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Supplementary Materials for

Compliant substratum guides endothelial commitment from human pluripotent stem cells

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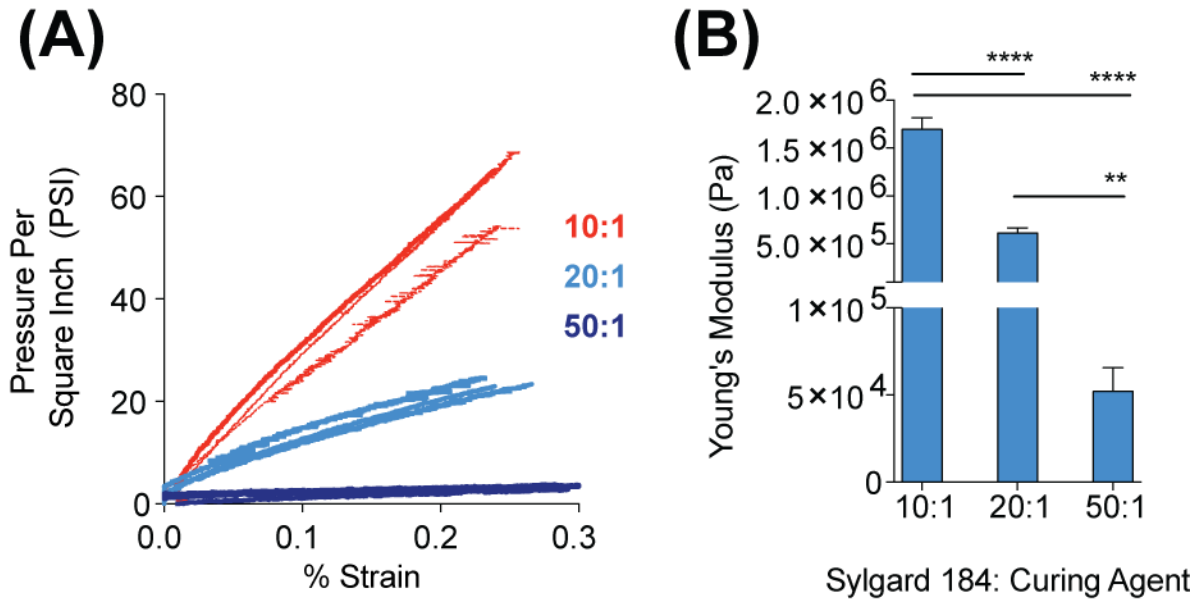

fig. S1. Development of compliant PDMS substrates. (**A**) Stress-strain curves for three different PDMS formulations from three independent experiments (n=3). (**B**) Calculated Young's Modulus of PDMS substrates as a function of the weight percent between elastomeric base and curing agent. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

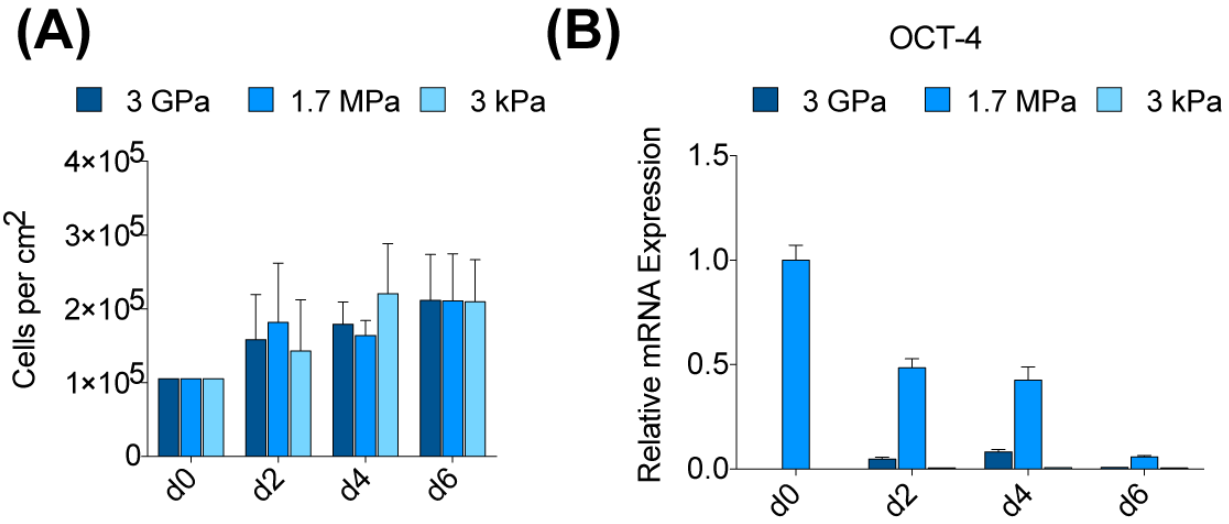

fig. S2. Differentiation and proliferation are supported on compliant silicone substrates. **(A)** Growth kinetics as a function of substrate stiffness**. (B)** Representative PCR analysis of mRNA expression from biological triplicates showing loss of self-renewal maker *OCT-4* for hiPSCs differentiated for six days on PDMS and E ~ 3 GPa surfaces. Differentiation kinetics normalized to *OCT-4* expression of undifferentiated hiPSCs (day 0).

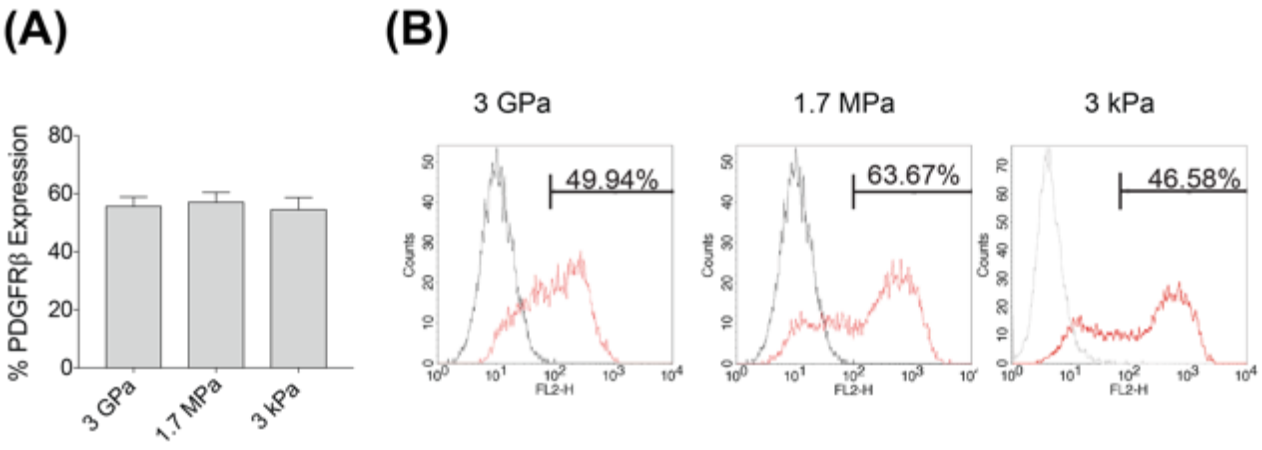

fig. S3. PDGFR-β expression from mesodermal differentiation in serum. (A) Average PDGFR-β expression as a function of substrate stiffness on day 12. **(B)** Representative flow cytometry histogram plots of PDGFR-β expression. Data are represented as mean ± S.E.M. from at least three independent differentiations.

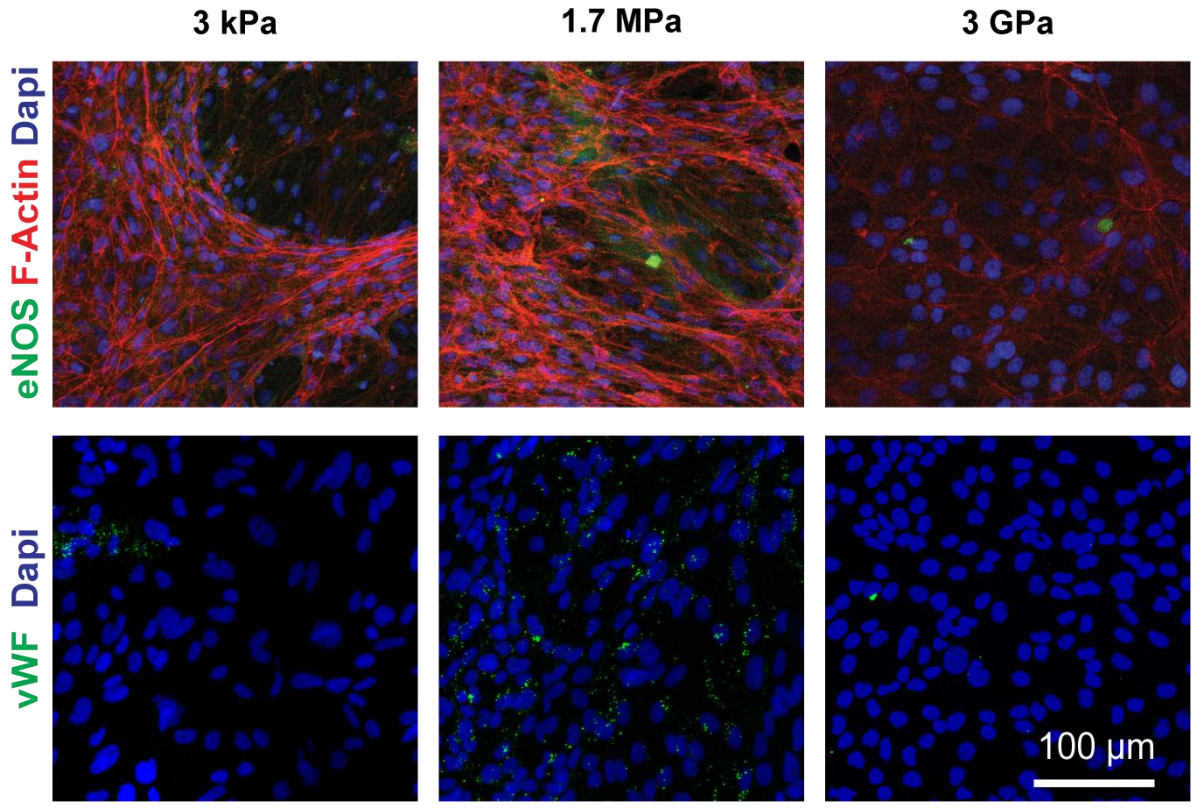

fig. S4. Immunofluorescence microscopy of mature EC markers after mesoderm stiffness priming. Representative immunofluorescent images of day 12 EVCs primed on compliant PDMS

substrates as well as E ~ 3 GPa (eNOS and vWF expression in green; F-actin in red; and nuclei in blue). At least three biological replicates were preformed.

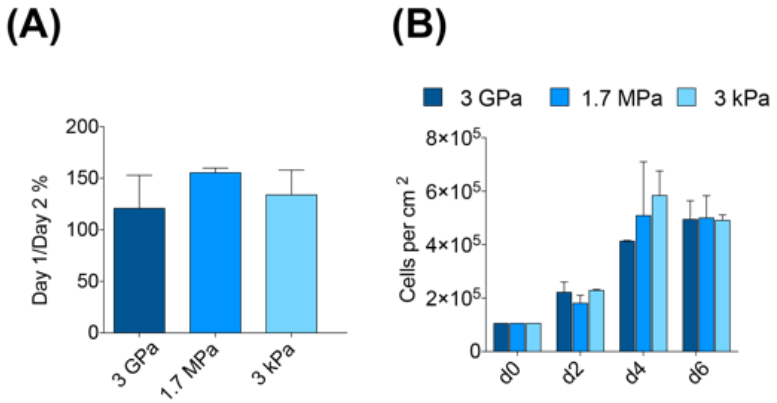

fig. S5. Differentiation and proliferation are supported on compliant silicone substrates in serum-free conditions. (A) Attachment efficiency across varied substrates in serum free conditions. **(B)** Cell counts during differentiation. Data are represented as mean ± S.E.M. from at least three independent differentiations.

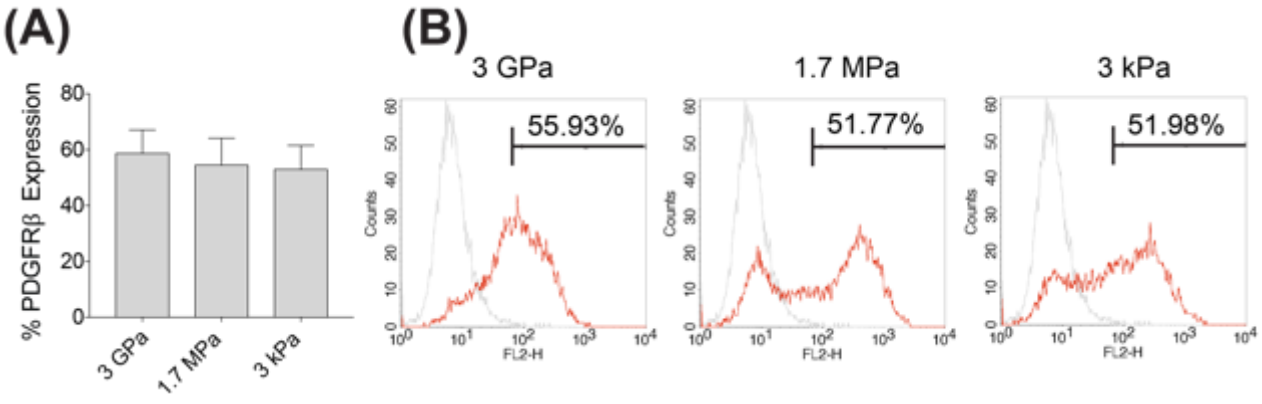

fig. S6. PDGFR-β expression from mesodermal differentiation in serum-free conditions. **(A)** Average PDGFR-β expression as a function of substrate stiffness on day 12. **(B)** Representative flow cytometry histogram plots of PDGFR-β expression. Data are represented as mean ± S.E.M. from at least three independent differentiations.

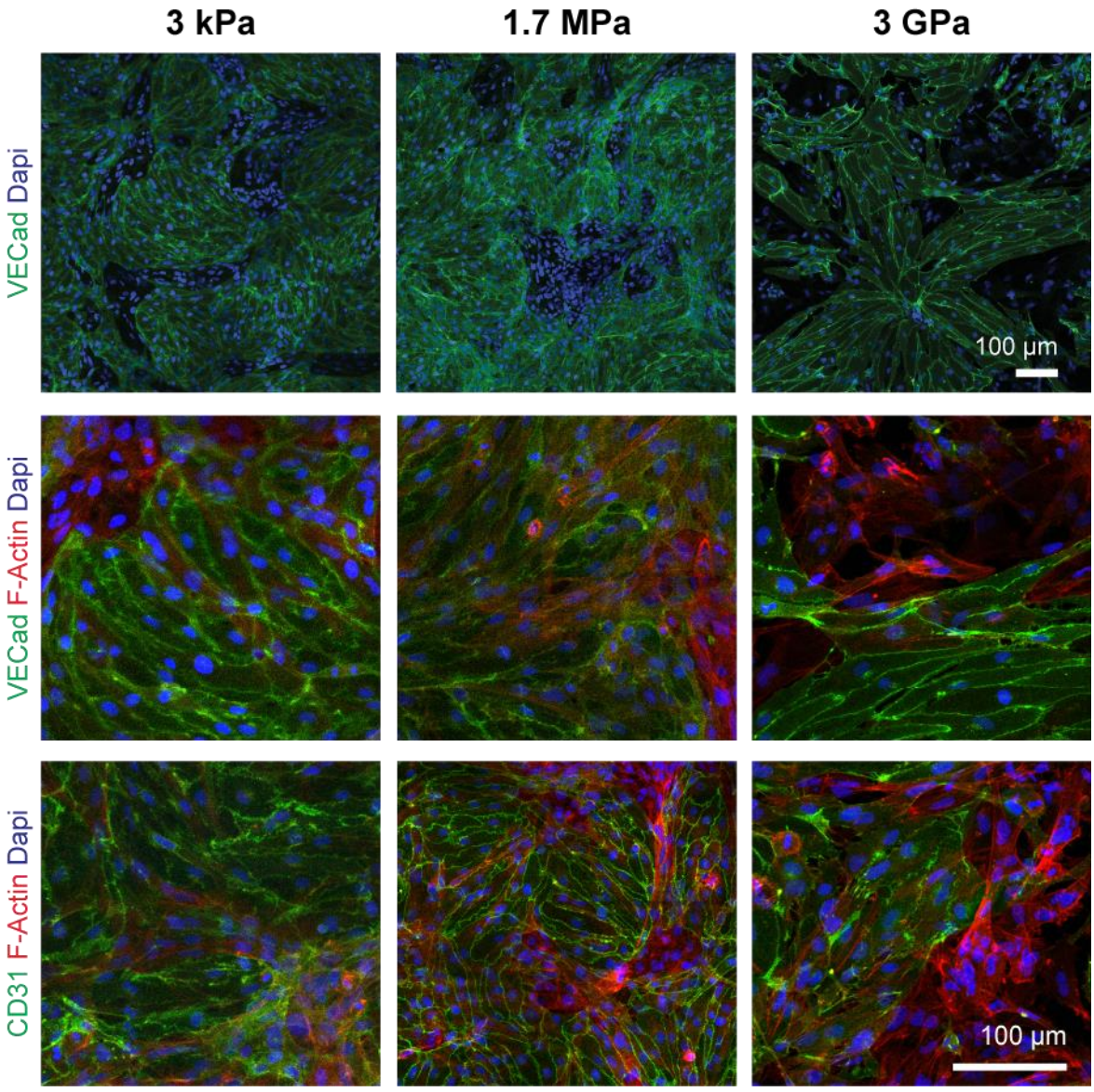

fig. S7. Immunofluorescence microscopy of EC markers after chemically defined mesoderm stiffness priming. Representative low magnification immunofluorescent images of VECad (green) and nuclei (blue) of day 12 EVCs primed on compliant PDMS substrates as well as E ~ 3 GPa substrates (**top**). High magnification images of VECad (green; **middle**) and CD31 (green; **bottom**) as well as F-actin (red) and nuclei (blue). At least three biological replicates were preformed.

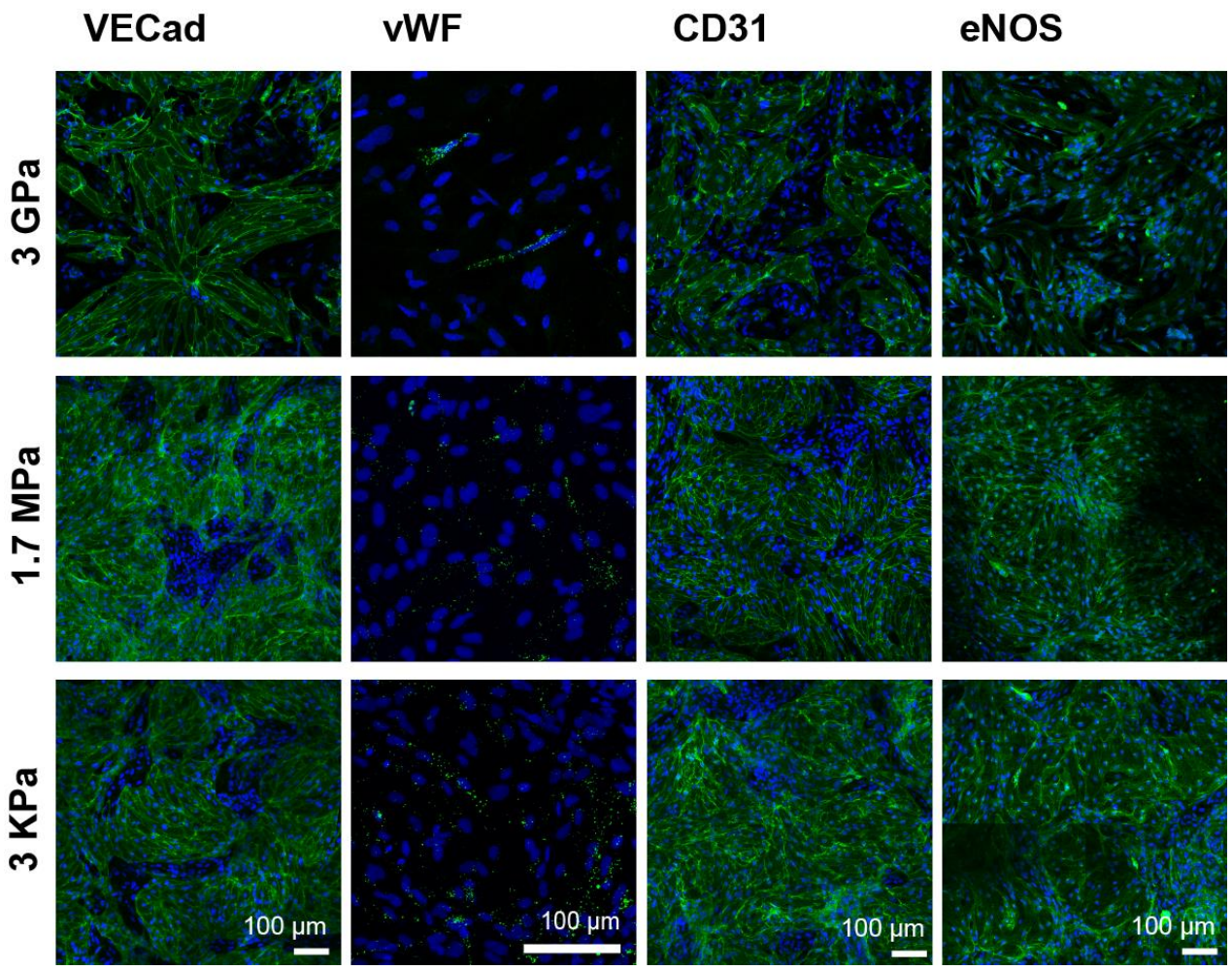

fig. S8. Immunofluorescence microscopy of EC markers after chemically defined mesoderm stiffness priming. Representative immunofluorescent images of day 12 EVCs primed on compliant PDMS substrates as well as E ~ 3 GPa substrates for the expression and localization of VECad, vWF, CD31 and eNOS (all in green; nuclei in blue).

table S1. Literature review of techniques to induce mesodermal specification from hiPSCs.

Mesoderm Induction	Days (D)	EC Differentiation Media	EC Differentiation Efficiency	Reference
30 ng/mL BMP-4, 25 ng/mL Activin A, 1.5 µM CHIR 99021	D:1-3	BPEL (bovine serum albumin (BSA) (without PVA), VEGF165 (50 ng/mL), B43152 (10 µM)	10-30% CD31+CD34+	[12]
LaSR basal medium (advanced DMEM/F12, 2.5 mM GluatMAX, 60 µg/mL ascorbic acid), 6-10 µM CHIR99021	D: 0 - 2	CD34+ purified Collagen IV coated dishes, EGM-2 medium (Lonza)	> 50% CD34+CD31+ EPCs	[36]
mTeSR1 media, activin A (10 ng/mL), FGF-2 (10 ng/mL), VEGF,165 (10 ng/mL), and BMP4 (10 ng/mL)	D: 1	Stemline II, FGF-2, VEGF,165, BMP4 (10ng/mL), - SB43152	CD31+-,CD144+-, KDR+- and NRP-1+-	[13]
+- Y-27632 (10 µM), aMEM, 10% FBS, .1% BME	D: 0-6	EGM (Promocell) VEGF,165 (50 ng/mL), SB43152 (10 µM)	20-50%	[24,26]
N1B27 medium (1:1 mixture of DMEM:F12 (1:1) with Glutamax and Neurobasal media with N2 and B27 (life technologies), BMP4 (25 ng/mL). VEGF (200 ng/mL), CHIR99021 (8 µM)/ CP21 (1µM) and forskolin (2 µM)	D: 0-4	StemPro-34 SFM, VEGF (50 ng/mL)	35% CD144+	[39]
DMEM F-12 basal media, Activin A (2 ng/mL), CHIR99021 (3 µM)	D: 0-2	Basal media, poly(vinyl) alcohol (2mg/ml), with mVEGF-A (20 ng/mL) or hVEGF-A (10 ng/ml)	35% $CD31^+$(control) to > 95% $CD31^+$ (via selective adhesion)	[41]
DMEM/F12 basal media, CHIR99021 (4 µM), VEGF, Activin A, BMP-4 (5 ng/mL)	D:0-4	Endothelial basal media (EGM), bFGF (5 ng/mL), VEGF-A (10 ng/mL)	> 80% $CD31^+/CD144^+$	[42]

table S2. Antibodies used in this study. IF = immunofluorescence; FC = flow cytometry

Antibody	Source	Catalog #	Purpose	Host Species & Reactivity	Working Concentration
Dapi	Roche	10236276	IF	Nucleus	1:10,000
AlexaFluor 488	Life Technologies	A-11008	IF	Goat anti-rabbit	1:1,000
AlexaFluor 546	Life Technologies	A-10036	IF	Donkey anti-mouse	1:1,000
AlexaFluor 647	Life Technologies	A-31573	IF	Donkey anti-rabbit	1:1,000
VECAD-PE	BD	560410	FC	Mouse anti-human	1:10
VECAD (F-8)	Santa Cruz	Sc-9989	IF	Mouse polyclonal	1:100
vWF (H-300)	Santa Cruz	Sc-14014	IF	Rabbit polyclonal	1:100
YAP/TAZ	Santa-Cruz	Sc-271134	IF	Mouse monoclonal	1:100
eNOS	BD	610297	IF	Mouse anti-human	1:100
PECAM-1/CD31	Dako	MO823	IF	Mouse anti-human	1:100