

Reporting Summary

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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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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

The following softwares were used to collect the data in this study:

- MetaMorph v7.10.1.161, Molecular Devices, LLC
- Olympus Fluoview v4.2a

Data analysis

The following softwares were used to analyze the data in this study:

- FIJI image processing software (NIH) - v2.0.0-rc-59/1.51n
- Prism v8.3.0 (GraphPad Software, Inc)
- FastQC v0.11.5
- Salmon v0.8.2
- R v3.5.0
- MATLAB 9.7
- Cytobank 7.2
- Bioconductor DESeq2 v3.5
- FlowJo software v10.4.1 (Tree Star, Inc.)
- Custom code used for bulk, single-cell and single-nucleus RNA-seq analysis is available from the corresponding authors upon request.

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

Source data files depicting the quantification values mentioned in the text or plotted in graphs shown in Figs. 1-3 and Extended Data Figs. 1-10, and the gene lists used for gene-set analysis in Table 1 and Table 2 are available in the online version of this paper at <http://www.nature.com/nature>. New RNA-seq data sets have been deposited online in the Gene Expression Omnibus (GEO database) under the accession number GSE141917. Previously published RNA-seq data sets can be found under the accession numbers GSE99743 and GSE104181. Custom code used to analyze the RNA-seq data and datasets generated and/or analyzed in the current study are available from the corresponding authors upon request.

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	Sample sizes were chosen on the basis of standard power calculations (with $\alpha = 0.05$ and power of 0.8) performed for similar experiments that were previously published. In general, statistical methods were not used to re-calculate or predetermine sample sizes.
Data exclusions	The ROUT test was used to identify and discard potential outliers. No exclusion criteria were pre-established. An outlier was identified and excluded in Extended Data Fig. 3e, in the Visudyne group (the outlier is indicated in the correspondent source data file).
Replication	Number of reliable reproductions of each experimental finding is stated in each Figure legend. Unless stated otherwise, main experimental findings were replicated at least once. Experimental replication was not attempted for negative data.
Randomization	Reported in Methods section, Statistical analysis and reproducibility subsection: Animals from different cages, but within the same experimental group, were selected to assure randomization.
Blinding	Reported in Methods section, Statistical analysis and reproducibility subsection: Experimenters were blinded to the identity of experimental groups from the time of euthanasia until the end of data collection and analysis for all the independent experiments.

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

The following primary antibodies were used for IHC:

- primary antibodies: rat anti-LYVE-1-eFluor660 or anti-LYVE-1-Alexa Fluor®488 (eBioscience, clone ALY7, 1:200), Armenian hamster anti-CD31 (Millipore Sigma, MAB1398Z, clone 2H8, 1:200), goat anti-IBA1 (Abcam, ab5076, polyclonal, 1:200), rat anti-CD68 (BioLegend, 137002, clone FA-11, 1:100), rat anti-LAMP-1 (Abcam, ab25245, clone 1D4B, 1:300), rabbit anti-fibrinogen (Dako, A0080, polyclonal, 1:200), rabbit anti-amyloid beta1-37/42 (Cell Signaling, 8243S, clone D54D2, 1:400) in PBS-T containing 0.5% BSA.

- secondary antibodies: rat, chicken, goat or donkey eFluor570 or Alexa Fluor® 488, 594, or 647 conjugated anti-rat, -goat, -rabbit, -mouse or -Armenian hamster IgG antibodies (Thermo Fisher Scientific, all at 1:500 in PBS-T).

The following antibodies were used for flow cytometry or fluorescence-activated cell sorting:

- meningeal lymphatic endothelial cell sorting: anti-CD45-BB515 (clone 30-F11, BD Biosciences), anti-CD31-Alexa Fluor® 647 (clone 390, BD Biosciences) and anti-Podoplanin-PE (clone 8.1.1, eBioscience) all at 1:200 in FACS buffer.
 - enrichment for brain myeloid (microglia/macrophage), blood endothelial and mural cells by cell sorting: anti-CD16/32 (101302, BioLegend); anti-CD13-FITC (558744, BD Biosciences), anti-Ly6G-PE (127608, BioLegend), anti-CD11b PerCP-Cy5.5 (550993, BD Biosciences), anti-CD31-APC (17-0311-80, eBioscience) and anti-CD45-APC-Cy7 (103116, BioLegend), all at 1:200 in FACS buffer, except for anti-CD13 (at 1:50).
 - enrichment for brain cortical myeloid cells by cell sorting: anti-CD16/32 (101302, BioLegend); anti-CD11b-PerCP-Cy5.5 (550993, BD Biosciences), anti-CD45-A700 (560510, BD Biosciences) and anti-Ly6G-BV421 (562737, BD Biosciences), all at 1:200 in FACS buffer.

The following antibodies were used for mass cytometry:

- anti-Ly6C-Nd-142 (clone HK1.4, Maxpar Ready, Biolegend, conjugated in-house), anti-CD169-Sm-147 (clone H1.2F3, Maxpar Ready, conjugated in-house), anti-XCR1-Eu-153 (clone ZET, BioLegend, conjugated in-house), anti-Siglec-H-Gd-160 (clone 551, Biolegend, conjugated in-house), anti-FcER1-Dy-161 (clone MAR-1, Maxpar Ready, conjugated in-house), anti-PD-1-Er-166 (clone CHBC0116081, R&D systems, conjugated in-house), anti-H-2Kb/Db-Yb-173 (clone 28-8-6, BioLegend, conjugated in-house), anti-CCR2-FITC (clone 475301, R&D Systems), anti-Thy1.2-PE (clone 30-H12, eBioscience), anti-CD45-Yb-89 (clone 30-F11, Fluidigm 3089005B), anti-CD11b-Nd-143 (clone M1/70, Fluidigm 3143015B), anti-FITC-Nd-144 (clone FIT22, Fluidigm 3144006B), anti-CD4-Nd-145 (clone RM4-5, Fluidigm 3145002B), anti-F4/80-Nd-146 (clone BM8, Fluidigm 3146008B), anti-Ly6G-Nd-148 (clone 1A8, Maxpar Ready, conjugated in-house), anti-CD19-Sm-149 (clone 6D5, Fluidigm 3149002B), anti-CD24-Nd-150 (clone M1/69, Fluidigm 3150009B), anti-CD64-Eu-151 (clone X54-5/7.1, Fluidigm 3151012B), anti-CD3e-Sm-152 (clone 145-2C11, Fluidigm 3152004B), anti-Ter119-Sm-154 (clone Ter119, Fluidigm 3154005B), anti-PE-Gd-156 (clone PE001, Fluidigm 3156005B), anti-CD103-Dy-162 (clone FIB504, Fluidigm 3162026B), anti-CD14-Dy-163 (clone M14-23, Biolegend, Ultra-LEAF, conjugated in-house), anti-CD62L-Dy-164 (clone MEL-14, Fluidigm 3164003B), anti-CD8a-Er-168 (clone SK1, Fluidigm 3168002B), anti-TCRb-Tm-169 (clone H57597, Fluidigm 3169002B), anti-NK1.1-Er-170 (clone PK136, Fluidigm, 3170002B), anti-CD44-Yb-171 (clone IM7, Fluidigm 3171003B), anti-CD86-Yb-172 (clone GL1, Fluidigm 3172016B), anti-I-A/I-E-Yb-174 (clone M5/114.15.2, Fluidigm 3174003B), anti-CD127-Lu-175 (clone A7R34, Fluidigm 3175006B), anti-B220-Yb-176 (clone RA3-682, Fluidigm 3176002B), anti-CD11c-Bi-209 (clone N418, Fluidigm 3209005B), anti-TNF-Pr-141 (clone MP6-XT22, Fluidigm 3141013B), anti-FOXP3-Gd-158 (clone FJK-16s, Fluidigm 3158003A) anti-RORgt-Tb-159 (clone B2D, Fluidigm 3159019B), anti-T-bet-Ho-165 (clone 4B10, Maxpar Ready, conjugated in-house) and anti-GATA3-Er-167 (clone TWAJ, Fluidigm 3167007A).

Validation

Each antibody was validated for the species (mouse or human) and application (immunohistochemistry, flow cytometry/cell sorting and mass cytometry) by the correspondent manufacturer. The usage was described in full detail the methods section of the manuscript.

Immunohistochemistry:

Tissue was rinsed in PBS and incubated with PBS 0.5% Triton X-100 (Thermo Fisher Scientific, PBS-T) for 30 min, followed by PBS-T containing 0.5% of normal serum (either goat or chicken) or 0.5% bovine serum albumin (BSA) for 30 min at room temperature (RT). This blocking step was followed by incubation with appropriate dilutions of primary antibodies (from the list described in the previous section) in PBS 0.5% Triton-X-100 containing 0.5% BSA overnight at 4°C. Whole mounts or tissue sections were then washed 3 times for 5 min at RT in PBS 0.5% Triton-X-100 followed by incubation with the appropriate secondary antibodies (from the list described in the previous section) for 1 hour at RT in PBS 0.5% Triton-X-100.

Flow cytometry:

Antibodies for flow cytometry or fluorescence-activated cell sorting (FACS) were purchased from BD Biosciences, eBioscience and BioLegend and used at a concentration of 1:200 unless recommended otherwise by the manufacturer, or optimized for usage at a different concentration. Briefly, the cell pellets were washed, resuspended in ice-cold FACS buffer (pH 7.4; 0.1 M PBS; 1 mM EDTA and 1% BSA), preincubated for 10 min at 4°C with Fc-receptor blocking solution (anti-CD16/32, 101302, BioLegend, 1:200 in FACS buffer) and stained for extracellular markers with a mix of primary antibodies from the list described in the previous section, for 25 minutes at 4°C.

Meningeal lymphatic endothelial cell sorting:

Briefly, after tissue digestion, cell suspensions were pelleted and resuspended in ice-cold FACS buffer containing anti-CD45-BB515 (1:200, BD Biosciences), anti-CD31-Alexa Fluor® 647 (1:200, BD Biosciences) and anti-Podoplanin-PE (1:200, eBioscience) for 15 minutes at 4°C.

Sorting of whole brain hemisphere myeloid (microglia/macrophage), blood endothelial and mural cells or of brain cortical myeloid cells:

Briefly, after tissue digestion, the cell pellets were washed, resuspended in ice-cold FACS buffer, preincubated for 10 min at 4°C with Fc-receptor blocking solution (anti-CD16/32, 101302, BioLegend, 1:200 in FACS buffer) and stained for extracellular markers with a mix of primary antibodies from the list described in the previous section, for 25 minutes at 4°C.

Mass cytometry:

Metal isotope-conjugated antibodies for mass cytometry were purchased from Fluidigm. Alternatively, purified antibodies were purchased from BioLegend and conjugated in-house to the appropriate metal isotopes using Maxpar antibody conjugation kits from Fluidigm, following the manufacturer's protocol. Antibodies were used at an appropriate concentration, based on recommendation by the manufacturer, or established upon optimization. All antibody concentrations can be provided by the authors upon request.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Adult (2–3 months-old) male C57BL/6J wild type (WT) mice were purchased from the Jackson Laboratory (JAX stock #000664, Bar Harbor, Maine, USA). Aged male (20–24 months-old) WT mice were provided by the National Institutes of Health/National Institute on Aging (Bethesda, MD, USA). All mice were maintained in the animal facility for habituation for at least one week prior

to the start of the experiment. Male hemizygous B6.Cg-Tg(APP^{SwFlon},PSEN1^{*M146L}*L286V)6799Vas/Mmjax (5x^{FAD}, JAX stock #008730), B6.Cg-Tg(PDGFB-APP^{SwInd})20Lms/2Mmjax (J20, JAX stock #006293) and B6.Cg-Tg(APP⁶⁹⁵)3Dbo (APP^{swe}, JAX stock #005866) were purchased from the Jackson Laboratory and bred in-house on a C57BL/6J background. In-house bred male transgene carriers and non-carrier (WT) littermates were used at different ages. 5x^{FAD} mice were used at ages ranging from 2-14 months; J20 mice were used at ages ranging from 14-16 months; APP^{swe} mice were used at ages ranging from 22-30 months. The genotype and age of mice from different strains are indicated in figure schemes or legends throughout the manuscript. Male mice were used in all the different experiments. Mice of all strains were housed in an environment with controlled temperature and humidity and on a 12-hour light/dark cycle (lights on at 7:00). All mice were fed with regular rodent's chow and sterilized tap water ad libitum.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All experiments were approved by the Institutional Animal Care and Use Committee of the University of Virginia.

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

Number of samples, brain regions, sex, age of death, proportions of APOE $\epsilon 4$ carriers and post-mortem interval. Demographic characteristics of donor brain samples used in single-nucleus RNA-seq experiments are depicted in Supplementary Table 3.

Recruitment

The Neuropathology Cores of the Charles F. and Joanne Knight Alzheimer's Disease Research Center (Knight-ADRC) and the Dominantly Inherited Alzheimer Network (DIAN) provided the parietal lobe tissue of postmortem brains for each sample. These samples were obtained with informed consent for research use and were approved by the review board of Washington University in St. Louis. AD neuropathological changes were assessed according to the criteria of the National Institute on Aging-Alzheimer's Association (NIA-AA). Their demographic, clinical severity, and neuropathological information are presented in Supplementary Table 3.

Ethics oversight

The brain samples used in the single-nucleus RNA-seq experiments were obtained with informed consent for research use and were approved by the review board of Washington University in St. Louis.

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

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Meningeal tissue for flow cytometry or mass cytometry:

Mice were injected with Euthasol (i.p.) and were transcardially perfused with ice cold PBS with heparin. Individual meninges were immediately dissected from the mouse's skull cap in ice-cold RPMI 1640. The tissues were digested for 20 min at 37°C with 1 mg/mL of Collagenase VIII, 1 mg/mL of Collagenase D and 50 U/mL of DNase I (all from Sigma Aldrich) in RPMI 1640. The same volume of RPMI with 5% FBS (Atlas Biologicals) and 10 mM EDTA (Thermo Fisher Scientific) was added to the digested tissue, which was then filtered through a 70 μ m cell strainer (Fisher Scientific). The cell pellets were washed and resuspended in ice-cold fluorescence-activated cell sorting (FACS) buffer (pH 7.4; 0.1 M PBS; 1 mM EDTA and 1% BSA), followed by incubation with FACS buffer containing the primary antibodies and/or dyes in the appropriate concentrations.

Meningeal tissue for lymphatic endothelial cell sorting:

Mice were euthanized by i.p. injection of Euthasol and transcardially perfused with ice cold PBS with heparin. To obtain a suspension of meningeal lymphatic endothelial cells (LECs), skull caps were quickly collected and meninges (dura mater and arachnoid) were dissected using Dumont #5 and #7 fine forceps in complete media composed of DMEM (Gibco) with 2% FBS (Atlas Biologicals), 1% L-glutamine (Gibco), 1% penicillin/streptomycin (Gibco), 1% sodium pyruvate (Gibco), 1% non-essential amino-acids (Gibco) and 1.5% Hepes buffer (Gibco). Individual brain meninges were then incubated with 1 mL of DMEM with 1 mg/mL of Collagenase VIII (Sigma-Aldrich) and 35 U/mL of DNase I (Sigma-Aldrich) for 15 min at 37°C. Cell suspensions from 10 individual meninges were then pooled into a single tube after filtration through a 70 μ m nylon mesh cell strainer. Suspensions of meningeal LECs were pelleted and resuspended in ice-cold FACS buffer, followed by incubation with FACS buffer containing the primary antibodies and/or dyes in the appropriate concentrations.

Whole brain hemisphere tissue for cell sorting:

Mice were euthanized by i.p. injection of Euthasol and transcardially perfused with ice cold PBS with heparin. The skulls were

collected, and the whole right brain hemisphere was carefully dissected into ice-cold advanced DMEM/F12 (Gibco, 12634010). Brain tissue was mechanically dissociated with sterile sharp scissors and digested for 45 min at 37°C in advanced DMEM/F12 with 1 mg/mL of Collagenase VIII, 50 U/mL of DNase I and 1% FBS. Every 15 min, the tissue suspensions were sequentially passed through a 10 mL serological pipette (10 times), followed by two passages through a 5 mL serological pipette (10 times each). Cellular suspensions were filtered through a 70 µm cell strainer, thoroughly mixed with an equal volume of 22% BSA in PBS and centrifuged at 1,000 × g for 10 min at RT (without break), to remove the myelin. After discarding the upper layer and supernatant containing the myelin and cell debris, the cell pellets were washed with advanced DMEM/F12 supplemented with 10 % FBS, resuspended in ice-cold FACS buffer, preincubated for 10 min at 4°C with Fc-receptor blocking solution and stained for extracellular markers.

Brain cortical tissue for cell sorting:

Mice were euthanized by i.p. injection of Euthasol and transcardially perfused with ice cold PBS with heparin. The skulls were collected, and the brain was carefully extracted into ice-cold HBSS medium (14025, Thermo Fisher Scientific). Under an articulated stereo microscope, pia and choroid plexus tissue contaminants were carefully discarded and the brain cortices were collected by macrodissection. Brain tissue was passed through a 5 mL pipette tip (10 times) and digested for 30 min at 37°C in HBSS with 1 mg/mL of Collagenase VIII, 1 mg/mL of Collagenase D, 50 U/mL of DNase I and 25 µg/mL Actinomycin D to inhibit transcription mediated by all RNA polymerases (all from MilliporeSigma). The same volume of DMEM/F12 (Gibco) with 5% FBS and 10 mM EDTA was added to the digested tissue, which was then passed through a 1 mL pipette tip (10 times) and filtered through a 70 µm cell strainer. The cell pellets were washed, resuspended in ice-cold FACS buffer, preincubated for 10 min at 4°C with Fc-receptor blocking solution and stained for extracellular markers.

Instrument

- Influx™ Cell Sorter (BD Biosciences), available at the University of Virginia Flow Cytometry Core Facility
- Helios Mass Cytometer, available at the University of Virginia Flow Cytometry Core Facility

Software

- FlowJo software v10.4.1 (Tree Star, Inc.)
- Zunder Lab single-cell debarcoder (<https://github.com/zunderlab/single-cell-debarcoder>) and MATLAB 9.7
- Cytobank 7.2

Cell population abundance

For each individual experiment, single-cell suspensions were incubated with viability dyes and aliquots from the unstained single-cell suspensions were incubated with ViaStain™ AOPI Staining Solution (CS2-0106, Nexcelom Bioscience) to provide accurate counts for each sample using Cellometer Auto 2000 (Nexcelom Bioscience). Live meningeal lymphatic endothelial cells (~1500 cells per sample) were directly sorted into a 96-well plate containing 100 µL of RNA extraction lysis buffer using the Influx™ Cell Sorter (BD Biosciences) that is available at the University of Virginia Flow Cytometry Core Facility. This procedure was thoroughly described in previous studies (Da Mesquita et al, Nature 560, 185-191, 2018; Louveau et al, Nat Neurosci 21, 1380-1391, 2018). Analysis of post-sort fractions was not performed, as all cells were collected for further transcriptome analysis by bulk RNA-seq.

Gating strategy

Mass cytometry:

Samples were debarcoded using the Zunder Lab single-cell debarcoder (<https://github.com/zunderlab/single-cell-debarcoder>) in MATLAB (9.7 version) and files uploaded in Cytobank (7.2 version). Raw data were subjected to arcsinh transformation (cofactor = 5), manually gated to exclude debris, doublets, dead cells, normalization beads (191/193Ir_DNA+, 195Pt_Cisplatin-, 140Ce_EQbeads-), corrected for Mahalanobis distance and CD45+ cell events selected (Extended data Fig. 3a).

Meningeal LEC sorting:

Sorting strategy, with representative gating, is described in detail in ref.5 (Da Mesquita et al, Nature 560, 185-191, 2018; Extended Data Fig. 6g).

Briefly, singlets were gated using the pulse width of the side scatter and forward scatter. The live LECs were then gated as DAPI-CD45-CD31+PDPN+ (using the exact same gating strategy as described in a previous publication - ref. 5) and sorted into a 96-well plate containing 100 µL of RNA extraction lysis buffer using the Influx™ Cell Sorter (BD Biosciences) that is available at the University of Virginia Flow Cytometry Core Facility.

Flow cytometry and FACS using brain tissue preparations:

Briefly, singlets were gated using the height, area and the pulse width of the forward and side scatters and then viable cells were selected as being negative for SYTOX or DAPI. Whole brain hemisphere myeloid (microglia/macrophage) cells were identified and sorted as DAPI-CD45+CD11b+Ly6G-. Blood endothelial and mural cells were identified and sorted as DAPI-CD45-CD11b-CD31+ and DAPI-CD45-CD11b-CD13+CD31-, respectively. Brain cortical myeloid cells were identified and sorted as SYTOX-Ly6G-CD45+CD11b+.

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