Supplementary Figure 3: Effect of phosphomimic substitutes (S1893E, S1981E) on radiation-induced ATM activation.

**A-C.** *In vitro* ATM kinase activity in cells transfected with phosphomimic mutants of ATM. Stable cell lines were established by transfection of AT1ABR cells with pMAT1 and the phosphomimic ATM mutants S1893E and S1981E in pMEP4. ATM protein was induced for 18hr with CdCl₂ and cells were irradiated with 6Gy of radiation, and incubated for 1 hr prior to preparation of extracts. Immunoprecipitation was carried out (1mg of extract) with anti-FLAG antibodies followed by determination of *in vitro* ATM kinase activity using GST-p53₁₄₄ as a substrate. ATM was determined by immunoblotting.

**D.** ATM phosphomimic mutants are not constitutively active but display radiation-inducible activity. Cell extracts were prepared as described in A-C above and immunoblotted with ATM, pS15-p53, p53, pS343-Nbs1, Nbs1, pT68-Chk2, Chk2, pS957-SMC1 and SMC1 antibodies.