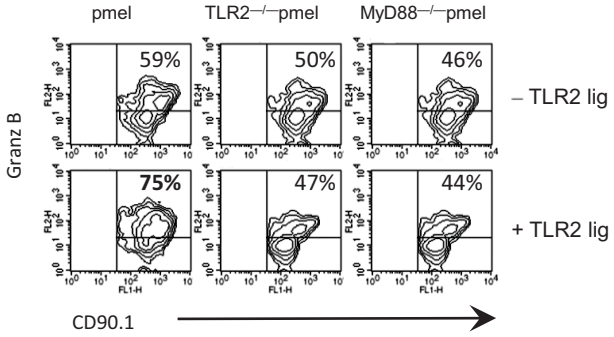

Supplemental 1. Activating TLR2-MyD88 signals in tumor-reactive CD8 T-cells augments granzyme B production. Purified pmel, TLR2$^{-/-}$pmel and MyD88$^{-/-}$pmel CD8 T-cells were activated with MyD88$^{-/-}$ splenocytes pulsed with mgp100 peptide with or without TLR2 agonist. Four days later the intracellular level of granzyme B were determined by flow cytometry. Shown on the upper right-hand of each plot are the percent of cytokine-positive cells. All data are representative of three or more independent experiments each yielding identical results.

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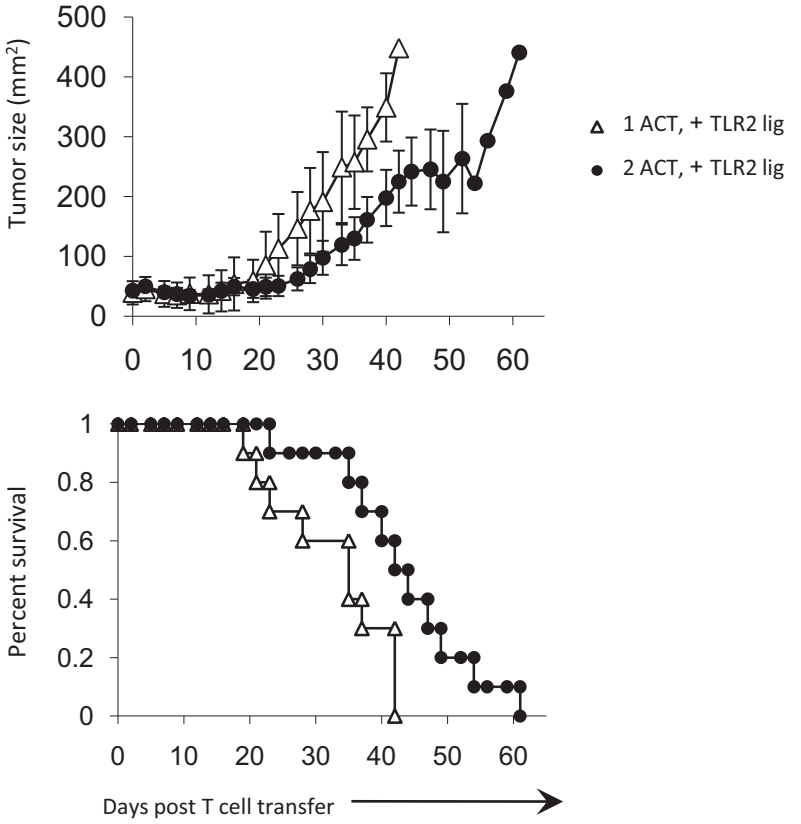

Supplemental-2. Antitumor effects of a second round of ACT. Wild type BL6 were injected (s.c.) with B16 melanoma cells and sublethally irradiated (400 cGy) when tumors reached a size of approximately of 50 mm^2. Mice were injected (i.v.; 1×10^6) with previously-activated pmel T cells one day after irradiation or were injected twice, one and fifteen days after irradiation. All mice were injected i.p. with $mgp100_{25\text{-}33}$ antigen and anti-CD40 antibody and weekly peritumioral s.c. injections of TLR2 ligand or control saline. Tumor sizes were calculated by measuring perpendicular by longitudinal diameter. Data are compiled from two independent experiments (n=5 experiment), each yielding identical trends; *P< 0.01.

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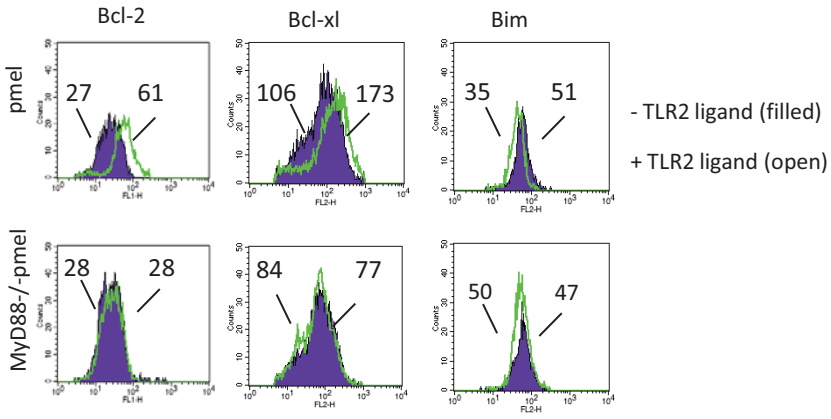

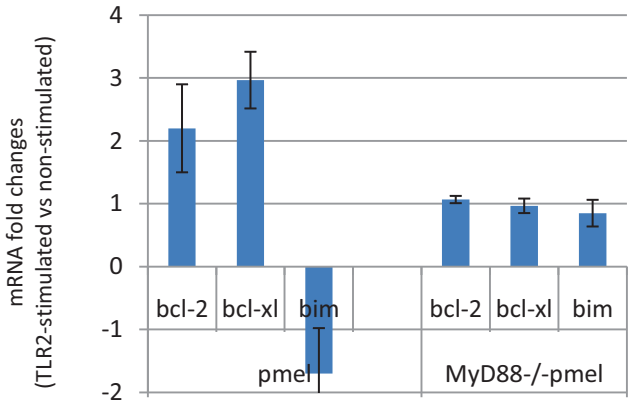

Supplemental 3. Activating TLR2-MyD88 signals in tumor-reactive CD8 T-cells alters apoptosis-related protein and transcript expression levels. Purified pmel and MyD88$^{-/-}$pmel CD8 T-cells were activated with MyD88$^{-/-}$splenocytes pulsed with mgp100 peptide with or without TLR2 agonist. Four days later the intracellular level of bcl-2, bcl-xl and bim were determined by flow cytometry or by real time PCR. (A) Histogram of intracellular levels of apoptosis-related proteins with (filled) or without TLR2 ligand (open). Mean fluorescence intensities are provided in each histogram. (B) Fold changes in mRNA transcript levels of TLR2-stimulated versus non-TLR stimulated T cells. All data are representative of three independent experiments each yielding identical trends.

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