

Supplemental Data for: DNA Polymerase η is regulated by competitive mono-ubiquitination and mono-NEDDylation

Natália Cestari Moreno^{1†}, Emilie J. Korchak^{2†}, Marcela Teatin Latancia¹, Dana A. D'Orlando¹, Temidayo Adegbenro¹, Irina Bezsonova^{2*}, Roger Woodgate^{1*}, Nicholas W. Ashton^{1*}

¹ Laboratory of Genomic Integrity, National Institute of Child Health and Human Development, National Institutes of Health, 9800 Medical Center Drive, Bethesda, MD 20892-3371, USA

² Department of Molecular Biology and Biophysics, UConn Health, Farmington, CT 06032, USA

[†] The first two authors contributed equally to this work

* To whom correspondence should be addressed:

Irina Bezsonova: Tel: +1 860-679-2769; Email: bezsonova@uchc.edu

Roger Woodgate: Tel: +1 301-435-4040; Email: woodgate@nih.gov

Nicholas W. Ashton: Tel: +1 617-582-9358; Email: nicholas_ashton@dfci.harvard.edu

Present Addresses:

Natália Cestari Moreno, Department of Cancer Biology, University of Kansas Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA

Nicholas W. Ashton, Department of Radiation Oncology, Dana-Farber Cancer Institute, Harvard Medical School, 4 Blackfan Street, Boston, MA 02215, USA

This document includes:

Supplemental Materials and Methods

Table S1

Figures S1 to S4

References for Supplemental Information

Supplemental Materials and Methods**Dot blot**

Recombinant human NEDD8 (R&D Systems #UL-812-500) or ubiquitin (R&D Systems #U-100H-10M) was diluted to $10 \mu\text{g } \mu\text{L}^{-1}$ in sample buffer (50 mM Tris pH 8.0, 150 mM NaCl and 0.1 mM EDTA) to prepare a stock, then further diluted to concentrations of 2, 1, 0.5, and 0.25 $\mu\text{g } \mu\text{L}^{-1}$. 0.5 μL of sample was spotted onto a nitrocellulose membrane and allowed to air dry for 30 minutes. Proteins were visualized with a reversible total protein stain (Revert total protein stain, LiCor #926-11011) and imaged on an Odyssey CLX infrared imaging system (Li-Cor) at 700 nM. Membranes were then destained, blocked, and immunoblotted with ubiquitin (E412J; Cell Signaling Technology #43124) or NEDD8 antibodies (E19E3; Cell Signaling Technology #2754). Primary antibodies were detected using IRDye 800CW-conjugated anti-rabbit fluorescent secondary antibodies (Li-Cor) and visualized at 800 nM.

Supplemental Table S1. Expression constructs used in this study

Plasmid	Mammalian/ E. coli	Figure(s)	Source	Addgene #
pCMV6-AN-DDK_ WT POLH (pJRM160)	Mammalian	1B-D, 2A-C, 3B-C, 6C	(1)	221897
pCMV6-AN-DDK_ K682A POLH (pNCM23)	Mammalian	1C	This work	221862
pCMV6-AN-DDK_ K709A POLH (pNCM24)	Mammalian	1C	This work	221863
pCMV6-AN-DDK_ K682A_K709A POLH (pNCM25)	Mammalian	1C	This work	221864
pCMV6-AN-DDK_ K682A_K686A_K694_K709A POLH (4KA) (pNCM26)	Mammalian	1C	This work	221865
pcDNA3.1(+)-N-HA _HA NEDD8 (pNCM18)	Mammalian	1D, 3C	This work	221859
pCMV6-AN-DDK_ WT POLH_ΔGG NEDD8 (pNCM21)	Mammalian	2B	This work	221860
pCMV6-AN-DDK_ D652A POLH (pNWA8)	Mammalian	3B, 3C	This work	222006
pCMV6-AN-HA_ Ubiquitin (pJRM147)	Mammalian	3B	(2)	131258
pEGFP-C1_NLS (pNCM36)	Mammalian	6A	This work	221867
pEGFP-C1_NLS_ WT POLH (pNCM37)	Mammalian	6A	This work	221868
pEGFP-C1_NLS_ WT POLH_ΔGG Ubiquitin (pNCM38)	Mammalian	6A	This work	221869

pEGFP-C1-NLS_ WT POLH_ΔGG NEDD8 (pNCM39)	Mammalian	6A	This work	221870
pEGFP-C1-NLS_ D652A POLH (pNCM40)	Mammalian	6A	This work	221871
pEGFP-C1-NLS_ K682A_K686A_K694_K709A POLH (4KA) (pNCM41)	Mammalian	6A	This work	221872
pEGFP-C1-NLS_ L704A_F707A_F708A POLH (PIP) (pNCM42)	Mammalian	6A	This work	221873
pcDNA3.1(+)-N_DYK_ WT PCNA (pNCM47)	Mammalian	6B	This work	221858
pcDNA3.1(+)-N_DYK_ K164R PCNA (pNCM48)	Mammalian	6B	This work	221874
pcDNA3.1(+)-N_DYK_ K164R PCNA_ΔGG Ubiquitin (pNCM49)	Mammalian	6B	This work	221875
pCMV6-AN-HA_ WT POLH (pJRM56)	Mammalian	6B	(3)	201671
pET15b_Pol η UBZ	E. coli	4A-D 5A-B	(4)	-
pET-15b_Ubiquitin	E. coli	4A-D	(5)	-
pET-28b(+)_N-His_NEDD8 (pNCM35)	E. coli	4A-D 5A-B	This work	221866

Supplemental Figures

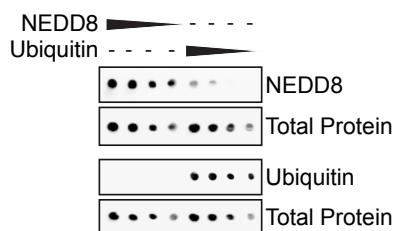


Figure S1: Specificity of detection of the NEDD8 antibody. A dot blot of recombinant human NEDD8 or ubiquitin (0.5 μL of 2, 1, 0.5, or 0.25 $\mu\text{g } \mu\text{L}^{-1}$ of protein). Membranes were stained to detect total protein, then immunoblotted with antibodies against NEDD8 or ubiquitin.

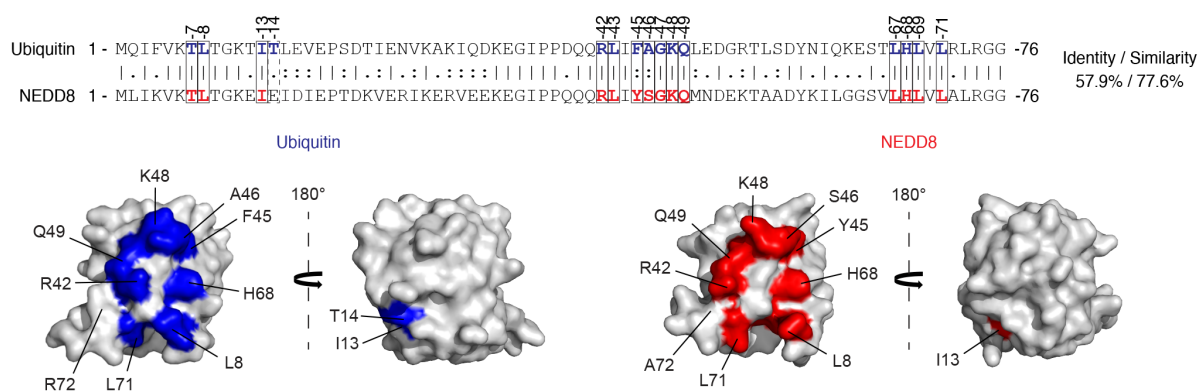


Figure S2: The UBZ-binding residues of ubiquitin are conserved in NEDD8. An alignment of the ubiquitin and NEDD8 primary sequences. The blue residues of ubiquitin are those which have previously been shown to be form the UBZ-binding surface (4). The corresponding residues of NEDD8 are shown in red where these residues are identical or similar. These UBZ-binding and corresponding residues are highlighted on the crystal structures of ubiquitin (PDB:1ubq) (6) or NEDD8 (PDB:1ndd) (7).

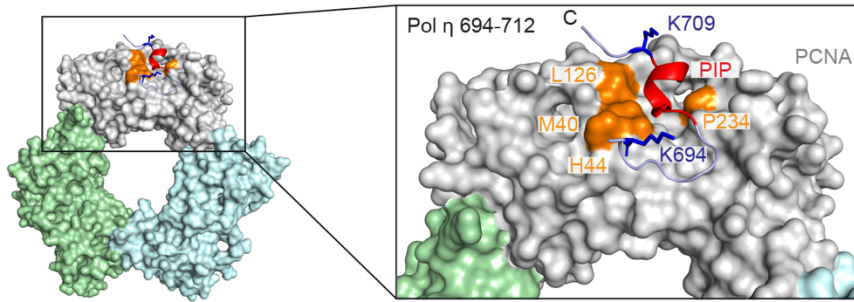


Figure S3: The sidechains of Pol η K694 and K709 do not interact with PCNA. A published crystal structure of a PIP-box containing Pol η peptide (amino acids 694-712) in complex with PCNA (PDB: 2zvz) (8) revealed that the side chains of K694 and K709 are directed away from the PCNA surface. The PCNA residues highlighted in orange define the PIP-binding universal binding site of PCNA.

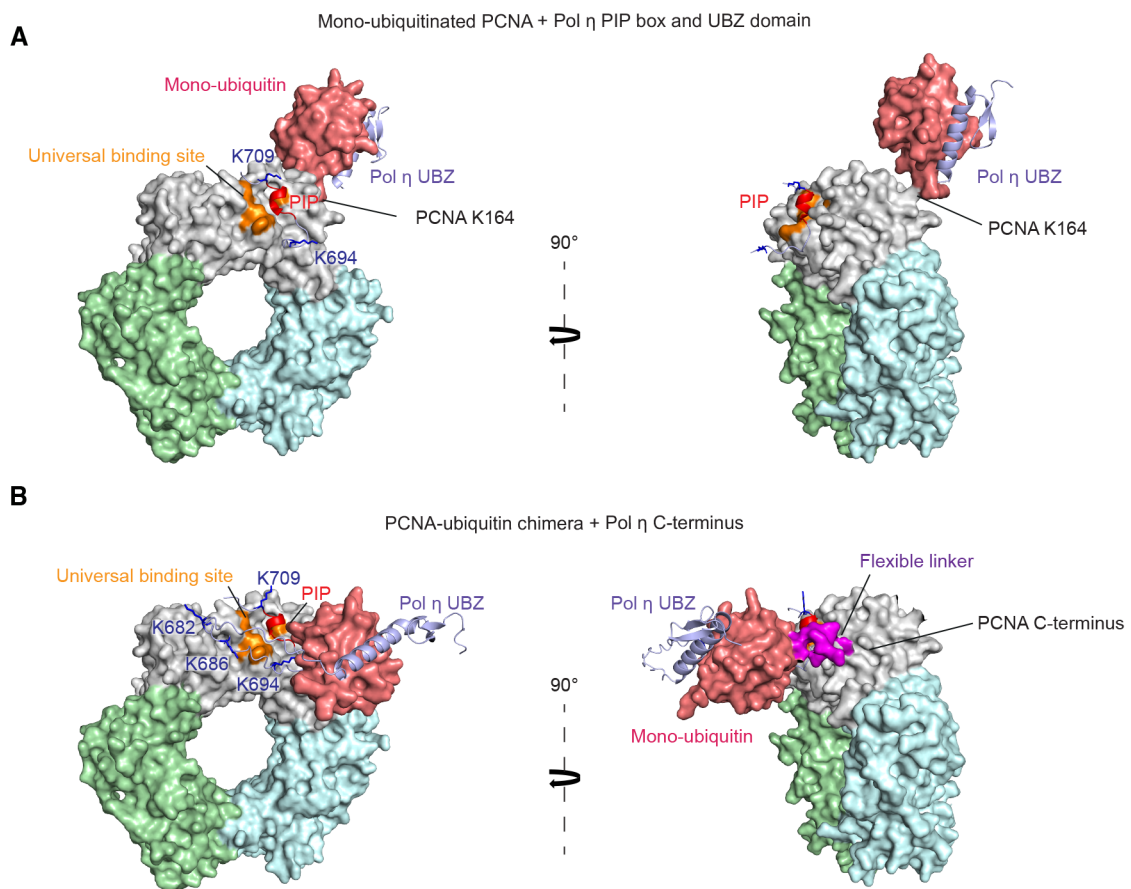


Figure S4: A PCNA-ubiquitin chimera mimics mono-ubiquitinated PCNA (A) A model of mono-ubiquitinated PCNA in complex with the PIP box (amino acids 694-712) and UBZ (amino acids 625-664) of Pol η . This model was assembled from a crystal structure of mono-ubiquitinated PCNA (PDB: 3tbl) (9), a crystal structure of the Pol η PIP box in complex with PCNA (PDB: 2zvz) (8), and an AlphaFold 3 (10) model of the Pol η UBZ domain bound to ubiquitin. **(B)** An AlphaFold model of the PCNA-ubiquitin chimera in complex with the C-terminus of Pol η (amino acids 634-713)

References

1. McIntyre, J., Sobolewska, A., Fedorowicz, M., McLenigan, M.P., Macias, M., Woodgate, R. and Sledziewska-Gojska, E. (2019) DNA polymerase ι is acetylated in response to S_N2 alkylating agents. *Sci Rep*, 9, 4789.
2. Ashton, N.W., Valles, G.J., Jaiswal, N., Bezsonova, I. and Woodgate, R. (2021) DNA polymerase ι interacts with both the TRAF-like and UBL1-2 domains of USP7. *J Mol Biol*, 433, 166733.
3. McIntyre, J., Vidal, A.E., McLenigan, M.P., Bomar, M.G., Curti, E., McDonald, J.P., Plosky, B.S., Ohashi, E. and Woodgate, R. (2013) Ubiquitin mediates the physical and functional interaction between human DNA polymerases η and ι . *Nucleic Acids Res*, 41, 1649-1660.
4. Bomar, M.G., Pai, M.T., Tzeng, S.R., Li, S.S. and Zhou, P. (2007) Structure of the ubiquitin-binding zinc finger domain of human DNA Y-polymerase η . *EMBO Rep*, 8, 247-251.
5. Ashton, N.W., Jaiswal, N., Cestari Moreno, N., Semenova, I.V., D'Orlando, D.A., Teatin Latancia, M., McIntyre, J., Woodgate, R. and Bezsonova, I. (2023) A novel interaction between RAD23A/B and Y-family DNA polymerases. *J Mol Biol*, 168353.
6. Vijay-Kumar, S., Bugg, C.E. and Cook, W.J. (1987) Structure of ubiquitin refined at 1.8 Å resolution. *J Mol Biol*, 194, 531-544.
7. Whitby, F.G., Xia, G., Pickart, C.M. and Hill, C.P. (1998) Crystal structure of the human ubiquitin-like protein NEDD8 and interactions with ubiquitin pathway enzymes. *J Biol Chem*, 273, 34983-34991.

8. Hishiki, A., Hashimoto, H., Hanafusa, T., Kamei, K., Ohashi, E., Shimizu, T., Ohmori, H. and Sato, M. (2009) Structural basis for novel interactions between human translesion synthesis polymerases and proliferating cell nuclear antigen. *J Biol Chem*, 284, 10552-10560.
9. Zhang, Z., Zhang, S., Lin, S.H., Wang, X., Wu, L., Lee, E.Y. and Lee, M.Y. (2012) Structure of monoubiquitinated PCNA: implications for DNA polymerase switching and Okazaki fragment maturation. *Cell Cycle*, 11, 2128-2136.
10. Abramson, J., Adler, J., Dunger, J., Evans, R., Green, T., Pritzel, A., Ronneberger, O., Willmore, L., Ballard, A.J., Bambrick, J. *et al.* (2024) Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature*, 630, 493-500.