Bortezomib Augments Natural Killer Cell Targeting of Stem-Like Tumor Cells

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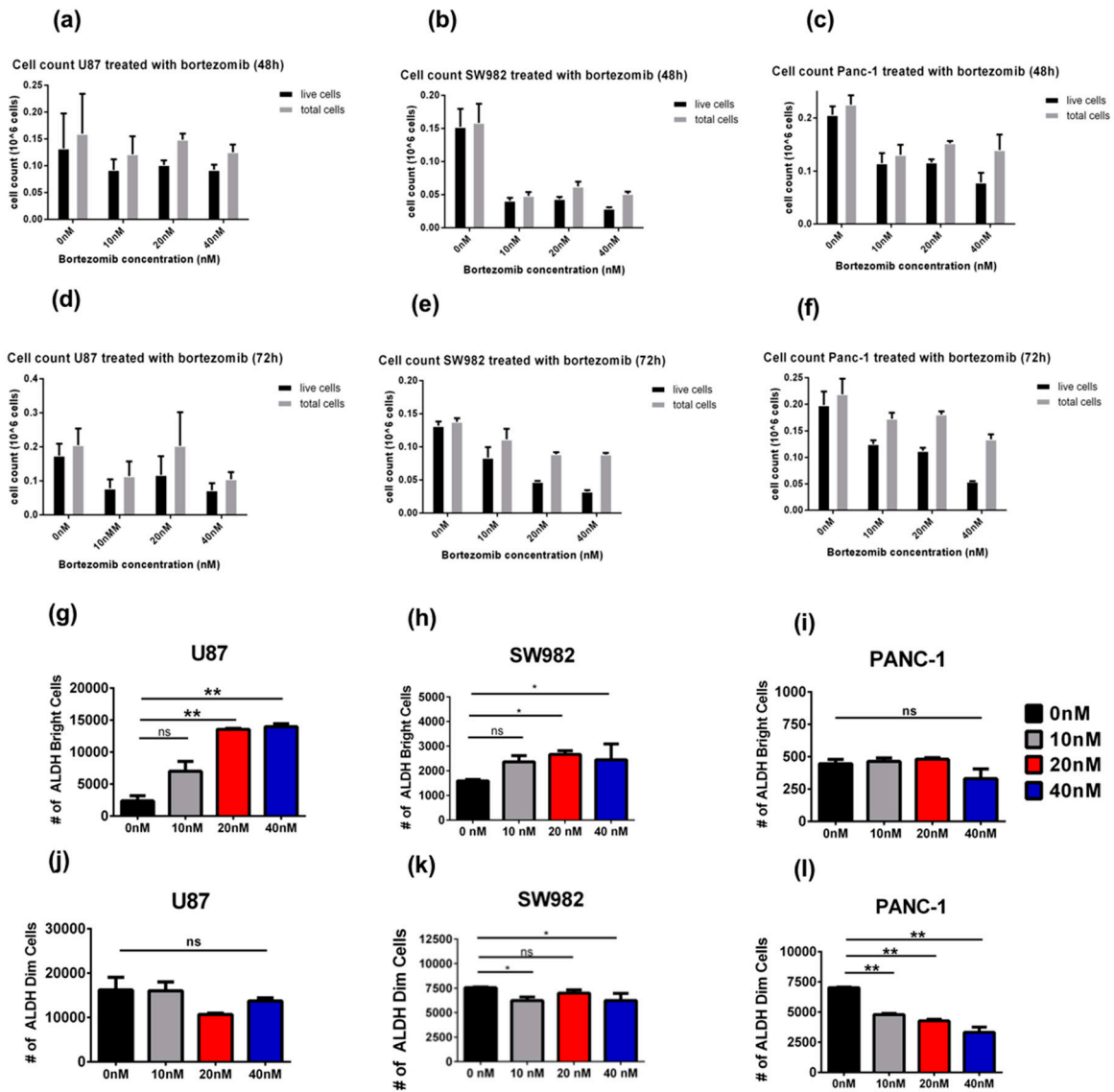

Figure S1. Cell counts and number of ALDH[brigh] and ALDH[dim] from bortezomib treated cells. Cell counts indicating total cell numbers and total live cells in U87 (**a**,**d**), SW982 (**b**,**e**), and PANC-1 (**c**,**f**) after 48 and 72 h, respectively. Numbers of ALDH[bright] and ALDH^{dim} cells in U87 (**g**,**j**), SW982 (**h**,**k**), and PANC-1 (**i**,**l**) cells treated with 0, 10, 20, and 40 nM bortezomib for 48 h. Cell numbers were calculated by flow cytometry based on aldefluor assay. (* = $p < 0.05$, ** = $p < 0.01$).

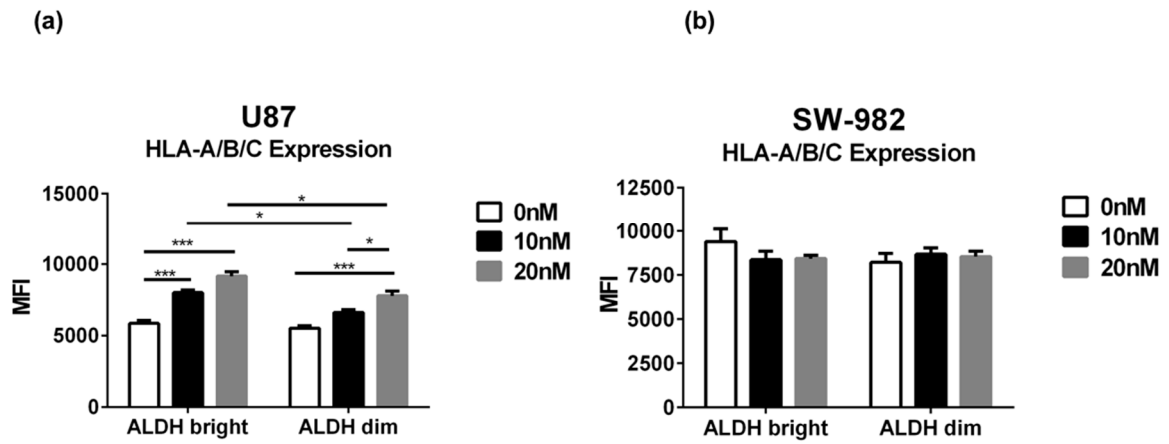**Figure S2.** HLA-ABC expression in ALDH[bright] and ALDH^{dim} cells treated with bortezomib. Bar graphs showing expression levels of HLA-ABC in U87 (**a**) and SW982 (**b**) treated with 0, 10, and 20 nM bortezomib for 48 h. (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

Figure S3. Bortezomib increases expression of cancer stem cell related genes and stress ligands on sorted ALDH[bright] and ALDH[dim] subpopulations in U87. (**a**) Bar graph showing MFI values comparing sorted ALDH[bright] and ALDH[dim] subpopulations, and unsorted cells including MFI for DEAB control. (**b**) Expression of *ALDH2* in ALDH[dim] treated with 0 and 20 nM; and (**c**) ALDH[bright] treated with 0 and 20 nM. (**d**,**f**) Expression of *MICA* and *MICB* in ALDH[dim] treated with 0 and 20 nM; and (**e**,**d**,**g**) ALDH^{bright} cells treated with 0 and 20 nM. (**h**) Bar graph showing expression levels of cancer stem cell related genes in ALDH[dim] treated with 0 and 20 nM and ALDH[bright] treated with 0 and 20 nM. Dotted lines indicate a two-fold expression threshold as indication of significant gene up-regulation. (* = $p < 0.05$, ** = $p < 0.01$).
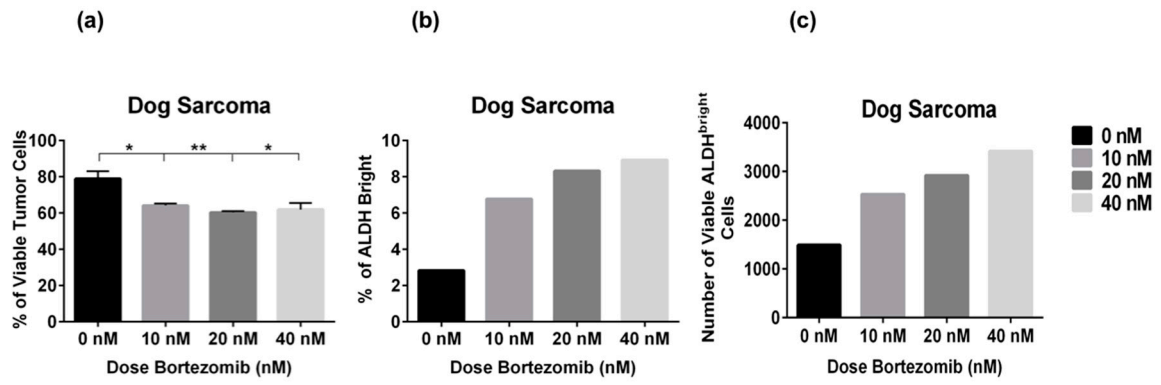
Figure S4. Bortezomib increases ALDH^{bright} cells by frequency and numbers and decreases the frequency of viable cells in dog sarcoma. (**a**) % of viable tumor cells, (**b**) % of ALDH^{bright} cells, and (**c**) number of ALDH^{bright} cells in dog sarcoma treated with bortezomib for 24 h. (* = $p < 0.05$, ** = $p < 0.01$).