# Supporting Information

## Kim et al. 10.1073/pnas.1203453109

### SI Materials and Methods

**MNase Analysis.** MNase analysis of bulk chromatin from yeast cells were performed as described (1). Briefly, cells were grown in 50 mL of YPD media to the cell density of $10^7$ cells/mL. Sphenoroplasting was done with 1 mg/mL zymolyase 100T (US Biological) at 30 °C for 5~10 min. To digest chromatin from $2 \times 10^6$ cells per mL, 1, 2, 4, or 8 U of MNase (Worthington) was used in 200 μL of digestion buffer (10 mM Hepes-Na at pH 7.5, 0.5 mM $MgCl_2$, and 0.05 mM $CaCl_2$) at 37 °C for 5 min. The reaction was then stopped by 25 μL of stop buffer containing 5 mg/mL of proteinase K, 1 mM EGTA, and 5% (wt/vol) SDS.

**Superhelical Density Analysis.** Plasmid DNA was extracted from 10 mL of culture in YPD, the density of which was $10^7$ cells per mL, as described in the literature (2). Twenty micrograms of extracted DNA was run on 0.8% of agarose gel in 1× TPE buffer containing 10 μg/mL chloroquine and was subject to Southern blot analysis by using a probe specific to *URA3* on the plasmid.

**Flow Cytometry Analysis.** Cells were prepared as described in *Current Protocols in Cytometry* (3). DNA content stained with propodium iodie was analyzed by FACSCalibur (BD Biosciences).


1. Zhang Z, Reese JC (2006) Isolation of yeast nuclei and micrococcal nuclease mapping of nucleosome positioning. *Methods Mol Biol* 313:245–255.
2. Morse RH (2009) Analysis of DNA topology in yeast chromatin. *Methods Mol Biol* 523: 93–108.
3. Lloyd D (2001) Flow cytometry of yeasts. *Curr Protoc Cytom*, 10.1002/0471142956.cy1110s09.


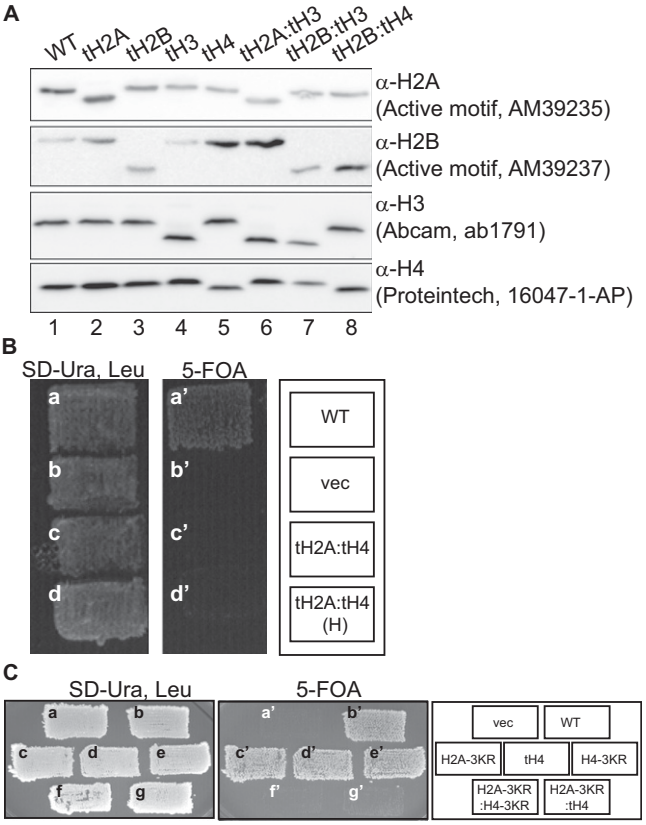


**Fig. S1.** (*A*) Immunoblots for core histones in wild-type and histone tail deletion strains. Whole-cell extracts from the histone tail deletes and wild-type cells were subjected to immunoblot analyses with indicated antibodies. (*B*) Viability of histone shuffle strains. Cells on the SD-ura, Leu plate carry mutant histone genes: wild-type H2A/H2B/H3/H4 (WT) (*a*), empty vector alone (vec) (*b*), tH2A/H2B/H3/tH4 (tH2A:tH4) (*c*), tH2A/H2B/H3/tH4 in pRS426 [tH2A:tH4 (H)] (*d*) as well as their wild-type counterparts in separate plasmids. Cells on the 5-FOA plate lacking wild-type histone genes in the *URA3* plasmid were marked with *a′*–*d′*. (*C*) Viability of histone shuffle strains containing tailless histones: empty vector (vec) (*a*), wild-type H2A/H2B/H3/H4 genes (WT) (*b*), H2A-3KR (K4,7,13R)/H2B/H3/H4 (H2A-3KR) (*c*), H2A/H2B/H3/tH4 (tH4) (*d*), H2A/H2B/H3/H4-3KR (K5,8,12R) (H4-3KR) (*e*), H2A-3KR/H2B/H3/H4-3KR (H2A-3KR:H4-3KR) (*f*), H2A-3KR/H2B/H3/tH4 (H2A-3KR:tH4) (*g*) as well as their wild-type counterparts in sepearate plasmids. Cells on the 5-FOA plate lacking wild-type histone genes in the *URA3* plasmid were marked with *a′*–*g′*.

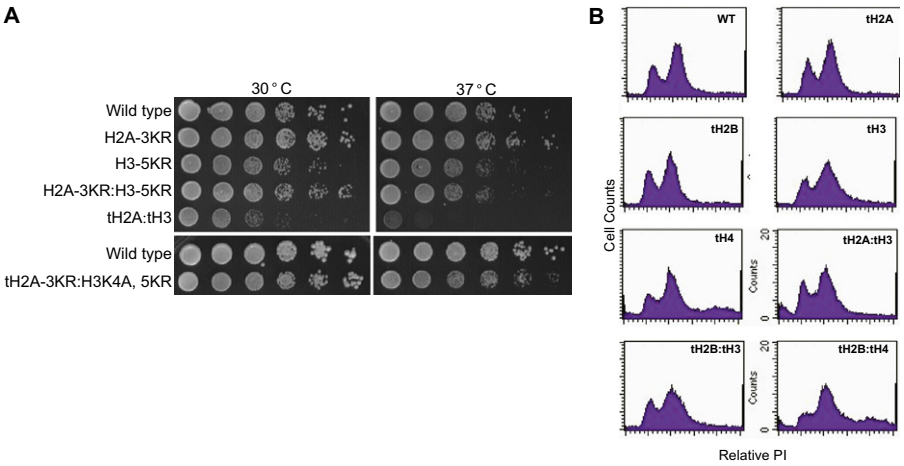


**Fig. S2.** (*A*) Growth of mutant strains at 30 °C and at 37 °C was tested by spotting 10-fold serially diluted cultures on YPD plates. (*B*) Cell cycle profiles of histone tail deletion mutants and wild-type cells were measured by cytometry. Note that the <1C DNA content in the tH2A:tH3 mutant did not consistently appear in independent experiments.

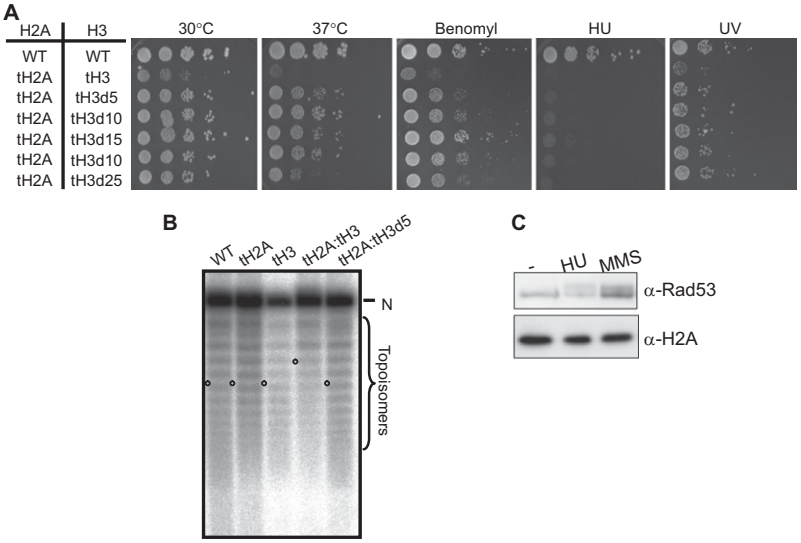


**Fig. S3.** (*A*) Spotting assays of mutant cells with tailles H2A (tH2A) and serially truncated H3 (tH3d5:Δ1-5, tH3d10:Δ1-10, tH3d15:Δ1-15, tH3d20:Δ1-20, tH3d25:Δ1-25) was done as described in Fig. 2. (*B*) Superhelical analysis of 2 μM plasmid (ectopically introduced pRS426) in histone tail deletes (tH2A, tH3, tH2A:tH3, tH2A:tH3d5) and wild-type cells. N, plasmids and the distributed topoisomers of the plasmids were marked with a bracket. The midpoint of topoisomer distribution in each lane is marked with an open circle. (*C*) tH2A mutant cells were treated with 100 mM HU or 0.2% MMS for 2 h. Whole-cell extracts from the mutant cells were then subjected to immnoblot analyses with the indicated antibodies against Rad53 and H2A.

**Table S1. Combinatory mutations on lysine residues within the H2A and H4 N-tails tested for the effect on cell viability**

| H2A residues | | | H4 residues | | | |
|---|---|---|---|---|---|---|
| K4 | K7 | K13 | K5 | K8 | K12 | Viability |
| R | R | R | R | R | R | Inviable |
| R | R | — | R | R | R | Viable |
| — | R | R | R | R | R | Viable |
| R | — | R | R | R | R | Viable |
| R | R | R | R | R | — | Viable |
| R | R | R | — | R | R | Viable |
| R | R | R | R | — | R | Viable |

Individual lysine residues substituted to arginines are marked with R in boxes.

**Table S2. Doubling time of histone tail delete-mutants in YPD media at 30 °C**

| Strain | Time, min |
|---|---|
| WT | 110 |
| tH2A | 119 |
| tH2B | 100 |
| tH3 | 150 |
| tH4 | 217 |
| tH2A:tH3 | 341 |
| tH2B:tH3 | 150 |
| tH2B:tH4 | 207 |

Overnight culture of each strain was transferred to liquid YPD media in a density of $1x10^6$ cells/mL. Cell numbers in individual cultures were counted with hemicytometer in every 15 min for 6 h.

**Table S3. Redundant roles for histone N-tails in pairs**

| | | Temperatures, °C | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| N-tail deletion | Viability | 16 | 30 | 37 | Benomyl | 6-AU | HU | Phleomycin | UV | Ref. |
| H2A/H2B | x | nd | nd | nd | nd | nd | nd | nd | nd | (1) |
| H3/H4 | x | nd | nd | nd | nd | nd | nd | nd | nd | (2) |
| H2A/H3 | | x | x | x | x | x | x | x | x | This study |
| H2A/H4 | x | nd | nd | nd | nd | nd | nd | nd | nd | This study |
| H2B/H3 | | | | | | x | x | | | This study |
| H2B/H4 | | | | | | | x | x | x | This study |

nd, not determined phenotypes; x, synergistic defective phenotypes detected. Blank cells indicate that no phenotypic defect was observed.

1. Schuster T, Han M, Grunstein M (1986) Yeast histone H2A and H2B amino termini have interchangeable functions. *Cell* 45:445–451.
2. Ling X, Harkness TA, Schultz MC, Fisher-Adams G, Grunstein M (1996) Yeast histone H3 and H4 amino termini are important for nucleosome assembly in vivo and in vitro: Redundant and position-independent functions in assembly but not in gene regulation. *Genes Dev* 10:686–699.

**Table S4. Yeast strains used in this study**

| Strain name | Genotypes | Used in |
|---|---|---|
| YJK341 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1-HHT2-HHF2*] p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] | Figs. 1 *B* and *D*, and S1 *B* and *C* |
| YJK346 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1-HHT2-HHF2*] p[*CEN LEU2*] | Figs. 1 *B* and *D*, and S1 *B* and *C* |
| YJK342 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 hta1Δ(1-20)-HTB1-HHT2-HHF2*] | Figs. 1*B*, S2, and S3 |
| YJK343 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 HTA1-htb1Δ(1-32)-HHT2-HHF2*] | Figs. 1*B* and S2 |
| YJK344 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 HTA1-HTB1-hht2Δ(1-30)-HHT2-HHF2*] | Figs. 1*B* and S2 |
| YJK363 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 HTA1-HTB1-HHT2-hhf2Δ(1-16)*] | Figs. 1*B*, S1C, and S2 |
| YJK367 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2Δ(1-30)-HHF2*] | Figs. 1*B* and S2 |
| YJK364 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 hta1Δ(1-20)-HTB1-HHT2-hhf2Δ(1-16)*] | Figs. 1 *B* and *D*, S1*B* and S2 |
| YJK345 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 HTA1- htb1Δ(1-32)- hht2Δ(1-30)-HHF2*] | Figs. 1*B* and S2 |
| YJK365 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 HTA1- htb1Δ(1-32)-HHT2-hhf2Δ(1-16)*] | Figs. 1*B* and S2 |
| YJK544 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 hta1K4,7,13R-HTB1-HHT2-HHF2*] | Fig. S1*C* |
| YJK545 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 HTA1-HTB1-HHT2-hhf2K5,8,12R*] | Fig. S1*C* |
| YJK546 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 hta1K4,7,13R-HTB1-HHT2-hhf2K5,8,12R*] | Fig. S1*C* |
| YJK551 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 hta1K4,7,13R-HTB1-HHT2-hhf2K5,8,12R*] | Fig. S1*C* |
| YJK540 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 hyb::hta1-HTB1-HHT2-hhf2K5,8,12HHF2*] | Fig. 1*D* |
| YJK541 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 HTA1-HTB1-HHT2-htb::HHF2*] | Fig. 1*D* |
| YJK542 | *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN URA3 HTA1-HTB1 HHT2-HHF2*] p[*CEN LEU2 hyb::HTA1-HTB1-HHT2-hyb::HHF2*] | Fig. 1*D* |
| CDAY251 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] | Ref. 1 figures 2–4 |
| YJK347 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-HHT2-HHF2*] | Figs. 2–4 |
| YJK348 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 HTA1-htb1Δ(1-32)-HHT2-HHF2*] | Figs. 2 and 3 |
| YJK349 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 HTA1-HTB1-hht2Δ(1-30)-HHT2-HHF2*] | Figs. 2 and 3 |
| YJK357 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 HTA1-HTB1-HHT2-hhf2 Δ(1-16)*] | Figs. 2 and 3 |
| YJK368 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1- hht2 Δ(1-30)-HHF2*] | Figs. 2–4 |
| YJK350 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 HTA1- htb1Δ(1-32)- hht2Δ(1-30)-HHF2*] | Figs. 2 and 3 |
| YJK369 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 HTA1- htb1Δ(1-32)-HHT2-hhf2Δ(1-16)*] | Figs. 2 and 3 |
| YJK389 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2Δ(1-5)-HHF2*] | Fig. 4 |
| YJK390 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2Δ (1-10)-HHF2*] | Fig. S3 |
| YJK391 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2Δ(1-15)-HHF2*] | Fig. S3 |
| YJK392 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2Δ(1-20)-HHF2*] | Fig. S3 |
| YJK393 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2Δ(1-25)-HHF2*] | Fig. S3 |
| YJK402 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 HTA1-HTB1-hht2Δ(1-5)-HHF2*] | Fig. 4 |

**Table S4. Cont.**

| Strain name | Genotypes | Used in |
|---|---|---|
| YJK411 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2S1A-HHF2*] | Fig. 4 |
| YJK412 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2K4A-HHF2*] | Fig. 4 |
| YJK413 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 h ht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2Q5A-HHF2*] | Fig. 4 |
| YJK556 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2R2A-HHF2*] | Fig. 4 |
| YJK557 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2T3A-HHF2*] | Fig. 4 |
| YJK463 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT set1Δ::URA3* p[*CEN LEU2 HTA1-HTB1-HHT2-HHF2*] | Fig. 4 |
| YJK558 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT* p[*CEN LEU2 HTA1-HTB1-hht2K4A-HHF2*] | Fig. 4 |
| YJK468 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT set1Δ::URA3* p[*CEN LEU2 HTA1-HTB1-hht2K4A-HHF2*] | Fig. 4 |
| YJK559 | *MAT***a** *his3-1 leu2-0 met15-0 ura3-0 hht1-hhf1::KAN hhf-2hht2::NAT hta1-htb1::HPH hta2-htb2::NAT set1Δ::URA3* p[*CEN LEU2 hta1Δ(1-20)-HTB1-hht2K4A-HHF2*] | Fig. 4 |

1. Ahn SH, et al. (2005) Sterile 20 kinase phosphorylates histone H2B at serine 10 during hydrogen peroxide-induced apoptosis in S. cerevisiae. *Cell* 120:25–36.