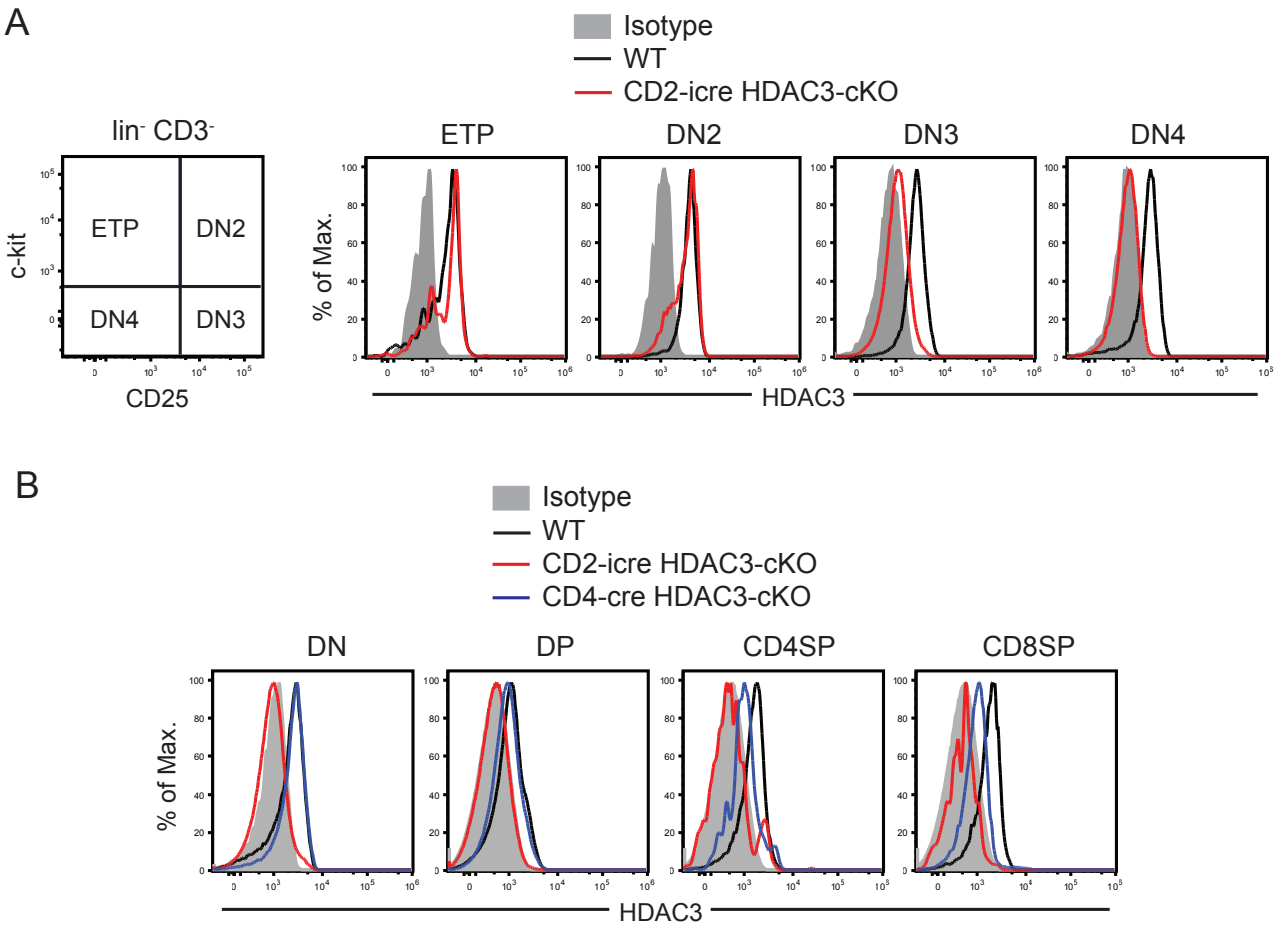
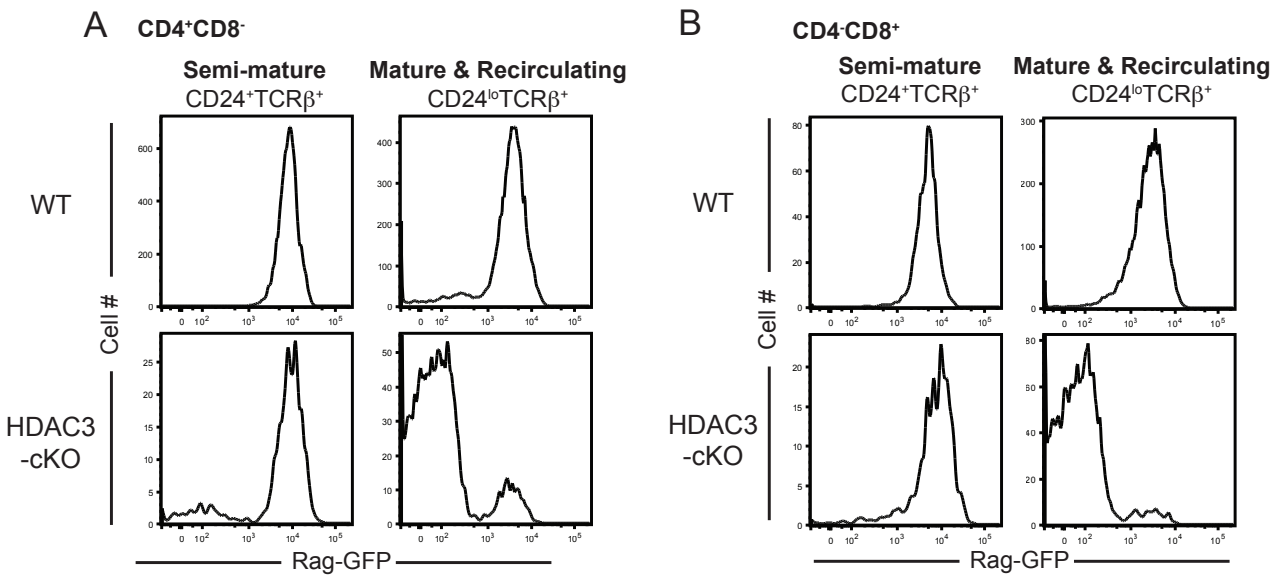
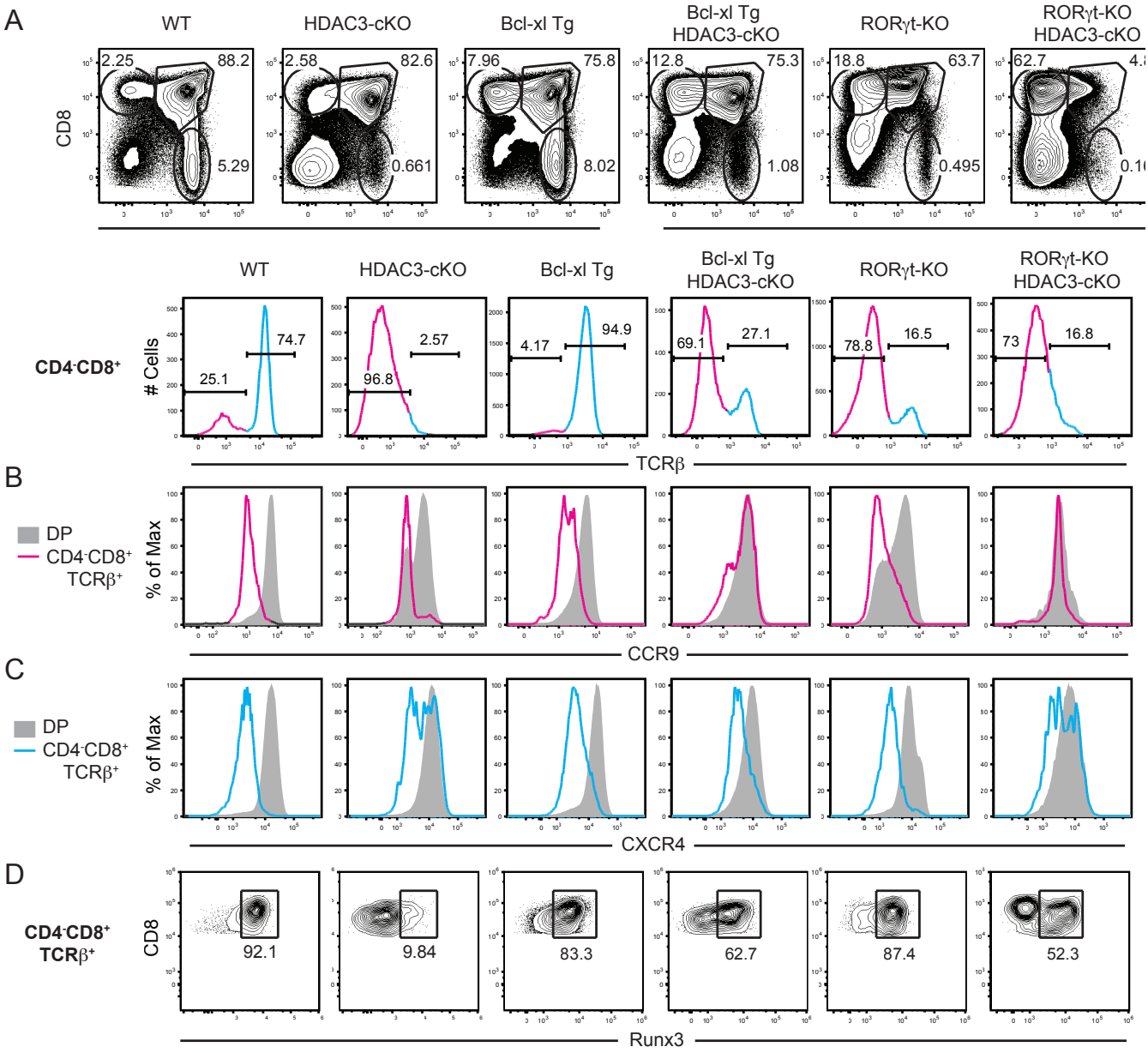
Supplementary Figures



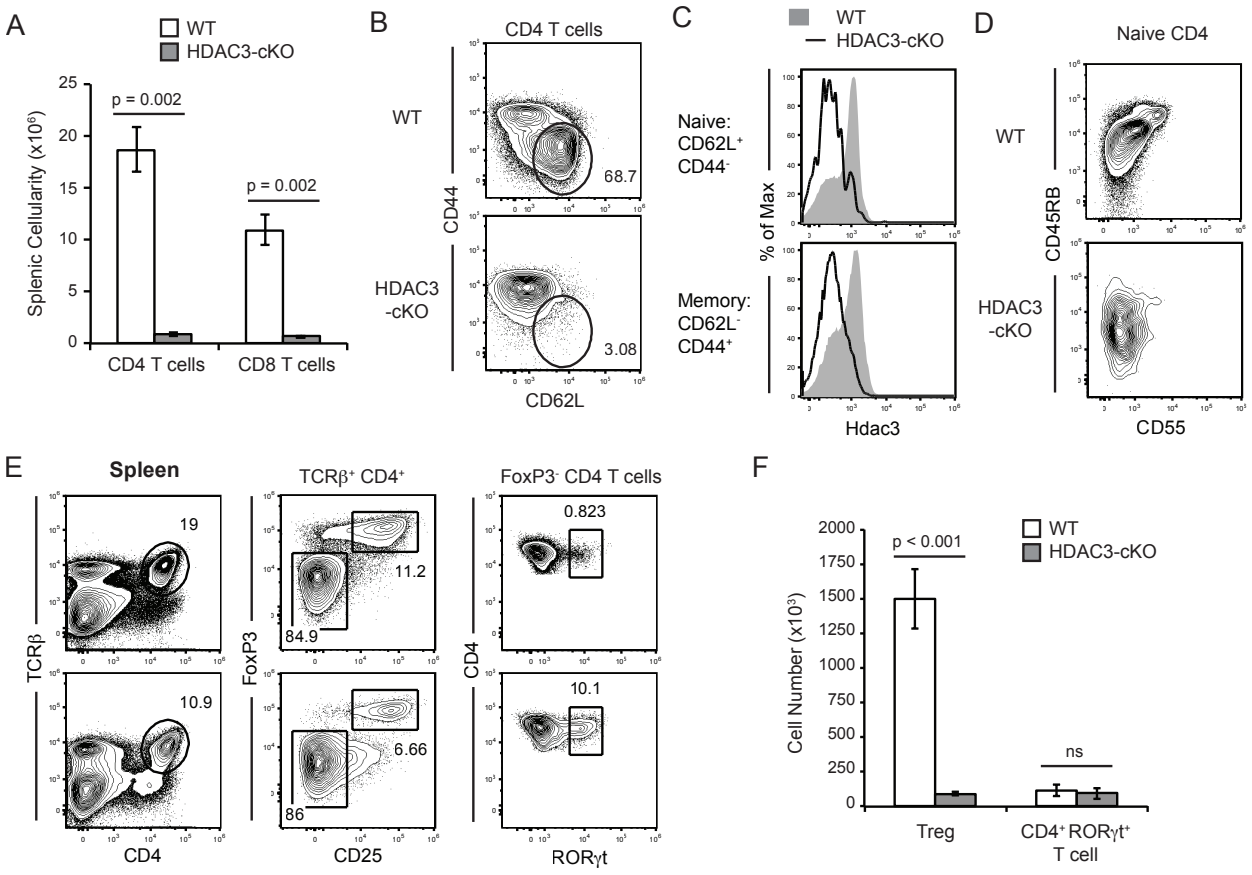
Supplementary Figure 1. HDAC3 protein is absent starting at DN3 thymocytes. (a) Intracellular staining of HDAC3 in DN subsets. DN thymocyte profiles using c-kit and CD25 from WT and HDAC3-cKO mice, previously gated on lin$^-$ (B220, CD11b, Gr-1, Ter119, CD19, CD11c, NK1.1, CD8α, CD4, TCRb) CD3$^-$ thymocytes to examine ETP (c-kit$^+$CD25$^-$), DN2 (c-kit$^+$CD25$^+$), DN3 (c-kit$^-$CD25$^+$), and DN4 (c-kit$^-$CD25$^-$) between WT and CD2-icre HDAC3-cKO mice. Filled grey histogram represents isotype control. (b) Intracellular expression of HDAC3 in DN (lin$^-$, CD3$^-$), DP, CD4SP, and CD8SP thymocytes from WT, CD2-icre HDAC3-cKO, and CD4-cre HDAC3-cKO mice. SP thymocytes from CD2-icre HDAC3-cKO mice were gated off a semi-mature gate (CD24$^+$ TCRβ$^+$), as many cells in the mature SP (CD24^{lo} TCRβ$^+$) gate are recirculating T cells (see Supplemental Figure 2). Data is representative of at least eleven mice per group from five independent experiments.



Supplementary Figure 2. Majority of SP thymocytes in HDAC3-cKO mice are recirculating T cells. Frequency of semi-mature SP thymocytes ($CD24^{+}$, $TCR\beta^{+}$, Rag-GFP^{+}), mature SP thymocytes ($CD24^{lo}$, $TCR\beta^{+}$, Rag-GFP^{+}), and recirculating T cells ($CD24^{lo}$, $TCR\beta^{+}$, Rag-GFP^{-}) from WT and HDAC3-cKO mice. Data is representative of ten mice per group from seven independent experiments.



Supplementary Figure 3. Bcl-xl Tg and RORγt-KO does not rescue development in HDAC3-cKO mice. (A) CD4-versus-CD8 profile and TCRβ surface expression of $CD4^{-}CD8^{+}$ thymocytes of WT, HDAC3-cKO, Bcl-xl Tg, Bcl-xl Tg HDAC3-cKO, RORγt-KO, and RORγt-KO HDAC3-cKO mice. (B-D) Surface expression of CCR9 on DP and $CD4^{-}CD8^{+}$ $TCR\beta^{-}$ thymocytes (B), surface expression CXCR4 on DP and $CD4^{-}CD8^{+}$ $TCR\beta^{+}$ thymocytes (C), and intracellular expression of Runx3 on $CD4^{-}CD8^{+}$ $TCR\beta^{+}$ thymocytes (D) from WT, HDAC3-cKO, Bcl-xl Tg, Bcl-xl Tg HDAC3-cKO, RORγt-KO, and RORγt-KO HDAC3-cKO mice. Data shown is the mean ± SEM of four mice from four independent experiments.



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	Naive CD4⁺ (x10⁶)	p value	Naive CD8⁺ (x10⁶)	p value
WT	11.68 ± 2.10		6.123 ± 1.27	
HDAC3-cKO	0.017 ± 0.011	p<0.01	0.037 ± 0.034	p<0.01
Bcl-xl Tg	24.99 ± 6.11	NS	12.90 ± 2.916	NS
Bcl-xl Tg HDAC3-cKO	0.046 ± 0.014	p<0.03	0.022 ± 0.007	p<0.04
RORγt-KO	5.266 ± 1.35	NS	2.436 ± 0.367	NS
RORγt-KO HDAC3-cKO	0.014 ± 0.008	p<0.03	0.004 ± 0.002	p<0.04
RORγt-KO Bcl-xl Tg	40.03 ± 2.586	p<0.001	36.57 ± 2.686	p<0.001
RB3	0.195 ± 0.127	p<0.002	0.117 ± 0.064	p<0.004

Supplementary Figure 4. Splenic T cells in HDAC3-cKO mice. (a) Cellularity of $CD4^+$ T cells ($TCR\beta^+$, $CD4^+$) and $CD8^+$ T cells ($TCR\beta^+$, $CD4^+$) in spleens from WT and HDAC3-cKO mice. Data shown are the means ± SEM of six mice per group from five independent experiments. (b) CD62L and CD44 expression on splenic $CD4^+$ T cells ($TCR\beta^+$, $CD4^+$) from WT and HDAC3-cKO mice. Gate identifies percentage of naïve T cells ($CD62L^+$, $CD44^-$). Data represents at lease five mice per group from five independent experiments. (c) Intracellular expression of HDAC3 in naïve ($CD62L^+$, $CD44^-$) and memory ($CD62L^-$, $CD44^+$) CD4+ T cells from WT and HDAC3-cKO mice. Data represents at least three mice from two independent experiments. (d) CD55-versus-CD45RB expression on splenic $CD4^+$ naïve T cells from WT and HDAC3-cKO mice. Data represents at least three mice from two independent experiments. (e) Frequency and (f) cell number of Tregs ($TCR\beta^+$, $CD4^+$, $CD25^+$, $FoxP3^+$) and $ROR\gamma t^+$ $CD4^+$ T cells ($TCR\beta^+$, $CD4^+$, $CD25^-$, $FoxP3^-$) from the spleen of WT and HDAC3-cKO mice. Data shown is the mean ± SEM of at least four mice per group from at least two independent experiments. (g) Number of naïve ($CD62L^+$ $CD44^-$) splenic $CD4^+$ and $CD8^+$ cells from each genotype was examined. Numbers represent average ± SEM from four WT, two HDAC3-cKO, two Bcl-xl Tg,

two Bcl-xl Tg HDAC3-cKO, two RORγt-KO, two RORγt-KO HDAC3-cKO, two RORγt-KO Bcl-xl Tg and four RB3 mice. P values shown are calculated for each population as compared to WT in an unpaired Student t test using GraphPad Prism. NS, not significant.