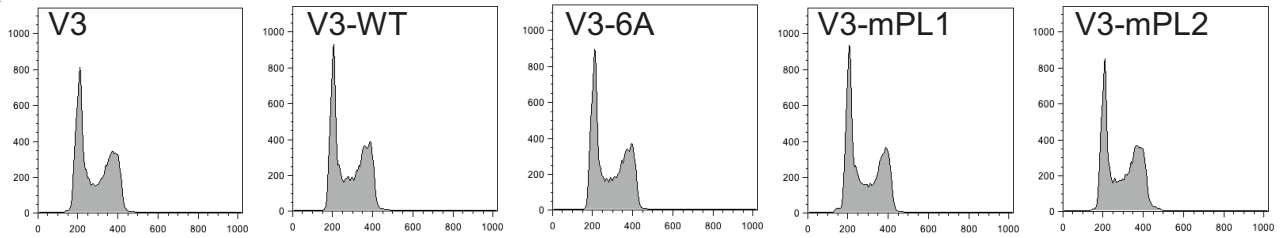
Supplemental Figure S1

A



	G1 (%)	S (%)	G2/M (%)
V3	37.4±0.3	26.8±1.4	36.6±1.6
V3-WT	35.5±0.4	28.2±0.5	36.8±0.8
V3-6A	37.3±0.1	27.7±0.6	36.2±0.6
V3-mPL1	36.7±0.5	30.0±1.3	33.0±1.9
V3-mPL2	32.6±1.0	28.5±1.1	37.8±1.3

B

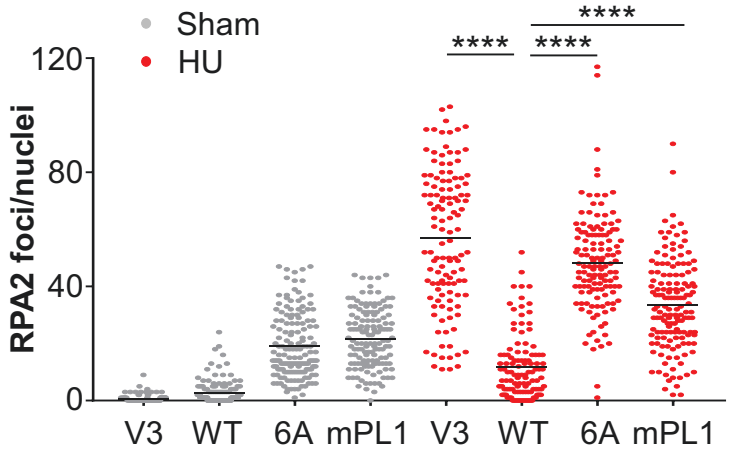


Figure S1. Cell cycle profiling and RPA foci of CHO V3 derivative cells. (A) Exponential growing V3 cells were fixed and stained with Propidium Iodide. Cell cycle distribution was analyzed by flow cytometry. V3 cells complemented with empty vector (V3), wild type DNA-PKcs (WT), and mutants carrying alanine substitutions at the T2609 cluster (V3-6A) or PL motif (V3-mPL1 and -mPL2). (B) V3 derived cells were pulse-labeled with 50 μM EdU, treated with HU (2 mM) for 2 h, pre-extracted with 0.1% TX-100, and stained against anti-RPA2 antibody and EdU. RPA2 nuclear foci were analyzed from EdU+ cell populations (N > 120) using the ImageJ software (NIH, USA). ****, P < 0.0001.

Supplemental Figure S2

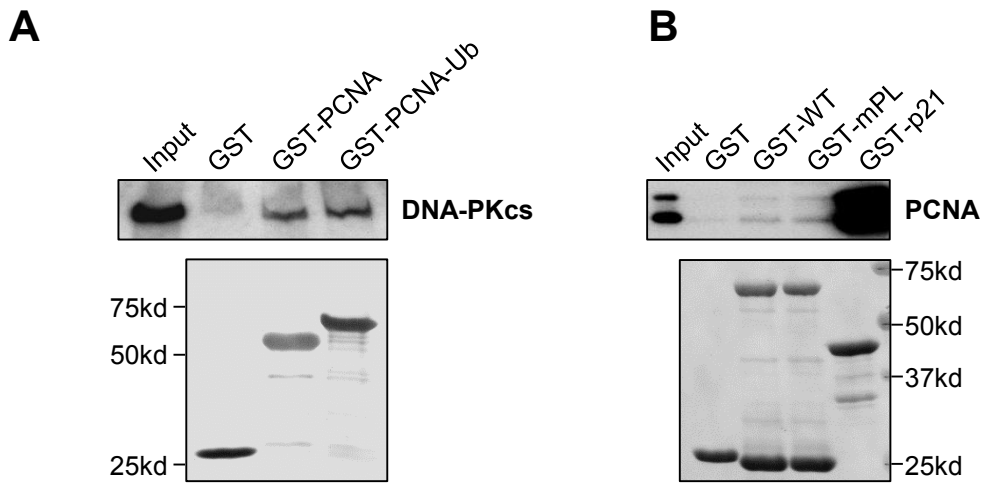


Figure S2. PIP-like motif of DNA-PKcs does not bind to PCNA. (A) GST-fused PCNA or PCNA-Ub (bottom, stained by Ponceau S) were incubated with purified human DNA-PKcs in the presence of Ethidium Bromide (EtBr, 40 ng/μL) followed by glutathione agarose pull down. The protein interactions were detected by western blot using anti-DNA-PKcs antibody. (B) GST-fused DNA-PKcs N-terminal fragments (1-403 a.a.) with wild-type or mutant PL motif were incubated with purified His-tagged PCNA in the presence of EtBr followed by glutathione agarose pull down. The protein interactions were detected by western blot using anti-PCNA antibody.

Supplemental Figure S3

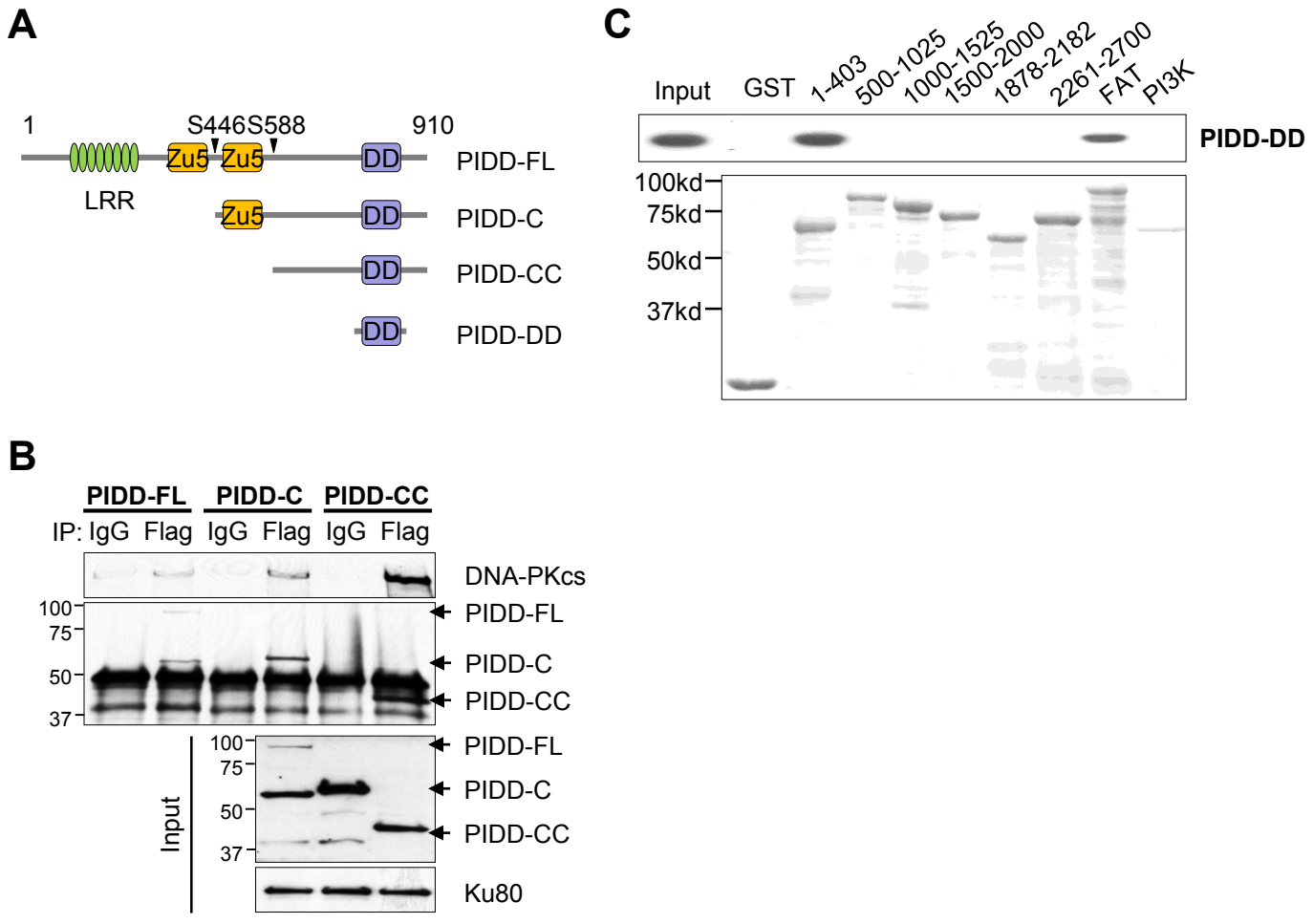
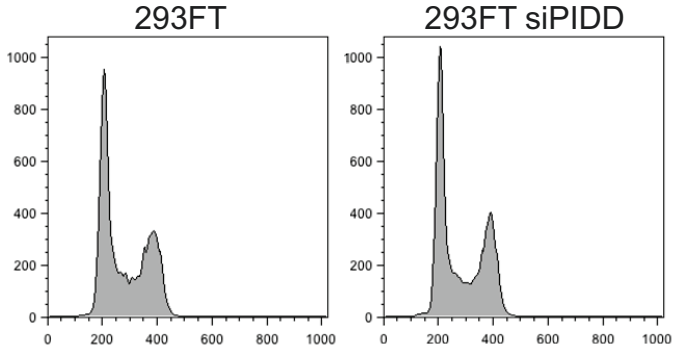


Figure S3. Protein-protein interaction between DNA-PKcs and PIDD. (A) PIDD domain organization: Leucine-rich repeats (LRR), ZO1/Unc5 (Zu5), and death domain (DD). PIDD is auto-cleaved at S446 and S588 and results in indicated PIDD fragments. (B) 293FT cells were transfected with a C-terminal FLAG-tagged PIDD-FL (amino acids 1-910), PIDD-C (amino acids 446-910), or PIDD-CC (amino acids 588-910) expression plasmid for 24 h. Whole cell lysates were subjected to immunoprecipitation with an anti-FLAG antibody followed by western blot against anti-DNA-PKcs or -FLAG antibodies. (C) GST-DNA-PKcs fragments were incubated with His-tagged PIDD DD domain (amino acids 778-873) followed by retrieval with Glutathion-sepharose beads. The bound PIDD was western blotted with anti-His antibody (top panel). The loading of various GST fusions was demonstrated by Ponceau S staining (bottom panel).

Supplemental Figure S4



	G1 (%)	S (%)	G2/M (%)
siCon	41.7±0.6	28.0±1.7	29.8±1.6
siPIDD	41.0±0.7	26.1±1.7	32.1±1.4

Figure S4. 293FT cells were transfected with siRNA control (siCon) and siRNA against PIDD (siPIDD #1) for 72 h and analyzed the cell cycle distribution with Propidium Iodide staining.

Supplemental Figure 5

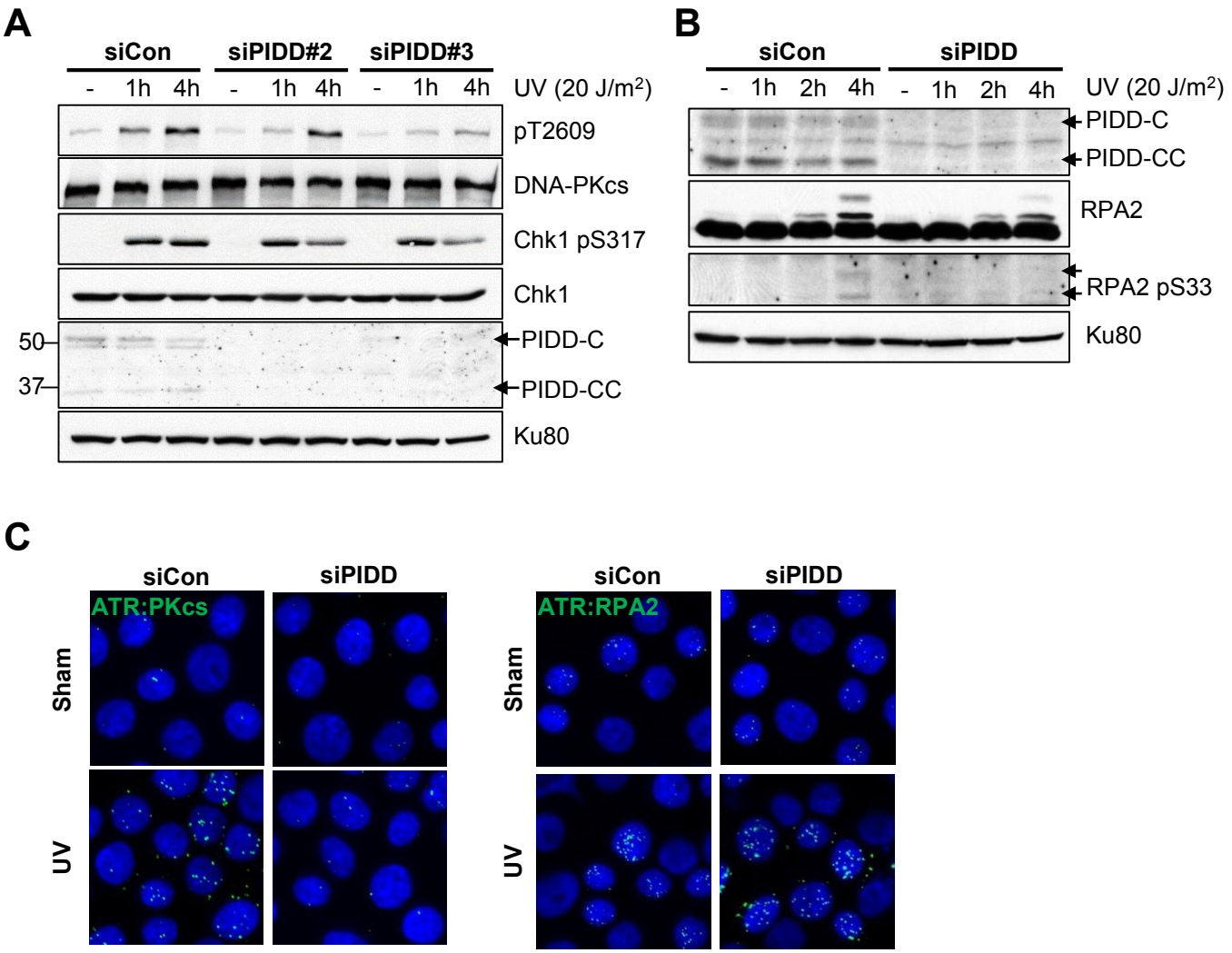


Figure S5. PIDD mediates DNA-PKcs association with ATR in response to UV. (A) Control (siCon) and siPIDD (#2 and #3) transfected 293FT cells were exposed to UV (20 J/m^2). Whole cell lysates were prepared at the indicated time points and subjected to western blot analysis. (B) Control and siPIDD (#1) transfected 293FT cells were exposed to UV (20 J/m^2) and harvested at the indicated time points. Whole cell lysates were subjected to western blot as indicated. (C) Control and siPIDD (#1) transfected HeLa cells were exposed to UV (20 J/m^2). Cells were fixed at 30 minutes and analyzed for proximity ligation assay (PLA, green) using rabbit anti-ATR antibody coupled with mouse anti-DNA-PKcs antibody (left panel) or mouse anti-RPA2 antibody (right panel).

Supplemental Figure S6

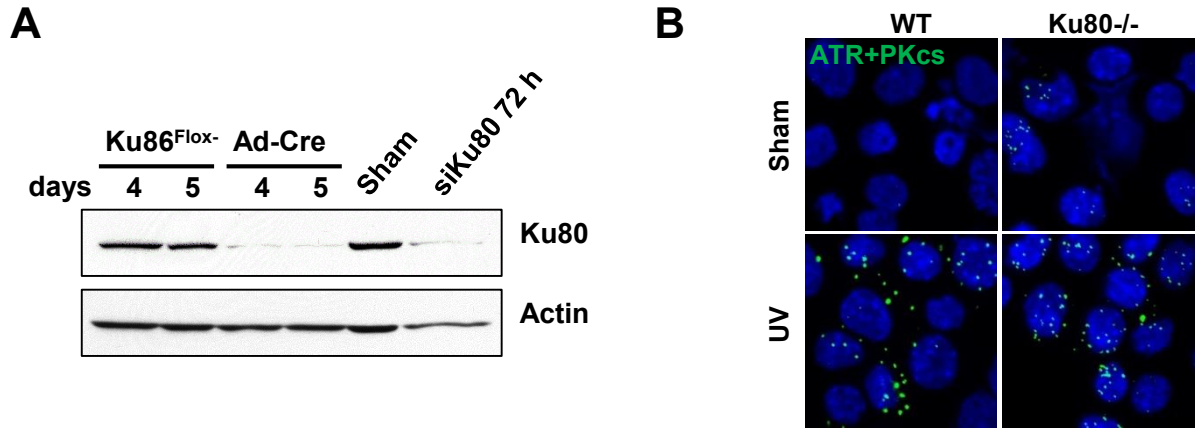


Figure S6. Ku80 is not required for DNA-PKcs and ATR association. (A) HCT116 $Ku86^{Flox/-}$ cells were infected with Cre recombinase-expressing adenovirus (Ad-Cre) or transfected with siRNA against Ku80. Cells were harvested at the indicated time points. Whole cell lysates were prepared for western blot analysis against anti-Ku80 or anti-Actin antibodies. (B) Mouse embryonic fibroblasts derived from wild-type (WT) or Ku80 knockout ($Ku80^{-/-}$) mice were exposed to UV (20 J/m^2) and fixed at 30 minutes followed by PLA (green) using rabbit anti-ATR and mouse anti-DNA-PKcs antibodies.

Supplemental Figure S7

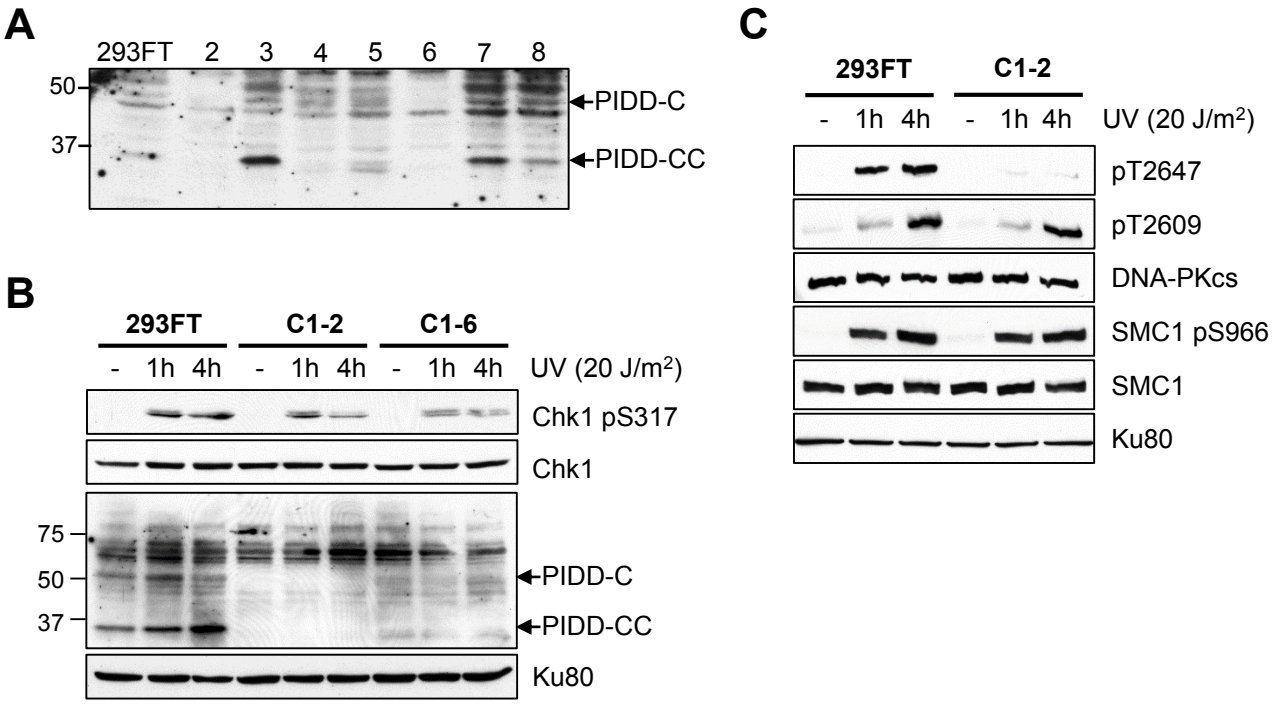


Figure S7. Knockout of endogenous PIDD gene via CRISPR/Cas9 approach.(A) Expression of endogenous PIDD in control 293FT cells and potential PIDD knockout cells via the CRISPR–Cas9 strategy. Cells were exposed to UV (20 J/m^2) and harvested at 1 hour. Whole cell lysate were prepared and subjected to western blot analysis. (B,C) Control 293FT and indicated PIDD knockout cells were UV irradiated. Whole cell lysates were prepared from sham or UV treated cells, and were subjected to western blot analysis.