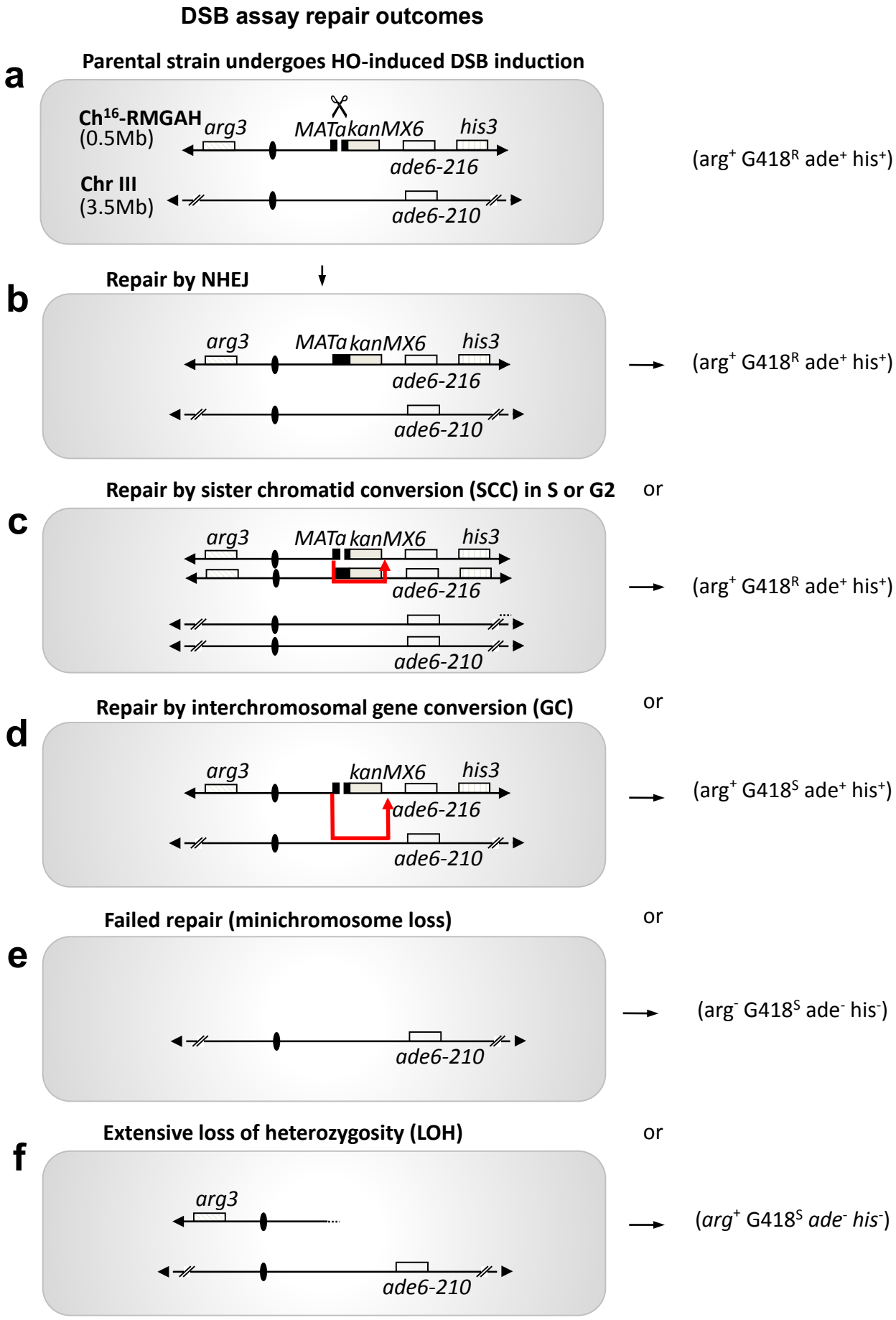
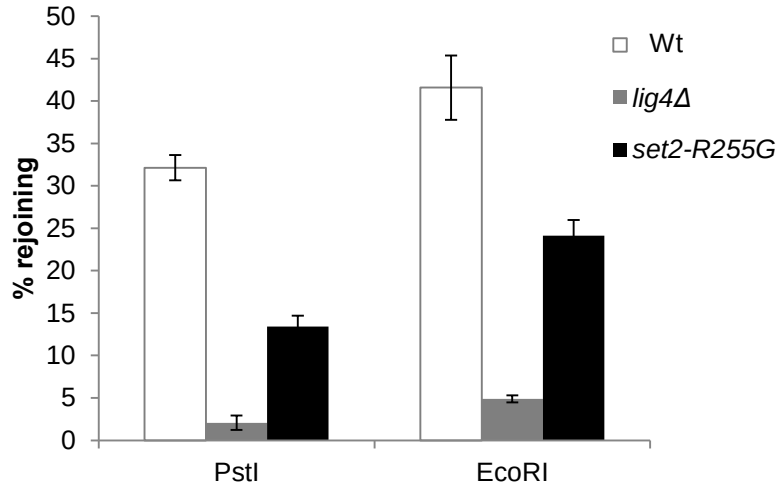
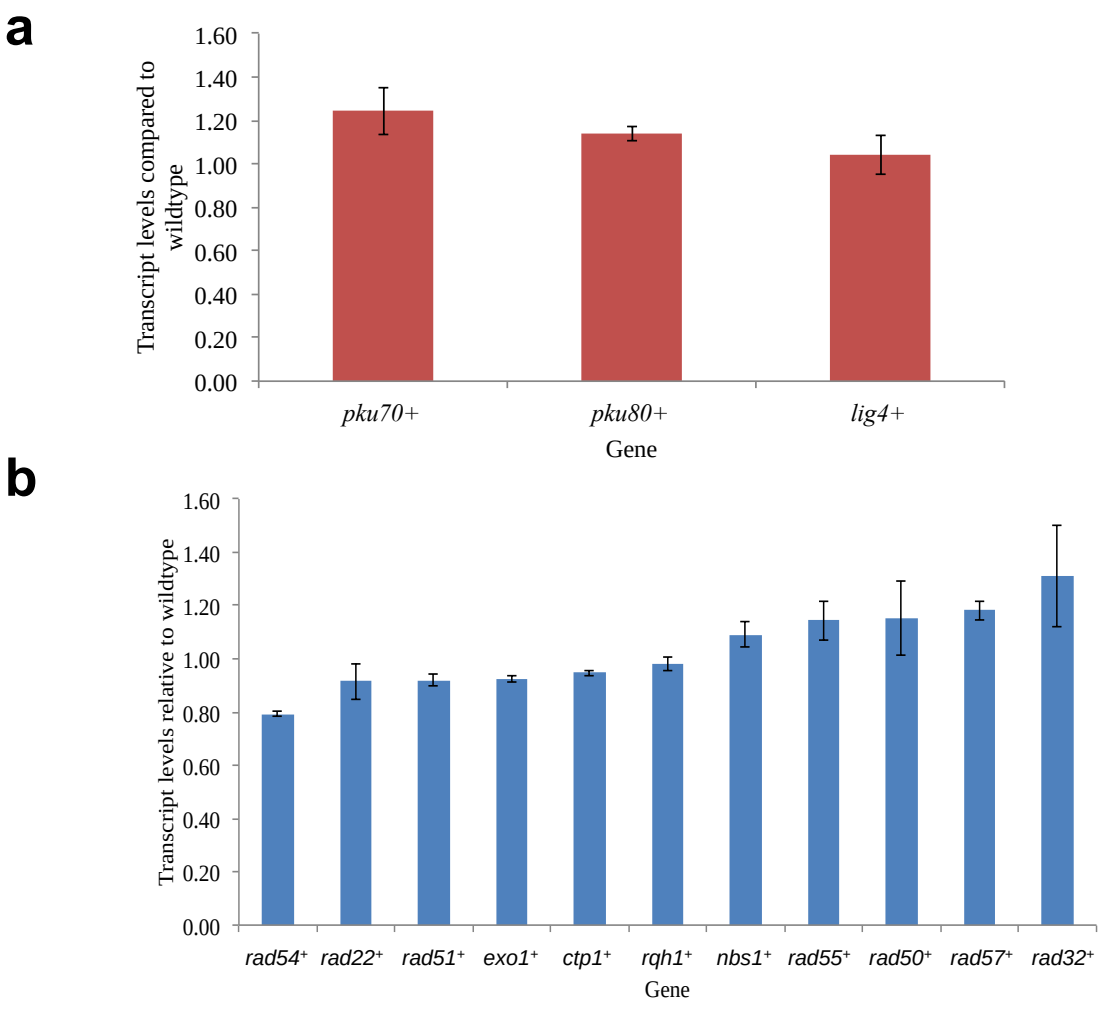

DSB assay repair outcomes
a
Parental strain undergoes HO-induced DSB induction
Ch16-RMGAH
(0.5Mb)
arg3
MATa
kanMX6
his3
ade6-216
Chr III
(3.5Mb)
ade6-210
(arg+ G418R ade+ his+)
b
Repair by NHEJ
arg3
MATa
kanMX6
his3
ade6-216
ade6-210
(arg+ G418R ade+ his+)
or
Repair by sister chromatid conversion (SCC) in S or G2
c
arg3
MATa
kanMX6
his3
ade6-216
ade6-210
(arg+ G418R ade+ his+)
or
Repair by interchromosomal gene conversion (GC)
d
arg3
kanMX6
his3
ade6-216
ade6-210
(arg+ G418S ade+ his+)
or
Failed repair (minichromosome loss)
e
ade6-210
(arg- G418S ade- his-)
or
Extensive loss of heterozygosity (LOH)
f
arg3
ade6-210
(arg+ G418S ade- his-)

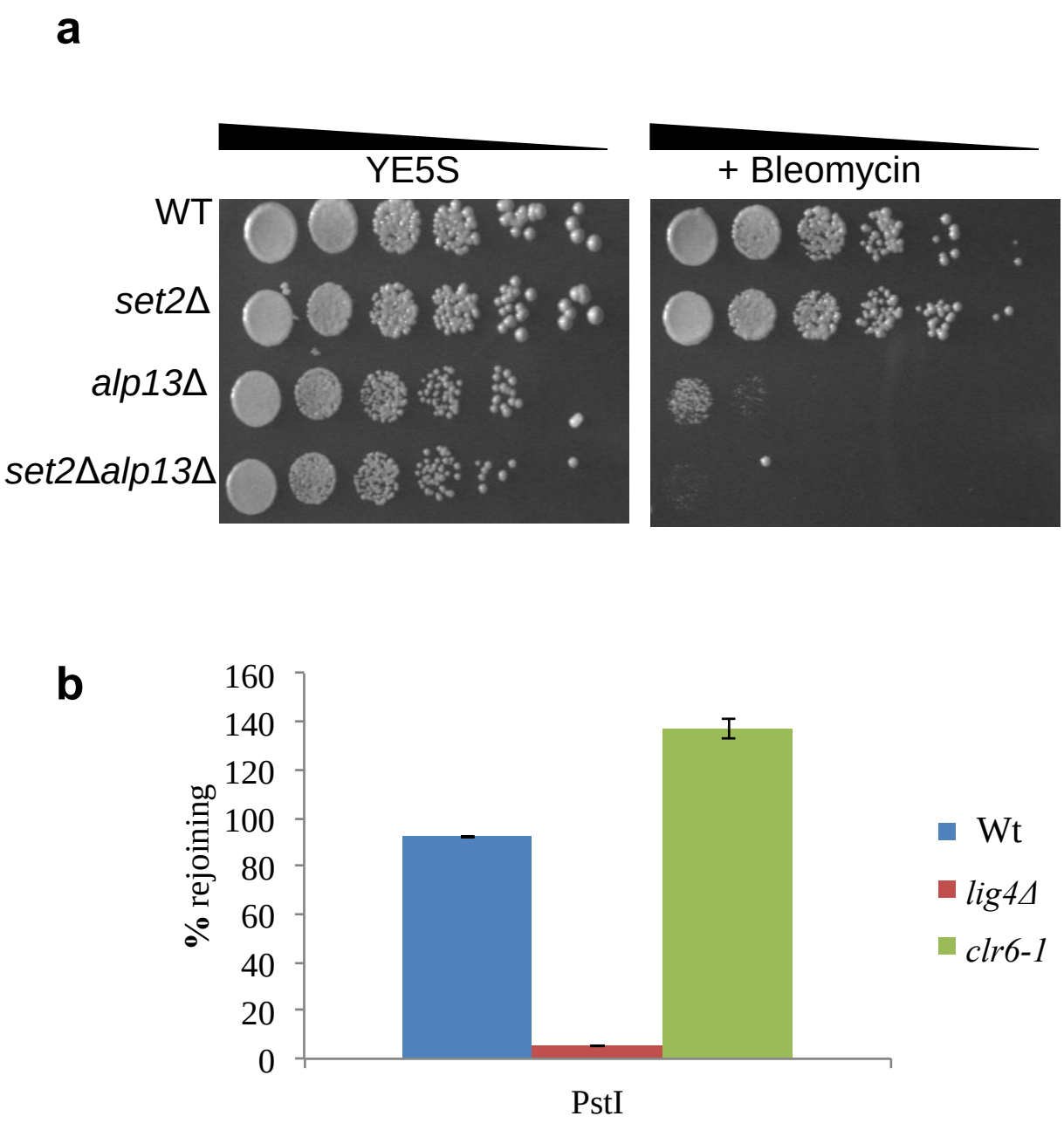
Supplementary Figure 1 DSB repair outcomes following a site specific DSB induction in Ch16-RMGAH.(**a**) Schematic of Ch^{16}-RMGAH. Ch^{16}-RMGAH, ChIII, centromeric regions (ovals), complementary heteroalleles (*ade6-M216* and *ade6-M210*; white), and the *his3* marker (white) was inserted ~50kb downstream from *ade6-M216*, as previously shown (Cullen et al., 2007)The *MATa* site (black) with an adjacent *kanMX6* resistance marker (grey) was inserted into *spcc23B6.06* ~ 30kb upstream from *ade6-M216*. The *arg3* marker was inserted into *spcc1795.09* on the left arm of the minichromosome. Derepression of pREP81X-HO (not shown) generates a DSB uniquely at the *MATa* target site (scissors). In Ch^{16}-RMYAH, *kanMX6* is replaced by *hph* (not shown). Consruction of Ch16-RMGAH and Ch16-RMYAH are as described (Tinline-Purvis et al., 2009) (**b**) Repair of HO-induced DSB by NHEJ results in retention of all markers resulting in an arg^+ $G418^R$/Hyg^R ade^+ his^+ phenotype as indicated. (**c**) DSB repair by sister chromatid conversion (SCC) during S or G2 phase, in which one of the two sister chromatids is intact, and used as a repair template results in retention of all markers, resulting in an arg^+ $G418^R$/Hyg^R ade^+ his^+ phenotype, as indicated. This is indistinguishable from NHEJ in a wild-type background. (**d**) DSB repair by interchromosomal gene conversion (GC) results in loss of the *KanMX* gene adjacent to the *MATa* break site while the other markers are retained resulting in an arg^+ $G418^S$/Hyg^S ade+ his^+ phenotype. (**e**) Failed DSB repair results in loss of the minichromosome and loss of all of the markers, resulting in an arg^- $G418^S$/Hyg^S ade- his^- phenotype, as indicated. (**f**). Extensive loss of heterozygosity (LOH) in which genetic material centromere-distal to the break-site is lost results in an arg^+ $G418^S$/Hyg^S ade- his^- phenotype, as indicated. LOH can result from cross-overs associated with gene conversion, break-induced replication, de novo telomere addition or isochromosome formation (Cullen et al., 2007; Tinline-Purvis et al., 2009).



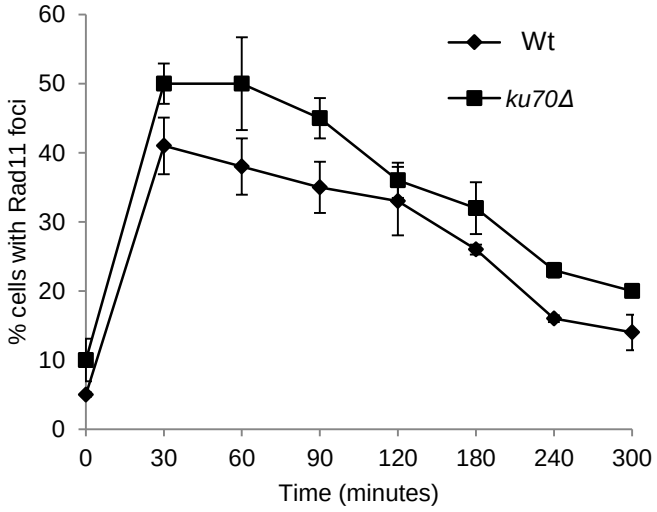
Supplementary Figure 2 Outcome of NHEJ plasmid-rejoining assay for wild type (Wt), *lig4*Δ and *set2-R255G* strains. Data are the mean of three experiments and error bars (± SE) are shown.



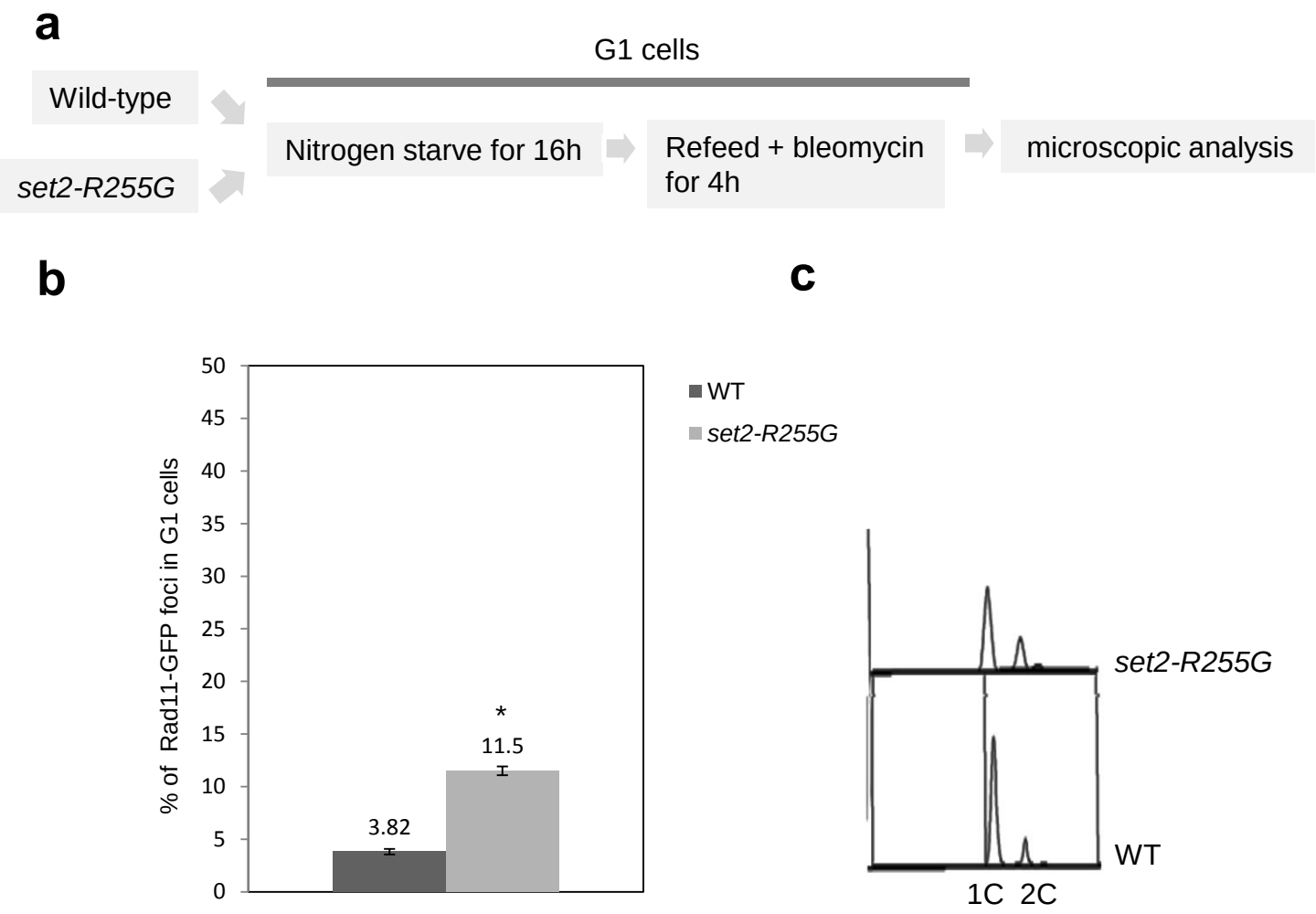
Supplementary Figure 3 HR and NHEJ gene expression is not significantly changed in *set2*Δ cells (**a**) NHEJ gene expression levels in *set2*Δ relative to wild type where the level in wild-type cells is 1.0. (**b**) HR gene expression levels in *set2*Δ cells relative to wild-type cells, where the level in wild-type cells is 1.0. Data represent the mean of two experiments with independently derived RNA and error bars are (±SE) are shown.



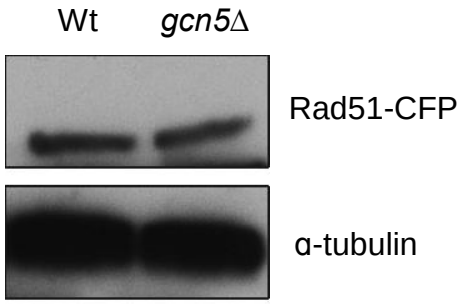
Supplementary Figure 4 The Clr6 HDAC complex is not required for the role of Set2 in NHEJ (**a**) 5-fold serial dilutions of wildtype, *alp13*Δ, *set2*Δ and *alp13*Δ *set2*Δ cells were grown on YE6S and YE6S + 1 µg/ml bleomycin. (**b**) Outcome of NHEJ plasmid-rejoining assay for *clr6-1* cells. Cells were grown at the restrictive temperature of 32°C. Data are the mean of three experiments and error bars (± SE) are shown.



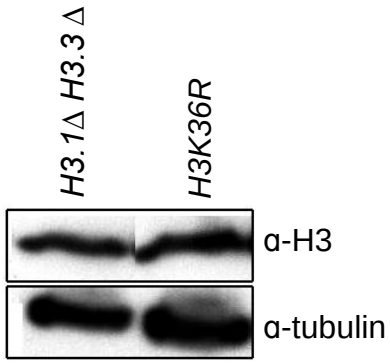
Supplementary Figure 5 Quantification of Rpa1(Rad11)-GFP foci in wild-type and *ku70*Δ strains strains following exposure to 50Gy IR treatment. Data are the mean of three experiments and error bars (± SE) are shown.



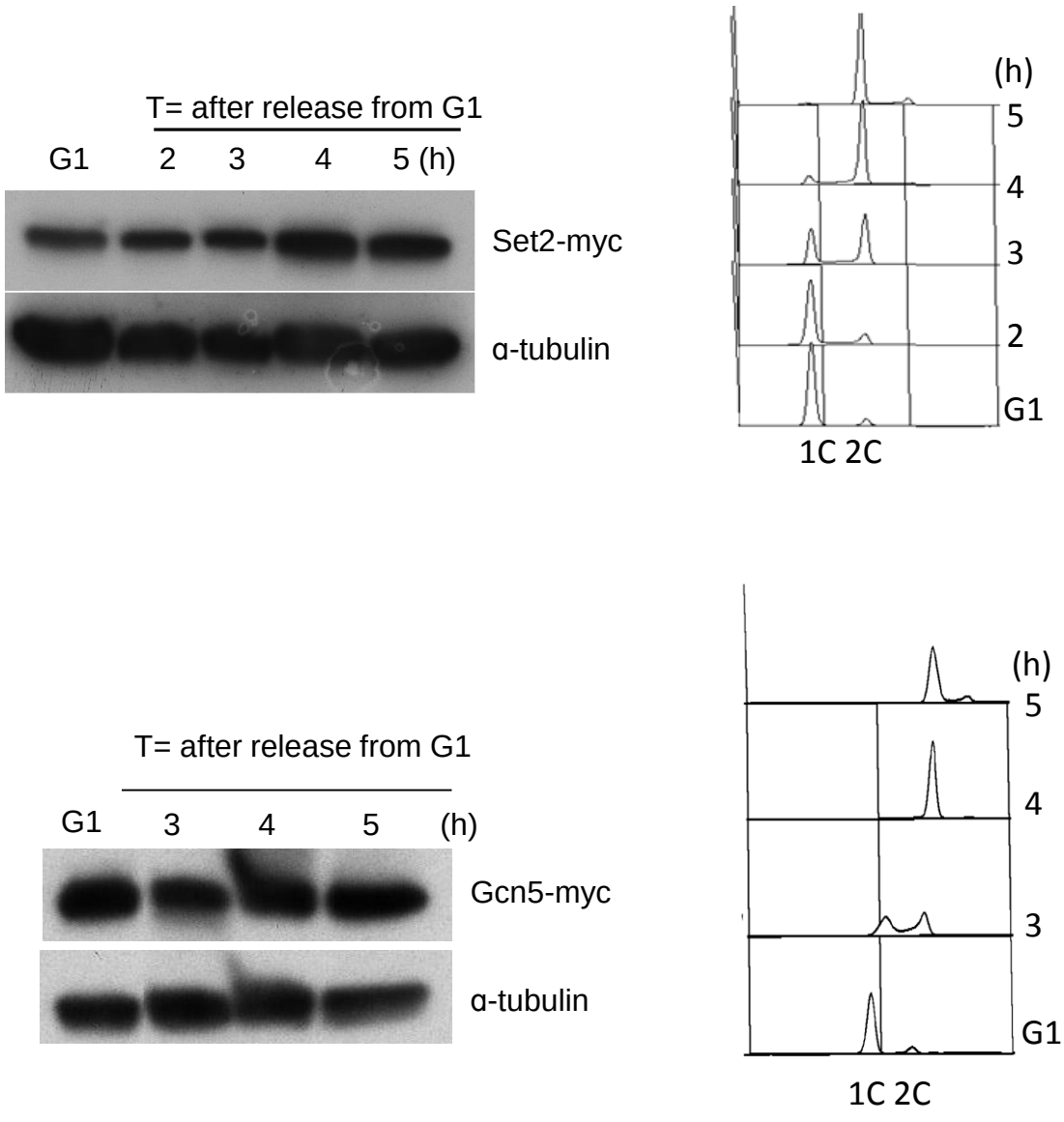
Supplementary Figure 6 Analysis of Rad11-GFP foci in G1 cells following DNA damage. (**a**) Cells were arrested in G1 and released into nitrogen-containing medium for 4h in the presence of 10ug/ml bleomycin (see schematic). (**b**) Quantification of Rad11-GFP foci from wild-type (WT) or *set2-R255G* cells. Star indicates significant difference (p=0.023 <0.05) (Student T-test). (**c**). FACS analysis of cells 4h after release from G1 and bleomycin treatment.



Supplementary Figure 7 Deletion of *gcn5*$^{+}$ does not affect Rad51-CFP expression. Western blot of Rad51-CFP and α-tubulin levels in wild-type (Wt) and *gcn5*Δ mutant

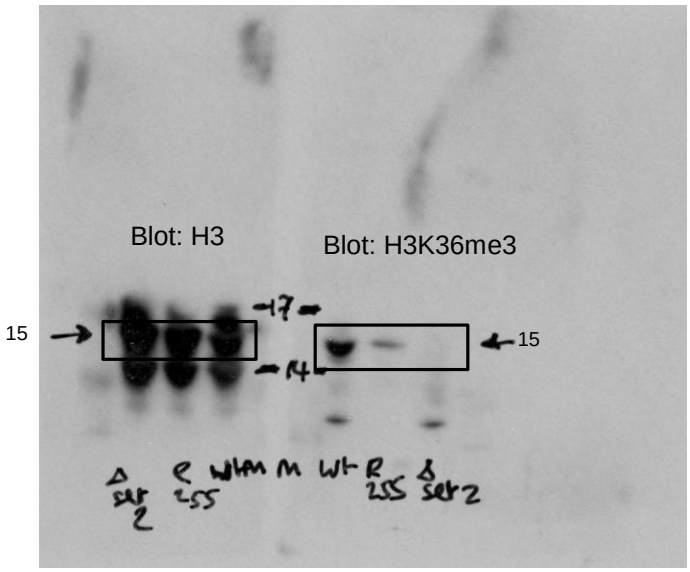


Supplementary Figure 8 Histone H3K36R mutation does not affect total histone H3 levels. Western blot of H3 and α-tubulin levels in *H3.1*Δ *H3.3*Δ and *H3.1*Δ *H3.3 H3K36R* (*H3K36R*) mutants.

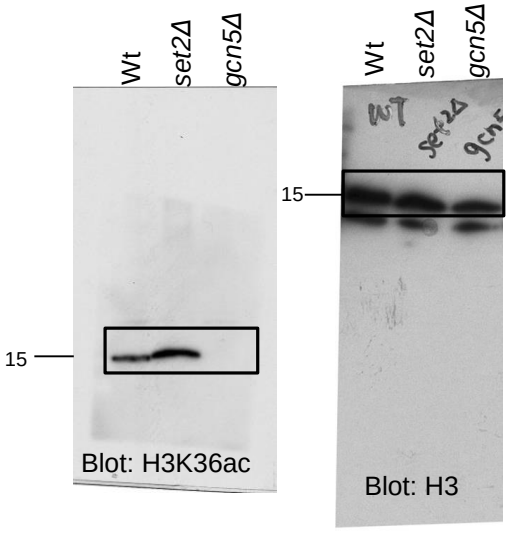


Supplementary Figure 9 Analysis of Set2 and Gcn5 levels across the cell cycle. Cells were arrested in G1 by nitrogen starvation, released and samples taken at time points indicated and subjected to Western blot and FACS analysis. Western blots were performed with anti-myc and anti α-tubulin antibodies as indicated.

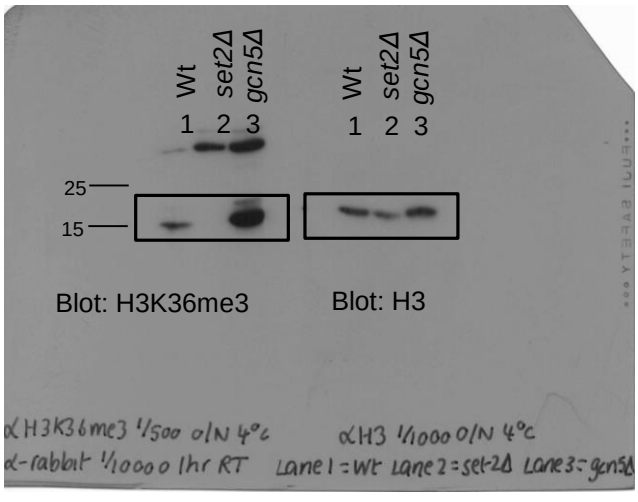
1d



3a

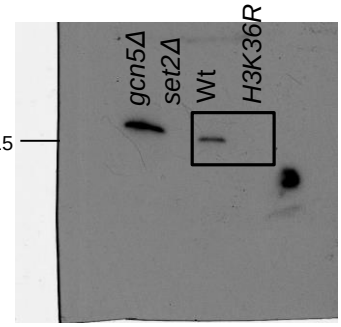


3b

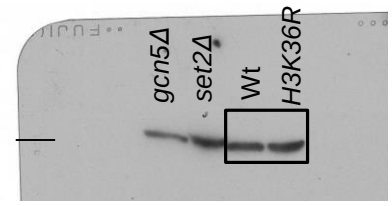


Supplementary Figure 10 Images of uncropped western blots used in the manuscript. The same protein extract was used to probe for H3 and H3K36me3 (1d); H3K36ac and H3 (3a) and H3K36me3 and H3 (3b). These data are each representative of more than three independent experiments.

4a

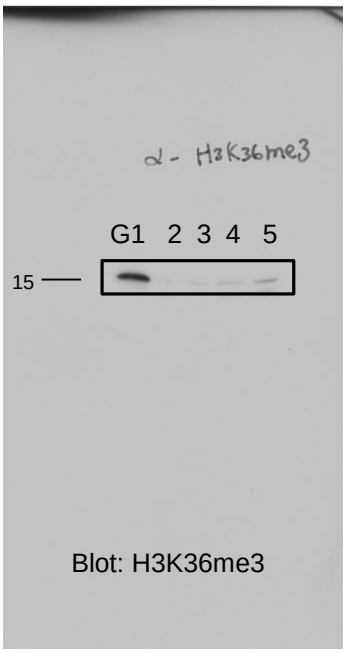


Blot: H3K36me3

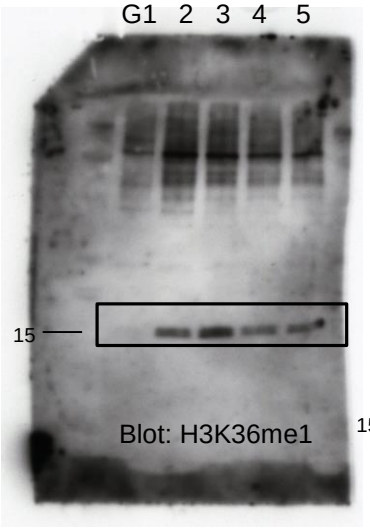


Blot: H3

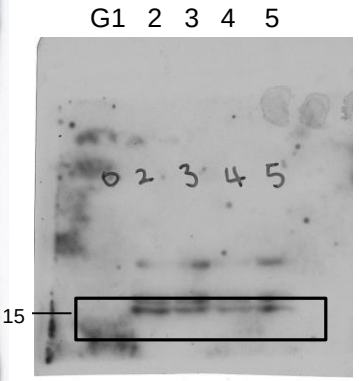
6b



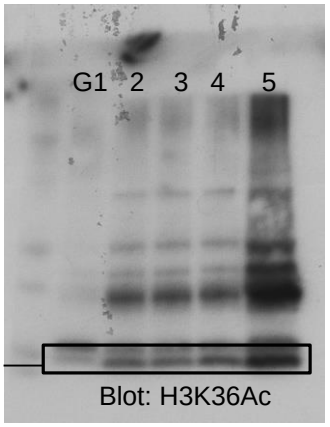
Blot: H3K36me3



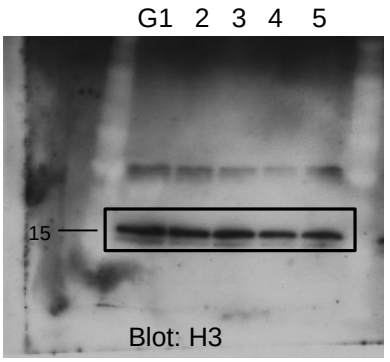
Blot: H3K36me1



Blot: H3K36me2



Blot: H3K36Ac



Blot: H3

Supplementary Figure 11 Images of uncropped western blots used in the manuscript. These western blots were all run from the same protein extract. These data are representative of more than three independent experiments.

Supplementary Table 1. Non-minichromosome strains used in this study

Strain	Genotype	Source
TH1069	*lig4::ura4 ade6-704 leu1-32 ura4-D18 h$^-$*	Tony Carr
TH2094	*arg3-D4 ade6-D1 ura4-D18 leu1-32 his3-D1 h$^-$*	Lab stock
TH3280	*gcn5::kanMX leu1-32 ura4-D18 ade6-M210 h$^-$*	Robin Allshire
TH5591	*alp13::kanMX ade6-210 arg3-D4 his3-D1 leu1-32 ura4-D18 h$^+$*	Shiv Grewal
TH6238	*H3.1/H4.1::his3 H3.3/H4.3::arg3 ura4-DS/E ade6-M210 leu1-32 his3-D1 arg3-D4 h$^-$*	Robin Allshire
TH6241	*H3.2K36R H3.1/H4.1::his3 H3.3/H4.3::arg3 ura4-DS/E ade6-M210 leu1-32 his3-D1 arg3-D4 h$^-$*	Robin Allshire
TH6874	*ade6-210 leu1-32 ura4-D18 h$^-$*	Lab stock
TH6237	*set2::kanMX ade6-210 leu1-32 ura4-D18 h$^-$*	Robin Allshire
TH6340	*set2::set2-R255G-ura4 ade6-D1 ura4-D18 leu1-32 his3-D1 h$^-$*	This study
TH6256	*urg1::urg1P-HO LE_L-HOcs-his3+-EU_R his3-D1 leu1-32 h$^-$*	Tony Carr
TH6367	*set2::set2-R255G-ura4 ade6-D1 ura4-D18 leu1-32 his3-D1arg3-D4 h$^+$*	This atudy
TH6318	*set2:ura4 gcn5::kanMX ade6-210 arg3-D4 his3-D1 leu1-32 ura4-D18 h$^+$*	This study
TH6525	*set2::ura4 alp13::kanMX ade6-210 arg3-D4 his3-D1 leu1-32 ura4-D18 h$^-$*	This study
TH7289-90	*gcn5::kanMX rad51::rad51-eCFP-ura4 ade6-M210 leu1-32 ura4-D18 his3-D1 h$^+$ /h$^+$*	This study
TH7396	*rad11::rad11-GFP-kanMX set2::set2-R255G-ura4 ade6-D1 leu1-32 his3-D1*	This study
TH8095	*set2::ura4 urg1P-HO LE_L-HOcs-his3+-EU_R his3-D1, ura4-D18, leu1-32*	This study
TH8443	*pku80-13myc-kanR, urg1::urg1P-HO LE_L-HOcs-his3+-EU_R his3-D1 leu1-32*	This study
TH8447	*set2::KanMX, pku80-13myc-kanR, urg1::urg1P-HO LE_L-HOcs-his3+-EU_R his3-D1 leu1-32*	This study

Supplementary Table 2. Minichromosome containing strains used in this study

Strain	Genotype	Parent strain	pREP81X-HO	pREP81X
TH2125/6	*leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMGAH	-	TH2130-2	TH2357
TH2654/3165	*leu1-32 arg3-D4 ade6-M210 ura4-D18 his3-D1* Ch^{16}-RMYAH	-	TH4104, 4121-2 (6815 pREP82X-HO)	TH4125
TH3779-81	*set2::ura4 ade6-210 arg3-D4 his3-D1 leu1-32 ura4-D18* Ch^{16}-RMGAH	TH3271	TH3841-3	TH4249
TH3785	*gcn5::kanMX ade6-210 arg3-D4 his3-D1 leu1-32 ura4-D18* Ch^{16}-RMYAH	TH3280	TH3864, 6338-9	TH3844
TH6380-2	*set2::set2-R255G-ura4 ade6-D1 ura4-D18 leu1-32 his3-D1 arg3-D4* Ch^{16}-RMGAH	TH6367	TH6415-17	TH6398
TH6512-3	*set2::ura4 gcn5::kanMX ade6-210 arg3-D4 his3-D1 leu1-32 ura4-D18* Ch^{16}-RMYAH	TH6318	TH6545-7	TH6548
TH6947-9	*gcn5::kanMX lig4::ura4 ade6-210 arg3-D4 his3-D1 leu1-32 ura4-D18* Ch^{16}-RMYAH	TH3785, 3444	TH6973-6	TH6978

Supplementary Table 3: qPCR primers used in this study

50bp on LHS of HO break	LS242 (HOhis3_50bp L_F1):CCCTGTAGAGAAATATAAAAGGTTAGGA LS243 (HOhis3_50bp-L_R1):GATGTGAGAACTGTATCCTAGCAAGATT
act1	qAct1-for:GGTTTCGCTGGAGATGATG qAct1-rev:ATACCACGCTTGCTTTGAG
fbp1	qFbp1-fwd:AAGGCGATATTAGCGATGTC qFbp1-rev:CAGTGTCCAAGGTGAAGC

SUPPLEMENTARY REFERENCES

1. Cullen, J.K. et al. Break-induced loss of heterozygosity in fission yeast: dual roles for homologous recombination in promoting translocations and preventing de novo telomere addition. *Mol. Cell. Biol.* **27**, 7745-57 (2007).
2. Tinline-Purvis, H. et al. Failed gene conversion leads to extensive end processing and chromosomal rearrangements in fission yeast. *EMBO J* **28**, 3400-12 (2009).