Expanded View Figures

Figure EV1. BIR efficiency is mildly decreased in annealing mutants.

A Schematic of the H-0 BIR assay. DSB is induced at a modified *MAT*a locus (Chr. III). Strand invasion occurs within the "Z" sequence (blue box) and DNA synthesis continues to the end of the chromosome.

B Representative Southern blots showing DSB repair products in WT, *rad59*Δ, *rad52-R70A*, and *rad59*Δ *rad52-R70A* cells. DNA was digested with *Bsp*1286I and probed with a *MAT*-distal sequence (yellow box in panel A).

C Viability of indicated strains is shown (mean ± SD; $n = 3$).

D Repair efficiency compared to parental *MAT*a at time 0 h (left) and repair efficiency of indicated mutants compared to WT by 6 h (right) (mean ± SD; $n \geq 3$).

E Representative Southern blots showing BIR repair product in H-0 system in WT and indicated mutant cells.

F Repair efficiency compared to parental *MAT*a at time 0 h (left) and repair efficiency of indicated mutants compared to WT by 6 h (right) (mean ± SD; $n \geq 3$).

G Model presenting the possible function of DNA binding/annealing domain of Rad52 in stabilizing the D-loop. Rad52 stabilizes/extends D-loop by three-strand exchange (left) or Rad52 anneals a 3′ invading strand unwound from its template back to disrupted, but still RPA-bound, D-loop (right).

Data information: Welch's unpaired *t*-test was used to determine the *P*-value in all panels. **P*-value 0.01 to 0.05, significant; ***P*-value 0.001 to 0.01, very significant; ****P*-value 0.0001 to 0.001, extremely significant; ****$P < 0.0001$, extremely significant; $P \geq 0.05$, not significant (ns).

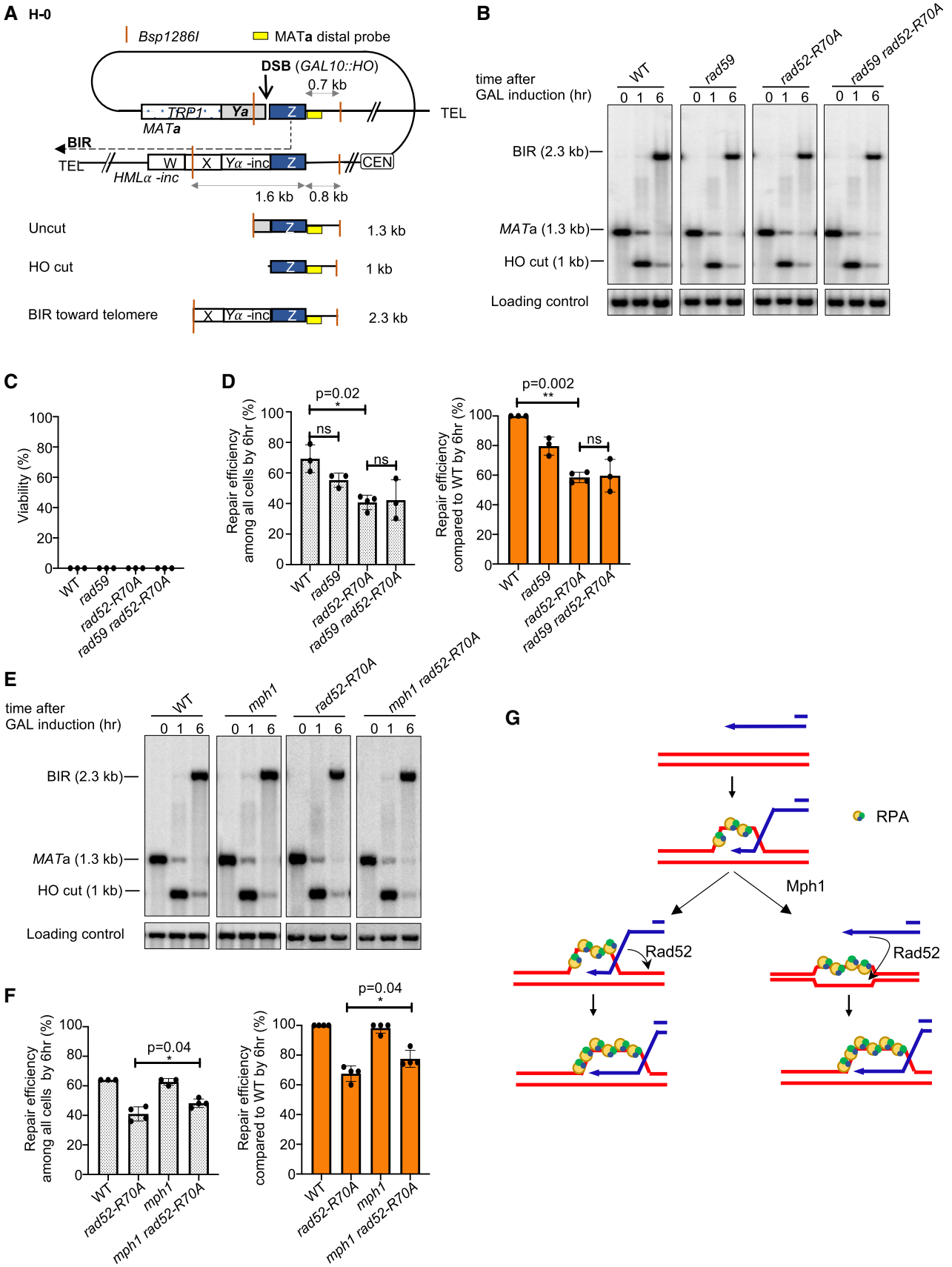
A H-0
Bsp1286I
MATa distal probe
DSB (GAL10::HO)
0.7 kb
TRP1
Ya
Z
MATa
TEL
BIR
TEL
W
X
Yα -inc
Z
CEN
HMLα -inc
1.6 kb
0.8 kb
Uncut
1.3 kb
HO cut
1 kb
BIR toward telomere
2.3 kb
B
time after GAL induction (hr)
WT
rad59
rad52-R70A
rad59 rad52-R70A
0 1 6
BIR (2.3 kb)
MATa (1.3 kb)
HO cut (1 kb)
Loading control
C
Viability (%)
D
Repair efficiency among all cells by 6hr (%)
p=0.02
ns
Repair efficiency compared to WT by 6hr (%)
p=0.002
E
mph1
mph1 rad52-R70A
F
p=0.04
G
RPA
Mph1
Rad52

Figure EV1.

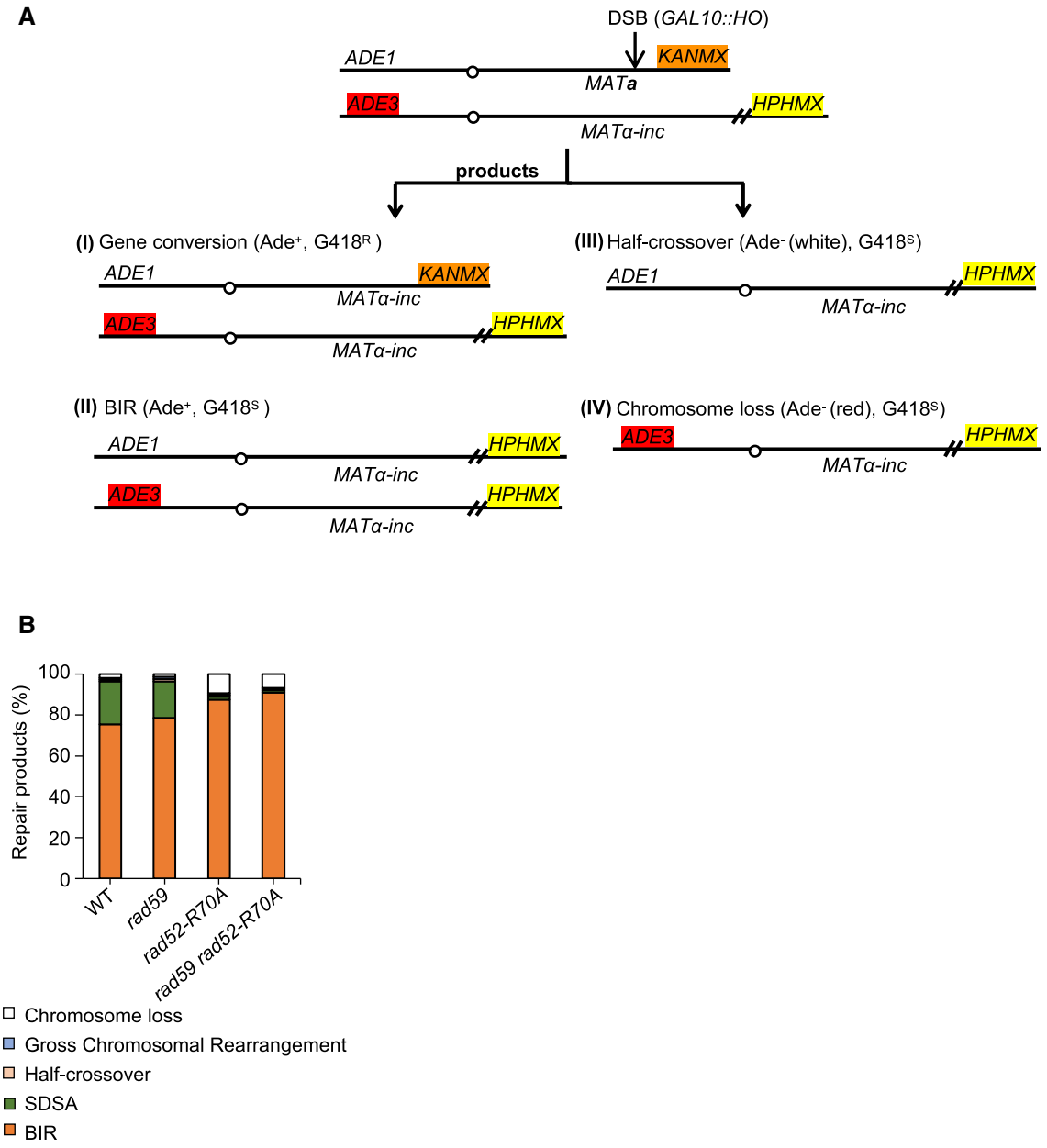

Figure EV2. Analysis of BIR efficiency in a disomic BIR system in annealing mutants.

A Schematic of the disomic BIR assay. DSB is induced at a modified *MAT*a locus on a truncated chromosome III, with only 46 bp homology to the right of the DSB. Strand invasion occurs within *MAT* sequences within the full-length chromosome III and replication continues until the end of the template chromosome. Chromosome ends are marked with *ADE1*, *ADE3*, *KANMX, and HPHMX* to distinguish different repair products or chromosome loss.

B Percentage of different repair products and chromosome loss. At least 500 colonies from YEP-Galactose plates were scored per mutant.

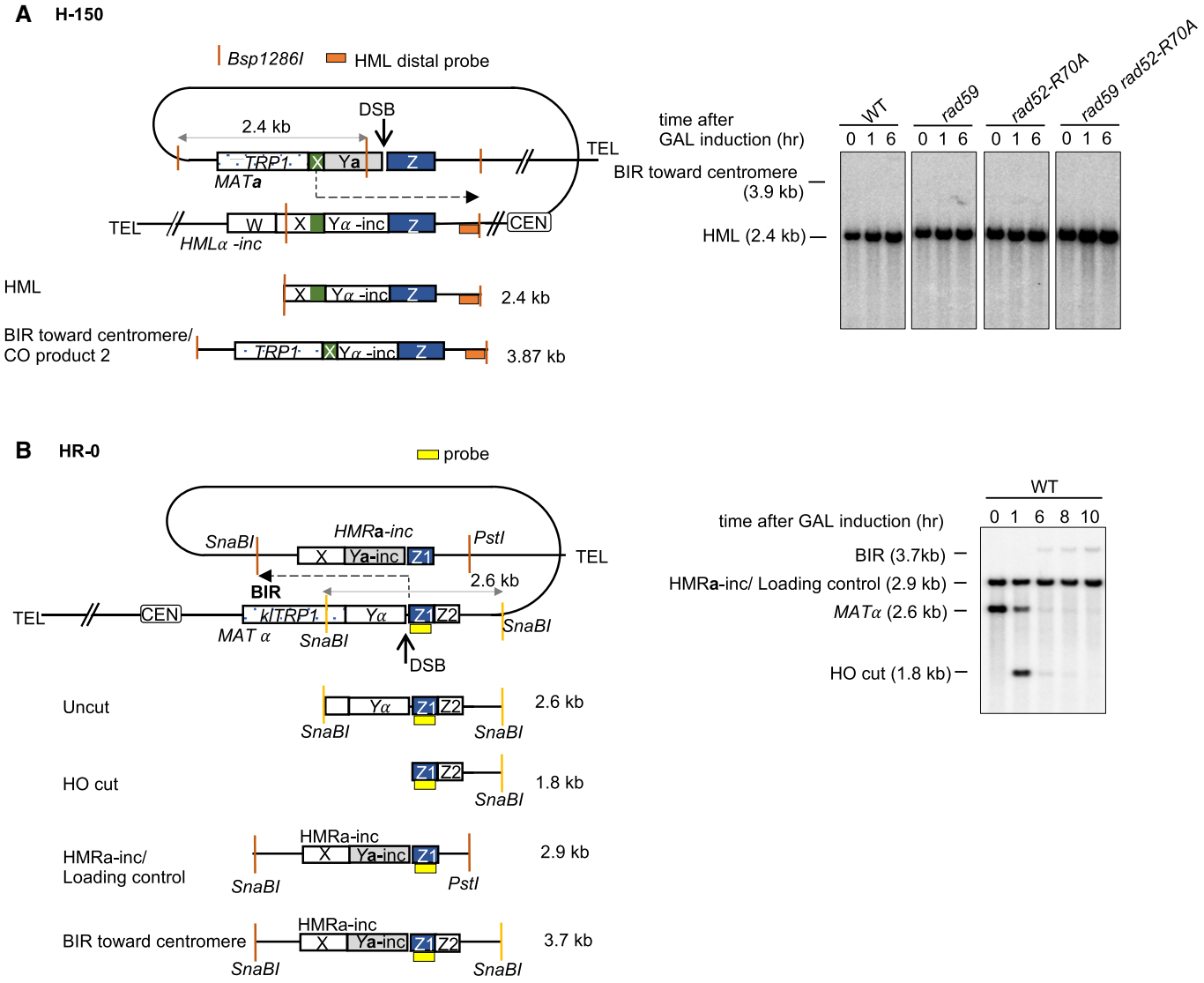

Figure EV3. Analysis of BIR toward the centromere.

A Schematic of H-150 assay with position of HML distal probe (left) and representative Southern blot (right) are shown. No BIR product was detected in the direction of the centromere.

B Schematic of HR-0 assay with sites of restriction enzymes, position of probe, and size of different bands (left) and representative Southern blot (right) are shown. Weak BIR product is visible.

Figure EV4. Analysis of Sir2 role in repair pathway choice when the template is within unsilenced region.

A Schematic of recombination assay on chromosome V, where a break is induced at *URA3* and is repaired with a partial *URA3* region ("*RA*") fragment inserted on the other arm of the chromosome V.

B Percentage of different repair products (left) and viability (right) of WT, *rad52-R70A, and sir2*Δ cells (mean ± SD; $n = 3$).

C Representative of Southern blots (left) and graph showing kinetics of BIR in H-0 assay (right) of WT and *sir2*Δ cells (mean ± SD; $n = 3$).

Data information: Welch's unpaired *t*-test was used to determine the *P*-value in all panels.

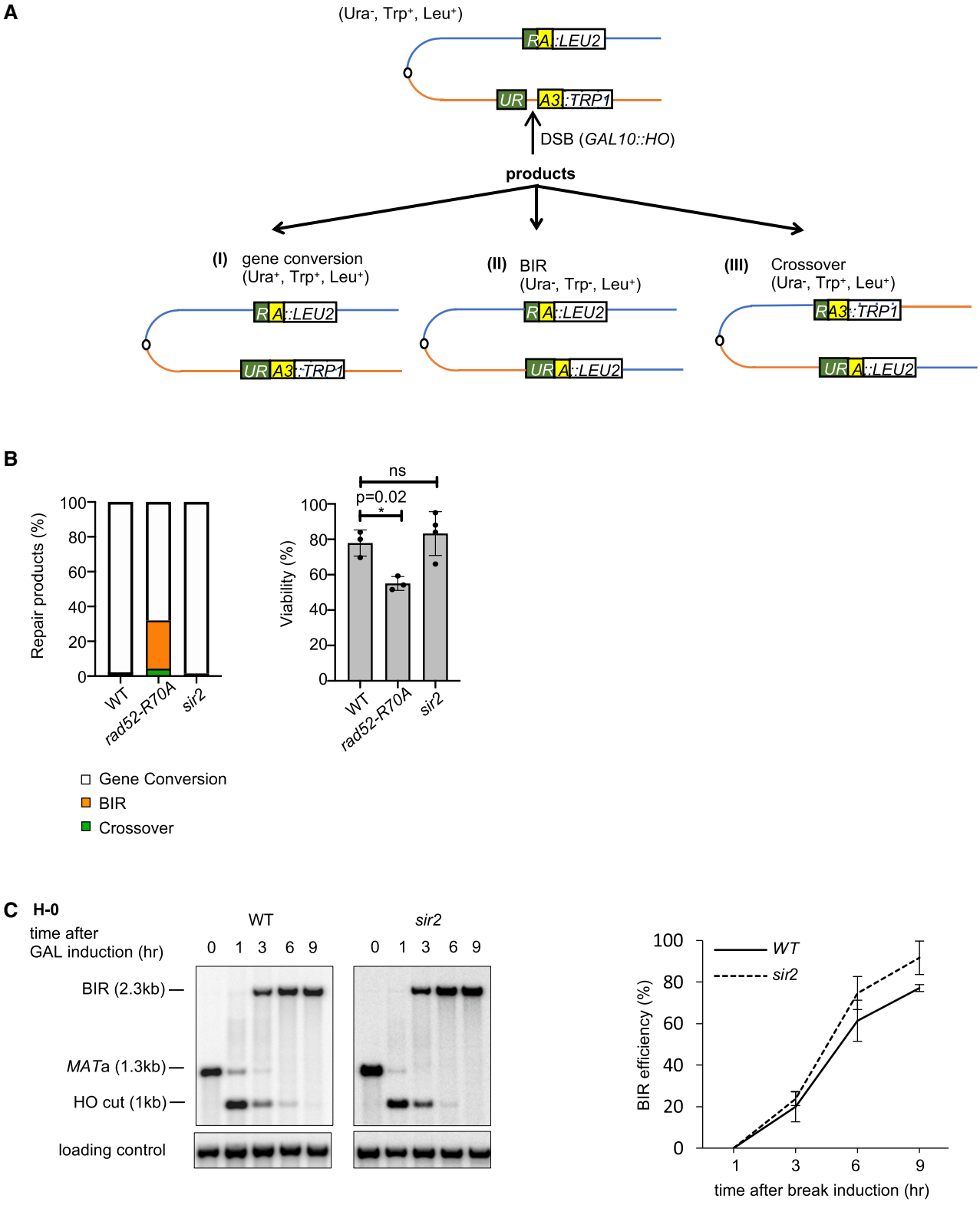

Figure EV4.

Figure EV5. MRX complex suppresses BIR in allelic recombination.

A Percentage of BIR and chromosome loss in WT and *mre11*Δ cells in allelic recombination. Chi-square test is used to determine the *P*-value, number of colonies tested per mutant is indicated in Appendix Table S1.

B Schematic of allelic assay with a *TRP1* probe (yellow box) used for Southern blot analysis. Ethidium bromide gel (top) and Southern blot with *TRP1* probe (bottom) showing chromosome III of haploid parental strains, diploid, and BIR products of WT (left) and mre*11*Δ cells (right). "*" depicts gross chromosomal arrangement event.

Data information: **P*-value 0.01 to 0.05, significant; ***P*-value 0.001 to 0.01, very significant; ****P*-value 0.0001 to 0.001, extremely significant; *****P* < 0.0001, extremely significant; $P \geq 0.05$, not significant (ns).

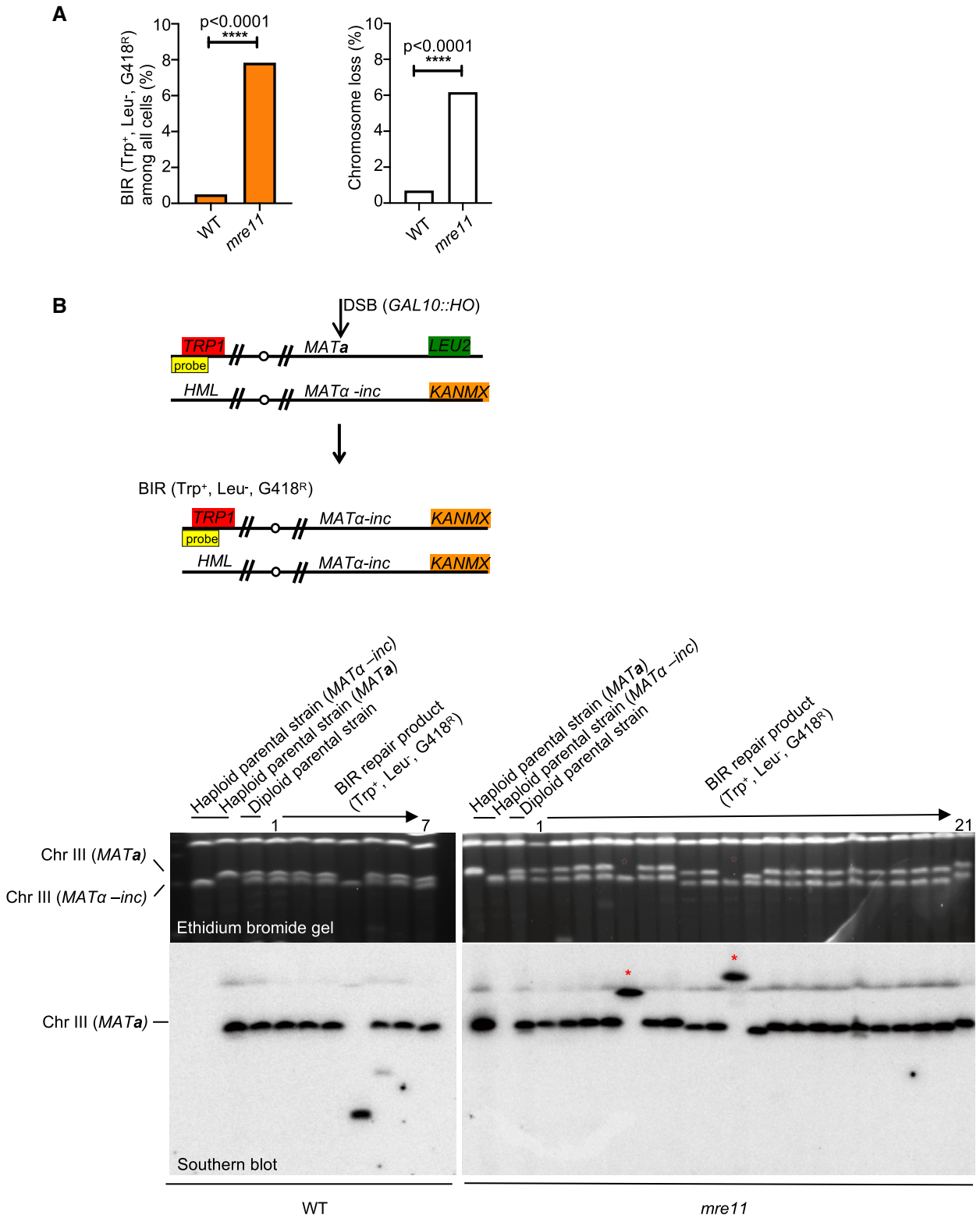

Figure EV5.