nature portfolio

Corresponding author(s): Martin Dichgans, Katalin Todorov-Völgyi
Last updated by author(s): Feb 2, 2024

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	For confocal imaging: Zeiss Confocal microscope (LSM800, Zeiss, 40x objective with Airy-scan detector), for Electron Microscopy: JEM 1400plus (JEOL) using the EMplified software (TVIPS, v 0.5.10), for Mass Spectrometry: nanoLC system (EASY-nLC 1200, Thermo Scientific, US) coupled online via a nanospray flex ion source (Proxeon – part of Thermo Scientific, US) equipped with a PRSO-V2 column oven (Sonation, Germany) to a Q-Exactive HF mass spectrometer (Thermo Scientific, US).
Data analysis	We used Maxquant (version 1.6.3.4, or 2.0.1.0) for proteomic data analysis and statistics; STRING (version 11.0) and Cytoscape (version 3.8.2) for protein network representation; ImageJ/Fiji (version 1.52p) for image processing and quantification; Excel (2016) and GraphPad (8.3.1) for additional data analysis, statistics and figure representation.

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE109 partner repository with the dataset identifiers Project accession: PXD045026 (Username: reviewer_pxd045026@ebi.ac.uk, Password: m4xYRerT); Project accession: PXD045006 (Username: reviewer_pxd045006@ebi.ac.uk, Password: lxbz6JxR); Project accession: PXD045004 (Username: reviewer_pxd045004@ebi.ac.uk, Password: eWml0Cei); Project accession: PXD044996 (Username: reviewer_pxd044996@ebi.ac.uk, Password: WeoaQaik); Project accession: PXD044993 (Username: reviewer_pxd044993@ebi.ac.uk, Password: 9wBe0DUA).

All proteomic data are under source data for each figure.

Research involving human participants, their data, or biological material

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

Reporting on sex and gender	not applicable
Reporting on race, ethnicity, or other socially relevant groupings	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

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	Sample sizes were determined based on results obtained in previous proteomic and immunohistochemical studies on brain vessels published by the authors (e.g. see Zellner et al. Acta Neuroptahologica 2018; Beaufort et al. Proc Natl. Acad Sci 2014).
Data exclusions	For the APOE-KO vs WT human iEC proteomic analysis n=1 APOE-KO sample was excluded because of incomplete APOE deficiency.
Replication	Animal-based experiments included over 4 independent animals per genotype, except proteomic analysis of BECs from AAV-treated mice which was performed on 4 independent BEC isolates derived from 2 mice per group. Replication of experiments was always sucessful.
Randomization	Mice were randomly selected after genotyping. There was no experimental intervention that would have required randomization. Vessel lysates for immunoblot analysis were randomly selected.
Blinding	Blinding was applied to immunohistochemical image analysis. For all other experiments, blinding was not possible in order to maintain the homogeneity of the measurements (tissue processing and sample analysis were conducted alternately from the samples of the different experimental groups, avoiding batch effect and reducing technical variance).

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

Anti-Col4 Ab (Southern Biotech 1340-01); ICC dilution 1:400
Anti- Arf6 Ab (Invitrogen PA1-093); ICC dilution 1:200; WB dilution 1:250
Ant-Tfrc Ab (Invitrogen 13-6800) ICC dilution 1:200; WB dilution 1:250
Anti-Cldn5 Ab (Thermo Fisher 352588) ICC dilution 1:150
Anti-Pecam1 Ab (Abcam ab215911) ICC dilution 1:150
Anti-Cdh5 Ab (R&D systems AF938) ICC dilution 1:200
Anti-Apoe Ab (Santa Cruz sc-393302) WB dilution 1:500
Anti-Mbp Ab (Millipore MAB386) IHC dilution1:200
Anti-SSEA4 Ab (Abcam ab16287) ICC dilution 1:500
Anti-NANOG Ab (Cell Signaling 4903) ICC dilution 1:500
Anti-Tra160 Ab (Millipore MAB4360) ICC dilution 1:500
Anti-Oct4 Ab (Stemgent 09-0023) ICC dilution 1:500
Anti-Pecam1 Ab (R&D System AF3628) IHC dilution 1:100
Anti-NeuN Ab (Abcam ab104225) IHC dilution 1:200
Anti-Alexa-488 (Jackson Laboratories 715-546-150) ICC/IHC dilution 1:500
Anti-Alexa-488 (Jackson Laboratories 711-545-152) ICC/IHC dilution 1:500
Anti-Alexa-488 (Jackson Laboratories 705-546-147) ICC/IHC dilution 1:500
Anti-Cy3 (Jackson Laboratories 715-165-150) ICC/IHC dilution 1:500
Anti-Cy3 (Jackson Laboratories 711-165-152) ICC/IHC dilution 1:500
Anti-Cy3 (Jackson Laboratories 705-165-147) ICC/IHC dilution 1:500
Anti-Alexa-647 (Jackson Laboratories 715-606-150) ICC/IHC dilution 1:500
Anti-Alexa-647 (Jackson Laboratories 711-606-152) ICC/IHC dilution 1:500
Anti-Alexa-647 (Jackson Laboratories 705-606-147) ICC/IHC dilution 1:500
Goat Anti-Mouse Immunoglobulins/HRP (Dako; #P0447) WB: 1:10000
Goat Anti-Rabbit Immunoglobulins/HRP Dako; #F026102-2) WB: 1:10000

Validation

All Abs are commercially available and were used according to the manufacturer´s instructions. Antibodies were validated by using only primary or secondary antibodies.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	A18944: purchased from Thermofisher (Cat No: A18945); HEK 293 T cells: ATCC (Cat No CRL-3216)
Authentication	A189044 Authenticated by manufacturer by flow cytometry (pluripotency), karyotype, pathogen test (for HIV-1, HIV-2, HTLV type I and II, HSV-1, HSV-2, CMV, EBV, HBV, HCV) and mycoplasma by qPCR.
Mycoplasma contamination	The line was regularly tested and confirmed negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	Not commonly misidentified lines were used

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, mixed males and females, C57BL/6J (3,6,12 and 18 months old), APOE-KO (3 months old), ARF6-KO (3 months old), ARF6-GFP-AAV or GFP-AAV treated C57BL/6J mice (3 months old). Animals were kept under standard conditions in a specific pathogen-free facility at 20-24°C and 45-65% humidity on a 12-h light/dark cycle and had access to food and water ad libitum.

Wild animals	The study did not involve wild animals.
Reporting on sex	Mouse-based analyses were performed on mixed gender experimental groups. The study was not designed for sex-based analyses and is not suitable for this purpose due to low sample size.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Animal experiments were performed in accordance with the German Animal Welfare Law (§4 TschG) and approved by the Government of Upper Bavaria (Vet_02-21-139).

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

Plants

Seed stocks	not applicable
Novel plant genotypes	not applicable
Authentication	not applicable