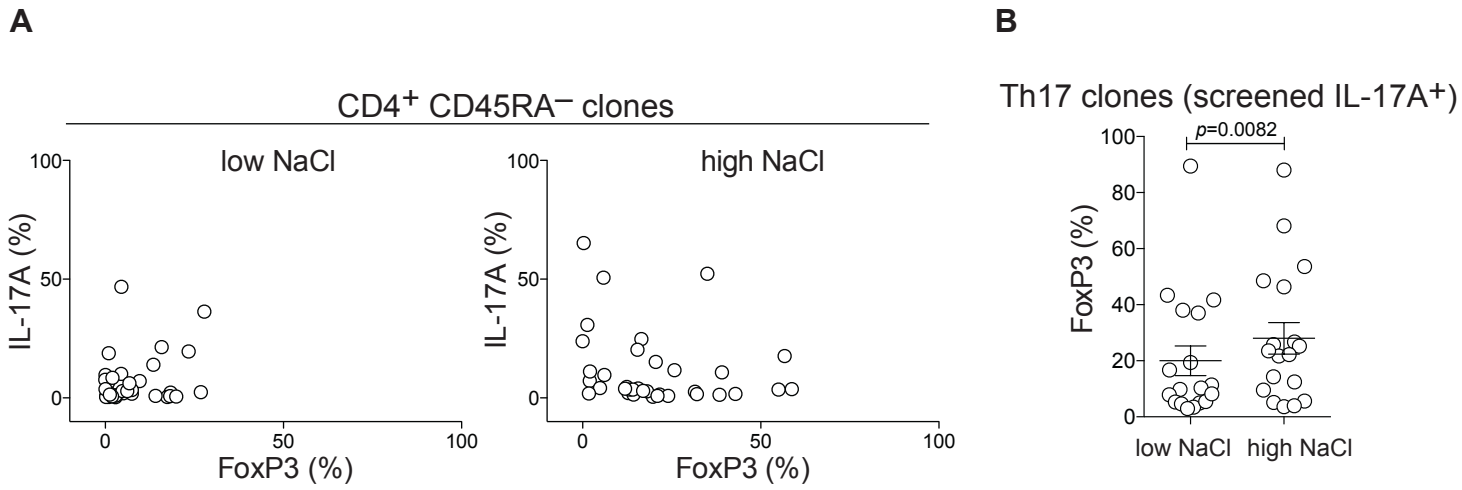

Figure S1. NaCl promotes an anti-inflammatory phenotype in human T cell clones. a, Resting T cell clones were generated from CD45RA$^-$ memory T helper cells and restimulated with CD3 and CD28 mAbs for 5 days (48 hours plate-bound) in low and high NaCl concentrations. Intracellular cytokine staining FACS analysis is shown. **b,** CD4$^+$CXCR3$^-$ CCR4$^+$CCR6$^+$ Th17 cells were sorted and cloned by limiting dilution plating. T cell clones expressing >20% IL-17A were restimulated and analyzed as in (a). Each circle represents an individual T cell clone. Student's two-tailed t test was used for comparisons between two groups.

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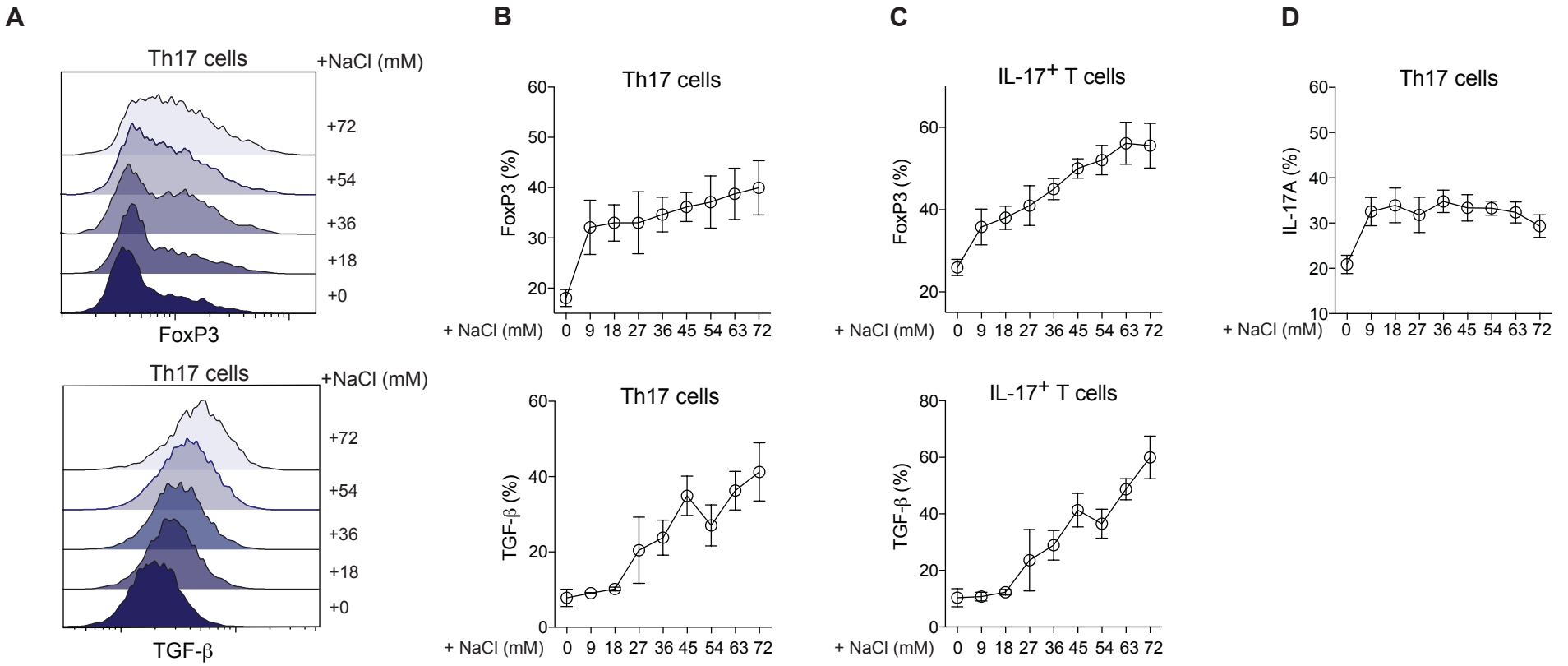

Figure S2. **NaCl exerts its anti-inflammatory effects on Th17 cells in a dose-dependent manner over a wide range of NaCl concentrations, whereas IL-17 upregulation reaches a plateau after only small increments of extracellular NaCl. a-d,** Th17 cells ($CD4^+CXCR3^-CCR4^+CCR6^+$) were sorted from human PBMC *ex vivo* and restimulated for 48 hours with CD3 and CD28 mAbs (48 hours plate-bound) in the presence of titrated concentrations of additional NaCl for a culture period of 5 days. Cytokine and transcription factor expression was determined by intracellular staining and FACS analysis after PMA and ionomycin restimulation for 5 hours. **a,** One representative experiment and **(b-d)** cumulative data for Th17 cells that were sorted according to their differential expression of chemokine receptors and that were gated on IL-17 positive T cells (c) are shown. Data show the mean ± SEM of four independent experiments (for the conditions +0 mM and +67.5 mM NaCl, n= 15).

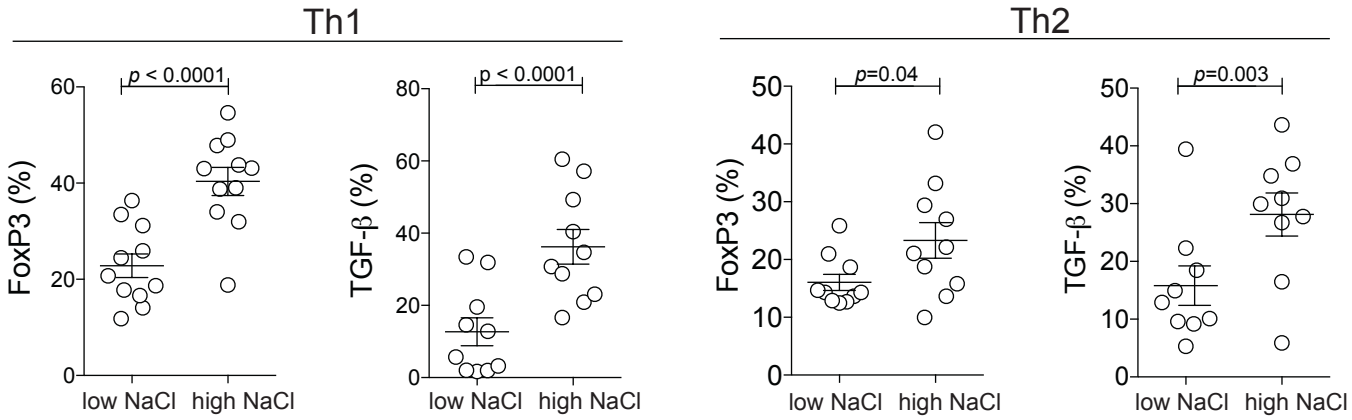

Figure S3. NaCl increases anti-inflammatory T cell effector functions across human T helper cell subsets. Th1 ($CXCR3^+CCR4^-CCR6^-$) and Th2 cells ($CXCR3^-CCR4^+CCR6^-$) were isolated *ex vivo* from human PBMC by flow cytometry and restimulated with CD3 and CD28 mAbs for 5 days (48 hours plate-bound) in low or high NaCl conditions before intracellular staining and FACS analysis after PMA and ionomycin restimulation. Each circle represents an individual blood donor. Student's two-tailed t test was used for comparisons between two groups.

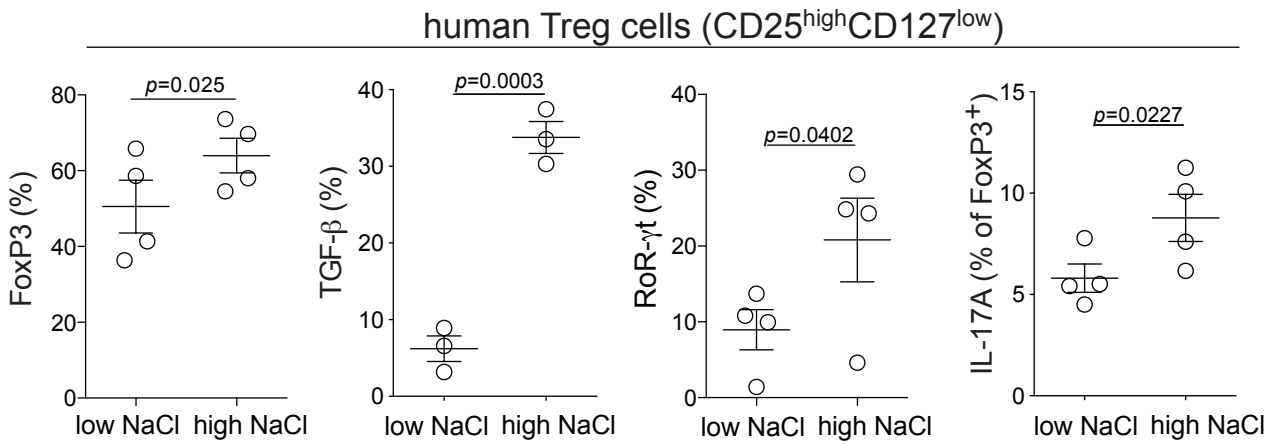

Figure S4. Treg cells acquire Th17 cell properties upon restimulation in high NaCl conditions despite maintaining an anti-inflammatory Treg signature. Human Treg cells were sorted as $CD25^{hi}CD127^{low}$ T cells and stimulated for 5 days with CD3 and CD28 mAbs (48 hours plate-bound) in high or low NaCl conditions before intracellular staining and FACS analysis after PMA and ionomycin restimulation for 5 hours. Each circle represents an individual blood donor. Student's two-tailed t test was used for comparisons between two groups.

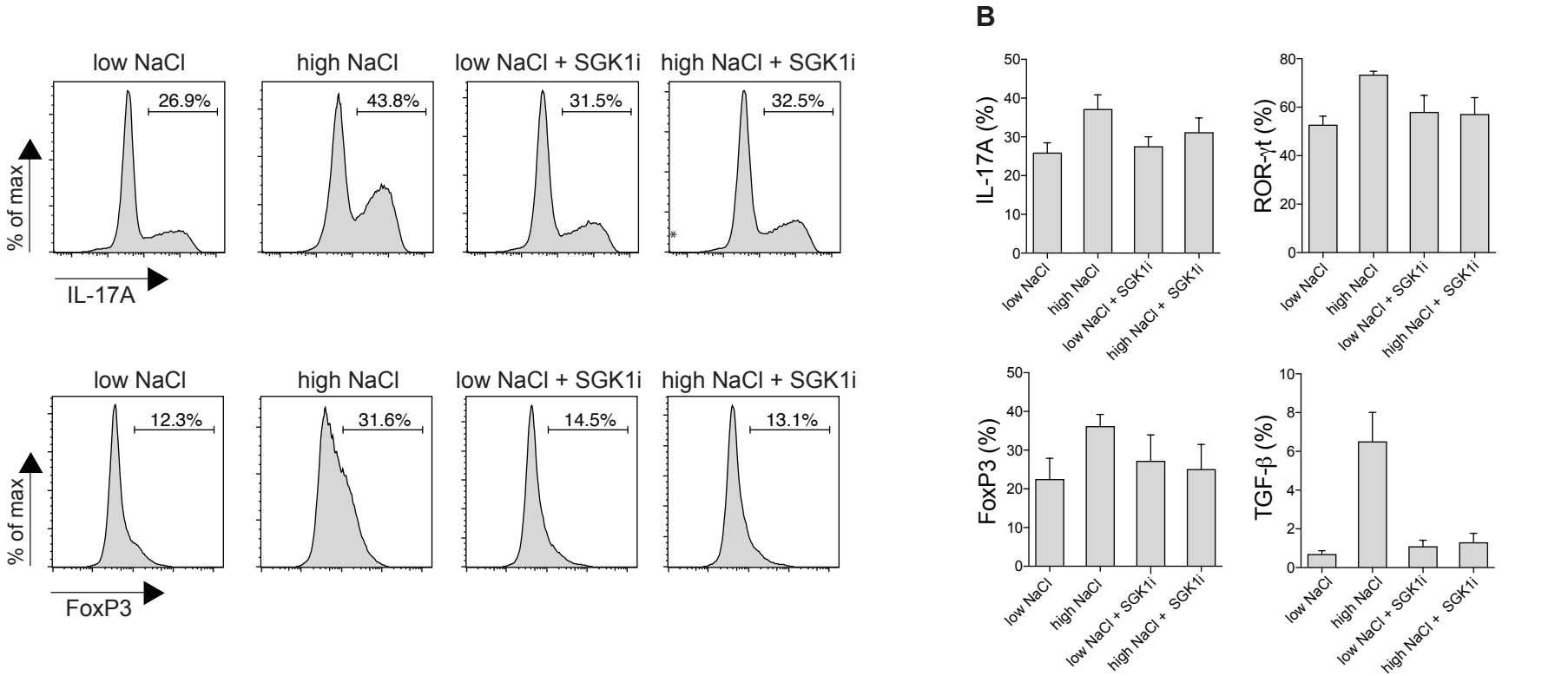

Figure S5. NaCl-mediated upregulation of IL-17 and FoxP3 expression in Th17 cells is abrogated by pharmacological inhibition of SGK1. Human Th17 cells (CXCR3$^-$ CCR4$^+$CCR6$^+$CD45RA$^-$) were isolated *ex vivo* by flow cytometry and stimulated for 48 hours with CD3 and CD28 mAbs in low or high NaCl conditions with or without the presence of the SGK1 inhibitor GSK650394 (SGK1i) at a concentration of $1x10^{-6}$ M. After PMA and ionomycin restimulation on day 5, intracellular staining and FACS analysis were performed. **a,** One representative experiment is shown. **b,** Data show the mean ± SEM of three to four independent blood donors ($p < 0.05$ for IL-17A, FoxP3, TGF-β; n.s. for ROR-γt, one-way ANOVA).

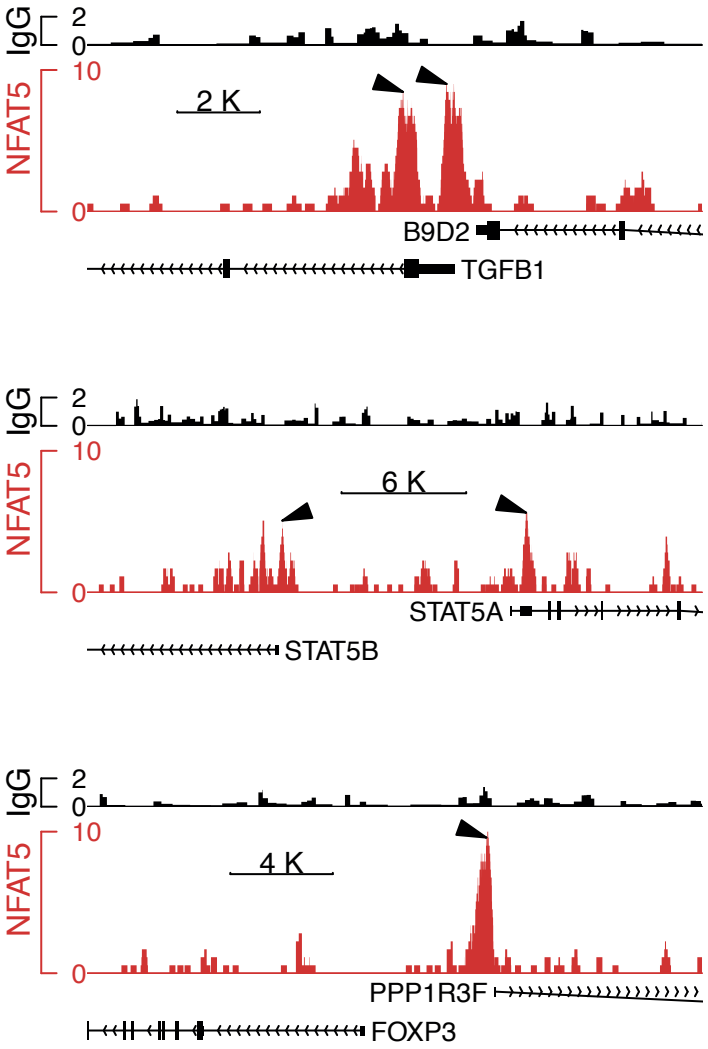

Figure S6. NFAT5 binds to the promoter regions of Treg-associated genes. ChIP-Seq analysis of NFAT5 binding sites in Treg-associated promoter regions. The arrowheads indicate the areas identified after peak calling, which show significant enrichment over the control experiment with IgG. The raw ChIP-Seq data for NFAT5 and IgG in the LoVo colon adenocarcinoma cell line were obtained from previously published work (GSE49402) (*35*). Reads were mapped to the human primary assembly genome hg38 (GRCh38.p12, Gencode release 30) with bowtie excluding multimapped reads and reporting only best alignments (*62*). Peaks were called using Homer v4.10 findPeaks tool in factor mode and the IgG experiment as the input control (*63*). Downstream analyses were performed with the statistical framework R. Genome browser tracks were produced with the help of the Bioconductor package trackViewer v1.18.3 (*64*).

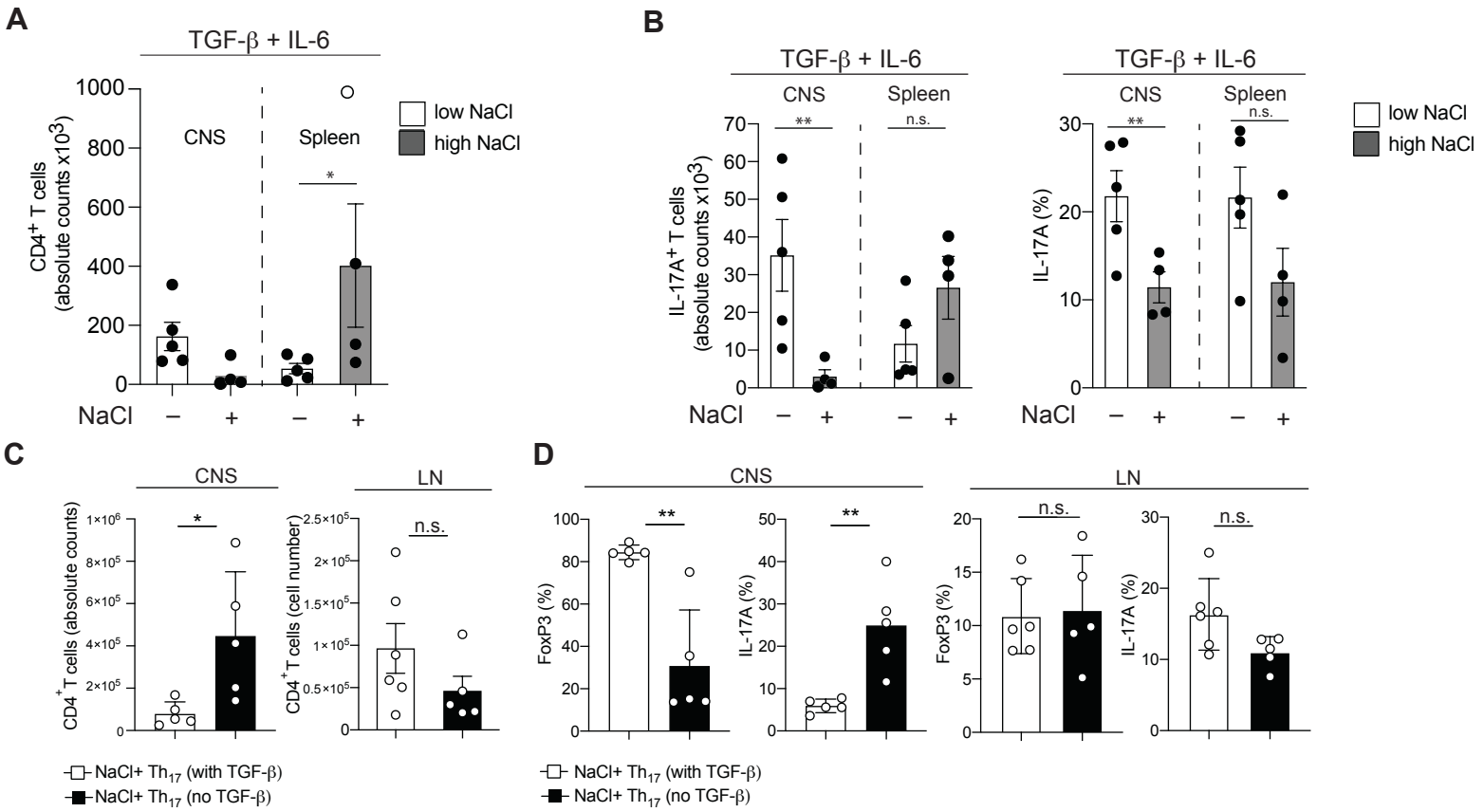

Figure S7. NaCl-induced enhancement or reduction in EAE disease scores correlates with the distribution of T cells in the CNS versus lymphoid organs and with their *ex vivo* phenotype. a, Absolute $CD4^+$ T cell numbers in the CNS and spleen of mice at peak of disease after adoptive T cell transfer (mean ± SEM, n=4-5, in high NaCl group one mouse died of disease before day 29). Two-way ANOVA with Sidak's multiple comparisons test (*$p<0.05$). **b**, The absolute numbers (left) and relative frequencies (right) of $CD4^+$ T cells producing IL-17 were determined by intracellular cytokine staining and flow cytometry at the peak of disease after 5 hours of PMA (50ng/ml) and ionomycin (1µg/ml) restimulation in the presence of monensin (1µg/ml). **c**, *Ex vivo* analysis of absolute numbers of T cells in the CNS and draining lymph nodes (LN) isolated at the peak of disease after adoptive transfer of T cells that were stimulated with high NaCl in TGF-β, IL-6 and IL-1β containing conditions (Th17 with TGF-β) versus with high NaCl in IL-6, IL-1β and IL-23 (Th17 no TGF-β) containing conditions. **d,** Analysis of FoxP3 and IL-17 expression by flow cytometry after PMA and ionomycin restimulation of T cells isolated as in c). Data show mean ± SEM. Student's two-tailed t test (n=5) was used for comparisons between two groups.

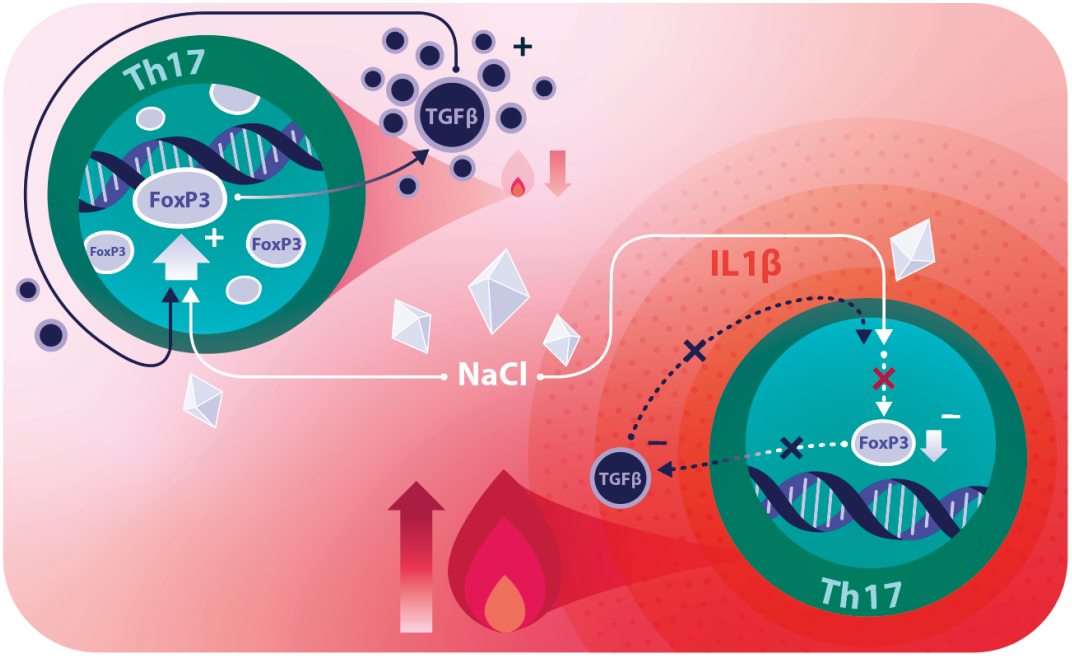

Figure S8. Model –pro- versus anti-inflammatory Th17 cell responses are promoted by NaCl through TGF-β regulation via the IL-1β switch. NaCl amplifies the anti-inflammatory Th17 cell fate in steady state conditions by enhancing the autocrine TGF-β loop, which promotes and stabilizes FoxP3 expression. A pro-inflammatory cytokine microenvironment including IL-1β abrogates the anti-inflammatory effect of NaCl by dominantly inhibiting FoxP3 expression and TGF-β production, thus promoting Th17 cell pathogenicity in settings of infection or inflammation. NaCl can therefore promote both the pro- and anti-inflammatory Th17 cell fate depending on cytokine switch factors in the microenvironment.