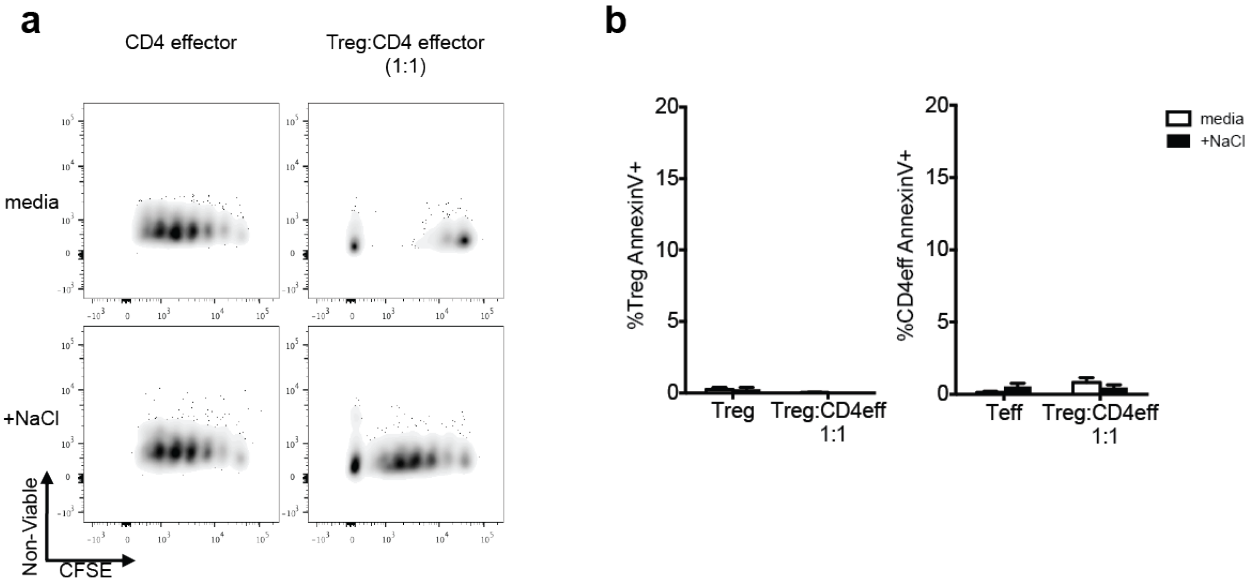
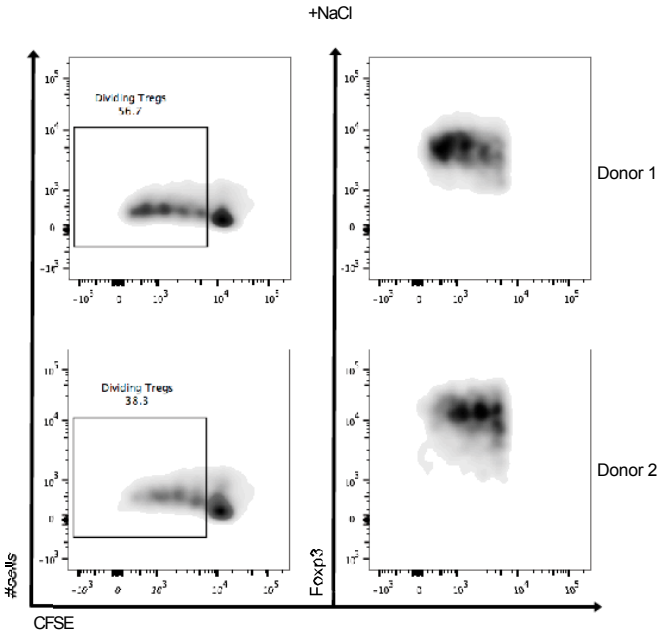
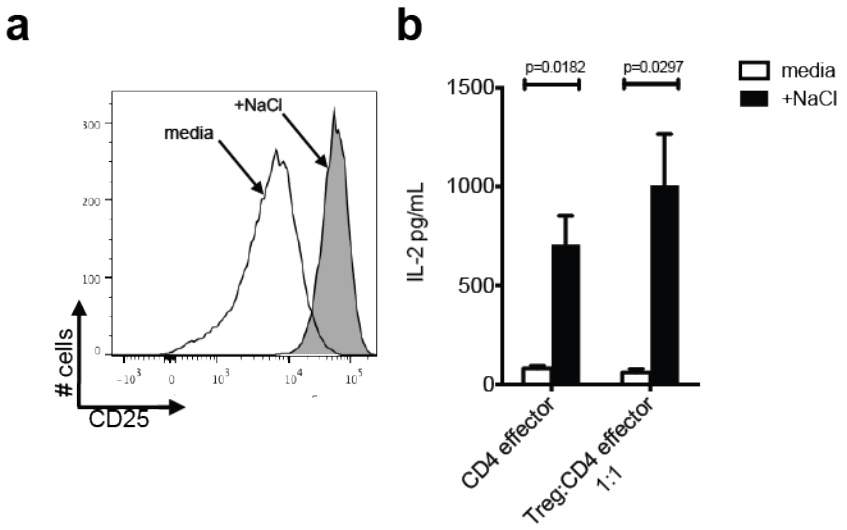
SUPPLEMENT



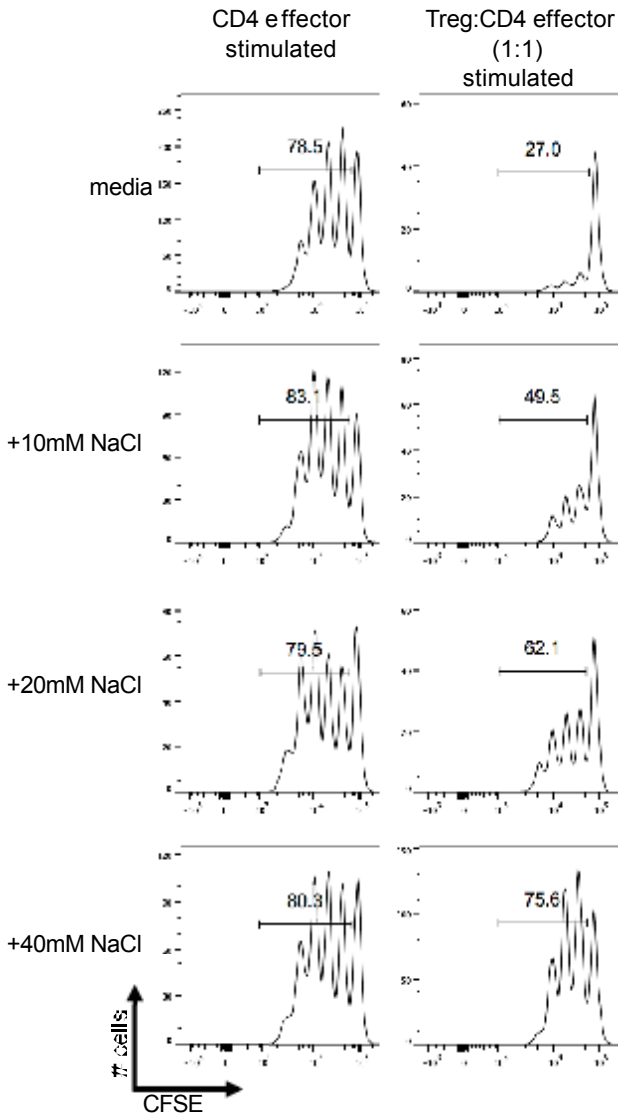
Supplementary Figure 1: $CD4^+$ naïve effector T cells (CD4 effector) were labeled with CFSE, stimulated with α-CD2/CD3/CD28 coated beads (at 2 beads/cell) and cultured alone or co-cultured with $CD4^+CD25^{high}CD127^{low}$ Regulatory T cells (Treg) at ratios as indicated. Cells were cultured either in media (media) or supplemented with an additional 40mM NaCl (+NaCl). CFSE dilution was measured by flow cytometry after 5 days. **(A)** Cells in the co-culture were stained for viability with live-dead dye. **(B)** Tregs alone and Teff alone or within co-culture system were analyzed for AnnexinV+ as a proxy for apoptosis (n=4). Statistical analyses were performed using paired student's t-test.



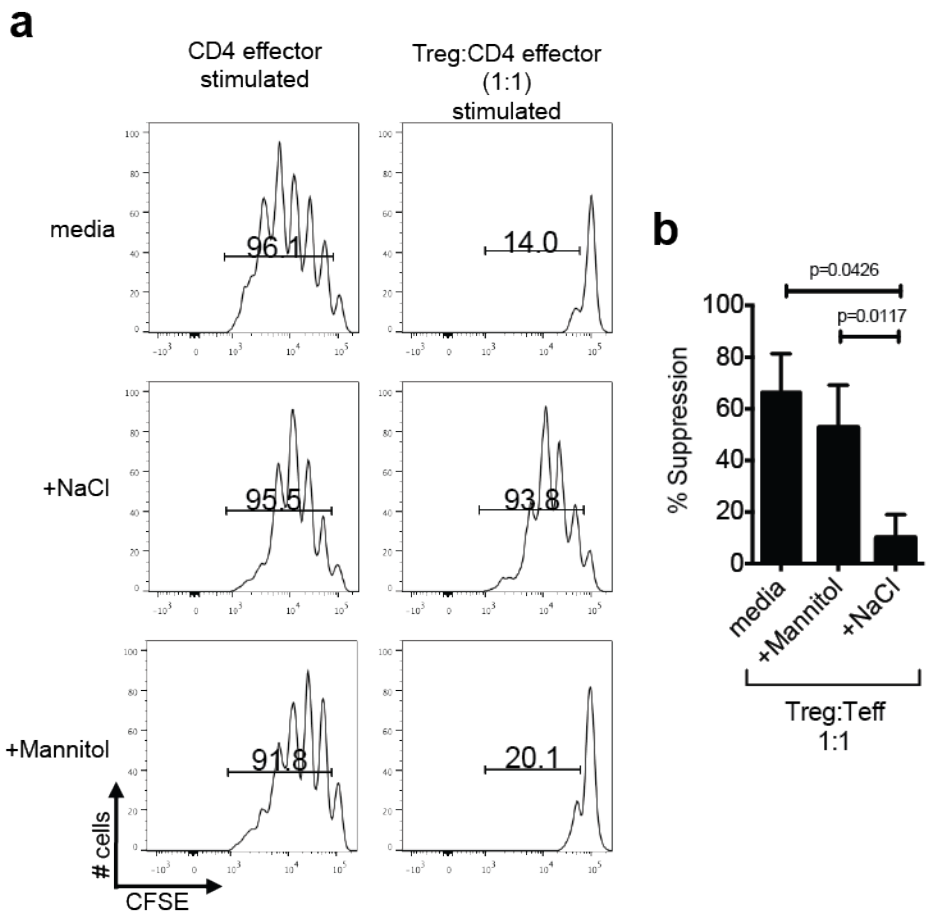
Supplementary Figure 2: Tregs were stained with CFSE and stimulated with α-CD3 (1μg/mL), α-CD28 (1μg/mL), and IL-2 (25 U/mL) and cultured either in normal media (media, data not shown) or media enriched with 40mM NaCl (+NaCl, data shown) for 4 days (n=2). Tregs were then analyzed by FACS and stained for viability, and Foxp3.



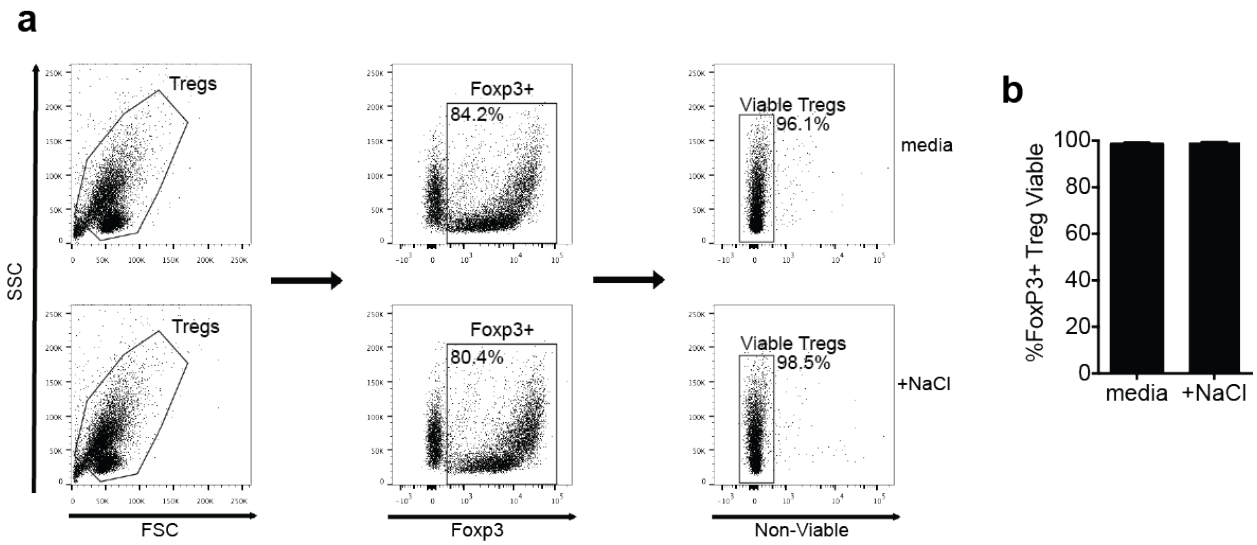
Supplementary Figure 3: $CD4^+$ naïve effector T cells (CD4 effector) were labeled with CFSE, stimulated with α-CD2/CD3/CD28 coated beads (at 2 beads/cell) and cultured alone or co-cultured with $CD4^+CD25^{high}CD127^{low}$ Regulatory T cells (Treg) at ratios as indicated. Cells were cultured either in media (media) or supplemented with an additional 40mM NaCl (+NaCl). CFSE dilution was measured by flow cytometry after 5 days. **(A)** Representative plot of Tregs from co-culture system were stained for CD25 and **(B)** Luminex analysis was performed on co-culture following 5 days (n=4). Statistical analyses were performed using paired student's t-test.



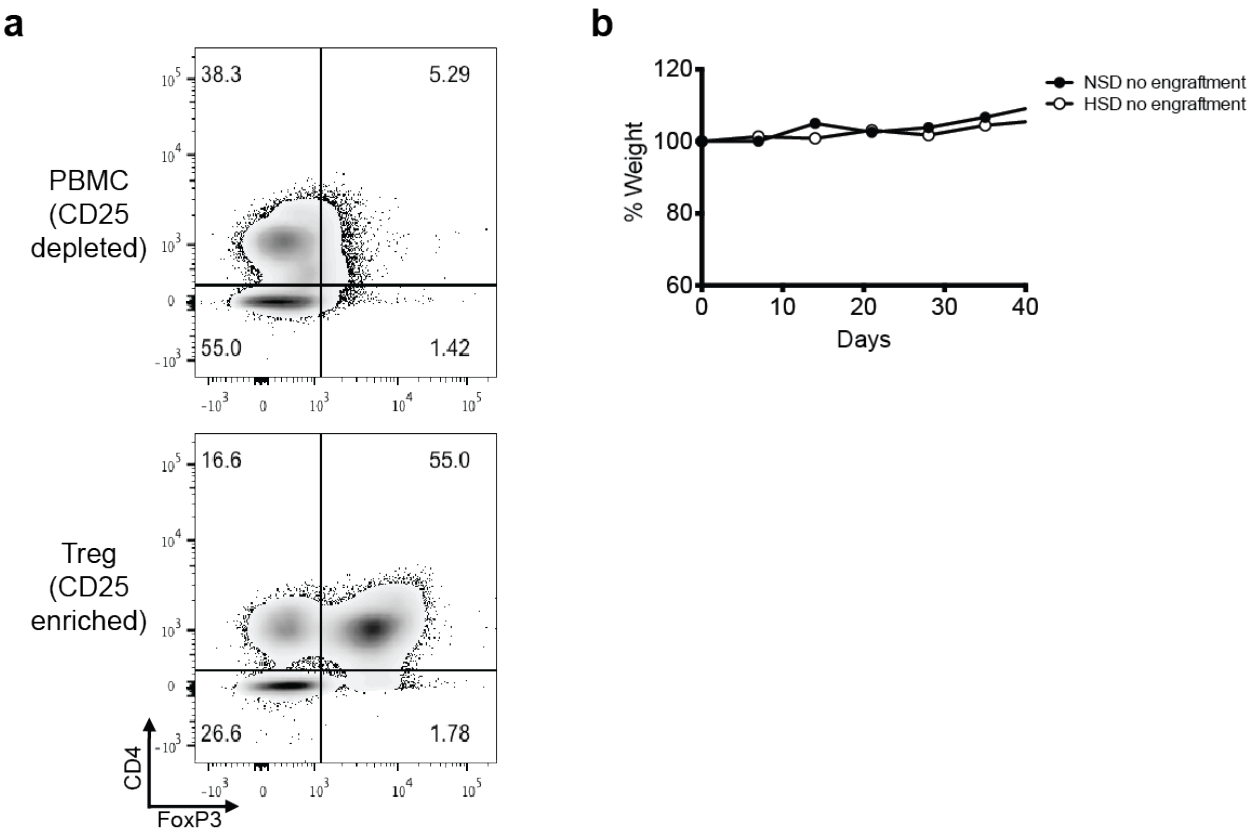
Supplementary Figure 4: CD4 effectors were labeled with CFSE, stimulated with α-CD2/CD3/CD28 coated beads (at 2 beads/cell) and cultured alone or co-cultured with Tregs at ratios as indicated. Cells were cultured either in media or with an additional 10mM NaCl, 20mM NaCl, or 40mM NaCl added at start of culture. CFSE dilution was measured by flow cytometry after 3.5 days. Histograms depict cellular proliferation and are gated on viable cells (n=1).



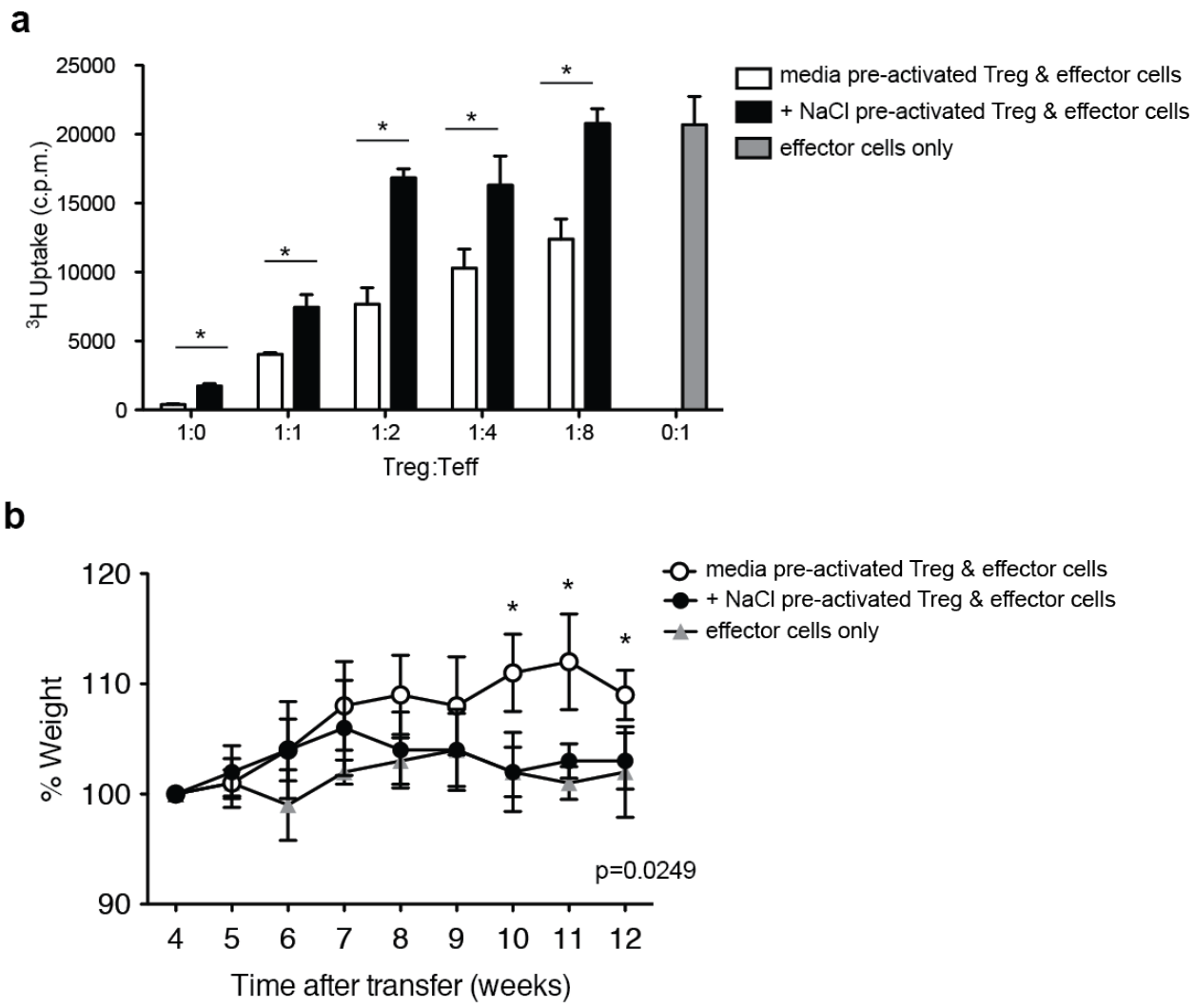
Supplementary Figure 5: $CD4^+$ naïve effector T cells (CD4 effector) were labeled with CFSE, stimulated with α-CD2/CD3/CD28 coated beads (at 2 beads/cell) and cultured alone or co-cultured with $CD4^+CD25^{high}CD127^{low}$ Regulatory T cells (Treg) at ratios as indicated. **(A)** Cells were cultured either in media (media), media supplemented with an additional 40mM NaCl (+NaCl), or media supplemented with an additional 80mM Mannitol (+Mannitol). CFSE dilution was measured by flow cytometry after 5 days. **(B)** The bar graph depicts a summary of experiments (n=3). Statistical analyses were performed using paired student's t-test.



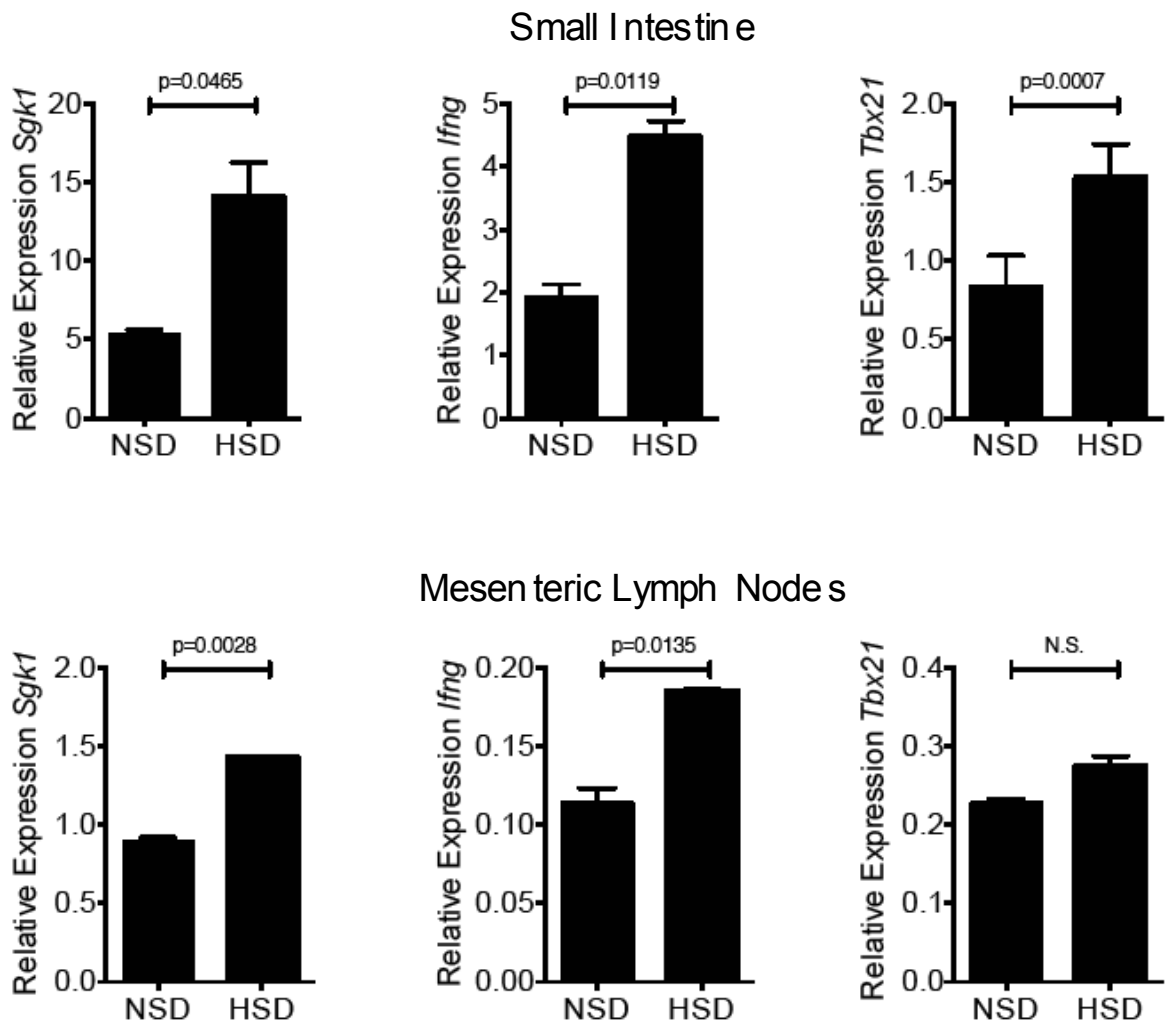
Supplementary Figure 6: Following 72-hour pre-activation protocol, an aliquot of Tregs was analyzed to confirm viability and FoxpP3 expression. **(A)** Representative plot with gating strategy. **(B)** The bar graph depicts a summary of experiments ascertaining viability (n=5). Statistical analyses were performed using paired student's t-test.



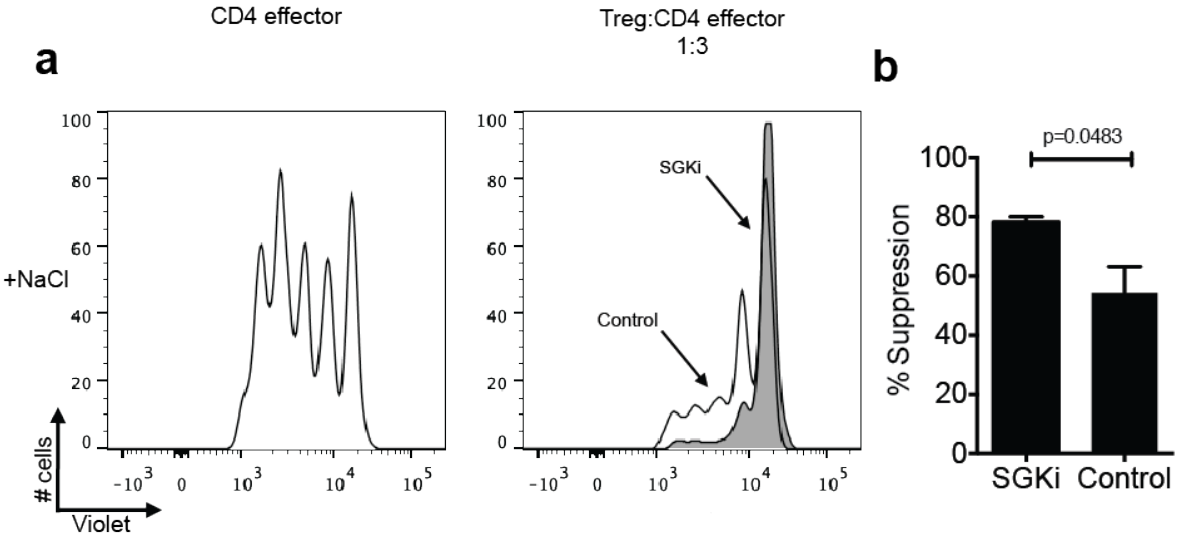
Supplementary Figure 7: 6-10 week old NSG mice received normal chow and tap water ad libitum (normal-salt diet, NSD) or sodium-rich chow containing 8% NaCl and tap water containing 1% NaCl ad libitum (high-salt diet, HSD). Animals were put on high-salt diet 2 weeks prior to engraftment of either $CD25^{-}$ human PBMC's (PBMC) alone or Tregs and PBMCs. Length of time to development of Graft versus Host Disease (GvHD) was observed in animals as proxy for Treg function. **(A)** Representative plots for Foxp3 and CD4 expression in CD25 depleted PBMC and Treg enriched preparations. **(B)** Animals receiving either HSD or NSD were left for duration of experiment without receiving any human lymphocytes (n=3 per diet and condition). Statistical analyses were performed using one-way ANOVA between respective dietary and adoptive transfer groups.



Supplementary Figure 8: Foxp3$^+$ (GFP$^+$) natural Tregs were isolated from *Foxp3*-GFP reporter mice and activated with αCD3, αCD28 with or without an additional 40mM NaCl in the cultural media for three days. Tregs were then cultured with WT naïve CD4$^+$ T cells in a suppression assay for five days. **(A)** Data shown are presented as mean [3H]-thymidine incorporation (n=7). **(B)** Normal or high-salt Tregs similarly pre-activated as in **(A)** were co-transferred into *Rag2*$^{-/-}$ mice with WT CD4$^+$CD25$^-$CD45RB^{hi} (n=7 per condition). Statistical analyses were performed using one-way ANOVA between respective dietary and adoptive transfer groups.



Supplementary Figure 9: Foxp3^{+} (GFP^{+}) natural Tregs were isolated from *Foxp3*-GFP reporter mice following 3 weeks diet of a normal chow and tap water ad libitum (normal-salt diet, NSD) or sodium-rich chow containing 8% NaCl and tap water containing 1% NaCl ad libitum (high-salt diet, HSD). Following 3 weeks of this diet GFP+ cells were isolated from mesenteric lymph nodes or lamina propria of the small intestine and gene expression was ascertained via qRT-PCR. The bar graphs represent a summary of independent experiments (n=3). Statistical analyses were performed using students paired t-test. N.S. = not significant



Supplementary Figure 10: (A) Tregs were activated with plate-bound αCD3 and soluble αCD28 at 1µg/mL in media enriched with 40mM NaCl and IL-2 (25 U/mL) for 72 hours. At start of culture NaCl media was supplemented either with a pharmacologic inhibitor for SGK1 (SGKi, grey) at 1uM (Tocris, GSK650394) or a DMSO control (control, white). Following incubation Tregs were washed, counted, and plated in a suppression assay in normal media with freshly sorted allogeneic CD4 effector cells stained with CFSE. CFSE dilution was measured via flow cytometry after 5 days. **(B)** The bar graph depicts a summary of experiments (n=3). Statistical analyses were performed using paired student's t-test.