



Supplemental Figure 3. Effects of T cell Receptor Activation on the Splenic T cell Immune Response to LPS.

Splenic cells were obtained from C57Bl/6 mice and T cells were isolated by negative selection column separation. Cells were stimulated *in vitro* with specific stimuli and analyzed by flow cytometry. Abbreviations for the five treatment groups of C57Bl/6 mice as detailed in Fig. 1. Mice studied at 24 hrs. following treatment. Gating: FSC/SSC, singlets, Live, CD90/CD4, CD90/CD8, CD69, Ki67, Granzyme B, IFN γ . Full gating strategy shown in Supp. Fig. 6. Data as mean \pm standard deviation. Significance determined using one-way ANOVA with Dunnett correction for multiple comparisons; * = $p < 0.05$ relative to Iso # = $p < 0.05$ LPSHi vs. CD3LPSLo. Figures representative of at least two independent experiments. A. Percent CD69⁺ of CD8 T cells following *in vitro* stimulation of total splenic cells or isolated T cells. N=4/group. B. Number of CD4 T cells obtained from the spleen. N=6/group. C. Percent CD69⁺ of splenic CD4 (Left) and CD8 (Right) T cells. N=6/group. D. Number of CD69⁺ CD4 T cells obtained from the spleen. N=6/group. E. CD69 MFI for splenic CD69⁺ CD4 T cells. N=3/group. F. Number of Ki67⁺ CD4 T cells obtained from the spleen. N=6/group. G. Percent Granzyme B⁺ of CD8 (Left) and CD4 (Right) T cells. N=6/group. H. Percent (Left) and number (Right) of CD4 T cells spontaneously producing IFN γ *ex vivo* without stimulation. Cells treated with Brefeldin A for five hours prior to intracellular cytokine staining. N=6-10/group. I. Flow cytometric plots following treatment showing IFN γ /Granzyme B in CD4 T cells. J. Percent (Left) and number (Middle) of splenic CD69⁺ CD4 T cells expressing PD1 and number of PD1⁺CD69⁺ CD4 T cells (Right) obtained from the spleen. N=3/group. K. Flow cytometric plots following treatment showing CD69/PD1 in CD4 T cells.