Supplementary figures

Resource Paper

Transcription factor Foxp3 and its protein partners form a complex regulatory network

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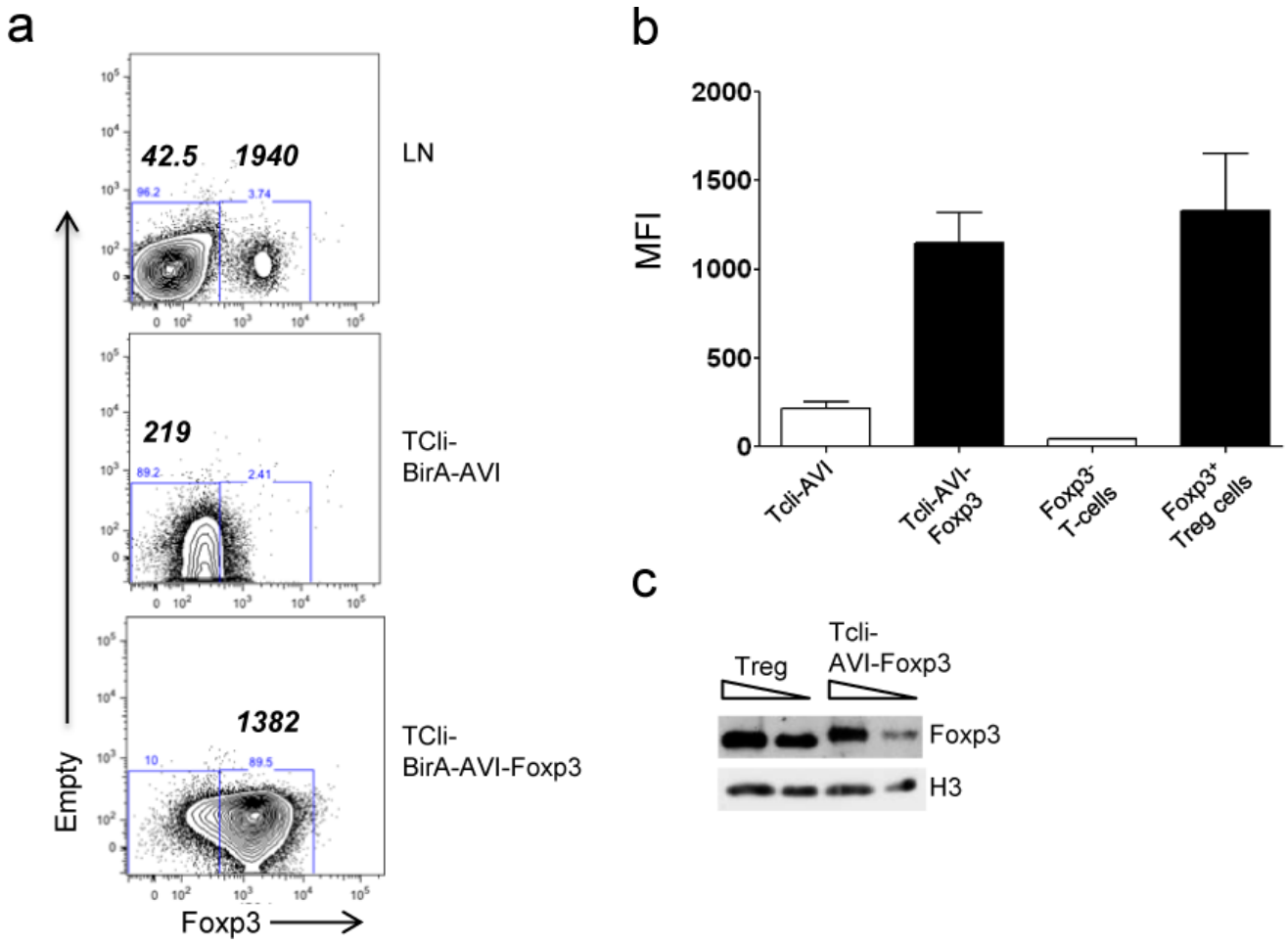
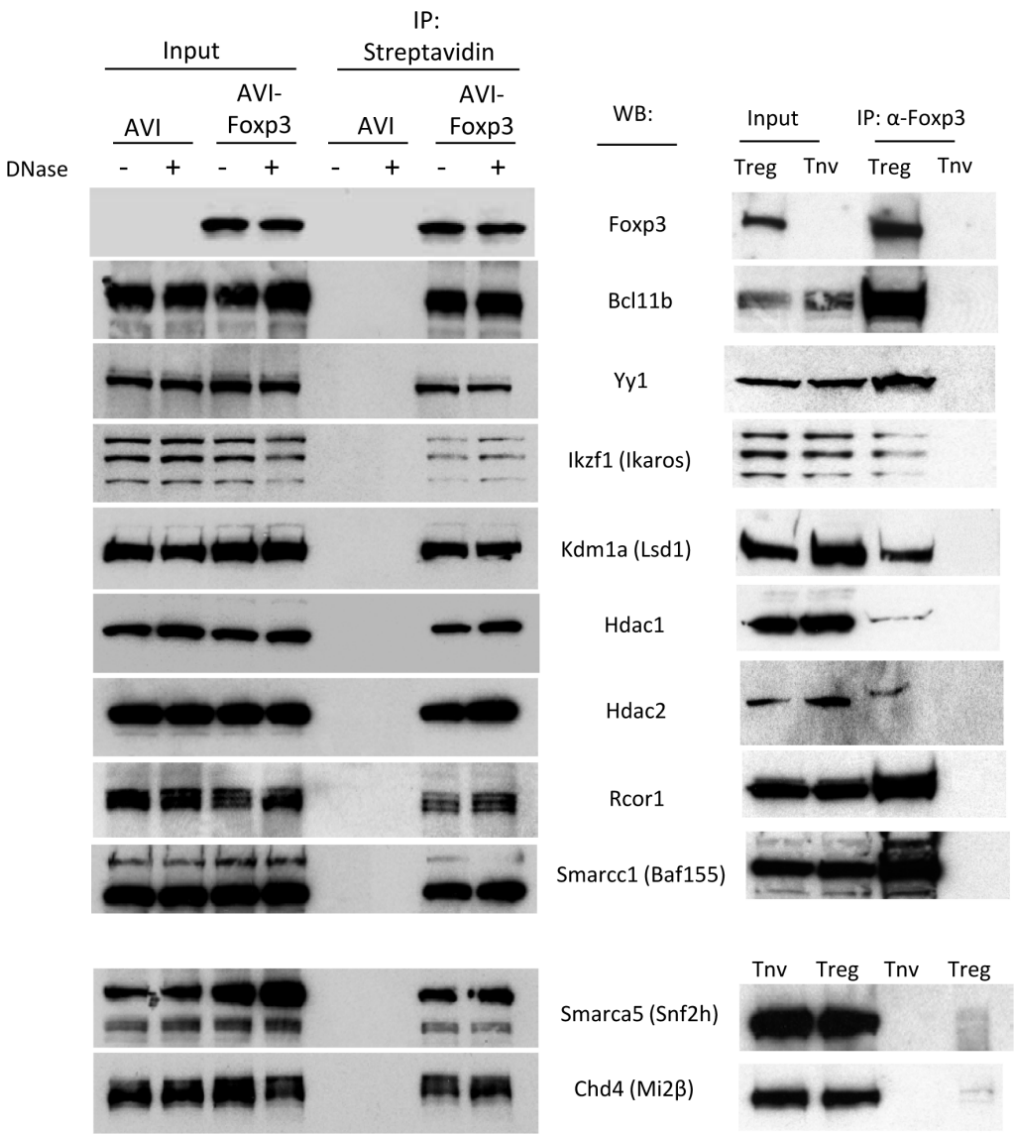
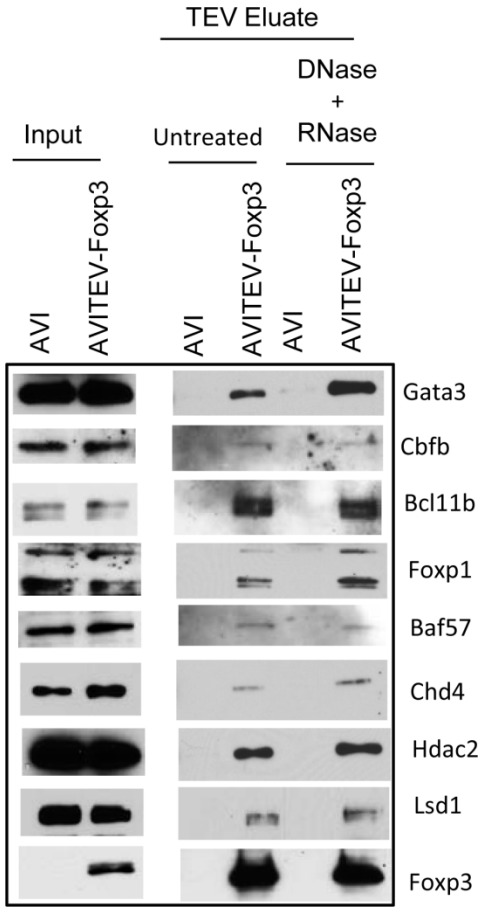


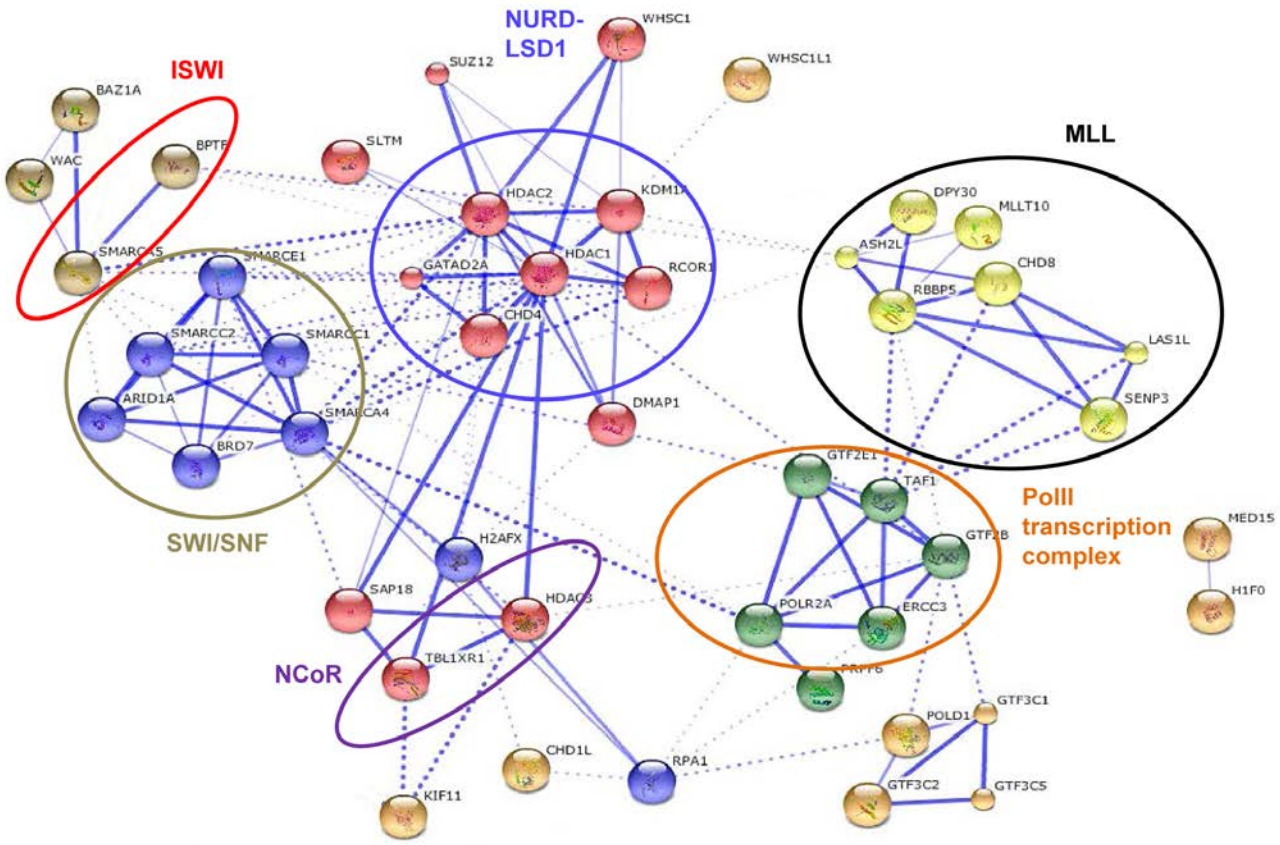
Supplementary Fig.1 Comparable expression of Foxp3 in retrovirally transduced TCli cell line and primary Treg cells. a) Flow cytometric analysis of Foxp3 expression in lymph node (LN) Treg cells and AVI or AVI-Foxp3 transduced TCli cells. Numbers in bold italics over the gated populations represent mean fluorescent intensity (MFI). b) An average Foxp3 MFI from three independent experiments described in (a). c) Western blot analysis demonstrating Foxp3 expression in Treg cells compared to TCli cells expressing AVI-Foxp3.



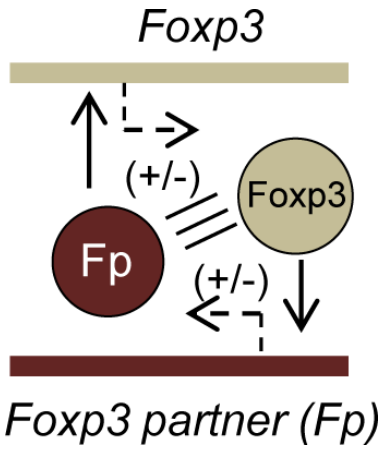
Supplementary Fig.2 Co-immunoprecipitation and western blot analysis of Foxp3 association with transcriptional-related nuclear factors identified by mass spectrometry. Foxp3 and its co-factors were immunoprecipitated using streptavidin- and Foxp3 antibody-conjugated magnetic beads from nuclear lysates of TCli-AVI-Foxp3 or primary Treg cells and probed using corresponding antibodies.



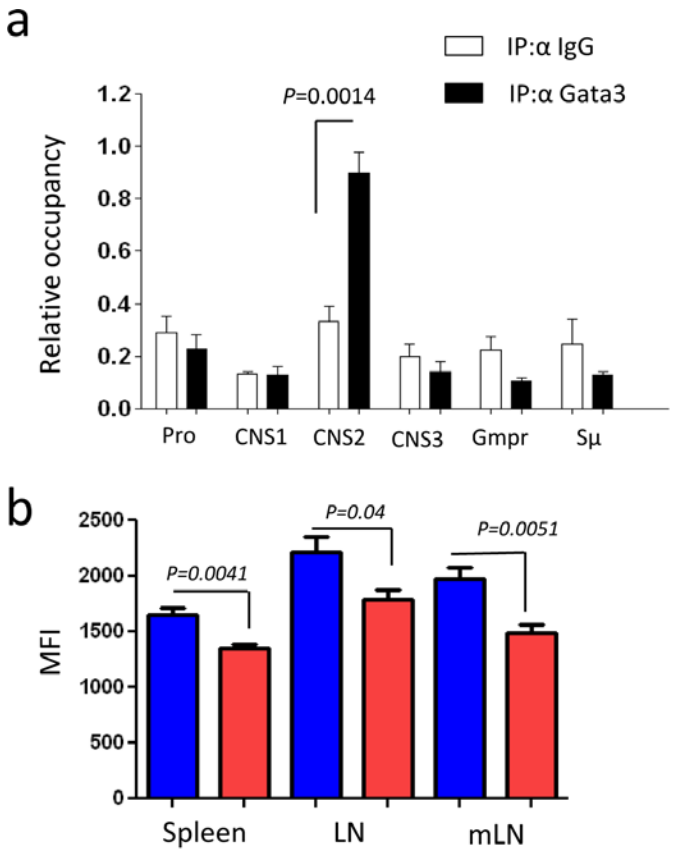
Supplementary Fig.3 Foxp3 complexes with its transcription-related partners are independent of DNA and RNA. Co-immunoprecipitation followed by Western blot analysis of the TEV eluted Foxp3 associated proteins after indicated treatments with DNase and RNase.



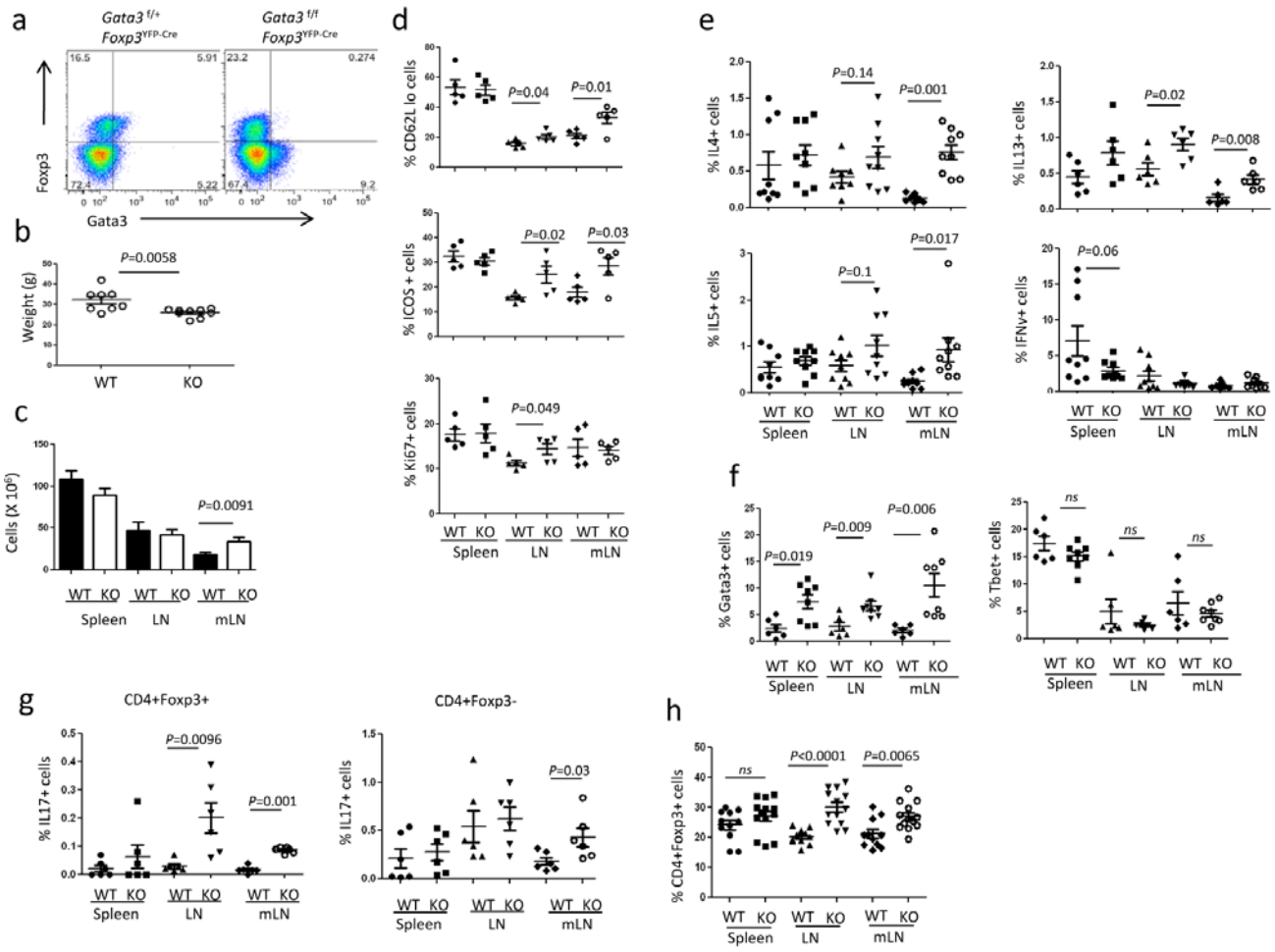
Supplementary Fig.4 Map of known interactions among Foxp3-associated transcriptional control-related proteins generated by STRING 9.0 software package. Interactions within known protein complexes are depicted as colored circles and ellipses.



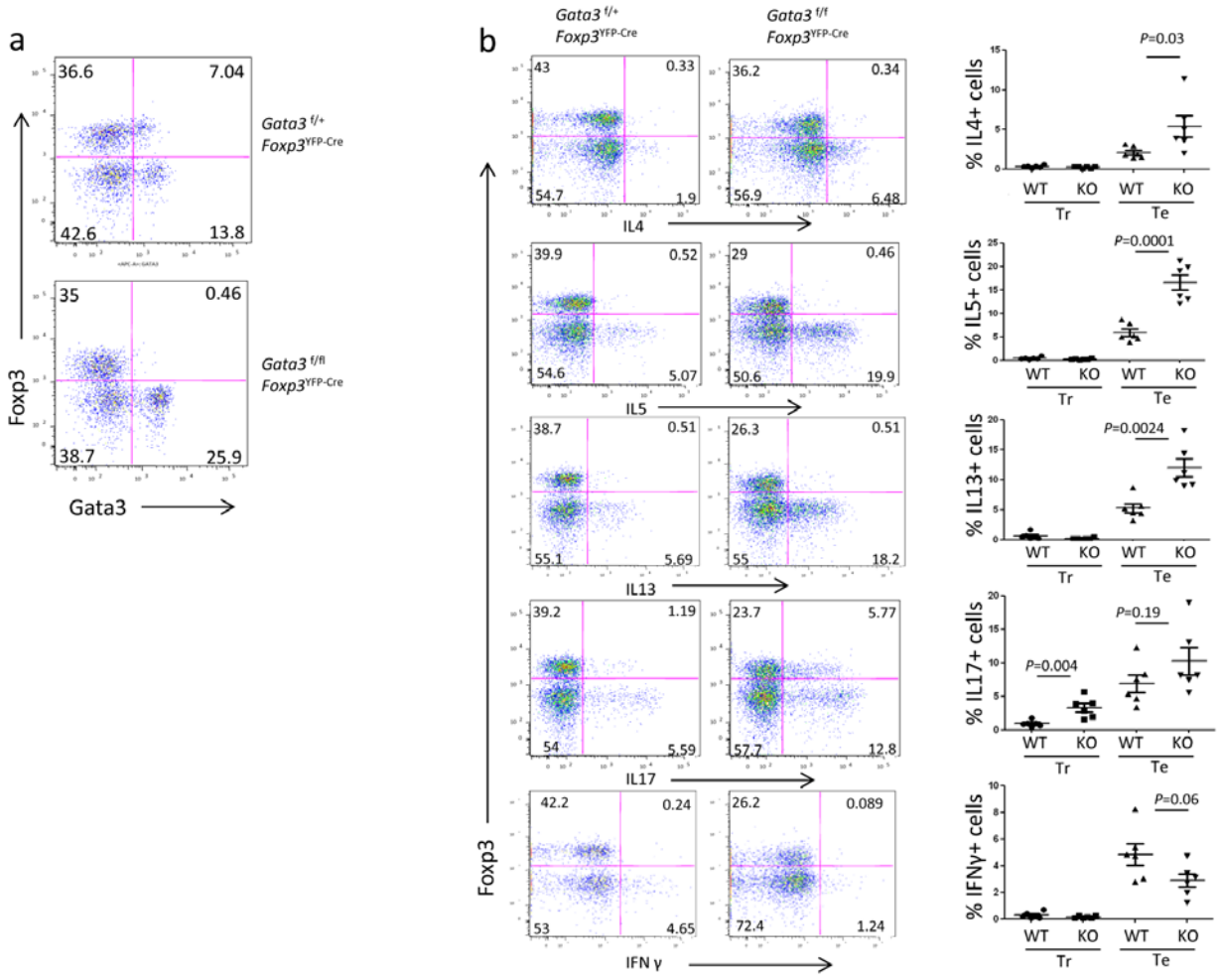
Supplementary Fig.5 A schematic depiction of regulatory relationships between Foxp3 and its partners. Parallel lines signify interactions between Foxp3 and its partners (Fp); the unidirectional arrows indicate binding to and regulation of corresponding genes. Broken arrows indicate gene expression affected in either positive or negative manner. Examples of Fp: Nfat, Runx/Cbfβ, GATA-3, Bcl-11b, Ets, Stat3.



Supplementary Fig.6 Gata3 binding to *Foxp3* CNS2 and Gata3-dependent potentiation of Foxp3 expression. a) ChIP-qPCR analysis of Gata3 binding to *Foxp3* regulatory elements in purified $CD4^{+}CD25^{+}$ Treg cells using Gata3 antibody and normal IgG as control. b) Mean fluorescence intensity (MFI) of Foxp3 expression assessed by flow cytometric analysis of $CD4^{+}CD8^{-}$ T cells from spleen, lymph nodes (LN) and mesenteric lymph nodes (mLN) of $Gata3^{fl/fl}Foxp3^{YFP\text{-}Cre}$ (red bars) and $Gata3^{fl/+}Foxp3^{YFP\text{-}Cre}$ (blue bars) littermate control mice (n=5 mice per group).



Supplementary Fig.7 Heightened spontaneous Th2 cytokine production in *Gata3*$^{f/f}$*Foxp3*$^{YFP\text{-}Cre}$ mice. a) Flow cytometric analysis of Gata3 expression in lymph node Treg and Foxp3^{-}CD4^{+} T cells from mice of indicated genotypes (at least 8 mice per group were analyzed). b) Body weights and c) lymph node (LN), spleen and mesenteric lymph nodes (mLN) cellularity of 5-6 months old *Gata3*$^{fl/fl}$*Foxp3*$^{YFP\text{-}Cre}$ (KO) mice and *Gata3*$^{fl/+}$*Foxp3*$^{YFP\text{-}Cre}$ (WT) control littermates. d) Expression of the indicated activation markers and e) cytokines by splenic, LN, and mLN CD4^{+}Foxp3^{-} T cells in 5-6 months old WT and KO mice. Data are representative of 2-3 independent experiments. f) Gata3 and T-bet expression in CD4^{+}Foxp3^{-} T cells from WT and KO mice. g) IL-17 production by splenic, LN, and mLN CD4^{+}Foxp3^{+} and CD4^{+}Foxp3^{-} T cells in WT and KO mice. h) Percentages of Foxp3^{+} cells within CD4^{+} T cell populations in LN, spleen, and mLN of WT and KO mice.



Supplementary Fig.8 Increased Th2 cytokine production by the large intestine lamina propria lymphocytes (LI-LPL) in *Gata3*$^{f/f}$*Foxp3*$^{YFP\text{-}Cre}$ mice. a) Representative FACS plots demonstrating the expression of Gata3 in LI-LPL in mice of indicated genotypes. b) Representative FACS plots and frequencies of indicated cytokines produced by LI-LPL in mice of indicated genotypes. The data are representative of six mice analyzed in two independent experiments.