nature portfolio

Corresponding author(s): Bhanu Priya Ganesh

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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: NA

Data analysis: NA

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

https://figshare.com/s/9355fa3158556b1458e9 (all the metabolomics data sets, IHC data sets, 16S rRNA sequencing data sets)
Metabolic workbench - metabolomics data sets are deposited under the ID: 4638 (In progress)

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	All participants provided written informed consent for the study and the subsequent use of the stored samples. The exclusion criteria for our study were patients younger than 18 years of age, patients with acute or chronic anemia requiring transfusion of ≥1 unit of packed red blood cells, patients with active cancer, and prisoners. We restricted our murine studies to male mice. Both male and female samples were included in the human studies, and all human female samples included in this study were from postmenopausal women.
Reporting on race, ethnicity, or other socially relevant groupings	Stroke (n=60) Controls (n=64) p value Demographics characteristics Sex, Female, n (%) 22 (36.6%) 21 (32.8%) 0.70a Age, mean± SD, y 62.38 ±13.24 58.29 ±14.88 0.25b Race, ethnicity n (%) 0.43c White/Caucasian 33 (55%) 42 (65.6%) Black/ African American 15 (25%) 8 (12.5%) Asian 3 (5%) 2 (3.1%) Other 9 (15%) 12 (18.7%) Hispanic (Other/White) 15 (25%) 14 (21.8%)
Population characteristics	Smoking (past or currently) 25 (41.6%) 8 (12.5%) 0.0003a Missing 5 (7.8%) Medical history, n (%) 0.76c Hypertension 53 (88.3%) 34 (53.1%) Missing 3 (4.68%) Diabetes 30 (50%) 13 (20.3%) Missing 4 (6.2%) Hypercholesterolemia 38 (63.3%) 23 (35.9%) Missing 4 (6.2%) Atrial fibrillation 13 (21.6%) 9 (14%) Missing 4 (6.2%)
Recruitment	ll participants provided written informed consent for the study and the subsequent use of the stored samples. The exclusion criteria for our study were patients younger than 18 years of age, patients with acute or chronic anemia requiring transfusion of ≥1 unit of packed red blood cells, patients with active cancer, and prisoners.
Ethics oversight	The use of Postmortem Human samples was deemed exempt by the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston (HSC-MS-22-0982 – UTHealth Neuropathology Core). Postmortem human brain tissue was obtained from the Neuropathology Core of the Alzheimer's Disease Research Center (ADRC) at the University of Pittsburgh. For the use of human samples for the metabolomics studies, the protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at Houston, Houston, Texas, USA (IRB No: HSC-MS-17-0452, HSC-MS-20-0780). All participants provided written informed consent for the study and the subsequent use of the stored samples. The exclusion criteria for our study were patients younger than 18 years of age, patients with acute or chronic anemia requiring transfusion of ≥1 unit of packed red blood cells, patients with active cancer, and prisoners.

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	The number of mice in each experiment (all Aims) will provide 80% power to detect (≥ 20%) differences, and these group sizes are sufficient and necessary for the decisive interpretation of the data (SPSS Sample Power; power of .8 and α of .05). This was determined with the help of preliminary data

Data exclusions	Animals that did not pass stroke surgery (NDS score) was excluded from the experiment
Replication	All the experiment mentioned in the manuscript were repeated more than 2 time independently for reproducibility of the data sets and sent to metabolomics together to minimize the handling error
Randomization	Since these animals (WT mice) were obtained from national institute of aging at an age of 18 months, they were housed for 3 weeks for microbiome changes to b e normalized and used evenly in the experiments. Similarly all the germ free mice used in the study were provided by Baylor college of Medicine germ free facility who controls for age and generation. All of the animals provided originated from the same facility and we randomly split the animals to maintain equal numbers between the groups and used them in our study. We used spare animals in case we encounter more animal loss during the surgery process since these animals were aged.
Blinding	All the experiments were blinded. The person who performed the stroke surgeries never took part in treatment and the treatment were given to animals by research technician who is blinded from the animals groups. The analysis of behavioral data sets were performed by an individual who did not take part in both surgery or treatment study design. Flow cytometry was performed using nomenclature that involves only numbers and after the analysis the team was unblinded just prior to statistical analysis.

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	Flow cytometry: antibodies pre-conjugated with fluorophores (BioLegend): CD45-eF450 (eBioscience, Cat#: 48-0451-82, Lot: 2005853), CD11b-APC (BioLegend, Cat#: 101212, Lot: B279418), Ly6CPerCP-Cy5.5 (BioLegend, Cat#: 128011, Lot: 292026), Tmem119-PE-Cy7 (eBioscience, Cat#: 25-6119-82, Lot: 2210260), P2RY12-PE (BioLegend, Cat#: 848003, B298459), and MHCII-APC-Fire750 (BioLegend, Cat#: 107652, Lot: B301025) pre-conjugated antibodies and Zombie Aqua (BioLegend, Cat#: 423102, Lot: B300004). For intracellular staining, cells were fixed and permeabilized (BioLegend, Cyto-Fast Fix/Perm kit, Cat#: 750000133) following the manufacturer's protocol. A pre-conjugated AHR-BV421 antibody (BD Horizon, Cat# 565791) was used for intracellular staining. Data were acquired using a Cytoflex-S cytometer (Beckman Coulter) and analyzed using FlowJo software (Treestar Inc.) IHC: IHC of formalin-fixed, paraffin-embedded human brain sections was performed as described previously66,67. Briefly, after deparaffinization, the sections were subjected to heat-induced antigen retrieval process by heating the samples (citrate buffer, pH 6.0; 99 °C; 20 min), then blocked with blocking buffer (5% donkey normal serum, 1% bovine serum albumin with 0.3% Triton X-100 in phosphate buffered saline [PBS]) for 1 h at room temperature. The sections were incubated with primary antibodies followed by detection using secondary antibodies. Primary antibody : AAhR: nsj Bioregents, cat#: RQ4534 (dilution 1:500); Iba1: Fujifilm, cat#: 013-27593; Mouse monoclonal antibody (dilution 1:200) followed by secondary antibody:Alexa Fluor 488-conjugated donkey anti-rabbit and Alexa Fluor 647-conjugated donkey anti-mouse (dilution 1:200, Jackson ImmunoResearch).
Validation	Every antibody was used to be validated with tissues containing no microglia for its specificity. Most of the microglial specific markers were validated. However, Ahr receptor antibody is validated using Ahr knock out mouse tissue or cells. Various dilution of primary antibody was performed before we choose the appropriate dilution for IHC.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HMC3 cells were maintained in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin in a humidified incubator at 37 ºC and 5% CO2. Fischer Scientific Cat No#: NC1278248 (ATCC Frozen HMC3 cell lines). The HMC3 cell line was established through SV40-dependent immortalization of a human fetal brain-derived primary microglia culture. Cells were purchased from Creative Biolabs HUMAN MICROGLIA CELL LINE HMC3, from Fischer Scientific
Authentication	The cells were purchased from the company and after 3 passages they were used in the experiment. We did not perform repeated passage. we did not genotype them.

Mycoplasma contamination	We performed mycoplasma contamination test at every step of our study. We are very sure that all the cell lines used in the study were mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

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). Permits should encompass collection and, where applicable, export.*
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.

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.

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	Mus Musculus, C57Bl6, WT strain - 3-months and 18 months of age, Germ free mice - 12 months of age.
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	All animals used were males
Field-collected samples	All animals, except germ-free (GF) mice, were group-housed in Tecniplast individually ventilated cage (IVC) racks, fed a commercially available irradiated, balanced mouse chow (no. 5058, LabDiet, St Louis, MO, USA), and provided corncob bedding. Animal rooms were maintained at 21–23 °C, under a 12:12-h light:dark cycle. All WT animals were maintained under specific pathogen-free conditions. All GF animals were maintained under sterile environment. The crates were assembled, bedded with Alpha-Dri bedding and autoclaved with lids closed. Mice were removed from the isolator in the gnotobiotic facility using sterile transfer bags, housed in shipping crates in a biosafety cabinet, and immediately transported. The two medical schools are only a few hundred yards apart, which precludes lengthy and stressful travel. To assess the "germ-free" status of the mice upon arrival at the UTHSCH, fecal samples were collected to confirm the absence of bacteria in the gut. Transient focal ischemia was induced under isoflurane anesthesia in young, aged, or GF mice for 60 min by occlusion of the right middle cerebral artery according to a previously described protocol71. Body temperature was maintained at 37.0 ± 1.0 °C throughout the surgery by an automated temperature control feedback system (TC1000, mouse, CWE Inc., USA). A midline ventral neck incision was made and unilateral MCAO was performed by inserting a Doccol monofilament (Doccol Corp, Redlands, CA, USA) into the right internal carotid artery. One hour after ischemia, the animals were re-anesthetized and reperfusion was established by withdrawal of the monofilament. The mice were then placed in recovery cages and euthanized 24 h after reperfusion. Sham controls were subjected to the same procedure, except that the sutures were not introduced into the middle cerebral artery. Animals were randomly assigned to the stroke and sham surgery groups, individually housed in their recovery cages for the first 2 h after surgery, and then returned to group housing. All analyses were performed by investigators who were blinded to the surgical conditions. Sham and stroke mice were housed separately to minimize the effects of microbiota contamination between experimental groups. Five mice were excluded from the study, because of either death during MCAO surgery (n=2), subarachnoid hemorrhage (n = 1), or no significant intra-ischemic neurological deficits (NDS = 0) after stroke (n = 2).
Ethics oversight	Animal experiments were performed according to the guidelines of the National Institute of Health, and all experiments were approved by the Institutional Animal Care and Use Committees of the University of Texas Health Science Center at Houston (UTHSCH) and the Baylor College of Medicine. All animal procedures were performed at an Association for Assessment and Accreditation of Laboratory Animal Care-accredited (AAALAC-accredited) facility and approved by the Animal Welfare Committee at the University of Texas Health Science Center in Houston, TX, USA."

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	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	Blood (mouse) Blood was drawn via cardiac puncture using heparinized needles. Red blood cell lysis was achieved by two consecutive 10-min incubations with Tris-ammonium chloride (Stem Cell Technologies). Brain (mouse) Mice were transcardially perfused with 60mL cold sterile PBS prior to aseptic removal of the spleen and brain tissues. Brain tissue was placed in complete Roswell Park Memorial Institute medium 1640 (Lonza) and mechanically and enzymatically digested in Collagenase/Dispase (1 mg/mL) and DNase (10 mg/mL; Roche Diagnostics) for 45 min at 37 °C. The cell suspension was filtered through a 70 µm filter. Leukocytes were harvested from the interphase of a 70-to-30% Percoll gradient. Skull bone marrow (mouse) To isolate the skull bone marrow, the meninges were gently peeled from the skull cup. The skull was then cut into small pieces using sterile scissors and mechanically dissociated in PBS, followed by filtration through a 70-µm cell strainer. After centrifugation at 500 g for 5 min, red blood cells were removed by adding 1 mL of ammonium chloride (Stem Cell Technologies, Cat #07850) lysis buffer for 2 min at room temperature, centrifugation at 500 g for 5 min, followed by resuspension of the cell pellet in PBS until use. Spleen (mouse) Spleen (whole) tissue was removed after PBS perfusion and passed through a 70 µm strainer and ammonium chloride solution (Stem Cell Technologies, Cat #07850) in 9:1 ratio was used for red blood cell lysis. Surface and intracellular staining Cells were washed and blocked with mouse FcR Block (BioLegend) before staining with antibodies pre-conjugated with fluorophores (BioLegend): CD45-eF450 (eBioscience, Cat#: 48-0451-82, Lot: 2005853), CD11b-APC (BioLegend, Cat#: 101212, Lot: B279418), Ly6CPerCP-Cy5.5 (BioLegend, Cat#: 128011, Lot: 292026), Tmem119-PE-Cy7 (eBioscience, Cat#: 25-6119-82, Lot: 2210260), P2RY12-PE (BioLegend, Cat#: 848003, B298459), and MHCII-APC-Fire750 (BioLegend, Cat#: 107652, Lot:

B301025) pre-conjugated antibodies and Zombie Aqua (BioLegend, Cat#: 423102, Lot: B300004). For intracellular staining, cells were fixed and permeabilized (BioLegend, Cyto-Fast Fix/Perm kit, Cat#: 750000133) following the manufacturer's protocol. A pre-conjugated AHR-BV421 antibody (BD Horizon, Cat# 565791) was used for intracellular staining. Data were acquired using a Cytoflex-S cytometer (Beckman Coulter) and analyzed using FlowJo software (Treestar Inc.). No less than 100,000 events were recorded for each sample/tissue. Cell type-matched fluorescence minus one (FMO) and unstained controls were used as gating strategies. Uniform Manifold Approximation and Projection (UMAP) plots were generated in FlowJo using the DownSample v3 plug-in (3,000 Live CD45+ cells per sample), followed by the UMAP v3.1 algorithm on all uncompensated parameters (except viability) using Euclidean distances, 15 nearest neighbors, a minimum distance of 0.5, and two components. Following the UMAP analysis, the Phenograph v3.0, plug-in was used with a K value of 174 (recommended by FlowJo) to generate 20 clusters of immune populations. Surface expression heatmaps for CD80, MHC-II, CD11b, P2RY12, and Tmem119 for all clusters are provided in Supplementary Fig 6). The identification markers for each cluster are shown in Supplementary Fig 7 of teh manuscript file).
Cell sorting
Single-cell suspension and surface staining were performed as described in the preceding section. After viability and singlet selection, MG gated as Live Tmem119+ (verified to be CD45intCD11b+) was sorted under an aseptic hood from a single-cell suspension prepared from naïve aged male brains (full brains, n=8) using BD FACSMelody. Sorting was performed from the tube directly into a 96-well plate for ex vivo experiments to preserve the cell counts. The cells were then washed with PBS, stained for surface markers and viability after OGD/R, and analyzed using flow cytometry.

Instrument: Cytoflex-S cytometer (Beckman Coulter), BD FACSMelody

Software: FlowJo software (Treestar Inc.)

Cell population abundance: No less than 100,000 events were recorded for each sample/tissue. Cell type-matched fluorescence minus one (FMO) and unstained controls were used to aid in gating strategy. tSNE plots were generated in FlowJo using DownSample plug-in (3,000 Live CD45+ cells per sample, i.e., 12,000 Live CD45+ for each of the six study groups) followed by tSNE algorithm on all compensated parameters (except viability and CD45) at 1,000 iterations, perplexity of 30, learning rate of 5040, and Barnes-Hut gradient algorithm

Gating strategy: Flow Cytometry: No less than 100,000 events were recorded for each sample/tissue. Cell type-matched fluorescence minus one (FMO) and unstained controls were used as gating strategies. Briefly, the bivatiat plots of forward scatter versus side scatter were used to select for particle size and granularity. Subsequently, forward scatter area versus length was used to identify and exclude the blood particles. Next gait was to select for viable cells using Zombie Aqua staining, negatively stained particles were selected as live singlet cells. Following the selection of the live cells, a plot of CD45 versus CD11b was used to select the classical gates of the myeloid populations in the brain. Intermediate staining of CD45 versus high staining of CD11b was determined as the microglia population, confirmed by double positive staining of P2RY12 and TMEM119. Additionally, microglia gait was confirmed to be negative for staining of Ly6C to exclude monocytic contaminations. High levels of CD45 staining along with high CD11b staining was identified as infiltrating myeloid cells a population comprised of monocytes, macrophages, dendritic cells. Lastly, on the same plot of CD45 versus CD11b, the CD45 high population with low CD11b staining was identified as an initial gait for the lymphocytes. B and T lymphocytes were subsequently identified from the initial lymphocyte gate using CD19 and CD3 pan markers, respectively.
Uniform Manifold Approximation and Projection (UMAP) plots were generated in FlowJo using the DownSample v3 plug-in (3,000 Live CD45+ cells per sample), followed by the UMAP v3.1 algorithm on all uncompensated parameters (except viability) using Euclidean distances, 15 nearest neighbors, a minimum distance of 0.5, and two components. Following the UMAP analysis, the Phenograph v3.0, plug-in was used with a K value of 174 (recommended by FlowJo) to generate 20 clusters of immune populations. Surface expression heatmaps for CD80, MHC-II, CD11b, P2RY12, and Tmem119 for all clusters are provided in Supplementary Fig 6). The identification markers for each cluster are shown in Supplementary Fig 7).
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☒ 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