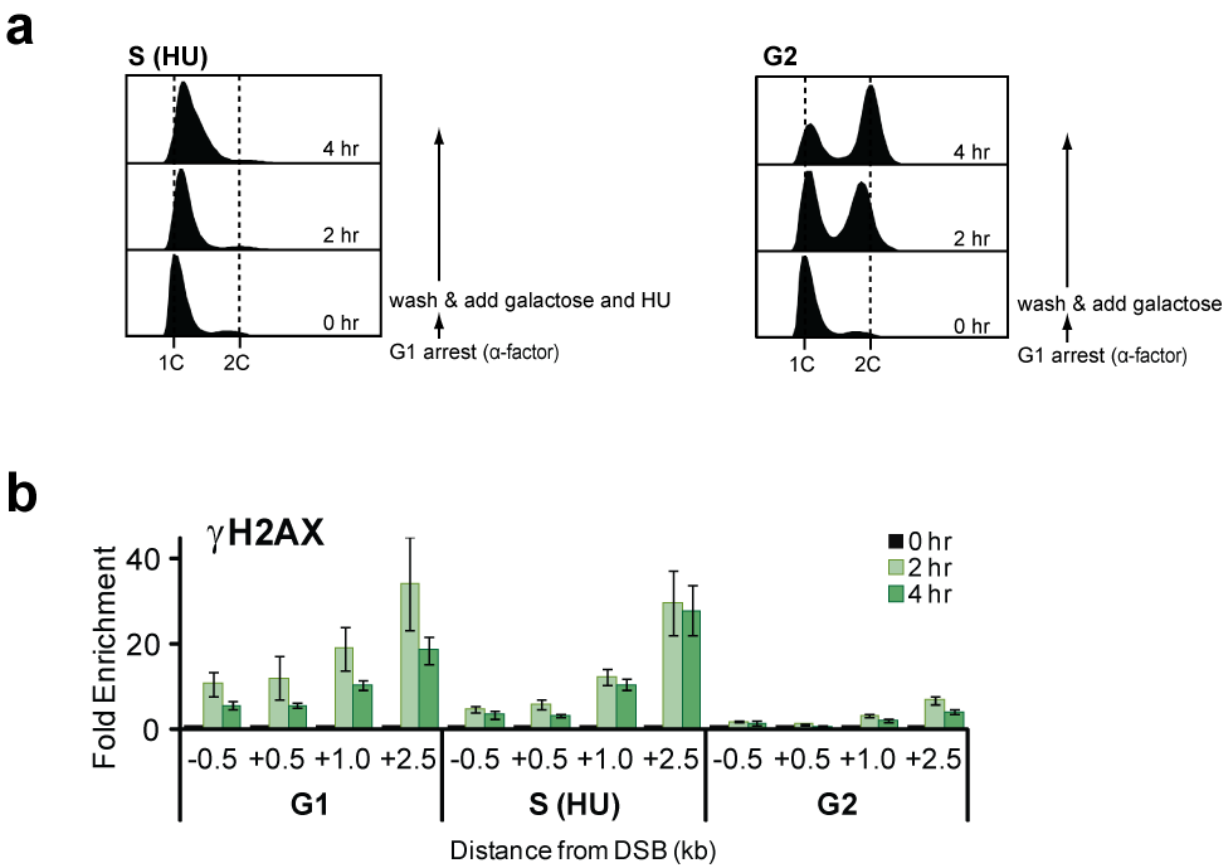
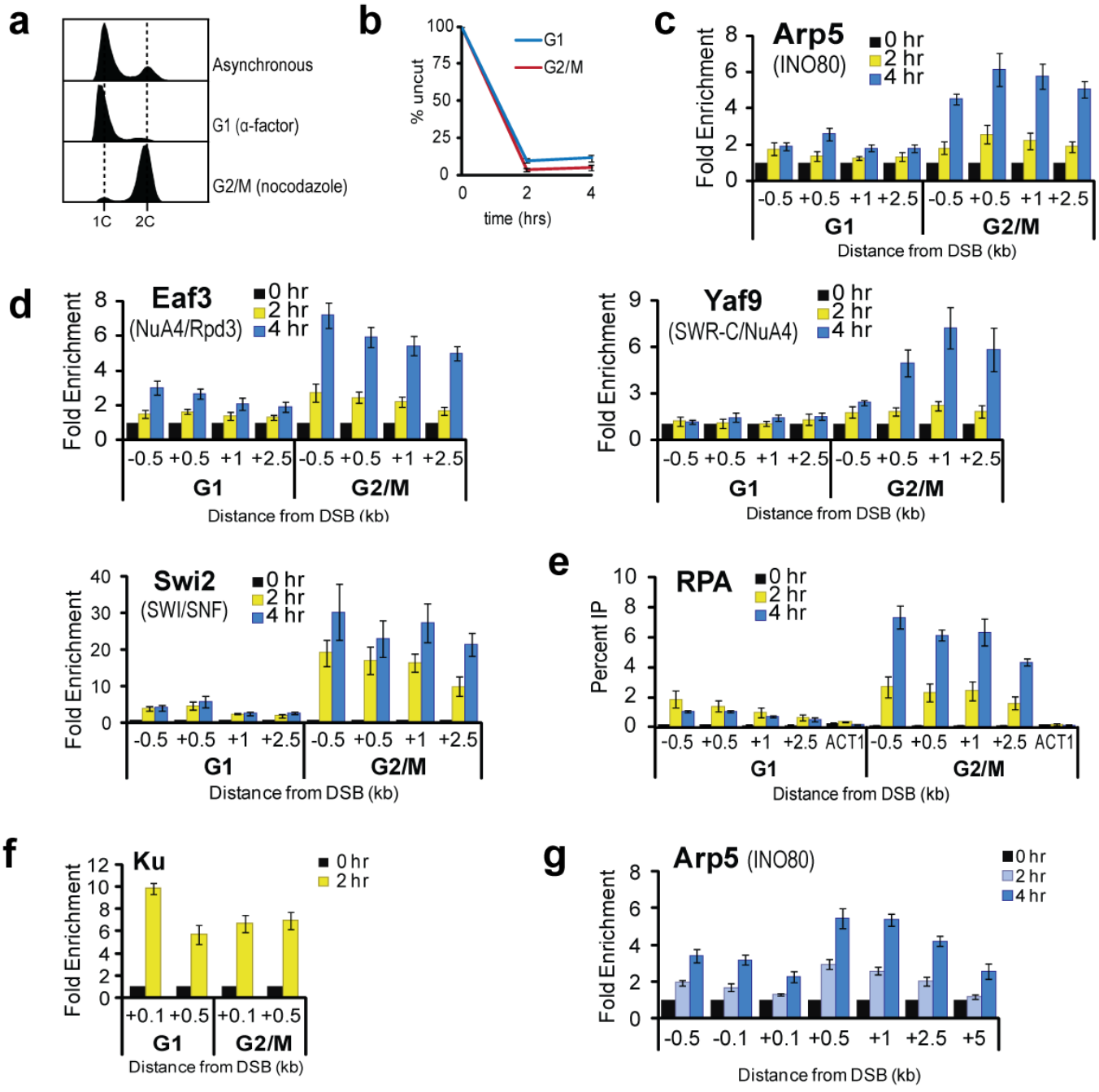
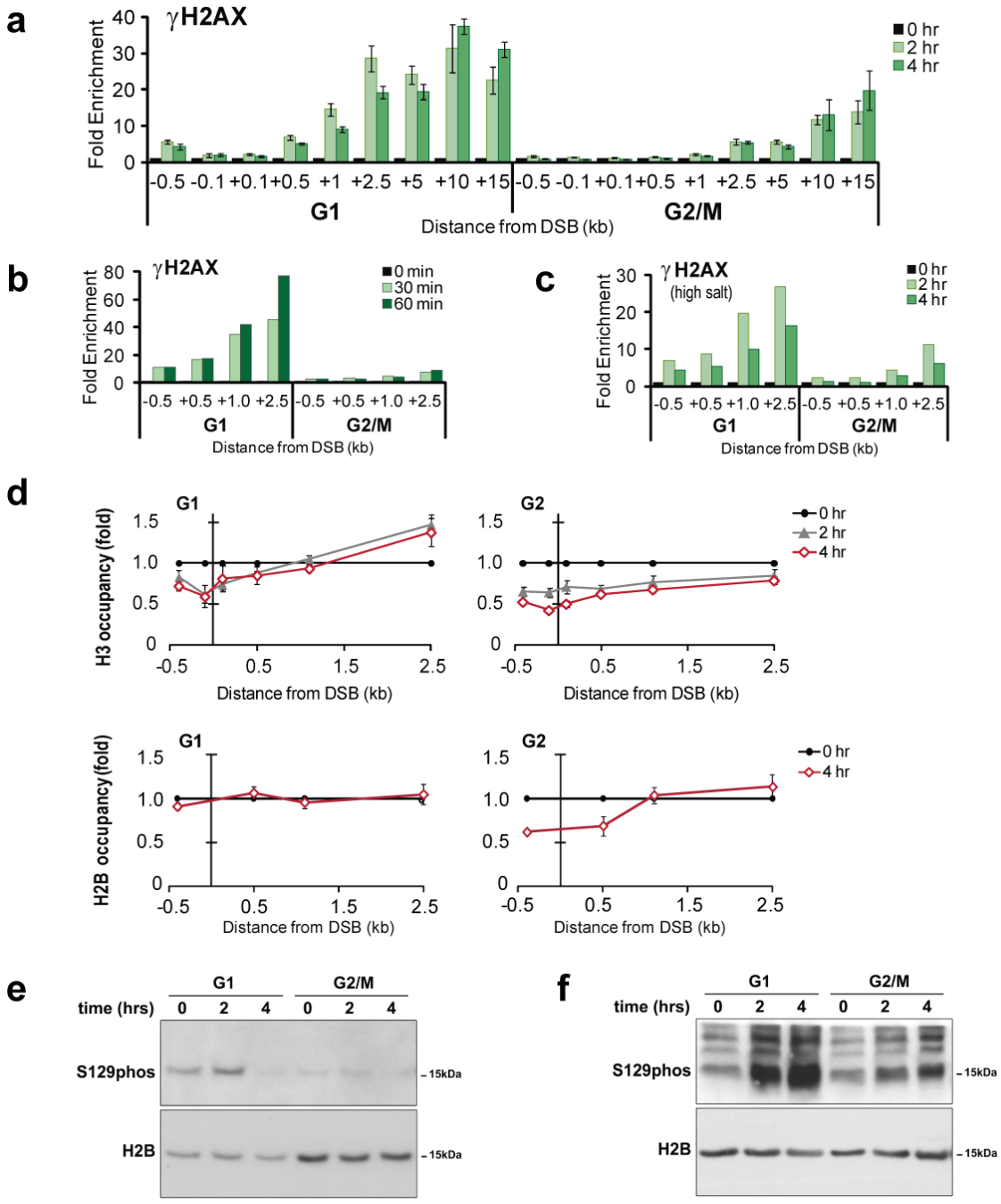


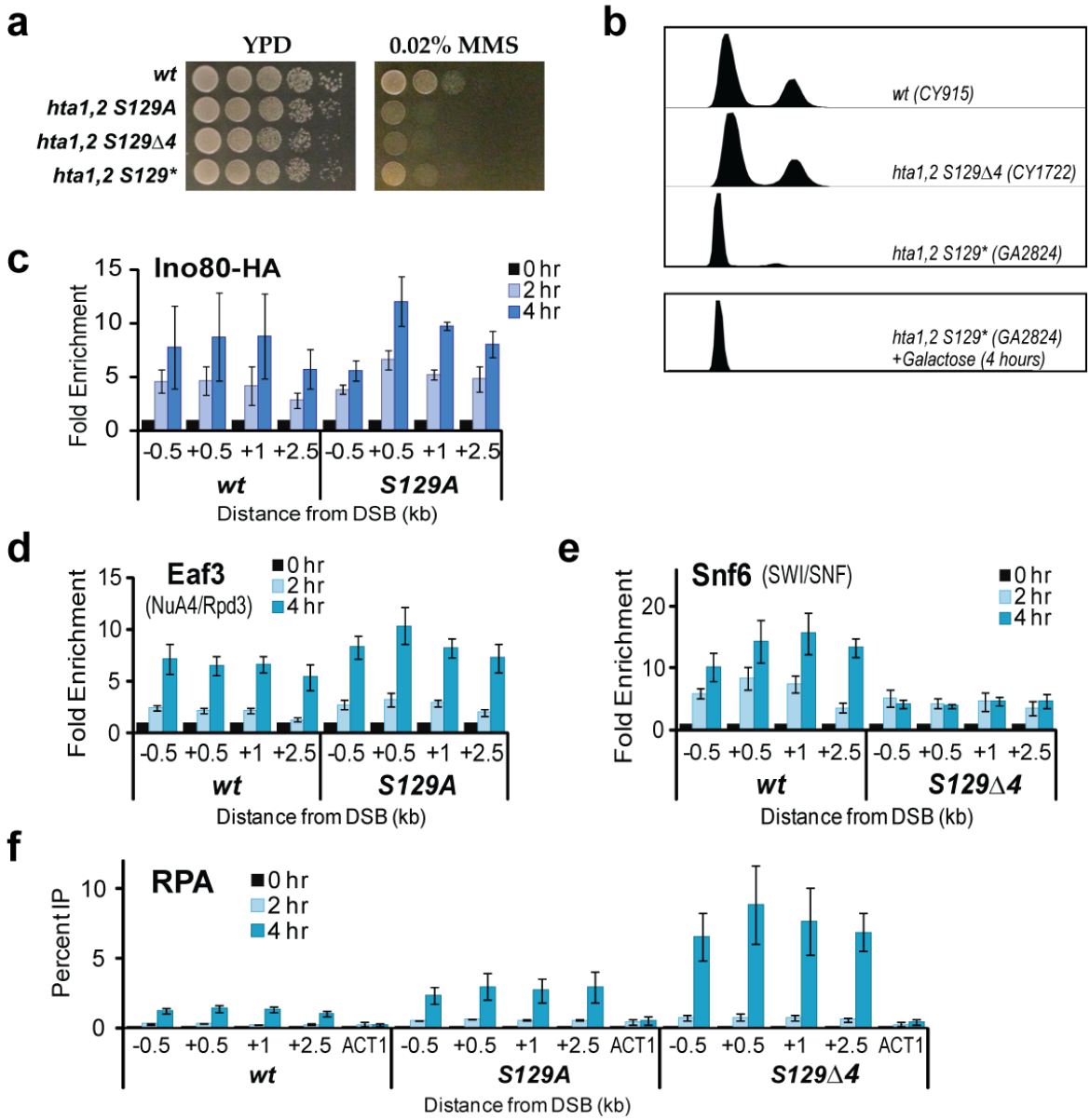
Supplementary Figure S1. **Cell cycle dependence of γH2AX.** Cells were treated as described in Figure 1b and (a) analyzed by flow cytometry for cell cycle distribution, and (b) analyzed by ChIP for levels of γH2AX surrounding the break site. Data represent at least two biological replicates; error bars represent s.e.m.



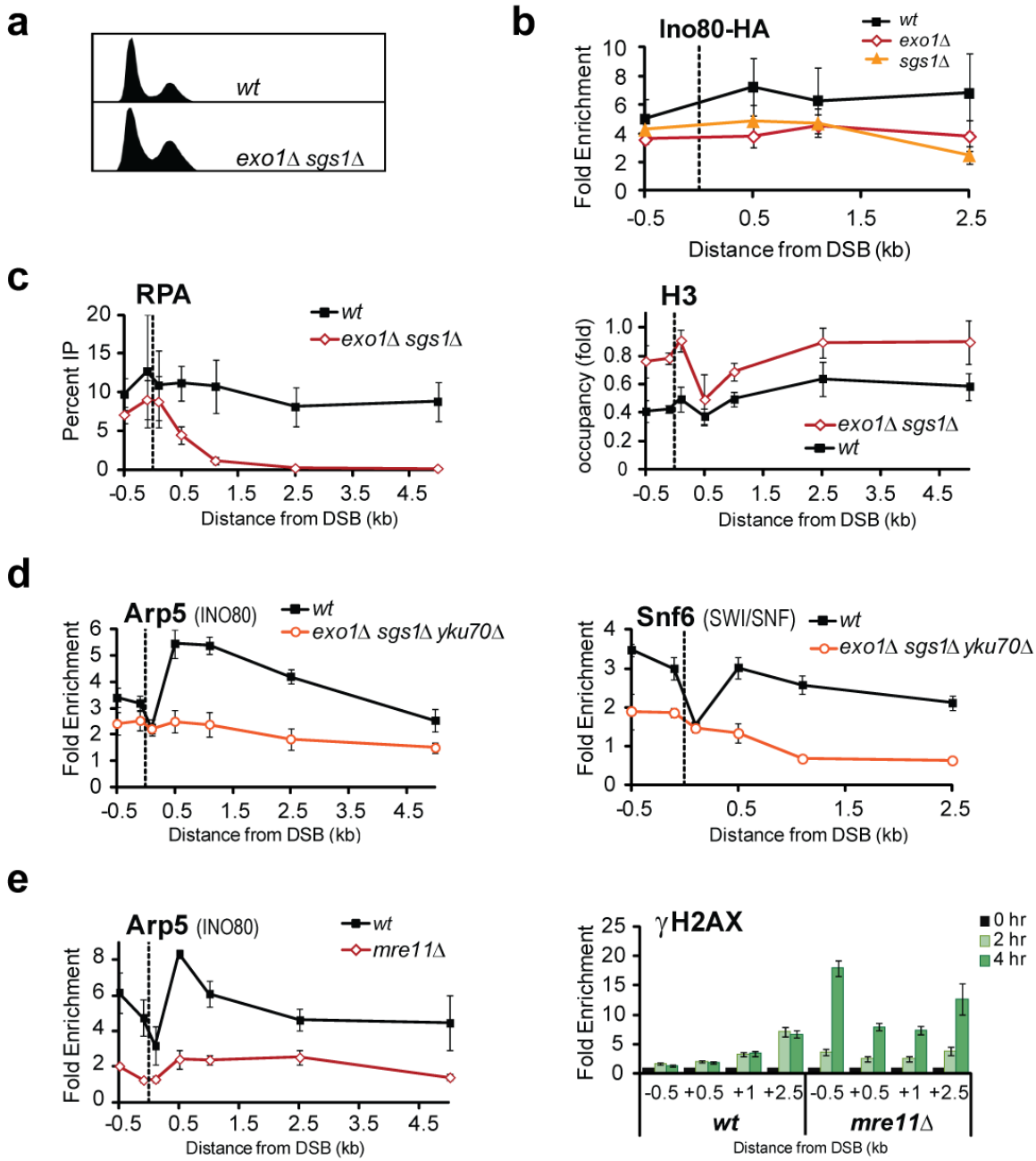
Supplementary Figure S2. Cell cycle dependent recruitment to a DSB. (a) Flow cytometry analysis showing effective cell cycle arrest using alpha-factor mating pheromone (α-factor) to arrest cells in G1, and nocodazole to arrest cells in G2/M. (b) Representative double-strand break induction for cell cycle arrested ChIP experiments. Percent uncut was calculated using qPCR signal achieved from primers spanning the HO cut site and normalized to a control region. (c-f) Cells were treated as described in Figure 1c and analyzed by ChIP for recruitment of the indicated proteins in the regions surrounding an induced DSB. (g) Wild-type, donorless cells were grown asynchronously and assayed by ChIP for recruitment of Arp5 to the region surrounding the DSB. Data shown represent at least two biological replicates; error bars represent s.e.m.



Supplementary Figure S3. Cell cycle regulation of H2A phosphorylation. (a,b) Cells were treated as described in Figure 1c and analyzed by ChIP for levels of γH2AX at the indicated time points after DSB induction. (c) As in (a) except that the initial antibody binding buffer conditions contained 500mM NaCl, rather than 140mM NaCl. (d) Cells were treated as described in Figure 1c and analyzed by ChIP for occupancy of histones H3 and H2B in the regions surrounding a DSB. ChIP data shown represent at least two biological replicates, with exception of panels (b) and (c) which represent one biological experiment each; error bars represent s.e.m. (e,f) Wild-type W303 (CY1343) or JKM139 (CY1508) cells, respectively, were arrested in G1 with αF or G2/M with nocodazole, then exposed to 10 µg/ml phleomycin and samples taken after two and four hours of exposure. Western-blot analysis was then performed using antibodies to phosphorylated histone H2A-S129 and umodified histone H2B.

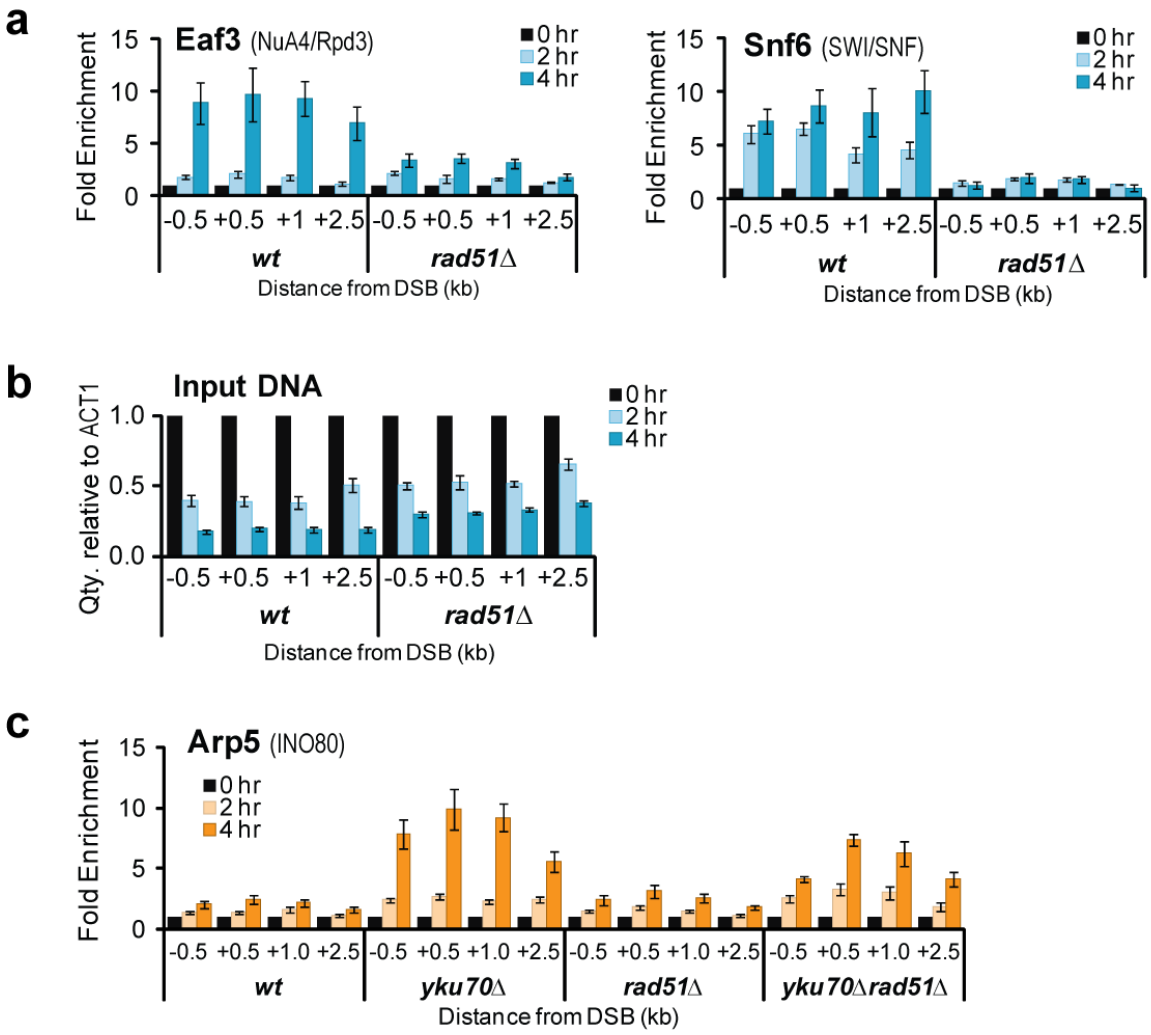


Supplementary Figure S4. Analysis of H2A phosphorylation-deficient mutants. (a) Phosphorylation mutants show expected sensitivity to methyl methanesulfonate (MMS). Serial dilutions of the indicated strains were spotted onto rich media or media containing 0.02% MMS. (b) Strain GA2824 accumulates in the G1 cell phase. FACS analysis in rich media containing 2% raffinose of wild-type (*wt*), our H2A C-terminal truncation strain (*hta1,2S129Δ4*; CY1722), the previously described *hta1,2-S129** strain (GA2824), and GA2824 after four hours of exposure to galactose. (c) H2A phosphorylation does not affect recruitment of INO80 to an induced DSB, regardless of cell cycle. A DSB was induced in freely cycling isogenic, donorless wild-type (*wt*) and *hta1,2-S129A* (*S129A*) cells and analyzed by ChIP for recruitment of an HA-tagged Ino80 to the region surrounding the DSB. (d) H2A phosphorylation does not affect recruitment of Eaf3 (NuA4/Rpd3) to an induced DSB. Cells were treated as in Figure 2b and analyzed by ChIP. (e) H2A phosphorylation does not affect recruitment of Snf6 (SWI/SNF) to an induced DSB. Cells were treated as in Figure 2c and analyzed by ChIP. (f) H2A phosphorylation inhibits resection. Cells were treated as in Figure 2a and recruitment of RPA surrounding the DSB was determined by ChIP. Data shown represent at least two biological replicates; error bars represent s.e.m.

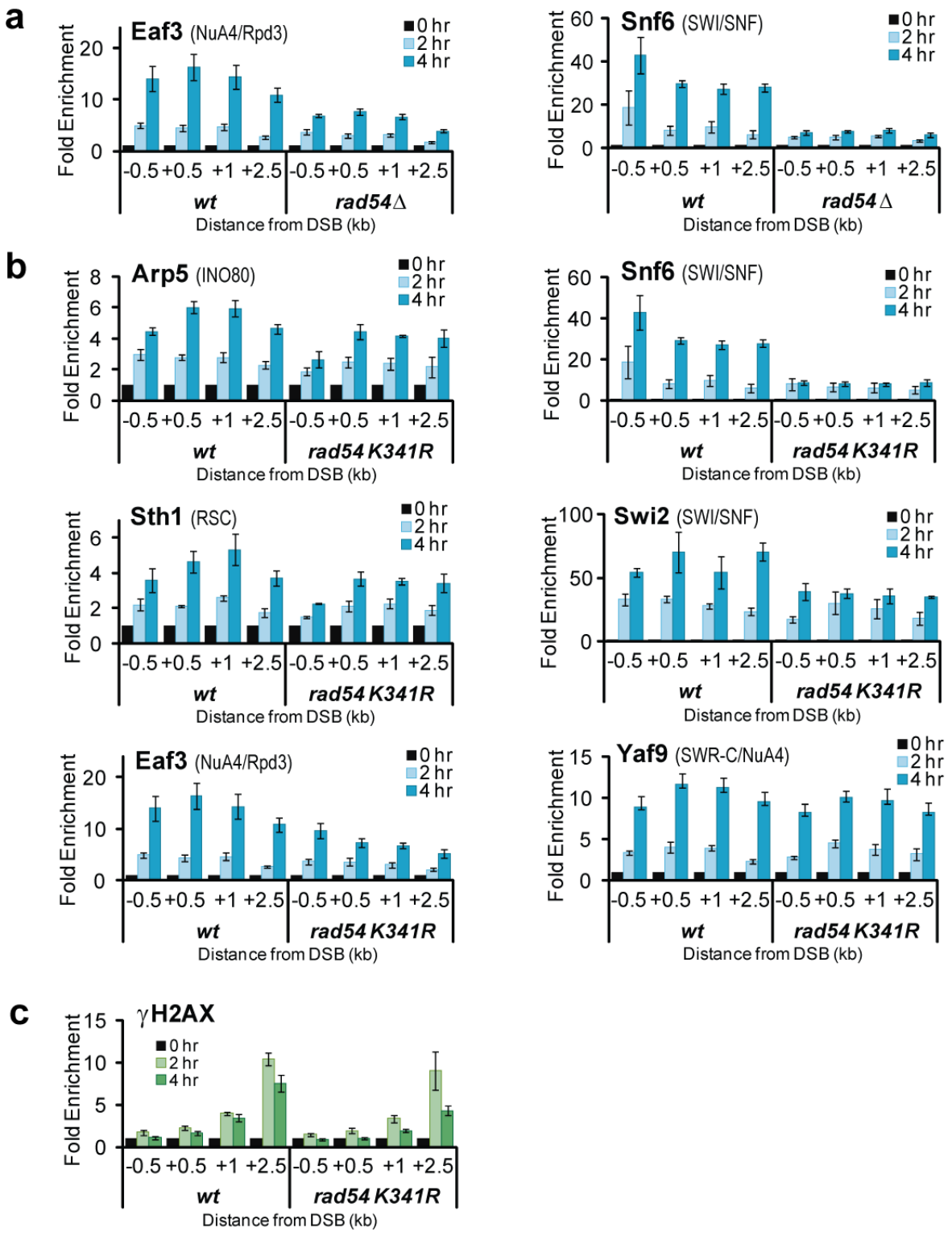


Supplementary Figure S5. DSB end processing is needed for chromatin modifying enzyme recruitment to a DSB. (a) Flow cytometry analysis confirms that wild-type (wt) and *exo1Δsgs1Δ* strains have identical cell cycle distributions. (b) Isogenic, donorless wild-type (*wt*), exo1Δ, and sgs1Δ cultures were grown asynchronously and assayed by ChIP for recruitment of HA-tagged Ino80 to regions surrounding an induced DSB. (c) Levels of RPA and histone H3 confirm a defect in long range resection. Cells treated as in Figure 3 and analyzed by ChIP. (d) Ku inhibition of recruitment is epistatic to end processing. Isogenic, donorless wild-type (*wt*) and *exo1Δsgs1Δ yku70Δ* cultures were grown asynchronously and analyzed by ChIP for recruitment of Arp5 and Snf6 to a DSB. (e) MRX promotes recruitment of chromatin modifying enzymes. Isogenic, donorless wild-type (*wt*) and *mre11Δ* cells were arrested in G2/M using nocodazole and analyzed by ChIP for levels of Arp5 and γH2AX to a DSB.

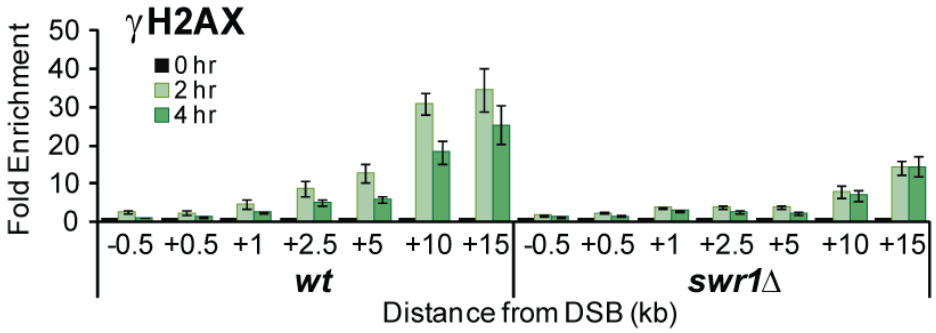
For all line graphs: data represent samples taken four hours post DSB induction; a dotted line indicates the HO cut site. Data shown represent at least two biological replicates; error bars represent s.e.m.



Supplementary Figure S6. Rad51 is required for recruitment to a DSB. (a) Cells were treated as in Figure 5a and analyzed by ChIP for Eaf3 and Snf6 to the DSB region. (b) Quantification by qPCR of input DNA from experiments described in Figure 5a relative to a control region (ACT1). (c) An independent recruitment pathway for INO80 exists in the G1 cell phase. Donorless, isogenic wild-type (*wt*), *rad51Δ*, *yku70Δ*, and *rad51Δyku70Δ* strains were arrested in G1 with αF and assayed by ChIP for recruitment of Arp5 surrounding an induced DSB. Data shown represent at least two biological replicates; error bars represent s.e.m.



Supplementary Figure S7. Effects of Rad54 on recruitment to a DSB. Isogenic, donorless wild-type (*wt*) and either (a) *rad54Δ* or (b) *rad54K341R* (a catalytically-dead version of Rad54) cultures were arrested in G2/M using nocodazole and analyzed by ChIP for recruitment of the indicated proteins to the DSB region. (c) γH2AX levels determined by ChIP in cultures described in (b). Data shown represent at least two biological replicates; error bars represent s.e.m.



Supplementary Figure S8. Effects of SWR-C on H2A phosphorylation surrounding a DSB. Isogenic, donorless wild-type (*wt*) and *swr1Δ* cultures were arrested in G2/M with nocodazole and assayed by ChIP for levels of γH2AX surrounding an induced DSB. Data shown represent two biological replicates; error bars represent s.e.m.

Supplementary Table S1. Genotypes of yeast strains used in this study

Strain #	Genotype	Parent strain	Source
CY915	*MATα Δho hml:ADE1 Δhmr:ADE1 ade1-100 leu2,3-112 lys5 trp1:hisG ura3-52 ade3::GAL10:HO*		ref. 20 (JKM179)
CY1276	*MAT**a** Δho Δhml::ADE1 Δhmr::ADE1 ade1-110 leu2,3-112 lys5 trp1::hisG ura3-52 ade3::GAL10:HO*		ref. 20 (JKM139)
CY1526	*MAT**a** Δho hml:ADE1 Δhmr:ADE1 ade1-100 leu2,3-112 lys5 trp1:hisG ura3-52 ade3::GAL10:HO*		ref. 28 (JKM139)
CY1343	*MATa leu2-3,112 ura3-1 his3-11,15 trp1-1 ade2-1 can1-100 bar1::hisG*	W303	
CY1508	*INO80-HA3::KanMX bar1Δ::URA3*	CY1276	*this work*
CY1574	*SWR1-HA3::KanMX bar1Δ::URA3*	CY1526	*this work*
CY1527	*exo1Δ::TRP1*	CY1526	ref. 28
CY1528	*exo1Δ::TRP1 sgs1Δ::KanMX*	CY1526	ref. 28
CY1529	*sgs1Δ::KanMX*	CY1526	ref. 28
CY1644	*exo1Δ::TRP1 sgs1Δ::KanMX yku70Δ::Hph*	CY1526	*this work*
CY1741	*swr1Δ::NatMX*	CY1526	*this work*
CY1509	*INO80-HA3::KanMX mre11Δ::TRP1*	CY1276	*this work*
CY1572	*INO80-HA3::KanMX bar1Δ::URA3 yku70Δ::Hph*	CY1276	*this work*
CY1910	*INO80-HA3::KanMX bar1Δ::URA3 rad51Δ::NatMX*	CY1276	*this work*
CY1911	*INO80-HA3::KanMX bar1Δ::URA3 yku70Δ::Hph rad51Δ::NatMX*	CY1276	*this work*
CY917	*rad51Δ::LEU2*	JKM179	J. Haber (Brandeis U.)
CY916	*rad54Δ::LEU2*	JKM179	J. Haber (Brandeis U.)
BY66	*rad54Δ::LEU2 rad54K341R*	CY915	ref. 35
CY1584	*INO80-HA3::KanMX*	CY915	*this work*
CY1217	*hta1,2 S129A*	CY915	ref. 22
CY1568	*hta1,2 S129A Ino80-HA3::KanMX*	CY915	*this work*
CY1722	*hta1,2 S129Δ4*	JKM179	J. Cote (Laval U.)
GA2824	*hta1S129* hta2S129* INO80-myc::KanMX4*	JKM179	ref. 18

Supplementary Table S2. Primer sequences used for ChIP-qPCR

Region	Mating type specificity	Name	Sequence
HO cut site	α	GB009.DSB al_F	TGTCTTCTCTGCTCGCTGAA
	a	GB011.DSB a_F	GCATAGTCGGGTTTTTCTTTT
		GB010.DSB_R	ATCCGTCCCGTATAGCCAAT
-0.5 kb	α	GB012.MATal -0.5 kb_F	TCGAAGCCTGCTTTCAAAAT
	α	GB013.MATal -0.5 kb_R	TCGAGAGGAAGGAACAGGAA
	a	GB014.MATa -0.5 kb_F	CAAGGATAGCCTTTGAATCAATTT
	a	GB015.MATa -0.5 kb_R	CCCTTTGGGCTCTTCTCTTT
-0.1 kb	**a**	GB075.MATa -0.2kb_F	AAAGAAGAAGTTGCAAAGAAATGTGG
	a	GB076.MATa -0.2kb_R	TGTTGCGGAAAGCTGAAACTAAAAG
	α	GB081.MATal -0.2kb_F	TCACAGGATAGCGTCTGGAAG
	α	GB082.MATal -0.2kb_R	TTTACACCGGAGCCAAACTG
+0.1 kb		GB077.MATZ_F	TGGTGACGGATATTGGGAAG
		GB078.MATZ_R	TTGGGAACAAGAGCAAGACG
+0.5 kb		GB037.MAT +0.5 kb_F	CATGCGGTTCACATGACTTT
		GB038.MAT +0.5 kb_R	CACCCAAGAAGGCGAATAAG
+1.0 kb		GB016.MAT +1.0 kb_F	CACAGATTGGACGGAGGACT
		GB017.MAT +1.0 kb_R	CAAGGATGCCCTTGTTTTGT
+2.5 kb		GB018.MAT +2.5 kb_F	AATCTGGATGTTCTAAGTGG
		GB019.MAT +2.5 kb_R	CGCATTTTCAACATGTTTGC
+5 kb		GB100.MAT +5.0 kb_F	CCGCAAGAATGATTCACAAC
		GB101.MAT +5.0 kb_R	CAGATTGCTTCAAAATCTGAGTG
+10 kb		GB090.MAT +10.0 kb_F	TGGCAGACTCCTTGTCTTTG
		GB091.MAT +10.0 kb_R	AGGTGAATTTGGACGGTGTG
+15 kb		GB098.MAT +15.0 kb_F	CAAACAGAGGCTCAAGATGG
		GB099.MAT +15.0 kb_R	TTCGGGCTTATCCTTTGGAG
ACT1 ORF		GB041.ACT1_R	GCCTTCTACGTTTCCATCCA
		GB042.ACT1_F	GGCCAAATCGATTCTCAAAA