Supplementary Information

Materials and Methods

Fibroblast Screening

Five lots of fibroblasts, designated A-E, were purchased from Lonza, Table S1, and screened for their vasculogenic potential. Screening was completed in simple vasculogenic devices. Vascular masters were poured with 3-4 mm of 10:1 PDMS and cured. Once cured the media ports were punched with a 4 mm biopsy punch and the gel ports were punched with a 1 mm biopsy punch. The feature side of the PDMS was then bonded to a square glass coverslip. HUVECs were seeded in the central channel to a final concentration of 6 x 10^6 cells/mL in a 3 mg/mL fibrin gel. Outer channels were seeded with each individual lot of fibroblasts (passage 7) at a final concentration of 3 x 10^6 cells/mL in a final concentration 3 mg/mL fibrin gel. Devices were fed EGM2MV (no additional cytokine supplements) for 5 days, perfused with 70 kDa FITC-dextran, Fig. S1, then fixed and stained with phalloidin. While phalloidin staining showed each fibroblast lot created vessel-like structures across the entire central channel, only two lots (NHLF-A and NHLF-E) had 100% of devices with any open ports creating partially perfusable vasculature. NHLF-B and NHLF-C had open ports in 50% of the total devices and NHLF-D had no perfusable devices. NHLF-A and NHLF-E were both tested in microphysiologic vascular lung-on-a-chip devices and the NHLF-E fibroblasts would consistently overgrow the microphysiologic device when cultured more than a week.

Neutrophil CellTracker Staining

Neutrophils are easily activated by too many centrifugation steps in the manufacturer's CellTracker protocol. Neutrophils were thawed following Astarte's protocols, once neutrophils were thawed, neutrophils were added to 9 mL of PBS with 1:1000 CellTracker Deep Red and centrifuged for 10 min at 200 g. Alternatively after centrifuging in PBS, resuspend the neutrophils in 1 mL of PBS with 1:1000 Deep Red and leave on ice for 15 minutes, count, dilute in media, and add to the plates without removing excess dye with a second centrifugation step.

Table S1. Cell Lot Information. Specific lots used for these studies with the commercially available donor information.

Material	Company	Cat #	Lot #	Age	Gender	Ethnicity	Smoker (Y/N)
NHLF-A	Lonza	CC-2512	580583	79	F	C	Y
NHLF-B	Lonza	CC-2512	608197	67	M	C	N
NHLF-C	Lonza	CC-2512	548315	52	M	C	Y
NHLF-D	Lonza	CC-2512	655309	56	M	B	N
NHLF-E	Lonza	CC-2512	615568	12	M	H	N
iPF-HLF	Lonza	CC-7231	627840	52	M	C	unknown
HUVEC	Lonza	C2519A	470896	Pooled	N/A	N/A	N/A
SAECs	Lonza	CC-2547S	677244	7	F	B	unknown
CF-HBE	Lonza	00196979	456504	38	M	C	unknown
Neutrophils	Astarte	1025	4058OC18	27	F	C	unknown

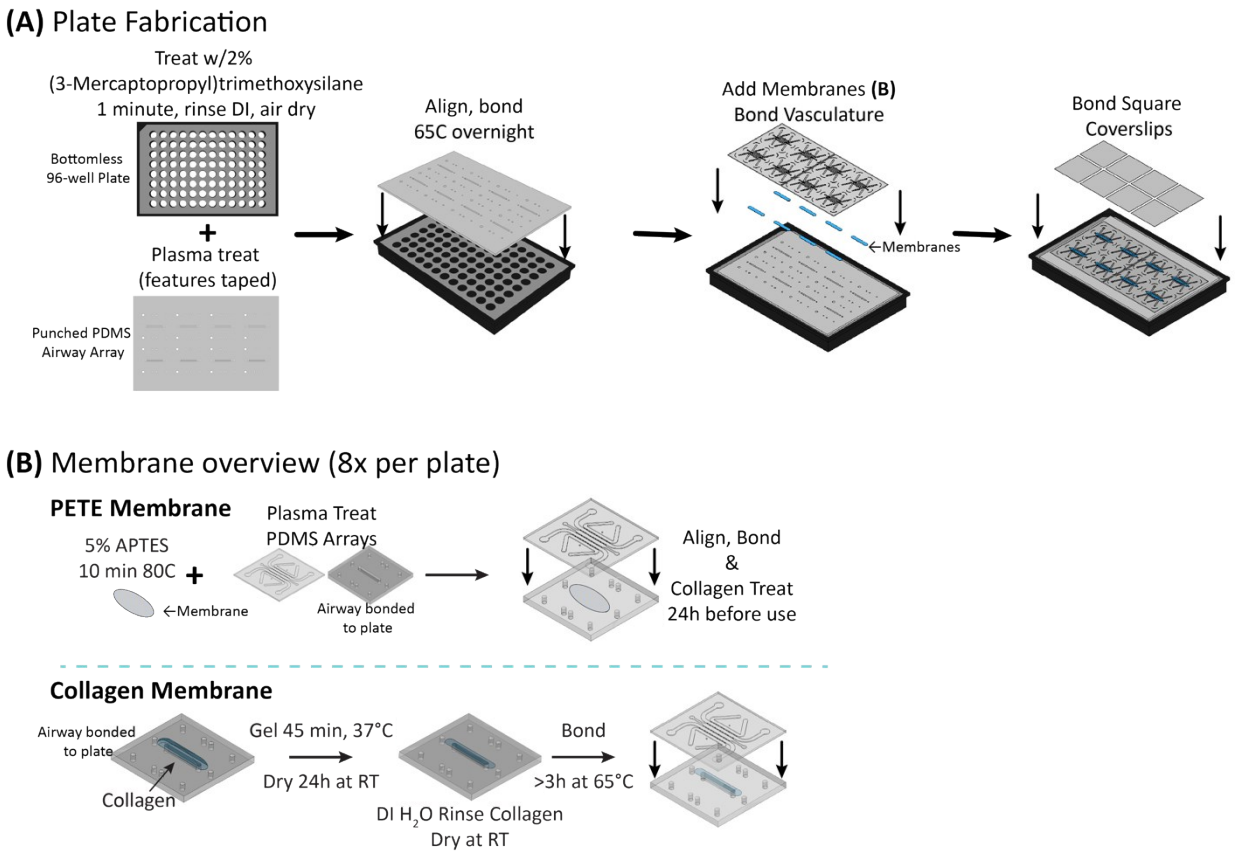

Fig. S1 Fabrication Overview. (A) Workflow for fabricating devices with a bottomless 96-well plate. (B) Process to add membranes for PETE or Collagen devices, all 8 devices are done simultaneously on each plate.

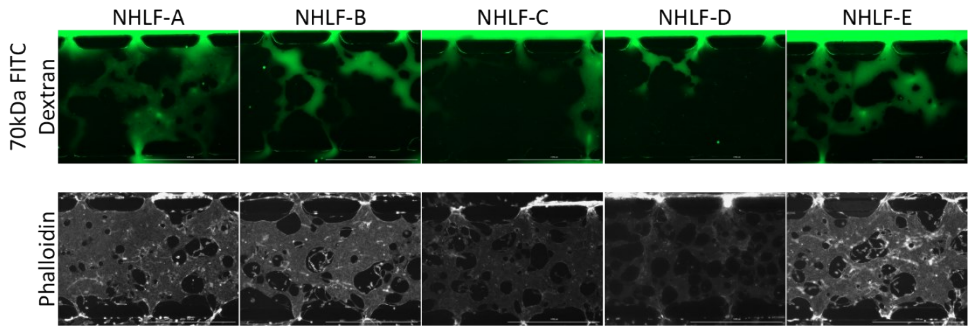

Fig. S2. Fibroblast lot screening. Five lots of fibroblasts, labeled A-E, were screened for vasculogenesis by testing perfusion in a vasculature-only device. Central channels were seeded with HUVECs while both outer channels were seeded with lots A-E. EGM-2MV media without additional VEGF/Ang1 supplements was used. Devices were perfused with 70kDa FITC Dextran, imaged, and then fixed/stained with phalloidin. Lots A and E had 100% of devices with a set of open ports perfusable across the devices, lot E often overgrew the 8-12 day cultures.

Table S2. Antibody List. Species: M-mouse, H-human, R-rat, G-goat, D-donkey

Target	Company	Cat #	Clone	Format	Species
α-SMA	ThermoFisher	53-9760-80	1A4	AF 488	M-Anti H/M/R
TUBB4	LSBio	LS-B12927	6C2	Primary	M-Anti H
Pro-Collagen I	Millipore	MAB1913	PCIDG10	Primary	M-Anti H
CD31	BioLegend	303126 303112	WM59	AF 594 AF 647	M-Anti H
555 Secondary	ThermoFisher	A32727	Polyclonal	AF 555	G-Anti M
488 Secondary	ThermoFisher	A21202	Polyclonal	AF 488	D-Anti M
IgG2a Iso.	ThermoFisher	53-4724-80	eBM2a	AF 488	M
CC10	Novus Bio.	NBP2-37439	3A8B8	Primary	M-Anti H
ZO-1	ThermoFisher	339188	1A12	AF 488	M-Anti H

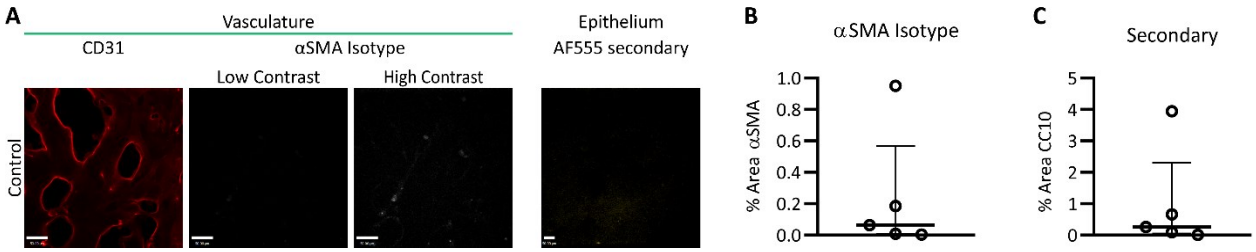

Fig. S3. (A) Control staining for αSMA isotype and secondary stain only for CC10 linearly contrasted identically to representative images in main text Figure 3, scale bar = 50 µm. (B) Percent area αSMA for the isotype device and (C) percent area for the CC10 secondary control device.

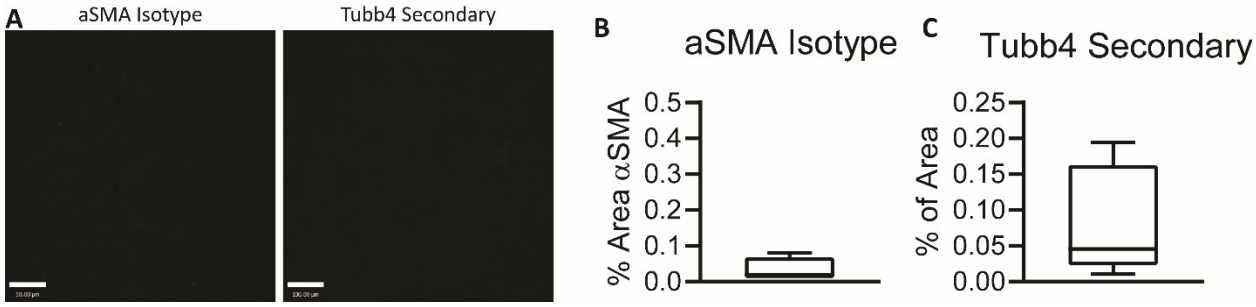

Fig. S4. (A) Tubb4 secondary stain control, linearly contrasted identically to representative images in main text Figure 4, scale bar aSMA isotype = 50 µm Tubb4 secondary =100 µm. (B) Percent area αSMA for the isotype device and (C) percent area for the Tubb4 secondary control device

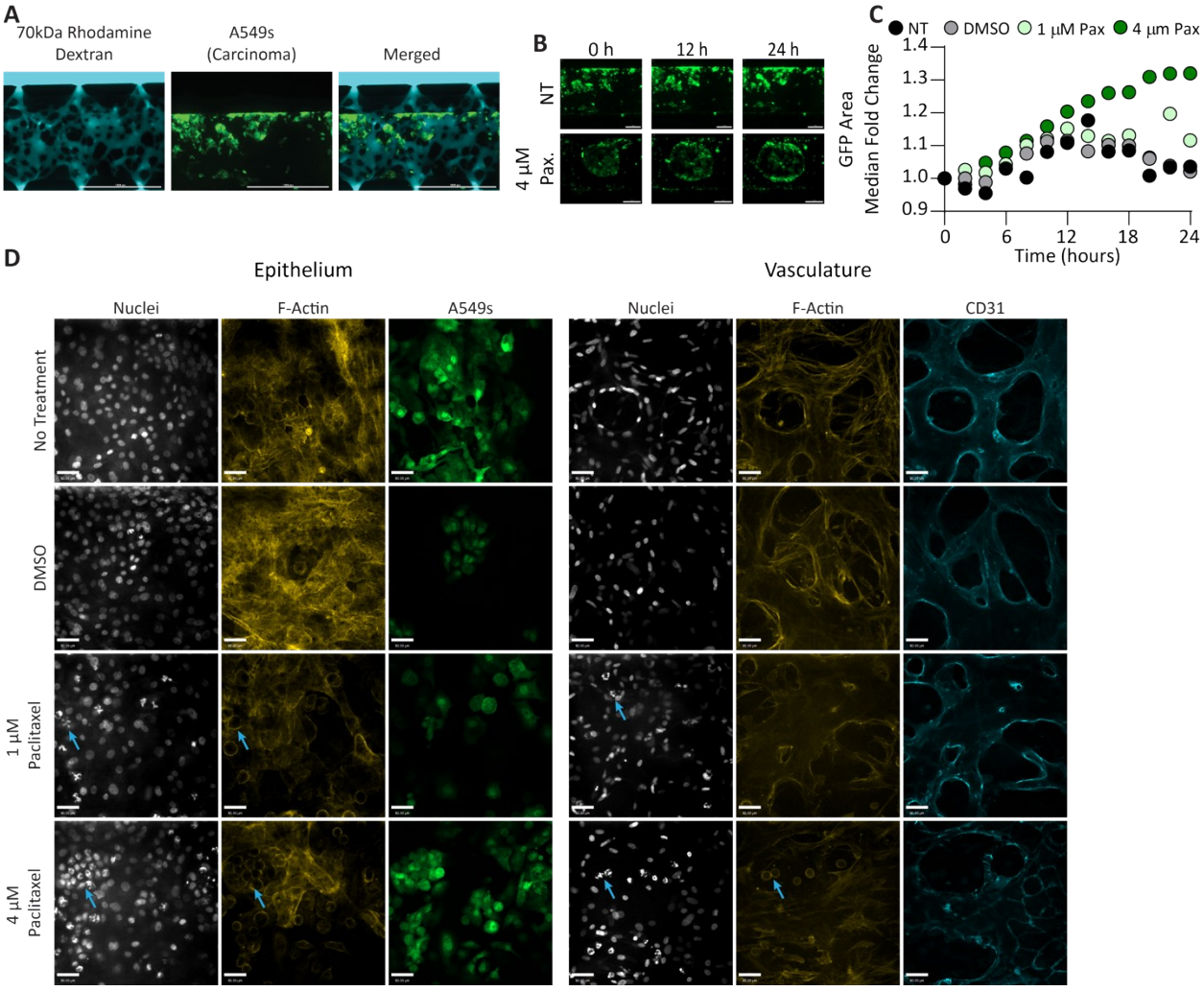

Fig. S5: A549 Cancer Model. NHLF-B and HUVECs were cultured in the central vascular channel with NHLF-A in the outer channels. Airways were seeded with 20:1 SAEC:A549-GFP. After 8 days of culture, 1 or 4 µM Paclitaxel (Pax, Cayman Chemical), a chemotherapeutic, was added to the media for 24 hours and airways were imaged using a Cytation 5 with BioSpa automated plate handler (n=4 per group); Gen5 was used to quantify 7 ROIs across each device during imaging (DMSO control matched the maximum volume of DMSO added in Pax groups). (A) Perfusable vascular networks formed below the A549 co-cultures. (B) Representative Cytation images (C) Median fold change of GFP area (n = 28) across the airways had limited changes over the 24 hours of imaging, however, Pax appeared to disrupt the A549s causing them to move resulting in a slight increase in GFP area, however there are no statistical differences. (C) Further confocal staining for nuclei, phalloidin, and CD31 shows nuclei in the airways and vasculature that appear to be dying in the Pax treatment groups (punctate nuclei and F-actin reorganization).