SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. RecQ4 binds single-stranded, but not duplex, DNA, and binding is stabilized in the presence of AMPPNP. Enzyme was incubated with duplex (open bars) and single-stranded (shaded bars) DNA substrates in the absence of nucleotide cofactor, or in the presence of ADP or AMPPNP. Double-filter binding assays were carried out by applying completed reactions by vacuum to treated nitrocellulose layered over DE81 paper, and then washing with 500mM potassium acetate. Protein bound substrate was retained by the nitrocellulose, and free substrate collected on the DE81 paper. The fraction of each reaction retained by the nitrocellulose was quantified by phosphorimaging analysis. Error bars indicate standard deviation (n=3).

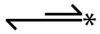
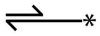
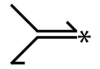
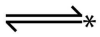
Supplemental Fig. 2. Wildtype and K898N mutant transgenes of *RecQ4-V5* have similar expression levels. Ovary homogenates were prepared, separated by SDS/PAGE, and Western blotting was carried out using an antibody against the V5 epitope tag. Actin was used as a loading control.

Supplemental Table 1. *(A)* Table indicating the sequences of DNA oligonucleotides used in substrate creation. *(B)* Table indicating the components of each substrate. The first column gives the name of each substrate. The second column gives the names of the oligonucleotide components of the substrate. The third column gives the diagram used to represent the substrate in figures.

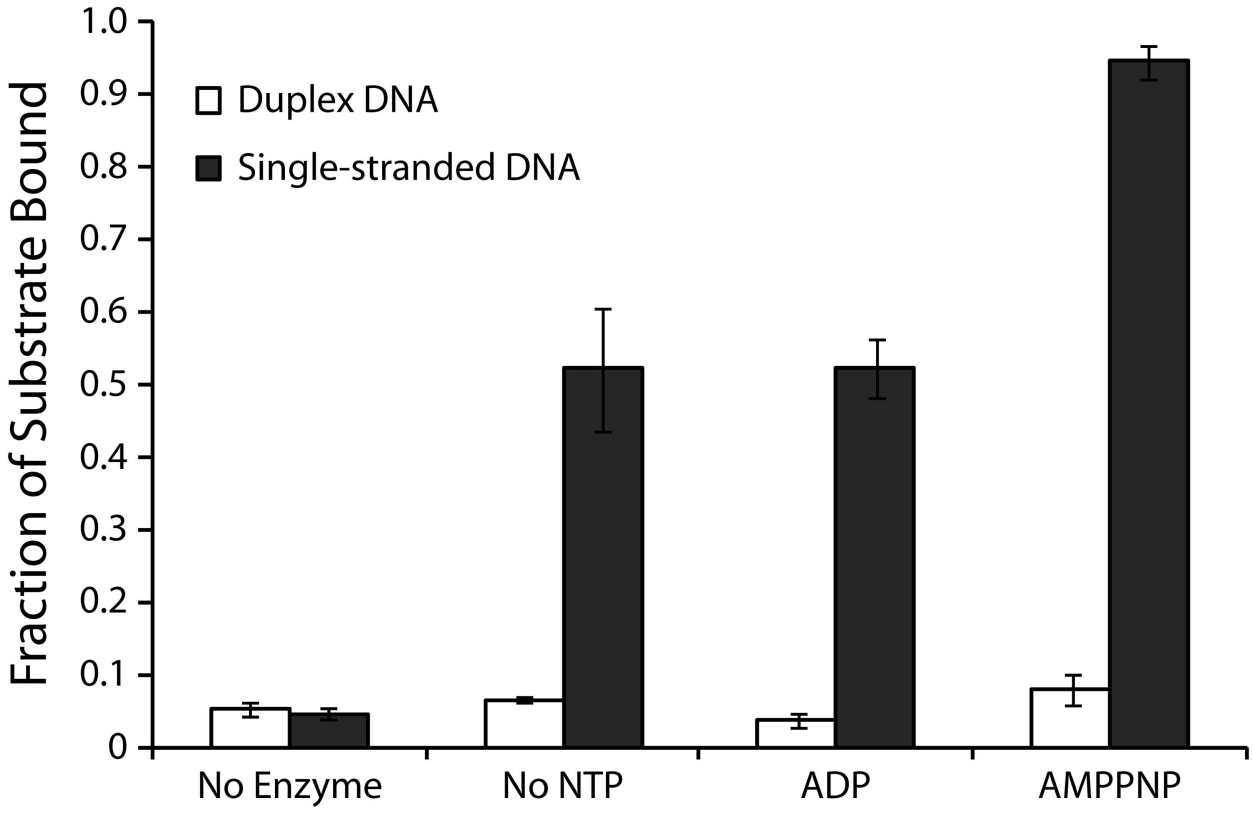
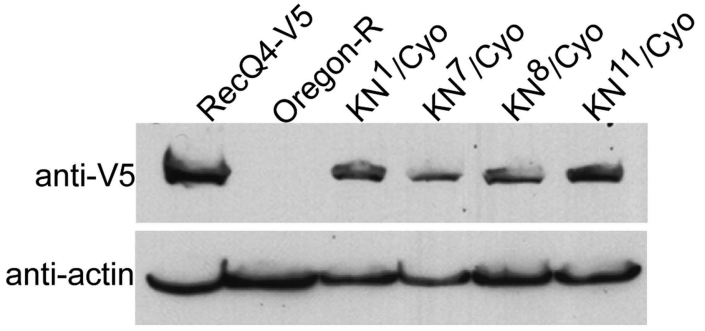
A

Name	Sequence (5′ to 3′)
7	CGAAGGCCATGATTGCGCACTGAATACATCCTGCCCTGTTATTAATTACGTTATCTTACG
8	GTGTTGCCGTCTACATGCTTGATTATTCTCGATGTATTCAGTGCGCAATCATGGCCTTCG
8s1	GATGTATTCAGTGCGCAATCATGGCCTTCG
9	CGTAAGATAACGTAATTAATAACAGGGCAGGATGTATTCAGTGCGCAATCATGGCCTTCG
9s1	CGTAAGATAACGTAATTAATAACAGGGCAG
10s1	GAGAATAATCAAGCATGTAGACGGCAACAC
dT30	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

B

Name	Component Oligos	Diagram
3′ extension	7, 8s1	
5′ extension	7, 9s1	
Fork	7, 8	
Duplex	7, 9	
Single Stranded	dT30	
3′ Flap	7, 8, 10s1	
5′ Flap	7, 8, 9s1	
Duplex Fork	7, 8, 9s1, 10s1	

Supplemental Figure 2



Supplemental Figure 2