SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Protein Purification

Sf9 cells were infected with recombinant baculovirus for expression of Exo1, Mre11 or Mre11/Rad50 human proteins and incubated at 25ºC for 72 hours. Exo1 was purified following the procedure described before (1). In brief, Sf9 cells expressing Exo1 were pelleted and then resuspended in a buffer containing 20 mM Tris-HCl (pH7.5), 2 mM DTT, 5 mM KCl, 0.5 mM PMSF, 0.5 μg/ml pepstatin A, 0.5 μg/ml leupeptin and 0.5 μg/ml antipain. The resuspension was homogenized and centrifuged. The pellet was solubilized in the above buffer containing 160 mM KCl and centrifuged again. The supernatant was loaded onto a Q sepharose column and Exo1 was eluted via a linear gradient of KCl (0.1 M-1 M). The fractions containing Exo1 were pooled, applied to a Heparin HiTrap column and a MonoS column chromatography sequentially.

The procedures for Mre11 or MR purification were described previously (2,3). Briefly, Sf9 cells were pelleted and then resuspended in a buffer containing 50 mM Na phosphate (pH 7.0), 0.5% Tween-20, 10% Glycerol, 20 mM β-mercaptoethanol, 20 mM imidazole, 2 mM PMSF and 0.5 M NaCl for Mre11 purification and 0.15 M NaCl for MR purification. The lysate was then homogenized, sonicated and centrifuged. The supernatant was incubated with Ni-NTA beads (QIAGEN) and proteins were eluted via an imidazole gradient. The fractions containing Mre11 were dialyzed into a buffer (50 mM NaCl; 20 mM Tris-HCl, pH 8.0, 0.05% Tween-20, 10% Glycerol and 1 mM DTT), loaded to a monoQ column and eluted with a linear gradient of NaCl (50 mM- 500 mM). Fractions containing Mre11 were confirmed by Western blot analysis using specific antibodies and nuclease assay. To get a homogenous MR complex, MR containing fractions from Ni-NTA were applied to MonoQ column and Superdex200 column chromatographies sequentially. The eluate containing both Rad50 and Mre11 at a peak fraction were used for the assays in this report. All the peak fractions subjected to SDS-PAGE and confirmed by Western Blot analysis.

Immunoprecipitation

Purified human Ku and Mre11 were incubated together at 4^{o}C for 1 hr in a binding buffer (10 μl) containing 40 mM Tris-HCl (pH 8.0), 40 mM KCl, 60 mM NaCl, 0.8 mM EDTA, 0.1 mM DTT, 0.4% NP-40 and 4% Glycerol. Protein G-Agarose (Roche), 40 μl (50% slurry), was incubated with either anti-Ku80 or anti-Mre11 (GeneTex) monoclonal antibody at 4^{o}C for 1 hr while tumbling. The beads were washed and incubated with the protein mixture in 200 μl binding buffer. The beads were pelleted, washed three times with lysis buffer and boiled with 1x sample buffer. Equal amount of input and samples were loaded to a 6% SDS-PAGE and analyzed by Western Blot using specific antibodies to Ku70 or Mre11.

Whole cell extract preparation and immunoblotting. Cells were trypsinized and washed twice in PBS. Cell pellets were resuspended in 50ul RIPA buffer and kept on ice for 30 min. The cells were snap frozen in liquid nitrogen, thawed immediately in a 37°C water bath, and the lysates were cleared by cetrifugation at 14,000 rpm at 4°C for 15 min. The supernatant was solubilized in 4X SDS buffer, resolved via an 8% SDS-PAGE gel and analyzed via Western blotting. Mre11 protein levels were detected via anti-Mre11 antibodies (GeneTex).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Protein purification of (A) Exo1, (B) Mre11 and (C) MR. Proteins were expressed and purified from Sf9 cells as described in the supplemental experimental procedures, resolved via SDS-PAGE and gels were Coomassie stained.

Supplemental Figure 2. Ku and Mre11 do not interact *in* vitro. Purified Ku and Mre11 were incubated together, immunoprecipitated with either Ku80 or Mre11 monoclonal antibody. Immunoprecipitated

proteins were separated via a 6% SDS-PAGE and analyzed by western blotting with Ku70 or Mre11 monoclonal antibody.

Supplemental Figure 3. Exonuclease activity of either purified Mre11 or Mre11-Rad50 complex. Exonulcease assay was performed as described in experimental procedures and for the time indicated in the figure. A. 12nM of Mre11 and 250nM of F-DNA were used in each reaction. B. 50nM of MR and 250nM of F-DNA were used in exonuclease assay. Each samples were stopped by adding formamide loading dye at time denoted in figure.

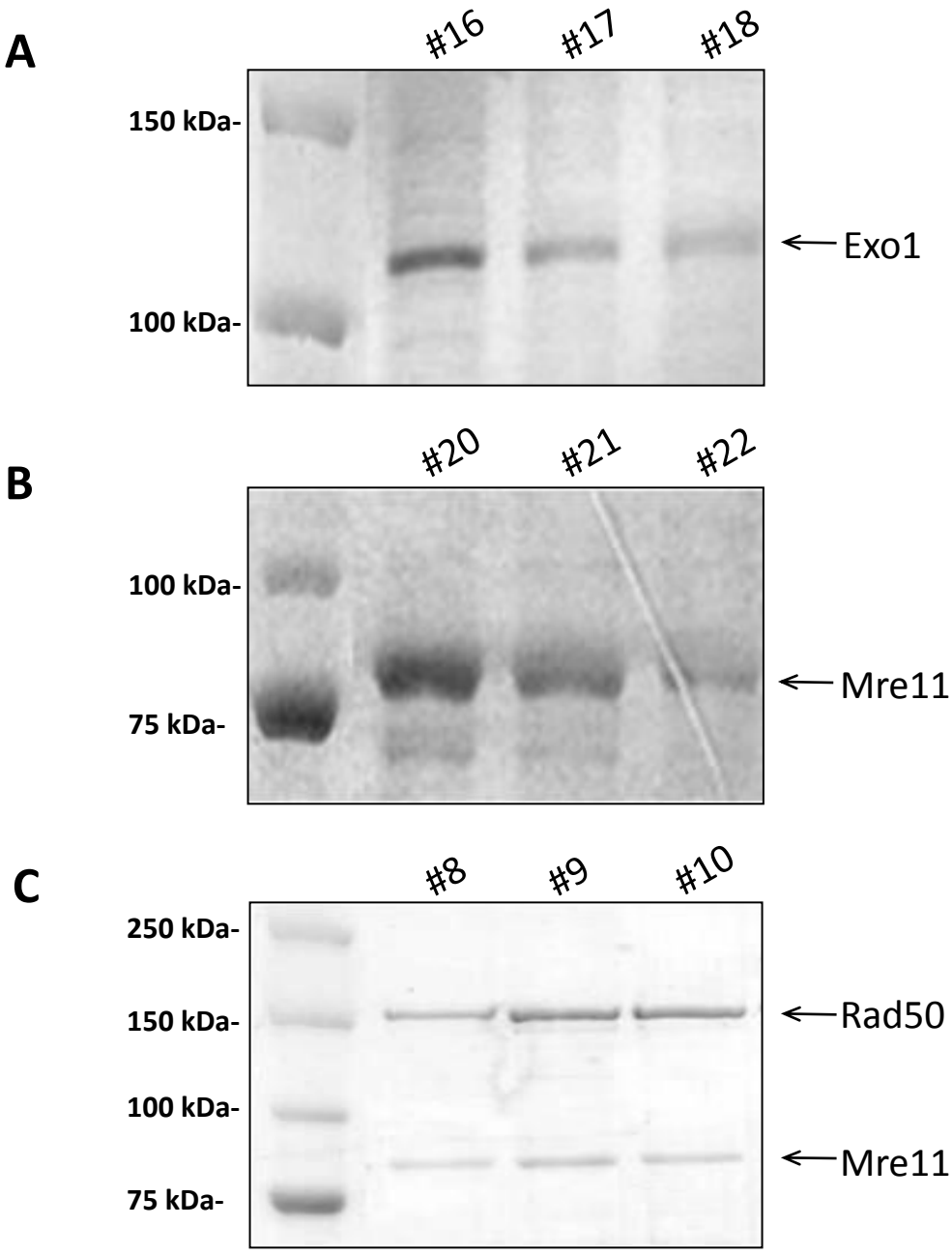
Supplemental Figure 4. Optimizing buffer conditions for nuclease activities of Exo1, Mre11, and the MR complex. Nuclease activities of Exo1, Mre11, or MR complex were tested in the buffers with different metal ions. The reactions were performed in a buffer containing different concentrations of $MnCl_2$ with or without $MgCl_2$. (A) Exo1-mediated DNA resection was similar in a buffer containing 5 mM $MgCl_2$ or 5 mM $MgCl_2$ and 0.5 mM $MnCl_2$ together but was inhibited by 1 mM $MnCl_2$. (B) DNA digestion mediated by Mre11 was similar with a buffer containing 1 mM $MnCl_2$ or 0.5 mM $MnCl_2$ and 5 mM $MgCl_2$ together. (C) MR exonuclease activity (bracket) without ATP and endonuclease activity (arrow) with ATP in the presence of 1 mM $MnCl_2$ was switched in a same buffer containing 0.5 mM $MnCl_2$. (D) MR activities were inhibited when incubating in a buffer containing 5 mM $MgCl_2$.

Supplemental Figure 5. Verification of Mre11 knock-down. Western blot analysis of control and Mre11 siRNA-transfected cells. YFP-Ku80-expressing U2OS cells were treated with control or Mre11 siRNA for 72 hours and harvested for Western blot analysis.

SUPPLEMENTAL REFERENCES

1. Genschel, J., Bazemore, L. R., and Modrich, P. (2002) *J Biol Chem* **277**, 13302-13311
2. Paull, T. T., and Gellert, M. (1998) *Mol Cell* **1**, 969-979
3. Lee, J. H., and Paull, T. T. (2006) *Methods Enzymol* **408**, 529-539

Supp Figure 1



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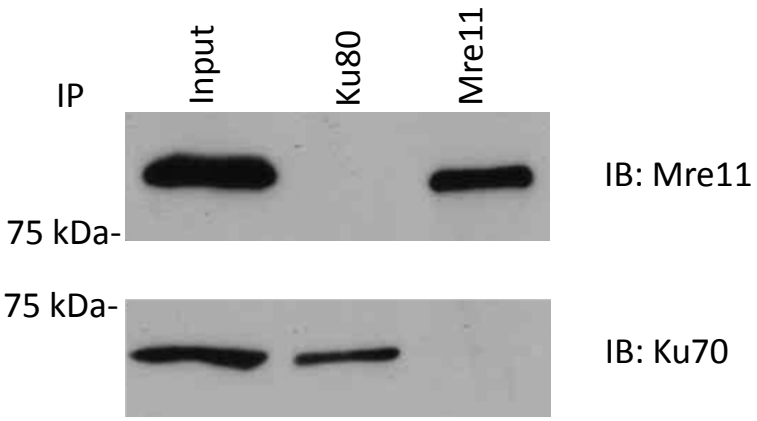

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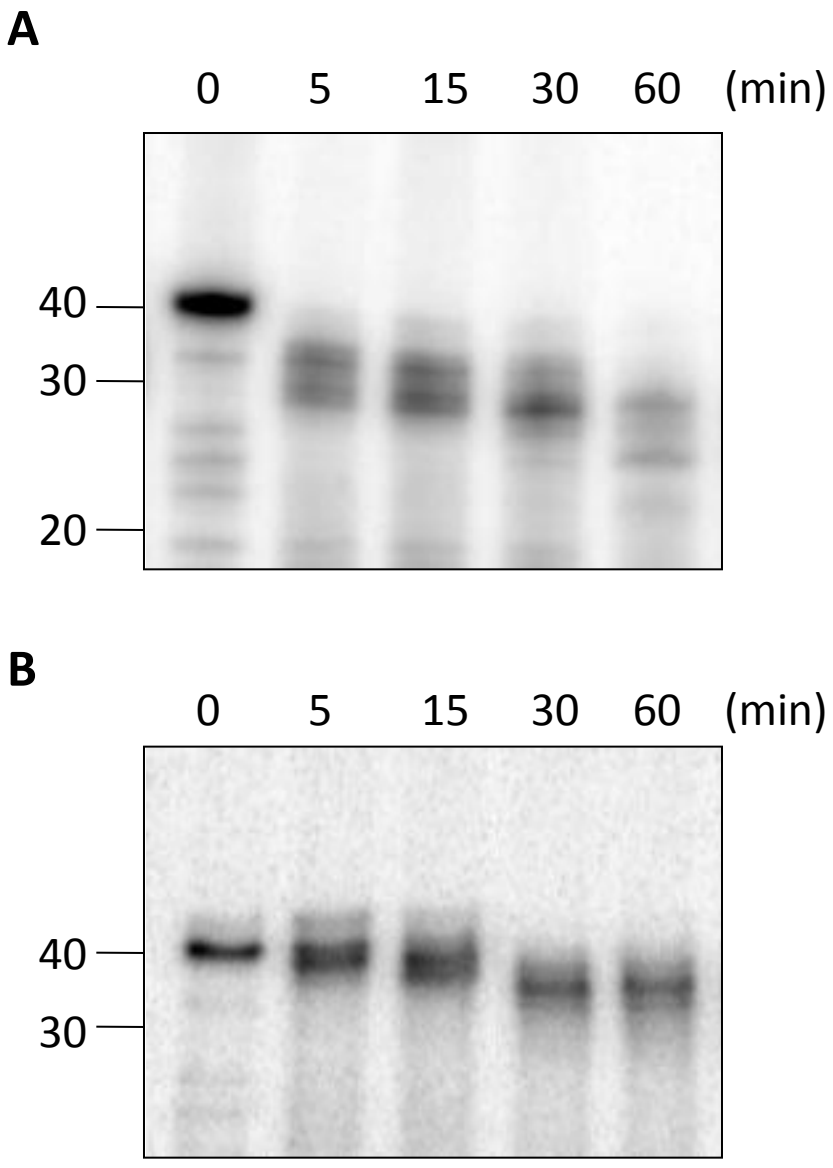

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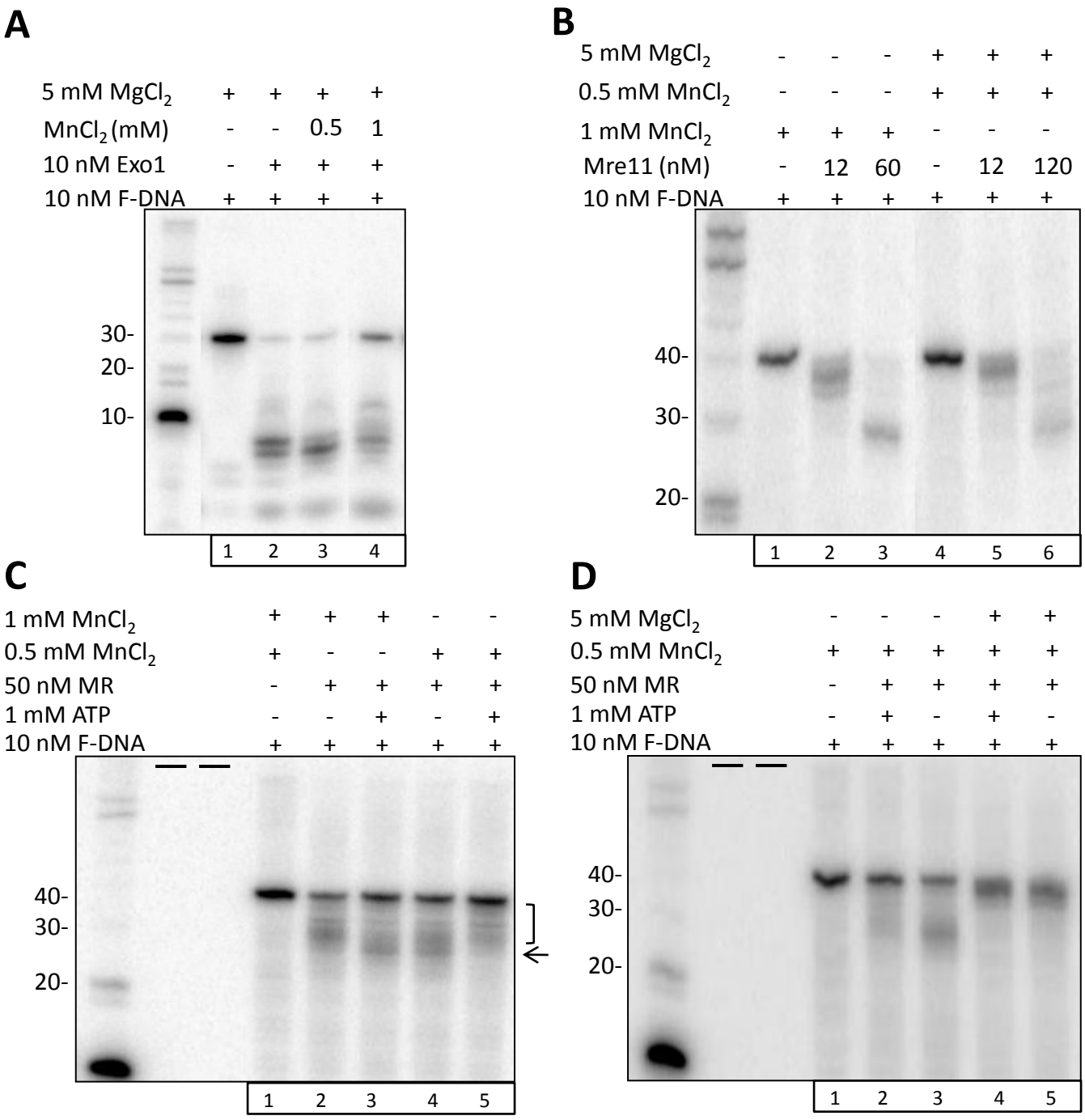

Supp Figure 5