**Supplementary Table 1**. Media treatment schedule for maintenance of 3D-oBRB.

| Day(s) | Basal Conditions | Apical Conditions |
|---|---|---|
| 1 | **Printing Medium** | **Printing Medium** |
| 2-7 | **VDM** | **VDM** |
| 8-14 | **VGM+AP** | **RPE-MM + AP** |
| 15-21 | **VGM** | **RPE-MM** |
| 22-29 | **VGM** | **RPE-MM + Prostaglandin E2** |
| 29-Fixation | **VMM** | **RPE-MM + Prostaglandin E2** |

**Supplementary Table 2.** Libraries generated for single cell RNA-seq.

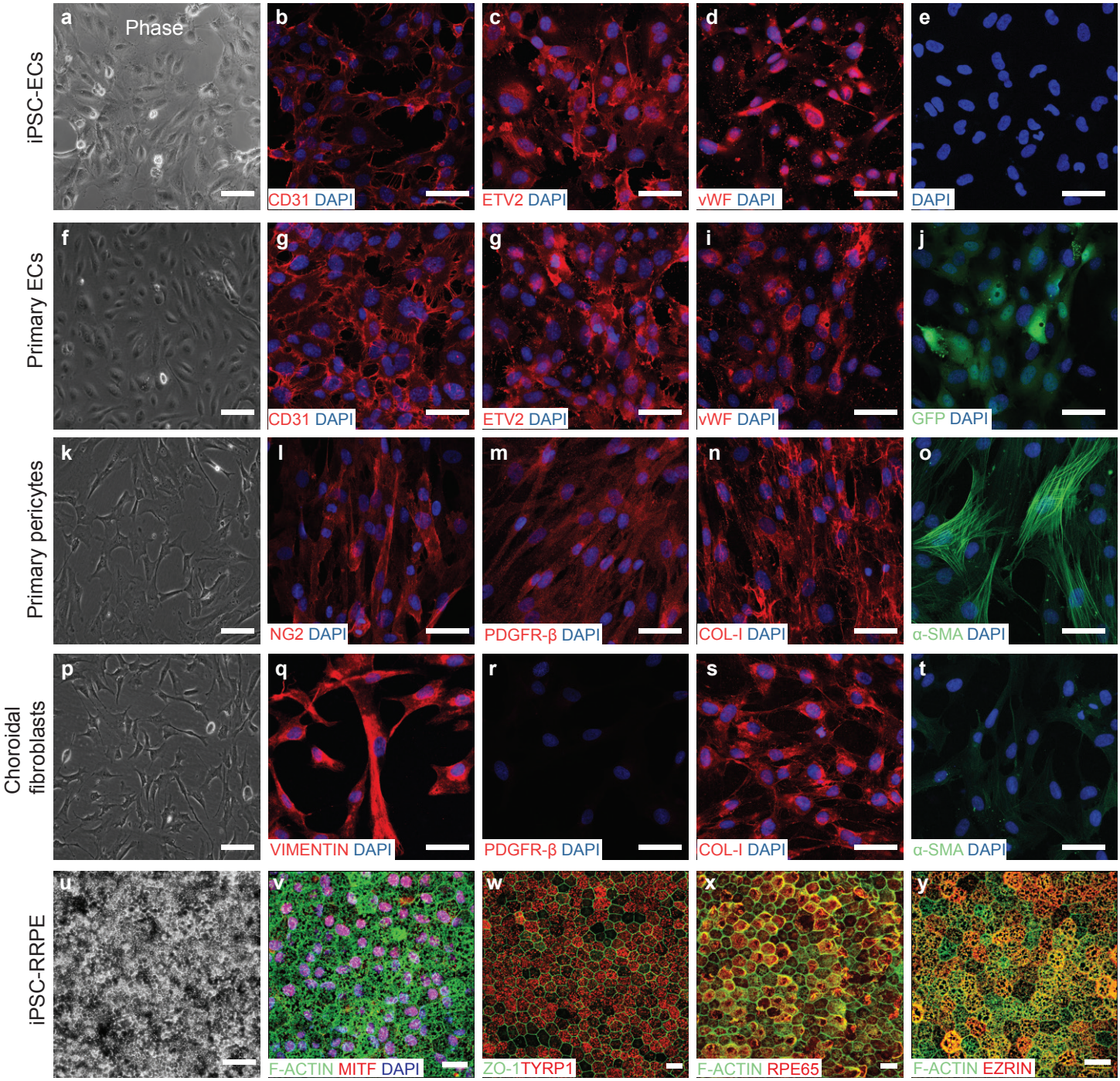
| **Library ID** | **Library Concentration (ng/ul)** | **Library Average Size (bp)** | **Sequence Modality(e.g. PE50-6-50)** | **Tube Label** | **Sample ID*** | **Sample Barcode Index1: I7 Sequence** |
|---|---|---|---|---|---|---|
| IS0011 | 2nM | 455 | (PE) 26-8-98mer | 2D iEndo | CT_3878_S1 | SI-GA-A6 |
| IS0011 | 2nM | 479 | (PE) 26-8-98mer | 2D iRPE | CT_3878_S2 | SI-GA-B6 |
| IS0011 | 2nM | 454 | (PE) 26-8-98mer | 3D iEndo | CT_3878_S3 | SI-GA-C6 |
| IS016 | 2nM | 508 | (PE) 26-8-98mer | 3D iRPE | | SI-GA-F7 |

**Supplementary Table 3.** Significant genes relevant to vascular maturation. p****<0.0001. Data depicts results from n = 5369 cells (2D iECs), and n = 1294 cells (3D-oBRB iECs).

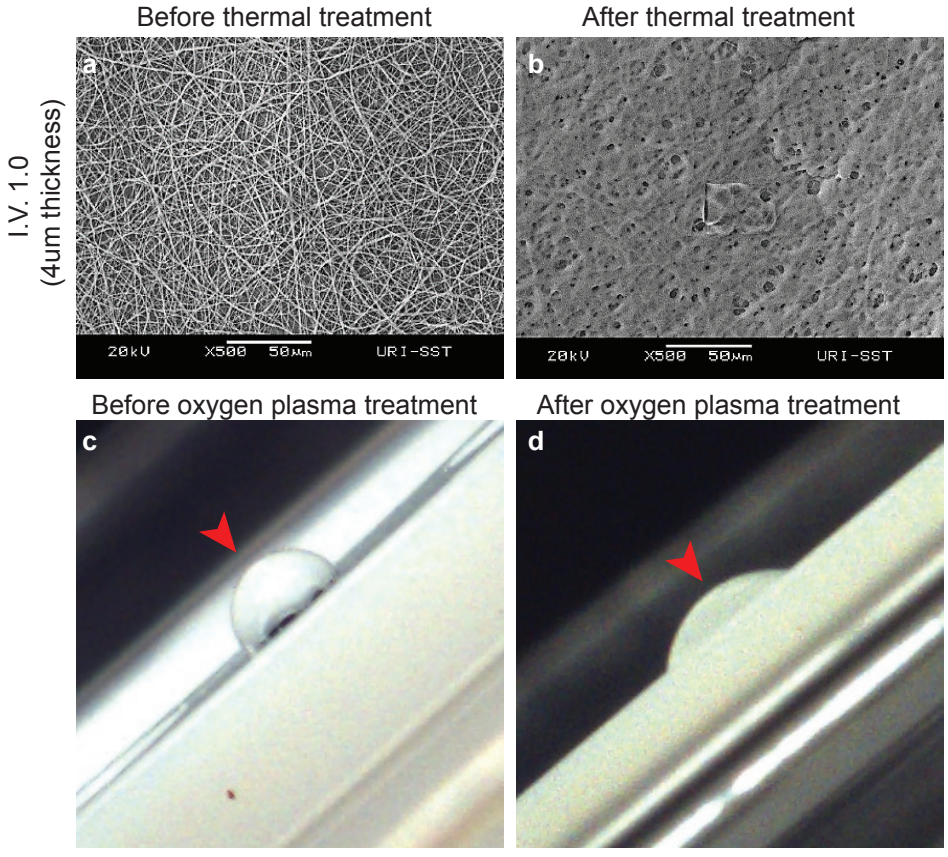
| Partially-Mature (PM) | |
|---|---|
| **Gene** | **Average LogFC** |
| TTR**** | 3.293097084 |
| PTGDS**** | 2.91023027 |
| SERPINF1**** | 2.746884668 |
| PMEL**** | 2.728151531 |
| TYRP1**** | 2.473397492 |
| SLC2A1**** | 2.447382476 |
| RBP1**** | 2.277384475 |
| DCT**** | 2.266739064 |
| APOE**** | 2.191424756 |
| ELN**** | 2.184219482 |
| CDKN1C**** | 2.184180543 |
| GPNMB**** | 2.155826338 |
| FRZB**** | 2.036587593 |
| TIMP3**** | 2.020232723 |
| NPC2**** | 1.971437309 |
| NEAT1**** | 1.877960021 |
| TRPM3**** | 1.868956372 |
| RPS4Y1**** | 1.748429142 |
| SFRP1**** | 1.71339181 |
| MIF**** | 1.626987544 |
| FOS**** | 1.61485332 |
| VEGFA**** | 1.604315395 |
| CRYAB**** | 1.596007132 |
| NDUFA4L2**** | 1.584758503 |
| EGR1**** | 1.583968974 |
| CST3**** | 1.583860234 |
| SCD**** | 1.576730172 |
| KCNQ1OT1**** | 1.569869951 |
| AKAP12**** | -1.509372337 |
| TUBB4B**** | -1.516117864 |
| LGALS1**** | -1.524191678 |
| TUBB6**** | -1.535855664 |
| SMS**** | -1.564620788 |
| GNG11**** | -1.573524516 |
| JPT1**** | -1.578619298 |
| ECSCR**** | -1.610296939 |
| TPM1**** | -1.705061753 |
| CAV1**** | -1.720755414 |
| CLIC1**** | -1.806758255 |
| MT1E**** | -1.829130034 |
| ARHGDIB**** | -1.833353979 |
| S100A16**** | -1.854543401 |
| SRGN**** | -1.855853186 |
| SERPINE1**** | -2.011057296 |
| TUBA1B**** | -2.085073696 |
| MT2A**** | -2.165154694 |
| HMGA1**** | -2.202244352 |
| MMP1**** | -2.466878261 |

| Fully-Mature (FM) | |
|---|---|
| **Gene** | **Average LogFC** |
| IGFBP3**** | 2.839701836 |
| ESM1**** | 2.603010821 |
| IGF2**** | 2.598543141 |
| INSR**** | 2.394917281 |
| HLA-B**** | 2.255402078 |
| TCF4**** | 2.052826873 |
| CD93**** | 2.036025929 |
| PECAM1**** | 1.99525718 |
| ACKR3**** | 1.990773556 |
| PLVAP**** | 1.970377307 |
| NEAT1**** | 1.965910456 |
| SPRY1**** | 1.962680299 |
| ANGPT2**** | 1.961169986 |
| RGCC**** | 1.929979405 |
| HSPG2**** | 1.871711824 |
| COL4A1**** | 1.851158264 |
| TP53I11**** | 1.834864472 |
| RFLNB**** | 1.820297661 |
| CXCR4**** | 1.812145964 |
| CD34**** | 1.811398119 |
| SPARC**** | 1.763536864 |
| DEPP1**** | 1.746571776 |
| PXDN**** | 1.745320416 |
| UNC5B**** | 1.703681255 |
| FLT1**** | 1.662451028 |
| B2M**** | 1.655103184 |
| COL4A2**** | 1.63152531 |
| ADGRF5**** | 1.620363791 |
| GJA1**** | 1.588797246 |
| SPP1**** | 1.559651451 |
| ANXA2**** | -1.532336179 |
| TUBA1B**** | -1.76975269 |
| MMP1**** | -1.885111478 |
| MT1E**** | -2.016514401 |
| MT2A**** | -2.294959755 |
| HMGA1**** | -2.481583463 |

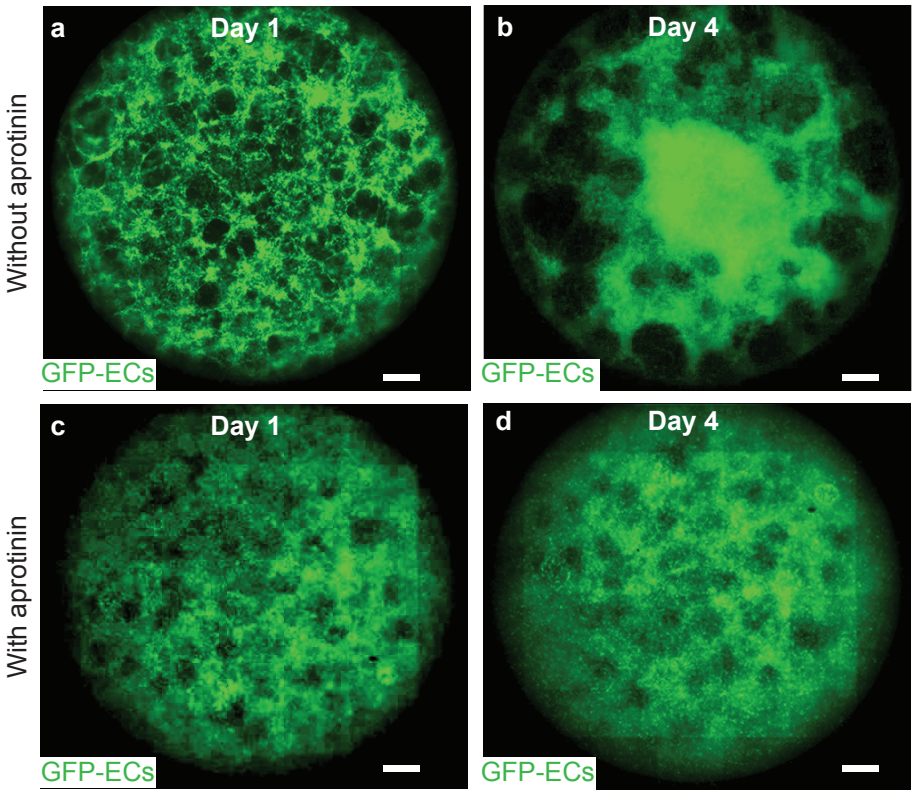
| Inflamed (Inf) | |
|---|---|
| **Gene** | **Average LogFC** |
| COL3A1**** | 3.790343782 |
| IGFBP5**** | 3.767910699 |
| COL1A2**** | 3.456215894 |
| MGP**** | 3.436031728 |
| STATH**** | 3.376360389 |
| COL1A1**** | 3.35150075 |
| TIMP1**** | 3.271068336 |
| STC1**** | 2.761122651 |
| COL6A2**** | 2.651220963 |
| SAA1**** | 2.634649398 |
| SERPINE2**** | 2.597995887 |
| COL6A1**** | 2.573399435 |
| AREG**** | 2.445094854 |
| COL6A3**** | 2.305247798 |
| DCN**** | 2.254222597 |
| FN1**** | 2.220866953 |
| IGF2**** | 2.062980648 |
| TFPI2**** | 1.856624892 |
| PLAC9**** | 1.830906964 |
| LUM**** | 1.806349544 |
| NDUFA4L2**** | 1.791817577 |
| LOX**** | 1.785051044 |
| CXCL2**** | 1.741603755 |
| TGM2**** | 1.731212221 |
| PDGFRB**** | 1.730017382 |
| CXCL1**** | 1.707399258 |
| MEG3**** | 1.706864124 |
| PLAT**** | 1.696673351 |
| SAA2**** | 1.675952576 |
| CXCL8**** | 1.661135865 |
| SPARC**** | 1.655768294 |
| PAPPA**** | 1.637425773 |
| EGR1**** | 1.628629962 |
| FDCSP**** | 1.57565212 |
| FOS**** | 1.574482144 |
| F3**** | 1.529745697 |
| COL5A2**** | 1.502431491 |
| ARHGDIB**** | -1.500379729 |
| STMN1**** | -1.526635755 |
| SMS**** | -1.555392149 |
| TPM1**** | -1.594545485 |
| ECSCR**** | -1.59696695 |
| JPT1**** | -1.607577918 |
| S100A16**** | -1.755430391 |
| TUBA1B**** | -1.910956266 |
| MMP1**** | -2.286670634 |
| HMGA1**** | -2.461526253 |


iPSC-ECs
Primary ECs
Primary pericytes
Choroidal fibroblasts
iPSC-RRPE
a Phase
b CD31 DAPI
c ETV2 DAPI
d vWF DAPI
e DAPI
f
g CD31 DAPI
g ETV2 DAPI
i vWF DAPI
j GFP DAPI
k
l NG2 DAPI
m PDGFR-β DAPI
n COL-I DAPI
o α-SMA DAPI
p
q VIMENTIN DAPI
r PDGFR-β DAPI
s COL-I DAPI
t α-SMA DAPI
u
v F-ACTIN MITF DAPI
w ZO-1TYRP1
x F-ACTIN RPE65
y F-ACTIN EZRIN

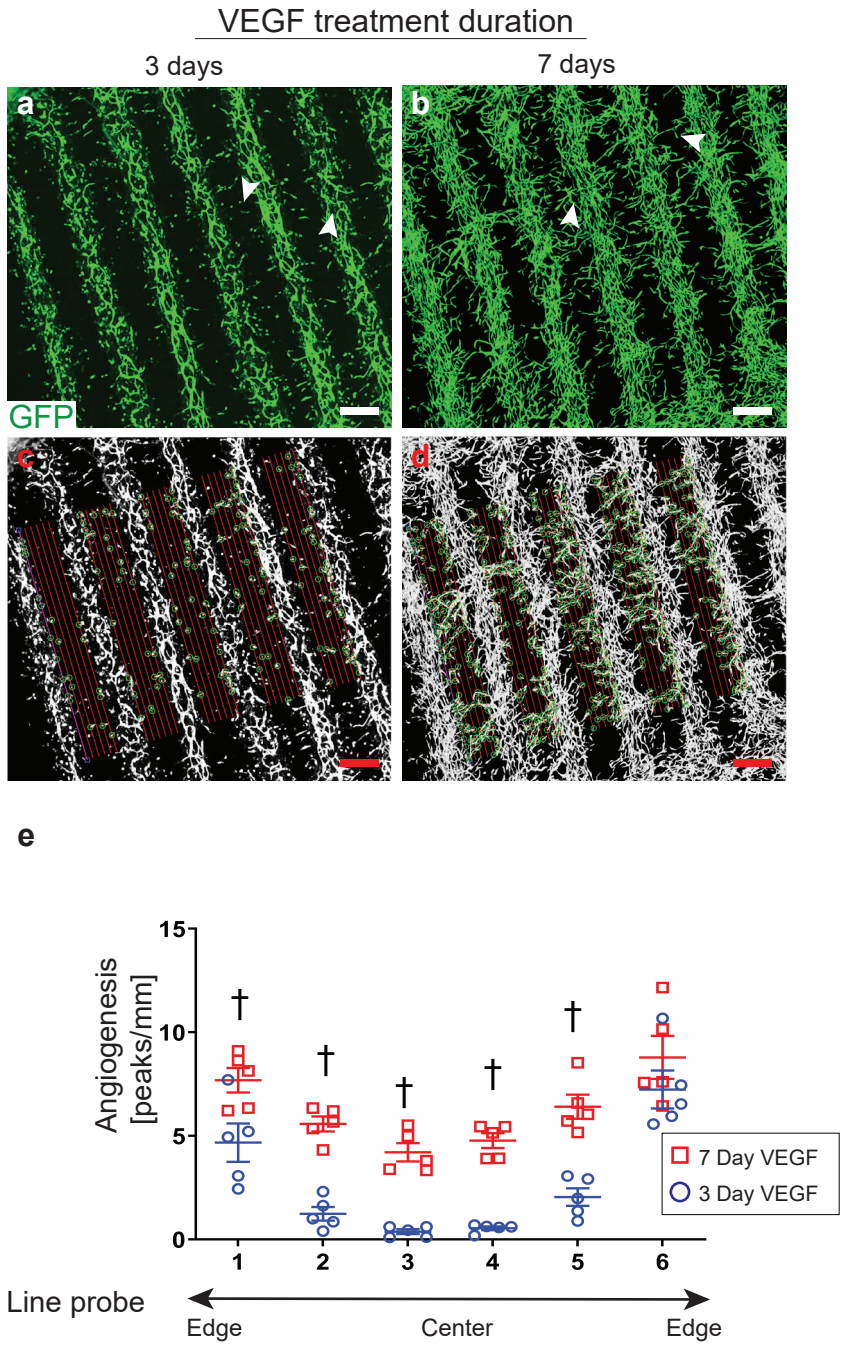
**Supplementary Fig. 1 | Representative images of iPSC-derived endothelial cells (iECs), primary endothelial cells (EC), retinal pericyte, choroid fibroblast, and iRPE. a-j,** Phase contrast images of iECs (**a**) (N=5) and primary ECs (**f**) (N=4). Immunostaining for iEC and GFP+ primary ECs with CD31 (**b,g**)(N=3), ETV2 (**c,h**) (N=3), vWF (**d,i**) (N=3). GFP expression in primary ECs (**j**) (N=4). DAPI (**b-e, g-j**). **k-o,** Phase contrast image of primary pericytes (**k**) (N=5). Immunostaining of primary pericytes with NG2 (**l**), PDGFR-β (**m**), COL-1 (**n**), and α-SMA (**o**) (**l-o** N=3). DAPI (**l-o**). **p-t,** Phase contrast image of choroidal fibroblasts (**p**) (N=5). Immunostaning of choroidal fibroblasts with VIMENTIN (**q**), lacking PDGFR-β expression (**r**), and positive expression for COL-1 (**s**) and lacking α-SMA (**t**) (**q-t** N=3). DAPI (**q-t**). **u-y,** Phase contrast image of iPSC-derived RPE (iRPE) cells (**u**). Immunostaining of iRPE cells with MITF (red) F-ACTIN (green) (**v**), ZO-1 (green) and TYRP1 (red) (**w**), F-ACTIN (green) and RPE65 (red) (**x**), and F-ACTIN green and Ezrin (red) (**y**). DAPI (**v-y**). Scale bars, 50 μm.



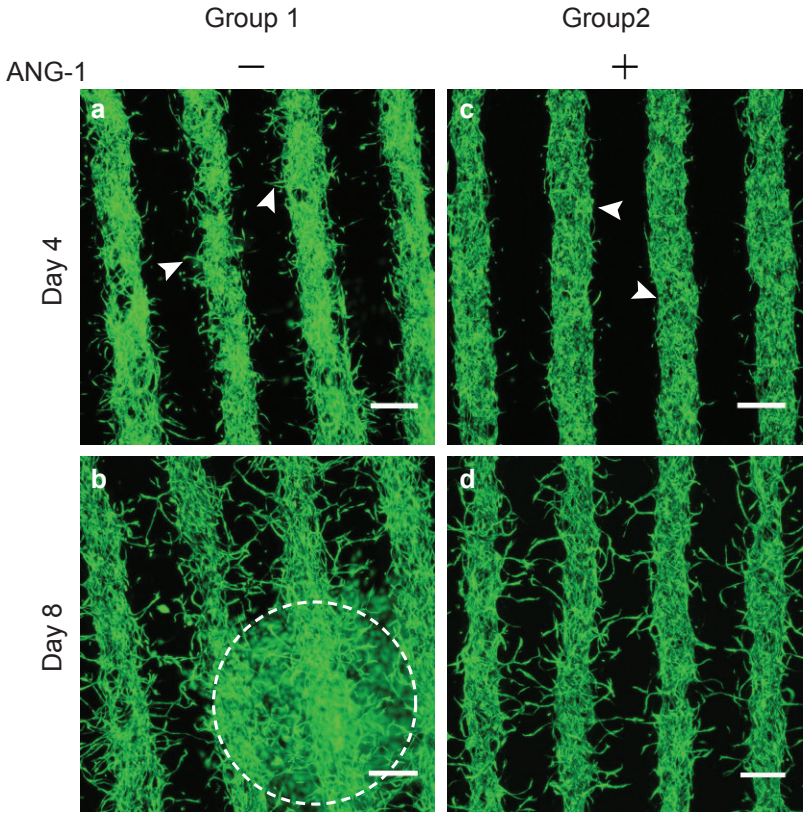

**Supplementary Fig. 2 | Scaffold preparation for bioprinting. a,b,** Microstructure of PLGA electrospun scaffolds without or with heat treatment **c,d,** Oxygen-plasma treatment-induced hydrophilicity on Teflon. Red arrowheads mark water droplet before and after the treatment. Scale bars, 50 µm. (n=3)



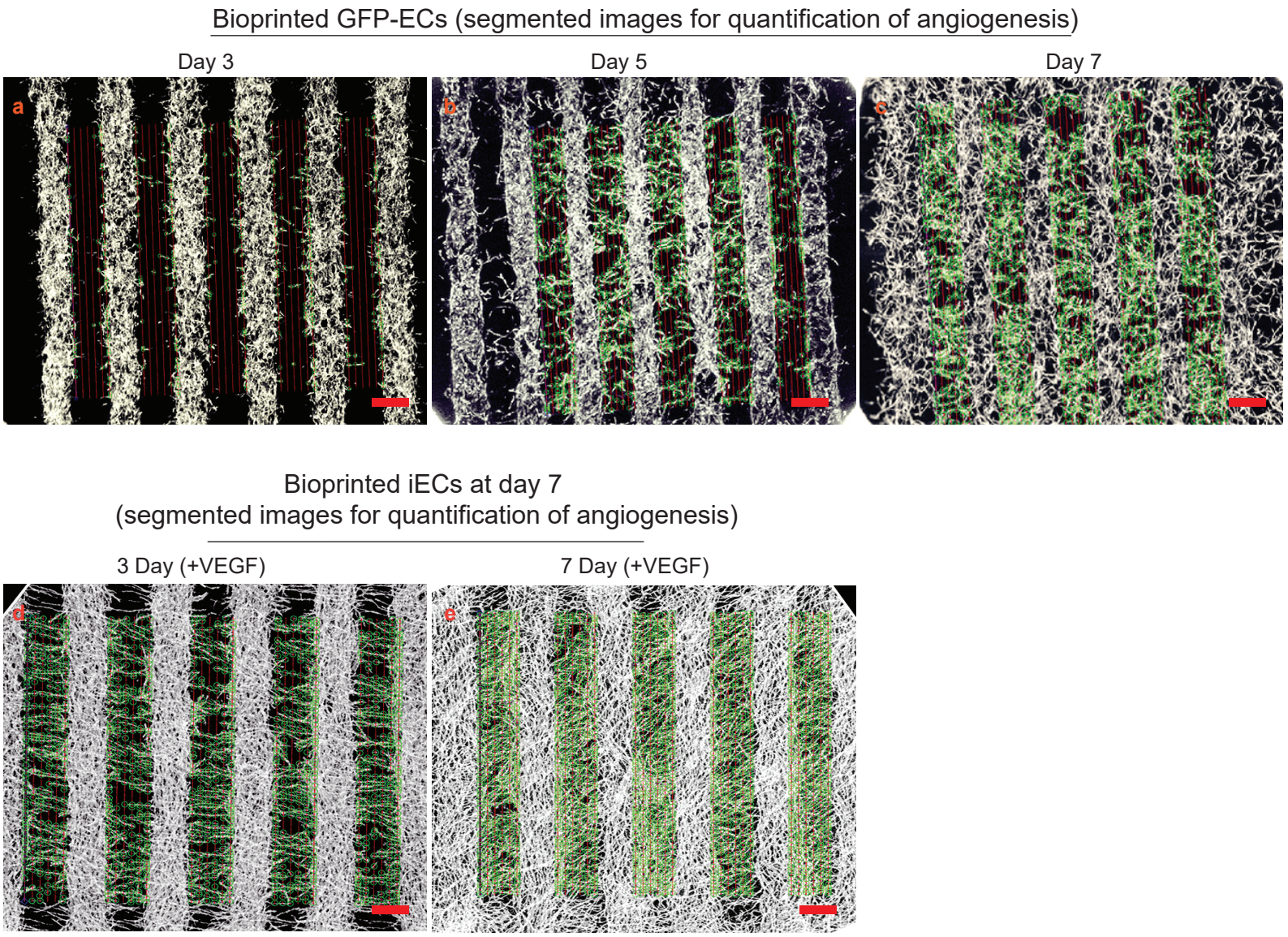

**Supplementary Fig. 3 | Aprotinin stabilizes 3D vasculature.** GFP positive endothelial cells were embedded in fibrin gels consisting of 2.5mg/ml of FIBRINOGEN and 0.5U/ml of THROMBIN. Cultures were incubated with or without aprotinin. Images represent entire well area in a 24 well plate. Images were taken at day 1 (**a,c**) and day 4 (**b,d**). Scale bars, 2 mm. (n=3)



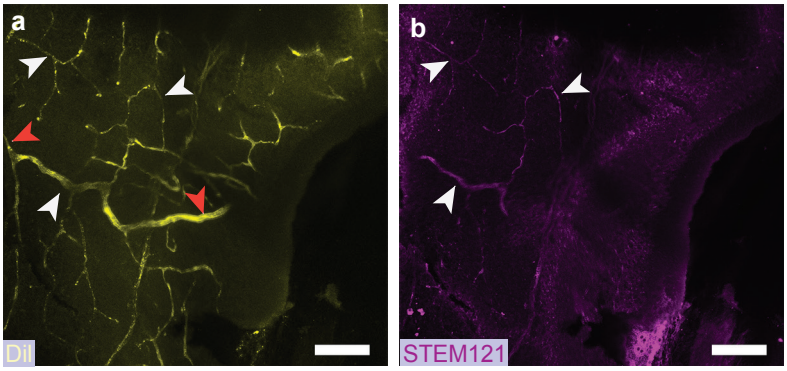

**Supplementary Fig. 4 | VEGF promotes angiogenesis printed tissue. a,b,** GFP positive ECs showing angiogenesis with 3 days (**a**) or 7 days (**b**) days of VEGF (85ng/mL) treatment. Arrow heads mark ECs derived capillaries expanding from the printed structure. Scale bars, 500μm. **c-e,** Image quantification of angiogenesis. Fluorescence intensity is measured along six red line probes between printed stripes. Statistical significance was attributed to values of $p<0.05$ as determined by two-way ANOVA and Sidak's multiple comparison test. †$p<0.05$, (n=5), error bars indicate STE.



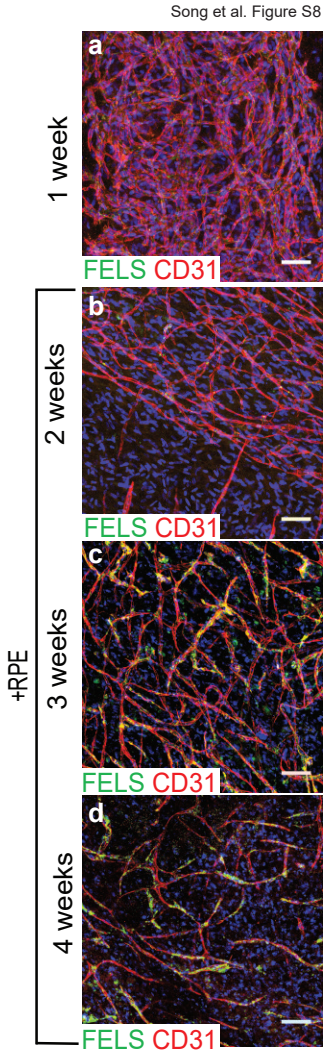

**Supplementary Fig. 5 | Angiopoietin-1 reduces EC migration. a-d,** Vascular development for 8 days with ANG-1 (100 ng/ml) treatment. Arrow heads mark branching out capillaries sprouting from the printed structure. Dotted-circle marks single cell migration. Scale bars, 500μm. n=3.



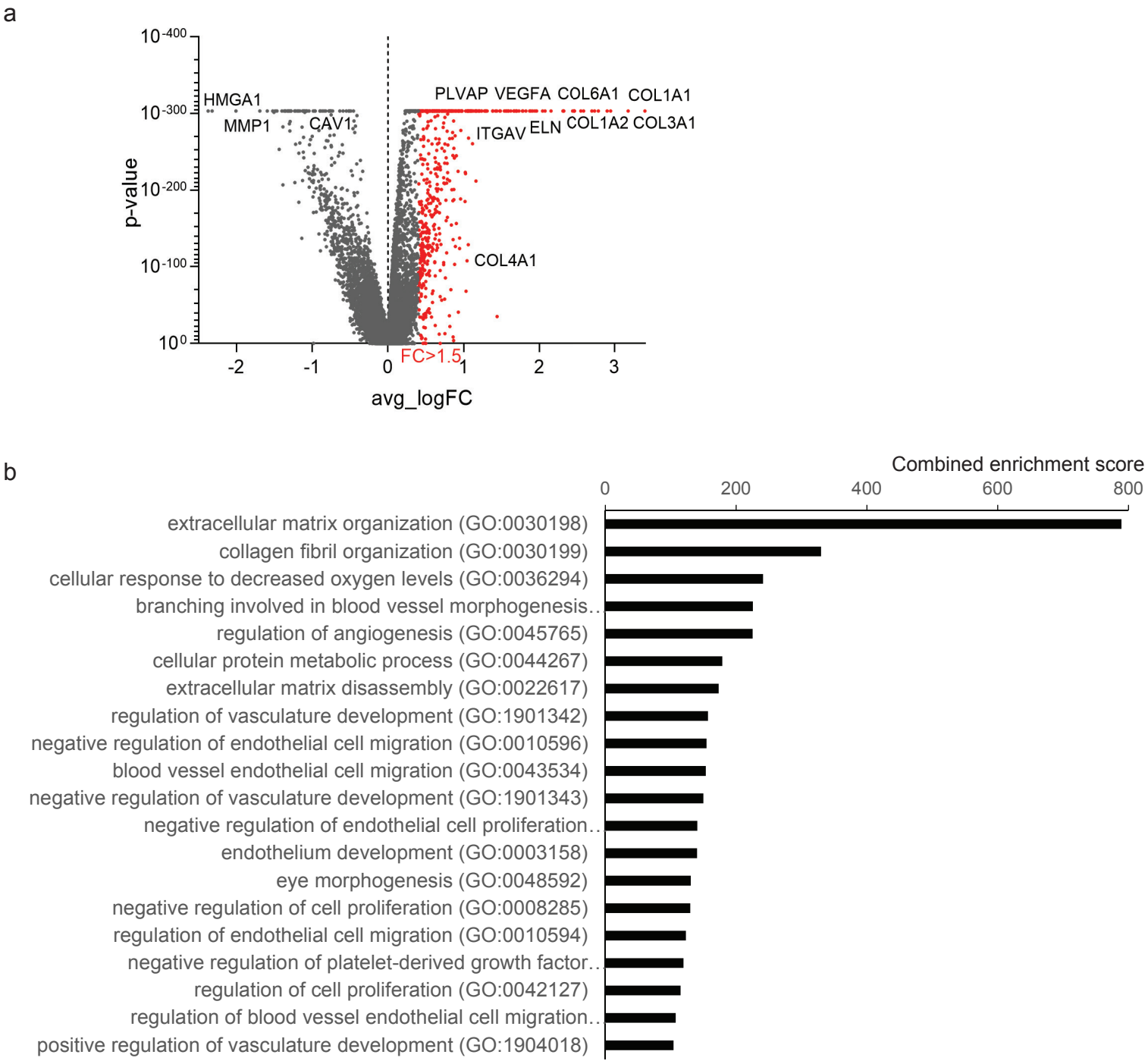

**Supplementary Fig. 6 | Angiogenesis quantification. a-e,** Fluorescence intensity is measured along six red line probes between printed stripes from day 3 to 7 of angiogenesis (**a-c**) and with 3 or 7 days of VEGF treatment (**d, e**). a-c, n=5. d,e, n=4. Line probes (red; 100μm apart) were placed in each gap between stripes of the printed geometry. Each line probe detects number of peaks of GFP intensity above a threshold. Line 1 and 6, 2 and 5, 3 and 4 mark edges, intermediate, and center of each gap, respectively. Scale bars, 500μm.



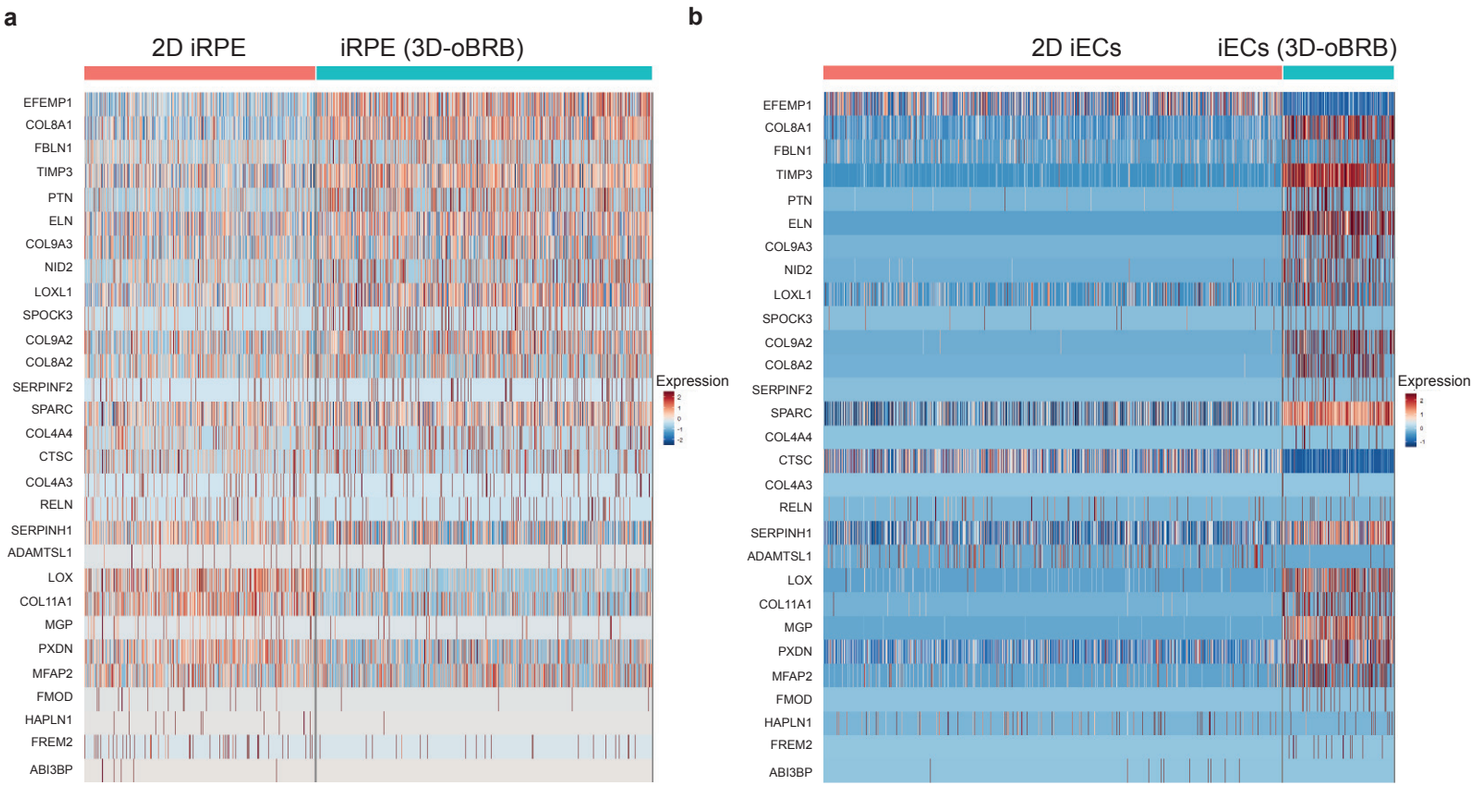

**Supplementary Fig. 7 | a,b,** DiI (yellow) signal marks rat capillaries and immunostaining for STEM121 (magenta) detects human capillaries integrated with rat capillaries.




**Supplementary Fig. 8 | Time course of fenestration marker expression in 3D-oBRB. a-d,** 3D vascular growth within tissues fixed at week 1 (**a**), week 2 (**b**), week 3 (**c**), and week 4 (**d**). Tissues were immunostained with FELS (green) and CD31 (red). Scale bars, 50μm. n=3.

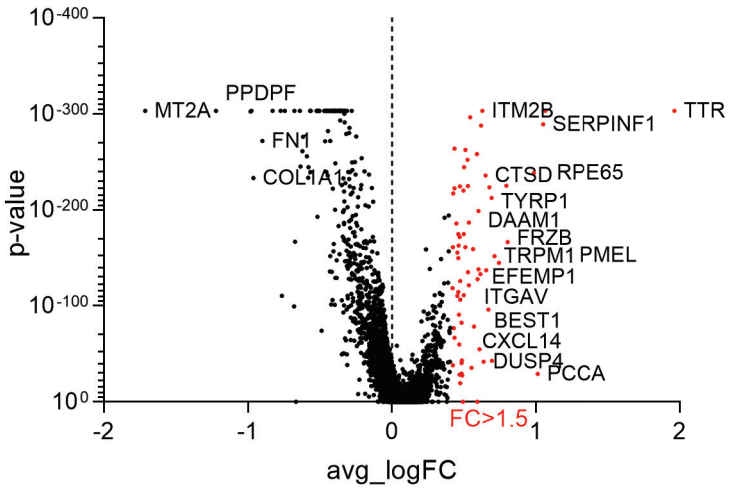



**Supplementary Fig. 9 | Comparative analysis of gene expression between 2D-iECs and iECs from 3D-oBRB. a,** Volcano plot of 21,792 genes. Red dots indicate genes that demonstrated greater than 1.5 log -fold change (531 genes) from 2D monoculture. **b,** Relevant Gene Ontology Biological Process categories (scores>100) using 531 genes (FC>1.5) by Enrichr20. Data depicts results from n = 5369 cells (2D iECs), and n = 1294 cells (3D-oBRB iECs).

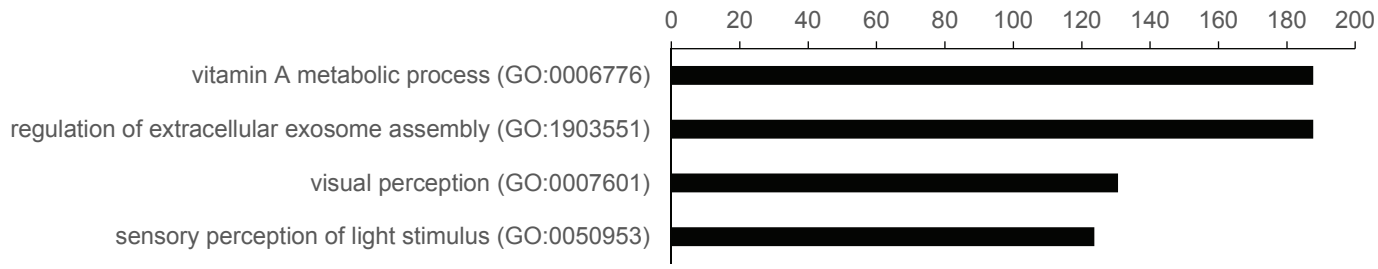



**Supplementary Fig. 10 | ECM related gene expressions in iEC and iRPE in 2D and in 3D-oBRB. a,** ECM gene expression comparisons between 2D iRPE and iRPE in 3D-oBRB. Average log fold change and significance calculations performed between RPE monocultures and oBRB performed using the Seurat gene analysis package. **b,** ECM gene expression comparisons between 2D iECs and iECs from 3D-oBRB. Average log fold change and significance ($p<0.05$) calculations performed between 2D iECs and 3D-oBRB performed using the Seurat gene analysis package. Data depicts results from n = 3012 cells (2D RPE), n = 4380 cells (3D-oBRB RPE), n = 5369 cells (2D iECs), and n = 1294 cells (3D-oBRB iECs).

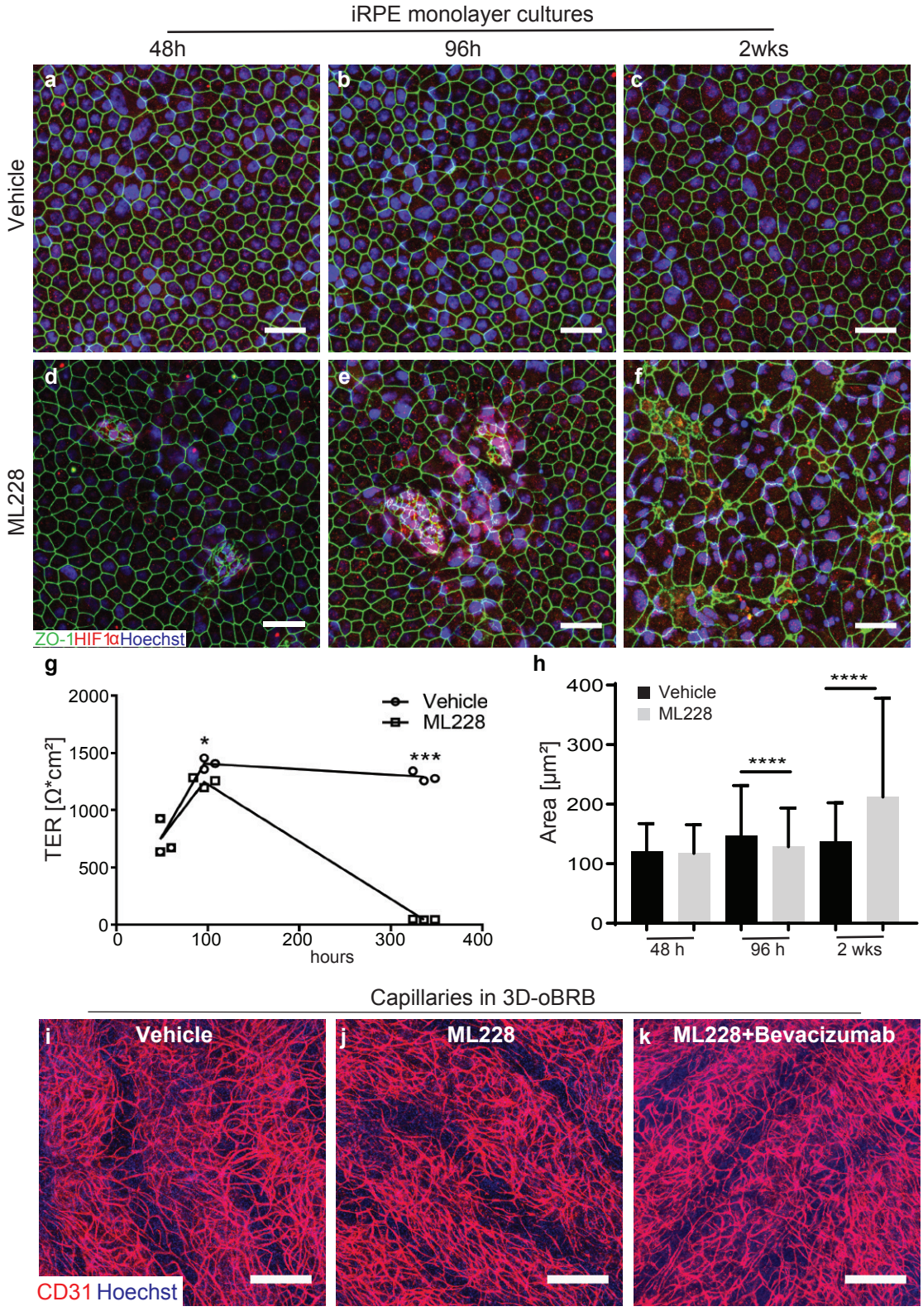
a




b




**Supplementary Fig. 11 | Comparative analysis of genes significantly different between 2D iRPE and iRPE cells from 3D-oBRB. a,** Volcano plot of 21,321 genes. Red dots indicate genes greater than 1.5 log-fold changes (69 genes) in iRPE from 3D-oBRB as compared to 2D-iRPE. **b,** Relevant Gene Ontology Biological Process categories (scores>100) using 69 genes (FC>1.5) by Enrichr20. Data depicts results from n = 3012 cells (2D RPE), n = 4380 cells (3D-oBRB RPE).


iRPE monolayer cultures
48h
96h
2wks
Vehicle
ML228
a
b
c
d
e
f
ZO-1HIF1αHoechst
g
TER [Ω*cm²]
2000
1500
1000
500
0
0
100
200
300
400
hours
Vehicle
ML228
*
***
h
Area [µm²]
400
300
200
100
0
Vehicle
ML228
****
****
48 h
96 h
2 wks
Capillaries in 3D-oBRB
i Vehicle
j ML228
k ML228+Bevacizumab
CD31 Hoechst

**Supplementary Fig. 12 | ML228 and Bevacizumab treatment on 2D iRPE and 3D-oBRB. a-f,** RPE monoculture at 48 hr (**a,d**), 96 hr (**b,e**), and 2weeks (**c,f**) from the beginning of ML228 (2μM; 96hr) treatment, immunostained with HIF-1α (red), ZO-1 (green), and Hoeschst (blue). Vehicle treatment consisted of DMSO. Scale bars, 30μm. (n=3) **g,** TER measurement of 2D iRPE without or with ML228 treatment (n=3). **h,** ZO-1 staining based morphometry analysis of individual cell area in vehicle and ML228 treated samples was performed, N=7495. Error bars indicate standard deviation. **i-k,** Images of deep choroidal regions of **i,** vehicle. **j,** ML228. **k,** ML228+bevacizumab treated 3D-oBRB, immunostained with CD31 (red) and Hoechst (blue) show no differences in deeper layers. Scale bars, 350μm. (n=4)

**Supplementary video legends**

**Supplementary Video 1.** Bioprinting of vascularized tissue with GFP positive ECs and VEGF dependent angiogenesis from day 4 to day 6.

**Supplementary Video 2.** Cross sectional slices of vascularized tissue with iECs at day 7

**Supplementary Video 3.** 3D-oBRB tissue model

**Supplementary Video 4.** ELASTIN and LAMININ formation in 3D-oBRB tissue model

**Supplementary Video 5.** Complement induced dry age-related macular degeneration model

**Supplementary Video 6.** HIF-1α induced CNV in 3D-oBRB with anti-VEGF (bevacizumab) treatment.