HAL open science

Cutting Edge: Eomesodermin Is Sufficient To Direct Type 1 Innate Lymphocyte Development into the Conventional NK Lineage

Olga Pikovskaya, Julie Chaix, Nyanza J. Rothman, Amelie Collins, Yen-Hua Chen, Anna M. Scipioni, Eric Vivier, Steven L. Reiner

To cite this version:

Olga Pikovskaya, Julie Chaix, Nyanza J. Rothman, Amelie Collins, Yen-Hua Chen, et al.. Cutting Edge: Eomesodermin Is Sufficient To Direct Type 1 Innate Lymphocyte Development into the Conventional NK Lineage. Journal of Immunology, 2016, 196 (4), pp.1449-1454. 10.4049/jimmunol.1502396 . hal-01438533

HAL Id: hal-01438533

https://hal.science/hal-01438533v1

Submitted on 8 Nov 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License

Cutting Edge: Eomesodermin Is Sufficient To Direct Type 1 Innate Lymphocyte Development into the Conventional NK Lineage

Olga Pikovskaya,[*,†,1] Julie Chaix,[*,†,1,2] Nyanza J. Rothman,[*,†] Amélie Collins,[*,†] Yen-Hua Chen,[*,†] Anna M. Scipioni,[*,†] Eric Vivier,[‡,§] and Steven L. Reiner[*,†]

Type 1 innate lymphocytes comprise two developmentally divergent lineages, type 1 helper innate lymphoid cells (hILC1s) and conventional NK cells (cNKs). All type 1 innate lymphocytes (ILCs) express the transcription factor T-bet, but cNKs additionally express Eomesodermin (Eomes). We show that deletion of *Eomes* alleles at the onset of type 1 ILC maturation using NKp46-Cre imposes a substantial block in cNK development. Formation of the entire lymphoid and nonlymphoid type 1 ILC compartment appears to require the semiredundant action of both T-bet and Eomes. To determine if Eomes is sufficient to redirect hILC1 development to a cNK fate, we generated transgenic mice that express Eomes when and where T-bet is expressed using *Tbx21* locus control to drive expression of Eomes codons. Ectopic Eomes induces cNK-like properties across the lymphoid and nonlymphoid type 1 ILC compartments. Subsequent to their divergent lineage specification, hILC1s and cNKs thus possess substantial developmental plasticity. ***The Journal of Immunology*****, 2016, 196: 1449–1454.**

Innate lymphoid cells are enriched at mucosal barriers in close proximity to epithelial surfaces, where they exert crucial functions during infection, tissue injury, and inflammation. Type 1 innate lymphocytes (ILCs) express the NK1.1 and NKp46 lineage Ags as well as the transcription factor T-bet. Type 1 ILCs can be further subdivided into discrete populations based on expression of Eomesodermin (Eomes), a close homolog of T-bet (1, 2). Eomes$^+$ conventional NK cells (cNKs) express integrin CD49b (DX5) and MHC class I–specific Ly49 receptors and circulate through lymphoid and nonlymphoid organs. In contrast, Eomes$^-$ type 1 helper innate lymphoid cells (hILC1s) express integrin CD49a and are anchored to their tissues and mucosal sites of origin (1–7). hILC1s and cNKs are thought to arise from separate lymphoid progenitors (6, 8). Nonetheless, type 1 ILC lineages are closely related transcriptionally and temporal deletion of Eomes from cNKs unveils latent hILC1-like characteristics (1, 9). We used genetic approaches to test the necessity and sufficiency of Eomes to determine the outcome of type 1 ILC maturation. Our results lead us to the conclusion that Eomes expression is a key determinant of cNK versus hILC1 maturation following lineage specification.

Materials and Methods

Mice

Mice were housed in specific pathogen-free conditions and used in accordance with the Columbia University Institutional Animal Care and Use Committee's guidelines. C57BL/6 *Eomes*$^{flox/flox}$, *Tbx21*$^{flox/flox}$, *Tbx21*$^{-/-}$ (10, 11), *NKp46-Cre*$^+$ (12), and *Id2*$^{hCD5/hCD5}$ reporter (13) mice were generated as previously described. They were bred locally to obtain the compound genotypes *Eomes*$^{flox/flox}$*NKp46-Cre*$^+$, *Tbx21*$^{flox/flox}$*NKp46-Cre*$^+$, and *Eomes*$^{flox/flox}$*NKp46-Cre*$^+$ *Tbx21*$^{-/-}$.

Detailed characterization of the *Tbx21-Eomes* transgenic (Tg) mouse strain will appear elsewhere. Briefly, Eomes cDNA followed by an SV40-PolyA site was inserted into the ATG translational start site of T-bet in the *Tbx21* bacterial artificial chromosome clone RP23-237M14 (CHORI) by Red/ET recombination technology (Gene Bridges). This strategy enabled T-bet regulatory elements to drive Eomes expression instead of T-bet expression from the Tg allele. The bacterial artificial chromosome construct was used for pronuclear injection of C57BL/6 zygotes. For some experiments, Tg mice were crossed to *Id2*$^{hCD5/hCD5}$ reporter mice that express human CD5 from an internal ribosome entry site–driven cassette knocked in to the 3′-untranslated region of *Id2* (13). Furthermore, Tg mice were crossed to *Tbx21*$^{-/-}$ mice for some analyses. Aged Tg mice had no evidence of skin lesions, intestinal inflammation, or hepatosplenomegaly.

Cell isolation and flow cytometry

Single-cell suspensions were prepared from liver, spleen, bone marrow, uterus, salivary gland, thymus, and lung tissues. Liver lymphocytes were

*Department of Microbiology and Immunology, College of Physicians and Surgeons, Columbia University, New York, NY 10032; †Department of Pediatrics, College of Physicians and Surgeons, Columbia University, New York, NY 10032; ‡Centre d'Immunologie de Marseille-Luminy, Aix-Marseille University UM2, INSERM U1104, CNRS UMR7280, Marseille 13288, France; and §Service d'Immunologie, Assistance Publique–Hôpitaux de Marseille, Marseille 13385, France

[1]O.P. and J.C. are cofirst authors.

[2]Current address: Centre d'Immunologie de Marseille-Luminy, Aix-Marseille University UM2, INSERM U1104, CNRS UMR7280, Marseille, France.

ORCIDs: 0000-0001-9146-7594 (A.M.S.); 0000-0002-1635-8619 (S.L.R.).

Received for publication November 11, 2015. Accepted for publication December 21, 2015.

This work was supported by National Institutes of Health Grants R01 AI061699 and R01 AI113365 (to S.L.R.) and F30 AI113963 (to O.P.), a European Research Council THINK Advanced Grant and the Equipe Labellisée Ligue Contre le Cancer (to E.V.), as well as by Institut Universitaire de France, INSERM, CNRS, and Aix-Marseille University (to Centre d'Immunologie de Marseille-Luminy).

Address correspondence and reprint requests to Dr. Steven L. Reiner, Department of Microbiology and Immunology, Columbia University, 701 West 168th Street, HHSC 912, New York, NY 10032. E-mail address: sr2978@cumc.columbia.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: cNK, conventional NK cell; Eomes, Eomesodermin; hILC1, type 1 helper innate lymphoid cell; ILC, innate lymphocyte; KLRG1, killer cell lectin–like receptor subfamily G, member 1; Tg, transgenic; WT, wild-type.

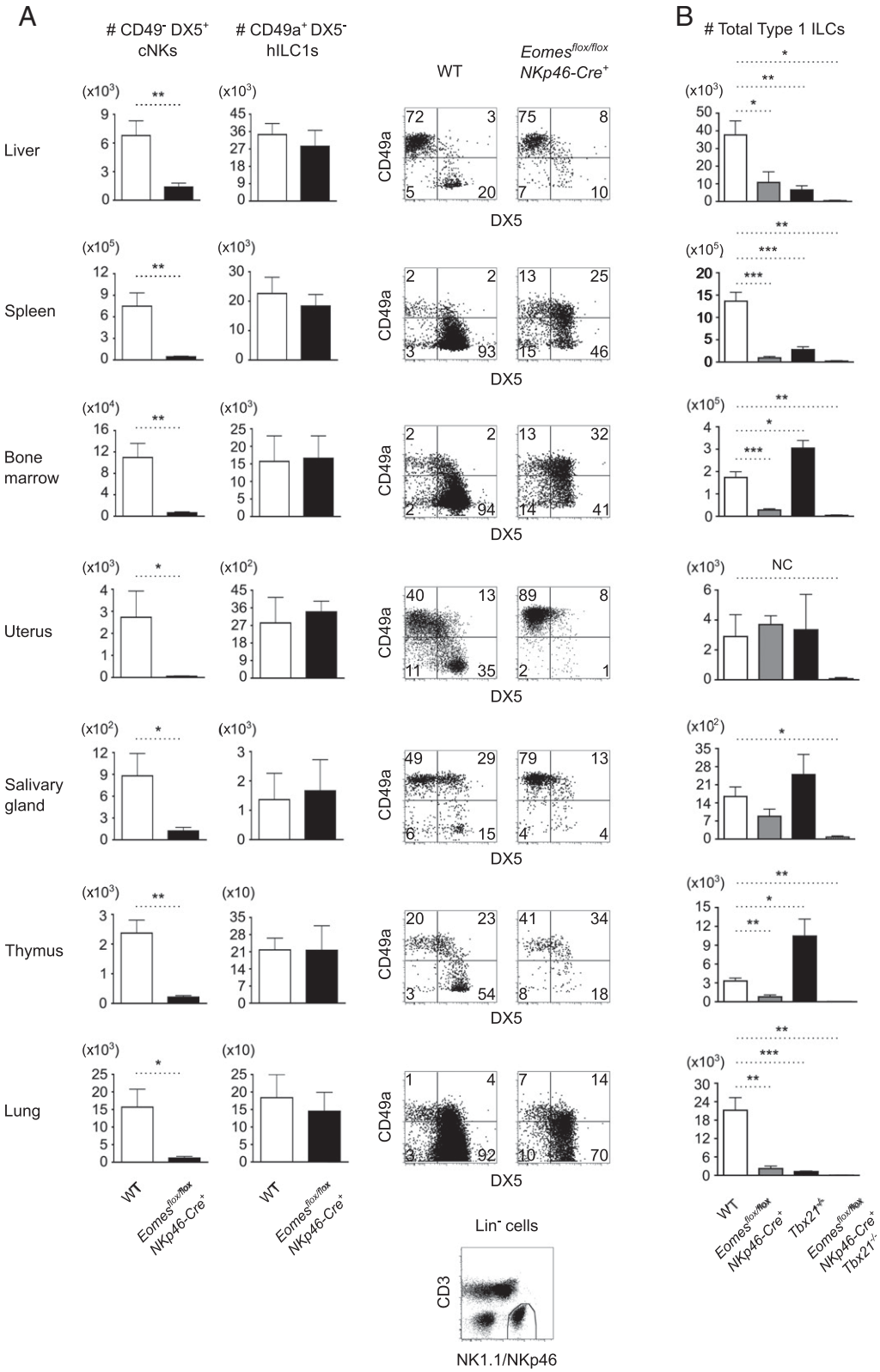


FIGURE 1. Loss of Eomes in NKp46⁺ ILCs impairs cNK development. (**A**) Representative flow cytometry of CD49a⁺DX5⁻ hILC1 and CD49a⁻DX5⁺ cNK subsets among type 1 ILCs (Lin [CD3, Gr-1, TER-119, CD19]⁻ NK1.1⁺NKp46⁺) from the indicated organs of WT and *Eomes*$^{flox/flox}$ *NKp46-Cre*⁺ mice (n = 4–5 mice per genotype). Absolute numbers of hILC1s and cNKs are indicated. The NK1.1/NKp46 gate is shown at the bottom. (**B**) Absolute numbers of total type 1 ILCs from the indicated organs of WT, *Eomes*$^{flox/flox}$*NKp46-Cre*⁺, *Tbx21*$^{-/-}$, and *Eomes*$^{flox/flox}$*NKp46-Cre*⁺ *Tbx21*$^{-/-}$ mice (n = 3–5 mice per genotype). Statistics were not calculated (NC) for the difference between WT and *Eomes*$^{flox/flox}$*NKp46-Cre*⁺ *Tbx21*$^{-/-}$ uterus because the *Eomes*$^{flox/flox}$*NKp46-Cre*⁺ *Tbx21*$^{-/-}$ uterus group represents the average of two mice. All other data are mean ± SEM representative of three to five independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

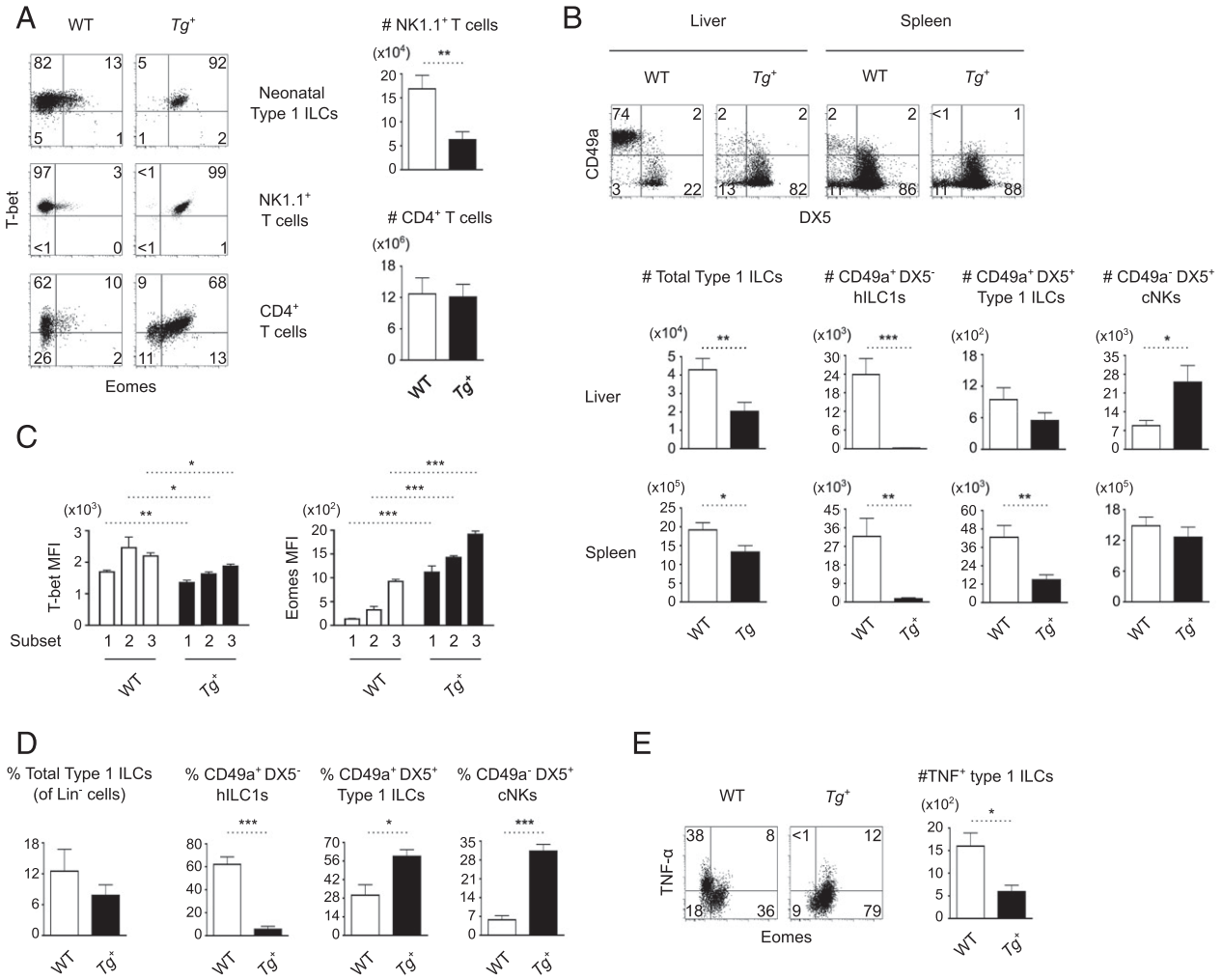


FIGURE 2. Eomes induces cNK attributes among type 1 ILCs. (**A**) *Tg*$^+$ Eomes expression in three cell populations (from at least three mice per genotype) that usually express T-bet but not Eomes: Lin$^-$NK1.1$^+$NKp46$^+$ type 1 ILCs from 3-d-old neonatal livers; NK1.1$^+$CD3$^+$ T cells from adult livers; and CD4$^+$ T cells from adult spleens after anti-CD3 and anti-CD28 stimulation for 48 h. Absolute numbers of NK1.1$^+$ T cells and CD4$^+$ T cells are indicated. Tg Eomes does not affect the development of CD4$^+$ T cells, but it results in moderate reduction of the number of hepatic NK1.1$^+$ T cells. (**B**) Flow cytometry of CD49a$^+$DX5$^-$, CD49a$^+$DX5$^+$, and CD49a$^-$DX5$^+$ type 1 ILC subsets from the livers and spleens of WT and *Tg*$^+$ mice (n = 5 mice per genotype). (**C**) T-bet and Eomes expression by hepatic CD49a$^+$DX5$^-$ (1), CD49a$^+$ DX5$^+$ (2), and CD49a$^-$DX5$^+$ (3) type 1 ILC subsets from WT and *Tg*$^+$ mice (n = 4 mice per genotype). (**D**) Frequency of total type 1 ILCs (among Lin$^-$ cells) and frequency of indicated subsets (among total type 1 ILCs) from the livers of WT and *Tg*$^+$ neonates (n = 4 mice per genotype). Tg Eomes does not affect the overall frequency of neonatal type 1 ILCs. (**E**) Flow cytometry of TNF-α expression by hepatic type 1 ILCs from WT and *Tg*$^+$ mice (n = 4 mice per genotype) after stimulation with 200 ng/ml PMA and 5 μg/ml ionomycin in the presence of GolgiPlug (Invitrogen) for 4 h. All data were obtained from mice that have one copy of the transgene (*Tg*$^+$). Data are mean ± SEM representative of two to five independent analyses. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. MFI, mean fluorescence intensity.

isolated at the interphase of a 40/60% Percoll gradient (1). Uterus and salivary gland were digested in the presence of DNase I and collagenase D or liberase TL (5, 7), whereas lung digestion additionally incorporated trypsin inhibitor. Cells were stained with a LIVE/DEAD fixable dead cell stain kit (Invitrogen) and blocked with Abs against CD16/CD32 (BD Biosciences) prior to staining with Abs for CD3, CD11b, CD27, CD43, CD122, human CD5, integrin α_v, killer cell lectin–like receptor subfamily G, member 1 (KLRG1), Ly49G2, Ly49H, NKp46, and TRAIL (eBioscience); and CD19, CD49a, CD49b, CD127, Ly49D, Ly-6G/Ly-6C (Gr-1), NK1.1, NKG2A/C/E, and TER-119 (BD Biosciences). Cells were treated with Cytofix/Cytoperm kits prior to staining with Abs for the transcription factors T-bet (eBioscience) and Eomes (eBioscience) or the cytokine TNF-α (BD Biosciences).

Statistical analysis

Statistics were calculated using Prism (GraphPad Software). Differences between groups were quantified using the unpaired t test. Statistical significance was reached at $p < 0.05$.

Results and Discussion

Deletion of Eomes in NKp46$^+$ ILCs blocks cNK differentiation

Within various lymphoid and nonlymphoid tissues examined, we could identify hILC1s and cNKs among lineage-negative, NK1.1$^+$NKp46$^+$ cells based on reciprocal expression of integrins CD49a and CD49b (DX5), respectively (Fig. 1A). We previously examined cNK development in mice with pan-hematopoietic deficiency of Eomes (1). To determine if Eomes expression at the onset of ILC development is necessary for cNK differentiation, we intercrossed *Eomes$^{flox/flox}$* and *NKp46-Cre*$^+$ mice (12). *Eomes$^{flox/flox}$NKp46-Cre*$^+$ mice had a substantial loss of CD49a$^-$DX5$^+$ cNKs (Fig. 1A), which supports the prior suggestion that Eomes is genetically upstream of CD49b induction (1). Remnant NK1.1$^+$NKp46$^+$ cells in Eomes conditional knockout mice were CD49a$^+$

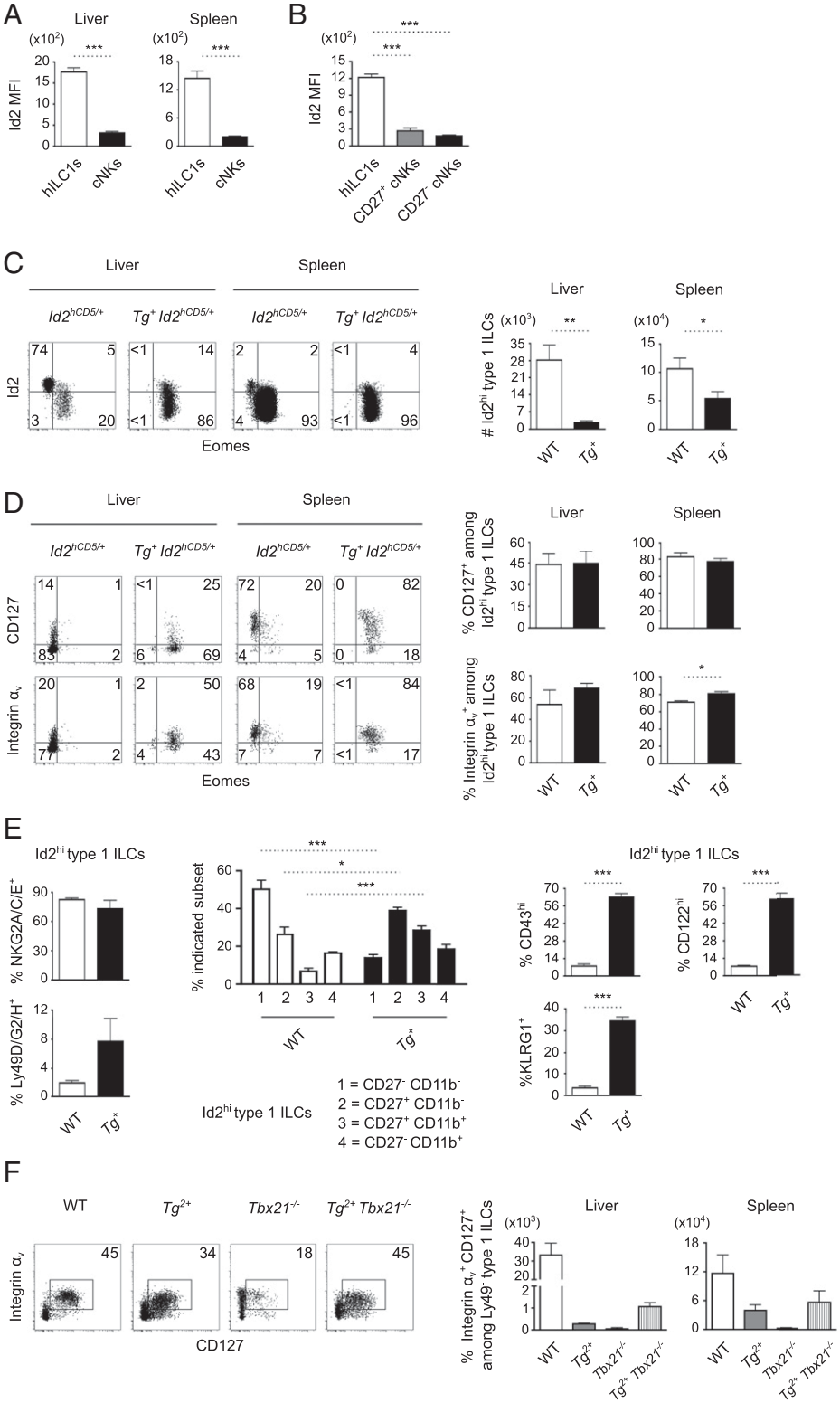


FIGURE 3. Eomes is permissive to some hILC1 attributes. (**A**) Id2 expression by CD49a$^+$DX5$^-$ hILC1s and CD49a$^-$DX5$^+$ cNKs among Lin$^-$ NK1.1$^+$NKp46$^+$ type 1 ILCs from the livers and spleens of *Id2*$^{hCD5/+}$ mice (n = 4 mice). (**B**) Id2 expression by CD49a$^+$Eomes$^-$ hILC1s, CD27$^+$ Eomes$^+$ immature cNKs, and CD27$^-$ Eomes$^+$ mature cNKs from the spleens of *Id2*$^{hCD5/+}$ mice (n = 4 mice). (**C**) Flow cytometry of Id2 and Eomes expression by NK1.1$^+$NKp46$^+$ type 1 ILCs from the livers and spleens of *Id2*$^{hCD5/+}$ and *Tg*$^+$*Id2*$^{hCD5/+}$ mice (n = 5 mice per genotype). Absolute numbers of Id2^{hi} cells are indicated. (**D**) Flow cytometry of CD127 and integrin α_v expression by Id2^{hi}NK1.1$^+$NKp46$^+$ type 1 ILCs from the livers and spleens of *Id2*$^{hCD5/+}$ and *Tg*$^+$*Id2*$^{hCD5/+}$ mice (n = 3–4 mice per genotype). (**E**) Frequency of NKG2A/C/E, Ly49D/G2/H, CD27, CD11b, CD43, KLRG1, and CD122 expression by Id2^{hi} NK1.1$^+$NKp46$^+$ type 1 ILCs from the livers of *Id2*$^{hCD5/+}$ and *Tg*$^+$*Id2*$^{hCD5/+}$ mice (n = 4 mice per genotype). (**F**) Flow cytometry of helper-like (CD127$^+$ integrin α_v^+) cells among Ly49$^-$ NK1.1$^+$NKp46$^+$ type 1 ILCs from the spleens of WT, *Tg*$^{2+}$, *Tbx21*$^{-/-}$, and *Tg*$^{2+}$*Tbx21*$^{-/-}$ mice. Absolute numbers of hILC1-like cells in the livers and spleens of n = 2 mice per genotype are summarized. (A–F) Data were obtained from mice that have 1 (*Tg*$^+$) or 2 (*Tg*$^{2+}$) copies of the transgene as indicated. Data are mean ± SEM representative of two to five independent experiments. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. MFI, mean fluorescence intensity.

DX5$^-$, CD49a$^+$DX5$^+$, or CD49a$^-$DX5$^+$ cells (Fig. 1A), and the persistence of these cells did not appear to result from inefficient deletion of Eomes (Supplemental Fig. 1A). These findings are consistent with a role for Eomes upstream of CD49a repression, which is supported by prior evidence of direct binding of Eomes to the locus encoding CD49a (14). Examination of TRAIL, CD127, and integrin α_v confirmed that deletion of Eomes shifts the balance of type 1 ILCs from cNKs to helper-like ILC1s (Supplemental Fig. 1B).

To test the contribution of T-bet to residual type 1 ILC development in Eomes conditional knockout mice, we examined *Eomes*$^{flox/flox}$*NKp46-Cre*$^+$*Tbx21*$^{-/-}$ mice (Fig. 1B). We used mice with a germline *Tbx21* deletion because *Tbx21*$^{flox/flox}$*NKp46-Cre*$^+$ mice underwent substantial development or expansion of hILC1s that escaped *Tbx21* deletion (Supplemental Fig. 1C, 1D). Compound deficiency of T-bet and Eomes resulted in almost complete elimination of the type 1 ILC compartment in all tissues examined (Fig. 1B).

Together with previous findings (1, 6, 7), the present results suggest that both T-bet and Eomes are required to develop the spectrum of lymphoid and nonlymphoid type 1 ILCs. Eomes, furthermore, seemingly plays its essential role in type 1 ILC maturation after the onset of NKp46 expression.

Eomes induces cNK properties among type 1 ILCs

Because loss of Eomes at the NKp46-expressing stage illustrates the necessity of Eomes for cNK development (Fig. 1A), we wished to test if Eomes expression at the onset of hILC1 lineage maturation would be sufficient to redirect hILC1s into a cNK identity. T-bet–dependent hILC1s lack endogenous Eomes expression (1, 2, 4, 6, 7). To enforce Eomes expression in developing hILC1s, we generated Tg mice in which *Tbx21* locus control elements drive expression of Eomes codons. Because T-bet is not expressed at substantial levels until type 1 ILCs acquire the lineage Ags NK1.1 and NKp46 (1, 15), we could determine the effect of Tg Eomes expression following divergent lineage specification from NK1.1$^-$NKp46$^-$ progenitors (6, 8).

We first verified that Tg Eomes is expressed when and where T-bet is expressed by examining the pattern of Eomes expression in three cell populations that ordinarily express T-bet but not Eomes: neonatal type 1 ILCs, NK1.1$^+$ T cells, and developing Th1 cells (1, 16). In each case, Tg cells expressed Eomes in proportion to T-bet (Fig. 2A). Analyses of type 1 ILCs revealed that Tg Eomes also perturbed the normal spatial and temporal pattern of hILC1 development. Tg Eomes caused a shift in frequency and absolute numbers toward CD49b (DX5) expression among NK1.1$^+$NKp46$^+$ type 1 ILCs in both lymphoid and nonlymphoid tissues of adult mice (Fig. 2B, Supplemental Fig. 2A, 2B). Examination of T-bet and Eomes levels revealed repressed T-bet expression in Tg type 1 ILC subsets (Fig. 2C, Supplemental Fig. 2C), suggesting that repression of T-bet by Tg Eomes may at least partly explain the phenotypic shift. In addition to the shift in adult ILC subsets, Tg neonates exhibited precocious development of DX5$^+$ type 1 ILCs, compared with the minor population evident in wild-type (WT) neonates (Fig. 2D). Finally, Eomes$^-$ hILC1s have been suggested to express higher levels of TNF-α than Eomes$^+$ cNKs (1, 6, 7). Consistent with the observed shift in integrin expression, we found that Tg Eomes attenuated TNF-α expression by hepatic type 1 ILCs (Fig. 2E). Eomes expression, thus, appears sufficient to redirect hILC1s into a more cNK-like fate.

Eomes is permissive to some hILC1 attributes

Emerging evidence suggests that progenitor cells of all helper ILCs are distinct from progenitor cells of cNKs and that helper ILC progenitors are marked by abundant expression of the transcription factor Id2 (6). We found that the descendant lineages of the distinct precursors, Eomes$^-$ hILC1s versus Eomes$^+$ cNKs, continue to express high versus intermediate levels of Id2, respectively (Fig. 3A, Supplemental Fig. 2D). Eomes$^-$ hILC1s expressed higher levels of Id2 than Eomes$^+$ cNKs regardless of their maturation (Fig. 3B). Examination of Tg NK1.1$^+$NKp46$^+$ type 1 ILCs revealed a subpopulation of Id2^{hi}Eomes$^+$ cells, suggesting the possibility that some cells arose from helper ILC precursors and were subsequently diverted into a cNK-like fate (Fig. 3C, Supplemental Fig. 2E). In support of the notion that the Id2^{hi} subpopulation may have arisen from helper ILC precursors, we detected other characteristics of hILC1s (1, 6, 17), including expression of CD127, integrin α_v (Fig. 3D, Supplemental Fig. 2F), NKG2A/C/E receptors, and a restricted Ly49 receptor repertoire (Fig. 3E, Supplemental Fig. 2G, 2H). The Id2^{hi} subset of Tg type 1 ILCs, nonetheless, also acquired numerous cNK features. Tg Eomes promoted upregulation of CD11b, CD43, KLRG1, and CD122 expression among Id2^{hi} cells in the liver and to a lesser degree in the spleen (Fig. 3E, Supplemental Fig. 2G, 2H). Despite increased maturity, Id2^{hi} Tg type 1 ILCs did not transition to the most terminal CD27$^-$ stages (18) of cNK maturation (Fig. 3E, Supplemental Fig. 2G, 2H). Together, these findings suggest that Eomes is sufficient to convert some but not all attributes of hILC1s to those of cNKs.

To determine whether the hILC1-like characteristics in a subset of Tg type 1 ILCs were T-bet dependent, we examined Tg mice with genetic deletion of both endogenous *Tbx21* alleles. In the absence of an *Id2* reporter allele, we used Ly49$^-$ NK1.1$^+$NKp46$^+$ as surrogate markers for hILC1s. Tg Eomes was sufficient to partially rescue the development of cells with hILC1 features (expression of CD127 and integrin α_v) in *Tbx21*$^{-/-}$ mice (Fig. 3F), indicating that the residual hILC1-like characteristics of the Tg line are not likely to be T-bet dependent. Therefore, the structural homology between the two transcription factors may enable Eomes to partially substitute for T-bet in hILC1 development. Similarly, T-bet may partially substitute for Eomes during cNK development insofar as *Eomes*$^{flox/flox}$*NKp46-Cre*$^+$ mice contain fewer cNK cells when intercrossed to T-bet–deficient mice (Fig. 1A, 1B).

Our findings suggest that type 1 ILCs with distinct lineage origins have the potential for substantial developmental plasticity. Our data are consistent with a model in which separate type 1 ILC lineages are specified from distinct precursors, and the absence or presence of Eomes subsequently directs or switches, albeit incompletely, their determination as hILC1s or cNKs. In an analogous manner, CD4$^+$ T cells can acquire cytotoxic T cell attributes and lose helper attributes when ThPOK expression decreases and Runx3 expression increases, yet they can still continue to express the CD4 marker (19, 20). Understanding the nature of the interrelatedness and plasticity between type 1 ILC subsets will help harness their therapeutic potential in infection, inflammation, and cancer.

Acknowledgments

We thank Dr. Jeff Zhu for advice in designing the transgene and Dr. Victor Lin of the Transgenic Mouse Shared Resource for microinjection and production of the Tg line.

Disclosures

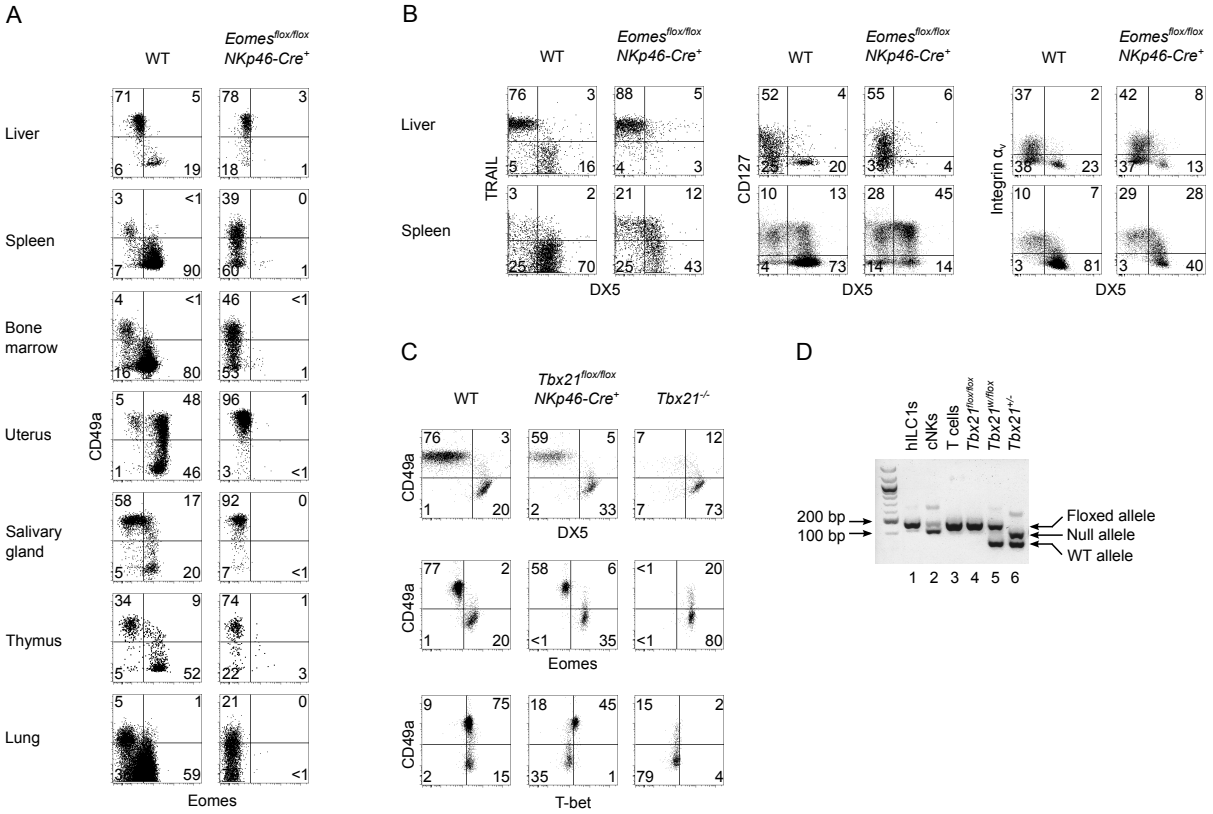
The authors have no financial conflicts of interest.

References

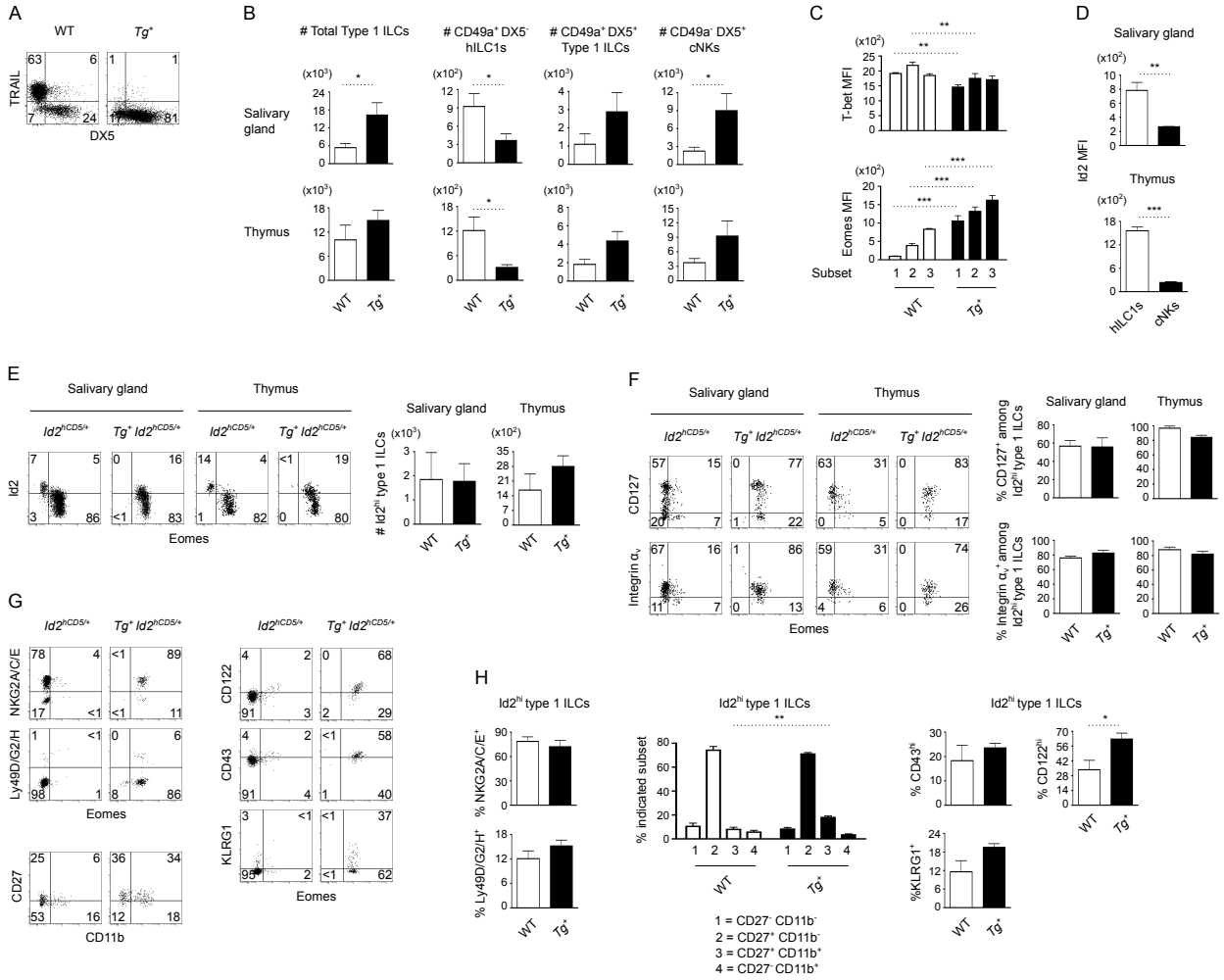
1. Gordon, S. M., J. Chaix, L. J. Rupp, J. Wu, S. Madera, J. C. Sun, T. Lindsten, and S. L. Reiner. 2012. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. *Immunity* 36: 55–67.
2. Daussy, C., F. Faure, K. Mayol, S. Viel, G. Gasteiger, E. Charrier, J. Bienvenu, T. Henry, E. Debien, U. A. Hasan, et al. 2014. T-bet and Eomes instruct the development of two distinct natural killer cell lineages in the liver and in the bone marrow. *J. Exp. Med.* 211: 563–577.
3. Fuchs, A., W. Vermi, J. S. Lee, S. Lonardi, S. Gilfillan, R. D. Newberry, M. Cella, and M. Colonna. 2013. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-γ-producing cells. *Immunity* 38: 769–781.

4. Peng, H., X. Jiang, Y. Chen, D. K. Sojka, H. Wei, X. Gao, R. Sun, W. M. Yokoyama, and Z. Tian. 2013. Liver-resident NK cells confer adaptive immunity in skin-contact inflammation. *J. Clin. Invest.* 123: 1444–1456.
5. Cortez, V. S., A. Fuchs, M. Cella, S. Gilfillan, and M. Colonna. 2014. Cutting edge: Salivary gland NK cells develop independently of Nfil3 in steady-state. *J. Immunol.* 192: 4487–4491.
6. Klose, C. S., M. Flach, L. Möhle, L. Rogell, T. Hoyler, K. Ebert, C. Fabiunke, D. Pfeifer, V. Sexl, D. Fonseca-Pereira, et al. 2014. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. *Cell* 157: 340–356.
7. Sojka, D. K., B. Plougastel-Douglas, L. Yang, M. A. Pak-Wittel, M. N. Artyomov, Y. Ivanova, C. Zhong, J. M. Chase, P. B. Rothman, J. Yu, et al. 2014. Tissue-resident natural killer (NK) cells are cell lineages distinct from thymic and conventional splenic NK cells. *eLife* 3: e01659.
8. Constantinides, M. G., B. D. McDonald, P. A. Verhoef, and A. Bendelac. 2014. A committed precursor to innate lymphoid cells. *Nature* 508: 397–401.
9. Robinette, M. L., A. Fuchs, V. S. Cortez, J. S. Lee, Y. Wang, S. K. Durum, S. Gilfillan, M. Colonna, L. Shaw, and B. Yu, et al. 2015. Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets. *Nat. Immunol.* 16: 306–317.
10. Intlekofer, A. M., N. Takemoto, E. J. Wherry, S. A. Longworth, J. T. Northrup, V. R. Palanivel, A. C. Mullen, C. R. Gasink, S. M. Kaech, J. D. Miller, et al. 2005. Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. *Nat. Immunol.* 6: 1236–1244.
11. Intlekofer, A. M., A. Banerjee, N. Takemoto, S. M. Gordon, C. S. Dejong, H. Shin, C. A. Hunter, E. J. Wherry, T. Lindsten, and S. L. Reiner. 2008. Anomalous type 17 response to viral infection by CD8+ T cells lacking T-bet and eomesodermin. *Science* 321: 408–411.
12. Narni-Mancinelli, E., J. Chaix, A. Fenis, Y. M. Kerdiles, N. Yessaad, A. Reynders, C. Gregoire, H. Luche, S. Ugolini, E. Tomasello, et al. 2011. Fate mapping analysis of lymphoid cells expressing the NKp46 cell surface receptor. *Proc. Natl. Acad. Sci. USA* 108: 18324–18329.
13. Jones-Mason, M. E., X. Zhao, D. Kappes, A. Lasorella, A. Iavarone, and Y. Zhuang. 2012. E protein transcription factors are required for the development of CD4(+) lineage T cells. *Immunity* 36: 348–361.
14. Teo, A. K., S. J. Arnold, M. W. Trotter, S. Brown, L. T. Ang, Z. Chng, E. J. Robertson, N. R. Dunn, and L. Vallier. 2011. Pluripotency factors regulate definitive endoderm specification through eomesodermin. *Genes Dev.* 25: 238–250.
15. Townsend, M. J., A. S. Weinmann, J. L. Matsuda, R. Salomon, P. J. Farnham, C. A. Biron, L. Gapin, and L. H. Glimcher. 2004. T-bet regulates the terminal maturation and homeostasis of NK and Valpha14i NKT cells. *Immunity* 20: 477–494.
16. Lazarevic, V., L. H. Glimcher, and G. M. Lord. 2013. T-bet: a bridge between innate and adaptive immunity. *Nat. Rev. Immunol.* 13: 777–789.
17. Takeda, K., E. Cretney, Y. Hayakawa, T. Ota, H. Akiba, K. Ogasawara, H. Yagita, K. Kinoshita, K. Okumura, and M. J. Smyth. 2005. TRAIL identifies immature natural killer cells in newborn mice and adult mouse liver. *Blood* 105: 2082–2089.
18. Chiossone, L., J. Chaix, N. Fuseri, C. Roth, E. Vivier, and T. Walzer. 2009. Maturation of mouse NK cells is a 4-stage developmental program. *Blood* 113: 5488–5496.
19. Mucida, D., M. M. Husain, S. Muroi, F. van Wijk, R. Shinnakasu, Y. Naoe, B. S. Reis, Y. Huang, F. Lambolez, M. Docherty, et al. 2013. Transcriptional reprogramming of mature CD4$^+$ helper T cells generates distinct MHC class II-restricted cytotoxic T lymphocytes. *Nat. Immunol.* 14: 281–289.
20. Reis, B. S., A. Rogoz, F. A. Costa-Pinto, I. Taniuchi, and D. Mucida. 2013. Mutual expression of the transcription factors Runx3 and ThPOK regulates intestinal CD4$^+$ T cell immunity. *Nat. Immunol.* 14: 271–280.

Supplemental Figures



Supplemental Figure 1. Evaluation of Eomes and T-bet expression in *NKp46-Cre*$^{+}$ mouse models. (**A**) Flow cytometry of NK1.1^{+} NKp46^{+} type 1 ILCs from WT and *Eomes*$^{flox/flox}$ *NKp46-Cre*$^{+}$ mice (n=4-5 mice per genotype) revealed efficient *Eomes* deletion in mutant mice. **(B)** Flow cytometry of TRAIL, CD127, and integrin α_v expression by type 1 ILCs from WT and *Eomes*$^{flox/flox}$ *NKp46-Cre*$^{+}$ mice (n=3 mice per genotype) suggested that loss of Eomes shifts the balance of type 1 ILCs from cNKs to helper-like ILC1s. (**C, D**) hILC1s escaped *Tbx21* deletion in *Tbx21*$^{flox/flox}$ *NKp46-Cre*$^{+}$mice. (C) Flow cytometry of hepatic type 1 ILCs from WT, *Tbx21*$^{flox/flox}$ *NKp46-Cre*$^{+}$ (T-bet cKO), and *Tbx21*$^{-/-}$ mice (n=3 mice per genotype). In contrast to *Tbx21*$^{-/-}$ mice with a germline deletion of T-bet, T-bet cKO mice had a substantial population of CD49a^{+} DX5^{-} Eomes^{-} hILC1s that expressed T-bet protein. (D) To determine whether these cells underwent *Tbx21* deletion but contained residual T-bet protein or escaped *Tbx21* deletion, we used genomic PCR to analyze the status of the *Tbx21* locus in 3 cell populations sorted from the same *Tbx21*$^{flox/flox}$ *NKp46-Cre*$^{+}$ mouse (n=2 mice): CD49a^{+} DX5^{-} hILC1s (lane 1), CD49a^{-} DX5^{+} cNKs (lane 2), and CD3^{+} T cells (lane 3). The following 3 primers were used to amplify wild-type and mutant *Tbx21* loci: (1) TAT GAT TAC ACT GCA GCT GTC TTC AG, (2) CAG GAA TGG GAA CAT TCG CCT GTG, and (3) CTC TGC CTC CCA TCT CTT AGG AGC. Comparison with control skin samples from mice that have WT, null, or floxed *Tbx21* alleles (lanes 4-6) revealed that the majority of hILC1s and a minor subset of cNKs in T-bet cKO mice escaped *Tbx21* deletion. Deletion did not occur in T cells. Data are mean ± SEM representative of 2-5 independent experiments.



Supplemental Figure 2. Transgenic Eomes is sufficient to convert some attributes of hILC1s to those of cNKs. (A) Flow cytometry of NK1.1$^+$ NKp46$^+$ type 1 ILCs from WT and *Tg*$^+$ mice (n=3 mice per genotype) revealed loss of TRAIL expression due to transgenic Eomes. **(B)** Absolute numbers of total type 1 ILCs and indicated subsets from the salivary gland and thymus of WT and *Tg*$^+$ mice (n=4 mice per genotype). **(C)** T-bet and Eomes expression by CD49a$^+$ DX5$^-$ (1), CD49a$^+$ DX5$^+$ (2), and CD49a$^-$ DX5$^+$ (3) type 1 ILC subsets from the spleens of WT and *Tg*$^+$ mice (n=4 mice per genotype). **(D)** Id2 expression by CD49a$^+$ DX5$^-$ hILC1s and CD49a$^+$ DX5$^-$ cNKs from the salivary gland and thymus of *Id2*$^{hCD5/+}$ mice (n=4 mice). **(E)** Flow cytometry of Id2 and Eomes expression by NK1.1$^+$ NKp46$^+$ type 1 ILCs from the indicated organs of *Id2*$^{hCD5/+}$ and *Tg*$^+$ *Id2*$^{hCD5/+}$ mice (n=4 mice per genotype). The number of Id2^{hi} cells is indicated. **(F)** Flow cytometry of CD127 and integrin α_v expression by Id2^{hi} NK1.1$^+$ NKp46$^+$ type 1 ILCs from the indicated organs of WT and *Tg*$^+$ *Id2*$^{hCD5/+}$ mice (n=3-4 mice per genotype). **(G)** Flow cytometry of NKG2A/C/E, Ly49D/G2/H, CD27, CD11b, CD43, KLRG1, and CD122 expression by Id2^{hi} NK1.1$^+$ NKp46$^+$ type 1 ILCs from the livers of WT and *Tg*$^+$ *Id2*$^{hCD5/+}$ mice (n=4 per genotype). **(H)** Summary of splenic data from (G). Data are mean ± SEM representative of 2-5 independent experiments; *p<0.05, **p<0.01, ***p<0.001.