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Corresponding author(s): Greg Lemke

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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 (*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	Zen Black and Zen Blue editions (ver 2.5) were used for collecting confocal images; Olympus VS-120 Virtual Slide Scanner and VS Desktop software (ver 2.8) was used for collecting thioS-labelled plaque burden data; QuantStudio Q5 was used for collecting qPCR data; TECAN Infinite® 200 PRO reader was used for collecting optical density reading for ELISA and BCA assay; Odyssey Gel Imaging System (Li-Cor) was used for acquired western blots; Microsoft Excel (ver 15.36) was used for compiling collected data; Med Associate Video Freeze system (SOF-843) was used to record and analyze mouse freezing behavior in fear conditioning assays. Single cell RNA Sequencing was conducted on FACS sorted brain immune cells following the user guide on the Next GEM single cell 3' v3.1 protocol. Briefly, single cell suspensions of approximately 14,000-16,000 cells from each sample was directly loaded onto microfluidic chip with barcoded beads to generate Gel Bead-in-Emulsions (GEMs) using 10X Genomics Chromium Single Cell Controller. Reverse transcription of GEMs for first strand cDNA synthesis and cDNA amplification were carried out according to 10X Next GEM single cell 3' v3.1 protocol. Following indexed scRNA-Seq library construction, the final library size distribution was determined using TapeStation (Agilent) and the concentration was measured by a Qubit fluorometer (ThermoFisher). The libraries were pooled in equal molar ratio, quantified by qPCR, and sequenced on Illumina NextSeq500 at 28 cycles for Read 1, 8 cycles for i7 index and 91 cycles for Read 2 at an average sequencing read depth of 33k-41k reads per cell. For more details, see Methods section in manuscript.
Data analysis	Fiji (ver 1.0) was used for all confocal image analysis and for two-photon microscopy stack images and time-lapse recordings; Imaris (ver 9.1.2; Bitplane, Zurich, Switzerland) with build-in MATLAB function was used for two-photon microscopy stack images for microglia and plaques reconstruction, distance analysis as well as intracellular Abeta content; ImageStudio (ver 5.2.5) was used for quantifying western blot data; statistics except for single cell RNAseq data were performed with GraphPad Prism (version 8.0) software. Flowjo (v10) was used for FACS analysis and gating the sorted cell population. For Raw sequencing data were aligned to the reference built using Ensembl primary assembly and annotation (release-93) that included both protein coding genes and polymorphic pseudogenes by cell ranger pipeline (v3.1.0). The resulting filtered gene expression matrix was further analyzed by "Seurat" (3.2.1.9002). Cells that had less than 10% of reads mapped to mitochondria genes and 200-10000 expressed genes were used in the analysis. Samples were integrated by Seurat standard workflow "FindIntegrationAnchors" and "IntegrateData" functions with default settings. After manually examining the Principle Component (PC) elbow plot, the top 25 PCs of the integrated data were used for clustering analysis and Uniform Manifold Approximation and Projection (UMAP)

analysis with default settings. Doublet scores were calculated by R package "scds" (v1.2.3) using the raw count matrix as input. The top 5% cells ranked by hybrid scores and clusters enriched (>50%) with doublets were removed from the downstream analysis. Data was re-scaled and re-clustered after doublet removal. The expression patterns of a large group of well-known immune markers (Supplementary Fig. 3b) were carefully examined to annotate the clusters. A second round of analysis was performed on microglia to further reveal the relationship of cells within the population. The top 20 PCs were used for clustering and UMAP analysis. One cluster that co-expressed both microglia markers (P2ry12 and Fcrls) and T/NK markers (Cd3g and Nkg7) was regarded as doublets and was filtered out. Clustering resolution was set at 0.2 because the clusters highly correlated with the UMAP topologies. Differential Expression (DE) analysis was performed by Seurat function "FindAllMarkers" and "FindMarkers" with default Wilcoxon Rank Sum test and logFC > 0.25 on pooled biological replicates. Genes with Bonferroni adjusted p-value < 0.05 were considered to be significant. When plotted, the adjusted p-value was log10 transformed. Each adjusted p-value was added an extremely small number (1e-310) to avoid infinite values before transformation. Then the value was signed by the up-/down-regulation of the gene. Supplemental Table 7 containing Trem2 positive expression from different stages of disease progression from Keren-Shaul et al. was used as validation dataset. Common symbols between this study and our top 10 DE genes ranked by logFC from each microglia cluster were used for heatmap plotting. Average expression values from both datasets were offset by 1 and log2 transformed and z-scaled. For more details, see Methods section in manuscript.

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 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

We declare that the main data supporting the findings of this study are available within the article and its Supplementary Information files. Sequencing data was deposited at Gene Expression Omnibus with the accession number GSE160523. Additional supporting raw data are available from the authors upon request.

Field-specific reporting

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☒ 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	In general, group sample sizes were chosen based on previous published studies from the Lemke lab and/or mice studies that used similar approaches and/or power analysis based on preliminary data. Biological sample number (denoted with 'n' in the manuscript) 3-8 per genotype per age group per condition was used in most studies. Whenever applicable, sex- and age-matched littermates mice were used as controls. For quantification of immunofluorescence imaging analysis (Mer, Axl, Gas6, LAMP1 and RTN3 mean fluorescence intensity), typically 3-5 field of view per section from 3-5 sections or whole brain tilescan were collected for confocal imaging per experiment from two-three independent experiments as suggested by previous publications (Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Traves, Nimmerjahn, Lemke, Nature 2016; Blanco-Suarez Allen Neuron 2017; Wang, Colonna JEM 2016). Additionally, the cohort effect size is supported by preliminary quantification showed the significant differences with narrow variability among biological groups. For western blot and ELISA quantification, cohort sample size was determined by analysis from preliminary results and previous publications (Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Traves, Nimmerjahn, Lemke, Nature 2016; Zagorska, Traves, Lemke, 2020 Life Science Alliance). For two-photon imaging experiments, multiple recorded volumes (4-7 per animal) and (8-10 per animal) imaging stacks in order to gain representativity within one biological sample and in biological sampling across 3-5 animals per genotype (Tufail, Nimmerjahn, Neuron 2017; Fourgeaud, Traves, Nimmerjahn, Lemke 2016 Nature; Bolmont Calhoun 2008 JNeurosci). 2 biological samples were chosen for non-transgenic WT and Axl-/-Mertk-/- age-matched littermates mice for recording microglia processes motility because 1) this has been extensive studied and published (Nimmerjahn Helmchen 2005 Science; Fourgeaud, Traves, Nimmerjahn, Lemke, Nature 2016), 2) our results from recordings from these mice were consistent with previous publications and 3) averaged processes motility per microglia basis was compared rather than per animal basis (Fig. 4g in the manuscript). For single cell RNAseq, 2 pooled cortices per sample, 2 biological replicates per group in total of 4 samples were used in the experiment (Frigerio, De Strooper 2019 Cell Rep). For behavioral studies, 12-20 mice per genotype were included and was determined by previous studies (Knafo DeFlipe 2009 Journal of Pathology; Suh, Tonegawa 2011 Science) and analysis from preliminary independent trials.
Data exclusions	No data exclusion
Replication	As stated above, wherever applicable, experiments have been repeated for at least two (mostly three) independent experiments with at least three technical replicates per experiments that represent at least 3 biological samples (except for two-photon imaging of only the non-transgenic groups and single cell RNAseq analysis with 2 biological replicates per group). All attempts of replication have been successful with a certain degree of variability.

Randomization

Littermates were used when possible; otherwise age-matched sex-matched mice or human samples were used. Mice of both genders were randomly allocated to experimental and control groups.
For human samples, sex- and age-matched AD (experimental group) and cognitively healthy (control group) postmortem brain sections were sectioned, prepared, randomly assigned by UCSD ADRC neuropathology core, and delivered to the experimenters who performed the IHC experiment labeling human Gas6 in AD plaque-loaded brains. Experimenters were blinded from the diagnosis while performing the experiments.

Blinding

Image analysis was done with the investigator blind with respect to genotypes and in a semi-automated fashion with set intensity thresholds for all conditions whenever applicable. Single cell preparation, sorting and library construction were handled blindly, alternated in group order during independent experiments and clustered in an unsupervised manner during analysis to ensure minimization of experimental error and bias. For behavioral studies, cages were randomized and numbered by an independent personnel and experimenters were blinded from group allocation while performing the behavioral experiments and 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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

Antibodies used were as follows:
anti-Axl (R&D AF854 Lot CTC0214101, polyclonal goat)
anti-Mer (eBioscience lot 4285684 clone: DS5MMER, monoclonal rat)
anti-Mer (R&D AF591, Lot DGS0517061 and Lot DGS0213111, polyclonal goat)
anti-mouse Gas6 (R&D AF986, lot: EFU0314121, polyclonal goat)
anti-human Gas6 (R&D AF885 lot GUS0218061 polyclonal goat)
anti-human-beta-amyloid, 1-16 antibody (Biolegend 803001, clone 6E10)
anti-Iba1 (Wako 019-19741, polyclonal rabbit)
anti-Iba1 (Novus NB100-1028, polyclonal goat)
anti-GFAP (Dako z-334, polyclonal rabbit)
anti-cleaved Casp3 (Cell Signaling 9661, lot 45, polyclonal rabbit)
anti-Tmem119 (Abcam AB209064, lot GR320057-1, monoclonal rabbit)
anti-RTN3 (EMD Millipore ABN1723 lot 3109186 polyclonal rabbit)
anti-LAMP1 (BD Biosciences, Cat# 553792, clone 1D4B, monoclonal rabbit)
anti-Trem2 (R&D systems, AF1729 and BAF 1729, polyclonal sheep)
anti-mouse CD16/32 (Biolegend 101301, rat IgG2a, lambda)
anti-CD45-PE, 2D1 (Invitrogen 12-9459-42, mouse IgG1, kappa)
anti-CD11b-FITC, M1/70 (Invitrogen 11-0112-85, rat IgG2b, kappa)
anti-CD31 (R&D, AF3628, polyclonal goat)
anti-laminin (Sigma, L-9393, polyclonal rabbit)
anti-vGlut1 (Millipore, AB5905, polyclonal guinea pig)
anti-PSD95 (Life Technologies, 51-6900, polyclonal rabbit)
anti-GAPDH (Millipore MAB374, clone 6C5, monoclonal mouse)
JRF AB042/26 for Abeta42 capture antibody and detection antibody JRF/AbN/25, unlabeled (made in-house from Janssen Pharmaceuticals)

Secondary antibodies for immunohistochemistry were fluorophore-conjugated
anti-rat (712- 545- 153 or 712- 165- 153 from Jackson ImmunoResearch),
anti-goat (A-11056 from Life Technologies, or 705-166-147 from Jackson ImmunoResearch),
anti-rabbit (A-11071 or A-21206 from Life Technologies),
anti-sheep (A21098 from Thermo Fisher Scientific)
anti-mouse (A-11029 from Life Technologies, 715-166-150 or 715- 176- 150 from Jackson ImmunoResearch)
IRDye 680RG IgG secondary antibodies were used for western blot li-cor detection.

Validation

For all primary antibodies below, WB = western blot, IHC = immunohistochemistry (including immunofluorescence), ICC = Immunocytochemistry, FC = flow cytometry and IP = immunoprecipitation. Validation were summarized from manufacturer's websites and from the usage in this manuscript.

anti-Axl (R&D AF854 Lot CTC0214101, polyclonal goat; WB on manufacturer's website and used in IHC in manuscript. Antibody validated for IHC and WB in Lemke lab and others using Axl knockout animal tissue sections. Zagorska Lemke Nature Immunology 2014; Lew Lemke 2014 ELife).
https://www.rndsystems.com/products/mouse-axl-antibody_af854

anti-Mer (eBioscience lot 4285684 clone: DS5MMER, monoclonal rat, FC on manufacturer's website and used in IHC in manuscript. Antibody validated for IHC and WB in Lemke lab and others using Mer knockout animal tissue sections. Zagorska Lemke Nature Immunology 2014; Lew Lemke 2014 ELife).
https://www.thermofisher.com/antibody/product/MERTK-Antibody-clone-DS5MMER-Monoclonal/14-5751-82

anti-Mer (R&D AF591, Lot DGS0517061 and Lot DGS0213111, polyclonal goat; WB, FC on manufacturer's website and used in IHC in manuscript), Antibody validated for IHC and WB by Lemke lab and others using Mer knockout animal tissue sections. Zagorska Lemke Nature Immunology 2014; Lew Lemke 2014 ELife).
https://www.rndsystems.com/products/mouse-mer-antibody_af591

anti-mouse Gas6 (R&D AF986, lot: EFU0314121, polyclonal goat; WB, ELISA on manufacturer's website and used in IHC in manuscript), Antibody validated for IHC and WB in Lemke lab and others using Gas6 knockout animal tissue sections. Zagorska Lemke Nature Immunology 2014; Lew Lemke 2014 ELife).
https://www.rndsystems.com/products/mouse-gas6-antibody_af986

anti-human Gas6 (R&D AF885 lot GUS0218061 polyclonal goat; IHC, WB and direct ELISA on manufacturer's website for human cancer tissues and samples and used in IHC in manuscript for detecting Gas6 in human AD postmortem samples). IHC studies performed in this manuscript were compared with age-matched controls and with no primary control tissues. Both of the negative controls showed no specific immunoreactivity whereas human AD samples show specific plaque-associated Gas6 expression.
https://resources.rndsystems.com/pdfs/datasheets/af885.pdf?v=20210303

anti-human-beta-amyloid, 1-16 antibody (Biolegend 803001, clone 6E10, WB, ELISA, IHC and IP on manufacturer's website and IHC in manuscript). The specificity to human Abeta is validated on WB using transgenic mouse and human brain lysate, recombinant mouse and human abeta by the manufacturer. And is validated in the Lemke lab comparing WT mouse (negative control) and transgenic AD mouse brain brain sections for IHC and tissue lysates for WB. Similar validation is performed by many others as well: Thakker DR, et al. 2009. Proc. Natl. Acad. Sci. USA. 106(11):4501-6.
https://www.biolegend.com/en-us/products/purified-anti-beta-amyloid-1-16-antibody-11228?GroupID=GROUP32

anti-Iba1 (Wako 019-19741, polyclonal rabbit, ICC and IHC on manufacturer's website and in manuscript). Previously validated in our lab for IHC (Fourgeaud, Traves, Lemke 2016 Nature) and by others (Ximerakis, M., et al.: Nat. Neurosci., 10, 1696(2019); Yin, C., et al.: Nat. Med. 3, 496(2019); Keren-Shaul, Amit, Cell 2017)
https://labchem-wako.fujifilm.com/us/product/detail/W01W0101-1974.html

anti-Iba1 (Novus NB100-1028, polyclonal goat, WB, IHC, ICC on manufacturer's website and used in IHC in manuscript). Previously validated in our lab for IHC (Fourgeaud, Traves, Lemke 2016 Nature) and by others (Imai, Y., & Kohsaka, S. (2002). Intracellular signaling in M-CSF-induced microglia activation: Role of Iba1. GLIA. https://doi.org/10.1002/glia.10149)
https://www.novusbio.com/products/aif-1-iba1-antibody_nb100-1028

anti-GFAP (Dako z-334, polyclonal rabbit, IHC on manufacturer's website and in manuscript). Previously validated as described on manufacturer's publication (Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). Neurochem Res 2000,25:1439-51) and in our lab for IHC (Fourgeaud, Traves, Lemke 2016 Nature).
https://www.agilent.com/en/product/immunohistochemistry/antibodies-controls/primary-antibodies/glial-fibrillary-acidic-protein-(dako-omnis)-76214#literature

anti-cleaved Casp3 (Cell Signaling 9661, lot 45, polyclonal rabbit, WB, IHC, ICC, FC on manufacturer's website and used in IHC in manuscript), validated in lab for IHC comparing 12mo. AD TAM-deficient brains with age-matched WT brain sections (negative control) and TAM-deficient 1mo. thymus sections (positive control) and also from previous publication (Fourgeaud, Traves, Lemke 2016 Nature). https://www.cellsignal.com/products/primary-antibodies/cleaved-caspase-3-asp175-antibody/9661

anti-Tmem119 (Abcam AB209064, lot GR320057-1, monoclonal rabbit, IHC on manufacturer's website and in manuscript)
Validated from literature for IHC in mouse brain tissue sections (Bennett Barres 2016 PNAS)
https://www.abcam.com/tmem119-antibody-28-3-microglial-marker-ab209064.html

anti-RTN3 (EMD Millipore ABN1723 lot 3109186 polyclonal rabbit; WB, IHC, IP on manufacturer's website and used in IHC in manuscript) Validation in AD mouse brain tissue sections from literature (Kraft, Lee FASEB 2013)
https://www.emdmillipore.com/US/en/product/Anti-RTN3-R458,MM_NF-ABN1723

anti-LAMP1 (BD Biosciences, Cat# 553792, clone 1D4B, IHC on manufacturer's website and used in IHC in manuscript). Validation in AD mouse brain tissue sections from literature (Yuan, Grutzendler, 2016 Neuron). https://www.bdbiosciences.com/us/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-mouse-antibodies/purified-rat-anti-mouse-cd107a-1d4b/p/553792

anti-Trem2 (R&D systems, AF1729 and BAF1729, WB and ICC on manufacturer's website and used in IHC in manuscript). Validation from C.Haass's and M. Colonna labs' publications and manufacturer's website. https://www.rndsystems.com/products/mouse-trem2-antibody_af1729

JRF AB042/26 and JRF/AbN/25 (from Janssen Pharmaceuticals) for capture and detection of Abeta1-42 were described in numerous publications (Schmidt, S.D., Mazzella, M.J., Nixon, R.A. & Mathews, P.M. Abeta measurement by enzyme-linked immunosorbent assay. Methods Mol Biol 849, 507-527 (2012)). Human Abeta42 specificity was validated in our hands compared brain extracts from transgenic animals vs. from non-transgenic animals as well as from synthetic Abeta1-42 standards.

anti-CD31 (R&D, AF3628, polyclonal goat, WB, FC, ICC, IHC/IF on manufacturer's website and used in IHC in manuscript) Ilan, N. and J.A. Madri (2003) Curr. Opin. Cell Biol. 15:515.; Gao, C. et al. (2003) Blood 102:169.; Wu, Y. et al. (2005) J. Immunol. 175:3484.
https://www.rndsystems.com/products/mouse-rat-cd31-pecam-1-antibody_af3628
anti-mouse CD16/32 (Biolegend 101301, rat IgG2a, lambda, FC on manufacturer's website and used in FC in manuscript)
https://www.biolegend.com/en-us/products/purified-anti-mouse-cd16-32-antibody-190?GroupID=GROUP20

anti-CD45-PE, 2D1 (Invitrogen 12-9459-42, mouse IgG1, kappa, FC on manufacturer's website and used in FC in manuscript)
https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-2D1-Monoclonal/12-9459-42

anti-CD11b-FITC M1/70 (Invitrogen 11-0112085, FC on manufacturer's website and used in FC in manuscript)
https://www.thermofisher.com/antibody/product/CD11b-Monoclonal-Antibody-M1-70-FITC-eBioscience/11-0112-85

anti-vGlut1 (Millipore, AB5905, polyclonal guinea pig, IHC on manufacturer's website and used in IHC in manuscript) Validation in literature for IHC in mouse brain sections (Blanco-Suarez, Allen 2018 Neuron; Farhy-Tselnicker, Allen 2017 Neuron).

anti-PSD95 (Life Technologies, 51-6900, polyclonal rabbit, IHC, ICC, IP, WB on manufacturer's website and used in IHC in manuscript) Validation in literature for IHC in mouse brain sections (Blanco-Suarez, Allen 2018 Neuron; Farhy-Tselnicker, Allen 2017 Neuron).

anti-GAPDH (Millipore MAB374, clone 6C5, monoclonal mouse, WB, ELISA, ICC, IHC, IF, IP on manufacturer's website and used in WB in manuscript) Validation in lab from previous publications for WB (Zagorska, Lemke 2014 Nat Imm; Lew, Lemke 2014, Elife)
https://www.emdmillipore.com/US/en/product/Anti-Glyceraldehyde-3-Phosphate-Dehydrogenase-Antibody-clone-6C5,MM_NF-MAB374

Animals and other organisms

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

Laboratory animals	Mouse strains used in this study included wild-type and mutant mice. C57BL/6J wild-type mice were obtained from The Jackson Laboratory. Mice were typically group-housed at approximately 22 degrees Celsius and provided with bedding and nesting material. All animals were maintained on a 12 hr light/dark cycle and given ad libitum access to standard rodent chow and water. Mouse facility is supplied with 100% fresh air with humidity equivalent to outside ambient air (30%-70%). The Axl-/-, Mertk-/-, Axl-/-Mertk-/- , and Cx3cr1GFP/+ strains have been described previously (Lu, Q. et al., Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. Nature 398 (6729), 723-728 (1999).and Jung, S. et al., Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Mol Cell Biol 20 (11), 4106-4114 (2000)). B6.Cg-Tg(APPSwePSEN1dE9) hemizygous mice (APP/PS1) (JAX number: 005864) were crossed with Axl-/-, Mertk-/- and/or Axl-/-Mertk-/- lines to generate APP/PS1 Axl-/-, APP/PS1Mertk-/-, and APP/PS1Axl-/-Mertk-/- mice which were harvested and analyzed at 2.5mo, 4mo, 7mo, 9.5mo, 12mo, 15mo and 18mo of age. For two-photon microscopy, APP/PS1 mice were crossed with Cx3cr1GFP/GFP or Cx3cr1GFP/GFP Axl-/-Mertk-/- strains. Only 15-16mo female APP/PS1Cx3cr1GFP/+ WT or Axl-/-Mertk-/- and healthy littermates were used in two-photon studies to avoid potential gender biases in pathology and microglial responses. APP41 mice, which express a 'Swedish' + 'London' (V717I) mutant human APP under the Thy-1 promoter (Rockenstein, E., Mallory, M., Mante, M., Sisk, A., & Masliaha, E., Early formation of mature amyloid-beta protein deposits in a mutant APP transgenic model depends on levels of Abeta(1-42). J Neurosci Res 66 (4), 573-582 (2001)), were a kind gift of Drs. Kuo-Fen Lee and Jiqing Xu from the Salk Institute. All lines have been backcrossed for >10 generations to and maintained on a C57BL/6 background. The APP41 mice and its littermates were analyzed at 15mo. All animal procedures were conducted according to protocols approved by the Salk Institute Animal Care and Use Committee (Protocol No. 17-00009). Mice of both genders were randomly allocated to experimental groups unless otherwise noted.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All animal procedures were conducted according to protocols approved by the Salk Institute Animal Care and Use Committee (Protocol No. 17-00009).

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	Paraffin-embeded brain sections from age-matched patients with clinical diagnosis of AD (BRAAK6) or with normal cognition were collected post-mortem and prepared by UCSD Alzheimer's Research Center (ADRC) neuropathology core. The samples were randomly assigned to the study by UCSD ADRC and delivered to the experimenters. Patient brain samples were matched with age (over 65 year olds), sex and postmortem hours. Both sexes were included in the post-mortem study.
Recruitment	The postmortem tissues used in this project were collected from the UCSD ADRC neuropathology core.
Ethics oversight	All participants consented to brain donation at the time of enrollment in the UCSD ADRC, as one of the 31 ADRC across the country.

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	Dissociated cells were resuspended in 300 ul of FACS buffer (2% FBS and 1mM EDTA in D-PBS, sterile). Fluorescence labeling procedures were then carried out on ice. Fc-receptors were blocked by addition of anti-CD16/32 antibody (1:100 dilution, Biolegend) for 15 min followed by the addition of labelled antibodies: anti-CD45-PE (1:25, Biolegend), anti-CD11b-FITC (1:25 Biolegend) and Hoechst 33258 (1:1000) for 1 hour. Finally, samples were washed twice with FACS buffer and immediately taken to the Salk Institute Flow Cytometry core facility (samples were kept on ice from this point onwards, or chilled at 4C while undergoing FACS purification).
Instrument	FACS purification was carried out on a BD FACS Aria Fusion sorter with 1x PBS for sheath fluid.
Software	FlowJo
Cell population abundance	Cells were purified using a 1-drop single cell sort mode (for counting accuracy). Approximately 20k cells were sorted per sample. These were directly deposited into a 1.5 ml Eppendorf tube without additional buffer to yield a sufficient concentration that permitted direct loading onto the 10x chip.
Gating strategy	FACS purification was carried out on a BD FACS Aria Fusion sorter with 1x PBS for sheath fluid. For high viability, concentrated cells suitable for downstream 10X Genomics analysis, an 85-µm nozzle was used with sheath pressure set to 45PSI. Live cells were gated first (Hoechst dye negative), followed by exclusion of debris using forward and side scatter pulse area parameters (FSC-A and SSC-A), exclusion of aggregates using forward and side scatter pulse width parameters (FSC-W and SSC-W), before finally gating on CD45+ cells to be isolated. Cells were purified using a 1-drop single cell sort mode (for counting accuracy). These were directly deposited into a 1.5 ml Eppendorf tube without additional buffer to yield a sufficient concentration that permitted direct loading onto the 10X chip.

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