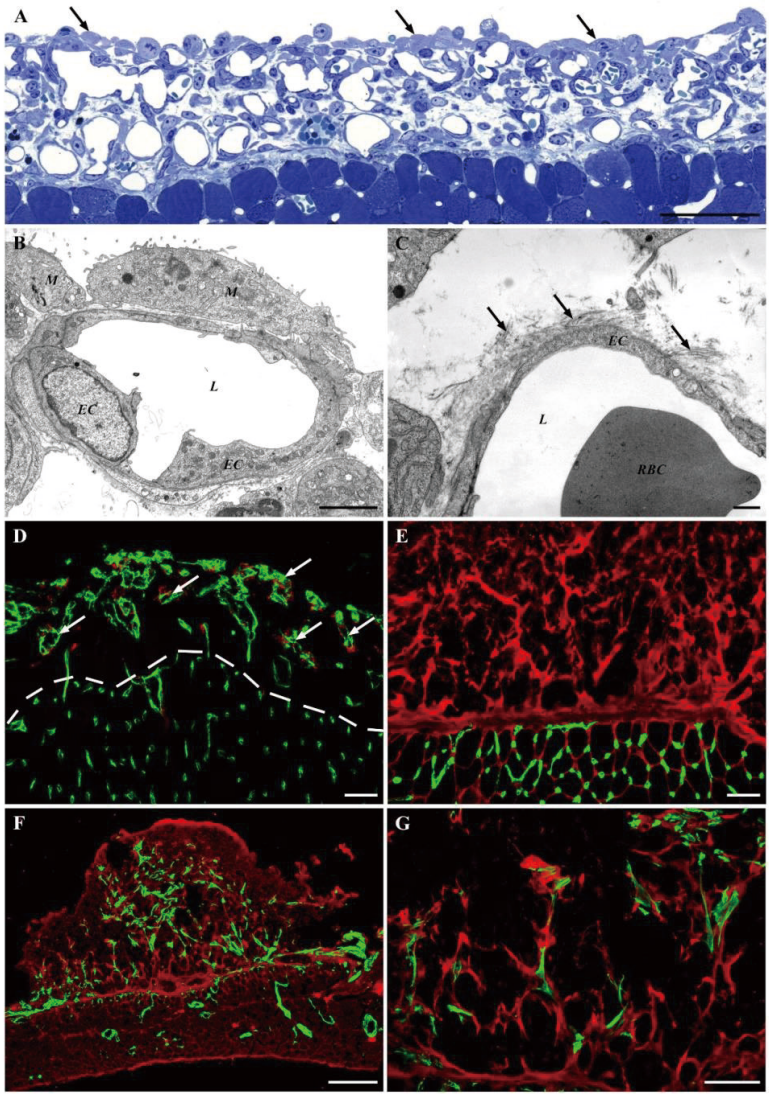

Figure S1 Processes of capillary plexus formation and extracellular matrix deposition in SPC111 tumor nodules. (A) Semi-thin cross section of a capillary plexus 35 days after SPC111 tumor cell injection. Tumor tissue is not present in this area. Vessels of the capillary plexuses elevated above the surface of the diaphragm. Arrows point at the mesothelial cover. Owing to perfusion fixation, the vessels are not collapsed and most of them do not contain erythrocytes. Scale bar: 50 µm. (B) Electron micrograph shows that the vessels are covered by mesothelial cells. Capillary lumen (L), endothelial cell (EC), mesothelial cell (M). Scale bar: 5 µm. (C) Collagen fibers (arrows) are visible around the elevated microvessel as a sign of matrix maturation. Endothelial cell (EC), capillary lumen (L), red blood cell (RBC). Scale bar: 1 µm. (D) Vascular proliferation (35 days after SPC111 tumor cell injection) elevated above the original surface (dashed line) of the diaphragm. Tumor tissue is not present in this area. The frozen section is stained for CD31 (green) and smooth muscle actin (SMA - red). Vessels of the diaphragm are negative for SMA. In contrast, the upper part shows the vessels of the capillary plexuses which are surrounded by SMA positive pericytes (arrows). Scale bar: 50 µm. (E) Avascular SPC111 tumor sample on day 21 stained for CD31 (green) and fibronectin (red). The extracellular matrix of the tumor contains fibronectin. At the lower part of the micrograph, the regularly arranged muscle fibers (appear black) are covered by fibronectin. Scattered capillaries are visible among the muscle fibers. Scale bar: 50 µm. (F) Late-stage (35 days) SPC111 sample stained for SMA (green) and collagen type I (red). The nodule contains collagen type I and SMA-positive myofibroblasts. In the diaphragm, only larger vessels are positive for SMA. Scale bar: 200 µm. (G) Late-stage (35 days) SPC111 sample stained for SMA (green) and collagen type I (red). The high power micrograph shows that the SMA-positive myofibroblasts are embedded in collagen type I containing matrix. Scale bar: 50 µm.

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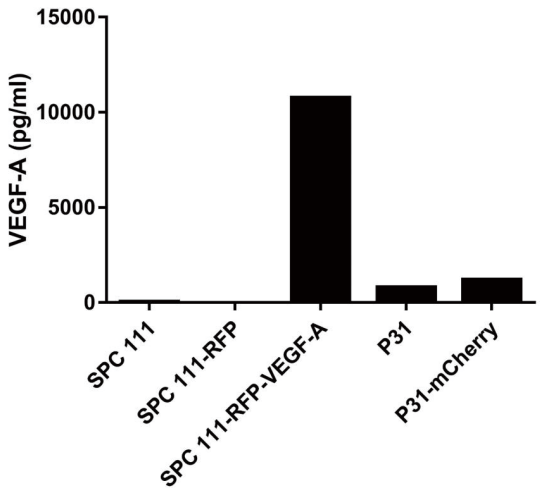

Figure S2 VEGF-A protein levels of the P31, SPC111 and transfected cell lines as measured by ELISA. Vascular endothelial growth factor A (VEGF-A) ELISA shows that compared with the control SPC111-RFP cells, SPC111-RFP-VEGF-A cells secreted 5.5×10^3-fold the amount of the key angiogenic factor, VEGF-A.

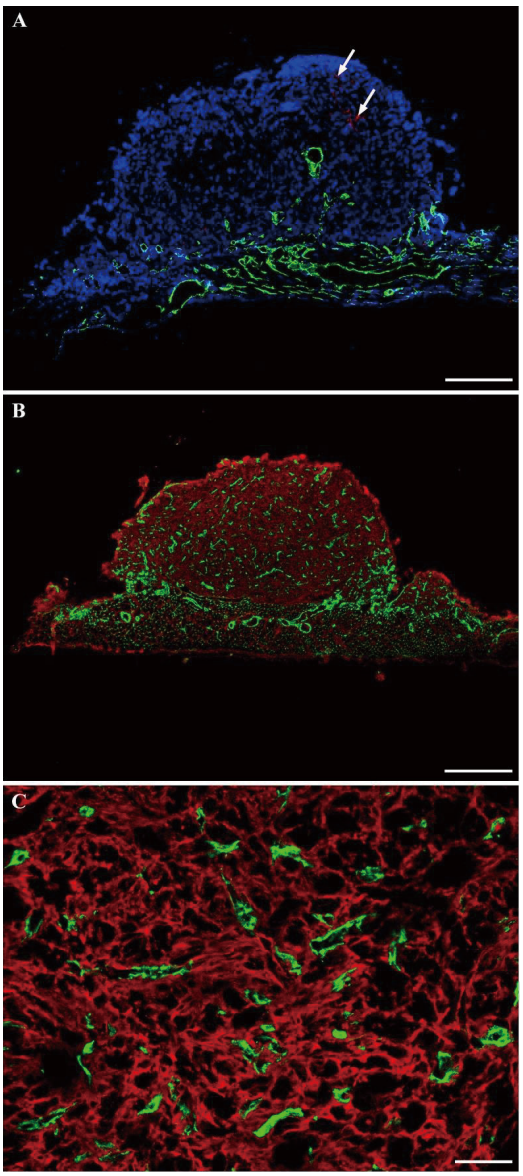

Figure S3 Extracellular matrix components of human origin are present is P31, while absent in SPC111 tumor nodules. (A) Late-stage SPC111 tumor nodule (35 days) stained for CD31 (green), human-specific collagen type I (red) and TOTO-3 (blue). The tumor is largely negative for human-specific collagen type I (red). Only a small amount of deposited human collagen type I is present (arrows). Scale bar: 200 μm. (B) P31 tumor nodule on day 42 is stained for CD31 (green) and fibronectin (red). The nodule is well-vascularized and contains large amount of fibronectin. Scale bar: 500 μm. (C) P31 tumor nodule on day 42 is stained for CD31 (green) and fibronectin (red). Higher magnification shows that large amount of fibronectin (red) is deposited evenly in the tumor and vessels are regularly arranged within the matrix. Scale bar: 50 μm.

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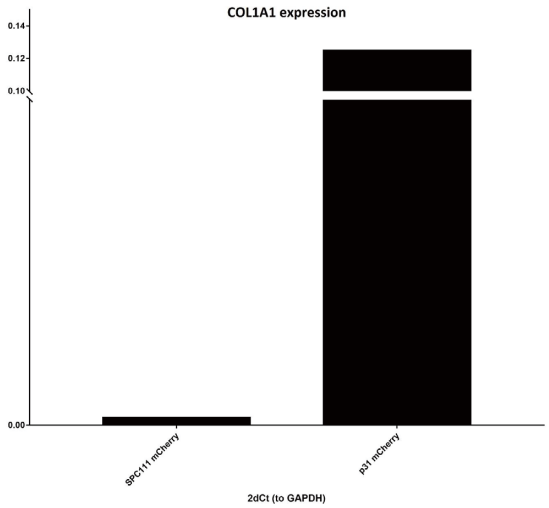

Figure S4 Comparison of COL1A1 transcript levels in P31 and SPC111 mCherry cell lines. Real-time PCR shows that the relative expression levels of COL1A1 is significantly higher in the P31 cell line.

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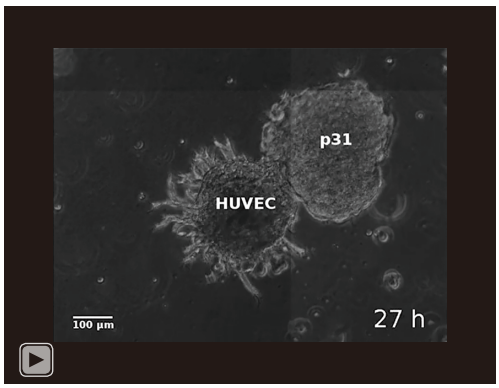
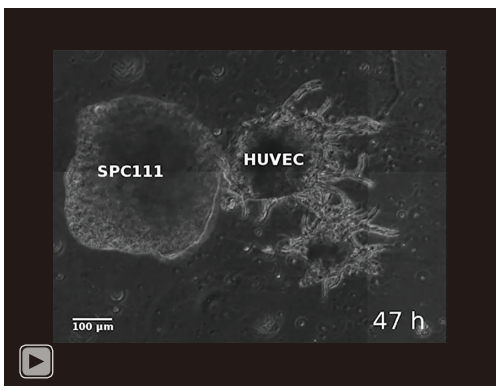
Supplementary videos

Keywords for videos: Time-lapse microscopy, live imaging, in vitro, MPM spheroid, endothelial sprouting



Video S1 Time-lapse video showing 4 days of sprout formation by HUVEC aggregate in fibrin extracellular matrix (ECM) gel in the presence of a P31 spheroid. Images were captured every hour by phase-contrast microscopy. Scale bar: 100 µm.



Video S2 Time-lapse video showing 4 days of sprout formation by HUVEC aggregate in fibrin ECM gel in the presence of a SPC111 spheroid. Images were captured every hour by phase-contrast microscopy. Scale bar: 100 µm.

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