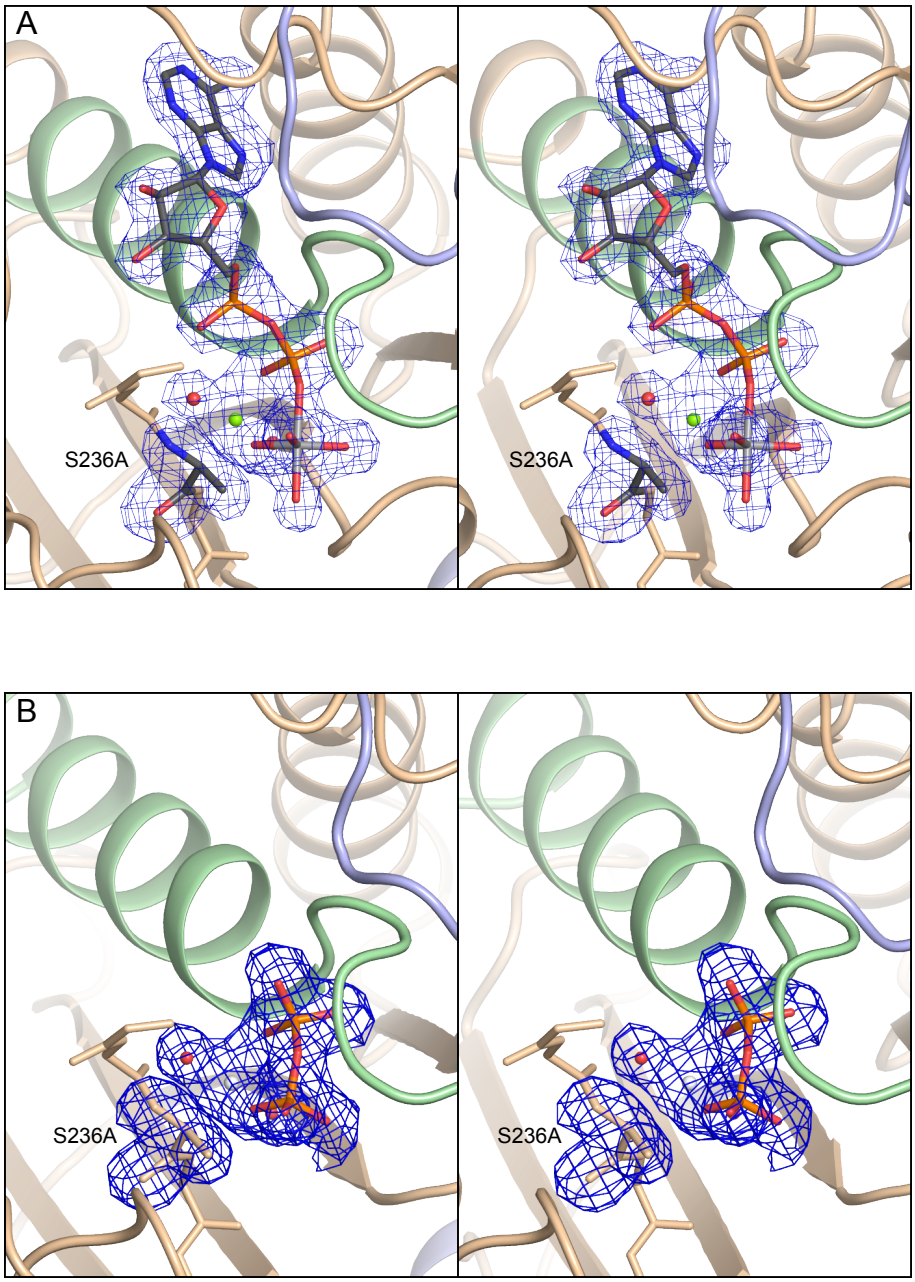
Supporting information

Supplementary Figure 1

Stereoview of the electron density for (a) $MgADPVO_4$ and (b) MgPPi in the S236A mutant protein complexes both at 2.0 Å resolution. The electron density maps which were contoured at 3σ were calculated from coefficients of the form F_o-F_c where the MgADP·VO_4, MgPPi, Ala236 and the associated water molecules were omitted from the phase calculation and refinement.

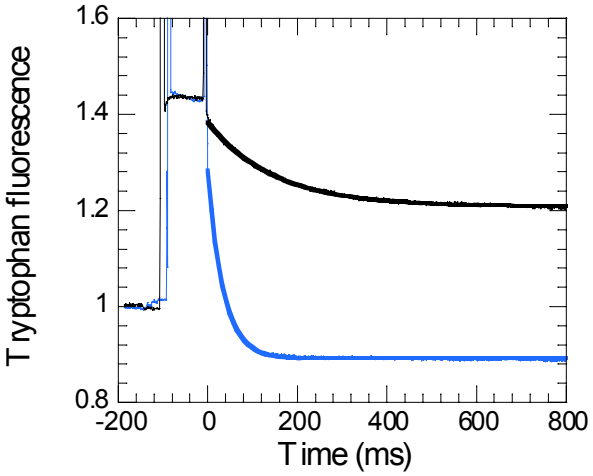
Supplementary Figure 2

(A) Determination of the ATP turnover rate constant by displacement with PPi. Tryptophan fluorescence was recorded for either 4 μM of wild-type S1dC (blue trace) or S236A construct (black trace) in an SLM 8000 fluorimeter with the signal normalised to 1 for the apo state. At minus 100 s, 100 μM ATP was added manually, followed by 5 mM PPi at 0 s. Single exponential fits to the decay phase are superposed (error in all fitted parameters was <1% in both cases) to yield rate constants of 0.028 s^{-1} (wild type) and 0.0068 s^{-1} (S236A). Note that the initial fluorescence enhancement of the steady-state phase (44%) is the same for both constructs, but the displacment with PPi gives a different end-point level. The latter is a reflection of the observation that PPi quenches the fluorescence of wild-type S1dC but enhances (22%) the fluorescence of S236A relative to the apo state. (B) Stopped-flow records showing the tryptophan fluorescence change on mixing 10 (black), 100 (blue) and 5000 (red) μM ATP with 1 μM S236A. (C) Plot of k_{obs} (fast phase) as a function of [ATP] for wild type (blue) and S3236A mutant (black). Buffer conditions were as in Fig. 5 of main text throughout.



Supplementary Figure 1, Frye et al., (2010)

A



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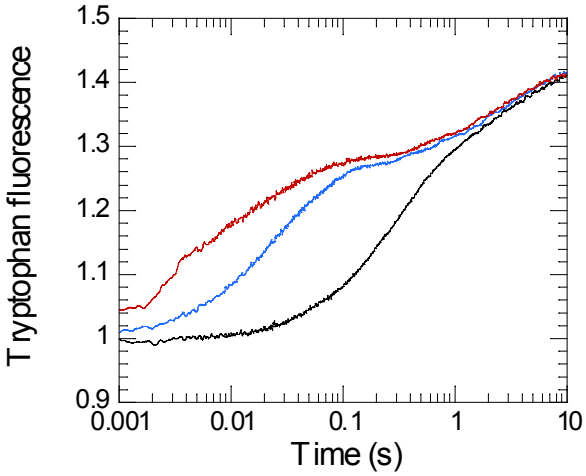

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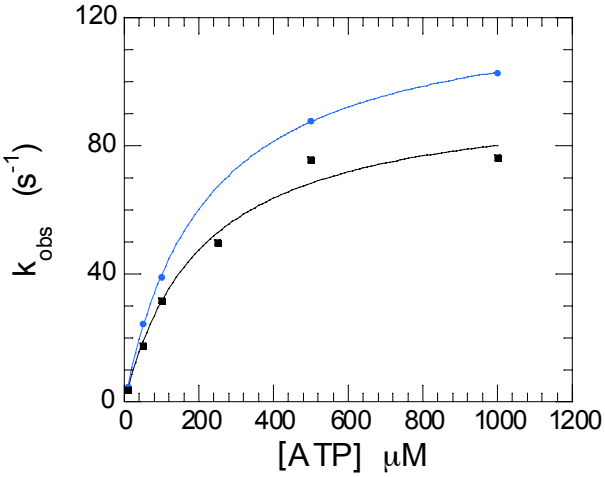

Supplementary Figure 2, Frye et al., (2010)