Supplemental Materials

Copper Availability Influences the Transcriptomic Response of *Candida albicans* to Fluconazole Stress

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Supplemental Materials and Methods

Total RNA isolation. Total RNA was isolated from frozen pellets using a Qiagen RNeasy Mini Kit, and DNase was treated with a Turbo DNA-free kit (Roche) according to the manufacturer's instructions. RNaseZap RNase Decontamination Solution (Invitrogen) was used on all materials and surfaces to preserve RNA integrity.

Preparation of libraries for sequencing. Extracted total RNA quality and concentration was assessed on a Fragment Analyzer (Agilent Technologies) and Qubit 2.0 (ThermoFisher Scientific), respectively. Only extracts with RNA Integrity Number (RIN) greater than 7 were processed for sequencing. RNA-seq libraries were prepared using the commercially available KAPA Stranded mRNA-Seq Kit. In brief, mRNA transcripts were first captured using magnetic oligo-dT beads, fragmented using heat and magnesium, and reverse transcribed using random priming. During the second strand synthesis, the cDNA:RNA hybrid was converted into double-stranded cDNA (dscDNA) and dUTP incorporated into the second cDNA strand, effectively marking the second strand. Illumina sequencing adapters were then ligated to the dscDNA fragments and amplified to produce the final RNA-seq library. The strand marked with dUTP was not amplified, allowing strand-specificity sequencing. Libraries are typically indexed using a dual indexing approach allowing for multiple libraries to be pooled and sequenced on the same sequencing lane on a NovaSeq 6000 Illumina sequencing platform. Before pooling and sequencing, fragment length distribution and library quality were first assessed on a Fragment Analyzer. All libraries were then pooled in equimolar ratio and sequenced. Multiplexing 75 libraries on one lane of an Illumina S1 NovaSeq flow cell will yield about 10 million 50 bp paired-end sequences per sample. Once generated, sequence data was demultiplexed and Fastq files generated using Bcl2Fastq conversion software provided by Illumina.

Processing of RNA-seq data. After quality filtering and adaptor trimming by Cutadapt (v 1.12),[1] the paired-read alignment was carried out against the transcriptomes of *Candida albicans* SC5314 (GCF_000182965.3), using STAR (v 2.5.3a).[2] To identify the expression differences across the treatment groups, the read counts were first filtered and normalized using the R package "RSEM" (v2.20).[3] Genes with read counts below a threshold of 10 in all samples were filtered out. The read counts of all genes for each sample were then estimated with RSEM. Subsequently, the raw count data was converted to counts per million (CPM) and normalized by adjusting with the effective library size via the 'calcNormFactors' function implemented in the R package edgeR (v. 3.26.7).[4] The significant DEGs, comparing to NT condition, were determined by using the negative binomial generalized linear models with quasi-likelihood tests ('glmQLFTest' functions in the edgeR package) with FDR *P* values of less than 0.05.

ICP-MS Measurements. ICP-MS measurements were performed by Dr. Martina Ralle in the OHSU Elemental Analysis Core with partial support from the NIH instrumentation grant S10RR025512. Measurements were performed using an Agilent 7700x equipped with an ASX 500 autosampler. The system was operated at a radio frequency power of 1550 W, an argon plasma gas flow rate of 15 L/min, and Ar carrier gas flow rate of 0.9 L/min. Elements were measured in in kinetic energy discrimination (KED) mode using He gas (4.3 ml/min). Data were quantified using an 11-point (0, 0.5, 1, 2, 5, 10, 20, 50, 100, 500, 1000 ppb (μg/kg)) for all elements except Mg and Ca (0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 50, 100 ppm

(µg /kg)) using a multi-element standard (CEM 2, (VHG-SM70B-100)) and single element standards for Mg (Inorganic Ventures, CGMG-1) and Ca (Inorganic Ventures, CGCA-1). For each sample, data were acquired in triplicates and averaged. A coefficient of variance (CoV) was determined from frequent measurements of a sample containing approximately 10 ppb of all elements except Ca (1 ppm). An internal standard (Sc, Ge, Bi) continuously introduced with the sample was used to correct for detector fluctuations and to monitor plasma stability. Accuracy of the calibration curve was assessed by measuring NIST reference material (water, SRM 1643f) twice during the measurement and found to within ± 4% for all determined elements.

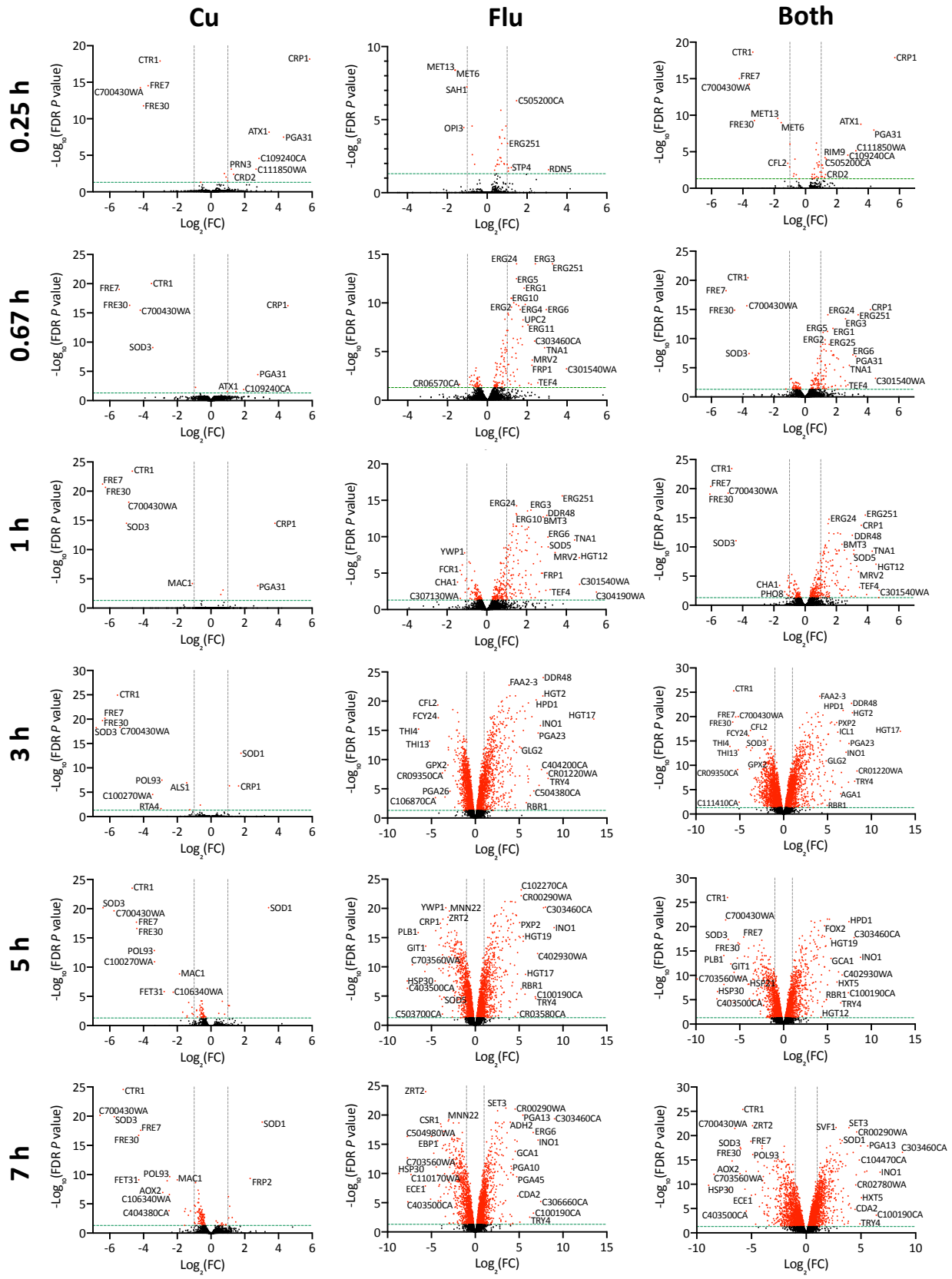

Fig. S1. Volcano plots of differentially expressed genes following treatment with Cu, fluconazole (Flu), or Both at all timepoints. Dashed green lines indicate the cut-off for FDR *P* values < 0.05 and dashed gray lines indicate boundaries for Log_2(FC) +/- 1. Points with FDR P values < 0.05 are red and those > 0.05 are black. Genes falling above the dashed green line and outside of the dashed gray lines were considered significantly changed. Significant DEGs had a log FC ≥ 1 or ≤ -1 and an FDR *P* value < 0.05.

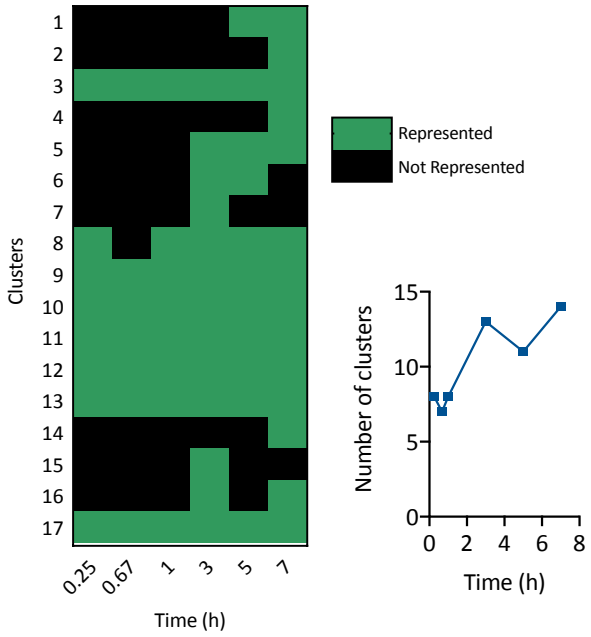

Fig. S2. Heat map illustrating representation of clusters at each timepoint. For each timepoint, clusters that were represented are denoted in green and those that were not represented are indicated in black. (Inset) Number of unique clusters represented at each timepoint.

Table S1. Metal content of YPD medium (µM) lot #2005064

	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
Conc ±	210 ±	131 ±	0.143 ±	11.2 ±	0.166 ±	22.1 ±	0.213 ±	0.143 ±
CoV	20	2	0.004	0.2	0.003	0.4	0.004	0.003

References

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