Supporting informations

The ubiquitin-binding domain of DNA polymerase η directly binds to DNA clamp PCNA and regulates translesion DNA synthesis

Kodavati Manohar¹,³⁺, Prashant Khandagale¹,⁴⁺, Shraddhaya Kumar Patel¹,²⁺, Jugal Kishor Sahu¹,², and Narottam Acharya¹,*

¹Laboratory of Genomic Instability and Diseases, Department of Infectious Disease Biology, Institute of Life Sciences, Bhubaneswar-751023, India.

²Regional Centre for Biotechnology, Faridabad, India.

³Present address: Department of Neurosurgery, Center for Neuroregeneration, Houston Methodist Research Institute, Houston, TX 77030, USA.

⁴Present address: Developmental Therapeutics Branch, Center for Cancer Research, NCI, NIH, Bethesda, Maryland 20892, USA

*Correspondence to:

Narottam Acharya, Phone: 91-674-2304278, Fax: 91-674-2300728

E-mail: narottam_acharya@ils.res.in; narottam74@gmail.com

⁺contributed equally.
Legends for supporting Figures

**Fig. S1:** Classification of three categories of Rad30 sequences from various organisms across kingdom. **A.** Category I Rad30s containing both ubz domain and pip motif(s). **B.** Category II Rad30s containing only pip motif. For better alignment Rad30 sequences from protists (i) and plants (ii) have been separated. **C.** Category III Rad30s containing only ubz domain. Critical motifs in the catalytic domains are shown in purple (Motif I to V), pip motifs in green and ubz domains in brown colours.

**Fig. S2** A control experiment where buffer was injected to PCNA in the cell and binding was analysed by ITC.

**Fig. S3:** (A) GST pull-down of His-PCNA mutants by CaPol\(\eta\). Beads of GST-CaPol was mixed with CaPCNA (lanes 1-3) or CaPCNA-79 mutant (lanes 4-6) in equilibration buffer and after incubation, the beads were washed and the bound PCNA was eluted with protein loading dye. The different fractions were resolved in 12 % SDS PAGE, blotted to the membrane and developed by the anti-His antibody. Lanes 1 and 4 are 10 % of the load; Lanes 2 and 5 are 10 % of the third washings; Lanes 3 and 6 are the total eluates. (B) Cells of genomic rad30\(\Delta\)rev3\(\Delta\) yeast strains harboring vector alone (YEP-ADH1p) or ScPol\(\eta\) or ScPol\(\eta\) pip (F627A,F628A) mutant or ScPol\(\eta\) H568A,H572A or CaPol\(\eta\) 1-512 aa or CaPol\(\eta\) 1-601aa or CaPol\(\eta\) D626A or CaPol\(\eta\) H624A,H628A or CaPol\(\eta\) or CaPol\(\eta\)-ScRad30pip or CaPol\(\eta\) H624A,H628A-ScRad3 pip or CaPol\(\eta\) D627A-ScRad30pip or CaPol\(\eta\) UBZ\(\Delta\)-ScRad30pip or ScPol\(\eta\) CTD\(\Delta\)-CaRad30ubz plasmids from an overnight SD-Ura culture were serially diluted and spotted onto SD-Ura plates. The culture plates were irradiated with the indicated doses of UV radiation, covered with aluminum foil, incubated at 30°C for 3 days and then photographed.
Fig. S1A: Category I DNA polymerase eta: DNA polymerase eta from fungi, animals and plant containing both UBZ and PCNA interaction motifs at the carboxyl terminal domain.
| Species | Sequence |
|---------|----------|
| CaRad30 (2) | SPIYRVNYGLSDCIYSINSESSLIDQRKAEEALSFVNNELSSEPIETIGVDKSN |
| CaRad30 (3) | ---------------------------------- |
| NcRad30 | ---------------------------------- |
| DhRad30 (1) | ---------------------------------- |
| UmRad30 | ---------------------------------- |
| YpRad30 | ---------------------------------- |
| CmRad30 | ---------------------------------- |
| StRad30 | ---------------------------------- |
| GcRad30 | ---------------------------------- |
| OoRad30 | SQ--TVPFVQ--RGRFPKHAIVQRQDGFLVLEQKQK-- |
| FoRad30 | ---------------------------------- |
| PgRad30 | ---------------------------------- |
| CsRad30 (3) | ---------------------------------- |
| NgRad30 | ---------------------------------- |
| McRad30 | ---------------------------------- |
| MmRad30 | ---------------------------------- |
| MrRad30 (3) | ---------------------------------- |
| DhRad30 (2) | ---------------------------------- |
| BgRad30 | ---------------------------------- |
| AfpRad30 | SN-- |
| SpRad30 | SPIYRVNYGLSDCIYSINSESSLIDQRKAEEALSFVNNELSSEPIETIGVDKSN--LAEISSLIVYDELENN |
| CaRad30 (2) | H-- |
| CaRad30 (3) | KNR-- |
| NcRad30 | ---------------------------------- |
| DhRad30 (1) | ---------------------------------- |
| UmRad30 | ---------------------------------- |
| YpRad30 | ---------------------------------- |
| CmRad30 | ---------------------------------- |
| StRad30 | ---------------------------------- |
| GcRad30 | ---------------------------------- |
| OoRad30 | KLAFlG-- |
| FoRad30 | ---------------------------------- |
| PgRad30 | ---------------------------------- |
| CsRad30 (3) | ---------------------------------- |
| NgRad30 | ---------------------------------- |
| McRad30 | ---------------------------------- |
| MmRad30 | ---------------------------------- |
| MrRad30 (3) | ---------------------------------- |
| DhRad30 (2) | ---------------------------------- |
| BgRad30 | ---------------------------------- |
| AfpRad30 | ---------------------------------- |
| SpRad30 | NSTSSAVYIKNENLRLKKLYGFLGISRIWSASRKRQGIGASLIDNLNLKFIYGYVISPFAEVAFSQPSESQGFIIWHSRNNSSKSLRTAYES |
PCNA – Buffer titration
A.

| GST-CaPol δ + His-CaPCNA | GST-CaPol δ + His-CaPCNA-79 | GST-CaPol δ + His-CaPCNA-90 |
|---------------------------|-----------------------------|-----------------------------|
| Load                      | Load                        | Load                        |
| Wash                      | Wash                        | Wash                        |
| Elution                   | Elution                     | Elution                     |
| Lane 1                    | Lane 2                      | Lane 3                      |
| kDa                       | 30                          | 25                          |
| 25                        | 30                          | kDa                         |

PCNA

B.

rad30Δ rev3Δ

| Vector | ScPol δ F627A,F628A | ScPol δ H568A,H572A | CaPol δ 1-538aa | CaPol δ 1-601aa | CaPol δ H624A,H628A | CaPol δ D626A | CaPol δ ScRad30PIP | CaPol δ H624,H628A~ScRad30PIP | CaPol δ D626A~ScRad30PIP | CaPol δ UBZΔ~ScRad30PIP | ScPol δ CTDΔ~CaRad30UBZ |
|--------|---------------------|---------------------|-----------------|-----------------|---------------------|-----------------|---------------------|---------------------------------|----------------------------|---------------------|---------------------|
| UV     | 0 J/m²              | 8 J/m²              | 16 J/m²         | 32 J/m²         |                     |                 |                     |                                 |                            |                     |                     |

Figure S3