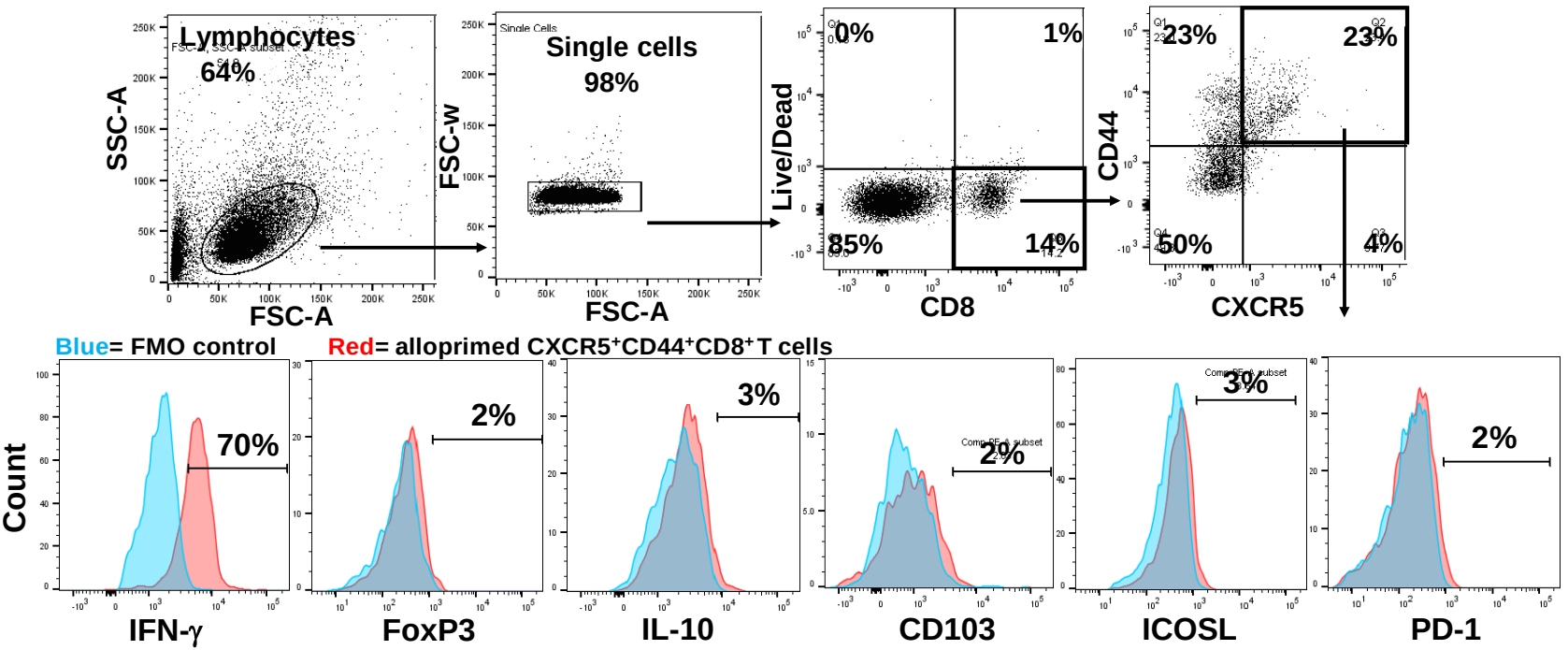

Figure S1. Alloprimed antibody-suppressor CD8$^+$ T cells express CXCR5$^+$IFN-γ$^+$CD8$^+$ phenotype. C57BL/6 mice (H-2^b) were transplanted with FVB/N (H-2^q) hepatocytes on day 0. On day 7, splenocytes were analyzed by flow cytometry. Cells were gated sequentially for lymphocytes, single cells, live CD8$^+$ T cells, and CD44$^+$CXCR5$^+$ cells. The CXCR5$^+$CD44$^+$CD8$^+$ T cell population was analyzed for intracellular and extracellular markers. CXCR5$^+$CD44$^+$CD8$^+$ T cells expressed IFN-γ, but did not express FoxP3, IL-10, CD103, ICOSL, or PD-1. Fluorescence minus one (FMO) controls were used for setting the positive gates and indicating background staining for plots (representative data shown, n=4).

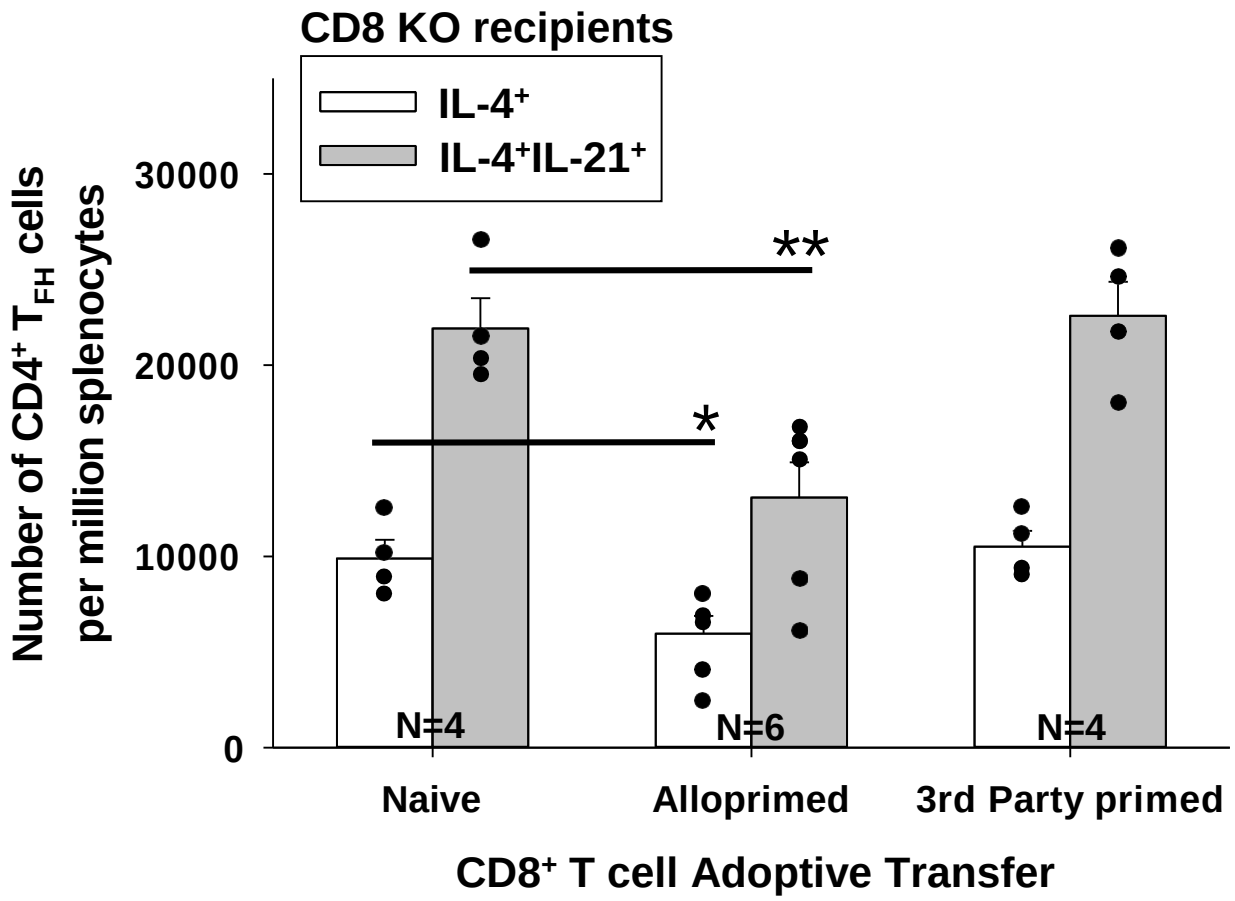

Figure S2. CD8$^+$ T cell-mediated suppression of CD4$^+$ T_{FH} cells in vivo is allospecific. CD8 KO recipients (H-2^b) were transplanted with FVB/N (H-2^q) hepatocytes on day 0. On the same day, cohorts of wild- type C57BL/6 mice were transplanted with FVB/N or B10.BR (H-2^k) hepatocytes to serve as sources for alloprimed CD8$^+$ T cells and third-party (3rd) primed CD8$^+$ T cells, respectively. On day 5, alloprimed and third-party (3rd) primed CD8$^+$ T cells were collected from C57BL/6 recipients and adoptively transferred (10 million cells) into CD8 KO recipients (day 5 following FVB/N hepatocyte transplant). Adoptive transfer of naïve CD8$^+$ T cells was used as a negative control. Spleens were harvested 48 hours later and the number of IL-4$^+$ or IL4$^+$IL-21$^+$CD4$^+$ T_{FH} cells were quantified by flow cytometry. Adoptive transfer of alloprimed CD8$^+$ T cells significantly reduced the number of IL-4$^+$CD4$^+$ T_{FH} cells post transplant (6,000±900 cells per million splenocytes, p=0.001, as depicted by "*") compared to CD8 KO recipients that received naïve CD8$^+$ T cells (9,900±1,000 cells per million splenocytes). Similarly, adoptive transfer of alloprimed CD8$^+$ T cells significantly reduced the number of IL4$^+$IL-21$^+$CD4$^+$ T_{FH} cells post transplant (13,000±1,800 cells per million splenocytes, p=0.003, as depicted by "**") compared to CD8 KO recipients that received naïve CD8$^+$ T cells (22,000±1,600 cells per million splenocytes). Adoptive transfer of third-party primed CD8$^+$ T cells did not reduce the number of IL-4$^+$ (11,000±800 cells per million splenocytes) or IL4$^+$IL-21$^+$ (23,000±1,800 cells per million splenocytes) CD4$^+$ T_{FH} cells in vivo (p=ns). Data was combined from duplicate experiments (n=4-6 mice per group). Error bars represent standard error.

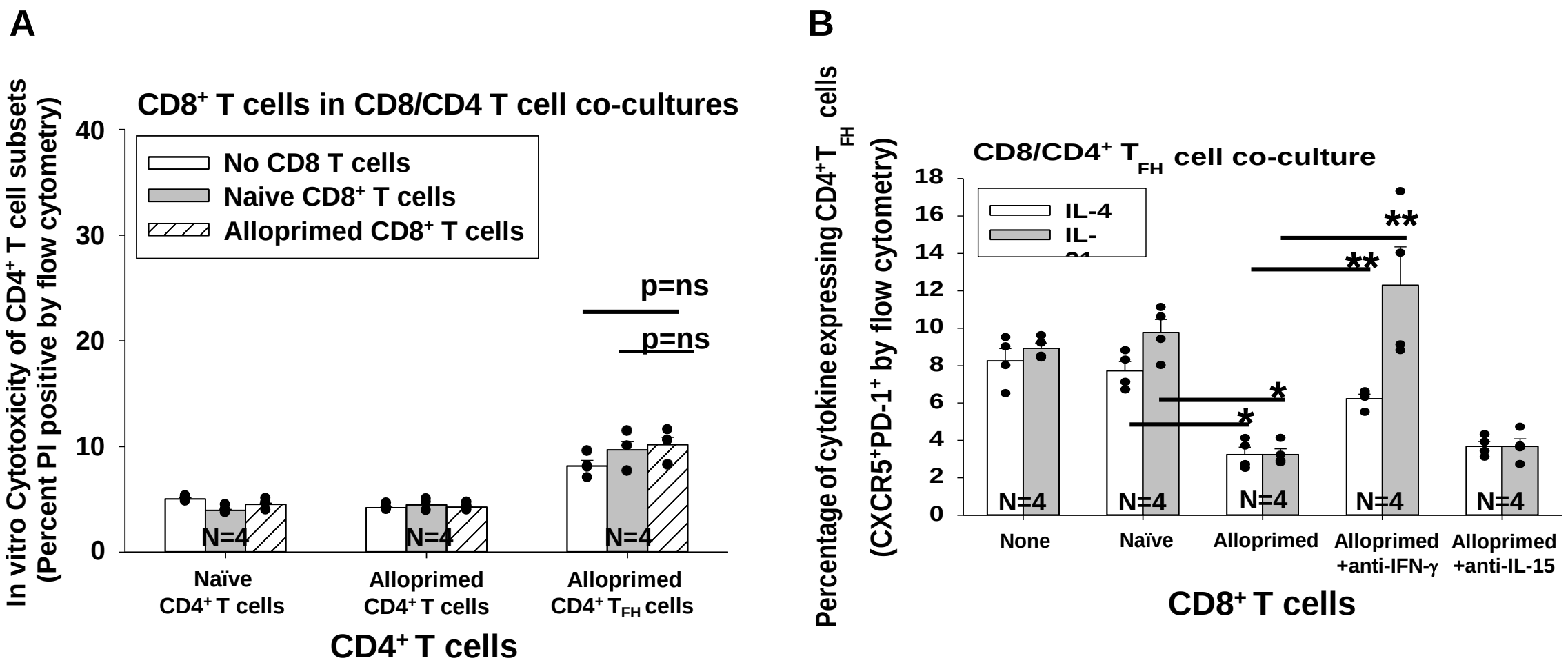

Figure S3. Alloprimed CD8⁺ T cells mediate IFN-γ-dependent non cytotoxic inhibition of CD4⁺ T_{FH} cells in co-culture. C57BL/6 mice were transplanted with allogeneic (FVB/N, H-2^q) hepatocytes. On day 7, alloprimed CD8⁺ T cells and CD4⁺ T cells were retrieved from recipient spleens. CD4⁺ T_{FH} cells (CXCR5⁺PD-1⁺CD4⁺ T cells) were isolated by flow cytometric cell sorting. **A)** Alloprimed CD8⁺ T cells were co-cultured with CFSE-labeled naïve, bulk alloprimed CD4⁺ T cells, or alloprimed CD4⁺ T cells that were flow sorted for CD4⁺ T_{FH} cells (CXCR5⁺PD-1⁺CD4⁺). After 4 hours of co-culture, CFSE-labeled CD4⁺ T cells were collected and analyzed by flow cytometry for apoptosis by propidium iodide (PI) uptake. No significant cytotoxicity was observed in any CD8/CD4 T cell co-cultures compared to cultures without CD8⁺ T cells (p=ns). Error bars represent standard error. **B)** CD4⁺ T_{FH} cells and CD8⁺ T cell were co-cultured for 4 hours followed by intracellular cytokine staining and flow cytometric analysis of CD4⁺ T_{FH} cells. CD4⁺ T_{FH} cell expression of IL-4 (3.3±0.4%) and IL-21 (3.3±0.3%) was significantly reduced in co-cultures with alloprimed CD8⁺ T cells compared to co-cultures with control naïve CD8⁺ T cells (8.3±0.7% and 8.9±0.3%, respectively, $p<0.0002$, "*****"). Addition of anti-IFN-γ mAb (clone XMG1.2 eBioscience) to co-cultures restored the expression of IL-4 (6.2±0.2%) and IL-21 expression (12.3±2.0) ($p<0.01$ for both "******" compared to alloprimed CD8⁺ T cell group; p=ns for both compared to naïve/no CD8⁺ T cell control groups). Addition of anti-IL-15 mAb (clone AI0.3 eBioscience) to co-cultures had no effect on CD8-mediated inhibition of CD4⁺ T_{FH} cell IL-4 (3.7±0.3%) and IL-21 (3.7±0.4%) expression (p=ns for both).