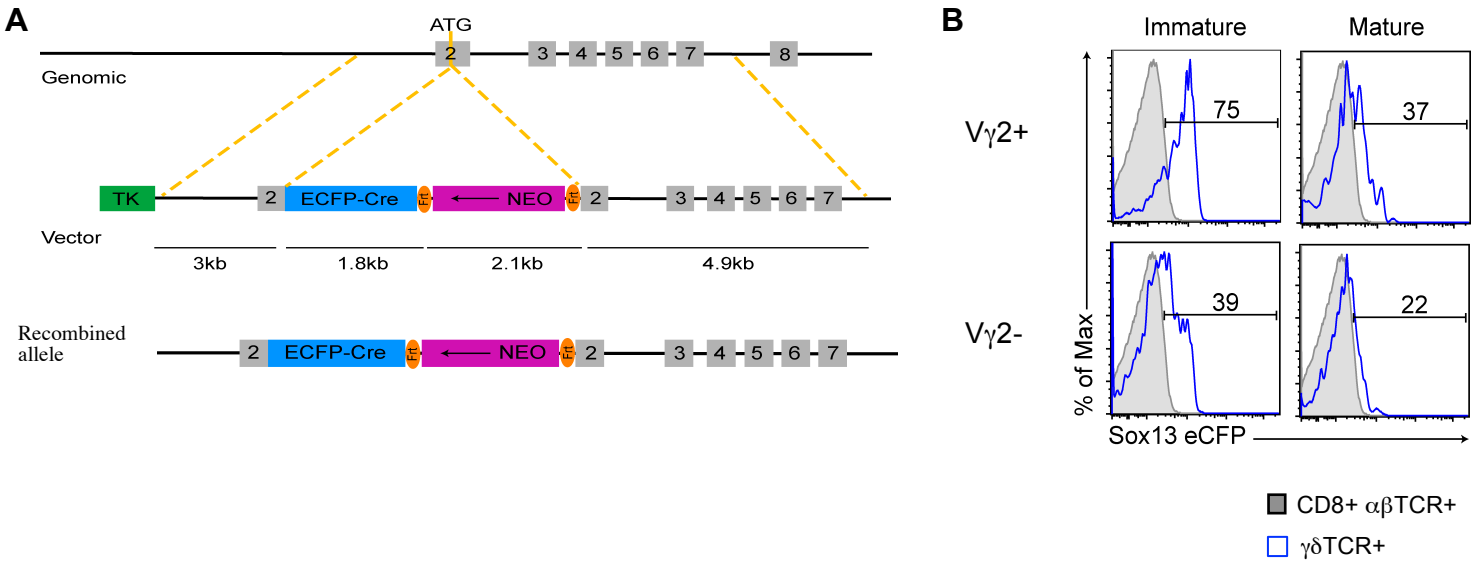

A
ATG
Genomic
TK
ECFP-Cre
NEO
Vector
3kb
1.8kb
2.1kb
4.9kb
Recombined allele
B
Immature
Mature
Vγ2+
Vγ2-
75
37
39
22
% of Max
Sox13 eCFP
CD8+ αβTCR+
γδTCR+
C
αβ T
Stage 1 NKT
Stage2/3 NKT
WT
Sox13 KI
0.32
25
6.4
Sox13 eCFP
D
LSK
Lin^neg
ckit
Sca1
YFP
E
No Culture
Culture No Stim
Culture PMA/Iono
Gated: TCRδ+
CD44
CD25
CD24
c-Kit
TCRδ
No Culture (2128)
Culture, No Stim (1842)
Culture, PMA/Iono (1616)
F
DN1d
DN1e
ETP
Total Vγ2+
<1%
2%
38%
EGFP
WT B6
Il17a^egfp/egfp
G
Vγ2+ CD24+
Vγ2+ CD24-
WT B6
Il17a^egfp/egfp
EGFP
CCR6
H
WT B6
Il17a^egfp/egfp
Vγ2+
Vγ1.1- Vγ2-
CD73
% of gated population
Vγ2+
Vγ2-Vγ1.1-
EGFP+CD73-
EGFP+CD73+

Figure S1. Related to Figure 1 and Figure 2. In the thymus, only innate lymphoid effectors and DN1 precursors express *Sox13*, while only γδ cells express *Il17a*-EGFP. (**A**) *Sox13* locus modified by a "knock-in" reporter-Cre fusion construct by homologous recombination in AB1 ES (129 background) cells. The drug resistance gene (*neo*) used to positively select clones with the vector insertion was removed by Flp recombinase targeting Frt sites prior to blastocyst injections. Numbers in boxes represent exons. The knock-in allele cannot produce normal SOX13. $Sox13^{+/-}$ mice have no significant alterations in Tγδ17 cell differentiation. TK, thymidine kinase, a negative selection marker. (**B**) Reporter expression in $\gamma\delta TCR^{+}$ thymocytes confirms the highest *Sox13* expression in immature ($CD24^{+}$) $V\gamma2^{+}$ T cells. *Sox13* expression is extinguished upon effector T cell maturation as previously reported. (**C**) Reporter expression in $\alpha\beta TCR^{+}$ thymocytes confirms that *Sox13* is not expressed in developing αβ T cells, except in early $CD44^{-}NK1.1^{-}CD1d{:}\alpha GalCer\ tetramer^{+}$ (stage 1) $V\alpha14^{+}$ invariant NKT cells, confirming published data (Cohen et al., 2013). (**D**) To determine the developmental onset of *Sox13* expression in hematopoietic cells *Sox13* reporter mice, in which the reporter is a fusion protein of ECFP and CRE recombinase, was crossed to the ROSA-YFP Cre reporter mouse line. In this model, progenitors that first express Sox13 and all their descendants will be YFP^{+}, irrespective of whether differentiated cells continue to express *Sox13* or not. All long-term repopulating bone marrow hematopoietic stem cells ($Lin\text{-}Sca1^{+}c\text{-}Kit^{+}$, LSK) were YFP^{+}, indicating that during hematopoiesis *Sox13* is first expressed in LSKs. By transcriptome analysis and ECFP expression in the reporter strain, *Sox13* is largely turned off in most lineage committed progenitors such as LMPPs and CLPs (data not shown), only to be reinduced in DN1 thymocytes. **(E)** Instability of DN1 cells after *in vitro* PMA/Ionomycin stimulation for ICS. Ex vivo thymocytes from 1wk old mice (not cultured) or cultured as indicated for 2-3h and then analyzed via FACS. CD24 and c-Kit staining of DN1 cells is significantly altered after PMA/Iono stimulation, and TCRδ is down regulated on γδ thymocytes (TCRδ MFI indicated in parentheses). **(F)** Neonatal DN1d, DN1e, and ETPs and $V\gamma2^{+}$ γδ thymocytes were analyzed for EGFP (driven by an IRES inserted immediately after the *Il17a* stop codon) expression via FACS. The same cell population of WT B6 mice was used to establish EGFP channel background fluorescence. **(G)** $V\gamma2^{+}$ γδ thymocytes were gated into immature ($CD24^{+}$) and mature ($CD24^{-}$) populations, and *Il17a-egfp* reporter and CCR6 expression was assessed. *Il17a* transcriptional activity is first observed in immature cells, and is highly active in mature cells including many that have yet to express detectable surface CCR6 protein. **(H)** $V\gamma2^{+}$ and $V\gamma1.1^{-}V\gamma2^{-}$ (mostly $V\gamma4^{+}$ in early post-natal mice) γδ thymocytes were analyzed

for expression of *Il17a-egfp* and CD73, which has been proposed to be induced by γδTCR signaling and upregulated prior to γδ effector lineage commitment. Greater than 90% of *Il17a-egfp*$^{+}$ Vγ2^{+} cells do not express CD73, suggesting minimal involvement of TCR signaling in establishing *Il17a* transcriptional capacity. Data are representative of 6 animals (2 independent experiments) analyzed at 5-7 d after birth. *** p<.001 by two-way ANOVA with Sidak's multiple comparisons test.

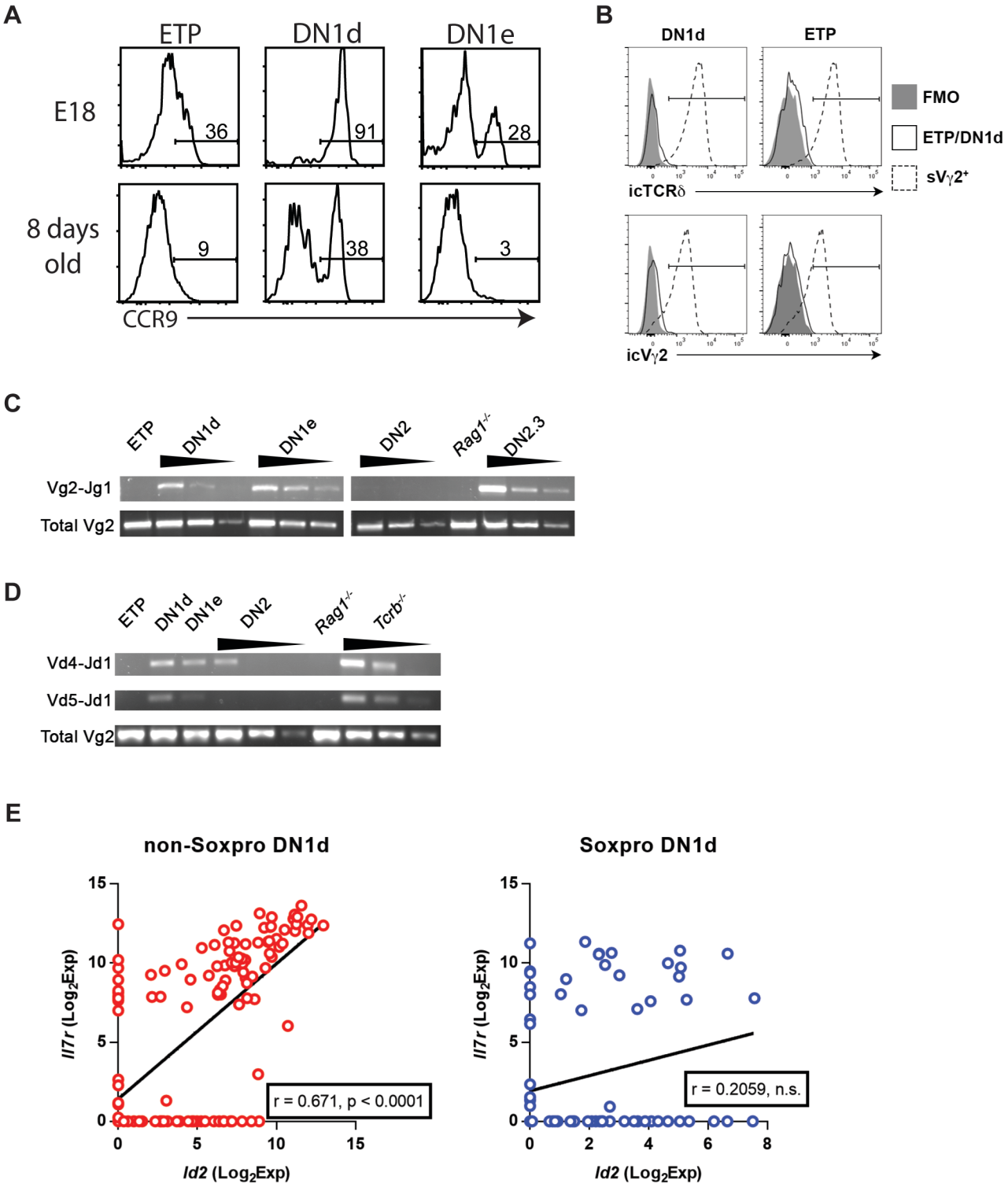
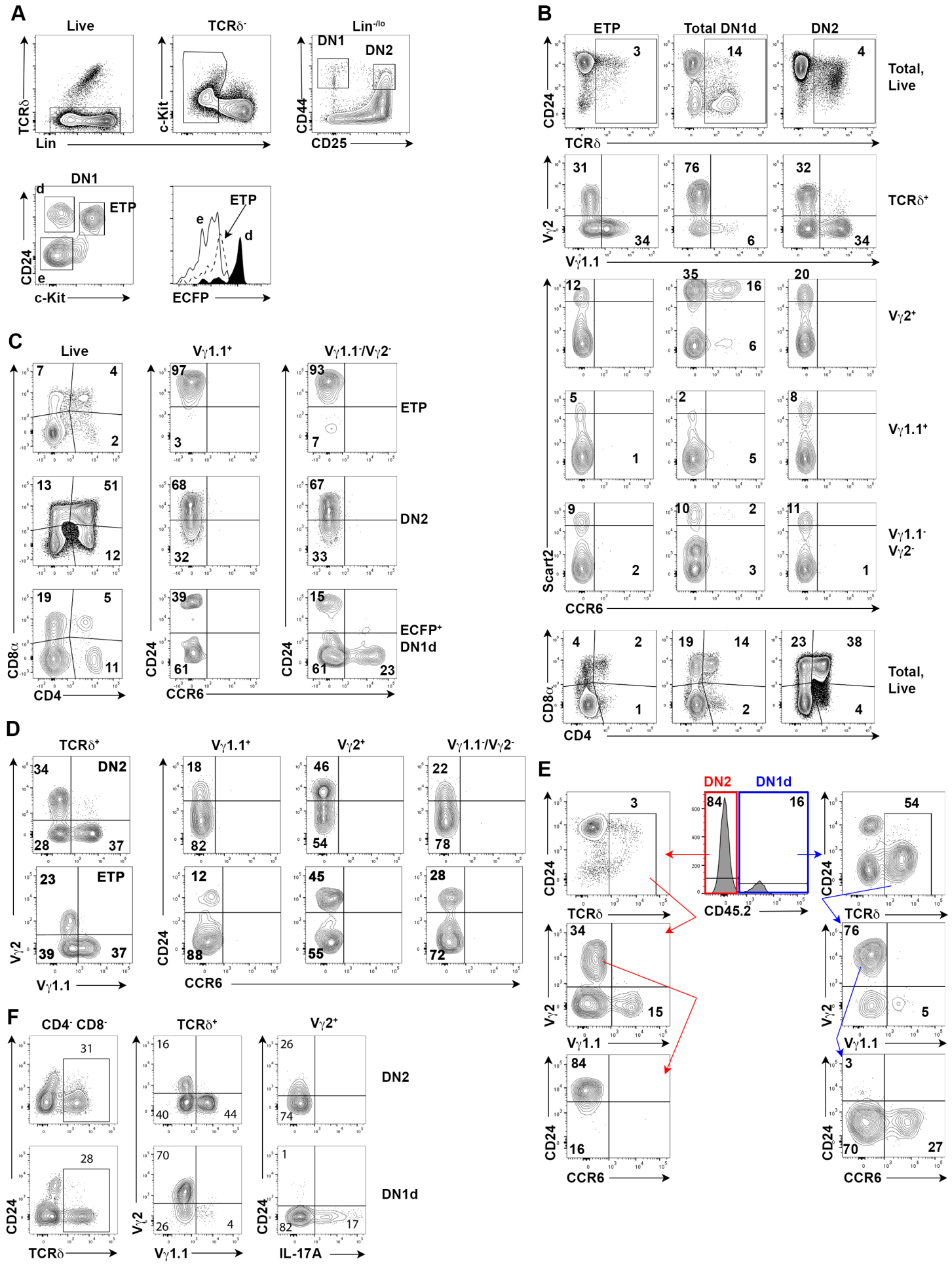


Figure S2. Related to Figure 3. Markers delineating heterogeneity among DN1d cells and TCR gene rearrangement status in DN1 progenitors. (**A**) Thymocyte progenitors at the indicated age were analyzed for expression of CCR9, a marker identified as highly enriched in Soxpro versus non-Soxpro DN1d cells. **(B)** DN1d cells do not express intracellular TCRγ and δ chains. Cells were surface stained with Ab cocktails to identify γδ T cells and DN1 subsets and then fixed and permeabilized and stained for intracellular (ic) TCRδ (top panels) or Vγ2

(bottom panels). Gray filled, fluorescence minus one (FMO) control; Solid histogram, indicated population stained for icTCR; Dashed histogram, icTCR expression in surface Vγ2 TCR^{+} γδ T cells, overlayed as a positive control. Data are representative of samples from one of six mice analyzed in two independent experiments. **(C-D)** Partial rearrangement of TCRγ and δ genes in DN1d cells. Genomic DNA was isolated from thymic progenitors or control cells and PCR performed to detect **(C)** Vγ2-Jγ1 or **(D)** Vδ4-Jδ1 and Vδ5-Jδ1 gene rearrangements. Lanes were loaded with 2000 cell equivalents or where indicated (filled triangles), with serial 4-fold dilutions starting at 2000 cell equivalents. *Rag1*$^{-/-}$ and *Tcrb*$^{-/-}$ total thymocytes or DN2.3 cell line (Vγ2-Jγ1 rearranged) were used as negative and positive controls, respectively. Total unrearranged Vγ2 gene segment was used as an internal control for all reactions. DN1d cells exhibit ~25% of rearrangement detected in the Vγ2^{+} DN2.3 γδ T cell line, with similar extents of Vδ4 and Vδ5 rearrangements (compared to thymocytes of *Tcrb*$^{-/-}$ mice). Low levels (<10% of the extent found in thymocytes of *Tcrb*$^{-/-}$ mice) of Vγ1.1-Jγ4 rearrangements are observed in DN1d cells (data not shown). DN2 thymocytes showed only Vδ4 gene rearrangements while TCR γ or δ gene rearrangements were undetectable in ETPs, as expected. **(E)** Correlation of *Id2* and *Il7r* expression in single DN1d cells. Soxpro and non-Soxpro DN1d cells were segregated as in **Figure 3**, and expression of the indicated genes critical for innate lymphoid cell (ILC) development plotted for each cell. Data suggest that some non-Soxpro DN1d cells may retain ILC developmental potential while *Id2* expression is relatively low in "Soxpro" cluster. Inset lines were calculated by least squares fit and correlation values and significance measures determined by Spearman correlation using GraphPad Prism.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Live
TCRδ
Lin
TCRδ-
c-Kit
Lin-/lo
DN1
DN2
CD44
CD25
DN1
d
ETP
e
CD24
c-Kit
ETP
e
d
ECFP
B
ETP
Total DN1d
DN2
CD24
TCRδ
Total, Live
TCRδ+
Vγ2
Vγ1.1
Vγ2+
Vγ1.1+
Vγ1.1-Vγ2-
Scart2
CCR6
CD8α
CD4
Total, Live
C
Live
Vγ1.1+
Vγ1.1-/Vγ2-
ETP
DN2
ECFP+ DN1d
CD8α
CD4
CD24
CCR6
D
TCRδ+
Vγ1.1+
Vγ2+
Vγ1.1-/Vγ2-
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E
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DN1d
CD24
TCRδ
CD45.2
Vγ2
Vγ1.1
CCR6
F
CD4- CD8-
TCRδ+
Vγ2+
DN2
DN1d
CD24
TCRδ
Vγ2
Vγ1.1
IL-17A

Figure S3. Related to Figure 4. Developmental potential of DN1d cells in hFTOC. (**A**) Representative gating strategy for sorting DN subsets from *Sox13*$^{ECFP/+}$ knock-in mice for hFTOC. Sox13-ECFP was detected and the positive fraction sorted on a FACSAria. Total, live cells were first gated strictly on TCRδ^{-}, then on cells that include those expressing low levels of Lin markers (Materials and Methods). Lin$^{-/lo}$ were segregated based on CD25 and CD44 expression into four classical DN subsets, with DN1 and DN2 cells containing T cell progenitors. DN1 cells were further stratified into ETP, DN1d, and DN1e subsets based on c-Kit and CD24 expression. Note that ECFP fluorescence intensity is significantly higher in DN1d cells using the FACSAria "enhanced ECFP detection" (Materials and Methods) in comparison to the conventional analytic cytometer LSRII, but there still exist ~10% of DN1d cells not expressing the reporter in 10 day old mice. (**B**) Development of "total" DN1d cells (not sorted for ECFP^{+} cells, in contrast to **Figure 4A**) in hFTOC. Alymphoid fetal thymi were repopulated with the indicated progenitor population from WT B6 mice and then cultured for 8 days prior to analysis. Shown are generative capacities of different progenitors for Tγδ17 phenotype cells (CCR6^{+}Scart2^{+}) among γδ T subtypes defined by expression of different TCRγ chains. CD4^{+}CD8^{+} double positive (DP) thymocytes can be generated from Soxpro cells but they are distinct from the corresponding cells from conventional T cell progenitors, with some expressing Scart2 that is not found on normal DP cells (not shown). **(C)** DP and other γδ T cell subset development from ECFP^{+} DN1d cells. Progenitors were sorted and hFTOC performed as above. DP generation is more restricted from ECFP^{+} DN1d (compare to **Figure S3B** assay using total DN1d cells), suggesting that some of the αβ T cell potential may be present in the ECFP^{-} DN1d fraction. Paucity of ECFP^{-} DN1d cells precluded direct development hFTOC assays of these cells. CCR6^{+} Vγ4^{+} (Vγ1.1^{-}/Vγ2^{-}) thymocytes are also generated from ECFP^{+} DN1d cells while ETP or DN2 did not generate CCR6^{+} Vγ4 Tγδ17 cells. **(D)** Extended duration of DN2 or ETP cell hFTOC do not alter developmental outcomes. To determine whether DN2 or ETP cells can generate Tγδ17 cells with a delayed kinetic compared to DN1d cells, DN2 and ETP progenitors from WT neonatal (d7) B6 mice were assayed in hFTOC after up to 3wk in culture. TCRVγ usage is comparable to early culture days (compare to **Figure S3B**) and most cells have matured as indicated by CD24 downregulation, but no CCR6^{+} cells are observed. **(E)** DN1d cells were sorted from WT B6 (CD45.2^{+}) mice and DN2 cells were sorted from CD45.1^{+} B6 congenic mice, mixed at equal numbers, and then used to repopulate alymphoid fetal thymic lobes. Repopulated lobes were cultured under hFTOC conditions for 1wk prior to analysis. Data are representative of 2 independent expeirments. **(F)** CD24 by TCRδ staining of Live/CD4^{-}

/CD8^{-} cells, Vγ chain analysis of TCRδ^{+} cells, and CD24 by IL-17A analysis of Vγ2^{+} cells from the same experiment shown in **Figure 4B**. Note that some CD24 downregulation is observed following PMA/Ionomycin stimulation (compare vs. **Figure 4A, Figure S3C**).

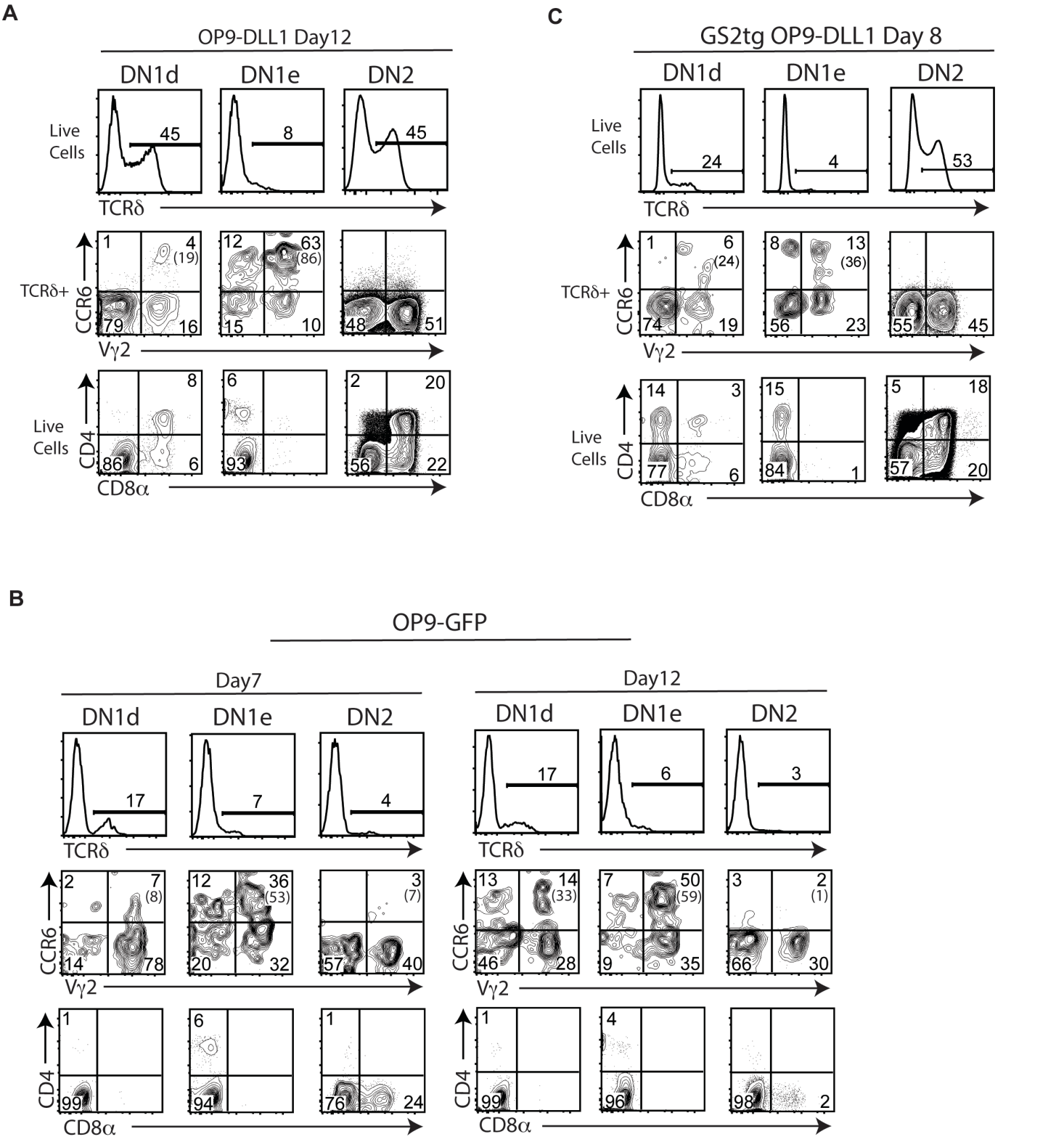
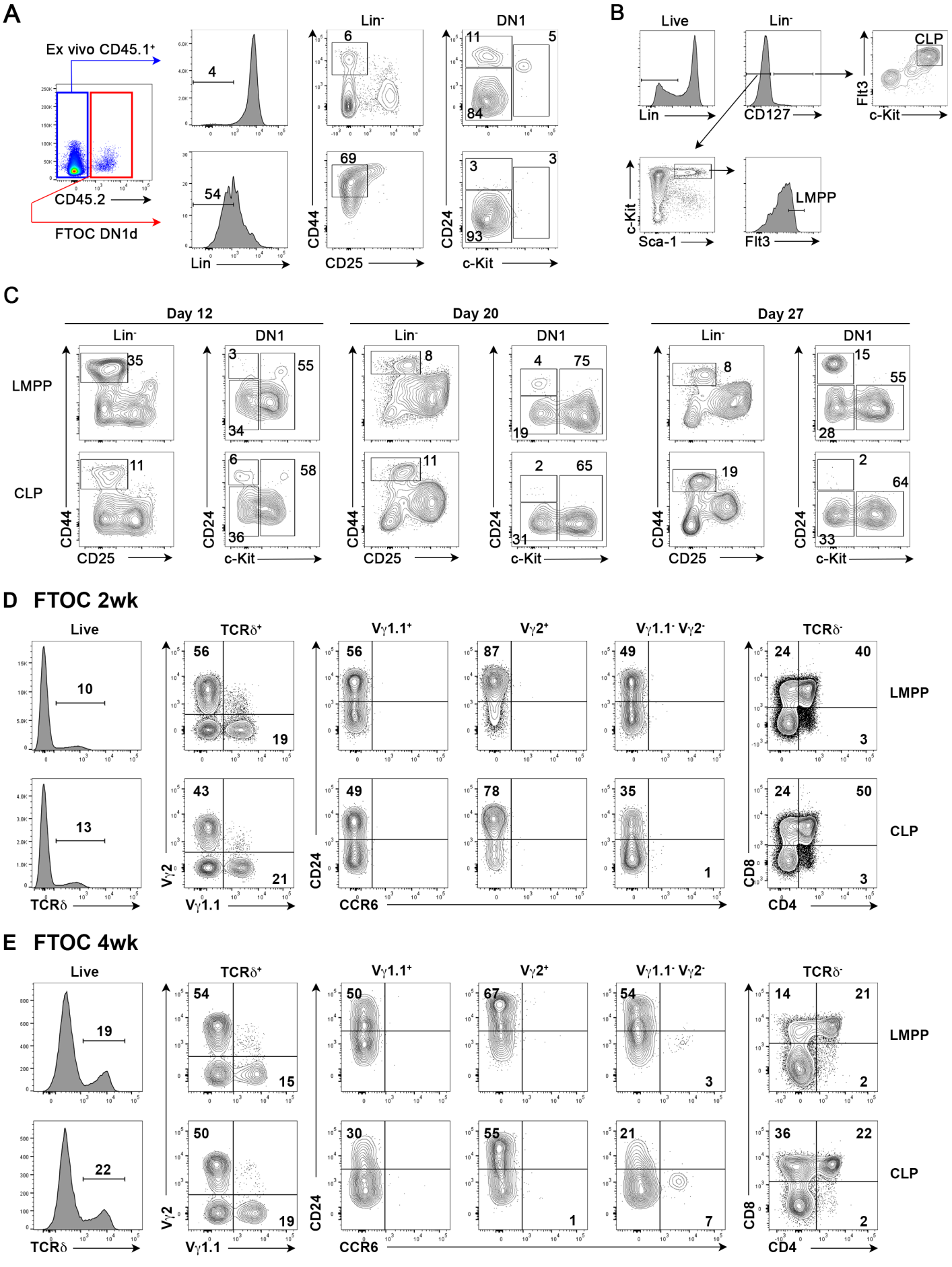


Figure S4. Related to Figure 4. Characteristics of γδ T cells generated *in vitro* from T precursor cell subsets.

(**A**) Tγδ17 cells generated from DN1d and DN1e cells are observed at longer-term cultures on OP9-DLL1 stroma. Shown is a longer duration of culture (day 12) of experiments of **Figure 4C**. , Inset parenthetical value in CCR6^{+}Vγ2^{+} quadrant indicates percent of CCR6^{+} cells among Vγ2^{+} cells (also for panel C). (**B**) Tγδ17 cells are

generated from DN1d and DN1e cells on OP9 cells without DLLs. Shown are representative cultures analyzed at day 7 and day 12 of indicated precursor subsets from *Tcrb*$^{-/-}$ mice. DP thymocytes are not generated/maintained and DN2 proT cells are significantly impaired in generating γδ T cells (compare to A) when Notch signaling is absent in culture. (**C**) Provision of rearranged TCRγ chain gene in developing T precursor subsets enhance the generation of Tγδ17 cells. Shown are analyses of OP9-DLL4 cultures at two time points initially seeded with sorted DN1d cells, DN1e cells, or DN2 proT cells from G8 TCRγ transgenic mice (Kang et al., 1998). By day 12, >70% of Vγ2^{+} cells express CCR6 whereas DN2 proT cells do not differentiate into Tγδ17 cells.


A
Ex vivo CD45.1+
CD45.2
FTOC DN1d
Lin
CD25
CD44
c-Kit
CD24
Lin-
DN1
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Live
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CD127
Flt3
c-Kit
CLP
Sca-1
LMPP
C
Day 12
Day 20
Day 27
LMPP
CLP
D FTOC 2wk
Live
TCRδ+
Vγ1.1+
Vγ2+
Vγ1.1- Vγ2-
TCRδ-
TCRδ
Vγ2
Vγ1.1
CD24
CCR6
CD8
CD4
E FTOC 4wk

Figure S5. Related to Figure 4. Development of fetal liver LMPP and CLP in hFTOC. (A) Analysis of DN1d potential to develop into DN1e cells. DN1d cells were sorted from thymuses of 7-10d old WT B6 and used to repopulate alymphoid fetal thymic lobes. Lobes were then cultured under hFTOC conditions for 3d prior to analysis. As described in *Materials and Methods*, hFTOC cells were mixed with $1x10^6$ thymocytes from 4wk old $CD45.1^+$ B6 congenic mice to facilitate gating. **(B)** Gating scheme for sorting LMPP and CLP from fetal liver. Red blood cell-depleted fetal liver cells were labeled with the indicated Ab for cell sorting. Live ($7\text{-}AAD^-$) cells were first gated on Lin^- (Lin = CD3ε/CD4/CD8α/CD19/B220/Gr-1/Ter-119, notably CD11b is *not* included in the Lin cocktail for fetal progenitors) and then on $CD127^{+/-}$. Common lymphoid progenitors (CLP) were sorted as $CD127^+/Flt3^{hi}/c\text{-}Kit^{hi}$. $CD127^-$ cells were gated on $Sca\text{-}1^+/c\text{-}Kit^{hi}$, and then lymphoid-primed multipotent progenitors (LMPP) were selected as those cells exhibiting the highest 25% of Flt3 expression. **(C)** DN subset analysis throughout CLP/LMPP hFTOC culture. While DN1 cells were present throughout the culture, DN1d-like cells were more variable. CLP generated a small number of $CD24^+/c\text{-}Kit^-$ DN1d-like cells by Day 12, but these cells were not maintained through Day 20 or 27. In contrast, LMPP did not generate a distinct population of $CD24^+/c\text{-}Kit^-$ DN1d-like cells until Day 20, but these cells were further enriched by Day 27. **(D-E)** Analysis of γδ cells developing in hFTOC lobes repopulated with LMPP or CLP 2 wk **(D)** or 4 wk **(E)** after cultures were established.

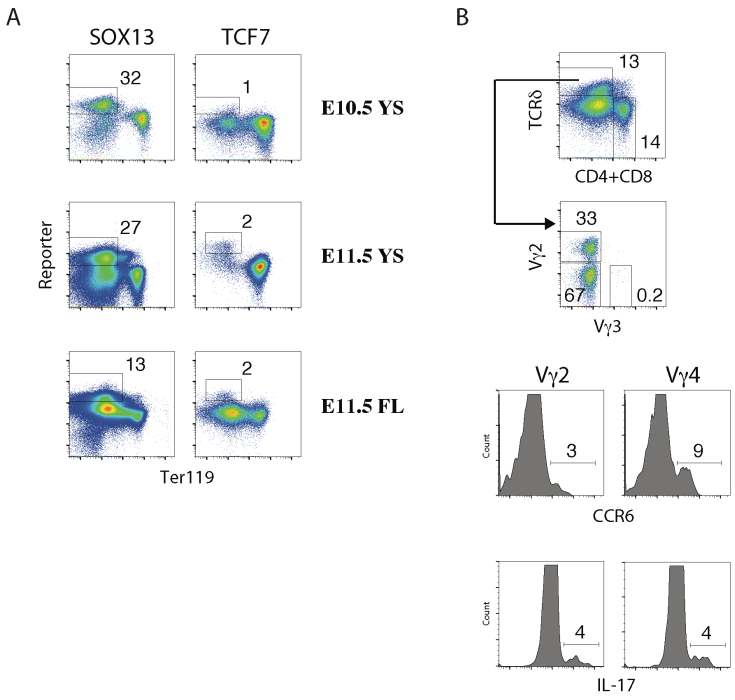
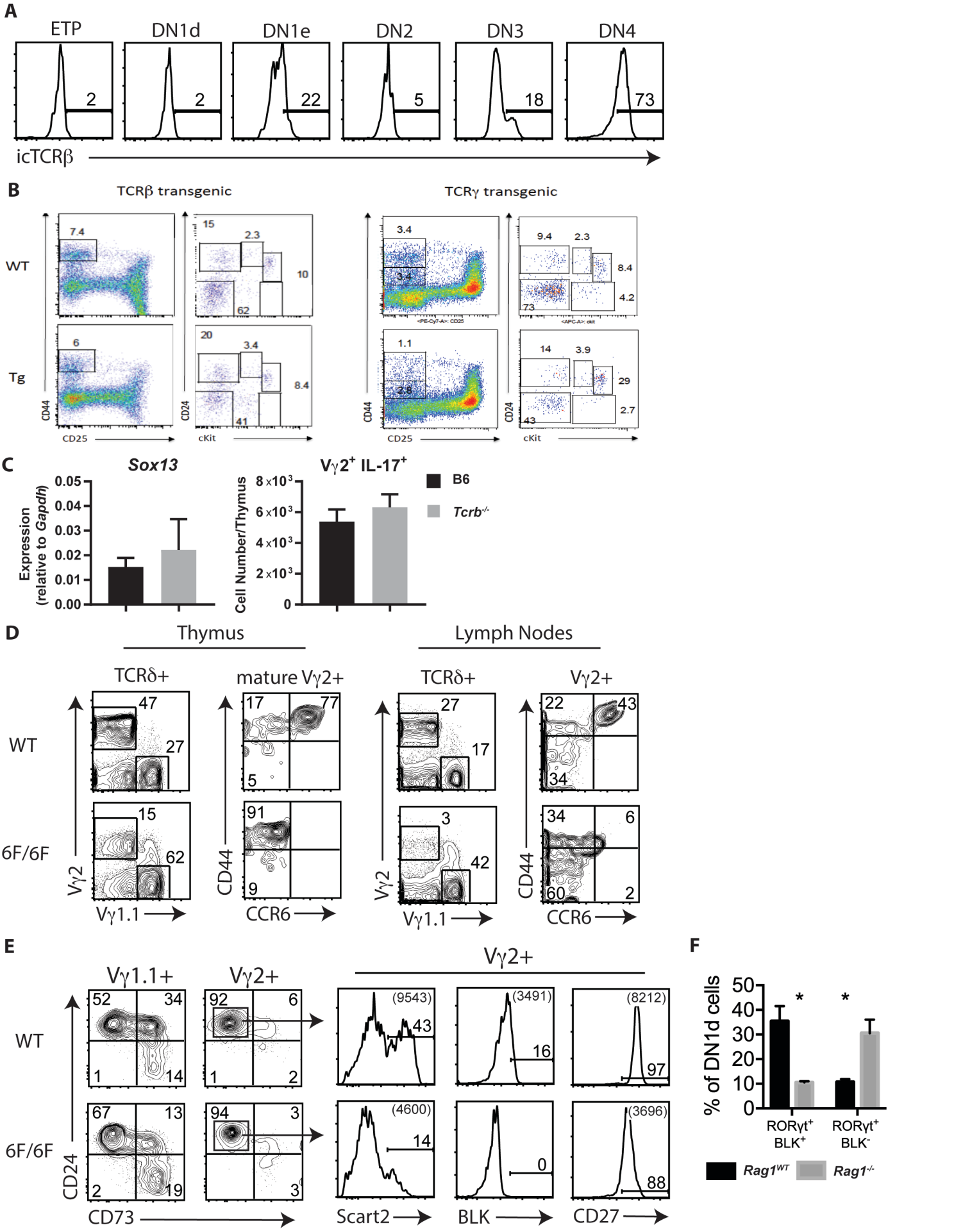


Figure S6. Related to Figure 4. Evidence for potential generation of Tγδ17 cells from embryonic yolk sac progenitors. **(A)** *Sox13-ECFP* and *Tcf7-EGFP* reporter expression in non-erythroid cells (Ter119^{neg}) at E10.5 and E11.5 yolk sac (YS) compared to E11.5 fetal liver (FL) cells. ECFP expression is based on intracellular staining (ICS) with anti-CFP antibody as many cells expressed low amounts of the reporter that were not reliably detected by non-ICS FACS. Representatives of five and two experiments for *Sox13* and *Tcf7*, respectively. Note that the ICS protocol removes the majority of Ter119^{+} cells. **(B)** *YS cells are highly biased to generate Tγδ17 thymocytes in FTOCs.* Cells from FTOCs reconstituted with E10.5 YS cells were analyzed for Tγδ17 cells and IL-17 production. E10.5 total YS cells were primed on OP9-DL1 stroma then used to reconstitute alymphoid E14.5 fetal thymuses in hanging drop cultures for 1 day followed by conventional FTOCs with IL-7 supplemented media for 16-21 days. IL-17 ICS was performed after the standard PMA/Ionomycin stimulation for 2hrs. Shown are TCRδ^{+} and CD4 and/or CD8 expressing cells (Top), Vγ2^{+} and Vγ3^{+} cells amongst γδTCR^{+} thymocytes (middle), followed by histograms depicting CCR6^{+} cells amongst Vγ2^{+} and Vγ4^{+} cells (Tγδ17 cells) and IL-17 production from the same cells. Vγ4^{+} cells are identified as Vγ2/3/1.1^{neg} cells. Remarkably, YS cells generated near exclusively the γTCR repertoire associated with Tγδ17 cells, as there were negligible fetal Vγ3^{+} and neonatal Vγ1.1^{+} cells generated from these embryonic cells in FTOCs (compare to **Figure 4, Figure S5, Figure S7**). Data are from one of three experiments with pooled lobes (6 or 10 combined).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icTCRβ
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TCRβ transgenic
TCRγ transgenic
WT
Tg
CD44
CD25
CD24
cKit
C
Sox13
Vγ2+ IL-17+
Expression (relative to Gapdh)
Cell Number/Thymus
B6
Tcrb-/-
D
Thymus
Lymph Nodes
TCRδ+
mature Vγ2+
Vγ2+
WT
6F/6F
Vγ2
Vγ1.1
CD44
CCR6
E
Vγ1.1+
Vγ2+
CD24
CD73
Scart2
BLK
CD27
F
% of DN1d cells
RORγt+ BLK+
RORγt+ BLK-
Rag1WT
Rag1-/-

Figure S7. Related to Figure 5. Ectopic TCR expression or TCR signaling deficiency does not impact DN1d progenitor frequency. (**A**) Intracellular (ic) expression of TCRβ chain in indicated DN subsets of WT B6 mice showing that DN1d cells have not assembled full length TCRβ chain, consistent with previous studies that have shown that DN1d cells have not rearranged *Tcrb* gene segments. (**B**) DN and DN1 subset distributions in TCRβ (LN3b, (Serwold et al., 2007)) and TCRγ (GS2tg) transgenic mice show that DN1d cells are present under conditions of ectopically early expression of the indicated TCR chain. In the models, rearranged TCRβ and TCRγ genes are expressed under the control of endogenous regulatory elements and most DN1 cells express the supplied TCRs (icTCRβ (Serwold et al., 2007) and TCRγ transcript assays, data not shown). DN1d cells do not express preTα chain (*Ptcra*, RNAseq) and thus do not have the potential to generate preTCR. **(C)** Quantitative PCR analysis of *Sox13* expression by DN1d from WT B6 and *Tcrb*$^{-/-}$ mice (left graph, n=5/genotype) and Vγ2^{+} IL-17^{+} cell numbers in the thymus of WT B6 and *Tcrb*$^{-/-}$ mice (right graph, n=3/genotype). Analyses were performed at 7-10d of age. **(D)** Analysis of thymic and skin-draining lymph node γδ T cells in WT B6 and 6F/6F mice. TCRVγ usage is abnormally skewed in 6F/6F mice, leading to a near absence of Tγδ17 (CD44^{+}CCR6^{+}) cells in both the thymus and lymph nodes. **(E)** The indicated subsets of γδ thymocytes from WT B6 or 6F/6F mice were analyzed first for CD73 expression among mature and immature populations, and then for Scart2, BLK, and CD27 expression by the CD24^{+}CD73^{-} population. Numbers in brackets represent mean fluorescence intensity (MFI). Prior to CD73 induction, expression of all tracked cell surface antigen is impaired when TCR signaling is compromised. **(F)** Summary of all mice analyzed as in **Figure 5F**. B6 n=20, *Rag1*$^{-/-}$ n=14. *, p<.05 by two-way ANOVA.

Supplementary Tables

Table S1. Related to Figure 2. Differentially expressed genes (DEGs) between neonatal DN1d and ImmV2 subsets. A complete list of genes (ordered by fold change) whose expression (in RPKM count) is changed 2-fold or more ($p \leq 0.05$) between DN1d versus immature ($CD24^{hi}$) $V\gamma2^{+}$ thymocytes (ImmV2). Each column represents an independently sorted replicate for indicated subsets.

Table S2. Related to Figure 2. Differentially expressed genes between neonatal DN1d and DN1ab subsets. A complete list of genes (ordered by fold change) whose expression (in RPKM count) is changed 2-fold or more ($p \leq 0.05$) between DN1d versus DN1ab (ETPs).

Table S3. Related to Figure 2. Differentially expressed genes between neonatal DN1d and DN1e subsets. A complete list of genes (ordered by fold change) whose expression (in RPKM count) is changed 2-fold or more ($p \leq 0.05$) between DN1d versus DN1e.

Table S4. Related to Figure 2. Differentially expressed transcription factors (TFs) across DN1ab, DN1d, DN1e and ImmV2 subsets. A complete list of TFs whose expression (in RPKM count) is changed 2-fold or more ($p \leq 0.01$) in pairwise comparisons among DN1ab, DN1d, DN1e and ImmV2 thymocytes. Heat maps representing this cluster is shown in Fig 2b. Gene expression values in DN1 cells segregated on c-Kit expression only (DN1 $ckit^{+}$), DN2 and DN4 cells are also shown for comparison.

Target	Forward	Reverse	Application
Bcl11b	CTGGGGGACAGCAATCCTTTC	TGTGGGTCCAAGTGATGGC	sc
Bhlhe40	ACGGAGACCTGTCAGGGATG	GGCAGTTTGTAAGTTTCCTTGC	sc
Blk	CGTTCCCTACCCAGGAATGA	AAGTCCTCCAACACCGACTG	sc
Ccr9	ATCTCTGGTCTGCCTTTGCC	TGCCAGGAATAAGGCTTGTGA	sc
Cd163l1	CTGGCCTCTGAGTTTAGGGTC	CCCTTGGTGTCGAACCAGC	sc
Cdh1	CAGGTCTCCTCATGGCTTTGC	CTTCCGAAAAGAAGGCTGTCC	sc
Cdk6	GGCGTACCCACAGAAACCATA	AGGTAAGGGCCATCTGAAAACT	sc
Cpa3	AATTGCTCCTGTCCACTTTGAC	TCACTAACTCGGAAATCCACAGT	sc
Csf1r	GAGCCTACCAGAAGACCCAC	GCAGGTTAGCATAGTCCTGGTC	sc
Csf2rb2	TCCAGCCAGATCGTGACCT	AATCCCCAAGAGATACACTCCA	sc
Dna2	GGGTGGAGCTACTTCGGAAGA	CTCCTCGGCTCAGAACTGTCT	sc
E2f2	ACGGCGCAACCTACAAAGAG	GTCTGCGTGTAAAGCGAAGT	sc
Egr2	GTGGCGGGAGATGGCATGAT	CACCAGGGTACTGTGGGTCA	sc
Ets1	CTACGGTATCGAGCATGCTCAGTG	AAGGTGTCTGTCTGGAGAGGGTCC	sc
Etv5	TCAGTCTGATAACTTGGTGCTTC	GGCTTCCTATCGTAGGCACAA	sc
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA	sc, q
Gata3	CTCGGCCATTCGTACATGGAA	GGATACCTCTGCACCGTAGC	sc
Gp49a	AGTGTCGTCACAAAAATAAGGCT	CCTGGGCGTACACAATTCCC	sc
Gpr183	CTAGCCACCTTCTGATATTGACTGT	ATTGCCATGAGACGTTGCCA	sc
Gzma	TGCTGCCCACTGTAACGTG	GGTAGGTGAAGGATAGCCACAT	sc
Id2	GGACTCGCATCCCACTATCG	TCAGATGCCTGCAAGGACAG	sc
Il17re	CCCTCTCTTGGTGAGGAAATC	GCCTAGCAGCTTCCTCTGG	sc
Il7r	GCCAAAAACGAGTCTGAATGTGA	CTGGCTGTGCAGGAAGATCA	sc
Lef1	GACTTCAGGTACAGGTCCCA	GTCAGTGTTCCTTGGGGTCA	sc
Maf	GTGCAGCAGAGACACGTCCT	CAACTAGCAAGCCCACTC	sc
Mcm2	ATCCACCACCGCTTCAAGAAC	TACCACCAAACTCTCACGGTT	sc

Myb	AGACCCCGACACAGCATCTA	CAGCAGCCCATCGTAGTCAT	sc
Nfil3	GGAGCAGAACCACGATAACCC	CCTCGTCCTACAGACCGGAT	sc
Notch1	CCCTTGCTCTGCCTAACGC	GGAGTCCTGGCATCGTTGG	sc
Rag2	CTGGCCTTCAGTGCCAAAAT	TGACCCACTGTTACCATCTGC	sc
Rorc	TGCAAGACTCATCGACAAGG	AGGGGATTCAACATCAGTGC	sc
Runx1	GCAGGCAACGATGAAAACTACT	GCAACTTGTGGCGGATTTGTA	sc
Scart2	ACCTGCTGGACTCTCGTTC	GCCGTCTGCACACATAAGG	sc
Sfpi1	ATGTTACAGGCGTGCAAAATGG	TGATCGCTATGGCTTTCTCCA	sc
Smo	GAGCGTAGCTTCCGGGACTA	CTGGGCCGATTCTTGATCTCA	sc
Sox13	GCTTTACCTATTCAGCCCAT	ACCTCTTCACCACAGGGG	sc, q
Sox4	CCCTTCTTAAAATTTCTTTTTCTGC	AGGTCCCCATGTCCATTTC	sc
Syk	GTGCTGGACTTACGATGTGGA	TGATCGCTTGGGAATCCGTG	sc
Tbx21	CATGCCAGGGAACCGCTTAT	TTGGAAGCCCCCTTGTTGTT	sc
Tcf12	ATGTGCTACGAAACCATGCAG	GCCATTGAGACTGACTGAATCTT	sc
Tcf3 E12	GGGAGGAGAAAGAGGATGA	GCTCCGCCTTCTGCTCTG	sc
Tcf3 E47	GGGAGGAGAAAGAGGATGA	CCGGTCCCTCAGGTCCTTC	sc
Tcf7	GCCAGCCTCCACATGGCGTC	GCTGCCTGAGGTCAGAGAATAA	sc
Tox	GCTCCCGTTCCATCCACAAA	TCCCAATCTCTTGCATCACAGA	sc
Vd4-Jd1	TGGACTGCACCTATGACACA	AGTCACTTGGGTTCCTTGTCC	sc
Vd5-Jd1	ATGCACAGCACATCCTTGTC	AGTCACTTGGGTTCCTTGTCC	sc
Vg2-Jg1	CTGGGAAGCCAACCTGGCAGATGA	CTTACCAGAGGGAATTACTATGAG	sc, g
Zbtb16	CCCAGTTCTCAAAGGAGGATG	TTCCCACACAGCAGACAGAAG	sc
Vd4-Jd1	AAATCGGGAGACGGACCAA	GGACAAGGAACCCAAGTGACT	g
Vd5-Jd1	TTGGAGAATCCGCATTGTACGTGC	GGACAAGGAACCCAAGTGACT	g
Vg2 (total)	CTGGGAAGCCAACCTGGCAGATGA	GCTTCGTCTTCTTCCTCCAAGG	g

Table S5. Related to STAR Methods. Primers used in this study. For application, sc = single-cell; q = standard RT-qPCR; g = genomic DNA.