

Supplemental material

Nagasawa et al., <https://doi.org/10.1084/jem.20190490>

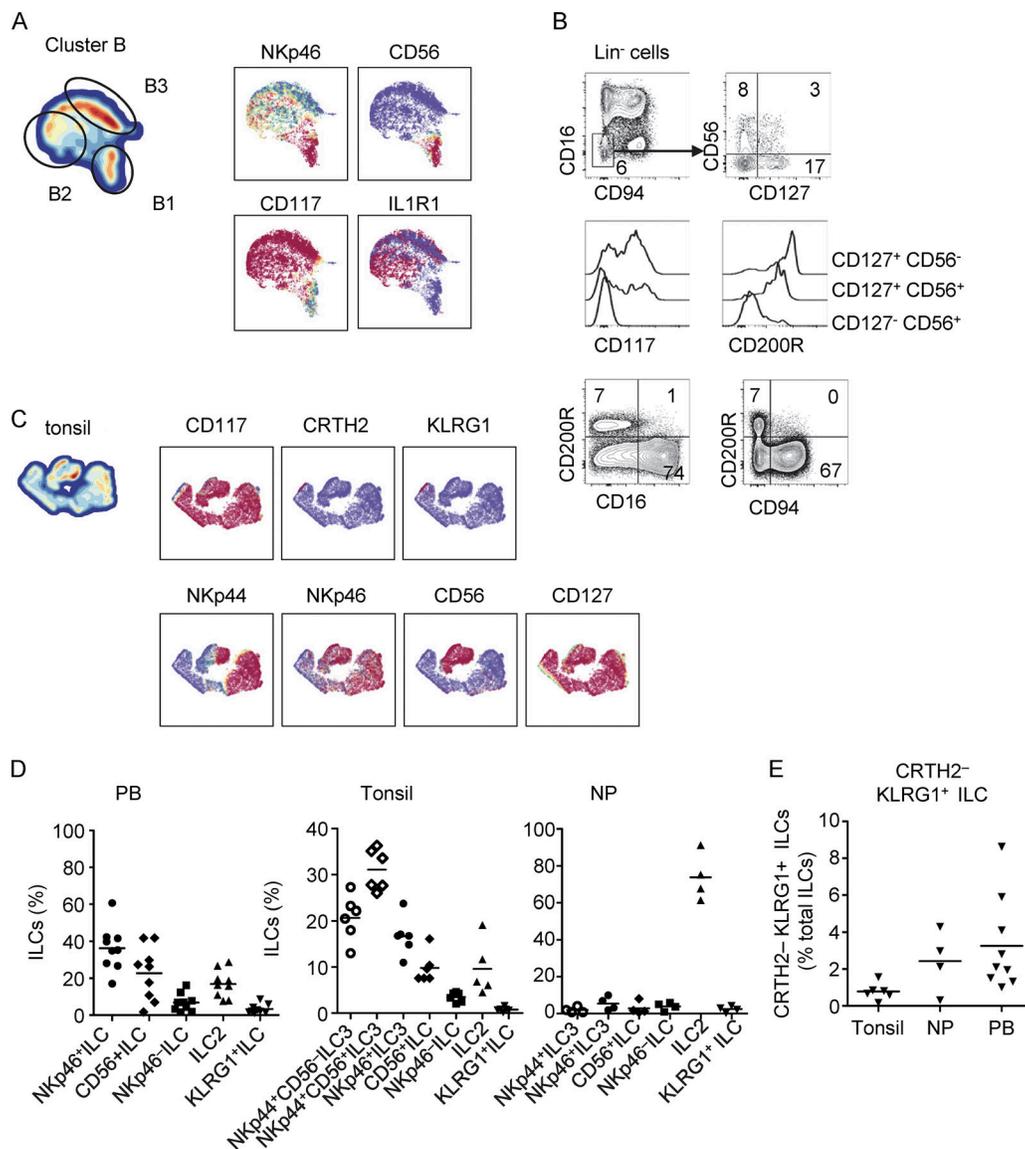


Figure S1. **Characterization of ILCs in PB, tonsil, and NP.** (A) HSNE analysis of zoom-in of ILC cluster B (from Fig. 1 A; $n = 8$). (B) Expression of CD127 and CD56 in PB Lin⁻CD94⁺CD16⁻ cells and expression of CD117 and CD200R in CD127⁺CD56⁻, CD127⁺CD56⁺, and CD127⁻CD56⁺ cells ($n = 6$). (C) HSNE analysis of the ILC population in tonsil ($n = 4$). (D) Frequency of each ILC subset within PB, tonsil, and NP. (E) Frequency of CRTH2⁻ KLRG1⁺ ILCs within tonsil, NP, and PB. All data are verified in at least two independent experiments.

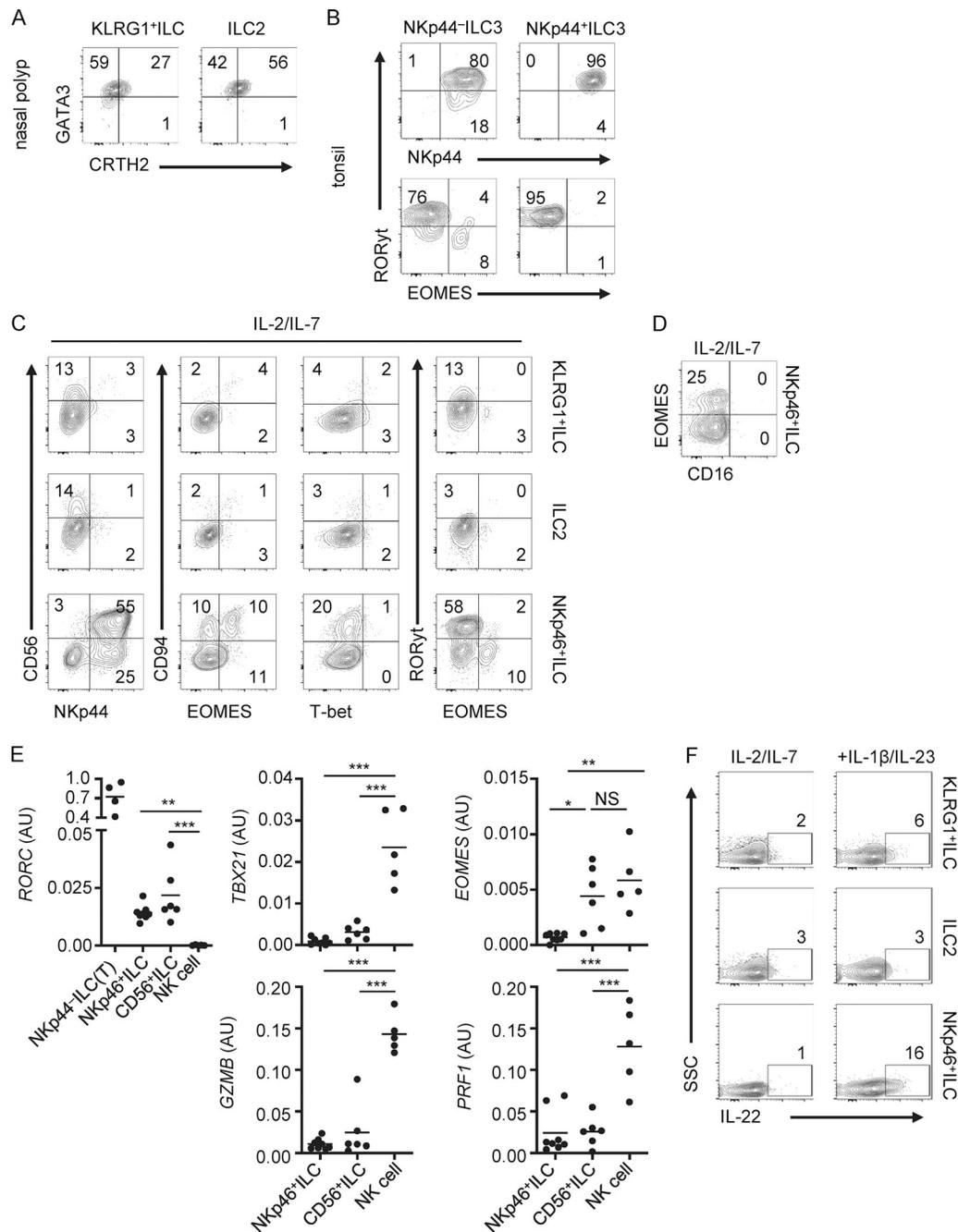


Figure S2. **Differentiation capacity of KLRG1⁺ ILCs and NKp46⁺ ILCs.** (A and B) Expression of CRTH2 and GATA3 in KLRG1⁺ ILCs and ILC2s isolated from NP (*n* = 3; A) NKp44⁻ ILC3s and NKp44⁺ ILC3s (both NKp46⁺ CD56⁻) isolated from tonsils (B) after culture for 7 d on OP9-DL1 in the presence of IL-2 (20 U/ml) and IL-7 (20 ng/ml; *n* = 3). (C) Expression of CD56, NKp44, RORyt, and EOMES in PB isolated ILC subsets after culture for 5 d on OP9-DL1 in the presence of IL-2 and IL-7 (*n* = 6). (D) Expression of EOMES and CD16 in PB isolated NKp46⁺ ILCs after cultured as in C (*n* = 3). (E) Quantification of relative RORC, EOMES, TBX21, GZMB, and PRF1 expression as compared with GAPDH in different ILC subsets isolated from tonsil (T) and PB. Each dot represents one donor. (F) Intracellular IL-22 expression after culture for 7 d on OP9-DL1 in the presence of IL-2, IL-7, IL-1β, and IL-23 (*n* = 3). *, *P* < 0.05; **, *P* < 0.001; ***, *P* < 0.0001 (one-way ANOVA). All data are verified in at least two independent experiments. AU, area under the curve. SSC, side scatter.

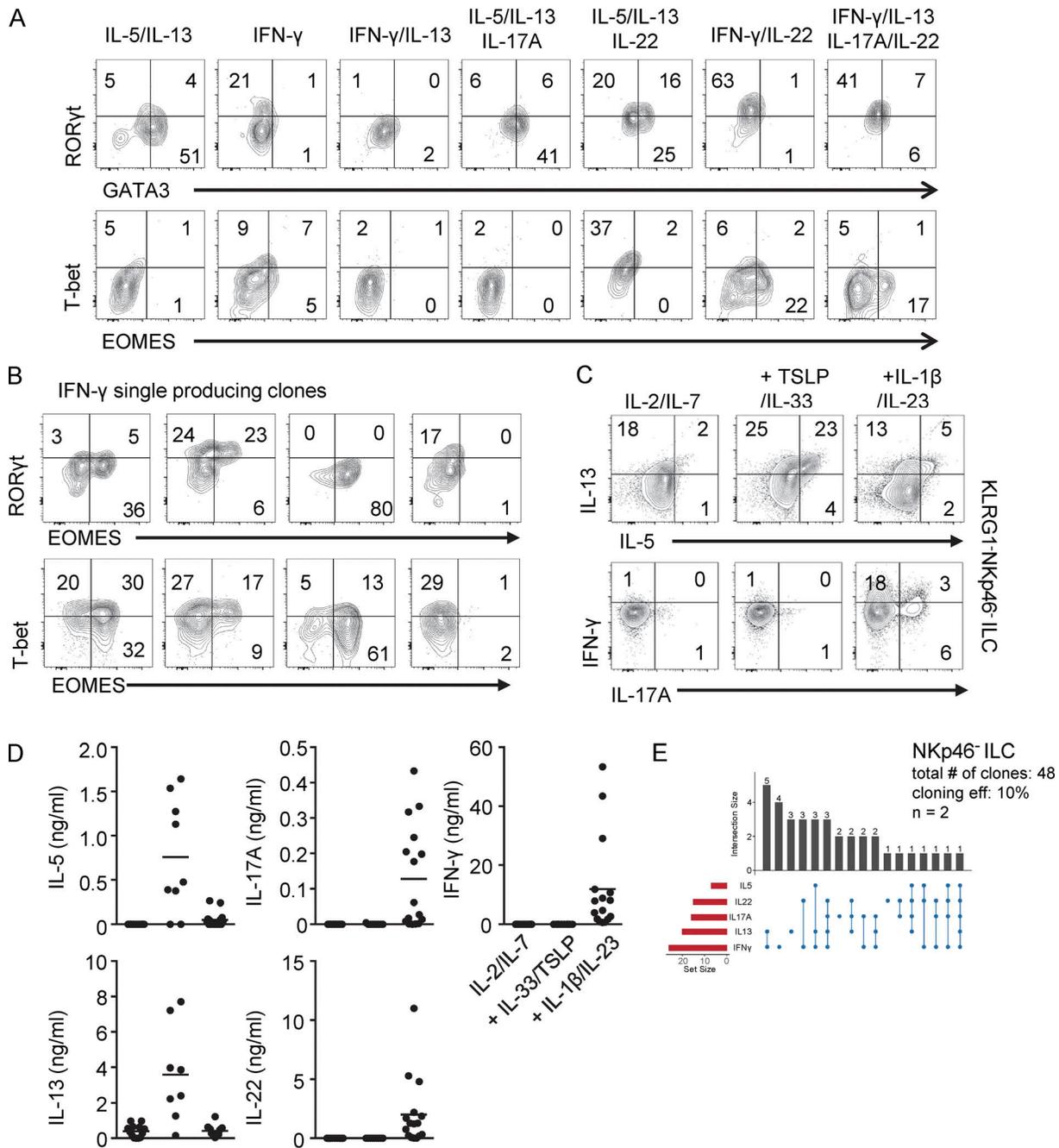


Figure S3. **Clonal analysis of CD117⁺ PB ILC subsets show broad functional differentiation capacities.** (A) Representative flow cytometric analysis of intracellular GATA3, ROR γ t, T-bet, and EOMES expression in clones obtained from KLRG1⁺ ILCs cultured for 14–21 d on OP9-DL1 with IL-2 (20 U/ml), IL-7, IL-1 β , and IL-23 (20 ng/ml each). (B) Representative flow cytometric analysis of NKp46⁺ ILC clones producing IFN- γ and their TF expression profile. (C) Representative flow cytometric analysis of intracellular IL-5, IL-13, IFN- γ , and IL-17A in KLRG1⁺ NKp46⁻ ILCs after 7 d cultured on OP9-DL1 cells with IL-2 and IL-7 with or without TSLP and IL-33 or IL-1 β and IL-23. (D) Quantification of cytokine production by ELISA in culture supernatants from cells stimulated as in C. The concentration is adjusted to 5,000 cells. (E) Summary of numbers and type of cytokines produced by NKp46⁻ ILC clones. Data in A–C are representative of at least three donors from two independent experiments.

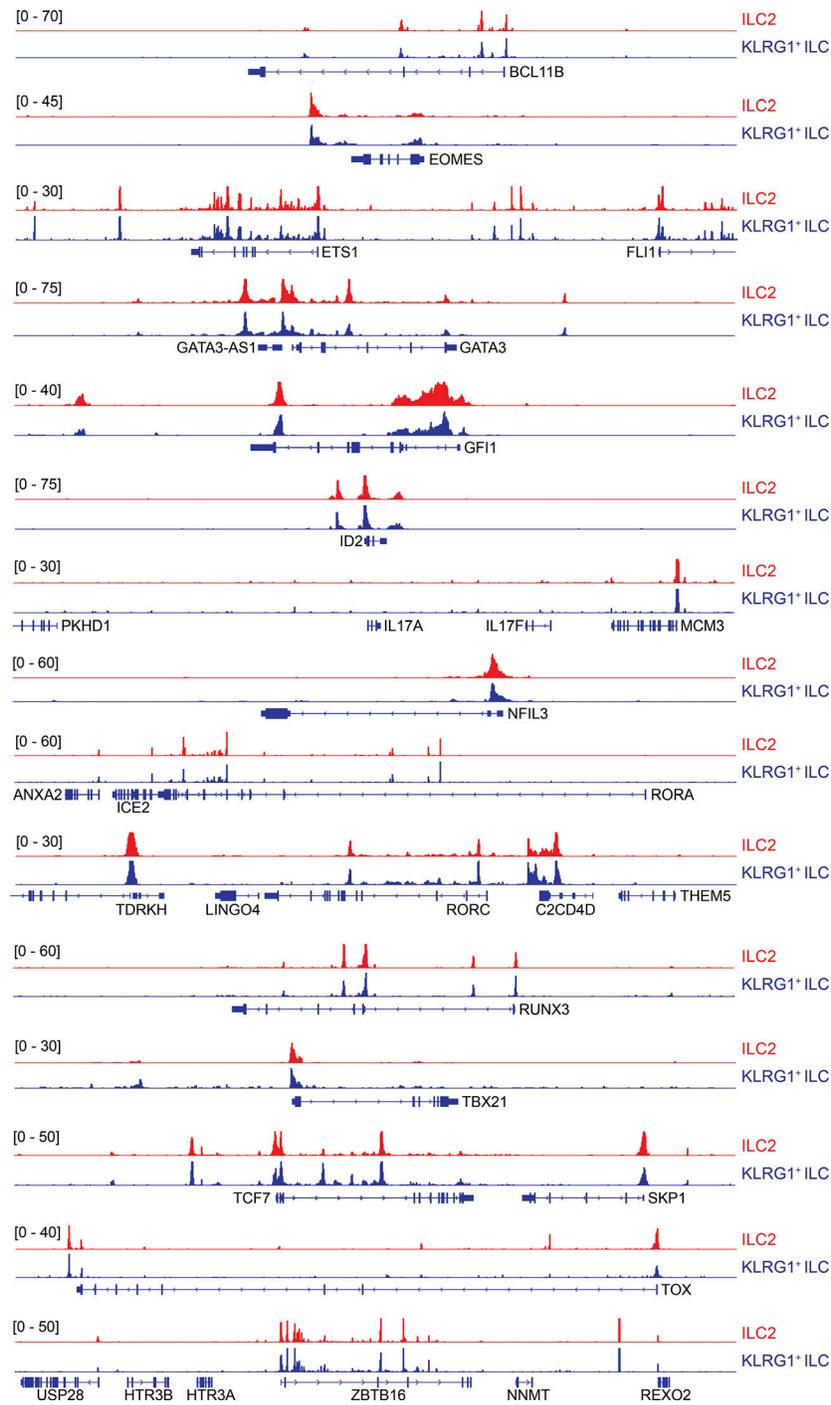


Figure S4. **Chromatin accessibility as measured by ATAC-Seq at key loci related to ILC biology.** Additional genome browser shots of ATAC-Seq signals across key loci relevant for ILC development and differentiation.

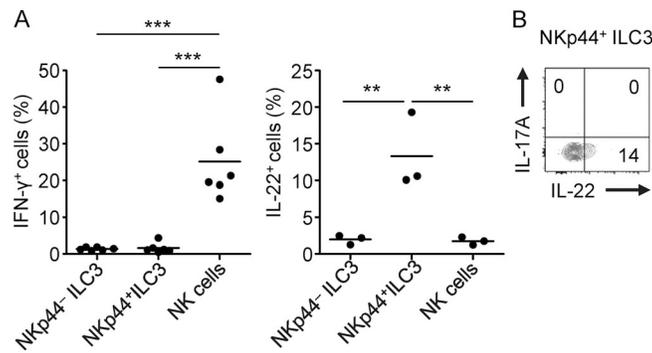


Figure S5. **Cytokine production profile of freshly isolated NKp44⁻ ILC3 and NKp44⁺ ILC3 from tonsil.** (A) IFN- γ and IL-22 production by freshly isolated NKp44⁻ ILC3s and NKp44⁺ ILC3 from tonsils were evaluated by intracellular cytokine staining after stimulation with PMA/ionomycin for 6 h. NK cells were used as a reference. Each dot represents an individual donor. (B) Representative flow cytometry plot of IL-17A and IL-22 production by freshly isolated NKp44⁺ ILC3s after stimulation with PMA/ionomycin for 6 h ($n = 6$). **, $P < 0.001$; ***, $P < 0.0001$ (one-way ANOVA).

Table S2. **Key resources**

Reagent or resource	Source	Identifier
Antibodies		
anti-human CD1a FITC (HI149)	BioLegend	Cat#300104, RRID:AB_314018
anti-human CD3 FITC (OKT3)	BioLegend	Cat#317306, RRID:AB_571907
anti-human CD4 FITC (RPA-T4)	BioLegend	Cat#300506, RRID:AB_314074
anti-human CD5 FITC (UCHT2)	BioLegend	Cat#300606, RRID:AB_314092
anti-human CD14 FITC (HCD14)	BioLegend	Cat#325604, RRID:AB_830677
anti-human CD16 FITC (3G8)	BioLegend	Cat#555406, RRID:AB_395806
anti-human CD19 FITC (HIB19)	BioLegend	Cat#302206, RRID:AB_314236
anti-human CD34 FITC (581)	BioLegend	Cat#343504, RRID:AB_1731852
anti-human CD94 FITC (DX22)	BioLegend	Cat#305504, RRID:AB_314534
anti-human CD123 FITC (6H6)	BioLegend	Cat#306014, RRID:AB_2124259
anti-human FcER1a FITC (AER37)	BioLegend	Cat# 334608, RRID:AB_1227653
anti-human TCRαβ FITC (IP26)	BioLegend	Cat#306706, RRID:AB_314644
anti-human TCRγδ FITC (B1)	BioLegend	Cat#331208, RRID:AB_1575108
anti-human BDCA2 FITC (201A)	BioLegend	Cat#354208, RRID:AB_2561364
anti-human CD161 PE (HP-3G10)	BioLegend	Cat#339904, RRID:AB_1501083
anti-human NKp44 PE (P448)	BioLegend	Cat#325108, RRID:AB_756100
anti-human IL-5 PE (JES1-39D107)	BioLegend	Cat#500904, RRID:AB_315139
anti-human CD45 AF700 (HI30)	BioLegend	Cat#304024, RRID:AB_493761
anti-human CD3 AF700 (UCHT1)	BioLegend	Cat#300424, RRID:AB_493741
anti-human IL-17A AF700 (BL168)	BioLegend	Cat#512318, RRID:AB_2124868
anti-human CD161 BV421 (HP-3G10)	BioLegend	Cat#339914, RRID:AB_2561421
anti-human IL-5 BV421 (JES1-39D10)	BioLegend	Cat#504311, RRID:AB_2563161
anti-human IFNγ BV510 (4S.B3)	BioLegend	Cat#502544, RRID:AB_2563883
anti-human CRTH2 PE-CF594 (BM16)	BioLegend	Cat#350126, RRID:AB_2572053
anti-human IL-13 APC (JES10-5A2)	BioLegend	Cat#501903, RRID:AB_315198
Anti-human CD5 APC Cy7(L17F12)	BioLegend	Cat#364010, RRID:AB_2564506
Anti-human CD161 APC/Fire 750 (HP-3G10)	BioLegend	Cat#339944, RRID:AB_2617016
Anti-human NKp46 BV421 (9E2)	BioLegend	Cat#331914, RRID:AB_2563853
Anti-human T-bet BV605 (4B10)	BioLegend	Cat#644817, RRID:AB_11219388
Anti-human CCR6 BV605(G034E3)	BioLegend	Cat#353420, RRID:AB_2561449
Anti-human CD56 BV650 (HCD56)	BioLegend	Cat#318344, RRID:AB_2563838
Anti-human CD3 Biotin (OKT3)	BioLegend	Cat#317320, RRID:AB_10916519
Anti-human CD19 Biotin (HIB19)	BioLegend	Cat#302204, RRID:AB_314234
Anti-human CD14 Biotin (62D3)	BioLegend	Cat#367106, RRID:AB_2566618
Anti-human CD16 Biotin (3G8)	BioLegend	Cat#302004, RRID:AB_314204
anti-human T-bet PE-Cy7 (4B10)	eBioscience	Cat#12582582, RRID:AB_925761
anti-human GATA3 PE (TWA)	eBioscience	Cat#12996642, RRID:AB_1963600
anti-human KLRG1 APC (13F12F2)	eBioscience	Cat#17948842, RRID:AB_2573303
Anti-human IL-22 PE Cy7(22URT1)	eBioscience	Cat#25-722-42
Anti-human IL1R1 PE	eBioscience	Cat#FAB269p, RRID:AB_2124912
Anti-human EOMES PE-eFluor610(WD1928)	eBioscience	Cat#61-4877-42, RRID:AB_2574616
anti-human CD127 PE-Cy7 (R34.34)	Beckman Coulter	Cat#A64618

Table S2. **Key resources (Continued)**

Reagent or resource	Source	Identifier
anti-human CD117 PE-Cy5 (104D2D1)	Beckman Coulter	Cat# IM2733, RRID:AB_131178
anti-human CRTH2 AF647 (BM16)	Beckman Dickinson	Cat#558042, RRID:AB_2112699
anti-human CD45 APC-Cy7 (2D1)	Beckman Dickinson	Cat#368518, RRID:AB_2616705
anti-human RoRyT AF647 (Q21-559)	Beckman Dickinson	Cat#563620, RRID:AB_2738324
Bacterial and virus strains		
N/A		
Biological samples		
Healthy human peripheral blood	Sanquin Bloodbank Amsterdam	N/A
Human nasal tissue	Amsterdam UMC	N/A
Human tonsil tissue	Amsterdam UMC/OLVG hospital Amsterdam	N/A
Human AB serum	Merck	Cat#H4522
Chemicals, peptides, and recombinant proteins		
Recombinant human IL-1b	R&D Systems	Cat#201-LB
Recombinant human IL-2	R&D Systems	Cat#202-IL-500
Recombinant human IL-7	Peptotech	Cat# 200-07
Recombinant human IL-23	R&D Systems	Cat#1290-IL-010
Recombinant human IL-33	R&D Systems	Cat#3625-IL-010
Recombinant human TSLP	R&D Systems	Cat#1398-TS-010
Phorbol 12-Myristate 13-acetate (PMA)	Sigma-Aldrich	Cat#P8139
Ionomycin	Merck	Cat#407950
BD Golgiplug Protein Transport Inhibitor	BD Biosciences	Cat#555029
Iscove's Modified Dulbecco's Medium	Gibco	Cat#21980-065
Roswell Park Memorial Institute (RPMI)1640	Gibco	Cat#52400-041
Anti-PE microbeads	Miltenyi	Cat#130-048-801
Anti-FITC microbeads	Miltenyi	Cat#130-048-701
AutoMACS Running Buffer – MACS Separation Buffer	Miltenyi	Cat#130-091-221
MACS separation columns LS	Miltenyi	Cat#130-042-401
MACS separation columns LD	Miltenyi	Cat#130-042-901
Pre-Separation Filters (30 µm)	Miltenyi	Cat#130-041-407
MojoSort streptavidin Nanobeads	BioLegend	Cat#480016
Lymphoprep	Stemcell Technologies	Cat#07861
Liberase TM	Roche	Cat#5401127001
DNase I	Roche	Cat#11284932001
Penicillin-Streptomycin	Roche	Cat#11074440001
HyClone™ FetalClone™ I Serum	ThermoFisher Scientific	Cat#SH30080.03
TD1 transposase and 2x transposase buffer	illumina	Cat#FC-121-1030
Spermine	Sigma-Aldrich	Cat#S3256
Spermidine	Sigma-Aldrich	Cat#S2626
6AA	Sigma-Aldrich	Cat#A7824
Sucrose	Sigma-Aldrich	Cat#S7903
Critical commercial assays		
Foxp3/Transcription Factor Staining Buffer Set	ThermoFisher Scientific	Cat#00-5523-00
Ready-Set-Go IL-5 ELISA kit	ThermoFisher Scientific	Cat#88-7056-77
Ready-Set-Go IL-13 ELISA kit	ThermoFisher Scientific	Cat#88-7439-88

Table S2. **Key resources (Continued)**

Reagent or resource	Source	Identifier
Ready-Set-Go IL-17A ELISA kit	ThermoFisher Scientific	Cat#88-7176-76
Ready-Set-Go IL-22 ELISA kit	ThermoFisher Scientific	Cat#88-7522-88
Human IFN Gamma uncoated ELISA kit	ThermoFisher Scientific	Cat#88-7316-88
NucleoSpin RNA XS kit	Macherey-Nagel	Cat#740902.250
High-Capacity cDNA Reverse Transcription Kit	ThermoFisher Scientific	Cat#4368813
Clariom S pico Human HT 24-array plate	ThermoFisher Scientific	Cat#902963
QIAzol Lysis Reagent	Qiagen	Cat#79306
RNeasy kit	Qiagen	Cat#73404
GeneTitan™ Hybridization, Wash, and Stain Kit	Thermo Fisher	Cat#901622
iQ™SYBR® Green supermix	BioRad	Cat#64084532
Min-elute PCR purification kit	Qiagen	Cat#28004
Deposited data		
Micro-array data	This paper/MAD Dutch Genomics Service & Support Provider	GSE123817
ATAC-seq data	This paper	GSE124054
Experimental models: Cell lines		
Mouse: OP9 stromal cell line	Nakano, T. Osaka, Japan	N/A
Mouse: OP9-DL1 stromal cell line	Dontje et al, 2006	N/A
Experimental models: Organisms/strains		
N/A		
Oligonucleotides		
Primer: GATA3 forward: ACCACAACCACACTCTGGAGGA	This paper	N/A
Primer: GATA3 reverse: TCGTTTCTGGTCTGGATGCCT	This paper	N/A
Primer: RORC forward: AATCTGGAGCTGGCCTTTCA	This paper	N/A
Primer: RORC reverse: CTGGAAGATCTGCAGCCTTT	This paper	N/A
Primer: TBX21 forward: ATTGCCGTGACTGCCTACCAGA	This paper	N/A
Primer: TBX21 reverse: GGAATTGACAGTTGGGTCCAGG	This paper	N/A
Primer: EOMES forward: AAATGGGTGACCTGTGGCAA GC	This paper	N/A
Primer: EOMES reverse: CTCCTGTCTCATCCAGTGGGAA	This paper	N/A
Primer: GZMB forward: CGACAGTACCATTGAGTTGTGCG	This paper	N/A
Primer: GZMB reverse: TTCGTCCATAGGAGACAATGCCC	This paper	N/A
Primer: PRF1 forward: ACTCACAGGCAGCCAACCTTTC	This paper	N/A
Primer: PRF1 reverse: CTCTTGAAGTCAGGGTGCAGCG	This paper	N/A
Primer: GAPDH forward: GTCTCCTCTGACTTCAACAGCG	This paper	N/A
Primer: GAPDH reverse: ACCACCCTGTTGCTGTAGCCAA	This paper	N/A
Recombinant DNA		
N/A		
Software and algorithms		
GraphPad Prism 7.0	GraphPad	www.graphpad.com
FlowJo V10	FlowJo	www.flowjo.com
RStudio	RStudio	www.rstudio.com
Cytosplore ^{+HSNE}	Cytosplore	www.cytosplore.org
BioRad CFX Manager	BioRad	www.biorad.com
Other		

Table S2. **Key resources (Continued)**

Reagent or resource	Source	Identifier
Yssel's Medium	In house	N/A

Table S1 is provided online as an Excel file.