







Supplementary information, Figure S4. ATM Y370 is a major EGFR-mediated phosphorylation site. **(A)** Characterization of the p-ATM Y370 antibody. Various amounts of peptides were spotted on PVDF membrane and detected by p-ATM Y370 antibody or amido black staining. IB: immuno-blotting. ATM pY370: ATM phospho-Y370 peptide. ATM Y370: ATM non-phospho-Y370 peptide. MCM7 pY600: MCM7 phospho-Y600 peptide. Lyn pY32: Lyn phospho-Y32 peptide⁵. **(B)** HEK 293T cells were transfected with plasmids as indicated and harvested for Co-IP assay followed by Western blot analysis after IR stimulation. **(C)** ATM association with Myc-tagged EGFR wild type (WT) or domain deletion mutants. Dead, kinase dead mutant; KD, kinase domain only; Δ CR, C-terminal regulatory region deletion mutant. (+) or (-) shown on the right respectively indicates positive or negative interaction with ATM or Y370 phosphorylated ATM. ECD: extracellular domain; TM: transmembrane; JM: juxtamembrane; KD: kinase domain; CR: C-terminal regulatory region; ICD: intracellular domain; Dead: EGFR kinase dead mutant. **(D)** HEK 293T cells were transfected with plasmids as indicated and harvested for Co-IP assay followed by Western blot analysis after IR stimulation. WCE: whole cell extract. **(E and F)** p-ATM S367 and S2996 IRIF staining of ATM-depleted (shATM) HeLa cells with reconstitution of Flag-tagged ATM wild type (WT) or Y370F mutant.

Quantitation of p-ATM S367 IRIF is presented as mean values \pm SD. $n = 55$. $**p < 0.01$.
Quantitation of p-ATM S296 IRIF is presented as mean values \pm SD. $n = 50$. $**p < 0.01$. **(G)** p-KAP1 IRIF staining of ATM-depleted (shATM) HeLa cells with reconstitution of Flag-tagged ATM wild type (WT) or Y370F mutant. Quantitation of p-KAP1 IRIF is presented as mean values \pm SD. $n = 51$. $**p < 0.01$. **(H)** ATM-depleted HeLa cells were transfected with Flag-tagged ATM WT, Y370F, or vector alone. Cell transfectants were treated with or without IR, harvested and analyzed by Western blot with indicated antibody.