Electronic Supplementary Information

On-chip perivascular *niche* supporting stemness of patient-derived glioma cells in a serum-free, flowable culture

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Table. S1: Table summarizing selected microfluidic-based models developed to study GBM cells, or their interactions with vasculature. This table compares published GBM-on-a-chip models to the present work.

	Ayuso et al., 2016 *Scientific Reports*	**Xu et al, 2016** *Scientific Reports*	**Ayuso et al., 2017** *Neuro-oncology*	**Ma et al., 2018** *Biomedical Microdevices*	**Truong et al., 2018** *Biomaterials*	**Xiao et al., 2019** *Advanced Science*	**Current Work**
Main Research Topics	• Drug penetration • Drug toxicity • Glucose and oxygen profile	• Cancer cell–Blood-Brain Barrier (BBB) interactions • Evaluation of therapeutic response in glioma brain tumour	• Generating pseudopalisades • Enhancing aggressiveness through blood vessel obstruction events	• Glioblastoma aggressiveness • Drug evaluation	• Glioma stem cell–vascular interactions	• Ex vivo dynamics of GBM cells • Microvasculature-on-a-chip • Examine function of primary patient-derived BTSCs	• Stem cell niche-on-a-chip • Physical and biochemical crosstalk between CSCs and brain microvessels
System Characteristics	Polystyrene with 2000μm wide central microchamber flanked by two 700 μm wide lateral microchambers with the chamber depth of 250 μm	PDMS device on glass wafer with an array of 16 functional units connected by micro-channels	Polystyrene device with perfusion of hermetic connections to the microdevice inlets and outlets	PDMS on PDMS-coated glass slide; device with concentration gradient generator channels	PDMS on glass slide with three concentric semicircles comprising of tri-layer tumour, stroma and vasculature regions	Chip (AIM biotech) adapted from prof. Roger Kamm Design	PDMS on glass slide device with gravity-drive perfusion
Cell Types	U251	U87, A549, MDA-MB-231, M624 and BEL-7402 cells	C6 or U251	U87	Patient-derived cell lines co-cultured with HUVECs	U87, patient-derived glioma stem-like cells and fresh patient-derived GBM cells; co-cultured with HUVECs	U87, patient-derived glioma cell line, normal brain cells; all co-cultured with hCMEC/D3, HUVECs, or HMVEC-L
ECMs	1.5 mg/ml collagen I	6 mg/mL collagen I	1.5 mg/ml collagen I	1.5 mg/ml collagen I	Matrigel	2.5 mg/ml fibrin	2 mg/ml collagen I with laminin

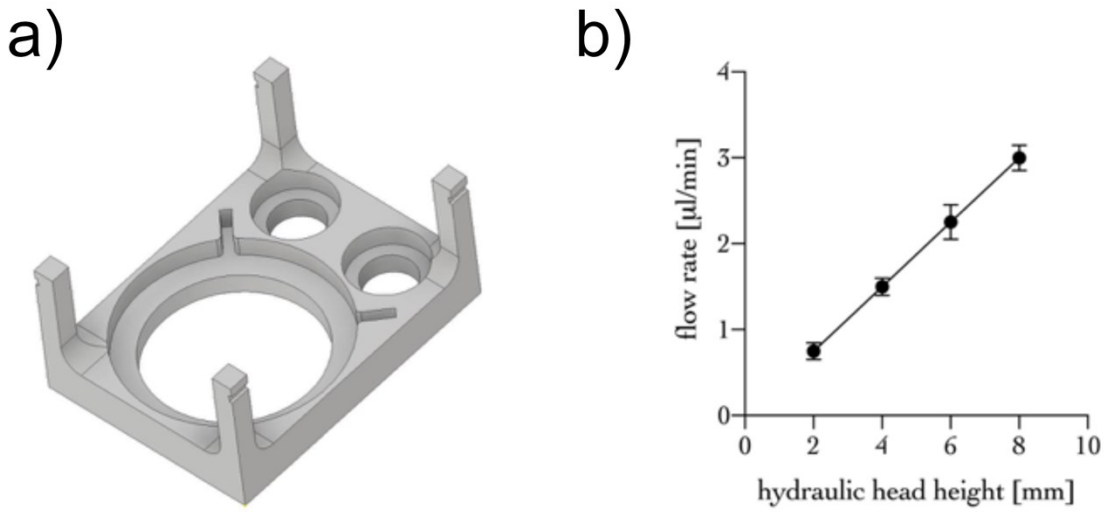

Fig. S1: Gravity-driven flow setup. a) design of the gravity-driven pump stage which allows two microfluidic devices and the pump reservoir to be connected easily and safely; b) flow rates obtained from four different hydraulic head heights in the microfluidic device. The graph represents data obtained from three independent experimental runs presented by mean±SEM;

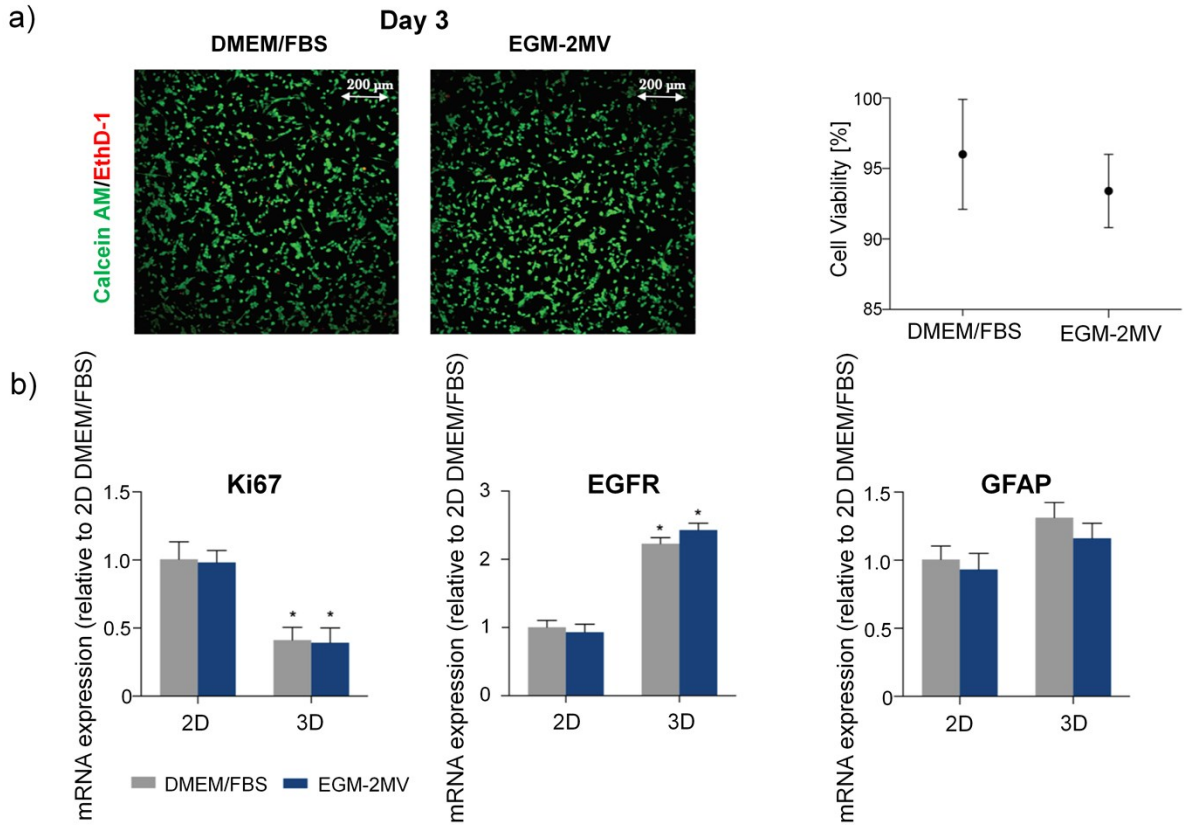

Fig. S2: U87 cells can survive in both DMEM/10% FBS and EGM-2MV media whilst cultured in 3D collagen I-based hydrogels in u-slides. a) left side: example images of calcein AM/EthD-1 staining of U87 cells at 3 days in culture; right side: graph presenting viability of U87 cells. Data presented as mean±SD (percentage of live cells); b) mRNA expression normalised to 2D DMEM/10%FBS samples. Results obtained from three independent experimental runs are presented by mean±SEM. One-way ANOVA with Tukey's post-hoc test was used for significance

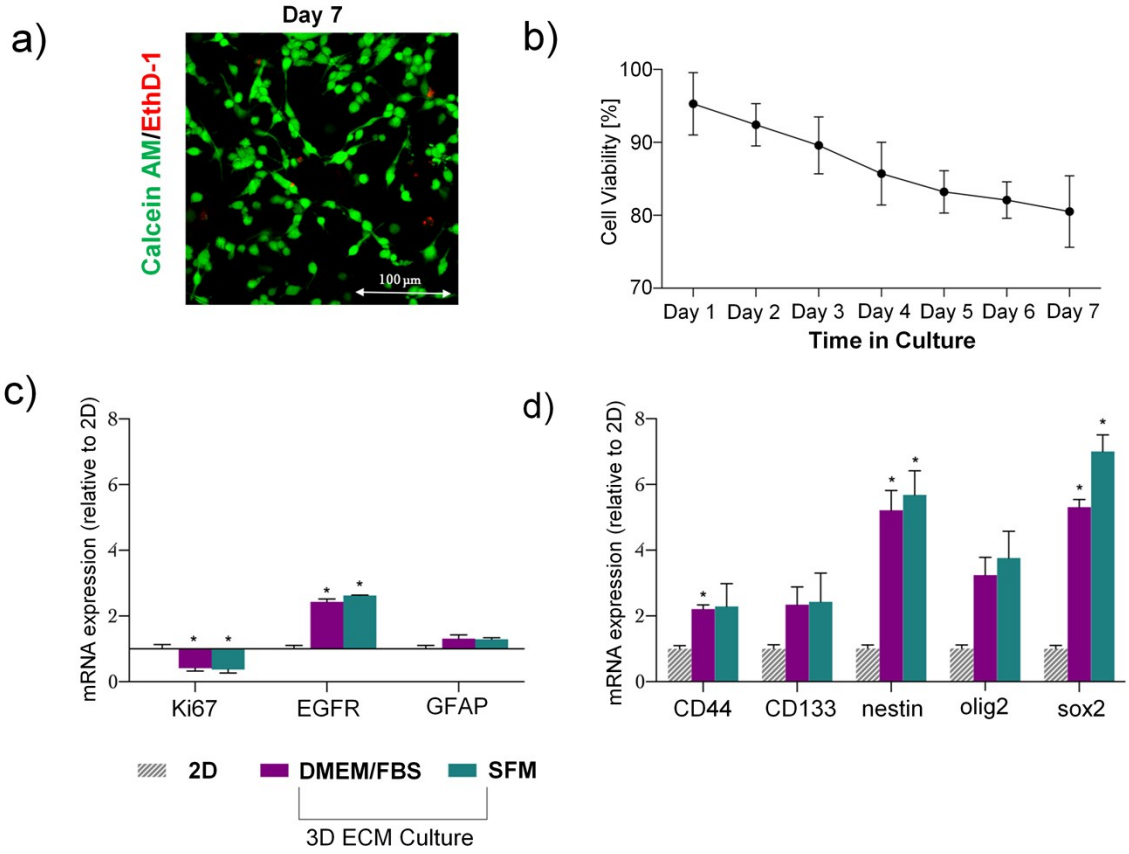

Fig. S3: U87 cells cultured in 3D collagen I-based ECM with serum-free medium express increased stemness-related genes compared to 2D culture. a) Representative image of calcein AM/EthD-1 staining of U87 cells at day-7 culture; b) Viability of U87 cells over seven days in 3D cell culture. Data presented as mean±SD (percentage of live cells). c) Ki67 EGRF and GFAP, and d) mRNA expression of U87 cells grown in 3D ECM versus 2D culture for three days (GAPDH was used as housekeeping gene). Results are from three independent experimental runs are presented by mean±SEM. One-way ANOVA with Tukey's post-hoc test was used for significance.

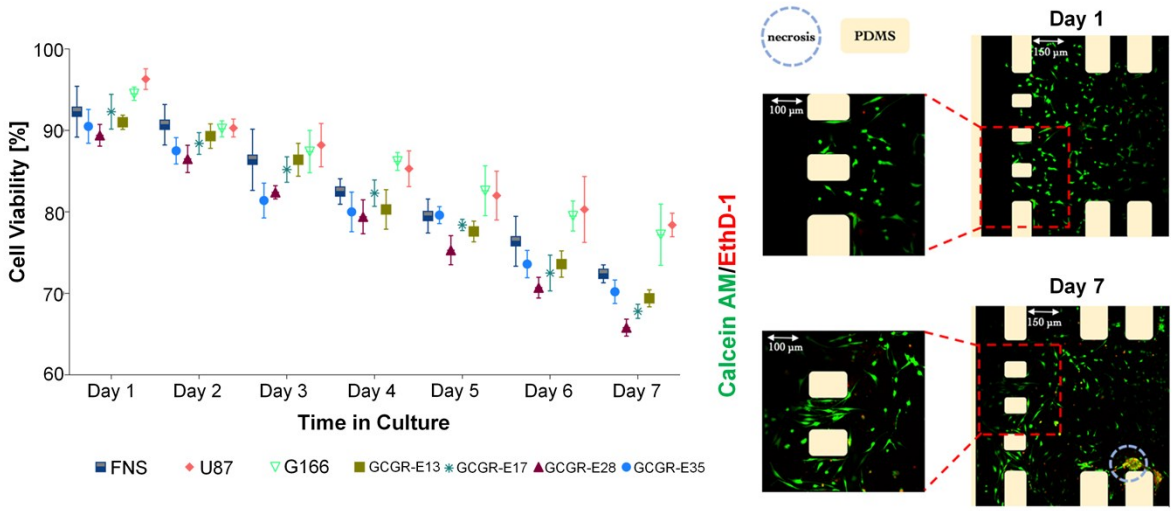

Fig. S4: Left: graph presenting viability of six different GSCs and one foetal neural stem cell line over seven days in cell culture; Right: Example images of calcein AM/EthD-1 staining of U87 cells at day 1 and day 7 in culture. At day seven in culture, cells migrated into the outermost side channels, and formed necrotic clusters in the centre of the chamber. The graph shows percentage viability values – data is presented as mean±SD, obtained from three independent experimental runs.

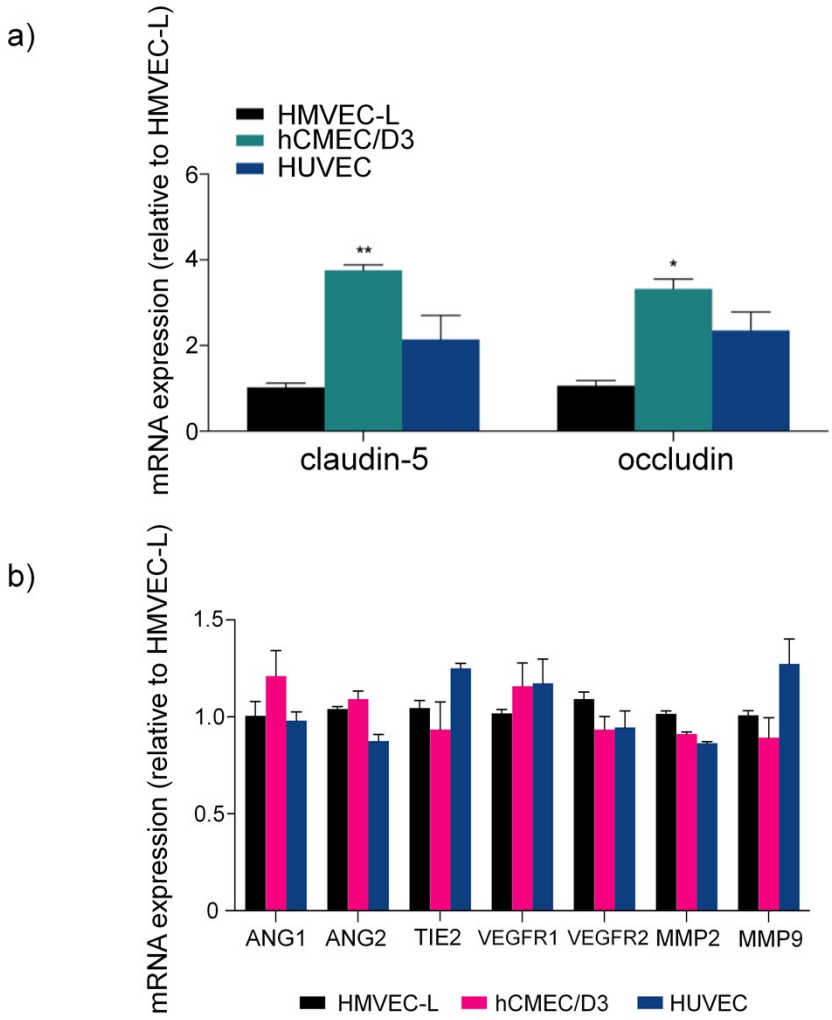

Fig. S5: a) mRNA expression normalised to HMVEC-L as sample of the lowest expression of all tested genes. Results obtained from three independent experimental runs are presented by mean±SEM. One-way ANOVA with Tukey's post-hoc test was used for significance; b) In monoculture of microvessels, endothelial cells of different tissue origin are characterised by similar levels of expression of neovascularisation-related genes. mRNA expression normalised to HMVEC-L microvessel. Results obtained from three independent experimental runs are presented by mean±SEM.

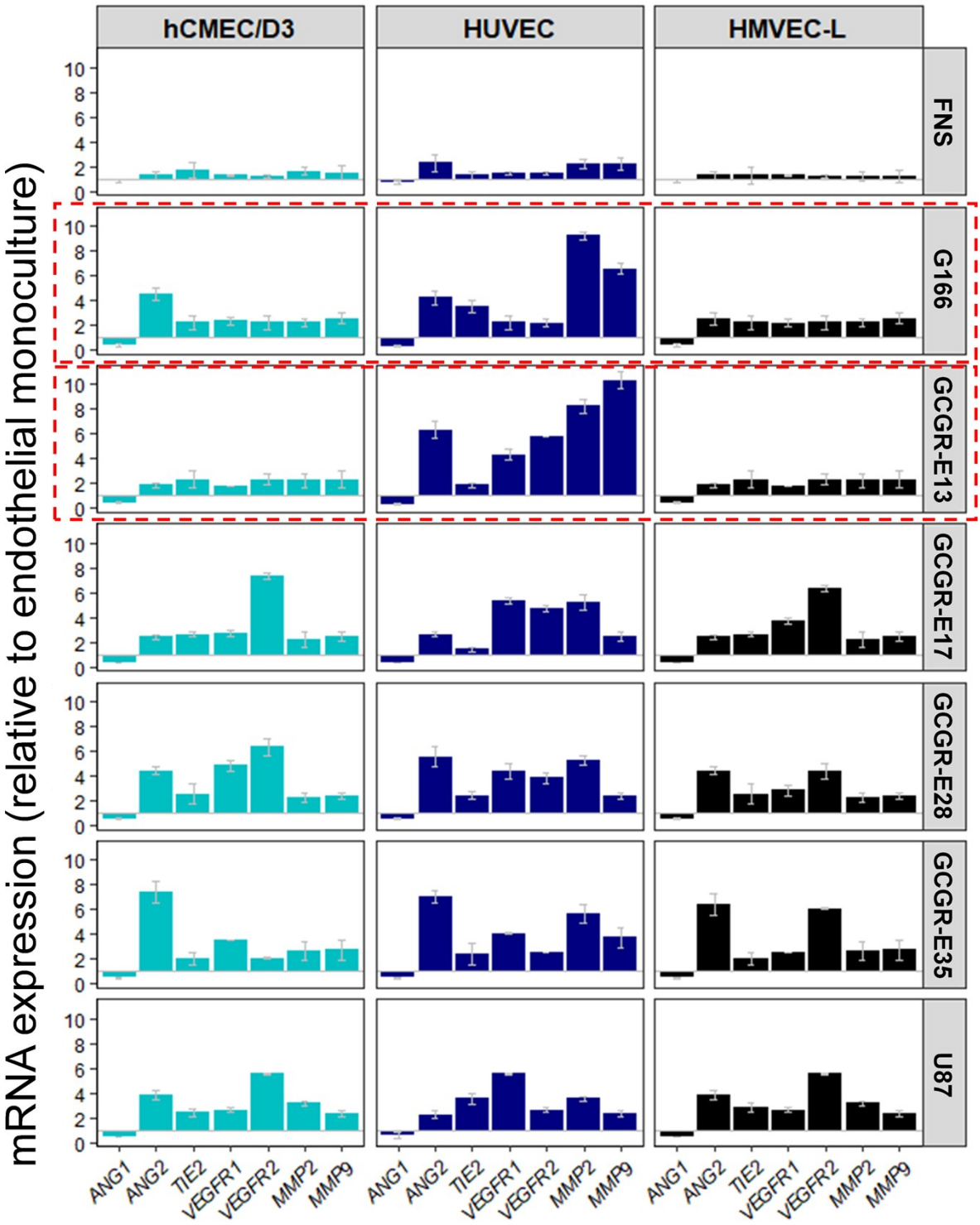

Fig. S6: Gene expression in endothelial cells changes under the influence of brain cancer cells in organ-specific manner. Graphs were plotted in RStudio after the analysis of mRNA expression normalised to endothelial monoculture. Results obtained from three independent experimental runs are presented by mean±SEM.

Table S2 a): qPCR primers sequences

gene	*primer sequence 5'->3'*
ANG1	forward (fw): GCCTACACTTTCATTCTTCCAGA
	reverse (rv): TCTTCCTTGTGTTTTCCTTCCAT
ANG2	fw: GGCAGCGTTGATTTTCAGAGGACT
	rv: TTTAATGCCGTTGAACTTATTTGT
CD31	fw: CCCAGCCCAGGATTTCTTAT
	rv: ACCGCAGGATCATTTGAGTT
CD44	fw: CCAGAAGGAACAGTGGTTTGGC
	rv: ACTGTCCTCTGGGCTTGGTGTT
CD133	fw: ATTGACTTCTTGGTGCTGTTGA
	rv: GATGGAGTTACGCAGGTTTCTC
claudin-5	fw: ACACTAATACGAAGGCACTCCA
	rv: CTCCTGGAAGATGGTGATGG
GAPDH	fw: GTCTCCTCTGACTTCAACAGCG
	rv: ACCACCCTGTTGCTGTAGCCCAA
GFAP	fw: GTACCAGGACCTGCTCAAT
	rv: CAACTATCCTGCTTCTGCTC
Ki67	fw: GAGAATCTGTGAATCTGGGTAA
	rv: CAGGCTTGCTGAGGGAAT
MMP2	fw: CCCCAAAACGGACAAAGAG
	rv: CACGAGCAAAGGCATCATCC
MMP9	fw: CACTGTCCACCCCTCAGAGC
	rv: GCCACTTGTCGGCGATAAGG
nestin	fw: CAGCGTTGGAACAGAGGTTGG
	rv: TGGCACAGGTGTCTCAAGGGTAG
occludin	fw: TCAGGGAATATCCTCACTTCAG
	rv: CATCAGCAGCAGCCATGTACTCTTCAC
olig2	fw: ATGCACGACCTCAACATCGCCA
	rv: ACCAGTCGCTTCATCTCCTCCA
sox2	fw: ATGCACCGCTACGACGTGA
	rv: CTTTTGCACCCCTCCCATTT

TIE2	fw: TACTAATGAAGAAATGACCCTGG
	rv: GGAGTGTGTAATGTTGGAAATCT
VE-cad	fw: AAGACCGATTAACCATGTCA
	rv: ATGTCAGGCTTTCTGGATTA
VEGFR1	fw: CAGGCCCAGTTTCTGCCATT
	rv: TTCCAGCTCAGCGTGGTCGTA
VEGFR2	fw: CCAGCAAAAGCAGGGAGTCTGT
	rv: TGTCTGTGTCATCGGAGTGATATCC
ZO-1	fw: ATTCCTTAGTGTCCAA
	rv: CCTGAGCAGTATCTT

Table S2: b): Primary antibodies.

Antigen	Host	Dilution IF	Dilution WB	Supplier
CD31	mouse	1:200	1:1000	Invitrogen
CD44	rabbit	1:500	1:2000	abcam
CD133	rabbit	1:200	1:500	abcam
GAPDH	goat	-	1:1000	abcam
GFAP	mouse	-	1:1000	Sigma
Ki67	rabbit	-	1:500	abcam
nestin	mouse	1:500	1:1000	Millipore
olig2	mouse	1:500	1:1000	Invitrogen
sox2	mouse	1:200	1:500	R&D Systems
VE-cad	mouse	1:200	1:100	Novus Biologicals
ZO-1	rabbit	1:500	1:1000	abcam

Table S2 c): Secondary antibodies and fluorescent probes

Secondary Antibodies

Antigen	Host	Conjugation	Dilution	Supplier
goat IgG	donkey	HRP	1:5000	R&D Systems
mouse IgG	sheep	HRP	1:5000	R&D Systems
rabbit IgG	donkey	HRP	1:5000	GE Healthcare
mouse IgG	donkey	Alexa Fluor® 488	1:1000	abcam
rabbit IgG	goat	Alexa Fluor® 488	1:1000	abcam
mouse IgG	goat	Alexa Fluor® 647	1:1000	abcam
rabbit IgG	donkey	Alexa Fluor® 647	1:1000	abcam

Fluorescent probes

Names	Dilution	Supplier
Alexa Fluor 488® phalloidin	1:5000	Invitrogen
CyTRAK Orange™	1:1000	abcam

Table S3: Endothelial permeability measurements for cells in both serum-containing medium and serum-free medium 12 and 24 hours after seeding;

Serum-containing Medium

cell type	12 hr	24 hr	
hCMEC/D3	10.5±1.4	8.2±0.9	
HUVECs	15.0±3.6	12.0±2.4	$x10^{-3}$ cm/min
HMVEC-L	14.8±2.9	11.7±2.6	

Serum-free Medium

cell type	12 hr	24 hr	
hCMEC/D3	11.2±0.8	8.7±1.1	
HUVECs	15.4±2.9	12.8±3.2	$x10^{-3}$ cm/min
HMVEC-L	15.0±3.5	12.4±2.4	