Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Manufacturing Human Pluripotent Stem Cells Derived Endothelial Cells in Scalable and Cell-friendly Microenvironments

Haishuang Lin, Qian Du, Qiang Li, Ou Wang, Zhanqi Wang, Christian Elowsky, Kan Liu, Chi Zhang, Soonkyu Chung, Bin Duan and Yuguo Lei

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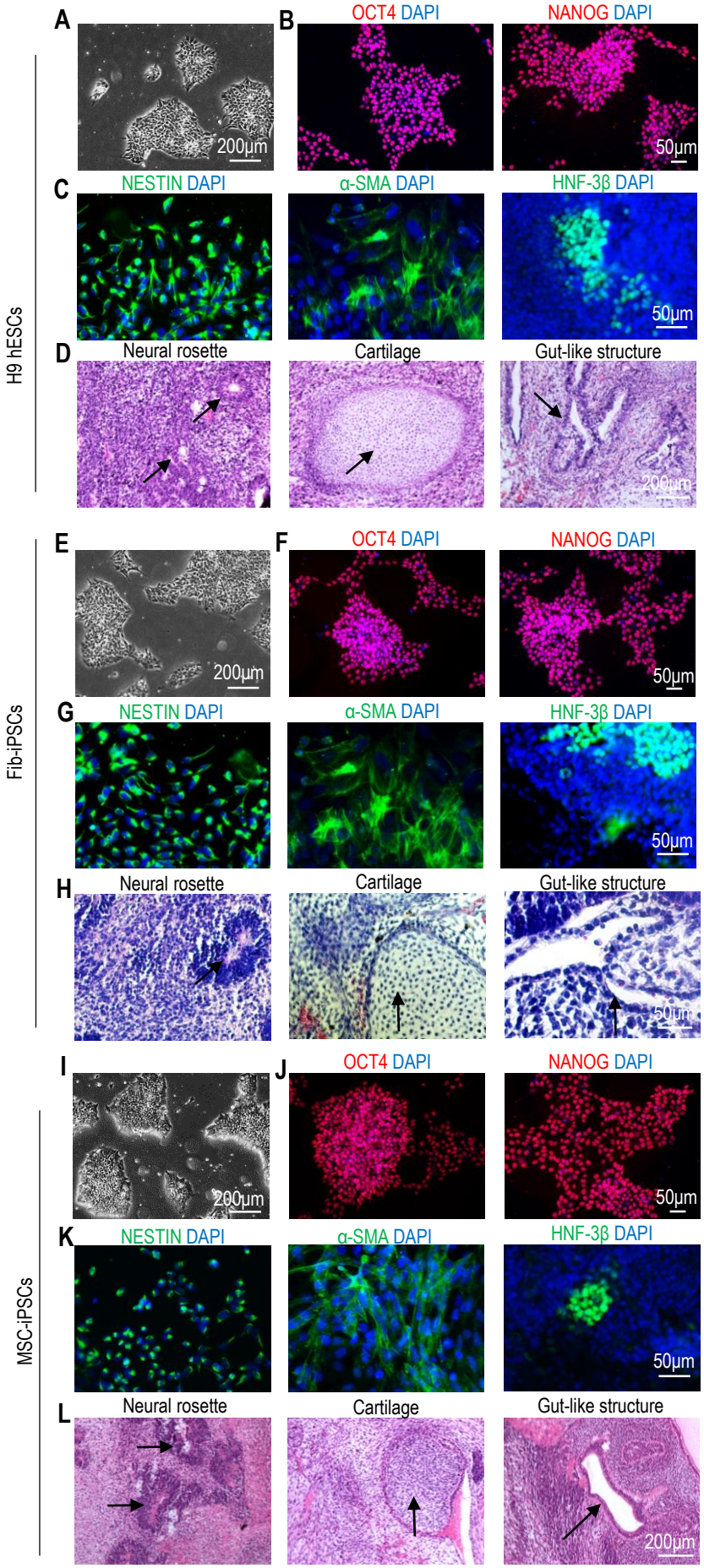


Figure S1. Starting hPSCs (H9s, Fib-iPSCs and MSC-iPSCs). (**A, E, I**) Phase images. (**B, F, J**) Majority of the cells expressed the pluripotency markers, OCT4 and NANOG. (**C, G, K**) They could be differentiated into all three germ layer cells such as NESTIN+ ectodermal, α-SMA+ mesodermal and HNF-3β+ endodermal cells in EB assay. (**D, H, L**) They formed teratomas containing all three germ layer tissues (arrows) in SCID mice.

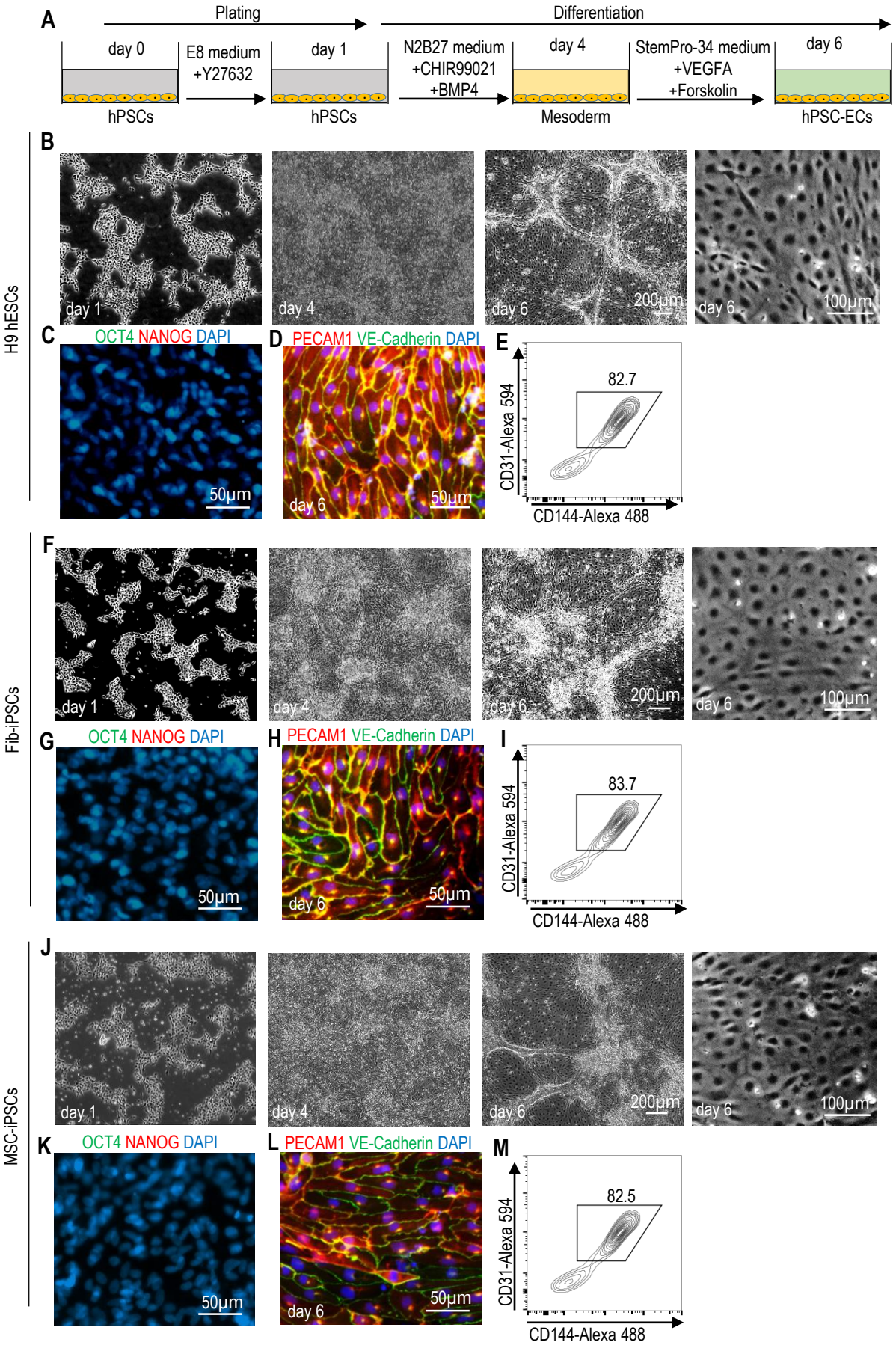


Figure S2. Differentiate hPSCs (H9s, Fib-iPSCs and MSC-iPSCs) into ECs in 2D cultures. (**A**) Illustration of the EC differentiation protocol. (**B, F, J**) Phase images of day 1, 4 and 6 cells during EC differentiation. (**C, G, K**) Immunostaining of day 6 cells for hPSCs markers, OCT4 and NANOG. (**D, H, L**) Immunostaining of day 6 cells for EC markers, PECAM1 and VE-Cadherin. (**E, I, M**) Flow cytometry analysis of day 6 hPSC-ECs for PECAM1 and VE-Cadherin.

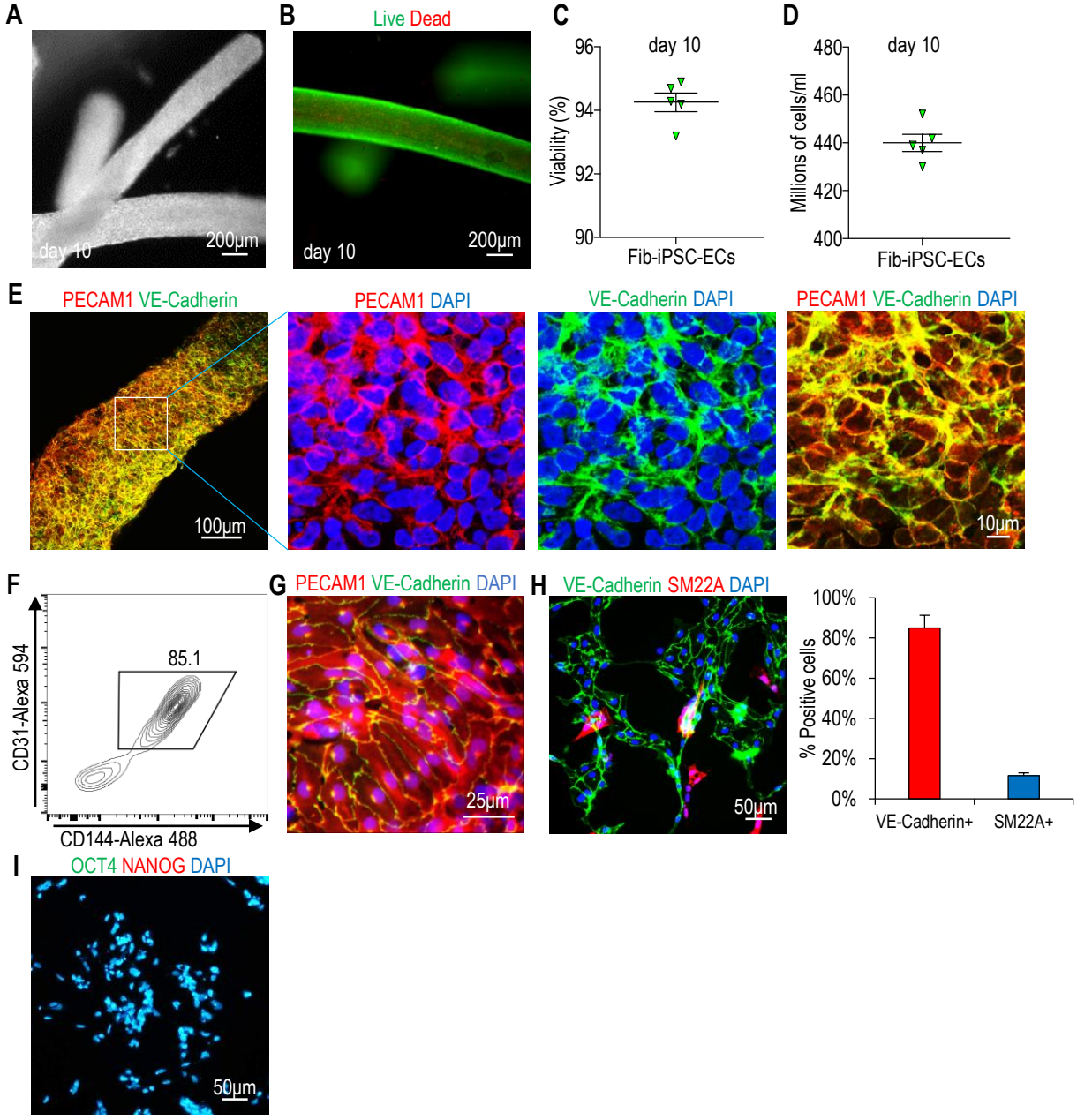


Figure S3. Differentiate Fib-iPSCs into ECs in alginate hydrogel tubes. (**A**) Phase images of day 10 cells. (**B, C**) Live/Dead staining and cell viability analysis of day 10 cells. Data are represented as mean ± SD (n=5). (**D**) Volumetric yield on day 10. Data are represented as mean ± SD (n=5). (**E, F**) Immunostaining and flow cytometry analysis of EC markers PECAM1 and VE-Cadherin on day 10 cells. (**G-I**) The day 10 cell masses were dissociated into single cells and plated on 2D surface overnight. Immunostaining showed majority of the cells were PECAM1+/VE-Cadherin+ (**G**), and detected ~10% SM22A+ cells (**H**), but no OCT4+/NANOG+ undifferentiated Fib-iPSCs (**I**).

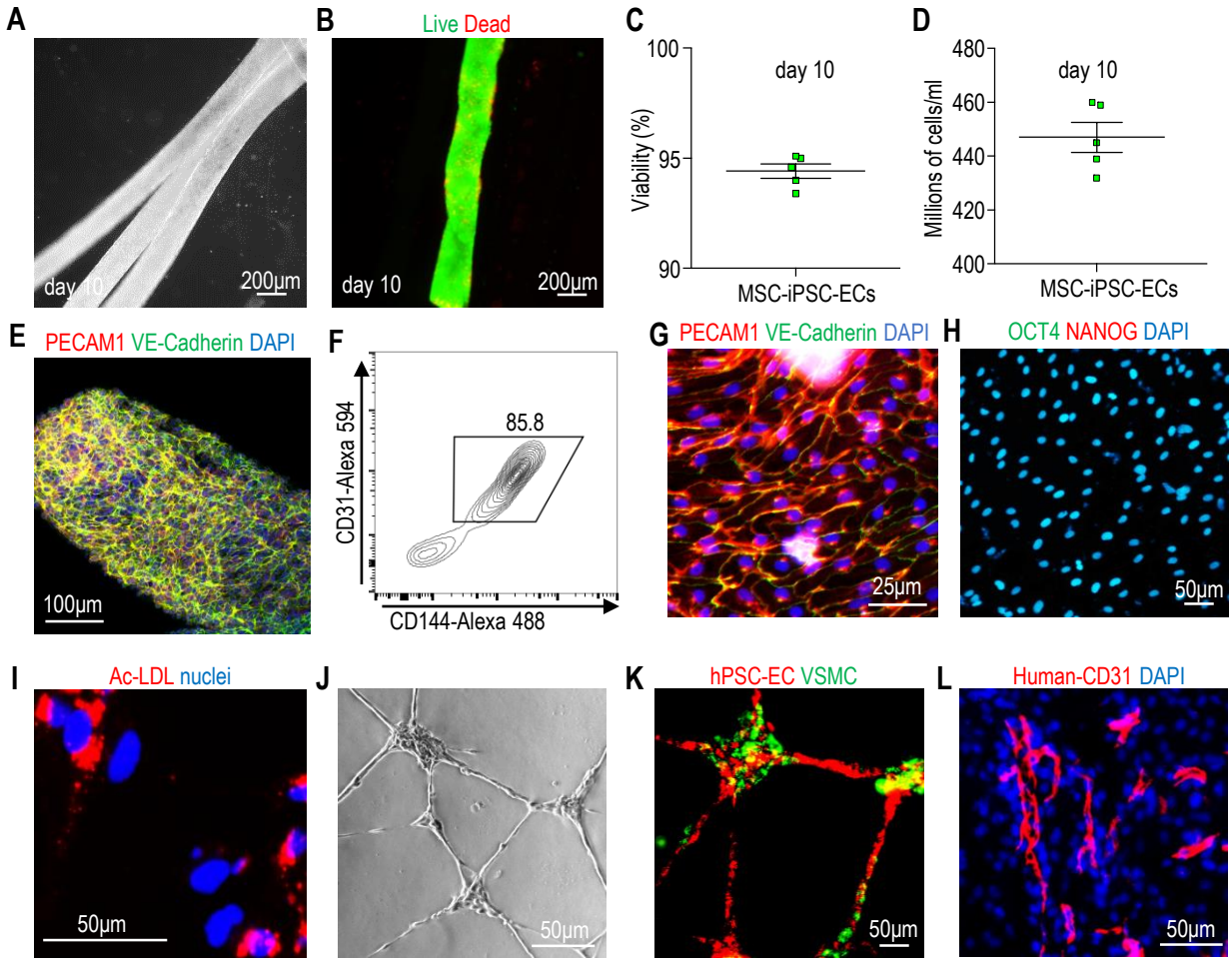


Figure S4. Differentiate MSC-iPSCs into ECs in alginate hydrogel tubes. (**A**) Phase images of day 10 cells. (**B, C**) Live/Dead staining and viability analysis of day 10 cells. Data are represented as mean ± SD (n=5). (**D**) Volumetric yield on day 10. Data are represented as mean ± SD (n=5). (**E, F**) Immunostaining and flow cytometry analysis of EC markers PECAM1 and VE-Cadherin on day 10 cells. (**G, H**) The day 10 cell masses were dissociated into single cells and plated on 2D surface overnight. Immunostaining showed majority of the cells were PECAM1+/VE-Cadherin+ (**G**), and detected no OCT4+/NANOG+ undifferentiated MSC-iPSCs (**H**). (**I**) ECs efficiently uptaked fluorescence-labelled acetylated LDL (Ac-LDL). (**J, K**) ECs formed tubular network when plated on Matrigel for 24 hours. The co-plated vascular smooth muscle cells attached to the EC network. (**L**) When transplanted subcutaneously with a Matrigel matrix, ECs formed nice vascular structures.

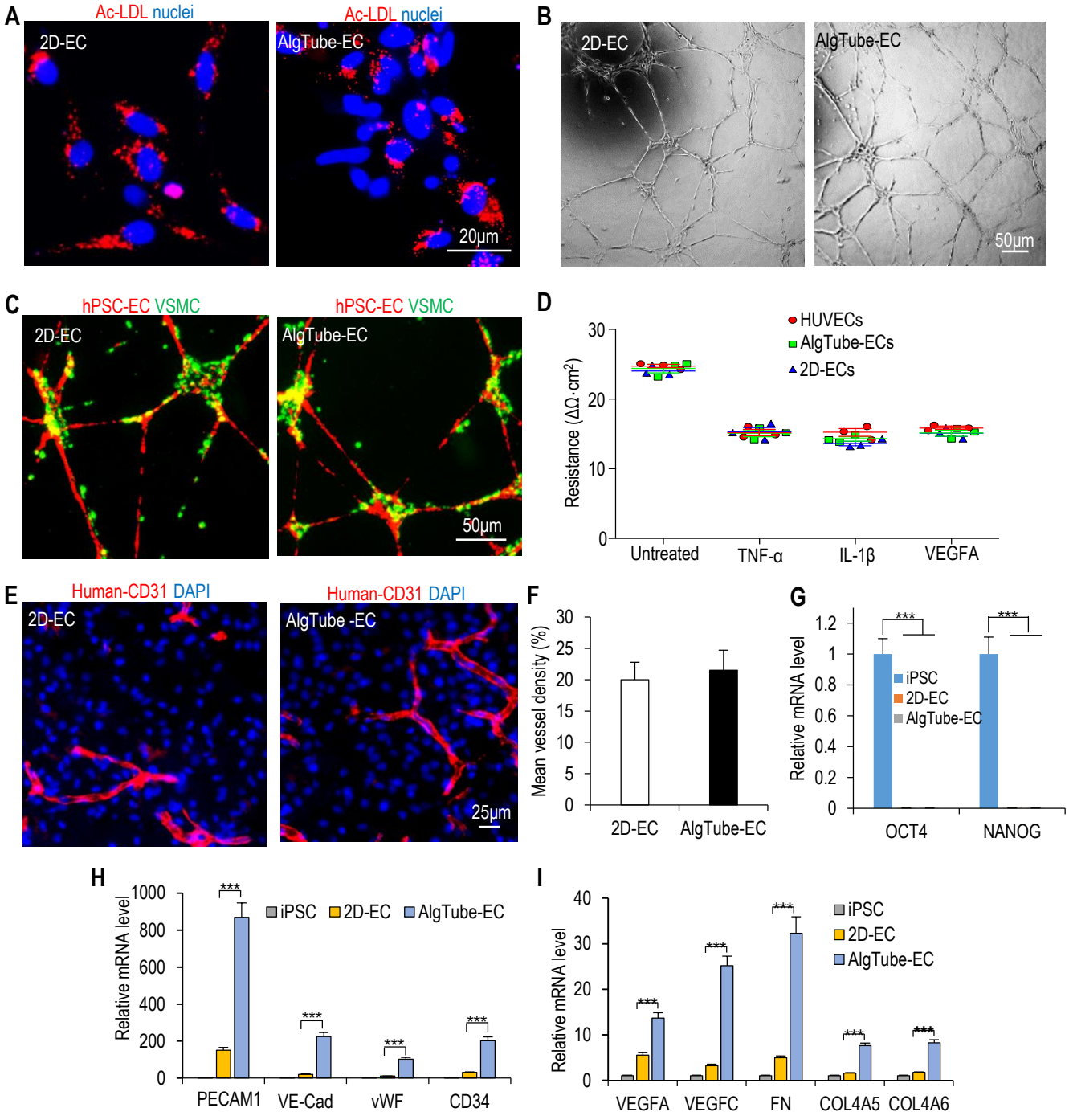


Figure S5. Properties of ECs derived from Fib-iPSCs in alginate hydrogel tubes (AlgTube-ECs) and 2D culture (2D-ECs)**.** (**A**) Both ECs efficiently uptaked fluorescence-labelled acetylated LDL (Ac-LDL). (**B, C**) Both ECs formed tubular network when plated on Matrigel for 24 hours. The co-plated vascular smooth muscle cells attached to the EC network. (**D**) TEER properties, either untreated or treated with 100 ng/ml TNF-α, 100 ng/ml IL-1β or 100 ng/ml VEGF-A, of HUVECs, AlgTube-ECs and 2D-ECs were similar. Data are represented as mean ± SD (n=3). (**E, F**) When transplanted subcutaneously with a Matrigel matrix, both ECs formed nice vascular structures with similar vessel density. (**G**) qRT-PCR of pluripotency markers OCT4 and NANOG of H9s, 2D-ECs and AlgTube-ECs. (**H, I**) qRT-PCR showed AlgTube-ECs had higher expression of some key genes related to ECs. Data are represented as mean ± SD (n=3). *** *P*<0.001.

A **Top 20 upregulated GO terms in AlgTube-ECs**

GO ID	GO term name	Gene number	P value
GO:0001568	blood vessel development	84	2.45E-21
GO:0001944	vasculature development	86	2.54E-21
GO:0072358	cardiovascular system development	86	7.22E-21
GO:0048514	blood vessel morphogenesis	73	8.64E-19
GO:0001525	angiogenesis	65	4.91E-18
GO:0072359	circulatory system development	99	9.76E-16
GO:0007399	nervous system development	151	6.41E-09
GO:0010631	epithelial cell migration	31	1.82E-07
GO:0001667	ameboidal-type cell migration	37	2.99E-07
GO:0001822	kidney development	32	4.19E-07
GO:0050880	regulation of blood vessel size	22	5.30E-07
GO:0007266	Rho protein signal transduction	25	5.67E-07
GO:0048762	mesenchymal cell differentiation	26	7.61E-07
GO:0035239	tube morphogenesis	38	7.97E-07
GO:0060562	epithelial tube morphogenesis	35	1.28E-06
GO:0001655	urogenital system development	35	1.28E-06
GO:0003151	outflow tract morphogenesis	15	1.29E-06
GO:0032835	glomerulus development	14	1.35E-06
GO:0003007	heart morphogenesis	29	1.41E-06
GO:0072001	renal system development	32	1.82E-06

B **Top 20 upregulated GO terms in 2D-ECs**

GO ID	GO term name	Gene number	P value
GO:1903047	mitotic cell cycle process	101	5.45E-23
GO:0000819	sister chromatid segregation	48	9.35E-21
GO:0098813	nuclear chromosome segregation	55	1.48E-20
GO:0000280	nuclear division	63	5.49E-20
GO:0140014	mitotic nuclear division	48	5.58E-19
GO:0000070	mitotic sister chromatid segregation	35	1.01E-17
GO:0006468	protein phosphorylation	158	1.09E-17
GO:0044772	mitotic cell cycle phase transition	66	2.47E-14
GO:0044770	cell cycle phase transition	68	2.87E-14
GO:0016310	phosphorylation	170	4.42E-14
GO:0042981	regulation of apoptotic process	122	8.03E-14
GO:0048513	animal organ development	224	1.1E-13
GO:1902850	microtubule cytoskeleton organization involved in mitosis	27	3.8E-13
GO:0006915	apoptotic process	143	8.58E-13
GO:0072359	circulatory system development	90	7.05E-12
GO:0007062	sister chromatid cohesion	27	1.27E-11
GO:0000226	microtubule cytoskeleton organization	54	2.14E-11
GO:0007052	mitotic spindle organization	23	3.04E-11
GO:0051783	regulation of nuclear division	30	1.04E-10
GO:0042325	regulation of phosphorylation	110	1.92E-10

Figure S6. Top 20 upregulated GO terms in AlgTube-ECs (**A**) and 2D-ECs (**B**).

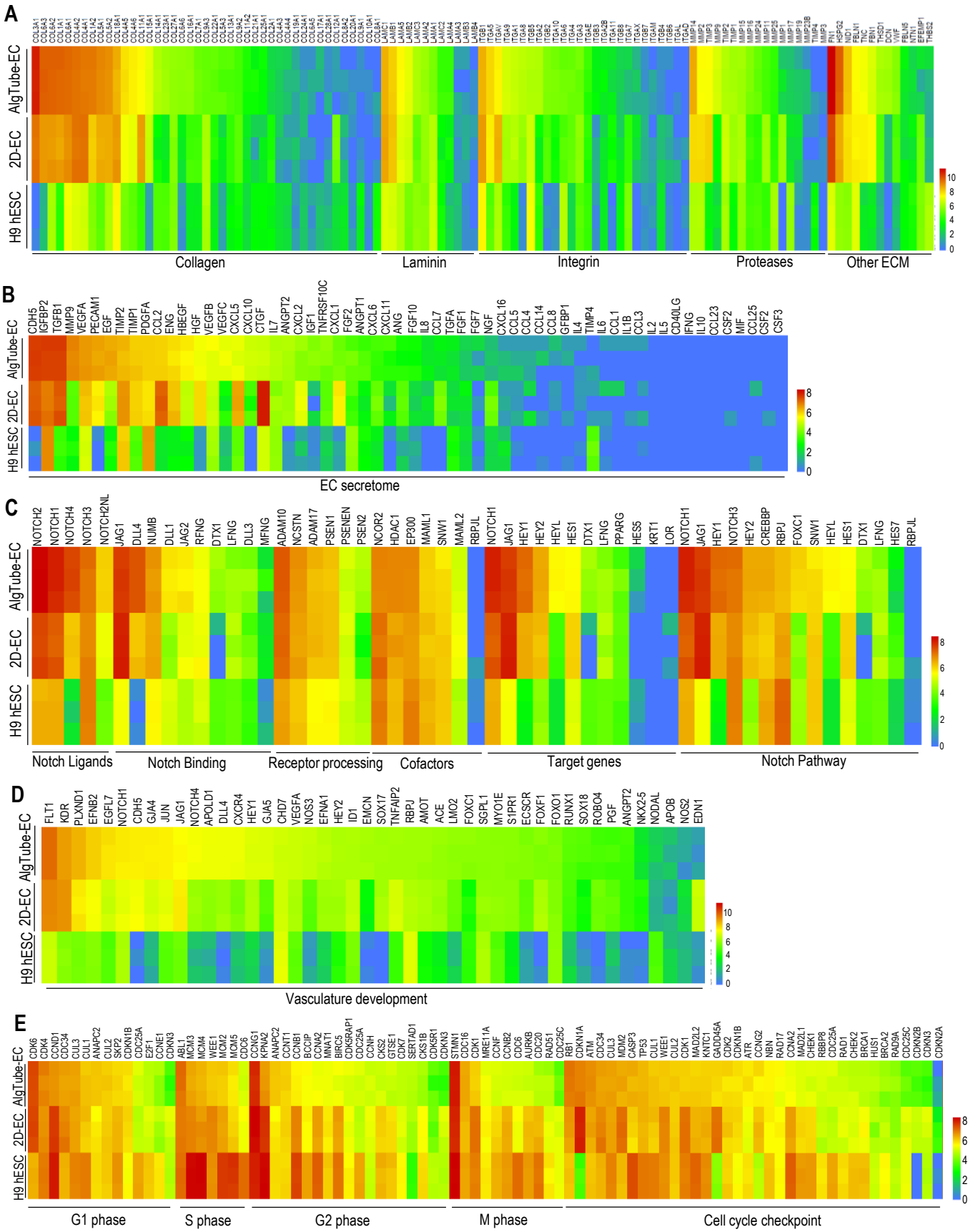


Figure S7. Differential gene expression analysis between AlgTube-ECs and 2D-ECs derived from H9s. Heat map of genes related to ECMs (**A**), EC secretome (**B**), Notch signaling (**C**), vasculature development (**D**) and cell cycle (**E**).

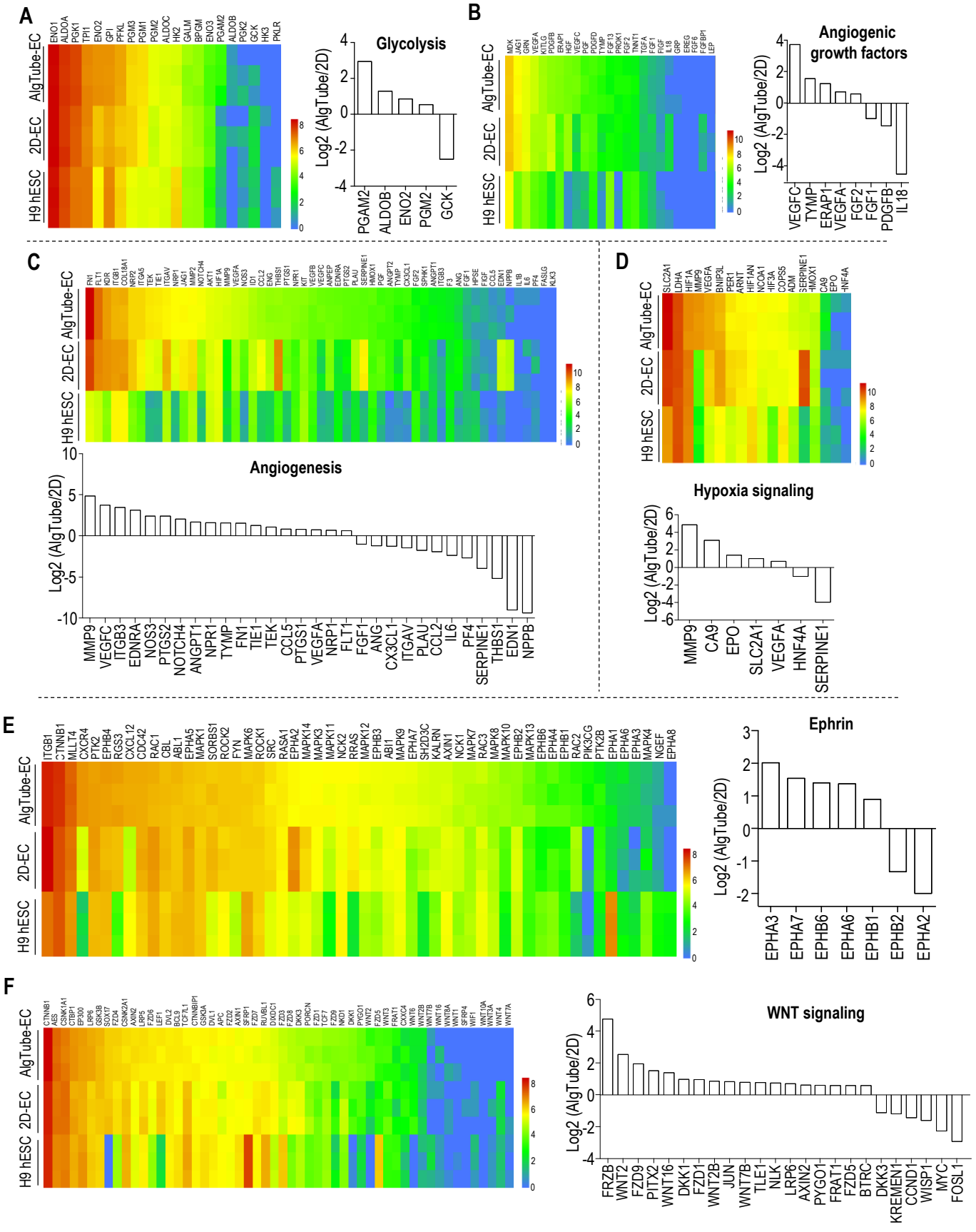
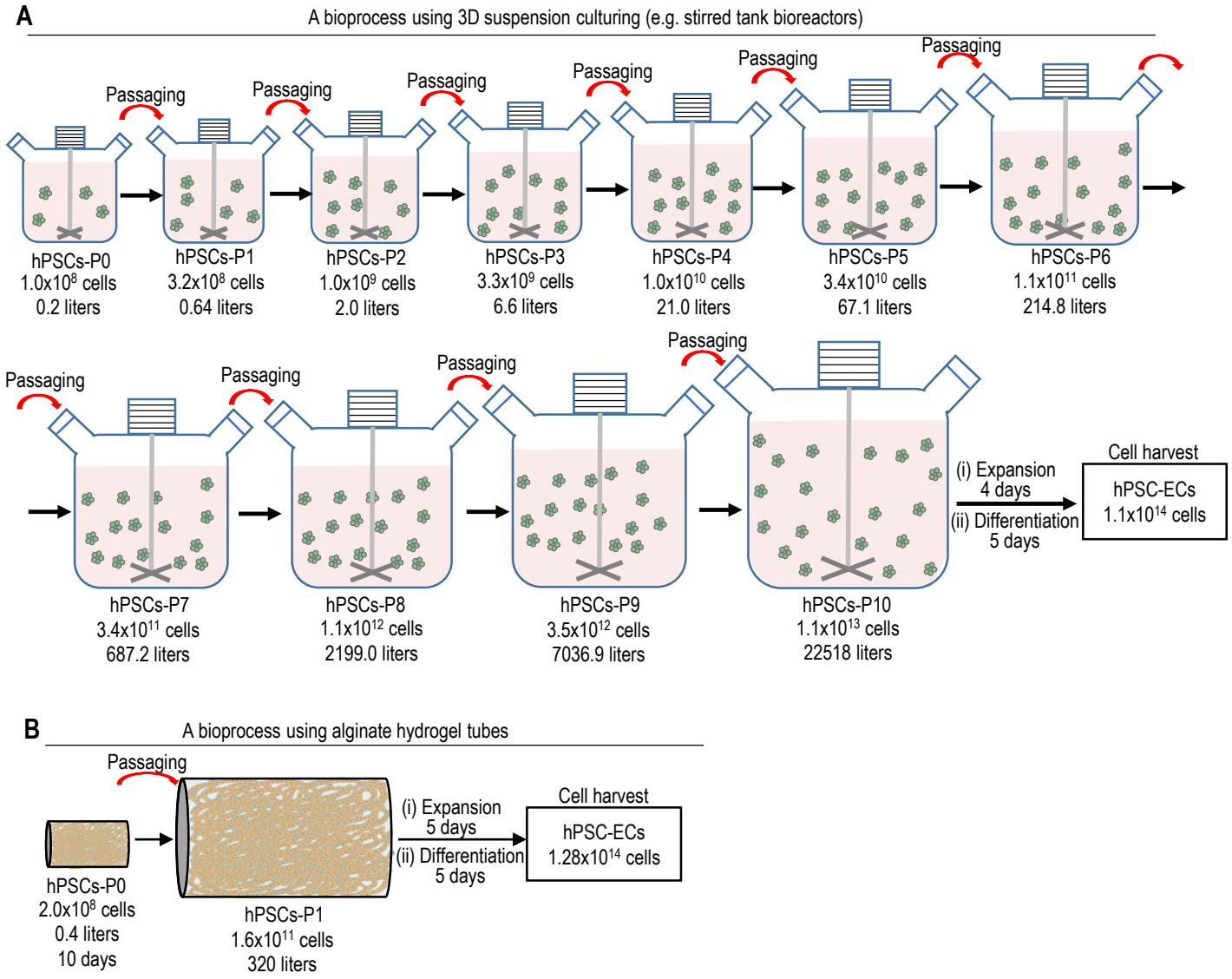


Figure S8. Differential gene expression analysis between AlgTube-ECs and 2D-ECs derived from H9s. (**A-F**) Heat map and Log2 (expression level in AlgTube-ECs/expression level in 2D-ECs) of genes related to glycolysis (**A**), angiogenic growth factors (**B**), angiogenesis (**C**), hypoxia signaling (**D**), ephrin (**E**) and WNT signaling (**F**).



C

	Stirred tank bioreactors	Alginate hydrogel tubes
Starting cells	1.0×10^{8}	2.0×10^{8}
Total produced cells (hPSC-ECs)	1.1×10^{14}	1.28×10^{14}
Production time (days)	49	20
Passaging operations	10	1
Total bioreactor volume (liter)	32753	320
Assumptions: (i) passaging efficiency: 80%; (ii) seeding density: 500000 cells/mL; (iii) 4-fold expansion/4 days per passage for 3D suspension culturing; (iv) 1000-fold expansion per 10 days per passage for alginate hydrogel tubes.		

Figure S9. Illustration of two bioprocesses for manufacturing ~10^{14} hPSC-ECs from ~10^{8} hPSCs. (**A**) Using 3D suspension culturing (e.g. stirred tank bioreactors), ~1.1×10^{14} hPSC-ECs are generated through serial expansion of hPSCs (with 10 passaging operations), followed by a 5 day differentiation. The total culture volume is 32753 liters and the total culture time is 49 days (the number of cells seeded, culture volume and culture time are shown at each passage). (**B**) Using alginate hydrogel tubes, ~1.28×10^{14} hPSC-ECs can be produced with 320 liters alginate hydrogel tubes, 1 passaging operations and 20 days. (**C**) Summaries of the two bioprocesses.

Table S1. Antibodies used in this study (Related to Figure 1, 3, 4, 6, 7 and Figure S2, 3, 4, 5, 6).

Antibody	Supplier	Catalog. No	Species	Dilution
VE-Cadherin	Santa Cruz	sc-9989	Mouse	1:100 (IF&FC)
α-SMA	Santa Cruz	sc-130616	Mouse	1:100 (IF&FC)
PECAM1	Santa Cruz	sc-1506-R	Rabbit	1:100 (IF&FC)
SM22A	Abcam	ab14106	Rabbit	1:100 (IF&FC)
SSEA4	R&D System	962648	Mouse	1:200 (IF&FC)
ALP	R&D System	962647	Mouse	1:200 (IF&FC)
HNF-3β	Santa Cruz	sc-101060	Mouse	1:200 (IF)
OCT4	R&D System	962649	Goat	1:200 (IF&FC)
NANOG	R&D System	963488	Goat	1:200 (IF&FC)
NESTIN	BioLegend	809801	Mouse	1:500 (IF&FC)
Human-CD31	BD Biosciences	555444	Mouse	1:200
Ki67	ThermoFisher	PA5-16785	Rabbit	1:200 (IF&FC)
FN	Abcam	ab2413	Rabbit	1:200 (WB)
NOTCH4	SAB	37195	Rabbit	1:200 (WB)
ITGA2	Santa Cruz	sc-136257	Mouse	1:200 (WB)
GAPDH	ProteinTech	10494-1-AP	Rabbit	1:20,000 (WB)
Secondary antibody	Jackson ImmunoResearch	711-585-152	Donkey	1:500
Secondary antibody	Jackson ImmunoResearch	711-165-152	Donkey	1:500
Secondary antibody	Jackson ImmunoResearch	705-605-147	Donkey	1:500

Table S2. Real time qPCR primers used in this study.

Primer Name	Forward 5'-3'	Reverse 5'-3'
PECAM1 (CD31)	GCAGCATCGTGGTCAACATAA	GCAGGACAGGTTCAGTCTTTCA
VE-Cadherin (CD144)	AAGGACATAACACCACGAAACG	CAAACTGCCCATACTTGACTGTG
vWF	TCGGGCTTCACTTACGTTCT	CCTTCACTCGGACACACTCA
CD34	GCCATTCAGCAAGACAACAC	AAGGGTTGGGCGTAAGAGAT
VEGFA	TCACAGGTACAGGGATGAGGACAC	TCCTGGGCAACTCAGAAGCA
VEGFB	GCTTAGAGCTCAACCCAGACACC	CAAGTCACCCTGCTGAGTCTGAA
VEGFC	CAGCACGAGCTACCTCAGCAAG	TTTAGACATGCATCGGCAGGAA
Fibronectin	GCACCACAGCCATCTCACAT	TCCAACGGCCTACAGAATTT
Collagen4A5	AAAAGAGCCCACGGTCAAG	GGGGTAGAGAGCCAGTAAGAA
Collagen4A6	ACCCTGCTGAGATCTGCTGT	GGCCCATCAAATCTTTCTGA
OCT4	CCCCAGGGCCCCATTTTGGTACC	ACCTCAGTTTGAATGCATGGGAGAGC
NANOG	TACCTCAGCCTCCAGCAGAT	CCTTCTGCGTCACACCATT
GAPDH	TCGACAGTCAGCCGCATCTTCTTT	ACCAAATCCGTTGACTCCGACCTT