Supplementary Information

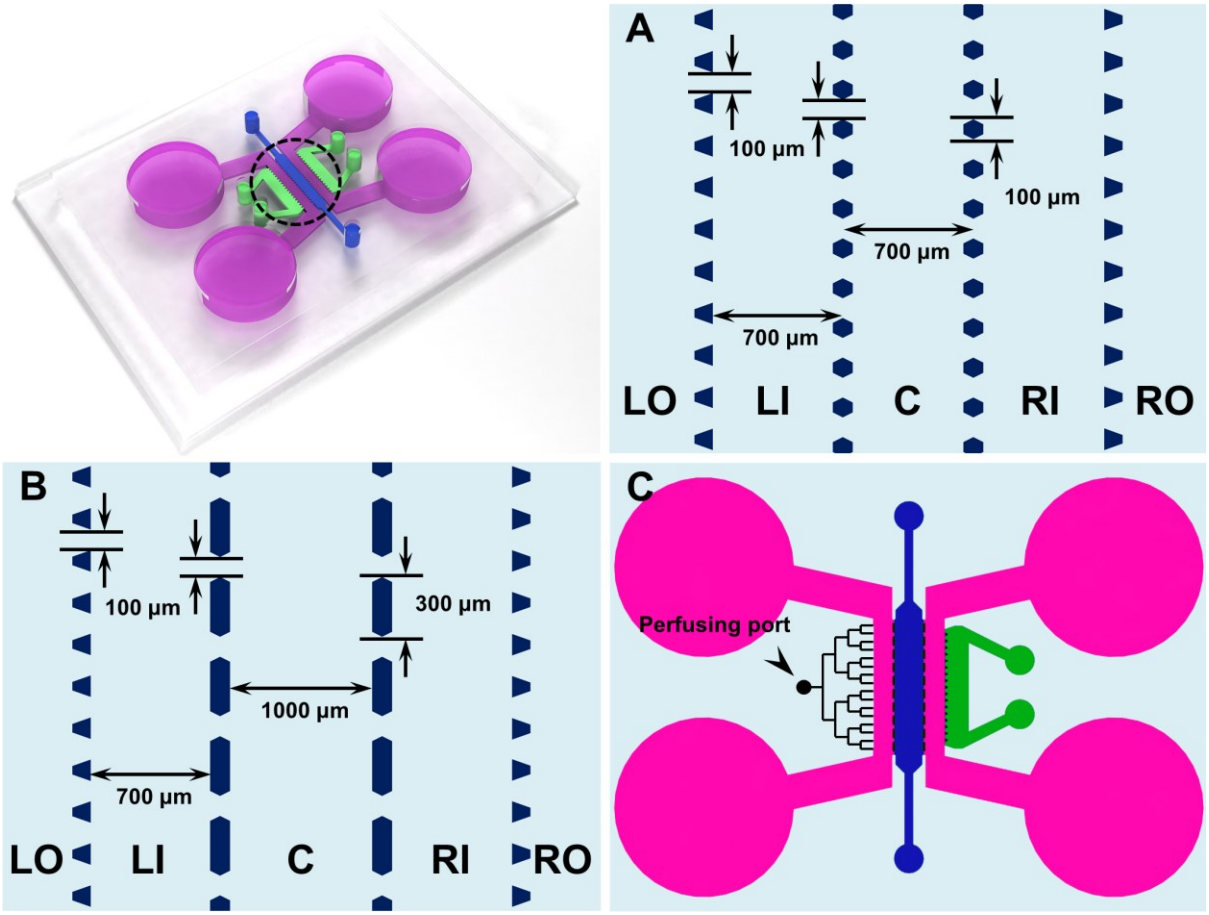

Fig. S1 Microfluidic chip channel configurations for vasculogenesis, angiogenesis and fluid perfusion experiments on a chip. **(a)** Channel configuration for vasculogenesis on a chip involves 1,000 µm wide channel, which is partitioned with 300 µm long hexagonal microposts spaced every 100 µm. The chips were fabricated to have 250 µm high structures to provide sufficient cell population at a given HUVEC density (2 or 3 million/ml), resulting in a rapid and optimized vascular network formation. **(b)** For angiogenic sprout formation, narrower central channels, having widths of 700 µm, were fabricated. Hexagonal microposts with 100 µm diameter were placed to have 100 µm interpost gaps between them. We fabricated a master with structures of 100 µm height. **(c)** To perform fluid perfusion experiment,

we fabricated a chip which has a flow inlet. Using green and blue channel denoted in the schematic diagram, we established perfused vessel network with the same scheme for angiogenesis experiment in this paper. A small tube, which is connected to the syringe pump, was plugged into the device to introduce continuous media through the vessel lumens.

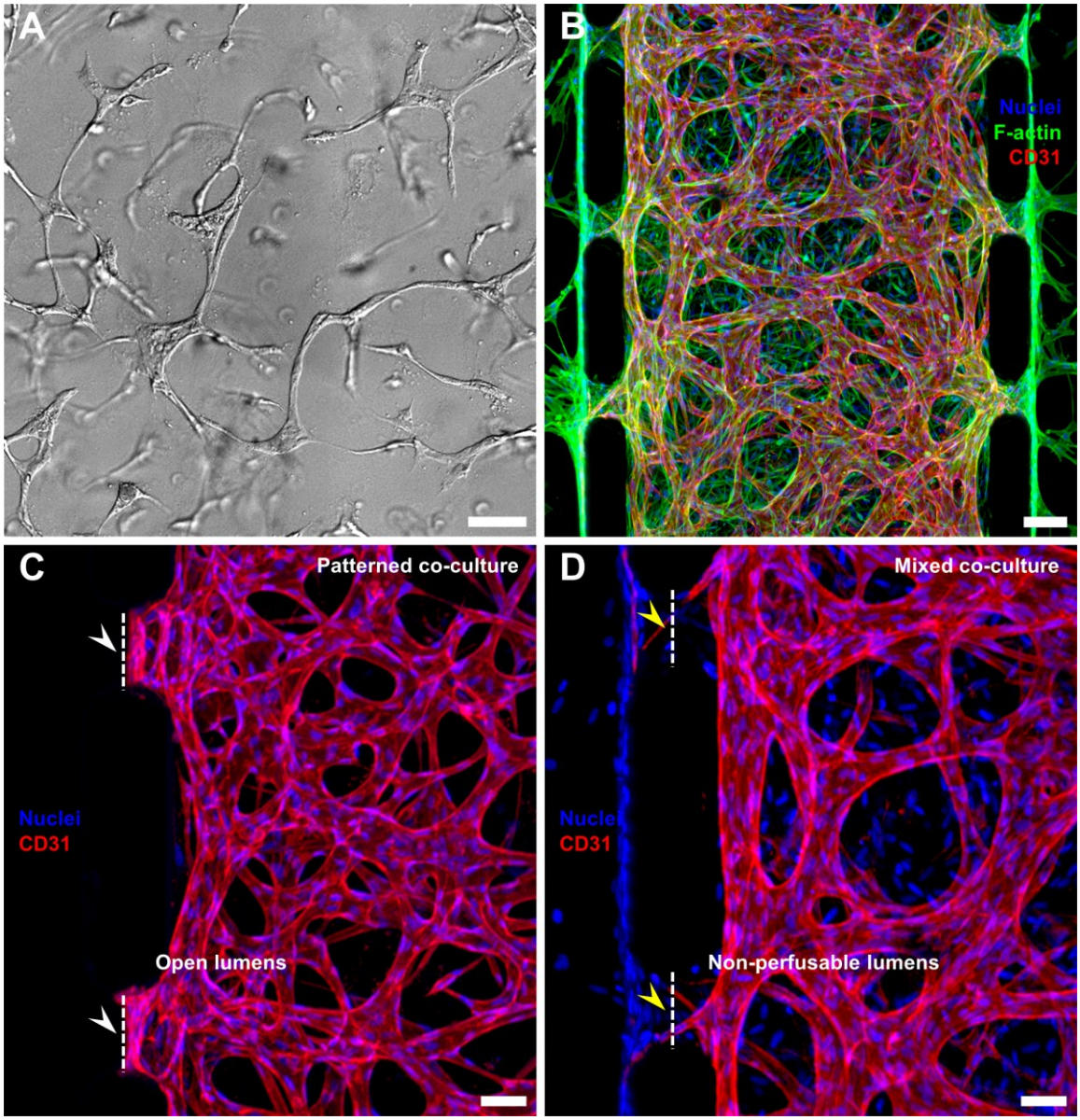

Fig. S2 Requirement for patterned co-culture of HUVECs and LFs in vasculogenesis on a chip. **(a)** Without LFs at the stromal cell culture channels(LO and RO), 4 days of monoculture of HUVECs embedded in a fibrin matrix failed to develop normal vascular networks as in the HUVEC-LF co-culture condition, exhibiting less tubule densities and interconnections, and lack of lumenized vasculature. **(b)** HUVECs form interconnected vascular networks by 5 days in the mixed co-culture with LFs in a ratio of 5 to 1 (3 million/ml of HUVECs and 0.6 million/ml of LFs). **(c)** In a patterned co-culture condition at day 4, tubules near the interpost openings developed

perfusable openings (white arrowheads) at the fibrin matrix/media interfaces (denoted as dashed line). **(d)** Tubules observed in a mixed co-culture condition, grown for 5 days, rarely extended toward the interfaces, and displayed closed, non-perfusable lumens (yellow arrowheads) within fibrin matrix. Scale bars, 100 μm.

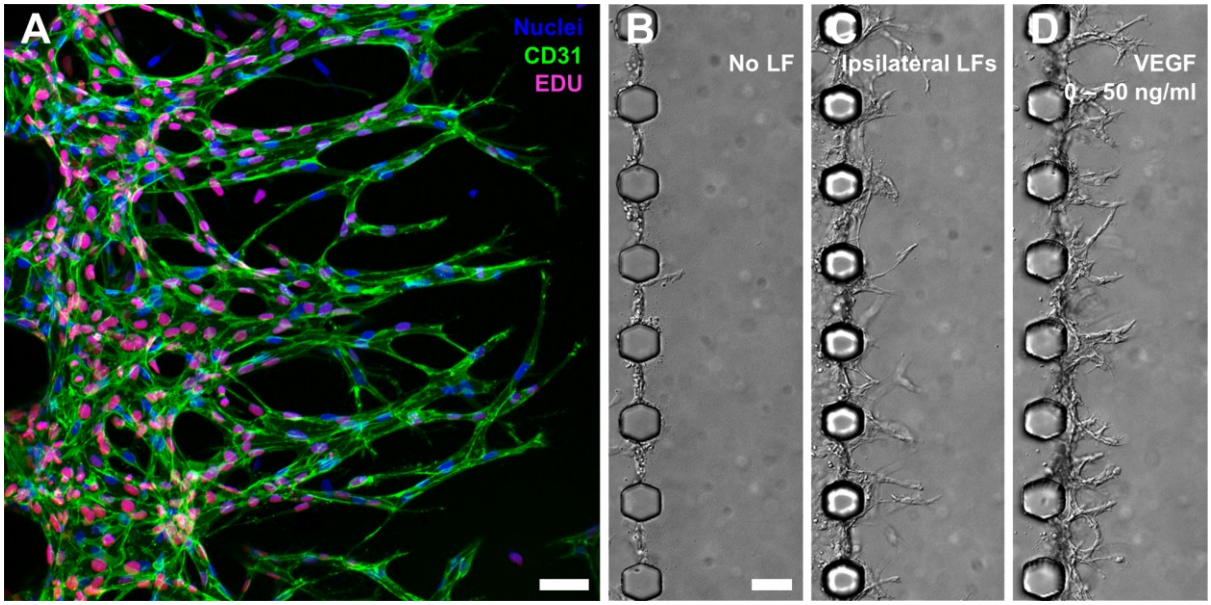

Fig. S3 Requirement for patterned co-culture of HUVECS and LFs in angiogenesis on a chip. **(a)** EDU proliferation assay performed for growing angiogenic sprouts revealed that most endothelial proliferation (purple nuclei) occurred at the stalk regions of sprouts, and tip cells at the fronts were characterized by non-proliferating phenotype (blue nuclei). Scale bar, 50 μm. **(b,c)** HUVECs cultured without LFs or with ipsilateral LF-seeding exhibited little or no sprout formation after 3 days of culture. Scale bar, 100 μm. **(d)** Angiogenic sprouts, grown for 3 days induced by VEGF gradient (0 – 50 ng/ml) exhibited relatively inefficient length extension and lumenization compared to the LF-induced sprouts.

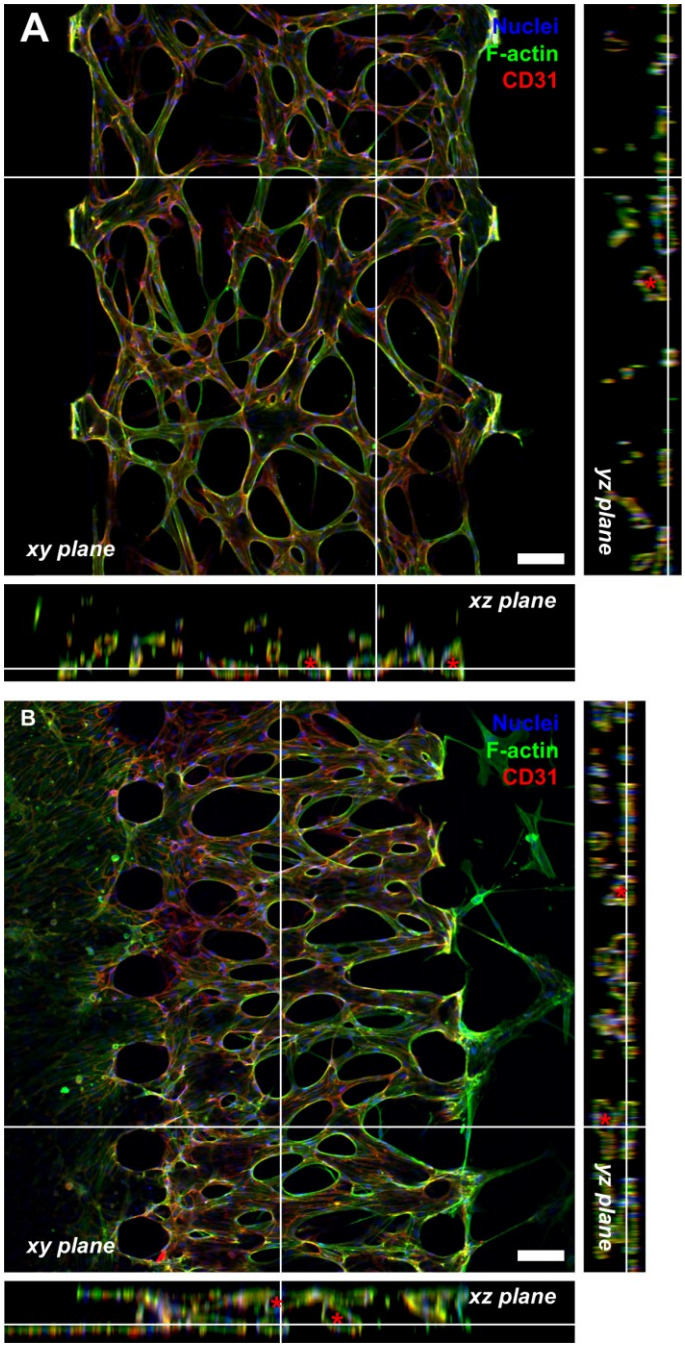

Fig. S4 Patency of 3D hollow lumens comprising microvascular networks. Confocal sections of the vasculatures formed via vasculogenesis (a) and angiogenesis (b) exhibited presence of hollow lumens (as indicated with asterisks), forming well-interconnected networks. Scale bars, 100 μm.

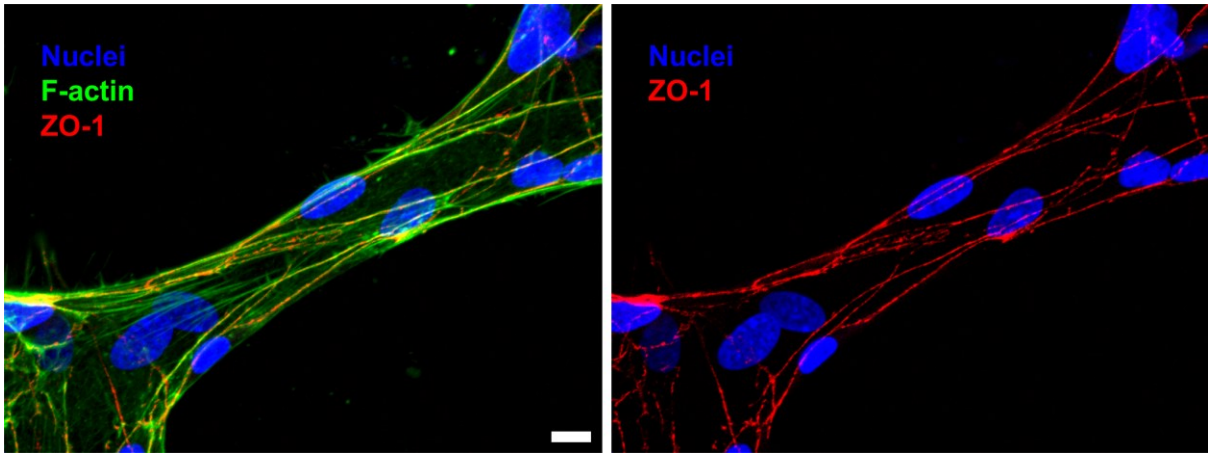

Fig. S5 Expression of tight junction protein ZO-1 at cell-cell contacts. Confocal micrograph of a blood vessel immunostained against ZO-1 (red) displayed localization of continuous and intact intercellular connections. Scale bar, 20 μm.

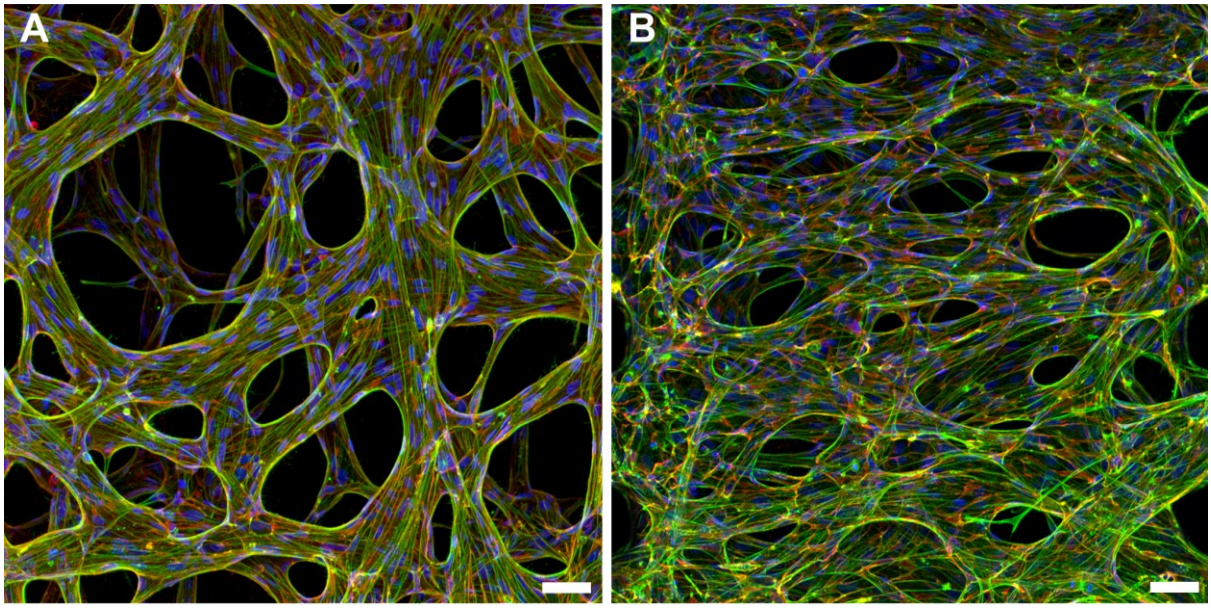

Fig. S6 Long-term stability of patent and perfusable lumens. Microvascular networks formed both by vasculogenesis **(a)** and angiogenesis **(b)** maintained perfusable and patent lumen for more than 7 days after perfusion had established, without discernible vessel regression or endothelial apoptosis. Scale bars, 50 μm.

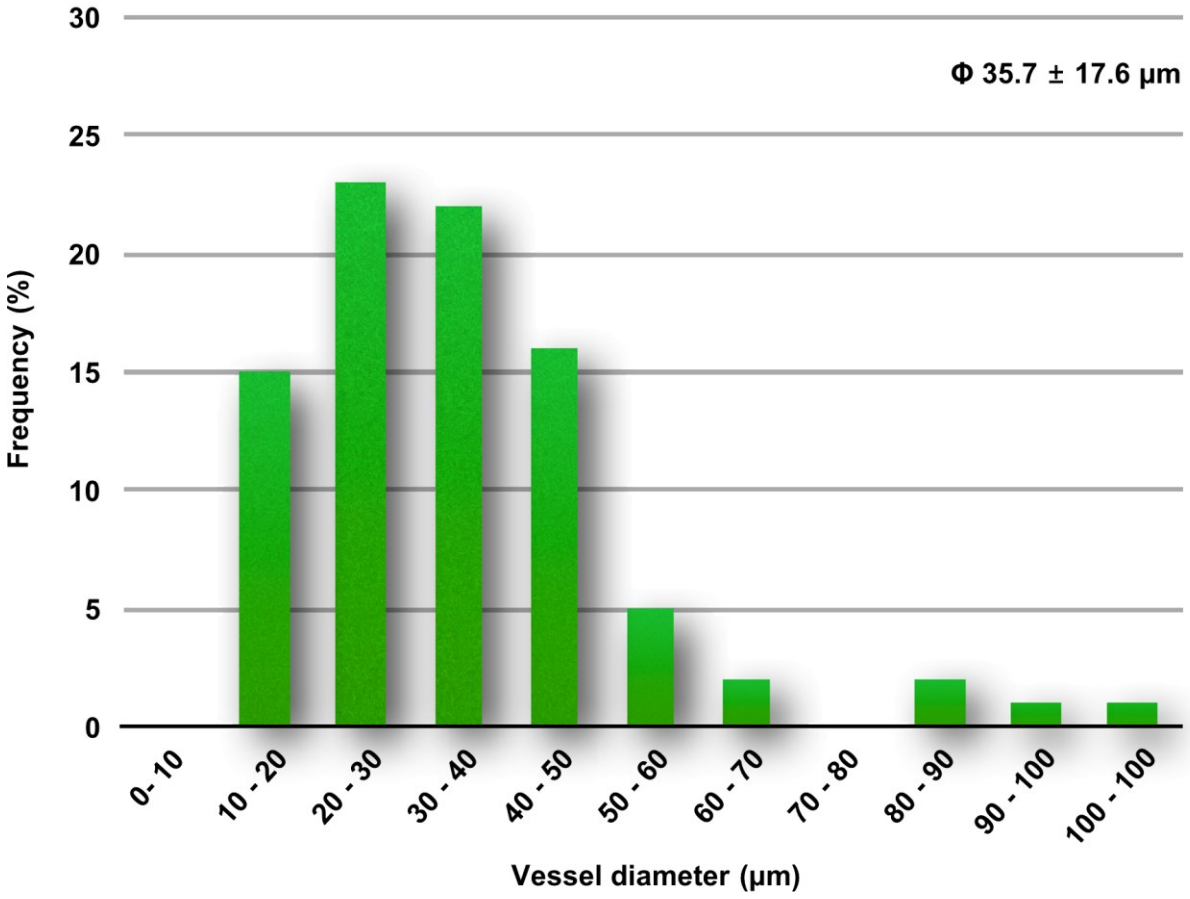

Fig. S7 Distribution of vessel diameter constituting perfusable microvascular network formed via angiogenic process.

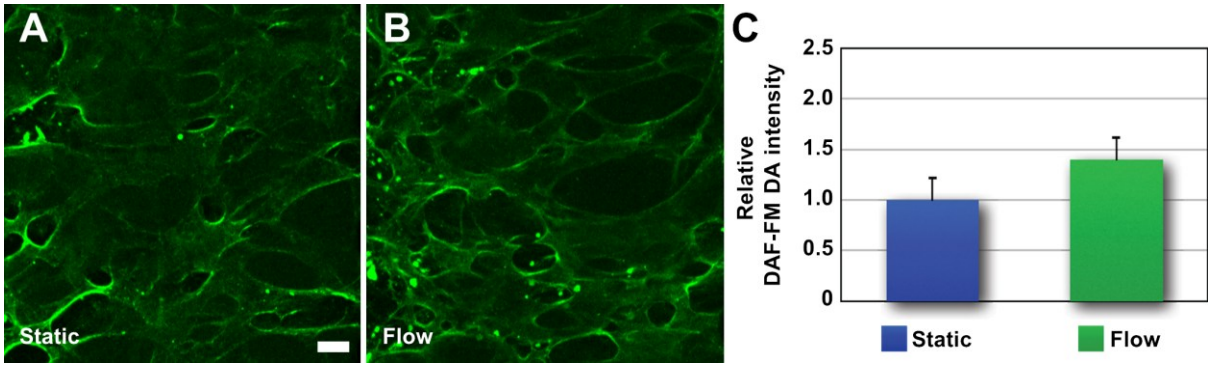

Fig. S8 Requirement for L-arginine supplement in perfusate in flow-mediated activation of endothelial nitric oxide synthesis. Without L-arginine supplement in perfusate, NO synthesis quantitatively assessed by DAF-FM DA (green) fluorescence intensity showed only insignificant increase in response to luminal fluid flow (n = 5 for static, n = 6 for flow condition). Error bars represent SEM. Scale bar, 50 μm.

Movie S1

Growing angiogenic sprouts forming open lumens with the flanking media channel. By live-imaging the angiogenic sprout for about 20 hours (day 3 ~ 4), we could see the angiogenic sprout reaches the flanking media channel and start to form open lumen at the fibrin matrix/media interface. This lumen is connected to the media channel, establishing perfusable networks of blood vessels.

Movie S2

Microbeads flowing through the lumen of the microvascular network formed by vasculogenic process. Real-time video shows free-flowing 7 μm polystyrene beads that move through the lumen of the microvascular network, visualizing hydrostatic pressure-driven luminal flow. The beads at the fluidic channel flow into the vascular network and navigate along the intra-luminal paths, then subsequently enter into the opposite fluidic channel.

Movie S3

Microbeads flowing through the lumen of the microvascular network formed by angiogenic process. Along with the luminal flow induced by hydrostatic pressure differential, 7 μm polystyrene beads introduced from the fluidic channel move through the intra-luminal paths to enter into the opposite fluidic channel.

Movie S4

HL-60 adhesion and migration on the apical surface of TNF-α-stimulated blood vessels. Stimulation with inflammatory cytokine TNF-a, the endothelium (red) allow the adhesion of differentiated neutrophil-like HL-60 cells (green), which were

introduced along with the luminal flow, on the apical surface. The HL-60 cells arrested on the endothelial wall actively migrate with polarized morphology.