

Appendix. Jaiswal et al. ATM/Wip1 activities at chromatin control Plk1 re-activation to determine G2 checkpoint duration

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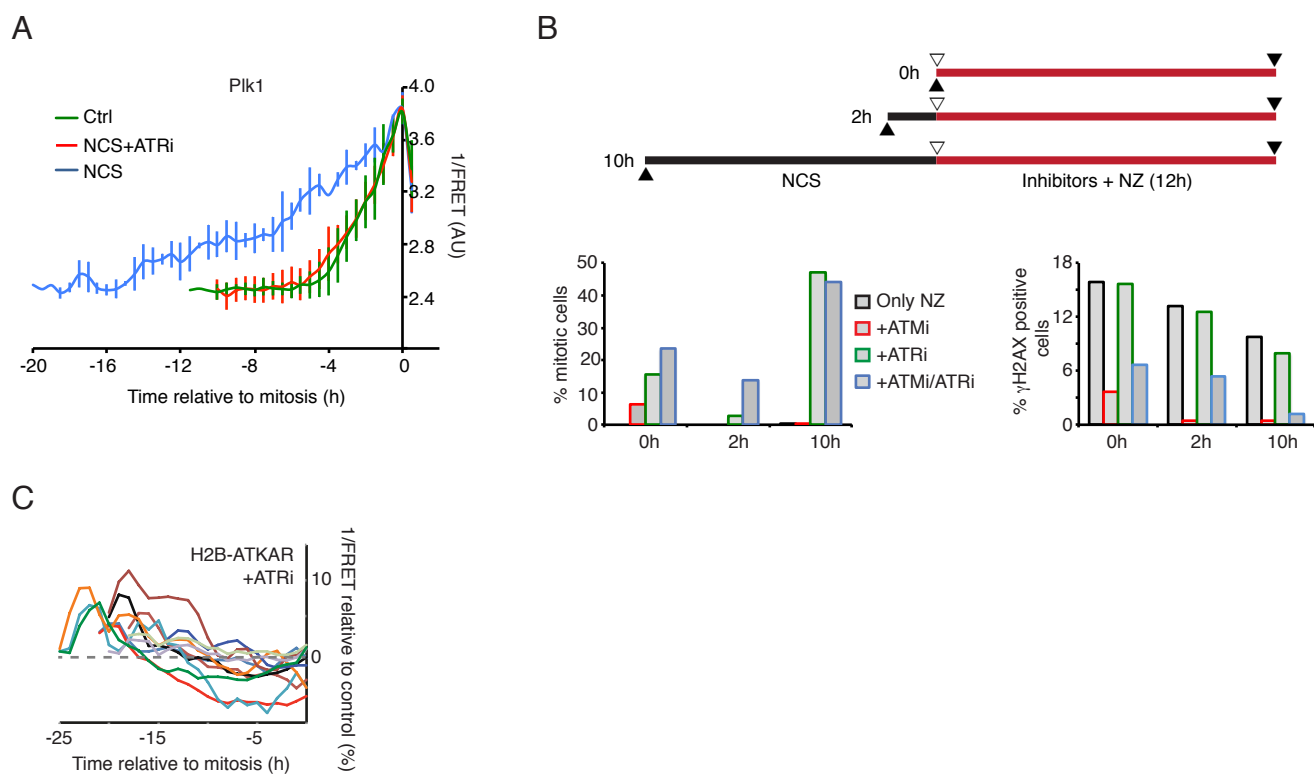
**Appendix Figure S1.** ATM inhibits Plk1 during the early phases of a DDR.

**(A)** ATR inhibits Plk1 activity during checkpoint recovery in RPE cells. RPE cells expressing a Plk1 FRET-probe were transfected with p53 siRNA. NCS (8 nM) and ATRi (1  $\mu$ M) were added as indicated. Graph shows average and SD of 15 cells (ATRi and Ctrl) or 2 cells (NCS; spontaneous recovery) synchronized *in silico* in mitosis.

**(B)** Synergistic effect of ATM and ATR inhibition early after NCS. U2OS cells were treated with NCS (1 nM) for 0, 2, and 10 h, and subsequently incubated for 12 h with nocodazole and inhibitors as indicated. Cells were fixed, stained for pS10-histone H3 and  $\gamma$ H2AX and analyzed by FACS.

**(C)** H2B-ATKAR is dephosphorylated before cells enter mitosis in presence of ATR inhibitor. Quantification of 1/FRET of U2OS cells expressing H2B-ATKAR after treatment with VE821 (1  $\mu$ M, 30 min) and NCS (2 nM). Each line represents a single cell that is synchronized in mitosis *in silico*. The FRET-ratio change of each cell relative to the FRET-ratio before NCS addition is shown.

# Appendix Figure S1



**Appendix Figure S2. ATM activity is detected throughout chromatin upon localized DNA damage**

(A, B) DSBs are restricted to the laser microirradiated area.  $\gamma$ H2AX and BRCA1 remain in laser microirradiated area in U2OS (A) and RPE cells (B). Images show immunofluorescence stainings with indicated antibodies in laser microirradiated and neighbouring non-irradiated cells.

(C) Quantification of spread of H2B-ATKAR FRET-change after laser micro-irradiation in RPE cells. Measurements were performed distal to the laser-micro-irradiated area. Graph shows average and SD of at least 6 cells per condition, performed as in Fig 5B.

# Appendix Figure S2

