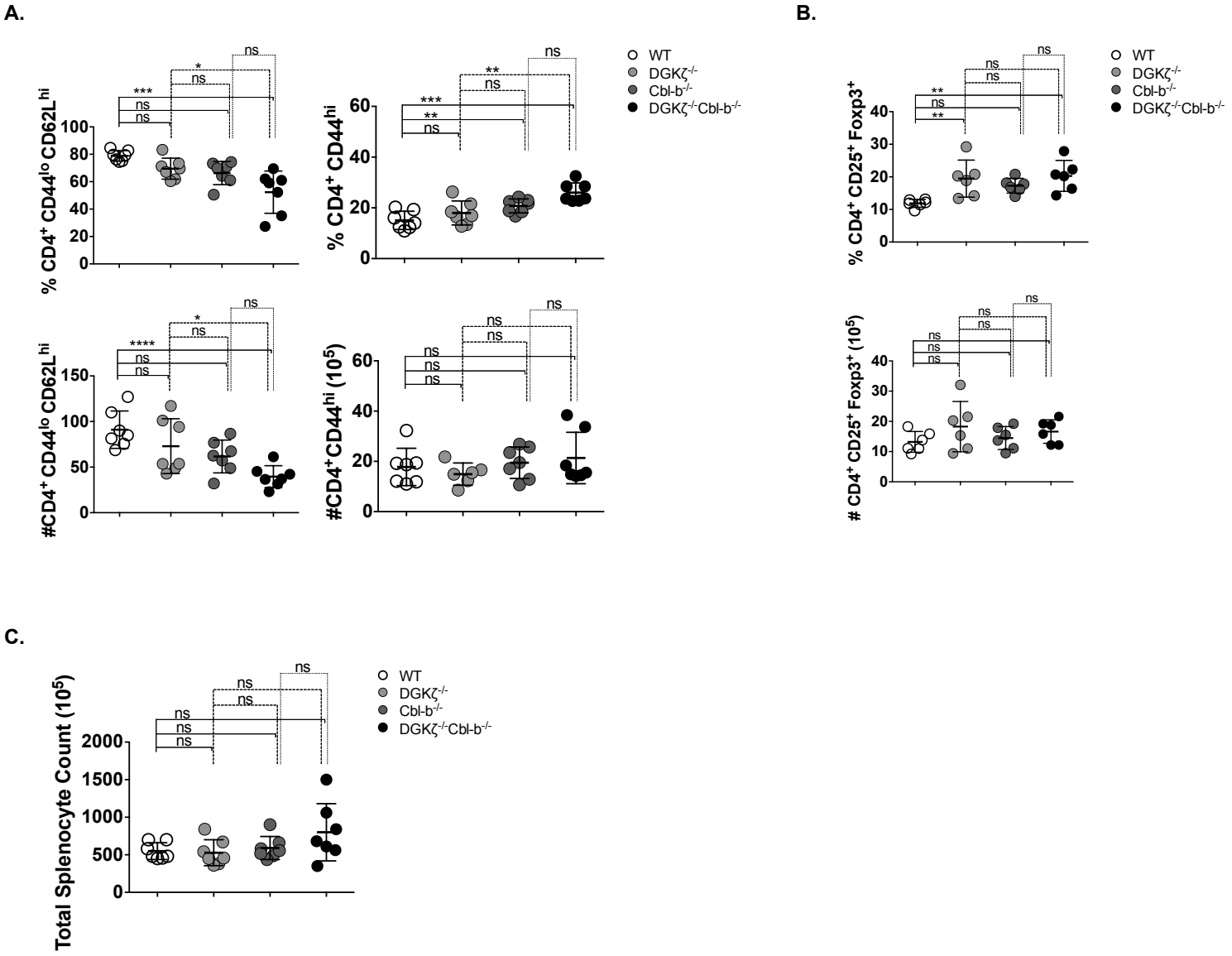
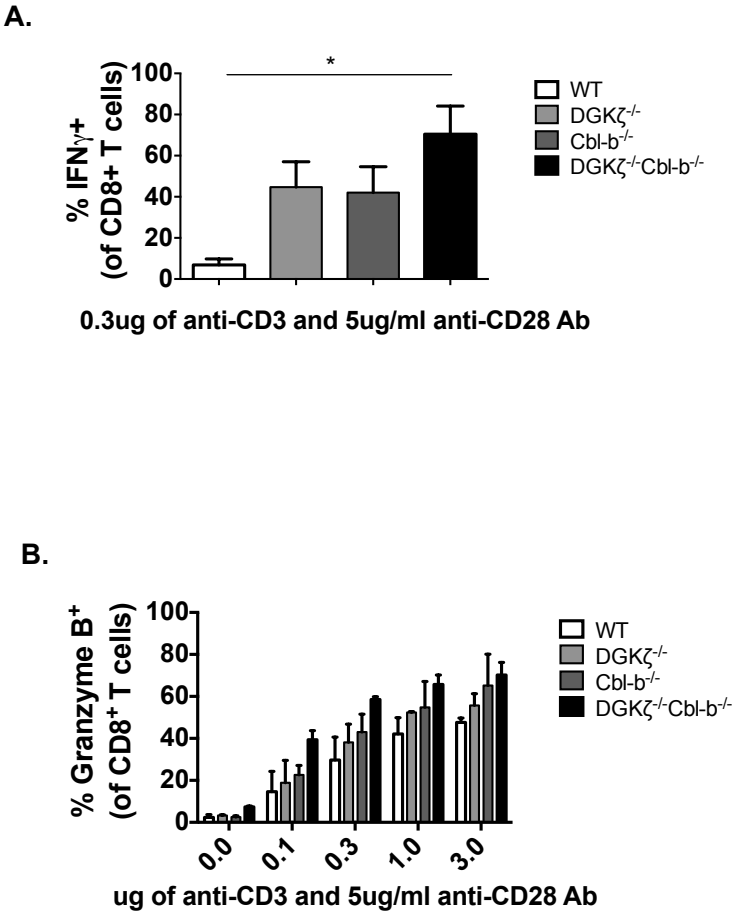
Supplemental Figure 1.



Supplemental Figure 1. Increased percentage of CD4$^+$ T cells with an activated phenotype in DGKζ and Cbl-b deficient mice. (A) Splenocytes were stained for cell surface markers CD4, CD8, and cell activation markers CD44 and CD62L, and the percentage and absolute number of naïve ($CD4^+CD44^{lo}CD62L^{hi}$) or activated ($CD4^+CD44^{hi}$) cells were determined after gating for $CD4^+$ (n=7 for each group). **(B)** Splenocytes were stained for cell surface markers CD4 and CD25, and intracellular FoxP3, and the percent and absolute number of $FoxP3^+CD25^+$ was determined after gating for $CD4^+$ T cells. **(C)** Total number of splenocytes from mice of each genotype was quantified.

Supplemental Figure 2.



Supplemental Figure 2. Increased cytokine production in DGKζ and Cbl-b deficient mice at minimal CD3 concentrations. 1x10^6 MACS-purified T cells were incubated with 5ug/ml anti–CD28 antibodies and either 0.3 (A) or indicated (B) μg of anti-CD3 at 37°C for 18 hrs. Cells were surface-stained for viability, CD8 and CD4, fixed and then stained for IFNγ **(A)** or granzyme B **(B)**. Depicted graphs represent percentage of $CD8^+$ cells staining positive for the indicated marker as assessed by flow cytometry.