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Supplementary Materials for

A model of guided cell self-organization for rapid and spontaneous formation of functional vessels

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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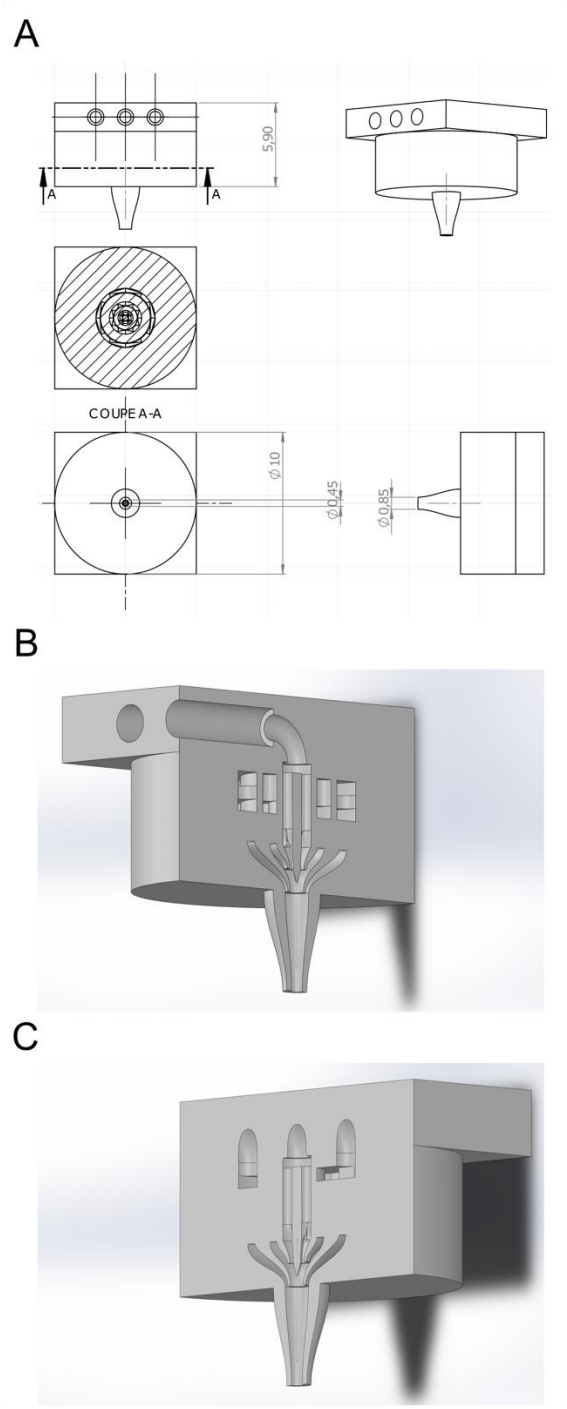

Fig. S1. Dimension and shape of the coextrusion chip. (**A**) Drawing of the chip and size (5.90×10 mm). (**B-C**) Cross-section views of the chip. The corresponding STL file is available as a separate and downloadable file as Supplementary File S1).

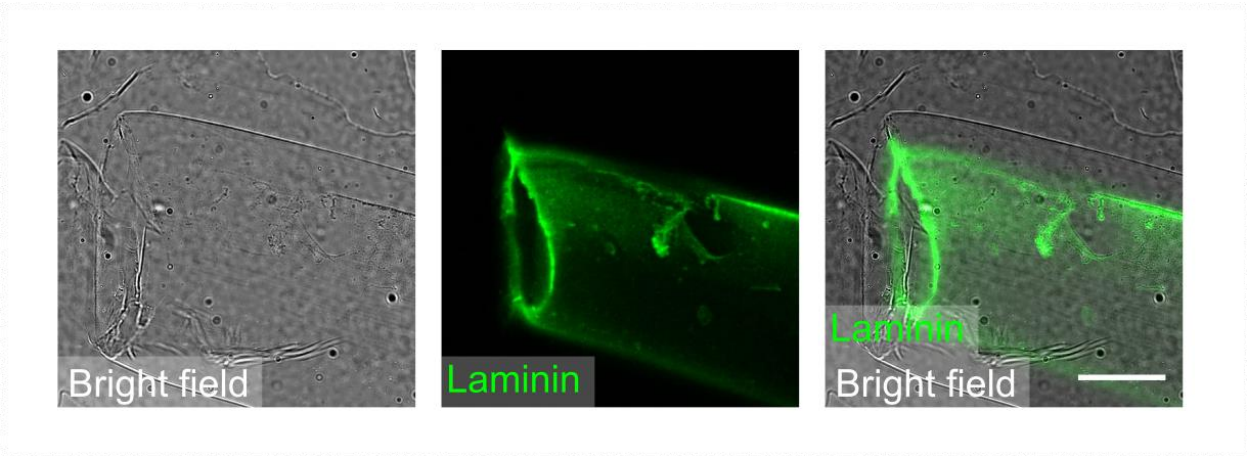

Fig. S2. Uniform laminin coating of the inner alginate wall. Immunostaining of Laminin was performed on alginate tubes internally coated with Matrigel. We used a 450 µm coextrusion device, the CCS was formulated with Matrigel and EGM2 medium in order to produce cell-free vesseloids with a uniform inner coating of Matrigel. Bar: 200 µm.

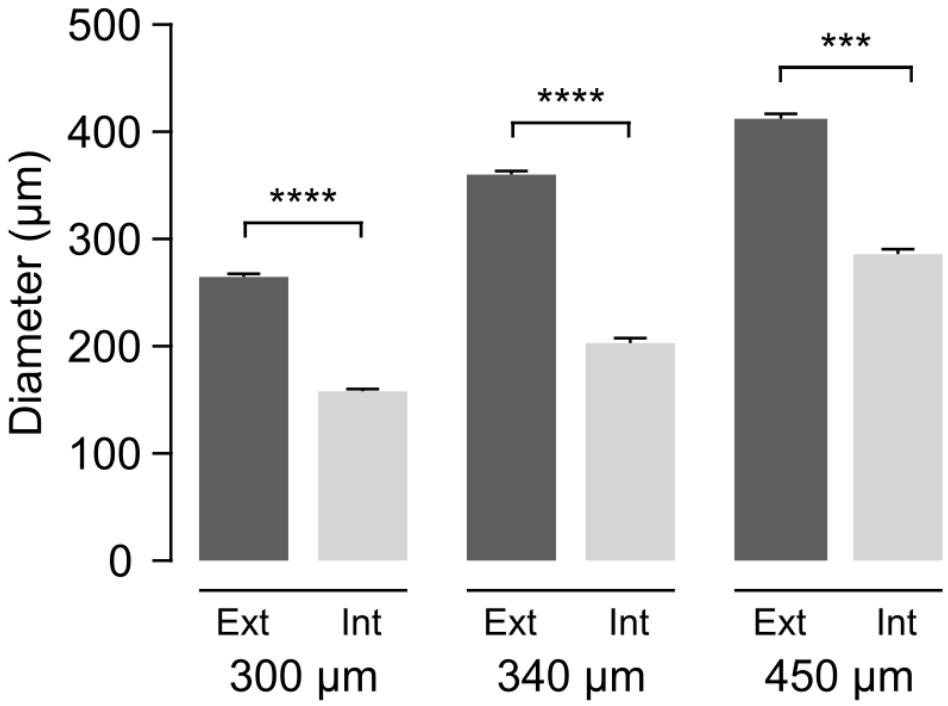

Fig. S3. Diameter tube measurement using three coextrusion devices of different nozzle sizes. Vesseloids were produced as described in the materials with three different co-extrusion devices. The sizes of the nozzle exits were 300 µm, 350 µm and 450 µm. The injection flow rates of the alginate solution (AL), the intermediate solution (IS) and cell core solution (CCS) were respectively: 2 $mL.h^{-1}$, IS 1 $mL.h^{-1}$, and 1 $mL.h^{-1}$. External diameter (with alginate walls) and internal diameter (lumen) were measured in n=12 tube sections (for 300 and 350 µm devices) or n=16 tube sections (for 450 µm device).

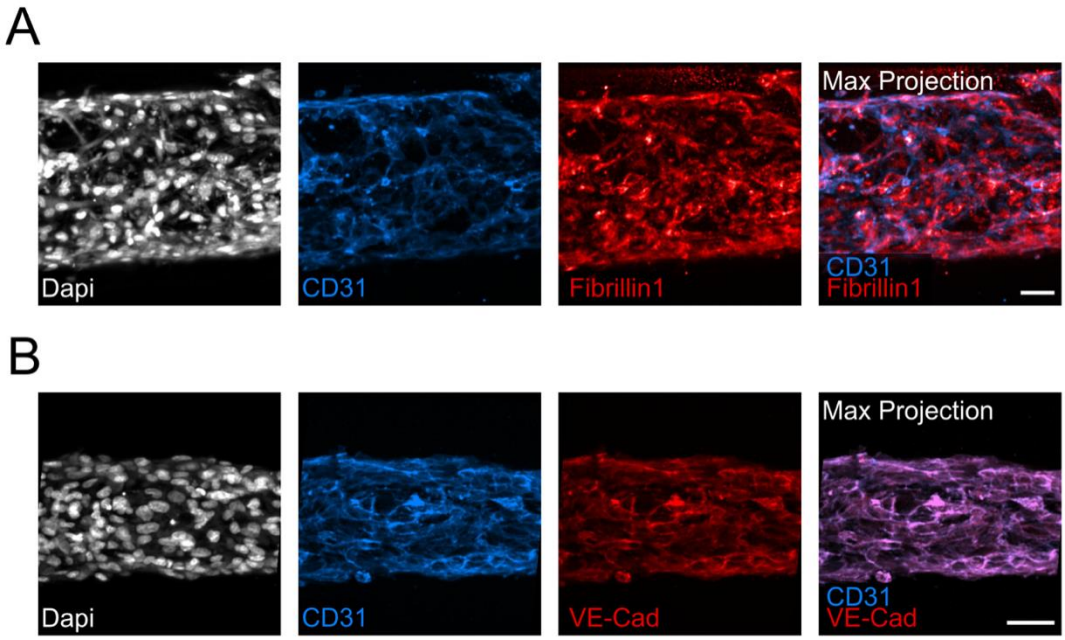

Fig. S4. Immunostainings of CD31, fibrillin-1, and VE-cadherin were performed on vesseloids at day 1. Nuclei were stained with Dapi. Bars: 50 µm

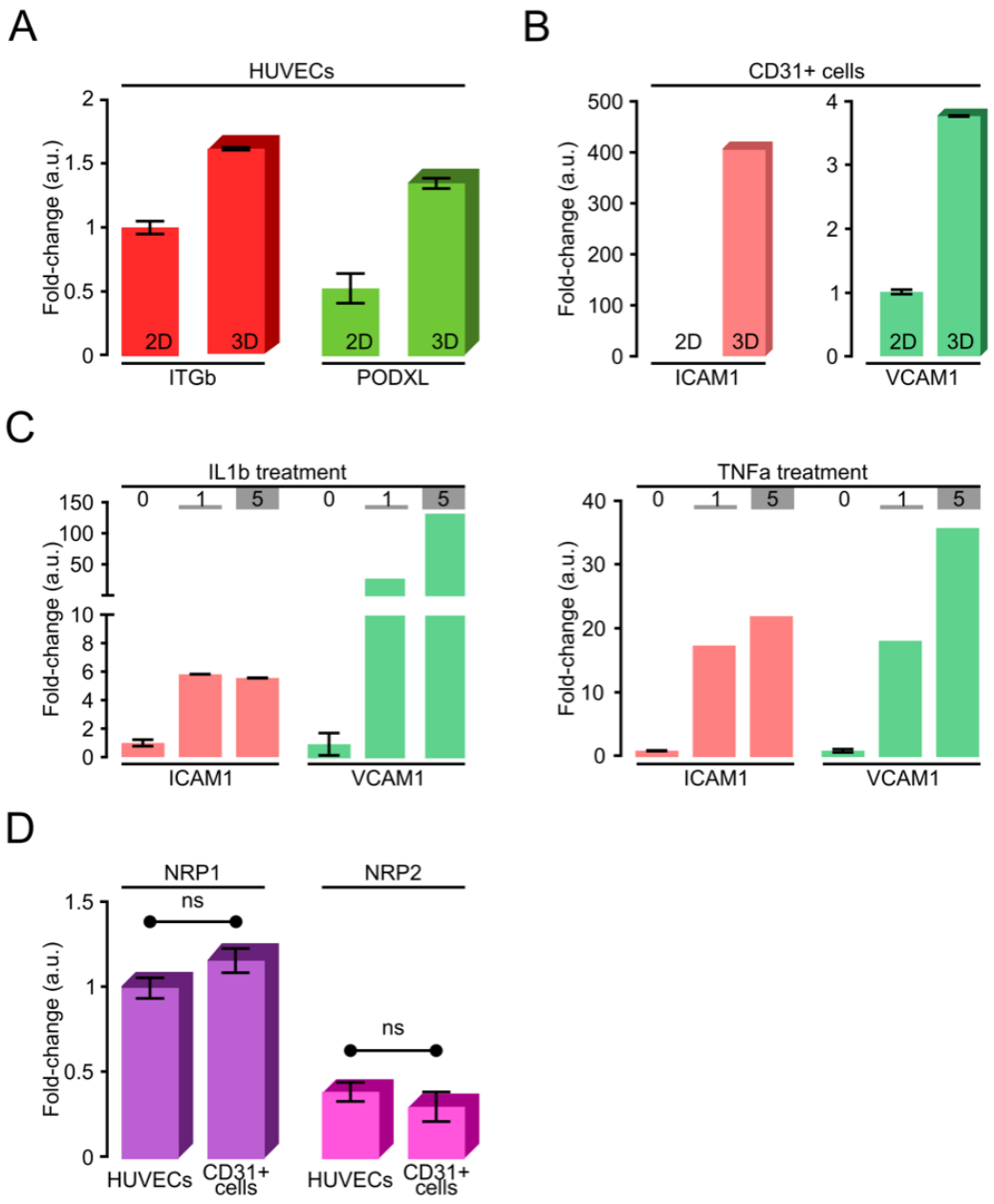

Fig. S5. Expression measurement of (i) basal and luminal markers, (ii) arterial and venous markers, and (iii) endothelial activation markers in regular culture conditions and in response to inflammatory stimuli. (A) ECs were collected from HUVECs only (in 2D mono-culture on Matrigel coating versus 3D mono-culture in vesseloids) before ITGβ/PODXL RT-qPCR analysis. **(B)** ECs were collected from CD31+ cells after CD31 magnetic bead cell-sorting (HUVECs in co-culture with vSMCs on Matrigel-coated 2D culture versus HUVEC in co-culture with vSMCs in vesseloids) before ICAM-1/VCAM-1 RT-qPCR analysis. **(C)** Inflammation was induced in vesseloids at day one, by TNFα or Il1β 12 hours treatment in a dose-dependent manner (0/1/5 ng.ml^{-1}) before ICAM-1 and VCAM-1 RT-qPCR analysis. **(D)** Expression of arterial (NRP1) and venous markers (NRP2) was analyzed between HUVEC cells in 3D with (CD31+) or without vSMC cells (HUVECs).

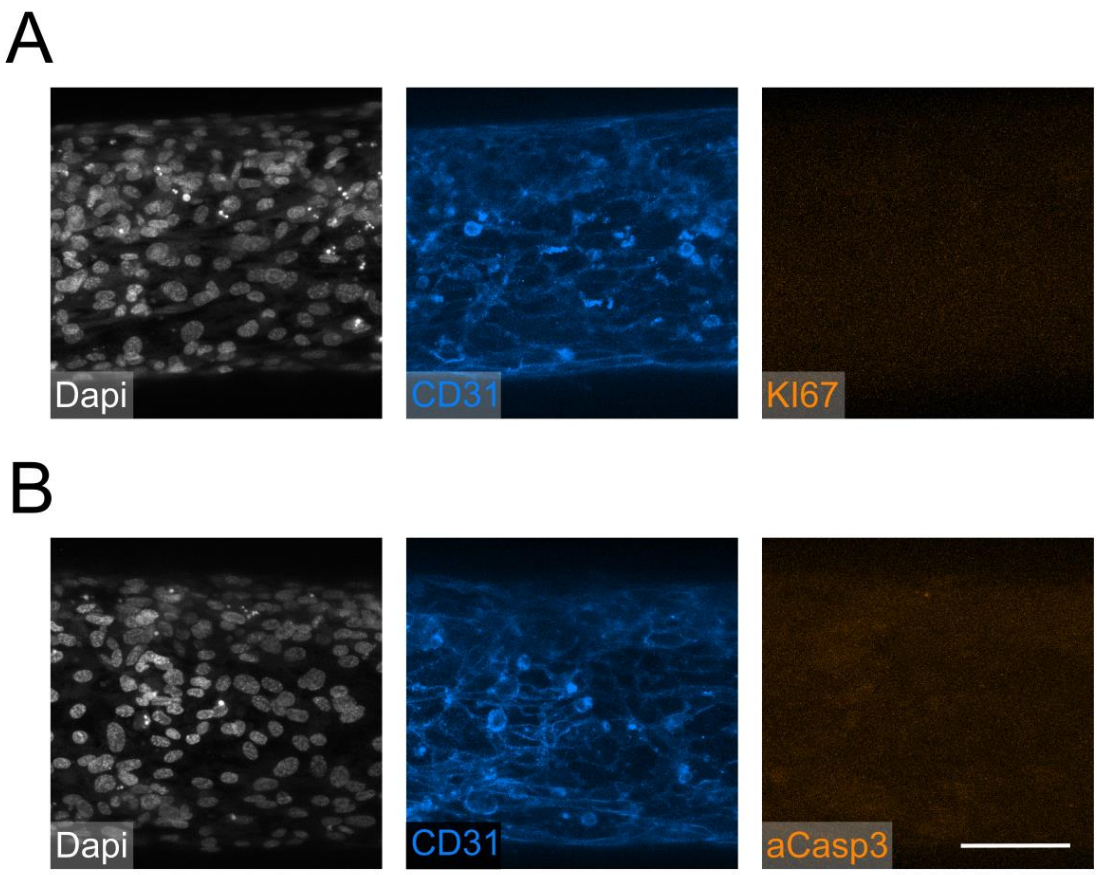

Fig. S6. Quiescent cells at day 1 by loading the maximum of cells in the vesseloids. Vesseloids were produced as described in the method section with a 450 µm nozzle exit co-extrusion device by heavily loading the cell suspension (38 % of cells in Matrigel). The injection flow rates of the alginate solution (AL), the intermediate solution (IS) and cell core solution (CCS) were respectively: 2 $mL.h^{-1}$, 1 $mL.h^{-1}$, and 1 $mL.h^{-1}$. Confocal fluorescent images of KI67 nuclear signal and activated-Caspase3 signal at day 1. At this high cell concentration in the tube during formation, quiescent cells are already found at day one (no proliferation, no apoptosis). Bar: 100 µm.

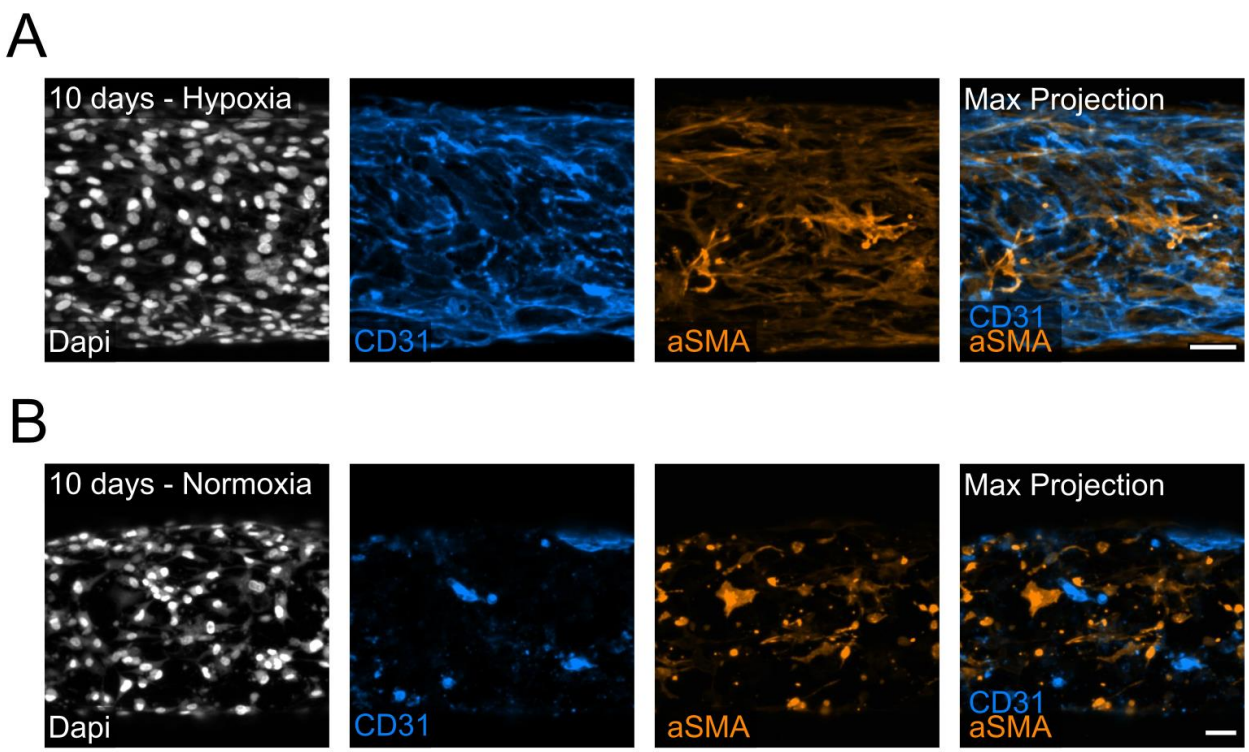

Fig. S7. Hypoxia culture stabilizes the vesseloids. Vesseloids were produced as described in the method section with a 450 µm nozzle exit co-extrusion device by heavily loading the cell suspension (38 % of cells in Matrigel). The injection flow rates of the alginate solution (AL), the intermediate solution (IS) and cell core solution (CCS) were respectively: 2 $mL.h^{-1}$, 1 $mL.h^{-1}$, and 1 $mL.h^{-1}$. Confocal fluorescent images of CD31 and aSMA were performed after 10 days of culture in normoxia conditions (37°C, 5% CO2, 20% O2) or hypoxia conditions (37°C, 5% CO2, 0.1% O2). Bar: 50 µm.

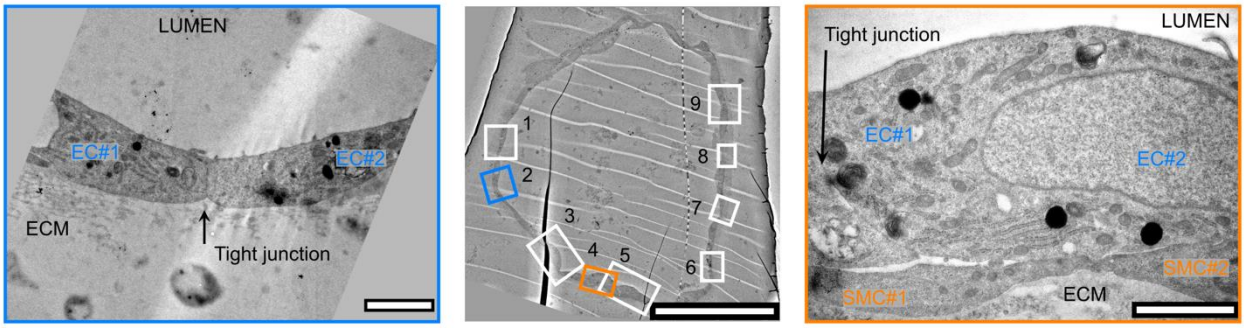

Fig. S8. Vesseloid imaged by TEM at high magnification (×25,000 and ×50,000) after 1 day of culture. At high magnification (x25000 and x50000) after one day of culture. The whole section is shown in the center and close-ups depict an area with only ECs (left) or with both ECs & SMCs (right). ECM: extracellular matrix. Bars: 50 µm (middle) / 2 µm (left & right).

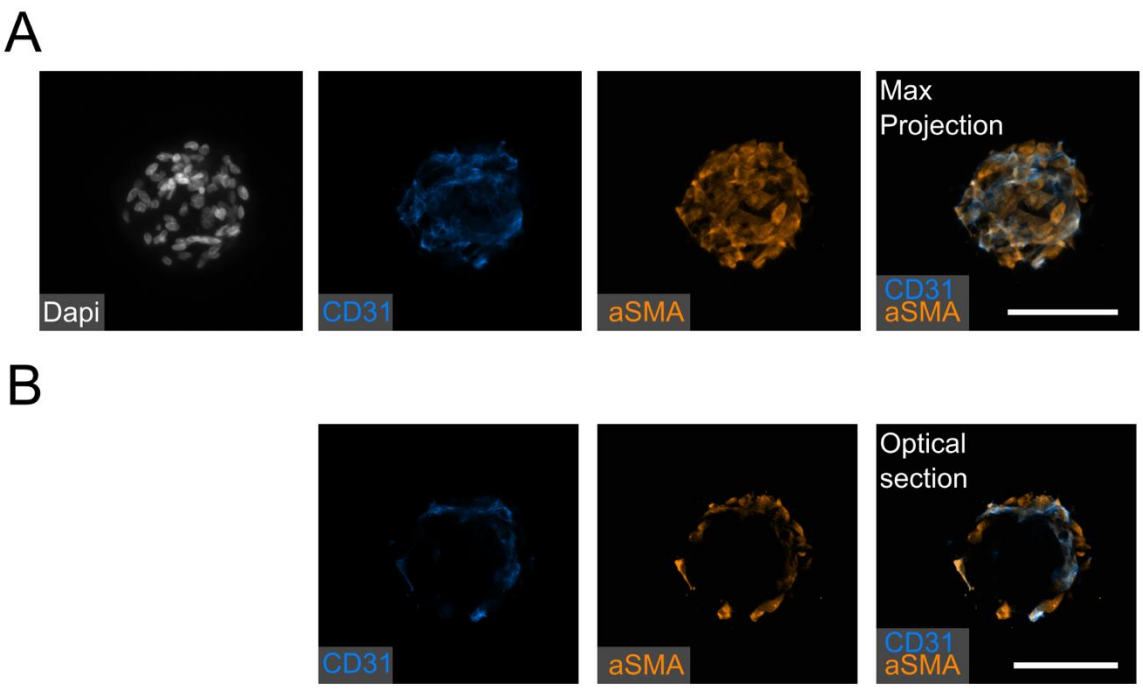

Fig. S9. Lumenization with EC lining and formation of an external SMC layer in spherical capsules. Spherical capsules containing ECs and SMC and Matrigel were produced in the same condition than vesseloids, but with a specific capsule-microfluidic chip (*16*). Confocal imaging of vesseloids immunostainings was performed at day 1. Nuclei are grey (Dapi), CD31 is blue, and αSMA labelling is orange. Images correspond to a maximal intensity projection along the z axis or optical sections in order to visualize the cell self-organisation inside the capsule. Bar: 200 µm.

Data file S1. STL file of the coextrusion chip.

Movie S1. ECs and SMCs are dynamic after encapsulation. HUVECs (transduced in blue, nuclear eBFP2) and SMCs (transduced in red, nuclear Tomato), were imaged during 26 hours from 2 HPE to 28 HPE. HPE: hour post-encapsulation.

Movie S2. The 20-kDa FITC-dextran perfusion. Vesseloid was produced with HUVEC and SMC and perfused at day one using a 20 kDa FITC-Dextran.

Movie S3. ET-1 induces vesseloid contraction. Vesseloid diameter was measured before and after ET-1 addition.

Movie S4. ET-1 induces intracellular calcium raise in both ECs and SMCs. Fluo4 measurement was performed during 10 min. ET-1 was added in the medium at t=1 min.