Supplemental Materials

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Supplementary Table:

Table S1: Binding coefficients of RPA and SOSS1 on single-stranded DNA substrates from EMSA data

Supplementary Methods
Figure S1. Recombinant proteins used in this study. RPA, SOSS1, MRN, Exo1, and nuclease-deficient Exo1 (D173A) were separated by SDS-PAGE and stained with Coomassie Blue.
Figure S2. RPA and SOSS binding to ssDNA substrates is more stable with increasing length of ssDNA. (A) A schematic representation of (dT)$_{16+42}$. The ssDNA tail contains 58 nt and may allow two RPA to bind with the 30 nt binding mode. Cy3 and Cy5 was separated by 16 nt as in (dT)$_{16+24}$. (B) FRET efficiency histograms for (dT)$_{16+42}$ DNA only and RPA binding to (dT)$_{16+42}$ in 2 and 72 nM RPA, and the time evolution of the FRET histogram after incubating with 72 nM RPA and flushing out the excess unbound RPA. Similar to the data for (dT)$_{16+24}$ (Figure 2B), the peaks centered at 0.52 and 0.29 FRET represent 0 and 2 RPA binding to (dT)$_{16+42}$ respectively. (C) A representative single-molecule FRET-time trace for (dT)$_{16+42}$ obtained after incubating with 72 nM RPA and flushing out the excess unbound RPA. (D) FRET efficiency histograms for (dT)$_{16+46}$ DNA only and SOSS1 binding to (dT)$_{16+42}$ in 30 nM SOSS, and the time evolution of the FRET histogram after incubating with 30 nM SOSS1 and flushing out the excess unbound SOSS1. The peaks centered at 0.52 and 0.38 FRET represents 0 and 1 SOSS1 binding to (dT)$_{16+42}$ respectively. (E) A representative single-molecule FRET-time trace for (dT)$_{16+42}$ obtained after incubating with 30 nM SOSS1 and flushing out the excess unbound SOSS1. (F) A schematic representation of (dT)$_{32+39}$ The ssDNA tail contains 71 nt and may allow two RPA to bind with the 30 nt binding mode and one RPA bind with the 10 nt binding mode. (G) FRET efficiency histograms for (dT)$_{32+39}$ DNA only and RPA binding to (dT)$_{32+39}$ in 2 and 72 nM RPA, and the time evolution of the FRET histogram after incubating with 72 nM RPA and flushing out the excess unbound RPA. The peak centered at 0.08 FRET may represent 2 or 3 RPA binding. (H) A representative single-molecule FRET-time trace for (dT)$_{32+39}$ obtained after incubating with 72 nM RPA and flushing out the excess unbound RPA. (I) FRET efficiency histograms for (dT)$_{32+39}$ DNA only and SOSS1 binding to (dT)$_{32+39}$ in 30 nM SOSS, and the time evolution of the FRET histogram after incubating with 30 nM SOSS1 and flushing out the excess unbound SOSS1. (J) A representative single-molecule FRET-time trace for (dT)$_{16+42}$ obtained after incubating with 30 nM SOSS1 and flushing out the excess unbound SOSS1. (K) FRET histograms for (dT)$_{69+8}$ in the absence and presence of 30 nM SOSS1. (L) Test for duplex destabilization by SOSS1. A partial duplex with a 58 nt tail was used where Cy3 and Cy5 were placed next to each other at the ss-dsDNA junction such that high FRET (~ 0.9) is observed for DNA only. This DNA construct and
(dT)$_{16+42}$ only differ in fluorophore labeling locations. Any significant local breathing (or melting) of the duplex DNA at the junction that lasts longer than our experimental time resolution (30 ms) would be reported as a drop in FRET. A representative single-molecule FRET-time trace is shown, which was obtained after incubating with 30 nM SOSS1 and flushing out the excess unbound SOSS1.
Figure S3. RPA limits long-range resection by Exo1. Resection assays were performed in vitro with RPA (200 nM), Exo1 (1.0 nM), MRN (5 nM), and 0.67 nM linearized plasmid DNA substrate in the presence of 25 mM MOPS, 2 mM ATP, 0.1 mM DTT, 100 μg/mL BSA, 0.05% Triton X-100, and 5 mM MgCl₂, (similar to previously published conditions (Nimonkar et al, 2011)). Reaction products were analyzed by SYBR green staining (top), by non-denaturing southern blot using a probe for the 3' strand (middle), or by qPCR (bottom). Right panel: Resection assays were performed as in the left panel except with nuclease-deficient MRN and Exo1 as indicated. Reaction products were analyzed by SYBR green staining (top) and by non-denaturing southern blot using a probe for the 3' strand (bottom).
Figure S4. **SOSS1 does not stimulate MRN nuclease activity.** Resection assays were performed in vitro as in Figure 3 with SOSS1 (14, 42, and 126 nM), Exo1 (0.4 nM), and MRN (4.5 nM). Reaction products were analyzed by SYBR green staining (top) or by non-denaturing southern blot using a probe for the 3’ strand. "++" indicates highest level of SOSS1 used in this reaction.
**Figure S5. Exo1 generates single nucleotide products.** Wild-type human Exo1 (4, 12, and 36 nM) was incubated with a 700 bp duplex, internally labeled with $^{32}$P. Products from the reaction were separated by thin-layer chromatography (top panel) and quantified (bottom panel).
Figure S6. Dna2/BLM is stimulated by MRN and RPA. (A) Resection assays were performed in vitro as in Figure 3 except with Dna2 (2 nM), BLM helicase (10 nM) in addition to RPA (80 nM), MRN (30 nM) and SOSS1 (80 nM). (B) Resection assays were performed in vitro as in Figure 7B except with 0.067 nM DNA and analyzed by qPCR.
Figure S7. SOSS1 does not stimulate Exo1 cleavage of a branched DNA structure. Nuclease assays were performed using a double-stranded DNA substrate composed of two oligonucleotides (TP2622 annealed to TP1152) with a flap region of 15 nt and a duplex of 35 bp, as shown (top) with TP2622 labeled on the 3’ end with $^{32}$P. Reactions included 0.45, 1.35, or 4 nM Exo1 and 42 nM SOSS1 and were analyzed by denaturing polyacrylamide gel electrophoresis followed by phosphorimager analysis.
Figure S8. Exo1 binds preferentially to a branched DNA structure. DNA-binding assays were performed with 3’[^32]P-labeled oligonucleotide DNA substrates (TP74/TP504 and TP74/TP2681, respectively) as shown. DNA (2 nM) was incubated with Exo1 (8, 16, 32, or 64 nM) complexes as indicated and analyzed by native gel mobility shift assay as in Fig. 1.
Summary of SOSS1 and RPA binding coefficients for single-stranded DNA substrates

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<th>SOSS</th>
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<td></td>
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<tr>
<td></td>
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<td>Internal</td>
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<td>Kd</td>
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Table S1. Equilibrium binding constants (Kd, nM) and Hill coefficients (h) calculated from binding data in Fig. 1. The data was fit by nonlinear regression and standard error is shown.
Supplementary Methods:

Protein expression and purification

The SOSS1(T117E) complex was expressed in Sf21 insect cells using the baculovirus expression system. Lysis of the cell pellet was performed as described (Lee & Paull, 2006), with the following modifications. The complex was eluted from Nickel-NTA resin (Qiagen) with low-salt NiB buffer (50 mM KCl, 50 mM potassium phosphate pH 7.0, 10% glycerol, 20mM β-mercaptoethanol, and 125 mM imidazole). Fractions containing the SOSS1 complex were loaded onto a 1 mL Hitrap GST column (G.E.) and washed with buffer A (25 mM Tris pH 8.0, 100 mM NaCl, 10% glycerol, and 1 mM DTT). The complex was eluted with buffer A containing 10 mM glutathione. The fractions containing SOSS1 were loaded onto a 1 mL HiTrap SP column (G.E.) and washed with buffer A then eluted with buffer A containing 500 mM NaCl. The fractions containing SOSS1 were loaded onto a Superdex 200 gel filtration column (G.E.) equilibrated in buffer A and fractions containing SOSS1 were aliquoted and stored at -80 °C.

RPA was expressed and purified as previously described (Henricksen et al, 1994) with these modifications: the protein eluted from Affi-Gel blue was loaded directly onto a 5 ml Hydroxyapatite column and washed sequentially with HI buffer (50 mM Tris pH 8.0, 2 mM DTT, 10% Glycerol, 0.01% NP40, and 0.25 mM EDTA) containing 50 mM KCl and then HI buffer without KCl. The protein was eluted with a 10 ml gradient of HI buffer containing 0 to 250 mM potassium phosphate pH 7.0. RPA-containing fractions were diluted 4 fold into HI buffer, loaded onto a Mono-Q column (G.E.) which was washed with A buffer. The column was eluted with A buffer containing 500 mM KCl. The
protein eluted from the Mono-Q was separated by gel filtration over a Superdex 200 column (G.E.) and RPA-containing fractions were aliquoted and stored at -80 °C.

The human MRN complex was purified as described previously (Bhaskara et al, 2007). Human Exo1 was purified as described previously (Genschel et al, 2002) with the following modification: After purification through Q Sepharose (G.E.), Heparin (G.E.), and SP resin (G.E.), hExo1 was loaded onto a Superdex 200 gel filtration column (G.E.) equilibrated with buffer A and the fractions containing hExo1 were aliquoted and stored at -80 °C. Purification of human Ku70/80 complex was performed as with MRN (Bhaskara et al, 2007) but the eluate from the nickel resin was loaded onto a 1 mL HiTrap Q column (G.E.) prewashed with buffer A. The Ku70/80 complex was eluted with buffer A containing 500 mM NaCl. Concentrated fractions of Ku70/80 were loaded onto a Superdex 200 equilibrated with buffer A and fractions containing Ku were aliquoted and stored at -80 °C. Cell pellets with BLM were lysed as described for MRN and the protein was purified by Nickel-NTA and 1mL HiTrap SP as described above. The protein was then loaded onto a column containing ~2 mL M2 anti-Flag antibody-conjugated agarose resin (Sigma) that was prewashed with buffer A. The protein was eluted from the Flag column with 5 mL buffer A containing 0.1mg/mL Flag peptide (Sigma). Dna2 protein was expressed in Sf21 insect cells using the baculovirus expression system. Dna2 expressing cells were lysed by homogenization and sonicated three times for 20 seconds in A buffer containing 0.5% tween-20 and 1mM PMSF (phenylmethylsulfonyl fluoride). The lysate was centrifuged for 1 hour at 35,000 rpm at 4°C. The supernatant was incubated with ~1mL M2 anti-Flag antibody-conjugated agarose resin (Sigma) with rotation at 4°C for 1 hour. After incubation the lysate with resin was centrifuged for 3
min at 1000g. After removing the supernatant, the remaining resin was washed with 20mL of A buffer twice and was eluted with 5mL of A buffer containing 0.8mg/mL 3X Flag peptide (Sigma). The peptide was incubated with the resin for 20 min before elution. The Flag eluent was then loaded onto 1 mL HiTrap SP column (G.E.) and washed with buffer A then eluted with buffer A containing 500 mM NaCl. The eluted protein fractions were dialyzed in A buffer and the dialyzed fractions were aliquoted, frozen in liquid nitrogen, and stored at -80°C.

**Oligonucleotide DNA substrates**

The substrate in Figure 1A consisted of 5’[^32P]-labeled TP46 (5’-CTGCAGGGTTTTTGTCCAGTCTGTAGCACTGTGTGAAGACACAGGCCAGATCCCA TG CTGTCCACGTGCCCAGGTCACTGA-3’) annealed to TP494 (5’-ACACAGTGCTACAGACTG GAACAAAAACCCTGCAG-3’). Substrates used in the binding experiments quantified in Figure 1B include the 3’ overhang substrate used in Figure 1A, a 5' overhang substrate consisting of TP46 annealed to TP2756 (5’-TCAGTGACCTGGCAGTGACTGACAGCATGGGATCTG-3’), and duplex DNA consisting of 5’[^32P]-labeled TP74 (5’-CTGCAGGGTTTTTGTCCAGTCTGTAGCACTGTGTGAAGACACAGGCCA-3’) annealed to TP504 (5’-TGGCCTGCTCTACAGTGCTACAGACTGGGAACAAAAACCCTGCAG-3’).

Substrates used for binding assays quantified in Figure 1C included 5’[^32P]-labeled TP70 (5’-CGGAATTCCATGTAATCCCTCGATGAGG-3’), TP144 (5’-CCTTTGGCCCTGAAGTAGATGCTTACTAGG-3’), TP236 (5’-GACGGATCCGATCCAGCATGATAAGATACATTG-3’), and 5’[^32P]-labeled TP8
(5’-
GACCTGGCACGTAGGACACGATGGGATCTGGCCTGTCTTACACAGTGCTACA
GACTGGAACAAAAACCTGCA-G-3’) annealed to TP74, TP2999 (5’-
CTGCAGGGGTTTTTGTTCAGTCTGTAAGCCTGTAAGCAGG-3’), or TP507
(5’- CTGCAGGGGTTTTTGTTCAGTCTGTAAGCAGG-3’).

The substrate in Figure 1D and E consisted of 5’ [32P]-labeled TP158 (5’-
CATGTAATCCCTCGATCGGGTTTTAGTCTAGCAGACAGACAGC-3’)
TGCACATCTGGCCTGTCTTACACAGTGCTACACTGGAACAAAAACCCCTGC
AG-3’) annealed to TP2791 (5’- AGACCTCGAGGGGATTACAT-3’) and TP2572
(5’- CTGCA GGG TTTTTGTTCAGTCTGTAAGCAGG-3’). The substrate in Fig. S7
consisted of 3’ [32P]-labeled TP2622 (5’-
CTGCAGGGGTTTTTGTTCAGTCTGTAAGCAGGCCAGAG-3’)
annealed to TP1152 (5’-
CATCTGGCCTGTCTTACACAGTGCTACACTGGAAGTTCTCAGAG-3’).

The substrate in Figure S8 consisted of 5’ [32P]-labeled TP74 annealed to TP504 and
TP74 annealed to TP2681 (5’-
CTAGCTACTTGACACAGTGCACAGTGCACACTGGAACTGACATGCTA-3’).

5’ labeling was performed with T4 polynucleotide kinase (NEB) and γ-[32P] ATP; 3’
labeling was done with terminal deoxytransferase (Roche) and [32P] cordycepin (Perkin
Elmer).

Oligonucleotides used in single-molecule FRET experiments included:

1. 5’- /Cy3/ GCC TCG CTG CCG TCG CCA - /biotin/ - 3’
2. 5’- TGG CGA CGG CAG CGA GCC (T)_{16}/ Cy5/ (T)_{24} - 3’
3. 5' - /Cy5/ GCC TCG CTG CCG TCG CCA -/biotin/- 3'
4. 5' - TGG CGA CGG CAG CGA GGC (T)₁₅/iAmMC₆T/ (T)₄₂ - 3'
5. 5' - TGG CGA CGG CAG CGA GGC (T)₃₁/iAmMC₆T/ (T)₃₉ - 3'
6. 5' - TGG CGA CGG CAG CGA GGC (T)₆₈/iAmMC₆T/ (T)₈ - 3'

The amine-modified thymine (/iAmMC₆T/) shown in the sequence enables the oligonucleotides to be labeled with the monofunctional NHS ester form of Cy3 dyes (G.E.). /Cy5/ represents the Cy5 dye that was inserted directly to the DNA backbone using phosphoramidite chemistry. The partial duplex DNA substrates (18 bp dsDNA) with poly(T) single-stranded tails carrying fluorescence dyes were annealed by mixing ~5 μM of biotinylated strand and ~7 μM of poly(T) strand in 10 mM Tris:HCl (pH 8.0) and 50 mM NaCl followed by slow cooling from 90°C to room temperature for ~2 hours.

References cited:


