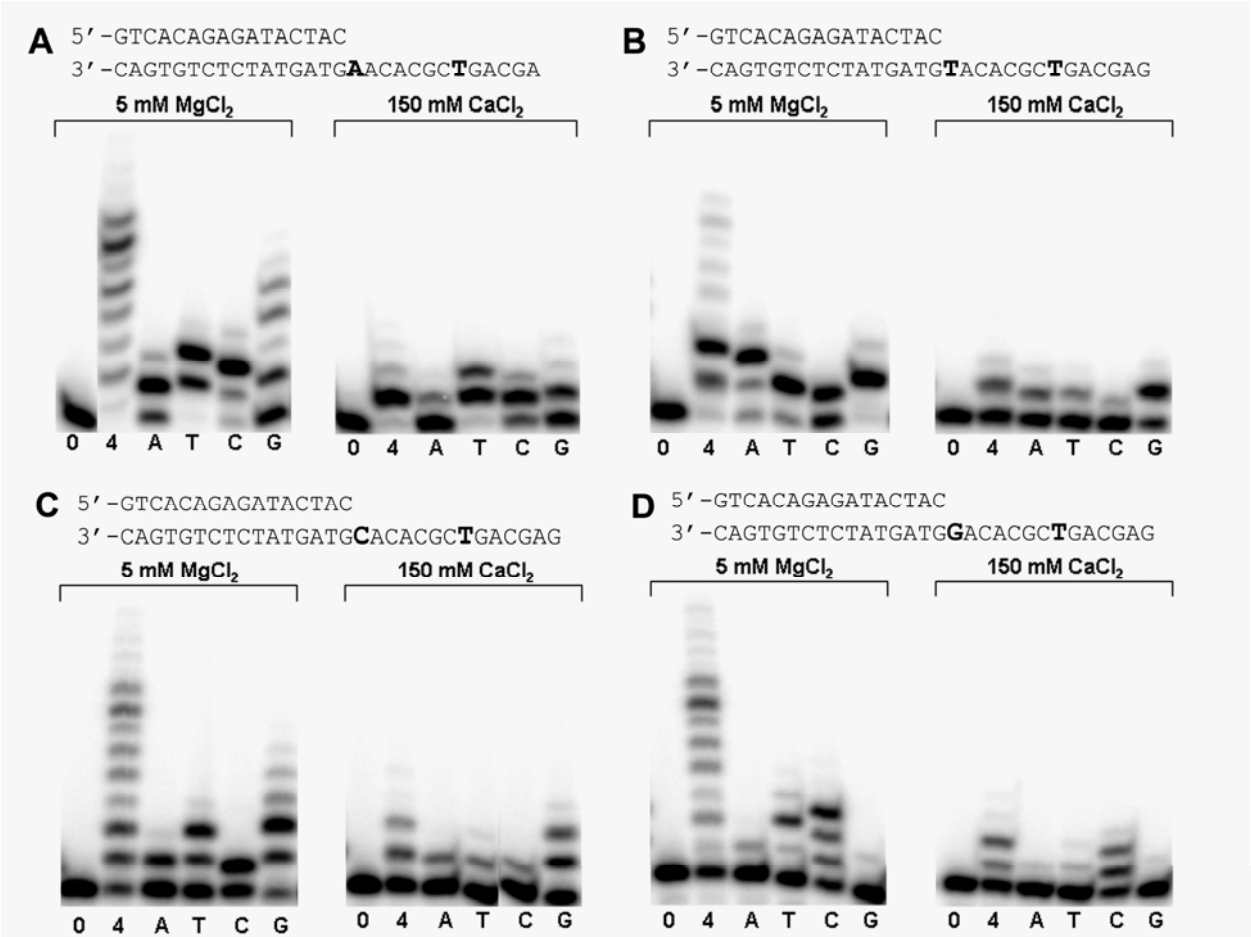

Figure S1 Effect of calcium to the replication of pol ι

Primer extension assays of pol ι in the presence and absence of 100 mM $CaCl_2$ have been carried in parallel, showing that Ca2+ does not change the nucleotide incorporation specificity of polι. Polι was incubated with DNA and either no nucleotides (0), all four dNTPs (4), or individual dNTPs (A,T,C,G) as labeled below the each lanes.

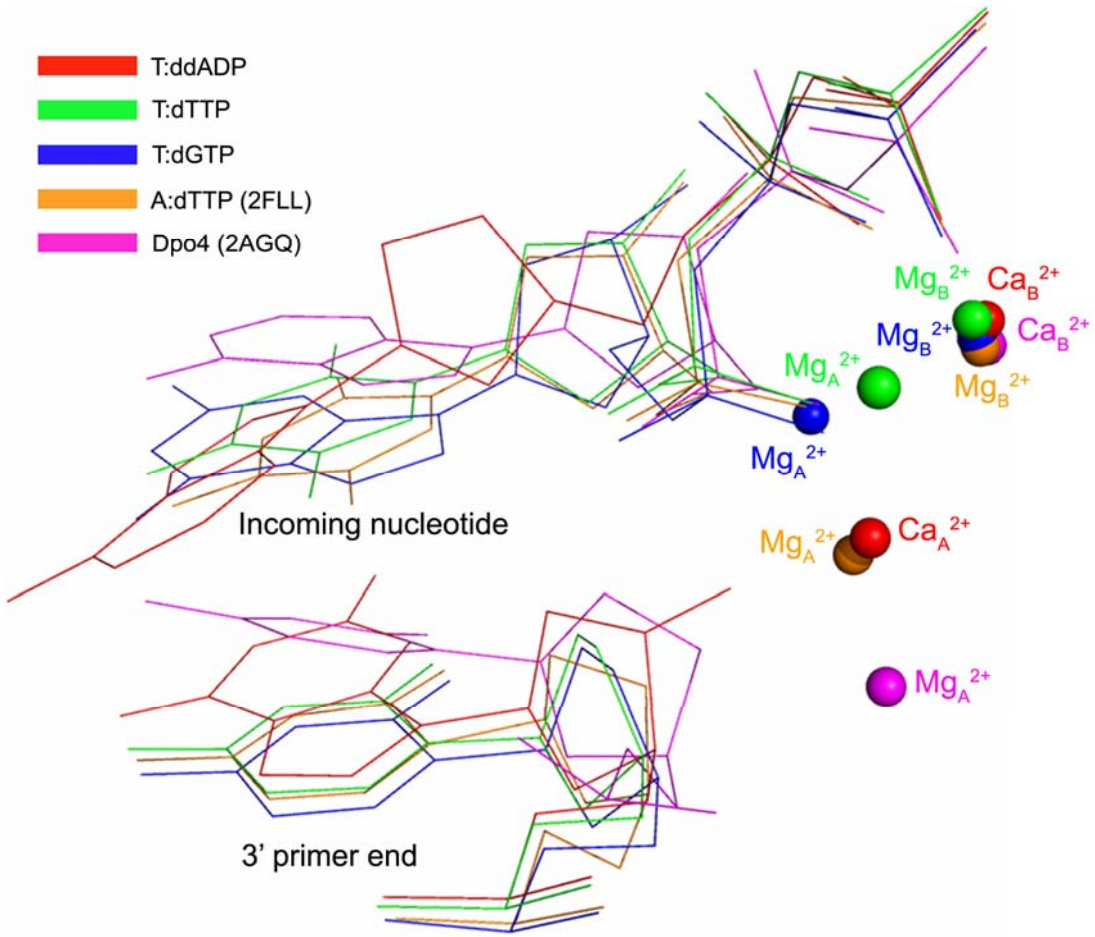

Figure S2 Comparison of positions of the metal ions at the active sites of Y-family polymerases. All three T template structures are superimposed with a purine template pol ι (2FLL) and a Dpo4 ternary complex (2AGQ). The metal ions are in spheres with unique colors labeled as color bar legends for all five structures in the right-up corner.

```
                 Finger                                  Thumb                                   Little Finger
                 59 61 62                 78             237      244                            307                    347
H. sapiens       PLGVQQKYLVVTCNYEARKLGVKKLMNVRDAKEKCP SLNHIKEIPGIGYKTAKCLEALG GPPQSFSEEDSFKKCSS---PHTVRLIIRRYSS-EKHYG
M. musculus      PLGVQQKYLVVTCNYEARKLGVRKLMNVRDAKEKCP SLNHIKEIPGIGYKTAKRLEVLG GPPQSFSEEDTFKKCSS---PHTVRLVIRRYS--DKHCN
B. taurus        PLGVQQKYLVVTCNYEARKLGVKKLMSVRDAKEKCP SLNHIKEMPGIGYKTTKRLEALG GPPQSFSEEDSFKKCSS---PHTIRLIIRRYSS-EKHYN
E. caballus      PLGVQQKSLMVTCNYEARKLGVKKCMNIRDAKEKCP SLNHIKEIPGIGYKTAKRLEALG GPPQSFSEEDSFKKCSS---PHTIRLTIRRNSS-ESYYS
M. domestica     PLGVQQKYLVVTCNYEARKLGVKKLMSVRDAKEKCP SLDHIKQMPGIGFKTTKRLESLG GPPQSFSEEDSFKKCSS---PHTIRLIIRRFSS-DRHFN
O. anatinus      PLGVQQKYLVVTCNYDARKLGVKKLMSIRDAKEKCP SLDHIKQMPGIGFKTTKRLESLG GPPQSFSEEDSIRKCST---PHTIRLTIRRFSA-EKSFN
D. rerio         PLGIQQKYIIVTSNYVARELGVNKLMSVTDAVEKCP SLTGLIKVPGIGYKTREKLKALG GPPQSLSDEDSFKKMST---PHTLRLTIRKYTVTNRWFN
S. salar         PLGIQQKYIIVTCNYVARDLGVTKLMLVTDAKEKCP SLNGLRKVPGVGHQTAKRLQALG GAPQSLSDEDSFKKMSS---PFTLRLTIRRYSATNKWFS
X. tropicalis    PLGIQQKYIVVTCNYEARKFGVTKLMLIKDAREKCP SLDHVKQIPGIGYKTSKRLESLG GPPQSISDEDSFKKCST---PHTLRLTIRQFSPSNKWFN

Dpo4             PVVVCVG-AVATANYEARKFGVKAGIPIVEAKKILP RELDIADVPGIGNITAEKLKKLG RVRKSIGRIVTMKRNSR---AIHVVAVTEDLDIVSRGRT
                   FSGRFEDS
```

Figure S3 Amino acid sequence alignment for three domains from polι of various species and Dpo4. Residues involved in stabilizing the U-turn DNA are highlighted in cyan (finger domain), green (thumb domain), and purple (little finger domain). Glutamine 59, which stabilizes mismatched G is highlighted in red. Residues that have similar properties and analogous functions are highlighted in yellow.

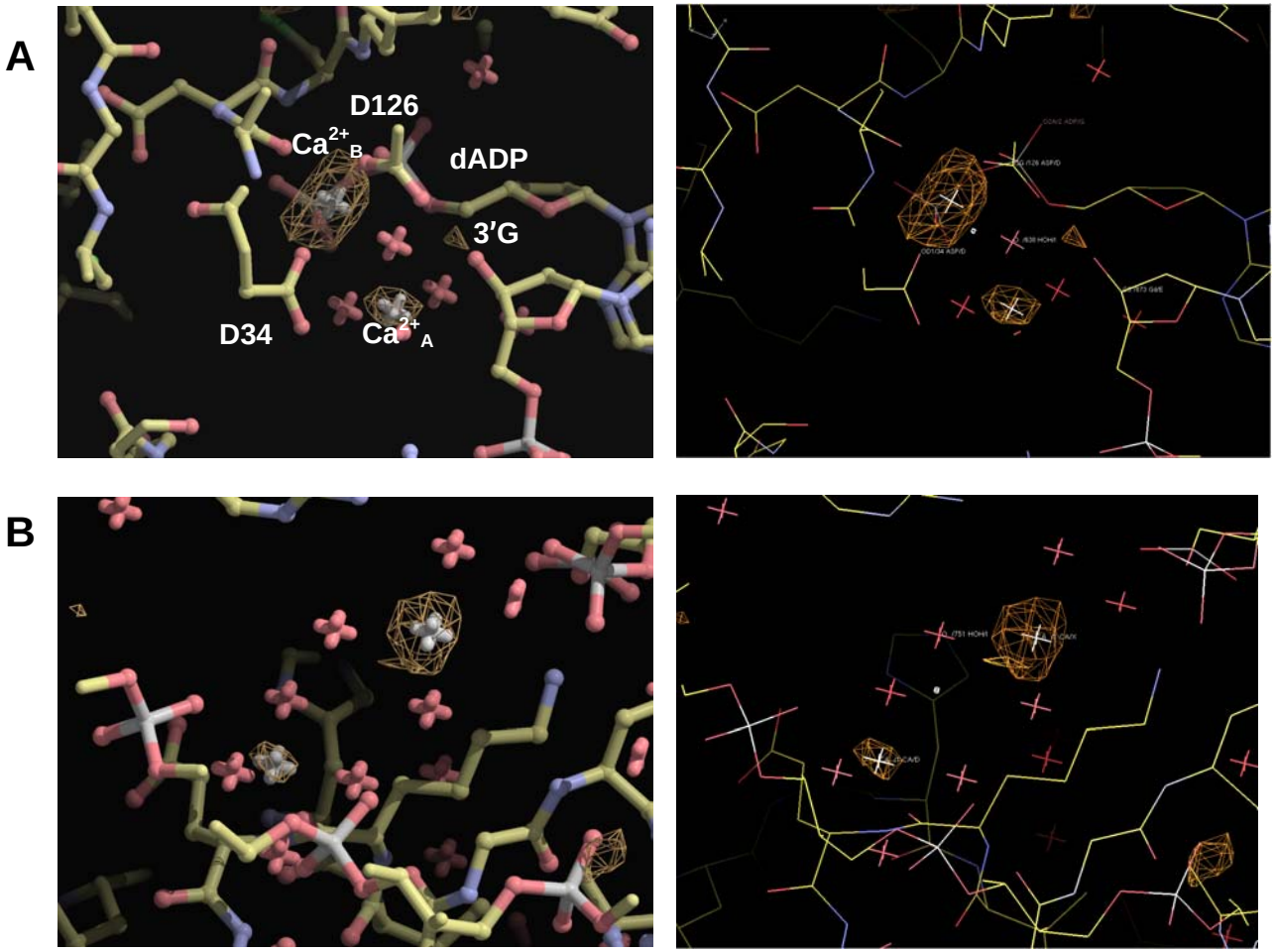

Figure 4S Anomalous map contoured at 3.3 s in the structure T:ddADP which was crystallized in the buffer containing 150mM $CaCl_2$, using the data collected at the wavelength of 0.9832 Å. (**A**) the Ca^{2+} ions (grey stars) at the active site with active site components labeled. (**B**) the Ca^{2+} ions are not in the active site. The images were taken from COOT.

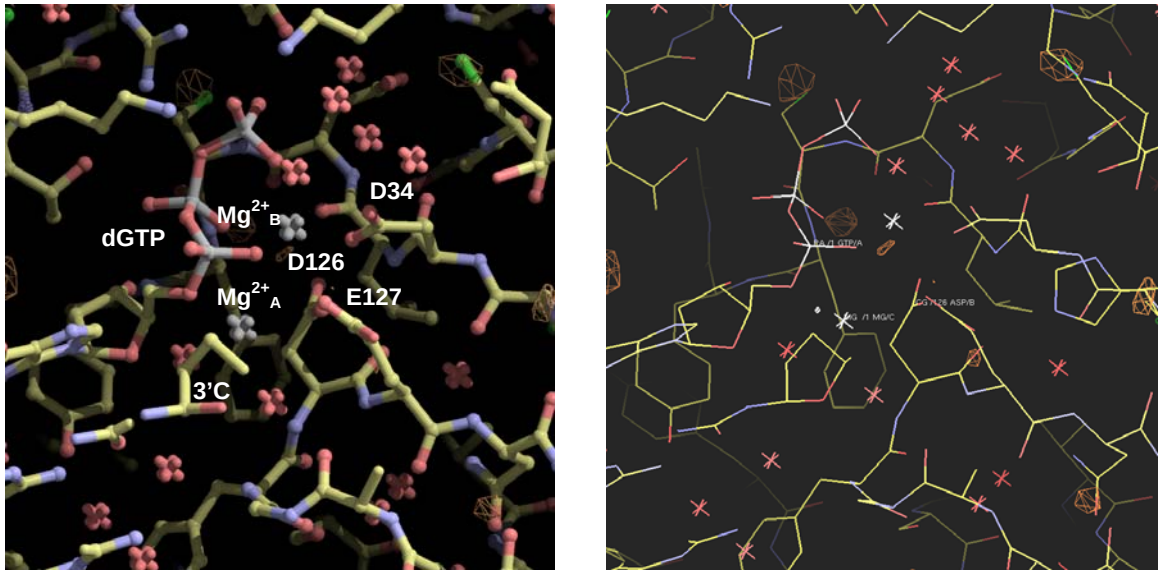

Figure 5S Anomalous map (brown) contoured at 3.3 s with the data collected at the wavelength 1.076 Å. The Mg^{2+} ions (grey stars) are at the active site of T:dGTP which was crystallized without $CaCl_2$. The three catalytic residues D34, D126, E127 are labeled. There is no anomalous density on the metal atoms at either A or B sites. The images are taken from COOT.