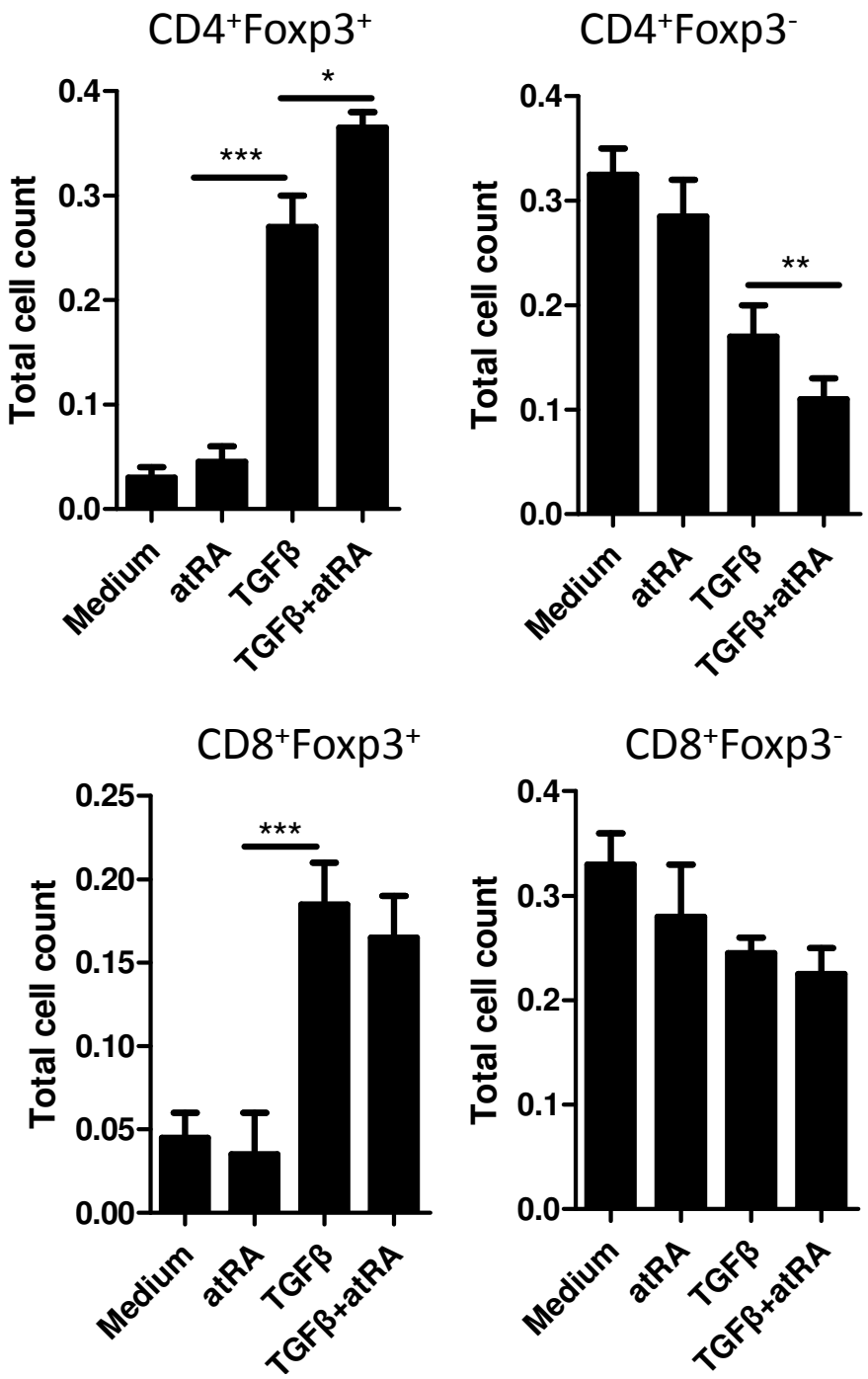
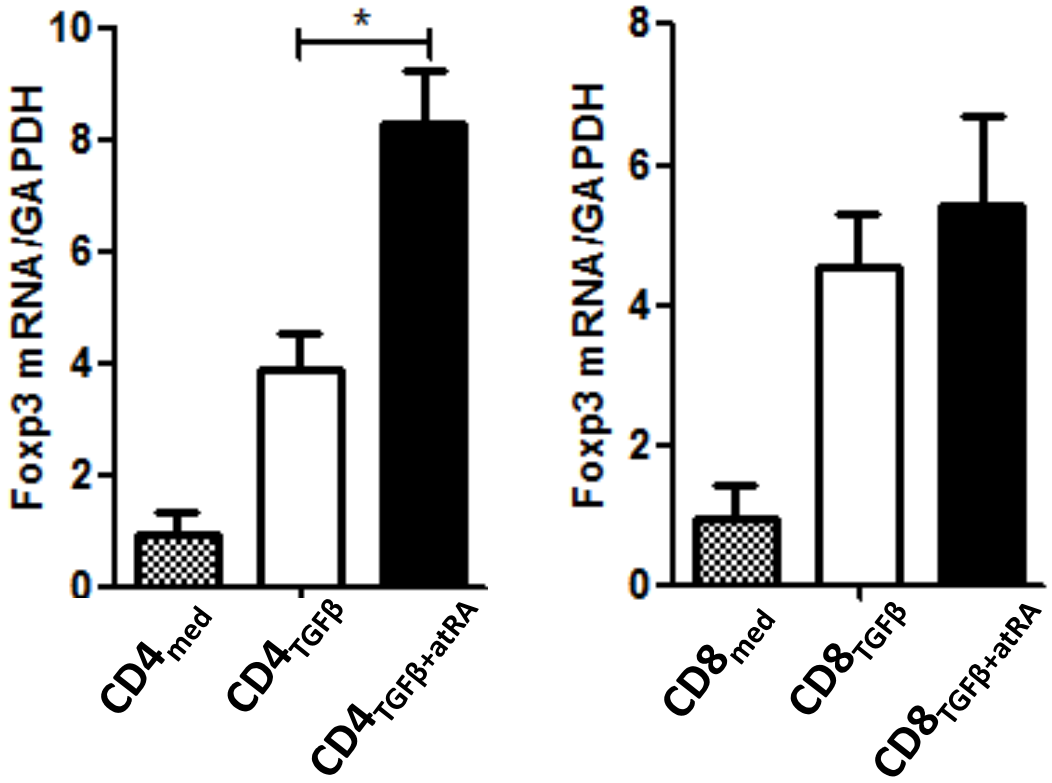
# S Figure 1



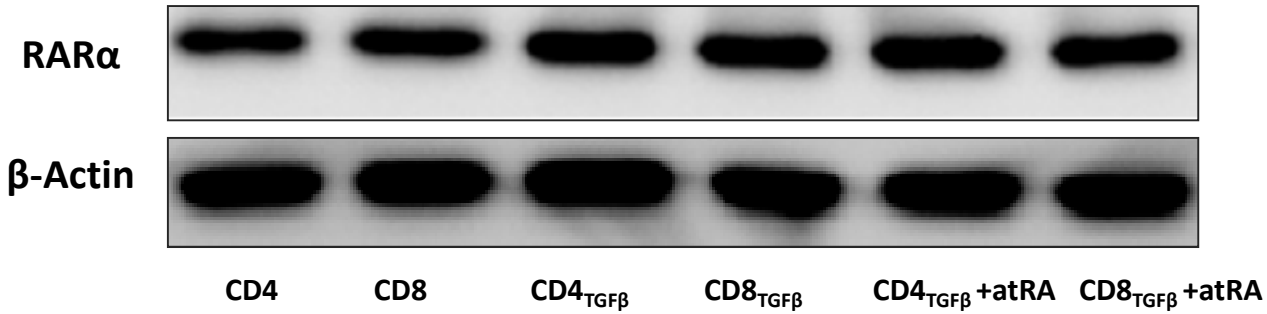

**S figure 1 . atRA enhances total Foxp3+ cells in CD4+ but not in CD8+ cell populations**. $2x10^5$ CD4+CD62L+CD25-Foxp3-(GFP-) or $2x10^5$ CD8+CD62L+CD25-Foxp3-(GFP-) cells isolated from C57BL/6 $Foxp3^{gfp}$ reporter mice were stimulated with immobilized anti-CD3 (1 µg/ml), soluble anti-CD28 (1 µg/ml), IL-2 (100 U/ml) ± TGF-β (2 ng/ml), and with ($CD4_{TGFβ+atRA}$ or $CD8_{TGFβ+atRA}$) or without atRA (50nM) ($CD4_{TGFβ}$ or $CD8_{TGFβ}$) for 3 days. Foxp3 (GFP) expression was examined by flow cytometry. The data are summary of three independent experiments showing the total cell number of Foxp3+ and Foxp3- cells from TGF-β-primed CD4+ or CD8+ cells (x $10^6$) , * $p<0.05$, ** $p<0.01$, *** $p<0.001$, NS, no significance.

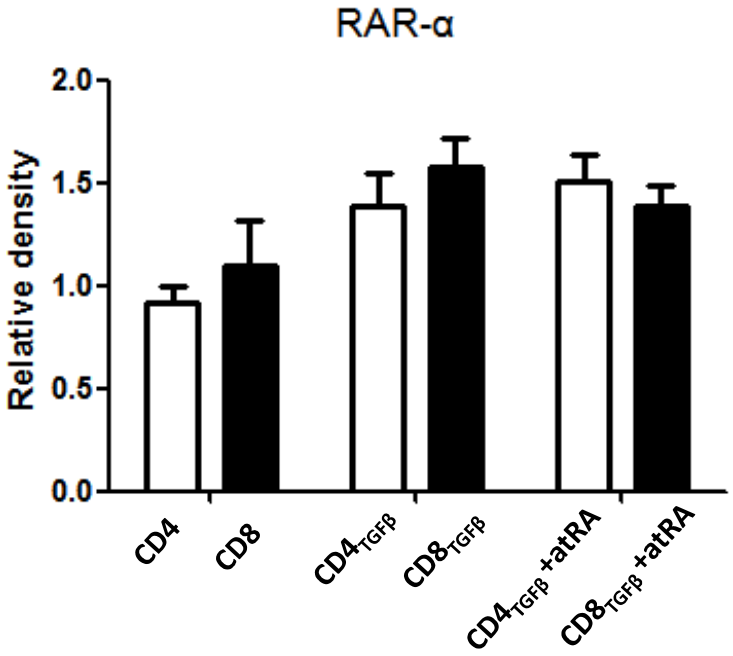
# S Figure 2




**S Figure 2. atRA enahces Foxp3 mRNA expression on TGF-β-primed CD4+ but not on TGF-β-primed CD8+ cells**. The cells were isolated and cultured as s Figure 1. The expression of Foxp3 mRNA was determined by quantitative RT-PCR on various CD4+ and CD8+ cells activated with TCR ± TGF-β ±atRA. Data are Mean ± SEM of three separate experiments. * $p<0.05$.

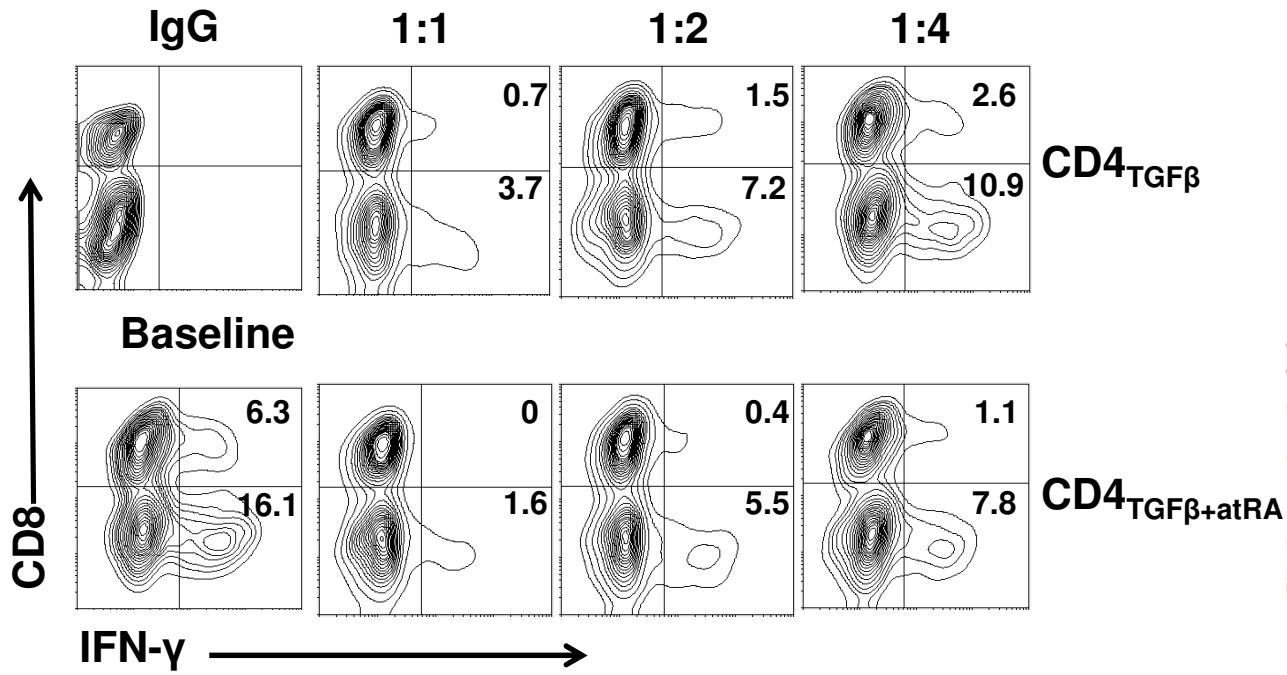
# S Figure 3

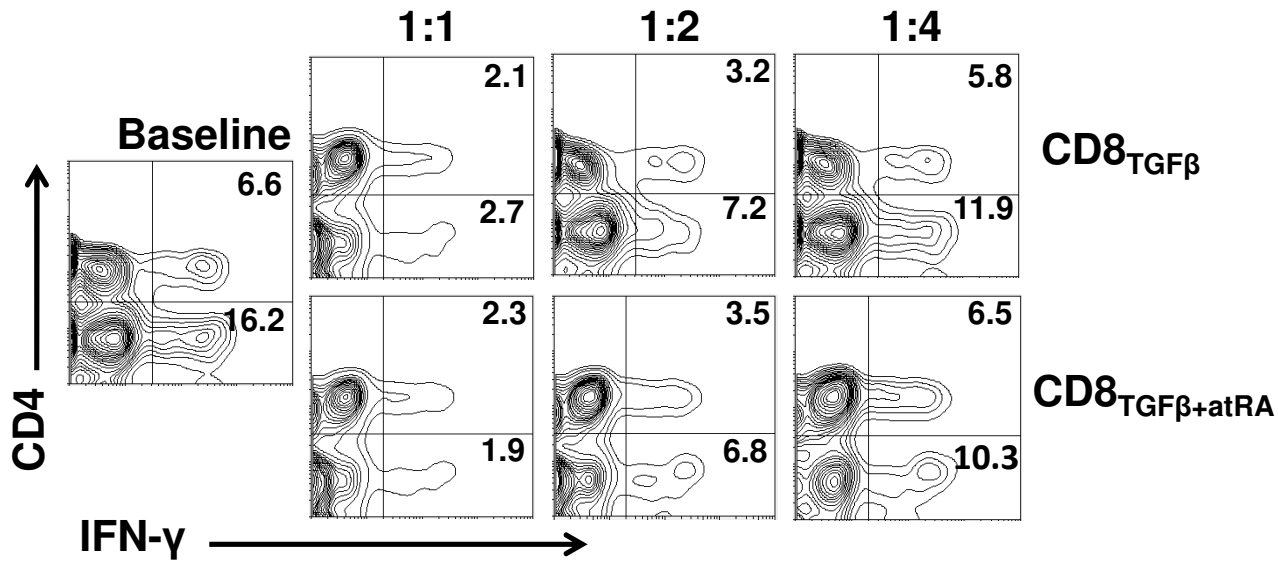


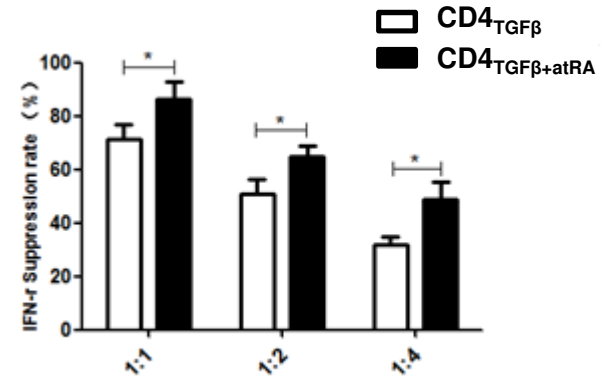



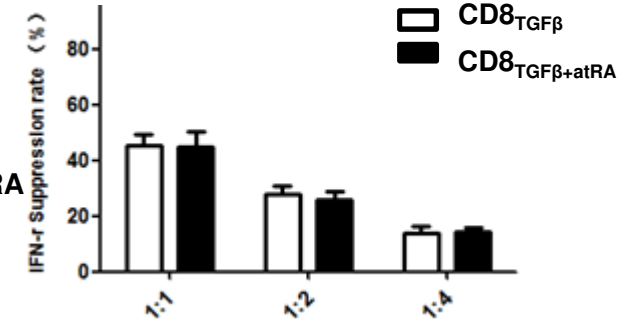

**S Figure 3. Both CD4+ and CD8+ cells similarly express RARα protein**. Naïve or activated CD4+ and CD8+ cells that had been stimulated with TCR ± TGF-β for three days were subjected to Western blot analysis for atRA receptor α chain (RARα) and β-Actin. The levels of RARα were normalized to β-Actin, with the ratio of naïve CD4+ cells being arbitrarily assigned a value of 1. The results shown are representative of the 3 independent experiments (top) and summary of three experiments (mean ± SEM) (bottom).

# S Figure 4




**S Figure 4. Addition of atRA enhances the suppressive ability of TGFβ primed CD4+ but not CD8+ cells against IFN-γ production of T responder cells**. Naïve CD4+ or CD8+ cells were co-cultured with soluble anti-CD3(1 ug/ml), anti-CD8 (1 ug/ml), anti-IL-4 (5 ug/ml), rm-IL-12 (10 ng/ml), APC, in the presence of 1:1, 1:2, 1:4 ratios of $CD4_{TGFβ}$, $CD4_{TGFβ+atRA}$ or $CD8_{TGFβ}$, $CD8_{TGFβ+atRA}$. Cells were stimulated at day3, then harvested and restimulated with PMA (0.25 mg/ml) and ionomycin (0.25 mg/ml) for 1 hr , then together with brefeldin A (5 mg/ml) for another 4 hours. IFN-γ+ cells on the gate of CD8 or CD4 was determined by FACS staining. Left are representative and right are value of three separate experiments.