

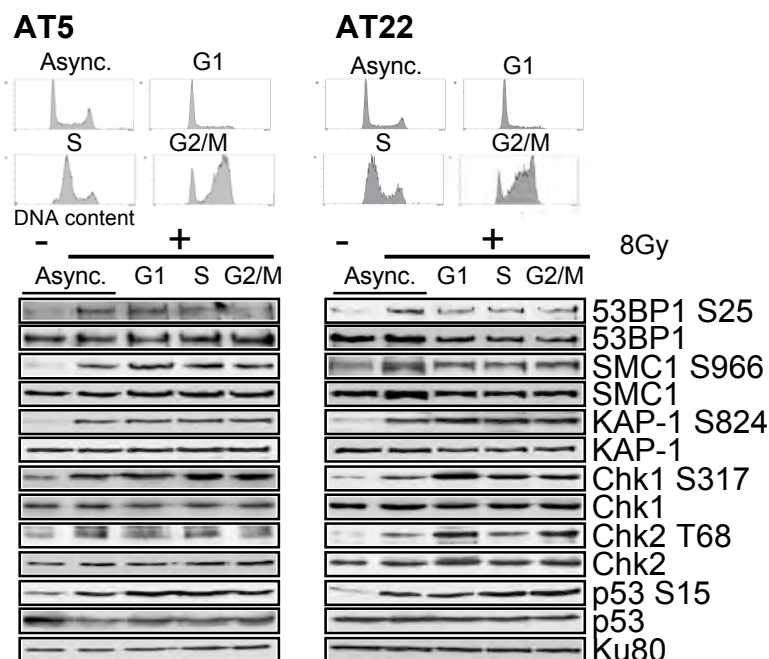
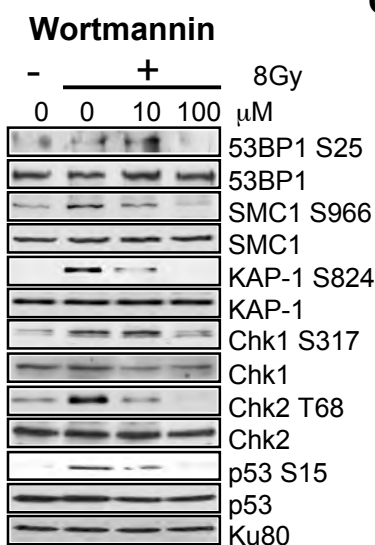
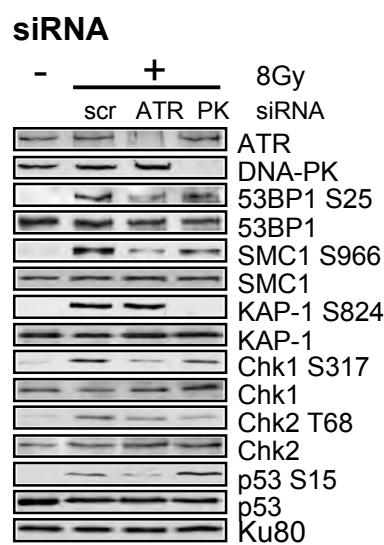
## Supplementary information

### **Distinct roles of ATR and DNA-PKcs in triggering DNA damage responses in ATM-deficient cells**

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**Fig. S1****A****B****C**

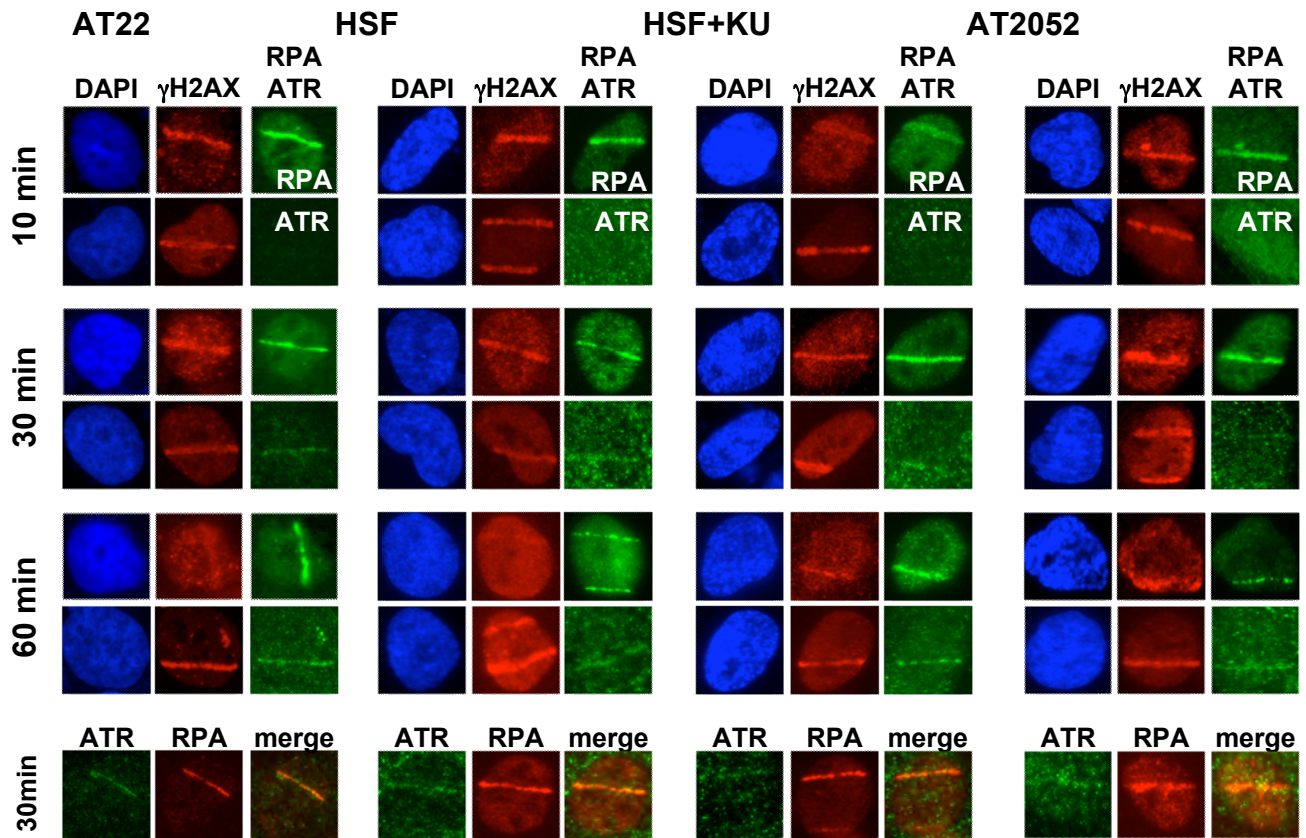
**Fig. S1. Phosphorylation of key DDR proteins by ATR in ATM-deficient cells.** (A) Synchronized AT5BIVA or AT22IJE-T cells were irradiated with 8 Gy of gamma rays during G1, S, or G2 phases and assayed by Western blotting with phospho-specific antibodies after 1 hr; cell synchronization was confirmed by single-parameter flow cytometry after PI staining for DNA content (PI; x axis). Please note that while an earlier paper (Kaneko *et al*) reported low levels of Chk1 in G1 cells, we do not see any cell cycle differences in Chk1 levels and, in this, our results are similar to that reported by Bassermann *et al* and Feijoo *et al*. This variability could be because of different cell lines used in these studies. (B) Phosphorylation of DDR proteins in AT22IJE-T cells irradiated with 8 Gy of gamma rays was assayed after pretreatment with increasing doses of wortmannin or (C) after siRNA-mediated knockdown of ATR or DNA-PKcs.

## REFERENCES

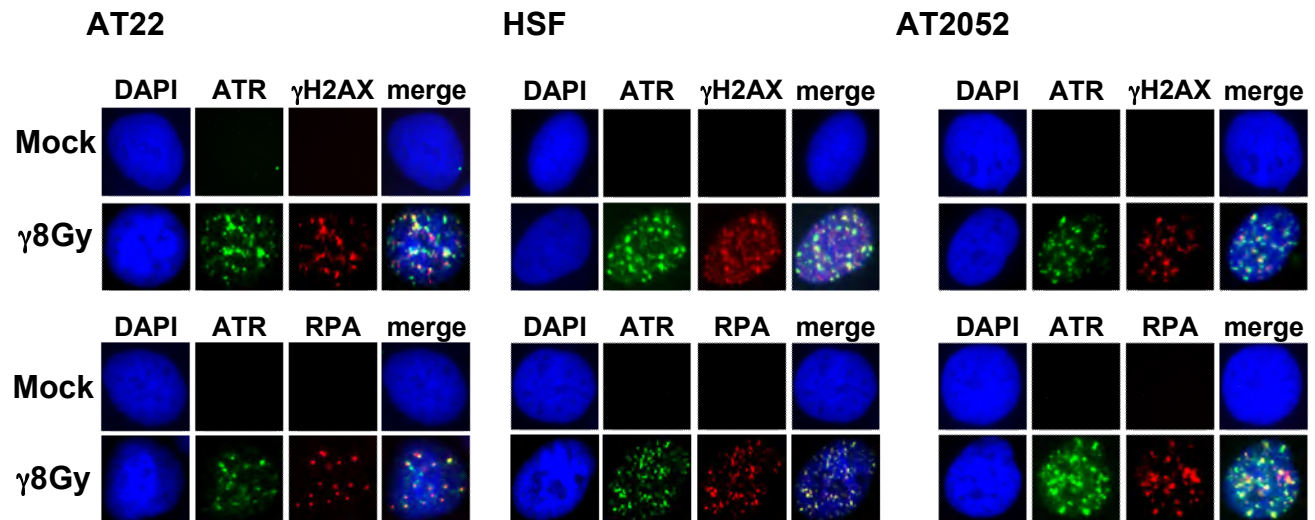
1. Bassermann F, Frescas D, Guardavaccaro D, Busino L, Peschiaroli A, Pagano M (2008) The Cdc14B-Cdh1-Plk1 axis controls the G2 DNA-damage-response checkpoint. *Cell* **134**(2): 256-267
2. Feijoo C, Hall-Jackson C, Wu R, Jenkins D, Leitch J, Gilbert DM, Smythe C (2001) Activation of mammalian Chk1 during DNA replication arrest: a role for Chk1 in the intra-S phase checkpoint monitoring replication origin firing. *J Cell Biol* **154**(5): 913-923
3. Kaneko YS, Watanabe N, Morisaki H, Akita H, Fujimoto A, Tominaga K, Terasawa M, Tachibana A, Ikeda K, Nakanishi M (1999) Cell-cycle-dependent and ATM-independent expression of human Chk1 kinase. *Oncogene* **18**(25): 3673-3681

**Fig. S2**

**A**



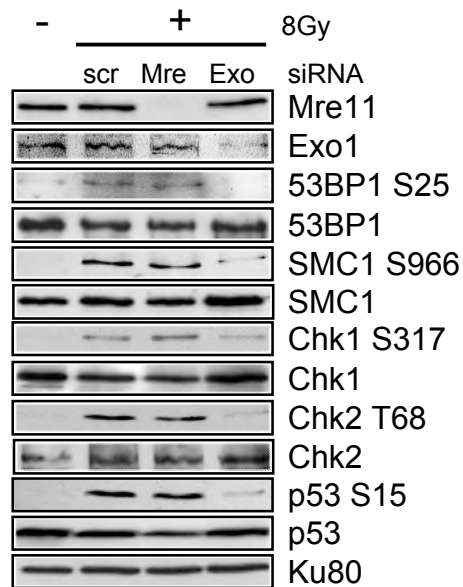
**B**



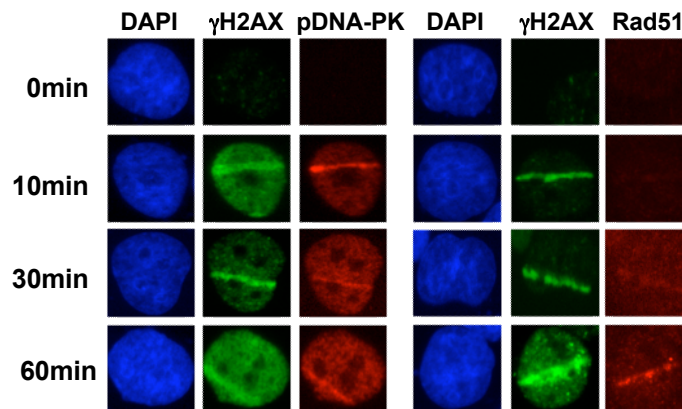
**Fig. S2. Sequential recruitment of RPA and ATR to DSBs in ATM-deficient cells. (A)** AT22IJE-T cells, HSFs, HSFs pre-treated with KU55933, or AT2052 cells were laser micro-irradiated and co-immunofluorescence stained with anti- $\gamma$ H2AX antibody and either anti-RPA antibody (top panel) or anti-ATR antibody (bottom panel) at the indicated times. These cells were also co-immunofluorescence stained with anti-RPA antibody and anti-ATR antibody at 30min. **(B)** AT22IJE-T, HSF, or AT2052 cells were immunofluorescence stained 1 hr after irradiation with 8 Gy of gamma rays.

## Fig. S3

**A**



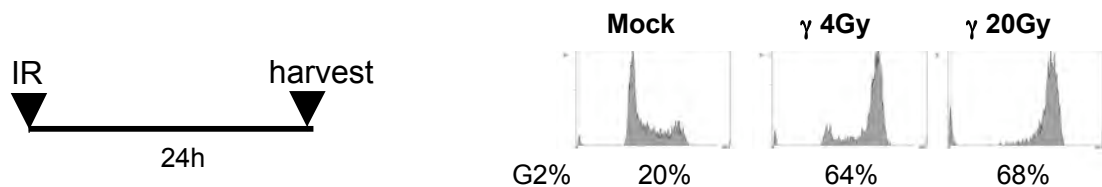
**B**



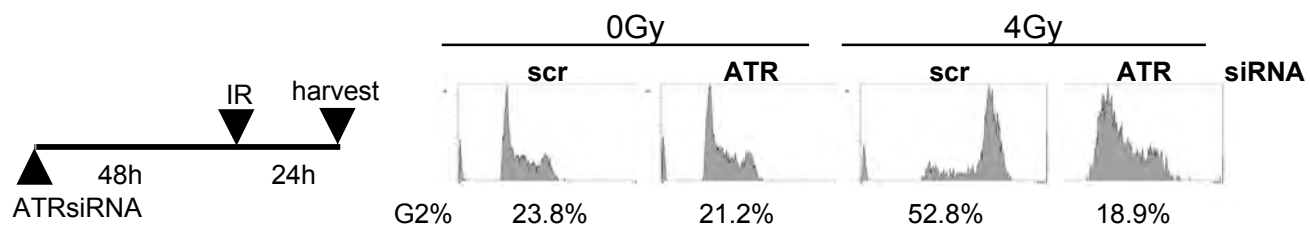
**Fig. S3. Exo1-dependent recruitment of ATR and activation of DDR signaling.** (A) Phosphorylation of DDR proteins in AT221JE-T cells irradiated with 8 Gy of gamma rays was assayed by Western blotting after siRNA-mediated knockdown of Mre11 or Exo1. (B) AT221JE-T cells were laser micro-irradiated and co-immunofluorescence stained with anti- $\gamma$ H2AX antibody and either anti-pDNA-PKcs(S2056) or anti-Rad51 antibody at the indicated times.

**Fig. S4**

**A**



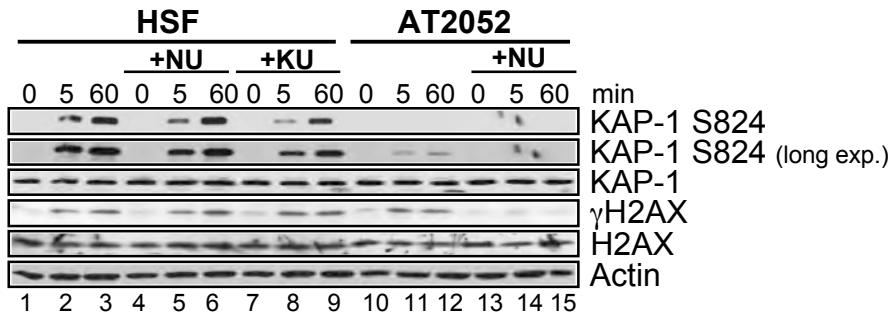
**B**



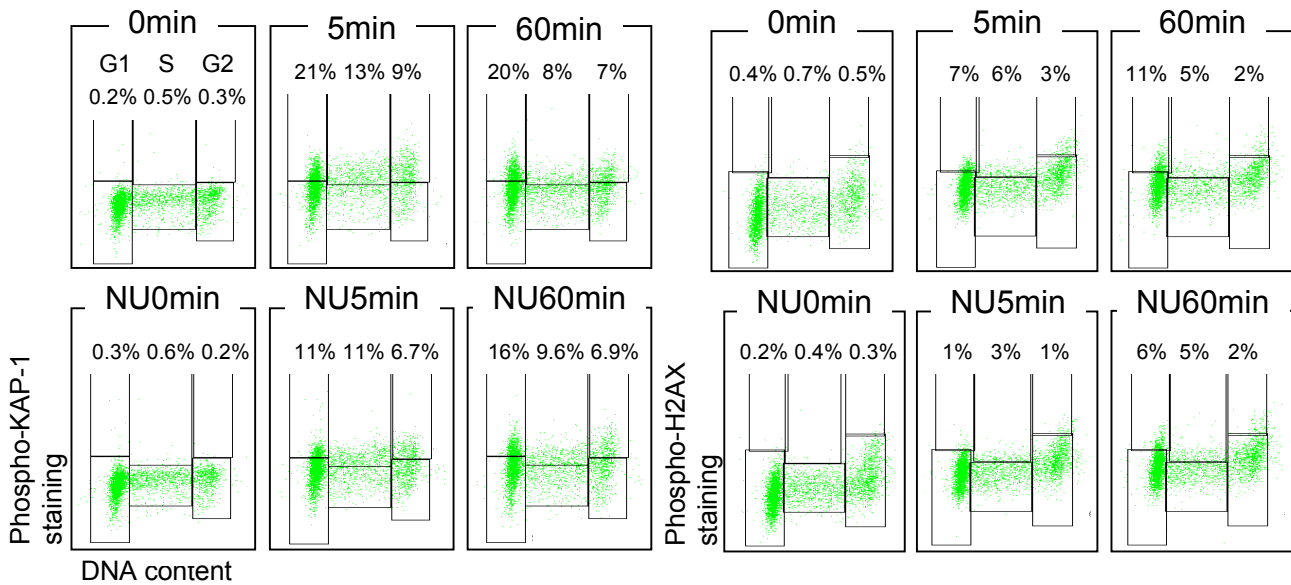
**Fig. S4. Implementation of G2/M checkpoints in ATM-deficient cells by ATR.** (A) Late G2 accumulation of AT22IJE-T cells (24 hr after irradiation) was quantified by single-parameter flow cytometry after PI staining for DNA content (PI; *x* axis). Percent cells in G2 are indicated at the bottom of each plot. (B) Lack of late G2 accumulation in AT22IJE-T cells with siRNA-mediated knockdown of ATR.

**Fig. S5**

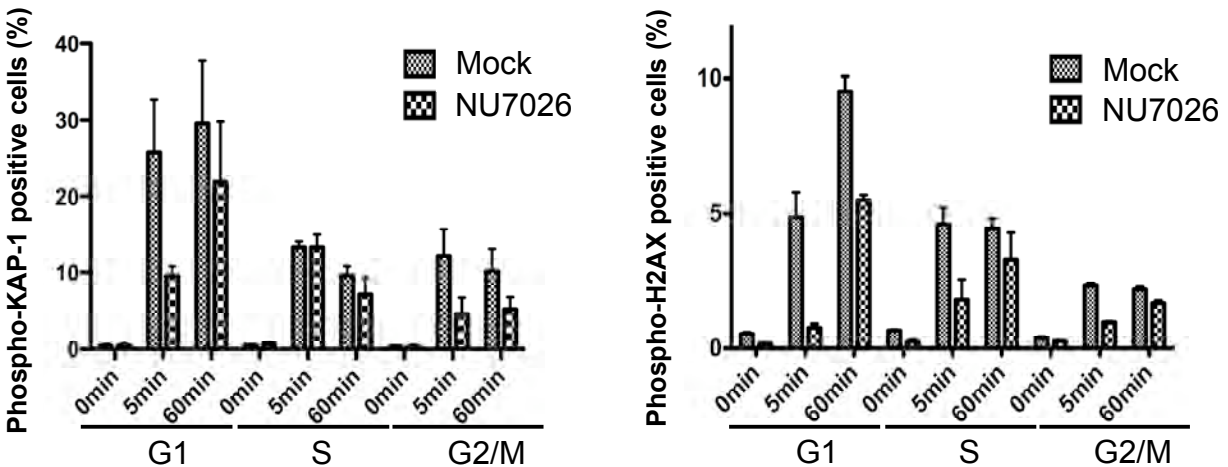
**A**



**B**



**C**



**Fig. S5. Phosphorylation of KAP-1 by both ATM and DNA-PKcs.** (A) HSF or AT2052 cells were irradiated with 8 Gy of gamma rays in the presence or absence of NU7026 or KU5933 and assayed by Western blotting with anti-pKAP-1 or anti-γH2AX antibody. Longer exposures were necessary for visualization of KAP-1 phosphorylation in ATM-deficient cells (long exp.) (B) Phosphorylation of KAP-1 or H2AX in different stages of the cell cycle (in the presence or absence of NU7026) was assayed by dual-parameter flow cytometry of 1BR3 cells. Staining for DNA content (x axis) and for KAP-1 or H2AX phosphorylation (y axis) is shown. Percent cells with phospho-KAP-1 or γH2AX signals are indicated for each cell cycle stage. (C) Percent cells with phospho-KAP-1 or γH2AX signals are plotted against the respective cell cycle stage and treatment condition.