The Hsp90 Co-Chaperone Sgt1 Governs Candida albicans Morphogenesis and Drug Resistance

Rebecca S. Shapiro1, Aimee K. Zaas2,3, Marisol Betancourt-Quiroz2, John R. Perfect2,3, Leah E. Cowen1*

1 Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada, 2 Department of Medicine, Duke University Medical Center, Durham, North Carolina, United States of America, 3 Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, United States of America

Abstract

The molecular chaperone Hsp90 orchestrates regulatory circuitry governing fungal morphogenesis, biofilm development, drug resistance, and virulence. Hsp90 functions in concert with co-chaperones to regulate stability and activation of client proteins, many of which are signal transducers. Here, we characterize the first Hsp90 co-chaperone in the leading human fungal pathogen, Candida albicans. We demonstrate that Sgt1 physically interacts with Hsp90, and that it governs C. albicans morphogenesis and drug resistance. Genetic depletion of Sgt1 phenocopies depletion of Hsp90, inducing yeast to filament morphogenesis and invasive growth. Sgt1 governs these traits by bridging two morphogenetic regulators: Hsp90 and the adenyl cyclase of the cAMP-PKA signaling cascade, Cyr1. Sgt1 physically interacts with Cyr1, and depletion of either Sgt1 or Hsp90 activates cAMP-PKA signaling, revealing the elusive link between Hsp90 and the PKA signaling cascade. Sgt1 also mediates tolerance and resistance to the two most widely deployed classes of antifungal drugs, azoles and echinocandins. Depletion of Sgt1 abrogates basal tolerance and acquired resistance to azoles, which target the cell membrane. Depletion of Sgt1 also abrogates tolerance and resistance to echinocandins, which target the cell wall, and renders echinocandins fungicidal. Though Sgt1 and Hsp90 have a conserved impact on drug resistance, the underlying mechanisms are distinct. Depletion of Hsp90 destabilizes the client protein calcineurin, thereby blocking crucial responses to drug-induced stress; in contrast, depletion of Sgt1 does not destabilize calcineurin, but blocks calcineurin activation in response to drug-induced stress. Sgt1 influences not only morphogenesis and drug resistance, but also virulence, as genetic depletion of C. albicans Sgt1 leads to reduced kidney fungal burden in a murine model of systemic infection. Thus, our characterization of the first Hsp90 co-chaperone in a fungal pathogen establishes C. albicans Sgt1 as a global regulator of morphogenesis and drug resistance, providing a new target for treatment of life-threatening fungal infections.

Introduction

Fungal pathogens have emerged as a leading cause of human mortality, particularly among the growing population of immunocompromised individuals. The frequency of invasive fungal infections has increased by over 200% in recent years [1], with staggering economic and public health costs; treatment expenditures in the United States alone exceed $2.6 billion and mortality rates range from 30–90%, depending on the pathogen and patient population [2,3]. The opportunistic pathogen Candida albicans reigns as the leading cause of fungal infection and the fourth most common cause of hospital-acquired infection, with mortality rates approaching 50% [4]. C. albicans thrives within the oral cavity and gastrointestinal tracts of healthy humans, and can cause superficial infections of oral and vaginal epithelial cells, as well as lethal systemic infections through bloodstream dissemination and organ invasion in immunocompromised individuals [5].

The pathogenic prowess of C. albicans is achieved through numerous virulence traits. Factors influencing virulence include the expression of surface adhesins that mediate epithelial cell adherence [6], the secretion of host cell damaging proteases [7], the capacity to produce drug-resistant biofilms [8], and the ability to transition between yeast and filaments [9]. Morphogenetic flexibility is often coupled with virulence, as C. albicans mutants locked as either yeast or filaments are typically attenuated in virulence [10,11,12,13,14,15], though there are cases where these traits may be uncoupled [16]. Morphogenesis is critical for biofilm formation [17,18], and genes that govern morphogenesis are frequently co-regulated with those encoding virulence factors such as proteases and adhesins [19], highlighting the importance of morphogenesis for C. albicans pathogenicity. The transition from yeast to filamentous growth is induced by diverse environmental cues including serum, nutrient limitation, carbon dioxide, temperature, and pH, and complex cellular signaling pathways govern response to these cues [9,20]. Amongst these pathways, the cAMP-protein kinase A (PKA) pathway plays a key role in mediating the transition from yeast to filamentous growth [9]. The mechanism by which some morphogenetic cues are sensed by the cAMP-PKA cascade is known, such as binding of the adenyl
cyclase Cyr1 to peptidoglycan in serum [21], though the mechanism underpinning responses to other cues, such as temperature, remains enigmatic.

The treatment of invasive fungal infections, including those caused by C. albicans, is notoriously challenging. This can largely be attributed to the close evolutionary relationship between fungal pathogens and their human hosts [22,23], and the resulting limited selection of clinically effective antifungal drugs. The most widely deployed class of antifungal drugs in clinical use is the azoles, which inhibit lanosterol 14α-demethylase, encoded by ERG11 in C. albicans, thereby blocking the biosynthesis of ergosterol, the predominant sterol of fungal membranes [24,25]. Azole-mediated inhibition of Erg11 further results in the accumulation of a toxic sterol intermediate, produced by Erg3, which disrupts membrane integrity and exerts a severe membrane stress on the cell [24,25]. The widespread deployment and prophylactic use of azoles, coupled with their fungistatic activity, results in conditions that favour the evolution of antifungal drug resistance [24,26]. Resistance mechanisms that minimize the impact of the drug on the cell include overexpression of drug efflux pumps, or alteration of the drug target, Erg11. Resistance mechanisms that minimize drug toxicity include alterations in the ergosterol biosynthesis pathway, including loss of function of Erg3, which blocks the accumulation of the toxic sterol [24,25]. Mechanisms that reduce drug toxicity are frequently dependent on cellular stress responses that are crucial for tolerating the membrane stress exerted by azoles [24,25]. The newest class of antifungal in clinical use is the echinocandins, which inhibit (1,3)-β-D-glucan synthase, thereby disrupting integrity of the fungal cell wall and resulting in cell wall stress [24,25]. The most common mechanism of echinocandin resistance is mutation in FKS1, encoding the catalytic subunit of (1,3)-β-D-glucan synthase [27]. Cellular stress responses are required for Fks1-mediated resistance, as well as for basal tolerance to the cell wall damage exerted by the echinocandins [9], further illustrating the paradigm in which stress response circuitry enables crucial responses to antifungal drug exposure.

A key mediator of circuitry controlling nearly all facets of C. albicans pathobiology, including morphogenesis, biofilm formation, virulence and antifungal drug resistance is the molecular chaperone Hsp90 [9,28]. Hsp90 is a highly conserved and essential chaperone that regulates the form and function of diverse client proteins in all eukaryotes [29,30]. Many Hsp90 client proteins are key regulators of cellular signaling, including protein kinases and transcription factors [29,31], rendering Hsp90 poised to govern diverse cellular processes. In C. albicans, inhibition of Hsp90 induces a temperature-dependent morphogenetic transition from yeast to filamentous growth via several cellular circuits, including the cell cycle regulation [32], the Pho83-Pcl1-Hms1 pathway [33], and the cAMP-PKA signaling cascade [34]. Though key components of PKA signaling are required for filamentous growth induced by Hsp90 depletion [34], the molecular mechanism by which Hsp90 represses cAMP-PKA signaling remains unknown. In addition to effects on morphogenesis, inhibiting Hsp90 function in C. albicans blocks the emergence and maintenance of resistance to azoles, and enhances the efficacy of azoles in vivo [35,36]. Compromise of Hsp90 function also abrogates azole resistance of C. albicans biofilms, though in a manner distinct from effects on Cna1 and Mkc1 stability [28]. Consistent with its central role in mediating these pathogenicity traits, depletion of C. albicans Hsp90 attenuates virulence in a murine model of systemic fungal infection [34].

Importantly, Hsp90 does not function autonomously, but rather interacts with a battery of co-chaperones that regulate its activity as well as its interaction with client proteins. Hsp90 co-chaperones can perform different functions, from regulating Hsp90’s ATPase activity to altering its localization and trafficking, and can have dramatic effects on Hsp90 client folding and function, though the mechanisms by which they do so remain largely enigmatic [39]. For instance, mammalian Hsp90 co-chaperones Ahal and p23 differentially modulate Hsp90-dependent stability of the mutant cystic fibrosis transmembrane conductance regulator (CFT) [40]. Many Hsp90 co-chaperones act as client adaptor proteins, by specifically recruiting select client proteins to Hsp90. For instance, recruitment of protein kinases to Hsp90 requires the co-chaperone Cdc37, which interacts simultaneously with the kinase and Hsp90, and modulates Hsp90’s ATPase activity [41,42]. The co-chaperone Sgt1 also functions as an adaptor protein, and binds Hsp90 concurrent with binding a diverse set of client proteins, including Skp1, an essential component of the kinetochore complex [43,44], as well as the adenyl cyclase Cyr1 in the model yeast Saccharomyces cerevisiae [45]. Hsp90 co-chaperones such as Cdc37 and Sgt1 have been identified in diverse eukaryotes, including plants, animals and model fungi, but have yet to be characterized in C. albicans. Given that Hsp90 is a global regulator of C. albicans biology and disease, the next frontier is to identify co-chaperones modulating Hsp90 function in this fungal pathogen.

Here, we characterize the first Hsp90 co-chaperone in the fungal pathogen C. albicans. We establish that Sgt1 regulates C. albicans morphogenesis and drug resistance, providing a powerful new therapeutic target for the treatment of life-threatening fungal infections.

Results

Genetic depletion of Sgt1 induces filamentation, wrinkly colony morphology and invasive growth

As the mechanism by which Hsp90 modulates C. albicans morphogenesis via the cAMP-PKA cascade remained elusive, we assessed whether Hsp90 repressed PKA signaling via co-chaperones. Our previous work suggested that Hsp90 might interact with the protein kinase PKA or the adenyl cyclase Cyr1 [34]. As a consequence, we focused on Cdc37 and Sgt1, co-chaperones that are well-established in other species to mediate Hsp90’s interaction with kinases and an adenyl cyclase, respectively [45,46]. As both CDC37 and SGT1 are essential in S. cerevisiae [44,47], we created genetically repressible conditional mutants of CDC37 and SGT1 in C. albicans. For CDC37 depletion, we engineered a strain in which the only allele of CDC37 was regulated by the MAL2 promoter, where transcription was repressed by glucose and induced by maltose (data not shown). For SGT1 depletion, we engineered a strain in which the only allele of SGT1 was regulated by the tetO promoter, where transcription was repressed by tetracycline or the analog doxycycline. Cdc37 depletion upon growth of the MAL2p-CDC37/deC37 strain in the presence of glucose did not induce filamentation (Figure S1A), suggesting that it is not the link between Hsp90 and cAMP-PKA signaling. In contrast, doxycycline-mediated depletion of Sgt1 in the tetO-SGT1/tet1 strain induced a transition from yeast to filamentous growth, in the absence of other inducing cues (Figure 1A and S1B). Notably, the filaments observed upon Sgt1 depletion bore a
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Sgt1 interacts with Hsp90 in C. albicans

The phenotypic similarities between genetic depletion of Hsp90 and Sgt1, suggest that Sgt1 may provide the link between Hsp90 and cAMP-PKA signaling. To determine if Sgt1 physically interacts with Hsp90 in C. albicans, as it does in species as diverse as S. cerevisiae, humans, and plants [43,48,49], we performed reciprocal co-immunoprecipitation between epitope-tagged versions of Hsp90 and Sgt1. We utilized an allele tagged at the C-terminus with a tandem affinity purification (TAP) tag [37], consisting of a calmodulin binding peptide (CBP), a tobacco etch virus (TEV) protease cleavage site, and Protein A, which binds IgG; the Sgt1 allele was tagged at the C-terminus with a haemagglutinin (HA) tag. Immunoprecipitation with anti-IgG agarose, followed by TEV protease cleavage, co-purified TAP-tagged Hsp90, as well as HA-tagged Sgt1; for the control strain lacking the tagged HSP90 allele, Sgt1-HA was present in the input, but was not immunoprecipitated (Figure 2, left panel). To further validate this interaction, we performed the reciprocal co-immunoprecipitation using HA-tagged Sgt1 and untagged Hsp90. Immunoprecipitation with anti-HA agarose co-purified HA-tagged Sgt1, as well as wild-type Hsp90; for the control strain lacking the tagged Sgt1 allele, Hsp90 was present in the input, but was not immunoprecipitated (Figure 2, right panel). Thus, reciprocal co-immunoprecipitation reveals a physical interaction between Hsp90 and Sgt1, and identifies the first putative Hsp90 co-chaperone in C. albicans.

Depletion of Sgt1 activates cAMP-PKA signaling, and Sgt1 interacts with the adenyl cyclase Cyr1

Since depletion of Hsp90 induces filamentous growth by activating cAMP-PKA signaling [34], we assessed whether depletion of Sgt1 might also stimulate this pathway to induce filamentous growth. To determine whether PKA signaling was modulated, we performed a PKA activity assay, which has been successfully used to monitor PKA signaling in C. albicans [50]. We used whole-cell protein extracts derived from wild-type, tetO-SGT1/sgt1Δ and tetO-HSP90/hsp90Δ cells grown in the presence or absence of doxycycline, to deplete Sgt1 or Hsp90. In this assay, a fluorescently labeled peptide that is a highly specific substrate for PKA is phosphorylated, causing a change in charge. The peptide is then separated into phosphorylated and non-phosphorylated forms by electrophoresis, and quantified by spectrophotometry. We found that genetic depletion of Sgt1, or depletion of Hsp90, both led to significantly increased PKA activity, relative to the wild type (P<0.01, ANOVA, Bonferroni’s Multiple Comparison Test, Figure 3A). Therefore, depletion of Sgt1 phenocopies depletion of Hsp90, leading to activation of PKA signaling.

To determine the mechanism by which Hsp90 and Sgt1 modulate PKA signaling, we investigated whether Sgt1 physically interacted with the adenyl cyclase Cyr1, as these two proteins are known to interact in S. cerevisiae [45]. To determine whether Sgt1 interacts with Cyr1 in C. albicans, we performed reciprocal co-immunoprecipitation between epitope-tagged Cyr1 and Sgt1. We utilized an allele of CTR1, N-terminally tagged with 6X-His-FLAG-Myc (HFM) [51], and the HA-tagged Sgt1 strain described...
Sgt1 enables basal tolerance and erg3-mediated resistance to azoles

Having determined that depletion of Sgt1 phenocopies depletion of Hsp90 in terms of morphogenesis, we next assessed whether Sgt1 played a role in antifungal tolerance and resistance, as Hsp90 enabled crucial cellular responses to drug-induced stress [35,37]. To assess the impact of depletion of Sgt1 on tolerance to the most widely deployed class of antifungals, the azoles, we assayed growth of a wild-type strain, a strain heterozygous for $\Delta er\text{g}3$ and an erg3 strain, as well as the $\Delta er\text{g}3$ strain (tetO-SGT1/sgt1$\Delta$) over a gradient of concentrations of theazole fluconazole relative to a drug-free control. The wild-type strain was tolerant to all concentrations of fluconazole, in the presence of absence of a constant concentration of doxycycline, and then spotted cells on rich medium without any inhibitors. All strains tested were able to growth overnight in 20 \mu g/ml doxycycline, followed by subculture in fresh medium with 20 \mu g/ml doxycycline and growth until mid-log phase. Data are normalized against the level of PKA activity of wild type cell extracts, and are displayed as means ± standard deviations for triplicate samples. * Indicates P<0.01, ANOVA. (B) Sgt1 and Cyr1 physically interact as measured by reciprocal co-immunoprecipitation of epitope-tagged proteins. Left panel, immunoprecipitation of Sgt1-HA with anti-HA agarose co-purified 6x-His-FLAG-Myc (HFM)-Cyr1, while HFM-Cyr1 did not immunoprecipitate with anti-HA agarose in control cells harbouring only untagged Sgt1. HFM-Cyr1 did not immunoprecipitate with anti-IgG agarose in control cells harbouring only untagged Hsp90. For the control strain lacking the tagged allele, HFM-Cyr1 and Hsp90 were present in the input, but were not immunoprecipitated (Figure 3B, left panel). To further validate this interaction, we performed the reciprocal co-immunoprecipitation. Immunoprecipitation with anti-Nickel (Ni-NTA) agarose co-purified Sgt1-HA, while Sgt1 did not immunoprecipitate with anti-IgG agarose in control cells harbouring only untagged Sgt1. Right panel, immunoprecipitation of HFM-Cyr1 relative to the control immunoprecipitation with only untagged Cyr1. doi:10.1371/journal.pone.0044734.g003

Figure 2. Sgt1 physically interacts with Hsp90 in \textit{C. albicans}.

Hsp90 and Sgt1 physically interact as measured by reciprocal co-immunoprecipitation of epitope-tagged proteins. Left panel, immunoprecipitation of Hsp90-TAP with anti-IgG agarose co-purified Sgt1-HA, while Sgt1-HA did not immunoprecipitate with anti-IgG agarose in control cells harbouring only untagged Hsp90. Right panel, immunoprecipitation of Sgt1-HA with anti-HA agarose co-purified Hsp90, while Hsp90 did not immunoprecipitate in control cells harbouring only untagged Sgt1. doi:10.1371/journal.pone.0044734.g002

Figure 3. Depletion of Sgt1 activates cAMP-PKA signaling, and Sgt1 interacts with the adenylyl cyclase Cyr1.

(A) Depletion of Sgt1 activates cAMP-PKA signaling. PKA activity was measured for cell extracts derived from the indicated strains. Sgt1 or Hsp90 levels were reduced by growth overnight in 20 \mu g/ml doxycycline, followed by subculture in fresh medium with 20 \mu g/ml doxycycline and growth until mid-log phase. Data are normalized against the level of PKA activity of wild type cell extracts, and are displayed as means ± standard deviations for triplicate samples. * Indicates P<0.01, ANOVA. (B) Sgt1 and Cyr1 physically interact as measured by reciprocal co-immunoprecipitation of epitope-tagged proteins. Left panel, immunoprecipitation of Sgt1-HA with anti-HA agarose co-purified 6x-His-FLAG-Myc (HFM)-Cyr1, while HFM-Cyr1 did not immunoprecipitate with anti-HA agarose in control cells harbouring only untagged Sgt1. Hsp90 also co-purified with Sgt1-HA, and was not purified in the controls with only untagged Sgt1. Right panel, immunoprecipitation of HFM-Cyr1 with anti-Nickel agarose co-purified Sgt1, while Sgt1 did not immunoprecipitate in the immunoprecipitation of HFM-Cyr1 relative to the control immunoprecipitation with only untagged Cyr1. doi:10.1371/journal.pone.0044734.g003

as the tetO-SGT1/sgt1$\Delta$ strain and the tetO-SGT1/sgt1$\Delta$ strain with both alleles of $\Delta er\text{g}3$ deleted (tetO-SGT1/sgt1$\Delta$ $\Delta er\text{g}3$), over a gradient of concentrations of fluconazole. Consistent with our previous findings, the wild-type strain was tolerant to all concentrations of fluconazole tested, while the tetO-SGT1/sgt1$\Delta$ strain had increased susceptibility; doxycycline had little impact (Figure 4B). The $\Delta er\text{g}3$ strain and tetO-SGT1/sgt1$\Delta$ $\Delta er\text{g}3$ mutants were both robustly resistant to fluconazole; doxycycline-mediated depletion of Sgt1 rendered the tetO-SGT1/sgt1$\Delta$ $\Delta er\text{g}3$ strain sensitive to fluconazole, while doxycycline had no effect on the control $\Delta er\text{g}3$ strain (Figure 4B). Thus, Sgt1 is required for erg3-mediated azole resistance.

Inhibition of Hsp90 function transforms fluconazole from fungistatic to fungicidal [36]. To determine if the same was true for deletion of Sgt1, we exposed cells to a gradient of concentrations of fluconazole, in the presence of absence of a constant concentration of doxycycline, and then spotted cells on rich medium without any inhibitors. All strains tested were able to
grow on rich medium following exposure to all concentrations of fluconazole tested, although doxycycline-mediated depletion of Sgt1 in the tetO-SGT1/sgt1Δ strain did reduce recovery following exposure to fluconazole (Figure 4C). Thus, depletion of Sgt1 has only a minor impact on the fungistatic activity of azoles, despite the central role for Sgt1 in basal tolerance and resistance to azoles.

Depletion of Sgt1 enhances susceptibility to echinocandins, and transforms them from fungistatic to fungicidal

To determine if Sgt1 affects tolerance and resistance to the newest class of antifungal drug, the echinocandins, we assayed growth of a wild-type strain, a strain heterozygous for SGT1 (SGT1/sgt1Δ), as well as the SGT1 doxycycline-repressible strain (tetO-SGT1/sgt1Δ) over a gradient of concentrations of the echinocandin micafungin. The wild-type strain and SGT1/sgt1Δ mutant displayed tolerance to the gradient of micafungin, while the tetO-SGT1/sgt1Δ strain showed increased susceptibility to micafungin, even in the absence of doxycycline (Figure 5A), similar to what was observed with the azoles. Doxycycline-mediated depletion of Sgt1 had little further effect on susceptibility of the tetO-SGT1/sgt1Δ strain (Figure 5A). Therefore, reduced expression of Sgt1 increases echinocandin susceptibility.

Next, we determined if Sgt1 enabled echinocandin resistance conferred by mutation of FKS1, which is the most prevalent mechanism of echinocandin resistance [27,54]. We utilized a well-characterized FKS1 mutation (T1922G), shown to confer resis-
To assess the impact of depletion of Sgt1 on FKS1-mediated resistance to the echinocandins, we assayed growth of a wild-type strain and an FKS1/FKS1T1922C echinocandin-resistant strain, as well as the tetO-SGT1/sgt1Δ strain containing the FKS1/FKS1T1922C mutation, over a gradient of concen- trations of micafungin. The FKS1/FKS1T1922C mutant was resis- tance to the echinocandins [55]. To assess the impact of depletion of Sgt1 on FKS1-mediated resistance to the echinocandins, we assayed growth of a wild-type strain and an FKS1/FKS1T1922C echinocandin-resistant strain, as well as the tetO-SGT1/sgt1Δ strain containing the FKS1/FKS1T1922C mutation, over a gradient of concentrations of micafungin. The FKS1/FKS1T1922C mutant was resistant to micafungin relative to the wild-type strain (Figure 5B). The tetO-SGT1/sgt1Δ strain had increased susceptibility to micafungin relative to the wild type, while the tetO-SGT1/sgt1Δ FKS1/FKS1T1922C had resistance comparable to the FKS1/ FKS1T1922C mutant (Figure 5B). Doxycycline-mediated depletion of Sgt1 in the tetO-SGT1/sgt1Δ FKS1/FKS1T1922C strain abrogated echinocandin resistance (Figure 5B), implicating Sgt1 as a key regulator of FKS1-mediated resistance to the echinocandins.

We next assessed if depletion of Sgt1 creates a fungicidal combination with echinocandins. Though the echinocandins are generally considered fungicidal against C. albicans [27], C. albicans can grow vigorously at intermediate echinocandin concentrations under laboratory conditions, and inhibition of Hsp90 transforms echinocandins from fungistatic to fungicidal [37]. We exposed cells to a gradient of concentrations of micafungin, in the presence or absence of a constant concentration of doxycycline, and then spotted cells onto YPD medium and incubated at 30°C for 48 hours before plates were photographed.
type strain and FKS1/FKS1<sup>T1922C</sup> mutant were able to grow on rich medium following exposure to all concentrations of micafungin, in the presence or absence of doxycycline (Figure 5C). Genetic compromise of Sgt1 in the tetO-<i>SGT1</i>/<i>sgt1Δ</i> and micafungin-resistant tetO-<i>SGT1</i>/<i>sgt1Δ</i> FKS1/FKS1<sup>T1922C</sup> strains was cidal in combination with almost any dose of micafungin tested, even in the absence of doxycycline (Figure 5C). Therefore, genetic reduction of Sgt1 increases echinocandin susceptibility, and creates a fungidical combination.

The Hsp90 client protein calcineurin is dependent on Sgt1 for activation, but not stabilization

To assess the mechanism by which depletion Sgt1 influences antifungal drug resistance, we focused on a key Hsp90 client protein implicated in drug resistance of <i>C. albicans</i>, calcineurin. The catalytic subunit of the protein phosphatase calcineurin, Cna1, depends on Hsp90 for its stability (Figure S3A, [37]). To assess whether Sgt1 was also required for Cna1 stability, we monitored protein levels of TAP-tagged Cna1 in a wild-type strain, the tetO-<i>SGT1</i>/<i>sgt1Δ</i> strain, as well as the MAL2-<i>SGT1</i>/<i>sgt1Δ</i> strain under conditions where Sgt1 is depleted. In the absence of Sgt1 depletion, all strains had comparable levels of Sgt1 and Cna1 (Figure 6A and Figure S3B). Genetic depletion of Hsp90 leads to a corresponding depletion of Cna1 (Figure S3A, [37]). However, depletion of Sgt1 had no impact on levels of Cna1 (Figure 6A and Figure S3B), indicating that Sgt1 is not required for Cna1 stability, in contrast to Cna1 dependence on Hsp90.

To determine if Sgt1 was required for activation, rather than stability of Cna1, we monitored transcript levels of a Cna1-dependent gene, UTR2 [56], which is known to be upregulated upon Cna1 activation by azoles or echinocandins [37,38]. In a wild-type strain, treatment with fluconazole or micafungin activated Cna1 (<i>P</i>&lt;0.01, ANOVA, Bonferroni’s Multiple Comparison Test), as measured by an increase in UTR2 transcript levels (Figure 6B). While doxycycline caused a modest reduction of UTR2 induction in response to fluconazole in the wild-type strain (<i>P</i>&lt;0.001, ANOVA), doxycycline-mediated depletion of Sgt1 in the tetO-<i>SGT1</i>/<i>sgt1Δ</i> strain blocked calcineurin activation in response to fluconazole (<i>P</i>&lt;0.01, ANOVA, Figure 6B). Doxycycline had no impact on UTR2 induction in response to micafungin, but doxycycline-mediated depletion of Sgt1 in the tetO-<i>SGT1</i>/<i>sgt1Δ</i> strain blocked calcineurin activation in response to micafungin (<i>P</i>&lt;0.01, ANOVA, Figure 6B). Thus, Sgt1 does not affect stability of the Hsp90 client protein calcineurin, but is required for calcineurin activation in response to drug-induced stress, providing a mechanism by which Sgt1 regulates drug resistance.

Genetic depletion of <i>C. albicans</i> Sgt1 leads to reduced fungal burden in a murine model of disseminated infection

Sgt1 is a promising therapeutic target given its importance for morphogenesis and drug resistance. To assess the impact of depletion of Sgt1 on virulence, we employed a murine model of systemic infection, in which inoculum of <i>C. albicans</i> is delivered via tail vein injection, and progresses from the bloodstream to deep-seated infection in organs such as the kidney [34,36]. Mice were infected with the wild-type strain or the tetO-<i>SGT1</i>/<i>sgt1Δ</i> strain, with or without tetracycline in the drinking water, and fungal burden in the mouse kidneys was monitored three days after infection. Tetracycline had no impact on kidney fungal burden of mice infected with the wild-type strain, but significantly reduced fungal burden of mice infected with the tetO-<i>SGT1</i>/<i>sgt1Δ</i> strain (Figure 7, <i>P</i>&lt;0.05, Kruskal-Wallis test). Similarly, mice treated with tetracycline had significantly reduced kidney fungal burden when infected with tetO-<i>SGT1</i>/<i>sgt1Δ</i>, compared to those infected with the wild type (Figure 7, <i>P</i>&lt;0.0001, Kruskal-Wallis test). Thus, depletion of Sgt1 leads to reduced kidney fungal burden, implicating Sgt1 as a potential therapeutic target for the treatment of <i>C. albicans</i> infections.

Discussion

Our results identify Sgt1, the first Hsp90 co-chaperone characterized in <i>C. albicans</i>, as a global regulator of circuitry controlling morphogenesis and drug resistance, with broad therapeutic potential. Genetic depletion of Sgt1 phenocopies Hsp90 deficiency of <i>H. capsulatum</i>, inducing a yeast to filament transition, wrinkly colony morphology and invasive growth (Figure 1). Sgt1 likely controls these morphogenetic traits by bridging two important regulators of morphogenesis: Hsp90 and the cAMP-PKA signaling cascade. Sgt1 physically interacts with both Hsp90 and the adenylyl cyclase Cyr1 (Figure 2 and Figure 3), and depletion of Sgt1 phenocopies Hsp90 deficiency of <i>H. capsulatum</i>, activating PKA signaling (Figure 3), thus providing a molecular mechanism by which Hsp90 orchestrates morphogenesis via PKA signaling. Sgt1 is also a key regulator of Hsp90-dependent resistance to the two most widely
deployed classes of antifungals, the azoles and the echinocandins (Figure 4 and Figure 5). Sgt1 enables both tolerance and erg3-mediated resistance to azoles (Figure 4). Depletion of Sgt1 abrogates basal tolerance and FKS1T1922C-mediated resistance to echinocandins, and transforms echinocandins from fungistatic to fungicidal (Figure 5). Though Sgt1 and Hsp90 share a conserved impact on drug resistance phenotypes, the mechanism involved are distinct. Depletion of Hsp90 leads to destabilization of a key client protein implicated in drug resistance, calcineurin [37], while depletion of Sgt1 does not alter calcineurin stability (Figure 6). Sgt1 is required for activation of calcineurin in response to drug-induced stress (Figure 6), suggesting a mechanism by which Sgt1 regulates antifungal drug resistance. Similar to Hsp90, Sgt1 also plays an important role in fitness in a murine model of infection (Figure 7), thus establishing this co-chaperone as a promising target for antifungal therapy.

Together, this work implicates Sgt1 as a central regulator of the Hsp90 chaperone machine. Importantly, though Hsp90 co-chaperones remain largely uncharacterized in C. albicans, it is apparent that not all Hsp90 co-chaperones influence these Hsp90-dependent traits, as depletion of the co-chaperone Cdc37 does not impact on C. albicans morphogenesis (Figure S1). Notably, Hsp90 does not regulate C. albicans morphogenesis and antifungal drug resistance via a single pathway, but rather has pleiotropic effects, consistent with its function as a central hub of protein homeostasis and regulatory circuitry. In S. cerevisiae, Hsp90 interacts with ~10% of the proteome [57], and the chaperone network is equally complex in C. albicans [58]. Elucidating the impact of Sgt1 and other co-chaperones on Hsp90 targets on a more global scale will reveal fundamental insight into the mechanisms modulating function of the Hsp90 chaperone machine.

Through its interactions with both Cyr1 and Hsp90, C. albicans Sgt1 couples temperature sensing with morphogenesis. Hsp90, Sgt1, and Cyr1 may interact directly or indirectly as part of a larger complex of proteins. Although not specifically implicated in morphogenesis in other species, Sgt1 associates with factors important for cell cycle progression [59,60], which has a fundamental impact on C. albicans morphogenesis [32,61]. In S. cerevisiae, Sgt1 is required for both the G1/S and G2/M transitions in the cell cycle, and together with Hsp90, interacts with key cell cycle regulators Skp1 and the Skp-Cullin-F-box (SCF) ubiquitin ligase complex to mediate kinetochore assembly and function [59,60]. This phenotype is conserved in mammals, where cells lacking Sgt1 experience a delay in mitosis, and Sgt1 and Hsp90 are required for proper kinetochore assembly [62]. In Drosophila, Sgt1 stabilizes the mitotic kinase Polo to allow proper centrosome maturation, as well as entry and progression through the cell cycle [63]. Notably, depletion of the Polo-like kinase Cdc5 in C. albicans induces Cyr1-dependent filamentous growth [64], suggesting that Sgt1 could similarly function to stabilize Cdc5 in C. albicans, as depletion of either protein promotes filamentation. Sgt1 also interacts with Cyr1 in S. cerevisiae [45], and Cyr1 is a key morphogenetic regulator in C. albicans [9]. Further, in S. cerevisiae, a physical interaction between Hsp90 and Sgt1 with Cyr1 plays a key role in regulating gene expression, including genes involved in polarized morphogenesis [65]. We find that Sgt1 interacts with Cyr1 and represses cAMP-dependent PKA activity in C. albicans (Figure 3), whereas in S. cerevisiae Sgt1 activates, rather than represses, Cyr1-mediated signaling [45], thus reflecting functional divergence in the Sgt1-dependent circuitry between these species. Future studies could test our model that in C. albicans, filamentation as well as PKA activation observed upon depletion of Sgt1 is dependent on the adenyl cyclase Cyr1 by deletion of CRY1 in the tetO-SGT1/sgt1Δ strain.

Sgt1 governs not only C. albicans morphogenesis, but also resistance to the azoles and echinocandins (Figures 4 and 5). Though Sgt1 and Hsp90 have a conserved impact on drug resistance phenotypes, the mechanisms involved are distinct. Depletion of Hsp90 leads to destabilization of a drug resistance regulator calcineurin [37]; while depletion of Sgt1 does not alter calcineurin stability, it is required for its activation, suggesting a mechanism by which Sgt1 controls antifungal drug resistance (Figure 6). This is reminiscent of drug resistance of C. albicans biofilms, where compromising Hsp90 function abrogates resistance of biofilms to azoles, independent of Cna1 stabilization [28]. A similar signature of dependence on Hsp90 for stability and Sgt1 for activation is observed with disease resistance in plants, where depletion of Sgt1 prevents cellular responses associated with Nod1 activation, while having no affect on Nod1 stability, while depletion of Hsp90 results in Nod1 destabilization [66]. While compromising Hsp90 function can block the evolution of drug resistance [35], prior exposure to Hsp90 inhibitors can actually enable the evolution of resistance; pre-treatment with an Hsp90 inhibitor can promote the emergence ofazole resistance through the induction of aneuploidy, due to the role of Hsp90 in mediating kinetochore assembly [67]. That Sgt1 is known to act with Hsp90 to mediate kinetochore assembly and function [59,60], suggests that Sgt1 may also have powerful and opposing effects on the evolution of drug resistance depending on whether the co-chaperone function is compromised prior to or during drug exposure.

Sgt1’s capacity to govern morphogenesis, regulate resistance to antifungal drugs, and influence fitness of C. albicans in a murine model, suggests that it may provide a promising therapeutic target for fungal infections. Targeting Sgt1 may provide a novel strategy to compromise function of the Hsp90 chaperone machine, as depletion of Sgt1 or Hsp90 has conserved phenotypic effects. Targeting Hsp90 has emerged as a powerful strategy for the treatment of fungal infections; compromising Hsp90 function enhances the efficacy of azoles and echinocandins against C. albicans in metazoan models of infection [36]. While Hsp90 inhibitors in clinical trials as anti-cancer agents are effective at systemic exposure to Hsp90 inhibitors in a mammalian model of biofilm infection where the drug and infection are localized [28], there is toxicity associated with systemic exposure to Hsp90 inhibitors in a mammalian model of
YPD with or without 20 and 30% similarity for cells were grown overnight in 20 at 30°C (maltose). Overnight cultures were grown in 5 ml of YPD or YPM cultured on YPD (2% bacto-peptone, 1% yeast extract, and 2% glucose) until mid-log phase. To monitor fluconazole (FL, Sequoia Research Products) treatment, cells were diluted to OD600 of 0.2, and grown in fresh medium with 20 g/mL DOX, subcultured to OD600 of 0.2, and grown in mid-log phase (DOX, BD Biosciences). For treatment of diverse infectious diseases, neurodegenerative disease, and cancer.

**Materials and Methods**

**Ethics Statement**

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Duke University according to the guidelines of the Animal Welfare Act, The Institute of Laboratory Animal Resources Guide for the Care and Use of Laboratory Animals, and Public Health Service Policy.

**Strains and Culture Conditions**

Strains used in this study are listed in Table S1 and their construction is described in the Text S1. C. albicans strains were cultured on YPD (2% bacto-peptone, 1% yeast extract, and 2% glucose) or YPM (2% bacto-peptone, 1% yeast extract, and 2% maltose). Overnight cultures were grown in 5 ml of YPD or YPM at 30°C unless otherwise indicated. For doxycycline treatment, cells were grown overnight in 20 μg/mL DOX, subcultured to OD600 of 0.2, and grown in fresh medium with 20 μg/mL DOX and growth until mid-log phase (DOX, BD Biosciences). For glucose-mediated depletion, cells were grown overnight in 50% YPM and 50% YPD, subcultured to OD600 of 0.2, and grown in fresh medium consisting of 50% YPM and 50% YPD and growth until mid-log phase.

**Plasmids**

Plasmids used in this study are listed in Table S2 and their construction is described in the Supplemental Methods. Plasmids were sequenced to verify the absence of any nonsynonymous mutations. Primers used in this study are listed in Table S3.

**qRT-PCR**

To monitor SGTT transcript levels, cells were grown overnight in YPD at 30°C, diluted to OD600 of 0.2, and grown overnight again with or without doxycycline, as indicated. Cells were again diluted to OD600 of 0.2 in the same conditions and grown to mid-log phase. To monitor UTR2 transcript levels in response to fluconazole (FL, Sequoia Research Products) treatment, cells were grown overnight in YPD at 30°C, diluted to OD600 of 0.2 in YPD with or without 20 μg/mL of doxycycline (DOX) and grown overnight at 30°C. Cells were diluted again to OD600 of 0.3 in YPD with or without 20 μg/mL of doxycycline (DOX) and grown for 2 hours at 30°C. After 2 hours, cells were either untreated, treated with 16 μg/mL of fluconazole (FL), or treated with 30 ng/ ml maficafungin (MF) and grown for an additional 3 hours. Cultures were then pelleted at 1300 rcf for 5 min and frozen overnight at –80°C. RNA was isolated using the QIAGEN RNaseasy kit and RNasefree DNase (QIAGEN), and cDNA synthesis was performed using the AffinityScript Multi Temperature cDNA Synthesis Kit (Agilent Technologies). PCR was performed using Fast SYBR® Green Master Mix (Applied Biosystems) and the StepOnePlus Real-Time PCR System (Applied Biosystems) with the following cycling conditions: 95°C for 20 s; then 95°C for 3 min and 60°C for 30 s, for 40 cycles. Reactions were performed in triplicate, with oLC752 and oLC753 (GPD1); oLC2030 and oLC2031 (SGTT); and oLC1434 and oLC1435 (UTR2). Data were analyzed with StepOne™ Software v2.2 (Applied Biosystems).

**Immunoprecipitation**

Immunoprecipitations were performed essentially as described [37]. Briefly, yeast cultures were grown overnight in YPD at 30°C. Cells were diluted to OD600 of 0.2 in 40 ml and grown to mid-log phase. Cells were washed with sterile H2O and resuspended in 500 μl of lysis buffer containing 20 mM Tris pH 7.5, 100 mM KCl, 5 mM MgCl2 and 20% glycerol, with one protease inhibitor cocktail (complete, EDTA-free tablet, Roche Diagnostics) per 10 ml, 1 mM PMSF (EMD Chemicals) and 20 mM sodium molybdate (Sigma Aldrich Co.) added fresh before use. Cells were transferred to a 2 ml screw-cap tube and the tube was filled with acid-washed glass beads and additional lysis buffer until the beads were just below the meniscus at the top of the tube to reduce foaming during bead beating. Cells were disrupted by bead beating twice for 4 minutes with a 5-minute break on ice between cycles. Lysates were recovered by piercing a hole in the bottom of each tube, placing each tube in a larger tube, and centrifuging at 1308 rcf for two 5-minute cycles at 4°C, recovering the lysates at each interval. Total collected lysates were cleared by centrifugation at 15,339 rcf for 10 minutes at 4°C and protein concentrations were determined by Bradford analysis.

Anti-IgG immunoprecipitations were done using rabbit IgG agarose (Sigma Aldrich Co.). Protein was diluted to 1 mg/ml in lysis buffer with 0.2% Tween-20, and incubated rotating at 4°C overnight with IgG agarose that had been washed three times with lysis buffer prior to use. Unbound material was removed by washing three times with 1 ml lysis buffer with 0.1% Tween-20, centrifuging at 78 rcf for 5 minutes at 4°C. Protein sample was then incubated with AcTEV Protease (Invitrogen), rotating for 3 hours at 4°C. Protease-treated samples were centrifuged at 78 rcf for 5 minutes at 4°C, and the supernatant was added to Ni-NTA agarose (QIAGEN), that had been washed three times with lysis buffer prior to use. Protein was incubated with Ni-NTA agarose, rotating for 2 hours at 4°C. Samples were then centrifuged at 2817 rcf for 1 minute at 4°C, and protein was eluted by boiling the sample in one volume of 6X sample buffer, containing 0.35 M Tris-HCl, 10% (v/v) SDS, 36% glycerol, 5% β-mercaptoethanol, and 0.012% bromophenol blue.

Anti-HA immunoprecipitations were done using the ProFound™ HA Tag IP/Co-IP Kit (Thermo Scientific). Briefly, protein was diluted to 600 μg in 830 μl of lysis buffer and incubated with 6 μl of anti-HA agarose slurry, rotating overnight at 4°C. Unbound material was removed by washing three times with tris buffered saline containing 0.05% Tween-20 (TBST), pulse centrifuging for 10 seconds. Protein was eluted by boiling in 25–100 μl of 2× non-reducing sample buffer, 2-mercaptoethanol was added after boiling.

Anti-nickel immunoprecipitations (to immunoprecipitate the His tag) were done using Ni-NTA agarose (QIAGEN). Protein was diluted to 1 mg/ml in lysis buffer with 0.2% Tween-20, and incubated rotating at 4°C overnight with Ni-NTA agarose that had been washed three times with lysis buffer prior to use.

Sgt1 Governs Fungal Morphology and Drug Resistance

Disseminated fungal infection [36]. This toxicity is likely attributed to the deleterious effects of inhibiting a key regulator of signaling and immune responses in the host [60]. A challenge associated with developing fungal selective Hsp90 inhibitors is the high degree of conservation between fungal and human Hsp90 (69% identity and 83% similarity for S. cerevisiae [44]). In contrast, fungal Sgt1 bears remarkably less similarity to human Sgt1 (26% identity and 30% similarity for S. cerevisiae [44]), potentially facilitating development of fungal-selective inhibitors. The therapeutic potential of targeting Sgt1 is poised to extend to other eukaryotic pathogens for which targeting Hsp90 has therapeutic potential, including the filamentous fungus Aspergillus fumigatus [36], the malarial parasite Plasmodium falciparum [70], and the African sleeping sickness agent, Trypanosoma evansi [71]. Sgt1 provides a powerful strategy for the development of inhibitors targeting the Hsp90 chaperone machine, with broad implications for the treatment of diverse infectious diseases, neurodegenerative disease, and cancer.
Unbound material was removed by washing three times with 1 ml lysis buffer with 0.1% Tween-20, centrifuging at 2817 rcf for 1 minute at 4°C. Protein was eluted by incubating with 250 mM imidazole for 5 minutes on ice. Eluted sample was removed from Ni-NTA agarose following centrifugation at 2415 rcf for 30 seconds at room temperature, and 2X sample buffer, containing 2-mercaptoethanol was added and the sample was boiled for 5 minutes.

Immune Blot Analysis

Immune blot analysis was performed essentially as described [53]. Protein was obtained either from immunoprecipitation protocols above, or alternatively, cells were grown overnight in YPD at 30°C, diluted to OD_{600} of 0.2, and grown overnight again with or without doxycycline, as indicated, then cells were subcultured into fresh medium with or without doxycycline and grown until mid-log phase; at this point, they were harvested by centrifugation and washed with sterile water. Cell pellets were resuspended in lysis buffer containing 30 mM HEPEs pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1 mM PMSF, and protease inhibitor cocktail (complete, EDTA-free tablet, Roche Diagnostics). Cells suspended in lysis buffer were mechanically disrupted by adding acid-washed glass beads and bead beating for 3 minutes. Protein concentrations were determined by Bradford analysis. Protein samples were mixed with one-sixth volume of 6X sample buffer for SDS-PAGE. Samples were boiled for 5 minutes and then separated by SDS-PAGE using an 8–10% acrylamide gel. Proteins were electrotransferred to PVDF membranes (Bio-Rad Laboratories, Inc.) and blocked with 5% skimmed milk in phosphate buffered saline (PBS) with 0.1% Tween. Blots were hybridized with antibodies against CaHsp90 (1:1000 dilution, Santa Cruz Biotechnology, Inc), TAP (1:5000, Open Biosystems), generously provided by Brian Larsen [72]), actin (1:1000 dilution, aprotinin) and mechanically disrupted by adding acid-washed glass beads and bead beating for 3 minutes. Protein concentrations were determined by Bradford analysis. PKA activity was established essentially as described [50]. Briefly, 10 μg of total protein was used, and PKA activity was measured following the guidelines of the PepTag® Assay for Non-Radioactive Detection of AMP-Dependent Protein Kinase (Promega) in a total volume of 25 μl, 20 mM Tris-HCl pH 7.4, 10 mM MgCl₂, 1 mM ATP, 2 μg of PepTag® Al Peptide and 10 μM cAMP. Reactions were incubated at 30°C for 30 minutes, inactivated at 95°C for 10 minutes and separated on a 0.8% agarose gel made with 50 mM Tris-HCl pH 8.0, run at 100 V for 15 minutes. Quantification of the phosphorylated peptide fractions excised from the gels was performed by spectrophotometry in a 96-well plate at 570 nm.

Minimum Inhibitory Concentration Assays

Minimum inhibitory concentration (MIC) assays were performed in flat-bottom, 96-well microtiter plates (Sarstedt) using a modified broth microdilution protocol, essentially as described [35]. MIC tests were set up in a total volume of 0.2 ml/well with two-fold dilutions of fluconazole in YPD (FL, Sequoia Research Products) or micafungin (MF, generously provided by Julia R. Kohler). Gradients of fluconazole were from 256 μg/ml down to 0 with the following concentration steps in μg/ml: 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0, and gradients of micafungin were from 2 or 8 μg/ml down to 0 with the following concentration steps in μg/ml: 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0 or 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, 0.0078125, 0.00390625, 0.001953125, 0. Doxycycline was included in plates at a constant concentration of 20 μg/ml, as indicated. Strains were grown overnight in YPD at 30°C. Cell densities of overnight cultures were determined and dilutions were prepared such that ~10⁵ cells were inoculated into each well. Plates were incubated in the dark at 30°C for 48 hours or 72 hours, as indicated, at which point plates were sealed and re-suspended by agitation. Absorbance was determined at 600 nm using a spectrophotometer ( Molecular Devices) and was corrected for background from the corresponding medium. Each strain was tested in duplicate on at least two occasions. MIC data were quantitatively displayed with colour using the program Java TreeView 1.1.3 (http://jtreeview.sourceforge.net).

Cidality Assays

Cidality was established essentially as previously described [38]. Briefly, MIC assays were performed as described above, and incubated at 30°C for 48 hours or 72 hours, as indicated. Cells from the MIC assay were then spotted onto solid YPD medium using a spotter, incubated at 30°C for 48 hours, and photographed using a Canon PowerShot A640 digital camera.

Murine Model of _C. albicans_ Infection

To prepare inoculum, cultures of wild type or _tetO-SGT1/sgt1Δ_ were started from frozen stocks onto Sabouraud dextrose agar plates and incubated at 35°C for 48 hours. Colonies were suspended in sterile pH 7.4 phosphate-buffered saline (PBS), centrifuged at 324 rcf for 5 minutes, washed with sterile PBS one time and diluted to the desired concentration (2.1×10⁶ cells/ml) as verified by counting on a Neubauer hemocytometer. Concentration was verified by serial dilution and culture on Sabouraud dextrose plates. For murine exposure, male CD1 mice (Charles River Laboratories, Wilmington, MA) age 8 weeks (weight 20–34 g) were infected via the tail vein with 100 μl of a 2.1×10⁶ CFU/ml of yeast suspension. For the wild-type strain SN95
m; filamentation. Sgt1 levels were reduced by growth overnight in 20 μg/mL DOX, as indicated. (B) Genetic depletion of Sgt1 induces subculture in fresh medium with 20 μg/mL DOX, followed by subculture in fresh medium with 20 μg/mL DOX and growth until mid-log phase, the conditions used for most assays. Cells were plated on to YPD following growth overnight with or without DOX, and after growth until mid-log, with or without DOX, as indicated. (C) Cells remain viable after transcriptional repression of Sgt1 in cultures grown in well-aerated tubes with continuous agitation when Sgt1 levels were reduced by growth overnight in 20 μg/mL DOX, followed by subculture in fresh medium with 20 μg/mL DOX and growth until mid-log phase, the conditions used for most assays. Cells were plated on to YPD following growth overnight with or without DOX, and after growth until mid-log, with or without DOX, as indicated, and colony forming units (CFUs) were counted.

Figure S3 Hsp90 client protein Cna1 retains stability upon depletion of Sgt1. (A) Cna1 is destabilized upon depletion of Hsp90. Hsp90 levels were reduced by growth overnight in 50% YPM and 50% YPD, followed by subculture in fresh medium consisting of 50% YPM and 50% YPD and growth until mid-log phase. First panel, immune blot analysis of Hsp90 levels (5 μg protein loaded per well); and second panel, immune blot analysis of Cna1-TAP levels (50 μg protein loaded per well). Actin was used as a loading control. (B) Cna1 retains stability upon depletion of Sgt1. Sgt1 levels were reduced by growth overnight in 50% YPM and 50% YPD, followed by subculture in fresh medium consisting of 50% YPM and 50% YPD and growth until mid-log phase. First panel, immune blot analysis of Sgt1 levels (50 μg protein loaded per well); and second panel, immune blot analysis of Cna1-TAP levels (50 μg protein loaded per well). Actin was used as a loading control.

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Author Contributions
Conceived and designed the experiments: RSS LEC. Performed the experiments: RSS AKZ MBQ. Analyzed the data: RSS. Contributed reagents/materials/analysis tools: JRP. Wrote the paper: RSS LEC.
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