SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Identification of SART3 as a Polη-associated protein. HEK 293T cells were transfected with a Flag-Polη expression vector. The cell lysates were harvested and immunoprecipitated with an anti-Flag M2 agarose affinity gel. The final eluates were separated by SDS–PAGE and revealed by silver staining. The bands (indicated with a black rectangle) were excised and analyzed via mass spectrometry analysis. The top candidate proteins identified were shown at the indicated region.

Figure S2. SART3 depletion impairs UV-induced PCNA-mUb.

(A) SART3 deficiency decreases PCNA-mUb after UV irradiation. U2OS cells were transfected with siNC or two different siSART3 oligos (targeted either the CDS or UTR regions of SART3), then irradiated with 15 J/m^2 UVC or not, further incubated for 4 h. The triton-insoluble fractions (TIF) and whole-cell lysates (WCL) were harvested and detected with anti-PCNA or anti-GFP antibody. Tubulin: loading control.

(B) SART3 ablation attenuates the UV-inducible PCNA-mUb formation in an Polη-independent pathway. SiNC or siSART3 oligos were transfected into MRC5 and XP30RO (Polη-deficient) cells. Then the cells were irradiated with 15 J/m^2 UVC or not, and further incubated. The triton-insoluble fractions (TIF) and whole-cell lysates (WCL) were harvested and checked with indicated antibodies.

Figure S3. SART3 modulates DNA replication and UV-induced CPD lesion bypass

(A) U2OS cells were transfected with siSART3 or siNC. Forty-eight hours later, cells were pulsed labelled with EdU (10 μM) for 1 h followed by reaction with Alexa488-azide. The cells were imaged by fluorescence microscopy. The proportion of EdU positive cells was measured, and at least 200 cells were counted. Data represent means ± SEM from three independent experiments. Statistical analyses were performed using a two-tailed Student's *t*-test.

(B) Representative images of cells stained with DAPI or antibody against BrdU after UV irradiation. GFP or GFP-SART3 stably overexpressed cells were transfected with siNC or siSART3 siRNA, irradiated with 20 J/m^2 UVC and further cultured. The cells were permeabilized

with 0.5% Triton X-100 in solution buffer for 5 min, denatured with 2 M HCl after fixation, then immunostained with anti-BrdU antibody and mounted with DAPI.

(C) Representative images of cells stained with DAPI or antibody against CPDs after UV exposure. GFP or GFP-SART3 stably expressing cells were transfected with siNC, siPolη or siSART3 siRNAs. 72 h later, the cells were irradiated with 10 J/m^2 UVC and further incubated. Then the cells were fixed and immunostained for CPDs in nuclei (DAPI). 'Denatured' means denaturation of DNA with 2 M HCl during CPD immunostaining.

Figure S4. SART3 interacts with both RAD18 and Polη independently.

(A) GST pulldown experiments show that the N terminus of Polη is responsible for its association with SART3. 293T cells were transfected with a series of Flag- Polη truncations. 48 h after transfection, the cells were lysed and the cell lysates were incubated with purified GST or GST-SART3. Western blot analysis was performed as indicated.

(B) GST pulldown experiments further confirm the association of RAD18 with SART3. Purified GST or GST-RAD18 were incubated with 293T cell lysates, endogenous SART3 bound with GST-RAD18 was detected by anti-SART3 immunoblotting.

(C) GFP-RAD18-ΔPID fails to bind Polη. Flag-Polη was co-transfected with either GFP-RAD18-FL (FL: full length) or GFP-RAD18-ΔPID (PID: Polη interaction domain, residues 401-445) into 293T cells. Cell lysates were immunoprecipitated with Flag M2 beads, and detected with anti-GFP or anti-Flag antibody. NS: non-specific band.

(D) The binding of RAD18 with SART3 is independent on its interaction with Polη. Flag-SART3 was co-transfected with GFP-RAD18-FL or GFP-RAD18-ΔPID into 293T cells. Co-IP assay was performed with Flag M2 beads, and western blot was performed with anti-GFP or anti-Flag antibody.

(E) SART3 deleted of coiled-coil domain still localizes in the nucleus. U2OS cells were transfected with either GFP-SART3-WT (WT: wild type) or GFP-SART3-ΔCC (CC: coiled-coil, residues 559-620), 48 h later, the cells were fixed and mounted with DAPI.

Figure S5. SART3-V591M does not lose its ability to promote UV-induced Polη recruitment and PCNA-mUb formation.

(A-C) SART3-V591M could rescue the decreased GFP-Polη focus formation caused by SART3 depletion after UV irradiation. U2OS cells transfected with siNC or siSART3-3′ UTR were further co-transfected with Flag, Flag-SART3 or Flag-SART3-V591M with GFP-Polη. (A) Cells were treated with 15 J/m^2 UVC, repaired for 10 h. The proportion of GFP-Polη cells with more than 30 foci was measured, and at least 200 cells were counted. Data represent means ± SEM from three independent experiments. (B) The cell pellets were harvested 72hr later, lysed by RIPA buffer and checked with the indicated antibodies. (C) The reduction at PCNA-mUb level caused by SART3 depletion could be rescued by stable expression of WT or V591M mutated GFP-SART3. U2OS cells stably expressing GFP, GFP-SART3 or GFP-SART3-V591M were transfected with two different oligos of siSART3 (target 3′ UTR regions) or siNC, and irradiated with 15 J/m^2 UVC. The triton-insoluble fractions (TIF) and whole-cell lysates (WCL) were harvested and analyzed with antibodies against SART3, PCNA and Tubulin. Tubulin: loading control.

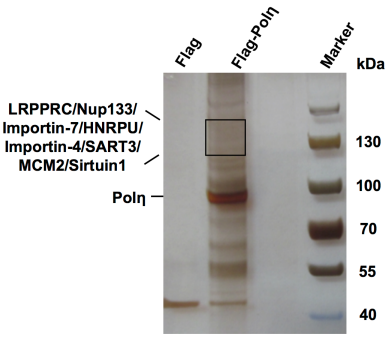
Figure S6. Several cancer-derived SART3 mutants exhibit less stimulatory effect on RAD18-Polη interaction.

(A) Cancer-derived SART3 mutants still form homodimers. Flag-SART3 and a series of GFP-SART3 mutants were transiently co-transfected into 293T cells followed by precipitating with Flag M2 beads (n = 2 biological replicates). (B) Several cancer-derived SART3 mutants show less stimulatory effect on RAD18-Polη interaction. The indicated plasmids were transfected into 293T cells. Co-IP was performed using Flag M2 beads, and detected with the indicated antibodies (n = 2 biological replicates). (C) The levels of SART3 mutants on chromatin after UV irradiation are reduced. U2OS cells were transfected with a series of GFP-SART3 mutants, then treated with 15 J/m^2 UVC and further cultured. The triton-insoluble fractions (TIF) and whole-cell lysates (WCL) were harvested and separated by SDS-PAGE. Levels of relative SART3 were analyzed by western blot and quantified using Image J. Data represent means from three independent experiments. (D) SART3-R580I still forms homodimers. 293T cells were co-transfected with GFP-SART3 or GFP-SART3-R580I and Flag-SART3. Co-IP was performed using Flag M2 beads followed by immunoblotting with anti-GFP and anti-Flag antibodies. (E) R580I mutation does not abrogate SART3 chromatin binding after UV exposure.

U2OS cells transfected with WT or R580I mutated GFP-SART3 were treated with 15 J/m^2 UVC and further cultured. The triton-insoluble fractions (TIF) and whole-cell lysates (WCL) were harvested and separated by SDS-PAGE. Levels of relative SART3 were analyzed by western blot and quantified using Image J.

Figure S7. The mutation counts of cancer patients with SART3 coding mutations or wild-type SART3. Median with interquartile range is shown and the individual values for each cancer are shown with dots. Npat indicates the number of patients. Mann-Whitney test was used for statistical analysis.

Huang M, et al., Fig. S1



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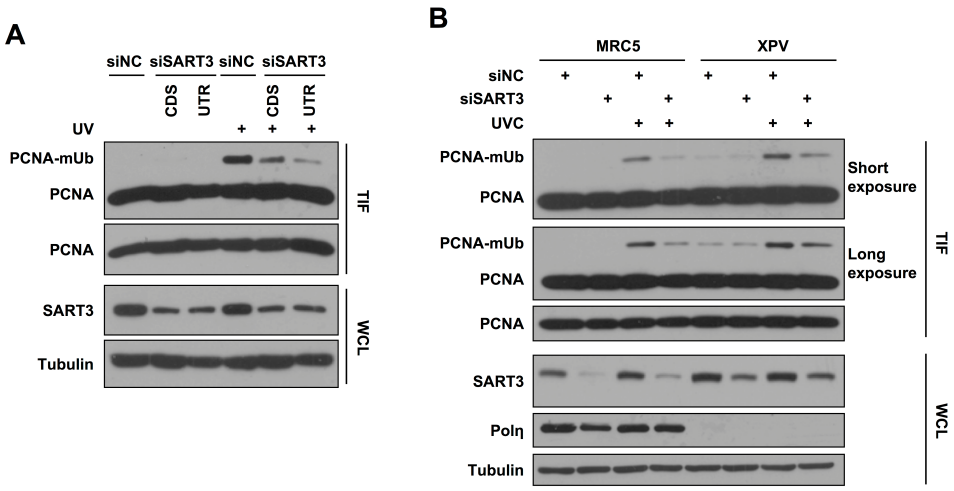
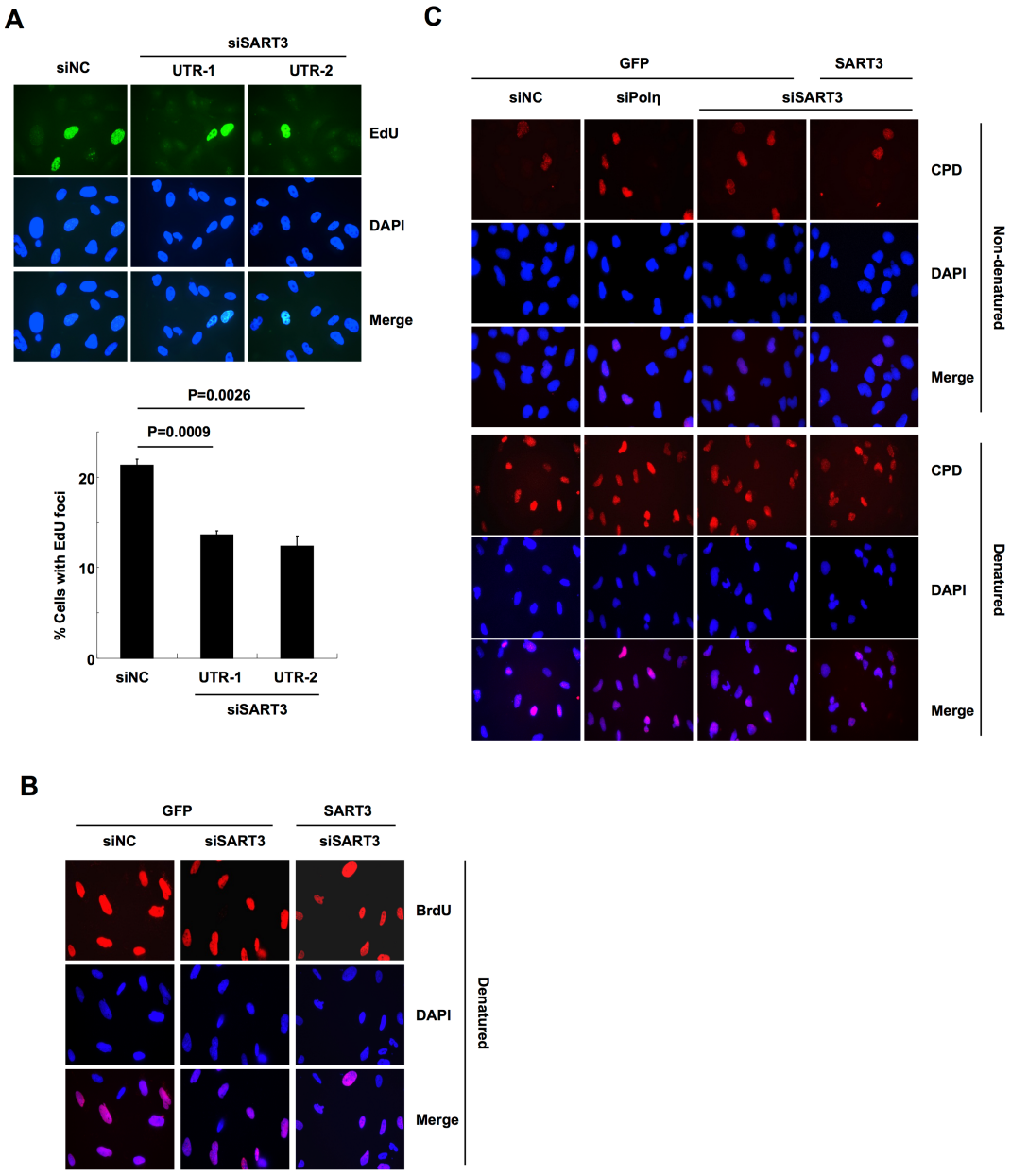


Huang M, et al., Fig. S3



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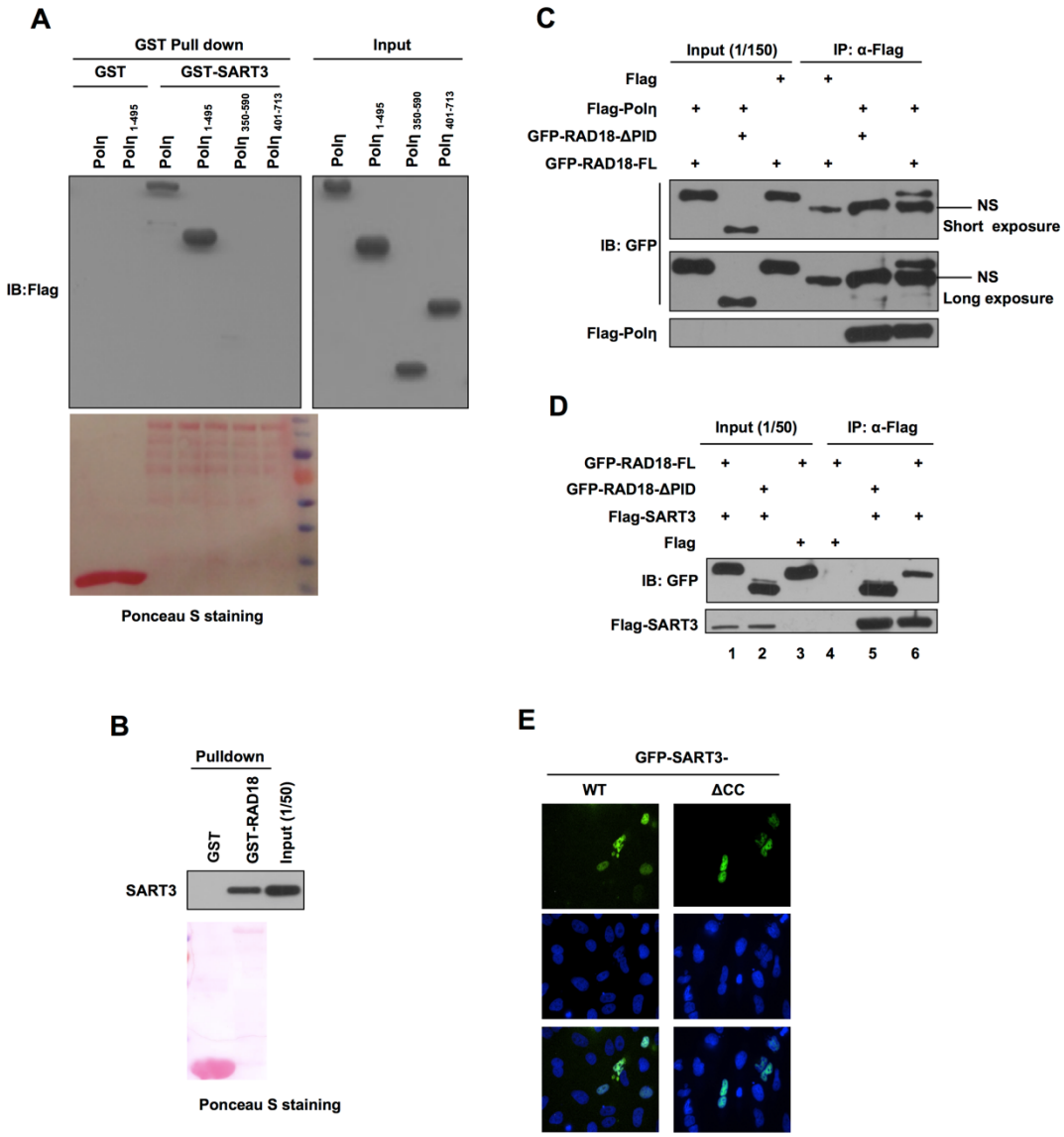

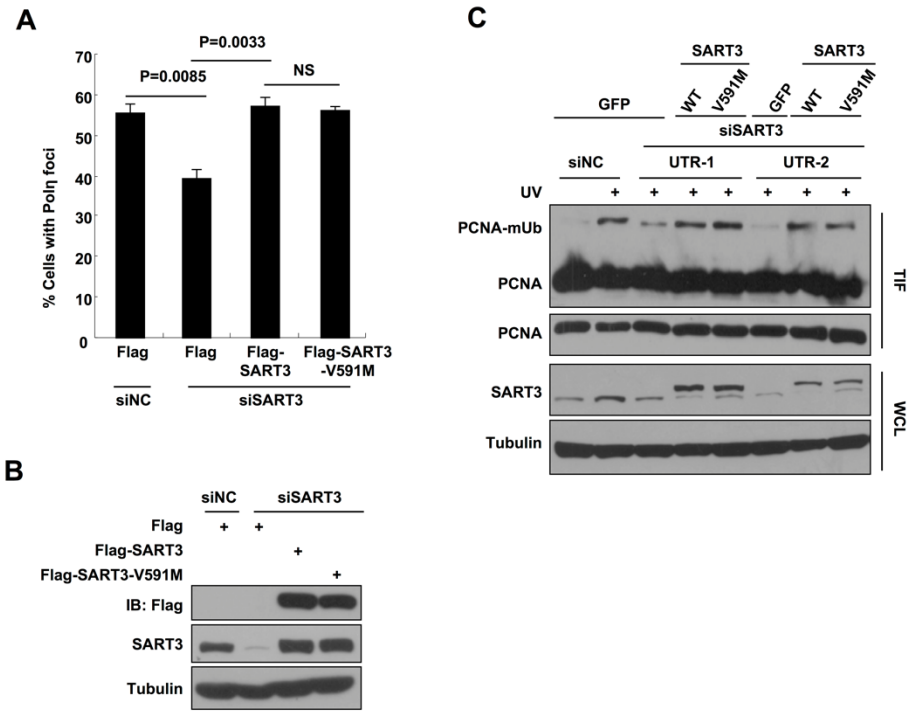
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40
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B
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siSART3
Flag
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Flag-SART3-V591M
IB: Flag
SART3
Tubulin
C
SART3
SART3
GFP
WT
V591M
GFP
WT
V591M
siSART3
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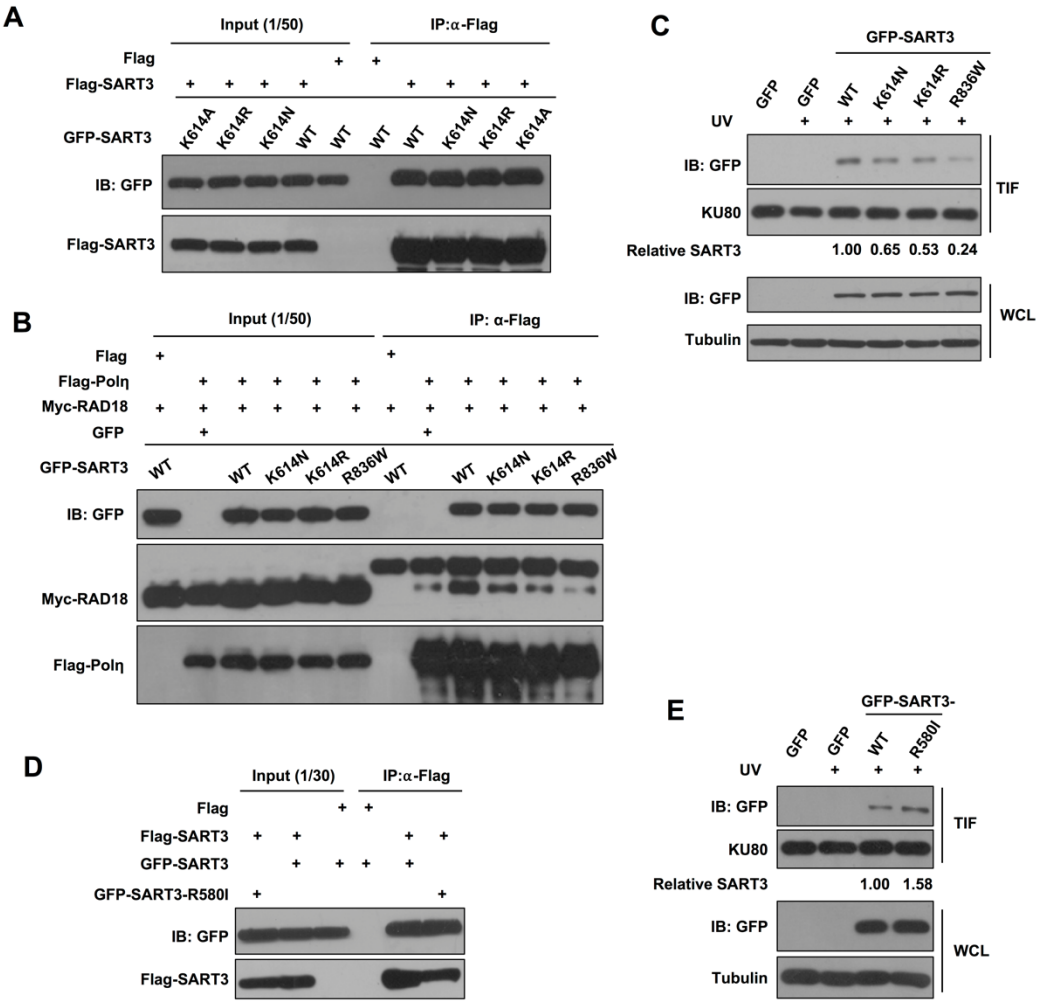
TIF
WCL

Huang M, et al., Fig. S6



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