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Supplemental Information

An Iron Homeostasis Regulatory Circuit with Reciprocal Roles in *Candida albicans* Commensalism and Pathogenesis

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Supplemental Experimental Procedures

Heat Maps

Heat maps of Sef1, Sfu1, and Hap43 enrichment at promoter sequences (Figure S1D) were generated by subtracting the background Cy3 and Cy5 median of the two replicate experiments and calculating the mean of $\log_2$ probe signal values of IP/whole cell extract. Genomic coordinates were extracted for 1kb upstream and downstream of the start codon of each ORF and 80bp wide bins were created in these extractions. Probe values were assigned to the bins based on the genomic coordinate of the center of each probe.

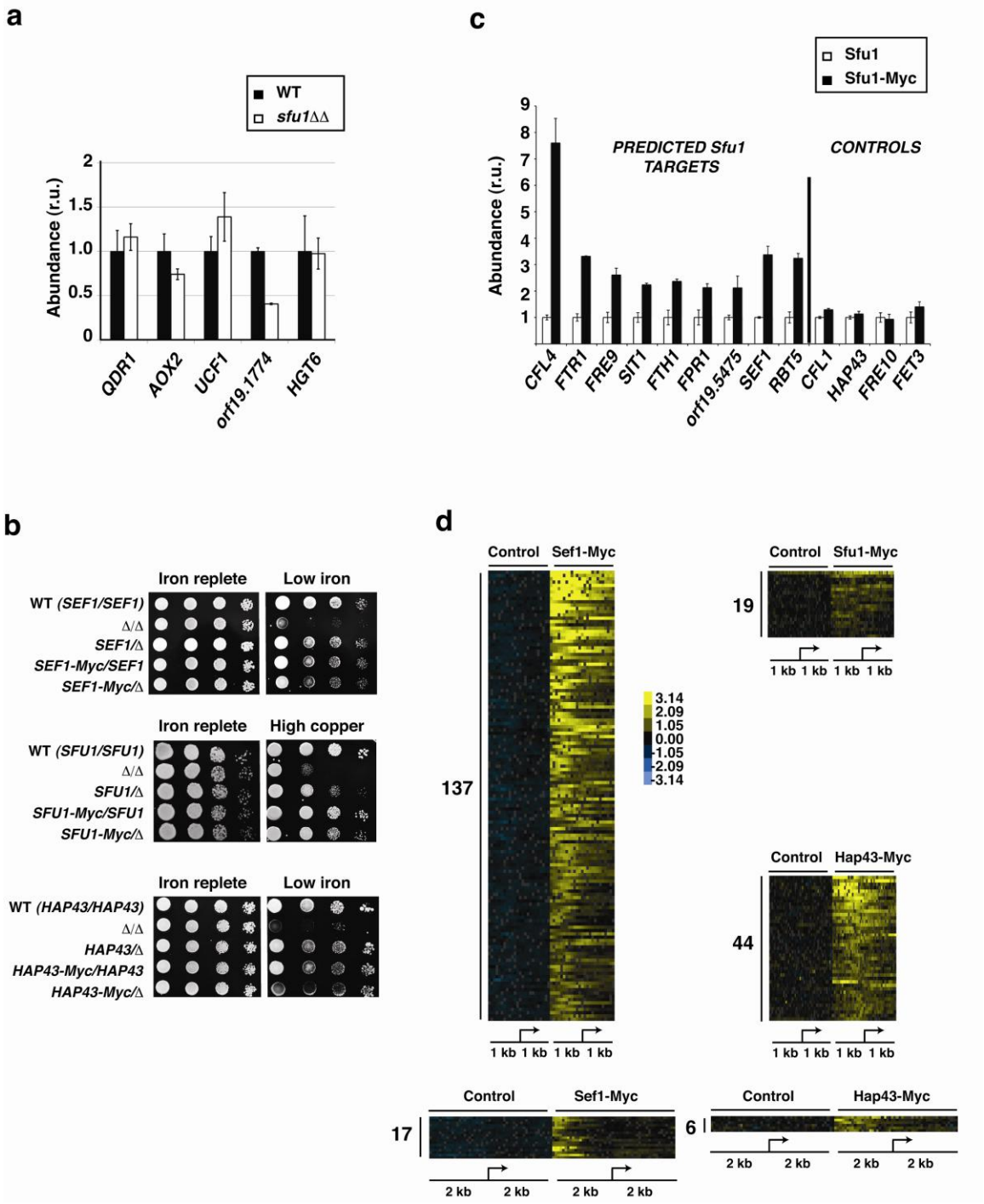

Figure S1. **Transcriptional Regulatory Activities of Sef1, Sfu1, and Hap43, Related to Figure 2**

a) Validation of *sfu1ΔΔ* microarray results using RT-PCR. *sfu1ΔΔ* and wild type strains were grown in iron-replete medium vs. iron-depleted medium, and RT-PCR was used to compare the expression of the 5 genes that were found to be highly downregulated in a previous analysis of *sfu1ΔΔ* (Lan et al.,

2004) but not in our own analysis. Error bars depict the standard deviation among triplicate samples.

b) Complementation analysis using Myc-tagged alleles of Sef1, Sfu1, and Hap43. Wild type, homozygous knockout, heterozygous knockout, and complemented strains were spotted onto the indicated media and incubated at 30°C. Sef1 and Hap43 activity were assessed on low iron medium, whereas Sfu1 activity was assessed on high copper medium (that is predicted to promote the assembly of Fet3/iron permease complexes and to increase the uptake of environmental iron).

c) Validation of predicted direct DNA binding targets of Sfu1-Myc. Four additional ChIP experiments were done comparing strains with and without Sfu1-Myc. qPCR was used to quantify the abundance of immunoprecipitated DNA in each extract corresponding to each of the 9 predicted Sfu1 direct binding targets (*CFL4, FTR1, FRE9, SIT1, FTH1, FRP1, orf19.5475, SEF1,* and *RBT5*; *PGA7* was not included since it shares a promoter region with *RBT5*), as well as 4 negative controls (*CFL1, HAP43, FRE10,* and *FET3).* White bars correspond to the control strain, black bars the epitope-tagged strain. Error bars depict the standard deviation among quadruplicate samples. Each of the 9 direct targets was at least 2 times as abundant in ChIP pellets from the epitope-tagged extracts, but none of the control promoters differed significantly between the tagged and control extracts.

d) Heat maps of promoters bound by Sef1, Sfu1, and Hap43. For each transcription factor, ChIP results in the untagged control extract are presented on the left, and results for the Myc-tagged strain are on the right. Promoter sequences include 1 kb upstream and downstream of the start codon, except in cases of larger promoters, in which 2kb upstream and downstream of the start codon are included. Note that all direct binding events are included in these maps, not just those with corresponding changes in RNA expression in the deletion mutants.

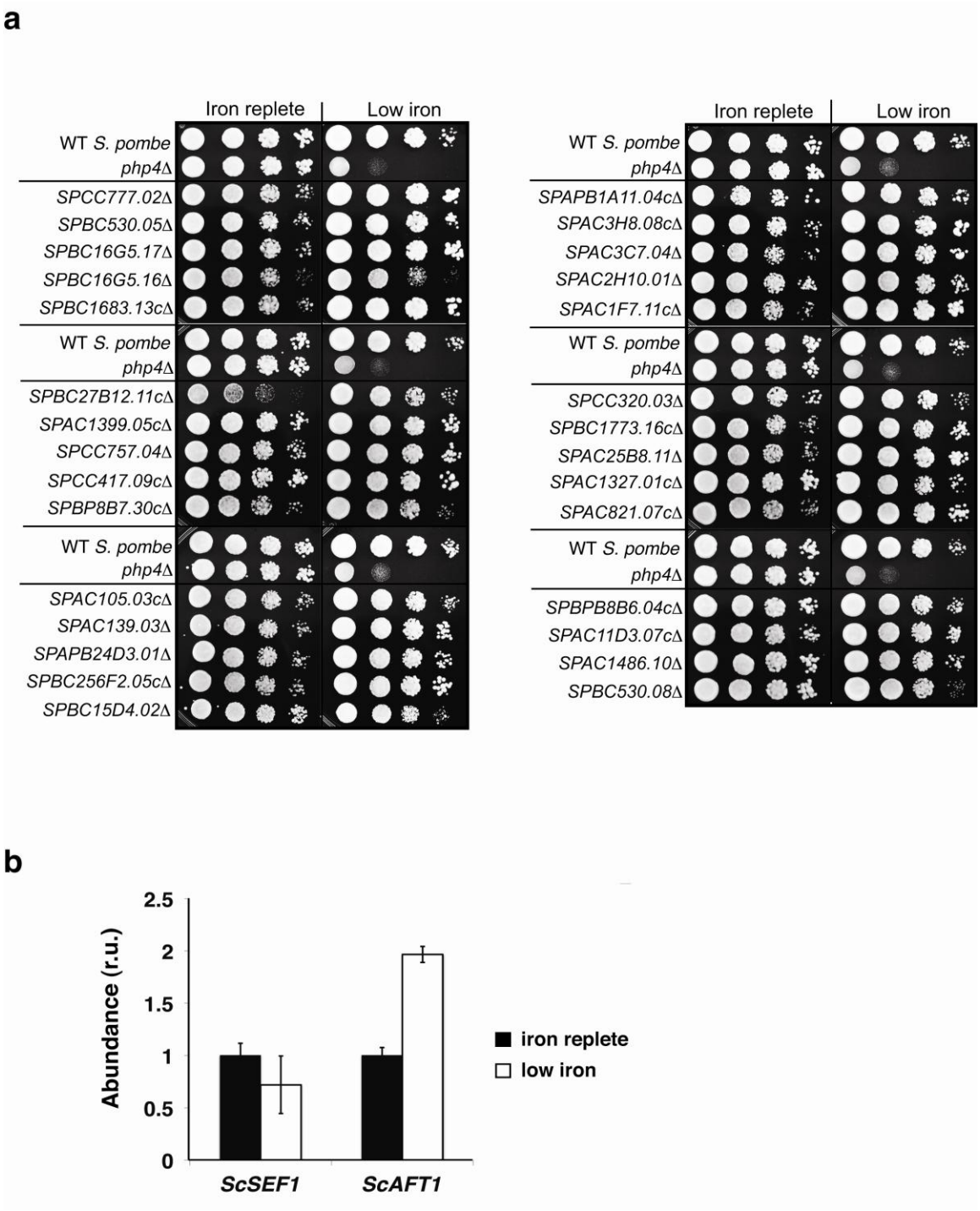

Figure S2. **Analysis of Transcription Factor Orthologs in *C. albicans, S. cerevisiae*, and *S. pombe*, Related to Figure 3**

a) Growth of viable knockouts of *S. pombe* zinc binuclear proteins on iron-depleted medium. Wild type *S. pombe*, the *php4*Δ mutant (that disrupts the CCAAT-binding complex), and 29 viable *S. pombe* zinc binuclear finger knockout mutants were plated on iron replete (YES) and iron-depleted (YES + 140 μM

DIP) media and incubated at 30°C. Wild type and *php4Δ* were included as resistant and sensitive controls.

b) *S. cerevisiae SEF1* is not induced in low iron medium. RT-PCR was used to assess transcript levels in wild type *S. cerevisiae* (S288C) grown in YEPD (black bars) vs. YEPD+ 100 μM BPS (white bars). Unlike *ScSEF1*, the *S. cerevisiae AFT1* gene was 2-fold induced in low iron medium. Error bars depict the standard deviation among triplicate samples.