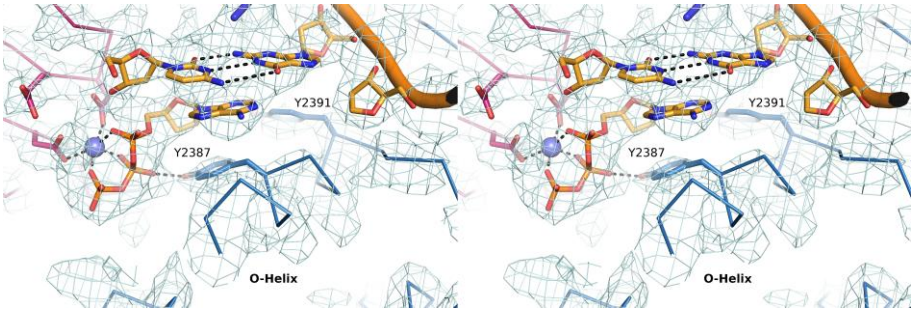
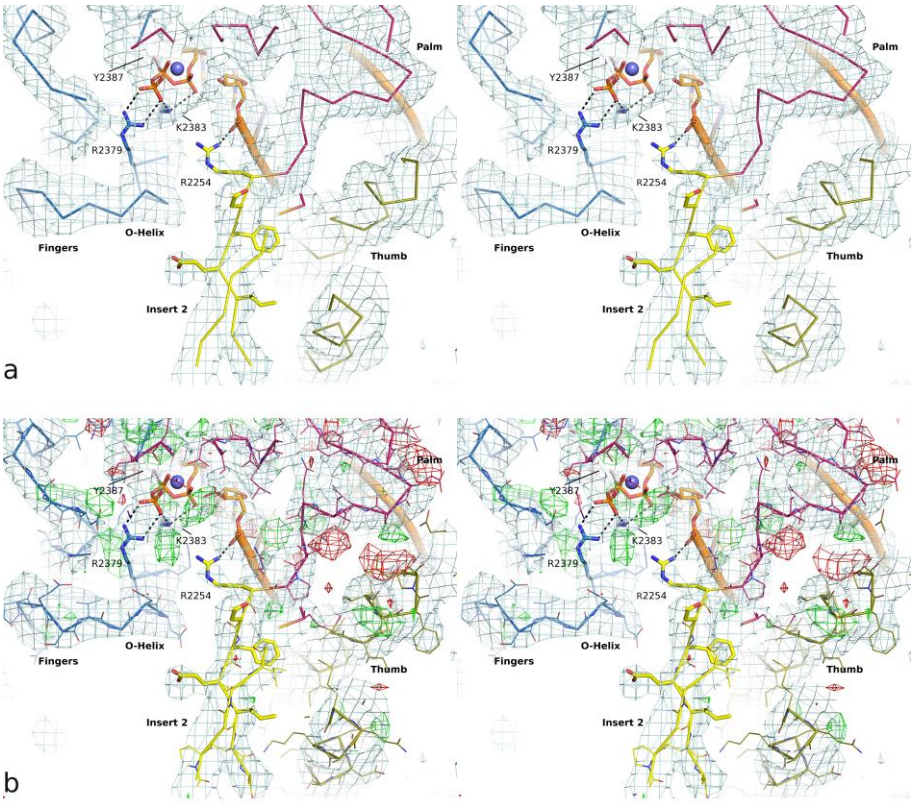


Supplementary Figure 1

Pol θ helix O and incoming ddATP.

The cross-crystal NCS-averaged density-modified 2mFo-DFc electron density map (light blue mesh), calculated between THF–ddATP and dCMP–ddGTP data sets, is shown at a level of 1.3 σ in a wall-eyed stereo view about the active site, including the C-terminal region of helix-O, where a potential hydrogen bond is indicated (black dashes) between the hydroxyl group of Y2387 and the β-phosphate of the incoming ddATP.



Supplementary Figure 2

Pol θ active site and insert 2.

(a) The NCS averaged 2mFo-DFc electron density map (light blue mesh), calculated with regard to each of the four molecules of the ASU in the THF–ddATP structure, is contoured at 1.0 σ about insert 2 and the triphosphate tail of the incoming ddATP, and displayed in a wall-eyed stereo view. Interactions from conserved basic residues R2379 and K2383 are shown (black dashes). The putative salt bridge between R2254 of insert 2 (yellow) and the 3'-terminal phosphate of the primer strand stabilizes the primer terminus. **(b)** Thin amino acid side chains and the NCS averaged mFo-Dfc residual electron density map (red and green meshes), contoured at a level of ±3.5 σ, are added to the view appearing in panel a. The residual peak evident near the primer terminal 3'-OH suggests that an unmodeled metal ion might reside with partial occupancy in metal binding site A.

Supplementary Table 1

Subdomain	Insertion loop	Insertion length (aa)	Residue start	Residue end
N-terminal Exo-like			S1819	N2090
	Exo1	36	I1859	T1898
	Exo2	22	Q1918	T1940
Thumb			G2093	E2217
	Insert 1	31	L2143	F2177
	Insert 2	58	R2254	S2313
Palm (segment 1)			K2218	P2253
…continued			M2314	S2332
Fingers			Q2333	Q2474
Palm (segment 2)			G2475	V2590
	Insert 3	33	S2497	G2531

Delimitation of subdomains and insertion elements in human pol θ

Supplementary Table 2

Insertion loop	Sequence
Exo1	IRSLTSSKTATIGSRFKQASSPQEIPIRDDGFPIKGCDDT
Exo2	QKEQKHSEISASLVPPSLDPSLT
Insert 1	LPPNREMKNQGSKKTLGSTRRGIDNGRKLRLGRQF
Insert 2	RDFEIKMPTLVGESPPSQAVGKGLLPMGRGKYKKGFSVNPRCQAQMEERAADRG MPFSIS
Insert 3	STFKSHGHREGMLQSDQTGLSRKRKLQGMFCPIRG

Amino acid sequences of human pol θ insertion elements