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Supplemental Information

Phosphorylation of Histone H4T80

Triggers DNA Damage Checkpoint Recovery

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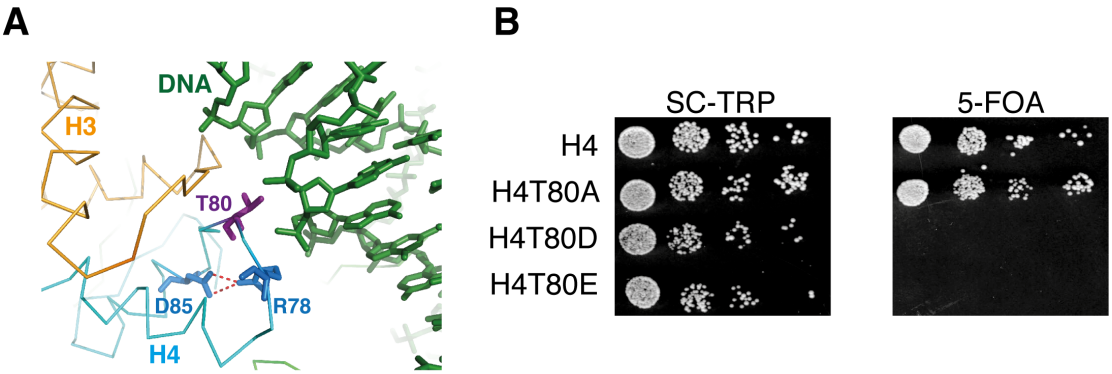

Figure S1. Histone H4T80 phosphorylation levels must be tightly regulated. Related to Figure 1. (A) Ribbon structure of H3 L1 loop and H4 L2 loop indicating hydrogen bonds between H4R78 and H4D85. **(B)** Spot test for cell viability. 10-fold serial dilutions were used.

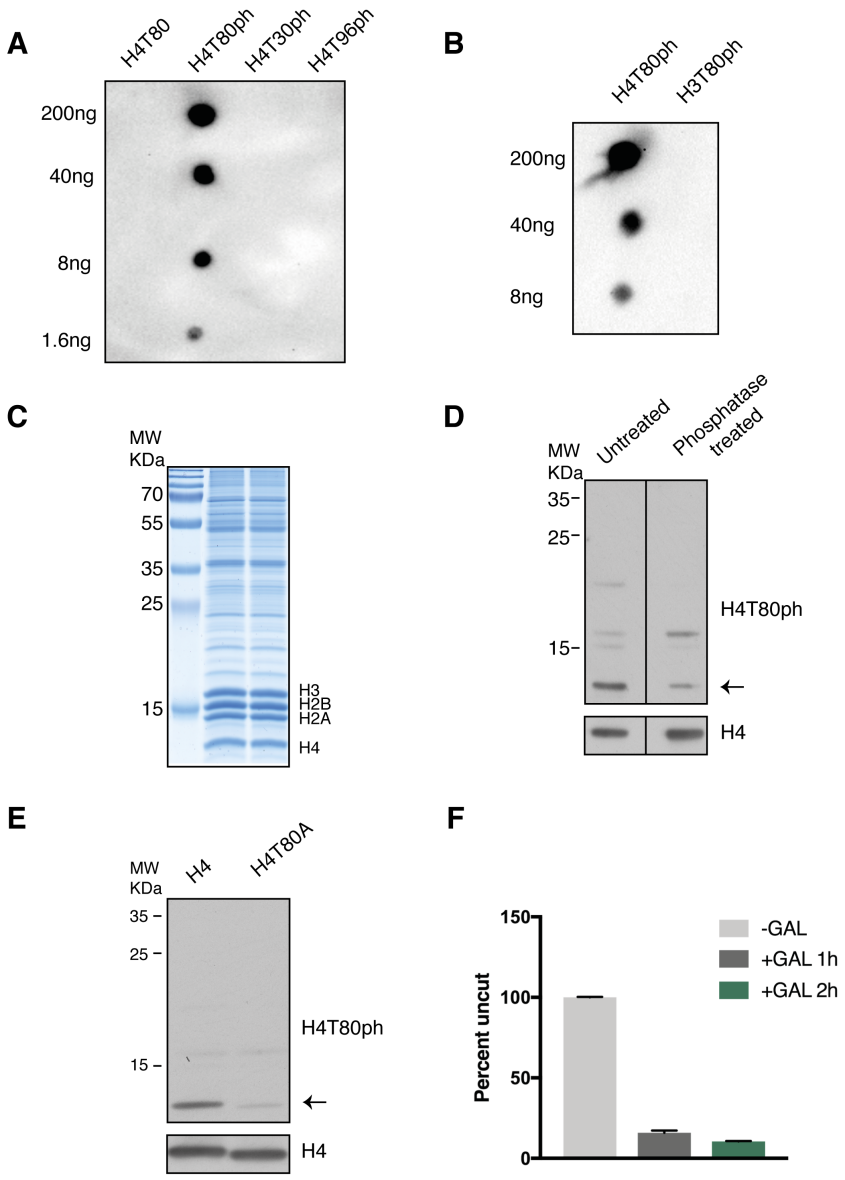

Figure S2. Anti-H4T80ph antibody specifically recognizes H4T80ph in yeast. Related to Figure 1. (A) and **(B)** Dot-blot analysis using H4T80ph specific antibody. 5-fold serial dilutions of different peptides, as indicated, were spotted onto a PVDF membrane. **(C)** Coomassie blue stained gel showing purified yeast histones separated by SDS-PAGE in 17% acrylamide gels. **(D)** Immunoblot analysis of purified yeast histones separated by SDS-PAGE in 17% acrylamide gels. Nitrocellulose membranes were treated or not with alkaline phosphatase. Blots were probed with anti-H4T80ph antibody and then re-probed with anti-H4 antibody as indicated. ← Corresponds to histone H4. **(E)** Immunoblot analysis of purified yeast histones separated by SDS-PAGE in 17% acrylamide gels. Both wild type and H4T80A mutant cells were grown to exponential phase. Blots were probed with anti-H4T80ph antibody and then re-probed with anti-H4 antibody as indicated. **(F)** HO cutting efficiency was measured by qPCR using primers encompassing the HO cut site and normalized to the *PRP8* reference locus. The mean of the non-induced samples was calculated as 100% uncut. Graphs show mean +SEM of three biological replicates.

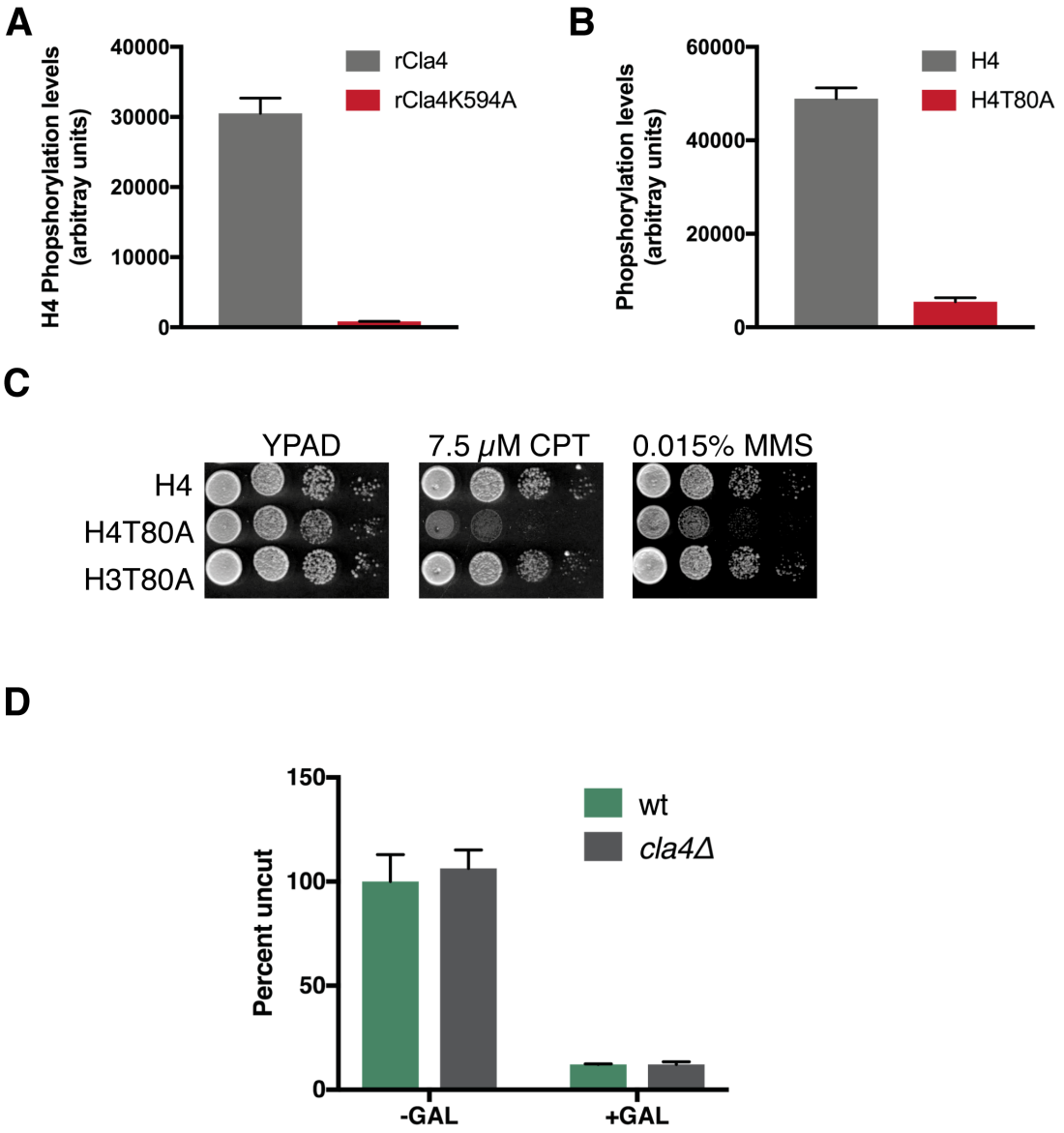

Figure S3. Cla4 phosphorylates H4T80 *in vivo* and *in vitro*. Related to Figure 2 and Figure 3. (A) and **(B)** Quantification of kinase experiments shown in Figure 2D and 2E respectively. Data are represented as mean +SEM of two independent experiments. **(C)** Spot test for DNA damage sensitivity of different yeast histone point mutants as indicated. 10-fold serial dilutions were used. **(D)** HO cutting efficiency was measured by qPCR using primers encompassing the HO cut site and normalized to the *PRP8* reference locus. The mean of the non-induced samples was calculated as 100% uncut. Graphs show mean +SEM of three biological replicates.

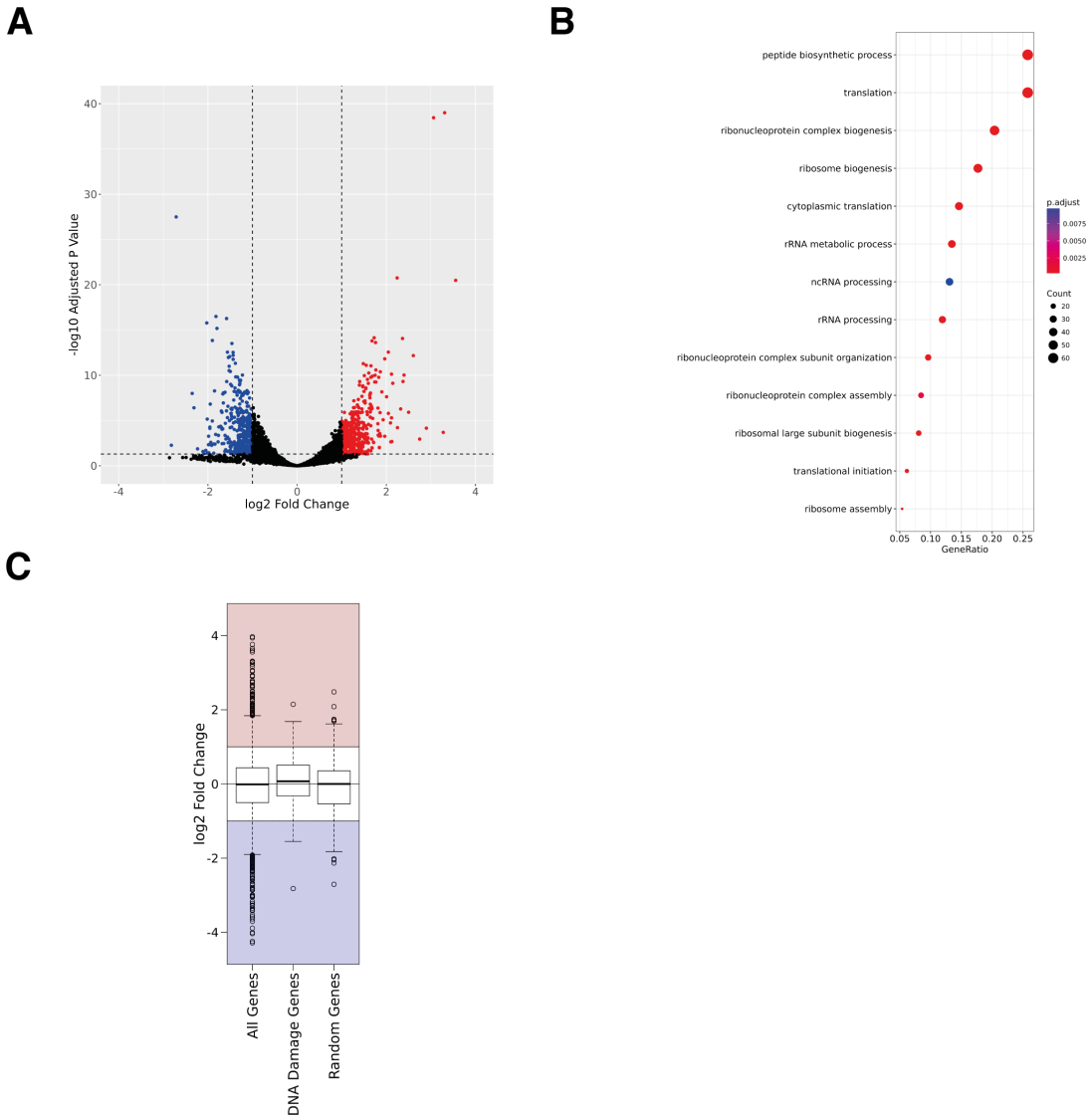

Figure S4. H4T80A mutation does not particularly impair the expression of genes involved in the DDR. Related to Figure 4. (A) Volcano plot showing the global differential expression between H4T80A mutant and wild type samples. The x-axis represents the log2 fold change and the y-axis represents the -log10 of the adjusted p-value from the DESeq2 differential expression analysis. Significantly up-regulated (fold change > 2, adjusted p value < 0.05) and down-regulated (fold change < 2, adjusted p value < 0.05) genes are highlighted in red and blue respectively. **(B)** Dot-plot of gene ontology terms enriched for genes down-regulated in H4T80A mutant cells compared to wild type. For each term, GeneRatio represents the proportion of genes in a given GO term that are significantly down-regulated. The size of the dots is representative of the total number of genes, whilst the colour is representative of the p value from the over-representation test. Genes up-regulated in the H4T80A mutant show no enrichment in the GO analysis. **(C)** Boxplot showing the distribution of log2 fold change values from the DESeq2 differential expression analysis for; all genes, 206 genes involved in the DNA damage response (based on GO term GO:0006974), and a random subset of 206 genes. Genes up- or down-regulated greater than 2-fold are highlighted in red or blue respectively.

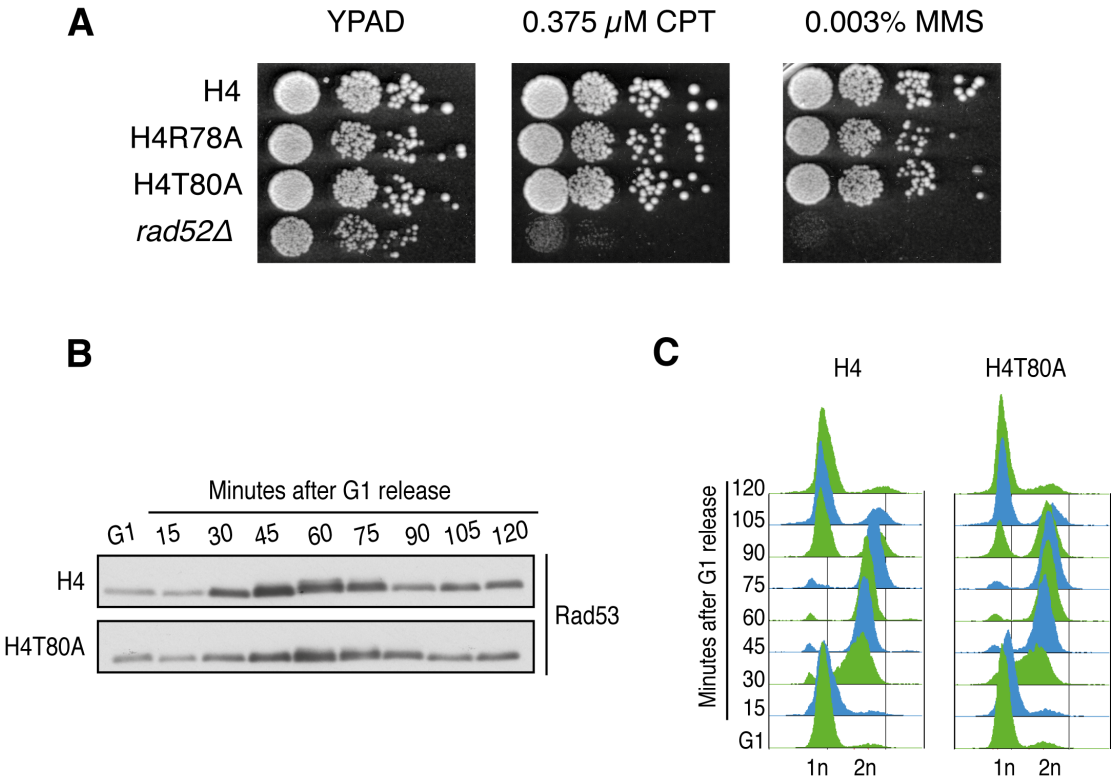

Figure S5. H4T80A mutation does not impair cell cycle progression. Related to Figure 4. **(A)** Spot test for DNA damage sensitivity of different yeast mutants as indicated. 10-fold serial dilutions were used. **(B)** Immunoblot analysis of Rad53 phosphorylation levels evaluated by mobility shift in SDS-PAGE gels. Wild type or H4T80A mutant cells were arrested in G1 using α-factor, and then released for 120 min. Samples were taken every 15 min. α-factor was added again after 45 min to arrest cells in G1. **(C)** Flow cytometry analysis of the same samples taken in B.

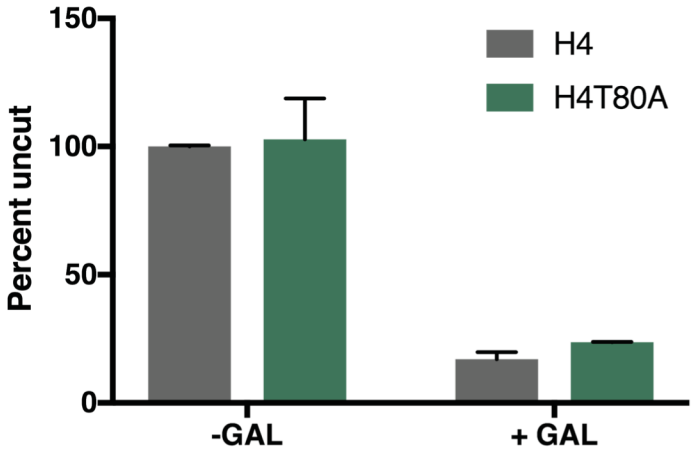

Figure S6. DSB is induced to the same extent in H4T80A mutant and wild type cells. Related to Figure 5. HO cutting efficiency was measured by qPCR using primers encompassing the HO cut site and normalized to the *PRP8* reference locus. The mean of the non-induced samples was calculated as 100% uncut. Graphs show mean +SEM of two biological replicates.

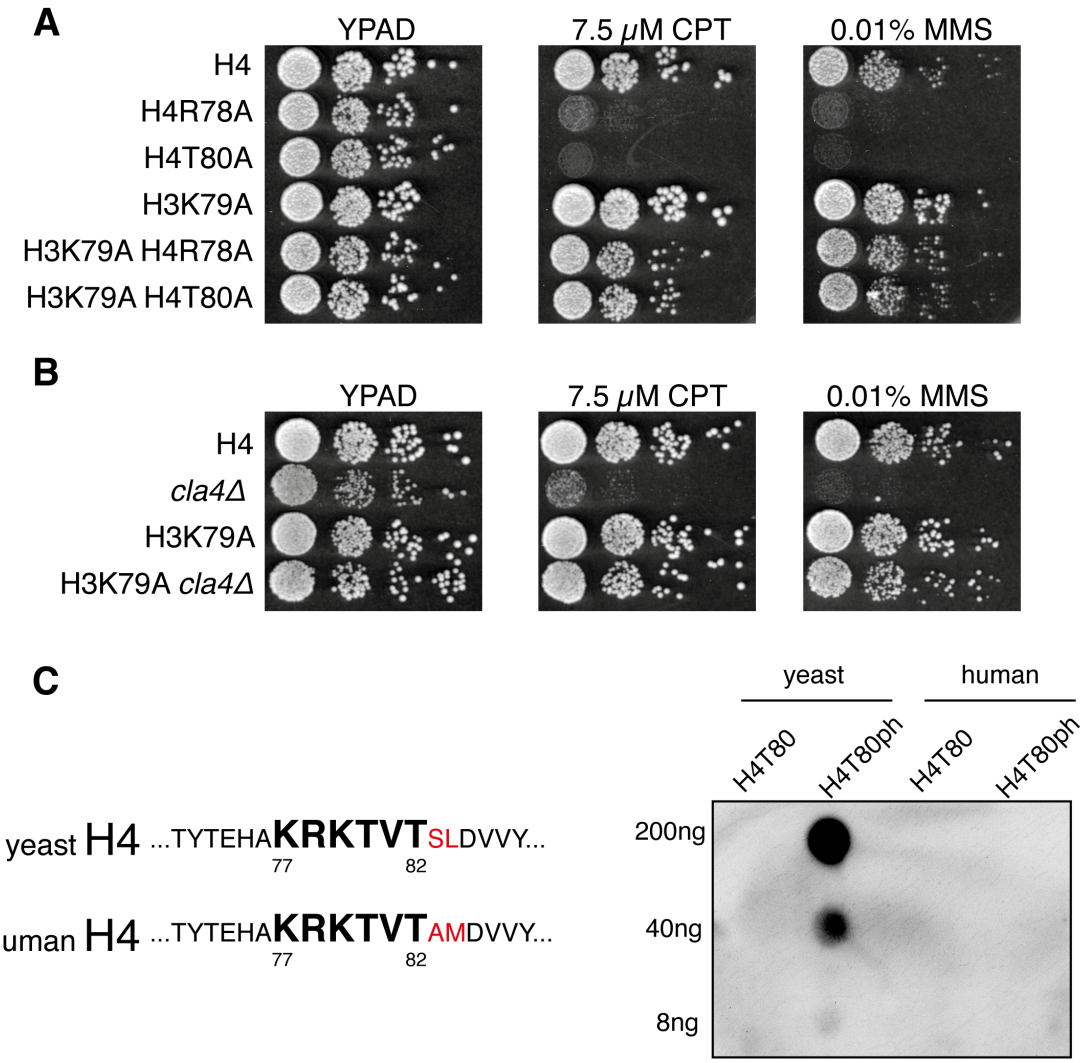

Figure S7. Mutation of H3K79A supresses H4T80A and *cla4Δ* mutant hypersensitivity to DNA damaging agents. Related to Figure 6. (A) and **(B)** Spot test for DNA damage sensitivity of different yeast mutants as indicated. 10-fold serial dilutions were used. **(C) Left** Comparison of yeast and human histone H4 L2 loop sequence. **Right** Dot-blot analysis using H4T80ph specific antibody. 5-fold serial dilutions of different peptides, as indicated, were spotted onto a PVDF membrane.

Strain	Genotype	Source
PTY1	*Mat a hht2,hhf2::natMX4*, *hht1,hhf1::kanMX4* pURA3-*HHT1-HHF1*	(Tessarz et al., 2014)
GMY041	PTY1 *cla4::hphMX4*	This study
GMY042	PTY1 *skm1::hphMX4*	This study
GMY043	PTY1 *ste20::hphMX4*	This study
GMY044	PTY1 *rad52::hphMX4*	This study
GMY045	PTY1 *RAD9-3HA::HIS3*	This study
GMY046	PTY1 *RTT107-3HA::HIS3*	This study
GMY047	PTY1 *dot1::HIS3*	This study
GMY048	PTY1 *cla4::hphMX4*, *dot1::HIS3*	This study
PTY2	*Mat a hht2,hhf2::KanMX4*, *hht1,hhf1::HIS3*, *hta1,htb1::NatMX6, hta2, htb2::hphMX6* pURA3-*HHT1-HHF1*	(Tessarz et al., 2014)
JKM179	*MATα ade1 leu2*-3,112, *lys5, trp1::hisG, ura3*-52, *hml::ADE1 hmr::ADE1 ade3::GAL::HO*	(Lee et al., 1998)
GMY049	JKM179 *CLA4-6HA::kanMX4*	This study
GMY050	JKM179 *cla4::hphMX4*	This study
GMY051	JKM179 *hht2,hhf2::natMX4*, *hht1,hhf1::kanMX4*	This study
GMY052	GMY051 *RTT107-6HA::hphMX4*	This study
GMY053	*Mat a hht2,hhf2::natMX4*, *hht1,hhf1::LEU2* pURA3-*HHT1-HHF1*	This study
GMY053	GMY053 *rad53-R605A::kanMX6*	
MKY5	*MATα ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1*	(Leung et al., 2016)
MKY1671	MKY5, *rtt107::kanMX6* *pRS315, RTT107-3XFLAG::natMX6*	(Leung et al., 2016)
MKY1672	MKY5, *rtt107::kanMX6* *pRS315, rtt107-K887M-3XFLAG::natMX6*	(Leung et al., 2016)
MKY1731	MKY5, *rtt107::KANMX6* *pRS315, rtt107-K426M-3XFLAG::NATMX6*	(Leung et al., 2016)

Table S1. Yeast Strains used in this study. Related to Key Resources Table. Strains are derivatives of W303. Histone point mutants were shuffled into yeast by counter-selection on 5-FOA.