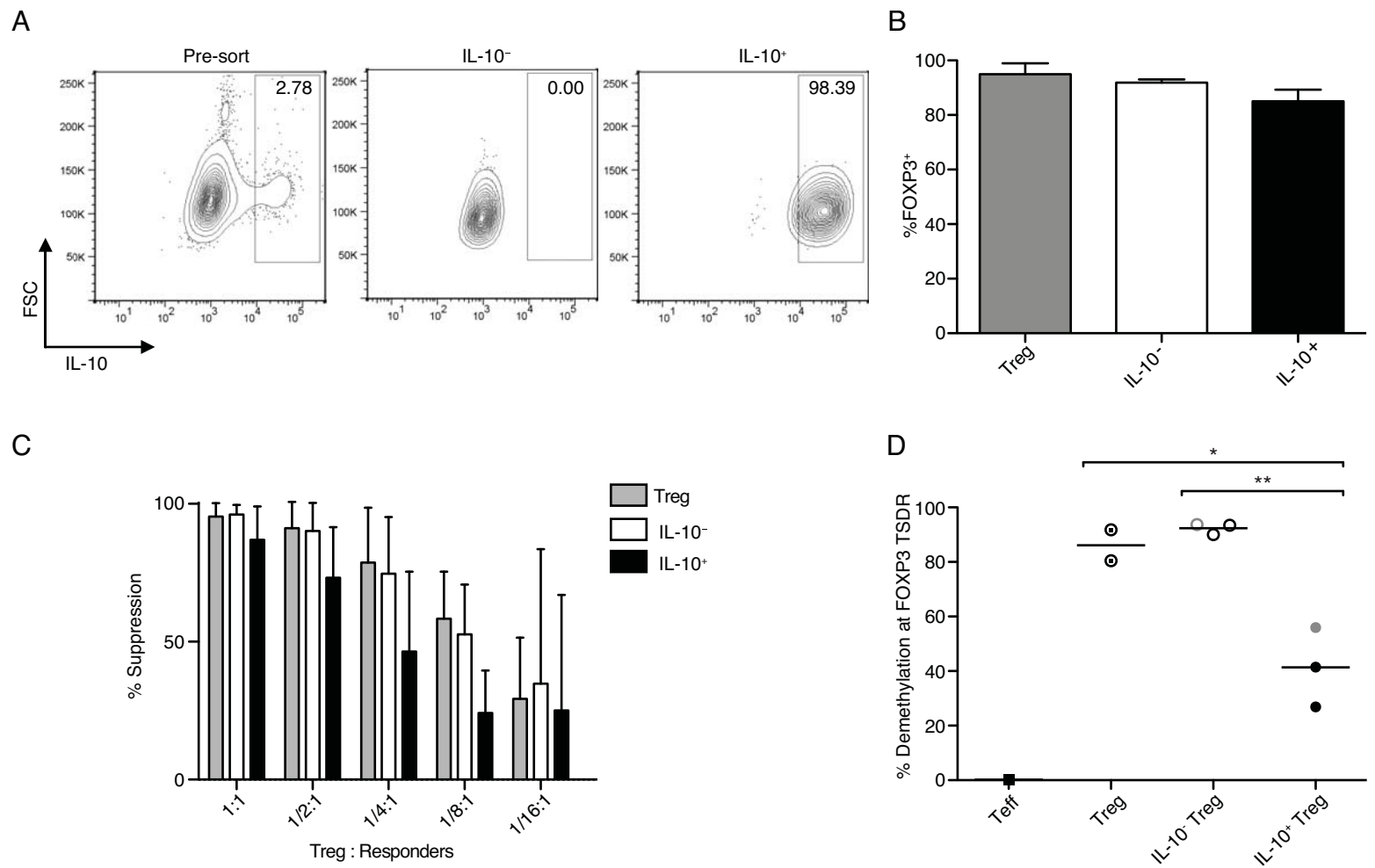
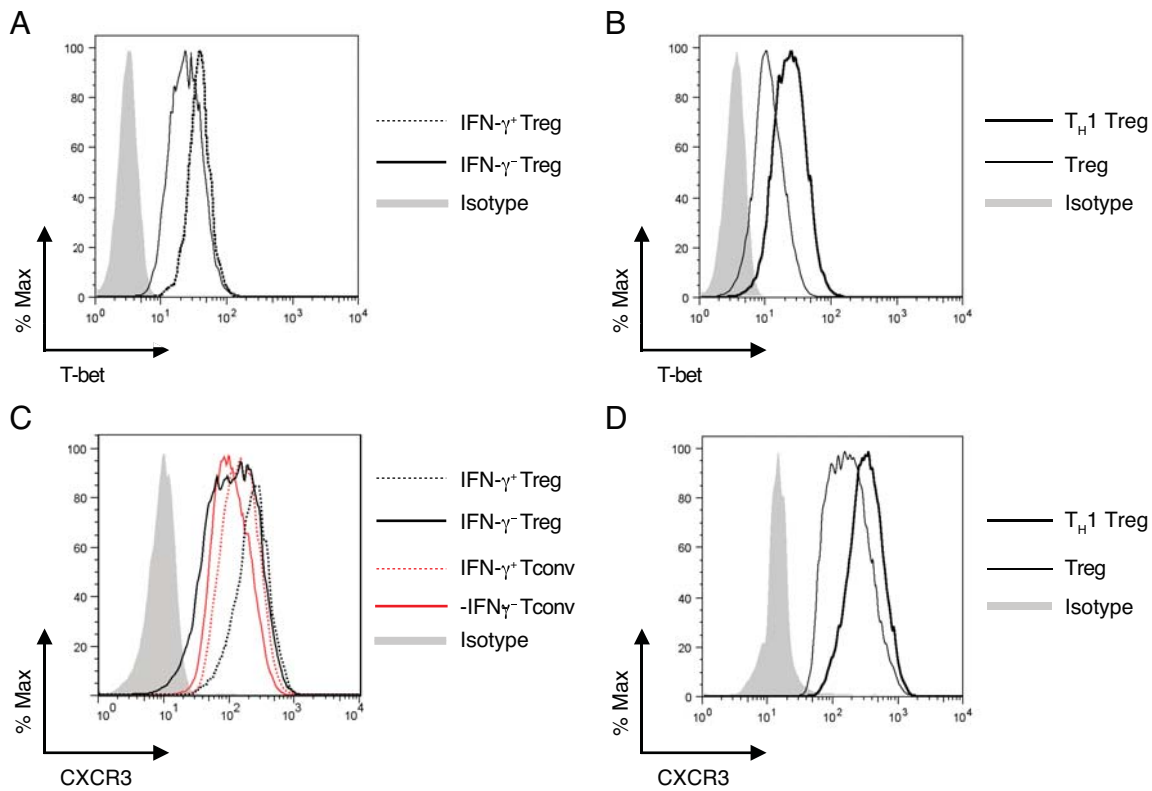


Supplemental Figure 1. IFN- γ ⁺ Tregs do not make IL-2. Representative flow cytometric analysis of IL-2 and IFN- γ following 14 d expansion and 4 h PMA/ionomycin stimulation (n = 3). Cytokine staining in total (top plots), or FOXP3⁺ subset (bottom plots) of CD4⁺CD127^{hi}-CD25⁺ Tregs (right) or CD4⁺CD127⁺CD25⁻ Tconv cells (left). Middle plots show gating of FOXP3⁺ subset in each population. Numbers represent frequency of cells in each quadrant.



Supplemental Figure 2. The IL-10⁺ subset retains FOXP3 protein expression and suppressive function, but exhibits increased methylation at the FOXP3 TSDR. A. Representative purities of populations before (left) and after cytokine capture and FACS-sorting of IL-10⁻ (center) and IL-10⁺ (right) cells from expanded CD4⁺CD127^{low}CD25⁺ Tregs. B. Percentage of expanded, PMA/ionomycin-stimulated cells expressing FOXP3, assessed by FACS. Shown are the mean + SD (n = 2). C. Percent suppression of T responder cell proliferation in vitro, as determined by incorporation of ³H-thymidine. Shown are the mean + SD (n = 2). D. Percentage of cells demethylated at the FOXP3 Treg-specific demethylated region (TSDR). IL-10⁻ and IL-10⁺ subsets (open and closed circles, respectively) were isolated following stimulation with PMA/ionomycin and resorting either by IL-10 capture (black) or by fixation and intracellular FOXP3 and IL-10 staining (gray). Significance was determined by ANOVA. *P<0.05, **P<0.01.



Supplemental Figure 3. CXCR3 and T-bet are upregulated in T_H1 -like Tregs. *A, B*, Histograms show T-bet expression in $CD4^+CD127^{lo/-}CD25^+CD45RA^+$ naïve Tregs after 8 days expansion, as assessed by FACS. Comparison of T-bet in *A*, Tregs gated as $FOXP3^+IFN-\gamma^-$ (*solid*) or $FOXP3^+IFN-\gamma^+$ (*dotted*) and *B*, in naïve Tregs cultured in neutral (*light*) or T_H1 -skewing (*heavy*) conditions. *C*, CXCR3 expression following PMA/ionomycin stimulation in expanded $CD4^+CD127^{lo/-}CD25^+$ Tregs (*black*) or $CD4^+CD127^+CD25^-$ Tconv cells (*red*) gated on intracellular IFN- γ expression, as assessed by FACS. *D*, Histogram shows CXCR3 expression in expanded $CD4^+CD127^{lo/-}CD25^+CD45RA^+CD45RO^-$ naïve Tregs cultured in neutral (*light*) or T_H1 -skewing (*heavy*) conditions.