Supporting Information

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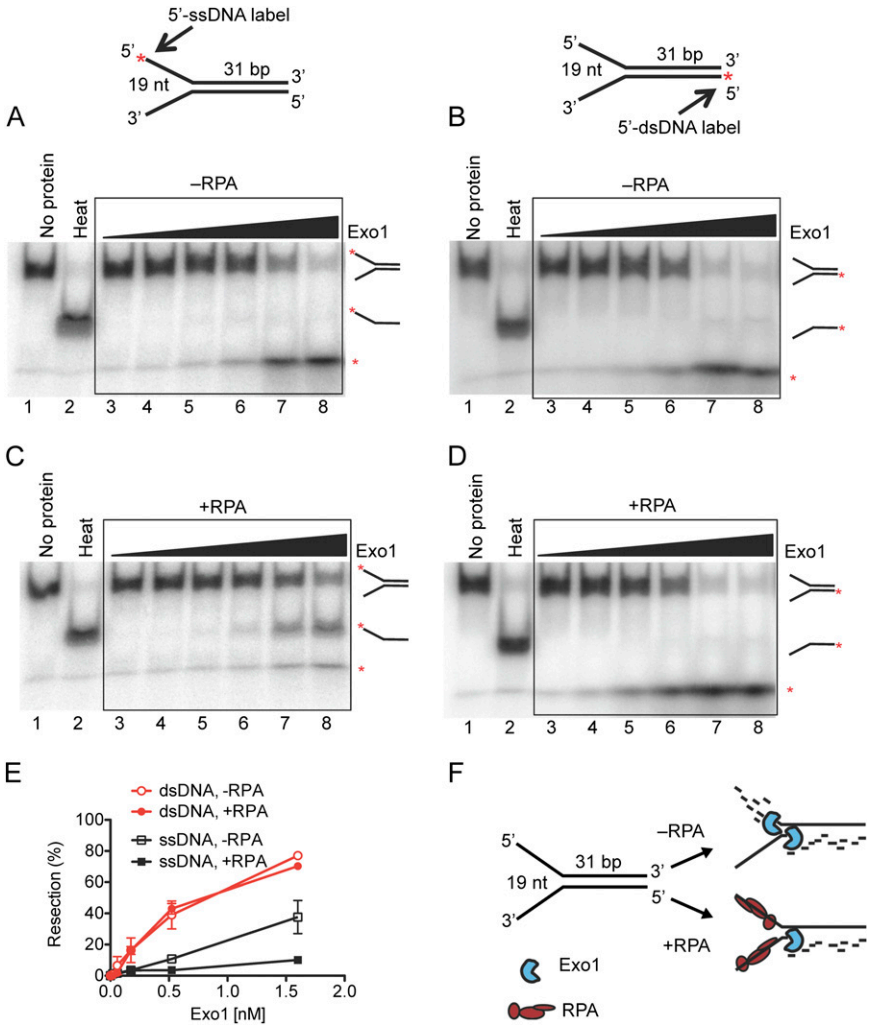

Fig. S1. Exo1 preferentially degrades dsDNA over ssDNA, but replication protein A (RPA) inhibits degradation of ssDNA. Shown is a schematic representation of the Y-structure DNA substrate with positions of ^{32}P label indicated by arrows. (*A* and *B*) Nuclease assays with increasing amount of Exonuclease 1 (Exo1) (0.02, 0.06, 0.2, 0.5, 1.6, and 4.7 nM) in the absence of RPA. The Y-structure DNA (1 nM) was labeled at the 5′ end of the ssDNA (*A*) or at the 5′ end of the dsDNA (*B*). (*C* and *D*) Nuclease assays as in *A* and *B*, but in the presence of RPA (22.5 nM). (*E*) Quantification of experiments as shown in *A*–*E*. Error bars show SE. (*F*) Model based on results from *A*–*D* showing nuclease activity of Exo1 on Y-structure DNA, in the presence or absence of RPA.

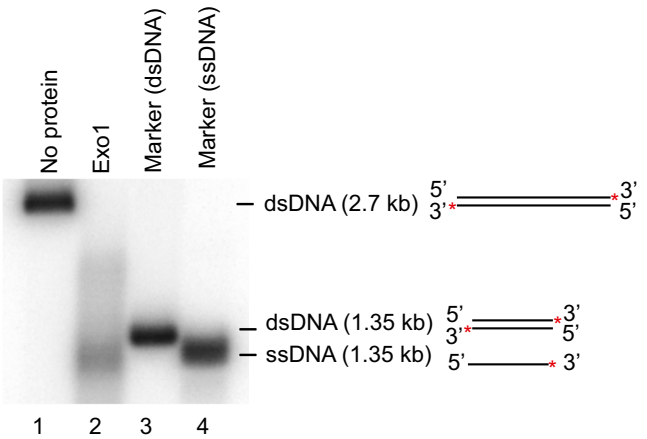

Fig. S2. Resection of dsDNA at high concentrations of Exo1 produces ssDNA of approximately half the length of the dsDNA. Lane 2, nuclease assays with Exo1 (50 nM) in the presence of RPA (0.4 μM) using 2.7 kb dsDNA (blunt, 1 nM) ^{32}P labeled at the 3′ end. Lane 1, substrate; lanes 3 and 4, markers for half-length dsDNA and ssDNA (1.35 kb), respectively.

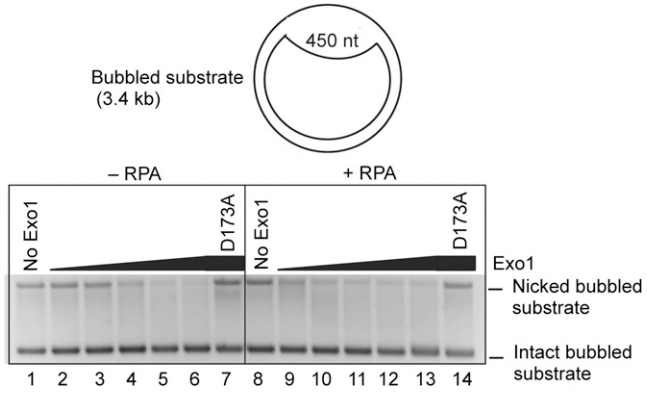

Fig. S3. Exo1 lacks endonuclease activity on covalently closed dsDNA with an ssDNA heteroduplex bubble. Shown are nuclease assays with Exo1 (0.5, 1.5, 4.5, 10, and 20 nM) or Exo1 D173A (20 nM) on a 3.4-kb dsDNA substrate (7.6 nM) containing a 450-nt bubble of noncomplementary ssDNA in the absence (lanes 1–7) or presence (lanes 8–14) of RPA (3 μM). Exo1 did not degrade covalently closed DNA. A fraction of the dsDNA substrate was nicked during substrate preparation due to handling of the individual ssDNA components; Exo1 readily degraded this nicked substrate fraction.

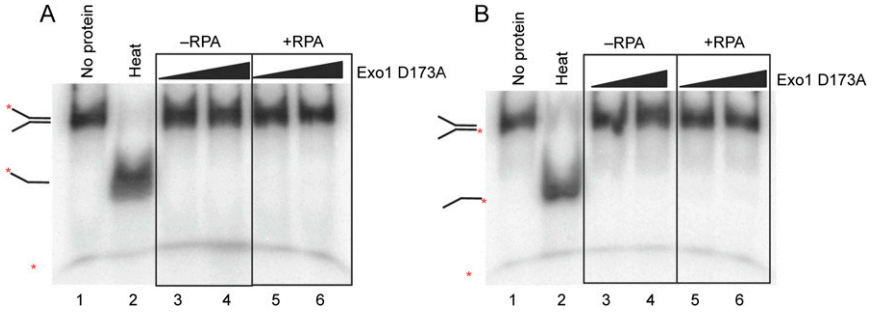

Fig. S4. Exo1 D173A shows no nucleolytic activity in the presence or absence of RPA. (*A*) Nuclease assays were performed with the Y–structure substrate (1 nM) ^{32}P labeled at the 5′ end of the ssDNA fork, with 1.6 and 4.7 nM Exo1 D173A, respectively. (*B*) As in *A*, but with substrate radioactively labeled at the 5′ end of the dsDNA region.

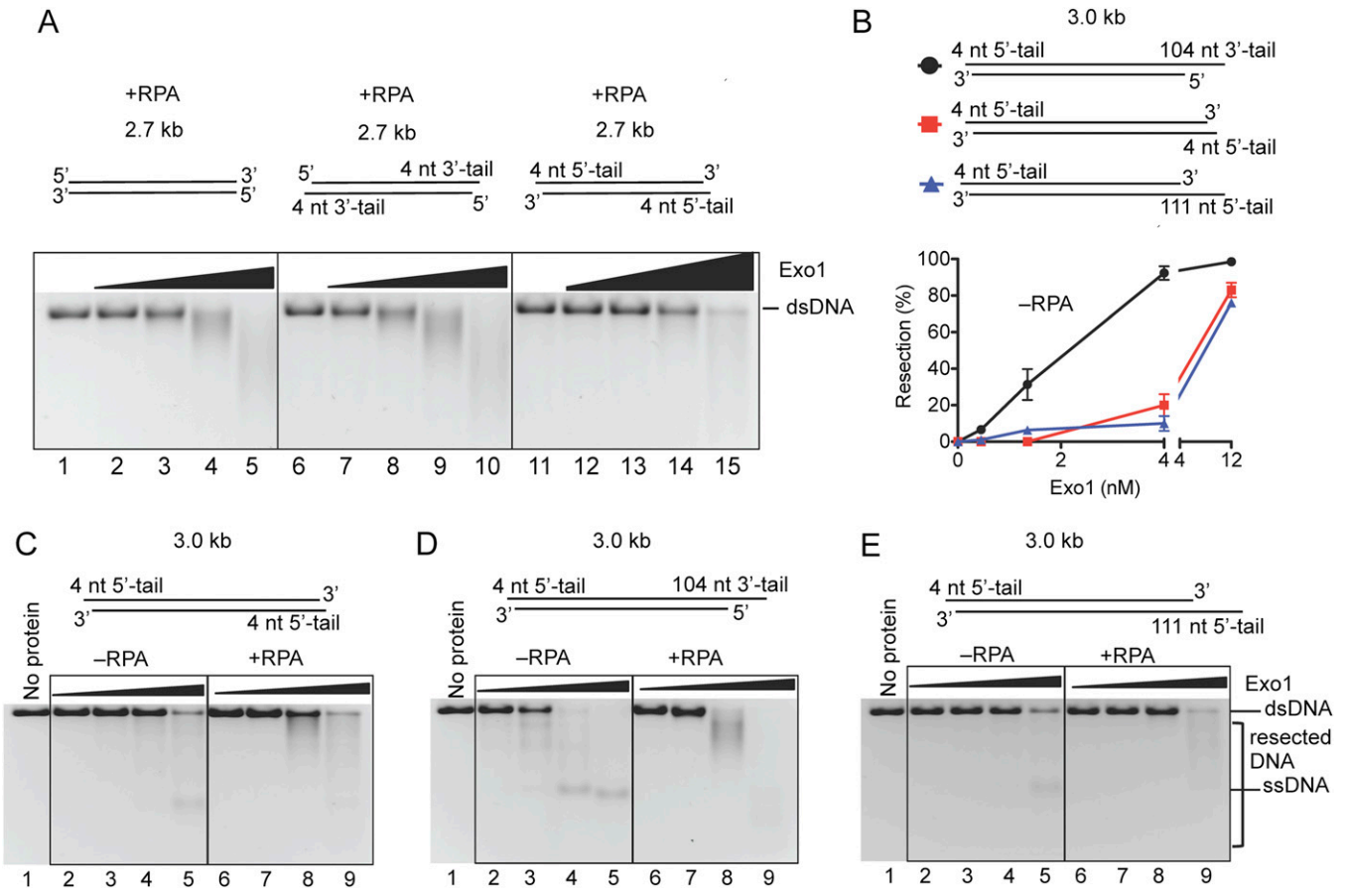

Fig. S5. Exo1 preferentially resects dsDNA that is resected at the 5′ end to have an ssDNA overhang at the 3′ end. (*A*) Nuclease assays using unlabeled dsDNA (7.6 nM) that either was blunt ended (lanes 2–5) or had an ssDNA overhang at the 3′ ends (4 nt, lanes 7–10) or an ssDNA overhang at the 5′ ends (4 nt, lanes 12–15), as a function of increasing amounts of Exo1 (1, 2, 4, and 6 nM in lanes 2–5 and 7–10 and 2, 4, 6, and 10 nM in lanes 12–15, respectively). The reaction was carried out in the presence of RPA (3 μM). (*B*) Quantification of Exo1 nuclease activity on 3.0 kb unlabeled DNA containing a ssDNA overhang of ∼100 nt with either a 3′ or a 5′ end or a ssDNA overhang of 4 nt at the 5′ ends (7.6 nM each). The reactions were carried out in the absence of RPA. Error bars show SE. (*C*–*E*) Nuclease reactions were carried out with Exo1 (0.45, 1.35, 4, and 12 nM) in the absence or presence of RPA (3 μM). The unlabeled DNA substrates (7.6 nM each) are shown above the lanes and contain an ssDNA overhang of ∼100 nt with either a 3′ or a 5′ end or a ssDNA overhang of 4 nt at the 5′ ends. All gels show an inverted image of EtBr-stained DNA.

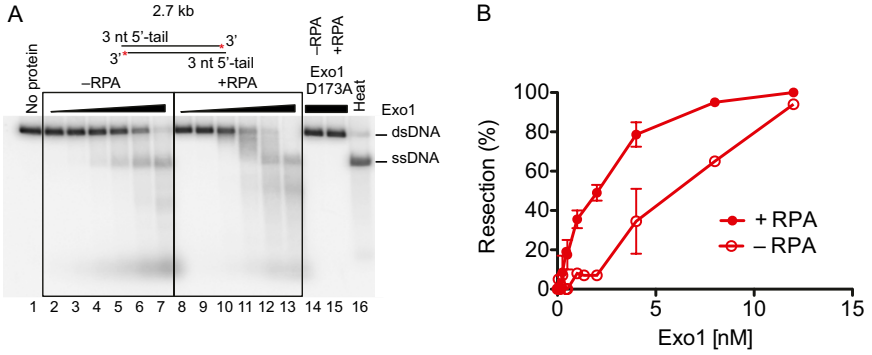

Fig. S6. RPA stimulates Exo1 nuclease activity. (*A*) Nuclease assay with Exo1 (0.05, 0.15, 0.45, 1.35, 4, and 12 nM) either without (lanes 2–7) or with RPA (3 μM, lanes 8–13). DNA, ^{32}P labeled at the 3′ end, with an ssDNA overhang of 3 nt at the 5′ ends was used as a substrate (7.6 nM). Lane 1 is identical to lane 1 in Fig. 3*B*, lanes 8–13 are identical to lanes 2–7 in Fig. 3*B*, and lanes 14–16 are identical to lanes 8–10 in Fig. 3*B*, respectively. (*B*) Quantification of experiments as shown in *A*. Error bars show SE.

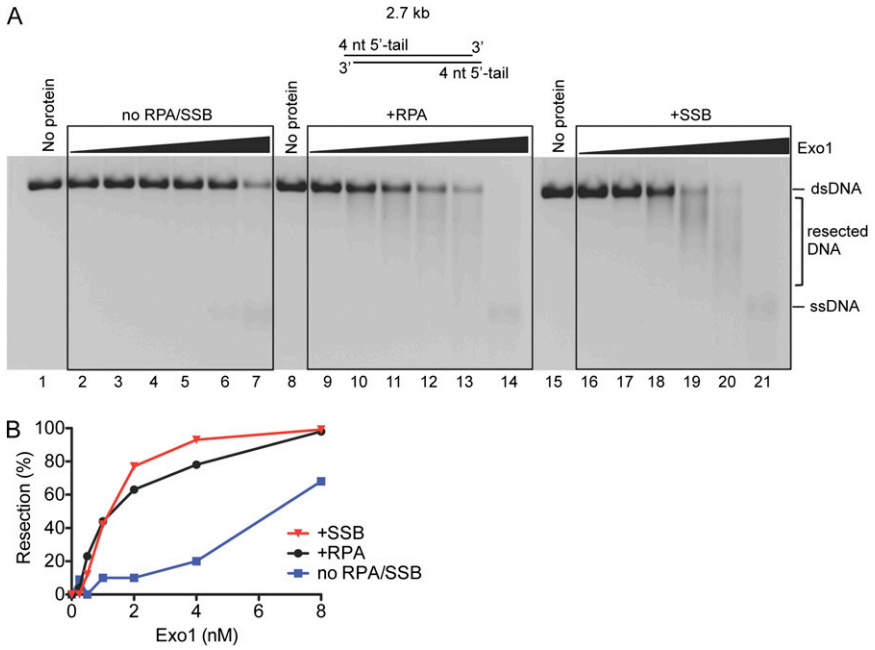

Fig. S7. Stimulatory effect of RPA on the nuclease activity of Exo1 is not species-specific. (*A*) Shown is nuclease assay with the indicated substrate (unlabeled 2.7 kb dsDNA with an ssDNA overhang of 4 nt at the 5′ ends, 7.6 nM) and increasing amounts of Exo1 (0.25, 0.5, 1, 2, 4, and 8 nM) either without (lanes 2–7) or in the presence of RPA (lanes 9–14, 3 μM) or ssDNA-binding (SSB) protein (lanes 16–21, 3.7 μM). (*B*) Quantification of the experiment shown in *A*.

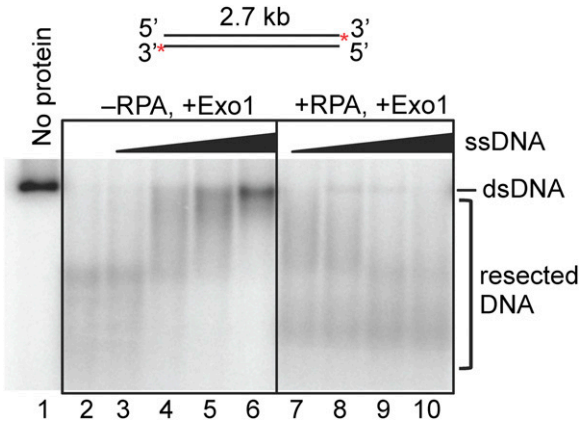

Fig. S8. RPA stimulates Exo1 by preventing nonspecific binding to ssDNA. DNA resection by Exo1 (1 nM) was analyzed in the presence of increasing amounts of circular ssDNA (1, 10, 25, and 50 ng), either in the absence or in the presence of a saturating concentration of RPA (1.1 μM). Blunt-ended DNA (2.7 kb), ^{32}P labeled at the 3′ end (1 nM), was the substrate.

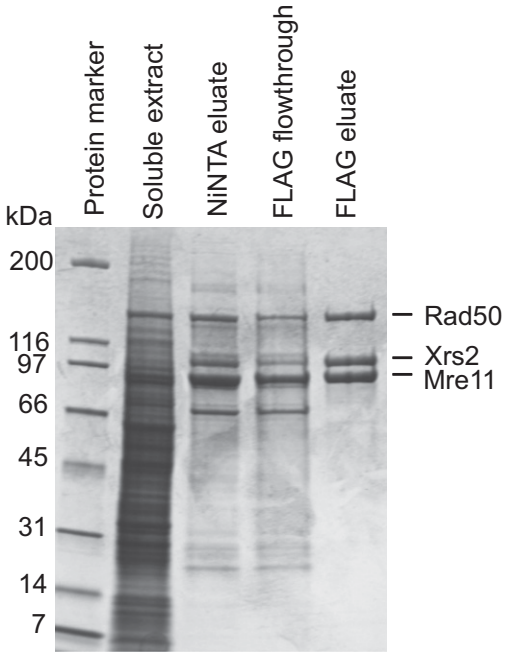

Fig. S9. Purification of the Mre11-Rad50-Xrs2 complex. The protein complex was expressed in Sf9 cells and purified by affinity chromatography. Representative fractions were analyzed by electrophoresis.

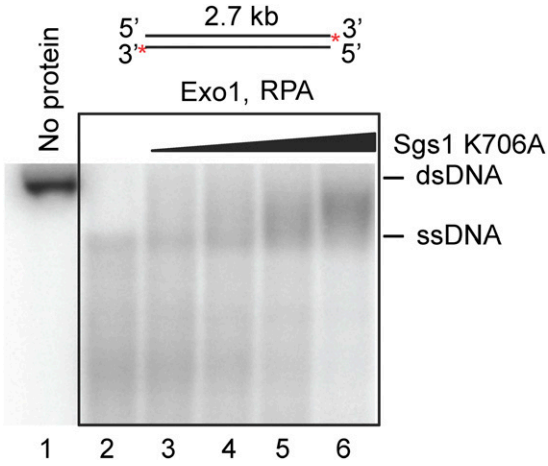

Fig. S10. Helicase-dead Sgs1 (K706A) inhibits resection of dsDNA by Exo1 in a concentration-dependent manner. DNA resection by Exo1 (1 nM) was analyzed in the presence of increasing amounts of helicase-dead Sgs1 (K706A, 0.5, 2, 8, and 20 nM). Blunt-ended DNA (2.7 kb), ^{32}P labeled at the 3′ end (1 nM), was the substrate. All reactions contained RPA (0.4 μM).

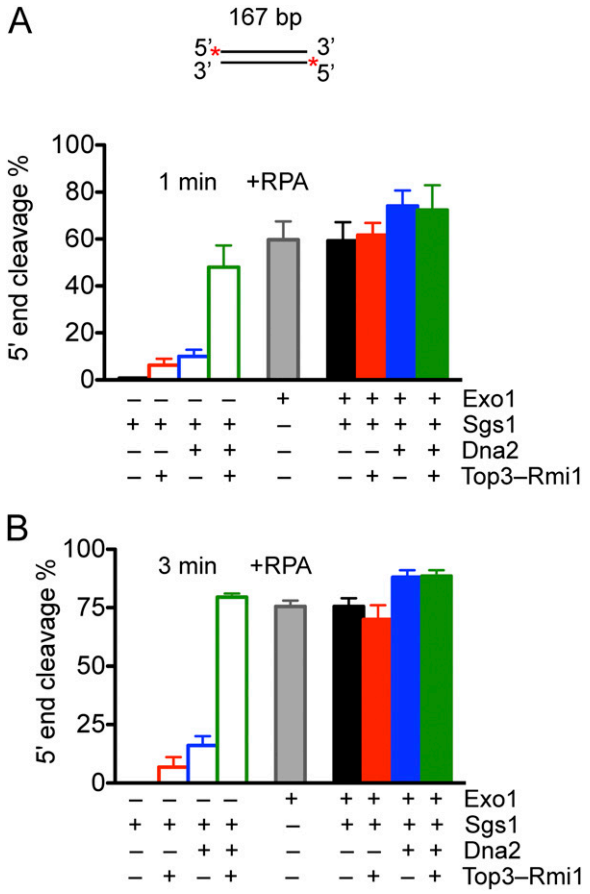

Fig. S11. Effect of Exo1 on resection of dsDNA by Sgs1, Dna2, and Top3-Rmi1. (*A*) Quantification of experiments where blunt-ended dsDNA (167 bp), ^{32}P labeled at the 5′ end (1 nM), was incubated for 1 min with Exo1 (6 nM), Sgs1 (1 nM), Dna2 (1 nM), and Top3-Rmi1 (3 nM) in the presence of RPA (50 nM). (*B*) As in *A*, but with incubation time of 3 min. Error bars show SE.