Supplementary Data For:

Structural evidence for an essential Fe-S cluster in the catalytic core domain of DNA polymerase ε

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Content:

Supplemental Table 1: Data collection and refinement statistics.

	$Pol2_{CORE}$-1-1228	$Pol2_{CORE}$-1-1187	$Pol2_{CORE}$-yeast-Anom
Data Collection	PDB ID 6h1v	PDB ID 6qib	PDB ID 6h1v
Spacegroup	C2	C2	C2
Cell parameter			
a,b,c (Å)	148.2, 70.0, 149.9	158.4 70.4 154.2	146.9, 70.3, 149.5
α,β,γ (°)	90, 109.4, 90	90, 113.0, 90	90, 109.1, 90
Wavelength	0.9840	0.96770	1.7360
Resolution range (Å)	73.0-2.70	78.5-2.80	19.9-6.00
Outer shell (Å)	2.80-2.70	2.92-2.80	8.00-6.00
Completeness (%)[a]	98.6(97.5)	98.5(99.2)	94.9(95.3)
Redundancy[a]	2.9(2.7)	4.3(4.5)	1.8(1.8)
I/σ(I)[a]	5.1(0.9)	7.8(1.3)	24.0(20.0)
R_{meas} (%)[a]	13.8(156.0)	14.4(123.0)	3.3(4.3)
CC1/2[a]	0.972(0.144)	0.995(0.447)	0.998(0.997)
SigAno[a]	-	-	1.21 (1.10)
Refinement			
Resolution (Å)	2.7	2.8	-
No. of reflections	39250	36257	-
R_{work}(%)/R_{free}(%)	23.9 (26.6)	22.2 (28.6)	-
No. of atoms			
Protein	8428	8608	-
DNA	528	528	-
dATP	30	30	-
Metal	1	1	-
Water	6	0	-
4Fe-4S	8	8	
B factors			
Protein	65.8	74.2	-
DNA	55	59.3	-
dATP	31.5	41.9	-
Metal	44.1	46.4	-
Water	38.6	-	-
4Fe-4S	73.5	77.7	
R.m.s deviations			
Bond length (Å)	0.005	0.003	-
Bond angle (°)	0.717	1.057	-
Ramachandran plot			
Most favored (%)	93.8	94.2	-
Additional allowed (%)	6.1	5.2	-

[a]Values in parentheses are for the outer shell

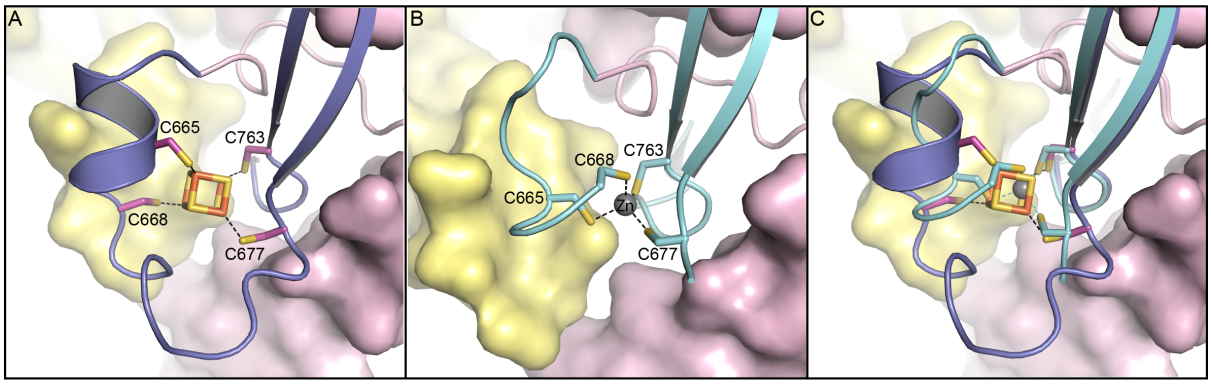

Figure S1. Comparison of the CysX-site with or without a bound Fe-S cluster. A) Zoomed-in view of the $Pol2_{CORE}$ structure (PDB ID: 6qib) showing the four cysteines of the CysX motif (as magenta sticks) in the P-domain (in slateblue cartoon) coordinating the four Fe atoms of the [4Fe-4S] cluster. The N-terminal domain and the palm domain are in yellow and pink surface representations, respectively. (B) The previously solved structure (PDB ID: 4m8o (1)) in a similar orientation shows the four cysteines of the CysX motif in the P-domain (in cyan) coordinating a Zn ion. C) The previous structure with Zn^{2+} (PDB ID: 4m8o (1)) superimposed over the new structure containing the Fe-S cluster (PDB ID: 6qib). Formation of an helical structure places two cysteine residues, Cys665 and Cys668 in perfect geometry to allow cluster binding.

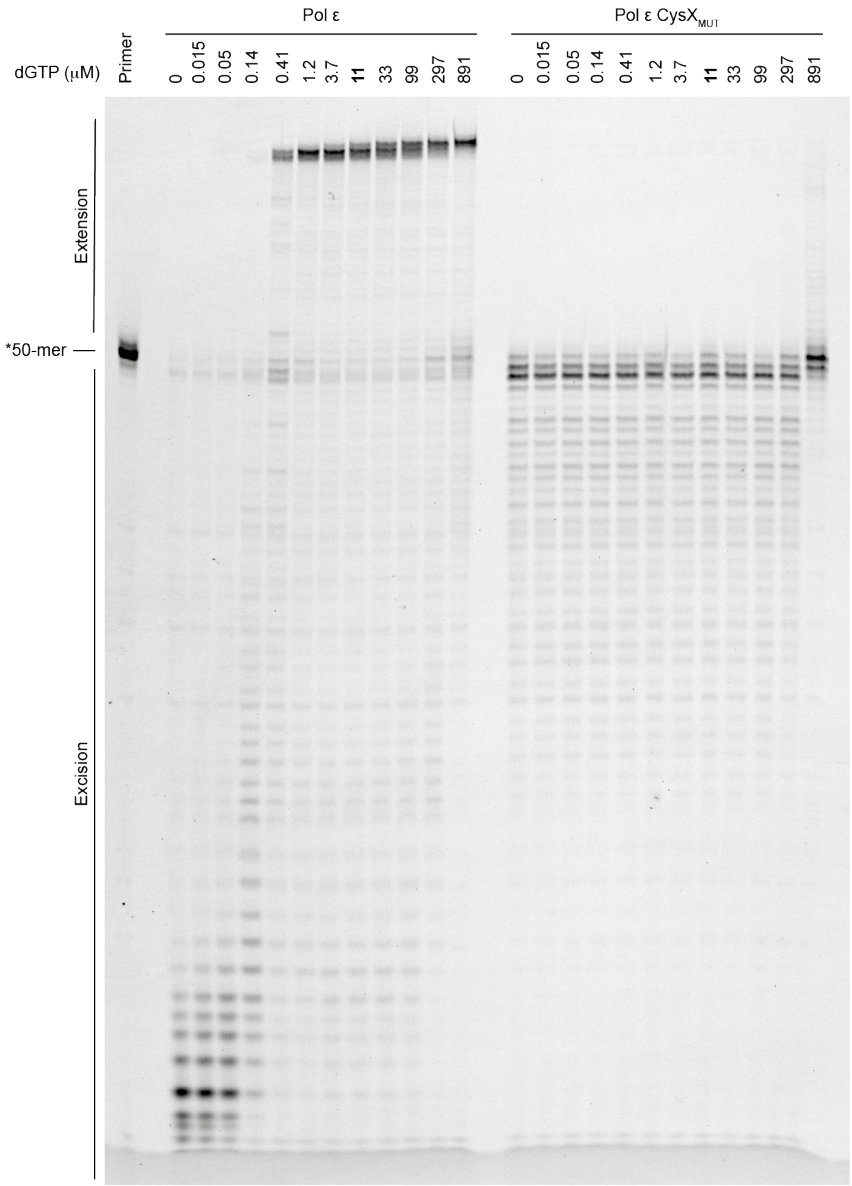

Fig. S2: Effect of dNTP concentration in primer extension assays with Pol ε and Pol ε CysX$_{MUT}$. The exonuclease activity in Pol ε CysX$_{MUT}$ can be suppressed but only at the highest dNTP concentrations. At 11 μM dGTP, there is also 22 μM dATP, 39 μM dCTP and 66 μM dTTP in the reaction mix, which mimics the physiological concentrations for yeast (2). The effect of a three-fold change in concentration for all four dNTPs is observed in each lane. Primer extension assays in the presence of varying amounts of dNTPs with a DNA substrate consisting of a fluorescently labeled 50-mer primer annealed to a perfectly matched 80-mer template.

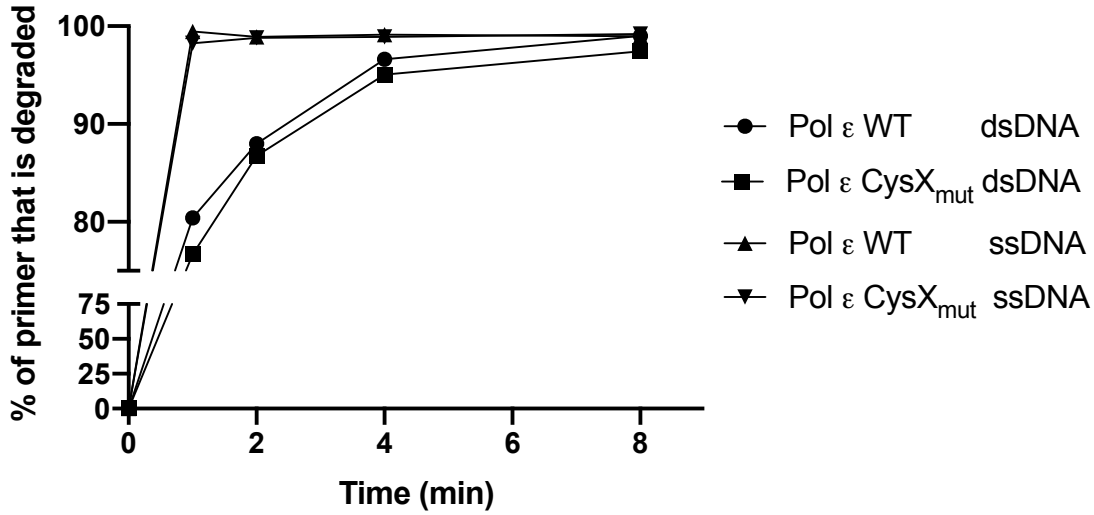

Fig. S3: The shortening of a full-length oligonucleotide occurs at similar rates by Pol ε $CysX_{MUT}$ and Pol ε $CysX_{MUT}$. Quantification of the observed exonuclease activity of Pol ε and Pol ε $CysX_{MUT}$ on a correctly matched 50/80-mer (dsDNA) or a single stranded 50-mer (ssDNA) substrate in figure 5.

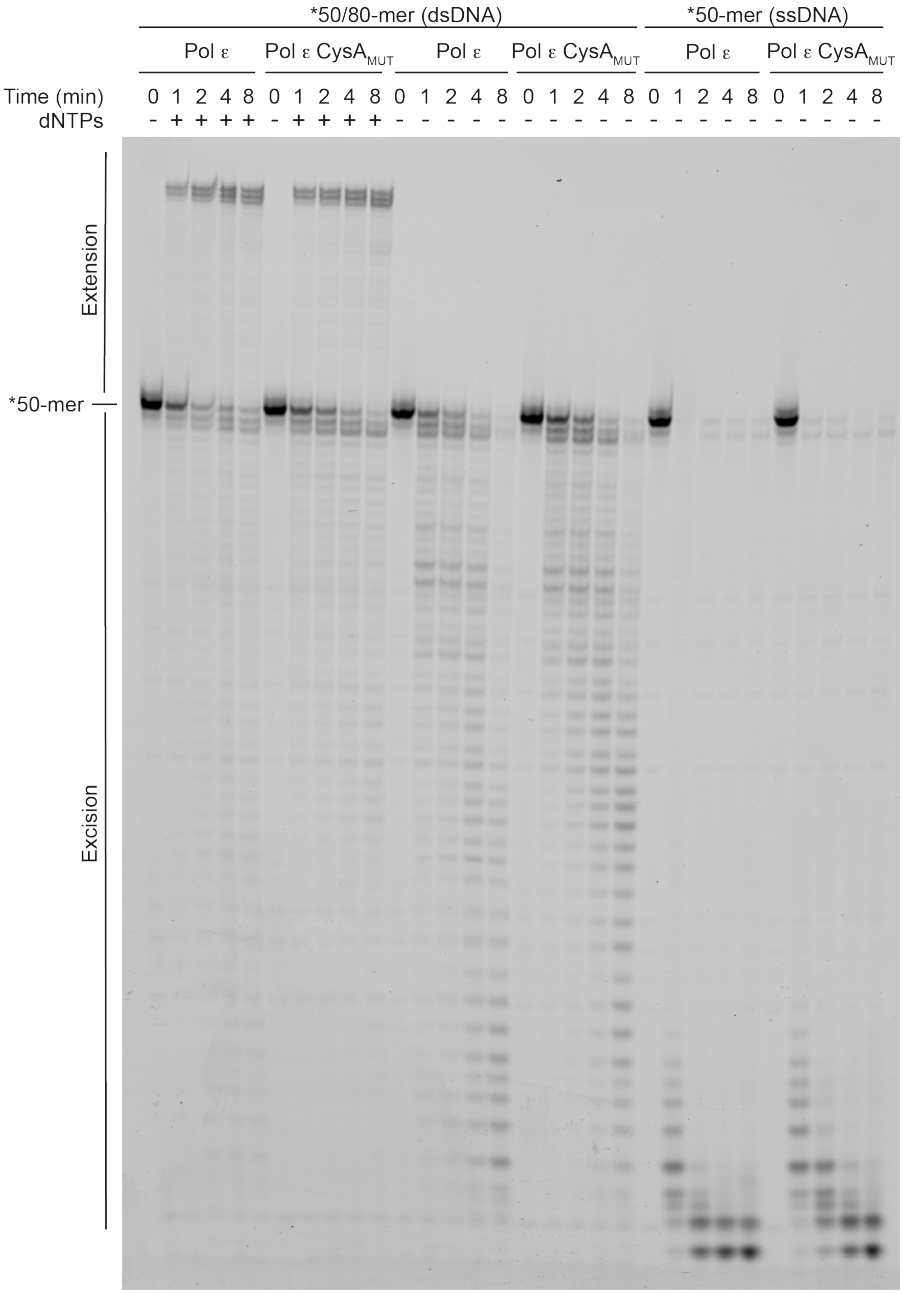

Figure S4. Impact of the substitutions in the CysA motif (C2111S/C2133S) on Pol ε polymerase and exonuclease activity. Primer extension assays in the presence of dNTPs with a DNA substrate consisting of a fluorescently labeled 50-mer primer annealed to a perfectly matched 80-mer template. The exonuclease activity was assayed in the absence of dNTPs using the same double-stranded DNA substrate or only the single-stranded 50-mer.

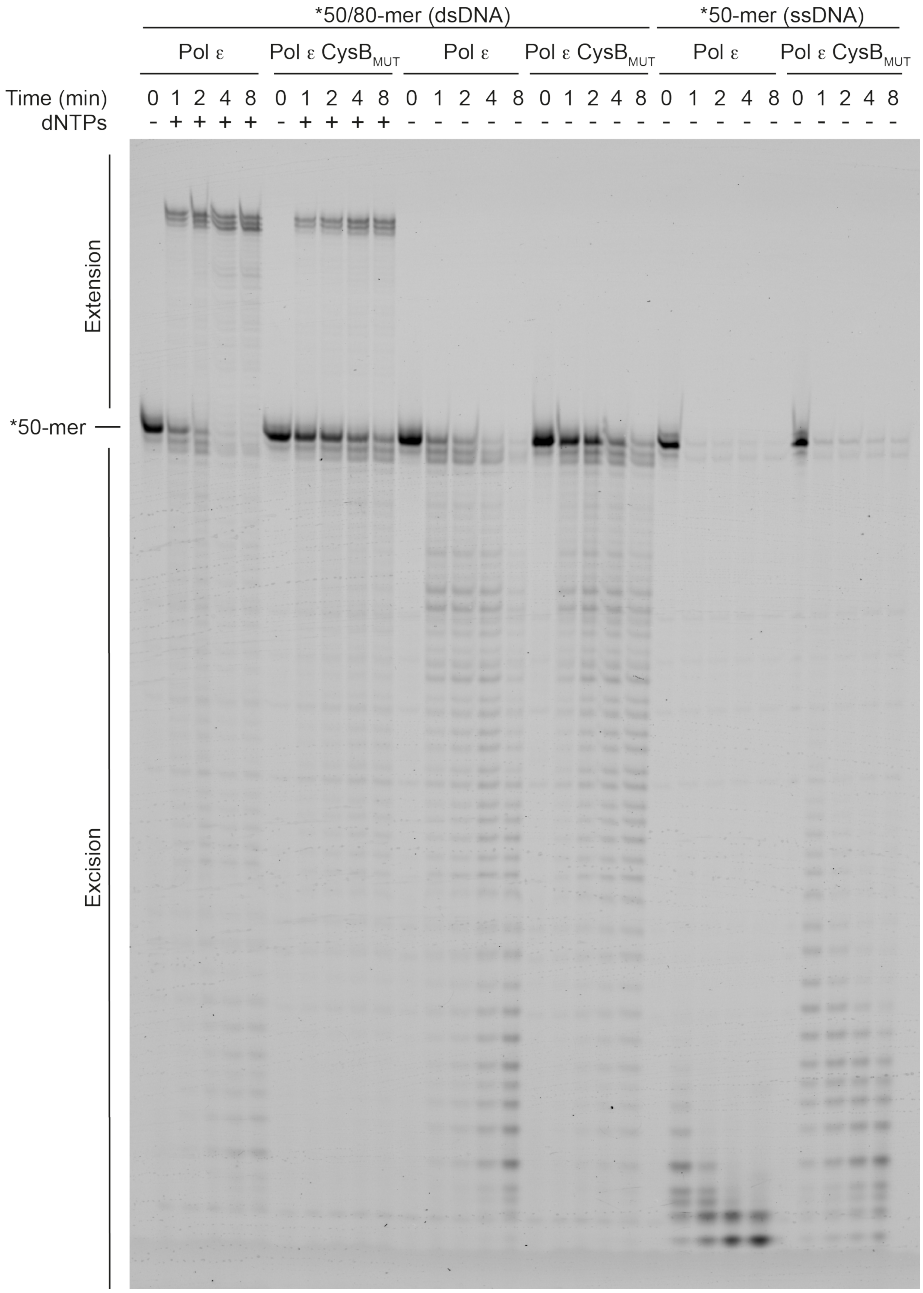

Figure S5. Impact of the substitutions in the CysB motif (C2167S/C2181S) on Pol ε polymerase and exonuclease activity. Primer extension assays in the presence of dNTPs with a DNA substrate consisting of a fluorescently labeled 50-mer primer annealed to a perfectly matched 80-mer template. The exonuclease activity was assayed in the absence of dNTPs using the same double-stranded DNA substrate or only the single-stranded 50-mer.

References

1. Hogg,M., Osterman,P., Bylund,G.O., Ganai,R.A., Lundström,E.-B., Sauer-Eriksson,A.E. and Johansson,E. (2014) Structural basis for processive DNA synthesis by yeast DNA polymerase ε. *Nat. Struct. Mol. Biol.*, **21**, 49–55.

2. Sabouri,N., Viberg,J., Goyal,D.K., Johansson,E. and Chabes,A. (2008) Evidence for lesion bypass by yeast replicative DNA polymerases during DNA damage. *Nucleic Acids Res.*, **36**, 5660–5667.