Supporting Information

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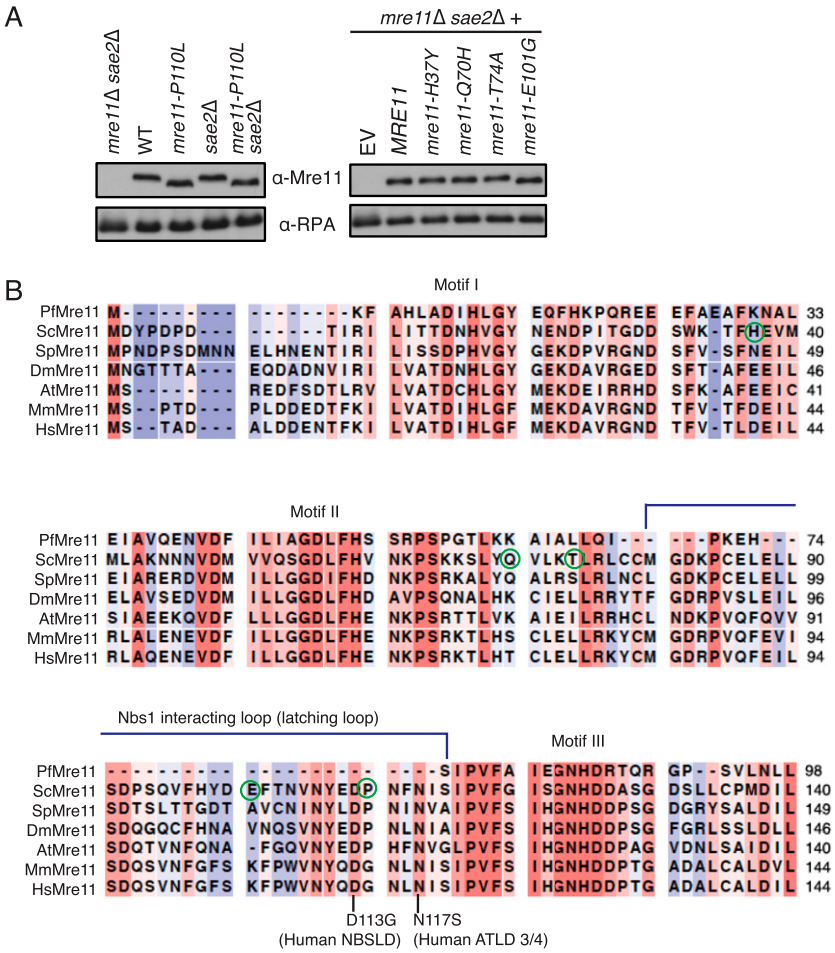
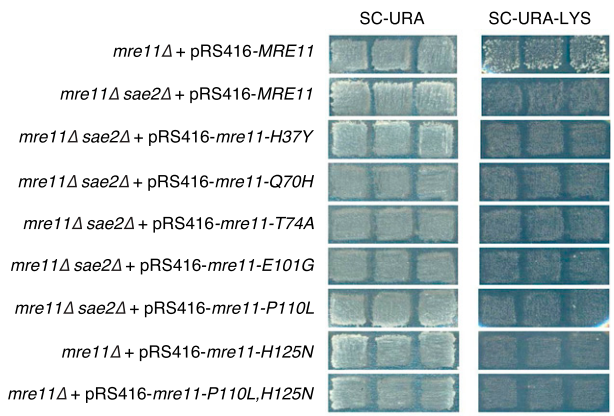


Fig. S1. The *sae2*Δ-suppressing *mre11* alleles: protein level and location. (*A*) Protein level of Mre11 and Mre11^{P110L} expressed from the endogenous *MRE11* locus in wild-type and *sae2*Δ derivatives (*Left*) and Mre11, Mre11^{H37Y}, Mre11^{Q70H}, Mre11^{T74A}, and Mre11^{E101G} expressed from pRS416 in *mre11*Δ *sae2*Δ cells (*Right*). (*B*) Sequence alignment of the N-terminal region of Mre11 from different species. Sequence conservation is indicated by colors, with red representing 100% and blue representing 0%. The Mre11 nuclease-related phosphoesterase motifs I–III, the Nbs1 interacting loop, and two human disease-associated *mre11* mutations are indicated. Residues that suppress *sae2*Δ DNA damage sensitivity when mutated are marked by green circles.

A



B

diploid strain	# tetrads / # total cells	sporulation efficiency
SAE2/SAE2 MRE11/MRE11	114/614	18.6%
sae2/sae2 MRE11/MRE11	0/578	0
SAE2/SAE2 P110L/P110L	106/635	16.7%
sae2/sae2 P110L/P110L	0/524	0
sae2/sae2 P110L/Δ	0/547	0

Fig. S2. The *sae2*Δ-suppressing *mre11* alleles do not restore Mre11 endonuclease activity. (*A*) Indicated *mre11* alleles were expressed from a plasmid in *mre11*Δ or *mre11*Δ *sae2*Δ cells harboring the *lys2-AluIR* and *lys2*-Δ5′ ectopic recombination reporter. Cells were patched as triplicates on SC-URA medium to maintain the plasmid and replicated to SC-URA-LYS medium to score for Lys^+ recombinants. (*B*) Sporulation efficiency from indicated diploid cells were determined by scoring number of tetrads/number of total cells by microscopy 2 d after growing cells on sporulation medium at 30 °C.

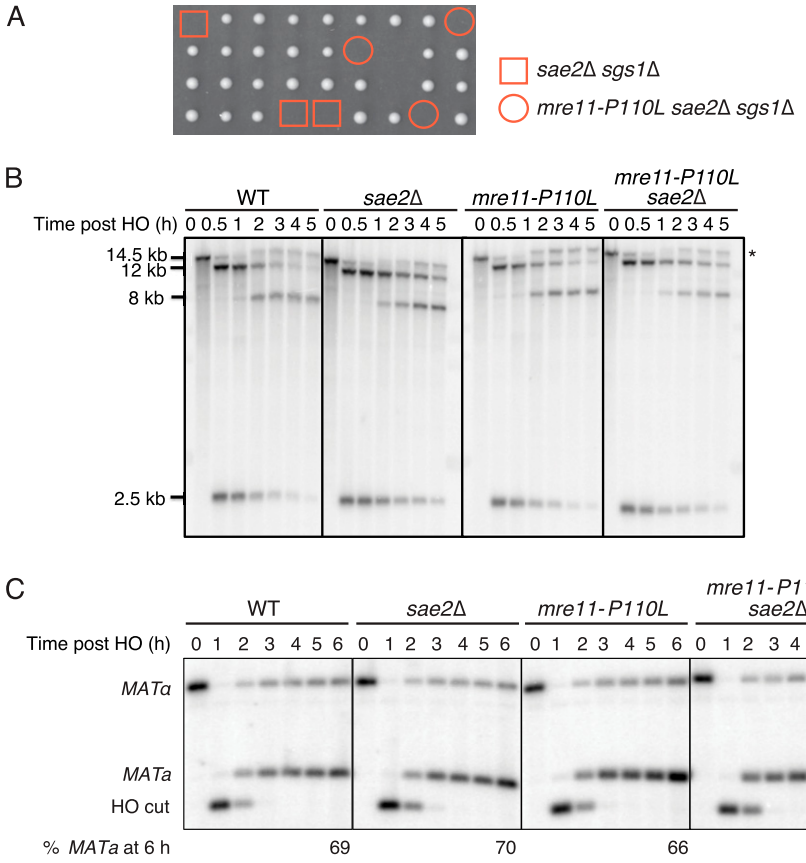


Fig. S3. *mre11-P110L* does not restore end resection or affect HR. (*A*) Spores from tetrad dissection showing that *mre11-P110L* does not suppress the *sae2*Δ *sgs1*Δ synthetic lethality. (*B*) Southern blot analysis of KpnI-digested genomic DNA from indicated strains taken at different time points after HO induction in G_2/M arrested cells. Uncut fragment (14.5 kb), cut fragments (12 and 2.5 kb), and the SSA product (8 kb) were detected by a *leu2* probe. A ssDNA-resection intermediate is marked by the asterisk. (*C*) A mating type switching assay was used to monitor HR efficiency on a HO-induced DSB at the *MATα* locus. Southern blot was performed and the ratio of *MAT***a** product among total DNA at 6-h after HO induction is indicated.

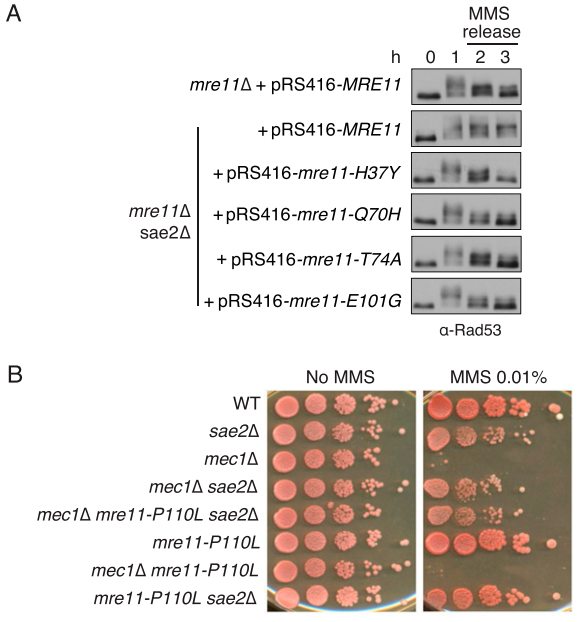


Fig. S4. The *mre11* alleles suppress the checkpoint recovery defect in *sae2*Δ. (*A*) Western blot analysis showing Rad53 phosphorylation and dephosphorylation in response to MMS. Log-phase growing cells ($t = 0$) from indicated strains were treated with 0.015% MMS for 1 h and released into fresh media ($t = 1$ h). Protein samples from different time points before and after MMS treatment were analyzed by using anti-Rad53 antibodies. (*B*) Tenfold serial dilutions of log-phase cultures of the indicated strains were spotted onto YPD medium with no MMS or 0.01% MMS.

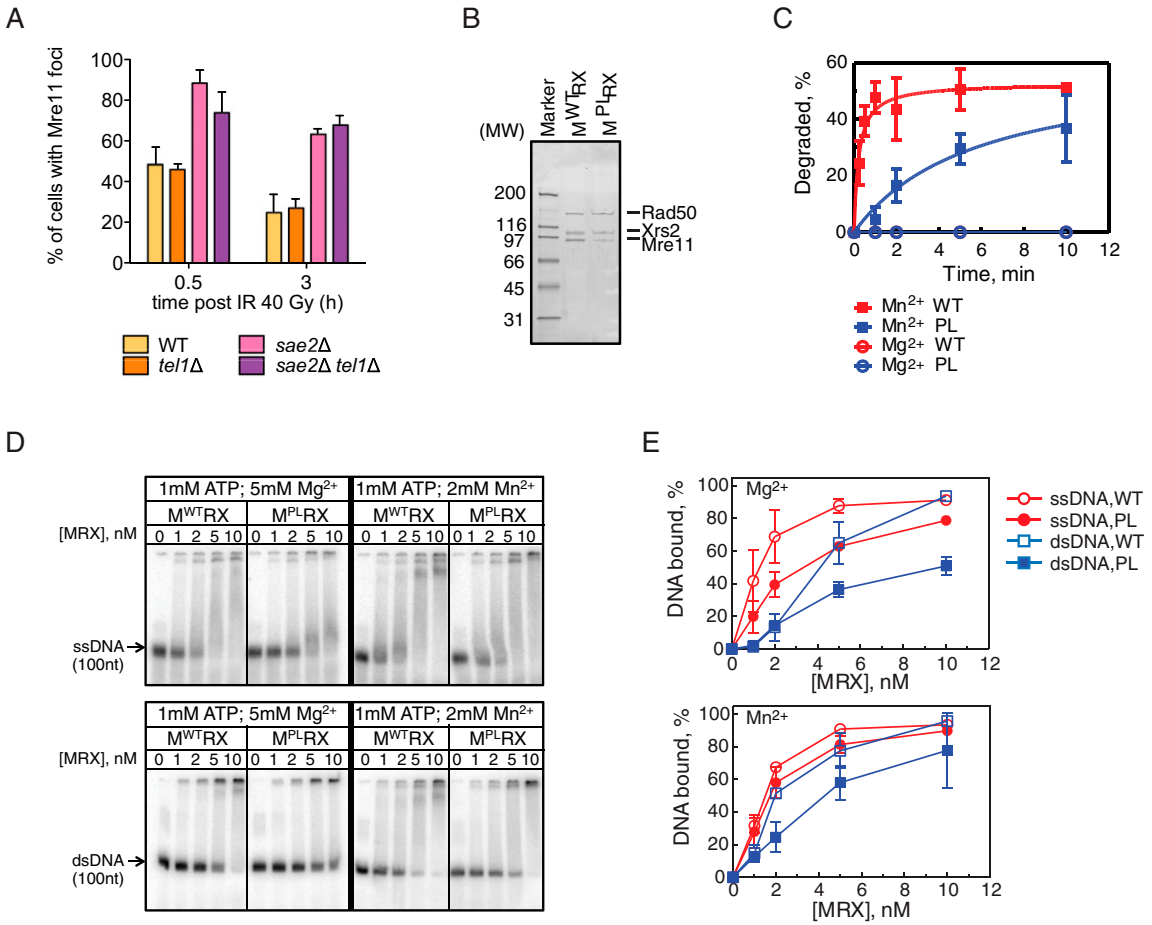


Fig. S5. Nuclease and DNA binding activity of the MRX and $M^{P110L}RX$ complexes. (*A*) Quantification of Mre11-YFP foci from indicated strains. The percentage of cells with one or more YFP foci at different time points after IR was quantitated. The mean values from three independent trials are plotted, and error bars show SD. (*B*) Purified MRX and $M^{P110L}RX$ protein complexes were run on a 4–15% gel and stained with SYPRO-Orange. (*C*) The $M^{P110L}RX$ proteins have weaker 3′-5′ exonuclease activity than MRX. The mean values from two independent trials are plotted, and error bars show SE. (*D*) DNA binding activity of WT and mutant complexes in the presence of ATP and 5 mM Mg^{2+} or 2 mM Mn^{2+}. (*E*) Quantification of the data shown in *D*. The mean values from two independent trials are plotted, and error bars show SE.

Table S1. Yeast strains

Strain	Genotype	Source
W1588-4C	*MATa*	1
LSY1091	*MATa sae2::KanMX6*	2
LSY2954-2B	*MATa mre11-P110L-NatMX4*	This study
LSY2882-10A	*MATa mre11-P110L-NatMX4 sae2::KanMX6*	This study
LSY2872-2A	*MATα rad50S::URA3*	This study
LSY2872-1A	*MATa mre11-P110L-NatMX4 rad50S::URA3*	This study
LSY779	*MATa mre11::LEU2*	3
LSY2719-2A	*MATα mre11::LEU2 sae2::KanMX6*	This study
ALE94	*MATα ade5-1 his7-2 leu2-3,112::p305L3 (LEU2) trp1-289 ura3 lys2::AluIR*	4
ALE108	*MATα ade5-1 his7-2 leu2-3,112::p305L3 (LEU2) trp1-289 ura3 lys2::AluIR sae2::HgrB*	4
LSY2930	*MATα ade5-1 his7-2 leu2-3,112::p305L3 (LEU2) trp1-289 ura3 lys2::AluIR mre11::TRP1*	This study
LSY2931	*MATα a ade5-1 his7-2 leu2-3,112::p305L3 (LEU2) trp1-289 ura3 lys2::AluIR mre11::TRP1 sae2::HgrB*	This study
LSY2933-2B	*MATα sae2::KanMX6 exo1::URA3 mre11-P110L-NatMX4*	This study
LSY2938	*MATα sae2::KanMX6 exo1::URA3*	This study
LSY3049-3B	*MATa sae2::KanMX6 yku70::HIS3 RAD5*	This study
LSY2954-1C	*MATa mre11-P110L-NatMX4 yku70::HIS3 sae2::KanMX6*	This study
YMV45	*MAT::hisG hml::ADE1 hmr::ADE1 ade1 lys5 ura3-52 trp1 ho ade3::pGAL-HO leu2-cs leu2 fragment inserted 4.6 kb upstream of leu2-cs*	5
LSY1951 (YMV45 *sae2*)	*MAT::hisG hml::ADE1 hmr::ADE1 ade1 lys5 ura3-52 trp1 ho ade3::pGAL-HO leu2-cs leu2 fragment inserted 4.6 kb upstream of leu2-cs sae2::KanMX6*	6
LSY3089	*MAT::hisG hml::ADE1 hmr::ADE1 ade1 lys5 ura3-52 trp1 ho ade3::pGAL-HO leu2-cs leu2 fragment inserted 4.6 kb upstream of leu2-cs mre11-P110L-NatMX4*	This study
LSY3086	*MAT::hisG hml::ADE1 hmr::ADE1 ade1 lys5 ura3-52 trp1 ho ade3::pGAL-HO leu2-cs leu2 fragment inserted 4.6 kb upstream of leu2-cs sae2::KanMX6 mre11-P110L-NatMX4*	This study
LSY2917	*MATa bar1::LEU2*	This study
LSY2944-6A	*MATa bar1::LEU2 mre11-P110L-NatMX4*	This study
LSY2918	*MATa bar1::LEU2 sae2::KanMX6*	This study
LSY2919	*MATa bar1::LEU2 sae2::KanMX6 mre11-P110L-NatMX4*	This study
LSY1996	*MATa tel1::HphMX4*	E. Mimitou
LSY2870-3A	*MATa sae2::KanMX6 tel1::HphMX4*	This study
LSY3051-4B	*MATa sae2::KanMX6 tel1::HphMX4 mre11-P110L-NatMX4*	This study
LSY3051-3D	*MATα tel1::HphMX4 mre11-P110L-NatMX4*	
LSY2363-28C	*MATa mec1::TRP1 sml1::HIS3*	This study
LSY2363-32B	*MATα mec1::TRP1 sml1::HIS3 sae2::KanMX6*	This study
LSY2988-1D	*MATa mec1::TRP1 sml1::HIS3 sae2::KanMX6 mre11-P110L-NatMX4*	This study
LSY2988-10C	*MATa mec1::TRP1 sml1::HIS3 mre11-P110L-NatMX4*	This study
W3483-10A	*MATa ADE2 MRE11-YFP bar1::LEU2*	7
LSY3036	*MATa ADE2 MRE11-YFP bar1::LEU2 sae2::KanMX6*	This study
LSY3037	*MATa ADE2 mre11-P110L-YFP bar1::LEU2 sae2::KanMX6*	This study
LSY3128	*MATa ADE2 mre11-H37Y-YFP bar1::LEU2 sae2::KanMX6*	This study
LSY3029	*MATa ADE2 mre11-P110L-YFP bar1::LEU2*	This study
LSY3085	*MATa ADE2 mre11-H37Y-YFP bar1::LEU2*	This study
LSY3245-11A	*MATa ADE2 MRE11-YFP bar1::LEU2 tel1::HphMX4*	This study
LSY3245-1C	*MATα ADE2 MRE11-YFP bar1::LEU2 sae2::KanMX6 tel1::HphMX4*	This study
LSY3079-10D	*MATa leu2::pGAL-HO-LEU2 hml::oripRS hmr::ampR*	This study
LSY3079-16A	*MATa leu2::pGAL-HO-LEU2 hml::oripRS hmr::ampR sae2::KanMX6*	This study
LSY3079-1B	*MATa leu2::pGAL-HO-LEU2 hml::oripRS hmr::ampR mre11-P110L-NatMX4*	This study
LSY3079-13C	*MATa leu2::pGAL-HO-LEU2 hml::oripRS hmr::ampR mre11-P110L-NatMX4 sae2::KanMX6*	This study
LSY3088	*MATα lys2::pGAL-ISceI ade2-ISIR-14MH*	This study
LSY3141-16D	*MATα lys2::pGAL-ISceI ade2-ISIR-14MH sae2::KanMX6*	This study
LSY3173-4A	*MATα lys2::pGAL-ISceI ade2-ISIR-14MH mre11-P110L-NatMX4*	This study
LSY3173-1C	*MATα lys2::pGAL-ISceI ade2-ISIR-14MH sae2::KanMX6 mre11-P110L-NatMX4*	This study
LSY785	*MATa yku70::HIS3*	2
LSY3181-4B	*MATα sae2::KanMX6 yku70::HIS3 exo1::URA3*	This study
LSY3180-2B	*MATα ade3::pGAL-HO sae2::KanMX6*	This study
LSY3180-3C	*MATα ade3::pGAL-HO mre11-P110L-NatMX4*	This study
LSY3180-1A	*MATα ade3::pGAL-HO mre11-P110L-NatMX4 sae2::KanMX6*	This study
LSY1009	*MATα ade3::pGAL-HO*	2
LSY3184	*MATa/MATα*	This study

Table S1. Cont.

Strain	Genotype	Source
LSY3185	*MATa/MATα sae2::KanMX6/sae2::KanMX6*	This study
LSY3186	*MATa/MATα mre11-P110L-NatMX4/mre11-P110L-NatMX4*	This study
LSY3187	*MATa/MATα sae2::KanMX6/sae2::KanMX6 MRE11/mre11-P110L-NatMX4*	This study
LSY3188	*MATa/MATα sae2::KanMX6/sae2::KanMX6 mre11-P110L-NatMX4/mre11-P110L-NatMX4*	This study
LSY3189	*MATa/MATα sae2::KanMX6/ sae2::KanMX6 mre11- P110L-NatMX4/mre11::LEU2*	This study
LSY3190	*MATa/MATα sae2::KanMX6/sae2::KanMX6 MRE11/mre11::LEU2*	This study

Most strains are of the W303 background (*trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112 ade2-1 RAD5*), only the mating type and differences from this genotype are shown. The full genotypes are shown for all of the non-W303 strains (ALE94, ALE108, LSY2930, LSY2931, YMV45, LSY1951, LSY3089, and LSY3086).

1. Zou H, Rothstein R (1997) Holliday junctions accumulate in replication mutants via a RecA homolog-independent mechanism. *Cell* 90(1):87–96.
2. Mimitou EP, Symington LS (2010) Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. *EMBO J* 29(19):3358–3369.
3. Moreau S, Ferguson JR, Symington LS (1999) The nuclease activity of Mre11 is required for meiosis but not for mating type switching, end joining, or telomere maintenance. *Mol Cell Biol* 19(1):556–566.
4. Lobachev KS, Gordenin DA, Resnick MA (2002) The Mre11 complex is required for repair of hairpin-capped double-strand breaks and prevention of chromosome rearrangements. *Cell* 108(2):183–193.
5. Vaze MB, et al. (2002) Recovery from checkpoint-mediated arrest after repair of a double-strand break requires Srs2 helicase. *Mol Cell* 10(2):373–385.
6. Clerici M, Mantiero D, Lucchini G, Longhese MP (2005) The Saccharomyces cerevisiae Sae2 protein promotes resection and bridging of double strand break ends. *J Biol Chem* 280(46): 38631–38638.
7. Lisby M, Barlow JH, Burgess RC, Rothstein R (2004) Choreography of the DNA damage response: Spatiotemporal relationships among checkpoint and repair proteins. *Cell* 118(6): 699–713.