Thangalev et al., http://www.jcb.org/cgi/content/full/jcb.201406100/DC1

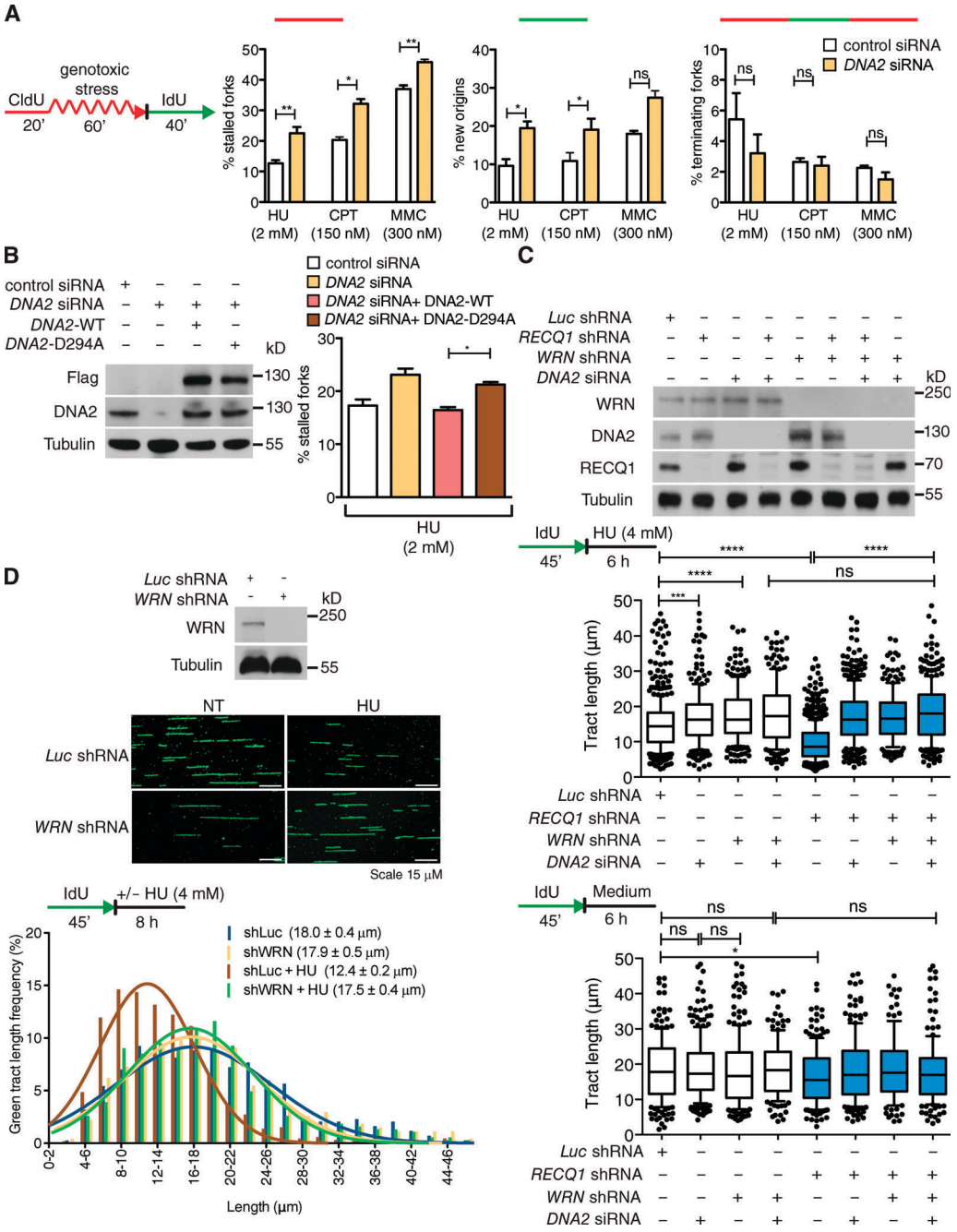

Figure S1. **DNA2 and WRN function in stalled fork processing.** (A, left) Schematic of DNA fiber tract analysis. (right) Quantification of red tracts (stalled forks), green tracts (new origins), and contiguous red–green–red tracts (termination events). Proper quantification of stalled forks is complicated by the fact that termination events might also lead to red tracts if termination occurs before the addition of the second label. Mean shown, $n = 3$. Error bars, standard error. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (paired Student's *t* test). (B, left) Expression of Flag-tagged WT (DNA2-WT) or nuclease-dead (DNA2-D294A) DNA2 in DNA2-depleted U-2 OS cells. (right) Quantification of stalled forks in DNA2-depleted cells expressing DNA-WT or DNA2-D294A. Mean shown, $n = 3$. Error bars, standard error. ns, not significant; *, $P < 0.05$ (paired *t* test). (C, top) Expression of RECQ1, WRN, DNA2, and tubulin in U-2 OS cells transfected with the indicated shRNA or siRNA. (middle) Statistical analysis of IdU tracts from U-2 OS cells depleted for the indicated proteins in the presence of 4 mM HU. (bottom) Statistical analysis of IdU tracts from U-2 OS cells depleted for the indicated proteins in the absence of drug treatment. Whiskers indicate the 10th and 90th percentiles. ns, not significant (Mann-Whitney test). $n \geq 300$ scored for each dataset. (D, top) Expression of WRN after WRN knockdown and representative fiber tract images in Luc- and WRN-depleted U-2 OS cells. Bar, 15 µm. Representative IdU tracts in WRN-depleted U-2 OS cells in the presence or absence of HU (out of 2 repeats; $n \geq 700$ scored for each dataset).

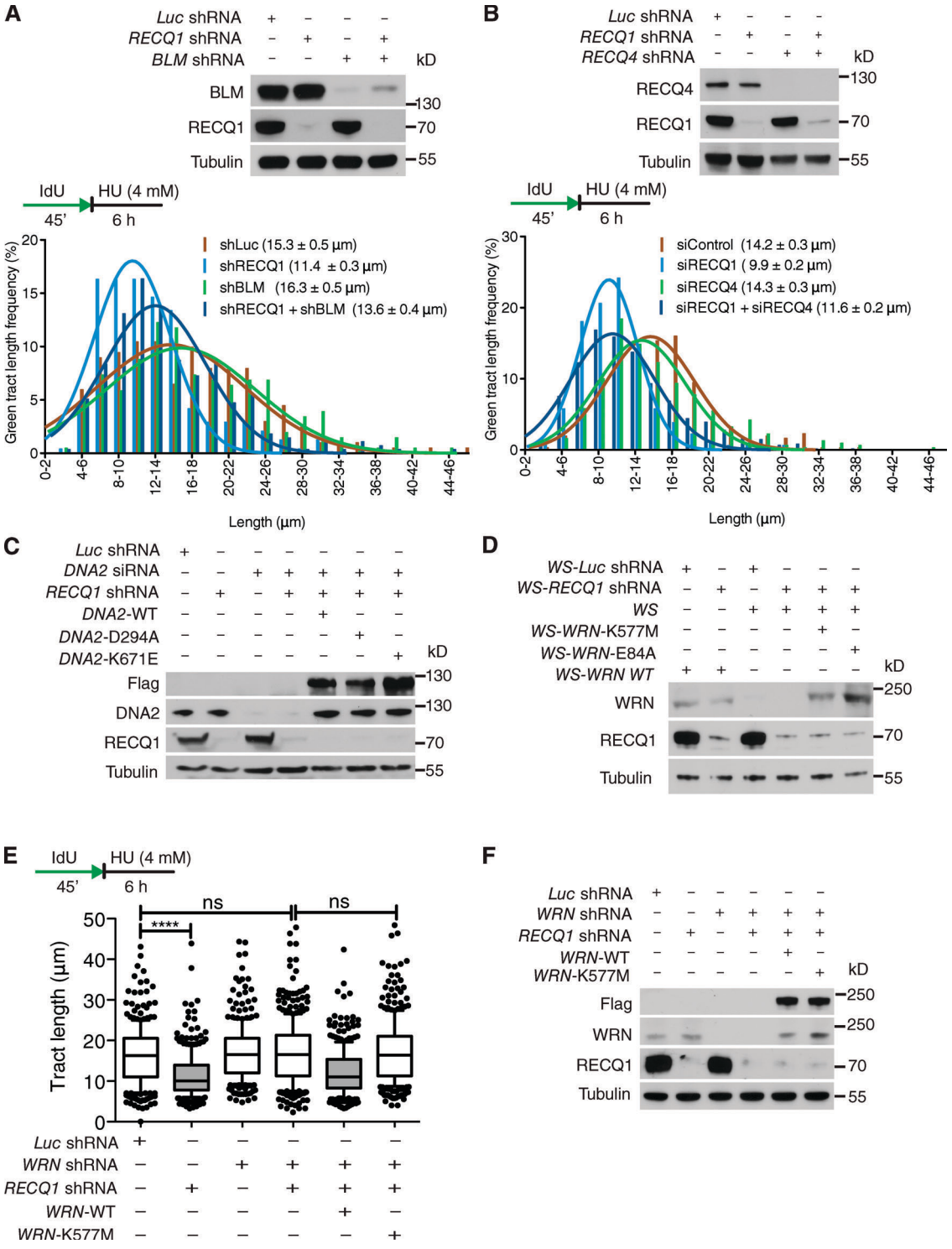

Figure S2. **BLM or RECQ4 depletion does not have a significant effect on stalled fork processing.** (A) Representative IdU tracts in Luc-, RECQ1-, BLM-, or RECQ1/BLM-codepleted U-2 OS cells in the presence of HU (out of 2 repeats; $n \geq 300$ scored for each dataset). (top) Expression of RECQ1, BLM and tubulin in U-2 OS cells transfected with indicated shRNA. (B) Representative IdU tracts in control, RECQ1-, RECQ4-, or RECQ1/RECQ4-codepleted U-2 OS cells in the presence of HU (out of 2 repeats; $n \geq 350$ scored for each dataset). (top) Expression of RECQ1, RECQ4 and tubulin in U-2 OS cells transfected with indicated shRNA. (C) Expression of RECQ1, DNA2-WT, DNA2-K671E, and DNA2-D294A in U-2 OS cells transfected with the indicated shRNA or siRNA. (D) Expression of RECQ1, WRN-WT, WRN-K577M, and WRN-E84A in WS cells. (E) Statistical analysis of IdU tracts from RECQ1-, WRN-, or RECQ1/WRN-codepleted U-2 OS cells. The RECQ1/WRN-codepleted cells were complemented with WT or ATPase-deficient (K577M) WRN, where indicated. Whiskers indicate the 10th and 90th percentiles. ns, not significant; ****, $P < 0.0001$ (Mann-Whitney test). (F) Expression of RECQ1, WRN-WT, and WRN-K577M in U-2 OS cells transfected with the indicated shRNA.

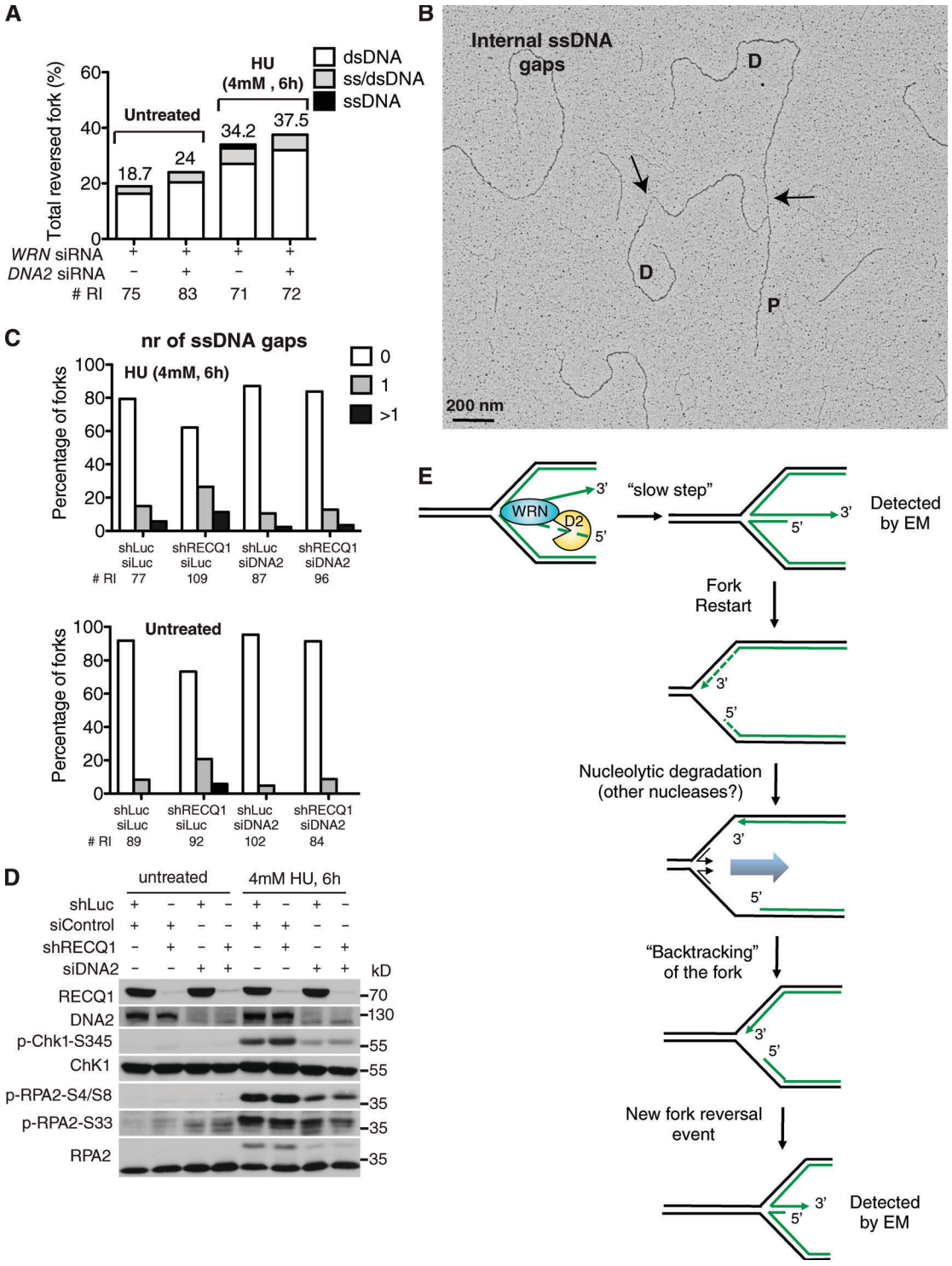

Figure S3. **DNA2 promotes ssDNA gap accumulation on replicated duplexes and the ATR-mediated checkpoint activation.** (A) Frequency of fork reversal and ssDNA composition of the reversed arms in WRN- and/or DNA2-depleted U-2 OS cells in the presence and absence of HU. The percentage values are indicated on the top of the bar. "# RI" indicates the number of analyzed replication intermediates. (B) Representative electron micrographs of replication forks displaying ssDNA gaps on the replicated duplexes or at the replication fork junction observed on genomic DNA in shRECQ1 U-2 OS cells upon HU-treatment. The black arrows point to ssDNA gaps. D, Daughter strand; P, Parental strand. (C) Statistical distribution of ssDNA gaps on newly replicated duplexes in RECQ1- and/or DNA2-depleted U-2 OS cells treated with HU (top) or in unperturbed conditions (bottom). "# RI" is the number of analyzed replication intermediates. (D) Western blot analysis of ATR-checkpoint activation (pChk1 and pRPA) in RECQ1- and/or DNA2-depleted U-2 OS cells with or without HU treatment. Total Chk1 and RPA level are displayed and used as loading control. (E) Schematic of the different structures detected by EM and DNA fibers. EM is a static method, which enriches for snapshots of the "slow steps" of a reaction (i.e., partially resected reversed forks). After fork restart, the nucleolytic degradation quickly proceeds to degrade nascent strands behind the junction. Reannealing of the parental strands leads to "backtracking" of the fork. A new reversal event arises as a consequence of asymmetric degradation, and thus ssDNA accumulation in proximity to the fork. Backtracking is easily detected by DNA fiber, but not by EM because a reversed fork formed after degradation and backtracking is indistinguishable from the original reversed fork present before initial degradation.

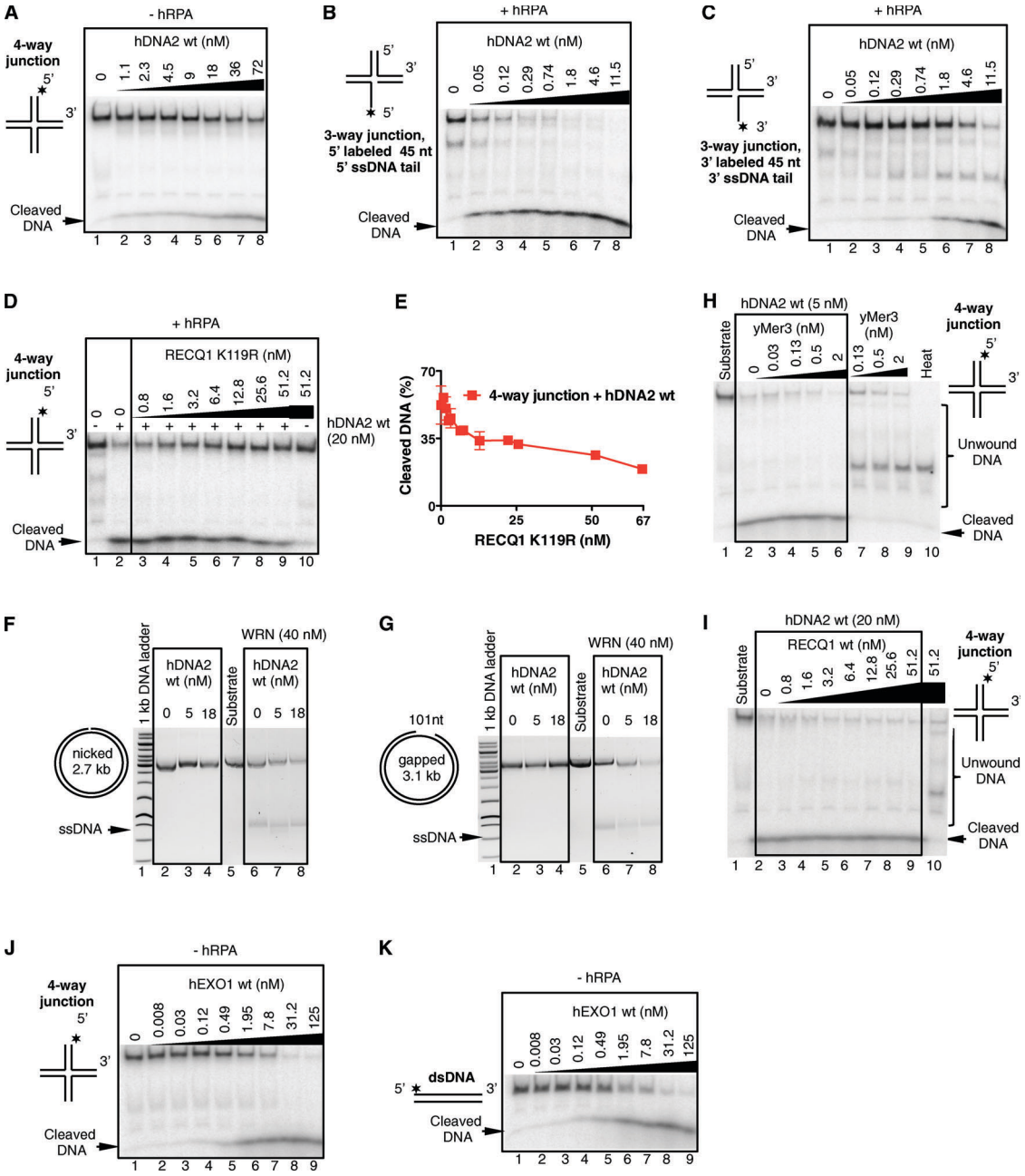

Figure S4. **Human DNA2 but not EXO1 preferentially degrades branched DNA.** (A) Degradation of a four-way junction by hDNA2 without human RPA (hRPA). Reaction products were separated on a native polyacrylamide gel (6%); *, position of the ^{32}P label. (B) Degradation of a three-way junction with a 5′ ssDNA tail by hDNA2 in the presence of hRPA (22.3 nM). Reaction products were separated on a native polyacrylamide gel (6%); *, position of the ^{32}P label. (C) Same experiment as in B, but with a junction containing a 3′ ssDNA tail. (D) RECQ1 K119R (ATPase-dead) inhibits four-way junction degradation by hDNA2. Increasing concentrations of RECQ1 (K119R) were preincubated with the substrate, and then hDNA2 (20 nM) was added to the reaction mixture. All reactions contained hRPA (65 nM). Reaction products were separated on a native polyacrylamide gel (6%); *, position of the ^{32}P label. (E) Quantitation of data from D. Averages shown ± SEM, $n = 2$. (F) Synergistic action of hDNA2 and WRN on a nicked plasmid based DNA substrate. The reactions contained 614 nM hRPA and were incubated at 37°C for 60 min. Products were separated on a 1% agarose gel and stained with GelRed. WRN helicase promotes degradation of nicked DNA by hDNA2. (G) Same experiment as in F, but with a gapped DNA substrate. (H) Degradation of a four-way junction by hDNA2 and *S. cerevisiae* Mer3. hDNA2 only degrades ssDNA unwound by Mer3, no synergy in DNA degradation was observed. All reactions contained hRPA, and were analyzed on a native polyacrylamide gel (6%). (I) Degradation of four-way junction by hDNA2 is not stimulated by WT hRECQ1. All reactions contained hRPA (65 nM). Reaction products were separated on a native polyacrylamide gel (6%). (J) Degradation of a four-way junction by hEXO1. Reaction products were separated on a native polyacrylamide gel (6%); *, position of the ^{32}P label. (K) Same experiment as in J, but with dsDNA.

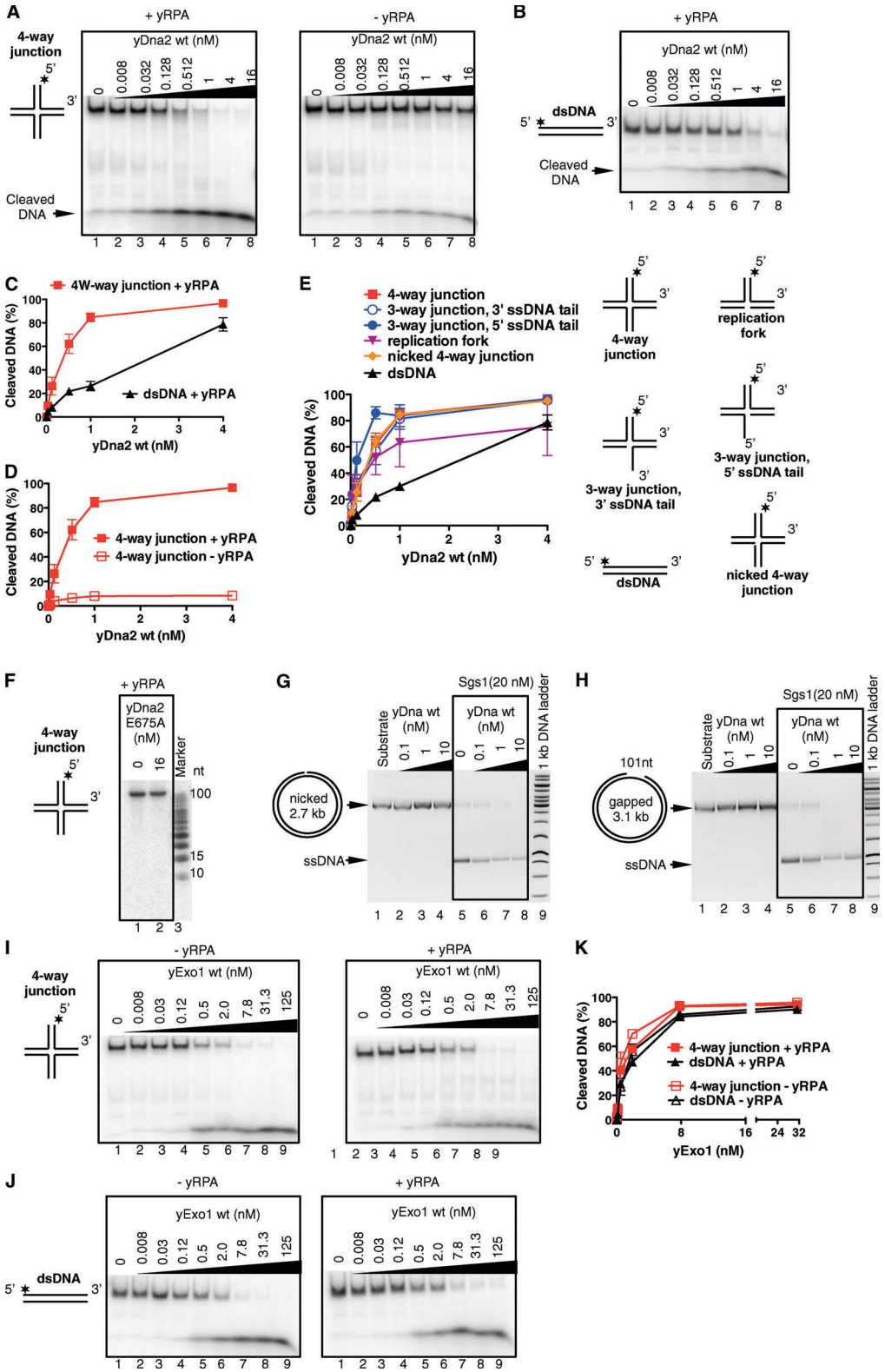

Figure S5. **Yeast Dna2 but not Exo1 preferentially degrades branched DNA.** (A) Degradation of a four-way junction by yDna2 in presence (left) or absence (right) of yeast RPA (yRPA). Reactions were separated on a native polyacrylamide gel (6%), *, position of the ^{32}P label. (B) Experiment as in A, but with dsDNA and yRPA. (C) Yeast Dna2 preferentially degrades four-way junctions in the presence of yRPA. Quantitation of data from A and B. Averages shown ± SEM; *n* = 2. (D) yRPA promotes DNA degradation by yDna2. Quantitation of data from A. The data points representing the degradation of a four-way junction in the presence of yRPA are identical to those from C. Mean shown ± SEM, *n* = 2. (E) Yeast Dna2 preferentially degrades branched structures over dsDNA. Quantitation of degradation of various DNA substrates as indicated (cartoons on the right) by yDna2 WT in presence of yRPA. Averages shown ± SEM; *n* = 2. (F) Denaturing 20% polyacrylamide gel showing that nuclease-dead yDna2 E675A variant does not degrade the four-way junction substrate. (G) Synergistic action of yDna2 and Sgs1 helicase on a nicked dsDNA plasmid based substrates. The reactions contained 770 nM yRPA and were incubated at 30°C for 60 min before being separated on a 1% agarose gel containing GelRed. (H) Experiment as in G, but with gapped DNA substrate. (I) Degradation of a four-way junction by yExo1 in the presence (right) or absence (left) of yRPA. (J) Same experiment as in I, but with dsDNA. (K) Quantitation of data from I and J. Averages shown ± SEM, *n* = 2.