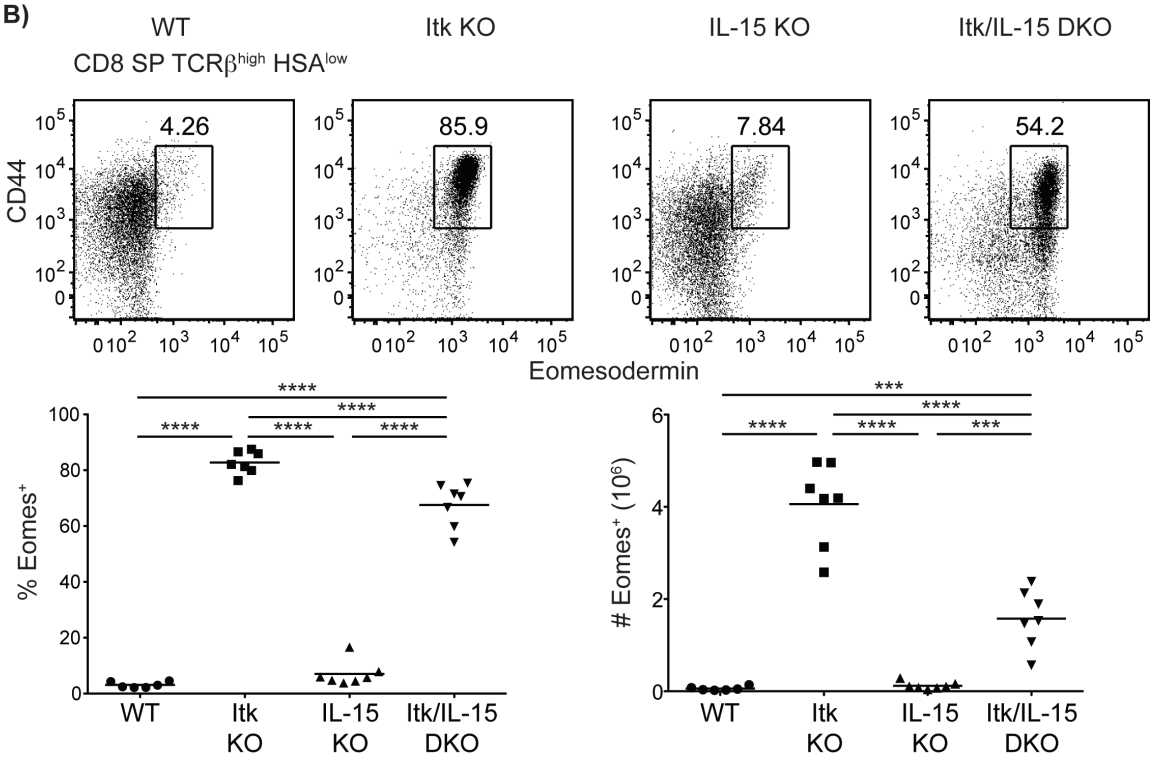
Supplemental Data

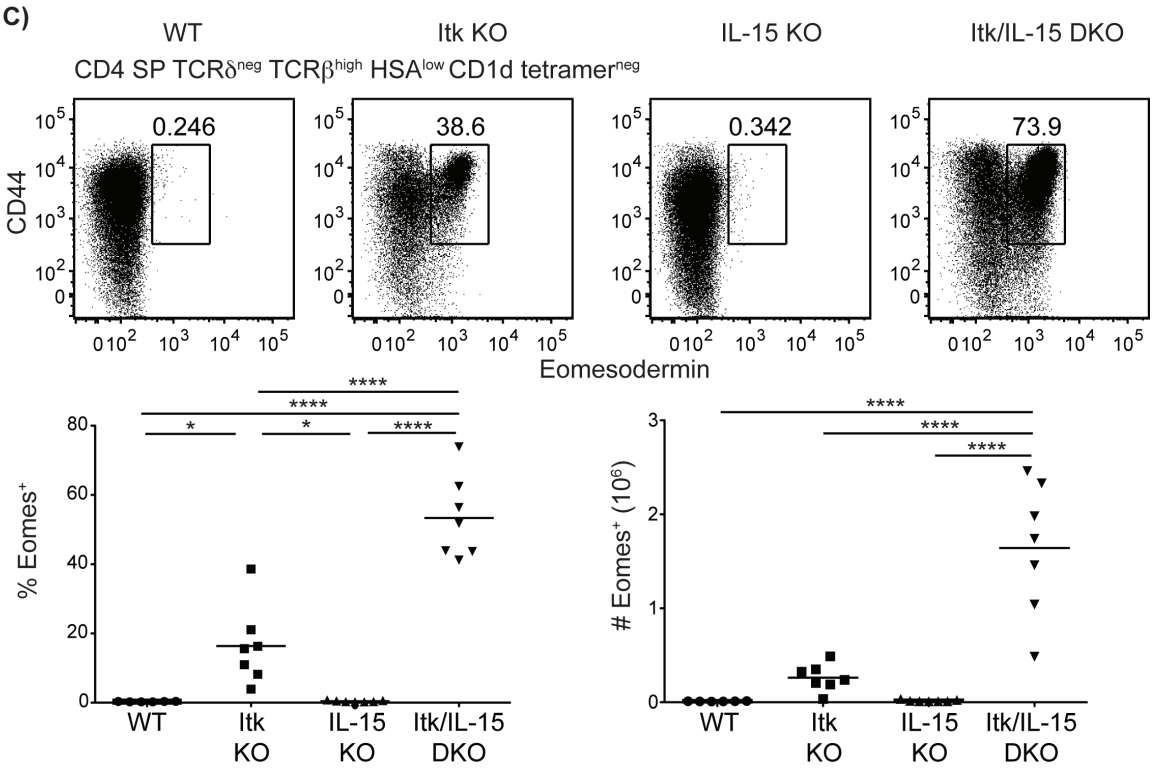
A)

	WT	Itk KO	IL-15 KO	Itk/IL-15 DKO
Thymic Cellularity	1.58×10^8 $\pm 6.12 \times 10^7$	1.03×10^8 $\pm 3.84 \times 10^7$	1.39×10^8 $\pm 3.14 \times 10^7$	7.49×10^7 $\pm 2.07 \times 10^7$

B)



C)



Supplemental Figure 1: IL-15 regulates the expansion of Eomesodermin^{+} αβ T cells.

Thymocytes from WT, *itk*$^{-/-}$, *il15*$^{-/-}$, and *itk*$^{-/-}$*il15*$^{-/-}$ mice were stained with CD1d-tetramer and antibodies to CD4, CD8, TCRδ, TCRβ, HSA (CD24), CD44, and Eomesodermin. Dot-plots show Eomes versus CD44 staining, and graphs show compilations of the percentages and absolute numbers of Eomes^{+} cells.

(A) Total thymic cellularity. Significant differences were seen between WT and *itk*$^{-/-}$ mice ($p < 0.005$) and between *il15*$^{-/-}$ and *itk*$^{-/-}$*il15*$^{-/-}$ mice ($p < 0.05$).

(B) Gated on CD8SP TCRβ^{high} HSA^{low} thymocytes.

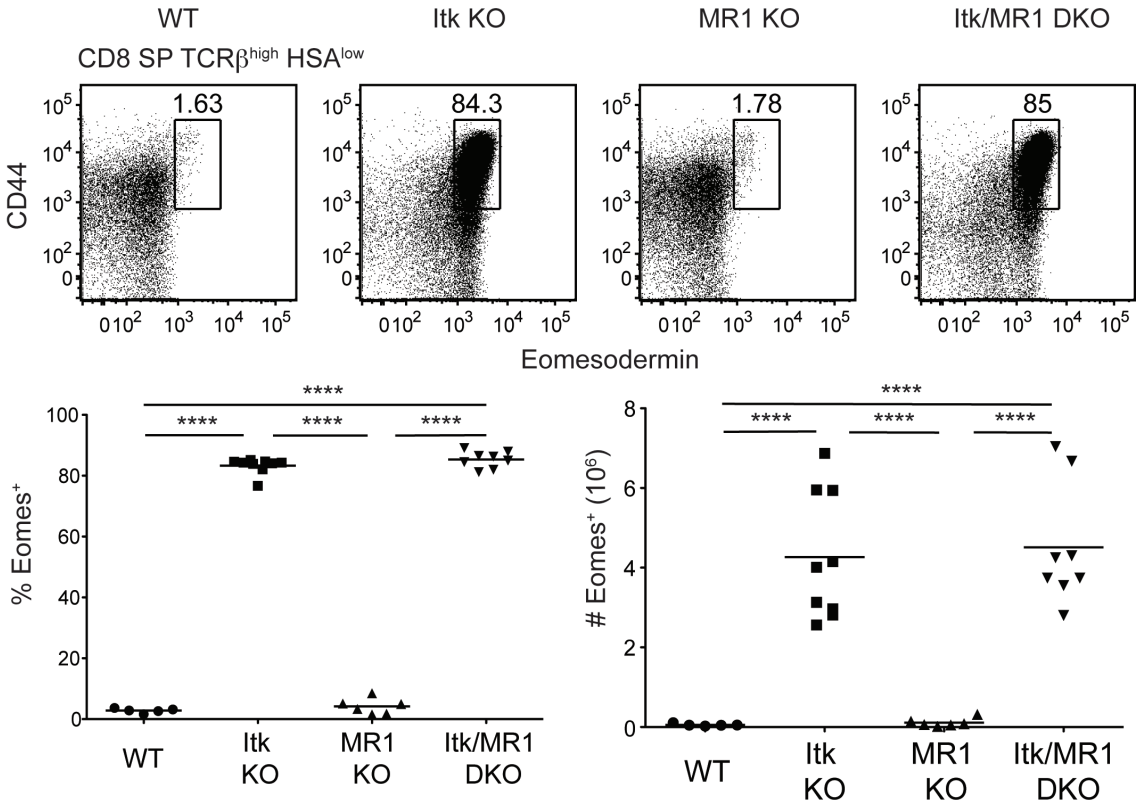
(C) Gated on CD4SP TCRδ^{neg} TCRβ^{high} HSA^{low} CD1d-tetramer^{neg} thymocytes.

n = 6-7 mice per group. Results are from three independent experiments. Statistical analysis performed using a one-way ANOVA. *$p < 0.05$ **$p < 0.05$ ***$p < 0.0005$ ****$p < 0.0001$

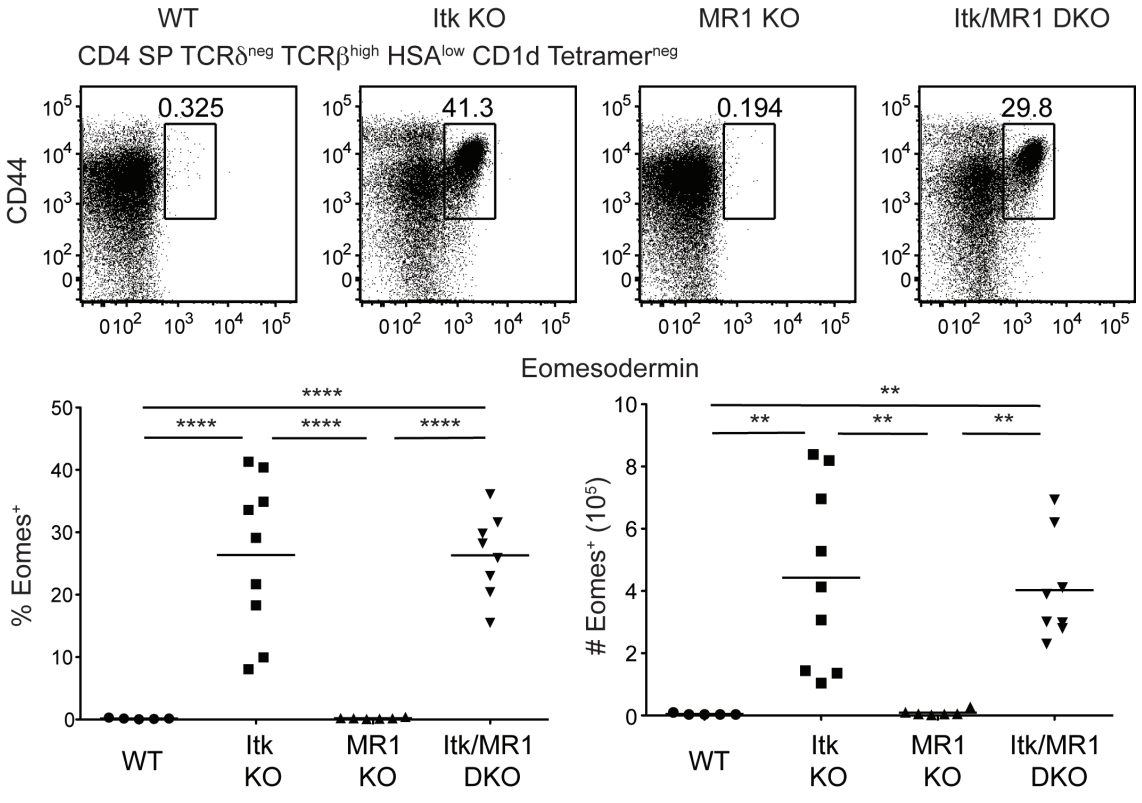
A)

	WT	Itk KO	MR1 KO	Itk/MR1 DKO
Thymic Cellularity	1.27×10^8 $\pm 5.23 \times 10^7$	7.42×10^7 $\pm 2.14 \times 10^7$	1.58×10^8 $\pm 7.62 \times 10^7$	7.81×10^7 $\pm 3.70 \times 10^7$

B)



C)



Supplemental Figure 2: *itk*$^{-/-}$ Eomesodermin^{+} αβ T cells develop independently of MAIT cells.

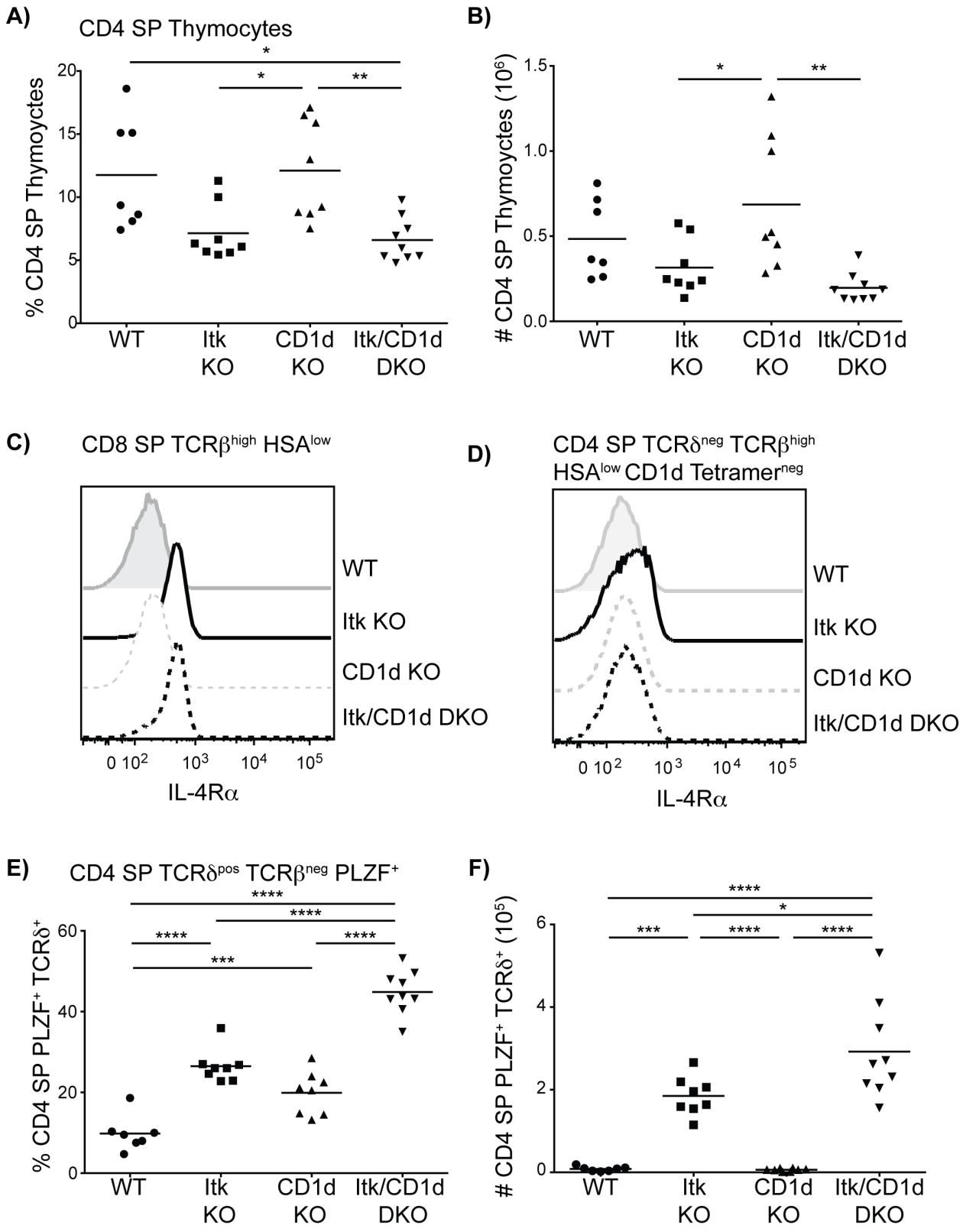
Thymocytes from WT, *itk*$^{-/-}$, *MR1*$^{-/-}$, and *itk*$^{-/-}$*MR1*$^{-/-}$ mice were stained with CD1d-tetramer and antibodies to CD4, CD8, TCRδ, TCRβ, HSA, CD44, and Eomesodermin. Dot-plots show Eomes versus CD44 staining, and graphs show compilations of the percentages and absolute numbers of Eomes^{+} cells.

(A) Total thymic cellularity. Significant differences were seen between *itk*$^{-/-}$ and *MR1*$^{-/-}$ mice ($p < 0.05$) and between *MR1*$^{-/-}$ and *itk*$^{-/-}$*MR1*$^{-/-}$ mice ($p < 0.05$).

(B) Gated on CD8SP TCRβ^{high} HSA^{low} thymocytes.

(C) Gated on CD4SP TCRδ^{neg} TCRβ^{high} HSA^{low} CD1d-tetramer^{neg} thymocytes.

n = 5-8 mice per group. Results are representative of three independent experiments. Statistical analysis performed using a one-way ANOVA. **$p < 0.005$ ****$p < 0.0001$


A)
CD4 SP Thymocytes
% CD4 SP Thymoyctes
WT
Itk KO
CD1d KO
Itk/CD1d DKO
B)
CD4 SP Thymoyctes (10^6)
C)
CD8 SP TCRβ^high HSA^low
WT
Itk KO
CD1d KO
Itk/CD1d DKO
IL-4Rα
D)
CD4 SP TCRδ^neg TCRβ^high
HSA^low CD1d Tetramer^neg
E)
CD4 SP TCRδ^pos TCRβ^neg PLZF+
% CD4 SP PLZF+ TCRδ+
F)
CD4 SP PLZF+ TCRδ+ (10^5)

Supplemental Figure 3: αβ NKT cells promote the development of Eomesodermin^{+} T cells.

Thymocytes from WT, *itk*$^{-/-}$, *cd1d*$^{-/-}$, and *itk*$^{-/-}$*cd1d*$^{-/-}$ mice were stained with CD1d-tetramer and antibodies against CD4, CD8, TCRδ, TCRβ, HSA, IL-4Rα (CD124), and Eomesodermin.

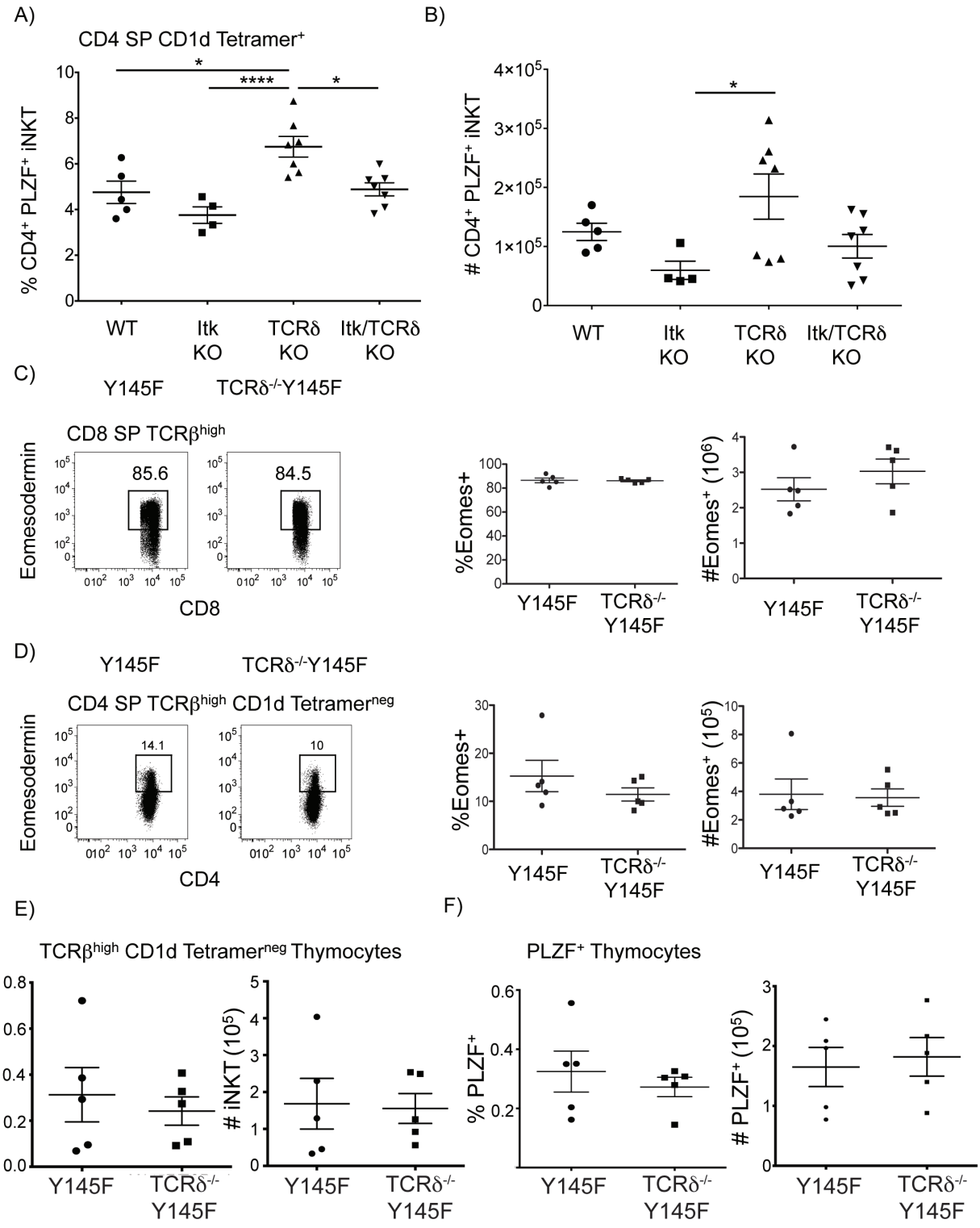
(A-B) Graphs show a compilation of four independent experiments indicating the percentage (A) and the number (B) of CD4 SP thymocytes. *n* = 7-9 mice per group.

(C) Histograms of IL-4Rα (CD124) expression on mature CD8 SP thymocytes (CD8 SP TCRβ^{high} HSA^{low}). Graphs represent one experiment with 2-3 mice per group.

(D) Histograms of IL-4Rα (CD124) expression on mature CD4 SP thymocytes (CD4 SP TCRδ^{neg} TCRβ^{high} HSA^{low} CD1d-tetramer^{neg}). Graphs represent one experiment with 2-3 mice per group.

(E-F) Graphs show a compilation of four independent experiments indicating the percentage (E) and the number (F) of CD4SP TCRδ^{pos} TCRβ^{neg} PLZF^{+} (γδ NKT) thymocytes. *n* = 7-9 mice per group.

Statistical analysis performed using a one-way ANOVA. *p < 0.05 **p < 0.005 ***p < 0.0005 ****p < 0.0001


A)
CD4 SP CD1d Tetramer+
% CD4+ PLZF+ iNKT
WT
Itk KO
TCRδ KO
Itk/TCRδ KO
B)
CD4+ PLZF+ iNKT
C)
Y145F
TCRδ-/-Y145F
CD8 SP TCRβhigh
Eomesodermin
85.6
84.5
CD8
%Eomes+
#Eomes+ (10^6)
D)
CD4 SP TCRβhigh CD1d Tetramerneg
14.1
10
CD4
#Eomes+ (10^5)
E)
TCRβhigh CD1d Tetramerneg Thymocytes
% iNKT
# iNKT (10^5)
F)
PLZF+ Thymocytes
% PLZF+
# PLZF+ (10^5)

Supplemental Figure 4: Eomesodermin^{+} T cells develop independently of γδ T cells.

(A-B) Thymocytes from WT, *itk*$^{-/-}$, *tcrd*$^{-/-}$, and *itk*$^{-/-}$*tcrd*$^{-/-}$ mice were stained with CD1d-tetramer and antibodies to CD4 and CD8. Graphs show compilations of the percentages (A) and absolute numbers (B) of αβ iNKT cells that are gated on CD4 SP CD1d-tetramer^{+} thymoyctes. *n* = 4-7 mice per group. Results are representative of two independent experiments. Statistical analysis was performed using a one-way ANOVA. *p < 0.05, ****p < 0.0001

(C-F) Thymocytes from SLP-76(Y145F) and SLP-76(Y145F)*tcrd*$^{-/-}$ mice were stained with a cell viability dye, CD1d-tetramer, and antibodies to CD4, CD8, TCRβ, and Eomesodermin. *n* = 5 mice per group. Results are representative of two independent experiments. Statistical significance was analyzed in GraphPad Prism using a student's t test.

Dot-plots show (C) Eomes versus CD8 staining or (D) Eomes versus CD4, and graphs show compilations of the percentages and absolute numbers of Eomes^{+} cells.

(C) Gated on CD8SP TCRβ^{high} thymocytes.

(D) Gated on CD4SP TCRβ^{high} CD1d-tetramer^{neg} thymocytes.

(E) Graphs show the frequency (right) and number (left) of αβ iNKT cells (Gated on live thymocytes that are TCRβ^{high} and CD1d-tetramer^{pos}).

(F) Graphs show the frequency (right) and number (left) of total CD4^{+} PLZF^{+} thymocytes (Gated on live PLZF^{+} thymocytes).