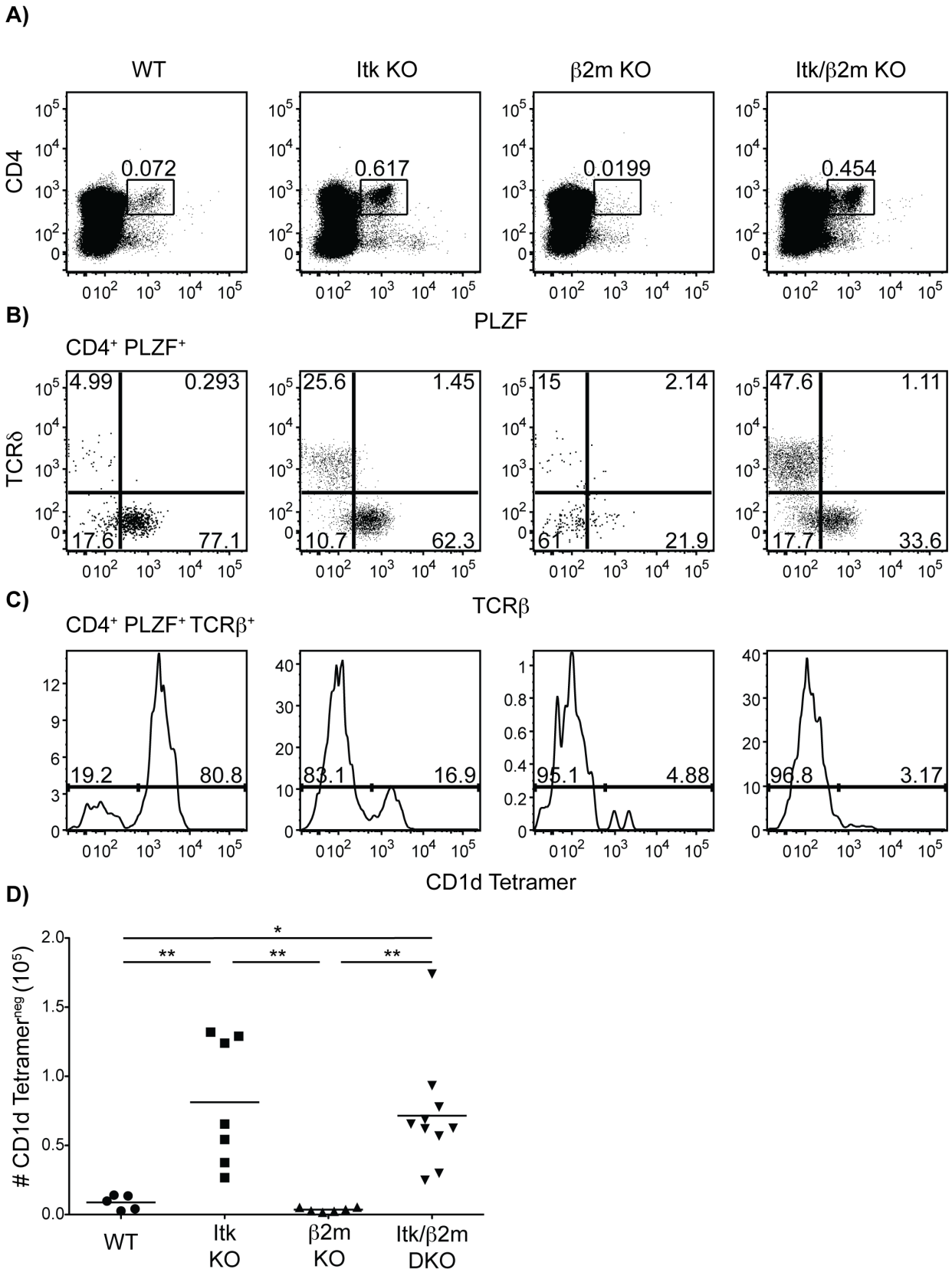
Supplemental Data



Supplemental Figure 1: The development of *itk*$^{-/-}$ CD4^{+} PLZF^{+} αβ T cells is not dependent on β2-microglobulin.

Thymocytes from WT, *itk*$^{-/-}$, *β2m*$^{-/-}$ and *itk/β2m*$^{-/-}$ mice were isolated and stained with CD1d-tetramer and antibodies to CD4, TCRδ, TCRβ, and PLZF.

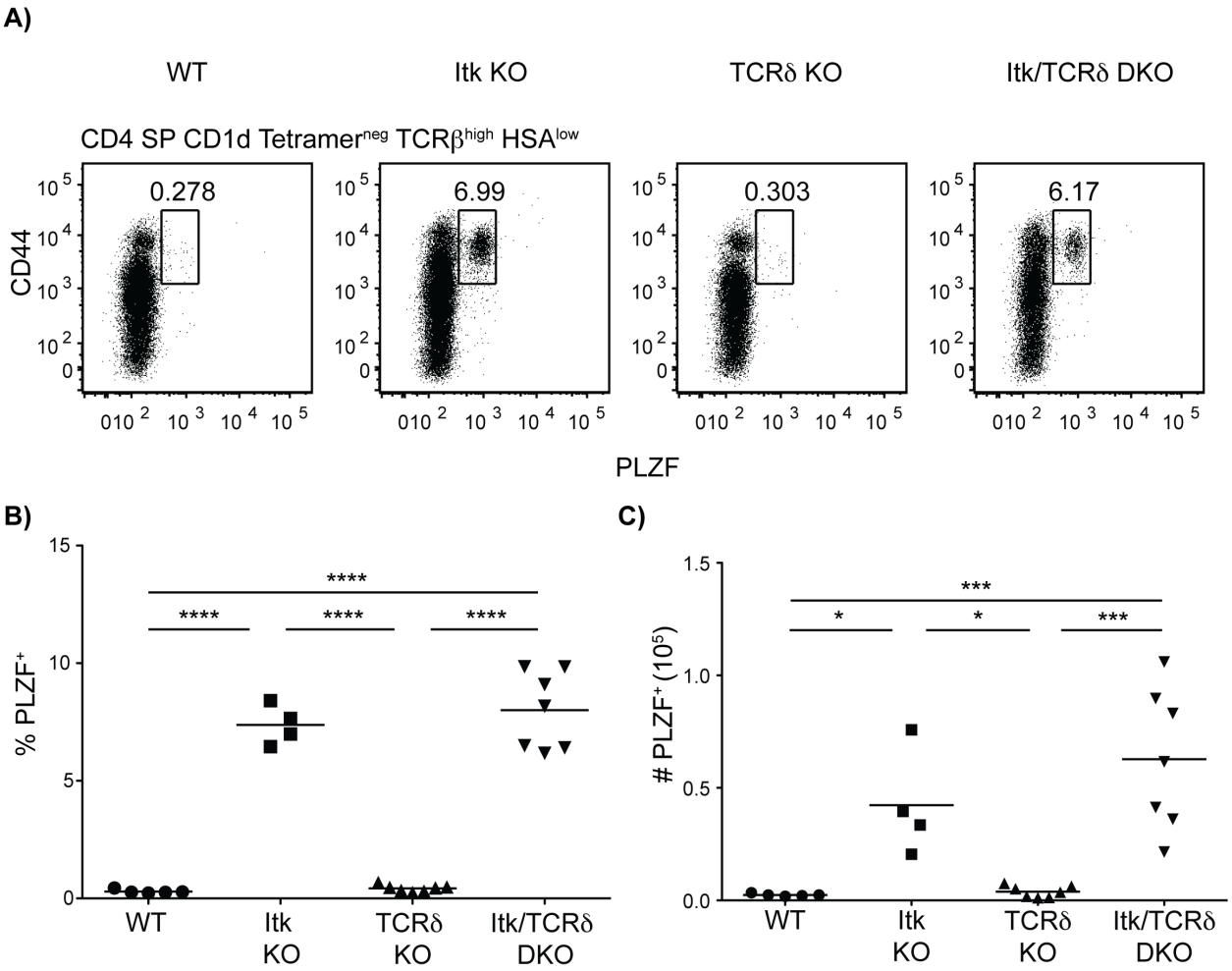
(A) Dot-plots show CD4 versus PLZF staining of total thymocytes; numbers indicate the percentages of CD4^{+} PLZF^{+} cells.

(B) Dot-plots show TCRβ versus TCRδ staining on CD4^{+} PLZF^{+} thymocytes. Numbers indicate the percentages of cells in each quadrant.

(C) Histograms show CD1d-tetramer staining on CD4^{+} PLZF^{+} TCRβ^{+} thymocytes.

(D) Graph shows a compilation of data indicating absolute numbers of CD4^{+} PLZF^{+} TCRβ^{+} CD1d-tetramer^{neg} thymocytes.

n = 5-10 mice per group. Results are representative of three independent experiments. Statistical analysis was performed using a one-way ANOVA. *p < 0.05, **p < 0.005, ****p < 0.0001



Supplemental Figure 2. The development of *itk*$^{-/-}$ innate PLZF^{+} CD4^{+} cells is independent on γδ T cells.

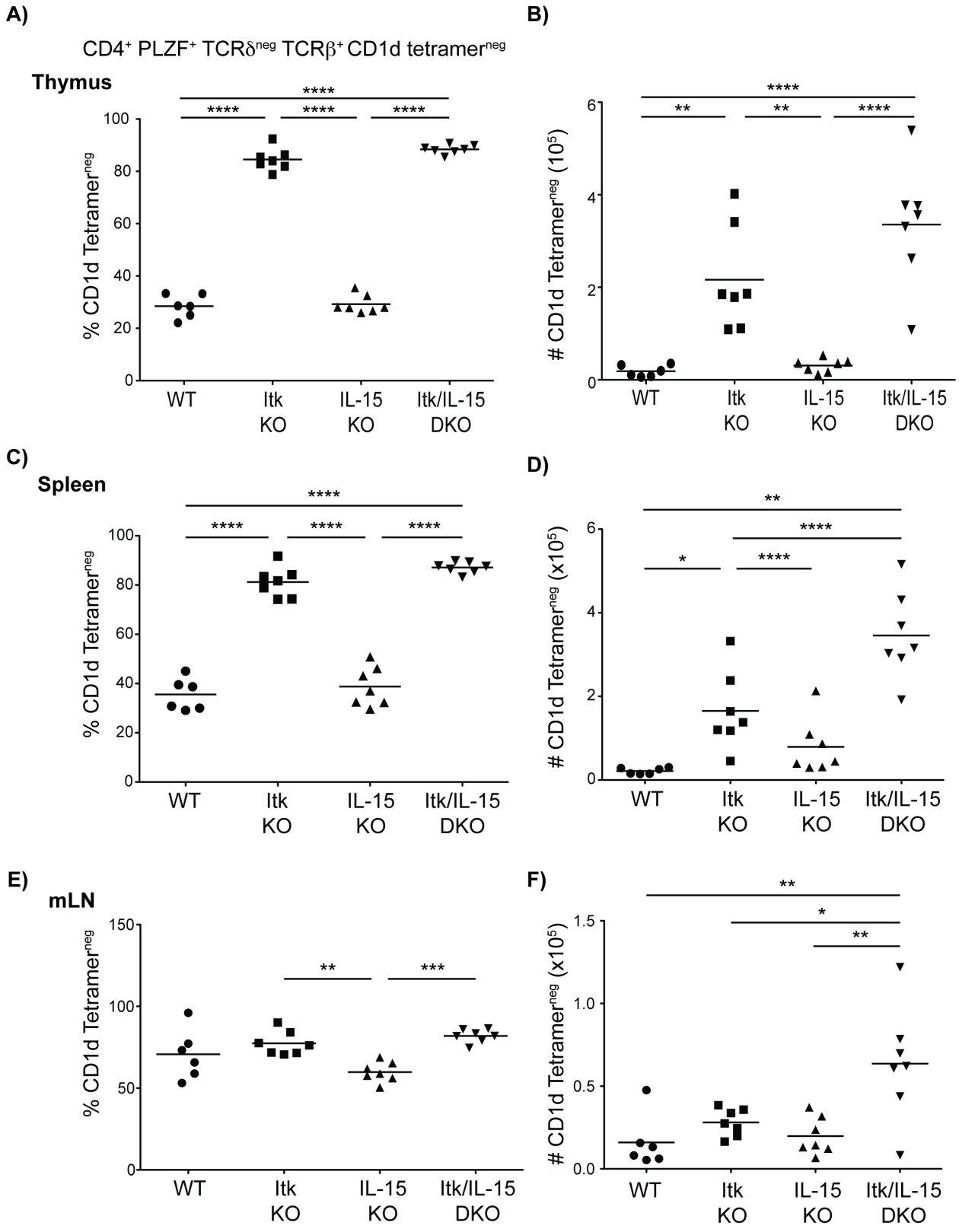
Thymocytes from WT, *itk*$^{-/-}$, *tcrd*$^{-/-}$ and *itk/tcrd*$^{-/-}$ mice were isolated and stained with CD1d-tetramer and antibodies to CD4, CD8, TCRβ, HSA (CD24), and PLZF.

(A) Dot-plots show CD44 versus PLZF staining of CD4SP CD1d Tetramer^{neg} TCRβ^{high} HSA^{low} thymocytes

(B) Frequency of CD44^{high} PLZF^{+} CD4SP CD1d Tetramer^{neg} TCRβ^{high} HSA^{low} thymocytes

(C) Number of CD44^{high} PLZF^{+} CD4SP CD1d Tetramer^{neg} TCRβ^{high} HSA^{low} thymocytes

n = 4-7 mice per group. Results are from two independent experiments. Statistical analysis was performed using a one-way ANOVA. *p < 0.05, ***p < 0.0005, ****p < 0.0001

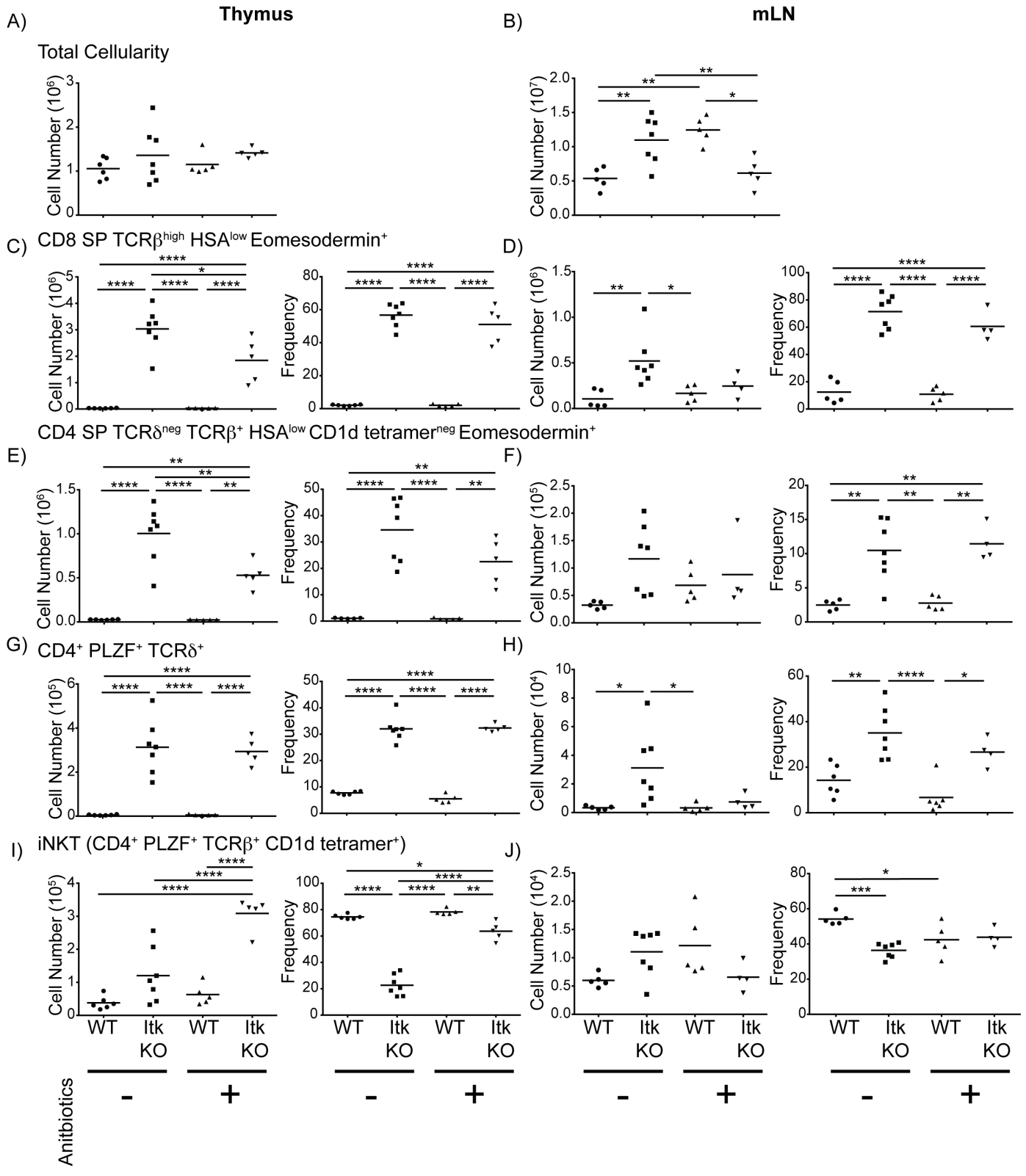

A)
CD4+ PLZF+ TCRδneg TCRβ+ CD1d tetramerneg
Thymus
% CD1d Tetramerneg
B)
CD1d Tetramerneg (10^5)
C)
Spleen
% CD1d Tetramerneg
D)
CD1d Tetramerneg (x10^5)
E)
mLN
% CD1d Tetramerneg
F)
CD1d Tetramerneg (x10^5)
WT
Itk KO
IL-15 KO
Itk/IL-15 DKO

Supplemental Figure 3. Peripheral expansion of *itk*$^{-/-}$ innate PLZF^{+} CD4^{+} αβ T cells increases in the absence of IL-15. Thymocytes from WT, *itk*$^{-/-}$, *il15*$^{-/-}$, and *itk/il15*$^{-/-}$ mice were harvested, processed, and stained with CD1d tetramer and with antibodies against CD4, TCRδ, TCRβ, and PLZF.

(A, C, E) Graphs show compilation of indicating the frequency of CD4^{+} PLZF^{+} TCRδ^{neg} TCRβ^{+} CD1d tetramer^{neg} thymocytes (top), splenocytes (middle), or mesenteric lymphocytes (bottom).

(B, D, F) Graphs show compilation of indicating the number of CD4^{+} PLZF^{+} TCRδ^{neg} TCRβ^{+} CD1d tetramer^{neg} thymocytes (top), splenocytes (middle), or mesenteric lymphocytes (bottom).

n = 6-7 mice per group. Results are representative of three independent experiments. Statistical analysis was performed using a one-way ANOVA. *p < 0.05, **p < 0.005, ***p < 0.0005, ****p < 0.0001


A)
Thymus
B)
mLN
Total Cellularity
Cell Number (10^6)
Cell Number (10^7)
C)
CD8 SP TCRβ^high HSA^low Eomesodermin^+
D)
Cell Number (10^6)
Frequency
CD4 SP TCRδ^neg TCRβ^+ HSA^low CD1d tetramer^neg Eomesodermin^+
E)
F)
Cell Number (10^5)
G)
CD4^+ PLZF^+ TCRδ^+
H)
Cell Number (10^4)
I)
iNKT (CD4^+ PLZF^+ TCRβ^+ CD1d tetramer^+)
J)
WT
Itk KO
Anitbiotics
-
+

Supplemental Figure 4. *itk*$^{-/-}$ innate PLZF^{+} CD4^{+} T cells promote the expansion of innate-like lymphocytes.

Thymocytes and mLN from WT and *itk*$^{-/-}$ mice untreated or treated with antibiotics were isolated and stained with CD1d-tetramer and antibodies to CD4, CD8, TCRδ, TCRβ, HSA, CD44, Eomes, and PLZF.

(A,B) Graph shows a compilation of data indicating the total cellularity of the thymus (A) or mesenteric lymph nodes (B).

(C,D) Graph shows a compilation of data indicating the absolute number (right) or frequency (left) of CD8^{+} TCRβ^{+} HSA^{low} Eomes^{+} lymphocytes from the thymus (C) or mesenteric lymph node (D).

(E,F) Graph shows a compilation of data indicating the absolute number (right) or frequency (left) of CD4^{+} TCRδ^{neg} TCRβ^{+} CD1d tetramer^{neg} HSA^{low} Eomes^{+} lymphocytes from the thymus (E) or mesenteric lymph node (F).

(G,H) Graph shows a compilation of data indicating the absolute number (right) or frequency (left) of CD4^{+} TCRδ^{+} PLZF^{+} lymphocytes from the thymus (G) or mesenteric lymph node (H).

(I,J) Graph shows a compilation of data indicating the absolute number (right) or frequency (left) of iNKT (CD4^{+} TCRδ^{neg} TCRβ^{+} CD1d tetramer^{pos} PLZF^{+}) lymphocytes from the thymus (I) or mesenteric lymph node (J).

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 < 0.005 ***p < 0.0005 ****p < 0.0001