Supporting Information

Nimonkar *et al.* 10.1073/pnas.0809380105

SI Materials and Methods

Enzymes and Reagents. EcoRI, HindIII, T4 polynucleotide kinase, and Klenow fragment (3′→5′ exo$^-$) were purchased from New England Biolabs. ATP was purchased from Sigma and dissolved as a concentrated stock at pH 7. The concentration of the stock solution was determined by using an extinction coefficient of 15,400 M^{-1} cm^{-1}. [α-^{32}P]dATP (3,000 Ci/mmol) and [γ-^{32}P]ATP (6,000 Ci/mmol) were purchased from NEN. Proteinase K was purchased from Roche.

DNA and Proteins. Plasmid DNA (pUC19) was purified with a protocol that used lysis at a neutral pH (1). Its concentration was determined by using an extinction coefficient of 6.6×10^3 M^{-1} cm^{-1}. Oligodeoxyribonucleotides X12-3 (5′-GACGTCATAGACGATTACATTGCTAGGACATGCTGTCTAGAGACTATCGC-3′) and X12-4 (5′-GCGATAGTCTCTAGACAGCATGTCCTAGCAAGCCAGAATTCGGCAGCGTC-3′) were synthesized by using Operon and purified by electrophoresis with a 20% denaturing polyacrylamide gel. Their concentrations were determined by using extinction coefficients of 4.84×10^3 and 4.83×10^3 M^{-1} cm^{-1}, respectively. Plasmid pJK1 (for overexpression of BLM) was a gift from Ian Hickson (Oxford University, U.K.). BLM protein was overexpressed and purified by using a 2-step chromatography procedure as described in ref. 2. The concentration of the purified protein was determined by using a Coomassie assay. The protein was free of contaminating ssDNA and dsDNA nucleases as determined by the resistance of 5′ end-labeled ssDNA and dsDNA fragments to resection. hExo1a, hExo1b, and hExo1b(D173A) were purified as described (3, 4). Two preparations of Exo1b were used in this study and are termed preparations A and B (the latter being ≈3-fold more active); unless otherwise indicated, experiments were conducted by using hExo1 preparation A. WRN helicase was a gift from David Chen (University of Texas Southwestern, Dallas, TX). Human RecQ1 and hRecQ5 were generous gifts from Ian Hickson. Human Q4 was a gift from Patrick Sung (Yale University, New Haven, CT). *Escherichia coli* RecQ (5) (Behzad Rad, University of California, Davis), hRad51 (6), hRPA (A. Carreira, University of California, Davis), *Saccharomyces cerevisiae* Rad51 (7), and *E. coli* RecA (8) were purified according to published protocols. λ exonuclease was from USB Corp.

Substrate for Nuclease Assays. EcoRI-linearized pUC19 (2.7 kbp) was ^{32}P-labeled at the 3′ ends by using [α-^{32}P]dATP and Klenow fragment according to the vendor's protocol. The unincorporated [α-^{32}P]dATP was separated from the labeled product by using a G-25 spin column (GE Healthcare). The concentration of the labeled product was determined by assuming ≈95% recovery from G-25 spin column. DNA concentration of the substrate is expressed in molar ends as well as nucleotides (nt). Radiolabeled size marker was prepared by digesting λ-DNA with HindIII. Fragments were ^{32}P-labeled at the 3′ ends by using [α-^{32}P]dATP and Klenow fragment according to the vendor's protocol. The unincorporated [α-^{32}P]dATP was separated from the labeled product by using a G-25 spin column.

Substrates for Helicase Assays. A forked substrate (50 nt long, with 19 unpaired bases and 31 paired bases) was generated by annealing oligodeoxyribonucleotides X12-3 and X12-4, which are partially complementary. X12-3 (^{32}P-labeled at the 5′ end by using T4 polynucleotide kinase) was mixed with cold X12-4 in a molar ratio of 1:3 in a buffer containing 10 mM Tris, 1 mM EDTA, 100 mM NaCl. The reaction mixture (50 μl) was heated to 95 °C for 5 min and gradually cooled (5–6 h) to room temperature to allow the complementary strands to anneal. DNA concentration of forked substrate is expressed as moles of nucleotide.

Helicase Assays. Helicases (100 nM) were incubated with forked substrate (10 μM nt) in a buffer containing 20 mM Na-Hepes (pH 7.5), 1 mM ATP, 6 mM $MgCl_2$, 0.1 mM DTT, 100 μg/ml BSA, and 0.05% Triton X-100. Reactions were incubated at 37 °C for 30 min, stopped by the addition of termination buffer (final concentration: 7 μg/μL proteinase K, 50 mM EDTA, 2% SDS), and further incubated at 37 °C for 30 min. The reaction products were resolved by electrophoresis in a 6% polyacrylamide gel using TAE [40 mM Tris-acetate (pH 8.0), 1 mM EDTA] buffer at 4.5 V/cm for 2 h. After electrophoresis, the gels were dried on DE81 paper (Whatman), visualized, and quantified with a Molecular Dynamics Storm 860 with ImageQuant version 5.2.

1. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY), 2nd Ed.
2. Karow JK, Chakraverty RK, Hickson ID (1997) The Bloom's syndrome gene product is a 3′–5′ DNA helicase. *J Biol Chem* 272:30611–30614.
3. Genschel J, Bazemore LR, Modrich P (2002) Human exonuclease I is required for 5′ and 3′ mismatch repair. *J Biol Chem* 277:13302–13311.
4. Dzantiev L, *et al.* (2004) A defined human system that supports bidirectional mismatch-provoked excision. *Mol Cell* 15:31–41.
5. Harmon FG, Kowalczykowski SC (1998) RecQ helicase, in concert with RecA and SSB proteins, initiates and disrupts DNA recombination. *Genes Dev* 12:1134–1144.
6. Sigurdsson S, Trujillo K, Song B, Stratton S, Sung P (2001) Basis for avid homologous DNA strand exchange by human Rad51 and RPA. *J Biol Chem* 276:8798–8806.
7. Zaitseva EM, Zaitsev EN, Kowalczykowski SC (1999) The DNA binding properties of *Saccharomyces cerevisiae* Rad51 protein. *J Biol Chem* 274:2907–2915.
8. Mirshad JK, Kowalczykowski SC (2003) Biochemical characterization of a mutant RecA protein altered in DNA-binding loop 1. *Biochemistry* 42:5945–5954.

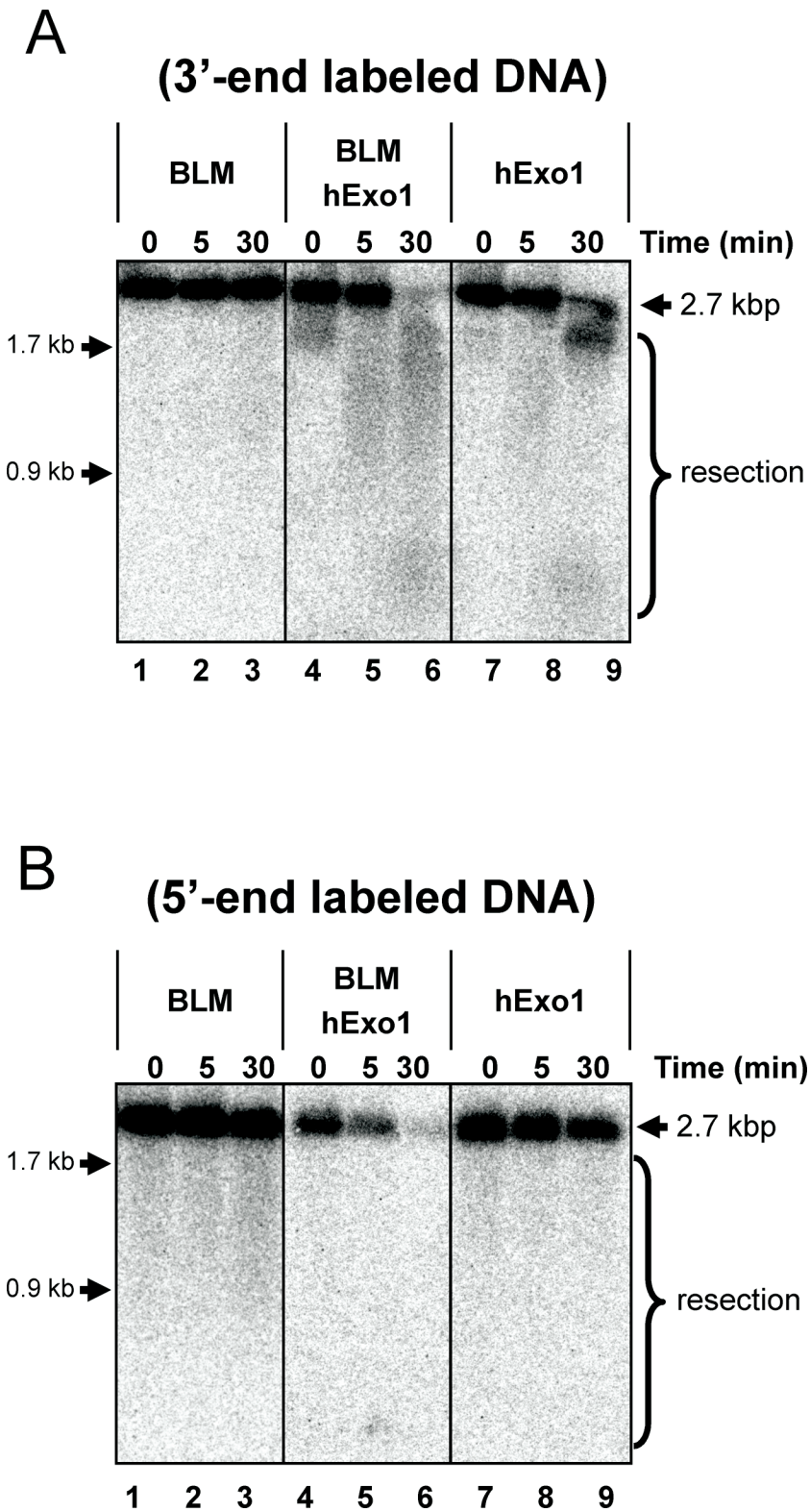

Fig. S1. Analysis of DNA resection by hExo1 using alkaline agarose-gel electrophoresis. Nuclease reactions were performed as described in *Materials and Methods*, except that products were analyzed by electrophoresis using a 1% alkaline agarose gel at 4.5 V/cm for 12 h. (*A*) DNA substrate labeled at the 3′ end and (*B*) substrate labeled at the 5′ end. Shown are time courses with BLM alone (lanes 1–3), hExo1 and BLM (lanes 4–6), and hExo1 alone (lanes 7–9). The positions of the intact substrate (2.7 kbp), resection products, and molecular size standards (5′ end-labeled PCR fragments) are indicated.

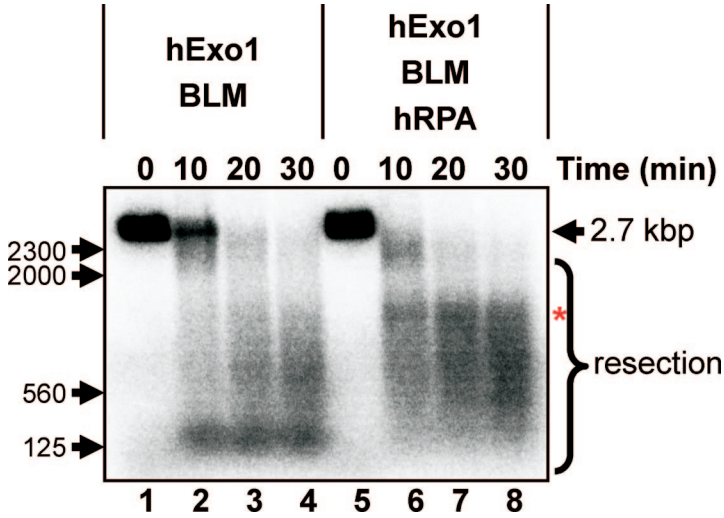

Fig. S2. RPA lowers the extent of BLM-stimulated resection of DNA by hExo1. Nuclease reactions were performed as described in *Materials and Methods*, except that the hExo1 and BLM concentrations were 40 nM and 80 nM, respectively, in the absence or presence of added hRPA (200 nM). Image shows reaction products: time course with hExo1 and BLM (lanes 1–4) and with hExo1, BLM and hRPA (lanes 5–8). The positions of the intact substrate (2.7 kbp), resection products, and molecular size standards are indicated. The asterisk marks position of higher molecular mass resection intermediates seen in the presence of RPA.

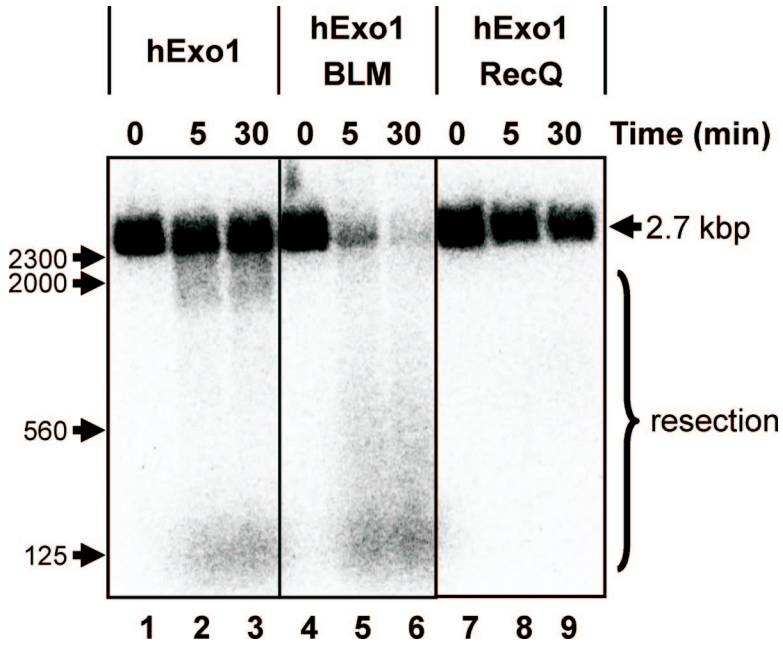

Fig. S3. Human Exo1 is not stimulated by *E. coli* RecQ. Nuclease reactions were performed as described in *Materials and Methods*, except BLM was replaced with an equimolar amount of *E. coli* RecQ. Image shows reactions products: time course with hExo1 alone (lanes 1–3), hExo1 and BLM (lanes 4–6), and hExo1 and *E. coli* RecQ (lanes 7–9). The positions of the intact substrate (2.7 kbp), resection products, and molecular size standards are indicated.

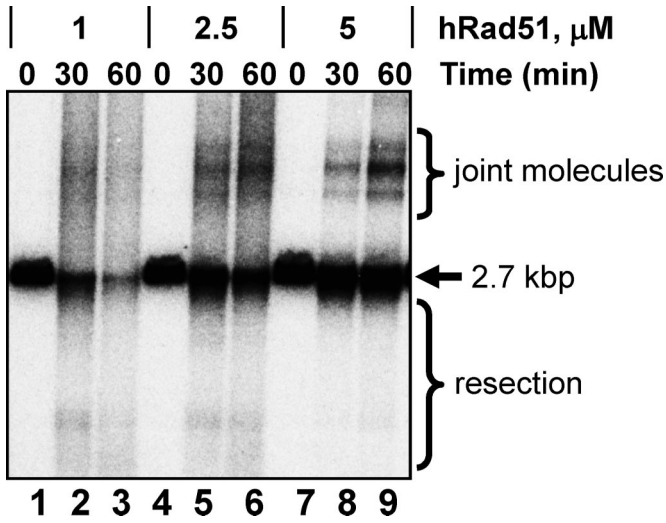

Fig. S4. The rate and extent of joint molecule formation are a function of hRad51 concentration. Joint molecule reactions were performed as described in *Materials and Methods*. Image shows reaction products: time course with 1.0 μM hRad51 (lanes 1–3), 2.5 μM hRad51 (lanes 4–6), and 5.0 μM hRad51 (lanes 7–9). The positions of intact DNA (2.7 kbp), resection products, and joint molecules are indicated.

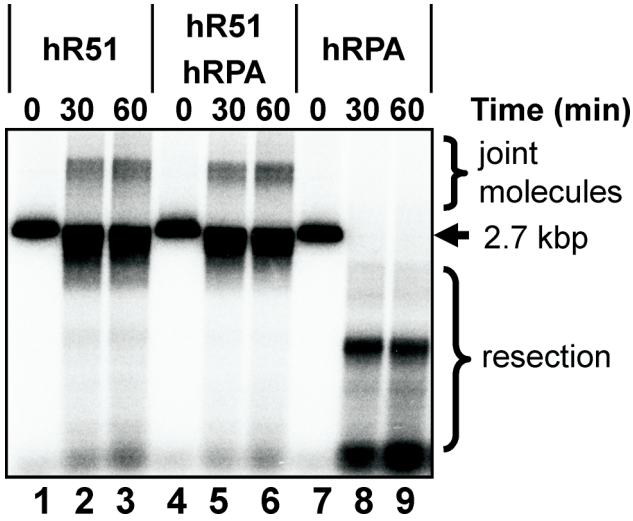

Fig. S5. RPA negligibly enhances hRad51-mediated joint molecule formation. Joint molecule reactions were performed as described in *Materials and Methods*. Image shows reaction products: time course with hRad51 alone (lanes 1–3), hRad51 and hRPA (lanes 4–6), and hRPA alone (lanes 7–9). The positions of intact DNA (2.7 kbp), resection products, and joint molecules are indicated.

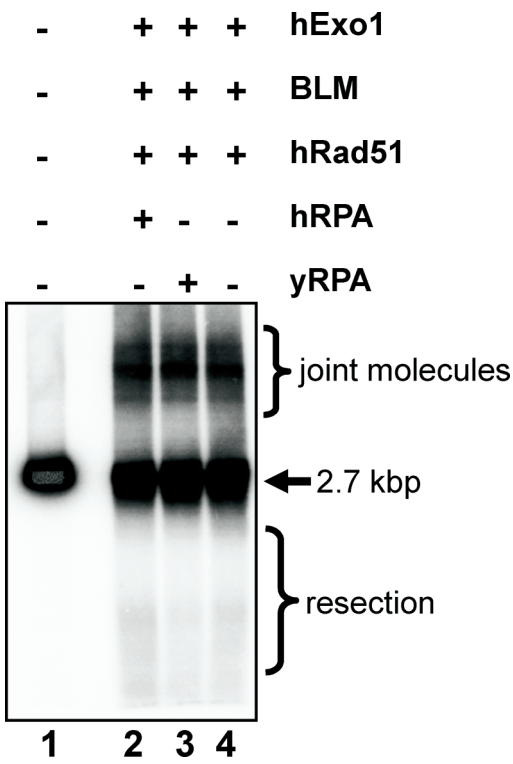

Fig. S6. hRad51 can mediate joint molecule formation in the presence of a heterologous RPA. Joint molecule reactions were performed as described in *Materials and Methods*. Lane 1, control, substrate incubated in the absence of any protein; lanes 2–5, pairing reactions in the presence of hRPA, yRPA, and no RPA, respectively. The positions of intact DNA (2.7 kbp), resection products, and joint molecules are indicated.