nature portfolio

Corresponding author(s): Lena Claesson-Welsh, Yi Jin

Last updated by author(s): Oct 20, 2022

# 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<br>*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<br>*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 | Confocal microscope images were taken with LAS X software (3.5.7.23225) from Leica. Western blot images were taken with Image Lab software (6.1) from BioRad. Quantitative PCR data were collected by SFX Maestro 1.1 from BioRad. |
| Data analysis | Microscopic images were analyzed with Fiji (ImageJ) software. Western blot images were analyzed with Image Lab software (6.1). Statistical analysis was performed using GraphPad Prism software (9.2). Flow simulation from the images of retinal vasculature was done using the PolNet platform (ref. 30). For computational analysis of retinal EC distribution, a python-based workflow 2D-coordinate system was employed, that can be accessed on github (https://github.com/wgiese/retina-vein-artery-cs). Quantitative PCR data were analyzed with SFX Maestro 1.1. Single cell-RNA sequencing data was processed with cellranger (v5.0.1) using the mm10 reference genome. Raw counts were normalized with a pooling size factor-based strategy as implemented in scran (v.1.18.7). Subtype annotations were generated using scmap (v.1.16.0) using the cluster assignment described in the original publication. Differential gene expression analysis was carried out using MAST as implemented in Seurat (v.4.1.1). The effects of dropouts in the data were reduced with imputation with the magic python package (v.0.1.1; k = 9, ka = 3, t = 1) and graphs were generated with ggplot2 (v.3.3.6). |

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

Single cell RNA-sequencing data generated from WT P6 and P10 retinas was obtained from the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra), accession number SRP322112. Additional data supporting the findings in this study are included in the main article and associated files. Source data are provided with this paper.

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | N/A |
| Population characteristics | N/A |
| Recruitment | N/A |
| Ethics oversight | N/A |

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 | No statistical methods were used to predetermine sample size. Sample sizes were chosen based on experience combined with animal welfare consideration and ethical permit constraints. Sample size for each experiment can be found in the related figure legends. |
| Data exclusions | In the GTPase activity assay, data that deviated from the average value for more than 2XSE were considered outliers and were excluded from statistics. No data was excluded from all other analyses. |
| Replication | For in vitro experiments, all presented results/quantifications are derived from at least three replicates. For in vivo experiments, all procedures were performed on at least three animals with multiple repeats. Number of replicates for each experiment is given in the related figure legend. |
| Randomization | Experimental animals were grouped by their genotypes in most experiments and sometimes grouped by their treatments. Randomization was not used. |
| Blinding | The investigators were not blinded to allocation during most experiment procedures and outcome assessments. Blinding was not performed due to limited human resources. However, blinding was applied in the vessel leakage analyses. |

# 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 |

## Methods

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

# Antibodies

Antibodies used

For immunofluorescence, Antibodies against pVE-cadherin Y685 or Y731 were generated by immunizing rabbits with phosphopeptides of the corresponding regions of mouse VE-cadherin (New England Peptide). The pY658 antibody was a kind gift from Dr Elisabetta Dejana, IFOM, Milano, Italy and Uppsala University, Sweden). The antibodies were purified and precleared by incubation on fixed and permeabilized Cdh5 null mouse ECs before use. The commercial antibodies used were goat anti-mouse VE-cadherin (AF1002, R&D Systems, 1:500), Mouse anti-VE-cadherin-alexa-647 (561567, Becton Dickinson, 1:500) goat anti-mouse CD31 (AF3628, R&D Systems, 1:500), chicken anti-GFP (ab13970, Abcam, 1:1000), rabbit anti-ERG (ab92513, Abcam, 1:500), mouse anti-Yes (610376, BD Biosciences, 1:400), mouse anti-Src (Clone GD11, Merck Millipore, 1:400).
For western blot, the following primary antibodies were used: rabbit anti-VE-cadherin Y685 (CP1981, ECM Biosciences, 1:1000); goat anti-mouse VE-cadherin (AF1002, R&D Systems, 1:1000); rabbit anti-pSrc (Y418) (44-660G, Thermo Fisher Scientific, 1:1000); mouse anti-Yes (610376, BD Biosciences, 1:1000); and mouse anti-p120-Catenin (610133, BD Biosciences, 1:1000); Mouse anti-c-Src (05-184, Millipore, 1:1000), Rabbit anti-GAPDH (2118, Cell signaling, 1:5000).
For antibody feeding assay, antibody against the VE-cadherin extracellular domain (Clone BV6, MABT134, Merck Millipore) was used.

Validation

Validation of antibodies against pVE-cadherin Y685 and Y731 were done by immunostaining in VE-Cad Y685F and Y731F mutant mice. Results are described in the manuscript (supplementary figure 1).
Validation of pVE-cadherin Y658 antibody was done in the previous publication from Dr Elisabetta Dejana.
Goat anti-mouse VE-cadherin (AF1002, R&D Systems) was validated by western blot and immunohistochemistry by the vendor. Species reactivity: mouse, approximately 30% cross-reactivity with recombinant human VE-Cadherin.
Goat anti-mouse CD31 (AF3628, R&D Systems) was validated by western blot, flow cytometry and immunohistochemistry by the vendor. Species reactivity: mouse, rat, approximately 10% cross-reactivity with recombinant human CD31.
Chicken anti-GFP (ab13970, Abcam) was validated by western blot and immunofluorescence by the vendor.
Rabbit anti-ERG (ab92513, Abcam) was validated by western blot, flow cytometry and immunohistochemistry by the vendor. Species reactivity: mouse, rat, human. The antibody also detects Fli-1.
Mouse anti-YES (610376, BD Biosciences) was validated by western blot and immunohistochemistry by the vendor. Species reactivity: human, mouse, rat, chicken, dog.
Mouse anti-SRC (Clone GD11, Merck Millipore) was validated by western blot by the vendor. Species reactivity: human, mouse, rat.
Rabbit anti-VE-cadherin Y685 (CP1981, ECM Biosciences) was validated by western blot by the vendor. Species reactivity: human.
Rabbit anti-pSRC (Y418) (44-660G, ThermoFisher Scientific) was validated by western blot and immunohistochemistry by the vendor. Species reactivity: human, mouse, chicken.
Mouse anti-p120-Catenin (610133, BD Biosciences) was validated by western blot and immunofluorescence by the vendor. Species reactivity: human, mouse, rat, chicken, dog.
Mouse anti-human VE-cadherin (Clone BV6, MABT134, Merck Millipore) was validated by western blot and immunofluorescence by the vendor. Species reactivity: human.
Rabbit anti-GAPDH (2118, Cell signaling) was validated by western blot, immunohistochemistry and flow cytometry by the vendor. Species reactivity: human, mouse, rat, monkey, bovine, pig.

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | Human primary umbilical vein endothelial cells (HUVECs) were from PromoCell. |
| Authentication | All cells are tested for cell morphology and cell-type specific markers using flow cytometric analyses by the vendor. |
| Mycoplasma contamination | Cells are free of mycoplasma contamination (tested by the vendors). |
| Commonly misidentified lines (See ICLAC register) | No cell lines used in this study are found in the database of commonly misidentified cell lines (ICLAC and NCBI Biosample). |

# Palaeontology and Archaeology

| | |
|---|---|
| Specimen provenance | N/A |
| Specimen deposition | N/A |

| | |
|---|---|
| Dating methods | N/A |

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

| | |
|---|---|
| Ethics oversight | N/A |

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

## 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 | All mice used were C57/Bl6 genetic background. Both males and females are included. For postnatal studies, P6, P7, P15 mice were used. For studies in adult mice, 6-12 week old mice were used. |
| Wild animals | N/A |
| Reporting on sex | Both males and females mice were included in the experiments. Sex-related effects were not observed in the previous studies using the same experimental methods, therefore data from males and females were not analyzed separately. |
| Field-collected samples | N/A |
| Ethics oversight | Animal husbandry and procedures were in accordance with institutional guidelines and approved by the Institutional Review Board for Animal Experimentation at Uppsala University (permit 5.8.18–06789/2018). |

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

## Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

| | |
|---|---|
| Clinical trial registration | N/A |
| Study protocol | N/A |
| Data collection | N/A |
| Outcomes | N/A |

## Dual use research of concern

Policy information about dual use research of concern

### Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

| No | Yes | |
|---|---|---|
| ☒ | ☐ | Public health |
| ☒ | ☐ | National security |
| ☒ | ☐ | Crops and/or livestock |
| ☒ | ☐ | Ecosystems |
| ☒ | ☐ | Any other significant area |

## Experiments of concern

Does the work involve any of these experiments of concern:

| No | Yes | |
|---|---|---|
| ☒ | ☐ | Demonstrate how to render a vaccine ineffective |
| ☒ | ☐ | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| ☒ | ☐ | Enhance the virulence of a pathogen or render a nonpathogen virulent |
| ☒ | ☐ | Increase transmissibility of a pathogen |
| ☒ | ☐ | Alter the host range of a pathogen |
| ☒ | ☐ | Enable evasion of diagnostic/detection modalities |
| ☒ | ☐ | Enable the weaponization of a biological agent or toxin |
| ☒ | ☐ | Any other potentially harmful combination of experiments and agents |

# ChIP-seq

## Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

| | |
|---|---|
| Data access links<br>*May remain private before publication.* | N/A |
| Files in database submission | N/A |
| Genome browser session<br>(e.g. UCSC) | N/A |

## Methodology

| | |
|---|---|
| Replicates | N/A |
| Sequencing depth | N/A |
| Antibodies | N/A |
| Peak calling parameters | N/A |
| Data quality | N/A |
| Software | N/A |

# 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 | N/A |
| Instrument | N/A |
| Software | N/A |
| Cell population abundance | N/A |

Gating strategy N/A

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

## Magnetic resonance imaging

### Experimental design

Design type N/A

Design specifications N/A

Behavioral performance measures N/A

### Acquisition

Imaging type(s) N/A

Field strength N/A

Sequence & imaging parameters N/A

Area of acquisition N/A

Diffusion MRI ☐ Used ☐ Not used

### Preprocessing

Preprocessing software N/A

Normalization N/A

Normalization template N/A

Noise and artifact removal N/A

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

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 |