

SUPPLEMENTARY MATERIAL

Yeast DNA polymerase ζ maintains consistent activity and mutagenicity across a wide range of physiological dNTP concentrations

Olga V. Kochenova^{1†}, Rachel Bezalel-Buch^{2†}, Phong Tran³, Alena V. Makarova^{2‡}, Andrei Chabes³, Peter M. J. Burgers² and Polina V. Shcherbakova^{1*}

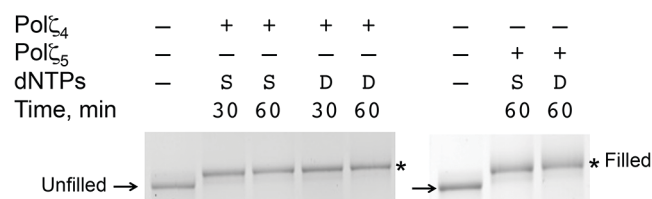
¹ Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68198, USA

² Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, USA

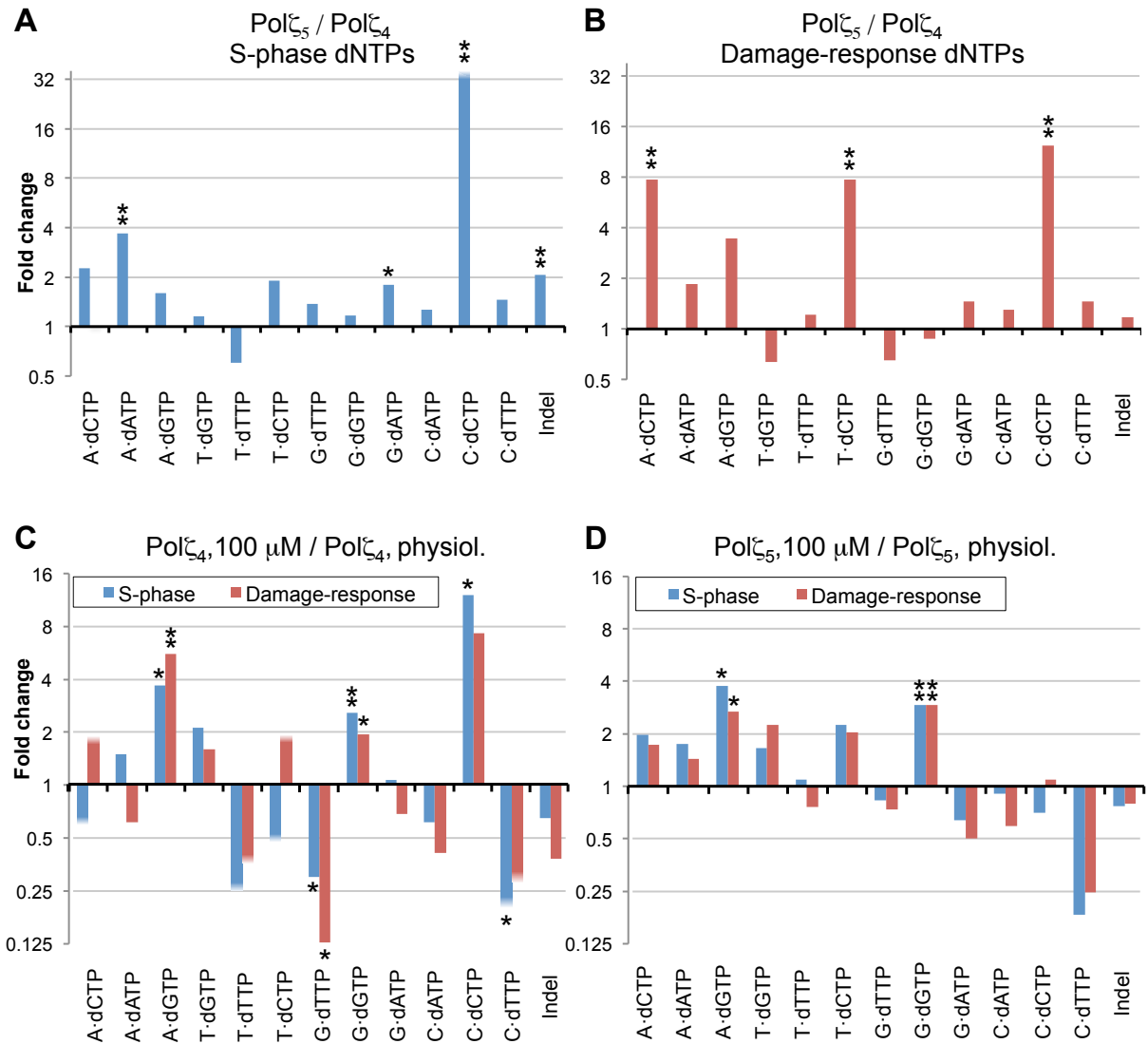
³ Department of Medical Biochemistry and Biophysics and Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, SE 90187 Umeå, Sweden

[†] These authors contributed equally to this work

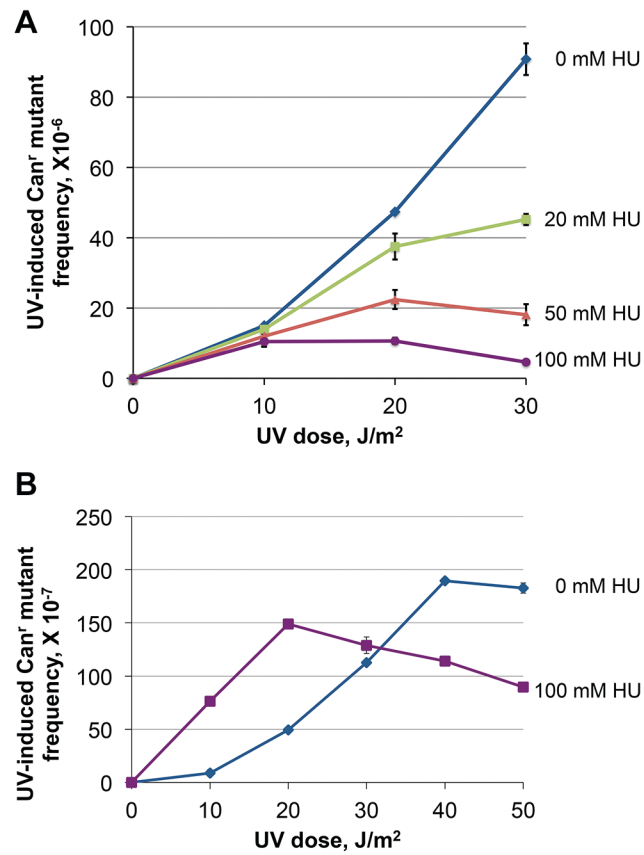
[‡] Present address: Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov Sq. 2, Moscow 123182, Russia



Supplementary Figure S1. Analysis of the gap-filling reactions by agarose gel electrophoresis. Reactions were treated with Proteinase K and then subjected to electrophoresis in 0.8% agarose gel in TAE buffer containing 0.5 $\mu\text{g/mL}$ ethidium bromide. Electrophoresis was carried out at 4 $^{\circ}\text{C}$ at 70 V for 20 h. Gap-filling reactions incubated for 30 and 60 min are shown for Pol ζ_4 , however, only 60-min reactions showing complete or nearly complete gap-filling products, were used to determine the fidelity and error specificity of Pol ζ complexes. Arrows and asterisks indicate the unfilled and filled gapped DNA substrate, respectively. *S*, S-phase dNTPs; *D*, damage-response dNTPs.



Supplementary Figure S2. Fold changes in the rate of single-base errors in *in vitro* reactions with Pol ζ_5 vs. Pol ζ_4 and at equimolar vs. intracellular dNTP concentrations. **(A)** Pol ζ_5 , S-phase dNTPs vs. Pol ζ_4 , S-phase dNTPs. **(B)** Pol ζ_5 , damage-response dNTPs vs. Pol ζ_4 , damage-response dNTPs. **(C)** Pol ζ_4 , 100 μ M dNTPs vs. Pol ζ_4 , intracellular dNTPs. **(D)** Pol ζ_5 , 100 μ M dNTPs vs. Pol ζ_5 , intracellular dNTPs. When no mutants of a particular type were observed in one of the conditions being compared, minimal estimates of the fold change are shown as bars with a color gradient at the end. **, $p < 0.01$; *, $p < 0.05$. The p values were determined by Fisher's exact test as described in Materials and Methods.



Supplementary Figure S3. The effect of HU treatment on the mutagenicity of UV light in the wild-type yeast strain. **(A)** Overnight cultures of the wild-type strain were plated onto selective and complete media with indicated HU concentrations and then irradiated with UV light. **(B)** The effect of HU pre-treatment on UV-induced mutagenesis. Overnight cultures of the wild-type strain were diluted ten-fold and grown to the logarithmic stage in the presence or absence of 100 mM HU and then plated onto selective and complete media with or without 100 mM HU, respectively. Each data point is an average frequency of UV-induced Can⁺ mutants for three independent determinations. Standard errors are shown where the size of the error bar exceeds the size of the plot symbol.