A Conserved Transcriptional Regulator Governs Fungal Morphology in Widely Diverged Species

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ABSTRACT Fungi exhibit a large variety of morphological forms. Here, we examine the functions of a deeply conserved regulator of morphology in three fungal species: *Saccharomyces cerevisiae*, *Candida albicans*, and *Histoplasma capsulatum*. We show that, despite an estimated 600 million years since those species diverged from a common ancestor, Wor1 in *C. albicans*, Ryp1 in *H. capsulatum*, and Mit1 in *S. cerevisiae* are transcriptional regulators that recognize the same DNA sequence. Previous work established that Wor1 regulates white–opaque switching in *C. albicans* and that its ortholog Ryp1 regulates the yeast to mycelial transition in *H. capsulatum*. Here we show that the ortholog Mit1 in *S. cerevisiae* is also a master regulator of a morphological transition, in this case pseudohyphal growth. Full-genome chromatin immunoprecipitation experiments show that Mit1 binds to the control regions of the previously known regulators of pseudohyphal growth as well as those of many additional genes. Through a comparison of binding sites for Mit1 in *S. cerevisiae*, Wor1 in *C. albicans*, and Wor1 ectopically expressed in *S. cerevisiae*, we conclude that the genes controlled by the orthologous regulators overlap only slightly between these two species despite the fact that the DNA binding specificity of the regulators has remained largely unchanged. We suggest that the ancestral Wor1/Mit1/Ryp1 protein controlled aspects of cell morphology and that movement of genes in and out of the Wor1/Mit1/Ryp1 regulon is responsible, in part, for the differences of morphological forms among these species.

THE fungal kingdom collectively represents enormous evolutionary diversity, with estimates of >1 million species. Fungi exhibit a large variety of morphological forms ranging from single, budding yeast cells to hyphae (long chains of highly elongated cells) to mushrooms. Morphological differences among species can be seen at the single-cell level, but also encompass differences in the appearance of communities of cells such as colonies on solid medium. In this article, we examine aspects of morphological diversity over a limited range of fungal species including *Saccharomyces cerevisiae* (baker's and brewer's yeast), *Candida albicans* (a normal resident of the human gut but also the most prevalent fungal pathogen), and *Histoplasma capsulatum* (the cause of histoplasmosis in humans). These three species represent an estimated 200 million to 1.2 billion years of evolutionary divergence from a common ancestor (herein simplified to 600 million years) (Taylor and Berbee 2006). In all three species, a conserved, orthologous transcriptional regulator (Mit1/Wor1/Ryp1) governs an aspect of morphology, but these morphologies differ between species.

The *C. albicans* Wor1 protein is a master regulator of a phenomenon known as white–opaque switching. White–opaque switching refers to the transition between two distinctive cell types, termed "white" and "opaque", each of which is heritable for many generations (Lohse and Johnson 2009; Soll 2009). Switching between these cell types occurs stochastically approximately once every 10^4 cell generations under standard laboratory conditions, but environmental signals can influence the direction of the switch (Rikkerink *et al.* 1988). The two cell types are easily distinguishable: opaque cells are more elongated than white cells, have larger vacuoles, and have different cell wall structures when visualized by electron microscopy (Anderson and Soll 1987;

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Slutsky *et al.* 1987). Moreover, the appearance of white and opaque colonies is different on a wide variety of media: white cells form rounded, glossy, white-colored colonies, whereas opaque cells form flatter, dull, tan-colored colonies. The two cell types also differ in their mating competence (Miller and Johnson 2002), in their preferred environmental niche in the host (Kvaal *et al.* 1997, 1999; Lachke *et al.* 2003), and in their recognition by cells of the innate immune system (Geiger *et al.* 2004; Lohse and Johnson 2008). Most, if not all, of these differences arise through the differential expression of ~20% of the genome between the two cell types (Lan *et al.* 2002; Tsong *et al.* 2003; Tuch *et al.* 2010). Wor1 is a transcriptional regulator that contains a novel DNA-binding domain termed WOPR (Lohse *et al.* 2010); full genome chromatin immunoprecipitation (ChIP) experiments revealed that Wor1 binds to ~170 intragenic regions (Zordan *et al.* 2007), including many that lie upstream of opaque-specific genes. *C. albicans* ΔΔ*wor1* cells are locked in the white form, whereas ectopically expressed *WOR1* drives white cells into the opaque form (Huang *et al.* 2006; Srikantha *et al.* 2006; Zordan *et al.* 2006).

H. capsulatum, another human fungal pathogen, diverged from a common ancestor with *C. albicans* ~600 million years ago (Taylor and Berbee 2006). In the soil, *H. capsulatum* exists primarily as mycelia—mats composed of tangled hyphae. When mycelial cells break off and are inhaled by a human, they rapidly convert to the disease-causing budding yeast form (Woods 2002; Holbrook and Rappleye 2008). This transition, triggered by a shift in temperature to 37°, involves differential expression of ~20% of the genome (Nguyen and Sil 2008). *RYP1*, the ortholog of *WOR1* in *H. capsulatum*, has been identified as a master regulator of this transition as Δ*ryp1* strains are locked in the mycelial form (Nguyen and Sil 2008).

In this article, we show that Ryp1 and Wor1 are both transcriptional activators and that both act through the same DNA sequence motif despite the large evolutionary distance between the proteins. *C. albicans* and *H. capsulatum*, however, are too distantly related to meaningfully compare the target genes of these regulators; in particular, the orthology relationships between many genes in the two species are currently ambiguous. To address the question of transcriptional circuit diversification, we turned to a comparison between *C. albicans* and a much more closely related species, *S. cerevisiae*, with an estimated divergence time of between 100 and 600 million years (herein stated as 200 million years) (Taylor and Berbee 2006).

S. cerevisiae has two orthologs of *WOR1*, *MIT1* (*YEL007W*) and *YHR177W*, as a result of a whole genome duplication that occurred before the *Saccharomyces* clade diverged. Two previous results suggested these genes were involved in the *S. cerevisiae* pseudohyphal growth program. First, overexpression of the *C. albicans* homolog *WOR1* induced haploid invasive growth in the *S. cerevisiae* S288c laboratory strain, which is normally incapable of this behavior (Li and Palecek 2005; Huang *et al.* 2006). Second, inactivation of *MIT1* in the "wild" strain Σ1278b compromised haploid invasive growth (E. Summers and G. R. Fink, unpublished communication). When the Σ1278b strain is starved for nutrients, its cells become elongated and remain attached to one another, forming chains of cells (filaments) known as pseudohyphae. These pseudohyphae grow outward and penetrate into the agar (Gimeno *et al.* 1992; Gagiano *et al.* 2002); this behavior can be monitored by plating Σ1278b on nutrient-limited agar, washing away the colonies, and visualizing the proliferation of cells trapped in the agar. Commonly described as a foraging response, this type of growth has been proposed to allow *S. cerevisiae* to explore new environments. Many common laboratory strains of *S. cerevisiae* do not exhibit this behavior due to mutations that accumulated during strain domestication (Liu *et al.* 1996). The inability of common laboratory strains to form pseudohyphae likely explains the sparsity of data for *MIT1* or *YHR177W* in the many high-throughput data sets generated for *S. cerevisiae*.

Filamentous growth in *S. cerevisiae* Σ1278b requires a number of previously identified transcriptional regulators, including Mga1, Phd1, Sok2, Ste12, Tec1, and Flo8, and genome-wide ChIP analyses have shown that these six regulators regulate one another and a network of hundreds of downstream targets (Borneman *et al.* 2006, 2007a,b; Monteiro *et al.* 2008). Here we show that Mit1 is a central transcriptional regulator of the *S. cerevisiae* filamentous growth program. Mit1 is required for filamentous growth and its ectopic expression can drive filamentous growth under environmental conditions that normally disfavor it. Genome-wide ChIP experiments show that Mit1 directly binds to, and is bound by, most of the previously known transcriptional regulators of filamentous growth and can therefore be considered a core regulator (Borneman *et al.* 2006). Thus Wor1, Ryp1, and Mit1 are all central regulators of morphological transitions that are different from one species to the next.

Materials and Methods

Growth conditions

Standard laboratory media were used (Guthrie and Fink 1991). Cultures for all microarray and ChIP experiments were grown in YEP media supplemented with 2% glucose, except for the ChIP of Wor1 that was performed under SD −URA conditions to select for Wor1 expression. β-Galactosidase assays were performed in synthetic complete dextrose (SCD) media lacking URA, HIS, TRP, URA and TRP, or URA and HIS to maintain selection for plasmids. Synthetic low ammonium dextrose (SLAD) nitrogen starvation media for diploid invasive growth assays were prepared as previously described, washing agar three times to remove traces of nitrogen (Gimeno *et al.* 1992).

Strain construction

A list of yeast strains used in this study can be found in Supporting Information, Table S13. Strains were constructed in the *S. cerevisiae* Σ2000 background (a Σ1278b

derivative), a gift from Hiten Madhani. Selectable markers for deleting genes were amplified with 50 bp of homology on each side of the gene to be replaced and transformed using standard methods (Longtine *et al.* 1998). Mit1-GFP was generated using a fusion of a S65T GFP cassette in frame at the C terminus of the protein, and nuclear localization was visualized as previously described (Huh *et al.* 2003).

Plasmids

A list of plasmids used in this study can be found in Table S14. A list of oligos can be found in Table S15. Plasmids for LacZ expression for β-galactosidase assays were derivatives of pLG669z. The previously reported plasmids P_{FLO11} 2/1 to P_{FLO11} 15/14 (Rupp *et al.* 1999) were used to analyze activity from 440-bp fragments of the *FLO11* promoter. To further localize the region of Mit1 activity, reporter plasmids with 50-bp fragments, arranged to provide 25-bp overlap, were synthesized with *Xho*I compatible ends, annealed, and ligated into pLG669z digested with *Xho*I.

Versions of a selectable CEN-linked P_{TEF}-containing plasmid were used for ectopic expression of proteins (Mumberg *et al.* 1995). *RYP1* was overexpressed by amplifying its coding region from *H. capsulatum* G217B and ligating into a CEN-linked P_{TEF} vector. The *WOR1* overexpression vector was previously reported (Lohse *et al.* 2010). To create the *MIT1* overexpression construct, the N-terminal portion of *MIT1* extending to a *Hin*dIII site was PCR amplified and cloned into a CEN-linked P_{TEF} vector. To complete the open reading frame, plasmid YGPM-25i24, containing a genomic fragment encompassing the entire *MIT1* ORF, was obtained as a gift from the laboratory of Greg Prelich (Jones *et al.* 2008), digested with *Hin*dIII and *Sna*BII, and cloned in frame into the P_{TEF}-5′*MIT1* plasmid. This plasmid was transformed into XL1-Gold cells (Stratagene, La Jolla, CA).

To create 6-His–tagged Yhr177w and Mit1 protein expression plasmids, regions corresponding to amino acids 6–201 (Yhr177w) or 5–251 (Mit1) were amplified from genomic DNA and ligated into pLIC-H3 between *Xma*I and *Xho*I sites. pLIC-H3 is a pET28b derivative and was a gift from the laboratory of Jeff Cox at University of California, San Francisco.

Protein expression and purification

MBP-Wor1 1–321 expression and purification were performed as previously described (Lohse *et al.* 2010). 6-His–tagged Yhr177w 6–201 aa and Mit1 5–251 aa were expressed in LB media. Cells were induced at an OD of 0.5 with 0.4 mM IPTG and grown for 4 hr at 25°.

For purification, cells were resuspended in lysis buffer (50 mM NaH_2PO_4, 600 mM NaCl, 10 mM imidazole, pH 8.0) supplemented with 5 mM β-mercaptoethanol, 1 mg/ml lysozyme, and one Complete Mini Protease inhibitor cocktail tablet, EDTA-free (Roche, Basel, Switzerland) per 10-ml vol. Resuspended cells were incubated at 4° for 20 min, sonicated, and incubated with 50 units/ml DNaseI for 30 min at 4°. The lysate was centrifuged and then run over Ni-NTA agarose columns (QIAGEN, Valencia, CA), which were washed with 20 column volumes of wash media (50 mM NaH_2PO_4, 600 mM NaCl, 20 mM imidazole, pH 8.0) followed by 5 column volumes of elution buffer (50 mM NaH_2PO_4, 600 mM NaCl, 250 mM imidazole, pH 8.0). Elution fractions were stored in 10 mM Tris, pH 7.4, 100 mM NaCl, 5 mM DTT, and 50% glycerol.

Electromobility gel shift assays

Electromobility gel shift Assays were performed as previously described (Lohse *et al.* 2010), using the 50-bp 1175–1225 fragment from *S. cerevisiae* P_{FLO11} or the mutated version of this fragment.

Invasion assays

Haploid invasion assays were performed as previously described (Lo and Dranginis 1998). Briefly, haploid invasion was assayed by plating cells on YEPD media for 2 days, washing them gently under a steady stream of running water for 20 sec, and photographing the remaining cells. Diploid pseudohyphal growth was tested by plating cells at a low density on SLAD agar, allowing individual colonies to form over 7 days, and then photographing colonies under a dissection scope. Ectopic induction of the pseudohyphal growth program was analyzed by patching diploid cells carrying *MIT1*, *WOR1*, or *RYP1* overexpression plasmids on selectable SD media for 2 days and then washing them under a steady stream of running water. Pseudohyphal microcolonies were observed by plating cells at low density under a coverslip on a slide topped with selectable SD agar media. Microcolonies were allowed to form overnight and then were photographed at 63× magnification on an inverted Zeiss Axiovert 200 M microscope (Carl Zeiss, Oberkochen, Germany), using Axiovision software.

β-galactosidase assays

β-galactosidase assays were performed in triplicate using a standard protocol (Rupp 2002). Strains were grown in selectable media to maintain selection for plasmids. For each strain, cultures were grown overnight, diluted back, and allowed to reach log phase before harvesting.

Expression microarrays

A full discussion of this topic is presented in File S3. Microarray data have been uploaded to NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) under accession nos. GSE32558 (transcription arrays and ChIP-chip data) and GSE32550 (transcription arrays only).

Chromatin immunoprecipitation

Chromatin immunoprecipitation experiments were performed as previously described (Nobile *et al.* 2009). Briefly, 200 ml of log-phase culture was cross-linked with 1% formaldehyde, quenched with 125 mM glycine, and harvested. Polyclonal anti-GFP antibody (ab290) was purchased from Abcam (Cambridge, MA) and was used for Mit1-GFP experiments. Additional antibodies were generated against N-terminal and C-terminal peptide sequences from Mit1 and

Yhr177w, by Bethyl (Montgomery, TX). C-terminal antibodies were used for all experiments with Yhr177w. Antibodies against Wor1 have been previously reported (Zordan *et al.* 2006, 2007). Yeast Chip on Chip arrays containing ~244k probes with an average resolution of ~50 nt were ordered from Agilent Technologies (Santa Clara, CA). These arrays were designed against the *S. cerevisiae* S288c reference strain, as the Σ genome sequence had not yet been completed; however, the minor sequence variation between these genomes would not impact our experiments. Mit1 and Yhr177w experiments and controls were each performed in duplicate; the Wor1 experiment and control were each performed once. Experimental analysis was performed using Agilent Chip Analytics Version 1.2 software and MochiView Version 1.45 (Homann and Johnson 2010). This process is explained in detail in File S3.

ChIP-chip data have been uploaded to NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/) under accession nos. GSE32558 (ChIP-chip data and transcription arrays) and GSE32557 (ChIP-chip data only).

Binding site comparisons

Binding data for Sok2, Tec1, and Ste12 were taken from previously published studies (Borneman *et al.* 2007b). All data sets for binding site comparisons were processed in the following manner, using MochiView Version 1.45. This process is explained in detail in File S3.

Motif analysis

DNA motif analysis of Mit1 binding sites was performed using MochiView v1.45. Comparisons between the Mit1 and Wor1 motifs were performed in MochiView v1.45, which uses the approach presented in Gupta *et al.* (2007). A full discussion of this topic is presented in File S3.

Orthology mapping

The *C. albicans* Wor1 binding site list was previously reported (Zordan *et al.* 2007). Comparisons between the Mit1/Wor1 target lists were made using gene annotations from the *Saccharomyces* Genome Database (SGD) (http://www.yeastgenome.org/) and *Candida* Genome Database (CGD) (http://www.candidagenome.org/). Orthology annotation was obtained from the Fungal Orthogroups Repository (http://www.broadinstitute.org/regev/orthogroups/), which was generated by the SYNERGY algorithm (Wapinski *et al.* 2007). All files were downloaded on September 30, 2009. On the basis of additional information (Schweizer *et al.* 2000; Kadosh and Johnson 2001), changes were made to the mapping for three sets of genes prior to further analysis. These changes are discussed in detail in File S3; they do not substantially change the results of the bootstrap statistical analysis described below.

Bootstrap analysis of target overlap

We applied bootstrap statistics to test the hypothesis that the shared orthology between the *S. cerevisiae* and *C. albicans* gene lists exceeded the overlap that might be expected by chance. We considered only the 66 *S. cerevisiae* and 118 *C. albicans* targets with orthology mapping between the two species (Table S11 and Table S12).

After applying the hand annotations described above to the orthology annotations, 13 of the 66 *S. cerevisiae* orthology-mapped Mit1 targets share orthology with one or more genes in the Wor1 target list. Sets of 66 *S. cerevisiae* genes were sampled from the group of all *S. cerevisiae* genes with ortholog mappings (4524 genes) and tested to determine whether the number of genes in the set with shared orthology to genes in the Wor1 target list equaled or exceeded the observed 13-gene Mit1-to-Wor1 mapping. Only one sampled set met this standard, confirming a significant z (n = 1,000,000; $P = 1 \times 10^{-6}$). A reciprocal test yielded similar results, comparing the benchmark of 10 of 118 Wor1 targets that share orthology with the Mit1 gene list against sets of 118 genes sampled from the 4393 *C. albicans* genes with ortholog mappings (n = 1,000,000; $P = 3.7 \times 10^{-5}$). These statistical analyses also hold true without the hand-annotated changes to the orthology mapping (File S3).

Results

MIT1 is required for haploid invasive growth and diploid pseudohyphal growth

We deleted *MIT1* in the Σ2000 background (a derivative of Σ1278b), which resulted in both a recapitulation of the unpublished invasion defect in haploid cells and a pseudohyphal growth defect in diploid cells (Figure 1, A and B). Deletion of *YHR177W*, the second *S. cerevisiae* ortholog of *WOR1*, had no effect on either phenotype. Previous studies have shown that expression of the cell wall floculin *FLO11* (*MUC1*) is critical for filamentous growth (Lo and Dranginis 1998), and whole-genome expression experiments showed that *FLO11* expression was at least 40-fold lower in a haploid Δ*mit1* strain relative to the unmodified parent strain (Table S1, File S1). When the *FLO11* coding sequence was replaced with GFP, the Δ*mit1* strain exhibited a dramatic reduction in GFP production compared to the parent strain, providing further evidence that *MIT1* is required for *FLO11* expression (Figure 1C). The decrease in *FLO11* expression likely accounts, at least in part, for the filament-defective phenotype of the Δ*mit1* strain.

We next determined whether ectopic expression of *MIT1* was sufficient to induce pseudohyphal growth under conditions where it does not normally take place. Indeed, ectopic expression of *MIT1* from the *TEF* promoter drives diploid cells to invade agar in rich media conditions (Figure 1D). Thus, *MIT1* fulfills the requirements of a "master regulator" of filamentous growth in that deletion of *MIT1* prevents filamentous growth and ectopic expression of *MIT1* drives it.

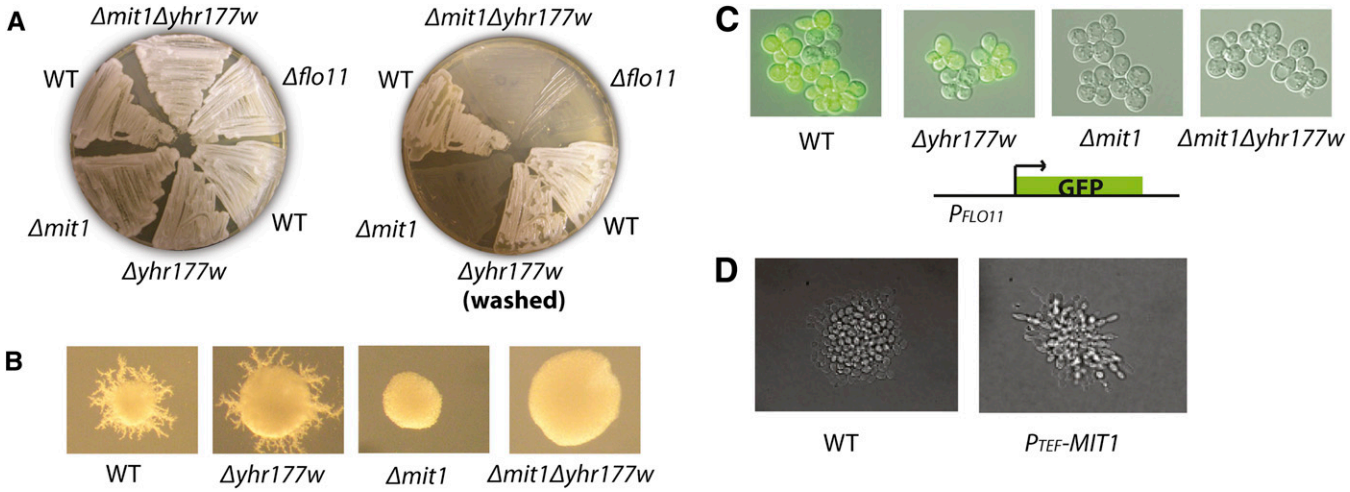

Figure 1 *MIT1* is required for haploid invasive growth, diploid pseudohyphal growth, and expression of *FLO11* in *S. cerevisiae*. (A) Haploid invasive growth assay. Haploid **a** cells of the indicated genotypes (Σ2000 background) were plated on YPD agar plates, grown for 2 days at 30°, and washed. Pre- (left) and post- (right) wash images are shown. (B) Diploid pseudohyphal growth assay. Individual **a**/α diploid cells (Σ2000 background) were plated on SLAD agar plates, grown for 7 days, and then photographed. A representative colony from each plate is shown. Δ*mit1* and Δ*mit1*Δ*yhr177w* colonies were never observed to display pseudohyphal growth in this assay. (C) Expression of *FLO11* is dependent on *MIT1*. The *FLO11* coding sequence was replaced with GFP (S65T). Haploid **a** cells were grown in YPD to log phase, placed under a coverslip, and then photographed at 40× magnification. (D) *MIT1* expressed from the *TEF* promoter drives diploid pseudohyphal growth in SD media conditions. Individual diploid Σ2000 cells were plated on an SD agar pad on a glass microscopy slide and allowed to grow under a coverslip at 30° for 16 hr, and then individual microcolonies were visualized at 40× magnification. Wild-type cells were not observed to display pseudohyphal growth while cells containing a plasmid expressing *MIT1* from the *TEF* promoter displayed elongated morphology and agar penetration consistent with the pseudohyphal growth phenotype.

Mit1 is a core member of the S. cerevisiae filamentous growth circuit

To better understand the role of Mit1 in regulating filamentous growth, we utilized chromatin immunoprecipitation combined with whole genome tiling microarrays (ChIP-Chip) to identify its direct downstream targets. A GFP tag was introduced to the C-terminal portion of Mit1 at its endogenous locus; GFP-tagged Mit1 was fully competent to promote invasive growth (not shown). Cross-linking, followed by DNA shearing and immunoprecipitation with antibodies directed against GFP, identified 74 discrete binding locations across the genome, corresponding to 68 intergenic regions positioned upstream of 94 genes (Figure 2A, Table S2, Table S3, Table S4, File S1, and File S2). These 74 locations were rigorously identified from two highly reproducible high-density tiling microarray experiments, minimizing false positives (see *Materials and Methods*). Four of the 5 known flocculin genes (*FLO1*, *FLO9*, *FLO10*, and *FLO11* but not *FLO5*) are bound by Mit1, with the strongest enrichment detected upstream of *FLO11*. Transcriptional regulators were strongly enriched in the Mit1-bound gene set; Mit1 binding was found upstream of 21 genes encoding sequence-specific DNA-binding proteins, including *MIT1* itself and most of the regulators previously implicated in filamentous growth (see below). We examined Mit1-bound locations to determine the DNA sequence specifically recognized by Mit1; we identified a motif that is present in ~70% of Mit1-bound sites, using a stringency level that produces false positive hits in 20% of a control set of promoter regions (Figure S1, Table S5, File S1, and File S3). This DNA motif is nearly identical to the previously reported Wor1 motif (Lohse *et al.* 2010) (Figure S1).

We also examined binding by the Mit1 paralog, Yhr177w, using two peptide-derived antibodies, but we did not observe any significant enrichment in a wild-type Σ2000 strain. However, *YHR177W* expression is upregulated approximately eightfold when *MIT1* is deleted (Table S1), and in this strain we identified 24 intergenic regions bound by Yhr177w, positioned upstream of 34 genes (Table S6, Table S7, File S1, and File S4). Most of these regions (16/24) were also bound by Mit1 in the experiment described above, indicating that Yhr177w and Mit1 regulate many of the same genes and probably share DNA-binding specificities. It is likely that Yhr177w regulates pseudohyphal growth in some environments, but it did not play an obvious role under the conditions examined here; for example, the Δ*mit1* strain exhibited the same filament-deficient phenotype as the Δ*mit1*Δ*yhr177w* strain (Figure 1A) and ectopic expression of *YHR177W* had no visible phenotype in either wild-type or Δ*mit1* backgrounds.

We next examined whether Mit1 was an integral part of the transcriptional network regulating filamentous growth in *S. cerevisiae*. Previous ChIP-chip analysis of the regulators Phd1, Sok2, Mga1, Flo8, Tec1, and Ste12 identified a highly interactive regulatory network where each factor regulates, and is regulated by, most of the other transcriptional regulators (Figure 2B) (Borneman *et al.* 2006). All six of these proteins were previously reported to bind the upstream region of *MIT1* (Monteiro *et al.* 2008) and the immunoprecipitation experiments presented in this article revealed that

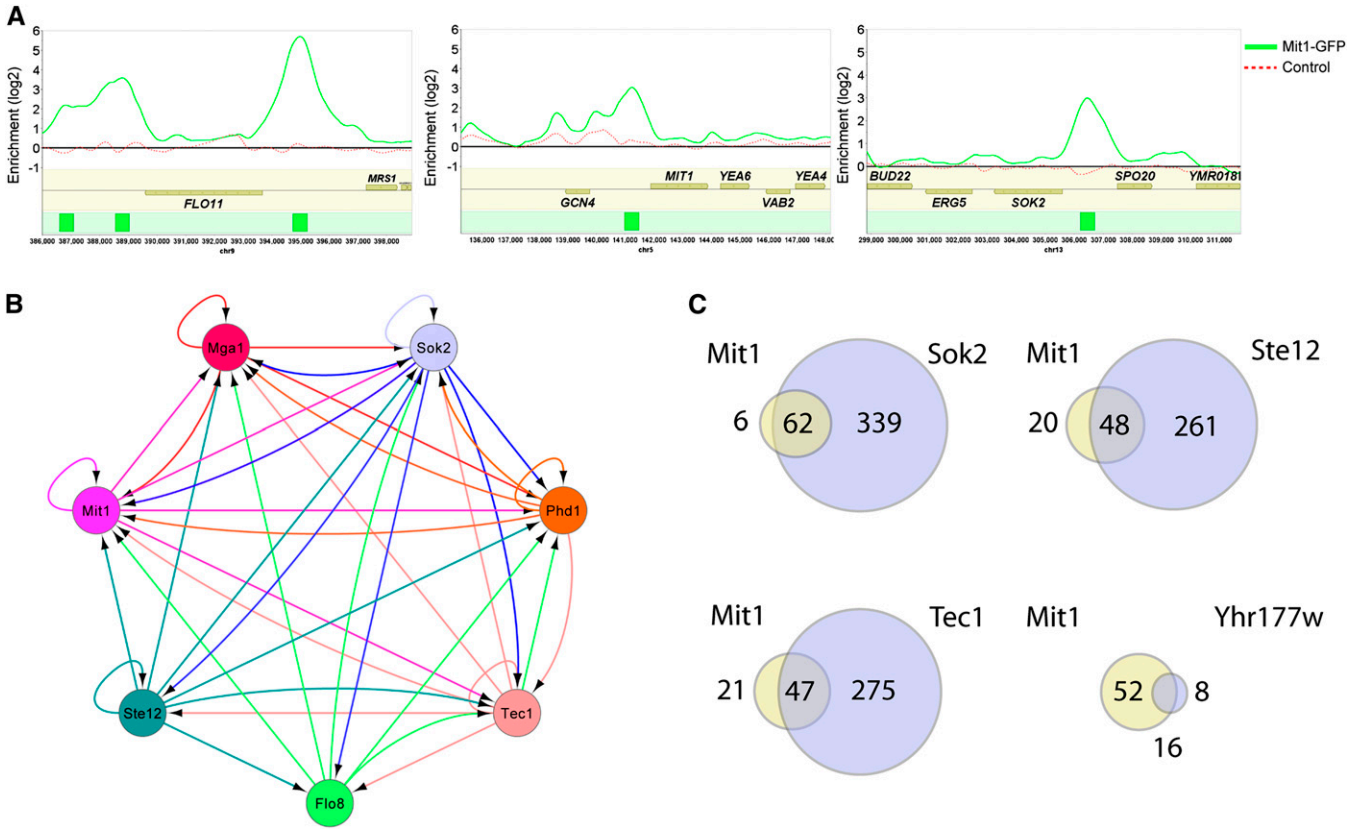

Figure 2 *MIT1* is a central transcriptional regulator of filamentous growth. (A) ChIP-chip plots illustrate binding of Mit1-GFP in Σ2000 haploid **a** cells to promoters of *FLO11*, *MIT1*, and *SOK2*. Experimental enrichment data are presented as a solid green line in each panel and control data from a wild-type untagged strain are presented as a dashed red line. The green boxes in the bottom track represent the called Mit1 binding sites. Data were visualized using Mochiview 1.45, with the *x*-axis representing the genomic location and the *y*-axis representing $\log_2$ enrichment. (B) The network of interactions between Mit1 and previously known diploid pseudohyphal growth regulators. Arrows indicate binding of a regulator at the promoter of a given regulator. Connections represent those previously reported for the other six regulators (Borneman *et al.* 2006, 2007b) and those from the Mit1 ChIP-chip data reported here. (C) A genome-wide binding analysis comparison of Mit1 and Yhr177w bound intergenic regions with those previously reported for Sok2, Ste12, and Tec1 (Borneman *et al.* 2007b) reveals a high degree of target gene overlap.

Mit1 binds to its own upstream region, as well as those of *PHD1*, *SOK2*, *MGA1*, and *TEC1* (Figure 2B). Thus, Mit1 appears to be as integral a part of the pseudohyphal growth circuit as any of the previously known regulators.

To determine the extent to which Mit1 shares targets with the previously identified regulators of filamentous growth, we compared the Mit1 ChIP data to the existing high-resolution data for Ste12, Tec1, and Sok2 (Borneman *et al.* 2007b). We found that 91% (62/68, $P = 2.59E\text{-}62$) of the Mit1 bound intergenic regions were also bound by Sok2, 69% (47/68, $P = 3.65E\text{-}44$) were bound by Tec1, and 71% (48/68, $P = 1.00E\text{-}46$) by Ste12 (Figure 2C, Table S8, and File S1). This strong overlap among downstream targets further supports the conclusion that Mit1 is a central regulator of the filamentous growth program.

Mit1 acts on a specific motif within the FLO11 promoter

To test whether Mit1 association with DNA, as determined by ChIP, was functionally relevant, we analyzed Mit1's binding to the promoter of the *FLO11* gene in detail. To identify the minimal DNA sequence required for Mit1 to activate expression of *FLO11*, we utilized a set of constructs containing overlapping 440-bp fragments of the *FLO11* promoter cloned into a P_{CYC1}-LacZ plasmid construct lacking its native upstream activating sequence (Figure 3A). These plasmids were previously used to identify regions of the *FLO11* upstream region required for activation by Ste12, Tec1, and Flo8 (Rupp *et al.* 1999). We transformed this plasmid set into both wild-type and Δ*mit1* haploid Σ2000 *S. cerevisiae* strains and identified two overlapping 440-bp fragments that possessed Mit1-dependent transcriptional activity (Figure 3B). The 240-bp overlap between these fragments is distinct from the sites of action of Ste12 and Tec1, includes the site of action of Flo8, and corresponds to the most highly enriched site for Mit1 binding in the ChIP experiment.

To further refine a DNA element required for activation by Mit1, we generated a series of plasmids containing overlapping 50-bp fragments of this 240-bp region. Two fragments showed Mit1-dependent transcriptional activation, corresponding to 1025–1075 bp and 1175–1225 bp upstream of the start codon (Figure 3C). Both fragments contained a high-scoring DNA motif recognized by the *C.*

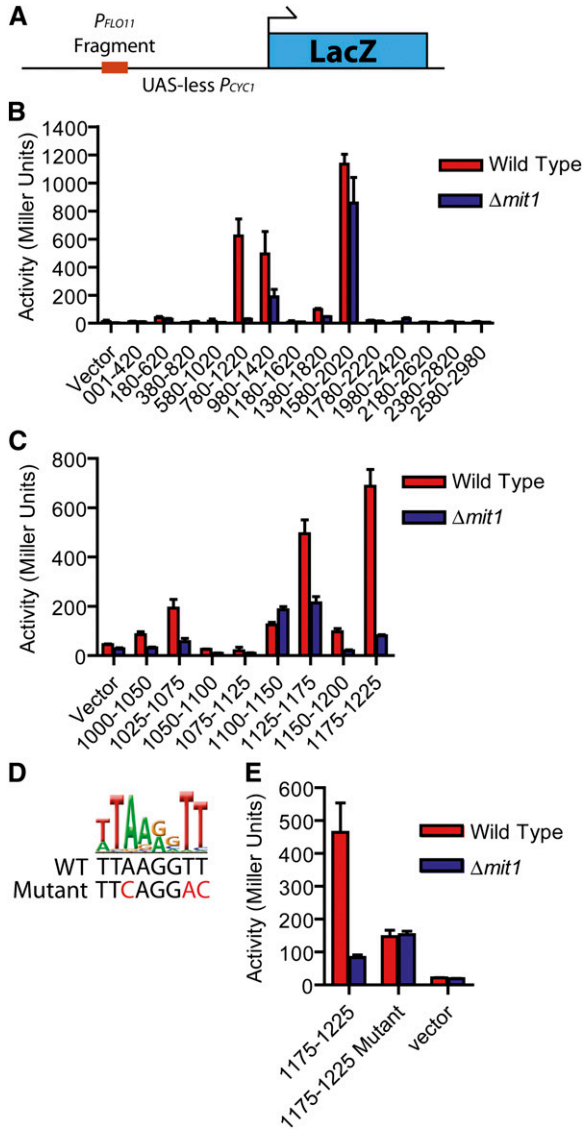

Figure 3 Mit1 acts on a discrete location within the *FLO11* promoter corresponding to the Mit1/Wor1 DNA-binding motif. (A) Schematic of the *UAS*-less P_{CYC1} plasmid with a *FLO11* promoter fragment used for the β-galactosidase activation assays. (B) Fragments (440 bp) of the *FLO11* promoter were inserted into a *UAS*-less *CYC1* promoter upstream of a LacZ reporter construct, as previously reported (Rupp *et al.* 1999). These plasmids were transformed into either wild-type haploid **a** cells (red) or Δ*mit1* haploid **a** cells (blue), and standard β-galactosidase assays were performed in selective SD media. β-Galactosidase units of activity (Miller units) are plotted on the *y*-axis, and fragments of the *FLO11* promoter are indicated on the *x*-axis (numbers represent the position of individual fragments relative to the *FLO11* start codon). (C) Fragments (50 bp) of the *FLO11* promoter were inserted into the LacZ reporter plasmid and β-galactosidase activity was measured for strains grown under the same conditions as in B. Fragment 1175–1225 displayed the strongest activity, and this activity was Mit1 dependent. Axis labeling is as in B. (D and E) A sequence resembling the Mit1/Wor1 recognition motif (displayed as a logo) was identified in the 1175–1225 *FLO11* promoter fragment. Three bases of this motif were mutated as shown (red), and the corresponding plasmid ("1175–1225 Mutant") exhibited reduced Mit1-dependent β-galactosidase activity when transformed into yeast. Axis labeling is as in B. Error bars in B, C, and E represent the standard deviation from two or three experiments.

albicans ortholog Wor1 (Lohse *et al.* 2010). To test whether this motif was needed for Mit1-dependent activation, we changed three critical nucleotides in the central core of this motif in the 1175–1225 fragment (Figure 3D). When assayed in the reporter system described above, these mutations reduced Mit1-dependent transcriptional activity (Figure 3E), revealing that the motif is recognized by Mit1. Moreover, these results indicate that Mit1 functions as an activator of transcription, at least in this minimal system.

Mit1, Wor1, and Ryp1 recognize the same DNA sequence

To further test the idea that Mit1 and Wor1 can recognize the same DNA sequence, we ectopically expressed *C. albicans WOR1* in a Δ*mit1*Δ*yhr177w S. cerevisiae* strain. Wor1 activated transcription from the 1175–1225 sequence of the *FLO11* promoter to high levels (Figure 4A), and point mutations in the binding site destroyed Wor1-dependent activation. Similar results were obtained for *H. capsulatum* Ryp1 (Figure 4B). Furthermore, ectopic expression of either *WOR1* or *RYP1* induced haploid invasive growth in the absence of *MIT1* and *YHR177W* (Figure S2).

Finally, we directly showed that Wor1, Mit1, and Yhr177w bind to the *S. cerevisiae* Mit1 site by performing electrophoretic mobility shift experiments with *Escherichia coli* purified MBP-Wor1 (1–321 aa), 6× His-Mit1 (5–251 aa), and 6× His-Yhr177w (6–201 aa). All three proteins bind the 1175- to 1225-bp fragment of the *FLO11* promoter, and this binding is lost when the Mit1 motif is mutated (Figure 4C).

The DNA-binding experiments described above predict that Wor1, if ectopically expressed in *S. cerevisiae*, should occupy at least some of the genomic sites normally occupied by Mit1. To test this idea, we expressed *WOR1* (under control of the *TEF* promoter) in the Δ*mit1*Δ*yhr177w S. cerevisiae* strain and, through full genome chromatin immunoprecipitation, found that it associates not only with the *FLO11* promoter, but also with at least six additional Mit1 target upstream regions (Figure 4D, Table S9, Table S10, File S1, and File S5). The levels of enrichment were lower for the Wor1 experiment than for the Mit1 experiment, so we cannot state with precision the actual overlap of target genes; however, it appears very high.

Thus, the DNA-binding specificity of the WOPR domain in Mit1, Ryp1, and Wor1 has been preserved over at least 600 million years. Such a result is not unprecedented; for example, the DNA-binding specificity of a MADS-box protein (Mcm1) has remained constant over this same evolutionary distance (Tuch *et al.* 2008). On the other hand, there are examples where DNA-binding specificity of a conserved regulator has changed significantly over much shorter evolutionary time periods (Baker *et al.* 2011).

Comparison of Mit1 and Wor1 target genes

Given that Mit1, Wor1, and Ryp1 recognize the same DNA sequence, it seemed unlikely that the different cellular phenotypes governed by these orthologs could be explained by

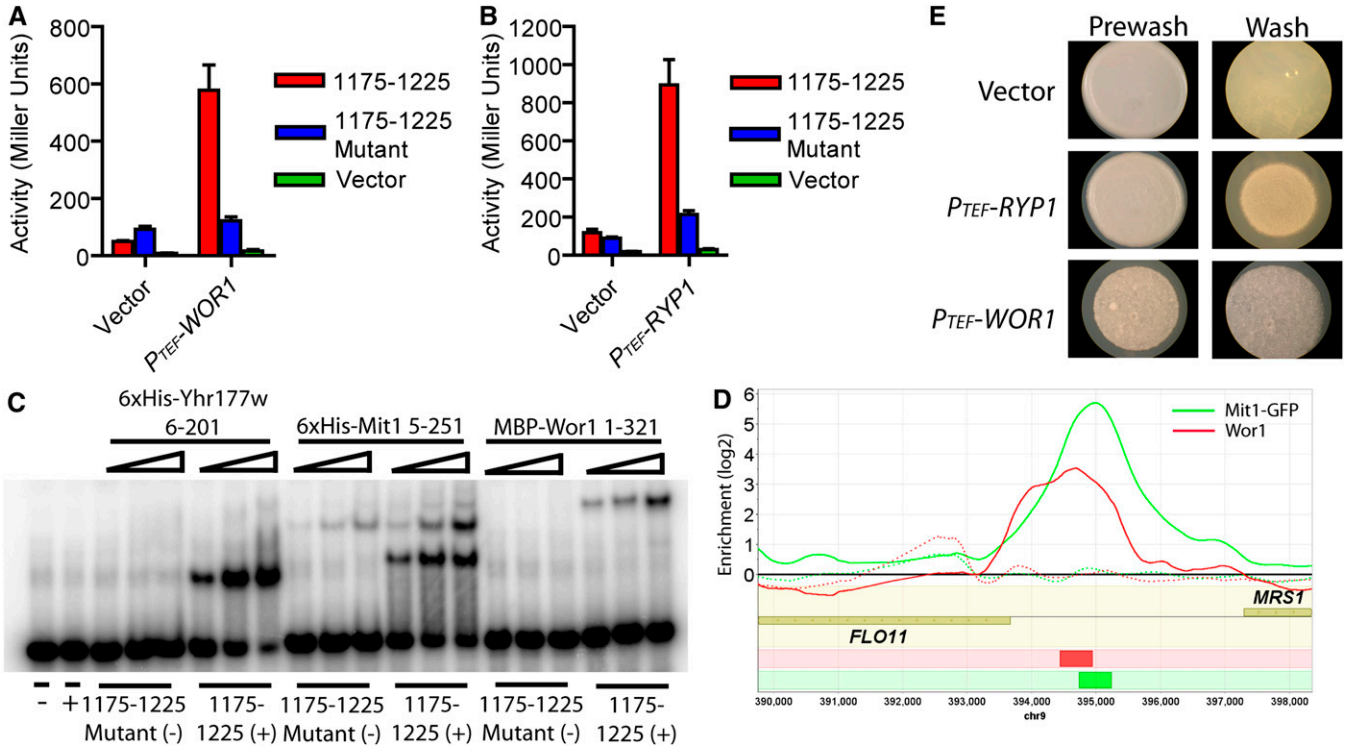

Figure 4 DNA binding and activation functions are conserved among *S. cerevisiae* Mit1, *C. albicans* Wor1, and *H. capsulatum* Ryp1. (A and B) Reporter plasmids carrying a functional upstream 1175–1225 *FLO11* promoter fragment (1175–1225, red), the nonfunctional mutated version of the fragment (1175–1225 mutant, blue), or no insert (vector, green) were cotransformed with a plasmid containing the *TEF* promoter driving an empty vector, (A) *C. albicans WOR1*, or (B) *H. capsulatum RYP1*. Both P_{TEF}-*WOR1* and P_{TEF}-*RYP1* activate transcription from the wild-type but not the mutant *FLO11* fragment-*CYC1* construct. β-Galactosidase units of activity (Miller units) are plotted on the *y*-axis, and error bars in A and B represent the standard deviation from two or three experiments. (C) Yhr177w, Mit1, and Wor1 bind to the 1175–1225 fragment from the *FLO11* promoter, as monitored by gel shift assays. Mit1 (5–251 aa), Yhr177w (6–201 aa), and MBP-Wor1 (1–321 aa) were incubated with either the *FLO11* 1175–1225 promoter fragment (1175–1225, +) or the *FLO11* 1175–1225 mutant fragment (1175–1225 mutant, −). All three proteins bind to the *FLO11* 1175–1225 promoter fragment but not to the mutated fragment, indicating that the binding of Mit1, Yhr177w, and Wor1 to the *FLO11* promoter is sequence specific. Yhr177w and Mit1 concentrations range from 0.5 to 4 nM, and MBP-Wor1 concentrations range from 2 to 8 nM. (D) A ChIP-chip plot showing ectopically expressed Wor1 associating with the *FLO11* promoter *in vivo*. Wor1 was immunoprecipitated from a Δ*mit1*Δ*yhr177w* strain carrying the P_{TEF}-*WOR1* plasmid (solid red line), and the control is a Δ*mit1*Δ*yhr177w* strain carrying P_{TEF}-vector plasmid (dashed red line). ChIP data for the Mit1-GFP experiments in a Mit1-GFP strain (solid green line) or in a control strain lacking GFP (dashed green line) are included for comparison. The red box in the bottom track represents a called Wor1 binding site and the green box represents a called Mit1 binding site. Data were visualized using Mochiview 1.45, with the *x*-axis representing the genomic location and the *y*-axis representing $\log_2$ enrichment. (E) Ectopic expression of *WOR1* or *RYP1* induces invasive growth on SD media. **a**/α diploid Σ2000 cells were spotted on SD agar, grown for 2 days at 30°, and then washed under a stream of water. Pre- (left) and post- (right) wash images are shown.

a major divergence in their protein function. Instead, we hypothesized that the sets of genes controlled by each regulator have changed significantly during the ~600 million years since they diverged from a common ancestor (Taylor and Berbee 2006). We tested this idea in two ways. First, the ectopic expression of either *WOR1* or *RYP1* in *S. cerevisiae* activates the pseudohyphal growth program (Figure 4E, Figure S2), consistent with the idea that the biochemical functions of Wor1, Ryp1, and Mit1 have remained largely conserved. Second, where possible, we mapped the 94 Mit1 target genes in *S. cerevisiae* (as determined by ChIP-chip) to their *C. albicans* orthogroups (see *Materials and Methods*). Sixty-six of the *S. cerevisiae* Mit1 target genes had at least one ortholog in *C. albicans*, but only 13 of these were also bound by Wor1 in *C. albicans* (Zordan *et al.* 2007) (Figure 5, Table 1, Table S11, Table S12, and File S1). We performed the same analysis in the opposite direction—mapping Wor1 targets to *S. cerevisiae* orthologs—and, as expected, found similar results (Figure 5, Table 1, Table S11, Table S12, and File S1). These observations are consistent with the high level of rewiring documented in the ascomycete lineage (Borneman *et al.* 2007a; Tuch *et al.* 2008) and indicate that the Wor1 and Mit1 circuits have undergone considerable diversification.

Although the overlap between these circuits is small, it is not likely to have occurred by chance ($P = 1 \times 10^{-6}$ by bootstrap analysis, see *Materials and Methods*), indicating that a small portion of an ancestral circuit has likely been preserved in *C. albicans* and *S. cerevisiae* (Figure 5, Figure S3, Table 1, Table S11, and Table S12). Nine of the 13 overlapping targets are transcriptional regulators (Table 1). While transcriptional regulators are enriched overall in

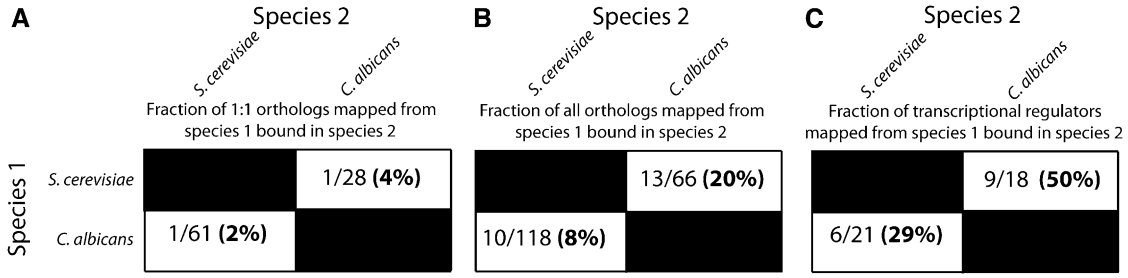

Figure 5 A comparison of the Mit1 regulon in *S. cerevisiae* to the Wor1 regulon in *C. albicans* indicates there is a small conserved set of orthologous genes that code for transcriptional regulators that are bound by both Mit1 and Wor1. (A–C) The number of Mit1- or Wor1-bound genes in species 1 that are also bound in species 2, as a fraction of the total genes bound in species 1 that can be mapped to orthologs in species 2 when (A) only 1:1 orthologous relationships are considered, (B) all orthologous relationships (*i.e.*, 1:1, 2:1, 3:5) are considered, or (C) only Mit1- or Wor1-bound genes that encode sequence-specific DNA-binding proteins are considered.

the Mit1-bound set, this bias for conserved transcription factors in the overlap of the two data sets remains significant [$P = 0.008$, Fisher's exact test (two-tailed)]. Seven of the 9 transcription factors that are conserved targets of Mit1/Wor1 are also targets of all six core members of the core filamentous growth network in *S. cerevisiae* (Monteiro *et al.* 2008), suggesting that a network of interactions among transcriptional regulators has been conserved across hundreds of millions of years of evolution while the "downstream" target genes of these regulators have rapidly moved in and out of the regulons.

Discussion

A great deal of evolutionary diversity (nominally >1 billion years) is embodied in the fungal kingdom. On a phenotypic level, this diversity can be easily visualized in the many different types of morphological transformations observed across fungal species. In this article, we consider three species of fungi—*S. cerevisiae* (baker's yeast), *C. albicans* (the major human fungal pathogen), and *H. capsulatum* (the cause of histoplasmosis)—each of which undergoes a specialized morphological program. Despite the fact that the morphologic transitions considered are different from one species to the next (pseudohyphal growth in *S. cerevisiae*, white–opaque switching in *C. albicans*, and the mycelia to yeast transition in *H. capsulatum*), an orthologous regulator (Mit1 from *S. cerevisiae*, Wor1 from *C. albicans*, and Ryp1 from *H. capsulatum*) is a major regulator of each. The three proteins all contain a WOPR domain, a 200-aa region that folds into a sequence-specific DNA-binding domain. We show that, despite an estimated 600 million years of

Table 1 Conserved targets of the Mit1/Wor1 regulon

S. cerevisiae bound target	*C. albicans* bound target	Transcriptional regulator	Gene description in *S. cerevisiae*	Gene description in *C. albicans*
MIT1	*WOR1*	Yes	Master regulator of pseudohyphal growth	Master regulator of the white–opaque switch
YHR177w	*WOR1*	Yes	No known function	Master regulator of the white–opaque switch
TOS8	*CUP9*	Yes	Bound by all seven pseudohyphal growth regulators	Repressed upon yeast–hyphal transition
CUP9	*CUP9*	Yes	Regulator of peptide transporters	Repressed upon yeast–hyphal transition
PHD1	*EFG1*	Yes	Overexpression enhances pseudohyphal growth	Regulates white–opaque switch, filamentation, cell wall-related genes
SOK2	*EFG1*	Yes	Deletion enhances pseudohyphal growth, positively regulates meiosis	Regulates white–opaque switch, filamentation, cell wall–related genes
ROX1	*RFG1*	Yes	Repressor of hypoxic genes, required for pseudohyphal growth	Repressor of filamentous growth
GAT2	*GAT2*	Yes	Repressed by leucine	Deletion has severe filamentation defects
RSF2	*orf19.5026*	Yes	Involved in glycerol-based growth	No known function
CLN1	*HGC1*	No	G1 cyclin, late G1-specific expression promotes S phase	Hypha-specific G1 expression, required for hyphal growth
SPS4	*orf19.7502*	No	Induced during sporulation	No known function, Hap43 induced
SUN4	*SUN41*	No	Glucanase possibly involved in cell septation	Cell wall glycosidase, hyphal induced, Efg1 regulated, involved in biofilm formation and cell separation
UTH1	*SIM1*	No	Mitochondrial outer membrane and cell wall localized SUN family member involved in cell wall biogenesis	Putative adhesin-like protein involved in cell wall maintenance

divergence, these orthologs bind to and activate transcription from the same DNA sequence motif. We also show that Wor1 (from *C. albicans*) and Ryp1 (from *H. capsulatum*) can drive the pseudohyphal growth of *S. cerevisiae* when overexpressed in that species. We conclude from our analysis that Mit1, Wor1, and Ryp1 are clear orthologs, that they retain their ancestral DNA-binding specificity, that they can activate transcription, and that, in some cases, the protein from one species can substitute for its ortholog in another species.

Although Mit1, Wor1, and Ryp1 appear to be similar biochemically, they each control a distinctive type of morphological change in their respective species. We hypothesized that evolutionary changes in the genes under control of each regulator—rather than changes to the regulators themselves—are the major contributor to the different physiological responses. We tested this idea directly by considering genes bound by each regulator in each species. ChIP-chip and transcriptional profiling data are available for both Wor1 in *C. albicans* (Lan *et al.* 2002; Tsong *et al.* 2003; Zordan *et al.* 2007; Tuch *et al.* 2010) and Ryp1 in *H. capsulatum* (A. Sil, unpublished data) and, in this article, we generated comparable data for Mit1 in *S. cerevisiae*. We found that the set of genes bound by an ortholog in one species is considerably different from the set in another species. We were able to carry out a meaningful comparison between *S. cerevisiae* and *C. albicans* orthologs and found that—of genes controlled by Mit1 in *S. cerevisiae* that had a clear ortholog in *C. albicans*—only 20% were controlled by Wor1 in *C. albicans*. As described in *Results*, the overlapping target genes are made up predominately of other transcriptional regulators, indicating that a portion of an ancestral circuit where Wor1/Mit1 controlled other transcriptional regulators was retained in both *S. cerevisiae* and *C. albicans*. However, the majority of the orthologous target genes regulated in one species are not regulated by the ortholog in the other species. We suggest that this diversification of target genes explains, in part, why the Mit1, Wor1, and Ryp1 proteins have retained many ancestral characteristics, yet they regulate very different morphological transitions. It also explains why ectopic overexpression of *WOR1* (from *C. albicans*) or *RYP1* (from *H. capsulatum*) in *S. cerevisiae* drives the *S. cerevisiae* pseudohyphal growth program rather than aspects of the morphological program specific to the species of origin.

In conclusion, this article shows that three orthologous regulators (one from each of three fungal species) possess very similar biological properties (*e.g.*, they recognize the same DNA sequence and activate transcription), yet they are master regulators of three distinct morphological transitions. We suggest, in accord with other examples (Prud'homme *et al.* 2007; Carroll 2008; Tuch *et al.* 2008), that changes in *cis*-regulatory sequences within target gene promoters are, at least in part, responsible for the different phenotypic outputs in different species produced by orthologous and biochemically similar regulators.

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GENETICS

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A Conserved Transcriptional Regulator Governs Fungal Morphology in Widely Diverged Species

Christopher W. Cain, Matthew B. Lohse, Oliver R. Homann, Anita Sil, and Alexander D. Johnson

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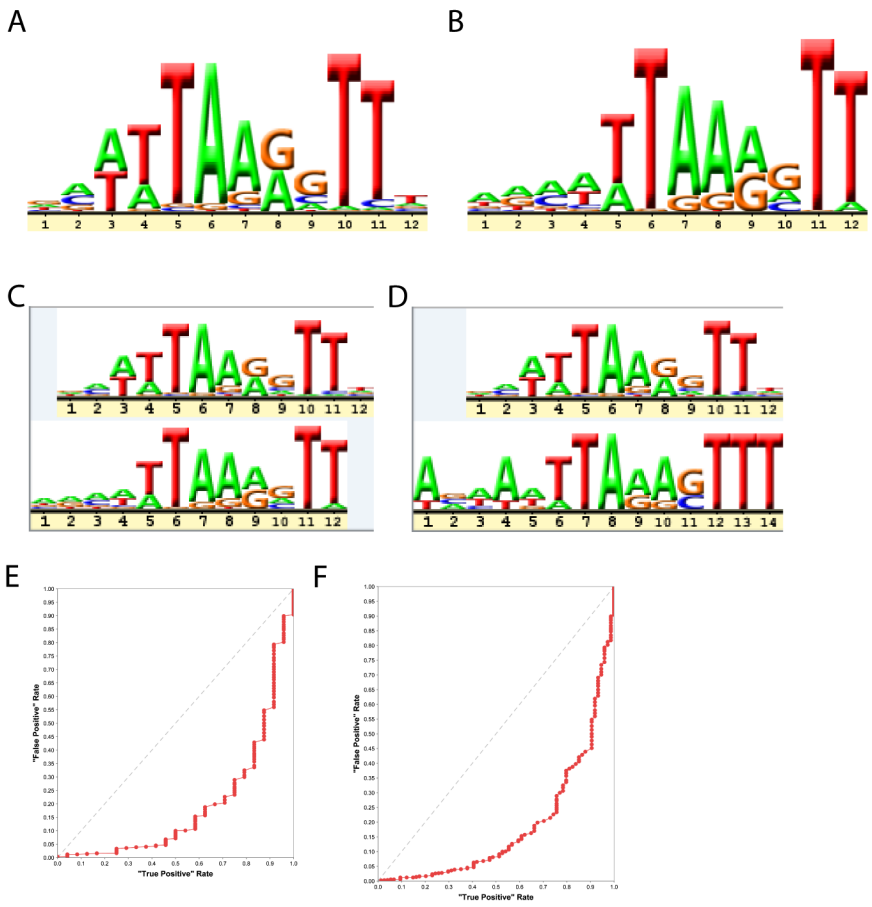

Figure S1 The DNA sequence recognized by Mit1 is similar to that recognized by Wor1. **(A)** Motif recognized by Mit1 developed using the lowest 50 scoring binding sites. Binding site determination and motif analysis performed using MochiView v1.45. **(B)** Motif recognized by Mit1 developed using all 74 binding sites. **(C)** A comparison of the motif from **(A)** to the motif from **(B)**, the motifs match with an E-value of 0 (as determined by MochiView v1.45, using an approach based on that presented in Gupta *et al*. 2007 (Gupta *et al.* 2007)). **(D)** A comparison of the motif from **(A)** to the previously published Wor1 motif (Lohse *et al.* 2010), the motifs match with an E-value of 0.0033. **(E)** ROC Enrichment plot for the Mit1 motif from **(A)**, the fraction of the experimental set (24 Mit1 binding sites not used to make the motif) bound is plotted on the x-axis ("True Positive"). The bound fraction of a control set of 500bp regions randomly selected from promoters not bound by Mit1 is plotted on the y-axis ("False Positive"). Plots were made using MochiView v1.45 using an approach similar to that previously reported (Lohse *et al.* 2010). **(F)** ROC Enrichment plot for the Mit1 motif from **(A)**, using the entire Mit1 binding set (74 sites) for the experimental set.

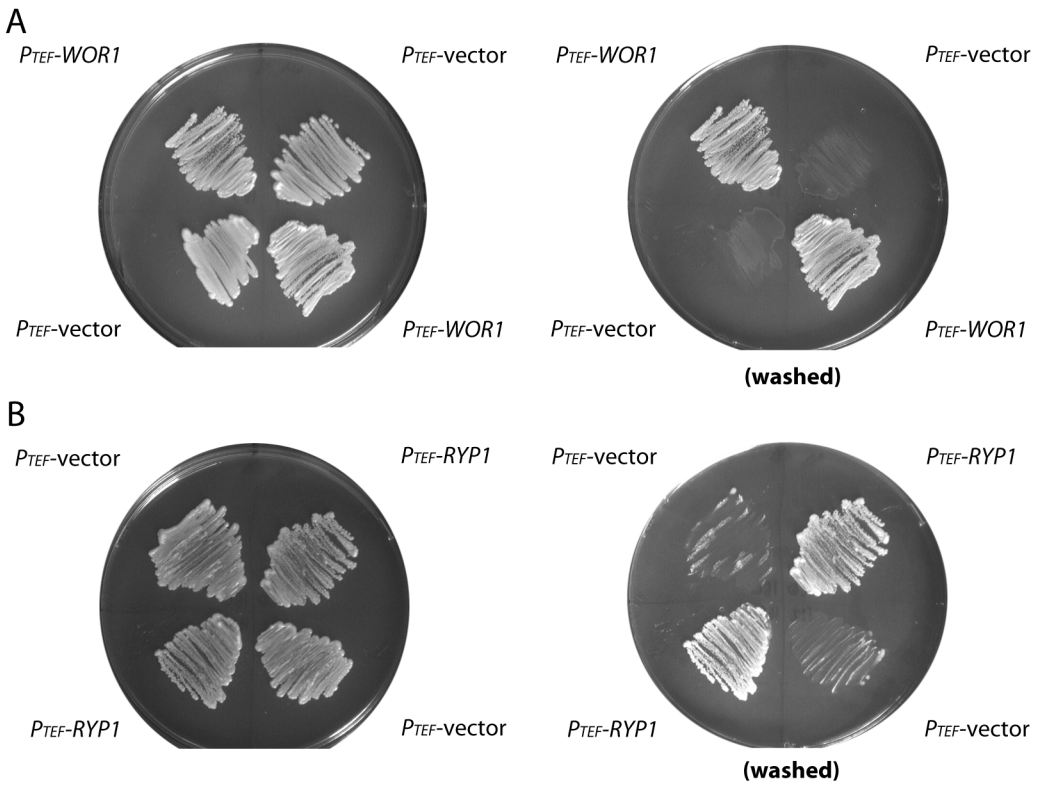

Figure S2 Ectopic expression of either *WOR1* or *RYP1* can induce the haploid invasive growth program independent of *MIT1* or *YHR177W*. **(A)** Haploid invasive growth assay for cells of the indicated genotype in a *Δmit1Δyhr177w* background. Cells were plated on SD –Ura –His plates, grown for 3 days at 30°C, and photographed (left panel). Plates were then washed under a gentle stream of water for 15 seconds and photographed again (right panel). **(B)** Haploid invasive growth assay for cells of the indicated genotype in a *Δmit1Δyhr177w* background. Cells were plated on SD –Ura –Trp plates, grown for 3 days at 30°C, and photographed (left panel). Plates were then washed under a gentle stream of water for 15 seconds and photographed again (right panel).

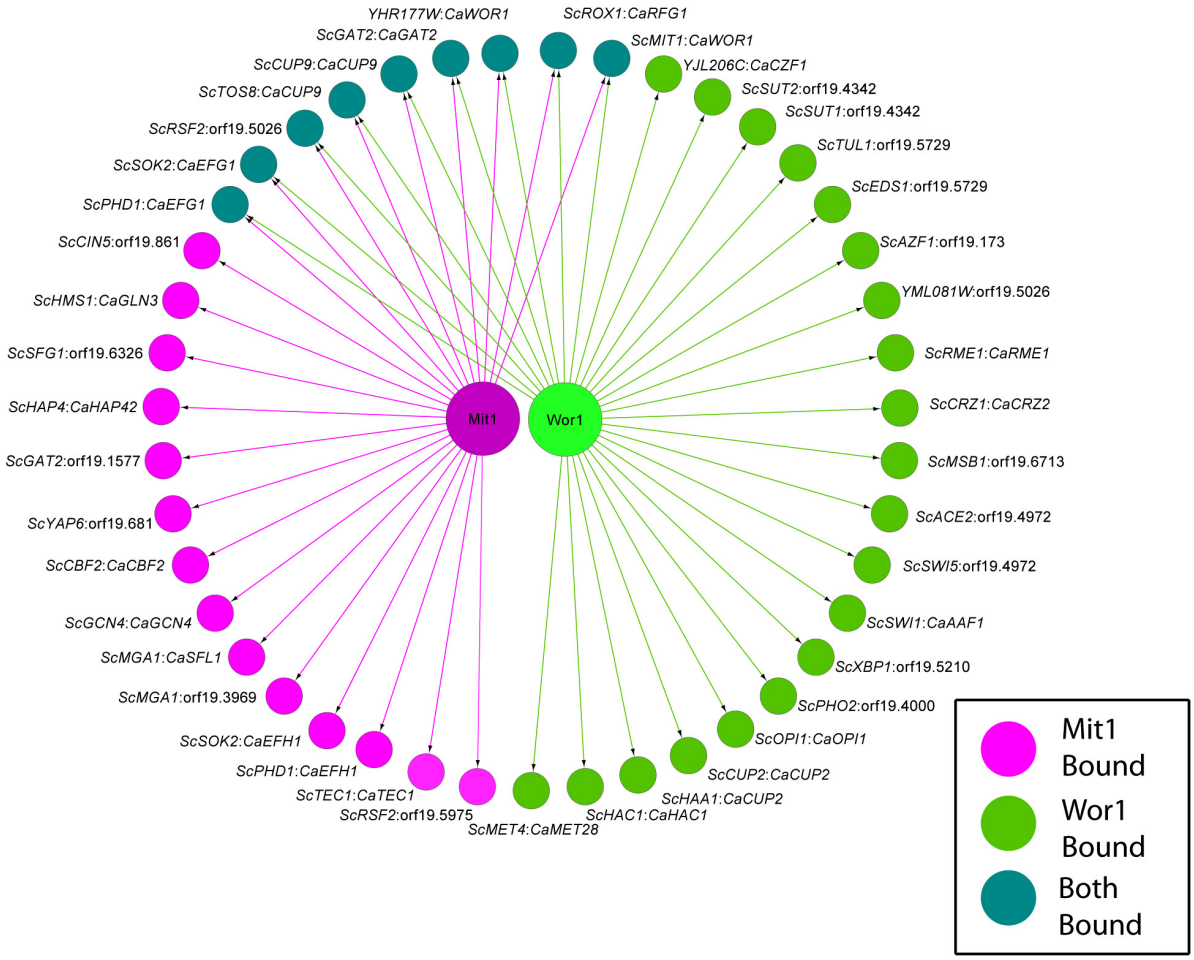

Figure S3 A comparison of the conserved transcription regulator targets of the Mit1 regulon in *S. cerevisiae* and the Wor1 regulon in *C. albicans*. Each node represents one orthologous pair of factors, where at least one member of the pair is bound by either Wor1 or Mit1. Orthologous pairs bound in both species are indicated in blue, those bound only in *S. cerevisiae* are in pink, and those bound only in *C. albicans* are in green. For example: *ScSOK2* and *ScPHD1* participate in a 2:2 relationship with *CaEFG1* and *CaEFH1*. Therefore there are 4 possible pairs of interactions – *ScSOK2:CaEFG1*, *ScSOK2:CaEFH1, ScPHD1:CaEFG1*, and *ScPHD1:CaEFH1*. Two of these pairs are represented as blue nodes bound by both Mit1 and Wor1, because *SOK2* and *PHD1* are bound by Mit1, and *EFG1* is bound by Wor1. Two of these pairs are represented as pink nodes bound only by Mit1, because *EFH1* is not a Wor1 target.

File S1

Supporting Data Tables

Available at http://www.genetics.org/cgi/content/full/genetics.111.134080/DC1.

This Excel File contains all of the data from Tables S1-S12. It contains the top 20 genes upregulated or downregulated upon deletion of *MIT1* (Table S1), a list of Mit1 binding sites and motif scores (Table S2), a list of Mit1 bound intergenic regions and promixal genes (Table S3), the top 10 Mit1 bound genes upregulated or downregulated upon deletion of *MIT1* (Table S4), the Position Specific Weight Matrix for the Mit1 motif in Figure S1a (Table S5), a list of Yhr177w binding sites and motif scores (Table S6), a list of Yhr177w bound intergenics and promixal genes (Table S7), an analysis of the overlap in Mit1 bound intergenic regions with intergenic regions bound by Sok2, Ste12, Tec1, Yhr177w, and Wor1 (Table S8), a list of Wor1 binding sites and motif scores (Table S9), a list if Wor1 bound intergenics and promixal genes (Table S10), Orthology Mapping, *S. cerevisiae* to *C. albicans* (Table S11), Orthology Mapping, *C. albicans* to *S. cerevisiae* (Table S12). Each table is included as a separate worksheet within the file. In addition, the file contains a combined version of Tables S3 and S8.

File S2

Mit1-GFP Binding Sites

Available at http://www.genetics.org/cgi/content/full/genetics.111.134080/DC1.

Mit1-GFP Binding Sites: Plots for the 74 Mit1 binding sites. Smoothed enrichment data for the Mit1-GFP strain is shown in green, for the GFP-less control strain in red. 500bp called Mit1 binding sites are indicated by the green boxes in the lower track in each image. Peaks are arranged in order of decreasing enrichment. Enrichment (log2) is indicated on the left y-axis. File produced using the SnapShot Function in MochiView v1.45 (HOMANN *et al.* 2010).

File S3

Supporting Materials and Methods

Available at http://www.genetics.org/cgi/content/full/genetics.111.134080/DC1.

This file contains discussions of the following topics:

- Mit1 DNA motif identification and analysis
- Experimental and analytical methods for the Expression Microarray results
- Experimental and analytical methods for the Chromatin Immunoprecipitation results
- Binding site comparisons with Sok2, Tec1, and Ste12
- Hand Annotation of Orthology Mapping
- Further bootstrap analysis of target overlap

File S4

Yhr177w Binding Sites

Available at http://www.genetics.org/cgi/content/full/genetics.111.134080/DC1.

Yhr177w Binding Sites: Plots for the 24 Yhr177w binding sites. Smoothed enrichment data for the *YHR177w Δmit1* strain is shown in red, for the *Δyhr177w Δmit1* control strain in green. Called Yhr177w binding sites are indicated by the dark grey boxes in the lower track in each image. Peaks are arranged in order of decreasing enrichment. Enrichment values of 5 [*FLO11/MRS1*] or 4 [*SOK2/SPO20*, *YBL029C-A/YBL029W*] were manually input for the three hand called peaks in this data set in order to ensure proper y-axis scaling for those peaks. All subsequent peaks are arranged in order of decreasing enrichment. Enrichment (log2) is indicated on the left y-axis. File produced using the SnapShot Function in MochiView v1.45 (HOMANN *et al.* 2010).

File S5

Wor1 Binding Sites

Available at http://www.genetics.org/cgi/content/full/genetics.111.134080/DC1.

Wor1 Binding Sites: Plots for the 9 Wor1 binding sites. Smoothed enrichment data for the P_{TEF}-*WOR1* strain is shown in solid red, for the control strain in dashed red. Smoothed enrichment data for the Mit1-GFP strain is shown in solid green, for the GFP-less control strain in dashed green. 500bp called Wor1-binding sites are indicated by the dark grey boxes in the lower track in each image, 500bp called Mit1 binding sites are indicated by the orange boxes in the lower track in each image. Enrichment (log2) is indicated on the left y-axis. File produced using the SnapShot Function in MochiView v1.45 (HOMANN *et al.* 2010).

Table S1 Top 20 Genes Upregulated or Downregulated upon deletion of *MIT1*

Gene	Name	*Δmit1*:WT (log2)	*Δmit1Δyhr177w*:WT (log2)	Notes
YKL208W	CBT1	2.28	2.14	
YHR177W		1.80	-0.67	a
YER033C	ZRG8	1.74	1.86	
YJL078C	PRY3	1.73	1.75	
YDL245C	HXT15	1.46	0.98	
YAR060C		1.28	0.83	
Q0085	ATP6	1.10	0.93	
YOR107W	RGS2	1.10	1.17	
YDR259C	YAP6	1.09	1.47	
YDL246C	SOR2	1.09	1.65	
YHR212C		1.02	0.83	
Q0055	AI2	1.02	0.94	
YIL169C		1.01	1.48	
Q0250	COX2	0.99	0.76	
YFL051C		0.96	0.68	
YAR062W		0.96	0.89	
YER158C		0.95	1.03	
YHR213W		0.92	0.85	
YJR159W	SOR1	0.88	1.46	
YBR085W	AAC3	0.86	1.05	
YBL072C	RPS8A	-2.24		
YIL099W	SGA1	-2.29	-1.45	
YDR408C	ADE8	-2.31	-2.16	
YDL215C	GDH2	-2.31	-2.05	
YBL053W		-2.34	-1.69	
YDL071C		-2.48	-1.87	
YDL096C	OPI6	-2.57	-3.55	
YEL007W	MIT1	-2.67	-1.16	b
YJL052C-A		-2.67	-1.91	
YLR062C	BUD28	-2.68	-1.95	
YPL261C		-2.89	-2.84	
YDL222C	FMP45	-2.90	-2.26	
YJL120W		-2.97	-2.87	
YMR063W	RIM9	-3.04	-2.62	
YLR438W	CAR2	-3.50	-2.83	
YIL066C	RNR3	-3.60	-2.84	
YER091C-A		-3.68	-3.29	
YNL057W		-3.77	-2.80	
YER106W	MAM1	-4.13	-3.11	

Gene	Name	*Δmit1*:WT (log2)	*Δmit1Δyhr177w*:WT (log2)	Notes
YIR019C	FLO11 (MUC1)	-5.45		c

Notes

a	Deleted in *Δmit1Δyhr177w* experiment
b	Deleted in *Δmit1* and *Δmit1Δyhr177w* experiments
c	Probe Flagged for Insufficient signal in *Δmit1Δyhr177w*:WT experiment

Table S2 Mit1 binding sites and motif scores

SEQ_NAME	START	END	STRAND	Enrichment	Max. Score Mit1 50 site motif [Maximum Value 4.529]
chr1	28716	29215	+	2.82	3.90
chr1	198200	198699	+	3.969	3.66
chr1	202734	203233	+	1.687	1.92
chr10	26119	26618	+	2.022	3.46
chr10	223716	224215	+	1.67	4.13
chr10	293744	294243	+	1.786	2.71
chr10	606041	606540	+	1.796	4.33
chr10	639462	639961	+	1.5	4.59
chr10	662557	663056	+	1.788	2.92
chr10	663381	663880	+	1.617	2.44
chr11	230543	231042	+	2.38	1.99
chr11	355472	355971	+	2.876	2.24
chr11	518426	518925	+	1.763	2.12
chr11	642168	642667	+	1.939	3.16
chr11	645009	645508	+	2.049	4.15
chr12	36754	37253	+	2.011	3.38
chr12	370198	370697	+	2.203	2.41
chr12	448204	448703	+	2.26	3.49
chr12	809153	809652	+	1.856	3.61
chr13	279910	280409	+	1.565	4.13
chr13	287374	287873	+	1.817	2.94
chr13	306205	306704	+	2.996	3.69
chr13	540575	541074	+	2.432	3.47
chr13	661345	661844	+	3.32	2.97
chr14	301429	301928	+	1.777	2.52
chr14	351975	352474	+	1.87	4.40
chr14	500901	501400	+	1.681	3.77
chr14	739177	739676	+	2.357	2.92
chr15	24505	25004	+	1.928	2.51
chr15	109845	110344	+	2.028	4.01
chr15	383146	383645	+	1.998	3.80
chr15	384678	385177	+	2.471	2.73
chr15	391606	392105	+	1.692	3.73
chr15	671119	671618	+	1.845	3.76
chr15	904080	904579	+	3.542	3.90
chr16	214764	215263	+	2.626	2.94
chr16	586603	587102	+	3.662	3.93
chr16	590987	591486	+	1.589	3.64

SEQ_NAME	START	END	STRAND	Enrichment	Max. Score Mit1 50 site motif [Maximum Value 4.529]
chr16	678959	679458	+	1.843	2.53
chr16	828832	829331	+	1.942	3.74
chr2	165099	165598	+	4.062	1.56
chr2	408469	408968	+	1.918	1.76
chr4	386171	386670	+	4.264	2.43
chr4	465114	465613	+	2.556	1.81
chr4	599701	600200	+	2.75	2.88
chr4	976134	976633	+	3.428	2.66
chr4	1080490	1080989	+	1.605	2.42
chr4	1345161	1345660	+	1.57	3.02
chr4	1489961	1490460	+	2.636	3.46
chr5	77719	78218	+	2.365	2.56
chr5	140986	141485	+	3.043	3.26
chr5	152528	153027	+	1.731	3.04
chr5	221391	221890	+	1.734	3.04
chr5	238030	238529	+	1.77	3.86
chr5	491111	491610	+	3.028	3.05
chr6	19364	19863	+	2.38	3.85
chr7	323753	324252	+	2.468	3.18
chr7	701056	701555	+	2.144	3.32
chr7	701904	702403	+	1.751	3.13
chr7	702871	703370	+	1.765	3.66
chr7	766006	766505	+	1.927	3.21
chr7	788264	788763	+	1.883	3.47
chr7	919615	920114	+	2.066	3.39
chr7	987221	987720	+	2.117	4.02
chr7	989756	990255	+	3.297	3.64
chr8	17016	17515	+	2.941	3.29
chr8	455419	455918	+	2.472	4.03
chr9	138318	138817	+	4.09	3.68
chr9	141535	142034	+	2.723	3.60
chr9	338789	339288	+	1.65	4.01
chr9	386588	387087	+	2.193	2.90
chr9	388534	389033	+	3.59	3.98
chr9	394738	395237	+	5.705	4.27
chr9	427308	427807	+	1.786	3.38

Table S3 Mit1 bound intergenic regions and promixal genes

SEQ_NAME	START	END	STRAND	Enrichment	Gene #1	Gene #1 Name	Gene #2	Gene #2 Name
chr1	27970	31567	+	2.82	YAL063C	FLO9	YAL062W	GDH3
chr1	196180	203393	+	3.969, 1.687	YAR050W	FLO1		
chr10	26087	26886	+	2.022	YJL216C		YJL214W	HXT8
chr10	223025	224751	+	1.67	YJL105W	SET4		
chr10	293842	294361	+	1.786	YJL078C	PRY3		
chr10	605349	608001	+	1.796	YJR094C	IME1	YJR094W-A	RPL43B
chr10	638747	639636	+	1.5	YJR115W		YJR113C	RSM7
chr10	662755	663694	+	1.788, 1.617	YJR127C	RSF2		
chr11	229523	231868	+	2.38	YKL110C	KTI12	YKL109W	HAP4
chr11	354721	356388	+	2.876	YKL043W	PHD1		
chr11	518588	518908	+	1.763	YKR042W	UTH1		
chr11	642134	645984	+	2.049, 1.939	YKR102W	FLO10		
chr12	36361	37331	+	2.011	YLL052C	AQY2		
chr12	370100	371620	+	2.203	YLR110C	CCW12	YLR113W	HOG1
chr12	448316	448703	+	2.26	YLR154C	RNH203	YLR154W-A	TAR1
chr12	808819	809996	+	1.856	YLR342W	FKS1		
chr13	279682	280589	+	1.565	YMR006C	PLB2		
chr13	286317	288077	+	1.817	YMR011W	HXT2		
chr13	305593	307487	+	2.996	YMR016C	SOK2	YMR017W	SPO20
chr13	540056	541197	+	2.432	YMR135C	GID8	YMR136W	GAT2
chr13	661529	662642	+	3.32	YMR199W	CLN1		
chr14	300647	302676	+	1.777	YNL180C	RHO5	YNL178W	RPS3
chr14	352015	352410	+	1.87	YNL145W	MFA2		
chr14	500254	501511	+	1.681	YNL066W	SUN4		
chr14	738541	739945	+	2.357	YNR060W	FRE4		
chr15	24294	25271	+	1.928	YOL157C		YOL156W	HXT11
chr15	109889	110295	+	2.028	YOL109W	ZEO1		
chr15	382822	383531	+	1.998				
chr15	384420	386823	+	2.471	YOR028C	CIN5	YOR030W	DFG16
chr15	391075	392174	+	1.692	YOR032C	HMS1	YOR032W-A	
chr15	670242	671844	+	1.845	YOR178C	GAC1		
chr15	902868	904752	+	3.542	YOR313C	SPS4	YOR315W	SFG1
chr16	213962	216010	+	2.626	YPL177C	CUP9		
chr16	585581	590277	+	3.662	YPR013C			
chr16	591022	592326	+	1.589	YPR015C			
chr16	678316	679687	+	1.843	YPR063C		YPR065W	ROX1
chr16	828135	829911	+	1.942	YPR148C		YPR149W	NCE102
chr2	164735	166095	+	4.062	YBL029C-A		YBL029W	

SEQ_NAME	START	END	STRAND	Enrichment	Gene #1	Gene #1 Name	Gene #2	Gene #2 Name
chr2	407126	409124	+	1.918	YBR082C	UBC4	YBR083W	TEC1
chr4	385584	387508	+	4.264	YDL037C	BSC1		
chr4	464993	465914	+	2.556	YDR011W	SNQ2		
chr4	599683	600786	+	2.75	YDR077W	SED1		
chr4	975774	976707	+	3.428	YDR259C	YAP6		
chr4	1080191	1081118	+	1.605	YDR309C	GIC2		
chr4	1345051	1345663	+	1.57	YDR441C	APT2		
chr4	1489787	1490583	+	2.636	YDR524C-B		YDR525W-A	SNA2
chr5	77432	78052	+	2.365	YEL040W	UTR2		
chr5	139764	141890	+	3.043	YEL009C	GCN4	YEL007W	MIT1
chr5	150978	153518	+	1.731	YEL001C		YER001W	MNN1
chr5	221287	221844	+	1.734	YER033C	ZRG8	YER034W	
chr5	238016	238458	+	1.77	YER044C	ERG28		
chr5	490574	491524	+	3.028	YER158C			
chr6	18681	20846	+	2.38				
chr7	323232	325331	+	2.468	YGL096W	TOS8		
chr7	701053	703636	+	2.144, 1.765, 1.751	YGR108W	CLB1	YGR106C	
chr7	765609	767430	+	1.927	YGR138C	TPO2	YGR140W	CBF2
chr7	787782	789032	+	1.883	YGR148C	RPL24B	YGR149W	
chr7	919468	920576	+	2.066	YGR213C	RTA1	YGR214W	RPS0A
chr7	986743	988051	+	2.117	YGR249W	MGA1		
chr7	989423	991178	+	3.297				
chr8	16118	19084	+	2.941				
chr8	455349	456586	+	2.472	YHR177W			
chr9	137875	139748	+	4.09	YIL119C	RPI1	YIL118W	RHO3
chr9	141567	142924	+	2.723	YIL117C	PRM5	YIL116W	HIS5
chr9	336418	339340	+	1.65	YIL009W	FAA3	YIL009C-A	EST3
chr9	385699	389568	+	3.59, 2.193	YIR018C-A			
chr9	393673	397290	+	5.705	YIR019C	FLO11	YIR021W	MRS1
chr9	424511	430493	+	1.786	YIR038C	GTT1		

Table S4 Top 10 Mit1 Bound Genes Upregulated or Downregulated upon deletion of *MIT1*

Gene	Name	*Δmit1*:WT (log2)	*Δmit1Δyhr177w*:WT (log2)	Notes
YHR177W		1.80	-0.67	a
YER033C	ZRG8	1.74	1.86	
YJL078C	PRY3	1.73	1.75	
YDR259C	YAP6	1.09	1.47	
YKL109W	HAP4	0.82	1.10	
YHR102W	FLO10	0.68	1.27	
YJL105W	SET4	0.67	0.77	
YBR083W	TEC1	0.54	0.48	
YJL214W	HXT8	0.51	0.64	
YGL096W	TOS8	0.48	0.21	
YGR108W	CLB1	-0.41	-0.66	
YNR060W	FRE4	-0.47	-0.56	
YDR525W-A	SNA2	-0.52	-0.43	
YMR199W	CLN1	-0.57	-0.59	
YGR148C	RPL24B	-0.66	-0.49	
YKL043W	PDH1	-0.74	-0.53	
YGR214W	RPS0A	-0.83	-0.37	
YDR077W	SED1	-1.21	0.05	
YEL007W	MIT1	-2.67	-1.16	b
YIR019C	FLO11 (MUC1)	-5.45		c

Notes

a	Deleted in *Δmit1Δyhr177w* experiment
b	Deleted in *Δmit1* and *Δmit1Δyhr177w* experiments
c	Probe Flagged for Insufficient signal in *Δmit1Δyhr177w*:WT experiment

Table S5 Mit1 Position Specific Weight Matrix

	A	C	G	T
1	0.477	0.183	0.141	0.200
2	0.908	0.006	0.062	0.023
3	0.967	0.006	0.003	0.023
4	0.046	0.595	0.200	0.160
5	0.006	0.497	0.003	0.494
6	0.026	0.144	0.043	0.788
7	0.006	0.046	0.003	0.945
8	0.947	0.026	0.023	0.003
9	0.693	0.026	0.003	0.278
10	0.477	0.006	0.003	0.513
11	0.046	0.183	0.337	0.435
12	0.261	0.340	0.102	0.298

Table S6 Yhr177w binding sites and motif scores

SEQ_NAME	START	END	STRAND	Enrichment	Max. Score Mit1 50 site motif [Maximum Value 4.529]	Notes
chr1	28521	29020	+	1.082	3.90	
chr1	198305	198804	+	2.367	3.66	
chr10	223639	224138	+	1.284	4.13	
chr10	662551	663050	+	1.371	2.92	
chr11	355463	355962	+	2.43	2.24	
chr11	644932	645431	+	1.444	4.15	
chr13	305552	307423	+	0	3.69	a
chr13	915024	915523	+	0.869	3.12	
chr14	495242	495741	+	1.111	3.35	
chr14	763870	764369	+	1.227	3.05	
chr15	384689	385188	+	2.439	2.73	
chr15	391490	391989	+	0.984	1.55	
chr16	920951	921450	+	1.017	1.99	
chr2	164574	166206	+	0	2.99	b
chr4	976119	976618	+	1.039	2.66	
chr4	1055879	1056378	+	1.039	2.37	
chr6	32079	32578	+	1.853	2.41	
chr7	206906	207405	+	2.039	3.30	
chr7	766084	766583	+	1.637	3.21	
chr7	919622	920121	+	1.09	3.39	
chr8	77092	77591	+	1.239	4.62	
chr9	141587	142086	+	1.516	3.60	
chr9	393807	396262	+	0	4.27	c
chr9	427338	427837	+	2.648	3.38	

Notes

a	Manually added
b	Manually added
c	Manually added

Table S7 Yhr177w bound intergenics and promixal genes

SEQ_NAME	START	END	STRAND	Enrichment	Gene #1	Gene #1 Name	Gene #2	Gene #2 Name	Mit1 bound?	Notes
chr1	27970	31567	+	1.082	YAL063C	FLO9	YAL062W	GDH3	1	
chr1	196180	203393	+	2.367	YAR050W	FLO1			1	
chr10	223025	224751	+	1.284	YJL105W	SET4			1	
chr10	662755	663694	+	1.371	YJR127C	RSF2			1	
chr11	354721	356388	+	2.43	YKL043W	PHD1			1	
chr11	642134	645984	+	1.444	YKR102W	FLO10			1	
chr13	305593	307487	+	0	YMR016C	SOK2	YMR017W	SPO20	1	a
chr13	914538	917577	+	0.869	YMR319C	FET4			0	
chr14	494999	495697	+	1.111	YNL069C	RPL16B			0	
chr14	762588	765369	+	1.227	YNR069C	BSC5	YNR070W		0	
chr15	384420	386823	+	2.439	YOR028C	CIN5	YOR030W	DFG16	1	
chr15	391075	392174	+	0.984	YOR032C	HMS1	YOR032W-A		1	
chr16	920482	921853	+	1.017	YPR192W	AQY1			0	
chr2	164735	166095	+	0	YBL029C-A		YBL029W		1	b
chr4	975774	976707	+	1.039	YDR259C	YAP6			1	
chr4	1055884	1056541	+	1.039	YDR297W	SUR2			0	
chr6	30541	33271	+	1.853	YFL051C				0	
chr7	205641	207035	+	2.039	YGL158W	RCK1			0	
chr7	765609	767430	+	1.637	YGR138C	TPO2	YGR140W	CBF2	1	
chr7	919468	920576	+	1.09	YGR213C	RTA1	YGR214W	RPS0A	1	
chr8	77311	77425	+	1.239	YHL014C	YLF2			0	
chr9	141567	142924	+	1.516	YIL117C	PRM5	YIL116W	HIS5	1	
chr9	393673	397290	+	0	YIR019C	FLO11	YIR021W	MRS1	1	c
chr9	424511	430493	+	2.648	YIR038C	GTT1			1	

Notes

a	Manually added
b	Manually added
c	Manually added

Table S8 Mit1 bound intergenic region overlap

SEQ_NAME	START	END	STRAND	Sok2 Bound	Ste12 Bound	Tec1 Bound	Yhr177w Bound	Wor1 bound
chr1	27970	31567	+	1	0	0	1	0
chr1	196180	203393	+	1	0	0	1	0
chr10	26087	26886	+	1	1	1	0	0
chr10	223025	224751	+	1	1	1	1	0
chr10	293842	294361	+	1	0	0	0	0
chr10	605349	608001	+	1	1	1	0	0
chr10	638747	639636	+	1	1	1	0	0
chr10	662755	663694	+	1	1	1	1	0
chr11	229523	231868	+	1	1	1	0	0
chr11	354721	356388	+	1	1	1	1	0
chr11	518588	518908	+	1	1	1	0	0
chr11	642134	645984	+	1	1	0	1	0
chr12	36361	37331	+	1	1	1	0	0
chr12	370100	371620	+	1	1	1	0	0
chr12	448316	448703	+	1	0	0	0	0
chr12	808819	809996	+	1	1	1	0	0
chr13	279682	280589	+	0	1	1	0	0
chr13	286317	288077	+	1	0	0	0	0
chr13	305593	307487	+	1	1	1	1	0
chr13	540056	541197	+	1	1	1	0	0
chr13	661529	662642	+	1	1	1	0	0
chr14	300647	302676	+	1	1	1	0	0
chr14	352015	352410	+	0	0	0	0	0
chr14	500254	501511	+	1	0	0	0	0
chr14	738541	739945	+	0	1	1	0	0
chr15	24294	25271	+	1	0	0	0	0
chr15	109889	110295	+	1	0	0	0	0
chr15	382822	383531	+	1	1	1	0	0
chr15	384420	386823	+	1	1	1	1	0
chr15	391075	392174	+	1	1	1	1	0
chr15	670242	671844	+	1	1	1	0	0
chr15	902868	904752	+	1	1	1	0	0
chr16	213962	216010	+	1	1	1	0	0
chr16	585581	590277	+	1	1	1	0	1
chr16	591022	592326	+	1	1	1	0	0
chr16	678316	679687	+	1	1	1	0	0
chr16	828135	829911	+	1	1	1	0	0
chr2	164735	166095	+	1	1	1	1	0

SEQ_NAME	START	END	STRAND	Sok2 Bound	Ste12 Bound	Tec1 Bound	Yhr177w Bound	Wor1 bound
chr2	407126	409124	+	0	1	1	0	0
chr4	385584	387508	+	1	0	0	0	1
chr4	464993	465914	+	1	0	1	0	0
chr4	599683	600786	+	1	1	1	0	0
chr4	975774	976707	+	1	1	1	1	0
chr4	1080191	1081118	+	1	1	1	0	0
chr4	1345051	1345663	+	1	1	1	0	0
chr4	1489787	1490583	+	1	1	1	0	0
chr5	77432	78052	+	1	1	1	0	0
chr5	139764	141890	+	1	1	1	0	1
chr5	150978	153518	+	1	0	1	0	0
chr5	221287	221844	+	1	0	0	0	0
chr5	238016	238458	+	1	0	0	0	0
chr5	490574	491524	+	1	1	1	0	0
chr6	18681	20846	+	1	0	0	0	0
chr7	323232	325331	+	1	1	1	0	1
chr7	701053	703636	+	0	1	0	0	0
chr7	765609	767430	+	1	0	0	1	0
chr7	787782	789032	+	0	0	0	0	0
chr7	919468	920576	+	1	0	0	1	0
chr7	986743	988051	+	1	1	1	0	0
chr7	989423	991178	+	1	1	1	0	1
chr8	16118	19084	+	1	1	1	0	1
chr8	455349	456586	+	1	0	0	0	0
chr9	137875	139748	+	1	1	1	0	0
chr9	141567	142924	+	1	1	1	1	0
chr9	336418	339340	+	1	0	0	0	0
chr9	385699	389568	+	1	1	1	0	0
chr9	393673	397290	+	1	1	1	1	1
chr9	424511	430493	+	1	1	0	1	0

Table S9 Wor1 binding sites and motif scores

SEQ_NAME	START	END	STRAND	Enrichment	Max. Score Mit1 50 site motif [Maximum Value 4.529]
chr11	7754	8253	+	2.184	2.65
chr16	586396	586895	+	2.505	3.93
chr4	386241	386740	+	2.105	2.51
chr5	141302	141801	+	3.786	2.63
chr6	148446	148945	+	3.084	3.66
chr7	324037	324536	+	2.47	1.65
chr7	989785	990284	+	2.22	3.64
chr8	16650	17149	+	4.127	3.29
chr9	394440	394939	+	3.529	4.27

Table S10 Wor1 bound intergenics and promixal genes

SEQ_NAME	START	END	STRAND	Enrichment	Gene #1	Gene #1 Name	Gene #2	Gene #2 Name	Mit1 Bound?
chr11	7530	9091	+	2.184					0
chr16	585581	590277	+	2.505	YPR013C				1
chr4	385584	387508	+	2.105	YDL037C	BSC1			1
chr5	139764	141890	+	3.786	YEL009C	GCN4	YEL007W	MIT1	1
chr6	148455	149104	+	3.084	YFR001W	LOC1			0
chr7	323232	325331	+	2.47	YGL096W	TOS8			1
chr7	989423	991178	+	2.22					1
chr8	16118	19084	+	4.127					1
chr9	393673	397290	+	3.529	YIR019C	FLO11	YIR021W	MRS1	1

Table S11 Orthology Mapping, *S. cerevisiae* to *C. albicans*

Gene	Name	Ortholog bound in *C. albicans*?	Orthology type	Orthologous Genes	Hand Annotation Notes
YOL157C			7-to-2	orf19.7668 (MAL2) orf19.3982	
YJL216C			6-to-2	orf19.7668(MAL2),orf19.3982	
YER001W	MNN1		4-to-1	orf19.6313 (MNT4)	
YGR138C	TPO2		3-to-3	orf19.6577(FLU1),orf19.7148(TPO2),orf19.4737(TPO3)	
YBR082C	UBC4		3-to-2	orf19.7571(UBC4),orf19.1085	
YLL052C	AQY2		3-to-1	orf19.2849(AQY1)	
YGR249W	MGA1		2-to-2	orf19.454(SFL1),orf19.3969	
YJR127C	RSF2	YES	2-to-2	orf19.5026,orf19.5975	
YKL043W	PHD1	YES	2-to-2	orf19.610(EFG1),orf19.5498(EFH1)	
YMR199W	CLN1	YES	2-to-2	orf19.3207(CCN1),orf19.6028(HGC1)	
YMR016C	SOK2	YES	2-to-2	orf19.610(EFG1),orf19.5498(EFH1)	
YEL007W	MIT1	YES	2-to-1	orf19.4884(WOR1)	a
YAL062W	GDH3		2-to-1	orf19.4716(GDH3)	
YDR077W	SED1		2-to-1	orf19.6321(PGA48)	
YDR441C	APT2		2-to-1	orf19.1448(APT1)	
YGR108W	CLB1		2-to-1	orf19.1446(CLB2)	
YGR148C	RPL24B		2-to-1	orf19.3789(RPL24A)	
YGR214W	RPS0A		2-to-1	orf19.6975(YST1)	
YIR021W	MRS1		2-to-1	orf19.3264(CCE1)	
YJL105W	SET4		2-to-1	orf19.7221(SET3)	
YJR094W-A	RPL43B		2-to-1	orf19.3942.1(RPL43A)	
YLR342W	FKS1		2-to-1	orf19.2929(GSC1)	
YMR017W	SPO20		2-to-1	orf19.117	
YNL066W	SUN4	YES	2-to-1	orf19.3642(SUN41)	
YOR028C	CIN5		2-to-1	orf19.861	
YOR178C	GAC1		2-to-1	orf19.7053(GAC1)	
YPL177C	CUP9	YES	2-to-1	orf19.6514(CUP9)	
YPR149W	NCE102		2-to-1	orf19.5960	
YKR042W	UTH1	YES	2-to-1	orf19.5032 (SIM1)	
YHR177W		YES	2-to-1	orf19.4884(WOR1)	b
YGL096W	TOS8	YES	2-to-1	orf19.6514(CUP9)	
YIR038C	GTT1		1-to-4	orf19.6998(GTT1),orf19.6947(GTT11),orf19.359(GTT12),orf19.356(GTT13)	
YLR110C	CCW12		1-to-3	orf19.2767(PGA59),orf19.4765(PGA6),orf19.2765(PGA62)	
YBL029W			1-to-2	orf19.4995,orf19.4996	
YDR525W-A	SNA2		1-to-2	orf19.3606,orf19.4132	
YMR136W	GAT2	YES	1-to-2	orf19.4056(GAT2),orf19.1577	

Gene	Name	Ortholog bound in *C. albicans*?	Orthology type	Orthologous Genes	Hand Annotation Notes
YOR313C	SPS4	YES	1-to-2	orf19.7502,orf19.7568	
YEL001C			1-to-2	orf19.1782 orf19.1372	
YBR083W	TEC1		1-to-1	orf19.5908(TEC1)	c
YDR259C	YAP6		1-to-1	orf19.861	
YEL009C	GCN4		1-to-1	orf19.1358(GCN4)	
YEL040W	UTR2		1-to-1	orf19.1671(UTR2)	
YER033C	ZRG8		1-to-1	orf19.1519	
YER044C	ERG28		1-to-1	orf19.2016	
YGR140W	CBF2		1-to-1	orf19.5311	
YGR149W			1-to-1	orf19.988	
YIL116W	HIS5		1-to-1	orf19.4177(HIS5)	
YIL118W	RHO3		1-to-1	orf19.3534(RHO3)	
YJR113C	RSM7		1-to-1	orf19.4018	
YKL109W	HAP4		1-to-1	orf19.1481(HAP42)	
YKL110C	KTI12		1-to-1	orf19.2385(KTI12)	
YLR113W	HOG1		1-to-1	orf19.895(HOG1)	
YMR135C	GID8		1-to-1	orf19.3806	
YNL178W	RPS3		1-to-1	orf19.6312(RPS3)	
YNL180C	RHO5		1-to-1	orf19.6237(RAC1)	
YOR030W	DFG16		1-to-1	orf19.881(DFG16)	
YOR032C	HMS1		1-to-1	orf19.3912(GLN3)	
YOR315W	SFG1		1-to-1	orf19.6326	
YPR015C			1-to-1	orf19.4670(CAS5)	
YPR063C			1-to-1	orf19.131.2	
YPR148C			1-to-1	orf19.2304	
YBL029C-A			1-to-1	orf19.3663.1	
YGR106C			1-to-1	orf19.1597 (AGB1)	
YIL009C-A	EST3		1-to-1	orf19.5423 (EST3)	
YLR154W-C	TAR1		1-to-1	orf19.6834.10 (TAR1)	
YPR065W	ROX1	YES	1-to-1		d
YAL063C	FLO9		0-to-0		
YAR050W	FLO1		0-to-0		
YDL037C	BSC1		0-to-0		
YDR011W	SNQ2		0-to-0		
YDR309C	GIC2		0-to-0		
YER034W			0-to-0		
YER158C			0-to-0		
YGR213C	RTA1		0-to-0		
YIL009W	FAA3		0-to-0		

Gene	Name	Ortholog bound in *C. albicans*?	Orthology type	Orthologous Genes	Hand Annotation Notes
YIL117C	PRM5		0-to-0		
YIL119C	RPI1		0-to-0		
YIR019C	FLO11		0-to-0		
YJL078C	PRY3		0-to-0		
YJL214W	HXT8		0-to-0		
YJR094C	IME1		0-to-0		
YKR102W	FLO10		0-to-0		
YMR006C	PLB2		0-to-0		
YMR011W	HXT2		0-to-0		
YNL145W	MFA2		0-to-0		
YNR060W	FRE4		0-to-0		
YOL109W	ZEO1		0-to-0		
YPR013C			0-to-0		
YDR524C-B			0-to-0		
YIR018C-A			0-to-0		
YJR115W			0-to-0		
YLR154C	RNH203		0-to-0		
YOL156W	HXT11		0-to-0		
YOR032W-A			0-to-0		

Notes

a	Changed from 2:2 to 2:1 since of19.4231(Pth2) not the same family member
b	Changed from 2:2 to 2:1 since of19.4231(Pth2) not the same family member
c	Changed from 4:1 to 1:1 since orf19.5908(Tec1) is only ortholog to YBR083W(Tec1)
d	Changed from 0:0 to 1:1 with orf19.2823 (Rfg1)

Table S12 Orthology Mapping, *C. albicans* to *S. cerevisiae*

Gene	Name	Ortholog bound in *S. cerevisiae*?	Orthology type	Orthologous Genes	Hand Annotation Notes
orf19.5302	PGA31		6-to-1	YIR013C(GAT4)	
orf19.4980	HSP70		5-to-2	YDL229W(SSB1),YNL209W(SSB2)	
orf19.7218	RBE1		5-to-2	YJL079C(PRY1),YKR013W(PRY2)	
orf19.1544			5-to-1	YMR173W(DDR48)	
orf19.4476			5-to-1	YPL088W	
orf19.5729	FGR17		4-to-2	YBR033W(EDS1),YKL034W(TUL1)	
orf19.272	FAA21		4-to-1	YER015W(FAA2)	
orf19.1978	GIT2		4-to-1	YCR098C(GIT1)	
orf19.24	RTA2		4-to-1	YOR049C(RSB1)	
orf19.6734	TCC1		4-to-1	YJL016W	
orf19.5170	ENA21		3-to-4	YDR040C(ENA1),YDR039C(ENA2),YDR038C(ENA5), YGL167C(PMR1)	
orf19.7436	AAF1		3-to-1	YPL016W(SWI1)	
orf19.656	DPP1		3-to-1	YDR284C(DPP1)	
orf19.3707	YHB1		3-to-1	YGR234W(YHB1)	
orf19.7382	CAM1		2-to-2	YPL048W(CAM1),YKL081W(TEF4)	
orf19.610	EFG1	YES	2-to-2	YKL043W(PHD1),YMR016C(SOK2)	
orf19.6028	HGC1	YES	2-to-2	YMR199W(CLN1),YPL256C(CLN2)	
orf19.6002	RPL8B		2-to-2	YHL033C(RPL8A),YLL045C(RPL8B)	
orf19.413.1	RPS27A		2-to-2	YKL156W(RPS27A),YHR021C(RPS27B)	
orf19.1585	ZRT2		2-to-2	YGL255W(ZRT1),YLR130C(ZRT2)	
orf19.4972			2-to-2	YLR131C(ACE2),YDR146C(SWI5)	
orf19.5026		YES	2-to-2	YJR127C(RSF2),YML081W	
orf19.2356	CRZ2		2-to-1	YNL027W(CRZ1)	
orf19.3127	CZF1		2-to-1	YJL206C	
orf19.7219	FTR1		2-to-1	YER145C(FTR1)	
orf19.4056	GAT2	YES	2-to-1	YMR136W(GAT2)	
orf19.3668	HGT2		2-to-1	YFL040W	
orf19.2003	HNM1		2-to-1	YGL077C(HNM1)	
orf19.7550	IFA14		2-to-1	YER007W(PAC2)	
orf19.5307	JEN2		2-to-1	YKL217W(JEN1)	
orf19.7046	MET28		2-to-1	YNL103W(MET4)	
orf19.1727	PMC1		2-to-1	YGL006W(PMC1)	
orf19.7610	PTP3		2-to-1	YER075C(PTP3)	
orf19.5994	RHB1		2-to-1	YCR027C(RHB1)	
orf19.1189			2-to-1	YOL155C(HPF1)	
orf19.2724			2-to-1	YGL215W(CLG1)	
orf19.3218			2-to-1	YJR124C	

Gene	Name	Ortholog bound in *S. cerevisiae*?	Orthology type	Orthologous Genes	Hand Annotation Notes
orf19.4346			2-to-1	YPL085W(SEC16)	
orf19.4699			2-to-1	YMR313C(TGL3)	
orf19.5267			2-to-1	YHR143W(DSE2)	
orf19.6713			2-to-1	YOR188W(MSB1)	
orf19.7502		YES	2-to-1	YOR313C(SPS4)	
orf19.220	PIR1		1-to-4	YJL159W(HSP150),YKL164C(PIR1),YKL163W(PIR3),YJL160C	
orf19.5001	CUP2		1-to-2	YGL166W(CUP2),YPR008W(HAA1)	
orf19.6514	CUP9	YES	1-to-2	YPL177C(CUP9),YGL096W(TOS8)	
orf19.7592	FAA4		1-to-2	YOR317W(FAA1),YMR246W(FAA4)	
orf19.5493	GSP1		1-to-2	YLR293C(GSP1),YOR185C(GSP2)	
orf19.5383	PMA1		1-to-2	YGL008C(PMA1),YPL036W(PMA2)	
orf19.3415	PTK2		1-to-2	YKL198C(PTK1),YJR059W(PTK2)	
orf19.5118	SDS24		1-to-2	YGL056C(SDS23),YBR214W(SDS24)	
orf19.3669	SHA3		1-to-2	YPL026C(SKS1),YDR247W(VHS1)	
orf19.5032	SIM1	YES	1-to-2	YJL116C(NCA3),YKR042W(UTH1)	
orf19.3642	SUN41	YES	1-to-2	YIL123W(SIM1),YNL066W(SUN4)	
orf19.1232	VRG4		1-to-2	YER039C(HVG1),YGL225W(VRG4)	
orf19.6494	WHI3		1-to-2	YNL197C(WHI3),YDL224C(WHI4)	
orf19.4884	WOR1	YES	1-to-2	YEL007W(MIT1),YHR177W	a
orf19.2459			1-to-2	YLR353W(BUD8),YGR041W(BUD9)	
orf19.4342			1-to-2	YGL162W(SUT1),YPR009W(SUT2)	
orf19.2823	RFG1	YES	1-to-1	YPR065W(ROX1)	b
orf19.7551	ALO1		1-to-1	YML086C(ALO1)	
orf19.1847	ARO10		1-to-1	YDR380W(ARO10)	
orf19.3122	ARR3		1-to-1	YPR201W(ARR3)	
orf19.4927	BNI1		1-to-1	YNL271C(BNI1)	
orf19.1960	CLN3		1-to-1	YAL040C(CLN3)	
orf19.3239	CTF18		1-to-1	YMR078C(CTF18)	
orf19.4322	DAP2		1-to-1	YHR028C(DAP2)	
orf19.7564	DPB2		1-to-1	YPR175W(DPB2)	
orf19.5999	DYN1		1-to-1	YKR054C(DYN1)	
orf19.1907	EMC9		1-to-1	YGR089W(NNF2)	
orf19.767	ERG3		1-to-1	YLR056W(ERG3)	
orf19.3675	GAL7		1-to-1	YBR018C(GAL7)	
orf19.646	GLN1		1-to-1	YPR035W(GLN1)	
orf19.2432	HAC1		1-to-1	YFL031W(HAC1)	
orf19.7585	INO1		1-to-1	YJL153C(INO1)	
orf19.4769	IPT1		1-to-1	YDR072C(IPT1)	
orf19.4885	MIR1		1-to-1	YJR077C(MIR1)	

Gene	Name	Ortholog bound in *S. cerevisiae*?	Orthology type	Orthologous Genes	Hand Annotation Notes
orf19.5117	OLE1		1-to-1	YGL055W(OLE1)	
orf19.1543	OPI1		1-to-1	YHL020C(OPI1)	
orf19.655	PHO84		1-to-1	YML123C(PHO84)	
orf19.3663	PHO91		1-to-1	YNR013C(PHO91)	
orf19.5171	PMT1		1-to-1	YDL095W(PMT1)	
orf19.7549	PMT5		1-to-1	YDR307W	
orf19.695	RGS2		1-to-1	YOR107W(RGS2)	
orf19.4438	RME1		1-to-1	YGR044C(RME1)	
orf19.2654	RMS1		1-to-1	YDR257C(RKM4)	
orf19.568	SPE2		1-to-1	YOL052C(SPE2)	
orf19.696	STE2		1-to-1	YFL026W(STE2)	
orf19.768	SYG1		1-to-1	YIL047C(SYG1)	
orf19.6496	TRS33		1-to-1	YOR115C(TRS33)	
orf19.2209	YVC1		1-to-1	YOR087W(YVC1)	
orf19.1285			1-to-1	YPR091C	
orf19.1301			1-to-1	YDL110C(TMA17)	
orf19.1486			1-to-1	YGR146C	
orf19.173			1-to-1	YOR113W(AZF1)	
orf19.1959			1-to-1	YHL013C(OTU2)	
orf19.2002			1-to-1	YFR002W(NIC96)	
orf19.2333			1-to-1	YHR009C	
orf19.2639			1-to-1	YKR085C(MRPL20)	
orf19.2822			1-to-1	YGR120C(COG2)	
orf19.3122.2			1-to-1	YDR363W-A(SEM1)	
orf19.4000			1-to-1	YDL106C(PHO2)	
orf19.4347			1-to-1	YKL116C(PRR1)	
orf19.4349			1-to-1	YOL070C(NBA1)	
orf19.4793			1-to-1	YOR252W(TMA16)	
orf19.4820			1-to-1	YKL162C	
orf19.4883			1-to-1	YBR203W(COS111)	
orf19.5210			1-to-1	YIL101C(XBP1)	
orf19.5991			1-to-1	YDL031W(DBP10)	
orf19.6555			1-to-1	YKL084W(HOT13)	
orf19.6736			1-to-1	YOR205C(GEP3)	
orf19.6769			1-to-1	YHL029C(OCA5)	
orf19.6770			1-to-1	YLL038C(ENT4)	
orf19.6805			1-to-1	YDR089W	
orf19.7041			1-to-1	YAL043C(PTA1)	
orf19.7043			1-to-1	YLR050C	

Gene	Name	Ortholog bound in *S. cerevisiae*?	Orthology type	Orthologous Genes	Hand Annotation Notes
orf19.721			1-to-1	YLL035W(GRC3)	
orf19.850			1-to-1	YJR062C(NTA1)	
orf19.933			1-to-1	YDR092W(UBC13)	
orf19.868	ADAEC		0-to-0		
orf19.4505	ADH3		0-to-0		
orf19.935	AGA1		0-to-0		
orf19.7414	ALS6		0-to-0		
orf19.723	BCR1		0-to-0		
orf19.3282	BMT3		0-to-0		
orf19.218	BUD20		0-to-0		
orf19.111	CAN2		0-to-0		
orf19.6000	CDR1		0-to-0		
orf19.1932	CFL4		0-to-0		
orf19.3895	CHT2		0-to-0		
orf19.7586	CHT3		0-to-0		
orf19.4688	DAG7		0-to-0		
orf19.2942	DIP5		0-to-0		
orf19.1120	FAV2		0-to-0		
orf19.3674	GAL102		0-to-0		
orf19.4304	GAP1		0-to-0		
orf19.4456	GAP4		0-to-0		
orf19.7565	GNP3		0-to-0		
orf19.2946	HNM4		0-to-0		
orf19.3664	HSP31		0-to-0		
orf19.4975	HYR1		0-to-0		
orf19.4072	IFF6		0-to-0		
orf19.570	IFF8		0-to-0		
orf19.5604	MDR1		0-to-0		
orf19.4279	MNN1		0-to-0		
orf19.2881	MNN4		0-to-0		
orf19.2602	OPT1		0-to-0		
orf19.5144	PGA28		0-to-0		
orf19.2833	PGA34		0-to-0		
orf19.4651	PGA53		0-to-0		
orf19.4698	PTC8		0-to-0		
orf19.2945	PUT4		0-to-0		
orf19.5124	RBR3		0-to-0		
orf19.7521	REP1		0-to-0		
orf19.3708	SAP2		0-to-0		

Gene	Name	Ortholog bound in *S. cerevisiae*?	Orthology type	Orthologous Genes	Hand Annotation Notes
orf19.5145	SSP96		0-to-0		
orf19.2652	TEF4		0-to-0		
orf19.4646	UEC1		0-to-0		
orf19.1409	VAC7		0-to-0		
orf19.5992	WOR2		0-to-0		
orf19.2882	XUT1		0-to-0		
orf19.3618	YWP1		0-to-0		
orf19.1286			0-to-0		
orf19.1728			0-to-0		
orf19.177			0-to-0		
orf19.1821			0-to-0		
orf19.1906			0-to-0		
orf19.1958			0-to-0		
orf19.206			0-to-0		
orf19.2210			0-to-0		
orf19.2247			0-to-0		
orf19.2332			0-to-0		
orf19.258			0-to-0		
orf19.2638			0-to-0		
orf19.2653			0-to-0		
orf19.2725			0-to-0		
orf19.2943.5			0-to-0		
orf19.2962			0-to-0		
orf19.3216			0-to-0		
orf19.3238			0-to-0		
orf19.3336			0-to-0		
orf19.3694			0-to-0		
orf19.3713			0-to-0		
orf19.3714			0-to-0		
orf19.3897			0-to-0		
orf19.413			0-to-0		
orf19.4280			0-to-0		
orf19.4321			0-to-0		
orf19.4394			0-to-0		
orf19.450			0-to-0		
orf19.467			0-to-0		
orf19.4792			0-to-0		
orf19.4818			0-to-0		
orf19.4900			0-to-0		

Gene	Name	Ortholog bound in *S. cerevisiae*?	Orthology type	Orthologous Genes	Hand Annotation Notes
orf19.4906			0-to-0		
orf19.5069			0-to-0		
orf19.5308			0-to-0		
orf19.5495			0-to-0		
orf19.5728			0-to-0		
orf19.5843			0-to-0		
orf19.5933			0-to-0		
orf19.6027			0-to-0		
orf19.6311			0-to-0		
orf19.6556			0-to-0		
orf19.6715			0-to-0		
orf19.6874			0-to-0		
orf19.6968			0-to-0		
orf19.6996			0-to-0		
orf19.7042			0-to-0		
orf19.7054			0-to-0		
orf19.7055			0-to-0		
orf19.7151			0-to-0		
orf19.7152			0-to-0		
orf19.7380			0-to-0		
orf19.7381			0-to-0		
orf19.7513			0-to-0		
orf19.7522			0-to-0		
orf19.849			0-to-0		
orf19.851			0-to-0		
orf19.867			0-to-0		
orf19.4231	PTH2		0-to-0		c
orf19.3643			0-to-0		d

Notes

a	Changed from 2:2 to 1:2 since orf19.4231(Pth2) removed
b	Changed from 0:0 to 1:1 with YPR065W (ROX1)
c	Changed from 2:2 to 0:0 since Mit1/YHR177w correspond to orf19.4884(Wor1)
d	Changed from 4:1 to 0:0 since orf19.5908(Tec1) is only ortholog to YBR083W(Tec1)

Table S13 Strains Used in this Study

Description	Strain	Source	Genotype
Activation Assays			
Wild Type, 001-420	CC88	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 2/1 (001-420)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 180-620	CC89	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 3/2 (180-620)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 380-820	CC90	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 4/3 (380-820)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 580-1020	CC91	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 5/4 (580-1020)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 780-1220	CC92	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 6/5 (780-1220)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 980-1420	CC93	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 7/6 (980-1420)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1180-1620	CC94	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 8/7 (1180-1620)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1380-1820	CC95	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 9/8 (1380-1820)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1580-2020	CC96	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 10/9 (1580-2020)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1780-2220	CC97	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 11/10 (1780-2220)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1980-2420	CC183	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 12/11 (1980-2420)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 2180-2620	CC99	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 13/12 (2180-2620)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 2380-2820	CC100	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 14/13 (2380-2820)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 2580-3020	CC101	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} 15/14 (2580-2980)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, Vector	CC102	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG
Δmit1, 001-420	CC109	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 2/1 (001-420)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 180-620	CC110	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 3/2 (180-620)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 380-820	CC117	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 4/3 (380-820)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 580-1020	CC81	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 5/4 (580-1020)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 780-1220	CC82	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 6/5 (780-1220)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 980-1420	CC83	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 7/6 (980-1420)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG**,** *leu2*
Δmit1, 1180-1620	CC84	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 8/7 (1180-1620)-*LacZ*; *URA3*; 2µ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*

Description	Strain	Source	Genotype
Δmit1, 1380-1820	CC85	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 9/8 (1380-1820)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 1580-2020	CC86	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 10/9 (1580-2020)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 1780-2220	CC111	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 11/10 (1780-2220)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 1980-2420	CC112	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 12/11 (1980-2420)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 2180-2620	CC113	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 13/12 (2180-2620)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 2380-2820	CC114	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 14/13 (2380-2820)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 2580-3020	CC115	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} 15/14 (2580-2980)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, Vector	CC116	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Wild Type, 1000-1050	CC132	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (1000-1050)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1025-1075	CC133	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (1025-1075)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1050-1100	CC134	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (1050-1100)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1075-1125	CC135	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (1075-1125)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1100-1150	CC153	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (1100-1150)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1125-1175	CC136	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (1125-1175)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1150-1200	CC137	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (1150-1200)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1175-1225	CC138, CC191	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (1175-1225)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, 1175-1225 Mutant	CC192	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (with 1175-1225 three mutations)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Wild Type, Vector	CC140, CC190	This Study	*MAT**a***, P_{CYC1}-*ΔUAS*::P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG
Δmit1, 1000-1050	CC141	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} (1000-1050)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, leu2
Δmit1, 1025-1075	CC142	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} (1025-1075)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 1050-1100	CC143	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} (1050-1100)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 1075-1125	CC144	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} (1075-1125)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 1100-1150	CC154	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} (1100-1150)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 1125-1175	CC145	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS*::P_{FLO11} (1125-1175)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*

Description	Strain	Source	Genotype
Δmit1, 1150-1200	CC146	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS::*P_{FLO11} (1150-1200)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 1175-1225	CC147, CC194	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS::*P_{FLO11} (1175-1225)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, 1175-1225 Mutant	CC195	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS::*P_{FLO11} (with 1175-1225 three mutations)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Δmit1, Vector	CC149, CC193	This Study	*MAT**a***, *mit1*::LeuGFP, P_{CYC1}-*ΔUAS::*P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ, *ura3*, *trp1*::hisG, *his3*::hisG, *leu2*
Complementation Activation Assays			
Δmit1, *Δyhr177w*, Vector, 1175-1225	CC185	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-(empty vector); *HIS3*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (1175-1225)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, Vector, 1175-1225 Mutant	CC186	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-(empty vector); *HIS3*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (with 1175-1225 three mutations)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, P_{TEF}-*WOR1*, 1175-1225	CC188	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-*WOR1*; *HIS3*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (1175-1225)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, P_{TEF}-*WOR1*, 1175-1225 Mutant	CC189	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-*WOR1*; *HIS3*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (with 1175-1225 three mutations)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, Vector, Vector	CC184	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-(empty vector); *HIS3*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, P_{TEF}-*WOR1*, Vector	CC187	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-*WOR1*; *HIS3*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, Vector, 1175-1225	CC161	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-(empty vector); *TRP1*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (1175-1225)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, Vector, 1175-1225 Mutant	CC179	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-(empty vector); *TRP1*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (with 1175-1225 three mutations)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, P_{TEF}-*RYP1*, 1175-1225	CC159	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-*RYP1*; *TRP1*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (1175-1225)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, P_{TEF}-*RYP1*, 1175-1225 Mutant	CC178	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-*RYP1*; *TRP1*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (with 1175-1225 three mutations)-*LacZ*; *URA3*; 2μ

Description	Strain	Source	Genotype
Δmit1, *Δyhr177w*, Vector, Vector	CC162	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-(empty vector); *TRP1*; *CEN*, P_{CYC1}-*ΔUAS*::P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, P_{TEF}-*RYP1*, Vector	CC160	This Study	*MAT**a***, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-*RYP1*; *TRP1*; *CEN*, P_{CYC1}-*ΔUAS*::P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ
ChIP-chip Experiments			
Mit1-GFP (Mit1 Chip-chip)	CC43	This Study	*MAT**a***, *MIT1*-GFP-KanMX, Sigma 2000 background (Derived from MY1384)
No GFP Control Strain (Mit1 Chip-chip control)	MY1384	Madhani Lab	*MAT**a***, wild type
Δmit1 (Yhr177w ChIP-chip)	CC19	This Study	*MAT**a***, *mit1*::KanMX
Δmit1, *Δyhr177w* (Yhr177w ChIP-chip control)	CC40	This Study	*MAT**a***, *mit1*::hphMX, *yhr177w*::KanMX
P_{TEF}-Wor1 (Wor1 ChIP-chip)	CC165	This Study	*MAT**a***, *ura3*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-*WOR1; URA3*; *CEN*
P_{TEF} Vector (Wor1 ChIP-chip control)	CC169	This Study	*MAT**a***, *ura3*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-(empty vector)*; URA3*; *CEN*
Haploid Invasive Growth			
Wild Type [WT]	MY1384	Madhani Lab	*MAT**a***, wild type
Δmit1	CC19	This Study	*MAT**a***, *mit1*::KanMX
Δmit1, *Δyhr177w*	CC40	This Study	*MAT**a***, *mit1*::hphMX, *yhr177w*::KanMX
Δyhr177w	CC28	This Study	*MAT**a***, *yhr177w*::KanMX
Δflo11	LY6603	Madhani Lab	*MAT**a***, *ura-52*,*his3*::hisG, *leu2*:hisG, *flo11*:LEU2
Diploid Pseudohyphal Growth			
Wild Type [WT], prototroph diploid	MY1432	Madhani Lab	MAT**a**/α, *ura3/+, leu2/+. His3::hisG/+. Trp1::hisG/+*
Δmit1	CC33	This Study	MAT**a**/α, *mit1*::kanMX/*mit1*::NatMX, *ura3/+, leu2/+. His3::hisG/+. Trp1::hisG/+*
Δyhr177w	CC34	This Study	MAT**a**/α, *yhr177w*::kanMX/*yhr177w*::hphMX, *ura3/+, leu2/+. His3::hisG/+. Trp1::hisG/+*
Δmit1, *Δyhr177w*	CC36	This Study	MAT**a**/α, *yhr177w*::kanMX/*yhr177w*::hphMX, *mit1*::hphMX/*mit1*::NatMX, *ura3/+, leu2/+. His3::hisG/+. Trp1::hisG/+*
P_{FLO11}-GFP expression			
Wild Type [WT] P_{FLO11}-GFP	CC13	This Study	*MAT**a***, *flo11*::GFP-KanMX
Δmit1 P_{FLO11}-GFP	CC14	This Study	*MAT**a***, *mit1*::NatMX, *flo11*::GFP-KanMX

Description	Strain	Source	Genotype
Δyhr177w P_{FLO11}-GFP	CC15	This Study	*MAT***a**,*yhr177w*::hphMX, *flo11*::GFP-KanMX
Δmit1, *Δyhr177w* P_{FLO11}-GFP	CC14	This Study	*MAT***a**, *mit1*::NatMX, *yhr177w*::HphMX, *flo11*::GFP-KanMX
Diploid Induced Invasion			
P_{TEF}-Vector *LEU CEN*	CC172	This Study	*MAT***a**/α, *ura3/ura3, leu2/leu2, his3::hisG/his3::hisG, trp1::hisG/trp1::hisG,* P_{TEF}-(empty vector)*; LEU2; CEN*
P_{TEF}-*MIT1 LEU CEN*	CC173	This Study	*MAT***a**/α, *ura3/ura3, leu2/leu2, his3::hisG/his3::hisG, trp1::hisG/trp1::hisG,* P_{TEF}-*MIT1; LEU2; CEN*
P_{TEF}-Vector *TRP CEN*	CC175	This Study	*MAT***a**/α, *ura3/ura3, leu2/leu2, his3::hisG/his3::hisG, trp1::hisG/trp1::hisG,* P_{TEF}-(empty vector)*; TRP1; CEN*
P_{TEF}-*RYP1 TRP CEN*	CC174	This Study	*MAT***a**/α, *ura3/ura3, leu2/leu2, his3::hisG/his3::hisG, trp1::hisG/trp1::hisG,* P_{TEF}-*RYP1*; *TRP1; CEN*
P_{TEF}-Vector *HIS CEN*	CC177	This Study	*MAT***a**/α, *ura3/ura3, leu2/leu2, his3::hisG/his3::hisG, trp1::hisG/trp1::hisG,* P_{TEF}-(empty vector)*; HIS3; CEN*
P_{TEF}-*WOR1 HIS CEN*	CC176	This Study	*MAT***a**/α, *ura3/ura3, leu2/leu2, his3::hisG/his3::hisG, trp1::hisG/trp1::hisG,* P_{TEF}-*WOR1; HIS3; CEN*
Haploid Induced Invasion			
Δmit1, *Δyhr177w*, Vector, Vector	CC184	This Study	*MAT***a**, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-(empty vector); *HIS3*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, P_{TEF}-*WOR1*, Vector	CC187	This Study	*MAT***a**, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-*WOR1*; *HIS3*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, Vector, Vector	CC162	This Study	*MAT***a**, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-(empty vector); *TRP1*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ
Δmit1, *Δyhr177w*, P_{TEF}-*RYP1*, Vector	CC160	This Study	*MAT***a**, *ura3*, *leu2*, *trp1*::hisG, *his3*::hisG, *mit1*::LeuGFP, *yhr177w*::KanMx, P_{TEF}-*RYP1*; *TRP1*; *CEN*, P_{CYC1}-*ΔUAS::*P_{FLO11} (empty vector)-*LacZ*; *URA3*; 2μ

Table S14 Plasmids used in this study

Called	Name	Description	Reference
Protein Expression Plasmids			
Mit1 5-251	pMBL313	pLIC-H3 [pET28 varient] with 6xHis-Mit1 5-251	This Study
Yhr177w 6-201	pMBL303	pLIC-H3 [pET28 varient] with 6xHis-YHR177w 6-201	This Study
MBP Wor1 1-321	bRZ94	Modified pET28 with 6xHis-MBP-Wor1 1-321	a
Overexpression plasmids			
b3909 Vector	p413TEF	P_{TEF}-(empty vector); *HIS3*; *CEN*; AmpR	b
b3911 Vector	p414TEF	P_{TEF}-(empty vector); *TRP1*; *CEN*; AmpR	b
b3915 Vector	p416TEF	P_{TEF}-(empty vector); *URA3*; *CEN*; AmpR	b
b3913 Vector	p415TEF	P_{TEF}-(empty vector); *LEU2*; *CEN*; AmpR	b
pTEF-Ryp1	CC39	P_{TEF}-*RYP1*; *TRP1*; *CEN*; AmpR	This Study
pTEF-Wor1	CC35	P_{TEF}-*WOR1*; *HIS3*; *CEN*; AmpR	a
pTEF-Wor1	CC24	P_{TEF}-*WOR1*; *URA3*; *CEN*; AmpR	This Study
pTEF-Mit1	CC43	P_{TEF}-*MIT1*; *LEU2*; *CEN*; AmpR	This Study
YGPM-25i24	YGPM-25i24	fragment of chromosome V, bp 140960-144888, contains *MIT1* ORF	c
Activation Assay Plasmids			
001-420 [2/1]	BES137	P_{CYC1}-*ΔUAS*-P_{FLO11} *2/1-LacZ*; *URA3*; *2μ*; AmpR	d
180-620 [3/2]	BES138	P_{CYC1}-*ΔUAS*-P_{FLO11} *3/2-LacZ*; *URA3*; *2μ*; AmpR	d
380-820 [4/3]	BES139	P_{CYC1}-*ΔUAS*-P_{FLO11} *4/3-LacZ*; *URA3*; *2μ*; AmpR	d
580-1020 [5/4]	BES140	P_{CYC1}-*ΔUAS*-P_{FLO11} *5/4-LacZ*; *URA3*; *2μ*; AmpR	d
780-1220 [6/5]	BES141	P_{CYC1}-*ΔUAS*-P_{FLO11} *6/5-LacZ*; *URA3*; *2μ*; AmpR	d
980-1420 [7/6]	BES160	P_{CYC1}-*ΔUAS*-P_{FLO11} *7/6-LacZ*; *URA3*; *2μ*; AmpR	d
1180-1620 [8/7]	BES161	P_{CYC1}-*ΔUAS*-P_{FLO11} *8/7-LacZ*; *URA3*; *2μ*; AmpR	d
1380-1820 [9/8]	BES142	P_{CYC1}-*ΔUAS*-P_{FLO11} *9/8-LacZ*; *URA3*; *2μ*; AmpR	d
1580-2020 [10/9]	BES143	P_{CYC1}-*ΔUAS*-P_{FLO11} *10/9-LacZ*; *URA3*; *2μ*; AmpR	d
1780-2220 [11/10]	BES144	P_{CYC1}-*ΔUAS*-P_{FLO11} *11/10-LacZ*; *URA3*; *2μ*; AmpR	d
1980-2420 [12/11]	BES164	P_{CYC1}-*ΔUAS*-P_{FLO11} *12/11-LacZ*; *URA3*; *2μ*; AmpR	d
2180-2620 [13/12]	BES162	P_{CYC1}-*ΔUAS*-P_{FLO11} *13/12-LacZ*; *URA3*; *2μ*; AmpR	d
2380-2820 [14/13]	BES145	P_{CYC1}-*ΔUAS*-P_{FLO11} *14/13-LacZ*; *URA3*; *2μ*; AmpR	d
2580-2980 [15/14]	BES146	P_{CYC1}-*ΔUAS*-P_{FLO11} *15/14-LacZ*; *URA3*; *2μ*; AmpR	d
Vector	BES168	P_{CYC1}-*ΔUAS*-P_{FLO11} (empty vector)-*LacZ*; *URA3*; *2μ*; AmpR	d
1000-1050	CC26	P_{CYC1}-*ΔUAS*-P_{FLO11} *1000-1050-LacZ*; *URA3*; *2μ*; AmpR	This Study
1025-1075	CC27	P_{CYC1}-*ΔUAS*-P_{FLO11} *1025-1075-LacZ*; *URA3*; *2μ*; AmpR	This Study
1050-1100	CC28	P_{CYC1}-*ΔUAS*-P_{FLO11} *1050-1100-LacZ*; *URA3*; *2μ*; AmpR	This Study
1075-1125	CC29	P_{CYC1}-*ΔUAS*-P_{FLO11} *1075-1125-LacZ*; *URA3*; *2μ*; AmpR	This Study
1100-1150	CC30	P_{CYC1}-*ΔUAS*-P_{FLO11} *1100-1150-LacZ*; *URA3*; *2μ*; AmpR	This Study
1125-1175	CC31	P_{CYC1}-*ΔUAS*-P_{FLO11} *1125-1175-LacZ*; *URA3*; *2μ*; AmpR	This Study
1150-1200	CC32	P_{CYC1}-*ΔUAS*-P_{FLO11} *1150-1200-LacZ*; *URA3*; *2μ*; AmpR	This Study

Called	Name	Description	Reference
1175-1225	CC33	P_{CYC1}-*ΔUAS*-P_{FLO11} *1175-1225-LacZ*; *URA3*; *2μ*; AmpR	This Study
1175-1225 MUT	CC34	P_{CYC1}-*ΔUAS*-P_{FLO11} *1175-1225* (with 3 mutations)-*LacZ*; *URA3*; *2μ*; AmpR	This Study

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Table S15 Oligonucleotides used in this study

Description	Primer sequence 5'-3'
EMSA Primers [Forward primer listed only]	
PFLO11 1175-1225	tttttgggtgtgcctggaaagttcaaattaaggtttttttcttctgtttt
PFLO11 1175-1225 Mutant	T TTT TGG GTG TGC CTG GAA AGT TCA AAT TcA GGacTT TTT CTT CTG TTT T
Protein Expression Plasmid Construction	
Yhr177w (AA6) 5', XmaI	Gttaca cccggg CCT ACC TGT TAT GGG TAC ATC
Yhr177w (AA201) 3' XhoI-Stop	CTC GGA CTC GAG TTA ATG AGC TTT GGT TCT GCC
Mit1 (AA5) 5' XmaI	Gttaca cccggg CCT ACT TTC AAA GGG TAC ATT G
Mit1 (AA251) 3' XhoI-Stop	CTC GGA CTC GAG TTA GTT GCT TTT ATT GTT TAT ATT ATT AGG ACC
***PFLO11* Fragments for Activation Assays**	
1000-1050 F	TCGAGaacttttttttctttttttgtcatccttttctttggggctaagaatggacC
1000-1050 R	TCGAGgtccattcttagccccaaagaaaaggatgacaaaaaaaaagaaaaaaagttC
1025-1075 F	TCGAGaaattaggctttactggtacgagttaacttttttttctttttttgtcatcC
1025-1075 R	TCGAGgatgacaaaaaaaaagaaaaaaagttaactcgtaccagtaaagcctaatttC
1050-1100 F	TCGAGttctctggggctagcgatcactgcaaaattaggctttactggtacgagttC
1050-1100 R	TCGAGaactcgtaccagtaaagcctaattttgcagtgatcgctagccccagagaaC
1075-1125 F	TCGAGagtgggtccttttttgtctttagtccttctctggggctagcgatcactgcaC
1075-1125 R	TCGAGtgcagtgatcgctagccccagagaaggactaaagacaaaaaggacccactC
1100-1150 F	TCGAGagtttcggccgttattttcctatcgagtgggtccttttttgtctttagtccC
1100-1150 R	TCGAGggactaaagacaaaaaggacccactcgataggaaaataacggccgaaactC
1125-1175 F	TCGAGcttaacaagaaaatgtcgcccaaagagtttcggccgttattttcctatcgC
1125-1175 R	TCGAGcgataggaaaataacggccgaaactctttgggcgacattttcttgttaagC
1150-1200 F	TCGAGaattaaggtttttttcttctgttttcttaacaagaaaatgtcgcccaaagC
1150-1201 R	TCGAGctttgggcgacattttcttgttaagaaaacagaagaaaaaaaccttaattC
1175-1225 F	TCGAGtttttgggtgtgcctggaaagttcaaattaaggtttttttcttctgttttC
1175-1225 R	TCGAGaaaacagaagaaaaaaaccttaatttgaactttccaggcacacccaaaaaC
1175-1225 Mutant F	TCGAG T TTT TGG GTG TGC CTG GAA AGT TCA AAT TcA GGacTT TTT CTT CTG TTT T C
1175-1225 Mutant R	TCGAG AAA ACA GAA GAA AAA GTC CTG AAT TTG AAC TTT CCA GGC ACA CCC AAA AA C
Deletion Constructs	
Mit1 Forward	TTCCACAAAACAACATTTCAACTAGACACAAGGACAACGTAAATTTCTAACGGATCCCCGGGTTAATTAA
Mit1 Reverse	AGGCAAGGAGAAGAAAAGAAAATCGGAGTACTTTTTTAAAATATATTTACGAATTCGAGCTCGTTTAAAC
Yhr177w Forward	AATTAATCAAACAATAGATAGGAAAAGGAGATCGACGAGTAAGCGTAAGACGGATCCCCGGGTTAATTAA

Description	Primer sequence 5'-3'
Yhr177w Reverse	TGAGCGGTACTTTAGGTGAACCTTTCAGCAATTGAAAGGATGCTGATCTTGAATTCGAGCTCGTTTAAAC
Flo11 GFP Replace Forward	AATTAAAATATACTTTTGTAGGCCTCAAAAATCCATATACGCACACTATGAGTAAAGGAGAAGAACTTTTCAC
Flo11 GFP Replace Reverse	AATTAAAATATACTTTTGTAGGCCTCAAAAATCCATATACGCACACTATGAGTAAAGGAGAAGAACTTTTCAC
Mit1 GFP Forward [deletion]	TTCCACAAAACAACATTTCAACTAGACACAAGGACAACGTAAATTTCTAAATGAGTAAAGGAGAAGAAC
Mit1 GFP Reverse [deletion]	AGGCAAGGAGAAGAAAAGAAAATCGGAGTACTTTTTTAAAATATATTTACTGCATAGGCCACTAGTGGATCTG
Mit1-GFP Fusion Forward	AATGATACGATGAACAACCTCAATACAAACACTTCAACTACTACACAATAAGGGATCCAAGGGTTAATTAA
Mit1-GFP Fusion Reverse	AGGCAAGGAGAAGAAAAGAAAATCGGAGTACTTTTTTAAAATATATTTACTGCATAGGCCACTAGTGGATCTG
Ectopic Expression Plasmid Construction	
Ryp1 Forward	aaaGGATCCatgggcaacggcacagctgc
Ryp1 Reverse	aaaGAATTCtcaacctgttgcagccgtat
Mit1 Forward	aattcctctagaggatccatggatatcgagcctacttt
Mit1 fragment Reverse	gtt tgc act agt aaa ttg att ac