Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Differentiating Human Pluripotent Stem Cells into Vascular Smooth Muscle Cells in Three Dimensional Thermoreversible Hydrogel

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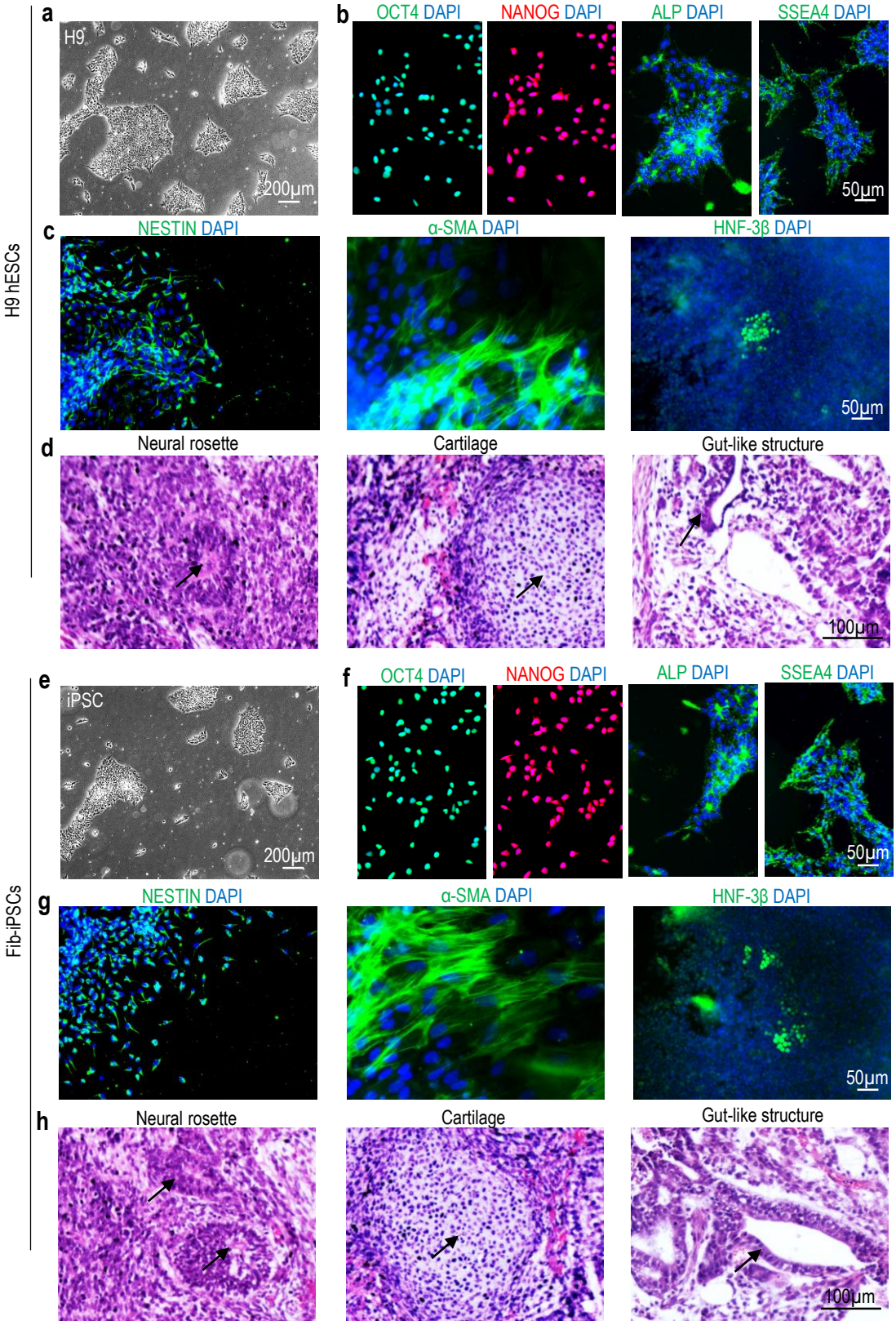

Figure S1. Starting hPSCs (H9s and Fib-iPSCs). (**a, e**) Phase images. (**b, f**) Majority of the cells expressed the pluripotency markers, OCT4, NANOG, ALP and SSEA4. (**c, g**) They could be differentiated into all three germ layer cells such as NESTIN+ ectodermal, α-SMA+ mesodermal and HNF-3β+ endodermal cells in EB assay. (**d, h**) They formed teratomas containing all three germ layer tissues (arrows) in SCID mice.

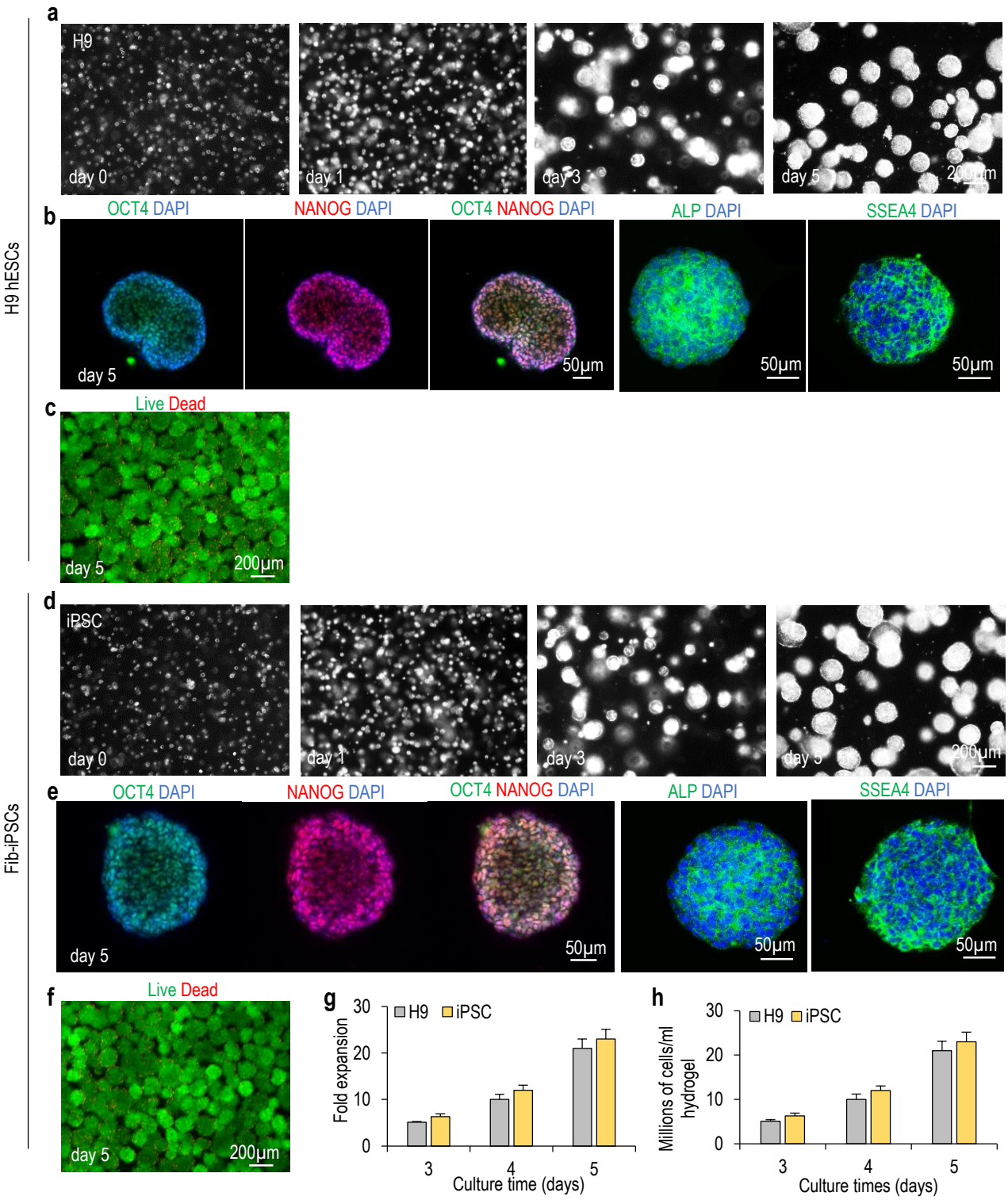

Figure S2. Culture hPSCs (H9s and Fib-iPSCs) in 3D thermoreversible hydrogels. (**a, d**) Phase images of day 0, 1, 3 and 5 hPSCs. (**b, e**) Immunostaining of day 5 hPSC spheroids for pluripotency markers OCT4, NANOG, ALP and SSEA4. (**c, f**) Live/dead cell staining of harvested day 5 hPSC spheroids. (**g, h**) About 5-, 10- and 20-fold expansion, and ~5, 10 and 20 million cells per milliliter of hydrogel were achieved on day 3, 4 and 5 respectively. Data are represented as mean ± SD of three biological replicates (n=3).

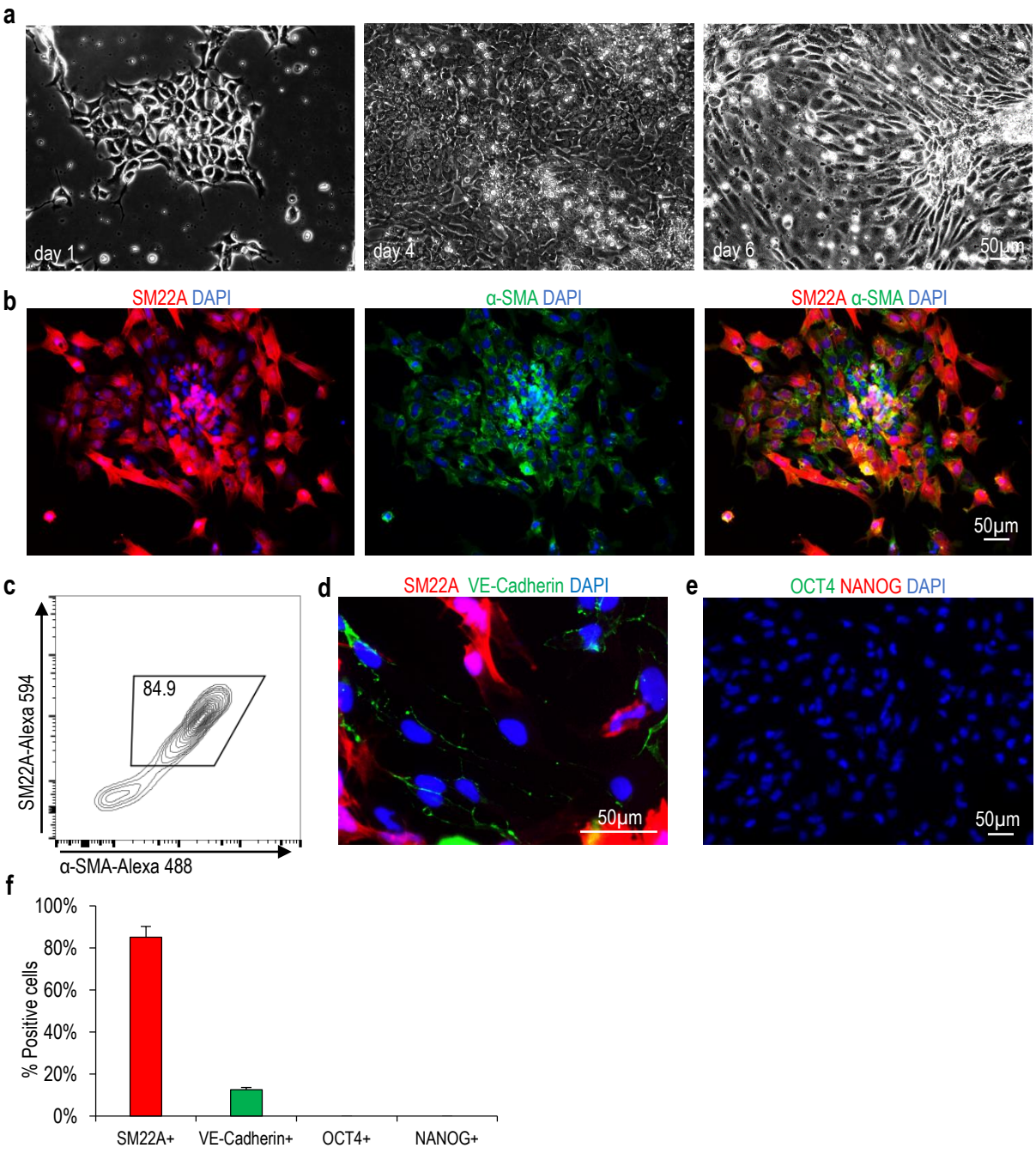

Figure S3. Differentiating Fib-iPSCs into VSMCs in 2D cultures. (**a**) Phase images of day 1, 4 and 6 cells. (**b, c**) Immunostaining and flow cytometry analysis of VSMCs marker genes SM22A and α-SMA on day 6 cells. (**d-f**) The day 6 cells contained a small fraction of VE-Cadherin+ cells (**d, f**), but no OCT4+/NANOG+ undifferentiated Fib-iPSCs (**e, f**). Data are represented as mean ± SD of three biological replicates (n=3).

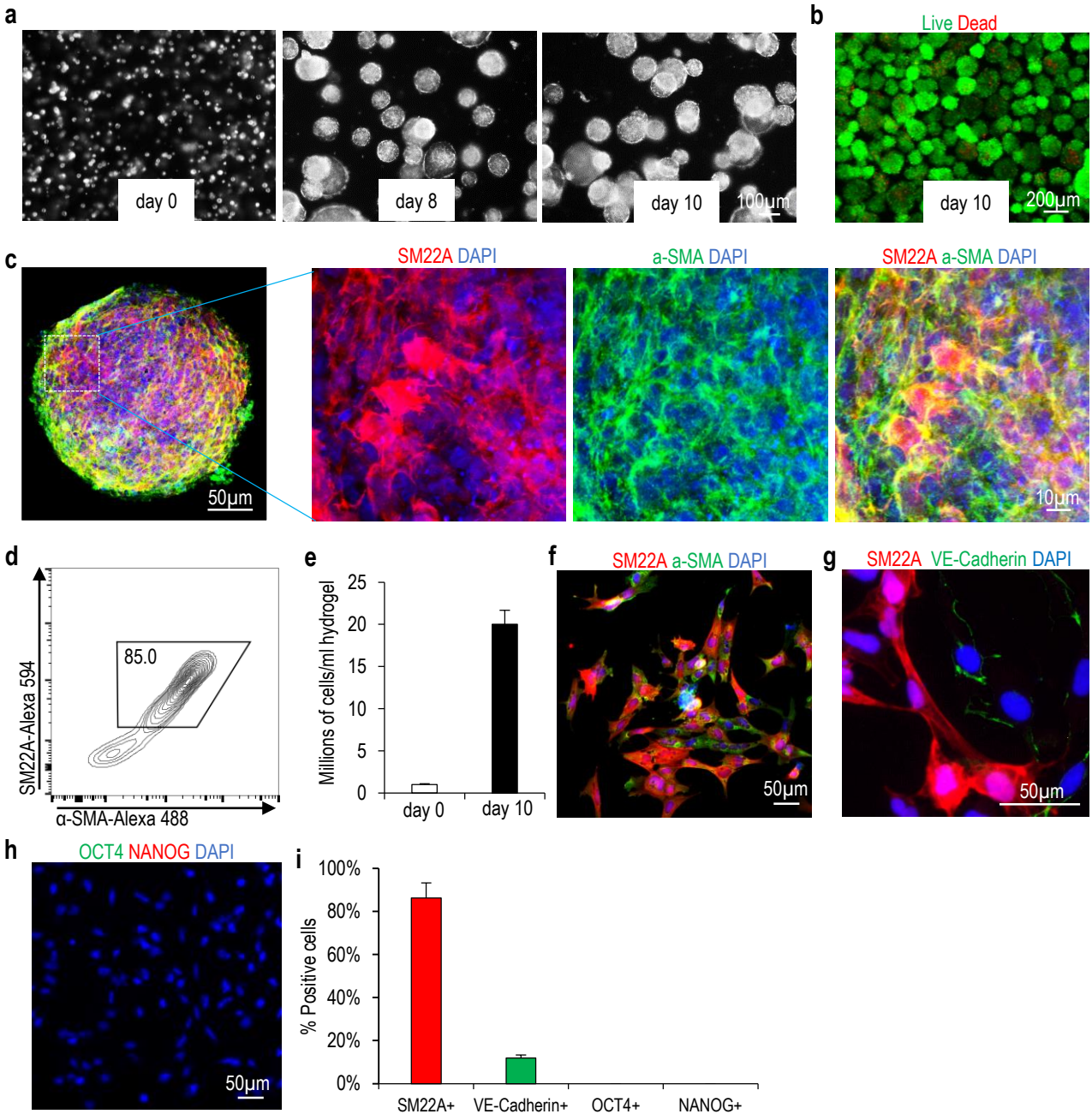

Figure S4. Differentiating Fib-iPSCs into VSMCs in 3D thermoreversible hydrogel. (**a**) Phase images of day 0, 8 and 10 cells. (**b**) Live/dead cell staining of harvested day 10 cells. (**c, d**) Immunostaining and flow cytometry analysis of VSMCs marker genes SM22A and α-SMA on day 10 cells. (**e**) ~2x10^7 VSMCs/mL hydrogel were produced. Data are represented as mean ± SD of three biological replicates (n=3). (**f-i**) When the day 10 spheroids were dissociated into single cells and plated on 2D surface overnight, immunostaining showed majority cells were SM22A+/α-SMA+ VSMCs (**f, i**), a small fraction of VE-Cadherin+ endothelial cells (**g, i**), but no undifferentiated OCT4+/NANOG+ iPSCs (**h, i**). Data are represented as mean ± SD of three biological replicates (n=3).

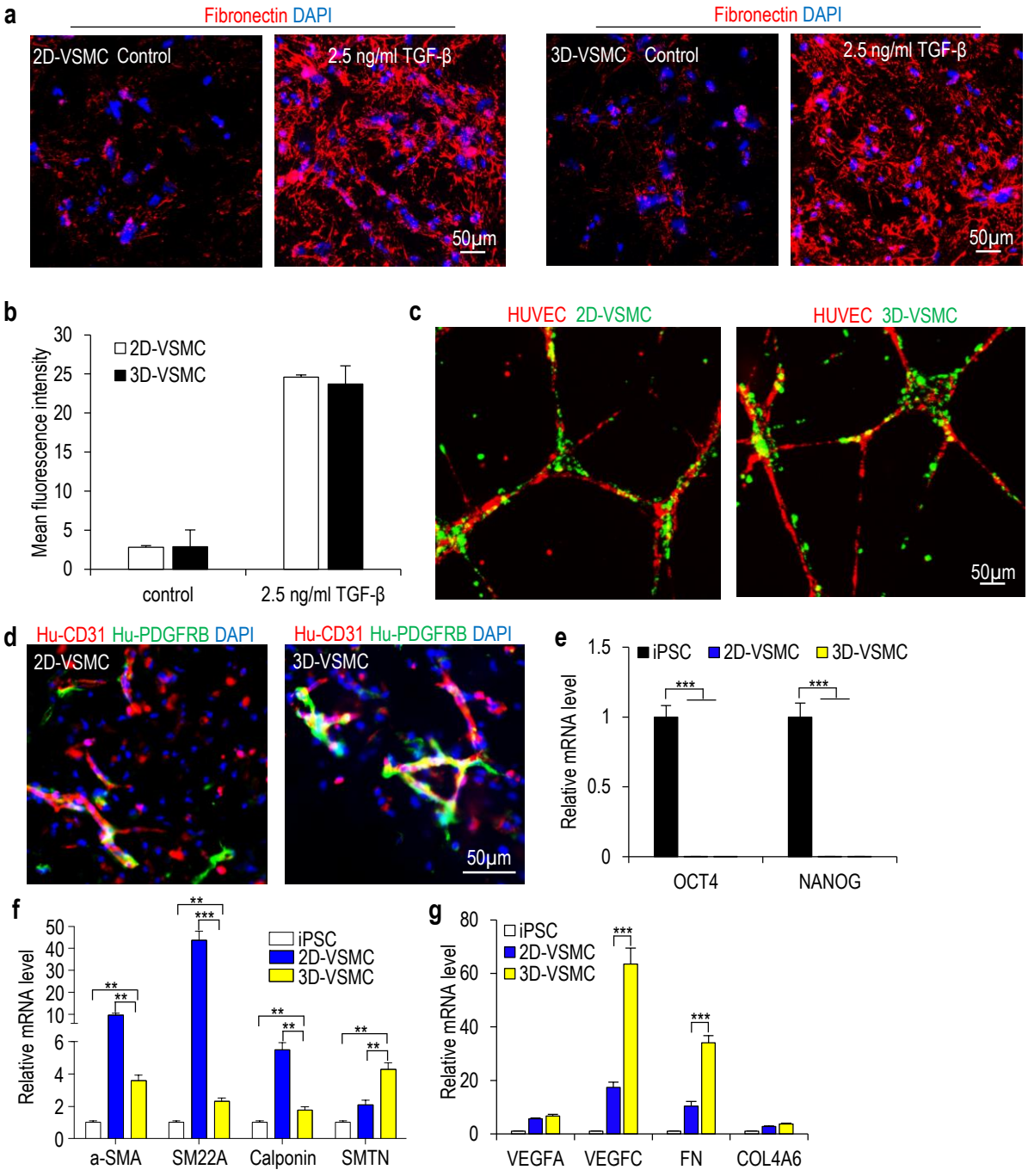

Figure S5. Properties of iPSCs derived VSMCs made in 3D hydrogel (3D-VSMCs) and 2D culture (2D-VSMCs). (**a, b**) Fibronectin production after 24 hours of 2.5 ng/mL TGF-β treatment. Data are represented as mean ± SD of three biological replicates (n=3). (**c**) Co-culture of VSMCs and HUVECs. (**d**) When VSMCs and HUVECs were co-transplanted subcutaneously, they formed nice vascular structures. (**e-g**) qRT-PCR analysis of 2D-VSMCs, 3D-VSMCs and Fib-iPSCs for pluripotency markers OCT4 and NANOG, synthetic VSMC marker α-SMA, SM22A and Calponin, contractile VSMC marker SMTN and other VSMCs related genes including growth factors VEGFA and VEGFC, and ECM genes FN and COL4A. Data are represented as mean ± SD of three biological replicates (n=3). ***P*<0.01, ****P*<0.001.

Supplementary tables

Table S1. Antibodies used in this study.

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)
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)
Fibronectin	Abcam	ab2413	Rabbit	1:500 (IF)
Human-PDGFRB	ThermoFisher	PA5-14718	Rabbit	1:100 (IF)
Human-CD31	BD Biosciences	555444	Mouse	1:200
Secondary antibody	Jackson ImmunoResearch	715-545-151	Donkey	1:500
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'**
SM22A	TGAAGGCGGCTGAGGACTAT	TCTGTTGCTGCCCATCTGAA
α-SMA	CTGGGACGACATGGAAAA	ACATGGCTGGGACATTGA
Calponin	AGCATGGCGAAGACGAAAGGAA	CCCATCTGCAGGCTGACATTGA
SMTN	CGGCCTGCGCGTGTCTAATCC	CTGTGACCTCCAGCAGCTTCCGAA
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