

SUPPLEMENTAL MATERIAL

Table S1 – *Saccharomyces cerevisiae* strains used in this study

| STRAIN | GENOTYPE ^(a) | ASSAY | ORIGIN |
|-------------|--|----------------------------|----------------------|
| ABM325 | <i>MATa::LEU2 his3-Δ3'-HOcs-URA3-his3-Δ5' trp1::GAL-HO-kanMX</i> | DSB-DRR ^(b) | This study |
| ABM326 | <i>MATa::LEU2 his3-Δ3'-HOcs-URA3-his3-Δ5' trp1::GAL-HO-kanMX rad52::TRP1</i> | DSB-DRR | This study |
| ABM507 | <i>MATa::LEU2 his3-Δ3'-HOcs-URA3-his3-Δ5' trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-hygMX-ADH1</i> | DSB-DRR | This study |
| ABX3479-1A | <i>MATα his3-Δ3'-URA3-his3-Δ5'</i> | S-DRR ^(c) | This study |
| ABX3479-2C | <i>MATa his3-Δ3'-URA3-his3-Δ5' rad52::TRP1</i> | S-DRR | This study |
| ABX3479-3A | <i>MATa his3-Δ3'-URA3-his3-Δ5' rad52::TRP1 adh1::HsRAD52-hygMX-ADH1</i> | S-DRR | This study |
| ABX3566 | <i>MATa/α HIS3/his3-11, 15 URA3/ura3-1</i> | IRR ^(d) | Manthey et al., 2017 |
| ABX3568 | <i>MATa/α HIS3/his3-11, 15 URA3/ura3-1 rad52::TRP1/rad52::TRP1</i> | IRR | Manthey et al., 2017 |
| ABX3666-37B | <i>MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX</i> | DSB-EGC ^(e) | Manthey et al., 2017 |
| ABX3684-12B | <i>MATα LEU2 adh1::HsRAD52-FLAG-hygMX-ADH1</i> | Western ^(f) | This study |
| ABX3697-82D | <i>MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad52::TRP1</i> | DSB-EGC | Manthey et al., 2017 |
| ABX3720-6C | <i>MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-G59R-FLAG-kanMX-hygMX-ADH1</i> | DSB-EGC | This study |
| ABX3782-2D | <i>MATα URA3 adh1::HsRAD52-G59R-FLAG-kanMX-hygMX-ADH1</i> | Western | This study |
| ABX3817-6C | <i>MATa ADE2 trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-hygMX-ADH1</i> | MTI ^(g) | This study |
| ABX3817-7D | <i>MATa ADE2 trp1::GAL-HO-kanMX rad52::TRP1</i> | MTI | This study |
| ABX3817-15B | <i>MATa ADE2 trp1::GAL-HO-kanMX</i> | MTI | This study |
| ABX3885-17B | <i>MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1</i> | DSB EGC | Manthey et al., 2017 |
| ABX3933-46C | <i>MATa hmr::hygMX trp1::GAL-HO-kanMX</i> | MTI | This study |
| ABX3943-3B | <i>MATa hmr::hygMX trp1::GAL-HO-kanMX rad52::TRP1</i> | MTI ChIP ^(h) | This study |

| | | | |
|-------------|--|----------------------------------|---------------------|
| ABX3943-7A | <i>MATa hmr::hygMX trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1</i> | MTI ChIP RS ⁽ⁱ⁾ | This study |
| ABX3961-4C | <i>MATa hmr::hygMX trp1::GAL-HO-kanMX URA3 RAD52-FLAG-kanMX</i> | MTI ChIP RS | This study |
| ABX3970-88A | <i>MATa::LEU2 his3-Δ3'-HOcs-URA3-his3-Δ5' trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-G59R-FLAG-kanMX-hygMX-ADH1</i> | DSB-DRR | This study |
| ABX3974-11C | <i>MATa ADE2 adh1::HsRAD52-S346X-FLAG-kanMX-hygMX-ADH1</i> | Western | This study |
| ABX3975-15A | <i>MATa::LEU2 his3-Δ3'-HOcs-URA3-his3-Δ5' trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-S346X-FLAG-kanMX-hygMX-ADH1</i> | DSB-DRR | This study |
| ABX-3976-2A | <i>MATa::LEU2 his3-Δ3'-HOcs LEU2-his3-ΔMsc I trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-S346X-FLAG-kanMX-hygMX-ADH1</i> | DSB-EGC | This study |
| ABX3985-55B | <i>MATa HIS3 hmr::hygMX trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-G59R-FLAG-kanMX-hygMX-ADH1</i> | MTI ChIP RS | This study |
| ABX3994-18D | <i>MATa hmr::hygMX trp1::GAL-HO-kanMX rad52::TRP1 adh1::HsRAD52-S346X-hygMX-ADH1</i> | MTI ChIP RS | This study |
| ABX4129 | <i>MATa/α HIS3/his3-11, 15 URA3/ura3-1 rad52::TRP1/rad52::TRP1 adh1::HsRAD52-G59R-FLAGkanMX--hygMX-ADH1/adh1::HsRAD52-G59R-FLAG-kanMX-hygMX-ADH1</i> | IRR | This study |
| ABX4130 | <i>MATa/α HIS3/his3-11, 15 URA3/ura3-1 rad52::TRP1/rad52::TRP1 adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1/adh1::HsRAD52-FLAG-kanMX-hygMX-ADH1</i> | IRR | This study |
| ABX4131 | <i>MATa/α HIS3/his3-11, 15 URA3/ura3-1 rad52::TRP1/rad52::TRP1 adh1::HsRAD52-S346X-FLAG-kanMX-hygMX-ADH1/adh1::HsRAD52-S346X-FLAG-kanMX-hygMX-ADH1</i> | IRR | This study |
| R113a | <i>MATa ADE2 CAN1 HIS3 LEU2 TRP1 URA3 tyr1</i> | MTI tester | Rodney Rothstein |

(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

(b) Double-strand break stimulated direct repeat recombination

(c) Spontaneous direct repeat recombination

(d) Ionizing radiation resistance

(e) Double-strand break stimulated ectopic gene conversion

- (f) Western blot
- (g) Mating type interconversion
- (h) Chromatin immuno-precipitation
- (i) Repair synthesis

Table S2 – Plasmids used in this study

| NAME | DESCRIPTION | SOURCE |
|---------------------|--|----------------------|
| pGBT9 | Multi-copy bacterial-yeast shuttle vector containing a <i>TRP1</i> selectable marker and the coding sequence of Gal4 DNA binding domain | Clontech |
| pGBT9-HsRAD52 | Derivative of pGBT9 for expression of wild-type HsRAD52-Gal4 DNA binding domain fusion | Manthey et al., 2017 |
| pGBT9-HsRAD52-G59R | Derivative of pGBT9-HsRAD52 for expression of mutant HsRAD52-G59R-Gal4 DNA binding domain fusion | This study |
| pGBT9-HsRAD52-S346X | Derivative of pGBT9-HsRAD52 for expression of mutant HsRAD52-S346X-Gal4 DNA binding domain fusion | This study |
| pGAD424 | Multi-copy bacterial-yeast shuttle vector containing a <i>LEU2</i> selectable marker and the coding sequence of Gal4 transcription activation domain | Clontech |
| pGAD424-HsRAD52 | Derivative of pGAD424 for expression of wild-type HsRAD52-Gal4 transcription activation domain fusion | Manthey et al., 2017 |
| pLAY700 | Derivative of pGAD424-HsRAD52 for expression of mutant HsRAD52-G59R-Gal4 transcription activation domain fusion | This study |
| pLAY701 | Derivative of pGAD424-HsRAD52 for expression of mutant HsRAD52-S346X-Gal4 transcription activation domain fusion | This study |
| pET28b | Plasmid containing <i>kanR</i> selectable marker for expression of N-terminally 6-His-tagged proteins in <i>E. coli</i> | Novagen |
| pLAY855 | pET28b expressing N-terminally 6-His-tagged wild-type HsRAD52 ₍₁₋₂₁₂₎ | This study |
| pLAY970 | pET28b expressing N-terminally 6-His-tagged mutant HsRAD52-S346X | This study |
| pLAY971 | pET28b expressing N-terminally 6-His-tagged mutant HsRAD52-G59R ₍₁₋₂₁₂₎ | This study |

Table S3 – Oligonucleotide primers used in this study

| NAME | SEQUENCE | APPLICATION |
|-------------|--------------------------------------|---|
| MATYaF-1812 | 5' – CAA TAT CAC CCC AAG CAC G – 3' | <i>MATa</i> recipient ChIP |
| MATYaR-1922 | 5' – GAA GTG GAG TAA TGC CAC – 3' | <i>MATa</i> recipient ChIP |
| HMLz2F-3852 | 5' – CAG ACT CAA GCA AAC AAT C – 3' | <i>HMLα</i> donor ChIP |
| HMLR-4040 | 5' – GAG GGC ACA AGG AAC ACG – 3' | <i>HMLα</i> donor ChIP |
| SAMF-(-192) | 5' - CAC TCT GGT AGC GAT GAA A – 3' | <i>SAM1</i> reference for ChIP and repair synthesis |
| SAMR-(-106) | 5' - CGA TGA ATA ACA GAC AAC AC – 3' | <i>SAM1</i> reference for ChIP and repair synthesis |
| Slxt-f | 5' - GCA GCA CGG AAT ATG GGA CT – 3' | Repair synthesis |
| Slxt-r | 5 – ATG TGA ACC GCA TGG GCA GT – 3' | Repair synthesis |

Table S4 – Master Data

| Genotype | IRR ^(a) | DSB-DRR ^(b) | S-DRR ^(c) | MTI ^(d) | DSB-EGC ^(e) |
|--|---|---|----------------------------------|--|--|
| WT | 33.2 (30.0, 36.5) ^(f) [1] | 2.27x10 ⁻¹ (1.82, 2.72) [1] | 3.86x10 ⁻⁵ [1] | 5.96x10 ⁻¹ (5.53, 6.40) [1] | 1.72x10 ⁻³ ^(g) (1.37, 2.07) [1] |
| <i>rad52</i> Δ | 4.3x10 ⁻² (2.2, 6.3) [-767] ^(h) | 3.41x10 ⁻² (2.18, 4.65) [-6.7] | 1.94x10 ⁻⁶ [-19.9] | 8.17x10 ⁻⁴ (5.81, 10.53) [-729] | 9.21x10 ⁻⁷ ^(g) (6.52, 11.90) [-1868] |
| <i>rad52</i> Δ <i>HsRAD52</i> | n.d. ⁽ⁱ⁾ | 2.26x10 ⁻¹ (1.50, 3.01) [+1.0] | 7.10x10 ⁻⁵ [+1.8] | 6.49x10 ⁻² (4.53, 8.45) [-9.2] | 7.20x10 ⁻⁵ ^(g) (5.73, 8.68) [-23.9] |
| <i>rad52</i> Δ <i>HsRAD52-FLAG</i> | 8.8x10 ⁻¹ (7.1, 10.4) [-37.7] | n.d. | n.d. | 5.66x10 ⁻² (3.07, 8.25) [-10.5] | 3.96x10 ⁻⁵ ^(g) (1.13, 6.79) [-43] |
| <i>rad52</i> Δ <i>HsRAD52-G59R-FLAG</i> | 2.9x10 ⁻² (1.7, 4.1) [-1145] | 1.74x10 ⁻¹ (1.25, 2.24) [-1.3] | n.d. | 1.17x10 ⁻³ (0.58, 2.07) [-509] | 2.67x10 ⁻⁶ (1.80, 3.55) [-644] |
| <i>rad52</i> Δ <i>HsRAD52-S346X-FLAG</i> | 4.7x10 ⁻² (2.6, 6.7) [-706] | 3.82x10 ⁻¹ (2.18, 5.46) [+1.7] | n.d. | 1.74x10 ⁻³ (0.62, 3.11) [-343] | 1.16x10 ⁻⁶ (0.50, 1.83) [-1483] |

^(a) Ionizing Radiation Resistance (% viable cells after exposure to 320 Gy of X-ray radiation)

^(b) Double-strand Break Stimulated Direct Repeat Recombination Frequency (recombinants/viable cell)

^(c) Spontaneous Direct Repeat Recombination Rate (events/cell/generation)

^(d) Mating Type Interconversion Frequency (recombinants/viable cell)

^(e) Double-strand Break Stimulated Ectopic Gene Conversion Frequency (recombinants/viable cell)

^(f) 95% confidence interval

^(g) From Manthey et al, 2017

^(h) Fold difference from wild-type

⁽ⁱ⁾ Not Determined

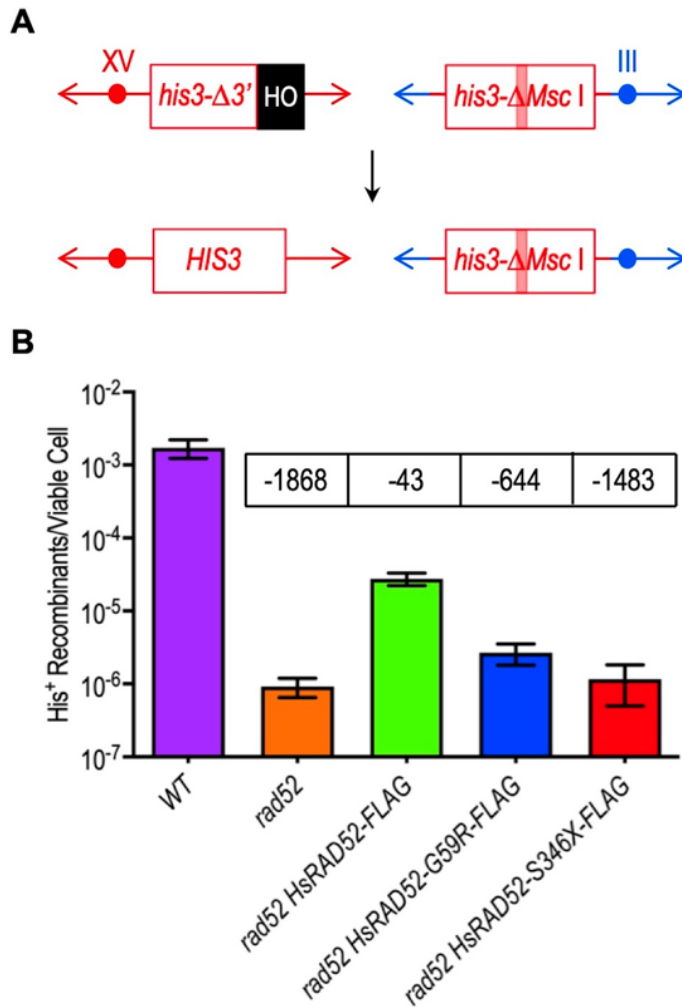
Table S5 – Y2H Data

| Plasmids | β-Galactosidase Activity (Miller Units) |
|---|---|
| pGAD424/pGBT9 | 0.010 (0.008, 0.012) ^(a) [1] |
| pGAD424/pGBT9-HsRAD52 | 0.008 (0.005, 0.011) [-1.3] ^(b) |
| pGAD424-HsRAD52/pGBT9 | 0.012 (0.006, 0.017) [+1.2] |
| pGAD424-HsRAD52/ pGBT9-HsRAD52 | 0.795 (0.642, 0.948) [+80] |
| pGAD424-HsRAD52-G59R/ pGBT9-HsRAD52-G59R | 1.039 (0.773, 1.306) [+104] |
| pGAD424-HsRAD52-S346X/ pGBT9-HsRAD52-S346X | 0.878 (0.646, 1.111) [+88] |

^(a) 95% confidence interval

^(b) Fold difference from wild-type

Figure S1

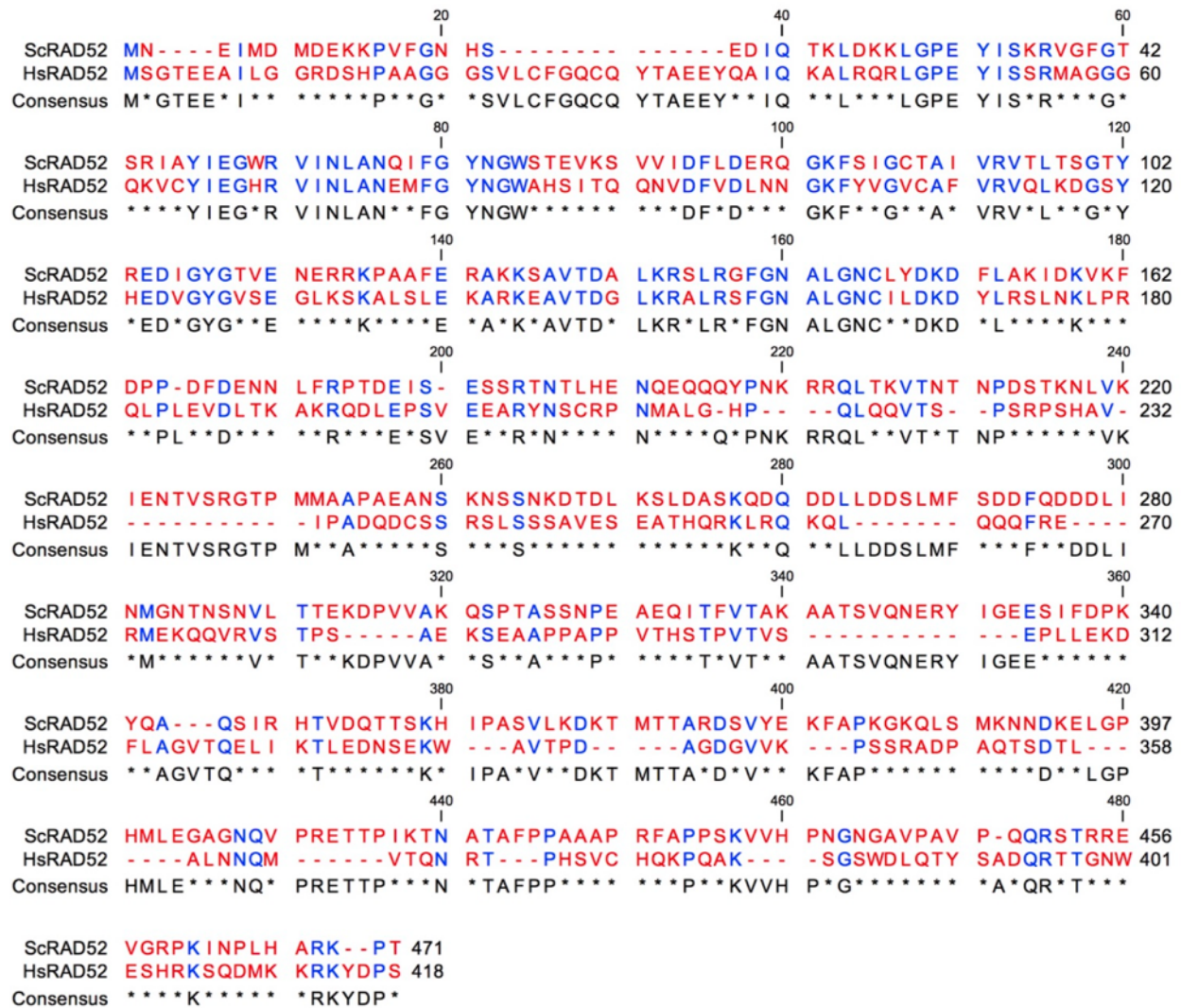


The *adh1::HsRAD52-G59R-FLAG* and *adh1::HsRAD52-S346X-FLAG* alleles do not suppress the ectopic gene conversion defect of *rad52* mutant yeast cells

- A. Cartoon depicting DSB-stimulated *his3* ectopic gene conversion (EGC) assay The *his3-Δ3'-HOcs* substrate (white "*his3Δ-3'*" box) at the *HIS3* locus on chromosome XV (red 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) proximal to the *LEU2* locus on chromosome III (blue 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 (pink bar). Repair of a 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.
- B. The *adh1::HsRAD52-G59R-FLAG* and *adh1::HsRAD52-S346X-FLAG* alleles confer defects in DSB-stimulated EGC Single colonies of haploid wild-type (ABX3666-37B), *rad52* (ABX3697-82D), *rad52 adh1::HsRAD52-FLAG* (ABX3885-17B), *rad52 adh1::HsRAD52-G59R-FLAG* (ABX3720-6C), and *rad52 adh1::HsRAD52-S346X* (ABX3976-2A) strains carrying the EGC assay components were used to inoculate at least

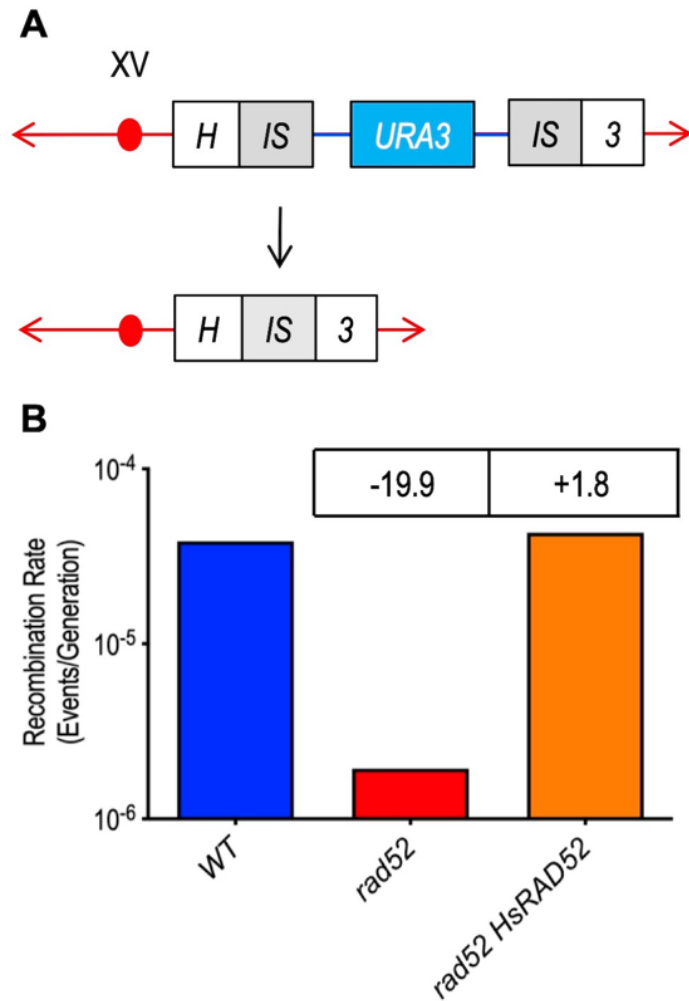
10 one milliliter YPGL cultures and grown overnight. Following a period of expression of HO endonuclease, appropriate dilutions were plated onto YPD medium to determine viability or medium lacking histidine to select for recombinants and incubated for three days at 30°. Colonies were counted and frequencies of EGC determined by dividing the number of His⁺ recombinants by the number of viable cells plated. Mean frequencies of EGC and 95% confidence intervals were plotted against genotype. Fold differences below (-) wild-type are in boxes above the plot of each mean frequency.

Figure S2



Alignment of primary amino acid sequences of yeast and human RAD52 proteins 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.

Figure S3

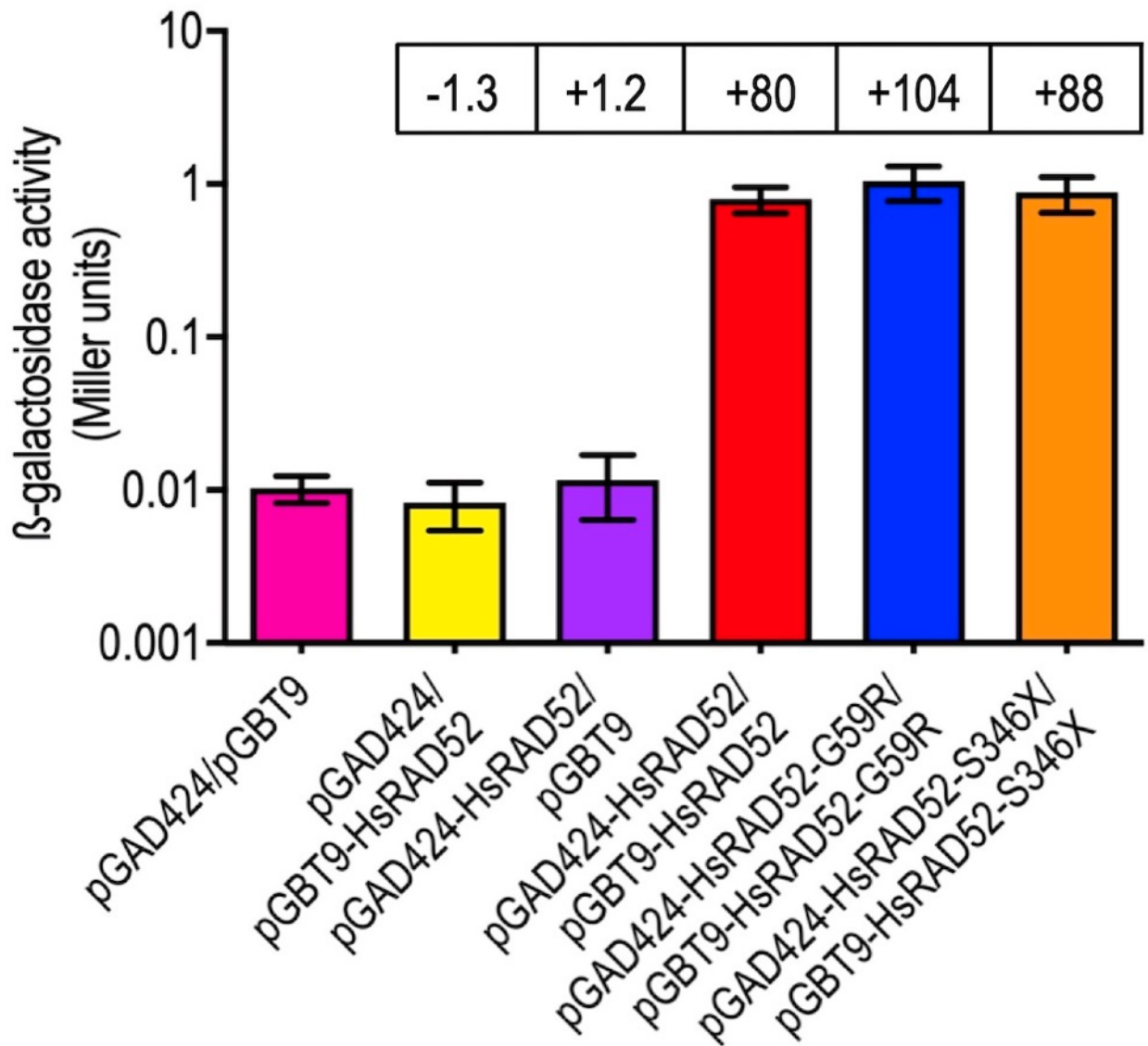


The *adh1::HsRAD52* allele complements the loss of spontaneous direct repeat recombination in *rad52* mutant yeast cells

A. Cartoon depicting assay for spontaneous recombination between non-tandem direct repeats Spontaneous recombination between duplicate segments of the *HIS3* coding sequence (gray *IS* boxes) flanking a 5kb *URA3*-marked (aqua box) plasmid (blue bar) inserted into the *HIS3* locus on chromosome XV creates an intact *HIS3* gene while deleting the plasmid.

B. The *adh1::HsRAD52* allele supports wild-type rates of spontaneous DRR in *rad52* mutant cells Single colonies of haploid wild-type (ABX3479-1A), *rad52* (ABX3479-2C), and *rad52 adh1::HsRAD52-FLAG* (ABX3479-3A) strains were used to inoculate a minimum of 10 one milliliter cultures of synthetic medium lacking uracil and grown overnight. Appropriate dilutions were plated onto solid YPD medium to determine viability, and onto medium lacking histidine to select for recombinants. Following incubation for three days at 30° colonies were counted and frequencies of DRR determined by dividing the number of His⁺ recombinants by the number of viable cells plated. Rates of DRR were plotted against genotype. Fold differences above (+) and below (-) wild-type are in boxes above the plot of each mean frequency.

Figure S4



HsRAD52-G59R and HsRAD52-S346X display wild-type levels of self-association in the yeast two-hybrid assay Single colonies of the yeast strains bearing plasmids containing genes encoding N-terminal fusions of wild-type or mutant HsRAD52 to the transcriptional activation and DNA binding domains of Gal4 were used to inoculate at least 10 five milliliter cultures of synthetic medium lacking leucine and tryptophan and grown to saturation at 30°C. Fold differences above (+) and below (-) background (pGAD424/pGBT9) mean β -galactosidase activity levels are in boxes above the plot.