SUPPLEMENTAL DATA

Kinetic and Structural Impact of Metal Ions and Genetic Variations on Human DNA Polymerase ι

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TABLE OF CONTENTS

FIGURE S1. **Pre-steady-state kinetics of dCTP incorporation opposite normal G by human pol ι (26−445), pol ι (1−445), and R96G pol ι (1−445) enzymes in the presence of either Mn^{2+} or Mg^{2+}.** *A*, each of a 10-fold excess (1 μM) of pol ι (1–445), pol ι (26–445), and R96G pol ι (1–445) enzymes was incubated with 100 nM 18-mer/36-mer primer/template DNA in the rapid quench-flow instrument and mixed with increasing dCTP concentrations at various time intervals in the presence of either 2 mM $MgCl_2$ or 0.15 mM $MnCl_2$ to perform the single turnover reactions, as described in the Experimental Procedures section. The results were fitted to a single-exponential equation to obtain the observed rates of nucleotide incorporation (k_{obs}) for each dCTP concentrations. *B* and *C*, each plot of the k_{obs} values *versus* dCTP concentration for pol ι (1–445, ■), pol ι (26–445, ▲), and R96G pol ι (1–445, ▼) enzymes was fit to a hyperbolic equation, and the estimated values of k_{pol} (the maximal rate of nucleotide incorporation) and $K_{d,dCTP}$ (the equilibrium dissociation constant for dCTP) are listed in the Table 1. *B*, 2 mM $MgCl_2$; *C*, 0.15 mM $MnCl_2$.

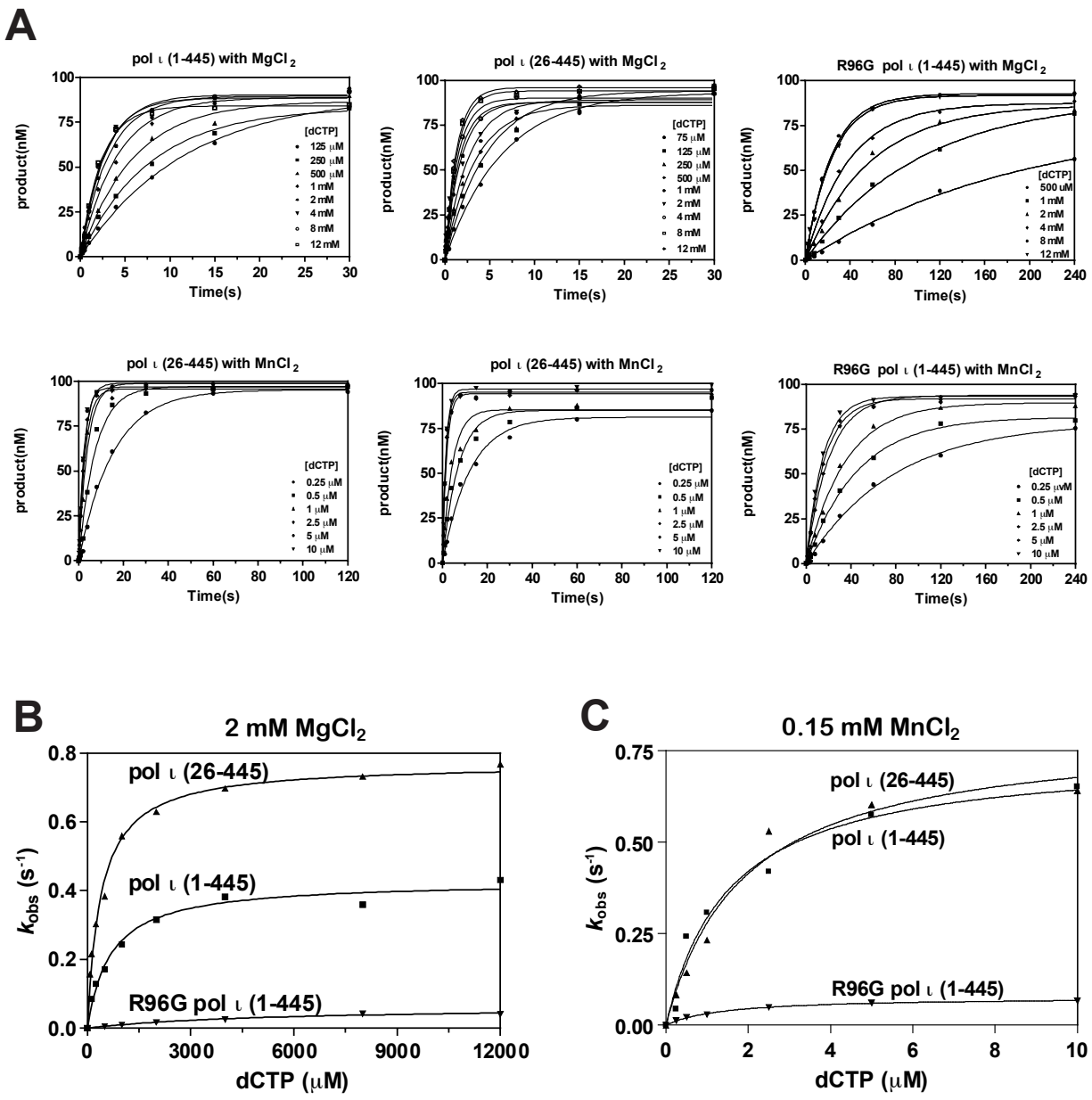

FIGURE S2. **Structural comparison of pol ι (26–445), pol ι (1–445), and R96G pol ι (1–445) ternary complexes with DNA and incoming nucleotide in the presence of Mg^{2+} or Mn^{2+}.** *A*, superposition of the overall structures of pol ι (26-445)·G·dCMPNPP·Mg^{2+} (blue), pol ι (1-445)·G·dCMPNPP·Mg^{2+} (gray), and R96G pol ι (1-445)·G·dCMPNPP·Mg^{2+} (yellow) complexes. Pol ι is shown as ribbons, DNA is shown as tube and ladder, the nucleotide is shown as sticks, and metal ions are shown as spheres. *B*, superposition of the overall structures of pol ι (26-445)·G·dCMPNPP·Mn^{2+} (red), pol ι (1-445)·G·dCMPNPP·Mn^{2+} (gray), and R96G pol ι (1-445)·G·dCMPNPP·Mn^{2+} (yellow) complexes. *C*, superposition of the active sites of pol ι (26-445)·G·dCMPNPP·Mg^{2+} (blue), pol ι (1-445)·G·dCMPNPP·Mg^{2+} (gray), and R96G pol ι (1-445)·G·dCMPNPP·Mg^{2+} (yellow) complexes. Active site residues, DNA, and nucleotide are shown as sticks, and metal ions are shown as spheres. *D*, superposition of active sites of pol ι (26-445)·G·dCMPNPP·Mn^{2+} (red), pol ι (1-445)·G·dCMPNPP·Mn^{2+} (gray), and R96G pol ι (1-445)·G·dCMPNPP·Mn^{2+} (yellow) complexes.