nature portfolio

Corresponding author(s): Erdinc Sezgin
Luca A. Andronico

Last updated by author(s): Jul 30, 2026

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	Flow cytometry data for cell phenotyping and cell sorting were acquired using BD FACSDiva software (v8.0.1 and v9.0.1, respectively). Confocal microscopy images were acquired using Zeiss Zen software (v2012 SP5). RNA-sequencing libraries were sequenced on an Illumina NextSeq 2000 platform using instrument-native software. For Proximity Network Analysis (PNA), sequencing libraries generated using the Pixelgen Proxiome kit (PROXIMM001) were sequenced on a DNBSEQ T7RS platform (v5.1 chemistry) using BCS software (v1.5.1.324). No proprietary or unpublished software was used for raw data acquisition other than the instrument-native software packages listed above.

Data analysis	Plasma membrane order quantification from microscopy images was performed using the previously published open-source software VISION (https://doi.org/10.1242/jcs.262166). NK-cell migration was analyzed using the open-source TrackMate plugin for FIJI (v1.54). Flow cytometry data were processed and analyzed using FCS Express 7 (DeNovo Software, v7.28.0035), including UMAP dimensionality reduction analyses. Statistical analyses and data visualization for PM-order measurements, flow-cytometry data, migration assays and killing assays were performed using GraphPad Prism 10 (v10.6.1).Transcriptomic analyses employed bcl2fastq (v2.20.0.422) for demultiplexing, FastQC (v0.12.0) for quality control, CutAdapt (v3.5) for adapter trimming, STAR (v2.7.9a) for alignment, featureCounts (v1.5.1) for read quantification, and DESeq2 (v1.46.0) within R (v4.4.3) for differential expression analysis. Data visualization and downstream analyses were performed using pcaExplorer (v3.0.0), ggplot2 (v3.5.1), pheatmap (v1.0.12), GSEA (v4.4.0), clusterProfiler (v4.14.6), pathview (v1.46.0), and Cytoscape (v3.10.4) with the EnrichmentMap (v3.5.0) and AutoAnnotate (v1.5.2) plugins.Proximity Network Analysis (PNA) sequencing data were processed using the Pixelator pipeline (nf-core/pixelator v2.3.1) and analyzed using pixelatorR (v0.16.0), Seurat (v5.2.1), Harmony (v1.2.3), ggplot2 (v3.5.2), and ComplexHeatmap (v2.22.0). Custom code used for transcriptomic analyses is available at https://github.com/CSI-Nano-Lab/GP_RNAseq. Figures were assembled using Inkscape (v1.4.4), and schematics were created using BioRender. The analysis pipeline used to analyse transcriptomic data is available at the GitHub repository https://github.com/CSI-Nano-Lab/GP_RNAseq. All data associated with this manuscript are publicly available in the Figshare repository (DOI: 10.17044/scilifelab.31882975)

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

All data associated with this manuscript are publicly available in the FigShare repository (DOI: 10.17044/scilifelab.31882975) and in the Supporting Information.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Not Applicable
Reporting on race, ethnicity, or other socially relevant groupings	Not Applicable
Population characteristics	The study included PBMCs from 14 healthy donors, 14 Long COVID patients, and 5 patients with chronic lymphocytic leukaemia (CLL). Healthy donors ranged from 27-44 years of age. Long COVID participants had PCR-confirmed SARS-CoV-2 infection with persistent symptoms and/or organ dysfunction lasting more than 12 weeks and ranged from 35-64 years of age. CLL patients had early-stage untreated disease and were sampled 3 months after their second COVID-19 vaccination. No additional demographic, genotypic, treatment, or clinical covariate information was collected. No additional demographic variables (e.g., sex) or genotypic information were collected, as the primary aim of the study was methodological development and validation rather than covariate-based subgroup analyses.
Recruitment	Healthy donor samples were obtained from the Karolinska University Hospital biobank, while peripheral blood samples from individuals with Long COVID and chronic lymphocytic leukaemia (CLL) were provided by clinical collaborators based on sample availability. Participants were therefore not randomly selected, and recruitment was based on availability and willingness to contribute samples, which may introduce selection bias. No additional demographic or clinical metadata (e.g., age, sex, or other clinical covariates) were collected. Because the primary aim of this study was to establish and validate a methodology for profiling plasma membrane order in immune cells rather than to perform population-level or stratified clinical analyses, such potential biases are unlikely to affect the main methodological conclusions, although they may limit the generalizability of findings to broader patient populations.
Ethics oversight	The study was approved by the Swedish Ethical Review Authority (Etikprövningsmyndigheten; Dnr 2021-03293). All participants provided informed consent.

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

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	No statistical methods were used to prospectively predetermine sample size. Instead, sample sizes were determined by the availability of donor material and the primary aim of the study, which was to establish and validate a methodological framework for profiling plasma membrane order in human immune cells. A total of 13 healthy donors, 13 individuals with Long COVID, and 5 patients with Chronic Lymphocytic Leukaemia were included, reflecting the number of high-quality samples that could be obtained from the corresponding clinical sources during the study period. Because the objective was to demonstrate proof-of-concept rather than to perform population-level comparisons or derive clinical associations, these sample sizes were considered sufficient to show technical feasibility, robustness of the measurement pipeline, and reproducibility of membrane-order phenotyping across diverse immune cell types.
Data exclusions	No data were excluded from the analyses
Replication	All key experimental findings were reproduced across independent biological samples. Donors were analyzed in batches on different days to account for potential inter-day variability in sample handling, staining and instrument performance. Plasma membrane order measurements, immune-cell phenotyping, NK-cell biophysical sorting, migration assays, killing assays, transcriptomic profiling and proximity network analyses were performed using independently collected donor samples (n ≥ 2 independent samples per experiment). Repeated experiments yielded consistent qualitative trends. Standardized calibration procedures, including daily flow cytometer voltage normalization using DOPC-coated beads and BD CS&T beads, were used to ensure technical consistency across acquisition days. No major discrepancies were observed between independent experiments.
Randomization	Samples were not randomly allocated to experimental groups. Group membership was determined by predefined biological or clinical criteria, including donor status (healthy donor, Long COVID, or CLL) and objective experimental measurements such as plasma membrane order or cell-surface marker expression. For functional assays, NK-cell subsets were isolated using predefined sorting gates based on plasma membrane order (highest and lowest percentiles of the distribution). Because group assignment was based on intrinsic sample characteristics rather than investigator-directed intervention, randomization was not applicable to this study. Standardized sample processing, staining, acquisition, and analysis procedures were used across all groups to minimize potential confounding effects.
Blinding	Investigators were not blinded to group allocation during data collection or analysis. Blinding was not considered necessary because group assignment was based on objective experimental measurements (e.g., plasma membrane order and cell-surface marker expression) rather than subjective assessment. Data acquisition and analysis were performed using standardized instrument settings, predefined gating strategies, automated image-analysis workflows, and established bioinformatic pipelines, minimizing the potential for observer bias.

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 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 & 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 and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Panel for PBMCs from healthy donors and patients: PBMCs were stained with anti-Human CD45-FITC (clone HI30, BioLegend, cat. no. 982316, 1:400 dilution), CD3-BV605 (clone SK7, BD Biosciences, cat. no. 563219, 1:100 dilution), CD8-PE-FIRE700 (clone SK1, BioLegend cat. no. 344766, 1:200 dilution), CD14-BV711 (clone M5E2, BioLegend, cat. no. 301838, 1:200 dilution), CD19-PE-Cy7 (clone SJ25C1, BioLegend, cat. no. 363012, 1:200 dilution), CD56-APC-R700 (clone NCAM16.2, BD Biosciences, cat. no. 565140, 1:200 dilution), CD11c-PE (clone Bu15, BioLegend, cat. no. 980602, 1:200 dilution), FVS660 (BD Biosciences, cat. no. 564405, 1:1,3x104 dilution) for cell viability. Panel for NK sorting and killing assay: Enriched NK cells were stained with CD3-PerCP-Cy5.5 (clone SK7, BioLegend, cat. no. 344808, 1:3000 dilution), CD56-PE (clone HCD56, BioLegend, cat. no. 318306, 1:150 dilution) and FVS780 (BD Biosciences, cat. no. 565388, 1:3x104 dilution) for cell viability. After incubation with K562 tumour cells, sorted NK cells were stained with CD56-PE (1:200 dilution), CD107a-Alexa Fluor647 (clone H4A3, BioLegend, cat. no. 328612, 1:400 dilution) and FVS780 (1:2000 dilution) for cell viability. Panel for apoptosis study: PBMCs from healthy donors were stained with CD3-PerCP-Cy5.5 (1:400 dilution), CD56-PE (1:200 dilution), CD19-PE-Cy7 (1:400 dilution), Annexin-V-Alexa Fluor647 (Thermofisher, cat. no. A23204, 1:100 dilution) and FVS780 (1:2000 dilution) for cell viability. Panel for assessing the effect of freezing-thawing on PM order: PBMCs from healthy donors were stained with CD3-PerCP-Cy5.5 (1:70 dilution), CD56-PE (1:150 dilution), CD19-PE-Cy7 (1:150 dilution), CD11c-APC-R700 (clone 3.9, BD Biosciences, cat. no. 566609, 1:150 dilution), CD14-Alexa Fluor647 (clone M5E2, BioLegend, cat. no. 301812, 1:150 dilution), CD8-BV605 (clone SK1, BD Biosciences, cat. no. 564116, 1:150 dilution), Lactadherin-FITC (Prolytix, cat. no. BLAC-FITC, 1:100 dilution) and FVS780 (1:100 dilution) for cell viability.

NK cells were isolated by negative magnetic selection according to manufacturer protocol using a MojoSort human NK cell isolation kit (cat. no.480054; BioLegend), whereas mouse anti-human CD107a Alexa Fluor 647 (BD Bioscience, clone H4A3, 2 µL/well) was uised during degranulation assay.

For migration assay, NK cells were stained with CellTrace Violet (CTV, ThermoFisher, cat. no. C34571, 1µM)

For Proximity Network Analysis the Pixelgen Proxiome Kit (PROXIMM001) was used for staining of surface proteins.

Validation

All primary antibodies used in this study were commercially obtained and validated by the manufacturers for the reported applications. Validation data, including specificity testing and recommended applications, are available in the corresponding manufacturers' datasheets and product webpages.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The CEM/C1 cell line (ATCC, cat. no. CRL-2265) were used during optimization of Pro12A staining, and the K562 tumor cells (ATCC, cat. no. CCL-243) were used for the killing assay.

Authentication

None of the cell lines were authenticated.

Mycoplasma contamination

Cell lines were tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

Not Applicable

Palaeontology and Archaeology

Specimen provenance

Not Applicable

Specimen deposition

Not Applicable

Dating methods

Not Applicable

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

Ethics oversight

Not Applicable

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

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

For laboratory animals, report species, strain 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 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.

Reporting on sex	*Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*
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.

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.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks: Not Applicable

Novel plant genotypes: Not Applicable

Authentication: Not Applicable

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.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session
(e.g. UCSC)

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

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

Immune staining: Frozen PBMCs were thawed by adding a 10-fold volume of RPMI-1640 (Sigma-Aldrich) supplemented with 10% FBS (Sigma-Aldrich) and Benzonase Nuclease (10 units/mL, Sigma-Aldrich), pelleted at 400 g for 8 minutes, resuspended in 5 mL medium and allowed to recover. After 1h minimum recovery, PBMCs were pelleted at 400 g for 6 minutes and resuspended in Staining Buffer (eBioscience, ThermoFisher) at a concentration of 3x106 cells/mL. Aliquots of 200 µL (i.e., 6x105 cells) in 0.5 mL Eppendorf were used to prepare single stained controls and multi-colour samples for flow cytometry. Optimal volumes of antibodies were obtained from calculation of staining indexes, and each aliquot was stained with either single antibody (for single stained controls) or a cocktail of antibodies. For panels containing more than one Brilliant Violet

(BV) dye, 30 µL of Brilliant Stain Buffer (ThermoFisher) was added to prevent dye aggregation. Cells were incubated at 4 ℃ in the darkness for 30 minutes, washed 2x with PBS (Gibco™, pH 7.4) and resuspended in 300 µL. Then, cells were stained with 1 µL of Fixable Viability Stain 660 (BD Bioscience, stock solution: 5 ng/µL in DMSO) at room temperature for 15 minutes, washed 2x in PBS and resuspended to a final volume of 400 µL in the flow tubes.

Sorting of NK: Before sorting, NK cells were enriched from PBMCs. Specifically, primary human NK cells were isolated by negative magnetic selection according to manufacturer protocol using a MojoSort human NK cell isolation kit (480054; BioLegend). The purity of enriched cells was >90%. Cells were counted (BioRad TC20) and checked for viability using trypan blue staining. After pre-enrichment, NK cells were stained with the specific antibodies cocktail and sorted based on their PM order. Cell sorting was performed on a BD FACSAria™ Fusion Flow Cytometer (BD Biosciences) equipped with 5 lasers, utilizing an 85 µm nozzle and running on FACS DIVA software.

Killing assay: sorted NK cells were resuspended in complete PRMI medium (1,2x106 live cells/mL), transferred in a V-bottom 96-well plates and co-cultured with K562 cells (ATCC) at 1:1 effector-to-target ratio in the presence of mouse anti-human CD107a Alexa Fluor 647 (BD Bioscience, clone H4A3, 2 µL/well) for 6 hours at 37°C. To block intracellular protein transport, BD GolgiStop (BD Bioscience) was added at a final concentration of 1:1000 during the assay after 1 hour of incubation. Once the 6 hours of incubation were completed, cells were washed twice in PBS + 2% FBS and stained with a cocktail of antibodies as described above for 30 minutes at 4°C in the dark, followed by two washes in PBS and staining with fixable Viability Stain (final concentration 1:1000) for 15 minutes at room temperature. Stained cells were washed in PBS once, stained with Pro12A and analysed in flow cytometry.

Instrument

Immune cells phenotyping was performed using a BD LSRFortessa 16-colour or a FACSCanto II Flow Cytometer whereas NK cell sorting was performed using a BD FACSAria™ Fusion Flow Cytometer

Software

Flow cytometry data during cell phenotyping and cell sorting were acquired using BD FACSDiva software (v8.0.1 or v9.0.1)

Cell population abundance

After CD56, CD14 and CD3 staining, pre-enriched NK cells showed a purity above 90% before sorting, (i.e. negative for CD3 and CD14 but positive for CD56).

Gating strategy

Flow cytometry data were analyzed by first gating on singlets, non-debris, and live cells negative for the viability dye. After these initial steps, further gating was performed according to the specific markers that define each cell population. Monocytes-derived Dendritic cells dim for CD11c (Mo-DCs (dim)) were identified as $CD3^-CD14^+CD11c^+$, while Monocytes-derived Dendritic cells bright for CD11c (Mo-DCs (bright)) were defined as $CD3^-CD14^{++}CD11c^+$. Dendritic cells dim for CD11c (DCs (dim)) were gated as $CD3^-CD14^-CD56^-CD19^-CD11c^+$, whereas Dendritic cells bright for CD11c (DCs (bright)) were defined as $CD3^-CD14^-CD56^-CD19^-CD11c^{++}$. B cells were identified as $CD3^-CD14^-CD56^-CD19^+$. NK cells dim for CD56 (NK cells (dim)) corresponded to $CD3^-CD14^-CD19^-CD56^+CD8^-$, and NK cells bright for CD56 (NK cells (bright)) to $CD3^-CD14^-CD19^-CD56^{++}CD8^-$. $CD8^+$ NK cells were defined as $CD3^-CD14^-CD19^-CD56^+CD8^+$. Within the T-cell compartment, $CD8^-$ T cells were gated as $CD3^+CD14^-CD56^-CD8^-$, and Cytotoxic T cells ($CD8^+$ T cells) as $CD3^+CD14^-CD56^-CD8^+$. Finally, NK-like T cells were defined by $CD3^+CD14^-CD56^+CD8^-$, and $CD8^+$ NK-like T cells by $CD3^+CD14^-CD56^+CD8^+$.

☒ 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

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See Eklund et al. 2016)

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.