Electronic Supplementary Information

(ESI)

Candida albicans Reprioritizes Metal Handling During Fluconazole Stress

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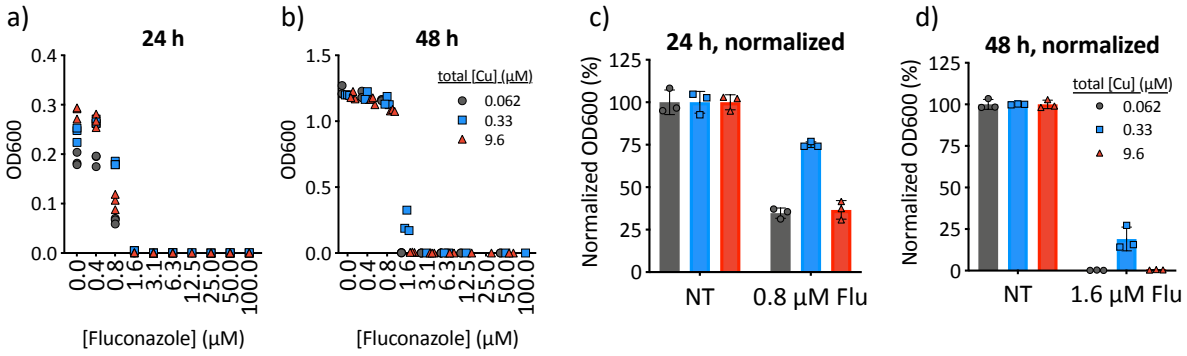
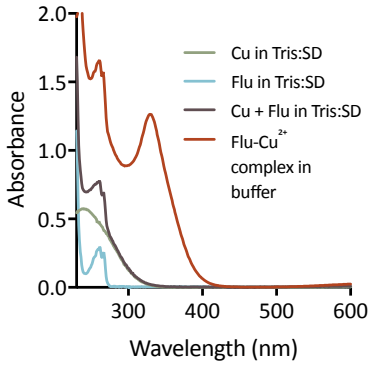
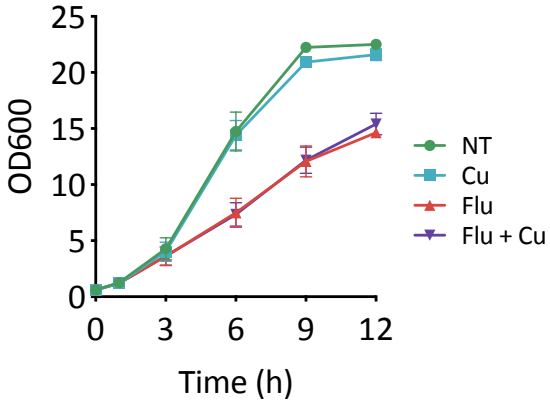


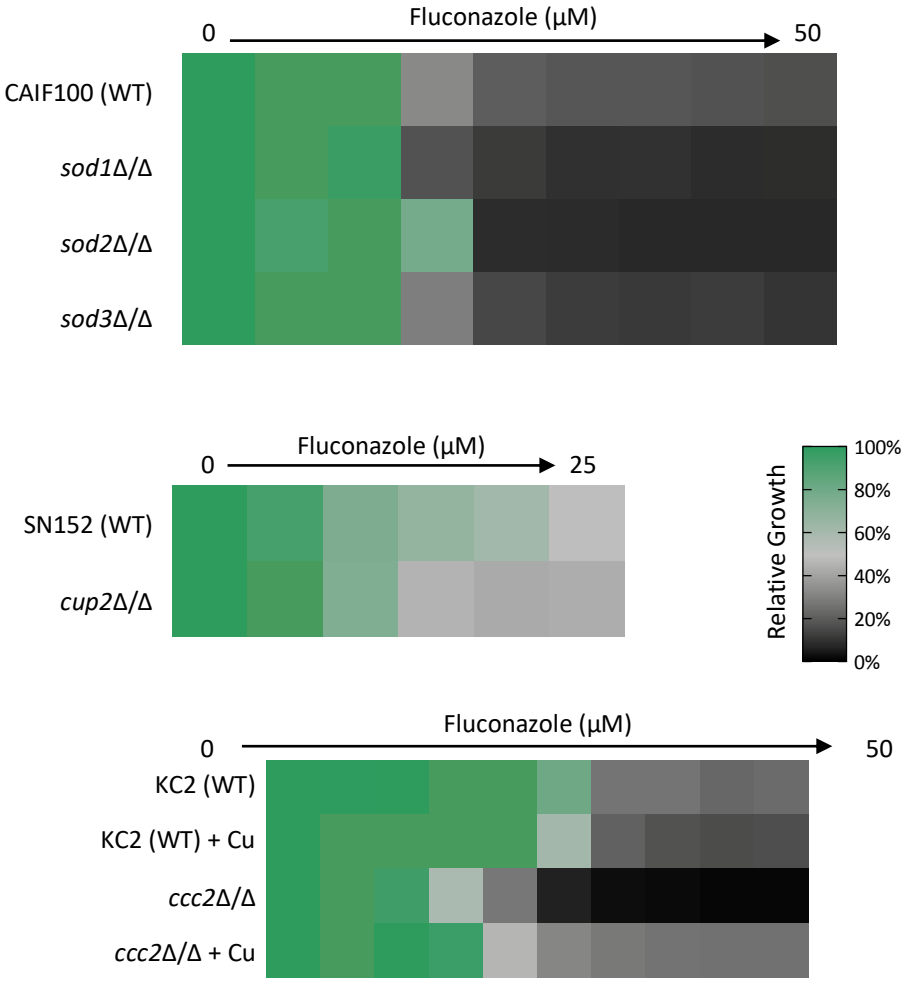
Supplementary Figure 1 | Growth of *C. albicans* in Chelex-treated YPD is abnormally slow, even after Mg, Ca, Cu, Fe, Mn, Zn, Ni, and Co levels have been restored, but Cu depletion or supplementation still potentiates fluconazole activity. OD600 values after 24 h (**a**) and 48 h (**b**) and Normalized OD600 values as percentages after 24 h (**c**) and 48 h (**d**) for *C. albicans* cells treated with 0–100 µM fluconazole in Cu deplete (gray dots or bars), Cu basal (blue squares or bars), or Cu-supplemented (red triangles or bars) YPD media. In comparison, OD600 readings greater than 1 are typically obtained for untreated cells at 24 h in normal YPD that has not been treated with Chelex. Data are reported as Mean ± SD for 3 replicates. Data normalization was performed relative to the untreated control for each Cu condition. Preparation of Chelex-treated YPD containing deplete, basal, or supplemental Cu levels is described in **Methods**. Metal content of each Cu formulation is provided in **Suppl. Tables 2–4**.



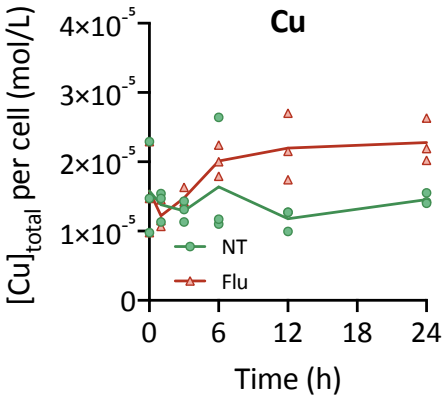
Supplementary Figure 2 | Fluconazole does not bind Cu(II) in Tris:SD medium. UV-Vis spectra of fluconazole and Cu in Tris:SD. Fluconazole (light blue trace) and Cu (green trace) have distinct signals in Tris:SD. Addition of both fluconazole and Cu (gray trace) did not result in a charge transfer band at 327 nm. The spectrum of the Cu(II)-fluconazole complex formed in HEPES buffer is overlaid for comparison (red trace). Conditions: In Tris:SD medium, pH 7.4 [Flu] = 400 µM, [$CuSO_4$] = 200 µM. In 50 mM HEPES buffer, pH 7.4 [Flu] = 1000 µM, [$CuCl_2$] = 800 µM.



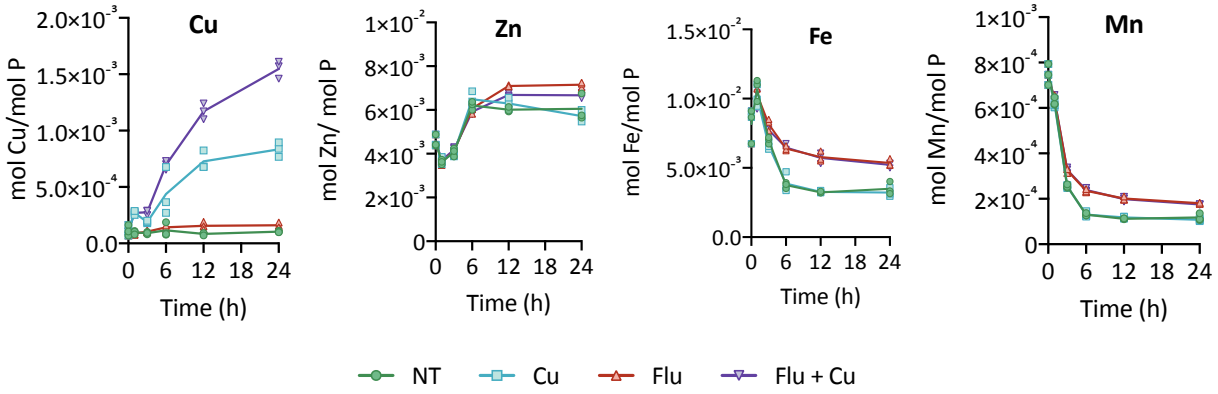
Supplementary Figure 3 | Growth curve of *C. albicans* cells treated at mid-log phase. 50-mL cultures of *C. albicans* cells at mid-log phase (OD~0.6) were treated with nothing (green circles), 10 µM Cu (blue squares), 10 µM fluconazole (red triangles), or both fluconazole and Cu (purple inverted triangles) and growth was determined over the course of 12 h by measuring OD600. Data are reported as average ± SD (*n* = 3).



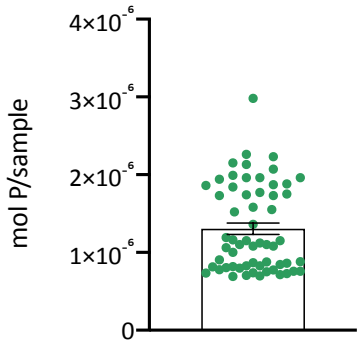
Supplementary Figure 4 | Susceptibility of additional *C. albicans* deletion strains to fluconazole. *C. albicans* deletion strains were treated with 0–50 μM fluconazole (diluted 2-fold) in YPD medium at 30 °C for 48 h then growth was determined by measuring OD600. Data are normalized to the untreated control for each strain. For the *ccc2*Δ/Δ strain, Cu supplementation reversed sensitivity to fluconazole. Cu supplementation did not strongly potentiate activity of fluconazole in the wild-type (WT) KC2 strain. Shown are representative heat maps from n = 2 biologically independent experiments.



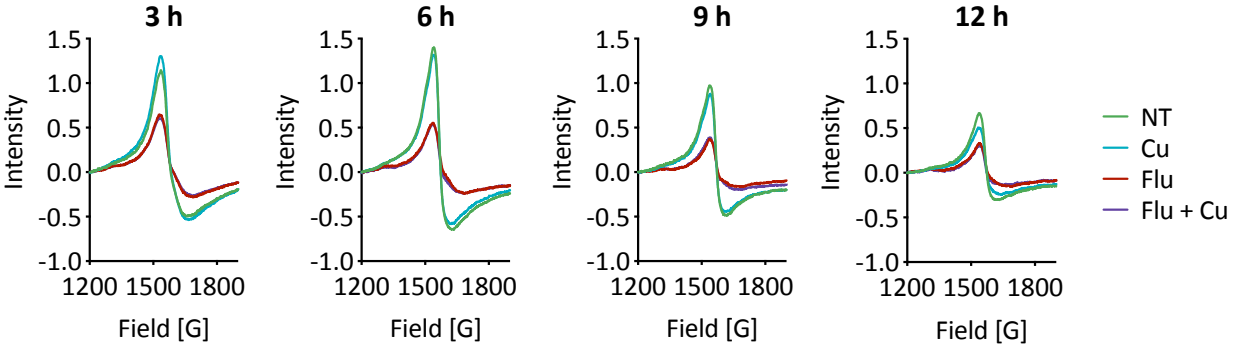
Supplementary Figure 5 | Expanded view of Fig. 4a from main text. Treatment with 10 µM fluconazole (red line) increases levels of cell-associated Cu relative to untreated cells (green line). Cell-associated metal levels were analyzed by ICP-MS. Lines represent the average from *n* = 3 biologically independent samples per timepoint.



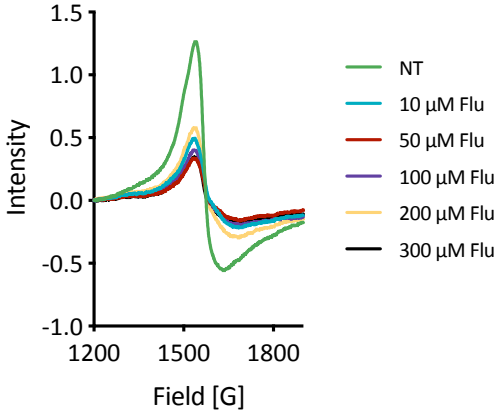
Supplementary Figure 6 | Metal content of *C. albicans* cells reported as mol Cu/mol P. Conditions: [Flu] = 10 µM, [$CuSO_4$] = 10 µM. Cells were grown in YPD medium for time indicated in figure legends. Cell-associated metal levels were analyzed by ICP-MS. Lines represent the average from *n* = 3 biologically independent samples per timepoint.



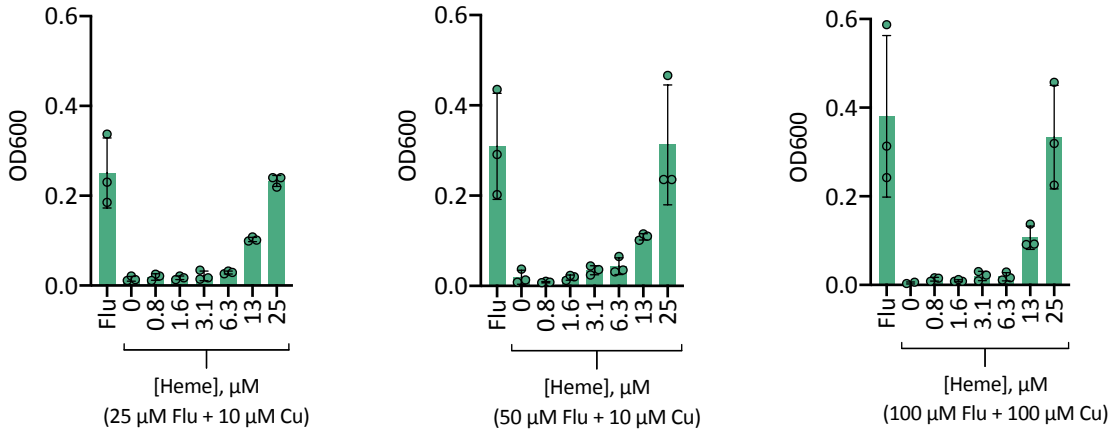
Supplementary Figure 7 | Analytical phosphorus content of cell samples as determined by ICP-MS. There was an average of 1.30 ± 0.07 x 10^{-6} mol P per sample. Data are reported as Mean ± SEM (*n* = 59).



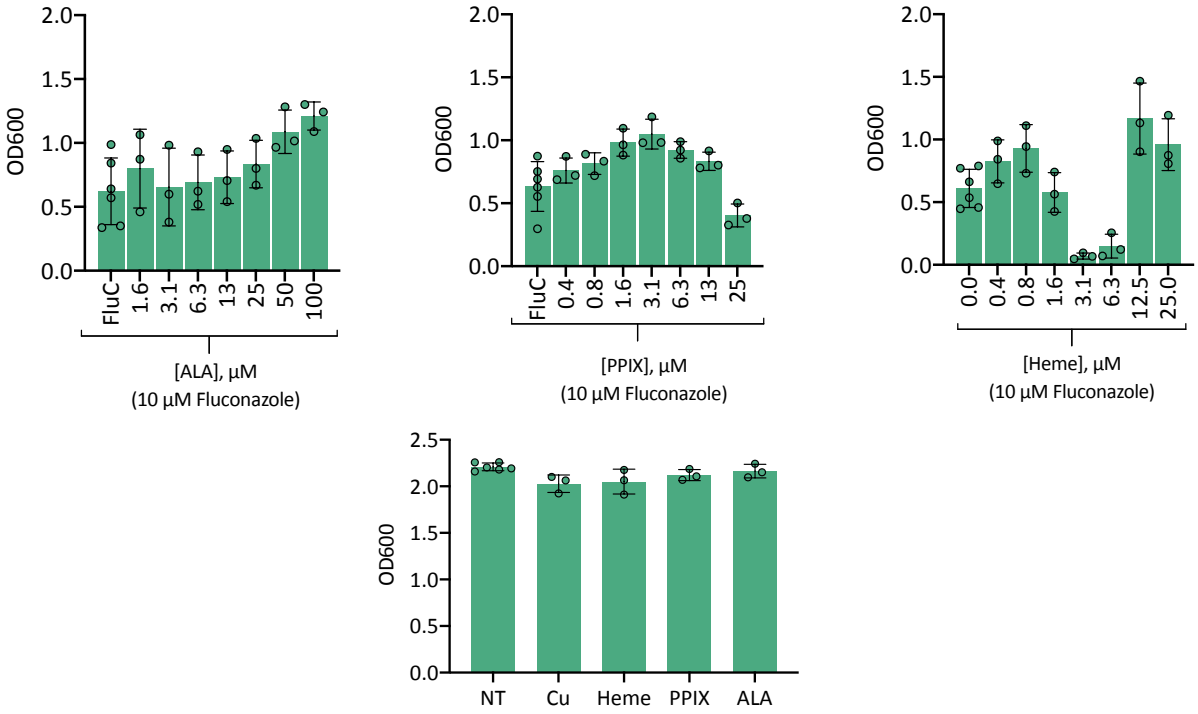
Supplementary Figure 8 | Time-course whole cell EPR spectra for *g* = 4.3 signal. Fluconazole treatment +/- Cu supplementation depletes EPR-detectable Fe by 3 h of treatment. The signal remains attenuated for these conditions through 12 h. When the cells reach stationary phase around 12 h, the signal is lower for untreated and Cu-supplemented cells relative to earlier timepoints.



Supplementary Figure 9 | Impact of fluconazole concentration on attenuation of signal at *g* = 4.3. Increasing the concentration of fluconazole above 10 µM does not further deplete the labile Fe signal at *g* = 4.3.



Supplementary Figure 10 | Sub-stoichiometric levels of heme rescue growth of cells treated with fluconazole and Cu. 48-h growth of *C. albicans* treated with fluconazole alone, fluconazole and Cu, or fluconazole and Cu plus heme at concentrations indicated in figure legends. Data are reported as average ± SD.



Supplementary Figure 11 | Controls for ALA, PPIX, and heme supplementation growth assays. (**top**) Supplementation of fluconazole-treated cells with ALA, PPIX, or heme promotes 48-h growth under most concentrations tested. (**bottom**) Treatment with Cu, heme, PPIX, or ALA does not impact 48-h growth of *C. albicans*. Conditions: [$CuSO_4$] = 10 µM, [Heme] = 25 µM, [PPIX] = 25 µM, [ALA] = 100 µM in YPD medium. Data are reported as average ± SD.

Supplementary Table 1 | Metal content of Chelex-treated YPD medium (µM) prepared from lot # 1882445

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	< 1.0	< 0.8	0.0609	0.920	< 0.002	0.850	0.102	0.0170
2	< 1.0	< 0.8	0.0333	1.13	< 0.002	0.900	0.107	0.0160
3	< 1.0	< 0.8	0.0282	1.03	< 0.002	0.692	0.106	0.0140

Supplementary Table 2 | Metal content of Chelex-treated YPD medium "Cu deplete" (µM) prepared from lot # 1882445

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	120	73.0	0.0528	7.16	0.265	43.2	0.169	0.118
2	123	74.9	0.0771	7.04	0.277	43.5	0.177	0.117
3	122	73.7	0.0574	6.99	0.279	43.5	0.163	0.110

Supplementary Table 3 | Metal content of Chelex-treated YPD medium "Cu basal" (µM) prepared from lot # 1882445

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	121	73.4	0.322	7.20	0.285	43.4	0.174	0.114
2	119	72.1	0.330	6.97	0.286	43.3	0.166	0.108
3	122	73.7	0.337	9.41	0.284	44.0	0.165	0.108

Supplementary Table 4 | Metal content of Chelex-treated YPD medium "Cu suppl." (µM) prepared from lot # 1882445

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	123	74.8	9.84	6.83	0.287	42.8	0.165	0.111
2	123	74.4	9.43	6.82	0.268	42.6	0.159	0.113
3	120	72.6	9.54	6.81	0.272	42.8	0.176	0.116

Supplementary Table 5 | Metal content of YPD medium (µM) lot #2005064

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	206	131	0.143	11.2	0.166	22.1	0.213	0.143
2	198	127	0.132	10.6	0.159	20.9	0.201	0.135
3	208	135	0.136	11.0	0.170	21.8	0.207	0.138

Supplementary Table 6 | Metal content of YPD medium (µM) lot #2044606

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	126	100	0.165	8.69	0.303	17.4	0.340	0.0795
2	122	96.7	0.168	8.46	0.290	16.6	0.327	0.0776
3	125	100	0.161	8.63	0.305	17.3	0.334	0.0780

Supplementary Table 7 | Metal content of Tris:SD Cu Drop-Out medium (µM)

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	2200	705	0.0433	0.829	3.11	2.46	0.00240	0.0171
2	2130	682	0.0417	0.794	3.01	2.33	0.00192	0.0170
3	2160	692	0.0429	0.826	3.06	2.41	0.00190	0.0178

Supplementary Table 8 | Metal content of Tris:SD Fe Drop-Out medium (µM)

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	2120	674	0.221	0.0211	3.00	2.31	0.00201	0.0168
2	2110	664	0.221	0.0169	2.98	2.31	0.00191	0.0169
3	2080	658	0.219	0.0157	2.95	2.33	0.00181	0.0170

Supplementary Table 9 | Metal content of Tris:SD Mn Drop-Out medium (µM)

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	2170	675	0.231	0.520	0.0144	2.35	0.00181	0.0178
2	2100	658	0.218	0.504	0.0139	2.28	0.00170	0.0180
3	2110	658	0.221	0.503	0.0134	2.30	0.00180	0.0180

Supplementary Table 10 | Metal content of Tris:SD Zn Drop-Out medium (µM)

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	2260	701	0.216	0.534	3.15	0.0785	0.00193	0.0193
2	2080	650	0.199	0.497	2.89	0.0629	0.00224	0.0181
3	2190	674	0.207	0.532	3.06	0.0604	0.00205	0.0184

Supplementary Table 11 | List of Primers Used in this Study

Primer Name	Sequence	Comments
EFB1-F	AGATGTCGACTTGTTCGGTTCTG	*EFB1* qRT-PCR primer forward
EFB1-R	CGATAGCTTTGACGTTGGT	*EFB1* qRT-PCR primer reverse
CTR1-F	GACACGAAGGTCATATGCA	*CTR1* qRT-PCR primer forward
CTR1-R	AAATGAAGCTGTAAGCCACAT	*CTR1* qRT-PCR primer reverse
CRP1-F	ACACAATGGTGACGAAACCGT	*CRP1* qRT-PCR primer forward
CRP1-R	AATCTGAATCCGTCGACTTCTTC	*CRP1* qRT-PCR primer reverse
CUP1-F	AATTCGAATTAGTTAACTACGCA	*CUP1* qRT-PCR primer forward
CUP1-R	TTGGAAGCACATTTGCATTCAGT	*CUP1* qRT-PCR primer reverse
SOD1-F	ATGGTTAAAGCTGTCGCTGTTGT	*SOD1* qRT-PCR primer forward
SOD1-R	GGATTGAAATGAGGACCAGCA	*SOD1* qRT-PCR primer reverse
SOD3-F	GGATCAGGTTGGGCATTTAT	*SOD3* qRT-PCR primer forward
SOD3-R	CAGTTGATCACGTTCCAAATTGC	*SOD3* qRT-PCR primer reverse

Supplementary Table 12 | List of Strains Used in this Study

Name	Yeast	Genotype	Source/Description
SC5314	*C. albicans*	Wild-type	Obtained from the American Type Culture Collection. Wild-type strain used in the *C. albicans* sequencing project.(60)
*ctr1*Δ/Δ	*C. albicans*	*ctr1Δ::loxP/ctr1Δ::loxP*	Obtained from the Brown lab at the University of Aberdeen. Originally reported by Mackie et al.(11)
SN152	*C. albicans*	*his1Δ/his1Δ*, *leu2Δ/leu2Δ*, *arg4Δ/arg4Δ*, *URA3/ura3Δ::imm434*, *IRO1/iro1Δ::imm434*	Obtained from the Fungal Genetics Stock Center.(57) Originally reported by Noble et al.(56)
*mac1*Δ/Δ	*C. albicans*	*mac1Δ::LEU2/mac1Δ::HIS1*	Obtained from the Fungal Genetics Stock Center.(57) Originally reported by Homann et al.(29)
*cup2*Δ/Δ	*C. albicans*	*cup2Δ::LEU2/cup2Δ::HIS1*	Obtained from the Fungal Genetics Stock Center.(57) Originally reported by Homann et al.(29)
CA-IF100	*C. albicans*	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ::cmLEU2*, *his1Δ/his1Δ::cdHIS1*, *URA3/ura3Δ*	Obtained from the Culotta lab at Johns Hopkins University. Originally reported by Frohner et al.(61)
*sod1*Δ/Δ	*C. albicans*	*sod1Δ::cmLEU2/sod1Δ::cdHIS1*	Obtained from the Culotta lab at Johns Hopkins University. Originally reported by Frohner et al.(61)
*sod2*Δ/Δ	*C. albicans*	*sod2Δ::cmLEU2/sod2Δ::cdHIS1*	Obtained from the Culotta lab at Johns Hopkins University. Originally reported by Frohner et al.(61)
*sod3*Δ/Δ	*C. albicans*	*sod3Δ::cmLEU2/sod3Δ::cdHIS1*	Obtained from the Culotta lab at Johns Hopkins University. Originally reported by Frohner et al.(61)
KC2	*C. albicans*	*ura3Δ::imm434/ura3Δ::imm434*	Obtained from the Culotta lab at Johns Hopkins University. Also called CAF3-1. Originally reported by Fonzi and Irwin.(62)
*crp1*Δ/Δ	*C. albicans*	*crp1Δ::hisG/crp1Δ::hisG*	Obtained from the Culotta lab at Johns Hopkins University. Originally reported by Weissman et al.(30)
*cup1*Δ/Δ	*C. albicans*	*cup1Δ::hisG/cup1Δ::hisG*	Obtained from the Culotta lab at Johns Hopkins University. Originally reported by Weissman et al.(30)
*crp1*Δ/Δ *cup1*Δ/Δ	*C. albicans*	*crp1Δ::hisG/crp1Δ::hisG*, *cup1Δ::hisG/cup1Δ::hisG*	Obtained from the Culotta lab at Johns Hopkins University. Originally reported by Weissman et al.(30)
*ccc2*Δ/Δ	*C. albicans*	*ccc2Δ::hisG/ccc2Δ::hisG*	Obtained from the Culotta lab at Johns Hopkins University. Originally reported by Weissman et al.(63)