Supplementary Material and Methods

DNA substrates. The "bubble" DNA was prepared as described previously[1]; the relaxed duplex DNA (in Supplementary Fig. 3b) was pUC19. The oligonucleotide-based substrates were prepared as described previously[2].

Proteins. The Dna2 constructs used in Supplementary Fig. 2 were expressed from a yeast expression vector[3] and purified as described previously[4].

ATPase assay. The ATPase assay was carried out as described previously[2], except for the reaction buffer contained 1.7 mM magnesium acetate and 30 mM sodium acetate.

Nuclease assays. The nuclease assays with the Y-structure DNA substrate were carried out for 30 minutes at 30°C in a buffer containing, unless indicated otherwise, 50 mM Tris HCl pH 7.5, 2 mM dithiothreitol, 2 mM $MgCl_2$, 250 μg/ml BSA (New England Biolabs), 150 pM (molecules) DNA substrate, unless indicated otherwise, and proteins as indicated. The assays in Supplementary Fig. 3 were carried out in 25 mM Tris acetate (pH 7.5), 1 mM dithiothreitol, 2 mM magnesium acetate, 250 μg/ml BSA, 1 mM ATP, 1 mM phosphoenolpyruvate (Sigma), 80 U/ml pyruvate kinase (Sigma), 200 ng of the respective DNA substrate, and the protein concentrations as indicated.

Supplementary References

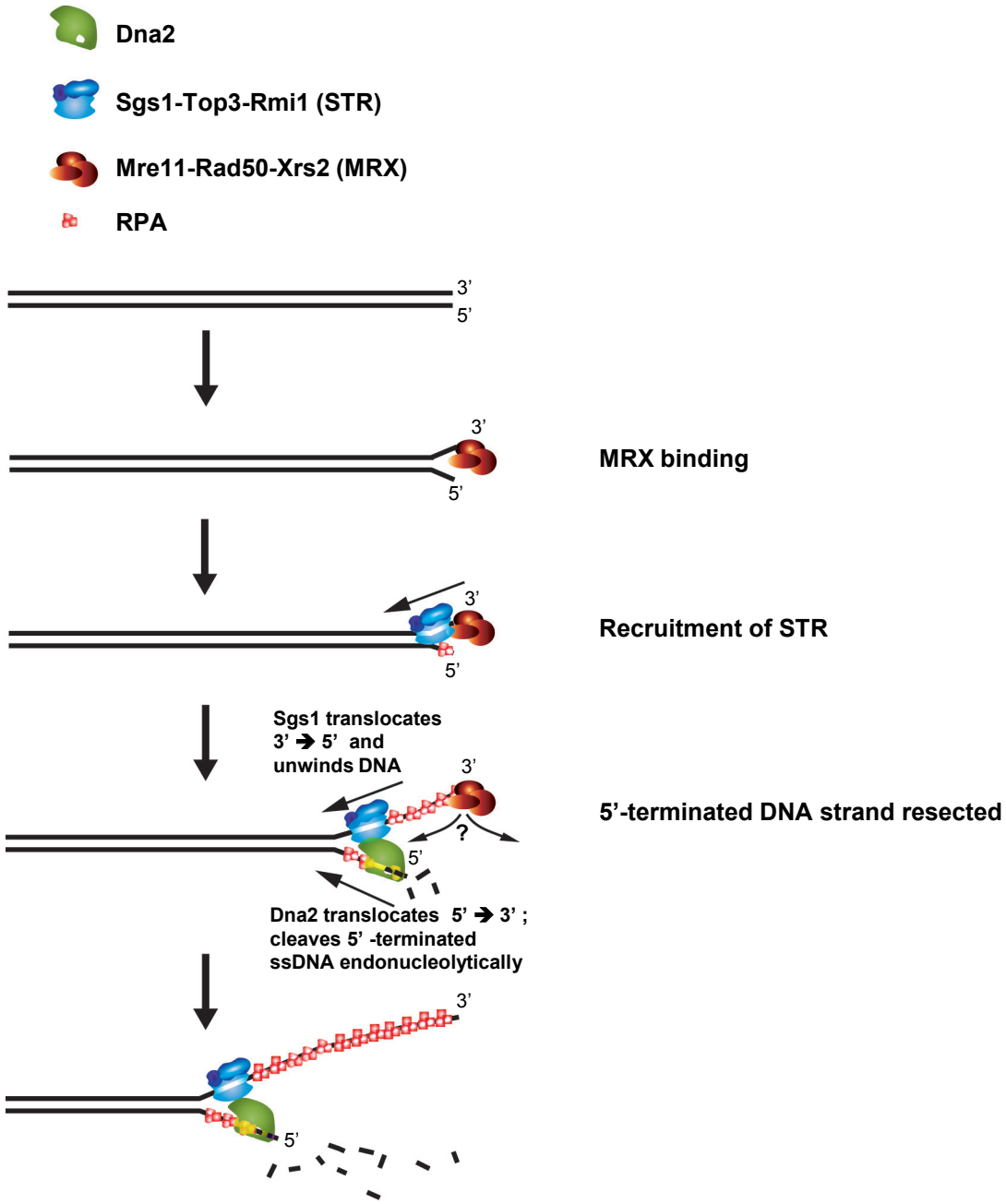
1 Hsieh, T. S. & Plank, J. L. Reverse gyrase functions as a DNA renaturase: annealing of complementary single-stranded circles and positive supercoiling of a bubble substrate. *J. Biol. Chem.* **281**, 5640-5647, (2006).

2 Cejka, P. & Kowalczykowski, S. C. The full-length Saccharomyces cerevisiae Sgs1 protein is a vigorous DNA helicase that preferentially unwinds holliday junctions. *J. Biol. Chem.* **285**, 8290-8301, (2010).

3 Pokharel, S. *et al.* Matching active site and substrate structures for an RNA editing reaction. *J. Am. Chem. Soc.* **131**, 11882-11891, (2009).

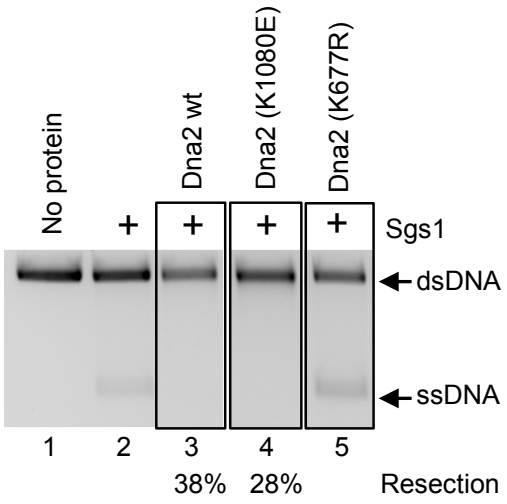
4 Budd, M. E., Choe, W. & Campbell, J. L. The nuclease activity of the yeast DNA2 protein, which is related to the RecB-like nucleases, is essential in vivo. *J. Biol. Chem.* **275**, 16518-16529, (2000).

5 Harmon, F. G., DiGate, R. J. & Kowalczykowski, S. C. RecQ helicase and topoisomerase III comprise a novel DNA strand passage function: a conserved mechanism for control of DNA recombination. *Mol. Cell* **3**, 611-620, (1999).

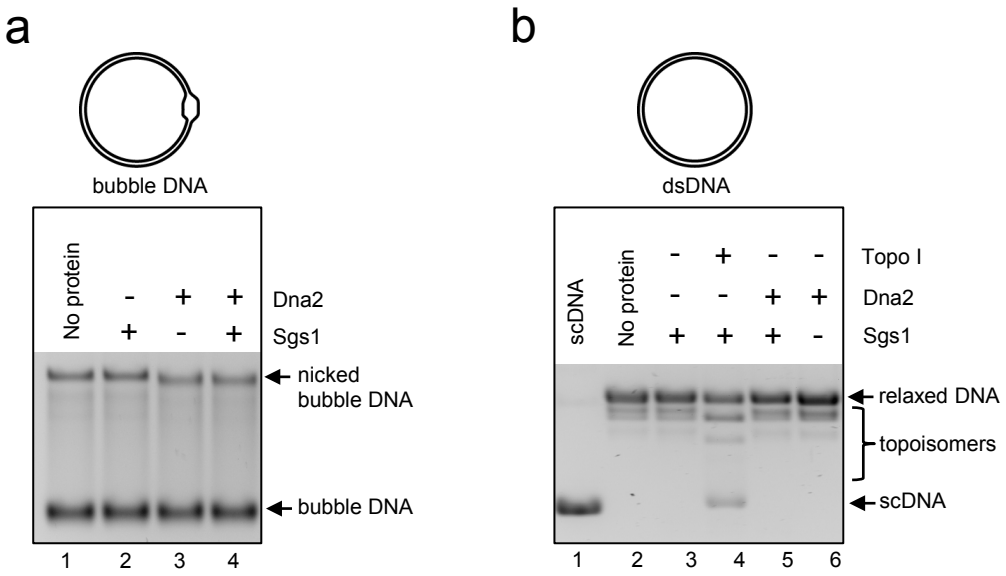


Supplementary Figure 1. Model for resection of a DNA double-strand break by Dna2, Sgs1, Top3, Rmi1, Mre11, Rad50, Xrs2, and RPA.

See text for details.

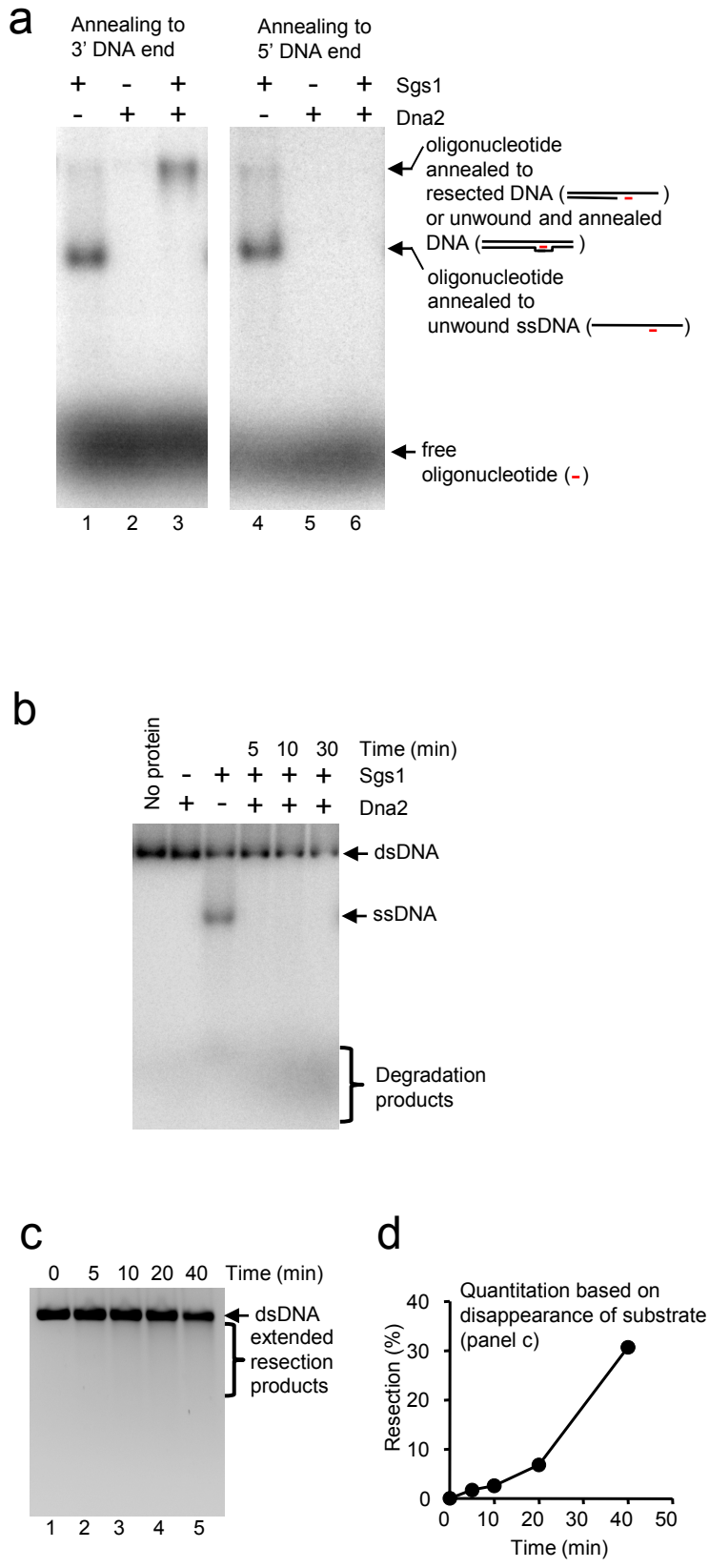


Supplementary Figure 2. The nuclease, but not the helicase activity of Dna2 is required for DNA end resection. The linear pUC19 DNA was incubated with 50 nM of either wild type Dna2, helicase-dead Dna2 (K1080E) or nuclease-dead Dna2 (K677R) in the presence of RPA (3 μM). All three proteins used in this experiment were purified from yeast cells using an identical procedure (see SI Material and Methods). The specific activity of the wt Dna2 was about 3-fold lower than that of the helicase-dead Dna2 (K1080E) mutant as judged based on the nuclease activity on a Y-structure oligonucleotide based substrate. Taking into account the difference in specific activity, wt Dna2 is about 4-fold more efficient in DNA end resection than helicase-dead Dna2 (K1080E). The nuclease activity of Dna2 was absolutely required for resection (lane 5). The extent of resection (in per cent) that is indicated below the lanes is the average from 2 independent experiments.



Supplementary Figure 3. Dna2 strictly requires a free end to degrade DNA.

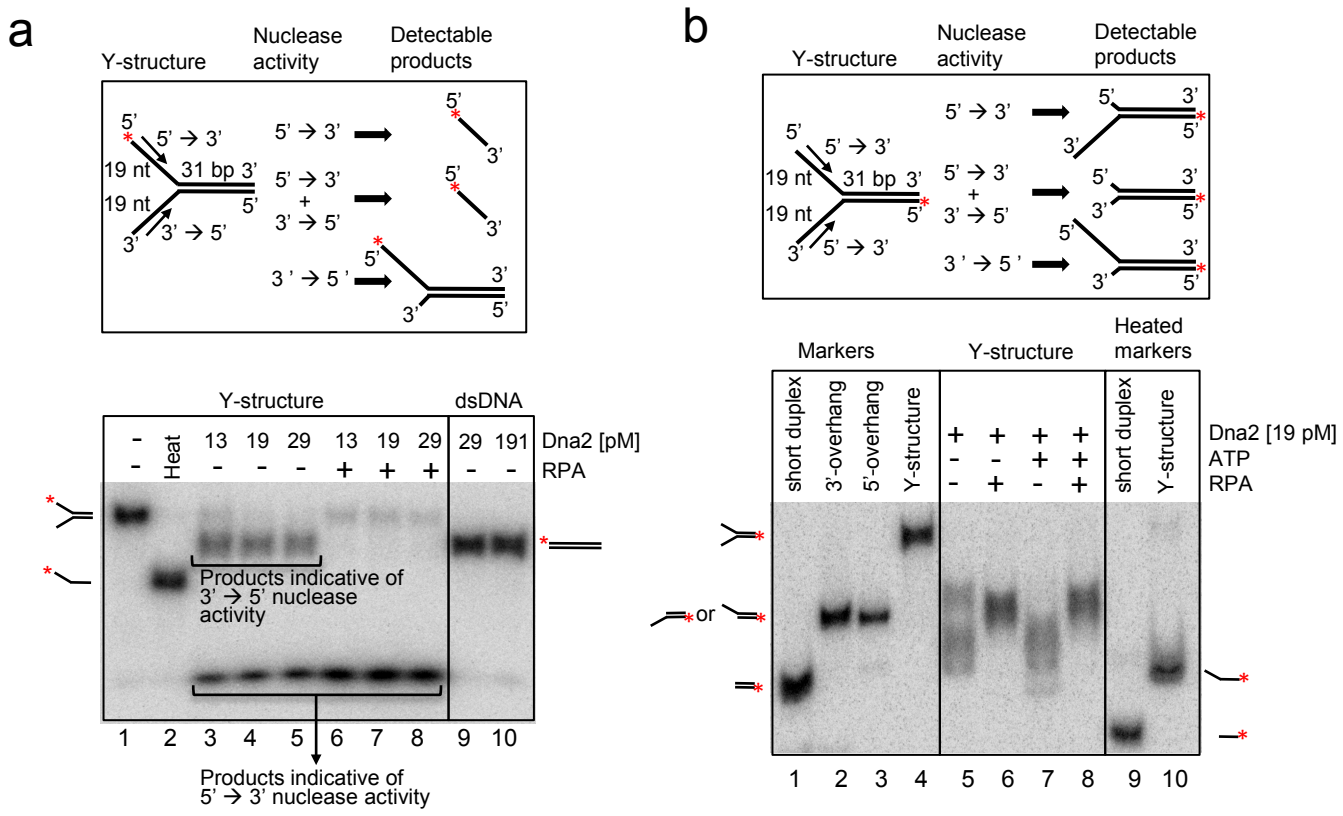
a, DNA containing 450 nt of unpaired ssDNA ("bubble DNA") was incubated with Sgs1 (1 nM) and/or Dna2 (1 nM), as indicated, and RPA (3 μM). The reaction products were separated on 1% agarose gel in the presence of 0.5 μg/ml ethidium bromide. Dna2 does not degrade bubble DNA, irrespectively of the presence of Sgs1. A fraction of the DNA substrate is nicked within the ssDNA region; limited degradation of the nicked bubble substrates by Dna2 is observed. **b**, Relaxed duplex DNA was incubated with Sgs1 (70 nM) and/or Dna2 (1 nM), as indicated, and RPA (3 μM). The reaction products were separated by electrophoresis in 1% agarose, and stained afterwards with 0.05 μg/ml ethidium bromide. Sgs1 can unwind dsDNA without a free end (P. Cejka, J.L. Plank, and S.C. Kowalczykowski, in preparation), as reveled by the change in superhelicity in the coupled reaction with Topo I (lane 4)[5]. Dna2 does not cleave the circular ssDNA produced by the Sgs1-catalyzed unwinding of the covalently closed dsDNA.



Supplementary Figure 4. Control experiments for the end resection analysis by hybridization.

a, Control experiment showing annealing of radioactively labeled probe oligonucleotides to ssDNA located 300 nt away from dsDNA break. See Fig. 2 for experimental scheme

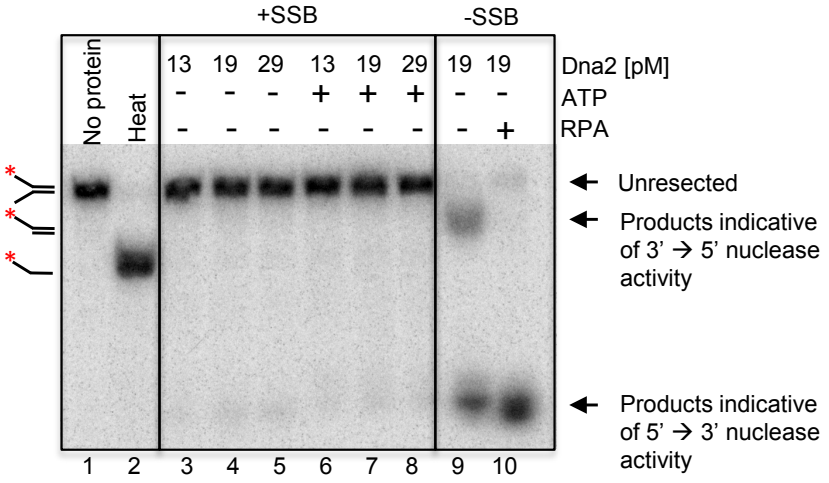
and more information. The reactions contained RPA (3 μM), Sgs1 (0.5 nM) and/or Dna2 (0.5 nM), as indicated, and were terminated after 20 minutes. Both 5'-and 3'-terminated strands are present when the dsDNA was unwound by Sgs1 in the presence of RPA, but only 3'-terminated strand is present in Sgs1-Dna2-RPA-containing reactions. **b**, The DNA end resection experiment was carried out with Sgs1 (50 pM), Dna2 (200 pM), RPA (250 nM) and 3' ^{32}P end-labeled pUC19 DNA (1 nM, molecules). The assay shows a rapid loss of signal and the appearance of fast migrating degradation products, indicating degradation occurring at the 3' terminated DNA strand. **c**, Resection products identical to those analyzed in Fig. 2 were separated by electrophoresis and visualized by ethidium bromide staining. No significant decrease in staining was observed within the first 20 minutes, indicating that resection is largely limited to the vicinity of DNA ends (compare with Fig. 2c). Therefore, the resection from the opposite end does not interfere with the hybridization analysis (Fig. 2). **d,** Quantification of the data from panel **c**.



Supplementary Figure 5. RPA stimulates the 5'→3' degradation by Dna2 and inhibits 3'→5'degradation.

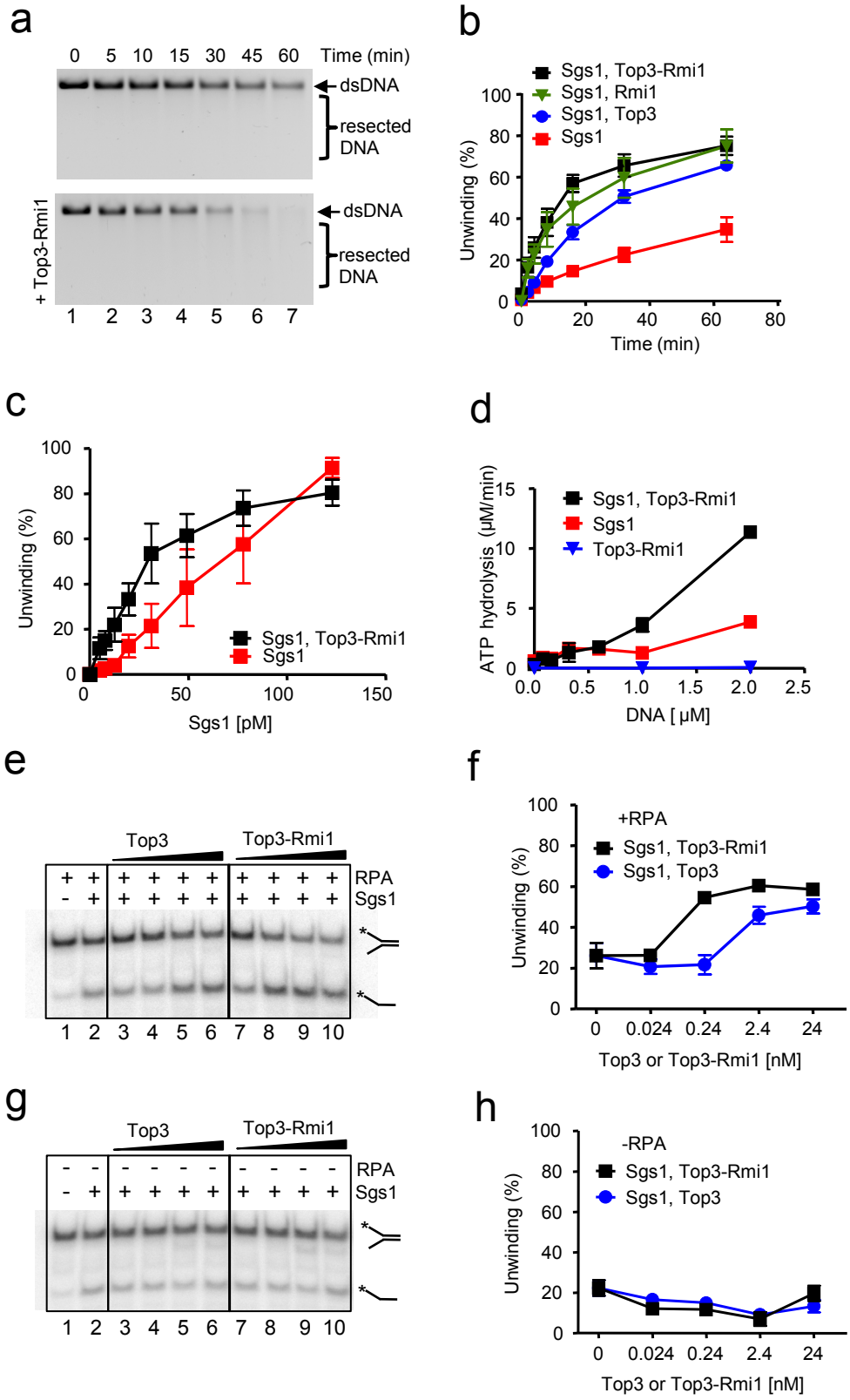
a, The synthetic Y-structure DNA substrate was ^{32}P-labeled at the 5'-terminus of one ssDNA arm (red asterisk), and was used to analyze 5'→3' and 3'→5' nuclease activities of Dna2. The reaction and expected detectable products are schematically shown at the top. The indicated concentrations of Dna2 were incubated with 150 pM DNA (molecules) with or without RPA (2.25 nM). Fully dsDNA (150 pM, molecules; 50 bp in length) was used as a control where indicated; "Heat" is heat-denatured substrate. The reaction products were separated by native 10% polyacrylamide gel electrophoresis and detected by autoradiography. Without RPA, Dna2 processes this substrate to form an intermediate product whose mobility is between the substrate and dsDNA control (lanes 3-5). The retention of the label indicates that this intermediate is the result of a 3' →5' nuclease activity; the loss of ~50% of the label shows that the 5'→3' activity is comparable. Addition of RPA resulted in the nearly complete degradation of the Y-structure DNA, with only a residual amount of substrate remaining (lanes 6-8). These results show that RPA stimulated at least the 5'→3' nuclease activity of Dna2, but the nature of the substrate could not reveal any effect on 3'→5' nuclease activity. **b**, The Y-structure DNA

was radioactively labeled at its 5'-terminus of the dsDNA region (see experimental scheme at the top) and analyzed as above. Dna2 acting without RPA brought about substantial degradation of the DNA (lanes 5 and 7), with most of the substrate molecules losing a part of both 5'- and 3'-ssDNA arms, as revealed by products that migrate below the respective 5'- or 3'-overhang control substrates (lanes 2 and 3). This result shows that Dna2 displays both nuclease polarities in the absence of RPA. Adding RPA results in products with a slower electrophoretic mobility (compare lanes 6 *vs*. 5, and 8 *vs*. 7). Because panel **a** clearly showed that RPA stimulated 5' → 3' nuclease activity, the inhibition of nuclease activity by RPA observed in lanes 6 and 8 thus can only be the consequence of RPA inhibiting the 3' → 5' nuclease activity. Lanes 5 and 6 show that even though the nucleolytic activity of Dna2 is enhanced by ATP, the effects of RPA are independent of ATP.



Supplementary Figure 6. SSB inhibits the nuclease activities of Dna2.

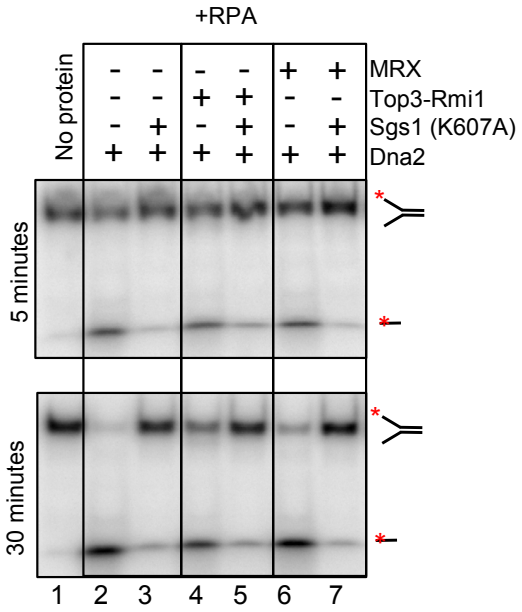
The Y-structure oligonucleotide-based DNA substrate was ^{32}P-labeled at the 5'-terminus of one ssDNA arm (red asterisk), and was used to analyze 5'→3' and 3'→5' nuclease activities of Dna2. See Supplementary Fig. 4a for experimental scheme. The indicated concentrations of Dna2 were incubated with 150 pM DNA (molecules), ATP (1 mM), where indicated, and either SSB or RPA (2.8 or 2.25 nM, respectively), where indicated. The reaction products were separated by native 10% polyacrylamide gel electrophoresis and detected by autoradiography.



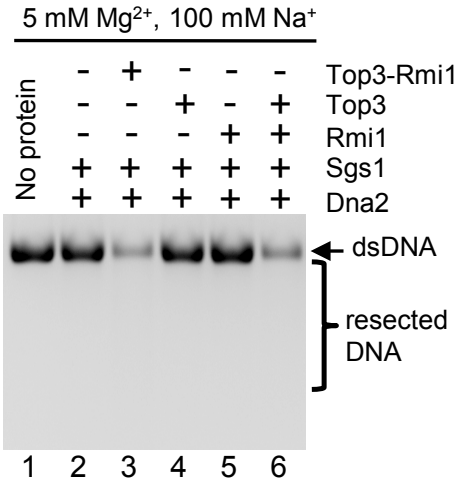
Supplementary Figure 7. Top3 and Rmi1 proteins stimulate DNA end resection by promoting DNA unwinding by Sgs1.

a, A representative experiment showing the kinetics of DNA resection by Sgs1 (0.3 nM), Dna2 (1 nM), and RPA (3 μM), with or without Top3-Rmi1 heterodimer (10 nM). **b**,

DNA unwinding by Sgs1 (24 pM) is stimulated by Top3 (2.4 nM), Rmi1 (2.4 nM) or Top3-Rmi1 heterodimer (2.4 nM). All reactions contained RPA (2.25 nM). The substrate is the Y-structure DNA used previously[2]. The products were separated by native 10% polyacrylamide gel electrophoresis, detected by autoradiography, and quantified. **c**, Y-structure DNA was used in helicase assays with a range of Sgs1 concentrations, RPA (2.25 nM), and Top3-Rmi1 (3 nM, where indicated) analyzed as in panel **b**. **d**, DNA-dependent ATP hydrolysis of Sgs1 is stimulated to Top3-Rmi1. The reactions contained Sgs1 (5 nM), RPA (113 nM), ATP (1mM), the indicated concentrations of Y-structure DNA (in nucleotides), and Top3-Rmi1 heterodimer (15 nM). **e**, Y-structure DNA was used in helicase assays in the presence of RPA (2.25 nM), with Sgs1 (24 pM) and either Top3 or Top3-Rmi1 (0.024, 0.24, 2.4 and 24 nM, respectively). **f**, Quantification of data from experiments such as those shown in panel **d**. **g**, Y-structure DNA was used in helicase assays in the absence of RPA, with Sgs1 (12 pM) and Top3 or Top3-Rmi1 (0.024, 0.24, 2.4 and 24 nM, respectively). **h**, Quantification of the data from experiments such as those shown in panel **f**.

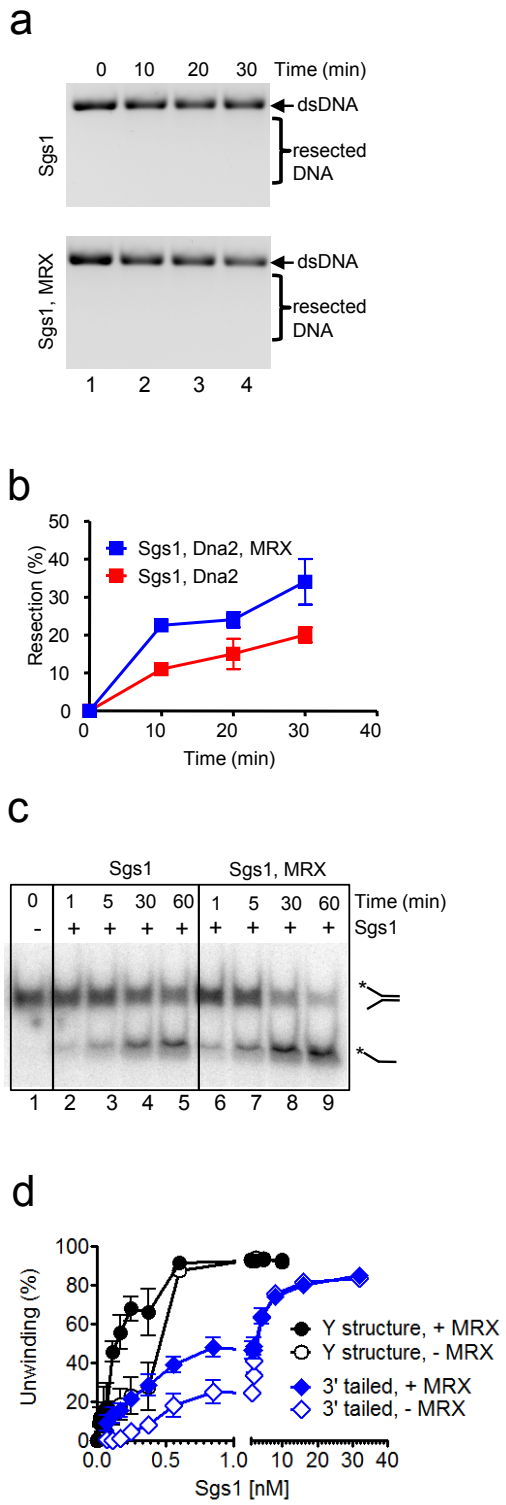


Supplementary Figure 8. Neither Sgs1, Top3-Rmi1 nor MRX stimulate the nuclease activity of Dna2. A 5' ^{32}P-labeled Y-structure DNA substrate (600 pM, molecules) was used to monitor the effects of helicase-dead Sgs1 (K706A, 3 nM), Top3-Rmi1 (3 nM) and MRX (3 nM), as indicated, on the nuclease activity of Dna2 (19 pM) in the presence of RPA (9 nM). Sgs1 strongly and Top3-Rmi1 and MRX modestly inhibited the nuclease activity of Dna2, likely through competition for DNA binding. This suggests that the stimulation of DNA end resection by Top3-Rmi1 and MRX is not mediated through promotion of Dna2 nuclease activity.



Supplementary Figure 9. At suboptimal reaction conditions, both Top3 and Rmi1 are needed to stimulate DNA end resection.

Representative experiment showing resection reactions in high salt buffer (5 mM Mg^{2+} and 100 mM Na^{+}) with Sgs1 (6 nM), Dna2 (1 nM), RPA (3 μM), Top3 (15 nM), Rmi1 (15 nM), and Top3-Rmi1 heterodimer (15 nM), as indicated.



Supplementary Figure 10. The MRX complex stimulates DNA end resection by promoting DNA unwinding by Sgs1.

a, A representative experiment showing the kinetics of DNA resection by Sgs1 (5.7 nM), Dna2 (1 nM), and RPA (3 μM), with or without MRX complex (20 nM) in high salt buffer (5 mM Mg^{2+} and 100 mM Na^{+}). **b**, Quantification of the data from experiments such as those shown in panel **a. c**, A representative experiment showing kinetics of DNA unwinding by Sgs1 (200 pM) in high salt buffer, without the MRX complex (lanes 2-5) or with MRX (5 nM, lanes 6-9). All reactions contained RPA (2.25 nM). The substrate is the Y-structure DNA described previously[2]. **d,** Quantification of helicase assays using Y-structure or 3' ssDNA-overhang substrates (150 pM, molecules) in high salt buffer, with Sgs1 concentrations as indicated, with or without the MRX complex (5 nM). All reactions contained RPA (2.25 nM).