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Last updated by author(s): Jun 14, 2022

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	BD FACSDiva™ (v8.0.1), SpectroFlo (v3.03), Olympus Fluoview FV32S-SW (v2.3.1.163) , Illumina NovaSeq 6000 system was used for libraries sequencing.
Data analysis	Cell ranger (v5 and v3.1), kallisto (v0.46.0) bustools (v0.39.3), Python (v3.9.7), Scrublet (v0.2.3), scvi-tools (v0.14.6), using Scanpy (v1.8.2), FlowJo (10.8.1), , CITE-seq-Count (v1.4.4), Seurat (v4.0), Fiji (ImageJ v2.1.0), All code used for analysis in this manuscript is available at https://github.com/nygctech/Kedmi-CITEseq.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data generated for this project are available at the Gene Expression Omnibus with the accession code GSE190372. Published data GSE176282 was used for analysis. Refdata-gex-mm10-2020-A reference library provided by 10x Genomics was used to generate gene expression count matrices.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Three or more mice per group were used in each experiment. The precise number of animals used are given in the figure legend. The sample size was determined from our previous experience and from what is accepted in the field.
Data exclusions	No samples were excluded from analysis.
Replication	All the findings on the main figures were replicate at least twice. The precise number of repeats used are given in the figure legend. All attempts were successful.
Randomization	Allocation into sample groups was random. In addition all control mice were from the same litter . Both males and females were used.
Blinding	Experiment were not perform blindly, except for RNA scope that was performed blindly, since it required scoring.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

CD3 biotinylated (145-2C11), Tonbo Bio 30-0031-U500, 1:100;CD3 TotalSeq™-A0182 (17A2),BioLegend 100251, 1:100;CD4 AF700(RM4-5), Thermo Scientific 56-0042-82, 1:400;CD4 BUV395(GK1.5), BD 563790, 1:400;CD25 APC (PC61), Thermo Scientific 17-0251-82, 1:200;CD25 TotalSeq™-A0097 (PC61), BioLegend 102055, 1:100;CD25 BV421 (PC61), BioLegend 102033, 1:200;CD25 PE (PC61), Invitrogen 12-0251-83, 1:200;CD44 PerCP-Cyanine5.5 (IM7), Thermo Scientific 45-0441-82, 1:400;CD44 AF700 (IM7), BD 560567, 1:200;CD45.1 BV650(A20), BD563754, 1:400;CD45.1 BV421(A20), BD 563983, 1:200;CD45.2 AF594 (104), BioLegend 109850, 1:200;CD45.2 FITC (104), eBioscience 11-0454-85, 1:400;CD90.1 APC-e780(HIS51), Thermo Scientific 47-0900-82, 1:400;CD90.2 PerCP-Cyanine5.5 (53-2.1), BioLegend 140316, 1:200;CD90.2 FITC (30-H12), BD 553013, 1:400;CD90.2 APC e780 (53-2.1), eBioscience 47-0902-82, 1:200;CD90.2 TotalSeq™-A0075 anti-mouse (30-H12), BioLegend 105345, 1:100;CD19 PerCP-Cyanine5.5 (1D3), Tonbo Bioscience 65-0193-U100, 1:400;CD45R/B220 PerCP-Cyanine5.5 (RA3-6B2), Invitrogen 45-0452-82, 1:400;CD45R/B220 BV510 (RA3-6B2), BD B563103, 1:400;CD45R/B220 biotinylated (RA3-6B2), Thermo Fisher 13-0452-85, 1:100;CD45R/B220 BV785 (RA3-6B2), BioLegend 103246, 1:200;CD127 PE (A7R34), eBioscience 12-1271-83, 1:100;CD127 BV650 (A7R34), BioLegend 135043, 1:100;CD127 PerCP-Cyanine5.5 (A7R34), BioLegend 135021, 1:50;CD127 (IL-7Rα) TotalSeq™-A0198 (A7R34), BioLegend 135045, 1:100;CD51 PE (RMV-7), BioLegend 104106, 1:100;CD51 BUV395 (RMV-7), BD 747834, 1:100;CD51 TotalSeq™-A1008 (RMV-7), BioLegend 104111, 1:100;MHCII I-A/I-E AF700 (56-5321-82), eBioscience 56-5321-80, 1:200;MHCII I-A/I-E BV421 (M5/114.15.2), BioLegend 107631, 1:400;CCR6 BV711(140706), BD 740646, 1:400;CCR6 BV421(29-2L17), Biolegend 129817, 1:150;CCR6 BUV395(140706), BD 747831, 1:50;CCR6 TotalSeq™-A0225 (29-2L17), BioLegend 129825, 1:100;NKp46 AF467 (29A1.4), BioLegend 137628, 1:200;NK1.1 PerCP-Cyanine5.5 (PK136), Invitrogen 45-5941-82, 1:200;CD62L APC (MEL-14), Fisher Scientific 50-150-09, 1:400;CXCR5 APC (L138D7), BioLegend 145506, 1:200;CXCR5 TotalSeq™-A0846 (L138D7), BioLegend 145535, 1:100;TCRβ BV711 (H57-597), BD 563135, 1:200;TCRβ APC e780 (H57-597), Invitrogen 47-5961-82, 1:200;TCRβ BV510 (H57-597), BD 563221, 1:200;TCRβ biotinylated (H57-597), Thermo Fisher 13-5961-85, 1:100;TCRβ FITC (H57-597), BioLegend 109206, 1:200;TCRg/d BV510 (GL3), BioLegend 118131, 1:200;TCRg/d FITC (GL3), BioLegend, 118105, 1:200;TCRg/d PerCP-Cyanine5.5 (GL3), BioLegend 118117, 1:400;TCR Vβ6 PerCP-eFluor 710, (RR4-7), Thermo Scientific 46-5795-82, 1:400;TCR Vβ6 FITC (RR4-7), BD 553193, 1:400;Bcl-6 BV421 (K112-91), BD 563363, 1:50;Foxp3 PE-Cy7 (FJK-16s), eBioscience 25-5773-82, 1:200;Foxp3 e660 (FJK-16s), Thermo Scientific

50-5773-82, 1:200;RORγt BV421 (Q31-378), BD 562894, 1:200;RORγt PE (B2D), Thermo Scientific 12-6981-82, 1:200;T-bet PE-CF594 (O4-46), BD 562467, 1:70;IL-17A AF700 (TC11-18H10.1) BioLegend 506914, 1:200;IFN-γ PE-Cy7 (XMG1.2), BioLegend 505826, 1:200;CD11c PE-Cy7 (N418), BioLegend 117318, 1:400;CD11c BV711 (HL3), BD 563048, 1:100;CD11c TotalSeq™-A0106 (N418), BioLegend 117355, 1:100;CD11b BUV395 (M1/70), BD 563553, 1:400;CD11b APC-e780 (M1/70), Invitrogen 47-0112-82, 1:400;CX3CR1 PE (SA011F11), BioLegend 149006, 1:200;CX3CR1 TotalSeq™-A0563 (SA011F11), Biolegend 149041, 1:100;Ly6c PerCP (HK1.4), BioLegend 128028, 1:200;SIRPa FITC (P84), BioLegend 144006, 1:400;Ly6G PerCP-Cyanine5.5 (1A8), BioLegend 127616, 1:400;CD273 APC (TY25), BioLegend 107210, 1:100; Clec12a PE (5D3), BD 562773, 1:200;CD103 BV421 (M290), BD 562771, 1:100;CD103 PE/dazzle 594 (2E7), Biolegend 121430, 1:200;XCR1 APC Cy7 (ZET), Biolegend 148224, 1:200;CCR7 PE(4B12), BD 560682, 1:100;CCR7 BV421(4B12), BioLegend 120119, 1:100;CXCR6 PE/dazzle 594 (SA051D1), biolegend 151117, 1:200;CXCR6 APC (DANID2), Thermo Scientific ebioscience 17-9186-82, 1:400;CXCR6 PE-Cy7 (SA051D1), biolegend 151118, 1:200;CD40 biotinylated (HM40-3), eBioscience, 1:100;CD40 biotinylated (3/23), BioLegend 124606, 1:100;TER-119 (TER-119) biotinylated, Thermo Fisher 13-5921-85, 1:200;CD16/32 (2.4G2), Bio X Cell BE0307, 1:400;CD64 TotalSeq™-A0202 (X54-5/7.1), BioLegend 139325, 1:100;CD14 TotalSeq™-A0424 (Sa14-2), BioLegend 123333, 1:100;CD115 TotalSeq™-A0105 (AFS98), BioLegend 135533, 1:100;Ly-6G/Ly-6C TotalSeq™-A0116 (RB6-8C5), BioLegend 10845910, 1:100;CD192 (CCR2) TotalSeq™-A0426 (SA203G11), BioLegend 150625, 1:100;TotalSeq™-A0301 anti-mouse Hashtag 1 Antibody (M1/42; 30-F11), BioLegend 155801 , 1:100;TotalSeq™-A0302 anti-mouse Hashtag 2 Antibody (M1/42; 30-F11) BioLegend 155803, 1:100;TotalSeq™-A0303 anti-mouse Hashtag 3 Antibody (M1/42; 30-F11) BioLegend 155805, 1:100;TotalSeq™-A0304 anti-mouse Hashtag 4 Antibody (M1/42; 30-F11) BioLegend 155807, 1:100;TotalSeq™-A0305 anti-mouse Hashtag 5 Antibody (M1/42; 30-F11) BioLegend 155809, 1:100;TotalSeq™-A0306 anti-mouse Hashtag 6 Antibody (M1/42; 30-F11) BioLegend 155811, 1:100;TotalSeq™-A0307 anti-mouse Hashtag 7 Antibody (M1/42; 30-F11) BioLegend 155813, 1:100;TotalSeq™-A0308 anti-mouse Hashtag 8 Antibody (M1/42; 30-F11) BioLegend 155815, 1:100; streptavidin BV650, BD 563855, 1:400; 'Home-made' barcoded antibodies: SIRPa_CD172a (P84), GAGTAGCACATAAAA; SiglecH (551), CGTGATTGAAGGAAA; SiglecF (S17007L), CGAAGAGGCCTTAAA; PDL1 (10F), TAGGAATGCTCGAAA; PDCA1_CD137 (17B5), TTCGTACAGTTCAAA; PD1 (RMP1-14), TGCTTCGCATGGAAA; NK1.1 (PK136), AGCAAGCCTCATAAA; MERTK (2B10C42), AGCTGCCACTACAAA; Ly6D (49-H4), CTTGTATGTAGGAAA; IL7Ra_CD127 (A7R34), CGGAGTAGTAATAAA; IA/IE (M5/114.15.2), TGGCTGGCTAGAAAA; Flt3 (A2F10), TAGCCGATCACGAAA; F4/80 (BM8), GTCGCTTAGCACAAA; CX3CR1 (SA011F11), CTCTACTTGCCGAAA; CSF1R_CD115 (AFS98), TTGATCGACCGTAAA; CLEC9A_DNGR1_CD370 (7H11), TGAGCCTCACTTAAA; CLEC12A_CD371 (5D3/CLEC12A), GAACTTCTGGCGAAA; cKit (2B8), CCTCGGATACTAAAA; CD86 (GL-1), ATGTCTAGGTACAAA; CD80 (16-10A1), AGTCATAGCCGCAAA; CD8 (5H10),CCGATCGTATGCAAA; CD74 (In1/CD74), TATACGGACGTGAAA; CD71 (RI7217),TAGGCTGCTTAAAAA; CD69 (H1.2F3), ACGGCTAATCACAAA; CD54 (YN1/1.7.4), GGACATTACCACAAA; CD44 (IM7), CTCAGATCTACCAAA; CD40 (x 03/23), CAGTACGTATTCAAA; CD4 (RM4-5), TGACGTAACACTAAA; CD38 (90), GGCCGAGTCTAAAAA; CD370 (7H11), GATCGTGTTGGCAAA; CD317 (REA818), GTCTGTAGGCATAAA; CD29 (HMB1-1),TTCACTGGCTAAAAA;CD273 (TY25), GACGTGGCCTAAAAA; CD26 (H194-112), GCTTAGCTCGGAAAA; CD24 (M1/69), TAGTGCTAGGCGAAA; CD209a (MMD3), CAATAGCAGCTCAAA; CD205 (NLDC-145), TCTGGAGGACAAAAA; CD16/32 (93), CAGTTGCTCTGAAAA; CD154 (MR1), CTCGAGTGAATCAAA; CD14 (Sa14-2), AAGGTGTCAGGCAAA; CD127 (A7R34), CGTACAAGCCACAAA; CD11c (N418), CGTAAGAACCGTAAA; CD11b (M1/70), TCAATTGCGTGCAAA; CD103 (2E7), CCGCGTTACACAAAA; CCR9_CD199 (L053E8), ACCGATCTCAGCAAA; CD62L (MEL-14), AATCGCTCCGGAAAA; Ly6B2 (7/4), TTGTATCTCCACAAA; ESCAM (AF2827), ATAGGTCATGCGAAA; Notch1(HMN1-12), GCTCAGATTAGTAAA; TNF,TCTCTCAAGTCCAAA;Notch2 (HMN2-35), CATACGCGAAGGAAA. All antibodies were obtained from BioLegend, except for Ly6D that was obtained from BD Pharmingen, flt3 that was obtained from eBioscience and ESAM and TNF that were purchased from R&D systems.

Validation

Antibodies were used according to recommendations of the manufacturer. CITEseq antibodies that showed patterned staining were considered as valid.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

All transgenic mice were bred and maintained in the Alexandria Center for Life Sciences West Tower Vivarium in specific pathogen-free conditions.C57BL/6 mice (Jax# 000664), Batf3-/-(B6.129S(C)-Batf3tm1Kmm/J #Jax 013755), Itgavf/f (B6.129P2(Cg)-Itgavtm2Hyn/J Jax# 032297, CD45.1 mice (B6.SJL-Ptprca Pepcb/BoyJ, Jax# 002014), CD4-Cre (Tg(Cd4-cre)1Cwi/BfluJ, Jax# 017336), CD11c-Cre (B6.Cg-Tg(Itgax-cre)1-1Reiz/J #Jax 008068), Ccr7-/-(B6.129P2(C)-Ccr7tm1Rfor/J, Jax# 006621), I-ABf/f (B6.129X1-H2-Ab1tm1Koni/J #Jax 013181), Zbtb46-Cre (B6.Cg-Zbtb46tm3.1(cre)Mnz/J #Jax 028538), Zbtb46-eGFP (B6.129S6(C)-Zbtb46tm1.1Kmm/J #Jax 027618), tdTomatoLSL (B6;129S6-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J #Jax 007908), Airef/f (B6.Cg-Airetm1Dfil/J #Jax 031409), Nur77-eGFP (C57BL/6-Tg(Nr4a1-EGFP/cre)820Khog/J #Jax 016617), CD90.1 (B6.PL-Thy1a/CyJ #Jax 000406), RORgt-Cre, Hh7-2tg, BAC-transgenic Rorc(t)-GfpTG, huLangerin (CD207)-DTA, Ccr7f/f, mKate2LSL, I-AB lox-STOP-lox, Aire-DTR, H2-DMa1f/f ,Itgb8-tdTomato, Adig, Female and male were used equally in th study. Mice 6-12 weeks old were used for experiments. All mice were housed in a 6am-6pm light on-off cycle facility with a temperature of 18-24 degree C and humidity maintained between 30-70%.

Wild animals

This study did not involve wild animals

Field-collected samples

This study did not involve samples collected from the field

Ethics oversight

All animal procedures were performed in accordance with protocols approved by the Institutional Animal Care and Usage Committee of New York University School of Medicine.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cell isolation: After removal of caecal patches, large intestine tissues were sequentially treated with PBS containing 1mM DTT at room temperature for 10min, twice with 5mM EDTA at 37°C for 10min to remove epithelial cells, and then minced and dissociated in digestion buffer (RPMI containing collagenase (1mg ml−1 collagenase D; Roche), DNase I (100µg ml−1; Sigma), dispase (0.1U ml−1; Worthington) and 10% FBS) with constant stirring at 37°C 55min. Leukocytes were collected at the interface of a 40%/80% Percoll gradient (GE Healthcare). Lymph nodes were mechanically disrupted for lymphocyte isolation. For isolation of myeloid cells and ILC, lymph nodes were mechanically disrupted with digestion buffer with constant stirring at 37°C 30min. staining: Live/dead fixable blue (ThermoFisher) was used to exclude dead cells. For transcription factor staining, cells were stained for surface markers, followed by fixation and permeabilization before nuclear factor staining according to the manufacturer's protocol (Foxp3 staining buffer set from eBioscience).
Instrument	Flow cytometric analysis was performed on an LSR II (BD Biosciences) or an Aria II (BD Biosciences)
Software	We used FACSDiva™ software to collect the data , and performed analyzes using FlowJo software (Tree Star).
Cell population abundance	Sort purity was determined to be 95% by running post sort sample.
Gating strategy	Hh7-2 gating: FSC, SSC; Live Dead-,singlets, Dump- (B220,TCRgd, Ly6G), MHCII-, CD4+, TCRb+, VB6+, CD45.1+ or CD90.1+ cDC gating: FSC, SSC; Live Dead-,singlets, Dump- (B220,TCRgd, Ly6G), CD11c+ and CD11b+ , SIRPa low-moderate (remove CD11c-, SIRPA high) cDC2 gating: FSC, SSC; Live Dead-,singlets, Dump- (B220,TCRgd, Ly6G), CD11c+ and CD11b+ , SIRPa low-moderate (remove CD11c-, SIRPA high), Clec12a- SIRPa migratory cDC2 gating: FSC, SSC; Live Dead-,singlets, Dump- (B220,TCRgd, Ly6G), CD11c+ and CD11b+ , SIRPa low-moderate (remove CD11c-, SIRPA high), Clec12a- SIRPa, PDL2+ Lti-like ILC3 gating: FSC, SSC; Live Dead-,singlets, Dump- (B220,TCRgd, Ly6G), TCRb- , CD90+, Il7R+, RORgt+, CCR6+, CD25+ Ncr1+ ILC3 gating: FSC, SSC; Live Dead-,singlets, Dump- (B220,TCRgd, Ly6G), TCRb- , CD90+, Il7R+, RORgt+, CCR6-, Ncr1+

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.