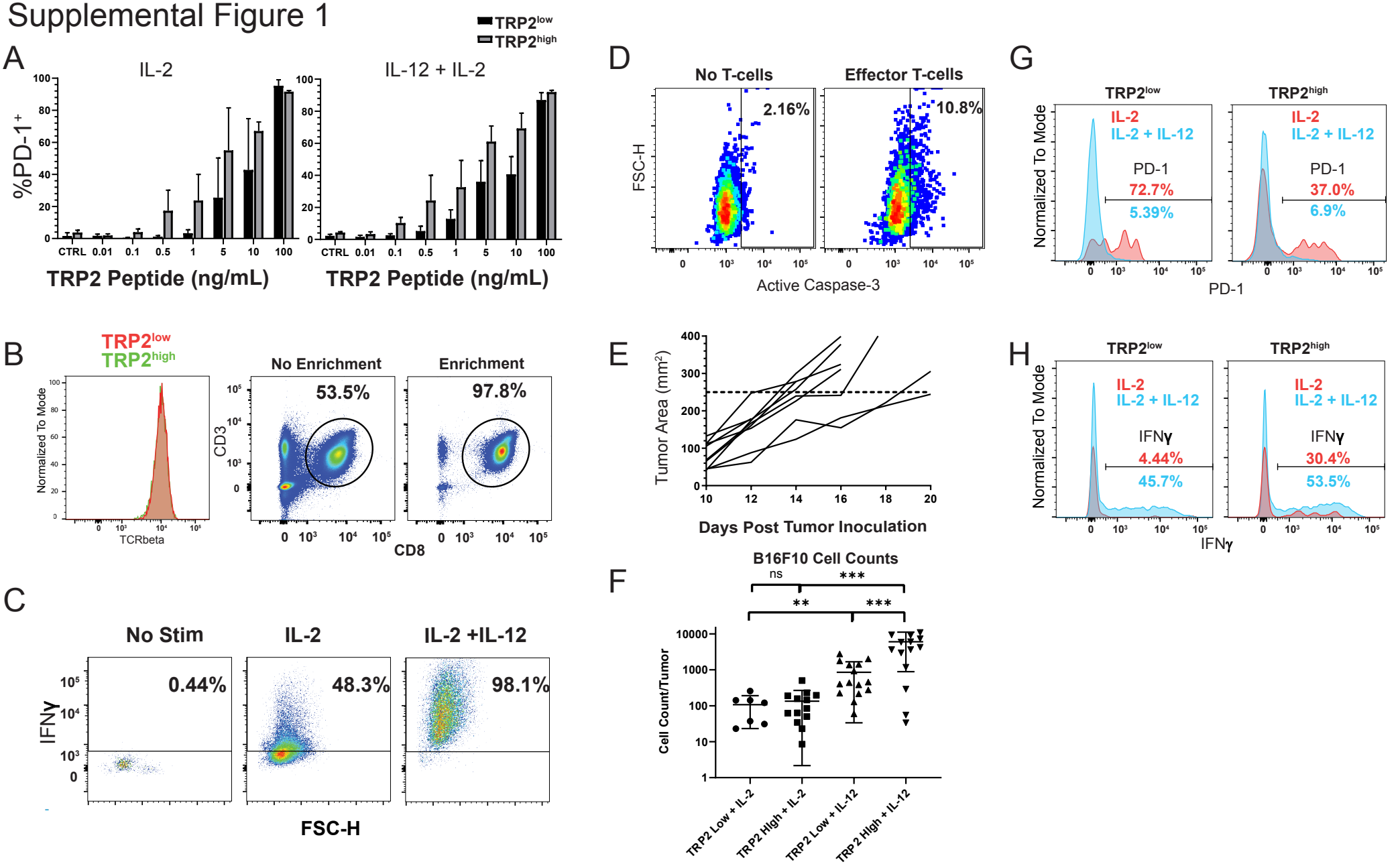
Supplemental Figure 1



(A)TRP2^{low} and TRP2^{high} splenocytes were placed into complete RPMI along with varying concentrations of TRP2 or irrelevant (CTRL; Ova) peptide and stimulated for 60 hours in 48 well plates. Cells were analyzed for PD-1 expression by flow cytometry. (B, left) Isolated TRP2^{low} and TRP2^{high} CD8^{+} T-cells were activated with plate bound anti-CD3 and recombinant B7-1 and assayed for TCRbeta levels after three days of activation. (B, right) CD8^{+} T-cells were isolated by magnetic enrichment, and stained before and after with anti-CD3 and anti-CD8. Shown are the percent double positive by flow cytometry. (C)TRP2^{high} T-cells were isolated by magnetic enrichment and stimulated for three days with anti-CD3 and recombinant B7-1 or media, and supplemented with IL-2 or IL-2 + IL-12. Cells were stained by intracellular cytokine staining for IFNγ after three days of stimulation. Representative FACS plots are shown. (D) B16F10 tumor targets were allowed to adhere to 24 well plates overnight and then activated T-cells were placed into co-culture with B16F10 targets for 12 hours. Tumor cells were analyzed by for intracellular caspase-3 staining. (E) 0.25x10^{6} B16F10 tumor cells were implanted subcutaneously into C57BL/6 female mice. Tumors were followed for growth and mice were euthanized when tumor area exceeded 250 mm^{2} per IACUC recommendations. (F) TRP2low and TRP2high T-cells were adoptively transferred to B16F10 tumor bearing animals. Mice were euthanized at D9 post transfer and cell counts were performed by FACS using PKH26 reference beads. (G) Mice bearing B16F10 tumors were treated with IL-2 or IL-2 plus IL-12 activated TRP2^{low} and TRP2^{high} T-cells at a ratio of 2:1. Mice were euthanized at D3 post adoptive T-cell transfer and T-cells were isolated by Ficoll enrichment in the presence of Golgi Stop and re-stimulated with plate bound anti-CD3 and recombinant murine B7-1 for four hours. T-cells were intracellularly stained for IFNγ. Representative FACS plots are shown. (H) Experimental set up was similar to (G) and mice were euthanized on D9 post adoptive T-cell transfer and T-cells were analyzed for PD-1 expression. Representative FACS plots are shown.