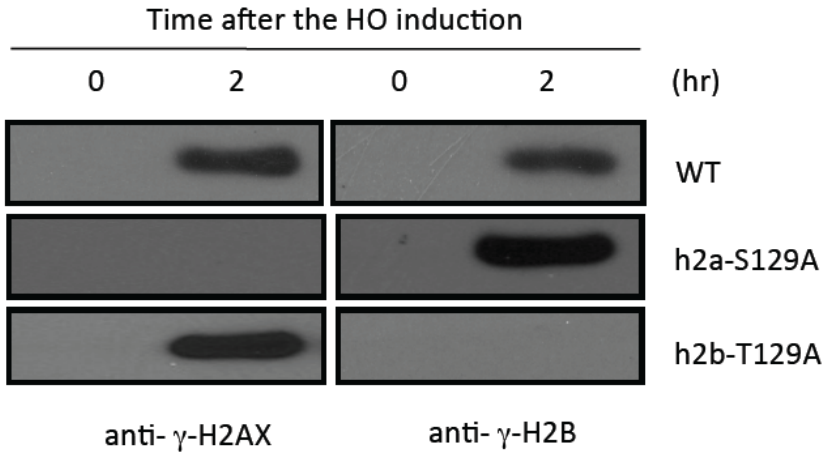
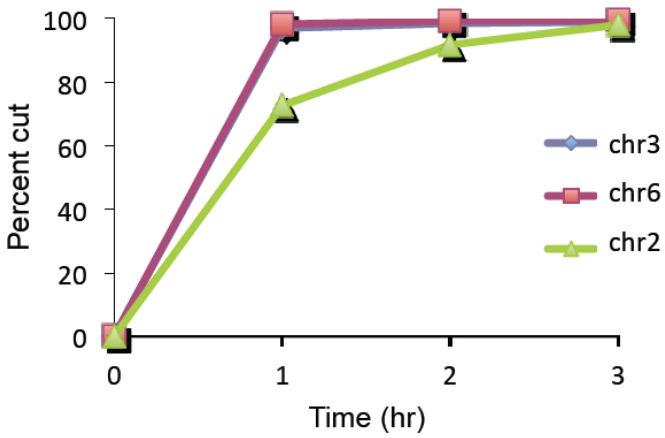
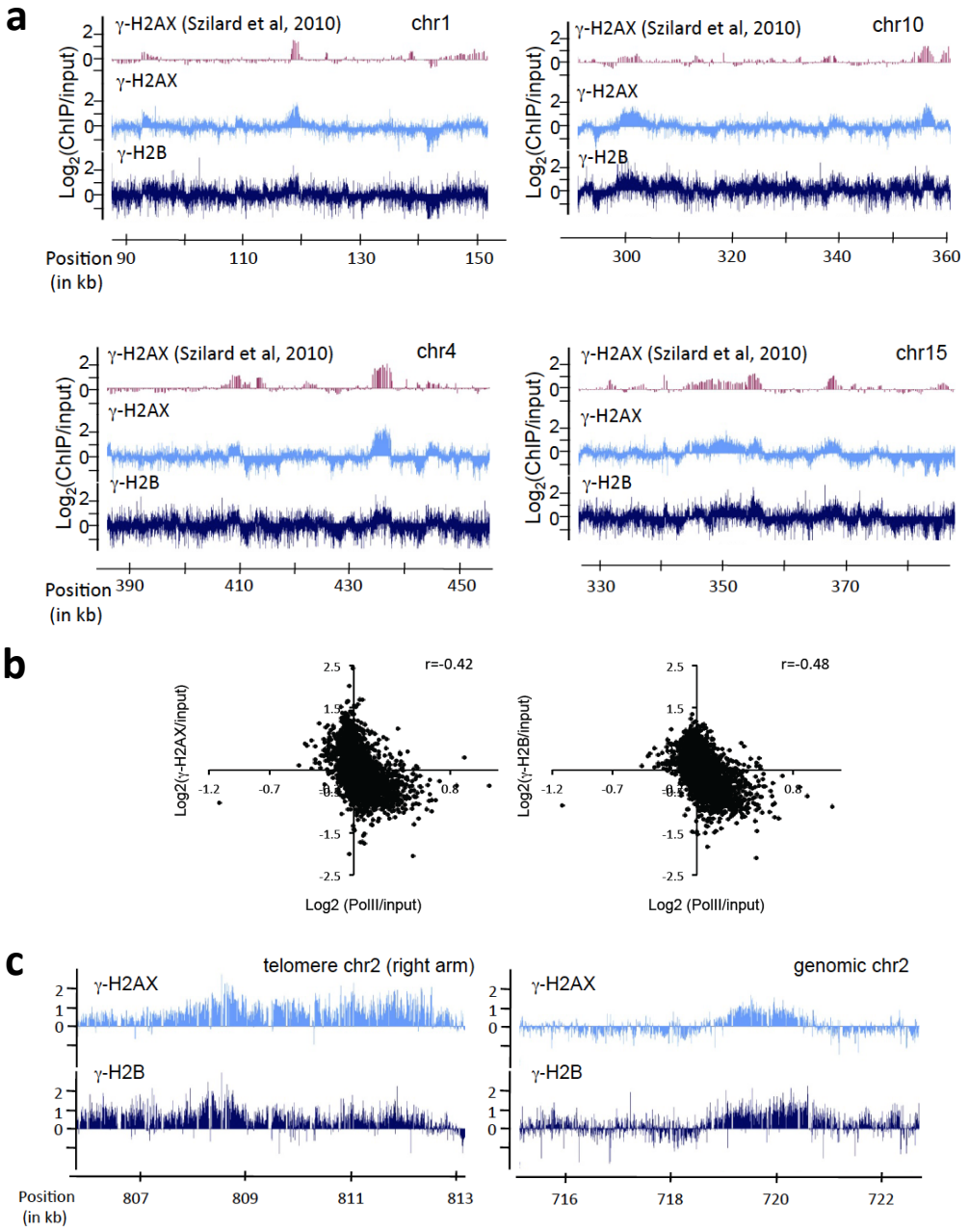
a



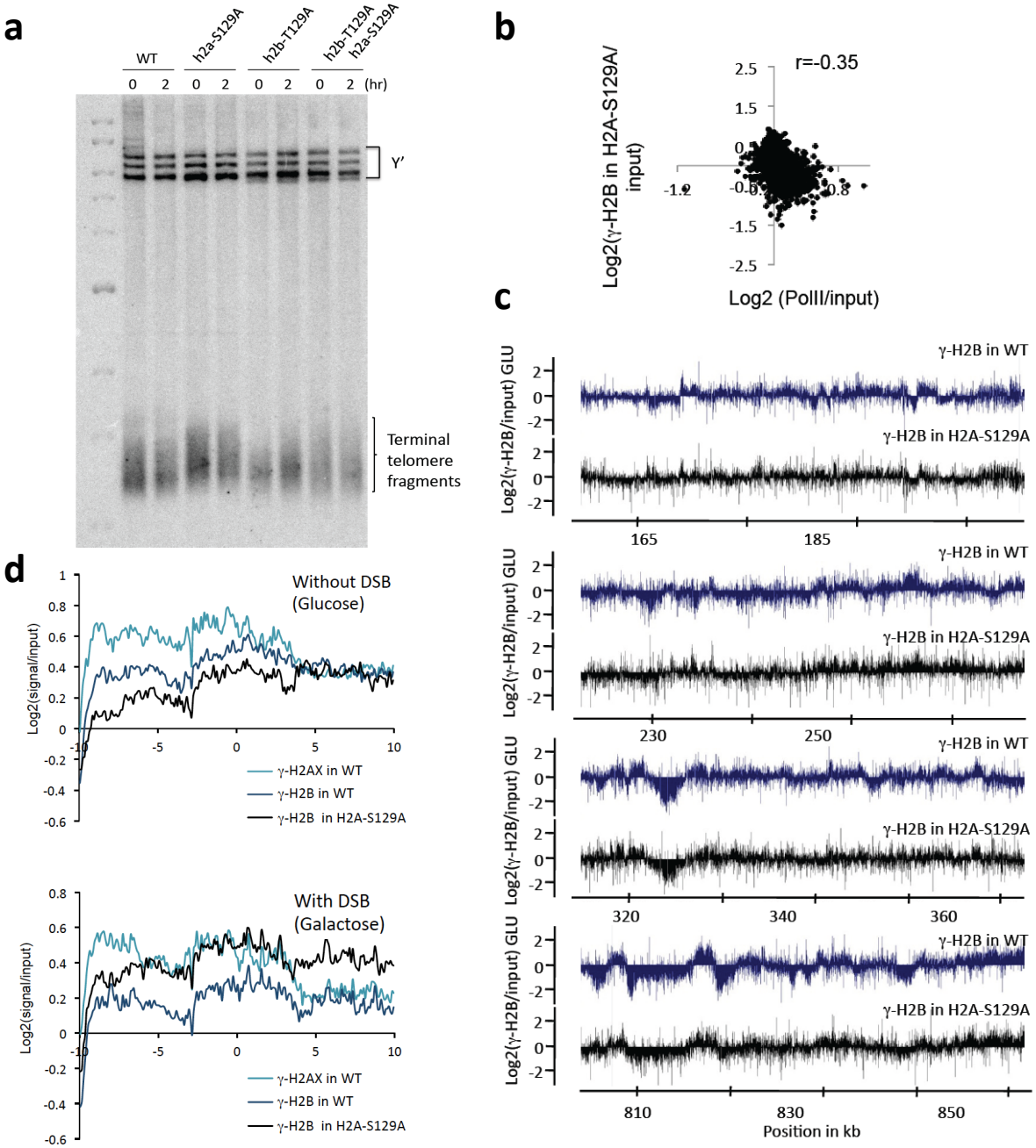
b



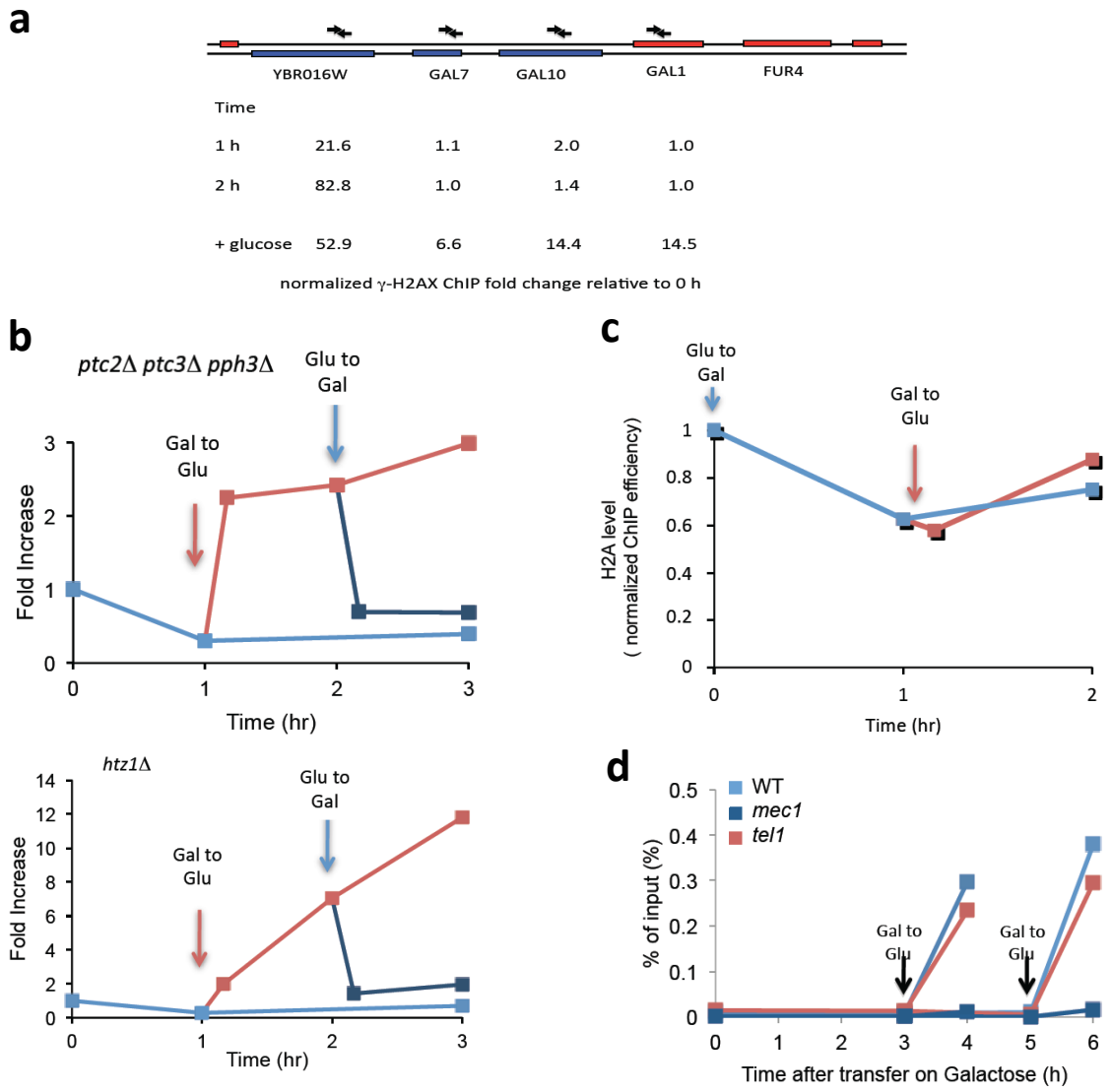
Supplementary Figure S1: (a) Characterization of γ-H2B antibody. A single HO-induced DSB creates an abundant phosphorylation of both γ-H2AX and γ-H2B in wild type cells. The γ-H2B antibody fails to react on a Western blot in a strain in which the only H2B gene carries H2B-T129A. Conversely γ-H2A antibody fails to react in a strain in which H2A-S129 is mutated to alanine. Western blot gels were run and the histone-sized region excised and stained. **(b)** Kinetics of HO cleavage of DSB sites on Chr2, Chr3, and Chr6. Cutting was monitored by the level of qPCR product of two primers flanking each cut site, relative to the level of amplification prior to induction of HO endonuclease.



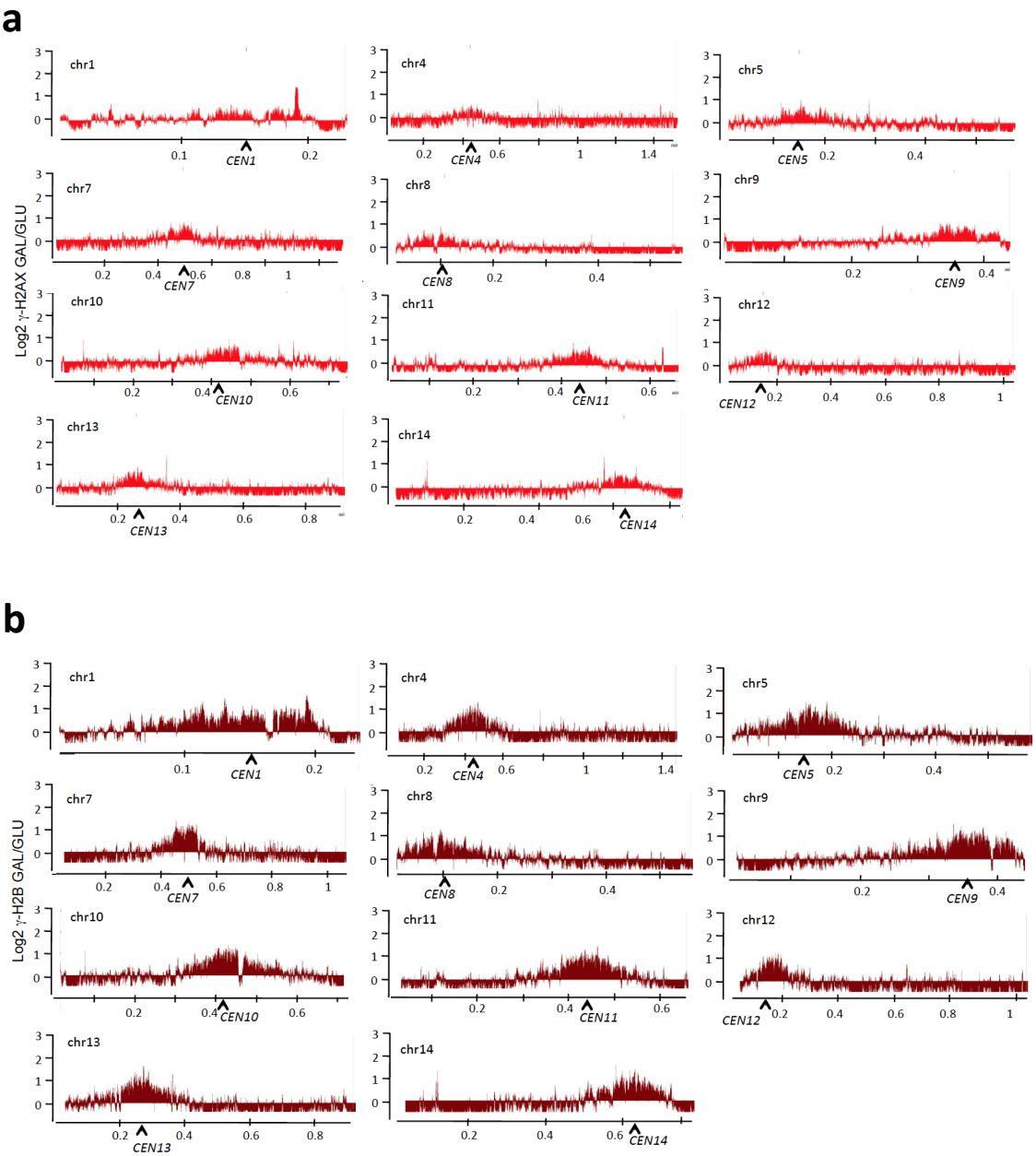
Supplementary Figure S2: (a) Profiles of γ-H2AX and γ-H2B in undamaged cells. ChIP-chip were performed with antibodies against γ-H2AX (light blue) and γ-H2B (dark blue), using yeast grown on glucose containing media (no DSB). The profile of γ-H2AX published earlier by Szilard et al, 2010 (purple), is also shown, for comparison. The log2 ratio of ChIP/input signals across four genomic regions are shown. **(b)** γ-H2AX and γ-H2B are enriched on silent genes in undamaged cells. For each genes of the yeast genome (sacSer1), the averaged γ-H2AX (left panel) or γ-H2B (right panel) signal was calculated on the entire gene length and plotted against the averaged PolII enrichment (retrieved from David et al, 2006). **(c)** Differential enrichment of γ-H2AX and γ-H2B at sub telomeric regions. The profiles of γ-H2AX (light blue) and γ-H2B (dark blue) obtained at the left telomere of chromosome 2 (left panel) and on a genomic region farther on the same chromosome (right panel) are shown. Note that, similarly to the signals observed on chr1, shown Fig. 3a, γ-H2B is less enriched than γ-H2AX at the telomere, while both signals are equivalent further away.



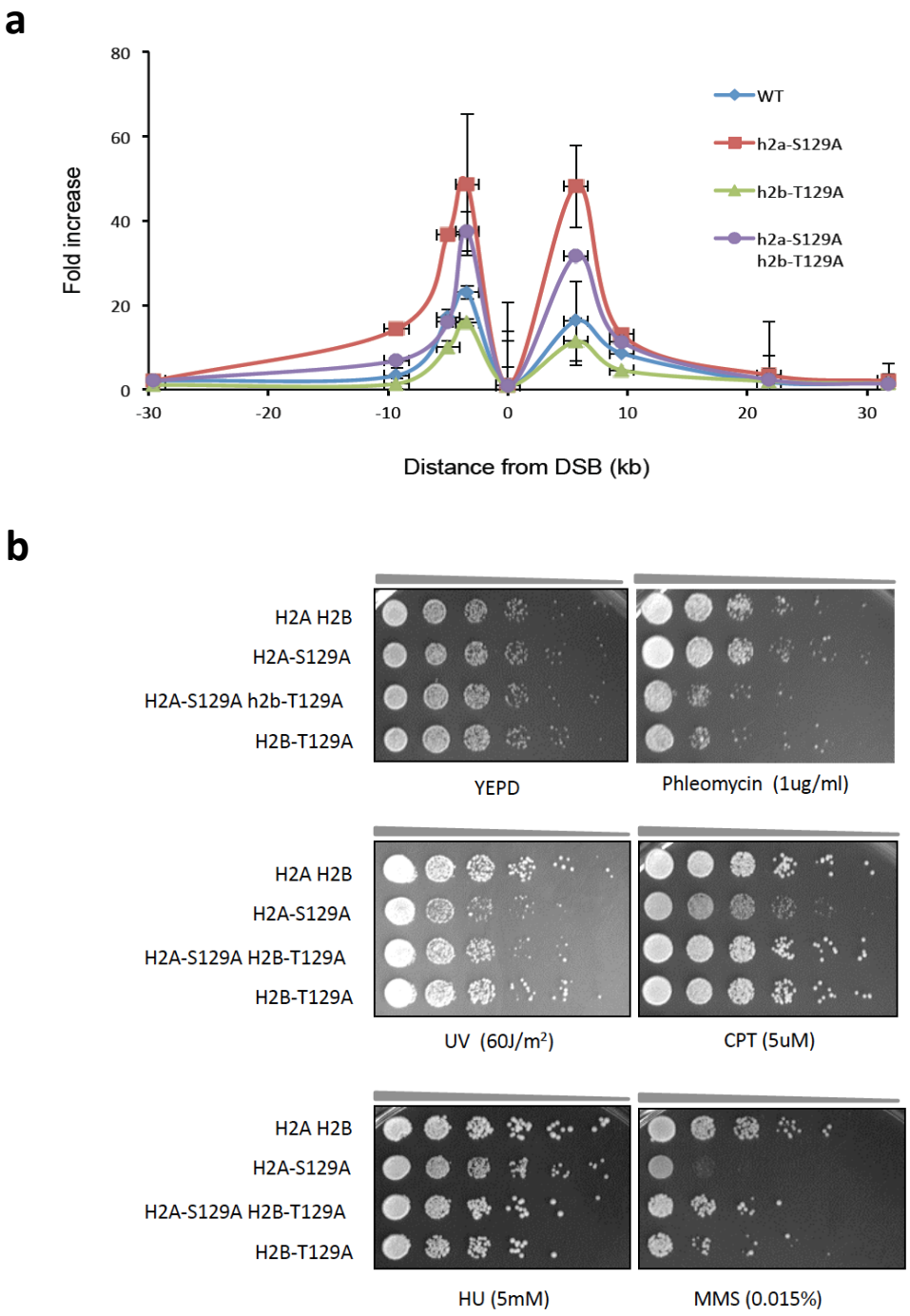
Supplementary Figure S3: (a) Effect of γ-H2AX and γ-H2B on telomere length. DNA from logarithmically growing cells was purified and digested with *Xho*I that cleaves within the Y' subtelomeric element. A Southern blot was probed with a Y' probe that hybridizes with the terminal (telomere containing) fragment and several sizes of internal subtelomeric Y' repeats. **(b)** γ-H2B profile is similar in WT and in a H2A-S129A mutant strain on undamaged chromosomes. For each gene of the yeast genome (sacSer1), the averaged γ-H2B signal in H2A-S129A mutant was calculated on the entire gene length and plotted against the averaged PolII enrichment (retrieved from David et al, 2006) **(c)** Examples of the profiles of γ-H2B signal obtained upon Glucose growth (no DSB), both in WT (blue) and in the H2A mutant strain (black). **(d)** γ-H2AX and γ-H2B profiles near telomeres in cells without and with a single unrepaired DSB. γ-H2AX and γ-H2B ChIP-chip signals in WT or H2A-S129A mutant strain obtained upon growth on Glucose (no DSB) or Galactose (HO cut) were averaged on 20kb at all chromosomes ends (left and right arms combined).



Supplementary Figure S4: (a) γ-H2AX at several positions within the *GAL* gene cluster, as represented in the Saccharomyces Genome Database. Fold increase, which are calculated by normalizing IP value to input and then to 0hr, for γ-H2AX ChIP are shown for the primer pairs (arrows) at times after adding galactose to induce HO cleavage of a DSB approximately 20 kb to the left of these genes. At 1 h, glucose was added to an aliquot of the culture to repress *GAL* gene transcription for another one hour. In this experiment, cells were not first washed free of galactose and the levels of increase in γ–H2AX modification are lower than in Fig. 5. **(b)** Restoration and loss of γ-H2AX from the *GAL10* gene as a function of repression or re-induction does not depend on three checkpoint phosphatases or on histone H2A.Z. These changes occur even when three phosphatases implicated in checkpoint regulation and in dephosphorylating γ-H2AX are ablated or when histone H2A.Z is removed by deleting *HTZ1*. **(c)** H2A level on *GAL10* gene does not undergo dramatic changes upon transfer on Glucose. Histone H2A levels were determined by ChIP at the *GAL10* gene under the conditions described in Fig. 5d. ChIP efficiency is calculated by normalizing IP value to input and then to 0 hr. **(d)** Mec1-dependency on restoration of γ-H2AX at *GAL10* after turning off transcription. Cells were arrested with nocodazole prior to galactose-mediated induction of an unrepairable DSB near the centromere of chromosome 2. γ-H2AX was measured at the *GAL10* gene after transcription was turned off at the times indicated by transferring cells to dextrose-containing medium, as described in Figure 5.



Supplementary Figure S5: (a) γ-H2AX accumulates at centromeric region of undamaged chromosomes after induction of DSB in the 3-cut strain. Profile of the γ-H2AX signal obtained upon Galactose growth (DSB induction) versus Glucose growth (no DSB). All chromosomes except chr2, 3, and 6 (shown Fig. 2) and chr15, 16 (shown Fig. 6a) are shown. Centromeres are indicated by an arrow. **(b)** γ-H2B accumulates at centromeric region of undamaged chromosomes after induction of DSB in the 3-cut strain. Profile of the γ-H2B signal obtained upon Galactose growth (DSB induction) versus Glucose growth (no DSB). Centromeres are indicated by an arrow.



Supplementary Figure S6: (a) Effect of preventing phosphorylation of H2A and H2B on 5' to 3' resection around a DSB. Two hr after HO-induction the extent of 5' to 3' resection was measured by chromatin immunoprecipitation of the largest subunit of RPA (Rfa1). The more extensive resection seen in h2a-S129A is suppressed by h2b-T129A.. **(b)** Effect of preventing phosphorylation of H2A and H2B on sensitivity to DNA damaging agents. Serial dilutions were plated on YEPD plates and on agar plates containing various DNA damaging agents or after exposure to UV light. The h2b-T129A mutation suppresses the hyper-resistance of h2a-S129A to phleomycin and the sensitivity of that mutation to MMS.

YCSL004	Chr6: 97749 nt::HPH:HOcs Chr2: 252kb::HOcs-*URA3*
YCSL024	Chr2: 252kb::HOcs-HPH *mec1*Δ::NAT *sml1*Δ::KAN
YCSL034	Chr2: 252kb::HOcs-*URA3* *mat*Δ *LEU2*
YCSL035	Chr2: 625kb::HOcs-*URA3* *mat*Δ *LEU2*
YCSL120	Chr2: 252kb::HOcs-HPH *tel1*Δ::*TRP1*
YCSL271	Chr2: 252kb::HOcs-*URA3* *htz1*Δ::NAT *mat*Δ *LEU2*
YCSL286	Chr2: 252kb::HOcs-*URA3* *ptc2*Δ::HPH *ptc3*Δ::KAN *pph3*Δ::NAT *mat*Δ *LEU2*
YKH124	*hta1-htb1*Δ::*LEU2 hta2-htb2*Δ::*TRP1* [p*hta1-S129A-HTB1*]
YKH127	*hta1-htb1*Δ::*LEU2 hta2-htb2*Δ::*TRP1* [*phta1-S129A-htb1-T129A*]
YKH128	*hta1-htb1*Δ::*LEU2 hta2-htb2*Δ::*TRP1* [p*HTA1-HTB1*]
YKH129	*hta1-htb1*Δ::*LEU2 hta2-htb2*Δ::*TRP1* [*pHTA1-htb1-T129A*]
YKH130	*rad9*Δ::KAN *hta1-S129A hta2-S129A*
R726	*hta1-S129A hta2-S129A*
YSL60	*rad9*Δ:::KAN

Supplementary Table S1: Strains used in this study.
Strains were derivatives of JKM179 (*ho*Δ *hml*Δ*::ADE1 MAT*α *hmr*Δ*::ADE1 ade1-100 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL10::HO*)

GAL10 p3	AGTCTTCAACCTGCAACTCGGTC
GAL10 p4	TGGAACTTGGGTTCCGGTAAAGG
GAL10 p11	GGTAATTAATCAGCGAAGCGATG
GAL10 p12	TTTCTCCTTGACGTTAAAGTATAGAGG
MNN2 p1	TCATCATGGTAGCCTTCAACGCC
MNN2 p2	AAACGAAGCATTTGGCTACGGCTC
RER2 p1	GATTCAACTTCCCGTGAGCTCC
RER2 p2	CATGGATGGGAACAGGAGATTCGC
ECM15 p1	AGGCCCTTCAATTGTTGTTCCTGC
ECM15 p2	TGTTTAGCGGACGTGTGTATGGTC
SCO2 p1	GTGCTGACGCATCTTGCCAATTC
SCO2 p2	TGTCGCCAATAGGGCAACCTTAG
ETR1 p1	CCCTTGATTTGCCTGCAATGGG
ETR1 p2	TATCGCCGGTAATGAGGGTGTC
CDS1 p1	TACACCATGCATGACCCGATGC
CDS1 p2	AGAAGAACTCCACAAGGACGCC
FUR4 p1	TAAAGCCGTCTTGGTTCCGTTTGC
FUR4 p2	AGAGCCATGAGGTTGTACATCGG
KAP104 p1	AGAAGCTTCCAGGAGAACGATGG
KAP104 p2	AAGTGGCTATTGAAGCCTGCGAG
CSL175-Ch2 252kb::HOcs p3	TGTTAGAGGTAATAGCTGTCTTAGATCGGC
CSL176-Ch2 252kb::HOcs p4	GATGAATTGAAAAGGTGGTATGGTGCACTC
CSL177-Yalpha p1	CTCACAGTTTGGCTCCGGTG
MAT8	GAGCAAGACGATGGGGAGTTTC
GAT1p1B	GCTCAGTGTGCGTTATGCTT
CSL178-TEFprom p1	TGTCAAGGAGGGTATTCTGGG
IMG1 p1	TGGATCATGGACAAGGTCCTAC
IMG1 p2	GGCGAAAACAATGGCACTCT
PWP2 p1	GACACACTTTACTTTGGCTTGGTT
PWP2 p2	GACTTCCAAAGACTTAAGCGCA
RAD18 p1	TGTCATCGTTGGGACTGTCA
RAD18 p2	GAAACATAACCATCCATCCTTTCC
ERS1 p1	ACACCCTGAATGGGGAAAC
ERS1 p2	CTGCATGGGTGCTTGATG
MAT3	ATTCTGCCATTCAGGGACAGCG
MAT4	CGTGGGAAAAGTAATCCGATGC
MAT13	TCAGGGTCTGGTGGAAGGAATG
MAT14	CAAAGGTGGCAGTTGTTGAACC
MAT15	CGTCTTCTCAGCGAACAACAGC
MAT16	GCAATAACCCACGGAAACACTG
MAT19	TCGTCGTCGCCATCATTTTC
MAT20	GCCCAAGTTTGAGAGAGGTTGC
YCR043C p1	CCAAGGAACTAATGATCTAAGCACA
YCR043C p2	ACCAGCAGTAATAAGTCGTCCTGA
ARS300 p1	CAAATTGCTTGAACGGATGC
ARS300 p2	GGCTCATAACCTGTTGACGTAA
YCL073C p1	CTGTACCATGTAAAATGAGCGG
YCL073C p2	TCAGCGTGCCCAAAAAA

Supplementary Table S2: Sequence of the primers used in this study.