Supplementary Information

Structural basis of high fidelity DNA synthesis by yeast DNA polymerase delta

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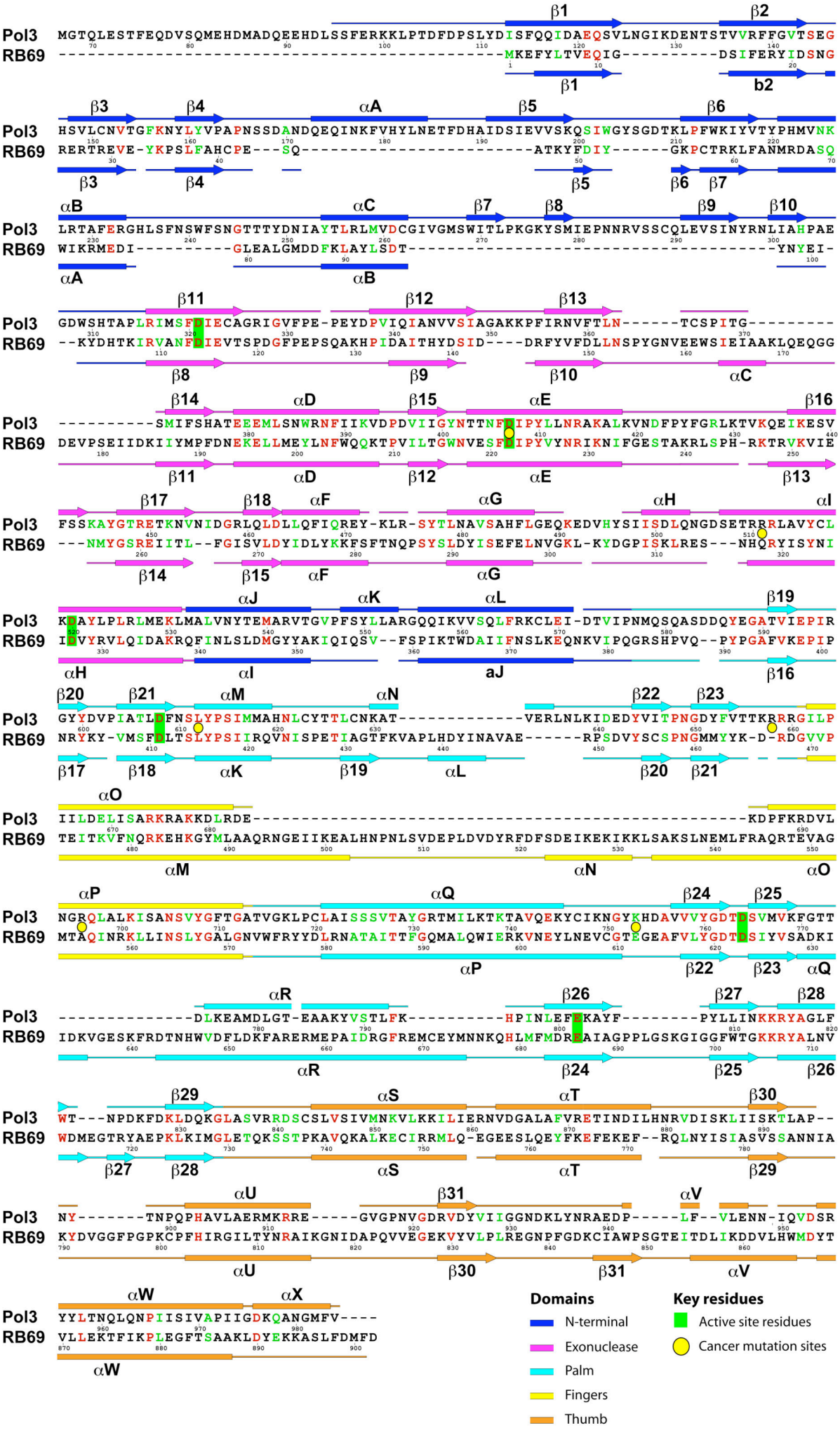
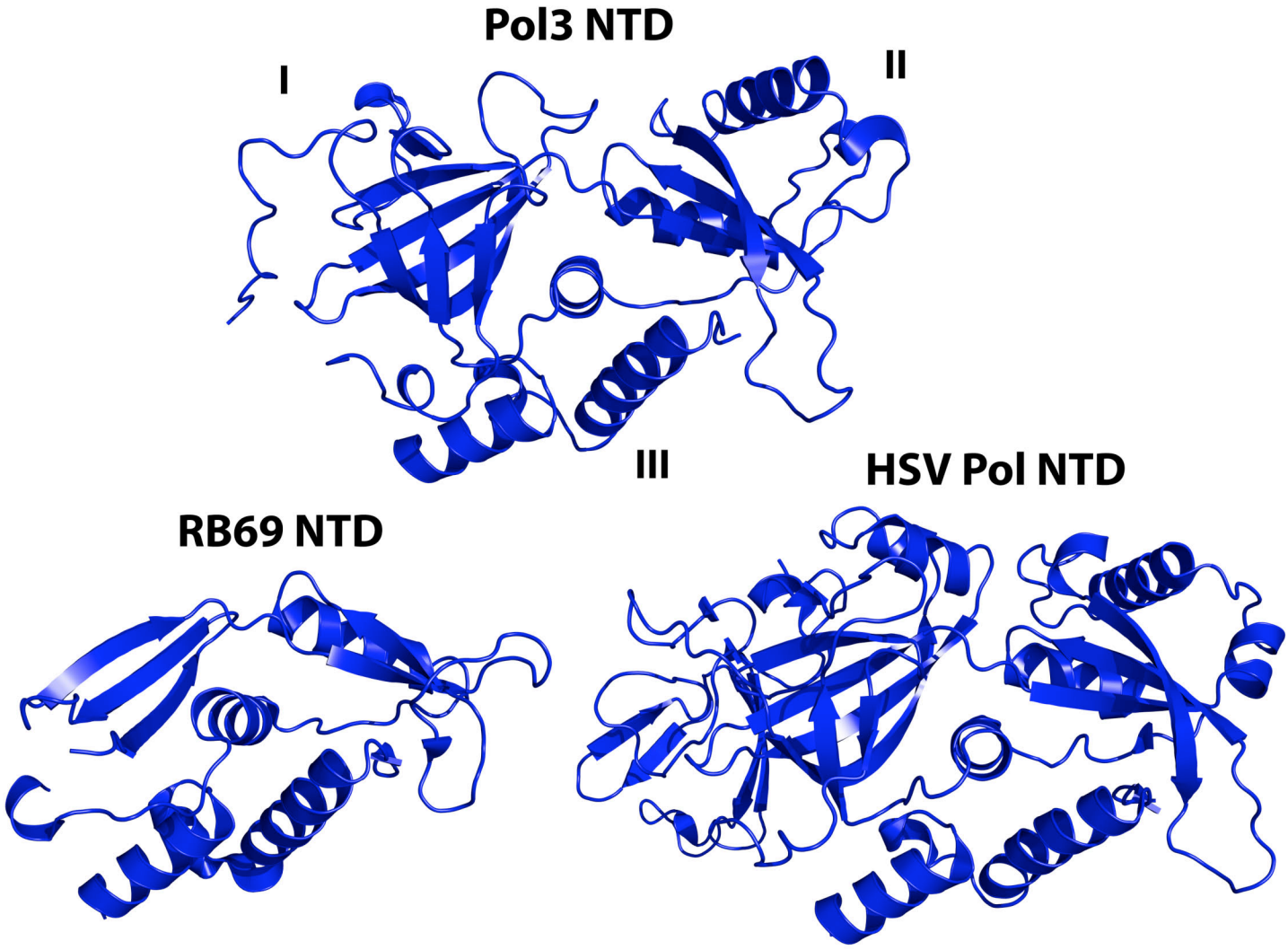
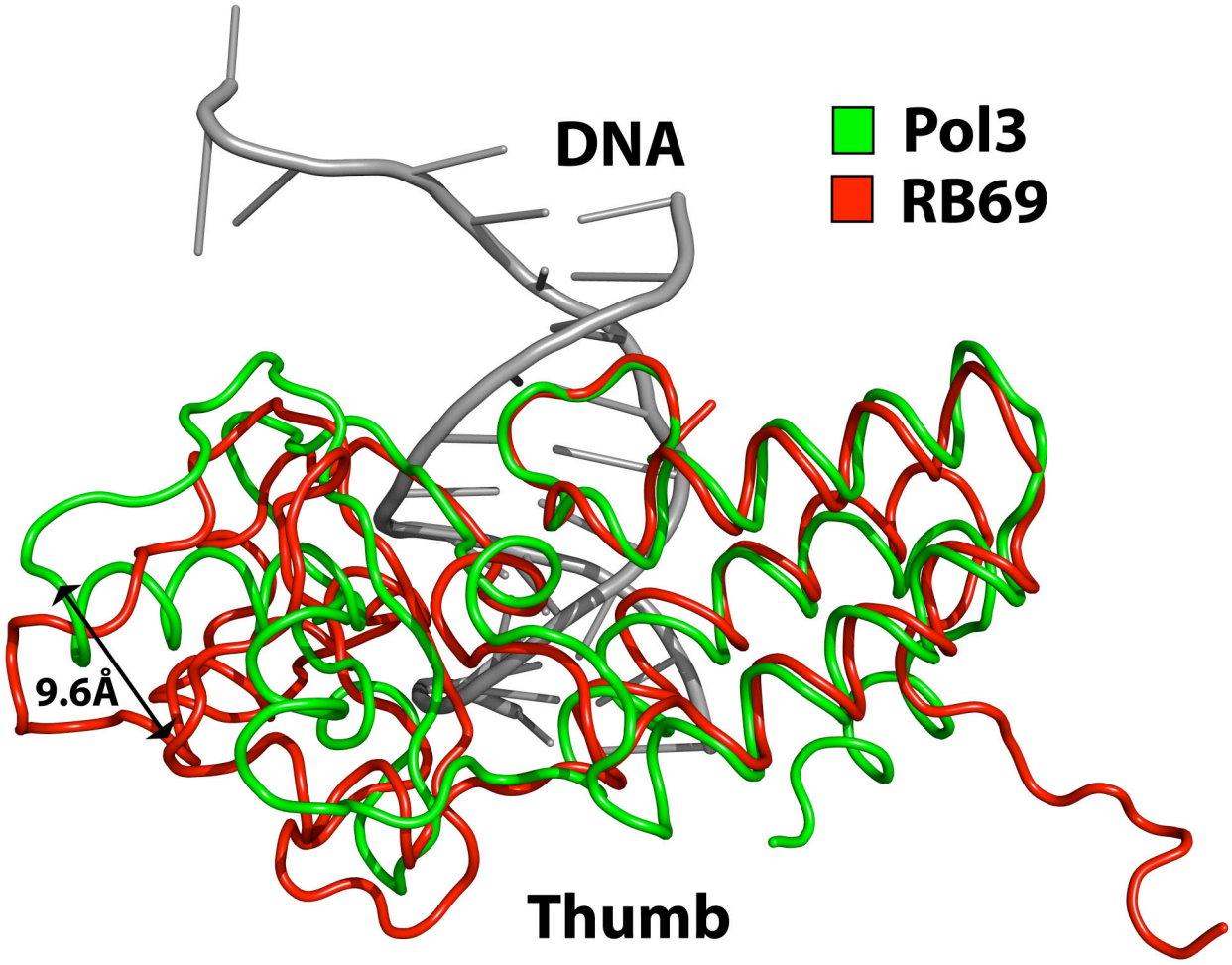


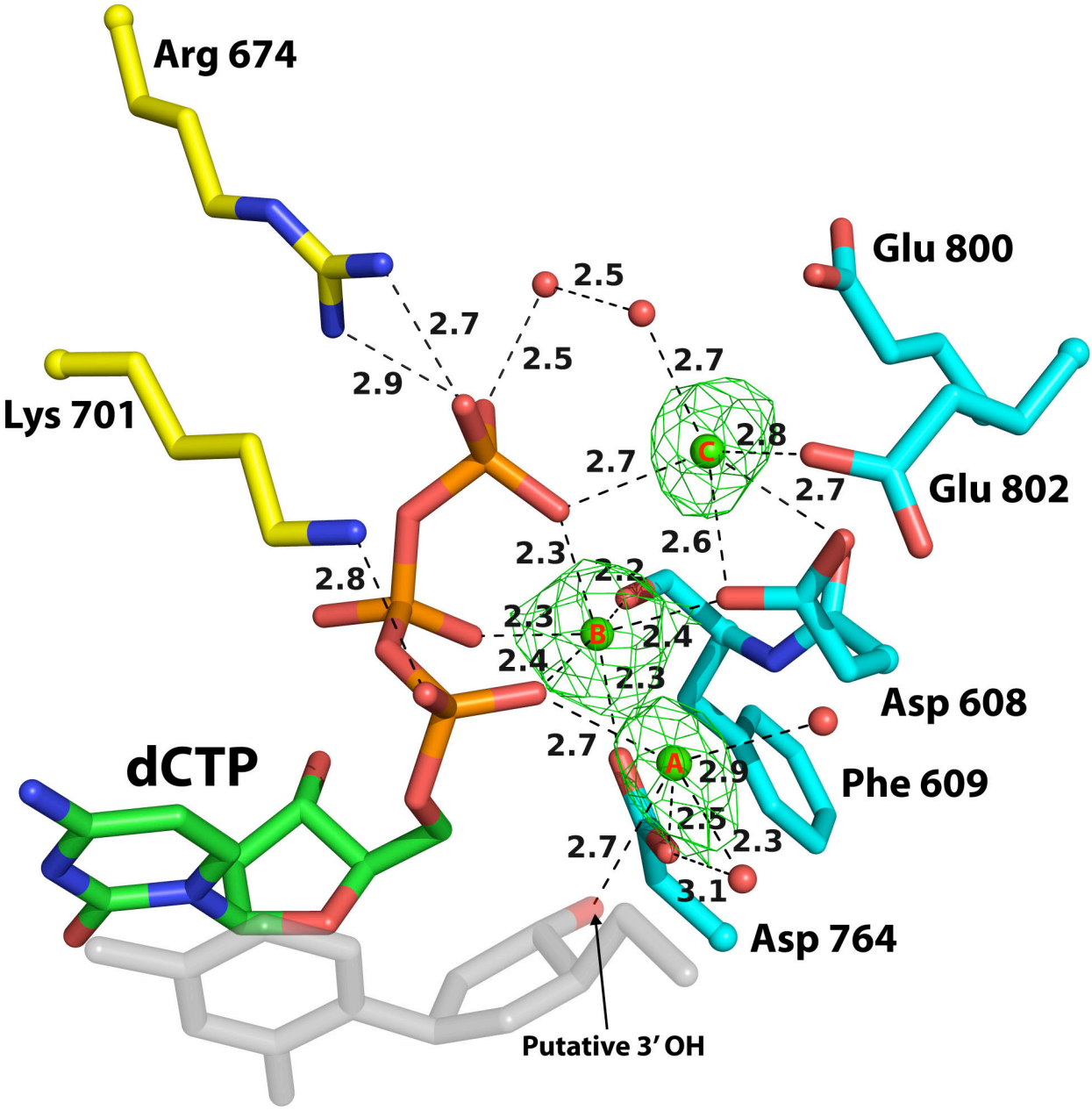
Supplementary figure 1: Structure based sequence alignment of Pol3 and RB69 Pol. The secondary structural elements are displayed above (Pol3) or below (RB69) the sequence. Identical residues are highlighted in red and semi-conserved residues are shown in green. Also highlighted are the active site residues in Pol3 and RB69, and the cancer mutations in Pol3.



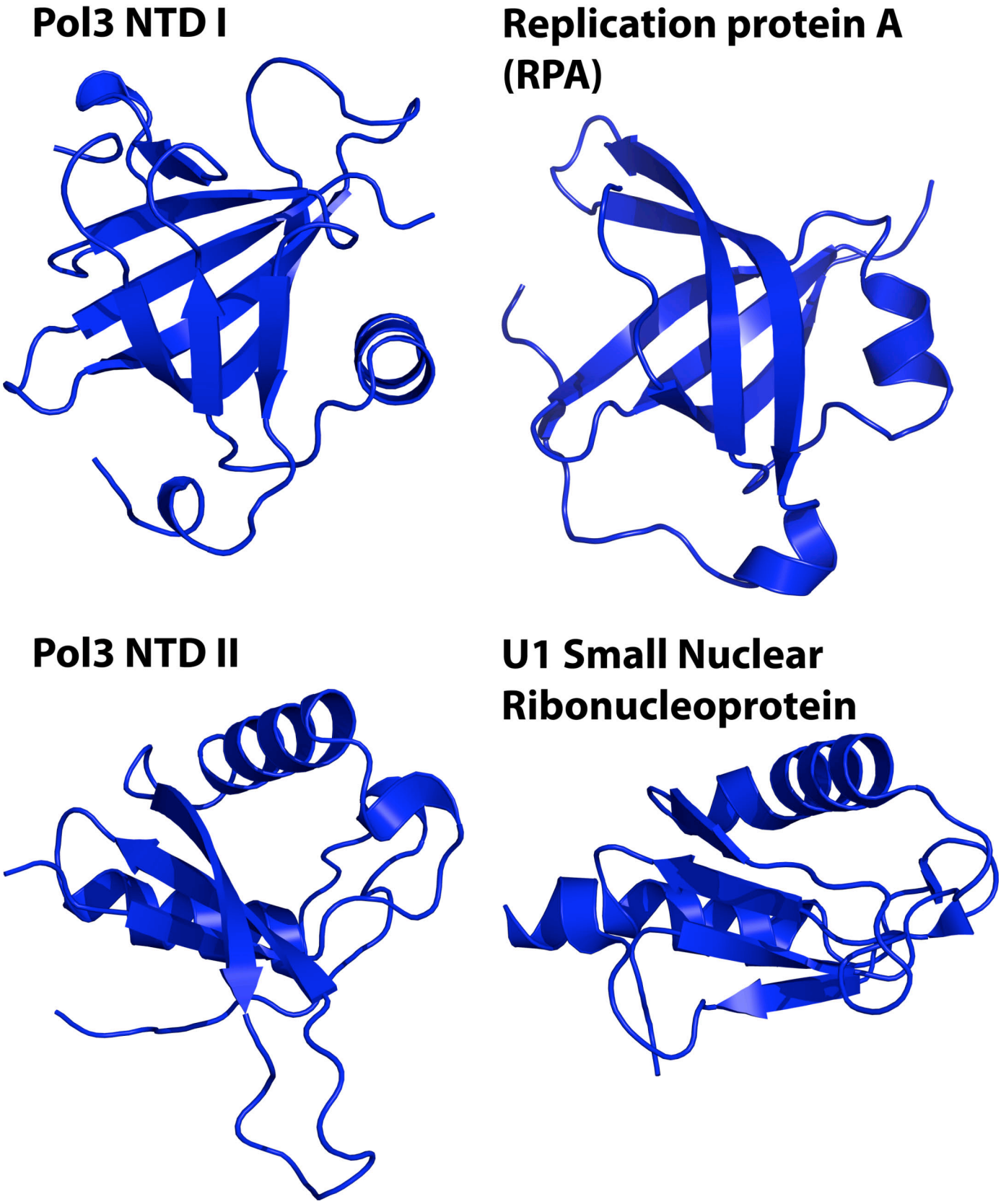
Supplementary figure 2: Comparison of the NTD in Pol3, RB69 Pol, and the Herpes Simplex Virus 1 (HSV) Pol. The NTD in Pol3 is composed of three motifs (I, II and III). It is much more elaborate and extended than in RB69 Pol and bears a strong resemblance to the NTD in the HSV Pol.



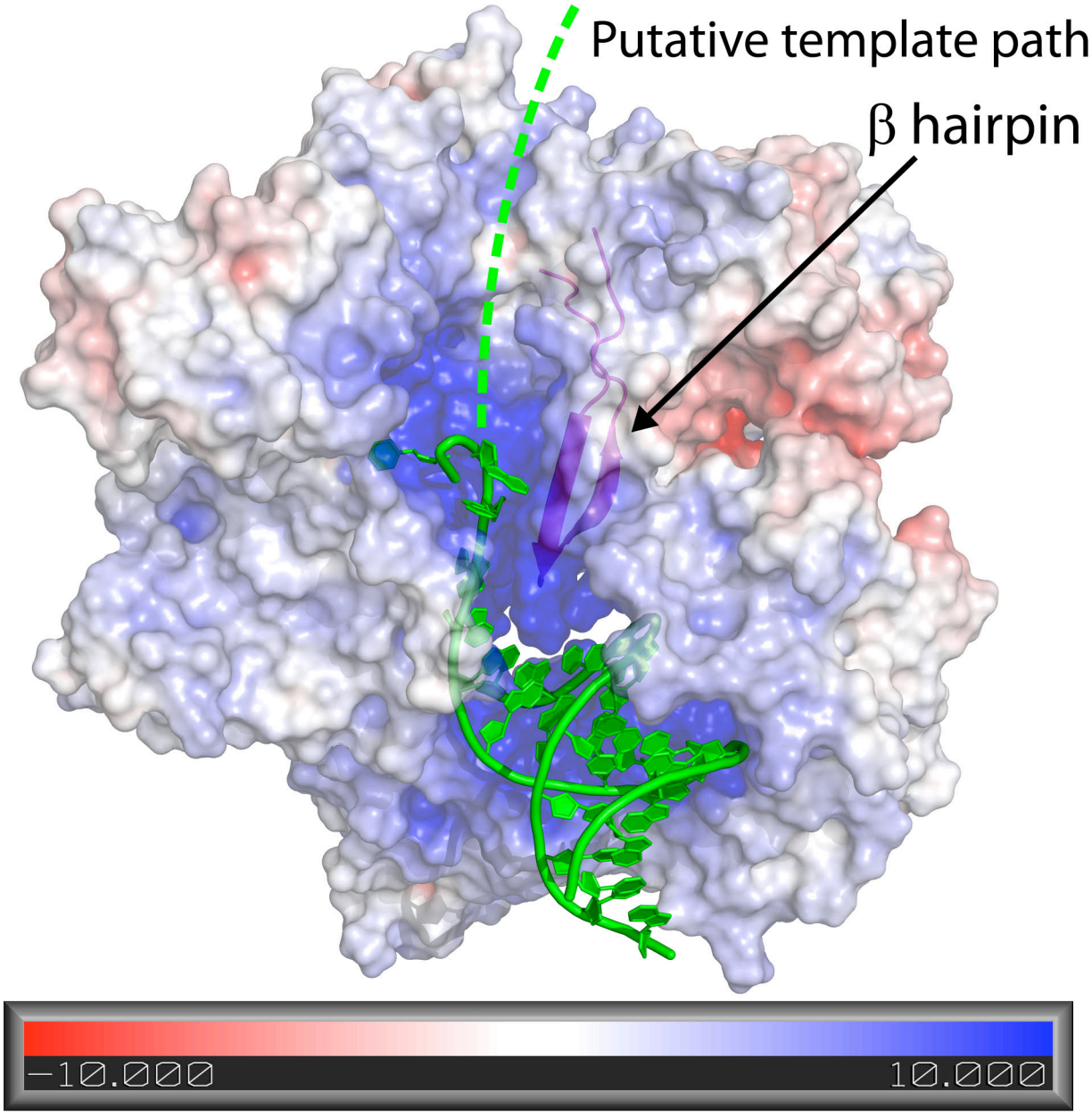
Supplementary figure 3: Comparison of Pol3 and RB69 Pol thumb domains. Compared to RB69 Pol, the tip of the thumb in Pol3 is shifted "upwards" towards the 5' end of the template by >9Å.



Supplementary figure 4: Close-up view of metal ion coordination in the Pol3 active site. The putative Ca2+ ions (A, B and C) are shown in green with composite Fo-Fc omit map (contoured at 1.5σ; 2.0Å resolution) density. Highlighted and labeled are incoming dCTP, the basic residues that interact with dCTP (R674 and K701), and the acidic D608, D764, E800, and E802. The water molecules are shown as red spheres and the putative 3'OH is shown in pink. The ligation distances are in Angstroms.



Supplementary figure 5: NTD motifs I and II. Motif I in Pol3 has a similar structure and topology to the single stranded DNA binding domain of replication protein A (RPA; PDB code 2B3G); motif II in Pol3 resembles the RNA binding motif (RNP or RRM) in the U1 small nuclear ribonucleoprotein (PDB code 2NZ4).



Supplementary figure 6: Putative path of the template strand in Pol3. The electrostatic potential is mapped on the Pol3 surface with increasing blue signifying increasing electropositivity and increasing red indicating increasing electronegativity. The template-primer is shown in green, and the hypothetical path of the "upstream" unpaired template strand is shown as a dashed line passing through a channel in motif I of the NTD, with the β-hairpin (highlighted in purple) acting as a "guide rail" along part of the channel.

Supplementary Table 1. Kinetics of nucleotide incorporation opposite templates G and T by exonuclease deficient yeast wild type Pol3 and the $E_{800}A$ $E_{802}A$ mutant Pol3

Pol3 exo-	Template: incoming dNTP	kcat (min^{-1}) x 10^{-2}	K_m (μM)	Catalytic efficiency k_{cat}/K_m	Efficiency relative to wild type
Wild type	G :dC	1.6 ± 0.2	0.9 ± 0.4	1.8×10^{-2}	-
$E_{800}A$ $E_{802}A$	G :dC	0.45 ± 0.03	10.7 ± 2.6	4.2×10^{-4}	2.3×10^{-2} (↓43x)
Wild type	T :dA	2.6 ± 0.1	0.3 ± 0.05	8.7×10^{-2}	-
$E_{800}A$ $E_{802}A$	T :dA	0.48 ± 0.01	1.1 ± 0.2	3.6×10^{-3}	4.1×10^{-2} (↓24x)
Wild type	T :dG	3.3± 0.4	490 ± 140	6.7×10^{-5}	-
$E_{800}A$ $E_{802}A$	T :dG	n.d.[a]	≥ 500	1.5×10^{-6}	2.2×10^{-2} (↓45x)

[a] Since the amount of product formed remained linear throughout the dNTP concentrations, the efficiency was determined from the slope of the line.