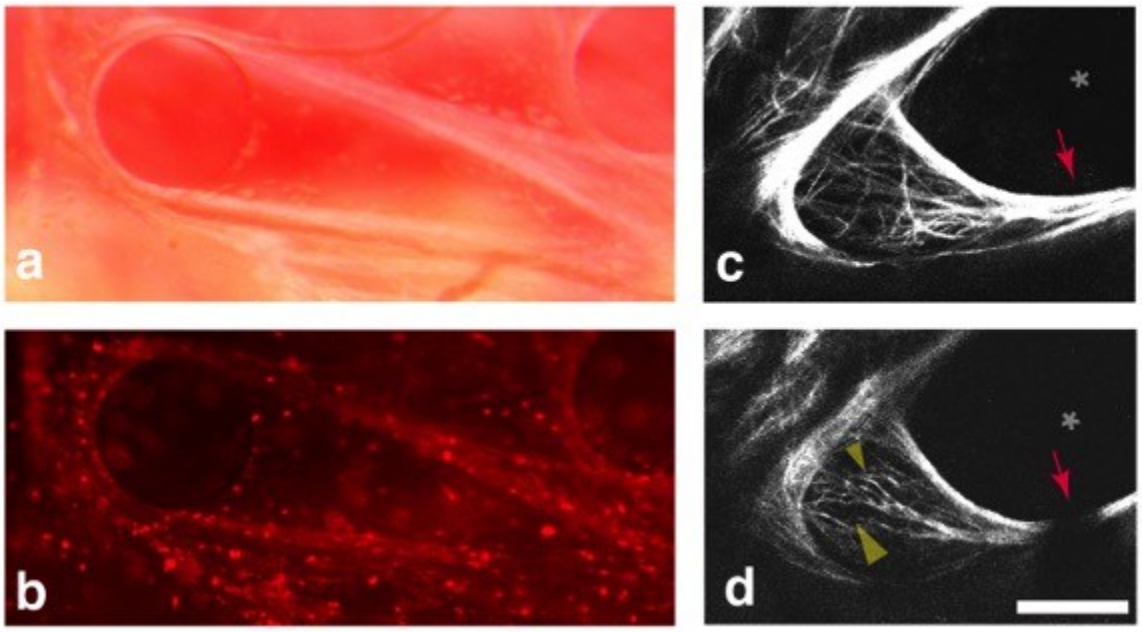
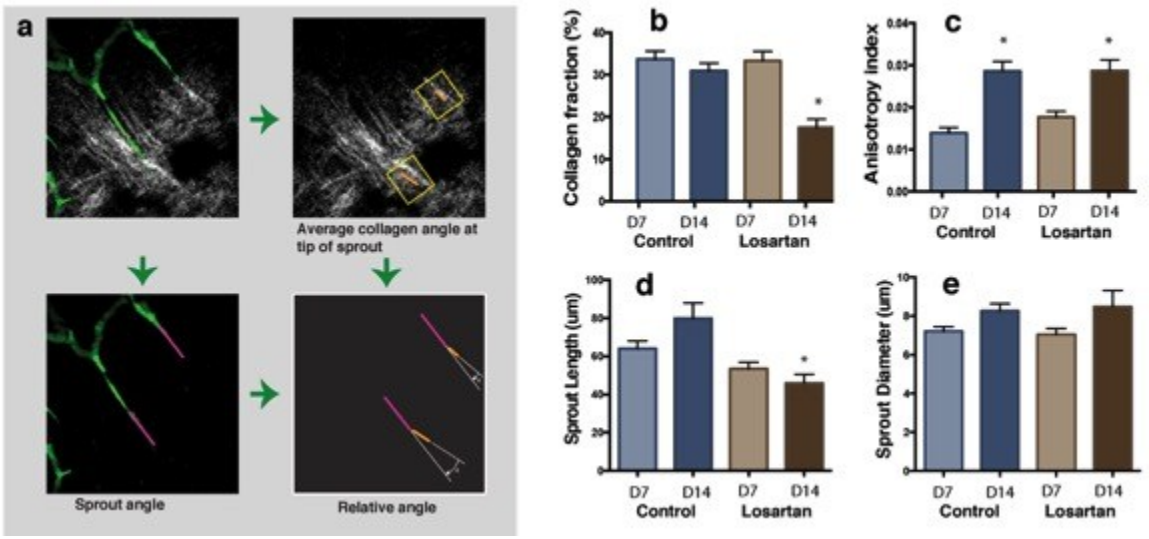
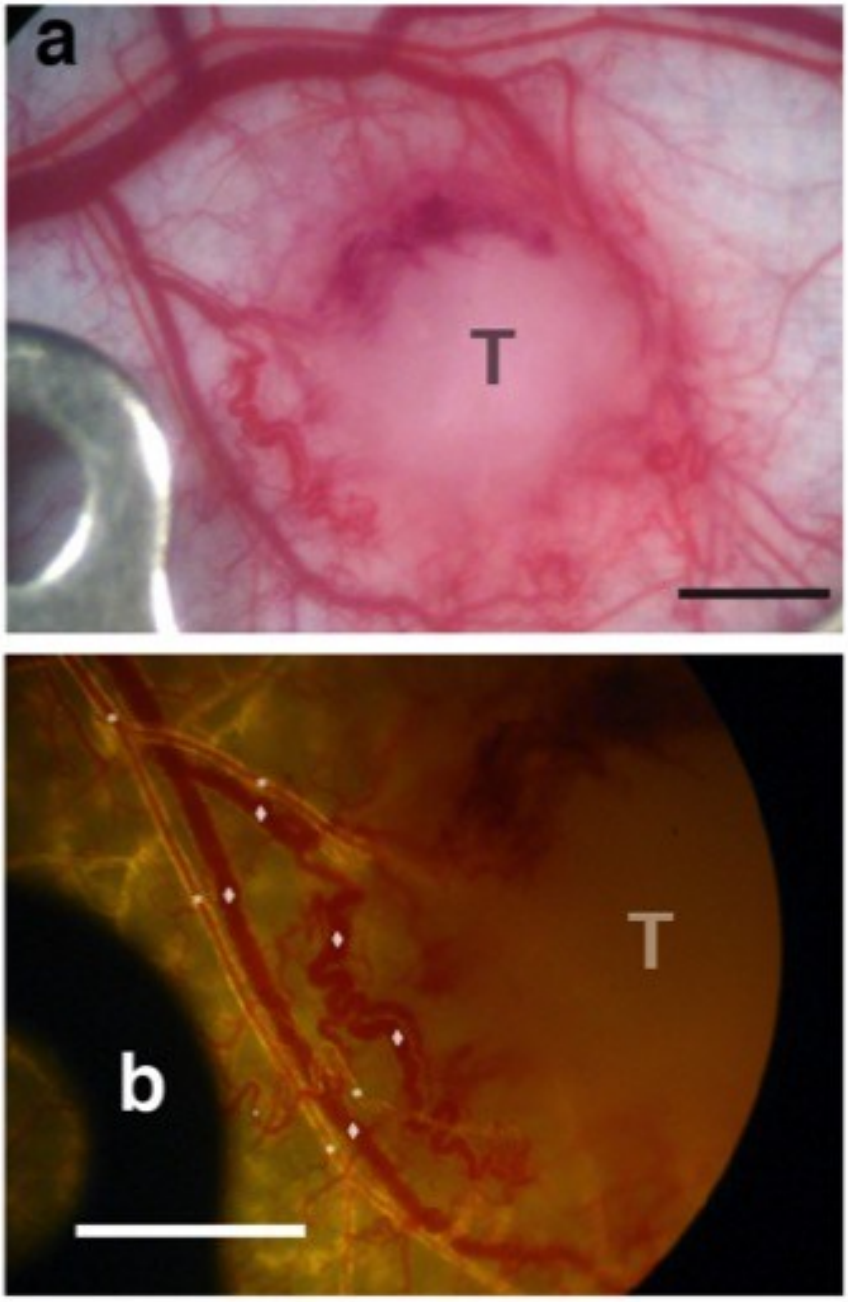
Electronic Supplementary Material (ESI) for Lab on a Chip.



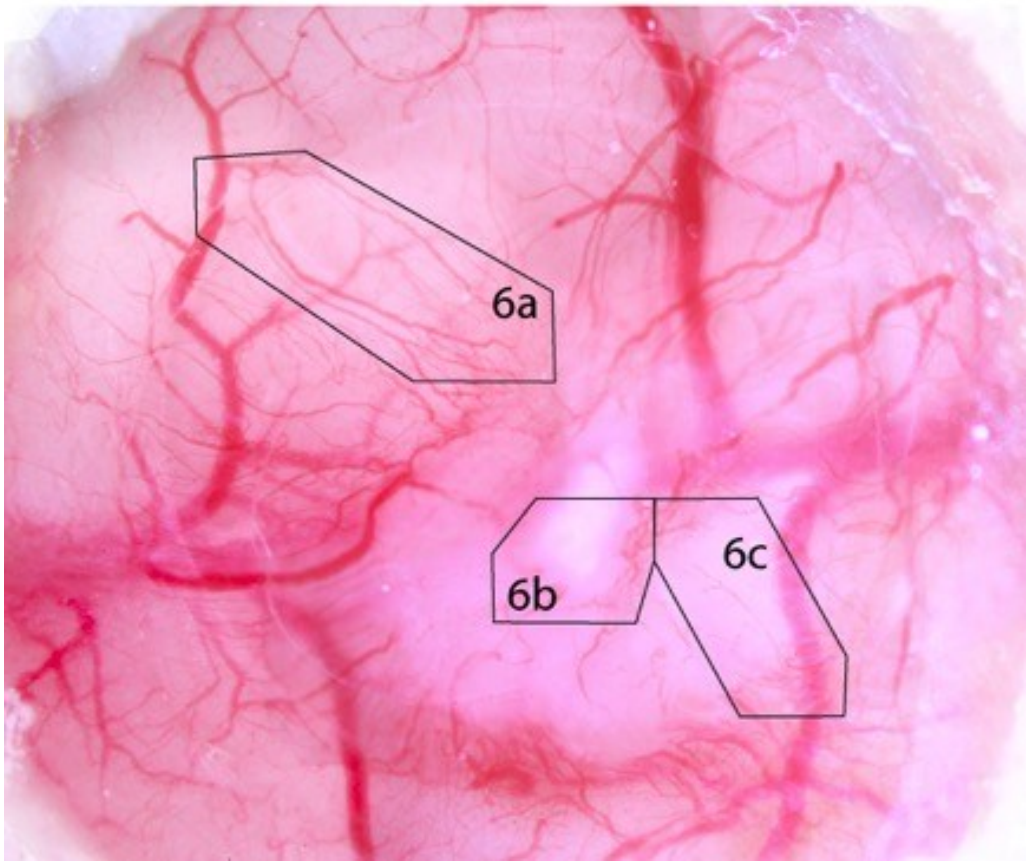
Supplementary Figure 1. Collagen tension around the TIC pillars. a) Brightfield image of collagen stretching around a pillar structure in a TIC with a 4T1 tumor. b) Corresponding fluorescence image of the αSMA-expressing cells embedded in the collagen. c, d) SHG images before (c) and 85 minutes after (d) laser ablation of a selected bundle of collagen fibers (red arrow) near a pillar structure (*). Some of the collagen fibers relax following laser ablation (yellow arrow heads). Scale bar = 200 μm.



Supplementary Figure 2. Effect of losartan on fiber and sprout properties. Measuring relative angles and the effect of Losartan treatment on collagen and sprouts. a) Using the ImageJ plugin FibrilTool plugin[1], we create regions of interest (ROIs) that extend past the tip of an identified sprout process (top right panel). The algorithm was applied to the SHG (collagen) channel, and returned the average angle of fibers in each ROI as well as the index of anisotropy. The orange line in the figure indicates the average fiber angle, and the length is proportional to the anisotropy of the fibers. The angle of each sprout was then determined (bottom left panel), and the relative angle calculated by subtraction (bottom right panel). The results using this method are presented in the main text, Figure 6f. b) Collagen fraction, measured by autothresholding the SHG images and calculating the fraction of positive pixels, significantly decreased with Losartan treatment at Day 14. c) The anisotropy index was also measured over the entire collagen matrix (rather than only at sprout tips). Anisotropy increased with tumor growth, indicating more fiber alignment. This enhanced directional alignment of fibers was not affected by losartan treatment. d) The length of sprouts decreased in the losartan group at day 14, compared to buffer-treated controls. e) Sprout diameter was not significantly different between groups.



Supplementary Figure 3. Tumor growth in the dorsal window chamber without a TIC. a) AK4.4 pancreatic tumor (T) imaged through the window chamber of a αSMA-DsRed / Tie2-GFP mouse. There is significant angiogenesis at this time point (day 4), and new vessels accumulate at the tumor periphery. b) fluorescence imaging of the same tumor, showing αSMA-DsRed-positive arterial walls, which appear yellow. Vessels appear red due to blood hemoglobin. Identifiable arteries and veins are indicated (* and ◊, respectively). Some angiogenic processes are visible, but most are obscured by the thick tumor tissue. Scale bar: 1mm.



Supplementary Figure 4. Regions of the TIC imaged in Supplementary Videos 6a-c. The MMTV tumor appears as the white, diffuse mass. The mouse was treated with Losartan for 7 days and perfused with DiO labeled RBCs to visualize the vasculature.

Supplementary Videos

Supplementary Video 1a. Vascular bundle advancing over the edge of the PDMS on day 13 after implantation with AK4.4 tumor fragment and PDMS "raft." The venules terminate at the capillary bed, out of the field of view at top. The single arteriole is fed by a distant artery to the left (see Figure 2, main text). The diameters of the larger venules are in the range 30-50μm.

Supplementary Video 1b. Lower magnification video of the vascular structure in Video 1a. The view shows the connections of the venules with the host capillary bed; the flexing motion is due to muscle contractions in the skin underlying the PDMS.

Supplementary Video 2. Intravital imaging of blood flow in an advancing angiogenic network. On day 21 in a DSFC chamber with a AK4.4 tumor, sprouting tip cells (visualized by Tie-2 GFP signal) can be observed extending from perfused looping structures are produced by progressive connection of sprouts at the leading edge. The process ensures that the tip cells are always near a supply of flowing blood (visualized by injection of DiO-labeled RBCs). Note that the sprouting cells quickly develop patent lumens, and platelets or RBCs can be observed pulsating with the structures.

Supplementary Video 3. Intravital imaging of blood flow in an advancing angiogenic network at lower magnification. On day 21 in a chamber with an AK4.4 tumor, multiple tip cells (visualized by Tie-2 GFP signal) extend from the edge of the new network. To visualize the blood flow, the mouse was injected with DiO labeled RBCs.

Supplementary Video 4. Animated z-stack of an image volume near the entrance to the TIC without a tumor implant. The dark shadow marks the inner boundary of the PDMS spacer. On Day 1, fibroblasts (red, αSMA-DsRed) have entered the chamber and a collagen matrix is evident (SHG signal, white). Angiogenic sprouts (FITC-dextran, green) are migrating within the collagen. The depth of the stack, 45μm, spans the height of the middle chamber of the TIC. Width of field=630x630 μm.

Supplementary Video 5. Time lapse movie of the maturing vascular network in a TIC in a Tie2-GFP/αSMA- DsRed mouse at day 8 with no tumor implant. Blood appears green due to DiO labeled RBCs. One of the new vessels, an arteriole transporting blood from the periphery towards the center of the TIC, has several αSMA+ cells incorporated in its wall and undergoes vasoconstriction (arrow head). In contrast, the large venule at bottom has more sparse αSMA^{+} cell coverage. $Tie2^{+}$ macrophages are often very active, migrating on the surface and the matrix.

Supplementary Video 6a. Intravital imaging in a TIC with a MMTV tumor and treated with Losartan (Day 7). Arrows indicate blood flow direction in the major arterioles and venules, and the dashed line marks the boundary of the inner chamber. The video starts near the edge of the chamber and scans to a region near the tumor (see the map in Supplemental Figure 4 and Figure 8 of the main text). Note the normal vascular morphology and blood flow near the TIC entrance, and the change in these features as the vasculature approaches the tumor. Blood flow is visualized by injection of DiO-labeled RBCs.

Supplementary Video 6b. Intravital imaging in a TIC with a MMTV tumor and treated with Losartan (Day 7). This video is within the tumor, at the interface of the avascular tumor core (see Supplemental Figure 4). Note the characteristic tumor vessel morphology with highly dilated and tortuous vessels and non-uniform blood velocities. Blood flow is visualized by injection of DiO-labeled RBCs.

Supplementary Video 6c. Intravital imaging in a TIC with a MMTV tumor and treated with Losartan (Day 7). This video starts within the tumor, where vessel density is non-homogeneous

and there are avascular regions. It scans down to the entrance of the chamber, where the angiogenic vessels interface the tumor. Here, we see the typical dense accumulation of vessels associated with tumor angiogenesis (see map in Supplemental Figure 4). Blood flow is visualized by injection of DiO-labeled RBCs.

Reference:

1. A. Boudaoud, A. Burian, D. Borowska-Wykret, M. Uyttewaal, R. Wrzalik, D. Kwiatkowska and O. Hamant, Nature protocols, 2014, 9, 457-463.