nature research

Corresponding author(s): Reiner A. Wimmer
Josef M. Penninger

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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	ZEN Blue Edition software V2.0 (Zeiss) was used to collect fluorescent images. FACSDIVA software V8.01 (BD) was used to collect FACS data.
Data analysis	FIJI software V1.52b was used for quantification of fluorescence signals. Statistical analysis was performed using GraphPad Prism version 7.00. FACS data was analysed using FlowJo software V10.3.

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 upon request. 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

RNAseq data and GSEA analysis data have been deposited to NCBI Expression Omnibus and are accessible through the GEO accession numbers GSE89475, GSE92724 and GSE117469.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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 size for in vitro and animal experiments were determined according to the minimal number of independent biological replicates that significantly identified an effect. Experiments were performed at least in triplicate independent measurements or more as indicated in the text.
Data exclusions	No data were excluded
Replication	All experiments in this study were independently replicated, with biological and technical replicates listed in the legends of the corresponding figures with similar results.
Randomization	In animal diabetes experiments and inhibitor-treatment experiments, mice were randomly allocated into each experimental group.
Blinding	Investigators were not blinded to allocation during experiments and outcome assessment. Blinding was not possible as the same investigator processed the organoids/animals and analysed the data.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

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

Antibodies

Antibodies used	For immunocytochemistry following antibodies and reagents were used: anti-human CD31 1:100 (hCD31, DAKO, M082329, Lot.20049471), anti-ICAM-1 1:50 (Sigma, HPA002126, Lot.B89962), anti-PDGFR-β 1:50 (CST, 3169S, Lot.13; R&D systems AF385, Lot.BIWO517081), anti-SMA 1:200 (Sigma, A2547;Lot.077M4846V Abcam, ab5694, Lot.GR3183259-5), anti-Calponin 1:200 (Abcam, AB46794, Lot.GR144432-7, DAKO M35556,Lot.10110188), anti-Collagen Type IV 1:250 (Merck AB769,Lot.2483202), anti-Laminin 1:100 (Merck, AB19012, Lot.2620142), anti-MYH11 1:50 (Sigma HPA014539, Lot.R06879), anti-Fibronectin 1:100 (Sigma F3648, Lot. 103M4818V), anti-Perlecan 1:50 (ThermoFisher 13-4400, Lot.RE215492), anti-von Willebrand factor 1:50 (DAKO, A008229, Lot.20031848), anti-Notch3 1:50 (Abcam, ab23426,

Lot.GR3194355-2), anti-Dll4 1:50 (Abcam, ab183532, Lot.GR162316-13) and Rhodamine labeled Ulex europaeus agglutinin I (UEA-I) 1:25 (Vector labs, RL-1062, Lot.Z0314), VE-Cadherin antibody 1:25 (Santa Cruz sc-9989 ,Lot.I1714), anti-human CD34 1:50 (Invitrogen, 07-3403 Lot.QE220240), anti-SM22 1:100 (Abcam, ab14106, Lot.GR309734-1), anti-NG2 1:50 (Abcam, ab129051, Lot.GR315521-3), anti-Hes5 1:50 (Merck, AB5708, Lot. 2893315), anti-Hey1 1:50 (Invitrogen PA5-40553, Lot.TB2523532), anti-EphrinB2 1:50 (Merck, MABN482, Lot. Q2297391), anti-Dll4 1:50 (Abcam, ab 183532, Lot.GR162316-3), anti-EphB4 1:50 (Invitrogen 371800, Lot.QF216343), anti-CoupTF2 1:50 (Abcam, ab41859, Lot.GR253380-17)

For FACS experiments following antibodies were used:
anti-CD31 1:200 (BD, 558094, Lot.5107596), anti-VE-Cadherin 1:20 (BD, 565672, Lot.7005701), anti-PDGFR-β 1:10 (BD, 558821), anti-CD90 1:20 (Biolegend, 328117 Lot.B212881), anti-CD44 1:10 (BD, 550989 Lot.7104817) anti-CD45 1:20 (ebioscience, 11-0459-41), and anti-CD73 1:20 (BD 742633, Lot.6357896, BD 561254, Lot.4233747)

Validation

All antibodies used in this study are commercially available and have been validated for the application by the manufacturer. Specific references for each antibody can be found on the suppliers homepage.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

The embryonic stem cell line H9 was purchased from WiCell.
The iPSC line NC8 was provided by Manfred Boehm, NIH.
The iPSC#2 and iPSC#3 lines were generated by in house reprogramming using Cytotune 2.0 (see Methods).
Primary human umbilical vein endothelial cells (HUVEC) (#C-12203) and primary human pericytes from placenta (#C-12980) were purchased from Promocell.
The immortalized human dermal endothelial cell line G1S1 was provided by the Kerjaschki lab.

Authentication

None of the cell lines were authenticated

Mycoplasma contamination

The cell lines used in this study were tested negative for mycoplasm.
Testing was performed on a monthly routine.

Commonly misidentified lines
(See ICLAC register)

None of the used cell lines are listed in the ICLAC database of commonly misidentified cell lines.

Animals and other organisms

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

Laboratory animals

12-15 weeks old immunodeficient NSG (NOD scid gamma) mice were used in this study.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve field-collected samples.

Human research participants

Policy information about studies involving human research participants

Population characteristics

This study includes RNA sequencing and immunohistological data of a total of 13 patients diagnosed with Type 2 diabetes (T2D) and 13 patients without T2D as controls.
All patients were recruited at the Medical University of Vienna, Austria.
Mean age: T2D 54,6 ± 16 years, Control 47,4 ±15,5 years
More details about the population can be found in Supplementary table 1.

Recruitment

Prerequirement for studying diabetic microangiopathy was the reliable and correct clinical diagnosis of diabetes mellitus and associated microangiopathy. During this recruitment period, physical, serological and organ-specific diagnosis of type 2 diabetes was carried out by our medical cooperation partners at the clinical level. Careful monitoring of patients was performed, including a list of clinical criteria. Eligible patients were then included into the study, unless these patients were not willing to give informed consent. All samples were taken during necessary surgical interventions.
Strategies to prevent bias were the following: Samples were immediately provided with coded ID-numbers. Samples were analyzed at the same time to ensure identical experimental conditions and to prevent time-dependent bias. Immunohistological stainings were assessed and evaluated without knowledge of disease status. Molecular analyses were performed in a blinded fashion, i.e., without the knowledge of the clinical status (T2D or non-T2D) of the individual patients.

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	Please refer to Methods
Instrument	Please refer to FACS analysis of vascular organoids or Human patient-derived endothelial cell preparations within the methods section
Software	Data were analyzed using FlowJo v10.0.6 software (Tree Star)
Cell population abundance	Endothelial cells and pericytes were purified by FACS sorting. Purity was assessed to be >90% using FACS analysis
Gating strategy	To gate samples for FACS analysis or sorting, cells were initially gated by FSC-A vs SCS for the exclusion of debris. For single cells, samples were further gated by FSC-A vs FSC-H. Finally, DAPI staining was used to exclude dead cells

☒ 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	Mice were scanned in vivo, structural and perfusion scan. For structural scan a MSME Spine Echo sequence was acquired. Perfusion: A pre-bolus injection of 0.05 ml of 0.01 mol/l gadolinium-based contrast agent (Magnevist, Berlex) was injected to correct for contrast agent leakage. Dynamic susceptibility contrast (DSC) perfusion MRI was collected using fast imaging with steady-state precession (FISP) with 500.6 ms temporal resolution following tail vein injection of 0.05 mL of 0.25 mol/L Magnevist.
Design specifications	n/a
Behavioral performance measures	n/a

Acquisition

Imaging type(s)	Structural, Perfusion
Field strength	15.2 Tesla Bruker
Sequence & imaging parameters	Structural: MSME, Spin Echo, 30*30mm, 256*256, 0.5mm 30 slices, axial, V-C, head-supine, TE 5.8 -81.18 (14 echoes), TR 3000 Perfusion: FISP, 30*30mm, 64*64, 1mm 1 slice, axial, V-D, head supine, TE 1.7, TR3.42, AV 2, repetitions 360, FA 5, temporal resolution 500.6ms,
Area of acquisition	Full coverage of left and right kidney

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	Processing was done using ImageJ (rsbweb.nih.gov/ij/), and the DSCoMAN plugin (https://dblab.duhs.duke.edu/wysiwyg/downloads/DSCoMAN_1.0.pdf)
Normalization	n/a
Normalization template	n/a
Noise and artifact removal	During acquisition scans were gated by the respiration of the mouse
Volume censoring	n/a

Statistical modeling & inference

Model type and settings	Analysis consisted of truncating the first 5 time points in the DSC-MRI time series to ensure steady-state magnetization, calculating the pre-bolus signal intensity () on a pixel-wise basis, converting the truncated DSC-MRI time series to a relaxivity-time curve (), is dynamic signal intensity curve, and correcting for the gadolinium leakage (K2)
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