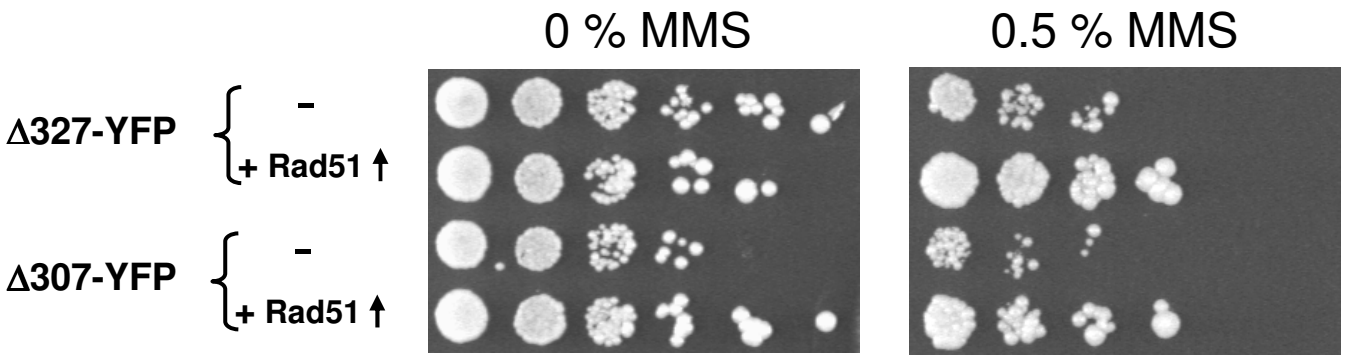
Figure S1

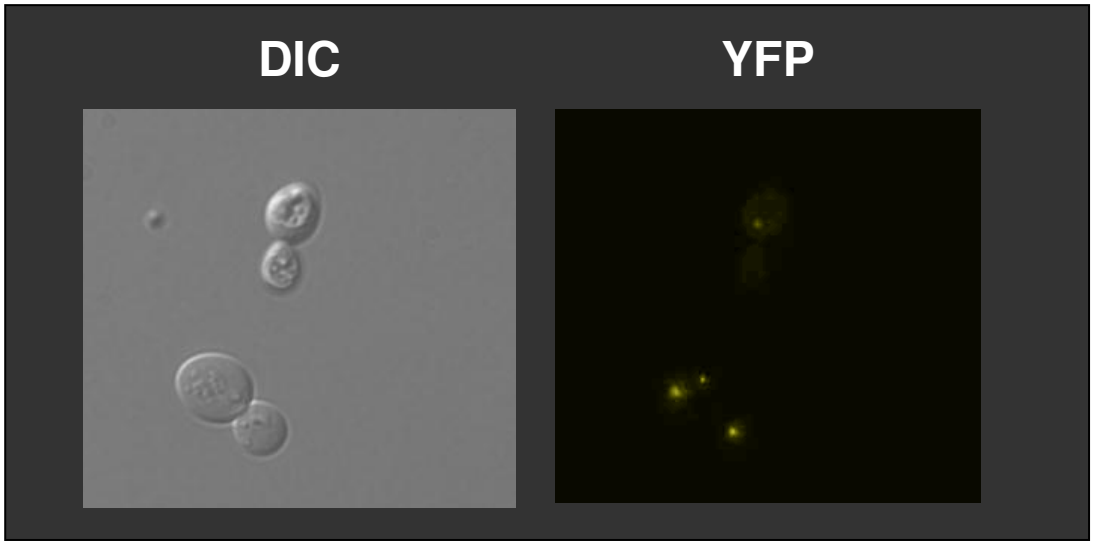


The MMS sensitivity of strains *rad52-Δ327-YFP* and *rad52-Δ327-YFP* strains is suppressed by overexpression of Rad51.

Cells transformed with either empty plasmid (-) or a Rad51 overexpression plasmid pYESS10Rad51 (+ Rad51↑) were either treated with 0% or 0.5% MMS for 10 minutes before plating as 10-fold serial dilutions on SC-His containing 2% galactose as the sole carbon source.

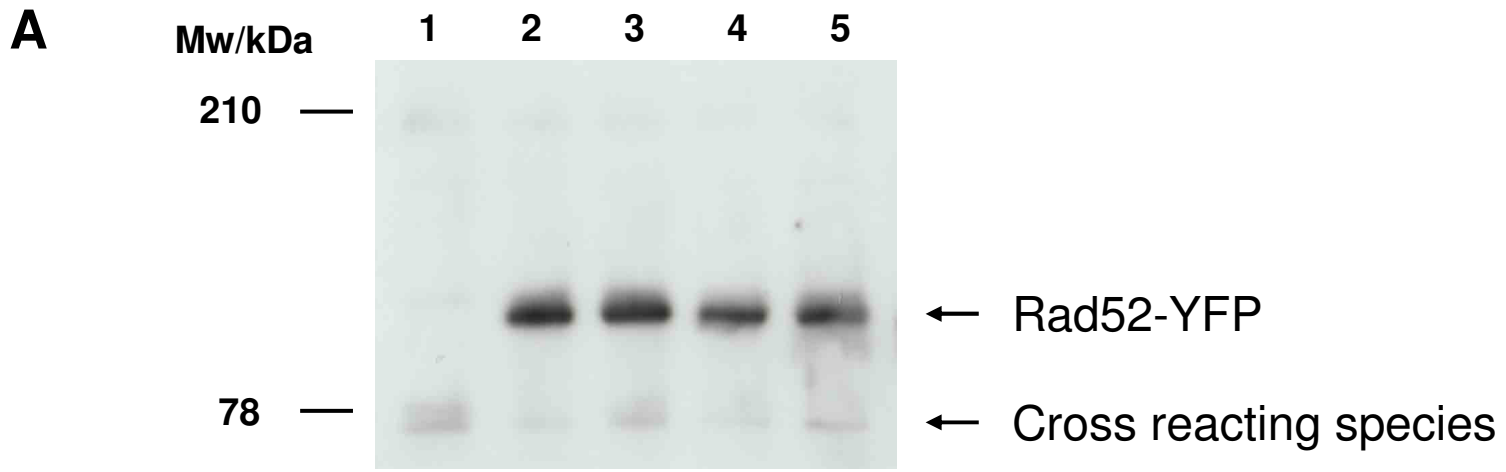
For pYESS10Rad51, see Jiang, H., Xie, Y., Houston, P., Stemke-Hale, K., Mortensen, U.H., Rothstein, R. and Kodadek. T. (1996) J. Biol. Chem. 271(52), 33181-33186.

Figure S2



KlRad52 forms repair foci in *S. cerevisiae* after exposure to MMS
K. lactis Rad52 expressed in a *S. cerevisiae rad52::HIS5* strain.
The strain was treated with 0.5% MMS for 15 minutes followed by 30 minutes of recovery in SC-His prior to microscopy.

Figure S3

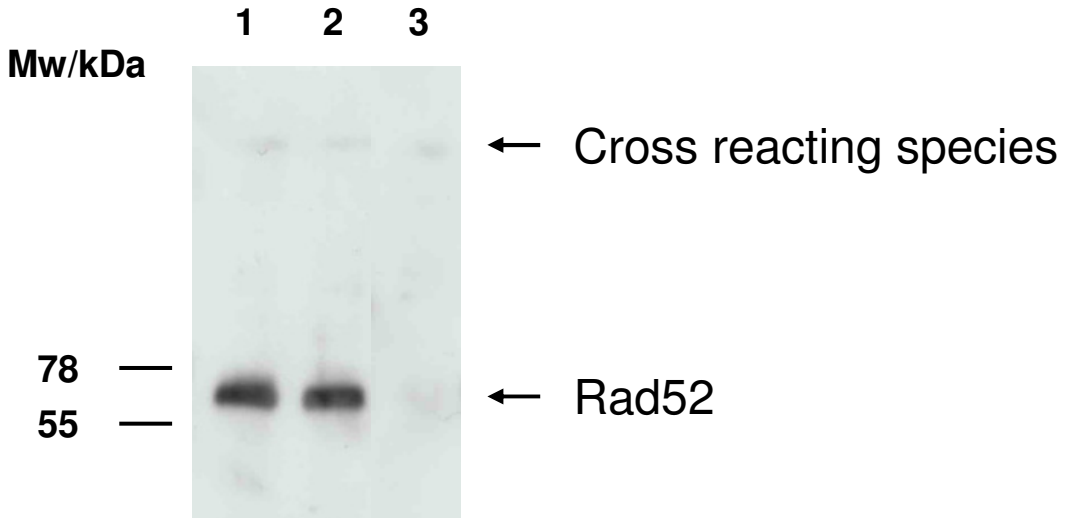


Rad52 levels are similar in strains expressing wild-type and mutant *rad52* alleles.
Protein extracts were prepared as described in Lettier et al. (2006) PLoS Genet. 2, e194; and proteins separated by SDS-PAGE (10%) and transferred to a nitrocellulose-membrane. The membrane was probed with a polyclonal Rad52 antibody (Sung (1997) J. Biol. Chem. 272 (45) 28194-28197) and immunocomplexes visualized using an ECL-Advanced Western Blotting Detection Kit (GE Healthcare). The position of Rad52 is indicated by the lower arrow to the right. Upper arrow points to cross reacting non-Rad52 protein that serves as loading control. Mw-markers are indicated to the left. Extracts were made from strain UM101-15B (*rad52*Δ; see Table S1) transformed with CEN based plasmids (see Material and Methods) to express the different *rad52* alleles from the *RAD52* promoter.

Extracts obtained from strains transformed with a plasmid expressing:
Lane 1: Empty plasmid (*rad52Δ)*
Lane 2: *RAD52-YFP*
Lane 3: *rad52-DS299-300AA-YFP*
Lane 4: *rad52-SDD304-306AAA-YFP*
Lane 5: *rad52-QDDD308-311AAAA-YFP*

Figure S3

B

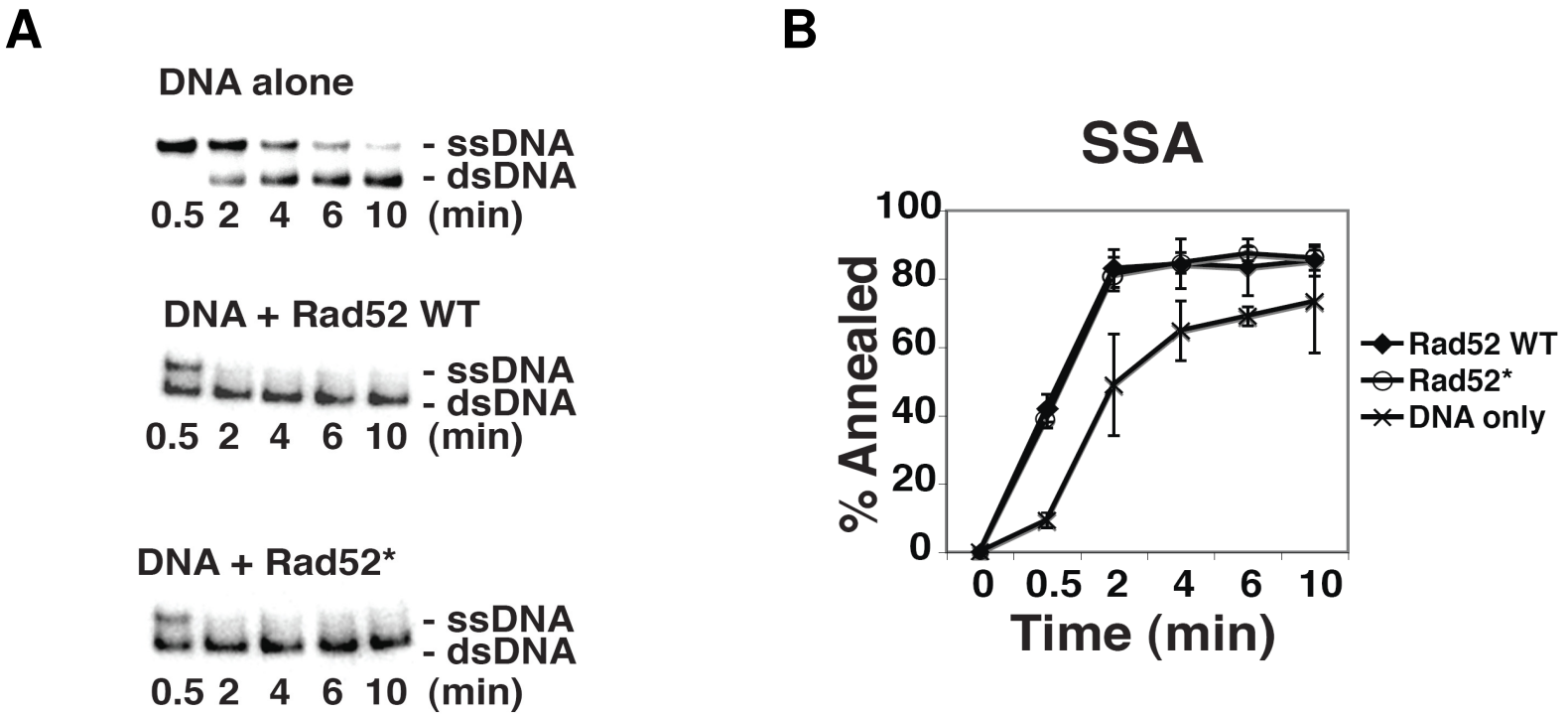


Rad52 levels are similar in wild-type and *rad52-QDDD308-311AAAA* strains.
Protein extracts and western blotting was performed as described in the legend to Figure S3A. The position of Rad52 is indicated by the lower arrow to the right. Upper arrow points to cross reacting non-Rad52 protein that serves as loading control. Mw-markers are indicated to the left.

Extracts were obtained from strains containing integrated *rad52* mutations:

Lane 1: Wild-type *RAD52*
Lane 2: *rad52-QDDD308-311AAAA*
Lane 3: *rad52Δ*

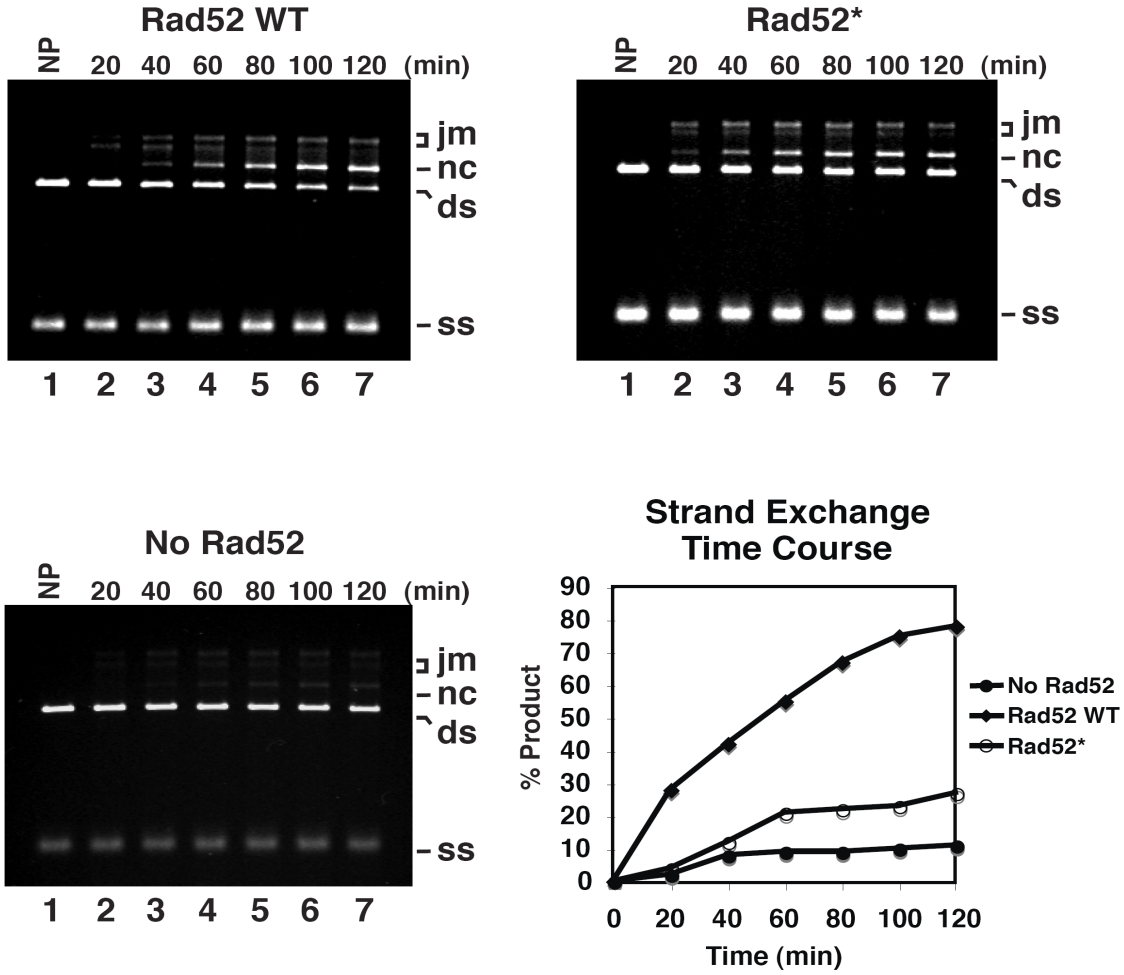
Figure S4



Single-strand annealing activity of Rad52.
(**A**) The ability of either Rad52 or Rad52-QDDD308-311AAAA (Rad52*) to anneal a radiolabeled 83mer to its complement was tested. DNA annealing in the absence of Rad52 was also examined.
The results from the annealing reactions are plotted in (**B**).

Single-strand DNA Annealing Assay. Oligo 1 (6 µM nucleotides) and radiolabeled Oligo 2 (6 µM nucleotides) were incubated in separate tubes at 37°C for 2 min in 12 µl of buffer D. The Rad52 protein species (75 nM) were added in 1 µl to Oligo 1 before mixing with Oligo 2. The completed reactions (25 µl) were incubated at 25°C, and at the indicated times, 7 µl of the reactions was removed and treated with 0.5% SDS, 0.5 mg/ml proteinase K, and an excess of unlabeled Oligo 2 (20 µM) at 25°C for 5 min in 15 µl. The samples (6 µl) were resolved in 12% native polyacrylamide gels run in TAE buffer at 4ºC. The dried gels were subjected to phosphorimaging analysis as above.

Figure S5



Time course analysis of recombination mediator activity. The experiments are done using the concentrations of wild-type and mutant Rad52 that produce maximum levels of product formation. Hence, the recombination mediator activity of Rad52 (1.4 μM) and the mutant Rad52-QDDD308-311AAAA (Rad52*) (2.8 μM) were examined. The % product (nc/(nc + ds) x 100) for each reaction was determined and plotted.