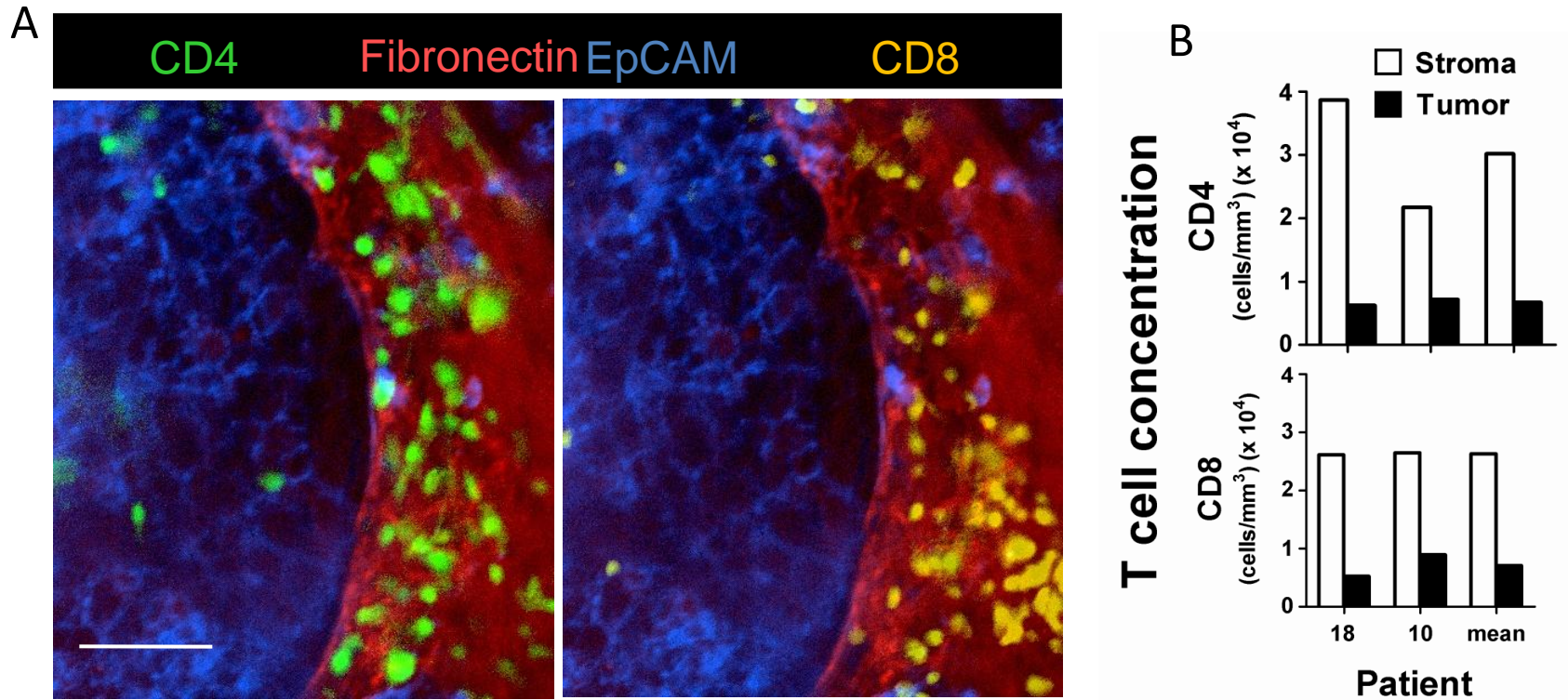


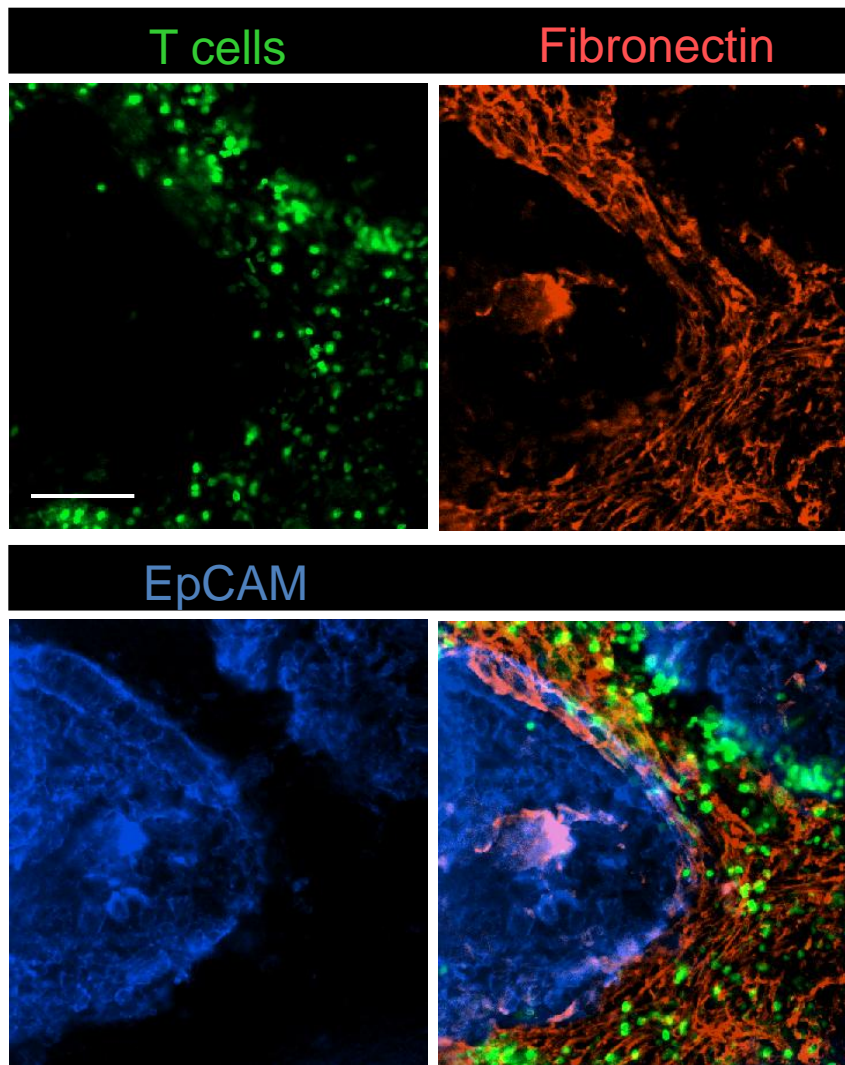
Fig S1



CD4 and CD8 T cells show a similar accumulation in the tumor stroma.

Fluorescently-labeled CD4 (CMFDA, green) and CD8 (Hoechst, yellow) T cells were added to a human lung tumor slice that was subsequently stained for fibronectin and EpCAM to respectively identify the stroma (red) and the tumor epithelial cell regions (blue). Each image is the maximum projection of 4 images spanning 60 μm in the z direction beneath the cut surface of the slice. Bar, 100 μm . (B) Concentration of CD4 (top panel) and CD8 T cells (bottom panel) in the stroma and the tumor epithelial cell regions. Data give the values obtained in slices from 2 different human lung tumors and the mean.

Fig S2



T cells preferentially migrate into the tumor stroma of a human colon tumor.

Fluorescently-labeled T cells (Hoechst, green) were added to a human colon tumor slice that was subsequently stained for fibronectin and EpCAM to respectively identify the stroma (red) and the tumor epithelial cell regions (blue). Each Image is the maximum projections of 4 images spanning 60 μm in the z direction beneath the cut surface of the slice. Bar, 100 μm .

Fig S3

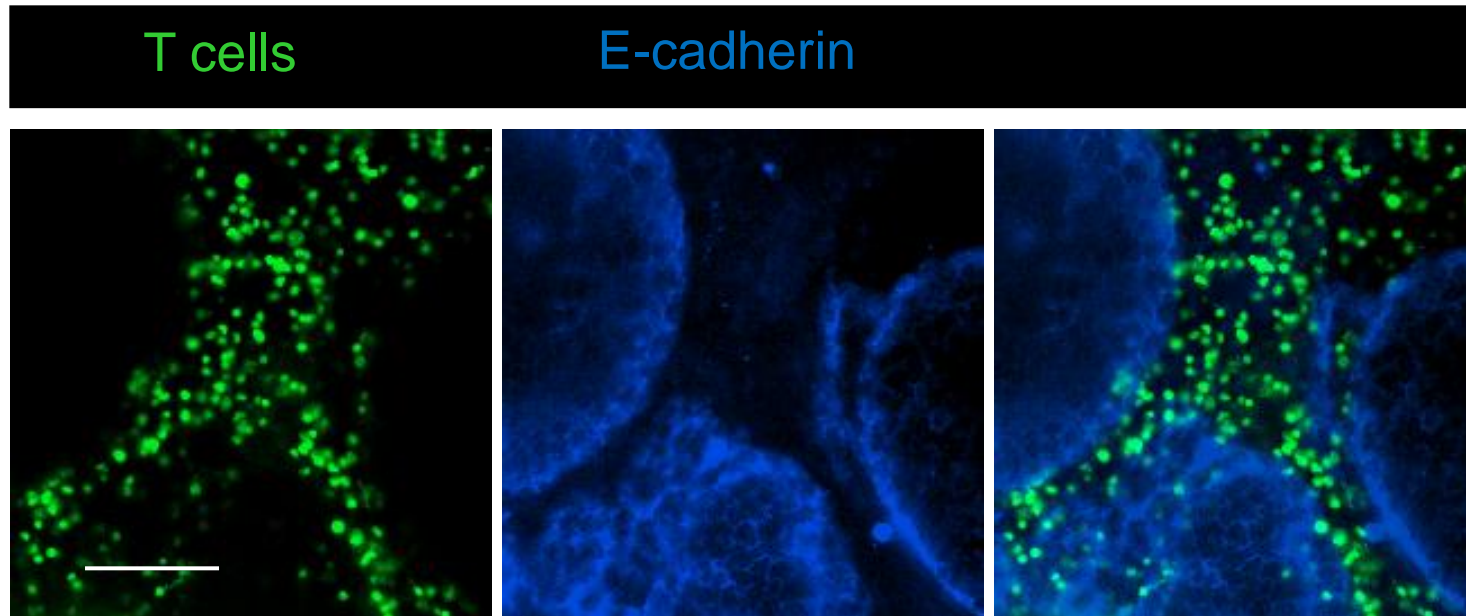
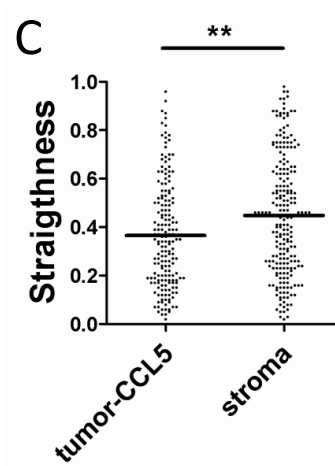
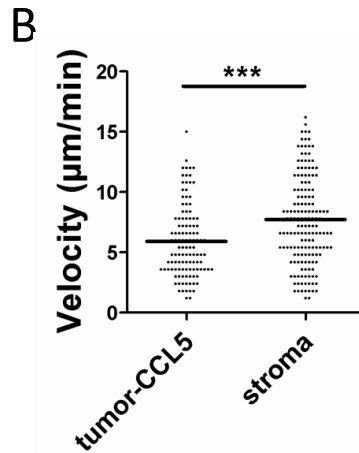
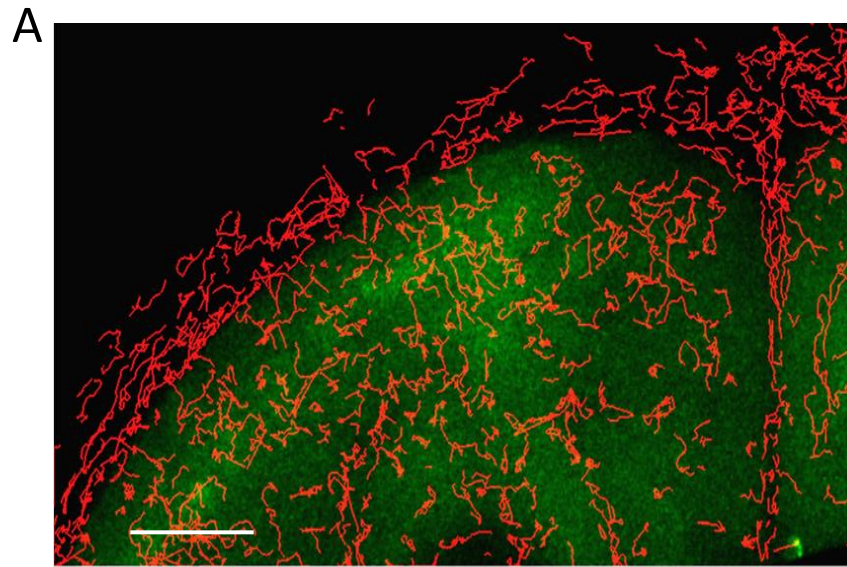


Figure 3. T cells preferentially migrate into the tumor stroma of a xenograft system.

Fluorescently-labeled T cells (CMFDA, green) from a tumor-specific clone were plated onto a slice of an autologous lung tumor engrafted into an immune incompetent mouse. Tumor cells were identified by staining for E-cadherin (blue). Bar, 100 μ m. Data is representative of more than 5 experiments.

Fig S4

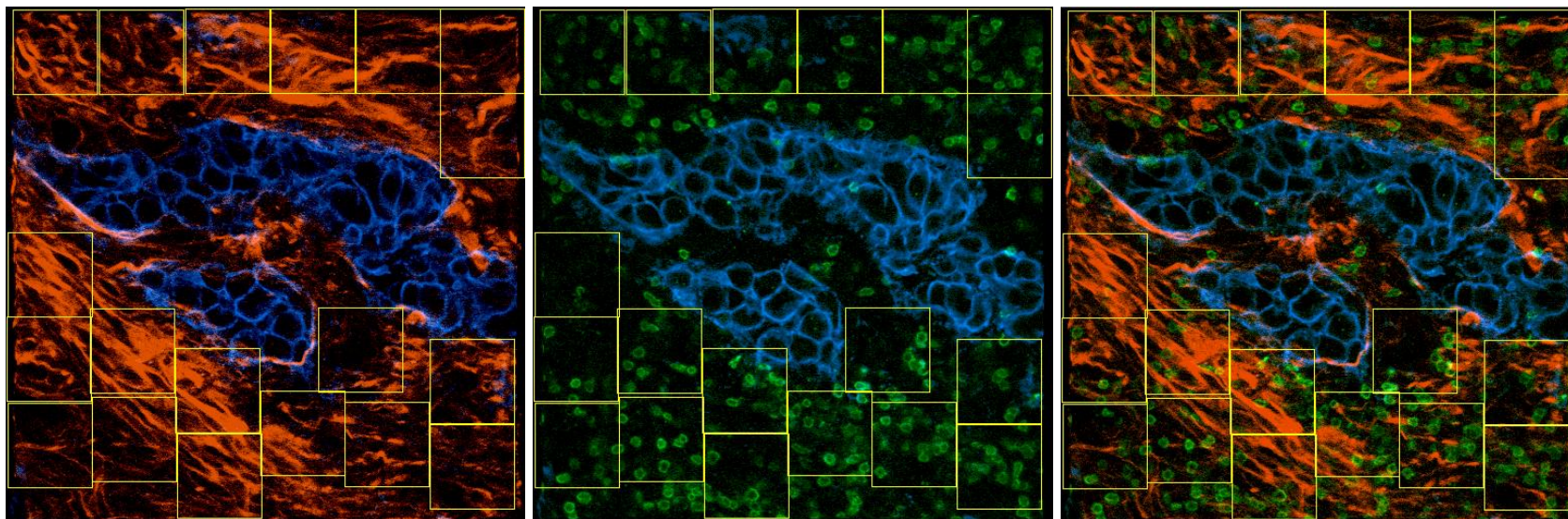


Motile behaviour of T cells in the stroma and tumor islets expressing CCL5.

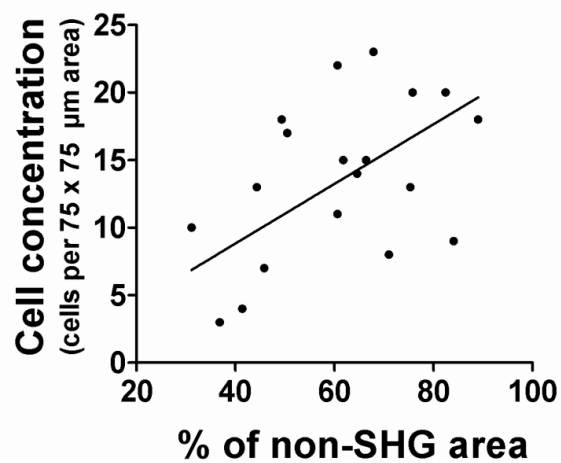
Trajectories of individual T cells in the stroma and in the tumor epithelial cell region expressing CCL5-GFP (green). Fluorescently-labeled T cells introduced into a tumor slice of a xenograft system were imaged during 20 min. Trajectories were superimposed to fluorescent images from the GFP. Bar, 50 µm. See also Video S2. (B and C) The graphs show the velocities of individual T cells and the straightness index of individual tracks in the stroma and in the tumor epithelial cell region expressing CCL5-GFP. Low-straightness indices correspond to confined trajectories. Results are representative of three independent experiments.

Fig. S5

A



B

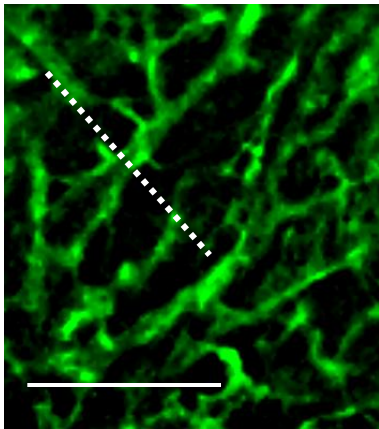


Relationship between the concentration of T cells and the density of collagen structures.

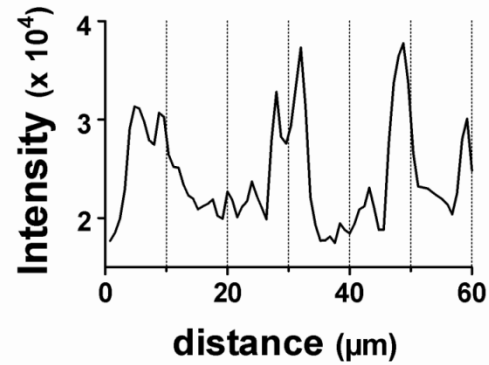
Second harmonic signal (red) from a human lung tumor slice also stained for CD3 (green) and EpCAM (blue). Adjacent 75x75 μm stromal regions are delineated by yellow squares. (B) the number of T cells and the percentage of SHG-free areas were automatically calculated in each square shown in (A).

Fig S6

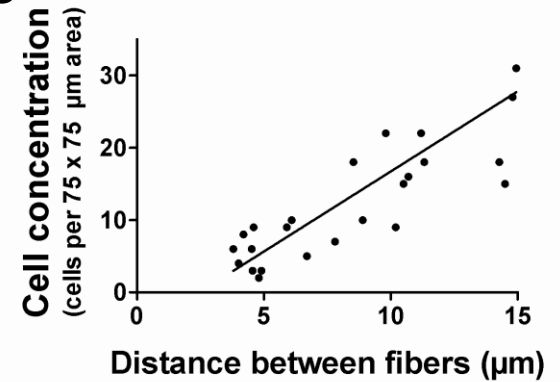
A



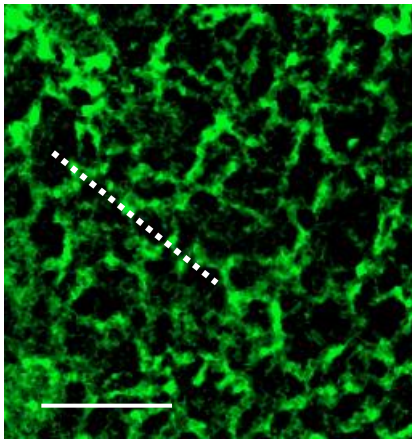
B



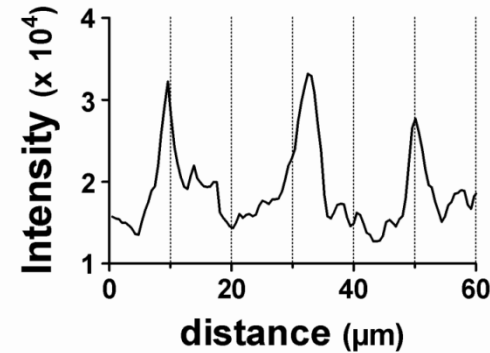
C



D



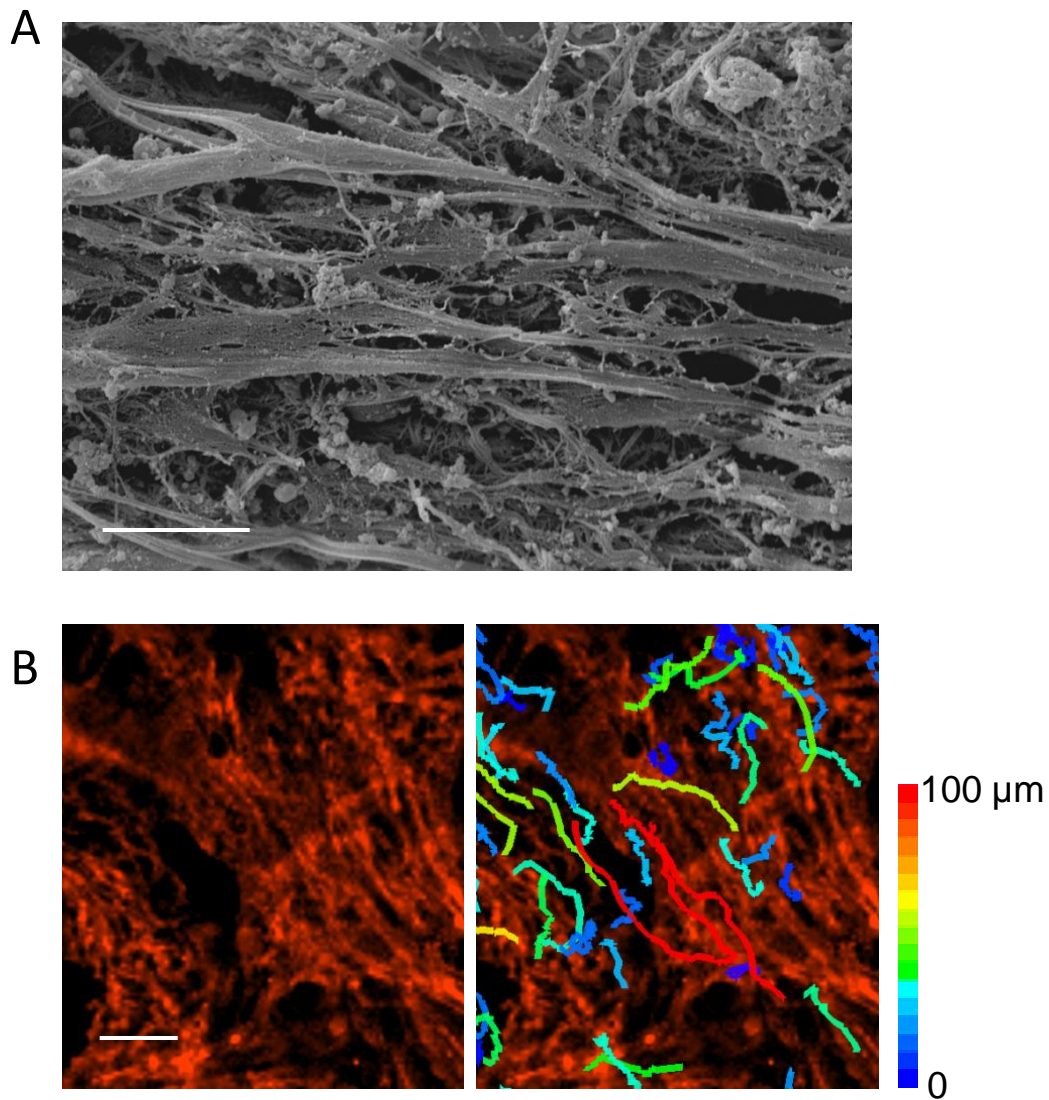
E



Pro-migratory regions of human lung tumors share structural features with the reticular network of human lymph nodes.

(A) A loose fibronectin stromal region (higher magnification of Fig. 4C) of a human lung tumor. (B) Fluorescence intensity of fibronectin along the white line shown in A. (C) The number of resident T cells was scored in 75 x 75 μm zones showing different distances between fibers. Results were obtained on slices from 3 different lung tumors. (D) A human lymph node slice was stained for fibronectin (green). (E) Fluorescence intensity of fibronectin along the white line shown in A. Bar, 50 μm in A and D.

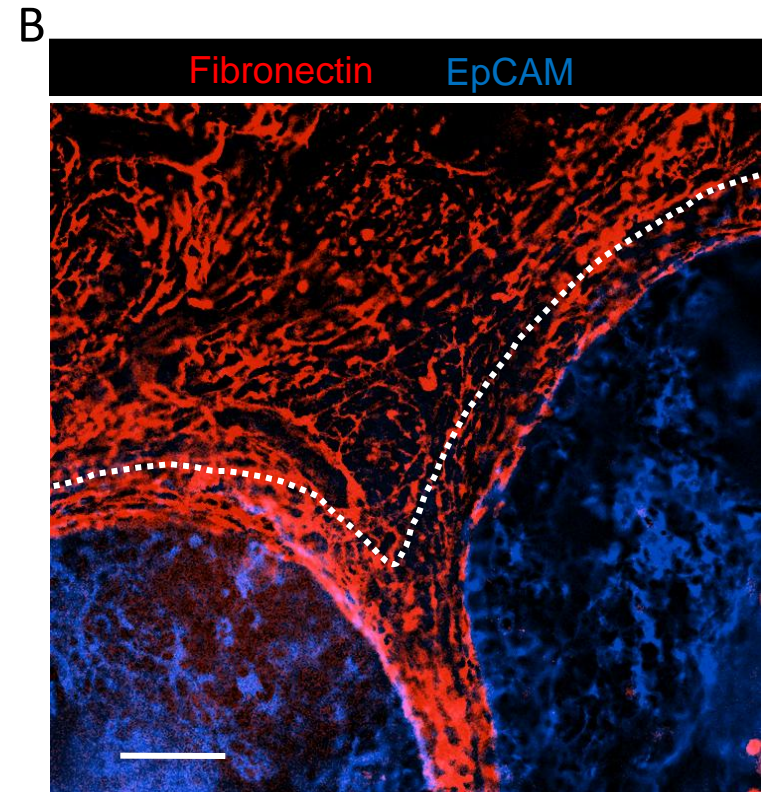
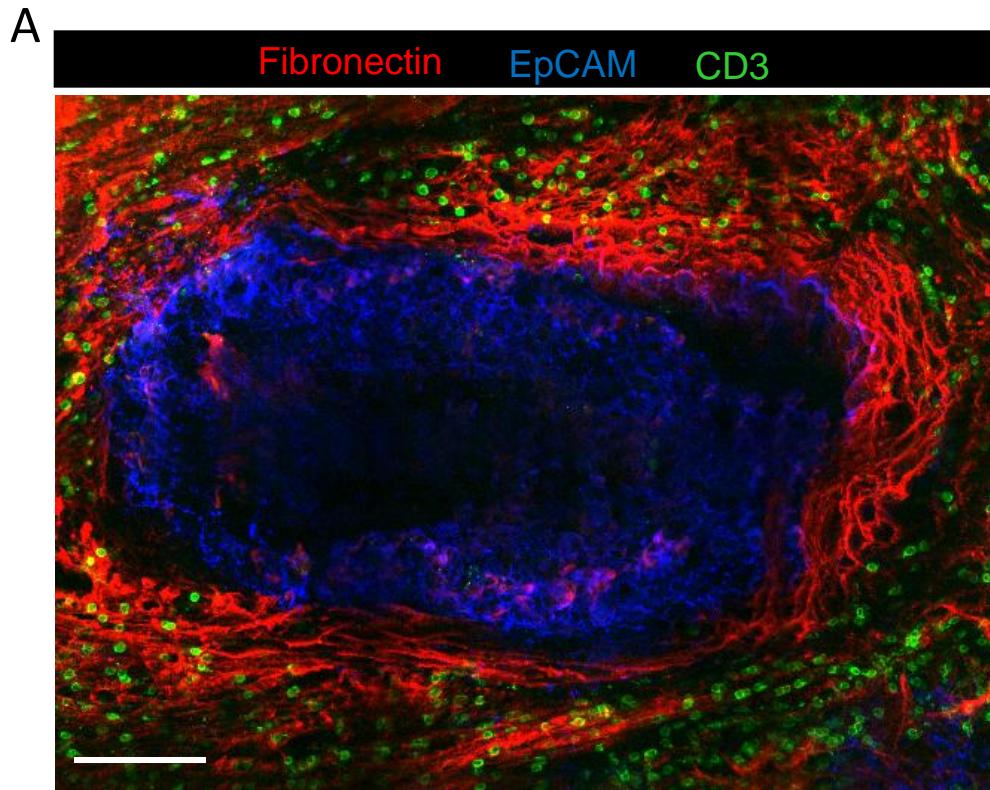
Fig. S7



T cells preferentially migrate along fibronectin fibers

(A) A representative SEM picture of a human lung tumor slice showing parallel strands in the stroma. Bar, 50 μm . (B) Trajectories of individual T cells, throughout a 20 min imaging session, in a human lung tumor slice post-stained for fibronectin (red). Tracks are color-coded according to the cell displacements which are the connecting lines between starting points and end points of each track. Bar, 50 μm .

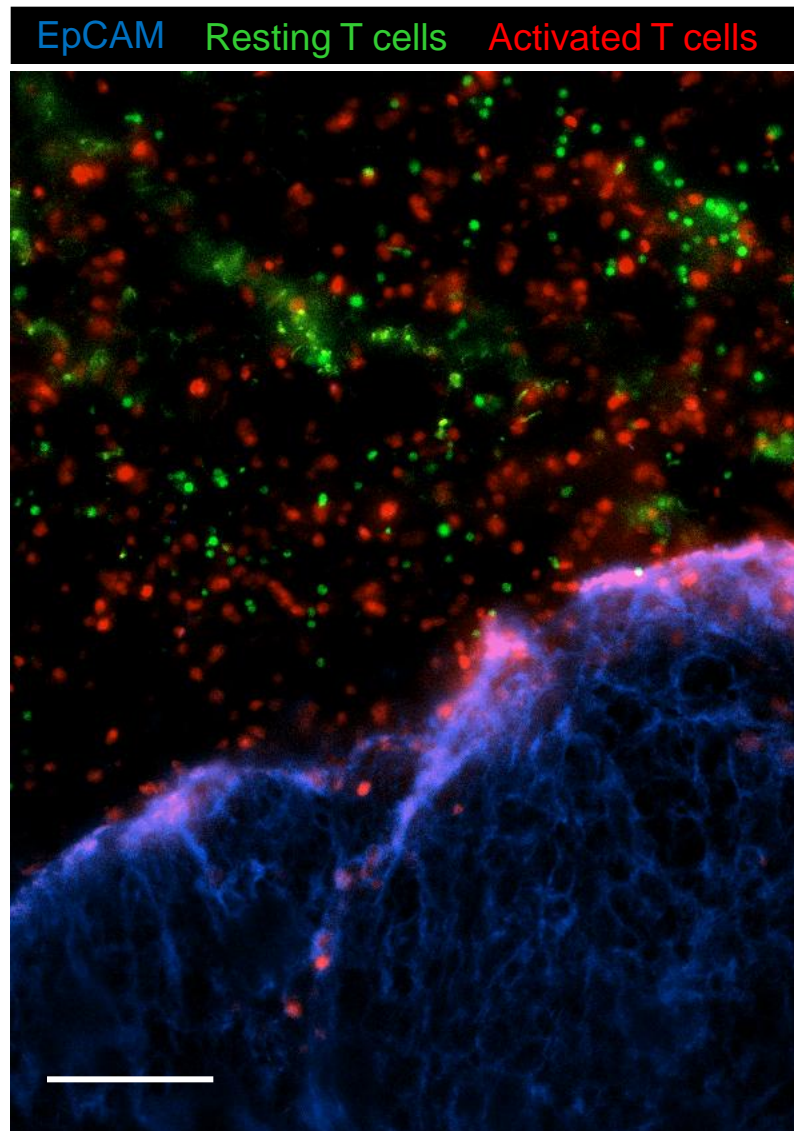
Fig. S8



Dense and tight fibronectin fibers immediately adjacent to the tumor mass.

Two human lung tumor slices were stained for fibronectin (green) and EpCAM (blue). The dotted white line delineates the first 50 μm of the stromal region adjacent to the tumor islet. Bar, 100 μm .

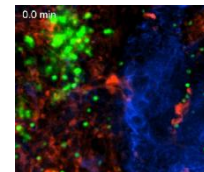
Fig. S9



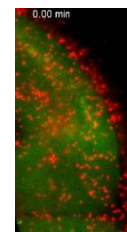
Activated T lymphocytes infiltrate the tumor stroma much more efficiently than resting T lymphocytes.

Fluorescently-labeled resting (CMFDA, green) and activated (SNARF, red) T cells were added to a human lung tumor slice that was subsequently stained for EpCAM to identify the tumor epithelial cell region (blue). The image is the maximum projections of 4 images spanning 60 μm in the z direction beneath the cut surface of the slice. Bar, 100 μm . Data is representative of more than 3 experiments.

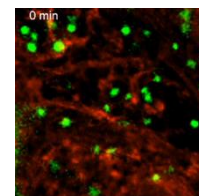
Video S1. T cells accumulate and migrate actively in the tumor stroma. Fluorescently-labeled T cells (green) introduced into a human tumor slice were imaged during 16 min with a wide-field microscope, after which the slice was stained for fibronectin (red) and EpCAM (blue). Frame intervals, 20 sec. The animation represents a 60 μm z-projection. A still image with T cell tracks is shown in Fig. 1D.



Video S2. Migratory behaviour of T cells in tumor expressing CCL5. Fluorescently-labeled T cells (red) introduced into a slice of an engrafted tumor expressing CCL5-GFP (green). Frame intervals, 20 sec. The animation represents a 60 μm z-projection. A still image with T cell trajectories is shown in Fig. S4A

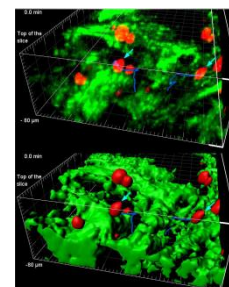


Video S3. T cells actively migrate in loose fibronectin regions. Fluorescently-labeled T cells (green) introduced into a human tumor slice were imaged during 19 min with a wide-field microscope, after which the slice was stained for fibronectin (red). Last frames show T cell trajectories which are color-coded according to the cell displacements ranging from blue (0-20 μm) to red (100 μm). The animation represents a 60 μm z-projection. Frame intervals, 20 sec.

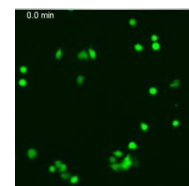


Video S4. T cells actively migrate in loose collagen regions.

The movie shows a 4D view of T cells (red) within a human lung tumor slice in relation to SHG structures (green). A stack of 20 optical sections with an interval of 4 μm was captured with a two-photon microscope. The movie was created from 3D reconstruction images using Imaris software. The left panel shows the raw imaging data while the right panel shows a volume rendering of the same data set. Examples of T cells migrating in porous SHG structures are indicated with arrows. T cell trajectories are in blue. Frame intervals, 30 sec.



Video S5. Activated T cells spontaneously migrate on ICAM-1. Fluorescently-labeled *in vitro* activated PBT (J15) were plated on ICAM-1 and imaged during 10 min. Frame intervals, 20 sec.



Video S6 and S7. Linear migration of T cells in perivascular regions of the stroma. Fluorescently-labeled T cell (green) introduced into human lung tumor slices were imaged during 19 min with a wide-field microscope, after which slices were stained for fibronectin (red) and CD31 (blue). T cell trajectories are in green. The animation represents a 60 μm z-projection. Frame intervals, 20 sec.

