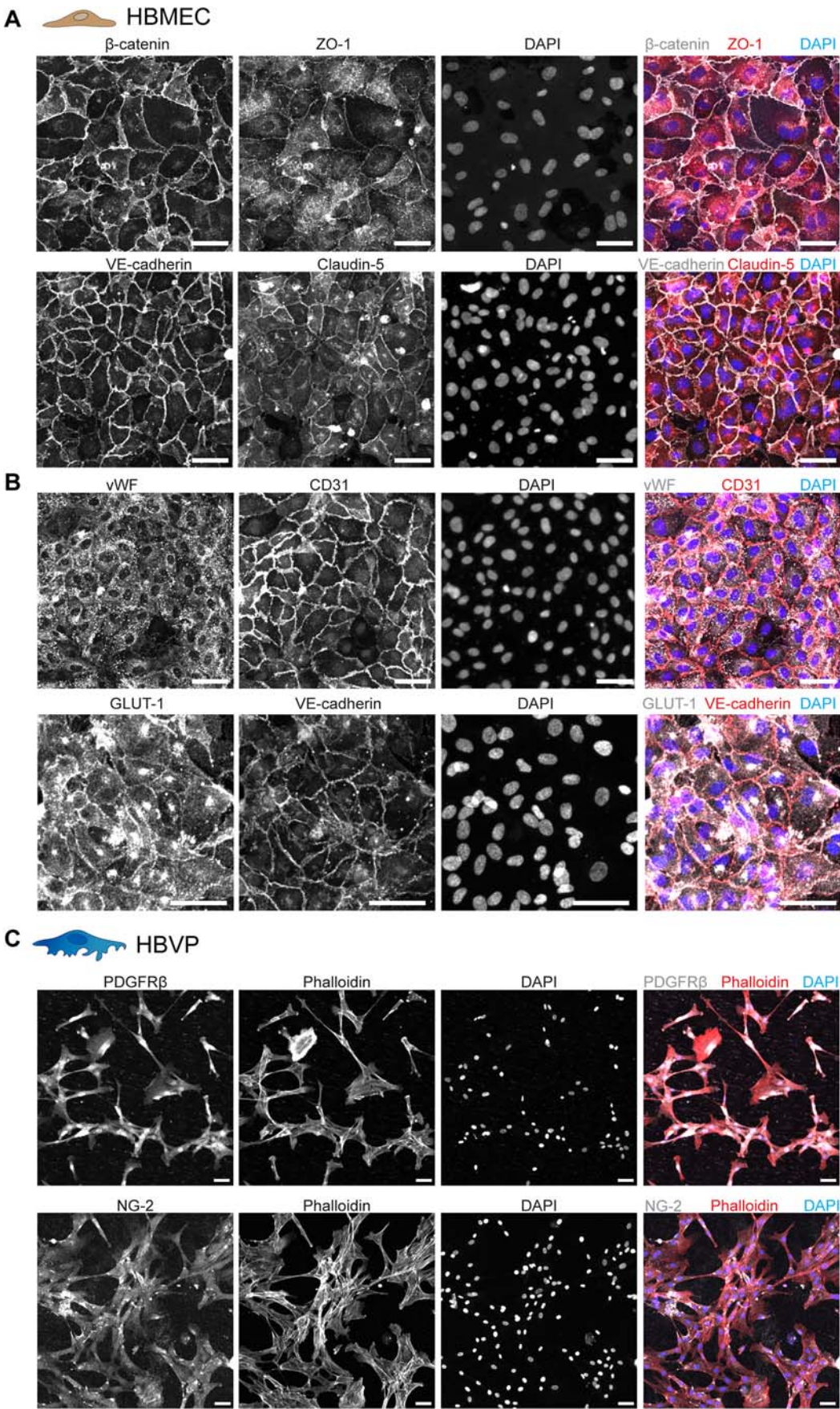
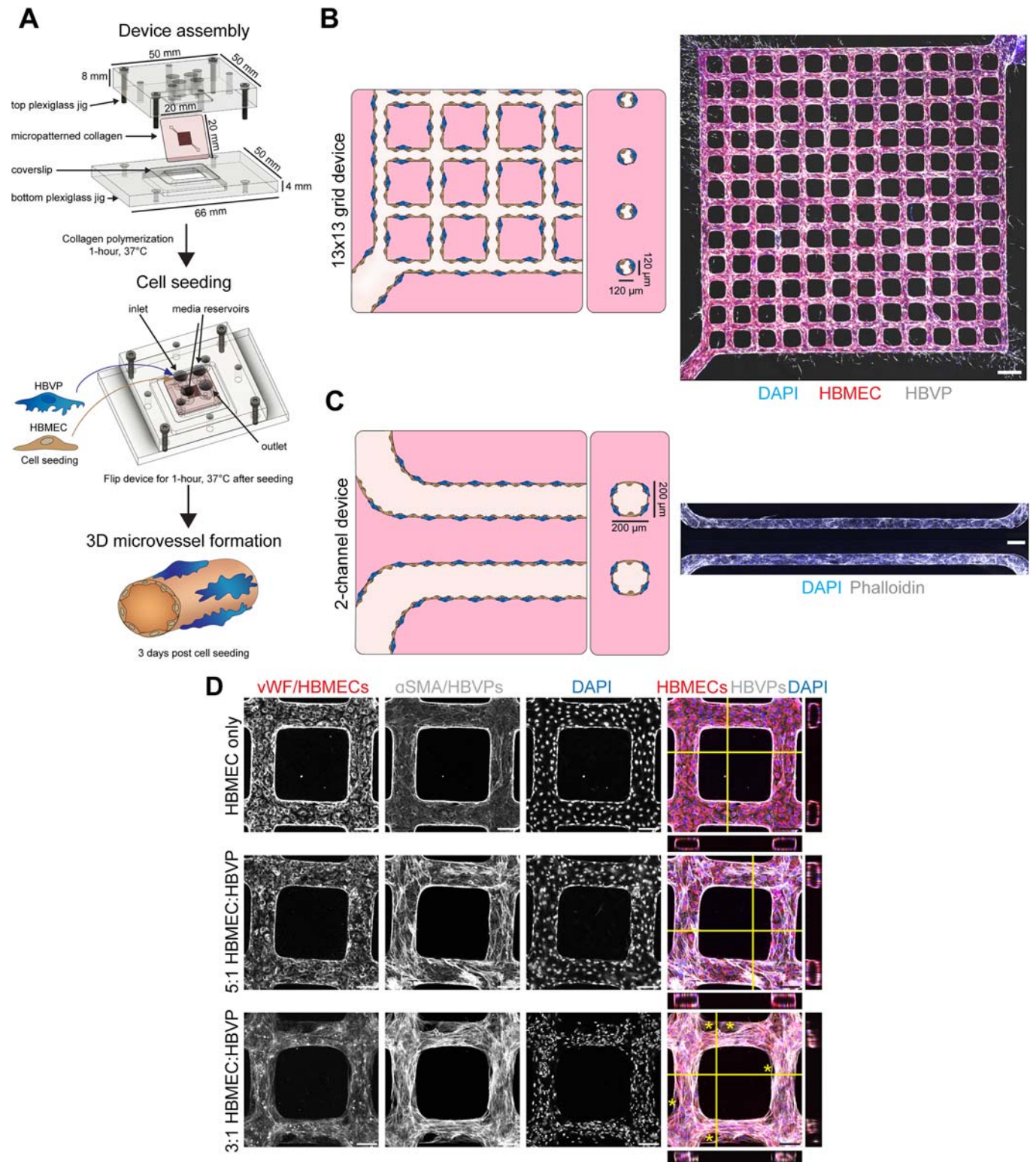


Expanded View Figures

Figure EV1. Characterization of brain-specific endothelial and pericyte marker expression and secretion of angiopoietin-Tie axis components.

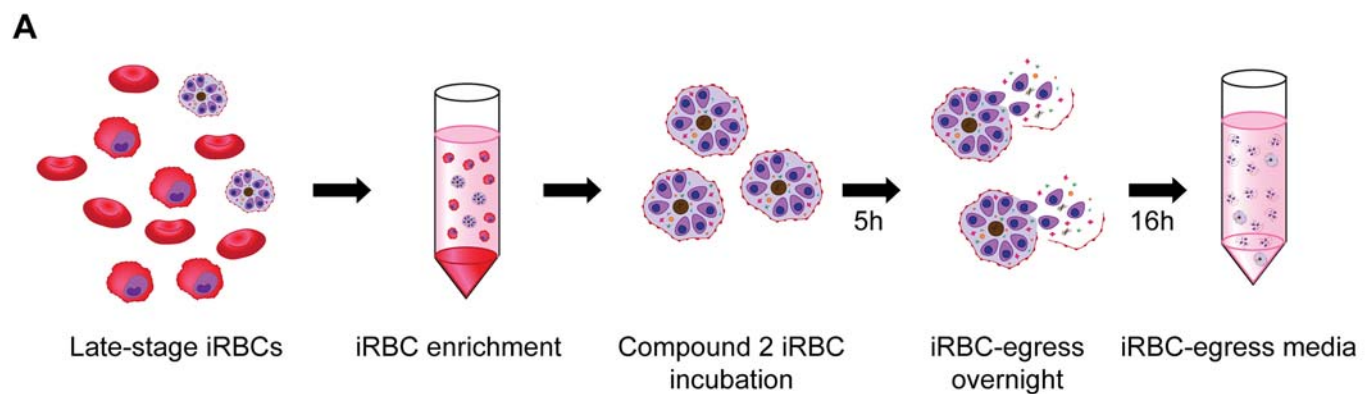
(A) Immunofluorescence maximum z-projection of a 2D HBMEC monolayer stained for adherens and tight junctional markers: β -catenin, VE-cadherin, ZO-1 (top) and claudin-5 (bottom), and 4', 6-diamidino-2-phenylindole (DAPI). The merged image includes the adherens junction markers (white), tight junction markers (red) and DAPI labeling (blue). Scale bars: 50 μ m. (B) Immunofluorescence maximum z-projection of a 2D HBMEC monolayer stained for vWF, brain glucose transporter GLUT-1, CD31, VE-cadherin and DAPI. The merge image includes vWF or GLUT-1 (white), CD31 or VE-cadherin (red) and DAPI labeling (blue). Scale bars: 50 μ m. (C) Immunofluorescence maximum z-projection of a 2D HBVP monolayer stained for PDGFR β (top) and NG-2 (bottom), Phalloidin and DAPI. The merge staining includes the pericyte markers (white), phalloidin (red) and DAPI labeling (blue). Scale bars: 50 μ m. (D) Concentration of secreted angiopoietin-1 (top) and angiopoietin-2 (bottom) in supernatant obtained from either HBVP-HBMEC co-culture or HBMEC-only monolayers grown in a transwell model. Data is presented as mean $\pm$ standard deviation ($n = 3$ independent experiments for angiopoietin-1 measurement and 4 for angiopoietin-2 measurement, Mann-Whitney U test).



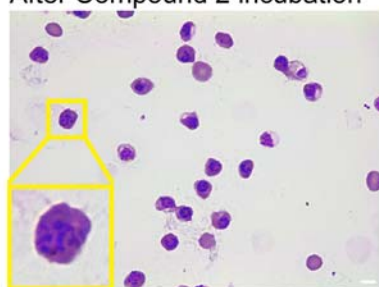


**Figure EV2. Fabrication of the 3D brain microvessel model.**

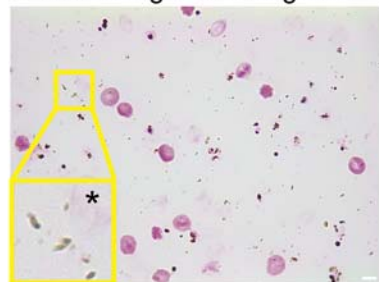
(A) Schematic representation of the protocol to fabricate the 3D brain microvessel model including device assembly, cell seeding and 3D microvessel formation. (B) Schematic overview and cross section of the 13×13 grid microvessel network (left). IFA maximum z-projection of the complete grid microvessel network stained for vWF for endothelial cells (red), mCherry for pericytes (white) and DAPI (blue). Scale bar: 500 μm . (C) Schematic overview and cross section of the 2-channel microvessel network (left). IFA maximum z-projection of the complete grid microvessel network stained for phalloidin (white) and DAPI (blue). Scale bar: 200 μm . (D) Immunofluorescence assay (IFA) maximum z-projection of microvessels fabricated using HBMEC-only, a 3:1 or 5:1 HBMEC to HBVP ratio labeled with vWF for HBMEC (red), α SMA for HBVP (white) and DAPI (blue). Orthogonal views display the presence or absence of a perfusable microvessel lumen and yellow asterisks represent regions of microvessel detachment. Scale bar: 100 μm .



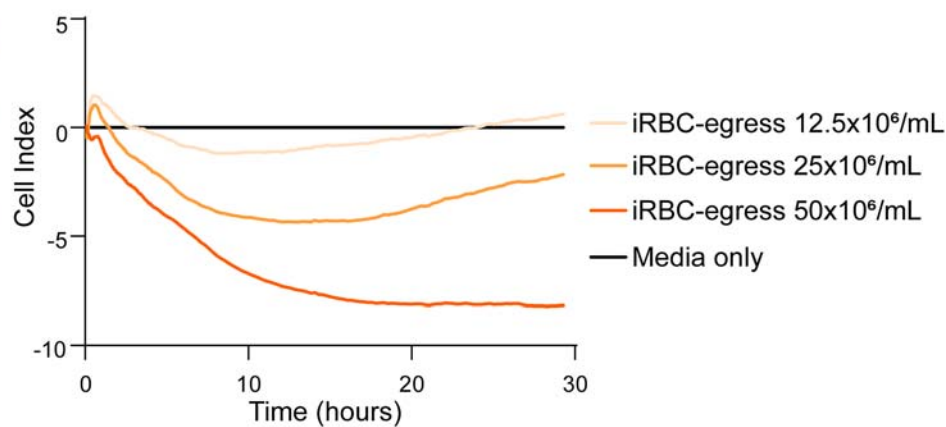
B After Compound 2 incubation



After overnight iRBC-egress



C



D



E

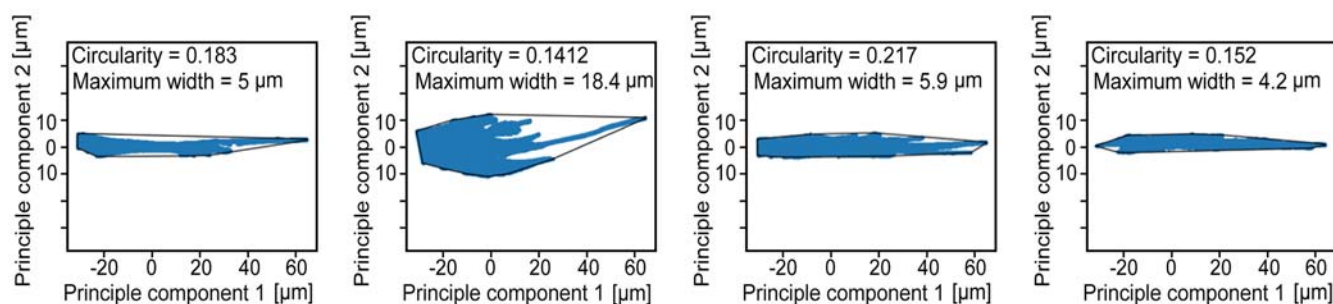
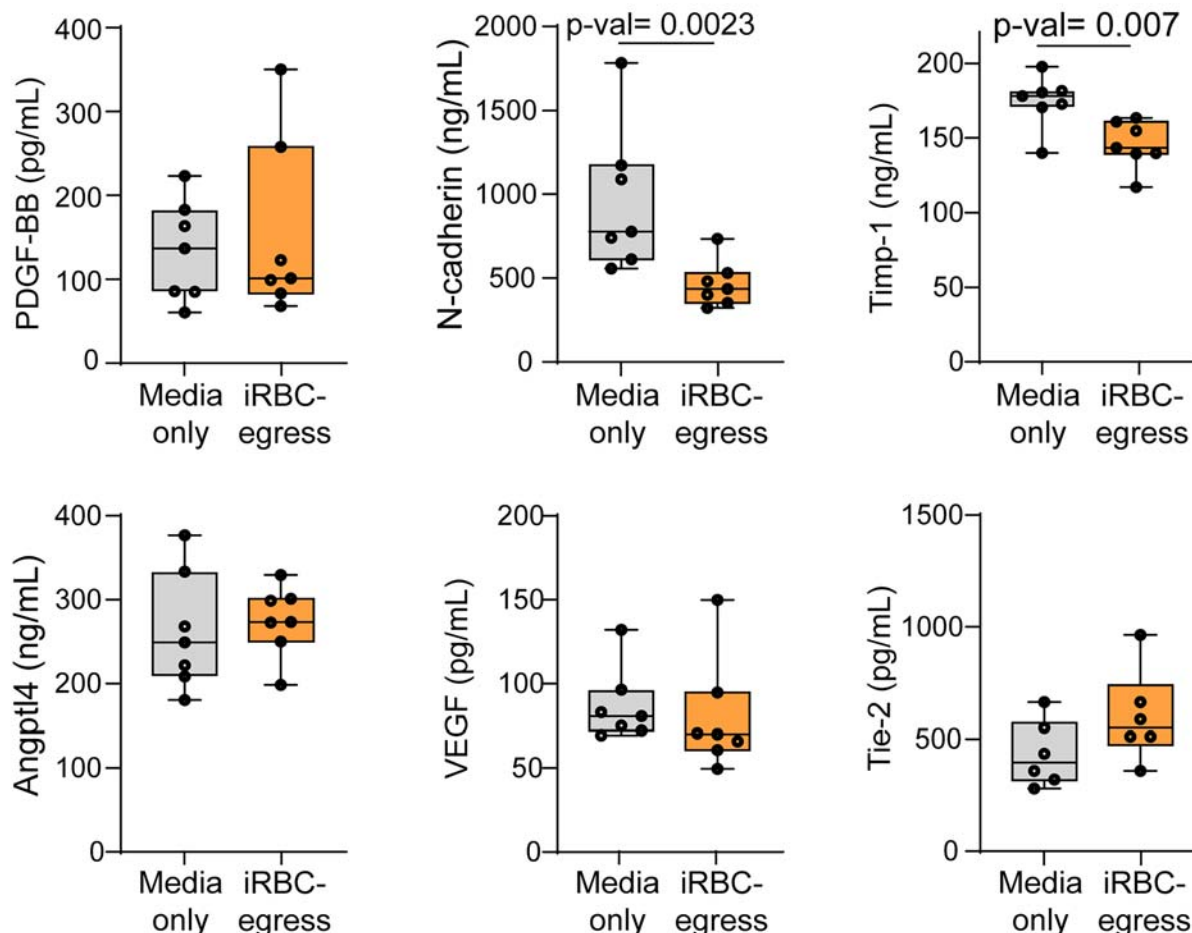


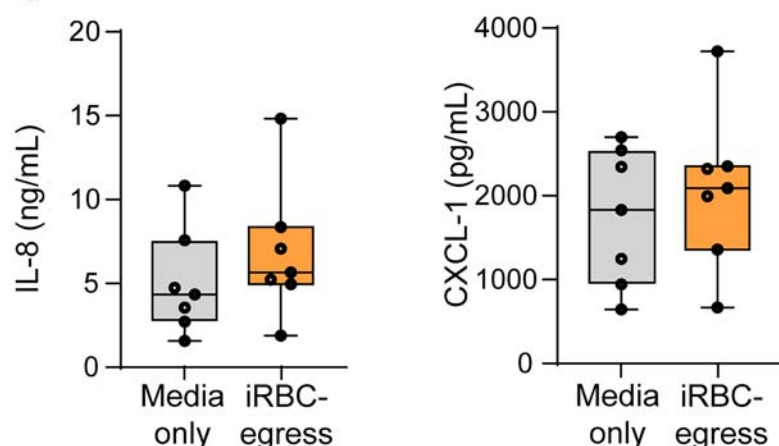
Figure EV3. Generation of endothelial barrier disruptive iRBC-egress media and a pipeline for analysis of pericyte morphological features.

(A) Schematic representation of the protocol to make iRBC-egress media. In short, late-stage *P. falciparum*-iRBC are purified by a gelaspan gradient separation and then incubated for 5 h with compound-2 to synchronize them at the point of egress. Compound-2 is removed and the *P. falciparum*-iRBC are resuspended in vascular growth media and left overnight on a shaker at 50 rpm to egress. (B) A thin smear of tightly synchronized schizonts before the removal of Compound-2 (yellow inset highlights a ROI with a schizont-stage iRBC) or the resultant iRBC-egress media before centrifugation (yellow inset highlights a ROI with free hemozoin particles next to an iRBC ghost, denoted with an asterisk) stained with Giemsa. Scale bars: 5 μ m. (C) Representative recording data of xCELLigence measurements on 2D HBMEC monolayers in the absence of HBVP taken after addition of iRBC-egress media at different concentrations. Data is normalized to the media-only control. (D) The analysis pipeline begins with the extraction of 3D segmented pericyte meshes. Shown here are four representative pericytes. Scale bars: 10 μ m. (E) Pericyte geometry is flattened into two principal dimensions using PCA by a Python-based image analysis pipeline. The 2D geometry's shape is then determined using convex hull analysis. To describe pericyte morphology, pericyte cell borders are analyzed for circularity, calculated as $4\pi \cdot \text{Area} / \text{Perimeter}^2$, where a value of 1 indicates a perfect circle and values approaching 0 indicate increasingly elongated shapes, and maximum width.

A Vascular factors



B Cytokines



C EGM-2MV + growth factors

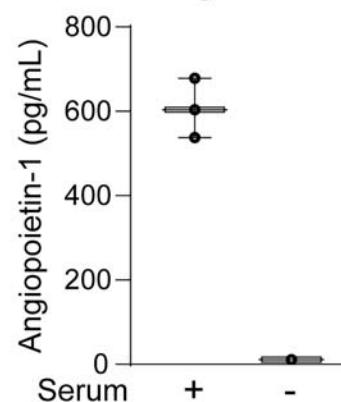


Figure EV4. iRBC egress products cause alteration of endothelial cell-pericyte interaction markers.

(A) Concentrations of vascular factors PDGF-BB, N-cadherin, Timp-1, Angptl4, VEGF and Tie-2 measured by Luminex from 3D brain microvessels supernatants treated with media-only or iRBC-egress media for 18-h. Box and whisker plots display the median, 25th and 75th percentiles and the minimum and maximum data points. ($n = 7$ supernatants pooled from 2-3 devices each). (B) Concentrations of released cytokines IL-8 and CXCL-1 measured by Luminex from 3D brain microvessels supernatants treated with media-only or iRBC-egress media for 18-h. Box and whisker plots display the median, 25th and 75th percentiles and the minimum and maximum data points. ($n = 7$ supernatants pooled from 2-3 devices each). (C) Concentrations of angiopoietin-1 measured by Luminex from freshly made growth factor-containing EGM-2MV with or without serum. Box and whisker plots display the median, 25th and 75th percentiles and the minimum and maximum data points. ($n = 3$, where angiopoietin-1 in serum-free vascular media was below the limit of detection). Data information: Statistical significance is analyzed by Mann-Whitney U test (A, B).

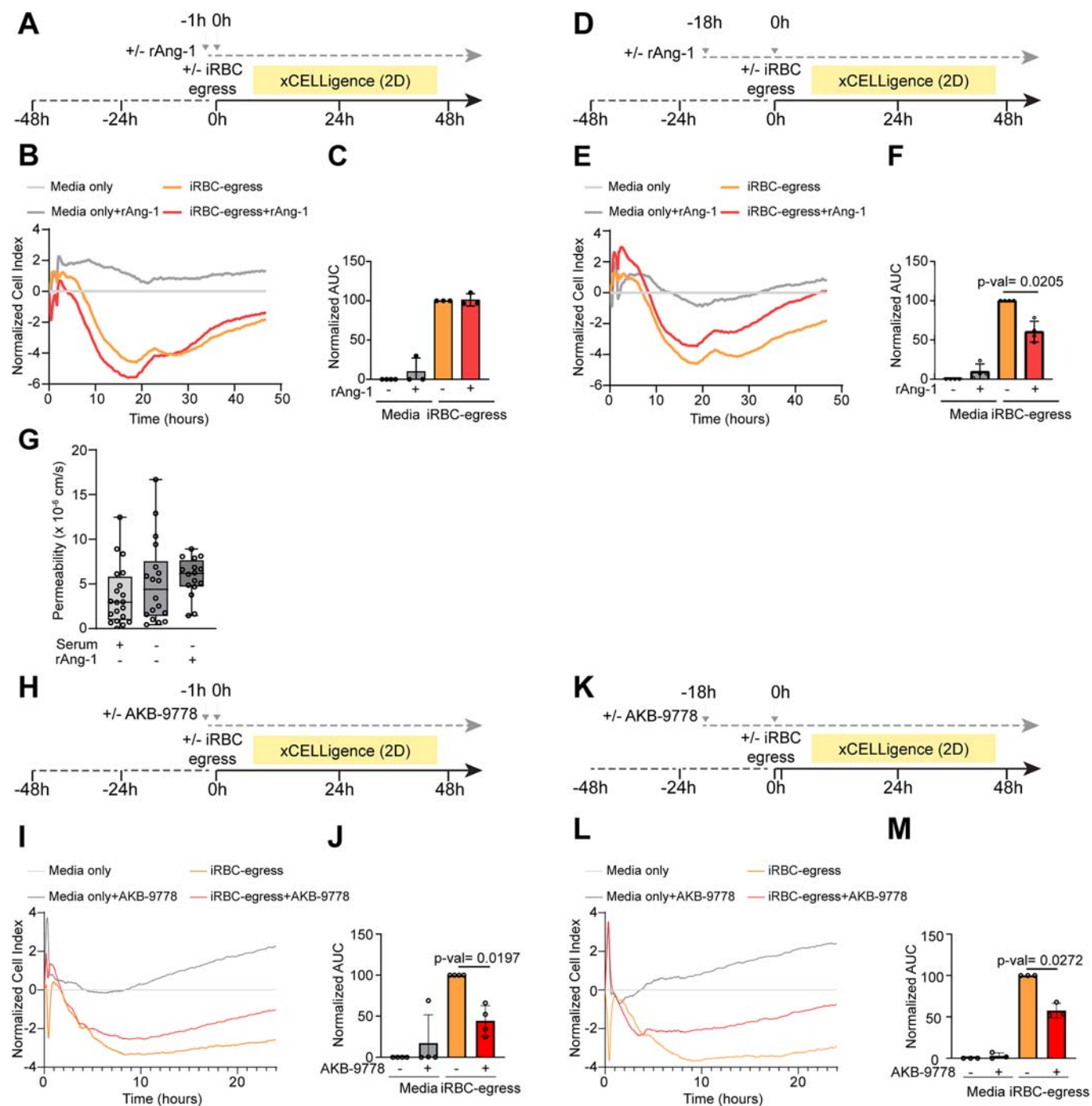


Figure EV5. Recombinant Ang-1 or AKB-9778 pre-treatment partially protects against 2D endothelial monolayer permeability increase induced by iRBC egress products.

(A) Experimental outline of short-term (1-h) incubation with rAng-1 in a 2D HBMEC monolayer in the absence of HBVP. (B) Representative recording data of xCELLigence measurements taken after a 1-h +/-rAng-1 pre-treatment followed by +/- iRBC-egress media addition. All conditions were normalized to the media-only control. (C) Area under the curve analysis with iRBC-egress media normalized as 100 ($n = 3$ independent experiments run in triplicate). (D) Experimental outline of long-term (18-h) incubation with rAng-1 in a 2D HBMEC monolayer in the absence of HBVP. (E) Representative recording data of xCELLigence measurements taken after an 18-h +/-rAng-1 pre-treatment followed by +/- iRBC-egress media addition. All conditions were normalized to the media-only control. (F) Area under the curve analysis with iRBC-egress media normalized as 100. ($n = 4$ independent experiments run in triplicate). (G) Apparent permeability of 70 kDa FITC-dextran in microvessels pre-treated with serum containing media, serum-free media or serum-free media+rAng-1 ($n = 10$ individual devices for serum containing media incubation, 9 for serum-free media incubation and 8 for serum-free media+rAng-1 incubation). (H) Experimental outline of short-term (1-h) incubation with AKB-9778 in a 2D HBMEC monolayer in the absence of HBVP. (I) Representative recording data of xCELLigence measurements taken after a 1-h +/-AKB-9778 pre-treatment followed by +/- iRBC-egress media addition. All conditions were normalized to the media-only control. (J) Area under the curve analysis with iRBC-egress media normalized as 100 ($n = 4$ independent experiments run in triplicate). (K) Experimental outline of long-term (18-h) incubation with AKB-9778 in a 2D HBMEC monolayer in the absence of HBVP. (L) Representative recording data of xCELLigence measurements taken after an 18-h +/-rAng-1 pre-treatment followed by +/- iRBC-egress media addition. All conditions were normalized to the media-only control. (M) Area under the curve analysis with iRBC-egress media normalized as 100. ($n = 3$ independent experiments run in triplicate). Data information: In (C, F, J, M), data are presented as bar charts displaying the mean +/- standard deviation (Repeated measures one-way ANOVA test with Dunnett's multiple comparisons test. In (G), box and whisker plots display the median, 25th and 75th percentiles and the minimum and maximum data points (Kruskal-Wallis test corrected for multiple comparisons using the Benjamini, Krieger and Yekutieli method).