SUPPLEMENTARY FILES

Supplementary Table 1 Yeast and human homologous recombination factors

FACTOR	HUMAN	YEAST
BRCA1	DSB resection RAD51 loading Conservative HR Non-conservative HR	NA
BRCA2	RAD51 mediator Conservative HR	NA
RAD51	Strand exchange Conservative HR	Strand exchange Conservative HR
RAD52	Single-strand annealing Conservative HR Non-conservative HR	Rad51 mediator Single-strand annealing Conservative HR Non-conservative HR
RAD54	RAD51 mediator Conservative HR	Rad51 mediator Conservative HR

a NA – Not applicable

Supplementary Table 2 *Saccharomyces cerevisiae* strains used in this study

STRAIN	GENOTYPE [a]
ABX3600	*MAT**a**/α HIS3/his3-11, 15 LEU2/leu2-3, 112 URA3/ura3-1 RAD52/RAD52-FLAG-kanMX ADH1/adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1*
ABX3566	*MAT**a**/α HIS3/his3-11, 15 URA3/ura3-1*
ABX3568	*MAT**a**/α HIS3/his3-11, 15 URA3/ura3-1 rad52::TRP1/rad52::TRP1*
ABX3569	*MAT**a**/α HIS3/his3-11, 15 URA3/ura3-1 adh1::HsRAD52-hygMX-ADH1/adh1::HsRAD52-hygMX-ADH1*
ABX3570	*MAT**a**/α HIS3/his3-11, 15 URA3/ura3-1 rad52::TRP1/rad52::TRP1 adh1::HsRAD52-hygMX-ADH1/adh1::HsRAD52-hygMX-ADH1*
ABX3666-37B	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX*
ABX3678-49B	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad51::hisG-URA3-hisG*
ABX3697-82D	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad52::TRP1*
ABX3703-36B	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX URA3 adh1::HsRAD52-hygMX-ADH1*
ABX3728-11C	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad51::LEU2 rad52::TRP1 adh1::HsRAD52-hygMX-ADH1*
ABX3728-14A	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad51::hisG-URA3-hisG rad52::TRP1*
ABX3728-28A	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad51::hisG-URA3-hisG adh1::HsRAD52-hygMX-ADH1*

Supplementary Table 2 *Saccharomyces cerevisiae* strains - continued

STRAIN	GENOTYPE
ABM537	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-hygMX-ADH1*
ABM562	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad54::LEU2*
ABM563	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad52::TRP1 rad54::LEU2 adh1::HsRAD52-hygMX-ADH1*
ABM564	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad54::LEU2 adh1::HsRAD52-hygMX-ADH1*
ABM568	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad55::LEU2 adh1::HsRAD52-hygMX-ADH1*
ABM570	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad52::TRP1 rad55::LEU2 adh1::HsRAD52-hygMX-ADH1*
ABM571	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad55::LEU2*
ABX3761-10C	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1*
ABX3841-35C	*MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1*
W961-5A	*MAT**a** HIS3*
ABM559	*MATa::LEU2 his3-Δ3'-HOcs trp1::GAL-HO-kanMX RAD52-FLAG-kanMX*
ABX3684-12B	*MATa LEU2 rad52::TRP1 adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1*
ABT821	*MAT**a** HIS3* pLAY836 (*adh1::HsRAD51-MYC URA3*)
ABT822	*MATa::LEU2 his3-Δ3'-HOcs trp1::GAL-HO-kanMX RAD52-FLAG-kanMX* pLAY836 (*adh1::RAD51-MYC URA3*)

Supplementary Table 2 *Saccharomyces cerevisiae* strains - continued

STRAIN	GENOTYPE
ABT823	*MAT**a** HIS3* pLAY837 (*adh1::RAD52-MYC URA3*)
ABT824	*MATa::LEU2 his3-∆3'-HOcs trp1::GAL-HO-kanMX RAD52-FLAG-kanMX* pLAY837 (*adh1::RAD52-MYC URA3*)
ABT836	*MATa LEU2 rad52::TRP1 adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1* pLAY836 (*adh1::RAD51-MYC URA3*)
ABT838	*MATα LEU2 rad52::TRP1 adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1* pLAY837 (*adh1::RAD52-MYC URA3*)
ABT839	*MAT**a** HIS3* pLAY850 (*adh1::HsRAD52-MYC URA3*)
ABX3834-2D	*MATa::LEU2 his3-∆3'-HOcs LEU2-his3-∆Msc I*
ABX3844-23D	*MATa::LEU2 his3-∆3'-HOcs LEU2-his3-∆Msc I trp1::GAL-HO-kanMX RAD52-FLAG-kanMX*
ABX3879-17B	*MATa::LEU2 his3-∆3'-HOcs LEU2-his3-∆Msc I trp1::GAL-HO-kanMX rad51::hisG-URA3-hisG RAD52-FLAG-kanMX*
ABX3885-17B	*MATa::LEU2 his3-∆3'-HOcs LEU2-his3-∆Msc I trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1*
ABX3885-32C	*MATa::LEU2 his3-∆3'-HOcs LEU2-his3-∆Msc I trp1::GAL-HO-kanMX rad51::hisG-URA3-hisG rad52::TRP1 adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1*
ABT875	*MATa::LEU2 his3-∆3'-HOcs LEU2-his3-∆Msc I trp1::GAL-HO-kanMX rad51::LEU2 RAD52-FLAG-kanMX* pLAY606 (*URA3*)
ABT876	*MATa::LEU2 his3-∆3'-HOcs LEU2-his3-∆Msc I trp1::GAL-HO-kanMX rad51::LEU2 RAD52-FLAG-kanMX* pLAY864 (*URA3 adh1-HsRAD52-FLAG*)

[a] All strains are isogenic and possess the following genotype unless otherwise noted: *ade2-1 can1-100 his3-11,-15 leu2-3,-112 trp1-1 ura3-1*

Supplementary Table 3 Plasmids constructed for this study

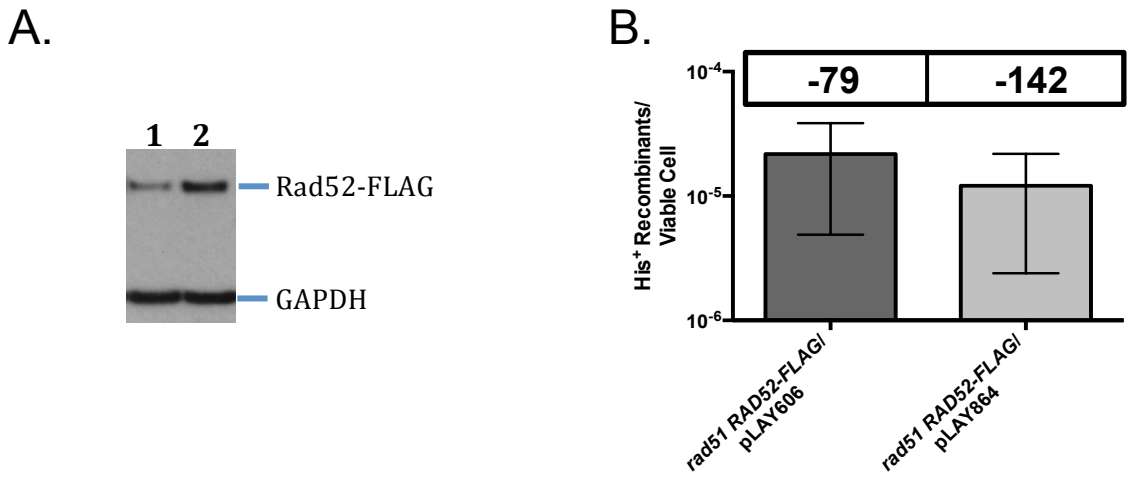
PLASMID	DESCRIPTION
pLAY606	*ADH1* promoter and terminator sequences flanking Not I site in a *URA3*-marked centromere plasmid
pLAY831	Coding sequence of *RAD51* inserted into the multiple cloning sequence of pGBT9
pLAY832	Coding sequence of *RAD51* inserted into the multiple cloning sequence of pGAD424
pLAY833	*ADH1* promoter and terminator sequences flanking Not I site that is in-frame with G8-9MYC epitope tag in the *URA3*-marked centromere plasmid pLAY606
pLAY836	Coding sequence of *RAD51* inserted into pLAY833
pLAY837	Coding sequence of *RAD52* inserted into pLAY833
pLAY850	Coding sequence of *HsRAD52* inserted into pLAY833
pLAY864	Coding sequence of *RAD52-FLAG* placed under the control of the *ADH1* promoter and terminator in pLAY606

Supplementary Table 4 Master Data

Genotype	IRR [a]	DSB-EGC [b]
WT	1.02×10^{2} (0.6, 1.41) [c] [1] [d]	1.72×10^{-3} (1.37, 2.07) [1]
RAD52-FLAG	n.d. [e]	2.92×10^{-3} (1.87, 3.96) [+1.7]
HsRAD52	8.6×10^{1} (7.4, 17.9) [-1.2]	2.32×10^{-3} (1.68, 2.97) [+1.4]
*rad51*Δ	n.d.	4.76×10^{-6} (1.62, 5.13) [-361]
*rad51*Δ *RAD52-FLAG*	n.d.	7.58×10^{-6} (6.28, 8.87) [-227]
*rad51*Δ *RAD52-FLAG/* pLAY606	n.d.	2.17×10^{-5} (0.49, 3.86) [-79]
*rad51*Δ *RAD52-FLAG/* pLAY864	n.d.	1.21×10^{-5} (0.24, 2.18) [-142]
*rad51*Δ *HsRAD52*	n.d.	1.19×10^{-4} (0.99, 1.39) [-14.5]
*rad52*Δ	4.22×10^{-1} (1.9, 8.99) [-242]	9.21×10^{-7} (6.52, 11.90) [-1868]
*rad52*Δ *HsRAD52*	4.6 (3.4, 6.1) [-22]	7.20×10^{-5} (5.73, 8.68) [-23.9]
*rad52*Δ *HsRAD52-FLAG*	n.d.	3.96×10^{-5} (1.13, 6.79) [-43]
*rad54*Δ	n.d.	3.47×10^{-5} (2.60, 4.30) (-49.6)
*rad54*Δ *HsRAD52*	n.d.	4.87×10^{-5} (3.41, 6.33) [-35.3]
*rad55*Δ	n.d.	9.81×10^{-5} (8.88, 10.75) [-17.5]
*rad55*Δ *HsRAD52*	n.d.	9.98×10^{-5} (6.47, 13.50) [-17.2]
*rad51*Δ *rad52*Δ	n.d.	6.69×10^{-8} (4.29, 9.10) [-25710]
*rad51*Δ *rad52*Δ *HsRAD52*	n.d.	5.77×10^{-5} (3.85, 7.68) [-29.8]
*rad52*Δ *rad54*Δ *HsRAD52*	n.d.	1.26×10^{-5} (1.11. 1.41) [-136.5]
*rad52*Δ *rad55*Δ *HsRAD52*	n.d.	5.25×10^{-5} (1.65. 8.86) [-32.8]

[a] Ionizing Radiation Resistance (% viable cells)
[b] Double-strand Break Stimulated Ectopic Gene Conversion Frequency (recombinants/viable cell)
[c] 95% confidence interval
[d] Fold difference from wild-type
[e] Not Determined

Supplementary Figure 1: Alignment of primary amino acid sequences of yeast and human RAD52 The amino acid sequences of Rad52 and HsRad52 were aligned using the CLC Sequence Viewer (CLC Bio, Aarhus, Denmark). identical residues are highlighted in blue.



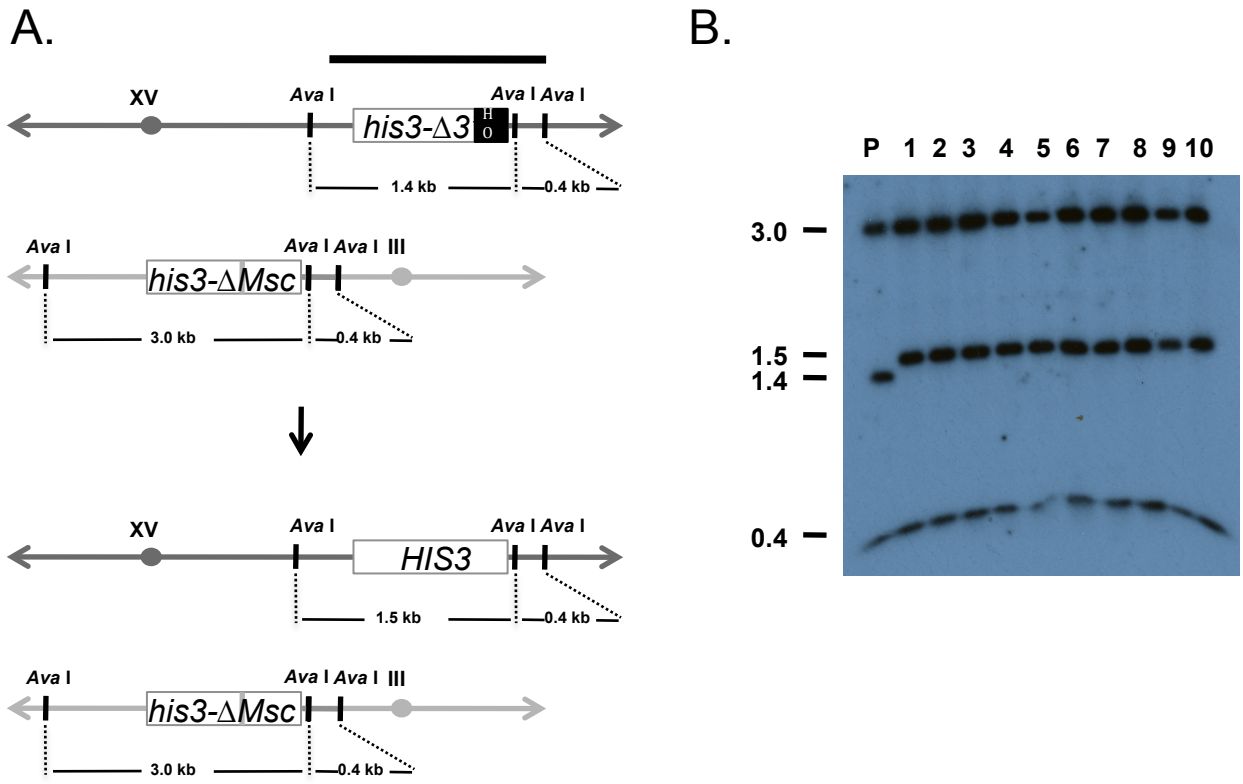
Supplementary Figure 2: Supplementary expression of *RAD52-FLAG* in a *rad51* mutant strain fails to stimulate frequencies of ectopic gene conversion

A.Western blot of strains containing plasmids that do, or do not facilitate expression of *RAD52-FLAG*

A haploid *rad51 RAD52-FLAG* strain was transformed with either pLAY606 (empty expression vector) or pLAY864 (expressing *RAD52-FLAG*). Aliquots of whole cell extracts were separated on gels, electroblotted, and probed with anti-FLAG or anti-GAPDH antibodies. Signals corresponding to the 55 kDa Rad52-FLAG and 37 kDa GAPDH proteins are denoted on the right side of the figure. Lanes: 1 – ABT875 (*rad51 RAD52-FLAG*/pLAY606); 2 – ABT876 (*rad51 RAD52-FLAG*/pLAY864)

B. EGC assays with strains containing plasmids that do, or do not facilitate expression of *RAD52-FLAG*

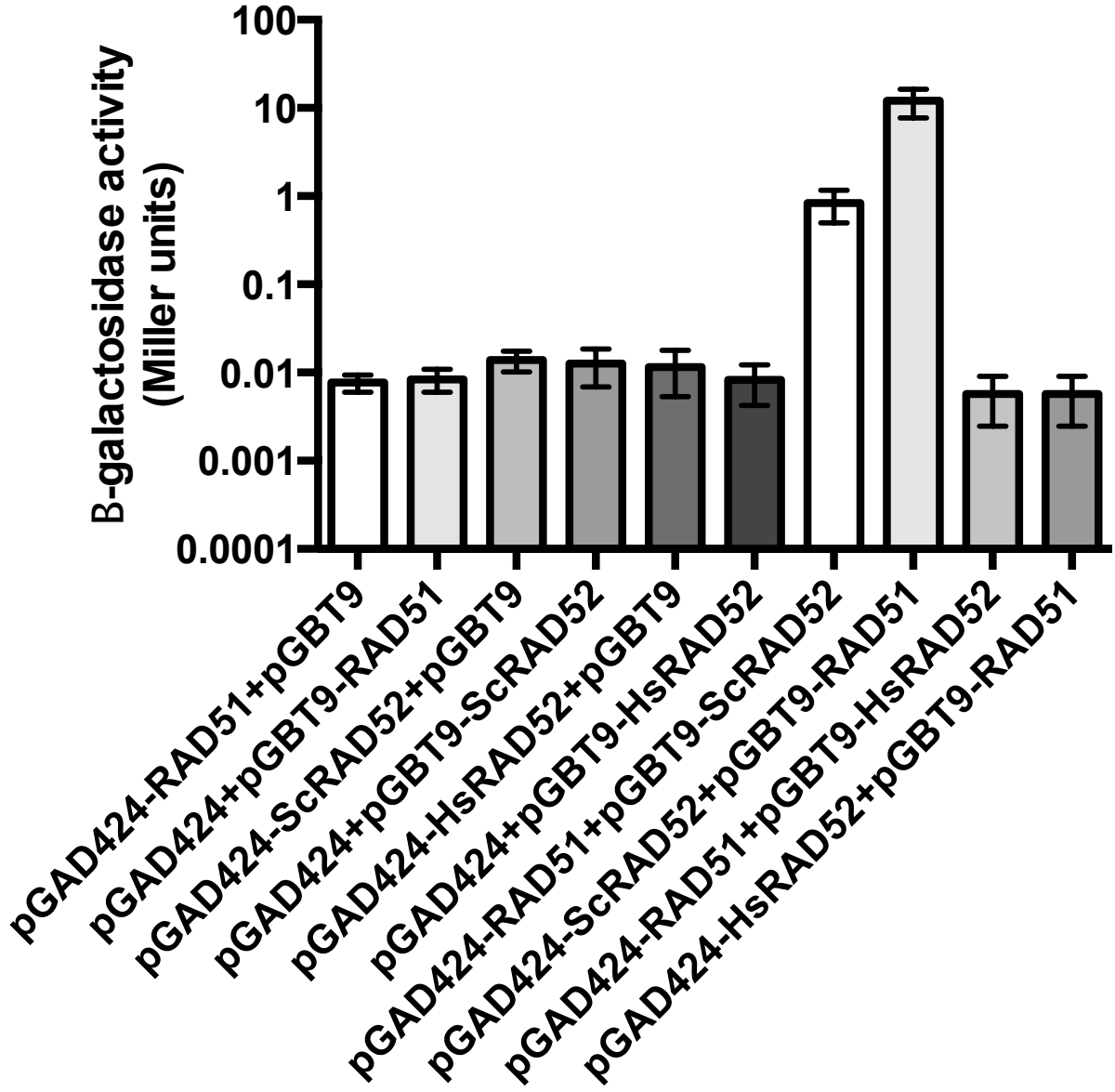
Single colonies of each strain were used to inoculate at least 10 one milliliter SD-Ura Glycerol/Lactate cultures and grown overnight. After inducing HO endonuclease cells were plated onto YPD to determine viability and SD-His to select for recombinants. Frequencies of EGC were determined by dividing the number of recombinants by the number of cells plated. Mean recombination frequencies and 95% confidence levels were plotted. Fold differences below wild-type are indicated in the boxes. Strains used in this analysis: ABT875 (*rad51 RAD52-FLAG*/pLAY606); ABT876 (*rad51 RAD52-FLAG*/pLAY864)



Supplementary Figure 3: Detailed analysis of the *his3* ectopic gene conversion assay

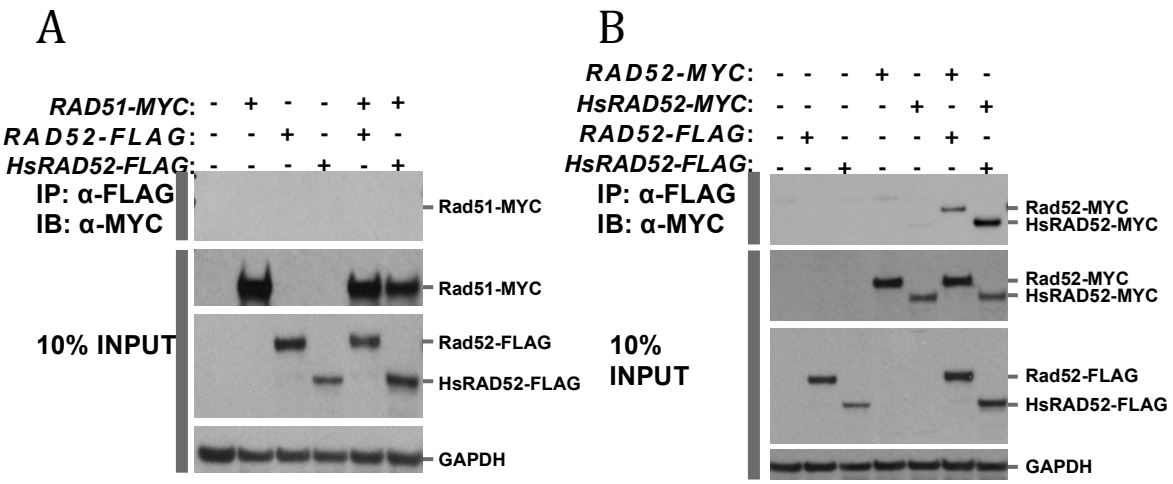
A. Graphic depiction of the *his3* ectopic gene conversion assay components The *his3-Δ3'-HOcs* substrate (white "*his3Δ-3'*" box) at the *HIS3* locus on chromosome XV (dark gray double ended arrow) substitutes a 127 bp DNA fragment containing an HO cut site (black "HO" box) for 238 bp of the 3' end of the *HIS3* coding sequence and flanking DNA. The *his3-ΔMsc* I substrate (white "*his3-ΔMsc* I" box) at the *LEU2* locus on chromosome III (light gray double ended arrow) is comprised of a 1.8 kb genomic clone containing the *HIS3* gene that has been disrupted by the insertion of a 10 bp *Not* I linker into the *Msc* I site in the coding sequence. Repair of an HO-catalyzed DSB at the *his3-Δ3'-HOcs* substrate by unidirectional transfer of information from the *his3-ΔMsc* I substrate (black arrow) creates an intact *HIS3* gene. The genomic sequence corresponding to the probe used to reveal the *his3* sequences in the genomic Southern blot is indicated by the solid black line above the white *his3Δ-3'* box. The positions of the *Ava* I restriction sites flanking the *his3Δ-3'* and *his3-ΔMsc* I substrates, and their distances from one another in kb are depicted.

B.Genomic Southern blot of parental and recombinant strains Genomic DNA prepared from a His^- parental strain, and 10 independent His^+ recombinants was digested with *Ava* I restriction endonuclease, run on an agarose gel, electroblotted to nylon, probed with a ^{32}P-labeled *HIS3* sequence, and complementary sequences visualized by exposure to X-ray film. Lanes carrying DNA from His^- parental (P) and His^+ recombinant (1-10) strains are marked at the top of the figure. Positions of the 1.4 and 0.4 kb *his3-Δ3'* substrate bands, 3.0 and 0.4 kb *his3-ΔMsc* I bands, and 1.5 and 0.4 *HIS3* recombinant product bands are denoted on the left side of the figure.



Supplementary Figure 4: Yeast two-hybrid studies of interactions between Rad51, and Rad52 or HsRAD52

The yeast strain Y187 was transformed with the plasmids pGBT9 and pGAD424, or their derivatives containing *RAD51*, *RAD52*, or *HsRAD52* sequences and grown on synthetic complete medium lacking leucine and tryptophan from which whole cell extracts prepared. β-galactosidase specific activities, in Miller units were determined in the extracts of a minimum of 10 independent cultures of each transformant. Mean specific activities and 95% confidence are plotted.



Supplementary Figure 5: Co-immunoprecipitation analysis of intermolecular interactions between Rad51, Rad52 and HsRAD52

Whole cell extracts were prepared from haploid yeast strains with or without chromosomal copies of *RAD52-FLAG*, or *adh1::HsRAD52-FLAG*, and with or without plasmid-borne copies of *RAD51-MYC*, *RAD52-MYC*, or *adh1::HsRAD52-FLAG*. Aliquots of extracts were exposed to anti-FLAG antibody, and the immunoprecipitated proteins separated on gels, electroblotted, and probed with anti-MYC antibody. Aliquots of extract 10% of the volume submitted to immunoprecipitation were run directly on gels, electroblotted, and probed with anti-MYC, anti-FLAG or anti-GAPDH antibodies. Proteins were visualized by treating blots with HRP-conjugated secondary antibodies and chemiluminescent detection reagents, and exposure to X-ray film.

A. Yeast Rad52 protein can interact with yeast Rad51 protein but human RAD52 protein cannot Presence of genomic copies of the *RAD52-FLAG* or *HsRAD52-FLAG* fusion genes, and/or a plasmid copy of *RAD51-MYC* in the yeast strains used to make the whole cell extracts are denoted with a (+) at the top of the figure. The top panel depicts proteins immunoprecipitated (IP) by anti-FLAG antibody and immunoblotted (IB) with anti-MYC antibody. The bottom three panels depict the proteins in 10% INPUT. Signals corresponding to the 55 kDa Rad52-FLAG, 49 kDa HsRAD52-FLAG, 44 kDa Rad51-MYC, and 37 kDa GAPDH proteins are denoted on the right side of the figure.

B. Both the yeast Rad52 and human RAD52 proteins self-interact Presence of genomic copies of the *RAD52-FLAG* or *HsRAD52-FLAG* fusion genes, and/or plasmid copies of *RAD52-MYC* or *HsRAD52-MYC* in the yeast strains used to make the whole cell extracts are denoted with a (+) at the top of the figure. The top panel depicts proteins immunoprecipitated (IP) by anti-FLAG antibody and immunoblotted (IB) with anti-MYC antibody. The bottom three panels depict the proteins in 10% INPUT. Signals corresponding to the 55 kDa Rad52-FLAG, 49 kDa HsRAD52-FLAG, 53 kDa Rad52-MYC, 47 kDa HsRAD52-MYC, and 37 kDa GAPDH proteins are denoted on the right side of the figure.