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Supplemental Information

Rad52 Restrains Resection at DNA Double-Strand Break Ends in Yeast

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Supplemental Information

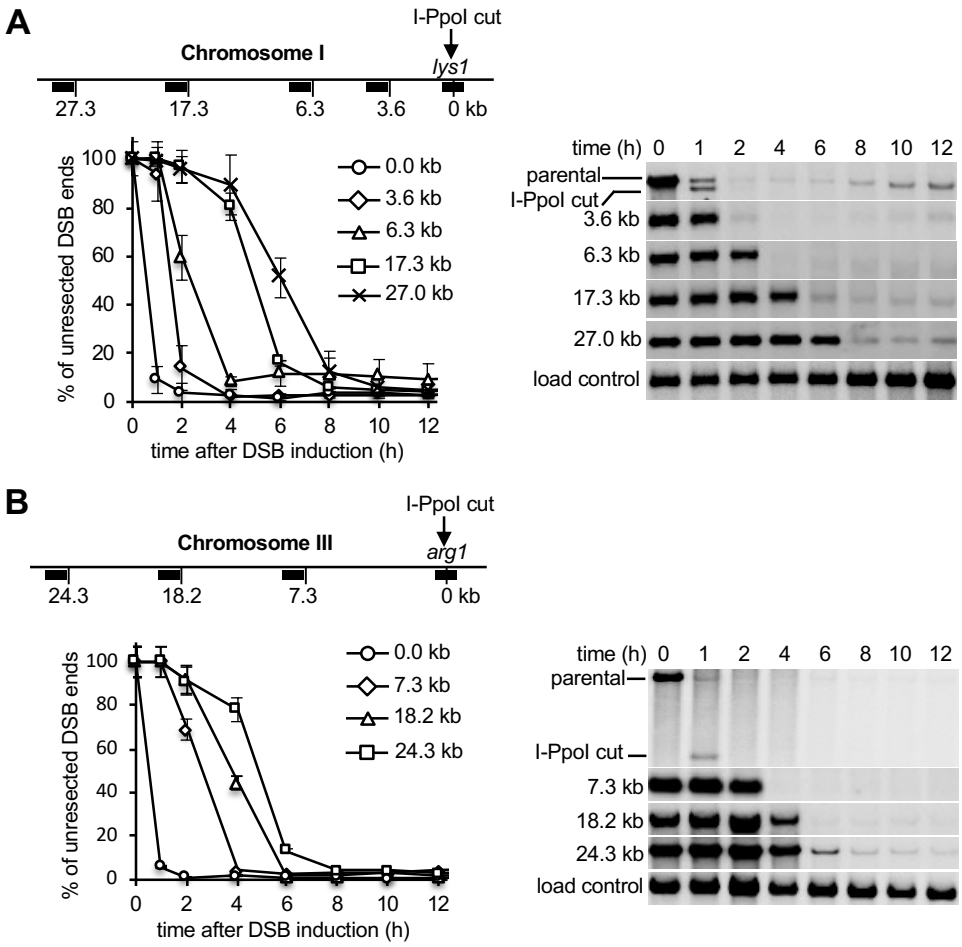


Figure S1. Measurement of DSB ends resection at *lys1* and *arg1* loci. Related to Figure 1. Schematic of I-*Ppo*I cleavage sites and the position of probes used to follow resection at different distances from a DSB at *lys1* (**A**) and *arg1* (**B**) loci. Southern blot analysis and kinetics of resection are shown. Primers used to prepare DNA probes for Southern blotting and restriction enzymes used to digest genomic DNA are presented in Table S3. Error bars denote SD (n=3).

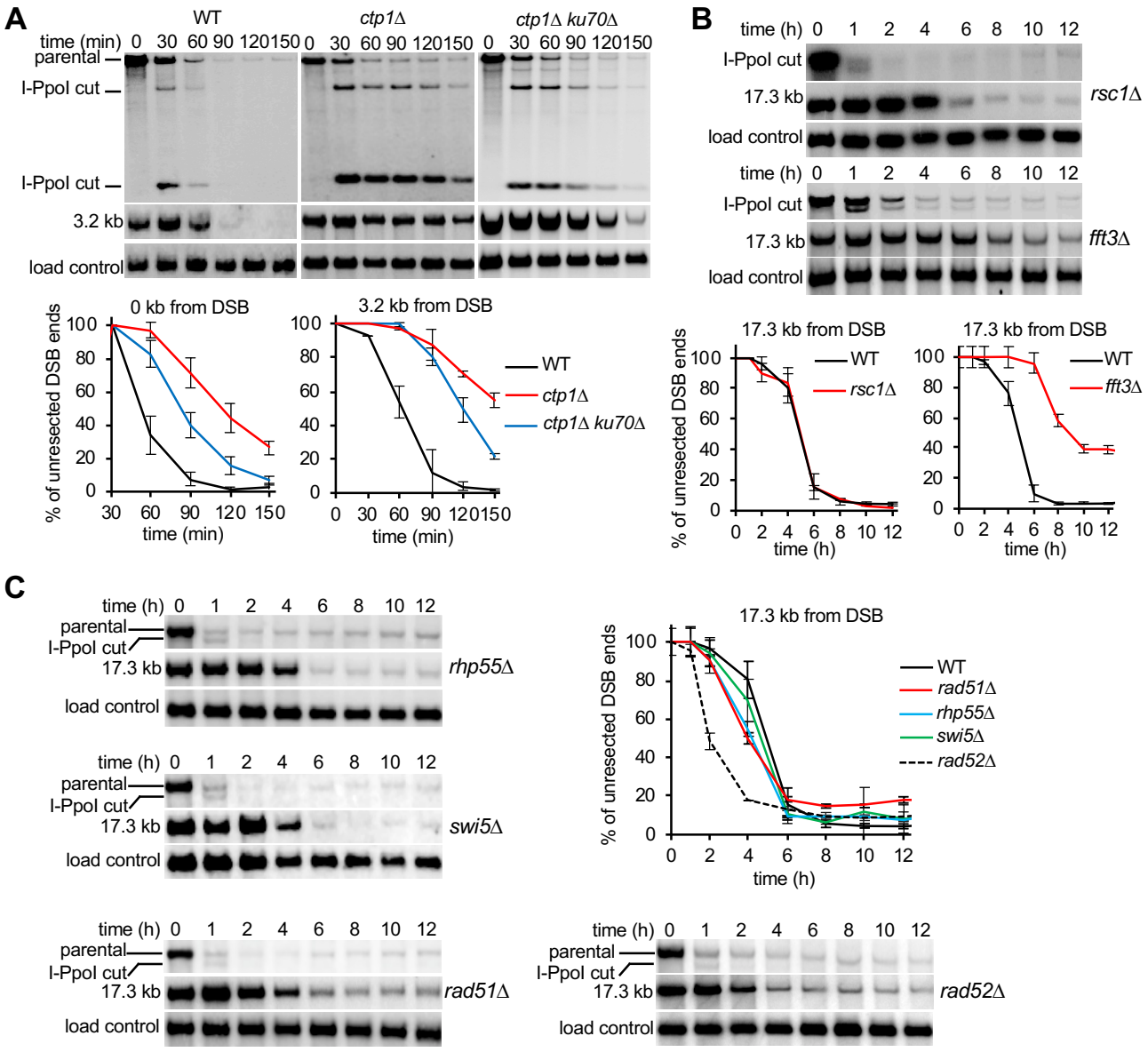


Figure S2. Analysis of resection in cells deficient in chromatin remodeling factors, mediators of Rad51 loading, Rad51 and Ctp1. Related to Figure 1 and 2. (A-C) Southern blot analysis and quantification of initial and extensive resection in indicated mutants in fission yeast at *lys1* locus. Error bars denote SD (n=3).

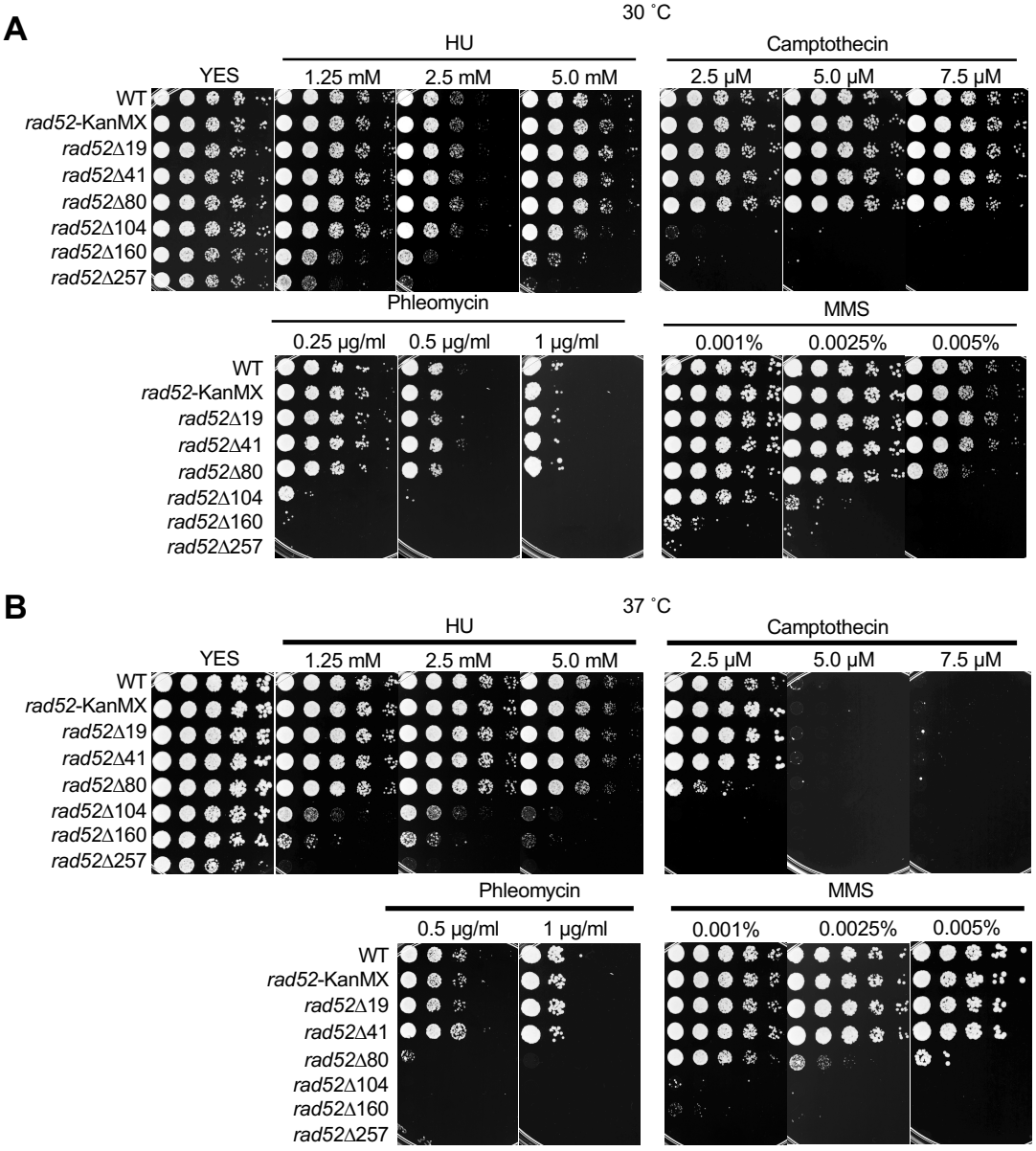


Figure S3. Analysis of DNA damage sensitivity of Rad52 mutants. Related to Figure 3. Spot assay analysis of DNA damage sensitivity of indicated Rad52 mutants at 30 °C **(A)** or 37 °C **(B)**. All truncated rad52 mutants carry *Kan*MX marker after stop codon. rad52-*Kan*MX denotes the wild type rad52 gene followed by *Kan*MX gene. Fivefold serial dilutions were made, and 2 μl were spotted onto YES or YES with hydroxyurea (HU), camptothecin (CPT), methyl methanesulfonate (MMS), or phleomycin.

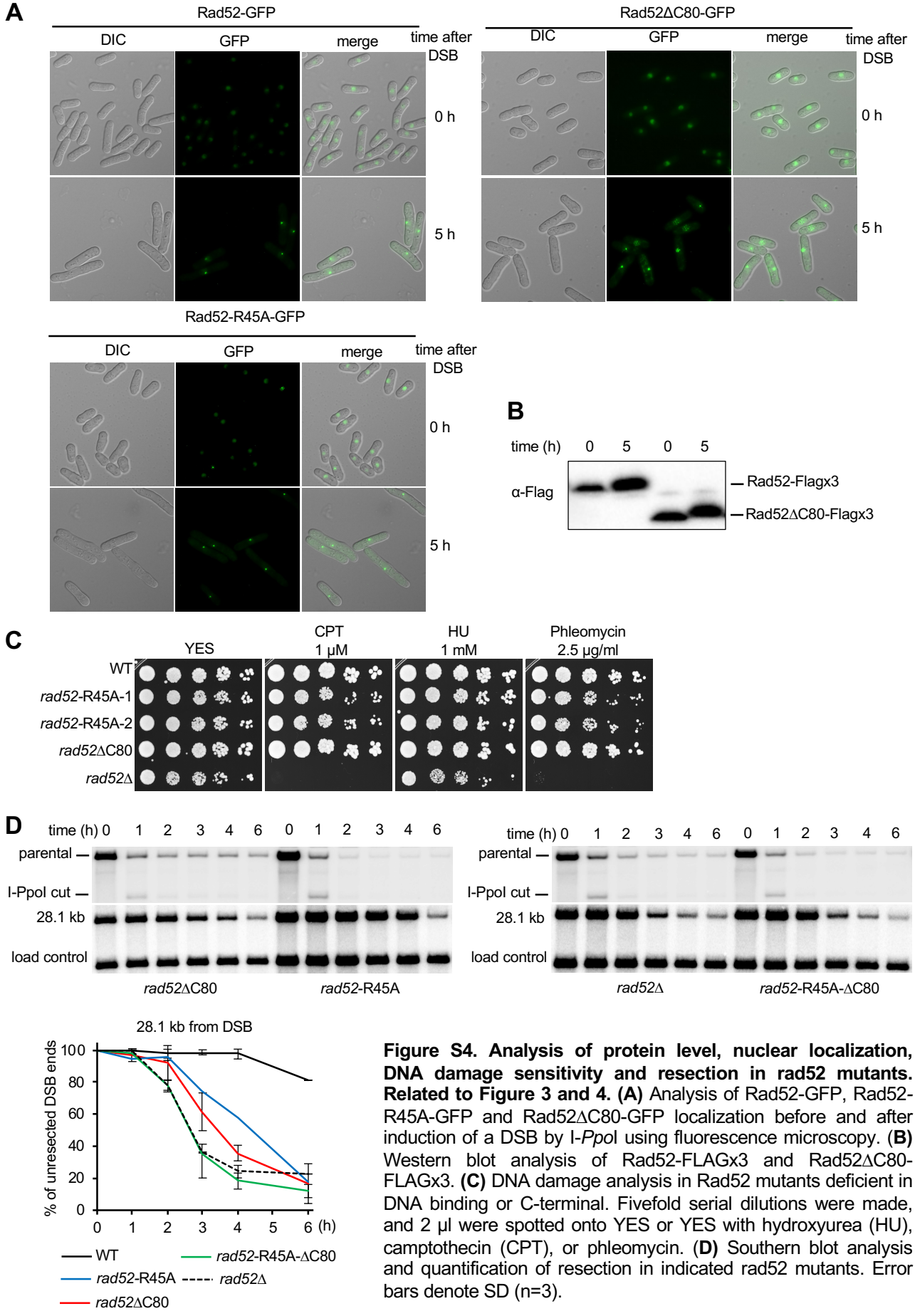


Figure S4. Analysis of protein level, nuclear localization, DNA damage sensitivity and resection in rad52 mutants. Related to Figure 3 and 4. (A) Analysis of Rad52-GFP, Rad52-R45A-GFP and Rad52ΔC80-GFP localization before and after induction of a DSB by I-*Ppo*I using fluorescence microscopy. **(B)** Western blot analysis of Rad52-FLAGx3 and Rad52ΔC80-FLAGx3. **(C)** DNA damage analysis in Rad52 mutants deficient in DNA binding or C-terminal. Fivefold serial dilutions were made, and 2 μl were spotted onto YES or YES with hydroxyurea (HU), camptothecin (CPT), or phleomycin. **(D)** Southern blot analysis and quantification of resection in indicated rad52 mutants. Error bars denote SD (n=3).

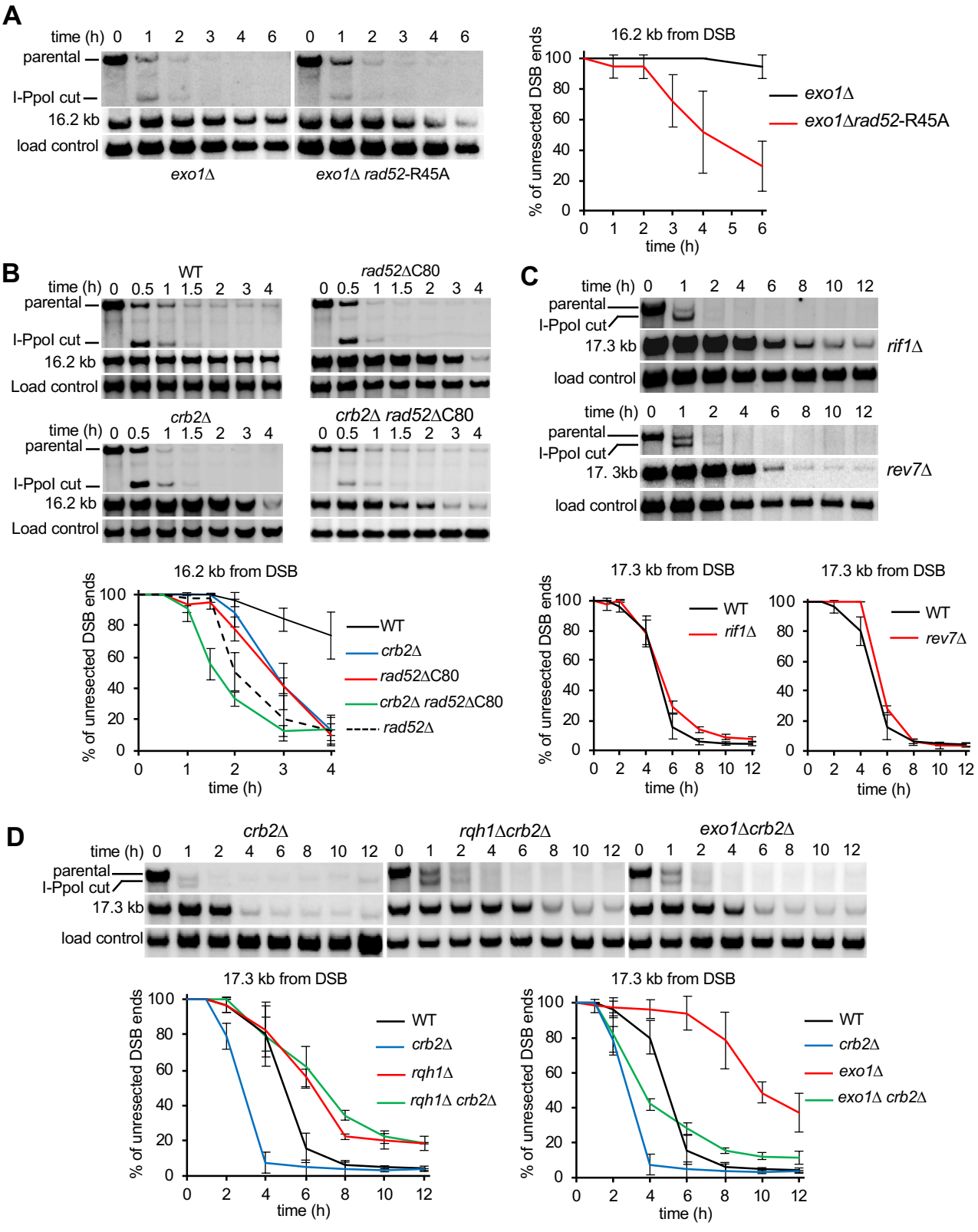


Figure S5. Epistasis analysis between Rad52 and Crb2, between Crb2 and major resection enzymes and analysis of resection in *rif1* and *rev7* mutants. Related to Figure 5. Southern blot analysis and quantification of resection in indicated mutants at *lys1* locus in fission yeast. Error bars denote SD (n=3).

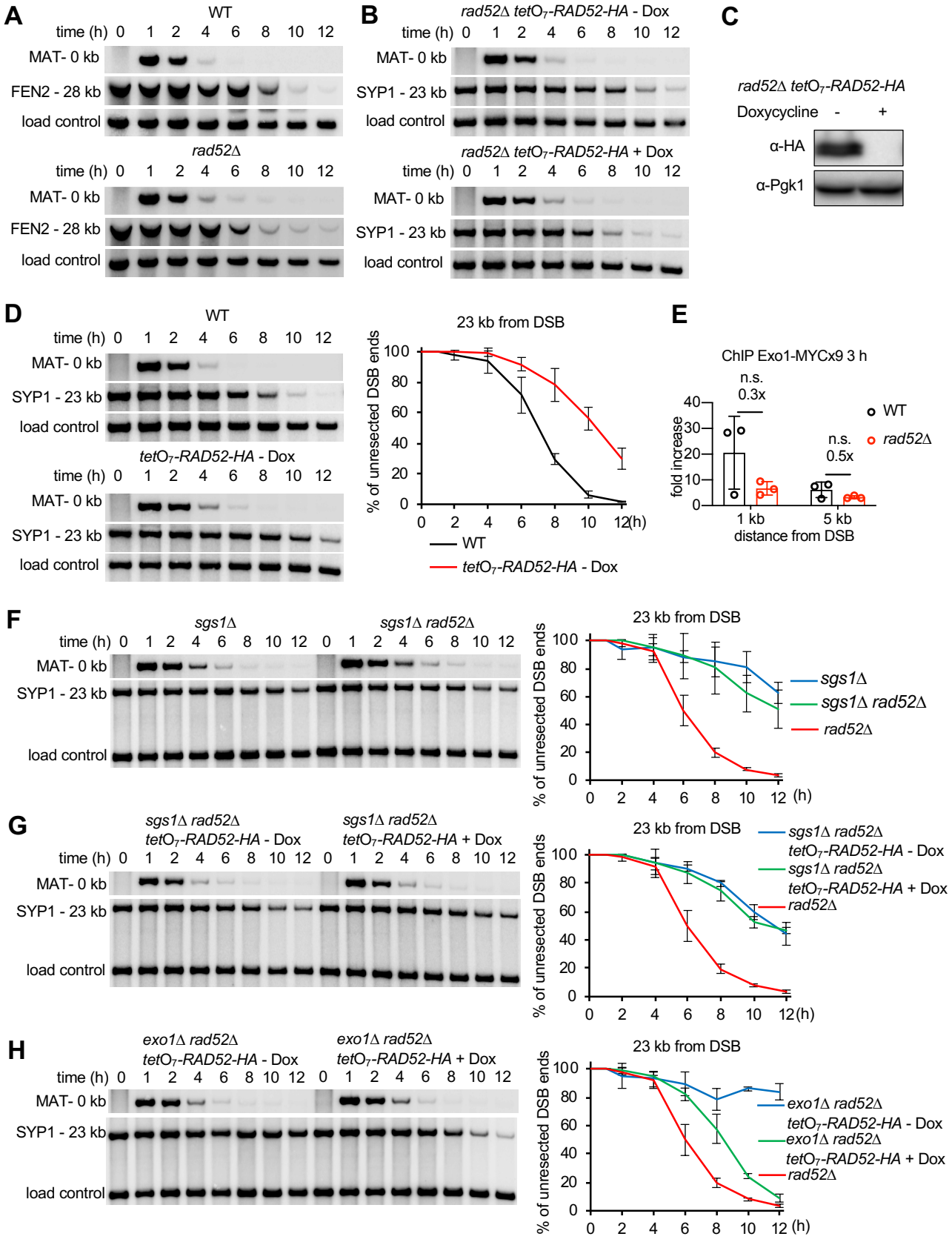
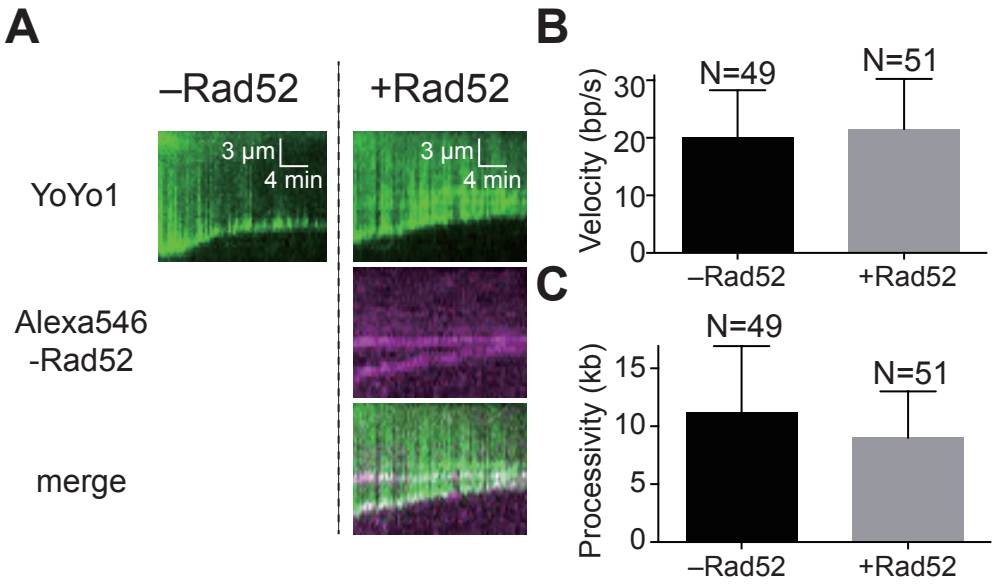


Figure S6. Analysis of resection in budding yeast cells that lack Rad52, overexpress Rad52 and analysis of Exo1 recruitment to DSB ends. Related to Figure 6. (**A,B,D,F,G,H)** Southern blot analysis and quantification of resection in indicated mutants at *MAT* locus in budding yeast. Error bars denote SD (n=3). **(C)** Western blot analysis of Rad52 protein level. **(E)** ChIP-qPCR analysis of Exo1 recruitment to DSB ends at *MAT* locus. Error bars denote SD (n=3).



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Exo1 velocity

–Rad52	20.0 ± 8.2 bp/sec (N=49)	p=0.39
+1nM Rad52	21.5 ± 8.7 bp/sec (N=51)	

Exo1 processivity

–Rad52	11.1 ± 5.7 kb (N=49)	p=0.0293
+1nM Rad52	9.0 ± 4.0 kb (N=51)	

Sgs1 velocity

–Rad52	83 ± 38 bp/sec (N=104)	p<0.0001
+1nM Rad52	38 ± 38 bp/sec (N=102)	

Sgs1 processivity

–Rad52	10.2 ± 4.9 kb (N=104)	p=0.0015
+1nM Rad52	8.1 ± 4.4 kb (N=102)	

Figure S7. Comparison of Rad52 effect on Exo1- and Sgs1-mediated end resection assays. Related to Figure 6 and 7. (A) Kymographs showing the resection of YOYO1-stained stained dsDNA (green) by Exo1 in the absence (right) and presence of 1 nM Alex546-Rad52 (magenta; left); note, we are unable to look at higher concentrations (i.e. 4 nM) of Rad52 in these assays, because the combined presence of Rad52 and YOYO1 causes extensive compaction of the DNA substrates (for unknown reasons), which is not seen with Rad52 alone. (**B)** Velocity distribution of Exo1 DNA end resection with or without 1 nM Alexa546-Rad52. Error bars represent 68% confidence intervals. (**C)** Processivity of Exo1 DNA end resection with or without 1 nM Alexa546-Rad52. Error bars represent 68% confidence intervals. (**D)** Velocity and processivity comparison of the effect of Rad52 on Exo1- and Sgs1-mediated DNA end resection. Values for the Sgs1-mediated end resection correspond to experiments and data presented in Figure 7A-C.

Supplemental Tables

Table S1. List of fission yeast strains used in the study. Related to STAR methods.

Strain name	Parental strain	Genotype	Source
ySK103		*leu1-32: :pDUAL-TETp-I-PpoI ura4-D18 rDNA-I-Ppolmt ade6-216*	this study
ySK113		*leu1-32: :pDUAL-TETp-I-PpoI ura4-D18 rDNA-I-Ppolmt ade6-216 lys1::I-PpoI^{CS}-hph*	this study
ySK121	ySK103	*leu1-32: :pDUAL-TETp-I-PpoI ura4-D18 rDNA-I-Ppolmt ade6-216 arg1::λ1-I-PpoI^{CS}-λ2-hph*	this study
ySK122	ySK113	*leu1-32: :pDUAL-TETp-I-PpoI ura4-D18 rDNA-I-Ppolmt ade6-216 lys1::I-PpoI^{CS}-hph IMR-L::ura4*	this study
ySK124	ySK122	*rad52::KanMX6*	this study
ySK128	ySK122	*exo1::KanMX6*	this study
ySK131	ySK122	*rqh1::NatMX4*	this study
ySK132	ySK128	*exo1::KanMX6 rqh1::NatMX4*	this study
ySK134	ySK122	*rqh1-3xFlag::KanMX6*	this study
ySK135	ySK122	*exo1-3xFlag::KanMX6*	this study
ySK137	ySK122	*crb2::KanMX6*	this study
ySK139	ySK122	*rif1::KanMX6*	this study
ySK142	ySK131	*crb2::KanMX6 rqh1::NatMX4*	this study
ySK143	ySK122	*rsc1::KanMX6*	this study
ySK145	ySK128	*crb2::NatMX6 exo1::KanMX6*	this study
ySK152	ySK122	*ssb1-3xFlag::KanMX6*	this study
ySK157	ySK122	*fft3::KanMX6*	this study
ySK170	ySK122	*mre11::KanMX6*	this study
ySK180	ySK122	*rad51::KanMX6*	this study
ySK187	ySK121	*rad52::KanMX6*	this study
ySK192	ySK122	*ctp1::KanMX6*	this study
ySK194	ySK122	*rad52::212Stop-KanMX6*	this study
ySK212	ySK122	*rad52::309Stop-KanMX6*	this study
ySK213	ySK122	*rad52::389Stop-KanMX6*	this study
ySK214	ySK122	*rad52::428Stop-KanMX6*	this study
ySK220	ySK122	*rad52::450Stop-KanMX6*	this study
ySK221	ySK213	*rad52::389Stop-KanMX6 rqh1::NatMX4*	this study
ySK224	ySK213	*rad52::389Stop-KanMX6 exo1::NatMX4*	this study
ySK232	ySK122	*rad52-GFP::KanMX6*	this study
ySK235	ySK122	*rad52::389Stop-GFP::KanMX6*	this study
ySK238	ySK122	*rad52::469Stop-KanMX6*	this study

ySK249	ySK122	*rhp55::KanMX6*	this study
ySK250	ySK122	*swi5::KanMX6*	this study
ySK251	ySK122	*rev7::KanMX6*	this study
ySK252	ySK134	*rqh1-3xFlag::KanMX6 rad52::NatMX4*	this study
ySK253	ySK135	*exo1-3xFlag::KanMX6 rad52::NatMX4*	this study
ySK258	ySK122	*rad52::365Stop-KanMX6*	this study
yZY002	ySK181	*Ku70::KanMX6 ctp1::NatMX4*	this study
yZY003	ySK121	*λ2-ura4 (21 kb upstream of arg1)*	this study
yZY006	yZY003	*rad52::389Stop-KanMX6 λ2-ura4 (21 kb upstream of arg1)*	this study
yZY025	yZY003	*rad52-R45A::KanMX6 λ2-ura4 (21 kb upstream of arg1)*	this study
yZY031	yZY003	*rad51::NatMX4 λ2-ura4 (21 kb upstream of arg1)*	this study
yZY032	yZY006	*rad51::NatMX4 rad52::389Stop-KanMX6 λ2-ura4 (21 kb upstream of arg1)*	this study
yZY036	yZY025	*rad51::NatMX4 rad52-R45A::KanMX6 λ2-ura4 (21 kb upstream of arg1)*	this study
yZY026	ySK122	*rad52-R45A::KanMX6*	this study
yZY040	ySK122	*nmt1-rad52*	this study
yZY043	ySK122	*rad52-3xFlag::KanMX6*	this study
yZY041	ySK122	*rad52::389Stop-NatMX4*	this study
yZY044	yZY041	*rad52ΔC80-3xFlag::KanMX6*	this study
yZY051	yZY026	*exo1::NatMX4 rad52-R45A::KanMX6*	this study
yZY053	yZY026	*rad52-R45A::NatMX4*	this study
yZY055	yZY053	*rad52-R45A-3xFlag::KanMX6*	this study
yZY057	yZY026	*rad52-R45A::389Stop::NatMX4*	this study

Table S2. List of budding yeast strains used in the study. Related to STAR methods.

Strain name	Parental strain	Genotype	Source
JKM139		*MATa ho hml::ADE1 hmr::ADE1 ade1-100 leu2-3,112 lys5 trp1::hisG ura3-52 lys5 ade3::GAL10::HO*	(Lee et al., 1998)
yAP485	JKM139	*rad52::KanMX*	this study
yYY478	yAP485	*rad52::KanMX tetO$_7$::RAD52-HA::URA3*	this study
yYY475	JKM139	*tetO$_7$::RAD52-HA::URA3*	this study
yZZ203	JKM139	*SGS1*-9xMyc-*TRP1*	(Zhu et al, 2008)
yZY058	yZZ203	*SGS1*-9xMyc-*TRP1 rad52::KanMX*	this study
yZZ042	JKM139	*EXO1*-9xMyc-*TRP1*	(Chen et al, 2012)
yZY059	yZZ042	*EXO1*-9xMyc-*TRP1 rad52::KanMX*	this study
yGI200	JKM139	*sgs1::KanMX*	(Zhu et al, 2008)
yZY064	yGI200	*sgs1::KanMX rad52::TRP1*	this study

yZY065	yYY478	*exo1::TRP1 rad52::KanMX tetO$_7$::RAD52-HA::URA3*	this study
yZY069	yZY064	*sgs1::KanMX rad52::TRP1 tetO$_7$::RAD52-HA::URA3*	this study

Table S3. Primers used to prepare DNA probes for Southern blotting and restriction enzymes used to digest genomic DNA in fission yeast. Related to STAR methods.

Primer name	Restriction enzyme(s)	Sequence
lys1_0kb	*Hpa*I/*Stu*I or *Eco*RI	5'- CAGAACAGCGGGCAGTTCGG-3' 5'- GGTTGTAACGAGACTTGGGGTAAGG-3'
lys1_3.6kb_*Hpa*I/*Stu*I (3.2 kb for *Eco*RI)	*Hpa*I/*Stu*I or *Eco*RI	5'- CCATGTTTAGAATGTGAACGAGATGC-3' 5'- GGACGTGGCATGAAGAATGTC-3'
lys1_6.3 kb_*Hpa*I/*Stu*I	*Hpa*I/*Stu*I	5'- GTAGCTCTCAGTATAGTCATTTGATTGC-3' 5'- CATTGGGAAGAGCGCTTAGTC-3'
lys1_17.3 kb_*Hpa*I/*Stu*I (16.2 kb for *Eco*RI)	*Hpa*I/*Stu*I or *Eco*RI	5'- GTATACGTTACTTGACATGTGCACACC-3' 5'- CGAGCTAAAATACTTAGTGCCTCTGGAC-3'
lys1_27.0kb_*Hpa*I/*Stu*I (28.1kb for *Eco*RI)	*Hpa*I/*Stu*I or *Eco*RI	5'- CCTACGCAACTGAGTCTAATAAGTC-3' 5'- CCTTTGCTCCAGTTTGTTAAGC-3'
lys1_58.6 kb	*Eco*RI	5'- CTCTTGGCTTTTTGCGTAAAGGAAGCTC-3' 5'- CGATTCACAACCATTGAAGGTCTTCTAGC-3'
lys1_load_control _*Hpa*I/*Stu*I	*Hpa*I/*Stu*I	5'- CGTGAGGTTGCTAGGAGAATCAAACTTC-3' 5'- CTCTTCAAGCTCACCACTCCCG-3'
lys1_load_control _*Eco*RI	*Eco*RI	5'- GGACTTCAAGGGCCCGTTAAC-3' 5'- CGTGGCAGCTTATAGTTGTGAAGGAG-3'
arg1_0kb	*Eco*RI	5'- GCGTGGCGTTAAGTGGTCAG-3' 5'- AAGATCTCAATCCCCGCCAGATGATAAG-3'
arg1_7.3kb	*Eco*RI	5'- CTGGAGGTGGAAGCGTGCC-3' 5'- GCTCGAATTCTCTCGTCGAAAAGGC-3'
arg1_18.2kb	*Eco*RI	5'- CATGCAAAAGTAGTTGGTTTTCAAAGGTAC-3' 5'- GATTGCCGGCCGTATCGTCTTG-3'
arg1_24.3kb	*Eco*RI	5'-CCCGTTGCTTGGTGTCATG-3' 5'- GCTGCGGAAGTTCACTGGATG-3'
arg1_load_control	*Eco*RI	5'- GATGAACCTATTTCTCACGAACAGGAGG-3' 5'- CGTCGTTTAGTTCATCTCCGGCTTC-3'
SSA_lambda	*Bsp*HI	5'- CATTGAGCAGTGCAGCGAAC-3' 5'- CCCGCCAGATGATAAGCATC-3'

Table S4. Primers used to prepare DNA probes for Southern blotting in budding yeast. Related to STAR methods.

Primer name	Sequence
MAT_0kb	5'-CTTGATTGTTTGCTTGAGTCTG-3' 5'-ACTAACAATACTTCAGTTTA-3'
FEN2_28kb	5'-CACCAATGCATATATATCCG-3' 5'-GAATAGTCGACCAGTCTAAC-3'
SYP1_23kb	5'-GAGTGCATTCAGATTCAACTC-3' 5'-CGAAGCATTATTAGGAACTAC-3'
HO_load_control	5'GTCCTAATACGACTTTTCAAATTGTCCTTTATGTCCGTCA-3' 5'ATACTTGTAAGCACTCTTCCTGTAGTGAATATCACTTTTG-3'
*Eco*RI was used in all the budding yeast southern blotting	

Table S5. Primers used in ChIP-qPCR. Related to STAR methods.

Primer name	Sequence
sp_lys1_1kb	5'- CCGCGGCGATCCTGCAAGC-3' 5'- GGTCATTGACTGGAGCGAGGC-3'
sp_lys1_10kb	5'- GGTTCAACACAACGTCCATTCAACCATGC-3' 5'- GCAGAGTTACTTCCTTGCACTCAAAAAATAGAAGCTC-3'
sp_lys1_58kb	5'- GAGTATTTCGGTAAGCAAGTATTACATCGGCTCT-3' 5'- CAATGGAGAAGGCACCTGCGGACATC-3'
sp_act1	5'- CAACAATCTTGACCTTCATGGAGCTAGGAGC-3' 5'- CCTGAAGCTCTTTTCCAACCCTCAGC-3'
sc_MATa_1kb	5'- GGTAGGCGAGGACATTATCTATCA-3' 5'- GAAGAATACCAGTTTATCTCGCATTCAAATC-3'
sc_MATa_5kb	5'- CCTGTGATGTGTAATGGAATGGC-3' 5'- CAAGTATCCATTGCCTAAGCCAA-3'
sc_ACT1	5'- TCGTTCCAATTTACGCTGGTT-3' 5'- CGGCCAAATCGATTCTCAA-3'