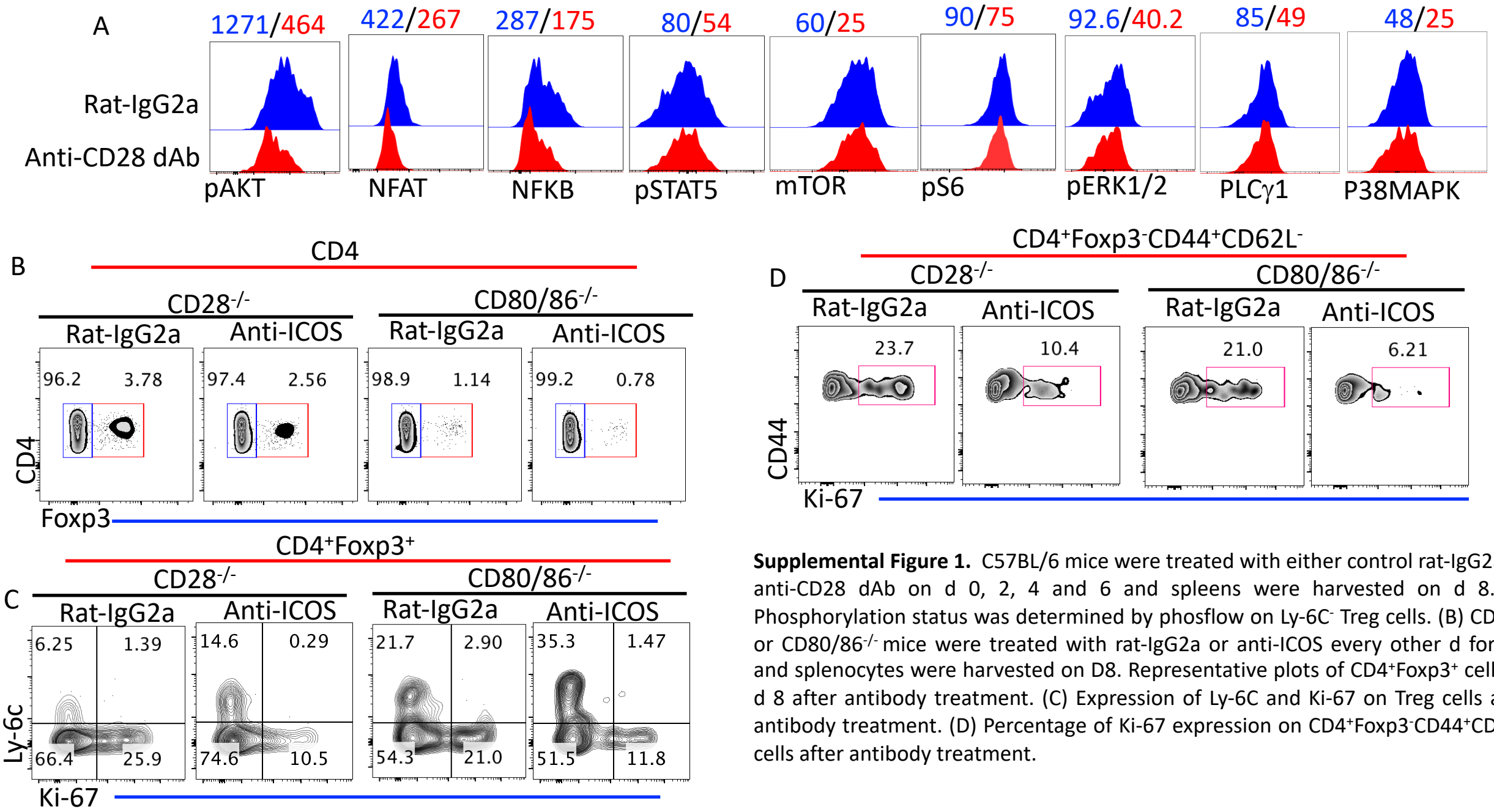
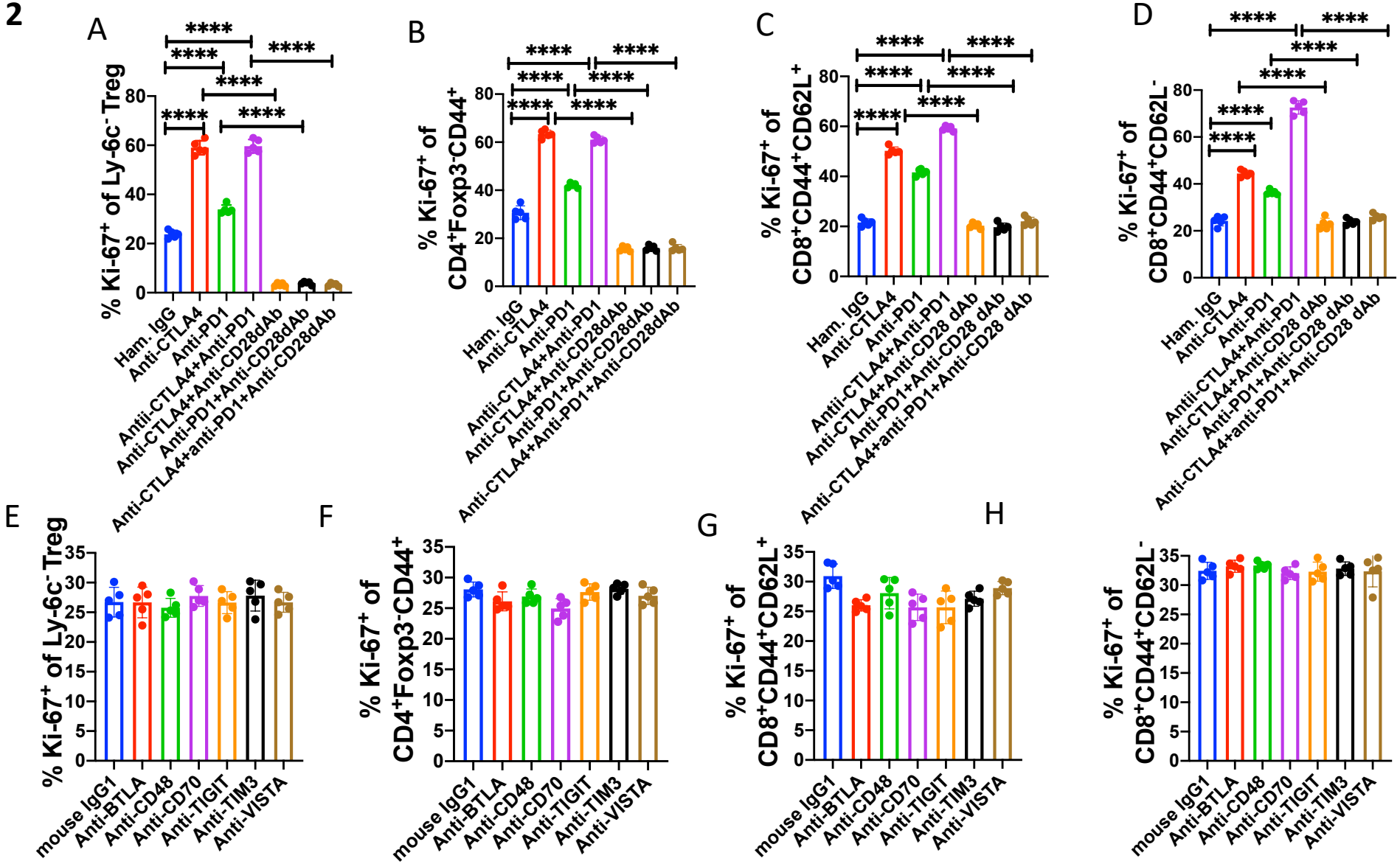
**Supplemental Fig. 1**



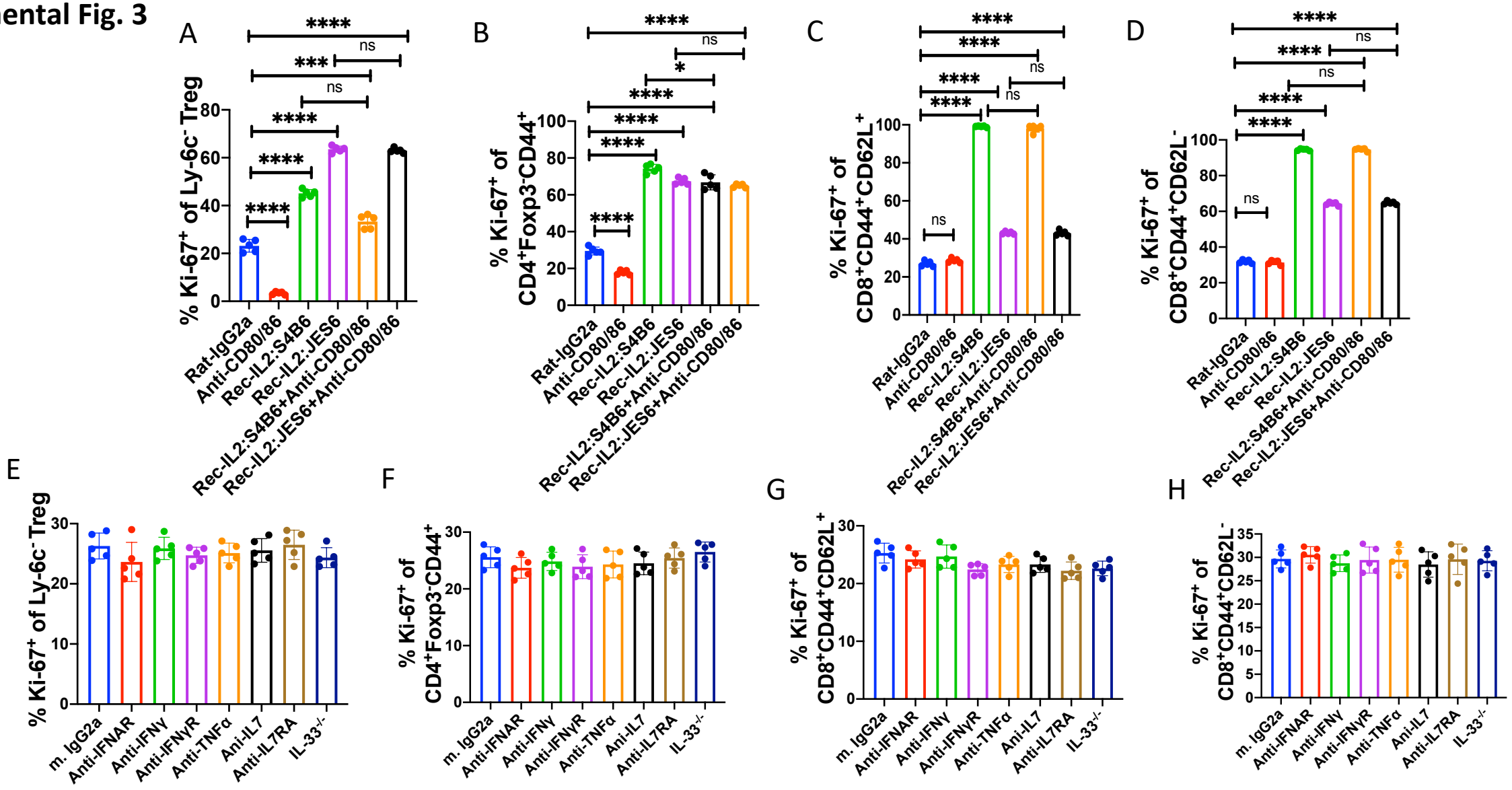

**Supplemental Figure 1.** C57BL/6 mice were treated with either control rat-IgG2a, or anti-CD28 dAb on d 0, 2, 4 and 6 and spleens were harvested on d 8. (A) Phosphorylation status was determined by phosflow on Ly-6C$^-$ Treg cells. (B) CD28$^{-/-}$ or CD80/86$^{-/-}$ mice were treated with rat-IgG2a or anti-ICOS every other d for 6 d and splenocytes were harvested on D8. Representative plots of CD4$^+$Foxp3$^+$ cells on d 8 after antibody treatment. (C) Expression of Ly-6C and Ki-67 on Treg cells after antibody treatment. (D) Percentage of Ki-67 expression on CD4$^+$Foxp3$^-$CD44$^+$CD62L$^-$ cells after antibody treatment.

**Supplemental Fig. 2**



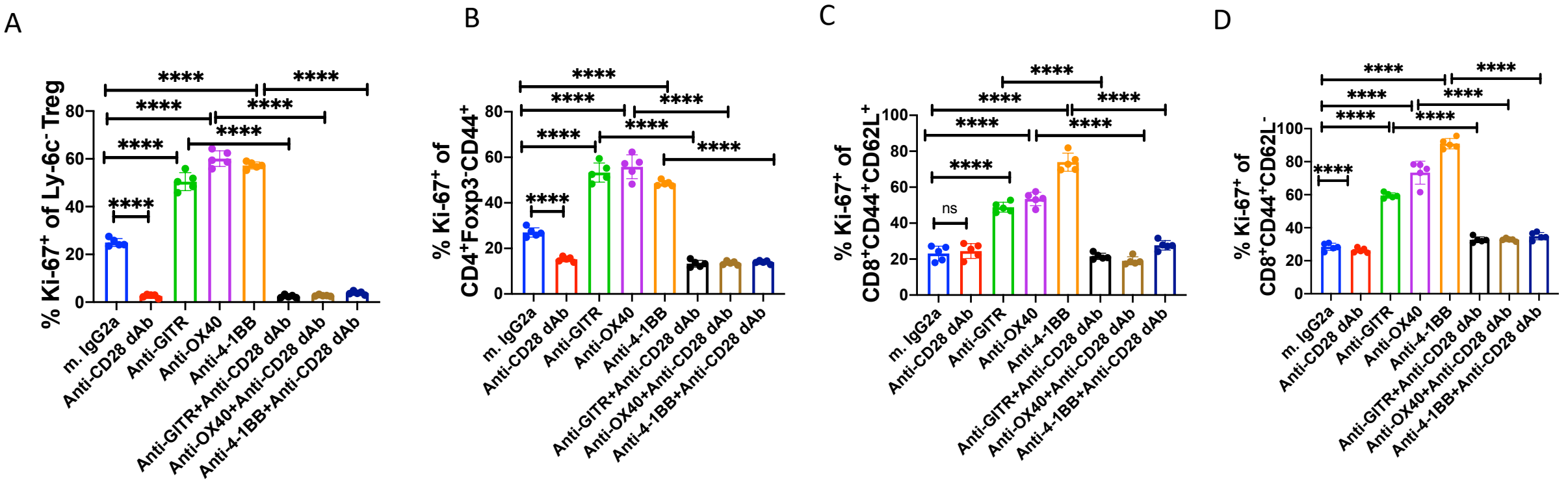

**Supplemental Figure 2.** C57BL/6 mice were treated (i.p.) either anti-CTLA4, anti-PD1 or both or together with anti-CD28 dAb every other day for 6 d and splenocytes were harvested on d 8. Percentage of Ki-67 expression among: (A) Ly-6C$^-$ Treg, (B) CD4$^+$Foxp3$^-$CD44$^+$, (C) CD8$^+$CD44$^+$CD62L$^+$, and (D) CD8$^+$CD44$^+$CD62L$^-$ subsets after antibody treatment. C57BL/6 mice were treated with mouse-IgG1, anti-BTLA, anti-CD48, anti-CD70, anti-TIGIT (agonistic) , anti-TIM3 and anti-VISTA every other d for 6 d and immunophenotyping of splenocytes was performed on d 8. Percentage of Ki-67 expression on (E) Ly-6C$^-$Treg, (F) CD4$^+$Foxp3$^-$CD44$^+$, (G) CD8$^+$CD44$^+$CD62L$^+$, and (H) CD8$^+$CD44$^+$CD62L$^-$ subsets in treated mice.

**Supplemental Fig. 3**




**Supplemental Figure 3.** C57BL/6 mice were treated with (i.p.) either IL2:S4B6, IL2:JES6 alone in combination with anti-CD80/86 on d 0, 2, 4 and 6 and splenocytes were isolated on d 8 for immune phenotyping. Percentage of Ki-67 expression among (A) Ly-6C$^-$ Treg, (B) CD4$^+$Foxp3$^-$CD44$^+$ (C) CD8$^+$CD44$^+$CD62L$^+$ and (D) CD8$^+$CD44$^+$CD62L$^-$ subsets after antibody treatment. C57BL/6 mice were treated with mIgG2a, anti-IFNAR, anti-IFNγ, anti-IFNγR, anti-TNFα, anti-IL-7 and anti-IL7Ra (250µg/dose) i.p every other d for 6 d and immunophenotyping of splenocytes was performed on d 8. Percentage of Ki-67 expression on (E) Ly-6C$^-$Treg, (F) CD4$^+$Foxp3$^-$CD44$^+$ (G) CD8$^+$CD44$^+$CD62L$^+$ and (H) CD8$^+$CD44$^+$CD62L$^-$ subsets in mAb treated or IL-33$^{-/-}$ mice.

**Supplemental Fig. 4**




**Supplemental Figure 4.** C57BL/6 animals were injected (i.p.) with anti-GITR, anti-OX40, anti-4-1BB (250μg/dose) alone or together with anti-CD28 dAb (100μg/dose) on d 0, 2, 4 and 6 and splenocytes were harvested on d 8. Percentage of Ki-67 expression among (A) Ly-6C-Treg, (B) $CD4^+Foxp3^-CD44^+$ (C) $CD8^+CD44^+CD62L^+$ and (D) $CD8^+CD44^+CD62L^-$ subsets after antibody treatments.