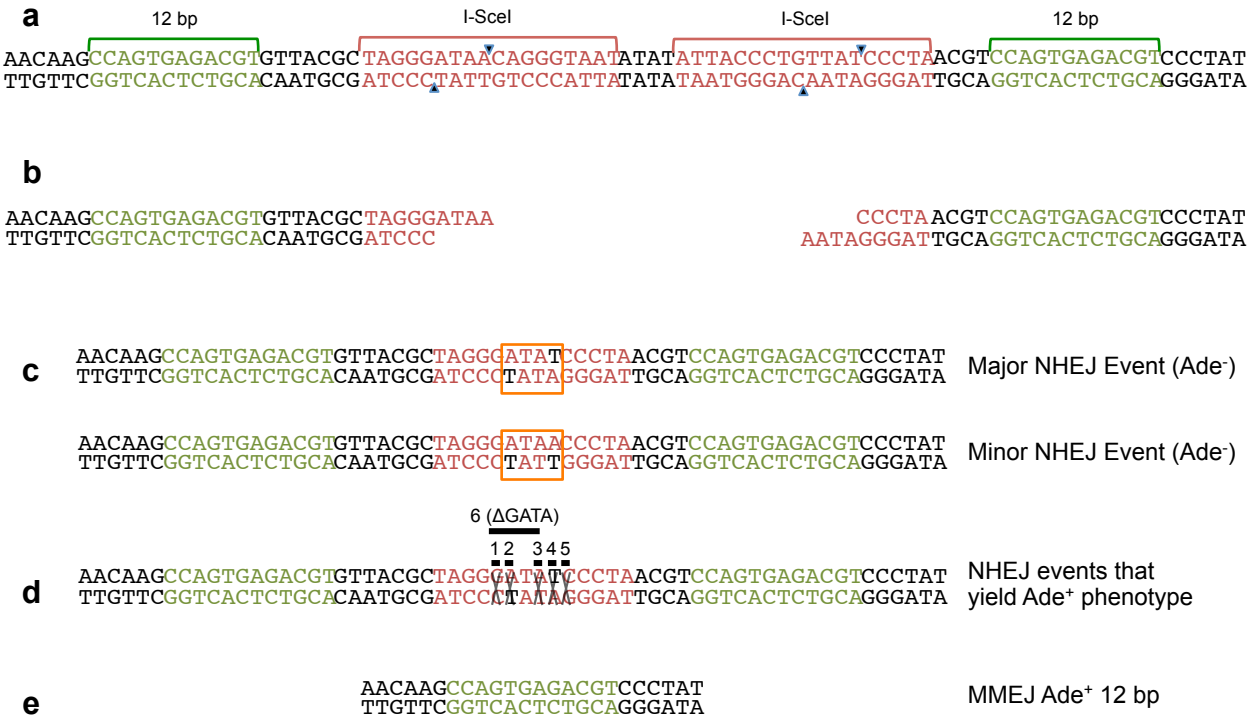

Figure S1. **I-*Sce*I cut site and repair sequences.**
a, Sequence of Inverted I-*Sce*I site and 12 bp microhomology **b**, I-*Sce*I cut site with resulting overhangs **c**, Main classes of NHEJ events **d**, NHEJ events that yield an Ade+ phenotype. Deletion of any one of the five indicated nucleotides or GATA (4 consecutive nucleotides) restores the *ADE2* reading frame to yield Ade+ NHEJ events **e**, MMEJ using 12 bp microhomology

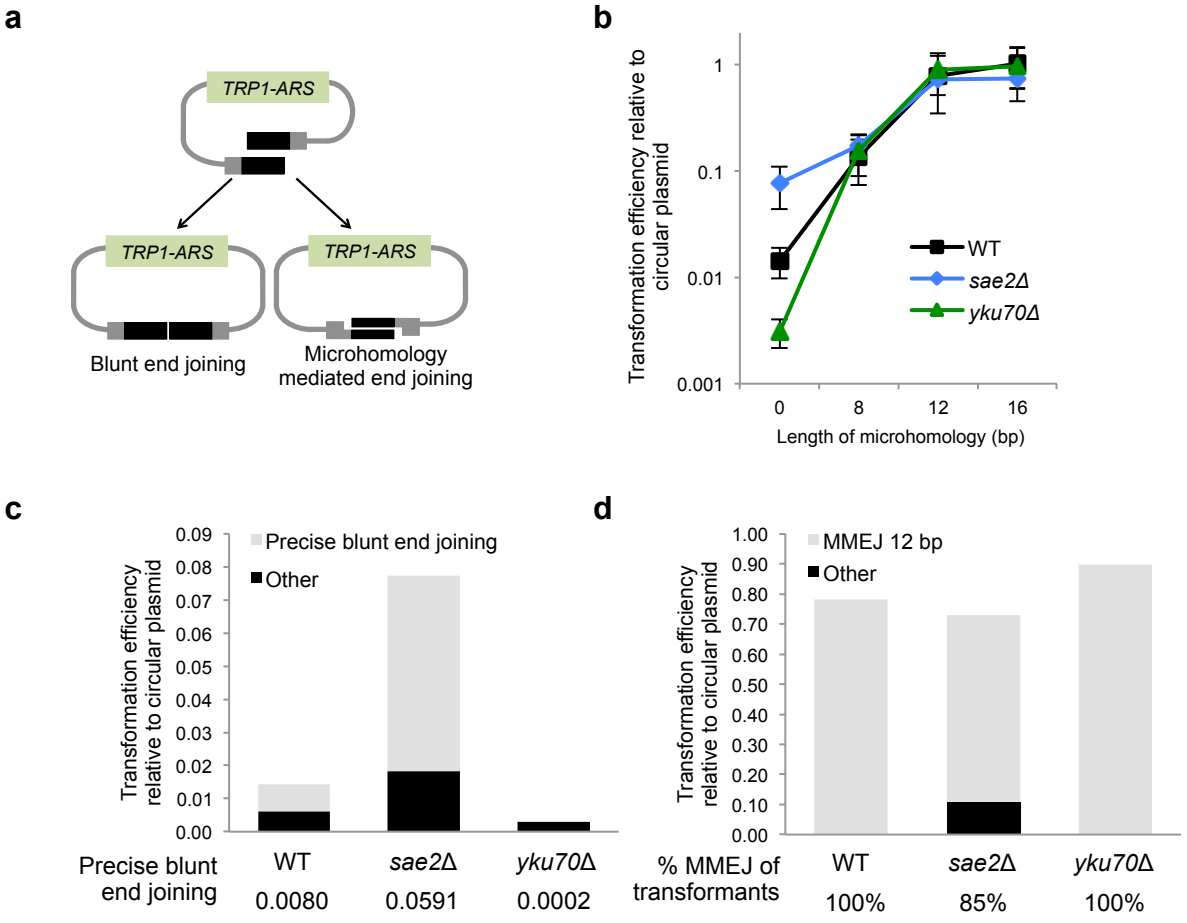

Figure S2. MMEJ is Ku-independent and partially Sae2 dependent.
a, Linear substrate for blunt end joining (0 MH) or with a direct repeat (8-16 bp) to promote repair by MMEJ. **b**, Graph of end joining frequencies of the indicated strains; transformations were performed in triplicate and error bars show s.d. **c**, Graph showing the fraction of precise blunt end joining as determined by sequencing at least 16 independently derived Trp$^+$ clones of the indicated genotypes. **d**, Graph showing the percent of circularization events that utilize the 12 bp MH as determined by sequencing 15-27 clones.

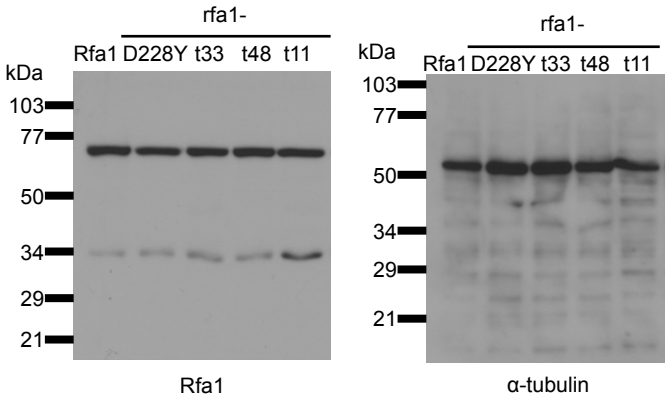

Figure S3.
Western blot of extracts prepared from the indicated strains probed with anti-Rfa1 or anti-α tubulin antibodies (cropped version shown in Figure 2b).

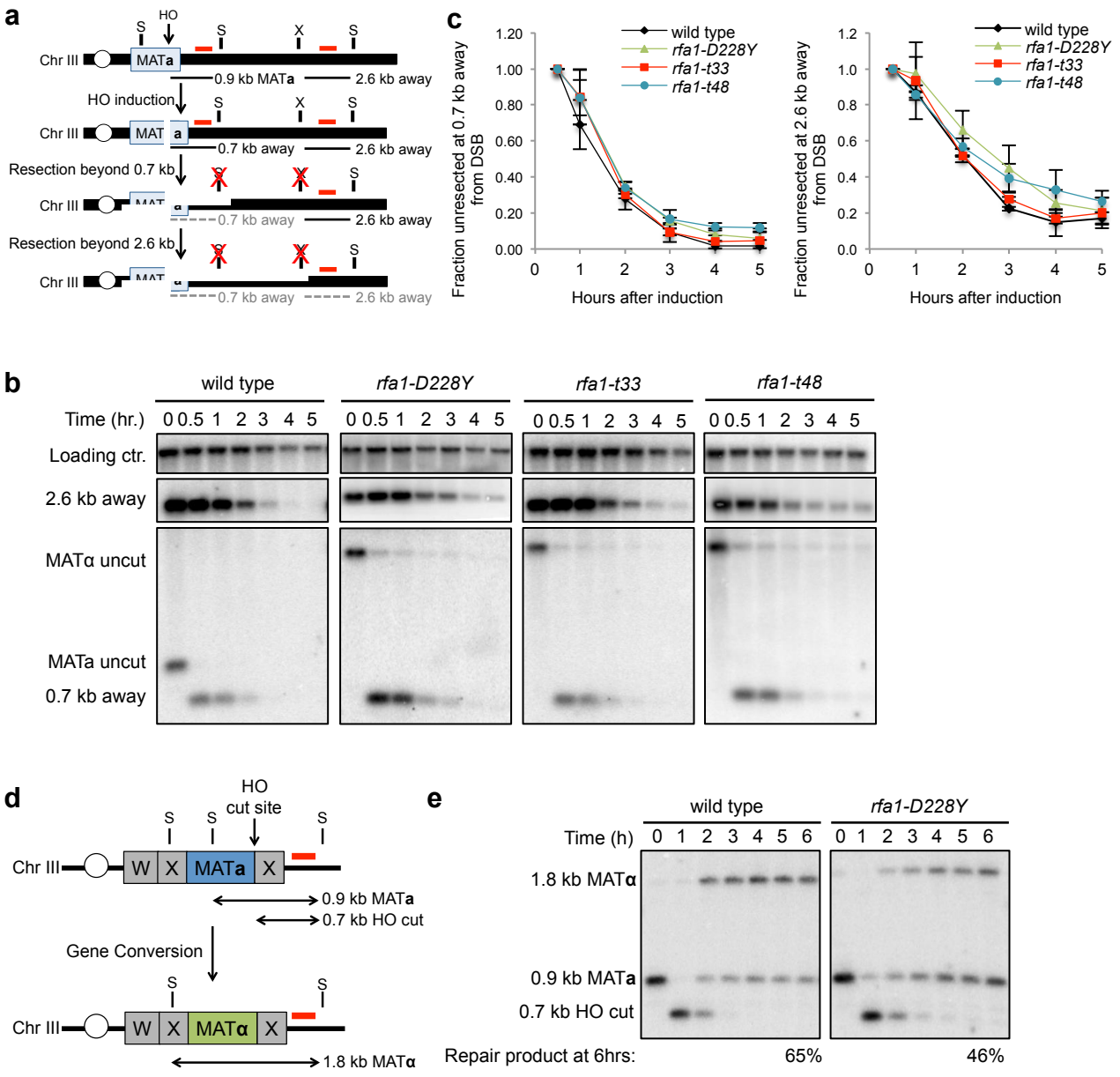

Figure S4. End resection and strand invasion in the *rfa1* mutants.
a, Schematic representation of the band disappearance resection assay. Loss of the 0.7 and 2.6 kb away fragments indicate resection has proceeded beyond the *Sty*I (S*)* and *Xba*I (X) restriction sites rendering them single-stranded and resistant to digestion. The red lines indicate the location of probes used to detect the 0.7kb and 2.6 kb distal restriction fragments. **b**, Southern blot of *Sty*I and *Xba*I digested genomic DNA isolated from wild type, *rfa1-D228Y*, *rfa1-t33*, and *rfa1-t48* cells. **c**, Quantification of resection 0.7 kb and 2.6 kb away from the HO cut site. **d**, Schematic for the mating-type switching assay: the 0.9 kb *Sty*I *MAT***a** fragment is cleaved upon HO induction to yield a 0.7 kb cut fragment. The 0.7 kb fragment disappears as resection proceeds and the break is repaired resulting in a 1.8 kb *MATα* fragment. **e**, Representative Southern blot of *Sty*I-digested genomic DNA from wild type and *rfa1-D228Y* cells at different times after HO induction.

Table S1A. Major microhomologies used

	Microhomology[1]	Distance from break (bp)
12 bp MH	CCA GTG AGA CGT	12 / 9
16 bp iMH	AGC AG**G** T**C**C CCA AAG TGT	32 / 5377

Table S1B. Alternative microhomologies used

Genotype	no. of events	Microhomology[1]	Distance from break (bp)
yku70Δ	1	GCT ATT **C**CT TCA	4828 / 5539
	1	ATG C**A**G AA	304 / 2107
	1	AAA TGA GGC CTA TTG	4374 / 2277
dnl4Δ	2	AAA TGA GGC CTA TTG	4374 / 2277
rfa1-t33	1	ATT TT**T** **G**AC GCC C**T**T CT	1383 / 5279
rfa1-D228Y	1	TGA TCA AGA ATG T	4812 / 4067
	1	AAA TGA GGC CTA TTG	4374 / 2277
rfa1-t48	1	ACA GG	0 / 4194
	1	CCA **G**GC C**G**G T	19 / 3241

[1] Bold font indicates a mismatch

Table S2: Yeast Strains

Strain	Genotype	Source
LSY0678	*MAT***a**	R. Rothstein
LSY0679	*MATα*	R. Rothstein
LSY1099	*MAT***a** *TRP1*	[1]
LSY2000	*MAT***a** *trp1ars416::kanMX6*	This study
LSY2013-1D	*MAT***a** *trp1ars416::kanMX6 yku70::HIS3*	This study
LSY2577-8A	*MATα trp1ars416::kanMX6 sae2::kanMX6*	This study
LSY2956-8D	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI*	This study
LSY2957-2B	*MAT***a** *ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI sae2::kanMX6*	This study
LSY2999-6C	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI yku70::HIS3*	This study
LSY3006-13B	*MAT***a** *ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI dnl4::kanMX6*	This study
LSY3013-3C	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI sgs1::hphMX6*	This study
LSY3013-15B	*MAT***a** *ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI exo1::URA3*	This study
LSY3013-16C	*MAT***a** *ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI sgs1::hphMX4 exo1::URA3*	This study
LSY3014-1C	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI rad51::LEU2*	This study
LSY3014-3B	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI rad52::TRP1*	This study
LSY3014-17C	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI rad51::LEU2 rad52::TRP1*	This study
LSY2997-12D	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI rfa1-D228Y::natMX4*	This study
LSY2996-1A	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI rfa1-t33::natMX4*	This study
LSY2995-6C	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI rfa1-t48::natMX4*	This study
LSY3012-1A	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI rfa1-t11*	This study
LSY3006-3B	*MAT***a** *ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI mre11-H125N-URA3*	This study
LSY3011-1D	*MATα ade2*-ISIR-12MH *lys2::P_{GAL}-I-SceI mre11::KlURA3*	This study
LSY3015-21C	*MATα ade2*-ISIR-12MH *lys2::pGAL-ISceI rfa1-D228Y::natMX4 rad51::LEU2*	This study
LSY3015-33D	*MATα ade2*-ISIR-12MH *lys2::pGAL-ISceI rfa1-D228Y::natMX4 rad52::TRP1*	This study
LSY3010-15A	*MAT***a** *ade2*-ISIR-12MH *lys2::pGAL-ISceI rfa1-t33::natMX4 sae2::kanMX6*	This study
LSY2900-13A	*MAT***a** *ade3::P_{GAL}-HO*	This study
LSY2900-5D	*MAT***a** *ade3::P_{GAL}-HO rfa1-D228Y*	This study
LSY2629-9A	*MATα hmlΔ hmrΔ ade3::P_{GAL}-HO rfa1-D228Y*	This study
LSY2509-2D	*MAT***a** *hmlΔ hmrΔ bar1::URA3 ade3::P_{GAL}-HO*	This study
LSY2726-38A	*MATα hmlΔ hmrΔ ade3::P_{GAL}-HO rfa1-t33*	This study
LSY2727-7C	*MATα hmlΔ hmrΔ ade3:: P_{GAL}-HO rfa1-t48*	This study

*All strains are of the W303 genotype (*leu2-3, 112 trp1-1 ura3 can1 his3-11, 15 ade2-1*), only mating type and other relevant genotypes are listed.

1. Langston, L.D. & Symington, L.S. Gene targeting in yeast is initiated by two independent strand invasions. *Proc Natl Acad Sci U S A* **101**, 15392-7 (2004).