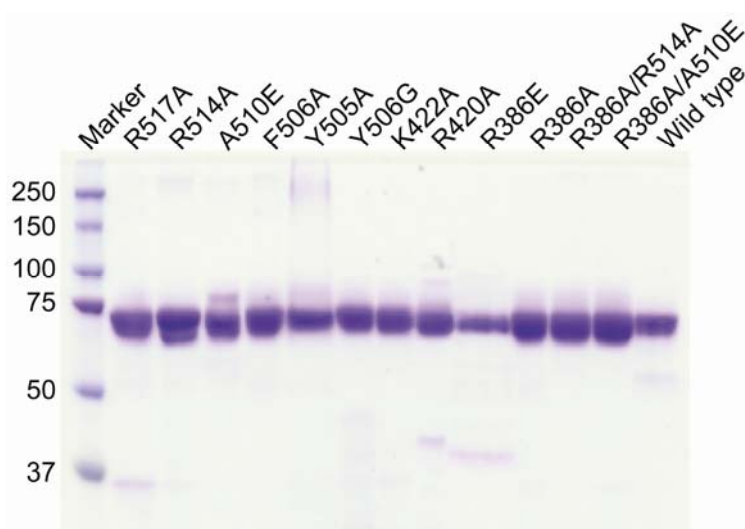


Supplementary Data

Identification of Critical Residues for the Tight Binding of Both Correct and Incorrect Nucleotides to Human DNA Polymerase λ

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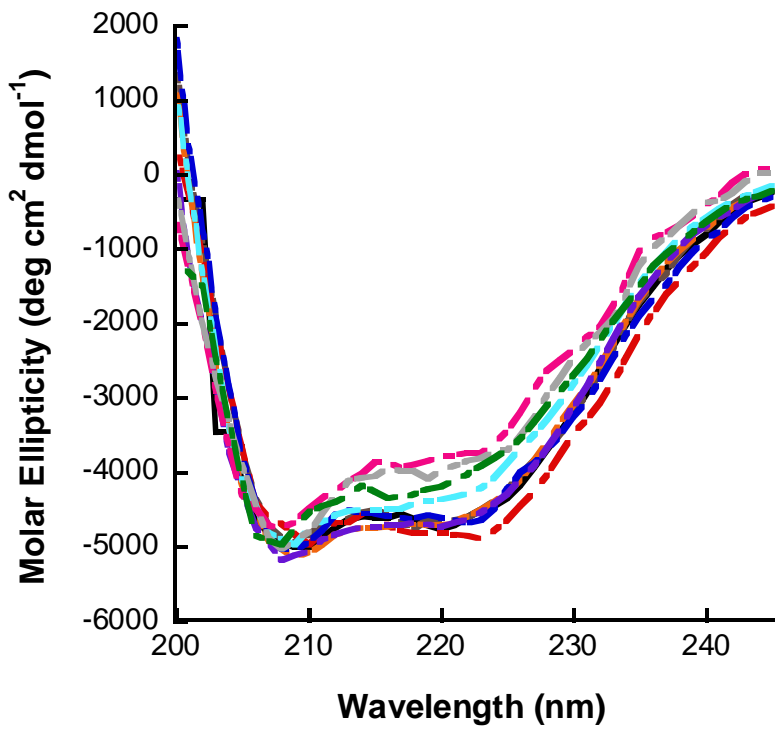
Supplementary Figure 1

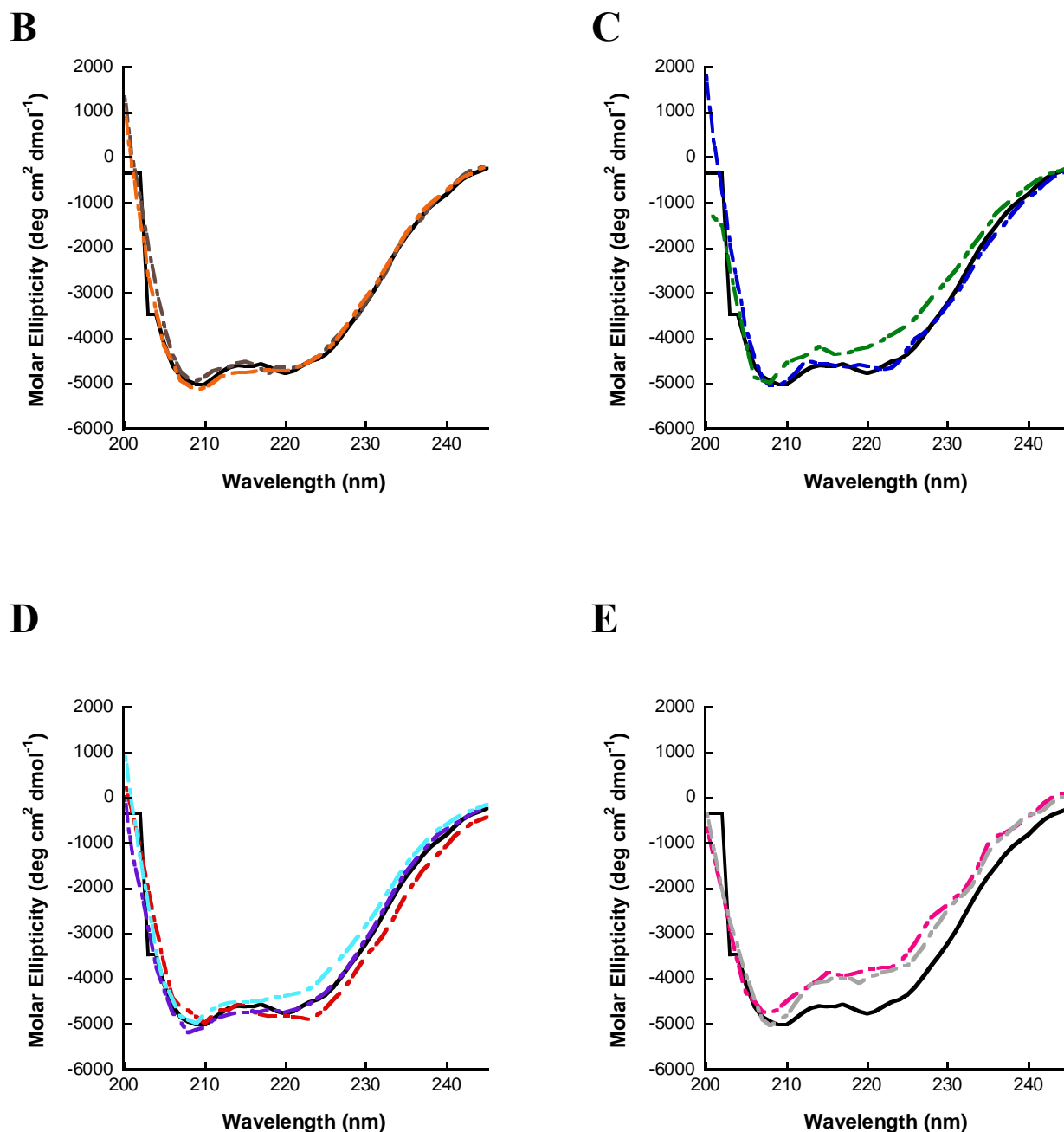


Supplementary Figure 1. Coomassie blue-stained SDS-PAGE of wild-type and mutant Pol λ . The purity of each enzyme was estimated by resolving 5 μ g of each protein on an 8% SDS-PAGE gel that was stained with Coomassie blue. The molecular weights (kDa) of the marker are listed along the left side.

Supplementary Figure 2

A

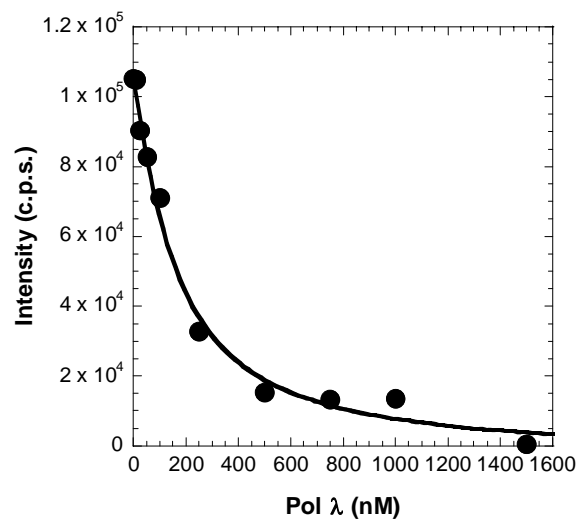




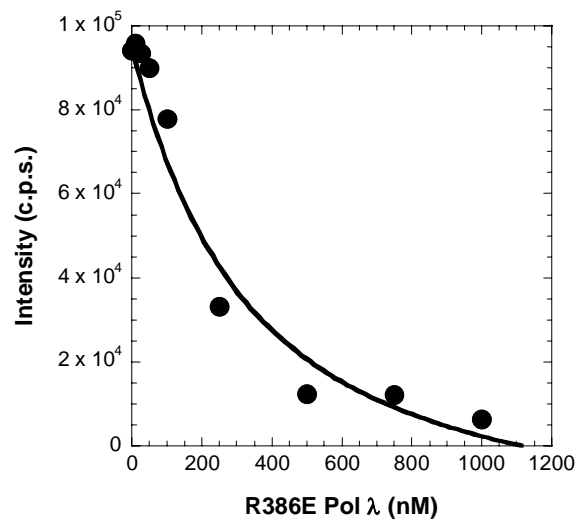
Supplementary Figure 2. Circular dichroism spectra for WT and mutant Pol λ . The CD spectra were collected and overlaid for WT (black), R386A (brown), K422A (orange), Y505A (green), F506A (blue), A510E (cyan), R514A (purple), R517A (red), R386A/A510E (pink), and R386A/R514A (gray). The CD spectra indicated that all the proteins were folded. Individual spectra overlaid with wild-type Pol λ are shown for (B) R386A and K422A; (C) Y505A and F506A; (D) A510E, R514A, and R517A; and (E) R386A/A510E and R386A/R514A. The CD spectra for WT, Y505A, and F506A Pol λ are from the work of Brown *et al.*¹

Supplementary Figure 3

A



B



Supplementary Figure 3. Equilibrium dissociation constant for the dissociation of the binary complex Pol λ•DNA. A plot of 2-aminopurine intensity versus the concentration of (A) WT Pol λ or (B) R386E Pol λ was fit to equation 1 which resolved a $K_d^{\text{DNA}} = 110 \pm 20$ nM and $K_d^{\text{DNA}} = 300 \pm 100$ nM for WT and the R386E mutant, respectively.

REFERENCE

1. Brown, J. A., Fiala, K. A., Fowler, J. D., Sherrer, S. M., Newmister, S. A., Duym, W. W. & Suo, Z. (2010). A Novel Mechanism of Sugar Selection Utilized by a Human X-Family DNA Polymerase. *J Mol Biol* **395**, 282-290.