SAGA Complex Subunits in *Candida albicans* Differentially Regulate Filamentation, Invasiveness and Biofilm Formation

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ABSTRACT

SAGA complex subunits in *Candida albicans* differentially regulate Filamentation, Invasiveness and Biofilm formation

Saima Rashid

SAGA (Spt-Ada-Gcn5-acetyltransferase) is a highly conserved, multiprotein co-activator complex that consists of five distinct modules. It has two enzymatic functions, a histone acetyltransferase (HAT) and a deubiquitinase (DUB) and plays a central role in processes such as transcription initiation, elongation, protein stability and telomere maintenance. We analysed conditional and null mutants of the SAGA complex module components in the fungal pathogen *Candida albicans*; Ngg1, (the HAT module); Ubp8, (the Dub module); Tra1, (the recruitment module), Spt7, (the architecture module) and Spt8, (the TBP interaction unit), and assessed their roles in a variety of cellular processes. We observed that *spt7Δ/Δ* and *spt8Δ/Δ* strains have a filamentous phenotype, and both are highly invasive in yeast growing conditions as compared to the wild type, while *ngg1Δ/Δ* and *ubp8Δ/Δ* are in yeast-locked state and non-invasive in both YPD media and filamentous induced conditions compared to wild type. RNA-sequencing-based transcriptional profiling of SAGA mutants reveals upregulation of hyphal specific genes in *spt7Δ/Δ* and *spt8Δ/Δ* strains and downregulation of ergosterol metabolism pathway. As well, *spt7Δ/Δ* and *spt8Δ/Δ* confer susceptibility to antifungal drugs, to acidic and alkaline pH, to high temperature, and to osmotic, oxidative, cell wall and DNA damage stresses, indicating that these proteins are important for genotoxic and cellular stress responses. Despite having similar morphological phenotypes (constitutively filamentous and invasive) *spt7* and *spt8* mutants displayed variation in nuclear distribution where *spt7Δ/Δ* cells were frequently binucleate and *spt8Δ/Δ* cells were consistently mononucleate. We also observed that *spt7Δ/Δ* and *spt8Δ/Δ* mutants were quickly engulfed by macrophages compared to *ngg1Δ/Δ* and *ubp8Δ/Δ* strains. All these findings suggest that the SAGA complex modules can have contrasting functions where loss of Spt7 or Spt8 enhances filamentation and invasiveness while loss of Ngg1 or Ubp8 blocks these processes.

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Contribution of authors

Chapter 2 and 3:

Tuana Mesquita is responsible for GRACE library screening of SAGA mutants and other related experiments.

Raha Parvizi Omran is responsible for RNA isolation and purification.

Dr Vanessa Dumeaux conducted preprocessing of RNASeq analysis.

Dr Pablo Godoy is responsible for the macrophage engulfment assay.

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5-fluoroorotic acid	5FOA
Cyclic AMP- protein kinase A	cAMP-PKA
Clustered regularly interspaced short palindromic repeats	CRISPR
Endoplasmic reticulum	ER
Gene replacement and conditional expression	GRACE
Mitogen-activated protein kinase	MAPK
Mating type-like	MTL
Phosphate-buffered saline	PBS
Polymerase chain reaction	PCR
Room type	RT
Tetracycline	TET
Wild type	WT
Yeast extract, peptone, dextrose	YPD
FRAP, ATM, TRRAP	FAT
FRAP, ATM, TRRAP, C-terminus	FATC FRB
Histone Acetyltransferase	HAT
Huntingtin, Elongation factor 3, A subunit of protein phosphatase 2A and Tor1	HEAT
Spt-Ada-Gcn5-acetyltransferase	SAGA
SAGA-like complex	SLIK
TRansformation/tRanscription domain-Associated Protein TTT Tel2-Tti1-Tti2	TRRAP
Nucleosomal Acetyltransferase of histone H4	NuA4

Preinitiation complex	PIC
Phosphatidylinositol-3-kinase	PIK
Phosphatidylinositol-3-kinase related kinase	PIKK
Synthetic Complete	SC
TATA-binding protein associated factor	TAF
TATA-binding protein	TBP
Transcription Factor II	TFII
Calcofluor White	CFW
4′,6-diamidino-2-phenylindole	DAPI
RNA polymerase II	PolII
SuPressor of Ty	SPT
Transcription Factor	TF
Fetal Calf Serum	FCS
Hydrogen peroxide	H_2O_2
Endoplasmic Reticulum	ER
Dithiothreitol	DTT
Methyl methane sulfonate	MMS
Hydroxyurea	HU

Introduction

The fungal kingdom is vast, covering approximately from 1.5 million to as many as 6 million diverse species globally (Fisher et al. 2012). It includes distinct species such as unicellular yeasts, filamentous fungi, mushrooms, and lichens, with notably varied life histories that make crucial contributions to the biosphere, to human industry and to medicine and research (Heitman 2011). The fungi are more closely related to animals than plants and shared a common ancestor ~1 billion years ago (Hedges et al. 2004). Some fungal species pose a major threat to human health, food security and biodiversity (Fisher et al. 2012; Fisher et al. 2020) Fungi can do considerable damage to human food stability; from generating epidemics in staple crops to contaminating food supplies with cancer-causing toxins (Avery et al. 2019; Fones et al. 2020) On the other hand, fungi can also be extremely beneficial. They are valuable in producing alcohol for consumption and for industrial use, and for ripening of soft cheeses and other fermented dairy products like kefirs, sour cream, and yogurt (Maicas 2020). Fungi can be engineered to produce immunosuppressive drugs to facilitate organ transplantation, drugs to reduce the risk of heart disease and anti-malarial drugs (Singh 2000; Tiffert et al. 2000). Fungi are important for food production and play crucial roles in maintaining health of plants (Buckley 2008). They form mutualistic and symbiotic relationship with plants e.g., mycorrhizae - a fungal association that facilitates the uptake of water and ions to plant roots during environmental stresses. They also fix the organic nitrogen for the plants – fungi take up inorganic nitrogen from the soil to form nitrates, nitrites, and urea that become readily available for the plants to use. The fungus increases the surface area, helping the plant to take up mineral nutrients, and in return the plant provides the fungus with sources of fixed carbon produced during photosynthesis (Govindarajulu et al. 2005). Finally, saprophytic fungi play a critical role in our ecosystem by decomposing dead organic matter and converting it into recyclable nutrients which can be reused by other organisms (Crowther, Boddy, and Hefin Jones 2012).

Fungi like yeasts can serve as model organisms for the study of molecular genetics of eukaryotes, because species like the budding yeast *Saccharomyces cerevisiae* can be easily grown in laboratory conditions and genetically customised by manipulations that can even involve incorporating human genes into the fungal cells (Laurent et al. 2016). Scientists can also make use of this organism to gain deeper understanding of various disease conditions in humans by studying the function of homologous genes in *S. cerevisiae*. Thus, even though some fungi can cause severe damage, directly threatening human health or disturbing

environmental biodiversity, their overall benefits clearly outweigh these negative aspects. Therefore, studying fungi at a broader level will be exciting to develop the in-depth knowledge of the organism and various complex life processes.

Like certain bacteria, some fungi are members of normal microflora of the human skin, oral, gastrointestinal and urinogenital tracts. The microbiome of human body consists of members distributed in three phyla – Phylum Ascomycota, Phylum Basidiomycota and Phylum Zygomycota - concentrated at various regions of the body (Ghannoum et al. 2010; Zhang et al. 2011; van Woerden et al. 2013; Limon, Skalski, and Underhill 2017). Out of diverse 6 million fungal species, over 600 fungal species are associated with humans, either as commensals and members of our microbiome or as opportunists that cause some severe infectious diseases, including blood stream infections that are often fatal. The most common fungal species which result in such severe infections include *Candida, Pneumocystis*, *Cryptococcus* and *Aspergillus spp.* Infections usually occur in patients with weakened immune system such as those undergoing chemotherapy or organ transplants or infected with HIV. With a global increase of the rate of invasive fungal infections and the rise and spread of fungal pathogens resistant to all classes of antifungals available, these fungal species pose a great risk to human health (Fisher et al. 2012; Fisher et al. 2020).

Because of their significant presence in the human body, their unique cellular architecture and better survivability in the harsh conditions of the GI tract, fungi can also serve as potential candidates for probiotics to treat various disease conditions due to the imbalance of microflora in the body like IBD (Brown and Gow 1999), diarrhea, and urinary tract infections. The most effective and clinically approved probiotic yeast is Saccharomyces boulardii (Guslandi et al. 2000; Guslandi, Giollo, and Testoni 2003). Recently, a yeast probiotic strain was engineered by adding the human GPCR protein known as purinergic receptor (P2Y2) into yeast to detect extracellular adenosine triphosphate (eATP) production. Linkage of this activated human receptor to the secretion of ATP- degrading enzyme -potato apyrase resulted in yeast strain that is successful at detecting and reducing pro-inflammatory levels of eATP (Scott et al. 2021).

1.1 Candida species and their virulence

Candida albicans is the predominant cause of fungal infections in humans ranging from mucosal to systemic infections (Fidel, Vazquez, and Sobel 1999). Based on Linnaean taxonomy, *Candida albicans* belongs to:

Kingdom	Fungi
Division	Ascomycota
Class	Saccharomycetes
Order	Saccharomycetales
Family	Saccharomycetaceae
Genus	*Candida*
Species	*albicans*

(Schoch et al. 2020).

Candida comes from the Latin word *candidus*, meaning "white." Its species name, *albicans*, has also been derived from the Latin word *albico* meaning "white." The yeast forms white colonies when cultured on agar surface, and in the case of certain infections, like oral thrush, it can produce white patches visible on human oral epidermal/mucosal membranes.

Candida albicans represents the most prevalent opportunistic fungal pathogen (Brand 2012) that forms a part of our natural microflora — the microorganisms that commonly live in or on our bodies. As a commensal yeast, it can colonise niches such as the skin, the oral cavity, and the gastrointestinal and urinogenital tracts of healthy adults (Schulze and Sonnenborn 2009; Angebault et al. 2013). However, as an opportunistic pathogen it can cause severe infections in immunocompromised hosts (Odds 1987) such as patients who are on long course treatments of antibiotics or steroids, patients immunosuppressed to facilitate organ transplants, people undergoing chemotherapy and HIV patients (Denning et al. 1991). *C. albicans* is the 3rd most common nosocomial pathogen isolated from blood cultures in hospitalised patients and systemic Candidiasis is associated with mortality rates of up to 50% (Wisplinghoff et al. 2004; Tournu and Van Dijck 2012; Mathé and Van Dijck 2013). Most women suffer from vaginal Candidiasis at least once in their lifetime (Zeng et al. 2018).

1.2 Candida Morphology and Infection

Morphological forms of *C. albicans* play crucial roles in *C. albicans* biology including reproduction, virulence, evading host immune response, and resistance to antifungal drugs. Also, yeast and filamentous forms can create extracellular communities called biofilms under certain conditions (Chandra et al. 2001). There are different phenotypic forms of *C. albicans* - white, opaque (Slutsky et al. 1987), gray (Tao et al. 2014) and a filamentous form. Normally, *C. albicans* exist in the yeast form but under certain conditions *C. albicans* can proliferate and invade the host epithelium. Thus, it switches its morphological state to the hyphal form, a property that is key to pathogenesis and biofilm formation (Jabra-Rizk, Falkler, and Meiller 2004; Ramage et al. 2005; Calderone and Fonzi 2001; Chauvel et al. 2012). However, the interaction of *C. albicans* and mammalian host tissues is highly complex, and *Candida* infection occurs in a series of sequential steps:

- Adhesion of fungal yeast cells to an epithelial surface by the expression of adhesins is required to initiate colonisation of the host cell surface (Preliminary candidiasis).
- Penetration into the epithelial cell surface is the limit of the infectious process in most cases, causing superficial candidiasis; normally the fungi are capable of further invasion into an immunologically intact host when switched to hyphal cells by expression of invasins which mediate uptake of fungus by the host cell through induced endocytosis.
- The attachment of the fungal cells to host cell surfaces give rise to formation of biofilms with yeast cells at the surface and hyphal cells at the top.
- When fungi penetrate the endothelial cells, they face the cellular host defense system. However, due to hyphal nature of the cells *C. albicans* can evade the host immune response and invade the tissues to gain access to the blood stream and spread to other organs in people with weakened immune system (Mayer, Wilson, and Hube 2013).

Antifungal drugs are mainly used to treat fungal infections. However, as a commensal yeast, *C. albicans* poses a challenge for antifungal therapy (Brand 2012). In comparison to anti-bacterial agents, there are limited anti-fungal drug classes available. This is in part because fungi are eukaryotic, which makes it difficult for the drug target to eliminate the pathogen from the host without toxicity to the host. Currently, there are only four classes of drugs available for the treatment of fungal infections based on the mode of action and cellular targets: azoles, polyenes, echinocandins and allylamines (Ghannoum and Rice 1999;

Williams and Lewis 2011; Mathé and Van Dijck 2013). Azoles, which inhibit the ergosterol biosynthetic pathway by targeting lanosterol 14-α-demethylase, are the most common antifungals. Targeting this demethylase inhibits ergosterol production which results in the accumulation of toxic sterols; this disturbs the cell membrane stability and can cause leakage of cell contents and eventually leads to cell death. Polyenes interact with sterols, mainly ergosterol, in the cell membrane, which causes disruption of membrane permeability, and as a result the cell starts leaking its contents which eventually leads to cell death. Echinocandins inhibit (1, 3)-β-D-glucan synthase causing fungal cell wall stress and leading to loss of cell wall integrity. Allylamines inhibits ergosterol biosynthesis by disrupting the enzyme squalene epoxidase responsible for the formation of fungal cell membrane (Robbins, Caplan, and Cowen 2017).

1.3 *Candida albicans* yeast to filamentous switching

The ability of *C. albicans* to transition from commensal to pathogen is in part due to the most common morphological switching, that between yeast and hyphal forms (Brand 2012). Both yeast and filamentous forms play an important role in virulence (Grant et al. 1997). The white yeast form allows the *C. albicans* to enter the endothelial cells to quickly occupy the essential organs and results in *Candidemia* (Jacobsen et al. 2012). However, a transition to the hyphal form allows invasive growth within deep niches of the organs, causing challenges for clinical treatment. The hyphal/filamentous form can invade host tissues *in vivo* or agar medium *in vitro*. Upon induction with different factors such as increased temperature, nutrient limitation or the presence of serum, the white yeast form cells of *C. albicans* switches to the invasive hyphal form (Brown and Gow 1999). The yeast cells commence the invasion of tissues and organs through formation of hyphae or pseudo-hyphae upon induction of host signaling. After host tissue invasion, the pathogen then gains access to the blood stream to spread to other organs of the host (Csank et al. 1998; Felk et al. 2002).

Filamentation in *C. albicans* is regulated by several pathways. However, the cyclic-AMP/ protein kinase A (cAMP/PKA) pathway and a mitogen activated protein kinase (MAPK) pathway play key roles in regulation of filamentation in *C. albicans* (Sonneborn, Bockmühl, and Ernst 1999; Cullen and Sprague 2012; Angebault et al. 2013).

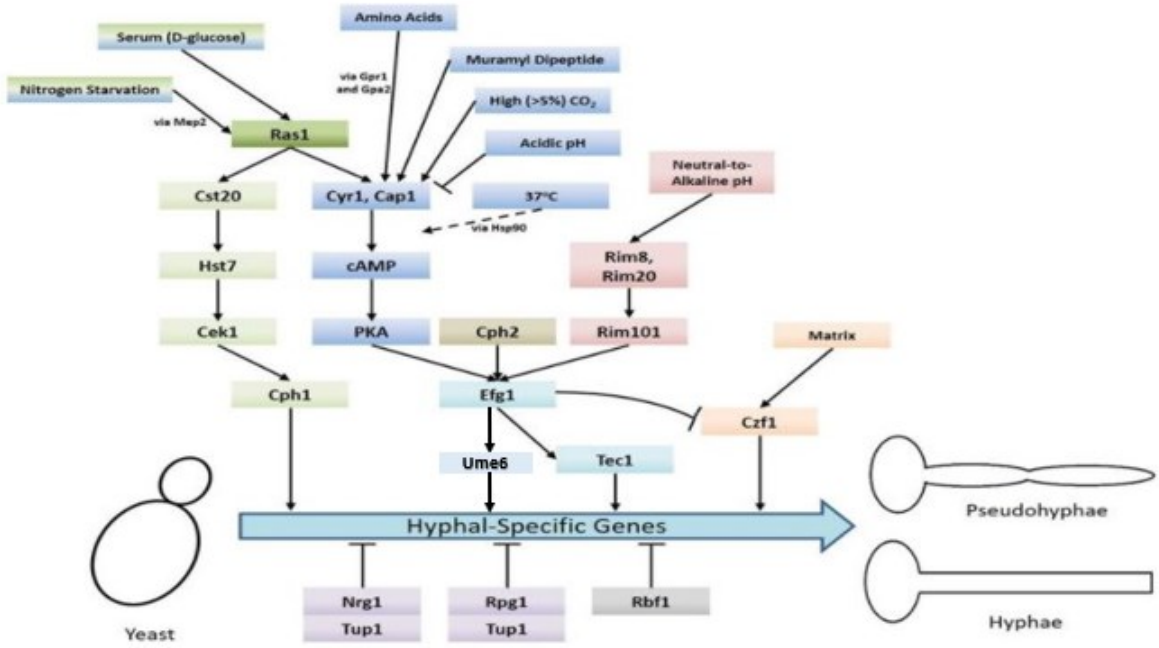


Figure 1. Schematic representation of signaling pathways that regulate the yeast-hyphae morphogenetic switch in *C. albicans*. cAMP/PKA (centre bluish scheme) and MAPK (left greenish scheme) pathways function in filamentation (Berman and Sudbery 2002). The arrows represent activation and the lines with bar represents repression in the filamentation pathway.

1.4 Biofilm formation and development

The vast majority of *C. albicans* infections have been linked with biofilm formation (Jabra-Rizk, Falkler, and Meiller 2004; Nett et al. 2007; Finkel and Mitchell 2011; Tournu and Van Dijck 2012; Mathé and Van Dijck 2013). The morphological switch from the yeast form to the filamentous form of *C. albicans* plays a key role in both its pathogenesis and in its ability to form a biofilm (Calderone and Fonzi 2001; Jabra-Rizk, Falkler, and Meiller 2004; Ramage et al. 2005; Chauvel et al. 2012). Biofilms represent a 3D structured surface-linked fungal population embedded in extracellular matrix of polysaccharide which provides protection and a structural framework to the biofilm cells (O'Toole, Kaplan, and Kolter 2000; Lewis 2001; Fux et al. 2005; Ghannoum et al. 2015). Thus, in a biofilm, fungal cells have a stable environment where they can resist extremely high concentration of antifungals. The cells can disperse from biofilms and migrate into the bloodstream and cause systemic infections with high mortality and morbidity (Finkel and Mitchell 2011). Biofilm formation in *C. albicans* has been shown to occur in series of sequential steps *in vitro* over a period of 24h-48h (Řičicová et al. 2010; Mathé and Van Dijck 2013) (Figure 2).

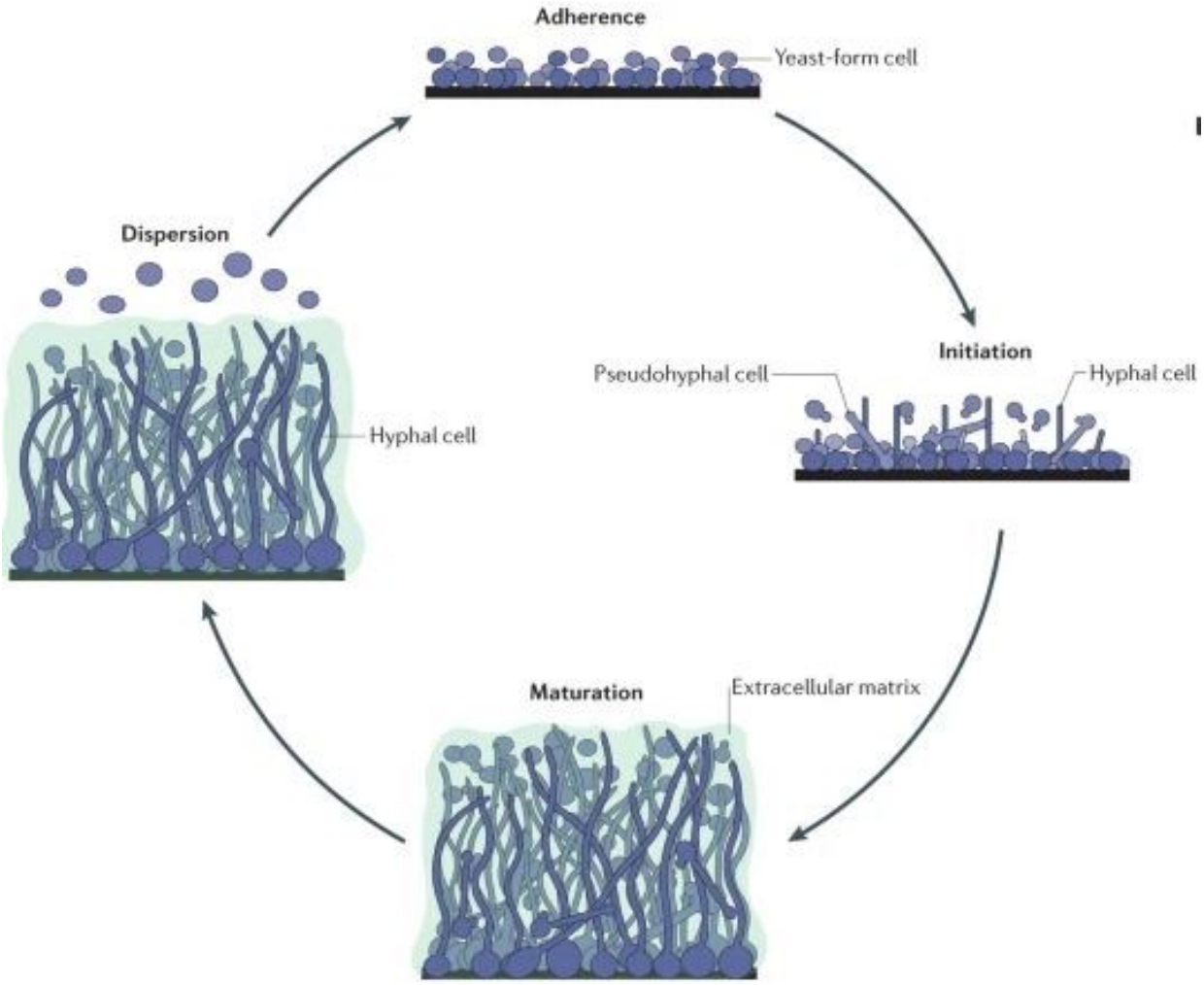


Figure 2. Schematic representation of the biofilm developmental stages in *C. albicans* (Lohse et al. 2018). Life cycle of a *C. albicans* biofilm begins with the attachment of free yeast form cells to the surface (adhesion step) followed by proliferation and germ tube formation (initiation step). This is followed by hyphae formation and accretion of extracellular matrix (maturation step). Non-adherent yeast cells from the biofilms are released and dispersed to initiate biofilm formation at new sites (dispersal step).

As established from *in vitro* studies, biofilm development comprises of four sequential steps:

- Adhesion: The first and foremost step in the biofilm development is the attachment of free yeast cells to the substrate to form a fungal basal layer (Nett et al. 2007; Finkel and Mitchell 2011; Nobile et al. 2012).
- Initiation: This is followed by proliferation and transformation of yeast cells to hyphal form.
- Maturation: The hyphal formation is the significant step for the biofilm formation followed by accretion of extracellular matrix as the biofilm matures.

- Dispersion: In the final step, non-adherent yeast cells of the biofilm are released into the surroundings where they can invade new sites for biofilm development. This has a great clinical significance as dispersed cells become a source of new biofilms, or spread into host tissues, and thus they are linked with candidemia or other invasive diseases (Tournu and Van Dijck 2012).

1.5 Transcriptional Regulation

Despite the widespread studies undertaken to unravel transcriptional regulator networks in *C. albicans*, we still lack a full, comprehensive understanding of the transcriptional regulation in this opportunistic fungus. Regulation of gene expression is an important biological process for the appropriate functioning of the cell in response to a variety of intra- and extra cellular signals. Gene expression maintains specific cell states that are controlled by transcription factors, cofactors, and chromatin regulators. To work properly, a cell needs to synthesize required proteins at proper times in response to cell development, differentiation, and environmental changes. For eukaryotic gene expression, transcription initiation is a key regulatory step. The formation of a pre-initiation complex (PIC) at the promoter is a primary step in transcription activation (Wandelt and Grummt 1983). Co-activator complexes like SAGA, the NuA4 complex and the ADA complex play important roles in this process, through regulating PIC assembly through interactions with transcription factors, by ATP dependent nucleosome remodelling and by covalent histone modifications (Green 2005). The transcription factors involved in PIC assembly comprise TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH, which are recruited at the promoter site by the TATA binding protein (TBP) (Reinberg, Horikoshi, and Roeder 1987; Ranish, Yudkovsky, and Hahn 1999). For recruiting RNA polymerase II (Pol II) to the promoter, TBP, TFIIB and TFIIF are required (Killeen, Coulombe, and Greenblatt 1992). Thus, co-activator modifying complexes dynamically remove or deposit post transcriptional modifications (PTMs) on histones thus creating or removing docking site for transcriptional factors (Yun et al. 2011; Bannister and Kouzarides 2011). Generally, histone acetylation takes place at multiple lysine residues and histone acetyltransferase complexes (HATs) play a role in this process (Brown et al. 2000).

1.6 Transcriptional regulator complex: SAGA

The SAGA complex is a multifunctional co-activator complex that is highly conserved among eukaryotes, from *Saccharomyces cerevisiae* to humans (Koutelou, Hirsch, and Dent 2010; Gurskiĭ et al. 2013; Srivastava et al. 2015). SAGA stands for Spt7p-Ada1p-Gcn5p

adaptor; it was first characterized as a histone acetyltransferase (HAT) but also has an enzymatic role as a histone deubiquitinase (Dub) (Grant et al. 1997; Henry et al. 2003). The SAGA complex was first identified in *S. cerevisiae* and is a large 1.8 MDa complex with 18-20 subunits. It also has structural functions and is involved in recruiting TBP to gene promoters to modulate gene transcription. In addition, it can be anchored to promoter regions to repress transcription (Belotserkovskaya et al. 2000; Warfield, Ranish, and Hahn 2004).

SAGA is subdivided into five main modules based on their enzymatic or structural function – the HAT module (Gcn5, Ada2, Ngg1,Sgf29) responsible for Histone H3 acetylation (Brownell et al. 1996; Grant et al. 1997); the Dub module (Ubp8, Sus1, Sgf73, Sgf11) responsible for Histone H2B de-ubiquitination (Henry et al. 2003); the Recruitment module (Tra1), which interacts with transcriptional activators (Brown et al. 2001); the architecture module (Taf5, Taf6, Taf9, Taf10, Taf12, Ada1, Spt20, Spt7) helps in maintaining structural integrity of the complex (Grant et al. 1997; Belotserkovskaya et al. 2000; Wu and Winston 2002); and the TBP interaction unit (Spt8, Spt3), which recruits the TATA-binding protein to the promoters in order to regulate transcription (Mohibullah and Hahn 2008; Gurskiĭ et al. 2013) (Figure 3,Table 1). Tra1, the only largest essential protein of the complex forms a large compartment, or separate TF binding module, which links with the SAGA complex. Architecture module compartment transmit signals to HAT module and Dub module. Upon activation, SAGA is recruited on to promoters, as directed by Tra1, where Gcn5, a HAT subunit, acetylates lysines K9, K14, K18, and K23 in histone protein H3 and is involved to some degree in acetylation of histone proteins H4 and H2B. Additionally, the ubiquitin protease module Ubp8 regulates levels of H2B- ubiquitin, which acts as a key determinant of histone methylation levels in transcribed regions and transcription elongation (Lee et al. 2000).

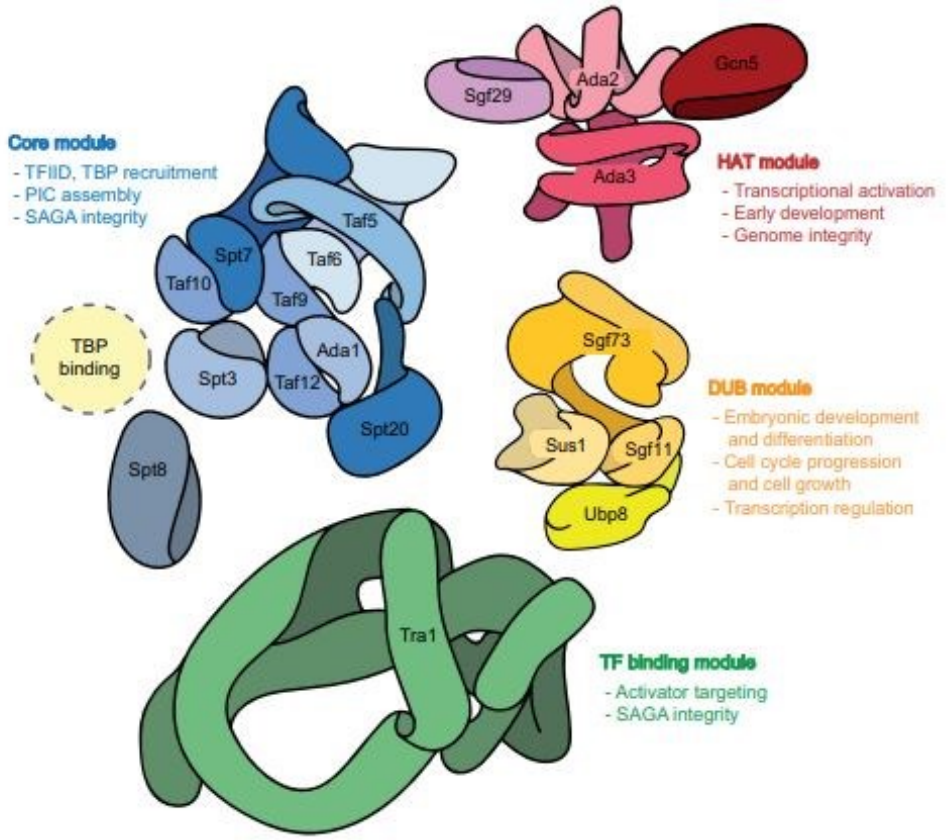


Figure 3. Schematic representation of the modular structure of the coactivator SAGA complex (Cheon et al. 2020). The diagram represents the key functions of each module and shows the most recent structural data found from *S. cerevisiae* (Helmlinger et al. 2011; Liu et al. 2019; Wang, Dienemann, et al. 2020).

1.6.1 TF-binding module/Recruitment module

The recruitment module includes only the essential protein Tra1p. It forms the largest subunit (433kD) of the SAGA complex. It belongs to phosphoinositide 3 kinase-related kinase (PIKK) HEAT (Huntingtin, elongation factor 3, a subunit of phosphatase PR65/A, and Tor), which acts as a docking site for binding acidic transcriptional factors such as Gcn4 and Gal4, FAT (FRAP, ATM, and TRRAP) domain; and the PIKK domain (Sharov et al. 2017). Earlier studies observing the effect of modifying the FATC domain of Tra1 established that cells containing mutations in this domain exhibit slow growth under stress conditions or are inviable (Hoke et al. 2010). Tra1 is thought to play an integral role in transcriptional initiation through interactions with specific gene activators within SAGA complex (Fishburn, Mohibullah, and Hahn 2005).

Tra1p also forms part of NuA4 complex as part of the activator-targeting module (Cheung and Díaz-Santín 2019). The only exception to this pattern is in the fission yeast *Schizosaccharomyces pombe,* where two paralogous genes *TRA1* and *TRA2* encode the Tra1. function; here Tra1 is exclusively found in SAGA and Tra2 in NuA4. In *S. pombe*, Tra1 depleted cells are viable where as Tra2 depleted cells are not (Avery et al. 2019).

Table 1. Subunits of co-activator SAGA complex in four representative model organisms.

Module	*Saccharomyces cerevisiae*	*Schizosaccharomyces pombe*	*Drosophila melanogaster*	*Homo sapiens*
HAT module	Gcn5p	Gcn5	dKAT2 (dGcn5)	KAT2A (GCN5) /KAT2B (PCAF)
	Ada2p	Ada2	dAda2b	TADA2b
	Ada3p (Ngg1p)	Ada3 (Ngg1)	dAda3	TADA3
	Sgf29p	Sgf29	Sgf29	SGF29 (CCDC101)
Core module	Taf5p	Taf5	Wda	TAF5L (PAF65β)
	Taf6p	Taf6	Saf6	TAF6L (PAF65α)
	Taf9p	Taf9	dE(y)1 (Taf9)	TAF9/TAF9b
	Taf10p	Taf10	Taf10b	TAF10 (STAF28)
	Taf12p	Taf12	Taf12	TAF12
	Ada1p	Ada1	Ada1	TADA1 (STAF42)
	Spt7p	Spt7	dSpt7	SUPT7L (STAF65γ)
	Spt20p	Spt20	Spt20	SUPT20H
	Spt3p	Spt3	dSpt3	SUPT3H
	Spt8p	Spt8	–	–
TF-binding module	Tra1p	Tra1	Nipped-A (dTra1)	TRRAP
DUB module	Ubp8p	Ubp8	dNonstop	USP22 (UBP22)
	Sgf11p	Sgf11	dSgf11	ATXN7L3
	Sgf73p	Sgf73	dATXN7	ATXN7 (SCA7)
	Sus1p	Sus1	dE(y)2	ENY2
Splicing module	–	–	Sf3b3	SF3B3
	–	–	Sf3b5	SF3B5

1.6.2 HAT module

The HAT module of the SAGA complex involves the Ngg1(Ada1), Ada2, Gcn5 and Sgf29 subunits (Horiuchi et al. 1997; Balasubramanian et al. 2002; Samara et al. 2010) As the name indicates, this module of SAGA is involved in the histone acetylation of lysine residues at K9, K14, K18, and K23 in histone H3 and is involved to some degree in H4 and H2B acetylation (Brownell et al. 1996; Grant et al. 1997). Gcn5 is the main catalytic subunit responsible for the HAT activity of SAGA as it is involved in the acetylation of histone tails on its own but is not sufficient to acetylate nucleosome histones alone (Brownell et al. 1996; Kuo et al. 1996). Gcn5 is necessary for filamentous and invasive growth in *C. albicans* and *gcn5/gcn5* mutant cells also showed sensitivity to cell wall stress (Chang, Fan, and Chen

2015). Ada2, another subunit of the HAT module, helps in the complexes' catalytic activity by activating Gcn5's catalytic activity without directly interacting with histones (Sun et al. 2018). Ada2 acetylates nucleosomal histones H3 and H2B. Ada2 plays a key role in cell wall integrity, cell adhesion, hyphal development, and pathogenesis (Sellam et al. 2009). Ngg1, which is part of the HAT module, has also been shown to be an important regulator of Gcn5 acetylation activity (Balasubramanian et al. 2002). In addition, Spt7 has also been shown to be a crucial factor for Gcn5 acetylation activity (Belotserkovskaya et al. 2000). Besides transcription activation, acetylation of histones is involved in transcription elongation (Govind et al. 2007).

1.6.3 Architecture/Core module

The architecture module consists of 10 subunits which forms the largest module of SAGA complex. It plays a critical role in the assembly of preinitiation complex (PIC) by recruiting TBP. It includes SPT (SuPressor of Ty) and TBP -Associated Factors (TAFs) and Ada1, which helps in maintaining the structural integrity of the SAGA complex. The structural module comprises of Spt7 and Spt20; while the TAFs include Taf5, 6, 9, 10, and 12 are shared between SAGA and Pol II transcription factor TFIID in *S. cerevisiae*. In *S. cerevisiae*, Ada1p, Spt7p and Spt20p are important for proper assembly of the SAGA complex (Grant et al. 1998; Belotserkovskaya et al. 2000). Spt7 deletion in *S. cerevisiae* showed a loss of SAGA complex integrity, establishing the importance of the Spt7 subunit in SAGA assembly (Wu and Winston 2002).

1.6.4 TBP interaction module

The Spt3 and Spt8 proteins recruit TATA- binding proteins within the SAGA complex (Eisenmann et al. 1992; Eisenmann et al. 1994). Both Spt3 and Spt8 have been shown to interact with TBP proteins directly, load them on promoters and facilitate assembly of PIC (Dudley, Rougeulle, and Winston 1999; Bhaumik and Green 2002). Intriguingly, Spt3 and Spt8 are involved in transcription repression (Belotserkovskaya et al. 2000). How Spt3 and Spt8 differentiate between transcription activation and suppression remains elusive.

1.6.5 Dub module

The SAGA complex also controls the gene expression through H2B ubiquitination and its role in transcription regulation has been studied thoroughly. The Dub module in the SAGA complex comprises of Ubp8 (Henry et al. 2003), Sgf73, Sgf11 (Gavin et al. 2002;

Helmlinger et al. 2004; Powell et al. 2004; Sanders et al. 2002) and Sus1 (Rodríguez-Navarro et al. 2004). Ubp8 is the catalytic protein involved in the de-ubiquitination of H2BK123 in yeast. It also forms a part of the SLIK complex (SAGA-like complex) in yeast and its activity is also required to modulate H3 methylation at some SAGA promoters (Daniel et al. 2004). Sgf11, along with Ubp8 is required for H2BK123 de-ubiquitination and forms a structural and/or functional submodule in SAGA (Ingvarsdottir et al. 2005; Lee et al. 2005). Next, Sus1 was identified as a subunit of both SAGA and the mRNA export complex-TREX-2. The recruitment of Sus1 to SAGA depends on Ubp8 and Sgf11. Sus1 has also been shown to be required for the H2B-deubiquitylation activity of Dub module (Rodríguez-Navarro et al. 2004; Köhler et al. 2008). Sgf73 is a transcriptional adaptor linking the core SAGA Gcn5-mediated acetylation to the Dub module (Lee et al. 2009; Morgan et al. 2016). It also recruits TREX-2 to SAGA. A physical interaction between SAGA and TREX-2 is indispensable for bringing transcriptional machinery to the nuclear pore complex, a phenomenon known as "gene gating" (Köhler et al. 2008; Lee et al. 2009). Studies have demonstrated that Ubp8 alone lacks deubiquitylation activity, suggesting that it requires the presence of other module components to bring the conformational change in the Ubp8 protein to carry out the deubiquitylation reaction (Lee et al. 2005; Bonnet et al. 2008). The minimal Dub module structure that confers full deubiquitylation of the nucleosome comprises of Ubp8, Sgf11, Sus1 and the N-terminal region of Sgf73 (Köhler et al. 2008; Lee et al. 2009).

1.7. Thesis objective

There are many subunits of the SAGA complex in *Candida albicans* that have not been investigated. In this study we were interested in establishing the role of some of these subunits in different cellular processes - morphogenetic changes, growth, invasiveness, biofilm formation, as well as in checking the importance of these subunits under various cellular and genotoxic conditions. The main objective of this research was to investigate whether mutations in different modules of the SAGA complex generate different phenotypes, or whether loss of a component of any module created the same result. In this work, we investigated conditional and null mutants of components of each of the five SAGA complex modules; Ngg1 of the HAT module, Ubp8 of the Dub module, Tra1 of the recruitment module, Spt7 of the architecture module, and Spt8 of the TBP interaction unit to assess their role in processes such as filamentation, invasiveness, and biofilm formation. It appears Tra1 like its orthologue in *S. cerevisiae,* is essential, as we failed to get the homozygous deletion of this gene. We identified that SAGA complex modules can appear to work in opposition –

loss of HAT and DUB module subunits direct the cells into the yeast mode of proliferation, while loss of architecture and interaction module components direct cells into filamentous growth. We also showed that Spt7 and Spt8 have an important regulatory role in response to cell-wall, osmotic, temperature and cellular and genotoxic stresses while as Ngg1 and Ubp8 has a regulatory role in response to high temperature. All these outcomes imply that Spt7 and Spt8 are indispensable for regulation of characteristics such as cell morphology, cell cycle division, genotoxic and cellular stress responses and responses to antifungal drugs.

Chapter 2. Materials and Methods

2.1 *Candida albicans* strains and their derivations

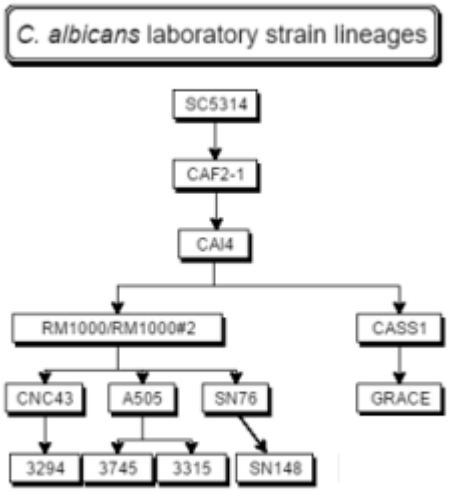


Figure 4. Derivatives of *C. albicans* strains used in this study. SC5314 is a wild type strain, belongs to *Candida albicans* clade and is representative of 40% clinical isolates world-wide. SC5314 has been sequenced to use as reference sequence. Wild type strains used in this study are derived from SC5314.

2.2 The GRACE Library

The GRACE™ (Gene Replacement And Conditional Expression) library is a collection of 2357 conditional mutants. These strains are prepared in the CaSS1 background strain (a derivative of SC5314) with the aim of finding drug targets and essential genes in the diploid *C. albicans* (Roemer et al. 2003). The GRACE library strains have been formed by replacing one copy of a gene with a *HIS3* cassette and placing the other copy of this gene under control of a conditional tetracycline promoter. The parental background strain CaSS1, and all the resultant strains, express a chimeric transactivation fusion protein - *E. coli* tetR binding domain linked with the *S. cerevisiae* Gal4 activation domain; this cassette, also containing the selectable *URA3* marker, is integrated at the *C. albicans LEU2* locus (Figure 5). Conditional repression of the mutants can be attained either by adding tetracycline/doxycycline or by allowing them to grow on medium supplemented with 5- fluororotic acid (5-FOA). In the presence of 5-FOA, cells with *URA3* gene convert it into 5- fluorouracil, which is toxic. Thus, growing the strains on 5- FOA plates can select for cells that have lost the *URA3* linked tetR transactivator module which in turn shuts off the tetR promoter-activated genes (Roemer et al. 2003).

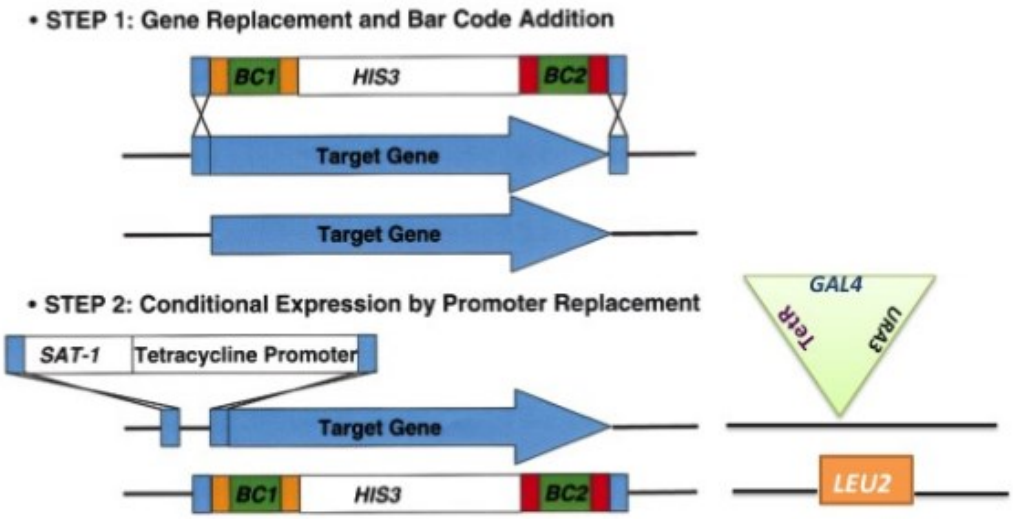


Figure 5. Schematic representation of the construction of GRACE library strains (Roemer et al. 2003).

2.3 Strains and oligonucleotides

The starting strain used for the construction of the SAGA deletion mutants was SN148 (Noble & Johnson, 2005). The knock-out mutants of *SPT7*, *SPT8, NGG1* and *UBP8* were built using the CRISPR/Cas9 method as described (Vyas, Barrasa, and Fink 2015). The *TRA1* mutant was attempted by classic homologous recombination replacement using *HIS1* and *URA3* as selectable markers. Table 2 contains the genotype descriptions for all the mutants constructed and strains mentioned. PCR and DNA sequencing were used to confirm all mutants mentioned in this work. Oligos and plasmids used in order to obtain and confirm the knock-out mutations are listed in Table 3.

Table 2. Strains used in this study.

Strains	*Parental*	*Genotypes*	*Reference*
CASS1	*CAI4*	*his3::hisG/his3::hisG leu2::tetRGAL4AD-URA3/LEU2*	*Roemer et al. (2003)*
NGG1tetR	*CASS1*	*ngg1::his3::hisG/his3::hisG leu2::tetRGAL4AD-URA3/LEU2*	*Roemer et al. (2003)*
TRA1tetR	*CASS1*	*tra1::his3::hisG/his3::hisG leu2::tetRGAL4AD-URA3/LEU2*	*Roemer et al. (2003)*
SPT7tetR	*CASS1*	*spt7::his3::hisG/his3::hisG leu2::tetRGAL4AD-URA3/LEU2*	*Roemer et al. (2003)*
SPT8tetR	*CASS1*	*spt8::his3::hisG/his3::hisG leu2::tetRGAL4AD-URA3/LEU2*	*Roemer et al. (2003)*
SN148	SN76	*arg4/arg4 leu2/leu2 his1/his1 ura3::imm434/ura3::imm434 iro1::imm434/iro1::imm434*	Noble & Johnson (2005)
*ngg1*Δ/Δ	SN148	ngg1::NGG1*/ngg1::NGG1* (CRISPR/CAS9) *arg4/arg4 leu2/leu2 his1/his1 ura3::imm434/ura3::imm434 iro1::imm434/iro1::imm434*	This study
*spt7*Δ/Δ	SN148	spt7::SPT7*/spt7::SPT7* (CRISPR/CAS9) *arg4/arg4 leu2/leu2 his1/his1 ura3::imm434/ura3::imm434 iro1::imm434/iro1::imm434*	This study
*spt8*Δ/Δ	SN148	spt8::SPT8*/spt8::SPT8* (CRISPR/CAS9) *arg4/arg4 leu2/leu2 his1/his1 ura3::imm434/ura3::imm434 iro1::imm434/iro1::imm434*	This study
*ubp8*Δ/Δ	SN148	ubp8::URA3/ubp8::URA3 (CRISPR/CAS9) *arg4/arg4 leu2/leu2 his1/his1 iro1::imm434/iro1::imm434*	This study

*Insertion of 3 stop codons in the repair DNA

Table 3. Primers used in this study.

Name	Sequence (5′ to 3′)
NGG1_sgRNA_F	atttgAGAATTAACACCAGAACACCg
NGG1_sgRNA_R	aaaacGGTGTTCTGGTGTTAATTCTc
NGG1_HR_F	ATTCAAAAACTTCCGAAAACGATAAAAAACGTAAAAATGAAGAATT CACATGATAACACC
NGG1_HR_R	TTCTTTGTTGCGCTCATAGGCACTTCGTCTTCATCGTCATGGTGTTATCA TGTGAATTCT
NGG1_Ex_F	GACTGATGCGCACTCTGTGTC
NGG1_Ex_R	CTCTTCCGACCAAAGATCCGC
SPT8_sgRNA_F	atttgAAATGAAGACGAGGAAGGTGg
SPT8_sgRNA_R	aaaacCACCTTCCTCGTCTTCATTTc
SPT8_HR_F	GGCGATGAAGATGAAGAAATGGCAGATGAAGATGGCGCATATGAAG ACTAGTAAGGTG
SPT8_HR_R	GCTCGTATCTTCTTCATCTTCTTCTTCTTCTTCTTACTCACCTTACTAGTCT TCATAT
SPT8_Ex_F	CATCAATCGAACAAGACGATC
SPT8_Ex_R	GTTAATGGTTGTTCATTTTCC
SPT7_sgRNA_F	atttgAATGAGAACAACGAGAGTGCg
SPT7_sgRNA_R	aaaacGCACTCTCGTTGTTCTCATTc
SPT7_HR_F	CCCGAAGATAAGAATGACAAACCTGAGACTCTAGACACCAATGAGAAC TAATAGAGTGC
SPT7_HR_R	ATCTCTATCACTACTATTGTTCTGAATTCTCTCACCAACAGCACTCTCGTTG TTCTCATT
SPT7_Ex_F	GCGATCTCTATGAAAAGCAAC
SPT7_Ex_R	CATCTTCATCTTCGTCCTCG
UBP8_sgRNA_F	atttgTGCCACCAATAATATCAATg
UBP8_sgRNA_R	aaaacCATTGATATTATTGGTGGCa
UBP8_HR_URA3_F	taatatataaATGCCTTCTGATGAAACAATATCTAAATAAAATGGCAATATCCACA TTGCCACGAATTCTATCTAATGAGGTTTATATACCGCCCCTTTT
UBP8_HR_URA3_R	CTCGTACAAAATTTATTTTATTATGCAATAATGTTGATGATTGATCAGAAAT GTCTAGTTGATCTGTAGTAGTAGGACCACCTTTGATTGTAAATAGT
UBP8_Ex_F	AACATCCATCTTCTCCTTGGCA
UBP8_Ex_R	CTTCTCCTCGTCGTGTTCACCT
URA3-F	TTGGGCAGATATTACCAATGC
URA3-R	GCTAAAGAAACCACCACCAA

2.4 Media

Yeast colonies were grown in yeast-peptone-dextrose YPD media (1% w/v yeast extract, 2% w/v Bacto peptone, 2% w/v dextrose, 80mg/L uridine with the addition of 2% w/v agar for solid medium) for 48hours at 30˚C. Yeast cells were cultured overnight from fresh single colonies and diluted in YPD liquid media to a starting OD_{600} of 0.2, and cultured for 4h for normal growing strains and 6h for slow growing strains at 30˚C, 220rpm. Hyphal colonies were induced in 10% serum supplemented YPD media plates containing 2% agar and in Spider media (1% Difco nutrient broth, 1% mannitol, 0.2% dibasic potassium phosphate, pH 7.2) plates containing 2% agar for 5 days at 37˚C. Hyphal cells were induced from overnight YPD cultures diluted in 10% serum supplemented YPD and Spider media from a starting OD_{600} of 0.2 and incubated for 4h for normal growing strains and 6h for slow growing strains at 37˚C respectively, 220rpm. All assays performed with the conditional repressed mutants were supplemented with 100µg/mL tetracycline.

2.5 Microscopy

For cell morphology, overnight cultures were grown in non-inducing media - YPD at 30°C and inducing media - Spider media and 10% fetal calf serum and were subjected to phase differential interference contrast microscopy. Cell morphology was assessed under 100x magnification. 1000 cells were counted and divided into three morphological categories: yeast, pseudo-hyphae, and hyphae. Four biological replicates were made for each mutant from the SAGA complex. The results were analyzed, and the graphs were made using Microsoft. For nuclear segregation analysis, DAPI staining of live cells were performed without permeabilization. Overnight cultures were resuspended at a starting optical density 600 nm (OD600) = 0.2 in YPD medium (1 % w/v yeast extract, 2 % w/v Bacto peptone, 2 % w/v dextrose, 80 mg/L uridine, and incubated for 4 to 6 hours (until successful completion of first cellular division of both wild type and mutant strains). To visualise DNA, cells were washed twice with 1X PBS followed by the addition of 3µg/mL DAPI (Sigma-Aldrich) into each tube. To visualise cell membrane and chitin distribution, Calcofluor staining (1.5µg/mL) was performed using similar strategy. Cells were examined by DIC and fluorescent microscopy at 100X magnification using a Leica DM 6000 microscope (Leica Microsystems Canada, Richmond Hill, ON, Canada) equipped with a Hamamatsu-ORCA ER camera (Hamamatsu Photonics, Hamamatsu City, Japan) and the HCX PLFLUO TAR 100× NA 1.30–0.6 oil objectives. Differential interference contrast optics or epifluorescence with DAPI (460nm) filters were utilized. Images were captured with Volocity software

(Improvision, Perkin-Elmer, Waltham, MA) and images were analysed using ImageJ/Fiji software.

2.6 Phenotypic sensitivity

To test sensitivity phenotypes, mutant strains from the SAGA complex were subjected to different stress conditions. The strains were inoculated from single colonies in 5mL YPD and incubated at 30˚C, overnight, and were diluted to OD 0.2. The starting dilution was used in a subsequent 1:10 serial dilution and 3 µL of each dilution were spotted onto the stress plates containing YPD agar media supplemented with menadione (0.15 mM) and hydrogen peroxide (7.5 mM) were used for oxidative stress assays; methyl methane sulfonate (MMS, 0.01 v/v), hydroxyurea (15mM) were used in DNA damage stress assays; and, Congo red (200 µg/mL) and antifungal caspofungin (0.75 µg/mL)were used to test the mutants for cell wall stress; dithiothreitol (DTT 30 mM) provided ER stress by forcing the accumulation of unfolded proteins; NaCl (1.5M),$CaCl_2$ (400mM) and glycerol (250 mM)were used for osmoticstress; fluconazole (10µg/mL), hygromycinB (100µg/mL), and anidulafungin(0.25µg/mL) treatments were used to trigger antifungal drug response. All YPD Plates were incubated at 30°C for 4 days except for caspofungin (200µg/mL) containing plates that were incubated for 7 days at 30 °C. To test for the mutants' ability to grow under temperature stress, YPD agar plates grown at 37˚C and 42˚C. YPD agar plates with pH 8.3 and pH 5 were used to test the mutants for response to alkaline and acidic stresses and incubated at 30°C for 48h.

2.7 Invasiveness assay

Overnight cultures from fresh single colonies were grown in liquid YPD at 30˚C and 220 rpm and diluted to an OD_{600} of 1.0. 3µL samples were spotted on YPD agar plates and Spider media plates and incubated at 30˚C and 37˚C for 120h. The resulting colonies were then washed gently under running water for 15 seconds to remove the non-adherent surface cells, and the invasiveness of the samples was observed. The colonies that remained on the plates after washing were considered invasive (invaded the agar and remained after washing) and those washed away (the cells that didn't invade the agar) were counted non-invasive. Two biological replicates were prepared for each sample. The plates were scanned before washing and after washing at 600 dots per inch (dpi) using an Epson Perfection v500 photo scanner.

2.8 Biofilm assay

The strains were inoculated in 5mL of liquid Spider media and incubated at 24˚C for 24h. 4 x 10^7 cells of each sample were added to 1mL of Lee's media in a 24-well flat-bottom plate. The media was discarded, and the biofilms were washed three times with 1mL DPBS buffer. The plates were allowed to dry, and the biofilms were stained with 325µL of 0.4% crystal violet for 45 minutes. The staining solution was washed 3 times with 1mL of sterile miliQ water and allowed to dry. The biofilms were de-stained with 500µL 95% ethanol. The amount of biofilm was measured based on the absorbance at 595nm. For each sample, three biological replicates were prepared. Results were analyzed and graphs made with GraphPad Prism (version 6.0).

2.9 Macrophage engulfment assay

The RAW 264.7 murine macrophage cell line was kindly provided by Dr. Albert Descoteaux (INRS-Armand-Frappier, Laval, QC, Canada). Macrophages were cultured in DMEM medium supplemented with 10% FBS, penicillin/streptomycin and HEPES. Macrophages were seeded at $2x10^5$ and grown for 48h at 37^0 C and 5% CO_2. Once cells reached 80% confluency, macrophages were collected with Trypsin-EDTA and centrifuged for 10 mins at 10,000 x rpm, at room temperature. Then cells were stained with trypan blue and counted. An aliquot of $1.2x10^6$ cells/mL was prepared for further macrophage engulfment assays.

Knock-out mutants *ngg1*Δ/Δ, *spt7*Δ/Δ, *spt8*Δ/Δ and *ubp8*Δ/Δ, derived from the parental strain SN148 were grown overnight in 5mL YPD medium at 37°C, 220x rpm shaking incubation. An aliquot of each mutant and the parental strains was taken to grow again for 3h prior to assay. An aliquot of each SAGA knock out mutant and the parental strain, were spun down, the media removed and cell pellet washed 3 times with PBS (phosphate buffer saline) and adjusted to a final fungal cell concentration of 1 x 10^8 cells/mL .Fungal cells were then stained with 50mg/mL of Calcofluor White (CFW, Sigma), and incubated for 10min at RT ,then washed three times in PBS and finally a 1/100 dilution in PBS was prepared for each strain in the assay.

Both macrophages and fungal cells for each strain were mixed in a ratio 1:10 (Candida cell: macrophage) in a well of a 96-well plate and then visualized in a high-content screening microscope ImageXpress XSL wide-field (Molecular Devices). The plate was placed in a chamber equilibrated at 37°C and 5%CO_2. Images were captured at 40x objective magnification on two channels (transmitted light and DAPI), at time point 0 and then every 5

min for 4h total running time. After, for every SAGA strain and macrophage images, a time-lapse video was generated, using the MetaXpress high content imaging acquisition and analysis software (Version 6.1.1, Molecular Devices). Fungal cells (budding or filament forms) engulfed by macrophages, were counted for every time-point, and normalized to the ratio: [number of fungal cells at time point 0/number of fungal cells for every time point] x 100. Macrophage engulfment kinetic curves, histograms, and statistical analysis were analyzed with GraphPad Prism (Version 6.0).

2.10 RNAseq analysis

The deletion mutant strains and control SN148 cultures were grown in YPD media from a starting OD_{600} of 0.1 and the cultures were allowed to grow at 30˚C, 220 rpm until OD_{600} 1.0. Total RNA was extracted using the Qiagen RNeasy minikit. The quality of RNA was assessed via Agilent 2100 Bioanalyzer using the Agilent RNA 6000 Nano kit. The RNAseq was performed by McGill University and Genome Quebec Innovation Centre using an Illumina MiSeq. Raw reads were pre-processed with the sequence-grooming tool cutadapt version 0.4.1 (Martin 2011)(Martin 2011) with the following quality trimming and filtering parameters (`--phred33 --length 36 -q 5 --stringency 1 -e 0.1`). Each set of paired ends read 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. SAMtools was then used to sort and convert SAM files. The read alignments and *C. albicans* SC5314 genome annotation were provided as input into 13 StringTie v1.3.3 (Pertea et al. 2015), which returned gene abundances for each sample.

2.11 Statical analysis

Data are presented as means ± standard errors of the means from separate experiments and were compared using one-way analysis of variance (ANOVA) and students *t*-test. The level of significance was set at a P value of <0.05. All statistical analyses were performed using GraphPad Prism (version 6) statistical software (GraphPad Software, San Diego, CA) and Microsoft Excel.

Chapter 3. Results

3.1 SAGA mutants can have opposing consequences for filamentation and invasiveness

To investigate mutants belonging to different modules of the SAGA complex in *C. albicans*, we initially made use of conditionally repressed mutants from the GRACE™ library (Roemer et al. 2003) including *TRA1 – ORF19.139* (recruitment module), *NGG1 – ORF19.3023* (HAT module), *SPT7 – ORF19.7572* (architecture unit) and *SPT8 – ORF19.4312* (TBP-associated unit); these genotypes are described in Table 1. We assessed the role of conditional SAGA mutants in filamentation and invasiveness in presence or absence of tetracycline (100 µg/mL). We found that conditional mutants *spt7* and *spt8* were filamentous and invasive in both filamentous inducing and non-inducing conditions while *tra1*Δ and *ngg1*Δ were in a yeast-locked state compared to wild type. As well, similar to *spt3*Δ/Δ and *spt20*Δ/Δ deleted strains in *C. albicans* (Laprade et al.,2002; Tan X et al., 2014), we found cells of the *spt7* and *spt8* conditional mutants did not separate properly during cell division and appeared clumped together. Also, the *spt7* and *spt8* repressed mutants formed wrinkled colonies in both inducing and non-inducing conditions- indication of filamentous cells while the *ngg1* and *tra1* repressed mutants formed smooth colonies even in the hyphal-inducing conditions of either 10% fetal calf serum (FCS) or Spider media at 37˚C for 5 days compared to the wild type CaSS1 strain (data not shown).

However, there are numerous issues associated with repression of gene transcription – the possibility of promoter leakage, the fact that the non-repressed mutants might have increased expression of routinely low expressed genes and therefore activate biological processes that in a wild type background would not be active, and ultimately the necessity of addition of tetracycline or doxycycline, which are iron chelators, and might act as a source of stress to the mutant (Samaranayake and Hanes 2011; Fiori and Van Dijck 2012). Also, the GRACE™ library is not comprehensive, as some SAGA modules (like the Dub module) do not have representatives in the collection. Therefore, we created null mutants for Spt7 from the architecture unit, Spt8 from the TBP-binding module, Ngg1 from the HAT-module, and Ubp8 from the de-ubiquitination module; these genotypes are described in Table 2. We failed to create a Tra1 null mutant strain despite repeated attempts. However, the Tra1 GRACE library strain was viable under repressing conditions, and when this library strain was grown on 5-FOA media to create a null mutant by removing the trans-activator tetR binding domain cassette linked with the *URA*3 gene, we were able to get colonies on 5-FOA agar media. The removal of the trans-activator domain was confirmed through PCR. These

observations had suggested that Tra1 may not be essential in *C. albicans*; this would be unprecedented, because the Tra1 function is essential in all other organisms investigated, and there is no evidence for a duplicated gene in *C. albicans*. However, when we attempted to remove the FATC domain (C-terminal domain) of Tra1, which plays an important role in cellular viability as part of its orthologue in *S. cerevisiae* (Hoke et al. 2010), we failed to get homozygous deletion of the domain. This suggests that Tra1 is in fact an essential protein in the SAGA complex, as previously found by *in vivo* transposon mutagenesis and machine learning analysis in a stable haploid isolate of *C. albicans* (Segal et al. 2018), true Tra1 inactivation leads to inviability in the fungal pathogen as in other systems where it has been investigated.

We assessed the colony morphology of SAGA mutants in normal yeast growing conditions (YPD) and both rich and starvation hyphal-inducing conditions specifically, 10% fetal calf serum (FCS) and mannitol-based Spider media at 37˚C. As shown in Figure 6, under yeast-growing conditions, both *spt7*Δ/Δ and *spt8*Δ/Δ null mutants generate wrinkled, crenulated colonies, and this wrinkled phenotype intensified when we induced hyphae by growth on 10% FCS or Spider media. The wrinkled and crenulated colonies suggest the presence of filamentous cells. These mutant cells are very filamentous when grown in liquid YPD media, showing a mix of hyphae and pseudo hyphae, but mainly pseudo-hyphae with branched filaments. The same phenotype was observed when the mutants were induced to form hyphae in 10% FCS or Spider media at 37˚C for 3h. The *spt8*Δ/Δ phenotype is consistent with that of the hyper-filamentous Spt3 deleted strain in *C. albicans*, which removes a subunit of the same module (Laprade et al. 2002). Similar to *spt3*Δ/Δ and *spt20*Δ/Δ deleted strains in *C. albicans* (Laprade et al. 2002; Desai and Mitchell 2015), we also found *spt7* and *spt8* mutant cells didn't separate properly during cell division and appeared clumped together. In contrast, the colonies of the deleted *ngg1* strain were smooth on either hyphae-inducing medium. The cells also appear mainly yeast or pseudo-hyphal in liquid media after of hyphae induction at 37˚C in both hyphal inducing conditions. The *ubp8* null from the deubiquitination module shows classic yeast morphology in both non-inducing- YPD media and hyphae- inducing media -10% FCS or Spider media at 37˚C. (Figure3.1). Overall, these results indicate that filamentation of *spt7*Δ/Δ and *spt8*Δ/Δ under both inducing and non-inducing conditions might influence a common, core component of the cellular machinery that plays a role hyphal formation, most likely a component downstream of multiple different signaling pathways.

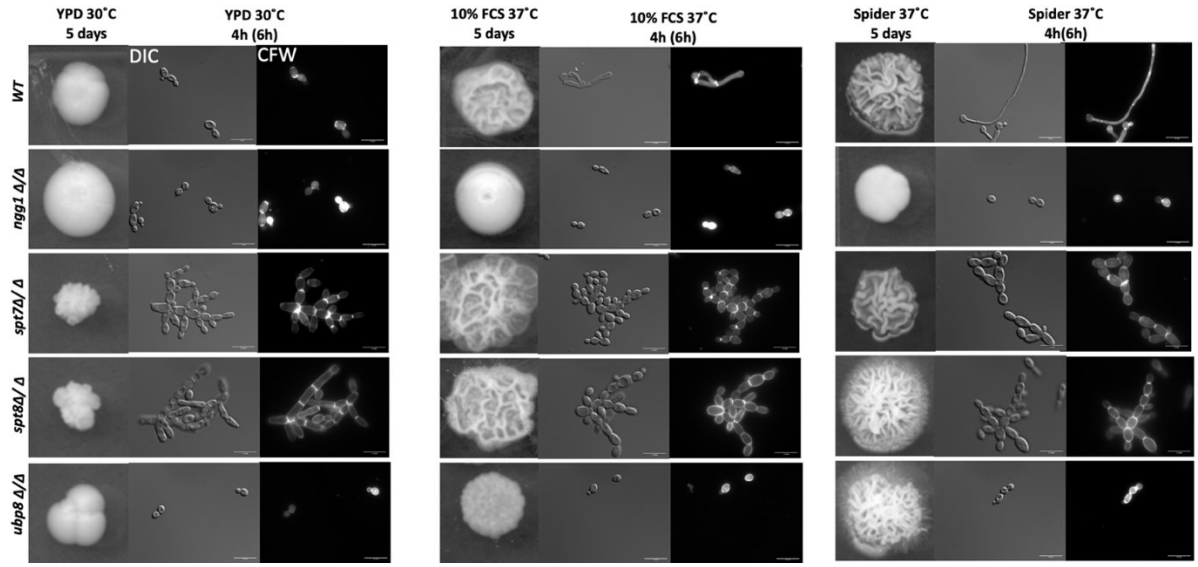


Figure 6. Colony and cellular morphology of SAGA mutants in *C. albicans.* 1:10 serial dilution of overnight culture of mutants were spotted on to yeast-growing conditions – YPD agar and hyphae-inducing conditions – 10% fetal calf serum media (FCS) and Spider media plates. The colony morphology was assessed after 5 days of incubation. Cells from liquid media were inoculated at starting OD_{600} of 0.2 and grown in liquid YPD, 10% FCS supplemented YPD and Spider medium, at 220 rpm, and 30˚C or 37˚C for 4h for normal growing strains (WT, *ngg1*Δ/Δ and *ubp8*Δ/Δ) and 6h for slow growing strains (*spt7*Δ/Δ and *spt8*Δ/Δ). The cells were washed with 1x PBS twice and stained with 2µg/mL calcofluor white (CFW). The cells were observed with the Leica DM6000 microscope at 100x magnification- DIC (Differential Interference Contrast). Scale bar = 15µm. *spt7*Δ/Δ and *spt8*Δ/Δ appear more hyphal compared to control and are mostly in pseudo hyphal state in inducing medium whereas *ngg1*Δ/Δ and *ubp8*Δ/Δ appear in yeast locked state. The control switches its morphology upon changed conditions while the SAGA mutants remain in their initial states upon induction.

We have extended the assessment of the phenotypes of these 4 non-essential SAGA module components. *spt7*Δ/Δ and *spt8*Δ/Δ deleted mutants grow slower compared to the background strain (SN148), so their growth was observed in rich YPD and SD media at 30˚C at different time intervals. As shown in Figures 7A and 7B, the *spt7*Δ/Δ and *spt8*Δ/Δ mutant strains grew considerably slower compared to the wild type during the first 30h of growth in YPD at 30˚C. The *ubp8*Δ/Δ mutant strain has the opposite behavior, growing slightly faster than the wild type SN148 strain in rich media. There is also description of the hyper-filamentous, slow-growing *spt3*Δ/Δ deleted mutant in *C. albicans* (Laprade et al. 2002) similar to the *spt8*Δ/Δ deleted mutant affecting another subunit of same SAGA module. This evidence suggests a role of negative regulation on filamentation of the TBP-interaction unit. This supports our observation that Spt7 and Spt8 act in the negative regulation of filamentation whereas Ngg1 and Ubp8 appear to function in positive regulation of hyphal development.

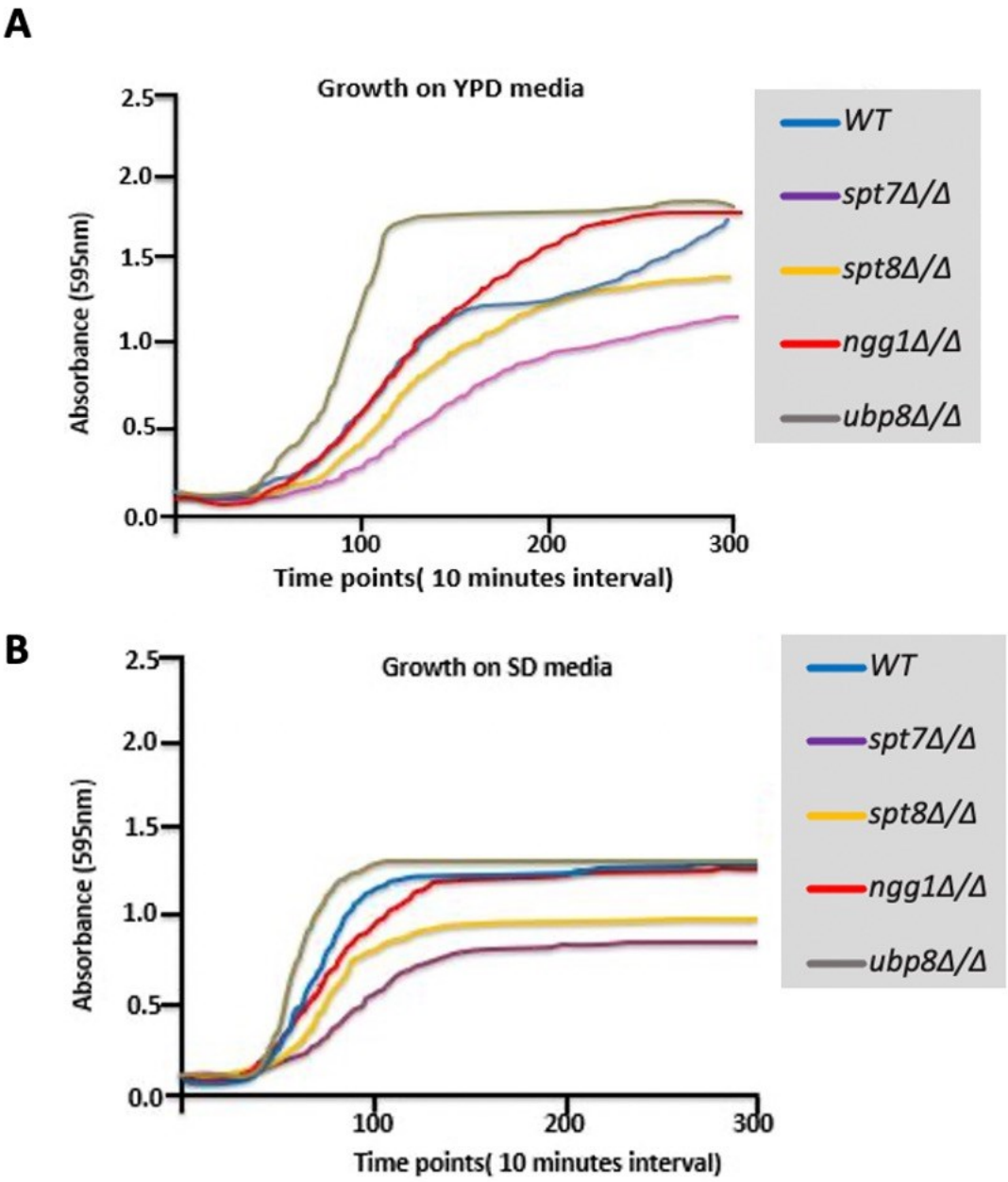


Figure 7. Growth curves of SAGA mutants. (A, B) Graphs showing growth rates of mutants on YPD and SD media. Growth rate of each strain was assessed using Sunrise™ TECAN plate reader over a period of 5 days by following the growth of 7 biological replicates from a starting OD_{600} 0.001 in 200µl YPD in 96 well plates at 30˚C. Results were analyzed, and graphs were plotted. *spt7Δ/Δ* and *spt8Δ/Δ* strains grew slowly compared to WT; *ngg1Δ/Δ* and ubp8*Δ/Δ* grew normally compared to WT.

Since filamentation (and ultimately invasiveness) in *C. albicans* is often associated with virulence, we tested the null strains for invasion in a plate-washing assay. An overnight

grown culture was spotted onto YPD agar at 30^0C and Spider media at 37^0C respectively and incubated for 120h followed by washing with a stream of milliQ water for 15 seconds. As shown in Figure 8A, after 120h incubation the *spt7*Δ/Δ and *spt8*Δ/Δ mutants were more invasive than the control in both yeast-growing and filamentous conditions at 30˚C and 37˚C whereas *ngg1*Δ/Δ and *ubp8*Δ/Δ were non-invasive like the wildtype (Figure3.2.2). The Spt7 and Spt8 knock out mutants were the most constitutively invasive, consistent with their hyper-filamentous phenotype. The plate-washing assay on YPD media at 30˚C showed the invasive phenotype when there was no inducing signal present, suggesting the *spt7*Δ/Δ and *spt8*Δ/Δ deleted mutants are constitutively activated. The spots for *spt7*Δ/Δ and *spt8*Δ/Δ were considerably more invasive compared to non-invasive conditions (Figure 8A), indicating a strong role of Spt7 and Spt8 on the negative regulation of invasion while Ngg1 and Ubp8 act positively as does Gcn5 which is a member of the HAT module as is Ngg1 (Chang, Fan, and Chen 2015).

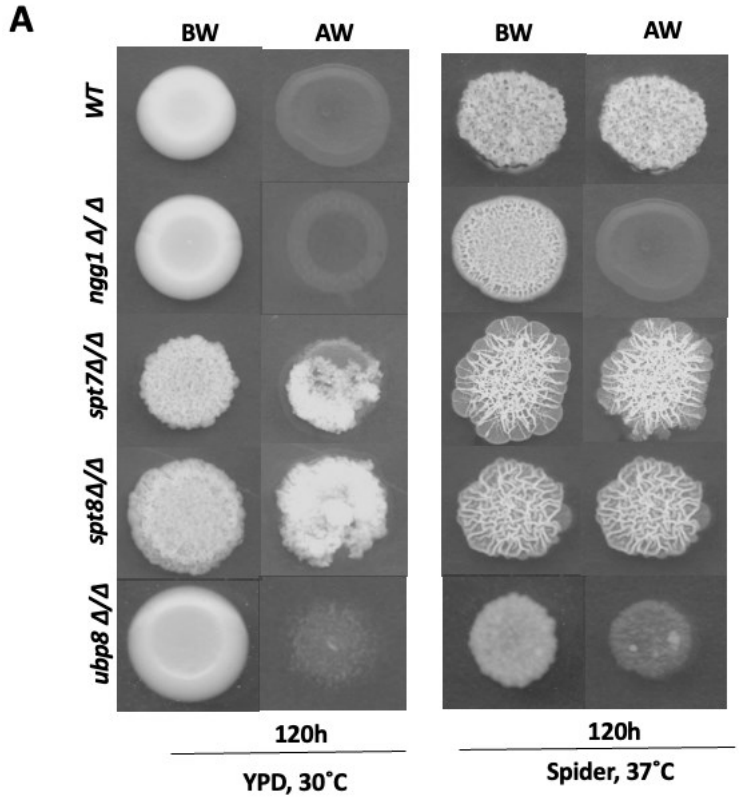


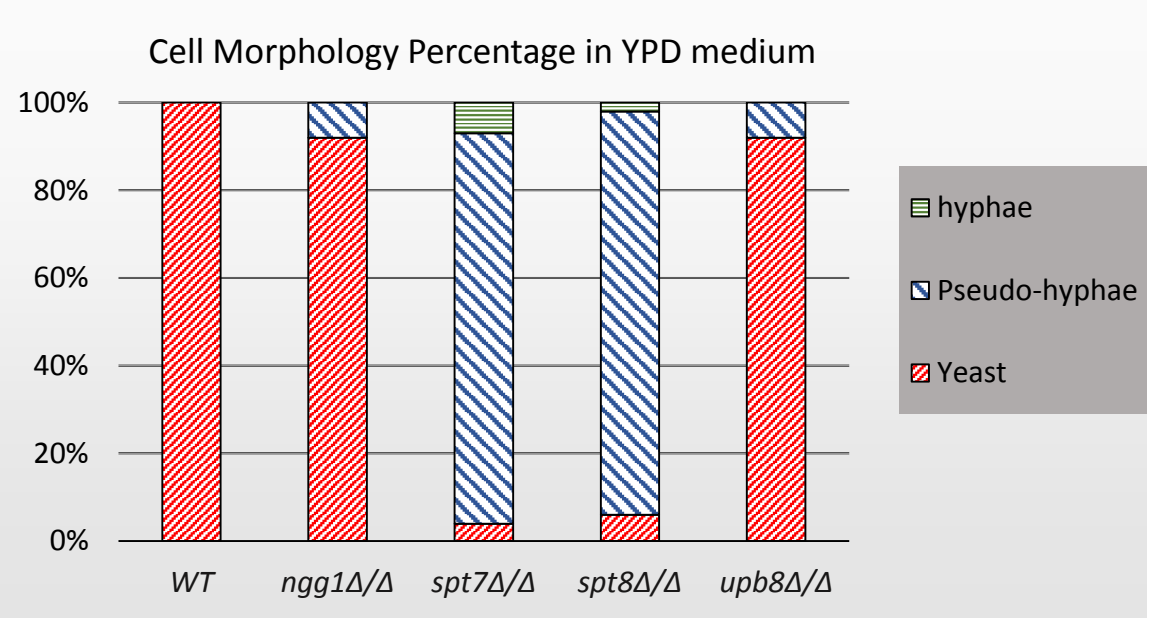
Figure 8. Invasiveness of SAGA mutants in *C. albicans*. Overnight cultures from fresh single colonies were grown in liquid YPD at 30˚C and 220 rpm and diluted to an OD_{600} of 1.0. 3µL samples were spotted on YPD agar plates and Spider media plates and incubated at 30˚C and 37˚C for 120h. The resulting colonies were then washed gently under running water for 15 seconds to remove the non-adherent surface cells, and the invasiveness of the samples was observed. The *spt7Δ/Δ* and *spt8Δ/Δ* knock-out mutants

were found to be invasive. However, *ngg1*Δ/Δ and *ubp8*Δ/Δ were non-invasive in both conditions compared to its control.

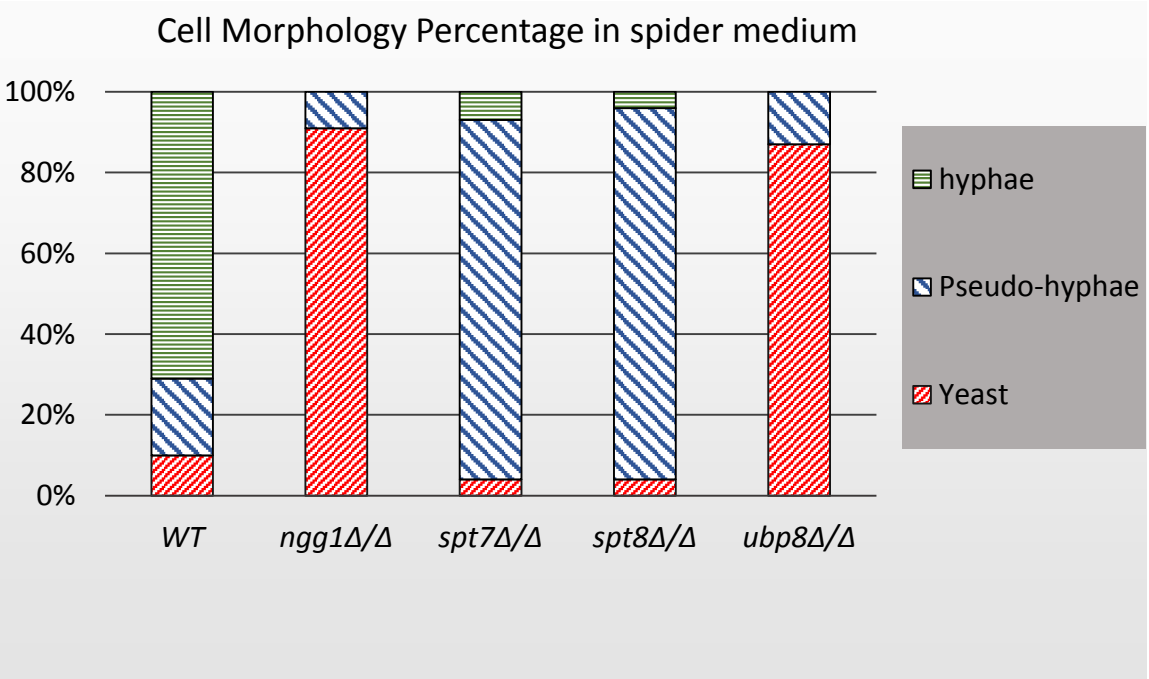
3.2 Cellular characteristics of SAGA mutants

To study the cellular phenotypes of SAGA mutants, we grew the mutants in non-inducing (YPD media) and filamentous inducing liquid media (Spider media and 10% FCS) at 30^0C and 37^0C respectively. We found that *spt7Δ/Δ* and *spt8Δ/Δ* cells displayed abnormal phenotypes in both filamentous inducing and non-filamentous inducing media where individual mutant cells were morphologically abnormal ranging from enlarged and elongated yeast-like cells to pseudo hyphal cells. The *ngg1Δ/Δ* and *ubp8Δ/Δ* cells were in an enlarged yeast-locked state compared to their isogenic wild type. There were 7% (±1%) hyphal, 88% (±2.5%) pseudo hyphal and 5% (± 0.5%) yeast variants in the *spt7* deleted mutant strain cultured in YPD media; 7% (± 1%) hyphal, 89% (±3%) pseudo hyphal and 4% (±0.5%) yeast variants in the *spt7* knock out strain cultured in Spider media and 5% (0.5%) hyphal, 93% (±3%) pseudo hyphal and 2%((±0.5%) yeast forms in the *spt7* deleted strain grown in 10% (±1%) FCS. Similarly, for *spt8* deleted mutants, there were 2% (±0.5%) hyphal, 92% (±2.5%) pseudo-hyphal and 6% (±0.5%) yeast variants in YPD media: 4% (±0.5%) hyphal, 92% (±2.5%) pseudo-hyphal and 4% (±0.5%) yeast variants in Spider media and 4% (±0.75%) hyphal, 91% (±1.5%) pseudo-hyphal and 5% (±0.5%) yeast variants in serum media. In dramatic contrast, for the *ngg1* deleted strain, there were 0% hyphal, 8% (±1%) pseudo-hyphal and 92% (±1.5%) yeast variants in YPD media, 0% hyphal, 9% (±1.5%) pseudo hyphal and 91% (±1.75%) yeast variants in Spider media and 0% hyphal, 11% (±1.75%) pseudo-hyphal and 91% yeast variants in serum media. A similar pattern was observed for the *ubp8* knock out mutant strain; there were 0% hyphal, 9% (±1.75%) pseudo-hyphal and 91% (±2.5%) yeast variants in YPD media, 0% hyphal, 13% (±1.5%) pseudo-hyphal and 87% (±2%) yeast variants in Spider media and 0% hyphal, 14% (±2%) pseudo-hyphal and 86% (±3%) yeast variants in serum media. For the wild type cells - 100% were yeast form cells when grown in YPD media; 71% (±3%) hyphal, 19% (±2%) pseudo-hyphal and only 10% (±1.5%) yeast variants when grown in Spider media and 69% (±2.5%) hyphae, 19% (±1.5%) pseudo hyphae and 12%(±1%) yeast cells when grown in serum media (Figure 9A-9C).

(A)


Cell Morphology Percentage in YPD medium
100%
80%
60%
40%
20%
0%
WT
ngg1Δ/Δ
spt7Δ/Δ
spt8Δ/Δ
upb8Δ/Δ
hyphae
Pseudo-hyphae
Yeast

(B)


Cell Morphology Percentage in spider medium
100%
80%
60%
40%
20%
0%
WT
ngg1Δ/Δ
spt7Δ/Δ
spt8Δ/Δ
ubp8Δ/Δ
hyphae
Pseudo-hyphae
Yeast

(C)

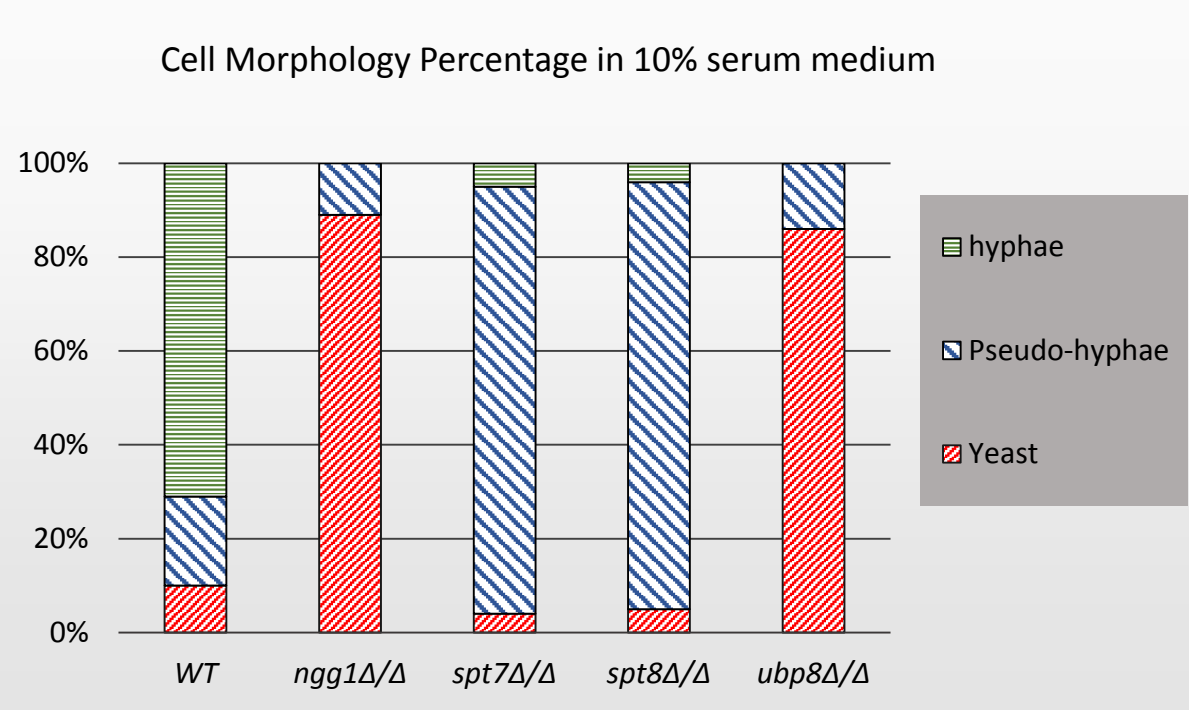


Figure 9. Cell morphology percentage and cell growth of SAGA mutants. (A, B, C) Clustered column graphs showing percentage of different cell morphologies displayed by mutants grown in YPD, Spider media and FCS. Cell morphologies were assessed using 100x magnification. 1000 cells were counted, quantified, and divided into the morphological categories- yeast, pseudo hyphae and hyphae. Four biological replicates were made for each mutant. *spt7* and *spt8* mutants showed pseudohyphal state in all conditions compared to its control and *ngg1* and *ubp8* mutants showed yeast locked state in both inducing and non-inducing state.

We further measured the cell size of the SAGA mutants in log phase yeast growth conditions (n=220). The yeast-locked cells of *ngg1* and *ubp8* deleted mutants were in range of 11.2-59.8 μm^2 and 11.1-28.732.1 μm^2 respectively. However, the *spt7* and *spt8* deleted cells grew as clusters with cell areas in the range of 22.19-101.3μm^2 and 21.44- 95.68 μm^2 respectively and are four to five times the size of the wild type which were in range of 6.4-23.98 µm2 (Figure 10). These results indicate that deletion of SAGA subunits has significant impact on the cellular morphology of *C. albicans.*

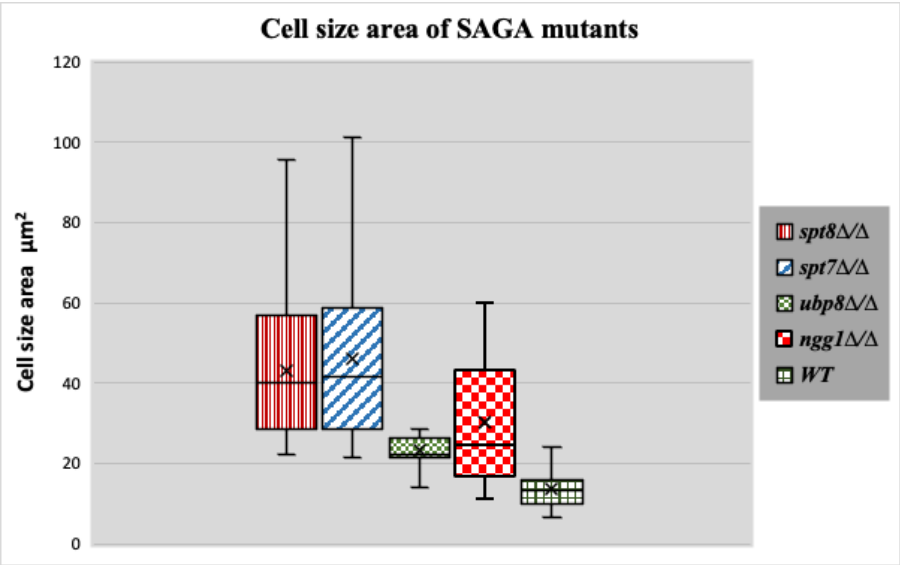


Figure 10. Box and whisker plot showing the cell size area of the SAGA mutants. Log phase yeast cells were stained with calcofluor white and measured in the DAPI channel using ImageJ Fiji 1.0 to assess the cell size area of each cell individually. The graph shows the *spt7* and *spt8* mutant cells are four-five times the size of WT. A majority of the cells of the *spt7Δ/Δ* and *spt8Δ/Δ* strains were in the range of 28-58µm² with means of 43 and 45µm² respectively. A majority of the cells of *ngg1Δ/Δ* are in the range of 16.5-43µm² with mean of 29.9µm² and *upb8Δ/Δ* cells were in the range of 22.1-26.4µm² with mean of cell 23.2µm² compared to WT which has a range of 6.4-13.8 µm² with a mean of 13.8µm².

As the SAGA *spt7* and *spt8* deletion mutants grew slowly on plates and liquid media, we measured the cell density of the SAGA mutants after 24 hours incubation by direct haemocytometer counting to avoid inaccurate quantification in spectrophotometric growth measurements due to filamentation. We started a cell count at 1 x 10^7 cells/mL; the *spt7* and *spt8* deleted mutants reached a density of 20 x 10^7 cells/mL and 25 x 10^7 cells/mL respectively after a 24-hour incubation at 30°C in liquid YPD media. The *ngg1* and *ubp8* deleted cells reached 75 x 10^7 cells/mL and 91 x 10^7 cells/mL respectively in the same conditions, compared to the wild type which reached 84 x 10^7 cells/mL (Figure 11). Furthermore, we checked the cell densities of the SAGA mutants using spectrophotometer (OD 600nm) - the wild type reached an OD of 1 starting from 0.1 within 6 hours, while the *ngg1* and *ubp8* deleted mutants reached an OD of 1 in 5-6 hours, and the *spt7* and *spt8*

deleted mutants took 16 hours to reach an OD of 1. These results highlight the impact of Spt7 and Spt8 on the growth of *C albicans.*

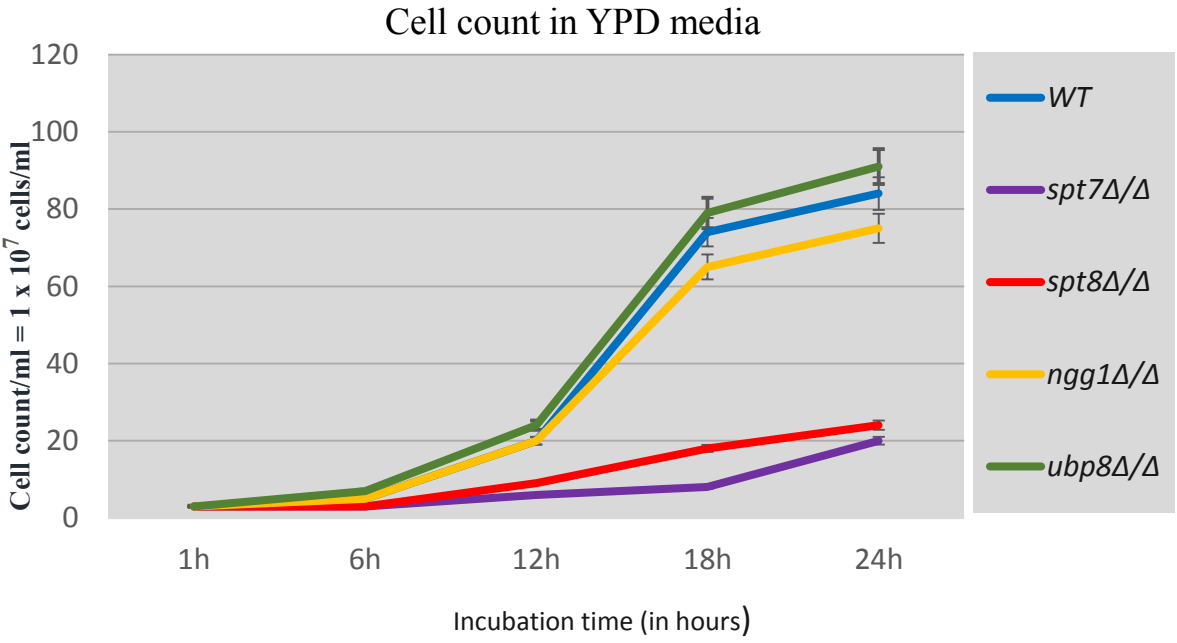


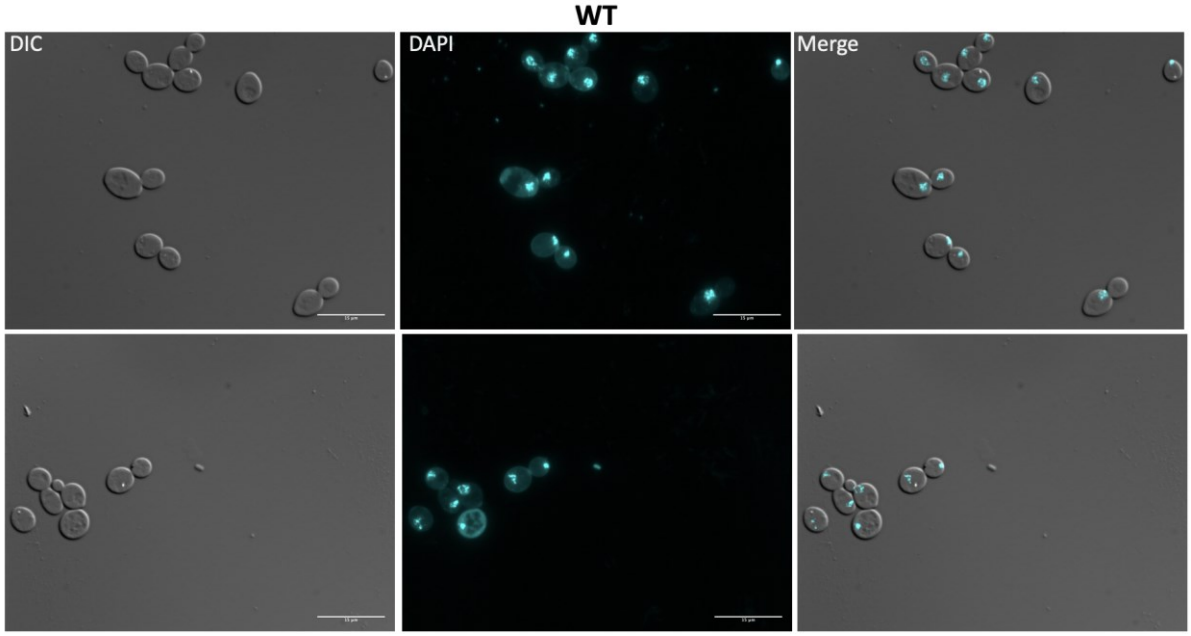
Figure 11. A line graph showing cell counts of mutants and wild type cultures in YPD at 30°C for 24 h. The initial cell count was started at 1 × 10^7 cells per ml. Both *spt7* and *spt8* mutants doubled their first cell population at the 6-hour mark while the *ngg1, upb8* mutants and the wild type doubled every 2 hours. Error bars are based on the standard deviation from two biological replicates at each time point.

3.3 Spt7 and Spt8 mutant strains displayed cell cycle related defects

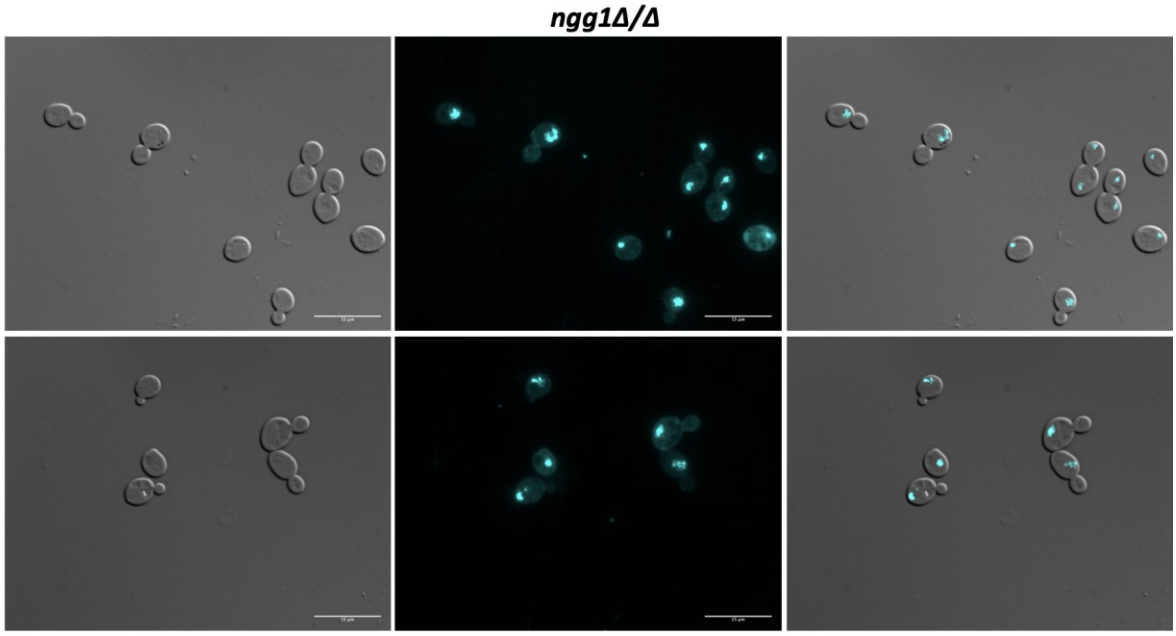
To study the effects of SAGA mutants on nuclear segregation, mutant cells of each SAGA subunit (n=210) and wild type cells (n=210) were stained with DAPI and observed under a microscope. The *spt7* and *spt8* deleted mutants showed similar phenotypes of cell clumping and difficulties in separation, so we investigated the patterns of nuclear distribution. Intriguingly, they showed considerable differences in their nuclear distribution, as 34% of *spt7*Δ/Δ cells were binucleate (n=72), 18% have diffuse nuclei (n=37), and 48% were mononucleate (n=100), whereas 95% of the *spt8*Δ/Δ deleted cells were mononucleate (n= 199). Both *ngg1*Δ/Δ and *ubp8*Δ/Δ mutant cells showed normal patterns of yeast cell morphogenesis where 94% large, budded cells have two nuclei, one in each bud cell and mother cell (n=98), while 90% (n=94) of small, budded cells have nuclei at the junction of bud neck and mother cell similar to wild type strain where 90% (n=94) of large budded cells have 2 nuclei, one in each bud and mother cell while 92% (n=97) of small budded cells have

nuclei at the junction of the bud neck and mother cell (Figure 12C). These results suggest that in the filamentous phenotypes of SAGA mutants, the cells might be in late S/G2 phase or defective/late M phase, reasonably due to failure in DNA repair machinery which resulted in abnormal nuclear content and an increased cell size. For nuclear segregation between mother and daughter cells, septal ring formation is required (Berman 2006). Further we tested the SAGA deleted mutants for the chitin composition of cell wall and septa using calcofluor staining. All the strains showed uniform chitin distribution in their cell walls and at septal junctions having well prominent and distinct septa like its wild type (Figure 12F). All these morphological defects in *spt7* and *spt8* deleted strains (slow growth, enlarged cell size, filamentation and abnormal nuclear segregation) indicate that Spt7 and Spt8 are needed for normal cellular physiology in *C. albicans.*

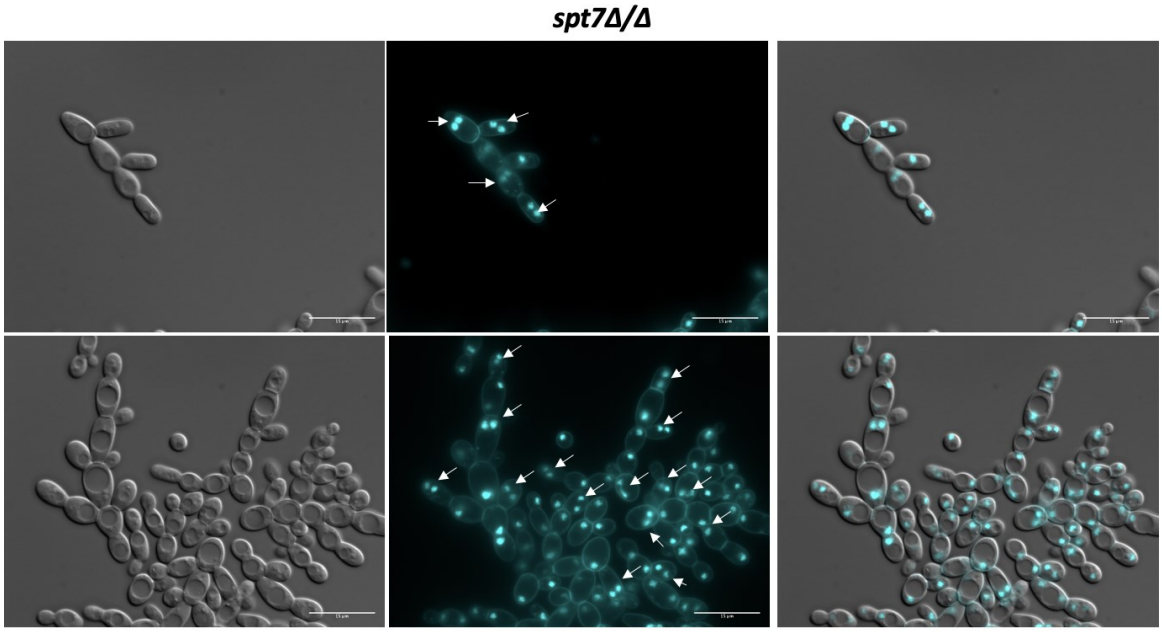
(A)



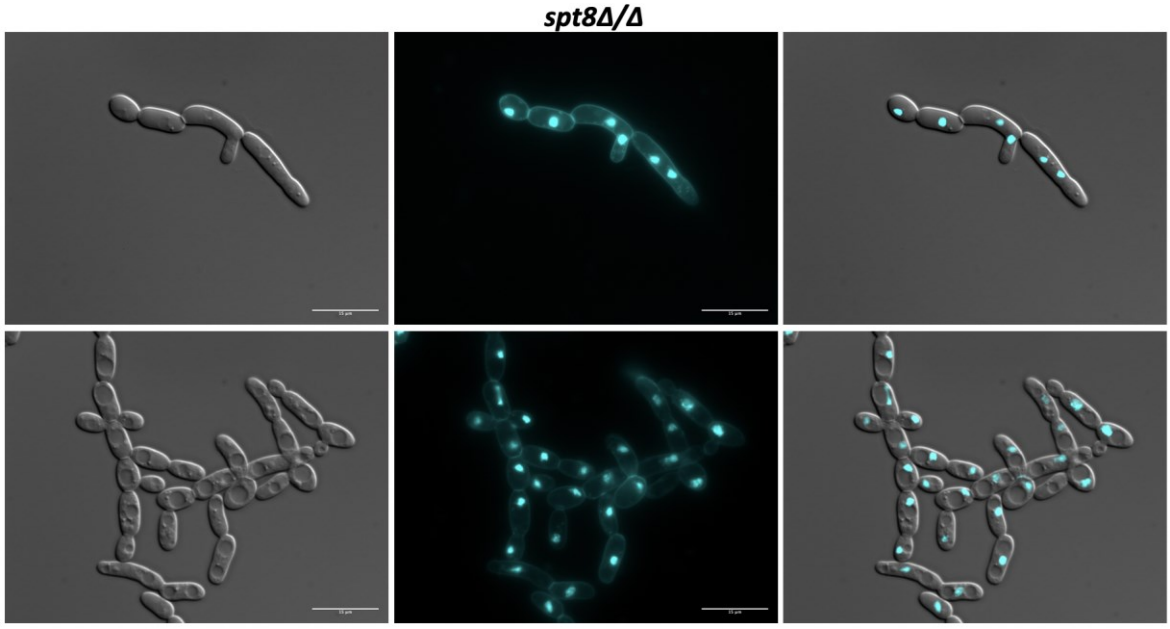
(B)



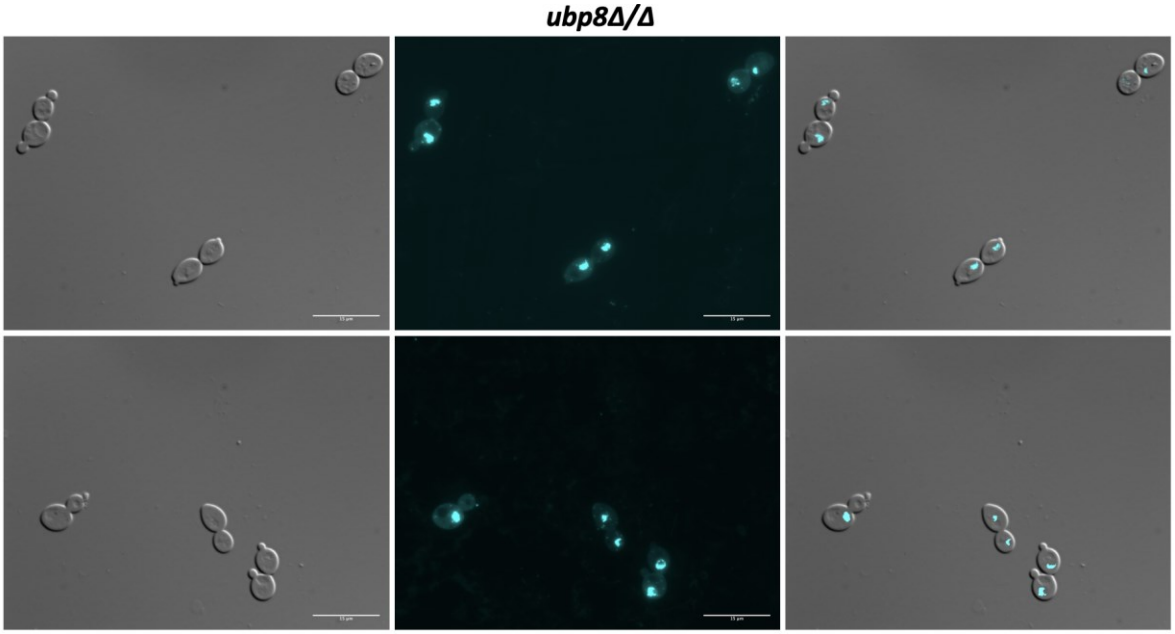
(C)



(D)



(E)



(F)

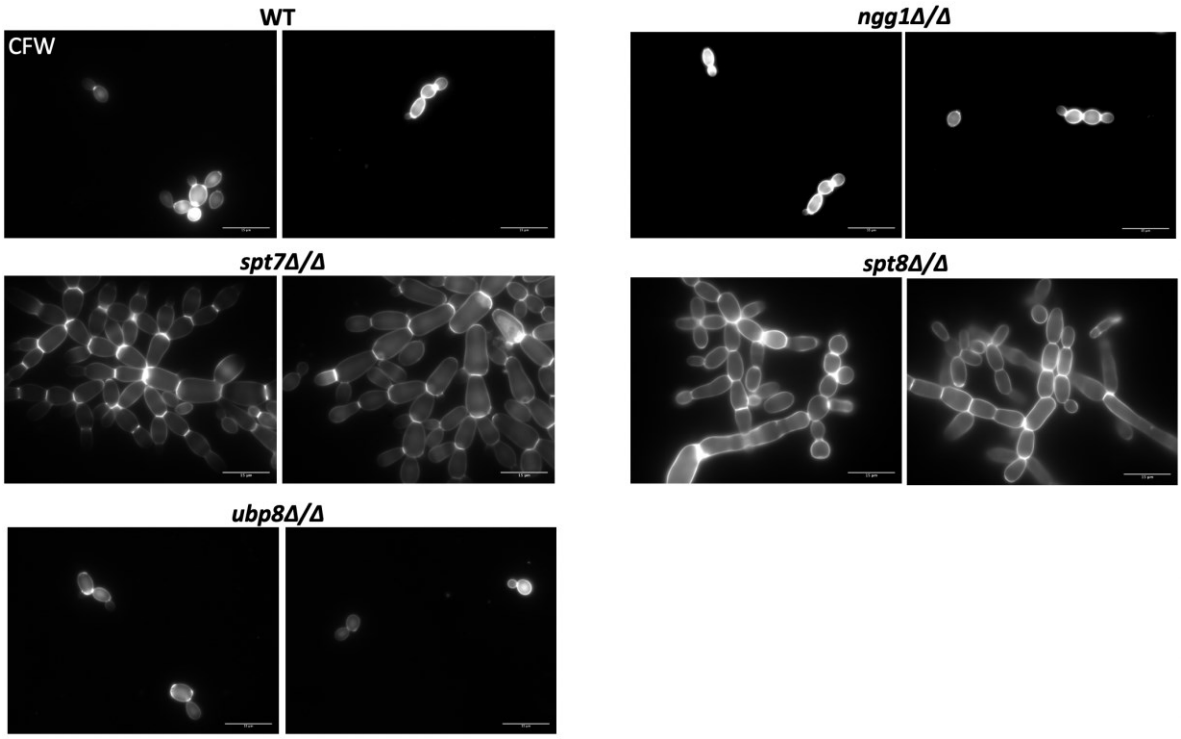


Figure 12. Staining of the SAGA mutants with DAPI and calcofluor white. Cultures were allowed to grow for 4 hours for wild type, *ngg1*Δ/Δ, *ubp8*Δ/Δ and for 6 hours for *spt7*Δ/Δ and *spt8*Δ/Δ under yeast growth conditions. Cells were washed twice with 1x PBS and then stained with 3µg/ml DAPI, or with 1.5µg/ml calcofluor and mounted on slides. Individual cells were examined under 100x magnification using LEICA DM 6000 microscope, scale bar 15µm. (A-E) All SAGA mutant cells showed normal nuclear segregation with each individual cell carrying single nuclei except (C) *spt7*Δ/Δ mutants were frequently binucleate (shown by white arrow heads) compared to its wild type. (F) Calcofluor white (CFW) stained cells displayed even chitin distribution and highly noticeable septa in all SAGA mutants similar to the wild type strain.

3.4 SAGA complex subunits appear to differentially influence biofilm regulation

Filamentation is often associated with the ability to form biofilms, which is considered an important factor for hospital-acquired infections (Chandra et al. 2001; Kojic and Darouiche 2004; Desai and Mitchell 2015; Tsui, Kong, and Jabra-Rizk 2016). We tested the deleted mutants for biofilm formation in Lee's medium after 48h of growth. While the *spt7*Δ/Δ and *spt8*Δ/Δ mutants shared many phenotypic similarities, it appears that the *spt8*Δ/Δ strain showed a somewhat increased biofilm formation compared to WT, while the *spt7*Δ/Δ strain showed decreased biofilm formation. We did not observe any significant difference in the *ngg1* and *ubp8* deleted strains tested in the regular biofilm induction. (Figure 13).

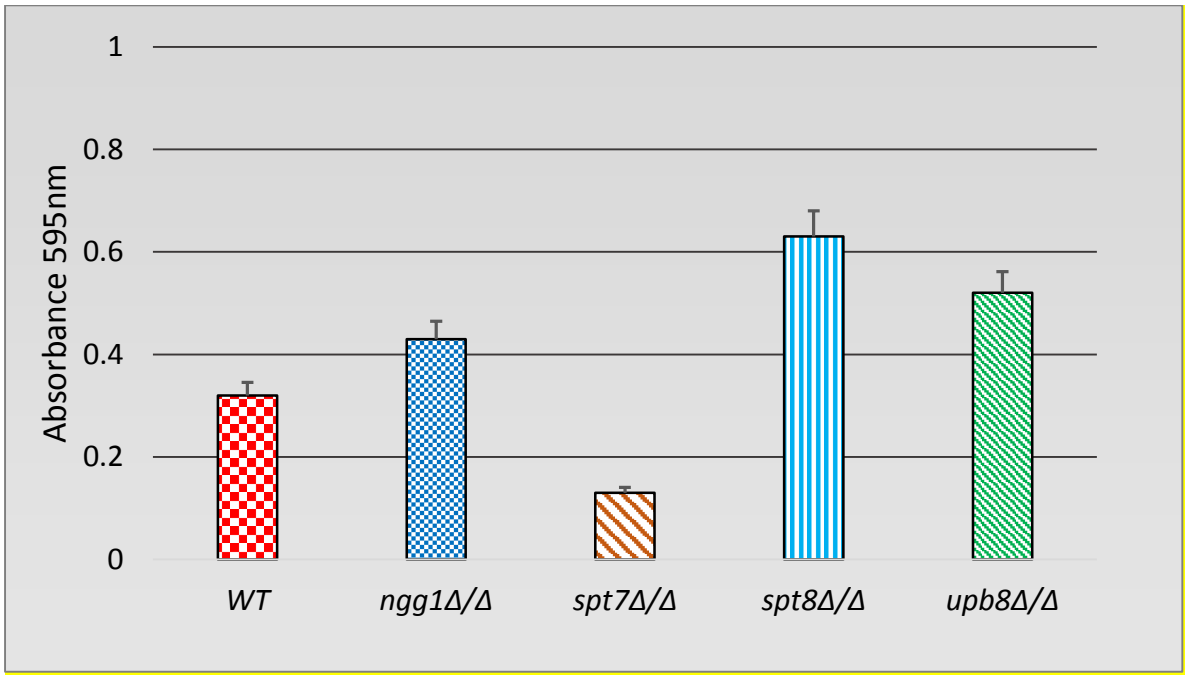


Figure 13. Biofilm formation of SAGA mutants in *C. albicans*. Quantification of biofilm formation in de-staining solution Deleted mutants of SAGA complex were tested for biofilm formation in Lee's medium after 48h. It appears *spt8Δ/Δ* enhances biofilm

formation whereas *spt7Δ/Δ* showed a decrease in biofilm formation compared to its control. Error bars indicates standard deviation.

3.5 Oxidative, osmotic, cell wall and temperature stresses in C. albicans are differentially influenced by SAGA sub-modules

Environmental stresses are often associated with SAGA complex influence in *S. cerevisiae* (Huisinga and Pugh 2004), so we investigated the consequences of the subunit mutations on response to a variety of stress conditions. SAGA knock-out mutants led to sensitivity to high temperature stress in *S. cerevisiae* as proved for *ngg1* and *ubp8* mutants through classical genetics and *spt7* and *spt8* mutants through large scale survey (Amerik, Li, and Hochstrasser 2000; Sinha et al. 2008; Ruiz-Roig et al. 2010). We did spot assays on YPD agar media and plates were incubated at 37˚C and 42˚C to assess the consequences of SAGA subunit loss in *C. albicans*. We found *ngg1*Δ/Δ and *ubp8*Δ/Δ were resistant to 42˚C incubation. However, *spt7*Δ/Δ and *spt8*Δ/Δ were sensitive at 37˚C compared to wildtype (Figure 14A). This result supports the idea that significant functional rewiring has taken place within this complex between the two species. In *S. cerevisiae, ngg1* and *ubp8* mutants are temperature sensitive whereas in *C. albicans ngg1* and *ubp8* mutants are resistant to high temperature. This suggests that the elements that are positively influencing temperature stress response in one organism have a negative influence on the same stress in a closely related fungus. This further highlight that Spt7 and Spt8 are required to cope up with increased temperature in *C. albicans.*

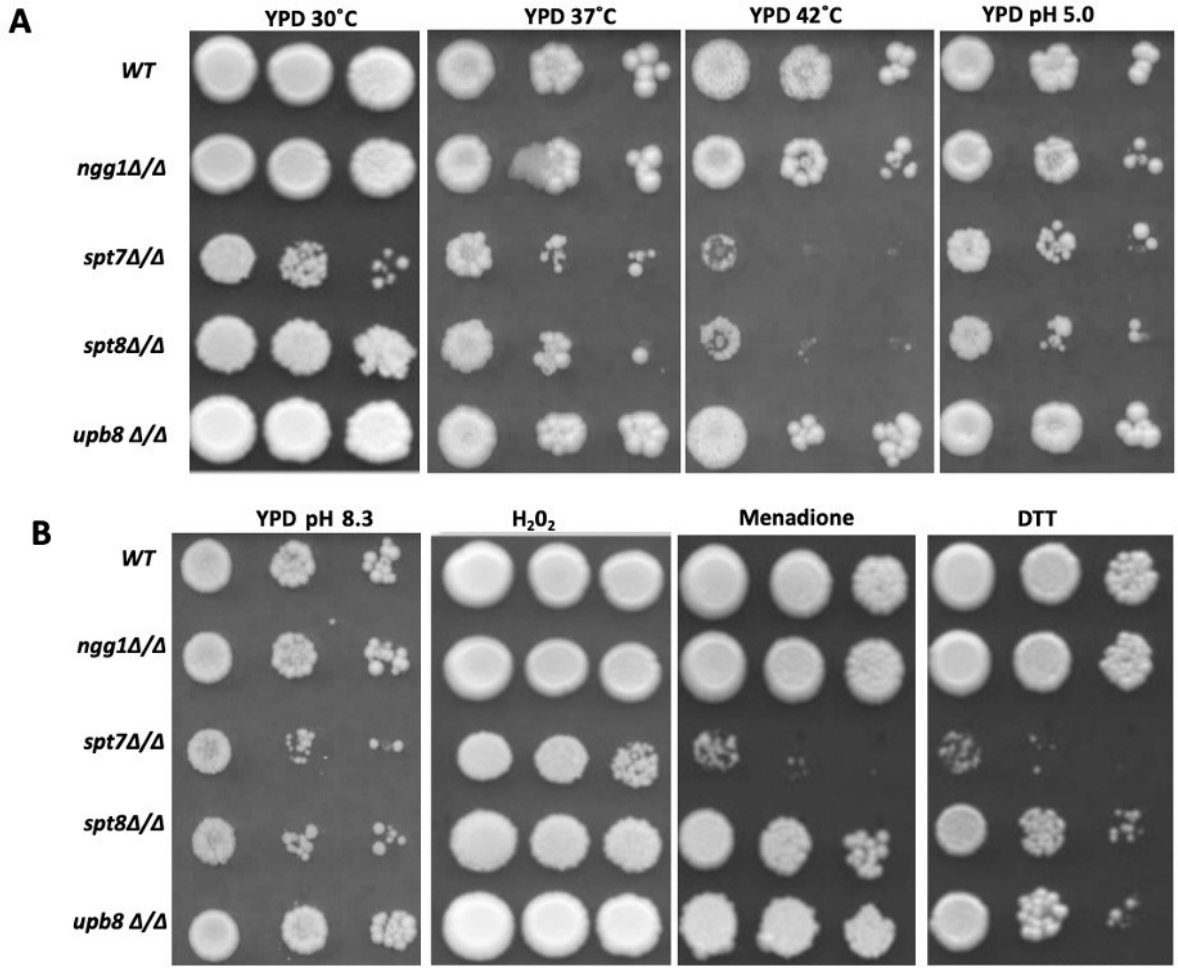
To evaluate the ability of the knock-out mutants to grow under oxidative stress, we tested the mutants on YPD media supplemented with different concentrations of hydrogen peroxide and menadione (Figure 14B). At 0.15 mM menadione, the mutants *spt7*Δ/Δ showed susceptibility compared to its wild type, while in 7.5mM H_2O_2 SAGA module subunits didn't exhibit any phenotypic change compared to its wild type. This finding suggests that Spt7 might play important roles in mediating oxidative stress resistance.

We examined the response to osmotic stress agents NaCl, $CaCl_2$ and glycerol. Interestingly, the *spt7*Δ/Δ and *spt8*Δ/Δ were susceptible to each of 400 mM calcium chloride, 1.5 M sodium chloride and 250 mM glycerol compared to the wild type. No phenotypic aberration was seen in the Ngg1 and Ubp8 mutants. Therefore, disruption of structural module and TBP interaction module subunits reduces osmotic response in *C. albicans* and suggests that both Spt7 and Spt8 play a key role in maintaining osmotolerance (Figure 14C).

Antifungal drugs such as caspofungin and chemicals such as Congo Red are often used to induce cell wall stress in *C. albicans* (Wiederhold et al. 2005; Eisman et al. 2006). Caspofungin and Congo Red interfere with β-glucan synthase and chitin synthase respectively (Roncero and Durán 1985; Ghannoum and Rice 1999). Based on previous descriptions of the *ada2*Δ/Δ and *gcn5*Δ/Δ mutants (Bruno et al. 2006; Chang, Fan, and Chen 2015), we tested our mutants against the cell-wall stressors caspofungin and Congo Red at different concentrations. The HAT-module *ngg1*Δ/Δ mutant was sensitive to 200µg/mL Congo Red, similar to the *gcn5*Δ/Δ mutant that also compromised the HAT module. The *spt7*Δ/Δ mutant was highly sensitive to both caspofungin and Congo Red, while the *spt8*Δ/Δ and *ubp8*Δ/Δ strains showed a WT response (Figure 14D).

Several studies describe filamentation as a potential phenotypic alteration in response to DNA damage in *C. albicans* (Reichow et al. 2007; Loll-Krippleber et al. 2014; Bachewich, Nantel, and Whiteway 2005). Since the SAGA knock-out mutants have altered filamentation, we exposed the mutants to genotoxic-stress-causing agents including the alkylating agent methyl methane sulfonate (MMS) and the DNA replication inhibitor hydroxyurea (HU). The *spt7*Δ/Δ, *spt8*Δ/Δ and upb8Δ/*Δ* mutants showed sensitivity at a concentration of 0.01% MMS, while the *ngg1*Δ/Δ mutant was comparable to wild type. Rich media containing 15mM HU showed the *ngg1*Δ/Δ strain to be resistant whereas other SAGA mutants exhibited sensitivity compared to wild type (Figure 14C).

We also analysed mutant strains in spot assays in media supplemented with different antifungal drugs - the ergosterol biosynthesis inhibitor fluconazole, the glucan synthase inhibitor anidulafungin, and the aminoglycoside antibiotic protein translation inhibitor hygromycin B. Spt7p appears to be a crucial component when it comes to response to drug treatments, as the *spt7*Δ/Δ strain showed sensitivity to 10µg/mL fluconazole, 100µg/mL hygromycin B and 0.25µg/mL anidulafungin; followed by *spt8*Δ/Δ that was not sensitive to hygromycin B, and lastly by *ngg1*Δ/Δ that conferred sensitivity to hygromycin B whereas *ubp8*Δ/Δ behaves like WT. It appears that the mechanisms of drug response regulation by the SAGA complex is drug-dependent, modulated by the different modules. Also, *ubp8*Δ/Δ shows resistance in the presence of the antifungal drugs (Figure 14D). This indicates that both Ubp8 and Ngg1 could act as potential drug targets, a point recently supported experimentally (Zhu et al. 2021).


A
YPD 30˚C
YPD 37˚C
YPD 42˚C
YPD pH 5.0
WT
ngg1Δ/Δ
spt7Δ/Δ
spt8Δ/Δ
upb8 Δ/Δ
B
YPD pH 8.3
H2O2
Menadione
DTT
WT
ngg1Δ/Δ
spt7Δ/Δ
spt8Δ/Δ
upb8 Δ/Δ

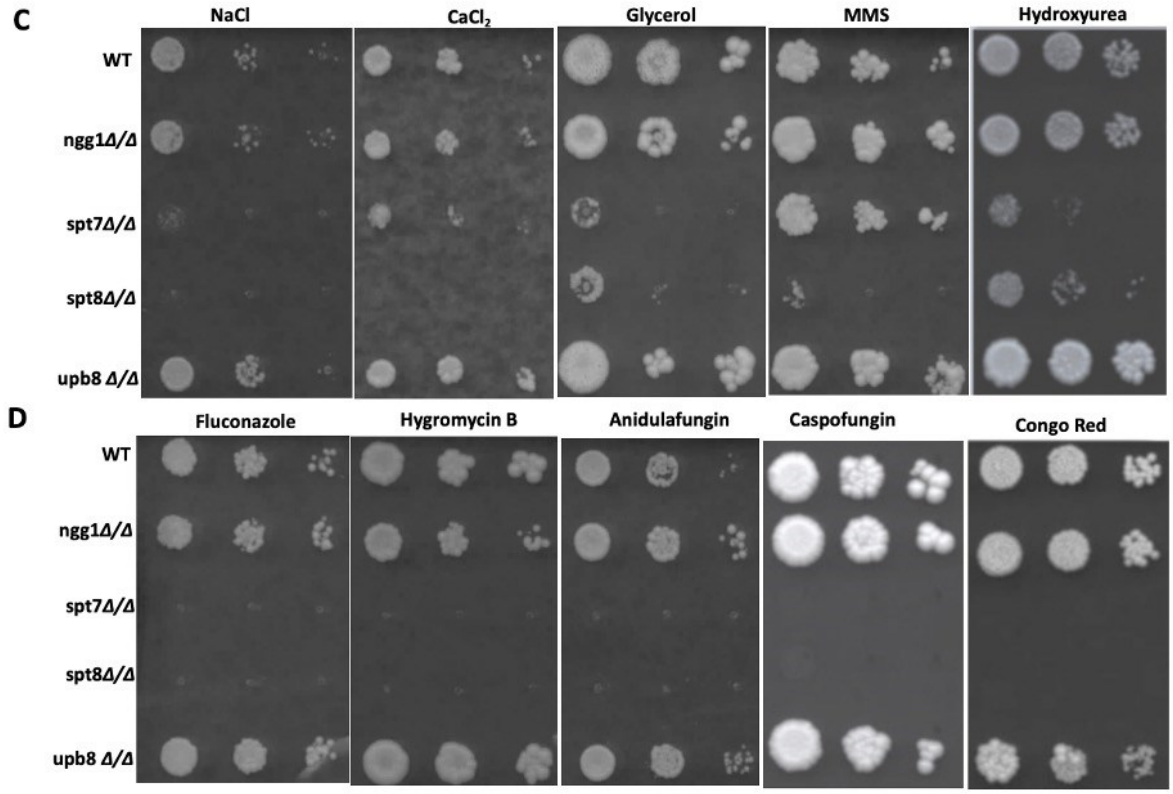


Figure 14. Genotoxic and cellular stress assay and antifungal drugs response of SAGA mutants in *C. albicans*. A 1:10 serial dilution of Overnight cultures grown in yeast growth conditions were spotted starting with OD of 0.2 onto YPD agar plates containing different chemicals and were incubated at 30°C for 4 days except for caspofungin (7 days) strains response to heat (37°C and 42°C) and alkaline medium (pH 8.3). Strains were subjected to acidic stress (pH 5.0), oxidative stress (Hydrogen peroxide 7.5 mM, 0.15mM Menadione) and ER stress (DTT- Dithiothreitol (30mM) (C) Strains were subjected to osmotic stress (NaCl 1.5mM, $CaCl_2$ 400mM, glycerol 250mM) and genotoxic stress of Methyl Methane Sulfonate (0.01% v/v), Hydroxy Urea (15mM) and Hydrogen peroxide (7.5mM), (D) to determine resistance to different cell membrane damaging drugs, fluconazole (10µg/mL), hygromycin B (100µg/mL), anidulafungin (0.25µg/mL), and cell wall stress caused by caspofungin (0.75µg/mL) and Congo red (200 µg/mL). Experiment was repeated 3 times for each sample.

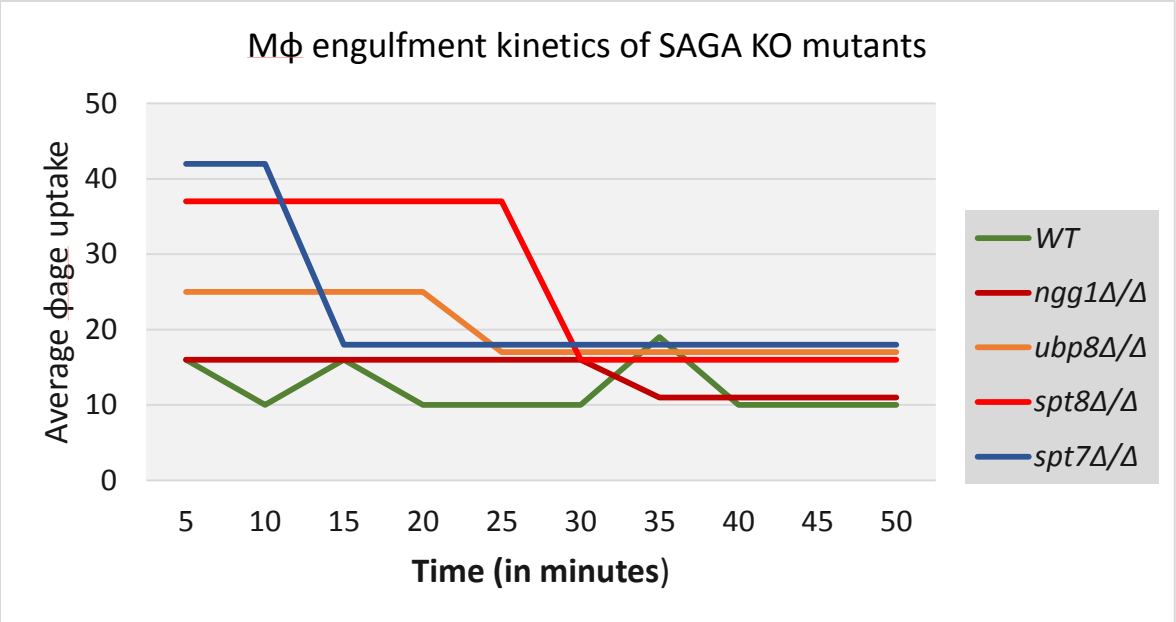
We also subjected the knock-out mutants to alkaline pH 8.3 and acidic pH 5.0 conditions (Vylkova et al. 2011). *spt7*Δ/Δ and *spt8*Δ/Δ show phenotypic change indicating that architecture module and TBP interaction unit play a role in regulating acidic/alkaline stresses compared to WT (Figure 14A and 14B). However, *ngg1*Δ/Δ and *ubp8*Δ/Δ showed normal growth comparable to WT. When we subjected mutant strains to 30mM DTT to generate ER stress through the accumulation of unfolded protein; strains with the *spt7*Δ/Δ mutation

showed sensitivity (Figure14B), suggesting that Spt7 is required for resistance to ER stress in *C. albicans*.

3.6 Macrophage engulfment assay shows a faster engulfment of filamentous strains in *C. albicans*

Macrophages are a first line of defense against *C. albicans* to prevent the host from developing infections (Krysan, Sutterwala, and Wellington 2014; Lorenz, Bender, and Fink 2004). To investigate the function of the SAGA complex in the *C. albicans/*macrophage interaction we tested the knock-out mutants of *ngg1*Δ/Δ, *spt7*Δ/Δ, *spt8*Δ/Δ and *ubp8*Δ/Δ in a macrophage engulfment assay. We assessed the rate of macrophage engulfment of the different mutants from the SAGA complex which showed that most of the engulfment by macrophages occurred in the first 50 minutes of interaction between fungal and immune cells (Figure 15A), compared to the wild type which showed lower rate of engulfment. This interaction (Candida-macrophage cells) starts at very early timepoints, and the macrophage recognition and further internalization vary among the SAGA mutants. In the first five minutes of interaction, *spt7*Δ/Δ showed a higher rate of engulfment when compared with the wild type (Figure 15B), while the *ngg1*Δ/Δ mutant showed a considerably lower rate of engulfment during this period. These results indicate that differences in the cellular morphology might play a role in the variance of macrophage engulfment assays where filamentous strains *spt7*Δ/Δ and *spt8*Δ/Δ were quickly recognised and engulfed by macrophages. However, yeast locked strains *ngg1*Δ/Δ and *ubp8*Δ/Δ showed lower rate of engulfment. This likely explains that SAGA complex subunits might play a role in pathogenicity.

(A)


Mϕ engulfment kinetics of SAGA KO mutants
Average ϕage uptake
50
40
30
20
10
0
5
10
15
20
25
30
35
40
45
50
Time (in minutes)
WT
ngg1Δ/Δ
ubp8Δ/Δ
spt8Δ/Δ
spt7Δ/Δ

(B)

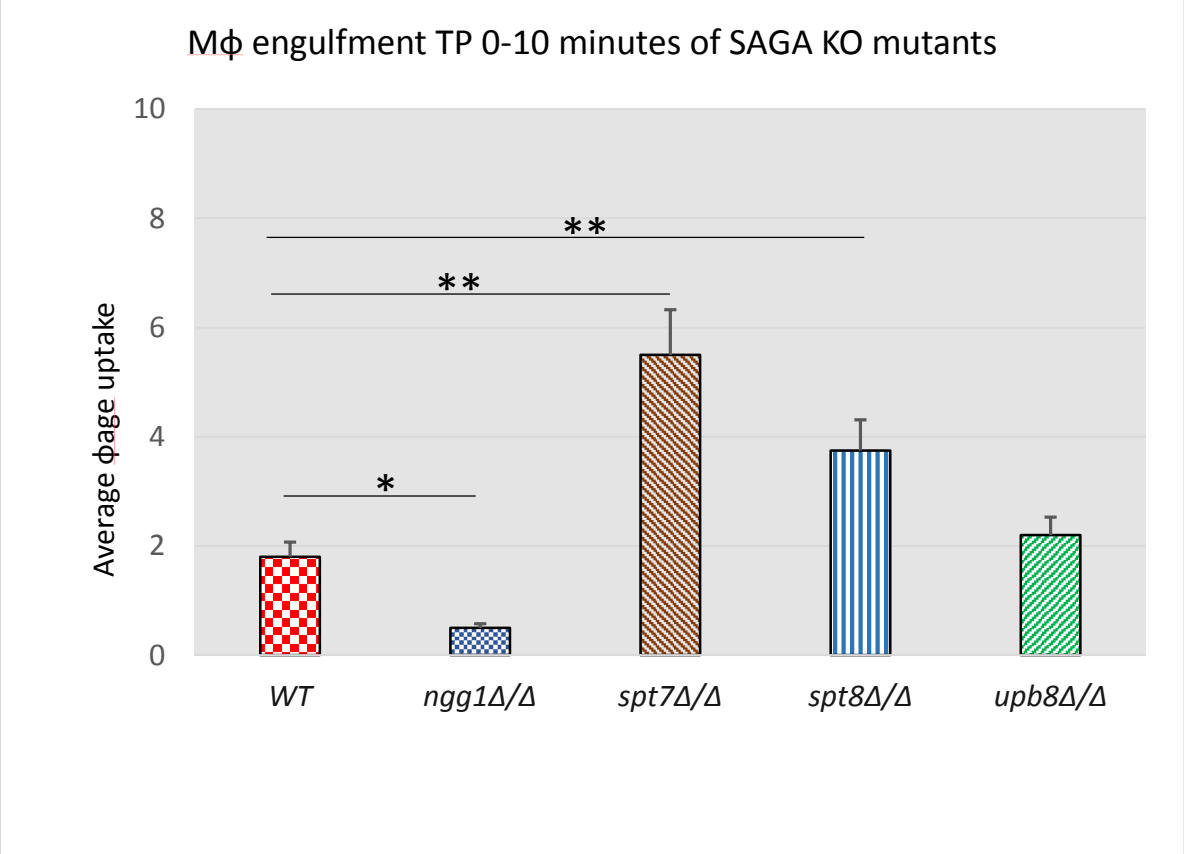

Mϕ engulfment TP 0-10 minutes of SAGA KO mutants
Average ϕage uptake
10
8
6
4
2
0
**
**
*
WT
ngg1Δ/Δ
spt7Δ/Δ
spt8Δ/Δ
upb8Δ/Δ

Figure 15. Macrophage engulfment of wildtype and SAGA mutant strains of *C. albicans*. Figure A and B show the time taken for RAW 264.7 murine macrophages to ingest live wildtype and mutant strains following initial cell-cell contact plotted versus the average macrophage uptake. The average time taken for engulfment of the *spt7Δ/Δ* and *spt8Δ/Δ* mutant strains was significantly less while as *ngg1Δ/Δ* was notably slower than for the wildtype control. t-test, **= p <0.01, *=p<0.001. Three biological replicates were made for each mutant. The results from the macrophage engulfment assay suggest that the core structural module subunit Spt7 mutant and the TBP interaction unit component Spt8 mutant could be less virulent, as the constitutively filamentous mutant strains *spt7*Δ/Δ and *spt8*Δ/Δ were quickly recognised and engulfed by macrophages relative to the Ngg1 and Upb8 mutants. Based on the previous evidence, the macrophage engulfment assay supports that SAGA complex sub-modules may work in opposing directions.

3.7 Expression analysis of SAGA module subunits

As specific null mutants of SAGA complex have a considerable effect on the functioning of *C. albicans* in properties such as invasiveness, filament formation, growth rates, drug resistance and biofilm formation, we performed RNA sequencing for the knock-out strains *ngg1*Δ/Δ, *spt7*Δ/Δ, *spt8*Δ/Δ, *ubp8*Δ/Δ compared to wild type strain cultured in yeast growth conditions to assess differences in gene expression. Using a statistical-significance analysis with a P value less than 0.05, we selected the up-regulated or down-regulated genes with a transcription ratio higher than 1.5-fold change or lower than -0.5-fold change relative to the wild type. We found that in the *spt7*Δ/Δ strain 104 genes were up and 280 genes were down regulated, and in the *spt8*Δ/Δ strain 94 genes were up and 318 genes were downregulated. In *ngg1* mutant strains 138 genes were upregulated and 133 genes were down regulated. *ubp8* null mutants had less of an effect on general gene expression 21 up and 7 genes down-regulated (Table S3-S6).

Analysis using the Candida Genome Database GO Term Finder revealed that among up-regulated genes in the *spt7* mutant, 42% (44/104 genes) were related to carbohydrate transport which is a significant enrichment (p-value 2.2 $x10^{-17}$), 46% (48/104 genes) were involved in organic substance transport (2 x $x10^{-5}$), while 6% (6/104 genes) were involved in arginine biosynthesis (p- value $4.1x10^{-8}$).In *ngg1* mutants, 19% (26/138) genes were involved in carbohydrate metabolic process (p- value 2.5 $x10^{-11}$), 13% (15/138) genes were involved in cellular response to chemical stress ((p- value $3.5x10^{-5}$), 30% (42/138) genes were involved in proteolysis (p- value $8.3x10^{-5}$) and 12% (16/138) genes were involved in ergosterol

biosynthetic pathway (p- value $0.02x10^{-3}$). In contrast, no particular functional class was dramatically enriched in either the *spt8* or the *upb8* mutants based on the GO term analysis of up-regulated genes. Among the downregulated genes in the *spt7* mutant 11% (36/318) down-regulated genes were related to ergosterol biosynthetic pathway which is enriched at p-value of $7.3x10^{-12}$,13% (39/318) genes were related to carbohydrate metabolism which is enriched at p-value $4.2x10^{-11}$, 5.4% (49/318) genes were related to organic hydroxy compound metabolic process with p-value of $2.94x10^{-15}$ and 30% (96/318) genes were related to small molecule metabolic process with enriched p-value of $8.2x\ 10^{-19}$. In *spt8* mutant 30% (83/280) of downregulated genes were involved in small molecule metabolic process which is enriched at p-value of $2.2x10^{-11}$, 14% (39/280) genes were related to organic hydroxy compound metabolic process with p-value of $1.42x10^{-13}$ and 12.5% (35/280) down-regulated genes were related to ergosterol biosynthetic pathway which is enriched at p-value of $1.5x10^{-12}$. Since SAGA mutants have significant effect on the number of cellular processes, we found that several genes increase or decrease their expression during the yeast-hyphal transition, adhesion, biofilm formation, stress responses, lipid and carbohydrate metabolic processes (Table 4).

As shown in Table 3, some specific classes of genes were upregulated in SAGA complex mutants compared to the reference strain SN148 (Crowther, Boddy, and Hefin Jones). The first class of genes include core filament genes and cell wall adhesion genes which include *HWP1, HGT12, UME6, PGA10, PGA13, PGA31, PGA58, ALS2, ALS3, ALS4* and *ALS9.* Hwp1 (hyphal wall protein 1) was highly upregulated in *spt7* and *spt8* mutants (9 fold and 12-fold respectively); this protein is important for adhesion to host cells, hyphal development, biofilm formation and virulence (Bruno et al. 2006; Sellam et al. 2009; Orsi et al. 2014). The upregulation of Hwp1 is consistent with the filamentous phenotype of the *spt7* and *spt8* mutants. Pga13 was 3.5-fold and 2.7-fold upregulated in *spt7* and *spt8* mutants, respectively. This is the key player in *C. albicans* morphogenesis and virulence (Gelis et al. 2012). Further, Hgt12 was 6.2- fold and 4.5- fold upregulated in *spt7* and *spt8* mutants respectively, this protein is required for expression of glucose transporter genes and plays a role in induced hyphal growth (Biswas, Van Dijck, and Datta 2007). Furthermore, Hgc1 (Hypha-specific G1 cyclin-related protein) was 4.5-fold upregulated in the *spt8* mutant; it plays a key role in hyphal development (Zheng, Wang, and Wang 2004; Buckley 2008). A second class consists of genes encoding transcription factors that positively regulate hyphal development and enhance biofilm formation via Hgc1-Ume6 (Banerjee et al. 2013). Ume6, a

true hyphae transcription factor (Zeidler et al. 2009) is 2.5-fold upregulated in the *spt8* mutant; Ume6 also plays a role in hyphal extension and virulence (Banerjee et al. 2008).

Intriguingly, in *spt8* mutants a group of box C/D type snoRNAs (small nucleolar RNAs) representing about 25% of all C/D box type snoRNAs in *C. albicans,* was upregulated from 2-fold to 6.5-fold. snoRNAs are non-coding RNAs involved in the single nucleotide modifications of other RNAs (Reichow et al. 2007), and are implicated in nucleolytic processing of ribosomal RNA precursors, telomeric DNA synthesis and alternative splicing (Maxwell and Fournier 1995; Tollervey and Kiss 1997; Matera, Terns, and Terns 2007). There are two classes of snoRNAs - box C/D type snoRNAs and box H/ACA type that are distinguished by structure and their involvement in specific chemical modifications (Balakin, Smith, and Fournier 1996; Tollervey and Kiss 1997). Box C/D types have a stem loop structure which guides 2-O'-methylation of ribose sugars, and while box H/ACA types have a double stem loop structure involved in pseudo-uridylation of uracil residues in target RNAs (Darzacq et al. 2002). It will be interesting to investigate how SAGA complex mutant *spt8* influences expression of a set of box C/D type snoRNAs.

Certain classes of genes were downregulated in SAGA complex mutants compared to the reference strain SN148 (Crowther, Boddy, and Hefin Jones). The notable class of genes encodes ergosterol biosynthesis elements *ERG3*, *ERG251* and *UPC2*. *Erg3* is -2.9-fold down in *spt7* mutant; *ERG3* is an important gene in ergosterol biosynthesis pathway and has key role in azole drug resistance (Zhou et al. 2018). Also, *Upc2* is -2.8-fold down in the *spt7* mutant and -1.3fold down in *spt8* mutant; *Upc2* is a transcription factor that is central to the regulation of ergosterol biosynthesis and plays a role in azole resistance (Vasicek et al. 2014) which is evident in our fluconazole assay where *spt7* and *spt8* mutants showed sensitivity. Also, we noted that the genes involved in cell cycle were downregulated in *spt7* and *spt8* mutants. In the *spt7* mutant, *FKH2* was downregulated -1.6-fold. *FKH2* is a fork-head transcription factor/morphogenesis regulator involved in S/G2 cell cycle arrest and polarisation; required for wild-type hyphal transcription, cell separation required (Berman 2006) and in *spt8* mutant, *HOF1* is downregulated -1.8-fold. *HOF1* plays a role in cytokinesis and DNA damage repair (Feng et al. 2020).

Table 4. Table showing expression analysis of SAGA complex subunits.

Upregulated genes >1.8 log2FC

Processes	***spt7 Δ/Δ***	***spt8Δ/Δ***	***ngg1Δ/Δ***	***ubp8Δ/Δ***
Filamentation	*HWP1, HGT12, PGA13*	*HWP1, ALS3, HGC1, HGT12, PGA13, UME6*	-	-
Cell wall Adhesins	*PGA58, PGA13, PGA31*	*ALS3, PGA13*	*ALS2, ALS4, ALS9, PGA10, PGA13*	-
Ergosterol biosynthesis	-	-	*ERG1, ERG3, ERG5, ERG11, ERG251, UPC2*	-
C/D Box snoRNA genes	*C2_02080W_A, C1_08970W_A, C1_08960W_A*	*C2_05600W_A, C2_02080W_A, C5_01160C_A, CR_06210C_A, C2_10800W_A, C1_12990W_A, CR_06220C_A, C1_13000W_A, CR_10460W_A, C1_08970W_A, C1_08960W_A*	-	-

Down regulated genes < - 0.6 log2FC

Processes	***spt7 Δ/Δ***	***spt8Δ/Δ***	***ngg1Δ/Δ***	***ubp8Δ/Δ***
Ergosterol biosynthesis	*ERG1, ERG3, ERG4, ERG5, ERG251, UPC2*	*ERG13, ERG11, ERG5, ERG4, ERG251, UPC2*	-	-
Sulfur / Methionine biosynthesis	*MET6, MET28*	*MET3, MET4, MET28, SUL2*	-	-
Carbohydrate metabolism	*MNN22, MNN1, OYE23, GDH2, PFK2, FBA1, TYE7*	*OYE23, OSM2, TYE7, PFK2*	-	-

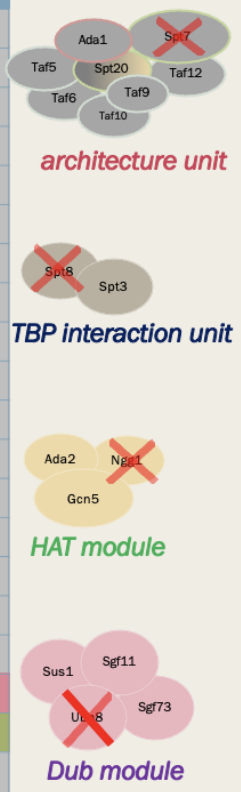
Chapter 4. Discussion

The SAGA complex and related complexes SLIK and ADA, are very well-studied transcriptional regulators in eukaryotic organisms such as *S. cerevisiae*, *Drosophila melanogaster* and humans (Gurskiĭ et al. 2013; Srivastava et al. 2015; Koutelou, Hirsch, and Dent 2010). In a variety of studies, rewiring has been observed as a recurrent event differentiating transcriptional regulation in *C. albicans* compared to the baker's yeast. Most of investigations refer to "rewiring" when specific orthologous transcriptional regulators have evolved in different species to activate or repress unrelated biological processes (Whiteway et al. 2015). However, this can also occur in general transcriptional regulators; in the SAGA complex Spt3 (TBP binding module) negatively modulates filamentation in *C. albicans*, opposite to its role in *S. cerevisiae*, while Gcn5 (HAT module) influences morphogenesis in a similar manner in both species, raising questions about the possible role of rewiring on this co-activator complex (Laprade et al. 2002).

In this study, we have provided an overview of different components of SAGA complex regulating growth, morphogenesis, invasiveness, biofilm formation and response to environmental stresses in *C. albicans* (Table 5).

Table 5. Table showing the results of different stress conditions in *C. albicans.*

Type of stress	Method of stress	ngg1Δ/Δ	spt7Δ/Δ	spt8Δ/Δ	ubp8Δ/Δ
Temperature	37°C		sensitivity	sensitivity	
	42°C		sensitivity	sensitivity	
pH	pH 5.0		sensitivity	sensitivity	
	pH 8.5		sensitivity	sensitivity	
Oxidative	H_2O_2 7.5mM				
	Menadione 0.15mM		sensitivity		
ER stress	DTT 30mM		sensitivity		
Osmotic	NaCl		sensitivity	sensitivity	
	$CaCl_2$		sensitivity	sensitivity	
	Glycerol		sensitivity	sensitivity	
DNA damage	MMS 0.01%			sensitivity	
	HU 30mM		sensitivity		
Antifungal	FLZ 10µg/mL		sensitivity	sensitivity	
	HygB 100µg/mL		sensitivity	sensitivity	
	Anidulafungin 0.25µg/mL		sensitivity	sensitivity	
Cell wall	Caspofungin 0.75µg/mL		sensitivity	sensitivity	
	Congo Red 200µg/mL		sensitivity	sensitivity	
Cell morphology	Filamentation	decreased	increased	increased	decreased
	Growth rate		slow	slow	fast
	Invasiveness		increased	increased	
	Biofilm	increased	decreased	increased	increased



We noted that the Spt7 (Core structural module) and Spt8 (TBP binding unit) components of SAGA complex are involved in the negative regulation of filamentation, as was previously noted for Spt3 (TBP binding module) (Laprade et al. 2002), while Ngg1 (HAT module) and Ubp8 (Dub module) appear to positively modulate filamentation, as was also found for Gcn5 of the HAT module (Chang, Fan, and Chen 2015). We also observed that wild type (control) can change its morphological state depending on the conditions; in non-inducing conditions it is in normal yeast form and in hyphal inducing media it can switch to hyphal state. However, none of the SAGA mutants can switch morphological state upon changing conditions, they are all locked into their respective morphological states. This supports a 'common' function for the SAGA complex as a whole- where HAT module (*ngg1*Δ/Δ) and Dub module (*ubp8*Δ/Δ) are in yeast locked state and architecture module (*spt7*Δ/Δ) and TBP interaction unit (*spt8*Δ/Δ) showed filamentation. Also, Spt7 and Spt8 play an important role in aspects of cell division as both *spt7*Δ/Δ and *spt8*Δ/Δ cells showed cytokinesis defects where mother and daughter cells fail to separate properly and appear 'clumped' together (Figure 6), as previously described for both *Caspt20*Δ/Δ and Sc*spt20*Δ/Δ (Core structural module) (Laprade

et al. 2002; Desai and Mitchell 2015). This evidence strongly suggests that architecture module and TBP interaction module plays a crucial role in cytokinesis and suggests an important role in maintaining cell wall integrity for the SAGA complex. Further, our results indicate that architecture module and TBP interaction unit might struggle in G1 re-entry or have G2/M cell cycle arrest or delay which leads to constitutive pseudo hyphal form and difficulty in cell separation. Although *spt7* and *spt8* mutant cells showed similarities in their phenotypes, *spt7*Δ/Δ cells were often binucleated whereas *spt8*Δ/Δ were mononucleated which strongly indicates that filamentous cells have perturbed cell cycles causing abnormal nuclear content and increased cell size compared to wild type cells. These results suggest that in the filamentous phenotypes of SAGA mutants, the cells might be in late S/G2 phase or defective/late M phase, reasonably due to failure in DNA repair machinery which resulted in morphological switch, abnormal nuclear content and an increased cell size (Berman 2006).

SAGA mutants of *C. albicans* have differences in growth rates - with the slow growers *spt7*Δ/Δ and *spt8*Δ/Δ, similar to that of the *S. cerevisiae* mutants *Scspt7*Δ, *Scspt20*Δ (Core structural module) and *Scspt3*Δ (TBP binding module)(Laprade et al. 2002); wild type growers like *ngg1*Δ/Δ similar to *gcn5*Δ/Δ (Chang, Fan, and Chen 2015) and apparently somewhat faster growers, *upb8*Δ/Δ (Dub module). The increased growth in rich medium of *upb8*Δ/Δ suggests that the control of growth rate may represent a balance between the selective advantages of fast growth and the need to maintain the integrity of the genome (Pir et al. 2012). Also, it appears that Spt7 and Spt8 play an important role in maintaining SAGA integrity and are critical for normal growth in *C. albicans,* as has been seen previously with mutants in *S. cerevisiae* Spt7 and Spt8 (Gansheroff et al. 1995; Belotserkovskaya et al. 2000; Wu and Winston 2002; Wang, Dienemann, et al. 2020). Our study suggests that the HAT module (Ngg1) is not critical for normal cell growth in *C. albicans* as the *ngg1*Δ/Δ strain grew at rates comparable to the wild-type strain (Figure 7A and 7B), consistent with the observation that deletion of Gcn5 of the same module also had no impact on cell growth (Chang, Fan, and Chen 2015).

A feature of *C. albicans'* pathogenicity is its ability to form hyphae, which is important for both invasiveness and for biofilm formation. Hyphal forms are critical for invading epithelial and endothelial cells and for evading host immune response. Analysis of colony and cell morphology of SAGA mutants shows that null mutants of *SPT7* and *SPT8* form wrinkled

colonies with branched filaments on solid media which is an indication of filamentous cells (comparable to *spt3*Δ/Δ*),* whereas *ngg1* and *ubp8* mutants form smooth colonies, similar to those of *gcn5*Δ/Δ (Chang, Fan, and Chen 2015). Previous analysis of the *gcn5* mutant showed altered hyphae when the strain was induced by 10% serum at 37˚C. We observed similar phenotypes for *ngg1*Δ/Δ from the HAT module of the protein complex. This implies that, similar to Gcn5, Ngg1 is essential to hyphal elongation in sensing serum (Chang, Fan, and Chen 2015). Intriguingly, *spt7*Δ/Δ *and spt8*Δ/Δ strains are constitutively hyphal in inducing and non-inducing conditions as was also seen for *spt3*Δ/Δ (Figure 6) (Belotserkovskaya et al. 2000; Lee et al. 2000). Our results strongly suggest that the HAT module (Ngg1) and the Dub module (Ubp8) play a role in invasiveness, compared to the core structural module (Spt7) and the TBP binding module (Spt8) which act as invasiveness repressors in both inducing and non-inducing conditions (Figure 8A). Our investigation of SAGA module suggests that the core structural module (Spt7) plays a role in biofilm formation. However, TBP binding module (Spt8), HAT module (Ngg1) and Dub module (Ubp8) seems to act as repressors of biofilm formation.

We tested the sensitivity of SAGA mutants for different stress conditions. Our investigation showed that, similar to the situation in baker's yeast, Ngg1 from the HAT module positively regulates transcriptional response to cell wall perturbations and negatively regulates response to heat stress (Piña et al. 1993). However, it also negatively regulates response to DNA damage stress, another example of rewired regulatory circuitry during the evolution of these fungi. It appears that the role of the HAT module (Ngg1) in response to oxidative stress is not dependent on the HAT domain since *gcn5*Δ/Δ mutants of the same module are described to be normal in responding to H_2O_2, CPT, MMS, and HU. Also, deletion of HAT domain from Gcn5 confers cell-wall stress sensitivity (Chang, Fan, and Chen 2015). Deletion of *NGG1, SPT7* and *SPT8* also confer cell-wall sensitivity suggesting that the encoded proteins might play important structural roles for the HAT function in the SAGA complex. It appears that SAGA has two opposing forces within itself regulating cell wall stress, oxidative stress, DNA damage stress, osmotic stress, high-temperature stress, morphological changes while Ngg1 and Ubp8 opposes the complex partners in temperature stress.

To characterize in more detail the relevance of SAGA for survival at high osmolarity, we performed a comprehensive phenotypic analysis of SAGA mutants for growth at high osmolarity (NaCl, $CaCl_2$ and glycerol). As shown in Fig.14C, deletion of *SPT7* and *SPT8*

strongly affected cell growth at high osmolarity. In *S. cerevisiae*, the HOG1 pathway controls the synthesis and storage of glycerol which increases intracellular osmotic/turgor pressure to make the cells adapt to high osmotic environments (Saito and Tatebayashi 2004). It is worth mentioning that mutations that affect SAGA structural integrity plays a critical role for survival at high osmolarity (Daniel and Grant 2007). In hyperosmotic condition, it activates the HOG1 MAP kinase pathway which recruits SAGA to the modified chromosome and promotes RNA polymerase II binding at the promoter region to initiate transcription (Proft and Struhl 2002; Wang, Chen, et al. 2020).

Antifungal drugs such as caspofungin and chemicals such as Congo red are often used to induce cell wall stress in *C. albicans* (Wiederhold et al. 2005; Eisman et al. 2006; Plaine et al. 2008). Caspofungin and Congo red interfere with β-glucan synthase and chitin synthase respectively. When we submitted the SAGA mutants to cell wall stress, *spt7*Δ/Δ*,* s*pt8*Δ/Δ *and ngg1*Δ/Δ showed hypersensitivity to Congo red whereas *spt7*Δ/Δ and *spt8*Δ/Δ were sensitive to caspofungin, comparable to *Scspt20*Δ/Δ *and Caspt20*Δ/Δ (Lesage et al. 2004; Desai and Mitchell 2015). This suggests that *spt7*Δ/Δ*, spt8*Δ/Δ *and ngg1*Δ/Δ membrane defects are related to the cell wall or cell membrane. Our findings showed that Core structural module (*spt7*Δ/Δ*)* and *spt8*Δ/Δ (TBP binding module) are susceptible to antifungal agents - hygromycin B and anidulafungin B. Interestingly, our results also revealed hypersensitivity of Core structural module (*spt7*Δ/Δ) and TBP interaction unit (*spt8*Δ/Δ) to azoles particularly fluconazole (Figure 14D) which is consistent with our RNASeq data that showed decreased expression of ergosterol biosynthetic genes mainly *ERG1,ERG3,ERG251,UPC2* that play significant functions in the sensitivity of Candida to antifungals (Borecká-Melkusová et al. 2009). Our results also revealed that HAT module (*ngg1*Δ/Δ) and Dub module (*ubp8*Δ/Δ) are resistant to azoles which signifies that it might act as potential drug targets in *C. albicans* which is supported by recent observation where effect of fluconazole on mice infected with ubp8 mutant cells was greater (60% of mice survived after 16 days of treatment) than mice infected with wild type cells (only 20% of mice survived)(Zhu et al. 2021). Intriguingly, *spt7*Δ/Δ showed hypersensitivity to ER stress agent DTT which indicates that core structural module subunit-Spt7 is required for ER stress resistance in *C. albicans.*

Macrophages form the first line of immune response in host against developing Candida infections (Krysan, Sutterwala, and Wellington 2014; Lorenz, Bender, and Fink 2004). SAGA mutants showed differences in their cellular morphology phenotype that likely

explains the variance in the macrophage engulfment kinetics. *spt7Δ/Δ* and *spt8Δ/Δ* are filamentous that is quickly recognized by macrophages. However, *spt8Δ/Δ* that forms longer filaments escapes engulfment by macrophages at start, while *spt7Δ/Δ* forms shorter branched pseudo-hyphae that possibly results in quick engulfment by macrophages. On the contrary, *ngg1Δ/Δ* were least engulfed by macrophages as it was in yeast locked form (Figure 15B). It is likely that macrophage engulfment of fungal cells is dependent on type and size of hyphae i.e., true hyphae or pseudo-hyphae and yeast form (Lewis et al. 2012). The Dub module subunit *ubp8Δ/Δ* also showed reduced macrophage engulfment compared to *spt7Δ/Δ and spt8Δ/Δ*. *In vivo* assays have shown that SAGA complex modules play role in virulence; where TBP interaction module subunit-*spt3Δ/Δ and* HAT module subunit- *gcn5Δ/Δ* has shown avirulent behavior whereas HAT module subunits-*ada2Δ/Δ*, *ngg1Δ/Δ*; TBP interaction unit component- *spt20Δ/Δ* and Dub module subunit- *ubp8Δ/Δ, sus1Δ/Δ* has revealed attenuated virulence in candidemia systemic infection mice model Laprade et al. (2002); (Sellam et al. 2009; Chang, Fan, and Chen 2015; Desai and Mitchell 2015; Xiao et al. 2018; Shivarathri et al. 2019; Zhu et al. 2021).The virulence nature of *ngg1* mutants in *C. albicans* have been corroborated (Li et al. 2017). However, the group has failed to reconstruct a mutant strain of *NGG1*. Based on previous studies in *C. albicans'* virulence *in vivo*, *spt3Δ/Δ* and *gcn5Δ/Δ* were avirulent compared to *spt20Δ/Δ, ada2Δ/Δ, ngg1Δ/Δ, ubp8Δ/Δ and sus1Δ/Δ* which showed attenuated virulence and combined with macrophage engulfment results and filamentation, it strongly appears that Core structural module-Spt7 and TBP binding module-Spt8 are avirulent, and HAT module- Ngg1 and Dub module-Ubp8 play roles in attenuated virulence.

Overall, we found that the single subunit of the recruitment module, Tra1, is essential as we failed to obtain a homozygous deletion of *TRA1*. We also found that both the core structural module subunit Spt7 and TBP interaction subunit Spt8 act as repressors of filamentation and invasiveness whereas HAT module subunit Ngg1 and Dub module subunit Ubp8 act as positive regulators. Further, we have shown that both Spt7 and Spt8 play important roles in maintaining SAGA integrity and are critical for growth in *C. albicans.* Also, both *spt7* and *spt8* mutants have shown cell cycle related defects as mother and daughter cells fail to separate during cytokinesis (Figure 1). Furthermore, we have shown that Spt7 and Spt8 are critical for normal *C. albicans* response to DNA damage stress and thus play key roles in maintaining genome integrity. Additionally, Spt7 and Spt8 are vital for the normal response of cells to cell wall, heat, ER, and alkaline stress. Our study also reveals that filamentous

strains *spt7*Δ/Δ and *spt8*Δ/Δ are quickly engulfed by macrophages which indicates that SAGA might play a role in pathogenicity in *C. albicans.* All these outcomes propose that core structural module and TBP interaction unit are indispensable for cell morphology, genotoxic and cellular stress responses whereas HAT module plays a role in cell wall stress response. Also, from our findings it appears that HAT module Ngg1 and Dub module Ubp8 might serve as potential drug targets in *C. albicans.*

4.1 Future directions

It is clear from the above that SAGA complex modules play an important role in regulating multiple cellular processes in *C. albicans*, some of which includes filamentation, invasion, biofilm formation, cell stress response and antifungal resistance. In this work, we have deleted only one subunit from each module of SAGA complex so it will be interesting to delete two subunits from the same module or two subunits from different modules of the SAGA complex to check their effect on phenotypes and viability. Also, *spt8* mutants showed an upregulation of 25% of C/D box snoRNAs in RNASeq analysis, so in depth experimental studies may shed further light on the role of SAGA complex in regulation of C/D box snoRNAs. Finally, it will be interesting to study in detail the cell division process in both *spt7* and *spt8* mutants, both of which have difficulty in cell separation. Although both *spt7* and *spt8* mutant have generally similar phenotypes they differ in nuclear distribution where *spt7* mutant cells were frequently binucleate and *spt8* mutant cells were consistently mononucleate. In the RNASeq analysis, we did see that *FKH2* was downregulated in the Spt7 mutant cells. *FKH2* is morphogenetic regulator which plays a role in S/G2 cell cycle arrest, (Berman 2006) so over production of *FKH2* in *spt7* mutant cells yield the phenotype which may not have defect in nuclear distribution and cytokinesis. Similarly, in the *spt8* mutant cells we saw that *HOF1*, which plays a role in cytokinesis and DNA damage repair) is downregulated. So over expressing of *HOF1* may help to establish the genes involved in morphogenesis and cytokinesis in *C. albicans.*

Chapter 5. Bibliography

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Appendices

Table S1. RNASeq Analysis of NGG1 mutant (Upregulated genes)

ORF	Candida Gene name	log2 (Fold change)	P value adjusted	Description
C3_00600W_A	*IFF11*	5	7.07E-01	Secreted protein required for normal cell wall structure and for virulence; member of the IFF family; Hap43p-repressed gene
C4_00450C_A	*PGA10*	3.41	5.66E-01	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
C6_04380W_A	*ALS2*	3.12	3.54E-01	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
CR_09140C_A		2.97	2.49E-03	Protein with a role in directing meiotic recombination events to homologous chromatids; induced by ciclopirox olamine; positively regulated by Sfu1; Hog1, fluconazole-repressed; Hap43-induced; Spider biofilm induced
C6_04130C_A	*ALS4*	2.75	3.30E-03	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
C1_13480W_A	*HSP70*	2.6	3.54E-04	Putative hsp70 chaperone; role in entry into host cells; heat-shock, amphotericin B, cadmium, ketoconazole-induced; surface localized in yeast and hyphae; antigenic in host; farnesol-downregulated in biofilm; Spider biofilm induced
C6_03710W_A	*ALS9*	2.5	4.10E-02	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
C2_07630C_A		2.41	6.40E-01	Possible stress protein; increased transcription associated with CDR1 and CDR2 overexpression or fluphenazine treatment; regulated by Sfu1, Nrg1, Tup1; stationary phase enriched protein; Spider biofilm induced

CR_08800W_A	*ITS2*	2.37	1.71E-05	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
CR_08790W_A	*RDN58*	2.18	5.38E-05	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
C2_09220W_A	*DDR48*	2.14	1.98E-02	Immunogenic stress-associated protein; filamentation regulated; induced by benomyl/caspofungin/ketoconazole or in azole-resistant strain; Hog1, farnesol, alkaline repressed; stationary phase enriched; Spider, flow model biofilm induced
CR_08890C_A	*ASR2*	2.1	9.81E-02	Adenylyl cyclase and stress responsive protein; induced in cyr1 or ras1 mutant; stationary phase enriched protein; Spider biofilm induced
C2_05180W_A	*WH11*	2.07	2.55E-02	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
C2_06870C_A	*PST1*	2.05	7.69E-02	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
CR_08780W_A	*ITS1*	2.05	4.70E-03	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
CR_08510W_A	*PGA13*	2.02	8.97E-03	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
CR_00200W_A	*PCK1*	2.01	2.71E-03	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
C1_07330W_A	*RME1*	1.98	1.29E-01	Zinc finger protein; controls meiosis in S. cerevisae; white-specific transcript; upregulation correlates with clinical development of fluconazole resistance; Upc2-regulated in hypoxia; flow model biofilm induced; Spider biofilm repressed

C3_00030C_A		1.95	7.69E-02	Protein with a predicted DEAD-like DNA/RNA helicase domain; shows colony morphology-related gene regulation by Ssn6; overlaps orf19.5472; Spider biofilm repressed
C2_07570W_A	*RNR22*	1.93	1.47E-01	Putative ribonucleoside diphosphate reductase;colony morphology-related gene regulation by Ssn6; transcript regulated by tyrosol and cell density; Hap43-repressed; Spider biofilm induced
C1_08590C_A	*ERG1*	1.86	1.47E-01	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
CR_08810W_A	*RDN25*	1.86	1.96E-03	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)
CR_06570C_A		1.82	2.05E-01	Protein of unknown function; Spider biofilm induced
C2_05700W_A	*OSM1*	1.78	1.12E-01	Putative flavoprotein subunit of fumarate reductase; soluble protein in hyphae; caspofungin repressed; stationary phase enriched protein; flow model biofilm induced; Spider biofilm repressed
C3_00010C_A		1.77	1.70E-01	Protein of unknown function; induced by Mnl1 under weak acid stress; transcript detected on high-resolution tiling arrays; Spider biofilm repressed
C2_02860W_A	*SUR2*	1.74	1.78E-01	Putative ceramide hydroxylase; predicted enzyme of sphingolipid biosynthesis; regulated by Tsa1, Tsa1B under H2O2 stress conditions; Spider and flow model biofilm induced
C5_02080C_A	*HSP12*	1.74	1.70E-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
C6_01650C_A	*FMP27*	1.74	5.17E-02	Putative mitochondrial protein; mRNA binds She3
C5_03510C_A		1.73	2.78E-02	Protein of unknown function; mRNA binds to She3; Hap43-repressed; rat catheter and flow model biofilm induced
C2_03320W_A	*CHK1*	1.72	1.67E-01	Histidine kinase; 2-component signaling, cell wall synthesis; hyphal growth defect; avirulent in mouse, not rat vaginal infection; phagocytosis rate increased; Spider biofilm induced; required for RPMI biofilm; Bcr1-induced in a/a biofilm

C3_00550C_A	*HRK1*	1.72	1.93E-01	Putative serine/threonine kinase; predicted role in cellular ion homeostasis; Spider biofilm repressed
C5_02110W_A		1.71	1.70E-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
C3_01540W_A		1.68	7.69E-02	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
CR_10840C_A	*XYL2*	1.68	2.58E-01	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
C1_06940C_A	*ATC1*	1.67	3.04E-01	Cell wall acid trehalase; catalyzes hydrolysis of the disaccharide trehalose; similar to S. cerevisiae vacuolar acid trehalase (Ath1p); Hap43p-repressed gene
C1_05300C_A	*PRD1*	1.65	1.00E-01	Putative proteinase; transcript regulated by Nrg1, Mig1, and Tup1; Hogp-induced; stationary phase enriched protein; Hap43-repressed; rat catheter biofilm repressed
C1_09250W_A	*CRP1*	1.65	1.90E-01	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
C1_11200W_A		1.65	7.69E-02	Predicted mucin-like protein; ketoconazole-induced; fluconazole-repressed; induced in cyr1 mutant; colony morphology-related gene regulation by Ssn6; flow model biofilm induced; Spider biofilm induced
C3_00480C_A	*DOT5*	1.61	2.50E-01	Putative nuclear thiol peroxidase; alkaline downregulated; sumoylation target; Spider and flow model biofilm induced
C3_04060C_A	*HEM13*	1.59	2.32E-01	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
C1_09690W_A	*MLS1*	1.58	2.32E-01	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
C4_02100C_A	*GPI14*	1.58	4.39E-01	Catalytic subunit of glycosylphosphatidylinositol-alpha 1,4 mannosyltransferase I, involved in GPI anchor biosynthesis; regulated by Tsa1p, Tsa1Bp under H2O2 stress conditions
CR_05340C_A	*IFE2*	1.56	1.93E-01	Putative alcohol dehydrogenase; yeast-enriched transcript; Efg1-regulated; induced by prostaglandins, Hog1, fluconazole; rat catheter biofilm induced
C4_02110W_A		1.53	3.54E-01	Protein of unknown function; Hap43-repressed gene
C1_01930W_A		1.52	3.96E-01	Protein of unknown function; regulated by Nrg1, Tup1; Spider and flow model biofilm induced
C4_05590W_A		1.52	1.67E-01	Ortholog of S. cerevisiae : YPR117W, C. glabrata CBS138 : CAGL0D04510g, C. dubliniensis CD36 : Cd36_45200, C. parapsilosis CDC317 : CPAR2_500480 and Candida tenuis NRRL Y-1498 : CANTEDRAFT_120679
C4_01250W_A	NAT4	1.5	3.54E-01	Putative histone acetyltransferase; involved in regulation of white-opaque switch; early-stage flow model biofilm induced; Spider biofilm induced
C5_04810W_A	PFK1	1.5	2.58E-01	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

Table S2. RNASeq Analysis of NGG1 mutant (Downregulated genes)

ORF	*Candida* Gene names	log2 Fold Change	P-value adjusted	Characteristics
C2_04280W_A		-4.59	2.46E-05	Ortholog(s) have protein serine/threonine kinase activity, protein serine/threonine/tyrosine kinase activity, protein tyrosine kinase activity
C1_13870W_A	*MET3*	-2.41	1.64E-02	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
C5_02600W_A	*PUT1*	-2.13	2.55E-02	Putative proline oxidase; alkaline upregulated by Rim101; flow model biofilm induced; Spider biofilm induced
CR_02020C_A	*OPT1*	-2.02	2.98E-06	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
CR_09920W_A		-1.67	1.47E-01	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
C1_13720W_A		-1.63	6.33E-02	Ortholog of S. cerevisiae : KEL3, C. glabrata CBS138 : CAGL0A01067g, C. dubliniensis CD36 : Cd36_12710, C. parapsilosis CDC317 : CPAR2_201590 and Candida tenuis NRRL Y-1498 : cten_CGOB_00106
C2_06680W_A	*FRP3*	-1.59	2.88E-02	Putative ammonium transporter; upregulated in the presence of human neutrophils; fluconazole-downregulated; repressed by nitric oxide; Spider biofilm induced; rat catheter biofilm repressed
CR_01860W_A	*OPT9*	-1.57	7.69E-02	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
C1_13760W_A	*AGM1*	-1.56	1.08E-01	Phosphoacetylglucosamine mutase (N-acetylglucosamine-phosphate mutase); enzyme of UDP-N-acetylglucosamine (UDP-GlcNAc) biosynthesis
C5_04490C_A	*CAR1*	-1.5	2.58E-01	Arginase; arginine catabolism; transcript regulated by Nrg1, Mig1, Tup1; colony morphology-related regulation by Ssn6; alkaline induced; protein decreased in stationary phase; sumoylation target; flow model biofilm induced
C5_04880C_A	*PUT2*	-1.5	3.27E-02	Putative delta-1-pyrroline-5-carboxylate dehydrogenase; alkaline upregulated; protein present in exponential and stationary growth phase yeast cultures; flow model biofilm induced; Spider biofilm induced
C3_07300W_A	*NOP13*	-1.41	4.84E-01	Ortholog of S. cerevisiae Nop13; a nucleolar protein found in preribosomal complexes; Hap43-induced gene; rat catheter biofilm induced
CR_06660W_A	*SEO1*	-1.4	4.39E-01	Protein with similarity to permeases; Sfu1-repressed; flucytosine induced; induced by Mnl1 under weak acid stress; flow model biofilm repressed
C1_14230C_A	*IRR1*	-1.38	3.43E-01	Putative cohesin complex subunit; cell-cycle regulated periodic mRNA expression

C1_13780W_A	*MYO2*	-1.36	1.47E-01	Class V myosin; nonessential; sole class V myosin in C. albicans; required for WT actin cytoskeletal polarity, nuclear organization, migration, hyphal growth; conserved myosin ATPase/tail domains; Hap43-induced; flow model biofilm repressed
C1_13900C_A	*LCB2*	-1.32	9.81E-02	Putative serine palmitoyltransferase component; mutation confers hypersensitivity to aureobasidin A
C1_14090W_A		-1.3	2.58E-01	Ortholog(s) have chaperone binding, unfolded protein binding activity and role in chaperone-mediated protein complex assembly, protein folding, protein import into mitochondrial intermembrane space, protein refolding
C1_14240W_A		-1.3	3.29E-02	Putative RSC chromatin remodeling complex component; possibly an essential gene, disruptants not obtained by UAU1 method
C1_14100W_A	*YPT52*	-1.25	3.18E-01	Rab-family GTPase involved in vacuolar trafficking, colocolizes with Vps1p and Ypt53p in late endosome
C1_13830C_A	*PDX1*	-1.23	1.47E-01	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
C1_14320C_A		-1.21	2.50E-01	Putative fumarylacetoacetate hydrolase; induced by nitric oxide independent of Yhb1; regulated by Sef1, Sfu1, Hap43; flow model biofilm induced
C1_04660W_A	*DUR1,2*	-1.19	5.71E-01	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
C1_13840W_A		-1.18	2.32E-01	Ortholog(s) have inorganic cation transmembrane transporter activity and role in cellular cobalt ion homeostasis, cellular manganese ion homeostasis, cobalt ion transport, manganese ion transport
C1_14120C_A	*RBE1*	-1.18	2.58E-01	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
C1_04640W_A		-1.16	4.94E-01	Ortholog(s) have hydrolase activity, acting on ester bonds, triglyceride lipase activity, role in lipid homeostasis and lipid droplet localization
C4_02020W_A	*CAT2*	-1.15	5.05E-01	Major carnitine acetyl transferase; intracellular acetyl-CoA transport; localized in peroxisomes and mitochondria; induced in macrophages; Hog1-repressed; stationary phase enriched; farnesol-upregulated in biofilm; Spider biofilm induced
C1_14170W_A		-1.14	1.88E-01	Ortholog(s) have ubiquitin-protein transferase

				activity and role in histone catabolic process, histone ubiquitination
CR_05660W_A	*SDA1*	-1.14	5.45E-01	Predicted nuclear protein involved in actin cytoskeleton organization, passage through Start, 60S ribosome biogenesis; rat catheter biofilm induced; Hap43-induced
C1_14080W_A		-1.13	2.50E-01	Nucleolar protein; component of the small subunit processome containing the U3 snoRNA; involved in pre-18S rRNA processing; flow model biofilm repressed
C2_05440W_A	*PEX6*	-1.13	3.40E-01	Ortholog(s) have ATPase activity, protein heterodimerization activity
C2_06470W_A	*RTA2*	-1.11	4.91E-01	Flippase involved in sphingolipid long chain base release; mediates calcineurin-dependent ER stress response and resistance to azoles; Plc1p, Ca2+, calcineurin-regulated;
C6_03790C_A	*HGT10*	-1.11	6.77E-01	Glycerol permease involved in glycerol uptake; member of the major facilitator superfamily; induced by osmotic stress, at low glucose in rich media, during cell wall regeneration; 12 membrane spans; Hap43p-induced gene
CR_08990C_A	*SLP3*	-1.11	6.02E-01	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
C6_00960W_A	*CAN1*	-1.1	3.93E-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
CR_00330C_A	*PXA1*	-1.1	7.20E-01	Putative peroxisomal, half-size adrenoleukodystrophy protein (ALD or ALDp) subfamily ABC family transporter
C1_06870C_A		-1.09	3.83E-01	Ortholog of C. parapsilosis CDC317 : CPAR2_208910, Candida tenuis NRRL Y-1498 : CANTEDRAFT_114047, Debaryomyces hansenii CBS767 : DEHA2D14388g and Pichia stipitis Pignal : PICST_37629
C2_06170C_A	*ECM17*	-1.09	5.66E-01	Putative sulfite reductase beta subunit; role in cell wall biogenesis; regulated by Tsa1/Tsa1B in H2O2 stress; Gcn4-regulated; Tbf1-activated; Hap43-repressed; Spider, flow, F-12/CO2 model biofilm induced
C3_03640W_A	*DAL9*	-1.09	2.58E-01	Putative allantoate permease; fungal-specific (no human or murine homolog)
C6_00790C_A	*CTR1*	-1.08	6.69E-01	Copper transporter; transcribed in low copper; induced Mac1, Tye7, macrophage interaction, alkaline pH via Rim101; 17-beta-estradiol repressed; complements S. cerevisiae ctr1 ctr3 copper transport mutant; flow model/Spider biofilm induced

C1_13670W_A	*OSM2*	-1.07	5.78E-01	Putative mitochondrial fumarate reductase; regulated by Ssn6p, Gcn2p, and Gcn4p; Hog1p-downregulated; stationary phase enriched protein; Hap43p-repressed gene
C1_13880C_A		-1.07	4.40E-01	C2H2 transcription factor; Spider biofilm induced
CR_01280C_A		-1.06	6.06E-01	Ortholog(s) have role in intracellular sterol transport and extracellular region, fungal-type vacuole lumen localization
C1_14220C_A	*FTR2*	-1.05	1.93E-01	High-affinity iron permease; probably interacts with ferrous oxidase; regulated by iron level, ciclopirox olamine, amphotericin B, caspofungin; complements S. cerevisiae ftr1 iron transport defect; Hap43-repressed; Spider biofilm induced
C6_01060C_A	*CAN2*	-1.05	4.15E-01	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
CR_05520W_A	*NOC2*	-1.05	6.21E-01	Putative nucleolar complex protein; Hap43-induced; transposon mutation affects filamentous growth; mutation confers hypersensitivity to 5-fluorouracil (5-FU), tubercidin (7-deazaadenosine); repressed in core stress response
C4_00200C_A	*MET15*	-1.04	5.83E-01	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
C1_14340C_A	*RIM101*	-1.01	1.67E-01	Transcription factor; alkaline pH response; required for alkaline-induced hyphal growth; role in virulence in mice; activated by C-terminal proteolytic cleavage; mediates both positive and negative regulation; Spider biofilm induced
CR_08340W_A	*CYS3*	-1.01	5.83E-01	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

Table S3. RNASeq Analysis of SPT7 mutant (Upregulated genes)

ORF	*Candida* Gene names	log2 Fold Change	p-value adjusted	Description
C7_00120W_A		10.16	3.51E-10	Putative mitochondrial outer membrane protein membrane fission effector; possibly an essential gene, disruptants not obtained by UAU1 method
C4_03570W_A	*HWP1*	9.3	3.54E-08	Hyphal cell wall protein; host transglutaminase substrate; opaque-, a-specific, alpha-factor induced; at MTLa side of conjugation tube; virulence complicated by URA3 effects; Bcr1-

				repressed in RPMI a/a biofilms; Spider biofilm induced
C7_02000C_A		9.14	7.29E-08	Putative allantoate permease; fungal-specific (no human or murine homolog)
C3_04160W_A	*DAL8*	9.08	1.27E-07	Putative allantoate permease; fungal-specific (no human or murine homolog)
C6_02890C_A	*HPD1*	8.9	2.38E-07	3-hydroxypropionate dehydrogenase; involved in degradation of toxic propionyl-CoA; rat catheter and Spider biofilm induced
C3_06450W_A	*GLG2*	8.19	8.48E-06	Putative self-glucosylating initiator of glycogen synthesis; expression regulated upon white-opaque switch; hypha-induced; Spider biofilm induced
C2_08170W_A		7.98	1.83E-05	Putative sterol deacetylase; flow model biofilm induced; rat catheter biofilm repressed
C2_02570W_A		7.57	1.25E-04	Predicted membrane transporter; member of the drug:proton antiporter (12 spanner) (DHA1) family, major facilitator superfamily (MFS); mRNA binds She3
C5_03050C_A	*PGA58*	7.4	2.43E-04	Putative GPI-anchored protein; transcription is positively regulated by Tbf1p
CR_00620C_A	*ARG1*	6.66	2.49E-54	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
CR_01220W_A		6.27	1.67E-05	Putative transporter; mutation confers hypersensitivity to toxic ergosterol analog; fungal-specific (no human or murine homolog)
C7_00280W_A	*HGT12*	6.16	4.76E-10	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
C4_04030W_A	*JEN2*	5.5	6.09E-08	Dicarboxylic acid transporter; regulated by glucose repression; induced by Rgt1; disruptants not obtained by UAU1 method; rat catheter and Spider biofilm induced
CR_10640W_A		5.09	4.89E-57	Has domain(s) with predicted antiporter activity, role in drug transmembrane transport and membrane localization
C1_01740W_A	*CTN1*	4.95	8.38E-09	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
CR_07130C_A	*ALK8*	4.86	7.28E-05	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
C5_04140W_A		4.84	4.88E-05	Protein of unknown function; decreased transcription is observed upon fluphenazine treatment or in an azole-resistant strain that overexpresses CDR1 and CDR2; transcription is repressed in response to alpha pheromone in SpiderM medium
CR_08670C_A		4.53	4.11E-16	Protein with an enoyl-CoA hydratase related domain; Spider biofilm induced
C6_02950C_A		4.49	2.80E-04	Protein of unknown function; expression downregulated in an ssr1 null mutant
CR_08310C_A		4.42	7.77E-05	S. pombe ortholog SPBC460.04c is a predicted sulfonate/alpha-ketoglutare dioxygenase; induced by nitric oxide; Spider biofilm induced
C4_06320C_A		4.38	1.04E-04	Ortholog of Candida albicans WO-1 : CAWG_03194
C3_04200W_A	*AFP99*	4.09	2.80E-12	Protein related to arginases; downregulated upon adherence to polystyrene; regulated by Gcn2p and Gcn4p
C6_02660C_A		3.96	1.07E-04	Has domain(s) with predicted amidase activity, carbon-nitrogen ligase activity, with glutamine as amido-N-donor activity
C6_02450W_A		3.93	4.31E-21	Ortholog of S. pombe SPCC550.08, an N-acetyltransferase; transcript induced during growth in the mouse cecum
C4_00080C_A		3.86	5.11E-06	Protein of unknown function; Hap43-repressed; Spider biofilm induced
C1_02630C_A	*EXG2*	3.84	4.37E-42	GPI-anchored cell wall protein, similar to S. cerevisiae exo-1,3-beta-glucosidase Exg2p; predicted Kex2p substrate; induced during cell wall regeneration; possibly an essential gene, disruptants not obtained by UAU1 method; Hap43p-repressed
C7_02920W_A		3.74	3.52E-27	Has domain(s) with predicted amidase activity, carbon-nitrogen ligase activity, with glutamine as amido-N-donor activity
C1_02270C_A		3.73	9.78E-15	Putative oxidoreductase; Spider biofilm induced
C3_02360C_A		3.69	7.12E-10	Has domain(s) with predicted catalytic activity, sulfuric ester hydrolase activity and role in metabolic process
C4_06910W_A		3.66	3.61E-05	PDR-subfamily ABC transporter (half-size); similar to WHITE subfamily proteins; repressed by fluphenazine treatment or in an azole-resistant strain that overexpresses CDR1 and CDR2; induced by nitric oxide; rat catheter biofilm induced
C2_02080W_A		3.53	1.13E-05	C/D box small nucleolar RNA (snoRNA)
CR_08510W_A	*PGA13*	3.5	3.80E-36	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
C1_11950W_A		3.43	2.32E-04	Protein of unknown function; rat catheter biofilm induced
C3_01930W_A	*PXP2*	3.42	4.21E-11	Putative acyl-CoA oxidase; enzyme of fatty acid beta-oxidation; induced during macrophage infection; opaque specific transcript; putative peroxisome targeting signal; Spider biofilm induced
C4_01070W_A	*HGT17*	3.36	4.39E-05	Putative MFS family glucose transporter; 20 members in C. albicans; 12 probable membrane-spanning segments; induced at low (0.2%, compared to 2%) glucose in rich media; Spider biofilm induced
C4_01550C_A	*CPA1*	3.25	8.61E-06	Putative carbamoyl-phosphate synthase subunit; alkaline repressed; rat catheter, Spider and flow model biofilm induced
CR_01330W_A	*CPA2*	3.19	6.96E-26	Putative arginine-specific carbamoylphosphate synthetase; protein enriched in stationary phase yeast cultures; rat catheter biofilm induced; Spider biofilm induced
C2_06590C_A	*GIT1*	3.18	3.97E-08	Glycerophosphoinositol permease; involved in utilization of glycerophosphoinositol as a phosphate source; Rim101-repressed; virulence-group-correlated expression
C2_05950C_A	*TES15*	3.08	6.16E-05	Putative acyl-CoA thioesterase; Hap43-repressed; Spider biofilm induced
C4_04080C_A	*PGA31*	3.08	3.24E-11	Cell wall protein; putative GPI anchor; expression regulated upon white-opaque switch; induced by Congo Red and cell wall regeneration; Bcr1-repressed in RPMI a/a biofilms
CR_04870C_A		3.04	1.48E-36	Trimethylaminobutyraldehyde dehydrogenase, the third enzyme of the carnitine biosynthesis pathway
C5_02430W_A	*ZCF22*	2.99	1.50E-06	Predicted Zn(Gurskiĭ et al.)2Cys6 transcription factor
C2_06700W_A	*AMO2*	2.96	2.36E-41	Protein similar to A. niger predicted peroxisomal copper amino oxidase; mutation confers hypersensitivity to toxic ergosterol analog; F-12/CO2 early biofilm induced
C4_02990C_A	*GST2*	2.94	4.50E-26	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
C3_00010C_A		2.78	6.05E-27	Protein of unknown function; induced by Mnl1 under weak acid stress; transcript detected on high-resolution tiling arrays; Spider biofilm repressed
C4_05070C_A	*ARG8*	2.64	3.14E-07	Putative acetylornithine aminotransferase; Gcn2, Gcn4 regulated; rat catheter biofilm induced; Spider biofilm induced

C3_07330W_A		2.57	2.19E-06	Predcted glucose 1-dehydrogenase (NADP+); rat catheter biofilm repressed
C4_03710C_A		2.54	1.29E-11	Predicted membrane protein; rat catheter biofilm induced
CR_08800W_A	*ITS2*	2.53	1.77E-38	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
C1_09290C_A	*ARG5,6*	2.5	2.28E-21	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
C4_06780C_A	***OYE32***	2.46	1.46E-08	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
C1_03000W_A	*HOL1*	2.42	7.00E-06	Putative MFS transporter; regulated by Nrg1; macrophage/pseudohyphal-repressed; induced by alpha pheromone in SpiderM medium; possibly an essential gene, disruptants not obtained by UAU1 method
C1_13160W_A	*PSA2*	2.42	1.52E-11	Mannose-1-phosphate guanyltransferase; Hap43, macrophage-repressed; stationary phase enriched protein; Spider biofilm induced; rat catheter biofilm repressed
C6_02200C_A		2.41	2.92E-04	Protein of unknown function; mRNA binds She3; transcript regulated upon yeast-hypha switch; induced in oralpharyngeal candidasis
CR_08790W_A	*RDN58*	2.41	5.11E-48	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
CR_08780W_A	*ITS1*	2.35	1.17E-21	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
C4_05560C_A	*ARO9*	2.3	3.18E-06	Aromatic transaminase; Ehrlich fusel oil pathway of aromatic alcohol biosynthesis; Rim101-dependent pH-regulation (alkaline induced); Hap43-induced gene
C3_00030C_A		2.29	2.22E-22	Protein with a predicted DEAD-like DNA/RNA helicase domain; shows colony morphology-related gene regulation by Ssn6; overlaps orf19.5472; Spider biofilm repressed
C2_08055W_A	*RPR1*	2.26	8.36E-05	Putative ortholog of S. cerevisiae RNAse P RNA; gene transcribed by RNA Pol III

CR_08810W_A	*RDN25*	2.25	3.19E-43	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)
C4_05130C_A	*ALD6*	2.24	1.60E-04	Putative aldehyde dehydrogenase; stationary phase enriched protein; expression regulated upon white-opaque switch; rat catheter biofilm induced; rat catheter and Spider biofilm induced
C6_03880W_A		2.23	1.08E-06	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
CR_05220C_A	*GUT1*	2.18	7.05E-09	Putative glycerol kinase; downregulated upon adherence to polystyrene; greater mRNA abundance observed in a cyr1 homozygous null mutant than in wild type
C4_00390W_A		2.17	2.04E-17	Ortholog(s) have chromatin binding activity
C1_04500W_A	*ICL1*	2.16	6.91E-05	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
C4_04520W_A		2.16	5.01E-05	Ortholog(s) have enzyme activator activity, telomerase inhibitor activity, role in box C/D snoRNA 3'-end processing, negative regulation of telomere maintenance via telomerase and nucleolus, nucleoplasm localization
C2_02970C_A	*ALD5*	2.12	2.65E-44	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
CR_00090C_A		2.11	7.40E-06	Protein of unknown function; stationary phase enriched protein; induced upon yeast-hypha transition; benomyl or caspofungin induced; Hap43-repressed; Spider biofilm induced
C4_02040W_A		2.09	5.86E-16	Ortholog(s) have polyamine oxidase activity and role in pantothenate biosynthetic process, spermine catabolic process
C4_03530W_A		2.07	3.89E-07	Ortholog of Candida albicans WO-1 : CAWG_03455
CR_08920W_A		2.07	9.52E-09	Protein with predicted oxidoreductase and dehydrogenase domains; Hap43-repressed; Spider biofilm induced
C1_08970W_A		2.06	2.80E-16	C/D box small nucleolar RNA (snoRNA)
C2_00630C_A		2.06	1.87E-05	Ortholog(s) have role in allantoin catabolic process
C7_02150C_A	*ECM42*	2.03	1.14E-04	Ornithine acetyltransferase; Gcn2, Gcn4-regulated; clade-specific gene expression; possibly essential gene, disruptants not obtained by UAU1 method; Spider biofilm induced

Table S4. RNASeq Analysis of SPT7 mutant (Downregulated genes)

ORF	*Candida* Gene names	log2 Fold-change	p-value adjusted	Description
C4_01340W_A		-8.16	7.39E-06	Protein similar to GPI-linked cell-wall proteins; induced in low iron; Spider biofilm induced; regulated in Spider biofilms by Bcr1, Tec1, Ndt80, Brg1
C6_01360W_A		-7.17	3.72E-09	Protein of unknown function; ketoconazole-repressed
CR_04210C_A	*QDR1*	-7.11	1.16E-30	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
C2_08290C_A	*UCF1*	-6.09	3.52E-11	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
C4_04770C_A	*MNN22*	-5.9	7.94E-51	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
CR_02920C_A	*AQY1*	-5.88	2.25E-79	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
C2_08300C_A		-5.7	1.12E-27	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
C1_14120C_A	*RBE1*	-5.36	1.18E-17	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
CR_10100C_A	*INO1*	-4.7	1.14E-06	Inositol-1-phosphate synthase; antigenic in human; repressed by farnesol in biofilm or by caspofungin; upstream inositol/choline regulatory element; glycosylation predicted; rat catheter, flow model induced; Spider biofilm repressed
C1_10360C_A		-4.66	2.20E-36	Putative protein of unknown function; Hap43p-repressed gene; increased transcription is observed upon fluphenazine treatment; possibly transcriptionally regulated by Tac1p; induced by nitric oxide; fungal-specific (no human/murine

				homolog
C1_08790W_A	*TPO3*	-4.51	1.45E-134	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
C2_07630C_A		-4.38	8.96E-12	Possible stress protein; increased transcription associated with CDR1 and CDR2 overexpression or fluphenazine treatment; regulated by Sfu1, Nrg1, Tup1; stationary phase enriched protein; Spider biofilm induced
C5_02110W_A		-4.06	1.08E-54	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
C4_03430W_A	*MOH1*	-4.03	4.86E-05	Ortholog of S. cerevisiae Moh1, essential for staionary phase growth; induced by alpha pheromone in SpiderM medium and by Mnl1 under weak acid stress; possibly essential (UAU1 method); flow model biofilm induced; Spider biofilm induced
C1_06870C_A		-4.02	2.86E-14	Ortholog of C. parapsilosis CDC317 : CPAR2_208910, Candida tenuis NRRL Y-1498 : CANTEDRAFT_114047, Debaryomyces hansenii CBS767 : DEHA2D14388g and Pichia stipitis Pignal : PICST_37629
C5_02080C_A	*HSP12*	-4	1.64E-53	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
C1_10400C_A	*FGR41*	-3.96	9.69E-24	Putative GPI-anchored adhesin-like protein; transposon mutation affects filamentous growth; Spider biofilm repressed
C2_05180W_A	*WH11*	-3.75	2.72E-45	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
C2_04010C_A	*HSP21*	-3.67	5.69E-06	Small heat shock protein; role in stress response and virulence; fluconazole-downregulated; induced in cyr1 or ras1 mutant; stationary phase enriched protein; detected in some, not all, biofilm extracts; Spider biofilm induced
C1_10450W_A	*GLY1*	-3.62	7.96E-58	L-threonine aldolase; complements glycine auxotrophy of S. cerevisiae shm1 shm2 gly1-1 triple mutant; macrophage/pseudohyphal-induced; the GLY1 locus has an RFLP and is triploid in strain SGY269; flow model biofilm induced
C5_02630C_A	*MNN1*	-3.46	3.16E-06	Putative alpha-1,3-mannosyltransferase; of the mannosyltransferase complex; negatively

				regulated by Rim101; transcript elevated in chk1 and nik1 mutants, but not in sln1 mutant; Spider and flow model biofilm induced
C6_01510W_A	*OYE23*	-3.42	1.06E-06	Putative NAPDH dehydrogenase; induced by nitric oxide, benomyl; oxidative stress-induced via Cap1; Hap43p-repressed; rat catheter biofilm induced
CR_06860C_A	*ARO10*	-3.39	8.88E-43	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
C6_02010C_A	*GPD2*	-3.24	2.10E-44	Surface protein similar to glycerol 3-P dehydrogenase; binds host Factor H, FHL-1, plasminogen; regulated by Ssn6, Nrg1, Efg1; induced by cell wall regeneration, macrophage/pseudohyphal growth, core stress response; Spider biofilm induced
C2_07570W_A	*RNR22*	-3.23	2.75E-40	Putative ribonucleoside diphosphate reductase;colony morphology-related gene regulation by Ssn6; transcript regulated by tyrosol and cell density; Hap43-repressed; Spider biofilm induced
C1_11850W_A		-3.2	1.45E-38	Protein of unknown function; Hap43-repressed gene; mRNA binds to She3; repressed in hyphae; Efg1 and Efh1 regulated; 5'-UTR intron; induced by Mnl1 under weak acid stress; rat catheter biofilm induced
C1_13480W_A	*HSP70*	-3.19	7.56E-39	Putative hsp70 chaperone; role in entry into host cells; heat-shock, amphotericin B, cadmium, ketoconazole-induced; surface localized in yeast and hyphae; antigenic in host; farnesol-downregulated in biofilm; Spider biofilm induced
C5_03480C_A		-3.16	7.64E-44	Spermidine transporter; induced in strains from HIV patients with oral candidiasis; alkaline repressed; amphotericin B induced; colony morphology regulated by Ssn6; reduced oral epithelial cell damage by mutant; Spider biofilm induced
C1_02120C_A	*SHA3*	-3.11	9.42E-36	Putative ser/thr kinase involved in glucose transport; Tn mutation affects filamentous growth; fluconazole-induced; ketoconazole-repressed; induced in by alpha pheromone in SpiderM; possibly essential; flow model biofilm induced
C7_00350C_A		-3.08	5.56E-30	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
C5_00390C_A		-3.07	9.28E-24	Protein of unknown function; transcript repressed upon yeast-hyphal switch; fluconazole-induced; Hap43-repressed; flow model biofilm induced
CR_01470W_A	*CSP37*	-3.06	3.97E-41	Hyphal cell wall protein; role in progression of mouse systemic infection; predicted P-loop,

				divalent cation binding, N-glycosylation sites; expressed in yeast and hyphae; hyphal downregulated; stationary-phase enriched; GlcNAc-induced
C1_12910W_A		-3.05	4.70E-05	Protein of unknown function; Spider biofilm repressed
C5_00890C_A	*GIT2*	-2.87	3.90E-07	Putative glycerophosphoinositol permease; fungal-specific; repressed by alpha pheromone in SpiderM medium; Hap43-repressed; Spider biofilm induced
C1_04770C_A	*ERG3*	-2.86	1.75E-21	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
C6_01670W_A	*MAE1*	-2.86	3.54E-10	Malic enzyme, mitochondrial; transcription regulated by Mig1, Tup1; colony morphology-related gene regulation by Ssn6; Hap43-repressed; Spider biofilm repressed
C2_07900W_A	*GDH2*	-2.82	7.18E-72	Putative NAD-specific glutamate dehydrogenase; fungal-specific; transcript regulated by Nrg1, Mig1, Tup1, and Gcn4; stationary phase enriched protein; flow model biofilm induced; Spider biofilm induced
C2_01020W_A	*HGT6*	-2.81	1.07E-33	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
C4_05900C_A		-2.8	8.28E-08	Protein of unknown function; Rgt1, Hap43-repressed; flow model biofilm induced; Spider biofilm induced
C1_08460C_A	*UPC2*	-2.76	6.43E-21	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
C7_01800C_A	*PFK2*	-2.76	5.56E-84	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
C1_09080C_A	*PGA6*	-2.74	1.62E-17	GPI-anchored cell wall adhesin-like protein; induced by high iron; upregulated upon Als2 depletion; mRNA binds She3 and is localized to hyphal tips; Spider biofilm repressed
C4_01530C_A	*ERG251*	-2.71	6.77E-26	C-4 sterol methyl oxidase; role in ergosterol biosynthesis; Hap43-induced; ketoconazole-induced; amphotericin B, caspofungin repressed; possibly essential gene, disruptants not obtained by UAU1 method; Spider biofilm repressed
C2_02860W_A	*SUR2*	-2.64	3.07E-32	Putative ceramide hydroxylase; predicted enzyme

				of sphingolipid biosynthesis; regulated by Tsa1, Tsa1B under H2O2 stress conditions; Spider and flow model biofilm induced
C3_07280C_A		-2.61	3.27E-14	protein with ENTH Epsin domain, N-terminal; Spider biofilm repressed
C2_10240W_A	*GPD1*	-2.6	1.04E-31	Glycerol-3-phosphate dehydrogenase; glycerol biosynthesis; regulated by Efg1; regulated by Tsa1, Tsa1B under H2O2 stress conditions; Sflow model and Spider biofilm induced
C5_03510C_A		-2.6	8.75E-12	Protein of unknown function; mRNA binds to She3; Hap43-repressed; rat catheter and flow model biofilm induced
C4_01750C_A	*FBA1*	-2.59	2.75E-50	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
C4_05730W_A		-2.58	3.14E-10	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
CR_07790C_A	*YHB1*	-2.55	2.20E-36	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
C1_07220W_A		-2.54	8.08E-05	Protein of unknown function; Plc1p-regulated; expression induced early upon infection of reconstituted human epithelium (Darzacq et al.), while expression of the C. dubliniensis ortholog is not; mutant is viable; Spider biofilm induced
C2_09950W_A	*FCY21*	-2.54	4.63E-24	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
C1_13140C_A	*TYE7*	-2.51	6.02E-57	bHLH transcription factor; control of glycolysis; required for biofilm formation; hyphally regulated by Cph1, Cyr1; flucytosine, Hog1 induced; amphotericin B, caspofungin repressed; induced in flow model biofilm and planktonic cultures
C3_04550C_A	*CMK1*	-2.5	8.74E-25	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
C6_01060C_A	*CAN2*	-2.49	6.45E-25	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
C1_05540C_A		-2.48	8.78E-18	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
C2_07440C_A		-2.47	4.63E-06	Ortholog(s) have sterol esterase activity, role in sterol metabolic process and integral component of membrane, lipid droplet localization
C6_03990C_A		-2.47	2.59E-04	Predicted ORF overlapping the Major Repeat Sequence on chromosome 6; member of a family encoded by FGR6-related genes in the RB2 repeat sequence; rat catheter biofilm repressed
C6_00290W_A		-2.46	9.81E-20	Protein of unknown function; regulated by yeast-hypha switch; induced by Mnl1 in weak acid stress; 5' UTR intron; repressed by chlamydospore formation in C. albicans and C. dubliniensis; rat catheter, Spider and flow model biofilm induced
C6_00480C_A	*FET31*	-2.45	5.18E-32	Putative multicopper oxidase; ketoconazole/caspofungin/amphotericin B repressed; Sef1/Sfu1/Hap43 regulated; reports differ if functional homolog of ScFet3; rat catheter and Spider biofilm induced
C1_02600W_A	*SNO1*	-2.42	3.23E-05	Protein with a predicted role in pyridoxine metabolism; stationary phase protein; regulated by Tup1, Efg1; Spider biofilm induced
C3_01900C_A		-2.42	1.20E-05	Protein with a predicted FYVE/PHD zinc finger domain; Hap43-repressed; Spider biofilm induced
C5_04810W_A	*PFK1*	-2.38	3.44E-56	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
CR_05210W_A		-2.38	7.39E-06	Protein of unknown function; Hap43-repressed; Spider biofilm repressed
C7_02610C_A		-2.37	2.85E-23	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
C2_09820W_A		-2.3	1.63E-05	Protein of unknown function; flow model biofilm induced; Spider biofilm induced
C7_01560C_A	*NUP*	-2.28	8.96E-16	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
C3_01950C_A		-2.27	2.38E-12	Has domain(s) with predicted integral component of membrane localization
CR_09460C_A		-2.26	2.53E-04	Protein of unknown function; Spider biofilm induced
C6_03700W_A	*ALS1*	-2.25	3.49E-17	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
C7_03200C_A		-2.25	6.46E-07	Putative pyridoxamine 5'-phosphate oxidase; planktonic growth and early-stage flow model biofilm induced
C3_01180C_A		-2.24	8.74E-16	Protein of unknown function; induced by Mnl1 under weak acid stress
C2_01380W_A	*PLB4.5*	-2.23	3.18E-22	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
C7_00730W_A	*MET28*	-2.23	3.35E-07	Predicted bZIP domain-containing transcription factor; protein induced during the mating process; possibly essential, disruptants not obtained by UAU1 method; Hap43-repressed; rat catheter biofilm induced
C1_09250W_A	*CRP1*	-2.21	1.15E-37	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
CR_10110W_A	*CHT3*	-2.21	8.08E-11	Major chitinase; secreted; functional homolog of S. cerevisiae Cts1p; 4 N-glycosylation motifs; possible O-mannosylation; putative signal peptide; hyphal-repressed; farnesol upregulated in biofilm; regulated by Efg1p, Cyr1p, Ras1p
C2_02590W_A	*ZRT2*	-2.2	1.40E-25	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
C3_04580C_A	*STP1*	-2.2	1.18E-19	Transcription factor; regulates SAP2, OPT1 expression and thereby protein catabolism for nitrogen source; activated via amino-acid-induced proteolytic processing; macrophage/pseudohyphal-repressed; Spider biofilm repressed
C2_08590W_A	*YWP1*	-2.19	6.18E-29	Secreted yeast wall protein; possible role in dispersal in host; mutation increases adhesion and biofilm formation; propeptide; growth phase, phosphate, Ssk1/Ssn6/Efg1/Efh1/Hap43 regulated; mRNA binds She3; flow and Spider biofilm repressed
C6_00330C_A	*GNP1*	-2.19	2.10E-44	Similar to asparagine and glutamine permease; fluconazole, caspofungin induced; regulated by Nrg1, Mig1, Tup1, Gcn2, Gcn4, and alkaline regulated by Rim101; repressed during chlamydospore formation; rat catheter, flow model biofilm induced
C2_08490W_A	*DSE1*	-2.18	1.29E-09	Essential cell wall protein involved in cell wall integrity and rigidity; periodic mRNA expression

				peaks at M/G1 phase; Ace2p-induced; required for virulence in a mouse model of infection
C4_01360W_A	*PGA53*	-2.18	1.06E-20	GPI-anchored cell surface protein of unknown function; greater mRNA abundance observed in a cyr1 homozygous null mutant than in wild type
C7_00930W_A	*GPH1*	-2.17	5.19E-25	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
C3_06860C_A		-2.15	4.23E-07	Putative xylose and arabinose reductase; flow model biofilm induced; Spider biofilm repressed
CR_09140C_A		-2.15	2.65E-04	Protein with a role in directing meiotic recombination events to homologous chromatids; induced by ciclopirox olamine; positively regulated by Sfu1; Hog1, fluconazole-repressed; Hap43-induced; Spider biofilm induced
C3_00800W_A	*MIH1*	-2.14	6.86E-12	Putative protein phosphatase of the PTP family (tyrosine-specific); ortholog of S. cerevisiae Mih1; mRNA binds She3
C1_08590C_A	*ERG1*	-2.1	2.11E-27	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
C4_06570C_A	*PDC11*	-2.1	1.64E-06	Pyruvate decarboxylase; antigenic; on hyphal not yeast cell surface; Hap43, Gcn4, Efg1, Efh1, Hsf1 regulated; fluconazole, farnesol induced; amino acid starvation repressed; flow model biofilm induced; Spider biofilm repressed
C2_06020W_A	*CNT*	-2.09	5.30E-20	CNT family H(+)/nucleoside symporter; transports adenosine, uridine, inosine, guanosine, tubercidin; variant alleles for high/low-affinity isoforms; S or G at residue 328 affects specificity; Spider, flow model biofilm induced
C3_02480C_A	*CCP1*	-2.06	3.56E-29	Cytochrome-c peroxidase N terminus; Rim101, alkaline pH repressed; induced in low iron or by macrophage interaction; oxygen-induced activity; regulated by Sef1, Sfu1, and Hap43; Spider biofilm induced; rat catheter biofilm repressed
C3_03420C_A	*NDE1*	-2.06	3.40E-42	Putative NADH dehydrogenase; may act alternatively to complex I in respiration; caspofungin repressed; rat catheter biofilm induced; Spider biofilm repressed
C5_04110W_A	*SCW11*	-2.03	1.06E-14	Cell wall protein; repressed in ace2 mutant; repressed in core caspofungin response; induced in high iron; possibly an essential gene, disruptants not obtained by UAU1 method; rat catheter and Spider biofilm repressed
C1_06850W_A	*PCL7*	-2.02	3.84E-11	Putative cyclin-like protein; possible Pho85 cyclin; hyphal repressed; induced by Mnl1 under weak acid stress
C1_08610C_A		-2	8.54E-14	Ortholog of S. cerevisiae Aim38/Rcf2, cytochrome

				c oxidase subunit; plasma membrane localized; Hap43-repressed; induced in oralpharyngeal candidasis; flow model biofilm induced; Spider biofilm repressed
C1_11080W_A		-2	1.24E-09	Putative 6-phosphofructo-2-kinase; catalyzes synthesis of fructose-2,6-bisphosphate; Hap43-repressed; flow model, rat catheter and Spider biofilm induced
C2_06860W_A	*LTE1*	-2	4.92E-08	Protein similar to GDP/GTP exchange factors; repressed by alpha pheromone in SpiderM medium; flow model biofilm repressed
C2_10430C_A	*FGR29*	-2	1.89E-04	Protein lacking an ortholog in S. cerevisiae; transposon mutation affects filamentous growth; rat catheter biofilm repressed

Table S5. RNASeq Analysis of SPT8 mutant (Upregulated genes)

ORF	*Candida* Gene names	log2 Fold-Change	P-value adjusted	Description
C4_03570W_A	*HWP1*	12.71	5.67E-08	Hyphal cell wall protein; host transglutaminase substrate; opaque-, a-specific, alpha-factor induced; at MTLa side of conjugation tube; virulence complicated by URA3 effects; Bcr1-repressed in RPMI a/a biofilms; Spider biofilm induced
C7_00120W_A		9.34	4.87E-08	Putative mitochondrial outer membrane protein membrane fission effector; possibly an essential gene, disruptants not obtained by UAU1 method
C3_04160W_A	*DAL8*	8.33	9.30E-06	Putative allantoate permease; fungal-specific (no human or murine homolog)
C2_07790C_A		7.41	4.08E-04	Protein of unknown function; induced by alpha pheromone in SpiderM medium
C2_00660C_A	*SOD4*	7.02	1.82E-03	Cu-containing superoxide dismutase; role in response to host innate immune ROS; regulated on white-opaque switch; ciclopirox olamine induced; caspofungin repressed; SOD1,4,5,6 gene family; yeast-associated; Spider biofilm induced
C3_06450W_A	*GLG2*	6.76	5.16E-03	Putative self-glucosylating initiator of glycogen synthesis; expression regulated upon white-opaque switch; hypha-induced; Spider biofilm induced
C1_05920W_A		6.62	7.23E-03	Protein of unknown function; induced during chlamydospore formation in both C. albicans and C. dubliniensis; Spider biofilm induced
C6_02890C_A	*HPD1*	6.58	8.03E-03	3-hydroxypropionate dehydrogenase; involved in degradation of toxic propionyl-CoA; rat catheter and Spider biofilm induced

C2_05600W_A		6.49	1.05E-02	C/D box small nucleolar RNA (snoRNA)
CR_00620C_A	*ARG1*	5.61	2.40E-31	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
C7_00110W_A	*SOD3*	4.88	1.46E-20	Cytosolic manganese-containing superoxide dismutase; protects against oxidative stress; repressed by ciclopirox olamine, induced during stationary phase when SOD1 expression is low; Hap43-repressed; Spider and flow model biofilm induced
C7_00280W_A	*HGT12*	4.6	7.55E-04	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
C7_03150W_A		4.55	4.27E-03	Protein of unknown function; rat catheter and Spider biofilm induced
C1_00780C_A	*HGC1*	4.54	7.58E-03	Hypha-specific G1 cyclin-related protein involved in regulation of morphogenesis, biofilm formation; Cdc28-Hgc1 maintains Cdc11 S394 phosphorylation during hyphal growth; required for virulence in mice; regulated by Nrg1, Tup1, farnesol
C2_09890W_A	*SMM1*	4.23	5.23E-03	Putative dihydrouridine synthase; Hap43-induced gene; rat catheter biofilm induced; Spider biofilm induced
C2_02080W_A		3.47	8.14E-05	C/D box small nucleolar RNA (snoRNA)
C1_02630C_A	*EXG2*	3.45	9.42E-35	GPI-anchored cell wall protein, similar to S. cerevisiae exo-1,3-beta-glucosidase Exg2p; predicted Kex2p substrate; induced during cell wall regeneration; possibly an essential gene, disruptants not obtained by UAU1 method; Hap43p-repressed
C4_00130W_A	*RBT5*	3.44	6.82E-07	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
C5_01160C_A		3.43	1.01E-04	C/D box small nucleolar RNA (snoRNA)
C2_06590C_A	*GIT1*	3.37	7.89E-07	Glycerophosphoinositol permease; involved in utilization of glycerophosphoinositol as a phosphate source; Rim101-repressed; virulence-group-correlated expression

C1_13160W_A	*PSA2*	3.35	8.78E-32	Mannose-1-phosphate guanyltransferase; Hap43, macrophage-repressed; stationary phase enriched protein; Spider biofilm induced; rat catheter biofilm repressed
C2_00680C_A	*SOD5*	3.23	1.07E-09	Cu-containing superoxide dismutase; protects against oxidative stress; induced by neutrophils, hyphal growth, caspofungin, osmotic/oxidative stress; oralpharyngeal candidiasis induced; rat catheter and Spider biofilm induced
C7_02280W_A		3.17	1.77E-04	Ortholog of C. parapsilosis CDC317 : CPAR2_808370, C. dubliniensis CD36 : Cd36_72070, Candida orthopsilosis Co 90-125 : CORT_0C00800 and Candida albicans WO-1 : CAWG_05577
C5_02220C_A		3.11	1.28E-02	Putative polyphosphate phosphatase; role in hydrolysis of diphosphorylated inositol polyphosphates and diadenosine polyphosphates; Spider biofilm induced
CR_08670C_A		3.05	1.18E-04	Protein with an enoyl-CoA hydratase related domain; Spider biofilm induced
CR_06210C_A		2.96	4.43E-03	C/D box small nucleolar RNA (snoRNA)
CR_06500C_A		2.95	6.08E-07	Protein of unknown function; Hap43-induced; regulated by Nrg1, Tup1; repressed by alpha pheromone in SpiderM medium; Spider biofilm induced; Bcr1-repressed in RPMI a/a biofilms
C2_10800W_A		2.89	1.40E-07	C/D box small nucleolar RNA (snoRNA)
CR_07070C_A	*ALS3*	2.86	2.72E-03	Cell wall adhesin; epithelial adhesion, endothelial invasion; alleles vary in adhesiveness; immunoprotective in mice; binds SspB adhesin of S. gordonii in mixed biofilm; induced in/required for Spider biofilm; flow model biofilm repressed
C1_12990W_A		2.81	2.68E-05	C/D box small nucleolar RNA (snoRNA)
CR_06220C_A		2.8	2.27E-03	C/D box small nucleolar RNA (snoRNA)
CR_09680C_A	*RTA4*	2.8	7.51E-03	Protein similar to S. cerevisiae Rsb1p, involved in fatty acid transport; transposon mutation affects filamentous growth; alkaline downregulated; caspofungin induced; possibly an essential gene; Hap43p-repressed
CR_10640W_A		2.72	2.15E-06	Has domain(s) with predicted antiporter activity, role in drug transmembrane transport and membrane localization
CR_04275W_A	*SCR1*	2.69	6.83E-08	Putative ortholog of S. cerevisiae signal recognition particle (SRP) RNA; gene transcribed by RNA Pol III
CR_08510W_A	*PGA13*	2.66	1.18E-10	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
C1_06280C_A	*UME6*	2.53	5.93E-03	Zn(Gurskiĭ et al.)2Cys6 transcription factor; has a long 5'-UTR that regulates translational efficiency and controls transition to filamentous growth; stability controlled by Grr1p, Ubr1p, Ptc2p in response to CO2 and O2 levels
C1_13000W_A		2.53	5.03E-03	C/D box small nucleolar RNA (snoRNA)
C6_00440C_A	*FET34*	2.51	2.71E-22	Multicopper ferroxidase; induced by low iron, ciclopirox olamine, ketoconazole, hypoxia; alkaline induced by Rim101; repressed in fluconazole-resistant isolate; Sfu1, Hog1 repressed; complements S. cerevisiae fet3; Spider biofilm induced
C7_03880C_A		2.47	4.00E-03	Protein required for virulence in reconstituted human epithelium (Darzacq et al.) model of ex vivo infection; decreased transcription is observed upon fluphenazine treatment; induced upon adherence to polystyrene
C1_02270C_A		2.37	1.74E-03	Putative oxidoreductase; Spider biofilm induced
C4_06780C_A	*OYE32*	2.37	9.59E-07	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
CR_10460W_A		2.34	1.43E-04	C/D box small nucleolar RNA (snoRNA)
CR_08810W_A	*RDN25*	2.27	9.88E-27	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)
C1_09290C_A	*ARG5,6*	2.23	3.09E-14	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
C1_08970W_A		2.19	2.11E-16	C/D box small nucleolar RNA (snoRNA)
C1_08810C_A	*MSS116*	2.18	3.82E-13	Putative DEAD-box protein; required for efficient splicing of mitochondrial Group I and II introns; Hap43-induced; rat catheter biofilm induced

C2_08055W_A	*RPR1*	2.17	8.35E-04	Putative ortholog of S. cerevisiae RNAse P RNA; gene transcribed by RNA Pol III
CR_09880W_A	*DEF1*	2.12	3.90E-31	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
CR_05370W_A		2.11	2.26E-05	Telomerase RNA; provides the template for the telomerase reverse transcriptase TERT/EST2
CR_08790W_A	*RDN58*	2.1	4.79E-22	5.8S ribosomal RNA; component of the large (60S) ribosomal subunit; encoded in about 55 copies of the rDNA repeat on Chromosome R
C2_00630C_A		2.06	9.33E-05	Ortholog(s) have role in allantoin catabolic process
CR_08800W_A	*ITS2*	2.05	1.78E-14	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

Table S6. RNASeq Analysis of SPT8 mutant (Downregulated genes)

ORF	Candida gene names	log2 Fold Change	p-value adjusted	Description
C4_01340W_A		-8.2	1.36E-05	Protein similar to GPI-linked cell-wall proteins; induced in low iron; Spider biofilm induced; regulated in Spider biofilms by Bcr1, Tec1, Ndt80, Brg1
C4_04770C_A	MNN22	-6.07	4.90E-48	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
C1_06870C_A		-5.33	1.19E-14	Ortholog of C. parapsilosis CDC317 : CPAR2_208910, Candida tenuis NRRL Y-1498 : CANTEDRAFT_114047, Debaryomyces hansenii CBS767 : DEHA2D14388g and Pichia stipitis Pignal : PICST_37629
C2_04010C_A	HSP21	-5.04	1.08E-06	Small heat shock protein; role in stress response and virulence; fluconazole-downregulated; induced in cyr1 or ras1 mutant; stationary phase enriched protein; detected in some, not all, biofilm extracts; Spider biofilm induced
C2_08300C_A		-4.79	8.18E-27	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
C2_08290C_A	UCF1	-4.36	6.15E-104	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
C6_01990W_A	PLB1	-4.31	4.80E-09	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
CR_02920C_A	AQY1	-4.1	6.73E-03	Arginase; arginine catabolism; transcript regulated by Nrg1, Mig1, Tup1; colony morphology-related regulation by Ssn6; alkaline induced; protein decreased in stationary phase; sumoylation target; flow model biofilm induced
C6_01060C_A	CAN2	-4.03	1.18E-30	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
C4_05730W_A		-3.7	8.09E-16	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
C1_07220W_A		-3.51	6.68E-07	Protein of unknown function; Plc1p-regulated; expression induced early upon infection of reconstituted human epithelium (Darzacq et al.), while expression of the C. dubliniensis ortholog is not; mutant is viable; Spider biofilm induced
C1_08790W_A	TPO3	-3.4	1.98E-03	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
C1_10400C_A	FGR41	-3.36	7.89E-22	Putative GPI-anchored adhesin-like protein; transposon mutation affects filamentous growth; Spider biofilm repressed
C3_03440C_A		-3.36	1.55E-04	Putative spermidine export pump; fungal-specific (no human or murine homolog)
C4_02740W_A		-3.29	1.59E-06	Protein of unknown function; flow model, rat catheter and Spider biofilm induced; Hap43-repressed
CR_06860C_A	ARO10	-3.15	2.89E-35	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
C2_01270W_A	CHA1	-3.1	2.89E-35	Similar to catabolic ser/thr dehydratases; repressed by Rim101; induced in low iron; regulated on white-opaque switch; filament induced; Tn mutation affects filamentation; flow model biofilm

				induced; Spider biofilm repressed
C1_11850W_A		-3.06	2.27E-25	Protein of unknown function; Hap43-repressed gene; mRNA binds to She3; repressed in hyphae; Efg1 and Efh1 regulated; 5'-UTR intron; induced by Mnl1 under weak acid stress; rat catheter biofilm induced
C7_01560C_A	NUP	-3.01	5.10E-17	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
C4_00440C_A	OPT7	-2.99	1.79E-08	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
C6_01360W_A		-2.94	5.34E-08	Protein of unknown function; ketoconazole-repressed
C1_14120C_A	RBE1	-2.92	9.88E-11	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
C1_13870W_A	MET3	-2.82	2.24E-25	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
C2_07900W_A	GDH2	-2.78	3.50E-48	Putative NAD-specific glutamate dehydrogenase; fungal-specific; transcript regulated by Nrg1, Mig1, Tup1, and Gcn4; stationary phase enriched protein; flow model biofilm induced; Spider biofilm induced
C5_00390C_A		-2.63	2.30E-16	Protein of unknown function; transcript repressed upon yeast-hyphal switch; fluconazole-induced; Hap43-repressed; flow model biofilm induced
C4_03960W_A		-2.61	1.87E-13	Protein of unknown function; ORF added to Assembly 21 based on comparative genome analysis; protein detected by mass spec in stationary phase cultures
C1_10360C_A		-2.6	7.97E-18	Putative protein of unknown function; Hap43p-repressed gene; increased transcription is observed upon fluphenazine treatment; possibly transcriptionally regulated by Tac1p; induced by nitric oxide; fungal-specific (no human/murine homolog
C3_03490W_A	RSN1	-2.55	1.05E-02	Protein of unknown function; flow model biofilm induced; Spider biofilm induced; induced during the mating process; Hap43-repressed
C3_04580C_A	STP1	-2.54	4.41E-22	Transcription factor; regulates SAP2, OPT1 expression and thereby protein catabolism for nitrogen source; activated via amino-acid-induced proteolytic processing; macrophage/pseudohyphal-repressed; Spider biofilm repressed

C2_08590W_A	YWP1	-2.45	6.10E-41	Secreted yeast wall protein; possible role in dispersal in host; mutation increases adhesion and biofilm formation; propeptide; growth phase, phosphate, Ssk1/Ssn6/Efg1/Efh1/Hap43 regulated; mRNA binds She3; flow and Spider biofilm repressed
CR_02060W_A		-2.41	5.37E-03	Protein of unknown function; Spider biofilm induced
C1_05160C_A		-2.35	1.39E-15	Putative phosphatidyl synthase; stationary phase enriched protein; transcript repressed by yeast-hypha switch; Hap43-repressed; rat catheter, Spider and flow model biofilm induced
C6_01510W_A	OYE23	-2.35	2.73E-04	Putative NAPDH dehydrogenase; induced by nitric oxide, benomyl; oxidative stress-induced via Cap1; Hap43p-repressed; rat catheter biofilm induced
C3_03640W_A	DAL9	-2.32	1.59E-07	Putative allantoate permease; fungal-specific (no human or murine homolog)
C5_00880C_A	GIT3	-2.29	1.08E-11	Glycerophosphocholine permease; white cell specific transcript; fungal-specific; alkaline repressed; caspofungin, macrophage/pseudohyphal-repressed; flow model biofilm induced; Spider biofilm induced
C1_09080C_A	PGA6	-2.28	3.29E-11	GPI-anchored cell wall adhesin-like protein; induced by high iron; upregulated upon Als2 depletion; mRNA binds She3 and is localized to hyphal tips; Spider biofilm repressed
C2_05640W_A		-2.26	1.19E-13	Putative helix-loop-helix (HLH) transcription factor with a role in filamentous growth
C3_01900C_A		-2.26	2.08E-06	Protein with a predicted FYVE/PHD zinc finger domain; Hap43-repressed; Spider biofilm induced
C1_12910W_A		-2.22	8.64E-03	Protein of unknown function; Spider biofilm repressed
C5_04490C_A	CAR1	-2.17	2.94E-25	Arginase; arginine catabolism; transcript regulated by Nrg1, Mig1, Tup1; colony morphology-related regulation by Ssn6; alkaline induced; protein decreased in stationary phase; sumoylation target; flow model biofilm induced
C5_05190W_A	PCL5	-2.17	1.47E-02	Putative cyclin for Pho85 kinase; Gcn4-induced; suppresses toxicity of C. albicans Gcn4 overproduction in S. cerevisiae via increased Pho85-dependent phosphorylation and degradation of Gcn4; rat catheter and Spider biofilm induced
C2_02860W_A	SUR2	-2.16	2.63E-23	Putative ceramide hydroxylase; predicted enzyme of sphingolipid biosynthesis; regulated by Tsa1, Tsa1B under H2O2 stress conditions; Spider and flow model biofilm induced
C2_02590W_A	ZRT2	-2.15	2.62E-21	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
C5_02600W_A	PUT1	-2.15	2.59E-10	Putative proline oxidase; alkaline upregulated by

				Rim101; flow model biofilm induced; Spider biofilm induced
C7_02810W_A	PRX1	-2.08	1.62E-17	Thioredoxin peroxidase; transcriptionally induced by interaction with macrophage; fluconazole induced; Fkh2p-downregulated; caspofungin repressed; protein present in exponential and stationary growth phase yeast cultures
CR_06300C_A	FGR50	-2.05	8.44E-03	Protein lacking an ortholog in S. cerevisiae; transposon mutation affects filamentous growth; Spider biofilm repressed
CR_02240C_A	OPT2	-2.03	4.64E-05	Oligopeptide transporter; induced upon phagocytosis by macrophage; macrophage/pseudohyphal-repressed after 16h; fluconazole-induced; virulence-group-correlated expression; Hap43-repressed
C1_02530C_A	DIP5	-2.02	2.77E-22	Dicarboxylic amino acid permease; mutation confers hypersensitivity to toxic ergosterol analog; induced upon phagocytosis by macrophage; Gcn4-regulated; upregulated by Rim101 at pH 8; rat catheter and Spider biofilm induced
C4_04000W_A	MET4	-2.01	2.67E-03	Putative transcription coactivator; predicted role in sulfur amino acid metabolism; required for yeast cell adherence to silicone substrate; Spider biofilm induced
C4_05880W_A	GAT1	-1.99	1.42E-08	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
C1_07350C_A	GPX3	-1.98	7.40E-03	Putative glutathione peroxidase involved in Cap1p-dependent oxidative stress response, required for Cap1p oxidation in response to H2O2; planktonic growth-induced
C6_00330C_A	GNP1	-1.98	2.89E-35	Similar to asparagine and glutamine permease; fluconazole, caspofungin induced; regulated by Nrg1, Mig1, Tup1, Gcn2, Gcn4, and alkaline regulated by Rim101; repressed during chlamydospore formation; rat catheter, flow model biofilm induced
C6_00480C_A	FET31	-1.98	6.75E-12	Putative multicopper oxidase; ketoconazole/caspofungin/amphotericin B repressed; Sef1/Sfu1/Hap43 regulated; reports differ if functional homolog of ScFet3; rat catheter and Spider biofilm induced
C3_05250C_A		-1.97	3.71E-04	Protein of unknown function; role in intracellular signal transduction; Spider biofilm induced
C1_04660W_A	DUR1,2	-1.96	6.07E-12	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
C1_10450W_A	GLY1	-1.94	1.78E-14	L-threonine aldolase; complements glycine

				auxotrophy of S. cerevisiae shm1 shm2 gly1-1 triple mutant; macrophage/pseudohyphal-induced; the GLY1 locus has an RFLP and is triploid in strain SGY269; flow model biofilm induced
C7_00730W_A	MET28	-1.94	7.95E-05	Predicted bZIP domain-containing transcription factor; protein induced during the mating process; possibly essential, disruptants not obtained by UAU1 method; Hap43-repressed; rat catheter biofilm induced
C1_13670W_A	OSM2	-1.9	1.25E-10	Putative mitochondrial fumarate reductase; regulated by Ssn6p, Gcn2p, and Gcn4p; Hog1p-downregulated; stationary phase enriched protein; Hap43p-repressed gene
C2_07570W_A	RNR22	-1.9	1.26E-14	Putative ribonucleoside diphosphate reductase;colony morphology-related gene regulation by Ssn6; transcript regulated by tyrosol and cell density; Hap43-repressed; Spider biofilm induced
C4_00450C_A	PGA10	-1.9	1.08E-02	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
CR_09920W_A		-1.9	1.40E-08	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
C1_13140C_A	TYE7	-1.86	1.98E-27	bHLH transcription factor; control of glycolysis; required for biofilm formation; hyphally regulated by Cph1, Cyr1; flucytosine, Hog1 induced; amphotericin B, caspofungin repressed; induced in flow model biofilm and planktonic cultures
C3_00800W_A	MIH1	-1.86	1.18E-08	Putative protein phosphatase of the PTP family (tyrosine-specific); ortholog of S. cerevisiae Mih1; mRNA binds She3
C7_04230W_A	NRG1	-1.86	2.49E-16	Transcription factor/repressor; regulates chlamydospore formation/hyphal gene induction/virulence and rescue/stress response genes; effects both Tup1 dependent and independent regulation; flow model biofilm induced; Spider biofilm repressed
C7_01800C_A	PFK2	-1.84	8.56E-32	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
C4_00370W_A	HOF1	-1.83	7.04E-05	Ortholog of S. cerevisiae Hof1; a protein localized to the bud neck and required for cytokinesis in S. cerevisiae; periodic mRNA expression, peak at cell-cycle G2/M phase; mutant is viable

C2_08120W_A	MAF1	-1.82	2.83E-06	Putative negative regulator of RNA polymerase III; decreased expression in hyphae vs yeast cells; caspofungin repressed; Spider biofilm repressed
C2_02680W_A	PDR17	-1.81	6.78E-04	Fungal-specific protein (no human or murine homolog); role in sensitivity to fluconazole, specifically
C3_01540W_A		-1.8	5.93E-11	
C4_02610C_A	SUL2	-1.8	4.58E-03	Putative sulfate transporter; transcript negatively regulated by Sfu1; amphotericin B induced; F-12/CO2 and Spider biofilm induced
C7_01490W_A		-1.8	3.93E-03	Protein of unknown function; Spider biofilm induced
C2_10240W_A	GPD1	-1.79	1.70E-12	Glycerol-3-phosphate dehydrogenase; glycerol biosynthesis; regulated by Efg1; regulated by Tsa1, Tsa1B under H2O2 stress conditions; Sflow model and Spider biofilm induced
C4_04230W_A		-1.79	4.54E-06	Putative transporter; fungal-specific; Spider biofilm induced
C7_00310C_A		-1.79	1.61E-04	Protein of unknown function; induced by nitric oxide; Spider biofilm repressed
C1_05520W_A		-1.78	6.80E-03	Protein of unknown function; induced by Sfu1; Spider biofilm induced
C4_03890W_A	PTP2	-1.78	1.31E-03	Predicted protein tyrosine phosphatase; involved in regulation of MAP kinase Hog1 activity; induced by Mnl1 under weak acid stress; rat catheter and Spider biofilm induced
C1_02980W_A	GOR1	-1.77	6.81E-10	Ortholog(s) have glyoxylate reductase activity, role in glyoxylate catabolic process and extracellular region localization
C4_05440C_A		-1.77	2.14E-03	Protein of unknown function; Spider biofilm induced
C6_03370W_A		-1.77	1.40E-13	Protein of unknown function; Plc1-regulated; induced by Mnl1 under weak acid stress; flow model biofilm induced
C2_06020W_A	CNT	-1.76	3.48E-11	CNT family H(+)/nucleoside symporter; transports adenosine, uridine, inosine, guanosine, tubercidin; variant alleles for high/low-affinity isoforms; S or G at residue 328 affects specificity; Spider, flow model biofilm induced
C3_01100W_A		-1.76	6.59E-03	Ortholog of C. dubliniensis CD36 : Cd36_81000, C. parapsilosis CDC317 : CPAR2_103050, Candida tenuis NRRL Y-1498 : CANTEDRAFT_116326 and Debaryomyces hansenii CBS767 : DEHA2G01958g
C2_06170C_A	ECM17	-1.75	3.00E-19	Putative sulfite reductase beta subunit; role in cell wall biogenesis; regulated by Tsa1/Tsa1B in H2O2 stress; Gcn4-regulated; Tbf1-activated; Hap43-repressed; Spider, flow, F-12/CO2 model biofilm induced
C1_04770C_A	ERG3	-1.74	1.32E-08	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

C2_01380W_A	PLB4.5	-1.72	6.70E-13	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
C5_04110W_A	SCW11	-1.72	3.07E-09	Cell wall protein; repressed in ace2 mutant; repressed in core caspofungin response; induced in high iron; possibly an essential gene, disruptants not obtained by UAU1 method; rat catheter and Spider biofilm repressed
C1_05540C_A		-1.69	2.94E-08	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
C5_02790C_A	GAP1	-1.69	2.46E-10	Amino acid permease; antigenic in human/mouse; 10-12 transmembrane regions; regulated by nitrogen source; alkaline, GlcNAc, phagocytosis induced; WT virulence in mice; Spider and flow model biofilm induced
C5_03870C_A		-1.69	1.78E-04	Protein of unknown function; Spider biofilm induced
	MEP1	-1.67	1.07E-11	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
C4_05900C_A		-1.66	1.20E-04	Protein of unknown function; Rgt1, Hap43-repressed; flow model biofilm induced; Spider biofilm induced
C3_04550C_A	CMK1	-1.63	6.98E-12	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
C1_11870W_A	MUP1	-1.62	4.34E-11	High affinity methionine permease; required for morphogenesis; alkaline upregulated by Rim101; Spider biofilm induced
C5_04880C_A	PUT2	-1.62	3.93E-04	Putative delta-1-pyrroline-5-carboxylate dehydrogenase; alkaline upregulated; protein present in exponential and stationary growth phase yeast cultures; flow model biofilm induced; Spider biofilm induced
C2_08490W_A	DSE1	-1.61	1.43E-05	Essential cell wall protein involved in cell wall integrity and rigidity; periodic mRNA expression peaks at M/G1 phase; Ace2p-induced; required for virulence in a mouse model of infection
C4_07080C_A		-1.61	2.58E-04	Protein with t-SNARE domains and a microtubule associated domain; Hap43-induced gene; repressed by alpha pheromone in SpiderM medium
C1_08070W_A	CDR4	-1.6	3.39E-13	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
C4_01750C_A	FBA1	-1.6	1.89E-13	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
C6_00960W_A	CAN1	-1.6	4.94E-11	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
CR_10110W_A	CHT3	-1.6	2.41E-05	Major chitinase; secreted; functional homolog of S. cerevisiae Cts1p; 4 N-glycosylation motifs; possible O-mannosylation; putative signal peptide; hyphal-repressed; farnesol upregulated in biofilm; regulated by Efg1p, Cyr1p, Ras1p
C7_01520W_A	FLU1	-1.59	1.05E-05	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)

Table S7. RNASeq Analysis of UPB8 mutant (Upregulated genes)

Genes	***Candida*** Gene names	**log2 fold-change**	**p-value adjusted**	**Description**
C1_05690C_A		3.76	1.00E+00	Protein of unknown function; Spider biofilm induced
C4_00290C_A		3.76	1.00E+00	Has domain(s) with predicted 2 iron, 2 sulfur cluster binding, iron ion binding, oxidoreductase activity, oxidoreductase activity and acting on paired donors, more
C1_01990W_A	*HSP30*	3.57	1.00E+00	Putative heat shock protein; fluconazole repressed; amphotericin B induced; Spider biofilm induced; rat catheter biofilm induced
C2_00660C_A	*SOD4*	3.57	1.00E+00	Cu-containing superoxide dismutase; role in response to host innate immune ROS; regulated on white-opaque switch; ciclopirox olamine induced; caspofungin repressed; SOD1,4,5,6 gene family; yeast-associated; Spider biofilm induced
C1_04440W_A		3.15	1.00E+00	Ortholog of Candida albicans WO-1 : CAWG_00951
C2_07710W_A		3.08	1.00E+00	Ortholog of C. dubliniensis CD36 : Cd36_21990, C. parapsilosis CDC317 : CPAR2_103660, Debaryomyces hansenii CBS767 : DEHA2F19756g and Pichia stipitis Pignal : PICST_61375
CR_06500C_A		2.9	3.08E-01	Protein of unknown function; Hap43-induced; regulated by Nrg1, Tup1; repressed by alpha pheromone in SpiderM medium; Spider biofilm induced; Bcr1-repressed in RPMI a/a biofilms
CR_07740W_A		2.86	1.00E+00	Ortholog of C. dubliniensis CD36 : Cd36_00150 and Lodderomyces elongisporus NRLL YB-4239 : LELG_01269

C4_01330W_A		2.78	7.96E-01	Protein of unknown function; Spider biofilm induced
C5_03520W_A		2.76	1.00E+00	Predicted ORF from Assembly 19; removed from Assembly 20; subsequently reinstated in Assembly 21 based on comparative genome analysis
C4_01340W_A		2.6	1.31E-02	Protein similar to GPI-linked cell-wall proteins; induced in low iron; Spider biofilm induced; regulated in Spider biofilms by Bcr1, Tec1, Ndt80, Brg1
C4_00930W_A	*tG(GCC)4*	2.56	1.00E+00	tRNA-Gly, predicted by tRNAscan-SE; GCC anticodon
C7_00480W_A	*FGR2*	2.56	1.00E+00	Protein similar to phosphate transporters; transposon mutation affects filamentous growth; expression is regulated upon white-opaque switching
C3_00020W_A		2.5	1.00E+00	Sef1p-, Sfu1p-, and Hap43p-regulated gene
C2_10780C_A	*PSO2*	2.29	1.00E+00	Putative DNA cross-link repair protein; expressed in opaque or white MTLa/MTLa or MTLalpha/MTLalpha, but not MTLa/MTLalpha cells; telomere-proximal gene; mutation does not affect white-to-opaque phenotypic switching
C2_08470C_A	*STN1*	2.22	1.00E+00	Protein involved in telomere maintenance; forms a complex with Ten1p
C2_05620W_A	*KTI11*	1.95	1.00E+00	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
CR_01220W_A		1.83	1.00E+00	Putative transporter; mutation confers hypersensitivity to toxic ergosterol analog; fungal-specific (no human or murine homolog)
C4_05990W_A	*tS(UGA)2*	1.78	1.00E+00	tRNA-Ser, predicted by tRNAscan-SE; UGA anticodon
CR_08750C_A	*tG(GCC)6*	1.78	1.00E+00	tRNA-Gly, predicted by tRNAscan-SE; GCC anticodon
C6_03670C_A		1.7	1.00E+00	Protein of unknown function; Spider biofilm induced

Table S8. RNASeq Analysis of UPB8 mutant (Downregulated genes)

ORF	Candida Gene names	log2 fold-change	P-value adjusted	Description
C6_01490C_A		-5.78	1.00E+00	Ortholog of Candida albicans WO-1 : CAWG_05215
CR_04610C_A		-5.4	1.00E+00	Ortholog of a S. cerevisiae Atg22; a vacuolar integral membrane protein required for efflux of amino acids during autophagic body breakdown in the vacuole; possibly an essential gene, disruptants not obtained by UAU1 method
C1_09550W_A		-3.82	1.00E+00	Protein of unknown function; transcript detected on high-resolution tiling arrays; rat catheter biofilm induced

C3_04350C_A		-1.99	1.00E+00	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
C3_04600C_A		-1.73	1.00E+00	Ortholog of Candida albicans WO-1 : CAWG_02773
C3_05130C_A	THI4	-1.59	1.00E+00	Thiamine biosynthetic enzyme precursor; repressed during the mating process; stationary phase enriched protein; Spider biofilm induced
C4_00720W_A	CSP2	-1.53	1.00E+00	Putative cell wall associated protein; C. albicans and C. dubliniensis specific gene highly induced during chlamydospore development in both species; localized to chlamydospore cell wall; Hap43-repressed; Spider biofilm induced
C1_06000W_A		-1.47	1.00E+00	Putative dicarboxylic amino acid permease; fungal-specific (no human or murine homolog); induced by alpha pheromone in SpiderM medium
CR_00050W_A	INO2	-1.35	1.00E+00	Transcriptional activator that forms a heterodimer with Ino4p; likely regulates genes involved in phosphatidylcholine and phosphatidylinositol biosynthesis, fatty acid beta-oxidation, and peroxisome biogenesis
C2_02060C_A	FMO1	-1.3	1.00E+00	Putative oxidoreductase; mutation confers hypersensitivity to toxic ergosterol analog, and to amphotericin B
C2_04970W_A	ABP2	-1.26	1.00E+00	Putative alpha-actinin-like protein; induced by alpha pheromone in SpiderM medium
C1_14190C_A		-0.96	1.00E+00	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
C6_03910C_A		-0.95	1.00E+00	Ortholog(s) have U2 snRNA binding activity, role in RNA folding, U2-type prespliceosome assembly and U2 snRNP, U2-type spliceosomal complex localization
C6_02400W_A		-0.91	1.00E+00	C/D box small nucleolar RNA (snoRNA)
CR_05420W_A		-0.85	1.00E+00	Dubious open reading frame
C3_06490W_A		-0.8	1.00E+00	Putative protein of unknown function; Hap43p-repressed gene; ortholog of S. cerevisiae YJL218W