Supplemental Information

FACT and Asf1 regulate nucleosome dynamics and coactivator binding at the *HO* promoter

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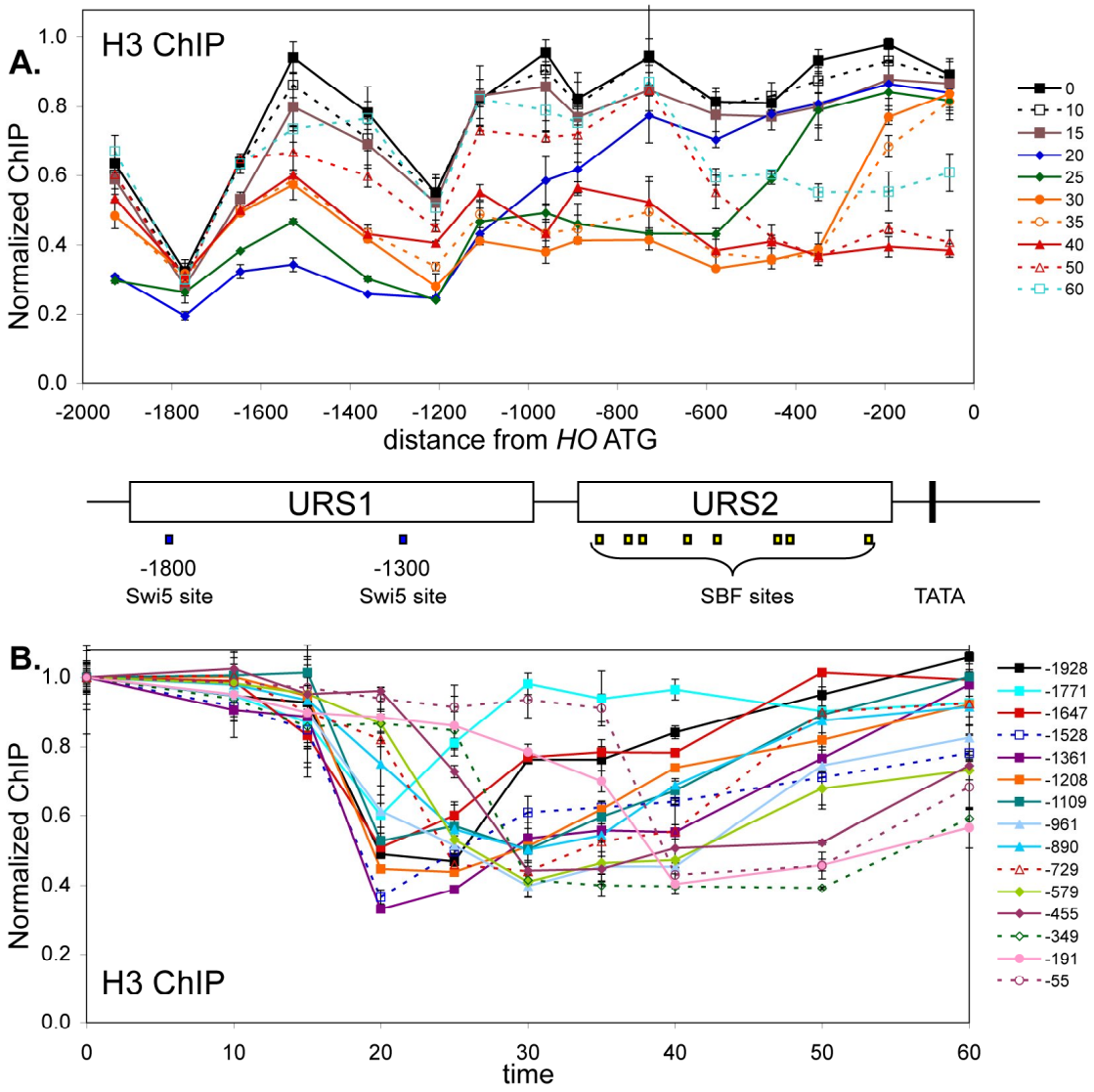
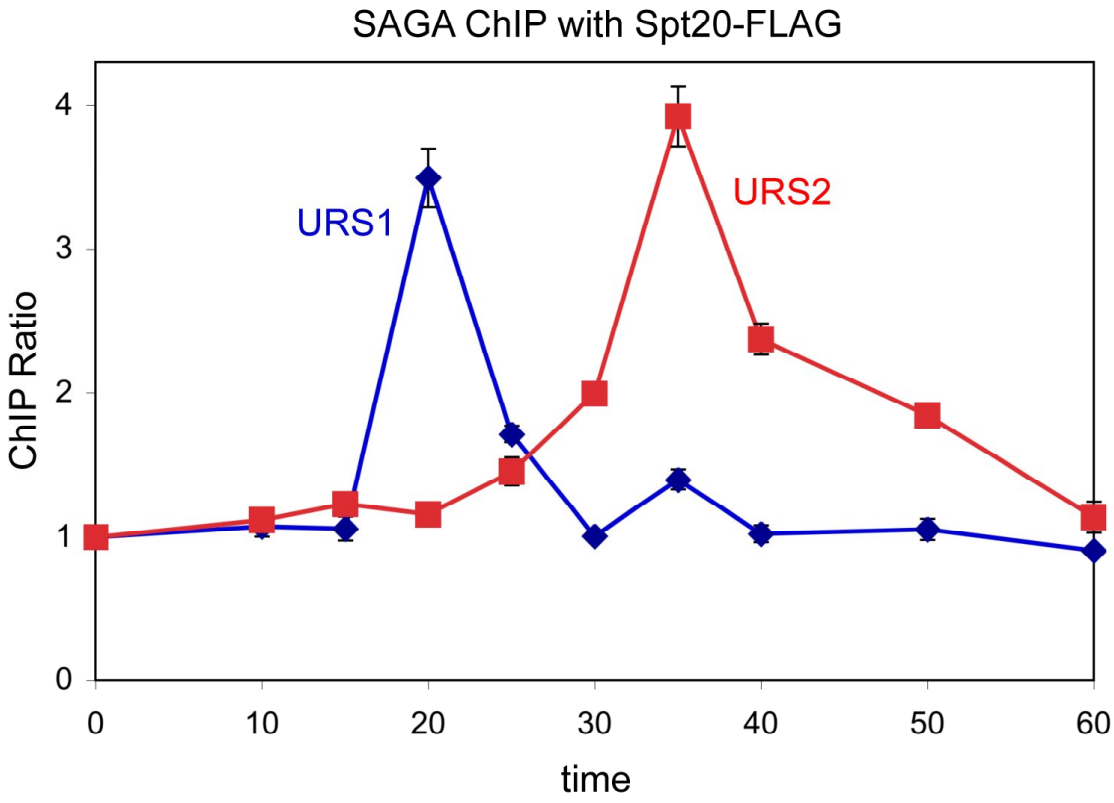


Supplemental Figure S1. Nucleosome eviction occurs in waves along the *HO* Promoter.

A. DY6669 cells (*GALp::CDC20*) with a *GALp::CDC20* allele were synchronized in mitosis by removing galactose, followed by release by addition of galactose (t = 0). The *CDC20* arrest is at the G2/M transition, and *HO* expression at 40 min following release corresponds to late G1 phase. Nucleosome occupancy was measured by H3 ChIP using samples taken at various times after the release. The DNA in the experiment was sheared to an average of 350 bp, as opposed to approximately 550 bp in the other ChIP

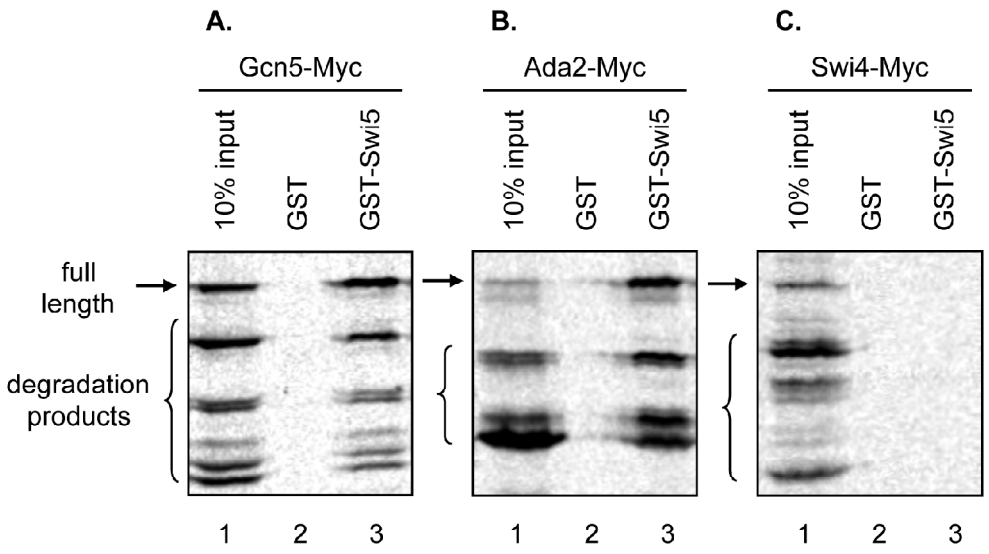
experiments. URS1, URS2, the Swi5 and SBF binding sites are shown for the *HO* promoter, where the ATG represents +1 and the transcription start site is at -20. Error bars in ChIP assays reflect the standard deviation of three replicate PCRs.

B. The data from panel A, is plotted as a function of time.



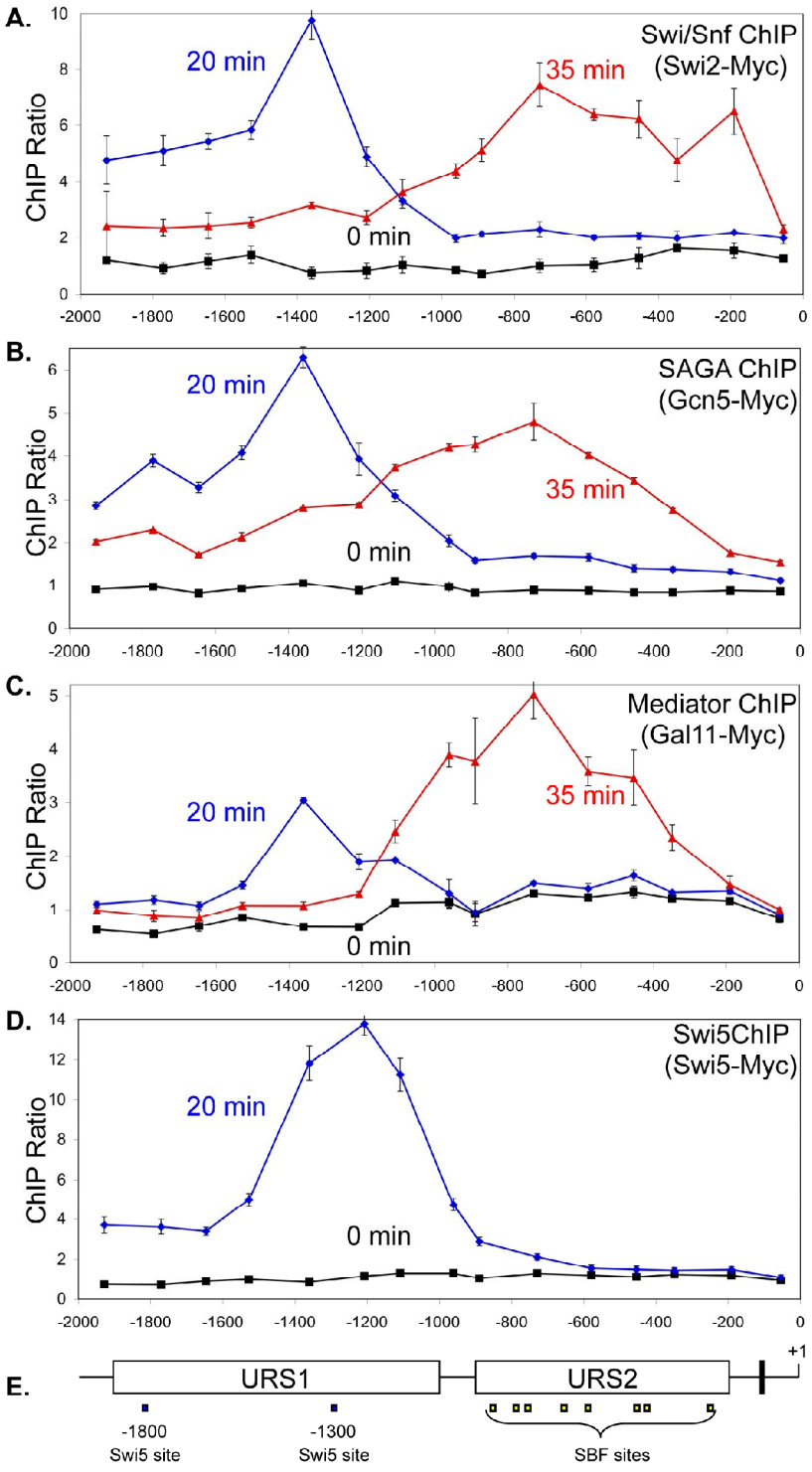
Supplemental Figure S2. The SAGA-specific Spt20 subunit binds to *HO*.

Binding of the Spt20-Flag subunit of SAGA to *HO* URS1 and URS2 was analyzed by ChIP in synchronized cells using strain DY13587 (*GALp::CDC20 SPT20-Flag*). Error bars in ChIP assays reflect the standard deviation of three replicate PCRs.



Supplemental Figure S3. SAGA binds to GST-Swi5.

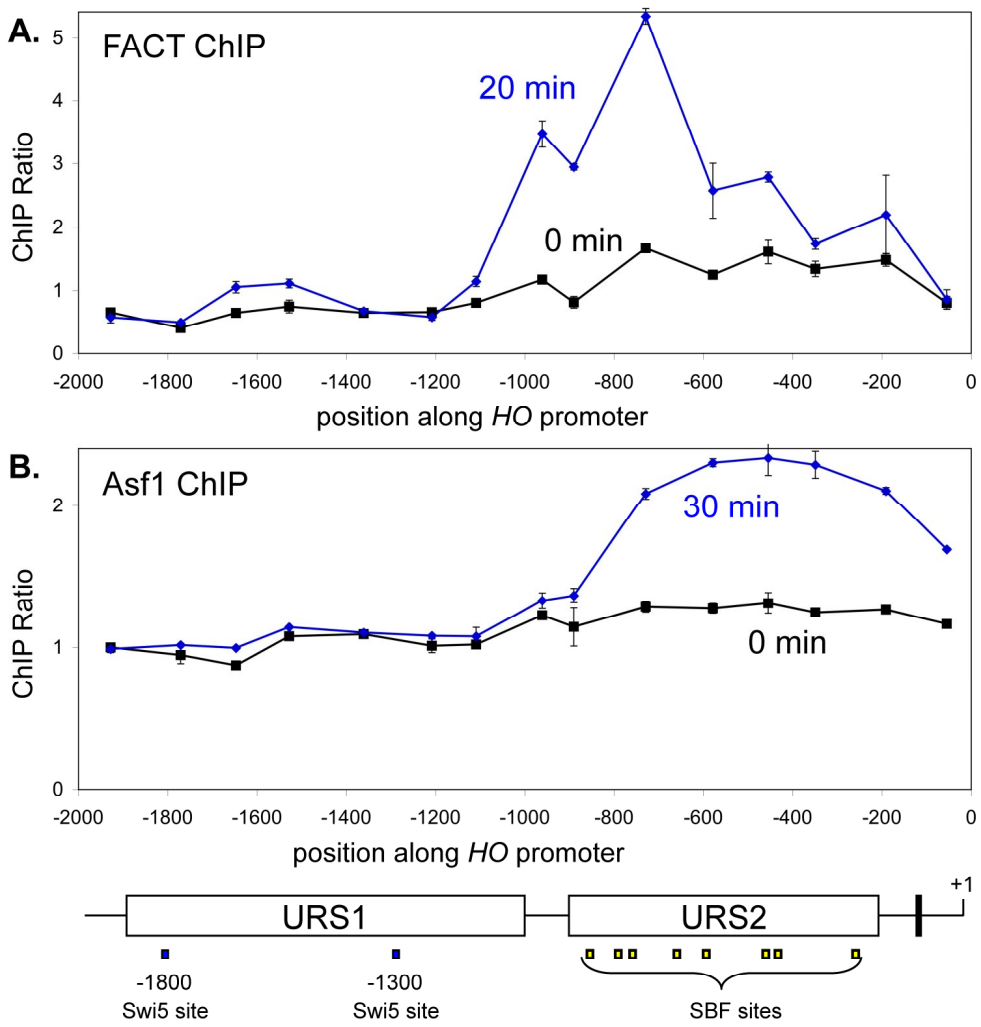
Extracts were prepared from strains DY7196 (*GCN5-Myc*) (Panel A), DY6152 (*ADA2-Myc*) (Panel B), and DY6241 (*SWI4-Myc*) (Panel C) and chromatographed on glutathione-agarose columns containing either GST-only or GST-Swi5. In each panel lane 1 contains the input extract (corresponding to 10% of the amount of material applied to the columns), lane 2 contains the material eluted from the GST-only column, and lane 3 contains the material eluted from the GST-Swi5 column.



Supplemental Figure S4. Location of coactivator binding at the *HO* promoter.

DY8602 (*SWI2-Myc GALp::CDC20*) (Panel A), DY12752 (*GCN5-Myc GALp::CDC20*) (Panel B), DY8460 (*GAL11-Myc GALp::CDC20*) (Panel C), and DY6546 (*SWI5-Myc*

GALp::CDC20) (Panel D), were synchronized by galactose withdrawal and readdition, and samples taken at 0, 20, and 35 min after release were processed for ChIP. ChIP samples were analyzed with fifteen pairs of PCR primers across the *HO* promoter, with an average PCR product size of 208 bp. The Swi5-Myc ChIP was not analyzed at the 35 min time point as previous work shows that there is no Swi5 binding in the nucleus at this time (Sbia et al., 2008), and also no Swi5 binding to DNA (Voth et al., 2007). The numbers on the X axis give the center of each PCR interval. Each ChIP sample was first normalized to an input DNA sample and then to the ChIP signal for a control region on chromosome I. Panel E contains a map of the *HO* promoter, indicating the URS1 and URS2 regions and the Swi5 and SBF binding sites. Error bars in ChIP assays reflect the standard deviation of three replicate PCRs.

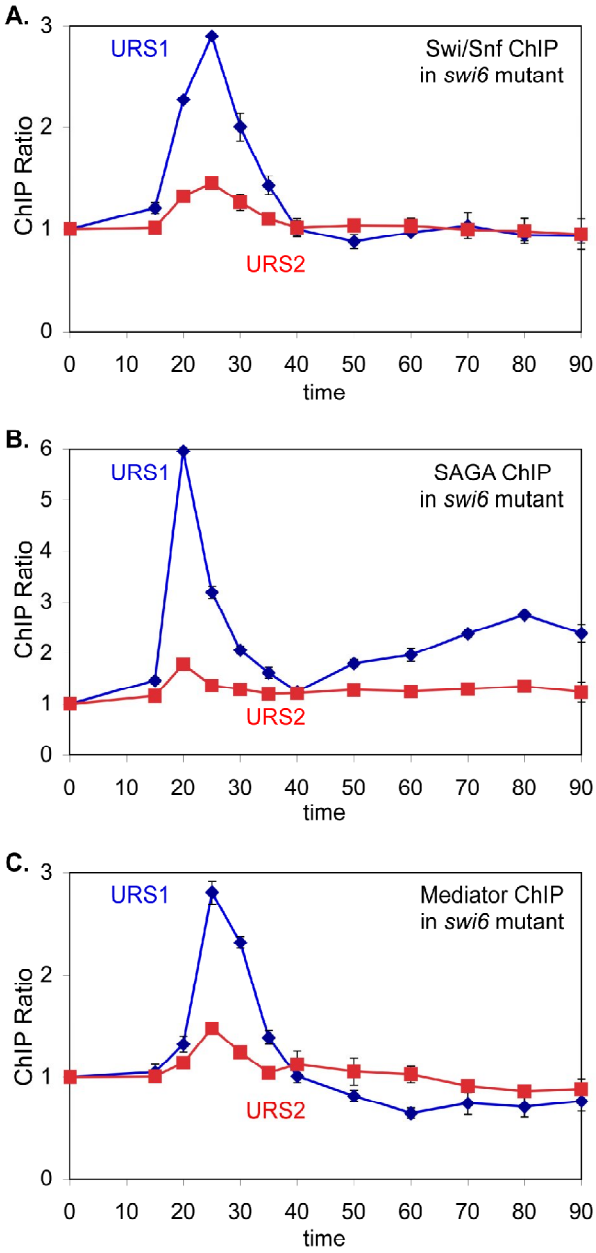


Supplemental Figure S5. Location of FACT and Asf1 binding at the *HO* promoter.

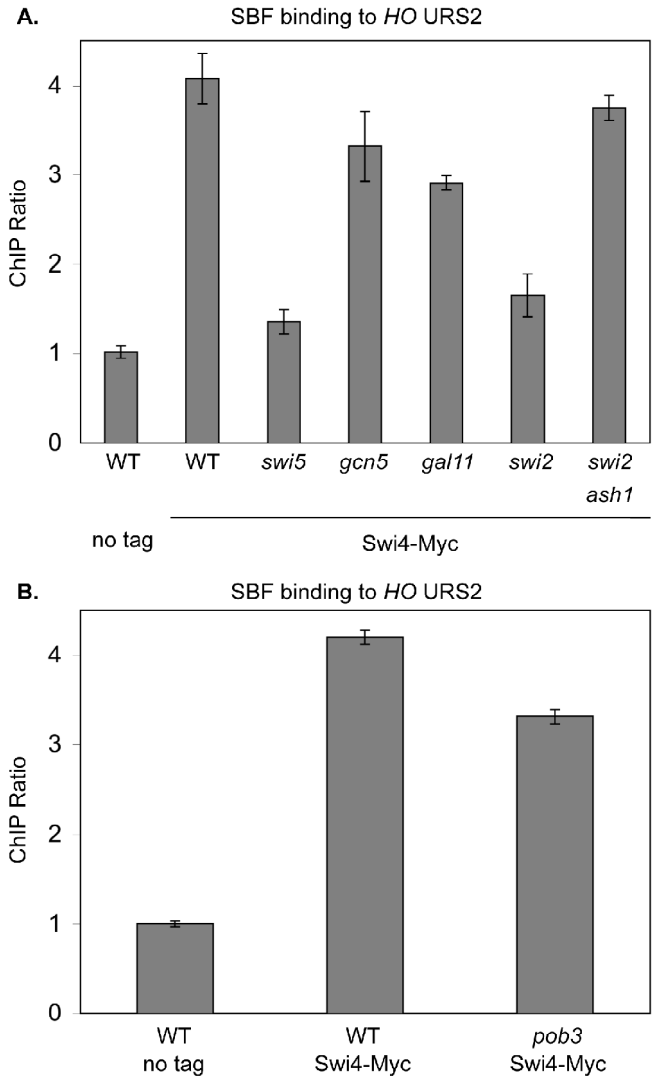
A. Strain DY10788 (*GALp::CDC20*) was synchronized by galactose withdrawal and readdition, and samples taken at 0 and 20 min after release were processed for ChIP using an antibody to the Spt16 subunit of FACT.

B. Strain DY13010 (*GALp::CDC20 ASF1-Myc*) was synchronized by galactose withdrawal and readdition, and samples taken at 0 and 30 min after release were processed for ChIP using an antibody to the Myc epitope.

ChIP samples were analyzed with fifteen pairs of PCR primers across the *HO* promoter, with an average PCR product size of 208 bp. Each ChIP sample was first normalized to an input DNA sample and then to the ChIP signal for a control region on chromosome I. The map shows the *HO* promoter, including the URS1 and URS2 regions and the Swi5 and SBF binding sites. Error bars in ChIP assays reflect the standard deviation of three replicate PCRs.



Supplemental Figure S6. A *swi6* mutation affects coactivator binding to URS2. Binding of Swi/Snf, SAGA, and Mediator to *HO* URS1 and URS2 were analyzed by ChIP in synchronized *swi6* mutant cells. The following cells were used: **A.** DY13529 (*GALp::CDC20 SWI2-Myc swi6*), **B.** DY13369 (*GALp::CDC20 GCN5-Myc swi6*), **C.** DY13532 (*GALp::CDC20 GAL11-Myc swi6*). Error bars in ChIP assays reflect the standard deviation of three replicate PCRs.



Supplemental Figure S7. A *swi5* or a *swi2* mutation reduces SBF binding to URS2. ChIP experiments were performed with extracts prepared from cells growing logarithmically at 25°C, and ChIP samples were analyzed for Swi4-Myc binding to *HO* URS2. Error bars in ChIP assays reflect the standard deviation of three replicate PCRs. **A.** Strains DY150 (no tag), DY6241(*SWI4-Myc::TRP1*), DY10593 (*SWI4-Myc::TRP1 swi5*), DY6244 (*SWI4-Myc::TRP1 gcn5*), DY7236 (*SWI4-Myc::TRP1 gal11*), DY11262 (*SWI4-Myc::TRP1 swi2*), and DY11264 (*SWI4-Myc::TRP1 swi2 ash1*) were used. **B.** Strains DY150 (no tag), DY6241(*SWI4-Myc::TRP1*), and DY13648 (*SWI4-Myc::TRP1 pob3*) were used.

Supplemental Table S1. Strain List.

DY150 *MAT***a** *ade2 can1 his3 leu2 trp1 ura3*

DY5628 *MAT***a** *gal11::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY5699 *MAT***a** *ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY5832 *MAT***a** *SWI5-Myc::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*

DY6152 *MAT***a** *ADA2-Myc::TRP1 ade2 can1 his3 leu2 trp1 ura3*

DY6241 *MAT***a** *SWI4-Myc::TRP1 ade2 can1 his3 leu2 trp1 ura3*

DY6244 *MAT***a** *SWI4-Myc::TRP1 gcn5::HIS3 ade2 can1 his3 leu2 trp1 ura3*

DY6542 *MAT***a** *SWI5-Myc::KanMX GALp::CDC20::ADE2 ace2::HIS3 ade2 can1 his3 leu2 trp1 ura3*

DY6546 *MAT***a** *SWI5-Myc::KanMX GALp::CDC20::ADE2 ade2 can1 his3 leu2 trp1 ura3*

DY6570 *MAT***a** *GALp::CDC20::ADE2 swi5::hisG-URA3-hisG ade2 can1 his3 leu2 trp1 ura3*

DY6650 *MAT***a** *GALp::CDC20::ADE2 gcn5::HIS3 ade2 can1 his3 leu2 trp1 ura3*

DY6669 *MAT***a** *GALp::CDC20::ADE2 ade2 can1 his3 leu2 trp1 ura3*

DY7196 *MAT***a** *GCN5-Myc::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*

DY7236 *MAT***a** *SWI4-Myc::TRP1 gal11::LEU2 ade2 can1 his3 leu2 trp1 ura3*

DY7379 *MAT***a** *pob3(L78R) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY7419 *MAT***a** *GALp::CDC20::ADE2 gal11:LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY7815 *MAT***a** *spt16-11 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY8341 *MAT***a** *SWI4-Myc::TRP1 ade2 can1 his3 leu2 trp1 ura3*

DY8353 *MAT***a** *SWI6-Myc::TRP1 ade2 can1 his3 leu2 trp1 ura3*

DY8460 *MAT***a** *GAL11-Myc::His3MX GALp::CDC20::ADE2 ade2 can1 his3 leu2 trp1 ura3*

DY8602 *MAT***a** *SWI2-Myc::TRP1 GALp::CDC20::ADE2 ade2 can1 his3 leu2 trp1 ura3*

DY9718 *MAT***a** *GALp::CDC20::ADE2 swi2(E834K) ade2 can1 his3 leu2 trp1 ura3*

DY10308 *MAT***a** *pob3(Q308K) ade2 can1 his3 leu2 trp1 ura3*

DY10593 *MAT***a** *SWI4-Myc::TRP1 swi5::LEU2 ade2 can1 his3 leu2 trp1 ura3*

DY10788 *MAT***a** *GALp::CDC20::ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY10790 *MAT***a** *SWI2-Myc::TRP1 GALp::CDC20::ADE2 pob3(L78R) ade2 can1 his3 leu2 lys2 trp1 ura3*

DY11246 *MAT***a** *GALp::CDC20::ADE2 pob3(L78R) ade2 can1 his3 leu2 lys2 trp1 ura3*

DY11262 *MAT***a** *SWI4-Myc::TRP1 swi2(E834K) ade2 can1 his3 leu2 trp1 ura3*

DY11264 *MAT***a** *SWI4-Myc::TRP1 swi2(E834K) ash1::LEU2 ade2 can1 his3 leu2 trp1 ura3*

DY12554 *MAT***a** *pob3(Q308K)::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*

DY12748 *MAT***a** *GAL11-Myc::His3MX GALp::CDC20::ADE2 pob3(L78R) ade2 can1 his3 leu2 lys2 trp1 ura3*

DY12750 *MAT***a** *GCN5-Myc::KanMX GALp::CDC20::ADE2 pob3(L78R) ade2 can1 his3 leu2 lys2 trp1 ura3*

DY12752 *MAT***a** *GCN5-Myc::KanMX GALp::CDC20::ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY12869 *MAT***a** *asf1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY12914 *MAT***a** *GALp::CDC20::ADE2 asf1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY12927 *MAT***a** *GCN5-Myc::KanMX GALp::CDC20::ADE2 asf1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY12964 *MAT***a** *SWI2-Myc::TRP1 GALp::CDC20::ADE2 asf1::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY12997 *MAT***a** *ASF1-Myc::KanMX ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY13010 *MAT***a** *ASF1-Myc::KanMX GALp::CDC20::ADE2 ade2 can1 his3 leu2 met15 trp1 ura3*

DY13137 *MAT***a** *GAL11-Myc::His3MX GALp::CDC20::ADE2 asf1::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY13145 *MAT***a** *ASF1-Myc::KanMX swi5::LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY13147 *MAT***a** *ASF1-Myc::KanMX swi6::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY13150 *MAT***a** *ASF1-Myc::KanMX pob3(L78R) ade2 can1 his3 leu2 met15 trp1 ura3*

DY13156 *MAT***a** *asf1::TRP1 pob3(L78R) ade2 can1 his3 leu2 lys2 trp1 ura3*

DY13162 *MAT***a** *ASF1-Myc::KanMX gal11::LEU2 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY13164 *MAT***a** *ASF1-Myc::KanMX gcn5::TRP1 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY13168 *MAT***a** *asf1::TRP1 pob3(Q308K)::KanMX ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY13176 *MAT***a** *ASF1-Myc::KanMX swi2(E834K) ade2 can1 his3 leu2 lys2 met15 trp1 ura3*

DY13178 *MAT***a** *asf1::TRP1 spt16-11 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY13369 *MATα GCN5-Myc::KanMX GALp::CDC20:: ADE2 swi6::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY13373 *MAT***a** *GALp::CDC20::ADE2 swi6::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*

DY13529 *MAT***a** *SWI2-Myc::TRP1 GALp::CDC20::URA3 swi6::ADE2 ade2 can1 his3 leu2 trp1 ura3*

DY13532 *MAT***a** *GAL11-Myc::HIS3MX GALp::CDC20::URA3 swi6::TRP1 ade2 can1 his3 leu2 trp1 ura3*

DY13587 *MAT***a** *SPT20-Flag::URA3(Kluyveromyces) GALp::CDC20::ADE2 ade2 can1 his3 leu2 trp1 ura3*

DY13648 *MATα SWI4-Myc::TRP1 pob3(L78R) ade2 can1 his3 leu2 trp1 ura3*

Supplemental Table S2. Oligonucleotide List.

RT-PCR oligos		
F1066	*HO*	AAATGGAGCGCTCTAAAGGAGAA
F1067	*HO*	CTAACCACAGACCAAGCATCCAA
F1719	*PIR1*	CCACCACCAATACTACTGTTGCTC
F1720	*PIR1*	ACCTTTACCGTCAGTCAGGATACC
F2043	*CLN2*	AATTCTTGATTGATGTATCCGCGTGG
F2044	*CLN2*	GTTAGGAATGGAAACAATGCCGTTCA
F2173	*RDN25* (control)	CGTTCCTTGTCTATGTTCCTTG
F2174	*RDN25* (control)	CACTGTACTTGTTCGCTATCG
ChIP oligos		
F1093	*HO* URS1 (-1293)	TATACCCAATCGCTGCGTGC
F1094	*HO* URS1 (-1293)	AGCCGCCACGAATCAAACTT
F1095	*HO* URS2 (-666)	GGCAAACCTAATGTGACCGT
F1096	*HO* URS2 (-666)	GGCAAACCTAATGTGACCGT
F1154	*HO* TATA	CCATATCCTCATAAGCAGCA
F1155	*HO* TATA	AAGCTCTGTGTTTGGTTTTT
F1759	*PIR1* UAS	CTGCCCAAATGCTTAAATACAGA
F1760	*PIR1* UAS	ATAATTCCTCGAAGCCAGAC
F1416	Chrom I control	GTTTATAGCGGGCATTATGCGTAGATCAG
F1417	Chrom I control	GTTCCTCTAGAATTTTTCCACTCGCACATTC
ChIP oligos tiled along the *HO* promoter		
F2083	*HO* Promoter -2033 to -1823	GAGTATTGTGTCATGTTCGAGACAAAC
F2084	*HO* Promoter -2033 to -1823	TTAAGTCCAAAGGCACAATTTTACG
F2085	*HO* Promoter -1872 to -1670	TTGATCTTTACCGTTTAGTTCCAAC
F2086	*HO* Promoter -1872 to -1670	GTAAAGCCTCCAGAACAGCTATG
F2087	*HO* Promoter -1742 to -1551	AAAGGCGGATCAAGATGTATGAAAG

F2088	*HO* Promoter -1742 to -1551	GGAACCATGTGATCTTACGTTGATATG
F2089	*HO* Promoter -1640 to -1416	TCCGAAAAGCAATTACTCTCTATGTT
F2090	*HO* Promoter -1640 to -1416	GCGATTGGGTATAATGAAGATTGTTA
F2091	*HO* Promoter -1471 to -1250	AAGCTAAGAATTTCACATGTTGTTG
F2092	*HO* Promoter -1471 to -1250	GTTGAGGTCTTTTCTATTTCTGATTG
F2093	*HO* Promoter -1295 to -1121	AATGCTGGAGCAAAAATTTCAATCAG
F2094	*HO* Promoter -1295 to -1121	GGAGCCCCTCAGACATTAGCC
F2115	*HO* Promoter -1204 to -1014	TCTACGGATGATCTGTGAGAA
F2116	*HO* Promoter -1204 to -1014	CTACGTTAAGACCTGTAACCGA
F2117	*HO* Promoter -1063 to -859	GAAAGAACCGCAGAGTGCTT
F2118	*HO* Promoter -1063 to -859	GAACCTGGTACGTATATTGTGGC
F2097	*HO* Promoter -983 to -796	TCGATCCGTTTGGCGTCTTT
F2098	*HO* Promoter -983 to -796	TAATCGACGACGGTCACATTAGGTT
F2099	*HO* Promoter -839 to -619 (-729)	TCATACCCTGACTTGGCAAAC
F2100	*HO* Promoter -839 to -619 (-729)	CTTAAGCCCTGTGTAGGATTGATT
F2101	*HO* Promoter -677 to -481	ATGCAGTTGAAGACATGTGCGTC
F2102	*HO* Promoter -677 to -481	CATAGAAACAGGACTTGCGAACCC
F2103	*HO* Promoter -573 to -336	ACGATTACCATGGAAATTAACGTACCT
F2104	*HO* Promoter -573 to -336	TCTATGAAAATGAATTGTTGCTCTGC
F2105	*HO* Promoter -448 to -250 (-349)	GGTTTACGAAATGATCCACGAAAATC
F2106	*HO* Promoter -448 to -250 (-349)	TTTCACACCTAATAACGCCCAGC
F2119	*HO* Promoter -307 to -75	ACCATTGGTACCTACTACTTTGAAT
F2120	*HO* Promoter -307 to -75	GCCATTTAGAATAGGAATTGAATAC
F2109	*HO* Promoter -165 to +56	GTTGAAGCATGATGAAGCGTTCTAAAC
F2110	*HO* Promoter -165 to +56	GCGATGTCTTTAATTTCACCGTTAGC
F1154	*HO* Promoter -43 to +176	CCATATCCTCATAAGCAGCA
F1155	*HO* Promoter -43 to +176	AAGCTCTGTGTTTGGTTTTT

REFERENCES

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