

Supplementary Information

DNA-PKcs and ATM epistatically suppress DNA end resection and hyperactivation of ATR-dependent G₂-checkpoint in S-phase irradiated cells

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Supplementary Figure Legends

Figure S1: (A and B) Representative plots showing the regions used for gating the BrdU positive, G₂-phase, A549 cells used to determine the signal plotted in Figure 1C. Upper panel: Cell cycle distribution determined by analyzing PI signal. Lower panel: Contour plots of BrdU *versus* PI signal used to define the gates for BrdU positive cells. (C and D) As in Figure S1A and S1B for AT5Biva cells. (E) Percentage of G₂-phase, AT hTert cells exposed to 10 Gy of IR in the presence or absence of ATRi.

Figure S2: (A) As in Figure 1A for DNA-PKcs deficient, HCT116 cells (HCT116^{DNA-PKcs^{-/-}}) exposed to 2 Gy of IR and treated with the indicated inhibitors. (B) As in Figure S2A for DNA-PKcs proficient HCT116 cells. (C) As in Figure S2A for A549 cells. (D) As in Figure S2A for DNA-PKcs deficient, HCT116 cells exposed to 10 Gy of IR and treated with the indicated inhibitors. (E) Left panels show the cell cycle distribution of S-phase enriched, parental A549 cells (A549^{wt}), at the time of exposure to 10 Gy IR (0 h), as well as 9 h after IR exposure. Right panels show similar results for a CRISPR/cas9 generated DNA-PKcs deficient A549 cells (A549^{DNA-PKcs^{-/-}}). (F) As in Figure 2SE for AT hTert cells.

Figure S3: (A) Quantitative analysis of DNA end resection using median values of RPA70 signal intensity. Other details as in Figure 5C (lower panels). (B) RPA70 signal intensity in EdU⁺, G₂-phase, A549 cells at 3 and 6 h after exposure of S-phase cells (EdU⁺) to 10 Gy X-rays, in the presence or absence of ATRi. (C) Quantitative analysis of DNA end resection using median values of BrdU signal intensity. Other details as in Figure 6D. (D) As in Figure S3B for AT hTert cells exposed to 10 Gy and analyzed at 3, 6, and 9 h post-irradiation in the presence or absence of ATRi. (E) RPA70 signal intensity in EdU⁺, G₂-phase, HCT116 cells, lacking DNA-PKcs activity, treated with indicated inhibitors and irradiated with 10 Gy. (F)

RPA70 signal intensity in EdU⁺, G₂-phase, M059J cells 6 h after exposure of S-phase cells (EdU⁺) to 10 Gy X-rays, in the presence or absence of CHK1i.

Figure S4: (A) Raw western blot data of experiments shown in Figure 1D. (B) Raw western blot data of experiments shown in Figure 3E. (C) Raw western blot data of experiments shown in Figure 3F.

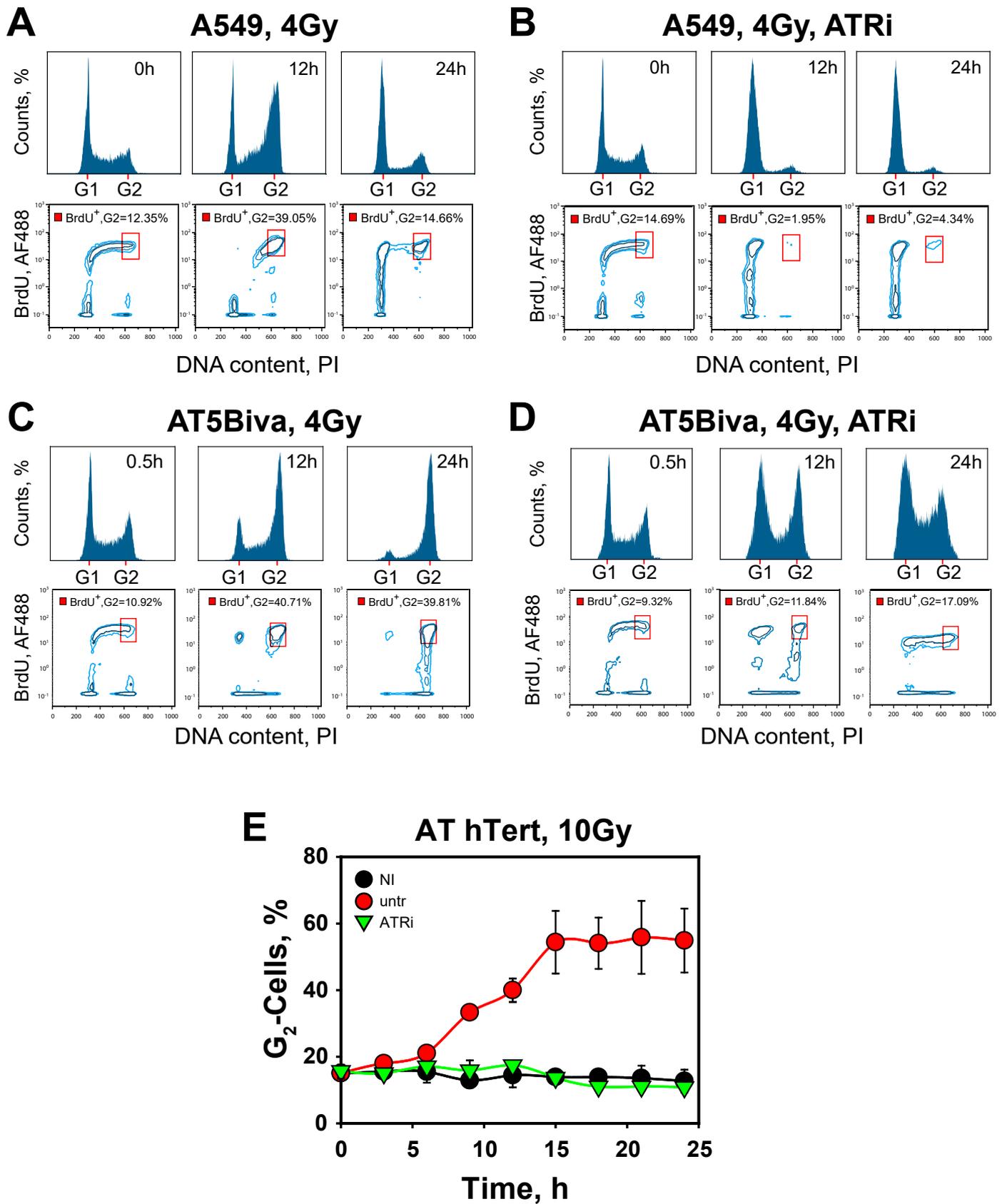


Figure S1

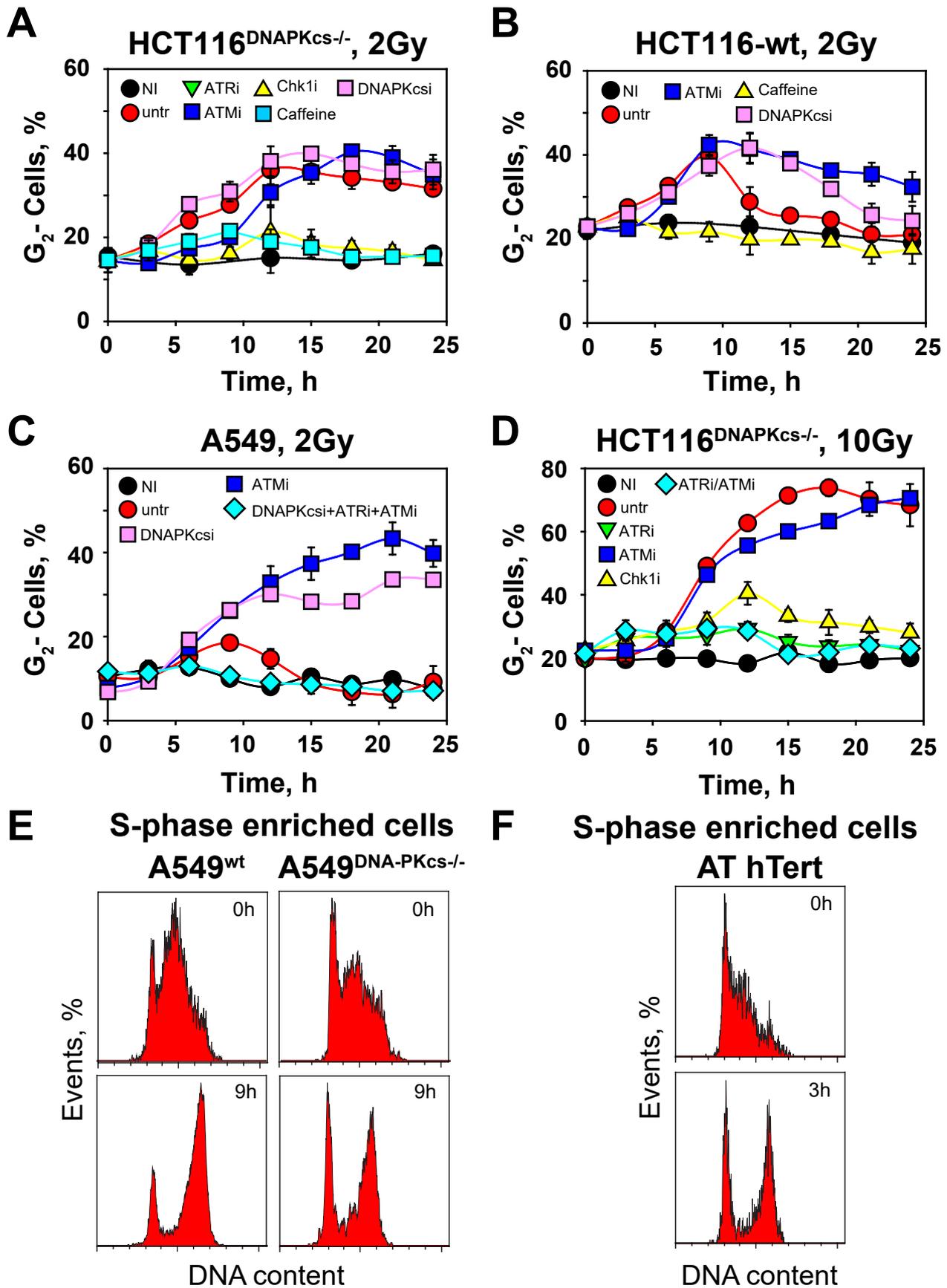


Figure S2

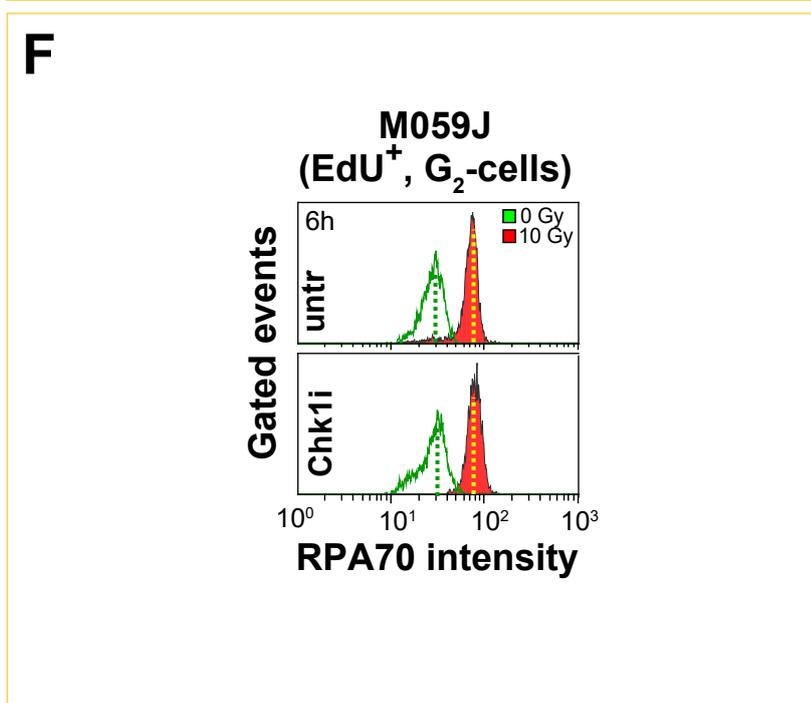
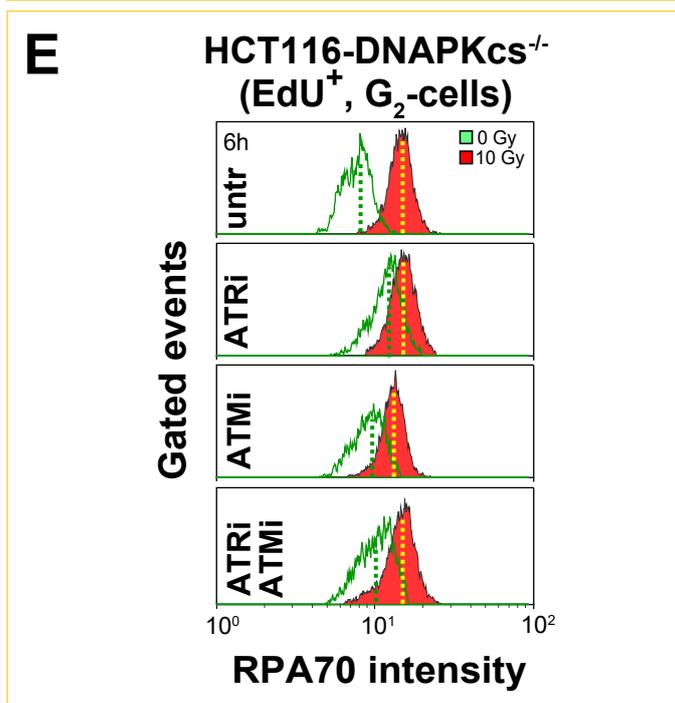
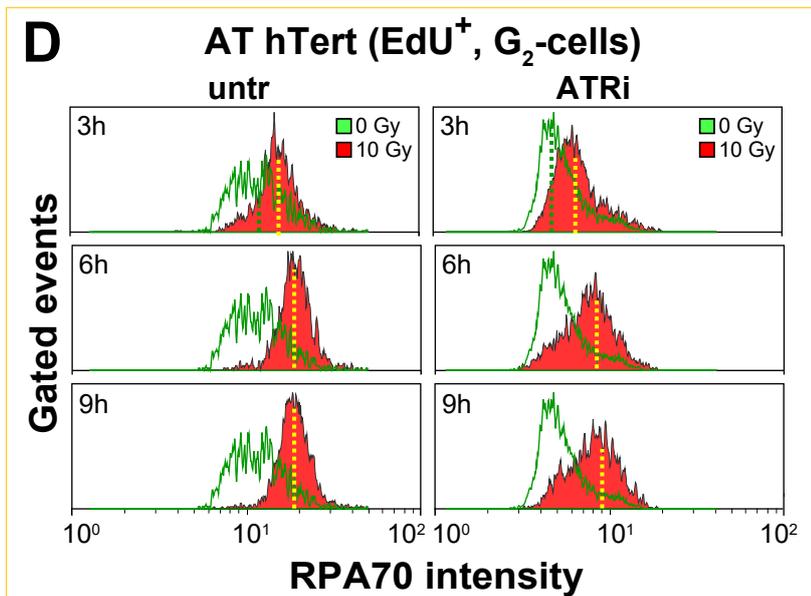
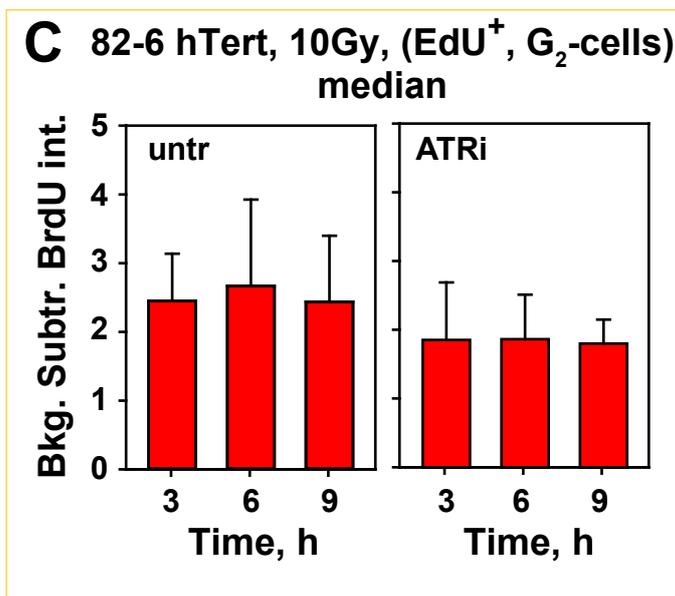
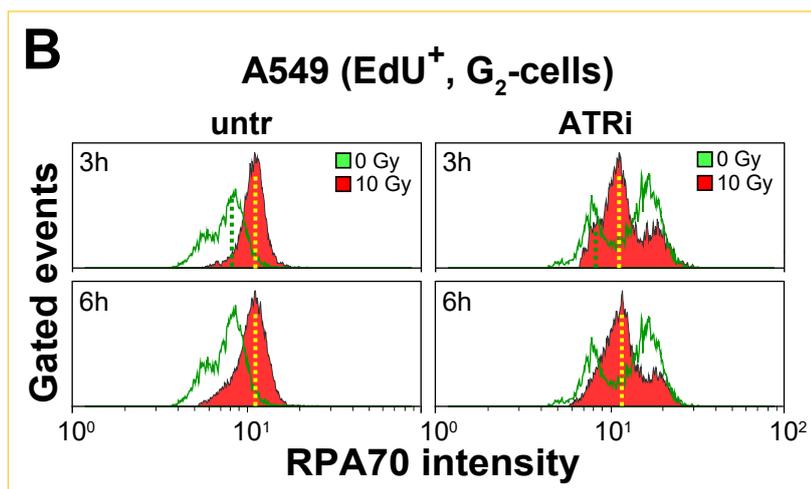
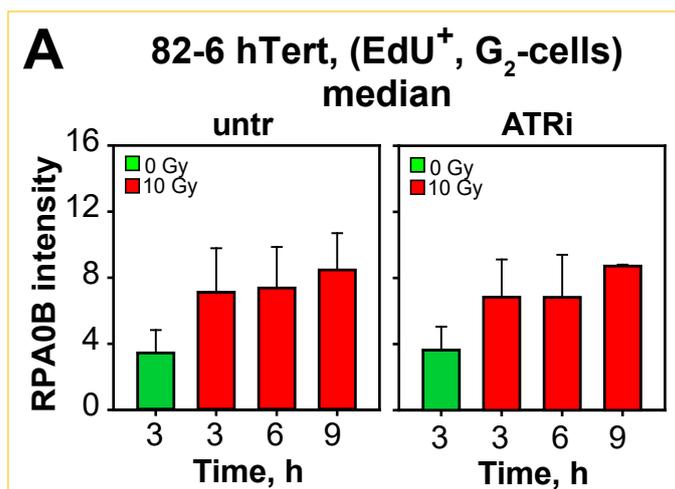


Figure S3

GM847-ATRkd

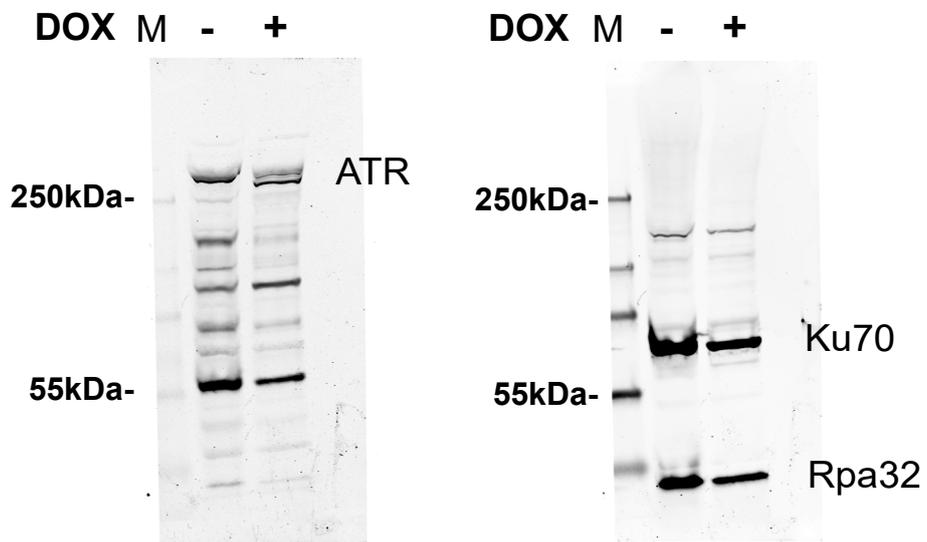


Figure 1D

S-phase enriched cells, 9h post IR

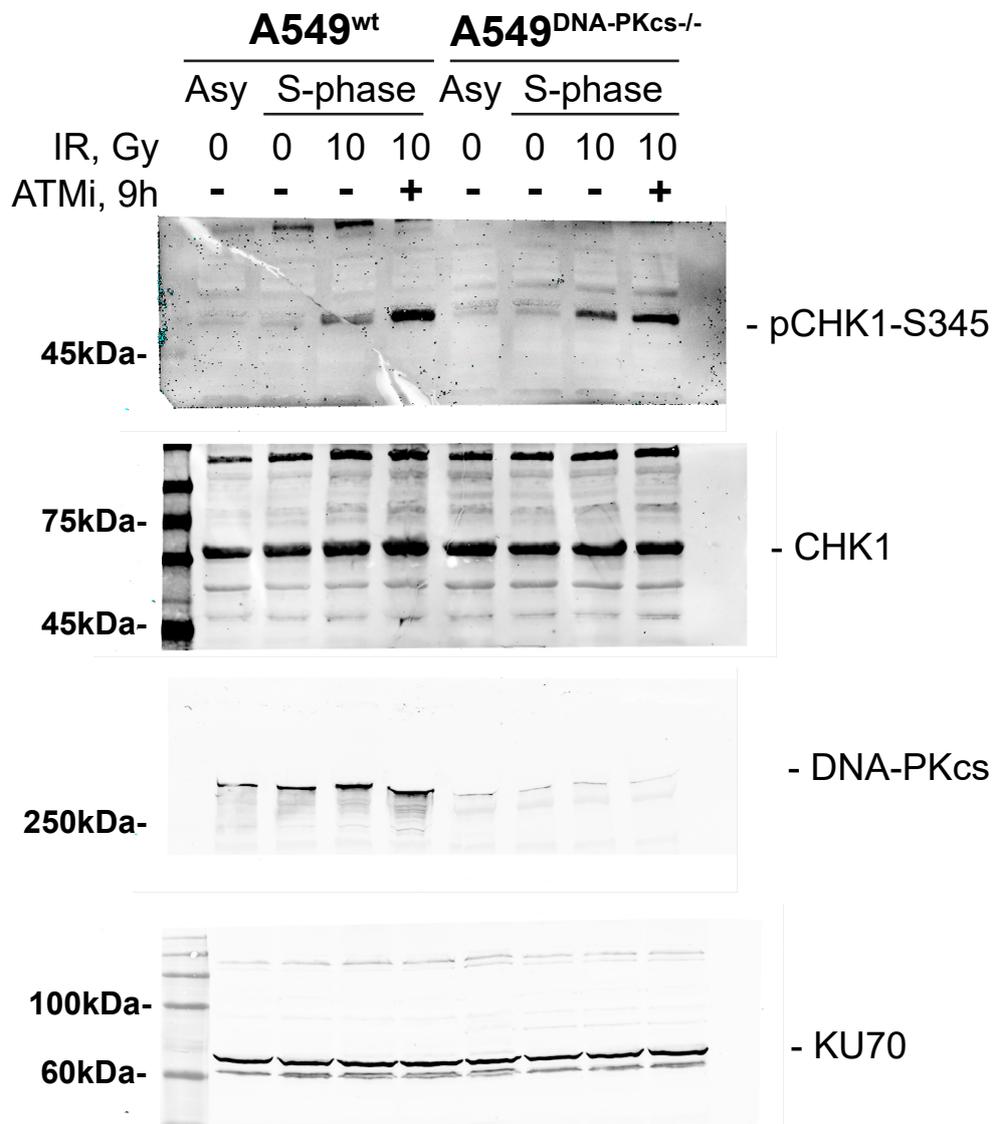


Figure 3E

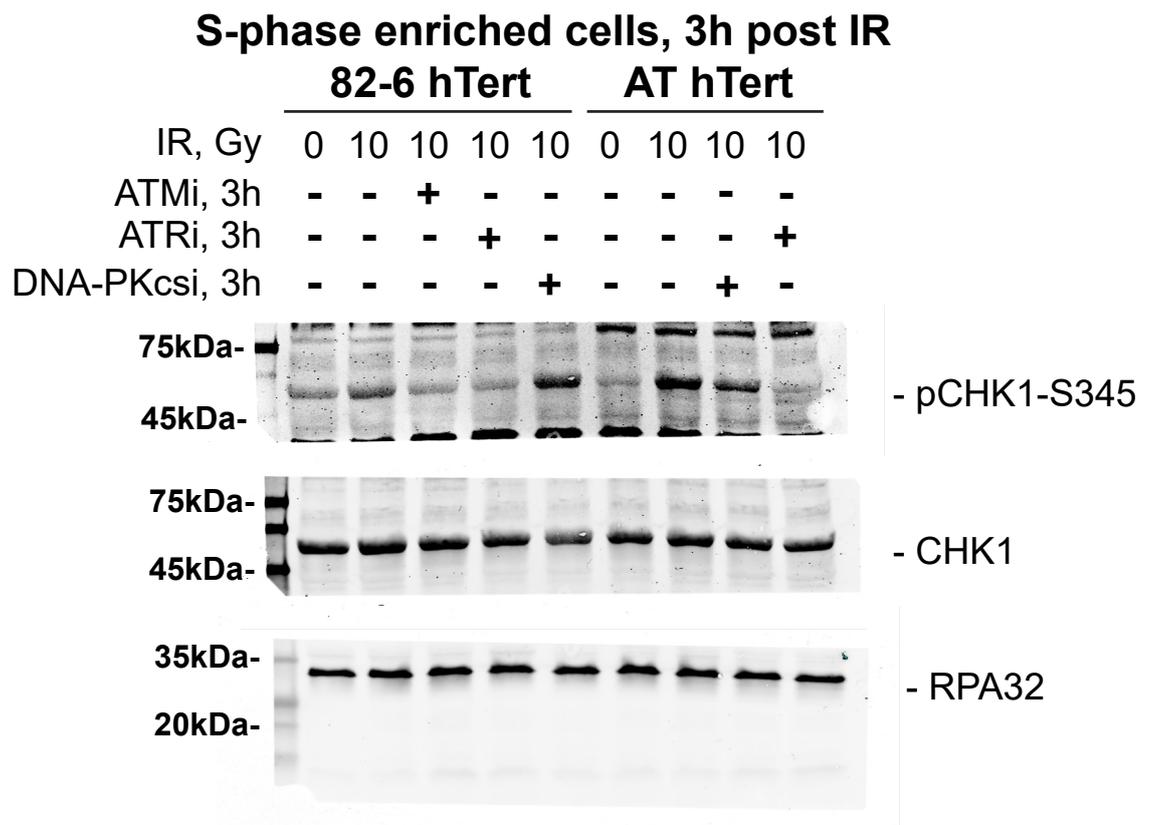


Figure 3F