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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	The following software were used for data collection: LSM image software (Carl Zeiss) Zen 2.3 software (Carl Zeiss) Preclinical Scan (MR Solutions) PC-SAM32 (SA instruments Inc) AcqKnowledge4.4 (BIOPAC Systems Inc) LabScribe v3 (iWorx).
Data analysis	The following software were used for data analysis: Zen 2.3 software (Carl Zeiss) ImageJ Software (NIH) MATLAB 2017a (The Mathworks) GraphPad Prism 8.0 (GraphPad Software) 3D rat brain model was visualized by ITK-SNAP and Para-View (Kitware) LabScribe v3 (iWorx).

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

All the data that support the findings of this study are available from the corresponding author upon reasonable 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 α=0.05 and power of 0.8) performed for similar experiments and statistical methods were not used to predetermine sample sizes as previously published (Louveau et al., Nature, 2015; Da Mesquita et al., Nature, 2018).
Data exclusions	No samples were excluded from the analysis.
Replication	Experiments were replicated at least once for all analyses and number of reproductions of each experimental finding is described in each figure legend. All attempts at experimental replication were successful.
Randomization	Animals from different cages, but within the same experimental group, were selected to assure randomization.
Blinding	The investigators were blinded during the experiments and quantifications.

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 and secondary antibodies were used in the immunostaining: anti-Prox1 (rabbit polyclonal, 11067-2-AP, Proteintech); anti-Prox1 (goat polyclonal, AF2727, R&D); anti-LYVE–1 (rabbit polyclonal, 11-034, Angiobio); anti-LYVE–1 (rat polyclonal, AF7939, R&D); Alexa Fluor 488-conjugated anti LYVE-1 (rat monoclonal, clone ALY7, 53-0443-82, eBioscience); anti-CD31 (rat monoclonal, clone MEC13.3, 557355, BD Biosciences); anti-CD31 (hamster monoclonal, clone 2H8, MAB1398Z, Merck); anti-VE–cadherin (goat polyclonal, AF1002, R&D); anti-Collagen IV (rabbit polyclonal, ab6586, Abcam); anti-Foxc2 (sheep polyclonal, AF6989, R&D); anti-Integrin α9 (goat polyclonal, AF3827, R&D); anti-αSMA–Cy3 (mouse monoclonal, clone 1A4,C6198, Sigma-Aldrich); anti-E–cadherin (goat polyclonal, AF748, R&D); anti-ER–TR7 (rat monoclonal, sc-73355, Santa Cruz); anti-fibronectin (rabbit polyclonal, AB2033, Merck); anti-CD3e (hamster monoclonal, 553058, BD Biosciences); anti-neurofilament (rabbit polyclonal, ab204893, Abcam); and Alexa Fluor 488-, 594-, 647-conjugated secondary antibodies were purchased from Jackson ImmunoResearch. Nuclei were stained with DAPI (Invitrogen). Primary antibodies were diluted at 1:400 and secondary antibodies were diluted at 1:1,000 for all the immunostainings.

Validation	All the antibodies were validated for the species (mouse or rat) and applications (immunohistochemistry) by the correspondent manufacturer, which is described in the manufacturer's website. Our usage was described in the Methods section of the manuscript as below. Immunofluorescence staining For both whole-mount and section staining, samples were permeabilized and blocked with blocking buffer containing 5% donkey (or goat) serum in 0.3% Triton-X 100 in PBS for 1 h at room temperature (RT). Samples were incubated with the primary antibody diluted in the blocking buffer overnight at 4°C. After several washes with PBS, samples were incubated for 4 h at 4°C with the fluorocrome-conjugated secondary antibodies diluted in the blocking buffer. After several washes with PBS, samples were mounted with Vectashield (Vector Laboratories).

Animals and other organisms

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

Laboratory animals	Prox1-GFP mice (Choi et al., Blood, 2011; provided by Dr. Young-Kwon Hong, University of Southern California), Prox1-CreERT2 mice (Bazigou et al., JCI, 2011; provided by Dr. Taija Mäkinen, Uppsala University) and Vegfr3flox/flox mice (Haiko et al., Mol Cell Biol., 2008; provided by Dr. Kari Alitalo, University of Helsinki) were transferred, established, and bred in SPF animal facilities at KAIST. Cre-ERT2 negative but flox/flox positive mice among the littermates of these mice were defined as control (WT) mice. All of these mice were maintained in the C57BL/6 background and for neonatal experiments, postnatal day (P) 4, P8, P10, P12, P18, and P28 neonates of both genders were analyzed. For adult mice experiments, adult mice aged 8~12 weeks of both genders were used for experiments except for those comparing young (3 months-old) mice with middle-aged (8~10 months-old) or aged (24~27 months-old) mice. Both genders of young (3 month-old), middle-aged (8~10 months-old) and aged (24~27 months-old) mice in the C57BL/6 background were puchased from the Animal Facility of Aging Science, Korea Basic Science Institute (Gwangju, Republic of Korea). No differences were observed between the genders in our experiments as described in the result section and Extended Data Fig. 8. Male Sprague-Dawley rats of 10 weeks of age weighing 350-400 g were purchased from the Koatech (Gyeonggi-do, Republic of Korea) for MRI experiments.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Animal care and experimental procedures were performed under the approval from the Institutional Animal Care and Use Committee (No. KA2017-42) of KAIST.

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

Magnetic resonance imaging

Experimental design

Design type	Resting state; block design.
Design specifications	Single baseline reference scan and 29 dynamic scans were performed with T2-weighted 2D Inversion recovery fast spin echo sequence (T2W 2D FLAIR). Each scan time was 4min 36secs. Single baseline reference scan and 13 dynamic scans (total 14 scans) were performed with T1-weighted 3D Fast low angle shot sequence(T1W 3D FLASH). Each scan time was 4min 6secs. Contrast agent was synchronized with the first scan, which was taken after the baseline scan.
Behavioral performance measures	n/a

Acquisition

Imaging type(s)	Structural: T1-weighted 3D FLASH technique. Diffusion/Perfusion of contrast agent : post contrast T2-weighted 2D FLAIR technique.
Field strength	3 Tesla
Sequence & imaging parameters	The scan parameters of T2 weighted 2D FLAIR sequence were as follows: scan direction = axial, TR=9500 ms, TEeff / TEbase = 85 ms / 17 ms, TI = 1800 ms, flip angle = 90°, number of averages = 1, field of view = 29 mm x 29 mm, matrix size = 128 x 256, slice thickness = 1 mm, gap = 0.1 mm, number of slices = 20, echo train length = 8, and scan time = 4 min 36 s. The FLAIR scan was repeated 29 times in total (excluding 0th single baseline scan) over 138 min with 4 min 36 s interval. A 3D FLASH sequence instead of the 2D FLAIR sequence was used in a separate rat to visualize infusion of contrast agent in 3D with higher spatial resolution. The scan parameters of T1 weighted 3D FLASH sequence were as follows: scan direction = axial, TR = 20 ms, TE = minimum, flip angle = 20°, number of averages = 1, field of view = 30 mm x 30 mm x 32 mm, matrix size = 256 x 192 x 64, and scan time = 4 min 6 s. The 3D FLASH imaging was dynamically performed and a total of 14 scans were taken over 57 min 24 sec with 4 min 6 sec interval.
Area of acquisition	Imaging area was set from frontal neocortex to the deep cervical lymph nodes. Imaging area was decided to cover the

Area of acquisition: region where the contrast agent in CSF could diffuse into and flow out through lymphatic system, especially subarachnoid cisterns, venous sinuses, skull foramina, and cervical lymph nodes.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software: Using MATLAB 2017a (The Mathworks Inc.) for zero-padding k-space from 128 X 256 to 256 X 256, motion-correction and region of interest based intensity analysis.

Normalization: After the head motion registration, the 2D volume images at each time point were normalized by the average intensity from the muscle region, which was not influenced by the contrast agent, to minimize the potential variation of MR signals by temperature and/or B0 (main magnetic field) changes. The normalized intensity of each scan was calculated by multiplying original signal intensity by average intensity of muscle region of interest from base image divided by average intensity of muscle region of interest from nth scan as described in the Online Methods.

Normalization template: Data were not normalized by template.

Noise and artifact removal: To correct the motion artifact through time series, we used image intensity based image registration.

Volume censoring: n/a

Statistical modeling & inference

Model type and settings: n/a

Effect(s) tested: n/a

Specify type of analysis: ☐ Whole brain ☒ ROI-based ☐ Both

Anatomical location(s): Region of interests (ROIs) were defined manually around superior sagittal sinus, cisterna magna, and skull basal region to evaluate basal outflow, deep cervical lymph node, and skull dorsal region. The variation in average intensity was measured within each ROI of target slices at the indicated time dimension.

Statistic type for inference (See Eklund et al. 2016): n/a

Correction: n/a

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☐	☒ Graph analysis
☒	☐ Multivariate modeling or predictive analysis

Graph analysis: We measured signal intensity of the indicated region of interest according to the MRI scan time.