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Supplemental Information

Negligible Effect of Sodium Chloride on the Development and Function of TGF-β-Induced $CD4^+$ $Foxp3^+$ Regulatory T Cells

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Supplemental Information

Supplemental figures

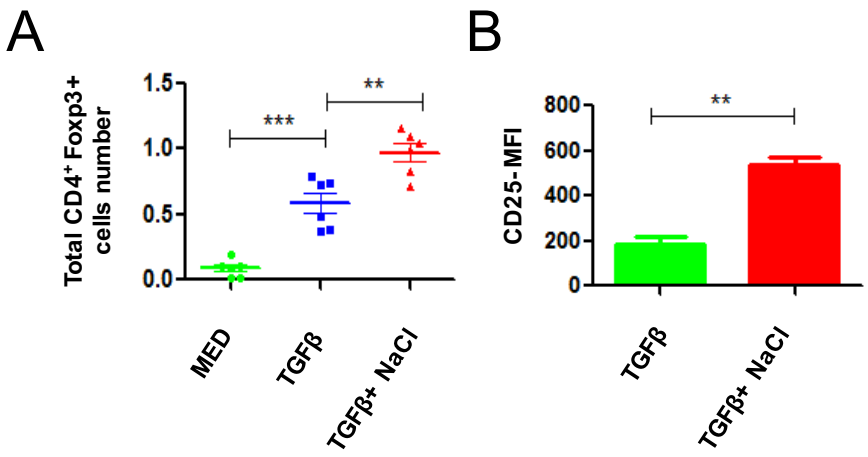

Figure S1. High-salt condition boosts iTreg cells proliferation and activation. **Related to Figure 1.** Splenic naïve CD4+ T cells were stimulated with anti-CD3/28 beads (5 cells per bead) in the presence of rmIL-2 (50 U/ml) and ±TGF-β (2 ng/ml) (iTreg/MED) in standard media, or with additional 40mM NaCl for 3 days. (A) Total GFP-Foxp3+ cells and mean fluorescence intensity (MFI) of CD25 expression (B) were analyzed. The data represent a summary of independent experiments (n=6). Statistical analyses were performed using one-way ANOVA. **P<0.01; ***P<0.001.

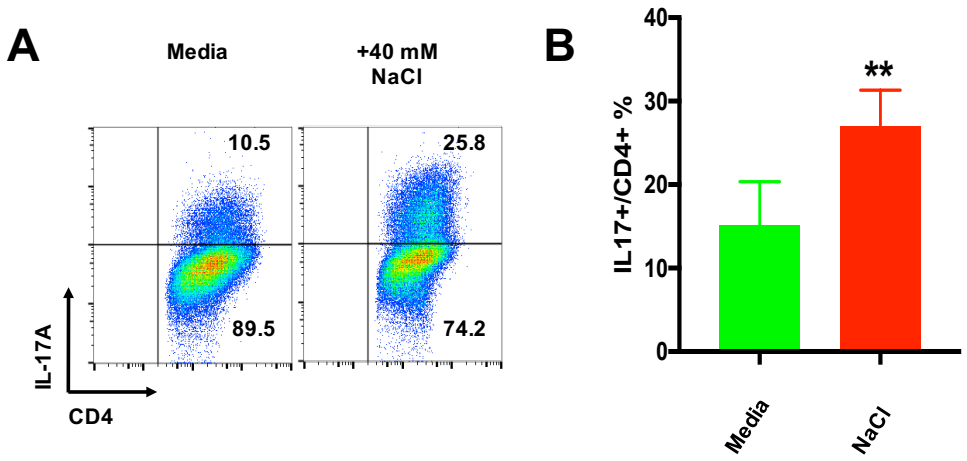

Figure S2. NaCl potentiates Th17 cells differentiation *in vitro*. Related to Figure 1.
(A) Naïve T cells were stimulated with 40 mM NaCl under Th17 differentiating conditions (TGF-β and IL-6) for 3 days, and analyzed by flow cytometry for IL-17A. (B) The bar graph depicts a summary of independent experiments (n=5). Statistical analyses were performed using Student's t test. **P<0.01.

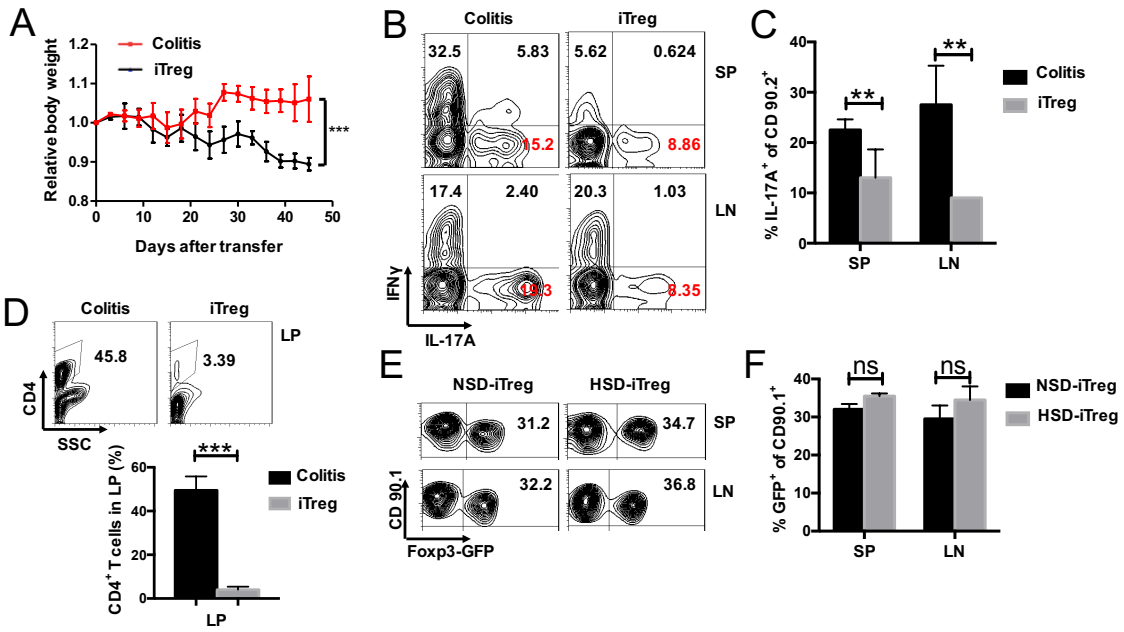

Figure S3. High-salt diet does not affect the suppressive function of iTregs *in vivo*. Related to Figure 3. iTregs were prepared from CD90.1$^+$ Foxp3- GFP knock-in mice. Naïve CD4$^+$ (CD90.2$^+$CD4$^+$CD25$^-$CD62L$^+$CD44$^-$) cells from C57BL/6 mice alone or together with CD90.1$^+$ iTregs were adoptively transferred into Rag1$^{-/-}$ mice i.p.. The mice were killed at 6 weeks after the cell transfer, and analyzed for disease severity (6 mice in each group in one experiment). (A) Body weight of the recipient mice was presented as a percentage of the initial weight. (B and C) CD4$^+$ cells from the spleen (SP) and mesenteric lymph nodes (LN) were examined. Flow cytometric analysis and frequencies of IL-17A$^+$CD90.2 $^+$ and IFNγ$^+$CD90.2$^+$ cells were examined in the respective mouse groups (6 mice in each group in one experiment). (D) The infiltrating cells in large intestines (LP) were analyzed on total CD4$^+$ T cells. (E and F) iTreg- Foxp3 loss were compared in SP and LN, cells were gated on CD90.1$^+$. Representative results (mean ± SEM) from four independent experiments are shown. Statistical analyses were performed using Student's t test. **P<0.01; ***P<0.001; ns, not significant.

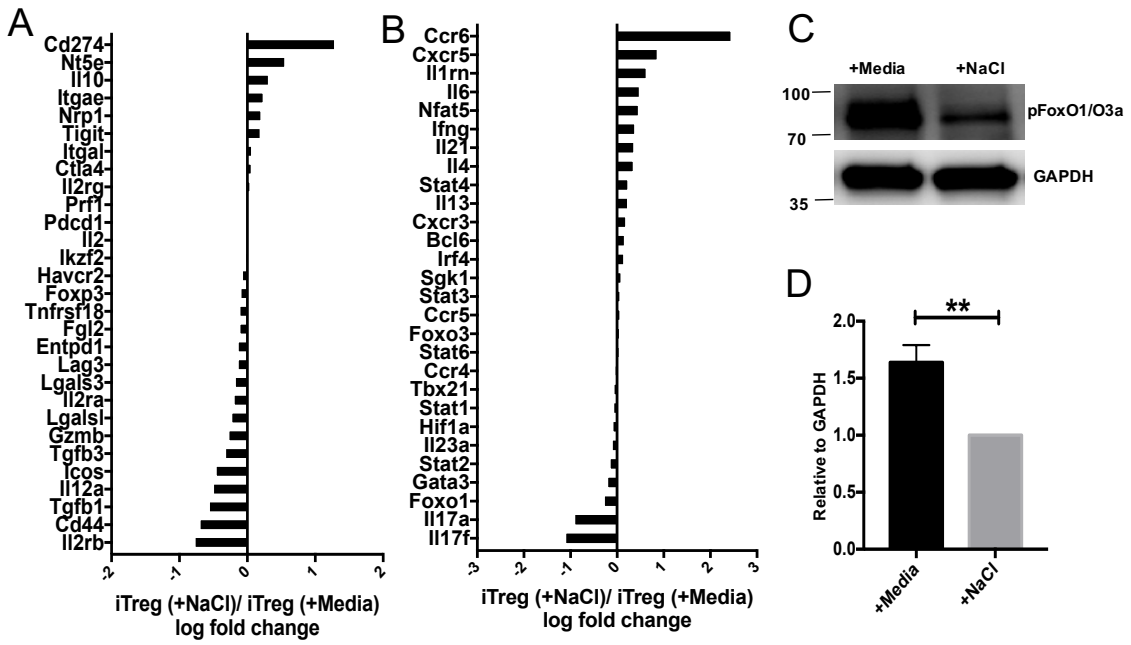

Figure S4. High-salt condition does not significantly change the RNA signatures related to immunoregulation and pro-inflammation features of iTreg subset. Related to Figure 1-4.
(A and B) iTregs were induced normally as in Fig. S1 and re-stimulated for 3 days with anti-CD3/CD28 microbeads (5 cells per bead) and IL-2 (50 U/ml) in the presence (+NaCl) or absence (Media) of an additional 40 Mm NaCl prior to being analyzed via RNAseq. (C and D) Protein level of phosphorylation of FoxO1/FoxO3a as determined by western blot after NaCl treatment. Representative results (mean ± SEM) from three independent experiments are shown. Statistical analyses were performed using Student's t test. **P<0.01.