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Supplementary Information for

Clutch mechanism of chemo-mechanical coupling in a DNA resecting motor-nuclease

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Figures S1, S2

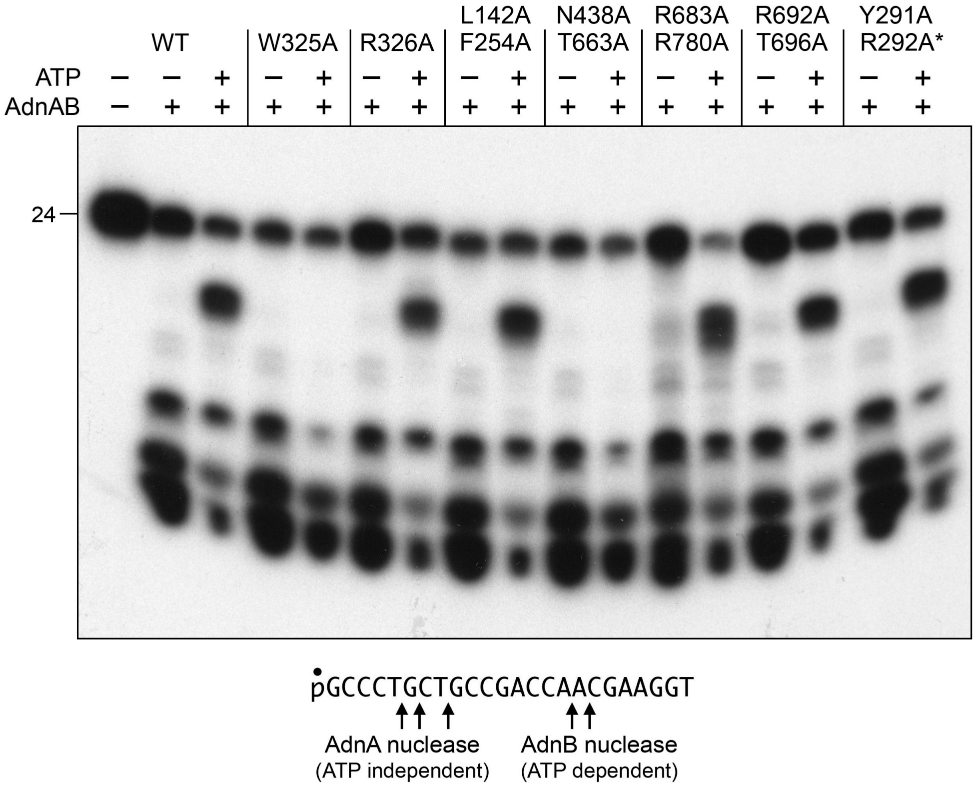

Figure S1. **AdnB mutations W325A and N438A-T663A uncouple ATP hydrolysis from ssDNA translocation.** Reaction mixtures (10 μl) containing 20 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$, 1 mM DTT, 0.1 μM 5' ^{32}P-labeled ssDNA substrate (shown at bottom), 1 mM ATP, and 0.1 pmol wild-type or mutant AdnAB (where indicated by +) were incubated for 20 min at 37°C. The reactions were quenched by adjustment of the mixtures to 45% formamide and 25 mM EDTA. The products were analyzed by electrophoresis through a 15-cm 18% polyacrylamide gel containing 7 M urea in 45 mM Tris-borate and 1.2 mM EDTA and then visualized by autoradiography. The sites of cleavage of the 24-mer ssDNA by the AdnA and AdnB nuclease domains are indicated by arrows at bottom.

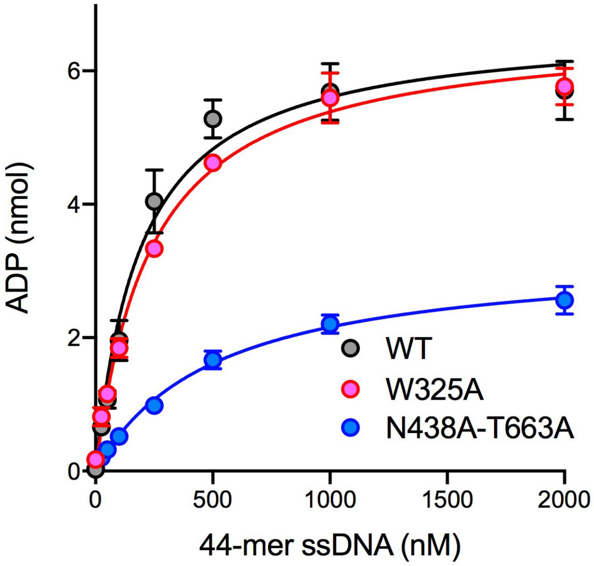

Figure S2. **Dependence of ATP hydrolysis on ssDNA concentration**. Reaction mixtures (10 µl) containing 20 mM Tris-HCl, pH 8.0, 1 mM DTT, 2 mM $MgCl_2$, 1 mM [α^{32}P]ATP, 25 nM wild-type (WT) or mutant AdnAB as specified, and 0, 25, 50, 100, 250, 500, 1000, or 2000 nM 44-mer ssDNA oligodeoxynucleotide were incubated at 37°C for 10 min. The extents of ATP hydrolysis are plotted as a function of ssDNA concentration. Each datum is the average of three independent titration experiments ±SEM. Nonlinear regression curve fits of the data to the Michaelis Menten equation are shown. The apparent K_m values for ssDNA (calculated in Prism) were as follows: wild-type AdnAB = 192 ± 33 nM; W325A = 236 ± 25 nM; N438A-T663A = 511 ± 74 nM. The extents of ATP hydrolysis extrapolated to saturating ssDNA (output as V_{max} in Prism) were: WT = 6.68 ± 0.34 nmol; W325A = 6.66 ± 0.22 nmol; N438A-T663A = 3.26 ± 0.18 nmol.