

MACROPHAGES

Macrophages restrict tumor immune infiltration by controlling collagen topography

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During tumorigenesis, the extracellular matrix is extensively remodeled. Whereas the impact of such remodeling on tumor growth and invasion is well described, the consequences on immune infiltration are not well understood. Combining tissue imaging and machine learning, we show that immune cell localization in tumors can be predicted by the local topography of fibrillar collagens. Such topographies are dictated by a fibrotic pathway driven by transcription factor 4 (*Tcf4*) in both cancer and stromal cells, which promotes collagen III deposition and results in intermingled collagen networks that favor intratumor infiltration of T cells and neutrophils. Macrophages inhibit this pathway, highlighting their key structural role in shaping the tumor extracellular matrix. Reanalysis of data from human solid tumors revealed a strong correlation between *TCF4*, *COL3A1*, and T cell and neutrophil signatures. Together, our data identify collagen network topographies as a key regulator of tumor-infiltrating immune cells.

INTRODUCTION

Tumor progression is dictated by the tug of war between cancer and immune cells, and shifting the balance toward immune cells has been successfully exploited in different immunotherapeutic strategies (1). Nevertheless, cancer remains the second leading cause of death worldwide (2), and immunotherapy successes are still limited, with lower success rates generally for solid tumors (3). T cells play a particularly pivotal role, as highlighted by their predictive power on prognosis (4, 5) and response to immunotherapies (6, 7). A central mechanism of antitumor immune responses is T cell-mediated cytotoxicity (8, 9), but their frequent exclusion from the tumor core promotes tumor immune escape (10, 11). One element of the tumor microenvironment mediating this exclusion is the dense extracellular matrix (ECM), in particular collagen, that surrounds tumor islets (12). Fibrillar collagen networks are remodeled during tumorigenesis and evolve over time, leading to distinct topographical organizations named tumor-associated collagen signatures (TACSs) (13, 14). At advanced stages, many tumors harbor a dense capsule of collagen fibers aligned to the tumor border (TACS-2), in which T cells tend to

accumulate, limiting their ability to act on cancer cells (12, 15–17). ECM remodeling also supports tumor progression directly by promoting cancer cell proliferation, survival, and metastasis (18). Reorientation of collagen fibers perpendicular to the tumor edge (TACS-3) has been shown to promote metastasis (13, 14, 19), whereas chaotic reorientation (TACS-6) promotes multidirectional migration of cancer cells (13); however, whether TACSs affect T cell infiltration remains unclear.

Recent studies have shed light on the interplay between tumor ECM remodeling and tumor-associated macrophages (TAMs) (20–22). TAMs promote ECM deposition indirectly by stimulating fibroblasts and directly by producing ECM-associated molecules (20–22) or degrading the ECM themselves (23–26). Whereas TAMs were originally identified as antitumor agents (27), subsequent studies revealed their diversity and more ambiguous role (28). Not only can macrophages impede antitumor immune responses by promoting an immunosuppressive environment (29), but they can also diminish the efficacy of immunotherapies, for example, by directly interacting with T cells and retaining them at the tumor periphery (30) or by capturing anti-PD-1 (programmed cell death protein 1) antibodies, thereby reducing their bioavailability for T cells (31). TAMs have also been shown to affect ECM biophysical properties (20, 21), prompting us to hypothesize that they could thereby regulate tumor immune infiltration.

In this study, we combined imaging and transcriptional analysis to test this hypothesis. We demonstrate that as tumors grow, fibrillar collagen topography dictates T cell infiltration and localization. Depleting macrophages in established fibrotic tumors modulated collagen topographies, rendering specific tumor areas more permissive to both T cells and neutrophils, whereas others only recruited T cells. This process depended on a fibrotic pathway of both tumor and stromal cells driven by the transcription factor (TF) *Tcf4*, which promoted the expansion of collagen III-rich disorganized fibrillar

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networks at the tumor periphery. This favored a TACS-2-like to TACS-6-like transition, which is favorable to immune cell infiltration. We further observed that the *TCF4-COL3A1* axis correlates with T cell and neutrophil numbers in human solid tumors (colorectal, stomach, bladder, and pancreatic), supporting the clinical relevance of our findings.

RESULTS

Macrophage depletion disrupts fibrillar collagen topography in tumors

To address whether TAMs could control T cell infiltration through ECM remodeling during tumorigenesis, we chose a mesenchymal

model (MC38) of colon adenocarcinoma (COAD). This model is known to exhibit a high macrophage infiltration (32), present an aligned collagen capsule reminiscent of TACS-2, and exclude T cells from the tumor core (33). MC38 cells were injected subcutaneously in the flanks of CD64-hDTR (CD64^{DTR}) mice that express the human diphtheria toxin (DT) receptor (DTR) under the control of the CD64 promoter expressed by macrophages and monocytes (34), enabling continuous TAM depletion for 5 days from established tumors (starting at day 9, ≈ 200 mm³; Fig. 1A and fig. S1A). Such prolonged depletion was essential because ECM remodeling is a relatively slow process (35). Macrophage depletion was efficient from day 10, with a 60% reduction in macrophage (F4/80⁺ cells) density, reaching more than 80% at day 14 (Fig. 1, B and C). GR1^{hi}

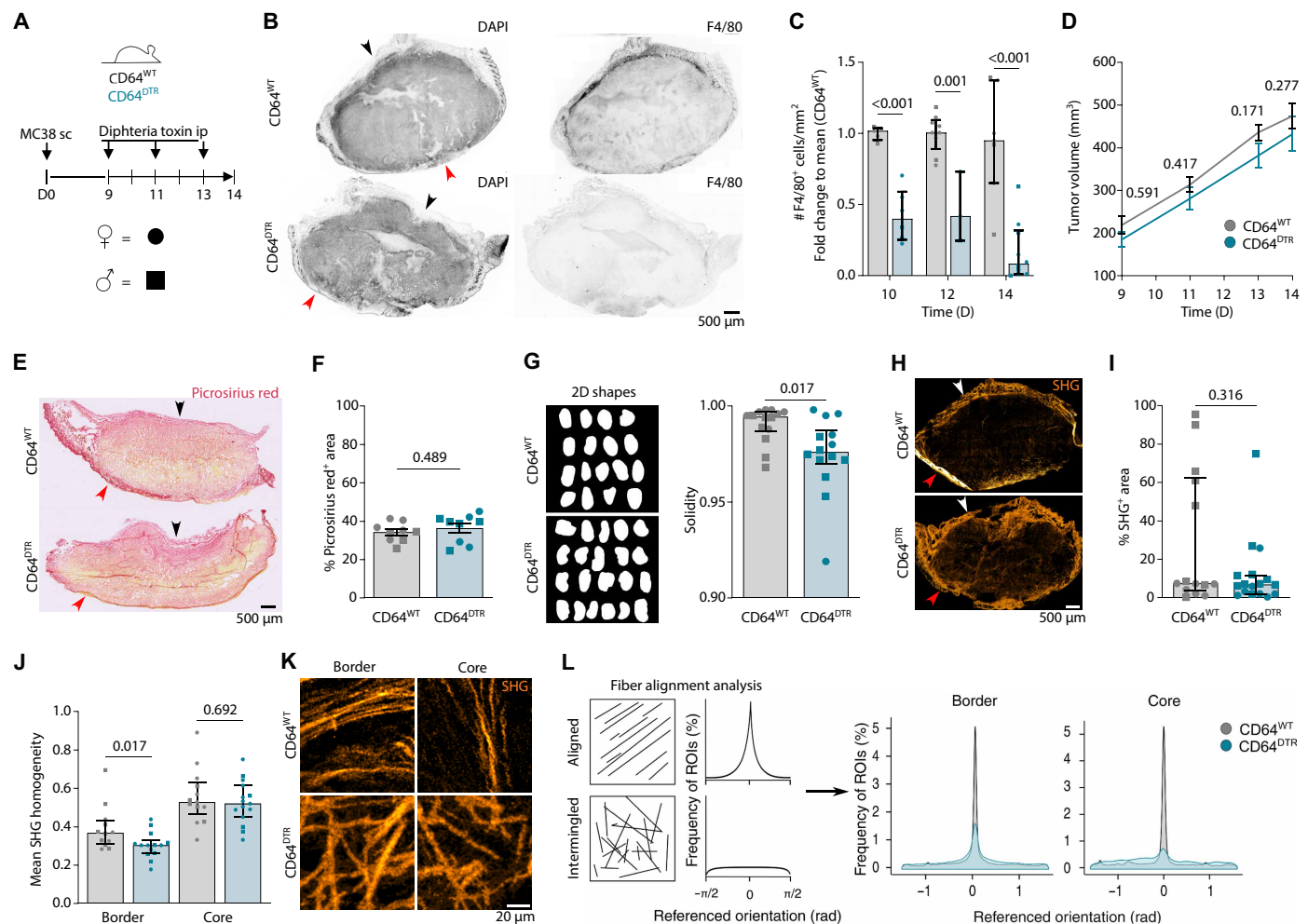


Fig. 1. Macrophage depletion disrupts fibrillar collagen topography in tumors. (A) CD64^{WT} and CD64^{DTR} mice were injected intraperitoneally (ip) with DT at days 9, 11, and 13 after MC38 subcutaneous (sc) injection and euthanized at day 14 (D14). (B) DAPI and F4/80 staining of CD64^{WT} and CD64^{DTR} tumors at day 14 (black arrowhead, tumor border; red arrowhead, skin). (C) Fold change (FC) values for the numbers of F4/80⁺ cells per square millimeter in CD64^{DTR} tumors over CD64^{WT} tumors at days 10, 12, and 14. (D) Tumor volumes from day 9 to day 14 of CD64^{WT} and CD64^{DTR} tumors ($n = 15$ CD64^{WT} and 16 CD64^{DTR}). (E) Picrosirius red (PR) staining and (F) percentages of picrosirius red-positive areas in CD64^{WT} and CD64^{DTR} tumors at day 14. (G) 2D shapes of tumor slices and solidity of CD64^{WT} and CD64^{DTR} tumors at day 14. (H) SHG signals of CD64^{WT} and CD64^{DTR} tumors at day 14. (I) Percentages of SHG⁺ areas in CD64^{WT} and CD64^{DTR} tumors at day 14. (J) Mean homogeneity of SHG images at the tumor borders and in the cores of CD64^{WT} and CD64^{DTR} tumors at day 14 (at least five 320 μ m-by-320 μ m ROIs per tumor). (K) SHG signals at the tumor borders and in the cores of CD64^{WT} and CD64^{DTR} tumors at day 14. (L) Analysis schematic and distribution of ROI referenced orientation at the border (0 to 1000 μ m from the border) and in the core (>1000 μ m) of the tumor ($n = 14$ CD64^{WT} and 18 CD64^{DTR}, 770,766 and 1,097,098 ROIs for CD64^{WT} and CD64^{DTR} tumors, respectively). In all graphs, each dot represents one tumor; squares indicate males, and circles indicate females. Statistics: (C) Median $\pm$ interquartile range (IQR), two-way ANOVA (multiple comparisons); (D) means $\pm$ SEM, multiple Mann-Whitney tests; [(F), (G), (I), and (J)] median $\pm$ IQR, Mann-Whitney test.

(gamma response gene 1–high) monocytes were also efficiently depleted in blood, with a maximum efficacy of 90% at day 10, whereas GR1^{lo} monocyte counts appeared unaffected (fig. S1, B to D). We observed a slight and transient, although nonsignificant, increase in neutrophil counts at day 12, which was resolved at day 14, ruling out potential systemic inflammation caused by macrophage depletion (fig. S1E). Macrophage depletion at such a late stage of tumor development did not induce any significant change in tumor growth (Fig. 1D and fig. S1F), allowing direct comparison of control [CD64 wild-type (WT) (CD64^{WT})] and macrophage-depleted (CD64^{DTR}) tumors, independently of tumor size.

Overall, collagen density was unaltered by macrophage depletion, as assessed by picrosirius red staining (Fig. 1, E and F). However, unexpectedly, the macroscopic shape of tumors was distorted, as shown by decreased shape solidity (Fig. 1G and fig. S1G). Further characterization of the fibrillar collagen architecture by second-harmonic generation (SHG) imaging revealed that CD64^{WT} tumors presented a homogeneous capsule of collagen fibers, all along the edge, on the side opposite to the skin (Fig. 1H, top), whereas CD64^{DTR} tumors exhibited a more heterogeneous capsule (Fig. 1H, bottom). Despite high variability, the percentage of SHG^{hi} regions was unaffected by macrophage depletion (Fig. 1I), confirming that overall fibrillar collagen content was not altered by macrophage depletion. To evaluate the homogeneity of the fibrillar collagen network, we performed gray-level co-occurrence matrix (GLCM) textural analysis of SHG images from both the tumor border and the tumor core. This analysis revealed that (i) the fibrillar collagen network was more homogeneous in the tumor core compared with the border and that (ii) macrophage depletion decreased fibrillar collagen network homogeneity at the tumor edge (Fig. 1J).

Upon closer examination of collagen fiber organization in CD64^{WT} tumors, we observed a thin capsule of aligned collagen fibers parallel to the tumor border (Fig. 1K, top), reminiscent of TACS-2 (14), consistent with the literature (33). By contrast, the capsule of CD64^{DTR} tumors harbored intermingled fibers, with no dominant orientation, resembling TACS-6, which has been defined as “chaotically aligned collagen fibers [...] without a clear tumor boundary” (Fig. 1K, bottom) (13). These observations are consistent with the loss of fiber orientation reported in an orthotopic MC38 model in which macrophages were depleted throughout tumorigenesis (20), as well as in established breast spontaneous tumors (21, 22), where TACSs were originally described (14). To quantify collagen fiber alignment, we used a structure tensor analysis to extract the local orientation of collagen fibers referenced to the mean orientation of a surrounding window (Fig. 1L, left) (36). In CD64^{WT} tumors, both the tumor core and edge exhibited a strong local dominant orientation of collagen fibers, consistent with the homogeneity quantified at a bigger scale by GLCM. Macrophage depletion led to a loss of fiber alignment in the tumor core. In addition, at the edge of macrophage-depleted tumors, we observed a mix of aligned and disorganized fibers, consistent with a decreased network homogeneity (Fig. 1L, right, and data file S2). This effect appeared specific to advanced tumor stages harboring highly organized fibrillar collagen, given that macrophage depletion in early-stage tumors (days 4 to 9; fig. S1, H to J, and data file S3) or in the B16F10 melanoma (days 9 to 14) model that shows markedly lower fibrillar density (fig. S1, K to N) did not alter collagen fiber organization.

Together, these results support a dynamic structural role of TAMs in the organization of fibrillar collagen networks. Their depletion

diversifies local collagen topographies and increases disorganized collagen networks in late-stage fibrotic tumors whose collagen architecture is normally highly organized.

Collagen remodeling is associated with increased T cell infiltration in specific network topographies

It has been proposed that the collagen capsule retains T cells at the edges of tumors, preventing their entry into the tumor core (10, 12). We therefore investigated whether the local changes in collagen organization observed upon macrophage depletion modify tumor permissiveness to T cell infiltration. We found that T cells infiltrated the tumor only at day 14 (Fig. 2A and fig. S2A), consistent with previous reports (33). This infiltration was enhanced by more than twofold upon macrophage depletion (Fig. 2, A and B), leading to increased tumor coverage by T cells (fig. S2B). T cells also penetrated deeper into the tumor (Fig. 2C). This was not due to increased levels of the T cell-attracting chemokines CXCL9 (CXC motif chemokine ligand 9) and CXCL10 in CD64^{DTR} tumors (fig. S2, C and D). In addition, macrophage depletion in early-stage tumors did not increase T cell density and infiltration in CD64^{DTR} tumors (fig. S2, E and F) nor in late B16F10 (fig. S2G), in which collagen was not remodeled.

We then characterized more precisely how infiltrating T cells distribute in advanced tumors. T cells tended to cluster in macrophage-depleted tumors, pointing to local signals driving their accumulation (Fig. 2D). In addition, T cells preferentially accumulated in collagen-rich regions at day 14, independently of macrophage depletion (>90%; Fig. 2E), including at different stages of tumor growth in C57B6/J mice (fig. S2H). Because tissue stiffness is known to be determined by collagen density and topography (37, 38), we measured local stiffness by atomic force microscopy (39). T cell-enriched regions were stiffer than T cell-poor regions, with a markedly stronger difference in CD64^{DTR} tumors (fig. S2I).

These observations prompted us to hypothesize that macrophage depletion renders the tumor more permissive to T cell infiltration by modifying collagen topography of the tumor capsule. We therefore fabricated synthetic three-dimensional (3D) structures of controlled topography by two-photon polymerization of polyethylene glycol diacrylate, coated with fibronectin to mimic the ECM found at the edges of MC38 tumors (40) (fig. S2J). These structures present either aligned (along the *x* axis) or intermingled fibers (Fig. 2F), mimicking TACS-2 and TACS-6, respectively. Ex vivo preactivated splenic T cells migrated with similar 2D speeds on both types of structures (Fig. 2, G and H). However, whereas T cell trajectories on intermingled fibers were isotropic in *x* and *y*, trajectories on aligned fibers exhibited migration anisotropy along the fiber axis (*x*). Such anisotropic migration shortened the distance traveled by T cells in the orthogonal direction (*y*) (Fig. 2I). These results show that T cells better explore their environment when migrating on intermingled fiber topographies and are consistent with our in vivo observations showing an increased penetrance of T cells in the core of CD64^{DTR} tumors exhibiting a TACS-6–like disorganized collagen capsule (Fig. 2C). ECM reorganization observed at day 14 was sufficient to facilitate T cell infiltration but insufficient to allow tumor invasion, given that we did not observe any signs of tumor invasion in draining lymph nodes, lungs, or livers, at later day 22 (fig. S2K).

Focusing on regions of T cell accumulation, we observed that whereas almost all T cell-enriched regions were collagen rich (Fig. 2E and fig. S2H), not all collagen-rich regions were colonized by T cells (Fig. 2J and fig. S2L). This applied to both CD64^{WT} tumors, which

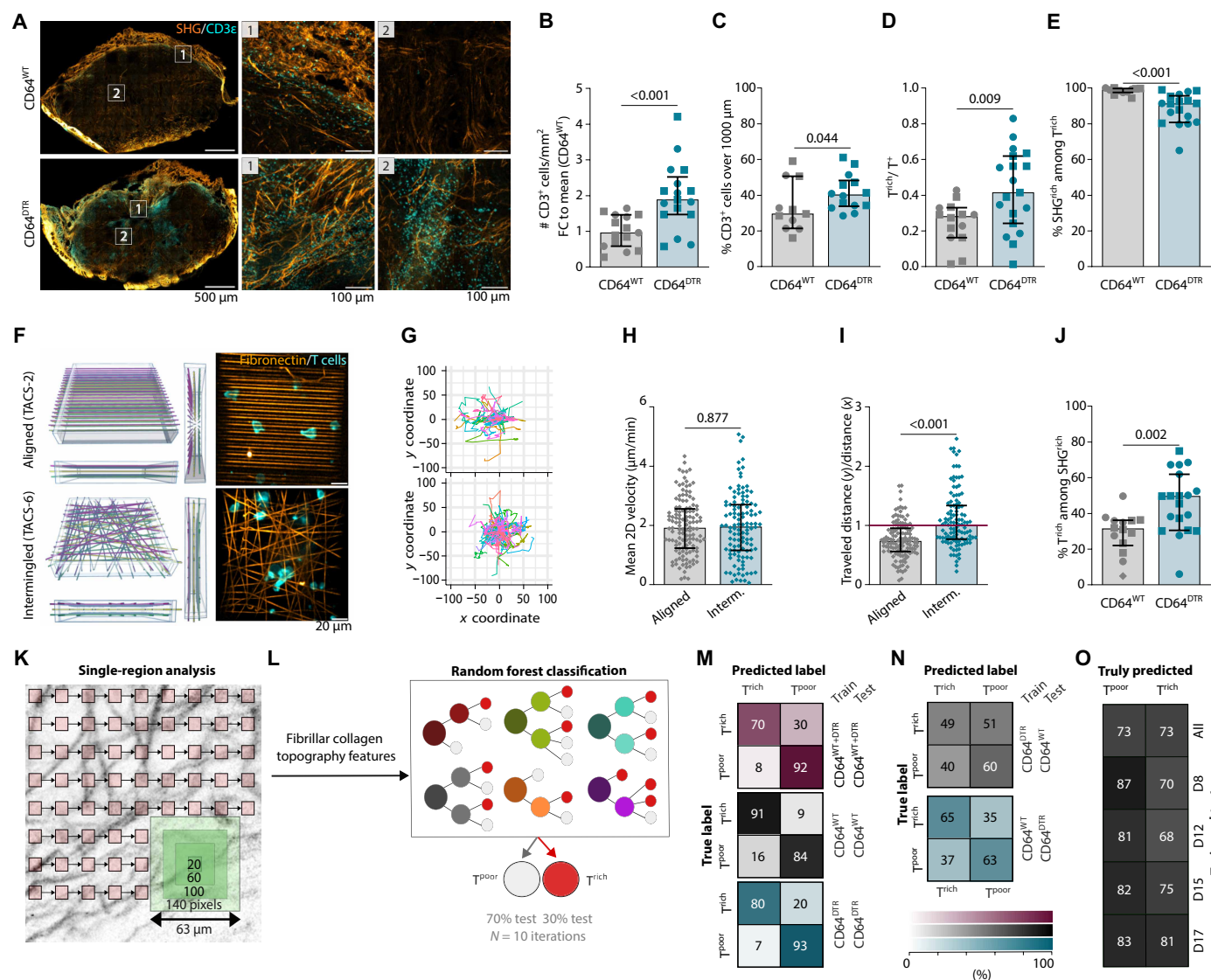


Fig. 2. Collagen remodeling is associated with increased T cell infiltration in specific network topographies. (A) SHG signals and CD3e staining at the borders (1) and in the cores (2) of CD64^{WT} and CD64^{DTR} tumors at day 14 (same sample as Fig. 1H). (B) FC values for the numbers of CD3⁺ cells per square millimeter in CD64^{DTR} tumors over CD64^{WT} tumors at day 14. (C) Percentages of T cells more than 1000 μ m from tumor, (D) ratios of T^{rich} over T⁺ regions, and (E) percentages of T^{rich} regions showing enrichment in SHG in CD64^{WT} and CD64^{DTR} tumors at day 14. (F) Design of 3D aligned or intermingled polyethylene glycol diacrylate fiber networks (left) and confocal images of splenic T cells, preactivated with anti-CD3/CD28 beads and seeded on aligned or intermingled fibers coated with fibronectin (right). (G) Subsampling of fifty 1-hour 2D tracks of T cells seeded in either aligned (top) or intermingled (bottom) structures. (H) Mean T cell 2D velocities in 1 hour in aligned or intermingled structures (dot indicates average of instantaneous speed for a single cell). (I) Ratios of maximum distance traveled by T cells in 1 hour in y axis over x axis in either aligned (top) or intermingled (bottom) structures (dot indicates cell). (J) Percentages of SHG^{rich} regions showing enrichment in T cells in CD64^{WT} and CD64^{DTR} tumors at day 14. (K) Image analysis strategy of two-photon, whole-slide, SHG/CD3 images and (L) of random forest classification of T^{rich} and T^{poor} areas using only collagen topographical features. (M) Confusion matrices (%) obtained after training and testing random forest classification on a CD64^{WT} and CD64^{DTR} merged dataset and on distinct CD64^{WT} or CD64^{DTR} datasets and (N) cross-trained and tested between CD64^{WT} or CD64^{DTR} datasets (n = 10 models). (O) Percentages of truly predicted T^{rich} and T^{poor} regions from confusion matrices obtained after training and testing random forest classification on MC38 tumors at days 8, 12, 15, and 17 combined or split by time point (n = 10 models). In all graphs, each dot represents one tumor unless otherwise indicated. Squares indicate males; circles indicate females. Statistics: Median $\pm$ IQR, Mann-Whitney test.

displayed 31% of collagen-rich regions enriched in T cells on average at day 14 (with a decrease over time from day 8 to day 17), and CD64^{DTR} tumors, in which this number reached 51%. This suggests that (i) collagen networks might locally exhibit specific traits that could favor T cell infiltration and/or accumulation and that (ii) those traits might be enriched in macrophage-depleted tumors. To assess whether collagen topography itself could dictate local T cell

enrichment, we used an unbiased approach that encompasses the complexity of local collagen network topography in tumors and its relationship with T cell infiltration. We analyzed CD3e/SHG images of control and macrophage-depleted tumors, divided in small regions of interest (ROIs) that covered the entire tumor. For each SHG⁺ ROI, we extracted a set of topographical parameters using a structure tensor analysis (36) at different window sizes, as well as T cell

numbers to classify regions as T^{rich} or T^{poor} (Fig. 2K). None of the individual parameters correlated linearly with local T cell numbers in CD64^{WT} and CD64^{DTR} tumors (fig. S3A). In addition, principal components analysis did not segregate T^{rich} and T^{poor} regions, regardless of the presence of macrophages, suggesting that the relationship between T cell enrichment and collagen topographies was nontrivial (fig. S3B).

We next developed a random forest model to classify T^{rich} and T^{poor} regions based exclusively on the SHG topographical features (Fig. 2L). We first tested whether collagen topography was predictive of T cell local enrichment on the merged dataset of both CD64^{WT} and CD64^{DTR} images. Our model could accurately predict T^{poor} regions (92%) and satisfactorily predict T^{rich} regions (70%) (Fig. 2M, top, and fig. S3C), demonstrating that association between T cells and specific collagen network features was not random. We then reasoned that the topographical differences in collagen networks observed in CD64^{WT} and CD64^{DTR} tumors (Fig. 1) and/or a different behavior of T cells could confuse the training of the model and thus dampen its predictive power on the merged datasets. We thus trained and tested separately the model on either CD64^{WT} or CD64^{DTR} images. This strategy increased the performances of T^{rich} prediction to 91 and 80% in CD64^{WT} and CD64^{DTR} tumors, respectively (Fig. 2M, bottom, and fig. S3C), suggesting that T cell enrichment has distinct topographical determinants in CD64^{WT} and CD64^{DTR} tumors. To confirm this finding, we cross-trained the model on one dataset (CD64^{WT} or CD64^{DTR}) and tested it on the other (CD64^{DTR} or CD64^{WT}, respectively), which led to close-to-random classification (Fig. 2N). To identify the ECM parameters that influence T cell localization in the collagen network, we determined the most relevant features of the model using a permutation analysis. In both CD64^{WT} or CD64^{DTR} images, the presence and the heterogeneity of fibers in terms of shape and intensity (energy), as well as their mutual organization (coherency, angle, and referenced orientation), were the main contributing features for efficient learning and accurate prediction (fig. S3D). Our random forest classifier was able to predict T cell local accumulation at days 8, 12, 15, and 17, independently of macrophage depletion, when trained and tested on the merged dataset of all time points, with increased performances when trained and tested on specific time points (Fig. 2O). Our results thus indicate that (i) fibrillar collagen organization dictates areas of T cell local accumulation and that (ii) macrophage depletion promotes a remodeling of fibrillar collagen networks, leading to an increase in T cell infiltration in specific topographical regions of the tumor.

Macrophage depletion induces a profibrotic program in cancer and stromal cells

We next investigated how macrophage depletion regulates the local topography of fibrillar collagen networks. Macrophage-derived transforming growth factor- β 1 (TGF- β 1) has been described as a master regulator of tumor-associated fibrosis (21), and its control of collagen deposition restricts CD8⁺ T cell infiltration (22). Quantification of free TGF- β 1 levels in tumor supernatants and of pSMAD2/3⁺ nuclei in CD64^{DTR} and CD64^{WT} tumors showed no significant difference (Fig. 3, A to C), suggesting that TGF- β 1 was not implicated in our findings. To comprehensively characterize the tumor microenvironment, we performed single-cell RNA sequencing (scRNA-seq) of whole CD64^{WT} and CD64^{DTR} tumors at day 14 (fig. S4A). At low resolution, we identified four cell clusters: nonimmune (*Ptprc*⁻),

lymphocytes (*Ptprc*⁺ and *Cd3e*⁺), monocytes/macrophages/dendritic cells (*Ptprc*⁺, *Itgam*⁺, *Fcgr1*⁺, and *H2-Ab1*⁺), and granulocytes (*Ptprc*⁺, *Csf3r*⁺, and *S100a8*⁺) (Fig. 3D, fig. S4B, and data file S4). Analysis of the monocyte/macrophage/dendritic cell cluster confirmed specific depletion of macrophages and revealed a classical tumor-associated signature (*Fcgr1*⁺, *Adgre1*⁺, *Mrc1*⁺, and *ApoE*⁺; fig. S4, C to F, and data file S5) (41, 42). We focused on ECM genes to identify candidates potentially responsible for regulating collagen networks upon macrophage depletion. As expected, the nonimmune cell cluster expressed the highest levels of core matrisome genes (43) and fibrillar collagen genes (44) (Fig. 3E and data file S6). By subclustering the nonimmune cluster, we identified one cluster of fibroblasts (high expression of *Col1a1*) and two clusters of cancer cells, both expressing the cancer marker *Bgn* and specific gene signatures: C1 (most differentially expressed gene: *Lgals3*) and C2 (most differentially expressed gene: *Airn*) (Fig. 3F, fig. S5A, and data file S7).

The C2 cluster was enriched in CD64^{DTR} tumors (Fig. 3G and fig. S5B) and expressed higher levels of fibrillar collagen genes (at similar level to fibroblasts) compared with the C1 cluster and the highest levels of core matrisome genes compared with the C1 and fibroblast clusters (Fig. 3H). We thus hypothesized that relative expansion of C2 underlies the changes in collagen topography observed in CD64^{DTR} tumors through the production of ECM-related molecules. To test this, we applied the multivariate information-based inductive causation (MIIC) algorithm, which infers direct and possibly causal relationships, as well as indirect pathways, in large networks (45, 46). First, we performed an unbiased MIIC analysis on the top 75 most informative TFs, defined as those whose expression variations best distinguish C1 from C2 signatures, independently of macrophage depletion. The top 535 non-TF genes, selected using the same mutual information threshold as for TFs, were included. Among those genes, collagen III (*Col3a1*) was the collagen gene sharing the highest mutual information with C1 versus C2 signatures, followed by *Col5a1*, *Col5a2*, and *Col8a1* (fig. S5C and data file S8). Increased collagen III abundance was shown to disrupt fibrillar collagen alignment and generate disorganized, wavy collagen networks lacking a dominant orientation in tumors (47, 48), a phenotype reminiscent of that observed in CD64^{DTR} tumors. In contrast, reduced collagen III levels favor the formation of thicker, more linearly aligned fibrils (47). Together, these findings indicate that collagen III is a compelling candidate to explain the loss of fiber alignment and the heterogeneous collagen architecture observed in macrophage-depleted tumors.

The MIIC algorithm proposed a main regulatory axis in the C2 cluster driven by the expression of *transcriptional repressor GATA binding 1* (*Trps1*), which, in turn, controls *T cell factor 4* (*Tcf4*), ultimately regulating the expression of *Col3a1* (Fig. 3I). To test whether this pathway could be engaged by macrophage depletion, we ran the MIIC algorithm on the TFs sharing the highest mutual information with the condition (CD64^{WT} or CD64^{DTR}) (top 50 or top 250 TFs) and total collagen genes [44 genes (43)] in both total cancer cells (C1 + C2) and fibroblasts. The MIIC algorithm returned very similar regulatory networks (Fig. 3, J and K, and fig. S5, D and E). *Col3a1* displayed the highest level of mutual information (fig. S5, F and G) and expression (fig. S5H) in cancer cells and fibroblasts compared with other fibrillar collagens, which was further increased by macrophage depletion (fig. S5H). Accordingly, macrophage depletion increased *Trps1* and *Tcf4* expression in all nonimmune clusters, with the highest levels observed in C2 (fig. S5I). Consistent with unchanged

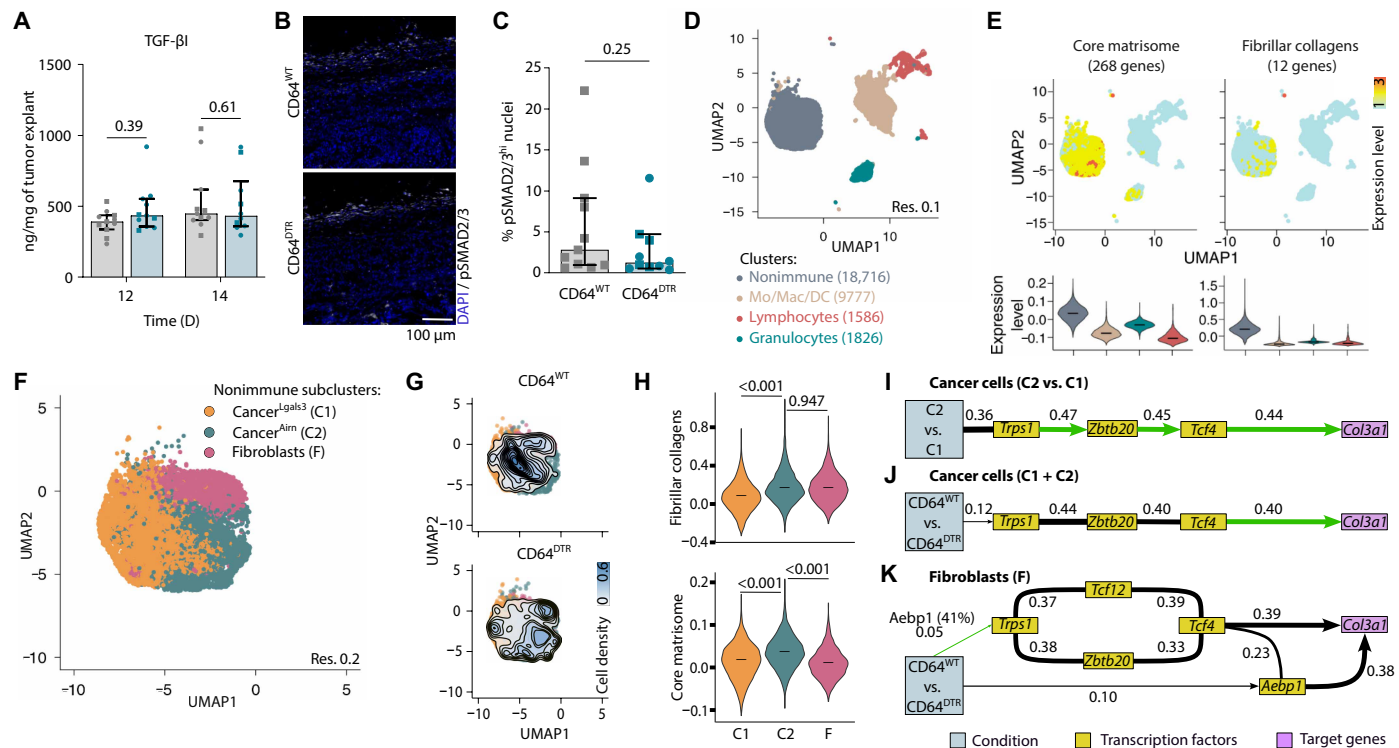


Fig. 3. Macrophage depletion induces a profibrotic program in cancer and stromal cells. (A) Enzyme-linked immunosorbent assay quantification of TGF-β1 in tumor supernatants from CD64^{WT} and CD64^{DTR} tumors at days 12 and 14. (B) DAPI and pSMAD2/3 staining and (C) percentages of pSMAD2/3⁺ nuclei in CD64^{WT} and CD64^{DTR} tumors at day 14. (D) UMAP (Uniform Manifold Approximation and Projection) showing low-resolution clustering of merged scRNA-seq data from CD64^{WT} and CD64^{DTR} tumors from two independent experiments. Mo/Mac/DC, monocyte/macrophage/dendritic cell. (E) UMAPs and violin plots showing core matrisome and fibrillar collagen signature expression levels in low-resolution clusters. (F) Subclustering of the nonimmune cluster from CD64^{WT} and CD64^{DTR} tumors. (G) UMAPs of nonimmune clusters showing cell densities in CD64^{WT} and CD64^{DTR} tumor datasets. (H) Core matrisome and fibrillar collagen signature expression levels in nonimmune clusters. (I) Transcriptional network induced by cancer cell clustering (C2 versus C1) independently of macrophage depletion identified by MIIC analysis (top 75 TFs + top 535 genes). (J) Transcriptional network induced by macrophage depletion in cancer cells (C1 + C2) and (K) in fibroblasts identified by MIIC analysis (top 50 TFs + 44 collagen genes). In (A) and (C), each dot represents one tumor (squares indicate males; circles indicate females). In (D) to (G), one dot represents one cell. Statistics: [(A) and (C)] Median ± IQR, Mann-Whitney test; (H) median, Wilcoxon test.

TGF-β1 levels in CD64^{WT} and CD64^{DTR} tumors, the TGF-β1 pathway (*Tgfb1*, *Tgfb1r1*, *Smad2*, and *Smad3*) was not significantly associated with *Tcf4* or *Col3a1* expression, suggesting that macrophage depletion favors a profibrotic ECM program in tumors independent of the classical TGF-β1-mediated pathway.

To understand how macrophages may control this pathway, we performed a cell-cell interaction analysis using the scRNA-seq data obtained from CD64^{DTR} and CD64^{WT} tumors. This analysis revealed that macrophages (*C1qa*) were the main “senders” among immune cells toward C1, C2, and fibroblast clusters (fig. S5J), with very similar patterns of ligand/receptor interactions (fig. S5K). Ligands included genes implicated in fibrosis (*Cacna1c* and *C1qb*) that could control production of collagen III (49, 50) and genes converging toward the *Wnt*/β-catenin pathway [*Lrp1* (51), *Lrp5* (52), *Sdc2* (53), and *Fgfr2* (54)], which could control *Tcf4* expression (fig. S5K). Binding motif enrichment analysis in the promoter regions of differentially up-regulated genes [$\log_2$ fold change [$\log_2$ (FC)] > 0.25] between CD64^{DTR} and CD64^{WT} in both fibroblasts and cancer cells (C1 + C2) showed that they all exhibited a significant enrichment in *Tcf4* binding motifs (fig. S5L and data file S9). This was also observed when comparing C2 over C1, independently from the condition. We also found enrichment of TCF3 and TCF12 binding motifs, which

showed a high degree of similarity with the TCF4 binding motif (fig. S5L). TCF4, TCF3, and TCF12 are all basic helix-loop-helix type I TFs involved in *Wnt* signaling (55, 56). Together, these results suggest that macrophages repress a *Tcf4*-mediated fibrotic pathway in cancer cells and fibroblasts, which reduces collagen III content in tumors and controls fibrillar collagen topography.

Collagen III-rich networks driven by *Tcf4* support tumor local T cell infiltration

To validate the regulatory pathway predicted by MIIC, we performed immunofluorescence staining for TCF4 and collagen III in tumors. The density of TCF4⁺ nuclei was increased in CD64^{DTR} tumors compared with CD64^{WT} tumors (Fig. 4, A and B), and TCF4⁺ nuclei spatially associated with perinuclear collagen III (Fig. 4C). Collagen III deposition was increased in CD64^{DTR} tumors at the tumor border (Fig. 4, D and E), and T cells localized in COL3^{rich} (collagen III-rich) regions (>88%; Fig. 4F).

However, similar to what we observed with SHG imaging, all COL3^{rich} regions were not enriched in T cells (Fig. 4G), underlining the contribution of other determinants. We thus hypothesized that the enrichment of both fibers (SHG^{rich}) and collagen III may be required. To test this hypothesis, we classified ROIs on the basis of

both SHG and collagen III content and compared the distributions of the various SHG/COL3 profiles in CD64^{WT} and CD64^{DTR} tumors (Fig. 4H, left). SHG^{poor}COL3^{poor} regions dominated in both control and macrophage-depleted tumors (70 and 49%, respectively) but were reduced in macrophage-depleted tumors. As predicted, COL3^{rich} areas (either SHG^{rich} or SHG^{poor}) were increased by twofold in CD64^{DTR} tumors compared with CD64^{WT}. The distribution of SHG/COL3 profiles of T^{rich} areas (Fig. 4H, right) markedly differed from those of tumor areas (Fig. 4H, left). The SHG^{rich}COL3^{rich} areas now largely dominated (50 and 71% in CD64^{WT} and CD64^{DTR} tumors, respectively), and SHG^{rich}COL3^{poor} followed (23 and 21%). No difference was observed in the SHG/COL3 profiles of regions colonized by T cells between CD64^{WT} and CD64^{DTR} tumors or in T cell distribution in WT tumors at days 8, 12, 15, and 17 (fig. S6A). These analyses further reinforced and refined the results from our random forest model: (i) T cells accumulate where fibers (SHG^{rich}) with specific topographies (COL3^{rich}) are, and (ii) the differences in predictions are more likely due to different topographies than to differential behavior of infiltrating T cells.

To formally establish the causal link between *Tcf4*, collagen III, and T cell infiltration, we combined macrophage depletion with the inhibition of *Tcf4*/ β -catenin-mediated transcription using ICG-001 (57). Although *Tcf4*/ β -catenin inhibition had no effect on tumor growth (fig. S6B), we observed a decrease in collagen III density at the tumor edge in CD64^{DTR} but not CD64^{WT} tumors (Fig. 4I), suggesting that this pathway might account for increased collagen III in CD64^{DTR} tumors. Accordingly, inhibition of *Tcf4*/ β -catenin also reduced T cell infiltration and tumor coverage by T cells in CD64^{DTR} tumors (Fig. 4J and fig. S6C), with no noticeable effect on CD64^{WT} tumors, further reinforcing this conclusion.

We next analyzed publicly available bulk RNA-seq databases of human colorectal tumors from The Cancer Genome Atlas (TCGA; COAD dataset). This analysis confirmed the correlation between not only effector T cell infiltration signature (data file S10) and *COL3A1* expression in tumors (fig. S6D), consistent with previous work (58), but also the expression of *TCF4* and *COL3A1*, as well as *TCF4* and effector T cell signatures (Fig. 4K and data file S10). *TCF4*, *COL3A1*, and effector T cell signatures also correlated with the signature of the C2 cluster on the basis of human orthologs of differentially expressed genes between C2 and C1 (Fig. 4L and data file S10). These correlations were not observed in juxtatumoral tissues, suggesting tumor-specific cues required for T cell recruitment. We then evaluated the same correlations in other human solid tumors exhibiting distinct fibrotic levels [assessed by expression of core matrisome, fibrillar collagens, and *COL3A1* [COAD, READ (rectum adenocarcinoma), STAD (stomach adenocarcinoma), BLCA (bladder urothelial carcinoma), and PAAD (pancreatic adenocarcinoma)]; fig. S6, E to G] and uncovered very similar patterns (Fig. 4M and fig. S6H).

Together, our results support a working model where macrophage depletion at a late stage of tumorigenesis drives a *Tcf4*-dependent fibrotic program in cancer cells and fibroblasts that remodels the topography of fibrillar collagen networks through the deposition of collagen III, favoring local T cell accumulation. They further suggest that this pathway may be engaged in several human solid tumors.

Fibrillar collagen topography directs T cell infiltration without affecting their transcriptional state

We then asked whether fibrillar collagen remodeling specifically influenced the recruitment of distinct T cell subsets or exerted a broader

effect on lymphocyte populations. To address this, we performed subclustering of the lymphocyte cluster and identified the following: CD8 exhausted T cells (*Cd3e*⁺, *Cd8a*⁺, *Pdcd1*⁺, *Lag3*⁺, *Havcr2*⁺, *Tox*⁺, and *Ctla4*⁺), a mix of CD8⁺ and CD4⁺ stemlike T cells (*Cd3e*⁺, *Tcf7*⁺, and *Lef1*⁺), B cells (*Cd3e*⁺ and *Cd19*⁺), regulatory T cells (T_{reg} cells; *Cd3e*⁺, *Cd4*⁺, *Pdcd1*⁺, *Lag3*⁺, *Havcr2*⁺, *Tox*⁺, *Il2ra*⁺, *Foxp3*⁺, *Ctla4*⁺, and *Itgae*⁺), and a cluster of natural killer cells (*Cd3e*⁺, *Klrbc1*⁺, *Gzma*⁺, and *Gzmb*⁺) (Fig. 5A, fig. S7A, and data file S11). Unexpectedly, differential gene expression analysis performed on T cells (CD3⁺) from CD64^{WT} and CD64^{DTR} tumors showed no marked difference in transcriptomes (Fig. 5B and data file S12). To examine whether macrophage depletion could alter the composition of infiltrating T cells, we performed multiplexed imaging to quantify different T cell clusters in CD64^{WT} and CD64^{DTR} tumors: CD8 T cells (CD3⁺, CD8⁺, and PD-1⁺), stemlike T cells (CD3⁺ and TCF1⁺), T_{reg} cells (CD3⁺, CD4⁺, and FOXP3⁺), T helper cells (CD3⁺, CD4⁺, and FOXP3⁺), and cycling T cells (CD3⁺ and KI67⁺). Regardless of macrophage depletion, most of the tumor-infiltrating T cells were CD8⁺ and exhibited a terminally exhausted phenotype, consistent with the lack of tumor control. Macrophage depletion did not change the proportion of each T cell cluster but rather increased all T cell numbers (Fig. 5, C to E). Macrophage depletion also did not affect the proportion of cycling T cells and only moderately increased their number, ruling out a contribution of local proliferation in CD64^{DTR} tumors. These results indicate that tumor permissiveness to T cells is not biased toward a specific cluster but rather appears to affect all T cell subtypes similarly. Consistently, *COL3A1* and *TCF4* expression also correlated with multiple T cell subset signatures (exhausted T cells and T helper 1–like cells, for example) in human tumors (Fig. 5F, fig. S7B, and data file S10). These results suggest that the topographical changes in collagen networks induced by late depletion of macrophages promote pan-T cell infiltration and uncouple the effect of TAMs on T cell activation and migration.

The absence of transcriptional reprogramming and stable dynamics between T cell clusters after macrophage depletion may explain the lack of tumor control, suggesting that a strong T cell infiltration alone is insufficient to boost tumor cell killing or drive macroscopic tumor regression. This aligns with the similar densities of cleaved caspase-3 (cCasp3)–positive areas in CD64^{WT} and CD64^{DTR} tumors at day 14 (Fig. 5, G and H). To further test this hypothesis, we combined macrophage depletion with either anti-PD-1 therapy alone or in combination with anti-CTLA4 (cytotoxic T lymphocyte-associated protein 4), given that both therapies have been shown to induce transcriptional reprogramming and change in T cell dynamics (59). Immune checkpoint inhibition combined with macrophage depletion was also insufficient to reduce macroscopic tumor size (Fig. 5, I and J). This could be explained by the reappearance of macrophages in the tumor at day 22 (fig. S7C). Accordingly, we observed that the capsule again presented highly aligned fibers (fig. S7D and data file S13), associated with similar T cell densities (Fig. 5, K and L). However, we detected increased apoptosis-induced killing with anti-PD-1/CTLA4 treatment in CD64^{DTR} tumors (Fig. 5, M and N), suggesting an additive effect of macrophage depletion and immune checkpoint inhibition on the cytotoxicity of T cells.

Fibrillar collagen topography dictates tumor permissiveness to immune infiltration with specific topographical determinants

We next investigated whether the increased tumor permissiveness in macrophage-depleted tumors was restricted to T lymphocytes or

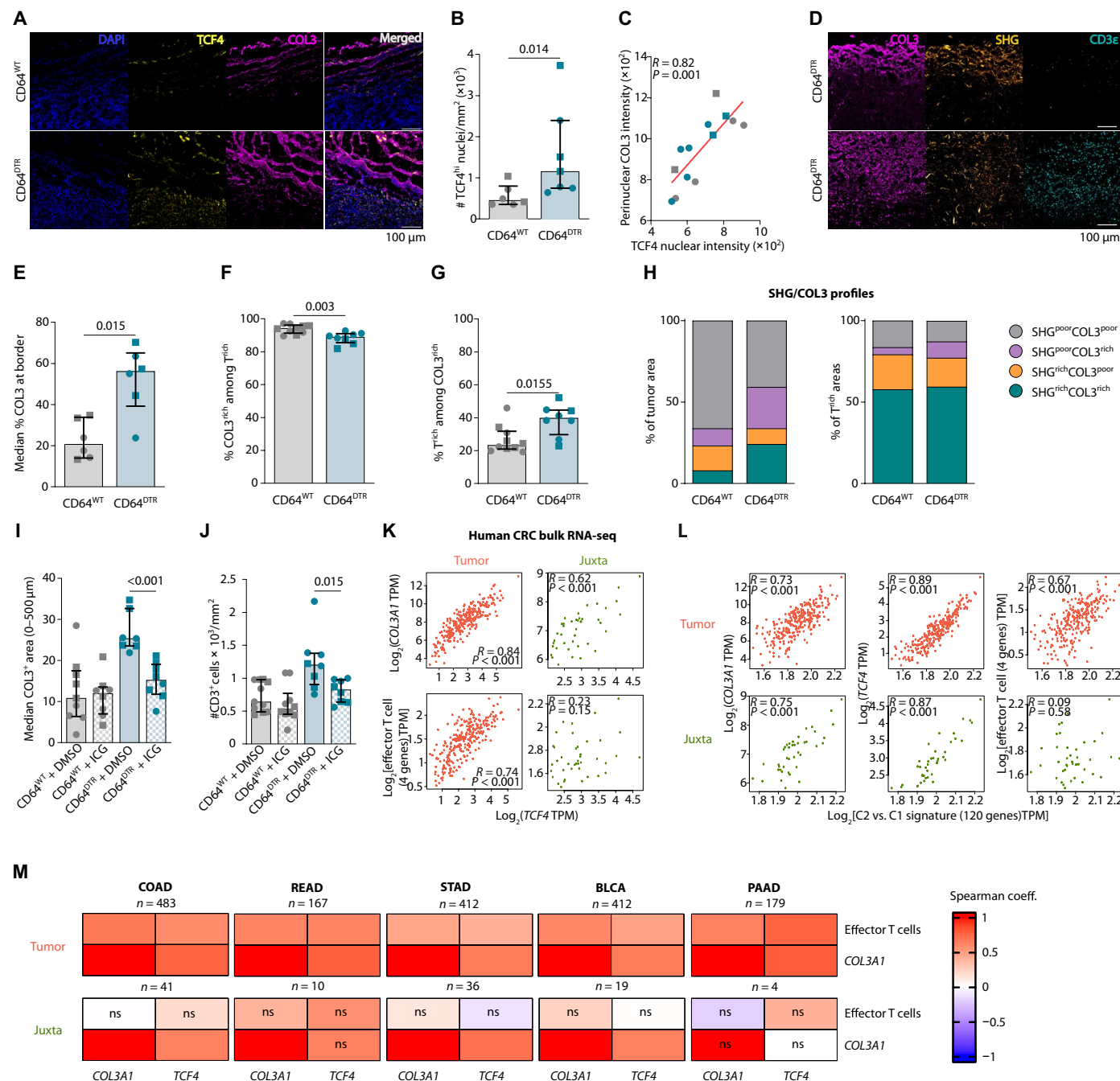


Fig. 4. Collagen III-rich networks driven by *Tcf4* support local T cell infiltration into tumors. (A) DAPI, TCF4, and COL3 staining at the tumor borders of CD64^{WT} and CD64^{DTR} tumors. (B) Numbers of TCF4^{high} nuclei in CD64^{WT} and CD64^{DTR} tumors ($N = 2$ independent experiments; median and interquartile range, Mann-Whitney test). (C) Spearman correlation of mean TCF4 nucleic intensities and mean COL3 perinuclear intensities (5 μ m around nucleus). TCF4 nucleic intensities and COL3 cytoplasmic intensities were calculated for each nucleus in the tumor and averaged by tumor. (D) Two-photon images of COL3, SHG, and CD3 from CD64^{WT} and CD64^{DTR} tumors at day 14. (E) Median percentages of COL3 in tiles located in the first 500 μ m from the tumor borders in CD64^{WT} and CD64^{DTR} tumors at day 14. (F) Percentages of T^{rich} regions showing enrichment in COL3 in CD64^{WT} and CD64^{DTR} tumors at day 14 and (G) percentages of COL3^{rich} regions showing enrichment in T cells in CD64^{WT} and CD64^{DTR} tumors at day 14. (H) Distributions of SHG^{rich}COL3^{rich}, SHG^{rich}COL3^{poor}, SHG^{poor}COL3^{rich}, and SHG^{poor}COL3^{poor} tiles in CD64^{WT} and CD64^{DTR} tumors at day 14 (left). Distributions of SHG^{rich}COL3^{rich}, SHG^{rich}COL3^{poor}, SHG^{poor}COL3^{rich}, and SHG^{poor}COL3^{poor} among T^{rich} tiles in CD64^{WT} and CD64^{DTR} tumors at day 14 (right) ($n = 7$ CD64^{WT} and 5 CD64^{DTR}). (I) Median percentages of COL3 in tiles located in the first 500 μ m from the tumor borders in CD64^{WT} and CD64^{DTR} tumors and (J) numbers of CD3⁺ cells per square millimeter in CD64^{WT} and CD64^{DTR} tumors at day 14 treated with the TCF4 inhibitor ICG-001 or DMSO. (K) Spearman correlation between TCF4, COL3A1, and effector T cell signature; (L) between C2 signature (C2 versus C1) expression and COL3A1, TCF4, and effector T cell signature in TCGA COAD; and (M) between expression of TCF4, COL3A1, and effector T cell signature in TCGA COAD, READ, STAD, BLCA, and PAAD tumors and juxtatumor tissue bulk RNA-seq dataset. In all graphs, each dot represents one tumor. Squares indicate males; circles indicate females. Statistics: Median $\pm$ IQR, Mann-Whitney test for all graphs unless otherwise indicated. ns, not significant.

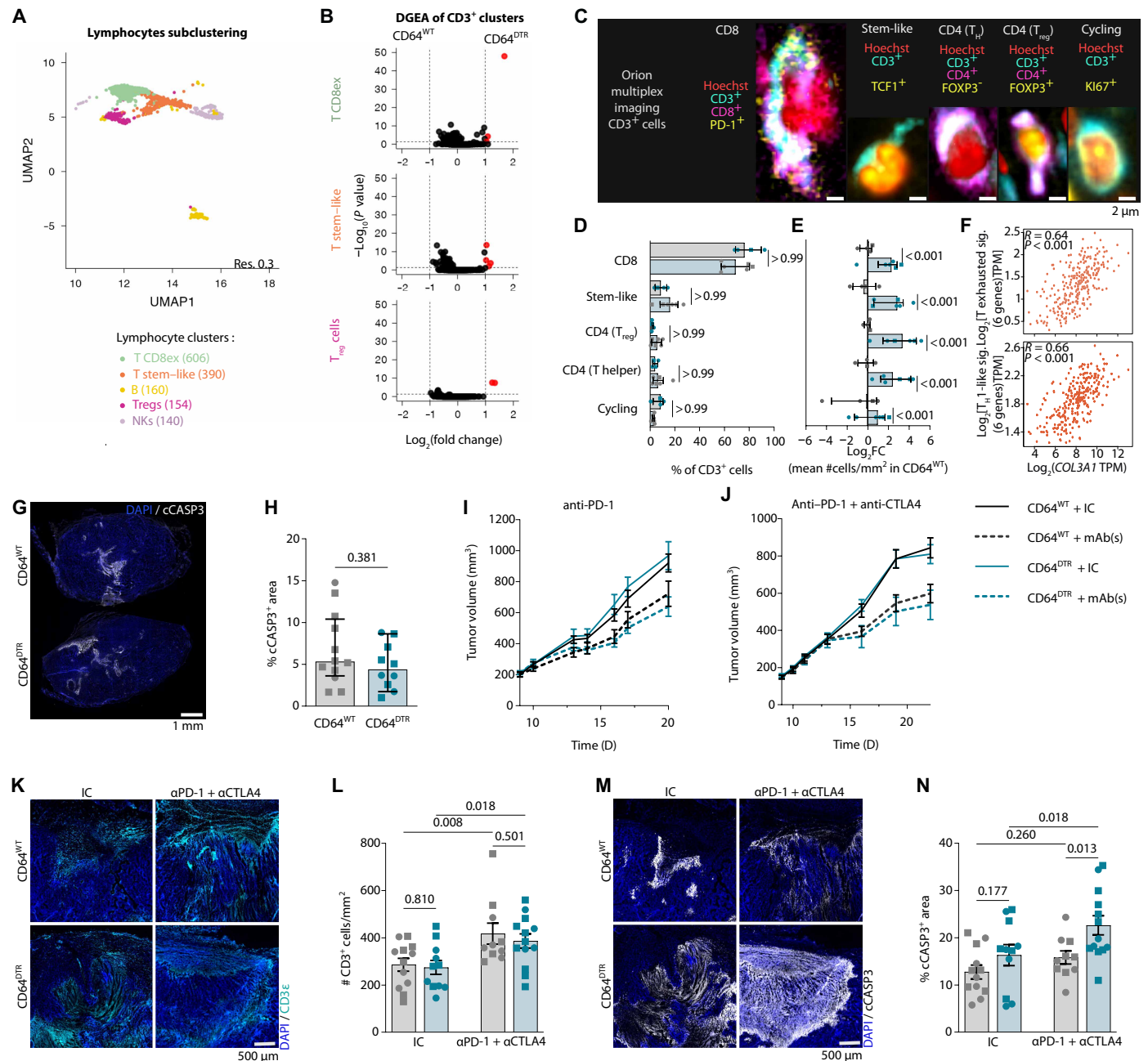


Fig. 5. Fibrillar collagen topography directs T cell infiltration without affecting transcriptional state. (A) UMAP showing subclustering of lymphocyte cluster from CD64^{WT} and CD64^{DTR} tumors. T CD8ex, CD8 exhausted T cells; T stemlike, a mix of CD8⁺ and CD4⁺ stemlike T cells; B, B cells; NKs, natural killer cells. (B) DGEA (differential gene expression analysis) of CD3⁺ clusters of CD64^{WT} and CD64^{DTR} tumors (black dots show genes with $\log_2(\text{FC}) < 1$ and adjusted $P > 0.05$, and red dots show genes with $\log_2(\text{FC}) > 1$ and adjusted $P < 0.05$). (C) Orion multiplex analysis of CD64^{DTR} and CD64^{WT} tumors showing examples of CD8, stemlike, regulatory, helper, and cycling T cells. (D) Proportions and (E) $\log_2(\text{FC})$ values for the numbers of CD3⁺ clusters per square millimeter in CD64^{DTR} and CD64^{WT} tumors. (F) Spearman correlation between expression of COL3A1 and exhausted T cell and T helper 1 (T_H1)-like cell signatures in the TCGA COAD tumor bulk RNA-seq dataset. (G) DAPI and cCASP3 staining and (H) percentages of cCASP3⁺ areas in CD64^{WT} and CD64^{DTR} tumors at day 14. (I) Tumor growth curves from day 9 to day 14 of CD64^{WT} and CD64^{DTR} tumors treated with anti-PD-1 antibody (Ab) or isotype control (IC) ($n = 14$ CD64^{WT} + anti-PD-1, $n = 16$ CD64^{WT} + IC, $n = 13$ CD64^{DTR} + anti-PD-1, and $n = 11$ CD64^{DTR} + IC) and (J) anti-PD-1 + anti-CTLA4 antibodies or ICs ($n = 12$ CD64^{WT} + Ab, $n = 12$ CD64^{WT} + IC, $n = 13$ CD64^{DTR} + Ab, and $n = 13$ CD64^{DTR} + IC). mAb, monoclonal antibody. (K) DAPI and CD3ε staining and (L) Number of CD3⁺ cells per square millimeter in CD64^{WT} and CD64^{DTR} tumors at day 22 treated with anti-PD-1 + anti-CTLA4 antibodies or ICs. (M) DAPI and cCASP3 staining and (N) percentages of cCASP3⁺ areas in CD64^{WT} and CD64^{DTR} tumors at day 22 treated with anti-PD-1 + anti-CTLA4 antibodies or ICs. In all graphs, each dot represents one tumor. Squares indicate males; circles indicate females. Statistics: [(D), (E), (L), and (N)] Median $\pm$ IQR, two-way ANOVA (multiple comparisons); [(I) and (J)] mean $\pm$ SEM; (H) median $\pm$ IQR, Mann-Whitney test.

whether it also applied to other immune cells. Our scRNA-seq revealed an increase in the infiltration of granulocytes in CD64^{DTR} tumors (fig. S8A), which we confirmed by immunofluorescence (Fig. 6, A and B). Similar to T cells (Fig. 2, A to E, and fig. S2, A and B), neutrophils tended to cluster together (Fig. 6C), covered larger areas of the tumor (fig. S8B), and localized in SHG^{hi} regions (Fig. 6D). However, as for T cells, not all SHG^{hi} regions were enriched in neutrophils (Fig. 6E). We therefore postulated that SHG^{hi} regions that were enriched in neutrophils may exhibit specific topographical features leading to their local accumulation, as observed for T cells. A “neutrophil-trained” random forest model on the merged dataset (CD64^{WT} + CD64^{DTR}) accurately predicted Neutrophil^{rich} and Neutrophil^{poor} regions (88 and 86%, respectively; Fig. 6F, top). Training the model separately on CD64^{WT} and CD64^{DTR} led to similar performance, except for the prediction of Neutrophil^{rich} regions by the model trained on CD64^{WT} only (64%), likely because of the smaller size of the dataset (Fig. 6F, bottom). This suggests that the determinants of Neutrophil^{rich} regions are shared between control and macrophage-depleted tumors and that increased neutrophil recruitment in the latter could be due to an increase in permissive areas.

We next investigated whether neutrophil infiltration also relied on the *Tcf4*–collagen III fibrotic pathway. Consistent with this hypothesis, *COL3A1*, *TCF4*, C2, and neutrophil signatures (data file S10) correlated in the human tumor TCGA dataset but not juxtatumor tissues (Fig. 6G and fig. S8, C and D). Chemical inhibition of *Tcf4*/β-catenin with ICG-001 prevented the neutrophil infiltration observed in CD64^{DTR} tumors and resulted in the loss of neutrophil coverage (Fig. 6H and fig. S8E), similar to what we observed for T cells. Unexpectedly, when comparing T cell and neutrophil localization, all neutrophil-rich areas overlapped with T cell-enriched areas, whereas only a minor fraction of T cell-enriched areas was also enriched in neutrophils (Fig. 6, I and J). Neutrophils tended to colonize more T^{rich} areas in CD64^{DTR} tumors when compared with CD64^{WT} or CD64^{DTR} tumors treated with ICG-001, although the variability in neutrophil infiltration compromised statistical significance. The reduced tumor coverage by neutrophils compared with T cells was not due to insufficient numbers of tumor-infiltrating neutrophils, given that T cell and neutrophil numbers were comparable (Fig. 6, A and B, and fig. S2A). Thus, some fibrillar regions present topographies permissive to both T cells and neutrophils, whereas others are specifically permissive to T cells, consistent with our

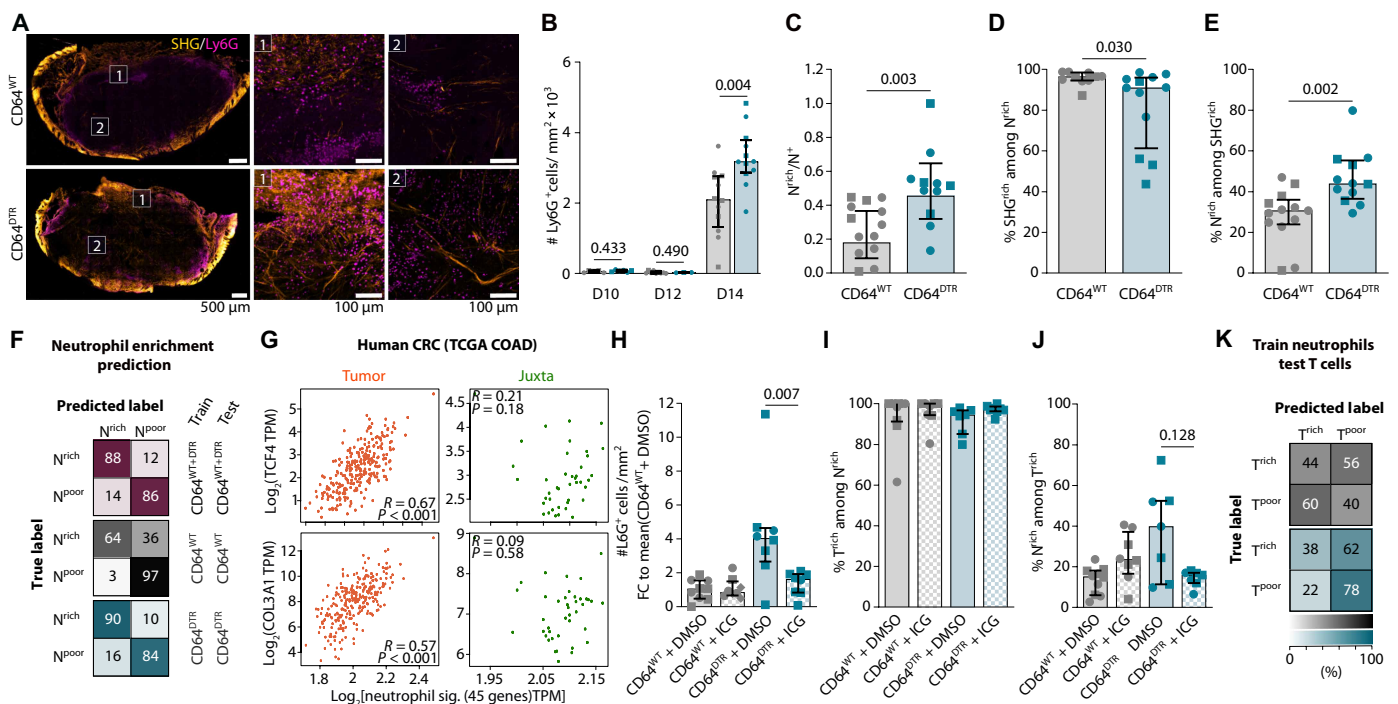


Fig. 6. Fibrillar collagen topography dictates tumor permissiveness to immune infiltration. (A) Two-photon images showing SHG and Ly6G staining at the borders (1) and in the cores (2) of CD64^{WT} and CD64^{DTR} tumors at day 14. (B) Numbers of Ly6G⁺ cells per square millimeter in CD64^{WT} and CD64^{DTR} tumors at days 10, 12, and 14. (C) Ratios of Neutrophil^{rich} (N^{rich}) over Neutrophil⁺ (N⁺) regions (100 μm-by-100 μm tiles) in CD64^{WT} and CD64^{DTR} tumors at day 14. (D) Percentages of Neutrophil^{rich} regions (100 μm-by-100 μm tiles) showing enrichment in SHG signal in CD64^{WT} and CD64^{DTR} tumors at day 14. (E) Percentages of SHG^{rich} regions enriched in neutrophils in CD64^{WT} and CD64^{DTR} tumors at day 14. (F) Confusion matrices (%) obtained after training and testing random forest classification on a CD64^{WT}- and CD64^{DTR}-merged dataset or on distinct CD64^{WT} or CD64^{DTR} datasets ($n = 10$ models). (G) Spearman correlation between expression of *TCF4*, *COL3A1*, and neutrophil signature in TCGA COAD tumors and juxtatumor tissue bulk RNA-seq dataset. (H) Numbers of neutrophils per square millimeter in CD64^{WT} and CD64^{DTR} tumors at day 14 treated with Tcf4 inhibitor ICG-001 or DMSO. (I) Percentages of Neutrophil^{rich} regions (100 μm-by-100 μm tiles) showing enrichment in T cells in CD64^{WT} and CD64^{DTR} tumors at day 14 treated with Tcf4 inhibitor ICG-001 or DMSO. (J) Percentages of T^{rich} regions (100 μm-by-100 μm tiles) showing enrichment in neutrophils in CD64^{WT} and CD64^{DTR} tumors at day 14 treated with the TCF4 inhibitor ICG-001 or DMSO. (K) Confusion matrices (%) obtained after training on Neutrophil^{rich} topographies and tested on T^{rich} topographies in CD64^{WT} or CD64^{DTR} datasets ($n = 10$ models). In all graphs, each dot represents one tumor. Squares indicate males; circles indicate females. Statistics: Median $\pm$ IQR, Mann-Whitney test.

neutrophil-trained random forest model not being able to predict T^{rich} regions (Fig. 6K).

Overall, our work shows that fibrillar collagen topographical features are sufficient to predict the localization of T cells and neutrophils in tumors. However, whereas the recruitment of both cell types is dependent on collagen fibers and collagen III, T cells and neutrophils do not share all topographical determinants, leading to colonization of different regions harboring specific topographies.

DISCUSSION

We show here that macrophage depletion results in a marked remodeling of fibrillar collagen in late-stage colorectal adenocarcinoma subcutaneous tumors, which is associated with increased permissiveness of the tumor to immune infiltration. This effect is specific to tumors with already established collagen capsule, as demonstrated by the absence of effects in early-stage tumors or poorly fibrotic tumors. We demonstrated that macrophage depletion leads to a switch from an aligned network of fibrillar collagen, reminiscent of TACS-2, to a network of intermingled fibers with no dominant orientation, resembling TACS-6. This rearrangement relies on increased deposition of collagen III driven by the TF *Tcf4* in both fibroblasts and cancer cells, a pathway that appears active in different solid tumors in humans. Topographical features of collagen networks alone were sufficient to predict local accumulation of both T cells and neutrophils.

Our work identifies ECM fine topography as a major determinant of the tumor immune landscape at two levels: the penetrance of immune cells in the tumor core and their subsequent local accumulation in specific areas. A dense, cross-linked collagen capsule at the tumor edge has long been considered a key barrier restricting T cell infiltration, with T cells accumulating at its periphery (12, 16, 60, 61). Disrupting the capsule integrity by degrading collagen *ex vivo* or inhibiting its cross-linking *in vivo* enables T cells to penetrate deeper into the tumor core (12, 60). Our results expand upon this concept, showing that macrophage depletion alters the collagen topography, effectively reshaping the tumor ECM into a noncanonical fibrillar collagen network enriched in collagen III. The preference of T cells for COL3^{rich} fiber-enriched regions was observed throughout tumor growth. This reorientation of collagen fibers creates new migratory paths for T cells to infiltrate the tumor, as supported by our *in vitro* experiments on synthetic fibers. Although macrophages have been shown to retain T cells at the tumor edge by directly interacting with them (30), our results indicate that they also play an indirect role by shaping ECM architecture. This permissive topography also enables neutrophil infiltration, revealing a broader immune accessibility.

Machine learning analysis further revealed that the specific accumulation of T cells and neutrophils in collagen-rich regions can be predicted by fibrillar collagen topographical features alone. Although some favorable topographies were shared between T cells and neutrophils, other collagen architectures were specifically associated with T cells, suggesting a broader spectrum of favorable networks for T cells compared with neutrophils. Furthermore, the predictive topographical features for T cells differed between control and macrophage-depleted tumors. This points to either the emergence of new collagen topographies permissive to T cells or a shift in their accessibility because of additional local cues such as chemokines. Deciphering the precise contribution of ECM topography versus chemokine-driven recruitment remains a challenge, given that many chemokines are ECM-bound (62–64).

Through the causative algorithmic (MIIC) analysis of the tumor transcriptome, we shed light on a profibrotic pathway likely resulting in collagen chaotic organization. Macrophage depletion enabled the up-regulation of *Tcf4* expression in cancer cells and fibroblasts, leading to an increased collagen III deposition, which promotes fibrillar collagen network disorganization (47, 48). Inhibiting *Tcf4* in macrophage-depleted tumors rescued the control phenotype. Our results differ from other studies of the MC38 orthotopic model and spontaneous breast tumors, where macrophage removal led to impaired fibrillogenesis through a decrease in collagen and cross-linking enzyme production by fibroblasts and macrophages themselves. These effects were mediated by loss of macrophage-derived TGF- β 1, which was not implicated in our model. Although TGF- β 1 is widely recognized as a master regulator of fibrosis, our findings shed light on an alternative profibrotic pathway mediated by *Tcf4*/*Wnt*- β -catenin signaling, usually repressed by macrophages. *Tcf4* has been previously reported as profibrotic in the liver (57), but, to the best of our knowledge, its role in cancer-associated fibrosis is unknown. Overall, our findings expand the understanding of fibrosis mechanisms governed by macrophages in cancer, highlighting the critical role of *Tcf4*.

We observed the same transcriptional profibrotic axis in both fibroblasts and cancer cells, suggesting that the *Tcf4*-collagen III pathway that we highlight could be a common trait of mesenchymal cells such as fibroblasts and the MC38 tumor model we used, consistent with *Tcf4* description as a pro-epithelial-to-mesenchymal transition (EMT) TF (65). This is consistent with the C2 versus C1 signature correlating with *TCF4* and *COL3A1* not only in tumor but also in juxtatumoral tissues, which suggests that the *TCF4*-*COL3A1* fibrotic axis is active in nontumor tissues. EMT-related TFs such as *Tcf4* can therefore act as double-edged swords. These factors may promote immune infiltration as reported here, but they also support cancer cell survival and invasion. For example, in immunodeficient mice, *Tcf4* inhibition leads to a reduction in tumor growth because of increased apoptosis of cancer cells (66, 67). The shift in ECM composition and increase in collagen III content could also modulate the balance between tumor growth and T cell activity. Collagen III is known to be cytostatic in some cancer models (47, 48, 68), whereas the interaction of T cells with collagen through the inhibitory receptor LAIR-1 (leukocyte-associated immunoglobulin-like receptor 1) was reported to favor T cell exhaustion (69). The exhausted profile of CD8⁺ T cells and their interactions with other immunomodulatory immune populations including neutrophils can also render them inefficient at tumor cell killing (70, 71). Unexpectedly, macrophage depletion did not affect T cell transcriptional profiles and T cell subset proportions. The pro- and antitumor consequences of macrophage depletion could therefore balance each other, preventing tumor reduction despite a massive infiltration of T cells, as we describe here. This could explain the poor clinical benefits of therapeutic strategies targeting macrophages and EMT pathways, including CSF1R (colony stimulating factor 1 receptor) and β -catenin/*TCF4* inhibition (72–74). Our results suggest that failure of such therapies could result from an increase in EMT pathways promoting cancer cell growth in anti-CSF1R therapies and a decrease in T cell infiltration/responses in therapies targeting the β -catenin/*TCF4* complex. However, combining transient macrophage depletion with immune checkpoint inhibitors led to increased T cell killing, revealing potential synergistic effects.

In conclusion, our findings uncover a previously undescribed interplay between macrophages, EMT pathways, and tumor ECM

topography. We demonstrated that macrophage depletion induces a transition from a canonical, aligned collagen TACS-2 capsule to an intermingled collagen III-rich TACS-6-like network, promoting immune infiltration of the tumor. Our work supports recent publications aimed at using collagens and TACSs as prognosis markers (75–77). However, this remains challenging, given that diverse TACSs tend to have dual effects on tumor and immune cells: TACS-2 would exclude T cells from the tumor but prevent tumor cells from exiting the tumor mass and forming metastases (13, 14), whereas TACS-6 would promote immune infiltration but allow multidirectional tumor cell migration (13). Unraveling how the diverse TACSs are established and evolve over tumor progression and characterizing their effects on both cancer and immune cell dynamics are essential to better understand the competition between the tumor and the immune system. This fundamental knowledge may open previously unrecognized avenues to not only improve immunotherapies and anti-EMT drugs but also design therapies directly targeting the tumor ECM.

MATERIALS AND METHODS

Study design

Our study aimed at uncovering the link between TAMs, ECM organization, and T cell infiltration at late stage of tumorigenesis, once the collagen capsule of the tumor represents a barrier for immune cells. To this end, we used a genetic mouse model allowing inducible macrophage depletion (CD64^{DTR} mice). The mice were injected subcutaneously with MC38 tumors, and TAMs were depleted for 5 days before imaging fibrillar collagen (by SHG) and analyzing T cells (immunofluorescence and confocal imaging). We developed a method to segment the images, extract ECM organization features, and predict T cell localization by a random forest model. To understand how topography would affect T cell migration in tumors, we designed an *in vitro* assay on synthetic fibrillar networks mimicking the ECM organization in tumors. We also tested *in vivo* the impact of macrophage depletion on the efficiency of immunotherapies by injecting anti-PD-1 alone or in combination with anti-CTLA4. To uncover how macrophages affected the collagen organization, we performed an scRNA-seq analysis of the whole tumor and analyzed our dataset with a mathematical algorithm (MIIC), enabling the inference of causal networks. We validated the candidate pathways (TCF4 and COL3) by immunofluorescence. Last, to examine the clinical relevance of our findings, we analyzed correlations between the signatures we identified in human datasets from the TCGA.

Mice

All animal experiments were conducted in accordance with European and French regulations (Directive 2010/63/EU; Decree 2013-118) and were approved by the French Ministry of Research (APAFIS #6092-2016072717343792 v5). CD64^{DTR} mice (34) were a gift of S. Henri and B. Malissen and were maintained on a C57BL/6J background. tdTomato/green fluorescent protein (GFP) mice were provided by L. Luo (Stanford University). C57BL/6J mice were purchased from Charles River Laboratories. Mice were housed under specific pathogen-free conditions at Institut Curie, under a 12-hour light-dark cycle with *ad libitum* access to osmotic water and irradiated chow (Mucedola, 4RF25SV). Both female and male mice aged between 8 and 14 weeks were used. Mice exhibiting signs of inflammation, infection, or substantial body weight loss were excluded from experiments.

Cell lines

MC38 COAD cells (gift from N. Haynes, Peter MacCallum Cancer Centre) and B16F10 cells (gift from C. Leclerc, Institut Pasteur, Paris, France) were cultured in RPMI GlutaMAX (Gibco, 61870036) supplemented with 10% fetal bovine serum (Biowest, S1810), 1% penicillin-streptomycin (Thermo Fisher Scientific, 15070063), and 0.1% β -mercaptoethanol. Cells were seeded at 0.2×10^6 cells/ml and split every 2 to 3 days at a 1:4 ratio using TrypLE Express (Gibco, 11528926). For *in vivo* injections, cells were plated at a 1:3 ratio the day before harvesting to achieve ~60% confluence at the time of injection.

In vivo tumor growth

Tumor growth was analyzed using a digital caliper and tumor volume was calculated using the following formula: $(\text{length} \times \text{width}^2)/2$. Tumors, draining lymph nodes, lungs, and liver were collected for subsequent analysis.

Macrophage depletion

Eight- to 14-week-old CD64^{WT} and CD64^{DTR} mice were injected with 0.5×10^6 MC38 or B16F10 cells in 100 μ l in phosphate-buffered saline (PBS) subcutaneously. Macrophage depletion started by intraperitoneal injection of DT (50 μ g/kg; Merck, 322326-1MG) and maintained every 48 hours with DT (17.5 μ g/kg) in PBS. Mice were euthanized 24 hours after the last DT injection (late depletion: DT at days 9, 11, and 13, analysis at day 14; early depletion: DT at days 3, 5, and 7, analysis at day 8).

TCF4 *in vivo* inhibition

CD64^{WT} and CD64^{DTR} mice were injected intraperitoneally with ICG-001 (10 mg/kg; MedChemExpress, HY-14428), resuspended in dimethyl sulfoxide (DMSO; Thermo Fisher Scientific, D139-1), and diluted in 30% polyethylene glycol (Sigma-Aldrich, 81169) 30% 1,2-propanediol (Sigma-Aldrich, 16033) at 9, 11, and 13 d.p.i. [days postinjection (of the tumor)] concomitantly with DT injections. Control mice were injected with the same amount of DMSO diluted in 30% polyethylene glycol and 30% 1,2-propanediol.

Immune checkpoint blockade

CD64^{WT} and CD64^{DTR} mice were then injected intraperitoneally with either anti-PD-1 (10 mg/kg; Leinco, P372) alone or in combination with anti-CTLA4 (10 mg/kg; Bio X Cell, #BE0131) or with the same dose of isotype controls (Euromedex, BX-BE0089 and BE0087, respectively) at days 10, 13, 16, and D19 and euthanized at day 22 d.p.i.

Time course analysis

Eight- to 14-week-old C57B6/J mice (females) were injected with 0.5×10^6 MC38 cells in 100 μ l in PBS subcutaneously. They were euthanized at 8, 12, 15, and 17 d.p.i. for tumor collection and subsequent analysis.

In vitro topotactic migration assay on fibrillar networks

Fibrillar network design, preparation, and loading

Deformable fiber arrays were fabricated as previously described (40). More details are provided in Supplementary Text. Preactivated T cells were used for the assay.

Live imaging and analysis

Imaging was performed on two spinning-disk confocal microscopes (Nikon Eclipse Ti-E, CSU-X1, Yokogawa). Images were acquired with a 561-nm laser for T cells and a 633-nm laser for fibers using Prime BSI or QuantEM cameras. Image stacks (180 μ m by 280 μ m by 31 μ m; voxel size, 0.16 μ m by 0.16 μ m by 1 μ m or 0.4 μ m by 0.4 μ m by 1 μ m) were collected over 5 hours at $\Delta T = 2.5$ - to 3-min

intervals. The analysis was performed using ImageJ (version from May 2017, provided by National Institutes of Health, MD, USA) and IMARIS (v9.2) and MATLAB (R2022b) to extract T cell tracks and compute 2D velocity and distance ratios over a 1-hour period.

Immunofluorescence

Slide preparation and staining

Tumors were fixed in 4% paraformaldehyde (Edimex, 3178-200-19), incubated for 24 hours at 4°C in obscurity, dehydrated in 15 and 30% sucrose (Thermo Fisher Scientific, 10634932), included in optimum cutting temperature (TissueTek, 4583), and stored at -20°C. Tumors were cut into 20-µm sections for SHG imaging or 7-µm sections for epifluorescence imaging. Sections were permeabilized using 0.2% Triton X-100 diluted in PBS, blocked using 1:100 Fc block (BD, 553142; 1:100), 1:20 goat serum (Jackson ImmunoResearch, 005-000-121) or donkey serum (Jackson ImmunoResearch, 0017-000-121) depending on the host of secondary antibodies, and 3% immunoglobulin G-free bovine serum albumin (Jackson ImmunoResearch, 001-000-162) in 0.05% Triton X-100 in PBS. They were stained with the following primary antibodies: anti-F4/80-AF647 (Bio-Rad, MCA497A647; 1:100), anti-CD3e (BioLegend, 100306; 1:200), anti-Ly6G (BioLegend, 127605; 1:200), anti-Col3a1 (SouthernBiotech, 1330-01; 1:300), anti-Tcf4 (ProteinTech, 16801875; 1:100), anti-fibronectin-AF488 (Biotechnie, NBP1-91258AF488; 1:100), and anti-cCasp3 (Cell Signaling Technology, 9664L; 1:100). Donkey anti-goat AF647 (Invitrogen, A21202; 1:200) and donkey anti-rabbit AF546 (Invitrogen, A10040; 1:200) secondary antibodies were used depending on the host of the primary antibody. Slides were mounted in Fluoromount-G with or without 4',6-diamidino-2-phenylindole (DAPI; Invitrogen, 15596276 or 15586276, respectively).

Imaging

Epifluorescence imaging was performed using an AxioScan Z1 microscope (Zeiss) at 20× (Zeiss Plan-Apochromat 20×/0.8 #420650-9901-000). Two-photon and confocal imaging was performed using an inverted two-photon laser scanning confocal microscope (Leica SP8), coupled with a femtosecond laser (Chameleon Vision II, Coherent Inc.) using a 20×/0.75-W HC IRAPO water immersion objective or an inverted Leica Stellaris 8 Dive equipped with a HC FLUOTAR L 25×/0.95-W VISIR 0.17 objective. The excitation wavelength was set at 940 nm, and signals were acquired using three nondescanned HyD detectors: 525/40 nm (GFP), 585/40 nm (tdTomato), and <492 nm (SHG). The acquisition was performed in resonant mode, with Z size of 10 µm and a Z step of 2 µm, and tiling arrays were performed on the whole tumor. Image stitching was performed using the LAS X software (Leica).

Image analysis

Cell detection and quantification were performed in QuPath (0.4.4). For regional analysis, tumors were tiled into 100-µm squares, and enrichment was defined relative to mean density in marker-positive regions. Tumor solidity was computed from manually drawn tumor masks in Fiji using the Shape Descriptor tool. Collagen texture was quantified by GLCMs in Python using scikit-image, with 256 gray levels, averaged across four orientations (0°, 45°, 90°, and 135°) and distances from 1 to 100 pixels (0.45 µm per pixel). GLCM homogeneity is a measure of the structural uniformity of the collagen fibers within a region, which increases as variation or contrast decreases.

SHG features including orientation, coherency, structure tensor energy, intensity, density, and distance to tumor margin were extracted

using the OrientationJ plugin (36) in ImageJ and custom Python scripts. More detailed information about image analysis is available in the Supplementary Materials.

Random forest classification

Random forest predictions were performed using a custom-made Python code. Seventy percent of the initial data were used for training the model, and the remaining 30% were used for the test set. In addition, for the prediction of T cell-rich regions in CD64^{WT} and CD64^{DTR} tumors at day 14, a stratified 10-fold cross-validation was performed on the training set, during which a RandomSearch ($N = 50$) was conducted to find the best combination of parameters based on the performance obtained on the validation set.

Single-cell RNA sequencing

MC38 tumors from CD64^{DTR} and CD64^{WT} were harvested at day 14 d.p.i., and scRNA-seq was performed with 10× technology (more details are provided in the Supplementary Materials). scRNA-seq data were analyzed with R using the Seurat suite (v4). Cells were then filtered out when expressing less than 1000 genes or when expressing more than 10% of mitochondrial genes. Doublets were identified using the DoubletFinder package (78). Differential analysis was done using the DESeq2 package (79), and gene set enrichment analysis was performed using the FGSEA package (80). Cell-cell interaction analysis was performed using LIANA package (81). Data management, quality control, and primary analysis were performed by the Bioinformatics platform of the Institut Curie.

MIIC analysis

MIIC network inference was performed on nonimmune cells using MIIC 2.0.3 version (45, 46). First, the top 50 and 250 TFs with the highest mutual information with the tested condition (CD64^{DTR} versus CD64^{WT}) were selected along with the list of collagen genes (44 genes). For cancer cells, the number of cells was subsampled to 4000 cells. For the analysis between the C1 and C2 cancer clusters, we selected the top 75 most informative TFs between C1 and C2 while including also the top 535 non-TF genes selected with the same mutual information threshold as the 75 TFs.

Human data analysis

Publicly available human transcriptomic data were analyzed using both web-based tools and R-based pipelines. Initial correlation analyses for TCGA COAD samples were performed using GEPIA2, applying Spearman correlation to evaluate pairwise gene-gene associations.

For extended analyses across multiple TCGA projects, all computations were performed in R (v4.3.2). Transcripts per million (TPM) expression values were retrieved from the Genomic Data Commons for five cancer types: COAD, READ, STAD, BLCA, and PAAD. Data collection was performed using the TCGAbiolinks package (v2.30.4).

Statistical analysis

Statistical tests used are indicated in the figure legend of each graph. Graphs and statistical analysis were performed using Prism Graph-Pad (v10.3.1) or R (v4.3.1) software. Mann-Whitney test was used to compare two conditions. Two-way analysis of variance (ANOVA) was performed when more than two conditions were compared. In all graphs, pooled biological replicates (mice for in vivo or cells for in vitro) from independent experiments (N) are plotted on the same graphs. Sample size (n) is indicated in figure or figure legend.

Supplementary Materials

The PDF file includes:

Supplementary Text

Figs. S1 to S8

Table S1

Legends for data files S1 to S13

References (83–86)

Other Supplementary Material for this manuscript includes the following:

Data files S1 to S13

MDAR Reproducibility Checklist

REFERENCES

- K. Esfahani, L. Roudaia, N. Buhlaiga, S. V. Del Rincon, N. Napneja, W. H. Miller Jr., A review of cancer immunotherapy: From the past, to the present, to the future. *Curr. Oncol.* **27**, S87–S97 (2020).
- D. S. Dizon, A. H. Kamal, Cancer statistics 2024: All hands on deck. *CA Cancer J. Clin.* **74**, 8–9 (2024).
- P. Guha, K. R. Heatherton, K. P. O'Connell, I. S. Alexander, S. C. Katz, Assessing the future of solid tumor immunotherapy. *Biomedicines* **10**, 655 (2022).
- J. Galon, A. Costes, F. Sanchez-Cabo, A. Kirilovsky, B. Mlecnik, C. Lagorce-Pagès, M. Tosolini, M. Camus, A. Berger, P. Wind, F. Zinzindohoué, P. Bruneval, P.-H. Cugnenc, Z. Trajanoski, W.-H. Fridman, F. Pagès, Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **313**, 1960–1964 (2006).
- M. Van den Eynde, B. Mlecnik, G. Bindea, T. Fredriksen, S. E. Church, L. Lafontaine, N. Haicheur, F. Marliot, M. Angelova, A. Vasaturo, D. Bruni, A. Jouret-Mourin, P. Baldin, N. Huyghe, K. Haustermans, A. Debucquoy, E. Van Cutsem, J.-F. Gigot, C. Hubert, A. Kartheuser, C. Remue, D. Léonard, V. Valge-Archer, F. Pagès, J.-P. Machiels, J. Galon, The link between the multiverse of immune microenvironments in metastases and the survival of colorectal cancer patients. *Cancer Cell* **34**, 1012–1026.e3 (2018).
- N. Scholler, R. Perbost, F. L. Locke, M. D. Jain, S. Turcan, C. Danan, E. C. Chang, S. S. Neelapu, D. B. Miklos, C. A. Jacobson, L. J. Lekakis, Y. Lin, A. Ghobadi, J. J. Kim, J. Chou, V. Plaks, Z. Wang, A. Xue, M. Mattie, J. M. Rossi, A. Bot, J. Galon, Tumor immune contexture is a determinant of anti-CD19 CART cell efficacy in large B cell lymphoma. *Nat. Med.* **28**, 1872–1882 (2022).
- L. Au, E. Hatipoglu, M. R. de Massy, K. Litchfield, G. Beattie, A. Rowan, D. Schnidrig, R. Thompson, F. Byrne, S. Horswell, N. Fotiadis, S. Hazell, D. Nicol, S. T. C. Shepherd, A. Fendler, R. Mason, L. Del Rosario, K. Edmonds, K. Lingard, S. Sarker, M. Mangwende, E. Carlyle, J. Attig, K. Joshi, I. Uddin, P. D. Becker, M. W. Sunderland, A. Akarca, I. Puccio, W. W. Yang, T. Lund, K. Dhillion, M. D. Vasquez, E. Ghorani, H. Xu, C. Spencer, J. I. López, A. Green, U. Mahadeva, E. Borg, M. Mitchison, D. A. Moore, I. Proctor, M. Falzon, L. Pickering, A. J. S. Furness, J. L. Reading, R. Salgado, T. Marafioti, M. Jamal-Hanjani, PEACE Consortium, G. Kassiotis, B. Chain, J. Larkin, C. Swanton, S. A. Quezada, S. Turajlic, TRACERx Renal Consortium, Determinants of anti-PD-1 response and resistance in clear cell renal cell carcinoma. *Cancer Cell* **39**, 1497–1518.e11 (2021).
- B. Breart, F. Lemaître, S. Celli, P. Bousso, Two-photon imaging of intratumoral CD8⁺ T cell cytotoxic activity during adoptive T cell therapy in mice. *J. Clin. Invest.* **118**, 1390–1397 (2008).
- A. Boissonnas, L. Fetler, I. S. Zeelenberg, S. Hugues, S. Amigorena, In vivo imaging of cytotoxic T cell infiltration and elimination of a solid tumor. *J. Exp. Med.* **204**, 345–356 (2007).
- I. Mellman, D. S. Chen, T. Powles, S. J. Turley, The cancer-immunity cycle: Indication, genotype, and immunotype. *Immunity* **56**, 2188–2205 (2023).
- G. Ortiz-Muñoz, M. Brown, C. B. Carbone, X. Pechuan-Jorge, V. Rouilly, H. Lindberg, A. T. Ritter, G. Raghupathi, Q. Sun, T. Nicotra, S. R. Mantri, A. Yang, J. Doerr, D. Nagarkar, S. Darmanis, B. Haley, S. Mariathasan, Y. Wang, C. Gomez-Roca, C. E. de Andrea, D. Spigel, T. Wu, L. Delamarre, J. Schöneberg, Z. Modrusan, R. Price, S. J. Turley, I. Mellman, C. Moussion, In situ tumour arrays reveal early environmental control of cancer immunity. *Nature* **618**, 827–833 (2023).
- H. Salmon, K. Franciskiewicz, D. Damotte, M.-C. Dieu-Nosjean, P. Validire, A. Trautmann, F. Mami-Chouaib, E. Donnadieu, Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J. Clin. Invest.* **122**, 899–910 (2012).
- G. Xi, W. Guo, D. Kang, J. Ma, F. Fu, L. Qiu, L. Zheng, J. He, N. Fang, J. Chen, J. Li, S. Zhuo, X. Liao, H. Tu, L. Li, Q. Zhang, C. Wang, S. A. Boppart, J. Chen, Large-scale tumor-associated collagen signatures identify high-risk breast cancer patients. *Theranostics* **11**, 3229–3243 (2021).
- P. P. Provenzano, K. W. Eliceiri, J. M. Campbell, D. R. Inman, J. G. White, P. J. Keely, Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC Med.* **4**, 38 (2006).
- H. Bougherara, A. Mansuet-Lupo, M. Alifano, C. Ngò, D. Damotte, M.-A. Le Frère-Belda, E. Donnadieu, E. Peranzoni, Real-time imaging of resident T cells in human lung and ovarian carcinomas reveals how different tumor microenvironments control T lymphocyte migration. *Front. Immunol.* **6**, 500 (2015).
- N. Hartmann, N. A. Giese, T. Giese, I. Poschke, R. Offringa, J. Werner, E. Ryschich, Prevailing role of contact guidance in intrastromal T-cell trapping in human pancreatic cancer. *Clin. Cancer Res.* **20**, 3422–3433 (2014).
- S. Ohno, M. Tachibana, T. Fujii, S. Ueda, H. Kubota, N. Nagasue, Role of stromal collagen in immunomodulation and prognosis of advanced gastric carcinoma. *Int. J. Cancer* **97**, 770–774 (2002).
- J. Prakash, Y. Shaked, The interplay between extracellular matrix remodeling and cancer therapeutics. *Cancer Discov.* **14**, 1375–1388 (2024).
- A. Brabrand, I. I. Kariuki, M. J. Engstrøm, O. A. Haugen, L. A. Dynnes, B. O. Åsvold, M. B. Lilledahl, A. M. Bofin, Alterations in collagen fibre patterns in breast cancer. A premise for tumour invasiveness? *APMIS* **123**, 1–8 (2015).
- R. Afik, E. Zigmond, M. Vugman, M. Klepfish, E. Shimshoni, M. Pasmanik-Chor, A. Shenoy, E. Bassat, Z. Halpern, T. Geiger, I. Sagi, C. Varol, Tumor macrophages are pivotal constructors of tumor collagenous matrix. *J. Exp. Med.* **213**, 2315–2331 (2016).
- O. Maller, A. P. Drain, A. S. Barrett, S. Borgquist, B. Ruffell, I. Zakharevich, T. T. Pham, T. Gruosso, H. Kuasne, J. N. Lakins, I. Acerbi, J. M. Barnes, T. Nemkov, A. Chauhan, J. Gruenberg, A. Nasir, O. Bjarnadottir, Z. Werb, P. Kabos, Y.-Y. Chen, E. S. Hwang, M. Park, L. M. Coussens, A. C. Nelson, K. C. Hansen, V. M. Weaver, Tumour-associated macrophages drive stromal cell-dependent collagen crosslinking and stiffening to promote breast cancer aggression. *Nat. Mater.* **20**, 548–559 (2021).
- K. M. Tharp, K. Kersten, O. Maller, G. A. Timblin, C. Stashko, F. P. Canale, R. E. Menjivar, M.-K. Hayward, I. Berestjuk, J. Ten Hoeve, B. Samad, A. J. Ironside, M. P. di Magliano, A. Muir, R. Geiger, A. J. Combes, V. M. Weaver, Tumor-associated macrophages restrict CD8⁺ T cell function through collagen deposition and metabolic reprogramming of the breast cancer microenvironment. *Nat. Cancer* **5**, 1045–1062 (2024).
- D. H. Madsen, D. Leonard, A. Masedunskas, A. Moyer, H. J. Jürgensen, D. E. Peters, P. Amorphimoltham, A. Selvaraj, S. S. Yamada, D. A. Brenner, S. Burgdorf, L. H. Engelholm, N. Behrendt, K. Holmbeck, R. Weigert, T. H. Bugge, M2-like macrophages are responsible for collagen degradation through a mannose receptor-mediated pathway. *J. Cell Biol.* **202**, 951–966 (2013).
- D. H. Madsen, H. J. Jürgensen, M. S. Siersbæk, D. E. Kuczek, L. Grey Cloud, S. Liu, N. Behrendt, L. Grøntved, R. Weigert, T. H. Bugge, Tumor-associated macrophages derived from circulating inflammatory monocytes degrade collagen through cellular uptake. *Cell Rep.* **21**, 3662–3671 (2017).
- D. H. Madsen, T. H. Bugge, Imaging collagen degradation in vivo highlights a key role for M2-polarized macrophages in extracellular matrix degradation. *OncoTargets Ther.* **2**, e27127 (2013).
- M.-L. Thorseth, M. Carretta, C. Jensen, K. Mølgaard, H. J. Jürgensen, L. H. Engelholm, N. Behrendt, N. Willumsen, D. H. Madsen, Uncovering mediators of collagen degradation in the tumor microenvironment. *Matrix Biol. Plus* **13**, 100101 (2022).
- R. Evans, P. Alexander, Cooperation of immune lymphoid cells with macrophages in tumour immunity. *Nature* **228**, 620–622 (1970).
- M. J. Pittet, O. Michielin, D. Migliorini, Clinical relevance of tumour-associated macrophages. *Nat. Rev. Clin. Oncol.* **19**, 402–421 (2022).
- A. L. Doedens, C. Stockmann, M. P. Rubinstein, D. Liao, N. Zhang, D. G. DeNardo, L. M. Coussens, M. Karin, A. W. Goldrath, R. S. Johnson, Macrophage expression of hypoxia-inducible factor-1 α suppresses T-cell function and promotes tumor progression. *Cancer Res.* **70**, 7465–7475 (2010).
- E. Peranzoni, J. Lemoine, L. Vimeux, V. Feuillet, S. Barrin, C. Kantari-Mimoun, N. Bercovici, M. Guérin, J. Biton, H. Ouakrim, F. Régnier, A. Lupo, M. Alifano, D. Damotte, E. Donnadieu, Macrophages impede CD8 T cells from reaching tumor cells and limit the efficacy of anti-PD-1 treatment. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E4041–E4050 (2018).
- S. P. Arlauckas, C. S. Garris, R. H. Kohler, M. Kitaoka, M. F. Cuccarese, K. S. Yang, M. A. Miller, J. C. Carlson, G. J. Freeman, R. M. Anthony, R. Weissleder, M. J. Pittet, In vivo imaging reveals a tumor-associated macrophage-mediated resistance pathway in anti-PD-1 therapy. *Sci. Transl. Med.* **9**, eaal3604 (2017).
- S. Hoves, C.-H. Ooi, C. Wolter, H. Sade, S. Bissinger, M. Schmittnaegel, O. Ast, A. M. Giusti, K. Wartha, V. Runza, W. Xu, Y. Kienast, M. A. Cannarile, H. Levitsky, S. Romagnoli, M. De Palma, D. Rüttinger, C. H. Ries, Rapid activation of tumor-associated macrophages boosts preexisting tumor immunity. *J. Exp. Med.* **215**, 859–876 (2018).
- N. J. Shields, E. M. Peyroux, A. L. Ferguson, M. Steain, S. L. Young, Late-stage MC38 tumours recapitulate features of human colorectal cancer – Implications for appropriate timepoint selection in preclinical studies. *Front. Immunol.* **14**, 1152035 (2023).
- A. Baranska, A. Shawket, M. Jouve, M. Baratin, C. Malosse, O. Voluzan, T.-P. Vu Manh, F. Fiore, M. Bajénoff, P. Benaroch, M. Dalod, M. Malissen, S. Henri, B. Malissen, Unveiling skin macrophage dynamics explains both tattoo persistence and strenuous removal. *J. Exp. Med.* **215**, 1115–1133 (2018).
- A. Naba, Ten years of extracellular matrix proteomics: Accomplishments, challenges, and future perspectives. *Mol. Cell. Proteomics* **22**, 100528 (2023).

36. Z. Püspöki, M. Storath, D. Sage, M. Unser, Transforms and operators for directional bioimage analysis: A survey. *Adv. Anat. Embryol. Cell Biol.* **219**, 69–93 (2016).
37. J. Comelles, V. Fernández-Majada, V. Acevedo, B. Rebollo-Calderon, E. Martínez, Soft topographical patterns trigger a stiffness-dependent cellular response to contact guidance. *Mater. Today Bio* **19**, 100593 (2023).
38. K. Riching, B. Cox, M. Salick, C. Pehlke, A. S. Riching, S. Ponik, B. R. Bass, W. Crone, Y. Jiang, A. M. Weaver, K. Eliceiri, P. Keely, 3D collagen alignment limits protrusions to enhance breast cancer cell persistence. *Biophys. J.* **107**, 2546–2558 (2014).
39. J. L. Hutter, J. Bechhoefer, Calibration of atomic-force microscope tips. *Rev. Sci. Instrum.* **64**, 1868–1873 (1993).
40. P. Ucla, J. L. L. Chénais, H. Ver Hulst, S. Xu, I. Calvente, E. Nematollahi, L. Leconte, J. Salameró, I. Bonnet, C. Monnot, H. D. Moreau, J. Landoulsi, V. Semetey, S. Coscoy, Quantifying cell traction forces at the single-fiber scale in 3D: An approach based on deformable photopolymerized fiber arrays. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2507677122 (2025).
41. L. Zhang, Z. Li, K. M. Skrzypczynska, Q. Fang, W. Zhang, S. A. O'Brien, Y. He, L. Wang, Q. Zhang, A. Kim, R. Gao, J. Orf, T. Wang, D. Sawant, J. Kang, D. Bhatt, D. Lu, C.-M. Li, A. S. Rapaport, K. Perez, Y. Ye, S. Wang, X. Hu, X. Ren, W. Ouyang, Z. Shen, J. G. Egen, Z. Zhang, X. Yu, Single-cell analyses inform mechanisms of myeloid-targeted therapies in colon cancer. *Cell* **181**, 442–459.e29 (2020).
42. M. Laviro, M. Petit, E. Weber-Delacroix, A. J. Combes, A. R. Arkal, S. Barthélémy, T. Courau, D. A. Hume, C. Combadière, M. F. Krummel, A. Boissonnas, Tumor-associated macrophage heterogeneity is driven by tissue territories in breast cancer. *Cell Rep.* **39**, 110865 (2022).
43. R. O. Hynes, A. Naba, Overview of the matrisome—An inventory of extracellular matrix constituents and functions. *Cold Spring Harb. Perspect. Biol.* **4**, a004903 (2012).
44. S. Ricard-Blum, The collagen family. *Cold Spring Harb. Perspect. Biol.* **3**, a004978 (2011).
45. L. Verry, N. Sella, S. Affeldt, P. P. Singh, H. Isambert, Learning causal networks with latent variables from multivariate information in genomic data. *PLOS Comput. Biol.* **13**, e1005662 (2017).
46. N. Sella, L. Verry, G. Uguzzoni, S. Affeldt, H. Isambert, MISC online: A web server to reconstruct causal or non-causal networks from non-perturbative data. *Bioinformatics* **34**, 2311–2313 (2018).
47. B. K. Brisson, E. A. Mauldin, W. Lei, L. K. Vogel, A. M. Power, A. Lo, D. Dopkin, C. Khanna, R. G. Wells, E. Puré, S. W. Volk, Type III collagen directs stromal organization and limits metastasis in a murine model of breast cancer. *Am. J. Pathol.* **185**, 1471–1486 (2015).
48. J. S. Di Martino, A. R. Nobre, C. Mondal, I. Taha, E. F. Farias, E. J. Fertig, A. Naba, J. A. Aguirre-Ghisio, J. J. Bravo-Cordero, A tumor-derived type III collagen-rich ECM niche regulates tumor cell dormancy. *Nat. Cancer* **3**, 90–107 (2022).
49. H. Li, A. Korcari, D. Ciufo, C. L. Mendias, S. A. Rodeo, M. R. Buckley, A. E. Loisele, G. S. Pitt, C. Cao, Increased Ca²⁺ signaling through Ca_v1.2 induces tendon hypertrophy with increased collagen fibrillogenesis and biomechanical properties. *FASEB J.* **37**, e23007 (2023).
50. T. Ogawa, S. Shichino, S. Ueha, S. Ogawa, K. Matsushima, Complement protein C1q activates lung fibroblasts and exacerbates silica-induced pulmonary fibrosis in mice. *Biochem. Biophys. Res. Commun.* **603**, 88–93 (2022).
51. A. Zilberberg, A. Yaniv, A. Gazit, The low density lipoprotein receptor-1, LRP1, interacts with the human frizzled-1 (HFZ1) and down-regulates the canonical Wnt signaling pathway. *J. Biol. Chem.* **279**, 17535–17542 (2004).
52. Q. Ren, J. Chen, Y. Liu, LRP5 and LRP6 in Wnt signaling: Similarity and divergence. *Front. Cell Dev. Biol.* **9**, 670960 (2021).
53. L. Naraghi, A. Koval, V. L. Katanaev, S. M. A. Najafi, Syndecan-2 positively regulates Wnt/β-catenin signaling in breast cancer cells. *Int. J. Biochem. Cell Biol.* **188**, 106853 (2025).
54. L. Bobzin, A. Nickle, S. Ko, M. Ince, A. Huang, A. Bhojwani, R. Roberts, A. E. Merrill, FGFR2 directs inhibition of WNT signaling to regulate anterior fontanelle closure during skull development. *Development* **152**, dev204264 (2025).
55. M. Wedel, F. Fröb, O. Elssesser, M.-T. Wittmann, D. C. Lie, A. Reis, M. Wegner, Transcription factor Tcf4 is the preferred heterodimerization partner for Olig2 in oligodendrocytes and required for differentiation. *Nucleic Acids Res.* **48**, 4839–4857 (2020).
56. J. H. van Es, A. Haegerbarth, P. Kujala, S. Itzkovitz, B.-K. Koo, S. F. Boj, J. Korving, M. van den Born, A. van Oudenaarden, S. Robine, H. Clevers, A critical role for the Wnt effector Tcf4 in adult intestinal homeostatic self-renewal. *Mol. Cell. Biol.* **32**, 1918–1927 (2012).
57. Y. Gao, S. Fan, P. Zhao, H. Li, C. Cai, X. Li, Y. Zhou, M. Huang, H. Bi, β-Catenin/TCF4 inhibitors ICG-001 and LF3 alleviate BDL-induced liver fibrosis by suppressing LECT2 signaling. *Chem. Biol. Interact.* **371**, 110350 (2023).
58. H. Zhang, C. Ding, Y. Li, C. Xing, S. Wang, Z. Yu, L. Chen, P. Li, M. Dai, Data mining-based study of collagen type III alpha 1 (COL3A1) prognostic value and immune exploration in pan-cancer. *Bioengineered* **12**, 3634–3646 (2021).
59. S. Kurtulus, A. Madi, G. Escobar, M. Klapholz, J. Nyman, M. Pawlak, D. Dionne, J. Xia, O. Rozenblatt-Rosen, V. K. Kuchroo, A. Regev, A. C. Anderson, Checkpoint blockade immunotherapy induces dynamic changes in PD-1⁺ CD8⁺ tumor-infiltrating T cells. *Immunity* **50**, 181–194.e6 (2019).
60. A. Nicolas-Boluda, J. Vaquero, L. Vimeux, T. Guilbert, S. Barrin, C. Kantari-Mimoun, M. Ponzo, G. Renault, P. Deptula, K. Pogoda, R. Bucki, I. Cascone, J. Courty, L. Fouassier, F. Gazeau, E. Donnadieu, Tumor stiffening reversion through collagen crosslinking inhibition improves T cell migration and anti-PD-1 treatment. *eLife* **10**, e58688 (2021).
61. H. Ueno, A. M. Jones, K. H. Wilkinson, J. R. Jass, I. C. Talbot, Histological categorisation of fibrotic cancer stroma in advanced rectal cancer. *Gut* **53**, 581–586 (2004).
62. T. Gerlza, M. Nagele, M. Gschwandtner, S. Winkler, A. Kungl, Designing an improved T-cell mobilising CXCL10 mutant through enhanced GAG binding affinity. *Protein Eng. Des. Sel.* **32**, 367–373 (2019).
63. A. L. Gray, R. Karlsson, A. R. E. Roberts, A. J. L. Ridley, N. Pun, B. Khan, C. Lawless, R. Luís, M. Szpakowska, A. Chevnigé, C. E. Hughes, L. Medina-Ruiz, H. L. Birchenough, I. Z. Mulholland, C. L. Salanga, E. A. Yates, J. E. Turnbull, T. M. Handel, G. J. Graham, T. A. Jowitt, I. Schiessl, R. P. Richter, R. L. Miller, D. P. Dyer, Chemokine CXCL4 interactions with extracellular matrix proteoglycans mediate widespread immune cell recruitment independent of chemokine receptors. *Cell Rep.* **42**, 111930 (2023).
64. X. Bao, E. A. Moseman, H. Saito, B. Petryniak, B. Petryaniuk, A. Thiriot, S. Hatakeyama, Y. Ito, H. Kawashima, Y. Yamaguchi, J. B. Lowe, U. H. von Andrian, M. Fukuda, Endothelial heparan sulfate controls chemokine presentation in recruitment of lymphocytes and dendritic cells to lymph nodes. *Immunity* **33**, 817–829 (2010).
65. E. Sánchez-Tilló, O. de Barrios, L. Siles, M. Cuatrecasas, A. Castells, A. Postigo, β-Catenin/TCF4 complex induces the epithelial-to-mesenchymal transition (EMT)-activator ZEB1 to regulate tumor invasiveness. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 19204–19209 (2011).
66. W. Wei, M.-S. Chua, S. Grepper, S. So, Small molecule antagonists of Tcf4/β-catenin complex inhibit the growth of HCC cells in vitro and in vivo. *Int. J. Cancer* **126**, 2426–2436 (2010).
67. S. H. Shin, D. Y. Lim, K. Reddy, M. Malakhova, F. Liu, T. Wang, M. Song, H. Chen, K. B. Bae, J. Ryu, K. Liu, M.-H. Lee, A. M. Bode, Z. Dong, A small molecule inhibitor of the β-catenin-TCF4 interaction suppresses colorectal cancer growth in vitro and in vivo. *EBioMedicine* **25**, 22–31 (2017).
68. D. C. Stewart, B. K. Brisson, B. Dekky, A. C. Berger, W. Yen, E. A. Mauldin, C. Loebel, D. Gillette, C.-A. Assenmacher, C. Quincey, D. Stefanovski, M. Cristofanilli, E. Cukierman, J. A. Burdick, V. F. Borges, S. W. Volk, Prognostic and therapeutic implications of tumor-restrictive type III collagen in the breast cancer microenvironment. *npj Breast Cancer* **10**, 86 (2024).
69. D. H. Peng, B. L. Rodriguez, L. Diao, L. Chen, J. Wang, L. A. Byers, Y. Wei, H. A. Chapman, M. Yamauchi, C. Behrens, G. Raso, L. M. S. Soto, E. R. P. Cuentes, I. I. Wistuba, J. M. Kurie, D. L. Gibbons, Collagen promotes anti-PD-1/PD-L1 resistance in cancer through LAIR1-dependent CD8⁺ T cell exhaustion. *Nat. Commun.* **11**, 4520 (2020).
70. Y. Tie, F. Tang, Y.-Q. Wei, X.-W. Wei, Immunosuppressive cells in cancer: Mechanisms and potential therapeutic targets. *J. Hematol. Oncol.* **15**, 61 (2022).
71. L. M. McLane, M. S. Abdel-Hakeem, E. J. Wherry, CD8 T cell exhaustion during chronic viral infection and cancer. *Annu. Rev. Immunol.* **37**, 457–495 (2019).
72. A. Moeller, R. Kurzrock, G. P. Botta, J. J. Adashek, H. Patel, S. Lee, S. Pabla, M. K. Nesline, J. Conroy, J. K. Sicklick, S. Kato, Challenges and prospects of CSF1R targeting for advanced malignancies. *Am. J. Cancer Res.* **13**, 3257–3265 (2023).
73. H. Colman, J. J. Raizer, T. Walbert, S. R. Plotkin, M. C. Chamberlain, E. T. Wong, V. K. Puduvalli, D. A. Reardon, F. M. Iwamoto, M. M. Mrugala, B. Johnson, K. Sonty, D. A. Karlin, M. Pelayo, M. Hutchinson, H. Hsu, Phase 1b/2 study of pexidartinib (PEX) in combination with radiation therapy (XRT) and temozolomide (TMZ) in newly diagnosed glioblastoma. *J. Clin. Oncol.* **36**, 2015 (2018).
74. M. Kahn, Can we safely target the WNT pathway? *Nat. Rev. Drug Discov.* **13**, 513–532 (2014).
75. K. S. Guo, A. S. Brodsky, Tumor collagens predict genetic features and patient outcomes. *NPJ Genom. Med.* **8**, 15 (2023).
76. M. Morkunas, D. Zilenaite, A. Laurinaviciene, P. Treigys, A. Laurinavicius, Tumor collagen framework from bright-field histology images predicts overall survival of breast carcinomas. *Sci. Rep.* **11**, 15474 (2021).
77. K. Mirsanaye, L. Uribe Castaño, Y. Kamaliddin, A. Golaraei, R. Augulis, L. Kontenis, S. J. Done, E. Žurauskas, V. Stambolic, B. C. Wilson, V. Barzda, Machine learning-enabled cancer diagnostics with widefield polarimetric second-harmonic generation microscopy. *Sci. Rep.* **12**, 10290 (2022).
78. C. S. McGinnis, L. M. Murrow, Z. J. Gartner, DoubletFinder: Doublet detection in single-cell RNA sequencing data using artificial nearest neighbors. *Cell Syst.* **8**, 329–337.e4 (2019).
79. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
80. G. Korotkevich, V. Sukhov, N. Budin, B. Shpak, M. N. Artyomov, A. Sergushichev, Fast gene set enrichment analysis. bioRxiv 060012 [Preprint] (2021). <https://doi.org/10.1101/060012>.
81. D. Dimitrov, D. Türe, M. Garrido-Rodríguez, P. L. Burmedi, J. S. Nagai, C. Boys, R. O. Ramirez Flores, H. Kim, B. Szalai, I. G. Costa, A. Valdeolivas, A. Dugourd, J. Saez-Rodriguez, Comparison of methods and resources for cell-cell communication inference from single-cell RNA-Seq data. *Nat. Commun.* **13**, 3224 (2022).

82. Z. Fusilier, H. Moreau, Macrophages restrict tumor permissiveness to immune infiltration by controlling local collagen topography through a Tcf4-Collagen3 fibrotic axis, *Zenodo* (2026); <https://doi.org/10.5281/zenodo.18610870>.
83. M. Mallocci, P. de Villemagne, P. Dorval, M. Feyeux, S. Blandin, G. Schmid, P. Hulin, P. P. Gilloteaux, Label-free imaging of large samples: 3D rendering and morphological analysis within histological workflows using serial block face imaging. *bioRxiv* 2022.05.03.488912 [Preprint] (2022). <https://doi.org/10.1101/2022.05.03.488912>.
84. R. M. Haralick, K. Shanmugam, I. Dinstein, Textural features for image classification. *IEEE Trans. Syst. Man Cybern.* **SMC-3**, 610–621 (1973).
85. S. van der Walt, J. L. Schönberger, J. Nunez-Iglesias, F. Boulogne, J. D. Warner, N. Yager, E. Gouillart, T. Yu, scikit-image contributors, scikit-image: Image processing in Python. *PeerJ* **2**, e453 (2014).
86. P. Bankhead, M. B. Loughrey, J. A. Fernández, Y. Dombrowski, D. G. McArt, P. D. Dunne, S. McQuaid, R. T. Gray, L. J. Murray, H. G. Coleman, J. A. James, M. Salto-Tellez, P. W. Hamilton, QuPath: Open source software for digital pathology image analysis. *Sci. Rep.* **7**, 16878 (2017).

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Macrophages restrict tumor immune infiltration by controlling collagen topography

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Editor's summary

Macrophages suppress antitumor immunity through multiple direct and indirect mechanisms, but how their interactions with the tumor extracellular matrix (ECM) affect infiltrating immune cells is not well understood. Using both microscopy to image murine tumors and machine learning, Fusilier *et al.* found that macrophages shape fibrillar collagen topography in late-stage tumors, which dictates T cell localization. Macrophage depletion activated a *Tcf4*-mediated fibrotic pathway that promoted the development of intratumor collagen III-rich networks that supported localized T cell and neutrophil infiltration. These findings demonstrate that macrophages can also affect the tumor immune microenvironment by shaping local ECM architectures. —Claire Olingy

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