## Extended Experimental Procedures

**Yeast strains and plasmids.** The yeast strains and plasmids used in this study are listed in Table S1 and Table S2, respectively. The oligonucleotide sequences are listed in Table S3. The yeast strains were constructed by a PCR-based method or standard genetic crosses. Briefly, to create a plasmid with the wild-type *htb1*$^+$ gene and a selective marker, a 1.4 kb DNA fragment containing the full-length *htb1*$^+$ gene was amplified by high-fidelity *pfu* DNA polymerase (Cellgen Biolab) with GF1 and GF2 primers and then cloned into pFA6a-KanMX6 digested with BamHI and BglII. A 350 bp homologous fragment downstream of the *htb1*$^+$ gene was also amplified with GF3 and GF4 primers and cloned into SacI and SacII sites in pFA6a-KanMX6. Site-directed mutagenesis of the *htb1*$^+$ plasmid was conducted according to standard protocols (Stratagene). To induce homologous recombination with the genomic *htb1*$^+$ gene, 10 μg of *htb1* DNA fragments with or without mutations were PCR-amplified from this plasmid with GF1 and GF4 primers and transformed into the wild-type strain by the lithium acetate method. G418-resistant colonies were isolated on YES plates containing 100 μg/ml of G418 (Sigma, A1720). A PCR analysis was performed to confirm whether homologous recombination occur, and DNA sequencing was performed to confirm the open reading frame (ORF) of *htb1*. For the histone H2B expression plasmid, the full-length *S. pombe* H2B ORF sequence was PCR-amplified with GF53 and GF54 primers and cloned into pET28a (Novagen) digested with BamHI and XhoI.

**Yeast growth and assays of genomic instability.** Cells of the fission yeast *S. pombe* were normally cultured in YES (yeast extract and supplements) or Edinburgh minimal 2 media (EMM) with supplements at 30°C. The cells cultured under replication stress conditions were first grown to middle log-phase ($OD_{600}$=0.4) and then treated with replication stress for the indicated times. For the assays of growth sensitivity to DNA damaging agents, the cells were grown overnight to saturation, and the culture was then diluted to $OD_{600}$=0.4 and grown for an additional 3 h. These log-phase cells were then harvested and plated at a five-fold serial dilution on YES plates containing the indicated DNA damaging agent. Clonal images were taken after three or four days of culture. The *Ura4* loss assay in rDNA was based on a previous report (1). The loss rate was determined with the fluctuation analysis calculator FALCOR and the median method (2).

**Generation and purification of antibodies.** Peptide sequences and antibody information are listed in Table S4 and Table S5, respectively. A rabbit polyclonal antibody was raised against a synthetic peptide of *S. pombe* H2B acetylated K33 (GL Biochem.) conjugated with keyhole limpet hemocyanin (KLH). This specific antibody was sequentially purified from crude serum with modified and unmodified H2BK33 peptides crosslinked to CNBr-activated Sepharose resin (GE Healthcare). The serum was first applied to a column of H2BK33 acetylation peptide. After a thorough washing, the antibody binding to the H2BK33 acetylation peptide was eluted with 50 mM glycine-HCl (pH 2.5) and immediately neutralized with 1 M Tris-HCl (pH 9.0).

The eluates were then applied to a second column of unmodified H2BK33 peptide. The flow-through fraction was collected and dialyzed against phosphate buffered saline (PBS). The antibody against *S. pombe* histone H2B was generated by immunizing rabbits with recombinant H2B. 6His-T7-H2B-6His was overexpressed in *Escherichia coli* (*E. coli*) BL21 (DE3) and purified with Ni-NTA columns (QIAGEN). The fractions containing H2B were dialyzed against PBS containing 40% glycerol and stored at -20°C.

**Chemical propionylation and mass spectrometry analysis of histone proteins.** To examine the histone modifications, chromatin extracts were subjected to PAGE-SDS electrophoresis to separate the histone proteins. The bands containing H2A, H2B, H3, and H4 were cut out, de-stained and dehydrated. MS analyses of histone proteins requires the proteins to be propionylated to neutralize the charge and block lysine residues. Thus, each gel slice was added to 200 µl propionic anhydride (Sigma, 240311) in $NH_4HCO_3$ (Sigma, A6141) buffer (pH 8.0) and incubated at 51°C for 30 min. The pH was immediately adjusted to 8 by adding concentrated ammonium hydroxide (Sigma). The propionylated histones were then digested with trypsin that only cleaves at arginine residues. The peptides were subjected to LC-electrospray ionization-tandem mass spectrometry. The collected data were searched with Sequest, analyzed by Proteome Discoverer (Thermo) and further validated manually.

**Cell cycle and flow cytometry analyses.** As described, we synchronized the *S. pombe* cells at the G1 phase with nitrogen starvation or at the G2/M phase with *cdc25-22* at 36°C for 3.5 h. After the cells were released, the cell cycle progression was monitored by measuring the septation index (a septum is formed when cytokinesis occurs) and analyzing the DNA content with fluorescence activated cell sorting (FACS). For the FACS analysis, the cells were first fixed with cold 70% ethanol and then incubated with 4 μg/ml propidium iodide (PI) in 50 mM sodium citrate containing 10 μg/ml RNase A for several hours. Immediately prior to the FACS analysis, the cells were briefly sonicated and then analyzed with a FACSCalibur (BD) system for PI detection (FL2-A).

**Preparation of whole-cell extracts and immunoblotting.** Whole-cell extracts were prepared by the following procedure. A 10-ml cell culture ($OD_{600}$=1.0 (10 ODs)) was harvested, washed once with 1/4 volume of ice-cold 100% trichloroacetic acid (TCA) and resuspended in 500 μl of 10% TCA and 500 μl of acid-washed glass beads (Sigma, G-8772). The cells were disrupted by vortexing for 10 min. After centrifugation at 10,000 x *g* for 10 min, the cell pellets were completely resuspended in the 2x SDS loading buffer with 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma P7626), 10 mM sodium butyrate (NaB, Sigma V900464) and 10 nM trichostatin A (TSA, Beyotime S1893). The pH was adjusted by adding 1/5 volume of Tris buffer (pH=11), and then the sample was boiled at 95 °C for 5 min, run on a Tris-glycine SDS-polyacrylamide gel and transferred to a 0.2-μm PVDF membrane

(Millipore, immobilon-$P^{SQ}$). The blots were probed with primary and secondary antibodies and detected via SuperSignal West Pico chemiluminescence (Thermo, 34078). For the Western blotting analysis of H2BK33ac, the H2BK33ac antibody was pre-incubated with 0.5 µg/ml unmodified H2BK33 peptide for 0.5 h at room temperature prior to the standard procedure. For the peptide competition assay, an approximately 20-fold molar excess of peptides (0.5 µg/ml) relative to antibodies was used. For the dot blots, 2 µl of the indicated amounts of histone H2B peptides were spotted onto 0.2-µm PVDF membranes and detected using the same method applied for immunoblotting.

**DNA combing analysis and microscopy imaging.** DNA combing was modified from previous methods (3, 4). Briefly, the strain J2172 was labeled with 10 µM 5-ethynyl-2'-deoxyuridine (EdU) (Molecular Probes, E10187), 200 µg/ml 5-iodo-2'-deoxyuridine (IdU) (Sigma I7125) or 200 µg/ml 5-chloro-2'-deoxyuridine (CIdU) (Sigma C6891) depending on the experimental requirements. The labelled cells were then embedded in agarose plugs, and genomic DNA was carefully extracted to prevent shearing. DNA adhered to the silanized glass coverslips (Genomic Vision, COV-001) in MES (Sigma, M2933) buffer (pH 6.2) and was stretched into fibers. Following heat crosslinking, the incorporated EdU was detected with the Click-iT EdU imaging kit (Molecular Probes, C10337). IdU and CIdU were detected by immunofluorescence using the indicated primary and secondary antibodies after DNA denaturation with 1M NaOH 22min. For staining with

calcofluor white (Sigma, 18909) or 4',6-diamidino-2-phenylindole (DAPI) (Sigma), the cells were fixed with cold 70% ethanol, stained with calcofluor white or DAPI and washed with PBS just prior to imaging. All of the images were observed using DeltaVision Elite microscopy (Applied Precision). Six sections of the *z*-axis were deconvolved and projected with maximum intensity in SoftWoRx (GE Healthcare), and the images were analyzed by Volocity (PerkinElmer). The number of DNA fibers calculated per sample were shown in figures and two biological replicates were scored and statistically analyzed.

**DNA combing analysis to measure the rate of stalled or collapsed forks**

J2172 strains with *cdc10-M17* and a *K33R/Q* or *clr6-1* mutation were first synchronized at G1 at 36.5 °C for 3 h, and then 12.5 mM HU was added to the culture for 1 h before release. Upon cell release at 25°C, IdU (red) was added for 3 h. Then, IdU and HU were washed out and CIdU (green) was added for an additional 0.5 h. Chromatin DNA was subjected to DNA combing assays. The percentages of stalled or collapsed forks were calculated as counts of DNA tracts that had incorporated only IdU (red) divided by the total counts of DNA tracts.

**Chromatin fractionation.** Chromatin was isolated as previously described (5). The growth of log-phase cells was stopped by the addition of 0.2% sodium azide ($NaN_3$) on ice, and the cells were then resuspended in 1.2 M sorbitol and subjected to lyticase (Cellgen Biolabs) digestion at 30°C for approximately 30 min until more than 90% of

the cells became “ghosts” in 1% Triton X-100. The spheroplasts were resuspended in buffer A (50 mM Tris-HCl, pH 8.0, 100 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, 10% glycerol, 0.05% NP-40, 1 mM PMSF, 1 mM DTT, protease inhibitor cocktail for yeast extracts (Sigma, P8215), 10 mM NaB, and 10 nM TSA and lysed with 1% Triton X-100 (TX-100) (Amresco). After removing the cell debris and intact cells by centrifugation at 700g for 10 min, the supernatants as WCE were loaded onto a 30% sucrose cushion and centrifuged at 35,000 x *g* for 10 min. The chromatin pellet was suspended and washed twice by incubating with buffer A containing 0.25% TX-100 for 5 min. Chromatin were further digested by 1 unit/μl Benzonase nuclease (Novagen, 70746) for 10 min to remove the nucleic acids and release the chromatin-bound proteins.

**Isolation of normal or HU-stalled replication forks.** Cells with *cdc25-22* were synchronized to G2/M phase by incubating at 36.5˚C for 3h and harvested as S phase cells with the highest septa percentage after releasing into 25˚C for 80-90min (in the case of *clr6-1* mutation, release into S phase after 120min due to slow growth). For HU treated S phase cells, treated with 12.5mM HU 3.5h upon blocked *cdc25-22* releasing. Then cells were crosslinked with 1% formaldehyde (Sigma, F8775) at 25°C for 20 min and stopped by adding 1/20 volume of 2.5 M glycine. Chromatin was isolated. Then chromatin DNA was sonicated by Bioruptor (Diagenode) to average 700bp fragments (in the case of isolating oligonucleosomes and histones around forks for Western blotting assays, the average DNA was 1000-2000bp) and saved as input

sample. Replisome was immunoprecipitated (IP) with anti-HA beads (sigma, A2095) to Rpa1-HA or anti-Flag beads (Sigma, A2220) to DNA polα-Spb70-3FLAG or DNA polδ-Cdc1-3FLAG, and eluted with 1% SDS as IP sample. Both input and IP protein samples were incubated at 65˚C for overnight for de-crosslinking and then boiled for immunoblotting.

**Chromatin immunoprecipitation and quantitative PCR (ChIP-qPCR).** The *cdc25-22* cells arrested at G2/M were released into the S phase with or without HU. Cell samples were taken at the very beginning of S phase (just appearance of Septa) (-HU) or at 3.5h (+12.5 mM HU) after G2/M release. After crosslinking with 1% formaldehyde (Sigma, F8775) at 25°C for 20 min and stopped by adding 1/20 volume of 2.5 M glycine, Chromatin was prepared and sonicated to ~500 bp. A 1/10 fraction was saved as the input sample, and the DNA products were electrophoresed on 2% agarose gels to confirm that the size of the sheared DNA fragments was ~500bp in average. After pre-clearing the sheared chromatin with protein-A beads (GE Healthcare, 17-5280-01), the chromatin sample was incubated with specific antibody-conjugated beads (Sigma A2095 for HA beads) for 2 h or overnight at 4°C. Following extensive washing with a high level of salt and detergent, the immunoprecipitated chromatin was eluted with 200 µl TE containing 1% SDS and the input sample was added to 1% SDS. Then the samples were incubated at 65°C overnight for reverse crosslinking. After treatment with 100 µg/ml proteinase K (NEB, P8102) and 100 µg/ml RNase A (Thermo, EN0531), the DNA was purified using a

MinElute PCR purification kit (Qiagen, 28004). Approximately 10 ng DNA templates were mixed with 10 µl of 2X SYBR Green PCR master mix (TaKaRa, RR420A) and 4 µl specific primer sets of DNA replication origins (each primer is 1 µM). The sample was run on the Roche LightCycler 96 Real-Time PCR System according to the manufacturer's instructions. Each sample from two independent experiments was quantified and averaged. The enrichment of immunoprecipitated DNA relative to the paired input DNA was determined by the comparative Ct ($2^{-Ct}$) method.

**Micrococcal nuclease (MNase) digestion of chromatin**. MNase digestion of chromatin was performed according to a previous method (6). Briefly, equal amounts of cells were digested with lyticase to nearly the same extent at 32°C for 30-60 min. The obtained spheroplasts were partially permeabilized with NP-40 buffer and digested with a series of increasing units of MNase (NEB, M0247) at 37°C for 10 min. After the reactions were stopped by adding 50 mM EDTA and 0.5% SDS, the DNA was purified by treating the samples with proteinase K and RNase A followed by phenol-chloroform extraction. The DNA concentration was measured using a NanoVue spectrophotometer (GE Healthcare), and equal amounts of total DNA were resolved on 1.5% agarose gels and visualized by ethidium bromide staining. The optical density of DNA was quantified with ImageJ software by calculating the integral area of density plot after background subtraction. The percentage of the indicated DNA amount of the lane from top to bottom of the gel was shown in figures.

**Yeast two-hybrid (Y2H) assay.** The Y2H experiment was based on Matchmaker Gal4 two-hybrid system 3 (Clontech). The indicated proteins were cloned in-frame into the pGADT7 vector with GAL4 activating domain (pAD) and the pGBKT7 vector with GAL4 DNA-binding domain (pBD). The pAD plasmid with leucine marker and pBD plasmid with tryptophan marker were co-transformed into *S. cerevisiae* strain AH109, and 8 independent transformants of each interacting pair with leucine and tryptophan markers were randomly selected and spotted onto the indicated test plates lacking leucine and tryptophan, and histidine or adenine. The experiments were independently replicated three times.

**MNase digestion assay of local chromatin around replication forks.** For each sample, 5L of cells at log phase with *cdc25-22* and *polδ-cdc1-3Flag* genetic background were first arrested at G2/M phase at a restrictive temperature of 36.5°C and then released into S phase in the absence or presence of HU at 26°C. After crosslinking with formaldehyde, the chromatin was then prepared, sonicated to an average size of ~2500 bp (a range of ~1000 to ~5000 bp), and subjected to immuneprecipitation with anti-Flag beads. The isolated chromatin, which was located around normal or stalling replication forks, was digested with MNase at 37°C for 10 min, in presence of RNase A and T1. The MNase digestion was then stopped with addition of 0.5% SDS and 50 mM of EDTA and kept at 65°C for 20 min, in presence of proteinase K. After de-crosslinking at 65°C for overnight and phenol-chloroform

extraction. The DNA in the water phase was lyophilized and subsequently dissolved in TE for gel electrophoresis.

**Phos-tag gel analysis of Cds1 phosphorylation.** Whole-cell extracts were prepared as previously described. The samples were run on a 8% SDS-polyacrylamide gel containing 10 μM Phos-tag (NARD institute, AAL-107). After electrophoresis, the gel was washed with buffer containing 20 mM EDTA to remove $Mn^{2+}$. Proteins were transferred to a 0.2-μm PVDF membrane (Millipore, immobilon-$P^{SQ}$) for subsequent Western blotting assay with a monoclonal antibody against HA-Cds1 (Bioeasytech, BE2008).

**Co-immunoprecipitation (co-IP) assay of Rad9 and Clr6 interaction.** Chromatin was prepared from 1L of cell cultures with His-tagged Rad9 and HA-tagged or untagged Clr6. The chromatin-bound proteins were extracted with 600 mM NaCl and subjected to immunoprecipitation with antibody against HA-Cr6, after the chromatin extracts were diluted to a salt concentration of 150 mM NaCl and DNase I treatment. Western blotting assay was followed for examining Clr6 and Rad9 in the brought-down samples.

## Supplemental Table S1. Yeast strains

| Strain | Genotype | Source |
|---|---|---|
| YGF0 | *h-, ura4-D18* | Lilin Du (LD330) |
| YGF1 | *h-, ura4-D18, htb1::kan* | This study (LD330 background) |
| YGF13 | *h-, ura4-D18, htb1-K33R::kan* | This study (LD330 background) |
| YGF30 | *h-, ura4-D18, htb1-K33A::kan* | This study (LD330 background) |
| YGF24 | *h-, ura4-D18, htb1-K33Q::kan* | This study (LD330 background) |
| YGF6 | *h-, ura4-D18, htb1-K5R::kan* | This study (LD330 background) |
| YGF188 | *h-, ura4-D18, htb1-K5Q::kan* | This study (LD330 background) |
| YGF189 | *h-, ura4-D18, htb1-K10R::kan* | This study (LD330 background) |
| YGF190 | *h-, ura4-D18, htb1-K10Q::kan* | This study (LD330 background) |
| YGF191 | *h-, ura4-D18, htb1-K15R::kan* | This study (LD330 background) |
| YGF192 | *h-, ura4-D18, htb1-K15Q::kan* | This study (LD330 background) |
| YGF31 | *h-, ura4-D18, htb1-K5RK10RK15R::kan* | This study (LD330 background) |
| YGF34 | *h-, ura4-D18, htb1-K5QK10QK15Q::kan* | This study (LD330 background) |
| YGF193 | *h-, ura4-D18, htb1-K5RK10RK15RK33R::kan* | This study (LD330 background) |
| YGF194 | *h-, ura4-D18, htb1-K5QK10QK15QK33Q::kan* | This study (LD330 background) |
| YGF70 | *h-, ura4-D18, htb1-FLAG::kan* | This study (LD330 background) |
| YGF104 | *h-, ura4-D18, ade6-704, cdc10-M17, leu1-32::[leu1$^+$adh1:hENT1], his7-366::[his7$^+$adh1:hsv-tk]* | Antony Carr (J2172) |
| YGF54 | *h-, ura4-D18, ade6-704, cdc10-M17, leu1-32::[leu1$^+$adh1:hENT1], his7-366::[his7$^+$adh1:hsv-tk], htb1::kan* | This study (J2172 background) |
| YGF55 | *h-, ura4-D18, ade6-704, cdc10-M17, leu1-32::[leu1$^+$adh1:hENT1], his7-366::[his7$^+$adh1:hsv-tk], htb1-K33R::kan* | This study (J2172 background) |

| | | |
|---|---|---|
| YGF56 | *h-, ura4-D18, ade6-704, cdc10-M17, leu1-32::[leu1$^{+}$adh1:hENT1], his7-366::[his7$^{+}$adh1:hsv-tk], htb1-K33Q::kan* | This study (J2172 background) |
| YGF122 | *h-, ura4-D18, ade6-704, cdc10-M17, leu1-32::[leu1$^{+}$adh1:hENT1], his7-366::[his7$^{+}$adh1:hsv-tk], clr3Δ::kan* | This study (J2172 background) |
| YGF123 | *h?, ura4-D18, cdc10-M17, leu1-32::[leu1$^{+}$adh1:hENT1], his7-366::[his7$^{+}$adh1:hsv-tk], clr6-1* | This study (J2172 background) |
| YGF105 | *h-, leu1-32* | NBRP (FY7950) |
| YGF106 | *h+, leu1-32, clr6-1* | NBRP (FY11920) |
| YGF107 | *h-, leu1-32, clr3Δ::kan* | NBRP (FY20012) |
| YGF108 | *h?, clr6-1, clr3Δ::kan* | Genevieve Thon |
| YGF109 | *h-, leu1-32, hos2Δ::kan* | NBRP (FY20013) |
| YGF110 | *h-, sir2Δ::kan* | NBRP (FY20704) |
| YGF111 | *h-, hst2Δ::kan* | NBRP (FY20703) |
| YGF112 | *h-, leu1-32, hst4Δ::kan* | NBRP (FY20710) |
| YGF113 | *h+, cdc25-22* | This study |
| YGF76 | *h+, cdc25-22, ssb1-3HA-kan* | This study |
| YGF121 | *h+, cdc25-22, cdc18-3HA-kan* | This study |
| YGF98 | *h?, cdc25-22, htb1-K33R::kan* | This study |
| YGF99 | *h?, cdc25-22, htb1-K33Q::kan* | This study |
| YGF109 | *h-, ade6-M210, ura4-D18, leu1-32, clr6-3HA-kan, clr3-13myc-kan* | Karl Ekwall (Hu67) |
| YGF145 | *h-, ade6-M210, ura4-D18, leu1-32, clr6-3HA-kan, clr3-13myc-kan, cdc25-22-nat* | This study (Hu67 background) |
| YGF95 | *h-, ade6-M210, ura4-D18, leu1-32, clr6-3HA-kan, clr3-13myc-kan, rad3Δ::hph* | This study (Hu67 background) |
| YGF152 | *h-, ade6-M210, ura4-D18, leu1-32, clr6-3HA-kan, clr3-13myc-kan, cds1Δ::hph* | This study (Hu67 background) |
| YGF146 | *h-, ade6-M210, ura4-D18, leu1-32, clr6-3HA-kan, clr3-13myc-kan, rad9Δ::hph* | This study (Hu67 background) |
| YGF227 | *h-, ade6-M210, ura4-D18, leu1-32, cdc25-22-nat, clr6-3HA-kan, clr3-13myc-kan, rad9Δ::hph* | This study (Hu67 background) |
| YGF148 | *h-, ade6-M210, ura4-D18, leu1-32, clr6-3HA-kan, clr3-13myc-kan, rad9-8his-hph* | This study (Hu67 background) |
| YGF83 | *h+, leu1-32, clr6-1, htb1-K33R::kan* | This study (FY11920 background) |
| YGF84 | *h+, leu1-32, clr6-1, htb1-K33A::kan* | This study (FY11920 background) |
| YGF85 | *h+, leu1-32, clr6-1, htb1-K33Q::kan* | This study (FY11920 background) |

| | | |
|---|---|---|
| YGF137 | *h?, clr6-1, cds1Δ::hph* | This study |
| YGF94 | *h-, cds1Δ::hph* | This study |
| YGF138 | *h90, ura4-D18, cds1Δ::ura4* | NBRP (FY7429) |
| YGF115 | *h-, leu1-32, ura4-D18, ade6-M210, his3-D1, rad3Δ::LEU2* | Lilin Du (LD297) |
| YGF116 | *h+, ade6-704, leu1-32, ura4-D18, cds1Δ::kan* | Antony Carr (Ets13) |
| YGF51 | *h+, ade6-704, leu1-32, ura4-D18, cds1Δ::kan, htb1-K33R::kan* | This study (Ets13 background) |
| YGF52 | *h+, ade6-704, leu1-32, ura4-D18, cds1Δ::kan, htb1-K33A::kan* | This study (Ets13 background) |
| YGF53 | *h+, ade6-704, leu1-32, ura4-D18, cds1Δ::kan, htb1-K33Q::kan* | This study (Ets13 background) |
| YGF117 | *h+, ade6-M210, leu1-32, ura4-D18, tel1Δ::LEU2* | NBRP (FY14071) |
| YGF118 | *h-, ade6-704, leu1-32, ura4-D18, chk1Δ::kan* | Antony Carr (Ets12) |
| YGF119 | *h90, ade6-216, leu1-32, lys1-131, ura4-D18, clr6-GFP-3HA::kan* | NBRP (FY15195) |
| YGF120 | *h90, ade6-216, leu1-32, lys1-131, ura4-D18, clr3-GFP-3HA::kan* | NBRP (FY15180) |
| YGF17 | *h-, leu1-, ura4-, cds1-2HA-6His::ura4* | NBRP (FY11064) |
| YGF131 | *h-, leu1-, ura4-, cds1-2HA-6His::ura4, htb1-K33R::kan* | This study (FY11064 background) |
| YGF132 | *h-, leu1-, ura4-, cds1-2HA-6His::ura4, htb1-K33Q::kan* | This study (FY11064 background) |
| YGF140 | *h?, cds1-2HA-6His::ura4, clr6-1* | This study (FY11064 background) |
| YGF139 | *h+, ade6-216, ura4-DS/E, leu1-32, Ylp2.4pUCura4.7* | Lilin Du (DY683) |
| YGF128 | *h+, ade6-216, ura4-DS/E, leu1-32, Ylp2.4pUCura4.7, htb1-K33R::kan* | This study (DY683 background) |
| YGF129 | *h+, ade6-216, ura4-DS/E, leu1-32, Ylp2.4pUCura4.7, htb1-K33Q::kan* | This study (DY683 background) |
| YGF199 | *h-, ura4-D18, leu1-32, his3-D1, cdc25-22* | Lilin Du (DY769) |
| YGF173 | *h+, ade-M216, leu1-32, ura4-D18, cdc25-22-nat, cdc45-6his-3HA-6his-ura4, spb70-3Flag-hph* | This study |
| YGF176 | *h+, ade-M216, leu1-32, ura4-D18, cdc25-22-nat, cdc45-6his-3HA-6his-ura4, spb70-3Flag-hph, htb1-K33R::kan* | This study (YGF173 background) |
| YGF177 | *h+, ade-M216, leu1-32, ura4-D18, cdc25-22-nat, cdc45-6his-3HA-6his-ura4, spb70-3Flag-hph, htb1-K33Q::kan* | This study (YGF173 background) |
| YGF184 | *h?, cdc25-22-nat, cdc45-6his-3HA-6his-ura4, spb70-3Flag-hph, clr6-1* | This study (YGF173 background) |
| YGF175 | *h+, ade-M216, leu1-32, ura4-D18, cdc25-22-nat, cdc45-6his-3HA-6his-ura4, cdc1-3Flag-hph* | This study |

| | | |
|---|---|---|
| YGF180 | *h+, ade-M216, leu1-32, ura4-D18, cdc25-22-nat, cdc45-6his-3HA-6his-ura4, cdc1-3Flag-hph, htb1-K33R::kan* | This study (YGF175 background) |
| YGF181 | *h+, ade-M216, leu1-32, ura4-D18, cdc25-22-nat, cdc45-6his-3HA-6his-ura4, cdc1-3Flag-hph, htb1-K33Q::kan* | This study (YGF175 background) |
| YGF187 | *h?, cdc25-22-nat, cdc45-6his-3HA-6his-ura4, cdc1-3Flag-hph, clr6-1* | This study (YGF175 background) |
| YGF198 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ* | AH109 |
| YGF200 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ,* [pGBKT7, pGADT7] | This study (AH109 background) |
| YGF201 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ,* [pGF58, pGF62] | This study (AH109 background) |
| YGF202 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ,* [pGF55, pGF61] | This study (AH109 background) |
| YGF203 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ,* [pGF55, pGBKT7] | This study (AH109 background) |
| YGF204 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ,* [pGADT7, pGF61] | This study (AH109 background) |
| YGF205 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ,* [pGF55, pGF62] | This study (AH109 background) |
| YGF206 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ,* [pGADT7, pGF62] | This study (AH109 background) |
| YGF207 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3::* | This study (AH109 background) |

| | | |
|---|---|---|
| | *MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGF55, pGF63] | |
| YGF208 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGADT7, pGF63] | This study (AH109 background) |
| YGF211 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGF55, pGF65] | This study (AH109 background) |
| YGF212 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGADT7, pGF65] | This study (AH109 background) |
| YGF213 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGF54, pGF56] | This study (AH109 background) |
| YGF214 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGF54, pGADT7] | This study (AH109 background) |
| YGF215 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGBKT7, pGF56] | This study (AH109 background) |
| YGF216 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGF54, pGF57] | This study (AH109 background) |
| YGF217 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGBKT7, pGF57] | This study (AH109 background) |
| YGF218 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGF54, pGF58] | This study (AH109 background) |
| YGF219 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3:: MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*, [pGBKT7, pGF58] | This study (AH109 background) |
| YGF222 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3,* | This study (AH109 background) |

| | | |
|---|---|---|
| | *GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3::*<br>*MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ,* [pGF54, pGF60] | |
| YGF223 | *MATa, trp1-901, leu2-3, ade2-112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3::*<br>*MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ,* [pGBKT7, pGF60] | This study (AH109 background) |

## Supplemental Table S2. Plasmids

| Plasmid | Description | Source |
|---|---|---|
| pFA6a | containing *kanMX6* | Susan Forsburg |
| pBluescript | Phagemid vector for cloning | Stratagene |
| pET-28a | Expression vector in *E. coli* | Novagen |
| pGF0 | pBluescript containing *ura4* at HindIII site | This study |
| pGF2 | pFA6a containing *htb1* | This study |
| pGF6 | pFA6a containing *htb1-K33R* | This study |
| pGF29 | pFA6a containing *htb1-K33A* | This study |
| pGF11 | pFA6a containing *htb1-K33Q* | This study |
| pGF17 | pFA6a containing *htb1-K5R* | This study |
| pGF49 | pFA6a containing *htb1-K5Q* | This study |
| pGF50 | pFA6a containing *htb1-K10R* | This study |
| pGF51 | pFA6a containing *htb1-K10Q* | This study |
| pGF52 | pFA6a containing *htb1-K15R* | This study |
| pGF53 | pFA6a containing *htb1-K15Q* | This study |
| pGF27 | pFA6a containing *htb1-K5RK10RK15R* | This study |
| pGF33 | pFA6a containing *htb1-K5QK10QK15Q* | This study |
| pGF45 | pFA6a containing *htb1-K5RK10RK15RK33R* | This study |
| pGF46 | pFA6a containing *htb1-K5QK10QK15QK33Q* | This study |
| pGF21 | pET-28a containing *htb1* | This study |
| pGF36 | pBluescript containing *cdc25-22-hph* | This study |
| pGF37 | pBluescript containing *cdc25-22-nat* | This study |
| pGF40 | pBluescript containing *hph* | This study |
| pGF41 | pBluescript containing *hph* and *rad3Δ* homologue sequences | This study |
| pGF42 | pBluescript containing *hph* and *cds1Δ* homologue sequences | This study |
| pGBKT7 | containing GAL4 DNA binding domain for yeast two-hybrid analysis | Clontech |
| pGADT7 | containing GAL4 activating domain for yeast two-hybrid analysis | Clontech |
| pGF54 | pGBKT7 containing *clr6* | This study |
| pGF55 | pGADT7 containing *clr6* | This study |
| pGF56 | pGADT7 containing *rad9* | This study |
| pGF57 | pGADT7 containing *rad1* | This study |
| pGF58 | pGADT7 containing *hus1* | This study |

| | | |
|---|---|---|
| pGF60 | pGADT7 containing *htb1* | This study |
| pGF61 | pGBKT7 containing *rad9* | This study |
| pGF62 | pGBKT7 containing *rad1* | This study |
| pGF63 | pGBKT7 containing *hus1* | This study |
| pGF65 | pGBKT7 containing *htb1* | This study |

## Supplemental Table S3. Oligonucleotides

| Oligonucleotide | Sequence (5' to 3') | Use |
|---|---|---|
| GF1 | CGGCCCGGATCCCTCGCA ATTTCAGCCTTTACGG | 5' Primer for *htb1* homologous seq. #1 into pFA6a-KanMX6 |
| GF2 | GCGGCGAGATCTTAATAT TCCATTCACCATCAATCTA AATCACC | 3' Primer for *htb1* homologous seq. #1 into pFA6a-KanMX6 |
| GF3 | CGGCCCGAGCTCTTATTCA CATTCAAAATATCCCAAA GAATCC | 5' Primer for *htb1* homologous seq. #2 into pFA6a-KanMX6 |
| GF4 | GGCGGCCCGCGGCTCGCT CAGTTTGCTTTACAC | 3' Primer for *htb1* homologous seq. #2 into pFA6a-KanMX6 |
| GF39 | GTATATAACCATCGCGTC GCC | 5' Primer for PCR *htb1* ORF for sequencing |
| GF40 | GTCGAAATGGAAGGCTGT GG | 3' Primer for PCR *htb1* ORF for sequencing |
| GF56 | CACCTACCATACTCGACTT GCTAC | 5' Primer for confirming *htb1* genomic insertion |
| GF57 | CCACTCCTGGGCATCTTGG | 3' Primer for confirming *htb1* genomic insertion |
| GF14 | GATAAGAAGCGTGGTAAA AACAGAAGGGAAACTTAT TCATCCTATAT | 5' Primer for mutagenesis of *htb1*-K33R |
| GF15 | ATATAGGATGAATAAGTT TCCCTTCTGTTTTTACCAC GCTTCTTATC | 3' Primer for mutagenesis of *htb1*-K33R |
| GF75 | CTGCTGATAAGAAGCGTG GTAAAAACAGAGCGGAAA CTTATTCATCCTA | 5' Primer for mutagenesis of *htb1*-K33A |
| GF76 | TAGGATGAATAAGTTTCC GCTCTGTTTTTACCACGCT TCTTATCAGCAG | 3' Primer for mutagenesis of *htb1*-K33A |
| GF28 | CTGCTGATAAGAAGCGTG GTAAAAACAGACAGGAAA CTTATTCAT | 5' Primer for mutagenesis of *htb1*-K33Q |

| | | |
|---|---|---|
| GF29 | ATGAATAAGTTTCCTGTCT GTTTTTACCACGCTTCTTA TCAGCAG | 3’ Primer for mutagenesis of *htb1*-K33Q |
| GF41 | AATGTCTGCTGCTGAAAG GAAACCCGCTTCCAAGG | 5’ Primer for mutagenesis of *htb1*-K5R |
| GF42 | CCTTGGAAGCGGGTTTCCT TTCAGCAGCAGACATT | 3’ Primer for mutagenesis of *htb1*-K5R |
| GF201 | TAAAAAATGTCTGCTGCT GAACAGAAACCCGCTTCC AAG | 5’ Primer for mutagenesis of *htb1*-K5Q |
| GF202 | CTTGGAAGCGGGTTTCTGT TCAGCAGCAGACATTTTTT A | 3’ Primer for mutagenesis of *htb1*-K5Q |
| GF203 | GAAAAGAAACCCGCTTCC AGGGCTCCCGCC | 5’ Primer for mutagenesis of *htb1*-K10R |
| GF204 | GGCGGGAGCCCTGGAAGC GGGTTTCTTTTC | 3’ Primer for mutagenesis of *htb1*-K10R |
| GF205 | AAACCCGCTTCCCAGGCT CCCGCCG | 5’ Primer for mutagenesis of *htb1*-K10Q |
| GF206 | CGGCGGGAGCCTGGGAAG CGGGTTT | 3’ Primer for mutagenesis of *htb1*-K10Q |
| GF47 | GCTCCCGCCGGTAGGGCT CCTAGG | 5’ Primer for mutagenesis of *htb1*-K15R |
| GF48 | CCTAGGAGCCCTACCGGC GGGAGC | 3’ Primer for mutagenesis of *htb1*-K15R |
| GF81 | AGGCTCCCGCCGGTCAGG CTCCTAG | 5’ Primer for mutagenesis of *htb1*-K15Q |
| GF82 | CTAGGAGCCTGACCGGCG GGAGCCT | 3’ Primer for mutagenesis of *htb1*-K15Q |
| GF69 | AAGGAAACCCGCTTCCAG GGCTCCCGC | 5’ Primer for mutagenesis of *htb1*-K5RK10R |
| GF70 | GCGGGAGCCCTGGAAGCG GGTTTCCTT | 3’ Primer for mutagenesis of *htb1*-K5RK10R |
| GF77 | TAAAAAATGTCTGCTGCT GAACAGAAACCCGCTTCC CAGGCTCCCGCC | 5’ Primer for mutagenesis of *htb1*-K5QK10Q |
| GF78 | GGCGGGAGCCTGGGAAGC GGGTTTCTGTTCAGCAGCA GACATTTTTTA | 3’ Primer for mutagenesis of *htb1*-K5QK10Q |
| GF53 | CGGCCCGGATCCGCTGCT GAAAAGAAACCCGC | 5’ Primer for expressing H2B protein in pET28a |
| GF54 | GCGGCGCTCGAGCTGAGC AGAAGAAGAATACTTGG | 3’ Primer for expressing H2B protein in pET28a |

| | | |
|---|---|---|
| GF85 | ATGGTAGATGGAGAAACGGG | 5’ Primer for ars2004 in ChIP-qPCR analysis |
| GF86 | CACGGCATCTTTCTTCACGA | 3’ Primer for ars2004 in ChIP-qPCR analysis |
| GF87 | TTCCTCAAATCACCCCACGT | 5’ Primer for ars2004-14kb in ChIP-qPCR analysis |
| GF88 | ATGTATAGCTGGAACGCCTG | 3’ Primer for ars2004-14kb in ChIP-qPCR analysis |
| GF91 | TTGGCGCTAAACAATCTCTG | 5’ Primer for ars3002 in ChIP-qPCR analysis |
| GF92 | TCCTTGTCGAACTCAATTGC | 3’ Primer for ars3002 in ChIP-qPCR analysis |
| GF93 | GAATCTTCAGACCTTGCAGC | 5’ Primer for ars3002-10kb in ChIP-qPCR analysis |
| GF94 | ACGGACTCCAACAACCATTTG | 3’ Primer for ars3002-10kb in ChIP-qPCR analysis |
| GF97 | ACTGACGCTATAGTCAATGA | 5’ Primer for PCR *rad3* deletion cassette |
| GF98 | TAAGTTTAATAATGGGTAGC | 3’ Primer for PCR *rad3* deletion cassette |
| GF99 | CCAAGTAAAAACTGCTTAGTAA | 5’ Primer for confirming *rad3* deletion |
| GF100 | GAAGGAAAATTACAAGAAATTGA | 3’ Primer for confirming *rad3* deletion |
| GF101 | GTTTGAAATACAAAGGAATG | 5’ Primer for PCR *cds1* deletion cassette |
| GF102 | AGAGTGGTTAGTGTAAACTT | 3’ Primer for PCR *cds1* deletion cassette |
| GF103 | TTTTGAATAATGGTAACAATTAAG | 5’ Primer for confirming *cds1* deletion |
| GF104 | ACATACAAGCGATTCTGTAAC | 3’ Primer for confirming *cds1* deletion |
| centromere-up-F | TTATTCCATAATAACACGACCAATC | 5’ Primer for centromere adjacent region in ChIP-PCR analysis |
| centromere-up-R | CGACGTTGAAAAGGAAATTAATTG | 3’ Primer for centromere adjacent region in ChIP-PCR analysis |
| mat3M-down-2.4kb-F | GTTGTGTTACTGAATTACCTTAG | 5’ Primer for mat3M adjacent region in ChIP-PCR analysis |
| mat3M-down-2.4kb-R | AACGGTAATGGTGTATCTTTG | 3’ Primer for mat3M adjacent region in ChIP-PCR analysis |
| subtel-F | AACAGCGTTGCTTATCGAA | 5’ Primer for subtelomere adjacent region in ChIP-PCR analysis |
| subtel-R | CTGTTGATTGCATGTCATC | 3’ Primer for subtelomere adjacent region in |

| | TAG | ChIP-PCR analysis |
|---|---|---|
| heterochromatin island9-F | ATGGTTGAAAATCAAGGGAATG | 5’ Primer for heterochromatin island9 region in ChIP-PCR analysis |
| heterochromatin island9-R | GTTGCGAATCCACGTATAAATC | 3’ Primer for heterochromatin island9 region in ChIP-PCR analysis |
| heterochromatin island6-F | ATGAAGTCCTCATCCGTG | 5’ Primer for heterochromatin island6 region in ChIP-PCR analysis |
| heterochromatin island6-R | GCGTGCCAATGATCTTTC | 3’ Primer for heterochromatin island6 region in ChIP-PCR analysis |
| heterochromatin island8-F | CAAGATAACGAGGTGGAATTATATTC | 5’ Primer for heterochromatin island8 region in ChIP-PCR analysis |
| heterochromatin island8-R | GATCTACTTGAGAAGACTCACTG | 3’ Primer for heterochromatin island8 region in ChIP-PCR analysis |
| heterochromatin island1-F | ACTATCGCTTGCAGAGAAAAG | 5’ Primer for heterochromatin island1 region in ChIP-PCR analysis |
| heterochromatin island1-R | GAACGTTTAGCATCGGAGG | 3’ Primer for heterochromatin island1 region in ChIP-PCR analysis |
| rad9-up-F | CAGCTAATGATACAAATACTTCAAAC | 5’ Primer for *rad9* adjacent region in ChIP-PCR analysis |
| rad9-up-R | TATTACTTGTAATTGCCCGAC | 3’ Primer for *rad9* adjacent region in ChIP-PCR analysis |
| rad9-F | CACTGTTTCAAATGTTAATCTTCG | 5’ Primer for *rad9* region in ChIP-PCR analysis |
| rad9-R | TTCTTGCTATTGGTAGTGACG | 3’ Primer for *rad9* region in ChIP-PCR analysis |
| hus1-up-F | GCTATAACACTACTCCCTCC | 5’ Primer for *hus1* adjacent region in ChIP-PCR analysis |
| hus1-up-R | AAACTACGAACGCGTAAAAG | 3’ Primer for *hus1* adjacent region in ChIP-PCR analysis |
| hus1-F | CAAGGATTAGCAACTTGTACAC | 5’ Primer for *hus1* region in ChIP-PCR analysis |
| hus1-R | CCGTTTACGTTAGTTAGGGC | 3’ Primer for *hus1* region in ChIP-PCR analysis |
| gcn5-up-F | CGTTTTAGCAGAGTCACTCC | 5’ Primer for *gcn5* adjacent region in ChIP-PCR analysis |
| gcn5-up-R | ATTTGCGCTCCAAAATACAATC | 3’ Primer for *gcn5* adjacent region in ChIP-PCR analysis |
| gcn5-F | CAAGTGTTGTAGACAGTACATC | 5’ Primer for *gcn5* region in ChIP-PCR analysis |
| gcn5-R | CGTTAGAAACAACACGAAATTG | 3’ Primer for *gcn5* region in ChIP-PCR analysis |
| clr6-up-F | GATGACCTTCCAAATTCTTTATG | 5’ Primer for *clr6* adjacent region in ChIP-PCR analysis |

| | | |
|---|---|---|
| clr6-up-R | GAATGTCTATTAAAATGTGCTGTATG | 3’ Primer for *clr6* adjacent region in ChIP-PCR analysis |
| clr6-F | CTATGATGGTACGTGTAAACTAG | 5’ Primer for *clr6* region in ChIP-PCR analysis |
| clr6-R | CAATATACTCATCGGTATGACAAC | 3’ Primer for *clr6* region in ChIP-PCR analysis |
| clr3-up-F | GACAAAGGGTGAATGTTTCC | 5’ Primer for *clr3* adjacent region in ChIP-PCR analysis |
| clr3-up-R | CGTTTAGTAAGGCTTTAAATAGAAG | 3’ Primer for *clr3* adjacent region in ChIP-PCR analysis |
| clr3-F | ATCCGATGATGCTGTAAACAC | 5’ Primer for *clr3* region in ChIP-PCR analysis |
| clr3-R | TAAAGTAGCTTCTCTAGCCGG | 3’ Primer for *clr3* region in ChIP-PCR analysis |
| act1-up-F | TAATAAAATAGGGACACGCGAG | 5’ Primer for *act1* adjacent region in ChIP-PCR analysis |
| act1-up-R | GAAATTGCTATATTTGAGCCAC | 3’ Primer for *act1* adjacent region in ChIP-PCR analysis |
| act1-F | GCGTTGGTTATTGATAATGGC | 5’ Primer for *act1* region in ChIP-PCR analysis |
| act1-R | TTGGATTTGGGGTTCAAAGG | 3’ Primer for *act1* region in ChIP-PCR analysis |
| ura4-up-F | GTTCAGCATCTATCTTCTTAATCG | 5’ Primer for *ura4* adjacent region in ChIP-PCR analysis |
| ura4-up-R | TGATTATCTTTTTCACCATGCC | 3’ Primer for *ura4* adjacent region in ChIP-PCR analysis |
| ura4-F | GATGAAAAATCCCATTGCCAAG | 5’ Primer for *ura4* region in ChIP-PCR analysis |
| ura4-R | CACTGTATGGCAATTTGTGATATG | 3’ Primer for *ura4* region in ChIP-PCR analysis |
| cds1-up-F | GATAACTTACCCGTGGCAATT | 5’ Primer for *cds1* adjacent region in ChIP-PCR analysis |
| cds1-up-R | GCGAAGCACGAGTAATATAAAC | 3’ Primer for *cds1* adjacent region in ChIP-PCR analysis |
| cds1-F | GAGGCACCACTACATGTTAG | 5’ Primer for *cds1* region in ChIP-PCR analysis |
| cds1-R | GACATTCTCAGATTCATCGG | 3’ Primer for *cds1* region in ChIP-PCR analysis |
| htb1-up-F | CTTACCACCAGATTTACCTCC | 5’ Primer for *htb1* adjacent region in ChIP-PCR analysis |
| htb1-up-R | GCTCCTATGTCTCCTTTTCC | 3’ Primer for *htb1* adjacent region in ChIP-PCR analysis |
| htb1-F | ATGTCTGCTGCTGAAAAG | 5’ Primer for *htb1* region in ChIP-PCR |

| | | |
|---|---|---|
| | AAAC | analysis |
| htb1-R | CTGAGCAGAAGAAGAATA CTTGG | 3’ Primer for *htb1* region in ChIP-PCR analysis |
| GF187 | AGCTACAAATCCCACTGG CTATATG | 5’ Primer for PCR 1.8kb *ura4* gene |
| GF188 | GTGATATTGACGAAACTTT TTGACATC | 3’ Primer for PCR 1.8kb *ura4* gene |

## Supplemental Table S4. Peptides

| Peptide | Sequence (N-terminus to C-terminus) | Description |
|---|---|---|
| GFP1 | RGKNRK(ac)ETYSC | H2BK33 acetylation |
| GFP2 | RGKNRKETYSC | Unmodified H2BK33 |
| GFP3 | KNRK(me2)ETYS | H2BK33 di-methylation |
| GFP4 | AAEK(ac)KPAS | H2BK5 acetylation |
| GFP5 | KKPASK(ac)AP | H2BK10 acetylation |
| GFP6 | SKAPAGK(ac)A | H2BK15 acetylation |
| GFP7 | AAEKKPASKAPAGKC | Unmodified H2BK5, K10, K15 |

## Supplemental Table S5. Antibodies

| Epitope | Source | Use | Note |
|---|---|---|---|
| spH2BK33ac | In-lab | IB: 1:500; ChIP: 1:100 | Primary antibody; rabbit pAb |
| spH2B | In-lab | IB:1:5000; ChIP: 1:200 | Primary antibody; rabbit pAb |
| spH3 | Abcam (ab1791) | IB: 1:5000 | Primary antibody; rabbit pAb |
| spH3 | Bioeasytech (BE3015) | IB: 1:5000 | Primary antibody; mouse mAb |
| spH3K9me3 | Abcam (ab8898) | IB: 1:500; ChIP: 1:200 | Primary antibody; rabbit pAb |
| spAct1 | ABclonal(AC026) | IB: 1:10000 | Primary antibody; rabbit mAb |
| spMcm3 | In-lab | IB: 1:1000 | Primary antibody; rabbit pAb |
| spMcm7 | In-lab | IB: 1:5000 | Primary antibody; rabbit pAb |
| spRpa1 | In-lab | IB: 1:1000 | Primary antibody; rabbit pAb |

| | | | |
|---|---|---|---|
| spRpa2 | In-lab | IB: 1:1000 | Primary antibody; rabbit pAb |
| spCdc18 | In-lab | IB: 1:1000 | Primary antibody; rabbit pAb |
| spPCNA | In-lab | IB: 1:1000 | Primary antibody; rabbit pAb |
| spCdc13 | Novus Biologicals (NB200-576) | IB: 1:500 | Primary antibody; mouse mAb |
| HA (HA-7) | Sigma (H3663) | IB: 1:1000 | Primary antibody; mouse mAb |
| HA | Bioeasytech (BE2008) | IB: 1:1000 | Primary antibody; rabbit pAb |
| FLAG (M2) | Sigma (F1804) | IB: 1:1000 | Primary antibody; mouse mAb |
| Myc (9E10) | Abcam (ab32) | IB: 1:1000 | Primary antibody; mouse mAb |
| His(6E8) | Bioeasytech (BE2019) | IB: 1:1000 | Primary antibody; mouse mAb |
| IdU | BD (347580) | IF: 1:200 | Primary antibody; mouse mAb |
| CIdU | Lifespan Bioscience (C188215) | IF: 1:50 | Primary antibody; rat mAb |
| Alexa Fluor 546 | Molecular probes (A11030) | IF: 1:200 | Secondary antibody; goat anti-mouse |
| Alexa Fluor 488 | Molecular probes (A11006) | IF: 1:200 | Secondary antibody; goat anti-rat |
| Alexa Fluor 488 azide | Molecular probes (C10337) | IF: N/A | EdU conjugates |
| IgG | Bioeasytech (BE0101) | IB: 1:10000 | Secondary antibody; goat anti-rabbit |
| IgG | Bioeasytech (BE0102) | IB: 1:10000 | Secondary antibody; goat anti-mouse |

**Supplemental figure legends**

**Supplemental Figure S1.** The validation of H2BK33ac and its antibody. (*A,B*) *S. pombe* H2BK33 is acetylated *in vivo*. Histone proteins were isolated and subjected to a mass spectrometry analysis (MS), which showed H2B acetylated at the K33 site. (*C,G*) Validation of antibodies recognizing *S. pombe* H2B or acetylated K33 of H2B. (*C*) Whole cell extracts from untagged and FLAG-tagged *htb1*$^{+}$ strains were immunoblotted using H2B or FLAG antibodies, respectively. (*D*) Dot blot of the H2BK33ac antibody against the indicated peptides *in vitro*. (*E,F*) Western blotting analysis of whole-cell extracts (WCE, *E*) or chromatin (*F*) from wild type (WT), H2BK33 mutants and recombinant *S. pombe* H2B (rH2B). (*G*) Western blotting analysis of WCE from the WT and H2BK33A cells using the indicated peptides as competitors. (*H*) Effect of the HDAC inhibitor sodium butyrate (NaB, 30mM) on H2BK33 acetylation. (*I*) The entire film of western blots in middle panel of Figure 1d were shown to support the use of highly specific H2BK33ac and H2B antibody applying to IP or ChIP experiments. (*J*) The validation of the H2BK33ac antibody for the ChIP assay and enrichment of ars2004 and ars3002 DNA in wt but not H2BK33R cells. The ChIP-enriched DNA was quantified by PCR reactions using primers to amplify the replication origins (ars2004 and ars3002). (*K*) Replication forks stalled randomly on chromatin in *htb1*-K33Q cells under HU.

**Supplemental Figure S2.** Phenotypes of H2BK33 mutants under normal and stress conditions. (*A*) Selective marker *kan* inserted downstream of the *htb1* gene does not affect cell growth or cellular response to HU and the DNA damaging agents CPT and MMS. (*B*) H2BK33 mutants were not sensitive to IR, UV, MPA, and environmental stress including heat, $H_2O_2$, and high salt. (*C*) Single mutation of either H2BK5, K10, or K15 to R or Q was not sensitive to replication stress but combined H2BK5QK10QK15Q mutation was. (*D*) The sensitivity of H2BK33Q to replication stress was almost the same as the combined H2BK5QK10QK15Q mutation, and no synthetic phenotype was observed. (*E*) FACS analysis of DNA replication speed (EdU incorporation) in the wt and H2BK33R cells. (*F*) H2BK33Q mutation but not H2BK33R in the DNA combing strain J2172 was also sensitive to replication stress. (*G*) Percentage of only CIdU fibers (green) in the wt and H2BK33 mutants under HU block and release experiments, which indicates the extent of late origin firing after release from HU treatment. The difference in CIdU fiber proportion was calculated between WT and H2BK33 mutants by Student's two-tailed *t*-test.

**Supplemental Figure S3.** (*A*) DNA combing strain J2172 harboring the *clr6-1* but not the *clr3Δ* mutation was also sensitive to HU. (*B*) ChIP analysis of Clr3-myc after HU treatment in S phase. (*C*) Clr6 is enriched at stalling replication forks. Stalling replication forks induced via HU presence was isolated by ChIP with antibody against RPA1. The levels of Clr6 and PCNA were measured by Western blotting. (*D*) An immunoprecipitation assay of the interaction between Clr6 and Rad9. A chromatin

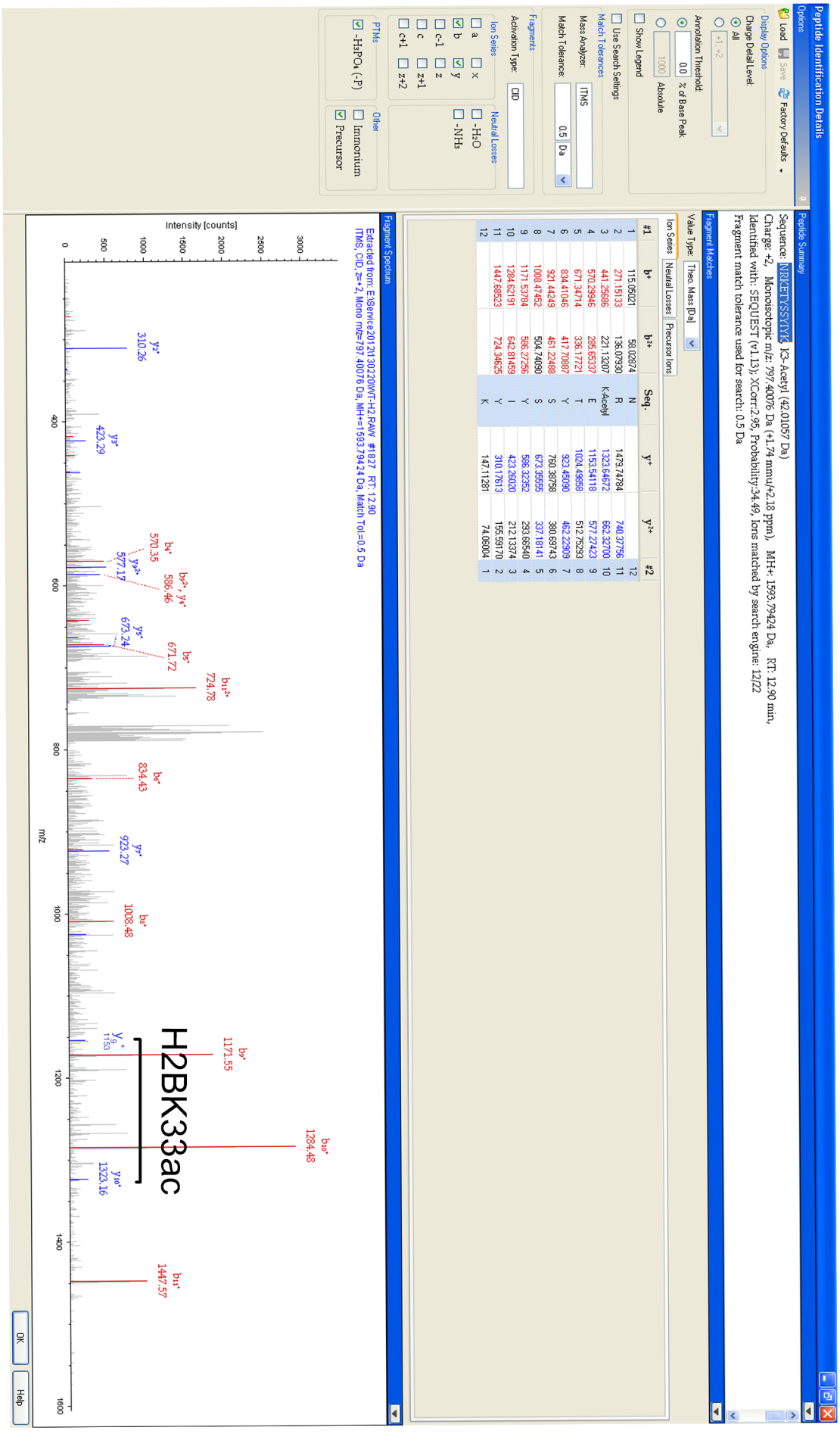
fraction was prepared from the cells treated with 12.5 mM HU for 3hrs and subjected to immunoprecipitation assay in the presence or absence of DNase I. (*E*) Clr6 association with Rad9-Hus1-Rad1 complex and also with H2B revealed by Y2H analysis. DO indicates dropout. T1-8 is 8 independent transformants.

**Supplemental Figure S4**. (*A*) Chromatin becomes more compacted in the HU-treated cells. MNase digestion of chromatin in *cdc25-22* cells that were synchronized at G2/M and then released into the S phase with or without the presence of 12.5 mM HU. WT: 90 min after releasing without HU or WT+HU2h; or 3.5h. HU was added to cultures immediately after G2/M releasing and the time counted. N1-4 denote mono, di, tri, tetra-nucleosome. The right panel is the quantification of the DNA amount percentage in the indicated lanes. (*B*) The growth assay of the *ura4* inserted rDNA, *ura4* deletion and *ura4* wt strains on URA- and FOA plates. (*C*) The loss of *ura4* gene was confirmed by PCR of independent colonies that did not grow even after 7 days of incubation on selective plates without uracil. The 1.8kb band was absent in *ura4* gene loss colonies (-) but present in silenced colonies (±, the full ura4 gene is at rDNA genes region) and normally grown WT colonies (+) that have a copy of full-length *ura4* gene at its native genomic locus. *ura4*-dS/E 1.4kb (a truncated ura4 gene at its native genomic locus) is not present in the WT strain (+). (*D*) *cdc45*-HA，*spb70*-Flag and *cdc1*-Flag do not alter the sensitivity of wt, htb1-K33R, htb1-K33Q, and clr6-1 cells to HU.

1. Irmisch A, Ampatzidou E, Mizuno K, O'Connell MJ, & Murray JM (2009) Smc5/6 maintains stalled replication forks in a recombination-competent conformation. *The EMBO journal* 28(2):144-155.
2. Hall BM, Ma CX, Liang P, & Singh KK (2009) Fluctuation analysis CalculatOR: a web tool for the determination of mutation rate using Luria-Delbruck fluctuation analysis. *Bioinformatics* 25(12):1564-1565.
3. Sabatinos SA, Green MD, & Forsburg SL (2012) Continued DNA synthesis in replication checkpoint mutants leads to fork collapse. *Molecular and cellular biology* 32(24):4986-4997.
4. Bianco JN, *et al.* (2012) Analysis of DNA replication profiles in budding yeast and mammalian cells using DNA combing. *Methods* 57(2):149-157.
5. Hu J, *et al.* (2012) The intra-S phase checkpoint targets Dna2 to prevent stalled replication forks from reversing. *Cell* 149(6):1221-1232.
6. Pidoux A, Mellone B, & Allshire R (2004) Analysis of chromatin in fission yeast. *Methods* 33(3):252-259.

# Figure S1

**A**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Options
Load
Save
Factory Defaults
Display Options
Charge Detail Level:
All
+1 +2
Annotation Threshold:
0.0
% of Base Peak
1000
Absolute
Show Legend
Use Search Settings
Match Tolerances
Mass Analyzer:
ITMS
Match Tolerance:
0.5
Da
Fragments
Activation Type:
CID
Ion Series
a
b
c-1
c
c+1
x
y
z
z+1
z+2
Neutral Losses
-H2O
-NH3
PTMs
-H3PO4 (-P)
Other
Immonium
Precursor
Peptide Summary
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Charge: +2, Monoisotopic m/z: 797.40076 Da (+1.74 mmu/+2.18 ppm), MH+: 1593.79424 Da, RT: 12.90 min,
Identified with: SEQUEST (v1.13); XCorr:2.95, Probability:34.49, Ions matched by search engine: 12/22
Fragment match tolerance used for search: 0.5 Da
Fragment Matches
Value Type:
Theo. Mass [Da]
Ion Series
Neutral Losses
Precursor Ions
#1 b+ b2+ Seq. y+ y2+ #2
1 115.05021 58.02874 N 1479.74784 740.37756 11
2 271.15133 136.07930 R 1323.64672 662.32700 10
3 441.25686 221.13207 K-Acetyl 1153.54118 577.27423 9
4 570.29946 285.65337 E 1024.49858 512.75293 8
5 671.34714 336.17721 T 923.45090 462.22909 7
6 834.41046 417.70887 Y 760.38758 380.69743 6
7 921.44249 461.22488 S 673.35555 337.18141 5
8 1008.47452 504.74090 S 586.32352 293.66540 4
9 1171.53784 586.27256 Y 423.26020 212.13374 3
10 1284.62191 642.81459 I 310.17613 155.59170 2
11 1447.68523 724.34625 Y 147.11281 74.06004 1
12 K
Fragment Spectrum
Extracted from: EiService20121302220WT-H2.RAW #1827 RT: 12.90
ITMS, CID, z=+2, Mono m/z=797.40076 Da, MH+=1593.79424 Da, Match Tol=0.5 Da
Intensity [counts]
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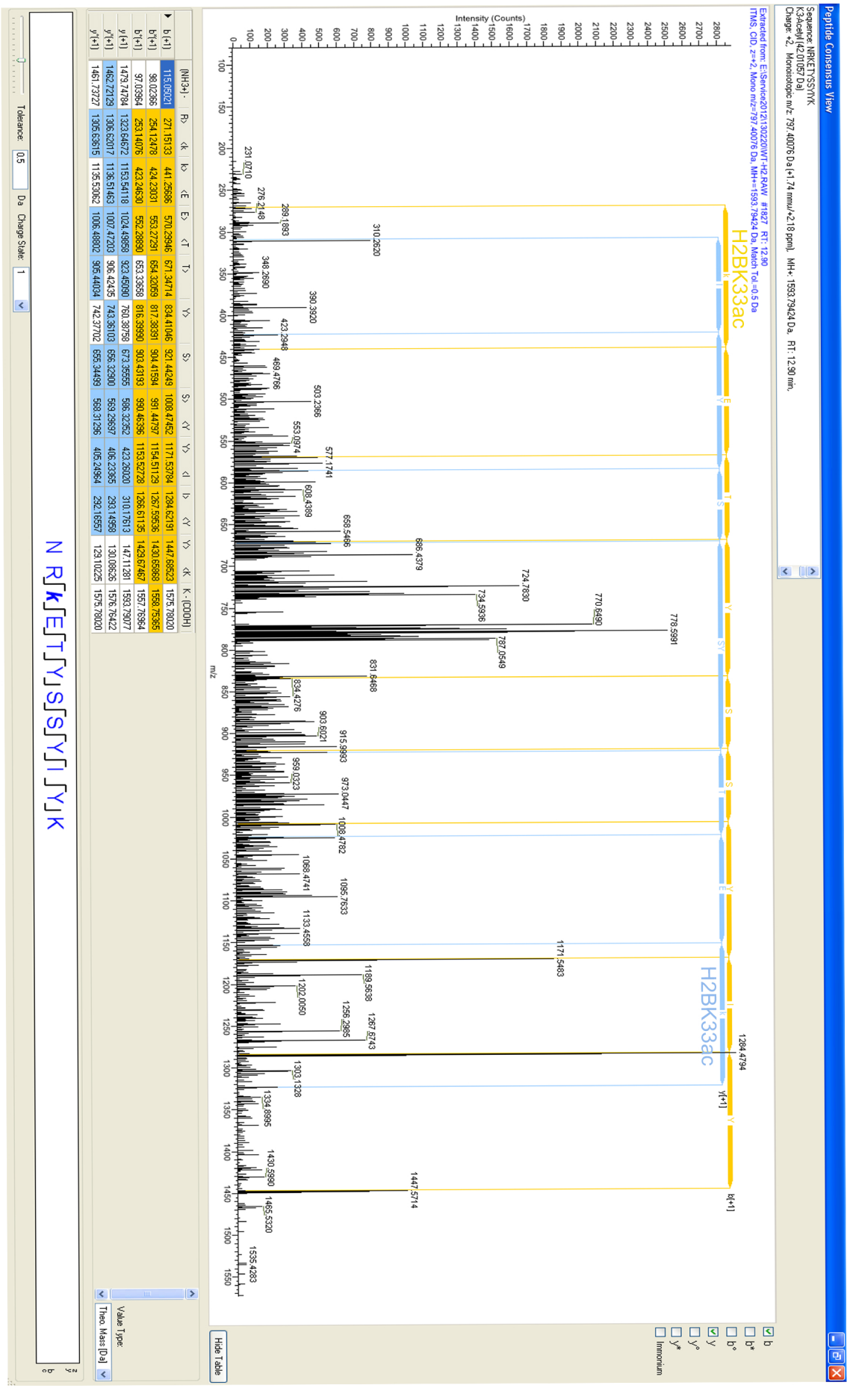
H2BK33ac
y2+ 310.26
y3+ 423.29
b4+ 570.35
y4+ 577.17
b5+, y4+ 586.46
y5+ 673.24
b5+ 671.72
b11+ 724.78
b6+ 834.43
y8+ 923.27
b8+ 1008.48
b9+ 1171.55
y9+ 1153
b10+ 1284.48
y10+ 1323.16
b11+ 1447.57
OK
Help

# Figure S1

**B**


Peptide Consensus View
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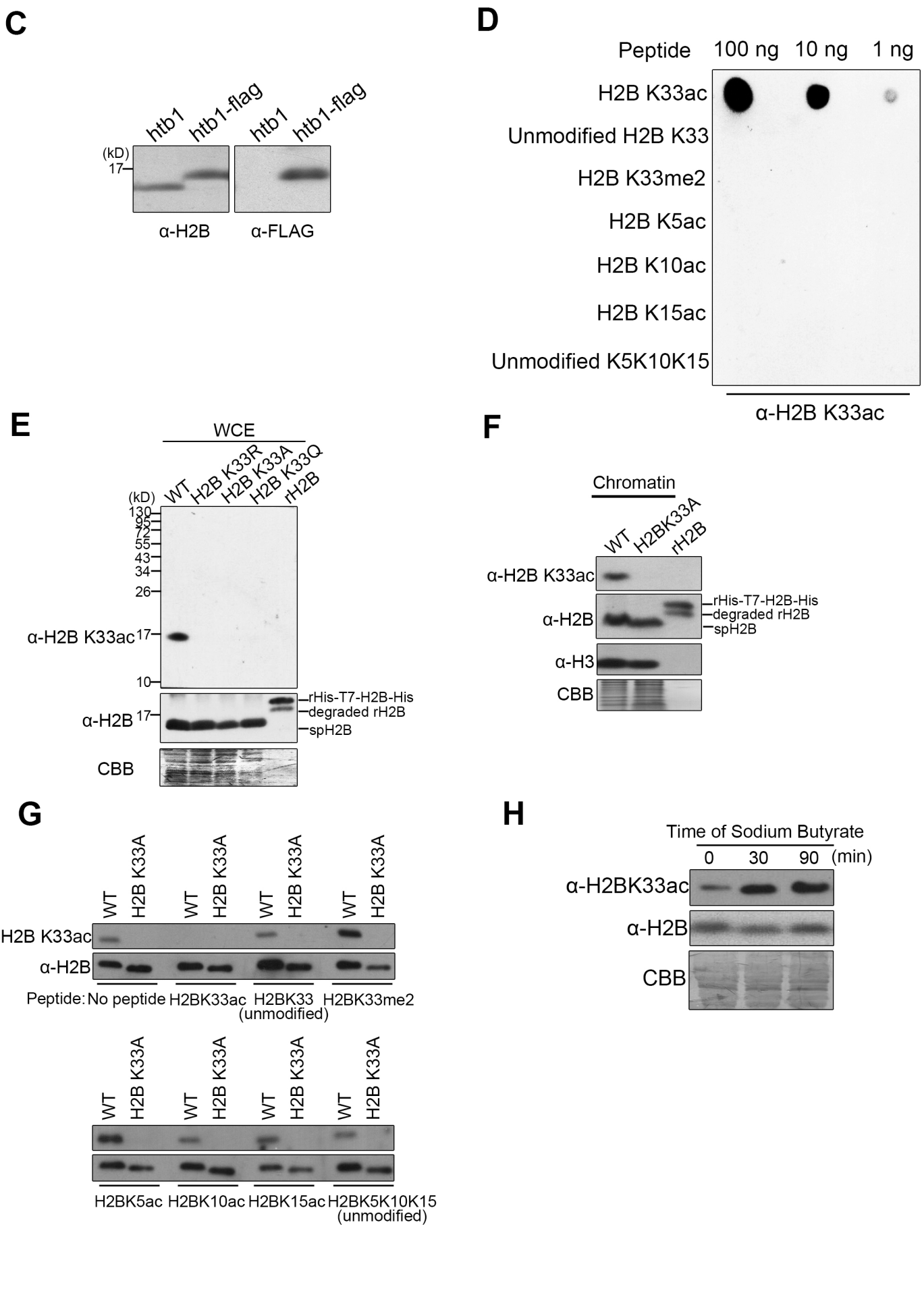
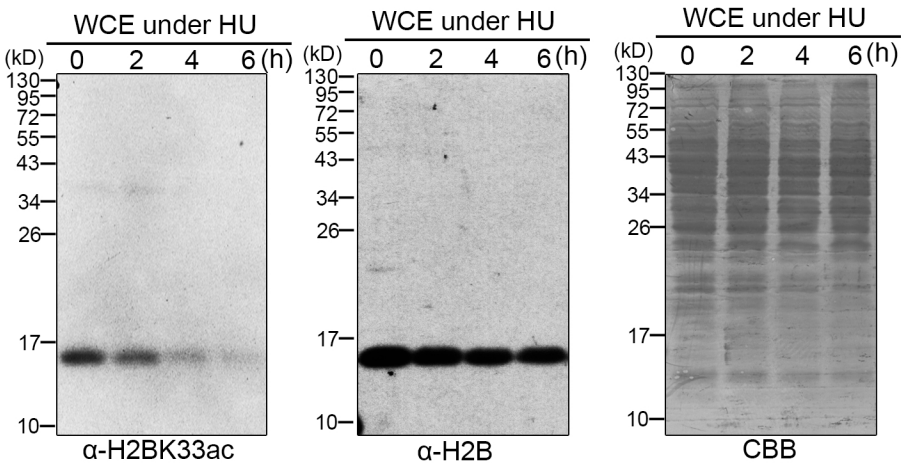
K3Ac-dep[142.01057 Da]
Charge: +2. Monoisotopic m/z: 797.40076 Da (+1.74 mmu/+2.18 ppm), MH+: 1593.79424 Da, RT: 12.90 min.
Extracted from: E:\Service2012\130220\WT-H2.RAW #1827 RT: 12.90
ITMS, CID, z=+2, Mono m/z=797.40076 Da, MH+=1593.79424 Da, Match Tol: ±0.5 Da
H2BK33ac
Intensity (Counts)
m/z
b[+1]
y[+1]
Hide Table
Tolerance: 0.5 Da Charge State: 1
Value Type: Theo. Mass [Da]

# Figure S1


C
htb1
htb1-flag
htb1
htb1-flag
(kD)
17
α-H2B
α-FLAG
D
Peptide
100 ng
10 ng
1 ng
H2B K33ac
Unmodified H2B K33
H2B K33me2
H2B K5ac
H2B K10ac
H2B K15ac
Unmodified K5K10K15
α-H2B K33ac
E
WCE
WT
H2B K33R
H2B K33A
H2B K33Q
rH2B
(kD)
130
95
72
55
43
34
26
17
10
α-H2B K33ac
α-H2B
CBB
rHis-T7-H2B-His
degraded rH2B
spH2B
F
Chromatin
WT
H2BK33A
rH2B
α-H2B K33ac
α-H2B
α-H3
CBB
rHis-T7-H2B-His
degraded rH2B
spH2B
G
WT
H2B K33A
α-H2B K33ac
α-H2B
Peptide: No peptide
H2BK33ac
H2BK33 (unmodified)
H2BK33me2
H2BK5ac
H2BK10ac
H2BK15ac
H2BK5K10K15 (unmodified)
H
Time of Sodium Butyrate
0
30
90
(min)
α-H2BK33ac
α-H2B
CBB

# Figure S1

**I**

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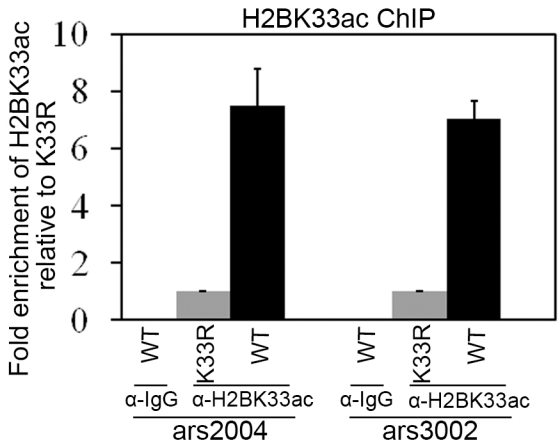


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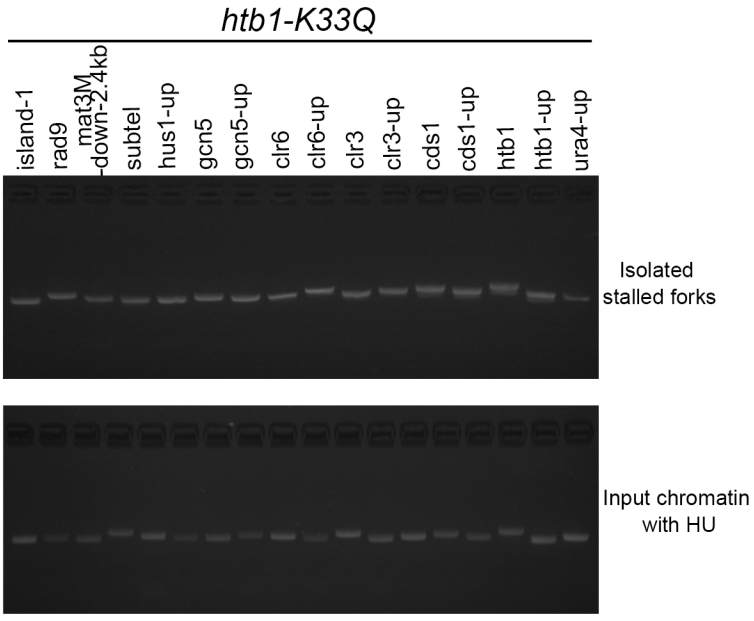
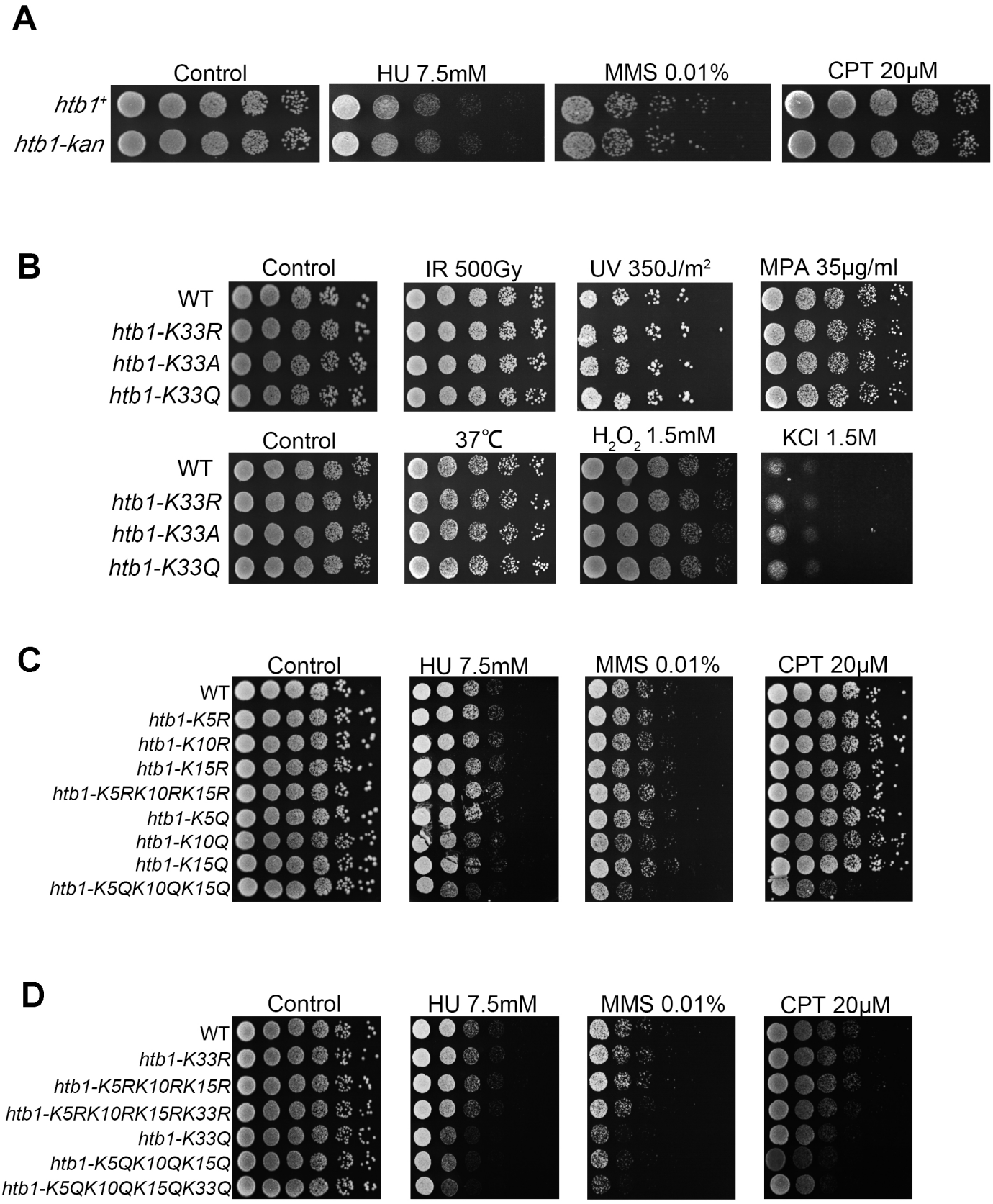


# Figure S2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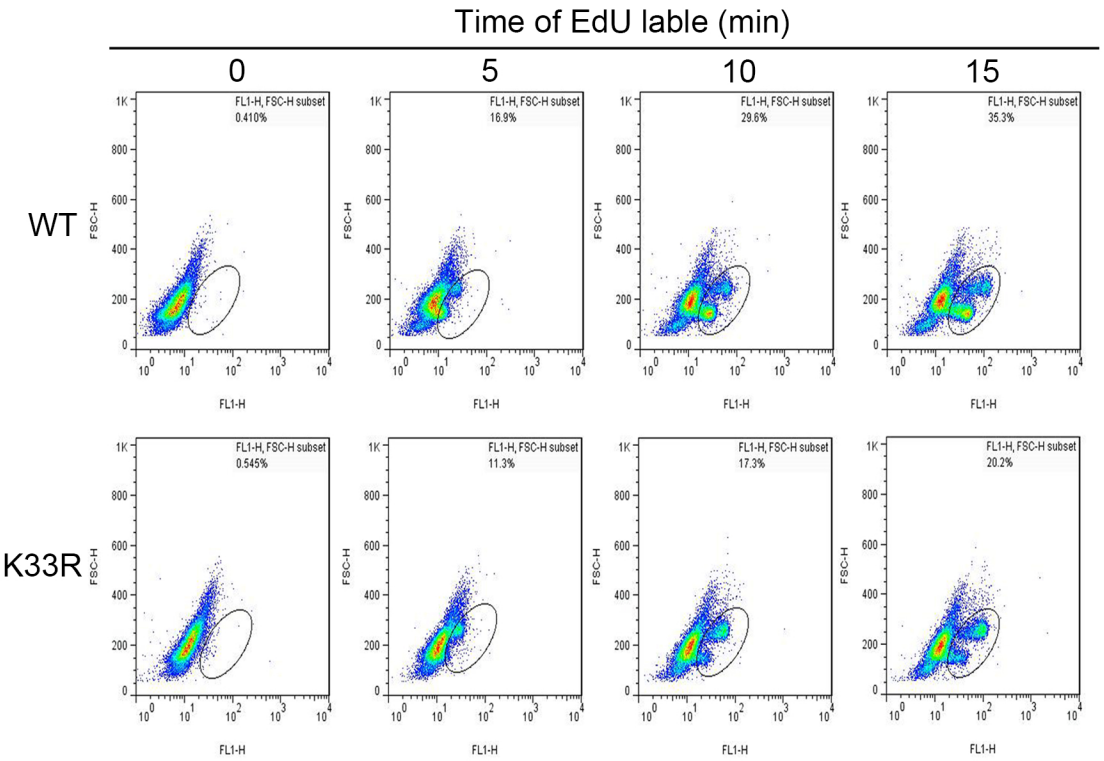
Control
HU 7.5mM
MMS 0.01%
CPT 20μM
htb1+
htb1-kan
B
Control
IR 500Gy
UV 350J/m2
MPA 35μg/ml
WT
htb1-K33R
htb1-K33A
htb1-K33Q
Control
37℃
H2O2 1.5mM
KCl 1.5M
WT
htb1-K33R
htb1-K33A
htb1-K33Q
C
Control
HU 7.5mM
MMS 0.01%
CPT 20μM
WT
htb1-K5R
htb1-K10R
htb1-K15R
htb1-K5RK10RK15R
htb1-K5Q
htb1-K10Q
htb1-K15Q
htb1-K5QK10QK15Q
D
Control
HU 7.5mM
MMS 0.01%
CPT 20μM
WT
htb1-K33R
htb1-K5RK10RK15R
htb1-K5RK10RK15RK33R
htb1-K33Q
htb1-K5QK10QK15Q
htb1-K5QK10QK15QK33Q

# Figure S2

**E**

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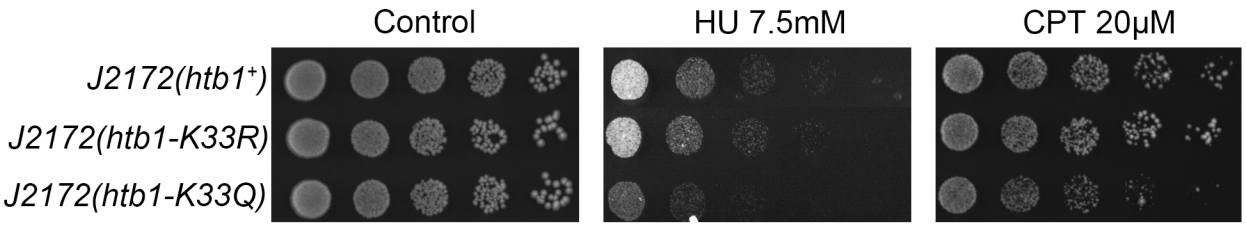
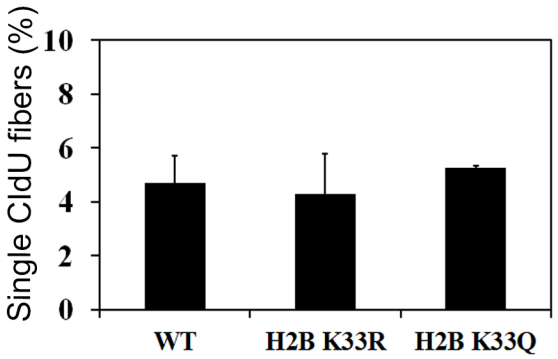
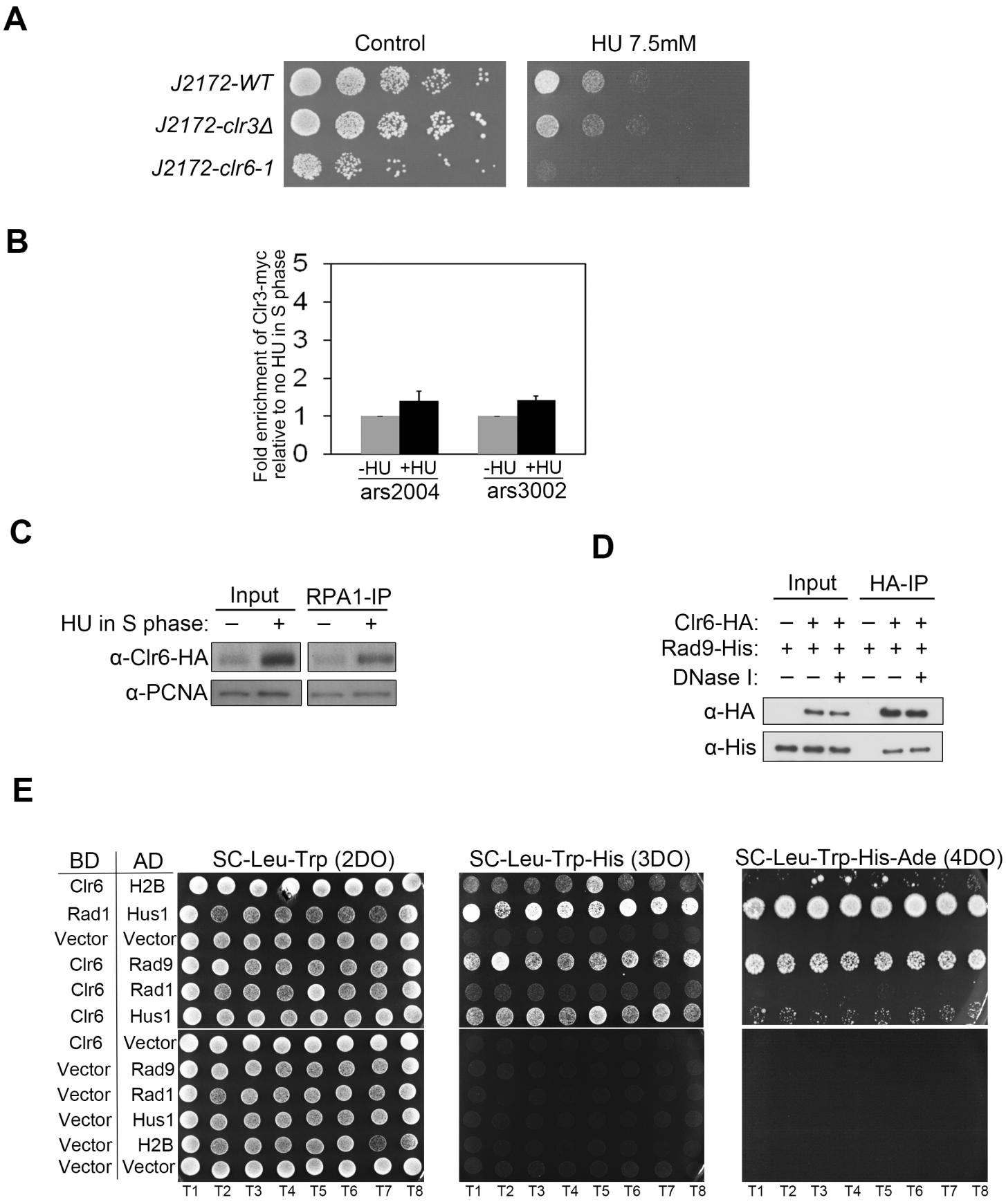



**G**

# Figure S3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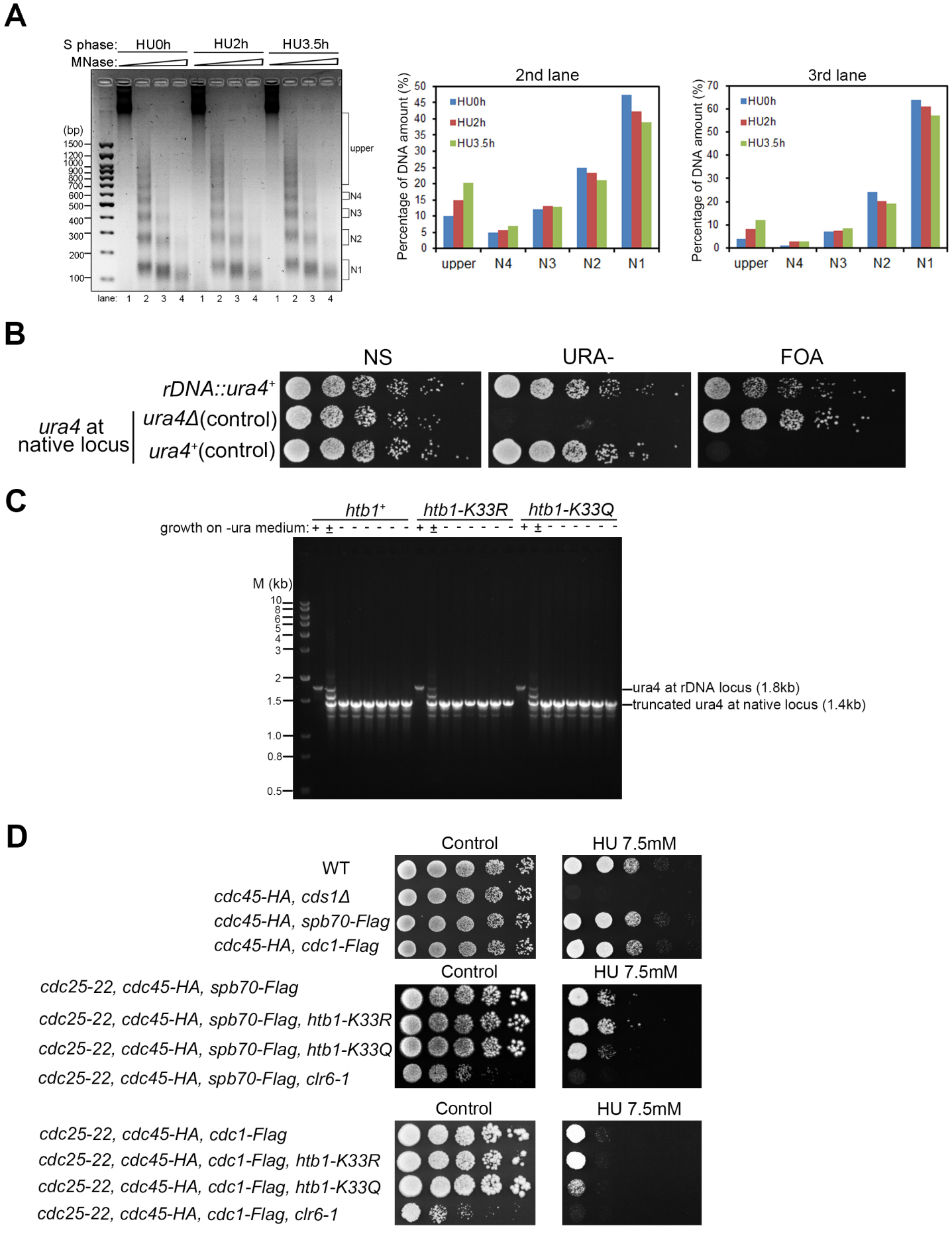
Control
HU 7.5mM
J2172-WT
J2172-clr3Δ
J2172-clr6-1
B
Fold enrichment of Clr3-myc relative to no HU in S phase
0
1
2
3
4
5
-HU +HU
ars2004
-HU +HU
ars3002
C
Input
RPA1-IP
HU in S phase: – + – +
α-Clr6-HA
α-PCNA
D
Input
HA-IP
Clr6-HA: – + + – + +
Rad9-His: + + + + + +
DNase I: – – + – – +
α-HA
α-His
E
BD
AD
SC-Leu-Trp (2DO)
SC-Leu-Trp-His (3DO)
SC-Leu-Trp-His-Ade (4DO)
Clr6 H2B
Rad1 Hus1
Vector Vector
Clr6 Rad9
Clr6 Rad1
Clr6 Hus1
Clr6 Vector
Vector Rad9
Vector Rad1
Vector Hus1
Vector H2B
Vector Vector
T1 T2 T3 T4 T5 T6 T7 T8

# Figure S4


A
S phase: HU0h HU2h HU3.5h
MNase:
(bp)
1500
1200
1000
900
800
700
600
500
400
300
200
100
upper
N4
N3
N2
N1
lane: 1 2 3 4 1 2 3 4 1 2 3 4
2nd lane
Percentage of DNA amount (%)
HU0h
HU2h
HU3.5h
upper N4 N3 N2 N1
3rd lane
Percentage of DNA amount (%)
HU0h
HU2h
HU3.5h
upper N4 N3 N2 N1
B
NS
URA-
FOA
rDNA::ura4+
ura4 at native locus
ura4Δ(control)
ura4+(control)
C
htb1+
htb1-K33R
htb1-K33Q
growth on -ura medium: + ± - - - - - - + ± - - - - - - + ± - - - - - -
M (kb)
10
8
6
5
4
3
2
1.5
1.0
0.8
0.5
ura4 at rDNA locus (1.8kb)
truncated ura4 at native locus (1.4kb)
D
Control
HU 7.5mM
WT
cdc45-HA, cds1Δ
cdc45-HA, spb70-Flag
cdc45-HA, cdc1-Flag
Control
HU 7.5mM
cdc25-22, cdc45-HA, spb70-Flag
cdc25-22, cdc45-HA, spb70-Flag, htb1-K33R
cdc25-22, cdc45-HA, spb70-Flag, htb1-K33Q
cdc25-22, cdc45-HA, spb70-Flag, clr6-1
Control
HU 7.5mM
cdc25-22, cdc45-HA, cdc1-Flag
cdc25-22, cdc45-HA, cdc1-Flag, htb1-K33R
cdc25-22, cdc45-HA, cdc1-Flag, htb1-K33Q
cdc25-22, cdc45-HA, cdc1-Flag, clr6-1