Supplemental Figures

Figure S1. Recombination mediator activity of various Rad52 species

(A) Full time course experiment of the standard reaction, involving pre-incubation of the ssDNA with Rad51 to allow formation of the presynaptic filament before RPA addition.

(B) Co-incubating the ssDNA with Rad51 and RPA resulted in severe inhibition of the DNA strand exchange reaction.

(C) to (F) Full time course of DNA strand exchange reactions conducted to examine the recombination mediator activity of the Rad52 species: Rad52 in panel C, Rad52-C in panel D, Rad52-N/C in panel E, and Rad52-M/C in panel F.

(G) The results from (A) to (F) were plotted.

Figure S2. EM analysis of the recombination mediator activity of Rad52 and Rad52-M/C

The parts show a representative field of the reactions described in Figure 5. DNA molecules that were covered only by RPA are circled.

Figure S3. Complementation of *rad52*_327 by Rad52 or Rad52-M/C tagged with His₆

(A) $rad52\Delta$ -327 cells harboring ADH-RAD52, ADH-His₆-RAD52, ADH-RAD52-His₆, ADH-RAD52-M/C, ADH-His₆-RAD52-M/C, or the empty ADH vector were serially diluted and spotted onto SC-Ura medium with or without 0.005% MMS. Note that the ability of Rad52 and Rad52-M/C to complement the $rad52 \Delta 327$ mutant is not affected by the (His)6-tag.

(B) Extracts of $rad52\Delta$ -327 cells harboring ADH-RAD52 (lane 1), ADH-His₆-RAD52 (lane 2), ADH-RAD52-His₆ (lane 3), ADH-RAD52-M/C (lane 4), ADH-His₆-RAD52-M/C (lane 5), or the empty ADH vector (lane 6) were subjected to immunoblot analysis with anti-Rad52 antibodies. Note that the Rad52 or Rad52-M/C protein level is not affected by the (His)6-tag.

Figure S4. Rad52-N does not enhance the recombination mediator activity of Rad52-M/C

(A) A standard strand exchange reaction (std) and RPA-inhibited strand exchange reactions with or without Rad52-N (0.3, 0.6, and 1.5 μ M in lanes 4 to 6 and lanes 8 to 10, respectively), Rad52-M/C (1.5 μ M), or the combination of Rad52N and Rad52-M/C, as indicated, were set up and allowed to proceed for 60 min. (B) Results from lanes 3 to 10 of (A) were quantified and plotted.

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Figure S1



Rad51+RPA



Rad51+RPA+Rad52-M/C



Rad51+RPA+Rad52



Figure S3



B



Figure S4



B

Α

