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Implantable Tissue Isolation Chambers for Analyzing Tumor Dynamics *In Vivo*

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Abstract

Recruitment of new blood vessels from the surrounding tissue is central to tumor progression and involves a fundamental transition of the normal, organized vasculature into a dense disarray of vessels that infiltrates the tumor. At present, studying the co-development of the tumor and recruited normal tissue is experimentally challenging because many of the important events occur rapidly and over short length scales in a dense three-dimensional space. To overcome these experimental limitations, we partially confined tumors within biocompatible and optically-clear tissue isolation chambers (TICs) and implanted them in mice to create a system more amenable to microscopic analysis. Our goal was to integrate the tumor into a recruited host tissue – complete with vasculature – and demonstrate that the system recapitulates relevant features of the tumor microenvironment. We show that the TICs allow clear visualization of the cellular events associated with tumor growth and progression at the host-tumor interface including cell infiltration, matrix remodeling and angiogenesis. The tissue within the chamber is viable for more than a month, and the process is robust in both the skin and brain. Treatment with losartan, an angiotensin II receptor antagonist, decreased collagen density and fiber length in the TIC, consistent with the known activity of this drug. We further show that collagen fibers display characteristic tumor signatures, and play a central role in angiogenesis, guiding the migration of tethered endothelial sprouts. The methodology combines accessible methods of microfabrication with animal models and will enable more informative studies of the cellular mechanisms of tumor progression.

Graphical abstract

Implantable tissue isolation chambers allow analyses of host-tumor interactions *in vivo*

Introduction

Drug discovery and development of new therapeutic strategies would benefit from functional assays that provide the appropriate anatomy, microenvironment and tissue-level physiology of human tumors. To control experimental conditions, much of this work is still being performed *in vitro* with highly simplified, artificial cell environments such as tissue culture plates or reconstituted biogels. These assays are useful for identifying and dissecting molecular pathways, but they lack crucial cell-cell and cell-matrix interactions that regulate tissue biology *in vivo*. Moreover, *in vitro* methodologies for creating vascularized tumors with nutrients supplied by flowing blood still under development. There is now widespread acknowledgment that cancer progression is inextricably linked to the stromal microenvironment of the tumor and its vascularization¹⁻⁴, and their absence makes it difficult to interpret *in vitro* studies of tumor biology.

More clinically relevant information can be obtained from *in vivo* mouse models, which allow measurement of growth rates or assessment of distant metastases using spontaneous, induced, or implanted tumors. Florescent reporter constructs have greatly enhanced the power of animal studies, allowing identification of specific cell populations and visualization of gene expression *in vivo*⁵⁻⁹. However, even when using transparent windows and sophisticated microscopy techniques, it is often difficult to track the underlying stromal remodeling processes involved in cancer progression such as fibrogenesis or the collusion between host and cancer cells. This is due to two major limitations. First, the limited depth penetration of optical microscopy generally restricts observations to a few hundred micrometers below the surface, thus missing important cellular dynamics that occur beneath the growing tumor. And second, structures and cell motion that extend in the *z* direction are difficult to detect or deconvolve when they overlap in the 3-D space.

To overcome these limitations, we implanted silicone elastomer tissue isolation chambers (TICs) beneath transparent windows in the brain or skin of mice. The implants are biocompatible and transparent, allowing long-term observation. They partially confine a centrally-placed tumor so that host-tumor interactions only occur in a relatively thin layer of tissue near the surface. The goals of the present study were to determine i) how the host tissue responds to contact with the PDMS of the TIC, ii) whether a tumor placed in the TIC can recruit host tissue and vasculature into the device, and iii) whether the stroma created within the device responds appropriately to pharmacological treatment.

We show that over a period of two weeks, host macrophages and fibroblasts enter the chamber and create the stromal matrix for the angiogenic vasculature, which integrates into the growing tumor. The TICs allow high resolution imaging of the events associated with tumor growth and progression at the host-tumor interface including cell infiltration, matrix dynamics and angiogenesis. The tissue within the chamber is viable for more than a month, and the process is robust in both the skin and brain. Treatment with losartan, an angiotensin II receptor antagonist, decreased collagen density and fiber length in the TIC, consistent with the known activity of this drug^{10, 11}.

We also demonstrate how the methodology can reveal processes that were previously difficult to discern, including distinct tumor collagen patterns and the direct association of angiogenic blood vessels with collagen fibers as they migrate to vascularize new tissue.

Results

Three TIC designs were used in this study (**Fig. 1**). All devices were constructed from PDMS, a biocompatible, optically clear elastomer using standard techniques of soft lithography¹²⁻¹⁶. To partially isolate a growing tumor, three approaches were used. First, in the “raft” design, a flat rectangular piece of PDMS (1.6×2.7mm; 75-100µm thick) was placed directly on the tissue under the glass of the dorsal window chamber, and a small fragment of an AK4.4 tumor explant was harvested from a donor mouse and placed on top (**Figs. 1a and 2, Supplementary Videos 1a and b**). Second, in the “hole” design, a PDMS disk (10mm in diameter; 100µm thick) was used for the bottom layer, and polystyrene beads were placed on top to maintain a fixed gap between the PDMS and glass cover. Holes were punched in the bottom layer to allow access to host cells and vessels (**Figs. 1b and 3, and Supplementary Videos 2 and 3**). Third, in the “pillar” design, a disc of PDMS was placed against the tissue, and a donut-shaped PDMS spacer was placed on top to create the inner chamber that contains the tumor (**Figs. 1c, 4-9, Supplementary Figs. 1-3 and Videos 4-6**). The system is closed with a PDMS cover and then a glass coverslip, which acts as the imaging window for the mouse chamber⁶. PDMS pillars in the base layer extend upward and contact the spacer, allowing access of host cells from the periphery, around the pillars. These pillars also provide a structural array to support the collagen network that forms. This design has the advantage that host tissue can only infiltrate the chamber from the periphery, so the time course of tumor integration is easy to follow. After implantation into the mouse skin or brain (**Fig. 1d**), we were able to observe the dynamics of macrophage and fibroblast infiltration, vessel sprouting, collagen and vessel remodeling, and formation of a stable vasculature in these systems.

Blood vessels and stromal cells are recruited to the host-PDMS interface

We first analyzed the tissue reaction to PDMS by placing a rectangular raft in the dorsal window chamber. Within 48hrs, there is dilation of host capillaries, migration of host cells to the PDMS surface, and deposition of matrix at the interface. By day 13, tissue structures containing vascular bundles create bridges between the host tissue and the PDMS surface (**Fig. 2**). Using α SMA⁺-DsRed/Tie-2⁺-GFP/FVB mice, it is possible to identify α SMA- and Tie-2-expressing cells using intravital microscopy. α SMA is generally expressed in vascular

smooth muscle cells and myofibroblasts, while Tie-2 is a marker of endothelial cells and macrophages. The bundles of perfused vascular loops have many outflow vessels that lead back to host capillaries and fewer inflow vessels that connect to arterialized capillaries with α SMA positive walls (**Fig. 2**). Thus, there was a surprisingly rapid transformation of capillary segments into an arteriole, likely accomplished by pruning of side branches and recruitment of smooth muscle cells. These new arterioles also express high levels of Tie-2 (**Fig. 2**). The new arterioles can be traced back to connections with high pressure arteries, far from the locations in the capillary bed where the blood exits the new tissue structure. This ability to observe arterialization and quantitatively trace the topology of the forming network is a major advantage, and it shows how the system develops in a way that ensures a large pressure difference to drive and flow through the new looping vasculature (**Supplementary Videos 1a and 1b**). The newly formed vascular bundle is well anchored in the tumor, forming a bridge that flexes during skin movement (**Supplementary Video 1b**). These observations showed interesting features of vascular morphogenesis and maturation, but also revealed a limitation of this “raft” design: the PDMS is able to drift relative to the tissue over a period of weeks or days, introducing stresses in the forming tissues. As a result, only 25% of raft implantations resulted in sustained vascularization of the tumor.

Creation of stroma within tumor chambers

To limit motion of the PDMS relative to the tissue, we next developed larger, circular devices with access holes or supporting pillars (**Fig. 1b, c**). These were then tested to determine whether the vasculature would extend sufficiently to interact with a centrally-implanted tumor. In the TICs with the “hole” design, vascular sprouts appear within the holes as early as three days after implantation and continue to migrate and mature. The sprouts form in a radial pattern above the PDMS layer, connect with each other and support the growth of a tumor placed on the PDMS (**Fig. 3**). Significant vascular remodeling is also observed in this system, and a predominant pathway usually evolves between adjacent holes, apparently due to flow-driven vascular adaptation. The developing vascular network advances by extension of sprouts and their frequent interconnection to form loops at the leading edge. This progressive addition of loops maintains a blood supply to the system during angiogenesis. It is possible to see the evolution of the vessel lumens, and pulsating flow is often observed in blind-ending sprout cells as new lumens fill (**Supplementary Videos 2, 3**). These are all features of angiogenesis described in other models such as the chick CAM and developing retina^{17, 18}, indicating that the events in the TIC recapitulate angiogenesis. These experiments showed that it is possible to form an extensive tumor vasculature above the PDMS layer and in the tumor. We next analyzed the host cells and matrix components during the process of chamber vascularization. The pillar design was more appropriate for these studies, because all host cells and vessels enter from the periphery, and then migrate radially into the central chamber. This geometry is more amenable to tracking the dynamics than the hole design, which allows access at multiple points under the tumor chamber. In the pillar design, the height of the chamber (150 μ m) is sufficient to allow a few cell layers and formation of a complex collagen matrix while minimizing overlap of structures in the z direction. Within 7 days after implantation, red α SMA⁺ cells (likely myofibroblasts) appear at the edge of the chamber and produce collagen, imaged using second harmonic generation (SHG) microscopy (**Fig. 4a**). The

collagen establishes a structural matrix that originates in the host tissue, anchors to the PDMS in some locations (**Fig. 4b**), and spans the height of the chamber (**Supplementary Video 4**).

Along with the α SMA expressing cells, Tie-2 positive cells (likely macrophages) enter the chamber, independent of the vasculature. Interestingly, vascularization occurs whether or not there is a tumor in the central chamber (**Fig. 4c**; **Supplementary Video 4**). The cell mobilization and formation of a fibrous matrix recapitulates the early stages of tumorigenesis in fibrotic tumors such as pancreatic, breast and liver^{10, 19-21}. As the activated stromal cells and collagen matrix populate the periphery of the chamber, the vascular sprouts follow behind (**Fig. 4c**). The sprouting tip cells extend from perfused loops at the leading front as they move into the device (**Fig. 5a, b**; **Supplementary Videos 2 and 3**). As in the hole design, angiogenic sprouts advance and make new connections, building an extensive network progressively at the leading edge. This sequence of host cell infiltration, collagen deposition and angiogenesis was reproducible, occurring in 21 out of 23 pillar design TICs.

Remodeling of the extending vascular network, and its maturation, happens continuously as the vasculature extends. Within 7-10 days, both the vasculature and collagen network have remodeled to resemble a typical stroma (dense and aligned collagen fibers and differentiated arteries and veins) in the region near the TIC entrance, away from the tumor (**Fig 5c and d**).

The arterialization seen in the early bridging structure (**Fig. 2**) also occurs here, as evidenced by the smooth muscle cell-invested walls (**Fig. 5a**, D6, arrow). These new arterioles are functional, exhibiting vasomotion (**Supplementary Video 5**). In later stages, the network of vessels and collagen assumes normal morphology (**Fig. 5c, d**). Interestingly, the distribution of collagen around the pillar structures often appears asymmetric, suggesting that the matrix fibers are under tension (**Fig. 5c arrowhead**). Indeed, if the fibers are cut with a high-intensity laser, the surrounding network relaxes (**Supplementary Fig. 1**). This indicates that there are activated myofibroblasts contracting the collagen, just as in other models of fibrotic tumors¹¹.

The host stroma integrates with the tumor tissue in the central tissue chamber

Within 7-14 days, the host stroma contacts the tumor in the central chamber. In the mosaic overview image of **Fig. 6** (showing day 7 after implantation of a MMTV tumor fragment in the cranial window TIC), the collagen network originates in the host tissue where it is still integrated with the underlying stroma. It extends over the edge of the PDMS and interfaces with the tumor, extending into, and encircling it.

Cells expressing α SMA associate with this matrix near the entrance to the chamber and precede the arrival of the angiogenic blood vessels. In addition to the main mass of the tumor, individual and groups of cancer cells often separate from the main mass. This type of overview image, which quantitatively shows – at subcellular resolution – all the host cells and vasculature interacting with and feeding the tumor is challenging to acquire with other methodologies.

There are also interesting features of the advancing vessel sprouts identified in the TICs. As they approach the central chamber, vascular sprouts are consistently associated with prominent collagen fibers (**Fig. 7a-c**). Extended tip cells emerge as Tie-2⁺ processes (**Fig. 7c**) and can be longer than 450 μm (80 μm mean length at day 14, **Supplementary Fig. 2d**). In regions where the collagen network has become sparse, the attachment of migrating sprouts to collagen fibers is more evident (**Fig. 7d, e**). Associated with the extension of sprouts is the appearance of tissue structures within the trailing network that resemble intussusceptive microvascular splitting²² (**Fig. 7d and e**).

To determine whether the sprouts preferentially migrate along collagen fibers, we measured the angles between the fibers and the extending sprouts (**Supplementary Fig. 2a**). We found that the angles clustered tightly around a relative angle of 0, indicating strong alignment (**Fig. 7f, control**). In Losartan treated mice, there were fewer sprouts, and a wider range of relative angles (**Fig. 7f**). We also found that Losartan decreased the collagen content (**Supplementary Fig. 2b**), as expected¹⁰. The anisotropy index increased (indicating less fiber alignment) from 7 to 14 days independent of Losartan treatment (**Supplementary Fig. 2c**). The length of sprouts decreased in the group treated with Losartan at day 14 (**Supplementary Fig. 2d**), whereas sprout diameter was not significantly different between groups (**Supplementary Fig. 2e**).

This demonstrates how the TIC can be used to distinguish cell-matrix interactions during tissue morphogenesis. The results show that angiogenic contact guidance requires specific structural and mechanical properties of the collagen matrix, and these properties were modified with Losartan treatment.

The new vasculature adapts into a hierarchical arterial-venous tree at the periphery but not inside the tumor

In traditional mouse models of cancer, tumors grow in direct contact with the host tissue, and the resulting angiogenic vasculature accumulates at the periphery and under the tumor (**Supplementary Fig. 3a**). In the Tie2-GFP αSMA -DsRed mice, it is possible to identify the large feeding vessels, but the thick tumor tissue obscures the tumor microvessels (**Supplementary Fig. 3b**). In contrast, tumors growing in the TIC have a well-delineated, pseudo-2D vascular network that enters radially from the edge of the chamber (**Fig. 8a**) and infiltrates the tumor (**Fig. 8b, c**). Feeding vessels can be tracked from their origin in the host tissue to the tumor, and blood flow can be observed throughout the network (**Fig. 8b, Supplementary Video 6a-c**). Near the periphery of the device, the vasculature matures, developing a hierarchical network with well-defined arteries and veins (**Fig. 8a, Supplementary Video 6a**). Near the tumor, however, the network has all the typical characteristics of tumor vasculature: highly tortuous, interconnected vessels with inconsistent diameters and heterogeneous flow (**Fig. 8c, Supplemental Videos 6b,c**)^{23, 24}. This shows that the vasculature and stroma within the TIC is subjected to the same tumor environment that leads to abnormal vasculature in other mouse models as well as human tumors²⁴.

The new collagen matrix exhibits the expected range of tumor associated collagen signatures

The production of collagen in and around tumors has attracted attention because of its potential role in tumor progression and invasion¹¹. In fibrotic tumors, infiltrating macrophages and fibroblasts cooperate to create a dense collagenous matrix that changes the mechanical properties and biology of the growing tumor. It has been shown that the amount of collagen – and its organization – correlates with tumor malignancy, and that tumor collagen can be classified into three “tumor-associated collagen signatures” (TACS)²⁵. TACS-1 indicates highly dense tumor collagen, and in TACS-2, tense collagen fibers wrap around the tumor tangentially²⁵. Perhaps most important is TACS-3, where collagen fibers are aligned normal to tumor boundary. Collagen fibers configured in TACS-3 potentially support cancer cell invasion, and this tumor collagen signature is associated with poor disease-specific and disease-free survival in breast cancer patients²⁶.

Interestingly, the ECM within our chambers displays these same collagen signatures (**Fig. 9**). Collagen organization appears diffuse and cross-linked far from the tumor, but there is highly dense, “loose” collagen within the tumor (TACS-1). Collagen fibers are also stretched around the main tumor mass (TACS-2) and there are often tense fibers extending radially into the tumor (TACS-3; **Figure 9, arrowheads**).

Discussion

Identification of the tissue interactions responsible for tumor progression has been difficult because of the challenges in analyzing cell and matrix dynamics in thick, 3D tissues. As the host tissue and tumor interact, there is mixing of extracellular matrix (ECM), vasculature and cells, all overlapping in 3D space, making the individual events difficult to track or interpret²⁷. To deconvolve these processes, we combined microfabricated chambers with intravital imaging to visualize matrix and vascular topologies in a tissue with limited height. Microfabricated devices have proven valuable for creating specific geometries or mechanical properties to probe cell behavior *in vivo*^{13, 15, 28, 29}. Unfortunately, it is not yet possible to faithfully reproduce the correct anatomy and physiology *in vitro*. By introducing microfabricated devices *in vivo*, we are able to specify the design, but populate it with vasculature and stroma supplied by the host tissue. In addition to providing a new tool for analyzing tumor dynamics, this technology also allows careful monitoring of the self-assembly of tissues *in vivo* and will be valuable for developing new paradigms for tissue engineering.

The advantages of two dimensional systems for studying cell dynamics and vascular development have long been recognized by the research community, as evidenced by the wide-spread use of the retinal vasculature and the chick chorioallantoic membrane models^{18, 30-34}. These models are naturally two-dimensional and can be manipulated so they are accessible for longitudinal studies. Systems with similar geometry – but designed for versatile studies of tumor progression in non-embryonic, adult tissue – would accelerate cancer research.

In our hybrid *in vitro* – *in vivo* approach, all of the constituents within the PDMS construct are native, including growth factors produced by the tumor and the stromal cells that respond to them. Furthermore, as opposed to *in vitro* studies that impose exogenous matrices such as collagen, fibronectin or matrigel, the ECM in our chambers is entirely fabricated by native stromal cells and remodeled by endogenous mechanisms. These early events –vessel sprout infiltration and matrix deposition-- can be initiated by the chamber itself^{35, 36}, thus accelerating the process of tumor integration. Similar approaches could be used to encourage vascularization in tissue engineering applications.

The sequence of events that results in tumor angiogenesis in our chambers closely follows that observed in spontaneous mouse and human tumors: the infiltration of activated stromal cells, the deposition of excess matrix and the extension of new vasculature into the tumor. The time course of vascularization is also similar to that in embedded tumor models in mice, where the vascular network reaches a maximum density within one or two weeks^{37, 38}. In the TICs, the vascular network is also extensive by day 14 and exhibits the same vascular tortuosity, vessel density variations, and flow abnormalities documented extensively in tumors^{39, 40}.

An interesting observation made in this study was the close association of migrating sprouts with taut bundles of collagen fibers. Guidance of cell migration by contact with matrix fibers has been documented previously in cell culture assays⁴¹⁻⁴³. In addition, angiogenesis by contact guidance has been described in other systems, including wound healing in the rat mesentery⁴⁴ and in collagen gels *in vitro*⁴⁵. However, evidence of matrix-guided sprouting has not been previously reported in the context of tumor angiogenesis. In the TICs, it was possible to detect and document the association between blood vessels and collagen fibers concomitantly with tumor growth.

We have shown that the system allows analysis of processes that are poorly accessible with other methods due to their low frequency, rapid dynamics or the short distances involved. For example, the migration of the host stromal cells into the tumor occurs over a distance of ~two millimeters in our device. In a tumor developing in a tissue bed, this distance might be only a few micrometers. Thus, by increasing the physical distance between the tumor and host, we increased the observation field and extended the vascularization and stroma migration processes for more detailed studies of host cell recruitment. Although not present in clinical or traditional *in vivo* animal models, this separation between the host and tumor tissues did not prevent the recruitment of the appropriate host cells, and can be controlled by modifying the device size or placement of holes.

The vasculature is especially amenable to analysis in the device because the extended distances needed for angiogenesis allows careful imaging of sprout cells and their associations with matrix fibers and trailing vasculature. The chamber geometry also forces vessel branches to align in the same plane. This allows clear identification of intussusceptive processes, which generally penetrate the lumen perpendicular to the plane of a bifurcation⁴⁶. In 3D tissues, these might be hidden; in our 2D geometry, many are visible (**Fig. 7d, e**).

Silicone elastomers have been used in the clinic for chronic catheters⁴⁷, biosensors⁴⁸ and tissue reconstruction⁴⁹ since the 1960s. As with any implanted, non-native material, there is a potential for the host tissue to respond to implanted elastomers in a “foreign body response,” which involves macrophage and fibroblast recruitment, and fibrosis at the interface⁵⁰. The foreign body response (FBR) can result in biofouling, diminished function of the implant, or chronic inflammation at the site^{50, 51}. Although silicone elastomers such as PDMS are highly biocompatible, further information about host-implant interactions should provide new opportunities and applications for microfabricated devices *in vivo*. Our results show that, although stromal cells and matrix components are recruited to the implant, there is also extensive vascularization of the deposited matrix that coats the PDMS. This is not generally observed in the FBR, and could greatly benefit implantable microsensors that need to maintain contact with blood-borne metabolites or other chemical species. Importantly, the vascularization was observed in both tissue sites – skin (**Fig. 2 and Supplementary Fig. 3**) and brain, (illustrated in the rest of the figures and supplementary videos) so appears to be robust. Furthermore, the PDMS did not produce inflammation in the host tissue.

The confinement of the tissue within a relatively thin compartment is optically advantageous, but restricts tumor growth in the vertical directions. The chamber heights used here – 150 μm in the pillar design and 300-400 μm in the hole design – are large enough to accommodate multiple cell layers and allow development of large feeding vessels. Furthermore, spontaneous tumors arising in tissue are also physically confined by the surrounding normal tissue. In this regard, tumors in the TIC, although confined in the *z* direction, have more freedom of movement in the *x-y* plane than tissue-embedded tumors, at least until the chamber is filled with infiltrating host tissue.

Another potential application of this methodology is the development of vascularized tissues for transplantation. A major challenge in tissue engineering is the lack of a supporting vasculature to supply the tissue quickly after implantation. In our system, a stable, mature vasculature developed and remained functional for more than two months post TIC implantation. Interestingly, the vascular network develops continuously and dynamically with the tumor, and small sprouts continue to form as late as 24-25 (**Fig. 7d and e**) and 63 days post implantation (**Fig. 7a, b and c**). It is possible that a similar strategy could be used to vascularize other engineered tissues designed to replace patient tissues. Although the process is robust in our tumor samples, further studies are necessary to determine whether other tissue types can be adequately vascularized in this way.

Many laboratories around the world study tumor biology using transparent window models, and even more groups have the ability to fabricate PDMS devices. The procedures here represent a straightforward integration of these technologies, and should be accessible to many researchers. We have presented three simple designs for isolating a tumor, but more sophisticated chambers could, for example, include subcompartments for collecting specific cell populations or access ports with varied dimensions to control gradients of tumor- or host-derived chemokines. Thus, this technology is easily adaptable and versatile, and has the potential to facilitate new discoveries in the fields of tumor biology and tissue engineering.

Materials and Methods

All animal experiments were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care. α SMA⁺-DsRed/Tie2⁺-GFP/FVB and Tie2⁺-GFP/Rag1 (for 4T1 tumor implantation) mice were used in these studies. In this transgenic strain, blood vessels express GFP under control of the TIE2 promoter, while α SMA-expressing cells express DsRed. The mice were implanted with transparent dorsal skinfold chambers (DSC) or cranial windows (CW) as described previously^{5, 55, 56}.

Tumor fragments

The AK4.4 pancreatic (Figs. 2, 3, Supplementary Fig. 3 and Supplementary Videos 1a and b, 2 and 3), 4T1 (**Figs. 5c, d, 7a-e, and Supplementary Fig. 1**) and MMTV (Figs. 6, 8, 9 Supplementary Figs. 2a and 4, Supplementary Videos 6a-c) breast cancer tumors were developed in a FVB/N (AK4.4 and MMTV) background and implanted in α SMA⁺-DsRed/Tie2⁺-GFP/FVB mice or developed in a Rag1 and implanted in Tie2⁺-GFP/Rag1 mice (4T1). For implantation in the chambers, the source tumors from donor mice were surgically excised and placed in a petri dish in sterile HBSS cell culture medium. The tumors were then cut into small fragments using sterile surgical scissors. Small fragments (~2mm in diameter) were placed in the center of the bottom layer of the TIC, covered with HBSS cell culture media, and either the glass coverslip or the PDMS cover was placed on top.

In vivo tissue isolation chambers (TICs)

TICs were fabricated from poly (dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning) at a ratio of base to curing agent of 12:1. A silicon wafer master was constructed using soft lithography to form the negative relief features. A spin coater was used at 800 rpm to create thin 75-100 μ m PDMS sheets. The PDMS sheets were then heated to 60°C overnight. The PDMS “raft”, a 1.6×2.7mm rectangular piece (**Fig. 2 and Supplementary Videos 1a and b**) was obtained by cutting a flat portion with PDMS with a sterile surgical scalpel. The dorsal window was created using standard procedures described previously⁵⁷. To place the “raft” in the window, the cover glass was removed, and the rectangular piece of PDMS was placed directly on the skin within the dorsal window. The tumor was then placed on top of the PDMS, and the glass coverslip was replaced to close the chamber. For the “hole” design, a PDMS disk of 10mm diameter was punched out with a circular punch (**Fig. 3 and Supplementary Video 2 and 3**). Randomly distributed access holes were then created with a 500 μ m biopsy punch. The resulting holes were 200-300 μ m in diameter due to deformation of the PDMS material during cutting. To maintain the space between the PDMS and glass cover slip, polystyrene beads (300-400 μ m in diameter) were placed on top of the PDMS before sealing the chamber. Finally, for the “pillar” design, PDMS disks were carefully cut out from the master using biopsy punches of various diameters and then sterilized by exposure to UV light for ~ 30 min (Figs. 4-9, Supplementary Figs. 1, 2 and 4, and Supplementary Videos 4-6). Three separate components, each 4 mm in diameter, were created for each device: i) a bottom layer with an array of circular pillars oriented vertically (each 200 μ m wide, 50 μ m in height, separated by 500 μ m); ii) an annular middle layer with a 3 mm diameter void, which served as a spacer and defined the central chamber; and iii) a top cover, comprised of a PDMS disk with no features; this layer contacted the glass

coverslip. The tumor fragment was placed on the pillars of the bottom layer and inside the annulus of the middle layer. The three PDMS layers were placed loosely on top of each other without being sealed. The bottom layer contained the pillar features, which created a space of 50 μm between the bottom and the middle donut-shaped spacer. The top layer was in direct contact with the glass coverslip. Therefore the sprouts could only enter the chamber through the 50 μm high space between the bottom and middle layer, around the PDMS pillars. Inside the chamber, within the spacer annulus, the height expands to 150 μm .

Intravital Microscopy

Multiphoton laser scanning microscope (MPLSM)—A custom-made multiphoton laser microscope was used at 810nm excitation wavelength⁵. Three dichroic mirrors and three filters of 405, 535 and 610 nm were used to simultaneously image second order harmonic generation (SHG), GFP and Ds-Red fluorescence signals respectively. The typical depth penetration limit of the MPLSM is 0.5-0.7mm, which allows imaging through the glass coverslip (100 μm thick) and 3 layers of PDMS (each 100 μm thick) all the way to the underlying native vasculature. Because the PDMS and glass are optically clear, the tissue thickness is the important determinant of light scattering. Tissue thickness was 150 μm in the center of the pillar design, and 300-400 μm in the hole design.

Stereo microscope—Bright field and fluorescence images were acquired with a Nikon SMZ1500 stereomicroscope (Nikon Instruments Inc., Melville, NY, USA) equipped with a Nikon D90 SLR photo camera and a QIClick^{TM} digital CCD camera (QImaging, Surrey, BC, Canada). The microscope was equipped with GFP and Ds-Red fluorescence filters, allowing visualization of the vasculature and the labeled RBCs *in vivo*. This setup was also used to acquire the supplemental intravital videos.

Visualization of the host vasculature in live mice—To assess the perfusion status of the vessels and visualize the blood flow *in vivo*, animals were perfused via retro-orbital injection with 100 μl of either FITC- or rhodamine- dextran solution or labeled red blood cells. For labeling, RBCs were collected from a donor mouse, labeled with highly lipophilic carbocyanine dyes (DiO or DiD) (Invitrogen^{TM} Life Technologies, Grand Island, NY)⁵. The RBC solution (100 μl , 50% hematocrit) was injected into the animal retro-orbitally. Fluorescence from the labeled RBCs was sufficiently bright to allow imaging for approximately 15 days.

Pharmacological treatment—Mice implanted with cranial window chambers and MMTV tumor fragments were treated with Losartan, an angiotensin receptor blocker at 40mg/kg (daily, day 1-7)^{10, 58} and then 80 $\mu\text{g/kg}$ (day 8-15), or an equal amount of PBS (control group) intraperitoneally.

Collagen fiber analysis—The orientation of the collagen fibers and blood vessels was determined using FibrilTool for ImageJ⁵⁹. The algorithm calculates the angle of aligned structures (fibers or vessels) and an index of anisotropy, which indicates the uniformity of the alignment⁵⁹. First, a z-projection was obtained from the entire stack of Fluoview images. Two similar size regions of interest (ROI) were chosen to include a sprout and the collagen

fibers at the top of the sprout. The software reports the predominant angle of the fiber structures in the ROI (either the collagen fibers or the vessels) and an index of anisotropy. The results are reported as the absolute value of the difference between the vessel and corresponding fiber angles and isotropy indices.

The FibrilTool plugin returns the average angle of fibers and the anisotropy. Repeating this procedure by tiling ROIs over the entire image gives a map of local collagen angles and anisotropy (Fig 5d). The relative angle between sprouts and ECM fibers was measured at day 7 (D7) and day 14 (D14) as described in the Supplementary methods section. Collagen fraction was calculated from the SHG images as the percentage area of collagen fibers in five fields of view oriented at four corners and the center of the image. The length of sprouts was measured from the top of a vascular loop to the tip of the vessel sprout. Only sprouts that were entirely located in the visual field were included in the length measurements. Sprout diameters were measured as the inner diameter marked by the fluorescent marker inside the sprout lumen.

Laser ablation of collagen fibers—The multiphoton laser microscope was used to ablate selected collagen fibers in the chambers. A preablation image was taken to identify the location and thickness of the collagen fibers. The laser beam was then programmed to scan a small rectangular region perpendicular to the collagen bundle. The laser power was then increased to the maximum voltage of 3V. The laser beam was focused to scan the collagen fibers repeatedly until the collagen fibers were interrupted as evidenced by empty space in between fibers. No visible damage was noticed in the adjacent tissue. The laser was returned to imaging range and a post-procedure image was acquired.

Statistical analysis—The numerical data in Figure 6 and Supplementary Figure 2 are expressed as mean $\pm$ SEM. Analysis of means was performed with a two-tailed paired (longitudinal comparisons) and unpaired (between groups) t-test (GraphPad Prism software, San Diego, CA, USA). Differences were considered significant at P values less than 0.05.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Duda DG, Duyverman AM, Kohno M, Snuderl M, Steller EJ, Fukumura D, Jain RK. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:21677–21682. [PubMed: 21098274]
2. Hielscher AC, Gerecht S. Cancer research. 2012; 72:6089–6096. [PubMed: 23172313]

3. Jing Y, Han Z, Liu Y, Sun K, Zhang S, Jiang G, Li R, Gao L, Zhao X, Wu D, Cai X, Wu M, Wei L. *PloS one*. 2012; 7:e43272. [PubMed: 22952657]
4. McAllister SS, Weinberg RA. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010; 28:4022–4028. [PubMed: 20644094]
5. Cheng G, Liao S, Kit Wong H, Lacorre DA, di Tomaso E, Au P, Fukumura D, Jain RK, Munn LL. *Blood*. 2011; 118:4740–4749. [PubMed: 21835951]
6. Jain RK, Munn LL, Fukumura D. *Nature reviews. Cancer*. 2002; 2:266–276. [PubMed: 12001988]
7. Chishima T, Miyagi Y, Wang X, Yamaoka H, Shimada H, Moossa AR, Hoffman RM. *Cancer research*. 1997; 57:2042–2047. [PubMed: 9158003]
8. Hoffman RM. *BioTechniques*. 2001; 30:1016–1022. 1024–1016. [PubMed: 11355337]
9. Hoffman RM. *Methods in molecular biology*. 2009; 515:45–61. [PubMed: 19378118]
10. Chauhan VP, Martin JD, Liu H, Lacorre DA, Jain SR, Kozin SV, Stylianopoulos T, Mousa AS, Han X, Adstamongkonkul P, Popovic Z, Huang P, Bawendi MG, Boucher Y, Jain RK. *Nature communications*. 2013; 4:2516.
11. Stylianopoulos T, Martin JD, Chauhan VP, Jain SR, Diop-Frimpong B, Bardeesy N, Smith BL, Ferrone CR, Hornicek FJ, Boucher Y, Munn LL, Jain RK. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:15101–15108. [PubMed: 22932871]
12. Jain A, Munn LL. *PloS one*. 2009; 4:e7104. [PubMed: 19768109]
13. Jain A, Munn LL. *Lab on a chip*. 2011; 11:2941–2947. [PubMed: 21773633]
14. Song JW, Bazou D, Munn LL. *Integrative biology : quantitative biosciences from nano to macro*. 2012; 4:857–862. [PubMed: 22673771]
15. Song JW, Munn LL. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:15342–15347. [PubMed: 21876168]
16. Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE. *Annu Rev Biomed Eng*. 2001; 3:335–373. [PubMed: 11447067]
17. Zeng G, Taylor SM, McColm JR, Kappas NC, Kearney JB, Williams LH, Hartnett ME, Bautch VL. *Blood*. 2007; 109:1345–1352. [PubMed: 17068148]
18. Shioda T, Munn LL, Fenner MH, Jain RK, Isselbacher KJ. *The American journal of pathology*. 1997; 150:2099–2112. [PubMed: 9176401]
19. Nakatsukasa H, Nagy P, Evarts RP, Hsia CC, Marsden E, Thorgerirsson SS. *The Journal of clinical investigation*. 1990; 85:1833–1843. [PubMed: 1693377]
20. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W, Yamauchi M, Gasser DL, Weaver VM. *Cell*. 2009; 139:891–906. [PubMed: 19931152]
21. Lisanti MP, Tsirogas A, Pavlides S, Reeves KJ, Peiris-Pages M, Chadwick AL, Sanchez-Alvarez R, Lamb R, Howell A, Martinez-Outschoorn UE, Sotgia F. *Cell cycle*. 2014; 13:580–599. [PubMed: 24434780]
22. Patan S, Munn LL, Tanda S, Roberge S, Jain RK, Jones RC. *Circulation research*. 2001; 89:723–731. [PubMed: 11597996]
23. Jain RK. *Nature medicine*. 2001; 7:987–989.
24. Jain RK. *Cancer research*. 1988; 48:2641–2658. [PubMed: 3282647]
25. Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ. *BMC medicine*. 2006; 4:38. [PubMed: 17190588]
26. Conklin MW, Eickhoff JC, Riching KM, Pehlke CA, Eliceiri KW, Provenzano PP, Friedl A, Keely PJ. *The American journal of pathology*. 2011; 178:1221–1232. [PubMed: 21356373]
27. Munn LL. *Physical biology*. 2013; 10:065003. [PubMed: 24304856]
28. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. *Science*. 1997; 276:1425–1428. [PubMed: 9162012]
29. Lim SY, Lee DG, Sivakumaran P, Crombie D, Slavin J, Dottori M, Conley B, Denham M, Leung J, Tee R, Dusting GJ, Pebay A, Dilley RJ. *Biochemical and biophysical research communications*. 2012; 422:75–79. [PubMed: 22560904]

30. Brem S, Brem H, Folkman J, Finkelstein D, Patz A. Cancer research. 1976; 36:2807–2812. [PubMed: 1277191]
31. Campochiaro PA, Hackett SF. Oncogene. 2003; 22:6537–6548. [PubMed: 14528278]
32. Cao Y, Arbiser J, D'Amato RJ, D'Amore PA, Ingber DE, Kerbel R, Klagsbrun M, Lim S, Moses MA, Zetter B, Dvorak H, Langer R. Sci Transl Med. 2011; 3:114rv113.
33. Ribatti D, Djonov V. Cancer letters. 2012; 316:126–131. [PubMed: 22197620]
34. Djonov V, Baum O, Burri PH. Cell and tissue research. 2003; 314:107–117. [PubMed: 14574551]
35. Gruionu G, Stone AL, Schwartz MA, Hoying JB, Williams SK. Journal of biomedical materials research. Part A. 2010; 95:811–818. [PubMed: 20734331]
36. Shepherd BR, Chen HY, Smith CM, Gruionu G, Williams SK, Hoying JB. Arteriosclerosis, thrombosis, and vascular biology. 2004; 24:898–904.
37. Fukumura D, Jain RK. Microvascular research. 2007; 74:72–84. [PubMed: 17560615]
38. Leunig M, Yuan F, Menger MD, Boucher Y, Goetz AE, Messmer K, Jain RK. Cancer research. 1992; 52:6553–6560. [PubMed: 1384965]
39. Munn LL. Drug discovery today. 2003; 8:396–403. [PubMed: 12706657]
40. Less JR, Skalak TC, Sevic EM, Jain RK. Cancer research. 1991; 51:265–273. [PubMed: 1988088]
41. Provenzano PP, Inman DR, Eliceiri KW, Trier SM, Keely PJ. Biophysical journal. 2008; 95:5374–5384. [PubMed: 18775961]
42. Dickinson RB, Guido S, Tranquillo RT. Annals of biomedical engineering. 1994; 22:342–356. [PubMed: 7998680]
43. Guido S, Tranquillo RT. Journal of cell science. 1993; 105(Pt 2):317–331. [PubMed: 8408268]
44. Anderson CR, Ponce AM, Price RJ. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 2004; 52:1063–1072. [PubMed: 15258182]
45. McLeod C, Higgins J, Miroshnikova Y, Liu R, Garrett A, Sarang-Sieminski AL. Journal of biomechanical engineering. 2013; 135:71002. [PubMed: 23722263]
46. Djonov VG, Kurz H, Burri PH. Developmental dynamics : an official publication of the American Association of Anatomists. 2002; 224:391–402. [PubMed: 12203731]
47. Zdrachala RJ, Zdrachala IJ. J Biomater Appl. 1999; 14:67–90. [PubMed: 10405885]
48. Graham AH, Bowen CR, Surguy SM, Robbins J, Taylor J. Med Eng Phys. 2011; 33:973–979. [PubMed: 21478042]
49. Gampfer TJ, Khoury H, Gottlieb W, Morgan RF. Ann Plast Surg. 2007; 59:581–590. [PubMed: 17992156]
50. Kenneth Ward W. J Diabetes Sci Technol. 2008; 2:768–777. [PubMed: 19885259]
51. Harding JL, Reynolds MM. Trends Biotechnol. 2014; 32:140–146. [PubMed: 24438709]
52. Jamshidi K, Shiradi MR. Am J Orthop (Belle Mead NJ). 2015; 44:E32–35. [PubMed: 25566563]
53. Muthusamy S, Subhawong T, Conway SA, Temple HT. Clin Orthop Relat Res. 2015; 473:742–750. [PubMed: 25212962]
54. Shemesh S, Kosashvili Y, Sidon E, Fichman S, Cohen N, Velkes S. J Bone Oncol. 2014; 3:80–83. [PubMed: 26909302]
55. Fukumura D, Yuan F, Endo M, Jain RK. The American journal of pathology. 1997; 150:713–725. [PubMed: 9033284]
56. Yuan F, Salehi HA, Boucher Y, Vasthare US, Tuma RF, Jain RK. Cancer research. 1994; 54:4564–4568. [PubMed: 8062241]
57. Fukumura D, Duda DG, Munn LL, Jain RK. Microcirculation. 2010; 17:206–225. [PubMed: 20374484]
58. Chauhan VP, Boucher Y, Ferrone CR, Roberge S, Martin JD, Stylianopoulos T, Bardeesy N, DePinho RA, Padera TP, Munn LL, Jain RK. Cancer cell. 2014; 26:14–15. [PubMed: 25026209]
59. Boudaoud A, Burian A, Borowska-Wykret D, Uyttewaald M, Wrzalik R, Kwiatkowska D, Hamant O. Nature protocols. 2014; 9:457–463. [PubMed: 24481272]

Figure 1. Implantable PDMS rafts and Tissue Isolation Chambers

a) A rectangular slab ("raft"; 1.6x2.7mm) of PDMS attracts host cells and vasculature when placed against mouse tissue. b) In the "hole" design, cancer cells are placed on top of a layer of PDMS. Host cells and vessels enter the chamber through holes (200-300 μm diameter) punched in the bottom PDMS layer. Polystyrene beads (300-400 μm diameter; Polysciences Inc., Warrington, PA) were used to create the space between the bottom PDMS layer and glass coverslip. After placing the tumor, the assembly is implanted in the mouse window preparation (dorsal skin or cranial). Host stromal cells including macrophages and fibroblasts (blue) enter the chamber and create an extracellular matrix that is anchored in the normal tissue. Blood vessels (red) migrate through the gap at the periphery created by the pillars to vascularize the tumor. c) In the "pillar" design, The TIC is formed from a base PDMS layer, which contains the molded pillars, a spacer with a central void that creates the tumor chamber and PDMS cover. d) Transparent window models allow visualization of the tissue in the dorsal skin (top) or brain (bottom).

Figure 2. Initial response of host tissue to PDMS

a) Low magnification imaging of a rectangular piece of PDMS device placed in the dorsal window chamber. The boundary of the PDMS is indicated by the dashed line. A vascular bundle reaches from the skin and extends over the surface of the PDMS. In this mouse, DsRed expression is driven by the α SMA promoter, so the smooth muscle cell-invested vessels appear red (middle panel). Note that one of the vessels associated with the new tissue structure is α SMA+ (yellow arrowhead). Tie-2 expressing cells are also identified in this mouse by GFP expression (bottom panel). There is colocalization of strong Tie-2 expression with the newly arterIALIZED α SMA+ vessel in the vascular bundle. b) Higher magnification view of the indicated region in (a). The looping system of vessels consists of the feeding arterial highlighted in (a) and a number of draining venules. At the leading edge, flow is stagnant in the blind-ending sprouts, and purple deoxygenated blood is visible (blue arrowhead). c) Region of origin of the new tissue structure. The large venule draining the new structure (*) enters the host capillary bed in a number of places. Some dilation and morphogenesis of the existing capillary bed is evident. The single arteriole (blue arrow) runs parallel to the large venule, and originates at an artery far from the capillary bed (arrow in a). See supplemental videos 1a, b.

Figure 3. Entry of vessel sprouts into a chamber device with access holes

a) In this design, vascular sprouts enter access holes (circles mark the locations of the access holes) and expand radially, connecting with sprouts from other access holes to establish blood flow in the chamber. A main connection generally develops between two holes (arrow). The AK4.4 pancreatic tumor appears as the whitish mass. Scale bar is 500 μm . Angiogenesis was robust in this design, with 4 out of 4 devices showing extensive vascularization in the tumor chamber.

Figure 4. Stromal cell recruitment, collagen deposition and entry of blood vessels

a) Seven days after TIC implantation, matrix fibers (white; visualized with SHG imaging) are visible at the entrance to the chamber, associated with red α SMA-positive cells. b) In many regions, it is apparent that the collagen is attached to the PDMS surface, and aligned, as if in tension. c) Day 2 and 3 near the entrance to a cranial window TIC without a tumor implant. The vascular sprouts enter the chamber where collagen has been deposited. The dark shadow is the boundary of the inner chamber of the TIC. In addition to the Tie-2 expressing endothelial cells, there are also green Tie2⁺ cells not associated with the vascular structures, likely macrophages. To further highlight the perfused vessels FITC-dextran was injected i.v. (green). By day 3, the sprouts have extended farther into the chamber, and some remodeling has occurred in the simple network. Scale bars: 100 μ m.

Figure 5. Vascular sprouts entering the TIC

a-c) Time sequence of new vasculature (green) migrating toward the top-left into a cranial window TIC, past the edge of the PDMS disk (dashed line) with no central tumor (imaged using MPLSM and SHG). This initial process is similar whether or not there is a tumor in the central chamber. Alignment of collagen fibers (white) is evident, and α SMA⁺ cells can be seen on the PDMS surface (red). The vasculature (green, FITC-dextran) extends by forming perfused loops and sprouts. As the matrix remodels, the vessels also remodel as they advance. a: D1: day 1, D3: day 3, D6: day 6 post TIC implantation. A pillar structure is indicated with *. **b)** MLPM image sequence of the expansion of vessel sprouts and vascular loops towards the center of the TIC (red: α SMA⁺ cells, green: blood vessels perfused with FITC-dextran, white: SHG- collagen fibers; * marks the location of a pillar) D1: day 1, D2: day2, D3: day 3, D4: day4, D6: day6. **c, d)** collagen (white) and vasculature (red, rhodamine-dextran injection) near the chamber periphery at day 19 after TIC and 4T1 tumor implantation. At this later stage, regions far from the tumor exhibit normal vascular morphology and collagen organization. The collagen network around many pillars (*) is asymmetrically deformed (**c**, arrowhead). Scale bars: 100 μ m.

Figure 6. Overview of MMTV breast tumor growing in the cranial window TIC

The GFP-expressing tumor cells were initiated in the center of the chamber, and are now being infiltrated by host α SMA⁺ cells (DsRed). The host stromal cells produce ECM fibers (white) that integrate the tumor into the newly forming tissue in the chamber. The migrating vasculature is visible at left (red, injected rhodamine dextran tracer). Such integration of the host tissue and tumor is robust, occurring in 13 out of 15 TICs implanted with the MMTV tumor. The width of the tumor fragment was approximately 2mm.

Figure 7. Collagen-sprout interactions and the effects of Losartan treatment

a) Merged fluorescence/ SHG image showing a rhodamine-dextran-filled tip cell tethered to a bundle of collagen fibers (white) at day 63 in a PDMS TIC implanted with a 4T1 tumor fragment. **b)** Brightfield image of the same sprout extending from a perfused looping segment. The ECM appears white in this image. **c)** Fluorescence image of the structure in a and b, showing the Tie-2 positive cells in green. This is the typical morphology, with a single sprouting process extending outward from a perfused loop, apparently tethered to matrix fiber(s). Scale bars: a-c: 100 μ m. **d)** Extension of a sprout process in the CW TIC at day 24. There is a 4T1 tumor (located out of the field of view, at right). A sprout (arrow) extends on the collagen fibers (white). The vasculature (red) is perfused with rhodamine-dextran. α SMA⁺ cells are also present. **e).** One day later at day 25, the sprout has advanced $\sim$ 100 μ m. There are several structures that resemble intussusceptive vessel splitting at the base of the sprout (arrowheads), which remodel significantly in the 24hour interval. **f)** Relative angle between sprouts and ECM fibers measured at day 7 (D7) and day 14 (D14) as described in the Supplementary Methods section (**Supplementary Fig. 2a**). In control chambers, the sprouts are more strongly aligned with fibers (relative angle = 0); in Losartan treated

chambers, there are fewer sprouts, and a wider range of relative angles. Scale bars: a: 500µm, b-e: 100 µm.

Figure 8. MMTV tumor vasculature in the cranial window pillar TIC

a) MPLSM/SHG image of the indicated region in the brightfield image (b). Near the edge of the PDMS, the vasculature extends radially into the central chamber. At this time point (day 7 after implantation), the vasculature is mature and has normal morphology in the regions far from the tumor. Four feeding arterioles (red arrowheads) and three venules (blue arrowheads) are indicated. These vessels have significant flow (see Supplementary Video 6a), and have acquired smooth muscle cells in their walls (red, α .SMA⁺-DsRed). Note that the arterioles generally have more α .SMA signal than the venules, as expected. c) The

vasculature near the growing tumor has dramatically different morphology and flow (see Supplementary Video 6b,c), as observed in other animal models and human tumors. The tumor was not fluorescently labeled in this group, but is visible as the whitish mass extending from the central tumor (T) in (b).

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Figure 9. Collagen organization in the TIC

a) Isolated collagen fiber image from Figure 6, showing the differences in collagen morphology in the chamber. In addition to normal collagen far from the tumor, we observe TACS 1-3 around the tumor. **B)** Magnified view of box in (a). Elongated matrix fibers are often observed entering the tumor (TACS-3), maintaining connections to the external ECM (arrowheads). It is thought that these allow invasion of tumor cells²⁵. All MMTV tumors examined for collagen formation (n=8) displayed similar collagen signatures around the tumor.