

# A Network of High-Mobility Group Box Transcription Factors Programs Innate Interleukin-17 Production

Nidhi Malhotra,<sup>1,4</sup> Kavitha Narayan,<sup>1,4</sup> Ok Hyun Cho,<sup>1</sup> Katelyn E. Sylvia,<sup>1</sup> Catherine Yin,<sup>1</sup> Heather Melichar,<sup>1</sup> Mehdi Rashighi,<sup>2</sup> Veronique Lefebvre,<sup>3</sup> John E. Harris,<sup>2</sup> Leslie J. Berg,<sup>1</sup> and Joonsoo Kang,<sup>1,\*</sup>  
The Immunological Genome Project Consortium<sup>5</sup>

<sup>1</sup>Department of Pathology

<sup>2</sup>Department of Medicine

University of Massachusetts Medical School, Worcester, MA 01655, USA

<sup>3</sup>Department of Cell Biology and Orthopaedic and Rheumatologic Center, Cleveland Clinic Lerner Research Institute, Cleveland, OH 44195, USA

<sup>4</sup>These authors contributed equally to this work

<sup>5</sup>A full list of Immunological Genome Project Consortium members may be found in the Acknowledgments

\*Correspondence: [joonsoo.kang@umassmed.edu](mailto:joonsoo.kang@umassmed.edu)

<http://dx.doi.org/10.1016/j.immuni.2013.01.010>

## SUMMARY

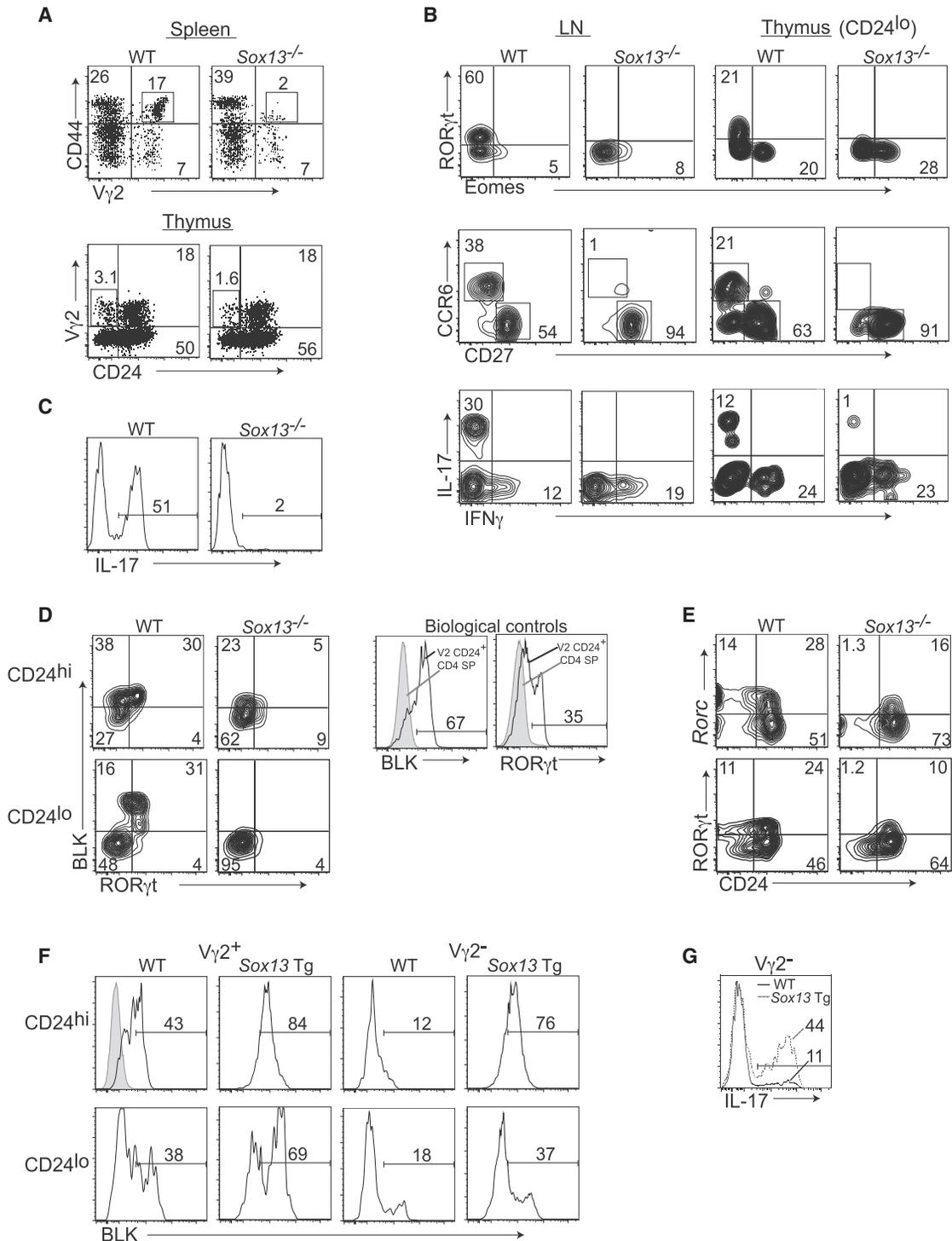
How innate lymphoid cells (ILCs) in the thymus and gut become specialized effectors is unclear. The prototypic innate-like  $\gamma\delta$  T cells ( $T\gamma\delta 17$ ) are a major source of interleukin-17 (IL-17). We demonstrate that  $T\gamma\delta 17$  cells are programmed by a gene regulatory network consisting of a quartet of high-mobility group (HMG) box transcription factors, SOX4, SOX13, TCF1, and LEF1, and not by conventional TCR signaling. SOX4 and SOX13 directly regulated the two requisite  $T\gamma\delta 17$  cell-specific genes, *Rorc* and *Btk*, whereas TCF1 and LEF1 countered the SOX proteins and induced genes of alternate effector subsets. The T cell lineage specification factor TCF1 was also indispensable for the generation of IL-22 producing gut NKp46<sup>+</sup> ILCs and restrained cytokine production by lymphoid tissue inducer-like effectors. These results indicate that similar gene network architecture programs innate sources of IL-17, independent of anatomical origins.

## INTRODUCTION

Innate lymphoid cells (ILCs) and innate-like T cells (ILTC) producing interleukin-17 (IL-17) and IL-22 have emerged as the central players in mucosal immunity (Spits and Di Santo, 2011). Upon infection or alterations in cellular environments, ILCs lacking clonal antigen receptors and T cells expressing  $\gamma\delta$  TCR rapidly produce effector cytokines and growth factors to promote pathogen clearance and tissue repair (O'Brien et al., 2009; Sonnenberg et al., 2011).  $\gamma\delta$ TCR<sup>+</sup> ILTCs, similar to adaptive  $\alpha\beta$  CD4<sup>+</sup> T cells, are segregated into effector subsets. However, unlike  $\alpha\beta$  T effectors,  $\gamma\delta$ TCR<sup>+</sup> effector subsets can be classified by the germline-encoded TCR chains and they are generated in the thymus (Jensen et al., 2008; Narayan et al., 2012; Ribot et al., 2009). More than half of V $\gamma$ 2 TCR<sup>+</sup> (designated as V2)  $\gamma\delta$  T cells are intrathymically programmed

to produce IL-17 ( $T\gamma\delta 17$ ) and express ROR $\gamma$ t (*Rorc*), the primary transcription factor (TF) controlling IL-17 and IL-22 expression in all lymphocytes (Ivanov et al., 2006). The emergent immature (CD24<sup>hi</sup>)  $\gamma\delta$  thymocyte subsets are further distinguished by TF networks that may specify effector fates. Most of these, including the high-mobility group (HMG) TFs SOX4 and SOX13, are expressed highly only at the early effector programming phase, their expression subsiding once effector capacity has been established at the mature (CD24<sup>lo</sup>) stage. Whether this “early” wave of TFs dominantly programs ILTC subset function was unknown.

In addition to  $T\gamma\delta 17$  ILTCs, there are at least four other ROR $\gamma$ t<sup>+</sup> ILTC and ILC subsets producing IL-17 and/or IL-22:  $\alpha\beta$ TCR<sup>+</sup> invariant NKT (Rachitskaya et al., 2008), lymphoid tissue inducer (LTI)-like (Sawa et al., 2010; Takatori et al., 2009), natural killer (NK) cell receptor expressing IL-22 producer (NKp46<sup>+</sup> NCR22; Luci et al., 2009; Sanos et al., 2009; Satoh-Takayama et al., 2008; Vonarbourg et al., 2010), and ILC17 cells (Buonocore et al., 2010), with the latter three subsets primarily localized in the gut associated lymphoid tissues (GALTs). How ILTCs and ILCs are programmed toward distinct effectors is not well understood. In particular, whether they share a unifying genetic blueprint for differentiation distinct from that specifying adaptive helper IL-17<sup>+</sup> T helper 17 cells (Th17) is unknown. To answer these questions, we determined the mechanism of innate effector programming of  $T\gamma\delta 17$  cells and its possible involvement in GALT ILC differentiation. We showed that the HMG TF TCF1 and LEF1, and their interacting partners SOX4 and SOX13, are expressed at particularly high amounts in the precursors of  $T\gamma\delta 17$  cells (Narayan et al., 2012). HMG TFs are transcription complex architectural proteins that bind to related sequences in the minor groove of DNA, and their cell-type-specific combinatorial clustering at target genes cooperatively controls transcription (Badis et al., 2009). TCF1, the nuclear effector of WNT signaling, is the best characterized HMG TF and is critical for T cell lineage specification downstream of Notch (Germar et al., 2011; Weber et al., 2011). We show here that the HMG TFs, not conventional TCR signaling, programmed IL-17 production in  $\gamma\delta$  ILTCs. Moreover, TCF1 controlled cytokine production in postnatal GALT ILCs and was absolutely



**Figure 1. SOX13 Is Essential for T $\gamma$  $\delta$ 17 Generation**

(A) Frequencies of activated and mature V $\gamma$ 2<sup>+</sup> T cells in  $\gamma$  $\delta$ TCR<sup>+</sup> cells in the spleen and thymus, respectively, of WT and *Sox13*<sup>-/-</sup> mice. Representative data (numbers within the gates represent percents of total) from one experiment of at least four are shown. Similar results were obtained with T-*Sox13*<sup>-/-</sup> mice (B6). (B) The defects in T $\gamma$  $\delta$ 17 generation originate in the thymus. LN and thymic mature (CD24<sup>lo</sup>) V $\gamma$ 2<sup>+</sup> cells from WT and *Sox13*<sup>-/-</sup> mice were analyzed for the expression of ROR $\gamma$ t and EOMES (an activator of *Ifng* transcription), cell surface CCR6 and CD27, and intracellular IL-17A and IFN- $\gamma$  in matV2 cells. Frequencies less than 0.5% are left as blanks.

(C) Intracellular staining for IL-17 in splenic V2 cells isolated from mice 4 hr post-Zymosan administration.

(D) Left shows intracellular and nuclear staining for the two markers of T $\gamma$  $\delta$ 17 cells, BLK and ROR $\gamma$ t, in V2 thymocytes from neonatal mice at different maturational stages. Right shows that staining of Abs to BLK and ROR $\gamma$ t in CD4<sup>+</sup>  $\alpha$  $\beta$  thymocytes was used as negative controls.

(legend continued on next page)

required for NCR22 ILC generation. These results identify shared gene network architecture centered on TCF1 underpinning IL-17 and IL-22 production by ILTCs and GALT ILCs.

## RESULTS

To determine whether a common gene network controls innate lymphoid effector differentiation, we first identified genes selectively required to generate V2  $\gamma\delta$ 17 cells. Emergent  $\gamma\delta$  ILTC subsets are marked with gene expression profiles predictive of their eventual effector functions upon thymic egress (Narayan et al., 2012). Hence, the  $\gamma\delta$  T effector subtype-specific core TF networks were candidates for specifying innate effector lineage fate.  $\gamma\delta$ 17 precursors, immature V2 (immV2, CD24<sup>hi</sup>) thymocytes, express genes encoding three HMG TFs, *Sox4*, *Sox13*, and *Tcf7* (encoding TCF1) at the highest amounts, whereas the HMG TF *Lef1* is expressed at similar amounts relative to other  $\gamma\delta$  cell subsets (Narayan et al., 2012). Aside from *Lef1*, the HMG TF expression precedes that of TCR (Melichar et al., 2007; Schilham et al., 1997; Verbeek et al., 1995; Weber et al., 2011; www.immgen.org). To determine whether this expression pattern programs  $\gamma\delta$ 17 cell differentiation and function, we examined  $\gamma\delta$  T cell subsets in *Sox13*<sup>-/-</sup> (Melichar et al., 2007), *Sox4*-deficient, and *Tcf7*<sup>-/-</sup> (Verbeek et al., 1995) mice and determined HMG TF chromatin occupancies in ex vivo  $\gamma\delta$ 17 precursors.

### Sox13 Programs V2 Cell $\gamma\delta$ 17 Differentiation

We found that *Sox13*-deficiency eliminated all V2  $\gamma\delta$ 17 cells, whereas other  $\gamma\delta$  effector subsets were largely intact (Figure 1). *Sox13* was identified as a  $\gamma\delta$  T cell-specific TF that interacts with TCF1 and LEF1 (Melichar et al., 2007), potentially modulating their function. Whereas all immature  $\gamma\delta$ TCR<sup>+</sup> thymocytes express *Sox13*,  $\gamma\delta$ 17 precursors express the protein at the highest level (see Figure S1A available online), with its expression rapidly extinguished upon thymic maturation (Narayan et al., 2012). Thus, all alterations in ILTCs associated with the absence of SOX13 must originate in the precursors or at the CD24<sup>hi</sup> stage. In *Sox13*<sup>-/-</sup> mice, the frequencies of CD44<sup>hi</sup> V2 cells were severely reduced in peripheral tissues, and CD24<sup>lo</sup> mature (mat) V2 thymocytes were diminished to ~50% of the wild-type (WT) (Figures 1A; Figures S1B and S1C). The numbers of other  $\gamma\delta$  effectors were only marginally lower (Figure S1C; data not shown). Critically, the V2 cells that were specifically absent in *Sox13*<sup>-/-</sup> mice were ROR $\gamma$ t<sup>+</sup>CCR6<sup>+</sup>CD27<sup>-</sup>CD44<sup>hi</sup>CD62L<sup>-</sup>  $\gamma\delta$ 17 cells (Narayan et al., 2012). Fetal and adult ROR $\gamma$ t<sup>+</sup> matV2 thymocytes, the immediate precursors of peripheral  $\gamma\delta$ 17 cells, were missing (Figure 1B; Figure S1D), while the number of immV2 cells was not significantly altered. The remaining V2 cells in *Sox13*<sup>-/-</sup> mice did not synthesize IL-17 (or IL-17F, data not shown) ex vivo (Figure 1B), even after stimulation with the TLR2 ligand, Zymosan (Figure 1C). These results demonstrate that the high SOX13 expression in devel-

oping immV2 thymocytes is a critical factor in  $\gamma\delta$ 17 cell differentiation.

The loss of V2  $\gamma\delta$ 17 cells occurred in both the fetal and adult *Sox13*<sup>-/-</sup> thymus. Fetal-derived V $\gamma$ 4<sup>+</sup> (V4)  $\gamma\delta$  T cells are the alternate IL-17 producers (Shibata et al., 2008). V $\gamma$ 4 gene rearrangements, which predominate in early fetal stages, precede that of V $\gamma$ 2 and the fetal V $\gamma$ 4 chain is paired with the germline encoded V $\delta$ 1TCR. While V4  $\gamma\delta$ 17 cells were negatively impacted in the fetal thymus by the absence of SOX13, these effectors were present in neonatal and adult *Sox13*<sup>-/-</sup> mice (Figures S1E–S1G). This result suggests that despite the lineage and functional relatedness (Narayan et al., 2012), developmental requirements for V2 and V4  $\gamma\delta$ 17 cells are distinct.

B lymphocyte kinase (BLK) is essential for  $\gamma\delta$ 17 development (Laird et al., 2010). Ectopic *Sox13* expression induces *Blk* expression in  $\alpha\beta$  thymocytes (Melichar et al., 2007) and among  $\gamma\delta$  T cells, BLK<sup>+</sup> cells are the sole source of IL-17 during pathogen challenge (Laird et al., 2010; Narayan et al., 2012). In *Sox13*<sup>-/-</sup> mice, V2  $\gamma\delta$ 17 precursors (immV2 cells) expressing normal amounts of BLK were depleted and the BLK and ROR $\gamma$ t coexpressors were specifically absent (Figure 1D). Analysis of *Rorc*<sup>Gfp/+</sup>:*Sox13*<sup>-/-</sup> mice showed decreased, but still significant, transcription of *Rorc* in the mutant immV2 cells (Figure 1E). These results suggested that SOX13-regulated BLK expression at the immature stage is critical for  $\gamma\delta$ 17 cell differentiation. In support of this interpretation, transgenic (Tg) expression of *Sox13* in all developing  $\gamma\delta$  cells (Melichar et al., 2007) increased the proportions of BLK<sup>+</sup>  $\gamma\delta$ TCR<sup>+</sup> cells, as well as the amount of BLK expression per cell, independent of TCR repertoire (Figure 1F). Correspondingly, more  $\gamma\delta$  T cells in peripheral tissues produced IL-17 (Figure 1G). This enhancement was pronounced for V4  $\gamma\delta$  T cells (V $\gamma$ 2<sup>-</sup>), whereas high ectopic *Sox13* expression was particularly detrimental for the survival of V2 cells that express the highest endogenous amount of *Sox13* (Melichar et al., 2007), confounding their analysis in the gain-of-function model system. The absence of V2  $\gamma\delta$ 17 cells in *Sox13*<sup>-/-</sup> mice and the increased IL-17 production from  $\gamma\delta$  T cells by the ectopic expression of SOX13 indicate that SOX13 is necessary for programming IL-17 production in ILTCs.

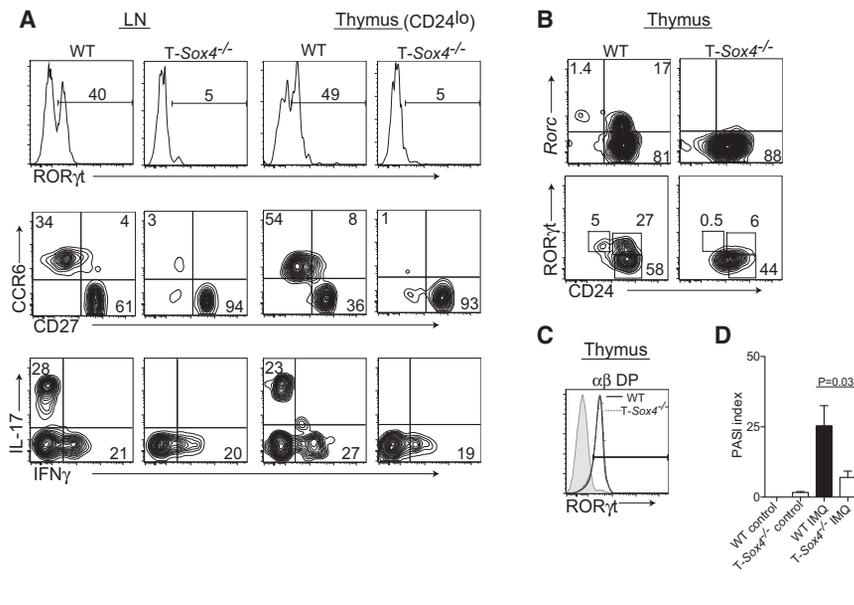
### Sox4 Regulates ROR $\gamma$ t Expression and Is Necessary for IL-17-Mediated Skin Inflammation

Thymic precursors lacking SOX4 also did not generate V2  $\gamma\delta$ 17 ILTCs in vivo (Figure 2). SOX4 is expressed highly in T and B cell precursors as well as in immature  $\alpha\beta$  CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) and  $\gamma\delta$  thymocyte subsets (www.immgen.org). SOX4 is a transcriptional activator and was shown to also physically interact with TCF1 and LEF1 (Sinner et al., 2007; data not shown). We generated T-cell-specific *Sox4*-deficient (T-*Sox4*<sup>-/-</sup>) mice to evaluate the function of SOX4 in ILTCs by breeding CD2 promoter-driven Cre (CD2p-Cre) transgenic (Tg) mice to *Sox4*<sup>fl/fl</sup> mice (Penzo-Méndez et al., 2007). Mature adaptive  $\alpha\beta$  thymocytes were generated in T-*Sox4*<sup>-/-</sup> mice.

(E) SOX13 partly regulates ROR $\gamma$ t expression in CD24<sup>hi</sup> immV2 thymocytes. A decrease in *Rorc* transcription (top) as indicated by GFP expression from *Rorc-Gfp* substrate introduced to *Sox13*<sup>-/-</sup> mice and intranuclear ROR $\gamma$ t protein expression (bottom) is shown. Representative data from one of two experiments are shown.

(F) Intracellular staining for BLK in two maturation stages of V $\gamma$ 2<sup>+</sup> and V $\gamma$ 2<sup>-</sup>  $\gamma\delta$  thymocytes from LCKp-*Sox13* Tg mice.

(G) Intracellular staining for IL-17A in *Sox13* Tg<sup>+</sup> LN  $\gamma\delta$  T cells. See also Figure S1.



**Figure 2. SOX4 Regulates ROR $\gamma$ t Expression during T $\gamma$  $\delta$ 17 Generation**

(A) LN and mature thymic V $\gamma$ 2<sup>+</sup> cells from WT (CD2p-Cre:Sox4<sup>+/+</sup>) and T-Sox4<sup>-/-</sup> mice were analyzed for the expression of ROR $\gamma$ t, CCR6, and CD27 and intracellular IL-17 and IFN- $\gamma$ . Representative data from one of four experiments is shown.

(B) SOX4 regulates ROR $\gamma$ t expression in immV2 thymocytes. The loss of *Rorc* transcription (top) as indicated by the loss of GFP expression from *Rorc-Gfp* substrate introduced to T-Sox4<sup>-/-</sup> mice and intranuclear ROR $\gamma$ t protein expression (bottom) is shown. Representative data from one of three experiments are shown.

(C) Overlaid histograms of ROR $\gamma$ t staining in  $\alpha\beta$  DP thymocytes in WT and T-Sox4<sup>-/-</sup> mice. The shaded histogram is the internal negative control for ROR $\gamma$ t staining, gated on  $\alpha\beta$  CD4<sup>+</sup> thymocytes that do not express *Rorc*.

(D) PASI scoring was used to quantify the severity of psoriatic inflammation in IMQ treated mice. See also Figure S2. Data is represented as mean  $\pm$  SEM.

Strikingly, V2 T $\gamma$  $\delta$ 17 ILTCs were not observed in T-Sox4<sup>-/-</sup> mice, whereas other  $\gamma\delta$  effector subsets were present (Figure 2A; data not shown). As in Sox13<sup>-/-</sup> mice, the V2 cells that were completely lost in peripheral lymphoid tissues were ROR $\gamma$ t<sup>+</sup>CCR6<sup>+</sup>CD27<sup>-</sup>CD44<sup>hi</sup>CD62L<sup>-</sup> T $\gamma$  $\delta$ 17 cells (Figures 2A and 2B). ROR $\gamma$ t<sup>+</sup>CCR6<sup>+</sup>CD27<sup>lo</sup> matV2 thymocytes were virtually undetectable in fetal and adult T-Sox4<sup>-/-</sup> mice (Figure 2A; data not shown), whereas immV2 cells were present in normal proportions (Figure S2A) and did not exhibit decreased rates of survival or proliferation (Figure S2B). Accordingly, the remaining differentiated V2 cells in T-Sox4<sup>-/-</sup> mice did not produce IL-17 ex vivo (Figure 2A). The block in T $\gamma$  $\delta$ 17 cells correlated with a loss of *Rorc* transcription (based on *Rorc*<sup>Gfp/+</sup>:T-Sox4<sup>-/-</sup> mice) and ROR $\gamma$ t protein expression beginning in immV2 thymocytes (Figure 2B; Figure S2C). This loss was nearly absolute, more severe than the decrease observed in Sox13<sup>-/-</sup> mice (Figure 1E). In contrast, ROR $\gamma$ t expression in  $\alpha\beta$  DP thymocytes was unaffected by the loss of SOX4 (Figure 2C), indicating that SOX4 is a  $\gamma\delta$  ILTC-specific modulator of ROR $\gamma$ t expression.

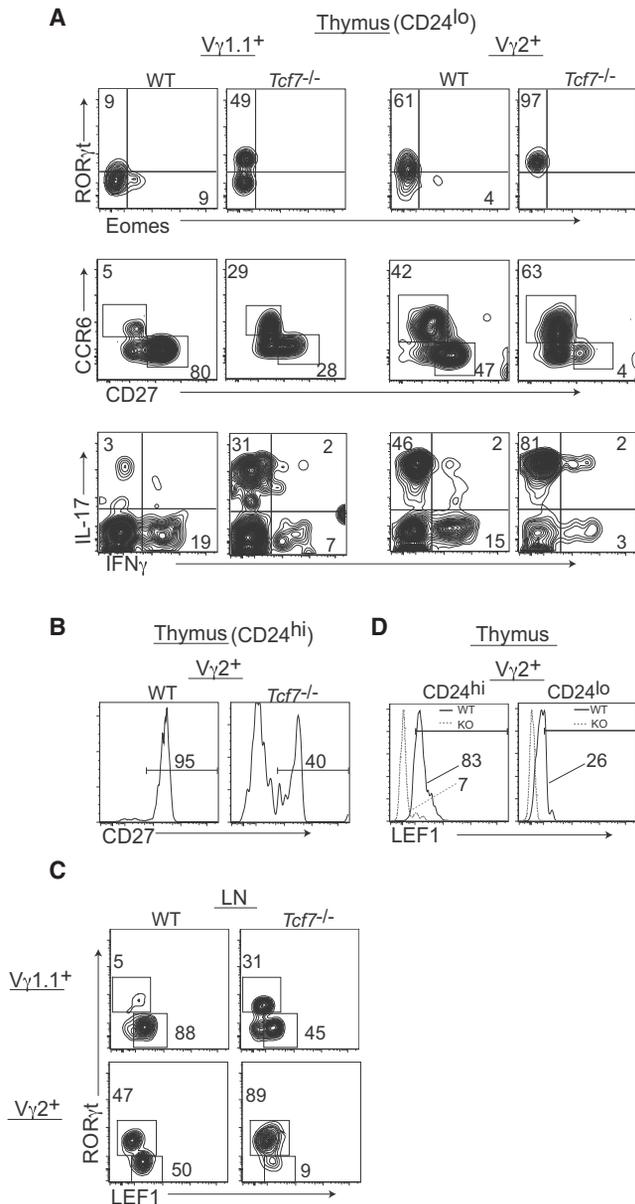
T $\gamma$  $\delta$ 17 cells have been implicated in the dermal inflammation-driven psoriasis-like disease in mice (Cai et al., 2011; Pantelyushin et al., 2012). The disease can be induced by the application of the TLR7 ligand Imiquimod (IMQ) to skin. T $\gamma$  $\delta$ 17 cells residing in the dermis have been shown to be the primary lymphoid responders responsible for the disease. To determine whether SOX4 is necessary to generate pathogenic dermal T $\gamma$  $\delta$ 17 cells, we first assessed the distribution of  $\gamma\delta$  T cell subsets in the dermis of T-Sox4<sup>-/-</sup> mice. V2, but not V4, T $\gamma$  $\delta$ 17 cells were greatly reduced in T-Sox4<sup>-/-</sup> dermal tissues before treatment (Figures S2D and S2E). After topical application of IMQ for 5 days, we observed significant thickening, scaling, and erythema in WT mice. However, T-Sox4<sup>-/-</sup> mice did not show overt inflammation (Figures S2F and S2G), which was quantified by the adapted Psoriasis Area and Severity Index (PASI) scoring system (Figure 2D). The lack of inflammation was correlated with significantly decreased proportions of V2 T $\gamma$  $\delta$ 17 cells (Figures

S2D and S2E) and CD11b<sup>+</sup>Gr-1<sup>+</sup> neutrophils (Figure S2H) in the treated T-Sox4<sup>-/-</sup> dermis.

Unlike the dermis, V2 T $\gamma$  $\delta$ 17 cells were a minor population relative to V $\delta$ 1<sup>+</sup>IL-17<sup>+</sup> V4 cells in skin draining LNs of resting WT mice (Figures S2I and S2J). V $\delta$ 1<sup>+</sup>IL-17<sup>+</sup> V4 cells were decreased in number in the fetal T-Sox4<sup>-/-</sup> thymus (~30% of the WT number), and reduced but substantial numbers of these fetal-derived effectors were found in adult T-Sox4<sup>-/-</sup> thymus and lymph nodes (LNs, Figure S2I; data not shown). V4 LN T cells responded to IMQ, as indicated by an increase in the proportion of IL-17 producers (Figure S2I). However, this response, and the persistence of dermal V4 cells in T-Sox4<sup>-/-</sup> mice (Figures S2D and S2E), was insufficient to precipitate the fulminant inflammatory condition in the skin. In conjunction with previously published reports (Cai et al., 2011; Pantelyushin et al., 2012), these results indicate that SOX4-dependent V2 T $\gamma$  $\delta$ 17 cells are the primary ILTCs mediating IMQ-mediated skin inflammation. In addition, the results showed that fetal-derived V4 T $\gamma$  $\delta$ 17 cells, while acutely dependent on SOX13 during gestation, have compensatory mechanisms to bypass the SOX4 requirement and replenish their numbers in peripheral tissues in the absence of either TFs. In contrast, the “late” V2 T $\gamma$  $\delta$ 17 cells are not produced in the absence of Sox4 or Sox13, reinforcing the conclusion that these two ILTC subsets are generated under distinct conditions and are not functionally interchangeable.

### TCF1 Restrains IL-17<sup>+</sup> Cell Generation

*Tcf7* is turned on by Notch signaling to specify the T cell fate (Gernar et al., 2011; Weber et al., 2011). Notch signaling also controls GALT ILC differentiation (Lee et al., 2012; Possot et al., 2011), raising the possibility that TCF1 is the core regulator of innate effector differentiation. In the absence of TCF1, development of thymic precursors and  $\alpha\beta$  T cells is aberrant (Verbeek et al., 1995). Although the total  $\gamma\delta$  thymocyte number is not significantly decreased in young *Tcf7*<sup>-/-</sup> mice (Verbeek et al.,



**Figure 3. TCF1 Constrains T $\gamma\delta$ 17 Generation**

(A) Deregulated IL-17 production in *Tcf7*<sup>-/-</sup> mice. Differentiation of T $\gamma\delta$ 17 thymocytes was examined by analyses of ROR $\gamma$ t and EOMES, CCR6 and CD27, and intracellular IL-17A and IFN- $\gamma$  expression in mature (CD24<sup>lo</sup>) V $\gamma$ 1.1<sup>+</sup> and V $\gamma$ 2<sup>+</sup>  $\gamma\delta$  T cells. Similar results were obtained when peripheral  $\gamma\delta$  T cell subsets were analyzed. Representative profiles from one of at least five independent experiments, each with minimum of three mice per genotype, are shown.

(B) Expression of CD27 expression on *Tcf7*<sup>-/-</sup> immV2 thymocytes. A similar trend for increased ratio of CCR6/CD27 was observed with other  $\gamma\delta$  thymic subtypes.

(C) Intranuclear staining for ROR $\gamma$ t and LEF1 in LN V $\gamma$ 1.1<sup>+</sup> (top) and V $\gamma$ 2<sup>+</sup> (bottom) T cells from WT and *Tcf7*<sup>-/-</sup> mice shows mutually exclusive expression of the TFs and the loss of LEF1<sup>+</sup>  $\gamma\delta$  T cells when TCF1 is nonfunctional. TCF1 expression, although biased, is not starkly separated from ROR $\gamma$ t expressors in any  $\gamma\delta$  T cell subsets. Staining controls are shown in Figure S3E.

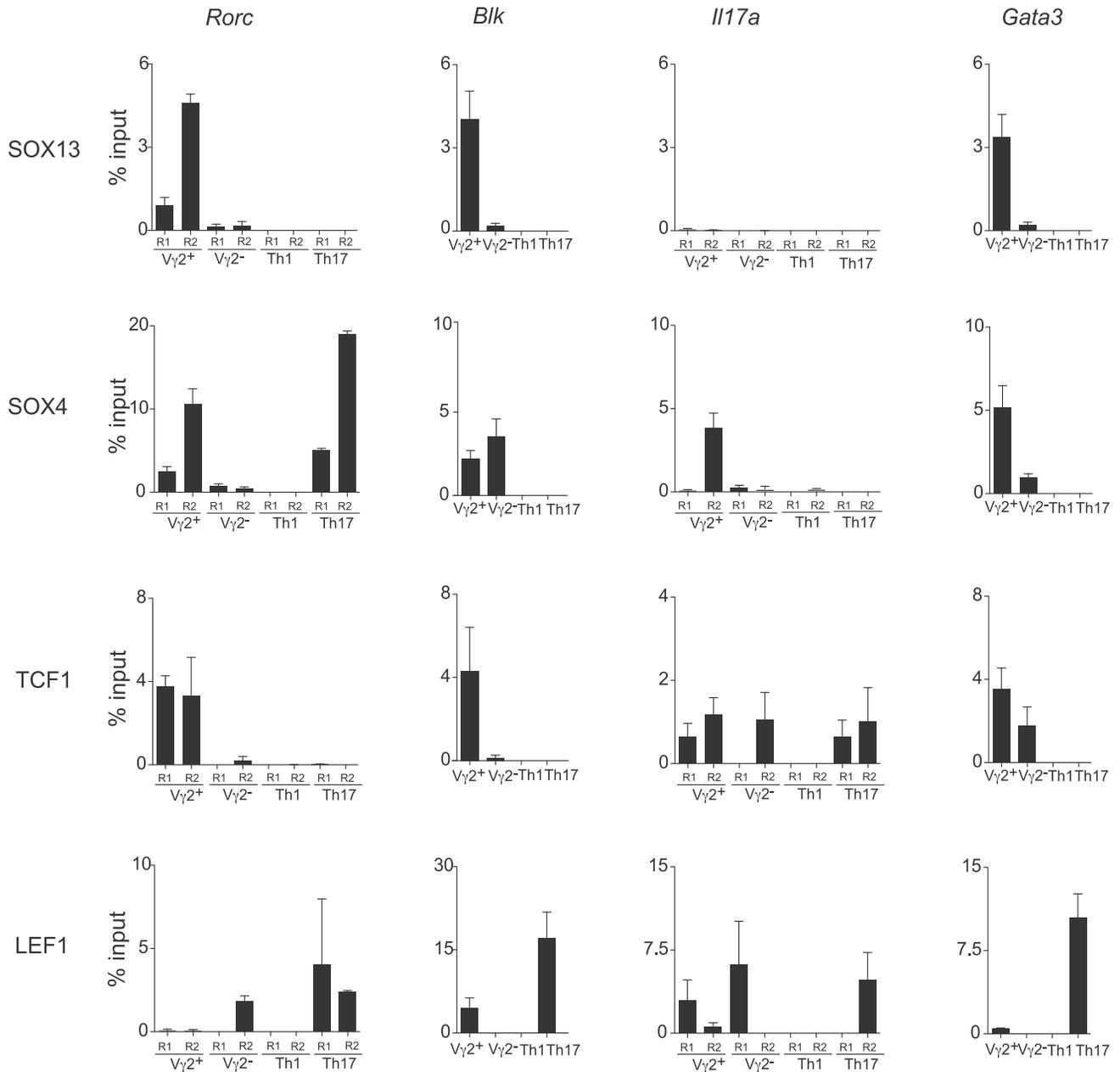
(D) Intranuclear staining for LEF1 in immature (CD24<sup>hi</sup>) and mature (CD24<sup>lo</sup>) V $\gamma$ 2<sup>+</sup> thymocytes from WT and *Tcf7*<sup>-/-</sup> mice. See also Figure S3.

1995), we found that the effector programs of  $\gamma\delta$  ILTCs were extensively distorted (Figures S3A and S3B). In particular, thymic and peripheral V2 cells exhibited skewed ratios of CCR6<sup>+</sup> (marking T $\gamma\delta$ 17 cells) to CD27<sup>+</sup> (IFN- $\gamma$  producers, [Ribot et al., 2009]) populations, with the latter subset being undetectable in some *Tcf7*<sup>-/-</sup> mice (Figure 3A). More than 80% of *Tcf7*<sup>-/-</sup> V2 cells produced IL-17, twice the frequency observed in WT V2 cells, and they were uniformly ROR $\gamma$ t<sup>+</sup> (Figure 3A). The bias toward IL-17 production was not V2 cell-specific because the number of thymic mature V $\gamma$ 1.1<sup>+</sup> (normally IFN- $\gamma$ <sup>+</sup>) cells expressing IL-17 was also increased by >10-fold (Figure S3C). This pattern was not observed in *Tcrb*<sup>-/-</sup> mice (N.M., data not shown), indicating that the deregulated effector programming is not simply a consequence of the decreased production of  $\alpha\beta$  thymocytes in *Tcf7*<sup>-/-</sup> mice.

In all thymic  $\gamma\delta$  subtypes, the decreased expression of CD27 was already evident at the immature stage (Figure 3B). This pattern, along with a relatively normal cell-cycle status of *Tcf7*<sup>-/-</sup>  $\gamma\delta$  thymocytes (Figure S3D), suggested that the proclivity of TCF1-deficient  $\gamma\delta$  ILTCs toward the IL-17 effector fate is an early developmental event, not a consequence of altered maintenance of mature effectors. This interpretation was further supported by the LEF1 expression pattern. LEF1 was a discriminatory regulator of  $\gamma\delta$  effectors, as evidenced by its mutually exclusive expression to ROR $\gamma$ t in  $\gamma\delta$  thymocytes (Figure 3C; Figure 3E) and the partial and complete loss of LEF1<sup>+</sup> subsets in V $\gamma$ 1.1<sup>+</sup> and V2 cells, respectively, when TCF1 was absent (Figures 3C and 3D), again starting at the immature stage of differentiation (Figure 3D). *Lef1* expression is primarily controlled by TCF1 (Driskell et al., 2007). Given the precedent that some TCF1 target gene expression can be inhibited by SOX13 (Marfil et al., 2010; Melichar et al., 2007), high amounts of SOX13 in immV2 thymocytes may interfere with TCF1-mediated induction of *Lef1*. Consistent with this, immature  $\gamma\delta$ TCR<sup>+</sup> thymocytes from *Sox13*Tg mice expressed significantly lower amounts of LEF1 and CD27 (Figure S3F). Taken together, TCF1 is necessary for pan- $\gamma\delta$  T cell development (Figures S3G and S3H), and it programs  $\gamma\delta$  T effector subset differentiation, promoting and inhibiting IFN- $\gamma$  and IL-17 production, respectively, whereas SOX4 and SOX13 have the opposite function.

### HMG TF Chromatin Occupancy

Fetal and adult immV2 thymocytes share the TF transcriptome (Figures S4A and S4B). To determine whether HMG TFs directly regulate the T $\gamma\delta$ 17 gene network, we examined their chromatin occupancies at three gene loci (*Blk*, *Rorc*, and *Il17a*) that are the hallmarks of T $\gamma\delta$ 17 cells, along with the ubiquitously active *Gata3* locus as a control, in ex vivo immature V $\gamma$ 2<sup>+</sup> and V $\gamma$ 2<sup>-</sup> thymocytes. First, we established epigenetic chromatin modifications of the gene loci. For comparison, in vitro differentiated adaptive Th1 and Th17  $\alpha\beta$  T cells were examined. Among  $\gamma\delta$  T cell subsets, *Blk* and *Rorc* are most abundantly expressed in V2 cells (Narayan et al., 2012). Accordingly, the *Blk* and *Rorc* loci were selectively enriched for active H3K4me3 (and acetylated H3, data not shown) modifications in immV $\gamma$ 2<sup>+</sup> cells. Conversely, the *Rorc* locus was repressed in immV $\gamma$ 2<sup>-</sup> cells as indicated by H3K27me3 markings (Figure S4C). In contrast, *Il17a* was decorated exclusively with repressive H3K27me3 chromatin marks in the immature  $\gamma\delta$  thymocytes, consistent



**Figure 4. ChIP Assay for TF Binding near the Transcriptional Regulatory Sites of *Rorc*, *Blk*, *Il17a*, and *Gata3* Loci**

Immature  $V\gamma 2^+$  and  $V\gamma 2^-$  thymocytes, the immediate precursors of mature thymic effectors, were compared to in vitro differentiated control Th1 and Th17 CD4  $\alpha\beta$  T cells. Analysis of mature  $\gamma\delta$  thymocytes was not possible due to their low numbers in mice. Graphs show quantitative PCR detection for relative enrichment of target DNA sequences from ChIP using Abs to indicated TF and control IgG (Figure S4). The regions examined are described in Figure S4 legend. Quantitative real-time PCR data are plotted as average percentage (%) of input  $\pm$  SD from two independent experiments. Binding of the TFs to TCF consensus sequences at the control *MyoG* promoter was undetectable in T cells (data not shown). See also Figure S4.

with its restricted expression in mature  $\gamma\delta$  thymocytes (Narayan et al., 2012). These results indicate that the distinct effector gene expression profiles of immature  $\gamma\delta$  cell subsets are foremost regulated at the chromatin level.

Next, we determined HMG TF occupancy of *Blk*, *Rorc*, and *Il17a* loci. SOX13 was localized to the *Blk* and *Rorc* loci in immature  $V\gamma 2^+$ , but not  $V\gamma 2^-$ , thymocytes (Figure 4). The low signals in  $V\gamma 2^-$  thymocytes can be accounted for by low SOX13 protein

expression in the cells (Figure S1A). Although SOX4 was detected at all four loci assessed in  $\gamma\delta$  thymocytes, only the docking at the *Rorc* loci was conserved in  $\alpha\beta$  Th17 cells. Moreover, SOX4 was particularly enriched at the Region 2 (R2) near the transcription start site dedicated for ROR $\gamma$ t production in  $V\gamma 2^+$  thymocytes (Ruan et al., 2011). These results supported the above finding (Figure 2) that SOX4 is an essential regulator of *Rorc* expression in T $\gamma\delta$ 17 cells.

TCF1 was enriched at the *Blk* and *Rorc* loci in immature  $V\gamma 2^+$  cells, but not in immature  $V\gamma 2^-$  cells. TCF1 and LEF1 occupancy at the *I17a* locus exhibited distinct modality with TCF1 preferentially enriched at the intronic R2, previously shown to be a docking region in  $\alpha\beta$  T cells (Yu et al., 2011), in both thymic subsets and LEF1 at the promoter upstream R1, particularly in immature  $V\gamma 2^-$  thymocytes. Consistent with the TCF1 chromatin occupancy in precursor cells (Weber et al., 2011), TCF1 was found docked onto the *Gata3* locus in  $\gamma\delta$  thymocyte subsets, but LEF1 was not. LEF1 was associated with the *Blk* locus in  $V\gamma 2^+$  thymocytes, presumably in the  $ROR\gamma t^-$  fraction, based on the mutually exclusive expression of  $ROR\gamma t$  and LEF1 in V2 thymocytes (Figure 3C). That LEF1 docking at the *Blk* locus is neutral to suppressive for transcription is supported by a selective enrichment at the locus in  $\alpha\beta$  Th17 cells that do not express *Blk*. As expected, LEF1 was excluded from the *Rorc* locus in  $V\gamma 2^+$  thymocytes. Integrated with the results from the genetic studies, these results indicate that the HMG TFs are direct transcriptional regulators of the  $T\gamma\delta 17$  genes. SOX13 and SOX4 cooperatively orchestrate  $T\gamma\delta 17$  differentiation by primarily controlling *Blk* and *Rorc* transcription, respectively. TCF1, implicated as a negative regulator of  $T\gamma\delta 17$  cells, was associated with all the loci examined. Its docking at the *Rorc* and *Blk* loci was  $V\gamma 2^+$  cell-type-specific, a pattern overlapping with SOX13 and SOX4, and raises the likelihood that a combinatorial assortment of HMG TFs at each target gene locus is directly controlling ILTC effector fate specification.

### Conventional TCR Signaling Alone Cannot Specify $T\gamma\delta 17$ Fate

A potential mechanistic explanation for the distinct global gene expression profiles of immature  $\gamma\delta$  effector subsets is that different  $\gamma$ TCR chains (for example,  $V\gamma 1.1/V\gamma 5$  versus  $V\gamma 2/V\gamma 4$ ) convey different signals to establish diverse differentiation programs. For extrathymic adaptive Th17 cell differentiation, TCR signaling-induced IRF4 (Brüstle et al., 2007) and ITK-NFAT (Gomez-Rodriguez et al., 2009) are critical regulators of *Rorc* and *I17a* expression, respectively. IRF4 is dispensable for  $T\gamma\delta 17$  cell differentiation (Powolny-Budnicka et al., 2011). The role of ITK in  $T\gamma\delta 17$  differentiation was unknown. Peripheral *Itk*<sup>-/-</sup>  $\gamma\delta$  T cells were impaired in their  $Ca^{2+}$  response when they were activated via TCR stimulation in vitro (Figure S5A), establishing ITK as a key signal integrator of  $\gamma\delta$ TCR signaling. In the absence of ITK, the transcriptomes of immV2 thymocytes converged with other  $\gamma\delta$  cell subsets, as indicated by principal component analysis (PCA, Figure 5A) that clusters related populations based on the major components of gene expression variability. ITK signaling was responsible for ~90% of the unique immV2 thymocyte transcriptome (Figure S5B). However, the characteristic TF profile of immV2 cells was mostly insulated from change when ITK was absent, as shown by hierarchical clustering (Figure 5B), correlating with the relatively normal generation of  $T\gamma\delta 17$  cells in *Itk*<sup>-/-</sup> mice (Figure S5C). These results demonstrate that while  $V\gamma 2$ TCR-ITK signaling is central to the establishment of distinct transcriptomes of  $\gamma\delta$  subsets it is not responsible for the wiring of  $\gamma\delta$  effector subset-specific TF networks at the immature stage. Together, these results indicate that the conventional TCR signaling pathways so far

implicated in adaptive Th17 differentiation do not dominantly specify the  $T\gamma\delta 17$  cell fate.

To directly assess the role of the  $\gamma\delta$ TCR in  $T\gamma\delta 17$  cell differentiation,  $\gamma\delta$  T effector development was tracked in  $V\gamma 2$  TCR Tg mice where nearly all  $\gamma\delta$  T cells express the identical  $V\gamma 2$  TCR chain (Kang et al., 1998).  $V\gamma 2$  TCR Tg expression on the cell surface is controlled by endogenous TCR $\delta$ -chain gene rearrangement and expression. If the TCR is deterministic for effector fate, expression of a functional  $V\gamma 2^+$  TCR in all  $\gamma\delta$  T cells should enhance the generation of  $T\gamma\delta 17$  cells. The Tg mice, however, did not produce a significantly enhanced number of IL-17<sup>+</sup>  $\gamma\delta$  cells, nor could the *Tcrg* Tg expression in *Sox13*<sup>-/-</sup> mice rescue the  $T\gamma\delta 17$  differentiation defect (Figure 5C). These results indicated that specific  $V\gamma 2$  TCR signaling per se is not the dominant determinant of effector lineage specification.

If the TCR signaling alone cannot specify ILTC effector fates, an alternate possibility was that distinct effector programs are preset at different developmental stages. To test whether T cell developmental intermediates possess unique effector generative capacity, we compared the ability of the early c-Kit (CD117)<sup>+</sup> T progenitors (ETP) versus the late c-Kit<sup>neg</sup> DN3 precursors to generate  $T\gamma\delta 17$  effectors. ETPs (fetal and adult) generated CCR6<sup>+</sup>CD27<sup>-</sup>  $T\gamma\delta 17$  V2 cells in the standard OP9-DL1 culture system (Figure 5D). However, no significant generation of CCR6<sup>+</sup>CD27<sup>-</sup> V2 cells was detectable from DN3 precursors. Further, the late precursors were markedly biased to produce V1 and V2 CD27<sup>+</sup>  $\gamma\delta$  cells (Figure S5D; data not shown). Together, these findings suggest that the maturational state of the precursors, and not TCR signaling alone, is a key determinant of  $T\gamma\delta 17$  effector lineage specification.

### TCF1 Is Necessary for GALT ILC Differentiation

One implication of the dominance of the HMG TF network in ILTC effector differentiation was that a similar regulatory gene network may operate to generate GALT ILCs that lack clonal antigen receptors. GALT ILCs express several HMG TFs at the messenger RNA level. These include *Tcf7*, *Sox4*, and *Tox* (Aliahmad et al., 2010), though not *Sox13* (Reynders et al., 2011; data not shown). To determine whether HMG TF networks control innate effector differentiation extrathymically, we first established the ILC subset-specific expression pattern of TCF1 and LEF1 proteins and *Axin2*, a canonical TCF1-WNT signaling target that can serve as a reporter of TCF1 as a transcriptional activator (Lustig et al., 2002). In mLN and splenic CD3<sup>-</sup>CD19<sup>-</sup> cells, TCF1 was expressed highly in IL-7R<sup>+</sup> subsets, with all IL-7R<sup>hi</sup>ROR $\gamma t^+$  LTi-like ILCs uniformly positive. The majority of mLN IL-7R<sup>+</sup> NKp46<sup>+</sup> NCR22 cells also expressed TCF1 (Figures 6A and 6B; Figure S6A), whereas <10% of the ROR $\gamma t^-$ IL-7R<sup>-</sup> was TCF1<sup>+</sup>. Expression of TCF1 and CD4, a classic marker of LTi cells, was mostly concordant, with only some CD4<sup>+</sup>IL-7R<sup>lo-neg</sup> cells lacking TCF1. LEF1, coexpressed with TCF1 in adaptive T cells, was not expressed in LTi-like or NCR22 ILCs (Figure 6A), paralleling its exclusion from  $\gamma\delta$  thymocytes fated for IL-17 production (Figure 3C). Similar results were obtained with neonatal intestinal lamina propria (LP) ILC subsets (data not shown). *Axin2* was variably expressed in TCF1<sup>+</sup> ILC subsets, with LTi-like ILCs (c-Kit<sup>+</sup>IL-7R<sup>+</sup>Lin<sup>-</sup>,  $\alpha 4\beta 7^{+/-}$ , >50% *Axin2*<sup>+</sup>) considerably enriched for WNT signaling activity than in NCR22 ILCs (~10% *Axin2*<sup>+</sup>) (Figure S6B). These results indicate

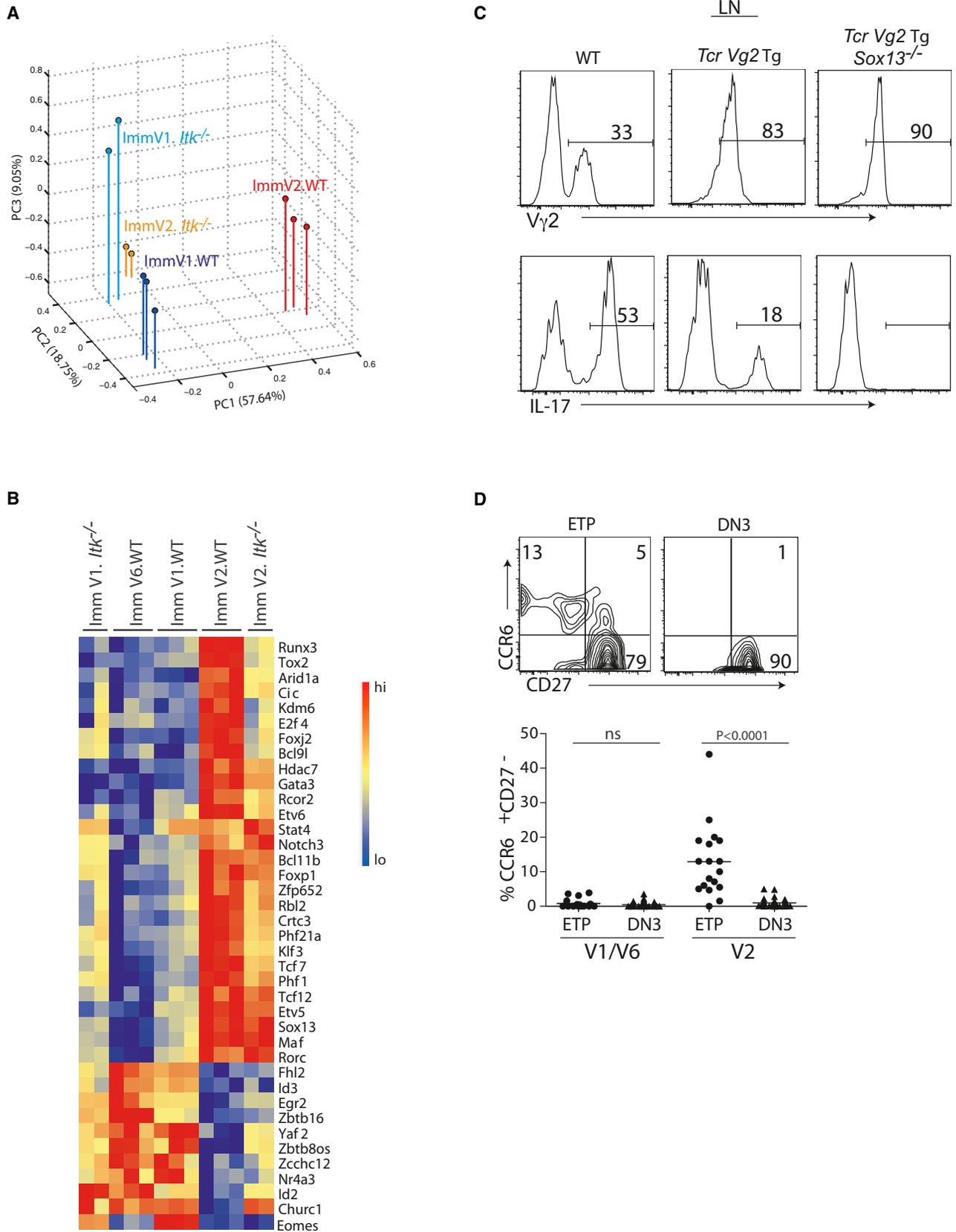


Figure 5. Constrained Impact of TCR Signaling in Effector Specification

(legend on next page)

that although TCF1 is expressed in most, but not all ILCs, it is likely to have broad activities beyond that of the canonical WNT signaling transcriptional activator.

To determine the range of TCF1 function, ILC subset composition, and function in the small intestine, mLN and spleen of *Tcf7*<sup>-/-</sup> mice were examined. In *Tcf7*<sup>-/-</sup> neonatal intestines and spleens, NKp46<sup>+</sup>IL-7R<sup>+</sup> NCR22 ILCs were specifically absent, whereas CD4<sup>+</sup> LTI-like ILCs were overrepresented proportionally, but only marginally increased in numbers (Figure 6C; data not shown). LTI-like ILCs are CCR6<sup>+</sup>CD25<sup>+</sup> (Sawa et al., 2010; Vonarbourg et al., 2010) and the frequency of the CCR6<sup>+</sup> fraction was elevated, a trend that was already evident in *Tcf7*<sup>+/-</sup> heterozygotes (Figure 6D). However, *Tcf7*<sup>-/-</sup> LTI-like ILCs lost CD25 expression, most likely indicating that as in T cell precursors (Weber et al., 2011), TCF1 may be a positive regulator of *Cd25* transcription in ILCs.

In 3- to 4-week-old *Tcf7*<sup>-/-</sup> mice, the number of ROR $\gamma$ T<sup>+</sup> ILCs was reduced to one third of normal in the mLN, whereas IL-7R<sup>+</sup> CD3<sup>-</sup>CD19<sup>-</sup>ROR $\gamma$ T<sup>neg-lo</sup> fraction was decreased to 10% of normal (Figure 6E). As in neonates, NKp46<sup>+</sup> ILCs were specifically depleted (Figures 6E and 6F; Figure S6C). These results show that TCF1 is absolutely required for NCR22 cell generation, whereas LTI-like ILC production per se appears less dependent on TCF1. During T $\gamma$  $\delta$ 17 differentiation, TCF1 dampened effector capacity (Figure 3). To determine whether TCF1 functions similarly in differentiated ILCs, we assessed the effector capacity of *Tcf7*<sup>-/-</sup> ILCs. All ROR $\gamma$ T<sup>+</sup> ILCs in *Tcf7*<sup>-/-</sup> mice expressed higher amounts of ROR $\gamma$ T per cell (Figure 6G) and were capable of enhanced IL-17 production (Figure 6H, top row). Upon activation with TLR2 agonist Zymosan, IL-17 and IL-22 production from *Tcf7*<sup>-/-</sup> LTI-like ILCs was significantly elevated (Figure 6H; Figure S6C). Thus, as in  $\gamma$  $\delta$  ILTC development, TCF1 has a dual function in ILC development, coordinating normal gene induction to ensure proper differentiation of ILC subsets and controlling effector function by restraining ROR $\gamma$ T expression and IL-17 and IL-22 production.

## DISCUSSION

We showed that dermal SOX13 and SOX4-dependent V2 T $\gamma$  $\delta$ 17 cells are the primary innate lymphoid mediators of psoriasis-like disease in C57BL/6 mice and they develop in the thymus under the control of a HMG TF regulatory network. Adaptive Th17 cell differentiation in peripheral tissues requires TCR signaling and its downstream targets ITK (Gomez-Rodriguez et al., 2009) and

IRF4 (Brüstle et al., 2007), the inflammatory cytokine IL-6 and its signal mediator STAT3 (Zhou et al., 2007), as well as TGF- $\beta$ -activated SMAD2 (Malhotra et al., 2010). None of these factors are essential for T $\gamma$  $\delta$ 17 ILTC development in the thymus (Lochner et al., 2008; Malhotra et al., 2010; Powolny-Budnicka et al., 2011). Instead, a complex network of HMG TFs cooperatively controls thymic ILTC differentiation by direct regulation of key genes involved in effector function. Among them, SOX4 and SOX13 are the central positive regulators of T $\gamma$  $\delta$ 17 differentiation, by primarily inducing ROR $\gamma$ T and BLK, respectively, and potentially localizing their interacting partner TCF1 to select chromatin sites. Given that HMG TFs operate in conjunction with cofactors, a detailed understanding of target gene-specific function of SOX4 and SOX13 awaits the full characterization of transcriptional complexes assembled by each factor.

During early fetal dendritic epidermal  $\gamma$  $\delta$  T cell (V $\gamma$ 3<sup>+</sup>) differentiation, cell surface SKINT signaling normally suppresses *Rorc* and *Sox13* expression to block IL-17 production (Turchinovich and Hayday, 2011), underscoring the importance of SOX13 in positively enforcing the IL-17 effector fate that must be circumvented to generate alternate innate effector cells in the fetus. SOX13 regulates several key factors of V2 T $\gamma$  $\delta$ 17 cell differentiation, including *Blk*, *Rorc*, and *Etv5* (K.E.S., data not shown). The exclusion of LEF1 from developing T $\gamma$  $\delta$ 17 cells is also likely to be established by SOX13, as suggested by the diminished *Lef1* expression in *Sox13*Tg mice. Although published studies to date support protein-protein interactions as the main regulatory mode of SOX13-TCF1 functions, it remains possible that each can impact chromatin occupancy of the other. For instance, the loss of TCF1 may result in more precursors with SOX13 bound to the *Rorc* locus, thereby leading to the enhanced generation of T $\gamma$  $\delta$ 17 cells. However, *Tcf7*<sup>-/-</sup>  $\gamma$  $\delta$  cells do not express LEF1, and the loss of LEF1-dependent effector developmental potential may also indirectly enhance T $\gamma$  $\delta$ 17 cell production. A systemic approach that can simultaneously track all relevant HMG TFs during T $\gamma$  $\delta$ 17 differentiation from thymic precursors will be necessary to define the rules governing functional connectivities of HMG TFs.

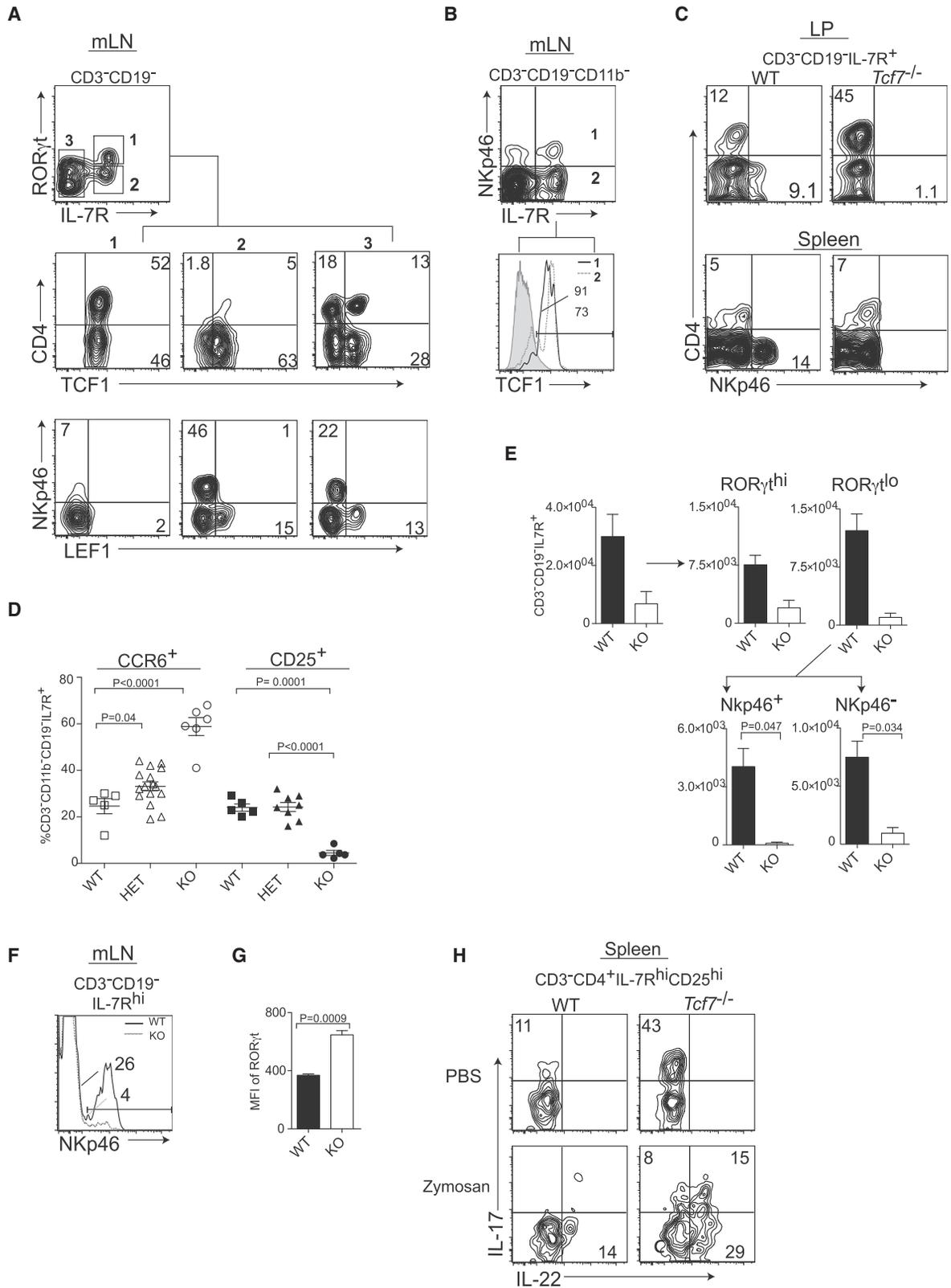
The mechanism by which the temporally disparate emergence of  $\gamma$  $\delta$  effector subtypes is linked to specific TCR $\gamma$  and  $\delta$  repertoire remains to be determined. We have identified ITK as a discriminatory signal mediator of TCR that is required for the molecular divergence of V2 cells from other  $\gamma$  $\delta$  cell subsets. Previously, it has been shown that some IL-17<sup>+</sup> V $\gamma$ 2<sup>+</sup> T cells can be generated in the absence of ligand recognition, whereas TCR triggering led

(A) PCA of the discriminatory gene signature of *Itk*<sup>-/-</sup> immV2 cells. PCA of the 15% most variable genes among the populations of cells shown (colors of bars and labels indicate population; MEV > 120 in at least one population; 1,433 genes). The first three principal components (PC1–PC3) are shown, along with the proportion of the total variability represented by each component (in parentheses along axes).

(B) A heat map of relative gene expression of TFs in immature  $\gamma$  $\delta$  subsets from WT and *Itk*<sup>-/-</sup> mice. Data were gene row normalized and hierarchically clustered by gene and subset. Genes are color coded (see legend) to display relative gene expression.

(C) LN cells from WT and *Tcrvg2* transgenic mice (with and without normal *Sox13*) that express a functional V $\gamma$ 2-J $\gamma$ 1-C $\gamma$ 1 (*Tcr Vg2 Tg*) chain in nearly all  $\gamma$  $\delta$  T cells were analyzed for the expression of V $\gamma$ 2 in  $\gamma$  $\delta$  T cells (top) or intracellular IL-17A in V $\gamma$ 2<sup>+</sup> T cells (bottom). Representative profiles from one of two experiments are shown, each with a minimum of three/group. Similar results were observed with thymocytes.

(D)  $\gamma$  $\delta$  T cell progenies of c-Kit<sup>hi</sup> ETPs and c-Kit<sup>lo</sup> DN3 (CD25<sup>+</sup>CD44<sup>-</sup>CD3<sup>-</sup>CD4<sup>-</sup>CD8<sup>-</sup>) precursors cultured on OP9-DL1 stromal monolayers were assayed for CCR6 and CD27 expression. Representative FACS plots of V2 cells (top) and a summary of the frequencies (bottom, Student t test p values) of CCR6<sup>+</sup>CD27<sup>-</sup> T $\gamma$  $\delta$ 17 cells generated from ETPs (10<sup>3</sup> cells per well) or DN3 (5 × 10<sup>3</sup> cells per well) precursors are shown. Similar results were obtained with varying cell numbers per well. Average cell numbers obtained from DN1 or DN3 were 3.5 × 10<sup>5</sup> or 4.6 × 10<sup>4</sup> per well, respectively. V1/V6 = V $\gamma$ 1.1<sup>+</sup> cells. Data are combined from three independent experiments, ETP n = 18; DN3 n = 38. See also Figure S5.



**Figure 6. TCF1 Regulates the Differentiation and Function of GALT ILCs**

(A) TCF1/LEF1 expression in CD3<sup>+</sup>CD19<sup>-</sup> mLN ILCs of adult mice. ILCs were segregated based on ROR $\gamma$ t and IL-7R expression. CD4, NKp46, intranuclear TCF1, and LEF1 expression was assessed on the three indicated subsets. Data shown are representative profiles from one of three independent studies.

(legend continued on next page)

to the capacity to produce IFN- $\gamma$  (Jensen et al., 2008). In vitro assays, however, showed that the cell surface expression of V $\gamma$ 2TCR itself is uniquely able to trigger signaling, akin to the pre-TCR signaling that generates  $\alpha\beta$  DP cells. Given the substantial, but constrained, alterations of V2 cells in *Itk*<sup>-/-</sup> mice, we propose that the V $\gamma$ 2TCR-ITK signaling constitutes a developmental checkpoint related to the  $\beta$  selection for  $\alpha\beta$  thymocytes but that this tonic signaling alone does not regulate the TF transcriptome that programs the T $\gamma\delta$ 17 effectors.

Published data (Jensen et al., 2008; Ribot et al., 2009; Turchinovich and Hayday, 2011) and our results from the in vitro cultures and TCR $\gamma$  Tg mice indicate at least two other factors that can contribute to the observed correlation between TCR repertoire and effector function: developmental timing and limiting permissive niches. In the OP9 culture system, DN3 cells and their progenies cannot generate T $\gamma\delta$ 17 cells, indicating a developmental stage-specific gene program, perhaps linked to an ordered *Tcrgv* gene rearrangement process. However, the enforced expression of V $\gamma$ 2TCR does not enhance the number of T $\gamma\delta$ 17 cells generated in vivo, indicating that V $\gamma$ 2TCR-specific signals alone cannot dictate effector fate and that there exists a limit to the number of T $\gamma\delta$ 17 cells that can be produced regardless of the TCR repertoire.

Recently, it has been concluded that most T $\gamma\delta$ 17 cells are generated during gestation (Haas et al., 2012). Although this can account for the correlation for V4 cells, whether V2 T $\gamma\delta$ 17 cells also originate exclusively during gestation remains to be clarified. The requirements for SOX4 and SOX13 in the generation of V2 and V4 T $\gamma\delta$ 17 cells are distinct, with V2 T $\gamma\delta$ 17 cells showing an absolute dependence, whereas V4 T $\gamma\delta$ 17 cells are mostly dependent on SOX13, but even here only the fetal thymic cellularity was significantly impacted by the loss of *Sox13*. These distinct developmental requirements between V2 and V4 cells are observed despite their overall molecular similarity at the gene expression level (Narayan et al., 2012), suggesting T cell-extrinsic environmental signals differentially affecting the early (V $\gamma$ 4<sup>+</sup>) versus late (V $\gamma$ 2<sup>+</sup>) T $\gamma\delta$ 17 cell development.

The high *Tcf7* expression is a unifying feature of developing thymocytes and GALT ILCs. Notch signaling has been shown to directly induce *Tcf7* transcription (Germar et al., 2011; Weber et al., 2011). On the basis of the known targets of TCF1 in T cells and their precursors, TCF1 may directly regulate the expression of several markers of GALT ILCs, including *Id2* (Germar et al., 2011; Rockman et al., 2001), *Il7r* (Germar et al., 2011), *Cd4* (Huang et al., 2006), and *Cd25* (Weber et al., 2011). For ILCs, *Tcf7*-deficiency led to the selective loss of NCR22 cells that

have been shown to be most dependent on Notch signaling for development (Lee et al., 2012). Other ROR $\gamma$ t<sup>+</sup> ILCs are mostly spared, although their functional profiles are altered when TCF1 is absent, as evidenced by the hyper production of cytokines, reminiscent of *Tcf7*<sup>-/-</sup>  $\gamma\delta$  ILCs. Thus, TCF1 is a negative regulator of IL-17 and IL-22 production in differentiated innate lymphoid effectors. An analogous (Yu et al., 2011) or distinct (Muranski et al., 2011) function of TCF1 in adaptive T cells has been proposed, but in vivo, TCF1 may primarily impact Th17 cell survival or renewal. This difference in the repertoire of TCF1 function in innate versus adaptive lymphocytes is likely linked to the dominance of TCR and cytokine receptor signaling in specifying adaptive effector differentiation, whereas the production of fast-acting innate lymphoid effectors is acutely dependent on intrinsic gene networks programmed in the tissues of origin. TCF1 may also be required for fetal LTi development as *Tcf7*<sup>-/-</sup> mice do not generate Peyer's patches (N.M. data not shown), similar to mice lacking the HMG TF *Tox* (Aliahmad et al., 2010). Together, these results suggest that the diversity of ROR $\gamma$ t<sup>+</sup> innate lymphoid subsets can be generated by unique combinatorial usage of HMG TFs in precursors arising in distinct tissues.

## EXPERIMENTAL PROCEDURES

### Mice

*Sox13*<sup>-/-</sup> (129/J), *Sox13* Tg (Melichar et al., 2007), *TcrVg2* Tg (C57BL/6) (Kang et al., 1998), *Axin2*<sup>2/+</sup> (H. Birchmeier, MDC Berlin), *Tcrb*<sup>-/-</sup>, *Rorc-Gfp* (JAX), *Itk*<sup>-/-</sup> (Felices et al., 2009), and *Tcf7*<sup>-/-</sup> mice (Verbeek et al., 1995) were previously described. *Sox4*<sup>fl/fl</sup> mice were generated by V. Lefebvre (Penzo-Méndez et al., 2007) and crossed to CD2p-CreTg mice. All mice were housed in a specific pathogen-free barrier facility and experiments performed were approved by the IACUC.

### Flow Cytometry

Antibodies (Abs) used are detailed in Supplemental Information. Data was acquired on a BD LSRII cytometer and was analyzed by using FlowJo (Treestar).

### Ex Vivo Stimulation, Zymosan Activation, and OP9 Culture

Freshly isolated thymic and LN cells were cultured ( $2 \times 10^6$ /well) with PMA (10 ng/ml) and Ionomycin (1  $\mu$ g/ml) for 4 hr at 37°C, with Golgi Stop and Golgi Plug (BD Biosciences) added after 1 hr. After stimulation, cells were stained for cell surface markers and intracellular cytokine production by using the Cytofix/Cytoperm kit (BD Biosciences). To activate innate effectors, we injected mice intraperitoneally with Zymosan (Sigma) in PBS (6 mg/mouse). Four hrs post-injection, lymphocytes were isolated from the mLNs and spleens. Cells were stimulated with PMA/Iono and intracellular staining for IL-22 and IL-17 in  $\gamma\delta$  ILCs and ILCs was performed. Intestinal LP lymphocytes from 10-day-old

(B) TCF1 expression in CD3<sup>-</sup>CD19<sup>-</sup>CD11b<sup>-</sup> mLN ILCs segregated based on Nkp46 and IL-7R was analyzed.

(C) TCF1 is required for the development of NCR22 cells. Intestinal LP and splenic ILCs (CD3<sup>-</sup>CD19<sup>-</sup>IL-7R<sup>+</sup>) from *Tcf7*<sup>-/-</sup> neonates were stained with CD4 and Nkp46 to track NCR22 cells. Data shown are representative profiles from one of four studies.

(D) Frequencies of CCR6<sup>+</sup> and CD25<sup>+</sup> in neonatal LP ILCs from *Tcf7*<sup>+/-</sup> heterozygotes (HET) and *Tcf7*<sup>-/-</sup> mice. Lines represent the mean, and Student t test p values are shown. Similar results were observed in mLN.

(E) Numerical reduction in the ILC subsets in the mLN of 3-week-old *Tcf7*<sup>-/-</sup> mice. The total cell number of IL-7R<sup>+</sup> ILCs, ROR $\gamma$ t<sup>hi</sup> and ROR $\gamma$ t<sup>lo</sup> ILCs, and Nkp46<sup>+</sup> and Nkp46<sup>-</sup> ROR $\gamma$ t<sup>lo</sup> ILCs is shown. Data are combined from two experiments, n = 5/group. Data are represented as mean  $\pm$  SEM.

(F) Representative histograms showing the frequency of NCR22 cells in the mLNs of 3-week-old *Tcf7*<sup>-/-</sup> mice.

(G) Increased expression of ROR $\gamma$ t in *Tcf7*<sup>-/-</sup> ILCs. Averages of MFI ( $\pm$ SEM) of ROR $\gamma$ t expression in the CD3<sup>-</sup>CD19<sup>-</sup>IL-7R<sup>hi</sup> spleen cells is provided (n = 5/group; one representative experiment of four).

(H) TCF1 restrains IL-17 and IL-22 production in the ILCs. Intracellular staining for IL-17 and IL-22 in the ex vivo splenic ILCs (CD3<sup>-</sup>CD19<sup>-</sup>IL-7R<sup>hi</sup>CD4<sup>+</sup>CD25<sup>+</sup>) was performed post-Zymosan administration. Unlike in other tissues, the number of splenic ROR $\gamma$ t<sup>+</sup> ILCs was marginally increased in adult *Tcf7*<sup>-/-</sup> mice. Profiles shown were obtained in two additional experiments. See also Figure S6.

mice were isolated as described (Qiu et al., 2012). Sorted fetal and adult ETPs (c-Kit<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup>CD25<sup>-</sup>CD44<sup>+</sup>) or c-Kit<sup>-</sup>DN3 (CD4<sup>-</sup>CD8<sup>-</sup>CD3<sup>-</sup>CD25<sup>+</sup>CD44<sup>-</sup>) cells were plated onto OP9-DL1 monolayers (J. C. Zuniga-Pflucker) at various concentrations in  $\alpha$ MEM media containing 20% FBS (GIBCO), 1 ng/ml IL-7 (R&D Systems), and 5 ng/ml Flt3L (R&D Systems). After 5–12 days of culture, cells were analyzed by flow cytometry.

### Psoriasis Induction

Aldara (5% Imiquimod, 3M Pharmaceuticals) or control vehicle cream was applied daily for five days on the back and ear. The disease severity in mice was scored by a modified PASI normally used to rank human psoriasis severity (Fredriksson and Pettersson, 1978). The scale thickness and erythema were scored from 0 to 4 (slight, moderate, severe, very severe), and the total area of the inflammation covering the back was scored from 0 to 6 (0%, 10%–29%, 30%–49%, 50%–69%, 70%–89%, 90%–100%). The scores for the scales and erythema were added and multiplied by the score for the body area to obtain the total score ranging from 0 (no disease) to 48. Dermal cells were obtained according to a published protocol (Suffia et al., 2005).

### Microarray Analysis

Samples were processed and analyzed according to the standard operating protocol of the Immunological Genome Project ([www.immgen.org](http://www.immgen.org); Supplemental Information); GEO:GSE15907.

### Chromatin Immunoprecipitation Assay

Sorted immature V $\gamma$ 2<sup>+</sup> and V $\gamma$ 2<sup>-</sup> thymocyte subsets were used to determine HMG TF chromatin occupancy at the *Blk*, *Rorc*, *Il17a*, and *Gata3* loci using commercially available Abs, reagents, and kits. In vitro differentiated Th1 and Th17 cells were used as controls. Detailed method is provided in Supplemental Information.

### SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures, one table, and Supplemental Experimental Procedures and can be found with this article online at <http://dx.doi.org/10.1016/j.immuni.2013.01.010>.

### ACKNOWLEDGMENTS

We thank M. Bix and E. Huseby for comments on the manuscript, the ImmGen core team (M. Painter, J. Ericson, S. Davis) for help with data generation and processing, eBioscience, Affymetrix, and Expression Analysis for support of the ImmGen Project, and H. Birchmeier and H. Clevers for mice. This work was supported by NIH/NCI CA100382 to J.K., NIH/NIAID AI084987 to L.J.B., AI072073 to ImmGen, and NIH/NIAMS AR54153 to V.L. Immunological Genome Project Consortium authors are as follows: E.L. Gautier, C. Jakubzick, G.J. Randolph, J.A. Best, J. Knell, A. Goldrath, J. Miller, B. Brown, M. Merad, V. Jojic, D. Koller, N. Cohen, P. Brennan, M. Brenner, T. Shay, A. Regev, A. Fletcher, K. Elpek, A. Bellemare-Pelletier, D. Malhotra, S. Turley, R. Jianu, D. Laidlaw, J. Collins, N. Malhotra, K. Sylvia, J. Kang, R. Gazit, B. Garrison, D.J. Rossi, F. Kim, T.N. Rao, A. Wagers, S.A. Shinton, R.R. Hardy, P. Monach, N.A. Bezman, J.C. Sun, C.C. Kim, L.L. Lanier, T. Heng, T. Kreslavsky, M. Painter, J. Ericson, S. Davis, D. Mathis, C. Benoist.

Received: July 15, 2012

Accepted: January 28, 2013

Published: April 4, 2013

### REFERENCES

- Aliahmad, P., de la Torre, B., and Kaye, J. (2010). Shared dependence on the DNA-binding factor TOX for the development of lymphoid tissue-inducer cell and NK cell lineages. *Nat. Immunol.* *11*, 945–952.
- Badis, G., Berger, M.F., Philippakis, A.A., Talukder, S., Gehrke, A.R., Jaeger, S.A., Chan, E.T., Metzler, G., Vedenko, A., Chen, X., et al. (2009). Diversity and complexity in DNA recognition by transcription factors. *Science* *324*, 1720–1723.
- Brüstle, A., Heink, S., Huber, M., Rosenplänter, C., Stadelmann, C., Yu, P., Arpaia, E., Mak, T.W., Kamradt, T., and Lohoff, M. (2007). The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. *Nat. Immunol.* *8*, 958–966.
- Buonocore, S., Ahern, P.P., Uhlig, H.H., Ivanov, I.I., Littman, D.R., Maloy, K.J., and Powrie, F. (2010). Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* *464*, 1371–1375.
- Cai, Y., Shen, X., Ding, C., Qi, C., Li, K., Li, X., Jala, V.R., Zhang, H.G., Wang, T., Zheng, J., and Yan, J. (2011). Pivotal role of dermal IL-17-producing  $\gamma\delta$  T cells in skin inflammation. *Immunity* *35*, 596–610.
- Driskell, R.R., Goodheart, M., Neff, T., Liu, X., Luo, M., Moothart, C., Sigmund, C.D., Hosokawa, R., Chai, Y., and Engelhardt, J.F. (2007). Wnt3a regulates Lef-1 expression during airway submucosal gland morphogenesis. *Dev. Biol.* *305*, 90–102.
- Felices, M., Yin, C.C., Kosaka, Y., Kang, J., and Berg, L.J. (2009). Tec kinase Itk in gammadeltaT cells is pivotal for controlling IgE production in vivo. *Proc. Natl. Acad. Sci. USA* *106*, 8308–8313.
- Fredriksson, T., and Pettersson, U. (1978). Severe psoriasis—oral therapy with a new retinoid. *Dermatologica* *157*, 238–244.
- Germar, K., Dose, M., Konstantinou, T., Zhang, J., Wang, H., Lobry, C., Arnett, K.L., Blacklow, S.C., Aifantis, I., Aster, J.C., and Gounari, F. (2011). T-cell factor 1 is a gatekeeper for T-cell specification in response to Notch signaling. *Proc. Natl. Acad. Sci. USA* *108*, 20060–20065.
- Gomez-Rodriguez, J., Sahu, N., Handon, R., Davidson, T.S., Anderson, S.M., Kirby, M.R., August, A., and Schwartzberg, P.L. (2009). Differential expression of IL-17A and -17F is coupled to TCR signaling via inducible T cell kinase. *Immunity* *31*, 587–597.
- Haas, J.D., Ravens, S., Duber, S., Sandrock, I., Oberdorfer, L., Kashani, E., Chennupati, V., Fohse, L., Naumann, R., Weiss, S., et al. (2012). Development of IL-17-Producing  $\gamma\delta$ T Cells Is Restricted to a Functional Embryonic Wave. *Immunity* *37*, 48–59.
- Huang, Z., Xie, H., Ioannidis, V., Held, W., Clevers, H., Sadim, M.S., and Sun, Z. (2006). Transcriptional regulation of CD4 gene expression by T cell factor-1/ $\beta$ -catenin pathway. *J. Immunol.* *176*, 4880–4887.
- Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor ROR $\gamma$  directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* *126*, 1121–1133.
- Jensen, K.D., Su, X., Shin, S., Li, L., Youssef, S., Yamasaki, S., Steinman, L., Saito, T., Locksley, R.M., Davis, M.M., et al. (2008). Thymic selection determines  $\gamma\delta$ T cell effector fate: antigen-naïve cells make IL-17 and antigen-experienced cells make IFN $\gamma$ . *Immunity* *29*, 90–100.
- Kang, J., Coles, M., Cado, D., and Raulet, D.H. (1998). The developmental fate of T cells is critically influenced by TCR $\gamma$  expression. *Immunity* *8*, 427–438.
- Laird, R.M., Laky, K., and Hayes, S.M. (2010). Unexpected role for the B cell-specific Src family kinase B lymphoid kinase in the development of IL-17-producing  $\gamma\delta$  T cells. *J. Immunol.* *185*, 6518–6527.
- Lee, J.S., Cella, M., McDonald, K.G., Garlanda, C., Kennedy, G.D., Nukaya, M., Mantovani, A., Kopan, R., Bradfield, C.A., Newberry, R.D., and Colonna, M. (2012). AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of Notch. *Nat. Immunol.* *13*, 144–151.
- Lochner, M., Peduto, L., Cherrier, M., Sawa, S., Langa, F., Varona, R., Riethmacher, D., Si-Tahar, M., Di Santo, J.P., and Eberl, G. (2008). In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ ROR $\gamma$  T cells. *J. Exp. Med.* *205*, 1381–1393.
- Luci, C., Reynders, A., Ivanov, I.I., Cognet, C., Chiche, L., Chasson, L., Hardwigsen, J., Anguiano, E., Banchereau, J., Chaussabel, D., et al. (2009). Influence of the transcription factor ROR $\gamma$  on the development of NKp46+ cell populations in gut and skin. *Nat. Immunol.* *10*, 75–82.
- Lustig, B., Jerchow, B., Sachs, M., Weiler, S., Pietsch, T., Karsten, U., van de Wetering, M., Clevers, H., Schlag, P.M., Birchmeier, W., and Behrens, J.

- (2002). Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. *Mol. Cell. Biol.* **22**, 1184–1193.
- Malhotra, N., Robertson, E., and Kang, J. (2010). SMAD2 is essential for TGF beta-mediated Th17 cell generation. *J. Biol. Chem.* **285**, 29044–29048.
- Marfil, V., Moya, M., Pierreux, C.E., Castell, J.V., Lemaigre, F.P., Real, F.X., and Bort, R. (2010). Interaction between Hhex and SOX13 modulates Wnt/TCF activity. *J. Biol. Chem.* **285**, 5726–5737.
- Melichar, H.J., Narayan, K., Der, S.D., Hiraoka, Y., Gardiol, N., Jeannet, G., Held, W., Chambers, C.A., and Kang, J. (2007). Regulation of gammadelta versus alphabeta T lymphocyte differentiation by the transcription factor SOX13. *Science* **315**, 230–233.
- Muranski, P., Borman, Z.A., Kerkar, S.P., Klebanoff, C.A., Ji, Y., Sanchez-Perez, L., Sukumar, M., Reager, R.N., Yu, Z., Kern, S.J., et al. (2011). Th17 cells are long lived and retain a stem cell-like molecular signature. *Immunity* **35**, 972–985.
- Narayan, K., Sylvia, K.E., Malhotra, N., Yin, C.C., Martens, G., Vallerskog, T., Kornfeld, H., Xiong, N., Cohen, N.R., Brenner, M.B., et al.; Immunological Genome Project Consortium. (2012). Intrathymic programming of effector fates in three molecularly distinct  $\gamma\delta$  T cell subtypes. *Nat. Immunol.* **13**, 511–518.
- O'Brien, R.L., Roark, C.L., and Born, W.K. (2009). IL-17-producing gammadelta T cells. *Eur. J. Immunol.* **39**, 662–666.
- Pantelyushin, S., Haak, S., Ingold, B., Kulig, P., Heppner, F.L., Navarini, A.A., and Becher, B. (2012). Ror $\gamma$ t+ innate lymphocytes and  $\gamma\delta$  T cells initiate psoriasisiform plaque formation in mice. *J. Clin. Invest.* **122**, 2252–2256.
- Penzo-Méndez, A., Dy, P., Pallavi, B., and Lefebvre, V. (2007). Generation of mice harboring a Sox4 conditional null allele. *Genesis* **45**, 776–780.
- Possot, C., Schmutz, S., Chea, S., Boucontet, L., Louise, A., Cumano, A., and Golub, R. (2011). Notch signaling is necessary for adult, but not fetal, development of ROR $\gamma$ t(+) innate lymphoid cells. *Nat. Immunol.* **12**, 949–958.
- Powolny-Budnicka, I., Riemann, M., Tänzler, S., Schmid, R.M., Hehlhans, T., and Weih, F. (2011). RelA and RelB transcription factors in distinct thymocyte populations control lymphotoxin-dependent interleukin-17 production in  $\gamma\delta$  T cells. *Immunity* **34**, 364–374.
- Qiu, J., Heller, J.J., Guo, X., Chen, Z.M., Fish, K., Fu, Y.X., and Zhou, L. (2012). The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. *Immunity* **36**, 92–104.
- Rachitskaya, A.V., Hansen, A.M., Horai, R., Li, Z., Villasmil, R., Luger, D., Nussenblatt, R.B., and Caspi, R.R. (2008). Cutting edge: NKT cells constitutively express IL-23 receptor and ROR $\gamma$ t and rapidly produce IL-17 upon receptor ligation in an IL-6-independent fashion. *J. Immunol.* **180**, 5167–5171.
- Reynders, A., Yessaad, N., Vu Manh, T.P., Dalod, M., Fenis, A., Aubry, C., Nikitas, G., Escalière, B., Renaud, J.C., Dussurget, O., et al. (2011). Identity, regulation and in vivo function of gut NKp46+ROR $\gamma$ t+ and NKp46+ROR $\gamma$ t- lymphoid cells. *EMBO J.* **30**, 2934–2947.
- Ribot, J.C., deBarros, A., Pang, D.J., Neves, J.F., Peperzak, V., Roberts, S.J., Girardi, M., Borst, J., Hayday, A.C., Pennington, D.J., and Silva-Santos, B. (2009). CD27 is a thymic determinant of the balance between iIFN $\gamma$ - and IL-17-producing  $\gamma\delta$ T cell subsets. *Nat. Immunol.* **10**, 427–436.
- Rockman, S.P., Currie, S.A., Ciavarella, M., Vincan, E., Dow, C., Thomas, R.J., and Phillips, W.A. (2001). Id2 is a target of the  $\beta$ -catenin/T cell factor pathway in colon carcinoma. *J. Biol. Chem.* **276**, 45113–45119.
- Ruan, Q., Kameswaran, V., Zhang, Y., Zheng, S., Sun, J., Wang, J., DeVirgiliis, J., Liou, H.C., Beg, A.A., and Chen, Y.H. (2011). The Th17 immune response is controlled by the Rel-ROR $\gamma$ -ROR $\gamma$  T transcriptional axis. *J. Exp. Med.* **208**, 2321–2333.
- Sanos, S.L., Bui, V.L., Mortha, A., Oberle, K., Heners, C., Johner, C., and Dieffenbach, A. (2009). ROR $\gamma$ t and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. *Nat. Immunol.* **10**, 83–91.
- Satoh-Takayama, N., Vosshenrich, C.A., Lesjean-Pottier, S., Sawa, S., Lochner, M., Rattis, F., Mention, J.J., Thiam, K., Cerf-Bensussan, N., Mandelboim, O., et al. (2008). Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. *Immunity* **29**, 958–970.
- Sawa, S., Cherrier, M., Lochner, M., Satoh-Takayama, N., Fehling, H.J., Langa, F., Di Santo, J.P., and Eberl, G. (2010). Lineage relationship analysis of ROR $\gamma$ t+ innate lymphoid cells. *Science* **330**, 665–669.
- Schilham, M.W., Moerer, P., Cumano, A., and Clevers, H.C. (1997). Sox-4 facilitates thymocyte differentiation. *Eur. J. Immunol.* **27**, 1292–1295.
- Shibata, K., Yamada, H., Nakamura, R., Sun, X., Itsumi, M., and Yoshikai, Y. (2008). Identification of CD25+ gamma delta T cells as fetal thymus-derived naturally occurring IL-17 producers. *J. Immunol.* **181**, 5940–5947.
- Sinner, D., Kordich, J.J., Spence, J.R., Opoka, R., Rankin, S., Lin, S.C., Jonatan, D., Zorn, A.M., and Wells, J.M. (2007). Sox17 and Sox4 differentially regulate  $\beta$ -catenin/T-cell factor activity and proliferation of colon carcinoma cells. *Mol. Cell. Biol.* **27**, 7802–7815.
- Sonnenberg, G.F., Fouser, L.A., and Artis, D. (2011). Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat. Immunol.* **12**, 383–390.
- Spits, H., and Di Santo, J.P. (2011). The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat. Immunol.* **12**, 21–27.
- Suffia, I., Reckling, S.K., Salay, G., and Belkaid, Y. (2005). A role for CD103 in the retention of CD4+CD25+ Treg and control of Leishmania major infection. *J. Immunol.* **174**, 5444–5455.
- Takatori, H., Kanno, Y., Watford, W.T., Tato, C.M., Weiss, G., Ivanov, I.I., Littman, D.R., and O'Shea, J.J. (2009). Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. *J. Exp. Med.* **206**, 35–41.
- Turchinovich, G., and Hayday, A.C. (2011). Skint-1 identifies a common molecular mechanism for the development of IFN $\gamma$ -secreting versus IL-17-secreting  $\gamma\delta$ T cells. *Immunity* **35**, 59–68.
- Verbeek, S., Izon, D., Hofhuis, F., Robanus-Maandag, E., te Riele, H., van de Wetering, M., Oosterwegel, M., Wilson, A., MacDonald, H.R., and Clevers, H. (1995). An HMG-box-containing T-cell factor required for thymocyte differentiation. *Nature* **374**, 70–74.
- Vonarbourg, C., Mortha, A., Bui, V.L., Hernandez, P.P., Kiss, E.A., Hoyler, T., Flach, M., Bengsch, B., Thimme, R., Hölscher, C., et al. (2010). Regulated expression of nuclear receptor ROR $\gamma$ t confers distinct functional fates to NK cell receptor-expressing ROR $\gamma$ t(+) innate lymphocytes. *Immunity* **33**, 736–751.
- Weber, B.N., Chi, A.W., Chavez, A., Yashiro-Ohtani, Y., Yang, Q., Shestova, O., and Bhandoola, A. (2011). A critical role for TCF-1 in T-lineage specification and differentiation. *Nature* **476**, 63–68.
- Yu, Q., Sharma, A., Ghosh, A., and Sen, J.M. (2011). TCF-1 negatively regulates expression of IL-17 family of cytokines and protects mice from EAE. *J. Immunol.* **186**, 3946–3952.
- Zhou, L., Ivanov, I.I., Spolski, R., Min, R., Shenderov, K., Egawa, T., Levy, D.E., Leonard, W.J., and Littman, D.R. (2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat. Immunol.* **8**, 967–974.