Figure S1, related to main Figure 2

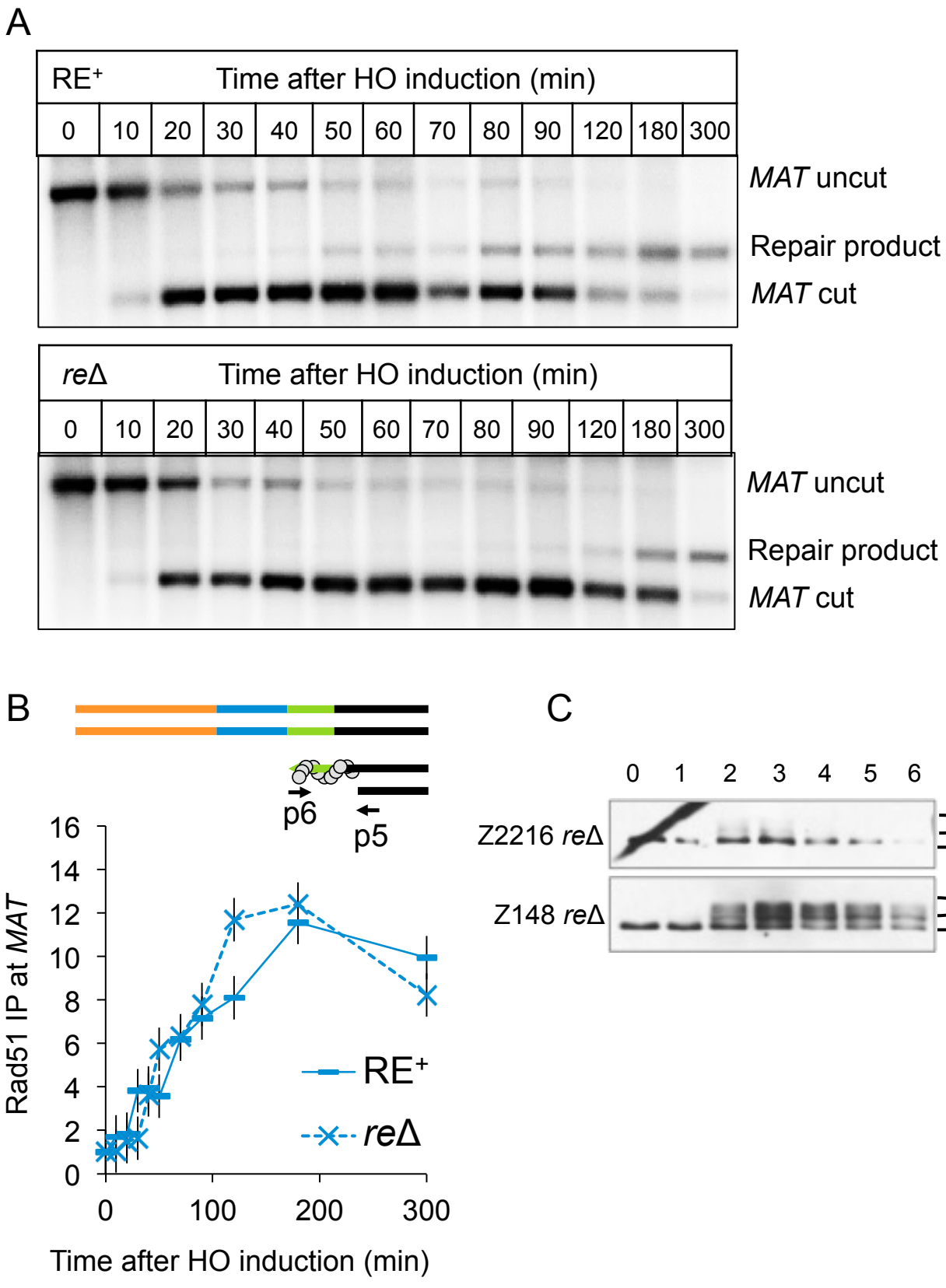
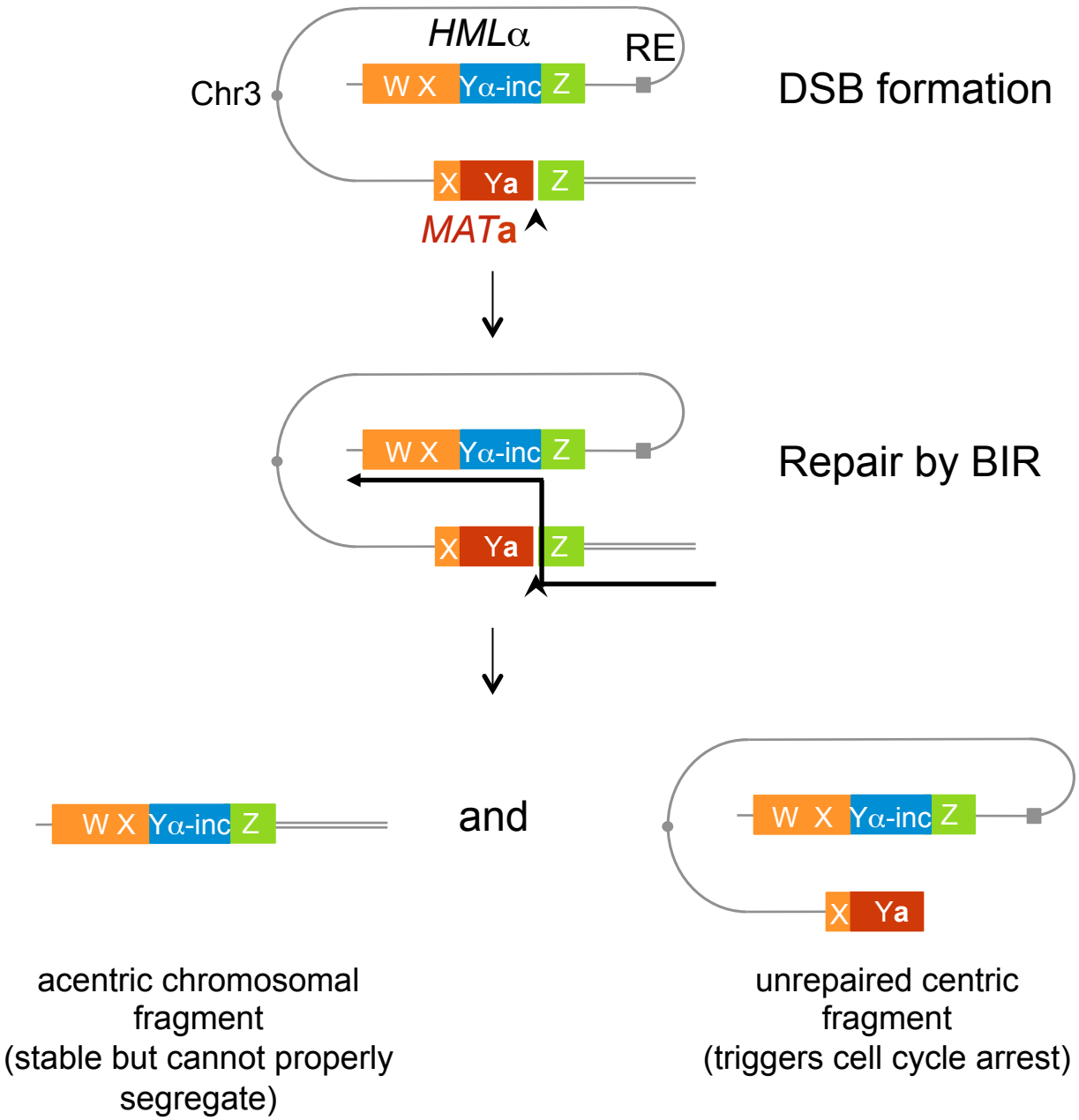


Figure S2, related to main Figure 4



Inviable BIR outcome

Figure S3, related to main Figure 4 and Figure 5

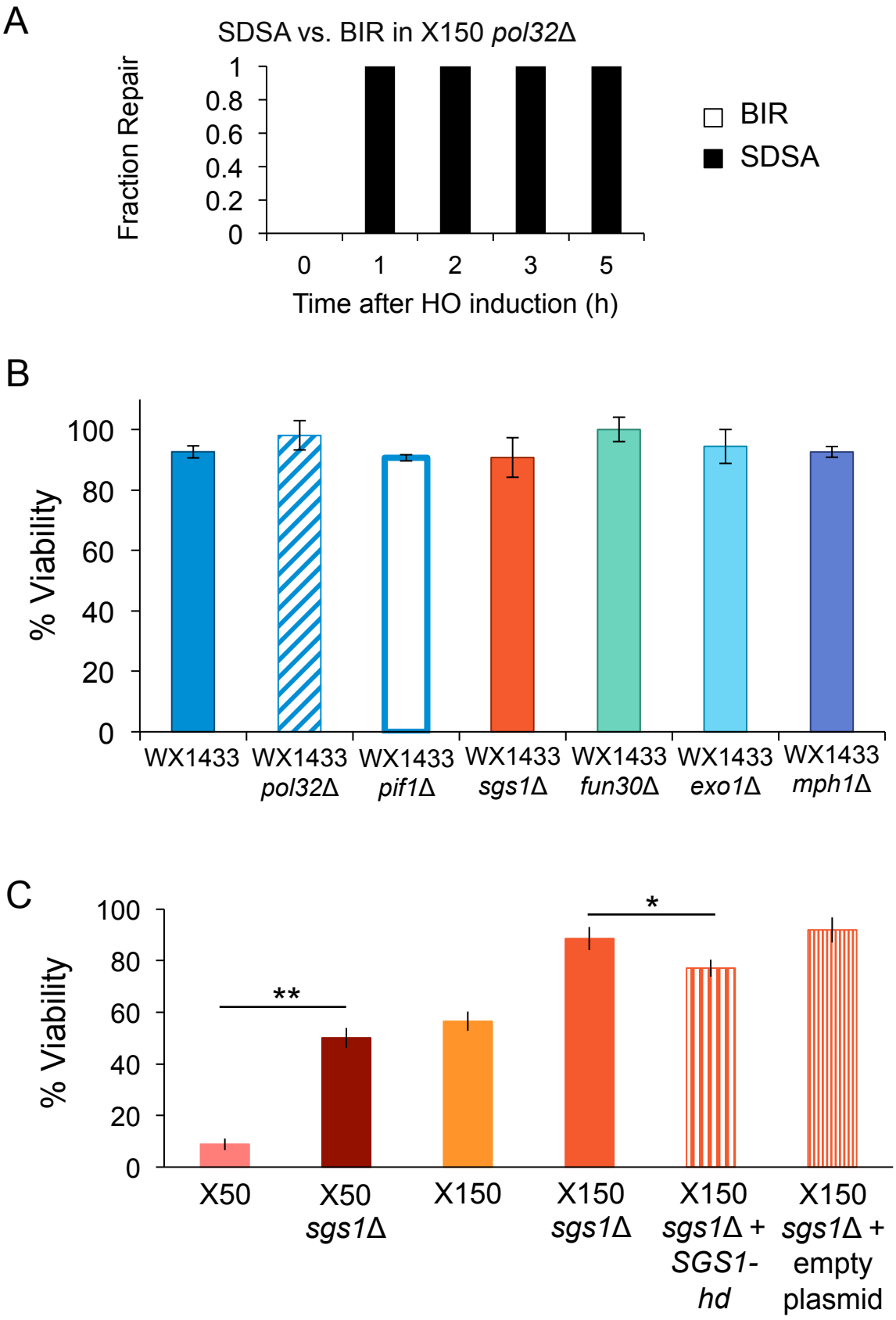
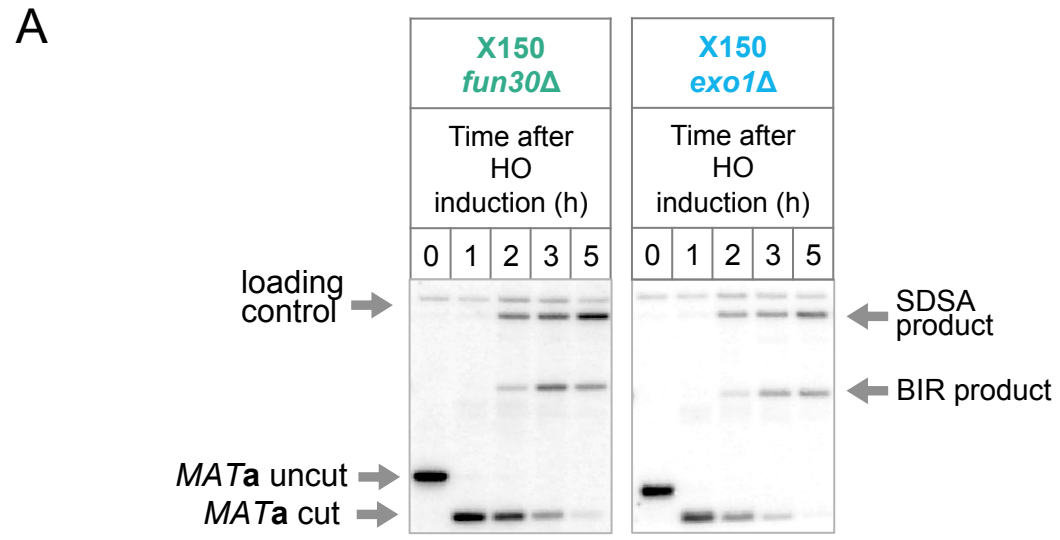
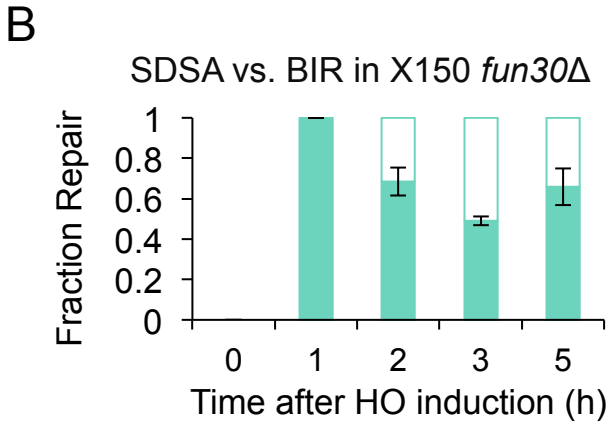
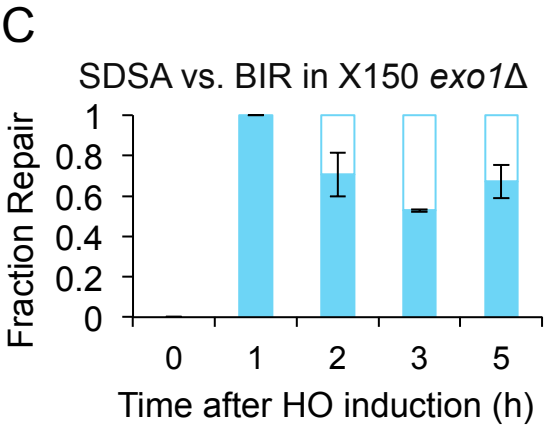
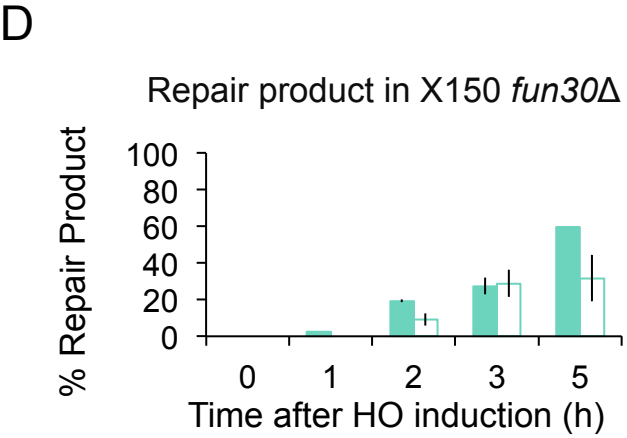


Figure S4, related to main Figure 4 and Figure 5









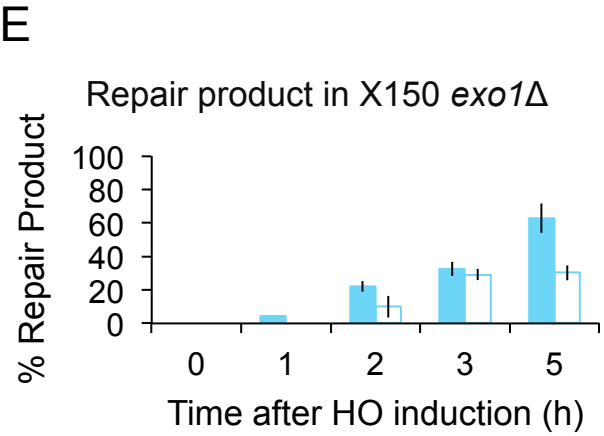
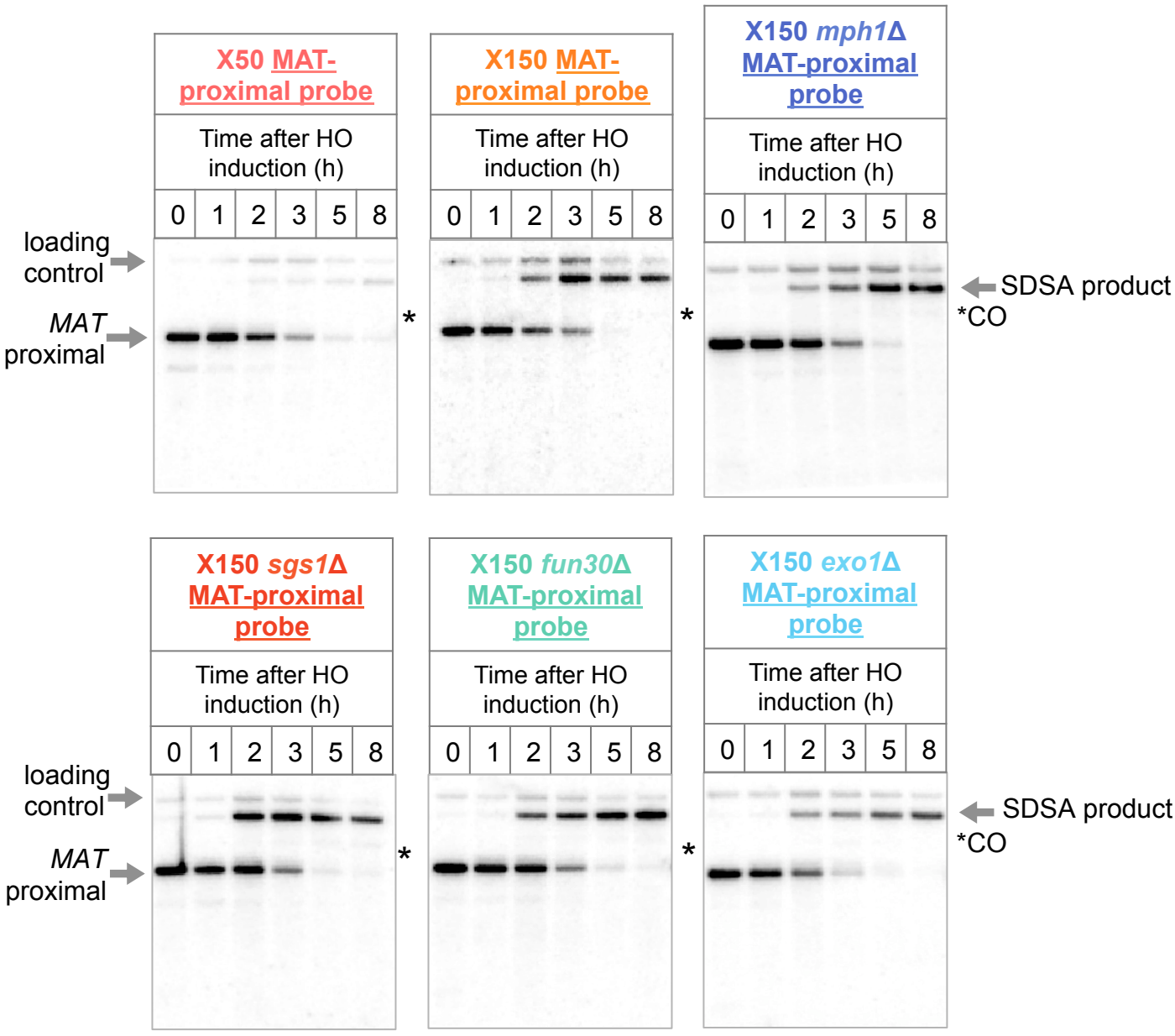


Figure S5, related to main Figure 4 and Figure 5



Supplemental Information

Supplemental Figure Legends

Figure S1, related to main Figure 2. Kinetics of repair in the absence of RE. (A) Southern blots showing kinetics of repair in RE^+ and *re*Δ. DNA was digested with AseI and probed with a *MAT* distal fragment. (B) Rad51 ChIP signal at the *MAT* locus in RE^+ and *re*Δ cells. The top panel is a schematic showing Rad51 nucleoprotein filament formation at DSB. Circles represent Rad51 protein, and arrows indicate the position of primers used for qPCR analysis. The bottom panels show quantification of the PCR product as a function of time following HO induction. IP signal from an independent *ARG5,6* locus was used to normalize for input DNA in the ChIP assay. (C) Representative Rad53 Western blot analysis in whole cell extracts of Z2216 *re*Δ and Z148 *re*Δ cells during DSB repair. Phosphorylated Rad53 (Rad53-ppp) migrates slowly compared to unphosphorylated Rad53.

Figure S2, related to main Figure 4. Schematic showing how BIR would result in an inviable outcome in this current intra-chromosomal repair system.

Figure S3, related to main Figure 4 and Figure 6. (A) Graph showing quantitative densitometric analysis of repair Southern blots for X150 *pol32*Δ. Data is represented as ratio of SDSA product (black graph) and BIR product (unfilled graph) over total product for a given time-point, which is normalized as 1. Data represent mean ± SD ($n = 3$). (B) Viability of WT and mutant WX1433 strains as denoted under the axes. Data represent mean ± SD ($n \geq 3$). (C) Viability of WT and mutant X150 strains as denoted under the axes. Data represent mean ± SD ($n \geq 3$). No significant change was observed in the WX1433 deletion mutants (Figure S3B). Two asterisks denote that the viabilities of the WT and marked mutant strains are significantly different with $P \leq 0.001$. "ns" indicates that the viabilities of the two strains are not significantly different.

Figure S4, related to main Figure 6. (A) Representative Southern blots showing DSB repair kinetics and products in (from left to right) X150, X150 *sgs1*Δ and X150 *mph1*Δ strains. DNA was digested with Bsp1286I and probed with a *MAT* distal fragment. (B) Graphs showing quantitative densitometric analysis of repair Southern blots shown in Figure S4A. Data is represented as ratio of SDSA product (solid color graph) and BIR product (unfilled graph) over total product for a given time-point, which is normalized as 1. Error bars represent ranges of two experiments.

Figure S5, related to main Figure 4 and Figure 6. (A) Representative Southern blots showing DSB repair kinetics and products in (from left to right) X150 *fun30*Δ and X150 *exo1*Δ strains. DNA was digested with Bsp1286I and probed with a *MAT* distal fragment. (B) Graphs showing quantitative densitometric analysis of repair Southern blots shown in Figure S5A. Data is

represented as ratio of SDSA product (solid color graph) and BIR product (unfilled graph) over total product for a given time-point, which is normalized as 1. Data represent mean ± SD (*n* = 3). (C) Graphs showing quantitative densitometric analysis of repair Southern blots shown in Figure S5A. The amount of product obtained from 5h time point was set to 100%. Data represent mean ± SD (n = 3).

Figure S6, related to main Figure 4 and Figure 6. Reducing non-invading X homology does not lead to GC repair resolved into COs. Southern blots showing repair in X50, X150, X150 *mph1*Δ, X150 *sgs1*Δ, X150 *fun30*Δ and X150 *exo1*Δ from top-left to bottom-right. Bsp1286I digested DNA was probed with a *MAT* proximal fragment. As resection proceeds through the Bsp1286I recognition sequence, the *MAT* proximal band disappears. Predicted size of the CO band is marked by asterisk (*).

Supplemental Tables

Table S1, related to STAR Methods. Yeast Strains used in this study

Strain name used in this study	Genotype	Lab Database name
Z327 (WT) RE^+, RE^+, WX1433 RE^+, WX1433, WX1433 (WT) RE^+	*hoΔ ade3::GAL-HO HML*α-inc *MAT***a** *hmr::ADE1 bar1Δ::ADE3 nej1Δ::KANMX ade1 leu2,3-112 trp1::hisG ura3-52 thr4 lys5*	YAM033
Z327 (WT) *reΔ*, *reΔ*, WX1433 (WT) *reΔ*, WX1433 *reΔ*	*reΔ::HPHMX*	YAM036a
Z2216 RE^+	*hoΔ ade3::GAL-HO HML YαΔ::NATMX-inc* – 2216bp*MATdistal - KANMX RE-HPHMX MAT***a** *hmr::LEU2 bar1Δ::ADE3 nej1Δ::KANMX ade1 leu2,3-112 trp1::hisG ura3-52 thr4 lys5*	YAM075
Z148 RE^+	*MAT Z2Δ*, *MAT Z1Δ*148bp::*Cg-TRP1*	YAM072
Z100 RE^+	*MAT Z2Δ*, *MAT Z1Δ*100bp::*Cg-TRP1*	YAM121
Z50 RE^+	*MAT Z2Δ*, *MAT Z1Δ*50bp::*Cg-TRP1*	YAM119
Z35 RE^+	*MAT Z2Δ*, *MAT Z1Δ*35bp::*Cg-LEU2*	YAB247
Z2216 *reΔ*	*hoΔ ade3::GAL-HO HML YαΔ::NATMX-inc* – 2216bp*MATdistal - KANMX reΔ::URA3MX MAT***a** *hmr::LEU2 bar1Δ::ADE3 nej1Δ::KANMX ade1 leu2,3-112 trp1::hisG ura3-52 thr4 lys5*	YAM138
Z148 *reΔ*	*reΔ::HPHMX MAT Z2Δ*, *MAT Z1Δ*148bp::*Cg-TRP1*	YAM112
Z100Z *reΔ*	*reΔ::HPHMX MAT Z2Δ*, *MAT Z1Δ*100bp::*Cg-TRP1*	YAM122
Z50 *reΔ*	*reΔ::HPHMX MAT Z2Δ*, *MAT Z1Δ*50bp::*Cg-TRP1*	YAM120
Z35 *reΔ*	*reΔ::HPHMX MAT Z2Δ*, *MAT Z1Δ*35bp::*Cg-TRP1*	YAB248
X250 RE^+	*MAT WΔ*, *MAT XΔ*250bp::*Cg-TRP1*	YAM097
X200 RE^+	*MAT WΔ*, *MAT XΔ*200bp::*Cg-TRP1*	YAM096
X150 RE^+, X150	*MAT WΔ*, *MAT XΔ*150bp::*Cg-TRP1*	YAM095
X250 RE^+	*MAT WΔ*, *MAT XΔ*100bp::*Cg-TRP1*	YAM094
X200 RE^+	*MAT WΔ*, *MAT XΔ*50bp::*Cg-TRP1*	YAM078
X250 *reΔ*	*reΔ::HPHMX MAT WΔ*, *MAT XΔ*250bp::*Cg-TRP1*	YAM102
X200 *reΔ*	*reΔ::HPHMX MAT WΔ*, *MAT XΔ*200bp::*Cg-TRP1*	YAM101
X150 *reΔ*	*reΔ::HPHMX MAT WΔ*, *MAT XΔ*150bp::*Cg-TRP1*	YAM100
X100 *reΔ*	*reΔ::HPHMX MAT WΔ*, *MAT XΔ*100bp::*Cg-TRP1*	YAM099
X50 *reΔ*	*reΔ::HPHMX MAT WΔ*, *MAT XΔ*50bp::*Cg-TRP1*	YAM079
WX1433 *pif1Δ*	*pif1Δ::URA3MX*	YAB056
X150 *pif1Δ*	*MAT WΔ*, *MAT XΔ*150bp::*Cg-TRP1 pif1Δ::URA3MX*	YAB058
X50 *pif1Δ*	*MAT WΔ*, *MAT XΔ*50bp::*Cg-TRP1 pif1Δ::URA3MX*	YAB057
WX1433 *fun30Δ*	*fun30Δ::NATMX*	YAB068
X150 *fun30Δ*	*MAT WΔ*, *MAT XΔ*150bp::*Cg-TRP1 fun30Δ::NATMX*	YAB070
WX1433 *sgs1Δ*	*sgs1Δ::NATMX*	YAM130
X150 *sgs1Δ*	*MAT WΔ*, *MAT XΔ*150bp::*Cg-TRP1 sgs1Δ::NATMX*	YAM129
X50 *sgs1Δ*	*MAT WΔ*, *MAT XΔ*50bp::*Cg-TRP1 sgs1Δ::NATMX*	YAM128

X150 *sgs1*Δ + *sgs1hd*	*MAT W*Δ, *MAT X*Δ150bp::*Cg-LEU2 sgs1*Δ*::NATMX pSJ23 (sgs1-hd – TRP1)*	YAB233
X150 *sgs1*Δ + empty plasmid	*MAT W*Δ, *MAT X*Δ150bp::*Cg-LEU2 sgs1*Δ*::NATMX pRS414 (TRP1* empty plasmid*)*	YAB245
WX1433 *exo1*Δ	*exo1*Δ*::HPHMX*	YAB204
X150 *exo1*Δ	*MAT W*Δ, *MAT X*Δ150bp::*Cg-TRP1 exo1*Δ*::HPHMX*	YAB206
X150 *exo1*Δ *fun30*Δ	*MAT W*Δ, *MAT X*Δ150bp::*Cg-TRP1 exo1*Δ*::HPHMX fun30*Δ*::NATMX*	YAB213
X150 *sgs1*Δ *fun30*Δ	*MAT W*Δ, *MAT X*Δ150bp::*Cg-TRP1 sgs1*Δ*::URA3MX fun30*Δ*::NATMX*	YAB216
X150 *sgs1*Δ *EXO1* O/E	*MAT W*Δ, *MAT X*Δ150bp::*Cg-TRP1 sgs1*Δ*::NATMX GAL::EXO1-LEU2*	YAB214
X150 *fun30*Δ *EXO1* O/E	*MAT W*Δ, *MAT X*Δ150bp::*Cg-TRP1 fun30*Δ*::NATMX GAL::EXO1-LEU2*	YAB215
WX1433 *mph1*Δ	*mph1*Δ*::NATMX*	YAM133
X150 *mph1*Δ	*MAT W*Δ, *MAT X*Δ150bp::*Cg-TRP1 mph1*Δ*::NATMX*	YAM132
X150 *mph1*Δ *sgs1*Δ	*MAT W*Δ, *MAT X*Δ150bp::*Cg-TRP1 mph1*Δ*::HPHMX sgs1*Δ*::NATMX*	YAB311
WX1433 *pol32*Δ	*pol32*Δ*::HPHMX*	YAM136
X150 *pol32*Δ	*MAT W*Δ, *MAT X*Δ150bp::*Cg-TRP1 pol32*Δ*::HPHMX*	YAM135
X50 *pol32*Δ	*MAT W*Δ, *MAT X*Δ50bp::*Cg-TRP1 pol32*Δ*::HPHMX*	YAM134
WX1433 *RAD51* O/E	*ADH1::RAD51-LEU2*	YAB249
X150 *RAD51* O/E	*MAT W*Δ, *MAT X*Δ150bp::*Cg-TRP1 ADH1::RAD51-LEU2*	YAM123
X50 *RAD51* O/E	*MAT W*Δ, *MAT X*Δ50bp::*Cg-TRP1 ADH1::RAD51-LEU2*	YAM124
Z50 *RAD51* O/E	*MAT Z2*Δ, *MAT Z1*Δ50bp::*Cg-TRP1 ADH1::RAD51-LEU2*	YAB244

Table S2, related to STAR Methods. Primers used in this study*

Name	5' → 3' sequence	Comments
AM5	AAACTCTTCTCAAAACCAAATTGCGCAAGGATTG ATTCAGTACAATTATGcggatccccgggttaattaa	to delete *RE* with *HPHMX*
AM6	CCTAGAATTTGGAATTGGATAATTTAACTCTTTAG AATATAACATCTACCgaattcgagctcgtttaaac	
p1 (MATZp2)	CGTCTTGCTCTTGTTCCCAA	to detect Rad51 at *HML*
p2 (HMLp3)	AGTAGCTTTCGGATGGCACA	
p5 (MATZp2)	CGTCTTGCTCTTGTTCCCAA	to detect Rad51 at *MAT*
p6 (MATDp8)	CGCATGGGCAGTTTACCTTT	
Arg5,6p1	CAAGGATCCAGCAAAGTTGGGTGAAGTATGGTA	normalization for qRT-PCR
Arg5,6p2	GAAGGATCCAAATTTGTCTAGTGTGGGAACG	
p3 (MATp11)	GGTTAAGATAAGAACAAAGAATGATGCT	primer extension assay (for all strains except Z2216 strains).
p4 (HOCSp3)	GACAAAATGCAGCACGGAAT	
p3 (AM43)	GCATATTGTGGAAATCAAAGATA	primer extension assay for Z2216 strains
p4 (HOCSp3)	GACAAAATGCAGCACGGAAT	
MatZ2+71	ATTGGCTATACGGGACGG	to make *MAT* distal Southern blot probe
MatZ2+493	CTTAGCTTGTACCAGAGG	
Act15'-89	TCTTCCCATCTATCGTCGGTAGAC	to make *ACT1* Southern blot probe
Act13'-807	GGTCAATACCGGCAGATTCC	
AM72	CTTATCGATACCGTCGACCTCG	to make *MAT* proximal Southern blot probe
bud5+1574	ACGACGACTTTTAGGCGC	
YS001 5' ADE2 500bp p1	TCATGTATAAATTGGTGCGTAAAATCG	normalization for agarose gel quantification of PCR
YS002 3'ADE2 500bp p2	GTTACTGGATATGTATGTATGTATAATAAGTGATC	
AM65	ttgtacatttggccttatagagtgtggtcgtggcggaggttgtttatcttTCGAG GTCGACGGTATC	to make Z148 strains by deleting partial *MAT* Z1/Z2 with *Cg-TRP1* using plasmid pYO2242.
AM78	tcaagatcgtttatggttaagataagaacaaagaatgatgctaagaattgaCGCT CTAGAACTAGTGGATC	
AM80	cgcaacagtataatttataaaccctggttttggttttgtagagtggttgaTCGAG GTCGACGGTATC	used with AM78 to make Z50 strains by deleting partial *MAT* Z1/Z2 with *Cg-TRP1* using plasmid pYO2242.
AM104	gacaaaatgcagcacggaatatgggactacttcgcgcaacagtataattttTCG AGGTCGACGGTATC	used with AM78 to make Z35 strains by deleting partial *MAT* Z1/Z2 with *Cg-LEU2* using plasmid pYO2241.

AM79	cgaataattatgctgaagtacgtggtgacggatattgggaagatgtgtttgTCGAGGTCGACGGTATC	used with AM78 to make Z100 strains by deleting partial *MAT* Z1/Z2 with *Cg-TRP1* using plasmid pYO2242.
AM67	gatatcttttaatgttgtctcactatcttgccaataagactctacccagatTCGAGGTCGACGGTATC	to make X50 strains by deleting *MAT* W and partial X with *Cg-TRP1* using plasmid pYO2242.
AM68	aataaaaagctcttttctatttgctgtaatttacctaagttaccagagagtCGCTCTAGAACTAGTGGATC	
AM74	ctagggccaacaaaaaccgtaagattagtgatgaggagaagaagttgttgcCGCTCTAGAACTAGTGGATC	used with AM67 to make X150 strains by deleting *MAT* W and partial X with *Cg-TRP1* using plasmid pYO2242.
AM73	tgtaacaacagaagaagaagttgaattaagggatatattaggattcttatcCGCTCTAGAACTAGTGGATC	used with AM67 to make X100 strains by deleting *MAT* W and partial X with *Cg-TRP1* using plasmid pYO2242.
AM75	caaacaacatctcaactcactactaccattactgtattactcaaagaaatgCGCTCTAGAACTAGTGGATC	used with AM67 to make X200 strains by deleting *MAT* W and partial X with *Cg-TRP1* using plasmid pYO2242.
AM76	gcgcagcatagaaaacgatagaagtaattatcaacttacacagaaaaataaCGCTCTAGAACTAGTGGATC	used with AM67 to make X250 strains by deleting *MAT* W and partial X with *Cg-TRP1* using plasmid pYO2242.

*Primer sequences for other gene deletions are available upon request. All gene disruptions/alterations were confirmed by PCR.