

SUPPLEMENTARY DATA

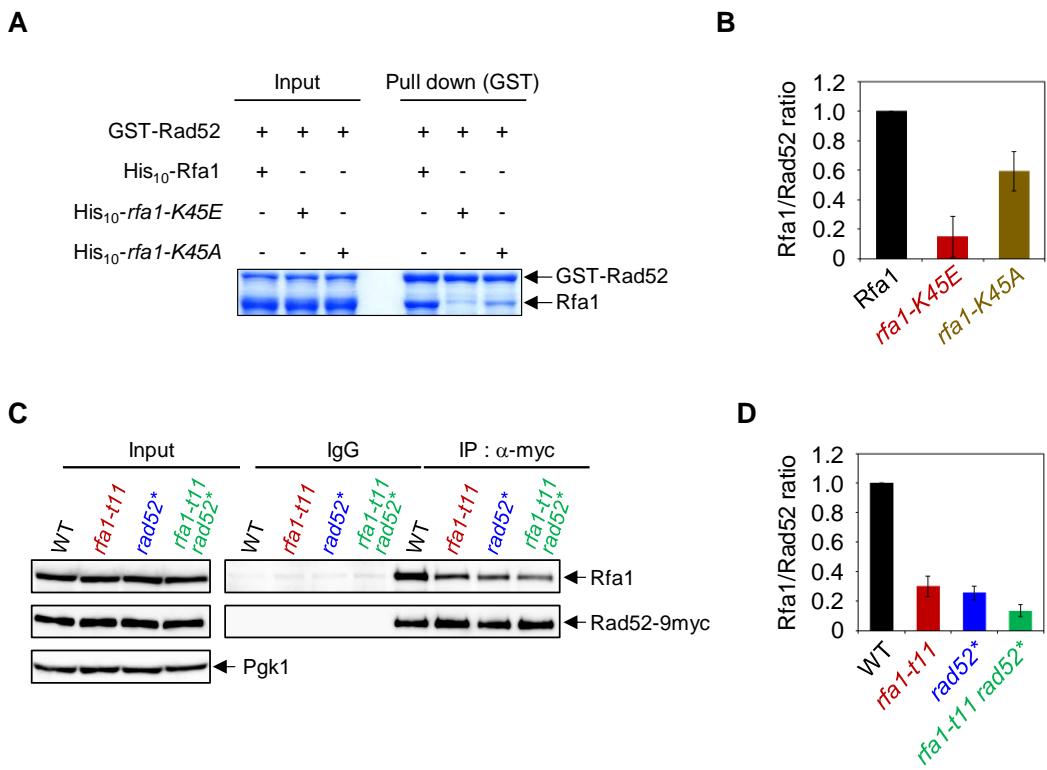
**RPA Interacts with Rad52 to Promote Meiotic Crossover and
Noncrossover Recombination**

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Supplementary Figure 1-17

Supplementary Table 1

Supplementary References



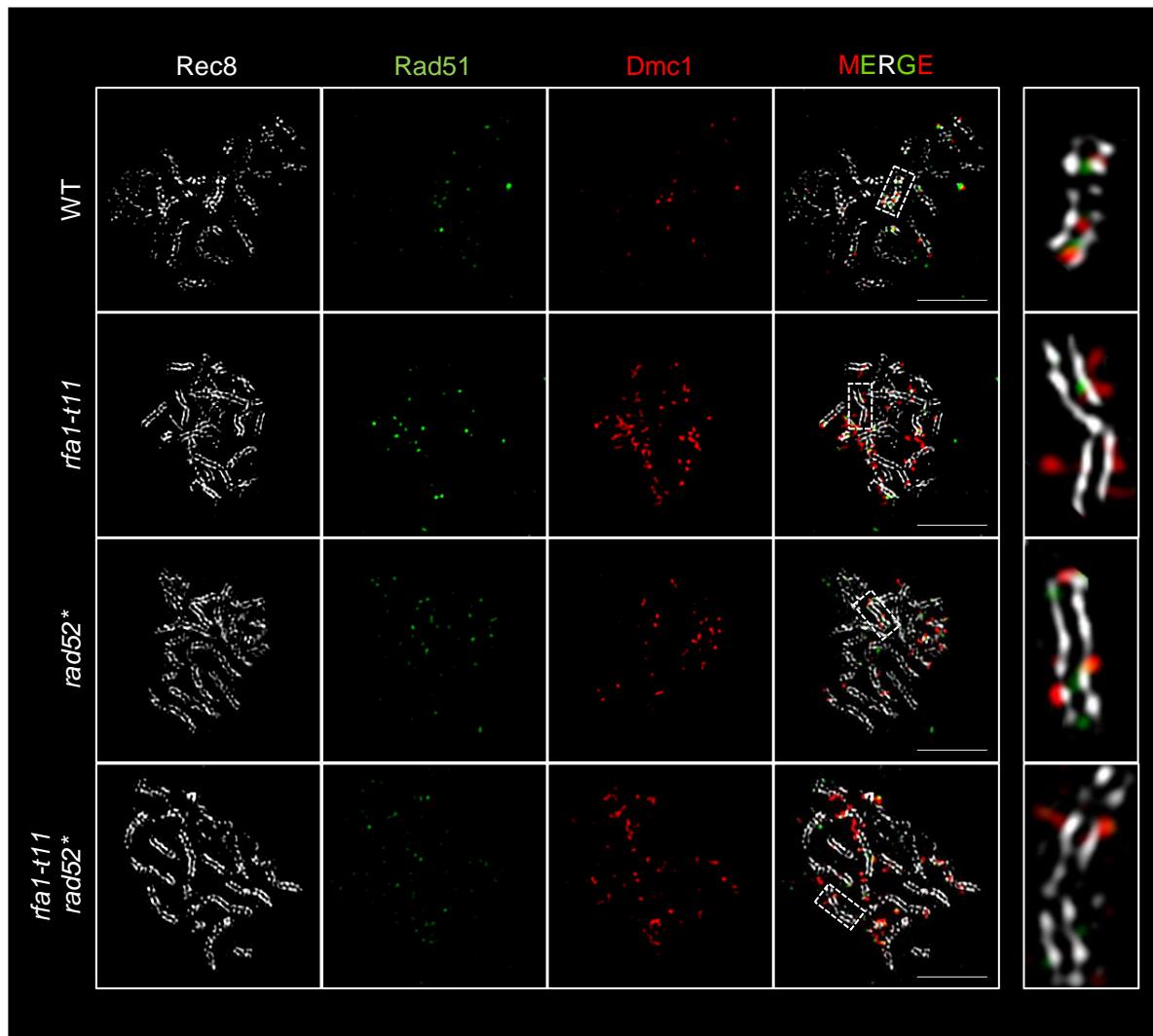
Supplementary Fig. 1. Protein interaction assay between Rad52 and Rfa1 in WT and mutants

(A) *In vitro* binding analysis for Rad52 and RPA. Rad52 proteins were incubated with wild-type (WT) Rfa1, rfa1-K45E, and rfa1-K45A, followed by co-purification using glutathione agarose resins. The protein mixtures were analyzed on SDS-PAGE gel and visualized by Coomassie Blue staining. GST, Glutathione S-transferase.

(B) Quantification of Rfa1 proteins from *in vitro* binding assay. Data are presented as the mean \pm SD (N = 4).

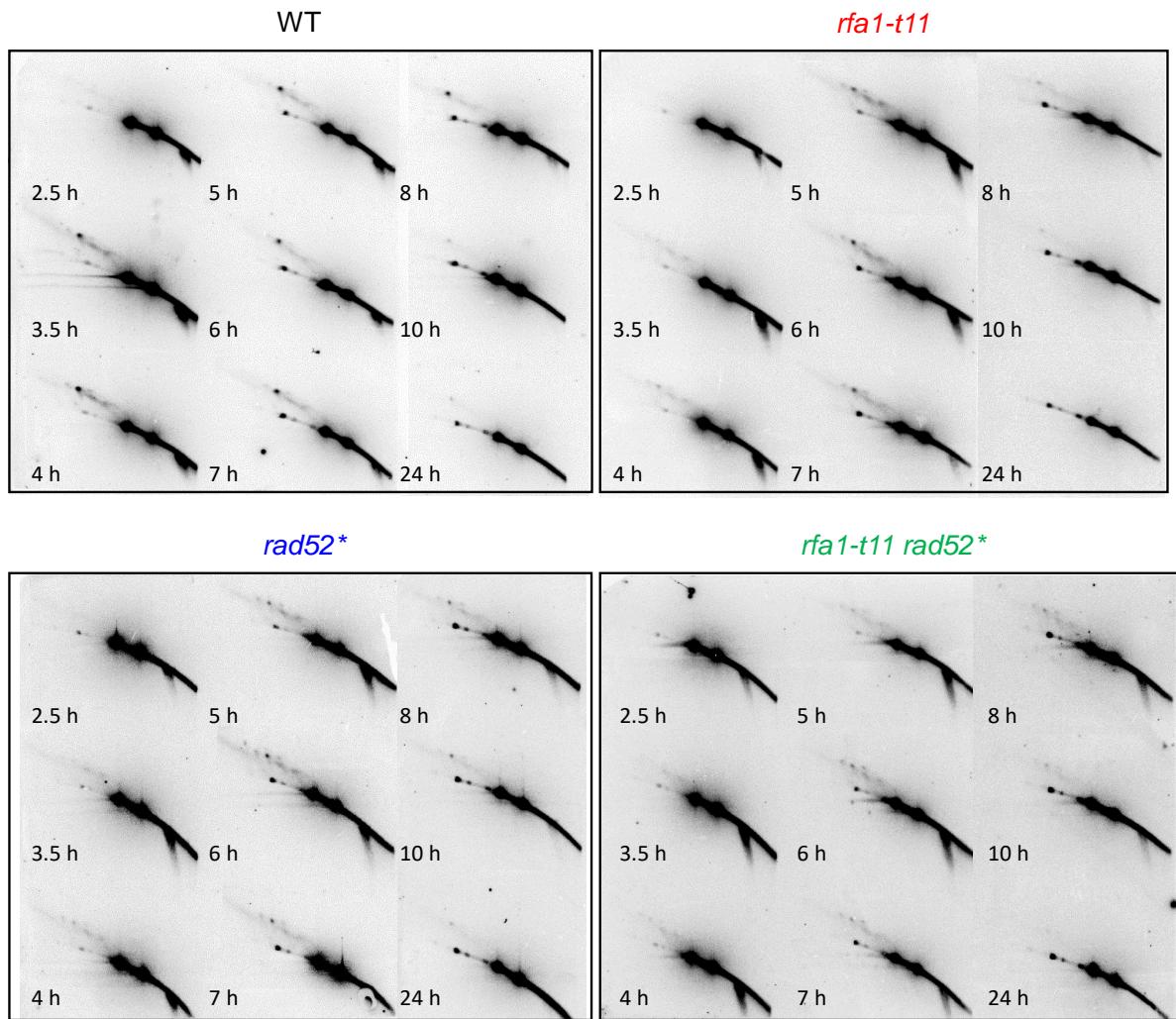
(C) Rad52-9myc were immunoprecipitated using anti-myc in WT, *rfa1-t11*, *rad52**, and *rfa1-t11 rad52**. Rfa1 was detected using anti-RPA antibodies.

(D) Quantification of Rfa1 protein signals from co-immunoprecipitation assay. Data are presented as the mean \pm SD (N = 3).



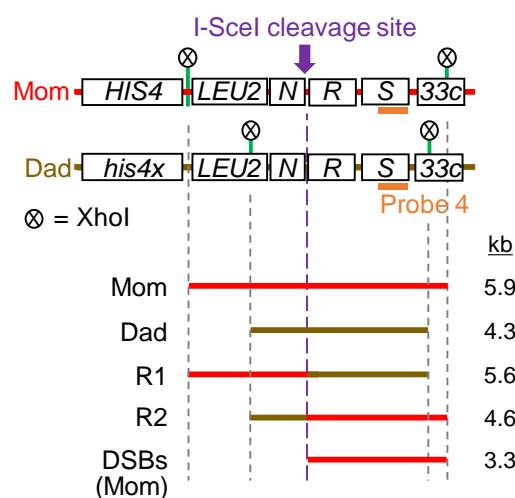
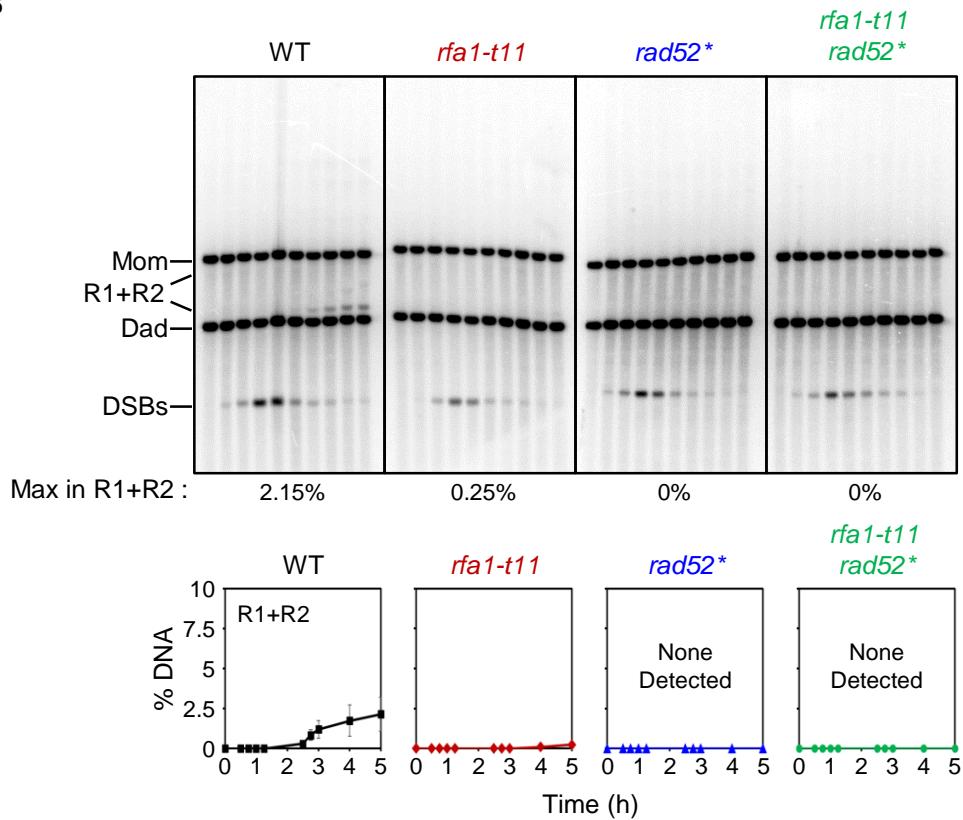
Supplementary Fig. 2. Localization of Rad51 and Dmc1 in WT, *rfa1-t11*, *rad52, and *rfa1-t11 rad52** cells**

SIM analysis of meiotic nuclear spread of WT (3 h), *rfa1-t11* (4 h), *rad52** (6 h), and *rfa1-t11 rad52** (6 h). Nuclei were immunostained with anti-Rad51, anti-Dmc1, and anti-HA (for Rec8) in spread chromosomes. The scale bar represents 2.5 μ m. Enlarged images of the boxed regions are shown to the right of each panel.



Supplementary Fig. 3. 2D gel images of WT and mutants

Representative 2D gel images in WT, *rfa1-t11*, *rad52**⁺, and *rfa1-t11 rad52**⁺.

A**B****Supplementary Fig. 4. Analysis of mitotic recombination in WT and mutants**

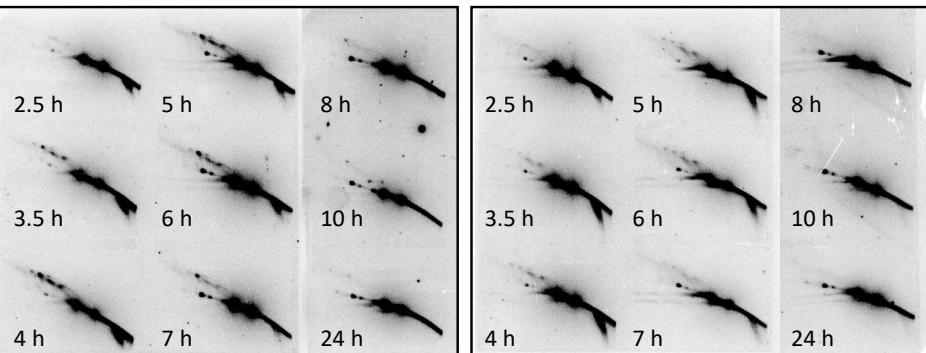
(A) I-SceI site-specific DSB assay system. The map of *HIS4LEU2* shows restriction enzyme sites and probe4 positions (1,2). R1, recombinant 1; R2, recombinant 2.

(B) One-dimensional (1D) gel analysis of recombinant formation in diploid cells following DSB-induction. Quantification of recombinants are shown in each gel of WT, *rfa1-t11*, *rad52**, and *rfa1-t11 rad52**. Data are presented as the mean \pm SD ($N = 3$).

RAD52-AID

-IAA

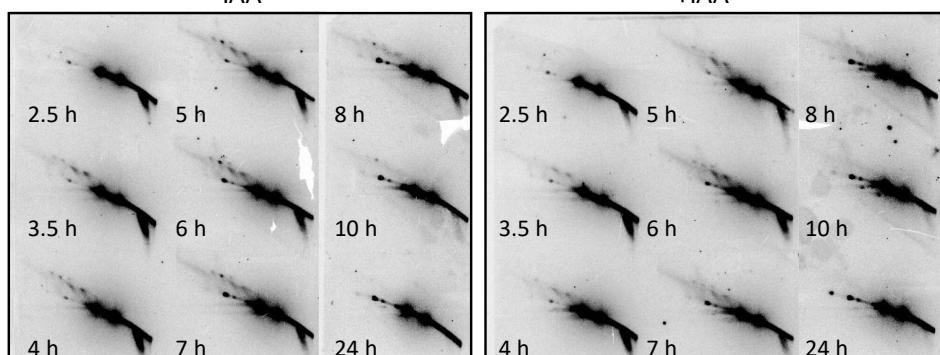
+IAA



rfa1-t11 RAD52-AID

-IAA

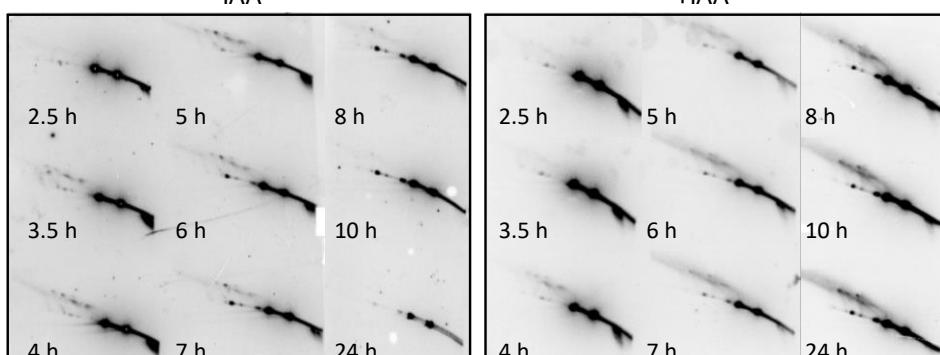
+IAA



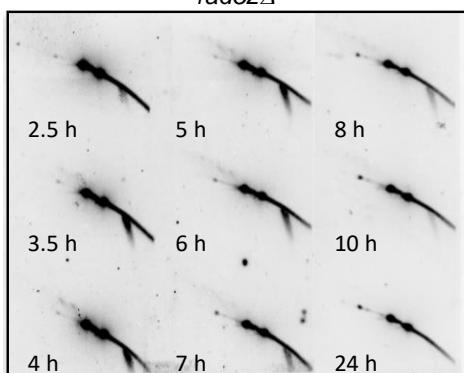
RFA1-AID

-IAA

+IAA

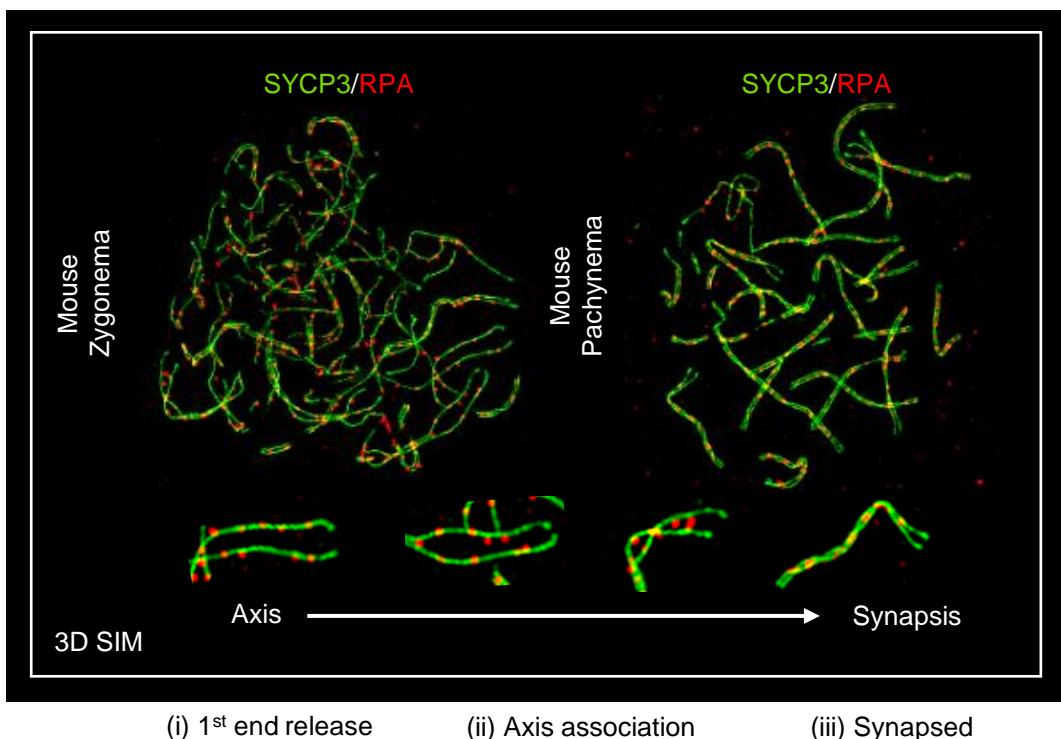


rad52Δ



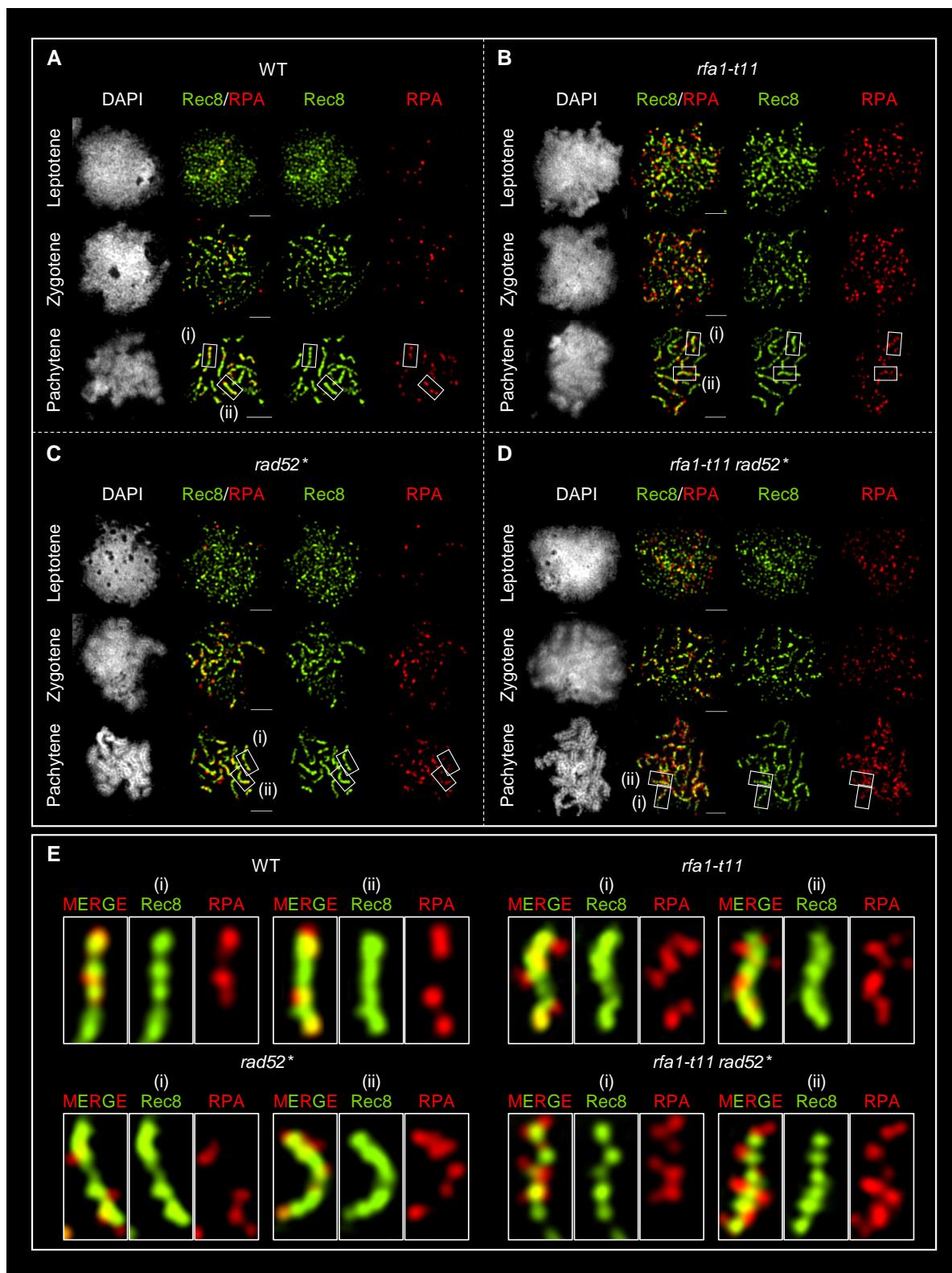
Supplementary Fig. 5. 2D gel images of *RAD52-AID*, *rfa1-t11 RAD52-AID*, *RFA1-AID*, and *rad52Δ* cells

2D gel images of *RAD52-AID*, *rfa1-t11 RAD52-AID*, *RFA1-AID* in the presence or absence of auxin, and *rad52Δ* cells.



Supplementary Fig. 6. Images of RPA and chromosome axis in mouse meiocyte

3D SIM images of mouse zygonema and pachynema chromosomes. The cells were immunostained with anti-RPA and anti-SYCP3 in spread chromosomes.

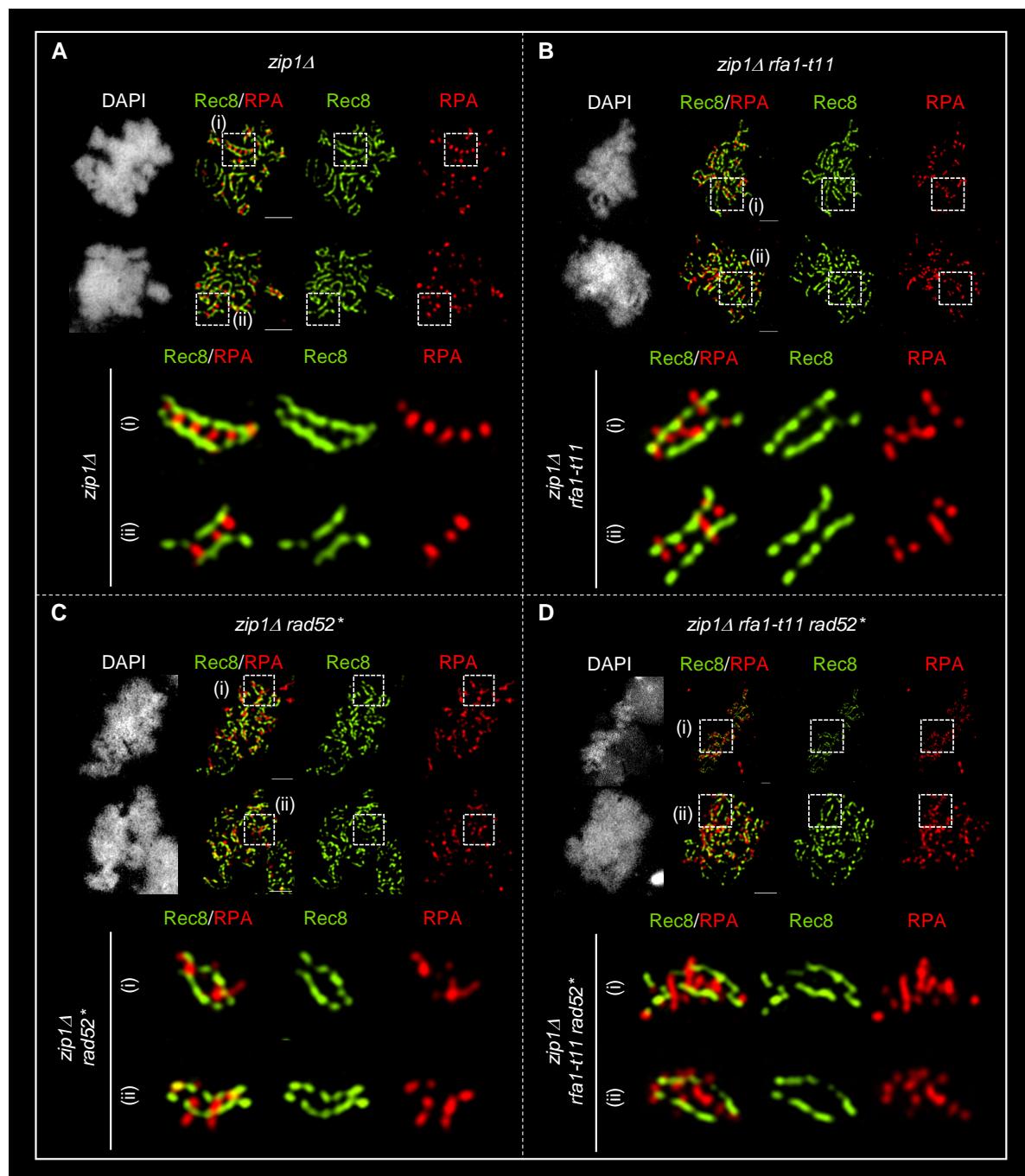


Supplementary Fig. 7. Localization of RPA-single stranded DNA on chromosome axis in WT, *rfa1-t11*, *rad52, and *rfa1-t11 rad52****

(A-D) Immunofluorescence analysis of Rfa1 and Rec8 on chromosome spread of WT, *rfa1-t11*, *rad52**,

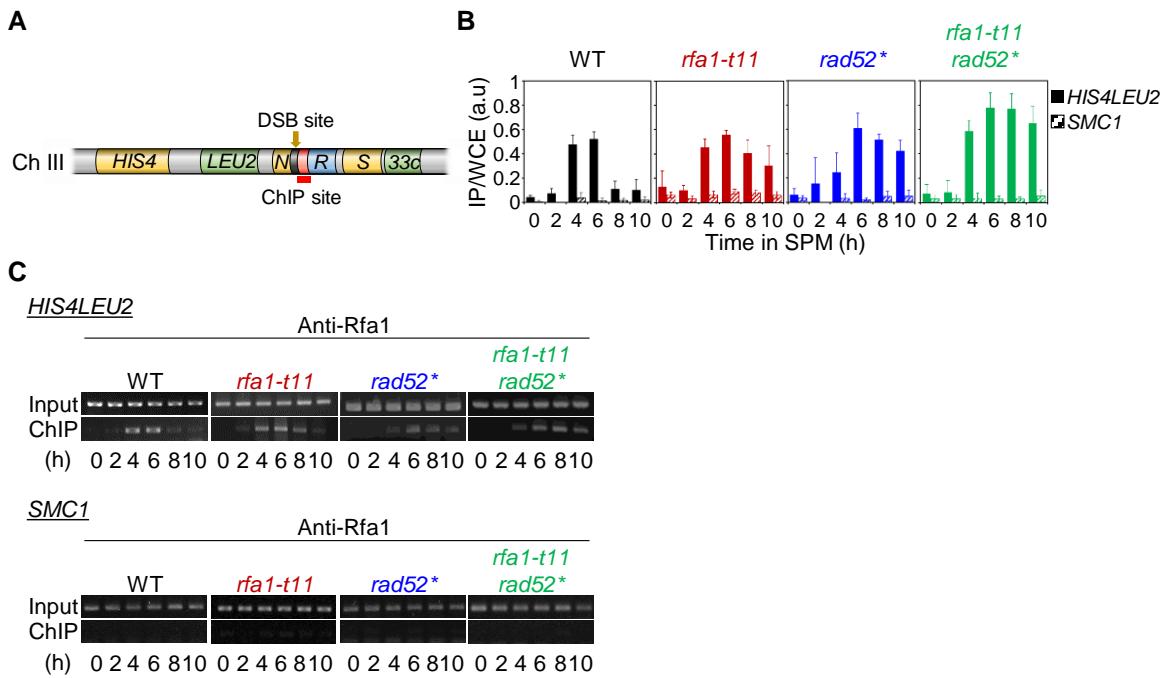
and *rfa1-t11 rad52**. The scale bar represents 2.5 μm .

(E) Boxed regions from Rfa1-Rec8 co-staining images enlarged in (A-D).



Supplementary Fig. 8. Localization of RPA-single stranded DNA on chromosome axis in *zip1Δ* cells

(A-D) Localization of Rec8 along with RPA assembly of spread chromosomes in *zip1Δ* cells immunostained for Rec8-3HA and RPA. The scale bars represent 2.5 μ m.

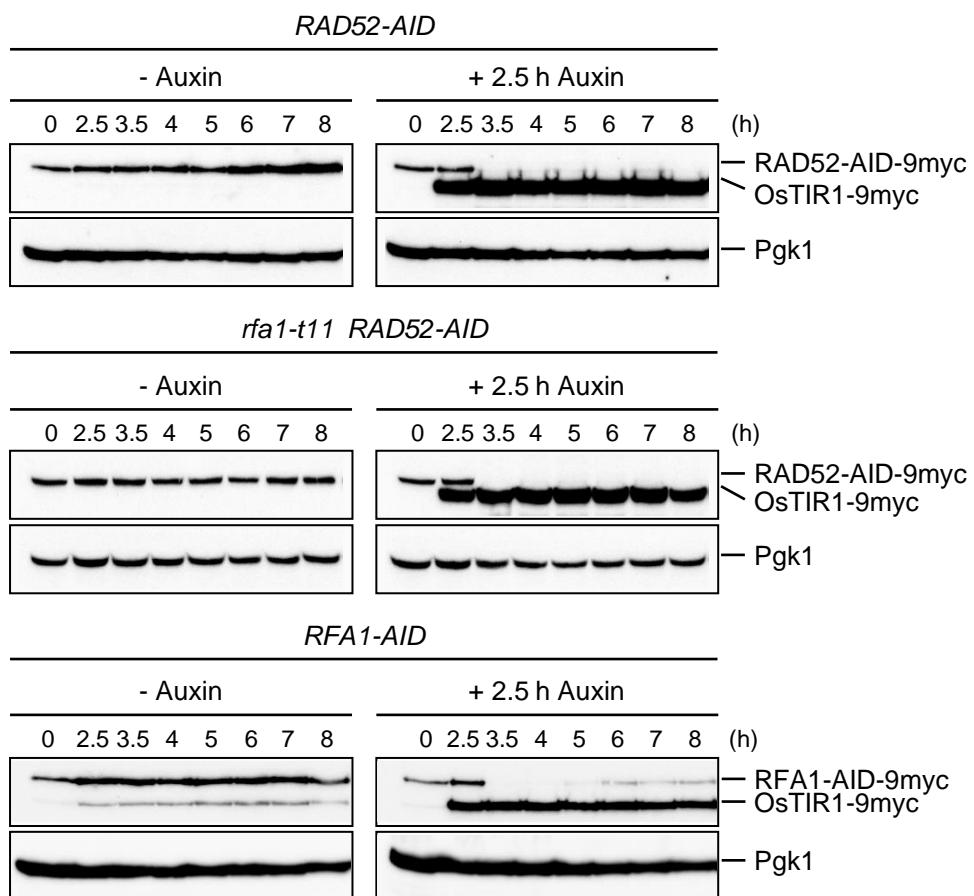


Supplementary Fig. 9. Chromatin immunoprecipitation assays at *HIS4LEU2* and *SMC1* locus

(A) Map of *HIS4LEU2* locus showing the DSB site and the *HIS4LEU2* ChIP sites.

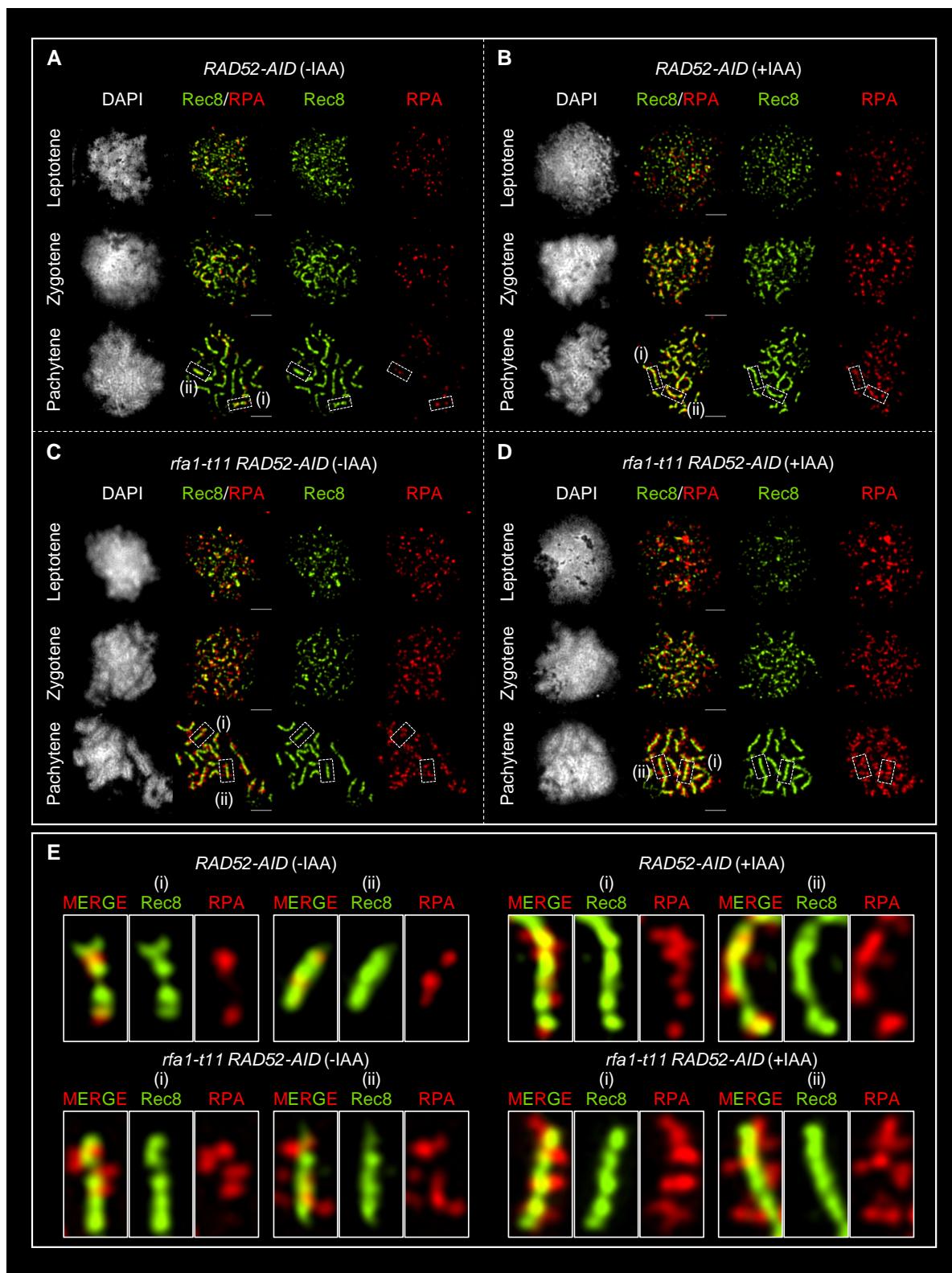
(B) Quantification of ChIP assay in the WT, *rfa1-t11*, *rad52**, and *rfa1-t11 rad52** strains. *HIS4LEU2* hotspot (closed bars) and a control region of the *SMC1* (diagonal bars) are shown. The error bars represent the SD of the mean.

(C) Representative gel image of ChIP assay at *HIS4LEU2* and *SMC1* locus.



Supplementary Fig. 10. Analysis of Rad52 and Rfa1 degradation in the presence or absence of auxin

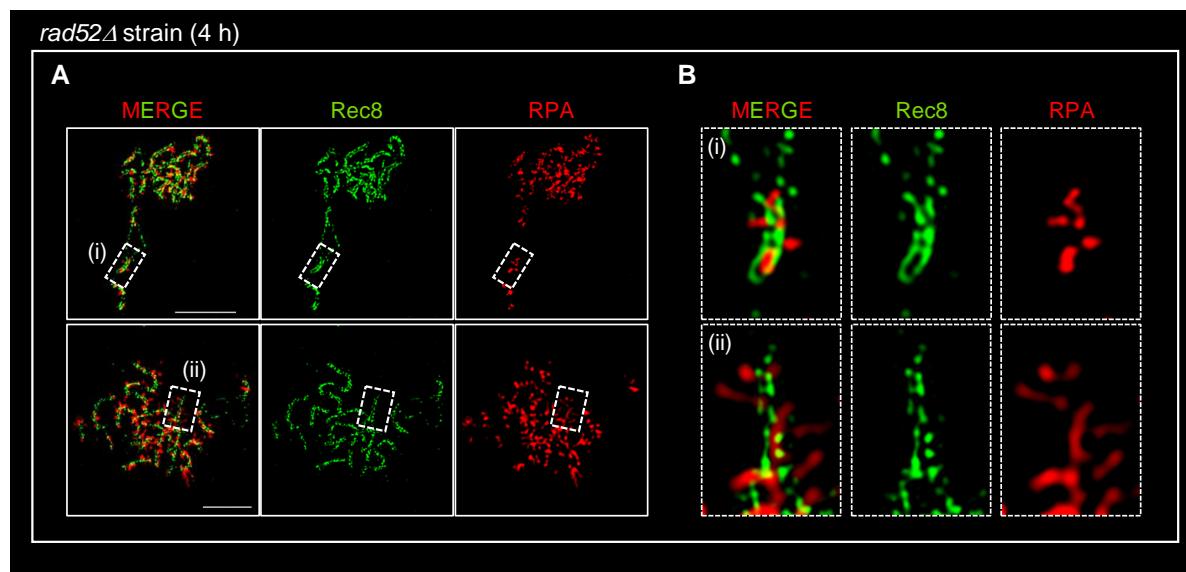
Analysis of Rad52 or Rfa1 protein degradation induced by auxin-degron. Cells were subjected to western blot using anti-myc (for detection of RAD52-AID-9myc and RFA1-AID-9myc) and anti-Pgk1 antibodies.



Supplementary Fig. 11. RPA localization in *RAD52-AID* and *rfa1-t11 RAD52-AID* cells

(A-D) Representative images of leptotene, zygote, and pachytene chromosomes. The cells were immunostained with anti-RPA and anti-HA (for Rec8-3HA) in spread chromosomes.

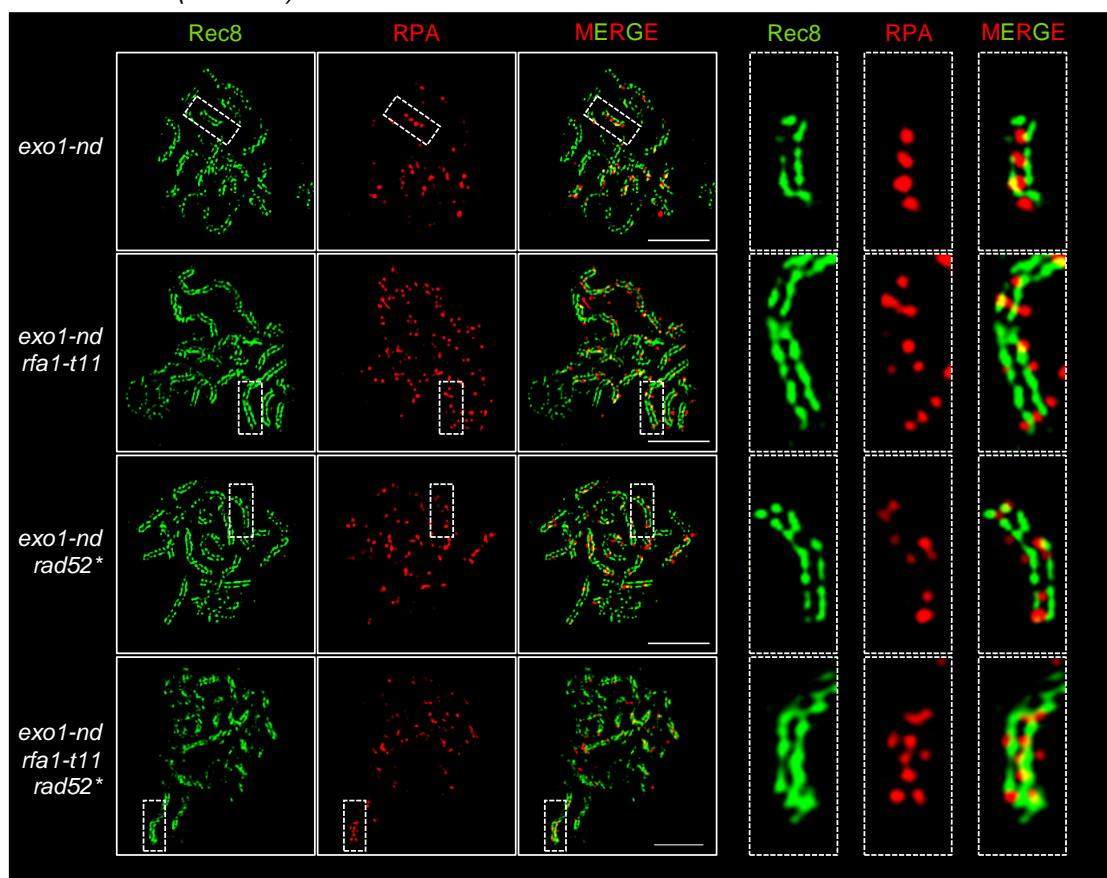
(E) Boxed regions from Rfa1-Rec8 co-staining images enlarged in **(A-D)**.



Supplementary Fig. 12. SIM analysis of RPA-single stranded DNA in *rad52Δ* cells

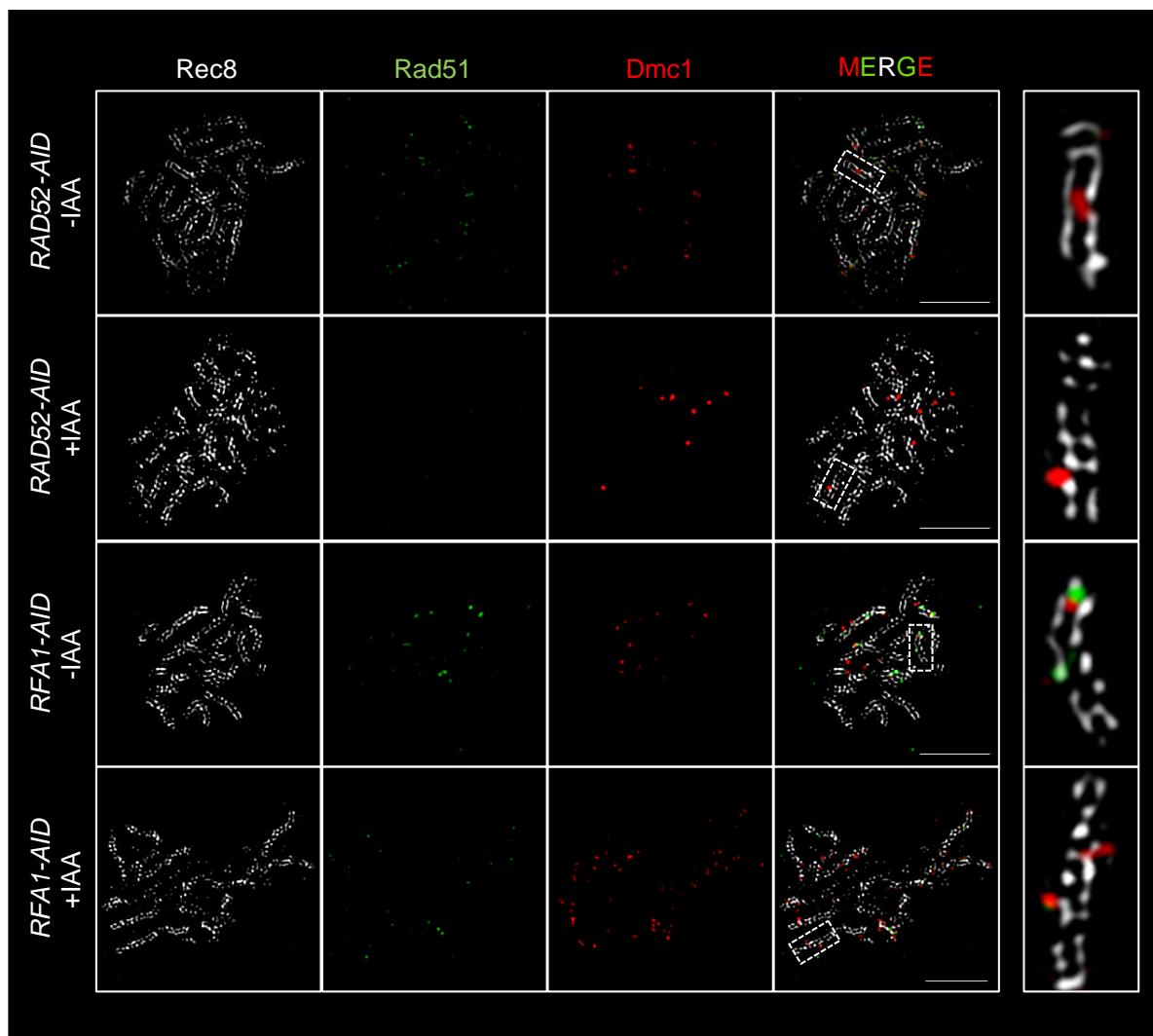
- (A) SIM Images of pachytene chromosome in *rad52Δ*. Nuclei were immunostained with anti-RPA and anti-HA (for Rec8-3HA) in spread chromosomes. The scale bar represents 2.5 μ m.
- (B) (i) and (ii) The boxed regions are magnified images from (A).

exo1-D173A (exo1-nd)



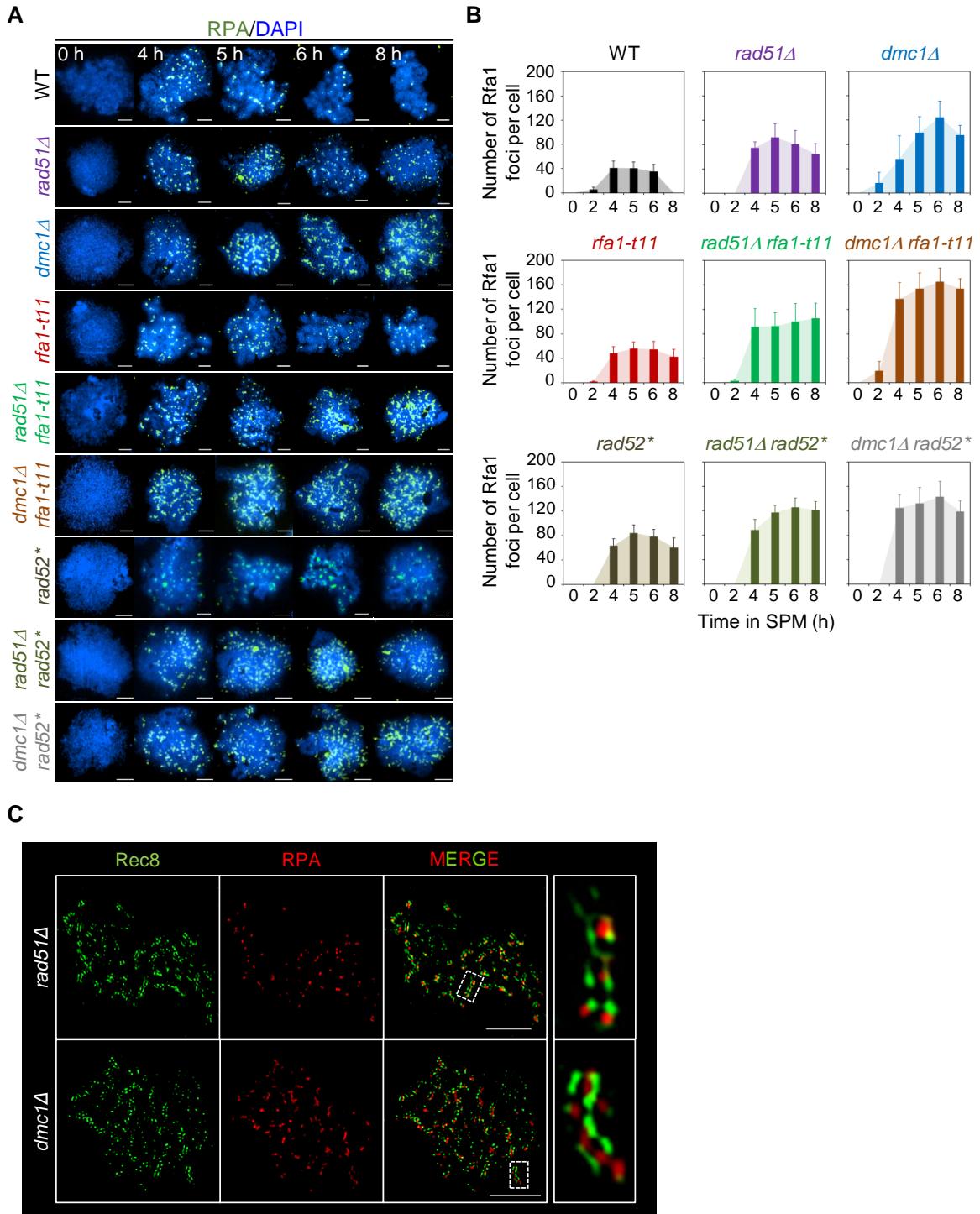
Supplementary Fig. 13. RPA-ssDNA filaments on chromosome axis in *exo1-D173A* cells

Localization of Rec8 along with RPA assembly of spread chromosomes in *exo1-D173A*, *exo1-D173A rfa1-t11*, *exo1-D173A rad52**, and *exo1-D173A rfa1-t11 rad52** cells immunostained for Rec8-3HA and RPA. The scale bars represent 2.5 μ m.



Supplementary Fig. 14. Localization of Rad51 and Dmc1 in *RAD52-AID* and *RFA1-AID* cells

SIM analysis of meiotic nuclear spread of *RAD52-AID* (-IAA, 4 h; +IAA, 4 h) and *RFA1-AID* (-IAA 4 h; +IAA 5 h). Nuclei were immunostained with anti-Rad51, anti-Dmc1, and anti-HA (for Rec8) in spread chromosomes. The scale bar represents 2.5 μ m. Enlarged images of the boxed regions are shown to the right of each panel.



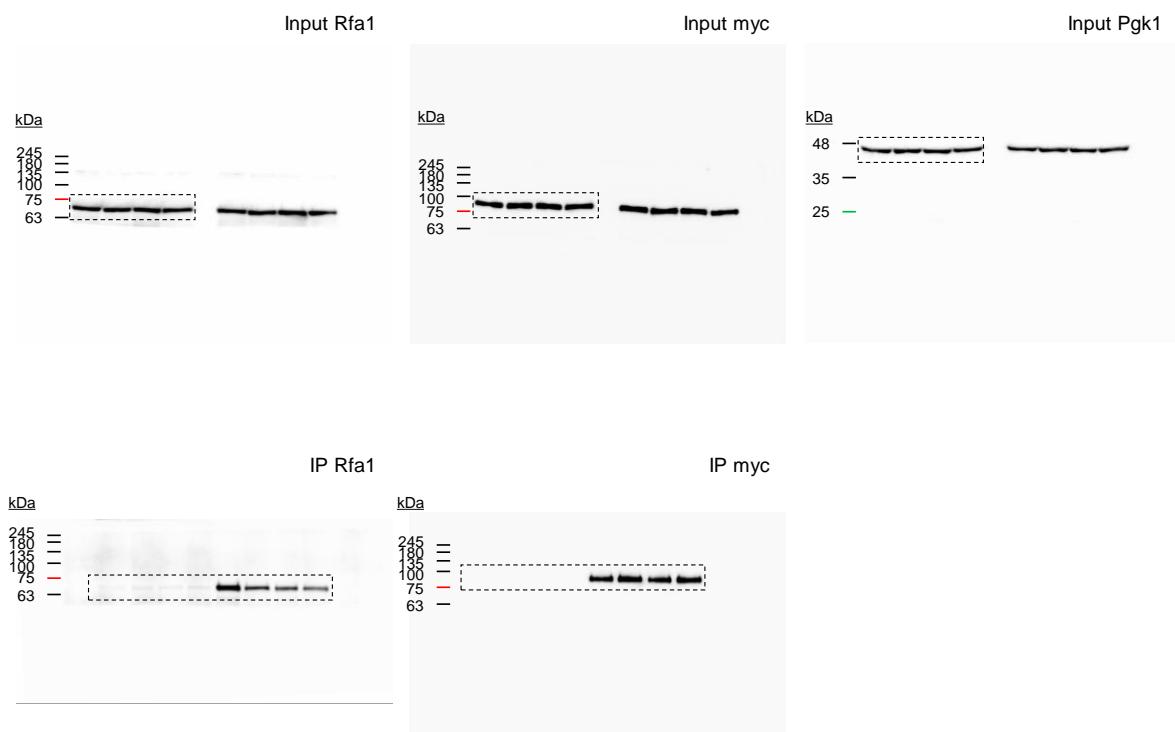
Supplementary Fig. 15. Assembly of RPA in *rad51Δ* and *dmc1Δ* strains

(A) Immunofluorescence analysis of Rfa1 focus formation in WT and mutant cells. RPA foci were observed on spread chromosomes from meiotic time course experiments. The nuclei are stained with DAPI.

(B) Quantitation of RPA foci numbers in (A). At least 20 nuclei were scored at each time point. The

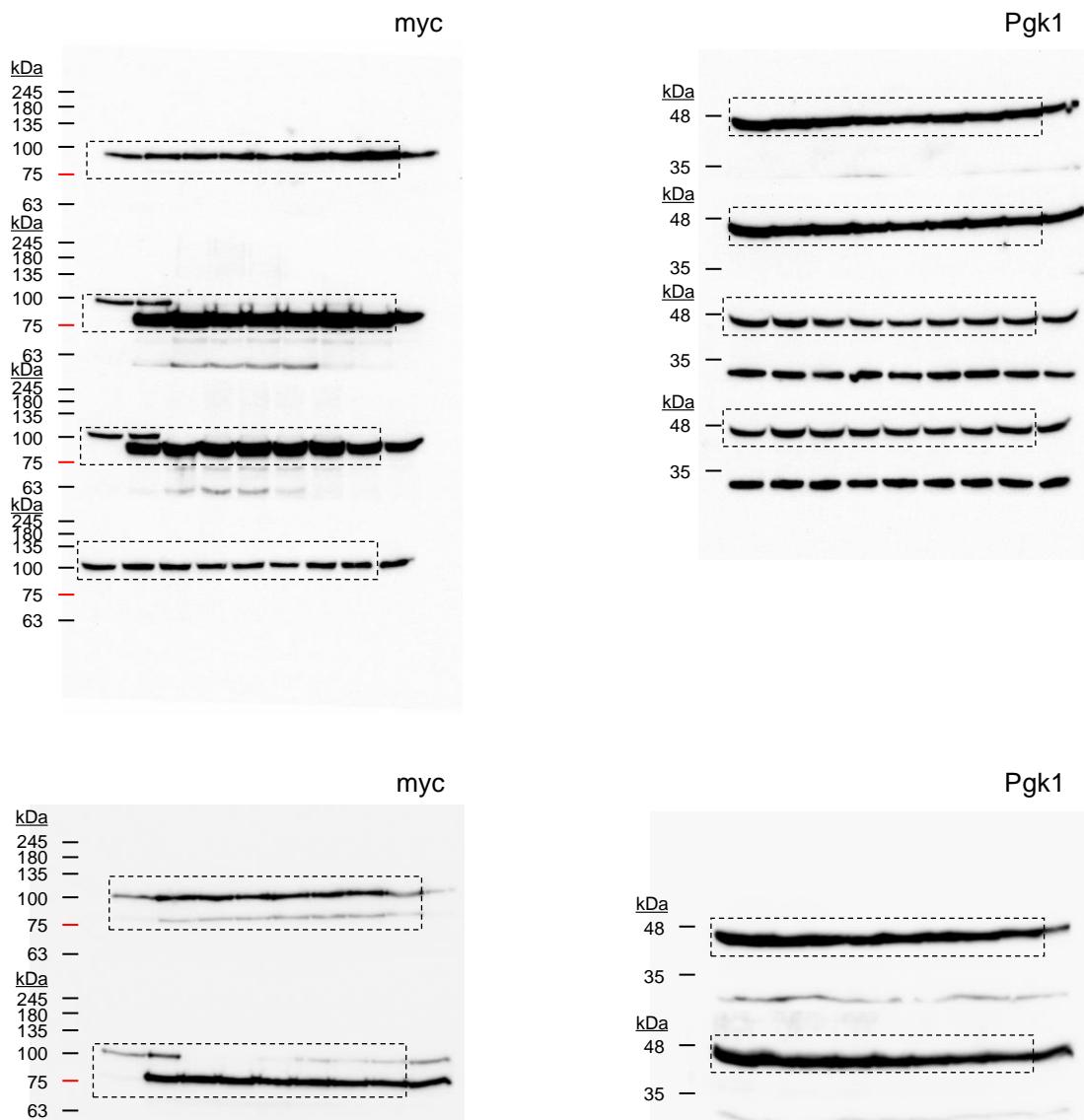
error bars represent the SD of the mean.

(C) Representative SIM images of *rad51Δ* and *dmc1Δ* mutant chromosomes. Nuclei were immunostained with anti-Rfa1 and anti-HA (for Rec8) in spread chromosomes. The scale bar represents 2.5 μm . Enlarged images of the boxed regions are shown to the right of each panel.



Supplementary Fig. 16. Uncropped western blot gel images

Western blot gel images corresponding to Figure S1.



Supplementary Fig. 17. Uncropped western blot gel images

Western blot gel images corresponding to Figure S10.

Supplementary Table 1. List of *S. cerevisiae* strains used in this study.

Strain [†]	Genotype [‡]
KKY4278	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3</i>
KKY3514	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, rfa1-t11/''</i>
KKY4564	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY4613	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, rfa1-t11/'', rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY5721	<i>MATa/MATa, REC8-3HA::URA3/REC8</i>
KKY3584	<i>MATa/MATa, REC8-3HA::URA3/REC8, rfa1-t11/''</i>
KKY4584	<i>MATa/MATa, REC8-3HA::URA3/REC8, rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY5117	<i>MATa/MATa, REC8-3HA::URA3/REC8, rfa1-t11/'', rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY3628	<i>MATa/MATa, spo11(Y135F)-HA::URA3/''</i>
KKY5520	<i>MATa/MATa, REC8-3HA::URA3/REC8, Zip3-13myc-HygB/Zip3</i>
KKY5527	<i>MATa/MATa, REC8-3HA::URA3/REC8, Zip3-13myc-HygB/Zip3, rfa1-t11/''</i>
KKY5521	<i>MATa/MATa, REC8-3HA::URA3/REC8, Zip3-13myc-HygB/Zip3, rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY5570	<i>MATa/MATa, REC8-3HA::URA3/REC8, Zip3-13myc-HygB/Zip3, rfa1-t11/'', rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY2856	<i>MATa/MATa, RAD52-AID-9myc-HygB/'', pCUP1-KanMX4-OsTIR1-9myc-URA3/''</i>
KKY4085	<i>MATa/MATa, RAD52-AID-9myc-HygB/'', pCUP1-KanMX4-OsTIR1-9myc-URA3/'', rfa1-t11/''</i>
KKY6440	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, rad52Δ::HygB''</i>
KKY4214	<i>MATa/MATa, RFA1-AID-9myc-HygB/'', pCUP1-KanMX4-OsTIR1-9myc-URA3/''</i>
KKY4583	<i>MATa/MATa, RAD52-AID-9myc-HygB/'', pCUP-KanMX4-OsTIR1-9myc-URA3/'', REC8-3HA::URA3/REC8</i>
KKY4582	<i>MATa/MATa, RAD52-AID-9myc-HygB/'', pCUP-KanMX-OsTIR1-9myc-URA3/'', REC8-3HA::URA3/REC8, rfa1-t11/''</i>
KKY4965	<i>MATa, Rad52-9myc-HygB</i>
KKY4967	<i>MATa, Rad52-9myc-HygB, rfa1-t11</i>
KKY5581	<i>MATa, rad52-QDDD-275-278-AAAA-9myc-KanMX4</i>
KKY5478	<i>MATa, rfa1-t11, rad52-QDDD-275-278-AAAA-9myc-KanMX4</i>
KKY940	<i>MATa/MATa, lys2::URA3-pGal1/10-SCEI/'', HIS4::LEU2(I-SceI-WT)/his4::LEU2(I-SceI-mt)</i>
KKY4150	<i>MATa/MATa, lys2::URA3-pGal1/10-SCEI/'', HIS4::LEU2(I-SceI-WT)/his4::LEU2(I-SceI-mt), rfa1-t11/''</i>
KKY6239	<i>MATa/MATa, lys2::URA3-pGal1/10-SCEI/'', HIS4::LEU2(I-SceI-WT)/his4::LEU2(I-SceI-mt)</i>

	<i>mt), rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY6241	<i>MATa/MATa, lys2::URA3-pGal1/10-SCEI'', HIS4::LEU2(I-SceI-WT)/his4::LEU2(I-SceI- mt), rfa1-t11'', rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY5463	<i>MATa/MATa, zip1Δ::KanMX4'', REC8-3HA::URA3/REC8</i>
KKY5465	<i>MATa/MATa, zip1Δ::KanMX4'', REC8-3HA::URA3/REC8, rfa1-t11/''</i>
KKY5467	<i>MATa/MATa, zip1Δ::KanMX4'', REC8-3HA::URA3/REC8, rad52-QDDD-275-278-AAAA- HygB/''</i>
KKY5469	<i>MATa/MATa, zip1Δ::KanMX4'', REC8-3HA::URA3/REC8, rfa1-t11'', rad52-QDDD-275- 278-AAAA-HygB/''</i>
KKY3295	<i>MATa/MATa, nuc1Δ::LEU2'', exo1-D173A'', Rec8-3HA::URA3/REC8</i>
KKY3296	<i>MATa/MATa, nuc1Δ::LEU2'', exo1-D173A'', Rec8-3HA::URA3'', rfa1-t11/''</i>
KKY5183	<i>MATa/MATa, nuc1Δ::LEU2'', exo1-D173A'', Rec8-3HA::URA3'', rad52-QDDD-275-278- AAAA-HygB/''</i>
KKY6140	<i>MATa/MATa, nuc1Δ::LEU2'', exo1-D173A'', Rec8-3HA::URA3'', rfa1-t11'', rad52- QDDD-275-278-AAAA-HygB/''</i>
KKY5017	<i>MATa/MATa, REC8-3HA::URA3/REC8, rad51Δ::HisG/''</i>
KKY6443	<i>MATa/MATa, REC8-3HA::URA3/REC8, dmc1Δ::KanMX4/''</i>
KKY1380	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, nuc1Δ::HygB/'', rad51Δ::HisG/''</i>
KKY1431	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, nuc1Δ::HygB/'', dmc1Δ::KanMX4/''</i>
KKY5571	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, nuc1Δ::HygB/'', rad51Δ::HisG'', rfa1-t11/''</i>
KKY3818	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, dmc1Δ::KanMX4/'', rfa1-t11/''</i>
KKY4744	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, nuc1Δ::HygB/'', rad51Δ::HisG'', rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY4819	<i>MATa/MATa, HIS4::LEU2-(BamHI)/his4x::LEU2-(NgoMIV)--URA3, nuc1Δ::HygB/'', dmc1Δ::KanMX4/'', rad52-QDDD-275-278-AAAA-HygB/''</i>
KKY4824	<i>MATa/MATa, nuc1Δ::HygB/'', rad52Δ::HygB/'', Rec8-3HA::URA3/REC8</i>

[†] All strains are isogenic derivatives of SK1 background.

[‡] All strains are also homozygous for the mutation *ho::hisG, leu2::hisG, ura3(ΔPstI-SmaI)*.

Supplementary references

1. Bzymek, M., Thayer, N.H., Oh, S.D., Kleckner, N. and Hunter, N. (2010) Double Holliday junctions are intermediates of DNA break repair. *Nature*, **464**, 937–941.
2. Lee, M.S., Yu, M., Kim, K.Y., Park, G.H., Kwack, K. and Kim, K.P. (2015) Functional validation of rare human genetic variants involved in homologous recombination using *Saccharomyces cerevisiae*. *PLoS One*, **10**, e0124152.