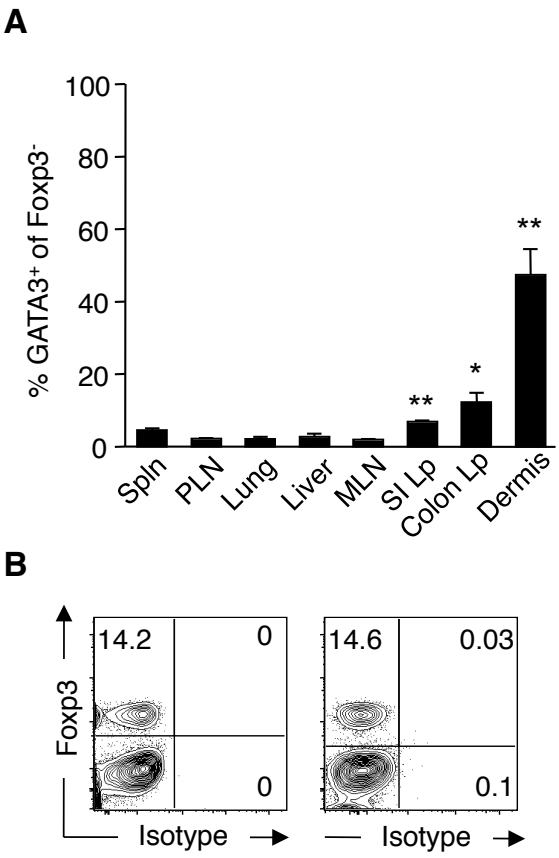

Figure S1. Expression of GATA3 by conventional T cells. (**A**) Cells were harvested from spleen (spln), peripheral lymph node (PLN), lung, liver, mesenteric lymph node (MLN), small intestinal lamina propria (SILp), colon Lp and skin and stained for the expression of CD4, TCRβ, GATA3 and Foxp3 and assessed by flow cytometry. Histogram shows GATA3 expression on live $CD4^+TCR\beta^+Foxp3^-$ cells. (n = 3) (* $p<0.05$, ** $p<0.008$). **(B)** Isotype control for GATA3 (*left panel*) and T-bet (*right panel*) in SILp $CD4^+TCR\beta^+$ cells. Data shown are representative of 3 independent experiments with similar results.

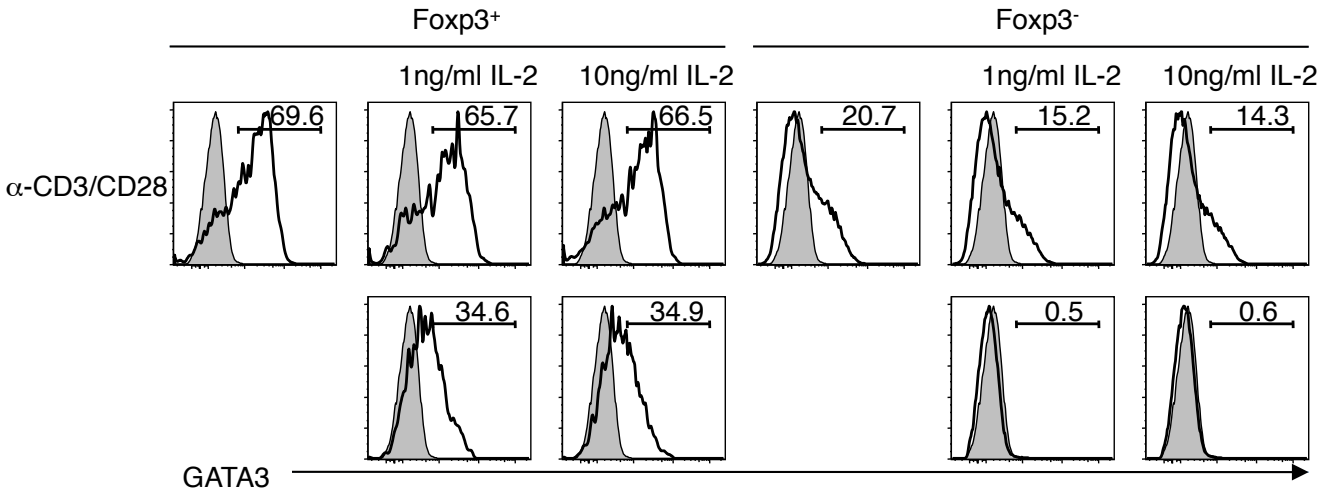

Figure S2. IL-2 signaling alone can upregulate GATA3 in T_{reg} cells. $CD4^+$ T cells were MACs sorted and cultured (*top panels*) in the presence of anti-CD3/CD28 (2μg/ml) alone or with various doses of rIL-2 alone for 24-hrs. Bottom histograms had no anti-CD3/CD28 added. Cells were harvested and stained for CD4, Foxp3 and GATA3. Histograms are gated on live $CD4^+Foxp3^+$ or $CD4^+Foxp3^-$ cells. Data shown are representative of 2 independent experiments with similar results.

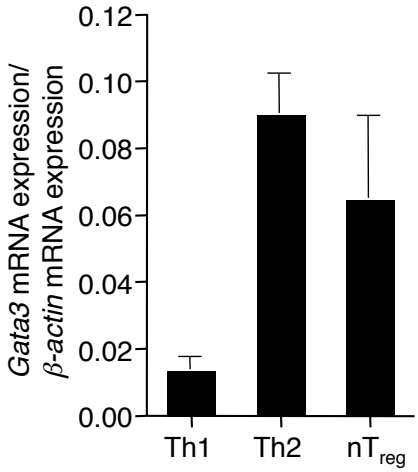

Figure S3. In vitro activated nT_{reg} cells express equivalent levels of *Gata3* compared to Th2 cells. $4x10^4$ $Foxp3^{eGFP+}$ T_{reg} cells (nT_{reg}) or $Foxp3^{eGFP+}CD44^{lo}$ naïve T cells were cultured for 96-hours *in vitro* with $4x10^4$ SpDCs in the presence of anti-CD3 antibody and 1ng/ml of rIL-2 and where indicated 10ng/ml rIL-12 and 10μg/ml of anti-IL-4 or 10ng/ml of rIL-4and 10μg/ml of anti-IFN-γ. Total RNA from Th1, Th2 and nT_{reg} cells were prepared. The expression of indicated genes was measured by quantitative RT-PCR. The relative expression was normalized by the expression of *β-actin*. Error bars represent means ± SD.

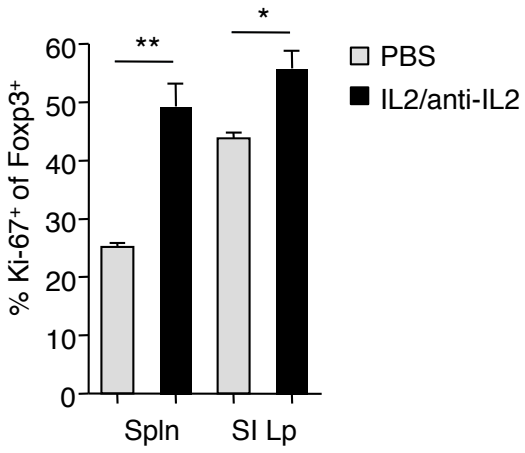

Figure S4. IL2/anti-IL2 complex increases proliferation of T_{reg} cells. Naïve C57BL/6 mice were treated with rIL-2/anti-IL-2 complexes for 6 days. Cells were harvested from the spleen (spln) and small intestinal lamina propria (SILp) and their expression of Ki-67 was evaluated by flow cytometry. Cells were stained for CD4, TCRβ, Foxp3 and Ki-67. Graph depicts the frequency of live $CD4^+TCR\beta^+Foxp3^+$ cells expressing Ki-67 in the spleen and SI Lp. (* $p \leq 0.02$, ** $p \leq 0.004$) (n = 3 mice per group).

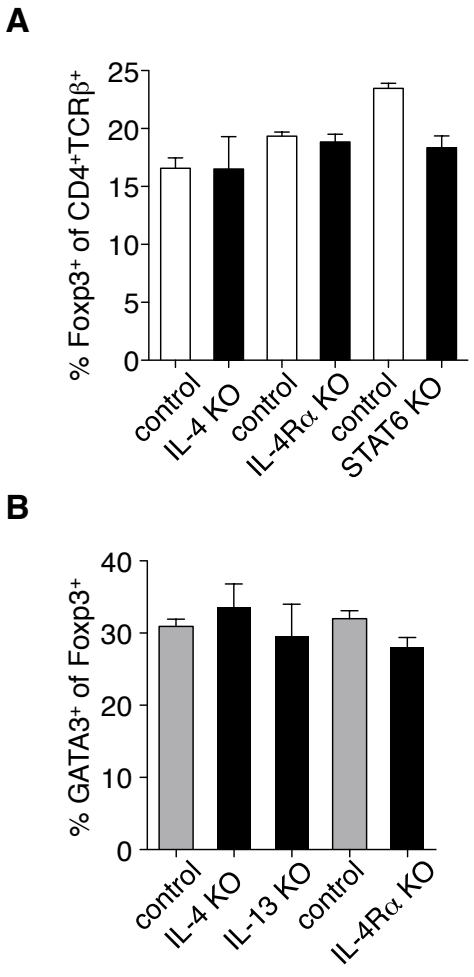

Figure S5. Expression of GATA3 and the frequency of T_{reg} cells is not dependent on Th2 factors in the GI tract. Cells were harvested from control, IL-4 KO, IL-4Rα KO, IL-13 KO and STAT6 KO mice from the small intestinal lamina propria and stained for live CD4, Foxp3, GATA3 and TCRβ. **(A)** Graph depicts the frequency of $CD4^+TCR\beta^+Foxp3^+$ cells found in the SILp. **(B)** Graph depicts the frequency of $GATA3^+$ $Foxp3^+$ cells in the SILp of the above knock out mice. (n = 3 mice per group).

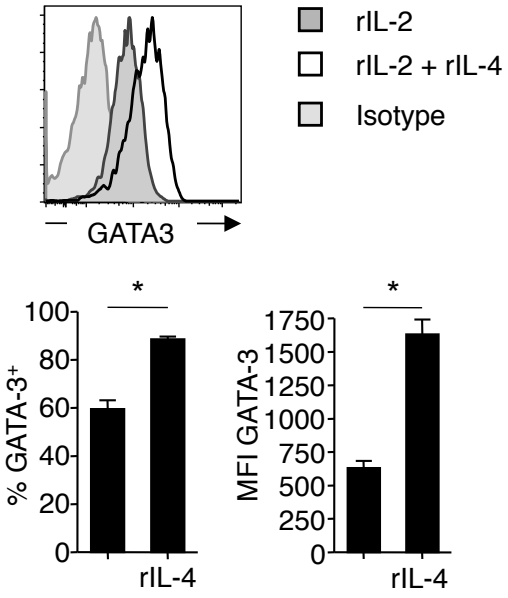

Figure S6. IL-4 enhances expression of GATA3 in T_{reg} cells. $4x10^4$ $Foxp3^{eGFP+}$ T cells were cultured for 96-hrs *in vitro* with $4x10^4$ SpDCs in the presence of anti-CD3 antibody and 1ng/ml of rIL-2 and where indicated 10ng/ml rIL-4. Cells were harvest and stained for expression of GATA3 and Foxp3 and assessed by flow cytometry. Plots are gated on live $CD4^+$ T cells. Graphical representation of data in plot. (* $p<0.02$). Data shown are representative of 3 independent experiments with similar results.

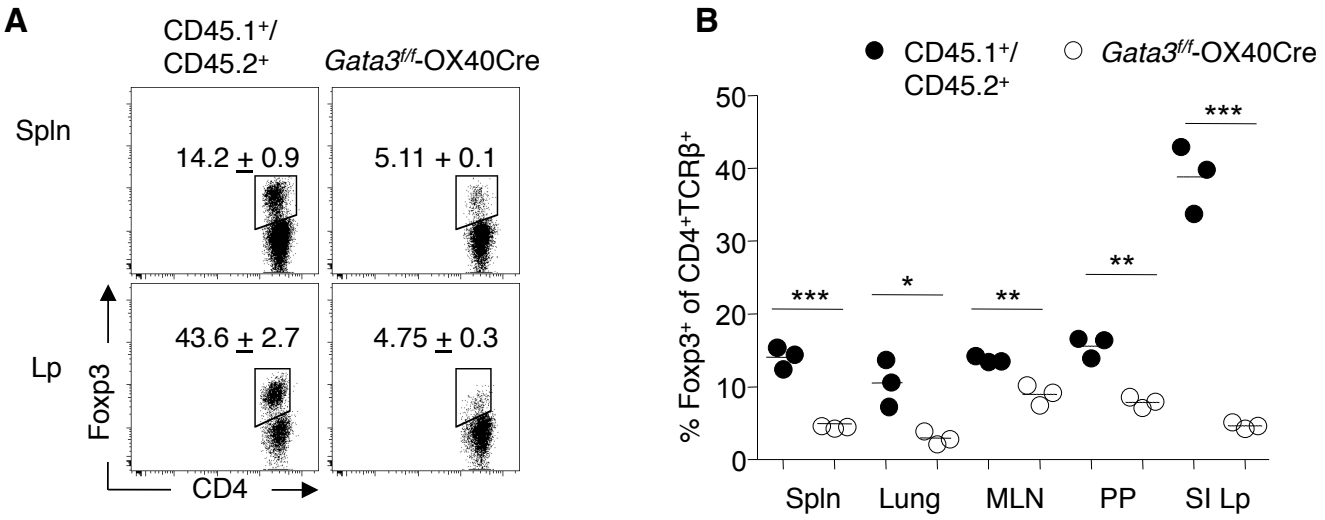

Figure S7. GATA3 confers a fitness advantage to Foxp3$^+$ T$_{reg}$. Congenic CD45.1$^+$ C57Bl/6 mice were lethally irradiated (950 rads) and reconstituted with bone marrow from CD45.1$^+$/CD45.2$^+$ C57Bl/6 mice and CD45.2$^+$ *Gata3$^{f/f}$*-OX40Cre mice. 8 weeks post reconstitution (**A**) Cells were harvested from the spleen, lung, mesenteric lymph node (mln), peyer's patches (pp) and small intestinal lamina propria (SI Lp) and stained for congenic markers CD45.1, CD45.2, CD4, Foxp3 and TCRβ. Histograms are gated on live CD4$^+$TCRβ$^+$ and appropriate congenic markers. (**B**) Graphical representation of percentage of Foxp3$^+$ CD4$^+$TCRβ$^+$ Each circle represents one mouse and crossbars depict the mean of three mice analyzed (* $p<0.05$; ** $p<0.01$; *** $p<0.001$ compared with CD45.1$^+$/CD45.2$^+$ T$_{reg}$). Data shown are representative of two independent experiments with similar results.

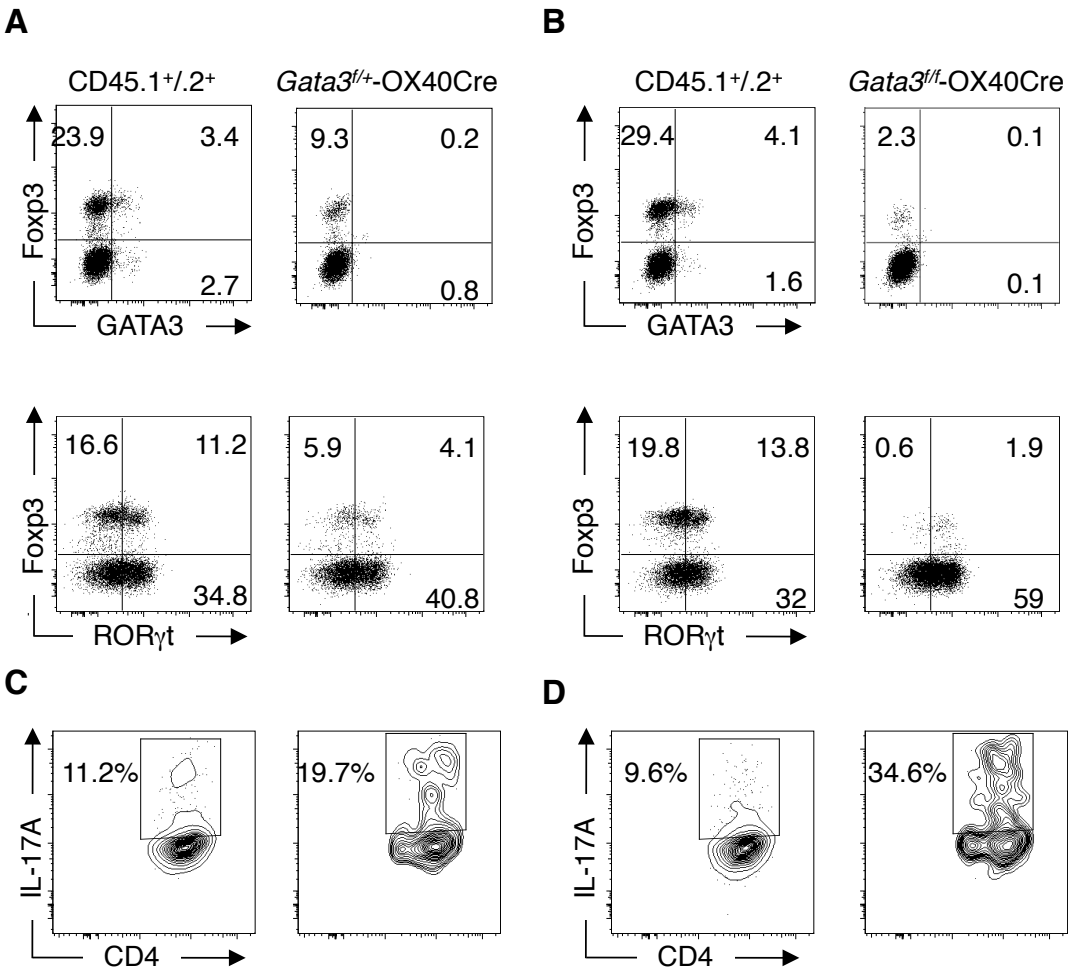

Figure S8. GATA3 restricts RORγt and IL-17A production in Foxp3$^+$ T_{reg} cells. Congenic CD45.1$^+$ C57Bl/6 mice were lethally irradiated (950 rads) and reconstituted with bone marrow from CD45.1$^+$/CD45.2$^+$ C57Bl/6 mice and (**A**) CD45.2$^+$ *Gata3$^{f/+}$*-OX40Cre, or (**B**) CD45.2$^+$ *Gata3$^{f/f}$*-OX40Cre mice. 8 weeks post reconstitution cells were harvested from the small intestinal lamina propria stained for congenic markers CD45.1, CD45.2, CD4, TCRβ, Foxp3, GATA3 and RORγt, or (**C**) SILp cells from CD45.1$^+$CD45.2$^+$ *Gata3$^{f/+}$*-OX40Cre or (**D**) CD45.1$^+$CD45.2$^+$ *Gata3$^{f/f}$*OX40 Cre were restimulated for 4 hrs with PMA/ionomycin and brefeldin A, stained for congenic markers CD45.1, CD45.2, CD4, TCRβ, Foxp3 and IL-17A and assessed by flow cytometry. Plots are gated on live Foxp3$^+$ CD45.1$^+$/CD45.2$^+$ and CD45.2$^+$ for *Gata3$^{f/+}$*-OX40Cre or *Gata3$^{f/f}$*-OX40Cre. Data shown are representative of two independent experiments with similar results.

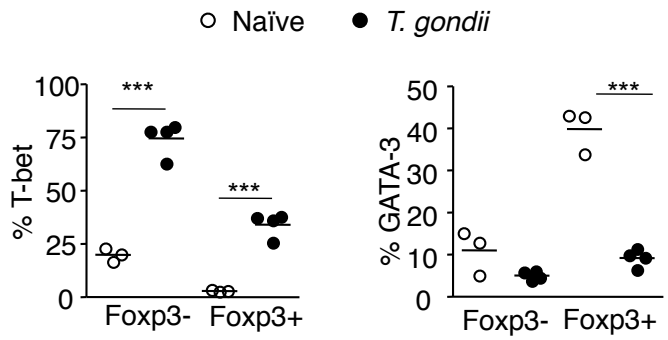

Figure S9. GATA3 expression in SI Lp T_{reg} cells is down regulated during acute *T. gondii* infection. Expression of GATA3 was analyzed in naïve C57BL/6 mice compared to age-matched female mice infected with 40 bradyzoites *T. gondii* clone C1 at 9 days post infection. Cells from the SI Lp were isolated and expression of Foxp3, T-bet and GATA3 was assessed by flow cytometry. Graphs represent populations gated on live $CD4^{+}TCR\beta^{+}$ cells. (***, $p < 0.0003$ compared to naïve). Data shown are representative of at least 3 independent experiments with similar results.

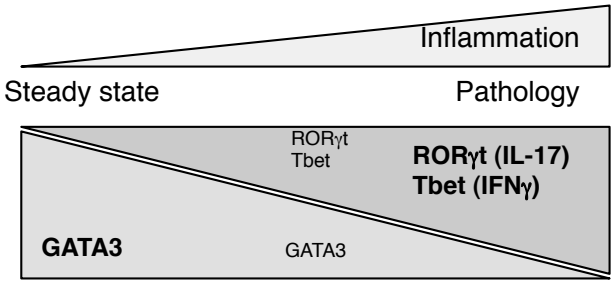

Figure S10. Expression of GATA3 limits excessive T_{reg} cell polarization in an inflammatory environment. GATA3 expression by T_{reg} cells limits the induction of potentially pathogenic effector cytokines induced in inflammatory milieus.

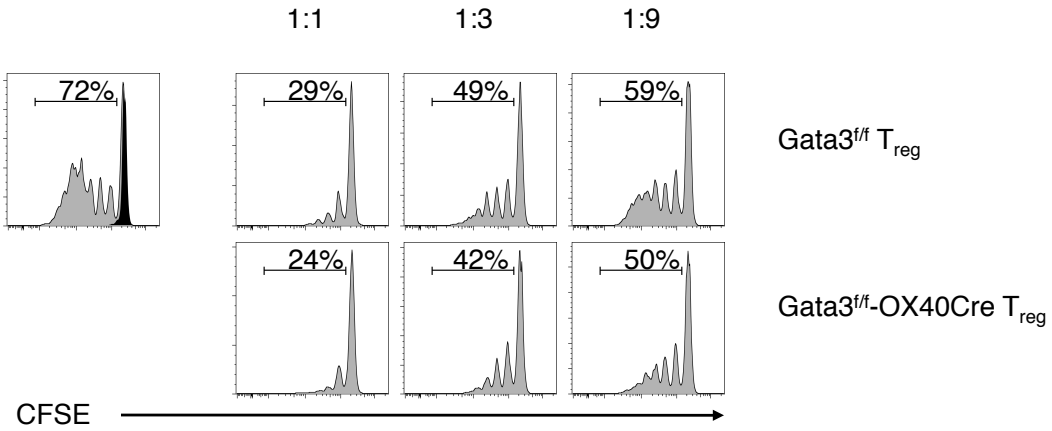

Figure S11. *Gata3$^{f/f}$*-OX40Cre T_{reg} cells have normal suppressive function in vitro. CD45.1^{+} naïve T cells (T_{resp}) were labeled with 1μM of CFSE and co-cultured with T cell depleted splenocytes in the presence of 1μg/ml of anti-CD3 antibody. CD4^{+}CD25^{bright} T_{reg} were sort purified from *Gata3$^{f/f}$* or *Gata3$^{f/f}$*-OX40Cre mice and co-cultured with CFSE labeled T_{resp} at the indicated ratios. Data shown are representative of three independent experiments with similar results.

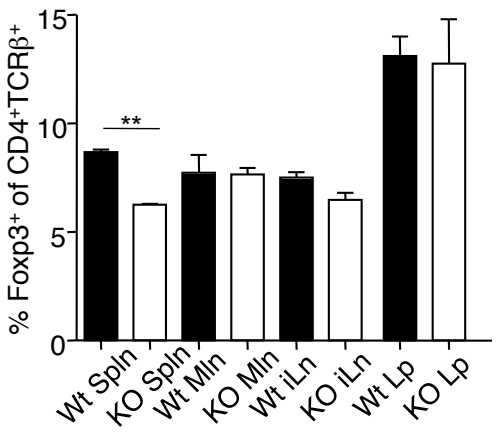

Figure S12 GATA3-deficient T_{reg} cells are at normal frequency prior to induction of EAE. Congenic CD45.1$^+$ C57Bl/6 mice were lethally irradiated (950 rads) and reconstituted with bone marrow from either CD45.2$^+$ *Gata3$^{f/+}$*-Foxp3Cre mice or *Gata3$^{f/f}$*-Foxp3Cre. 10 weeks post reconstitution cells were harvested from the spleen, mesenteric or inguinal lymph nodes and the small intestinal lamina propria (SI Lp) and stained for congenic markers CD45.2, CD4, CD25, Foxp3 and TCRβ. Graph depicts the frequency of Foxp3 of live CD4$^+$TCRβ$^+$. (n = 2 mice per group.)

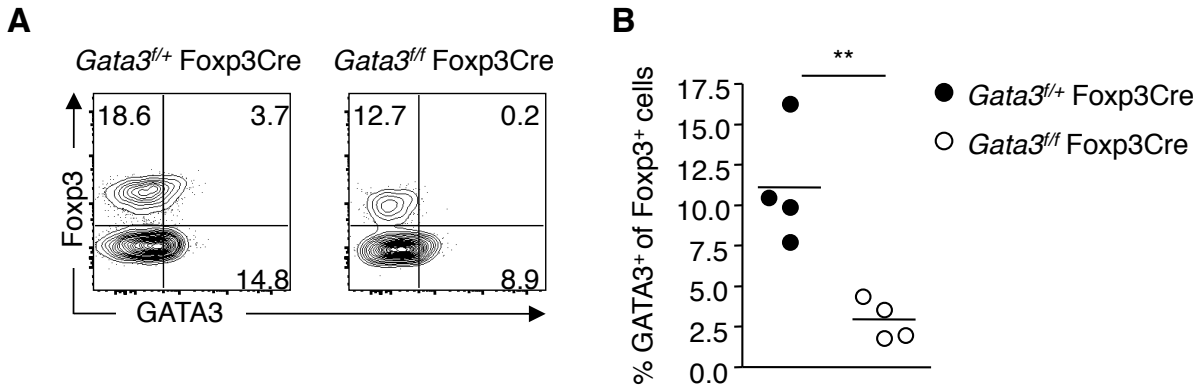

Figure S13 Expression of GATA3 in T_{reg} cells during EAE. Congenic CD45.1$^+$ C57Bl/6 mice were lethally irradiated (950 rads) and reconstituted with bone marrow from either CD45.2$^+$ *Gata3$^{f/+}$*-Foxp3Cre mice or *Gata3$^{f/f}$*-Foxp3Cre. 9 weeks post reconstitution mice were immunized for EAE as described in materials and methods. **(A)** Cells were harvested from the CNS and stained for congenic markers CD45.2, CD4, GATA3, Foxp3 and TCRβ. Plots are gated on CD4$^+$TCRβ$^+$ live cells. Numbers in quadrants refers to frequency of each subset. **(B)** Graph depicts the frequency of GATA3$^+$ T_{reg} cells of live Foxp3$^+$ CD4$^+$TCRβ$^+$. (n=4 mice per group, $p<0.005$).