nature portfolio

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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

Raw single-cell RNA sequencing data were processed using the Cell Ranger Single Cell Software Suite (10x Genomics, v6.1.2) with default parameters, and reads were aligned to the zebrafish reference genome GRCz11 (Ensembl release 105). Cell clustering, marker gene identification, differential expression analysis, and feature plot generation were performed using Seurat (v5). For quantitative RT–PCR, cycle threshold (Ct) values were obtained using a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific). Genome-wide DNA methylation profiles were generated using the Infinium MethylationEPIC BeadChip (Illumina) at the New York Brain Bank. Confocal image acquisition and colocalization analyses were performed using the ZEN software colocalization module (blue edition, version 3.2; Carl Zeiss, Jena, Germany). Automated cell counting was conducted using a Countess II FL Automated Cell Counter (Thermo Fisher Scientific). RNA integrity and quality were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies).

Data analysis	Imaging data were acquired using Zeiss AxioImager Z1, Zeiss LSM780, and Zeiss LSM800 confocal microscopes. Colocalization of immunohistochemical markers was quantified using the ZEN software colocalization module (Carl Zeiss, Germany). Multiplex immunofluorescence staining was performed using the COMET platform (Lunaphore Technologies), and image analysis was conducted using HORIZON Viewer (Lunaphore Technologies) and HALO software (Indica Labs). For bulk RNA sequencing analyses, quality-controlled reads were aligned to the human genome (GRCh38) using GSNAP, and differential expression analysis was performed with DESeq2. Single-cell RNA-seq datasets were processed using Seurat (v5). Cells with fewer than 200 expressed genes or genes expressed in fewer than three cells were excluded prior to downstream analyses. Following quality filtering, datasets were normalized, the top 2,000 variable genes were selected, and datasets were integrated using FindIntegrationAnchors, IntegrateData, IntegrateLayers, and JoinLayers functions. DoubletFinder was used to identify and remove doublets prior to downstream analyses. Cycle threshold (Ct) values for qRT–PCR experiments were obtained using a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific). Statistical analyses were performed using GraphPad Prism (v9.2.0).

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 sequencing datasets generated in this study are deposited to NCBI Gene expression Omnibus (GEO) with the following accession numbers: GSE268721, GSE268780, GSE268803, and GSE269111. Previous datasets used in this study are GSE108038 and GSE78117. Source data underlying all graphs in the main figures and Extended Data figures are provided with this paper. The unique reagents generated in this study are available from the corresponding author upon reasonable request.

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	na
Reporting on race, ethnicity, or other socially relevant groupings	na
Population characteristics	na
Recruitment	na
Ethics oversight	Human samples were sourced from the New York Brain Bank and anonymized to ensure that researchers had no access to the personal information of the donors. Approval from the Institutional Review Board at Columbia University Irving Medical Center was obtained prior to the generation of clinical data.

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	For single-cell sequencing experiments, SCOPIT was used to estimate sequencing depth and cell numbers. For experimental studies, sample sizes were guided by prior work in the field and, where appropriate, power calculations performed using G*Power or nQuery. For analyses of human cohorts, postmortem tissue, cerebrospinal fluid, proteomic, transcriptomic, and genetic datasets, all available eligible samples meeting predefined inclusion criteria were analyzed; no formal sample size calculations were performed for these observational studies. For imaging datasets involving nested measurements, the biological replicate (animal or donor) was predefined as the independent unit of inference.
Data exclusions	No animals, human donors, biological replicates, or experimental datasets were excluded from the analyses except where technical sample

Data exclusions	preparation or processing problems occurred, as described in the Methods. For sequencing experiments, low-quality cells and doublets were removed during standard quality-control processing before downstream analyses.
Replication	Experimental findings were independently replicated where appropriate using biological replicates, independent human cohorts, complementary experimental systems, and orthogonal methodologies. Representative microscopy and immunoblot images are from experiments repeated independently with similar results, as indicated in the figure legends. For experimental studies, a minimum of three biological replicates (individual animals or human donors) per condition was used where applicable. Multiple measurements (e.g., vessels or ROIs) obtained from the same individual were treated as nested observations and analyzed using cluster-aware statistical models, with the biological replicate defined as the independent unit of inference.
Randomization	Animals were randomly assigned to experimental and control groups where applicable to minimize selection bias. Experimental groups were balanced across independent experiments, and randomized block designs were used when appropriate. Human postmortem tissues, cerebrospinal fluid samples, and publicly available transcriptomic and genetic datasets were observational and therefore not subject to experimental allocation. Image acquisition and quantitative analyses were performed in a blinded manner, and statistical analyses accounted for relevant biological covariates where appropriate.
Blinding	Image acquisition and quantitative image analyses in zebrafish and mouse experiments were performed in a blinded manner. Tissue staining and labeling were performed by one investigator, while image quantification was performed independently with sample identities concealed until completion of the analysis. Blinding was not applicable to computational analyses of previously generated datasets or automated bioinformatic workflows, which were performed using predefined analysis pipelines.

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

For the immunohistochemistry (IHC), the primary antibodies used included:

FN1 (Proteintech, Cat.# 66042-1-Ig, dilution 1:250 and 1:300), (Sigma, Cat. #F3648, dilution 1:100),
CD31 (Abcam, Cat.# ab134168, dilution 1:250 and 1:300), CD31 (BD Biosciences, Cat # 550389, dilution 1:200)
GFAP (Thermo Fisher, Cat.# OPA1-06100, dilution 1:250), GFAP (Abcam, Cat.#4674, dilution 1:500), GFAP (Novus, Cat.# NBP1-05198, dilution 1:2500)
VEGF (R&D Systems, Cat.# MAB2932-100, dilution 1:200 and 1:300)
IGF-1 (Abcam, Cat.# ab106836, dilution 1:200 and 1:300), IGF1 (Abcam, Cat #ab9572, dilution 1:500)
HB-EGF (Thermo Fisher, Cat.# PA5-119187, dilution 1:200 and 1:300)
A-beta42 (Cell Signaling, Cat.# 8243S, dilution 1:200)
GS (Abcam, Cat.# ab176562, dilution 1:500)
MAP2 (Abcam Cat. #ab5392, dilution 1:1000)
MBP (Abcam Cat #ab133620, dilution 1:20000)
IBA1 (Abcam Cat# ab283319, dilution 1:500), (WAKO, Cat #011-27991, dilution 1:100)
CD140b (R&D Systems Cat #AF385, dilution 1:100)
4G8 (Biolegend Cat# 800701, dilution 1:100)
AQP4 (Abcam Cat #ab282586, dilution 1:10000), (Abcam Cat #ab284135 dilution 1:200), (Proteintech Cat #CL594-16473 dilution 1:500)
CDH5 (Thermo Fisher Cat #PA5-143232, dilution 1:200)
Fibrinogen (Abcam Cat #ab34269, dilution 1:100 and 1:300)
APOE (Millipore Sigma Cat #Calbiochem 178479, dilution 1:200), (Bioss Cat #bs-5039R, dilution 1:200)
B2M (Abcam Cat #ab75853, dilution 1:200)
ZO-1 (Thermo Fisher, Cat.# 33-9100, dilution 1:500), ZO1 (Proteintech, cat# 21773-1-AP, dilution factor 1:200)
GFP (Thermo Fisher, Cat.# PA1-9533, dilution 1:2000)
BLBP (Abcam, Cat.# ab281734, dilution 1:1000), BLBP (Abcam, Cat.# ab279649, dilution 1:500)
PDGFRB (R&D Systems, Cat. #AF385-SP, dilution 1:100)
FN1-EDA (Antibodies-online, Cat.# ABIN108413, dilution 1:200)
FN1-EDB (Cell Signaling, Cat.# 36349, dilution 1:200)
Fibronectin (Thermo Fisher, Cat.# PA5-29578, dilution 1:250)
Anti-Dextran Antibody, Clone DX1; Unconjugated, mouse mAb IgG1 (Stem Cell, Cat # 60026, dilution 1:500)

The secondary antibodies employed were:
Goat anti-mouse Alexa Fluor 448 (Thermo Fisher, Cat.# A11001, dilution 1:500)
Goat anti-chicken Alexa Fluor 448 (Thermo Fisher, Cat.# A11039, dilution 1:500)
Goat anti-rabbit Alexa Fluor 555 (Thermo Fisher, Cat.# A32732, dilution 1:500)
Goat anti-mouse Alexa Fluor 555 (Thermo Fisher, Cat.# A21127, dilution 1:500)
Goat anti-mouse Alexa Fluor 568 (Thermo Fisher, Cat.# A11019, dilution 1:250)
Goat anti-rabbit Alexa Fluor 647 (Thermo Fisher, Cat.# A32733, dilution 1:500)
Donkey anti-mouse Alexa Fluor 488 (Thermo Fisher, Cat.# A32766, dilution 1:500)
Donkey anti-goat Alexa Fluor 555 (Thermo Fisher, Cat.# A32816, dilution 1:500)
Donkey anti-rabbit Alexa Fluor 647 (Thermo Fisher, Cat.# A32795, dilution 1:500)
Donkey anti-Rabbit IgG Alexa Fluor 647(Thermo Fisher Scientific, #A31573, dilution 1:1000)
Donkey anti-Mouse IgG AF750 (Thermo Fisher Scientific, #SA000081, dilution 1:1000)
Donkey anti-Chicken IgY AF647 (Thermo Fisher Scientific, #A78952, dilution 1:1000)
Donkey anti-mouse IgG AF568 (Thermo Fisher Scientific, #A10037, dilution 1:1000)
Donkey anti-Chicken IgY AF488 (Thermo Fisher Scientific, #A78948, dilution 1:1000)
Donkey anti-Goat IgG Alexa 4Fluor 488 (Thermo Fisher Scientific, #A-11055 1:1000)
Donkey anti-Goat IgG AF647 (Jackson Immunoresearch, #705-606-147, dilution 1:250)
Donkey anti-rabbit F(ab)' AlexaFluor-647 (Jackson ImmunoResearch 711-605-152, dilution 1:250)
Donkey anti-rabbit F(ab)' AlexaFluor-488 (Jackson ImmunoResearch 715-545-150, dilution 1:250)

For Western Blot and Co-immunoprecipitation, following antibodies were used:
Fibronectin (Invitrogen PA5-29578, 1:1000), FN1 (Proteintech, Cat.# 66042-1-Ig, dilution 1:1000)
b-actin (Millipore Sigma A5316-100UL, dilution 1:1000)
APOE (Millipore Sigma/Calbiochem, Cat. #178479, dilution 1:1000)

Validation

Antibodies are validated by the manufacturer through wither western blots, immunization peptide inhibition or knockout validation. All antibodies were tested for efficacy and reliability with no-primary controls in human samples, cell cultures,and zebrafish before use.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Primary human fetal astrocytes were obtained from ScienCell Research Laboratories (Cat. #1800; sex not specified by the vendor). KOLF2.1J isogenic human iPSC lines (male donor) expressing APOEε3/3 or APOEε4/4 variants were used, together with additional previously published APOE isogenic iPSC lines derived from four independent human donors, as described in the Methods. iPSCs were maintained under feeder-free conditions in mTeSR1 medium and differentiated into astrocytes, endothelial cells, mural cells, and 3D vascular models as described. Differentiation efficiency was confirmed by immunostaining for lineage-specific markers.

Authentication

Primary human fetal astrocytes were commercially obtained from ScienCell Research Laboratories and authenticated by the vendor. KOLF2.1J and the additional APOE isogenic iPSC lines were authenticated by genotype verification and G-banded karyotyping, as described in the original publications and confirmed prior to use. All lines used in this study exhibited normal karyotypes.

Mycoplasma contamination

Cells are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi.

Commonly misidentified lines
(See ICLAC register)

The cell lines used in this study are not listed in the ICLAC register of commonly misidentified cell lines.

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

Human APOE targeted replacement mice on a C57BL/6J background (APOEε3 and APOEε4 lines) and APPNL-G-F knock-in mice (C57BL/6J background; Swedish, Arctic, and Iberian mutations) were used. Age- and sex-matched C57BL/6J wild-type mice served as controls where appropriate. Adult Tg(kdrl:GFP) zebrafish (Danio rerio; approximately 6 months of age; both sexes) were used for VEGFR inhibition studies. Tg(fli1a:EGFP) and Tg(her4.1:RFP) zebrafish lines were used for cell sorting and functional analyses.

Wild animals

na

Reporting on sex

Animals from both sexes were used for this study in equal ratios.

Field-collected samples

na

Ethics oversight

All mice used in this study were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Columbia University Medical Center Institutional Animal Care and Use Committee (IACUC) (approval number AABF3553 and AABR4602). Animals were maintained under temperature and humidity-controlled environment of Columbia University Medical Center on a 12/12-h light/dark cycle, with ad libitum access to food and water in compliance with the protocols approved by the Institutional Animal Care and Use Committee of Columbia University (IACUC). For zebrafish, all animal experiments were performed in accordance with the applicable regulations and approved by the Institutional Animal Care and Use Committee

(IACUC) at Columbia University (protocol number AC-AABN3554). Animals from the same fish clutch were randomly distributed for each experimental group. Animals were handled with caution to reduce suffering and overall animal numbers.

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

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.

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

Zebrafish brains were dissected and single-cell suspensions were prepared according to previously established protocols (https://doi.org/10.1016/j.xpro.2020.100042). The telencephalon of 6–16-month-old fish was dissected in ice-cold PBS and enzymatically dissociated using the Neural Tissue Dissociation Kit (Miltenyi Biotec, Cat. No. 130-092-628). After dissociation, cells were filtered through a 40 µm cell strainer into PBS containing 2% BSA, centrifuged at 300 g for 10 min, and resuspended in PBS with 4% BSA. Viability indicator dyes Sytox Blue (Invitrogen, Cat. No. S34857) and DyeCycle Ruby (Invitrogen, Cat. No. V10309) were used during fluorescence-activated cell sorting (FACS). Samples from different experimental groups were processed in parallel to minimize processing-related variability.

Instrument

Sony MA900-FP cell sorter (Sony Biotechnology).

Software

Sony Cell Sorter Software (Sony Biotechnology).

Cell population abundance

For VEGFR inhibition experiments, a total of 24,518 sorted cells were collected and analyzed. For fn1b$^{-/-}$ experiments, 66,848 sorted cells were collected across biological replicates.

Gating strategy

Sytox Blue–negative (viable) and DyeCycle Ruby–positive (proliferating) cell populations were gated for cell viability. FSC-A/SSC-A gates were used to select the cellular fraction, and FSC-H/FSC-A gating was applied to exclude doublets. Gates were established using unstained and single-color controls and applied consistently across all samples. Representative sorting histograms are provided in Supplementary Information 1.

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