Supplementary Materials and Methods

DNA oligonucleotides and ^{32}P labeling

PAGE-purified DNA substrates were purchased from Bioneer Co. (Daejeon, Korea). For crystallization, DNA molecules were further purified and annealed. DNA1 contained 5'-ATTCGACCTGGCACGTAGGACAGCAG-3' (template strand) and 5'-CTGCTGTCCTA CGTGCCAGGTCAAGA-3' (non-template strand). DNA2 contained 5'-GACCTGGCACG TAGGACAAAAA-3' (template) and 5'-GTCCTACGTGCCAGCTG-3' (non-template). The substrates were annealed in buffer containing 150 mM NaCl and, 15 mM sodium citrate buffer by boiling at 95°C for 5 min, followed by slow-cooling at 18°C overnight. For nuclease assays, 5'-^{32}P-labeled substrates were prepared with polynucleotide kinase (Roche Applied Science, Rockford, IL, USA) and γ-^{32}P-dATP (PerkinElmer, Waltham, MA, USA). 3'-^{32}P-labeled substrates were prepared with Terminal transferase (Roche Applied Science, Rockford, IL, USA) and α-^{32}P-dATP (PerkinElmer, Waltham, MA, USA). DAR134 (5'-C ATCCATGCCTACCTGACAGCTTA GCACATCGGACTGTCAGGTAGGCATG-3') was prepared as previously described (Paull and Gellert, 2000) and labeled with [^{32}P] at the 5′ end. The TP124/ TP580 substrate was prepared as described by Hopkins and Paull (2008) by annealing 3' [^{32}P]-labeled TP124 (5'-CATCTGGCCTGTCTTACACAGTGCTACAGACTG GAACAAAAACCCTGCAG-3') annealed to TP580 (5'-CTGCAGGGTTTTTGTTC CAGTCTGTAGCACTGTGTAAGACAGGCCsAsGsAsTsG-3'). Phosphorothioate bonds are indicated by "s" between the nucleotides. All labeled DNA was further purified by 10% native PAGE, electro-eluted in extraction buffer (500 mM ammonium acetate, 10 mM $MgCl_2$, 1 mM EDTA, and 0.1% SDS), ethanol-precipitated, and resuspended in H_2O. Both DAR134 and TP124/TP580 are annealed using a slow-cooling protocol as described above.

Mutagenesis

All the *Mj*Mre11 and *Sc*Mre11 mutants used in this study were created by PCR-based methods (Stratagene). Sequencing of the genes encoding the Mre11 mutant proteins confirmed that no additional mutation other than the targeted mutation was introduced. The *Mj*Mre11 mutant proteins were purified using affinity chromatography followed by Mono-S and gel filtration chromatography, as described above. Primer sequences used in mutagenesis are listed in Supplementary Table S3.

Yeast strains

All yeast strains used in this study are derivatives of SLY1A (*ho⊿ hml::ADE1 MAT***a** *hmr::ADE1 ade1 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL10::HO*) or BY4741 (*MAT***a** *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*). Gene deletion or replacement was constructed using the one-step gene replacement technique using a PCR-derived amplification of a selection gene cassette flanked by short terminal sequences homologous to the target gene (Wach et al, 1994). To construct *mre11* mutant, the amino terminal part (+1~1064 bp) of the *MRE11* coding sequence was cloned into pSC2 and modified by PCR based site-directed mutagenesis. Mutant mre11 was then integrated into *MRE11* genomic locus by replacing *mre11*::*URA3* with mutagenized *mre11* fragment harboring designated mutations. Mutations were confirmed by sequencing. A detailed list of strains used for this study is shown in Supplementary Table S4.

Analysis of yeast *mre11* mutants

Five-fold serial dilutions of nuclease deficient *mre11* (mre11-H125N) and *mre11* variants with mutations at putative DNA binding interface (K62A and R184A) or at Xrs2 binding (R83A) were spotted onto YEPD plates with the indicated dose of genotoxic drugs and incubated at 30^{o}C for 2-3 days before being photographed. Drug sensitivity assay was also performed in strains deleted for *SGS1* to examine the effect of *mre11* mutations on resection. CPT, camptothecin; methyl methane-sulfonate (MMS).

The amount of resection was monitored at various times post HO expression using Southern blot hybridization or quantitative PCR as described previously (Zhu et al, 2008; Zierhut and Diffley, 2008). The amount of ssDNA that is resistant to *Eco*RI or *Bsa*JslA1 cleavage flanking the HO induced DSB at the *MAT* locus from yeast strains lacking *HML* and *HMR* homologous donor sequences was measured to determine the resection rate. For Southern blot based resection assay, genomic DNA isolated from cells at indicated times post HO induction was digested by *Eco*RI, run on 1% agarose gel and then hybridized with radiolabeled DNA probe annealed to the *MAT* distal sequence. The intensity of the bands on Southern blots was analyzed by ImageQuant TL (Amersham Biosciences). DSB end resection beyond each *Eco*RI site was estimated as a percentage of the signal intensity hybridized to the *Eco*RI fragment at 1 hr after break induction, normalized by the band intensity hybridized to *TRA1* DNA probe.

For PCR based resection assay, genomic DNA was digested with *Bsa*JslA1 at 0.7 and 5.7 kb distal sites flanking a DSB, and the enzyme digests were subjected to qPCR using a series of appropriate primer sets that amplify regions flanking the *Bsa*J1 cleavage

sites. Successful resection at the given genomic locus will transform the region into single stranded DNA, and the region becomes resistant to *Bsa*JslA1 mediated cleavage to produce PCR products. The percent resection is determined by dividing the number of PCR signals from the restriction enzyme digests into the number of undigested samples normalized by the PCR signals of yet other location (*ARS501*) that do not harbor *Bsa*JslA1 recognition sequence. To detect resection at 0.7 kb, primers 0.7k-F (5'-CCTTCTTCATTACTAATTCATCTTCGCCACA-3) and 0.7k-R (5'-GGGTGATGTACACCCTAATCAACAGTTTTGT-3') were used, whereas to detect resection at 5.7 kb, primers 5.7k-F (5'- GTCCATAATTGAAATAACCGAGTCCGT-3') and 5.7k-R (5'-CTCCACTTGATGCTTCCTTATTGAGAG-3') were used. To detect ssDNA at ARS501, primers ARS501-F (5'-ACTTAGTTCTGGAGATTATCGCCTTATCG-3') and ARS501-R (5'-CTGTCTTTCGCTTAGTTCACCTCTACC-3') were used.

Electrophoretic mobility-shift assays

5' ^{32}P-labeled TP124/580 (20 nM) in the buffer (10 mM BTP-HCl pH 7.5, 50 mM NaCl, 5 mM DTT, 5% glycerol) was incubated at 37°C for 30 min with three different concentrations (1, 5, and 15 μM) of *Mj*Mre11 (wild-type, R55S, R89S, K129A, K132D, I302R, and I302Y), and the complexes were separated on 6% native PAGE gels in 0.5×TBE.

Gel filtration analysis

For gel filtration analysis, wild-type or each mutant *Mj*Mre11 (2 mg/ml) was loaded into a Superdex 200 column (Amersham Biosciences) equilibrated with buffer containing 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5% glycerol, and 5 mM 2-mercaptoethanol.

Yeast two-hybrid assay

Yeast two-hybrid system analysis was performed with a GAL4 DNA-binding domain (BD)-fused Mre11s and a GAL4 activation domain (AD)-fused Xrs2 or Rad50. The bait (BD-Mre11) and the prey (AD-Xrs2 or AD-Rad50) proteins were co-expressed in the yeast strain PBN204 (*MATa, pGAL1-lacZ, pGAL1-URA3, pGAL2-ADE2, trp1-901, leu2-3, gal4d, gal80d*). PBN204 contains *URA3*, *ADE2*, and beta-galactosidase as reporter genes and *trp1* and *leu2* as selection marker genes (Panbionet Inc.). Co-transformation of BD-polypyrimidine tract-binding protein (PTB) and AD-PTB served as a positive control for the protein-protein interaction. PTB is a homo-dimeric protein. pGBKT7 and pGADT7 were used as the negative control.

Supplementary References

Wach A, Brachat A, Pöhlmann R, Philippsen P (1994) New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast **13:**1793-1808.

Chen VB, Arendall WB 3rd, Headd JJ, Keedy DA, Immormino RM, Kapral GJ, Murray LW, Richardson JS, Richardson DC (2010) MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr. **66:**12-21

Supplementary Figure legends

Supplementary Figure S1. Simulated annealing omit maps of the DNA1-*Mj*Mre11 complex at 3.55 Å resolution. All maps (A–D) were calculated with phases after the model with the omitted region was subjected to simulated annealing refinement. (**A**) The $2F_o$-F_c map for the omitted DNA1. The map was contoured at 1.0 σ. (**B**) The $2F_o$-F_c map for the omitted Mg^{2+} ion, contoured at 4.0σ. (**C**) The $2F_o$-F_c map for the protein–DNA interface contoured at 1.0 σ. (**D**) The F_o-F_c map near the active site of the Mre11 C/D dimer, contoured at 2.4σ. The density shown here is present only in the protein–DNA1 complex, but not in the protein–DNA2 complex. We cannot precisely assign the source of the electron density due to the limited resolution of this structure. However, the density is similar than the mononucleotide we modeled in this figure. One density is present at the crevice near His85, His188, Tyr210, and the Mg^{2+} ion. About 10 Å away from this density, another density surrounded by a cluster of ring residues (Tyr13, Tyr16, and Tyr210) is observed. The nucleotide modeled in this density is at the trajectory of DNA (yellow) bound to *Mj*Mre11, which was derived from the Mre11 A/B–DNA1 complex after superposition.

Supplementary Figure S2. Schematic diagram of the protein–protein interface within the asymmetric unit. Mre11 A, B, C/D, and E/F are shown in cyan, pink, light green, and gold, respectively. H-bonds and ion-pairs are shown in a dotted line. **(A)** Close-up view of the interaction between Mre11 C and Mre11 A/B dimer, in which the capping domain and the C-terminal tail of Mre11 C bind to the four-helix-bundle region of Mre11 A/B. **(B)** Close-up view of the interaction between Mre11 F and Mre11 A/B within the asymmetric unit. Helices α5 and α4 of Mre11 F bind to loop α1–β2 and loop β12–β13 of Mre11 A, respectively

Supplementary Figure S3. Close-up view of the *Mj*Mre11-DNA complex

(A) Structural superposition of the Mre11 - DNA1 (cyan/ magenta/ orange/ yellow) and

Mre11 - DNA2 (gray) complexes. The figure is drawn in the same orientation as that of 1C. **(B)** Schematic diagram of the interactions between *Mj*Mre11 and DNA1 and **(C)** DNA2. Mre11 A and B are boxed and marked with A and B, respectively. Non-template and template strands are labeled. (**D**) Structural superposition of various Mre11 proteins; *Mj*Mre11 (cyan), *Pf*Mre11 (purple; 3DSC), *Sp*Mre11 (yellow; 4FBQ), *Hs*Mre11 (green; 3T1I), and *Tm*Mre11 (gray; 3QG5). A region containing residues Tyr13, His17, Arg55, and Arg89 of *Mj*Mre11 is highlighted; Tyr13 of *Mj*Mer11 is equivalent to Tyr13 (*Pf*), Tyr30 (*Sp*), Phe25 (*Hs*), and Val19(*Tm*); Asn17 of *Mj*Mer11 is equivalent to His17 (*Pf*), Lys33 (*Sp*), and Thr23(*Tm*); Arg55 of *Mj*Mer11 is equivalent to Arg55 (*Pf*), Lys71 (*Sp*), Lys66 (*Hs*), and Asn65 (*Tm*); and Arg89 of *Mj*Mer11 is equivalent to Gln89 (*Pf)*, Thr133 (*Hs*), Lys97 (*Tm*). For clarity, only a few residues, including those from *Mj*Mre11, are labeled. (**E**) Structural superposition of various Mre11 proteins. A region containing residues Lys129, Ser131, and Lys132 of *Mj*Mre11 (green) is highlighted. Arg87, Arg90, and Lys144 of *Pf*Mre11 (purple) are very close to this region. Arg196 of *Tm*Mre11 (gray) is close to the Lys132 of *Mj*Mre11. In addition, Arg190 and Arg193 in *Sp*Mre11 (yellow) and Arg188 and Arg191 in *Hs*Mre11 (green) are located in this basic loop.

Supplementary Figure S4. **Surface representation of Mre11 structures from various species reveals a structurally conserved basic region that binds to DNA ends**. A basic region that interacts with the duplex end is marked with an arrow and dashed circle. (**A**) *Mj*Mre11, (**B**) *Pf*Mre11 (3DSC), (**C**) *Tm*Mre11 (2Q8U) (**D**) *Sp*Mre11 (4FBQ), and (**E**) *Hs*Mre11 (3T1I).

Supplementary Figure S5. **Exonuclease activity of wild-type and mutant *Mj*Mre11 proteins toward TP124/580.**

Residues in the DNA-binding interface (Arg55, Arg89, Lys129, and Lys132) and capping-domain mutant (I302R and I302Y) are mutated. Reaction mixtures containing 20 nM P^{32}-labeled DNA substrate and *Mj*Mre11 (200 nM or 600 nM) were incubated at 55°C for 30 min. Standard molecular marker size is shown on the left. Quantitation of substrate cleavage is calculated and shown as in Figure 3D.

Supplementary Figure S6. DNA damage repair in cells expressing *mre11* mutants.
Five-fold serial dilutions of exponentially growing cells (two different backgrounds:
(A) BY4741 and **(B)** JKM139) spotted onto YEPD plates with the indicated amounts of DNA-damaging agents. Photographs were taken after 3 days of incubation at 30°C.

Supplementary Figure S7. Analysis of 5' resection by Southern blotting.
(**A**) The location of probe and the *Eco*RI restriction sites that were used to detect the formation of *Eco*RI resistant ssDNA are shown. (B) Results of Southern blot based resection assays performed on cells (JKM139 derivatives) with *SGS1* deletion but expressing wild type Mre11, mre11-K62A, -R184A or nuclease deficient mre11-H125N.

Supplementary Figure S8. PCR-based resection assay. A JKM139 strain lacking sequences homologous to *MAT***a** and *MATα* (*HMR, HML*) should repair a DSB break induced at the *MAT* locus by inefficient end joining or microhomology-mediated end joining (MMEJ) upon persistent HO expression and thus considered near "unrepairable". Unresected DNA is cleaved by the *Bsa*J1 restriction enzyme (Bs) and does not produce PCR products when amplified using primers flanking the restriction site (upper panel; no resection). Following DNA resection, the resulting ssDNA becomes resistant to restriction enzyme digestion, and is readily amplified by PCR using primers flanking the restriction site. Percent resection is determined by dividing the signal intensity of the PCR products from each time point by the intensity from cells in which HO was not induced.