Supplemental material

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Figure S1. Integration of the Atm D2899A mutant transgene in each founder occurs on a different chromosome than endogenous ATM. FISH of ATM genomic DNA on metaphase spreads from ATM^{TgD2899A} mice displays both BAC integration sites (marked with white arrowheads) and endogenous loci. Bar, 10 µm.



Figure S2. **ATM kinase activity is dispensable for ATM recruitment to DNA breaks.** HA-tagged WT or kinase-inactive D2870A mutant human ATM were transiently expressed in the ATM-deficient AT4BI immortalized patient fibroblast cell line, subjected to laser microirradiation to introduce DNA breaks, allowed to recover for 1 h, and processed for immunofluorescence with HA and NBS1 antibodies and goat anti-mouse Alexa Fluor 568 and goat anti-rabbit Alexa Fluor 488, respectively. In parallel, WT cells were treated with 10 mM KU55933 ATMi for 30 min before laser irradiation. Data are representative of two independent experiments. Images were acquired with a microscope (TCS SP2 AOBS; Leica) equipped with an HCX Plan Aprochromat ibd.BL 63×, NA 1.4 objective lens and camera (Leica) using Leica confocal software. Images were cropped in Photoshop (Adobe). Bars, 10 µm.



Figure S3. **FACS histograms of YFP expression in bone marrow and spleen subsets and Southern blot analysis of digested genomic DNA assaying for rearrangement of the pMX-INV-integrated substrate within v-Abl-transformed pre-B cell lines, indicative of RAG-dependent DNA recombination.** (A) Freshly isolated bone marrow and splenocytes were harvested and stained to assess YFP⁺ frequency within the indicated subsets described in Fig. 2 B. Shown are histograms with the indicated YFP⁺ frequency. Multiple experiments are presented as YFP⁺ frequency means in Fig. 2 B. (B) Southern blot analysis of EcoRV– Ncol-digested (top) and EcoRV-digested (bottom) genomic DNA assaying for rearrangement of the pMX-INV–integrated substrate within the indicated v-Abl-transformed pre-B cell lines treated for the indicated times with STI571 to induce RAG gene expression. Shown are unrearranged (UR) and coding end (CE) DNA cleavage intermediates as well as hybrid (hybrid joint [HJ]) and coding (coding joint [CJ]) products. Nucleic acid markers are in kilobases.