Supplemental Table 1. Oligonucleotides Used in This Study.

G77telo
A*GCTGAGCATGTCCAGACATGTCCTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG

G77scr
A*GCTGAGCATGTCCAGACATGTCCGTGAGTGTGAGTGTGAGTGTGAGTGTGAGTGTGAGTGTGAGTGTGAG

Biotinylated-G68telo
b-A*GCTGAGCATGTCCAGACATGTCCTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTA

C38telo
T*AACCCTAACCCTAGGACATGTCTGGACATGCTCAGCT

C38scr
A*CACTCATACTCACGGACATGTCTGGACATGCTCAGCT

C50telo
T*AACCCTAACCCTAACCCTAACCCTAGGACATGTCTGGACATGCTCAGCT

C50scr
A*CACTCACACTCACACTCATACTCACGGACATGTCTGGACATGCTCAGCT

C71telo
C*CCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAGGACATGTCTGGACATGCTCAGCT

C77telo
C*CCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAGGACATGTCTGGACATGCTCAGCT

C77scr
C*TCACACTCACACTCACACTCACACTCACACTCACACTCACACTCATACTCACGGACATGTCTGGACATGCTCAGCT

TOLA-G
TTAGGGTTAGGGTTAGGGTTAGGG

TOLA-C
CCCTAACCCTAACCCTAACCCTAA

All oligos are depicted in 5’ to 3’ orientation. * = phosphorothioate bond; “b” = 5’ linked biotin moiety.

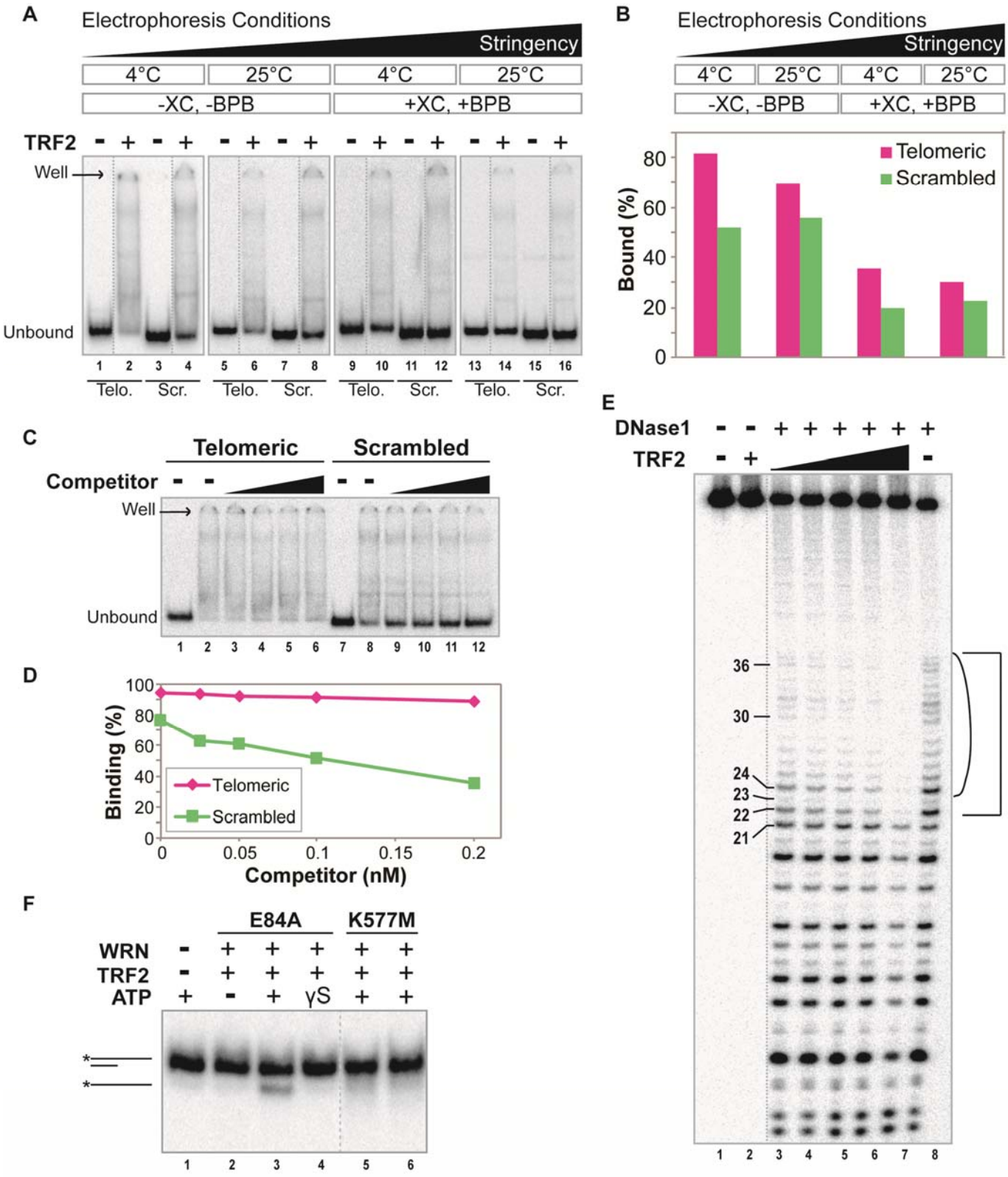
Supplemental Methods

DNase 1 footprinting. Telomeric overhang substrate (*G77/C38, 0.5 nM) was first incubated without or withTRF2 (5.8-29 nM) for 5 min at 4°C in the presence of 10 ng of salmon sperm DNA, followed by incubation with DNase 1 (0.02 U) for 2 min at 37°C. Reactions were terminated with equal volumes of formamide dyes (95% formamide, 20 mM EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanol). Subsequently, DNA products were heat-denatured at 90°C for 5 min, chilled on ice and separated by denaturing (14%) PAGE. Finally, gels were dried and labeled DNA products visualized by phosphorimaging.

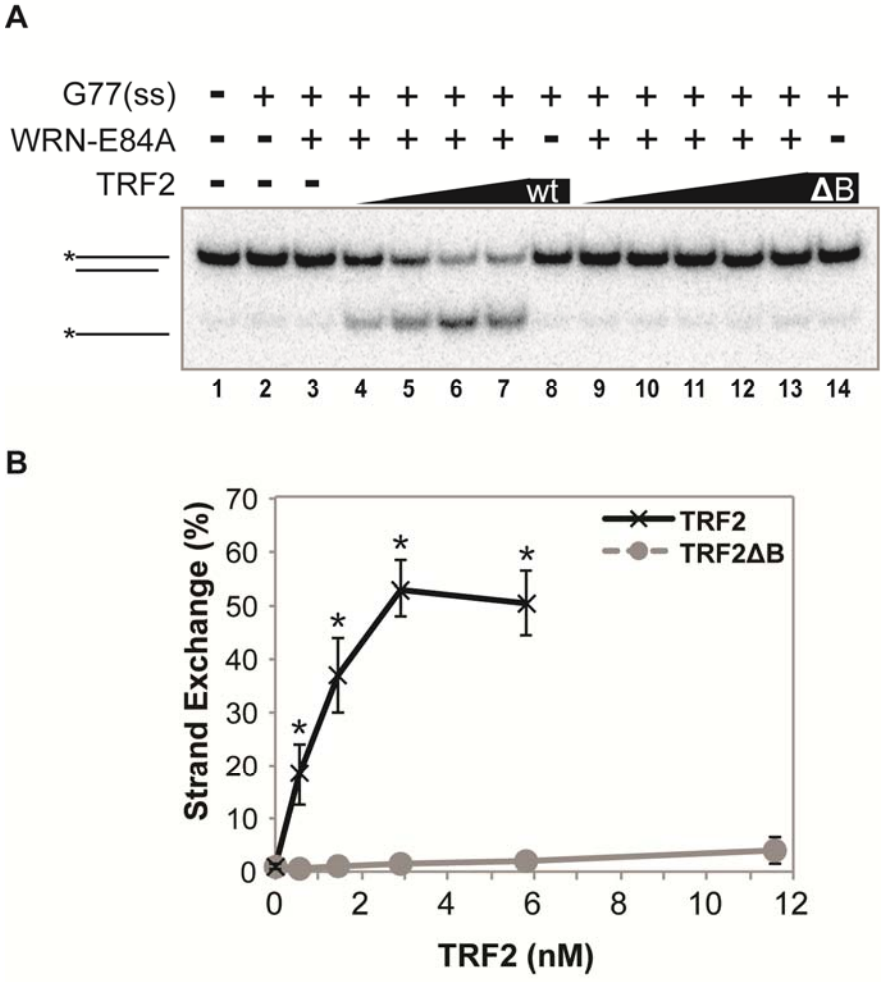
Supplemental Figure Legends

Supplemental Figure 1. Preferential TRF2 Binding to *G77telo/C38telo Compared to *G77scr/C38scr and ATP Dependence of WRN-mediated Unwinding . A) *G77telo/C38telo or *G77scr/C38scr (0.05 nM) was incubated in reaction buffer (pH 8.0) with TRF2 (2.9 nM) at 37°C for 10 min. Following the reaction, a volume of glycerol with or without xylene cyanol (XC) and bromophenol blue (BPB) was added, as indicated. Binding was analyzed by EMSA (performed under conditions specified at the top of the figure). **B)** Bar graph of data from *A* except the electrophoresis condition at 4°C without running dyes that shows the mean of 3 independent experiments. TRF2 binding to *G77telo/C38telo (*pink*) and *G77scr/C38scr (*green*) was calculated as described in Fig. 1C and plotted for each electrophoresis condition (*specified at top*). **C)** To further determine the preference of TRF2 binding, *G77telo/C38telo or *G77scr/C38scr (0.05 nM) was incubated with TRF2 at 37°C for 10 min without or with addition of unlabeled G77scr/C38scr competitor (0.03, 0.05, 0.1, or 0.2 nM = 0, 0.5, 1, 2, or 4-fold molar excess over labeled substrate) following initial binding step. EMSA was performed at 4°C without BPB and XC. **D)** Line graph representation of experiment shown in *C*. TRF2 binding to *G77telo/C38telo (*pink*) or *G77scr/C38scr (*green*) was calculated as described in Fig. 1C and plotted with respect to competitor (unlabeled G77scr/C38scr) concentration. **E)** DNase I footprinting was performed as described in Supplemental Methods on the telomeric overhang substrate *G77telo/C38telo (0.5 nM) with TRF2 (0, 5.8, 7.3, 9.7, 14.5, or 29 nM). Nucleotide size markers are depicted at left and the boundary of the TRF2 footprint (*right-angle bracket*) at the site of the telomeric duplex region (*curved bracket*) is shown at right. **F)** Helicase assays were performed by incubating *G77telo/C38telo (0.05 nM) at 37°C for 10 min with TRF2 (2.9 nM) and WRN-E84A (0.5 nM, lanes 2-4) or WRN-K577M (0.5, lane 5 or 1.0 nM, lane 6) in the absence or presence of ATP or ATPγS (1 mM) as indicated. Products were separated by native PAGE (8%, 29:1 acrylamide:bis-acrylamide) and visualized by phosphorimaging. DNA species are indicated at left.

Supplemental Figure 2. TRF2ΔB Does Not Stimulate WRN-mediated Strand Exchange Between Duplex and Single-stranded Telomeric DNA. A) *G77telo/C71telo (0.1 nM) and G77telo (1 nM) were incubated in reaction buffer (pH 8.0) containing WRN-E84A (3.6 nM) and wild-type TRF2 (0, 0.6, 1.5, 2.9, or 5.8 nM) or TRF2 ΔB (0, 0.6, 1.5, 2.9, 5.8, or 11.6 nM) at 37°C for 15 min. DNA products were separated using native PAGE, and relevant DNA species are indicated at left. **B)** Line graph of at least 3 independent experiments performed as in *A*. Strand exchange (%) was calculated as described in Fig. 2. Statistical analysis was performed using an unpaired, one-tailed t-test, and significant differences between reactions containing wild-type TRF2 and mutant TRF2 ΔB are indicated by asterisks (all p values $\leq$0.013).



Supplemental Figure 1



Supplemental Figure 2