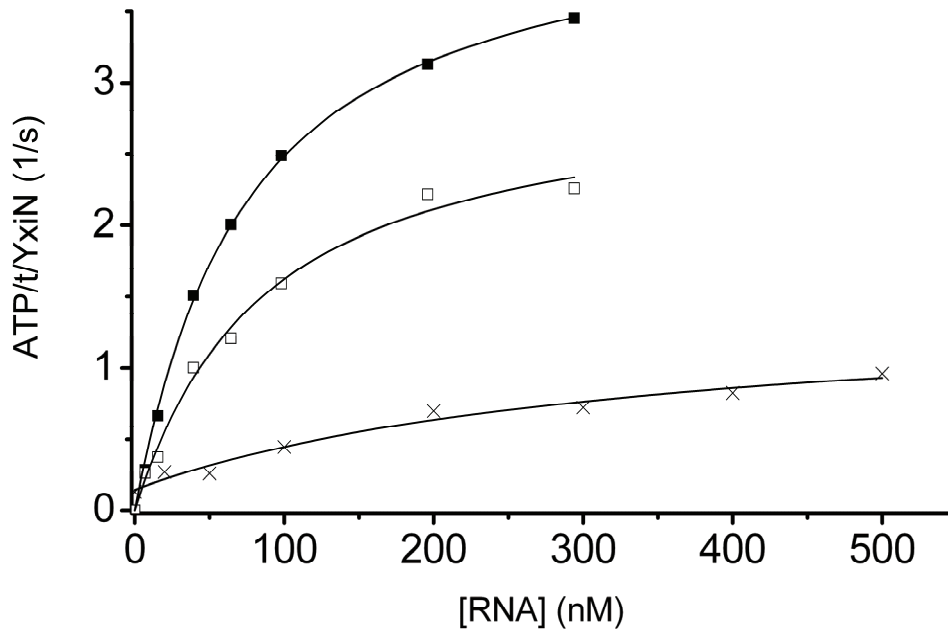


Supplementary Figure 1, Karow & Klostermeier

Steady state ATPase activity of constructs used for single molecule FRET experiments



Wild-type YxiN (filled squares) is a Michaelis-Menten enzyme with  $k_{\text{cat}} = 4.3 \text{ s}^{-1}$ , and  $K_{\text{app,RNA}} = 68 \text{ nM}$ . YxiN' (YxiN\_C61/267S\_A115/S229C, crosses), a construct we previously used for single molecule FRET experiments (1), has a reduced  $k_{\text{cat}}$  of  $1.3 \text{ s}^{-1}$ , and an elevated  $K_{\text{app,RNA}}$  of  $324 \text{ nM}$  (data taken from Ref. 1). In the present study, an improved single molecule FRET construct was used, in which the intrinsic cysteines C61 and C267 have been replaced by alanines instead of serines. YxiN' (YxiN\_C61/267A\_A115/S229C, open squares) shows wild-type like properties with  $k_{\text{cat}} = 3.0 \text{ s}^{-1}$  and  $K_{\text{app,RNA}} = 80 \text{ nM}$ .

1. Theissen, B., Karow, A. R., Kohler, J., Gubaev, A., & Klostermeier, D. (2008) Cooperative binding of ATP and RNA induces a closed conformation in a DEAD box RNA helicase. *Proc Natl Acad Sci U S A* **105**, 548-553.