Supplemental Materials

Supplemental Methods

In Vitro Nucleosome Stability Assay

The stability of H2A-H2B binding to the nucleosome was measured by Förster Resonance Energy Transfer (FRET) assay during NaCl-induced dissociation, as previously described (HOCH *et al.* 2007). Briefly, NCPs were reconstituted by salt dialysis using the 149mer 601 DNA positioning sequence and purified *Xenopus* histone proteins including WT H2B or the H2B-L103A mutant. Assays were conducted at 100 nM NCP using H4-V60W as the FRET donor and H2A-L108C-AEDANS as the FRET acceptor in FRET reaction buffer (20 mM Tris-Cl, pH 7.6; 0.1 mM EDTA, 0.1 mM b-mercaptoethanol) at 25 °C in varying salt concentrations.

Figure S1

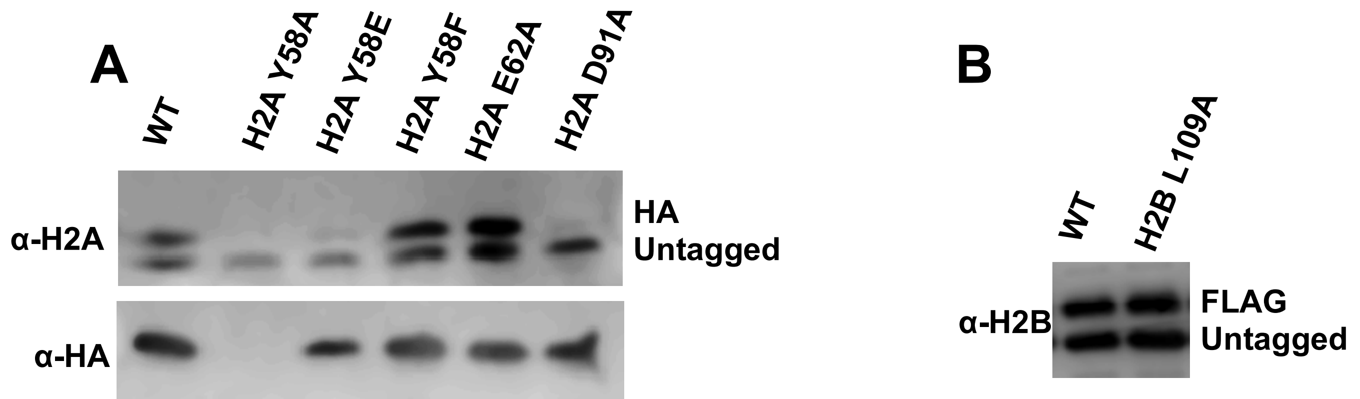


Figure S1. Analysis of expression of mutant histones by Western blot of yeast whole cell extracts. In all cases, an untagged wild type histone was co-expressed. (A) HA-tagged essential histone mutants analyzed with both the H2A (upper panel) and HA (lower panel) antibodies (B) FLAG-tagged H2B L109A analyzed using the anti-H2B antibody.

Figure S2

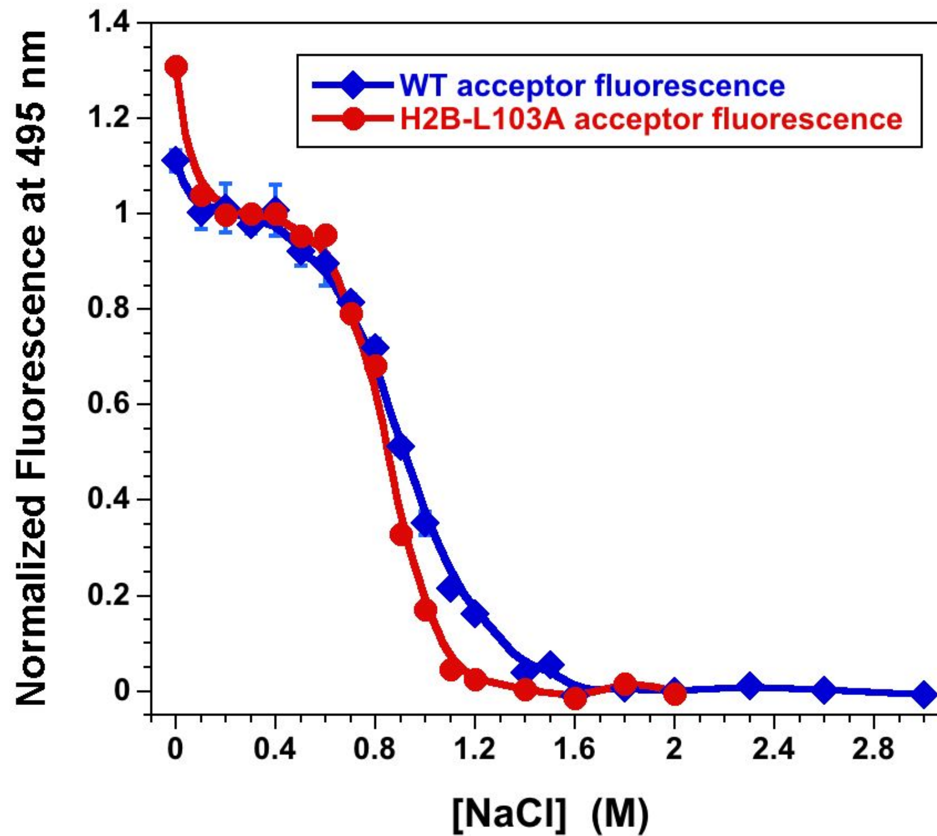
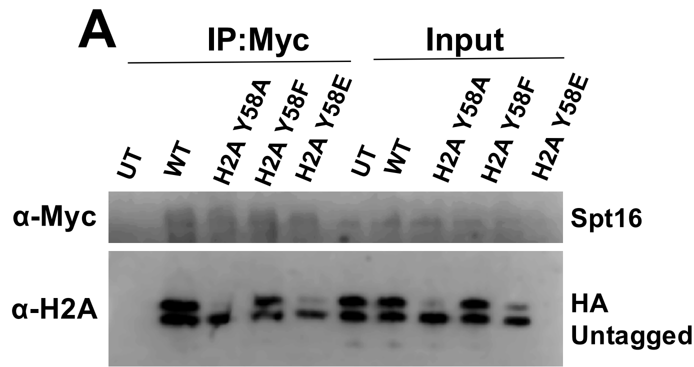
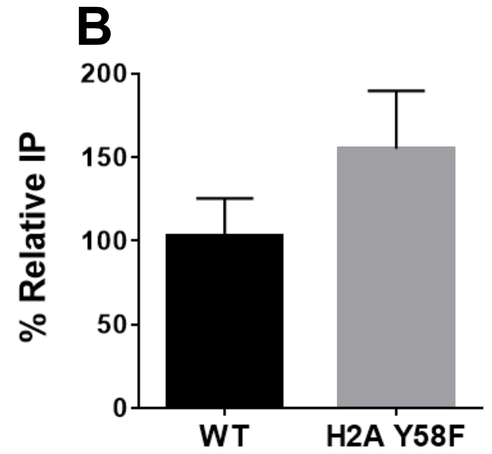
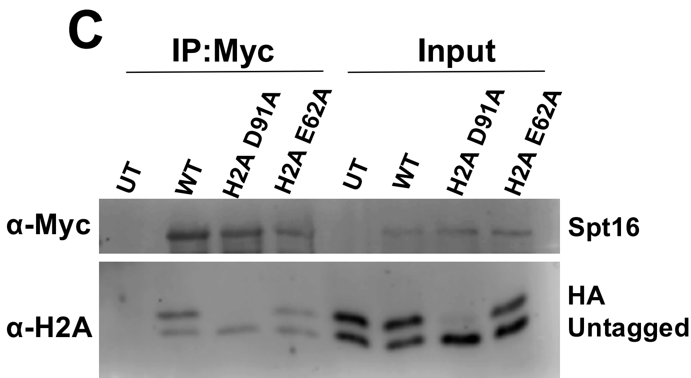


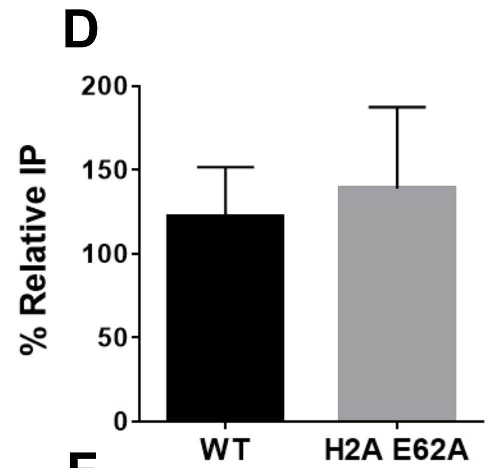
Figure S2. The *Xenopus* H2B L103A mutant (homologous to yeast H2B L109A) has only a minor effect on nucleosome stability *in vitro*. FRET analysis of the salt stability of *Xenopus* H2B L103A-containing nucleosomes. Fluorescence of the H2A-L108C-AEDANs acceptor was monitored at indicated salt concentrations for 100nM of WT or H2B L103A-containing NCPs. Error bars are shown or are equal or smaller than the size of the data points.

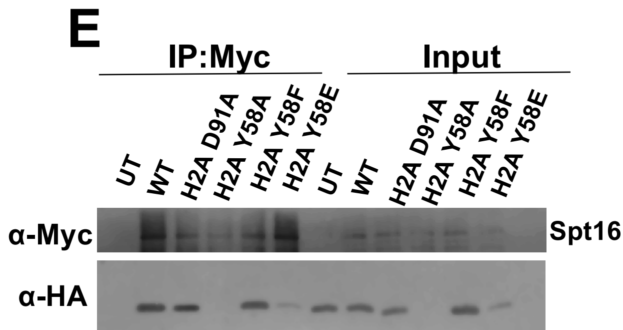
Figure S3











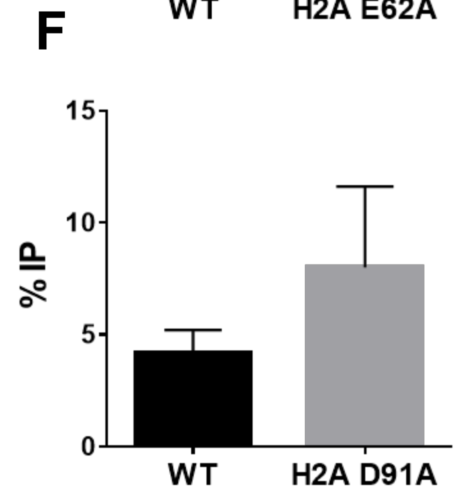


Figure S3. Other essential acidic patch residues are not important for binding FACT. Co-IP analysis using anti-Myc beads of nuclease treated yeast whole cell extracts containing Spt16-Myc and HA-tagged (A-B) H2A Y58A/F/E with the H2A antibody (C-D) E62A with the H2A antibody (E-F) H2A D91A and Y58A/F/E with the HA antibody. (B,D,F) Quantification of the % IP of the tagged band relative to the untagged band. Graphs represent the mean and SEM of at least 3 independent replicate experiments.

Figure S4

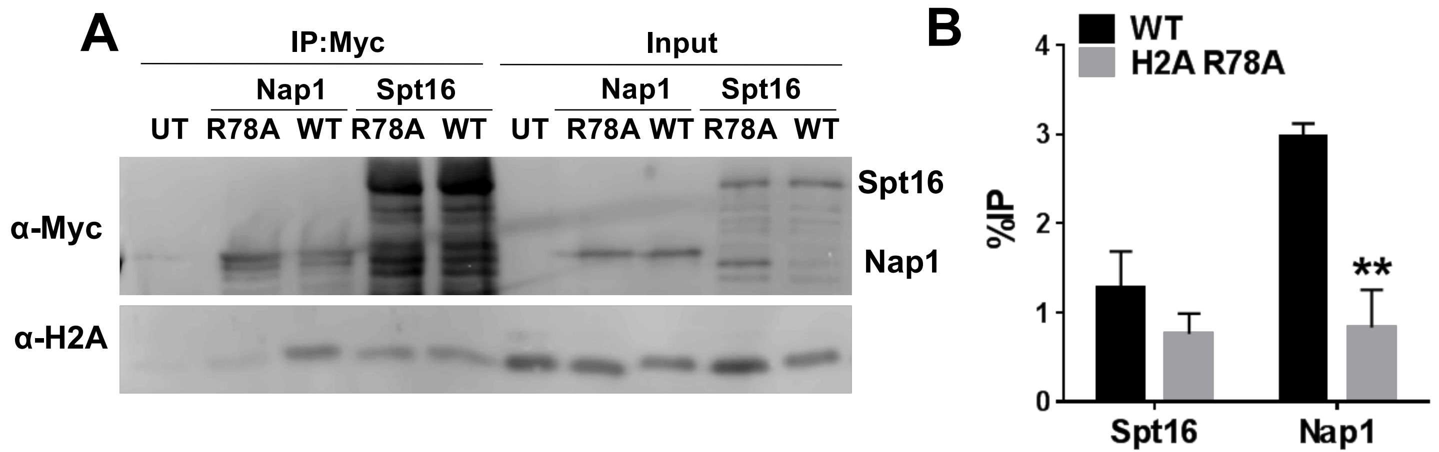


Figure S4. Analysis of FACT and Nap1 binding to H2A R78A expressed as the only histone copy. Co-IP analysis was conducted using anti-Myc beads and Spt16-Myc or Nap1-Myc in the absence of nuclease. Quantification of the % IP representing the mean and SEM of at least 3 independent replicates. **$P \leq 0.01$

Figure S5

Figure S5. (A) H2A R78E mutant is lethal in yeast. H2A R78E was expressed on a *LEU2* plasmid and wild type histones were expressed on a *URA3* plasmid that was shuffled out by FOA selection. (B) HA-tagged H2A R78E mutant analyzed using both anti-H2A (upper panel) and anti-HA (lower panel) antibodies. An untagged wild type H2A is co-expressed.

Figure S6

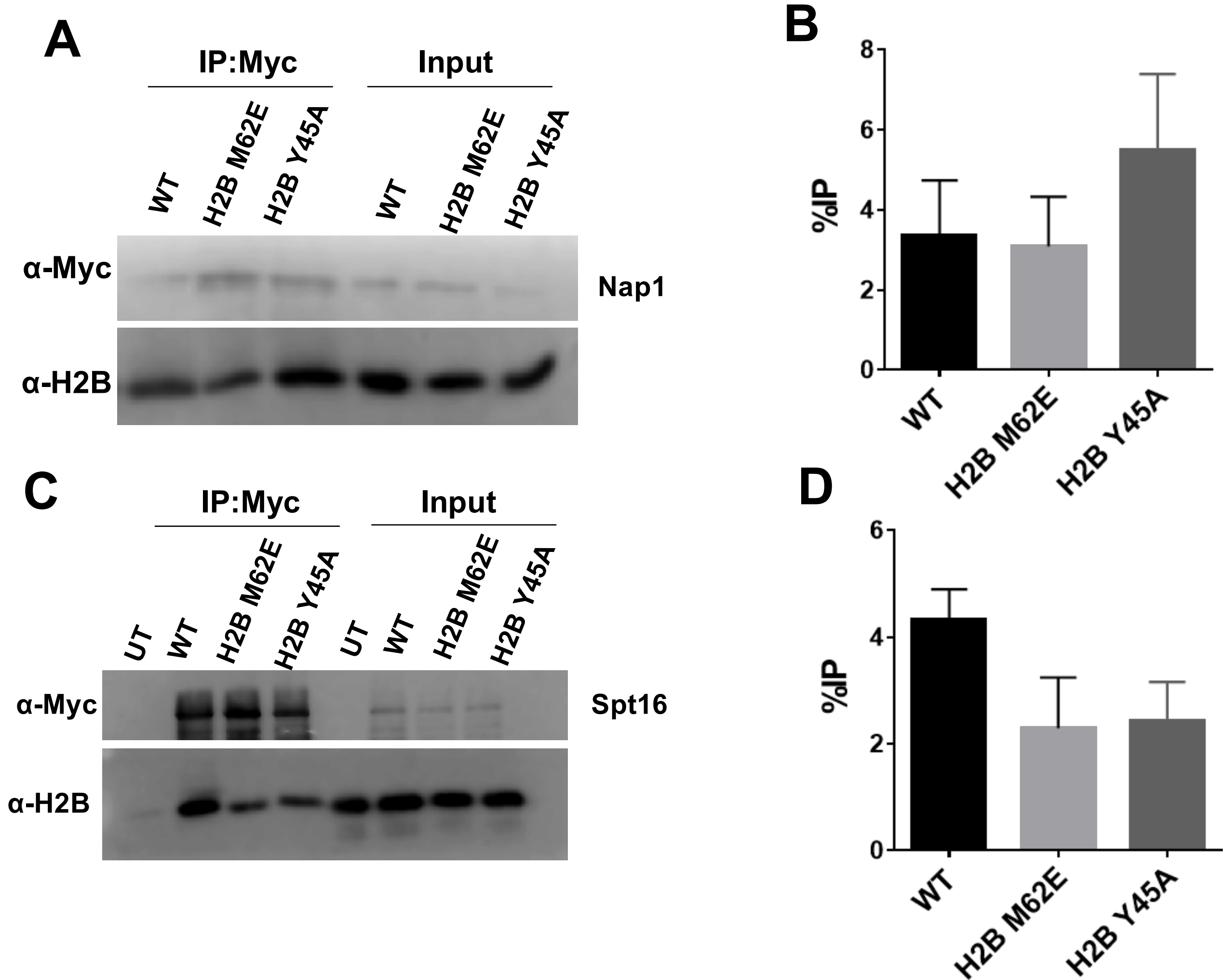


Figure S6. Analysis of FACT and Nap1 binding to H2B M62E and Y45A mutants when expressed as the only histone copy. Co-IP analysis with anti-Myc beads was conducted using Nap1-Myc (A-B) and using Spt16-Myc following nuclease treatment (C-D). Quantification of the % IP representing the mean and SEM of at least 3 independent replicates.

Table S1. Yeast Strains Used in This Study

Strain Name	Genotype	Figure(s)
WY500* (WT)	*MAT**a** his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf2-hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1-HHT2-HHF2*]	
YT01 (H2A D91A)	Isogenic to WY500; also includes p[*CEN LEU2 hta1_D91A-HTB1-HHT2-HHF2*]	1
YT02 (H2A E62A)	Isogenic to WY500; also includes p[*CEN LEU2 hta1_E62A-HTB1-HHT2-HHF2*]	1
YAH053 (HA-H2A Y58A)	Isogenic to WY500; also includes p[*CEN LEU2 hta1_HA Y58A-HTB1-HHT2-HHF2*]	1,S1
YT03 (H2B L109A)	Isogenic to WY500; also includes p[*CEN LEU2 HTA1-htb1_L109A-HHT2-HHF2*]	1
YAH059 (HA-H2A Y58F)	Isogenic to WY500; also includes p[*CEN LEU2 hta1_HA Y58F-HTB1-HHT2-HHF2*]	1, S1
YAH060 (HA-H2A Y58E)	Isogenic to WY500; also includes p[*CEN LEU2 hta1_HA Y58E-HTB1-HHT2-HHF2*]	1, S1
WY471.500 (WT Gal Shutdown WT)	Isogenic to WY500; URA plasmid replaced with p[*CEN HIS3 HTA1-pGAL-HTB1-HHT2-HHF2*] p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*]	1
WY471.1 (WT Gal Shutdown ΔL1)	Isogenic to WY500; URA plasmid replaced with p[*CEN HIS3 HTA1-pGAL-HTB1-HHT2-HHF2*] p[*CEN LEU2 hta1_Δ39-42-HTB1-HHT2-HHF2*]	1
WY471.3 (WT Gal Shutdown Leu2)	Isogenic to WY500; URA plasmid replaced with p[*CEN HIS3 HTA1-pGAL-HTB1-HHT2-HHF2*] p[*CEN LEU2*]	1
WY471.pT01 (WT Gal Shutdown H2A D91A)	Isogenic to WY500; URA plasmid replaced with p[*CEN HIS3 HTA1-pGAL-HTB1-HHT2-HHF2*] p[*CEN LEU2 hta1_D91A-HTB1-HHT2-HHF2*]	1
WY471.pT02 (WT Gal Shutdown H2A E62A)	Isogenic to WY500; URA plasmid replaced with p[*CEN HIS3 HTA1-pGAL-HTB1-HHT2-HHF2*] p[*CEN LEU2 hta1_E62A-HTB1-HHT2-HHF2*]	1
WY471.pT03 (WT Gal Shutdown H2B L109A)	Isogenic to WY500; URA plasmid replaced with p[*CEN HIS3 HTA1-pGAL-HTB1-HHT2-HHF2*] p[*CEN LEU2 HTA1-htb1_L109A-HHT2-HHF2*]	1
WY471.pT016 (WT Gal Shutdown H2A Y58A)	Isogenic to WY500; URA plasmid replaced with p[*CEN HIS3 HTA1-pGAL-HTB1-HHT2-HHF2*] p[*CEN LEU2 hta1_Y58A-HTB1-HHT2-HHF2*]	1
YT09 (Untagged ChIP control)	Isogenic to WY500; URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] p[*CEN URA3 HTA1-HTB1-HHT2-HHF2*]	1
YAH002 (Untagged WT FLAG-H2B L109A)	Isogenic to WY500; URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] p[*CEN URA3 HTA1-htb1_L109A_FLAG-HHT2-HHF2*]	1, S5
YAH050 (Untagged WT; HA-H2A)	Isogenic to WY500; also includes p[*CEN LEU2 hta1_HA-HTB1-HHT2-HHF2*]	1,3,4,S1, S3
YAH051 (Untagged WT; HA-H2A	Isogenic to WY500; also includes p[*CEN LEU2 hta1_D91A_HA-HTB1-HHT2-HHF2*]	1,3,4,S1, S3

D91A)		
YAH094 (Spt16-Myc; HA-H2A R78E+wt)	Isogenic to LT100; also includes p[*CEN LEU2 hta1-_R78E_HA-HTB1-HHT2-HHF2*]	1,S5
YAH001 (Untagged WT FLAG-H2B)	Isogenic to WY500; URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] p[*CEN URA3 HTA1-htb1_FLAG-HHT2-HHF2*]	1,2,4
WY504 (WT)	Isogenic to WY500; also includes p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*]	1,5
LT100 (Spt16-Myc)	Isogenic to WY500 except spt16::9xMyc::TRP1 trp1^::His3	
LT101 (Nap1-Myc)	Isogenic to WY500 except nap1::9xMyc::TRP1 trp1^::His3	
YAHN8 (Nap1-Myc; FLAG-H2B L109A)	Isogenic to LT101; URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] p[*CEN URA3 HTA1-htb1_L109A_FLAG-HHT2-HHF2*]	2
YAHS8 (Spt16-Myc; FLAG-H2B L109A)	Isogenic to LT100; URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] p[*CEN URA3 HTA1-htb1_L109A_FLAG-HHT2-HHF2*]	2, S1
YAHS103 (Spt16-Myc; FLAG-H2B)	Isogenic to LT100; URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] p[*CEN URA3 HTA1-HTB1_FLAG-HHT2-HHF2*]	2, S1, S6
YAHN103 (Nap1-Myc; FLAG-H2B)	Isogenic to LT101; URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] p[*CEN URA3 HTA1-HTB1_FLAG-HHT2-HHF2*]	2, S6
YAH101 (Spt16-Myc; HA-H2A L86A+wt)	Isogenic to LT100; also includes p[*CEN LEU2 hta1-_L86A_HA-HTB1-HHT2-HHF2*]	3
YAH098 (Spt16-Myc; HA-H2A E57A+wt)	Isogenic to LT100; also includes p[*CEN LEU2 hta1-_E57A_HA-HTB1-HHT2-HHF2*]	3
YAHS17 (Spt16-Myc; HA-H2A)	Isogenic to LT100; also includes p[*CEN LEU2 hta1_HA-HTB1-HHT2-HHF2*]	3,4,S3
YMAP004 (Spt16-Myc; H2B M62E+wt)	Isogenic to LT100; also includes p[*CEN LEU2 HTA1-htb1_M62E_FLAG-HHT2-HHF2*]	4
YMAP005 (Spt16-Myc; H2B Y45A+wt)	Isogenic to LT100; also includes p[*CEN LEU2 HTA1-htb1_Y45A_FLAG-HHT2-HHF2*]	4
YMAP006 (Spt16-Myc; H2A R78A+wt)	Isogenic to LT100; also includes p[*CEN LEU2 hta1-_R78A_HA-HTB1-HHT2-HHF2*]	4
YAP007 (H2A E57A, H2B M62E)	Isogenic to WY500, also includes [*CEN LEU2 hta1_E57A-htb1_M62E-HHT2-HHF2*]	5
ML73 (H2A E57A)	Isogenic to WY500; URA plasmid replaced with p[*CEN LEU2 hta1_E57A-HTB1-HHT2-HHF2*]	5
YAH034 (WT Flo8-His3 Fusion)	Isogenic to WY500 plus *FLO8::HIS3;* URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-*	5

	HHF2]	
LT354.M62E (H2B M62E Flo8-His3 Fusion)	Isogenic to YAH034 URA plasmid replaced with p[*CEN LEU2 HTA1-htb1_M62E-HHT2-HHF2*]	5
LT354.Y45A (H2B Y45A Flo8-His3 Fusion)	Isogenic to YAH034 URA plasmid replaced with p[*CEN LEU2 HTA1-htb1_Y45A-HHT2-HHF2*]	5
LT354.E57A (H2A E57A Flo8-His3 Fusion)	Isogenic to YAH034 URA plasmid replaced with p[*CEN LEU2 hta1_E57A-HTB1-HHT2-HHF2*]	5
YMW001 (Spt16-Myc; H2A M62E)	Isogenic to LT100; URA plasmid replaced with p[*CEN LEU2 HTA1-htb1_M62E-HHT2-HHF2*]	5, S6
YMW003 (Spt16-Myc; H2A Y45A)	Isogenic to LT100; URA plasmid replaced with p[*CEN LEU2 HTA1-htb1_Y45A-HHT2-HHF2*]	5, S6
YAHS22 (Spt16-Myc; HA-H2A Y58A)	Isogenic to LT100; also includes p[*CEN LEU2 hta1_Y58A_HA-HTB1-HHT2-HHF2*]	S1, S3
YAHS24 (Spt16-Myc; HA-H2A Y58F)	Isogenic to LT100; also includes p[*CEN LEU2 hta1_Y58F_HA-HTB1-HHT2-HHF2*]	S1, S3
YAHS25 (Spt16-Myc; HA-H2A Y58E)	Isogenic to LT100; also includes p[*CEN LEU2 hta1_Y58E_HA-HTB1-HHT2-HHF2*]	S1, S3
YAHS20 (Spt16-Myc; HA-H2A D91A)	Isogenic to LT100; also includes p[*CEN LEU2 hta1_D91A_HA-HTB1-HHT2-HHF2*]	S1, S3
YAHS21 (Spt16-Myc; HA-H2A E62A)	Isogenic to LT100; also includes p[*CEN LEU2 hta1_E62A_HA-HTB1-HHT2-HHF2*]	S1, S3
YMW002 (Nap1-Myc; H2A M62E)	Isogenic to LT101; URA plasmid replaced with p[*CEN LEU2 HTA1-htb1_M62E-HHT2-HHF2*]	S3
YMW004 (Nap1-Myc; H2A Y45A)	Isogenic to LT101; URA plasmid replaced with p[*CEN LEU2 HTA1-htb1_Y45A-HHT2-HHF2*]	S3
YAH019 (Spt16-Myc; H2A R78A)	Isogenic to LT100; URA plasmid replaced with p[*CEN LEU2 hta1_R78A-HTB1-HHT2-HHF2*]	S4
YAH020 (Spt16-Myc; WT)	Isogenic to LT100; URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*]	S4
YAH023 (Nap1-Myc H2A; R78A)	Isogenic to LT101; URA plasmid replaced with p[*CEN LEU2 hta1_R78A-HTB1-HHT2-HHF2*]	S4
YAH024 (Nap1-Myc; WT)	Isogenic to LT101; URA plasmid replaced with p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*]	S4

Table S2. MIQE

Item to check	Importance	Item to check	Importance
Experimental design		qPCR oligonucleotides	
Definition of experimental and control groups **In this study, we measured the tagged mutant histones against tagged wild type histones and control untagged histones.**	E	Primer sequences **PMA1** FWD: GATCCACCAAGAGACGATACTGCT RVS: ACCGCCACCTAGACCTAATCTTTC **PYK1** FWD: TGGTTGCTTTGAGAAAGGCTGG RVS: TTCGTGGTTTGGTGGGATTGG **ICS2** FWD: ACGTGCTACGGACGAAAAGT RVS: CAGGGATTATTACCTGGGACACA **GAL1** FWD: GACCATTGGCCGAAAAGTGC RVS: ACCAGGCGATCTAGCAACAAA	E
Number within each group **3 or more**	E	RTPrimerDB identification number	D
Assay carried out by the core or investigator's laboratory? **By the investigator's laboratory in the core**	D	Probe sequences	D[4]
Acknowledgment of authors' contributions	D	Location and identity of any modifications **None**	E
Sample		Manufacturer of oligonucleotides **Invitrogen/Fisher**	D
Description **Yeast whole cell extracts**	E	Purification method **Desalted**	D
Volume/mass of sample processed **100uL for IP, 10uL for input**	D	qPCR protocol	
Microdissection or macrodissection	E	Complete reaction conditions **ABI 7500 FAST Quantitative ΔΔCt** 95°→(95°→60° (40x))→95°→60°→95°→60° 4' 5s 30s 15s 1' 15s 15s	E
Processing procedure	E	Reaction volume and amount of	E

Item to check	Importance	Item to check	Importance
Following formaldehyde crosslinking, yeast cells were spun down, lysed by bead beating, sonicated, and IP'ed using FLAG antibody. Extracts were proteinase K digested, crosslinking was reversed, and DNA extracted by PCI followed by RNAseA digestion		cDNA/DNA **ddH_2O 2.05µL** **Eva Green 7.50µL** **10x Rox 0.45µL** **DNA 2µL**	
If frozen, how and how quickly? **Extracts were frozen at -80° by placing in the freezer**	E	Primer, (probe), Mg^{2+}, and dNTP concentrations **Primer: 0.5mM** **The rest as in commercial Eva Green Mastermix (Biotium)**	E
If fixed, with what and how quickly? **1% Formaldehyde for 15 minutes**	E	Polymerase identity and concentration **Cheetah hot-start Taq polymerase**	E
Sample storage conditions and duration (especially for FFPE[2] samples) **Yeast pellets and extracts were stored at -80°**	E	Buffer/kit identity and manufacturer **Fast Eva Green qPCR Master Mix; Biotium**	E
Nucleic acid extraction **PCI**		Exact chemical composition of the buffer	D
Procedure and/or instrumentation **ABI 7500 FAST Quantitative ΔΔCt** 95°→(95°→60°(40x))→95°→60°→95°→60° 4' 5s 30s 15s 1' 15s 15s	E	Additives (SYBR Green I, DMSO, and so forth) **10x ROX**	E
Name of kit and details of any modifications **Fast Eva Green qPCR Master Mix +0.3x Rox; Biotium**	E	Manufacturer of plates/tubes and catalog number **96-well plates from Fisher Scientific Cat# AB-1900**	D
Source of additional reagents used **N/A**	D	Complete thermocycling parameters **ABI 7500 FAST Quantitative ΔΔCt** 95°→(95°→60°(40x))→95°→60°→95°→60° 4' 5s 30s 15s 1' 15s 15s	E
Details of DNase or RNase treatment **Samples were treated with 0.33mg/mL RNAseA at 37° for 15-20 minutes**	E	Reaction setup (manual/robotic) **Manual**	D
Contamination assessment (DNA or RNA) **RNA is not visible following treatment on agarose gel**	E	Manufacturer of qPCR instrument **ABI 7500 fast from Applied Biosystems**	E
Nucleic acid quantification **~10-100ng/uL for IP, ~250-350ng/uL for input**	E	qPCR validation	

Item to check	Importance	Item to check	Importance
Instrument and method **ABI 7500 FAST Quantitative ΔΔCt** 95°→95°→60°→95°→60°→95° →60° 4' 5s 30s 15s 1' 15s 15s	E	Evidence of optimization (from gradients)	D
Purity (A_{260}/A_{280})	D	Specificity (gel, sequence, melt, or digest) **Melt Curve**	E
Yield **~10-100ng/uL for IP, ~250-350ng/uL for input**	D	For SYBR Green I, C_q of the NTC **NTC fails to amplify**	E
RNA integrity: method/instrument **N/A**	E	Calibration curves with slope and *y* intercept **Slope:-3.379** **Y intercept: 41.19**	E
RIN/RQI or C_q of 3′ and 5′ transcripts **N/A**	E	PCR efficiency calculated from slope **>97.676%**	E
Electrophoresis traces **RNA is not visible on agarose gel**	D	CIs for PCR efficiency or SE	D
Inhibition testing ($C_q$dilutions, spike, or other) **$C_q$dilutions**	E	r^2 of calibration curve **0.995**	E
Reverse transcription **N/A**		Linear dynamic range **9 logs**	E
Complete reaction conditions	E	C_q variation at LOD **Average SD of C_q for duplicate reactions 0.07 cycles**	E
Amount of RNA and reaction volume	E	CIs throughout range	D
Priming oligonucleotide (if using GSP) and concentration	E	Evidence for LOD **Amplification detected in 100% of input samples**	E
Reverse transcriptase and concentration	E	If multiplex, efficiency and LOD of each assay **N/A**	E
Temperature and time	E	Data analysis	
Manufacturer of reagents and catalogue numbers	D	qPCR analysis program (source, version) **ABI 7500 Software**	E
C_qs with and without reverse transcription	D[3]	Method of $C_q$determination **ΔΔCt**	E
Storage conditions of cDNA	D	Outlier identification and disposition **Data was not excluded**	E
qPCR target information		Results for NTCs **No amplification**	E
Gene symbol	E	Justification of number and	E

Item to check	Importance	Item to check	Importance
PMA1 **PYK1** **ICS2** **GAL1**		choice of reference genes **NA**	
Sequence accession number **NA**	E	Description of normalization method **%IP/Input**	E
Location of amplicon **PMA1** chrVII:480900-481067 **PYK1** chrI:71895+72109 **ICS2** chrII:554899+555019 **GAL1** chrII:279088+279176	D	Number and concordance of biological replicates **At least 3**	D
Amplicon length **PMA1 168bp** **PYK1 215bp** **ICS2 121bp** **GAL1 89bp**	E	Number and stage (reverse transcription or qPCR) of technical replicates **2 technical replicates on qPCR plate**	E
In silico specificity screen (BLAST, and so on) **BLAST**	E	Repeatability (intraassay variation) **SD =0.02-0.2 cycles**	E
Pseudogenes, retropseudogenes, or other homologs? **No**	D	Reproducibility (interassay variation, CV)	D
Sequence alignment **NA**	D	Power analysis **NA**	D
Secondary structure analysis of amplicon **NA**	D	Statistical methods for results significance **t-test**	E
Location of each primer by exon or intron (if applicable) **NA**	E	Software (source, version) **Graphpad Prism 6**	E

Supplemental References:

HOCH, D. A., J. J. STRATTON and L. M. GLOSS, 2007 Protein-protein Forster resonance energy transfer analysis of nucleosome core particles containing H2A and H2A.Z. J Mol Biol **371:** 971-988.