SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Walker A box mutant WRN-K577M lacks the ability to convert the specialized Holliday junction to a replication fork. Holliday junction substrate (~2 fmol) was incubated without (lane 1) and with ATPase- and helicase-deficient WRN-K577M protein (lanes 2-4, 6, 15, and 30 fmol) at 37°C for 30 min in WRN reaction buffer. Aliquots of these reactions were analyzed by both native PAGE (*top panel*) and denaturing PAGE (*bottom panel*) and the labeled DNA products visualized by phosphorimaging. Markers on native PAGE were *LeadD81/LagD84 (lane 5) and replication fork product = *LeadD81/LeadP122-LagD84/LagP122 (lane 6). Unlike WRN-E84A and wild type WRN proteins, WRN-K577M does not catalyze conversion of our model Holliday junction substrate to 4-stranded replication fork (*top panel*). However, WRN-K577M retains its inherent 3' to 5' exonuclease activity on this substrate (*bottom panel*).

Supplemental Figure 2. Effect of Mg^{+2} concentration on WRN-mediated unwinding of a 3' overhang partial duplex. A 3' overhang partial duplex substrate (*LagP70/LagD30, 0.25 nM) was incubated for 10 min at 37°C without (lane 1) or with 0.6 nM WRN-E84A (lanes 2-7) in reactions containing 250 μM ATP and the indicated concentrations of $MgCl_2$. DNA products were separated by native PAGE (*top*), then visualized and quantified by phosphorimaging. The percent of WRN-mediated duplex unwinding (with respect to total DNA/lane) for 2 independent experiments is plotted versus $MgCl_2$ concentration (*bottom*).

Supplemental Figure 3. Effect of Mg^{+2} concentration on BLM-mediated conversion of Holliday junction to four-stranded replication fork. A) Holliday junction substrate (~2 fmol) was incubated for 30 min at 37°C without or with BLM (12 fmol) in the presence of 1 mM ATP and the indicated concentration of $MgCl_2$. DNA products of these reactions (lanes 1-10) were separated by native PAGE along with markers (lane 11) for the replication fork, *LeadD81/LeadP122, *LeadD81/LagD84, and *LeadD81 substrates (*top panel*) and the replication fork product quantitated (*bottom panel*) as described in Experimental Methods. **B)** Holliday junction substrate (~2 fmol) was incubated for 30 min at 37°C without or with human RAD54 (10, 40, 100, 400 and 1000 fmol) in the presence of 1 mM ATP and either 1 or 4 mM $MgCl_2$ as indicated. DNA products of these reactions (lanes 1-11) and markers were separated as in *A*.

Supplemental Figure 4. Effect of RPA on conversion of Holliday junction to four-stranded replication fork by WRN-E84A. Holliday junction substrate (~2 fmol) was incubated for 30 min at 37°C in buffer containing 1 mM $MgCl_2$ and 1 mM ATP without (lanes 1, 9-11) or with (lanes 2-8) **(A)**WRN-E84A (24 fmol) or **(B)** BLM (24 fmol) in the absence (lanes 1-2) or presence (lanes 3-11) of human RPA at the indicated concentrations. DNA products were separated by native PAGE, along with markers (lane 12) for the four-stranded replication fork, *LeadD81/LeadP122, *LeadD81/LagD84, and single-stranded *LeadD81 and visualized by phosphorimaging.

Supplemental Figure 5. Human DNA pol δ dectectably extends 4-stranded replication forks converted from Holliday junctions by BLM. Holliday junction substrate (~2 fmol, lanes 1-8) was incubated with BLM (15-60 fmol) in the presence of ATP (1 mM) and dNTPs (100 μM) for

25 min at 37°C, followed by the addition of human DNA pol δ (30 fmol) where indicated and further incubation at 37°C for 5 min. In control reactions, 4-stranded fork (2 fmol, lanes 9 and 10), *LeadD81/LeadP122 (2 fmol, lanes 11 and 12) and *LeadD81/LagD84 (lanes 13 and 14) substrates were also incubated without or with pol δ (30 fmol) at 37°C for 5 min in the presence of dNTPs (100 μM) and ATP (1 mM). Products were analyzed by denaturing PAGE and visualized by phosphorimaging (top panel). The bottom panel is an exposure of the same gel optimized to show the small amounts of the fully extended 122-123 nt product (indicated by red arrows) only in the specific Holliday junction-containing reactions incubated with BLM followed by pol δ.