Supplemental Material

Increased processivity, misincorporation, and nucleotide incorporation efficiency in *Sulfolobus solfataricus* Dpo4 thumb domain mutations

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Running title: New properties of *S. solfataricus* Dpo4 mutants

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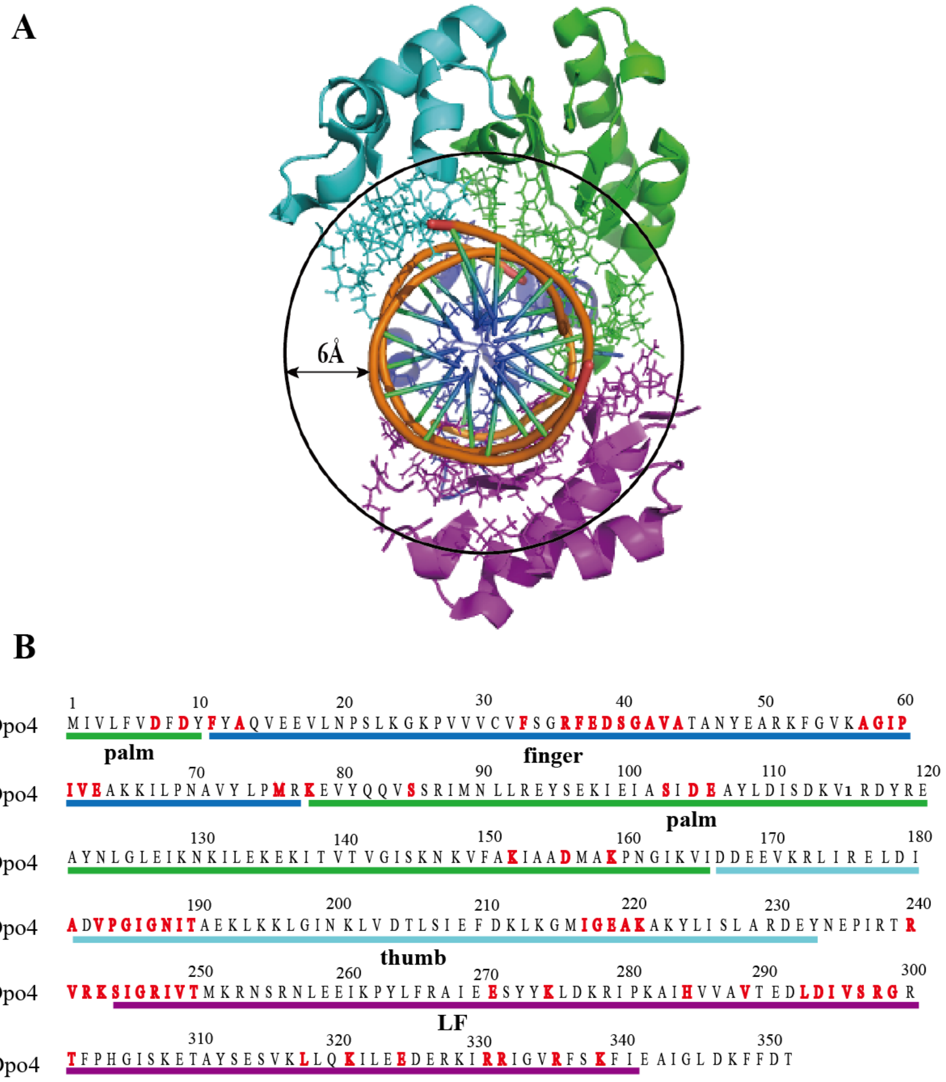

FIG S1 All amino acids residues located within 6Å of the DNA substrate. A, a view down the DNA-helical axis, residues located within 6Å of the DNA substrate were analyzed. B, total 74 residues located within 6Å of the DNA substrate were shown with red high light. Four structural domains in Dpo4 are color coded as follows: green, palm domain; cyan, thumb domain; blue, finger domain; magenta, little finger domain.

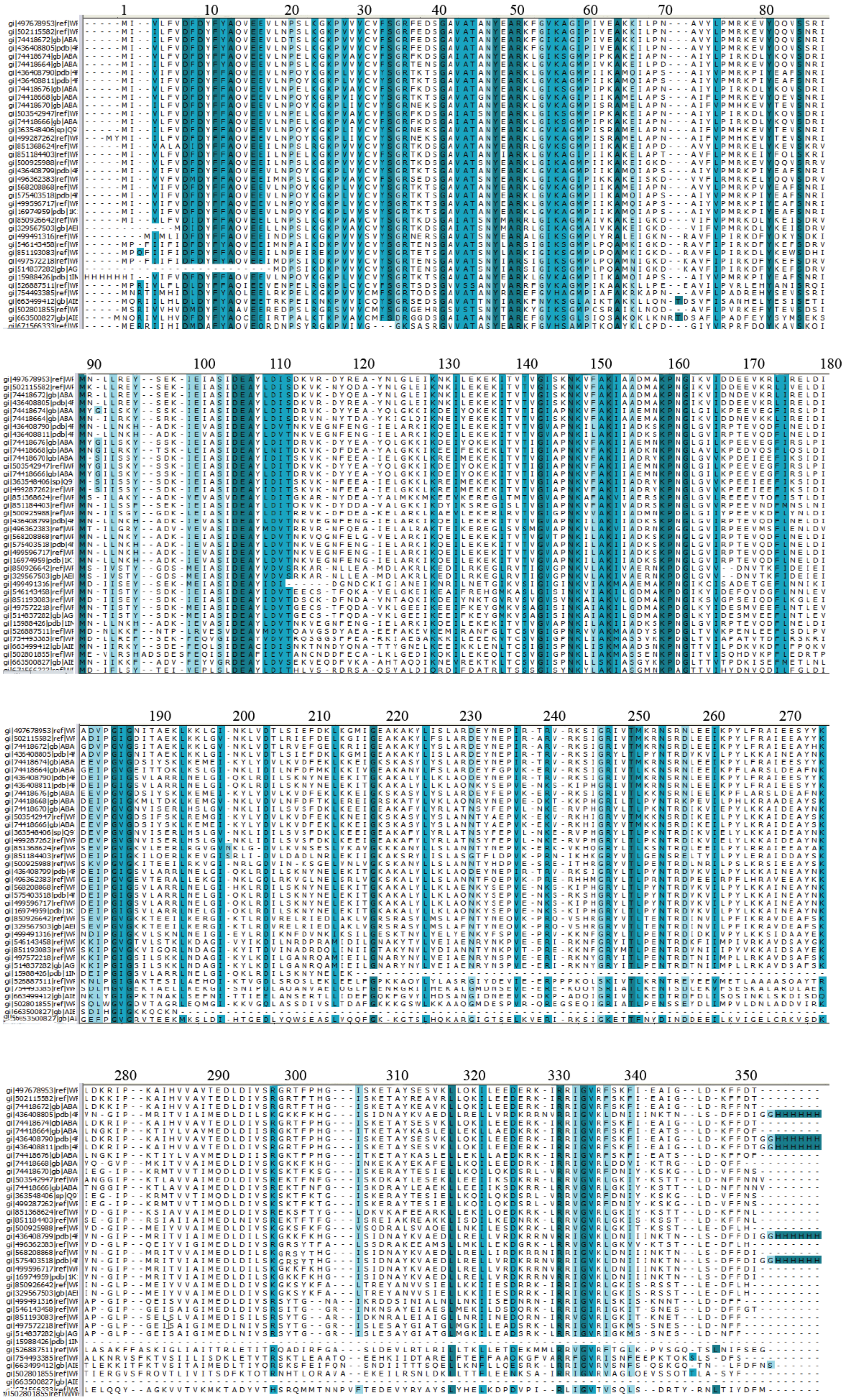
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FIG S2 The multiple sequence alignment of the Y-family of DNAPs. Members of the Y-family were identified by a Blast search in the NCBI database using amino acids Dpo4, from *S. solfataricus*, as the query. More than 500 sequences were initially obtained, but 38 sequences were finally present by the removal of sequences with over 40% identity and lower than 97% identity. Blue and light-blue characters indicate conserved and highly conserved residues surrounding the active site, respectively.

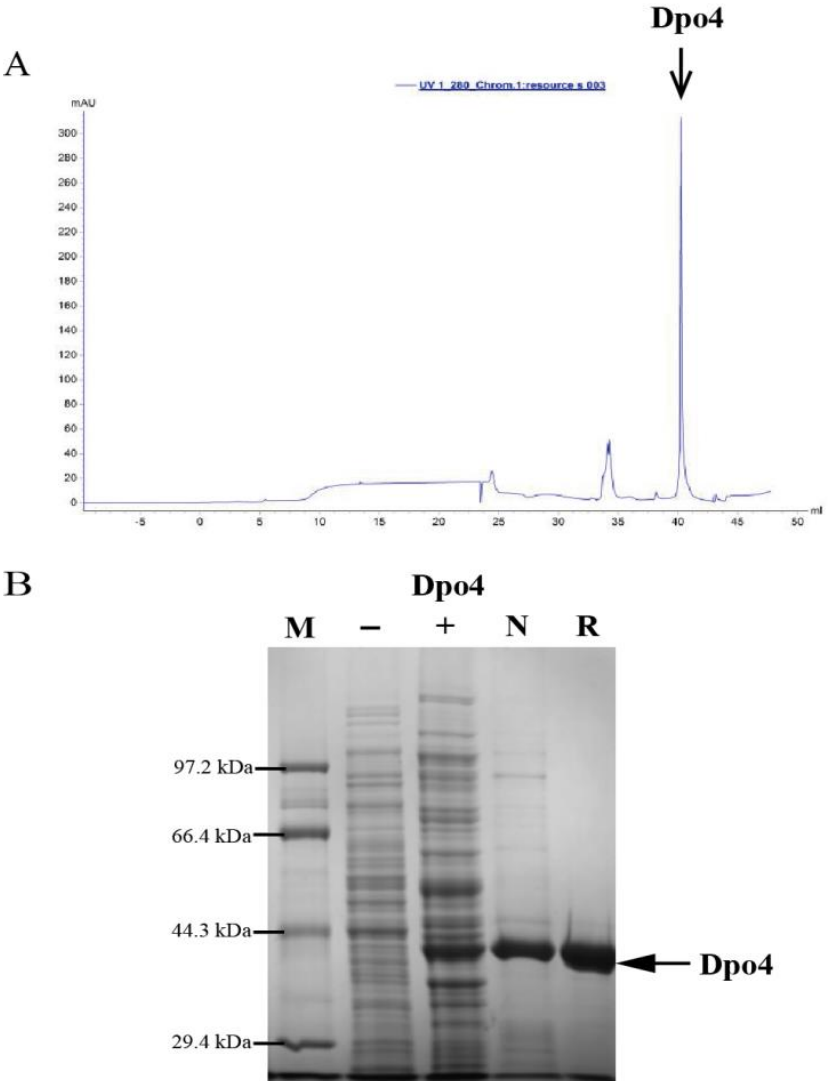

FIG S3 Purification of *S.solfataricus* Dpo4. A, UV$_{280}$ detection for Dpo4 fractions purified by a ResourceS cation-exchange column; B, SDS-PAGE analysis of proteins at various step of the purification are shown: M, protein marker; -, lysate of noninduced cells; +, lysate of IPTG-induced cells; N, Dpo4 fraction after Ni affinity column; R, Dpo4 fraction after ResourceS cation-exchange column.

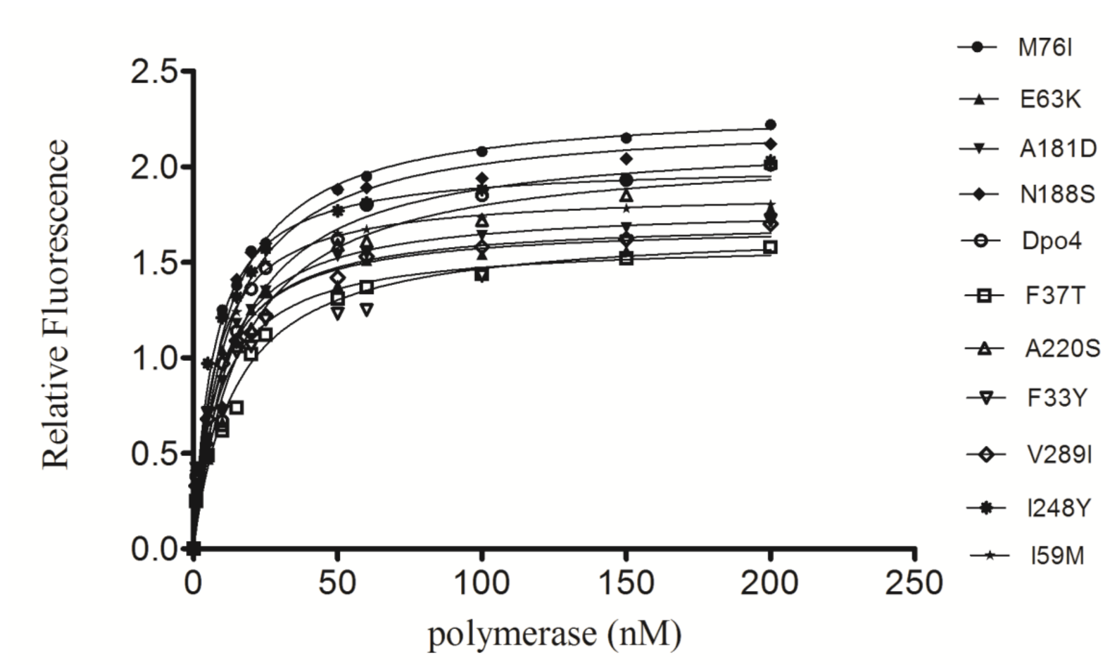

FIG S4 Equilibrium titrations of DNA substrate with Dpo4 and mutants. A constant concentration of 2AP-DNA (10 nM) was titrated with increasing concentration of Dpo4 or mutants (0~200 nM). The fluorescence were excited at 315 nm and emission at 370 nm. Each measurement was repeated three times, and the average value of the fluorescence intensity was recorded. A control experiment was performed with the non-fluorescent DNA under identical conditions. The fluorescence changes from the control experiments were subtracted from the data obtained with the 2AP-DNA, and the corrected values are plotted against polymerase. The analysis of the data yielded the dissociation constant K_d for wtDpo4 and mutants.

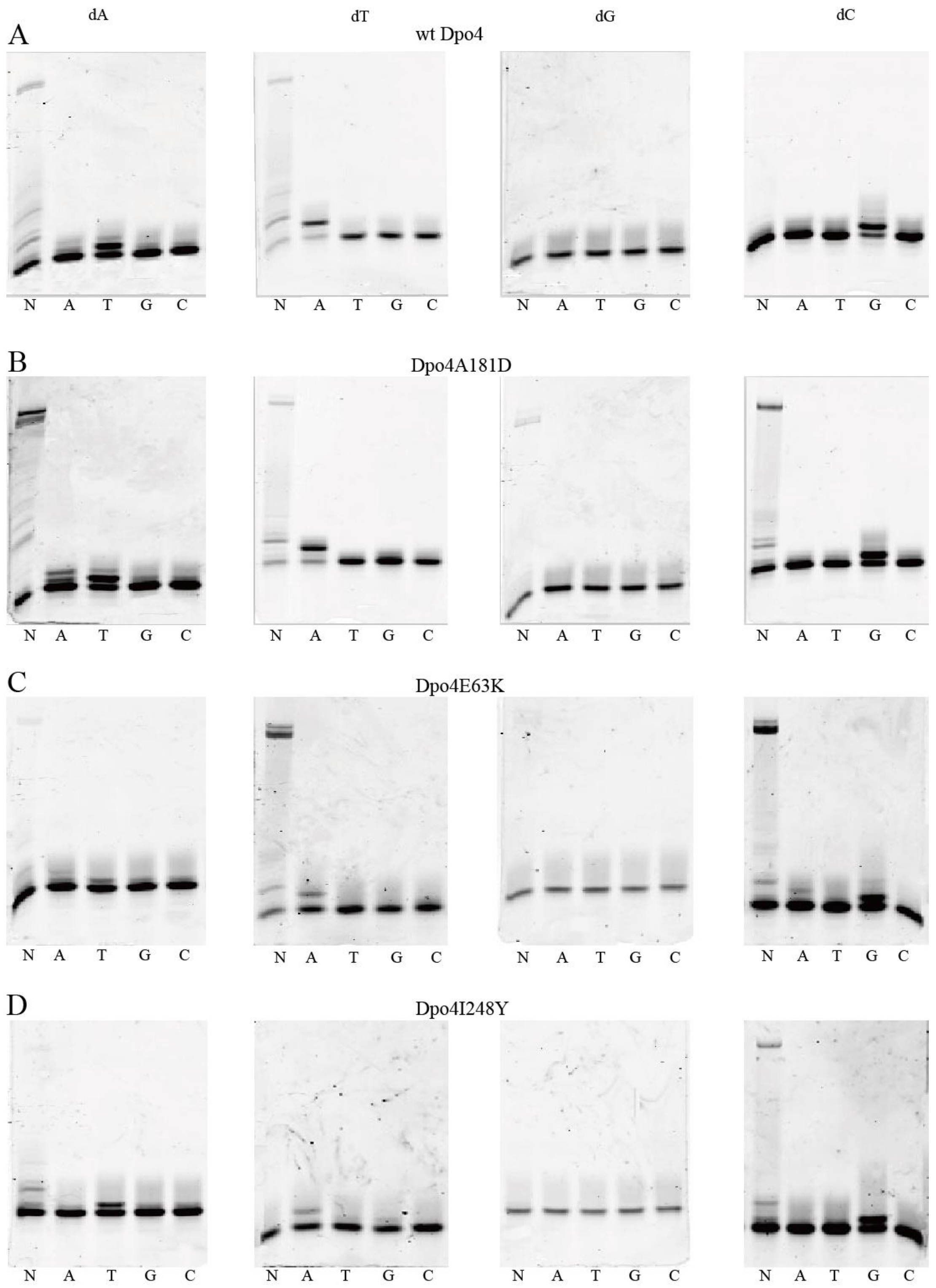

FIG S5 Fidelity profile of wtDpo4 and the mutants. The results for primer extension assay in which wtDpo4 (A), A181D (B), E63K (C), and I248Y mutant (D) were incubated with DNA duplexes (dA, dT, dG and dC) and various dNTPs are shown.

TABLE S1 Non-conserved residues within 6Å of the DNA binding site and mutation frequency

Position sites	Original AA	Mutation AA/frequency	Position sites	Original AA	Mutation AA/frequency
33	F/14	Y/23	220	A/20	S/10, K/4, N/1
36	R/37	K/1	221	K/30	R/5, F/1
37	F/8	T/21, G/2, N/4, S/3	240	R/24	K/12
38	E/16	K/18, S/1, Q/1, G/1, A/1	241	V/12	S/5, T/1, E/8,N/1, Q/2, P/2, I/4, K/1
39	D/18	T/10, N/4, K/3, R/1, H/1, S/1	242	R/20	K/8, V/3, I/2, P/1, A/1, S/1
40	S/36	R/2	243	K/22	I/4,V/3, T/1, H/1, S/2, Q/1, D/1, E/1
42	A/35	V/2, S/1	244	S/11	P/8, H/8, N/5, Q/4
43	V/31	I/7	245	I /14	H/9, Q/1, K/1, R/3 M/1, F/3, Y/3, L/1
44	A/36	S/2	246	G/34	S/2
57	A/24	S/14	247	R/34	K/2
58	G/37	A/1	248	I /13	Y/22, E/1
59	I/7	M/29, L/2	249	V/16	L/13, I/3, M/1, A/2, T/1
60	P/34	A/3, S/1	271	E/25	D/4, S/4, A/1, Q/1,K/1
61	I /31	L/5, T/1, C/1	275	K/34	R/1, Q/1
62	V/7	P/8, I/12, S/4, K/4, Y/1, Q/1, A/1	285	H/6	T/11, Y/6, A/4, S/7, G/1, V/1
63	E/4	K/20, R/5, Q/5, A/1, F/1, T/1, S/1	289	V/7	I/28, K/1
76	M/23	I/9, V/2, A/3, P/1	293	L/33	I/1, F/1, A/1
78	K/33	L/1, R/1, H/1, F/2	294	D/28	N/3, S/1, E/2, T/1, K/1
103	S/36	G/2	295	I /32	T/3, Y/1
156	D/25	E/9, S/2, G/2	296	V/24	L/7, I/3, Y/1, Q/1
181	A/3	D/14, G/4, E/3, S/8, N/2, K/4	297	S/31	T/4, Q/1
183	V/14	I/19, L/4, F/1	298	R/22	K/13, H/1
184	P/34	H/2, Y/1, W/1	299	G/19	S/11, E/4, Q/1, N/1
186	I /19	V/19	301	T/21	K/7, S/6, D/1, Q/1
188	N/5	S/11, K/9, D/6, E/3, T/1, A/1, R/1, P/1	321	K/18	E/10, Q/4, D/1, R/1, N/1, T/1
189	I /9	V/18, S/4, K/6, M/1	325	E/14	R/6, K/4, S/6, A/3, Q/1, P/1, T/1
190	T/14	L/15, I/8, Q/1			
217	I /14	V/6, T/7, L/4, F/5	332	R/35	L/1
219	E/10	K/17, R/3, N/3, P/1, Q/1, T/1	336	R/29	K/6, T/1
			339	K/21	N/11, D/1, G/2, Q/1

TABLE S2 The binding energy and binding affinity of mutants and wtDpo4 to DNA

Mutants	Binding energy ⊿G_{bind} (kcal/mol)	(⊿G_{bind})^{mutants} /(⊿G_{bind})^{wt}	Binding affinity K_d (nM)
wtDpo4	-3966.4	—	14.3±1.1
F33Y	-4066.6	1.03	8.9±0.9
F37T	-3962.9	0.99	14.5±0.7
I59M	-4175.3	1.05	7.1±0.6
E63K	-4119.1	1.04	8.5±0.7
M76I	-4055.1	1.02	10.8±1.2
A181D	-4071.5	1.03	8.8±0.8
N188S	-4030.2	1.02	11.7±1.0
A220S	-3932.7	0.99	16.2±1.0
I248Y	-4176.0	1.05	6.7±0.7
V289I	-4127.9	1.04	8.1±1.2

TABLE S3 The extension length and concentration of each product for each DNAP

DNAP	A	B	C	Average length
wtDpo4	7 nt (3.5%±3.0%)	15 nt (55.8%±1.4%)	20 nt (40.7%±1.6%)	16 nt
F33Y	8 nt (8.5%±2.1%)	15 nt (51.3%±4.2%)	21 nt (40.2%±4.3%)	16 nt
E63K	_	16 nt (37.5%±1.8%)	22 nt (62.5%±1.8%)	19 nt
A181D	_	15 nt (11.7%±5.5%)	21 nt (88.3%±4.8%)	20 nt
I248Y	8 nt (16.6%±0.6%)	16 nt (28.6%±2.4%)	21 nt (54.8%±1.8%)	17 nt
N188S	7 nt (3.7%±2.2%)	14 nt (10.5%±1.6%)	20 nt (85.8%±3.7%)	18 nt
F37T	8 nt (13.3%±3.5%)	15 nt (60.9%±5.2%)	20 nt (25.8%±1.7%)	15 nt
V289I	7 nt (13.5%±1.8%)	14 nt (70.0%±6.0%)	19 nt (16.5%±4.2%)	13 nt
I59M	7 nt (5.4%±0.4%)	14 nt (34.4%±3.4%)	19 nt (60.2%±3.8%)	16 nt
A220S	8 nt (15.1%±3.9%)	15 nt (64.2%±0.3%)	19 nt (20.7%±3.6%)	14 nt
M76I	9 nt (24.0%±1.4%)	15 nt (48.0%±0.7%)	19 nt (28.0%±2.1%)	14 nt