Figure S1. *CD4-cre R27Tg* mice exhibited autoimmune phenotypes. (A) FACS analysis and frequencies of Ki67^{+}, CD44^{hi}CD62L^{lo} subset and IFNγ^{+} cells in Foxp3^{-}CD4^{+} Tconv cells in spleen from *CD4-cre R27Tg* mice (>14 wks) and control littermates were shown. **(B)** H&E-stained sections of the lung, colon, and stomach (ST) from the indicated mice (bar, 50 μm). Data are representative of four independent experiments. Each symbol represents an individual mouse, and the bar represents the mean. *p<0.05, **p<0.01.

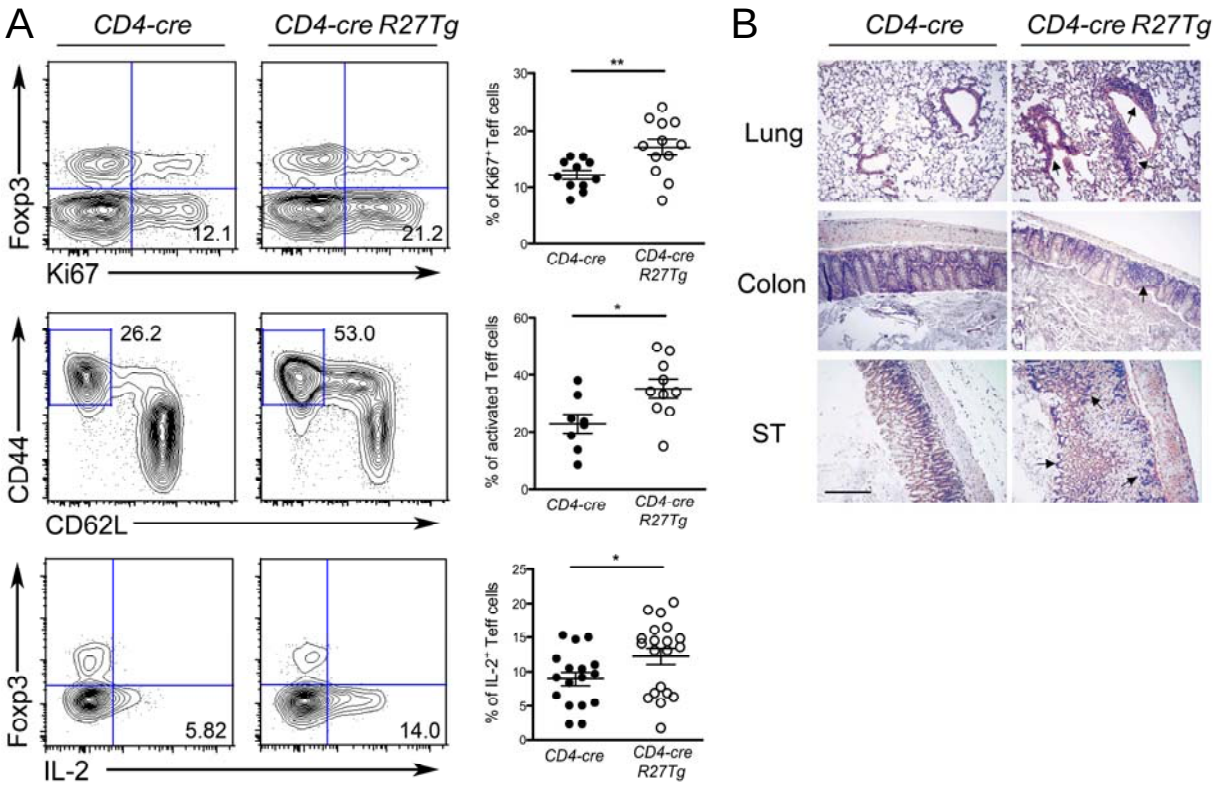

Figure S2. Overexpression of miR-27 in T cells led to reduced CD4⁺ T cell population in the periphery. FACS analysis and ratios of frequencies of different Ly5.1⁻ and Ly5.1⁺ **(A)** thymocyte subsets or **(B)** splenocyte populations within each donor-derived compartment 8 wks after BM transfer. FACS data are representative of three independent experiments. Each symbol represents an individual mouse, and the bar represents the mean. ***p<0.001.

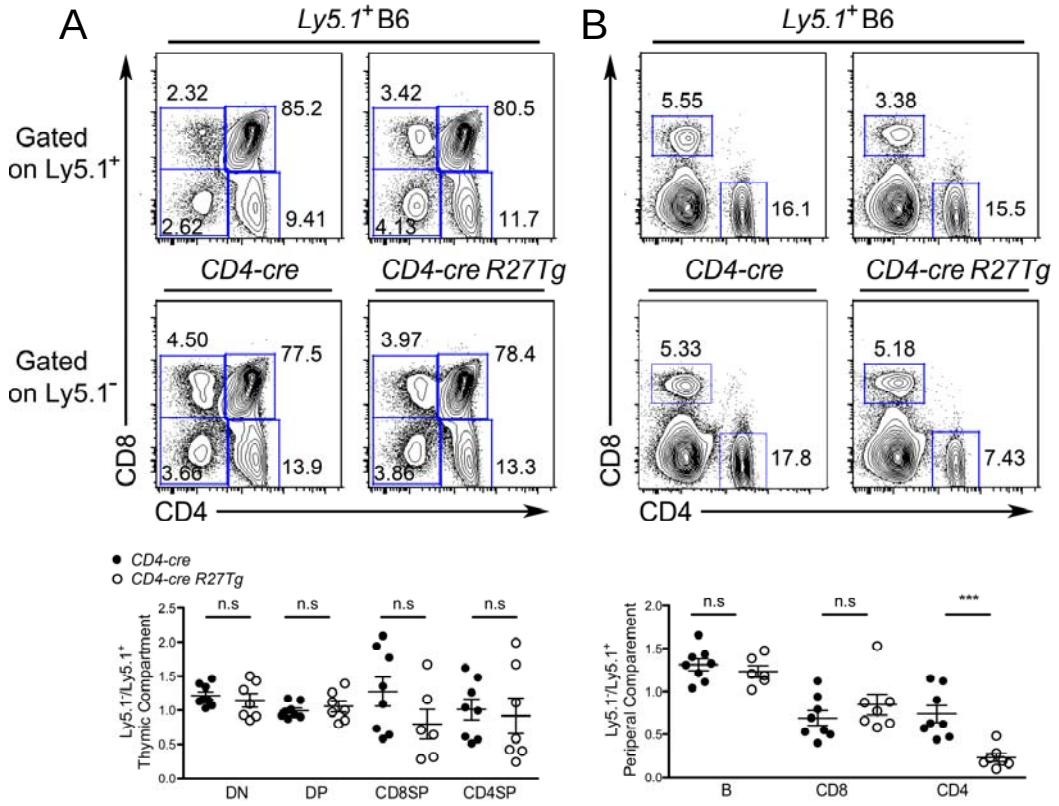

Figure S3. Diminished proliferation capacity in Treg cells with excessive miR-27 expression. FACS analysis and ratios of frequencies of **(C)** $Ly5.1^{-}Ki67^{+}$ and $Ly5.1^{+}Ki67^{+}$ splenic $Foxp3^{+}$ Treg cells. FACS data are representative of three independent experiments. Each symbol represents an individual mouse, and the bar represents the mean. ***p<0.001.

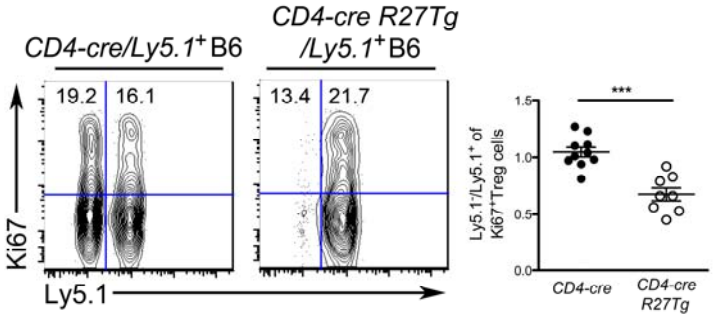

Figure S4. Expression of other miR-23~27~24 members in different thymocyte subsets. qPCR analysis of the expression of miR-23a/b and miR-24 in different thymocyte subsets. Data represent mean ± SD and are representative of three independent experiments (n=6).

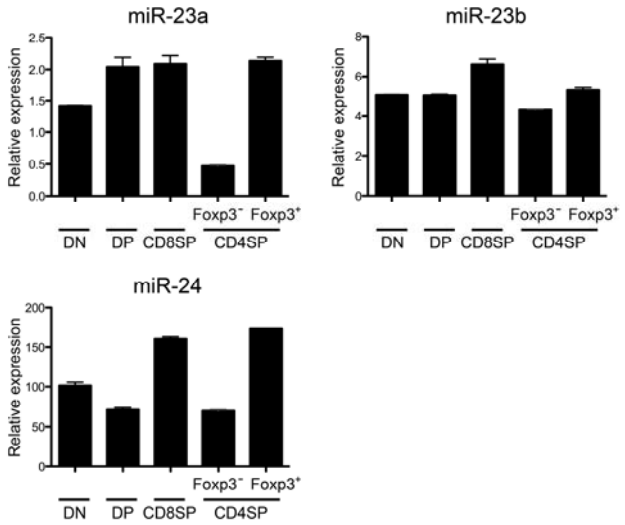

Figure S5. Minimal role of miR-27 in controlling Treg cell tissue trafficking. (A) Ratios of YFP-cre^{+} miR-27-overexpressing Treg cells and YFP-cre^{-} WT Treg cells in indicated tissues from *Foxp3$^{cre/+}$ R27Tg* mice. FACS analysis and ratios of MFI of **(B)** CCR7 in pLN Treg cells or **(C)** CCR9 and CD103 in LP Treg cells with or without miR-27 overexpression from *Foxp3$^{cre/+}$ R27Tg* mice and WT control mice. Data represent mean ± SD and are representative of three independent experiments. Each symbol represents an individual mouse, and the bar represents the mean. *p<0.05, ***p<0.001.

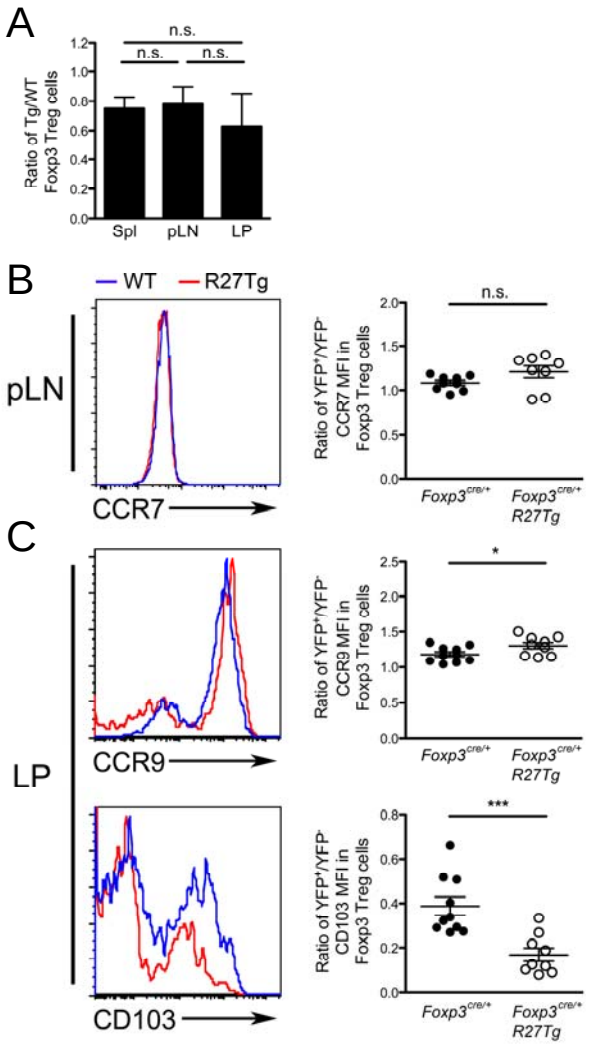

Figure S6. Protein expressions of previously identified miR-27 targets in T cells. Immunoblot analysis of Foxo1, Smad2/3 and Runx1 expression in T cells with or without miR-27 overexpression. Densitometric expression values of each molecule were normalized to β-actin expression values and n-fold increase on the basis of each corresponding WT. Data are representative of three independent experiments (n=3-6).

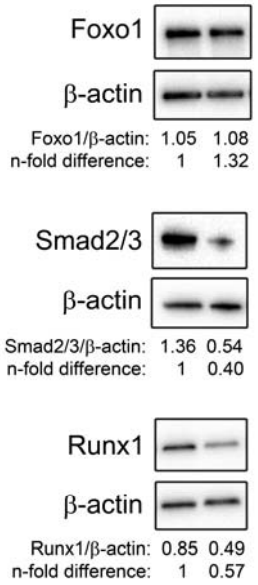

Figure S7 Excessive miR-27 expression resulted in mild reduction in Foxp3 expression in peripheral Treg cells. Ratios of MFI of Foxp3 between Ly5.1^{-} and Ly5.1^{+} **(A)** thymic or **(B)** splenic Foxp3^{+}CD4^{+} Treg cells. Data are representative of two independent experiments. Each symbol represents an individual mouse, and the bar represents the mean. ***p<0.001.

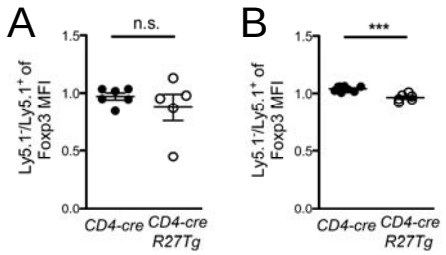

Figure S8. Treg cells with miR-27 overexpression exhibited comparable *in vitro* suppressor capacity. Treg cells (Tr) isolated from *CD4-cre R27Tg* mice or WT control littermates were subjected to *in vitro* suppression analysis at indicated ratios of responder T cells (Te). Data represent mean ± SD and are representative of three independent experiments (n=6).

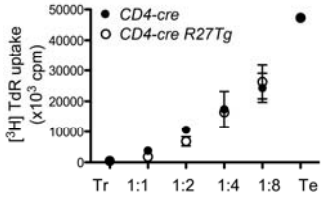

Figure S9. Transfer of miR-27-overexpressing Tconv cells failed to induce colitis. (A) Percentages of body weight change of *Rag1*$^{-/-}$ recipient mice after adoptive transfer of 4x10^5 (CD4$^+$CD45RB^{hi}CD25$^-$) WT or R27Tg T cells. **(B)** Frequencies of CD4$^+$ T cells isolated from lamina propria (LP) 12 wks after T cell transfer. Data represent mean ± SD and are representative of three independent experiments. Each symbol represents an individual mouse, and the bar represents the mean. **p<0.01.

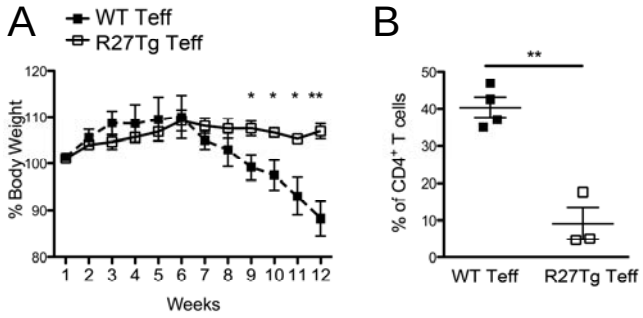

Figure S10. *Foxp3^{cre} R27Tg* mice exhibited elevated gut inflammation despite having normal Treg cell numbers. FACS analysis and frequencies of **(A)** Foxp3^{+} cells in total CD4^{+} T cells as well as **(B)** CD44^{hi}CD62L^{lo} cells, **(C)** Ki67^{+} and **(D)** IL-17^{+} cells in Tconv cells from LP in 6 wks old *Foxp3^{cre} R27Tg* mice or WT controls. Data represent mean ± SD and are representative of three independent experiments. Each symbol represents an individual mouse, and the bar represents the mean. *p<0.05.

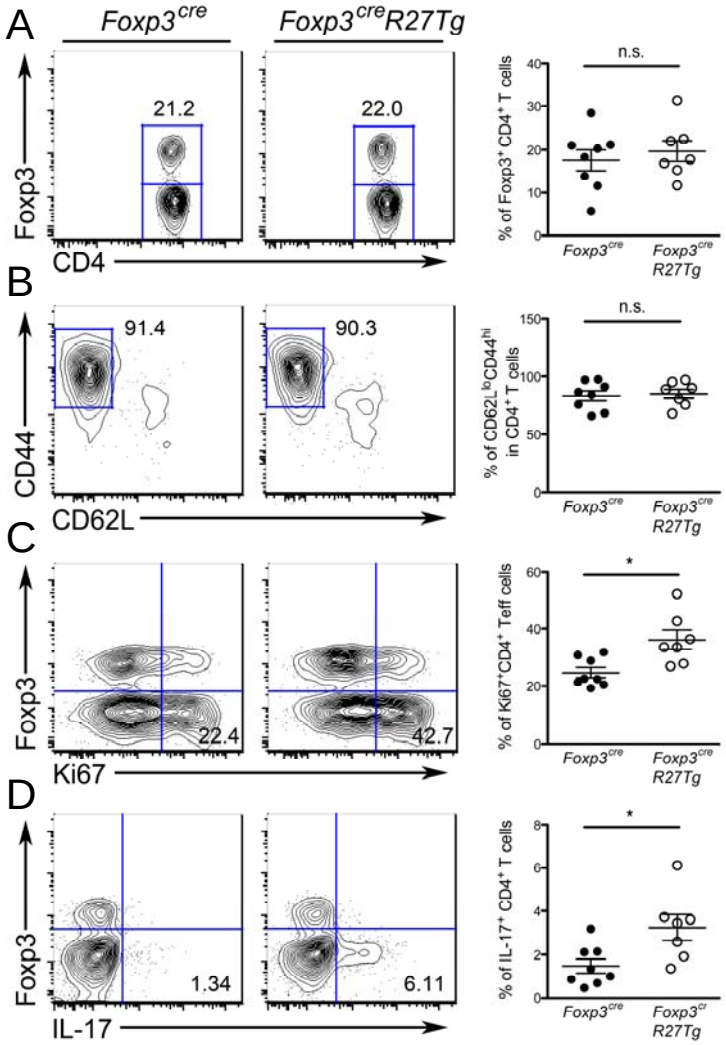

Figure S11. Excessive miR-27 expression broadly impacted genes associated to immune system process in Treg cells. Annotated gene ontology biological processes were assigned to genes differentially expressed in Treg cells with or without miR-27 overexpression as determined by RNA-seq.

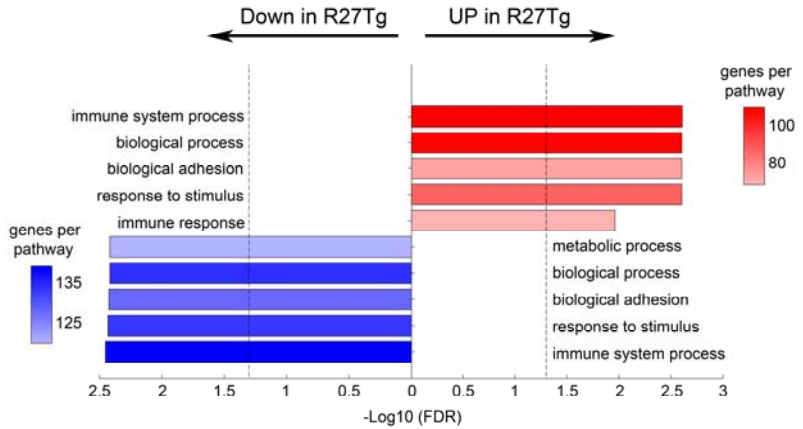

Figure S12. Excessive miR-27 expression inhibited IL-10 and GZMB expression in iTreg cells. FACS analysis of frequencies, and MFI of **(A)** IL-10 and **(B)** GZMB in R27Tg TGFβ induced-iTreg cells compared to WT controls. Data represent mean ± SD and are representative of three independent experiments. Each symbol represents an individual mouse, and the bar represents the mean. *p<0.05, ***p<0.001.