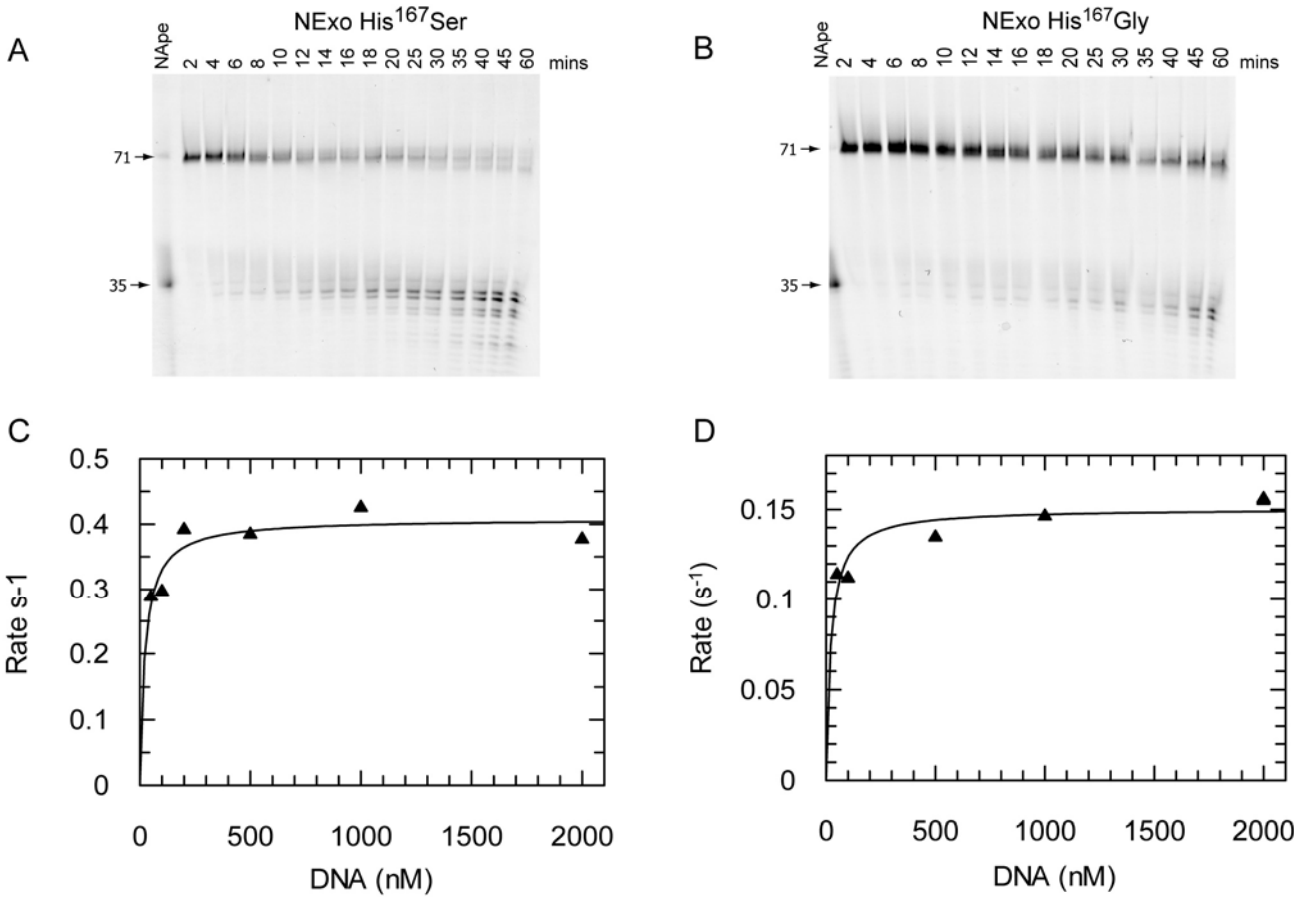
Supplemental data

Figure S1. Kinetic analysis of AP endonuclease activity of NExo His^{167}Ser and His^{167}Gly mutants



Assays were performed with substrate 71AP, a 71 bp DNA with an abasic furanose analogue at position 36. This longer substrate enables the separation of the AP endonuclease activity from the 5´-3´ exonuclease activity, as can be seen in panels A and B. Reactions were performed with substrate concentrations from 0.05 to 2 μM, and mutant enzyme concentrations 10- to 20-fold lower, for the times shown before stopping the reaction with formamide EDTA loading buffer and separation by 12% PAGE. A control lane is also shown where the same substrate was digested with NApe. Rates were determined by fitting the linear increase of abasic DNA products against time. Rates were then normalised by dividing by enzyme concentration, and plotted vs. enzyme concentration. Data are shown with the best fit to the Michaelis-Menten equation for (C) His^{167}Ser k_{cat} = 0.41 ± 0.02 s^{-1}, K_M = 24 ± 8 nM. (D) His^{167}Gly k_{cat} = 0.15 ± .01 s^{-1}, K_M = 22 ± 7 nM. Other conditions were as described in Materials & Methods