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Supplemental Data

Essential Role for DNA-PKcs

in DNA Double-Strand Break Repair

and Apoptosis in ATM-Deficient Lymphocytes

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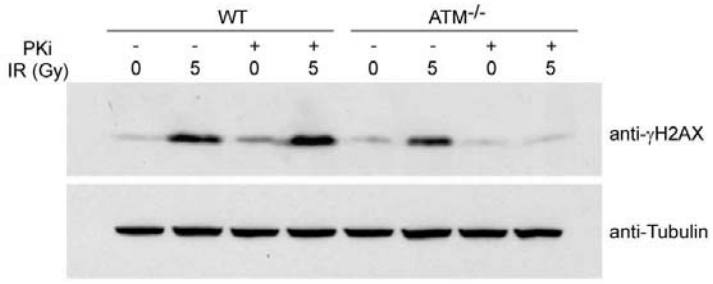
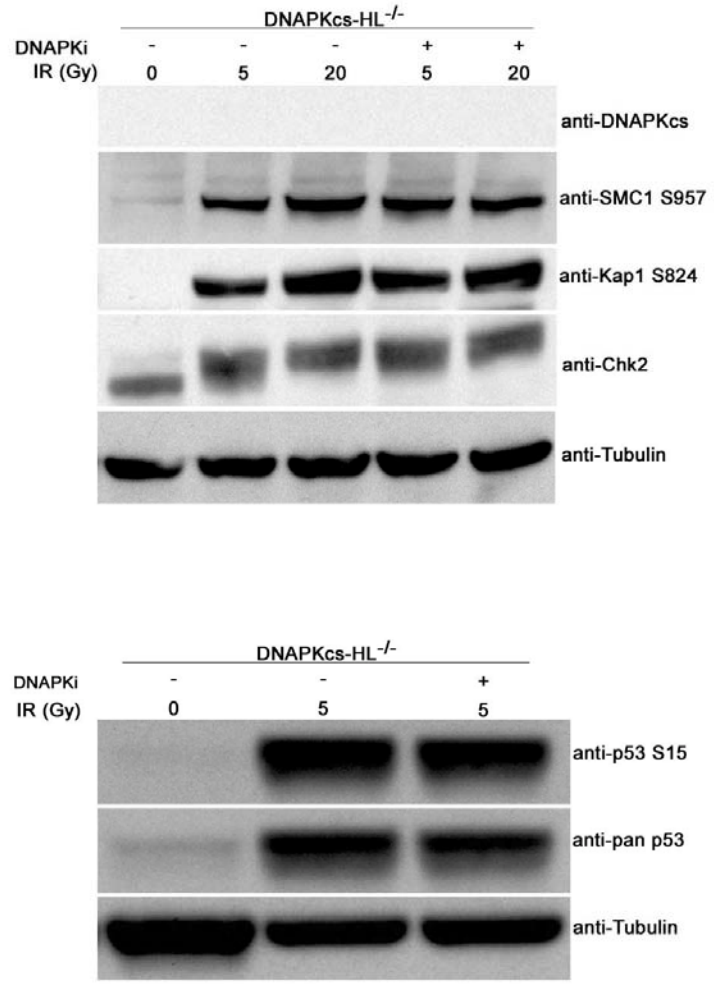
Figure S1



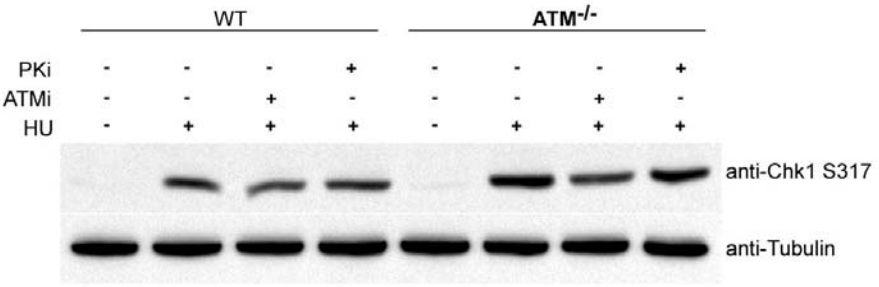
DNA-PKcs inhibition blocks γ-H2AX formation in primary ATM$^{-/-}$ Bcells
(A) Western blotting for phosphorylated H2AX on extracts from wild type or ATM$^{-/-}$ B cells 30 minutes after irradiation with 0 or 5 Gy. B cells with pre-treated with DNA-PKcs inhibitor Nu7026 as indicated.

Figure S2



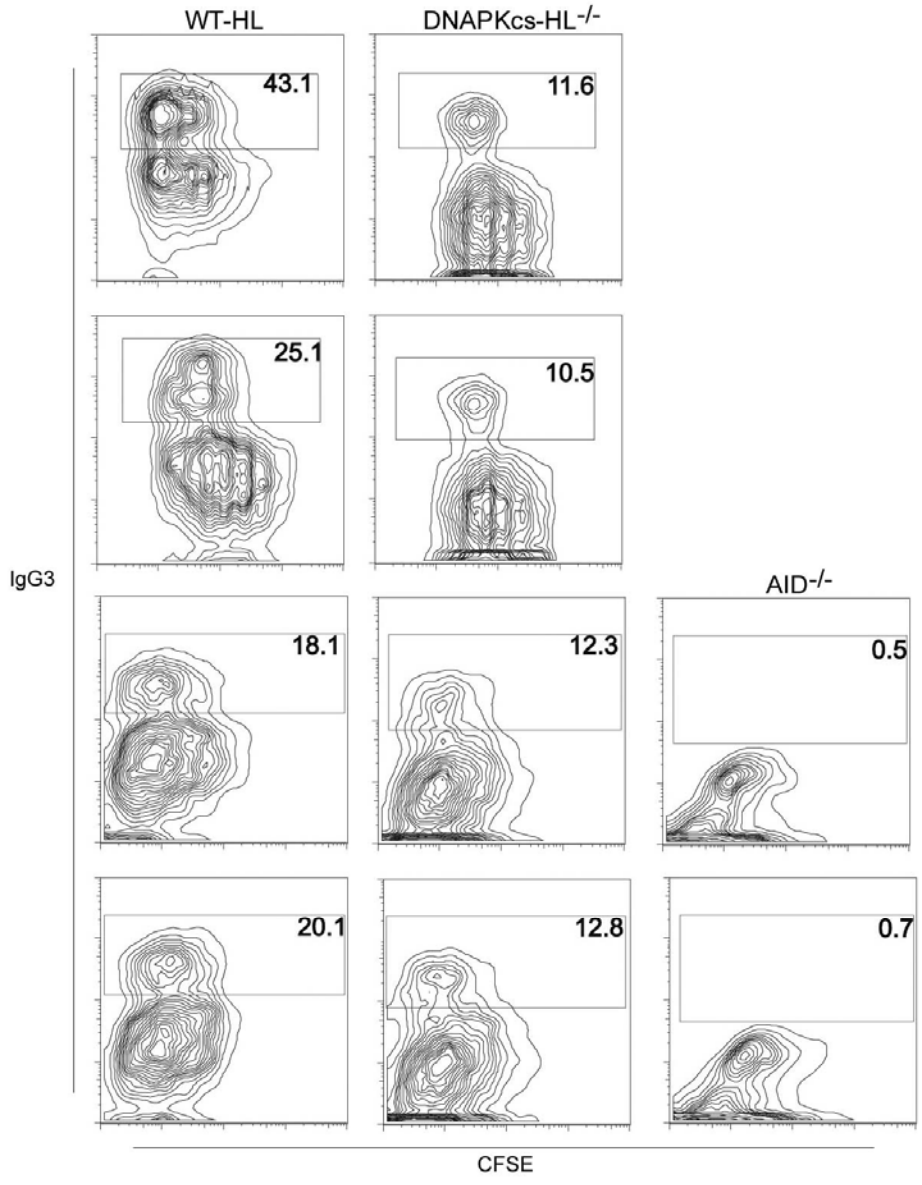
No off-target effects of PKi on ATM activity
(A) Treatment with Nu7026 does not alter the levels of phosphorylated Kap-1, Smc1 or Chk2 in DNAPKcs$^{-/-}$ B cells evaluated 30min after 5 or 20Gy of ionizing radiation. (B) Accumulation and phosphorylation of p53 protein 4 hours after IR in DNAPKcs$^{-/-}$ Bcells pre-treated with Nu7026

Figure S3



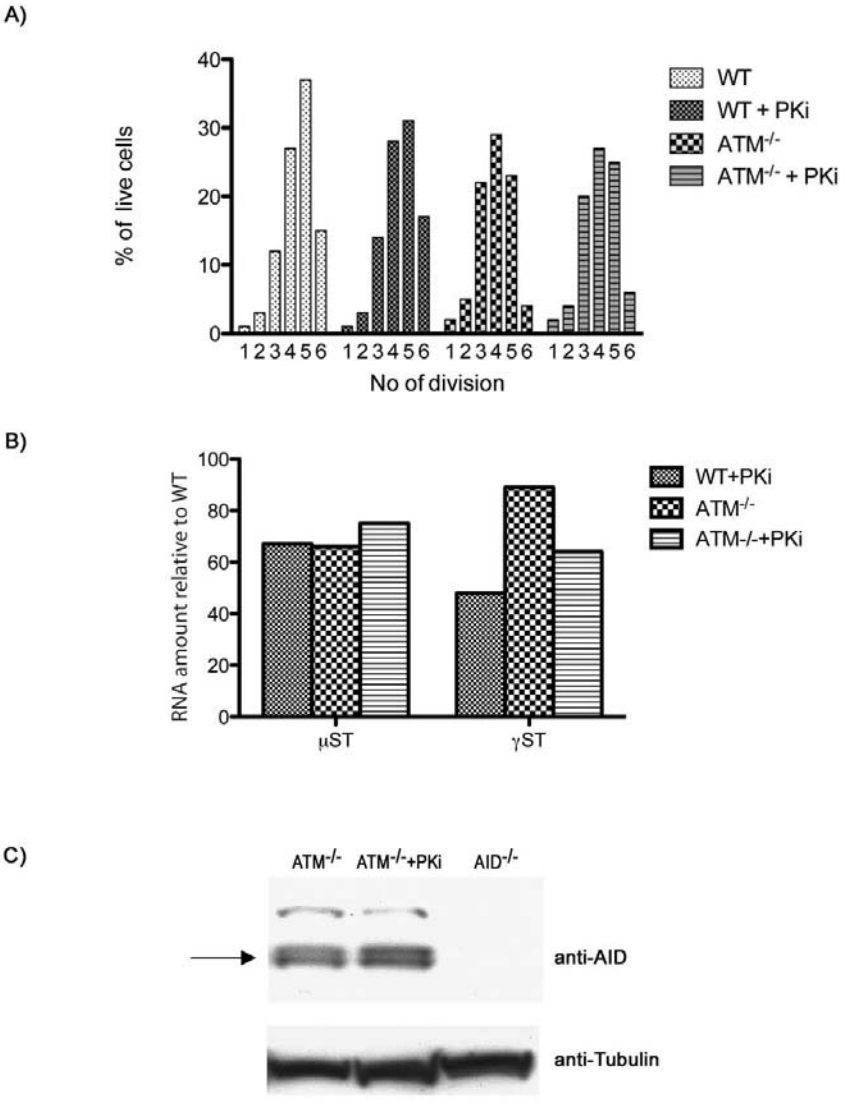
No off-target effects of PKi on ATR activity
WT and ATM$^{-/-}$ B cells were pre-treated with ATMi (KU55933) or PKi for 1h and then challenged with 2mM HU for 2h. Levels of ATR-dependent phosphorylated Chk1 were analyzed by western blotting.

Figure S4



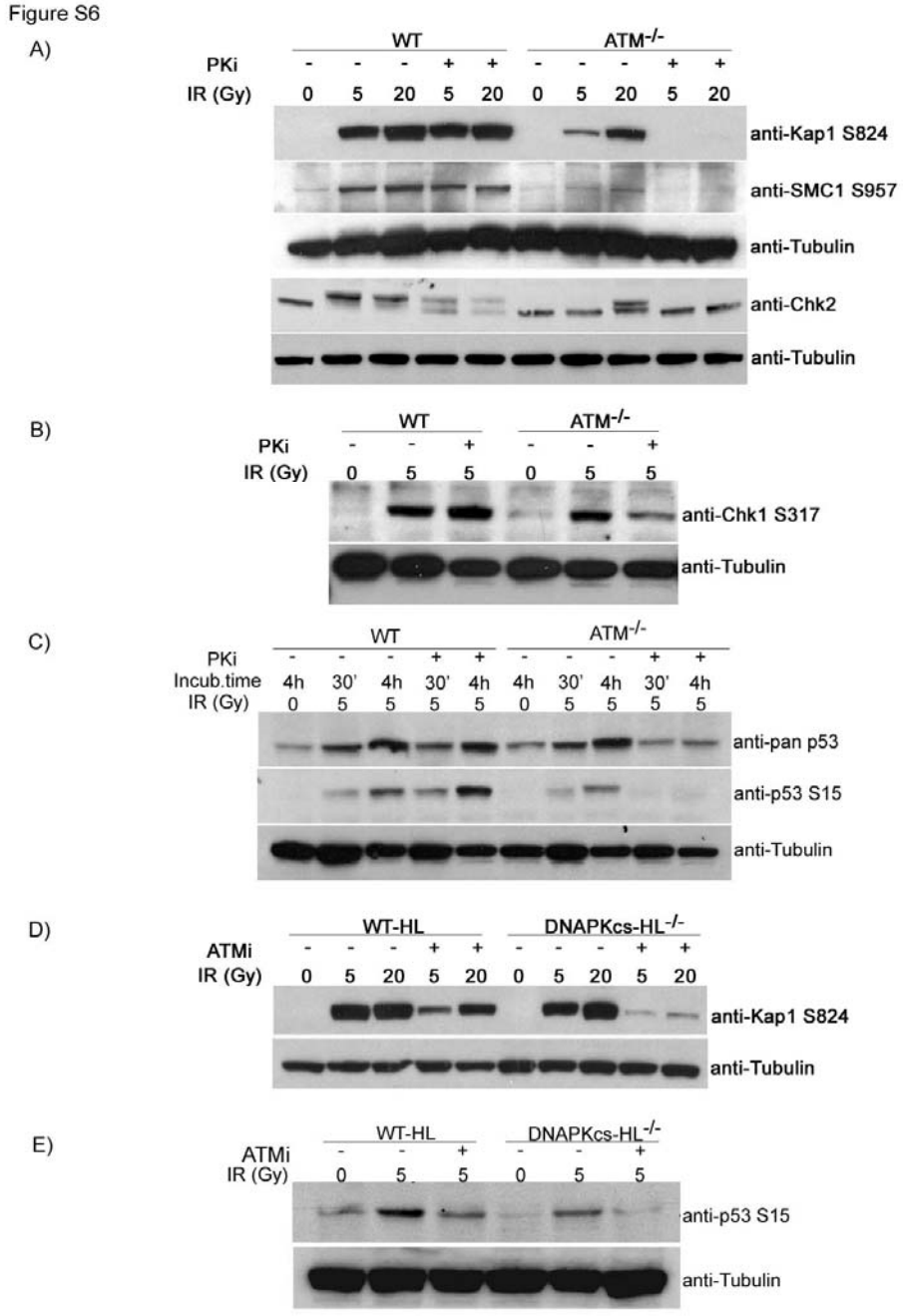
Defective CSR to IgG3 in DNAPKcs$^{-/-}$ Bcells
CSR to IgG3 was determined in DNAPKcs-HL$^{-/-}$, WT-HL littermates and AID$^{-/-}$ B cells five days after stimulation with LPS. Cells were labeled with CFSE to assess cell division.

Figure S5



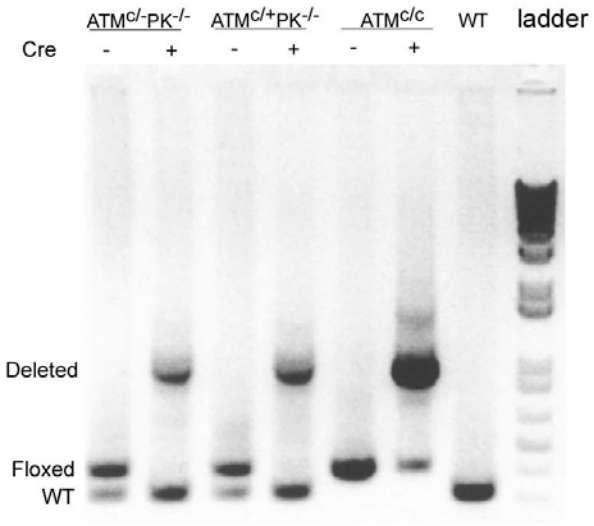
DNA-PKcs inhibition does not affect cell division, switch region transcription or AID protein levels
(A) Cell division was assessed three days after stimulation with LPS and IL4 by flow cytometry using CFSE dye dilution.
(B) Sterile Sμ and Sγ1 transcripts in B cells measured by real-time RT-PCR on day 3 after stimulation with LPS and IL4.
(C) AID protein levels measured by Western blotting on day 3.

Figure S6



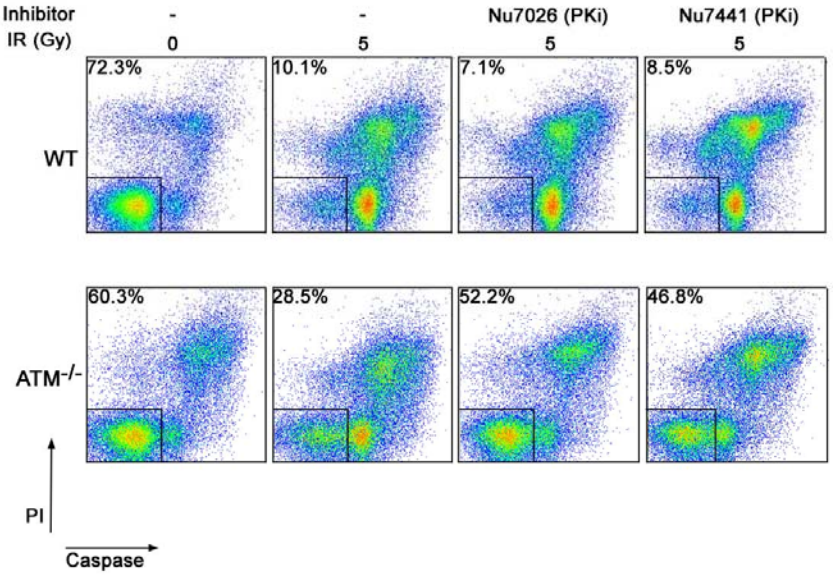
Impaired DNA damage signaling in ATM$^{-/-}$ thymocytes threated with PKi and DNAPKcs-HL$^{-/-}$ Bcells treated with ATMi
Wild type and ATM$^{-/-}$ thymocytes were mock or pre-treated with Nu7026 for 1hour, and then harvested for Western blot analysis of Kap-1, SMC1 and Chk2 phosphorylation (A) and Chk1 phosphorylation (B) 1 hour after IR. (C) IR induced p53 accumulation and phosphorylation in WT or ATM$^{-/-}$ thymocytes mock or pre-treated with PKi for 1 hour and the irradiated with 5Gy. Cells were harvested for Western blot analysis 30 minutes or 4hours after IR. Kap-1 (D) and p53 (E) phosphorylation in WT-HL and DNAPKcs-HL$^{-/-}$ B cells pre-treated for 1 hour with ATMi Ku55933, irradiated with 5 or 20Gy as indicated and analyzed by Western blotting.

Figure S7



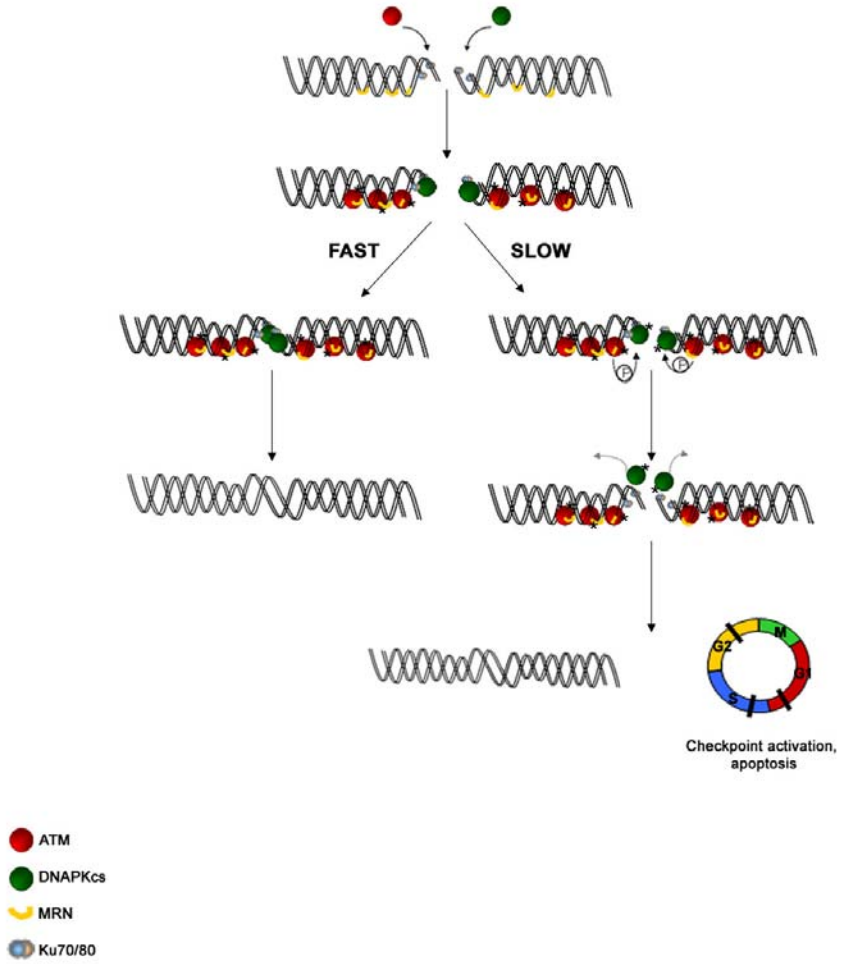
Efficiency of deletion of the ATM floxed allele after Cre infection
$ATM^{c/-}PK^{-/-}$, $ATM^{c/+}PK^{-/-}$ and $ATM^{c/c}$ B cells were infected with Cre-GFP or GFP empty vector, and sorted 4 days later. PCR analysis was performed as described (Zha et al., 2008)

Figure S8



IR induced apoptosis in WT and ATM$^{-/-}$ thymocytes
WT and ATM$^{-/-}$ were pre-treated or not with PKi (Nu7026) or PKi (Nu7441), irradiated with 5Gy and percentage of live cells was quantified by flow-cytometry 24 hours post-IR. Percentage of live cells (Propidium Iodide (PI) and Caspase 3-negative) are indicated.

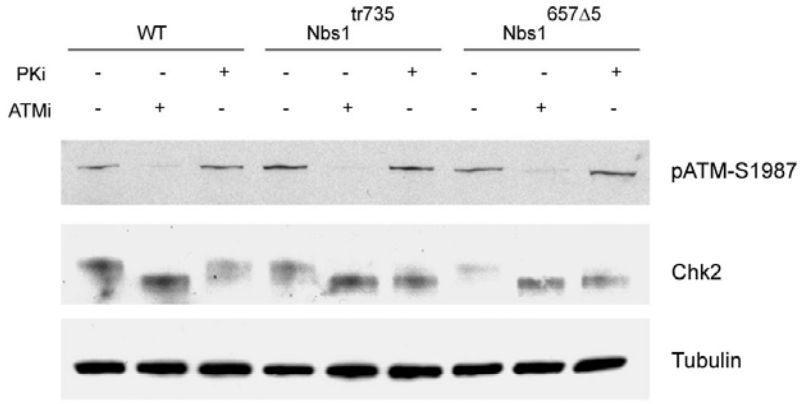
Figure S9



Model for overlapping roles of ATM and DNA-PKcs in non homologous end joining and apoptosis in lymphocytes

Physiological DSBs in B cells undergoing CSR as well as IR induced breaks in thymocytes occur primarily in the G1 phase of the cell cycle. The model shows that ATM is rapidly recruited to a DSB and flanking chromatin via interaction with DSB sensor Mre11/Rad50/Nbs1 (MRN). DNA-PKcs is also recruited to the end, but by Ku70/Ku80, and during CSR DNA-PKcs plays a non-catalytic role in NHEJ-dependent repair. ATM is activated and the lesion is normally repaired quickly via NHEJ. If that fails, and the lesion persists, ATM and DNA-PKcs both phosphorylate DNA-PKcs, which results in its removal from the break. ATM subsequently triggers cell cycle arrest, apoptosis or facilitates DNA repair by phosphorylating effectors that are independently recruited to the break. In the absence of ATM, DNA-PKcs remains associated with the lesion and phosphorylates substrates that are normally phosphorylated by ATM. In cell cycling populations such as fibroblasts where repair occurs in the S phase of the cell cycle, ATR is the predominant kinase that replaces ATM (Cimprich and Cortez, 2008).

Figure S10



DNA-PKcs contributes to Chk2 phosphorylation in a murine model of NBS ($Nbs1^{657\Delta5}$) and in mice lacking the Nbs1 C-terminus $Nbs1^{tr735}$.
B cells from wild type and mutant mice were irradiated with 5Gy and Chk2 phosphorylation (evidenced by mobility shift) was assessed 30 min post-irradiation. ATMi decreases Chk2 mobility shift in WT, $Nbs1^{657\Delta5}$ and $Nbs1^{tr735}$ mice and PKi decreases Chk2 mobility shift only in $Nbs1^{657\Delta5}$ and $Nbs1^{tr735}$ mice (but not WT) despite normal ATM phosphorylation.