
TPO4	1.5	0.0	Putative sperimidine transporter; fungal-specific (no human or murine homolog); Spider biofilm induced; promoter bound by Tec1 and Ndt80; Bcr1-repressed in RPMI a/a biofilms
YMX6	1.5	0.0	Putative NADH dehydrogenase; macrophage-downregulated gene; induced by nitric oxide; rat catheter biofilm induced

Appendix 18: RNA-seq result of Zcf24 -GOF. Differentially expressed Up-regulated genes in *Candida albicans* **Zcf24 -GOF**, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
ZCF24	5.8	0.0	Predicted Zn(II)2Cys6 transcription factor; caspofungin induced; Hap43-repressed
OPT4	5.3	0.0	Oligopeptide transporter; detected at germ tube plasma membrane
orf19.1691	5.1	0.0	Plasma-membrane-localized protein; filament induced; Hog1, ketoconazole, fluconazole and hypoxia-induced; regulated by Nrg1, Tup1, Upc2; induced by prostaglandins
RNR22	4.5	0.0	Putative ribonucleoside diphosphate reductase;colony morphology-related gene regulation by Ssn6
RME1	4.3	0.0	Zinc finger protein, controls asexual sporulation; white-specific transcript
orf19.4450.1	4.2	0.0	Protein conserved among the CTG-clade; 2 adjacent upstream SRE-1 elements; highly up-regulated in cecum-grown cells in a Cph2-dependent manner; Hap43-repressed
BLP1	4.1	0.0	Protein of unknown function, serum-induced

UCF1	4.1	0.0	Upregulated by cAMP in filamentous growth; induced in high iron, decreased upon yeast-hypha switch; downregulation correlates with clinical fluconazole resistance
PLB1	4.0	0.0	Phospholipase B; host cell penetration and virulence in mouse systemic infection; Hog1-induced
RHD3	4.0	0.0	GPI-anchored yeast-associated cell wall protein; induced in high iron; clade-associated gene expression; not essential for cell wall integrity; fluconazole-repressed; flow model and Spider biofilm repressed
TPO3	3.8	0.0	Putative polyamine transporter; MFS-MDR family; induced by Sfu1, regulated upon white-opaque
AQY1	3.8	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance; virulent in mice; flucytosine repressed; flow model/RPMI/Spider/rat catheter biofilm induced
STF2	3.7	0.0	Protein involved in ATP biosynthesis; repressed in hyphae; repressed by Efg1, Hap43
orf19.3910	3.5	0.0	Has domain(s) with predicted RNA binding, ribonuclease T2 activity
QDR1	3.5	0.0	Putative antibiotic resistance transporter; regulated by white-opaque switch, Nrg1, Tup1; Hap43, caspofungin repressed
RHR2	3.5	0.0	Glycerol 3-phosphatase; roles in osmotic tolerance, glycerol accumulation in response to salt; Spider/flow model biofilm induced
ADH5	3.4	0.0	Putative alcohol dehydrogenase; regulated by white-opaque switch; fluconazole-induced; antigenic in murine infection; regulated by Nrg1, Tup1
OSM1	3.4	0.0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae; caspofungin repressed; stationary phase enriched protein; flow model biofilm induced; Spider biofilm repressed
CDR4	3.3	0.0	Putative ABC transporter superfamily; fluconazole, Sfu1, Hog1, core stress response induced expression; rat catheter and flow model biofilm induced
GAP2	3.2	0.0	General broad specificity amino acid permease; ketoconazole, flucytosine repressed
orf19.1867	3.2	0.0	Putative malate permease; induced during macrophage infection; regulated by Gcn2 and Gcn4; putative peroxisome targeting signal; Hap43-repressed; Spider biofilm induced
orf19.1307	3.2	0.0	Predicted membrane protein; rat catheter biofilm induced
OPT3	3.2	0.0	Oligopeptide transporter; transcript induced by macrophage phagocytosis, BSA or peptides; fluconazole-induced; induced by Rim101 at pH 8; virulence-group-correlated expression; Hap43-repressed; Spider biofilm induced
DUR1,2	3.1	0.0	Urea amidolyase; hydrolyzes urea to CO2; use of urea as N source and for hyphal switch in macrophage; regulated by Nrg1/Hap43; required for virulence; promotes mouse kidney and brain colonization; rat catheter and flow model biofilm induced
orf19.5169	3.1	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
IFE2	3.1	0.0	Putative alcohol dehydrogenase; yeast-enriched transcript; Efg1-regulated; induced by prostaglandins, Hog1, fluconazole; rat catheter biofilm induced
CRP1	3.1	0.0	Copper transporter; CPx P1-type ATPase; mediates Cu resistance; similar to Menkes and Wilson disease proteins; copper-induced
HEM13	3.1	0.0	Coproporphyrinogen III oxidase; antigenic; on yeast cell surface, not hyphae; iron-regulated expression; Hap43, macrophage-repressed
CAN2	3.1	0.0	Basic amino acid permease; arginine metabolism; regulated by Nrg1/Tup1; caspofungin, flucytosine induced; colony morphology-related regulation by Ssn6
orf19.411	2.9	0.0	Protein similar to GTPase regulators; induced in low iron; transcript activated by Mnl1 under weak acid stress; Hap43-, Sfu1- and Sef1-regulated; flow model biofilm induced, Spider biofilm induced
OPT1	2.9	0.0	Oligopeptide transporter; transports 3-to-5-residue peptides; alleles are distinct, one has intron; suppresses S. cerevisiae ptr2-2 mutant defects; induced by BSA or peptides; Stp3p, Hog1p regulated; flow model biofilm induced
YHB1	2.8	0.0	Nitric oxide dioxygenase; acts in nitric oxide scavenging/detoxification; role in virulence in mouse; transcript activated by NO, macrophage interaction; Hap43, hypha repressed; mRNA binds She3
orf19.7027	2.7	0.0	Protein of unknown function; Spider biofilm induced
orf19.6690	2.7	0.0	Protein of unknown function; Hap43-repressed gene
CAN1	2.6	0.0	Basic amino acid permease; complements lysine transport mutation; 10 predicted transmembrane regions, 3 predicted N-glycosylation sites
GPM2	2.6	0.0	Putative phosphoglycerate mutase; repressed in hyphae; macrophage/pseudohyphal-repressed; induced by high levels of peroxide stress, farnesol
orf19.951	2.6	0.0	Protein of unknown function; transcript repressed upon yeast-hyphal switch; fluconazole-induced; Hap43-repressed; flow model biofilm induced
orf19.5626	2.5	0.0	Protein of unknown function; Plc1-regulated; induced by Mnl1 under weak acid stress; flow model biofilm induced
DAK2	2.5	0.0	Putative dihydroxyacetone kinase; repressed by yeast-hypha switch; fluconazole-induced; caspofungin repressed
MNN14	2.5	0.0	Predicted alpha-1,3-mannosyltransferase activity with a role in protein glycosylation; Hap43-repressed; Spider biofilm induced
GAT1	2.4	0.0	GATA-type transcription factor; regulator of nitrogen utilization; required for nitrogen catabolite repression and utilization of isoleucine, tyrosine and tryptophan N sources

SFT1	2.4	0.0	Putative Golgi v-SNARE; Plc1-regulated; Spider biofilm induced
ECM21	2.4	0.0	Predicted regulator of endcytosis of plasma membrane proteins; fluconazole induced, alkaline induced by Rim101; repressed by caspofungin and in azole-resistant strain overexpressing MDR1
PFK1	2.4	0.0	Phosphofructokinase alpha subinit; activated by fructose 2,6-bisphosphate, AMP, ATP inhibited; activity reduced on hyphal induction; phagocytosis-repressed
ITS1	2.4	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58
XYL2	2.3	0.0	D-xylulose reductase; immunogenic in mice; soluble protein in hyphae; induced by caspofungin, fluconazole, Hog1 and during cell wall regeneration
PHO113	2.3	0.0	Putative constitutive acid phosphatase; Rim101-repressed; DTT-extractable
OSM2	2.3	0.0	Putative mitochondrial fumarate reductase; regulated by Ssn6p, Gcn2p, and Gcn4p; Hog1p-downregulated; stationary phase enriched protein; Hap43p-repressed gene
orf19.787.1	2.3	0.0	Protein of unknown function; ORF added to Assembly 21 based on comparative genome analysis
ITS2	2.3	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25
BUB3	2.2	0.0	Protein similar to S. cerevisiae Bub3; a kinetochore checkpoint component; induced by hydroxyurea treatment; flow model biofilm induced; Spider biofilm induced
PHO15	2.2	0.0	HAD-family 2-phosphoglycolate phosphatase, likely involved in a metabolic repair system, not in protein dephosphorylation; involved in regulation of white-opaque switch; hyphal repressed; induced in core stress response
orf19.5114.1	2.2	0.0	Ortholog of C. dubliniensis CD36 : Cd36_02630, C. parapsilosis CDC317 : CPAR2_206910, Candida tenuis NRRL Y-1498 : cten_CGOB_00051 and Pichia stipitis Pignal : psti_CGOB_00173
orf19.1381	2.2	0.0	Ortholog of S. cerevisiae/S. pombe Lsb5; predicted role in actin cortical patch localization, actin filament organization, endocytosis; flow model biofilm induced; Spider biofilm repressed
orf19.6983	2.1	0.0	Protein of unknown function; Hap43-repressed gene; repressed by nitric oxide; Spider biofilm induced
MEP1	2.1	0.0	Ammonium permease; Mep1 more efficient permease than Mep2, Mep2 has additional regulatory role; 11 predicted transmembrane regions
PLB4.5	2.1	0.0	Phospholipase B; Hog1-induced; regulated by Ssn6; putative GPI-anchor; repressed during cell wall regeneration; clade-associated gene expression
GCY1	2.0	0.0	Aldo/keto reductase; mutation confers hypersensitivity to toxic ergosterol analog; farnesol-repressed; stationary phase enriched protein; flow model biofilm induced; Spider biofilm repressed
AGP2	1.9	0.0	Amino acid permease; hyphal repressed; white-opaque switch regulated; induced in core caspofungin response, during cell wall regeneration, by flucytosine
orf19.993	1.9	0.0	Protein of unknown function; rat catheter biofilm repressed
MAF1	1.9	0.0	Putative negative regulator of RNA polymerase III; decreased expression in hyphae vs yeast cells
orf19.2350	1.9	0.0	Protein similar to S. cerevisiae Yor378w; MFS family transporter
ZCF27	1.9	0.0	Putative Zn(II)2Cys6 transcription factor
PFK2	1.9	0.0	Phosphofructokinase beta subunit; fructose 2,6-bisphosphate, AMP activated; ATP inhibited; phagocytosis, hyphal repressed; fluconazole-induced
RDN58	1.9	0.0	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
GPD1	1.9	0.0	Glycerol-3-phosphate dehydrogenase; glycerol biosynthesis; regulated by Efg1
orf19.7445	1.9	0.0	Ortholog of S.c. Vid24; a peripheral membrane protein located at Vid (vacuole import and degradation) vesicles; regulated by Sef1, Sfu1, and Hap43; Spider biofilm induced
orf19.6225.1	1.8	0.0	Ortholog(s) have role in mitochondrial cytochrome c oxidase assembly and extrinsic component of matrix side of mitochondrial inner membrane, mitochondrial matrix localization
PCL7	1.8	0.0	Putative cyclin-like protein; possible Pho85 cyclin; hyphal repressed; induced by Mnl1 under weak acid stress
ISN1	1.8	0.0	Putative inosine 5'-monophosphate 5'-nucleotidase; fungal-specific (no human or murine homolog)
PIR1	1.8	0.0	1,3-beta-glucan-linked cell wall protein; N-mannosylated, O-glycosylated by Pmt1; cell wall defect in het mutant; Hog1/fluconazole/hypoxia induced
orf19.5136	1.8	0.0	Putative pyridoxamine 5'-phosphate oxidase; planktonic growth and early-stage flow model biofilm induced
CMK1	1.8	0.0	Putative calcium/calmodulin-dependent protein kinase II; expression regulated upon white-opaque switching
MSN4	1.8	0.0	Zinc finger transcription factor; similar to *S. cerevisiae* Msn4, but not a significant stress response regulator in *C. albicans*
IFM3	1.8	0.0	Protein with a 2-hydroxyacid dehydrogenase catalytic domain; Hap43-repressed; Plc1-regulated; overlaps orf19.2177
orf19.7473	1.8	0.0	Ortholog(s) have role in endocytosis and actin cortical patch localization
SYG1	1.8	0.0	Ortholog(s) have role in signal transduction and plasma membrane localization
orf19.1314	1.8	0.0	Protein of unknown function; planktonic growth-induced gene

PRD1	1.8	0.0	Putative proteinase; transcript regulated by Nrg1, Mig1, and Tup1; Hogp-induced; stationary phase enriched protein; Hap43-repressed; rat catheter biofilm repressed
RDN25	1.8	0.0	25S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
orf19.35	1.8	0.0	Predicted kinase; rat catheter, flow model, Spider biofilm induced
ZSF1	1.7	0.0	Ortholog od S. cerevisiae Tis11, a mRNA-binding protein
MSO1	1.7	0.0	Putative secretory protein involved in S. cerevisiae sporulation; repressed during pseudohyphal growth in the presence of lysed macrophages; Hap43-repressed; Spider biofilm induced
YTH1	1.7	0.0	Putative mRNA cleavage and polyadenylation specificity factor; transcription is regulated upon yeast-hyphal switch; decreased expression in hyphae compared to yeast-form cells
orf19.3004	1.7	0.0	Ortholog of *S. cerevisiae* : YDR262W, *C. glabrata* CBS138 : CAGL0M08734g, *C. dubliniensis* CD36 : Cd36_02920, *C. parapsilosis* CDC317 : CPAR2_108490 and *C. auris* B8441
GST2	1.7	0.0	Glutathione S transferase; induced by benomyl and in populations of cells exposed to fluconazole over multiple generations; regulated by Nrg1, Tup1
orf19.3881	1.7	0.0	Ortholog of *C. dubliniensis* CD36 : Cd36_31790, *C. parapsilosis* CDC317 : CPAR2_204900, C. auris B8441 : B9J08_000806 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_136864
HGT7	1.6	0.0	Putative MFS glucose transporter; glucose, fluconazole, Snf3 induced, expressed at high glucose; 20 member *C. albicans* glucose transporter family
DEF1	1.6	0.0	RNA polymerase II regulator; role in filamentation, epithelial cell escape, dissemination in RHE model; induced by fluconazole, high cell density
RBE1	1.6	0.0	Pry family cell wall protein; Rim101, Efg1, Ssn6, alkaline repressed; O-glycosylation; no GPI anchor predicted; ketoconazol induced; regulated by Sef1, Sfu1, Hap4
orf19.4589	1.6	0.0	Ortholog(s) have polyamine oxidase activity and role in pantothenate biosynthetic process, spermine catabolic process
IML2	1.6	0.0	Protein of unknown function; early-stage flow model biofilm induced
orf19.5572	1.6	0.0	Protein of unknown function; Spider biofilm repressed
orf19.321	1.6	0.0	Ortholog(s) have L-methionine transmembrane transporter activity and role in methionine import across plasma membrane
orf19.3302	1.6	0.0	Putative type-1 protein phosphatase targeting subunit; transcript repressed by yeast-hyphal switch; transcript induced by Mnl1p under weak acid stress; flow model biofilm induced
orf19.173	1.5	0.0	C2H2 transcription factor; induced by Mnl1 under weak acid stress
GDB1	1.5	0.0	Putative glycogen debranching enzyme; expression is regulated upon white-opaque switch
orf19.2529.1	1.5	0.0	Protein of unknown function; Spider biofilm repressed
PEX5	1.5	0.0	Pex5p family protein; required for PTS1-mediated peroxisomal protein import, fatty acid beta-oxidation
orf19.3712	1.5	0.0	Protein of unknown function; induced by Mnl1 under weak acid stress; flow model biofilm induced; Spider biofilm induced
orf19.4550	1.5	0.0	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family; flow model biofilm induced

Appendix 19: RNA-seq result of Zcf15-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf15-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
ZCF15	5.9	0.0	Predicted Zn(II)2Cys6 transcription factor of unknown function; rat catheter biofilm induced
REI1	4.5	0.0	Putative cytoplasmic pre-60S factor; Hap43-induced; repressed by prostaglandins
orf19.4634	3.8	0.0	Protein required for thiolation of uridine at wobble position of Gln, Lys, and Glu tRNAs; has a role in urmylation; S. cerevisiae ortholog has a role in invasive and pseudohyphal growth
NOG2	3.6	0.0	Putative nucleolar GTPase; repressed by prostaglandins; Hap43-induced, rat catheter and Spider biofilm induced
ENP2	3.6	0.0	Putative nucleolar protein; essential; heterozygous mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); Hap43-induced; Spider biofilm induced
orf19.1708	3.5	0.0	Protein of unknown function; Spider biofilm induced
NOP14	3.4	0.0	Putative nucleolar protein; Hap43-induced; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); heterozygous mutant is resistant to parnafungin; Spider biofilm induced
orf19.5020	3.3	0.0	Protein of unknown function; Hap43-induced; Spider biofilm induced
HGT2	3.1	0.0	Putative MFS glucose transporter; 20 member *C. albicans* glucose transporter family; 12 probable membrane-spanning segments; expressed in rich medium with 2% glucose; rat catheter and Spider biofilm induced
orf19.2934	3.1	0.0	Similar to S. cerevisiae Bud20; predicted role in cellular bud site selection; rat catheter and Spider biofilm induced
RSM22	3.0	0.0	Predicted mitochondrial small ribosomal subunit; rat catheter and Spider biofilm induced
NOP8	3.0	0.0	Ortholog of S. cereviiae Nop8; has a role in ribosomal large subunit biogenesis; rat catheter and Spider biofilm induced
MAK16	2.9	0.0	Putative constituent of 66S pre-ribosomal particles; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
BUD22	2.9	0.0	Protein with a predicted role in 18S rRNA maturation and small ribosomal subunit biogenesis; repressed in core stress response; repressed by prostaglandins
orf19.2320	2.8	0.0	Putative serine/threonine-protein kinase; possibly an essential gene, disruptants not obtained by UAU1 method
HIS3	2.8	0.0	Imidazoleglycerol-phosphate dehydratase, enzyme of histidine biosynthesis; functionally complements S. cerevisiae his3-1 mutation; hyphal-induced expression; regulated by Gcn2p and Gcn4p; fungal-specific (no human or murine homolog)
ENP1	2.8	0.0	Protein required for pre-rRNA processing and 40S ribosomal subunit synthesis; associated with U3 and U14 snoRNAs; transposon mutation affects filamentous growth; repressed by prostaglandins; Spider biofilm induced
orf19.494	2.8	0.0	Putative RNA-binding protein; role in assembly of box H/ACA snoRNPs and thus pre-rRNA processing; Spider biofilm induced
orf19.2167	2.8	0.0	Ortholog(s) have role in ribosomal large subunit biogenesis, ribosomal small subunit biogenesis and nucleolus localization
orf19.4921.1	2.7	0.0	Protein of unknown function; Spider biofilm repressed
orf19.7088	2.7	0.0	Ortholog(s) have N(6)-L-threonylcarbamoyladenine synthase activity, single-stranded telomeric DNA binding activity
ELP3	2.7	0.0	Predicted histone acetyltransferase; role in regulation of transcription, tRNA wobble uridine modification; Spider biofilm induced
DIM1	2.7	0.0	Putative 18S rRNA dimethylase; predicted role in rRNA modification and processing; Hap43-induced; likely to be essential for growth based on insertional mutagenesis strategy; F-12/CO2 early biofilm induced
orf19.5049	2.7	0.0	Putative U3-containing 90S preribosome processome complex subunit; Hap43-induced gene; rat catheter and Spider biofilm induced; F-12/CO2 early biofilm induced
orf19.2090	2.7	0.0	Ortholog of S. cerevisiae Ecm16, an essential DEAH-box ATP-dependent RNA helicase specific to the U3 snoRNP required for 18S rRNA synthesis; Hap43-induced; Spider biofilm induced
SAS10	2.7	0.0	Putative U3-containing small subunit processome complex subunit; Hap43p-induced gene; mutation confers resistance to 5-fluorocytosine (5-FC); repressed upon high-level peroxide stress
orf19.2386	2.7	0.0	Ortholog(s) have role in endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more
orf19.6175	2.6	0.0	Putative 35S rRNA processing protein; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
orf6.5536	2.6	0.0	Protein with a role in nucleolar integrity and processing of pre-rRNA; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); Hap43-induced; Spider biofilm induced
orf19.6886	2.6	0.0	Ortholog(s) have rRNA binding activity and role in maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA), rRNA processing, ribosomal large subunit export from nucleus

BRG1	2.6	0.0	Transcription factor; recruits Hda1 to hypha-specific promoters; Tn mutation affects filamentation; Hap43-repressed; Spider and flow model biofilm induced; required for Spider biofilm formation; Bcr1-repressed in RPMI a/a biofilms
orf19.7546	2.6	0.0	Protein involved in rRNA processing; required for maturation of the 35S primary transcript of pre-rRNA and for cleavage leading to mature 18S rRNA; Spider biofilm induced
HBR3	2.5	0.0	Essential protein; regulated by hemoglobin; S. cerevisiae ortholog is essential; Hap43p-induced gene
orf19.7197	2.5	0.0	Putative intranuclear transport and DNA replication mediator; heterozygous null mutant exhibits resistance to parnafungin in the *C. albicans* fitness test; Spider biofilm induced
orf19.1486	2.5	0.0	Protein with a life-span regulatory factor domain; regulated by Sef1, Sfu1, and Hap43; flow model biofilm induced; Spider biofilm induced
KRR1	2.5	0.0	Putative nucleolar protein; repressed benomyl treatment or in an azole-resistant strain that overexpresses MDR1; F-12/CO2 early biofilm induced
RRN3	2.5	0.0	Protein with a predicted role in recruitment of RNA polymerase I to rDNA; caspofungin induced; flucytosine repressed; repressed in core stress response; repressed by prostaglandins
NAN1	2.4	0.0	Putative U3 snoRNP protein; Hap43p-induced gene; physically interacts with TAP-tagged Nop1p
orf19.5802	2.4	0.0	Ortholog(s) have transferase activity, role in maturation of SSU-rRNA and cytoplasm localization
UTP18	2.4	0.0	Putative U3 snoRNA-associated protein; Hap43-induced; repressed in core stress response; physically interacts with TAP-tagged Nop1
orf19.6862	2.4	0.0	Hap43-induced gene; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine)
orf19.3470	2.3	0.0	Putative flavodoxin; similar to S. cerevisiae Tyw1, an iron-sulfur protein required for synthesis of wybutosine modified tRNA; predicted Kex2p substrate; Spider biofilm induced
SPB1	2.3	0.0	Putative AdoMet-dependent methyltransferase; Hap43-induced; repressed by prostaglandins; possibly essential gene, disruptants not obtained by UAU1 method; Spider biofilm induced
RRP15	2.3	0.0	Putative nucleolar protein; constituent of pre-60S ribosomal particles; Hap43-induced; repressed by prostaglandins
UTP21	2.3	0.0	Putative U3 snoRNP protein; Hap43-induce; physically interacts with TAP-tagged Nop1; Spider biofilm induced
TRM2	2.3	0.0	Putative tRNA methyltransferase; repressed by prostaglandins; Spider biofilm induced
SRP40	2.3	0.0	Putative chaperone of small nucleolar ribonucleoprotein particles; macrophage/pseudohyphal-induced; rat catheter biofilm induced
RRP9	2.3	0.0	Ribosomal protein; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); physically interacts with TAP-tagged Nop1; Hap43-induced; Spider biofilm induced
orf19.4459	2.3	0.0	Predicted heme-binding stress-related protein; Tn mutation affects filamentous growth; induced during chlamydospore formation in *C. albicans* and C. dubliniensis; Spider biofilm induced
IMP4	2.3	0.0	Putative SSU processome component; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
FRP3	2.3	0.0	Putative ammonium transporter; upregulated in the presence of human neutrophils; fluconazole-downregulated; repressed by nitric oxide; Spider biofilm induced; rat catheter biofilm repressed
orf19.7398	2.3	0.0	Protein of unknown function' Hap43-induced gene; repressed by prostaglandins
orf19.2330	2.3	0.0	Putative U3 snoRNA-associated protein; Hap43-induced; transposon mutation affects filamentous growth; repressed by prostaglandins
orf19.7397.1	2.2	0.0	Predicted ORF identical to NSA2
orf19.5038	2.2	0.0	Predicted tRNA (guanine) methyltransferase activity; Spider biofilm induced
HCA4	2.2	0.0	Putative role in regulation of cell wall biogenesis; Hap43p-induced gene; possibly an essential gene, disruptants not obtained by UAU1 method; flow model and rat catheter biofilm induced
CIC1	2.2	0.0	Putative proteasome-interacting protein; rat catheter biofilm induced
orf19.1388	2.2	0.0	Putative 66S pre-ribosomal particle component; Hap43-induced; F-12/CO2 early biofilm induced
NOP6	2.2	0.0	Putative ortholog of S. cerevisiae Nop6; role in ribosomal small subunit biogenesis; Spider biofilm induced
orf19.107	2.2	0.0	DEAH-box ATP-dependent RNA helicase, required for 18S rRNA synthesis; rat catheter biofilm induced
NOP15	2.2	0.0	Nucleolar ribosome biogenesis factor; hyphal-induced expression; Hap43-induced; rat catheter biofilm induced
orf19.7552	2.2	0.0	Putative U3-containing small subunit processome complex protein; Hap43-induced gene; repressed in core stress response; Spider biofilm induced
RPC19	2.1	0.0	Putative RNA polymerases I and III subunit AC19; Hap43-induced; rat catheter biofilm induced
NSA1	2.1	0.0	Putative 66S pre-ribosomal particles conmponent; Hap43-induced; repressed by prostaglandins
PPT1	2.1	0.0	Putative serine/threonine phosphatase; induced in high iron
RRP8	2.1	0.0	Ribosomal protein; Hap43-induced; F-12/CO2 early biofilm and rat catheter biofilm induced
orf19.3463	2.1	0.0	Putative GTPase; role in 60S ribosomal subunit biogenesis; Spider biofilm induced

DIP2	2.1	0.0	Putative small ribonucleoprotein complex; Tn mutation affects filamentous growth; physically interacts with TAP-tagged Nop1; heterozygous null mutant exhibits resistance to parnafungin; Hap43-induced gene; Spider biofilm induced
orf19.2319	2.1	0.0	Putative nucleolar protein with a predicted role in pre-rRNA processing; Hap43-induced gene; repressed in core stress response
DOT4	2.1	0.0	Protein similar to ubiquitin C-terminal hydrolase; localizes to cell surface of hyphal cells, but not yeast-form cells; repressed upon high-level peroxide; Hap43p-induced; rat catheter biofilm induced
ITS1	2.1	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58; in S. cerevisiae it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
orf19.3393	2.0	0.0	Putative DEAD-box helicase; Hap43-induced; Spider biofilm induced
SOF1	2.0	0.0	Putative protein with a predicted role in 40S ribosomal subunit biogenesis; rat catheter biofilm induced
NMD3	2.0	0.0	Putative nonsense-mediated mRNA decay protein; repressed in core stress response; repressed by prostaglandins
orf19.6828	2.0	0.0	Ortholog(s) have role in rRNA processing and preribosome, large subunit precursor localization
SPB4	2.0	0.0	Putative ATP-dependent RNA helicase; flucytosine repressed; Spider biofilm induced
YTM1	2.0	0.0	Protein similar to S. cerevisiae Ytm1p, which is involved in biogenesis of the large ribosomal subunit; transposon mutation affects filamentous growth; protein level decreases in stationary phase cultures; Hap43p-induced gene
orf19.809	2.0	0.0	Ortholog(s) have rRNA binding activity, role in maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA) and nucleolus, preribosome localization
orf19.6234	2.0	0.0	Putative U2 snRNP component; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); Hap43-induced, Spider biofilm induced
orf19.6829	2.0	0.0	Protein with a predicted mitochondrial ATPase expression domain; possibly an essential gene, disruptants not obtained by UAU1 method
LTV1	1.9	0.0	Putative GSE complex component; repressed by prostaglandins
orf19.6418	1.9	0.0	Ortholog(s) have unfolded protein binding activity and role in protein import into nucleus, ribosomal large subunit biogenesis
CHR1	1.9	0.0	Predicted DEAD-box ATP-dependent RNA helicase; functional homolog of *S. cerevisiae* Rok1; Hap43-induced; Spider biofilm induced
orf19.2362	1.9	0.0	Putative 90S preribosome component; Hap43p-induced gene; possibly an essential gene, disruptants not obtained by UAU1 method
TSR1	1.9	0.0	Component of 20S pre-rRNA processing unit; repressed by prostaglandins
orf19.1404	1.9	0.0	Predicted tRNA dihydrouridine synthase; Spider biofilm induced
UTP22	1.9	0.0	Putative U3 snoRNP protein; Ssr1-induced; repressed by prostaglandins; heterozygous null mutant is resistant to parnafungin
RRS1	1.9	0.0	Putative ribosome biogenesis and nuclear export protein; Hap43p-induced gene; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine)
UTP5	1.9	0.0	Putative U3 snoRNA-associated protein; Hap43p-induced gene; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); physically interacts with TAP-tagged Nop1p
orf19.3477	1.9	0.0	Putative pseudouridine synthase; predicted role in snRNA pseudouridine synthesis, tRNA pseudouridine synthesis; Spider biofilm induced
UTP20	1.9	0.0	Putative snoRNA-binding protein; *S. cerevisiae* Utp20 ortholog; likely essential for growth; repressed in core stress response; mutation confers resistance to 5-fluorocytosine (5-FC) and parnafungin
orf19.4492	1.9	0.0	Ortholog(s) have role in nuclear division, rRNA processing, ribosomal large subunit biogenesis and nuclear periphery, nucleolus, preribosome, large subunit precursor localization
RPL7	1.8	0.0	Ribosomal protein L7; repressed upon phagocytosis by murine macrophages; Hap43-induced; rat catheter and Spider biofilm induced
RPO41	1.8	0.0	Putative mitochondrial RNA polymerase; repressed in core stress response; Spider biofilm induced
NOP4	1.8	0.0	Putative nucleolar protein; Hap43-induced; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); represses in core stress response
orf19.2575	1.8	0.0	Putative S-adenosylmethionine-dependent methyltransferase; Hap43p-induced gene
SDA1	1.8	0.0	Predicted nuclear protein involved in actin cytoskeleton organization, passage through Start, 60S ribosome biogenesis; rat catheter biofilm induced; Hap43-induced
orf19.3990	1.8	0.0	Ortholog(s) have DNA-directed 5'-3' RNA polymerase activity, RNA polymerase III activity and role in tRNA transcription by RNA polymerase III, transcription initiation from RNA polymerase III promoter
NOC4	1.8	0.0	Putative nucleolar protein; Hap43-induced; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); Spider biofilm induced
ITS2	1.8	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25; in *S. cerevisiae* it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
orf19.3759	1.8	0.0	Putative elongator complex subunit; for modification of wobble nucleosides in tRNA; Spider biofilm induced

orf19.3304	1.8	0.0	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in the nucleus and cytoplasm; Spider biofilm induced
orf19.1646	1.8	0.0	Ortholog(s) have rRNA primary transcript binding activity
IPT1	1.8	0.0	Inositol phosphoryl transferase; catalyzes the synthesis of the most abundant sphingolipid, mannose-(inositol-P)2-ceramide, M(IP)2C, from MIPC; required for wild-type membrane localization of Cdr1; Spider biofilm induced
orf19.1687	1.8	0.0	Ortholog of *S. cerevisiae* Prp43, an RNA helicase in the DEAH-box family that functions in both RNA polymerase I and polymerase II transcript metabolism; Hap43-induced gene
orf19.4173	1.7	0.0	Ortholog(s) have role in peptidyl-diphthamide biosynthetic process from peptidyl-histidine
orf19.5991	1.7	0.0	Ortholog(s) have role in assembly of large subunit precursor of preribosome, maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more
orf19.5126	1.7	0.0	Putative adhesin-like protein
RPF1	1.7	0.0	Putative nucleolar protein with a predicted role in the assembly and export of the large ribosomal subunit; essential for growth; rat catheter and Spider biofilm induced
orf19.5356	1.7	0.0	Protein with a predicted role in cell wall integrity; repressed in core stress response
PDC2	1.7	0.0	Homeodomain-like transcription factor; regulator of pyruvate decarboxylase; contains a putative C-terminal activation domain, Glu- and Pro-rich; complements glucose utilization defect of *S. cerevisiae* pdc2 mutant
orf19.1791	1.7	0.0	Putative protein with a predicted role in 60S ribosomal subunit biogenesis; Hap43p-induced gene; ortholog of *S. cerevisiae* MAK11
RLI1	1.7	0.0	Member of RNase L inhibitor (RLI) subfamily of ABC family; predicted not to be a transporter; regulated by Sef1p, Sfu1p, and Hap43p
MPP10	1.7	0.0	Putative SSU processome and 90S preribosome component; repressed in core stress response; repressed by prostaglandins
SPE2	1.7	0.0	Putative S-adenosylmethionine decarboxylase; Hap43-induced gene; possibly adherence-induced; Spider biofilm induced
RCL1	1.7	0.0	Putative U3-containing 90S preribosome processome complex subunit; Hap43-induced; essential; *S. cerevisiae* ortholog is essential; represses in core stress response;
orf19.7618	1.7	0.0	Putative nucleolar protein with a predicted role in pre-18S rRNA processing; Plc1p-regulated; Spider biofilm induced
orf19.962	1.7	0.0	Protein with a fungal RNA polymerase I subunit RPA14 domain; proposed to play a role in the recruitment of pol I to the promoter; Hap43-induced gene
SGD1	1.6	0.0	Predicted small ribosomal subunit biogenesis protein; repressed in core stress response; transcript increases in populations of cells exposed to fluconazole over multiple generations; Spider biofilm induced
ELF1	1.6	0.0	Putative mRNA export protein; Walker A and B (ATP/GTP binding) motifs; required for wild-type morphology, growth; expressed in hyphal, pseudohyphal, and yeast form; Hap43-induced; Spider and flow model biofilm induced
UTP4	1.6	0.0	Putative U3 snoRNA-associated protein; Hap43-induced; physically interacts with TAP-tagged Nop1; Spider biofilm induced
orf19.4479	1.6	0.0	Putative U3-containing 90S preribosome subunit; Hap43-induced; repressed in core stress response; Spider biofilm induced
UTP13	1.6	0.0	Putative U3 snoRNA-associated protein; Hap43-induced; repressed in core stress response; physically interacts with TAP-tagged Nop1
CUP9	1.6	0.0	Transcription factor; represses SOK1 expression in response to farnesol inhibition; yeast-hypha switch repressed; ketoconazole-induced; Plc1-regulated; colony morphology-related Ssn6 regulation; Spider, flow model biofilm induced
HNM1	1.6	0.0	Putative choline/ethanolamine transporter; mutation confers hypersensitivity to toxic ergosterol analog; colony morphology-related gene regulation by Ssn6; clade-associated gene expression
orf19.6477	1.6	0.0	Ortholog(s) have tRNA (guanine-N7-)-methyltransferase activity, role in tRNA (guanine-N7)-methylation and cytosol, nucleus, tRNA (m7G46) methyltransferase complex localization
PUS7	1.6	0.0	Pseudouridine synthase; catalyzes pseudouridylation in U2 snRNA, 5S rRNA, cytoplasmic tRNAs and in pre-tRNA(Tyr); F-12/CO2 early biofilm induced
NOP13	1.6	0.0	Ortholog of *S. cerevisiae* Nop13; a nucleolar protein found in preribosomal complexes; Hap43-induced gene; rat catheter biofilm induced
orf19.177	1.6	0.0	Has domain(s) with predicted phosphatidylinositol binding activity and role in cell communication
NEP1	1.6	0.0	Ortholog(s) have rRNA (pseudouridine) methyltransferase activity
RPA34	1.6	0.0	Putative RNA polymerase I subunit; rat catheter biofilm induced
JIP5	1.6	0.0	Ortholog of *S. cerevisiae* Jip5; predicted role in biogenesis of the large ribosomal subunit; repressed in core stress response; Hap43-induced gene
PWP2	1.6	0.0	Putative 90S pre-ribosomal component; repressed in core stress response; repressed by prostaglandins; physically interacts with TAP-tagged Nop1; Hap43-induced
DRS1	1.6	0.0	Putative nucleolar DEAD-box protein; Hap43-induced; mutation confers hypersensitivity to 5-fluorouracil (5-FU), tubercidin (7-deazaadenosine); Tbf1-induced; repressed in core stress response

MAK5	1.5	0.0	Putative nucleolar DEAD-box RNA helicase; oxidative stress-repressed via Cap1; repressed by prostaglandins
orf19.1697	1.5	0.0	Ortholog(s) have role in cytoplasmic translation, poly(A)+ mRNA export from nucleus and cytoplasm localization
orf19.2527	1.5	0.0	Putative protein of unknown function; Hap43-induced; required for normal biofilm growth; F-12/CO2 early biofilm induced
DBP3	1.5	0.0	Putative ATP-dependent DEAD-box RNA helicase; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
orf19.1578	1.5	0.0	Ortholog of S. cereviaie Rrp5, an RNA binding protein involved in synthesis of 18S and 5.8S rRNAs; Hap43-induced gene
orf19.3556	1.5	0.0	Transportin or cytosolic karyopherin beta; Spider biofilm induced

Appendix 20: RNA-seq result of Zcf18-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf18-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
HGT12	19.5	0.0	Glucose, fructose, mannose transporter; major facilitator superfamily; role in macrophage-induced hyphal growth; detected at germ tube plasma membrane by mass spectrometry; Snf3p-induced; 12 probable transmembrane segments
orf19.2310	13.5	0.0	Predicted single-stranded nucleic acid binding protein; flow model biofilm induced
orf19.2247	13.2	0.0	Ortholog of C. dubliniensis CD36 : Cd36_21220, C. parapsilosis CDC317 : CPAR2_406700, *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_127772 and *Candida tropicalis* MYA-3404 : CTRG_01766
orf19.2049	6.6	0.0	Plasma membrane-associated protein; heterozygous null mutant displays sensitivity to virgineone; Spider biofilm induced
orf19.7279.1	6.2	0.0	Protein of unknown function; Spider biofilm induced
ZCF18	5.5	0.0	Putative Zn(II)2Cys6 transcription factor; heterozygous null mutant displays sensitivity to virgineone and decreased colonization of mouse kidneys
REI1	4.0	0.0	Putative cytoplasmic pre-60S factor; Hap43-induced; repressed by prostaglandins
GPX1	4.0	0.0	Putative thiol peroxidase; rat catheter and Spider biofilm induced
NOG2	3.6	0.0	Putative nucleolar GTPase; repressed by prostaglandins; Hap43-induced, rat catheter and Spider biofilm induced
orf19.4634	3.5	0.0	Protein required for thiolation of uridine at wobble position of Gln, Lys, and Glu tRNAs; has a role in urmylation; *S. cerevisiae* ortholog has a role in invasive and pseudohyphal growth
DIM1	3.4	0.0	Putative 18S rRNA dimethylase; predicted role in rRNA modification and processing; Hap43-induced; likely to be essential for growth based on insertional mutagenesis strategy; F-12/CO2 early biofilm induced
ENP1	3.1	0.0	Protein required for pre-rRNA processing and 40S ribosomal subunit synthesis; associated with U3 and U14 snoRNAs; transposon mutation affects filamentous growth; repressed by prostaglandins; Spider biofilm induced
orf6.5536	2.9	0.0	Protein with a role in nucleolar integrity and processing of pre-rRNA; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); Hap43-induced; Spider biofilm induced
NOP14	2.9	0.0	Putative nucleolar protein; Hap43-induced; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); heterozygous mutant is resistant to parnafungin; Spider biofilm induced
orf19.4921.1	2.9	0.0	Protein of unknown function; Spider biofilm repressed
ENP2	2.9	0.0	Putative nucleolar protein; essential; heterozygous mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); Hap43-induced; Spider biofilm induced
BUD22	2.9	0.0	Protein with a predicted role in 18S rRNA maturation and small ribosomal subunit biogenesis; repressed in core stress response; repressed by prostaglandins
NOP8	2.9	0.0	Ortholog of S. cereviiae Nop8; has a role in ribosomal large subunit biogenesis; rat catheter and Spider biofilm induced
orf19.131	2.9	0.0	Ortholog of C. dubliniensis CD36 : Cd36_61330, C. parapsilosis CDC317 : CPAR2_602800, C. auris B8441 : B9J08_005399 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_94450
NOP6	2.8	0.0	Putative ortholog of *S. cerevisiae* Nop6; role in ribosomal small subunit biogenesis; Spider biofilm induced
orf19.1708	2.8	0.0	Protein of unknown function; Spider biofilm induced
HIS3	2.8	0.0	Imidazoleglycerol-phosphate dehydratase, enzyme of histidine biosynthesis; functionally complements *S. cerevisiae* his3-1 mutation; hyphal-induced expression
MAK16	2.6	0.0	Putative constituent of 66S pre-ribosomal particles; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
orf19.4450.2	2.6	0.0	Ortholog of C. dubliniensis CD36 : Cd36_06690, C. auris B8441 : B9J08_002288, Debaryomyces hansenii CBS767 : DEHA2F26620g and Pichia stipitis Pignal : PICST_62738

orf19.2167	2.6	0.0	Ortholog(s) have role in ribosomal large subunit biogenesis, ribosomal small subunit biogenesis and nucleolus localization
orf19.2386	2.6	0.0	Ortholog(s) have role in endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more
orf19.6175	2.6	0.0	Putative 35S rRNA processing protein; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
orf19.2330	2.6	0.0	Putative U3 snoRNA-associated protein; Hap43-induced; transposon mutation affects filamentous growth; repressed by prostaglandins
ELP3	2.5	0.0	Predicted histone acetyltransferase; role in regulation of transcription, tRNA wobble uridine modification; Spider biofilm induced
RSM22	2.5	0.0	Predicted mitochondrial small ribosomal subunit; rat catheter and Spider biofilm induced
orf19.7546	2.5	0.0	Protein involved in rRNA processing; required for maturation of the 35S primary transcript of pre-rRNA and for cleavage leading to mature 18S rRNA; Spider biofilm induced
orf19.2320	2.4	0.0	Putative serine/threonine-protein kinase; possibly an essential gene, disruptants not obtained by UAU1 method
KRR1	2.4	0.0	Putative nucleolar protein; repressed benomyl treatment or in an azole-resistant strain that overexpresses MDR1; F-12/CO2 early biofilm induced
ABP140	2.4	0.0	Ortholog of *S. cerevisiae* actin-binding protein Abp140; Hap43-induced; F-12/CO2 early biofilm induced
orf19.2934	2.4	0.0	Similar to *S. cerevisiae* Bud20; predicted role in cellular bud site selection; rat catheter and Spider biofilm induced
orf19.7197	2.3	0.0	Putative intranuclear transport and DNA replication mediator
UTP21	2.3	0.0	Putative U3 snoRNP protein; Hap43-induce; physically interacts with TAP-tagged Nop1; Spider biofilm induced
TRM2	2.3	0.0	Putative tRNA methyltransferase; repressed by prostaglandins; Spider biofilm induced
UTP18	2.2	0.0	Putative U3 snoRNA-associated protein; Hap43-induced
HBR3	2.2	0.0	Essential protein; regulated by hemoglobin; *S. cerevisiae* ortholog is essential; Hap43p-induced gene
SAS10	2.2	0.0	Putative U3-containing small subunit processome complex subunit
RRP15	2.2	0.0	Putative nucleolar protein; constituent of pre-60S ribosomal particles
RRP9	2.2	0.0	Ribosomal protein; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); physically interacts with TAP-tagged Nop1
orf19.7552	2.2	0.0	Putative U3-containing small subunit processome complex protein
orf19.2090	2.2	0.0	Ortholog of *S. cerevisiae* Ecm16, an essential DEAH-box ATP-dependent RNA helicase specific to the U3 snoRNP required for 18S rRNA synthesis; Hap43-induced; Spider biofilm induced
NMD3	2.2	0.0	Putative nonsense-mediated mRNA decay protein; repressed in core stress response
orf19.5038	2.2	0.0	Predicted tRNA (guanine) methyltransferase activity; Spider biofilm induced
RRN3	2.1	0.0	Protein with a predicted role in recruitment of RNA polymerase I to rDNA
CIC1	2.1	0.0	Putative proteasome-interacting protein; rat catheter biofilm induced
ITS1	2.1	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58
NAN1	2.1	0.0	Putative U3 snoRNP protein; Hap43p-induced gene; physically interacts with TAP-tagged Nop1p
NIP7	2.1	0.0	Putative nucleolar protein with role in ribosomal assembly; hyphal-induced; Hap43-induced; Spider biofilm induced
orf19.1486	2.1	0.0	Protein with a life-span regulatory factor domain; regulated by Sef1, Sfu1, and Hap43
SPB1	2.1	0.0	Putative AdoMet-dependent methyltransferase; Hap43-induced; repressed by prostaglandins
LTV1	2.0	0.0	Putative GSE complex component; repressed by prostaglandins
NSA1	2.0	0.0	Putative 66S pre-ribosomal particles conmponent; Hap43-induced; repressed by prostaglandins
RPL7	2.0	0.0	Ribosomal protein L7; repressed upon phagocytosis by murine macrophages; Hap43-induced
CNS1	2.0	0.0	Putative co-chaperone; Hap43p-induced gene; mutation confers hypersensitivity to radicicol
orf19.3304	2.0	0.0	Exosome non-catalytic core component
HCA4	2.0	0.0	Putative role in regulation of cell wall biogenesis
orf19.3470	1.9	0.0	Putative flavodoxin; similar to *S. cerevisiae* Tyw1, an iron-sulfur protein required for synthesis of wybutosine modified tRNA; predicted Kex2p substrate; Spider biofilm induced
SRP40	1.9	0.0	Putative chaperone of small nucleolar ribonucleoprotein particles; macrophage/pseudohyphal-induced; rat catheter biofilm induced
orf19.7397.1	1.9	0.0	Predicted ORF identical to NSA2
orf19.1388	1.9	0.0	Putative 66S pre-ribosomal particle component; Hap43-induced; F-12/CO2 early biofilm induced
orf19.107	1.9	0.0	DEAH-box ATP-dependent RNA helicase, required for 18S rRNA synthesis; rat catheter biofilm induced

DIP2	1.9	0.0	Putative small ribonucleoprotein complex; Tn mutation affects filamentous growth; physically interacts with TAP-tagged Nop1; heterozygous null mutant exhibits resistance to parnafungin; Hap43-induced gene; Spider biofilm induced
IMP4	1.9	0.0	Putative SSU processome component; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
UTP22	1.9	0.0	Putative U3 snoRNP protein; Ssr1-induced; repressed by prostaglandins; heterozygous null mutant is resistant to parnafungin
NOP13	1.9	0.0	Ortholog of *S. cerevisiae* Nop13; a nucleolar protein found in preribosomal complexes; Hap43-induced gene; rat catheter biofilm induced
DOT4	1.9	0.0	Protein similar to ubiquitin C-terminal hydrolase; localizes to cell surface of hyphal cells, but not yeast-form cells; repressed upon high-level peroxide; Hap43p-induced; rat catheter biofilm induced
RPC19	1.8	0.0	Putative RNA polymerases I and III subunit AC19; Hap43-induced; rat catheter biofilm induced
NOC4	1.8	0.0	Putative nucleolar protein; Hap43-induced; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); Spider biofilm induced
JIP5	1.8	0.0	Ortholog of *S. cerevisiae* Jip5; predicted role in biogenesis of the large ribosomal subunit; repressed in core stress response; Hap43-induced gene
orf19.4492	1.8	0.0	Ortholog(s) have role in nuclear division, rRNA processing, ribosomal large subunit biogenesis and nuclear periphery, nucleolus, preribosome, large subunit precursor localization
orf19.1609	1.7	0.0	Ortholog(s) have role in endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA and LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA) and 90S preribosome, nucleolus localization
HNM1	1.7	0.0	Putative choline/ethanolamine transporter; mutation confers hypersensitivity to toxic ergosterol analog; colony morphology-related gene regulation by Ssn6; clade-associated gene expression
SOF1	1.7	0.0	Putative protein with a predicted role in 40S ribosomal subunit biogenesis; rat catheter biofilm induced
RRS1	1.7	0.0	Putative ribosome biogenesis and nuclear export protein; Hap43p-induced gene; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine)
TEC1	1.7	0.0	TEA/ATTS transcription factor
orf19.2319	1.7	0.0	Putative nucleolar protein with a predicted role in pre-rRNA processing; Hap43-induced gene; repressed in core stress response
orf19.5991	1.7	0.0	Ortholog(s) have role in assembly of large subunit precursor of preribosome, maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more
NOP4	1.7	0.0	Putative nucleolar protein; Hap43-induced; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); represses in core stress response
orf19.6829	1.7	0.0	Protein with a predicted mitochondrial ATPase expression domain; possibly an essential gene, disruptants not obtained by UAU1 method
GAL4	1.7	0.0	Zn(II)2Cys6 transcription factor; involved in control of glycolysis; ortholog of *S. cerevisiae* Gal4, but not involved in regulation of galactose utilization genes
TSR1	1.7	0.0	Component of 20S pre-rRNA processing unit; repressed by prostaglandins
orf19.6578	1.7	0.0	Predicted membrane transporter; vesicular neurotransmitter (VNT) family, major facilitator superfamily (MFS); repressed in core caspofungin response; induced in oralpharyngeal candidasis; Spider biofilm induced
RLI1	1.7	0.0	Member of RNase L inhibitor (RLI) subfamily of ABC family; predicted not to be a transporter; regulated by Sef1p, Sfu1p, and Hap43p
orf19.5126	1.6	0.0	Putative adhesin-like protein
orf19.3477	1.6	0.0	Putative pseudouridine synthase; predicted role in snRNA pseudouridine synthesis, tRNA pseudouridine synthesis; Spider biofilm induced
MAK5	1.6	0.0	Putative nucleolar DEAD-box RNA helicase; oxidative stress-repressed via Cap1; repressed by prostaglandins
ZCF1	1.6	0.0	Zn(II)2Cys6 transcription factor; transcript regulated during hypha formation
orf19.962	1.6	0.0	Protein with a fungal RNA polymerase I subunit RPA14 domain; proposed to play a role in the recruitment of pol I to the promoter; Hap43-induced gene
NEP1	1.6	0.0	Ortholog(s) have rRNA (pseudouridine) methyltransferase activity
MCD1	1.5	0.0	Alpha-kleisin cohesin complex subunit; for sister chromatid cohesion in mitosis and meiosis; repressed by alpha pheromone in SpiderM medium; periodic cell-cycle expression; Hap43-repressed
orf19.6418	1.5	0.0	Ortholog(s) have unfolded protein binding activity and role in protein import into nucleus, ribosomal large subunit biogenesis
ITS2	1.5	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25

Appendix 21: RNA-seq result of Zcf19-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf19-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001

Gene	log2FC	Adjusted P value	Description
orf19.3210	19.6	0.0	Predicted protein of unknown function; Plc1-regulated
ZCF19	8.6	0.0	Predicted Zn(II)2Cys6 transcription factor
orf19.1461	5.6	0.0	S. pombe ortholog SPCC576.01c is a predicted sulfonate dioxygenase; possibly transcriptionally regulated upon hyphal formation; Spider biofilm induced
orf19.4634	3.5	0.0	Protein required for thiolation of uridine at wobble position of Gln, Lys, and Glu tRNAs; has a role in urmylation; *S. cerevisiae* ortholog has a role in invasive and pseudohyphal growth
REI1	3.4	0.0	Putative cytoplasmic pre-60S factor; Hap43-induced; repressed by prostaglandins
DIM1	3.3	0.0	Putative 18S rRNA dimethylase; predicted role in rRNA modification and processing
NOP14	2.9	0.0	Putative nucleolar protein; Hap43-induced
ENP2	2.9	0.0	Putative nucleolar protein; essential
DAL1	2.6	0.0	Putative allantoinase; transcript regulated by Nrg1 and Mig1; macrophage/pseudohyphal-repressed
ROA1	2.5	0.0	Putative PDR-subfamily ABC transporter involved in sensitivity to azoles; Spider biofilm induced
orf19.2386	2.5	0.0	Ortholog(s) have role in endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more
NOP6	2.4	0.0	Putative ortholog of *S. cerevisiae* Nop6; role in ribosomal small subunit biogenesis; Spider biofilm induced
BUD22	2.4	0.0	Protein with a predicted role in 18S rRNA maturation and small ribosomal subunit biogenesis; repressed in core stress response; repressed by prostaglandins
orf19.2175	2.3	0.0	Mitochondrial apoptosis-inducing factor; induced by nitric oxide; Spider biofilm induced; rat catheter biofilm repressed
orf19.4921.1	2.3	0.0	Protein of unknown function; Spider biofilm repressed
TRM2	2.3	0.0	Putative tRNA methyltransferase; repressed by prostaglandins; Spider biofilm induced
orf19.6175	2.3	0.0	Putative 35S rRNA processing protein; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
orf19.5802	2.3	0.0	Ortholog(s) have transferase activity, role in maturation of SSU-rRNA and cytoplasm localization
KRR1	2.3	0.0	Putative nucleolar protein; repressed benomyl treatment or in an azole-resistant strain that overexpresses MDR1; F-12/CO2 early biofilm induced
HBR3	2.3	0.0	Essential protein; regulated by hemoglobin; *S. cerevisiae* ortholog is essential; Hap43p-induced gene
orf19.1486	2.2	0.0	Protein with a life-span regulatory factor domain; regulated by Sef1, Sfu1, and Hap43; flow model biofilm induced; Spider biofilm induced
orf19.2167	2.2	0.0	Ortholog(s) have role in ribosomal large subunit biogenesis, ribosomal small subunit biogenesis and nucleolus localization
UTP21	2.1	0.0	Putative U3 snoRNP protein; Hap43-induce; physically interacts with TAP-tagged Nop1; Spider biofilm induced
ITS1	2.1	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58; in *S. cerevisiae* it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
DIP2	2.1	0.0	Putative small ribonucleoprotein complex; Tn mutation affects filamentous growth
ELP3	2.1	0.0	Predicted histone acetyltransferase; role in regulation of transcription, tRNA wobble uridine modification; Spider biofilm induced
RPL7	2.1	0.0	Ribosomal protein L7; repressed upon phagocytosis by murine macrophages; Hap43-induced; rat catheter and Spider biofilm induced
SRP40	2.0	0.0	Putative chaperone of small nucleolar ribonucleoprotein particles; macrophage/pseudohyphal-induced; rat catheter biofilm induced
HCA4	1.9	0.0	Putative role in regulation of cell wall biogenesis; Hap43p-induced gene; possibly an essential gene, disruptants not obtained by UAU1 method; flow model and rat catheter biofilm induced
orf19.6829	1.8	0.0	Protein with a predicted mitochondrial ATPase expression domain; possibly an essential gene, disruptants not obtained by UAU1 method
RPA34	1.8	0.0	Putative RNA polymerase I subunit; rat catheter biofilm induced
FCY21	1.8	0.0	High affinity, high capacity, hypoxanthine-adenine-guanine-cytosine/H+ symporter; similar to *S. cerevisiae* Fcy2; mutation confers resistance to 5-fluorocytosine (5-FC); flow model biofilm induced
TEC1	1.7	0.0	TEA/ATTS transcription factor; white cell pheromone response, hyphal gene regulation; required for Spider and RPMI biofilm formation; regulates BCR1; Cph2 regulated transcript; alkaline, rat catheter, Spider, flow model biofilm induced
orf19.3477	1.7	0.0	Putative pseudouridine synthase; predicted role in snRNA pseudouridine synthesis, tRNA pseudouridine synthesis; Spider biofilm induced
orf19.5833	1.6	0.0	Ortholog(s) have SUMO binding, polyubiquitin modification-dependent protein binding, ubiquitin binding activity

IFM3	1.6	0.0	Protein with a 2-hydroxyacid dehydrogenase catalytic domain; Hap43-repressed; Plc1-regulated; overlaps orf19.2177
orf19.6578	1.6	0.0	Predicted membrane transporter; vesicular neurotransmitter (VNT) family, major facilitator superfamily (MFS); repressed in core caspofungin response; induced in oralpharyngeal candidasis; Spider biofilm induced
ALS7	1.5	0.0	ALS family protein; hypermutable contingency gene; growth-regulated, downregulated in biofilm; two variable repeat regions; expression in *S. cerevisiae* does not confer adhesiveness; ALS family includes adhesins, cell-surface glycoproteins

Appendix 22: RNA-seq result of Zcf21-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf21-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
HGT12	22.8	0.0	Glucose, fructose, mannose transporter; major facilitator superfamily; role in macrophage-induced hyphal growth; detected at germ tube plasma membrane by mass spectrometry; Snf3p-induced; 12 probable transmembrane segments
orf19.3210	19.7	0.0	Predicted protein of unknown function; Plc1-regulated
orf19.5611	15.8	0.0	Predicted 3-methylbutanol:NAD(P) oxidoreductase and methylglyoxal reductase (NADPH-dependent); role in ergosterol metabolic process; early stage flow model biofilm induced; Spider biofilm induced
orf19.7279.1	7.3	0.0	Protein of unknown function; Spider biofilm induced
ZCF21	5.0	0.0	Predicted Zn(II)2Cys6 transcription factor; mutants display increased colonization of mouse kidneys; Spider biofilm induced
PGA57	4.5	0.0	Putative GPI-anchored protein; Hap43p-induced gene
REI1	3.5	0.0	Putative cytoplasmic pre-60S factor; Hap43-induced; repressed by prostaglandins
orf19.4634	3.4	0.0	Protein required for thiolation of uridine at wobble position of Gln, Lys, and Glu tRNAs; has a role in urmylation; *S. cerevisiae* ortholog has a role in invasive and pseudohyphal growth
HIS3	3.0	0.0	Imidazoleglycerol-phosphate dehydratase, enzyme of histidine biosynthesis; functionally complements *S. cerevisiae* his3-1 mutation; hyphal-induced expression; regulated by Gcn2p and Gcn4p; fungal-specific (no human or murine homolog)
DIM1	3.0	0.0	Putative 18S rRNA dimethylase; predicted role in rRNA modification and processing; Hap43-induced; likely to be essential for growth based on insertional mutagenesis strategy; F-12/CO2 early biofilm induced
REP1	2.6	0.0	Putative transcription factor involved in transcription of N-acetylglucosamine-inducible genes; involved in negative regulation of MDR1 transcription; mutants show increased resistance to azole drugs
NOP14	2.6	0.0	Putative nucleolar protein; Hap43-induced; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); heterozygous mutant is resistant to parnafungin; Spider biofilm induced
NOP6	2.5	0.0	Putative ortholog of *S. cerevisiae* Nop6; role in ribosomal small subunit biogenesis; Spider biofilm induced
FRP3	2.4	0.0	Putative ammonium transporter; upregulated in the presence of human neutrophils; fluconazole-downregulated; repressed by nitric oxide; Spider biofilm induced; rat catheter biofilm repressed
SET6	2.4	0.0	Ortholog of *S. cerevisiae* Set6, a SET domain protein of unknown function; Hap43-induced; rat catheter and Spider biofilm induced
ELP3	2.4	0.0	Predicted histone acetyltransferase; role in regulation of transcription, tRNA wobble uridine modification; Spider biofilm induced
ENP2	2.4	0.0	Putative nucleolar protein; essential; heterozygous mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); Hap43-induced; Spider biofilm induced
ITS1	2.4	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58; in *S. cerevisiae* it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
orf19.1427	2.3	0.0	Putative transporter; fungal-specific; Spider biofilm induced
orf19.2167	2.3	0.0	Ortholog(s) have role in ribosomal large subunit biogenesis, ribosomal small subunit biogenesis and nucleolus localization
orf19.6175	2.3	0.0	Putative 35S rRNA processing protein; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
orf19.5038	2.2	0.0	Predicted tRNA (guanine) methyltransferase activity; Spider biofilm induced
RPC19	2.2	0.0	Putative RNA polymerases I and III subunit AC19; Hap43-induced; rat catheter biofilm induced
HBR3	2.1	0.0	Essential protein; regulated by hemoglobin; *S. cerevisiae* ortholog is essential; Hap43p-induced gene
orf19.2090	2.1	0.0	Ortholog of S. cerevisiae Ecm16, an essential DEAH-box ATP-dependent RNA helicase specific to the U3 snoRNP required for 18S rRNA synthesis; Hap43-induced; Spider biofilm induced

ITS2	2.1	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25; in *S. cerevisiae* it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
NIP7	2.1	0.0	Putative nucleolar protein with role in ribosomal assembly; hyphal-induced; Hap43-induced; Spider biofilm induced
orf19.1486	2.0	0.0	Protein with a life-span regulatory factor domain; regulated by Sef1, Sfu1, and Hap43; flow model biofilm induced; Spider biofilm induced
orf19.5070	1.9	0.0	Similar to cell-wall mannoproteins; induced in low iron; induced in cyr1 homozygous null; regulated by osmotic and oxidative stress via Hog1; Spider biofilm induced
YTM1	1.9	0.0	Protein similar to *S. cerevisiae* Ytm1p, which is involved in biogenesis of the large ribosomal subunit; transposon mutation affects filamentous growth; protein level decreases in stationary phase cultures; Hap43p-induced gene
SOF1	1.9	0.0	Putative protein with a predicted role in 40S ribosomal subunit biogenesis; rat catheter biofilm induced
orf19.4128	1.9	0.0	Ortholog of C. dubliniensis CD36 : Cd36_19390, C. parapsilosis CDC317 : CPAR2_209600, C. auris B8441 : B9J08_004606 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_134010
UTP18	1.9	0.0	Putative U3 snoRNA-associated protein; Hap43-induced; repressed in core stress response; physically interacts with TAP-tagged Nop1
orf19.3425	1.9	0.0	RING/FYVE/PHD zinc finger protein; Spider biofilm induced
RGT1	1.8	0.0	Zn(II)2Cys6 transcription factor; transcriptional repressor involved in the regulation of glucose transporter genes; ortholog of *S. cerevisiae* Rgt1; mutants display decreased colonization of mouse kidneys
orf19.6829	1.8	0.0	Protein with a predicted mitochondrial ATPase expression domain; possibly an essential gene, disruptants not obtained by UAU1 method
orf19.6578	1.8	0.0	Predicted membrane transporter; vesicular neurotransmitter (VNT) family, major facilitator superfamily (MFS); repressed in core caspofungin response; induced in oralpharyngeal candidasis; Spider biofilm induced
orf19.3625	1.8	0.0	Has domain(s) with predicted zinc ion binding activity
orf19.1800	1.8	0.0	Protein of unknown function; Spider biofilm induced
GAL4	1.7	0.0	Zn(II)2Cys6 transcription factor; involved in control of glycolysis; ortholog of *S. cerevisiae* Gal4, but not involved in regulation of galactose utilization genes; caspofungin repressed; Spider biofilm repressed
RDN58	1.6	0.0	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
RPL7	1.6	0.0	Ribosomal protein L7; repressed upon phagocytosis by murine macrophages; Hap43-induced; rat catheter and Spider biofilm induced
RDN25	1.6	0.0	25S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R; in some strains the gene may contain the self-splicing group I intron (LSU)
orf19.5126	1.6	0.0	Putative adhesin-like protein
ADR1	1.5	0.0	C2H2 transcription factor; ortholog of *S. cerevisiae* Adr1 but mutant phenotype suggests a different set of target genes; transposon mutation affects filamentous growth; Spider biofilm induced

Appendix 23: RNA-seq result of Zcf22-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf22-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.3210	15.7	0.0	Predicted protein of unknown function; Plc1-regulated
LIP3	15.3	0.0	Secreted lipase; gene family member whose members are expressed differentially in response to carbon source and infection; possible role in nutrition and/or in creating an acidic microenvironment; flow model biofilm induced
ZCF22	7.2	0.0	Predicted Zn(II)2Cys6 transcription factor
orf19.5169	6.1	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
orf19.1307	6.0	0.0	Predicted membrane protein; rat catheter biofilm induced
orf19.1691	4.6	0.0	Plasma-membrane-localized protein; filament induced; Hog1, ketoconazole, fluconazole and hypoxia-induced; regulated by Nrg1, Tup1, Upc2; induced by prostaglandins; flow model biofilm induced; rat catheter and Spider biofilm repressed
RNR22	3.8	0.0	Putative ribonucleoside diphosphate reductase;colony morphology-related gene regulation by Ssn6; transcript regulated by tyrosol and cell density; Hap43-repressed; Spider biofilm induced
orf19.1369	3.6	0.0	Protein with predicted peptidase domains; Hap43-repressed gene
PLB1	3.6	0.0	Phospholipase B; host cell penetration and virulence in mouse systemic infection; Hog1-induced; signal sequence, N-glycosylation, and Tyr phosphorylation site; induced in fluconazole-resistant strains; rat catheter biofilm repressed
ECM38	3.4	0.0	Putative gamma-glutamyltransferase; alkaline upregulated; Spider biofilm induced; possibly an essential gene, disruptants not obtained by UAU1 method
AQY1	3.3	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance; virulent in mice; flucytosine repressed; flow model/RPMI/Spider/rat catheter biofilm induced; required for RPMI biofilm formation; Bcr1-induced in a/a RPMI biofilms
OPT7	3.1	0.0	Putative oligopeptide transporter; possibly transports GSH or related compounds; Hog1-induced; expression of OPT6, -7, or -8 does not suppress defect of mutant lacking OPT1-3; Hap43-repressed; F-12/CO2 early biofilm induced
orf19.4450.1	3.0	0.0	Protein conserved among the CTG-clade; 2 adjacent upstream SRE-1 elements; highly up-regulated in cecum-grown cells in a Cph2-dependent manner; Hap43-repressed; rat catheter, Spider and flow model biofilm induced
ADH5	2.8	0.0	Putative alcohol dehydrogenase; regulated by white-opaque switch; fluconazole-induced; antigenic in murine infection; regulated by Nrg1, Tup1; Hap43, macrophage repressed, flow model biofilm induced; Spider biofilm induced
QDR1	2.8	0.0	Putative antibiotic resistance transporter; regulated by white-opaque switch, Nrg1, Tup1; Hap43, caspofungin repressed; repressed during chlamydospore formation; flow model biofilm induced; Spider biofilm repressed
orf19.4589	2.8	0.0	Ortholog(s) have polyamine oxidase activity and role in pantothenate biosynthetic process, spermine catabolic process
OPT3	2.7	0.0	Oligopeptide transporter; transcript induced by macrophage phagocytosis, BSA or peptides; fluconazole-induced; induced by Rim101 at pH 8; virulence-group-correlated expression; Hap43-repressed; Spider biofilm induced
RHD3	2.7	0.0	GPI-anchored yeast-associated cell wall protein; induced in high iron; clade-associated gene expression; not essential for cell wall integrity; fluconazole-repressed; flow model and Spider biofilm repressed
orf19.4612	2.6	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
STF2	2.5	0.0	Protein involved in ATP biosynthesis; repressed in hyphae; repressed by Efg1, Hap43; transcript upregulated in clinical isolates from HIV+ patients with oral candidiasis; rat catheter, flow model and Spider biofilm induced
OPT1	2.4	0.0	Oligopeptide transporter; transports 3-to-5-residue peptides; alleles are distinct, one has intron; suppresses *S. cerevisiae* ptr2-2 mutant defects; induced by BSA or peptides; Stp3p, Hog1p regulated; flow model biofilm induced
OSM1	2.3	0.0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae; caspofungin repressed; stationary phase enriched protein; flow model biofilm induced; Spider biofilm repressed
CDR4	2.1	0.0	Putative ABC transporter superfamily; fluconazole, Sfu1, Hog1, core stress response induced; caspofungin repressed; fluconazole resistance not affected by mutation or correlated with expression; rat catheter and flow model biofilm induced
snR42a	2.1	0.0	H/ACA box small nucleolar RNA (snoRNA)
orf19.1368	2.0	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
orf19.2371	2.0	0.0	Putative Gag protein of retrotransposon Tca2; separated by a stop codon from Pol protein orf19.2372; likely translated as single polyprotein that includes Gag, reverse transcriptase, protease, and integrase; rat catheter biofilm repressed
CRP1	1.9	0.0	Copper transporter; CPx P1-type ATPase; mediates Cu resistance

DAK2	1.8	0.0	Putative dihydroxyacetone kinase; repressed by yeast-hypha switch; fluconazole-induced; caspofungin repressed; protein enriched in stationary phase yeast cultures; flow model biofilm induced; rat catheter and Spider biofilm repressed
OSM2	1.8	0.0	Putative mitochondrial fumarate reductase; regulated by Ssn6p, Gcn2p, and Gcn4p; Hog1p-downregulated; stationary phase enriched protein; Hap43p-repressed gene
ECI1	1.8	0.0	Protein similar to *S. cerevisiae* Eci1p, which is involved in fatty acid oxidation; transposon mutation affects filamentous growth; expression is regulated upon white-opaque switching
GCY1	1.8	0.0	Aldo/keto reductase; mutation confers hypersensitivity to toxic ergosterol analog
MNN22	1.8	0.0	Alpha-1,2-mannosyltransferase; required for normal cell wall mannan
PLB4.5	1.7	0.0	Phospholipase B; Hog1-induced; regulated by Ssn6; putative GPI-anchor
orf19.2372	1.6	0.0	Pol protein of retrotransposon Tca2; separated by a stop codon from Gag protein orf19.2371
DLD2	1.6	0.0	Ortholog(s) have D-lactate dehydrogenase (cytochrome) activity, role in lactate catabolic process and mitochondrial inner membrane, mitochondrion localization
CPA2	1.5	0.0	Putative arginine-specific carbamoylphosphate synthetase
orf19.6305	1.5	0.0	Hydroxytrimethyllysine aldolase, the second enzyme in the carnitine biosynthesis pathway

Appendix 24: RNA-seq result of Zcf26-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf26-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.3210	19.0	0.0	Predicted protein of unknown function; Plc1-regulated
orf19.6919	16.7	0.0	Ortholog of C. dubliniensis CD36 : Cd36_71210, C. parapsilosis CDC317 : CPAR2_702710, C. auris B8441
ZCF26	9.2	0.0	Zn2-Cys6 transcription factor of unknown function; induced by alpha pheromone in Spider medium
orf19.5020	6.5	0.0	Protein of unknown function; Hap43-induced; Spider biofilm induced
orf19.3088	6.4	0.0	bZIP transcription factor; possibly transcriptionally regulated upon hyphal formation
REI1	5.3	0.0	Putative cytoplasmic pre-60S factor; Hap43-induced; repressed by prostaglandins
NOG2	5.0	0.0	Putative nucleolar GTPase; repressed by prostaglandins; Hap43-induced, rat catheter and Spider biofilm induced
orf19.4634	4.9	0.0	Protein required for thiolation of uridine at wobble position of Gln, Lys, and Glu tRNAs
MAK16	4.7	0.0	Putative constituent of 66S pre-ribosomal particles; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
ENP2	4.6	0.0	Putative nucleolar protein; essential; heterozygous mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU)
orf19.4273	4.4	0.0	Putative mitochondrial membrane protein; ortholog of *S. cerevisiae* Sls1
tG(GCC)6	4.4	0.0	tRNA-Gly, predicted by tRNAscan-SE; GCC anticodon
RSM22	4.4	0.0	Predicted mitochondrial small ribosomal subunit; rat catheter and Spider biofilm induced
NOP14	4.3	0.0	Putative nucleolar protein; Hap43-induced; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU)
KTI11	4.2	0.0	Zn-ribbon protein; required for synthesis of diphthamide on translation factor eEF2
SEN2	4.2	0.0	Putative tRNA splicing endonuclease subunit; mutation confers hypersensitivity to toxic ergosterol
HGT1	4.2	0.0	High-affinity MFS glucose transporter; induced by progesterone, chloramphenicol, benomyl
NOP8	4.1	0.0	Ortholog of S. cereviiae Nop8; has a role in ribosomal large subunit biogenesis; rat catheter and Spider biofilm induced
SMM1	4.0	0.0	Putative dihydrouridine synthase; Hap43-induced gene; rat catheter biofilm induced; Spider biofilm induced
orf19.5207	3.9	0.0	Predicted diphthamide biosynthesis protein; Spider biofilm induced
orf19.5905	3.9	0.0	Protein of unknown function; Hap43-induced; F-12/CO2 early biofilm induced
orf19.5220	3.8	0.0	Putative RNA exonuclease; induced in a ssr1 null mutant
orf19.1708	3.8	0.0	Protein of unknown function; Spider biofilm induced
ENP1	3.8	0.0	Protein required for pre-rRNA processing and 40S ribosomal subunit synthesis
orf19.3337	3.8	0.0	Protein of unknown function; merged with orf19.3338
DIM1	3.8	0.0	Putative 18S rRNA dimethylase; predicted role in rRNA modification and processing
orf19.8278	3.8	0.0	Predicted ORF from original SGTC Assembly 19 annotation, removed from the reduced ORF set by the SGTC
orf19.2090	3.7	0.0	Ortholog of *S. cerevisiae* Ecm16

orf19.2320	3.7	0.0	Putative serine/threonine-protein kinase; possibly an essential gene, disruptants not obtained by UAU1 method
POP3	3.7	0.0	Putative RNase MRP and nuclear RNase P component; decreased repressed by prostaglandins; Spider biofilm induced
orf19.7088	3.7	0.0	Ortholog(s) have N(6)-L-threonylcarbamoyladenine synthase activity, single-stranded telomeric DNA binding activity
orf19.4355	3.7	0.0	Ortholog of C. dubliniensis CD36
BUD22	3.6	0.0	Protein with a predicted role in 18S rRNA maturation and small ribosomal subunit biogenesis
RRN3	3.6	0.0	Protein with a predicted role in recruitment of RNA polymerase I to rDNA; caspofungin induced
RMP1	3.6	0.0	Ortholog of Rmp1
orf19.2330	3.6	0.0	Putative U3 snoRNA-associated protein; Hap43-induced; transposon mutation affects filamentous growth; repressed by prostaglandins
ELP3	3.6	0.0	Predicted histone acetyltransferase; role in regulation of transcription, tRNA wobble uridine modification; Spider biofilm induced
orf19.2934	3.6	0.0	Similar to *S. cerevisiae* Bud20; predicted role in cellular bud site selection; rat catheter and Spider biofilm induced
MTG1	3.5	0.0	Putative mitochondrial GTPase
snR57b	3.5	0.0	C/D box small nucleolar RNA (snoRNA)
orf19.6675	3.5	0.0	Protein of unknown function; from Assembly 19; removed from Assembly 20
orf19.2564	3.5	0.0	Putative nucleolar protein; implicated in ribosome biogenesis; rat catheter biofilm repressed
orf19.2167	3.5	0.0	Ortholog(s) have role in ribosomal large subunit biogenesis, ribosomal small subunit biogenesis and nucleolus localization
ALK2	3.5	0.0	N-Alkane inducible cytochrome P450
NCS2	3.5	0.0	Putative cytosolic thiouridylase subunit; Spider biofilm induced
RRP15	3.4	0.0	Putative nucleolar protein; constituent of pre-60S ribosomal particles; Hap43-induced; repressed by prostaglandins
orf19.5049	3.4	0.0	Putative U3-containing 90S preribosome processome complex subunit
orf19.6175	3.4	0.0	Putative 35S rRNA processing protein; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
UTP21	3.3	0.0	Putative U3 snoRNP protein; Hap43-induce; physically interacts with TAP-tagged Nop1; Spider biofilm induced
orf19.3470	3.3	0.0	Putative flavodoxin; similar to *S. cerevisiae* Tyw1
RRP9	3.3	0.0	Ribosomal protein; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine
BUD21	3.3	0.0	Small-subunit processome component; repressed by prostaglandins
orf19.2386	3.3	0.0	Ortholog(s) have role in endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript
TRM2	3.2	0.0	Putative tRNA methyltransferase; repressed by prostaglandins; Spider biofilm induced
snR8a	3.2	0.0	H/ACA box small nucleolar RNA (snoRNA)
orf19.5802	3.2	0.0	Ortholog(s) have transferase activity, role in maturation of SSU-rRNA and cytoplasm localization
NAN1	3.2	0.0	Putative U3 snoRNP protein; Hap43p-induced gene; physically interacts with TAP-tagged Nop1p
KRR1	3.2	0.0	Putative nucleolar protein; repressed benomyl treatment or in an azole-resistant strain that overexpresses MDR1
SAS10	3.2	0.0	Putative U3-containing small subunit processome complex subunit
orf19.107	3.1	0.0	DEAH-box ATP-dependent RNA helicase, required for 18S rRNA synthesis; rat catheter biofilm induced
orf19.3393	3.1	0.0	Putative DEAD-box helicase; Hap43-induced; Spider biofilm induced
HBR3	3.1	0.0	Essential protein; regulated by hemoglobin; *S. cerevisiae* ortholog is essential; Hap43p-induced gene
orf19.4964	3.1	0.0	Ortholog(s) have role in mRNA splicing, via spliceosome and RES complex, nucleus localization
RPP1	3.1	0.0	Putative ortholog of *S. cerevisiae* Rpp1; subunit of both RNase MRP and nuclear RNase P; rat catheter and Spider biofilm induced
orf19.7197	3.1	0.0	Putative intranuclear transport and DNA replication mediator
IMP4	3.1	0.0	Putative SSU processome component; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
TES1	3.1	0.0	Putative acyl-CoA thioesterase
orf19.6886	3.1	0.0	Ortholog(s) have rRNA binding activity and role in maturation of LSU-rRNA from tricistronic rRNA transcript
orf19.3304	3.1	0.0	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in the nucleus and cytoplasm

orf19.494	3.0	0.0	Putative RNA-binding protein; role in assembly of box H/ACA snoRNPs and thus pre-rRNA processing; Spider biofilm induced
UTP22	3.0	0.0	Putative U3 snoRNP protein; Ssr1-induced; repressed by prostaglandins; heterozygous null mutant is resistant to parnafungin
orf19.3831	3.0	0.0	Ortholog(s) have enzyme activator activity, telomerase inhibitor activity
orf19.2237.1	3.0	0.0	Ortholog of Slx9 required for pre-rRNA processing
orf19.5067	3.0	0.0	Predicted nuclear exosome-associated nucleic acid binding protein; rat catheter and Spider biofilm induced
orf19.6477	3.0	0.0	Ortholog(s) have tRNA (guanine-N7-)-methyltransferase activity
FYV5	3.0	0.0	Protein with a predicted role maturation of 18S rRNA; rat catheter biofilm induced
orf19.5216	3.0	0.0	Ortholog of C. dubliniensis CD36 : Cd36_20380, C. parapsilosis CDC317
CNS1	3.0	0.0	Putative co-chaperone; Hap43p-induced gene; mutation confers hypersensitivity to radicicol
orf19.7546	3.0	0.0	Protein involved in rRNA processing
NOP6	3.0	0.0	Putative ortholog of *S. cerevisiae* Nop6
UTP18	2.9	0.0	Putative U3 snoRNA-associated protein
orf19.4160	2.9	0.0	Ortholog(s) have N(6)-L-threonylcarbamoyladenine synthase activity
orf19.5704	2.9	0.0	Ortholog(s) have rRNA binding activity, role in RNA splicing, mitochondrial RNA processing
orf19.5038	2.9	0.0	Predicted tRNA (guanine) methyltransferase activity; Spider biofilm induced
DIP2	2.9	0.0	Putative small ribonucleoprotein complex; Tn mutation affects filamentous growth
DOT4	2.9	0.0	Protein similar to ubiquitin C-terminal hydrolase
orf19.2018	2.8	0.0	Protein with a predicted DnaJ chaperone domain and a CSL-type zinc finger; Spider biofilm induced
MCD1	2.8	0.0	Alpha-kleisin cohesin complex subunit; for sister chromatid cohesion in mitosis and meiosis
NOC4	2.8	0.0	Putative nucleolar protein; Hap43-induced
RPA12	2.8	0.0	Putative DNA-directed RNA polymerase I; induced upon adherence to polystyrene
orf19.7488	2.8	0.0	Component of the SSU processome; predicted role in pre-18S rRNA processing; Spider biofilm induced
CHR1	2.8	0.0	Predicted DEAD-box ATP-dependent RNA helicase
orf6.5536	2.8	0.0	Protein with a role in nucleolar integrity and processing of pre-rRNA
orf19.4563	2.8	0.0	Protein of unknown function; repressed by prostaglandins; Hap43-induced, Spider biofilm induced
PHO84	2.8	0.0	High-affinity phosphate transporter; transcript regulated by white-opaque switch; Hog1, ciclopirox olamine or alkaline induced
FAD3	2.8	0.0	Omega-3 fatty acid desaturase
orf19.4760	2.8	0.0	Putative protein-histidine N-methyltransferase; Spider biofilm induced
orf19.4895	2.8	0.0	Ortholog of *C. dubliniensis* CD36 : Cd36_09650, *C. parapsilosis* CDC317 : CPAR2_805060, C. auris B8441
orf19.2547	2.8	0.0	Has domain(s) with predicted RNA binding, ribonuclease activity
NSA1	2.8	0.0	Putative 66S pre-ribosomal particles conmponent; Hap43-induced; repressed by prostaglandins
RRP8	2.8	0.0	Ribosomal protein; Hap43-induced; F-12/CO2 early biofilm and rat catheter biofilm induced
NOP15	2.8	0.0	Nucleolar ribosome biogenesis factor; hyphal-induced expression
RRN11	2.8	0.0	Putative RNA polymerase I subunit; rat catheter biofilm induced; Spider biofilm induced
orf19.6862	2.8	0.0	Hap43-induced gene; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU)
PGA57	2.8	0.0	Putative GPI-anchored protein; Hap43p-induced gene
orf19.1404	2.8	0.0	Predicted tRNA dihydrouridine synthase; Spider biofilm induced
orf19.3626	2.8	0.0	Has domain(s) with predicted asparagine synthase (glutamine-hydrolyzing) activity
orf19.6976	2.7	0.0	Predicted MFS membrane transporter; member of the proton coupled folate transporter/heme carrier protein family
orf19.3759	2.7	0.0	Putative elongator complex subunit; for modification of wobble nucleosides in tRNA; Spider biofilm induced
orf19.1409.2	2.7	0.0	Protein of unknown function; Spider biofilm induced
orf19.5608	2.7	0.0	RNA polymerase III subunit; Spider biofilm induced
orf19.3477	2.7	0.0	Putative pseudouridine synthase; predicted role in snRNA pseudouridine synthesis, tRNA pseudouridine synthesis
KTI12	2.7	0.0	Protein similar to *S. cerevisiae* Kti12p

orf19.3204	2.7	0.0	Ortholog of C. dubliniensis CD36 : Cd36_51610, C. parapsilosis CDC317
orf19.3275	2.7	0.0	Ortholog of C. dubliniensis CD36 : Cd36_25870, C. parapsilosis CDC317
BUD23	2.7	0.0	Putative methyltransferase; Hap43-induced; repressed by prostaglandins
orf19.4128	2.7	0.0	Ortholog of C. dubliniensis CD36 : Cd36_19390, C. parapsilosis CDC317
orf19.3463	2.7	0.0	Putative GTPase; role in 60S ribosomal subunit biogenesis; Spider biofilm induced
orf19.6662	2.7	0.0	Putative coenzyme Q (ubiquinone) binding protein; transcript is upregulated in clinical isolates
orf19.6234	2.7	0.0	Putative U2 snRNP component; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU)
orf19.2066	2.7	0.0	Ortholog(s) have protein-lysine N-methyltransferase activity and role in peptidyl-lysine trimethylation
BRG1	2.7	0.0	Transcription factor; recruits Hda1 to hypha-specific promoters; Tn mutation affects filamentation
orf19.1287	2.7	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
orf19.3978	2.7	0.0	Protein required for maturation of 18S rRNA; rat catheter biofilm repressed
orf19.5431	2.6	0.0	Protein of unknown function; Hap43-repressed; Spider biofilm induced
orf19.4161	2.6	0.0	Ortholog(s) have SUMO transferase activity, role in DNA repair and Smc5-Smc6 complex localization
CIC1	2.6	0.0	Putative proteasome-interacting protein; rat catheter biofilm induced
orf19.1360	2.6	0.0	Ortholog(s) have role in mitochondrial genome maintenance, mitochondrion organization
orf19.6723	2.6	0.0	Protein of unknown function; Spider biofilm induced
SRP40	2.6	0.0	Putative chaperone of small nucleolar ribonucleoprotein particles
orf19.1388	2.6	0.0	Putative 66S pre-ribosomal particle component; Hap43-induced; F-12/CO2 early biofilm induced
PPT1	2.6	0.0	Putative serine/threonine phosphatase; induced in high iron
orf19.1642	2.6	0.0	Ortholog of *S. cerevisiae* Loc1, a nuclear protein involved in asymmetric localization of ASH1 mRNA in *S. cerevisiae*
NIP7	2.6	0.0	Putative nucleolar protein with role in ribosomal assembly; hyphal-induced; Hap43-induced; Spider biofilm induced
HCM1	2.6	0.0	Protein with forkhead domain; similar to *S. cerevisiae* Hcm1p; Hap43p-induced gene
HCA4	2.6	0.0	Putative role in regulation of cell wall biogenesis
TRM1	2.6	0.0	Putative N2,N2-dimethylguanine tRNA methyltransferase; induced upon adherence to polystyrene
MRR1	2.6	0.0	Putative Zn(II)2Cys6 transcription factor; regulator of MDR1 transcription
RPO41	2.6	0.0	Putative mitochondrial RNA polymerase; repressed in core stress response; Spider biofilm induced
orf19.3501	2.6	0.0	*S. cerevisiae* ortholog Pxl1 localizes to sites of polarized growth
orf19.7624	2.6	0.0	Ortholog(s) have role in maturation of SSU-rRNA from tricistronic rRNA transcript
orf19.773	2.5	0.0	Protein similar to *S. cerevisiae* Rsa3 predicted nucleolar protein involved in maturation of pre-60S ribosomal particles
SIR2	2.5	0.0	Required for wild-type lifespan, asymmetric inheritance of oxidatively damaged proteins, rDNA silencing
orf19.3481	2.5	0.0	Putative mitochondrial ATP-dependent RNA helicase of the DEAD-box family
orf19.2604	2.5	0.0	*S. pombe* ortholog SPAC2C4.06c is a predicted tRNA (cytosine-5-)-methyltransferase
RMS1	2.5	0.0	Putative lysine methyltransferase; Hap43-induced
SPB1	2.5	0.0	Putative AdoMet-dependent methyltransferase; Hap43-induced
OGG1	2.5	0.0	Mitochondrial glycosylase/lyase; repairs oxidative damage to mitochondrial DNA, contributes to UVA resistance, role in base-excision repair; Spider biofilm induced
orf19.4173	2.5	0.0	Ortholog(s) have role in peptidyl-diphthamide biosynthetic process from peptidyl-histidine
orf19.5126	2.5	0.0	Putative adhesin-like protein
SPB4	2.5	0.0	Putative ATP-dependent RNA helicase; flucytosine repressed; Spider biofilm induced
orf19.5678	2.4	0.0	Has domain(s) with predicted role in peptidyl-diphthamide biosynthetic process from peptidyl-histidine and cytoplasm localization
SPE2	2.4	0.0	Putative S-adenosylmethionine decarboxylase; Hap43-induced gene; possibly adherence-induced; Spider biofilm induced
SET6	2.4	0.0	Ortholog of *S. cerevisiae* Set6, a SET domain protein of unknown function; Hap43-induced; rat catheter and Spider biofilm induced
orf19.7552	2.4	0.0	Putative U3-containing small subunit processome complex protein

orf19.6227	2.4	0.0	Ortholog of C. dubliniensis CD36 : Cd36_06390, C. parapsilosis CDC317 : CPAR2_209040
RLI1	2.4	0.0	Member of RNase L inhibitor (RLI) subfamily of ABC family; predicted not to be a transporter
orf19.6416	2.4	0.0	Protein involved in N-glycosylation; Spider biofilm induced; rat catheter biofilm repressed
PRP39	2.4	0.0	Putative component of the U1 snRNP; involved in splicing; Hap43-induced gene; Spider biofilm induced
orf19.5609	2.4	0.0	Ortholog of C. dubliniensis CD36 : Cd36_63840, C. parapsilosis CDC317
NMD3	2.4	0.0	Putative nonsense-mediated mRNA decay protein; repressed in core stress response; repressed by prostaglandins
LTV1	2.4	0.0	Putative GSE complex component; repressed by prostaglandins
PTH2	2.4	0.0	Putative cAMP-independent regulatory protein; constitutive expression independent of MTL or white-opaque status
DBF4	2.3	0.0	Regulatory subunit of Cdc7p-Dbf4p protein kinase complex that acts as negative regulator of hyphal development
orf19.7376	2.3	0.0	Ortholog of C. dubliniensis CD36 : Cd36_85880, C. parapsilosis CDC317 : CPAR2_806870, C. auris B8441
orf19.3303	2.3	0.0	Ortholog(s) have tRNA methyltransferase activity and role in tRNA methylation, wybutosine biosynthetic process
HMX1	2.3	0.0	Heme oxygenase; utilization of hemin iron; transcript induced by heat, low iron, or hemin; repressed by Efg1
FLU1	2.3	0.0	Multidrug efflux pump of the plasma membrane; MDR family member of the MFS (major facilitator superfamily) of transporters; involved in histatin 5 efflux; fungal-specific (no human/murine homolog)
ABP140	2.3	0.0	Ortholog of *S. cerevisiae* actin-binding protein Abp140; Hap43-induced; F-12/CO2 early biofilm induced
YTM1	2.3	0.0	Protein similar to *S. cerevisiae* Ytm1p, which is involved in biogenesis of the large ribosomal subunit; transposon mutation affects filamentous growth; protein level decreases in stationary phase cultures; Hap43p-induced gene
orf19.1794	2.3	0.0	Ortholog(s) have mRNA 5'-UTR binding, pre-mRNA intronic binding, translation regulator activity and role in Group I intron splicing, mitochondrial mRNA processing, positive regulation of mitochondrial translation
UTP4	2.3	0.0	Putative U3 snoRNA-associated protein; Hap43-induced; physically interacts with TAP-tagged Nop1; Spider biofilm induced
orf19.4889	2.3	0.0	Predicted MFS family membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family; Spider biofilm induced
orf19.5206	2.3	0.0	Putative chaperone protein; role in the assembly of box H/ACA snoRNPs and thus for pre-rRNA processing; Spider biofilm induced
orf19.5391	2.3	0.0	Predicted RNA splicing and ER to Golgi transport protein; Hap43-induced gene
DBP8	2.3	0.0	Protein similar to *S. cerevisiae* Dbp8p, an ATP-dependent helicase involved in rRNA processing; oxidative stress-repressed via Cap1p
RCL1	2.3	0.0	Putative U3-containing 90S preribosome processome complex subunit; Hap43-induced
RPC19	2.3	0.0	Putative RNA polymerases I and III subunit AC19; Hap43-induced; rat catheter biofilm induced
SOF1	2.3	0.0	Putative protein with a predicted role in 40S ribosomal subunit biogenesis
DBP2	2.3	0.0	Putative DEAD-box family ATP-dependent RNA helicase; flucytosine induced
orf19.3556	2.3	0.0	Transportin or cytosolic karyopherin beta; Spider biofilm induced
orf19.4570	2.3	0.0	Ortholog of *C. dubliniensis* CD36
orf19.2778	2.3	0.0	Protein of unknown function; transcript is upregulated in clinical isolates from HIV+ patients with oral candidiasis
UTP13	2.2	0.0	Putative U3 snoRNA-associated protein
RRP42	2.2	0.0	Putative exosome non-catalytic core component
SGD1	2.2	0.0	Predicted small ribosomal subunit biogenesis protein
PWP2	2.2	0.0	Putative 90S pre-ribosomal component; repressed in core stress response; repressed by prostaglandins; physically interacts with TAP-tagged Nop1; Hap43-induced
orf19.131	2.2	0.0	Ortholog of C. dubliniensis CD36 : Cd36_61330
RPF1	2.2	0.0	Putative nucleolar protein with a predicted role in the assembly and export of the large ribosomal subunit; essential for growth; rat catheter and Spider biofilm induced
orf19.5356	2.2	0.0	Protein with a predicted role in cell wall integrity; repressed in core stress response
orf19.6903	2.2	0.0	Predicted RNA polymerase III subunit C37; Spider biofilm induced
UTP20	2.2	0.0	Putative snoRNA-binding protein; *S. cerevisiae* Utp20 ortholog
POP4	2.2	0.0	Ortholog of *S. cerevisiae* Pop4; a subunit of both RNase MRP and nuclear RNase P

orf19.81	2.2	0.0	Ortholog(s) have role in exonucleolytic trimming to generate mature 3'-end of 5.8S rRNA from tricistronic rRNA transcript
UTP9	2.2	0.0	Small-subunit processome protein; Ssr1-induced; repressed by prostaglandins; physically interacts with TAP-tagged Nop1
orf19.2319	2.2	0.0	Putative nucleolar protein with a predicted role in pre-rRNA processing; Hap43-induced gene; repressed in core stress response
orf19.6297	2.2	0.0	Ortholog(s) have pseudouridine synthase activity, role in mRNA pseudouridine synthesis, tRNA pseudouridine synthesis and cytoplasm, nucleus localization
CTF1	2.2	0.0	Putative zinc-finger transcription factor, similar to A. nidulans FarA and FarB; activates genes required for fatty acid degradation; induced by oleate; null mutant displays carbon source utilization defects and slightly reduced virulence
MSS116	2.2	0.0	Putative DEAD-box protein; required for efficient splicing of mitochondrial Group I and II introns; Hap43-induced; rat catheter biofilm induced
orf19.2639	2.2	0.0	Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit localization
SRR1	2.2	0.0	Two-component system response regulator; involved in stress response; Plc1-regulated; upregulated in cyr1 null mutant; flow model biofilm induced; Spider biofilm induced
orf19.29	2.2	0.0	Ortholog of *S. cerevisiae* Tah11, a DNA replication licensing factor required for pre-replication complex assembly; rat catheter, flow model and Spider biofilm induced
SKN1	2.2	0.0	Protein with a role in beta-1,6-glucan synthesis; probable N-glycosylated type II membrane protein
ADR1	2.2	0.0	C2H2 transcription factor; ortholog of *S. cerevisiae* Adr1 but mutant phenotype suggests a different set of target genes; transposon mutation affects filamentous growth; Spider biofilm induced
TSR1	2.1	0.0	Component of 20S pre-rRNA processing unit; repressed by prostaglandins
orf19.2891	2.1	0.0	Ortholog(s) have role in cellular response to oxidative stress
FRE10	2.1	0.0	Major cell-surface ferric reductase under low-iron conditions
orf19.3970	2.1	0.0	Putative ribosome biogenesis factor; possibly essential, disruptants not obtained by UAU1 method
orf19.7194	2.1	0.0	Protein required for virulence in reconstituted human epithelium (RHE) model of ex vivo infection
orf19.4078	2.1	0.0	Ortholog(s) have role in U1 snRNA 3'-end processing, U4 snRNA 3'-end processing and U5 snRNA 3'-end processing, more
orf19.1687	2.1	0.0	Ortholog of *S. cerevisiae* Prp43, an RNA helicase in the DEAH-box family that functions in both RNA polymerase I and polymerase II transcript metabolism; Hap43-induced gene
PMS1	2.1	0.0	Putative DNA mismatch repair factor; ortholog of *S. cerevisiae* PMS1 which is an ATP-binding protein involved in DNA mismatch repair
orf19.7397.1	2.1	0.0	Predicted ORF identical to NSA2
orf19.4963	2.1	0.0	Ortholog(s) have protein carrier activity, unfolded protein binding activity, role in ribosomal large subunit biogenesis and cytoplasm, nucleus localization
orf19.4097	2.1	0.0	Ortholog of C. dubliniensis CD36
orf19.3798	2.1	0.0	Ortholog(s) have tRNA (guanine-N7-)-methyltransferase activity
ELF1	2.1	0.0	Putative mRNA export protein; Walker A and B (ATP/GTP binding) motifs
orf19.4479	2.1	0.0	Putative U3-containing 90S preribosome subunit; Hap43-induced; repressed in core stress response
orf19.7398	2.1	0.0	Protein of unknown function' Hap43-induced gene; repressed by prostaglandins
orf19.1697	2.1	0.0	Ortholog(s) have role in cytoplasmic translation, poly(A)+ mRNA export from nucleus and cytoplasm localization
orf19.5066	2.1	0.0	Putative pre-60S pre-ribosomal particle subunit; essential gene; *S. cerevisiae* ortholog RRP17 is essential
orf19.6829	2.1	0.0	Protein with a predicted mitochondrial ATPase expression domain
JIP5	2.1	0.0	Ortholog of *S. cerevisiae* Jip5; predicted role in biogenesis of the large ribosomal subunit
orf19.1825	2.0	0.0	Protein of unknown function; mutants are viable; filament induced; regulated by Nrg1, Rfg1, Tup1
UTP5	2.0	0.0	Putative U3 snoRNA-associated protein; Hap43p-induced gene; mutation confers resistance to 5-fluorocytosine (5-FC)
orf19.6316.4	2.0	0.0	Protein of unknown function; Hap43-induced; rat catheter and Spider biofilm induced
orf19.6705	2.0	0.0	Putative guanyl nucleotide exchange factor with Sec7 domain; required for normal filamentous growth
orf19.6907	2.0	0.0	Ortholog(s) have DNA binding activity, role in regulation of DNA damage checkpoint and cytoplasm, nuclear periphery localization
ECM1	2.0	0.0	Putative pre-ribosomal factor; decreased mRNA abundance observed in cyr1 homozygous mutant hyphae; induced by heavy metal (cadmium) stress; Hog1p regulated
orf19.177	2.0	0.0	Has domain(s) with predicted phosphatidylinositol binding activity and role in cell communication
CSI2	2.0	0.0	Putative 66S pre-ribosomal particle component; Hap43-induced; essential for growth; transposon mutation affects filamentous growth; Spider biofilm induced

RPL7	2.0	0.0	Ribosomal protein L7; repressed upon phagocytosis by murine macrophages; Hap43-induced; rat catheter and Spider biofilm induced
orf19.3704	2.0	0.0	Ortholog(s) have role in maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA), maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)
NRM1	2.0	0.0	Transcriptional regulator of cell cycle gene expression; regulates expression of genes involved in DNA replication stress
orf19.5991	2.0	0.0	Ortholog(s) have role in assembly of large subunit precursor of preribosome, maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more
PDC2	2.0	0.0	Homeodomain-like transcription factor; regulator of pyruvate decarboxylase
orf19.6736	2.0	0.0	Protein required for mitochondrial ribosome small subunit biogenesis; role in maturation of SSU-rRNA; Spider biofilm induced
orf19.3797	2.0	0.0	Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit localization
RIM2	2.0	0.0	Putative mitochondrial carrier protein; induced by alpha pheromone in SpiderM medium; Spider biofilm induced
YVH1	2.0	0.0	Putative dual specificity phosphatase (phosphoserine/threonine and phosphotyrosine phosphatase); required for wild-type growth rate and for wild-type virulence in mouse model of systemic infection; Hap43p-induced gene
PRP5	2.0	0.0	Putative pre-mRNA processing RNA-helicase; induced upon adherence to polystyrene; rat catheter and Spider biofilm induced
orf19.7504	2.0	0.0	Ortholog of *S. cerevisiae* Rts3; a component of the protein phosphatase type 2A complex; Plc1-regulated; induced in core caspofungin response; Spider biofilm induced
RRS1	2.0	0.0	Putative ribosome biogenesis and nuclear export protein; Hap43p-induced gene; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine)
MET3	2.0	0.0	ATP sulfurlyase; sulfate assimilation; repressed by Met, Cys, Sfu1, or in fluconazole-resistant isolate; Hog1, caspofungin, white phase-induced; induced on biofilm formation, even in presence of Met and Cys; Spider, F-12/CO2 biofilm induced
orf19.1720	2.0	0.0	Ortholog(s) have role in mitotic recombination
orf19.1486	1.9	0.0	Protein with a life-span regulatory factor domain; regulated by Sef1, Sfu1, and Hap43; flow model biofilm induced; Spider biofilm induced
orf19.6828	1.9	0.0	Ortholog(s) have role in rRNA processing and preribosome, large subunit precursor localization
UTP15	1.9	0.0	Small subunit (SSU) processome component
APN2	1.9	0.0	Putative class II abasic (AP) endonuclease; flucytosine induced
NEP1	1.9	0.0	Ortholog(s) have rRNA (pseudouridine) methyltransferase activity
orf19.3089	1.9	0.0	Predicted mitochondrial intermembrane space protein
UTP8	1.9	0.0	Essential nucleolar protein; involved in tRNA export from the nucleus and ribosomal small subunit biogenesis
GST2	1.9	0.0	Glutathione S transferase; induced by benomyl and in populations of cells exposed to fluconazole over multiple generations
DED1	1.9	0.0	Predicted ATP-dependent RNA helicase; RNA strand annealing activity; Spider biofilm induced
PHA2	1.9	0.0	Putative prephenate dehydratase; Hap43p-repressed gene; expression downregulated in an ssr1 null mutant
orf19.2143	1.9	0.0	Ortholog(s) have tRNA (guanosine-2'-O-)-methyltransferase activity, role in endocytic recycling, tRNA methylation
orf19.6503	1.9	0.0	Ortholog(s) have structural constituent of ribosome activity
orf19.809	1.9	0.0	Ortholog(s) have rRNA binding activity
PGA32	1.9	0.0	Putative GPI-anchored adhesin-like protein; induced in high iron; Spider biofilm induced
PET127	1.9	0.0	Protein with a predicted role in 5'-end processing of mitochondrial RNAs; ortholog of *S. cerevisiae* Pet127; Hap43-induced; rat catheter and Spider biofilm induced
BMT3	1.9	0.0	Beta-mannosyltransferase
orf19.4492	1.9	0.0	Ortholog(s) have role in nuclear division, rRNA processing, ribosomal large subunit biogenesis and nuclear periphery, nucleolus, preribosome, large subunit precursor localization
orf19.1791	1.9	0.0	Putative protein with a predicted role in 60S ribosomal subunit biogenesis; Hap43p-induced gene
RPC53	1.9	0.0	Ortholog(s) have RNA polymerase III activity, role in tRNA transcription by RNA polymerase III and RNA polymerase III complex localization
orf19.6818	1.9	0.0	Has domain(s) with predicted ATP binding, helicase activity, nucleic acid binding activity
NOP4	1.8	0.0	Putative nucleolar protein; Hap43-induced; mutation confers hypersensitivity to 5-fluorocytosine (5-FC)
orf19.2362	1.8	0.0	Putative 90S preribosome component
orf19.4365	1.8	0.0	Has domain(s) with predicted RNA methyltransferase activity and role in RNA processing

SMC1	1.8	0.0	Protein similar to chromosomal ATPases; RNA abundance regulated by tyrosol and cell density; cell-cycle regulated periodic mRNA expression
orf19.6558	1.8	0.0	Ortholog(s) have GTPase activator activity and cytosol localization
PCL1	1.8	0.0	Cyclin homolog; transcript induced by filamentous growth; induced by alpha pheromone in SpiderM medium
orf19.3205	1.8	0.0	Mitochondrial ribosomal protein of the large subunit; rat catheter biofilm induced
DAL81	1.8	0.0	Zn(II)2Cys6 transcription factor; ortholog of *S. cerevisiae* Dal81, involved in the regulation of nitrogen-degradation genes
MPP10	1.8	0.0	Putative SSU processome and 90S preribosome component; repressed in core stress response; repressed by prostaglandins
MMS22	1.8	0.0	Putative adapter subunit of E3 ubiquitin ligase complex, acts with cullin subunit Rtt101p in response to DNA damage
orf19.6751	1.8	0.0	Ortholog(s) have tRNA (cytosine-2'-O-)-methyltransferase activity, tRNA (guanosine-2'-O-)-methyltransferase activity
orf19.2796	1.8	0.0	Ortholog(s) have DNA-directed DNA polymerase activity, role in DNA replication initiation
RPA190	1.8	0.0	Putative RNA polymerase I subunit A190; Hap43p-induced gene; flucytosine induced
NCE103	1.8	0.0	Carbonic anhydrase; converts of CO2 to bicarbonate; essential for virulence in host niches with limited CO2
LAS1	1.8	0.0	Putative bud formation and morphogenesis protein; mutation confers hypersensitivity to 5-fluorocytosine (5-FC)
orf19.7291	1.8	0.0	Ortholog(s) have tRNA (adenine-N1-)-methyltransferase activity, role in tRNA methylation and nucleus
CCJ1	1.8	0.0	Protein involved in cell cycle regulation; ortholog of S. pombe SPAC1071.09c DNAJ domain protein; Hap43-induced gene
LAC1	1.8	0.0	Ceramide synthase; required for biosynthesis of ceramides with C18:0 fatty acids, which serve as precursors for glucosylsphingolipids; caspofungin induced
orf19.6360	1.8	0.0	Protein involved in pre-mRNA splicing; Spider biofilm induced
orf19.3357	1.8	0.0	Ortholog(s) have structural constituent of ribosome activity and mitochondrial small ribosomal subunit, mitochondrion localization
orf19.1772	1.8	0.0	Ortholog of *S. cerevisiae* : MRX1, C. glabrata CBS138 : CAGL0J03278g
SEF1	1.8	0.0	Zn2-Cys6 transcription factor; regulates iron uptake; negatively regulated by Sfu1p, positively regulated by Tbf1
CLG1	1.8	0.0	Putative cyclin-like protein; transcription is regulated upon yeast-hyphal switch
orf19.4940	1.8	0.0	Putative histidine permease; fungal-specific (no human or murine homolog); Hap43p-induced gene
orf19.5465	1.8	0.0	Ortholog(s) have first spliceosomal transesterification activity and role in generation of catalytic spliceosome for first transesterification step
orf19.3581	1.8	0.0	Ortholog(s) have histone binding activity
orf19.6156	1.8	0.0	Ortholog of *S. cerevisiae* : AIM11, *C. glabrata* CBS138 : CAGL0I04928g, *C. dubliniensis* CD36 : Cd36_80770, *C. parapsilosis* CDC317 : CPAR2_102260 and *C. auris* B8441 : B9J08_002841
orf19.154	1.8	0.0	Putative ortholog of *S. cerevisiae* Utp30; a U3-containing 90S preribosome complex protein; Hap43-induced; Spider biofilm induced
orf19.2124	1.8	0.0	Predicted alcohol dehydrogenase; Spider biofilm induced
orf19.2575	1.8	0.0	Putative S-adenosylmethionine-dependent methyltransferase; Hap43p-induced gene
TRI1	1.8	0.0	Has domain(s) with predicted DNA binding activity
MAK5	1.8	0.0	Putative nucleolar DEAD-box RNA helicase; oxidative stress-repressed via Cap1; repressed by prostaglandins
CDC6	1.8	0.0	Putative ATP-binding protein with a predicted role in DNA replication
orf19.5510	1.8	0.0	Ortholog(s) have role in chromatin silencing at telomere, negative regulation of transcription from RNA polymerase II promoter by pheromones and CHRAC localization
orf19.1646	1.8	0.0	Ortholog(s) have rRNA primary transcript binding activity
orf19.4159	1.8	0.0	Ortholog(s) have magnesium ion transmembrane transporter activity
orf19.1609	1.8	0.0	Ortholog(s) have role in endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA
MTG2	1.8	0.0	Putative Obg family GTPase member; peripheral protein of the mitochondrial inner membrane
TEM1	1.8	0.0	Putative GTPase involved in mitotic exit and cytokinesis; induced under Cdc5p depletion
orf19.1335	1.7	0.0	Ortholog of *S. cerevisiae* Mtr4, an ATP-dependent 3'-5' RNA helicase of the DEAD-box family
orf19.429	1.7	0.0	Putative non-canonical poly(A) polymerase; repressed by nitric oxide; Spider biofilm induced
orf19.2711	1.7	0.0	Similar to *S. cerevisiae* Elp2, an Elongator complex subunit required for modification of wobble nucleosides in tRNA; repressed in core stress response

CCN1	1.7	0.0	G1 cyclin; required for hyphal growth maintenance (not initiation); cell-cycle regulated transcription (G1/S); Cdc28p-Ccn1p initiates Cdc11p S394 phosphorylation on hyphal induction; expression in *S. cerevisiae* inhibits pheromone response
GIN1	1.7	0.0	Protein involved in regulation of DNA-damage-induced filamentous growth; putative component of DNA replication checkpoint; ortholog of *S. cerevisiae* Mrc1p, an S-phase checkpoint protein; Hap43p-induced gene
orf19.6418	1.7	0.0	Ortholog(s) have unfolded protein binding activity and role in protein import into nucleus, ribosomal large subunit biogenesis
PRI2	1.7	0.0	Putative DNA primase; gene adjacent to and divergently transcribed with CDC68; Hap43-induced; Spider biofilm repressed
orf19.2657	1.7	0.0	Protein of unknown function
CHS3	1.7	0.0	Major chitin synthase of yeast and hyphae; synthesizes short-chitin fibrils; Chs4-activated; transcript induced at yeast-hyphal transition; Chs1 and Chs2, but not Chs3, are inhibited by the protoberberine HWY-289; Spider biofilm induced
ISW2	1.7	0.0	Ortholog of *S. cerevisiae* Isw2; an ATPase involved in chromatin remodeling
orf19.7422	1.7	0.0	Ortholog(s) have role in endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more
orf19.5541	1.7	0.0	Protein with similarity to S. pombe Nrd1p; transcription induced upon induction of hyphal growth
PAM18	1.7	0.0	Predicted component of the presequence translocase-associated import motor (PAM complex) involved in protein import into mitochondrial matrix; rat catheter biofilm induced
orf19.1757	1.7	0.0	Putative C2H2 transcription factor; expression upregulated in clinical isolates from HIV+ patients with oral candidiasis; Spider biofilm induced
MRPL3	1.7	0.0	Ribosomal protein of the large subunit, mitochondrial
orf19.547	1.7	0.0	Ortholog(s) have 5'-3' exonuclease activity, 5'-flap endonuclease activity, double-stranded DNA 5'-3' exodeoxyribonuclease activity, single-stranded DNA 5'-3' exodeoxyribonuclease activity
orf19.25	1.7	0.0	Ortholog(s) have tRNA (guanine(9)-N(1))-methyltransferase activity, tRNA (guanine) methyltransferase activity and role in tRNA N1-guanine methylation, tRNA methylation
orf19.4643	1.7	0.0	Ortholog of C. dubliniensis CD36: Cd36_41430, *Candida tropicalis* NEW ASSEMBLY : CTRG1_00187, *Candida tropicalis* MYA-3404 : CTRG_00187 and *Candida albicans* WO-1 : CAWG_03642
SMI1B	1.7	0.0	Putative cell wall assembly regulatory protein; Rim101-repressed; possibly an essential gene, disruptants not obtained by UAU1 method
SDH4	1.7	0.0	Succinate dehydrogenase, membrane subunit; induced in high iron
SDA1	1.7	0.0	Predicted nuclear protein involved in actin cytoskeleton organization, passage through Start, 60S ribosome biogenesis; rat catheter biofilm induced; Hap43-induced
PUS7	1.7	0.0	Pseudouridine synthase; catalyzes pseudouridylation in U2 snRNA, 5S rRNA, cytoplasmic tRNAs and in pre-tRNA (Tyr); F-12/CO2 early biofilm induced
NMA111	1.7	0.0	Putative serine protease and general molecular chaperone; macrophage-induced gene; repressed in core stress response; merged with orf19.3288.1 in Assembly 21
GIN4	1.7	0.0	Autophosphorylated kinase; role in pseudohyphal-hyphal switch and cytokinesis; phosphorylates Cdc11p on S395; necessary for septin ring within germ tube but not for septin band at mother cell junction; physically associates with septins
DBR1	1.7	0.0	Debranchase; homozygous mutant accumulates lariat intermediates of mRNA splicing; rat catheter biofilm repressed
orf19.5235	1.7	0.0	Putative mitochondrial ribosomal protein of the large subunit; Hap43-induced; mutants are viable; protein level decreases in stationary phase
TBF1	1.7	0.0	Essential transcription factor; induces ribosomal protein genes and the rDNA locus; acts with Cbf1 at subset of promoters; recruits Fhl1 and Ifh1 to promoters; role is analogous to that of *S. cerevisiae* Rap1; Spider biofilm induced
orf19.1578	1.7	0.0	Ortholog of S. cereviaie Rrp5, an RNA binding protein involved in synthesis of 18S and 5.8S rRNAs; Hap43-induced gene
orf19.6247	1.7	0.0	Ortholog(s) have chromatin binding activity
orf19.7618	1.7	0.0	Putative nucleolar protein with a predicted role in pre-18S rRNA processing; Plc1p-regulated; Spider biofilm induced
orf19.4455	1.6	0.0	Protein of unknown function; Spider biofilm induced
orf19.445	1.6	0.0	Protein of unknown function; repressed by prostaglandins
HEM1	1.6	0.0	Putative 5-aminolevulinate synthase; caspofungin repressed; induced by high iron, nitric oxide; regulated by Ssn6; Hap43-repressed; Spider biofilm induced
RTA3	1.6	0.0	7-transmembrane receptor protein involved in regulation of asymmetric lipid distribution in plasma membrane
orf19.2847	1.6	0.0	Ortholog(s) have RNA polymerase III activity, role in tRNA transcription by RNA polymerase III and RNA polymerase III complex localization
CLA4	1.6	0.0	Ste20p family Ser/Thr kinase required for wild-type filamentous growth
NRP1	1.6	0.0	Ortholog(s) have cytoplasmic stress granule localization

orf19.4176	1.6	0.0	Ortholog(s) have structural constituent of ribosome activity and mitochondrial small ribosomal subunit localization
RPA34	1.6	0.0	Putative RNA polymerase I subunit; rat catheter biofilm induced
CAS5	1.6	0.0	Transcription factor involved in regulation of cell wall homeostasis
TSR2	1.6	0.0	Protein with a predicted role in pre-rRNA processing; repressed by prostaglandins
orf19.7553	1.6	0.0	Protein of unknown function; rat catheter biofilm repressed
orf19.304	1.6	0.0	Putative transporter similar to MDR proteins; fungal-specific; Spider biofilm induced
orf19.3170	1.6	0.0	Ortholog(s) have role in RNA polymerase I assembly, RNA polymerase II core complex assembly
orf19.3972	1.6	0.0	Ortholog(s) have role in endoplasmic reticulum to Golgi vesicle-mediated transport, retrograde transport, endosome to Golgi
FAL1	1.6	0.0	eIF4A subfamily of DEAD-box ATP-dependent RNA helicases; predicted nucleolar protein required for maturation of 18S rRNA; Spider biofilm induced
orf19.7254	1.6	0.0	Ortholog(s) have role in mRNA metabolic process, mitochondrial translational initiation and extrinsic component of membrane, mitochondrial inner membrane localization
DRS1	1.6	0.0	Putative nucleolar DEAD-box protein; Hap43-induced; mutation confers hypersensitivity to 5-fluorouracil (5-FU), tubercidin (7-deazaadenosine); Tbf1-induced; repressed in core stress response
orf19.702	1.6	0.0	Ortholog(s) have DNA helicase activity, role in regulation of translational termination and cytoplasmic stress granule, polysome localization
orf19.2673	1.6	0.0	Ortholog(s) have SUMO transferase activity, role in DNA repair and Smc5-Smc6 complex localization
SAC7	1.6	0.0	Putative GTPase activating protein (GAP) for Rho1; repressed upon adherence to polystyrene; macrophage/pseudohyphal-repressed; transcript is upregulated in RHE model of oral candidiasis and in clinical oral candidiasis
BMS1	1.6	0.0	Putative GTPase; Hap43-induced gene; mutation confers resistance to 5-fluorocytosine (5-FC); flucytosine induced; repressed by prostaglandins; Spider biofilm induced
orf19.6984	1.6	0.0	Ortholog of C. dubliniensis CD36 : Cd36_85460, C. parapsilosis CDC317 : CPAR2_405760, C. auris B8441 : B9J08_000903 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_116052
RIX7	1.6	0.0	Putative ATPase of the AAA family; role in ribosomal subunit export from the nucleus; mutation impairs hyphal growth and biofilm formation
YOX1	1.6	0.0	Putative homeodomain-containing transcription factor; transcriptional repressor; periodic mRNA expression, peak at cell-cycle G1/S phase
MNN11	1.6	0.0	Ortholog(s) have alpha-1,6-mannosyltransferase activity, role in protein N-linked glycosylation, protein glycosylation and mannan polymerase complex localization
orf19.4150	1.6	0.0	Putative glutaredoxin; induced by nitric oxide; Spider biofilm induced
CDC13	1.6	0.0	Essential protein with similarity to *S. cerevisiae* Cdc13p, involved in telomere maintenance
PEA2	1.6	0.0	Putative coiled-coil polarisome; predicted role in polarized morphogenesis, cell fusion, and low affinity Ca2+ influx; rat catheter biofilm induced
orf19.7361	1.6	0.0	Ortholog(s) have tRNA-intron endonuclease activity, role in tRNA-type intron splice site recognition and cleavage and mitochondrial outer membrane, tRNA-intron endonuclease complex localization
orf19.3480	1.6	0.0	Ortholog(s) have structural constituent of ribosome activity and mitochondrial small ribosomal subunit localization
orf19.7215	1.6	0.0	Nucleolar protein; component of the small subunit processome containing the U3 snoRNA; involved in pre-18S rRNA processing; flow model biofilm repressed
orf19.6499	1.6	0.0	Predicted DNA-directed RNA polymerase; role in transcription; Spider biofilm induced
orf19.5575	1.5	0.0	Putative peripheral peroxisomal membrane peroxin
orf19.168	1.5	0.0	Ortholog(s) have role in U4 snRNA 3'-end processing, exonucleolytic trimming to generate mature 3'-end of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more
orf19.7011	1.5	0.0	Ortholog(s) have role in maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA) and nucleus, preribosome, small subunit precursor localization
SEO1	1.5	0.0	Protein with similarity to permeases; Sfu1-repressed; flucytosine induced; induced by Mnl1 under weak acid stress; flow model biofilm repressed
YBL053	1.5	0.0	Putative subunit of a replication fork-pausing checkpoint complex
NAM7	1.5	0.0	Putative role in nonsense-mediated mRNA decay; similar to *S. cerevisiae* Nam7p
orf19.4340.1	1.5	0.0	Ortholog(s) have poly(U) RNA binding, splicing factor binding activity and U1 snRNP, U2 snRNP, U4/U6 x U5 tri-snRNP complex, U5 snRNP, post-mRNA release spliceosomal complex, spliceosomal complex localization
orf19.3582	1.5	0.0	Ortholog(s) have protein-lysine N-methyltransferase activity and role in peptidyl-lysine dimethylation, peptidyl-lysine monomethylation, vesicle-mediated transport
orf19.962	1.5	0.0	Protein with a fungal RNA polymerase I subunit RPA14 domain
SMC5	1.5	0.0	Protein similar to *S. cerevisiae* Smc5p, which is involved in DNA repair

orf19.3487	1.5	0.0	Ortholog of C. dubliniensis CD36 : Cd36_33330, C. parapsilosis CDC317 : CPAR2_701980, C. auris B8441 : B9J08_000884, Debaryomyces hansenii CBS767 : DEHA2C15840g and Pichia stipitis Pignal : PICST_31066
ESC4	1.5	0.0	Protein similar to *S. cerevisiae* Esc4; a protein that represses transposition; transposon mutation affects filamentation; rat catheter biofilm repressed
orf19.5433	1.5	0.0	Ortholog(s) have protein folding chaperone activity, role in mitochondrial respiratory chain complex III assembly and mitochondrial matrix, mitochondrion localization
MTG2	19.0	0.0	Predicted protein of unknown function; Plc1-regulated
TEM1	16.7	0.0	Ortholog of C. dubliniensis CD36 : Cd36_71210, C. parapsilosis CDC317 : CPAR2_702710, C. auris B8441 : B9J08_002141 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_116256
orf19.1335	9.2	0.0	Zn2-Cys6 transcription factor of unknown function; induced by alpha pheromone in SpiderM medium
orf19.429	6.5	0.0	Protein of unknown function; Hap43-induced; Spider biofilm induced
orf19.2711	6.4	0.0	bZIP transcription factor; possibly transcriptionally regulated upon hyphal formation; Hap43; F-12/CO2 early biofilm induced; Spider biofilm induced
CCN1	5.3	0.0	Putative cytoplasmic pre-60S factor; Hap43-induced; repressed by prostaglandins
GIN1	5.0	0.0	Putative nucleolar GTPase; repressed by prostaglandins; Hap43-induced, rat catheter and Spider biofilm induced
orf19.6418	4.9	0.0	Protein required for thiolation of uridine at wobble position of Gln, Lys, and Glu tRNAs; has a role in urmylation; *S. cerevisiae* ortholog has a role in invasive and pseudohyphal growth
PRI2	4.7	0.0	Putative constituent of 66S pre-ribosomal particles; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
orf19.2657	4.6	0.0	Putative nucleolar protein; essential; heterozygous mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); Hap43-induced; Spider biofilm induced
CHS3	4.4	0.0	Putative mitochondrial membrane protein; ortholog of *S. cerevisiae* Sls1; coordinates expression of mitochondrially-encoded genes; Hap43-induced
ISW2	4.4	0.0	tRNA-Gly, predicted by tRNAscan-SE; GCC anticodon
orf19.7422	4.4	0.0	Predicted mitochondrial small ribosomal subunit; rat catheter and Spider biofilm induced
orf19.5541	4.3	0.0	Putative nucleolar protein; Hap43-induced; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); heterozygous mutant is resistant to parnafungin; Spider biofilm induced
PAM18	4.2	0.0	Zn-ribbon protein; required for synthesis of diphthamide on translation factor eEF2; involved in modification of wobble nucleosides in tRNAs; rat catheter and Spider biofilm induced
orf19.1757	4.2	0.0	Putative tRNA splicing endonuclease subunit; mutation confers hypersensitivity to toxic ergosterol analog and to amphotericin B; 5'-UTR intron; Hap43-induced; Spider biofilm induced
MRPL3	4.2	0.0	High-affinity MFS glucose transporter; induced by progesterone, chloramphenicol, benomyl; likely essential for growth; protein newly produced during adaptation to the serum; rat catheter and Spider biofilm induced
orf19.547	4.1	0.0	Ortholog of S. cereviiae Nop8; has a role in ribosomal large subunit biogenesis; rat catheter and Spider biofilm induced
orf19.25	4.0	0.0	Putative dihydrouridine synthase; Hap43-induced gene; rat catheter biofilm induced; Spider biofilm induced
orf19.4643	3.9	0.0	Predicted diphthamide biosynthesis protein; Spider biofilm induced
SMI1B	3.9	0.0	Protein of unknown function; Hap43-induced; F-12/CO2 early biofilm induced
SDH4	3.8	0.0	Putative RNA exonuclease; induced in a ssr1 null mutant
SDA1	3.8	0.0	Protein of unknown function; Spider biofilm induced
PUS7	3.8	0.0	Protein required for pre-rRNA processing and 40S ribosomal subunit synthesis; associated with U3 and U14 snoRNAs; transposon mutation affects filamentous growth; repressed by prostaglandins; Spider biofilm induced
NMA111	3.8	0.0	Protein of unknown function; merged with orf19.3338; rat catheter, flow and Spider model biofilm induced; promoter bound by Bcr1, Efg1, Ndt80, and Rob1; orf19.3338 Bcr1-repressed in RPMI a/a biofilms
GIN4	3.8	0.0	Putative 18S rRNA dimethylase; predicted role in rRNA modification and processing
DBR1	3.8	0.0	Predicted ORF from original SGTC Assembly 19 annotation, removed from the reduced ORF set by the SGTC; subsequently reinstated in Assembly 21 based on comparative genome analysis
orf19.5235	3.7	0.0	Ortholog of *S. cerevisiae* Ecm16, an essential DEAH-box ATP-dependent RNA helicase specific to the U3 snoRNP required for 18S rRNA synthesis; Hap43-induced; Spider biofilm induced
TBF1	3.7	0.0	Putative serine/threonine-protein kinase
orf19.1578	3.7	0.0	Putative RNase MRP and nuclear RNase P component; decreased repressed by prostaglandins; Spider biofilm induced
orf19.6247	3.7	0.0	Ortholog(s) have N(6)-L-threonylcarbamoyladenine synthase activity, single-stranded telomeric DNA binding activity

orf19.7618	3.7	0.0	Ortholog of C. dubliniensis CD36 : Cd36_29080, C. parapsilosis CDC317 : CPAR2_202490, *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_112323 and Debaryomyces hansenii CBS767 : DEHA2F19184g
orf19.4455	3.6	0.0	Protein with a predicted role in 18S rRNA maturation and small ribosomal subunit biogenesis; repressed in core stress response; repressed by prostaglandins
orf19.445	3.6	0.0	Protein with a predicted role in recruitment of RNA polymerase I to rDNA; caspofungin induced; flucytosine repressed; repressed in core stress response; repressed by prostaglandins
HEM1	3.6	0.0	Ortholog of Rmp1; subunit of RNase MRP subunit that processes pre-rRNA and has a role in cell cycle-regulated degradation of daughter cell-specific mRNAs; rat catheter and Spider biofilm induced
RTA3	3.6	0.0	Putative U3 snoRNA-associated protein; Hap43-induced; transposon mutation affects filamentous growth; repressed by prostaglandins
orf19.2847	3.6	0.0	Predicted histone acetyltransferase; role in regulation of transcription, tRNA wobble uridine modification; Spider biofilm induced
CLA4	3.6	0.0	Similar to *S. cerevisiae* Bud20; predicted role in cellular bud site selection; rat catheter and Spider biofilm induced
NRP1	3.5	0.0	Putative mitochondrial GTPase; likely essential for respiratory competence and in large ribosomal subunit assembly; mitochondrial translation; Spider biofilm induced
orf19.4176	3.5	0.0	C/D box small nucleolar RNA (snoRNA)
RPA34	3.5	0.0	Protein of unknown function; from Assembly 19; removed from Assembly 20; restored based on comparative genome analysis; F-12/CO2 early biofilm induced
CAS5	3.5	0.0	Putative nucleolar protein; implicated in ribosome biogenesis; rat catheter biofilm repressed
TSR2	3.5	0.0	Ortholog(s) have role in ribosomal large subunit biogenesis, ribosomal small subunit biogenesis and nucleolus localization
orf19.7553	3.5	0.0	N-Alkane inducible cytochrome P450
orf19.304	3.5	0.0	Putative cytosolic thiouridylase subunit; Spider biofilm induced
orf19.3170	3.4	0.0	Putative nucleolar protein; constituent of pre-60S ribosomal particles; Hap43-induced; repressed by prostaglandins
orf19.3972	3.4	0.0	Putative U3-containing 90S preribosome processome complex subunit; Hap43-induced gene; rat catheter and Spider biofilm induced; F-12/CO2 early biofilm induced
FAL1	3.4	0.0	Putative 35S rRNA processing protein; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
orf19.7254	3.3	0.0	Putative U3 snoRNP protein; Hap43-induce; physically interacts with TAP-tagged Nop1; Spider biofilm induced
DRS1	3.3	0.0	Putative flavodoxin; similar to *S. cerevisiae* Tyw1, an iron-sulfur protein required for synthesis of wybutosine modified tRNA; predicted Kex2p substrate; Spider biofilm induced
orf19.702	3.3	0.0	Ribosomal protein; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); physically interacts with TAP-tagged Nop1; Hap43-induced; Spider biofilm induced
orf19.2673	3.3	0.0	Small-subunit processome component; repressed by prostaglandins
SAC7	3.3	0.0	Ortholog(s) have role in endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA and LSU-rRNA), more
BMS1	3.2	0.0	Putative tRNA methyltransferase; repressed by prostaglandins; Spider biofilm induced
orf19.6984	3.2	0.0	H/ACA box small nucleolar RNA (snoRNA)
RIX7	3.2	0.0	Ortholog(s) have transferase activity, role in maturation of SSU-rRNA and cytoplasm localization
YOX1	3.2	0.0	Putative U3 snoRNP protein; Hap43p-induced gene; physically interacts with TAP-tagged Nop1p
MNN11	3.2	0.0	Putative nucleolar protein; repressed benomyl treatment or in an azole-resistant strain that overexpresses MDR1; F-12/CO2 early biofilm induced
orf19.4150	3.2	0.0	Putative U3-containing small subunit processome complex subunit; Hap43p-induced gene; mutation confers resistance to 5-fluorocytosine (5-FC); repressed upon high-level peroxide stress
CDC13	3.1	0.0	DEAH-box ATP-dependent RNA helicase, required for 18S rRNA synthesis; rat catheter biofilm induced
PEA2	3.1	0.0	Putative DEAD-box helicase; Hap43-induced; Spider biofilm induced
orf19.7361	3.1	0.0	Essential protein; regulated by hemoglobin; *S. cerevisiae* ortholog is essential; Hap43p-induced gene
orf19.3480	3.1	0.0	Ortholog(s) have role in mRNA splicing, via spliceosome and RES complex, nucleus localization
orf19.7215	3.1	0.0	Putative ortholog of *S. cerevisiae* Rpp1; subunit of both RNase MRP and nuclear RNase P; rat catheter and Spider biofilm induced
orf19.6499	3.1	0.0	Putative intranuclear transport and DNA replication mediator; heterozygous null mutant exhibits resistance to parnafungin in the *C. albicans* fitness test; Spider biofilm induced
orf19.5575	3.1	0.0	Putative SSU processome component; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
orf19.168	3.1	0.0	Putative acyl-CoA thioesterase

orf19.7011	3.1	0.0	Ortholog(s) have rRNA binding activity and role in maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA), rRNA processing, ribosomal large subunit export from nucleus
SEO1	3.1	0.0	Exosome non-catalytic core component; involved in 3'-5' RNA processing and degradation in the nucleus and cytoplasm; Spider biofilm induced
YBL053	3.0	0.0	Putative RNA-binding protein; role in assembly of box H/ACA snoRNPs and thus pre-rRNA processing; Spider biofilm induced
NAM7	3.0	0.0	Putative U3 snoRNP protein; Ssr1-induced; repressed by prostaglandins; heterozygous null mutant is resistant to parnafungin
orf19.4340.1	3.0	0.0	Ortholog(s) have enzyme activator activity, telomerase inhibitor activity, role in box C/D RNA 3'-end processing, negative regulation of telomere maintenance via telomerase and nucleolus, nucleoplasm localization
orf19.3582	3.0	0.0	Ortholog of Slx9 required for pre-rRNA processing
orf19.962	3.0	0.0	Predicted nuclear exosome-associated nucleic acid binding protein; rat catheter and Spider biofilm induced
SMC5	3.0	0.0	Ortholog(s) have tRNA (guanine-N7-)-methyltransferase activity, role in tRNA (guanine-N7)-methylation and cytosol, nucleus, tRNA (m7G46) methyltransferase complex localization
orf19.3487	3.0	0.0	Protein with a predicted role maturation of 18S rRNA; rat catheter biofilm induced
ESC4	3.0	0.0	Ortholog of C. dubliniensis CD36: Cd36_20380, C. parapsilosis
orf19.5433	3.0	0.0	Putative co-chaperone; Hap43p-induced gene; mutation confers hypersensitivity to radicicol

Appendix 25: RNA-seq result of Zcf27-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf27-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.3210	14.8	0.0	Predicted protein of unknown function; Plc1-regulated
orf19.2968	7.2	0.0	Protein of unknown function; Spider biofilm induced
IFF4	6.0	0.0	Adhesin-like cell surface protein; putative GPI-anchor; null mutant germ tubes show decreased adhesion to plastic substrate; mutants are viable; Hap43-repressed gene
orf19.6501	6.0	0.0	Ortholog of *C. dubliniensis* CD36 : Cd36_71960, Pichia stipitis Pignal : psti_CGOB_00136, *Candida tropicalis* NEW ASSEMBLY : CTRG1_05049 and Spathaspora passalidarum NRRL Y-27907 : SPAPADRAFT_63786
orf19.1277	5.0	0.0	Protein of unknown function; Rgt1, Hap43-repressed; flow model biofilm induced; Spider biofilm induced
orf19.6592	4.7	0.0	Predicted membrane transporter, member of the aromatic acid:proton symporter (AAHS) family, major facilitator superfamily (MFS)
MAL31	4.6	0.0	Putative high-affinity maltose transporter; transcript is upregulated in clinical isolates from HIV+ patients with oral candidiasis; alkaline induced; Spider biofilm induced
WH11	4.2	0.0	White-phase yeast transcript; expression in opaques increases virulence/switching; mutant switches as WT; Hap43, hypoxia, ketoconazol induced; required for RPMI biofilm; Bcr1-induced in RPMI a/a biofilm; rat catheter, Spider biofilm induced
orf19.3337	4.0	0.0	Protein of unknown function; merged with orf19.3338; rat catheter, flow and Spider model biofilm induced; promoter bound by Bcr1, Efg1, Ndt80, and Rob1; orf19.3338 Bcr1-repressed in RPMI a/a biofilms
ALS1	4.0	0.0	Cell-surface adhesin; adhesion, virulence, immunoprotective roles; band at hyphal base; Rfg1, Ssk1, Spider biofilm induced; flow model biofilm repressed; CAI-4 strain background effects; promoter bound Bcr1, Tec1, Efg1, Ndt80, and Brg1
GST2	4.0	0.0	Glutathione S transferase; induced by benomyl and in populations of cells exposed to fluconazole over multiple generations; regulated by Nrg1, Tup1; induced by nitric oxide; stationary phase enriched; Spider biofilm induced
OPT1	3.9	0.0	Oligopeptide transporter; transports 3-to-5-residue peptides; alleles are distinct, one has intron; suppresses *S. cerevisiae* ptr2-2 mutant defects; induced by BSA or peptides; Stp3p, Hog1p regulated; flow model biofilm induced
ZCF27	3.9	0.0	Putative Zn(II)2Cys6 transcription factor
orf19.3105	3.9	0.0	Putative cytochrome P450 protein; possibly an essential gene, disruptants not obtained by UAU1 method
BRG1	3.9	0.0	Transcription factor; recruits Hda1 to hypha-specific promoters; Tn mutation affects filamentation; Hap43-repressed; Spider and flow model biofilm induced; required for Spider biofilm formation; Bcr1-repressed in RPMI a/a biofilms
HGT1	3.6	0.0	High-affinity MFS glucose transporter; induced by progesterone, chloramphenicol, benomyl; likely essential for growth; protein newly produced during adaptation to the serum; rat catheter and Spider biofilm induced

SAP10	3.4	0.0	Secreted aspartyl protease; roles in adhesion, virulence (RHE model), cell surface integrity; distinct specificity from Sap9; at cell membrane and wall; GPI-anchored; induced in low iron; Tbf1-activated; Spider biofilm induced
orf19.1368	3.4	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
WOR3	3.4	0.0	Transcription factor; modulator of white-opaque switch; induced in opaque cells; promoter bound by Wor1; overexpression at 25 degr shifts cells to opaque state; deletion stabilizes opaque cells at higher temperatures; Spider biofilm induced
RNR22	3.3	0.0	Putative ribonucleoside diphosphate reductase;colony morphology-related gene regulation by Ssn6; transcript regulated by tyrosol and cell density; Hap43-repressed; Spider biofilm induced
AQY1	3.3	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance; virulent in mice; flucytosine repressed; flow model/RPMI/Spider/rat catheter biofilm induced; required for RPMI biofilm formation; Bcr1-induced in a/a RPMI biofilms
orf19.4476	3.2	0.0	Protein with a NADP-dependent oxidoreductase domain; transcript induced by ketoconazole; rat catheter and Spider biofilm induced
ADH5	3.2	0.0	Putative alcohol dehydrogenase; regulated by white-opaque switch; fluconazole-induced; antigenic in murine infection; regulated by Nrg1, Tup1; Hap43, macrophage repressed, flow model biofilm induced; Spider biofilm induced
PGA7	3.2	0.0	GPI-linked hyphal surface antigen; induced by ciclopirox olamine, ketoconazole, Rim101 at pH 8; Hap43, fluconazole; flow model biofilm induced; Spider biofilm induced; required for RPMI biofilm; Bcr1-induced in a/a biofilm
GCA2	3.1	0.0	Predicted extracellular glucoamylase; induced by ketoconazole; possibly essential, disruptants not obtained by UAU1 method; promotes biofilm matrix formation; Spider biofilm induced; Bcr1-induced in RPMI a/a biofilms
orf19.5169	3.0	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
orf19.4607	3.0	0.0	Possible Golgi membrane protein; Hap43-repressed; hypha induced; flow model biofilm induced; Spider biofilm induced
orf19.2281	2.9	0.0	Has domain(s) with predicted CoA-transferase activity and role in metabolic process
PLB4.5	2.7	0.0	Phospholipase B; Hog1-induced; regulated by Ssn6; putative GPI-anchor; repressed during cell wall regeneration; clade-associated gene expression; Hap43-induced; rat catheter and Spider biofilm repressed
TPO3	2.7	0.0	Putative polyamine transporter; MFS-MDR family; induced by Sfu1, regulated upon white-opaque; decreased expression in hyphae vs yeast-form cells; regulated by Nrg1; Spider biofilm repressed
IFE2	2.7	0.0	Putative alcohol dehydrogenase; yeast-enriched transcript; Efg1-regulated; induced by prostaglandins, Hog1, fluconazole; rat catheter biofilm induced
orf19.2691	2.6	0.0	Planktonic growth-induced gene
FRP1	2.6	0.0	Ferric reductase; alkaline-induced by Rim101; iron-chelation-induced by CCAAT-binding factor; fluconazole-repressed; ciclopirox-, hypoxia-, Hap43-induced; colony morphology-related regulation by Ssn6; Spider and flow model biofilm induced
RHR2	2.6	0.0	Glycerol 3-phosphatase; roles in osmotic tolerance, glycerol accumulation in response to salt; Spider/flow model biofilm induced; regulated by macrophage, stress, yeast-hyphal switch, pheromone, Gcn4, Hog1, Nrg1, Tup1
orf19.4612	2.6	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
orf19.4530.1	2.6	0.0	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
ASR2	2.6	0.0	Adenylyl cyclase and stress responsive protein; induced in cyr1 or ras1 mutant; stationary phase enriched protein; Spider biofilm induced
OPT3	2.5	0.0	Oligopeptide transporter; transcript induced by macrophage phagocytosis, BSA or peptides
RBT5	2.5	0.0	GPI-linked cell wall protein; hemoglobin utilization; Rfg1, Rim101, Tbf1, Fe regulated; Sfu1, Hog1, Tup1, serum, alkaline pH, antifungal drugs,
orf19.3460	2.5	0.0	Protein of unknown function; mRNA binds She3; transcript regulated upon yeast-hypha switch; induced in oralpharyngeal candidasis
orf19.3439	2.5	0.0	Protein of unknown function; Cyr1-repressed; rat catheter and Spider biofilm induced
RHD3	2.5	0.0	GPI-anchored yeast-associated cell wall protein; induced in high iron; clade-associated gene expression
FCR1	2.5	0.0	Transcription factor; repressor of fluconazole/ketoconazole/brefeldin A resistance; Tn mutation enhances filamentation
BIO3	2.4	0.0	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis
STF2	2.4	0.0	Protein involved in ATP biosynthesis; repressed in hyphae; repressed by Efg1, Hap43
PST3	2.4	0.0	Flavodoxin-like protein involved in oxidative stress protection and virulence
NUP	2.4	0.0	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not; similar to a nucleoside permease of S. pombe; possibly processed by Kex2p
BMT4	2.3	0.0	Beta-mannosyltransferase; for elongation of beta-mannose chains on the acid-labile fraction of cell wall phosphopeptidomannan
PHO113	2.3	0.0	Putative constitutive acid phosphatase; Rim101-repressed; DTT-extractable

orf19.4459	2.3	0.0	Predicted heme-binding stress-related protein; Tn mutation affects filamentous growth
OSM1	2.3	0.0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae; caspofungin repressed
CDR4	2.3	0.0	Putative ABC transporter superfamily; fluconazole, Sfu1, Hog1, core stress response induced; caspofungin repressed
RBR3	2.2	0.0	Cell wall adhesin-like protein; repressed by Rim101; possibly an essential gene, disruptants not obtained by UAU1 method
orf19.1395	2.2	0.0	Ortholog(s) have copper ion transmembrane transporter activity
PNC1	2.1	0.0	Putative nicotinamidase, involved in NAD salvage pathway
BUB3	2.1	0.0	Protein similar to *S. cerevisiae* Bub3; a kinetochore checkpoint component; induced by hydroxyurea treatment
orf19.1496	2.1	0.0	Putative transcription factor with zinc finger DNA-binding motif; Hap43p-repressed gene
RMS1	2.1	0.0	Putative lysine methyltransferase
orf19.6690	2.0	0.0	Protein of unknown function; Hap43-repressed gene
DAK2	2.0	0.0	Putative dihydroxyacetone kinase; repressed by yeast-hypha switch
RBT1	2.0	0.0	Cell wall protein with similarity to Hwp1; required for virulence; predicted glycosylation
GRE2	2.0	0.0	Putative reductase; Nrg1 and Tup1-regulated; benomyl- and hyphal-induced
RBE1	2.0	0.0	Pry family cell wall protein; Rim101, Efg1, Ssn6, alkaline repressed; O-glycosylation
GLK1	2.0	0.0	Putative glucokinase; transcript regulated upon yeast-hyphal switch; Efg1 regulated
ITS1	1.9	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58
OPT6	1.9	0.0	Putative oligopeptide transporter; fungal-specific (no human or murine homolog)
orf19.411	1.9	0.0	Protein similar to GTPase regulators; induced in low iron; transcript activated by Mnl1 under weak acid stress
orf19.3053	1.9	0.0	Protein of unknown function; present in exponential and stationary phase yeast
orf19.3004	1.9	0.0	Ortholog of *S. cerevisiae* : YDR262W, C. glabrata CBS138 : CAGL0M08734g, C. dubliniensis CD36
GDB1	1.9	0.0	Putative glycogen debranching enzyme; expression is regulated upon white-opaque switch; regulated by Nrg1, Tup1
orf19.7473	1.8	0.0	Ortholog(s) have role in endocytosis and actin cortical patch localization
ITS2	1.8	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25
HSP12	1.8	0.0	Heat-shock protein; induced by osmotic/oxidative/cadmium stress, fluphenazine treatment, low iron, CDR1 and CDR2 overexpression, or ssn6 or ssk1 null mutation; overexpression increases resistance to farnesol and azoles
GLK4	1.8	0.0	Putative glucokinase; decreased expression in hyphae compared to yeast-form cells
orf19.1887	1.8	0.0	Ortholog(s) have sterol esterase activity, role in sterol metabolic process and integral component of membrane, lipid droplet localization
orf19.4216	1.8	0.0	Putative heat shock protein; decreased expression in hyphae; transcription is increased in populations of cells exposed to fluconazole over multiple generations; overexpression increases resistance to farnesol and azoles
XYL2	1.8	0.0	D-xylulose reductase; immunogenic in mice; soluble protein in hyphae; induced by caspofungin, fluconazole, Hog1 and during cell wall regeneration; Mnl1-induced in weak acid stress; stationary phase enriched; flow model biofilm induced
GPM2	1.7	0.0	Putative phosphoglycerate mutase; repressed in hyphae; macrophage/pseudohyphal-repressed
orf19.7043	1.7	0.0	Ortholog of *S. cerevisiae*: YLR050C, C. glabrata CBS138 : CAGL0F01991g, *C. parapsilosis* CDC317 : CPAR2_703230, C. auris B8441 : B9J08_003284 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_117826
orf19.1461	1.7	0.0	S. pombe ortholog SPCC576.01c is a predicted sulfonate dioxygenase; possibly transcriptionally regulated upon hyphal formation; Spider biofilm induced
snR42a	1.7	0.0	H/ACA box small nucleolar RNA (snoRNA)
orf19.7085	1.7	0.0	Protein of unknown function; induced in core stress response; induced by cadmium stress via Hog1; oxidative stress-induced via Cap1; induced by Mnl1 under weak acid stress; macrophage-repressed; rat catheter and Spider biofilm induced
orf19.3639	1.7	0.0	Ortholog(s) have DNA-3-methyladenine glycosylase activity, alkylbase DNA N-glycosylase activity, damaged DNA binding activity
orf19.5431	1.7	0.0	Protein of unknown function; Hap43-repressed; Spider biofilm induced
XKS1	1.7	0.0	Putative xylulokinase; Hap43-repressed; induced by prostaglandins; rat catheter biofilm repressed
YHB1	1.7	0.0	Nitric oxide dioxygenase; acts in nitric oxide scavenging/detoxification; role in virulence in mouse
CRP1	1.7	0.0	Copper transporter; CPx P1-type ATPase

orf19.36.1	1.7	0.0	Ortholog of C. parapsilosis CDC317: CPAR2_104640, *Candida orthopsilosis* Co 90-125 : CORT_0B05695 and *Candida orthopsilosis* NEW ASSEMBLY : CORT1B05695
DAP1	1.7	0.0	Similar to mammalian membrane-associated progesterone receptors involved in DNA damage response; induced in core stress response; Hog1 regulated; clade-associated expression; Hap43-repressed
orf19.4589	1.6	0.0	Ortholog(s) have polyamine oxidase activity and role in pantothenate biosynthetic process, spermine catabolic process
PFK1	1.6	0.0	Phosphofructokinase alpha subinit; activated by fructose 2,6-bisphosphate, AMP, ATP inhibited; activity reduced on hyphal induction; phagocytosis-repressed; fluconazole, flow model biofilm induced; rat catheter and Spider biofilm repressed
OSM2	1.6	0.0	Putative mitochondrial fumarate reductase; regulated by Ssn6p, Gcn2p, and Gcn4p; Hog1p-downregulated; stationary phase enriched protein; Hap43p-repressed gene
YCP4	1.6	0.0	Flavodoxin-like protein involved in oxidative stress protection and virulence; flow model, rat catheter and Spider biofilm repressed
CZF1	1.6	0.0	Transcription factor; regulates white-opaque switch
orf19.5114.1	1.6	0.0	Ortholog of C. dubliniensis CD36 : Cd36_02630, C. parapsilosis CDC317 : CPAR2_206910, *Candida tenuis* NRRL Y-1498 : cten_CGOB_00051 and Pichia stipitis Pignal : psti_CGOB_00173
orf19.5572	1.6	0.0	Protein of unknown function; Spider biofilm repressed
GSY1	1.6	0.0	Glycogen synthase (UDP glucose/starch glucosyltransferase)
orf19.3360	1.6	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
GAC1	1.6	0.0	Putative regulatory subunit of ser/thr phosphoprotein phosphatase 1
ARO9	1.5	0.0	Aromatic transaminase; Ehrlich fusel oil pathway of aromatic alcohol biosynthesis; Rim101-dependent pH-regulation (alkaline induced); Hap43-induced gene
MSO1	1.5	0.0	Putative secretory protein involved in *S. cerevisiae* sporulation
ZSF1	1.5	0.0	Ortholog od *S. cerevisiae* Tis11, a mRNA-binding protein
orf19.3325	1.5	0.0	Putative glycogen synthesis initiator; regulated by Efg1 and Efh1

Appendix 26: RNA-seq result of Zcf31-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf31-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.3210	18.2	0.0	Predicted protein of unknown function; Plc1-regulated
orf19.7279.1	5.2	0.0	Protein of unknown function; Spider biofilm induced
AQY1	4.6	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance; virulent in mice
ZCF31	4.6	0.0	Zn(II)2Cys6 transcription factor of unknown function; mutant is sensitive to copper and SDS, and resistant to Calcofluor White; required for yeast cell adherence to silicone substrate
orf19.1691	3.5	0.0	Plasma-membrane-localized protein; filament induced
BLP1	3.2	0.0	Protein of unknown function, serum-induced
UCF1	2.9	0.0	Upregulated by cAMP in filamentous growth; induced in high iron, decreased upon yeast-hypha switch; downregulation correlates with clinical fluconazole resistance; Ras1-regulated; Hap43-repressed; flow model biofilm induced
RNR22	2.9	0.0	Putative ribonucleoside diphosphate reductase; colony morphology-related gene regulation by Ssn6; transcript regulated by tyrosol and cell density; Hap43-repressed; Spider biofilm induced
orf19.1353	2.8	0.0	Protein of unknown function; repressed by yeast-hypha switch; Ras1-regulated; oral infection induced; mutants defective in damage to oral epithelium; flow model biofilm induced; Spider biofilm induced
HGT6	2.8	0.0	Putative high-affinity MFS glucose transporter; 20 family members; induced in core stress response; fluconazole, oralpharyngeal candidasis induced; flow model biofilm induced; Spider biofilm induced
orf19.4450.1	2.6	0.0	Protein conserved among the CTG-clade; 2 adjacent upstream SRE-1 elements; highly up-regulated in cecum-grown cells in a Cph2-dependent manner; Hap43-repressed; rat catheter, Spider and flow model biofilm induced
STF2	2.5	0.0	Protein involved in ATP biosynthesis; repressed in hyphae; repressed by Efg1, Hap43; transcript upregulated in clinical isolates from HIV+ patients with oral candidiasis; rat catheter, flow model and Spider biofilm induced
orf19.1277	2.4	0.0	Protein of unknown function; Rgt1, Hap43-repressed; flow model biofilm induced; Spider biofilm induced
IFE2	2.2	0.0	Putative alcohol dehydrogenase; yeast-enriched transcript; Efg1-regulated; induced by prostaglandins, Hog1, fluconazole; rat catheter biofilm induced
ADH5	2.2	0.0	Putative alcohol dehydrogenase; regulated by white-opaque switch; fluconazole-induced; antigenic in murine infection; regulated by Nrg1, Tup1; Hap43, macrophage repressed, flow model biofilm induced; Spider biofilm induced

GIG1	2.1	0.0	Protein induced by N-acetylglucosamine (GlcNAc); localized in cytoplasm; mutation causes increased resistance to nikkomycin Z
OSM1	2.1	0.0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae; caspofungin repressed; stationary phase enriched protein; flow model biofilm induced; Spider biofilm repressed
orf19.411	2.0	0.0	Protein similar to GTPase regulators; induced in low iron; transcript activated by Mnl1 under weak acid stress; Hap43-, Sfu1- and Sef1-regulated; flow model biofilm induced, Spider biofilm induced
IHD1	2.0	0.0	GPI-anchored protein; alkaline, hypha-induced; regulated by Nrg1, Rfg1, Tup1 and Tsa1, Tsa1B in minimal media at 37; oralpharyngeal candidasis induced ; Spider biofilm induced; regulated in Spider biofilms by Tec1, Efg1, Ndt80, Rob1, Brg1
YHB1	2.0	0.0	Nitric oxide dioxygenase; acts in nitric oxide scavenging/detoxification; role in virulence in mouse; transcript activated by NO, macrophage interaction; Hap43, hypha repressed; mRNA binds She3
RHR2	1.9	0.0	Glycerol 3-phosphatase; roles in osmotic tolerance, glycerol accumulation in response to salt; Spider/flow model biofilm induced; regulated by macrophage, stress, yeast-hyphal switch, pheromone, Gcn4, Hog1, Nrg1, Tup1
HEM13	1.9	0.0	Coproporphyrinogen III oxidase; antigenic; on yeast cell surface, not hyphae; iron-regulated expression; Hap43, macrophage-repressed; farnesol-induced; possibly essential; flow model biofilm induced; rat catheter, Spider biofilm repressed
GPM2	1.8	0.0	Putative phosphoglycerate mutase; repressed in hyphae; macrophage/pseudohyphal-repressed; induced by high levels of peroxide stress, farnesol; flow model biofilm induced; rat catheter and Spider biofilm repressed
DAP1	1.5	0.0	Similar to mammalian membrane-associated progesterone receptors involved in DNA damage response; induced in core stress response; Hog1 regulated; clade-associated expression; Hap43-repressed

Appendix 27: RNA-seq result of Zcf35-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf35-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
TRY6	29.1	0.0	Helix-loop-helix transcription factor; regulator of yeast form adherence
orf19.3210	17.7	0.0	Predicted protein of unknown function; Plc1-regulated
orf19.3378	15.4	0.0	Protein of unknown function; regulated by Tsa1, Tsa1B in minimal media at 37 degrees C
ALK8	8.9	0.0	Alkane-inducible cytochrome P450; catalyzes hydroxylation of lauric acid to hydroxylauric acid
DUR3	8.6	0.0	High affinity spermidine transporter: expression is induced by urea; fungal-specific (no human or murine homolog)
MEP2	8.1	0.0	Ammonium permease and regulator of nitrogen starvation-induced filamentation; 11 predicted transmembrane regions
GPT1	7.4	0.0	GABA/polyamine transporter; 9 to 11 membrane spanning segments
BMT9	6.8	0.0	Beta-mannosyltransferase, 9-gene family that includes characterized genes BMT1, BMT2, BMT3, and BMT4 with roles in beta-1,2-mannosylation of cell wall phosphopeptidomannan; regulated by Sef1, Sfu1, Hap43; rat catheter biofilm repressed
OPT3	6.3	0.0	Oligopeptide transporter: transcript induced by macrophage phagocytosis, BSA or peptides; fluconazole-induced
orf19.7227	6.3	0.0	Protein phosphatase inhibitor
CDR4	6.2	0.0	Putative ABC transporter superfamily: fluconazole, Sfu1, Hog1, core stress response induced; caspofungin repressed
RBR1	6.1	0.0	Glycosylphosphatidylinositol (GPI)-anchored cell wall protein
orf19.1438	6.0	0.0	Protein with homology to NADH dehydrogenase; regulated by Sef1p-, Sfu1p-, and Hap43p
GAP2	5.7	0.0	General broad specificity amino acid permease: ketoconazole, flucytosine repressed
MOH1	5.7	0.0	Ortholog of *S. cerevisiae* Moh1, essential for staionary phase growth
orf19.164	5.6	0.0	Ortholog(s) have triglyceride lipase activity, role in triglyceride catabolic process and peroxisomal matrix localization
OPT2	5.5	0.0	Oligopeptide transporter; induced upon phagocytosis by macrophage; macrophage/pseudohyphal-repressed after 16h
orf19.5785	5.5	0.0	Protein of unknown function; upregulated in a cyr1 or ras1 null mutant; induced by nitric oxide
orf19.7279.1	5.4	0.0	Protein of unknown function; Spider biofilm induced
RME1	5.4	0.0	Zinc finger protein, controls asexual sporulation; white-specific transcript
IFE2	5.3	0.0	Putative alcohol dehydrogenase; yeast-enriched transcript; Efg1-regulated; induced by prostaglandins, Hog1, fluconazole; rat catheter biofilm induced
RNR22	5.3	0.0	Putative ribonucleoside diphosphate reductase;colony morphology-related gene regulation by Ssn6
orf19.6311	5.3	0.0	Protein of unknown function; Hap43-induced; rat catheter and Spider biofilm induced
TES15	5.1	0.0	Putative acyl-CoA thioesterase; Hap43-repressed; Spider biofilm induced

ZCF35	5.1	0.0	Zn(II)2Cys6 transcription factor; Hap43-induced; Spider biofilm induced
ARG1	5.0	0.0	Argininosuccinate synthase; arginine synthesis; Gcn4, Rim101 regulated
orf19.344	4.9	0.0	Protein of unknown function; upregulated by fluphenazine treatment or in an azole-resistant strain that overexpresses CDR1 and CDR2; transcript possibly regulated by Tac1
ADH5	4.9	0.0	Putative alcohol dehydrogenase; regulated by white-opaque switch; fluconazole-induced; antigenic in murine infection; regulated by Nrg1, Tup1; Hap43, macrophage repressed, flow model biofilm induced; Spider biofilm induced
orf19.3910	4.8	0.0	Has domain(s) with predicted RNA binding, ribonuclease T2 activity
orf19.2048	4.8	0.0	Proten of unknown function; transcript positively regulated by Sfu1; Hap43 repressed; Spider biofilm induced
orf19.6950	4.8	0.0	Putative vacuolar membrane transporter for cationic amino acids; Spider biofilm induced
SAP1	4.7	0.0	Secreted aspartyl proteinase; acts in utilization of protein as nitrogen source
orf19.4121	4.7	0.0	Predicted thioesterase/thiol ester dehydrase-isomerase; Spider biofilm induced
OPT1	4.7	0.0	Oligopeptide transporter; transports 3-to-5-residue peptides; alleles are distinct, one has intron
ALK6	4.6	0.0	Putative cytochrome P-450 of N-alkane-induced detoxification; macrophage-induced gene
ARG8	4.6	0.0	Putative acetylornithine aminotransferase; Gcn2, Gcn4 regulated; rat catheter biofilm induced; Spider biofilm induced
ATO6	4.6	0.0	Putative fungal-specific transmembrane protein
PGA10	4.5	0.0	GPI anchored membrane protein; utilization of hemin and hemoglobin for Fe in host
ALK2	4.5	0.0	N-Alkane inducible cytochrome P450
ADH2	4.5	0.0	Alcohol dehydrogenase; soluble in hyphae; expression regulated by white-opaque switching
BRG1	4.4	0.0	Transcription factor; recruits Hda1 to hypha-specific promoters; Tn mutation affects filamentation
OPT4	4.4	0.0	Oligopeptide transporter; detected at germ tube plasma membrane
FDH1	4.3	0.0	Formate dehydrogenase; oxidizes formate to CO2; Mig1 regulated; induced by macrophages
TPO4	4.3	0.0	Putative sperimidine transporter; fungal-specific (no human or murine homolog); Spider biofilm induced
OYE22	4.3	0.0	Putative NADPH dehydrogenase; rat catheter biofilm induced
DAL4	4.2	0.0	Putative allantoin permease; fungal-specific (no human or murine homolog)
PRC2	4.2	0.0	Putative carboxypeptidase; induced by human neutrophils; Spider biofilm induced
orf19.4607	4.2	0.0	Possible Golgi membrane protein; Hap43-repressed; hypha induced; flow model biofilm induced; Spider biofilm induced
orf19.3337	4.2	0.0	Protein of unknown function; merged with orf19.3338
GCA1	4.1	0.0	Extracellular/plasma membrane-associated glucoamylase; expressed in rat oral infection
GAT1	4.1	0.0	GATA-type transcription factor; regulator of nitrogen utilization; required for nitrogen catabolite repression and utilization of isoleucine, tyrosine and tryptophan N sources; required for virulence in a mouse systemic infection model
orf19.1307	4.1	0.0	Predicted membrane protein; rat catheter biofilm induced
MNN22	4.0	0.0	Alpha-1,2-mannosyltransferase; required for normal cell wall mannan; regulated by Tsa1, Tsa1B at 37 deg
orf19.6899	4.0	0.0	Putative oxidoreductase; mutation confers hypersensitivity to toxic ergosterol analog; rat catheter and Spider biofilm induced
DDR48	4.0	0.0	Immunogenic stress-associated protein; filamentation regulated
SAP10	4.0	0.0	Secreted aspartyl protease; roles in adhesion, virulence (RHE model), cell surface integrity; distinct specificity from Sap9
orf19.1867	3.9	0.0	Putative malate permease; induced during macrophage infection; regulated by Gcn2 and Gcn4
AMO1	3.9	0.0	Putative peroxisomal copper amine oxidase
BLP1	3.9	0.0	Protein of unknown function, serum-induced
UCF1	3.9	0.0	Upregulated by cAMP in filamentous growth; induced in high iron, decreased upon yeast-hypha switch
OPT7	3.9	0.0	Putative oligopeptide transporter; possibly transports GSH or related compounds
ARG3	3.9	0.0	Putative ornithine carbamoyltransferase; Gcn4-regulated; Hap43-induced; repressed in alkalinizing medium
orf19.4612	3.9	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
CRZ2	3.9	0.0	C2H2 transcription factor, involved in regulation of early adaptation to murine GI tract; Rim101-repressed at pH 8
STF2	3.8	0.0	Protein involved in ATP biosynthesis; repressed in hyphae; repressed by Efg1, Hap43

CPA2	3.8	0.0	Putative arginine-specific carbamoylphosphate synthetase; protein enriched in stationary phase yeast cultures
orf19.4783	3.8	0.0	Protein of unknown function; induced during chlamydospore formation in both *C. albicans* and C. dubliniensis
orf19.1395	3.8	0.0	Ortholog(s) have copper ion transmembrane transporter activity, inorganic phosphate transmembrane transporter activity and role in cellular copper ion homeostasis, copper ion transmembrane transport, phosphate ion transmembrane transport
GCA2	3.7	0.0	Predicted extracellular glucoamylase; induced by ketoconazole
orf19.6690	3.7	0.0	Protein of unknown function; Hap43-repressed gene
FUR4	3.7	0.0	Putative uracil permease
orf19.6627	3.7	0.0	Protein of unknown function; possibly transcriptionally regulated upon hyphal formation
orf19.1368	3.7	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
orf19.1353	3.6	0.0	Protein of unknown function; repressed by yeast-hypha switch; Ras1-regulated
ARG5,6	3.5	0.0	Arginine biosynthetic enzyme; processed in *S. cerevisiae* into 2 polypeptides with acetylglutamate kinase (Arg6) activity and acetylglutamate-phosphate reductase (Arg5) activity; Gcn4 regulated; alkaline repressed; Spider biofilm induced
OSM1	3.5	0.0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae
RBT1	3.5	0.0	Cell wall protein with similarity to Hwp1; required for virulence; predicted glycosylation
SOD6	3.5	0.0	Copper-containing superoxide dismutase; gene family includes SOD1, SOD4, SOD5, and SOD6
orf19.1350	3.5	0.0	Protein with a thioredoxin domain; predicted role in cell redox homeostasis; rat catheter and Spider biofilm induced
orf19.7495	3.4	0.0	Protein with NADPH oxidoreductase containing flavin mononucleotide (FMN) domain; induced by nitric oxide
GST2	3.4	0.0	Glutathione S transferase: induced by benomyl and in populations of cells exposed to fluconazole over multiple generations
DUR1,2	3.4	0.0	Urea amidolyase; hydrolyzes urea to CO2; use of urea as N source and for hyphal switch in macrophage; regulated by Nrg1/Hap43
YMX6	3.4	0.0	Putative NADH dehydrogenase; macrophage-downregulated gene; induced by nitric oxide; rat catheter biofilm induced
orf19.6660	3.3	0.0	Protein of unknown function; mRNA binds to She3; Hap43-repressed; rat catheter and flow model biofilm induced
AQY1	3.3	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance; virulent in mice; flucytosine repressed
CRP1	3.3	0.0	Copper transporter; CPx P1-type ATPase; mediates Cu resistance; similar to Menkes and Wilson disease proteins
orf19.36.1	3.3	0.0	Ortholog of C. parapsilosis CDC317: CPAR2_104640, *Candida orthopsilosis* Co 90-125 : CORT_0B05695 and *Candida orthopsilosis* NEW ASSEMBLY : CORT1B05695
LYS143	3.3	0.0	Zn(II)2Cys6 transcription factor; ortholog of *S. cerevisiae* Lys14 involved in the regulation of lysine biosynthesis genes
GCY1	3.3	0.0	Aldo/keto reductase; mutation confers hypersensitivity to toxic ergosterol analog; farnesol-repressed
XYL2	3.3	0.0	D-xylulose reductase; immunogenic in mice; soluble protein in hyphae
SLP3	3.3	0.0	Plasma membrane protein implicated in stress response; similar to stomatin mechanoreception proteins
orf19.3053	3.2	0.0	Protein of unknown function; present in exponential and stationary phase yeast
RSN1	3.2	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; induced during the mating process; Hap43-repressed
MRF1	3.2	0.0	Putative mitochondrial respiratory protein; induced by farnesol, benomyl, nitric oxide, core stress response
ATC1	3.2	0.0	Cell wall acid trehalase; catalyzes hydrolysis of the disaccharide trehalose; similar to *S. cerevisiae* vacuolar acid trehalase (Ath1p)
orf19.2838	3.2	0.0	Protein of unknown function; mutation confers hypersensitivity to amphotericin B; flow model biofilm induced
orf19.787.1	3.2	0.0	Protein of unknown function; ORF added to Assembly 21 based on comparative genome analysis
CPA1	3.2	0.0	Putative carbamoyl-phosphate synthase subunit; alkaline repressed; rat catheter, Spider and flow model biofilm induced
ICL1	3.2	0.0	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice; induced upon phagocytosis by macrophage; farnesol regulated
FCR1	3.2	0.0	Transcription factor; repressor of fluconazole/ketoconazole/brefeldin A resistance
NUP	3.1	0.0	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not
MNN14	3.1	0.0	Predicted alpha-1,3-mannosyltransferase activity with a role in protein glycosylation; Hap43-repressed; Spider biofilm induced

orf19.4530.1	3.1	0.0	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
orf19.4370	3.1	0.0	Protein of unknown function; induced by nitric oxide; oxidative stress-induced via Cap1; fungal-specific (no human or murine homolog)
orf19.419	3.1	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
PDR16	3.1	0.0	Phosphatidylinositol transfer protein; induction correlates with CDR1, CDR2 overexpression/azole resistance
orf19.7225	3.1	0.0	Ortholog of C. dubliniensis CD36 : Cd36_13150, C. parapsilosis CDC317 : CPAR2_700610, C. auris B8441 : B9J08_003318 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_135418
DAK2	3.1	0.0	Putative dihydroxyacetone kinase; repressed by yeast-hypha switch; fluconazole-induced; caspofungin repressed
DAL5	3.1	0.0	Allantoate permease; nitrogen catabolite repressed, induced in absence of preferred N sources; nitrogen source regulation requires Gat1
orf19.4229	3.1	0.0	Putative polyphosphate phosphatase; role in hydrolysis of diphosphorylated inositol polyphosphates and diadenosine polyphosphates
orf19.711	3.1	0.0	Protein of unknown function; induced by nitric oxide; predicted ORF from Assembly 19; removed from Assembly 20
orf19.5169	3.0	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
MEP1	3.0	0.0	Ammonium permease; Mep1 more efficient permease than Mep2, Mep2 has additional regulatory role
orf19.4680	3.0	0.0	Possile protease; mutation confers hypersensitivity to toxic ergosterol analog
PSA2	3.0	0.0	Mannose-1-phosphate guanyltransferase; Hap43, macrophage-repressed
SKN2	3.0	0.0	Protein with a potential role in beta-1,6 glucan biosynthesis; similarity to Kre6 and Skn1
SPS20	3.0	0.0	Peroxisomal 2,4-dienoyl-CoA reductase; stationary phase enriched protein; Spider biofilm induced
orf19.5508	3.0	0.0	Ortholog of *Candida albicans* WO-1 : CAWG_05700
YHB1	3.0	0.0	Nitric oxide dioxygenase; acts in nitric oxide scavenging/detoxification; role in virulence in mouse
orf19.5626	2.9	0.0	Protein of unknown function; Plc1-regulated; induced by Mnl1 under weak acid stress; flow model biofilm induced
orf19.2346	2.9	0.0	Putative protein of unknown function, transcription is positively regulated by Tbf1p
orf19.6983	2.9	0.0	Protein of unknown function; Hap43-repressed gene; repressed by nitric oxide; Spider biofilm induced
EHT1	2.9	0.0	Putative acyl-coenzymeA:ethanol O-acyltransferase; regulated by Sef1, Sfu1, and Hap43; induced by alpha pheromone in SpiderM medium; Spider biofilm induced; promoter bound by Ndt80
GDH3	2.8	0.0	NADP-glutamate dehydrogenase; Nrg1, Plc1 regulated; hypha, hypoxia, Efg1-repressed; Rim101-induced at pH 8; GlcNAc, ciclopirox, ketoconazole induced; exp and stationary phase protein; Spider biofilm repressed; rat catheter biofilm induced
orf19.3004	2.8	0.0	Ortholog of *S. cerevisiae*: YDR262W, C. glabrata CBS138 : CAGL0M08734g, C. dubliniensis CD36
AOX1	2.8	0.0	Alternative oxidase; low abundance; constitutively expressed; one of two isoforms (Aox1p and Aox2p)
orf19.4450.1	2.8	0.0	Protein conserved among the CTG-clade; 2 adjacent upstream SRE-1 elements; highly up-regulated in cecum-grown cells in a Cph2-dependent manner
orf19.732	2.8	0.0	Possible dehydrogenase: flow model biofilm induced; rat catheter biofilm induced; Spider biofilm induced
orf19.1411	2.8	0.0	Putative cytochrome P450; Hap43-repressed gene
ARE2	2.8	0.0	Acyl CoA:sterol acyltransferase; uses cholesterol and oleoyl-CoA substrates; protoberberine derivative drug inhibits enzyme activity; ketoconazole-induced; Hap43-repressed; flow model biofilm induced; Spider biofilm induced
RHR2	2.8	0.0	Glycerol 3-phosphatase; roles in osmotic tolerance, glycerol accumulation in response to salt; Spider/flow model biofilm induced; regulated by macrophage, stress, yeast-hyphal switch, pheromone, Gcn4, Hog1, Nrg1, Tup1
orf19.6758	2.8	0.0	Predcted glucose 1-dehydrogenase (NADP+); rat catheter biofilm repressed
GDB1	2.8	0.0	Putative glycogen debranching enzyme; expression is regulated upon white-opaque switch; regulated by Nrg1, Tup1; rat catheter biofilm repressed
PIR1	2.8	0.0	1,3-beta-glucan-linked cell wall protein
orf19.6816	2.8	0.0	Putative xylose and arabinose reductase; flow model biofilm induced; Spider biofilm repressed
GSY1	2.8	0.0	Glycogen synthase (UDP glucose/starch glucosyltransferase)
TPO3	2.7	0.0	Putative polyamine transporter; MFS-MDR family; induced by Sfu1, regulated upon white-opaque
BUB3	2.7	0.0	Protein similar to *S. cerevisiae* Bub3; a kinetochore checkpoint component
HGT20	2.7	0.0	Putative glucose transporter of the major facilitator superfamily
BNA32	2.7	0.0	Has domain(s) with predicted catalytic activity, pyridoxal phosphate binding activity and role in biosynthetic process

orf19.6637	2.7	0.0	Predicted glycosyl hydrolase; hypoxia induced; flow model biofilm induced
PLC2	2.6	0.0	Phosphatidylinositol (PtdIns)-specific phospholipase C (PI-PLC); predicted type 2 membrane protein; role in, and regulated by, filamentation, Nrg1 and Tup1; no mouse systemic virulence role; orf19.5797 and orf19.1586 are almost identical
orf19.5114.1	2.6	0.0	Ortholog of C. dubliniensis CD36 : Cd36_02630, C. parapsilosis CDC317: CPAR2_206910, *Candida tenuis* NRRL Y-1498 : cten_CGOB_00051 and Pichia stipitis Pignal : psti_CGOB_00173
orf19.2371	2.6	0.0	Putative Gag protein of retrotransposon Tca2; separated by a stop codon from Pol protein orf19.2372; likely translated as single polyprotein that includes Gag, reverse transcriptase, protease, and integrase; rat catheter biofilm repressed
GTT11	2.6	0.0	Glutathione S-transferase, localized to ER; induced in exponentially growing cells, under oxidative stress; induced by nitric oxide; Spider biofilm induced
orf19.6770	2.6	0.0	protein with ENTH Epsin domain, N-terminal; Spider biofilm repressed
EXO70	2.6	0.0	Predicted subunit of the exocyst complex, involved in exocytosis; localizes to a crescent on the surface of the hyphal tip
BNA31	2.6	0.0	Putative arylformamidase, enzyme of the NAD biosynthesis pathway; Gcn4p-regulated
ECM21	2.6	0.0	Predicted regulator of endcytosis of plasma membrane proteins; fluconazole induced, alkaline induced by Rim101; repressed by caspofungin and in azole-resistant strain overexpressing MDR1
ARG4	2.6	0.0	Argininosuccinate lyase, catalyzes the final step in the arginine biosynthesis pathway; alkaline downregulated; flow model biofilm induced; Spider biofilm induced
HEM13	2.6	0.0	Coproporphyrinogen III oxidase; antigenic; on yeast cell surface, not hyphae
YIM1	2.6	0.0	Protein similar to protease of mitochondrial inner membrane; increased transcription is observed upon benomyl treatment; macrophage-downregulated gene
orf19.2350	2.5	0.0	Protein similar to *S. cerevisiae* Yor378w; MFS family transporter
NPR1	2.5	0.0	Predicted serine/threonine protein kinase, involved in regulation of ammonium transport; induced in core stress response; Hap43p-repressed gene
PDK2	2.5	0.0	Putative pyruvate dehydrogenase kinase; mutation confers hypersensitivity to amphotericin B
orf19.6840	2.5	0.0	Protein of unknown function; transcript detected in high-resolution tiling arrays
SSP96	2.5	0.0	Putative flavin-containing monooxygenase; F-12/CO2 early biofilm induced
orf19.7566	2.5	0.0	Predicted amino acid transport domain; transcript upregulated in clinical strains from HIV+ patients with oral candidiasis; alkaline upregulated by Rim101
orf19.6941	2.5	0.0	Putative diacylglycerol acyltransferase; catalyzes the terminal step of triacylglycerol formation; flow model biofilm induced; Spider biofilm induced
GAC1	2.5	0.0	Putative regulatory subunit of ser/thr phosphoprotein phosphatase 1; fluconazole-induced; caspofungin repressed; transcript induced by Mnl1 under weak acid stress; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
FRP6	2.5	0.0	Putative ammonia transport protein; regulated by Nrg1 and Tup1; regulated by Ssn6; induced by human neutrophils
DAL9	2.5	0.0	Putative allantoate permease; fungal-specific (no human or murine homolog)
ATO2	2.5	0.0	Putative fungal-specific transmembrane protein; fluconazole repressed, Hap43-repressed; flow model biofilm induced; Spider biofilm induced
LYP1	2.5	0.0	Putative permease; amphotericin B induced; flucytosine repressed; possibly an essential gene, disruptants not obtained by UAU1 method
LYS12	2.5	0.0	Homoisocitrate dehydrogenase; catalyzes 4th step in the alpha-aminoadipate pathway of lysine biosynthesis; clade-associated gene expression; protein level decreases in stationary phase cultures; Spider biofilm repressed
orf19.1433	2.5	0.0	Protein of unknown function; Hap43-repressed; colony morphology-related gene regulation by Ssn6; Spider biofilm induced
MAF1	2.5	0.0	Putative negative regulator of RNA polymerase III; decreased expression in hyphae vs yeast cells; caspofungin repressed; Spider biofilm repressed
RHD3	2.4	0.0	GPI-anchored yeast-associated cell wall protein; induced in high iron; clade-associated gene expression; not essential for cell wall integrity; fluconazole-repressed; flow model and Spider biofilm repressed
GPM2	2.4	0.0	Putative phosphoglycerate mutase; repressed in hyphae; macrophage/pseudohyphal-repressed; induced by high levels of peroxide stress, farnesol; flow model biofilm induced; rat catheter and Spider biofilm repressed
orf19.5777	2.4	0.0	Protein of unknown function; F-12/CO2 early biofilm induced
SFT1	2.4	0.0	Putative Golgi v-SNARE; Plc1-regulated; Spider biofilm induced
ECM42	2.4	0.0	Ornithine acetyltransferase; Gcn2, Gcn4-regulated; clade-specific gene expression; possibly essential gene, disruptants not obtained by UAU1 method; Spider biofilm induced
ALS2	2.4	0.0	ALS family protein; role in adhesion, biofilm formation, germ tube induction; expressed at infection of human buccal epithelial cells; putative GPI-anchor; induced by ketoconazole, low iron and at cell wall regeneration; regulated by Sfu1p

MET15	2.4	0.0	O-acetylhomoserine O-acetylserine sulfhydrylase; sulfur amino acid synthesis; immunogenic; Hog1, adherence-induced; brown color of mutant in Pb(2+) medium a visual selection; chlamydospore formation induced, F-12/CO2 biofilm induced
orf19.1562	2.4	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; repressed by alpha pheromone in SpiderM medium
CAN1	2.4	0.0	Basic amino acid permease; complements lysine transport mutation; 10 predicted transmembrane regions, 3 predicted N-glycosylation sites; phagocytosis by macrophages induces transcript; rat catheter, Spider and flow model biofilm induced
STP4	2.4	0.0	C2H2 transcription factor; induced in core caspofungin response; colony morphology-related gene regulation by Ssn6; induced by 17-beta-estradiol, ethynyl estradiol; rat catheter and Spider biofilm induced
MSO1	2.4	0.0	Putative secretory protein involved in *S. cerevisiae* sporulation; repressed during pseudohyphal growth in the presence of lysed macrophages; Hap43-repressed; Spider biofilm induced
GLT1	2.4	0.0	Putative glutamate synthase; regulated by Sef1, Sfu1, and Hap43; rat catheter biofilm repressed
orf19.1381	2.3	0.0	Ortholog of *S. cerevisiae*/S. pombe Lsb5; predicted role in actin cortical patch localization, actin filament organization, endocytosis; flow model biofilm induced; Spider biofilm repressed
REX3	2.3	0.0	Ortholog(s) have 3'-5'-exoribonuclease activity
orf19.6117	2.3	0.0	S. pombe ortholog SPAC5D6.04 is a predicted auxin family transmembrane transporter; ketoconazole and hypoxia induced
LYS4	2.3	0.0	Homoaconitase; regulated by Gcn4, Gcn2; induced in response to amino acid starvation (3-AT); induced by human whole blood or PMNs; Hap43-repressed; flow model and Spider biofilm repressed
RIB3	2.3	0.0	3,4-Dihydroxy-2-butanone 4-phosphate synthase; homodimeric enzyme of riboflavin biosynthesis; converts ribulose 5-phosphate to L-3,4-dihydroxy-2-butanone 4-phosphate; transcription regulated on yeast-hyphal switch, macrophage interaction
PNG2	2.3	0.0	Putative peptide:N-glycanase; gene has variable numbers of 12-bp repeats; induced by caspofungin, ciclopirox olamine, ketoconazole or hypoxia; gene of core caspofungin response; Hap43-induced; Spider biofilm induced
orf19.2459	2.3	0.0	Protein of unknown function; mRNA binds to She3; Hap43 repressed gene; Spider biofilm induced
SCS7	2.3	0.0	Putative ceramide hydroxylase; regulated by Nrg1; induced in high iron; fluconazole-induced; Hap43-repressed; Spider biofilm induced
orf19.1797	2.3	0.0	D-arabinose 5-phosphate isomerase; has GutQ domain which is associated with phosphosugar binding; other biofilm induced; rat catheter and Spider biofilm induced; F-12/CO2 early biofilm induced
orf19.2047	2.3	0.0	Putative protein of unknown function; Hap43p-repressed gene; mutation confers hypersensitivity to toxic ergosterol analog, and to amphotericin B
orf19.1107	2.3	0.0	Protein of unknown function; Spider biofilm induced
HGT3	2.3	0.0	Putative glucose transporter of the major facilitator superfamily; the *C. albicans* glucose transporter family comprises 20 members
FGR22	2.3	0.0	Putative phosphatidylinositol-specific phospholipase C (PI-PLC); predicted type 2 membrane protein; no *S. cerevisiae* ortholog; role in, and regulated by, filamentation, Hap43p
orf19.4128	2.2	0.0	Ortholog of C. dubliniensis CD36 : Cd36_19390, C. parapsilosis CDC317 : CPAR2_209600, *C. auris* B8441 : B9J08_004606 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_134010
PFK1	2.2	0.0	Phosphofructokinase alpha subinit; activated by fructose 2,6-bisphosphate, AMP, ATP inhibited; activity reduced on hyphal induction; phagocytosis-repressed; fluconazole, flow model biofilm induced; rat catheter and Spider biofilm repressed
GPH1	2.2	0.0	Putative glycogen phosphorylase; role in glycogen metabolism; regulated by Ssk1, Mig1, Tup1, Hap43; fluconazole-induced; localizes to cell surface of hyphae, not yeast; stationary phase enriched protein; Spider biofilm induced
orf19.4791	2.2	0.0	Protein of unknown function; Spider biofilm induced
MODF	2.2	0.0	Has domain(s) with predicted ATP binding, ATPase activity, nucleoside-triphosphatase activity
FGR13	2.2	0.0	Protein encoded in retrotransposon Zorro3 with a potential zinc finger; lacks an ortholog in *S. cerevisiae*; transposon mutation affects filamentous growth
PGA13	2.2	0.0	GPI-anchored cell wall protein involved in cell wall synthesis; required for normal cell surface properties; induced in oralpharyngeal candidasis; Spider biofilm induced; Bcr1-repressed in RPMI a/a biofilms
orf19.5438	2.2	0.0	Putative ubiquitin-protein ligase; role in protein sumoylation, protein ubiquitination; Spider biofilm induced
LIP6	2.2	0.0	Secreted lipase, member of family of lipase genes expressed differentially in response to carbon source and during infection; may have a role in nutrition and/or in creating an acidic microenvironment; induced on adherence to polystyrene
orf19.2726	2.2	0.0	Putative plasma membrane protein; Plc1-regulated; Spider biofilm induced
orf19.7499	2.2	0.0	Putative nicotinic acid mononucleotide adenylyltransferase, involved in NAD salvage pathway; Spider biofilm repressed
DLD2	2.2	0.0	Ortholog(s) have D-lactate dehydrogenase (cytochrome) activity, role in lactate catabolic process and mitochondrial inner membrane, mitochondrion localization

MIT1	2.2	0.0	Mannosylinositol phosphorylceramide (MIPC) synthase catalytic subunit; sphingolipid biosynthesis; fluconazole, caspofungin induced; macrophage-repressed; Spider biofilm induced
ECM4	2.2	0.0	Cytoplasmic glutathione S-transferase; regulated by Nrg1, Tup1; induced in core stress response, in cyr1 or ras1 mutant (yeast or hyphal cells); Tn mutation affects filamentous growth; stationary phase enriched; Spider biofilm induced
orf19.7043	2.2	0.0	Ortholog of *S. cerevisiae* : YLR050C, C. glabrata CBS138 : CAGL0F01991g, C. parapsilosis CDC317 : CPAR2_703230, C. auris B8441 : B9J08_003284 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_117826
IFK2	2.2	0.0	Putative thiol-specific monooxygenase; mutant is viable; flow model biofilm induced
orf19.5552	2.2	0.0	Putative transcriptional regulator of ribonucleotide reductase genes; Spider biofilm induced
LEU1	2.2	0.0	3-isopropylmalate dehydratase; antigenic in humans; repressed in hyphae; alkaline repressed; upregulated by human whole blood or PMNs; regulated by Sef1, Sfu1, and Hap43; rat catheter biofilm induced, Spider biofilm repressed
orf19.302	2.2	0.0	Ortholog(s) have role in endoplasmic reticulum to Golgi vesicle-mediated transport and TRAPPI protein complex, TRAPPII protein complex, TRAPPIII protein complex localization
RBE1	2.1	0.0	Pry family cell wall protein; Rim101, Efg1, Ssn6, alkaline repressed; O-glycosylation; no GPI anchor predicted; ketoconazol induced; regulated by Sef1, Sfu1, Hap4
LEU2	2.1	0.0	Isopropyl malate dehydrogenase; leucine biosynthesis; induced by human whole blood or PMNs; protein level decreases in stationary phase; GlcNAc-induced protein; flow model biofilm repressed
LEU4	2.1	0.0	Putative 2-isopropylmalate synthase; regulated by Nrg1, Mig1, Tup1, Gcn4; induced by human whole blood or PMNs; macrophage/pseudohyphal-repressed after 16h; Spider biofilm repressed
AUT7	2.1	0.0	Putative autophagosome protein; acts synergistically with Ysy6p to regulate unfolded protein response and mitochondrial function under ER stress
orf19.5525	2.1	0.0	Putative oxidoreductase; protein levels affected by URA3 expression in CAI-4 strain background; Efg1, Efh1 regulated; Rgt1-repressed; protein present in exponential and stationary growth phase yeast; rat catheter biofilm repressed
orf19.675	2.1	0.0	Cell wall protein; induced in core stress response and core caspofungin response; iron-regulated; amphotericin B, ketoconazole, and hypoxia induced
orf19.1656	2.1	0.0	Protein with a predicted FYVE/PHD zinc finger domain; Hap43-repressed; Spider biofilm induced
orf19.4368	2.1	0.0	Has domain(s) with predicted hydrolase activity and role in cellular process
MUM2	2.1	0.0	Protein similar to *S. cerevisiae* Mum2, a protein essential for meiotic DNA replication and sporulation; induced by alpha pheromone in Spider medium; transcript regulated by Tup1
orf19.3813	2.1	0.0	Ortholog of *C. dubliniensis* CD36 : Cd36_44340, *C. parapsilosis* CDC317 : CPAR2_302240, *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_105331 and *Debaryomyces hansenii* CBS767 : DEHA2E03454g
GRE3	2.1	0.0	Putative D-xylose reductase; antigenic in murine systemic infection; soluble protein in hyphae; induced by farnesol, macrophage interaction and by Mnl1 under weak acid stress
orf19.2372	2.1	0.0	Pol protein of retrotransposon Tca2; separated by a stop codon from Gag protein orf19.2371; likely translated as single polyprotein with Gag, reverse transcriptase, protease, and integrase
orf19.2506	2.1	0.0	Protein of unknown function; opaque-specific transcript; induced during chlamydospore formation in both *C. albicans* and *C. dubliniensis*; Hog1-repressed; Spider biofilm induced
CBP1	2.1	0.0	Corticosteroid binding protein; transcription induced at late log-phase or upon adherence to polystyrene; not induced by corticosterone; contains a possible NAD/FAD binding region; regulated by Nrg1, Tup1; Spider biofilm induced
orf19.1314	2.0	0.0	Protein of unknown function; planktonic growth-induced gene
orf19.6769	2.0	0.0	Has domain(s) with predicted intracellular anatomical structure localization
orf19.1573	2.0	0.0	Ortholog(s) have fungal-type vacuole membrane, vacuole-mitochondrion membrane contact site localization
TPS1	2.0	0.0	Trehalose-6-phosphate synthase; role in hyphal growth and virulence in mouse systemic infection; induced in presence of human neutrophils; macrophage/pseudohyphal-repressed after 16h; stationary phase enriched protein; Hap43-repressed
orf19.6527	2.0	0.0	Pheromone-regulated protein (Prm10) of *S. cerevisiae*; colony morphology-related gene regulation by Ssn6; induced by Mnl1 under weak acid stress; possibly essential gene, disruptants not obtained by UAU1 method; Spider biofilm induced
orf19.5727	2.0	0.0	Ortholog of C. dubliniensis CD36 : Cd36_64130, C. parapsilosis CDC317 : CPAR2_601230, C. auris B8441 : B9J08_003898 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_115908
orf19.4901	2.0	0.0	Predicted methyltransferase; Spider biofilm induced
orf19.4795	2.0	0.0	Protein of unknown function; Sef1-, Sfu1-, and Hap43 regulated; Spider biofilm induced
APR1	2.0	0.0	Vacuolar aspartic proteinase; transcript equivalent in yeast-form and mycelial cells but is elevated at lower growth temperatures; upregulated iby human neutrophils; protein enriched in stationary phase; Spider biofilm induced
IFM3	2.0	0.0	Protein with a 2-hydroxyacid dehydrogenase catalytic domain; Hap43-repressed; Plc1-regulated; overlaps orf19.2177
DOG1	2.0	0.0	Putative 2-deoxyglucose-6-phosphatase; haloacid dehalogenase hydrolase/phosphatase superfamily; similar to *S. cerevisiae* Dog1, Dog2, Hor1, Rhr2; regulated by Nrg1, Tup1; Spider biofilm repressed

orf19.4550	2.0	0.0	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family; flow model biofilm induced
orf19.411	2.0	0.0	Protein similar to GTPase regulators; induced in low iron; transcript activated by Mnl1 under weak acid stress; Hap43-, Sfu1- and Sef1-regulated; flow model biofilm induced, Spider biofilm induced
UGA6	2.0	0.0	Putative GABA-specific permease; decreased transcription is observed upon benomyl treatment or in an azole-resistant strain that overexpresses MDR1
ARA1	2.0	0.0	D-Arabinose dehydrogenase; dehydro-D-arabinono-1,4-lactone synthesis; active on D-arabinose, L-fucose, L-xylose, L-galactose; inhibited by metal ions, thiol group-specific reagents; induced on polystyrene adherence; Spider biofilm induced
orf19.4609	2.0	0.0	Putative dienelactone hydrolase; protein abundance is affected by URA3 expression in the CAI-4 strain background; protein present in exponential and stationary growth phase yeast cultures
MET10	1.9	0.0	Sulfite reductase; role in sulfur amino acid metabolism; induced by human whole blood or PMNs; Hog1-induced; possibly adherence-induced; flow model, Spider model, F-12/CO2 biofilm induced
ALT1	1.9	0.0	Putative alanine transaminase; mutation confers hypersensitivity to 5-fluorocytosine (5-FC); rat catheter and flow model biofilm induced
GLC3	1.9	0.0	Putative 1,4-glucan branching enzyme; fluconazole-induced; colony morphology-related gene regulation by Ssn6; stationary phase enriched protein
POS5	1.9	0.0	Protein similar to *S. cerevisiae* Pos5p, a mitochondrial NADH kinase involved in the oxidative stress response; planktonic growth-induced gene; likely to be essential for growth, based on an insertional mutagenesis strategy
orf19.6318	1.9	0.0	Has domain(s) with predicted membrane localization
FBA1	1.9	0.0	Fructose-bisphosphate aldolase; glycolytic enzyme; antigenic in murine/human infection; regulated by yeast-hypha switch; induced by Efg1, Gcn4, Hog1, fluconazole; phagocytosis-repressed; flow model biofilm induced; Spider biofilm repressed
CAF16	1.9	0.0	ABC family protein, predicted not to be a transporter; Hap43, caspofungin repressed; rat catheter and Spider biofilm repressed
PNC1	1.9	0.0	Putative nicotinamidase, involved in NAD salvage pathway; decreased transcription is observed in an azole-resistant strain that overexpresses MDR1
orf19.3713	1.9	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; induced by Mnl1 under weak acid stress; transcript detected in high-resolution tiling arrays
orf19.4264	1.9	0.0	Protein of unknown function; induced during chlamydospore formation in both *C. albicans* and C. dubliniensis; flow model biofilm induced
orf19.225	1.9	0.0	Predicted 2-hydroxyacid dehydrogenase; Hap43-repressed gene
orf19.5157	1.8	0.0	Protein with a protein tyrosine phosphatase-like protein domain; putative membrane-spanning regions; rat catheter biofilm induced
IML2	1.8	0.0	Protein of unknown function; early-stage flow model biofilm induced; Hap43-repressed; Spider biofilm repressed
PFK2	1.8	0.0	Phosphofructokinase beta subunit; fructose 2,6-bisphosphate, AMP activated; ATP inhibited; phagocytosis, hyphal repressed; fluconazole-induced; stationary-phase enriched; flow model biofilm induced; rat catheter/Spider biofilm repressed
CUP1	1.8	0.0	Helix-loop-helix transcription factor; regulator of yeast form adherence; required for yeast cell adherence to silicone substrate; Spider and F-12/CO2 biofilm induced; repressed by alpha pheromone in Spider medium
orf19.4907	1.8	0.0	Predicted protein of unknown function; Plc1-regulated
UGA2	1.8	0.0	Protein of unknown function; regulated by Tsa1, Tsa1B in minimal media at 37 degrees C
orf19.7473	1.8	0.0	Alkane-inducible cytochrome P450; catalyzes hydroxylation of lauric acid to hydroxylauric acid; overproduction causes fluconazole resistance in WT and causes multidrug resistance in a cdr1 cdr2 double mutant; rat catheter biofilm repressed
orf19.2670	1.8	0.0	High affinity spermidine transporter: expression is induced by urea; fungal-specific (no human or murine homolog); not required for virulence in a mouse intravenous model
orf19.5114	1.8	0.0	Ammonium permease and regulator of nitrogen starvation-induced filamentation; 11 predicted transmembrane regions; in low nitrogen cytoplasmic C-terminus activates Ras/cAMP and MAPK signal transduction pathways to induce filamentation
TPI1	1.8	0.0	GABA/polyamine transporter; 9 to 11 membrane spanning segments; complements GABA uptake defect of an *S. cerevisiae* uga4 put4 gap1 triple mutant; complements growth of an *S. cerevisiae* spe1 mutant under polyamine limitation
MSN4	1.8	0.0	Beta-mannosyltransferase, 9-gene family that includes characterized genes BMT1, BMT2, BMT3, and BMT4 with roles in beta-1,2-mannosylation of cell wall phosphopeptidomannan
orf19.899	1.8	0.0	Oligopeptide transporter: transcript induced by macrophage phagocytosis, BSA or peptides; fluconazole-induced; induced by Rim101 at pH 8; virulence-group-correlated expression; Hap43-repressed; Spider biofilm induced
EFG1	1.8	0.0	Protein phosphatase inhibitor; Hap43-repressed; homozygous Tn insertion decreases colony wrinkling but does not block hyphal growth in liquid media; mutation confers hypersensitivity to toxic ergosterol analog; Spider biofilm induced
orf19.1461	1.8	0.0	Putative ABC transporter superfamily
orf19.321	1.8	0.0	Glycosylphosphatidylinositol (GPI)-anchored cell wall protein

orf19.173	1.8	0.0	Protein with homology to NADH dehydrogenase; regulated by Sef1p-, Sfu1p-, and Hap43p
ALS7	1.8	0.0	General broad specificity amino acid permease; ketoconazole, flucytosine repressed
orf19.449	1.8	0.0	Ortholog of *S. cerevisiae* Moh1, essential for staionary phase growth; induced by alpha pheromone in Spider medium and by Mnl1 under weak acid stress
TPS3	1.8	0.0	Ortholog(s) have triglyceride lipase activity, role in triglyceride catabolic process and peroxisomal matrix localization
orf19.4906	1.8	0.0	Oligopeptide transporter; induced upon phagocytosis by macrophage; macrophage/pseudohyphal-repressed after 16h; fluconazole-induced; virulence-group-correlated expression; Hap43-repressed
orf19.3661	1.8	0.0	Protein of unknown function; upregulated in a cyr1 or ras1 null mutant; induced by nitric oxide
orf19.4134	1.8	0.0	Protein of unknown function; Spider biofilm induced
STB3	1.8	0.0	Zinc finger protein, controls asexual sporulation; white-specific transcript
orf19.4534	1.8	0.0	Putative alcohol dehydrogenase; yeast-enriched transcript
orf19.1424	1.8	0.0	Putative ribonucleoside diphosphate reductase; colony morphology-related gene regulation by Ssn6
orf19.3325	1.7	0.0	Protein of unknown function; Hap43-induced; rat catheter and Spider biofilm induced
orf19.6554	1.7	0.0	Putative acyl-CoA thioesterase; Hap43-repressed; Spider biofilm induced
orf19.6316	1.7	0.0	Zn(II)2Cys6 transcription factor; Hap43-induced; Spider biofilm induced
orf19.3442	1.7	0.0	Argininosuccinate synthase; arginine synthesis; Gcn4, Rim101 regulated; induced by amino acid starvation (3-AT), benomyl treatment; stationary phase enriched protein; repressed in alkalinizing medium; rat catheter, Spider biofilm induced
PTC4	1.7	0.0	Protein of unknown function; upregulated by fluphenazine treatment or in an azole-resistant strain that overexpresses CDR1 and CDR2; transcript possibly regulated by Tac1
orf19.1180	1.7	0.0	Putative alcohol dehydrogenase; regulated by white-opaque switch
orf19.4883	1.7	0.0	Has domain(s) with predicted RNA binding, ribonuclease T2 activity
orf19.5103	1.7	0.0	Proten of unknown function; transcript positively regulated by Sfu1
BBC1	1.7	0.0	Putative vacuolar membrane transporter for cationic amino acids; Spider biofilm induced
ZCF27	1.7	0.0	Secreted aspartyl proteinase; acts in utilization of protein as nitrogen source
PSD2	1.7	0.0	Predicted thioesterase/thiol ester dehydrase-isomerase; Spider biofilm induced
orf19.3302	1.7	0.0	Oligopeptide transporter; transports 3-to-5-residue peptides; alleles are distinct, one has intron; suppresses *S. cerevisiae* ptr2-2 mutant defects
MNN13	1.7	0.0	Putative cytochrome P-450 of N-alkane-induced detoxification; macrophage-induced gene
orf19.4127	1.7	0.0	Putative acetylornithine aminotransferase; Gcn2, Gcn4 regulated; rat catheter biofilm induced; Spider biofilm induced
FLO9	1.7	0.0	Putative fungal-specific transmembrane protein
PRC3	1.7	0.0	GPI anchored membrane protein; utilization of hemin and hemoglobin for Fe in host; Rim101 at ph8/hypoxia/ketoconazole/ciclopirox/hypha-induced; required for RPMI biofilm formation, Bcr1-induced in a/a biofilm; rat catheter biofilm repressed
orf19.7196	1.7	0.0	N-Alkane inducible cytochrome P450
PHO113	1.7	0.0	Alcohol dehydrogenase; soluble in hyphae; expression regulated by white-opaque switching; regulated by Ssn6; indued by Mnl1 in weak acid stress; protein enriched in stationary phase yeast cultures; Spider biofilm induced
orf19.1800	1.7	0.0	Transcription factor; recruits Hda1 to hypha-specific promoters; Tn mutation affects filamentation; Hap43-repressed; Spider and flow model biofilm induced; required for Spider biofilm formation; Bcr1-repressed in RPMI a/a biofilms
orf19.4511	1.7	0.0	Oligopeptide transporter; detected at germ tube plasma membrane; transcript inducede during phagocytosis by macrophages; fungal-specific; Hap43-repressed; merged with orf19.2292 in Assembly 20; rat catheter and Spider biofilm induced
PGK1	1.7	0.0	Formate dehydrogenase; oxidizes formate to CO2; Mig1 regulated; induced by macrophages
orf19.4828	1.7	0.0	Putative sperimidine transporter; fungal-specific (no human or murine homolog)
PLB5	1.6	0.0	Putative NADPH dehydrogenase; rat catheter biofilm induced
orf19.7278	1.6	0.0	Putative allantoin permease; fungal-specific (no human or murine homolog)
orf19.3881	1.6	0.0	Putative carboxypeptidase; induced by human neutrophils; Spider biofilm induced
CDC19	1.6	0.0	Possible Golgi membrane protein; Hap43-repressed; hypha induced; flow model biofilm induced; Spider biofilm induced
CPH2	1.6	0.0	Protein of unknown function; merged with orf19.3338
RIM8	1.6	0.0	Extracellular/plasma membrane-associated glucoamylase; expressed in rat oral infection; regulated by carbohydrates, pH, galactose; promotes biofilm matrix formation
ZCF1	1.6	0.0	GATA-type transcription factor; regulator of nitrogen utilization; required for nitrogen catabolite repression and utilization of isoleucine, tyrosine and tryptophan N sources

POT1-2	1.6	0.0	Predicted membrane protein; rat catheter biofilm induced
TFS1	1.6	0.0	Alpha-1,2-mannosyltransferase; required for normal cell wall mannan; regulated by Tsa1, Tsa1B at 37 deg; repressed in core stress response; NO, Hog1 induced
orf19.3922	1.6	0.0	Putative oxidoreductase: mutation confers hypersensitivity to toxic ergosterol analog
SOD2	1.6	0.0	Immunogenic stress-associated protein; filamentation regulated; induced by benomyl/caspofungin/ketoconazole or in azole-resistant strain
STP1	1.6	0.0	Secreted aspartyl protease; roles in adhesion, virulence (RHE model), cell surface integrity; distinct specificity from Sap9; at cell membrane and wall; GPI-anchored
PGA14	1.6	0.0	Putative malate permease; induced during macrophage infection
UBC8	1.6	0.0	Putative peroxisomal copper amine oxidase
NCR1	1.6	0.0	Protein of unknown function, serum-induced
PRX1	1.6	0.0	Upregulated by cAMP in filamentous growth; induced in high iron, decreased upon yeast-hypha switch; downregulation correlates with clinical fluconazole resistance; Ras1-regulated; Hap43-repressed; flow model biofilm induced
ZCF10	1.6	0.0	Putative oligopeptide transporter; possibly transports GSH or related compounds; Hog1-induced; expression of OPT6, -7, or -8 does not suppress defect of mutant lacking OPT1-3; Hap43-repressed; F-12/CO2 early biofilm induced
XKS1	1.6	0.0	Putative ornithine carbamoyltransferase; Gcn4-regulated; Hap43-induced; repressed in alkalinizing medium; rat catheter and Spider biofilm induced
orf19.4589	1.6	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
BMT8	1.6	0.0	C2H2 transcription factor, involved in regulation of early adaptation to murine GI tract
orf19.4174	1.5	0.0	Protein involved in ATP biosynthesis; repressed in hyphae; repressed by Efg1, Hap43
VRP1	1.5	0.0	Putative arginine-specific carbamoylphosphate synthetase
orf19.6980	1.5	0.0	Protein of unknown function; induced during chlamydospore formation in both *C. albicans* and C. dubliniensis
ECM15	1.5	0.0	Ortholog(s) have copper ion transmembrane transporter activity, inorganic phosphate transmembrane transporter activity and role in cellular copper ion homeostasis, copper ion transmembrane transport, phosphate ion transmembrane transport
orf19.5572	1.5	0.0	Predicted extracellular glucoamylase; induced by ketoconazole; possibly essential, disruptants not obtained by UAU1 method; promotes biofilm matrix formation; Spider biofilm induced; Bcr1-induced in RPMI a/a biofilms
orf19.33	1.5	0.0	Protein of unknown function; Hap43-repressed gene
orf19.1890	1.5	0.0	Putative uracil permease
DOT5	1.5	0.0	Protein of unknown function; possibly transcriptionally regulated upon hyphal formation
PGM2	1.5	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
GAD1	1.5	0.0	Protein of unknown function; repressed by yeast-hypha switch; Ras1-regulated; oral infection induced; mutants defective in damage to oral epithelium; flow model biofilm induced; Spider biofilm induced
HGT7	1.5	0.0	Arginine biosynthetic enzyme; processed in *S. cerevisiae* into 2 polypeptides with acetylglutamate kinase (Arg6) activity and acetylglutamate-phosphate reductase (Arg5) activity; Gcn4 regulated; alkaline repressed; Spider biofilm induced
orf19.5103	29.1	0.0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae; caspofungin repressed; stationary phase enriched protein; flow model biofilm induced; Spider biofilm repressed
BBC1	17.7	0.0	Cell wall protein with similarity to Hwp1; required for virulence; predicted glycosylation; fluconazole, Tup1 repressed; farnesol, alpha factor, serum, hyphal and alkaline induced; Rfg1, Rim101-regulated
ZCF27	15.4	0.0	Copper-containing superoxide dismutase; gene family includes SOD1, SOD4, SOD5, and SOD6; gene may contain an intron; Hap43-repressed; flow model and rat catheter biofilm induced
PSD2	8.9	0.0	Protein with a thioredoxin domain; predicted role in cell redox homeostasis; rat catheter and Spider biofilm induced
orf19.3302	8.6	0.0	Protein with NADPH oxidoreductase containing flavin mononucleotide (FMN) domain; induced by nitric oxide
MNN13	8.1	0.0	Glutathione S transferase; induced by benomyl and in populations of cells exposed to fluconazole over multiple generations
orf19.4127	7.4	0.0	Urea amidolyase; hydrolyzes urea to CO2; use of urea as N source and for hyphal switch in macrophage; regulated by Nrg1/Hap43; required for virulence; promotes mouse kidney and brain colonization; rat catheter and flow model biofilm induced
FLO9	6.8	0.0	Putative NADH dehydrogenase; macrophage-downregulated gene; induced by nitric oxide; rat catheter biofilm induced
PRC3	6.3	0.0	Protein of unknown function; mRNA binds to She3; Hap43-repressed; rat catheter and flow model biofilm induced

orf19.7196	6.3	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance; virulent in mice; flucytosine repressed; flow model/RPMI/Spider/rat catheter biofilm induced; required for RPMI biofilm formation; Bcr1-induced in a/a RPMI biofilms
PHO113	6.2	0.0	Copper transporter; CPx P1-type ATPase; mediates Cu resistance; similar to Menkes and Wilson disease proteins; copper-induced; Tbf1-activated; suppresses Cu sensitivity of *S. cerevisiae* cup1 mutant; flow model biofilm induced
orf19.1800	6.1	0.0	Ortholog of C. parapsilosis CDC317: CPAR2_104640, *Candida orthopsilosis* Co 90-125 : CORT_0B05695 and *Candida orthopsilosis* NEW ASSEMBLY : CORT1B05695
orf19.4511	6.0	0.0	Zn(II)2Cys6 transcription factor; ortholog of *S. cerevisiae* Lys14 involved in the regulation of lysine biosynthesis genes
PGK1	5.7	0.0	Aldo/keto reductase; mutation confers hypersensitivity to toxic ergosterol analog; farnesol-repressed; stationary phase enriched protein; flow model biofilm induced; Spider biofilm repressed
orf19.4828	5.7	0.0	D-xylulose reductase; immunogenic in mice; soluble protein in hyphae; induced by caspofungin, fluconazole, Hog1 and during cell wall regeneration; Mnl1-induced in weak acid stress; stationary phase enriched; flow model biofilm induced
PLB5	5.6	0.0	Plasma membrane protein implicated in stress response; similar to stomatin mechanoreception proteins; overexpression induces apoptotic-like cell death; absent from hyphal cells; induced by Rgt1; rat catheter and Spider biofilm induced
orf19.7278	5.5	0.0	Protein of unknown function; present in exponential and stationary phase yeast; identified in extracts from biofilm and planktonic cells; flow model biofilm induced gene; GlcNAc-induced protein
orf19.3881	5.5	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; induced during the mating process; Hap43-repressed
CDC19	5.4	0.0	Putative mitochondrial respiratory protein; induced by farnesol, benomyl, nitric oxide, core stress response; oxidative stress-induced via Cap1; stationary-phase enriched protein; Spider biofilm induced
CPH2	5.4	0.0	Cell wall acid trehalase; catalyzes hydrolysis of the disaccharide trehalose; similar to *S. cerevisiae* vacuolar acid trehalase (Ath1p); Hap43p-repressed gene
RIM8	5.3	0.0	Protein of unknown function; mutation confers hypersensitivity to amphotericin B; flow model biofilm induced
ZCF1	5.3	0.0	Protein of unknown function; ORF added to Assembly 21 based on comparative genome analysis; protein detected by mass spec in stationary phase cultures
POT1-2	5.3	0.0	Putative carbamoyl-phosphate synthase subunit; alkaline repressed; rat catheter, Spider and flow model biofilm induced
TFS1	5.1	0.0	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice; induced upon phagocytosis by macrophage; farnesol regulated; Pex5-dependent peroxisomal localization; stationary phase enriched; rat catheter, Spider biofilm induced
orf19.3922	5.1	0.0	Transcription factor; repressor of fluconazole/ketoconazole/brefeldin A resistance; Tn mutation enhances filamentation; partially rescues *S. cerevisiae* pdr1 pdr3 fluconazole sensitivity; rat catheter biofilm induced/Spider biofilm repressed
SOD2	5.0	0.0	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not; similar to a nucleoside permease of S. pombe; possibly processed by Kex2p
STP1	4.9	0.0	Predicted alpha-1,3-mannosyltransferase activity with a role in protein glycosylation; Hap43-repressed; Spider biofilm induced
PGA14	4.9	0.0	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
UBC8	4.8	0.0	Protein of unknown function; induced by nitric oxide; oxidative stress-induced via Cap1; fungal-specific (no human or murine homolog)
NCR1	4.8	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
PRX1	4.8	0.0	Phosphatidylinositol transfer protein; induction correlates with CDR1, CDR2 overexpression/azole resistance; fluphenazine, 17-beta-estradiol, ethynyl estradiol, NO induced; farnesol-downregulated in biofilm; rat catheter biofilm induced
ZCF10	4.7	0.0	Ortholog of C. dubliniensis CD36 : Cd36_13150, C. parapsilosis CDC317 : CPAR2_700610, C. auris B8441 : B9J08_003318 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_135418
XKS1	4.7	0.0	Putative dihydroxyacetone kinase; repressed by yeast-hypha switch; fluconazole-induced; caspofungin repressed; protein enriched in stationary phase yeast cultures; flow model biofilm induced; rat catheter and Spider biofilm repressed
orf19.4589	4.7	0.0	Allantoate permease; nitrogen catabolite repressed, induced in absence of preferred N sources; nitrogen source regulation requires Gat1; possibly essential gene (by UAU1 method); Hap43-repressed
BMT8	4.6	0.0	Putative polyphosphate phosphatase; role in hydrolysis of diphosphorylated inositol polyphosphates and diadenosine polyphosphates; Spider biofilm induced
orf19.4174	4.6	0.0	Protein of unknown function; induced by nitric oxide; predicted ORF from Assembly 19; removed from Assembly 20; restored based on transcription data
VRP1	4.6	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
orf19.6980	4.5	0.0	Ammonium permease; Mep1 more efficient permease than Mep2, Mep2 has additional regulatory role; 11 predicted transmembrane regions; low mRNA abundance; hyphal downregulated; flow model biofilm induced

ECM15	4.5	0.0	Possile protease; mutation confers hypersensitivity to toxic ergosterol analog
orf19.5572	4.5	0.0	Mannose-1-phosphate guanyltransferase; Hap43, macrophage-repressed; stationary phase enriched protein; Spider biofilm induced; rat catheter biofilm repressed
orf19.33	4.4	0.0	Protein with a potential role in beta-1,6 glucan biosynthesis; similarity to Kre6 and Skn1; possibly essential, disruptants not obtained by UAU1 method; Hap43-induced; flow model biofilm induced; rat catheter biofilm repressed
orf19.1890	4.4	0.0	Peroxisomal 2,4-dienoyl-CoA reductase; stationary phase enriched protein; Spider biofilm induced
DOT5	4.3	0.0	Ortholog of *Candida albicans* WO-1 : CAWG_05700
PGM2	4.3	0.0	Nitric oxide dioxygenase; acts in nitric oxide scavenging/detoxification; role in virulence in mouse; transcript activated by NO, macrophage interaction; Hap43, hypha repressed; mRNA binds She3
GAD1	4.3	0.0	Protein of unknown function; Plc1-regulated; induced by Mnl1 under weak acid stress; flow model biofilm induced
HGT7	4.2	0.0	Putative protein of unknown function, transcription is positively regulated by Tbf1p
DOT5	4.2	0.0	Protein of unknown function; Hap43-repressed gene; repressed by nitric oxide; Spider biofilm induced

Appendix 28: RNA-seq result of Zcf38-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf38-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.1167	8.6	0.0	Ortholog(s) have sulfonate dioxygenase activity and role in sulfur compound catabolic process
ZCF38	7.1	0.0	Putative Zn(II)2Cys6 transcription factor
PGA13	5.6	0.0	GPI-anchored cell wall protein involved in cell wall synthesis; required for normal cell surface properties; induced in oralpharyngeal candidasis
FRP1	4.6	0.0	Ferric reductase; alkaline-induced by Rim101; iron-chelation-induced by CCAAT-binding factor; fluconazole-repressed
MLS1	4.5	0.0	Malate synthase; glyoxylate cycle enzyme; no mammalian homolog; regulated upon white-opaque switch; phagocytosis
orf19.3301	4.4	0.0	Putative ubiquitin ligase complex component; induced by heavy metal (cadmium) stress
orf19.7596	4.3	0.0	Protein with a phosphoglycerate mutase family domain; Hap43-repressed gene
PGA7	4.3	0.0	GPI-linked hyphal surface antigen; induced by ciclopirox olamine, ketoconazole, Rim101 at pH 8; Hap43, fluconazole
ICL1	4.1	0.0	Isocitrate lyase; glyoxylate cycle enzyme; required for virulence in mice
orf19.5785	3.9	0.0	Protein of unknown function; upregulated in a cyr1 or ras1 null mutant; induced by nitric oxide
LCB4	3.8	0.0	Putative sphingosine kinase; Tac1p-regulated expression; rat catheter biofilm induced
PLB1	3.2	0.0	Phospholipase B; host cell penetration and virulence in mouse systemic infection
orf19.1473	3.1	0.0	2-hydroxyacid dehydrogenase domain-containing protein; Hap43-repressed gene; induced by alpha pheromone in SpiderM medium
BIO5	2.9	0.0	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method
orf19.2962	2.8	0.0	Protein of unknown function; Spider biofilm induced
RCH1	2.7	0.0	Plasma membrane protein; involved in regulation of cytosolic calcium homeostasis
orf19.3395	2.7	0.0	Predicted MFS membrane transporter, member of the drug:proton antiporter (12 spanner) (DHA1) family
ALS2	2.7	0.0	ALS family protein; role in adhesion, biofilm formation, germ tube induction
SAP9	2.6	0.0	Secreted aspartyl protease; roles in adhesion, cell surface integrity
orf19.996	2.4	0.0	Protein with a predicted leucine-rich repeat domain; possibly an essential gene, disruptants not obtained by UAU1 method
orf19.4612	2.4	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
DOT6	2.4	0.0	Protein with a predicted role in telomeric gene silencing and filamentation; repressed by high-level peroxide stress; Spider biofilm induced
ITS1	2.3	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58
BUL1	2.3	0.0	Protein similar but not orthologous to *S. cerevisiae* Bul1; a protein involved in selection of substrates for ubiquitination
orf19.6222.1	2.3	0.0	Ortholog of C. parapsilosis CDC317 : CPAR2_208910, *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_114047
BIO2	2.3	0.0	Putative biotin synthase; induced by high iron
CAN3	2.3	0.0	Predicted amino acid transmembrane transporter; transcript regulated by white-opaque switch; Hap43-repressed gene

orf19.5258	2.1	0.0	Protein of unknown function; induced by nitric oxide
orf19.6888	2.1	0.0	Zn(II)2Cys6 domain transcription factor; regulated by Mig1 and Tup1; rat catheter and Spider biofilm induced
NDT80	2.1	0.0	Ortholog of Ndt80; meiosis-specific transcription factor
orf19.7567	2.0	0.0	Protein of unknown function; induced by alpha pheromone in SpiderM medium
BIO3	2.0	0.0	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis; transcription regulated by biotin availability and Vhr1p
ITS2	2.0	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25
GDH3	2.0	0.0	NADP-glutamate dehydrogenase; Nrg1, Plc1 regulated; hypha, hypoxia, Efg1-repressed; Rim101-induced at pH 8; GlcNAc, ciclopirox, ketoconazole induced; exp and stationary phase protein; Spider biofilm repressed; rat catheter biofilm induced
XKS1	2.0	0.0	Putative xylulokinase; Hap43-repressed; induced by prostaglandins; rat catheter biofilm repressed
IFR2	1.9	0.0	Zinc-binding dehydrogenase; induced by benomyl, ciclopirox olamine, alpha pheromone, Hap43; regulated by oxidative stress via Cap1, osmotic stress via Hog1; protein present in exponential and stationary phase; rat catheter biofilm repressed
SIT1	1.9	0.0	Transporter of ferrichrome siderophores, not ferrioxamine B; required for human epithelial cell invasion in vitro, not for mouse systemic infection; regulated by iron, Sfu1, Rfg1, Tup1, Hap43; rat catheter and Spider biofilm induced
TPS3	1.9	0.0	Predicted trehalose-phosphate synthase regulatory subunit; regulated by Efg1; regulated by Tsa1, Tsa1B under H2O2 stress conditions; flow model biofilm induced (reports differ)
ATC1	1.9	0.0	Cell wall acid trehalase; catalyzes hydrolysis of the disaccharide trehalose; similar to *S. cerevisiae* vacuolar acid trehalase (Ath1p); Hap43p-repressed gene
orf19.1654	1.9	0.0	Predicted membrane protein; induced by prostaglandins
GPI14	1.9	0.0	Catalytic subunit of glycosylphosphatidylinositol-alpha 1,4 mannosyltransferase I, involved in GPI anchor biosynthesis; regulated by Tsa1p, Tsa1Bp under H2O2 stress conditions
ARE2	1.9	0.0	Acyl CoA:sterol acyltransferase; uses cholesterol and oleoyl-CoA substrates; protoberberine derivative drug inhibits enzyme activity; ketoconazole-induced; Hap43-repressed; flow model biofilm induced; Spider biofilm induced
RAD16	1.9	0.0	Ortholog of *S. cerevisiae* Rad16; a protein that recognizes and binds damaged DNA; flucytosine induced; rat catheter and Spider biofilm induced
ALS4	1.9	0.0	GPI-anchored adhesin; role in adhesion, germ tube induction; growth, temperature regulated; expressed during infection of human buccal epithelial cells; repressed by vaginal contact; biofilm induced; repressed during chlamydospore formation
PSF2	1.8	0.0	Ortholog(s) have role in DNA-dependent DNA replication, double-strand break repair via break-induced replication, mitotic DNA replication
DOT5	1.8	0.0	Putative nuclear thiol peroxidase; alkaline downregulated; sumoylation target; Spider and flow model biofilm induced
orf19.7278	1.8	0.0	Similar to a region of the Tca2 (pCal) retrotransposon, which is present in strain hOG1042 as 50 to 100 copies of a linear dsDNA; rat catheter biofilm repressed
orf19.33	1.8	0.0	Predicted ORF from Assembly 19; removed from Assembly 20; restored based on transcription data; similar to orf19.7550
orf19.2371	1.8	0.0	Putative Gag protein of retrotransposon Tca2; separated by a stop codon from Pol protein orf19.2372; likely translated as single polyprotein that includes Gag, reverse transcriptase, protease, and integrase; rat catheter biofilm repressed
GPX2	1.7	0.0	Similar to glutathione peroxidase; induced in high iron; alkaline induced by Rim101; induced by alpha factor or interaction with macrophage; regulated by Efg1; caspofungin repressed; Spider biofilm induced
orf19.2261	1.7	0.0	Ortholog(s) have RNA binding activity, role in mRNA splicing, via spliceosome and U2 snRNP, U2-type prespliceosome localization
orf19.787.1	1.7	0.0	Protein of unknown function; ORF added to Assembly 21 based on comparative genome analysis
orf19.5525	1.7	0.0	Putative oxidoreductase; protein levels affected by URA3 expression in CAI-4 strain background; Efg1, Efh1 regulated
GDB1	1.7	0.0	Putative glycogen debranching enzyme; expression is regulated upon white-opaque switch; regulated by Nrg1, Tup1; rat catheter biofilm repressed
UGA6	1.7	0.0	Putative GABA-specific permease; decreased transcription is observed upon benomyl treatment or in an azole-resistant strain that overexpresses MDR1
orf19.2372	1.7	0.0	Pol protein of retrotransposon Tca2; separated by a stop codon from Gag protein orf19.2371
GSY1	1.7	0.0	Glycogen synthase (UDP glucose/starch glucosyltransferase); transcript repressed by yeast-hyphal switch, Efg1-regulated
RDN58	1.7	0.0	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
UBA4	1.7	0.0	Putative ubiquitin activating protein; Hap43-repressed; induced by prostaglandins; clade-associated gene expression

BIO4	1.6	0.0	Putative dethiobiotin synthetase; transcript upregulated in clinical isolates from HIV+ patients with oral candidiasis; Hap43-repressed; GlcNAc-induced protein; Spider biofilm induced; biotin-dependent transcription regulated by Vhr1p
orf19.1434	1.6	0.0	Ortholog(s) have DNA polymerase binding, protein kinase activator activity, signaling adaptor activity
ALS9	1.6	0.0	ALS family cell-surface glycoprotein; expressed during infection of human epithelial cells; confers laminin adhesion to *S. cerevisiae*; highly variable; putative GPI-anchor; Hap43-repressed
URA1	1.6	0.0	Dihydroorotate dehydrogenase; de novo pyrimidine biosynthesis; regulated by yeast-hypha switch, Nrg1/Mig1/Tup1; flow model biofilm induced; rat catheter and Spider biofilm repressed
orf19.6852.1	1.6	0.0	Protein of unknown function; Spider biofilm induced
orf19.6809	1.6	0.0	Putative phosphomutase-like protein; protein present in exponential and stationary growth phase yeast; Hap43-repressed; Spider biofilm repressed
HRK1	1.6	0.0	Putative serine/threonine kinase; predicted role in cellular ion homeostasis; Spider biofilm repressed
FGR51	1.6	0.0	Protein lacking an ortholog in *S. cerevisiae*; transposon mutation affects filamentous growth; Hap43p-repressed gene
SEC2	1.6	0.0	Guanyl-nucleotide exchange factor for the small G-protein Sec4; delivery of post-Golgi secretory vesicles to sites of polarized growth; phosphorylation by Cdc28 needed for normal hyphal growth; Hap43-repressed; flow model biofilm induced
CDC19	1.5	0.0	Pyruvate kinase at yeast cell surface; Gcn4/Hog1/GlcNAc regulated; Hap43/polystyrene adherence induced; repressed by phagocytosis/farnesol; hyphal growth role; stationary phase enriched; flow model biofilm induced; Spider biofilm repressed
RDN25	1.5	0.0	25S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R; in some strains the gene may contain the self-splicing group I intron (LSU)
orf19.6559	1.5	0.0	RNA polymerase III transcription initiation factor complex (TFIIIC) subunit; growth phase regulated protein; downregulaated in stationary phase yeast cultures; Hap43-repressed; flow model biofilm induced; Spider biofilm repressed

Appendix 29: RNA-seq result of Zcf5-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf5-GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
ZCF5	5.9	0.0	Zn(II)2Cys6 transcription factor; colony morphology-related gene regulation by Ssn6
MLS1	2.9	0.0	Malate synthase; glyoxylate cycle enzyme; no mammalian homolog; regulated upon white-opaque switch; phagocytosis, strong oxidative stress induced; stationary phase enriched; flow model biofilm repressed; rat catheter, Spider biofilm induced
orf19.6222.1	2.5	0.0	Ortholog of C. parapsilosis CDC317 : CPAR2_208910, *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_114047, Debaryomyces hansenii CBS767 : DEHA2D14388g and Pichia stipitis Pignal : PICST_37629
HGT20	2.5	0.0	Putative glucose transporter of the major facilitator superfamily; the *C. albicans* glucose transporter family comprises 20 members; 12 probable membrane-spanning segments; regulated by Nrg1
GAP2	2.4	0.0	General broad specificity amino acid permease; ketoconazole, flucytosine repressed; Ssy1-dependent histidine induction; regulated by Nrg1, Tup1; colony morphology-related gene regulation by Ssn6; Spider and flow model biofilm induced
ALS2	2.3	0.0	ALS family protein; role in adhesion, biofilm formation, germ tube induction; expressed at infection of human buccal epithelial cells; putative GPI-anchor; induced by ketoconazole, low iron and at cell wall regeneration; regulated by Sfu1p
AMO1	2.3	0.0	Putative peroxisomal copper amine oxidase
BIO5	2.2	0.0	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method; Spider biofilm induced; transcription regulated by biotin and Vhr1p
ITS1	2.1	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58; in *S. cerevisiae* it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
GPX2	2.1	0.0	Similar to glutathione peroxidase; induced in high iron; alkaline induced by Rim101; induced by alpha factor or interaction with macrophage; regulated by Efg1; caspofungin repressed; Spider biofilm induced
SKN2	2.1	0.0	Protein with a potential role in beta-1,6 glucan biosynthesis; similarity to Kre6 and Skn1; possibly essential, disruptants not obtained by UAU1 method; Hap43-induced; flow model biofilm induced; rat catheter biofilm repressed
CAN3	2.0	0.0	Predicted amino acid transmembrane transporter; transcript regulated by white-opaque switch; Hap43-repressed gene
SNZ1	1.9	0.0	Stationary phase protein; vitamin B synthesis; induced byyeast-hypha switch, 3-AT or in azole-resistant strain overexpressing MDR1; soluble in hyphae; regulated by Gcn4, macrophage; Spider biofilm induced; rat catheter biofilm repressed

ALS9	1.9	0.0	ALS family cell-surface glycoprotein; expressed during infection of human epithelial cells; confers laminin adhesion to *S. cerevisiae*; highly variable; putative GPI-anchor; Hap43-repressed
GPD1	1.9	0.0	Glycerol-3-phosphate dehydrogenase; glycerol biosynthesis; regulated by Efg1; regulated by Tsa1, Tsa1B under H2O2 stress conditions; Sflow model and Spider biofilm induced
orf19.1590	1.9	0.0	Ortholog of *C. dubliniensis* CD36 : Cd36_18970, *C. parapsilosis* CDC317 : CPAR2_212800, *Debaryomyces hansenii* CBS767 : DEHA2F08690g and *Candida guilliermondii* ATCC 6260 : PGUG_01488
BIO3	1.9	0.0	Putative adenosylmethionine-8-amino-7-oxononanoate transaminase involved in biotin biosynthesis; transcription regulated by biotin availability and Vhr1p
ADE13	1.8	0.0	Adenylosuccinate lyase; enzyme of adenine biosynthesis; soluble protein in hyphae; not induced during GCN response, in contrast to the *S. cerevisiae* ortholog; repressed by nitric oxide
YHB4	1.7	0.0	Protein related to flavohemoglobins; not required for wild-type nitric oxide resistance; has predicted globin, FAD-binding, and NAD(P)-binding domains but lacks some conserved residues of flavohemoglobins; Hap43p-repressed gene
orf19.33	1.7	0.0	Predicted ORF from Assembly 19; removed from Assembly 20; restored based on transcription data; similar to orf19.7550
ALS4	1.7	0.0	GPI-anchored adhesin; role in adhesion, germ tube induction; growth, temperature regulated; expressed during infection of human buccal epithelial cells; repressed by vaginal contact; biofilm induced; repressed during chlamydospore formation
ITS2	1.7	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25; in *S. cerevisiae* it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
HRK1	1.7	0.0	Putative serine/threonine kinase; predicted role in cellular ion homeostasis; Spider biofilm repressed
SCS7	1.6	0.0	Putative ceramide hydroxylase; regulated by Nrg1; induced in high iron; fluconazole-induced; Hap43-repressed; Spider biofilm induced
orf19.1656	1.6	0.0	Protein with a predicted FYVE/PHD zinc finger domain; Hap43-repressed; Spider biofilm induced
GPI14	1.6	0.0	Catalytic subunit of glycosylphosphatidylinositol-alpha 1,4 mannosyltransferase I, involved in GPI anchor biosynthesis; regulated by Tsa1p, Tsa1Bp under H2O2 stress conditions
orf19.3810	1.6	0.0	Ortholog(s) have methylenetetrahydrofolate dehydrogenase (NAD+) activity, role in folic acid-containing compound biosynthetic process, one-carbon metabolic process, purine nucleobase biosynthetic process and cytosol localization
GCV2	1.5	0.0	Glycine decarboxylase P subunit; protein of glycine catabolism; repressed by Efg1; Hog1-induced; induced by Rim101 at acid pH; transcript induced in elevated CO2; stationary phase enriched protein
RDN58	1.5	0.0	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
ADE1	1.5	0.0	Phosphoribosylaminoimadazole succinocarboxamide synthetase, enzyme of adenine biosynthesis; not induced in GCN response, unlike the *S. cerevisiae* ortholog; fungal-specific (no human or murine homolog); levels decrease in stationary phase
LYS12	1.5	0.0	Homoisocitrate dehydrogenase; catalyzes 4th step in the alpha-aminoadipate pathway of lysine biosynthesis; clade-associated gene expression; protein level decreases in stationary phase cultures; Spider biofilm repressed
RDN25	1.5	0.0	25S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R; in some strains the gene may contain the self-splicing group I intron (LSU)
ADE5,7	1.5	0.0	Phosphoribosylamine-glycine ligase and phosphoribosylformylglycinamidine cyclo-ligase; interacts with Vps34p; required for hyphal growth and virulence; flucytosine induced; not induced in GCN response, in contrast to *S. cerevisiae* ortholog
GCV1	1.5	0.0	Putative T subunit of glycine decarboxylase; transcript negatively regulated by Sfu1; Spider biofilm repressed

Appendix 30: RNA-seq result of Zcf6 -GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf6 -GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.1691	6.6	0.0	Plasma-membrane-localized protein; filament induced; Hog1, ketoconazole, fluconazole and hypoxia-induced; regulated by Nrg1, Tup1, Upc2; induced by prostaglandins; flow model biofilm induced; rat catheter and Spider biofilm repressed
PLB1	6.0	0.0	Phospholipase B; host cell penetration and virulence in mouse systemic infection; Hog1-induced; signal sequence, N-glycosylation, and Tyr phosphorylation site; induced in fluconazole-resistant strains; rat catheter biofilm repressed
RNR22	5.7	0.0	Putative ribonucleoside diphosphate reductase; colony morphology-related gene regulation by Ssn6; transcript regulated by tyrosol and cell density; Hap43-repressed; Spider biofilm induced
ZCF6	5.4	0.0	Putative transcription factor with zinc cluster DNA-binding motif; involved in virulence
OPT7	4.6	0.0	Putative oligopeptide transporter; possibly transports GSH or related compounds
ATO6	4.6	0.0	Putative fungal-specific transmembrane protein
ADH5	4.3	0.0	Putative alcohol dehydrogenase; regulated by white-opaque switch; fluconazole-induced
PGA10	4.1	0.0	GPI anchored membrane protein; utilization of hemin and hemoglobin for Fe in host; Rim101 at ph8/hypoxia/ketoconazole/ciclopirox/hypha-induced; required for RPMI biofilm formation, Bcr1-induced in a/a biofilm; rat catheter biofilm repressed
orf19.4612	4.0	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
AQY1	3.9	0.0	Aquaporin water channel; osmotic shock resistance, WT freeze tolerance; virulent in mice; flucytosine repressed; flow model/RPMI/Spider/rat catheter biofilm induced; required for RPMI biofilm formation; Bcr1-induced in a/a RPMI biofilms
SOD6	3.9	0.0	Copper-containing superoxide dismutase; gene family includes SOD1, SOD4, SOD5, and SOD6
RSN1	3.8	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; induced during the mating process; Hap43-repressed
RHR2	3.7	0.0	Glycerol 3-phosphatase; roles in osmotic tolerance, glycerol accumulation in response to salt
STF2	3.7	0.0	Protein involved in ATP biosynthesis; repressed in hyphae; repressed by Efg1, Hap43
OSM1	3.6	0.0	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae; caspofungin repressed; stationary phase enriched protein; flow model biofilm induced; Spider biofilm repressed
CRP1	3.6	0.0	Copper transporter; CPx P1-type ATPase; mediates Cu resistance; similar to Menkes and Wilson disease proteins; copper-induced; Tbf1-activated; suppresses Cu sensitivity of *S. cerevisiae* cup1 mutant; flow model biofilm induced
FGR23	3.6	0.0	Protein of unknown function; repressed by a1/alpha2 in white-phase cells, a-specific, alpha factor-induced; Hap43-repressed; flow model biofilm induced; Tn mutation affects filamentous growth;
orf19.4450.1	3.6	0.0	Protein conserved among the CTG-clade; 2 adjacent upstream SRE-1 elements; highly up-regulated in cecum-grown cells in a Cph2-dependent manner; Hap43-repressed; rat catheter, Spider and flow model biofilm induced
IFE2	3.5	0.0	Putative alcohol dehydrogenase; yeast-enriched transcript; Efg1-regulated; induced by prostaglandins, Hog1, fluconazole; rat catheter biofilm induced
RME1	3.4	0.0	Zinc finger protein, controls asexual sporulation; white-specific transcript; upregulation correlates with clinical development of fluconazole resistance; Upc2-regulated in hypoxia; flow model biofilm induced; Spider biofil
RHD3	3.3	0.0	GPI-anchored yeast-associated cell wall protein; induced in high iron; clade-associated gene expression; not essential for cell wall integrity; fluconazole-repressed; flow model and Spider biofilm repressed
HEM13	3.3	0.0	Coproporphyrinogen III oxidase; antigenic; on yeast cell surface, not hyphae; iron-regulated expression; Hap43, macrophage-repressed; farnesol-induced; possibly essential; flow model biofilm induced; rat catheter, Spider biofilm repressed
QDR1	3.3	0.0	Putative antibiotic resistance transporter; regulated by white-opaque switch, Nrg1, Tup1; Hap43, caspofungin repressed; repressed during chlamydospore formation; flow model biofilm induced; Spider biofilm repressed
DAK2	3.3	0.0	Putative dihydroxyacetone kinase; repressed by yeast-hypha switch; fluconazole-induced; caspofungin repressed; protein enriched in stationary phase yeast cultures; flow model biofilm induced; rat catheter and Spider biofilm repressed
orf19.7027	3.3	0.0	Protein of unknown function; Spider biofilm induced
MNN14	3.2	0.0	Predicted alpha-1,3-mannosyltransferase activity with a role in protein glycosylation; Hap43-repressed; Spider biofilm induced
CDR4	3.2	0.0	Putative ABC transporter superfamily; fluconazole, Sfu1, Hog1, core stress response induced; caspofungin repressed; fluconazole resistance not affected by mutation or correlated with expression; rat catheter and flow model biofilm induced
orf19.2371	3.1	0.0	Putative Gag protein of retrotransposon Tca2; separated by a stop codon from Pol protein orf19.2372

POL93	3.1	0.0	Predicted ORF in retrotransposon Tca8 with similarity to the Pol region of retrotransposons encoding reverse transcriptase, protease and integrase; downregulated in response to ciclopirox olamine; F-12/CO2 early biofilm induced
LAP3	3.1	0.0	Putative aminopeptidase; positively regulated by Sfu1; clade-associated gene expression
orf19.3021	3.0	0.0	Putative protein of unknown function; Hap43-repressed gene; Spider biofilm induced
orf19.411	3.0	0.0	Protein similar to GTPase regulators; induced in low iron; transcript activated by Mnl1 under weak acid stress; Hap43-, Sfu1- and Sef1-regulated; flow model biofilm induced, Spider biofilm induced
OPT3	3.0	0.0	Oligopeptide transporter; transcript induced by macrophage phagocytosis, BSA or peptides
TPO3	2.9	0.0	Putative polyamine transporter; MFS-MDR family; induced by Sfu1, regulated upon white-opaque
orf19.6690	2.8	0.0	Protein of unknown function; Hap43-repressed gene
XYL2	2.8	0.0	D-xylulose reductase; immunogenic in mice; soluble protein in hyphae; induced by caspofungin, fluconazole, Hog1 and during cell wall regeneration; Mnl1-induced in weak acid stress
GPM2	2.8	0.0	Putative phosphoglycerate mutase; repressed in hyphae; macrophage/pseudohyphal-repressed; induced by high levels of peroxide stress, farnesol; flow model biofilm induced
OPT1	2.8	0.0	Oligopeptide transporter; transports 3-to-5-residue peptides; alleles are distinct, one has intron; suppresses *S. cerevisiae* ptr2-2 mutant defects; induced by BSA or peptides; Stp3p, Hog1p regulated
PFK1	2.8	0.0	Phosphofructokinase alpha subinit; activated by fructose 2,6-bisphosphate, AMP, ATP inhibited; activity reduced on hyphal induction
RBE1	2.8	0.0	Pry family cell wall protein; Rim101, Efg1, Ssn6, alkaline repressed; O-glycosylation; no GPI anchor predicted; ketoconazol induced; regulated by Sef1, Sfu1, Hap4; flow model biofilm induced; rat catheter and Spider biofilm repressed
GCY1	2.7	0.0	Aldo/keto reductase; mutation confers hypersensitivity to toxic ergosterol analog; farnesol-repressed; stationary phase enriched protein; flow model biofilm induced; Spider biofilm repressed
HGT20	2.7	0.0	Putative glucose transporter of the major facilitator superfamily; the *C. albicans* glucose transporter family comprises 20 members; 12 probable membrane-spanning segments; regulated by Nrg1
PHO113	2.6	0.0	Putative constitutive acid phosphatase; Rim101-repressed; DTT-extractable; N-glycosylated; possibly an essential gene, disruptants not obtained by UAU1 method
orf19.2372	2.6	0.0	Pol protein of retrotransposon Tca2; separated by a stop codon from Gag protein orf19.2371; likely translated as single polyprotein with Gag, reverse transcriptase, protease, and integrase; rat catheter biofilm repressed
SAP10	2.6	0.0	Secreted aspartyl protease; roles in adhesion, virulence (RHE model), cell surface integrity; distinct specificity from Sap9; at cell membrane and wall; GPI-anchored; induced in low iron; Tbf1-activated; Spider biofilm induced
ALS2	2.6	0.0	ALS family protein; role in adhesion, biofilm formation, germ tube induction; expressed at infection of human buccal epithelial cells; putative GPI-anchor; induced by ketoconazole, low iron and at cell wall regeneration; regulated by Sfu1p
orf19.5169	2.6	0.0	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor, hydrolase activity
IFE1	2.5	0.0	Putative medium-chain alcohol dehydrogenase; rat catheter and Spider biofilm repressed
SKN2	2.5	0.0	Protein with a potential role in beta-1,6 glucan biosynthesis; similarity to Kre6 and Skn1; possibly essential, disruptants not obtained by UAU1 method; Hap43-induced; flow model biofilm induced; rat catheter biofilm repressed
NUP	2.5	0.0	Nucleoside permease; adenosine and guanosine are substrates, whereas cytidine, adenine, guanine, uridine, uracil are not; similar to a nucleoside permease of S. pombe; possibly processed by Kex2p
orf19.4376	2.4	0.0	Protein of unknown function; Spider biofilm induced
ZRT2	2.3	0.0	Zinc transporter, essential for zinc uptake and acidic conditions tolerance; transcript induced by amphotericin B, interaction with macrophages; induced in oralpharyngeal candidiasis; Spider biofilm induced
CAN2	2.3	0.0	Basic amino acid permease; arginine metabolism; regulated by Nrg1/Tup1; caspofungin, flucytosine induced; colony morphology-related regulation by Ssn6; Hap43-repressed; rat catheter and Spider biofilm induced; promoter bound by Efg1
snR42a	2.3	0.0	H/ACA box small nucleolar RNA (snoRNA)
FCY2	2.3	0.0	Purine-cytosine permease of pyrimidine salvage; mutation associated with resistance to flucytosine in clinical isolates; transposon mutation affects filamentation; farnesol-upregulated in biofilm
MNN22	2.3	0.0	Alpha-1,2-mannosyltransferase; required for normal cell wall mannan; regulated by Tsa1, Tsa1B at 37 deg; repressed in core stress response; NO, Hog1 induced; confers sensitivity to cell wall perturbing agents; Spider biofilm repressed
ITS1	2.3	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58; in *S. cerevisiae* it is transcribed as part of the 35S precursor that is processed during rRNA maturation to yield 18S, 5.8S, and 25S rRNA species
FBA1	2.3	0.0	Fructose-bisphosphate aldolase; glycolytic enzyme; antigenic in murine/human infection
YHB1	2.3	0.0	Nitric oxide dioxygenase; acts in nitric oxide scavenging/detoxification; role in virulence in mouse
ITS2	2.3	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25

orf19.6983	2.2	0.0	Protein of unknown function; Hap43-repressed gene; repressed by nitric oxide; Spider biofilm induced
ZCF27	2.2	0.0	Putative Zn(II)2Cys6 transcription factor
orf19.6480	2.2	0.0	Ortholog(s) have role in UDP-glucose transmembrane transport
PFK2	2.1	0.0	Phosphofructokinase beta subunit; fructose 2,6-bisphosphate, AMP activated
TPI1	2.1	0.0	Triose-phosphate isomerase; antigenic in mouse/human; mutation affects filamentation
orf19.36.1	2.1	0.0	Ortholog of C. parapsilosis CDC317 : CPAR2_104640, *Candida orthopsilosis* Co 90-125 : CORT_0B05695 and *Candida orthopsilosis*
PGK1	2.1	0.0	Phosphoglycerate kinase; localizes to cell wall and cytoplasm; antigenic in murine/human infection; flow model biofilm, Hog1-, Hap43-, GCN-induced; repressed upon phagocytosis; repressed in Spider biofilms by Bcr1, Ndt80, Rob1, Brg1
orf19.1562	2.1	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; repressed by alpha pheromone in SpiderM medium
ATC1	2.1	0.0	Cell wall acid trehalase; catalyzes hydrolysis of the disaccharide trehalose; similar to *S. cerevisiae* vacuolar acid trehalase (Ath1p); Hap43p-repressed gene
orf19.7278	2.1	0.0	Similar to a region of the Tca2 (pCal) retrotransposon, which is present in strain hOG1042 as 50 to 100 copies of a linear dsDNA; rat catheter biofilm repressed
orf19.302	2.1	0.0	Ortholog(s) have role in endoplasmic reticulum to Golgi vesicle-mediated transport and TRAPPI protein complex, TRAPPII protein complex, TRAPPIII protein complex localization
CDC19	2.0	0.0	Pyruvate kinase at yeast cell surface; Gcn4/Hog1/GlcNAc regulated; Hap43/polystyrene adherence induced; repressed by phagocytosis/farnesol; hyphal growth role
PHO15	2.0	0.0	HAD-family 2-phosphoglycolate phosphatase, likely involved in a metabolic repair system, not in protein dephosphorylation; involved in regulation of white-opaque switch
MEP1	2.0	0.0	Ammonium permease; Mep1 more efficient permease than Mep2, Mep2 has additional regulatory role; 11 predicted transmembrane regions; low mRNA abundance
PRD1	2.0	0.0	Putative proteinase; transcript regulated by Nrg1, Mig1, and Tup1; Hogp-induced; stationary phase enriched protein; Hap43-repressed; rat catheter biofilm repressed
PDC11	2.0	0.0	Pyruvate decarboxylase; antigenic; on hyphal not yeast cell surface; Hap43, Gcn4, Efg1, Efh1, Hsf1 regulated; fluconazole, farnesol induced
orf19.3053	2.0	0.0	Protein of unknown function; present in exponential and stationary phase yeast; identified in extracts from biofilm and planktonic cells; flow model biofilm induced gene; GlcNAc-induced protein
BUB3	2.0	0.0	Protein similar to *S. cerevisiae* Bub3; a kinetochore checkpoint component; induced by hydroxyurea treatment; flow model biofilm induced; Spider biofilm induced
orf19.993	2.0	0.0	Protein of unknown function; rat catheter biofilm repressed
PLB4.5	2.0	0.0	Phospholipase B; Hog1-induced; regulated by Ssn6; putative GPI-anchor; repressed during cell wall regeneration; clade-associated gene expression; Hap43-induced; rat catheter and Spider biofilm repressed
ERG1	1.9	0.0	Squalene epoxidase, epoxidation of squalene to 2,3(S)-oxidosqualene; ergosterol biosynthesis; allylamine antifungal drug target; NADH reducing cofactor but *S. cerevisiae* Erg1 uses NADPH; flow model biofilm induced; Spider biofilm repressed
ALS4	1.9	0.0	GPI-anchored adhesin; role in adhesion, germ tube induction; growth, temperature regulated; expressed during infection of human buccal epithelial cells; repressed by vaginal contact; biofilm induced; repressed during chlamydospore formation
GDB1	1.9	0.0	Putative glycogen debranching enzyme; expression is regulated upon white-opaque switch; regulated by Nrg1, Tup1; rat catheter biofilm repressed
CAN1	1.9	0.0	Basic amino acid permease; complements lysine transport mutation; 10 predicted transmembrane regions, 3 predicted N-glycosylation sites; phagocytosis by macrophages induces transcript; rat catheter, Spider and flow model biofilm induced
MAF1	1.9	0.0	Putative negative regulator of RNA polymerase III; decreased expression in hyphae vs yeast cells; caspofungin repressed; Spider biofilm repressed
TPS3	1.9	0.0	Predicted trehalose-phosphate synthase regulatory subunit; regulated by Efg1; regulated by Tsa1, Tsa1B under H2O2 stress conditions; flow model biofilm induced (reports differ)
GPM1	1.9	0.0	Phosphoglycerate mutase; surface protein that binds host complement Factor H and FHL-1; antigenic; fluconazole, or amino acid starvation (3-AT) induced, farnesol-repressed; Hap43, flow model biofilm induced; Spider biofilm repressed
ECM21	1.9	0.0	Predicted regulator of endcytosis of plasma membrane proteins; fluconazole induced, alkaline induced by Rim101; repressed by caspofungin and in azole-resistant strain overexpressing MDR1; flow model, rat catheter and Spider biofilm induced
RDN58	1.8	0.0	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
URA1	1.8	0.0	Dihydroorotate dehydrogenase; de novo pyrimidine biosynthesis; regulated by yeast-hypha switch, Nrg1/Mig1/Tup1; flow model biofilm induced; rat catheter and Spider biofilm repressed
orf19.7445	1.8	0.0	Ortholog of S.c. Vid24; a peripheral membrane protein located at Vid (vacuole import and degradation) vesicles; regulated by Sef1, Sfu1, and Hap43; Spider biofilm induced
orf19.787.1	1.8	0.0	Protein of unknown function; ORF added to Assembly 21 based on comparative genome analysis; protein detected by mass spec in stationary phase cultures

UPC2	1.8	0.0	Zn2-Cys6 transcript factor; regulator of ergosterol biosynthetic genes and sterol uptake; binds ERG2 promoter; induced by ergosterol depletion, by azoles, anaerobicity; macrophage/pseudohyphal-repressed; flow model biofilm induced
PGI1	1.7	0.0	Glucose-6-phosphate isomerase; enzyme of glycolysis; antigenic; Efg1-regulated; induced upon adherence to polystyrene; repressed by phagocytosis, human neutrophils; flow model biofilm induced; rat catheter and Spider biofilm repressed
CUP1	1.7	0.0	Metallothionein; involved in copper resistance; copper induced; Spider biofilm induced; flow model biofilm repressed
ADE13	1.7	0.0	Adenylosuccinate lyase; enzyme of adenine biosynthesis; soluble protein in hyphae; not induced during GCN response, in contrast to the *S. cerevisiae* ortholog; repressed by nitric oxide
orf19.2350	1.7	0.0	Protein similar to *S. cerevisiae* Yor378w; MFS family transporter; transposon mutation affects filamentous growth; null mutants are viable; fungal-specific (no human or murine homolog)
RDN25	1.7	0.0	25S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R; in some strains the gene may contain the self-splicing group I intron (LSU)
orf19.254	1.7	0.0	Protein of unknown function; Hog1p-repressed; Spider biofilm induced
ZCF1	1.7	0.0	Zn(II)2Cys6 transcription factor; transcript regulated during hypha formation; 5'-UTR intron; mutants show decreased colonization of mouse kidneys; flow model biofilm induced; Spider biofilm induced
TDH3	1.7	0.0	NAD-linked glyceraldehyde-3-phosphate dehydrogenase; binds fibronectin, laminin; at cell surface; antigenic in infection; farnesol-repressed; stationary phase-enriched; GlcNAc-induced; flow model biofilm induced; Spider biofilm repressed
SYG1	1.7	0.0	Ortholog(s) have role in signal transduction and plasma membrane localization
AGP2	1.7	0.0	Amino acid permease; hyphal repressed; white-opaque switch regulated; induced in core caspofungin response, during cell wall regeneration, by flucytosine; regulated by Sef1, Sfu1, and Hap43; rat catheter and Spider biofilm induced
ENO1	1.6	0.0	Enolase, involved in glycolysis and gluconeogenesis; also has transglutaminase activity involved in assembly of cell wall polysaccharides; major cell-surface antigen; binds host plasmin/plasminogen; immunoprotective; may be essential
orf19.7196	1.6	0.0	Putative vacuolar protease; upregulated in the presence of human neutrophils; Spider biofilm induced
APE2	1.6	0.0	Neutral arginine, alanine, leucine specific metallo-aminopeptidase; purified from cell wall/intracellular fractions; protein repressed during mating; Hog1, farnesol-induced; may be essential (UAU1 method); rat catheter biofilm repressed
ERG3	1.6	0.0	C-5 sterol desaturase; introduces C-5(6) double bond into episterol; some clinical isolates show increased azole resistance and defects in hyphal growth and virulence; Efg1p-repressed; fluconazole-induced
CMK1	1.6	0.0	Putative calcium/calmodulin-dependent protein kinase II; expression regulated upon white-opaque switching; biochemically purified Ca2+/CaM-dependent kinase is soluble, cytosolic, monomeric, and serine-autophosphorylated; Hap43p-repressed
RIB3	1.6	0.0	3,4-Dihydroxy-2-butanone 4-phosphate synthase; homodimeric enzyme of riboflavin biosynthesis; converts ribulose 5-phosphate to L-3,4-dihydroxy-2-butanone 4-phosphate; transcription regulated on yeast-hyphal switch, macrophage interaction
orf19.7459	1.6	0.0	Putative mitochondrial protein with a predicted role in respiratory growth; fluconazole-induced; ketoconazole-repressed; mutants display a strong defect in flow model biofilm formation; Spider biofilm induced
MUQ1	1.6	0.0	Putative choline phosphate cytidylyltransferase/phosphoethanolamine cytidylyltransferase; repressed in hyphae compared vs yeast; Hap43-repressed; flow model biofilm induced; Spider biofilm repressed
orf19.3712	1.6	0.0	Protein of unknown function; induced by Mnl1 under weak acid stress; flow model biofilm induced; Spider biofilm induced
orf19.5525	1.6	0.0	Putative oxidoreductase; protein levels affected by URA3 expression in CAI-4 strain background; Efg1, Efh1 regulated; Rgt1-repressed; protein present in exponential and stationary growth phase yeast; rat catheter biofilm repressed
ERG251	1.5	0.0	C-4 sterol methyl oxidase; role in ergosterol biosynthesis; Hap43-induced; ketoconazole-induced; amphotericin B, caspofungin repressed
GPD1	1.5	0.0	Glycerol-3-phosphate dehydrogenase; glycerol biosynthesis; regulated by Efg1; regulated by Tsa1, Tsa1B under H2O2 stress conditions; Sflow model and Spider biofilm induced
DES1	1.5	0.0	Putative delta-4 sphingolipid desaturase; planktonic growth-induced gene
PIR1	1.5	0.0	1,3-beta-glucan-linked cell wall protein; N-mannosylated, O-glycosylated by Pmt1; cell wall defect in het mutant; Hog1/fluconazole/hypoxia induced; iron/Efg1/Plc1/temp regulated; flow model biofilm induced; hyphal, Spider biofilm repressed

Appendix 31: RNA-seq result of Zcf8-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf8 -GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
DAL52	11.2	0.0	Putative allantoate permease; mutant is viable; similar but not orthologous to *S. cerevisiae* Dal5
ZCF8	5.2	0.0	Predicted Zn(II)2Cys6 transcription factor; repressed by fluphenazine treatment; required for yeast cell adherence to silicone substrate
orf19.1308	3.4	0.0	Predicted membrane transporter, member of the drug:proton antiporter (14 spanner) (DHA2) family, major facilitator superfamily (MFS)
FRP3	3.2	0.0	Putative ammonium transporter; upregulated in the presence of human neutrophils
orf19.6444	3.0	0.0	Ortholog(s) have GTPase activator activity
orf19.3139	2.9	0.0	Putative NADP-dependent oxidoreductase; Hap43-repressed; induced by benomyl treatment
orf19.6888	2.7	0.0	Zn(II)2Cys6 domain transcription factor; regulated by Mig1 and Tup1; rat catheter and Spider biofilm induced
YOR1	2.7	0.0	Protein similar to *S. cerevisiae* Yor1; ABC-type plasma membrane transporter involved in resistance to aureobasidin A
orf19.4612	2.7	0.0	Protein with a dienelactone hydrolase domain; Hap43-repressed gene
orf19.1287	2.7	0.0	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
MNN13	2.5	0.0	Predicted alpha-1,3-mannosyltransferase activity with a role in protein glycosylation; regulated by Sef1p-, Sfu1p-, and Hap43p
orf19.2686	2.5	0.0	Ortholog(s) have carboxypeptidase activity, role in nitrogen compound metabolic process, proteolysis involved in cellular protein catabolic process and fungal-type vacuole lumen localization
orf19.4122	2.5	0.0	Ortholog(s) have acyl-CoA hydrolase activity, role in fatty acid beta-oxidation, fatty acid oxidation and peroxisome localization
HMX1	2.5	0.0	Heme oxygenase; utilization of hemin iron; transcript induced by heat, low iron, or hemin; repressed by Efg1; induced by low iron; upregulated by Rim101 at pH 8; Hap43-induced; Spider and flow model biofilm induced
HGT20	2.5	0.0	Putative glucose transporter of the major facilitator superfamily; the *C. albicans* glucose transporter family comprises 20 members; 12 probable membrane-spanning segments; regulated by Nrg1
orf19.4128	2.5	0.0	Ortholog of C. dubliniensis CD36 : Cd36_19390, C. parapsilosis CDC317 : CPAR2_209600, C. auris B8441 : B9J08_004606 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_134010
GDT1	2.4	0.0	Golgi Ca2+/H+ exchanger, plays a compensatory role for the calcium pump Pmr1p in regulation of calcium homeostasis
YMX6	2.3	0.0	Putative NADH dehydrogenase; macrophage-downregulated gene; induced by nitric oxide; rat catheter biofilm induced
orf19.7648	2.3	0.0	Has domain(s) with predicted antiporter activity, xenobiotic transmembrane transporter activity, role in drug transmembrane transport and membrane localization
SMP3	2.3	0.0	Mannosyltransferase of glycosylphosphatidylinositol (GPI) biosynthesis; catalyzes mannosylation of Man3-GPI precursor; essential for viability; 8-9 transmembrane regions predicted; has HQEXRF motif; functional homolog of *S. cerevisiae* Smp3p
FAD3	2.2	0.0	Omega-3 fatty acid desaturase; production of alpha-linolenic acid, a major component of membranes; caspofungin induced; Plc1-regulated; colony morphology-related gene regulation by Ssn6; Spider biofilm induced, flow model biofilm repressed
orf19.6984	2.2	0.0	Ortholog of C. dubliniensis CD36 : Cd36_85460, C. parapsilosis CDC317 : CPAR2_405760, C. auris B8441 : B9J08_000903 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_116052
orf19.1309	2.2	0.0	Has domain(s) with predicted role in RNA catabolic process and ribonuclease H2 complex localization
BIO5	2.2	0.0	Putative transporter; Hap43, flucytosine repressed; possibly essential, disruptants not obtained by UAU1 method; Spider biofilm induced; transcription regulated by biotin and Vhr1p
orf19.6976	2.1	0.0	Predicted MFS membrane transporter; member of the proton coupled folate transporter/heme carrier protein family; virulence-group-correlated expression; Spider biofilm induced
REX3	2.1	0.0	Ortholog(s) have 3'-5'-exoribonuclease activity
orf19.4940	2.0	0.0	Putative histidine permease; fungal-specific (no human or murine homolog); Hap43p-induced gene
SEO1	2.0	0.0	Protein with similarity to permeases; Sfu1-repressed; flucytosine induced; induced by Mnl1 under weak acid stress; flow model biofilm repressed
DBP7	1.9	0.0	Putative ATP-dependent DEAD-box RNA helicase; Hap43-induced; rat catheter biofilm induced
orf19.177	1.9	0.0	Has domain(s) with predicted phosphatidylinositol binding activity and role in cell communication
orf19.5575	1.8	0.0	Putative peripheral peroxisomal membrane peroxin; required for regulating peroxisome size and maintenance; Spider biofilm induced
orf19.4370	1.8	0.0	Protein of unknown function; induced by nitric oxide; oxidative stress-induced via Cap1; fungal-specific (no human or murine homolog)
OPT8	1.8	0.0	Oligopeptide transporter; similar to Opt1 and to *S. cerevisiae* Ygl114wp, but not other OPTs; induced by nitric oxide, amphotericin B; expression of OPT6, 7, 8 does not complement mutants lacking Opt1, Opt2, and Opt3; Spider biofilm induced

MET14	1.8	0.0	Putative adenylylsulfate kinase; predicted role in sulfur metabolism; possibly adherence-induced; protein present in exponential and stationary growth phase yeast; F-12/CO2 biofilm induced
ADR1	1.8	0.0	C2H2 transcription factor; ortholog of *S. cerevisiae* Adr1 but mutant phenotype suggests a different set of target genes; transposon mutation affects filamentous growth; Spider biofilm induced
ECM38	1.7	0.0	Putative gamma-glutamyltransferase; alkaline upregulated; Spider biofilm induced; possibly an essential gene, disruptants not obtained by UAU1 method
CYS3	1.7	0.0	Cystathionine gamma-lyase; induced by alkaline, amphotericin B, cadmium stress, oxidative stress via Cap1; possibly adherence-induced; Hog1 regulated; reduced levels in stationary phase yeast cells; Spider and flow model biofilm induced
YIM1	1.7	0.0	Protein similar to protease of mitochondrial inner membrane; increased transcription is observed upon benomyl treatment; macrophage-downregulated gene
orf19.4609	1.7	0.0	Putative dienelactone hydrolase; protein abundance is affected by URA3 expression in the CAI-4 strain background; protein present in exponential and stationary growth phase yeast cultures; rat catheter biofilm repressed
orf19.850	1.7	0.0	Ortholog(s) have protein-N-terminal asparagine amidohydrolase activity, protein-N-terminal glutamine amidohydrolase activity and role in N-terminal protein amino acid modification, protein catabolic process
UTP22	1.7	0.0	Putative U3 snoRNP protein; Ssr1-induced; repressed by prostaglandins; heterozygous null mutant is resistant to parnafungin
orf19.6477	1.7	0.0	Ortholog(s) have tRNA (guanine-N7-)-methyltransferase activity, role in tRNA (guanine-N7)-methylation and cytosol, nucleus, tRNA (m7G46) methyltransferase complex localization
ECM331	1.7	0.0	GPI-anchored protein; mainly at plasma membrane, also at cell wall; Hap43, caspofungin-induced; Plc1-regulated; Hog1, Rim101-repressed; colony morphology-related regulated by Ssn6; induced by ketoconazole and hypoxia
PHA2	1.7	0.0	Putative prephenate dehydratase; Hap43p-repressed gene; expression downregulated in an ssr1 null mutant
RLM1	1.7	0.0	Transcription factor required for wild-type resistance to cell wall perturbation caused by caspofungin treatment; regulates caspofungin-induced transcription of SKO1
MXR1	1.7	0.0	Putative methionine sulfoxide reductase; Plc1-regulated; induced by human neutrophils, flucytosine; macrophage regulated (gene induced, protein decreased); possibly adherence-induced; Spider biofilm induced
orf19.5431	1.7	0.0	Protein of unknown function; Hap43-repressed; Spider biofilm induced
ATO2	1.6	0.0	Putative fungal-specific transmembrane protein; fluconazole repressed, Hap43-repressed; flow model biofilm induced; Spider biofilm induced
orf19.3869	1.6	0.0	Protein of unknown function; regulated by Tsa1, Tsa1B in minimal media at 37 degrees C; shows colony morphology-related gene regulation by Ssn6; Spider biofilm induced
orf19.3222	1.6	0.0	Predicted vacuolar protein; rat catheter biofilm repressed; flow model biofilm repressed
CTP1	1.5	0.0	Putative citrate transport protein; flucytosine induced; amphotericin B repressed, caspofungin repressed; Hap43p-induced gene

Appendix 32: RNA-seq result of Zcf9-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Zcf9 -GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
EFH1	21.2	0.0	APSES transcription factor; homodimer; minor role in transcriptional regulation vs Efg1; regulates filamentous growth, phenotypic switch
HGT1	9.2	0.0	High-affinity MFS glucose transporter; induced by progesterone, chloramphenicol, benomyl; likely essential for growth
AMO1	9.2	0.0	Putative peroxisomal copper amine oxidase
ZCF9	5.6	0.0	Putative Zn(II)2Cys6 transcription factor; hypersensitive to toxic ergosterol analog ECC69 and/or ECC1384
RBT5	5.2	0.0	GPI-linked cell wall protein; hemoglobin utilization; Rfg1, Rim101, Tbf1, Fe regulated; Sfu1, Hog1, Tup1, serum, alkaline pH, antifungal drugs, geldamycin repressed; Hap43 induced; required for RPMI biofilms; Spider biofilm induced
orf19.6596	4.2	0.0	Putative esterase; possibly transcriptionally regulated by Tac1; induced by Mnl1 under weak acid stress
orf19.7077	3.9	0.0	Putative ferric reductase; induced by Mac1 under copper starvation; Plc1-regulated; Rim101-repressed
orf19.6306	3.5	0.0	Trimethylaminobutyraldehyde dehydrogenase, the third enzyme of the carnitine biosynthesis pathway
orf19.6888	3.3	0.0	Zn(II)2Cys6 domain transcription factor; regulated by Mig1 and Tup1; rat catheter and Spider biofilm induced
FDH3	3.1	0.0	Glutathione-dependent formaldehyde dehydrogenase; glycine catabolism; repressed by Efg1 in yeast, not hyphal growth conditions
AMO2	2.9	0.0	Protein similar to A. niger predicted peroxisomal copper amino oxidase; mutation confers hypersensitivity to toxic ergosterol analog

CBR1	2.7	0.0	Putative cytochrome B5 reductase; plasma membrane-localized
CIS2	2.6	0.0	Putative role in regulation of biogenesis of the cell wall; upregulated in biofilm; Gcn4p-regulated
orf19.7098	2.5	0.0	Putative protein of unknown function; Hap43-repressed; repressed by nitric oxide; Spider biofilm induced
orf19.5517	2.4	0.0	Similar to alcohol dehydrogenases; induced by benomyl treatment, nitric oxide; induced in core stress response
DAO2	2.4	0.0	Putative D-amino acid oxidase; rat catheter biofilm induced
GIT2	2.2	0.0	Putative glycerophosphoinositol permease; fungal-specific; repressed by alpha pheromone in SpiderM medium
GRP1	2.0	0.0	Protein similar to dihydroflavonol-4-reductases
CTR1	2.0	0.0	Copper transporter; transcribed in low copper; induced Mac1, Tye7, macrophage interaction, alkaline pH via Rim101
CFL1	1.9	0.0	Protein similar to ferric reductase Fre10p; possible functional homolog of *S. cerevisiae* Fre1p (reports differ)
CYB5	1.8	0.0	Cytochrome b(5); ortholog of *S. cerevisiae* Cyb5; induced in high iron; fluconazole-induced; shows colony morphology-related gene regulation by Ssn6; mutants are viable
OPT8	1.7	0.0	Oligopeptide transporter; similar to Opt1 and to *S. cerevisiae* Ygl114wp, but not other OPTs; induced by nitric oxide, amphotericin B
orf19.6305	1.7	0.0	Hydroxytrimethyllysine aldolase, the second enzyme in the carnitine biosynthesis pathway; rat catheter biofilm repressed
PGA45	1.6	0.0	Putative GPI-anchored cell wall protein; repressed in core caspofungin response; Hog1-induced; regulated by Ssn6

Appendix 33: RNA-seq result of Hal9-GOF. Differentially expressed Up-regulated genes in *Candida albicans* Hal9 -GOF, RNAs must show log2 fold ≥ 1.5 in abundance with *P*-values <0.001.

Gene	log2FC	Adjusted P value	Description
orf19.3210	19.2	0.0	Predicted protein of unknown function; Plc1-regulated
orf19.6919	15.8	0.0	Ortholog of C. dubliniensis CD36 : Cd36_71210, C. parapsilosis CDC317 : CPAR2_702710, C. auris B8441 : B9J08_002141 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_116256
orf19.2310	13.0	0.0	Predicted single-stranded nucleic acid binding protein; flow model biofilm induced
OPT9	9.9	0.0	Probable pseudogene similar to fragments of OPT1 oligopeptide transporter gene
orf19.4476	6.4	0.0	Protein with a NADP-dependent oxidoreductase domain; transcript induced by ketoconazole; rat catheter and Spider biofilm induced
CSH1	5.9	0.0	Aldo-keto reductase; role in fibronectin adhesion, cell surface hydrophobicity; regulated by temperature, growth phase, benomyl, macrophage interaction
HAL9	5.6	0.0	Putative Zn(II)2Cys6 transcription factor; gene in zinc cluster region of Chr. 5
orf19.2583.2	5.5	0.0	Pseudogene; formerly an ORF Predicted by Annotation Working Group
orf19.4459	5.5	0.0	Predicted heme-binding stress-related protein; Tn mutation affects filamentous growth
orf19.3088	5.3	0.0	bZIP transcription factor; possibly transcriptionally regulated upon hyphal formation; Hap43; F-12/CO2 early biofilm induced; Spider biofilm induced
PTH2	5.0	0.0	Putative cAMP-independent regulatory protein; constitutive expression independent of MTL or white-opaque status; Spider biofilm induced
FET99	4.8	0.0	Multicopper oxidase family protein; similar to *S. cerevisiae* Fet3; does not complement *S. cerevisiae* fet3 mutant growth under low-iron; iron-repressed; regulated by Tup1, Rim101
AOX2	4.8	0.0	Alternative oxidase; cyanide-resistant respiration; induced by antimycin A, oxidants; growth; Hap43, chlamydospore formation repressed; rat catheter, Spider biofilm induced
GST2	4.7	0.0	Glutathione S transferase; induced by benomyl and in populations of cells exposed to fluconazole over multiple generations; regulated by Nrg1, Tup1; induced by nitric oxide
OPT1	4.7	0.0	Oligopeptide transporter; transports 3-to-5-residue peptides; alleles are distinct, one has intron
orf19.5785	4.1	0.0	Protein of unknown function; upregulated in a cyr1 or ras1 null mutant; induced by nitric oxide
orf19.344	4.1	0.0	Protein of unknown function; upregulated by fluphenazine treatment or in an azole-resistant strain that overexpresses CDR1 and CDR2; transcript possibly regulated by Tac1
WOR3	4.1	0.0	Transcription factor; modulator of white-opaque switch; induced in opaque cells; promoter bound by Wor1; overexpression at 25 degr shifts cells to opaque state
PGA13	3.9	0.0	GPI-anchored cell wall protein involved in cell wall synthesis; required for normal cell surface properties; induced in oralpharyngeal candidasis; Spider biofilm induced
snR76	3.9	0.0	C/D box small nucleolar RNA (snoRNA)
MRF1	3.7	0.0	Putative mitochondrial respiratory protein; induced by farnesol, benomyl, nitric oxide, core stress response; oxidative stress-induced via Cap1; stationary-phase enriched protein
orf19.5020	3.6	0.0	Protein of unknown function; Hap43-induced; Spider biofilm induced

orf19.3483	3.6	0.0	Putative phosphatidyl glycerol phospholipase C; Plc1-regulated; flow model biofilm induced; Spider biofilm induced
BMT4	3.4	0.0	Beta-mannosyltransferase; for elongation of beta-mannose chains on the acid-labile fraction of cell wall phosphopeptidomannan; 9-gene family member
orf19.6079	3.3	0.0	Predicted ORF in retrotransposon Tca8 with similarity to the Gag region encoding nucleocapsid-like protein; repressed by ciclopirox olamine; filament induced
PST3	3.3	0.0	Flavodoxin-like protein involved in oxidative stress protection and virulence
POL93	3.3	0.0	Predicted ORF in retrotransposon Tca8 with similarity to the Pol region of retrotransposons encoding reverse transcriptase, protease and integrase
orf19.4634	3.1	0.0	Protein required for thiolation of uridine at wobble position of Gln, Lys, and Glu tRNAs; has a role in urmylation; *S. cerevisiae* ortholog has a role in invasive and pseudohyphal growth
HRQ2	3.1	0.0	Protein of unknown function; mutants are viable; rat catheter and Spider biofilm induced
orf19.1667.1	3.1	0.0	Ortholog(s) have role in mitochondrial cytochrome c oxidase assembly, mitochondrial respiratory chain complex assembly and mitochondrial intermembrane space localization
RSM22	3.0	0.0	Predicted mitochondrial small ribosomal subunit; rat catheter and Spider biofilm induced
SSP96	2.9	0.0	Putative flavin-containing monooxygenase; F-12/CO2 early biofilm induced
orf19.7495	2.9	0.0	Protein with NADPH oxidoreductase containing flavin mononucleotide (FMN) domain; induced by nitric oxide
CZF1	2.9	0.0	Transcription factor; regulates white-opaque switch; hyphal growth regulator; expression in *S. cerevisiae* causes dominant-negative inhibition of pheromone response
orf19.2812	2.8	0.0	Protein of unknown function; Spider biofilm induced
orf19.2691	2.7	0.0	Planktonic growth-induced gene
orf19.29	2.7	0.0	Ortholog of *S. cerevisiae* Tah11, a DNA replication licensing factor required for pre-replication complex assembly; rat catheter, flow model and Spider biofilm induced
PDR16	2.7	0.0	Phosphatidylinositol transfer protein; induction correlates with CDR1, CDR2 overexpression/azole resistance; fluphenazine, 17-beta-estradiol, ethynyl estradiol, NO induced
LAP3	2.6	0.0	Putative aminopeptidase; positively regulated by Sfu1; clade-associated gene expression
RME1	2.6	0.0	Zinc finger protein, controls asexual sporulation; white-specific transcript
HSP78	2.6	0.0	Heat-shock protein; regulated by macrophage response, Nrg1, Mig1, Gcn2, Gcn4, Mnl1p; heavy metal (cadmium) stress-induced; stationary phase enriched protein
NOG2	2.6	0.0	Putative nucleolar GTPase; repressed by prostaglandins; Hap43-induced, rat catheter and Spider biofilm induced
snR40	2.5	0.0	C/D box small nucleolar RNA (snoRNA)
orf19.2547	2.5	0.0	Has domain(s) with predicted RNA binding, ribonuclease activity
orf19.4173	2.5	0.0	Ortholog(s) have role in peptidyl-diphthamide biosynthetic process from peptidyl-histidine
orf19.4478	2.5	0.0	Ortholog(s) have aspartate-tRNA ligase activity, role in mitochondrial aspartyl-tRNA aminoacylation and mitochondrion localization
orf19.6869	2.4	0.0	Putative lipid raft associated protein; Spider biofilm induced
OYE32	2.4	0.0	NAD(P)H oxidoreductase family protein; induced by nitric oxide, amphotericin B, oxidative stress via Cap1; upregulation associated with MDR1 overexpression or benomyl treatment
ECM331	2.4	0.0	GPI-anchored protein; mainly at plasma membrane, also at cell wall; Hap43, caspofungin-induced
orf19.95	2.4	0.0	Ortholog of *S. cerevisiae* : PRM5, *C. dubliniensis* CD36 : Cd36_60980, *C. parapsilosis* CDC317 : CPAR2_603060, C. auris B8441 : B9J08_003420 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_113703
HEM1	2.4	0.0	Putative 5-aminolevulinate synthase; caspofungin repressed; induced by high iron, nitric oxide
GCY1	2.4	0.0	Aldo/keto reductase; mutation confers hypersensitivity to toxic ergosterol analog
ZCF31	2.4	0.0	Zn(II)2Cys6 transcription factor of unknown function; mutant is sensitive to copper and SDS, and resistant to Calcofluor White; required for yeast cell adherence to silicone substrate
orf19.1825	2.4	0.0	Protein of unknown function; mutants are viable; filament induced; regulated by Nrg1, Rfg1, Tup1
ITS1	2.4	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN18 and RDN58
RRP9	2.4	0.0	Ribosomal protein; mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine); physically interacts with TAP-tagged Nop1
PST2	2.3	0.0	Flavodoxin-like protein involved in oxidative stress protection and virulence; putative NADH:quinone oxidoreductase; similar to 1,4-benzoquinone reductase
ELP3	2.3	0.0	Predicted histone acetyltransferase; role in regulation of transcription, tRNA wobble uridine modification; Spider biofilm induced
orf19.5431	2.3	0.0	Protein of unknown function; Hap43-repressed; Spider biofilm induced
orf19.5920	2.3	0.0	Ortholog of C. dubliniensis CD36 : Cd36_84570, C. parapsilosis CDC317 : CPAR2_404420, C. auris B8441 : B9J08_003958 and *Candida tenuis* NRRL Y-1498 : CANTEDRAFT_115339
orf19.7344	2.2	0.0	Ortholog(s) have DNA binding, chromatin binding

orf19.3970	2.2	0.0	Putative ribosome biogenesis factor; possibly essential, disruptants not obtained by UAU1 method
BMT1	2.2	0.0	Beta-mannosyltransferase, required for addition of the 1st beta-mannose residue to acid-stable fraction of cell wall phosphopeptidomannan
orf19.5678	2.2	0.0	Has domain(s) with predicted role in peptidyl-diphthamide biosynthetic process from peptidyl-histidine and cytoplasm localization
orf19.5465	2.2	0.0	Ortholog(s) have first spliceosomal transesterification activity and role in generation of catalytic spliceosome for first transesterification step
ALS9	2.2	0.0	ALS family cell-surface glycoprotein; expressed during infection of human epithelial cells; confers laminin adhesion to *S. cerevisiae*; highly variable; putative GPI-anchor; Hap43-repressed
RPD3	2.1	0.0	Histone deacetylase; regulates frequency of white-to-opaque and opaque-to-white switching
orf19.1887	2.1	0.0	Ortholog(s) have sterol esterase activity, role in sterol metabolic process and integral component of membrane, lipid droplet localization
FAD3	2.1	0.0	Omega-3 fatty acid desaturase; production of alpha-linolenic acid, a major component of membranes; caspofungin induced
BMT3	2.1	0.0	Beta-mannosyltransferase; adds 2nd beta-mannose to the acid-stable fraction of cell wall phosphopeptidomannan, elongation of beta-mannose chains on the phosphopeptidomannan acid-labile fraction; Hap43-induced; Spider biofilm induced
XKS1	2.1	0.0	Putative xylulokinase; Hap43-repressed; induced by prostaglandins; rat catheter biofilm repressed
YCP4	2.0	0.0	Flavodoxin-like protein involved in oxidative stress protection and virulence; flow model, rat catheter and Spider biofilm repressed
RTA3	2.0	0.0	7-transmembrane receptor protein involved in regulation of asymmetric lipid distribution in plasma membrane
ENP2	2.0	0.0	Putative nucleolar protein; essential; heterozygous mutation confers resistance to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU), and tubercidin (7-deazaadenosine)
orf19.4530.1	2.0	0.0	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
ECM3	2.0	0.0	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
CHK1	2.0	0.0	Histidine kinase; 2-component signaling, cell wall synthesis; hyphal growth defect
orf19.3470	2.0	0.0	Putative flavodoxin; similar to *S. cerevisiae* Tyw1
orf19.2459	2.0	0.0	Protein of unknown function; mRNA binds to She3; Hap43 repressed gene; Spider biofilm induced
PRC3	2.0	0.0	Putative carboxypeptidase Y precursor: transcript regulated by Nrg1 and Mig1; regulated by Gcn2 and Gcn4
orf19.3501	2.0	0.0	*S. cerevisiae* ortholog Pxl1 localizes to sites of polarized growth
OYE23	2.0	0.0	Putative NAPDH dehydrogenase; induced by nitric oxide, benomyl
orf19.1169	2.0	0.0	Putative DnaJ-like molecular chaperone; Spider biofilm induced
snR42a	2.0	0.0	H/ACA box small nucleolar RNA (snoRNA)
orf19.2657	2.0	0.0	Protein of unknown function
orf19.5070	1.9	0.0	Similar to cell-wall mannoproteins
SCH9	1.9	0.0	Protein kinase; involved in growth control, ribosomal protein synthesis, cell size, resistance to rapamycin
TES1	1.9	0.0	Putative acyl-CoA thioesterase
HCM1	1.9	0.0	Protein with forkhead domain; similar to *S. cerevisiae* Hcm1p; Hap43p-induced gene
SIR2	1.9	0.0	Required for wild-type lifespan, asymmetric inheritance of oxidatively damaged proteins, rDNA silencing
RRN3	1.9	0.0	Protein with a predicted role in recruitment of RNA polymerase I to rDNA; caspofungin induced
AAH1	1.9	0.0	Adenine deaminase; purine salvage and nitrogen catabolism; colony morphology-related regulation by Ssn6; Hog1, CO2-induced
RIM2	1.9	0.0	Putative mitochondrial carrier protein; induced by alpha pheromone in SpiderM medium
GCS1	1.9	0.0	Gamma-glutamylcysteine synthetase; glutathione synthesis, required for virulence;induced in low iron, H2O2, Cd, or presence of human neutrophils; possibly adherence-induced
PDC2	1.8	0.0	Homeodomain-like transcription factor; regulator of pyruvate decarboxylase
orf19.4792	1.8	0.0	Protein with a regulator of G-protein signaling domain; Plc1-regulated; Spider biofilm induced; rat catheter biofilm repressed
orf19.6556	1.8	0.0	Protein of unknown function; rat catheter, flow model and Spider biofilm induced
ROD1	1.8	0.0	Protein similar to *S. cerevisiae* Rod1;a membrane protein with a role in drug tolerance; repressed by Rgt1; mutant is viable
orf19.2639	1.8	0.0	Ortholog(s) have structural constituent of ribosome activity and mitochondrial large ribosomal subunit localization
XOG1	1.8	0.0	Exo-1,3-beta-glucanase; 5 glycosyl hydrolase family member; affects sensitivity to chitin and glucan synthesis inhibitors

SRR1	1.8	0.0	Two-component system response regulator; involved in stress response; Plc1-regulated; upregulated in cyr1 null mutant
orf19.2167	1.8	0.0	Ortholog(s) have role in ribosomal large subunit biogenesis, ribosomal small subunit biogenesis and nucleolus localization
ARG5,6	1.7	0.0	Arginine biosynthetic enzyme; processed in *S. cerevisiae* into 2 polypeptides
orf19.6175	1.7	0.0	Putative 35S rRNA processing protein; Hap43-induced; repressed by prostaglandins; Spider biofilm induced
UTP22	1.7	0.0	Putative U3 snoRNP protein; Ssr1-induced; repressed by prostaglandins; heterozygous null mutant is resistant to parnafungin
orf19.5438	1.7	0.0	Putative ubiquitin-protein ligase; role in protein sumoylation, protein ubiquitination; Spider biofilm induced
MRR1	1.7	0.0	Putative Zn(II)2Cys6 transcription factor; regulator of MDR1 transcription; gain-of-function mutations
orf19.177	1.7	0.0	Has domain(s) with predicted phosphatidylinositol binding activity and role in cell communication
orf19.2386	1.7	0.0	Ortholog(s) have role in endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript
orf19.3477	1.7	0.0	Putative pseudouridine synthase; predicted role in snRNA pseudouridine synthesis, tRNA pseudouridine synthesis
orf19.6156	1.7	0.0	Ortholog of *S. cerevisiae* : AIM11, *C. glabrata* CBS138 : CAGL0I04928g, *C. dubliniensis* CD36 : Cd36_80770
orf19.1395	1.7	0.0	Ortholog(s) have copper ion transmembrane transporter activity, inorganic phosphate transmembrane transporter
APE3	1.7	0.0	Putative vacuolar aminopeptidase Y,; regulated by Gcn2 and Gcn4; rat catheter and Spider biofilm repressed
orf19.3089	1.7	0.0	Predicted mitochondrial intermembrane space protein; predicted role in phospholipid metabolism
RPF1	1.7	0.0	Putative nucleolar protein with a predicted role in the assembly and export of the large ribosomal subunit
WSC2	1.7	0.0	Putative cell wall integrity and stress response protein; mRNA binds She3; Spider biofilm induced
orf19.3406	1.7	0.0	Predicted chloride transporter; member of conserved Mcm1 regulon; Spider biofilm repressed
YHB4	1.7	0.0	Protein related to flavohemoglobins; not required for wild-type nitric oxide resistance
COX11	1.7	0.0	Cytochrome oxidase assembly protein; transcript regulated by Nrg1; protein repressed during the mating process
HOT1	1.7	0.0	Putative transcription factor; required for inhibition of filamentous growth by farnesoic acid and for expression of PHO81
orf19.4656	1.6	0.0	Ortholog of *C. dubliniensis* CD36 : Cd36_41300, *C. parapsilosis* CDC317 : CPAR2_400390, C. auris B8441
orf19.4150	1.6	0.0	Putative glutaredoxin; induced by nitric oxide; Spider biofilm induced
orf19.6829	1.6	0.0	Protein with a predicted mitochondrial ATPase expression domain; possibly an essential gene, disruptants not obtained by UAU1 method
HPA2	1.6	0.0	Ortholog(s) have D-amino-acid N-acetyltransferase activity, N-acetyltransferase activity and role in D-amino acid metabolic process
orf19.6477	1.6	0.0	Ortholog(s) have tRNA (guanine-N7-)-methyltransferase activity
orf19.2244	1.6	0.0	Similar to oxidoreductases and to *S. cerevisiae* Yjr096wp; Sfu1 repressed; induced by benomyl treatment, Ssr1
ECM42	1.6	0.0	Ornithine acetyltransferase; Gcn2, Gcn4-regulated; clade-specific gene expression; possibly essential gene
orf19.1336.2	1.6	0.0	Ortholog(s) have role in mitochondrial respiratory chain complex assembly and mitochondrial intermembrane space localization
FUM11	1.6	0.0	Fumarate hydratase; induced in high iron; protein in exponential and stationary-phase yeast cells
orf19.4907	1.6	0.0	Putative protein of unknown function; Hap43p-repressed gene; increased transcription is observed upon fluphenazine treatment
NRM1	1.6	0.0	Transcriptional regulator of cell cycle gene expression
orf19.6225.1	1.6	0.0	Ortholog(s) have role in mitochondrial cytochrome c oxidase assembly and extrinsic component of matrix side of mitochondrial inner membrane, mitochondrial matrix localization
ATO2	1.6	0.0	Putative fungal-specific transmembrane protein; fluconazole repressed
orf19.5963	1.6	0.0	Putative prenyltransferase; essential gene in *S. cerevisiae*; Spider biofilm induced
FGR47	1.5	0.0	Protein lacking an ortholog in *S. cerevisiae*; transposon mutation affects filamentous growth
orf19.5126	1.5	0.0	Putative adhesin-like protein
CRH11	1.5	0.0	GPI-anchored cell wall transglycosylase, putative ortholog of *S. cerevisiae* Crh1p
ARE2	1.5	0.0	Acyl CoA:sterol acyltransferase; uses cholesterol and oleoyl-CoA substrates; protoberberine derivative drug inhibits enzyme activity
DED1	1.5	0.0	Predicted ATP-dependent RNA helicase; RNA strand annealing activity; Spider biofilm induced

PAN6	1.5	0.0	Ortholog(s) have pantoate-beta-alanine ligase activity and role in pantothenate biosynthetic process
HAP41	1.5	0.0	Putative Hap4-like transcription factor; Hap43-repressed; not required for response to low iron; induced by Mnl1 under weak acid stress
ITS2	1.5	0.0	Non-coding region in the 55 copies of rDNA repeat, between RDN58 and RDN25
orf19.4358	1.5	0.0	Putative protein of unknown function; Hap43p-repressed gene; *S. cerevisiae* ortholog YDL157C localizes to mitochondria
CPA2	1.5	0.0	Putative arginine-specific carbamoylphosphate synthetase; protein enriched in stationary phase yeast cultures