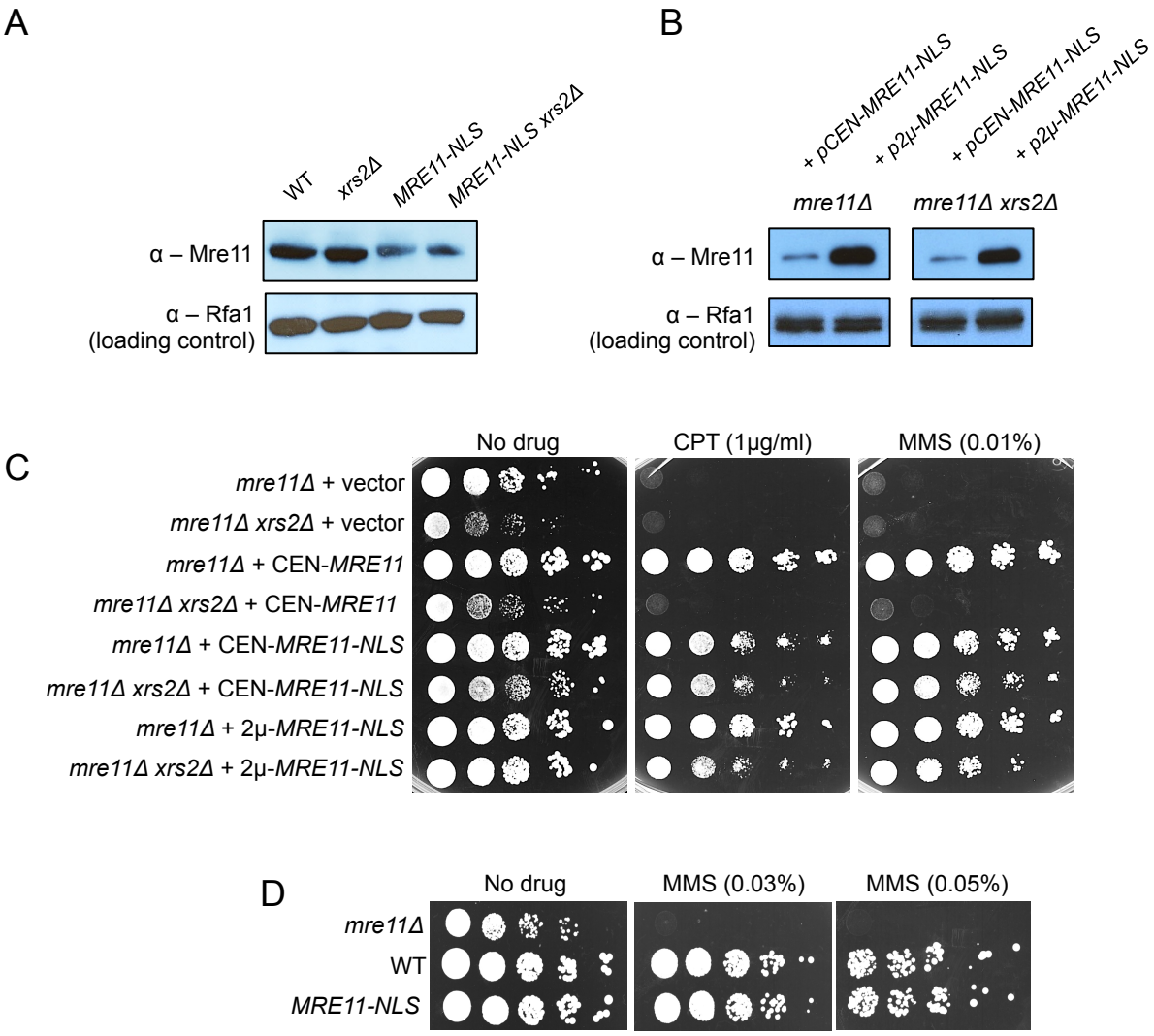
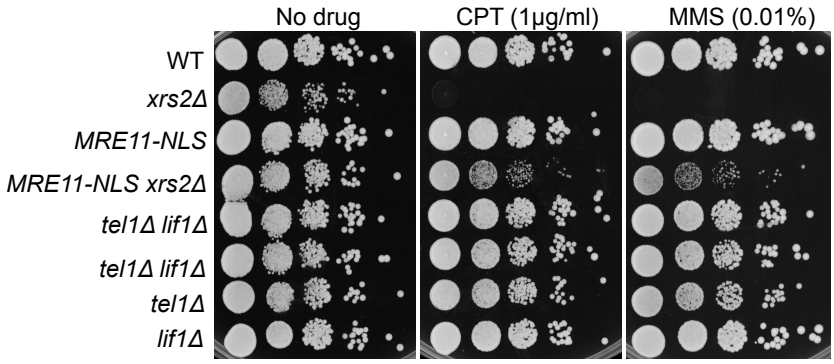
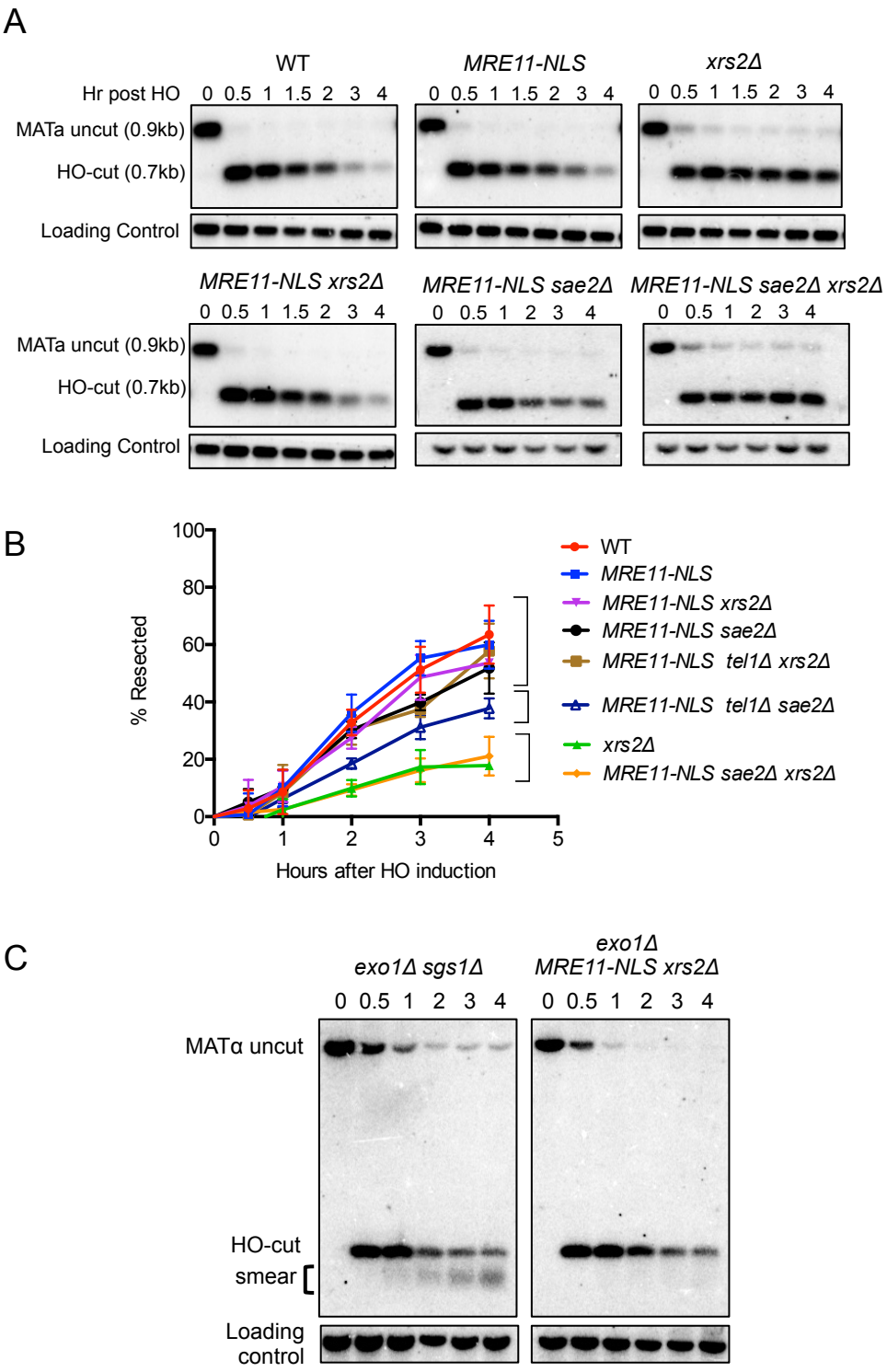


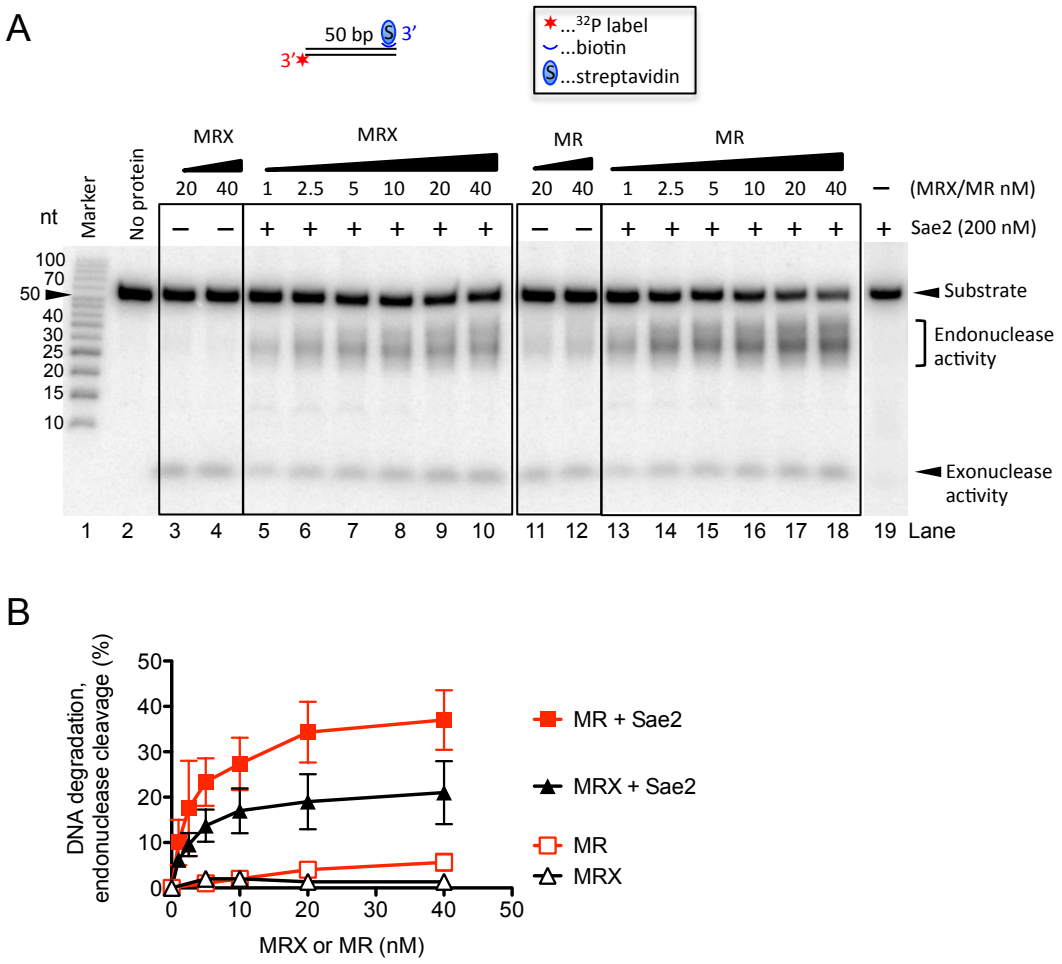
Supplementary Figure 1. Mre11-NLS is not limiting for DNA repair, related to Figure 1.
(A) Steady-state protein levels of Mre11 and Rfa1 (loading control) of indicated strains measured by western blot analysis.
(B) Overexpression of *MRE11-NLS* using a 2-micron plasmid construct compared with expression from a single copy (*CEN*) plasmid.
(C) Tenfold serial dilutions of the indicated strains harboring *CEN* or 2-micron plasmids were spotted onto selective plates containing 1μg/ml CPT or 0.01% MMS.
(D) Tenfold serial dilutions of the indicated strains were spotted onto selective plates containing 0.03% or 0.05% MMS



Supplementary Figure 2. The DNA damage sensitivity of the *MRE11-NLS xrs2Δ* mutant is not due to loss of Tel1 signaling and NHEJ, related to Figure 2.
Ten-fold serial dilutions of the indicated strains were spotted onto rich medium without drug, or medium containing CPT or MMS at the indicated concentrations.



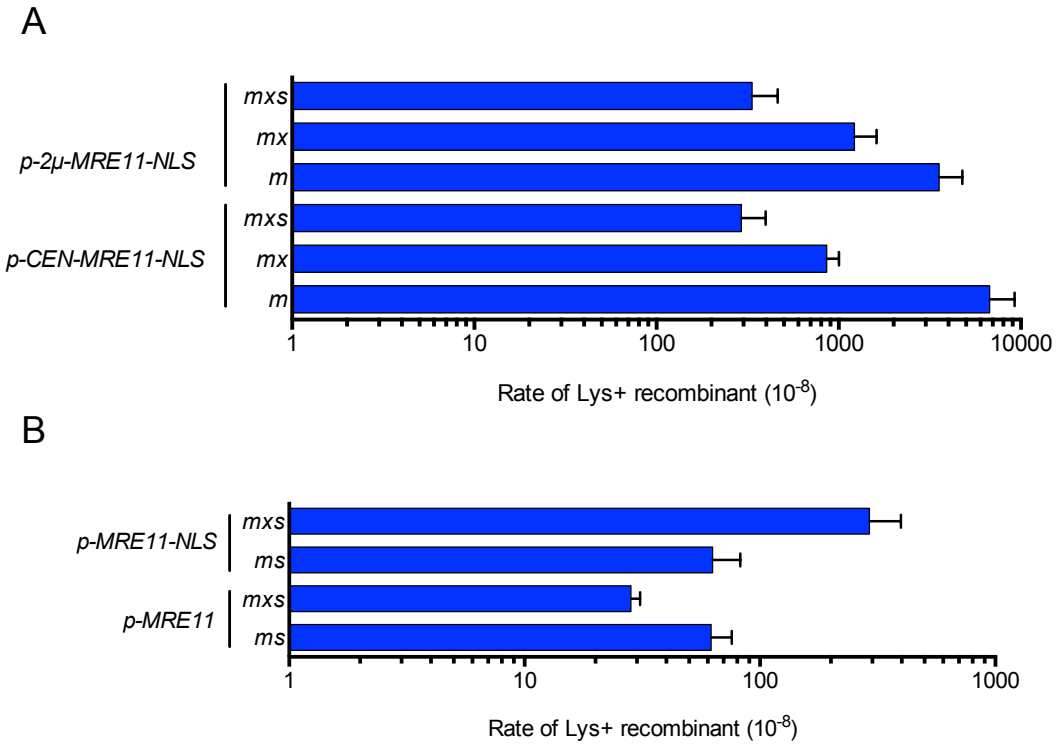
Supplementary Figure 3. Suppression of the *xrs2Δ* end resection defect by *MRE11-NLS* related to Figure 3.
(A) Southern blot analysis of the genomic DNA from the indicated strains.
(B) qPCR analysis of end resection in the indicated strains. Error bars indicate s.d. (n=3).
(C) Southern blot of StyI digested DNA from *exo1Δ sgs1Δ* and *exo1Δ MRE11-NLS xrs2Δ* strains. The smear indicates MRX-Sae2 cleavage products in the absence of extensive resection.



Supplementary Figure 4. **The effect of Xrs2 on the nuclease activities or Mre11- Rad50 in the presence of Sae2, related to Figure 4.**

(A) Nuclease assay with Mre11-Rad50 (MR), Mre11-Rad50-Xrs2 (MRX) and Sae2, as indicated.

(B) Quantitation of data from panel a. Error bars indicate SEM ($n = 2$).



Supplementary Figure 5. Hairpin cleavage by the MR complex, related to Figure 5.
(A) IR-stimulated recombination rates for the indicated strains with *MRE11-NLS* expressed from a single copy (*CEN*) or high copy number plasmid (2-micron).
(B) IR-stimulated recombination rates for p*MRE11* or p*MRE11-NLS* expressed in *mre11Δ sae2Δ* and *mre11Δ sae2Δ xrs2Δ* derivatives.

Supplementary Table1, related to Figures 1, 2, 3, 4 and 5.

Strain	Genotype	Source
LSY0678	*MATa*	R. Rothstein
LSY0679	*MATα*	R. Rothstein
LSY2992	*MATα xrs2::kanMX*	This study
LSY2993	*MATa xrs2::kanMX*	This study
LSY0779	*MATa mre11::LEU2*	Lab collection
LSY0780	*MATα mre11::LEU2*	Lab collection
LSY3000-5C	*MATa mre11:LEU2 xrs2::kanMX*	This study
LSY3000-2A	*MATα mre11:LEU2 xrs2::kanMX*	This study
LSY3289	*MATa MRE11-NLS*	This study
LSY3386-1A	*MATα MRE11-NLS*	This study
LSY3386-8D	*MATa MRE11-NLS xrs2::URA3*	This study
LSY3386-4A	*MATα MRE11-NLS xrs2::URA3*	This study
LSY3397-3B	*MATα MRE11-NLS rad50::URA3*	This study
LSY3397-10D	*MATα rad50::URA3 xrs2::URA3*	This study
LSY3397-9B	*MATa MRE11-NLS rad50::URA3 xrs2::URA3*	This study
LSY3475-5B	*MATa MRE11-NLS sae2::kanMX*	This study
LSY3398-4C	*MATα sae2::kanMX xrs2::URA3*	This study
LSY3516-4C	*MATα MRE11-NLS sae2::kanMX xrs2::URA3*	This study
LSY3516-12C	*MATa MRE11-NLS sae2::kanMX xrs2::URA3*	This study
LSY2363-28C	*MATa mec1::TRP1 sml1::HIS3*	Lab collection
LSY3399-9C	*MATα mec1::TRP1 sml1::HIS3 MRE11-NLS*	This study
LSY3399-20B	*MATα mec1::TRP1 sml1::HIS3 xrs2::URA3*	This study
LSY3399-16C	*MATa mec1::TRIP1 sml1::HIS3 MRE11-NLS xrs2::URA3*	This study
LSY3685-3B	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ HA-TEL1-URA3*	This study
LSY3685-5D	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ HA-TEL1-URA3 MRE11-NLS*	This study
LSY3685-5A	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ HA-TEL1-URA3 xrs2::kanMX*	This study
LSY3685-3D	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ HA-TEL1-URA3 MRE11-NLS xrs2::kanMX*	This study
LSY2611-23D	*MATa ade2-ISIR-10MH lys2::P_{GAL}-I-SceI*	This study
LSY3529-2C	*MATα ade2-ISIR-10MH lys2::P_{GAL}-I-SceI MRE11-NLS*	This study
LSY3529-1B	*MATα ade2-ISIR-10MH lys2::P_{GAL}-I-SceI xrs2::URA3*	This study
LSY3529-5A	*MATα ade2-ISIR-10MH lys2::P_{GAL}-I-SceI MRE11-NLS xrs2::URA3*	This study
LSY3529-15C	*MATa ade2-ISIR-10MH lys2::P_{GAL}-I-SceI MRE11-NLS xrs2::URA3*	This study
LSY3542	*MATa ade2-ISIR-10MH lys2::P_{GAL}-I-SceI xrs2-S47A-H50A*	This study
LSY3344-15D	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ*	This study
LSY3464-1A	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ*	This study
LSY3387-1D	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS*	This study
LSY3464-1C	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS*	This study
LSY3083-22D	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ xrs2::kanMX*	This study
LSY3464-4C	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ xrs2::kanMX*	This study
LSY3327	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS xrs2::kanMX*	This study
LSY3464-2C	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS xrs2::kanMX*	This study
LSY3494-5C, 18B	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS sae2::kanMX*	This study
LSY3387-9A	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS xrs2::kanMX sae2::kanMX*	This study
LSY3387-10A, 21B	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS xrs2::kanMX sae2::kanMX*	This study
LSY3565-1C, 2A	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS tel1::hphMX*	This study
LSY3565-3A	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS tel1::hphMX*	This study
LSY3589-1B, 3D	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS tel1::hphMX sae2::kanMX*	This study
LSY3589-2D	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ MRE11-NLS tel1::hphMX sae2::kanMX*	This study
LSY3576-5B	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 MRE11-NLS*	This study
LSY3576-12D	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 MRE11-NLS*	This study
LSY3576-2A	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 xrs2::kanMX*	This study

LSY3576-5D	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 xrs2::kanMX*	This study
LSY3576-1C	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 MRE11-NLS xrs2::kanMX*	This study
LSY3576-4B	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 MRE11-NLS xrs2::kanMX*	This study
LSY3576-1A, 12B	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 MRE11-NLS xrs2::kanMX sae2::kanMX*	This study
LSY3584-9A	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 MRE11-NLS sae2::kanMX*	This study
LSY3584-2C	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 MRE11-NLS sae2::kanMX*	This study
LSY3576-1A, 12B	*MATa leu2::Gal-HO-LEU2 hmlΔ hmrΔ DNA2-TEV-9MYC-HIS3 MRE11-NLS xrs2::kanMX sae2::kanMX*	This study
ALE94	*MATα ade5-1 his7-2 leu2-3,112::p305L3 (LEU2) trp1-289 ura3-Δ lys2::AluIR*	(Lobachev et al., 2002)
ALE108	*MATα ade5-1 his7-2 leu2-3,112::p305L3 (LEU2) trp1-289 ura3-Δ lys2::AluIR sae2::hgrB*	(Lobachev et al., 2002)
LSY2930	*MATα ade5-1 his7-2 leu2-3,112::p305L3 (LEU2) trp1-289 ura3-Δ lys2::AluIR mre11::TRP1*	(Chen et al., 2015)
LSY3109	*MATα ade5-1 his7-2 leu2-3,112::p305L3 (LEU2) trp1-289 ura3-Δ lys2::AluIR mre11::TRP1 xrs2::kanMX*	This study
LSY3174	*MATα ade5-1 his7-2 leu2-3,112::p305L3 (LEU2) trp1-289 ura3-Δ lys2::AluIR mre11::TRP1 xrs2::kanMX sae2::hgrB*	This study
ALE1	*MATα ade5-1 his7-2 leu2-3,112::p305L28 (LEU2) trp1-289 ura3-Δ lys2::AluIR*	(Lobachev et al., 2000)
LSY3553	*MATα ade5-1 his7-2 leu2-3,112::p305L28 (LEU2) trp1-289 ura3-Δ lys2::AluIR sae2::kanMX*	This study
LSY3557	*MATα ade5-1 his7-2 leu2-3,112::p305L28 (LEU2) trp1-289 ura3-Δ lys2::AluIR tel1::hphMX*	This study
LSY2346	*MATa lif1::kanMX*	This study
LSY1996	*MATa tel1::hphMX*	This study
LSY3543-4C	*MATa tel1::hphMX lif1::kanMX*	This study
LSY3543-8D	*MATα tel1::hphMX lif1::kanMX*	This study
LSY3299-5C	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ exo1::kanMX sgs1::hphMX*	Lab collection
LSY3519-5A	*MATα leu2::Gal-HO-LEU2 hmlΔ hmrΔ exo1::kanMX xrs2::kanMX MRE11-NLS*	This study

*All strains, except ALE94, ALE108, ALE1, LSY2930, LSY3109, LSY3174, LSY3553 and LSY3557 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.

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Supplemental Experimental Procedures

Yeast strain and plasmid construction: The strain containing *MRE11-NLS* integrated into the endogenous locus was constructed by one-step gene targeting. A PCR fragment containing a sequence encoding a monopartite NLS (CCAAAAAAGAAGAGAAAGGTC) in the 3' end of the *MRE11* ORF, along with 759bp of the upstream and 59bp of the downstream region of the *MRE11* locus, was transformed into an *mre11::URA3* strain, selecting for 5-fluoroorotic acid resistance. PCR and DNA sequencing were used to confirm clones with correct integration of the *MRE11-NLS* allele. The strain containing N-terminal HA-tagged *TEL1* was constructed by integration of pRS306-HA-TEL1 (Nakada et al., 2003) at the endogenous *TEL1* locus. Integration was confirmed by PCR and expression of HA-Tel1 was confirmed by western blotting. Other W303 derivatives were constructed by crossing isogenic strains present in our laboratory collection to produce the indicated genotypes. For non-W303 strains, one-step gene replacement with PCR products was used to construct desired mutations. pRS416-*mre11-H125N-NLS* was constructed by site-directed mutagenesis of pRS416-*MRE11-NLS* (Schiller et al., 2012).

Recombinant proteins. Mre11-Rad50 heterodimer was prepared using his-tagged Mre11 and untagged Rad50 constructs (Cannavo and Cejka, 2014). The soluble extract preparation and binding to Ni-NTA resin was carried out as described previously for the heterotrimer (Cannavo and Cejka, 2014). The Ni-NTA resin bound by Mre11-Rad50 was washed with Wash buffer I (Tris-HCl, pH 7.5, 50 mM; β-mercaptoethanol, 2 mM; NaCl, 0.2 M; phenylmethylsulphonyl fluoride, 1 mM; leupeptin, 10 $\mu g.ml^{-1}$; glycerol, 10%; imidazole, 25 mM), followed by Wash buffer II ($KHPO_4$, pH 7.4, 20 mM; β-mercaptoethanol, 2 mM; KCl, 80 mM; glycerol, 10%, phenylmethylsulphonyl fluoride, 1 mM) containing 25 mM imidazole. The heterodimer was eluted with Wash buffer II supplemented with 300 mM imidazole. Fractions containing Mre11-Rad50 were pooled and diluted with 5 volumes Wash buffer II without imidazole and 1 volume of H_2O. The sample was loaded on a pre-equilibrated 1 ml HiTrap SP column (GE Healthcare). The column was washed with SP buffer A ($KHPO_4$, pH 7.4, 20 mM;

dithiothreitol, 1 mM; KCl, 100 mM; glycerol, 10%, phenylmethylsulphonyl fluoride, 1 mM). The protein was eluted with a 20 ml gradient in the same buffer with increasing KCl concentration (0.1 to 1 M). Samples were analyzed on SDS-PAGE gels and fractions containing protein (1.8 ml) were diluted with 10 ml Wash buffer II without imidazole and 10 ml H_2O. The sample was loaded on a pre-equilibrated 1 ml HiTrap Q column (GE healthcare), washed and eluted as above but with only 12 ml KCl gradient. Fractions containing recombinant Mre11-Rad50 were pooled, frozen in liquid nitrogen and stored at -80°C. Xrs2 was similarly expressed in *S. frugiperda* 9 cells and prepared by applying the soluble extract on anti-FLAG M2 affinity resin (Sigma). The resin was extensively washed with de-gassed Wash buffer (Tris-HCl, pH 7.5, 30 mM; β-mercaptoethanol, 0.3 mM; NaCl, 0.3 M; phenylmethylsulphonyl fluoride, 1 mM; leupeptin, 10 $\mu g.ml^{-1}$; glycerol, 10%; NP40, 0.1%), and then with the same buffer without NP40. Xrs2 was eluted with wash buffer without NP40 but with FLAG peptide (Sigma, 200 $\mu g.ml^{-1}$). Fractions containing Xrs2 were pooled, frozen in liquid nitrogen and stored at -80°C.

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