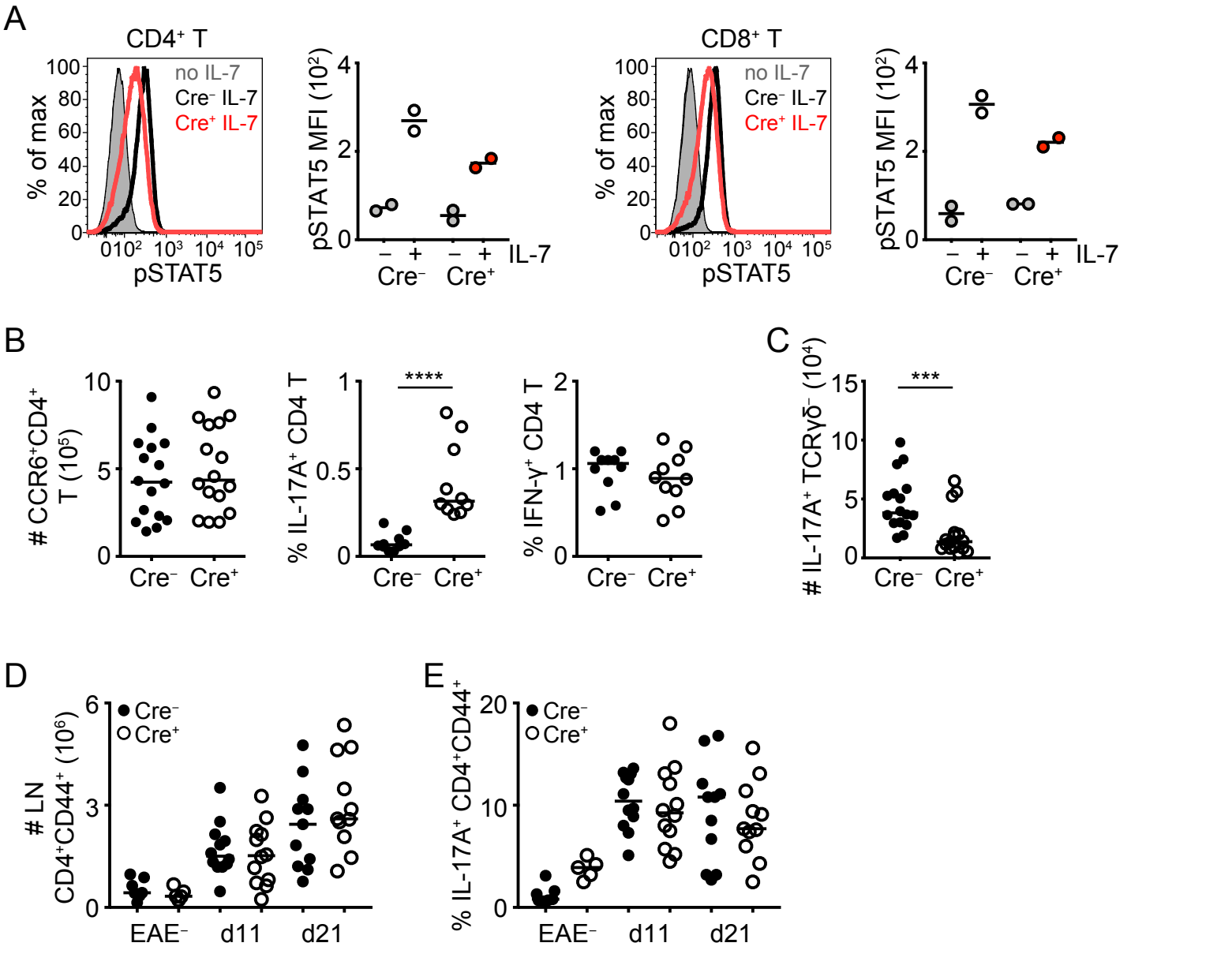
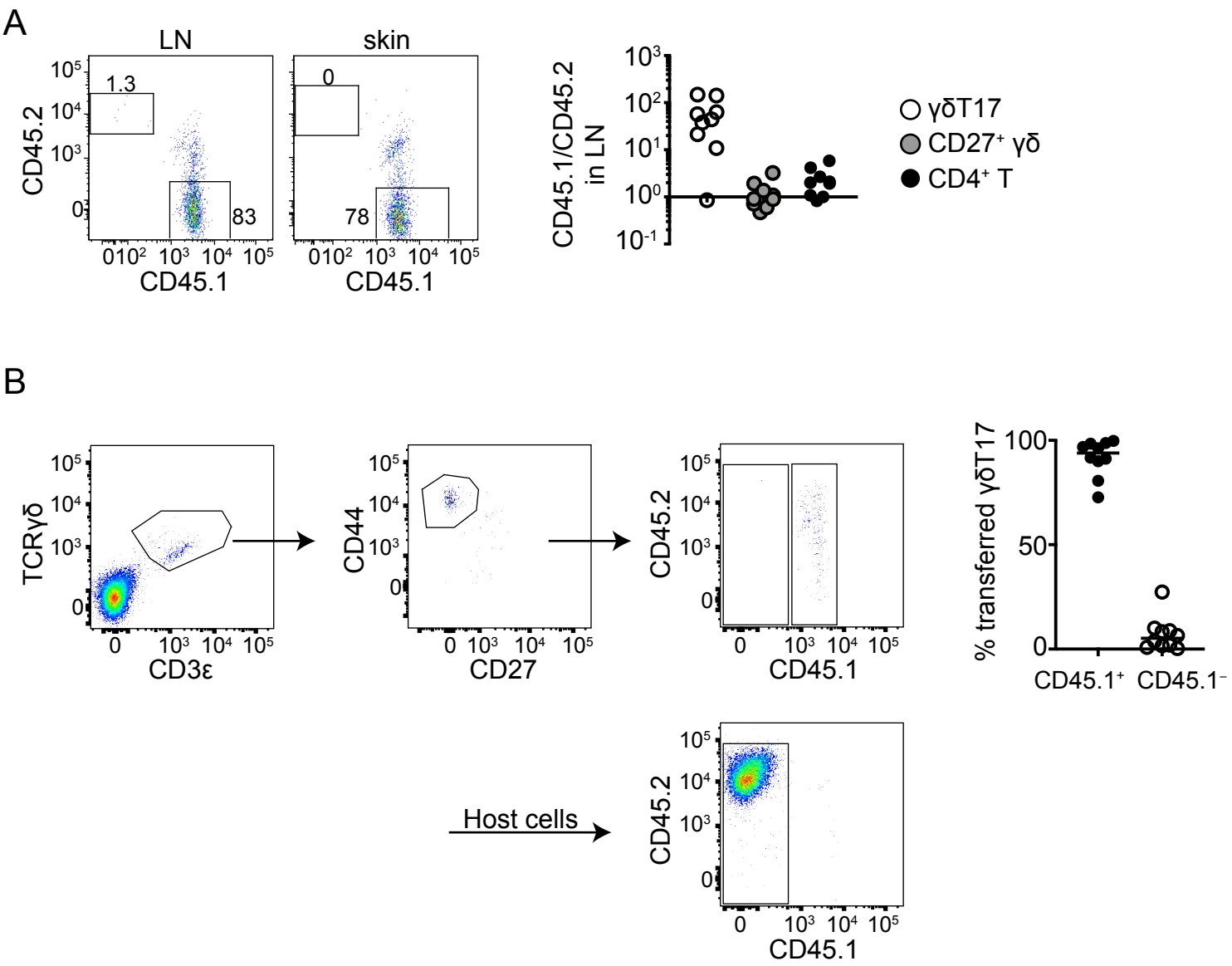
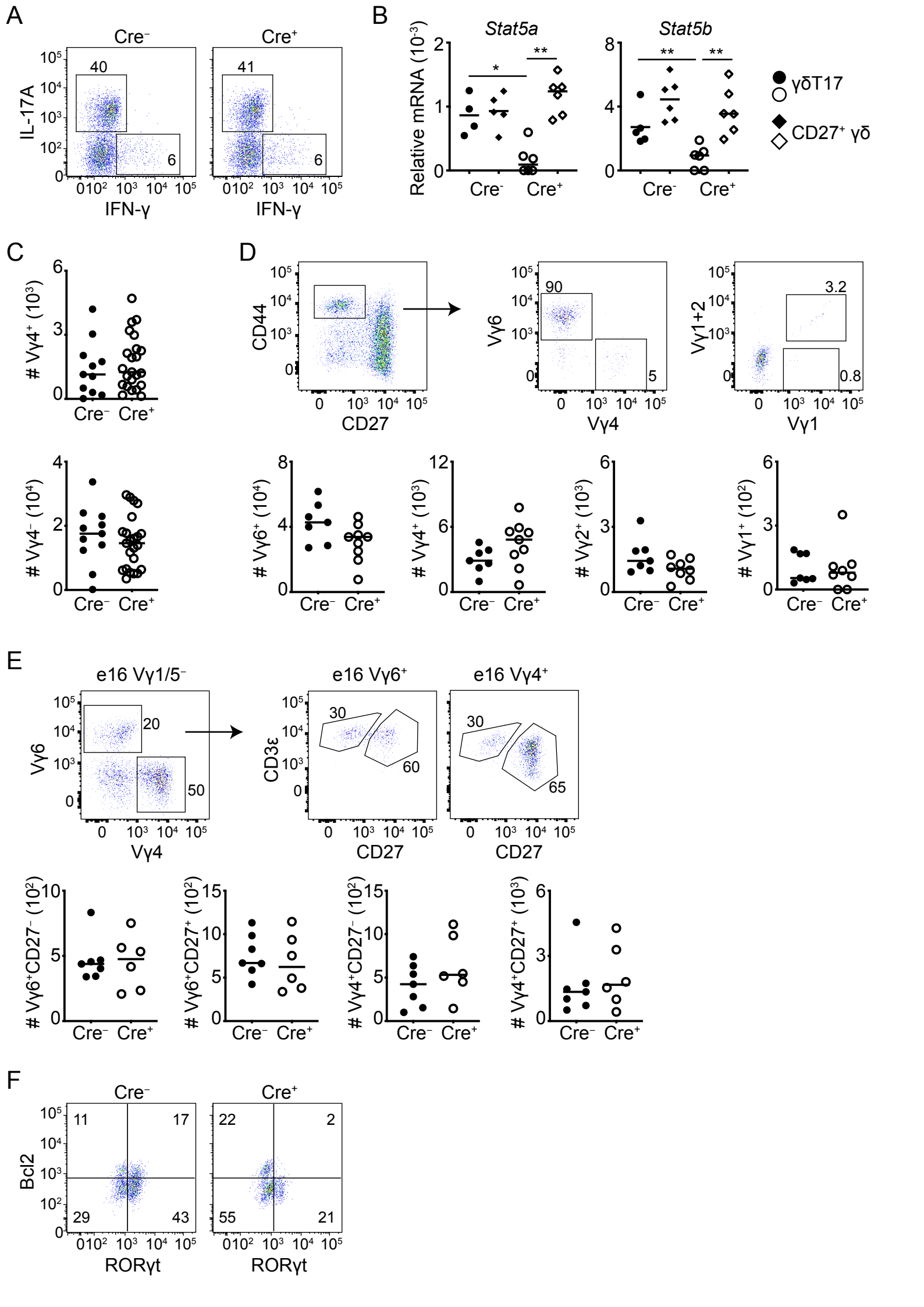


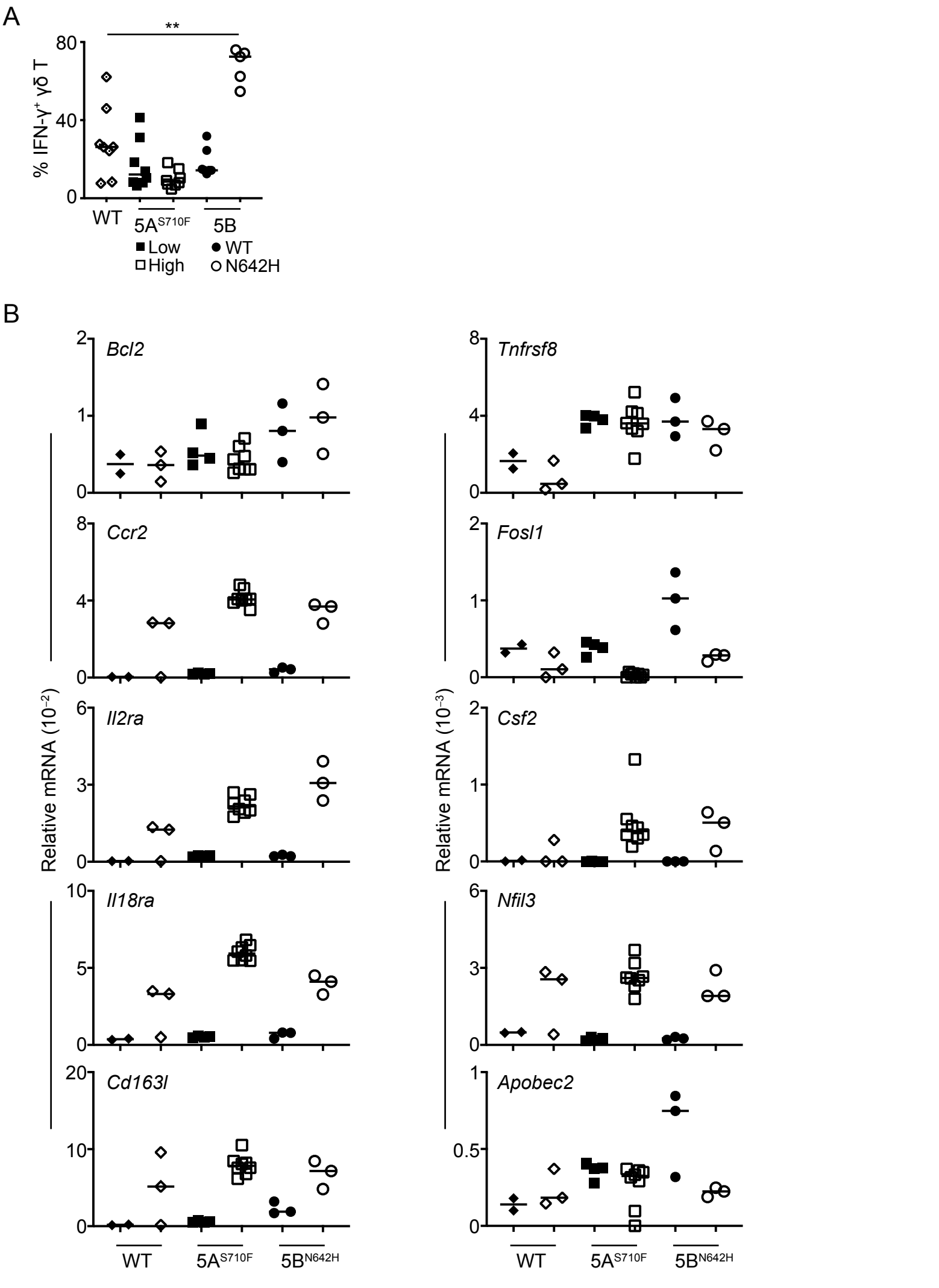
Supplemental Figure 1. CD4⁺, CD8⁺ and non-γδ innate T cells in RORγt^{CRE}-STAT5$^{F/F}$ mice. Flow cytometric analysis of LN CD4⁺, CD8⁺ and non-γδ T cells in RORγt^{CRE}-STAT5$^{F/F}$ (Cre⁺) and littermate control mice (Cre⁻). In graphs each symbol represents a mouse or experiment and line the median. ***p < 0.01, ****p < 0.0001 using Mann-Whitney test. (**A**) Expression of pSTAT5 (histograms) and pSTAT5 mean fluorescent intensity (MFI) in CD4⁺ (left) and CD8⁺ T cells (right) following a 30 minute stimulation with 20 ng/ml recombinant murine IL-7 (representative of 2 experiments). (**B**) Numbers of CD4⁺CCR6⁺ T cells (left), frequency of IL-17A⁺ CD4⁺ T cells (middle) and frequency of IFN-γ⁺ CD4⁺ T cells (right). (**C**) Number of IL-17A⁺ non-γδ T cells. (**D-E**) Numbers of CD4⁺CD44⁺ T cells (**D**) and frequency of IL-17A⁺ CD4⁺CD44⁺ T cells (**E**) in the LNs of unimmunized control mice (EAE⁻) and mice at days 11 and 21 after MOG-CFA immunization. (**A**) n = 2 experiments (pool of 4-5 mice per experiment); (**B** and **C**) n = 16 mice, 5 experiments or n = 10, 3 experiments (cytokines); (**D-E**) n = 5-12 mice, 3 experiments.



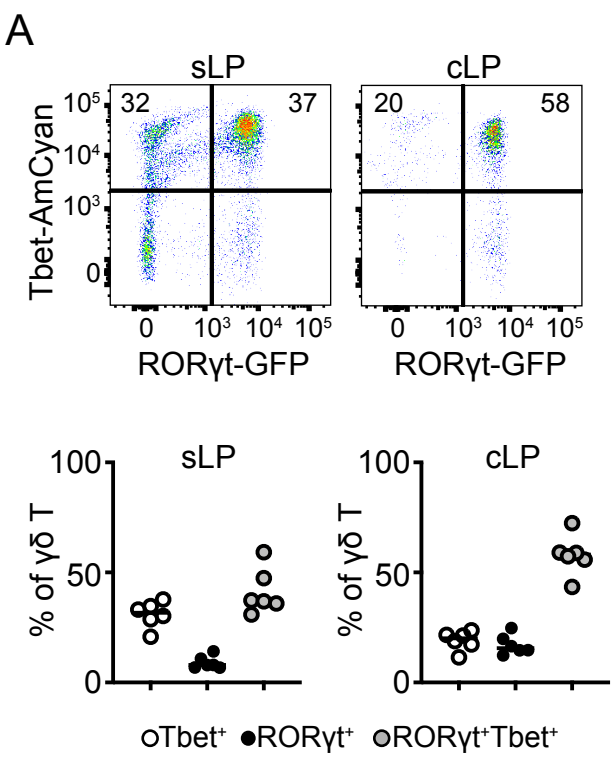
Supplemental Figure 2. Cell-intrinsic requirement of STAT5 in γδT17 cells. Flow cytometric analysis of γδ T cells after BM or neonatal thymic reconstitution. In graphs, each symbol represents a mouse and line the median. (**A**) Dot plots: frequency of CD45.1$^+$ (wild-type) and CD45.2$^+$ (STAT5-deficient) γδT17 cells from mixed BM chimera hosts (CD45.1$^+$CD45.2$^+$) in the LN and skin; graph shows the ratio of CD45.1$^+$/CD45.2$^+$ cells in the LN (each symbol represents a mouse); total of 9 chimeras. (**B**) Upper panel dot plots: frequency of CD45.1$^+$ (wild-type) and CD45.1$^-$ (STAT5-deficient) γδT17 cells in the LN of RAG1 mice reconstituted 3 weeks earlier with neonatal thymic γδ T cells. Lower panel dot plot indicates CD45.1 and CD45.2 in host cells; n = 10 mice, 2 experiments.

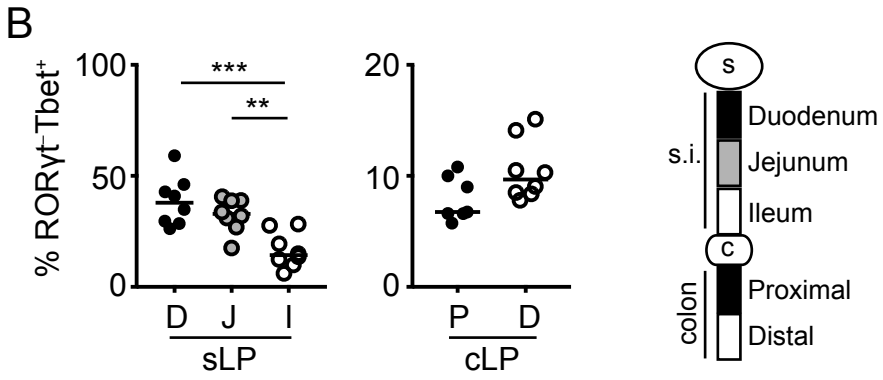

A
Cre⁻
Cre⁺
IL-17A
IFN-γ
40
6
41
6
B
Stat5a
Stat5b
Relative mRNA (10⁻³)
Cre⁻
Cre⁺
γδT17
CD27⁺ γδ
C
Vγ4⁺ (10³)
Vγ4⁻ (10⁴)
D
CD44
CD27
Vγ6
Vγ4
90
5
Vγ1+2
Vγ1
3.2
0.8
Vγ6⁺ (10⁴)
Vγ4⁺ (10³)
Vγ2⁺ (10³)
Vγ1⁺ (10²)
E
e16 Vγ1/5⁻
e16 Vγ6⁺
e16 Vγ4⁺
Vγ6
Vγ4
20
50
CD3ε
CD27
30
60
30
65
Vγ6⁺CD27⁻ (10²)
Vγ6⁺CD27⁺ (10²)
Vγ4⁺CD27⁻ (10²)
Vγ4⁺CD27⁺ (10³)
F
Bcl2
RORγt
11
17
29
43
22
2
55
21

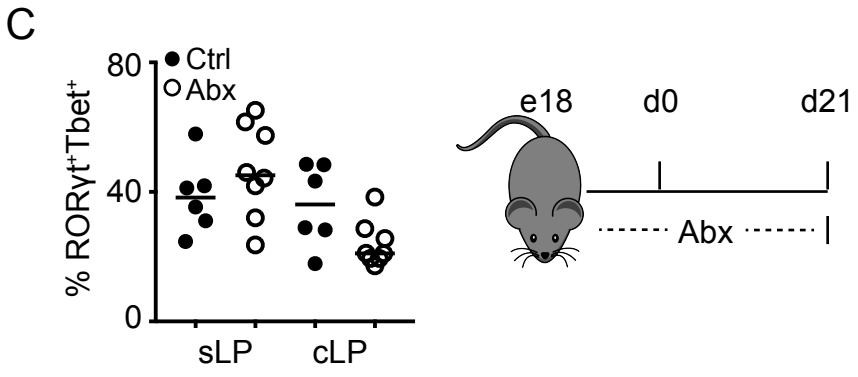
Supplemental Figure 3. Impact of STAT5 during embryonic and neonatal γδT17 cell development. Flow cytometric analysis of γδ T cells in RORγt^{CRE}-STAT5$^{F/F}$ (Cre^{+}) and littermate control mice (Cre^{-}). In graphs, each symbol represents a mouse and line the median. *p < 0.05, **p < 0.01 using Mann-Whitney test. (**A**) Expression of IL-17A and IFN-γ in thymic γδ T cells one day after birth (representative of two litters with 2-5 pups per genotype per litter). (**B**) Expression of *Stat5a* and *Stat5b* in sorted thymic CD27^{-}CD44^{+}CCR6^{+} (γδT17) and CD27^{+} γδ T cells one day after birth. (**C**) Numbers of Vγ4^{+} and Vγ4^{-} d2 thymic γδT17 cells. (**D**) Expression of the indicated Vγ chains in d2 γδT17 cells and numbers of Vγ6-, Vγ4-, Vγ2- and Vγ1-expressing cells γδT17. (**E**) Expression of Vγ6/Vγ4 and CD27/CD3 in Vγ5^{-}Vγ1^{-} γδ T cells at embryonic day e16 in the thymus and numbers of Vγ6- and Vγ4-expressing γδ T cells. (**F**) Expression of BCL2 and RORγt in γδ T cells from LN of day 7 old mice (numbers indicate percent of expression; representative of two litters with 3-5 pups per genotype per litter). (**B**) n = 4-6 mice, 2 experiments (sorts); (**C**) n = 11 (d2) and 9 (d7) Cre^{-} mice and 23 (d2) and 12 (d7) Cre^{+} mice, 3 experiments; (**D**) n = 7 Cre^{-} mice and 9 Cre^{+} mice, 3 experiments; (**E**) n = 7 Cre^{-} mice and 6 Cre^{+} mice, 2 experiments.

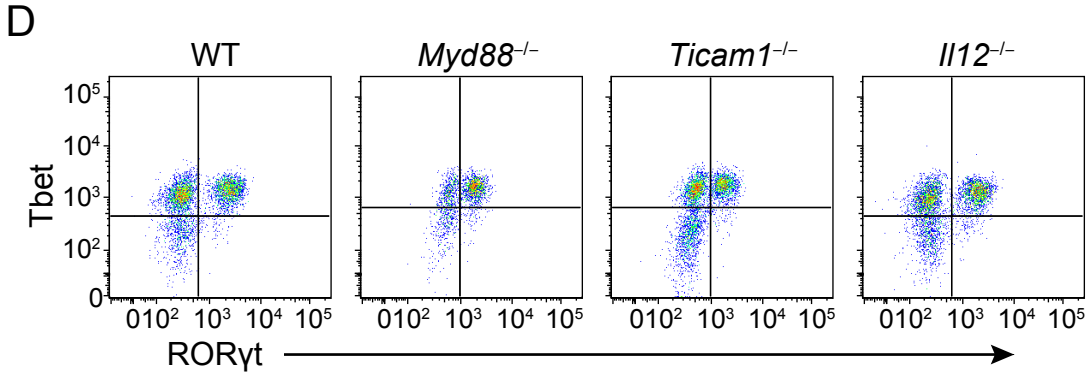

A
**
80
40
0
% IFN-γ⁺ γδ T
WT
5A S710F
5B
Low
High
WT
N642H
B
Bcl2
Tnfrsf8
Ccr2
Fosl1
Il2ra
Csf2
Il18ra
Nfil3
Cd163l
Apobec2
Relative mRNA (10⁻²)
Relative mRNA (10⁻³)
WT
5A S710F
5B N642H
CD27⁺ γδ: closed symbols
γδT17:open symbols

Supplemental Figure 4. Impact of hyperactive *STAT5A* and *STAT5B* in γδ T cells. Flow cytometric and gene expression analysis of γδ T cells in *STAT5A* and *STAT5B* hyperactive mutant mice as described in Figure 2. **p < 0.01 using Mann-Whitney test (**A**) Expression of IFN-γ within the LN γδ T cell compartment of the indicated *STAT5A* and *STAT5B* hyperactive mutant mice or WT control mice. (**B**) Expression of the indicated STAT5A/B target genes in sorted $CD27^{-}CD44^{+}CCR6^{+}$ (γδT17) and $CD27^{+}$ γδ T cells from $STAT5AS710F^{High}$ (n = 4-8), STAT5BN642H (n = 3-4) and WT (n = 2) mice.

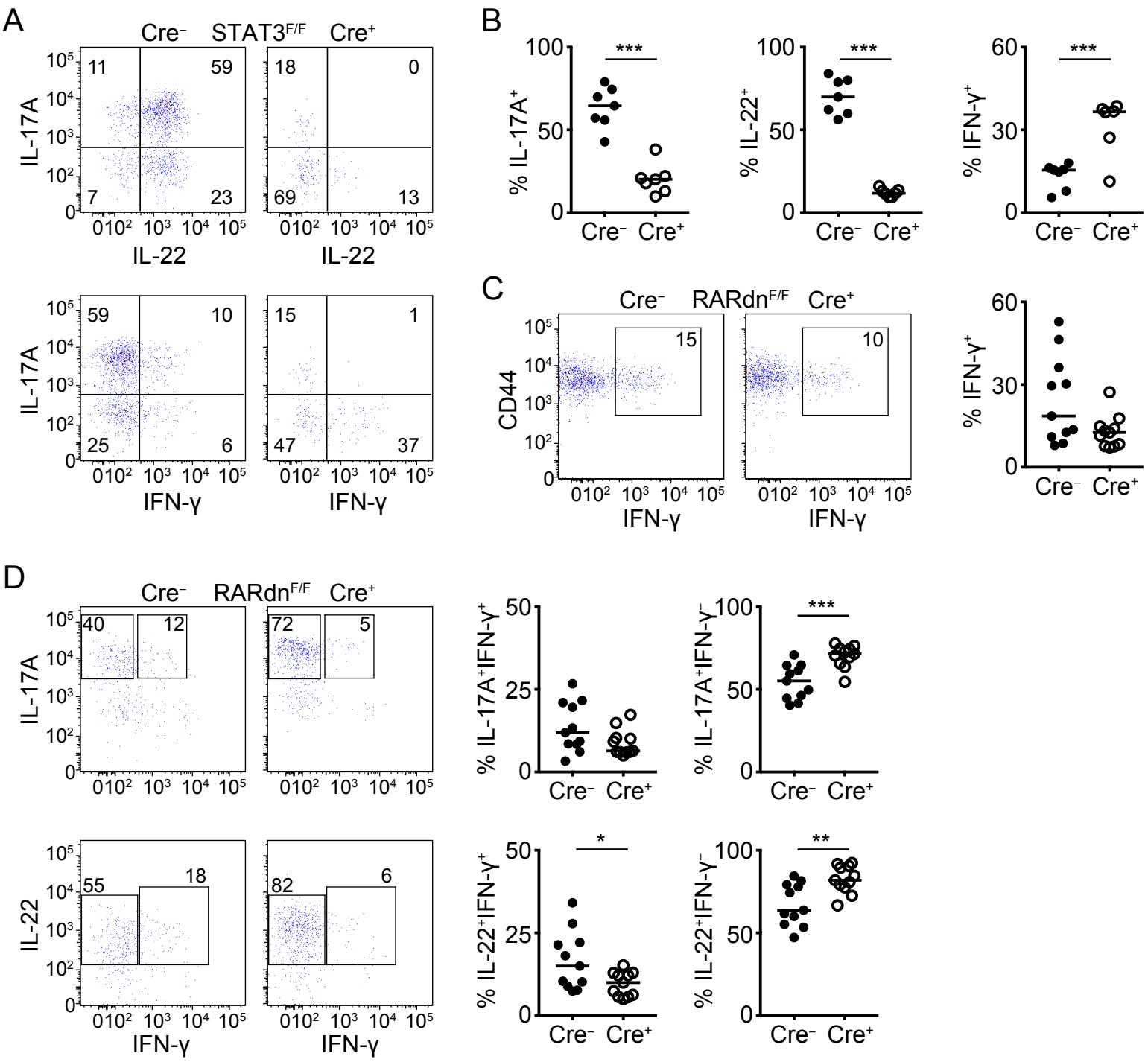

A
sLP
cLP
32
37
20
58
Tbet-AmCyan
RORγt-GFP
% of γδ T
Tbet+
RORγt+
RORγt+Tbet+


B
% RORγt-Tbet+
***
**
D J I
sLP
P D
cLP
s
Duodenum
s.i.
Jejunum
Ileum
c
colon
Proximal
Distal

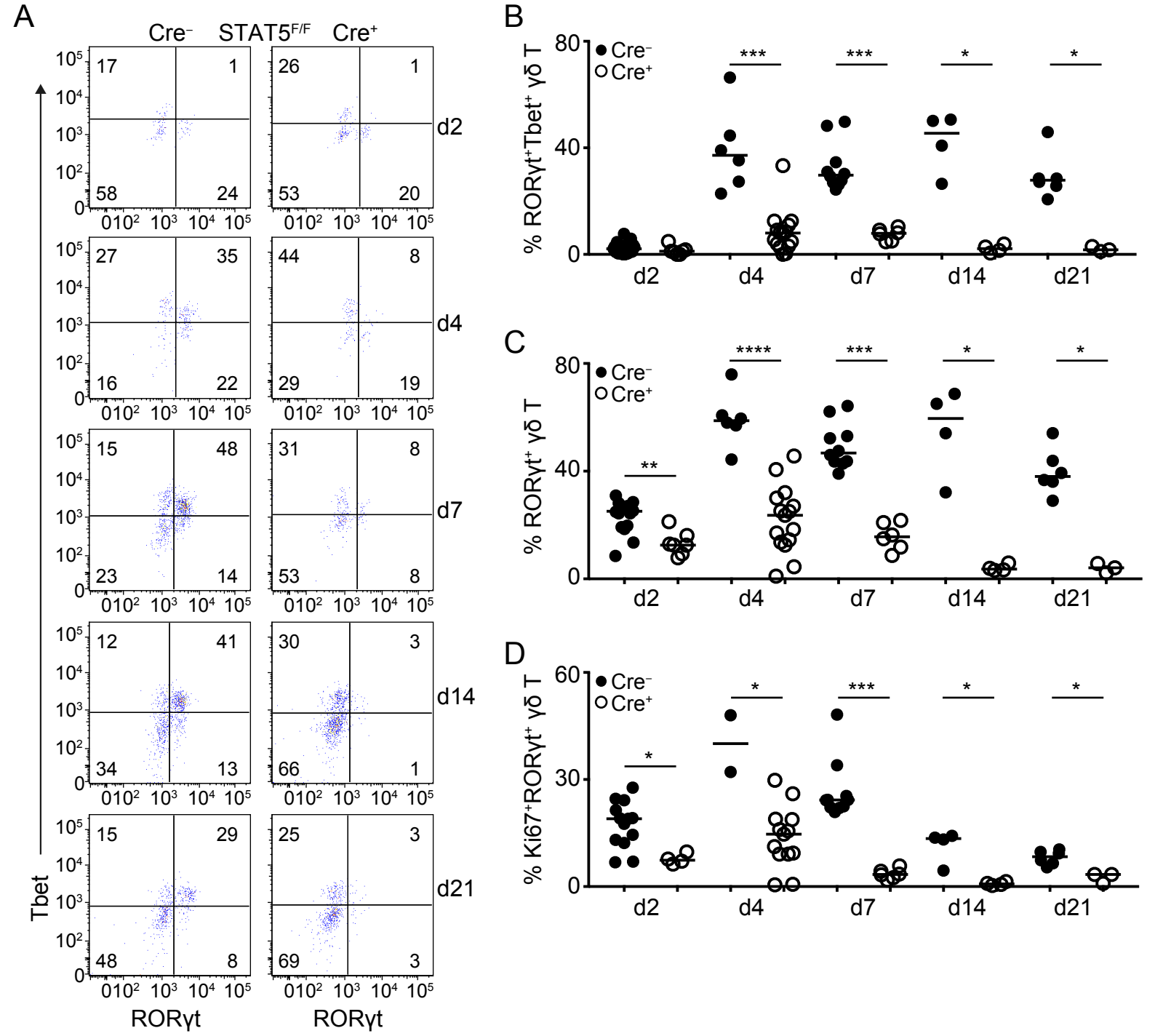

C
Ctrl
Abx
% RORγt+Tbet+
sLP
cLP
e18
d0
d21
Abx


D
WT
Myd88-/-
Ticam1-/-
Il12-/-
Tbet
RORγt

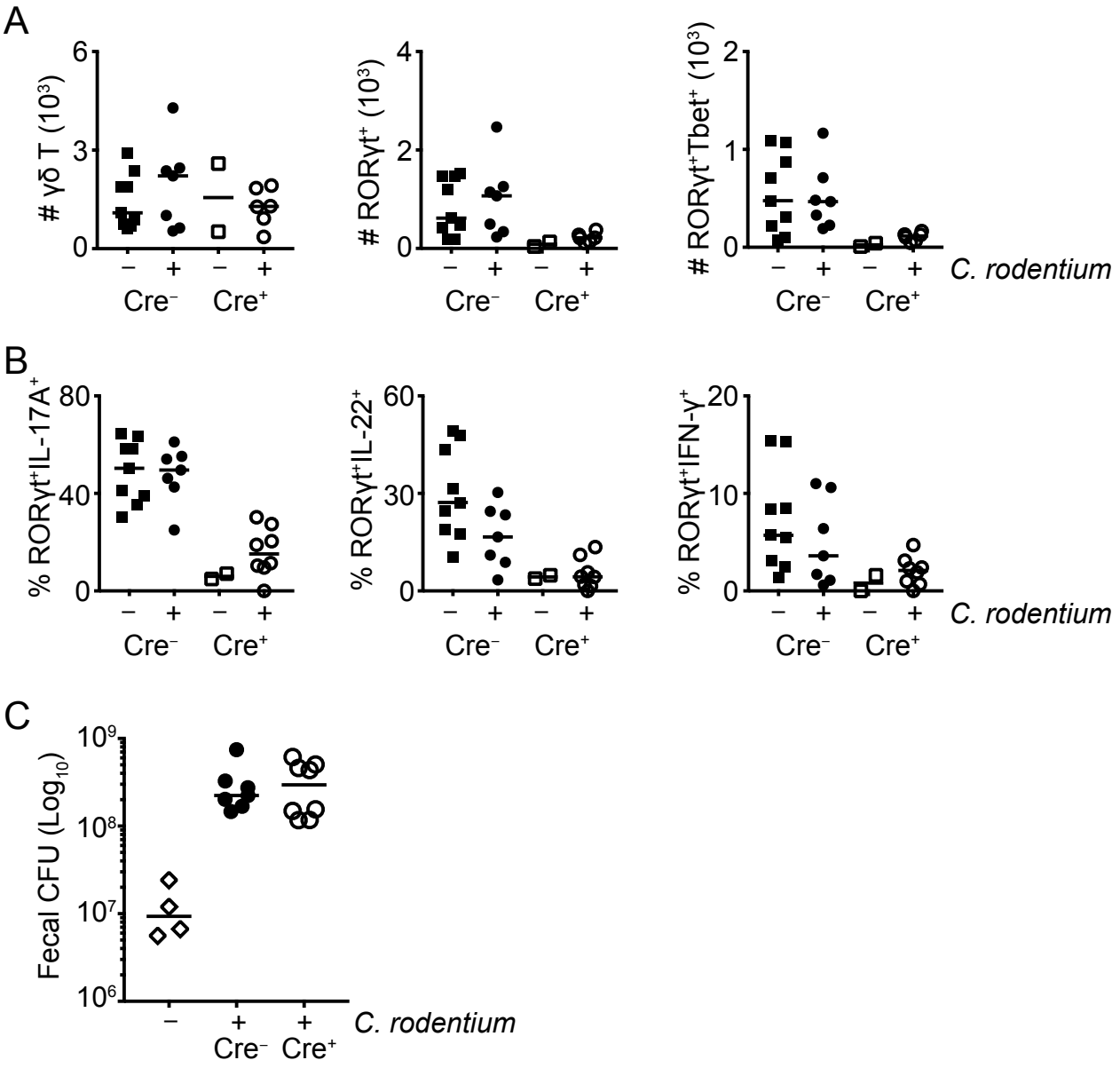
Supplemental Figure 5. Characterization of a novel Tbet^{+} γδT17 population in the intestinal lamina propria at steady-state. Flow cytometric analysis of intestinal γδ T cells from the indicated treatments and mouse strains. In graphs each symbol represents a mouse and line the median. **p < 0.01, ***p < 0.001, using Mann-Whitney test. (**A**) Dot plots: expression of AmyCyan and GFP in cLP and sLP γδ T cells from double transgenic mice reporting AmCyan under the control of the Tbet promoter and GFP under the control of the RORγt promoter; Graphs: frequency of RORγt^{+}Tbet^{+}, RORγt^{+} and Tbet^{+} γδ T cells in the sLP and cLP using the double-reporter. (**B**) Frequency of RORγt^{-}Tbet^{+} cells within the γδ T cell compartment in the indicated small intestinal and colonic segments of WT C57BL/6 mice. (**C**) E18 pregnant mice were treated with an antibiotics (Abx) cocktail (see Methods) in the drinking water until their pups were analyzed at 21 days old. Graph shows the frequency of RORγt^{+}Tbet^{+} cells within the sLP and cLP γδ T cell compartment in these pups. (**D**) Expression of RORγt and Tbet in the cLP γδ T cell compartment in the indicated knockout mouse strains. (**A**) n = 6 mice, 2 experiments; (**B**) n = 8 mice, 4 experiments; (**C**) n = 6-8 mice, 2 experiments; (**D**) n = 2-4 mice per strain.



Supplemental Figure 6. STAT3 and retinoic acid receptor signaling regulate cytokine production in intestinal γδT17 cells. Flow cytometric analysis of small intestinal γδ T cells in RORγt^{CRE}-STAT3$^{F/F}$ or RORγt^{CRE}-RARdn$^{F/F}$ (Cre^{+}) and littermate control mice (Cre^{-}) following IL-23 stimulation. In graphs each symbol represents a mouse and line the median. *p < 0.05, **p < 0.01, ***p < 0.001 using Mann-Whitney test. (**A**) Expression of IL-17A and IL-22 (top) or IL-17A and IFN-γ (bottom) in RORγt^{CRE}-STAT3$^{F/F}$ (Cre^{+}) and littermate control mice (Cre^{-}). Numbers indicate percent of positive expression. (**B**) Frequency of IL-17A^{+}, IL-22^{+} and IFN-γ^{+} γδ T cells in RORγt^{CRE}-STAT3$^{F/F}$ (Cre^{+}) and littermate control mice (Cre^{-}). (**C**) Expression of CD44 and IFN-γ (dot plots) and frequency of IFN-γ^{+} γδ T cells (graph) in RORγt^{CRE}-RARdn$^{F/F}$ (Cre^{+}) and littermate control mice (Cre^{-}). (**D**) Expression of IL-17A and IFN-γ (top dot plots) or IL-22 and IFN-γ (bottom dot plots) with graphical representation of the frequency of IL-17A^{+}IFN-γ^{+} and IL-17A^{+}IFN-γ^{-} or IL-22^{+}IFN-γ^{+} and IL-22^{+}IFN-γ^{-} γδ T cells in RORγt^{CRE}-RARdn$^{F/F}$ (Cre^{+}) and littermate control mice (Cre^{-}). (**A**-**B**) n = 7 mice, 2 experiments; (**C**-**D**) n = 11 mice, 5 experiments.



Supplemental Figure 7. STAT5 regulates the neonatal fate of intestinal Tbet⁺ γδT17 cells. Flow cytometric analysis of small intestinal γδ T cells in RORγt^{CRE}-STAT5$^{F/F}$ (Cre⁺) and littermate control mice (Cre⁻) during neonatal ontogeny. Day of birth is counted as day(d)1. In graphs each symbol represents a mouse and line the median. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 using Mann-Whitney test. (**A**) Expression of RORγt and Tbet within the γδ T cell compartment of sLP at the indicated days after birth. Numbers indicate percent of RORγt and Tbet expression. (**B**) Frequency of sLP RORγt⁺Tbet⁺ γδ T cells at the indicated days after birth. (**C**) Frequency of sLP RORγt⁺ γδ T cells (including Tbet⁺) at the indicated days after birth. (**D**) Frequency of sLP Ki67⁺RORγt⁺ γδ T cells at the indicated days after birth. (**A**-**D**) = 2-16 mice from at least 5 different litters.



Supplemental Figure 8. Intestinal γδT17 cells are not required for neonatal bacterial infection. Flow cytometric analysis of cLP γδ T cells in RORγt^{CRE}-STAT5$^{F/F}$ (Cre$^+$) and littermate control mice (Cre$^-$) before or after infection with *Citrobacter rodentium*. In graphs, each symbol represents a mouse and line the median. Cytokine detection was performed following IL-23 re-stimulation. (**A**-**B**) Day 10-12 old pups were infected orally with *C. rodentium* and cLP cells were analyzed 6 days later. (**A**) Numbers of total (left), RORγt$^+$ (middle) and RORγt$^+$Tbet$^+$ (right) γδ T cells in infected and uninfected mice. (**B**) Frequency of RORγt$^+$IL-17A$^+$ (left), RORγt$^+$IL-22$^+$ (middle) and RORγt$^+$IFN-γ$^+$ (right) γδ T cells in infected and uninfected mice. (**C**) Fecal colony forming units (CFU) in infected and uninfected mice detected by qPCR. (**A**-**B**) n = 5-9 mice, 3 experiments; (**C**) n = 4 control and 7-8 infected mice; 3 experiments.