Figure S4 Proliferation and cytokine production of intra-tumoral and splenic CD8+ and CD4+ T cells after re-stimulation. (A) Proliferation of intra-tumoral PD-1hiCD8+, PD-1lowCD8+, splenic PD-1hiCD8+, PD-1lowCD8+, intra-tumoral PD-1hiCD4+, PD-1lowCD4+ T cells, splenic PD-1hiCD4+, and PD-1lowCD4+ T cells. The tumor infiltrating leukocytes and splenocytes were subjected to plate-bound anti-CD3/anti-CD28 activation for 54 hours. The proliferation of T cells was determined by EdU incorporation during the last 6-hours of activation and the percentage of EdU+ CD8+ T cells among indicated CD8+ or CD4+ T-cell populations was shown. (n=3 mice). (B) Gating for flow cytometric analysis of intracellular cytokines in indicated T-cell populations. The tumor infiltrating leukocytes and splenocytes were subjected to plate-bound anti-CD3/anti-CD28 activation for 8 hours and harvested for the staining of surface markers and intracellular cytokines, followed by flow cytometric analysis. Brefeldin A (3 µg/ml) and Monensin (2 µM) were added to the cell culture media during the last 5-hrs of activation. The percentages of (C) TNFα single positive cells, (D) TNFα and IFNγ double positive cells and (E) TNFα and IL-2 double positive cells among the indicated cell populations were shown. (n=3 mice). ns, not significant; *P < 0.05, **P < 0.01 and ***P < 0.001 (unpaired Student’s t-test)