Systematic Identification and Characterization of Five Transcription Factors Mediating the Oxidative Stress Response in Candida Albicans

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Abstract

Background

Candida albicans is an opportunistic human fungal pathogen that can cause both superficial and systemic infections, especially in immunocompromised individuals. In response to *C. albicans* infections, innate immune cells of the host produce and accumulate reactive oxygen species (ROS) that can lead to irreversible damage and apoptosis of fungal cells. Prior studies have identified several transcription factors involved in the oxidative stress response of *C. albicans*. However, a systematic study to identify transcription factors mediating the oxidative response had not been previously conducted.

Results

In this study, we screened a comprehensive transcription factor mutant library consisting of 211 transcription factor deletion mutant strains in the presence and absence of H_2O_2, a potent inducer of ROS, and identified five transcription factors (Skn7, Dpb4, Cap1, Dal81 and Stp2) involved in the response to H_2O_2. Genome-wide transcriptional profiling revealed that H_2O_2 induced a discreet set of genes differentially regulated in common among the five transcription factor mutant strains identified. Functional enrichment analysis identified KEGG pathways pertaining to glycolysis/gluconeogenesis, amino sugar and nucleotide sugar metabolism, and ribosome synthesis as the most enriched pathways. Furthermore, among the most common differentially expressed genes, hexose catabolism and iron transport were the most enriched GO terms.

Conclusions

Our study is the first to systematically identify and characterize transcription factors involved in the response to H_2O_2. Based on the transcription factors identified, we found that exposure to H_2O_2 modulates several downstream gene classes involved in fungal virulence. Overall, this study sheds new light on the metabolism, physiological functions and cellular processes involved in the H_2O_2-induced oxidative stress response in *C. albicans*.

Background

Over the past twenty years, we have seen a significant increase in invasive fungal infections. *Candida* species are common commensals of the skin and mucosal surfaces of humans, but they can also lead to life-threatening systemic diseases, especially in immunocompromised individuals [1, 2]. Indeed, *Candida* species represent the fourth most common cause of nosocomial bloodstream infections in the United States [3]. The annual mortality rate of *Candida* bloodstream infections has been reported to be as high as 50% in adults and 30% in children [4, 5]. *Candida albicans* is the most prevalent *Candida* species responsible for infections in the clinic [6]. *C. albicans* has a number of important virulence factors that contribute to its capacity to cause infections in humans, such as its ability to undergo morphological transitions, its ability to form drug-resistant biofilms on mucosal surfaces and on implanted medical devices, and its ability to secrete numerous host-damaging enzymes [7-10].

In order to mitigate infection with *C. albicans*, the host innate immune system utilizes a number of defense strategies, such as the production of reactive oxygen species (ROS) to damage and kill invading *C. albicans* cells [11, 12]. ROS, which include superoxide, hydrogen peroxide (H_2O_2), hydroxyl radicals, singlet oxygen, nitrogen dioxide and peroxynitrite [13], are primarily released by host macrophages and neutrophils through a mechanism referred to as the respiratory or oxidative burst response [11]. ROS interact with nucleic acids, lipids, and proteins to induce irreversible damage and ultimately apoptosis of *C. albicans* cells [14]. In response to redox disturbances caused by the accumulation of ROS within macrophages, *C. albicans* cells are triggered to undergo filamentation [15], which allows *C. albicans* cells to escape the macrophage [11]. Consistently, cells of *C. albicans* mutant strains that are hypersensitive to ROS, are unable to form hyphae within macrophages and are thus unable to escape macrophage killing [11, 16]. Certain antifungal drugs that induce ROS, such as miconazole and the echinocandins, have been reported to be highly effective against *C. albicans*, even in its recalcitrant biofilm form [17, 18]. Thus, understanding how *C. albicans* mechanistically responds to ROS produced by the host innate immune cells is important for the development of novel antifungal strategies that may target this fungal response pathway.

ROS can activate the mitogen-activated protein kinase (MAPK) signaling pathway in *C. albicans* [19], and in turn, regulate various transcription factors (TFs), kinases, cyclin dependent protein kinases, and membrane proteins involved in MAPK signaling [11, 20-22]. Cap1 is perhaps the most well-known TF that mediates the response to ROS in *C. albicans* [23]. Genome-wide transcriptional profiling using microarrays demonstrated that Cap1 regulates genes associated with cellular antioxidant defense mechanisms and carbohydrate metabolism, among other pathways [24]. Several other TFs, such as Sfp1 [25] and Skn7 [26], have also been reported to regulate the response to ROS in *C. albicans*. Despite our knowledge of these important regulators of the *C. albicans* ROS response, a systematic study to identify TFs involved in the ROS response had not yet been performed.

Here, we systematically screened a comprehensive *C. albicans* TF mutant library consisting of 211 TF mutant strains [27] in the presence and absence of H_2O_2, a potent inducer of ROS. The results revealed that in addition to Cap1 and Skn7, three additional TFs, Dal81, Stp2 and Dpb4, were found to be involved in the *C. albicans* response to H_2O_2, based on the findings that their mutant strains were hypersensitive to H_2O_2. We note that we did not identify any TF mutant strains that were resistant to H_2O_2 in our screen. We also note that an *sfp1*Δ/Δ mutant strain was not included in this library, and thus we did not assess its sensitivity to H_2O_2. Genome-wide transcriptional profiling by RNA sequencing (RNA-seq) of these five TF mutant strains revealed that H_2O_2 induced significant expression changes in 63 key genes (15 genes were upregulated and 48 genes were downregulated) in common among the five H_2O_2 hypersensitive mutant strains. Functional enrichment analysis identified KEGG pathways pertaining to glycolysis/gluconeogenesis, amino sugar and nucleotide sugar metabolism, and ribosome synthesis as the most enriched pathways. Furthermore, among the most common differentially expressed genes, hexose catabolism and iron transport were the most enriched GO terms.

Results

Five *C. albicans* transcription factor mutants exhibited hypersensitivity to H_2O_2

To systematically identify *C. albicans* regulators of the ROS response, we screened a comprehensive TF mutant library consisting of 211 TF mutant strains in the presence and absence of H_2O_2 using spot assays on YPD plates. Prior to performing the genetic screen, we assessed the sensitivity of the isogenic wildtype (WT) strain (SN250) to concentrations of H_2O_2 ranging from 0.5-10.0 mM and found a dose-dependent relationship between increasing H_2O_2 concentration and increasing oxidative sensitivity (data not shown). When the H_2O_2 concentration reached 5 mM, the growth of the WT strain was hindered by approximately 50% (Fig. 1), and this concentration was chosen for the screen. We found that, in addition to the known hypersensitive TF mutant strains, c*ap1*Δ/Δ (TF140) and *skn7*Δ/Δ (TF083), three additional TF mutant strains, *dpb4*Δ/Δ (TF094), *dal81*Δ/Δ (TF155) and *stp2*Δ/Δ (TF162), showed significant sensitivity to H_2O_2 relative to the WT strain (Fig. 1). No *C. albicans* TF mutant strains were observed as clearly resistant to H_2O_2 in our screen (data not shown).

RNA-sequencing of the five TF mutant strains hypersensitive to H_2O_2

To determine the transcriptome of the five hypersensitive TF mutant strains, we performed RNA-seq on the five mutant strains and the isogenic WT strain in the presence and absence of H_2O_2. A total of 6,376 transcriptionally active regions were detected from the sequencing, 285 of which were novel transcriptionally active regions (nTARs, Dataset S1 Sheet A). The number of differentially expressed genes among relevant RNA-seq comparisons with fold changes of greater than or equal to twofold (Dataset S1 Sheet B and Fig. S1). For the WT strain SN250, 62 genes were significantly upregulated and 80 genes were significantly downregulated greater than or equal to twofold in the presence of H_2O_2 (Dataset S1 Sheet B and Fig. S1). Compared to that of SN250, the responses of the TF mutant strains to H_2O_2 were far more striking. 550, 240, 961, 148 and 105 genes were upregulated greater than or equal to twofold in the presence of H_2O_2 for the *skn7*Δ/Δ (TF083), *dpb4*Δ/Δ (TF094), c*ap1*Δ/Δ (TF140), *dal81*Δ/Δ (TF155) and *stp2*Δ/Δ (TF162) mutant strains, respectively (Dataset S1 Sheet B, Fig. S1, and Fig. 2). Additionally, in the presence of H_2O_2, 828, 778, 888, 278 and 214 genes were downregulated greater than or equal to twofold in these five TF mutant strains, respectively (Dataset S1 Sheet B, Fig. S1 and Fig. 2). Moreover, 15 genes were upregulated greater than or equal to twofold and 48 genes were downregulated greater than or equal to twofold in common among all five TF mutant strains in the presence of H_2O_2 (Fig. 2 and Fig. 3).

KEGG pathway enrichment and GO term analyses of the differentially expressed genes among the five TF mutant strains hypersensitive to H_2O_2

To gain an understanding of the biological pathways involved in response to H_2O_2 for the five hypersensitive TF mutant strains, we performed KEGG pathway enrichment analyses for each strain in the presence and absence of H_2O_2 (Fig. 4). The enriched pathway in common among these five mutant strains was amino sugar and nucleotide sugar metabolism. Interestingly, the patterns of enrichment for these five mutant strains appeared to be divided into two groups. In the first group, consisting of the *skn7*Δ/Δ (TF083), *dpb4*Δ/Δ (TF094) and c*ap1*Δ/Δ (TF140) mutant strains, we observed enrichment mainly in fatty acid metabolism, carbohydrate metabolism, proteasome metabolism, glutathione metabolism, and ribosome synthesis. In the second group, consisting of the *dal81*Δ/Δ (TF155) and *stp2*Δ/Δ (TF162) mutant strains, enrichment was mainly in meiosis and the cell cycle. Overall, glycolysis/gluconeogenesis, amino sugar and nucleotide sugar metabolism, and ribosome synthesis were the three most enriched KEGG pathways across all five TF mutant strains (Fig. 5).

We further performed GO term and KEGG enrichment analyses on the 15 upregulated genes and the 48 downregulated genes in common among all five of the TF mutant strains from Fig. 3. The GO term results included molecular function, biological process and cell component as enriched. The KEGG pathways results included metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, human diseases, and drug development as enriched. Tables 1 and 2 present the top five significantly enriched GO terms and KEGG pathways, respectively, of the commonly regulated genes among all five TF mutant strains in the presence of H_2O_2.

Confirmation of RNA-sequencing data by RT-qPCR

Based on the RNA-seq results presented in Fig. 3, we chose several genes of interest to validate by RT-qPCR. The selected genes were primarily divided into five functional categories: oxidation reduction, membrane, cell wall, transporters, and other (Table 3).

With the exceptions of *CFL2* and *CFL4* in the *dpb4*Δ/Δ (TF094) mutant strain, as well as *SOD4* in the *dal81*Δ/Δ (TF155) and *stp2*Δ/Δ (TF162) mutant strains, which trended in the right directly, but did not meet the significance threshold, the expression patterns of all genes as determined by RT-qPCR were consistent with the RNA-seq results. The transcription factor Cap1 is reported to regulate the catalase-encoding gene *CAT1* during oxidative stress [28]; however, we observed by RNA-seq and RT-qPCR that the expression of *CAT1* in the c*ap1*Δ/Δ mutant strain was, unexpectedly, not significantly changed in the presence of H_2O_2, relative to the WT strain. In the presence of H_2O_2, some genes in certain functional categories displayed clear expression level directions by RNA-seq and RT-qPCR, while others showed varied expression levels. For example, genes encoding membrane regulators/components (e.g. *ECM21* and *ATO1*) were significantly downregulated in all five TF mutant strains in the presence of H_2O_2 relative to the WT strain by RNA-seq and RT-qPCR. Conversely, genes encoding two transporters involved in antimicrobial resistance, *QDR1* and *ROA1*, showed varied expression patterns relative to the WT strain by RNA-seq and RT-qPCR.

Discussion

C. albicans is one of the most common opportunistic fungal pathogens of humans. Infection with *C. albicans* can cause life-threatening bloodstream infections with high mortality rates, especially in immunocompromised individuals [1, 2]. In response to infection with *C. albicans*, the host responds by

producing a variety of ROS that can damage and kill invading *C. albicans* cells [11, 13]. To counter ROS, *C. albicans* has evolved a number of oxidative stress response mechanisms that are known to be regulated by different transcription factors.

In the present study, we systematically screened a comprehensive 211 member *C. albicans* TF mutant library [29] in the presence and absence of H_2O_2, a potent inducer of ROS. Five mutant strains were severely defective (did not grow or barely grew) on YPD plates in the presence of 5 mM H_2O_2 (Fig. 1), and we define these strains as hypersensitive. Although two mutant strains, *ecm22Δ/Δ* (TF171) and *ino2Δ/Δ* (TF185), showed some resistance to H_2O_2 (data not shown), we did not follow up on these strains since their sensitivities were relatively minor. Among the five TFs identified, Cap1, a bZIP transcription factor of the AP-1 family, is known to be an important regulator of the oxidative stress response in *C. albicans* [24, 30, 31]. Skn7, a transcription factor that is part of a two-component regulatory system, is known to be activated via phosphorylation in response to oxidative stress [26, 32]. The remaining three TFs identified were not previously associated with the regulation of oxidative stress response pathways. Dpb4 is known to regulate nuclear DNA and mitochondrial DNA replication and maintenance, as well as rRNA and mitochondrial rRNA processing [33], while transcription factors Dal81 and Stp2 are reported to play roles in regulating nitrogen utilization via distinct amino acid metabolic pathways [34-36].

To date, the most well-known transcription factor regulating the oxidative stress response in *C. albicans* is Cap1 and the most widely reported signaling pathway mediating the response to oxidative stress is the Hog1-MAPK signaling pathway [20, 37, 38]. *CAT1*, encoding a key Cap1-dependent catalase, has been shown to be highly upregulated in the presence of ROS [28]. Interestingly, in our study, the expression levels of *CAT1* in the five TF mutant strains did not show significant differential regulation relative to the WT strain in the presence of H_2O_2 (Table 3 and Dataset S1 Sheet B). One possible explanation for this observed difference is that previous studies largely co-cultured *C. albicans* with phagocytes, while we directly added exogenous H_2O_2 to stimulate *C. albicans* [28,39]. One prior study found that Hog1 contributes to the oxidative stress response at a post-transcriptional level in *C. albicans* [38]. Consistent with this report, we observed no obvious changes in the expression level of *HOG1* in the WT strain in the presence of H_2O_2 (Table 3 and Dataset S1 Sheet B). Altogether, these findings suggest that other signaling pathways are likely involved in the *C. albicans* oxidative stress response to H_2O_2. The transcription factor Stp2 is reported to contribute to nutrient adaption/sensing and biofilm formation in *C. albicans* via targeting the rapamycin (TOR) signaling pathway [40-43]. Therefore, investigating whether our five identified transcription factors participate in the TOR signaling pathway in the presence of ROS is an intriguing area of future interest.

Glycolysis/gluconeogenesis, amino sugar and nucleotide sugar metabolism, and ribosome synthesis were the top three enriched KEGG pathways from the RNA-seq analyses for the five H_2O_2-hypersensitive TF mutant strains in the presence of H_2O_2, among which amino sugar and nucleotide sugar metabolism were the only enriched KEGG pathways in common among these five TF mutant strains (Fig. 4 and Fig. 5). The chitin deacetylase Cda2 [44], is a member of the amino sugar and nucleotide sugar metabolism pathway. Consistently, *CDA2* was significantly downregulated in all five of the H_2O_2-hypersensitive TF mutant strains in the presence of H_2O_2 (Fig. 3). Other than the KEGG pathways we found to be in common among the five H_2O_2-hypersensitive TF mutant strains, we also found two discreet enriched KEGG pathway groups for the five TF mutant strains (Fig. 4). The TF mutant strains *skn7*Δ/Δ (TF083), *dpb4*Δ/Δ (TF094) and c*ap1*Δ/Δ (TF140) were largely enriched in metabolism pathways, while *dal81*Δ/Δ (TF155) and *stp2*Δ/Δ (TF162) were largely enriched in meiosis and cell cycle pathways. These findings suggest that these five transcription factors likely play roles in the H_2O_2 -induced oxidative stress response of *C. albicans* via different mechanisms, which could intersect with *CDA2*. The relationship between the presence of chitin and the production of ROS has been previously established in bacteria and plants [45,46]. Interestingly, the chitin synthase encoding gene *CHS3* was also downregulated in all five H_2O_2-hypersensitive TF mutant strains in the presence of H_2O_2 (Dataset S1 Sheet B). Based on these observations, it seems likely that chitin content is important in the *C. albicans* H_2O_2-induced oxidative stress response, and possibly in the way the host detects the presence of a pathogen.

ROS can potentially damage almost every essential component of the cell, leading to enzyme inactivation, membrane disruption, functional mutations, and eventually, cell death [47]. We demonstrate that among the five hypersensitive transcription factor mutant strains we identified, H_2O_2 has considerable effects on the regulation of genes involved in *C. albicans* virulence, including genes encoding components of the plasma membrane, the cell wall, phosphate transporters and efflux pumps, heat shock proteins, ferric reductases, and superoxide dismutase isoenzymes, among others (Table 3). Future work will elucidate the specific roles that these TFs play in the *C. albicans* H_2O_2-induced oxidative stress response, and the mechanisms underlying the regulation of these transcription factors and their downstream target genes.

Conclusions

The present study is the first to systematically screen a *C. albicans* transcription factor mutant library for oxidative sensitivities to H_2O_2. Our findings provide a comprehensive understanding of the metabolism, physiological functions, and cellular processes of *C. albicans* to H_2O_2-induced oxidative stress.

Methods

Strains, media and reagents

All *C. albicans* strains were grown at 30°C on YPD plates (1% yeast extract, 2% peptone and 2% glucose). A *C. albicans* TF deletion mutant library containing 211 TF mutant strains [27], and the isogenic WT strain SN250 were used. H_2O_2 (Sigma-Aldric, 323381) was stored under dark conditions at room temperature. A working concentration of H_2O_2 was prepared fresh for each experiment.

Hydrogen peroxide-induced redox assay

The sensitivities of each transcription factor mutant strain to H_2O_2 were determined by oxidative stress response assays. In brief, for each transcription factor mutant strain, a single colony of *C. albicans* was inoculated onto YPD plates and grown overnight at 30°C with continuous shaking at 200 rpm. Cells were then diluted to an initial OD_{600} of 0.5 and 5μL aliquots of serial diluted yeast suspensions (10^7, 10^6, 10^5, 10^4, 10^3 and 10^2 cells per mL in PBS) were used for the spot assays. The spot assays were performed on YPD plates supplemented with and without H_2O_2 (5 mM) and incubated at 30°C for 48 hours. For each experiment, the WT strain (SN250) and *cap1*Δ/Δ (TF140) strain were used as controls. Each TF mutant strain was tested in triplicate.

RNA extractions

Total RNA from *C. albicans* cells grown in the presence and absence of H_2O_2 was extracted using the Yeast RNAiso Reagent Kit (TaKaRa, Tokyo, Japan), according to the manufacturer's instructions. The RNA was quantified using a NanoDrop2000 (Thermo Fisher Scientific, Carlsbad, CA, USA), and the quality was assessed using a BioAnalyzer 2100 (Aglient Technologies, Santa Clara, CA, USA). Only high-quality RNA samples (OD_{260}/OD_{280} =1.8~2.2, OD_{260}/OD_{230} >2.0, RNA integrity number (RIN) >8, 28S:18S >1.0, and total amount >2μg) were stored at -80°C for later use.

Library preparation and RNA sequencing

Library construction and sequencing were performed at Majorbio Biotech Co., Ltd. (Shanghai, China). The cDNA libraries were constructed using a TruSeq™ RNA sample preparation kit (Illumina, San Diego, CA, USA). The mRNA was isolated according to the polyA selection method with oligo(dT) beads and fragmented using fragmentation buffer. The cDNA was synthesized using a SuperScript double-stranded cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) with random hexamer primers (Illumina, San Diego, CA, USA), according to the manufacturer's protocol. The synthesized cDNA was subjected to end-repair, phosphorylation, and poly (A) addition. The cDNA library was amplified for 15 PCR cycles using Phusion DNA polymerase (New England Biolabs, Ipswich, MA, USA). After being quantified using a Qubit® 2.0 fluorometer and validated using the Bioanalyzer 2100 (Aglient Technologies, Santa Clara, CA, USA), paired-end sequencing was performed using the Illumina NovaSeq 6000 (2 × 150 bp read length). A total of 268.25 Gb of clean raw data was obtained, and Q30 was as high as 90.8%. Approximately 94.24%-96.34% of the sequenced reads were successfully mapped to the reference genome sequence (NCBI ASM18296v3). RNA-seq was performed for each sample in duplicate.

Analysis of differential gene expression

The raw paired-end reads were trimmed and quality controlled using SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) set to default parameters. The clean reads were then separately aligned to the *C. albicans* reference genome NCBI ASM18296v3 with the orientation mode using Tophat software (version 2.0.0). Differential gene expression was estimated as the number of fragments per kilobase of exon model per million mapped reads (FPKM). RSEM (http://deweylab.biostat.wisc.edu/rsem/) was used to quantify the gene abundances. EdgeR was utilized to identify the differentially expressed genes (DEGs), which were determined using thresholds of the Benjamini-corrected *P*-value of less than 0.05 and fold changes greater than or equal to two.

Bioinformatics analysis of the sequencing data

After annotation, sequencing data were subjected to KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology analysis) enrichment analyses. GO functional enrichment and KEGG pathway analyses were carried out using Goatools (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/home.do), respectively.

Quantitative RT-qPCR

The RNA-seq results were validated by quantitative RT-qPCR using the SYBR Premix Ex Taq Kit (Takara, Tokyo, Japan) and LightCycle 480 Real-time PCR System (Roche, Shanghai, China). *ACT1* was used as the internal control, and the relative expression of the genes of interest were analyzed using the $2^{-\Delta\Delta CT}$ method. All reactions were performed with the following settings: denaturation at 95°C for 30 seconds, followed by 40 cycles of five seconds at 95°C and 30 seconds at 60°C. Each sample was processed in triplicate, and threshold expression value changes greater than or equal to twofold were considered differentially expressed. All primer sequences are listed in Table S1.

Availability of data and materials

All datasets generated in this study are included in this published article and its supplementary information files.

Abbreviations

ROS: Reactive oxygen species

RNA-seq: Ribonucleic acid sequencing

RT-qPCR: Real-time quantitative polymerase chain reaction

TF: Transcription factor

KO: Transcription factor knockout strain

KO_H: H_2O_2 treated transcription factor knockout strain

WT: Wildtype strain

WT_H: H_2O_2 treated wild type strain

YPD: Yeast extract peptone dextrose

PBS: Phosphate-buffered saline

Declarations

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Contributions

CJ, YP, and CJN conceived the study. CJ and YP designed the experiments. YC, DW, DD, QN and TS performed the experiments. YC and CJ wrote the manuscript. CJN edited and revised the manuscript. YP supervised the study. All authors have read and approved the final manuscript.

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Ethics declaration

Consent for publication

Not applicable

Competing interests

Clarissa J. Nobile is a cofounder of BioSynesis, Inc., a company developing diagnostics and therapeutics for biofilm infections. The remaining authors declare that they have no competing interests pertaining to the work presented in this manuscript.

References

1. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20(1):133-63.
2. Calderone RAC, C.J. Candida and Candidiasis. ASM Press: Washington, DC, USA. 2012.
3. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2004; 39(3):309-17.
4. Negri M, Silva S, Henriques M, Oliveira R. Insights into Candida tropicalis nosocomial infections and virulence factors. Eur J Clin Microbiol Infect Dis. 2012; 31(7):1399-412.
5. Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP. National surveillance of nosocomial blood stream infection due to Candida albicans: frequency of occurrence and antifungal susceptibility in the SCOPE Program. Diagnostic microbiology and infectious disease. 1998; 31(1):327-32.
6. Cavalheiro M, Teixeira MC. Candida Biofilms: Threats, Challenges, and Promising Strategies. Frontiers in medicine2018; 5:28.

7. Silva S, Rodrigues CF, Araújo D, Rodrigues ME, Henriques M. Candida Species Biofilms' Antifungal Resistance. Journal of fungi (Basel, Switzerland). 2017; 3(1).
8. Kernien JF, Snarr BD, Sheppard DC, Nett JE. The Interface between Fungal Biofilms and Innate Immunity. Frontiers in immunology. 2017; 8:1968.
9. Hendrickson JA, Hu C, Aitken SL, Beyda N. Antifungal Resistance: a Concerning Trend for the Present and Future. Current infectious disease reports. 2019; 21(12):47.
10. Calderone RA, Fonzi WA. Virulence factors of Candida albicans. Trends Microbiol. 2001; 9(7): 327-35.
11. Dantas Ada S, Day A, Ikeh M, Kos I, Achan B, Quinn J. Oxidative stress responses in the human fungal pathogen, Candida albicans. Biomolecules. 2015; 5(1):142-65.
12. Kim J, Park S, Lee JS. Epigenetic Control of Oxidative Stresses by Histone Acetyltransferases in Candida albicans. Journal of microbiology and biotechnology. 2018; 28(2):181-9.
13. Powers SK, Ji LL, Kavazis AN, Jackson MJ. Reactive oxygen species: impact on skeletal muscle. Comprehensive Physiology. 2011; 1(2):941-69.
14. Halliwell B. Oxidative stress and cancer: have we moved forward? The Biochemical journal. 2007; 401(1):1-11.
15. Nasution O, Srinivasa K, Kim M, Kim YJ, Kim W, Jeong W, et al. Hydrogen peroxide induces hyphal differentiation in Candida albicans. Eukaryotic cell. 2008; 7(11):2008-11.
16. Enjalbert B, MacCallum DM, Odds FC, Brown AJ. Niche-specific activation of the oxidative stress response by the pathogenic fungus Candida albicans. Infection and immunity. 2007; 75(5):2143-51.
17. Delattin N, Cammue BP, Thevissen K. Reactive oxygen species-inducing antifungal agents and their activity against fungal biofilms. Future medicinal chemistry. 2014; 6(1):77-90.
18. Hao B, Cheng S, Clancy CJ, Nguyen MH. Caspofungin kills Candida albicans by causing both cellular apoptosis and necrosis. Antimicrobial agents and chemotherapy. 2013; 57(1):326-32.
19. Smith DA, Nicholls S, Morgan BA, Brown AJ, Quinn J. A conserved stress-activated protein kinase regulates a core stress response in the human pathogen Candida albicans. Molecular biology of the cell. 2004; 15(9):4179-90.
20. Komalapriya C, Kaloriti D, Tillmann AT, Yin Z, Herrero-de-Dios C, Jacobsen MD, et al. Integrative Model of Oxidative Stress Adaptation in the Fungal Pathogen Candida albicans. PloS one; 2015; 10(9):e0137750.
21. Cui S, Li M, Hassan RYA, Heintz-Buschart A, Wang J, Bilitewski U. Inhibition of Respiration of Candida albicans by Small Molecules Increases Phagocytosis Efficacy by Macrophages. mSphere. 2020; 5(2).
22. Kos I, Patterson MJ, Znaidi S, Kaloriti D, da Silva Dantas A, Herrero-de-Dios CM, et al. Mechanisms Underlying the Delayed Activation of the Cap1 Transcription Factor in Candida albicans following Combinatorial Oxidative and Cationic Stress Important for Phagocytic Potency. mBio. 2016; 7(2):e00331.
23. Patterson MJ, McKenzie CG, Smith DA, da Silva Dantas A, Sherston S, Veal EA, et al. Ybp1 and Gpx3 signaling in Candida albicans govern hydrogen peroxide-induced oxidation of the Cap1 transcription factor and macrophage escape. Antioxidants & redox signaling. 2013; 19(18):2244-60.
24. Wang Y, Cao YY, Jia XM, Cao YB, Gao PH, Fu XP, et al. Cap1p is involved in multiple pathways of oxidative stress response in Candida albicans. Free radical biology & medicine. 2006; 40(7):1201-9.
25. Lee SY, Chen HF, Yeh YC, Xue YP, Lan CY. The Transcription Factor Sfp1 Regulates the Oxidative Stress Response in Candida albicans. Microorganisms. 2019; 7(5).
26. Singh P, Chauhan N, Ghosh A, Dixon F, Calderone R. SKN7 of Candida albicans: mutant construction and phenotype analysis. Infection and immunity. 2004; 72(4):2390-4.
27. Nobile CJ, Fox EP, Nett JE, Sorrells TR, Mitrovich QM, Hernday AD, et al. A recently evolved transcriptional network controls biofilm development in Candida albicans. Cell. 2012; 148(1-2):126-38.
28. Fradin C, De Groot P, MacCallum D, Schaller M, Klis F, Odds FC, Hube B. Granulocytes govern the transcriptional response, morphology and proliferation of Candida albicans in human blood. Mol Microbiol. 2005; 56(2): 397-415.
29. Homann OR, Dea J, Noble SM, Johnson AD. A phenotypic profile of the Candida albicans regulatory network. PLoS Genet. 2009; 5(12): e1000783.
30. Alarco AM, Raymond M. The bZip transcription factor Cap1p is involved in multidrug resistance and oxidative stress response in Candida albicans. Journal of bacteriology. 1999; 181(3):700-8.
31. Zhang X, De Micheli M, Coleman ST, Sanglard D, Moye-Rowley WS. Analysis of the oxidative stress regulation of the Candida albicans transcription factor, Cap1p. Molecular microbiology. 2000; 36(3):618-29.
32. Basso V, Znaidi S, Lagage V, Cabral V, Schoenherr F, LeibundGut-Landmann S, et al. The two-component response regulator Skn7 belongs to a network of transcription factors regulating morphogenesis in Candida albicans and independently limits morphogenesis-induced ROS accumulation. Molecular microbiology. 2017; 106(1):157-82.
33. Khamooshi K, Sikorski P, Sun N, Calderone R, Li D. The Rbf1, Hfl1 and Dbp4 of Candida albicans regulate common as well as transcription factor-specific mitochondrial and other cell activities. BMC genomics. 2014; 15:56.
34. Turner SA, Ma Q, Ola M, Martinez de San Vicente K, Butler G. Dal81 Regulates Expression of Arginine Metabolism Genes in Candida parapsilosis. mSphere. 2018; 3(2).
35. Miramón P, Pountain AW, van Hoof A, Lorenz MC. The Paralogous Transcription Factors Stp1 and Stp2 of Candida albicans Have Distinct Functions in Nutrient Acquisition and Host Interaction. Infection and immunity 2020; 88(5).

36. Martínez P, Ljungdahl PO. Divergence of Stp1 and Stp2 transcription factors in Candida albicans places virulence factors required for proper nutrient acquisition under amino acid control. Molecular and cellular biology. 2005; 25(21):9435-46.
37. Ikner A, Shiozaki K. Yeast signaling pathways in the oxidative stress response. Mutat Res. 2005; 569(1-2): 13-27.
38. Dantas Ada S, Day A, Ikeh M, Kos I, Achan B, Quinn J. Oxidative stress responses in the human fungal pathogen, Candida albicans. Biomolecules. 2015; 5(1): 142-65.
39. Enjalbert B, MacCallum DM, Odds FC, Brown AJ. Niche-specific activation of the oxidative stress response by the pathogenic fungus Candida albicans. Infect Immun. 2007; 75(5):2143-51.
40. Böttcher B, Hoffmann B, Garbe E, Weise T, Cseresnyés Z, Brandt P, Dietrich S, Driesch D, Figge MT, Vylkova S. The transcription factor Stp2 is important for Candida albicans biofilm establishment and sustainability. Front Microbiol. 2020; 11:794.
41. García C, Tebbji F, Daigneault M, Liu NN, Köhler JR, Allen-Vercoe E, Sellam A. The human gut microbial Metabolome modulates fungal growth via the TOR signaling pathway. mSphere. 2017; 2(6): e00555-17.
42. Liu NN, Uppuluri P, Broggi A, Besold A, Ryman K, Kambara H, Solis N, Lorenz V, Qi W, Acosta-Zaldívar M, Emami SN, Bao B, An D, Bonilla FA, Sola-Visner M, Filler SG, Luo HR, Engström Y, Ljungdahl PO, Culotta VC, Zanoni I, Lopez-Ribot JL, Köhler JR. Intersection of phosphate transport, oxidative stress and TOR signalling in Candida albicans virulence. PLoS Pathog. 2018; 14(7): e1007076.
43. Lee YT, Fang YY, Sun YW, Hsu HC, Weng SM, Tseng TL, Lin TH, Shieh JC. THR1 mediates GCN4 and CDC4 to link morphogenesis with nutrient sensing and the stress response in Candida albicans. Int J Mol Med. 2018; 42(6): 3193-3208.
44. Zida A, Bamba S, Yacouba A, Ouedraogo-Traore R, Guiguemdé RT. Anti-Candida albicans natural products, sources of new antifungal drugs: A review. Journal de mycologie medicale. 2017; 27(1):1-19.
45. Ali M, Li QH, Zou T, Wei AM, Gombojab G, Lu G, Gong ZH. Chitinase gene positively regulates hypersensitive and defense responses of pepper to Colletotrichum acutatum infection. Int J Mol Sci. 2020; 21(18):6624.
46. Kawasaki T, Yamada K, Yoshimura S, Yamaguchi K. Chitin receptor-mediated activation of MAP kinases and ROS production in rice and Arabidopsis. Plant Signal Behav. 2017; 12(9): e1361076.
47. Costa V, Moradas-Ferreira P. Oxidative stress and signal transduction in Saccharomyces cerevisiae: insights into ageing, apoptosis and diseases. Molecular aspects of medicine. 2001; 22(4-5):217-46.

Tables

Table 1. Significant GO term enrichment of the commonly regulated genes in the presence of H_2O_2 (KO_H vs. KO)

GO ID	GO Term	*p* value
0019320	Hexose catabolic process	<0.0001
1901678	Iron coordination entity transport	0.0001
0046365	Monosaccharide catabolic process	0.0002
0030001	Metal ion transport	0.0004
0000041	Transition metal ion transport	0.0007

Table 2. Significant KEGG pathway enrichment of the commonly regulated genes in the presence of H_2O_2 (KO_H vs. KO)

Pathway ID	Description	*p* value
map00600	Sphingolipid metabolism	0.0115
map00051	Fructose and mannose metabolism	0.0246
map00561	Glycerolipid metabolism	0.0303
map00520	Amino sugar and nucleotide sugar metabolism	0.0307
map00620	Pyruvate metabolism	0.0464

Table 3. Changes of gene expression levels in the presence of H_2O_2 (KO_H vs. KO and WT_H vs WT)

Gene name	Gene ID	Description	Fold changes (Log_2)				
			(TF083_H/TF083)	(TF094_H/TF094)	(TF140_H/TF140)	(TF155_H/TF155)	(TF162_H/TF162)
Oxidation Reduction							
CFL2	gene3747	Oxidoreductase	-7.11±1.51	-1.83±0.03	-10.36±5.71	-4.73±0.34	-2.66±0.73
CFL4	gene4020	Ferric/cupric-chelate reductase	-3.33±0.59	-2.25±0.55	-3.48±1.16	-3.53±0.47	-2.36±0.25
SOD4	gene1465	Cu-containing superoxide dismutase	-4.77±1.32	-1.62±1.47	-5.02±2.44	-3.30±0.15	-2.15±0.17
SOD6	gene1424	Copper-containing superoxide dismutase	-3.02±0.25	-2.47±0.47	-2.97±0.65	-1.22±0.36	-0.42±0.16
PUT1	gene4135	proline dehydrogenase	-2.80±0.80	-4.91±1.74	-4.68±0.99	-0.98±0.86	-0.72±0.86
FMA1	gene425	Oxidoreductase	7.00±2.54	3.23±0.35	5.60±1.90	3.91±0.33	3.46±1.07
CAT1	gene653	Catalase A	-0.71±0.14	-1.96±1.23	0.98±0.76	0.78±0.70	1.45±0.57
Membrane							
ECM21	gene979	Regulator of endcytosis of plasma membrane proteins	-4.62±1.01	-7.12±2.80	-5.89±1.69	-1.10±0.52	-2.47±0.82
ATO1	gene2526	Fungal-specific transmembrane protein	-10.01±4.47	-6.54±2.36	-9.51±3.98	-2.80±0.58	-1.70±0.69
PGA34	gene5500	GPI-anchored protein	-2.88±0.60	-3.07±0.49	-4.70±1.51	-2.59±0.22	-4.99±1.31
Cell Wall							
SCW4	gene238	Cell wall protein	-1.98±0.34	-5.24±0.85	-2.54±0.06	-3.10±2.10	-2.26±1.63
RBR1	gene5666	Glycosylphosphatidylinositol (GPI)-anchored cell wall protein	5.07±1.85	6.47±1.89	5.28±1.90	2.10±0.05	0.68±0.49
Various transporters							
QDR1	gene5644	Multidrug transporter	9.77±4.47	4.25±0.93	1.36±0.36	2.19±0.40	2.98±0.34
HGT7	gene1496	MFS glucose transporter	7.16±1.39	2.09±0.04	1.01±0.21	1.90±0.10	2.31±0.35
ROA1	gene180	PDR-subfamily ABC transporter	-4.41±1.09	-3.10±1.25	-5.95±1.74	-2.42±0.79	-3.69±1.69
PHO89	gene3391	Phosphate permease	-12.58±8.5	-10.19±3.68	-9.07±4.32	1.36±0.36	1.33±0.94
FGR2	gene4892	Phosphate transporter	-4.96±1.64	-5.82±1.50	-7.04±3.05	3.8±0.43	-0.95±0.53
Others							
HOG1	gene1721	Mitogen-activated protein kinase	1.15±0.58	0.58±0.84	-1.32±0.58	0.80±0.47	0.37±0.93
GCY1	gene3145	glycerol 2-dehydrogenase (NADP(+))	6.67±2.21	4.74±0.74	3.34±0.60	1.33±0.46	2.50±0.30
LIP2	gene903	Secreted lipase	-1.56±0.56	-4.55±0.81	-4.61±1.38	-0.38±0.12	-2.10±0.89
HSP30	gene186	Heat shock protein	-7.79±3.27	-8.04±1.93	-6.94±2.36	-1.67±0.40	-3.87±1.17
OP4	gene1260	Ala- Leu- and Ser-rich protein	-5.84±2.14	-3.31±0.45	-7.85±2.81	-3.12±0.05	-3.35±0.49
MRV2	gene4281	Spider biofilm induced	-7.4±3.7	-6.86±1.38	-6.00±1.21	-4.83±0.75	-2.44±0.25

Figures

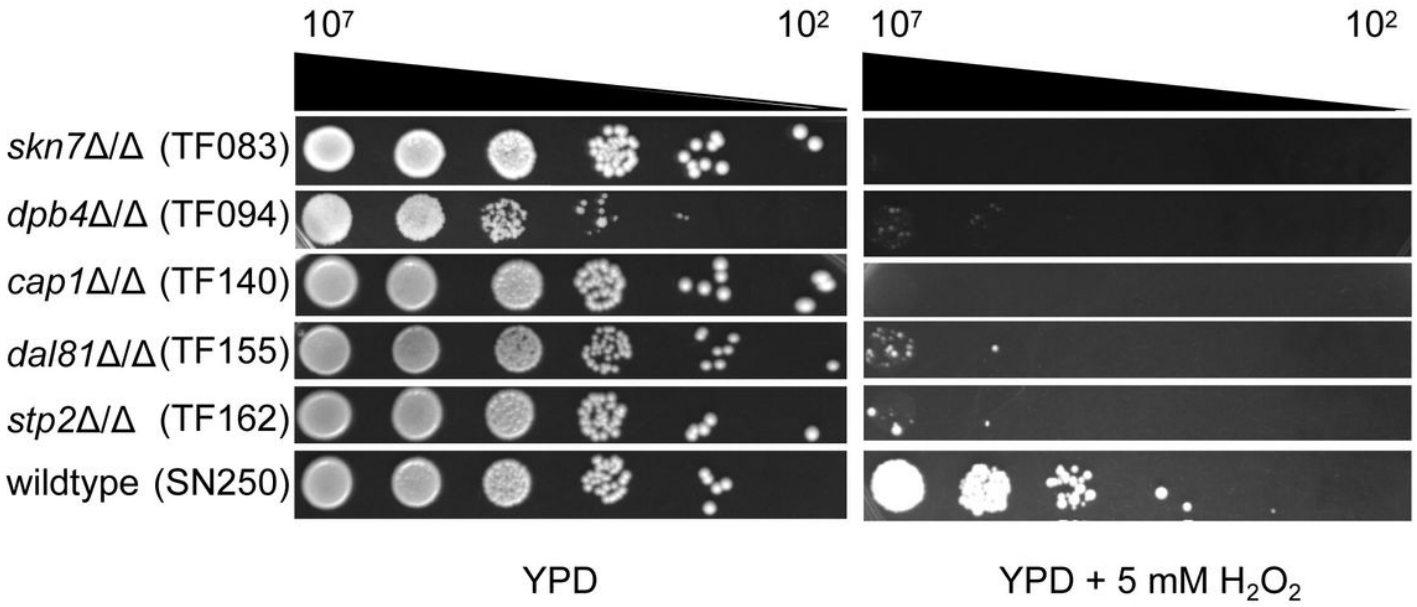


Figure 1

Sensitivity of C. albicans transcription factor mutants to H2O2. Legend: 5μL of serial diluted C. albicans suspensions (107-102/mL) were spotted onto YPD plates in the presence and absence of 5 mM H2O2 and were incubated at 30°C for 48 hours. SN250 was used as the isogenic WT strain. Each TF mutant strain was tested in triplicate.

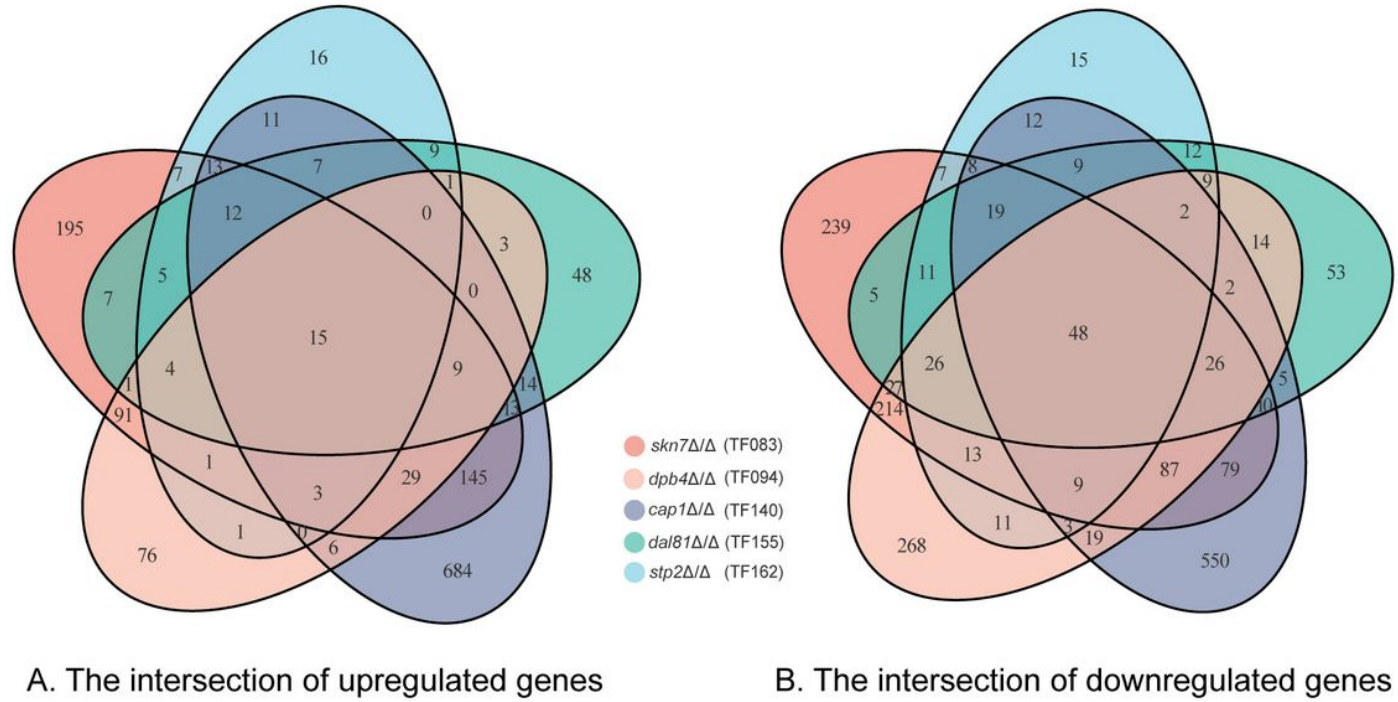
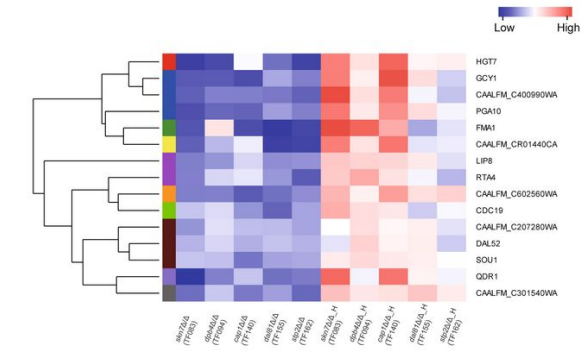


Figure 2

The number of differentially expressed genes in five C. albicans hypersensitive TF mutants in the presence of H2O2. Legend: RNA-seq was performed on the five TF mutant strains in the presence and absence of H2O2. Differentially expressed genes (DEGs) were defined as Benjamini-corrected p <0.05 and a fold-change greater than or equal to two. (A) Significantly upregulated DEGs in the five TF mutant strains in the presence of H2O2. (B) Significantly downregulated DEGs in the five TF mutant strains in the presence of H2O2.

A. heat map of upregulated genes



B. heat map of downregulated genes

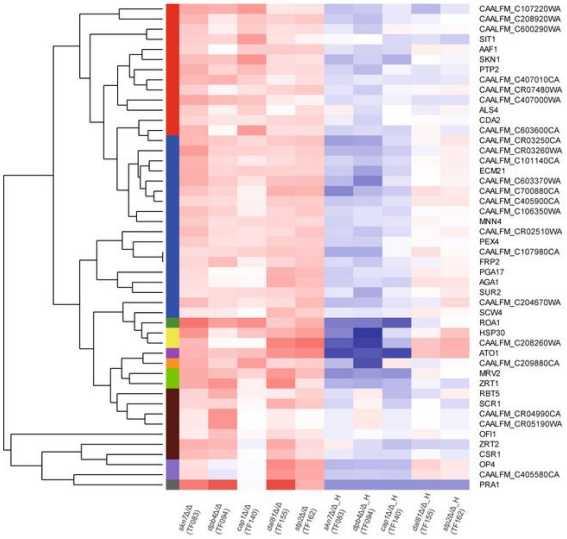


Figure 3

Heat map of the differentially expressed genes in common among the five C. albicans hypersensitive TF mutants in the presence of H2O2. Legend: RNA-seq was performed on the five TF mutant strains in the presence and absence of H2O2. Differentially expressed genes (DEGs) were defined as Benjamini-corrected p <0.05 and a fold-change greater than or equal to two. (A) Heat map of the significantly upregulated DEGs in common among the five TF mutant strains in the presence of H2O2. (B) Heat map of the significantly downregulated DEGs in common among the five TF mutant strains in the presence of H2O2. The darker the color gradient is, the greater the expression change is. Dendrogram of gene clustering is shown on the left. H is an abbreviation for H2O2.

Figure 4

Bubble chart of KEGG pathways enriched in the five C. albicans hypersensitive TF mutant strains in the presence of H2O2. Legend: RNA-seq was performed on the five TF mutant strains in the presence and absence of H2O2. KEGG pathways were analyzed using KOBAS. X-axis represents enrichment ratio, and Y-axis represents KEGG pathways. The size of each dot indicates the number of genes included. The bigger the dot is, the more genes are involved in the pathway. H is an abbreviation for H2O2.

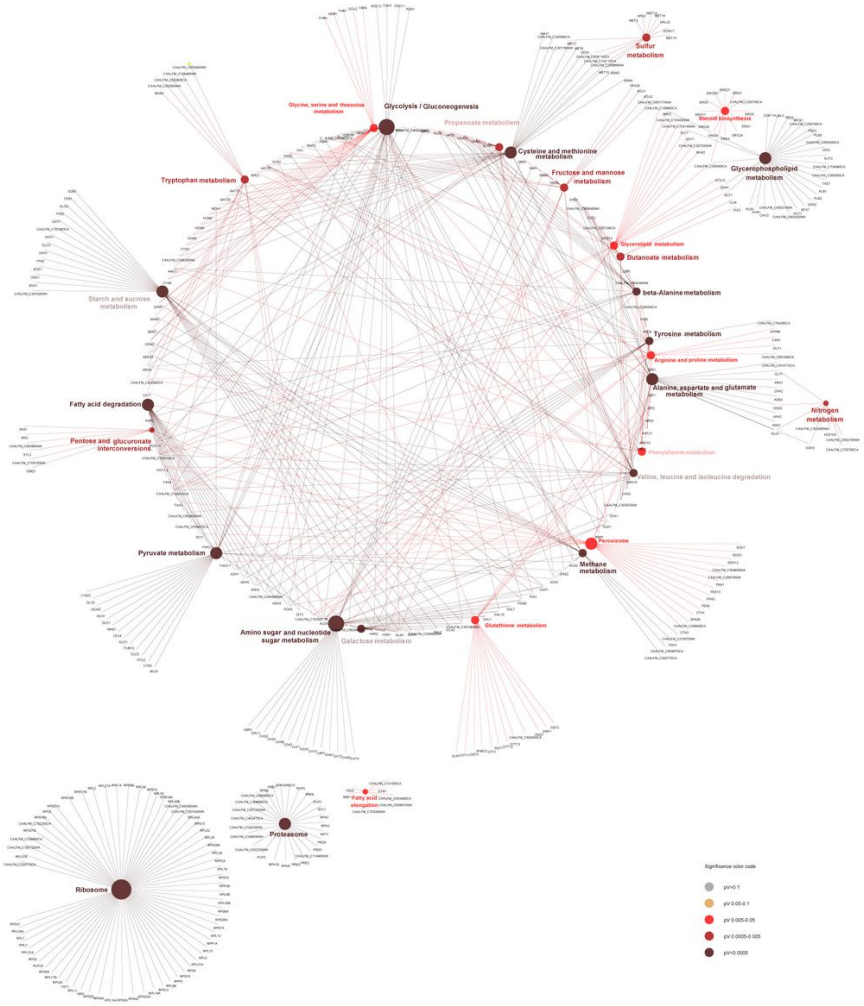


Figure 5

Correlation between enriched KEGG pathways and relevant genes in the five C. albicans hypersensitive TF mutant strains in the presence of H2O2. Legend: RNA-seq was performed on the five TF mutant strains in the presence and absence of H2O2. KEGG pathways were analyzed using KOBAS. The size of each dot indicates the number of genes included. The bigger the dot is, the more genes are involved in the pathway. The darker the color gradient is, the more enriched the pathway is.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
- DatasetS1.xlsx
- Fig.S1.pdf