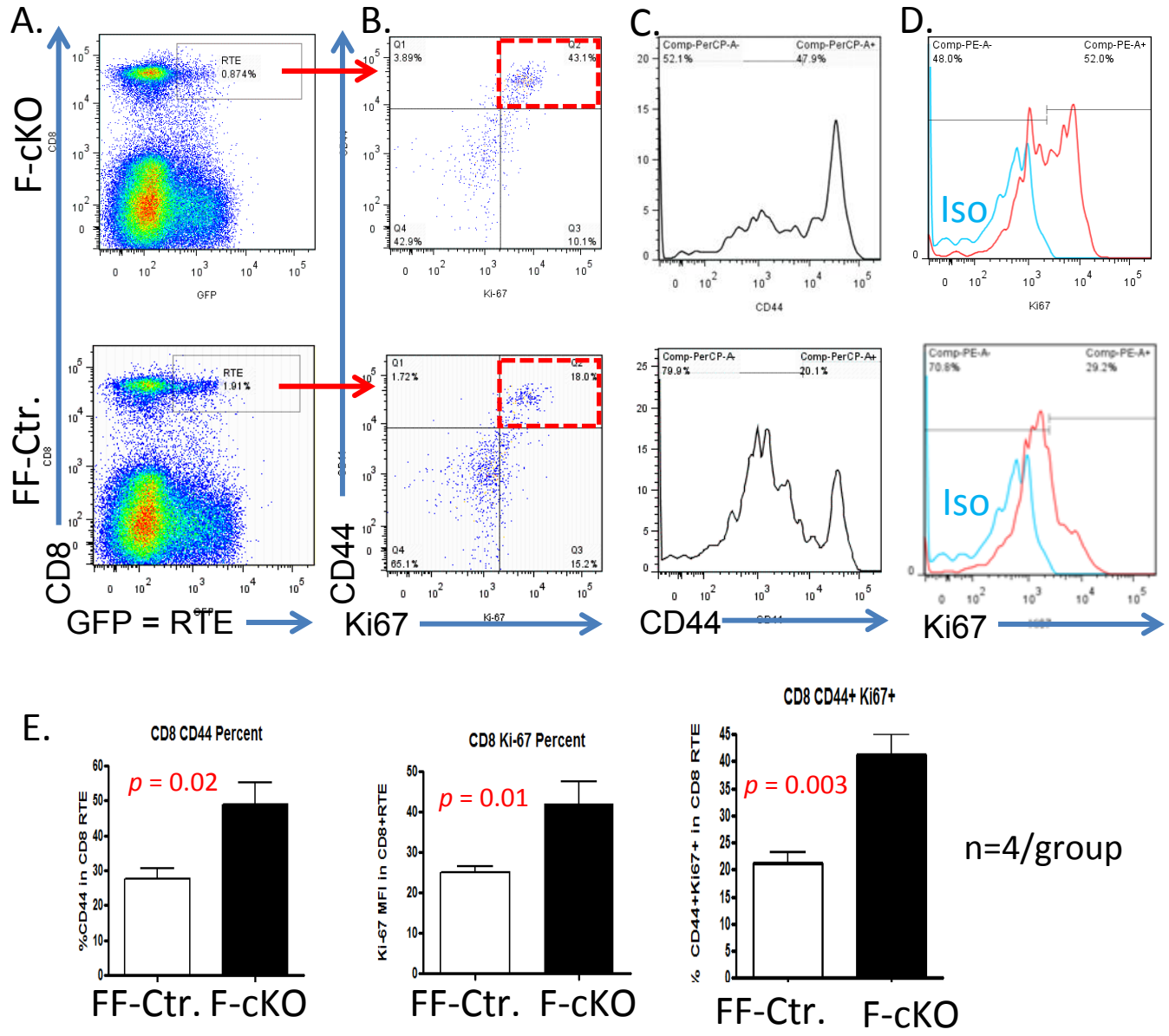
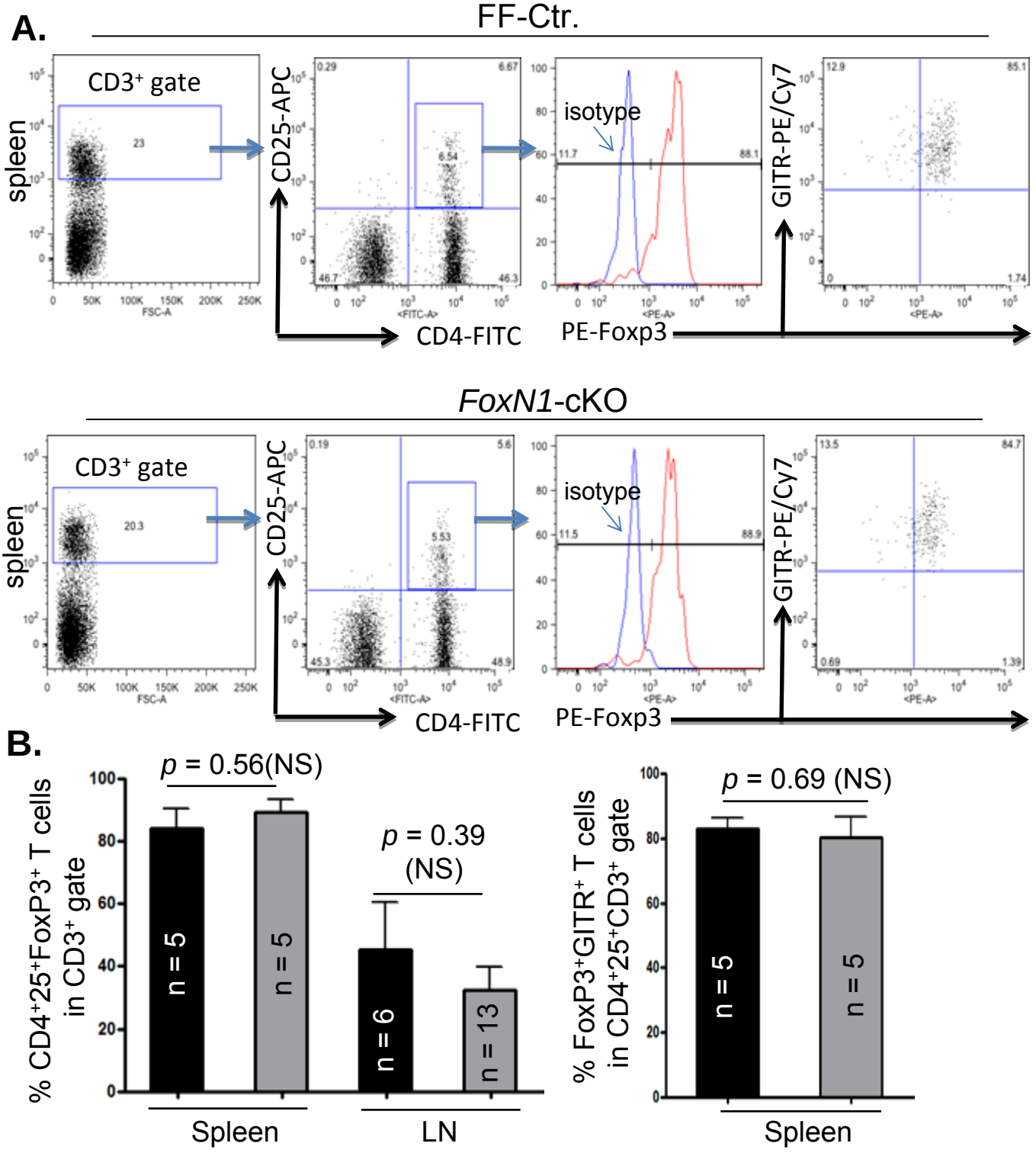
Supplemental Table S1. List of mouse colonies used in this work

Colony	Targeted gene and generation	Significance	Jackson Lab #
fx/fx-uCreER^{T}	Two loxp tags were inserted into the *FoxN1* gene as described in previous publication (30) .	Accelerated Thymic involution in a young mouse	#012941 crossed to #004682
fx/fx-only	Same as above but no Cre gene	Use as littermate controls	#012941
RAG-GFP	Green fluorescent protein (GFP)$^{+}$ reporter gene is driven by *Rag* gene	Used to identity T and B cells that have recently undergone RAG recombination; used here as marker of recent thymic emigrants (RTE)	#005688
fx/fx-uCreER^{T} or fx/fx-only carrying *Rag*-GFP	Crossbreeding fx/fx-uCreER^{T} with *Rag*-GFP mice	Tracking of RTEs derived from an involuted thymus	(#012941 crossed to #004682) crossed to #005688
RAG$^{-/-}$	*Rag1* gene knockout	Used as adoptive transplantation hosts due to without T and B lymphocytes	#002216
RIP-mOVA (Ovalbumi)	Chicken OVA driven by the RAT Insulin Promoter	Aire dependent mOVA expressed in mTEC as mock “self” antigen.	#005431
OT-II	Transgenic TcrαTcrβ recognizing chicken OVA in the context of I-A^{b}	TCR transgenic producing CD4 SP thymocytes that binds OVA:MHC complex strongly	#004194
Aire$^{-/-}$	*Aire* gene knockout	Used as positive controls of autoimmunity	#004743

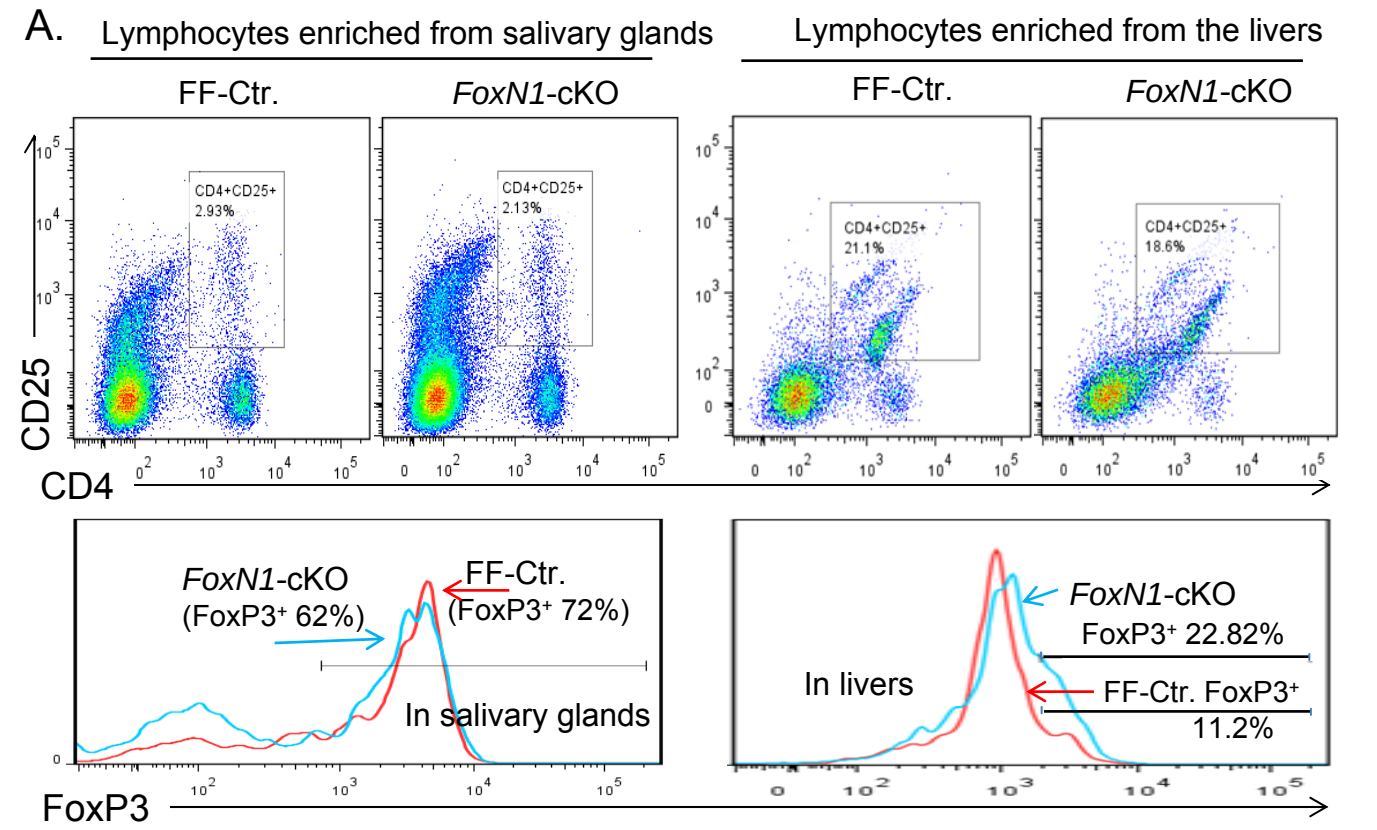
(All mice have C57BL/6 genetic background)

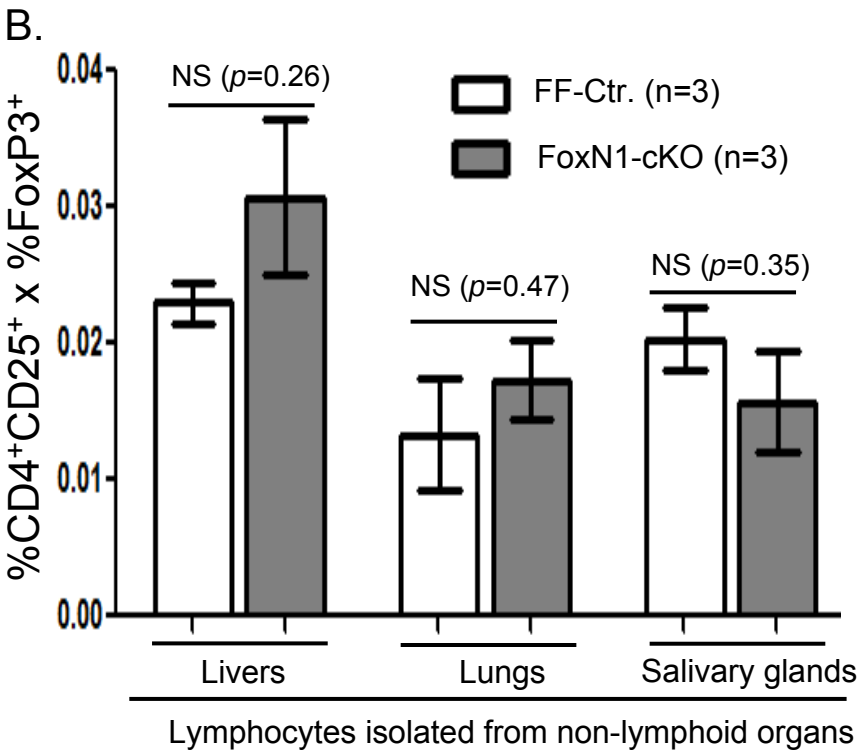


Supplemental Fig. S1. CD8 Recent Thymic Emigrants from the atrophied thymus acquired an activated immune cell phenotype. Peripheral spleen cells were freshly isolated from the F-cKO and FF-Ctr mice carrying RAG-GFP reporter. The cells were stained with CD8, CD44, and Ki67 antibodies, and $CD8^+GFP^+$ cells were defined as $CD8^+$ RTEs. **(A)** Representative dot plots show $CD8^+GFP^+$ gate; **(B)** $CD44^{hi}Ki67^+$ cell gates (red boxes) in $CD4^+$ RTEs from *FoxN1* cKO (top panels) and control (bottom panels) mice. **(C)** Representative histograms of $CD44^{hi}$; and **(D)** $Ki67^+$ in $CD8^+$ RTEs from *FoxN1* cKO (top panels) and control (bottom panels) mice. **(E)** Summarized results of % $CD44^{hi}$, $Ki67^+$, and $CD44^{hi}Ki67^+$ double positive cells in $CD8^+$ RTEs (from left to right panels). A Student *t*-test was used to determine statistical significance between groups. All data are expressed as mean ± SEM.



Supplementary Figure S2. CD4⁺ regulatory T cells in the periphery of F-cKO mice were not changed. (A) Representative flow cytometry plots of spleen cells show gating strategy of Treg cells from spleen of *FoxN1*-cKO and FF-Ctr control mice. **(B)** Summarized results of the % $CD4^+CD25^+FoxP3^+$ (left panel), and % $CD4^+FoxP3^+GITR^+$ (right panel) functional Treg cells in the spleen and lymph nodes (LN) from *FoxN1*-cKO (grey bars) and age-matched FF-Ctr control mice (black bars) mice (n = animal numbers, NS = not significant). A Student *t*-test was used to determine statistical significance between groups. All data are expressed as mean ± SEM.





Supplementary Fig. S3. % of CD4⁺ Treg cells in the liver, lung, and salivary glands were not significantly different between *FoxN1*-cKO and FF-Ctr mice. (**A**) Representative flow cytometry results from salivary gland and liver lymphocytes enriched through two-layer density gradient centrifuge, showing $CD4^+CD25^+$ and $FoxP3^+$ gates. (**B**) Summarized results of % T_{reg} cells ($CD4^+CD25^+FoxP3^+$) in the liver, lung, and salivary gland lymphocytes. (n = animal numbers, NS = not significant). A Student *t*-test was used to determine statistical significance between groups. All data are expressed as mean ± SEM.