Supplementary Materials

Table S1. Sequences of primers and oligonucleotides used in this study.

Primer name	Oligonucleotide sequence (5′→3′)	Tm
Cloning of *polTt72* gene to expression vectors		
polTt72-NdeI-F	CGAATTCCATATGGACAAAGACCTTTTGGGATAC	62.0 °C
polTt72-BamHI-R	CGCGGATCCTTAAAGAACATTATTAAAACATTCTTCAC	61.2 °C
polTt72-SalI-R	CGCGTCGACAAGAACATTATTAAAACATTCTTCAC	61.0 °C
Cloning of *polTt72* gene to pHSG576		
polTt72-PstI-F	CGATTCCTGCAGCTATGGACAAAGACCTTTTGGGATAC	66.6 °C
polTt72-BamHI-R	CGCGGATCCTTAAAGAACATTATTAAAACATTCTTCAC	61.2 °C
Cloning of *polTth* gene to pHSG576		
polTth-SalI-F	CGATTCGTCGACATATGGAGGCGATGCTTCCGCTCTTTGAACCCA	71.8 °C
polTth-EcoRI-R	GCGGAATTCCTAACCCTTGGCGGAAAGCCAGTCCTCCCCCATCC	74.8 °C
Cloning of *polTaq* gene to pHSG576		
polTaq-SalI-F	CGATTCGTCGACATATGAGGGGGATGCTGCCCCTCTTTGAG	71.5 °C
polTaq-EcoRI-R	GCGGAATTCTCACTCCTTGGCGGAGAGCCAGTCCTC	71.3 °C
Terminal transferase activity assay		
polTt72-Cy3	Cy3-TGGCTGCTTCTAAGCCAACATCCT	57.4 °C
polTt72-blunt	AGGATGTTGGCTTAGAAGCAGCCA	57.4 °C
Microscale thermophoresis		
polTt72-Cy5	Cy5-TGGCTGCTTCTAAGCCAACATCCT	57.4 °C
polTt72-long	CTAGTGAGGATGTTGGCTTAGAAGCAGCCA	63.0 °C

Table S2. Sequences of primers used in site-directed mutagenesis of *polTt72* gene.

Primer name:	Oligonucleotide sequences (5′→3′):	Codon change:
D15A_F	ATACAAAAAGATTGTTGGGATAGCTATAGAAACATGGGACGGTAAAC	GAT → GCT
D15A_R	GTTTACCGTCCCATGTTTCTATAGCTATCCCAACAATCTTTTTGTAT	GAT → GCT
E17A_F	GATTGTTGGGATAGATATAGCAACATGGGACGGTAAACGAG	GAA → GCA
E17A_R	CTCGTTTACCGTCCCATGTTGCTATATCTATCCCAACAATC	GAA → GCA
L27A_F	GGGACGGTAAACGAGGAGGATCAGCTGATCCTTATTATG	CTT → GCT
L27A_R	CATAATAAGGATCAGCTGATCCTCCTCGTTTACCGTCCC	CTT → GCT
D78A_F	GTTGGGCACAACCTAAAGTTTGCCCTTAAGTTCTTTTATATGAACA	GAC → GCC
D78A_R	TGTTCATATAAAAGAACTTAAGGGCAAACTTTAGGTTGTGCCCAAC	GAC → GCC
Y180A_F	GTGAAGCACAAAAAGAGGCCGCAATCAACGATGTTAAG	TAC → GCC
Y180A_R	CTTAACATCGTTGATTGCGGCCTCTTTTTGTGCTTCAC	TAC → GCC

D184A_F	CACAAAAAGAGTACGCAATCAACGCTGTTAAGTACTTAAAAGATTTAGC	GAT → GCT
D184A_R	GCTAAATCTTTTAAGTACTTAACAGCGTTGATTGCGTACTCTTTTTGTG	GAT → GCT
D384A_F	AGGCTATGTTCCTGTATTTGCTGCTTATTCTCAAGTAG	GAT → GCT
D384A_R	CTACTTGAGAATAAGCAGCAAATACAGGAACATAGCCT	GAT → GCT
E389A_F	GCTGATTATTCTCAAGTAGCATTAAGAATTCTTGCC	GAA → GCA
E389A_R	GGCAAGAATTCTTAATGCTACTTGAGAATAATCAGC	GAA → GCA
D615A_F	GGTATATGACATTGACAGTTCACGCTAGTATCTTTTTTGAG	GAT → GCT
D615A_R	CTCAAAAAAGATACTAGCGTGAACTGTCAATGTCATATACC	GAT → GCT
S616A_F	GGTATATGACATTGACAGTTCACGATGCTATCTTTTTTGAG	AGT → GCT
S616A_R	CTCAAAAAAGATAGCATCGTGAACTGTCAATGTCATATACC	AGT → GCT

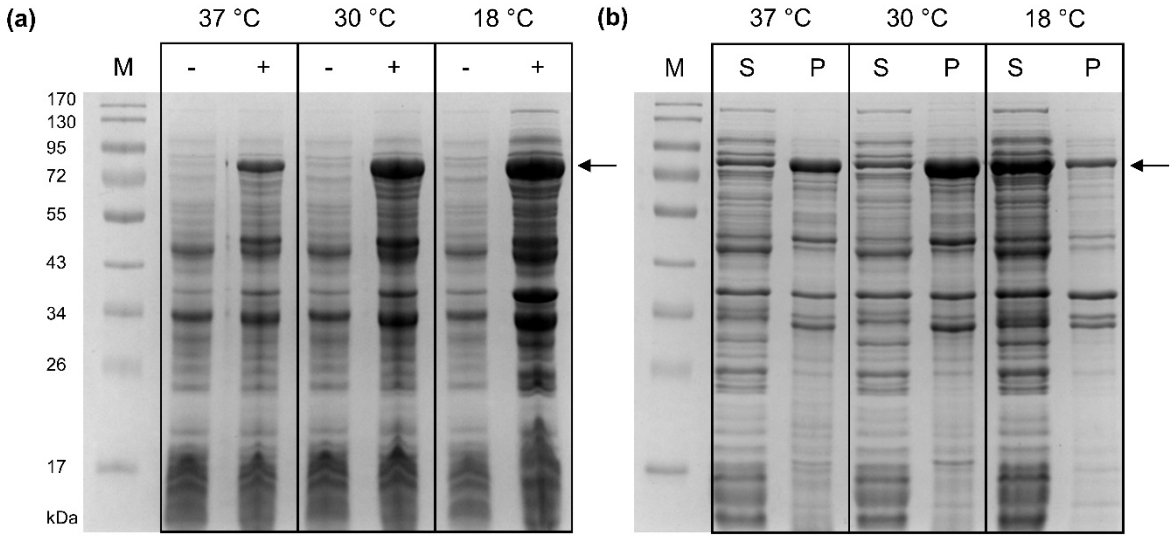

Figure S1. (**a**) Effect of temperature on overproduction of the Tt72 pol in *E. coli* Bl21(DE3)[pRARE][pET15b_polTt72]; (**b**) Solubility analysis of recombinant protein. Proteins were separated in 10% SDS-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue. An arrow indicates the position of the His-tagged recombinant Tt72 pol (82,650 Da); lane (-) before induction; (+) after induction with IPTG (1 mM); S, soluble fraction; P, insoluble fraction; M, protein molecular mass markers (PageRuler™ Prestained Protein Ladder, Thermo Scientific).

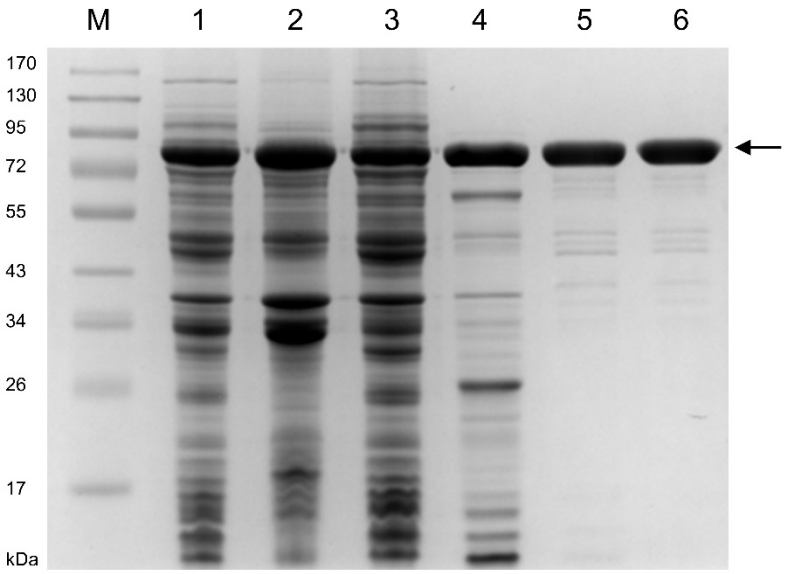

Figure S2. Successive steps in purification of the Tt72 pol. Proteins were separated in 10% SDS-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue. An arrow indicates the position of the His-tagged recombinant Tt72 pol (82.7 kDa); Lane 1, lysate from *E. coli* Bl21(DE3)[pRARE, pET15_polTt72]; 2, the fraction of insoluble proteins; 3, the fraction of soluble proteins; 4, clear cell-lysate after centrifugation and heat-treatment (60 °C, 20 min); (5) HiTrap™ TALON affinity chromatography fraction; (6) HiTrap™ Heparin HP affinity chromatography fraction; (M) protein molecular mass markers (PageRuler™ Prestained Protein Ladder, Thermo Scientific).

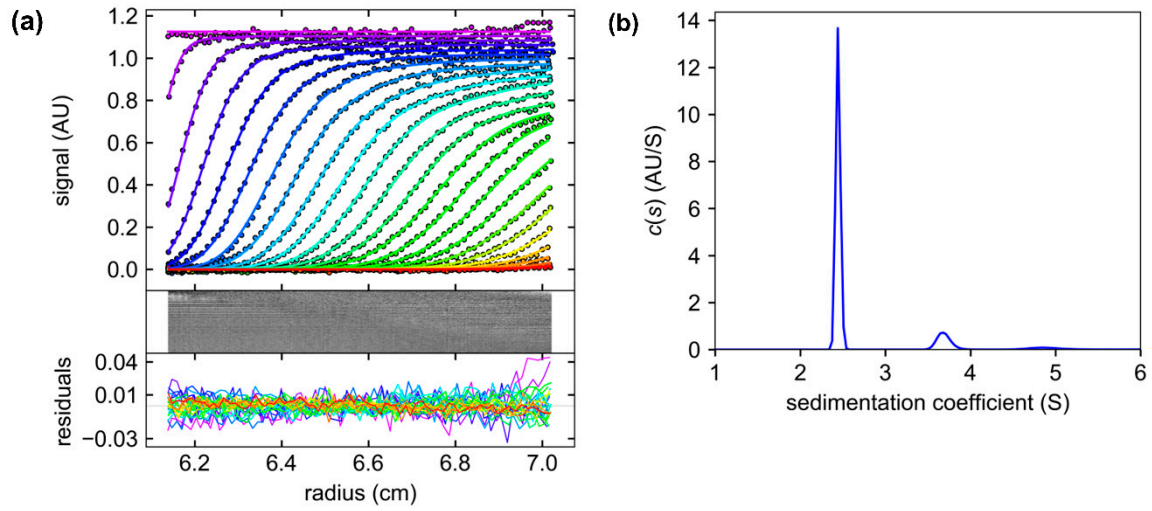

Figure S3. Determination of the Tt72 pol oligomeric state by analytical centrifugation. (**a**) Sedimentation-velocity data (dots) collected for Tt72 pol samples at concentration 1.0 mg/mL. Sedimentation velocity data were overlaid with the best-fit curves (lines) obtained from sedimentation coefficient distribution analysis. For clarity, only every third scan and every third data point is included. Below, the residuals of the experimental fits. (**b**) Sedimentation coefficient distributions [c(s)] of Tt72 pol.

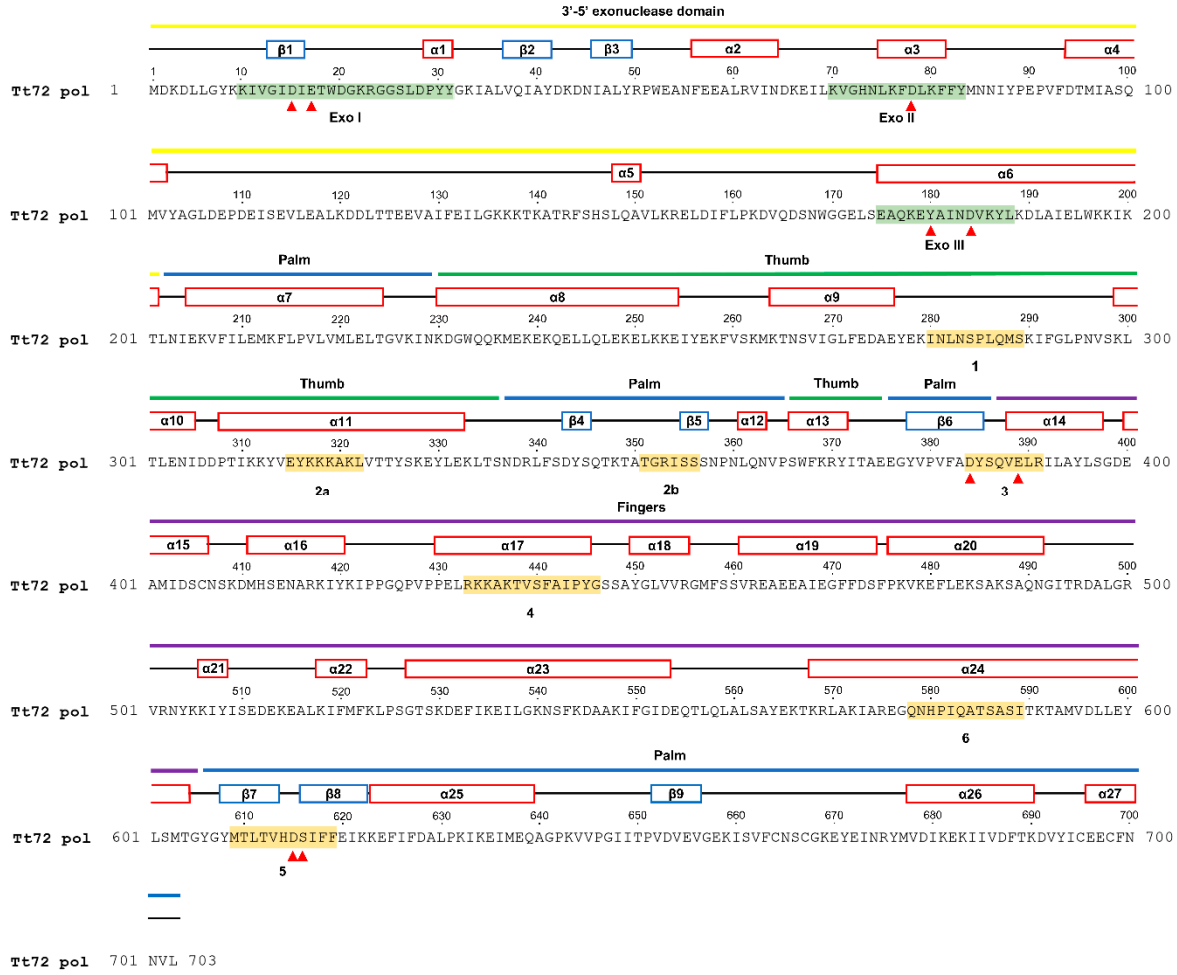

Figure S4. Secondary structure prediction of Tt72 pol. The amino acid sequence of Tt72 pol was subjected to secondary structure meta-server analysis [124], which was plotted as a line for coil, blue boxes for alpha-helices, and yellow boxes for beta-sheets. A colored line above the structural elements indicates the position of domains and subdomains. The yellow line indicates the 3′-5′ exo domain. Blue, green, and purple lines within the nucleotidyltransferase domain indicate palm, thumb, and fingers subdomains. The highly conserved motifs within the amino-acid sequence in both domains are indicated by colored unframed green and yellow boxes. The red triangle indicates the catalytic residues within the 3′-5′ exo and nucleotidyltransferase domain, which importance was tested using site-directed mutagenesis.

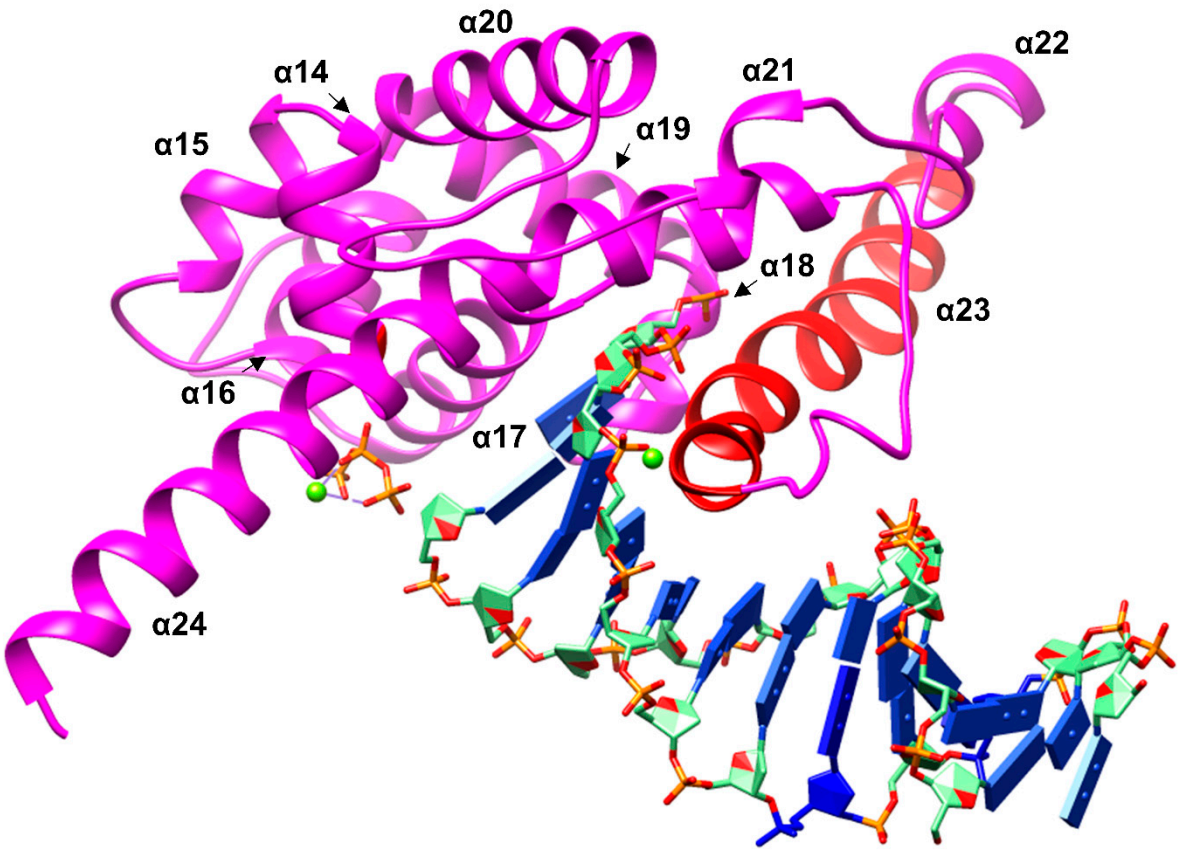

Figure S5. Structural model of subdomain fingers (387-605 aa) of Tt72 DNA polymerase. The unique secondary-structure element *α*-helix (*α*23, 527-553 aa) is shown in red.