Supplementary Table 1 | Catalytic efficiency of dC and dT incorporation opposite dG by WT and mutant Pol ν

DNA / Pol ν75	Correct (G – dCTP)			Incorrect (G – dTTP)			Fidelity
	K_M (μM)	k_{cat} (min^{-1})	k_{cat} / K_M (M^{-1} min^{-1})	K_M (μM)	k_{cat} (min^{-1})	k_{cat} / K_M (M^{-1} min^{-1})	dC/dT selectivity
13/19mer-G / WT	149±6	62.4±1.2	0.42 x 10^6	627±82	14.4±1.0	2.3 x 10^4	18.3
Coldspot / WT	16.7±0.8	54.8±0.8	3.3 x 10^6	294±17	5.8±0.1	2.0 x 10^4	165
Hotspot / WT	7.9±0.9	10.4±0.3	1.3 x 10^6	86±6	14.5±0.3	16.9 x 10^4	7.7
Coldspot / K679A	9.7±0.3	103±2	10.6 x 10^6	386±87	2.3±0.1	6.0 x 10^3	1782
Hotspot / K679A	8.8±0.4	15.1±0.2	1.7 x 10^6	422±47	11.2±0.6	2.7 x 10^4	63.0
Hotspot / E675R	3.7±0.3	1.4±0.1	0.38 x 10^6	35±5	2.7±0.1	7.7 x 10^4	4.9
Hotspot / ΔIns2	21.4±0.7	13.2±0.1	0.62 x 10^6	240±7	14.4±0.2	6.0 x 10^4	10.3

13/19mer-G	5' GAGTCAACAGGTC 3' CTCAGTTGTCCAG**G**TATGG
G(-66) coldspot	5' TGAGTGAGCTAACT 3' ACTCACTCGATTGA**G**TGTAATTAA
G(164) hotspot	5' GCGATCGGTGCGGGC 3' CGCTAGCCACGCCCG**G**AGAAGCGA

Supplementary Table 2 | Catalytic efficiency of WT and ΔIns2 Pol ν with looped out primers

	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (M^{-1} min^1)	Relative efficiency (%)	
Normal DNA				to WT & normal DNA	
WT	245±20	16.2±0.50	66.1 x 10^3	100	
ΔIns2	789±47	17.4±0.57	22.1 x 10^3	33.3	
P[3,1] DNA					to WT & P[3,1]
WT	251±12	7.0±0.1	27.9 x 10^3	42.2	100
ΔIns2	459±41	2.3±0.1	5.0 x 10^3	7.6	17.9
P[3,2] DNA					to WT / P[3,2]
WT	369±22	2.9±0.1	7.9 x 10^3	12.0	100
ΔIns2	310±53	0.4±0.02	1.3 x 10^3	2.0	16.4%