Supplemental Experimental Procedures

ATPase assay – Experimental procedures were adapted from previous publication (40,41). The reactions were carried out with increased amounts of proteins in 20 µL of system containing reaction buffer [25 mM Tris-HCl (pH 8.0), 50 mM KCl, 5 mM Mg_2Cl, 100 µM ATP, 1 mM DTT and 100 ng of BSA] including 0.05 mCi/mL [γ-32P]ATP (PerkinElmer) at 37˚C for 30 minutes. To determine the response to nucleotide, either 5 nM or 10 nM of 3WLRNA oligo was added into the indicated reaction. RNA oligo was purchased from Intergrated DNA Technologies (San Diego, CA). Reactions were terminated by adding 50 mM EDTA. One microliter of each reaction was spotted onto polyethyleneimine cellulose thin-layer chromatography (Sigma) and developed in 1 M formic acid with 0.5 M LiCl. Results were analyzed using Storm 320 Phospho Imager (Molecular Dynamics).

Gel mobility shift assay – The reactions were carried out with increased amounts of proteins in 20 µL of system containing reaction buffer [25 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM DTT, 1 mM EDTA, 40 ng/µl poly(dI-dC) and 100 ng of BSA]. 2 nM radioactively labeled oligo probes were added into each reaction and incubated at room temperature for 30 minutes. All RNA and DNA oligos were purchased from Intergrated DNA Technologies (San Diego, CA). T7 polynucleotide kinase (Ambion) was used to label 3WLRNA oligo on 5' with [γ-^{32}P]ATP (PerkinElmer) which was then purified by G-25 microspin column (GE Healthcare). Double-stranded substrates were obtained by hybridizing labeled 3WLRNA to equal amount of either T20DNA or T22RNA. The reactions were terminated by adding 5 µL of nondenaturing loading dye containing 25 mM Tris-HCl (pH 8.0), 20 mM EDTA, 0.1% Nonidet P-40, 0.1% bromophenol blue, 0.1% xylene cyanol and 25% glycerol. The reaction mixtures were loaded onto a 5% native polyacrylamide gels and electrophoresed at 4˚C. Results were analyzed using Storm 320 Phospho Imager.

Helicase assay – Briefly, all reactions were carried out with increased amounts of proteins in 20µL of system containing reaction buffer [25 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM $MgCl_2$, 3 mM ATP, 1 mM DTT and 100 ng of BSA]. 10 nM radioactively labeled 3WLRNA/T20DNA substrate and 100nM of unlabeled competing T20DNA strand were added into each reaction and incubated at 37˚C for 30 minutes. The reactions were terminated by adding 5µL of the nondenaturing loading dye used for gel mobility shift assays with 0.5% SDS. The reaction mixtures were then loaded onto a 15% native polyacrylamide gels. Results were analyzed using Storm 320 Phospho Imager.

Exoribonuclease assay – All reactions were carried out with increased amounts of proteins in 20µL of system containing reaction buffer [25 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM $MgCl_2$, 1 mM DTT and 100 ng of BSA]. 20 nM radioactively labeled oligo probes were added into each reaction and incubated at 37˚C for 30 minutes or indicated time points. The reactions were terminated by adding 5µL of denaturing loading dye containing 1 X TBE, 82% formamide, 0.1% bromophenol blue, 0.1% xylene cyanol and 25% glycerol. The reaction mixtures were then loaded onto a 14% denaturing polyacrylamide gels containing 1 X TBE, 10% formamide, and 7M urea. Results were analyzed using Storm 320 Phospho Imager.