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Supplemental Material to:

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APLP2 regulates the expression of MHC class I molecules on irradiated Ewing's sarcoma cells

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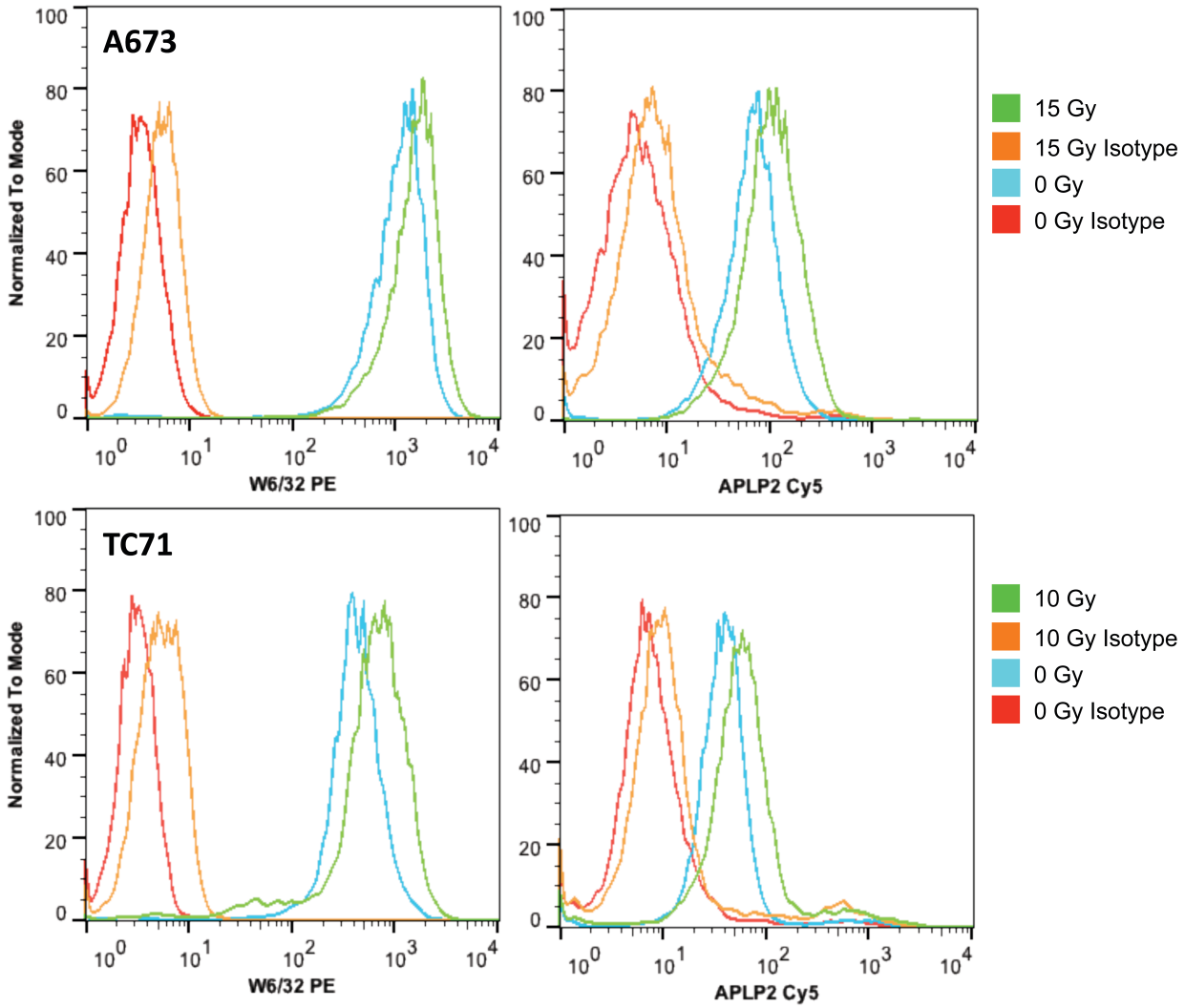
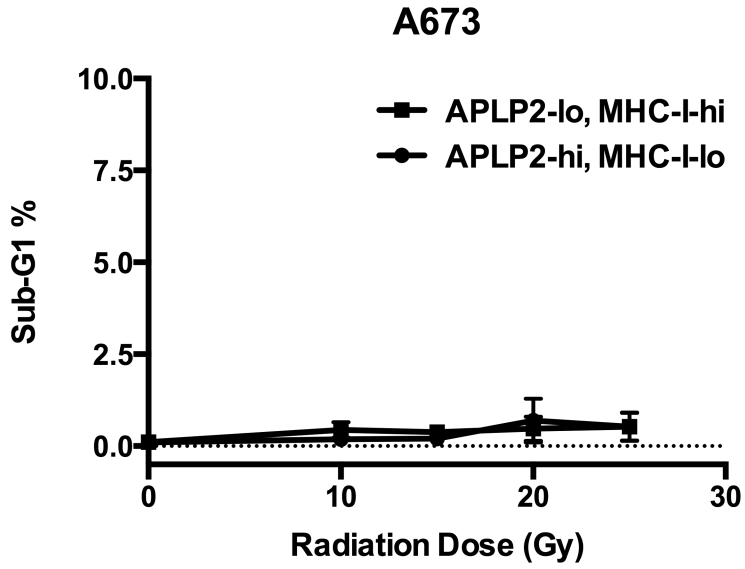
Supp. Fig. 1



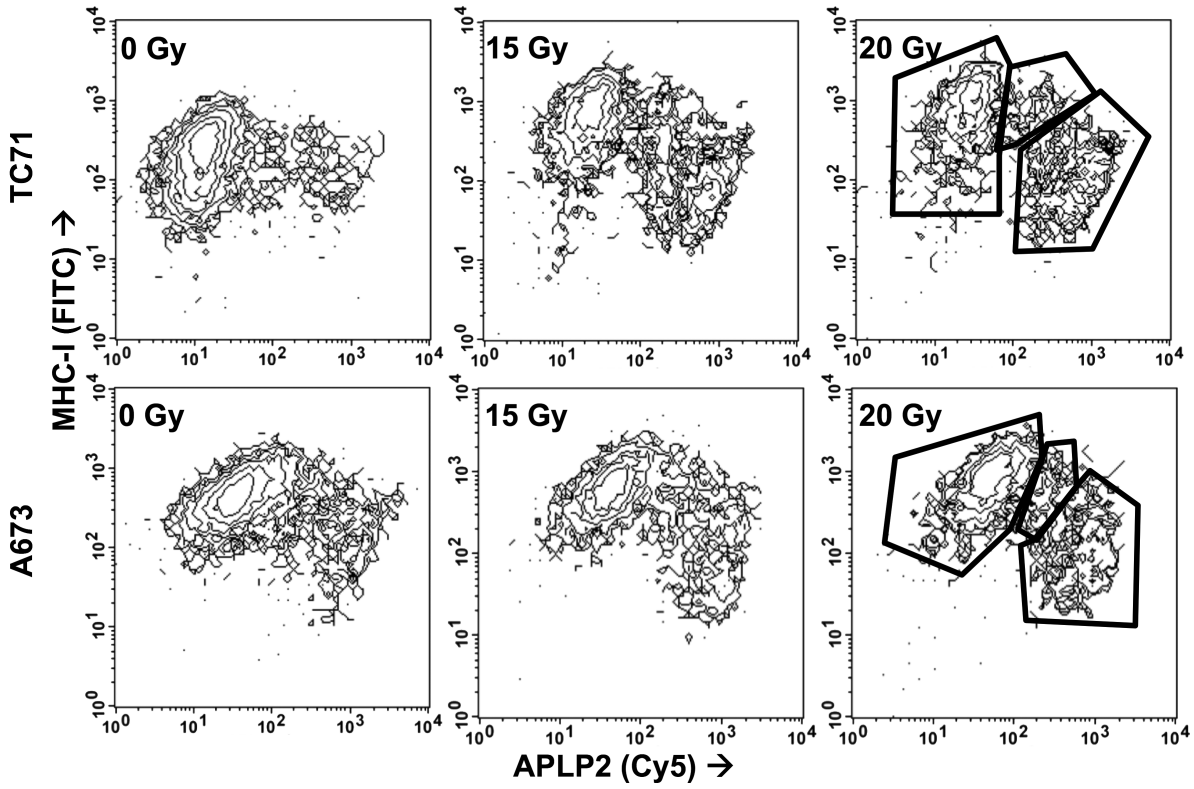
Supp. Fig. 1. EWS cell lines A673 (top row) and TC71 (bottom row) were irradiated at 15 and 10 Gy, respectively (green and orange histograms), as described in the Material and Methods. Cells not exposed to radiation served as baseline controls and are denoted as 0 Gy (red and blue histograms). Folded, MHC class I molecules were detected using W6/32 mouse monoclonal antibody culture supernatant and indirectly stained using PE-conjugated goat anti-mouse IgG (left column, green and blue histograms). Secondary antibody alone served as the negative control (left column, orange and red histograms). Surface APLP2 was detected using a rabbit polyclonal antibody and Cy5-conjugated goat anti-rabbit IgG (right column, green and blue histograms). As an isotype control, normal rabbit serum was substituted for the anti-APLP2 antibody at an equal volume (right column, orange and red histograms). Staining for APLP2 and MHC class I molecules remained separated from isotype controls, even after irradiation.

Supp. Fig. 2.



Supp. Fig. 2. Exposure of A673 EWS cells to radiation does not result in a significant increase in apoptosis. Log-phase A673 cells were seeded in tissue culture dishes for approximately 24 h before irradiation at the indicated doses (0, 10, 15, 20, and 25 Gy). At 24 h after irradiation, the cells were fixed and then stained with propidium iodide, and the DNA content of the cells was analyzed by flow cytometry. Analysis of the cell cycle distribution was performed on the data by the use of ModFit software. The data that are shown are the mean +/-S.D. (n=6) from 1 experiment that had results representative of the results from 2 separate experiments, and are consistent with observations of normal A673 cell morphology post-irradiation in several additional experiments.

Supp. Fig. 3.



Supp. Fig. 3. Representative dot plots showing APLP2 (x-axis) and MHC class I molecules (y-axis) co-expression, with gates displayed on cells irradiated at 20 Gy signifying three subpopulations: APLP2-high, MHC-I-low; APLP2-int, MHC-I-high; and APLP2-low, MHC-I-high. Graphs of data obtained from the same experiment are shown in Figure 3.

Supp. Table 1. Summary statistics used in the preparation of Figure 4B.

	TC71			A673		
	Mean	S.D.	n	Mean	S.D.	n
W6/32	108.6702	1.718	4	110.5598	9.240	6
BB7.2	104.5799	4.334	4	109.7258	5.675	7

For each cell line (TC71 or A673), the columns include the Mean (of percent change values = siAPLP2 MFI/siControl MFI x 100), the Standard Deviation, and the Number of Paired siAPLP2/siControl Samples (n)