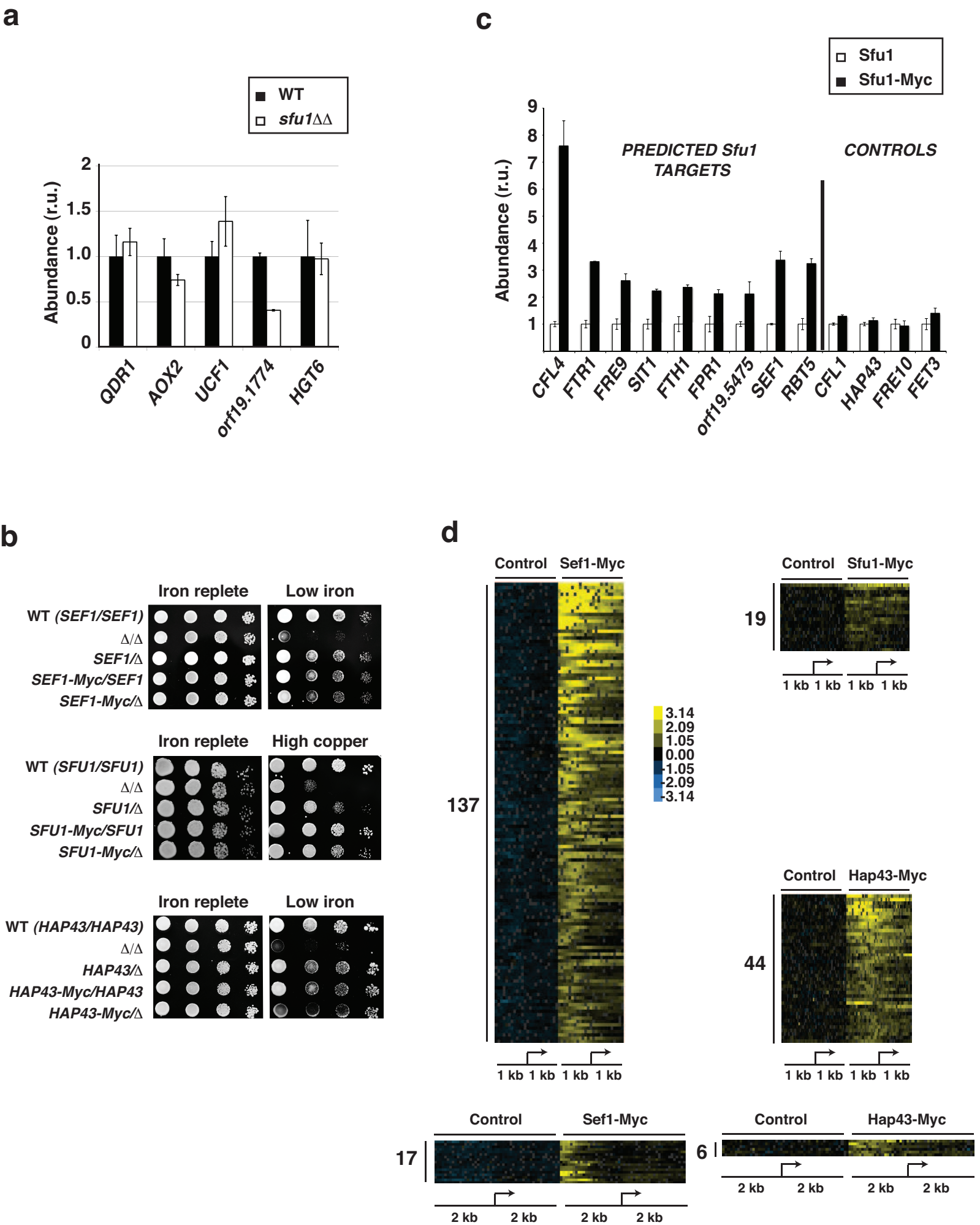
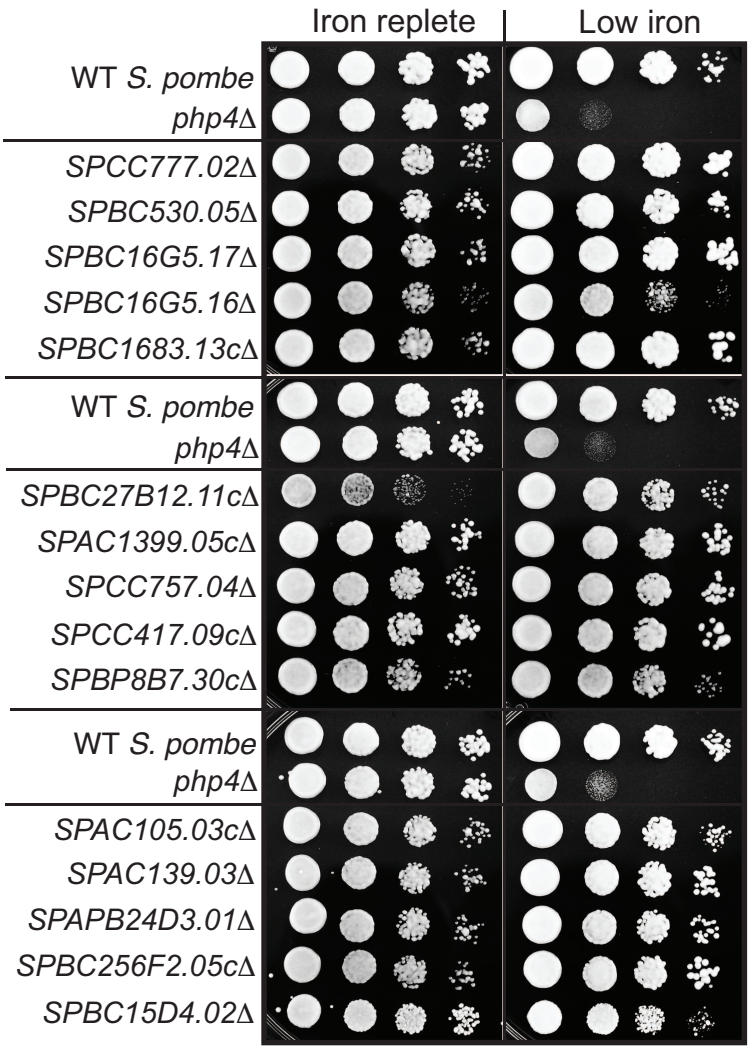

a
WT
sfu1ΔΔ
Abundance (r.u.)
2
1.5
1.0
0.5
0
QDR1
AOX2
UCF1
orf19.1774
HGT6
c
Sfu1
Sfu1-Myc
PREDICTED Sfu1 TARGETS
CONTROLS
Abundance (r.u.)
9
8
7
6
5
4
3
2
1
CFL4
FTR1
FRE9
SIT1
FTH1
FPR1
orf19.5475
SEF1
RBT5
CFL1
HAP43
FRE10
FET3
b
Iron replete
Low iron
WT (SEF1/SEF1)
Δ/Δ
SEF1/Δ
SEF1-Myc/SEF1
SEF1-Myc/Δ
Iron replete
High copper
WT (SFU1/SFU1)
Δ/Δ
SFU1/Δ
SFU1-Myc/SFU1
SFU1-Myc/Δ
Iron replete
Low iron
WT (HAP43/HAP43)
Δ/Δ
HAP43/Δ
HAP43-Myc/HAP43
HAP43-Myc/Δ
d
Control
Sef1-Myc
137
3.14
2.09
1.05
0.00
-1.05
-2.09
-3.14
1 kb 1 kb 1 kb 1 kb
Control
Sfu1-Myc
19
1 kb 1 kb 1 kb 1 kb
Control
Hap43-Myc
44
1 kb 1 kb 1 kb 1 kb
Control
Sef1-Myc
17
2 kb 2 kb 2 kb 2 kb
Control
Hap43-Myc
6
2 kb 2 kb 2 kb 2 kb

a


Iron replete
Low iron
WT S. pombe
php4Δ
SPCC777.02Δ
SPBC530.05Δ
SPBC16G5.17Δ
SPBC16G5.16Δ
SPBC1683.13cΔ
WT S. pombe
php4Δ
SPBC27B12.11cΔ
SPAC1399.05cΔ
SPCC757.04Δ
SPCC417.09cΔ
SPBP8B7.30cΔ
WT S. pombe
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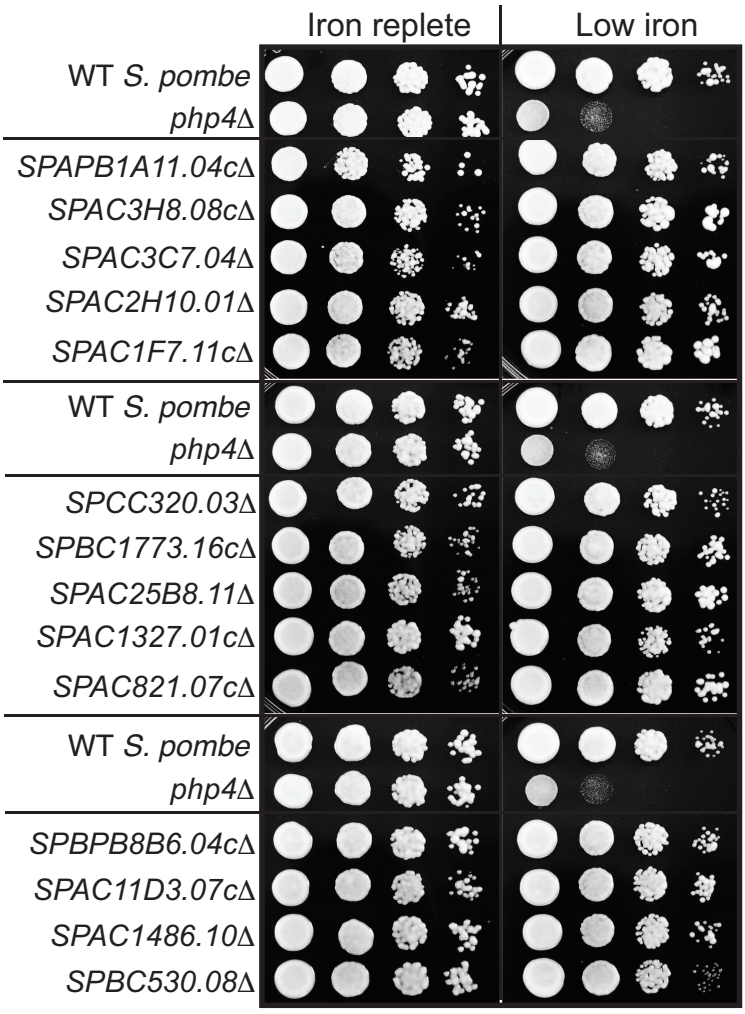
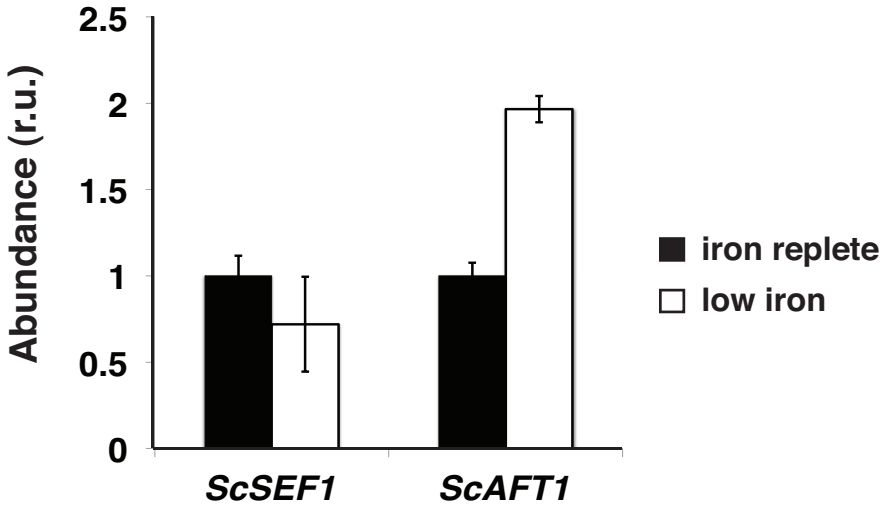
SPAC105.03cΔ
SPAC139.03Δ
SPAPB24D3.01Δ
SPBC256F2.05cΔ
SPBC15D4.02Δ


Iron replete
Low iron
WT S. pombe
php4Δ
SPAPB1A11.04cΔ
SPAC3H8.08cΔ
SPAC3C7.04Δ
SPAC2H10.01Δ
SPAC1F7.11cΔ
WT S. pombe
php4Δ
SPCC320.03Δ
SPBC1773.16cΔ
SPAC25B8.11Δ
SPAC1327.01cΔ
SPAC821.07cΔ
WT S. pombe
php4Δ
SPBPB8B6.04cΔ
SPAC11D3.07cΔ
SPAC1486.10Δ
SPBC530.08Δ

b


Abundance (r.u.)
2.5
2
1.5
1
0.5
0
ScSEF1
ScAFT1
iron replete
low iron

Supplementary Figures

Figure S1 Supporting data for Figure 2: Transcriptional regulatory activities of Sef1, Sfu1, and Hap43

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. 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 Supporting data for Figure 3: Analysis of transcription factor orthologs in *C. albicans, S. cerevisiae*, and *S. pombe*

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.

Supplementary Tables

Table S1a Expression microarray results depicted in Figure 2

Table S1b ChIP-Chip results depicted in Figure 2
The genomic coordinates of sequences used for MEME analysis appear in bold.

Table S2a Strains used in Figure 3 and throughout this study

Table S2b Primers used for construction of strains in Figure 3 and throughout this study

Supplementary Methods

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.

Table S1a Microarray results for Wild type *C. albicans* in iron-replete vs. iron-de

Systematic Name	Gene Name	Description	log2(iron-replete/iron-		
			Replicate 1	Replicate 2	Replicate 3
Up-regulated > 2-fold in YEPD vs. YEPD + BPS					
orf19.4674.1	***CRD2***	Metallothionein; role	6.2295	4.262	4.954
orf19.2475	***PGA26***	Putative GPI-anchor	3.945	3.751	2.759
orf19.4040	***ILV3***	Putative dihydroxyac	4.1475	3.258	2.609
orf19.238	***CCP1***	Similar to cytochrom	6.722	3.013	2.88
orf19.7498	***LEU1***	Protein described as	5.6995	2.121	3.201
orf19.1770	***CYC1***	Cytochrome c; comp	4.842	3.0725	2.4745
orf19.637	***SDH2***	Succinate dehydroge	3.973	3.0245	2.93
orf19.4495	***NDH51***	Subunit of nicotinam	4.703	2.9835	2.447
orf19.2871	***SDH12***	Protein with similarity	4.003	2.8785	2.4325
orf19.7590		Protein described as	3.898	2.8335	2.2005
orf19.2091		Predicted ORF in As	4.3225	3.0265	2.025
orf19.4365		Predicted ORF in As	2.7905	3.987	2.3685
orf19.3327	***TRM2***	Predicted ORF in As	2.948	3.9265	1.5645
orf19.1744	***HEM4***	Protein described as	3.727	3.057	2.2425
orf19.5077		Predicted ORF in As	3.4755	2.988	1.598
orf19.3223.1		Predicted ORF; desc	2.8655	2.8165	1.8295
orf19.6794	***FESUR1***	Protein described as	3.499	2.6505	2.423
orf19.3175		Predicted ORF in As	4.683	2.5325	2.616
orf19.4758		Predicted ORF in As	3.896	2.583	2.2365
orf19.6531	***NUC2***	Protein described as	3.698	2.562	2.106
orf19.1710		Protein described as	3.6305	2.523	1.997
orf19.6257	***GLT1***	Alkaline downregulat	4.2325	2.722	2.19
orf19.1517	***ARO3***	3-Deoxy-D-arabinoh	2.782	2.7755	2.0055
orf19.5893	***RIP1***	Protein described as	2.7305	2.618	1.67
orf19.5629	***QCR7***	Protein described as	2.48	2.547	1.7285
orf19.1625		Predicted ORF in As	2.775	2.4785	1.867
orf19.6385	***ACO1***	Protein described as	5.6095	2.525	2.1655
orf19.446.2		Predicted ORF in As	3.0705	2.434	1.909
orf19.287		Predicted ORF in As	2.827	2.4475	1.6615
orf19.1179		Transcriptionally regu	2.7435	2.5165	1.7395
orf19.2821		Predicted ORF in As	2.8575	2.6805	1.311
orf19.3527	***CYT1***	Protein described as	3.1615	2.342	1.865
orf19.2954		Predicted ORF in As	2.7615	2.574	1.5365
orf19.446.1		Predicted ORF in As	2.6245	2.405	1.334
orf19.4099	***ECM17***	Predicted enzyme of	3.5115	2.345	1.5825
orf19.3290		Predicted ORF in As	3.033	2.4075	1.6705
orf19.913.2		ORF Predicted by Ar	2.8875	2.353	1.553
orf19.1957	***CYC3***	Cytochrome c heme	2.324	2.3695	1.721
orf19.1549		Predicted ORF in As	2.6315	2.6635	1.6505
orf19.1873		Predicted ORF in As	3.055	2.5245	1.3415
orf19.4016		Predicted ORF in As	2.826	2.524	1.804
orf19.3366.1		ORF Predicted by Ar	3.296	2.4855	1.755
orf19.5521	***ISA1***	Reported to have po	3.521	2.2825	2.0735

Table S1b. ChIP-Chip results depicted in Figure 2

Sef1 binding targets				
Peak #	Gene name	Systematic name	Normalized log2 SEF1 enrichme	Agilent Score
1	SOD2	orf19.3340	5.7	10.371
2	ISU1	orf19.6548	5.1	9.9905
3	HGT2	orf19.3668	4.3	9.9135
4		orf19.4901	4.5	9.792
5		orf19.3610	4.25	9.7015
6	ACO1	orf19.6385	4.6	9.6295
7	SAL6	orf19.5758	4.5	9.6105
7		orf19.5757	4.5	9.6105
8	ATM1	orf19.1077	4	9.547
9	ISA1	orf19.5521	4	9.492
9	GCV1	orf19.5519	4	9.492
10	SNM1	orf19.1927	4.25	9.464
10	SEF2	orf19.1926	4.25	9.464
11	YHM2	orf19.4197	4.25	9.42
11	FCA1	orf19.4195.1	4.25	9.42
12	NBP35	orf19.747	4	9.348
12		orf19.746	4	9.348
13	CFL5	orf19.1930	3.9	9.152
14	HAP43	orf19.681	3.8	9.0535
15		orf19.2149	3.85	9.0355
15		orf19.2150	3.85	9.0355
16	HAP3	orf19.4647	5.88	9.024
17	TRR1	orf19.4290	3.8	8.998
18		orf19.6003	4.25	8.856
19	SOD4	orf19.2062	3.5	8.795
20	FRE9	orf19.3538	3.45	8.744
21		orf19.5952	3.7	8.6985
22	GDH3	orf19.4716	3.1	8.6185
23	HMX1	orf19.6073	3.25	8.5975
24	FTR1	orf19.7219	3.25	8.5365
24	RBE1	orf19.7218	3.25	8.5365
25		orf19.7445	3.75	8.4515
26	SMF12	orf19.2270	3.7	8.4475
27	orf19.22	orf19.22	3.25	8.404
28		orf19.5326	3.25	8.3685
29		orf19.4513	3	8.3455
30		orf19.7306	3	8.309
31	CAP1	orf19.1623	3.25	8.2825
31		orf19.1624	3.25	8.2825
32	SAP99	orf19.853	3.45	8.227
33		orf19.6793	3	8.0995
33	RRD1	orf19.6792	3	8.0995
34	CFL2	orf19.1264	3	8.0565
35		orf19.1486	2.75	7.7615
36	IDH1	orf19.4826	2.75	7.681

Table S2a. Strains used in Figure 3 and th

Strain	Species	Relevant Genotype
SN250	*C. albicans*	Wild type
SN425	*C. albicans*	Wild type (prototroph)
SN330	*C. albicans*	*sef1* D/D
SN452	*C. albicans*	*sef1* D/D (prototroph)
SN515	*C. albicans*	*sfu1* D/D
SN668	*C. albicans*	*sfu1* D/D (prototroph)
SN694	*C. albicans*	*hap43* D/D
SN802	*C. albicans*	*aft2* D/D
SN436	*C. albicans*	*SEF1*-complemented strain
SN664	*C. albicans*	*SFU1*-complemented strain
SN863	*C. albicans*	*HAP43*-complemented strain
SN423	*C. albicans*	*SEF1-Myc/SEF1*
SN646	*C. albicans*	*SFU1-Myc/SFU1*
SN840	*C. albicans*	*HAP43-Myc/HAP43*
SN830	*C. albicans*	*SEF1-Myc/ D*
SN858	*C. albicans*	*SFU1-Myc/ D*
SN860	*C. albicans*	*HAP43-Myc/ D*
BY4741	*S. cerevisiae*	Wild type
Weissman lab "WT"	*S. cerevisiae*	Wild type (control)
sef1 D	*S. cerevisiae*	*sef1* D
hap4 D	*S. cerevisiae*	*hap4* D
SN846	*S. cerevisiae*	*aft1* D
SP286	*S. pombe*	Wild type

Table S2b. Primers used for construction of strains in Figure 3 and through

Primer Na	Purpose	Sequence
Primers used in *C. albicans*		
SNO300	Common 5' verificati	CCGTTAATTAACCCGGGGATC
SNO301	Common 3' verificati	ggaacttcagatccactagttctagagc
SNO772	Common 5' verificati	attcagcgaacggggtgtac
SNO778	Common 3' verificati	gctatggcgcattcatcgacc
SNO460	SEF1-Myc Forward	TTAATAATGATAACCAAGATGACGACTTTTTGGGTTGGTT
SNO461	SEF1-Myc Reverse	ACTTATTCATTACAAAATCATATTAACATAATTACTAACTA
SNO503	SEF1-Myc 5' verifica	TGAAATCTTTTGATTCCAGCAAACC
SNO504	SEF1-Myc 3' verifica	CTTCTATTGTTCCACAAGGTGCCAG
SNO1016	HAP43-Myc 5' flank_	ggcgaattggagctccaccgcggtggcggccgctctagaactagtgGATCgttta
SNO1017	HAP43-Myc 5' flank_	GTTCACCGTTAATTAACCCGGGGATCCGATTATATGCTC
SNO1018	HAP43-Myc 13xMyc	CGAGAATTAGATAGAAGAGCATATAATcggatccccgggttaatta
SNO1019	HAP43-Myc 13xMyc	GTGTCGGAAATACTTCATACTGTAAGTCAAAGCGGCCGC
SNO1020	HAP43-Myc 3' flank_	agatccactagttctagagcggccgcTTTGACTTACAGTATGAAGTAT
SNO1021	HAP43-Myc 3' flank_	GTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAG
SNO1022	HAP43-Myc 5' verific	GACCAAGGATACTTCTTCTGATGG
SNO1023	HAP43-Myc 3' verific	GTGCAGGTAATACTATTGCTGGTG
SNO883	SEF1-Myc/SEF1D 5'	ggcgaattggagctccaccgcggtggcggccgctctagaactagtgGATCgttta
SNO884	SEF1-Myc/SEF1D 5'	CTCGAGGGGGGGCCCGGTACCCAAAGTTAAGGGAGGAC
SNO885	SEF1-Myc/SEF1D FI	ACAACCAATCGACTCCTCCCTTAACTttgggtaccgggcccccccc
SNO886	SEF1-Myc/SEF1D FI	TTTACATTCTAATGAGGTAGAATCGGCGGCCGCTCTAGA
SNO887	SEF1-Myc/SEF1D 3'	tccactagttctagagcggccgccGATTCTACCTCATTAGAATGTAA
SNO888	SEF1-Myc/SEF1D 3'	GTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAG
SNO901	SEF1-Myc/SEF1D 5'	CTGCACGACCTTGCATCATTAC
SNO898	SEF1-Myc/SEF1D 3'	CACAAGGTGCCAGAATATACACAG
SNO1070	SFU1-Myc/SFU1D 5'	ggcgaattggagctccaccgcggtggcggccgctctagaactagtgGATCgttta
SNO1071	SFU1-Myc/SFU1D 5	CTCGAGGGGGGGCCCGGTACCCAAATTCATAAACGGTG
SNO1072	SFU1-Myc/SFU1D F	ACAAAGTCAACCACCGTTTATGAATttgggtaccgggccccccctc
SNO1073	SFU1-Myc/SFU1D F	AAGGGGATTGTTTTGCATACTCGGCGGCCGCTCTAGAAC
SNO1074	SFU1-Myc/SFU1D 3'	tccactagttctagagcggccgccGAGTATGCAAAACAATCCCCTTT
SNO1075	SFU1-Myc/SFU1D 3	GTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAG
SNO1084	SFU1-Myc/SFU1D 5'	TCAAGTACCAGTTACTGTTTGAGAG
SNO1085	SFU1-Myc/SFU1D 3	AGGACGTAATGATGATGATGAAGG
SNO1076	HAP43-Myc/HAP43D	ggcgaattggagctccaccgcggtggcggccgctctagaactagtgGATCgttta
SNO1077	HAP43-Myc/HAP43D	CTCGAGGGGGGGCCCGGTACCCAACCGATTACTCGCTG
SNO1078	HAP43-Myc/HAP43D	GAAATCAGCGAGTAATCGGttgggtaccgggccccccctcgagga
SNO1019	HAP43-Myc/HAP43D	GTGTCGGAAATACTTCATACTGTAAGTCAAAGCGGCCGC
SNO1020	HAP43-Myc/HAP43D	agatccactagttctagagcggccgcTTTGACTTACAGTATGAAGTAT
SNO1021	HAP43-Myc/HAP43D	GTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAG
SNO1082	HAP43-Myc/HAP43D	GACAACAAAAGCAGTTCATTGG
SNO1083	HAP43-Myc/HAP43D	TCAAGTACCAGTTACTGTTTGAGAG
SNO1056	qPCR for Sef1 prom	CGGGTCTAGTAGTAAACAAAGC
SNO1057	qPCR for Sef1 prom	CTGTCAGGAAGAAAGGAAGAGA
SNO1062	qPCR for Rbt5 prom	TACGAGGTTTCGCTATTTCTTGAC
SNO1063	qPCR for Rbt5 prom	GTAGCAAATACTTATGCAGCTTGG
SNO1066	qPCR for SFU1 pron	TTCAAGTACCAGTTACTGTTTGAG