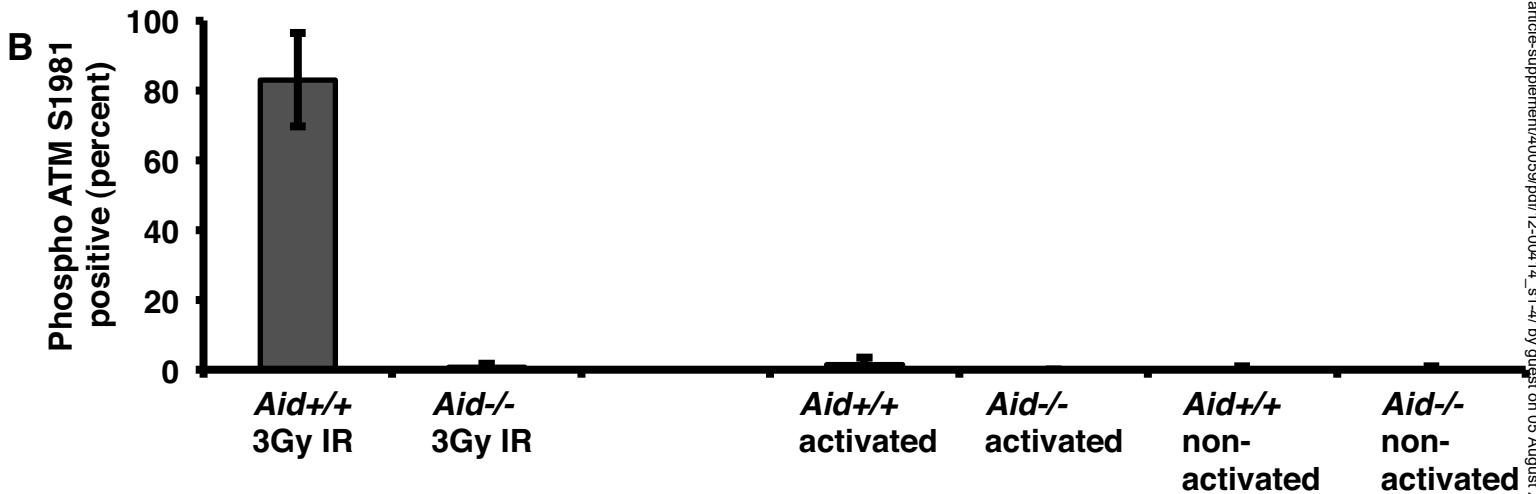
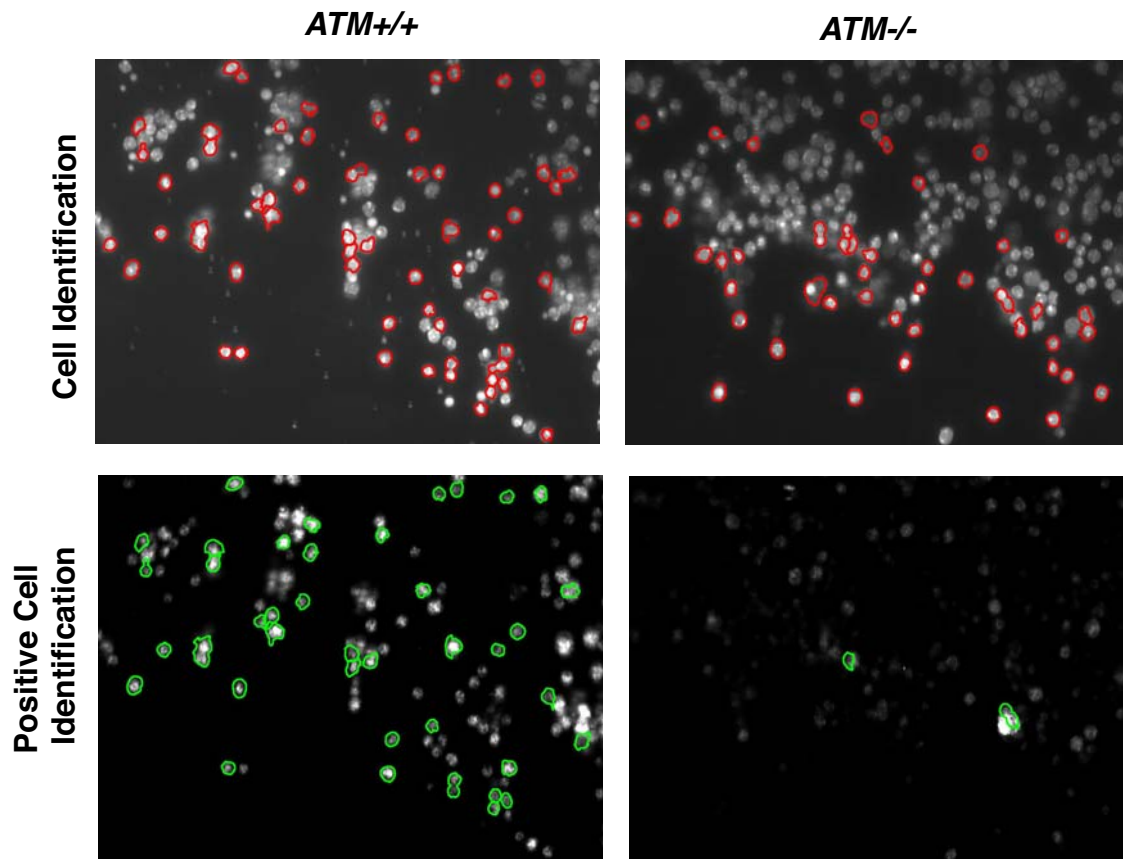


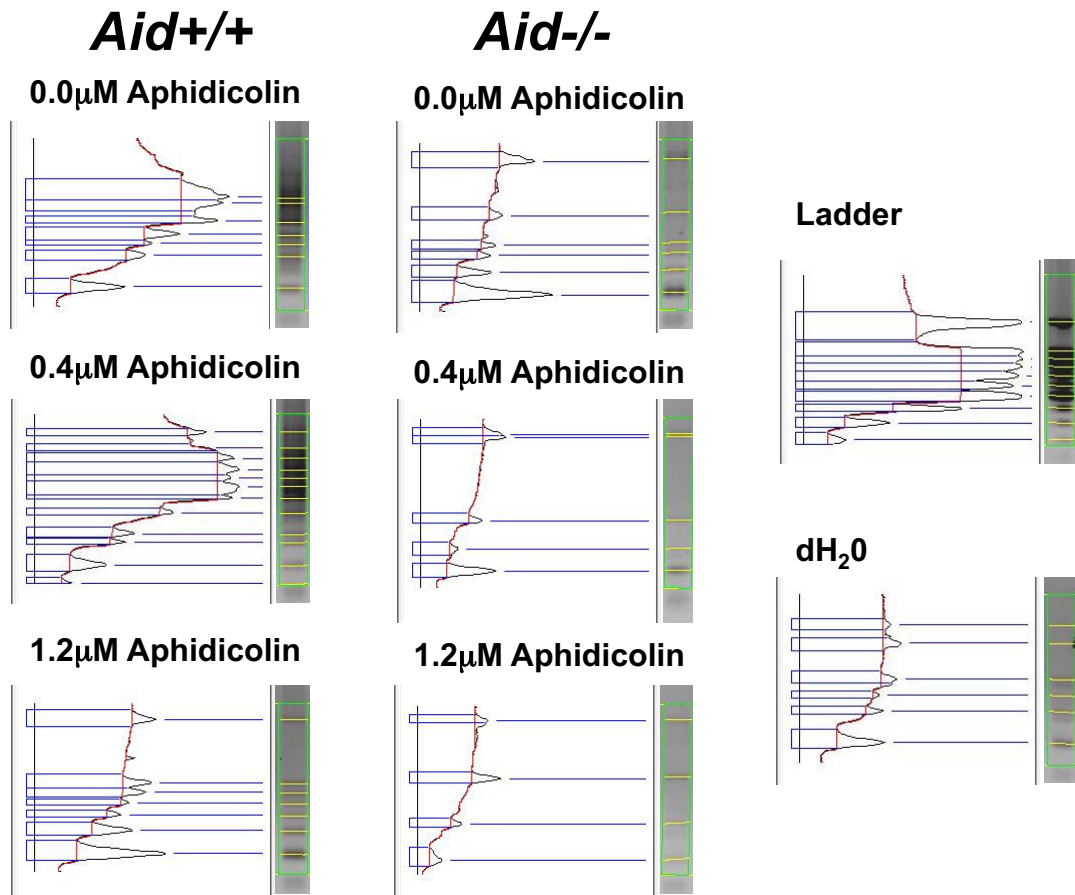
Supplementary Figure 1: Aphidicolin affects the cell cycle, but does not impinge on the viability or inhibit DNA repair capability of B-cells. *A*, MACS (Miltenyi, Inc) sorted, B220+IgM+, *Aid*^{+/+} or *Aid*^{-/-} splenic B-cell cultures were supplemented with 0 μ M, 0.4 μ M or 1.2 μ M aphidicolin (APH) and activated with α CD40 and IL-4 for 4 days. On day 3, B-cells were stained with propidium iodide, and the cell cycle distribution was measured by flow cytometry. *B*, B-cell cultures were supplemented with 0 μ M, 0.4 μ M or 1.2 μ M aphidicolin (APH) and activated with α CD40 and IL-4 for 4 days were counted for viable cells via Trypan Blue exclusion test, as detailed in the method section. Error bars show 95% confidence interval of four independent experiments. *C*, Schematic of experimental design. Maximum intensity projections were generated from 3 focal planes flanking the nuclear equator, and γ -H2AX foci were quantified in projection images. *D*, Representative wide-field image of B-cells stained for γ -H2AX foci at 0 minutes, 30 minutes, 6 hours, and 24 hours after ionizing irradiation (1Gy). *E*, Bar chart showing the average foci per cell from B-cell cultures that were treated with 0 μ M, 0.4 μ M or 1.2 μ M APH, and fixed after 30 minutes, 6 hours or 24 hours after 0 Gy or 1Gy ionizing irradiation. Error bars show 95% confidence interval of 3 independent experiments. The *p* value was calculated by student t-test. *F*, Representative wide-field image of NIH3T3 stained for γ -H2AX foci at 30 minutes, 6 hours, and 24 hours after ionizing irradiation (1Gy).

A

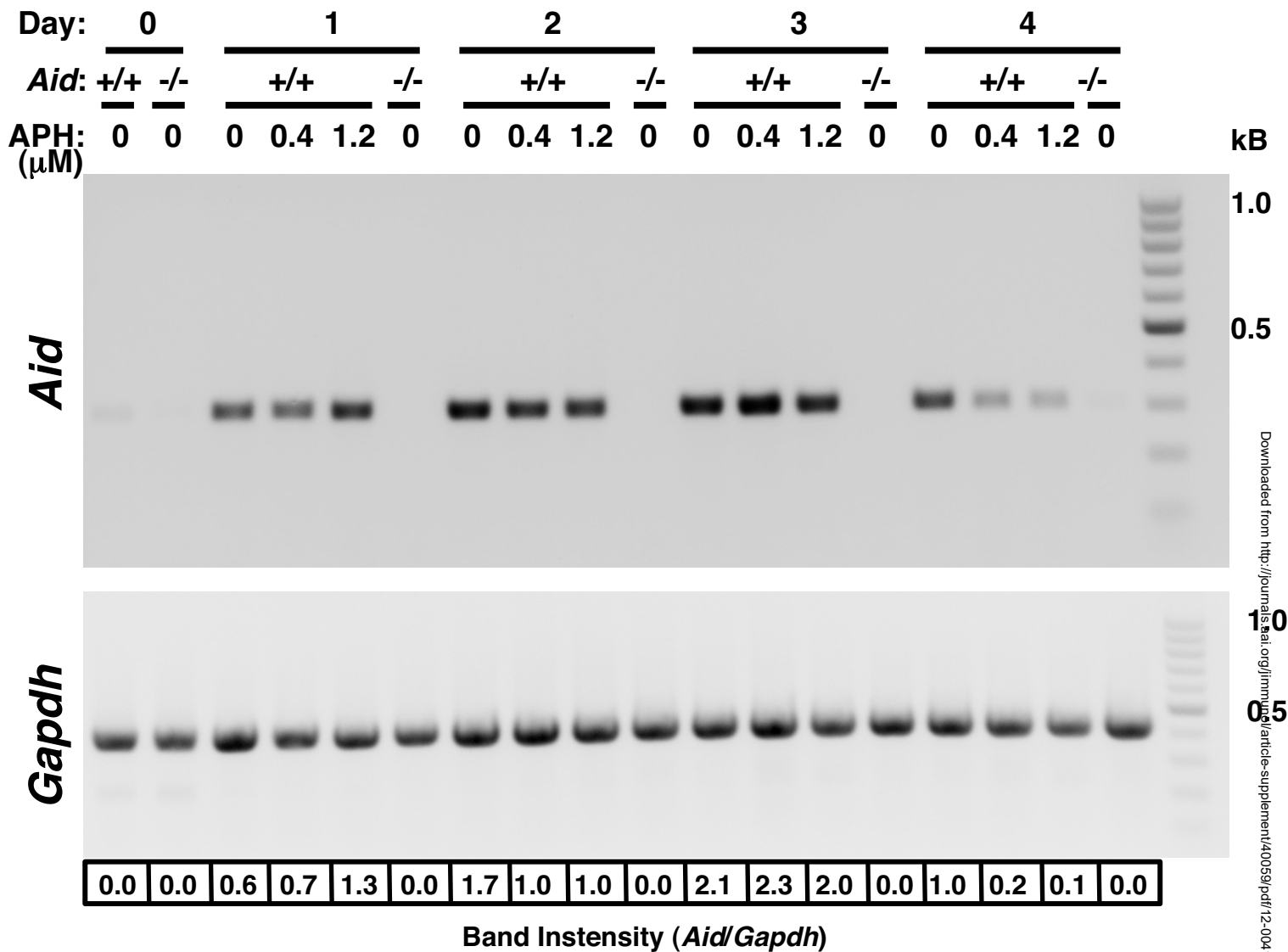


Supplementary Figure 2: Quantification of the ATM S1981 data. Determining threshold values:

Control images from each experiment were analyzed using CellProfiler (www.cellprofiler.com) to determine the positive and negative threshold values. The DAPI channel was used to identify individual cell nuclei. The identified nuclei were then used as masks for the pATM channel and the mean intensity of each identified cell was measured. Experimental data analysis: Image data from the experimental groups was analyzed using CellProfiler (www.cellprofiler.com) to identify the total number of cells in each image and the number of those identified cells that were pATM positive. The DAPI channel was used to identify individual cell nuclei. The identified nuclei were then used as masks for the pATM channel and the mean intensity of each identified cell was measured. Those mean intensity values were compared to the threshold values determined above, and the cells classified as positive or negative for pATM accordingly. *A*. Representative images of Image quantification. *B*. Bar chart showing the percent of cells expressing ATM S1981. IR: 3Gy irradiation dose. Error bars indicate 95% confidence interval of 3 experiments.



Supplementary Figure 3: Quantitation of S γ 1 to S μ recombination products Line scans for each lane gels were quantified by Syngene Tools (Invitrogen) for APH treated *Aid*^{+/+} and *Aid*^{-/-} activated B-cells. The 100bp DNA ladder and water are shown for reference. Quantified PCR bands are shown as yellow marks



Supplementary Figure 4: *Aid* expression in purified B-cells. Purified B-cells were activated for 4 days in the presence of aphidicolin. RNA from each sample was extracted using Qiagen RNAeasy micro Kit (Cat. No. 74004). RT-PCR for *Gapdh* is described in the Methods section. Primers for *Aid* RT-PCR are FWD: cagggacggcatgagacct REV: tcagccttgccgtcttcaca. (Morgan, et.al. (2004)) Cycling conditions: 50°C 30 min., 95°C 15 min. for the reverse transcription, 94°C 30 secs., 61°C 30 secs., 72°C 1 min., for 35 cycles, and the 72°C 10 mins. Bands were quantified by Syngene Tools (Invitrogen) as described in Supplementary Figure 5. The fold ratio is the amount of *Aid* transcript/*Gapdh* transcript. The 100bp (Promega) ladder is shown for band size reference.