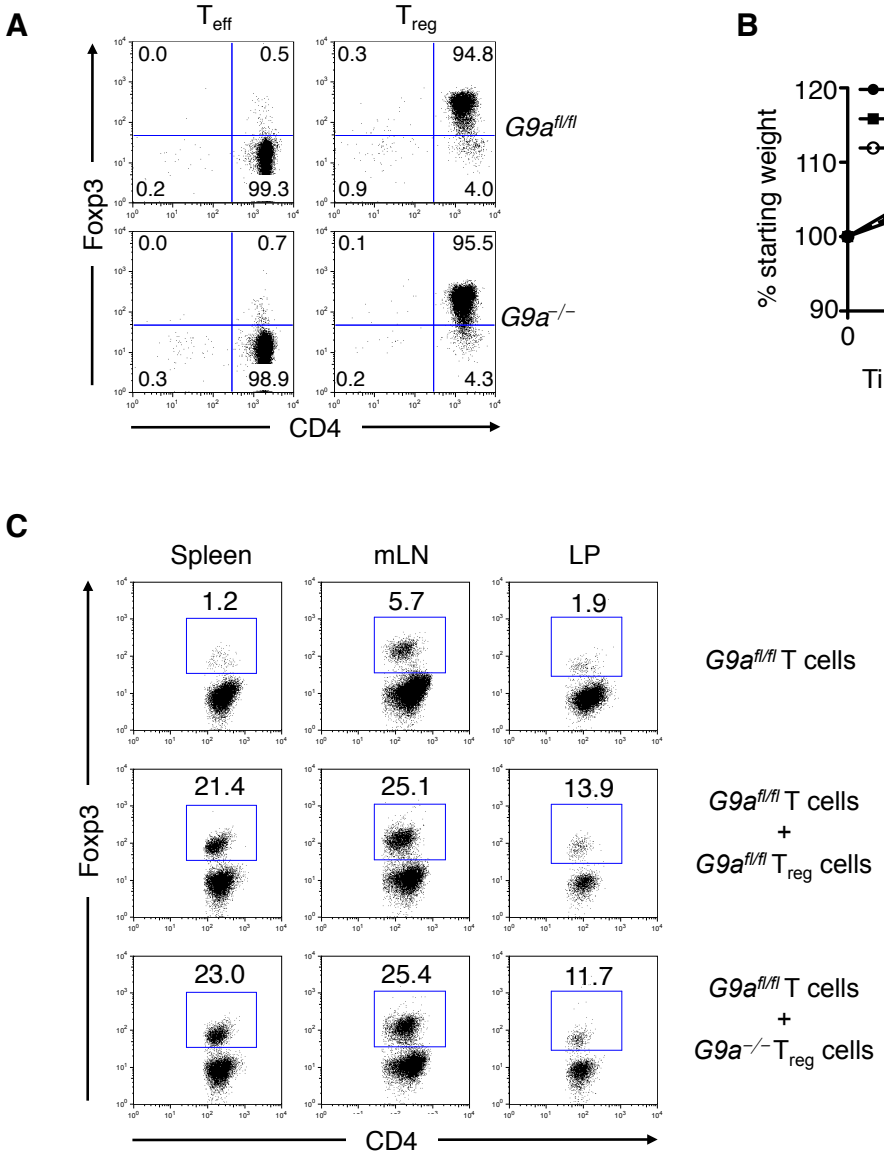
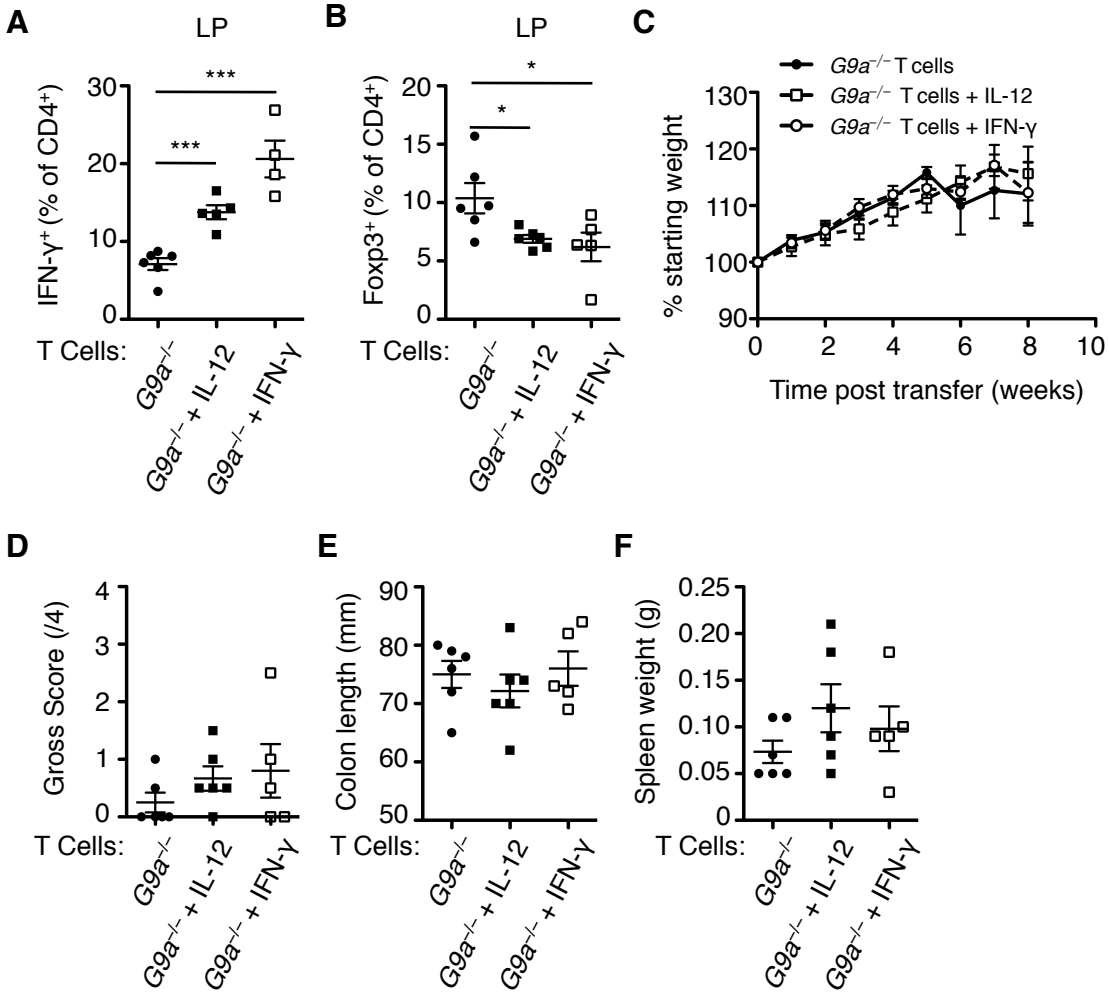
Supplemental Material

G9a regulates CD4 T cell differentiation during intestinal inflammation in mice
Antignano et al.



Supplemental Figure 1. Comparable expansion of *G9a*$^{fl/fl}$ and *G9a*$^{-/-}$ T_{reg} cells after transfer with *G9a*$^{fl/fl}$ CD4$^+$CD25$^-$CD45RB^{high} T cells into *Rag1*$^{-/-}$ mice. (**A**) Representative CD4 and Foxp3 staining of donor cells after FACS sorting. (**B**) CD4$^+$CD25$^-$CD45RB^{high} naïve *G9a*$^{fl/fl}$ T cells (4 x 10^5) were transferred into *Rag1*$^{-/-}$ alone or in the presence of *G9a*$^{fl/fl}$ or *G9a*$^{-/-}$ T_{regs} (2 x 10^5) and the mice were monitored for weight loss (percentage of initial weight) over 7 weeks. (**C**) Representative CD4 and Foxp3 staining of spleen, mLN and LP cells 7 weeks after transfer. Data shown is representative of three independent experiments (n=3 per experiment). Numbers represent frequency of CD4$^+$Foxp3$^+$ cells. Error bars indicate SEM.



Supplemental Figure 2. Treatment with either IFN-γ or IL-12 does not result in weight loss from transferred *G9a*$^{-/-}$ T cells. CD4^{+}CD25^{-}CD45RB^{high} naïve T cells (4 x 10^5) from *G9a*$^{-/-}$ mice were transferred into *Rag1*$^{-/-}$ mice and treated i.p. with 250 ng IFN-γ or IL-12 3x per week for the first 4 weeks. Mice were analyzed at 8 weeks post induction for (**A**) intracellular IFN-γ produced by LP CD4^{+} cells, (**B**) Foxp3-expression of LP CD4^{+} cells, (**C**) weight loss, (**D**) gross colon pathology, (**E**) colon length and (**F**) spleen weight. Data is from a single experiment with n=5-6 mice per group. *, $P < 0.05$; ***, $P < 0.001$. Errors bars indicate SEM.

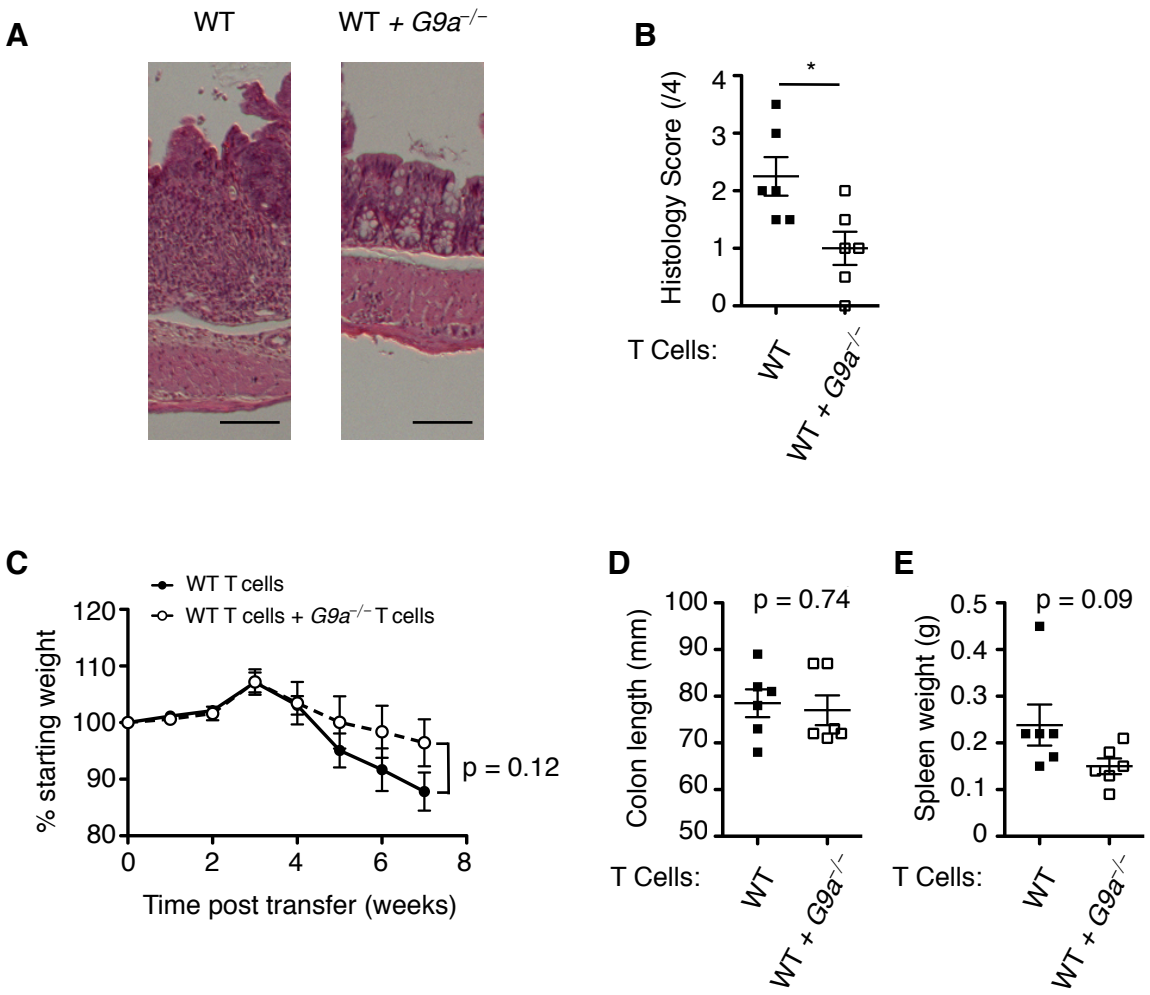
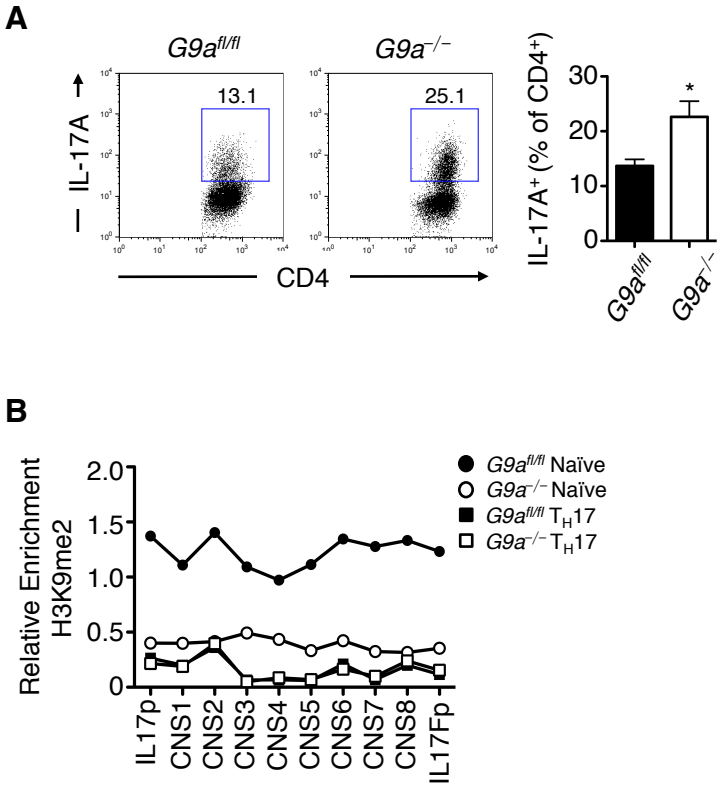
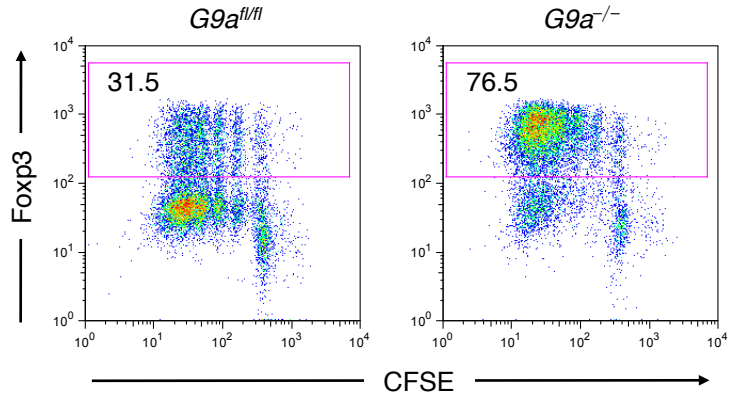


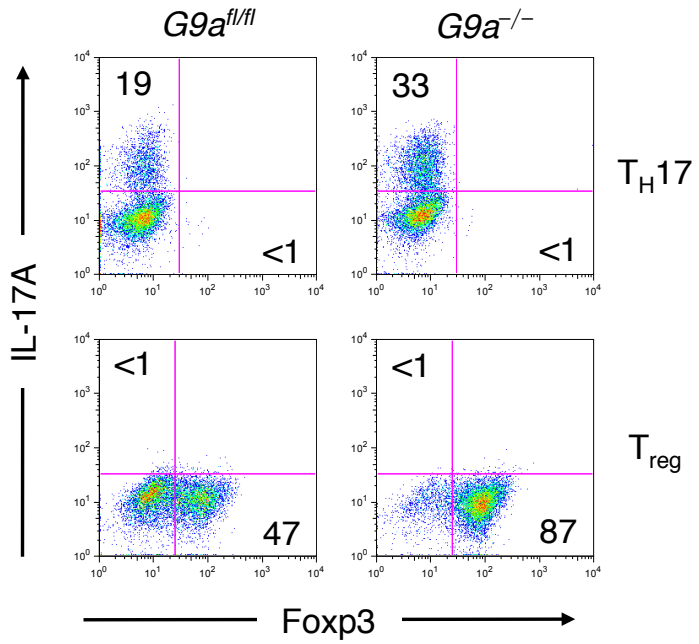
Figure 3. Co-transfer of *G9a*$^{-/-}$ and WT T cells into *Rag1*$^{-/-}$ recipients results in reduced colon damage but similar weight loss as WT T cells alone. *Rag1*$^{-/-}$ mice were reconstituted with either WT T cells alone or in combination with *G9a*$^{-/-}$ T cells at a 1:1 ratio. (**A)** Representative sections of H&E stained proximal colons. Bar, 50 μm. (**B**) Colitis was assessed histologically at 7 weeks post transfer and severity of inflammation was scored. (**C**) Weight loss was monitored for 7 weeks Mice were sacrificed at 7 weeks and (**D**) Colon length, and (**E**) spleen weight determined. Weight loss is from 2 independent experiments; otherwise data shown is one representative experiment of three (n=5-6 mice per experiment). Error bars indicate SEM. *, $P < 0.05$; *P* value is shown for non statistically significant results.



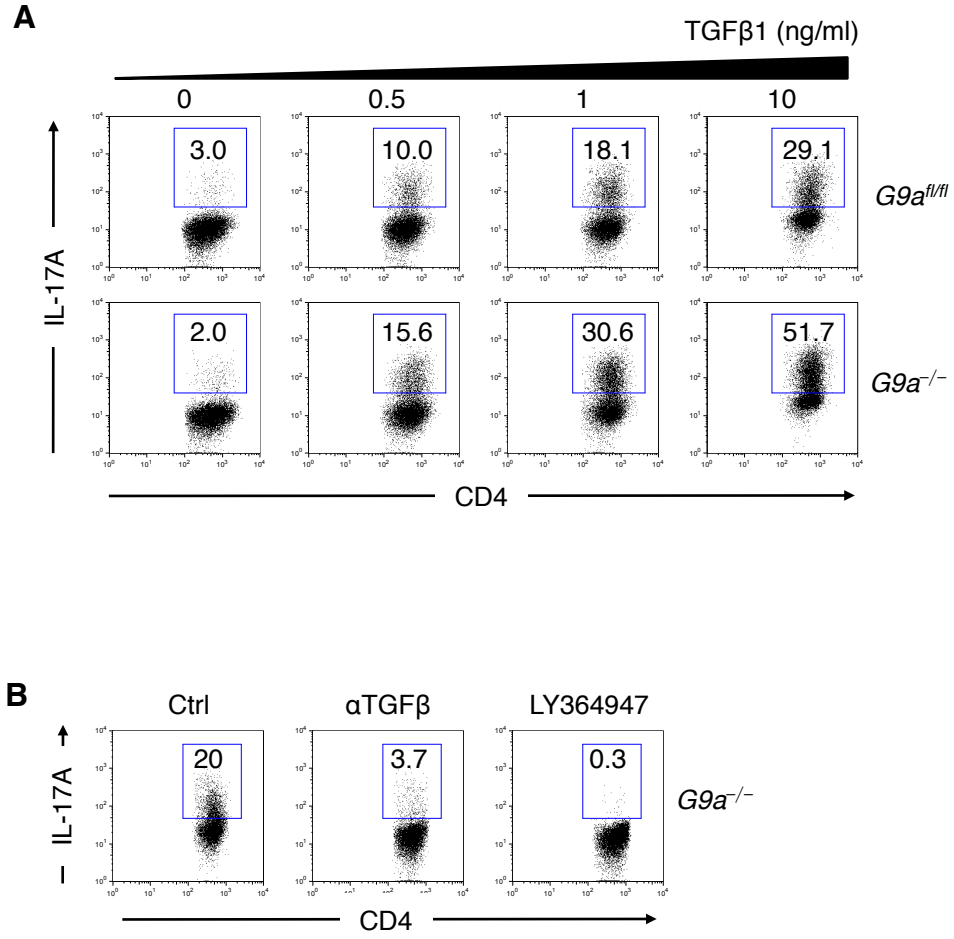
Supplemental Figure 4. G9a-dependent H3K9me2 is required to limit T_H17 cell differentiation in vitro. (**A**) Naïve $CD4^+$ T cells from *G9a*$^{fl/fl}$ or *G9a*$^{-/-}$ mice were differentiated for 6 days under T_H17 cell-promoting conditions and analyzed for IL-17A production. Representative flow cytometry data and quantification of IL-17A$^+$ CD4$^+$ cells (n=7) is shown. **(B)** *G9a*$^{fl/fl}$ or *G9a*$^{-/-}$ naïve $CD4^+$ cells were differentiated to T_H17 cells and analyzed by ChIP for H3K9me2 levels at the *Il17a/f* locus. Data shown is the mean from 3 independent experiments performed in duplicate. Numbers represent frequency of CD4$^+$IL-17A$^+$ cells. *, $P < 0.05$. Errors bars indicate SEM.



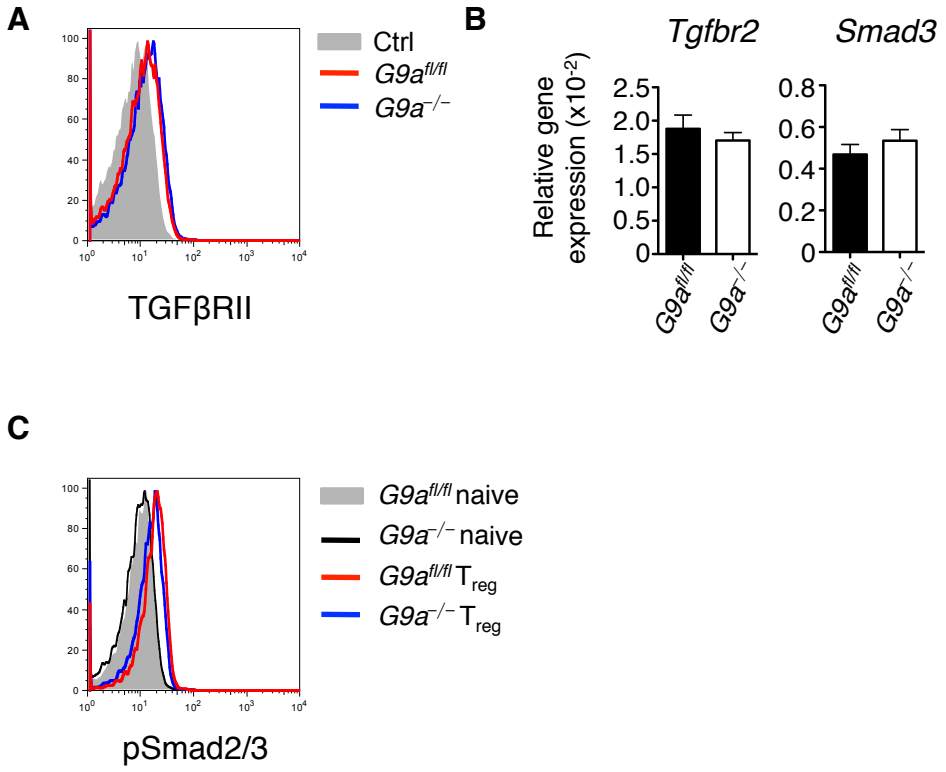
Supplemental Figure 5. G9a deficiency does not affect proliferation during T_{reg} cell differentiation. Naïve $CD4^+CD25^-$ T cells from *G9a*$^{fl/fl}$ or *G9a*$^{-/-}$ mice were stained with CFSE and differentiated for 4 days under T_{reg} cell promoting conditions and analyzed for Foxp3 expression and CFSE dilution. Data shown is representative of 3 independent experiments. Numbers represent frequency of $CD4^+Foxp3^+$ cells.



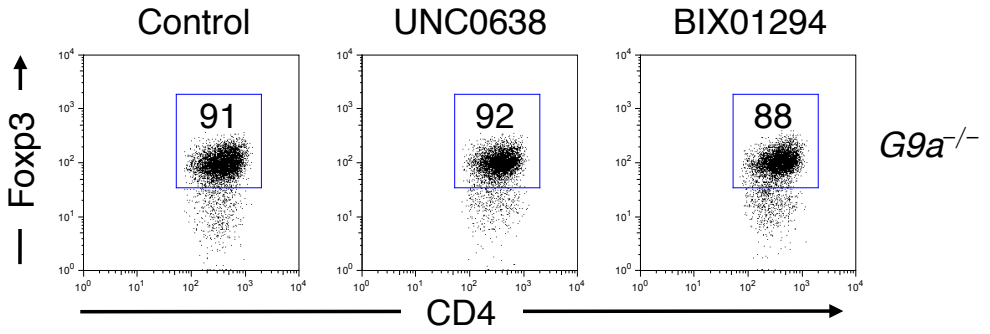
Supplemental Figure 6. G9a is not required for repression of lineage-promiscuous IL-17A or Foxp3 expression. *G9a*$^{fl/fl}$ and *G9a*$^{-/-}$ T_H cells were polarized to T_H17 or T_{reg} cells for 6 days and the cells were analyzed by flow cytometry for the expression of IL-17A and Foxp3. Data shown is representative of more than 3 independent experiments. Numbers represent frequency of cells in each quadrant.



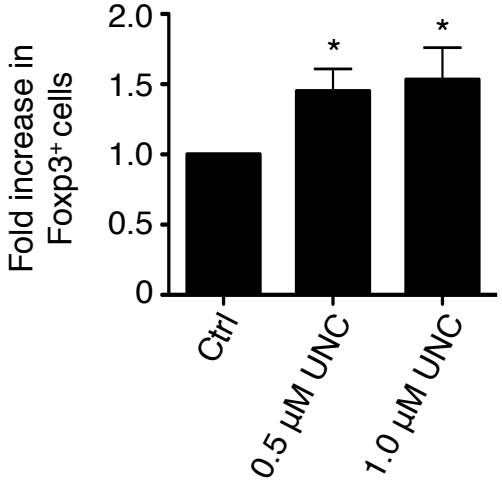
Supplemental Figure 7. Enhanced sensitivity to TGFβ1 leads to increased T_H17 cell differentiation from *G9a*$^{-/-}$ T cells. (**A**) *G9a*$^{fl/fl}$ and *G9a*$^{-/-}$ naïve CD4$^+$ cells were differentiated under T_H17 cell-promoting conditions with the indicated dose of TGFβ1 and analyzed at day 6 for intracellular IL-17A by flow cytometry. Numbers represent frequency of CD4$^+$IL-17A$^+$ cells. (**B**) Naïve *G9a*$^{-/-}$ T cells were differentiated under T_H17 cell-promoting conditions in the presence of vehicle, neutralizing antibody to TGFβ1 (10 μg/ml) or TGFβ signaling inhibitor (LY364947, 5μM). Data shown is representative of at least 3 independent experiments. Numbers represent frequency of CD4$^+$IL-17A$^+$ cells.



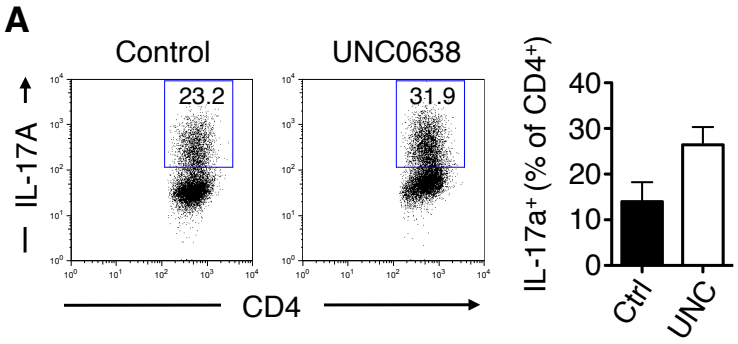
Supplemental Figure 8. G9a-deficiency does not affect expression of TGFβ signaling components. (**A**) Naïve *G9a*$^{fl/fl}$ and *G9a*$^{-/-}$ CD4$^+$ cells were analyzed by flow cytometry for the expression of TGFβRII (isotype control is shown in filled grey, *G9a*$^{fl/fl}$ in red and *G9a*$^{-/-}$ in blue). (**B**) *Tgfbr2* and *Smad3* mRNA expression in naïve *G9a*$^{fl/fl}$ or *G9a*$^{-/-}$ CD4$^+$ cells was analyzed by qRT-PCR. (**C**) *G9a*$^{fl/fl}$ and *G9a*$^{-/-}$ naïve and T_{reg} cells were analyzed by flow cytometry for phosphorylated Smad2/3. Data shown is representative of at least 2 independent experiments. Error bars indicate SEM.



Supplemental Figure 9. Inhibition of the methyltransferase activity of G9a has no effect on T_{reg} cell differentiation in G9a-deficient T cells. Naïve *G9a*$^{-/-}$ CD4^{+} T cells were differentiated under T_{reg} cell-promoting conditions for 6 days in the absence (control) or presence of the G9a-specific inhibitor UNC0638 (1 μM) or BIX01294 (0.5 μM) and analyzed by flow cytometry for the expression of CD4 and Foxp3. Data shown is representative of 3 independent experiments. Numbers represent frequency of CD4^{+}Foxp3^{+} cells.



Supplemental Figure 10. Inhibition of the methyltransferase activity of G9a in human T cells results in enhanced generation of Foxp3$^+$ cells. Naïve human conventional T cells were cultured for 13-14 days in the absence (Ctrl) or presence of the indicated concentration of the G9a-specific inhibitor UNC0638 (UNC) and analyzed by flow cytometry for the expression of Foxp3. Data is shown as a fold increase in Foxp3-expression above control for each of 8 healthy donors. *, $P < 0.05$. Errors bars indicate SEM.



Supplemental Figure 11. The methyltransferase activity of G9a is required to dampen T_H17 cell generation. Naïve $CD4^+$ T cells from C57BL/6 mice were differentiated under T_H17 cell-promoting conditions for 6 days in the absence (ctrl) or presence of the G9a-specific inhibitor UNC0638 (UNC, 0.5 μM) and analyzed by flow cytometry for the expression of CD4 and IL-17A. Results are from 3 independent experiments. Numbers represent frequency of $CD4^+IL\text{-}17A^+$ cells. Error bars indicate SEM.