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	A QuantStudio 3 PCR System was used for acquiring gene expression levels for qPCR analysis. iREC and iEC samples were outsourced to Novogene, who then used their own platforms to obtain the bulk RNA-seq data. A Nikon AX-R confocal microscope was used for acquiring in vivo and in vitro immunofluorescence images. An EVOM3 system and an STX-4 probe were used for acquiring TEER readings. A BioTek Synergy H1 was used for collecting plate reader data. Flow analysis was conducted on a BD FACSCanto flow cytometer and BD FACSDiva software was used to acquire flow cytometry data. Western Blot membranes were visualized with Clarity Western ECL Substrate (Bio-Rad) and imaged using Bio-rad ChemiDoc.
Data analysis	FlowJo software (v10.1) was used for flow data processing and analysis. Fiji (v.2.16.0) was used for junctional protein fluorescence intensity analysis. NIS-Elements AR (v6.02.03), Imaris (v9.6), and Fiji (v.2.16.0) were used for in vitro network analysis. Imaris (v10.0) and Fiji (v.2.16.0) were used for in vivo network analysis. NIS-Elements AR (v6.02.03) and Fiji (v.2.16.0) were used to develop immunofluorescence images and add scale bars. Trim Galore (v 0.6.7, cutadapt v 3.5), salmon 152, Bioconductor package DESeq2153 (v 1.34.0), EnhancedVolcano (v 1.12.0), tximport155 (v 1.22.0), and STRING database were used for RNA-Seq data analysis. Graphpad Prism (v10.2.3.) and JMP 17 were used for general statistical analysis and figure development.

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 data supporting the findings of this study are presented in the paper and the supplementary information. Source data are available through the Duke Research Data Repository (DOI: https://doi.org/10.7924/r4r482). All data supporting the findings of this study are available within the paper and its supplementary information. RNA-Seq data were deposited into the NCBI Gene Expression Omnibus database under accession number GSE284350. Pre-existing RNA-Seq data used in the study can be accessed under accession numbers GSE195519 and GSE228900.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	To achieve a high power of statistical significance for the in vivo studies, we targeted a sample size in treatment and control groups of more than seven for each experiment term. For in vitro studies, no sample size calculation was performed. Sample sizes were determined based on standard practice in the field and prior experience, targeting at least three biological replicates and two to three technical replicates per experimental group. Sample size is explicitly stated for each experimental group in figure captions and the methods section.
Data exclusions	No data excluded.
Replication	All experiments were performed in triplicate (technical and biological replicates) unless otherwise stated. The replication numbers are indicated in figure captions and the statistics section within the methods.
Randomization	Cells were randomly allocated into experimental groups. Animals were randomly allocated into experimental groups from a pool of animals.

Blinding	For all in vivo and in vitro experiments, investigators were not blinded because the nature of quantitative analysis is not subject to human bias.

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

All antibodies include information following the order antibody, vendor name, catalog number, application, species, and dilution.

In vitro
Calponin-1 SCBT sc-58707 ICC Mouse 1/100
PDGFRb SCBT sc-432 ICC Rabbit 1/200
CD13 SCBT sc-18899 ICC Mouse 1/100
Nestin SCBT NB300-265s ICC Rabbit 1/200
NG2 SCBT sc-166251 ICC Mouse 1/100
Transgelin (SM22α) Cell Signaling 40471s ICC Rabbit 1/100
CD31 Abcam ab28364 ICC Rabbit 1/100
VE-Cadherin SCBT sc-9989 ICC Mouse 1/100 or 1/4000
von Willebrand Factor SCBT sc-365712 ICC Mouse 1/100
Alexa Fluor 546 Phalloidin Thermo Fisher A22283 ICC N/A 1/400
Occludin Thermo Fisher Scientific 711500 ICC Rabbit 1/100
Claudin-5 Thermo Fisher Scientific 35-250-0 ICC Mouse 1/50; 1/200
ZO-1 Thermo Fisher Scientific 40-2200 ICC Rabbit 1/100
Claudin-2 Thermo Fisher Scientific 710221; 5600 ICC Rabbit; Mouse 1/200; 1/100
β-catenin SCBT sc-7199; sc-393501 ICC Rabbit; Mouse 1/100
Dylight-conjugated Ulex Europaeus Agglutinin Vector Laboratories DL-1068-1 ICC; 1/400
DAPI Thermo Fisher Scientific D1306 ICC; 1/1000
ICAM-1 R&D systems BBA3 ICC Mouse; 1/100

In vivo
Griffonia Simplicifolia Lectin I (GSL I) Isolectin B4, DyLight™ 594 Vector Laboratories DL-1207-.5 IF; 1/400
Anti-NG2, Alexa Fluor™488 Conjugate Antibody Millipore Sigma AB5320A4 IF Rabbit 1/200
Rhodamine-conjugated Ulex Europaeus Agglutinin Vector Laboratories RL-1062-2 IF 1/400

Flow cytometry
PE-Anti-Human CD140b BD Pharmingen 558821 Flow cytometry Mouse 1/10
APC-Anti-Human CD344 Miltenyi Biotec 130-106-614 Flow cytometry Mouse 1/10
PE-Anti-Human CD31 BD Pharmingen 555446 Flow cytometry Mouse 1/10
eBioscience Fixable Viability Dye eFluor 780 Invitrogen 65-0865-14 Flow cytometry 1/1000

Western Blot
P16-INK4A proteintech 10883-1-AP WB Rabbit 1/1000
P21 Waf1/Cip1 Cell Signaling 2947S WB Rabbit 1/1000
β-actin Cell Signaling 3700S WB Mouse 1/4000
Anti-mouse IgG, HRP-linked Cell Signaling 7076P2 WB Horse 1/2000
Anti-rabbit IgG, HRP-linked Cell Signaling 7074P2 WB Horse 1/2000

Validation

All antibodies were used without additional validation. The validation of all antibodies could be found from the corresponding supplier as listed below:

In vitro
Calponin-1 SCBT sc-58707 ICC Mouse 1/100; https://www.scbt.com/p/calponin-1-antibody-calp?srsltid=AfmBOoqyP3RaJ30B4tw_QVZHRPx9KG2UliBj8NcYgYgdaKltZl-PGoh7

PDGFRb SCBT sc-432 ICC Rabbit 1/200; https://www.scbt.com/p/pdgfr-beta-antibody-958?srsltid=AfmBOooa0vvZ1jRBKR1Smjm0Yv_GeAIAGPgE_JIibdRXdBwe6KyE3s5H
CD13 SCBT sc-18899 ICC Mouse 1/100; https://www.scbt.com/p/cd13-antibody-sj1d1?srsltid=AfmBOopIDG0ZGq1vyPTvf1GhO5EdF5LTZszX5NQMps5m9gQH_VROfAxJ

Nestin SCBT NB300-265s ICC Rabbit 1/200; https://www.novusbio.com/products/nestin-antibody_nb300265?srsltid=AfmBOoqTZyYlZ7s5uD_zgsKV_y4VvBOPPs0bNW6z6dSaohDsefj_xX15
NG2 SCBT sc-166251 ICC Mouse 1/100; https://www.scbt.com/p/ng2-antibody-g-9?srsltid=AfmBOorw6yEs5KxMBW2hDU6aA4X_DwfE9FI4dWo8EyeRRVEhEeWFp9lh

Transgelin (SM22α) Cell Signaling 40471s ICC Rabbit 1/100; https://www.cellsignal.com/products/primary-antibodies/transgelin-tagln-antibody/40471?srsltid=AfmBOor_cTerf1UghLgQU6XdomGEF8CF7X_r48NS5Ytf7W8JqSOafi8D
CD31 Abcam ab28364 ICC Rabbit 1/100; https://www.abcam.com/enus/products/primary-antibodies/cd31-antibody-ab28364

VE-Cadherin SCBT sc-9989 ICC Mouse 1/100 or 1/4000; https://www.scbt.com/p/ve-cadherin-antibody-f-8?srsltid=AfmBOorcpk9Jkocxef08yucAO_hlvY0da_AJvDcc_rWzt1A8qYRAaIdQ
von Willebrand Factor SCBT sc-365712 ICC Mouse 1/100; https://www.scbt.com/p/vwf-antibody-c-12?srsltid=AfmBOopvao4CKvmkvaxKLuvBH1aifM0USJJCWe4pdFSvDZAequY5D1le

Alexa Fluor 546 Phalloidin Thermo Fisher A22283 ICC N/A 1/400; https://www.thermofisher.com/order/catalog/product/A22283
Occludin Thermo Fisher Scientific 711500 ICC Rabbit 1/100; https://www.thermofisher.com/antibody/product/Occludin-Antibody-Polyclonal/71-1500

Claudin-5 Thermo Fisher Scientific 35-250-0 ICC Mouse 1/50; 1/200; https://www.thermofisher.com/antibody/product/Claudin-5-Antibody-clone-4C3C2-Monoclonal/35-2500
ZO-1 Thermo Fisher Scientific 40-2200 ICC Rabbit 1/100; https://www.thermofisher.com/antibody/product/ZO-1-Antibody-Polyclonal/40-2200

Claudin-2 Thermo Fisher Scientific 710221; 5600 ICC Rabbit; Mouse 1/200; 1/100; https://www.thermofisher.com/antibody/product/Claudin-2-Antibody-clone-3HCLC-Recombinant-Superclonal/710221
β-catenin SCBT sc-7199; sc-393501 ICC Rabbit; Mouse 1/100; https://www.scbt.com/p/beta-catenin-antibody-h-102?srsltid=AfmBOorE8BVXj32jHlwMts0kuZNJUloAYY6wohTfGqiiFQ13iXsSdPvZ
https://www.scbt.com/p/beta-catenin-antibody-a-5?srsltid=AfmBOopSFf6NuT-p3ddzESisiWWh6mUGjZi-4l5CJYqEoc5nj_5qi0F0

Dylight-conjugated Ulex Europaeus Agglutinin Vector Laboratories DL-1068-1 ICC; 1/400; https://vectorlabs.com/products/dylight-649-ulex-europaeus-agglutinin/?srsltid=AfmBOoo5A547eBOB5xAy_Aq2_hOaN8M_TuXcKGqShyGJIxABf_7_MTqU
DAPI Thermo Fisher Scientific D1306 ICC; 1/1000; https://www.thermofisher.com/order/catalog/product/H3569

ICAM-1 R&D systems BBA3 ICC Mouse; 1/100; https://www.rndsystems.com/products/human-icam-1-cd54-antibody-bbig-i1_bba3

In vivo
Griffonia Simplicifolia Lectin I (GSL I) Isolectin B4, DyLight™ 594 Vector Laboratories DL-1207-.5 IF; 1/400; https://vectorlabs.com/products/dylight-594-gsl-i-isolectin-b4/?srsltid=AfmBOoqogaL2Q4GFsOSkcSBFOh83ubRg0vsj5_DscqC8tXMIn6ppS-g7
Anti-NG2, Alexa Fluor™488 Conjugate Antibody Millipore Sigma AB5320A4 IF Rabbit 1/200; https://www.sigmaaldrich.com/US/en/product/mm/ab5320a4?srsltid=AfmBOopvtRCjR3sW5LrDVTMKTvgRcu3dBP1cT7QhAgaYvzsu7Yhl4clA

Rhodamine-conjugated Ulex Europaeus Agglutinin Vector Laboratories RL-1062-2 IF 1/400; https://vectorlabs.com/products/rhodamine-ulex-europaeus-agglutinin-i/?srsltid=AfmBOopzQ54_FcU5MbL65asT722eeE32ywOyyByNNfdirqadEp4jmrCe

Flow cytometry
PE-Anti-Human CD140b BD Pharmingen 558821 Flow cytometry Mouse 1/10; https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-mouse-anti-human-cd140b.558821?tab=product_details
APC-Anti-Human CD344 Miltenyi Biotec 130-106-614 Flow cytometry Mouse 1/10; https://www.miltenyibiotec.com/US-en/products/cd344-frizzled-4-antibody-anti-human-ch3a4a7.html#conjugate=pe:size=100-tests-in-1-ml

PE-Anti-Human CD31 BD Pharmingen 555446 Flow cytometry Mouse 1/10; https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-mouse-anti-human-cd31.555446?tab=product_details
eBioscience Fixable Viability Dye eFluor 780 Invitrogen 65-0865-14 Flow cytometry 1/1000; https://www.thermofisher.com/order/catalog/product/65-0865-14

Western Blot
P16-INK4A proteintech 10883-1-AP WB Rabbit 1/1000; https://www.ptglab.com/products/P16,P19-Antibody-10883-1-AP.htm?srsltid=AfmBOopOX2Z_ylZi1UwmOtfmwBvLcqktpsBOmFADPMv1fZJ1TXGo0-H1

P21 Waf1/Cip1 Cell Signaling 2947S WB Rabbit 1/1000; https://www.cellsignal.com/products/primary-
https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074?srsltid=AfmBOoqKyPFrnVsHYuENTSyY9vh4RCu7UXirI21zSN9cd03MBcUJRpHo

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Mono-allelic mEGFP-tagged TJP1 WTC (WTC-TJP1, AICS-0023) hiPSCs were sourced from the Coriell Institute for Medical Research. C1-2 iPSCs are derived from fibroblast from ATCC (CRL-2097) and were provided by the collaborator. Primary HRECs (ACBRI 181) and HRPCs (ACBRI 183 V) were sourced from Cell Systems. Both hiPSC lines were derived from male donors. Both primary cell lines were from mixed donors and the specific sex was not provided.
Authentication	WTC-TJP1 were authenticated by the Coriell Institute for Medical Research. C1-2 were karyotyped yearly. The primary HRECs and HRPCs were authenticated by Cell Systems.
Mycoplasma contamination	All cell lines are mycoplasma tested quarterly. All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell 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	Female and male C57BL/6J (Mus musculus; Jackson Laboratory) mice from postnatal day 7 (P7) to postnatal day P17 of both sex were used for the in vivo experiments. Mice were subject to high oxygen on P7, intravitreal injection was perfromed on P12, samples were collected, processed and analyzed on P17.
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex was not considered in the study design.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	Animal studies were conducted under the Duke University Institutional Animal Care and Use Committee-approved animal protocol A070-22-04.

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	hiPSC-derived cells were harvested for analysis using TrypLE dissociation buffer and collected in 100 µL of 0.1% BSA. Cells were then incubated with primary antibody for 30 minutes on ice. Cells were washed three times with 0.1% BSA and filtered through a 40-µm cell strainer for analysis.

For intracellular expression, hiPSC-derived cells were harvested using TrypLE dissociation buffer. Cells were centrifuged and fixed in 2% PFA for 15 minutes on ice and then washed two times with 0.1% BSA. Cells were then permeabilized with 0.1% TWEEN 20 in PBS for 10 minutes at room temperature, after which cells were washed two times with 0.1% BSA. Cells were incubated with primary antibody for 30 minutes on ice. Cells were washed three times with 0.1% BSA and filtered through a 40-µm cell strainer for analysis.

Instrument	BD FACSCanto flow cytometer
Software	BD FACSDiva software were used to acquire flow cytometry data. FlowJo software (v10.1) was used for flow data processing and analysis.
Cell population abundance	The iRECs were sorted based on CD31+ and Frizzled4+ after gating on FSC-A, and FSC-H (singlets), FSC-A, and SSC-A (cells). The iREC population was approximately 66.5%. The iRPCs were sorted based on PDGFRβ and Frizzled4+ after gating on FSC-A, and FSC-H (singlets), FSC-A, and SSC-A (cells). The iRPC population was approximately 96.3% on passage 1, approximately 92.5% on passage 5, and approximately 61.2% on passage 10.
Gating strategy	Doublets were gated out using FSC-A and FSC-H. Cells were gated using FSC-A and SSC-A. Subsequent gatings were performed using fluorescence minus one (FMO) controls.

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