Supplemental Data

Genome-Wide Dynamics of Htz1, a Histone H2A Variant that Poises Repressed/Basal Promoters for Activation through Histone Loss

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Supplemental Experimental Procedures

RNA Preparation and Microarray Analysis

Heat shock (HS) and diauxic shift experiments utilized YBC2128 (WT) and YBC1864 (*htz1Δ)*. For HS, cells were grown in YPD at 25°C to an OD_{600} of ~0.8, and a portion (control sample) of the culture was spun down and frozen in liquid nitrogen as T=0, no HS. The remainder were collected and resuspended in YPD pre-warmed at 37°C. Growth was continued at 37°C for 30 min and samples were collected (HS). Then the culture was shifted back to 25°C for 30 min and samples were collected (recovery). For diauxic shift experiments, cells were grown in YPD at 30°C to an OD_{600} of ~0.3 (T=0), and samples were collected every 2 hr for 24 hr. HS and diauxic shift experiments were performed as duplicate biological replicates. HS time course experiments (time points of 0, 5, 10, and 20 min following HS) were performed as triplicate biological replicates, and cultures were split for expression profiling and ChIP analysis (by qPCR). RNA preparation and microarray analysis was performed essentially as described by Zhang et al., 2004.

Chromatin Immunoprecipitation (ChIP)

ChIP experiments were performed as described by Roberts et al., 2003, with the following modifications. Strains were grown in YPD at 30°C to an OD_{600} of 0.6-0.8 and cells were cross-linked with 1% formaldehyde for 45 min at room temperature. For experiments aimed at determining Htz1 occupancy genome-wide in response to HS, samples were collected and treated as described above either prior to HS (T=0), following 30 min of HS, and following 30 min recovery at 25°C. For experiments aimed at determining Htz1 occupancy by qPCR analysis, samples were taken at the time points of 0 (no HS), 1, 2, 5, 10, 20, and 30 min following HS. For diauxic shift experiments, samples were taken at time points described above. All ChIPs were performed as described for HA-Htz1 ChIPs (Zhang et al., 2004) with the following modifications: for αHtz1 and αH3 ChIPs, 1 μl of polyclonal αHtz1 antibody (Abcam, catalog no. ab4626) or 2 μl of polyclonal αH3 antibody (Abcam, catalog no. ab1791) was used, respectively, with 35 μl of sheep anti-rabbit IgG M-280 Dynabeads (~ 2×10^7 beads, Dynal Biotech, catalog no. 112.04) and 500 μg of supernatant from the sheared chromatin; For Swr1-Myc, H2A-TAP and H3-TAP ChIPs, 1.33 μl of anti-Myc antibody (9E11, GeneTex, catalog no. GTX20056) or 2 μl anti-protein A antibody (Sigma, catalog no. P-2921) was used, respectively, with 50 μl of pan-mouse IgG Dynabeads (~ 2×10^7 beads, Dynal Biotech, catalog no. 110.23) and 500 μg of supernatant from the sheared chromatin. All ChIPs were performed in duplicate except the following, which were performed in triplicate: HA-Htz1 (αHA) ChIPs in strain YBC1867 and YBC2084; H2A-TAP (anti-protein A) ChIPs in strain YBC2200 and parallel untagged control ChIPs in strain YBC1894; HS time course experiments in which histone occupancy (HA-Htz1, H2A-TAP and H3) was analyzed by qPCR analysis; αHtz1 ChIPs in WT (YBC1894), *bdf1Δ* (YBC2512), *bdf2Δ* (YBC2513), *sas3Δ* (YBC1911), and *gcn5Δ* (YBC1662) mutants. The purified ChIP samples were subjected to either ChIP microarray (ChIP-chip) analysis or qPCR analysis.

Genome-wide Localization Experiments (ChIP-chip)

The amplification and labeling of ChIP materials, hybridization, scanning and analyzing of arrays were performed essentially as described previously (Roberts et al., 2003). Flagged spots (of poor quality) and background-subtracted spots whose intensity were < 5 in either Cy3 or Cy5 channel, or the sum of intensity in both channels were < 25, were removed. Then Cy3 intensities are normalized to Cy5 intensities such that the mean ratio of Cy5/Cy3 intensities is 1. The normalized ratios of Cy5 (labeled ChIP DNA) to Cy3 (labeled input DNA) were calculated.

Also, a percentile rank was assigned to each segment on the array based on the ChIP enrichment ratios as described in the text. The median ChIP ratios and median percentile ranks (MPR) are reported in Table S4.

Real-Time Quantitative PCR (qPCR) Analysis

qPCR was performed as described by Zhang et al., 2004, except that the average of three independent replicates was reported as relative amplification of each target of interest compared to a normalization control amplicon, which is within the non-promoter IGR iYMR325W, using primer set A. Sequences for all primer sets used in this study are provided in Table S2.

Chromatin Preparation

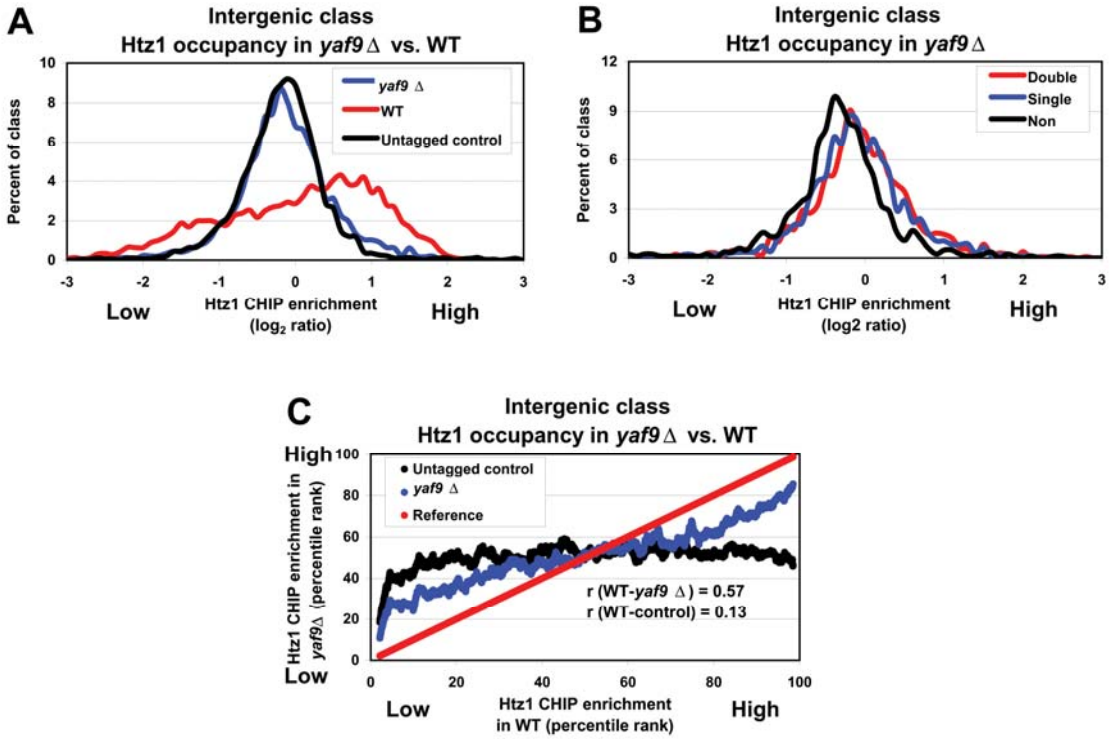
Strain YBC2228 (HA-Htz1, H2A-TAP) was grown in 1 L of YPD at 30°C to an OD_{600} of ~0.8. Cells were harvested by centrifugation and washed in 0.5 volume cold ddH2O and then 0.5 volume SB (20 mM Tris-HCl [pH 7.5], 1 M sorbitol), resuspended in 10 ml SB, frozen in liquid nitrogen and kept at –80°C until use. The cell slurry was thawed on ice, resuspended in 10 ml PSB (200 mM Tris-HCl [pH 7.5], 20 mM EDTA, 1 M NaCl, 100 mM 2-mercaptoethanol (2-ME)) and incubated at room temperature for 10 min. Cells were collected by centrifugation, washed in 50 ml of wash buffer (20 mM Tris-HCl [pH 7.5], 1 M NaCl) and 50 ml SB, and then resuspended in 10 ml SB containing 200 µl glusulase (PerkinElmer, catalog no. PC1128-0595) and incubated at 30°C for 1 hr or until the reading at A_{600} of the mixture dropped to 20-30% of original. The slurry was pelleted by centrifugation and spheroplasts were washed twice in 50 ml SB. The pellet was resuspended in 20 ml EBX-0.1 (20 mM Tris-HCl [pH 7.5], 0.1 M NaCl, 0.25% Triton X-100, 15 mM 2-ME, protease inhibitor cocktail (PI: 2 µg/ml chymostatin, 2 µM pepstatin A, 0.6 µM leupeptin, 2 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride), 50 mM Na-butyrate), and a 10% Triton X-100 solution was added to a final concentration of 0.5%. Suspensions were gently swirled for 10 min while on ice. Cell lysates were then layered over a 10 ml cushion of NIB (20 mM Tris-HCl [pH 7.5], 0.1 M NaCl, 1.2 M sucrose, 15 mM 2-ME, PI, 50 mM Na-butyrate) and centrifuge at 12,000 *g* for 20 min. The nuclear pellet was resuspended in 10 ml EBX-0.1 and nuclei were lysed by adding 10% Triton X-100 to a final concentration of 1%. Nuclear suspensions were swirled on ice for 15 min, split into 3 tubes, centrifuged at 15,000 *g* for 20 min. To test for salt resistance of Htz1, pellets were then separately washed twice in one of three buffers of increasing stringency: EBX-0.25 (same as EBX-0.1 but with 0.25 M NaCl), EBX-0.5 (0.5 M NaCl), or EBX-0.7 (0.7 M NaCl). All three were then separately washed with EBX-0.1 (0.5 mM 2-ME, no Na-butyrate) and resuspended in 1 ml EBX-0.1 (0.5 mM 2-ME, no Na-butyrate). Each was then digested to mononucleosomes with micrococal nuclease (Usb, catalog no. 70196Y), subjected to centrifugation, and the supernatant was collected. The supernatant was subjected to SDS-PAGE analysis and western analysis using αHA (12CA5) antibody and αH3 antibody. The same experiment was repeated with strain YBC1895 (untagged strain) and western blots were probed with polyclonal αHtz1 antibody, polyclonal αH2A antibody (Upstate, catalog no. 07-146) and αH3 antibody.

Supplemental References

Roberts, D.N., Stewart, A.J., Huff, J.T., and Cairns, B.R. (2003). The RNA polymerase III transcriptome revealed by genome-wide localization and activity-occupancy relationships. Proc. Natl. Acad. Sci. USA *100*, 14695–14700.

Zhang, H., Richardson, D.O., Roberts, D.N., Utley, R., Erdjument-Bromage, H., Tempst, P., Cote, J., and Cairns, B.R. (2004). The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation, and Htz1 replacement near telomeres. Mol. Cell Biol. *24*, 9424–9436.

Figure S1. Yaf9 Is Required for Efficient Htz1 Deposition

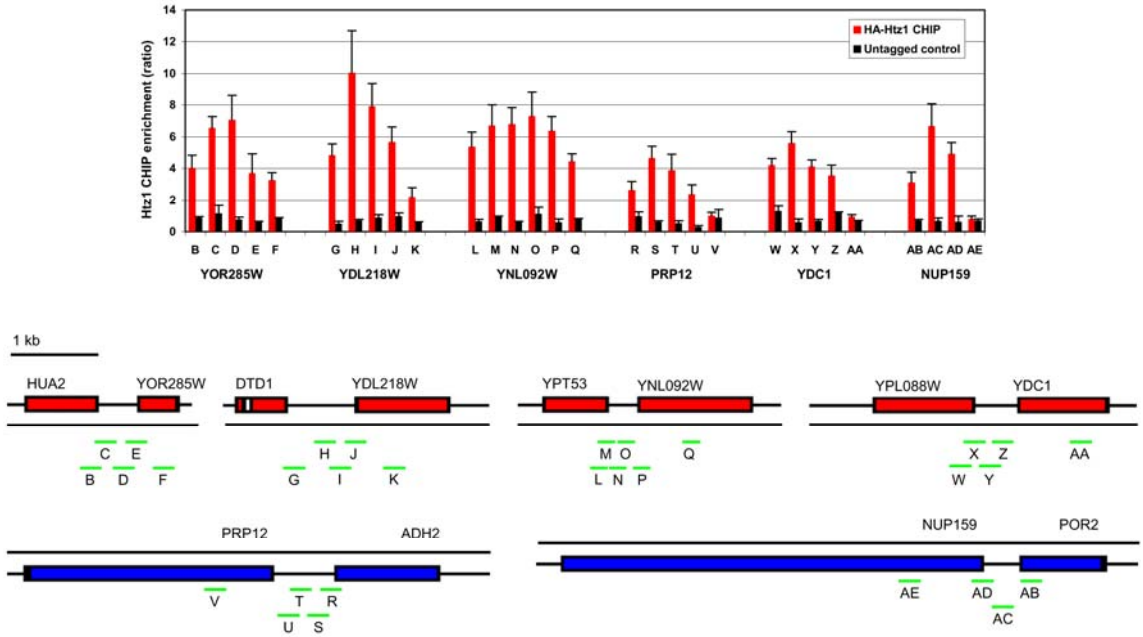


(A) Htz1 occupancy is highly reduced genome-wide in a strain lacking Yaf9. Distribution of median ratios of Htz1 ChIP at IGRs in a *yaf9Δ* strain was compared to that in WT and untagged control strains. Strains: *yaf9Δ* YBC2084; WT YBC1867; untagged YBC1895.

(B) Yaf9 is required for Htz1 promoter specificity. Distribution of the median ratios of Htz1 ChIP in a *yaf9Δ* strain for each promoter class.

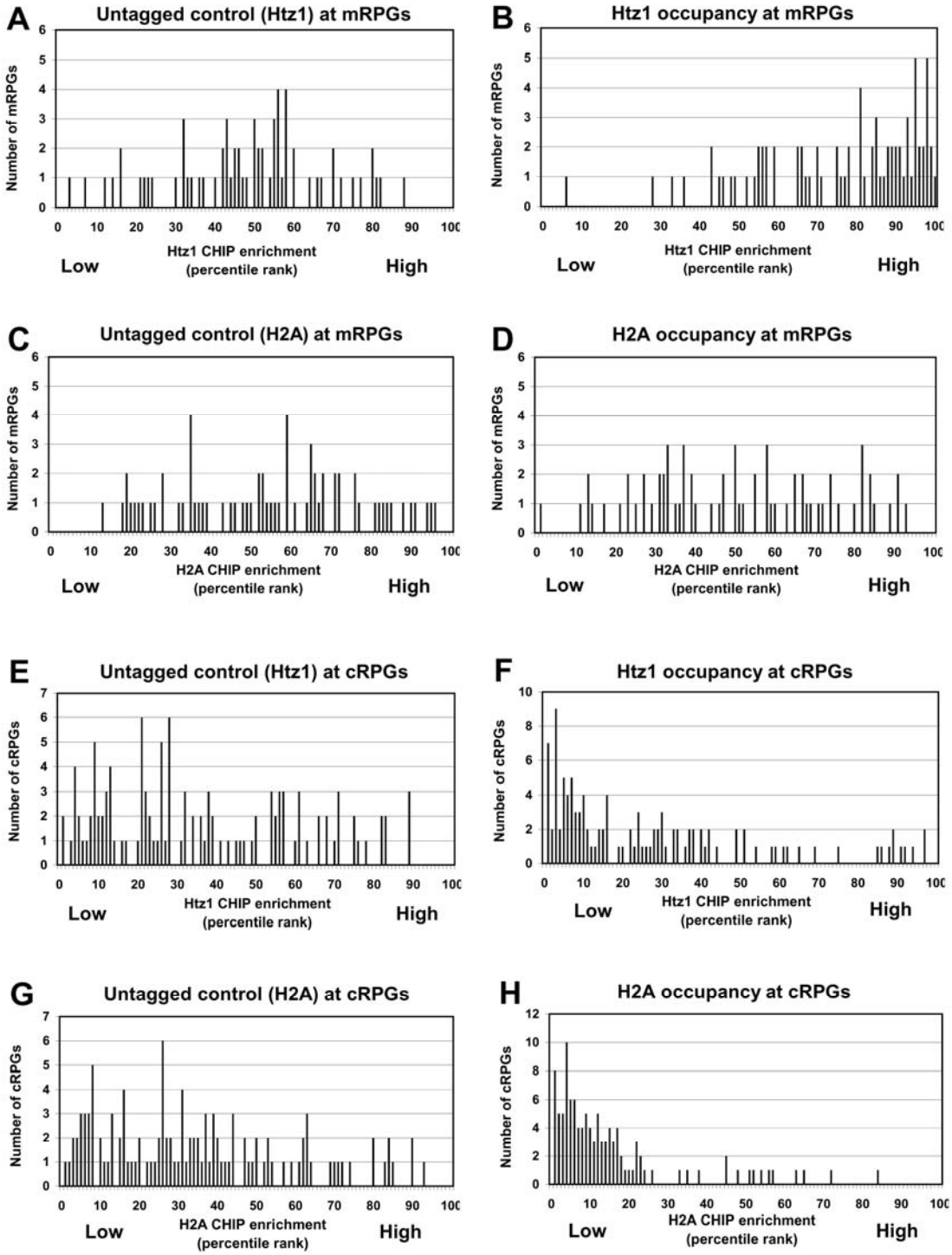
(C) Htz1 ChIP enrichment in WT (sorted by percentile ranks, x-axis) versus Htz1 occupancy in *yaf9Δ*, plotted as the moving average (window size 80, step 1) of percentile ranks (y-axis).

Figure S2. Tiling of Htz1 Occupancy at Promoters



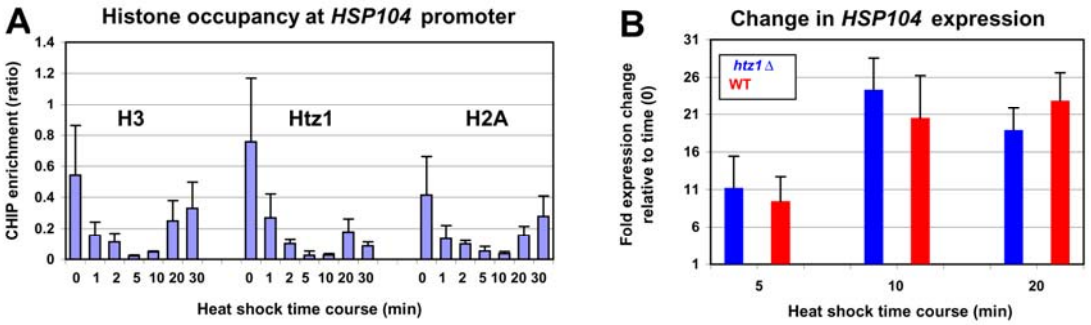
Htz1 occupancy at particular positions of six promoters was determined by qPCR of ChIP replicates. (p*YOR285W* and p*YDL218W* are TATA-containing promoters, with the predicted TATA located within amplicons D and I, respectively). Values are the average of three independent ChIPs with qPCR determination performed twice. Error bars: SD. Positions for each amplicon are as indicated on the physical map diagram. See Table S2 for primer sequences.

Figure S3. Htz1 Occupies the Promoters of mRPGs, but Is Deficient at the Promoters of cRPGs



Each histogram depicts the number of mRPGs (A-D) or cRPGs (E-H) (y-axis) within each percentile rank interval of Htz1 or H2A ChIP enrichment (x-axis) in tagged (HA-Htz1: B and F; H2A-TAP: D and H) and untagged (for Htz1: A and E; for H2A: C and G) strains. See Figures 1 and 2 for strain designations.

Figure S4. The *HSP104* Promoter Is Histone Deficient and Lacks Histones Entirely Following Heat Shock



(A) The *HSP104* promoter is not enriched for Htz1, and all tested histones are lost following HS. Histone ChIP enrichment was determined by qPCR. Values are the average of three independent ChIPs with qPCR determination performed twice. Error bars: SD.
(B) Deletion of *HTZ1* has no impact on *HSP104* activation during HS. Changes in *HSP104* expression during HS time course in WT and *htz1Δ* strains were quantified by microarray analysis. Error bars: SD.

Table S1. Yeast Strains Used in This Study

Strains*	Genotype	Source
YBC1662	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 gcn5Δ::KanMX*	Research Genetics
YBC1864	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 htz1Δ::KanMX*	Research Genetics
YBC1867	*MATα HHT1-HHF1 Δ(HHT2-HHF2) leu2-3,112 ura3-52 lys2Δ201 HA-HTZ1*	M. Smith
YBC1894	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	Research Genetics
YBC1895	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Research Genetics
YBC1911	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sas3Δ::KanMX*	Research Genetics
YBC2084	*MATα HHT1-HHF1 Δ(HHT2-HHF2) leu2-3,112 ura3-52 lys2Δ201 HA-HTZ1 yaf9Δ::URA3*	This work
YBC2128	*MATα leu2 ura3 HA-HTZ1*	This work
YBC2137	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1408(YCp-50 copy II; H3-H4 WT; URA3)]*	This work
YBC2150	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1425(CEN; H3 WT; H4-K16R; TRP1)]*	This work
YBC2151	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1423(CEN; H3 WT; H4-K16Q; TRP1)]*	This work
YBC2154	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1420(CEN; H3 WT; H4-K5,12R; TRP1)]*	This work
YBC2156	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1420(CEN; H3 WT; H4-K8,16R; TRP1)]*	This work
YBC2162	*MATα leu2 ura3 HA-HTZ1 swr1Δ::KanMX*	This work
YBC2170	*MATα leu2 ura3 HA-HTZ1 SWR1-Myc::KanMX*	This work
YBC2198	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 HHT2:TAP::his5+*	E.K. O'Shea and J.S. Weissman
YBC2200	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 HTA2:TAP::his5+*	E.K. O'Shea and J.S. Weissman
YBC2218	*MAT***a** *leu2 ura3 lys2-128∂ HA-HTZ1 set1Δ::HIS3MX6*	This work
YBC2223	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1435 (pRS314-H3K4A-H4WT (copy II); TRP1)]*	This work
YBC2224	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1436 (pRS314-H3K4R-H4WT (copy II); TRP1)]*	This work
YBC2228	*MATα leu2 ura3 HA-HTZ1 HTA2:TAP::his5+*	This work
YBC2460	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1415(H3K14Q-H4 WT; TRP1)]*	This work
YBC2461	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1416(H3K14G-H4 WT; TRP1)]*	This work
YBC2462	*MATα hht1-hhf1::pWZ405-F2F9-LEU2 Δ(HHT2-HHF2) leu2 his3Δ200 ura3-52 lys2 trp1Δ63 HA-HTZ1 [p1417(H3K14R-H4 WT; TRP1)]*	This work
YBC2512	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 bdf1Δ::KanMX*	Research Genetics
YBC2513	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 bdf2Δ::KanMX*	Research Genetics

*All strains are derivatives of the S288C genetic background.

Table S2. Primers Used in This Study

Primer Sets	Gene	Forward Primer	Reverse Primer	Amplicon Size	Annealing Tm (^{o}C)
A	iYMR325W	CAGTGTTTGTGGAGCATTTTCTG	AAGTGACGCATATTCTATACGACCC	235	56
B	YOR285Wp4	GGGCAAACAAGTGTCCAGTTCTAA	CACACAGTGTCCCGTTAATTTCA	264	57
C	YOR285Wp3	ATACAGCATACTAAGCGCTTTCCAG	CATTGCAAGTAGGAAAAGGACATACC	238	57
D	YOR285Wp2	CATAAGGCCCAAATCCAGATATCA	CGTTTCTTAGTCACAAACCCTGA	248	56
E	YOR285Wp1	GGAAGGAAGTAAGGGGAGAATTT	ACCACATTAGGATCATGCTTTCC	239	56
F	YOR285Wo1	CTCGATTGTTCATATTCCTGCTTCC	AAGTCAAGTTTATCACCCCCATGAG	261	58
G	YDL218Wp5	ACTAATGAAGGGCCCGTTACAATC	TTGGCGAAGGTTATTGAAGGAAG	252	58
H	YDL218Wp3	GTAAAAGTGAAAAAAGGGACGG	GTCATCAACGAGTATGCTTACTGGT	225	56
I	YDL218Wp2	GGGGATATATACCCTTAAATTGACG	AAAACCGTAAACTTTCGTACTGAGA	244	55
J	YDL218Wp1	AAGGTTCAACAAAGCGTCCGTT	TACCTCTATTTCCATAGGCGTGGA	229	56
K	YDL218Wo	TACTGCTGACAGTCATAGAAGAGCATG	CGGTCCTAACATTGGTGTTATCGTA	260	58
L	YNL092Wp5	ATAAGGCTCAAAACTGGGTCGAAG	GTCGTGTATTTTGTTCAGGACATGG	261	58
M	YNL092Wp4	GAAAGCACCAGCTGTACAAAATCTC	GTTTGCTGTTGTTTTGCTTGCAG	265	57
N	YNL092Wp3	CTACTCATGATCGCACAATTACCG	CCCGCCCCTAAAAAAAGTGTATT	280	57
O	YNL092Wp2	ACAGTTCGTAACAACAGCTGGAAGA	TCAGATCCTCACCAATTTTTGCC	235	58
P	YNL092Wp1	ACTGTTATTTTGGTGTACCAGCGG	CTGAGATCGATGCCCATTTTTTC	220	58
Q	YNL092Wo	CCTTCTCGTGGATCTTAGCAGAAT	ATCAACAAACGAGCCAGCACATA	259	58
R	PRP12p5	AGGTAGTTGGCTTATCCAGTTTACCAG	ATGGTGTTCACTCGTGCTAGCAAA	250	58
S	PRP12p4	TGACAGTGTTCGAAGTTTCACGA	GAACTTCGATCATTTCAATGCTGG	262	58
T	PRP12p3	TCCTCCAACAAACAGTCGGTTATAG	GGTGTGTACATTGCAGTGCATCAT	259	58
U	PRP12p2	TTTCACTTTTCGTCTTGACGTCC	CTGTATAGGCCCGCTATATTTTGGT	227	58
V	PRP12o1	ACTTACTCTCGTACTTCACTCGAGCTTC	ACAATTCTTGGCAGAAATGGCAC	254	58
W	YDC1 p5	GAGTGATCCAACTTTCAAGTCGTTAC	CTAACTTAACATCTTTGCCTCTGGG	279	56
X	YDC1 p4	TACAGCAAGAGTAGACGAAGCGATT	GGGACATGAAGTAATATGAGCCGA	229	57
Y	YDC1 p3	GACACCTGCTGATTTTTACTGATCG	GAAGCATGCAATAGAAAGAAAGCAA	238	58
Z	YDC1 p2	CCTGAAAGCAACTTATCCATCAG	CACTAGGTTCTATGATATATCCTTTTGC	221	56
AA	YDC1 o1	CACGTTCATGATTCATTTGCAAAG	GGAAAAATCCCCACCTCCATATAA	298	58
AB	NUP159p4	TAACCGCTATTTGCAAGTGGGA	GAGAACGATAGAGACCAGCCAAAT	255	57
AC	NUP159p2	TTATCGTGACCTACGACCAGTGT	GTGCGTTTCTCTGCTAAAATTG	260	56
AD	NUP159p1	CTCAGGTTAATTTGTCTCCTCTCCAAC	GGCAGTTTTTCATTGAAGGAAGG	230	58
AE	NUP159o1	AGACCGATGACGAAGTTTCATAC	GTTCAGAATCCTGGGAAGGTTC	256	55
AF	MRK1p2	CAAAGTCGTCCGATGAGGAACTAA	GAGATTATTTTCAAGTCCCTTCCCC	224	58
AG	MRK1o	GCCGCGTGTTGAAATTAAATTCT	TCGACCTGGTTTGAGTAATTAGTGG	261	58
AH	YNL116Wp	CCTTGCCCCAGTGTACACATATATAA	TGCGTTGCTATACTTTCTCGACTTC	272	58
AI	YNL116Wo	CTTGATATATGCTGCAAACCAGCC	GCCAGCCTTTCTTATAATCGGTTC	226	58
AJ	RIM11p	TACTACCAAGGGGTTCTGTAAGGCTT	GTTCTAGCGTTGCGTGATTAGTGTT	269	58
AK	RIM11o	CAGAGGTTACGTCATTTCGTCCAT	CTGGTGCTCTATAGTACCGTGAACAA	259	58
AL	RRP43 o1	AAATTGTAGTCTTGAGTCGCACTGG	TAATTGGCATTCGGGGTCTAACT	278	57
AM	RBK-RRP43 p1	GATGCCTGTGAAACTACTGTCAAAC	TATGCTGTTGGAACTTTGGGATG	259	57
AN	RBK o1	CAGATCGTATATAACCCCTCACCTTTC	TCTCGAACCCAAAGTCATAACCAC	255	58
AO	HSP104 p1	TGTGGCGAGAGTTTCATGGTTTA	ATTTGCAGTTCTTTGAGATGGGC	298	58
AP	HSP104 o1	CTGCTCAATTAGCCAAGCGTTACT	ATGAAGCTTCCTTCTGCCTAGCTAAC	240	58