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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Sequencing data was collected using Nextseq 500, Illumina. Flow cytometry data were collected using FACSCelesta flow cytometer, BD.
Data analysis	Data analysis used software: FlowJo, LLC, https://www.flowjo.com/ (FlowJo_V10) GraphPad Prism, GraphPad Software, Inc, https://www.graphpad.com/scientific-software/prism/ (GraphPad Prism 8) Tophat, Kim et al., 2013, https://ccb.jhu.edu/software/tophat/index.shtml SAMtools, Li et al., 2009, http://www.htslib.org R Statistical Computing Software, The R Foundation, https://www.r-project.org/ ESAT,Derr et al. 2016, https://github.com/garber-lab/ESAT DESeq2, Love et al., 2014, https://bioconductor.org/packages/release/bioc/html/DESeq2.html EdgeR, Robinson and Oshlack, 2010, https://bioconductor.org/packages/release/bioc/html/edgeR.html fastICA, Hyvärinen and Oja, 2000, https://cran.r-project.org/web/packages/fastICA/index.html Rtsne, Van Der Maaten, 2014, https://cran.r-project.org/web/packages/Rtsne/index.html Monocle, Trapnell et al., 2014, https://bioconductor.org/packages/release/bioc/html/monocle.html kknn, Samworth, 2012, https://cran.r-project.org/web/packages/kknn/index.html HOMER, Heinz S, 2010, http://homer.ucsd.edu/homer/index.html GSEA, Aravind S, 2005, https://genepattern.broadinstitute.org/gp/pages/index.jsf DEBrowser, Kucukural A, 2019, https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-018-5362-x.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Accession codes and links related with sequencing data in this study was provided as follows:
Datasets can be found under SuperSeries GSE122326 at
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE97727.
GSE97727: CD94- and CD94+ NK cell bulk and single cell RNA--Seq
GSE122324: CD94-CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells RNA-Seq
GSE122325: CD94-CD56dim NK cells, 1o stim and 5 day culture RNA-Seq
GSE122548: CD94-CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells ATAC-Seq
GSE122549: CD94-CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells CUT&RUN
Figures associated with raw data:
Fig. 2g; Fig. 3a-f; Fig. 4b-g; Fig. 5a-h; Fig. 6a-c and i; Fig. 7b-f; Extended Data Fig. 3a-c; Extended Data Fig. 4a-d; Extended Data Fig. 5a,c; Extended Data Fig. 6d.

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.

[X] 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

Sample size was determined based on previous studies of similar nature. No statistical methods were used to predetermine sample sizes.

Data exclusions

For PFA-fixed and paraffin-embedded rectosigmoid tissues blocks from UCSF, samples with small tissue size or high variability in tissue autofluorescence were blindly excluded, the submucosa and tunica muscularis were blindly excluded.
It is common for highly vascularized tissues that are collected by biopsy to contain residual RBC, when fixed with formalin, for histologic evaluation, do autofluoresce. Thus, when this occurs, in lieu of quantifying the entire mucosa, we set exclusion regions to ensure the RBCs, and other autofluorescent regions of the tissue, are not included in the quantification. In addition, because tissue sections, depending on the position of the tissue in the block and the residual amount of tissue in the block, do vary in the amount of tissue in any given section, and because the confidence in tissue quantification increases with the number of total cells quantified (ie the denominator), we excluded samples from quantification with a low number of total mucosal cells.

Replication

Flow cytometry experiments were replicated using 3-113 independent donors for each treatment, condition, marker or cell subset; Bulk and single cell RNA-Seq experiments were replicated using 2-4 independent donors; ATAC-Seq and CUT&RUN experiments were replicated using 2 independent donors. RT-PCR data were replicated using 3-4 independent donors. Histology experiments were replicated using 8-16 independent donors. The number of donors and repeat times of specific figure were included in the figure legend, all attempts at replication were successful.

Randomization

Leukopaks used to PBMCs were randomly picked. Samples of HIV-1 negative and positive individuals were randomly pull out from the storage.

Blinding

Samples from University of California, San Francisco SCOPE Cohort were tested blindly, sample information was known from Translational Medicine Core of the University of Massachusetts Center for AIDS Research Cohort, when samples were picking up or obtained.

Behavioural & social sciences study design

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

Study description

Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).

Research sample

State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.

Sampling strategy

Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to

Sampling strategy	*predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

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

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access and import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

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
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

Anti-Human CD3 Biolegend Cat# 317306 Clone: OKT3 (FITC) (1:200 dilution)
Anti-Human CD4 Biolegend Cat# 317408 Clone: OKT4 (FITC) (1:200 dilution)
Anti-Human TCRγ/δ Biolegend Cat# 331208 Clone: B1 (FITC) (1:200 dilution)
Anti-Human CD19 Biolegend Cat# 302206 Clone: HIB19 (FITC) (1:200 dilution)
Anti-Human CD20 Biolegend Cat# 302304 Clone: 2H7a (FITC) (1:200 dilution)
Anti-Human CD22 Biolegend Cat# 363508 Clone: S-HCL-1 (FITC) (1:200 dilution)
Anti-Human CD14 Biolegend Cat# 325604 Clone: HCD14 (FITC) (1:200 dilution)
Anti-Human CD16 Biolegend Cat# 302030 Clone: 3G8 (PerCP) (1:200 dilution)
Anti-Human CD16 Biolegend Cat# 980104 Clone: 3G8 (APC) (1:400 dilution)
Anti-Human CD16 Biolegend Cat# 302028 Clone: 3G8 (PerCP/Cy5.5) (1:200 dilution)
Anti-Human CD34 Biolegend Cat# 343504 Clone: 581 (FITC) (1:200 dilution)
Anti-Human CD94 Biolegend Cat# 305504 Clone: DX22 (FITC) (1:200 dilution)
Anti-Human CD94 Biolegend Cat# 305506 Clone: DX22 (PE) (1:200 dilution)
Anti-Human CD94 Biolegend Cat# 305516 Clone: DX22 (PE/Cy7) (1:200 dilution)
Anti-Human CD94 Biolegend Cat# 305514 Clone: DX22 (PerCP/Cy5.5) (1:200 dilution)
Anti-Human CD94 Biolegend Cat# 305508 Clone: DX22 (APC) (1:200 dilution)
Anti-Human FcεR1α Biolegend Cat# 334608 Clone: AER-37 (FITC) (1:200 dilution)
Anti-Human CD1a Biolegend Cat# 300104 Clone: HI149 (FITC) (1:200 dilution)
Anti-Human CD11c Biolegend Cat# 301604 Clone: 3.9 (FITC) (1:200 dilution)
Anti-Human CD123 Biolegend Cat# 306014 Clone: 6H6 (FITC) (1:200 dilution)
Anti-Human BDCA1 Biolegend Cat# 354208 Clone: 201A (FITC) (1:200 dilution)
Anti-Human TCRα/β Biolegend Cat# 306706 Clone: IP26 (FITC) (1:200 dilution)
Anti-Human B220 Biolegend Cat# 103206 Clone: RA3-6B2 (FITC) (1:200 dilution)
Anti-Human RORγT eBioscience Cat# 12-6988-82 Clone: AFKJS-9 (PE) (1:50 dilution)
Anti-Human RORγT eBioscience Cat# 17-6988-82 Clone: AFKJS-9 (APC) (1:50 dilution)
Anti-Human TBX21 eBioscience Cat# 25-5825-82 Clone: ebio4B10 (PE/Cy7) (1:200 dilution)
Anti-Human EOMES eBioscience Cat# 12-4877-42 Clone: WD1928 (PE) (1:200 dilution)
Anti-Human GATA3 eBioscience Cat# 25-9966-42 Clone: TWAJ (PE/Cy7) (1:200 dilution)
Anti-Human CRTH2 Biolegend Cat# 350110 Clone: BM16 (APC) (1:200 dilution)
Anti-Human CRTH2 Biolegend Cat# 350116 Clone: BM16 (PerCP/Cy5.5) (1:200 dilution)
Anti-Human IL-22 eBioscience Cat# 12-7229-42 Clone: 22URTI (PE) (1:200 dilution)
Anti-Human IFN-γ Biolegend Cat# 502512 Clone: 4S.B3 (APC) (1:200 dilution)
Anti-Human IFN-γ Biolegend Cat# 502509 Clone: 4S.B3 (PE) (1:200 dilution)
Anti-Human IFN-γ Biolegend Cat# 502528 Clone: 4S.B3 (PE/Cy7) (1:200 dilution)
Anti-Human CD2 Biolegend Cat# 309207 Clone: TS1/8 (PE) (1:200 dilution)
Anti-Human CD6 Biolegend Cat# 313906 Clone: BL-CD6 (PE) (1:200 dilution)
Anti-Human CD44 Biolegend Cat# 338806 Clone: BJ18 (APC) (1:200 dilution)
Anti-Human CD44 Biolegend Cat# 338808 Clone: BJ18 (PE) (1:200 dilution)
Anti-Human CD45 BD Cat# 560178 Clone: 2D1 (APC/H7) (1:200 dilution)
Anti-Human CD56 Biolegend Cat# 318306 Clone: HCD56 (PE) (1:200 dilution)
Anti-Human CD56 Biolegend Cat# 318310 Clone: HCD56 (APC) (1:200 dilution)
Anti-Human CD56 Biolegend Cat# 318322 Clone: HCD56 (PerCP/Cy5.5) (1:200 dilution)
Anti-Human CD56 Biolegend Cat# 318332 Clone: HCD56 (APC/Cy7) (1:200 dilution)
Anti-Human CD57 Biolegend Cat# 359623 Clone: HNK-1 (PE/Cy7) (1:200 dilution)
Anti-Human CD62L Biolegend Cat# 304805 Clone: DREG-56 (PE) (1:200 dilution)
Anti-Human CD62L Biolegend Cat# 304810 Clone: DREG-56 (APC) (1:200 dilution)
Anti-Human CD62L Biolegend Cat# 304822 Clone: DREG-56 (PE/Cy7) (1:200 dilution)
Anti-Human CD107a Biolegend Cat# 328619 Clone: H4A3 (APC) (1:200 dilution)
Anti-Human CD107a Biolegend Cat# 328607 Clone: H4A3 (PE) (1:200 dilution)
Anti-Human CD117 Biolegend Cat# 313213 Clone: 104D2 (PerCP/Cy5.5)
Anti-Human CD117 Biolegend Cat# 313206 Clone: 104D2 (APC) (1:200 dilution)
Anti-Human CD127 Biolegend Cat# 351316 Clone: A019D5 (APC) (1:200 dilution)
Anti-Human CD127 Biolegend Cat# 351304 Clone: A019D5 (PE) (1:200 dilution)
Anti-Human CD127 Biolegend Cat# 351320 Clone: A019D5 (PE/Cy7) (1:200 dilution)
Anti-Human Granzyme K Biolegend Cat# 370503 Clone: GM26E7 (Alexa Fluor 647) (1:200 dilution)
Anti-Human Granzyme K Biolegend Cat# 370513 Clone: GM26E7 (PerCP/Cy5.5) (1:200 dilution)

Anti-Human NKG2A MACS Cat# 130-098-813 Clone: REA110 (PE) (1:200 dilution)
Anti-Human FGFBP2 Biolegend Cat# 346603 Clone: TDA3 (PE) (1:200 dilution)
Anti-Human KIR2DL1 Biolegend Cat# 339505 Clone: HP-MA4 (PE) (1:200 dilution)
Anti-Human KIR2DL2/L3 Biolegend Cat# 312613 Clone: DX27 (PerCP/Cy5.5) (1:200 dilution)
Anti-Human KIR3DL1 Biolegend Cat# 312719 Clone: DX9 (PE/Cy7) (1:200 dilution)
Anti-Human TCF7 Cell Signaling Cat# 14456 Clone: C63D9 (PE) (1:200 dilution)
Anti-Human CXCR3 Biolegend Cat# 353705 Clone: G025H7 (PE) (1:200 dilution)
Anti-Human CXCR3 Biolegend Cat# 353708 Clone: G025H7 (APC) (1:200 dilution)
Anti-Human CXCR6 Biolegend Cat# 356006 Clone: K041E5 (APC) (1:200 dilution)
Anti-Human CXCR6 Biolegend Cat# 356003 Clone: K041E5 (PE) (1:200 dilution)
Anti-Human CXCR6 Biolegend Cat# 356009 Clone: K041E5 (PerCP/Cy5.5) (1:200 dilution)
Anti-Human Ki67 Biolegend Cat# 350519 Clone:Ki-67 (PerCP/Cy5.5) (1:200 dilution)
Anti-Human Ki67 Biolegend Cat# 350514 Clone:Ki-67 (APC) (1:200 dilution)
Anti-TCF7 Cell Signaling Cat#2203S Clone: C63D9
Anti-Human Annexin V ebioscience Cat# 88-8007-74 (APC) (1:200 dilution)
Anti-Human Annexin V Biolegend Cat# 640936 (PerCP/Cy5.5) (1:200 dilution)

Validation

No customized antibodies were used. All antibodies used to detect human proteins were validated in human, please refer to manufacture's website for validation details, antibody profiles and relevant citations.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

K562 and Jurkat cells were purchased from ATCC.

Authentication

Cell lines were not independently authenticated by our lab.

Mycoplasma contamination

Cells were confirmed for mycoplasma negative by mycoplasma detection kit (Lonza. Cat#LT27-318)

Commonly misidentified lines
(See ICLAC register)

Cell lines used in this study was not in commonly misidentified lines list of ICLAC database.

Palaeontology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

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

Laboratory animals

For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.

Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics

Age and gender of participants were deidentified by cohort provider.

Recruitment

All human blood and colon samples were collected from participants who had provided written informed consent for protocols

Recruitment	that included study of cellular immunity in HIV-1 infection, in accordance with procedures approved by the University of California, San Francisco (UCSF) Institutional Review Boards (clinical information were described in Supplementary Table 1) and the University of Massachusetts Medical School (UMMS) (clinical information were described in Supplementary Table 2). Routine screening colonoscopy was scheduled as medically indicated at UMMS. HIV-1- control individuals undergoing colonoscopy the same day were matched for gender and age. No selection were used in participants recruitment. For PFA-fixed and paraffin-embedded rectosigmoid tissues blocks from UCSF, samples with small tissue size or high variability in tissue autofluorescence were blindly excluded, the submucosa and tunica muscularis were blindly excluded (please see the rational above), therefore any relevent selection bias should be excluded.
Ethics oversight	University of Massachusetts Medical School

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	GSE122549: CD94-CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells CUT&RUN For reviewers: To review GEO accession GSE122549: Enter token adyhowaipbuvhib into the box.
Files in database submission	GSM3473668 cut and run sample1 CD94-CD56dim Rabbit IgG GSM3473669 cut and run sample1 CD94-CD56dim H3K4me1 GSM3473671 cut and run sample1 CD94-CD56dim H3K4me3 GSM3473672 cut and run sample1 CD94-CD56dim TCF7 GSM3473674 cut and run sample1 CD94+CD56dim Rabbit IgG GSM3473675 cut and run sample1 CD94+CD56dim H3K4me1 GSM3473677 cut and run sample1 CD94+CD56dim H3K4me3 GSM3473678 cut and run sample1 CD94+CD56dim TCF7 GSM3473680 cut and run sample1 CD94+CD56hi Rabbit IgG GSM3473681 cut and run sample1 CD94+CD56hi H3K4me1 GSM3473683 cut and run sample1 CD94+CD56hi H3K4me3 GSM3473684 cut and run sample1 CD94+CD56hi TCF7 GSM3473686 cut and run sample2 CD94-CD56dim Rabbit IgG GSM3473687 cut and run sample2 CD94-CD56dim H3K4me1 GSM3473689 cut and run sample2 CD94-CD56dim H3K4me3 GSM3473690 cut and run sample2 CD94-CD56dim TCF7 GSM3473692 cut and run sample2 CD94+CD56dim Rabbit IgG GSM3473693 cut and run sample2 CD94+CD56dim H3K4me1 GSM3473695 cut and run sample2 CD94+CD56dim H3K4me3 GSM3473696 cut and run sample2 CD94+CD56dim TCF7 GSM3473698 cut and run sample2 CD94+CD56hi Rabbit IgG GSM3473699 cut and run sample2 CD94+CD56hi H3K4me1 GSM3473701 cut and run sample2 CD94+CD56hi H3K4me3 GSM3473702 cut and run sample2 CD94+CD56hi TCF7 GSE122549_RAW.tar TDF
Genome browser session (e.g. UCSC)	To review GEO accession GSE122549: enter token adyhowaipbuvhib into the box.

Methodology

Replicates	Sorted CD94-CD56dim, CD94+CD56dim, and CD94+CD56hi NK cells were processed as described94–96. Cells were lysed in nuclear extraction buffer (20 mM HEPES-KOH, pH 7.9; 10 mM KCL; 0.5 mM spermidine; 0.1% Triton X-100; 20% glycerol).

Nuclei were precipitated by centrifugation and then resuspended and bound to Bio-Mag Plus Concanavalin A coated beads (Polysciences, cat# 86057), and incubated for 5 mins in blocking buffer (20 mM HEPES, pH 7.5; 150 mM NaCl; 0.5 mM spermidine; 0.1% BSA; 2 mM EDTA). Then nuclei were incubated overnight at 4°C with the following antibodies at 1:100 dilution: rabbit anti-human IgG (Control, abcam, cat# ab2410), rabbit anti-H3K4me1 (diagnode, cat# c15410194), rabbit anti-H3K27ac (diagnode, cat# c15410196), rabbit anti-H3K4me3 (diagnode, cat# c15410003), rabbit anti-TCF7 (cell signaling technology, cat# 2203S), or rabbit anti-H3K27me3 (diagnode, cat# c15410195). Protein A-micrococcal nuclease fusion protein, a gift from Thomas Fazzio, was added at 600 ug/ml for 1 hr at 4oC. CaCl2 was added to a final concentration 2 mM to activate the micrococcal nuclease, and 2X stop buffer (200 mM NaCl; 20 mM EDTA; 4 mM EGTA; 50 ug/ml RNase A; 40 ug/ml glycogen) was added 30 min later. Supernatant containing the released chromatin was subjected to pheno-chloroform-isoamyl extraction and DNA was precipitated with ethanol. The sequencing library was constructed according to the NEBNext Ultra II DNA library Prep kit for Illumina-based sequencing (NEB, cat#7645L), using NEBNext Multiplex Oligos (NEB, cat# E6609S). For donor 1, primers D1-D6 were used to amplify each antibody-enriched DNA preparation from CD94-CD56dimNK cells according to the antibody order mentioned above. Accordingly, primers D7-D12 were used for sorted CD94+CD56dimNK cells, and primers E1-E6 were used for sorted CD94+CD56hiNK cells. For donor 2, primers E7-E12 were used for sorted CD94-CD56dimNK cells, primers F1-F6 were used for sorted CD94+CD56dim NK cells, and primers F7-F12 were used for sorted CD94+CD56hiNK cells. Libraries were paired-end sequenced on a Nextseq 500 V2 (Illumina) using 45 cycles Read 1, 8 cycles Index 1, and 32 cycles Read 2.

Sequencing depth

The sequencing depth of CUT&RUN was around 5-10 million paired end reads for each sample. Since the CUT&RUN typically requires ~1/10th the sequencing depth as ChIP-Seq (Peter J Skene, 2017, eLife), the sequencing depth in this study was sufficient to generate high quality data for downstream analysis.

Antibodies

Anti-Human IgG	abcam Cat# ab2410
Anti-H3K4me1	diagnode Cat# c15410194
Anti-H3K4me3	diagnode Cat# c15410003
Anti-TCF7	Cell Signaling Cat#2203S Clone: C63D9

Peak calling parameters

For CUT&RUN, paired-end reads were removed where the average quality scores in window size 10 are less then 15 and trimmed where leading and trailing bases with quality scores less than 15 using trimmomatic version 0.32. Reads that were longer than 25 bases after trimming were kept for further analysis. The reads were then aligned to human reference genome hg19 using Bowtie2 with options --un-conc to filter out reads that align un-concordantly. Duplicated reads were filtered out using Picard's MarkDuplicates version 0.32. Peaks 26 were then called using MACS2. Alignment files were also converted to tdf format using IGVtools count function version 2.3.31 using -w 5 parameter.

Data quality

The methods to ensure data quality was mentioned in "Peak calling parameters" section (see above).

Software

Software used for CUT&RUN was listed in "Software and Code" section (see above).

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

Live and dead cells were discriminated using the Live and Dead violet viability kit (Invitrogen, L-34963). For cell surface molecule detection, the cells were resuspended in antibody-containing MACS buffer for 30-60 min at 4°C in the dark. To detect cytokine production, cells were stimulated with the indicated cytokines for 16 hrs, or with PMA and ionomycin (cell stimulation cocktail 00-4970-03, ebioscience) for 3-6 hrs. In both cases, protein transport inhibitor (00-4980-03, eBioscience) was present during the stimulation. For intracellular staining of transcription factors or cytokines, cells were fixed and permeabilized using Foxp3 staining buffer kit (eBioscience) and target intracellular molecules were stained as for surface staining.

Instrument

FACSCelesta flow cytometer, BD

Software

FlowJo, LLC, https://www.flowjo.com/
BD FACS Diva

Cell population abundance

The abundance of enrichment of sorted cells were checked after sorting before performing further experiment, the enrichment of sorted cells were above 99%.

Gating strategy | Specific gating strategy for indicated figure was described in figure legend.

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

Magnetic resonance imaging

Experimental design

Design type | *Indicate task or resting state; event-related or block design.*

Design specifications | *Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*

Behavioral performance measures | *State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s) | *Specify: functional, structural, diffusion, perfusion.*

Field strength | *Specify in Tesla*

Sequence & imaging parameters | *Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*

Area of acquisition | *State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software | *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*

Normalization | *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*

Normalization template | *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal | *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring | *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings | *Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*

Effect(s) tested | *Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016) | *Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*

Correction | *Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.