Supporting Information

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SI Discussion

Several studies have reported that RPA and *E. coli* SSB either stimulate (18, 19, 31) or inhibit Exo1-catalyzed DNA resection (5, 23, 66). Our results clarify how these proteins—both of which strip Exo1 from DNA—may nonetheless appear to stimulate resection. Exo1 binds DNA avidly (dwell-time >2,000 s; Fig. 1), but only a fraction of the nucleases resect DNA. The low enzyme activity is not evident in bulk resection assays, but can be measured in single-molecule experiments. These inactive Exo1 proteins block access to free DNA ends. RPA and SSB strip both active and inactive Exo1 from DNA (Fig. 2 and Fig. S7), promoting distributive resection that is catalyzed by a small subset of active nucleases. This effect is most apparent with yExo1, where ~15% of DNA-bound nucleases resect DNA. Notably, Cannavo et al. reported that yeast RPA (yRPA) and SSB both stimulate yExo1, especially at high ratios of free DNA ends to yExo1 (~0.25–8 nM yExo1 with 16 nM DNA ends in ref. 18). Similarly, hRPA modestly stimulated hExo1, but only when the nuclease was limiting (19). Our assays demonstrate that inactive Exo1 will block DNA ends for processing by active enzymes. In addition, RPA and SSB may limit Exo1 binding to competitor ssDNA, as proposed by Cannavo et al. Together, these activities may appear to stimulate Exo1 in a gel-based ensemble assay, especially when a significant percentage of the molecules is capable of binding but not resecting free DNA ends.

SI Experimental Procedures

Proteins and DNA.

Human Exo1-biotin and Exo1(D78A/D173A)-biotin. A pFastBac1 (Life Tech.) plasmid containing hExo1 was generously provided by Paul Modrich (4). Plasmid pIF7, which contains an avidity tag (GLNDIFEAQKIEWHE) at the C terminus, was created by inverse PCR using primers LM006 and LM008 (74). To create Exo1 (D78A/D173A), pIF7 was mutated by two rounds of QuikChange Mutagenesis (Agilent) using oligonucleotides TP2792 and TP2793 for D78A and TP3253 and TP3254 for D173A. Biotinylated Exo1 and Exo1(D78A/D173A) were purified as previously described (21) with the following modifications: Exo1-bio was expressed in Sf21 insect cells using the Bac-to-Bac (Life Tech.) expression system. Cells were coinfected with Exo1-Avidity tag and BirA (biotin ligase) viruses, and pellets were harvested 72 h after infection. To purify Exo1-biotin, cells were homogenized in buffer A [25 mM Tris·HCl, pH 8, 100 mM NaCl, and 10% (vol/vol) glycerol] containing 1 mM PMSF in a Dounce homogenizer (Kimble Chase; Kontes) before three rounds of sonication on ice (30 s each time with a 30-s recovery on ice). Insoluble matter was pelleted for 1 h at 100,000 × *g*, and the supernatant was added to Q beads in batch. The beads were rotated at 4 °C for 1 h, spun at 1,500 × *g* for 5 min, and washed 3× with buffer A. Exo1 was eluted from the Q beads by incubating the beads with 15 mL buffer B [25 mM Tris·HCl, pH 8, 1 M NaCl, and 10% (vol/vol) glycerol]. The supernatant was removed from the beads via a Poly-Prep Chromatography Column (BioRad) and incubated with SoftLink Soft Release Avidin Resin (Promega) for 1 h. The reversible Avidin resin was packed into a column, rinsed with buffer A on an ÄKTA fast protein liquid chromatography (FPLC), and eluted with 5 mM biotin over the course of 30 min. Exo1-containing fractions were bound to a 1 mL SP Hi-Trap column in buffer A and eluted in 500-μL fractions with buffer B. The most concentrated Exo1-containing fractions were separated by gel filtration using a Superdex-200 column (GE) in buffer A. Biotinylated Exo1 was snap-frozen in 3-μL aliquots and stored at −80 °C. Both WT and Exo1(D78A/D173A) purified with similar homogeneity.

We measured Exo1 biotinylation efficiency as previously described (72). Briefly, purified Exo1-bio was incubated with a large excess of streptavidin for 10 min on ice, mixed with loading dye, and run on an SDS/PAGE gel. The extraordinarily strong biotin-streptavidin interaction is not denatured by the SDS/PAGE gel conditions. We scored biotinylation efficiency by measuring the percentage of Exo1 proteins that shift up above the Exo1 molecular weight on the gel.

Human Exo1-Flag. A Flag epitope (DYKDDDDK) was inserted at the C terminus of hExo1 by inverse PCR with primers LM4 and LM7 to create pIF8. hExo1-Flag was purified as described above, but with an anti-FLAG M2 resin (Sigma) replacing the reversible Avidin resin. hExo1-Flag was eluted from the anti-FLAG beads by incubating with 100 μg/mL Flag peptide, as suggested by the manufacturer (Sigma). After elution from the Flag beads, hExo1-containing fractions were purified via SP and gel filtration columns, as described for Exo1-biotin (see above).

yExo1. A pFastBac1 (Life Tech.) plasmid containing yeast Exo1 was generously provided by R. Michael Liskay. Plasmid pTP3184, which contains an avidity tag (GLNDIFEAQKIEWHE) at the C terminus, was created by inverse PCR using primers IF190 and IF191. Biotinylated yExo1 was expressed and purified as above; however, instead of separating the most concentrated fractions via a Superdex-200 column, these fractions were pooled, dialyzed overnight in buffer A, aliquoted, snap-frozen, and stored at −80 °C.

Human RPA and RPA-GFP. Plasmids overexpressing human RPA (hRPA) and hRPA-GFP were generously provided by Mauro Modesti and purified essentially as described previously (44).

RPA70 A/B dimer. For purification, a pET28a plasmid overexpressing the human RPA70 A/B-DNA binding domains (pIF106) was transformed into BL21(DE3) cells (47). A single colony was inoculated into 50 mL lysogeny broth (LB) with 50 μg/mL kanamycin and incubated overnight at 37 °C with agitation. The next morning, the overnight preculture was diluted 100-fold into 6 L of LB + kanamycin and incubated at 37 °C with agitation until OD at 600 nm reached 0.6. Once this OD was reached, the solutions were cooled to 16 °C on ice in a cold room. Protein expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and the culture was incubated at 16 °C with agitation for 16–18 h. Cells were harvested by centrifuging for 15 min at 5,000 × *g*. The supernatant was discarded, and the cell pellet was resuspended in 40 mL PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4, 1 mM PMSF + 1 tablet of cOmplete-EDTA-free protease inhibitor; Roche). The cells were flash-frozen in liquid nitrogen and stored at −80 °C until needed.

The frozen cell paste corresponding to 3 L starter culture was thawed in lukewarm water and immediately placed on ice. All subsequent steps were performed at 4 °C. One volume of 2× lysis buffer [1 M NaCl, 60 mM Hepes, pH 7.8, 2 mM DTT, 40 mM imidazole, pH 8, 20% (vol/vol) glycerol, 2 mM PMSF, and 0.02% (vol/vol) Nonidet P-40 substitute] was added to the cells and resuspended by mixing with a pipette. The lysate was sonicated on ice for a total of 90 s (Fisher Scientific 705 Sonic Dismembrator at 75% amplitude; 15-s bursts with 90-s rests in between). The lysate was then centrifuged at 100,000 × *g* for 35 min at 4 °C (Ti-45 rotor in Optima XE ultracentrifuge; Beckman-Coulter). A 5-mL HisTrap FF column was pre-equilibrated with 1× lysis buffer using the ÄKTA FPLC (GE Healthcare). The clarified lysate was loaded, and the column was washed with

50 mL 1× lysis buffer. Protein was eluted with a gradient to 100% (vol/vol) elution buffer [500 M NaCl, 30 mM Hepes, pH 7.8, 1 mM DTT, 500 mM imidazole, pH 8, 10% (vol/vol) glycerol, 1 mM PMSF, and 0.01% (vol/vol) Nonidet P-40 substitute] over eight column volumes.

RPA70 A/B-containing fractions were loaded on a Sephacryl S-300 HR (GE Healthcare) column pre-equilibrated with buffer R [50 mM KCl, 20 mM Tris·HCl, pH 7.5, 1 mM DTT, 0.5 mM EDTA, and 10% (vol/vol) glycerol]. Next, protein-containing fractions were loaded onto a 1-mL Hitrap Q HP column pre-equilibrated with buffer R, washed with 15 mL buffer R, and then eluted with a gradient to 100% (vol/vol) buffer RE [500 mM KCl, 20 mM Tris·HCl, pH 7.5, 1 mM DTT, 0.5 mM EDTA, and 10% (vol/vol) glycerol] over 10 column volumes. Fractions were analyzed on a 10–12% SDS/PAGE gel. The purest RPA70 A/B fractions were pooled and dialyzed against 2 L storage buffer [10 mM Tris·HCl, pH 7.6, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, and 50% (vol/vol) glycerol] overnight at 4 °C before being aliquotted and flash-frozen in liquid nitrogen for storage at −80 °C.

All other truncation mutants of RPA were purified as described by Wyka et al. (47).

Yeast RPA. An overexpression plasmid containing all three subunits of yRPA was modified by introducing an intein-chitin binding domain (CBD) to the C terminus of the Rfa2 subunit of RPA (pIF65). Plasmid pIF65 was transformed into Rosetta/pLysS cells (Novagen), and a single colony was inoculated into 50 mL of LB with 50 μg/mL carbenicillin and 34 μg/mL chloramphenicol and incubated overnight at 37 °C with agitation. The next morning, the overnight preculture was diluted 100-fold into 6 L of LB + carbenicillin + chloramphenicol and incubated at 37 °C with agitation until OD at 600 nm reached 0.6. The solutions were cooled to 16 °C, and protein expression was induced with 1 mM IPTG at 16 °C for 16–18 h. Cells were harvested by centrifuging for 15 min at 5,000 × *g*. The supernatant was discarded, and the cell pellet was resuspended in 40 mL PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, and 1.47 mM KH_2PO_4, pH 7.4) with 1 mM PMSF. Cell pellets were flash-frozen in liquid nitrogen and stored at −80 °C until needed.

The frozen cell paste corresponding to 3 L starter culture was thawed in lukewarm water and immediately placed on ice. All subsequent steps were performed at 4 °C. One volume of 2× lysis buffer [500 M NaCl, 40 mM Tris·HCl, pH 7.5, 10 mM imidazole, pH 8, 20% (vol/vol) glycerol, and 1 mM PMSF] was added to the cells and resuspended by mixing with a pipette. The lysate was sonicated on ice for a total of 90 s (Fisher Scientific 705 Sonic Dismembrator at 75% amplitude; 15-s bursts with 90-s rests in between). The lysate was then centrifuged at 100,000 × *g* for 35 min at 4 °C (Ti-45 rotor in Optima XE ultracentrifuge; Beckman-Coulter). A 5-mL HisTrap FF column was pre-equilibrated with 1× lysis buffer using the ÄKTA FPLC (GE Healthcare). The clarified lysate was injected using a 50 mL SuperLoop (GE), and the column was washed with 50 mL 1× lysis buffer. Protein was eluted with a gradient to 100% (vol/vol) elution buffer [250 mM NaCl, 20 mM Tris·HCl, pH 7.5, 500 mM imidazole, pH 8, and 10% (vol/vol) glycerol] over eight column volumes. RPA-containing fractions were loaded on a 5-mL Chitin (New England BioLabs) column pre-equilibrated with buffer A (250 mM KCl, 20 mM Tris·HCl, pH 7.5, and 1 mM EDTA) and washed with 300 mL buffer A. To cleave the protein from the resin, the column was flushed with 60 mL elution buffer (buffer A+ 50 mM DTT) and left with no flow at 4 °C overnight. The next day the protein was eluted, and the RPA-containing fractions were diluted fivefold in buffer RA [20 mM Tris·HCl, pH 7.5, 0.5 mM EDTA, and 10% (vol/vol) glycerol]. The sample was loaded onto a 1 mL Hitrap Q HP column pre-equilibrated with buffer R, washed with 15 mL buffer R, and then eluted with a gradient to 100% (vol/vol) buffer RE [500 mM KCl, 20 mM Tris·HCl, pH 7.5, 1 mM DTT, 0.5 mM EDTA, and 10% (vol/vol) glycerol] over 10 column volumes. Fractions were analyzed on a 10–12% SDS/PAGE gel. The purest RPA fractions were pooled and dialyzed against 2 L storage buffer [10 mM Tris·HCl, pH 7.6, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, and 50% (vol/vol) glycerol] overnight at 4 °C before being aliquoted and flash-frozen in liquid nitrogen for storage at −80 °C.

T4 gp32. Plasmid pIF89 was generated by PCR amplifying the gene encoding gp32 from phage DNA using oligos IF025 and IF026. The PCR amplicon was digested with NdeI and SpeI, and ligated into a homemade pET-derived vector that contains a C-terminal intein-CBD. Plasmid pIF89 was transformed into BL21-ArcticExpress cells (Agilent), and a single colony was used to start a 100 mL overnight preculture. The overnight preculture was diluted 100-fold into 6 L LB + carbenicillin and incubated at 30 °C with agitation until OD_{600}~0.6. The cells were cooled to 12 °C, and protein expression was induced with 0.4 mM IPTG at 12 °C for 16–20 h. Cells were harvested by centrifuging for 10 min at 5,000 × *g* and 4 °C, and the cell pellet was resuspended in 35 mL resuspension buffer [50 mM Tris·HCl, pH 7.5, 500 mM NaCl, 1 mM ETDA, and 10% (vol/vol) sucrose] with 1 mM PMSF. Cell pellets were flash-frozen in liquid nitrogen and stored at −80 °C until needed.

The frozen cell pellet corresponding to 3 L culture was thawed in cold water and immediately placed on ice. The sample was sonicated on ice for a total of 90 s (Fisher Scientific 705 Sonic Dismembrator at 75% amplitude; 15-s bursts with 90-s rests in between). The lysate was then centrifuged at 100,000 × *g* for 35 min at 4 °C (Ti-45 rotor in Optima XE ultracentrifuge; Beckman-Coulter). The clarified lysate was loaded on a 10-mL Chitin (New England BioLabs) column pre-equilibrated with buffer C (50 mM Tris·HCl, pH 7.5, 500 mM NaCl, and 1 mM EDTA) and washed with 1 L buffer D (50 mM Tris·HCl, pH 7.5, 1 M NaCl, and 1 mM EDTA). To cleave the protein from the resin, the column was flushed with 30 mL elution buffer (buffer D + 50 mM DTT) and left in elution buffer at 4 °C overnight. The next day, gp32 fractions were pooled and concentrated to a concentration of ~90 μM using a 10K Amicon concentrator (Millipore). The protein was then dialyzed against 4 L storage buffer [20 mM Tris·HCl, pH 7.5, 150 mM NaCl, and 10% (vol/vol) glycerol] overnight at 4 °C before being aliquoted and flash-frozen in liquid nitrogen for storage at −80 °C.

SSB. SSB was amplified from genomic DNA and cloned into a pET-derived vector that contains a C-terminal intein-CBD. Plasmid pIF122 was transformed into BL21(DE3) cells. A starter culture from a single colony was used to inoculate 6 L LB + carbenicillin and grown to an OD_{600}~0.6 at 37 °C. On reaching an optical density of 0.6, the culture was induced with IPTG to 0.5 mM and grown overnight at 16 °C. Cells were harvested by centrifugation, resuspended in resuspension buffer, and lysed by sonication. The clarified lysate was applied to a 10-mL Chitin gravity column, washed, and cleaved as for gp32. The protein was then dialyzed into storage buffer [50 mM Tris·HCl, pH 7.4, 300 mM NaCl, and 50% (vol/vol) glycerol] and stored at −20 °C. The concentration was determined by comparing the concentration to a set of known BSA standards.

SOSS1. SOSS1 was purified according to previously published protocols (21). Briefly, the SOSS1 (hSSB1 T117E) complex was expressed in Sf21 insect cells, harvested, and lysed similarly to Exo1-Bio. Next, the lysate was purified via Nickel-NTA resin, a Hi-Trap GST column, and a Hi-Trap SP column. The most concentrated fractions were loaded on a Superdex 200 gel filtration column, and fractions from this containing SOSS1 were combined, aliquoted, and frozen at −80 °C.

Fluorescent Protein Labeling.

Conjugation of hExo1/yExo1 to streptavidin QDs. Exo1-bio (80 nM) and streptavidin QDs (100 nM) were incubated in 10 μL imaging

buffer (40 mM Tris·HCl, pH 8, 60 mM NaCl, 0.2 mg/mL BSA, 2 mM DTT, and 1 mM $MgCl_2$) for 10 min on ice. Saturating biotin was added to bind free streptavidin, and the mixture was diluted to 200 μL (4 nM Exo1, 5 nM QD). The Exo1-QD mixture was injected into the flowcells via a 100-μL injection loop (at a flow rate of 200 μL/min), and the flowcell was flushed thoroughly at a flow rate of 400 μL/min to remove all Exo1 molecules that did not associate with the DNA curtains.

Conjugation of Exo1 to anti-biotin antibodies. Exo1-bio (80nM) and anti-biotin CF488A antibody (1.2 μM; Biotium) were incubated in 10 μL imaging buffer as above for 10 min on ice. Saturating biotin was added to bind free antibody and the mixture was diluted to 200 μL (4 nM Exo1, 60 nM anti-biotin CF488). This mixture was injected as above.

SOSS1. SOSS1 (200 nM) was incubated with Alexa488-anti GST antibodies (200 nM; Cell Signaling) before being diluted to 1 nM. For short injections, 1 or 10 nM SOSS1 (as indicated) was injected through a 5-mL loop. For longer time points, 30 mL 1 nM SOSS1 was loaded into a syringe and injected continuously for 1 h.

Gel-based resection assays with streptavidin-conjugated hExo1-bio. Resection assays were conducted as described previously (21). Briefly, WT hExo1 (500 pM), hExo1-bio (500 pM), or hExo1-bio (500 pM) + streptavidin (1 μg) was incubated in imaging buffer with 30 ng linearized 4.4-kb DNA (4-nt 3′ overhang created with SphI-HF; NEB) for 30 min at 37 °C. The reactions were deproteinized with 2 μg Proteinase K for 20 min at 37 °C, and the resection products were run on a 1% agarose gel overnight at 25 V. The gels were stained with SYBR green and analyzed on a Typhoon FLA 9500 laser scanner (GE Healthcare).

Gel-based resection assays with Exo1 and RPA. Resection assays were conducted as above, but with a few modifications. hExo1-bio or yExo1-bio at indicated concentrations were incubated in imaging buffer with 10 ng PstI-HF (4-nt 3′ overhang; NEB) linearized 6.3-kb DNA in the presence or absence of hRPA or yRPA as indicated for 1 h at 37 °C or 30 °C. The reactions were deproteinized with 2 μg Proteinase K (NEB), run on a gel, stained, and analyzed as above.

DNA substrates for single-molecule experiments. DNA substrates were prepared by annealing oligos IF7 and LM003 to purified lambda DNA (New England Biolabs) (28). Briefly, ∼15 nM λ-phage DNA was heated to 65 °C, combined with ∼10 μM IF7 and LM003, and allowed to slowly cool to room temperature. After cooling, the reaction was supplemented with ATP to 1 mM, T4 DNA ligase (2,000 units; New England Biolabs) and incubated overnight at room temperature. To avoid adding nicks, the ligase was salt inactivated by supplementing the reaction with 100 μL 5 M NaCl to (final concentration: 1 M NaCl). To remove excess proteins and oligonucleotides, the reaction was passed over an S-1000 gel filtration column (GE), and the ligated DNA was stored at 4 °C. To reverse the DNA orientation, the same protocol was followed with oligos IF6 and LM024.

Single-Molecule Methods.

Microscope slide nanofabrication. To deposit diffusion barriers for DNA curtains, we developed a custom wafer-based nanofabrication process. Chrome diffusion barriers were made on 1.58-mm-thick, 101.6-mm-diameter ground and polished GE124 quartz discs (Technical Glass Products). To fit the glass into a wafer-processing holder, a flat was made by grinding 2 mm into the glass. Glass wafers were immersed in Piranha solution [3:1 sulfuric acid, 96%/hydrogen peroxide 30% (vol/vol)] for 8 min and spun-dried in a nitrogen flow at 2,000 rpm for 10 min (Spin Rinse Dryer Verteq, SRD, A182-39MLB 4713-7E). The wafers were spin coated in a Headway Spinner (PWM32) in two time steps for each layer (300 rpm for 10 s followed by 4,000 rpm for 60 s with 350-rpm/s ramp). A first layer of polymethylmethacrylate (PMMA), molecular weight 495K plus 1.5% (wt/vol) in anisole (MicroChem), was followed by a layer of Aquasave conducting polymer (Mitsubishi Rayon). The double coated wafer was set at 150 °C on a hotplate for 1 min. Electron beam lithography was performed in a Jeol 6000 FSE aligner. After writing the nanopattern, the Aquasave layer was rinsed off with deionized water and dried in N_2 flow. The PMMA layer was developed by rinsing the wafer in a 3:1 solution of isopropanol to methyl isobutyl ketone (MIBK; MicroChem) for 1 min. The wafer was rinsed in isopropanol and dried in N_2 flow. Electron beam evaporation (Cooke E-beam/sputter deposition system) was used to deposit a 13-nm layer of chromium (99.998% purity, Kurt J. Lesker) on the wafer. To lift off the PMMA, the chromium-coated wafer was sonicated in acetone for 30–60 min, rinsed in ethanol, and dried in N_2 flow. The wafers were covered with a clean-room rated silicon wafer tape (ICROS) and diced into six flowcell-sized (50 × 22 mm) substrates (using a Disco 321 dicing saw).

Flow cell preparation. We followed previously described protocols for assembling DNA curtains (28). Briefly, nano-fabricated flowcells were pre-equilibrated in buffer L (10 mM Tris·HCl, pH 7.8, and 100 mM NaCl) and covered with a ternary lipid bilayer composed of a mixture of DOPC (97.7 mol%), DOPE-biotin (0.3 mol%), and DOPE-mPEG2K (2 mol%; Avanti Lipids). Flowcells were next incubated in imaging buffer (40 mM Tris·HCl, pH 8, 2 mM DTT, 1 mM $MgCl_2$, 0.2 mg/mL BSA; fraction VI, Sigma-Aldrich) for 10 min. Next, streptavidin (Life Technologies; 0.1 mg/mL in imaging buffer) was injected into the flowcell. Finally, biotinylated λ-DNA was injected into the flowcell, and the flowcell was mounted on a custom-machined microscope stage for immediate fluorescent observation.

Single-Molecule Microscopy. Images were collected with a Nikon Ti-E microscope in a prism-TIRF configuration. The inverted microscope setup allowed for the sample to be illuminated by a 488-nm laser light (Coherent) through a quartz prism. To minimize spatial drift, the experiment was conducted on a floating TMC optical table. A 60× water immersion objective lens (1.2 NA; Nikon), two EMCCD cameras (Andor iXon DU897, −80 °C), and the Nis Elements software (Nikon) were used to collect the data at 1 Hz with a 100- or 200-ms exposure time. A computer-controlled shutter (700-μs opening time; Vincent Associates) was used to take 3,600 frames in 1 h. Frames were saved as TIFF files without compression, and further image analysis was done in ImageJ (National Institutes of Health). Fluorescent labels were tracked in ImageJ with a custom-written particle tracking script. The resulting trajectories were analyzed in Matlab (Mathworks). For each frame, the fluorescent particle was fit to a 2D Gaussian function to obtain trajectories with subpixel resolution. To correct for sample drifting on the XY-stage plane, a QD fixed to the slide surface was tracked as a reference, and such trajectories were subtracted from the molecule trajectories. To ensure that the trajectories corresponded to Exo1 on DNA, at the beginning of each experiment, the flow was stopped to see the characteristic recoiling motion of DNA-bound proteins. Only DNA-bound QDs were counted for statistical analysis. For individual Exo1 molecules, the velocity was determined by fitting the time-dependent motion along the DNA to a line. Some Exo1 molecules did not initiate translocation immediately on binding the DNA. Segments in the trajectory that corresponded to such paused Exo1 were not taken into account for calculating the molecules' velocities but were counted in the DNA-binding lifetimes. To determine Exo1 DNA-binding lifetimes, we measured the total amount of time that each Exo1 molecule was bound to DNA. Exo1 lifetime data were fit to single-exponential decays of the form

$$y(t) = Ae^{-t/\tau_1} + B,$$

where y is the number of DNA-bound Exo1 molecules at time t, τ is the lifetime, A is the normalized amplitude, and B corresponds to a constant offset.

For multiple turnover experiments, we found that the Exo1 survival probabilities had a biexponential distribution, and the resulting data were fit to

$$y(t) = Ae^{-t/\tau_1} + Be^{-t/\tau_2},$$

where τ_1 and A are the lifetime and amplitude of the first component, and τ_2 and B are the lifetime and amplitude of the second component, respectively. Automated fitting was done via a custom Matlab script (available on request). The quality of the fit, as determined by the coefficient of determination (R^2), is summarized in Table S1.

To verify that a biexponential fit was statistically appropriate, an F-test was applied to the multiple turnover data in Fig. 5 *C* and *D* (75). The significance of the fit between the single exponential model (model 1) and the double exponential model (model 2) was calculated by comparing the ratio $F(1,2)$

$$F(1,2) = \frac{(SSQ1 - SSQ2)(n - df2)}{SSQ2(df1)},$$

where $df1$ and $df2$ are the degrees of freedom for models 1 and 2. The residuals sums of squares *SSQ1* (single exponential) and *SSQ2* (double exponential) were calculated from the n data points for each model. The F-distribution was set at the 95% CI. As the data approach zero, we did not consider a constant term in either model 1 or model 2. Thus, the significance probability was determined from the F probability distribution with two degrees of freedom (*df1*) for the single exponential and four degrees of freedom (*df2*) for the double exponential fit. Our data consisted of $n = 55$ data points for hExo1 and $n = 62$ for yExo1. The resulting ratio, $F(1,2)$, was 362 for hExo1 (Fig. 5*C*) and 1,000 for yExo1 (Fig. 5*D*). To test the null hypothesis with 95% confidence, the F probability, F_{NULL}, was calculated with Matlab function finv(). F_{NULL} was 3 for both hExo1 and yExo1. Because $F(1,2) >> F_{NULL}$, the null hypothesis is not true, and the biexponential model fits the data more accurately than the single exponential model with at least 95% confidence.

In the absence of SSBs, Exo1 remained associated with the DNA for over an hour, and we are only able to report a lower bound on the lifetimes. For single-turnover reactions in the presence of SSBs, $t = 0$ was defined as the time when 90% of the maximum SSB concentration (as measured by GFP-RPA fluorescence signal) had entered the flowcell. For multiple turnover experiments, Exo1 was continuously coinjected with 1 nM RPA, and the lifetimes were measured from transient binding events on individual DNA molecules.

Observing Exo1-biotin on DNA curtains. All single-molecule experiments were carried out in imaging buffer containing 40 mM Tris·HCl, pH 8, 60 mM NaCl, 1 mM $MgCl_2$, 2 mM DTT, and 0.2 mg/mL BSA. For Fig. S2, 1 mM $MgCl_2$ was replaced with 1 mM EDTA. For single turnover assays, biotinylated Exo1 was incubated with a 1.5× molar excess of streptavidin QDs on ice for 10 min (emitting at 705 or 605 nm; Life Tech.). After incubation, the reaction was quenched with imaging buffer containing biotin, diluted to 4 nM for hExo1 or 1 nM for yExo1, and injected into the flowcell at a linear flow rate of 200 μL/s. After Exo1 bound the DNA, flow was increased to 400 μL/min. Constant flow was maintained with a continuous-drive syringe pump (Legato 210; KD Scientific). For multiple turnover experiments, two separate reactions were set up with biotinylated Exo1 and 1.5× molar excess of streptavidin QDs emitting at either 705 or 605 nm on ice for 10 min. These two reactions were quenched with imaging buffer containing biotin, mixed, and diluted to 500 pM for hExo1 or 200 pM for yExo1. The QD-Exo1 reaction was supplemented with 1 nM RPA and injected into the flowcell continuously for 10 min at 400 μL/min. All experiments with hExo1 were conducted at 37 °C, and the corresponding experiments with yExo1 were conducted at 30 °C. Constant temperature was maintained via an objective heater (Bioptechs) and a custom-built microscope stage heater.

Observing hExo1-Flag on DNA curtains. A modified labeling scheme was used to ensure a single fluorophore per hExo1-Flag enzyme. First, 4 nM hExo1-Flag was injected into preassembled DNA curtains at a flow rate of 200 μL/s. After excess hExo1-Flag was flushed out of the flowcell, 700 μL 1 nM QD-conjugated anti-FLAG M2 antibodies (Sigma) was injected at a flow rate of 400 μL/min. This strategy guarantees that each DNA-bound hExo1-Flag is labeled with, at most, one fluorophore. Imaging and data analysis were identical to that of hExo1-biotin.

Tissue Culture.

Cell culture conditions and treatments. U2OS cells were maintained in DMEM (Invitrogen) supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Transfections for siRNA were carried out with lipofectamine RNAiMax (Invitrogen) following the manufacturer's instructions. The siRNA used in this study are as follows: TP3802 for siControl, TP4115 for siRPA2, and TP4206 for Dna2 (Table S2). A mammalian expression vector containing Exo1-GFP was cloned by Gateway mutagenesis from a DNASU donor vector (HsCD00076255) into a destination vector containing a C-terminal GFP (Vivid Colors pcDNA6.2/C-EmGFP-DEST; Life Tech.). This vector (Exo1-GFP) was transfected by Fugene HD (Promega) according to the manufacturer's instructions. EdU (5-ethynyl-2′-deoxyuridine) incorporation was used to detect active DNA synthesis. After indicated siRNA treatments, 10 μM EdU was added into the medium for 30 min. EdU was detected by using the Click-iT EdU imaging kit (Invitrogen; C10340).

U2OS-ER-AsiSI (EV28) cells were cultured as previously described (12). A mammalian expression vector containing Exo1-V5 was cloned by Gateway mutagenesis from a DNASU donor vector (HsCD00076255) into a destination vector containing a C-terminal V5 tag (pLX304; Addgene). Transfer of the plasmid into the EV28 cells was done by lentiviral infection, and a clonal population was selected for by stable integration of the vector with blasticidin.

Protein extracts and Western blotting. For whole cell extracts, cells were collected with Laemmli buffer [4% (vol/vol) SDS, 20% (vol/vol) glycerol, and 120 mM Tris·HCl, pH 6.8] and sonicated in a Diagenode Bioruptor 300 for 10 min followed by boiling for 5 min at 95 °C before loading. Samples were resolved by SDS/PAGE and analyzed by standard Western blotting. Primary antibodies used for this study are anti-RPA32/RPA2 (Abcam; AB2175) and anti–β-tubulin (Abcam; ab6046). Blots were analyzed by standard chemiluminescence (GE Healthcare; Amersham ECL Prime system) using a Bio-Rad molecular imager ChemiDoc XRS+ system.

Laser microirradiation. Laser microirradiation was carried out with a FluoView 1000 confocal microscope (Olympus) as previously described (76). Briefly, U2OS cells were grown on glass-bottomed dishes (Willco Wells). After 24-h siRNA treatment, cells were transfected with the Exo1-GFP vector. Culture medium was replaced with normal medium containing 10 μM BrdU 8 h after transfection and incubated for 24 h. A 405-nm solid-state laser from the confocal microscope was used to generate BrdU-dependent DNA damage along the laser path. Following laser damage, cells were analyzed by live confocal fluorescent microscopy or immunofluorescence analysis as indicated.

Live-cell microscopy analysis. Experiments for live-cell microscopy were performed in a heated environmental chamber with 5% CO_2 on a Fluoview 1000 confocal microscope (Olympus) as previously described (77). For quantification of GFP-Exo1 recruitment after laser microirradiation, the average fluorescence intensity at the damage site and an undamaged control region from the same cell were directly recorded using the FV10-ASW3.1 software. Variation of the fluorescence intensity was quantified as the difference between the average fluorescence intensity in the damaged region vs. in the undamaged region from the same cell. Each curve corresponds to data obtained from >20 analyzed cells from two independent experiments.

Immunofluorescence. After indicated treatments, cells were treated and processed for immunofluorescence (IF) as previously described (78). Briefly, cells were fixed with 2% (vol/vol) formalin for 15 min at room temperature, permeabilized with 0.5% (vol/vol) Triton-X for 10 min, and then blocked with PBS containing 3% (wt/vol) BSA. After blocking, cells were incubated with the primary antibody for 1 h at room temperature, followed by secondary antibody incubation for 1 h at room temperature. The primary antibody used for IF was anti–phospho-histone H2A.X (Ser139) (Millipore; 04-263), and the secondary antibody was Alexa Fluor 594 goat anti-mouse IgG. For both the IF and EdU detection analyses, cells were imaged and analyzed with Z-stacked setting using the FV10-ASW3.1 software on a Fluoview 1000 confocal microscope (Olympus). For quantification of EdU experiment, >200 cells for each treatment were counted. Percentages of the EdU-positive cells were plotted from data obtained from two independent experiments.

ChIP. After indicated cell treatments, proteins were cross-linked to DNA by adding formaldehyde drop-wise directly to the media for a final concentration of 1% (vol/vol) and rotating gently at room temperature for 15 min. Next, glycine was added to a final concentration of 125 mM, and the cells were incubated for 5 min at room temperature. Cells were rinsed in PBS, harvested, and pelleted for 30 min at 1,000 × *g*. For lysis, the pellet was resuspended in 2 mL RIPA buffer [50 mM Tris·HCl, pH 8, 150 mM NaCl, 2 mM EDTA, pH 8, 1% (vol/vol) Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and protease inhibitors (miniComplete, Roche)]. Next, the lysate was sonicated in a Bioruptor for 15–30 min at high power with a 10 s on/10 s off cycle. The lysate was cleared for 30 s, 4 °C, and 18,928 × *g*, and the supernatant was transfered to new tube; 1 μL RNase A (0.5 mg/mL) was added to the input and incubated with shaking for 4 h at 65 °C. The chromatin was purified with one column of the Qiagen PCR purification kit and eluted with 50 μL water (heated at 50 °C and left for 30 min on column before spinning). DNA concentration was determined, and the quality of the shearing was analyzed by running 1 μg on a 1.5% agarose gel. The chromatin was diluted to 10 μg/mL with RIPA buffer, and the appropriate primary antibody was added as indicated and incubated overnight with rotation at 4 °C; 25 μL magnetic IgG+ IgA Dynabeads (12.5 μL of each) was added to all samples and immunoprecipitated 2 h with rotation at room temperature. The beads were washed once successively with 1 mL of each of the following buffers and incubating for 5 min at room temperature, followed by pelleting the Dynabeads: TSE-150 [1% (vol/vol) Triton X-100, 0.1% SDS, 2 mM EDTA, 20 mM Tris·HCl, pH 8, and 150 mM NaCl], TSE-500 [1% (vol/vol) Triton X-100, 0.1% SDS, 2 mM EDTA, 20 mM Tris·HCl, pH 8, and 500 mM NaCl], LiCl/detergent buffer [0.25 M LiCl, 1% (vol/vol) Nonidet P-40, 1% (wt/vol) 2,5-dimethoxy-4-chloroamphetamine, 1 mM EDTA, and 10 mM Tris·HCl, pH 8], and TE (10 mM Tris·HCl, pH 8, and 0.1 mM EDTA). The beads were again pelleted, and the supernatant was removed. DNA was eluted by adding 125 μL elution buffer [1% (wt/vol) SDS and 0.1 M sodium bicarbonate] to the protein A/G beads and shaking for 30 min at 30 °C. The supernatant was moved to a new tube and shaken overnight at 65 °C. DNA was purified with one column of the Qiagen PCR purification kit and eluted with 50 μL water.

In vivo resection assay and qPCR. The in vivo resection assay was performed as previously described (12). The AsiSI sites used for resection have been previously characterized (DSB1, Chr 1: 89231183; DSB2, Chr 1: 109838221). qPCR of DNA near genomic AsiSI sites was also done as described previously (12). Sites used for ChIP-qPCR in this study have been previously studied (79). DSB1 (DSB-2 cited) is located on chromosome 21 at AsiSI position 32167382. For 80 bp away from the break, primers TP3955 and TP3956 were used. For 800 bp away from the break, primers TP4002 and TP4003 were used. Similarly, DSB2 (DSB-II cited) is located on chromosome 22 at AsiSI position 37194040. For 80 bp away from the break, primers TP4028 and TP4029 were used. For 800 bp away from the break, primers TP4030 and TP4031 were used.

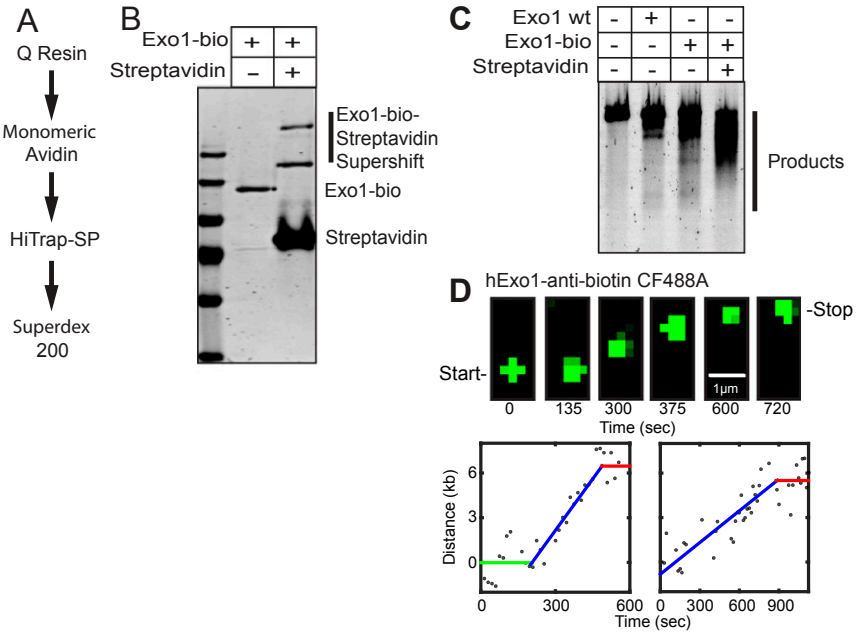

Fig. S1. Human Exo1-biotin (hExo1-bio) purification and labeling. (*A*) Purification scheme for hExo1-bio. (*B*) SDS/PAGE gel showing hExo1-bio and hExo1-bio + streptavidin. Gel shift of hExo1-bio-streptavidin conjugates are indicated. The complete disappearance of the hExo1-bio band indicates that nearly 100% of the purified nucleases are biotinylated. (*C*) Resection assay with untagged WT hExo1 (500 pM), hExo1-bio (500 pM), or hExo1-bio (500 pM) + streptavidin (1 μg). Proteins were incubated in imaging buffer with 30 ng linearized 4.4-kb DNA (4-nt 3′ overhang) for 30 min at 37 °C. Resected DNA was separated on a 1% agarose gel and stained with SYBR green. The resection protocol has also been described previously (21). Together, these assays indicate that streptavidin-conjugated hExo1-bio retains full resection activity. (*D*) Snapshots at indicated times (*Upper*) and single-particle tracking of two representative trajectories of resection by CF488-anti-biotin–labeled hExo1 (*Lower*). In both trajectories, hExo1 transitions between a resecting and a paused state. These results indicate that both states are intrinsic to hExo1 and are not dependent on the nature of the fluorophore.

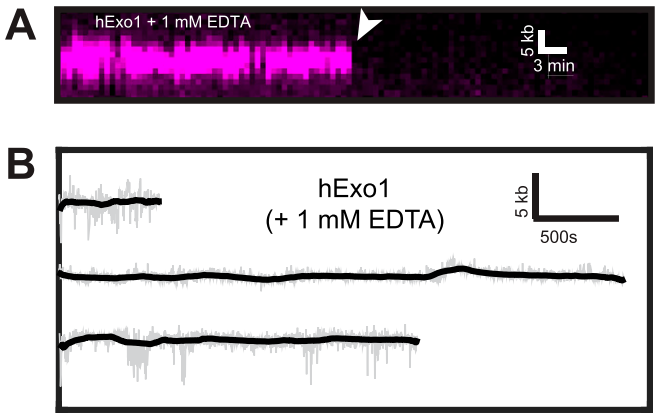

Fig. S2. hExo1 requires a divalent cation to move on DNA. (*A*) Kymograph of hExo1-bio (magenta) in the presence of 1 mM EDTA. The white arrow indicates when the protein dissociated from DNA. (*B*) Example single-molecule trajectories of hExo1-bio with 1 mM EDTA. The raw data are displayed in gray, and the smoothed data are shown in black (>5-s boxcar averaging sliding window). As expected, hExo1 did not move in the absence of Mg^{+2}, which is required for nuclease activity.

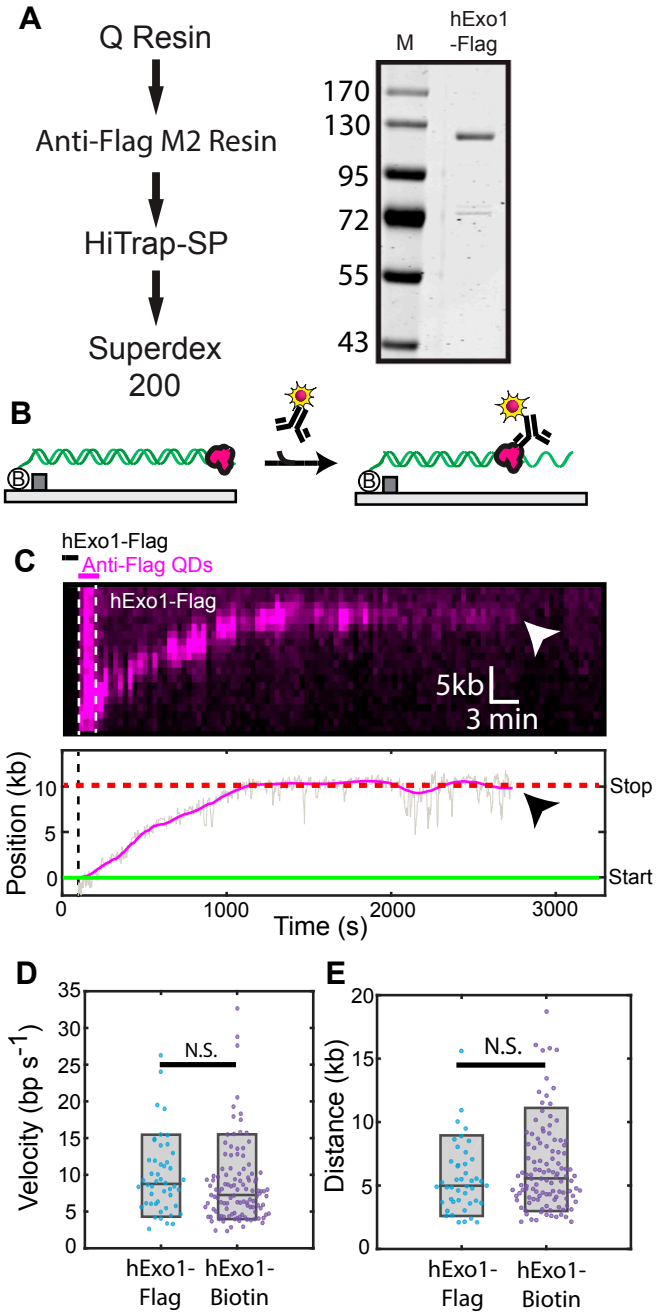

Fig. S3. Human Exo1-Flag (hExo1-Flag) resects DNA similarly to hExo1-bio. (*A*) Purification scheme and SDS/PAGE gel showing recombinant hExo1-Flag. (*B*) Cartoon illustration of the in situ hExo1-Flag labeling strategy. First, unlabeled hExo1-Flag is loaded on the DNA and excess protein is flushed out. Second, anti-Flag antibody-conjugated QDs are injected into the flowcell. This guarantees that hExo1 is labeled with, at most, one QD. (*C*) Kymograph (*Upper*) and single-particle trace of a resecting hExo1-Flag. Arrowheads indicate hExo1-Flag dissociation. The sequential hExo1-Flag and QD injection scheme is also shown in the kymograph. (*D*) Comparison of hExo1 velocity and (*E*) processivity. The mean hExo1-Flag velocity (9.8 ± 5.2 bp/s; $n = 50$) and processivity (5.6 ± 2.7 kb; $n = 50$) was statistically similar to hExo1-bio ($P = 0.18$ and $P = 0.15$, respectively). Within our experimental resolution, 78% ($n = 39/50$) of hExo1-Flag molecules paused at least once, with the remaining 22% ($n = 11/50$) resecting without pausing. Of those that paused, 44% ($n = 17/39$) paused before resection and 85% ($n = 33/39$) paused after resection.

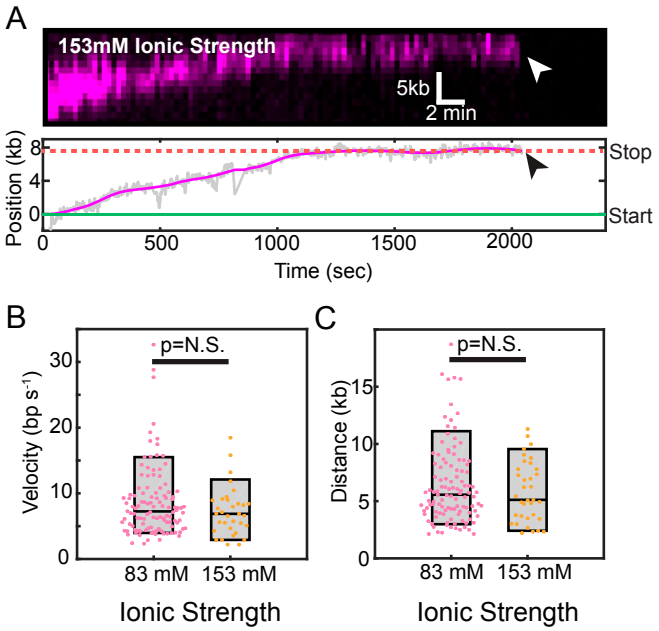

Fig. S4. hExo1 is a processive nuclease at a higher ionic strength. (*A*) Kymograph (*Upper*) and the corresponding particle-tracking trace (*Lower*) of a single hExo1-bio resecting a free DNA end in the presence of I = 153 mM total ionic strength (imaging buffer with 130 mM NaCl). hExo1 resects ~8 kb and pauses before dissociating from the DNA (arrowhead indicates dissociation). (*B*) Box plots of hExo1 velocities in a buffer with I = 83 mM (magenta, mean velocity = 8.6 ± 5.3 bp/s, $n = 113$) or with I = 153 mM (orange, mean velocity = 7.2 ± 3.7 bp/s, $n = 33$). (*C*) hExo1 is a processive nuclease at both low (I = 83 mM, magenta, mean processivity = 6.4 ± 3.4 kb, $n = 111$) and high (I = 153 mM, orange, processivity = 5.8 ± 2.6 kb, $n = 35$) total ionic strengths. The velocities and processivities from both nicks and ends are statistically indistinguishable ($P = 0.17$ for velocities, $P = 0.38$ for processivities). Box plots indicate the median, 10th, and 90th percentiles of the distribution.

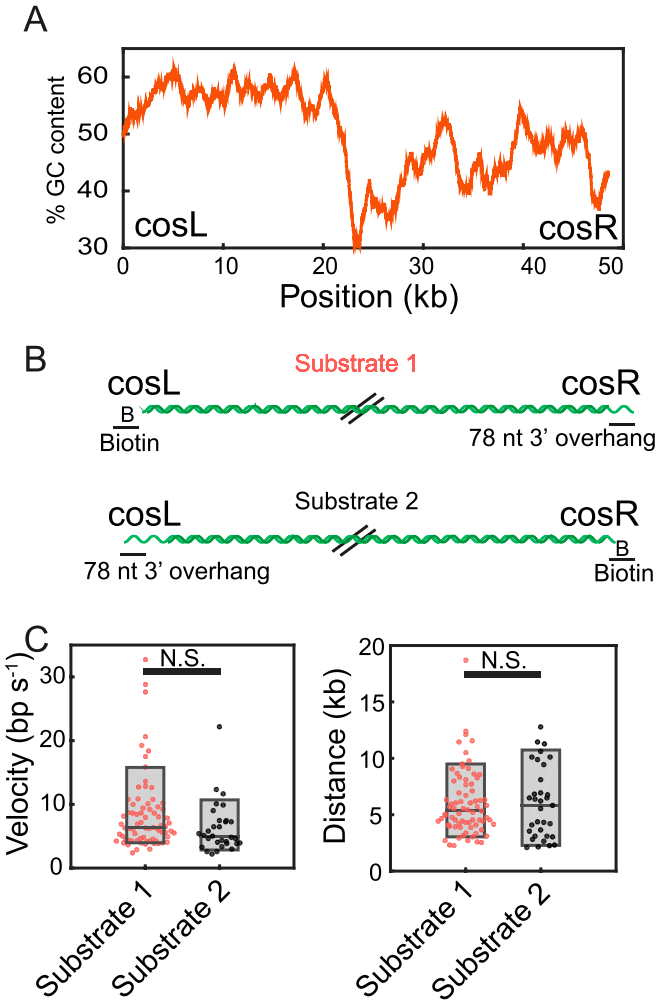

Fig. S5. hExo1 resection is sequence independent. (*A*) GC content of our DNA substrate (derived from λ-phage) measured using a 1-kb sliding window from cosL to cosR. The GC content is increased on the cosL side relative to the cosR side. (*B*) Model of substrates used for testing sequence-dependent hExo1 resection activity. Oligonucleotides were ligated to the ends of the DNA to biotinylate one end and create a 3′-ssDNA overhang on the other end. The two substrates load hExo1 on opposite ends of the λ-DNA. (*C*) Velocities (*Left*) and processivities (*Right*) of hExo1 molecules that started at the ends of substrates 1 (magenta) and substrate 2 (black). The boxplot represents the 10th, median, and 90th percentiles of each distribution. Substrate 1 is used throughout the manuscript, and its velocity and processivity are reported in Fig. 1. For substrate 2, the velocity of hExo1 was 6.3 ± 4 bp/s ($n = 31$) and the processivity was 6.0 ± 3.2 kb ($n = 33$). The velocities and processivity were not statistically different between the two substrates ($P = 0.07$ for velocity, $P = 0.98$ for processivity). Within our resolution, there are no major differences in hExo1 behavior on substrate 1 vs. substrate 2.

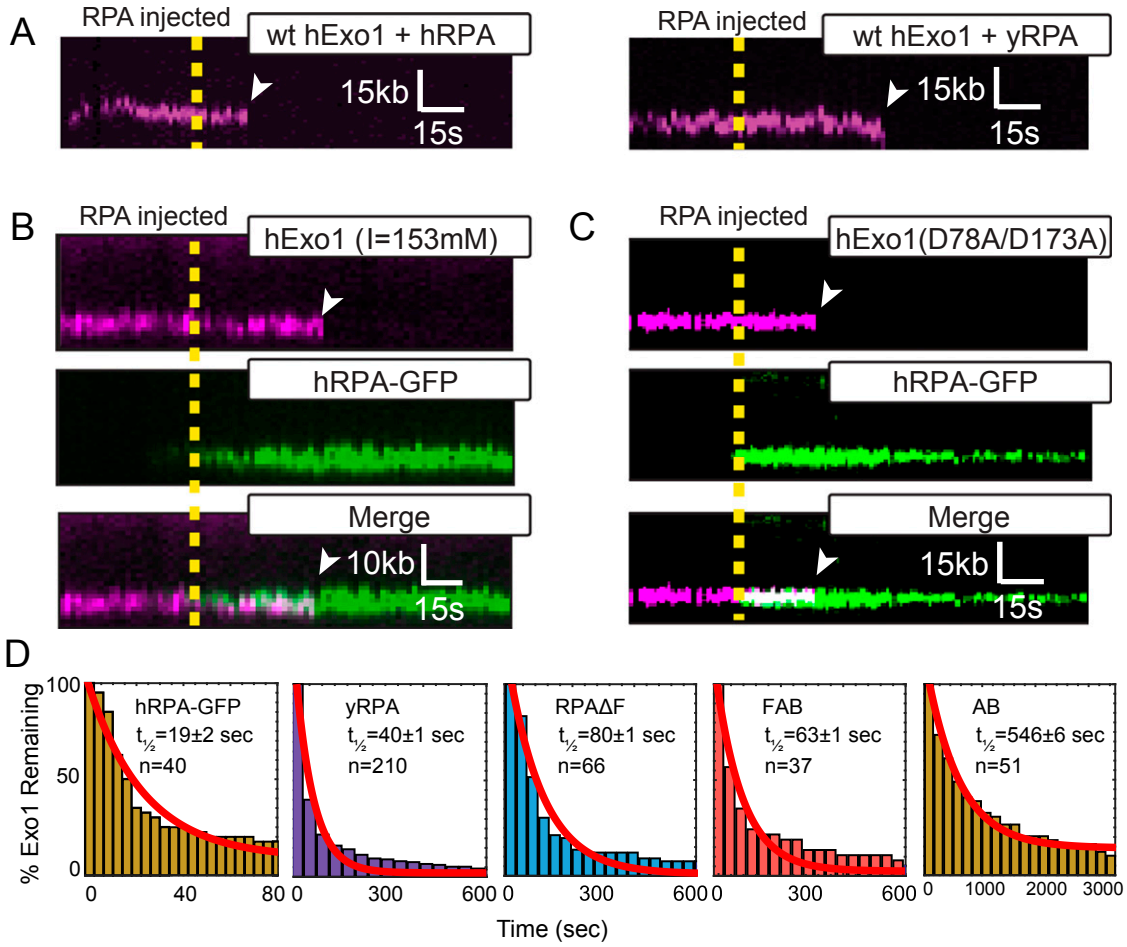

Fig. S6. hExo1 is rapidly removed by both human and yeast RPA. (*A*) Kymographs of hExo1-bio (magenta) when 1 nM hRPA (*Left*) or 1 nM yRPA (*Right*) is injected into the flowcell. In both kymographs, the WT RPAs are not labeled. The dashed line represents the time when RPA is injected and white arrowheads mark hExo1 dissociation events. Representative kymographs of (*B*) hExo1-bio at a high ionic strength (I = 153 mM) or (*C*) hExo1(D78A/D173A)-bio (magenta, *Top*) plus hRPA-GFP (green, *Middle*). (*Bottom*) Merged images. hRPA displaces WT and nuclease-dead hExo1. (*D*) Lifetime of hExo1 on DNA with various RPAs. Red line, fit to an exponential model. Table S1 summarizes hExo1 half-lives in the presence of various RPAs and prokaryotic SSBs.

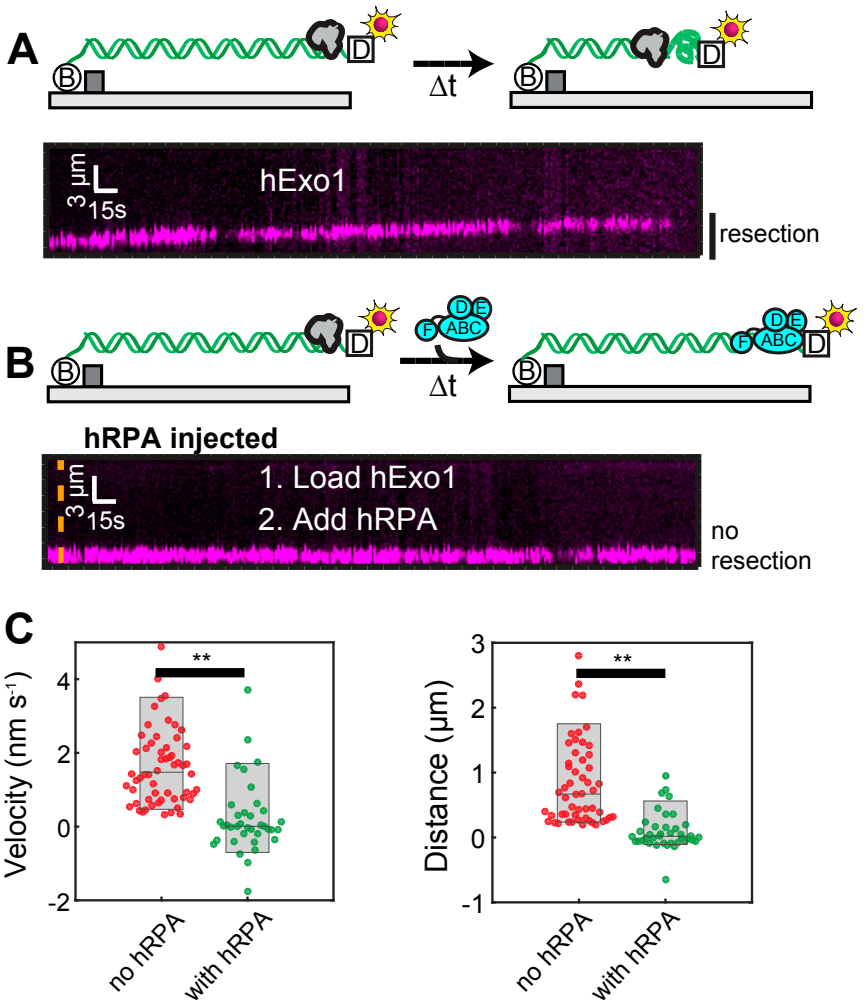

Fig. S7. Unlabeled hExo1 is inhibited by hRPA. (*A*) Cartoon illustration of the experiment (*Upper*). To monitor resection catalyzed by unlabeled hExo1, the DNA substrate was prepared with a 3′-72 nt polyT and was terminated with a digoxigenin (dig, white square in cartoon illustration). The 3′-ssDNA end was labeled with an anti-dig conjugated QD. hExo1-catalyzed resection converts dsDNA to ssDNA, which appears as an overall shortening of the DNA at these flow rates. Kymograph (*Lower*) hExo1-catalyzed resection on naked DNA. (*B*) Cartoon illustration of the experiment as above (*Upper*) after injection of 1 nM hRPA. After injection of hRPA, hExo1 is rapidly displaced by hRPA, and the QD does not move. Kymograph (*Lower*) shows unlabeled hExo1 resection after injection of 1nM RPA (orange line). (*C*) Velocity (*Left*) and processivity (*Right*) of the QD-labeled ssDNA in the absence (red) or with 1 nM RPA (green). The velocity of the QD was 1.9 ± 1.6 nm/s ($n = 60$) in the absence of RPA and 0.2 ± 1.6 nm/s ($n = 39$) with RPA. Likewise, the processivity was 0.9 ± 0.7 μm ($n = 54$) in the absence of RPA and 0.1 ± 0.3 μm ($n = 39$) in the presence of RPA. These were significantly different (**$P < 0.01$), indicating that RPA displaces unlabeled hExo1.

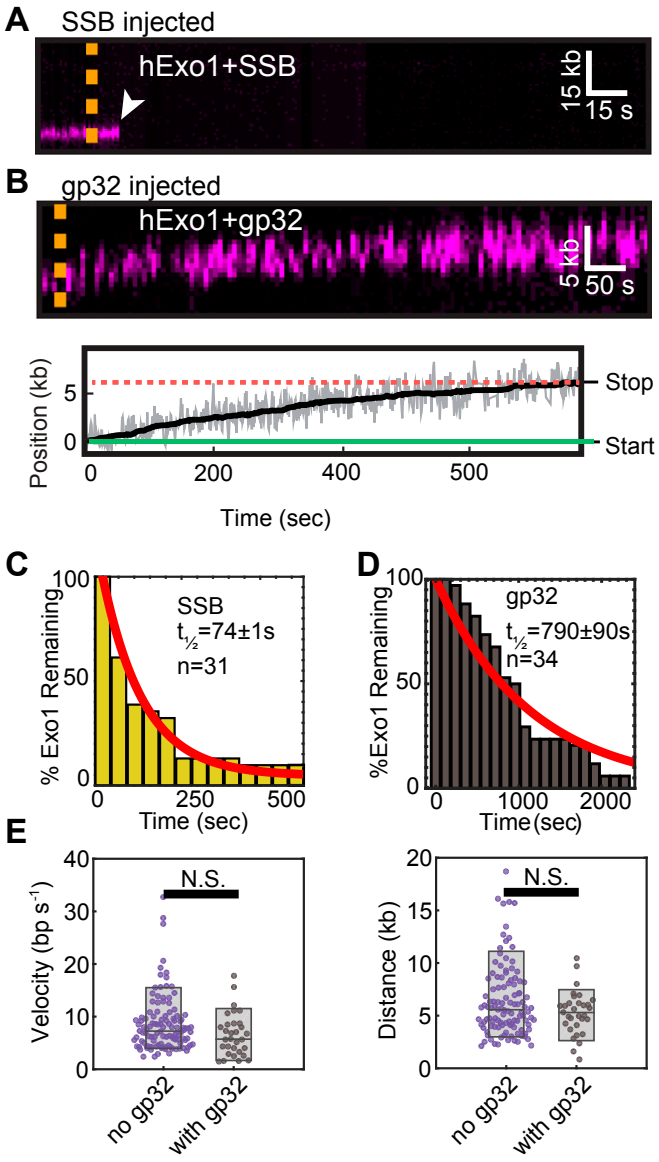

Fig. S8. Effects of SSB and gp32 on hExo1 resection. (*A*) Kymograph of hExo1 displacement by WT SSB. The dashed line indicates when SSB was injected and the white arrow indicates hExo1 dissociation. (*B*) Kymograph (*Upper*) and corresponding particle-tracking trace (*Lower*) of hExo1 on injection of gp32. Green and red lines indicate the start and stopping point of the molecule, respectively. (*C*) Lifetime of hExo1 in the presence of 1nM SSB (half-life = 74 ± 1.3 s, $n = 31$) and (*D*) 1 nM gp32 (half-life = 790 ± 90 s, $n = 34$). The red lines are single exponential fits of the data. Table S1 further summarizes the fit parameters. (*E*) Velocity (*Left*) and processivity (*Right*) of hExo1 in the absence (purple) or presence (gray) of gp32. The velocity of hExo1 in the presence of gp32 was 7.4 ± 3.8 bp/s ($n = 27$, $P = 0.06$), whereas the processivity was 5.5 ± 1.8 kb ($n = 29$, $P = 0.09$). These values were not statistically different from those of hExo1 in the absence of SSBs.

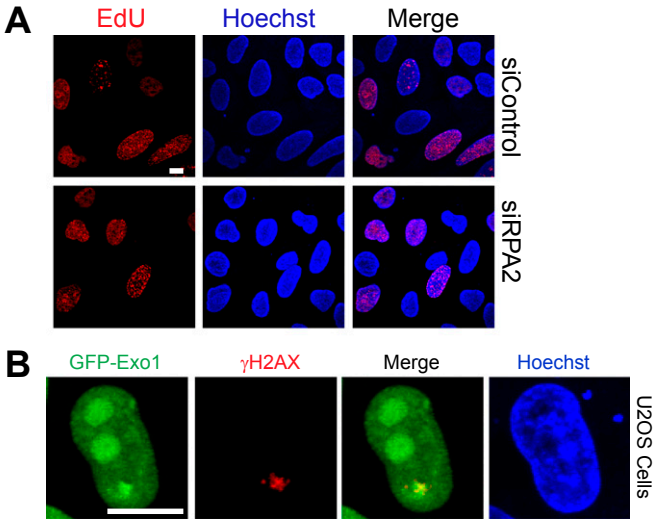

Fig. S9. Exo1-GFP colocalizes with DNA damage. (*A*) RPA2 knockdown does not affect the cell cycle. Representative images of EdU-stained DNA in siControl- and siRPA2-treated cells. Quantification of EdU foci for two biological replicates (>200 cells per replicate) is included in the main text (Fig. 3D). (Scale bar, 10 μm.) (*B*) Exo1-GFP colocalizes with the DNA damage marker γH2AX at sites of laser-induced DNA damage. (Scale bar, 10 μm.)

Table S1. hExo1 survival half-lives

Injection	hExo1 half-life (s)	Relative to hExo1 with hRPA	Coefficient of determination (R^2)
hRPA	18 ± 1	1	0.98
hRPA-GFP	19 ± 2	1	0.96
yRPA	40 ± 1	2	0.98
hRPAΔF	80 ± 1	4	0.97
FAB	63 ± 1	3	0.97
AB	546 ± 6	30	0.98
T4 gp32	790 ± 90	40	0.97
SSB	74 ± 1	4	0.98
SOSS1	>2,000	>100	ND
Mock	>2,000	>100	ND

ND, not determined.

Table S2. Oligonucleotides used in this study

Name	Sequence
LM3	[p]GGG CGG CGA CCT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
LM4	[p]TTA CTT GTC ATC GTC ATC CTT GTA ATC CTG GAA TAT TGC TCT TTG AAC ACG G
LM7	CAT ATG CAG ACT GCT GCA AAG C
LM6	[p]CTG AGC CTC GAA GAT GTC GTT CAG GCC CTG GAA TAT TGC TCT TTG AAC ACG
LM8	AAA ATC GAA TGG CAC GAA TAA CAT ATG CAG ACT GCT GCA AAG
LM24	[p]AGG TCG CCG CCC TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
NG1	[p]GG GCG GCG ACC TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT T[Dig]
TP2792	GGGATCAAGCCTATTCTCGTATTTGCTGGATGTACTTTACC
TP2793	GGTAAAGTACATCCAGCAAATACGAGAATAGGCTTGATCCC
TP3253	ATAATTACAGAGGACTCGgctCTCCTAGCTTTTGGCTGTAAAAAG
TP3254	CTTTTTACAGCCAAAAGCTAGGAGagcCGAGTCCTCTGTAATTAT
TP3802	AAUUCUCCGAACGUGUCACGUdTdT (includes complement)
TP3955	ATCGGGCCAATCTCAGAGG
TP3956	GCGACGCTAACGTTAAAGCA
TP4002	TGTCCTGTTCTTTTGTAGCGCA
TP4003	AAGCAAAAGCCTGAGGCTTAG
TP4028	CCGCCAGAAAGTTTCCTAGA
TP4029	CTCACCCTTGCAGCACTTG
TP4030	GGGTATGGAGCTGCCTCTAA
TP4031	GACAAAGATGGCTGGAGGAG
TP4115	ACCUAGUUUCACAAUCUGdTdT (includes complement)
TP4206	GGAAUGGAUAACCGGUACCdTdT (includes complement)
IF6	[p]GGG CGG CGA CCT[Bio]
IF7	[p]AGG TCG CCG CCC[Bio]
IF8	[p]AGG TCG CCG CCC[Dig]
IF9	[p]GGG CGG CGA CCT[Dig]
IF25	Aatcgcatatgtttaaacgtaaatctactgctgaa
IF26	AGGGCAACTAGTGCATCTCCCGTGATGCAGTAAAGGTCATTCAAAAGGTCATCCA
IF190	AGCGCAGAAAATTGAATGGCATGAATAACTCGAGGCATGCGGT
IF191	TCAAAAATATCGTTCAGGCCAAGCTTTTTACCTTTATAAACAAATTGAGAAAGCAAG

Bio, biotinylated; Dig, digoxigeninylated; p, phosphorylated.