Supporting Information

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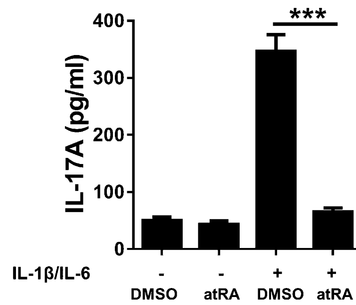

Fig. S1. Natural regulatory T cells (nTregs) primed with *all-trans* RA (atRA) no longer secrete soluble IL-17A. Human nTregs were sorted from healthy peripheral blood mononucleated cells (PBMCs) and expanded with anti-CD3/CD28–coated beads (1:3; one bead to three cells) and IL-2 (300 U/mL) with or without atRA solvent (DMSO) or atRA (0.1 μM) for 1 wk. These cells were restimulated with anti-CD3/CD28 beads with or without IL-6 (10 ng/mL) and IL-1β (10 ng/mL). Three days later, the supernatants were harvested and subjected to ELISA to measure soluble IL-17A. Values indicate mean ± SEM of four separate experiments (***$P < 0.001$).

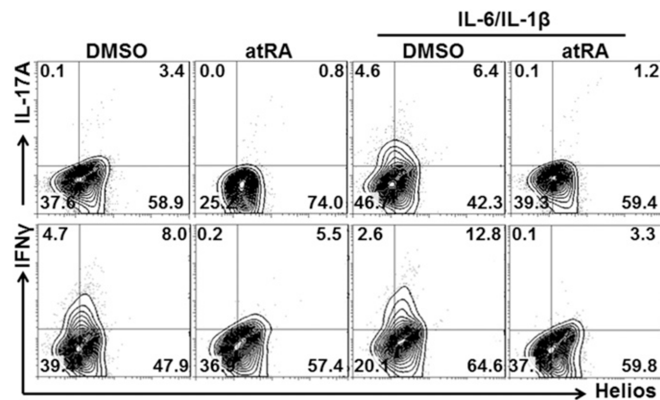

Fig. S2. atRA suppresses the expression of IL-17A and IFN-γ in $Helios^{+}$ $Foxp3^{+}$ nTregs. Human nTregs were sorted and expanded as described earlier for 1 wk. These cells were restimulated with anti-CD3/CD28 beads with or without IL-6 (30 ng/mL) and IL-1β (10 ng/mL). Three days later, cells were harvested and stained with IL-17A, IFN-γ, Helios, and Foxp3. Representative of Th1 or Th17 frequency from three separate experiments (flow data were gated on $Foxp3^{+}$ cells).

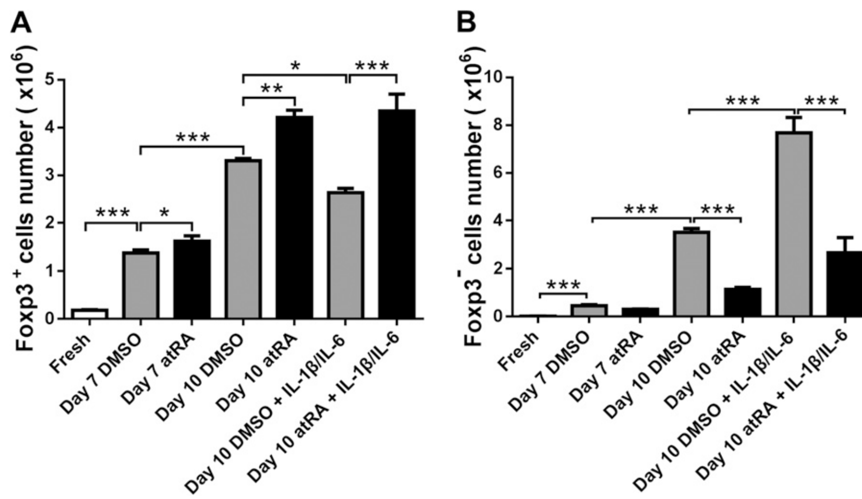

Fig. S3. atRA predominately expands Foxp3^{+} cells. Human nTregs were sorted from healthy PBMCs and expanded with anti-CD3/CD28–coated beads (1:3; one bead to three cells) and IL-2 (300 U/mL) with or without atRA solvent (DMSO) or atRA (0.1 μM) for 1 wk. These cells were restimulated with anti-CD3/CD28 beads with or without IL-6 (10 ng/mL) and IL-1β (10 ng/mL) for another 3 d. (*A*) Total numbers of Foxp3^{+} cells. (*B*) Total numbers of Foxp3^{-} cells. Values indicate mean ± SEM of four separate experiments (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

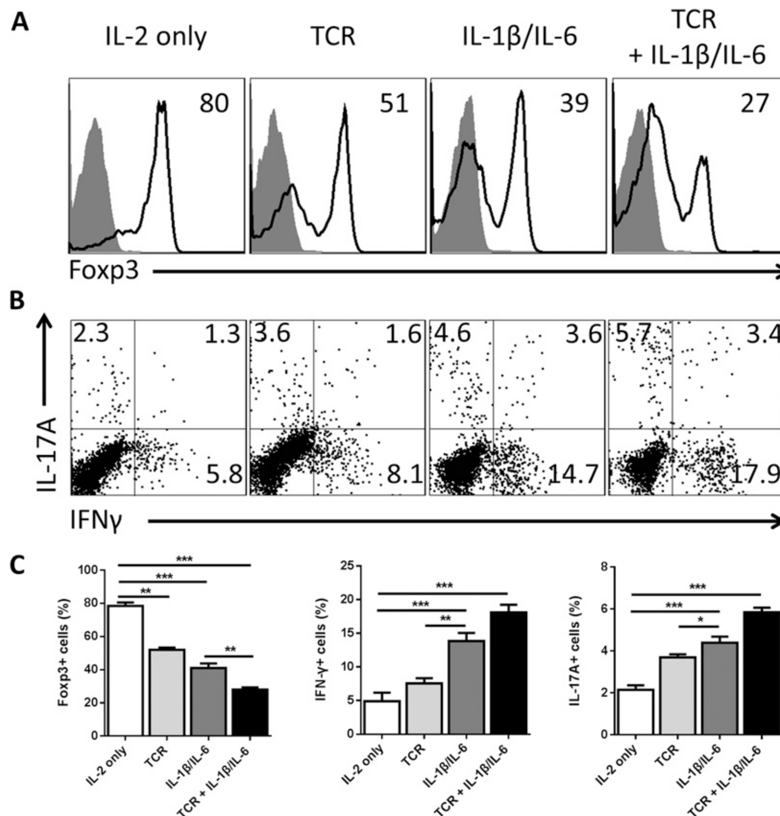

Fig. S4. IL-1β and IL-6 play a more important role than TCR stimulation in the stability of nTregs. Human nTregs were sorted from healthy PBMCs and expanded with anti-CD3/CD28–coated beads (1:3; one bead to three cells) and IL-2 (300 U/mL) for 1 wk. These cells were restimulated with anti-CD3/CD28 beads or IL-6 (10 ng/mL), IL-1β (10 ng/mL), or both together for an additional 3 d. IL-2 only was a control. Cells were stained with Foxp3, IFN-γ, and IL-17A for flow cytometry analysis. (*A*) Representative of Foxp3^{+} cell frequency from three separate experiments. (*B*) Representative of Th1 or Th17 frequency from three separate experiments. (*C*) Values are mean ± SEM of three independent experiments (**$P < 0.01$ and ***$P < 0.001$).

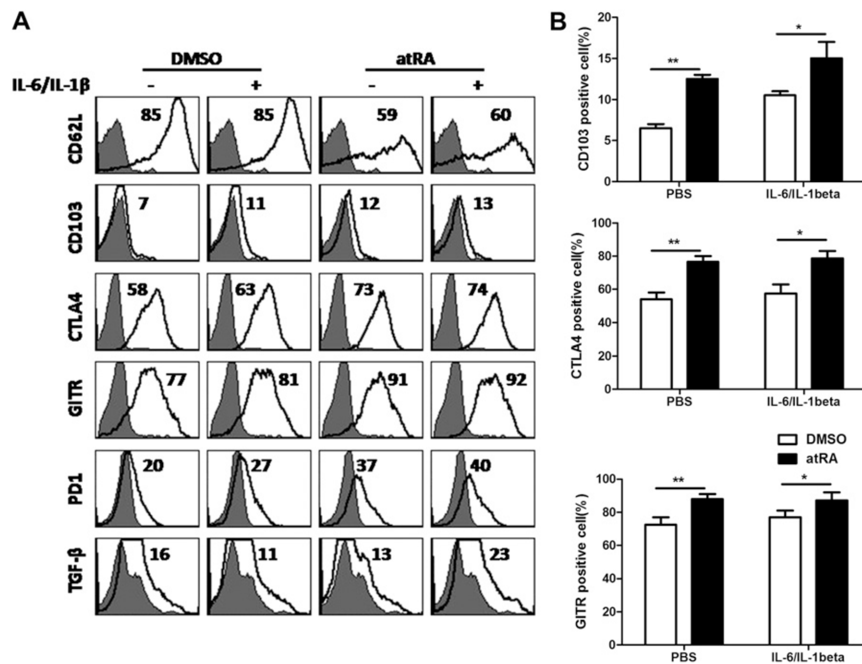

Fig. S5. Phenotypic characteristics of nTregs following the treatment of atRA. Human nTregs were expanded as described earlier, and the expression of Treg cell related makers (CD62L, CD103, CTLA4, GITR, PD1, and TGF-β) was examined with flow cytometry. (*A*) Data are representative of three separate experiments. (*B*) Values of CD103, CTLA-4, and GITR frequency on nTregs are mean ± SEM of three independent experiments ($*P < 0.05$ and $**P < 0.01$).

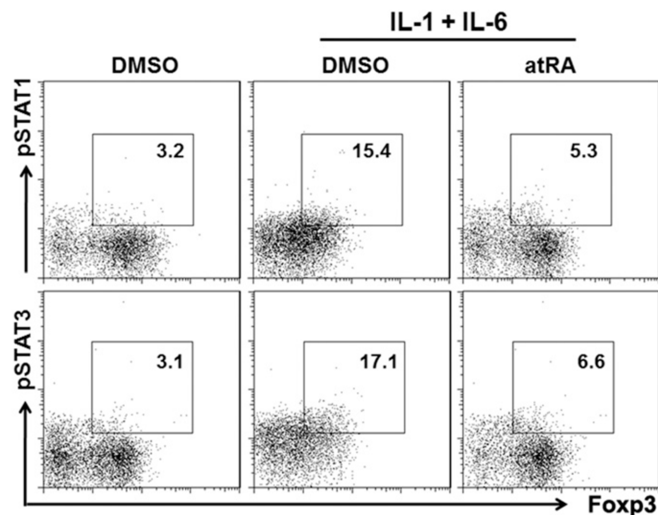

Fig. S6. atRA decreased STAT1 and STAT3 activation following IL-1 or IL-6 stimulation in $Foxp3^+$ cells. Human nTregs were sorted and expanded as described earlier for 1 wk. These cells were restimulated with anti-CD3/CD28 beads with or without IL-6 (10 ng/mL) and/or IL-1β (10 ng/mL). Three days later, cells were harvested and stained with pSTAT1, pSTAT3, and Foxp3. Representative of pSTAT1 or pSTAT3 frequency from three separate experiments (flow data were gated on $Foxp3^+$ cells).

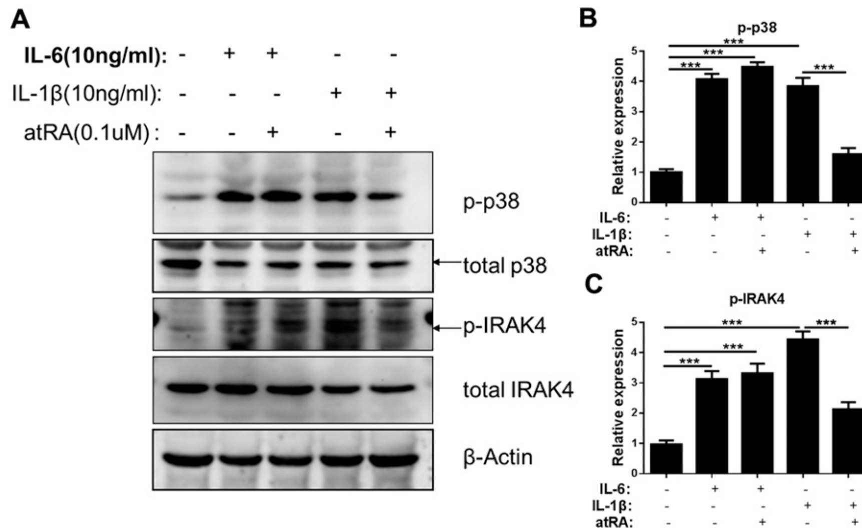

Fig. S7. atRA could suppress IRAK4 and p38 activation mediated by IL1-β. Human nTregs were sorted and expanded as described earlier for 1 wk. Then, cells were restimulated with anti-CD3/CD28 beads with or without IL-6 (10 ng/mL) and/or IL-1β (10 ng/mL) for 30 min. Last, cells were harvested for Western blot analysis of total p38, p-p38, total IRAK4, and p-IRAK4. (*A*) Data are representative of three separate experiments with similar results. β-Actin was used as the internal reference. (*B* and *C*) Values are mean ± SEM of three independent experiments (***$P < 0.001$).

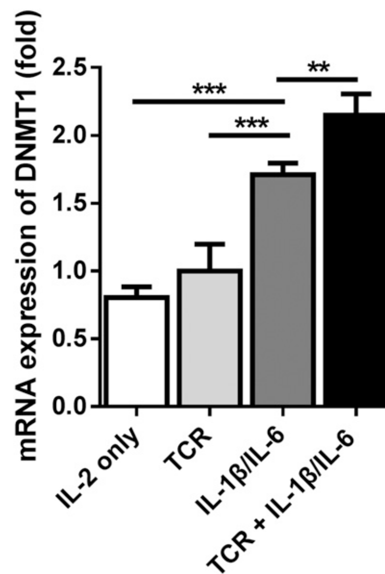

Fig. S8. IL-1β and IL-6 play a more important role than TCR stimulation in the DNMT1 expression. Human nTregs were sorted from healthy PBMCs and expanded with anti-CD3/CD28–coated beads (1:3; one bead to three cells) and IL-2 (300 U/mL) for 1 wk. These cells were restimulated with anti-CD3/CD28 beads or IL-6 (10 ng/mL), IL-1β (10 ng/mL), and both together for another 3 d. IL-2 only was a control. Cells were harvested for DNMT1 mRNA quantitative PCR (qPCR) assay. β-Actin was used as the internal reference. Fold changes were normalized with freshly isolated nTregs. Values indicate mean ± SEM of four separate experiments (**$P < 0.01$ and ***$P < 0.001$).

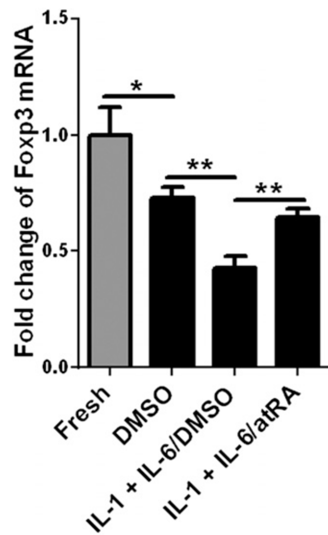

Fig. S9. atRA helps to maintain the expression of Foxp3 in expanded nTreg with the stimulation of IL-1 and IL-6. Human nTregs were sorted and expanded with or without atRA as described earlier for 1 wk. These cells were restimulated with anti-CD3/CD28 beads with or without IL-6 (10 ng/mL) and IL-1β (10 ng/mL). One day later, cells were harvested and the RNA was isolated. One-step qPCR was used to detect the expression of Foxp3 mRNA. β-Actin was used as the internal reference. Fold changes were normalized with freshly isolated nTreg. Values indicate mean ± SEM of four separate experiments (*$P < 0.05$ and **$P < 0.01$).

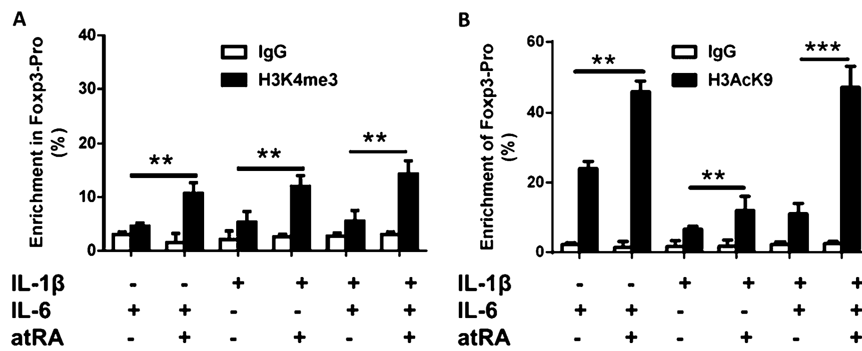

Fig. S10. atRA facilitates gene transcription permissive epigenetic modifications at Foxp3 promoter region in nTreg cells at inflammatory conditions. The percentage of trimethylation at lysine 4 (H3K4me3) (*A*) or acetylation at lysine 9 (H3AcK9) (*B*) of histone 3 in nTreg cell populations were analyzed with ChIP experiment described in *Materials and Methods*. ChIP qPCR with specific primers against Foxp3 promoter mentioned in *Materials and Methods* was performed, and the percentage values were normalized with the input DNA. The experiments were repeated twice with similar results.

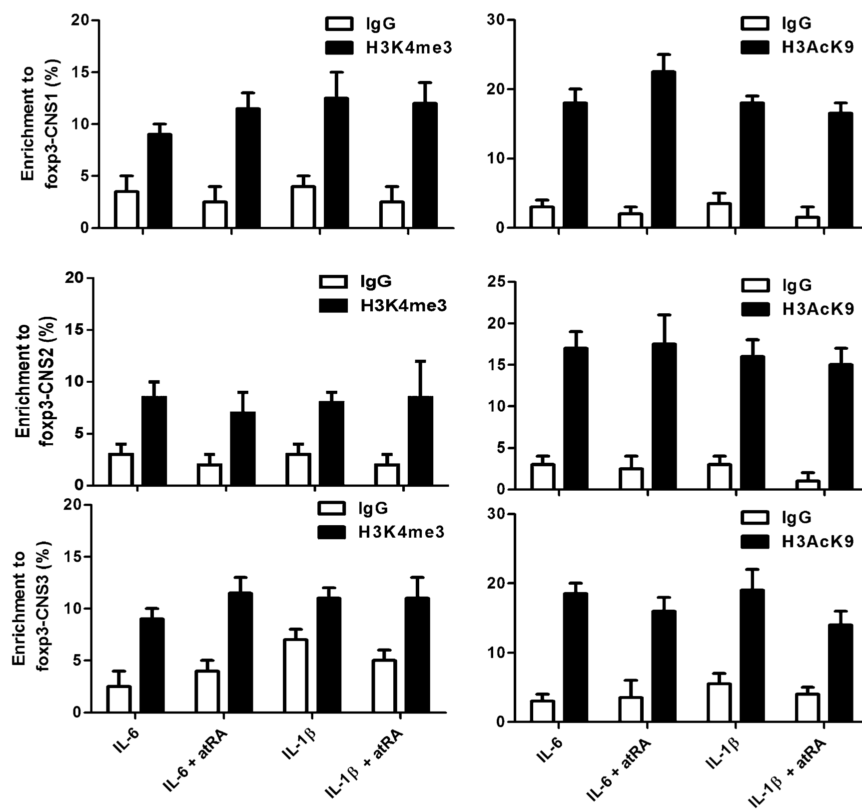

Fig. S11. atRA does not affect epigenetic modification status of H3K4me3 or H3AcK9 at Foxp3 CNS 1–3 regions. The percentages of H3K4me3 or acetylation H3AcK9 of histone 3 in nTreg cell populations were analyzed with ChIP as described in *Materials and Methods*. ChIP qPCR with specific primers against Foxp3 promoter as described in *Materials and Methods* was performed, and the percentage values were normalized with the input DNA. The experiments were repeated twice with similar results.