

Supplemental Information

NEDDylation antagonizes ubiquitination of Proliferating Cell Nuclear Antigen and regulates the recruitment of Polymerase η in response to oxidative DNA damage

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Fig. S1. PCNA is NEDDylated. (A) Immunoblotting analysis of PCNA NEDDylation in His-tagged NEDD8 conjugates enriched by Ni²⁺ pull-down (Ni-PD). (B) Analysis of the effect of NEDP1 on PCNA NEDDylation or its ubiquitination. In A-B, HEK293T cells were transfected with the indicated plasmids, and then PCNA NEDDylation or ubiquitination was examined by Ni-PD and western blotting with indicated antibodies. (C) Analysis of the interaction between RAD18 and UBC12. HEK293T cells were transfected with 3Flag-RAD18 and Myc-UBC12. At 48 h after transfection, cells were lysed in NP-40 lysis buffer and sonicated for 15 times. Co-IP was performed using antibody against anti-Flag or anti-Myc antibody. (D) *Ubc12*^{-/-} HEK293T cells were generated using the CRISPR/CAS9 method. The expression of UBC12 in WT or *Ubc12*^{-/-} HEK293T cells was analyzed by western blotting using anti-UBC12 antibody. (E) *Rad18*^{-/-} HEK293T cells were generated using the TALEN method. The expression of RAD18 in WT or *Rad18*^{-/-} HEK293T cells was analyzed by western blotting using anti-RAD18 antibody.

Fig. S2. NEDDylated PCNA accumulates in response to DNA damage. (A) A time course analysis of PCNA NEDDylation in His-tagged NEDD8 conjugates enriched by Ni-PD after UV treatment. (B) Analysis of the effect of UBC12-C111S on accumulation of NEDDylated PCNA under oxidative stress. In A and B, indicated plasmids were transfected into HEK293T cells and treated with 20 J/m² UV or 800 μM H₂O₂ for the indicated time. Then PCNA NEDDylation was detected by Ni-PD and western blotting with anti-PCNA antibody. (C) Analysis of the effect of MLN4924 on PCNA ubiquitination (upper panel) or its NEDDylation (lower panel) under oxidative stress. HEK293T cells were transfected with indicated plasmids and treated with DMSO or MLN4924, at 36 h after transfection, cells were treated with 800 μM H₂O₂ for the

indicated time. Then PCNA NEDDylation or ubiquitination was detected by Ni-PD and western blotting using anti-PCNA antibody. (D) A time course analysis of NEDDylated PCNA and ubiquitinated PCNA under UV-induced stress. HEK293T cells were transfected with indicated plasmids, after 36 h, cells were treated with 20 J/m² UV for the indicated time. Then His-tagged PCNA conjugates enriched by Ni-PD were detected by western blotting with anti-Myc, anti-HA or anti-PCNA antibodies.

Fig. S3. UBC12 knockout enhances PCNA ubiquitination. (A) Immunoblotting analysis of PCNA ubiquitination in HEK293T WT or *Ubc12*^{-/-} cells. (B) Analysis of PCNA ubiquitination in HEK293T WT or *Ubc12*^{-/-} cells under H₂O₂-induced oxidative stress. In A and B, indicated plasmids were transfected into HEK293T or *Ubc12*^{-/-} cells and treated with or without 800 μM H₂O₂ for the indicated time. Then PCNA NEDDylation was detected by Ni-PD and western blotting with anti-HA (A) or anti-PCNA (B) antibodies.

Fig. S4. USP1 inhibits ubiquitination but not NEDDylation of PCNA. (A) Immunoblotting analysis of the effect of USP1 on PCNA NEDDylation or its ubiquitination. (B) Analysis of the effect of USP1 on PCNA ubiquitination under H₂O₂-induced oxidative stress. (C) Analysis of the effect of USP1 on PCNA NEDDylation under H₂O₂-induced oxidative stress. In A-C, indicated plasmids were transfected into HEK293 cells and treated with or without 800 μM H₂O₂ for the indicated time. Then PCNA NEDDylation or ubiquitination was detected by Ni-PD and western blotting with indicated antibodies.

Fig. S5. Polη is highly expressed in skin cancer patients and correlated to poor prognosis. Bioinformatics analyses were performed to study the expression of polη (A) and UBC12 (C) in SKCM (Skin Cutaneous Melanoma), and the correlation between polη expression and survival

in SKCM (B), using SKCM and normal dataset from GEPIA (gene expression profiling interactive analysis) (<http://gepia.cancer-pku.cn/>).

Fig. S6. Cropped and uncropped western blot images of Fig. 2E.

Fig. S7. Cropped and uncropped western blot images of Fig. 3A

Fig. S1

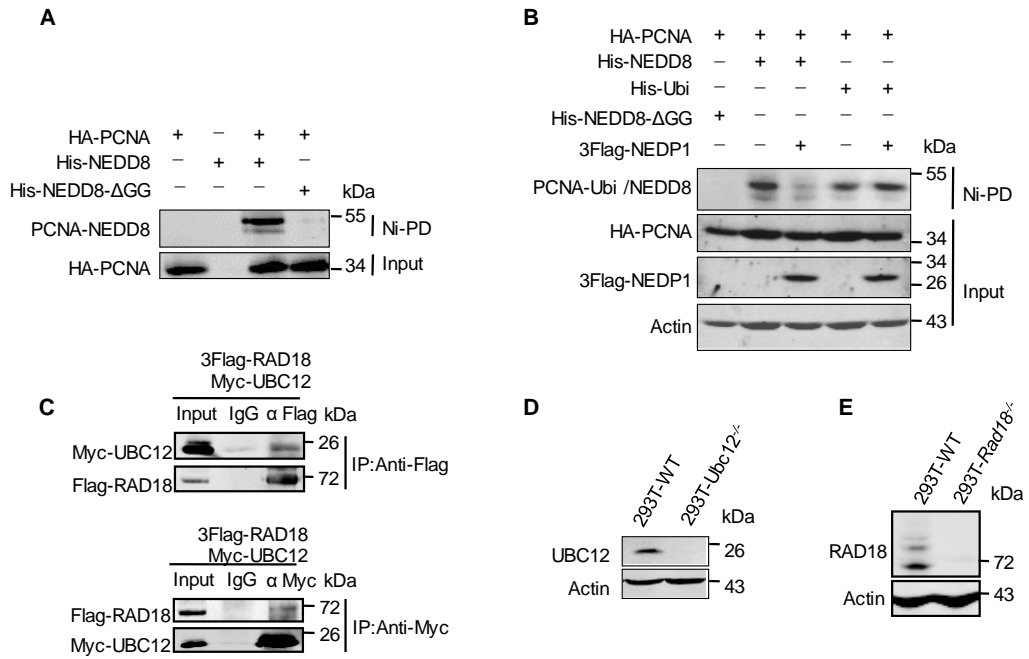


Fig. S2

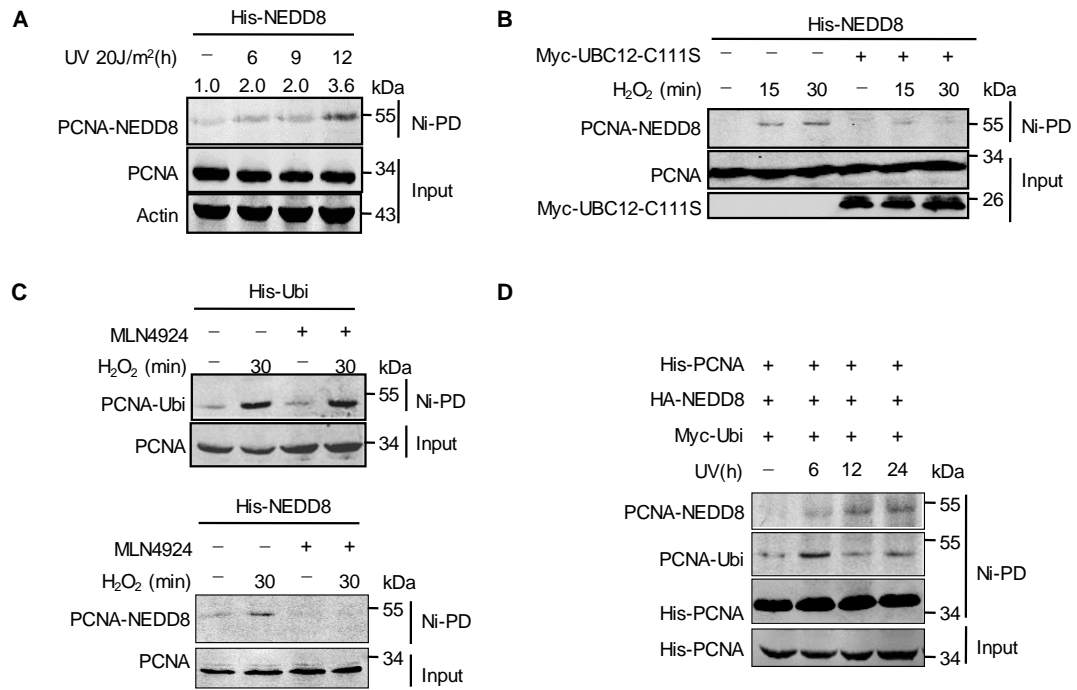


Fig. S3

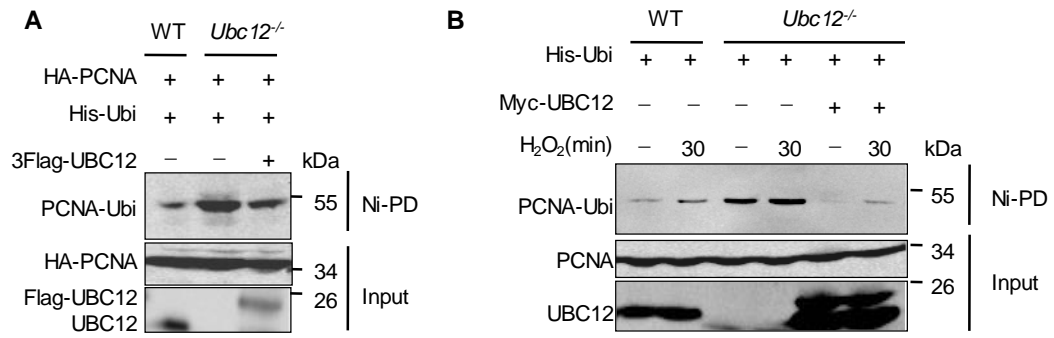


Fig. S4

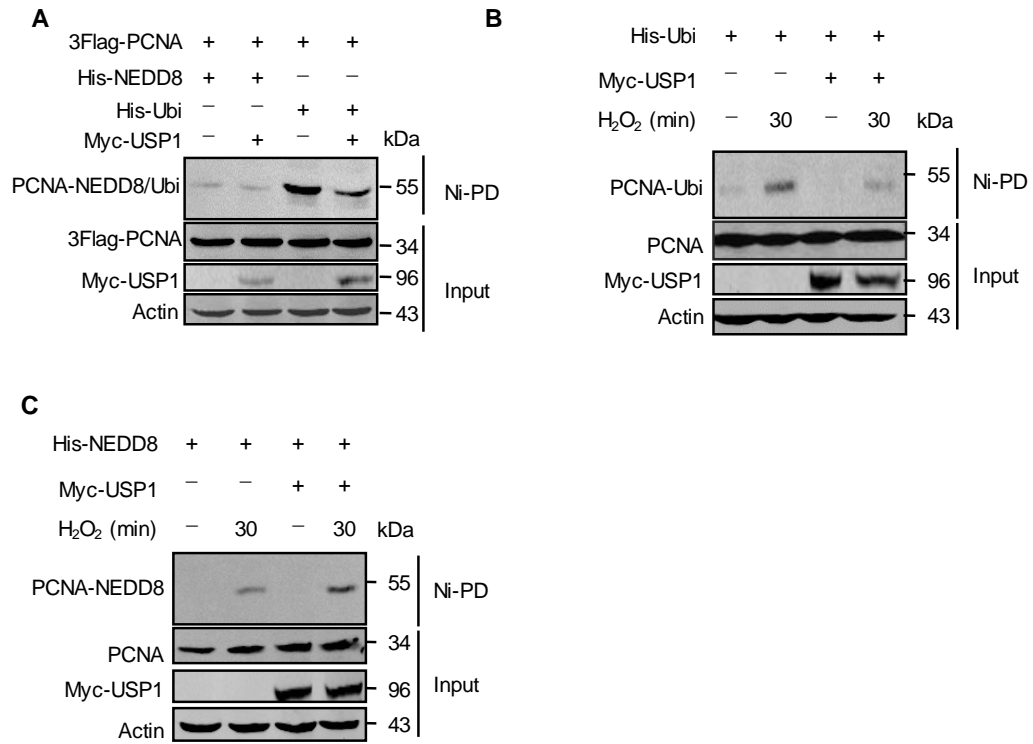


Fig. S5

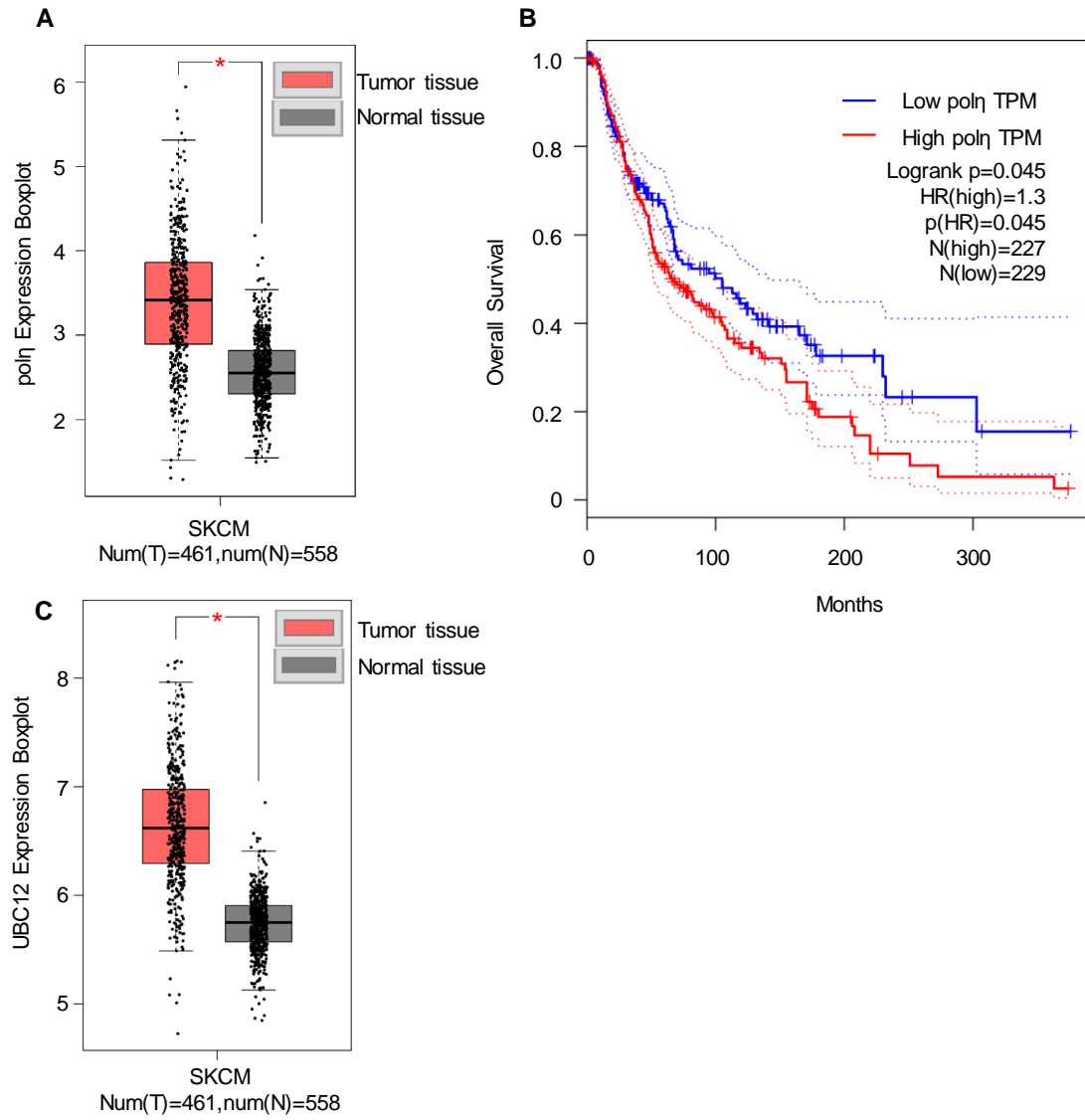


Fig. S6

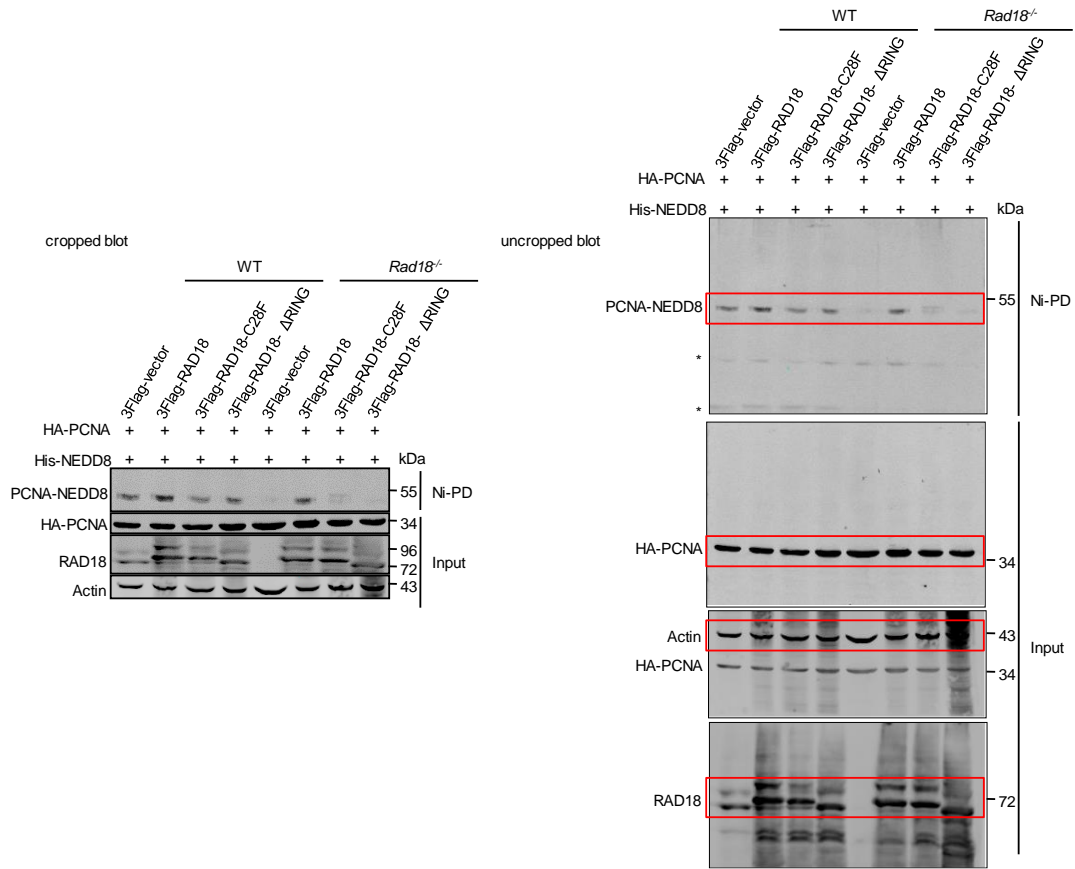


Fig. S7

