nature research

Corresponding author(s): Berislav V. Zlokovic

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   Online Methods, 'Statistical Analysis' section: "Sample sizes were calculated using nQUERY assuming a two-sided alpha-level of 0.05, 80% power, and homogeneous variances for the 2 samples to be compared, with the means and common standard deviation for different parameters predicted from published data and our previous studies."

2. Data exclusions

   Describe any data exclusions.

   Online Methods, Animals section: "Because previous studies in mice with deficient PDGFRβ signaling did not find the effect of gender on pericyte coverage, BBB integrity or blood flow regulation, both male and female mice at 2, 4-6, 12-16, and 36-48 weeks of age were used in the study... All animals were randomized for their genotype information and were included in the study."

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   For each series of experiments, all replication attempts were successful.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Online Methods, 'Animals' section: "All animals were randomized for their genotype information and were included in the study."

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Online Methods, 'Animals' section: "The operators responsible for experimental procedure and data analysis were blinded and unaware of group allocation throughout the experiments." and 'Statistical Analysis' section: "All analyses were performed using GraphPad Prism 7.04v software and by an investigator blinded to the experimental conditions."

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

Nature Medicine: doi:10.1038/nm.4482

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ 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 any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Matlab R2013a was used for MRI analyses. For DCE-MRI datasets, we used our GUI code running with Matlab (https://github.com/petmri/ROCKETSHIP). We also used statistical parametric mapping SMP8 running with Matlab too for volumetric studies and ROI identifications. We used FreezeFrame software for fear conditioning behavioral analysis. Flow cytometry data were acquired with FACSDiva 8.0.1 software and analyzed with FlowJo V10. ImageJ 1.48v was also used for MRI and histology image processing. Finally, GraphPad Prism 7.04v was used to analyze the data.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No unique materials were used.

Nature Medicine: doi:10.1038/nm.4482

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

For immunofluorescence analysis in human tissue samples, we used the following antibodies: for pericyte coverage - polyclonal goat anti-human PDGFRβ (R&D systems, AF385; 1:100), for fibrinogen and fibrin extravascular deposits - polyclonal rabbit anti-human fibrinogen (Dako, A0080; 1:500), and species-specific fluorochrome-conjugated secondary antibodies were incubated for 1 h at room temperature.

We used the following antibodies in mouse tissue samples: for pericyte coverage - polyclonal goat anti-mouse aminopeptidase N/ANPEP (CD13; R&D systems, AF2335; 1:100); for fibrinogen and fibrin extravascular deposits - polyclonal rabbit anti-human fibrinogen (Dako, A0080; 1:500) which recognizes both monomeric form of fibrinogen as well as fibrinogen-derived fibrin polymers and cross reacts with mouse fibrinogen and fibrin17; for myelin basic protein (MBP) - polyclonal goat anti-human MBP (Santa Cruz, sc-13914-R; 1:500,) which cross reacts with mouse MBP; for axons SMI-312 neurofilament - monoclonal mouse anti-mouse SMI-312 (SMI-312; BioLegend, SMI312; 1:500); for oligodendrocytes - polyclonal rabbit anti-mouse Olig2 (Millipore, AB9610; 1:200) or monoclonal mouse anti-Olig2 (ThermoFisher, MA5-15810; 1:200); for myelinated mature oligodendrocytes - monoclonal mouse anti-cyclic nucleotide phosphodiesterase (CNPase; Abcam, ab6319; 1:500); for oligodendrocyte progenitor cells - monoclonal rabbit anti-platelet-derived growth factor receptor α (PDGFRα; Cell Signaling, #3174; 1:200); for neurons - polyclonal rabbit anti-mouse NeuN (Millipore, ABN78; 1:500); for microglia - rabbit anti-mouse ionized calcium binding adaptor molecule 1 (Iba-1; Wako, 019-19741; 1:1,000); for astrocytes - rabbit anti-Glial Fibrillary Acidic Protein (GFAP; Dako, z0334; 1:500). For cultured pericytes, we used a goat anti-human platelet-derived growth factor receptor beta (PDGFRβ; R&D Systems, AF385; 1:100). After incubation in primary antibodies, sections were washed in PBS and incubated with fluorophore-conjugated secondary antibodies, and then mounted onto slides with fluorescence mounting medium (Dako).

We used the following antibodies in vitro: after OGD, mature oligodendrocytes were detected with an anti-mouse myelin basic protein (MBP) monoclonal antibody (SMI-99; Covance; 1:500), and pericytes with a goat anti-mouse PDGFRβ polyclonal antibody (R&D Systems, #AF1042; 1:500), followed by TUNEL and Dapi-Fluoromount-G (SouthernBiotech) staining. Secondary antibodies were donkey anti-mouse Alexa Fluor IgG 568 (Invitrogen; 1:500) for MBP-positive oligodendrocytes, and anti-goat Alexa Fluor IgG 568 (Invitrogen; 1:500) for PDGFRβ-positive pericytes. TUNEL assay (Roche) was performed after SMI-99 or PDGFRβ immunostaining.
Double immunostaining for fibrin(ogen) (rabbit anti-human polyclonal antibody cross reacts with mouse fibrinogen, DAKO, #A0080; 1:500) and MBP (as above) was performed to determine fibrin(ogen) accumulation in oligodendrocytes. Secondary antibodies were donkey anti-mouse Alexa Fluor IgG 568 (Invitrogen; 1:500) for MBP-positive cells and donkey anti-rabbit Alexa Fluor IgG 488 (Invitrogen; 1:500) for fibrinogen.
Triple staining with CytoID Autophagy Kit (ENZO Life Sciences, ENZ-51031-K200) on live cells followed by immunostaining for the active form of caspase 3 (rabbit anti-mouse polyclonal, Abcam, ab13847; 1:250) and MPB or PDGFRβ (as above) was done to visualize the formation of autophagosomes and activation of caspase 3 in oligodendrocyte and pericyte cultures, respectively, at different time points. Secondary antibody for active caspase 3 was donkey anti-rabbit 647 Alexa Fluor (Invitrogen; 1:500); secondary antibodies for MBP-positive oligodendrocytes and PDGFRβ-positive pericytes were as described above.
To access availability of oxygen to cultured oligodendrocytes in the presence of fibrin (0.1 mg/mL; i.e., the highest concentration used to treat cells), we employed Image-iT Hypoxia Reagent for live cells (5 µM, ThermoFisher, H10498), and determined whether fibrin can interfere with oxygen delivery to cells making them hypoxic. We also used Alexa 594-conjugated transferrin (25 µg/mL; ThermoFisher, T13343) to determine whether fibrin (0.01 and 0.1 mg/mL) or fibrinogen (1.5 mg/mL) interfere with uptake of transferrin from the culture medium by plated oligodendrocytes.

All details were provided within the Online Methods, 'Human Postmortem Studies - Histopathological Analyses', 'Immunohistochemistry', and 'Cell Cultures - Immunocytochemistry' sections, also including two summary tables of all antibodies (for both human and mouse analyses), dilutions, species, potential cross-reactions, catalog numbers, manufactures, and citations when possible.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Nature Medicine: doi:10.1038/nm.4482

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Platelet-derived growth factor receptor β (Pdgfrβ) F7/F7 mutant mice and their respective controls Pdgfrβ+/+ maintained on a 129S1/SvlmJ background were used. Mice at 2, 4-6, 12-16, and 36-48 weeks of age and of both sexes were used in the study. We also crossed Fibrinogen-deficient (alpha-chain) heterozygous mice (Fga+/-) maintained on a mixed genetic background with F7/F7 mice. Double transgenic F7/F7; Fga+/- mice and their F7/F7; Fga+/+ littermate controls were also used in the study. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California using US National Institutes of Health guidelines. See Online Methods, 'Animals' section.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

We used human tissue samples obtained from the Rush University Medical Center and the University of Southern California. Informed consent was obtained and the study approved by the Institutional Review Board of Rush University Medical Center and the University of Southern California. All autopsy cases underwent neuropathological evaluation of AD including assignment of Braak stages. Aged subjects that did not carry diagnosis of AD or another neurodegenerative disease and showed neuropathological findings within the normal range for age were used as age-matched controls. Mini-Mental State Examination information was available for most but not all individuals. A total of 15 controls and 16 AD individuals were used for histopathological analyses. The demographic information of all cases is provided in Supplementary Table 1. See also Online Methods, 'Human Postmortem Studies' section.

Nature Medicine: doi:10.1038/nm.4482

nature research

Corresponding author(s): Berislav V. Zlokovic

Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Wild type and F7/F7 animals were transcardially perfused with 0.01 M PBS + EDTA and brains immediately removed. White matter tissue (including corpus callosum, internal capsule, cingulum, and external capsule) was isolated from the brains, trypsinized for 30 min in 0.25% trypsin at 37°C and then further dissociated using a glass homogenizer. Cells were fixed in 4% PFA for 10 min, blocked in 10% NDS/0.1% Triton-X/1X PBS, stained with mature oligodendrocyte markers rabbit proteolipid protein (PLP; Abcam, ab105784; 1:2000) and mouse MBP (SMI-99; BioLegend; 1:500), followed by incubation in secondary 488- (Invitrogen; 1:200) and 647-Alexa Fluor (Invitrogen; 1:200) respectively. See Online Methods.
6. Identify the instrument used for data collection.	BD SORP FACSAria I (Becton-Dickinson).
7. Describe the software used to collect and analyze the flow cytometry data.	Data were acquired using FACSDiva 8.0.1 software and analyzed with FlowJo V10.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Purity was determined by relevant staining using flow cytometry. 10,000 events were originally collected from which positively-gated cells showed 95-98% purity.
9. Describe the gating strategy used.	Control stains (unstained and MBP- or PLP- single stained cells) were used to set gates. All samples were then FSC-A and SSC-A gated, followed by FSC-A/FSC-H gating to select singlet cells. Subsequent relevant gating was conducted. See Online Methods.

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

Nature Medicine: doi:10.1038/nm.4482

nature research

Corresponding author(s): Berislav V. Zlokovic

MRI Studies Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Experimental design

1. Describe the experimental design.	F7/F7 mice and littermate controls were scanned cross-sectionnaly (at 2, 4-6, 12-16, 36-48 weeks of age) with a Biospec 7T system (300 MHz, Bruker, Billerica, MA, USA) at the California Institute of Technology (Pasadena, CA, USA). The magnet is equipped with the standard B-GA12 gradient set (~12-mm inner diameter; 400 mT.m-1 maximum gradient) and a 35-mm internal diameter quadrature volume coil was used (M2M Imaging, Cleveland, OH). Fibrinogen-deficient and plasminogen-deficient F7/F7 mice and their littermate controls were scanned cross-sectionally (12-16 weeks of age) with our new MR Solutions 7T PET-MR system (MR Solutions Ltd., Guildford, UK) at the Zilkha Neurogenetic Institute (University of Southern California, Los Angeles, CA, USA). The MR Solutions magnet is equipped with the MRS cryogen-free MRI system (bore size ~24-mm, up to 600 mT.m-1 maximum gradient) and a 20-mm internal diameter quadrature bird cage mouse head coil. Comparable sequences and parameters were used with both MR scanners. See Online Methods.
2. 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.	Total imaging time was approximately 1.5 h per mouse. Mice were scanned cross-sectionnaly at 2, 4-6, 12-16, 36-48 weeks of age.
3. Describe how behavioral performance was measured.	Mice were anesthetized by 1-1.5% isoflurane/air. Respiration rate (80-120 breaths per minute) and body temperature (36.5 ± 0.5°C) are monitored during the experiments using an abdominal pressure-sensitive probe and a rectal temperature probe. The isoflurane dose and heated air flow was adjusted continuously to ensure stable and reproducible depth of anesthesia. ImageJ's Time Series Analyzer V3 plugin was used in real time to ensure good Gd-bolus intravenous injection for both DCE and DSC scans. See Online Methods.

Nature Medicine: doi:10.1038/nm.4482

Acquisition

4. Imaging

a. Specify the type(s) of imaging.	Structural (T2w, T1w, and T2*w), diffusion (DTI-PGSE) and perfusion (DCE and DSC).
b. Specify the field strength (in Tesla).	7T and 11.7T for in vivo and ex vivo scanning sessions, respectively.
c. Provide the essential sequence imaging parameters.	In vivo scans: Pre-contrast T1-values using a variable time repetition (VTR) spin-echo sequence (TR = 5000, 3000, 1500, 800, 400, and 200 ms, RARE factor 3, TE = 11 ms, 1 average, resolution 0.2x0.2x1 mm3), followed by a dynamic series of 800 T1-weighted images with identical geometry and a temporal resolution of 2.6 s (fast low angle shot (FLASH), TR/TE = 20.6/3.2 ms, 2 averages, flip angle 15°, 200x200x1000 µm3). Using a power injector, a bolus dose of 0.5 mmol/kg Gd-DTPA (Gadoliniumdiethylenetriamine pentaacetic acid, Magnevist®, diluted in saline 1:5) is injected via the tail vein (rate of 600 µL/min) at 5 min (volume injected 190 µL) and DCE images are collected for an additional 30 min after the injection. The DSC-MRI imaging is performed on the exact same geometry. A dynamic series of 160 T2*-weighted images is used, with a temporal resolution of 600 ms (FLASH, TR/TE = 18.9/5 ms, 1 average, flip angle 15°, resolution 200x200x1000 µm3). A second bolus dose of Gd-DTPA is injected via the tail vein (rate of 1000 µL/min) at 18 s (volume injected 200 µL) and DSC images are collected for an additional 80 s after the injection. About 1.5 hours scan session per mouse. Ex vivo scans: First, 3D-rapid acquisition with relaxation enhancement (RARE) anatomical images were acquired (TR/TE = 250/9 ms; RARE factor 8; 140x80x80 matrix; 28x16x16 mm FOV, 200 µm isotropic voxel size; 1 average). Then, DWIs were acquired using a conventional pulsed-gradient spin echo (PGSE) sequence (TR/TE = 300/16.2 ms, 350×200x200 matrix, 28x16x16 mm FOV, 80 µm isotropic voxel size, 1 average, δ = 3 ms, Δ = 8 ms, Gd = 1000 mT/m, nominal b-factor = 3000 s/mm2). Six diffusion weighted images were acquired in addition to one volume with no diffusion sensitization using an optimized six points icosahedral encoding scheme for a total imaging time of 24 h. Plus, an additional high-resolution T2*-weighted was acquired using a FLASH sequence (TR/TE = 50/5.19 ms, 400x200x240 matrix, 28x16x16 mm FOV, 50 µm isotropic voxel size, averages 18) for a total imaging scan of 12 h. See Online Methods.
d. For diffusion MRI, provide full details of imaging parameters.	DWIs were acquired ex vivo using a conventional pulsed-gradient spin echo (PGSE) sequence (TR/TE = 300/16.2 ms, 350×200x200 matrix, 28x16x16 mm FOV, 80 µm isotropic voxel size, 1 average, δ = 3 ms, Δ = 8 ms, Gd = 1000 mT/m, nominal b-factor = 3000 s/mm2). Six diffusion weighted images were acquired in addition to one volume with no diffusion sensitization using an optimized six points icosahedral encoding scheme for a total imaging time of 24 h.
5. State area of acquisition.	DCE and DSC where 2 slices (thickness 1-mm) were selected within the dorsal hippocampus territory and the prefrontal cortex to guarantee having WM areas such as corpus callosum, internal and external capsule, and cingulum, as well as common carotid arteries for arterial input functions. See Onlline Methods.

Nature Medicine: doi:10.1038/nm.4482

Preprocessing

6. Describe the software used for preprocessing.

To pre-process the raw ex vivo DWIs, we first corrected for eddy current distortions using the "eddy correct" tool in FSL (www.fmrib.ox.ac.uk/fsl). Extra cerebral tissue was removed using the "skull-stripping" Brain Extraction Tool from BrainSuite (http://brainsuite.org/). All resulting volumes were visually inspected and manually edited as needed. Then, all images were linearly aligned using FSL's "flirt" function with 12 degrees of freedom to allow for rotation, translation, scaling, and skewing in 3D. The gradient direction tables were rotated accordingly after each linear registration for the 6 diffusion volumes. Furthermore, each skull-stripped b0 images were elastically registered to a minimum deformation template created using all linearly registered images for both +/+ and F7/F7 mice. This was done to ensure that all scans were in the same space for further analysis.

To process in vivo T1-VTR and DCE scans, we used our in-house T1 mapping/DCE processing software (Rocketship) implemented in Matlab (Barnes, S. R. et al. ROCKETSHIP: a flexible and modular software tool for the planning, processing and analysis of dynamic MRI studies. BMC Med. Imaging 15, 19 (2015)). Available here: https://github.com/petmri/ROCKETSHIP. To process DSC perfusion data, we used another in-house Matlab script.

7. Normalization

a. If data were normalized/standardized, describe the approach(es).

N/A

b. Describe the template used for normalization/ transformation.

N/A

8. Describe your procedure for artifact and structured noise removal.

Linear alignement (for DWI) and motion correction (for DCE/DSC) were applied using FSL's "flirt" function and ImageJ's Stack Reg - Rigid Body plugin, respectively.

9. Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

N/A

Statistical modeling & inference

10. Define your model type and settings.

N/A

11. Specify the precise effect tested.

We measured BBB permeability and CBF dysfunction using DCE and DSC techniques, respectively; WM integrity and volumetric changes using 3D DWI datasets; and hemosiderin deposits using 3D T2*-weighted sequence.

12. Analysis

a. Specify whether analysis is whole brain or ROI-based.

The analysis is ROI-based except for DTI-tractography mapping where the whole brain was used.

b. If ROI-based, describe how anatomical locations were determined.

The brain regions-of-interest boundaries were manually drawn for each slice using ImageJ and a mouse brain anatomical atlas (Allen Mouse Brain Atlas).

13. State the statistic type for inference. (See Eklund et al. 2016.)

To test for group differences, a voxel-wise linear regression was run, with F7/F7 mice coded as 1 and +/+ mice coded as 0. See Online Methods for more details, as well as 1/ Daianu, M. et al. 7T Multi-shell Hybrid Diffusion Imaging (HYDI) for Mapping Brain Connectivity in Mice. Proc. SPIE-- Int. Soc. Opt. Eng. 9413, (2015) and 2/ Daianu, M., Jacobs, R. E., Weitz, T. M., Town, T. C. & Thompson, P. M. Multi-Shell Hybrid Diffusion Imaging (HYDI) at 7 Tesla in TgF344-AD Transgenic Alzheimer Rats. PloS One 10, e0145205 (2015).

Nature Medicine: doi:10.1038/nm.4482

14. Describe the type of correction and how it is obtained for multiple comparisons.	For DTI metrics and tractography mapping, a regional false discovery rate (FDR) correction was used to correct for multiple comparisons across voxels. Additionally, searchlight-based multivoxel pattern statistics were performed on the resulting probabilistic p-value maps from the regression in all cohorts.
15. Connectivity	
a. For functional and/or effective connectivity, report the measures of dependence used and the model details.	N/A
b. For graph analysis, report the dependent variable and functional connectivity measure.	N/A
16. For multivariate modeling and predictive analysis, specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.	N/A

Nature Medicine: doi:10.1038/nm.4482