Electronic Supplementary Material (ESI) for Lab on a Chip.

Table S1: Cell used in this study: control and PAH cells were collected from healthy donors or PAH patients.

PHBI patient ID	Sex (age in years)	Phenotype	Race	Type of Cells	Passages received	Passages used at the time of experiment
PHBI-ST-042	Male (53)	IPAH	White	*EC*	2	3~5
PHBI-ST-026	Male (40)	IPAH	White	*EC*	3	4~5
PHBI-CC-014	Male (45)	IPAH	White	*SMC*	2	4~6
PHBI-CC-017	Male (56)	IPAH	White	*SMC*	4	5~6
PHBI-ST-042	Male (53)	IPAH	White	*ADC*	1	3~4
PHBI-CC-016	Male (27)	IPAH	White	*ADC*	2	4~5
PHBI-AH-022	Male (49)	Control	White	*EC*	3	4~6
PHBI-AH-022	Male (49)	Control	White	*SMC*	2	4~6
PHBI-AH-022	Male (49)	Control	White	*ADC*	2	5~6
PHBI-VA-011	Female (32)	IPAH	White	*EC*	3	4~5
PHBI-ST-040	Female (16)	IPAH	White	*EC*	3	4~5
PHBI-ST-020	Female (56)	IPAH	White	*SMC*	2	4~6
PHBI-BA-019	Female (29)	IPAH	Black	*SMC*	3	5~6
PHBI-BA-003	Female (62)	IPAH	White	*ADC*	2	4~5
PHBI-VA-014	Female (33)	IPAH	White	*ADC*	3	5~6
PHBI-UA-014	Female (45)	Control	White	*EC*	3	4~6
PHBI-AH-014	Female (28)	Control	White	*SMC*	2	4~5
PHBI-UA-015	Female (36)	Control	White	*EC*	3	5~6

Table S2: Characteristics of control and PAH cells used in this study.

Types of cells	Expression of proteins
N-EC	CD31, VEGFR2
N-SMC	FSP1, α-SMA, SM-22α
N-ADC	FSP1, CD90
PAH-EC	CD31, VEGFR2, FSP1, α-SMA, SM-22α,
PAH-SMC	FSP1, α-SMA, SM-22α
PAH-ADC	FSP1, α-SMA, SM-22α, CD90

Table S3: Primary and secondary antibodies used in this study.

Antigen	Host	Dilution ratio		Source	Catalogue Number
		IF	WB		
CD31	Mouse	1:80		Santa Cruz Biotechnology, TX	sc-376764
VE-Cadherin	Mouse	1:50		Santa Cruz Biotechnology, TX	sc-9989
VEGFR2	Rabbit	1:100	1:2000	Cell Signaling, MA	2479
FSP1 (S100A4)	Rabbit	1:500	1:4000	EMD Millipore, MA	ABF32
α-SMA	Mouse	1:500	1:4000	Sigma Aldrich, MO	A2547
SM-22α	Rabbit	1:400		Abcam, MA	ab14106
CD90	Mouse	1:200		BioLegend, CA	328102
Human collagen Type I α1	Mouse	1:100		R&D Systems, MN	MAB6220
Human collagen Type IV	Mouse	1:100		R&D Systems, MN	MAB6308
Anti-mouse IgG Alexa 555	Goat	1:400		Life technologies, CA	A11008
Anti-mouse IgG Alexa 488	Goat	1:400		Life technologies, CA	A11001
Anti-rabbit IgG Alexa 488	Goat	1:400		Life technologies, CA	A21422
Anti-rabbit IgG Alexa 647	Goat	1:400		Life technologies, CA	A21244
Anti-rabbit IgG Alexa 594	Donkey	1:400		Life technologies, CA	A21207

IF; Immunofluorescence, WB; western blot

Supplemanatary figures

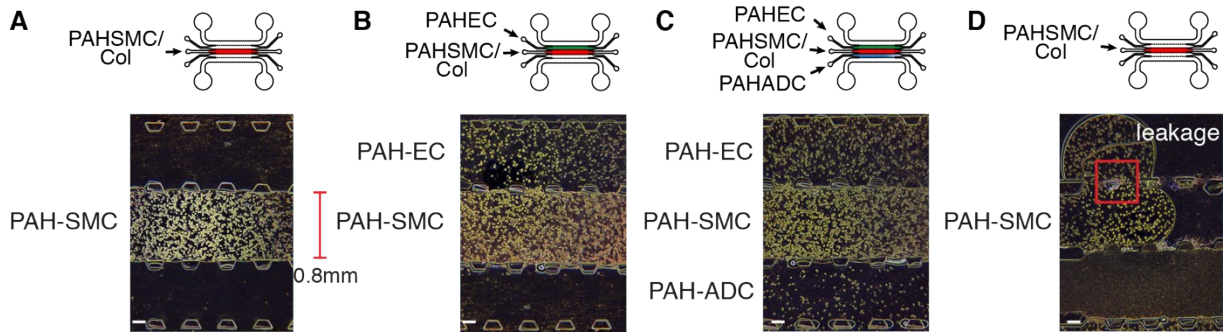

Figure S1. On-chip seeding process of PAH cells. (**A**) PAH-SMCs mixed with collagen were seeded on the medial layers, which gelled in 20 minutes and formed a medial layer of 0.8 mm. (**B**) PAH-ECs and (**C**) PAH-ADCs on the intimal and adventitial channels; ADCs were seeded 20 minutes after seeding of ECs. (**D**) A chip with damaged pillars (red box) and a leak, which was discarded. Scale bar 250 μm.

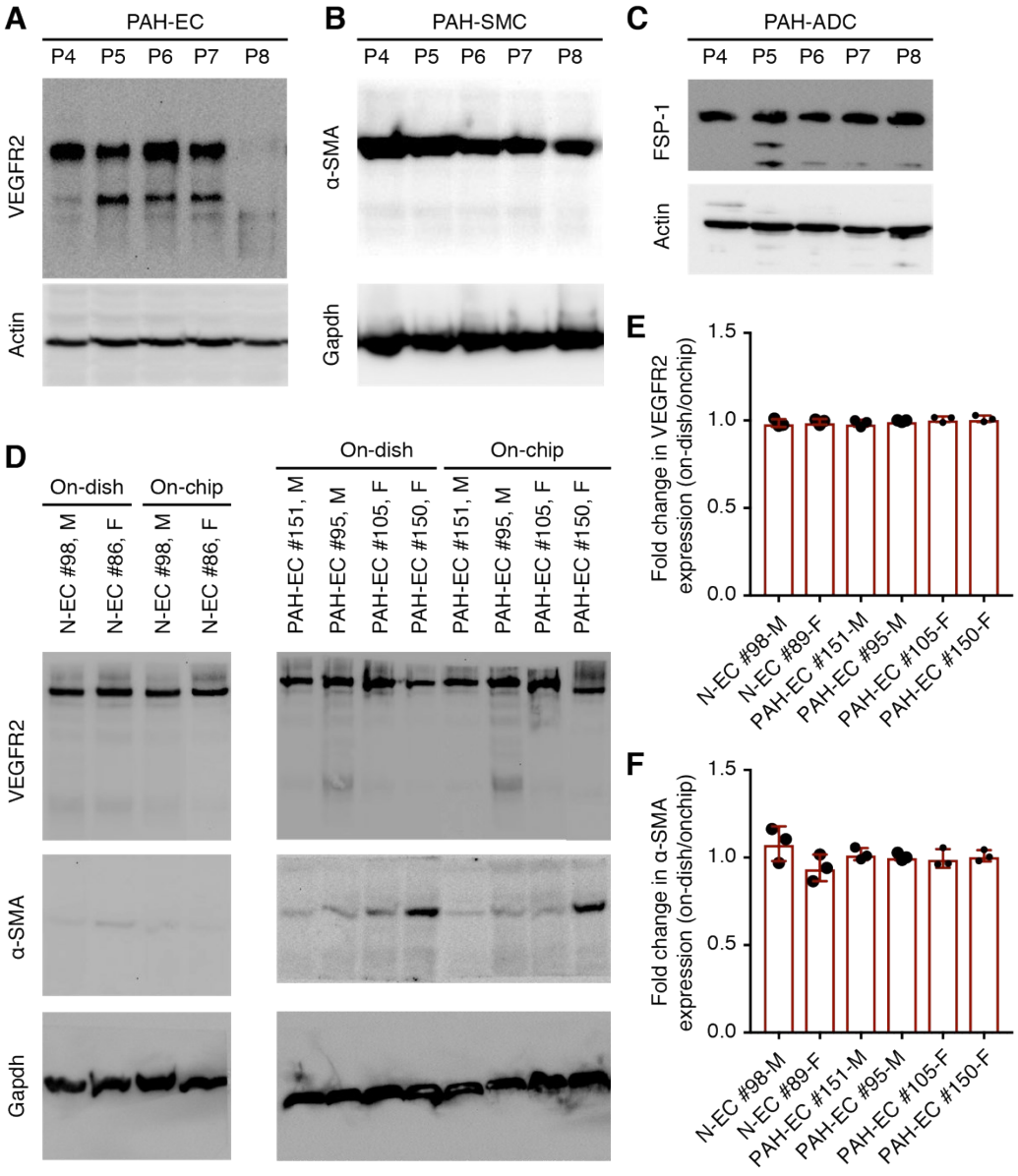

Figure S2. Cellular markers in various passages of PAH-ECs, PAH-SMCs, and PAH-ADCs. (**A, B, C**) VEGFR2, α-SMA, and FSP-1 expression patterns in various passages of PAH-ECs, PAH-SMCs, and PAH-ADCs, respectively. (**D, E, F**) No differences in the protein level of VEGFR2 and α-SMA expression after five days on-chip and on-dish culture of N-ECs and PAH-ECs collected from different donors or patients. Interestingly, PAH-EC #150, collected from female IPAH patients, had lower level of VEGFR2 but higher level of α-SMA compared to other PAH-ECs. Importantly, the level of expression remained unchanged when grown on-dish vs on-chip. m = male and f = female.

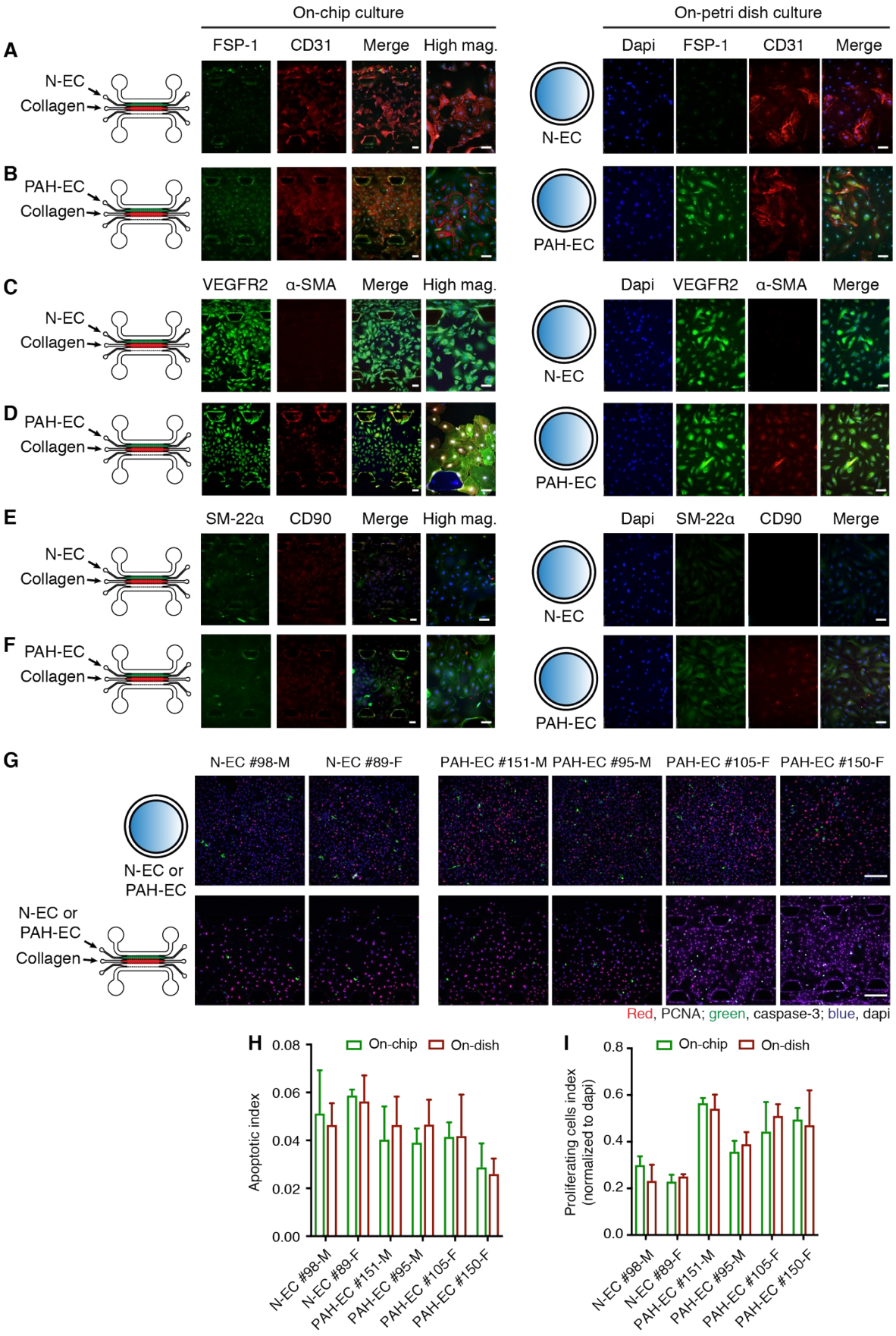

Figure S3. N-ECs and PAH-ECs grown on chips versus on-dishes showed no differences in the expression of various cellular markers. N-ECs and PAH-ECs grew and stayed within the intimal compartment

in the presence of collagen in the medial compartment. (**A, C, E**) On chip or on-dish culture of N-ECs showed expression of CD31 and VEGFR2, but not FSP-1, α-SMA, SM-22α, or CD90. In contrast, both on chip and on-dish cultures of PAH-ECs expressed (**B**) CD31 and FSP-1 or (**D**) VEGFR2 and α-SMA. (**E, F**) Neither N-ECs nor PAH-ECs expressed CD90, but PAH-ECs showed relatively higher expression of SM-22α than N-ECs. Both cells grew in monolayers. (**G, H, I**) The growth patterns of N-ECs and PAH-ECs. Despite some variability in the rates of apoptosis and proliferation among ells collected from different donors or patients, there were no differences in the apoptosis and proliferation of cells collected from a given patient regardless whether the cells were grown on-dish or on-chip. n = 3 chips prepared with the same cells from the same donors/patients. Scale bars 100 and 75 μm.

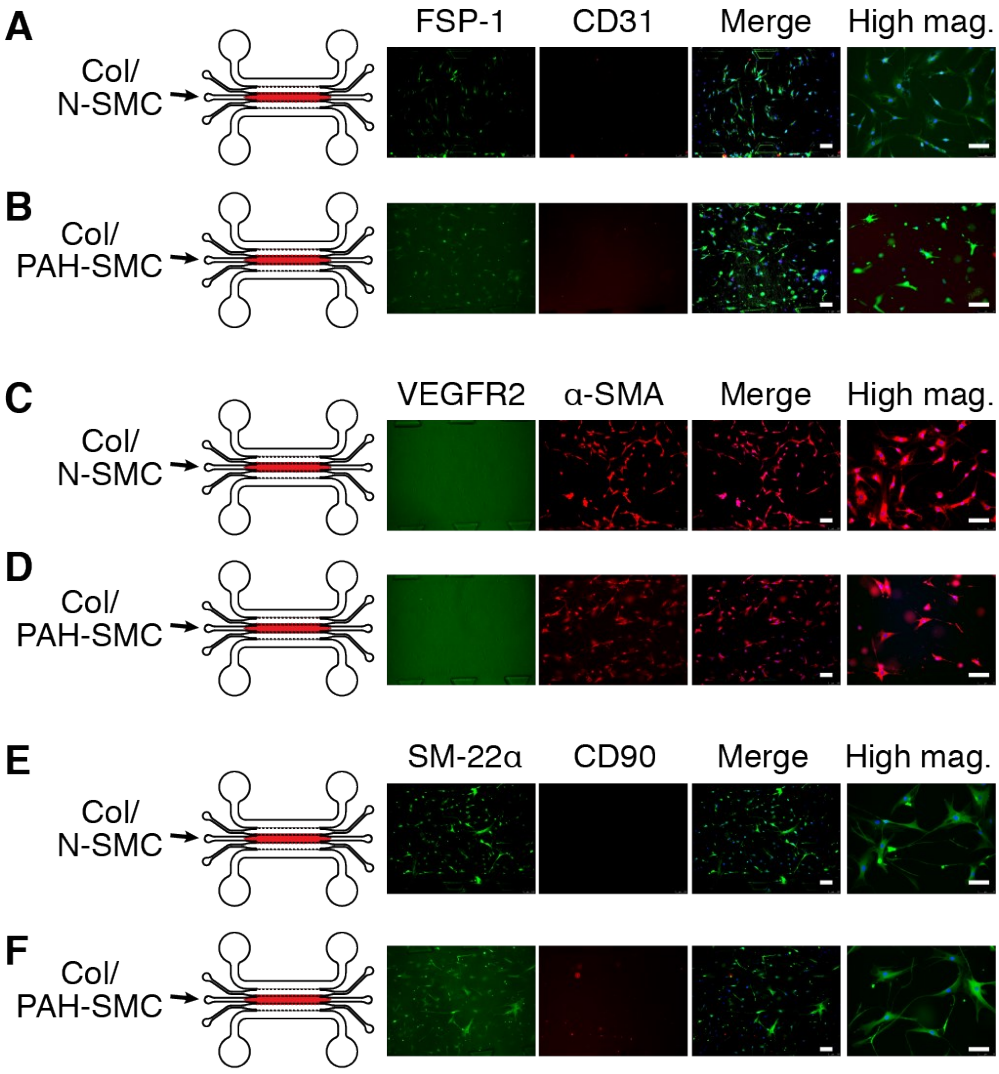

Figure S4. On-chip culture caused no alterations in the phenotypes of N-SMCs and PAH-SMCs. (**A-F**) Both N-SMCs and PAH-SMCs expressed FSP-1, α-SMA or SM-22α, but not CD31 or VEGFR2 or CD90. n = 3 chips prepared with the same cells from the same patients. Scale bars 100 and 75 μm.

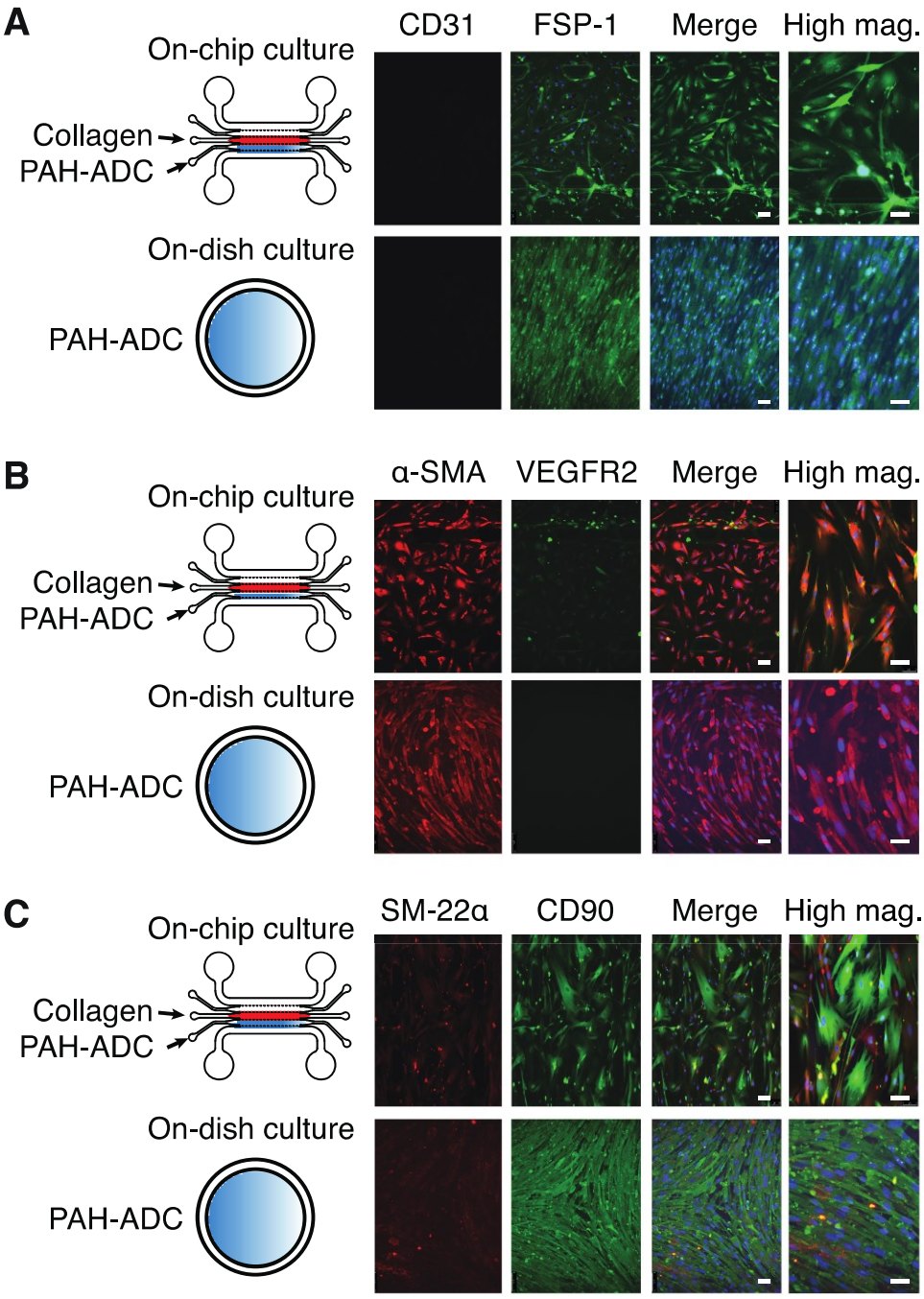

Figure. S5. The phenotypes PAH-ADCs remained unaltered when grown on chip versus on-dish. (**A, B**, **C**) PAH-ADCs expressed FSP-1, α-SMA, and CD90, but only slightly expressed SM-22α and showed no positive staining for CD31 and VEGFR2 in on-chip or in on-dish cultures. A physiologically relevant perpendicular growth pattern of PAH-ADCs was observed. On-petri dish, PAH-ADCs were cultured for 9 days until confluency but on-chip, the cells were cultured for 5 days before staining. n = 3 chips prepared with the same cells from the same donors/patients. Scale bars 100 and 75 μm.

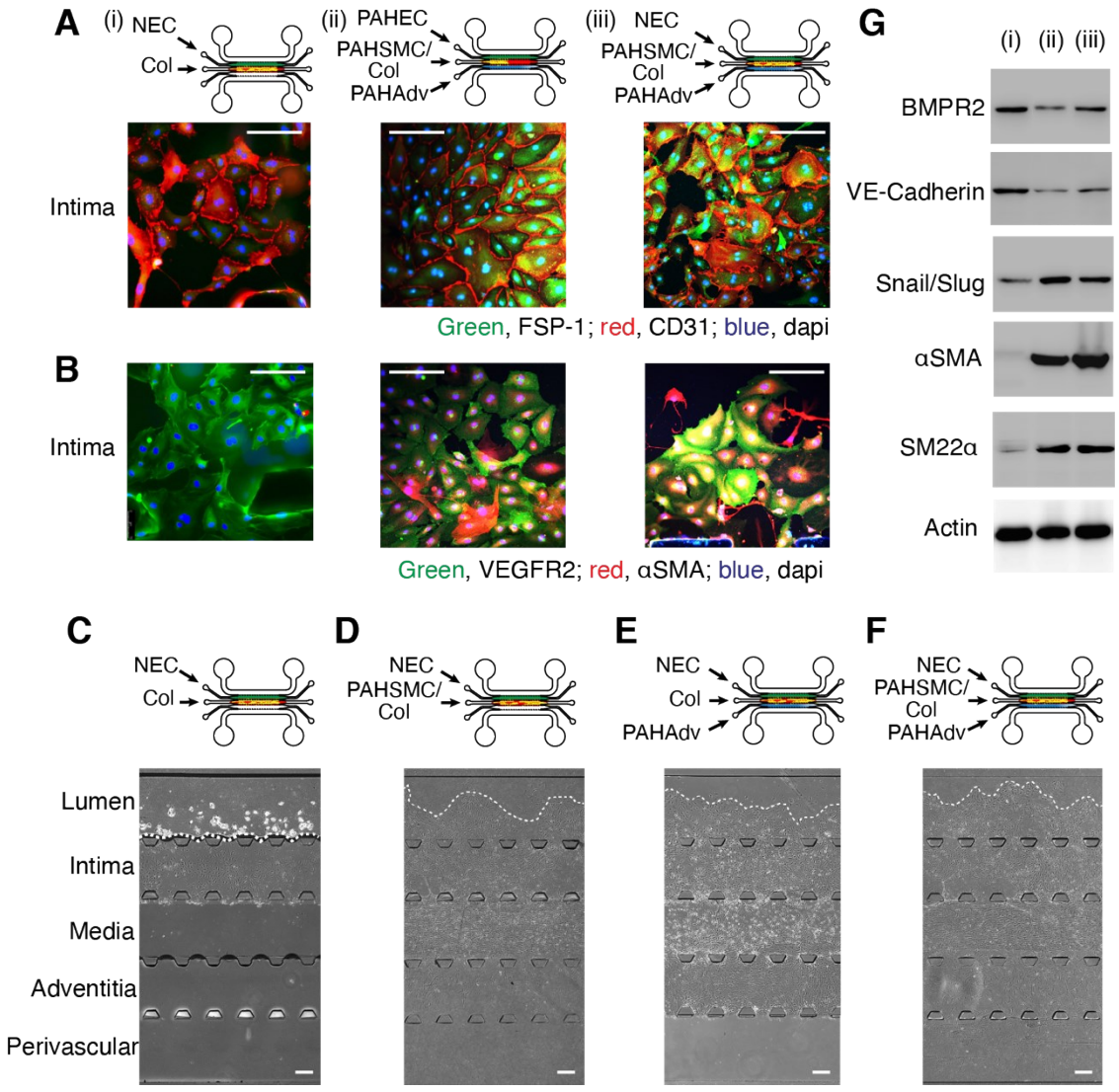

Figure S6. The device's pillar structure allows cellular cross-talk. (**A, B**) N-ECs expressed CD31 and VEGFR2, but not FSP-1 and α-SMA. N-ECs in the intimal channel changed their phenotype to SMC-like cells by expressing FSP-1 and α-SMA when grown along with PAH-SMCs and PAH-ADCs in the medial and adventitial channels. PAH-ECs expressed both FSP-1 and α-SMA when grown along with PAH-SMCs and PAH-ADCs in two other channels. Growth patterns of N-ECs when cultured (**C**) alone or in the presence of (**D**) PAH-SMCs, (**E**) PAH-ADCs, and (**F**) PAH-SMC/PAH-ADCs. In the presence of PAH-SMCs and/or PAH-ADCs, N-ECs grew more inside the lumen than in the absence of other cells. (**G**) The expression of different endothelial to mesenchymal markers when (i) N-ECs were seeded alone, (ii) PAH-ECs along with PAH-SMCs and PAH-ADCs, and (iii) N-ECs along with PAH-SMCs and PAH-ADCs. n = 3 chips prepared with the same cells from the same donors/patients. Scale bars 250 and 100 μm.

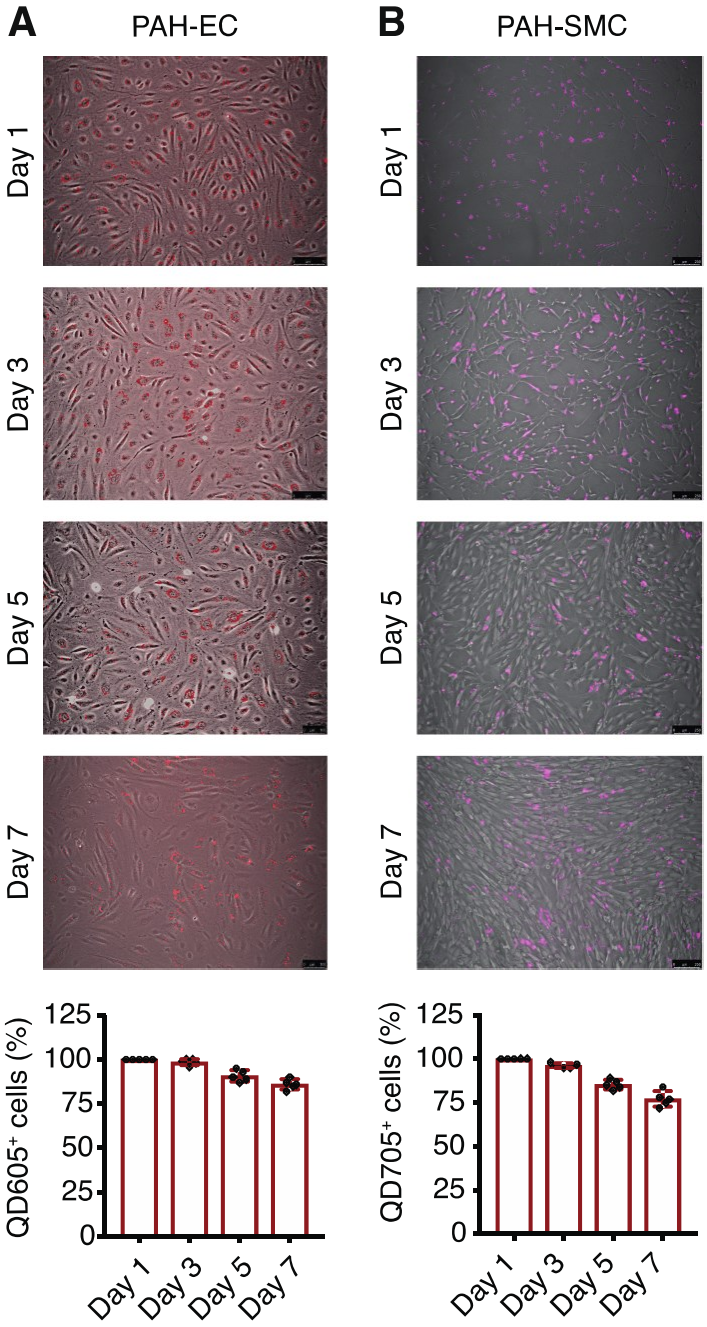

Figure S7. (**A**) PAH-ECs and (**B**) PAH-SMCs were labeled with Quantum Dot 605 (QD605) and QD705, respectively, and grown on dishes for seven days. Images were taken at 1, 3, 5, and 7 days after culture and the percentage of QD-positive cells were quantified using ImageJ®. n = 3, Scale bar 100 μm.

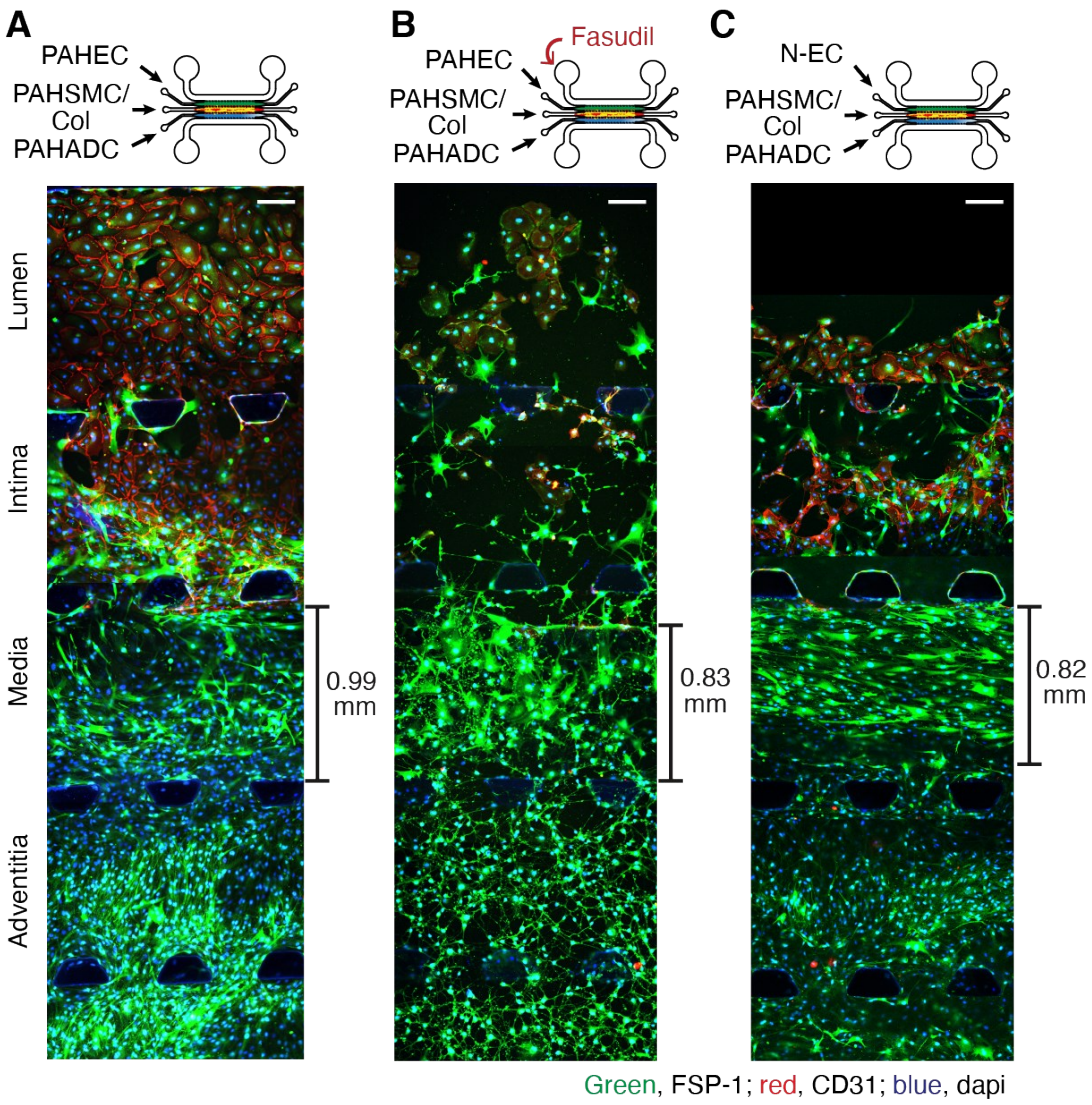

Figure S8. Fasudil affects arterial remodeling on three-layered PAH-cells-laden chips. (**A**) PAH-ECs underwent muscularization and the medial and intimal layers became thicker, when PAH-EC/SMC/ADCs were seeded in their respective layers. (**B**) Fasudil, when given via the luminal layer, prevented muscularization of PAH-ECs and reduced medial wall thickening. (**C**) When N-ECs were seeded, instead of PAH-ECs, the muscularization of the intimal layers and thickening of the medial and adventitial layers did not occur. n = 3 chips prepared with the same cells from the same donors/patients. Scale bars 100 μm.

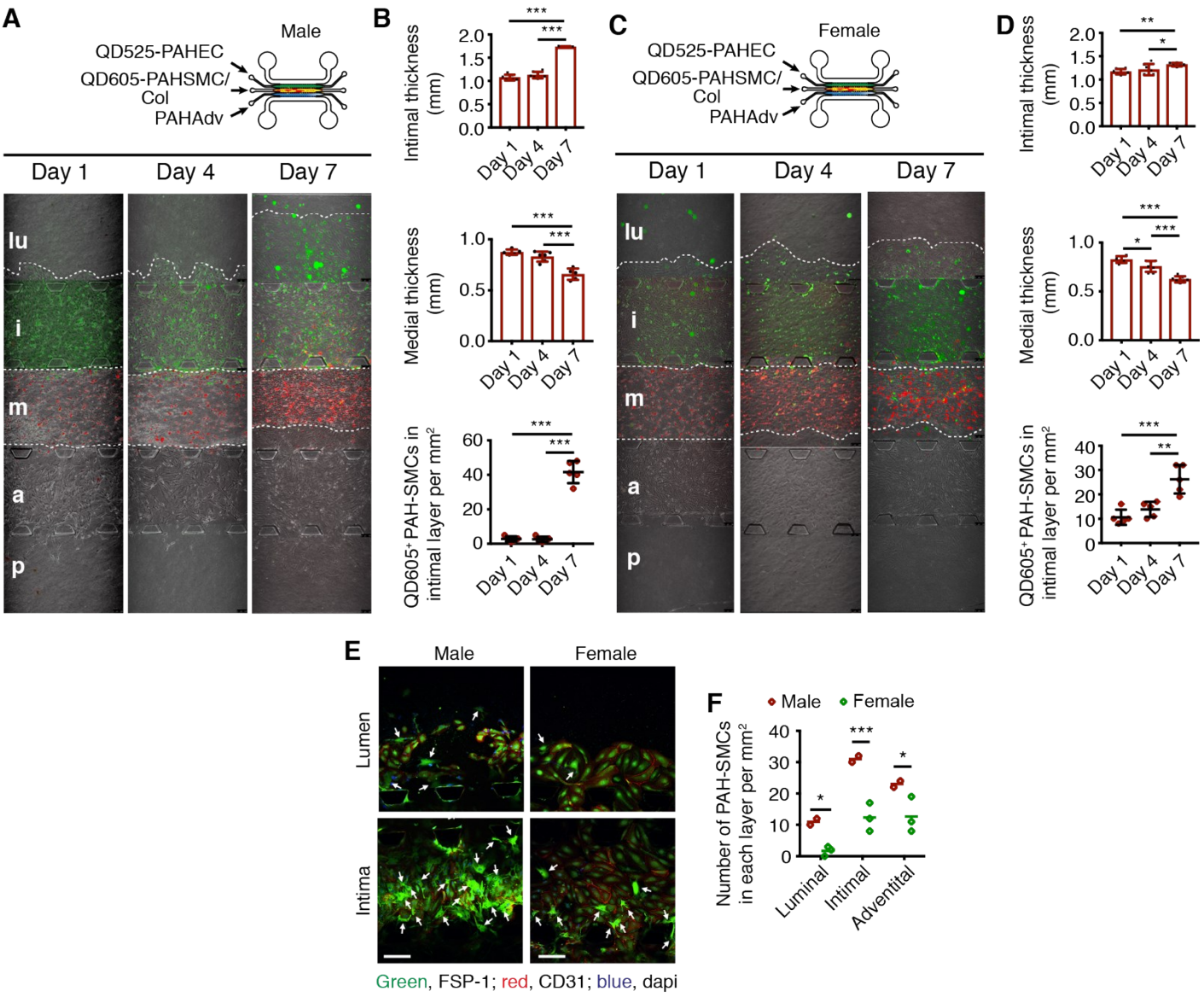

Figure S9. The device can simulate the PAH-afflicted pulmonary arteries of both sexes. Male and female PAH-ECs and PAH-SMCs were labeled with QD525 and QD605, respectively, and seeded in their respective channels along with PAH-ADCs in the adventitial layer of the chips. (**A, C**) Male and female chips, imaged on day 1, 4, and 7 to assess the arterial remodeling, showed varying degrees of arterial remodeling based on the patient's sex. (**B, D**) The intimal and medial thickness and the number of QD-labeled PAH-SMCs in male and female chips on different days. (**E, F**) Immunostaining and subsequent measurement of FSP-1 and CD31 indicate the presence of a greater number of FSP-1^{+}CD31^{-} PAH-SMCs in the luminal and intimal layers of the control male chips than female chips imaged on day 6. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; data represent mean

± SD of chips prepared with pulmonary arterial cells from two male and two female PAH patients. n = 5 chips prepared with the same cells from the same donors/patients. Scale bar 250 and 100 μm.

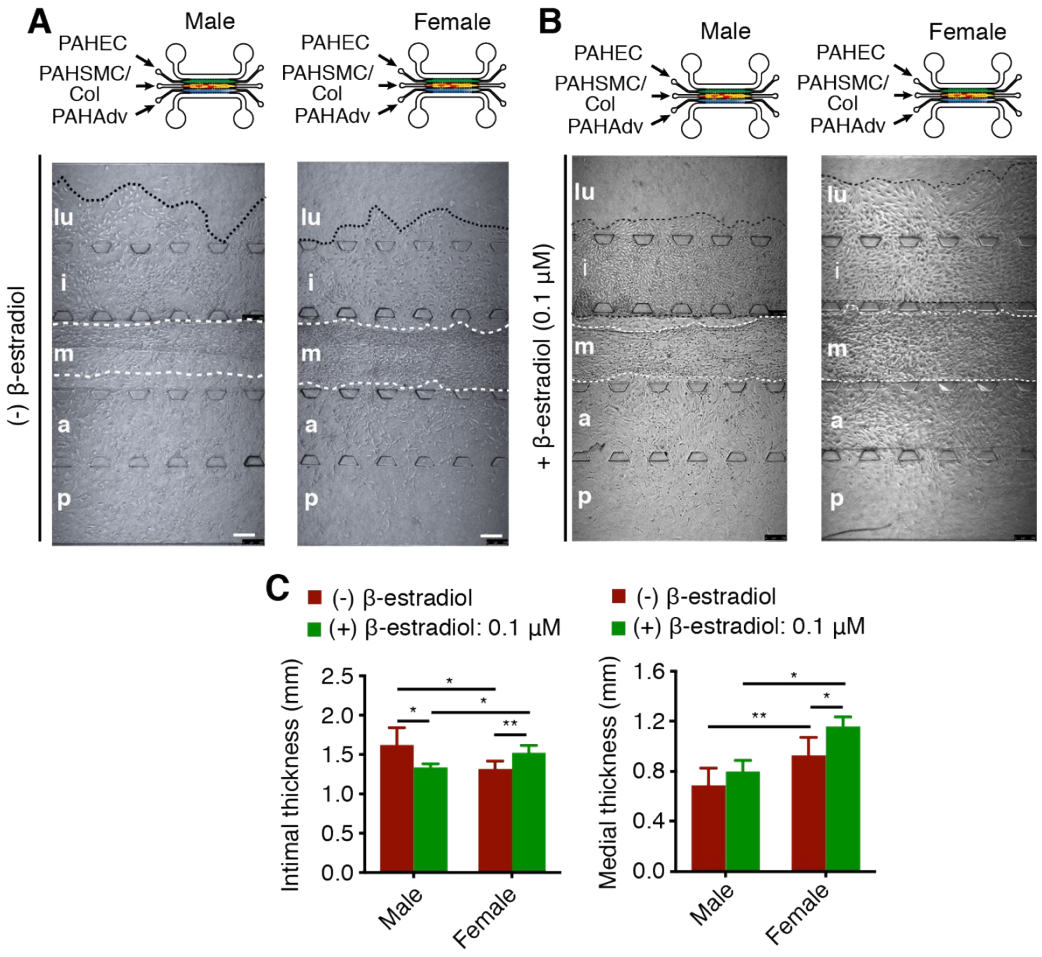

Fig. S10. Chips prepared with cells from male and female PAH patients respond differently to estrogen treatment. PAH-EC/SMC/ADCs of male and female patients were seeded in their respective channels. (**A, B, C**) When treated with 0.1 μM of β-estradiol, female chips showed a higher intimal and medial thickening than the control chips, but male chips showed reduced intimal thickening and no significant changes in medial thickening. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; data represent mean ± SD of pulmonary arterial cells from two male and two female PAH patients. n = 6 chips prepared with the same cells from the same donors/patients. Scale bars 250 and 100 μm.