An Essential Role of Transcription Factor Runx1 for T Cell Maturation

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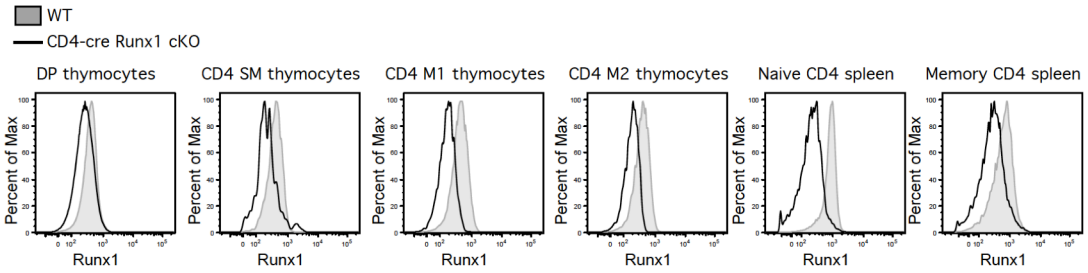
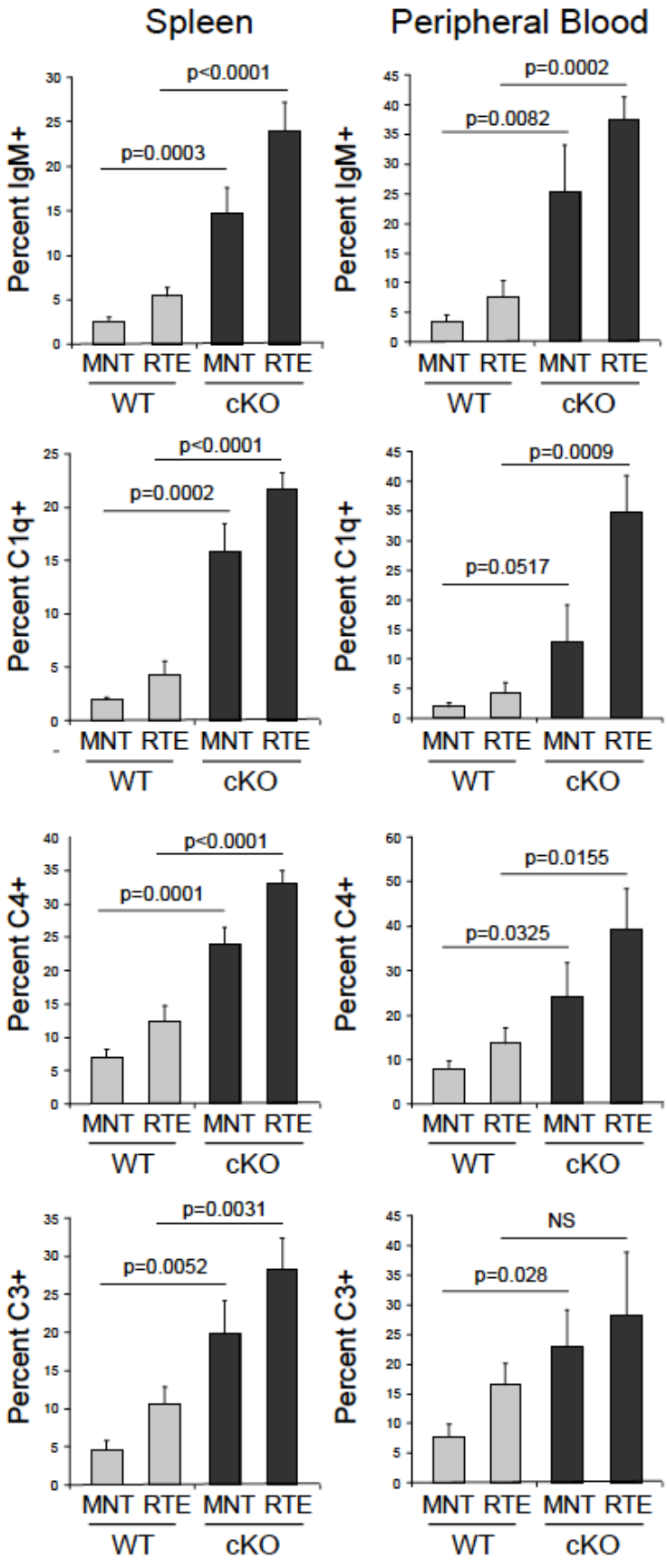


Supplemental Figure 1

Runx1 protein expression throughout T cell development in CD4-cre Runx1 cKO mice and WT mice

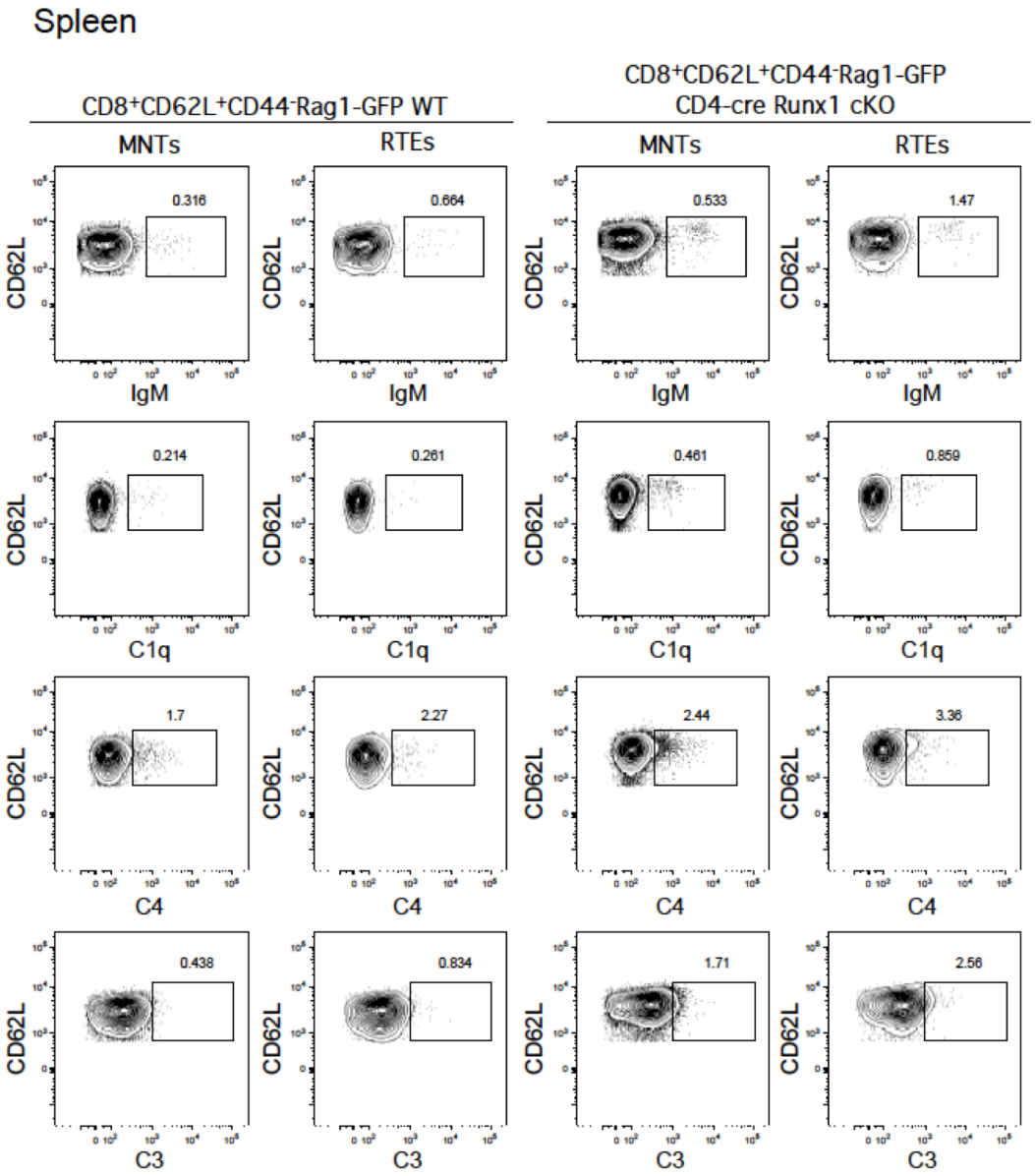
Intranuclear Runx1 staining was performed on thymocytes and splenocytes from WT and CD4-cre Runx1 cKO mice. The protein levels of Runx1 in DP ($CD4^+CD8^+$), CD4 SM ($CD4^+CD8^-CD24^{hi}CCR7^{lo}$), CD4 M1 SP ($CD4^+CD8^-CD24^{hi}CCR7^{hi}$) thymocytes, CD4 M2 SP ($CD4^+CD8^-CD24^{lo}$CCR7hi) thymocytes, naïve ($CD4^+CD62L^+CD44^-$) and memory ($CD4^+CD62L^-CD44^+$) T cells from both WT and CD4-cre Runx1 cKO mice were examined. As intranuclear staining destroys GFP fluorescence, expression of Runx1 in RTEs was not examined. Data shown are representative of five WT and five Rag1-GFP CD4-cre Runx1 cKO mice from five separate experiments.


Spleen
Peripheral Blood
Percent IgM+
p<0.0001
p=0.0003
p=0.0002
p=0.0082
Percent C1q+
p<0.0001
p=0.0002
p=0.0009
p=0.0517
Percent C4+
p<0.0001
p=0.0001
p=0.0155
p=0.0325
Percent C3+
p=0.0031
p=0.0052
NS
p=0.028
MNT RTE MNT RTE
WT cKO

Supplemental Figure 2

Quantitation of IgM and complement protein deposition on splenic and peripheral blood Runx1-deficient CD4$^+$ RTEs and MNTs as compared to WT

Deposition of IgM, C1q, C4 and C3 in splenic (left column) and peripheral blood (right column) CD4$^+$ RTEs and MNTs from Rag1-GFP WT and Rag1-GFP CD4-cre Runx1 cKO mice as shown in Figure 3 was quantified across several experiments. For analysis of complement deposition in splenic CD4$^+$ T cells, the results shown are quantified from at least 6 Rag-GFP WT and 5 Rag-GFP CD4-cre Runx1 cKO mice from 5 independent experiments. For the analysis of complement deposition in peripheral blood CD4+ T cells, the results shown are quantified from at least 6 Rag1-GFP WT and 3 Rag1-GFP CD4-cre Runx1 cKO mice. Error bars represent SEM, and significance was determined by unpaired Student's *t* test using GraphPad Prism.

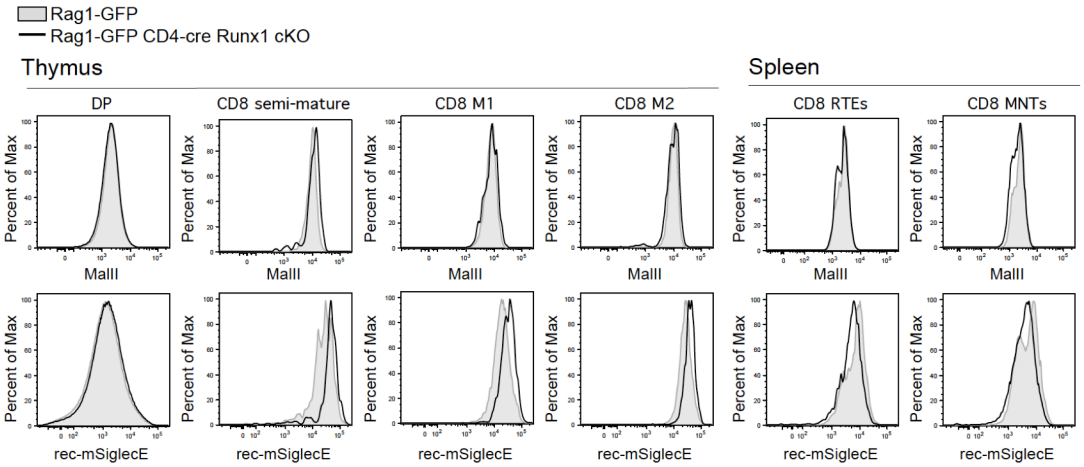


Supplemental Figure 3

IgM, C1q, C4 and C3 are not deposited on CD8$^+$ T cells from CD4-cre Runx1 cKO mice

Splenic CD8$^+$ RTEs and MNTs form Rag1-GFP WT and Rag1-GFP CD4-cre Runx1 cKO mice were examined for the deposition of IgM, C1q, C4 and C3 as denoted in the figure. Cells were incubated in GVB^{++} buffer prior to staining with indicated Abs. CD8$^+$ RTEs were

gated on $CD62L^{+}CD44^{-}$Rag1-GFP^{+} while $CD8^{+}$ MNTs were gated on $CD62L^{+}CD44^{-}$Rag1-GFP^{-}. Data shown are representative of six Rag1-GFP WT and six Rag1-GFP CD4-cre Runx1 cKO mice from five separate experiments.



Supplemental Figure 4

α2,3- and α2,8-sialylation on CD8 SP thymocytes and CD8$^+$ T cells is not altered from CD4-cre Runx1 cKO mice

DP (CD4$^+$CD8$^+$), SM CD8 SP (CD8$^+$CD24^{hi}CCR7^{lo}), M1 CD8 SP (CD8$^+$CD24^{hi}CCR7^{hi}), M2 CD8 SP (CD8$^+$CD24^{lo}CCR7^{hi}) thymocytes, splenic CD8$^+$ RTEs (CD8$^+$CD62L$^+$CD44$^-$Rag1-GFP$^+$), CD8$^+$ MNTs (CD8$^+$CD62L$^+$CD44$^-$Rag1-GFP$^-$) from Rag1-GFP WT (grey histogram) and Rag1-GFP CD4-cre Runx1 cKO mice (solid line) were examined for α2,8-sialylation by using recombinant mSiglec-E Fc chimera (rec mSiglec-E) and α2,3-sialylation using MalII. Thymocytes were first gated on expression of Rag1-GFP to exclude recirculating mature T cells. Representative FACS analysis from at least three mice in each group from three separate experiments is shown.