

1 **Supplementary information**

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3 **Modeling diabetic retinopathy early pathophysiological phenotypes in a human inner**

4 **blood-retinal barrier-on-a-chip**

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6 Maurissen et al.

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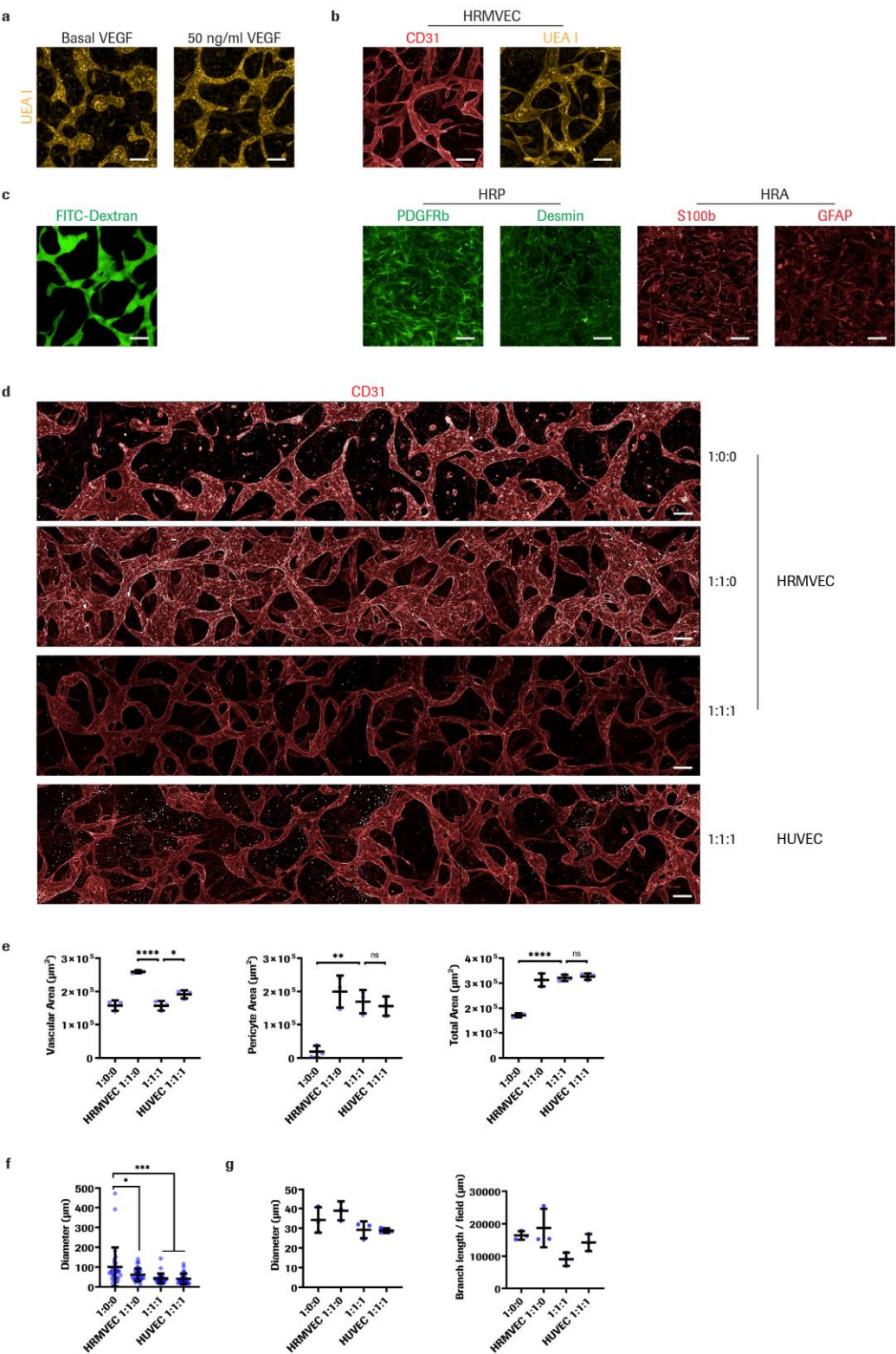
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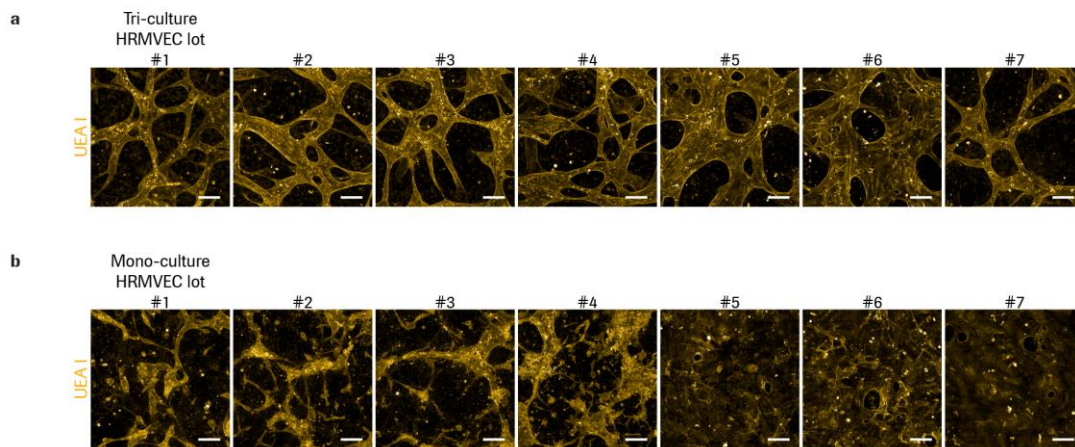
27 Supplementary Fig. 18 | Treatments analyte measurements.

28 Supplementary Table 1 | Information about cells used in this study.

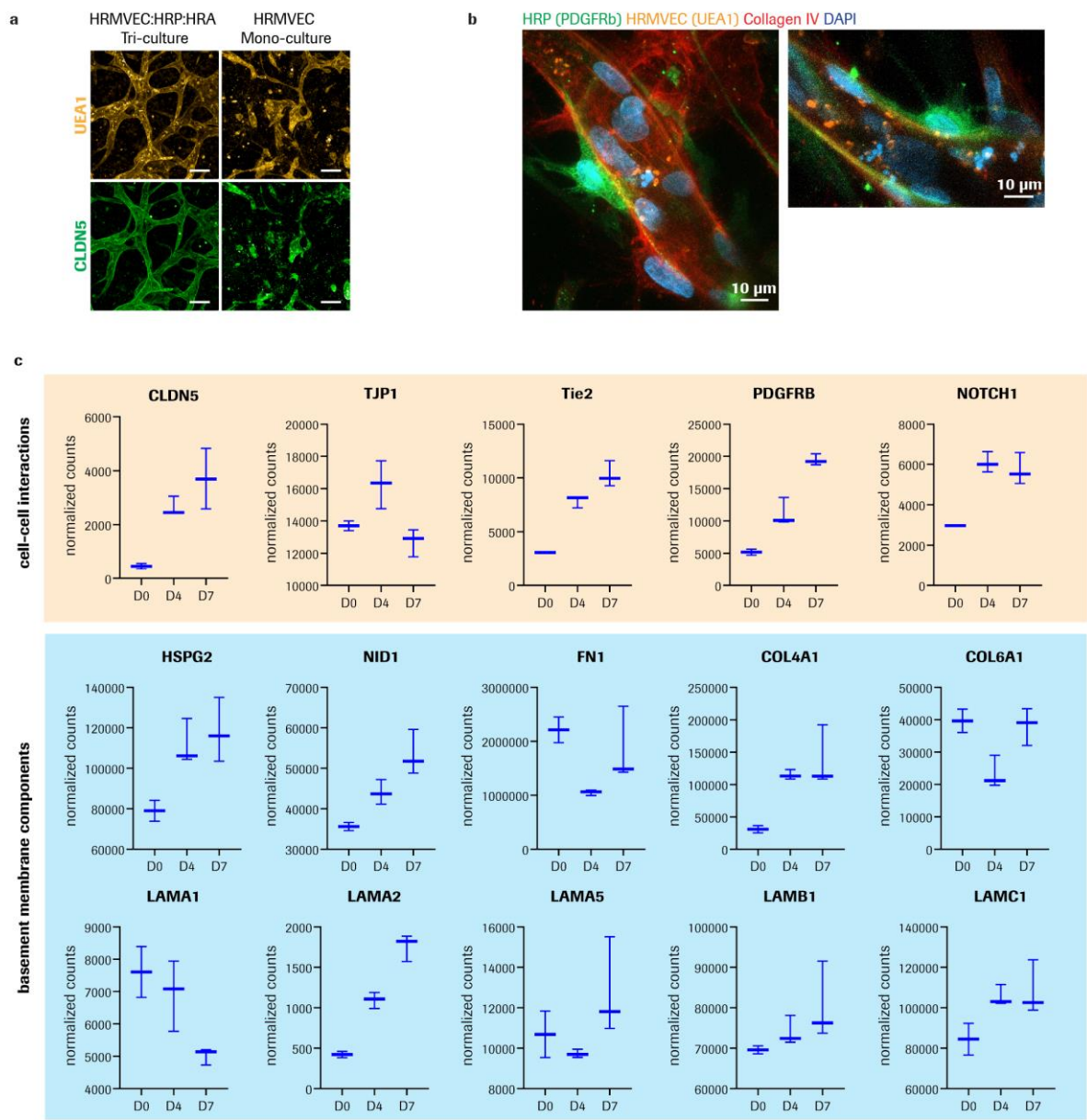
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Supplementary Fig. 1 | Characterization of iBRB MVN morphology. **a**, Representative images of endothelial networks (UEA I) cultured in medium with basal (5 ng ml⁻¹) VEGF (left) or in medium supplemented with 50 ng ml⁻¹ VEGF (right). **b**, Representative images of endothelial (HRMVEC) markers CD31 and UEA I (top), pericyte (HRP) markers PDGFR β and Desmin (bottom left) and astrocyte (HRA) markers S100b and GFAP (bottom right). Stainings were repeated in n = 3 independent experiments with similar results. **c**, Endothelial networks perfused with FITC-dextran (100 μ g ml⁻¹, 10 kDa). **d**, Global images of different HRMVEC:HRP:HRA cell ratios: monoculture (1:0:0), co-culture with HRMVECs and HRPs (1:1:0) and tri-culture with HRMVECs, HRPs and HRAs (1:1:1). Tri-culture with HUVECs, HRPs and HRAs (1:1:1) is shown as comparison. **e**, Quantification of mean vascular, pericyte and total areas per channel. n = 3 HRMVEC 1:0:0, n = 3 1:1:0, n = 3 1:1:1 and n = 3 HUVEC 1:1:1 whole channels analyzed from n = 3 independent experiments. **f**, Distribution of vessel diameters for one whole channel per condition quantified manually. n = 6 HRMVEC 1:0:0, n = 6 1:1:0, n = 6 1:1:1 and n = 6 HUVEC 1:1:1 network ROI analyzed. **g**, Quantification of mean vessel diameter and branch length per channel. n = 3 HRMVEC 1:0:0, n = 3 1:1:0, n = 3 1:1:1 and n = 3 HUVEC 1:1:1 whole channels analyzed from n = 3 independent experiments. Data are mean $\pm$ s.d. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; one-way ANOVA. Source data are provided as a Source Data file. All images show maximum intensity projections of 290 μ m Z-stacks. Scale bars, 100 μ m (**a-d**).



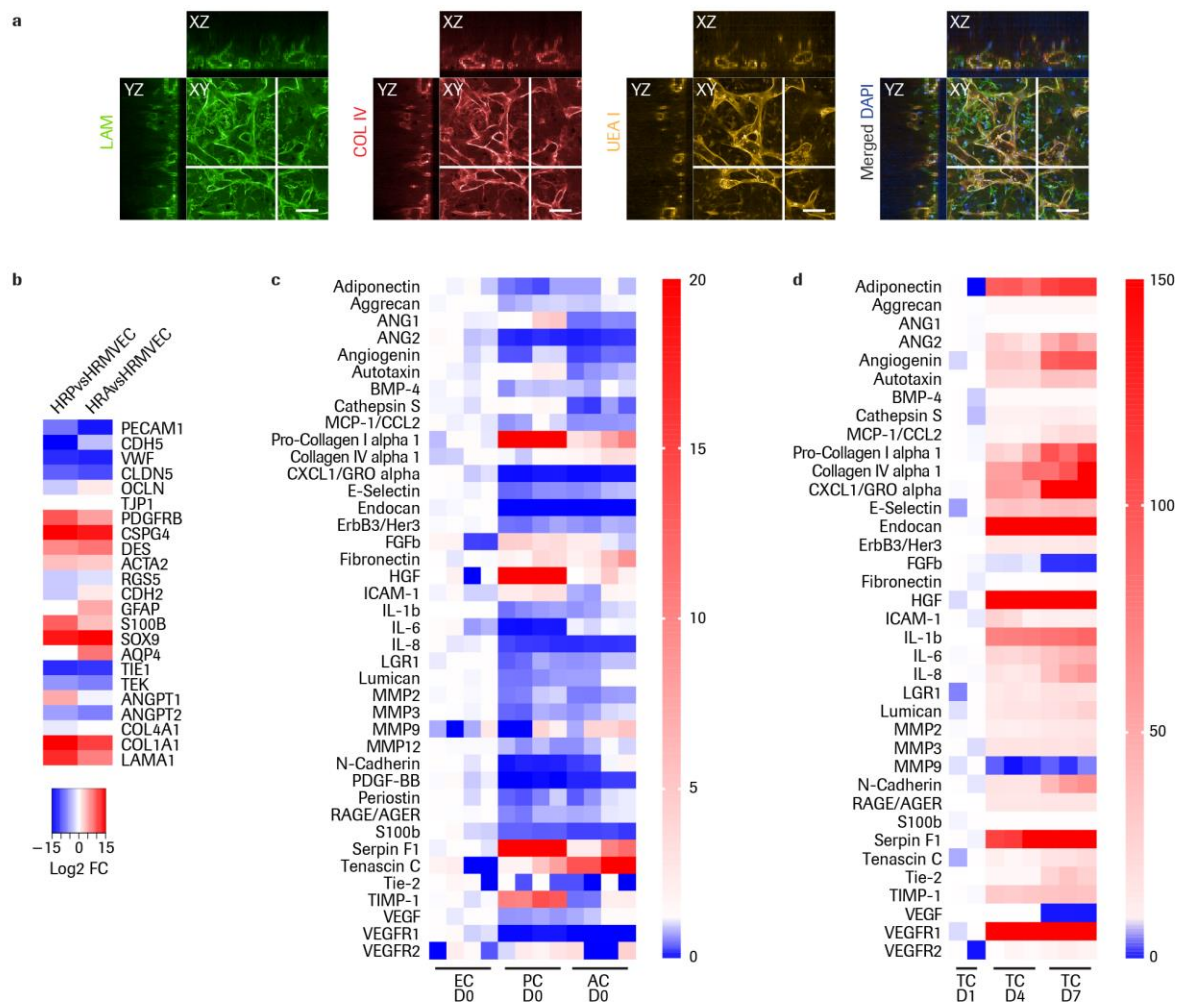
Supplementary Fig. 2 | Donor heterogeneity on iBRB MVN formation. **a**, Representative image of comparison between seven different lots of HRMVECs (UEA I) co-cultured with HRPs and HRAs. **b**, Comparison between different lots of HRMVECs (UEA I) in mono-culture. HRMVEC lots #5, #6 and #7 formed a monolayer. All images show maximum intensity projections of 290 μm Z-stacks. Scale bars, 100 μm (**a**, **b**).



Supplementary Fig. 3 | Characterization of iBRB MVN formation. **a**, Representative image comparison between 3D mono- and tri-cultures, and tight junction (CLDN5) integrity. Images show maximum intensity projections of 290 μ m Z-stacks. Scale bars, 100 μ m. **b**, Confocal images at 63x magnification showing pericytes (PDGFR β green) lining the vessels (UEA I orange). **c**, Normalized counts of genes involved in cell-cell interactions and basement membrane

67 composition during the maturation phase of the iBRB-on-a-chip (D0 to D7). Data are from n = 3
68 independent experiments.

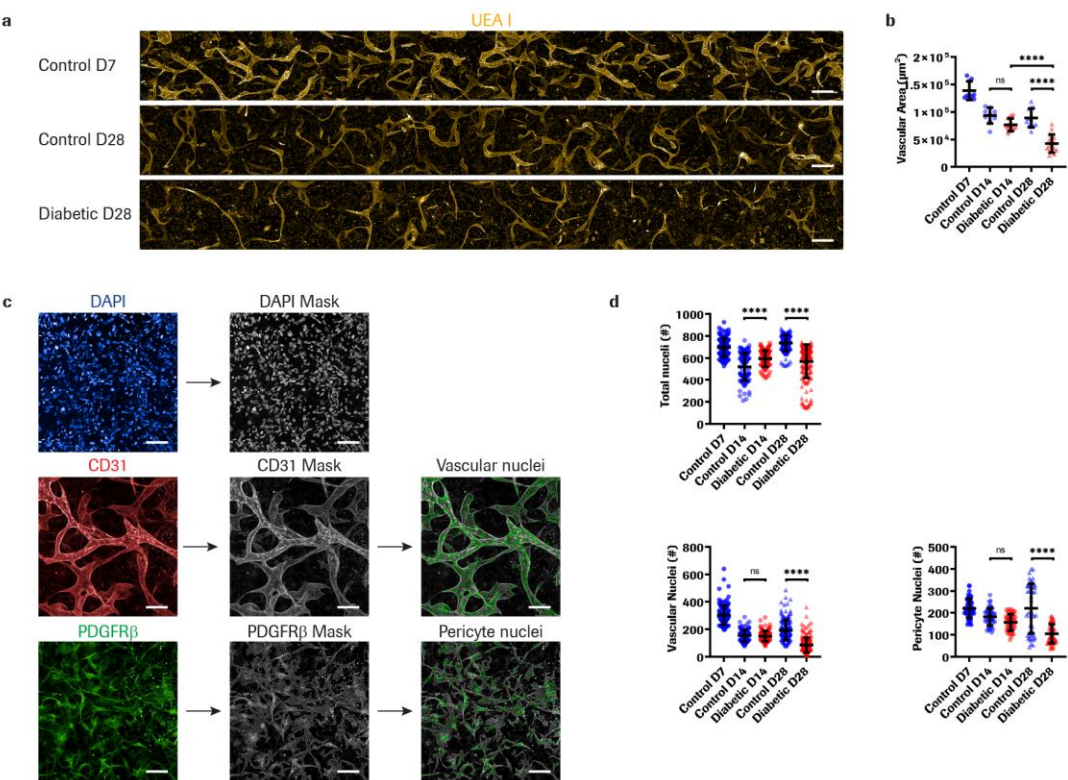
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Supplementary Fig. 4 | Characterization of iBRB maturity. **a**, Representative cross-section images of basement membrane proteins (LAM and COL IV). Images show orthogonal projections of 290 μ m Z-stacks. Stainings were repeated in n = 3 independent experiments with similar results. **b**, Heat map of differential gene expression between HRP and HRMVECs, and between HRAs and HRMVECs on D0. Results are from a characterization gene panel without cutoff. Data are RNA-Seq aggregated Log2 FC from n = 3 independent experiments. Genes with a high Log2 FC (red) are upregulated in HRPs or HRAs while genes with a low Log2 FC (blue) are upregulated in HRMVECs. **c**, Heat map of analyte measurements on HRMVEC (EC), HRP

80 (PC) and HRA (AC) supernatants on D0, showing analyte concentrations normalized to the mean
81 EC value on D0. **d**, Heat map of analyte measurements on tri-culture (TC) supernatants on D1,
82 D4 and D7, normalized to the mean TC value on D1. The scale indicates fold change to control.
83 Supernatants were obtained from n = 4 independent experiments. Source data are provided as a
84 Source Data file. Scale bars, 100 μ m (**a**).

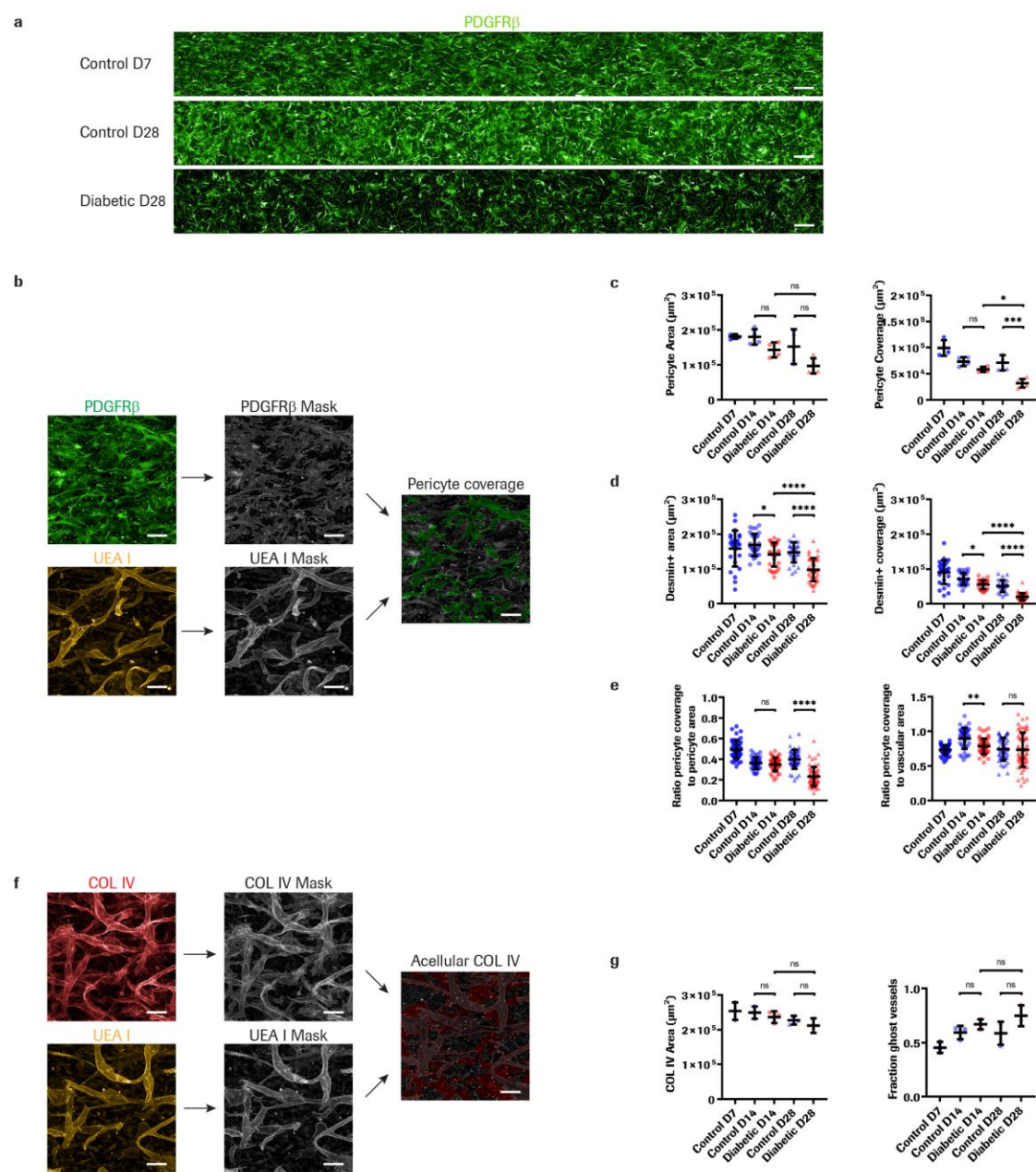
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Supplementary Fig. 5 | Chronic diabetic treatment causes vascular regression. a, Representative channel images of iBRB MVNs (UEA I) cultured in normal medium on D7 (top), D28 (middle) or in diabetic medium on D28 (bottom). Vascular regression is visible in the diabetic condition on D28. **b,** Quantification of mean vascular area per channel. $n = 9$ control D7, $n = 9$ D14, $n = 12$ D28, $n = 9$ diabetic D14 and $n = 15$ D28 whole channels analyzed from $n = 3$ independent experiments. **c,** Image analysis pipeline of maximum intensity projections obtained from whole-channel image acquisitions with fluorescence confocal microscopy. Nuclei (DAPI) were segmented, and ECs (CD31) and pericytes (PDGFR β) were thresholded to obtain masks and quantify respective areas. Nuclei included in each mask were also quantified. **d,** Quantification of the number of total (top), vascular (bottom left) and pericyte (bottom right) nuclei for control

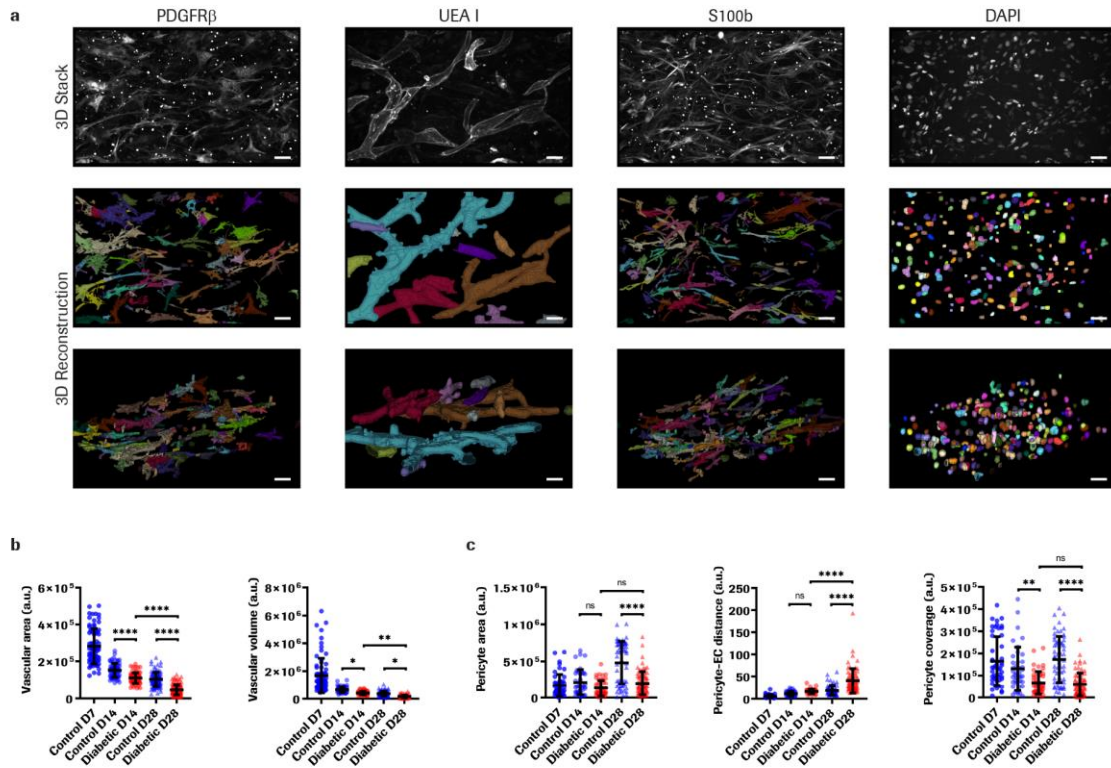
98 conditions on D7, D14 and D28 and diabetic conditions on D14 and D28. n = 113 control D7, n =
99 117 D14, n = 165 D28, n = 117 diabetic D14 and n = 203 D28 ROI networks analyzed for total
100 and vascular nuclei, and n = 49 control D7, n = 42 D14, n = 48 D28, n = 48 diabetic D14 and n =
101 61 D28 ROI networks analyzed for pericyte nuclei from n = 3 independent experiments. Data are
102 mean $\pm$ s.d. ****P<0.0001; one-way ANOVA. Source data are provided as a Source Data file.
103 Images show maximum intensity projections of 395 μ m Z-stacks. Scale bars, 100 μ m (**a**, **c**).

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Supplementary Fig. 6 | Chronic diabetic treatment causes reduced perivascular coverage. a, Representative channel images of pericytes (PDGFR β), with pericyte loss visible in the diabetic condition on D28. **b,** Image analysis for quantifying pericyte coverage. Maximum intensity projections of pericytes (PDGFR β) and ECs (UEA I) were thresholded to obtain masks, and the

pericyte coverage was calculated from the overlapping areas. **c**, Quantification of mean pericyte area and coverage per channel. n = 4 control D7, n = 4 D14, n = 4 D28, n = 4 diabetic D14 and n = 5 D28 whole channels analyzed from n = 3 independent experiments. **d**, Quantification of pericyte area (left) and coverage (right) based on the pericyte-specific marker Desmin, for control conditions on D7, D14 and D28 and diabetic conditions on D14 and D28. The results are consistent with pericyte quantifications using the PDGFR β marker. n = 26 control D7, n = 30 D14, n = 27 D28, n = 29 diabetic D14 and n = 40 D28 ROI networks analyzed from n = 2 independent experiments. **e**, Quantification of pericyte coverage to pericyte area ratio (left) and pericyte coverage to vascular area ratio (right). n = 49 control D7, n = 42 D14, n = 48 D28, n = 48 diabetic D14 and n = 61 D28 ROI networks analyzed from n = 3 independent experiments. **f**, Image analysis of ghost vessels. Maximum intensity projections of basement membrane (COL IV) and ECs (UEA I) were thresholded and ghost vessels were obtained by calculating the difference between COL IV and UEA I mask areas. **g**, Quantification of mean COL IV area and avascular area per channel. n = 3 control D7, n = 3 D14, n = 3 D28, n = 3 diabetic D14 and n = 3 D28 whole channels analyzed from n = 3 independent experiments. Data are mean $\pm$ s.d. *P<0.05; **P<0.01; ****P<0.0001; one-way ANOVA. Source data are provided as a Source Data file. Images show maximum intensity projections of 395 μ m Z-stacks. Scale bars, 100 μ m (**a**, **b**, **f**).

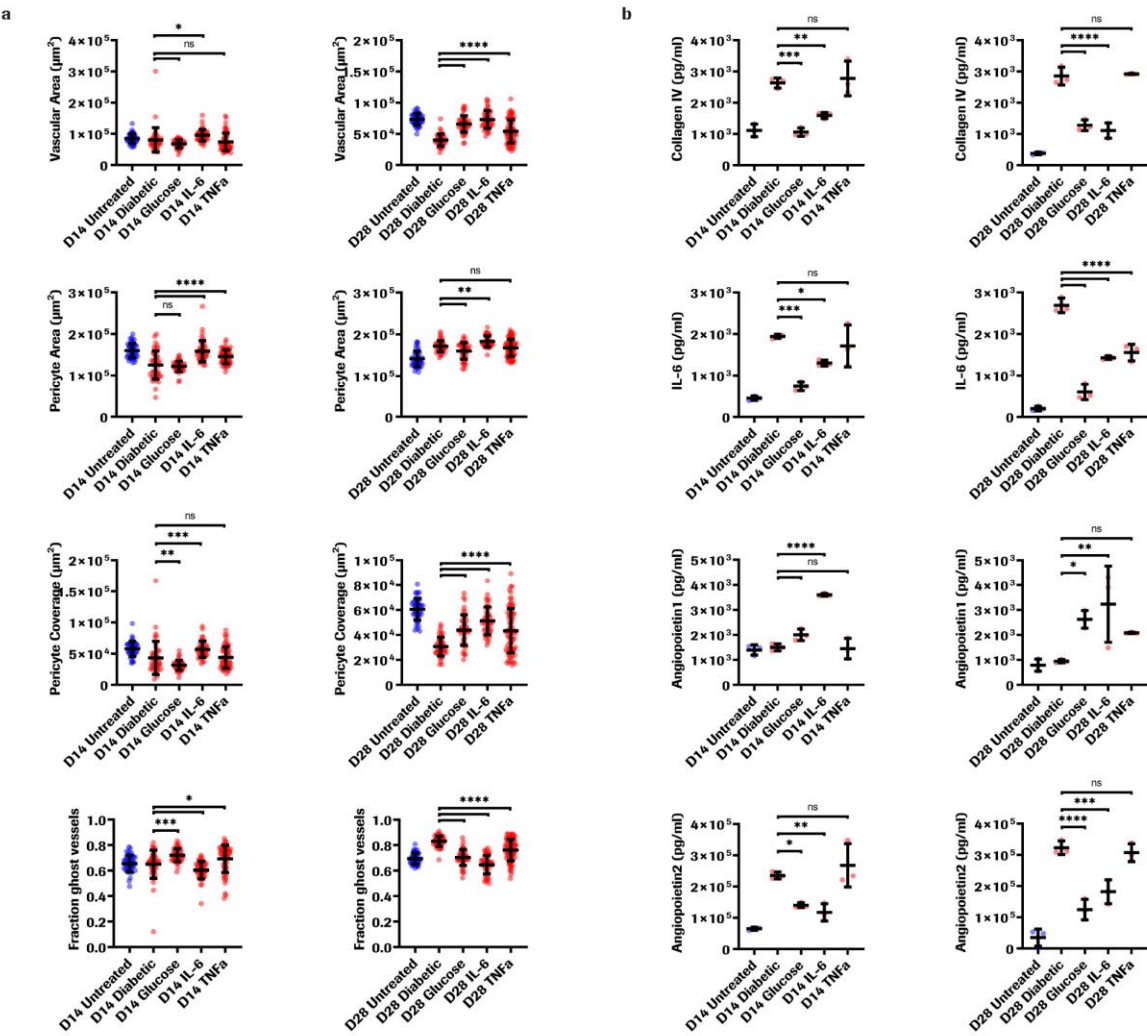


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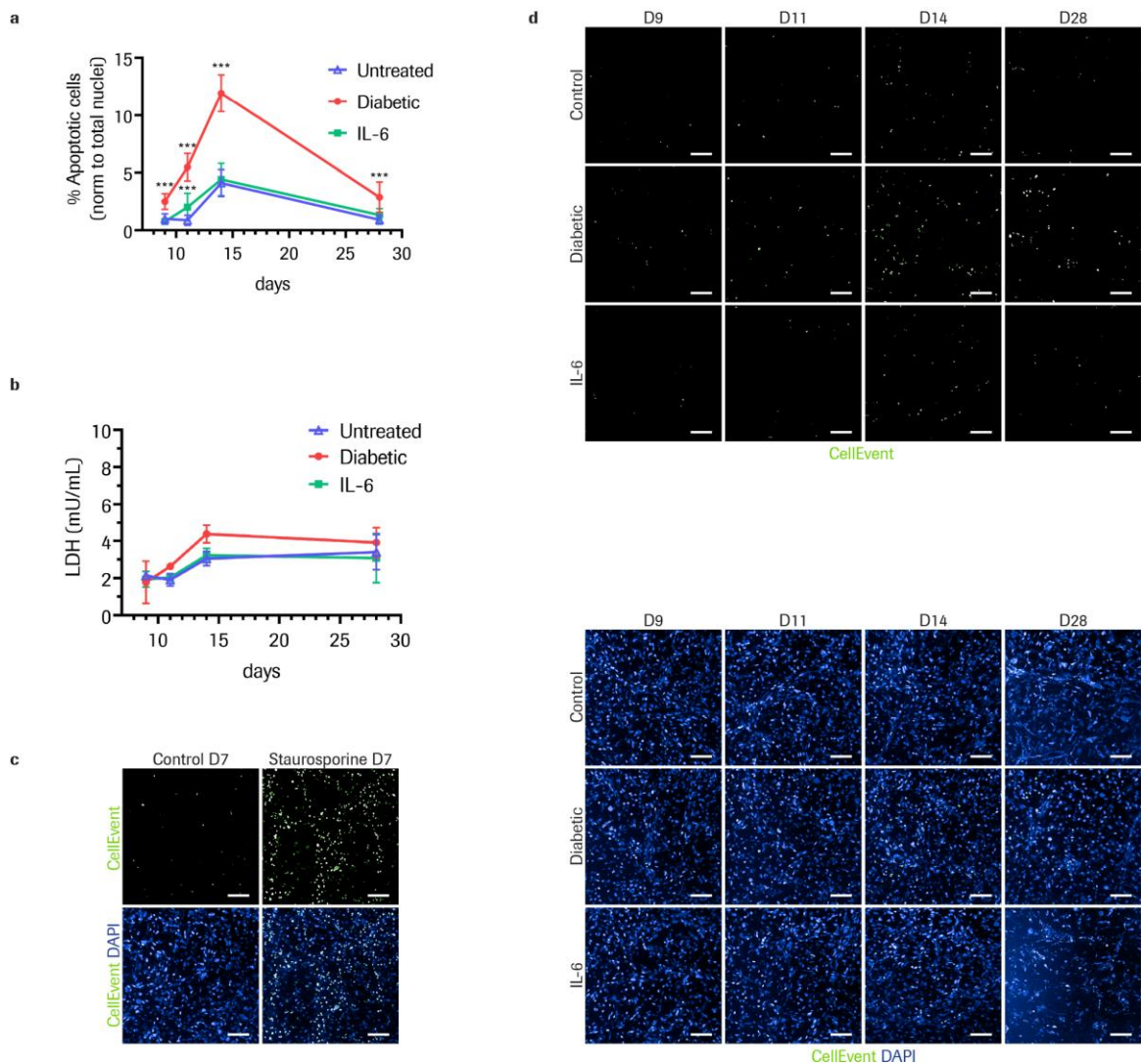
Supplementary Fig. 7 | 3D analysis confirms diabetic phenotypic changes. **a**, 3D image analysis through reconstruction and segmentation of pericyte- (PDGFR β), EC- (UEA I), astrocyte- (S100b) and nuclei-specific (DAPI) stainings. Fluorescent image stacks (top), and different views of image reconstructions (middle and bottom) are visualized. Images show 3D views of 395 μ m Z-stacks. **b**, 3D quantification of vascular area (left) and volume (right) for control conditions on D7, D14 and D28 and diabetic conditions on D14 and D28. $n = 75$ control D7, $n = 72$ D14, $n = 105$ D28, $n = 77$ diabetic D14 and $n = 130$ D28 ROI networks analyzed from $n = 3$ independent experiments. **c**, 3D quantification of pericyte area (left), and pericyte-EC distance (middle) and area (right). Direct pericyte-EC interactions are reduced with diabetic treatment. $n = 49$ control D7, $n = 42$ D14, $n = 63$ D28, $n = 48$ diabetic D14 and $n = 76$ D28 ROI networks analyzed from $n = 3$ independent experiments. Data are mean $\pm$ s.d. * $P < 0.05$; ** $P < 0.01$;

143 ****P<0.0001; one-way ANOVA. Source data are provided as a Source Data file. Images show
144 maximum intensity projections of 395 μm Z-stacks. Scale bars, 100 μm (**a**, **d**).

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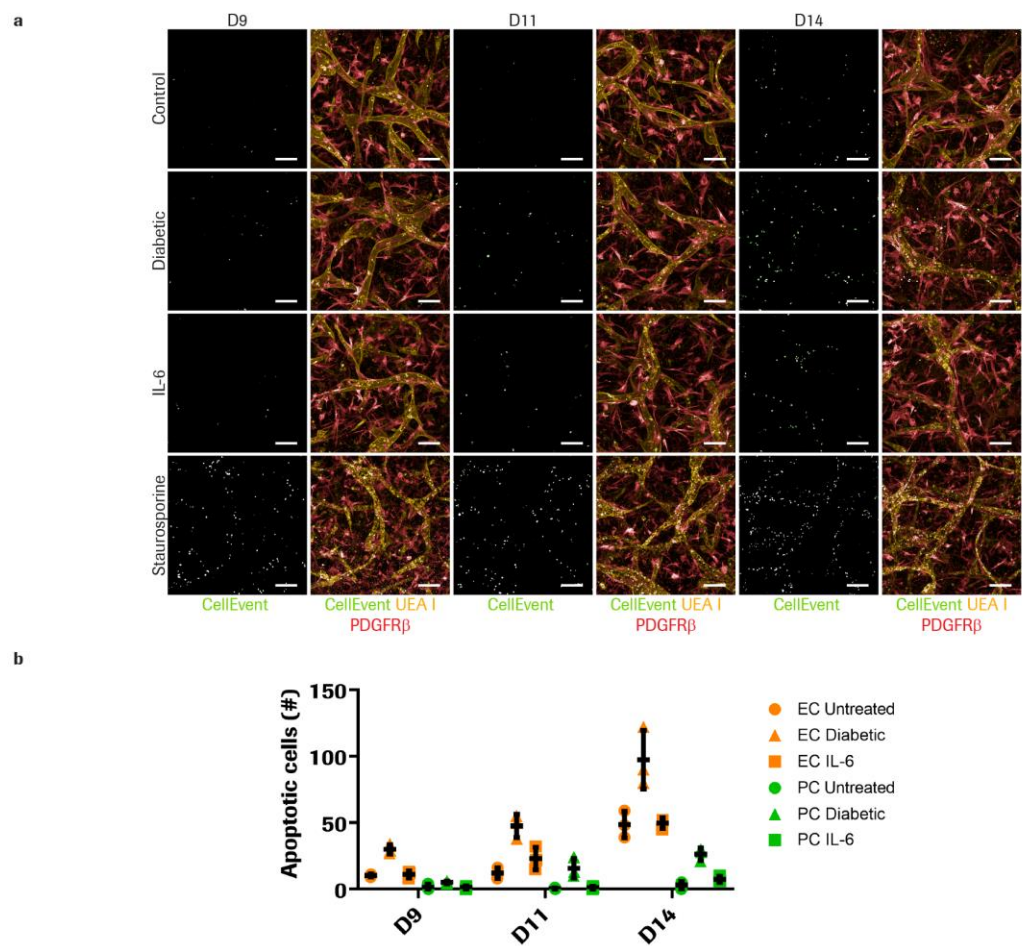
Supplementary Fig. 8 | Effects of diabetic cocktail components. **a**, Quantification of vascular area, pericyte area and coverage, and fraction ghost vessels for untreated, diabetic, glucose, IL-6 and TNF- α conditions on D14 and D28. n = 74 untreated D7, n = 45 D14, n = 44 D28, n = 44 diabetic D14, n = 43 D28, n = 44 glucose D14, n = 44 D28, n = 45 IL-6 D14, n = 44 D28, n = 59 TNF- α D14 and n = 75 D28 treated ROI networks analyzed from n = 3 replicate channels. **b**, Analyte measurements showing supernatant concentrations of human collagen IV alpha I, IL-6, Angiopoietin-1 and Angiopoietin-2 obtained from n = 3 replicate channels. Data are mean $\pm$ s.d. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; one-way ANOVA. Source data are provided as a Source Data file. **c**, Representative images of endothelial networks (UEA I) and overlay images with pericytes (PDGFR β) or basement membranes (COL IV) on D14 and D28, following 7 (D14) or 21 (D28) days of treatment respectively. Treatments with D-Glucose, IL-6 or TNF- α were tested individually, and in combination to produce the diabetic treatment (Diabetic). All images show maximum intensity projections of 245 μ m Z-stacks. Scale bars, 100 μ m (**c**).



Supplementary Fig. 9 | Cell viability assays. **a**, Quantification of the percentage of apoptotic cells (CellEvent) normalized to total nuclei (DAPI) per field for untreated, diabetic and IL-6-treated conditions over time. n = 45 untreated D9, D11, D14 and D28, n = 45 diabetic D9 and D14, n = 44 diabetic D11 and D28, n = 45 IL-6 D9, D14 and D28, and n = 44 IL-6 D11 treated ROI networks analyzed from n = 3 replicate channels. **b**, Quantification of LDH release for untreated, diabetic and IL-6 conditions over time obtained from n = 3 replicate channels for each condition. Data are mean $\pm$ s.d. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; one-way

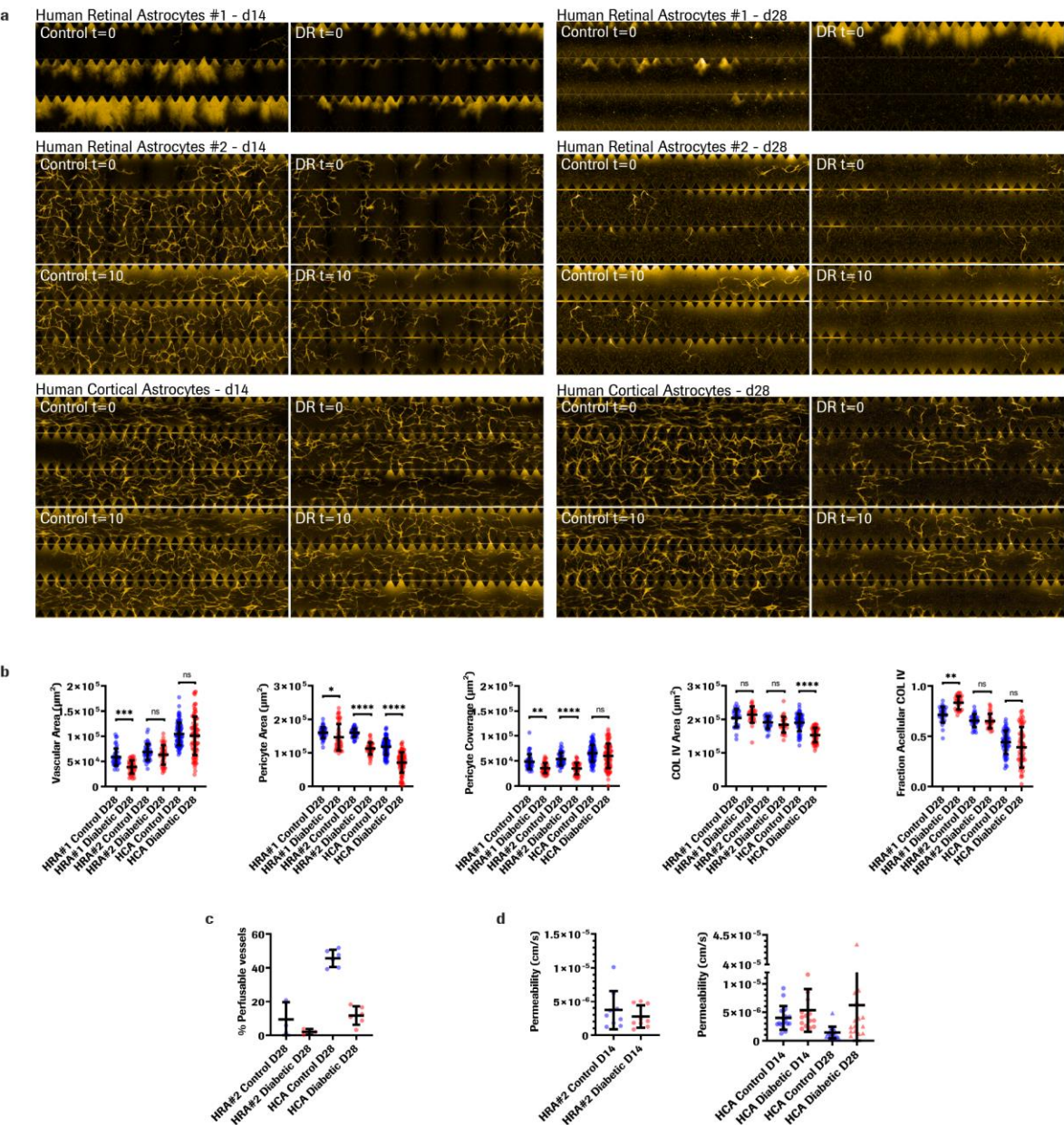
171 ANOVA. Source data are provided as a Source Data file. **c**, Representative images of untreated
172 control and Staurosporine-treated cells on D7. CellEvent marks apoptotic nuclei and DAPI all
173 nuclei. Staurosporine is a positive control inducing apoptosis. **d**, Representative images of
174 untreated control, diabetic cocktail, IL-6 alone, and Staurosporine control on D9, D11, D14 and
175 D28. All images show maximum intensity projections of 245 μm Z-stacks. Scale bars, 100 μm (**c**,
176 **d**).

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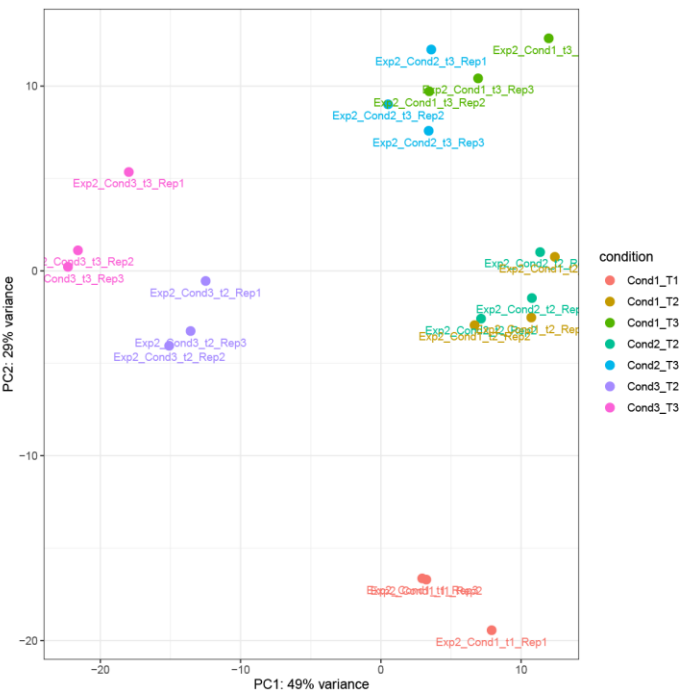
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180 **Supplementary Fig. 10 | Sequence of cell death events.** **a**, Representative images of apoptotic
181 nuclei (CellEvent), endothelial network (UEA I) and pericyte (PDGFR β) stainings on D9, D11
182 and D14 for untreated control, diabetic, IL-6 and Staurosporine-treated conditions (positive
183 control). **b**, Number of apoptotic cells by cell type; endothelial cells (EC) in orange and pericytes
184 (PC) in green on D9, D11 and D14 for untreated control, diabetic and IL-6 conditions. One
185 representative ROI per $n = 3$ replicate channels was quantified for each condition on D9, D11 and
186 D14. Source data are provided as a Source Data file. All images show maximum intensity
187 projections of 245 μm Z-stacks. Scale bars, 100 μm (**a**).

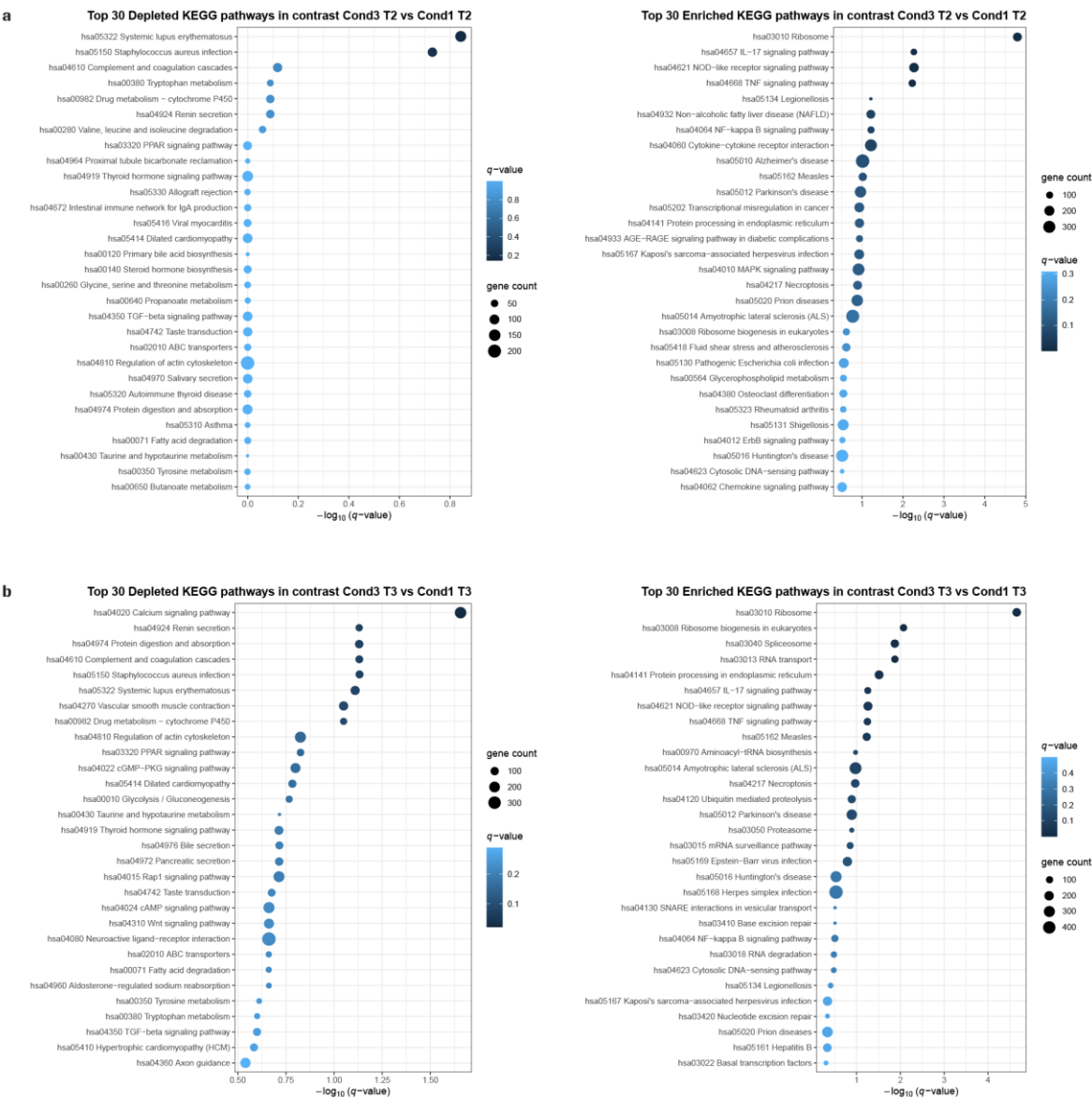


190 **Supplementary Fig. 11 | Perfusability and permeability assays. a**, Images of untreated control
191 and diabetic iBRB MVNs perfused with TRITC-labelled 70kDa dextran at t = 0 and t = 10 min.
192 Human retinal astrocytes (HRA) and human cortical astrocytes (HCA) from different donors
193 were compared on D14 and D28. Triplicate channels are shown for each condition. Images show
194 maximum intensity projections of 190 μm Z-stacks. Channel height visible is 1.3 mm. **b**,

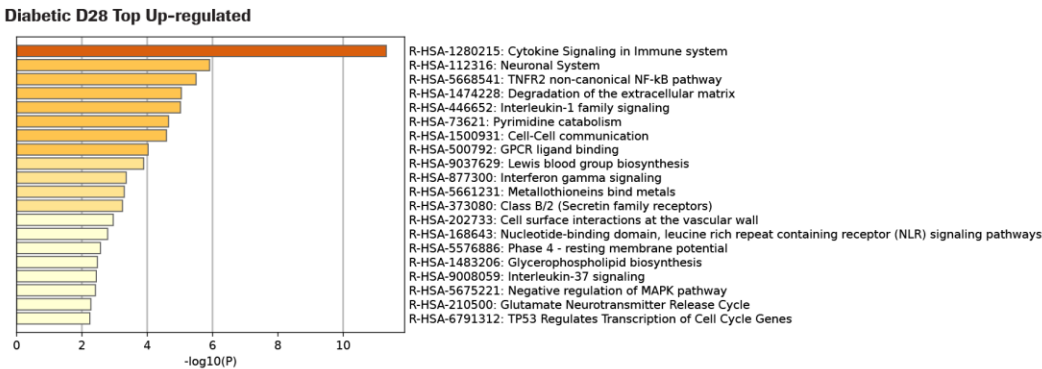
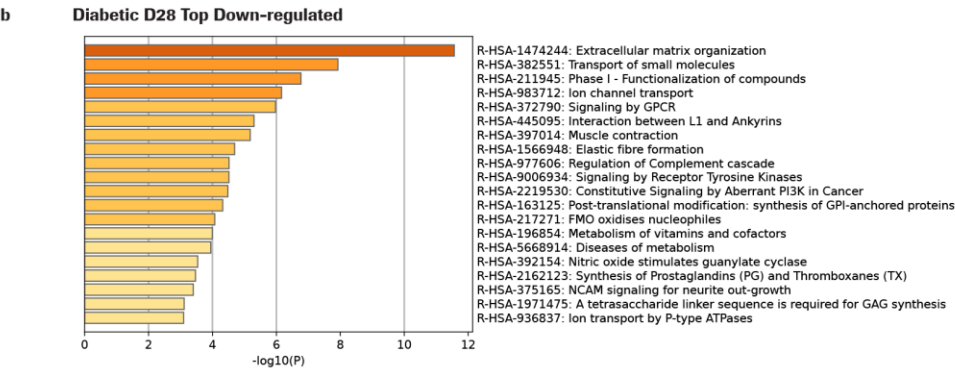
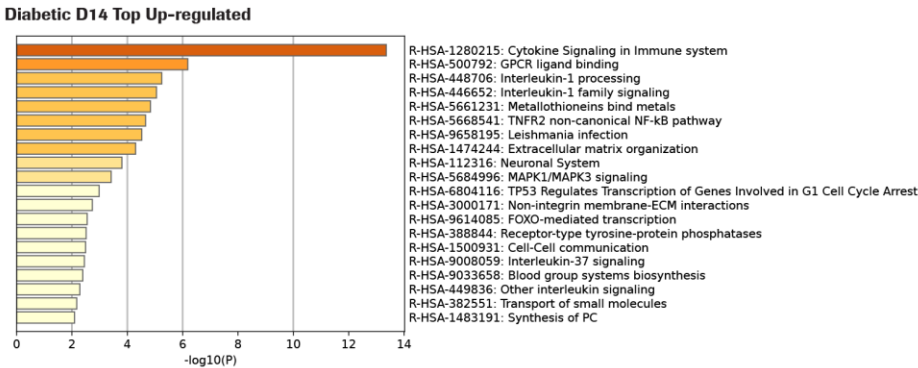
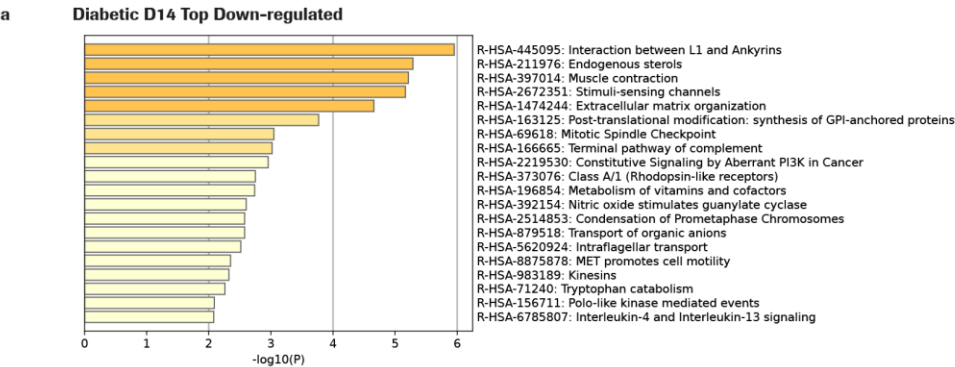
Quantifications of vascular area, pericyte area and coverage, and COL IV area and avascular area. n = 45 HRA#1 untreated, n = 45 diabetic D28, n = 45 HRA#2 untreated, n = 45 diabetic D28, n = 91 HCA untreated, n = 90 diabetic D28 treated ROI networks analyzed from n = 3 replicate channels. n = 91 HCA diabetic D28 treated networks for pericyte area. n = 30 HRA#1 untreated, n = 30 diabetic D28, n = 30 HRA#2 untreated, n = 30 diabetic D28, n = 60 HCA untreated, n = 58 diabetic D28 treated ROI networks for COL IV area and avascular area. Data are mean $\pm$ s.d. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; one-way ANOVA. **c**, Perfusability corresponding to the fraction of TRITC+ perfused vessels to UEA I+ total vessels on D28 for n = 3 HRA#2 and n = 6 HCA replicate channels per condition. **d**, Permeability values of perfusable untreated and diabetic MVNs containing HRA#2 or HCA in tri-cultures. n = 9 HRA#2 (3 channels), and n = 18 HCA (6 channels) network ROI analyzed per condition. Source data are provided as a Source Data file.



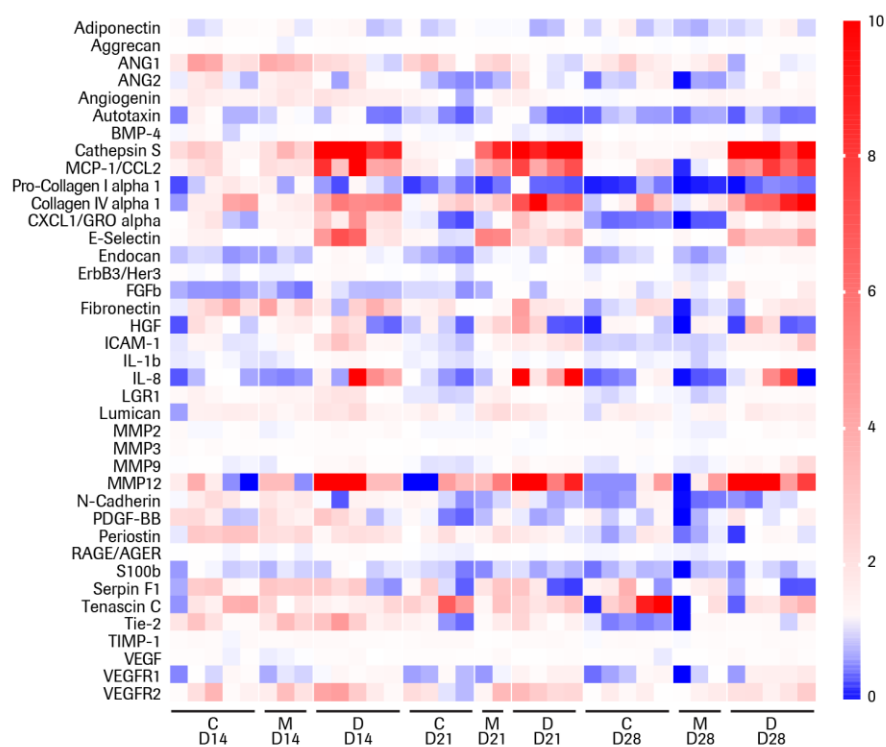
210 **Supplementary Fig. 12 | RNA-sequencing analysis.** Principal component analysis of RNA-
211 sequencing replicate samples from n = 3 independent experiments. Cond1, untreated control;
212 Cond2, osmotic control; Cond3, diabetic condition; T1, D7; T2, D14; T3, D28; Rep1-3, replicate
213 1-3.



217 **Supplementary Fig. 13 | Gene set enrichment analysis. a**, Top 30 differentially regulated
218 KEGG pathways between the diabetic condition and untreated control on D14. **b**, Top 30 KEGG
219 pathways on D28.



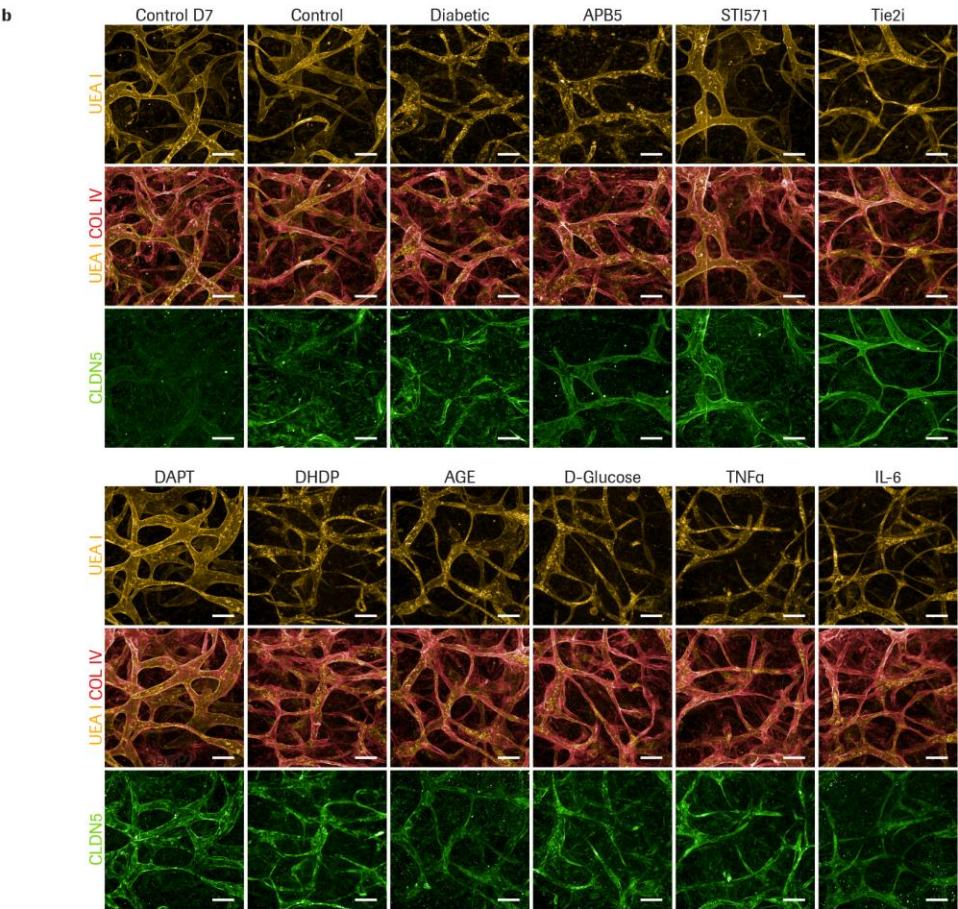
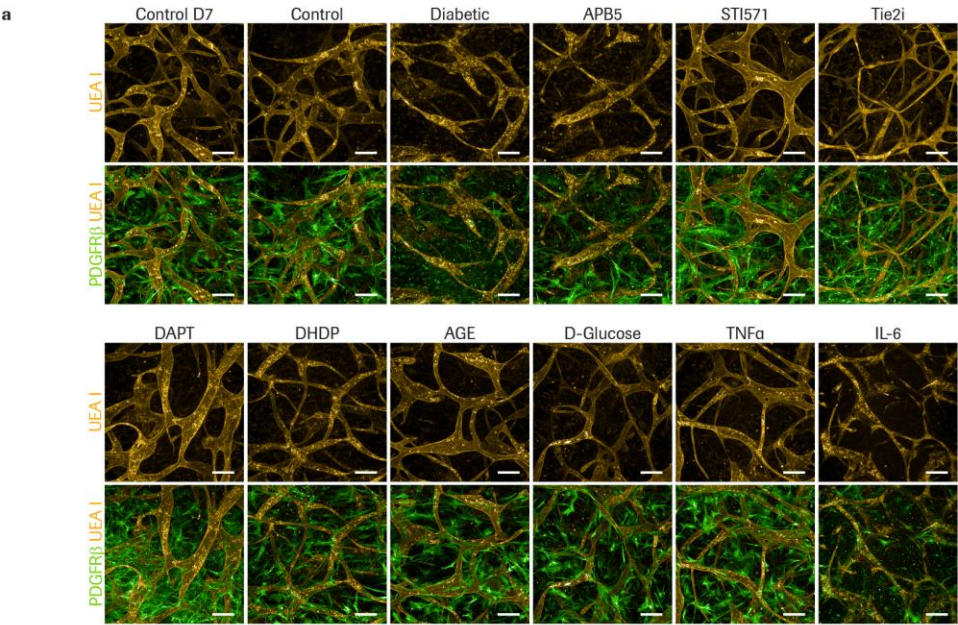
223 **Supplementary Fig. 14 | Pathway analysis. a**, Top 20 differentially regulated Reactome
224 pathways between the diabetic condition and untreated control on D14. As an input, differentially
225 regulated genes with an absolute Log2 FC > 1 were selected. **b**, Top 20 Reactome pathways on
226 D28.
227



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230 **Supplementary Fig. 15 | Diabetic analyte measurements.** Heat map of analyte measurements
231 on untreated control, osmotic control and diabetic supernatants on D14, D21 and D28, showing
232 analyte concentrations normalized to values on D7. The scale indicates fold change to control.
233 Supernatants were obtained from n = 5 independent experiments. Source data are provided as a
234 Source Data file.

235



Supplementary Fig. 16 | Inhibition of pericyte-endothelial cell communication pathways

cause microvascular alterations. a, Representative images of endothelial networks (UEA I) and

overlay images with pericytes (PDGFR β) on D14, following 7 days of treatment. Controls are

shown after iBRB MVN formation on D7 (Control D7) and on the treatment endpoint D14

(Control). All treatments were applied from D7 to D14. STI571, PDGFR β inhibitor. Treatments

with D-Glucose, TNF- α or IL-6 were tested individually, and in combination to produce the

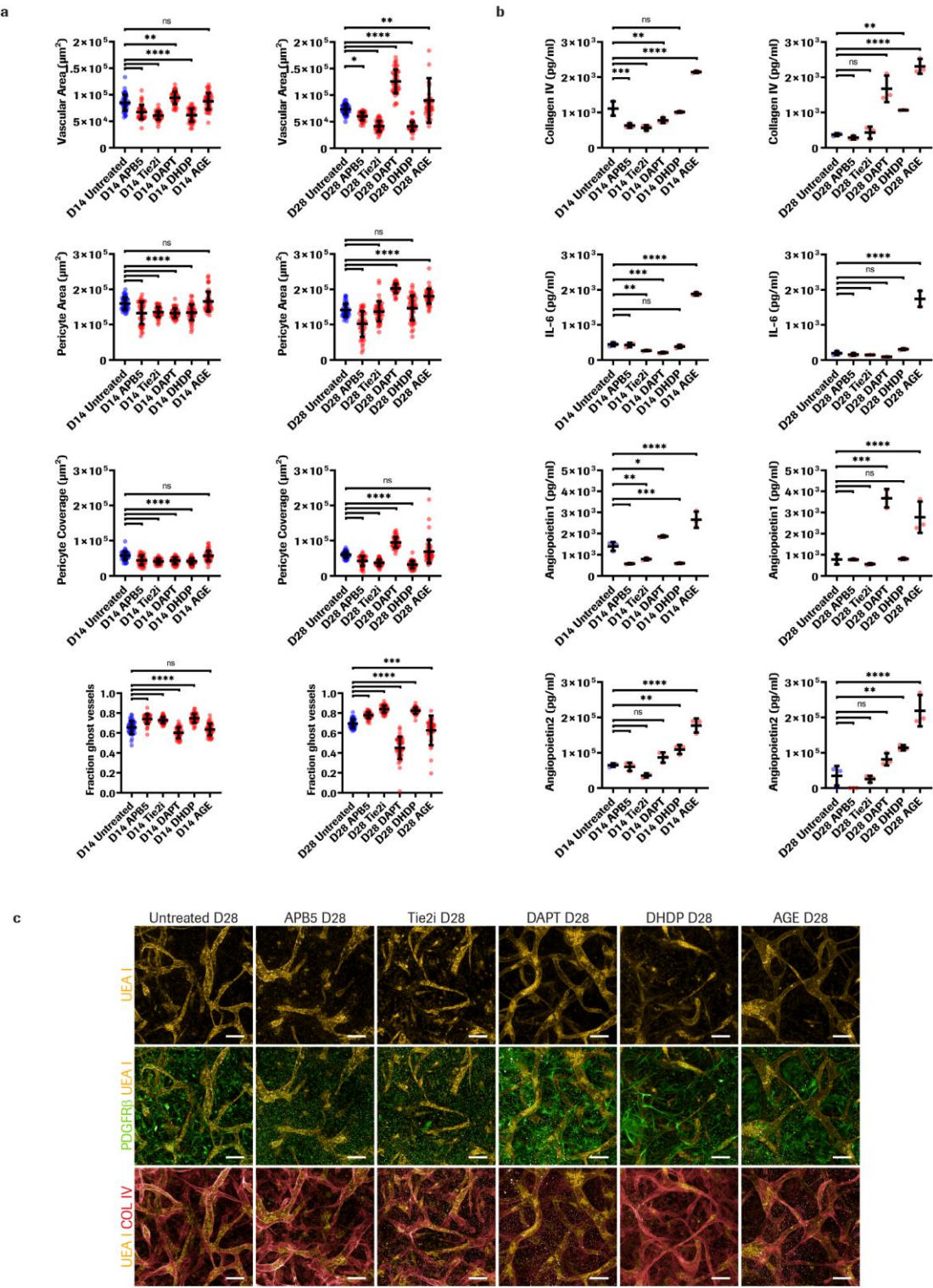
diabetic treatment (Diabetic). **b,** Representative images of iBRB MVNs (UEA I), overlay images

with basement membranes (COL IV), and images of tight junctions (CLDN5) on D14, following

7 days of treatment. Images show maximum intensity projections of 395 μ m Z-stacks, from n = 2

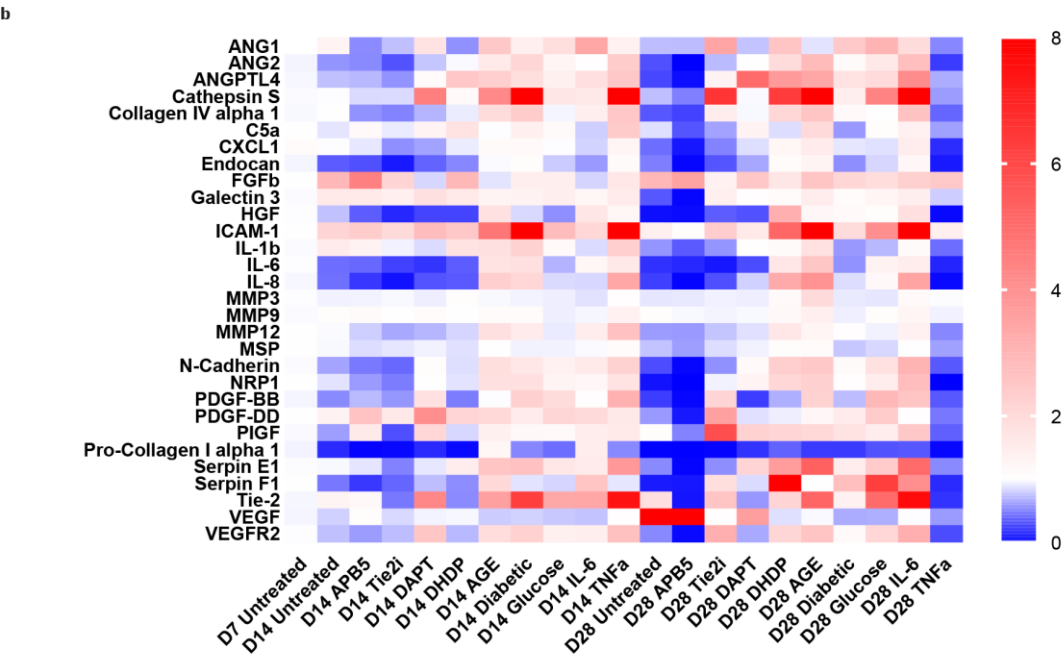
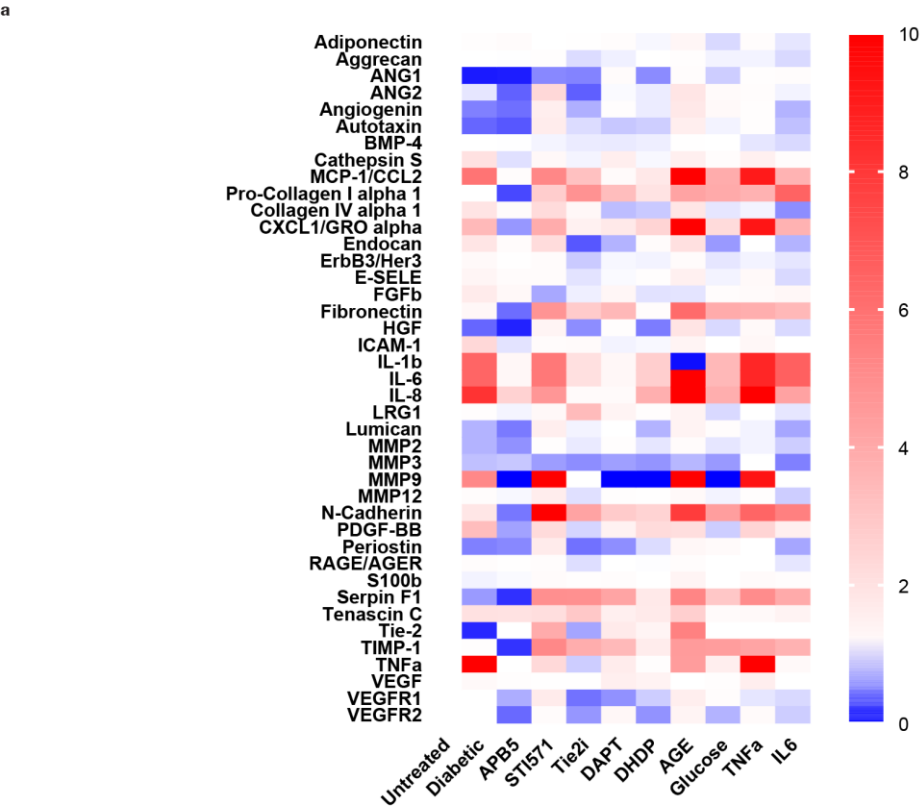
independent experiments and n = 1 experiment for D-Glucose, TNF- α and IL-6 conditions. Scale

bars, 100 μ m (**a, b**).



254 and AGE-treated conditions on D14 and D28. n = 45 untreated D14, n = 44 D28, n = 43 APB5
255 D14, n = 41 D28, n = 44 Tie2i D14, n = 45 D28, n = 44 DAPT D14, n = 45 D28, n = 44 DHDP
256 D14, n = 45 D28, n = 45 AGE D14 and n = 45 D28 treated ROI networks analyzed from n = 3
257 replicate channels. **b**, Analyte measurements of human collagen IV alpha I, IL-6, Angiopoietin-1
258 and Angiopoietin-2 obtained from n = 3 replicate channels. Data are mean $\pm$ s.d. *P<0.05;
259 **P<0.01; ***P<0.001; ****P<0.0001; one-way ANOVA. Source data are provided as a Source
260 Data file. **c**, Representative images of endothelial networks (UEA I) and overlay images with
261 pericytes (PDGFR β) or basement membranes (COL IV) on D28. All images show maximum
262 intensity projections of 245 μ m Z-stacks. Scale bars, 100 μ m (**c**).

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Supplementary Fig. 18 | Treatments analyte measurements. **a**, Heat map of analyte measurements for untreated, diabetic, APB5, STI571 (PDGFR β inhibitor), Tie2i, DAPT, DHDP, AGE, glucose, TNF- α and IL-6-treated conditions on D14, normalized to the untreated control on D14. STI571, PDGFR β inhibitor. Supernatants were pooled from n = 3 replicate channels. **b**, Heat map of analyte measurements for untreated conditions on D7, D14 and D28, and APB5, Tie2i, DAPT, DHDP, AGE, diabetic, glucose, IL-6 and TNF- α -treated conditions on D14 and D28, normalized to the untreated control on D7. The scale indicates fold change to control. Supernatants were obtained from n = 3 replicate channels. Source data are provided as a Source Data file.

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Supplementary Table 1 | Information about cells used in this study.

Cell type	Source	Type	Supplier	Cat number	Batch number	Donor information
HRMVEC - 1	retina	primary	Cell Sytems	ACBRI 181	181.04.01.02.02	NA
HRMVEC - 2	retina	primary	Pelo-Biotech	PB-CH-160-8511	QC-18B19F09	28 year-old female
HRMVEC - 3	retina	primary	Pelo-Biotech	PB-CH-160-8511	QC-03B18F04	NA
HRMVEC - 4	retina	primary	Cell Biologics	H-6065	F021518Ag	NA
HRMVEC - 5	retina	primary	Cell Biologics	H-6065	M021518	NA
HRMVEC - 6	retina	primary	Cell Biologics	H-6065	122118U	NA
HRMVEC - 7	retina	primary	Cell Biologics	H-6065	120117Ag	NA
HRP	retina	primary	Cell Sytems	ACBRI 183	183.02.01.01.10	NA
HRA - 1	retina	primary	ScienCell	1870	23521	20 week-old male
HRA - 2	retina	primary	ScienCell	1870	30020	56 year-old female
HCA	brain	immortalized	Innoprot	P10251-IM	NA	NA

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NA: not provided by the supplier

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