A DNA nick at Ku-blocked double-strand break ends serves as an entry site for exonuclease 1 (Exo1) or Sgs1-Dna2 in long-range DNA end resection

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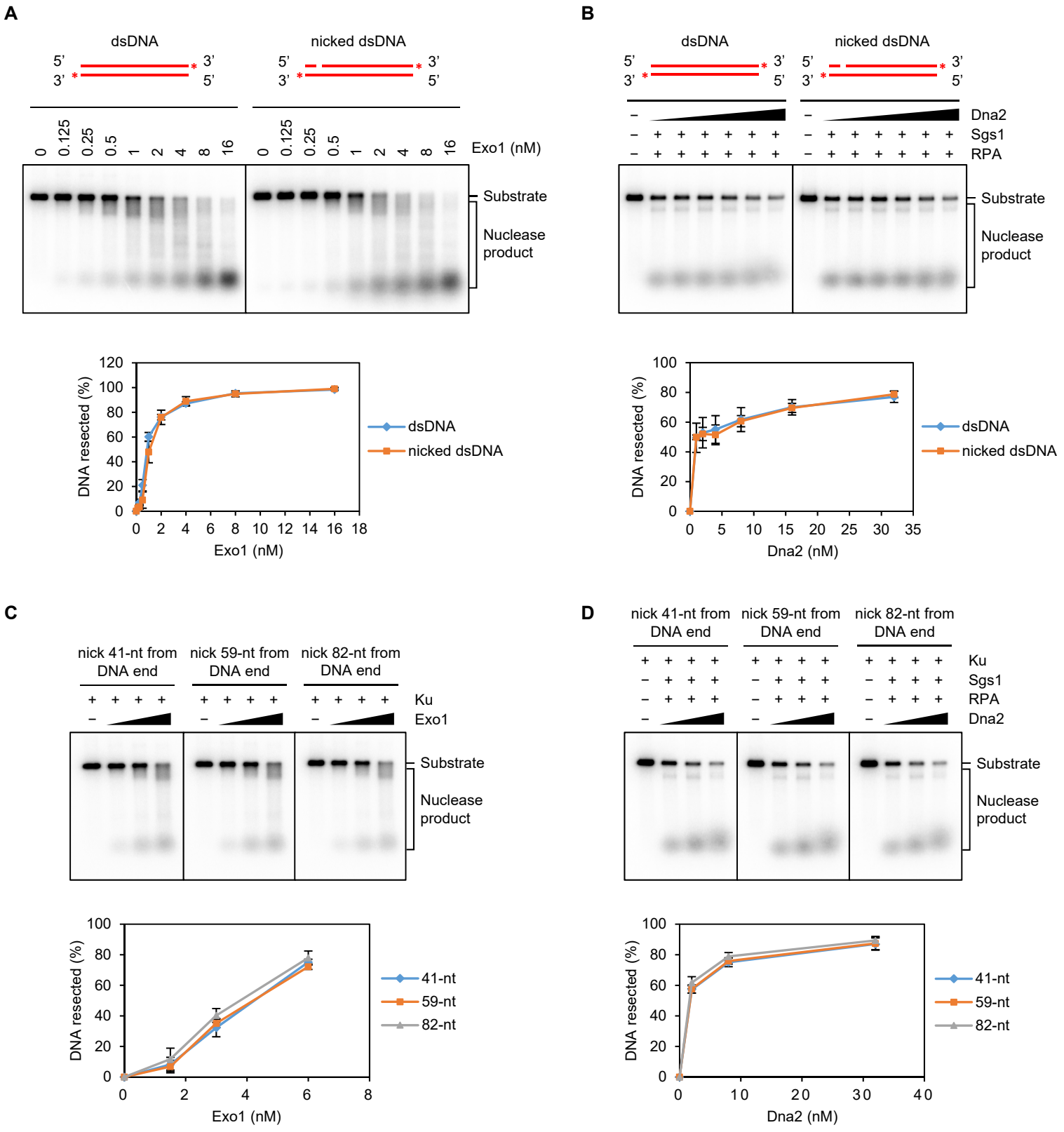
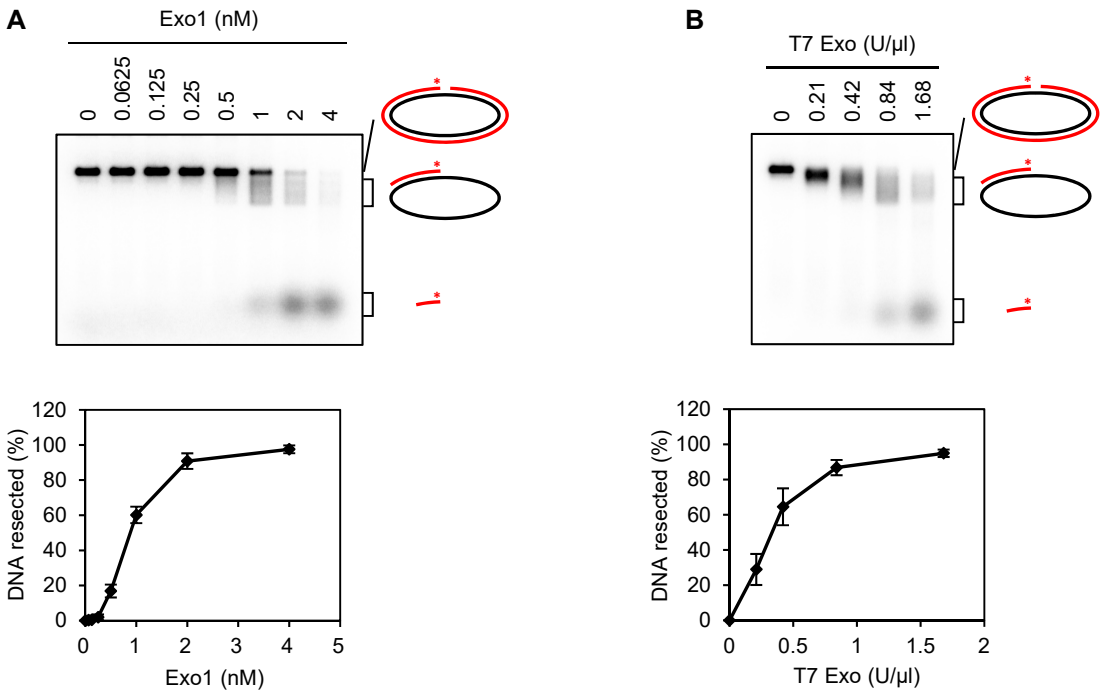
Supporting Information

Supplemental Figure S1



Supplemental Figure S1. Nuclease activity of Exo1 or Dna2 on linear dsDNA substrate with or without an incision site

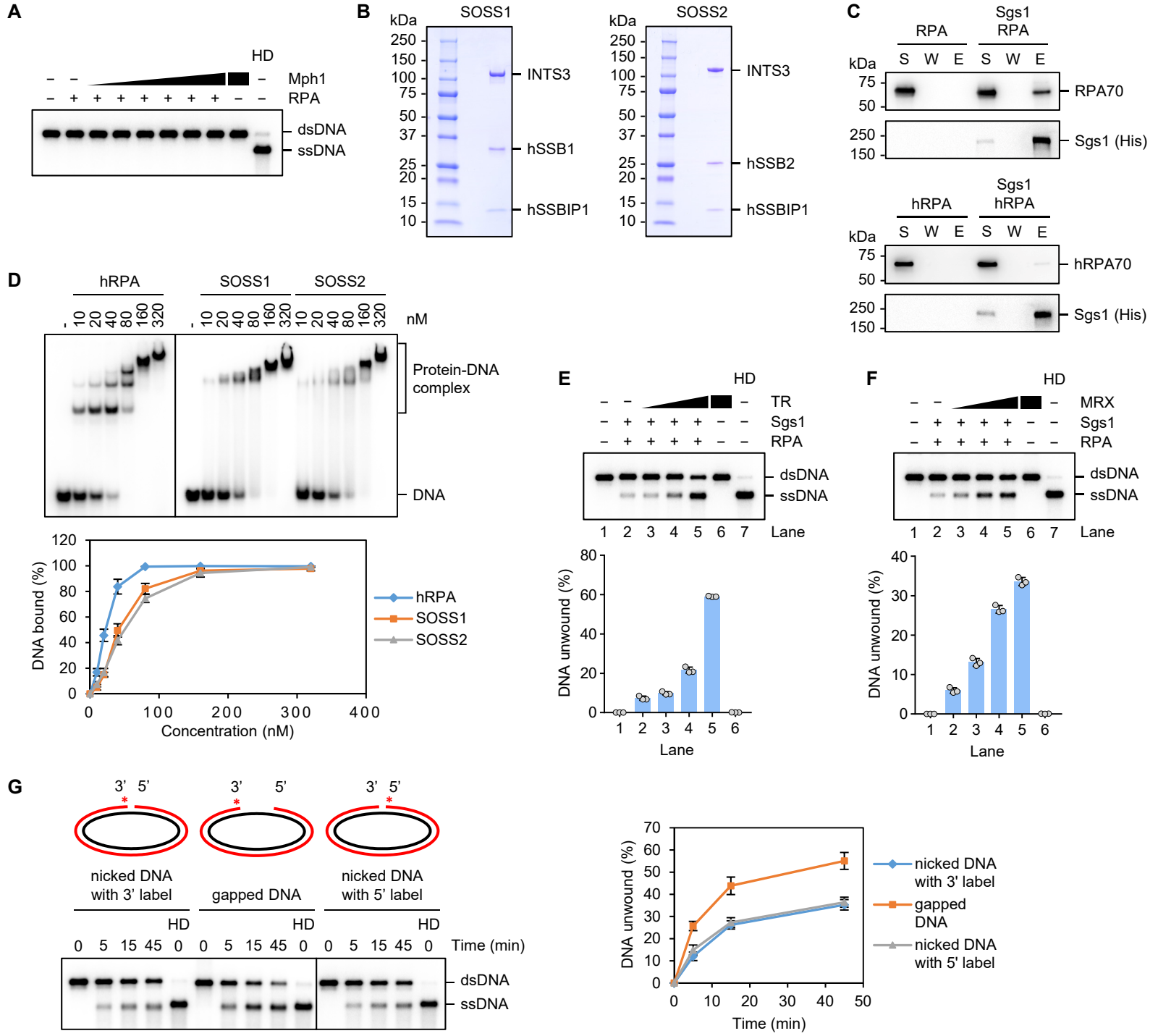
A, The indicated concentration of Exo1 was incubated with linear dsDNA substrate (1 nM) with or without a nick. The results from three independent experiments were graphed, with the error bars representing SD. B, DNA substrates used in A were incubated with Dna2 (1, 2, 4, 8, 16 and 32 nM) in the presence of Sgs1 (16 nM) and RPA (800 nM). The results were graphed as in A. The asterisk denotes the ^{32}P label in the substrate in all the figure parts. C, DNA substrate (1 nM) with a nick 41-nt, 59-nt or 82-nt away from the DNA end was pre-incubated with Ku (48 nM), followed by the addition of Exo1 (1.5, 3 and 6 nM). The results were quantified and presented as in A. D, Ku-blocked DNA substrates used in C were incubated with Dna2 (2, 8 and 32 nM) in the presence of Sgs1 (16 nM) and RPA (800 nM). The quantification of the results was shown as in A.



Supplemental Figure S2. Nick processing activities of Exo1 and T7 Exo on circular nicked dsDNA

A, Nuclease activity of the indicated concentrations of Exo1 was tested on 1 nM circular nicked dsDNA substrate. The asterisk denotes the ^{32}P label in the substrate. The results from three independent experiments were quantified and plotted, with the error bars representing SD. B, Nuclease assay was carried out as in A, except that the indicated concentrations of T7 Exo were used. The results were quantified and plotted as in A.

Supplemental Figure S3



Supplemental Figure S3. Helicase activity of Sgs1 on circular dsDNA substrate with a nick or a gap

A, Mph1 helicase (1, 2, 4, 8, 16 and 32 nM) failed to unwind the nick-containing circular DNA substrate with RPA (800 nM) being present. The assay was conducted as described in Figure 5B. HD, heat denaturation. B, SOSS1 and SOSS2 were analyzed by SDS-PAGE and Coomassie blue staining. C, Species-specific interaction between Sgs1 and yeast RPA. 6xHis-tagged Sgs1 was incubated with yeast or human RPA, followed by the addition of Ni-NTA resin to capture protein complexes. The supernatant (S) containing unbound proteins, the wash (W), and the eluate (E) fractions were analyzed by SDS-PAGE and immunoblotting using antibodies against the largest subunit of yeast or human RPA or against the poly-histidine tag. D, The indicated concentrations of hRPA, SOSS1 and SOSS2 were tested for their binding to 90-nt ssDNA (20 nM). The results were quantified and graphed, with the error bars representing SD. E, Stimulation of unwinding of the nick-containing DNA catalyzed by Sgs1 (8 nM) in conjunction with RPA (800 nM) by the TR complex (2, 4 and 8 nM). The results from three independent experiments were graphed, with the error bars representing SD. F, DNA unwinding by Sgs1-RPA was assessed with MRX (4, 8 and 16 nM) as in E. The results were graphed as in E. G, Time-dependent unwinding of the nick-containing and gapped circular DNA substrates (0.5 nM each) was tested with Sgs1 (8 nM) and RPA (400 nM). The results from three independent experiments were graphed, with the error bars representing SD. The asterisk denotes the ^{32}P label in the substrate.