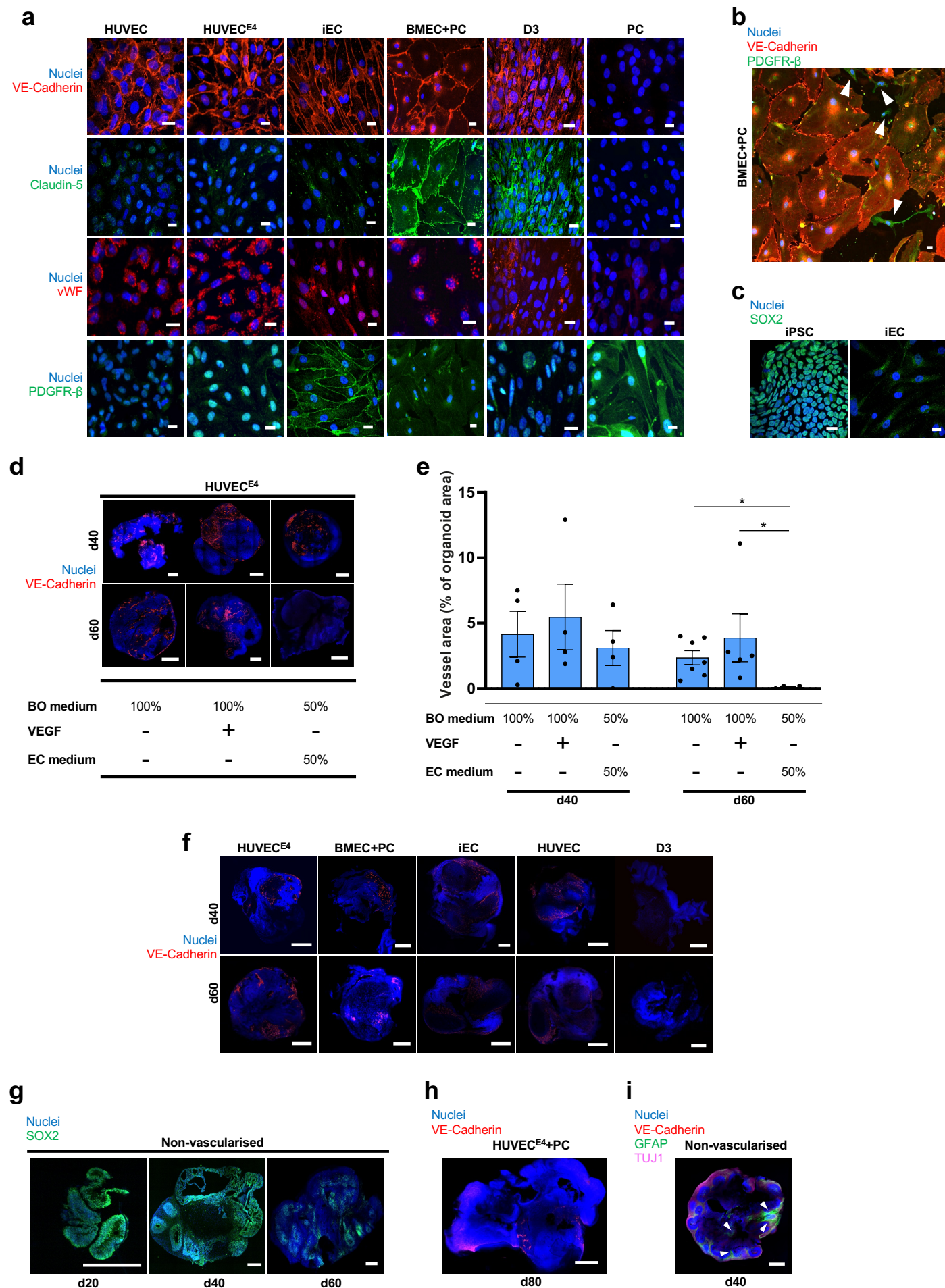
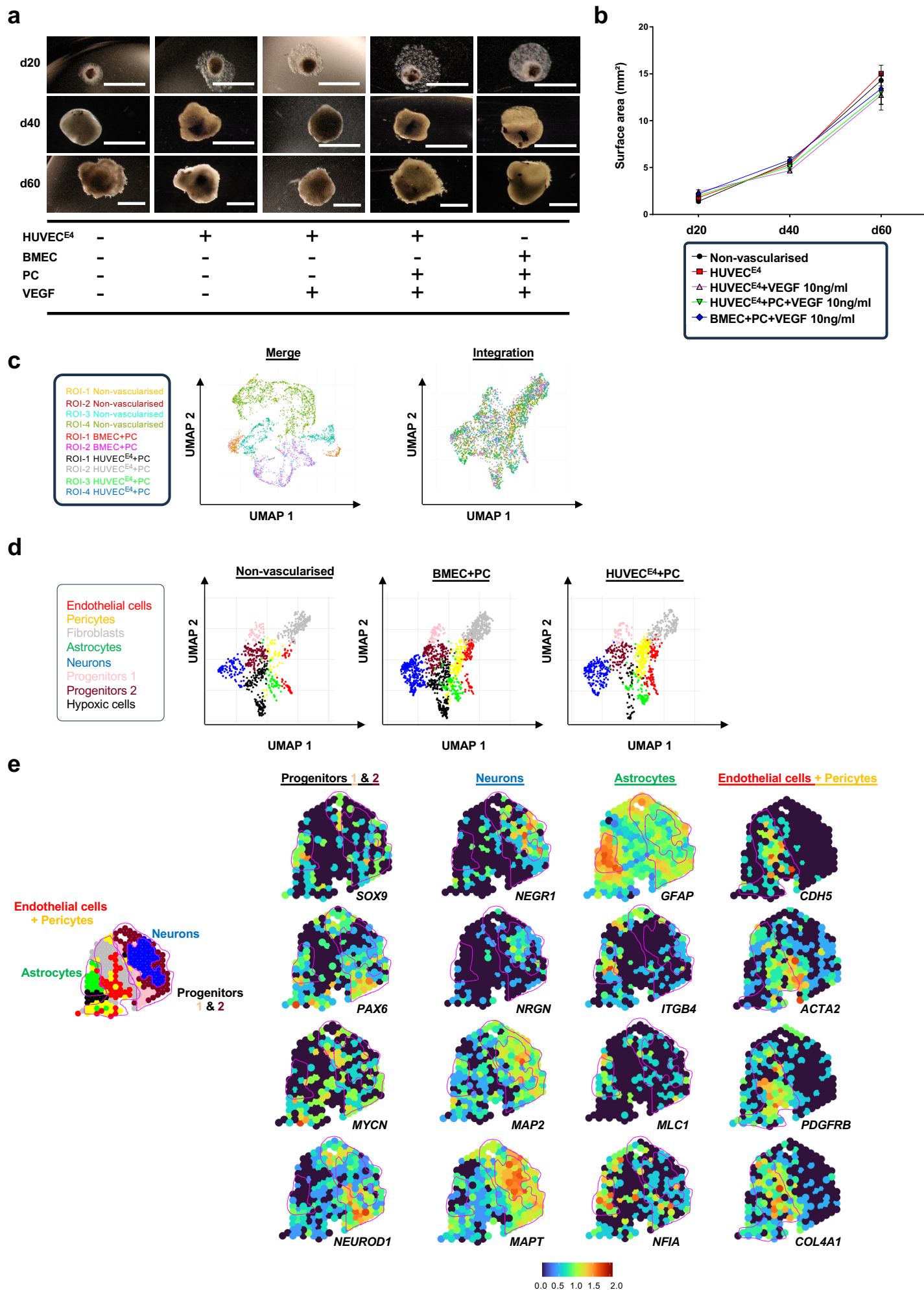




Extended Data Fig. 1

Extended Data Fig. 1 | Optimization of endothelial and culture conditions for robust vascularized brain organoids generation.

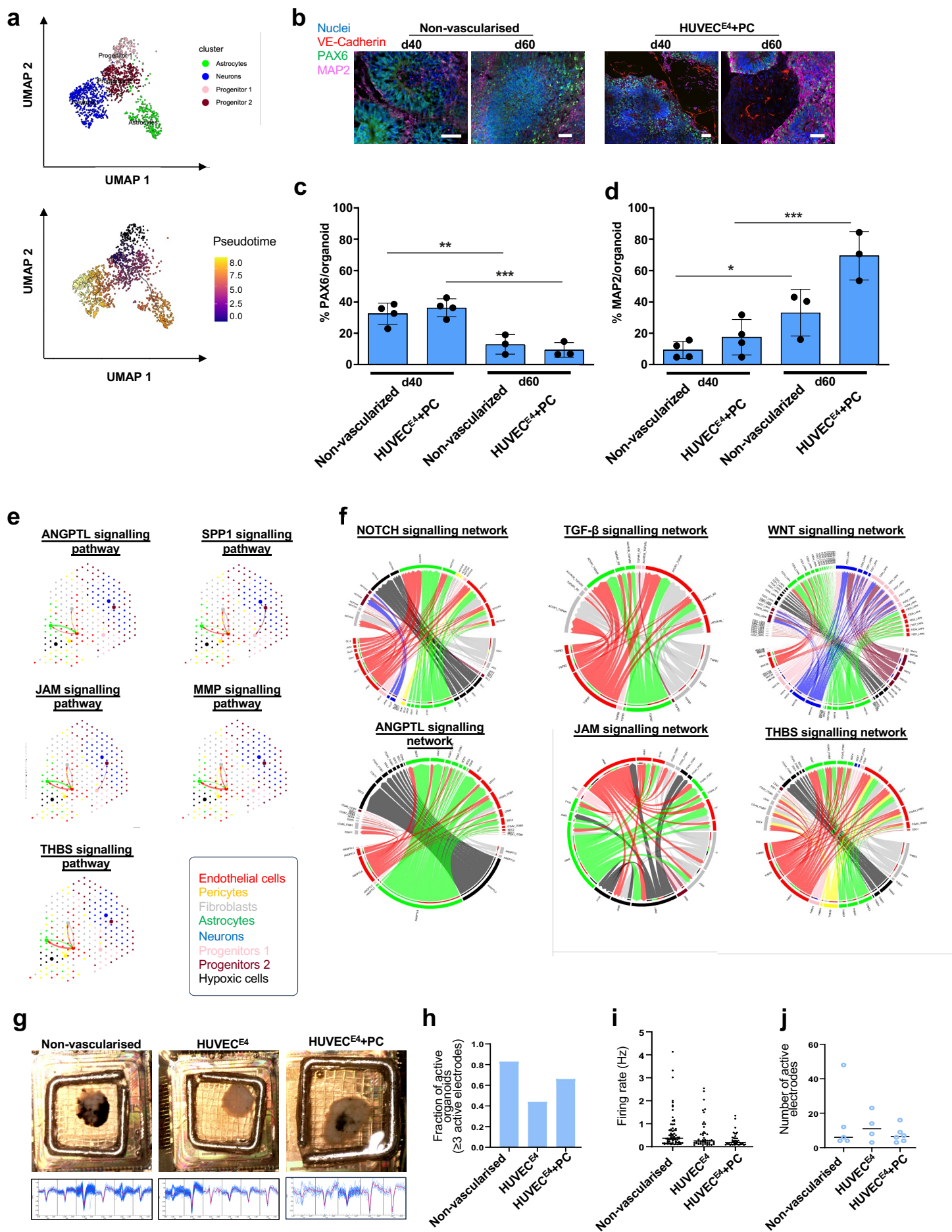
a, Expression of endothelial and mesenchymal markers in different human endothelial cell (EC) sources in 2D culture, as depicted (scale bars, 20 μm). **b**, Co-expression of VE-Cadherin (red) and PDGFR- β (green) in BMEC+PC. White arrowheads indicate PDGFR- β -positive PC (scale bars, 20 μm). **c**, Loss of the pluripotency marker SOX2 (green) in iEC compared with undifferentiated iPSC (scale bar, 20 μm). **d,e**, Optimization of the culture medium, assessed at d40 and d60. HUVEC^{E4} vBO were grown in BO medium alone, BO medium supplemented with VEGF (10 ng ml⁻¹) from d7 to d21, or a 1:1 mixture of BO and EBM2 medium: VE-Cadherin (red) networks (**d**; scale bars, 500 μm) and vessel area fraction per organoid (**e**). Each dot represents one organoid (At d40, n = 4 organoids per condition; at d60, n = 7, 5 and 4 organoids for BO maturation medium alone, BO maturation medium + VEGF and the 1:1 BO maturation medium/EBM-2 mixture, respectively, from at least three independent differentiations), bars show mean $\pm$ s.e.m.; two-sided Kruskal–Wallis test with Dunn's multiple comparisons test; *P < 0.05, **P < 0.01. **f**, VE-Cadherin (red) networks at d40 and d60 across the five EC sources (scale bars, 500 μm). **g**, Non-vascularized BO at d20, d40 and d60 stained for SOX2 (green), showing progressive neuroepithelial maturation and partial loss of apical organisation (scale bars, 300 μm). **h**, Extended culture of HUVEC^{E4}+PC vBO to d80 (scale bar, 500 μm). **i**, Astroglial compartment of non-vascularized BO at d40 stained for GFAP (green), VE-Cadherin (red) and TUJ1 (magenta). White arrowheads indicate emerging GFAP-positive astrocyte clusters (scale bar, 500 μm). Nuclei are counterstained with DAPI (blue) throughout. Images are maximum intensity projections of 10 μm z-stacks in **a**, **b**, **c** and **g**, and of 50 μm z-stacks in **d**, **f**, **h** and **i**.



Extended Data Fig. 2

Extended Data Fig. 2 | Growth kinetics and integration of the spatial transcriptomic datasets.

a,b, Growth of brain organoids from d20 to d60 across five conditions: non-vascularized, HUVEC^{E4}, HUVEC^{E4} with VEGF (10 ng ml⁻¹ from d7 to d21), HUVEC^{E4} + pericytes (PC) with VEGF, and BMEC+PC with VEGF: bright-field images (**a**; scale bars, 500 μ m) and organoid surface area (**b**). At each time point, values represent six organoids per condition sampled across multiple independent differentiations; organoids were not longitudinally tracked. Data are shown as mean $\pm$ s.e.m. Groups were compared separately at each time point using a two-sided Kruskal-Wallis test followed by Dunn's multiple-comparisons test; no significant differences were detected between conditions. **c**, UMAP embedding of the Visium spots before (left) and after (right) CCA-based integration. Each colour identifies one region of interest (ROI), with four regions for non-vascularized, two for BMEC+PC and four for HUVEC^{E4}+PC organoids. Before integration, spots segregate by region of interest; after integration they align into a single manifold. **d**, Integrated UMAP split by condition, coloured by annotated cell-type clusters. **e**, Spatial projection of lineage marker genes. Left, segmentation of the main compartments, outlined in magenta. Right, spatial expression of progenitor, neuron, astrocyte and vascular associated genes.



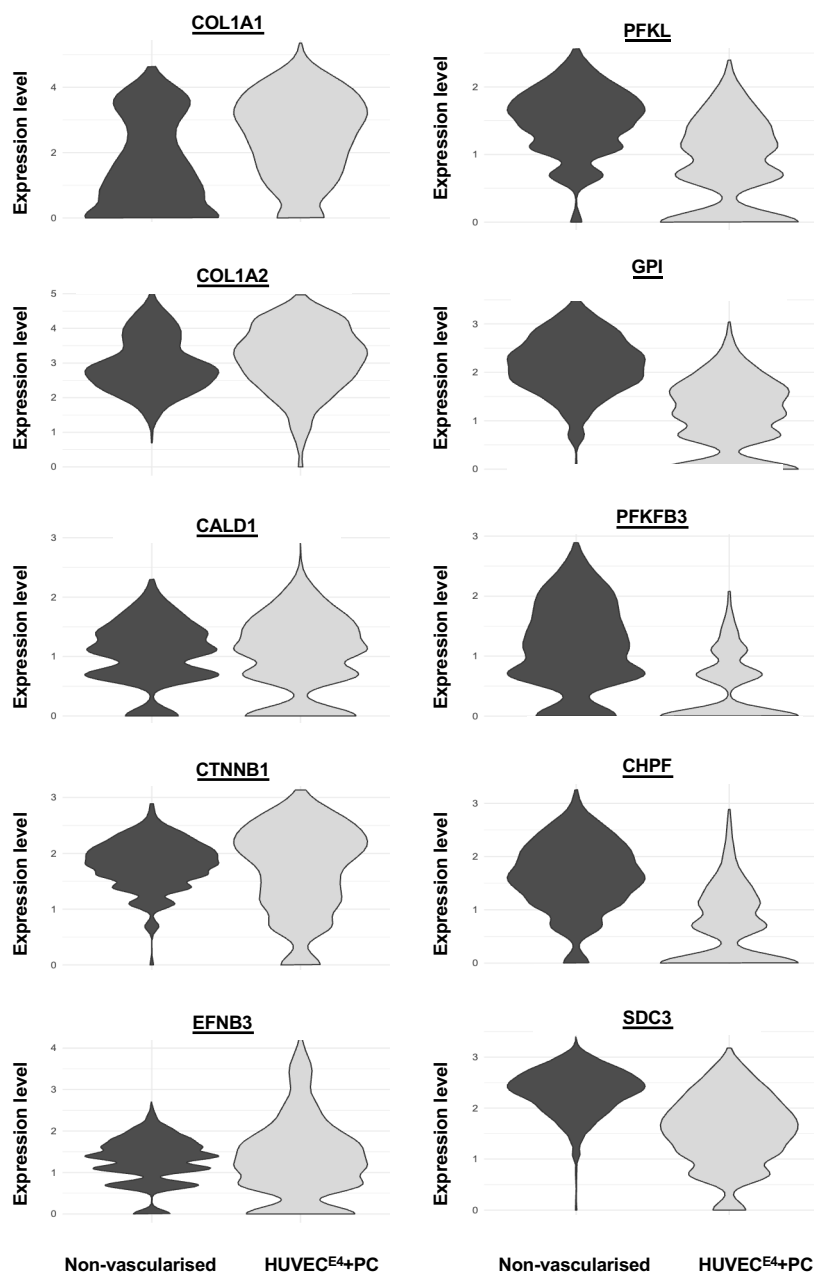
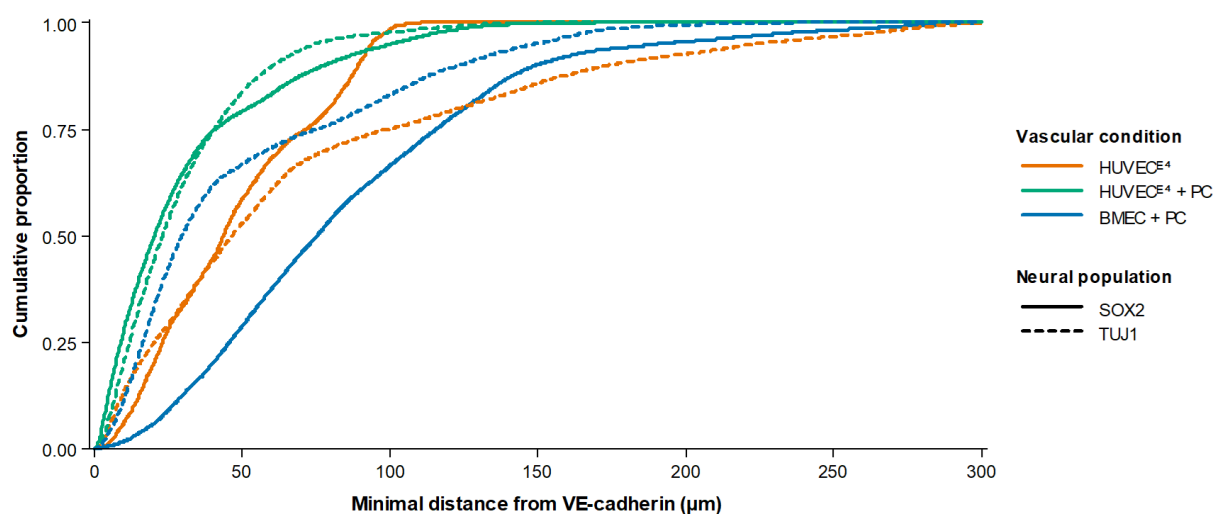
Extended Data Fig. 3

Extended Data Fig. 3 | Neural maturation and spontaneous network activity are preserved in vascularized brain organoids.

a, Integrated UMAP of the spatial transcriptomic spots restricted to the neural lineage, coloured by cluster (upper) and by pseudotime (lower). **b**, Immunofluorescence for the apical progenitor marker PAX6 (green), the neuronal marker MAP2 (magenta) and the endothelial marker VE-Cadherin (red) in non-vascularized BO and HUVEC^{E4} + pericyte (PC) vBO at d40 and d60 (scale bars, 50 μ m). **c,d**, Organoid area positive for PAX6 (**c**) and MAP2 (**d**) in the same conditions. Each dot represents one organoid ($n = 4$ organoids per condition at d40 and $n = 3$ organoids per condition at d60, from at least three independent differentiations), bars show mean $\pm$ s.e.m.; ordinary two-way ANOVA with time and vascularization as factors, followed by Šídák's multiple comparisons test; ** $P < 0.01$, *** $P < 0.001$. In **h–j**, $n = 6$ organoids per condition. One-way ANOVA after verification of normality (Shapiro–Wilk) and of equality of variances; no significant difference between conditions in **h–j**. **e**, Pathway-level CellChat networks inferred from the spatial transcriptomic data for the ANGPTL, SPP1, JAM, MMP and THBS pathways. Coloured dots are spatial spots annotated by cell type and arrows indicate the direction of inferred signalling, with arrow thickness proportional to the summed communication probability of all contributing ligand–receptor pairs. **f**, Chord diagrams of the NOTCH, TGF- β , WNT, ANGPTL, JAM and THBS pathways. Sector size is proportional to the total outgoing and incoming signalling strength of each population, chord thickness to the communication probability, and chord colour identifies the signalling source. **g**, High-density microelectrode array (HD-MEA) recordings at d80 in non-vascularized BO, HUVEC^{E4} vBO and HUVEC^{E4}+PC vBO. Upper, bright-field images of individual organoids on the HD-MEA platform. Lower, extracellular recordings of spontaneous multi-unit activity, with raw spike waveforms in blue and the averaged action potential waveform in red. **(h)** Fraction of active organoids, defined as at least three active electrodes per well ($n = 6$ organoids per condition). **(i)**, Distribution of firing-event frequencies recorded over a 5-min period; each dot represents one detected firing event. **(j)**, Number of active electrodes per organoid; each dot represents one organoid.

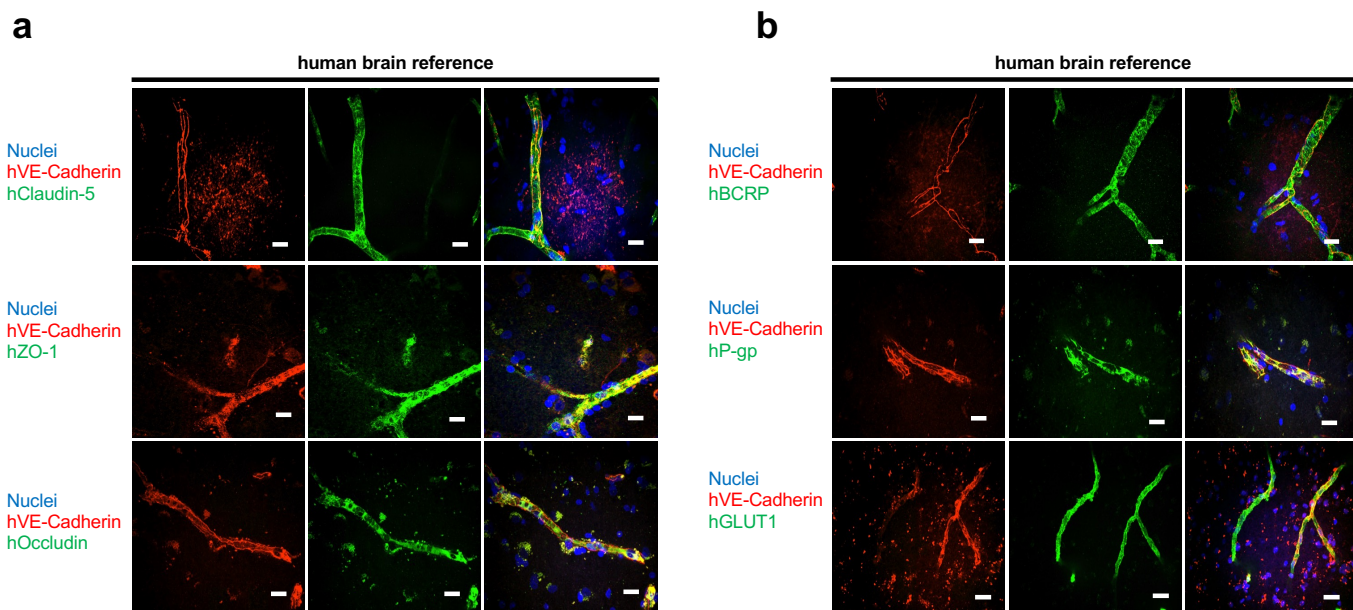
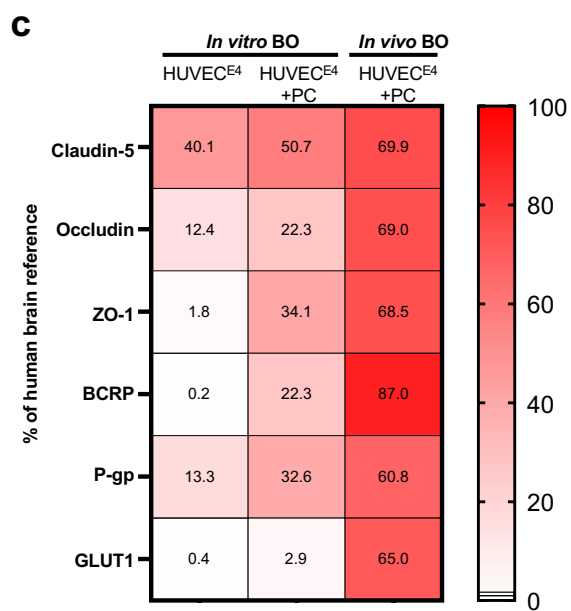
One-way ANOVA after verification of normality (Shapiro–Wilk) and of equality of variances; no significant difference between conditions in **h–j**.

Nuclei are counterstained with DAPI (blue) in **b**. Images in **b** are maximum intensity projections of 10 μ m z-stacks. In **c** and **d**, each dot represents one organoid and bars show mean $\pm$ s.e.m.

a**b**

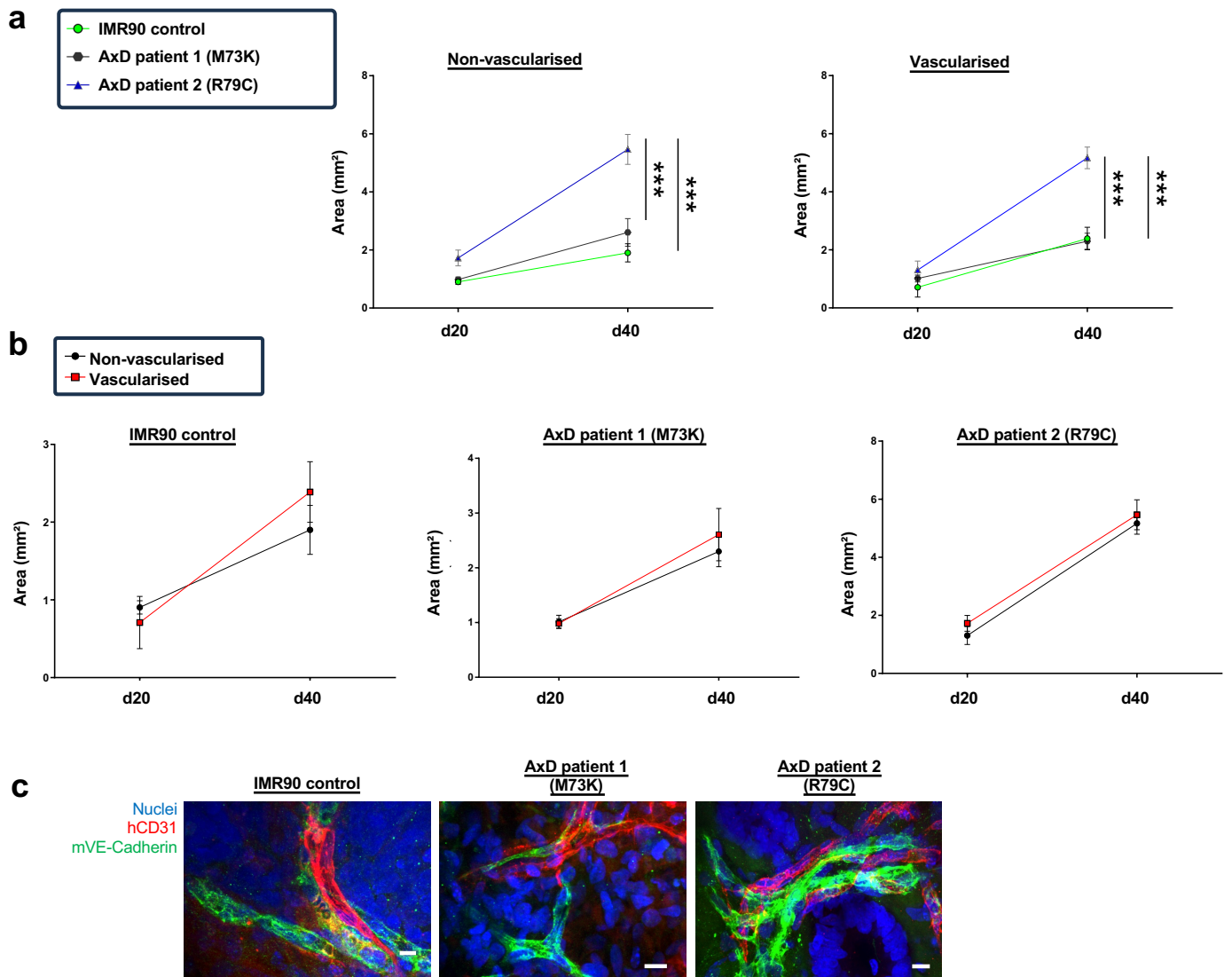
Extended Data Fig. 4 | Transcriptomic and spatial signatures of the neurovascular remodelling induced by vascularization.

a, Violin plots of normalized expression for ten genes differentially expressed between non-vascularized BO (dark grey) and HUVEC^{E4}+PC vBO (light grey), grouped by function: extracellular matrix composition and vascular structural components (COL1A1, COL1A2, CALD1, CHPF), glycolytic metabolism (PFKL, GPI, PFKFB3), WNT/ β -catenin signalling (CTNNB1), and cell adhesion and guidance signalling (SDC3, EFNB3). **b**, Cumulative distributions of the nearest-neighbour distance from VE-Cadherin-positive vascular-mask coordinates to SOX2-positive neural progenitors (solid lines) or TUJ1-positive neurons (dashed lines) in HUVEC^{E4} (orange), HUVEC^{E4}+PC (green) and BMEC+PC (blue) vBO at d40-d60. N = 2 organoids per condition. Each curve is computed from all segmented cells of that condition. SOX2 and TUJ1 measurements were obtained from separate immunostained tissue sections and therefore represent independent spatial analyses rather than paired measurements within the same section.



Extended Data Fig. 5 | Human cortical reference and quantitative assessment of BBB-associated vascular marker localization.

a,b, Immunofluorescence for blood–brain barrier (BBB) markers in adult human cortical tissue from four different patients, used as the reference for the comparisons shown in **c**. Human vessels labelled for VE-Cadherin (hVE-Cadherin, red) display junctional Claudin-5, ZO-1 and Occludin (**a**) and express the efflux transporters BCRP and P-gp and the glucose transporter GLUT1 (**b**), all in green (scale bars, 40 μ m). **c**, Vascular colocalization of BBB-associated markers with human VE-Cadherin in HUVEC^{E4} and HUVEC^{E4} + PC vBO in vitro and in HUVEC^{E4} + PC vBO after xenografting, normalized to the corresponding value measured in adult human cortical vessels and expressed as a percentage of the human brain reference. Nuclei are counterstained with DAPI (blue) in **a** and **b**. Images in **a** and **b** are maximum intensity projections of 30 μ m z-stacks. Values are means of n = 10 images per condition, and the human brain reference was established from four different patients; no statistical comparison was performed.



Extended Data Fig. 6

Extended Data Fig. 6 | Growth and vascular integration of Alexander disease brain organoids

a, Organoid surface area between d20 and d40 for IMR90 control, AxD patient 1 (M73K) and AxD patient 2 (R79C), in non-vascularized (left) and vascularized (right) conditions. **b**, The same data replotted by genotype to compare non-vascularized and vascularized conditions within each line. **c**, Human and murine vascular networks within xenografted AxD vBO, labelled for human CD31 (hCD31, red) and murine VE-Cadherin (mVE-Cadherin, green), showing human-murine vascular interfaces in all three cell lines (scale bars, 10 μ m). Nuclei are counterstained with DAPI (blue) in **c**. Images in **c** are maximum intensity projections of 30 μ m z-stacks.

In **a** and **b**, each point represents the mean of six organoids per condition and error bars denote s.e.m. In **a**, non-vascularized and vascularized organoids were analysed separately using ordinary two-way ANOVA with time and cell line as factors, followed by Šídák's multiple-comparisons test. In **b**, each cell line was analysed separately using ordinary two-way ANOVA with time and vascularization as factors, followed by Šídák's multiple-comparisons test. *** $P < 0.001$. No significant effect of vascularization on organoid growth was detected within any cell line.

Extended Data Table 1 | Vascularization success rate according to media type.

Medium type	Numbers of experiment	Vascularization success rate (%)
BO medium without VEGF	8	5/8 (62.5)
BO medium + VEGF 10 ng ml ⁻¹	14	14/14 (100.0)
1:1 ratio BO medium + EC medium	3	0/3 (0.0)

Comparison	P value
BO medium alone (5/8) vs BO medium + VEGF (14/14)	0.036
BO medium + VEGF (14/14) vs 1:1 mixture (0/3)	0.001
BO medium alone (5/8) vs 1:1 mixture (0/3)	0.180

Brain organoids were embedded with HUVEC^{E4} endothelial cells at d7 and cultured until d40 in the indicated medium, with VEGF (10 ng ml⁻¹) added from d7 to d21 where applicable. Vascularization success was scored as a binary outcome at d40, defined by the presence of three-dimensional VE-Cadherin-positive or CD31-positive endothelial structures within the neural tissue, irrespective of the extent of the vascular network. Experiments refers to independent differentiation batches. All experiments in this table were performed with HUVEC^{E4}; the 14 batches in the BO media + VEGF row are the same as those reported in the HUVEC^{E4} row of Extended Data Table 2. BO, brain organoid; EC, endothelial cell; VEGF, vascular endothelial growth factor. Success rates differed across the three media conditions (global exact test, $P = 0.0005$). Pairwise comparisons were performed using two-sided Fisher's exact tests; P values were not adjusted for multiple comparisons. HUVEC, human umbilical vein endothelial cells; HUVEC^{E4}, HUVEC transduced with the adenoviral E4ORF1 gene

Extended Data Table 2 | Vascularization success rate according to endothelial cell source.

Vascular cell preparation	Number of independent batches	Vascularization success rate (%)
HUVEC ^{E4}	14	14/14 (100.0)
BMEC+PC	7	4/7 (57.1)
iEC	4	2/4 (50.0)
HUVEC	4	2/4 (50.0)
D3	3	0/3 (0.0)

Comparison vs HUVEC ^{E4} (14/14)	P value
BMEC + PC (4/7)	0.026
iEC (2/4)	0.039
HUVEC (2/4)	0.039
D3 (0/3)	0.001

Brain organoids were embedded at d7 with the indicated endothelial cell type, alone or together with pericytes, and cultured until d40 in BO medium supplemented with VEGF (10 ng ml⁻¹) from d7 to d21. Vascularization success was scored as defined in Extended Data Table 1. Experiments refers to independent differentiation batches. The 14 batches in the HUVEC^{E4} row are the same as those reported in the BO media + VEGF row of Extended Data Table 1; all other batches are distinct. Success rates differed across the five endothelial cell preparations (global exact test, $P = 0.0006$). Pairwise comparisons with HUVEC^{E4} were performed using two-sided Fisher's exact tests; P values were not adjusted for multiple comparisons.

BMEC, brain microvascular endothelial cells; D3, hCMEC/D3 cell line; HUVEC, human umbilical vein endothelial cells; HUVEC^{E4}, HUVEC transduced with the adenoviral E4ORF1 gene; iEC, iPSC-derived vascular cells; PC, pericytes.

Supplementary Table 1. Key reagents, media, and materials used for the study

Reagent / Medium / Material	Application	Supplier / Manufacturer	Catalog No. / Reference
Matrigel® hESC-qualified	Coating and embedding of hiPSCs and EBs	Corning	11573560
mTeSR+™ medium	Maintenance of hiPSCs	STEMCELL Technologies	100-0276
EB Formation Medium	Formation of embryoid bodies	STEMCELL Technologies	8570
Neural Induction Medium	Early neural differentiation	STEMCELL Technologies	8570
Brain Organoid Maturation Medium	Organoid maturation phase	STEMCELL Technologies	8571
EBM-2 Endothelial Basal Medium + supplements	Endothelial cell culture	Lonza	CC-3202/6
Penicillin–Streptomycin (100×)	Antimicrobial supplement	Thermo Fisher Scientific	P0781
Fetal Bovine Serum (FBS), heat-inactivated	Supplement for pericyte and MSC culture	Sigma-Aldrich	F7524
ROCK inhibitor Y-27632	Maintenance of single-cell hiPSC viability	Axon Medchem	1683
Trypsin-EDTA 0.05%	Cell detachment	Thermo Fisher Scientific	T4049
HBSS (without Ca ²⁺ , Mg ²⁺)	Tissue washing and digestion	Thermo Fisher Scientific	H9394
DNase I	Enzymatic digestion of tissue	Thermo Fisher Scientific	D4527
Dispase II	Enzymatic digestion of tissue	Sigma-Aldrich	D4693
Collagenase type II	Enzymatic digestion of tissue	Sigma-Aldrich	C0130
Heparin sodium	Digestion and anti-coagulation	Sigma-Aldrich	H3149
RBC Lysis Buffer	Red blood cell lysis	Sigma-Aldrich	79217
Donkey Serum	Blocking agent for IF	Sigma-Aldrich	D9663
Tween-20	Permeabilization reagent	Sigma-Aldrich	P1379
TBS-azide buffer	Immunostaining buffer	Sigma-Aldrich	S7653 / T3253 / S2002
Human VEGF-165 Recombinant Protein	Endothelial cell culture	Thermo Fisher Scientific	17861763
Gentle Cell Dissociation Reagent	hiPSC passaging	STEMCELL Technologies	100-0485

Supplementary Table 2. Antibodies and probes used for immunofluorescence and vascular labelling

Antibody / Probe	Target	Host / Type	Supplier	Catalog no.	Dilution	Application
Anti-ZC4.3 (<i>Neisseria meningitidis</i>)	Bacteria (strain ZC4.3)	Rabbit polyclonal	Institut Cochin, Boudouous Lab	<i>Home-made</i>	1:500	IF
Anti-human α -SMA	Smooth muscle cells	Mouse monoclonal	Thermo Fisher Scientific	MA5-11547	1:400	IF
Anti-BCRP/ABC52	Efflux transporter (BBB marker)	Rabbit monoclonal	Abcam	ab229193	1:100	IF
Anti-human CD31	Endothelium	Mouse monoclonal	Daiko-Agilent	M0823	1:100	IF
Anti-mouse CD31	Endothelium	Rat monoclonal	Biologend	107502	1:100	IF
Anti-mouse VE-Cadherin	Endothelial junctions	Rabbit monoclonal	Abcam	ab205336	1:100	IF
Anti-mouse CD41	Platelets / thrombosis	Rat monoclonal	Abcam	ab33661	1:100	IF
Anti-human Claudin-5	Tight junctions	Rabbit polyclonal	Abcam	ab15106	1:100	IF
Anti-human Collagen IV	Vascular basement membrane	Mouse monoclonal	Abcam	ab6311	1:100	IF
Anti-mouse Endomucin	Endothelium	Rat monoclonal	SanctaCruz	sc-65495	1:100	IF
Anti-human GFAP	Astrocytes	Rabbit polyclonal	Signa-Aldrich	G9269	1:100	IF
anti-human-MAP2	Neuronal dendrites	Rabbit polyclonal	Biologend	840601	1:100	IF
Anti-human GLUT-1	Glucose transporter (BBB marker)	Rabbit polyclonal	Thermo Fisher Scientific	AGT-021-200UL	1:100	IF
Anti-human PDGFR-beta	Pericytes	Rabbit monoclonal	Signa-Aldrich	HPA028499	1:100	IF
Anti-human Occludin	Tight junctions	Mouse monoclonal	Biologend	NBP2-52972	1:100	IF
Anti-human P-glycoprotein (Pgp/ABC1)	Efflux transporter	Mouse monoclonal	Thermo Fisher Scientific	MA1-26528	1:100	IF
Anti-human SOX2	Neuroprogenitors	Goat monoclonal	R&D	AF2018	1:100	IF
anti-human-Tubulin $\beta 3$ (TUJ1)	Neuronal marker	Mouse monoclonal	Biologend	801202	1:100	IF
Anti-human VE-Cadherin	Endothelial junctions	Goat monoclonal	R&D	AF938	1:100	IF
Anti-human VWF (clone HIE2.34)	Endothelial / platelet marker	Mouse monoclonal	Merck Millipore	MA8T856	1:100	IF
Anti-human ZO-1	Tight junctions	Mouse monoclonal	Thermo Fisher Scientific	33-9100	1:100	IF
Anti-human PAX6	Neural progenitor cells	Rabbit polyclonal	Thermo Fisher Scientific	12323-1-AP	1:100	IF
Anti-human HuC/HuD	Neurons	Mouse monoclonal	Thermo Fisher Scientific	A-21271	1:100	IF
α -Rat IgG (H+L) AF 488	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A-21208	1:500	IF
α -Mouse IgG (H+L) Alexa Fluor Plus 488	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A32766	1:500	IF
α -Goat IgG (H+L) AFP 488	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A32814	1:500	IF
α -Goat IgG (H+L) AFP 555	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A32816	1:500	IF
α -Mouse IgG (H+L) AF 555	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A32773	1:500	IF
α -Rat IgG (H+L) AFP555	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A48270	1:500	IF
α -Rabbit IgG (H+L) AFP555	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A32794	1:500	IF
α -Rabbit IgG (H+L) AF 647	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A-31573	1:500	IF
α -Goat IgG (H+L) AF 647	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A-21447	1:500	IF
α -Rat IgG (H+L) AFP 647	Secondary antibody	Donkey polyclonal	Thermo Fisher Scientific	A48272	1:500	IF
Streptavidin AF 488	Biotin	Streptavidin conjugate	Thermo Fisher Scientific	S11223	1:500	IF
Streptavidin AF 555	Biotin	Streptavidin conjugate	Thermo Fisher Scientific	S32355	1:500	IF
Ulex europaeus agglutinin I (UEA-I), Rhodamine	Human-specific endothelium		Eurobio/ugap	FL-1061-2/3613325	<i>i.v. injection of 100 μl solution (2 mg/ml)</i>	In vivo perfusion / IF
Sulfo-NHS-Biotin EZ-link™	Vascular permeability probe		Thermo Fisher Scientific	21217	<i>i.v. injection of 100 μl solution (1 mg/ml)</i>	In vivo perfusion / IF
4',6'-diamidino-2-phenylindole (DAPI)	Nucleus		Signa-Aldrich	D8417	<i>1:500 of a solution at 200 μg/ml</i>	IF

Supplementary Table 3. Molecular biology / histology reagents

Reagent / Medium / Material Application		Supplier	Catalog No. / Reference
Paraformaldehyde 4%	Tissue fixation	Electron Microscopy Sciences	15710
10x Visium Spatial Gene Expression	Spatial transcriptomics	10x Genomics	PN-1000181
NovaSeq 6000	High-throughput sequencing	Illumina	—