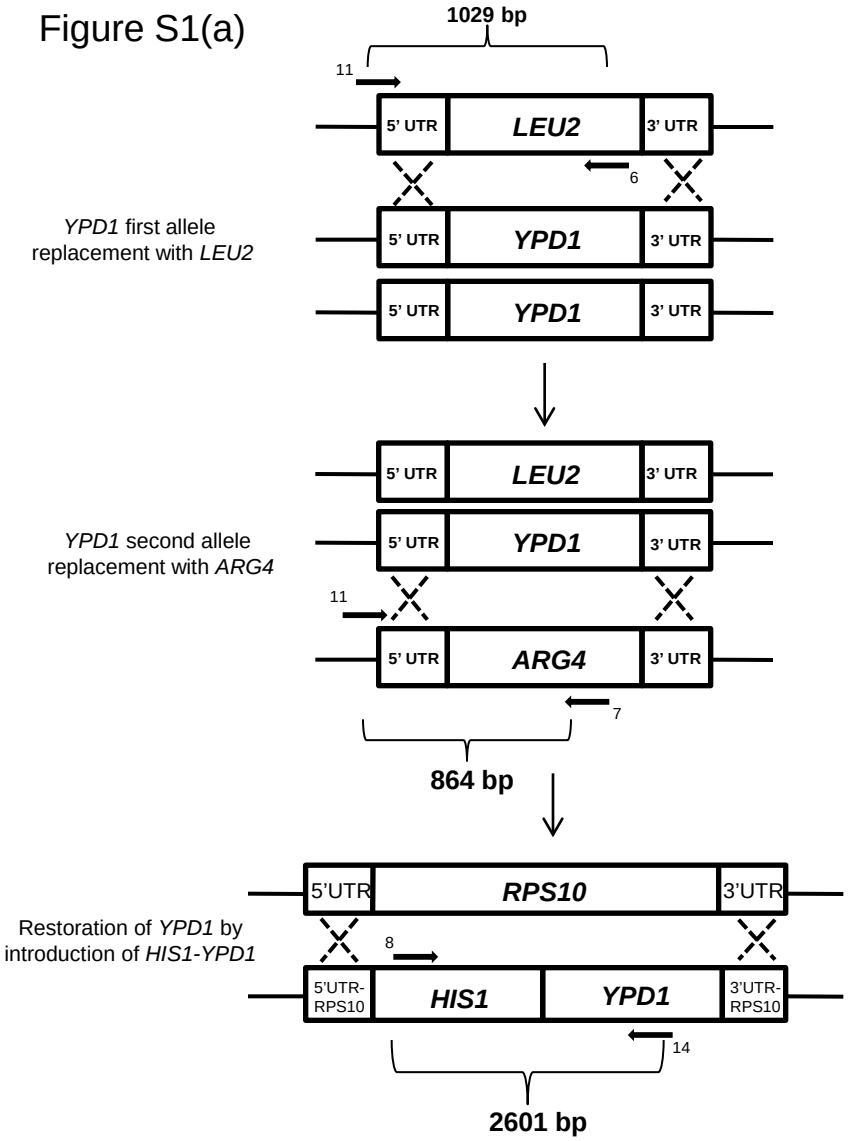
Figure S1(a)



S1(b)

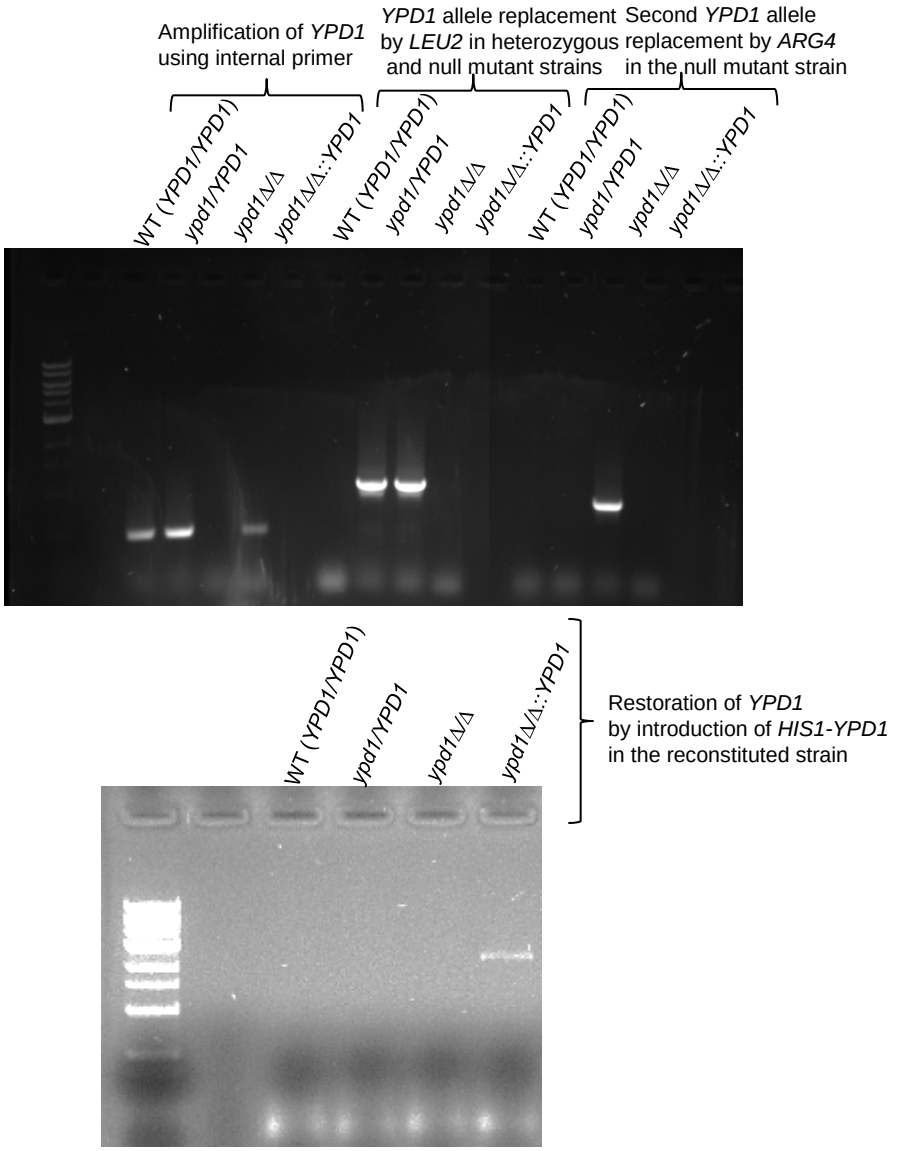


Figure S1. Verification of the *ypd1Δ/Δ* mutant and reconstituted strains (a) Schematic representation (size not to scale) of the construction of disruption cassettes to delete *YPD1* gene. (b) confirmation of *YPD1* deletion by PCR analysis. Lanes 1 to 4: (1) wild type SN425 (*YPD1/YPD1*), (2) heterozygous (*ypd1/YPD1*), (3) homozygous (*ypd1Δ/Δ*) and (4) reconstituted (*ypd1Δ/Δ::YPD1*) strains. Genomic DNA from these strains was used to confirm *YPD1* allele replacement and reconstitution. The PCR primer combinations are given on top of arrows and the expected band size is indicated by braces on top of each allele replacement schematic. The *ypd1Δ/Δ* was constructed by following the method of noble and Johnson (1). The *C. albicans* strains and plasmids used to disrupt *YPD1* are described in detail elsewhere (2-3).

Construction of *YPD1* deletion mutant and reconstituted strains in *C. albicans* SN148 (Ura^-) background

To ascertain whether Ypd1-GFP fusion protein is functional we performed a complementation assay. To perform this assay we first needed to create a *ypd1* null mutant strain in *C. albicans* SN148 (Ura^-) background. This was required because the Ypd1-GFP fusion plasmid contains *URA3* selection marker. The *ypd1*Δ/Δ strain described in the manuscript was created in SN152 (Ura^+) background and could not be used for complementation because *URA3* selection was not available. The *YPD1* was disrupted in SN148 (Ura^-) background by using the disruption cassettes (described in table S1) used to disrupt *YPD1* in SN152 (Ura^+) background. A gene-reconstituted strain was created by PCR amplifying the full length *YPD1*-ORF including its promoter and subcloning it into CIp10 plasmid vector (4). This plasmid was linearized with NcoI and transformed in a previously generated *ypd1*Δ/Δ homozygous null mutant strain to re-integrate *YPD1*-ORF at the RPS10 locus (ribosomal protein 10) in *C. albicans* genome. The heterozygous, null and gene-reconstituted strains created in SN148 background were confirmed by PCR analysis (data not shown).

The pACT1-*YPD1*-GFP plasmid, described in the methods section, was used to transform into the *ypd1*Δ/Δ (Ura^-) null mutant generated in the SN148 background. The recombinant plasmid containing *YPD1-GFP* fusion was linearized by using StuI prior to transformation into the *ypd1*Δ/Δ (Ura^-) null mutant. Transformants were selected for uridine prototrophy. Transformants for correct integration of the YPD1-GFP construct at the *RPS10* locus were confirmed by PCR analysis (data not shown).

Complementation assay

The *C. albicans ypd1*Δ/Δ strain expressing Ypd1-GFP, the wild-type strain (*YPD1*/*YPD1*), the null mutant strain *ypd1*Δ/Δ, and the gene-reconstituted strain (*ypd1*Δ/Δ::*YPD1*) were incubated at 30°C for 48 h on YPD agar plates containing 0.025% SDS. As described in the manuscript, the *ypd1*Δ/Δ strain was hypersensitive to SDS, while the sensitivity of the *ypd1*Δ/Δ::*YPD1*-GFP fusion strain to SDS was similar to that of the wild-type strain, suggesting that the GFP fusion protein restores the functional activity of Ypd1 after loss of the endogenous protein (see figure S2). Therefore, these data support the conclusion that the Ypd1-GFP fusion protein retains function in *C. albicans ypd1*Δ/Δ strain transformed with pACT1-*YPD1*-GFP and it complements the loss of Ypd1 (see figure S2).

Figure S2

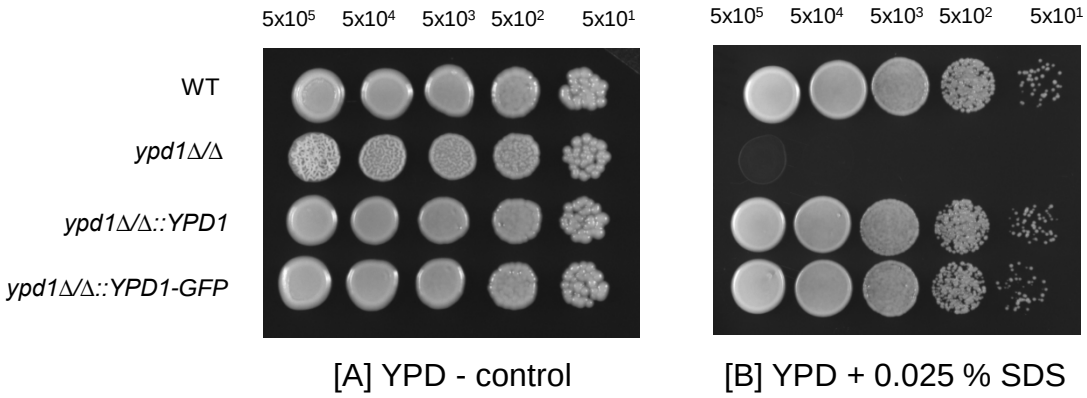


Figure S2. Growth of wild-type (WT) (*YPD1/YPD1*), *ypd1Δ/Δ*, *ypd1ΔΔ::YPD1* and *ypd1ΔΔ::YPD1-GFP* strains of *C. albicans* at 30°C for 48 h on YPD agar (control) (A) and YPD agar containing 0.025% SDS (B). Five-microliter cell dilutions ($5x10^5$ to $5x10^1$ cells) were spotted onto each plate.

References

1. Noble SM, Johnson AD. 2005. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. Eukaryot Cell. **4**(2):298-309.

2. Noble SM, French S, Kohn LA, Chen V, Johnson AD. 2010. Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat Genet. **42**(7):590-8.

3. Desai C, Mavrianos J, Chauhan N. 2011. *Candida albicans* SRR1, a putative two-component response regulator gene, is required for stress adaptation, morphogenesis, and virulence. Eukaryot Cell **10**:1370-1374.

4. Murad, A. M., P. R. Lee, I. D. Broadbent, C. J. Barelle, and A. J. Brown. 2000. CIp10, an efficient and convenient integrating vector for *Candida albicans*. Yeast **16:**325-7.

Supplementary Table S1. List of plasmids used in the present study

Plasmid	Description /genotype	Reference
pSN40	Cloning vector for construction of *C. albicans* gene deletion mutants; constructed by subcloning the *C. maltosa LEU2* ORF including promoter and terminator sequences into the pCR-Blunt II-TOPO vector (Invitrogen); *LEU2*, Kan^r markers	24
pSN69	Cloning vector for construction of *C. albicans* gene deletion mutants; constructed by subcloning the *C. dubliniensis ARG4* ORF including promoter and terminator sequences into the pCR-Blunt II-TOPO vector (Invitrogen); *ARG4*, Kan^r markers	24
pSN75	Cloning vector for reconstitution of *C. albicans* genes.	25
pNC-YPD1-5'UTR	Knock-out construct in which a 641bp SacI/SpeI PCR fragment carrying 5'-UTR with the promoter region of *YPD1* and a 460bp NotI/ApaI PCR fragment carrying the 3' UTR region of *YPD1* gene were cloned flanking the *LEU2* marker into pSN40	This study
pNC-YPD1-3'UTR	Knock-out construct in which a 641bp KpnI/SpeI PCR fragment carrying 5'-UTR with the promoter region of *YPD1* and a 460bp NotI/ApaI PCR fragment carrying the 3' UTR region of *YPD1* gene were cloned flanking the *ARG4* marker into pSN69	This study
pSN75-YPD1	A strain reconstitution plasmid construct in which a 1425 bp Xho1 fragment containing the complete 555 bp YPD1 gene ORF along with promoter/terminator regions were cloned into pSN75 vector.	This study
pACT1-GFP	pACT-GFP vector contains the codon-optimized yeast enhanced green fluorescent protein as reporter whose expression is driven by *CaACT1* promoter. pACT-GFP is based on parent plasmid CIp10 which provides homology for integration of target GFP-tagged genes at chromosomal RPS1 locus in *C. albicans*.	27
pACT1-YPD1-GFP	Reporter construct in which, 846 bp *YPD1* lacking a stop codon was amplified and cloned into HindIII site of pACT1-GFP. Resulting vector expresses a GFP-tagged Ypd1 protein.	This study

Supplementary Table S2. List of primers used in the present study.

No.	Primer	Sequence (5'-3')
1	YPD1-5'UTR-SacF	ATGCGAGCTCACGTCATTGAGGTGGAGTGAGGTT
2	YPD1-5'UTR-KpnF	ATGCGGTACCACGTCATTGAGGTGGAGTGAGGTT
3	YPD1-5'UTR-SpeR	ATGCACTAGTGCCGTCTTGATTGGTTATCACTGC
4	YPD1-3'UTR-NotF	ATGCGCGGCCGCATGGGTGTGCTTTGTTAGAGCAGC
5	YPD1-3'UTR-ApaR	ATGCGGGCCCTTTCCCTGCATCTTTCTTTTTACCGG
6	LEU2 reverse	CAATGCAATATCTTTCAATGCCATT
7	ARG4 reverse	TTTTACAAGTATGAAAGGAGGGGAAG
8	HIS1 forward	AATGCTGCAGCTTATTGAGCGGTGCC
9	Recon-XhoI-F	ATGCCTCGAGAACATATGCCTTGCTATTTTTTTAAAATATATC
10	Recon-XhoI-R	ATGCCTCGAGCTATTCGTAATATTCGTCCAATGCTCTTCT
11	YPD1-Outside 5'F	GTAATTCCATTTATTATTTTGTTGGTCTCCCTC
12	YPD1-Outside 3'R	CGAGAATTATGGTGCACCATTGCAAACATT
13	YPD1-Internal-F	TGTCGACTGGGCAGTGTTTAGTGA
14	YPD1-Internal-R	TTGGTCGAATCCATCTCTCGCCTT
15	YPD-ACT-GFP-F	AGTCAAGCTTATGTCAGAAGATAAATTACAAAAATTAC
16	YPD-ACT-GFP-R	AGTCAAGCTTTTCGTAATATTCGTCCAATG