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Corresponding author(s): Corresponding author: Kapil Bharti
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Reporting Summary

Nature Research 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 Research 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	Commercial imaging softwares associated with Leica and Zeiss microscopes were used fro data collection
Data analysis	ImageJ (an open source software), MATLAB, Prism, and Excel were used for data analysis. Details of custom codes is provided in the Methods section. Program code is available from the corresponding author upon request.

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 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 data supporting the findings of this study are available within the article and its Supplementary Information files or are available from the corresponding author upon 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	Statistical analysis was performed using two-way ANOVA or unpaired t-test. Both of these methods require a minimum of three samples in each treatment group. For that reason, it was ensured to have minimum of three samples in each treatment group.
Data exclusions	No data was excluded from the analysis
Replication	All experiments were replicated by independently by the first three authors of the paper.
Randomization	3D-oBRB were randomly selected into different treatment groups for dry and wet AMD modeling experiments.
Blinding	Blinding was done during the treatment of different groups. One operator prepared cell culture medium for the treatment and the other operator treated samples. For analysis, sample names were masked when possible.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Primary antibodies included Mouse anti-PLVAP (FELS, 1:50, Abcam, Cat# AB81719); Rabbit anti-CD31 (1:50, Abcam, Cat# AB28364); Mouse anti CD31 (1:50, Agilent, Cat# M0823); Rabbit anti-Laminin (1:50; Abcam, Cat# AB11575); Mouse anti E-cadherin (1:100; Abcam; Cat# AB40772); mouse anti-ZO-1 (1:100; Thermo Fisher Scientific, Cat# 33-9100); rabbit anti-ZO-1 (1:50; Thermo Fisher Scientific, Cat# 61-7300) Mouse anti-Collagen-IV (1:50; Abcam, Cat# AB6311); Rabbit anti-Elastin (1:50 Abcam, Cat# AB21610); Rabbit anti-APOE (1:50; Abcam, Cat# AB52607), Rabbit anti-VEGF (1:50; Thermo Fisher Scientific, Cat# P807), Mouse anti-STEM121 (1:100; Takara Bio, Cat# Y40410); Rabbit anti-VWF (1:100; Dako, Cat#GA52761-2); Rabbit anti-vWF (1:100, Dako, Cat# A0082); Mouse anti-CD31 (1:100, Dako, Cat# M0823); Rabbit anti-ETV2 (1:100, Abcam, Cat# AB181847); Mouse anti-aSMA (1:200, Sigma Aldrich, Cat# F3777); Mouse anti-NG2 (1:100, Thermo Fisher, Cat# 14-6504-82); Rabbit anti-PDGFR-b (1:100, Abcam, Cat# AB32570); Rabbit anti-Collagen-I (1:100, Thermo Fisher, Cat# PA5-95137); Rabbit anti-Vimentin (1:100, Abcam, Cat# AB92547)
Validation	Validation statement is described in each manufacturer's website including antibody information and citations

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Human placental microvascular pericytes (Angio-Proteomie, Cat# cAP-0029, Boston, MA) , iCell endothelial cells (Cellular Dynamics International, Cat# R1022), iCell Retinal Pigment Epithelial Cells (Cellular Dynamics International,Cat# R1101), Choroidal fibroblasts (Alabama Eye Bank), Primary Human Retinal Microvascular Endothelial Cells (Cell systems, Cat#ACBRI 181), GFP Expressing Human Retinal Microvascular Endothelial Cells (Angioproteomie, Cat#cAP-0010GFP)
Authentication	All cell lines were authenticated with immunostaining with markers. Data is provided in Fig. S1.

Mycoplasma contamination	All cell lines were routinely tested to be negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	n/a

Animals and other organisms

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

Laboratory animals	RNU rats were used for this study (males)
Wild animals	n/a
Field-collected samples	n/a
Ethics oversight	The use of rodent model was approved by the NIH Animal Care and Use Committee.

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