Vascularized tumor-on-a-chip to investigate immunosuppression of CAR-T cells

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Supplementary Data and Methods

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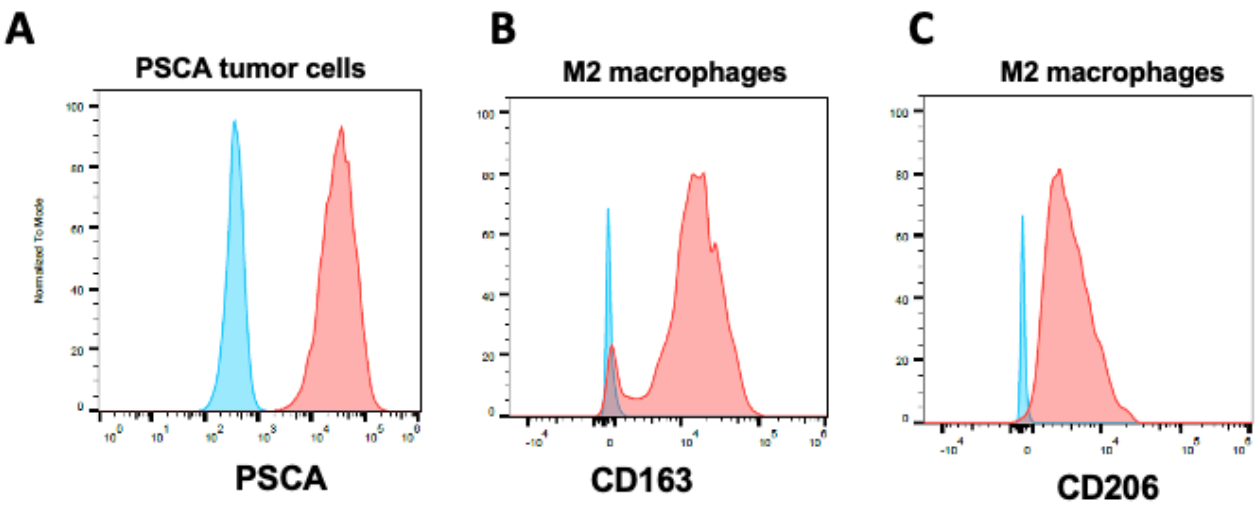

Figure S1. Flow cytometric analysis of cancer cells and M2 macrophages. A) PSCA transduced DU145 prostate cancer cells were stained with a mAb against PSCA antigen. M2-polarized macrophages differentiated from primary monocytes were analyzed for M2-macrophage specific markers B) CD163 and C) CD206. The cyan curves represent the negative control, while the red curve depicts the sample labeled with the respective mAb.

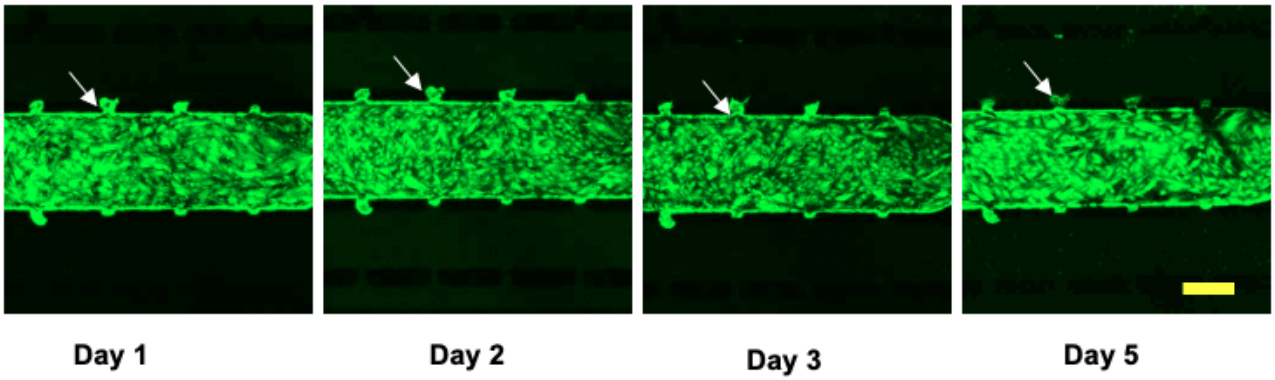

Figure S2. Endothelial-lined channel in the TOC over the duration of the experiment. TOC was loaded with the tumor cells and endothelial cells, the latter were transduced to constitutively express GFP. The endothelial cells (green) cover the surface of the microfluidic channel (ch1 – see Fig. 1) and cover the pores between ch1 and ch2 (white arrows). Scale bar 200 µm.

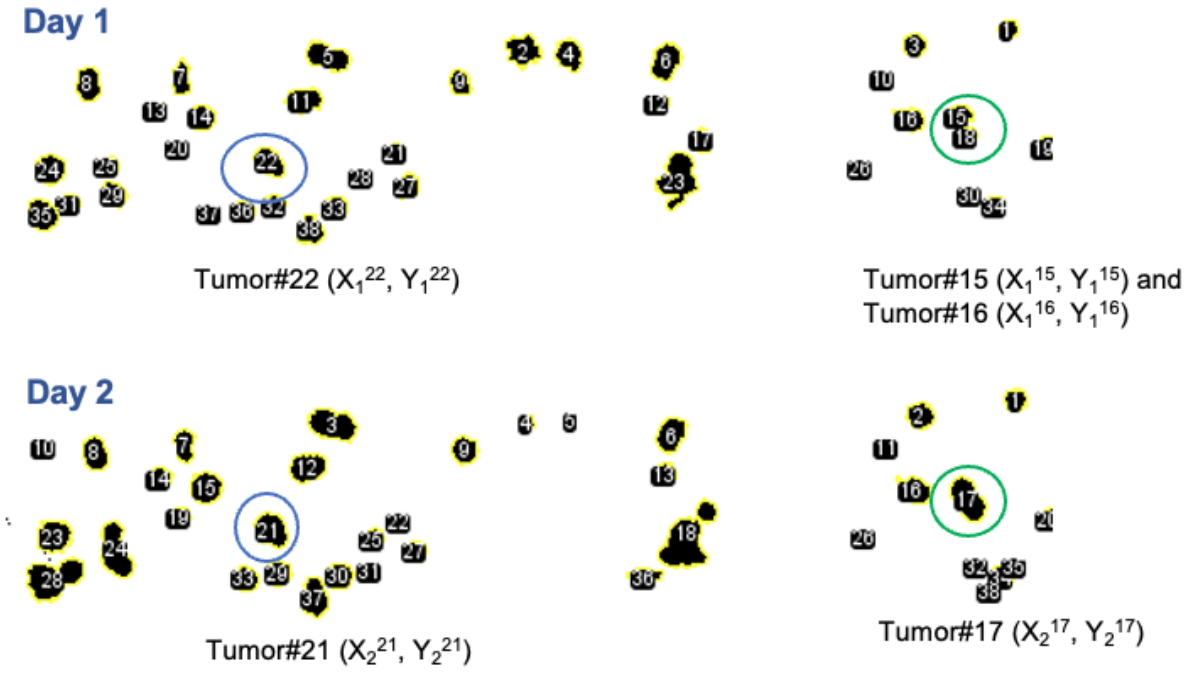

Figure S3. Algorithm to track growth of individual tumors in the TOC. The devices were loaded with fluorescently labelled DU145 tumor cells and images from two consecutive days are shown. The blue circles in the images show same tumor across two days. Using the tracking algorithm, the Euclidean distance between Tumor#22 on day 1 from all tumors on day 2 is calculated. Tumor#21 on day 2 is identified as the same tumor as Tumor#22 on Day 1 because it has the minimum Euclidean distance. Consequently, the tumor number on Day 2 (Tumor#21) is reassigned as Tumor #22. In cases where tumors merge (e.g., tumors highlighted by green circles), Tumor#15 and Tumor#18 on Day 1 are both found to have the minimum Euclidean distance to Tumor#17 on Day 2. The algorithm identifies these Day 1 tumors as merged into one tumor on Day 2.

The image processing for tracking growth of individual tumors follows these steps:

1. **Thresholding and Identification:**
   - Fluorescent images are thresholded in ImageJ.
   - Using particle analysis, ImageJ assigns a unique and sequential identification number to each tumor.

2. **Centroid Extraction:**
   - The X and Y coordinates of each tumor's centroid are stored in two separate two-dimensional arrays. These arrays are indexed by the tumor identification number and the day of the image.

3. **Euclidean Distance Calculation:**
   - Using the X and Y coordinates, the Euclidean distance between a tumor on Day 2 and each tumor on subsequent days is calculated.
   - The distances are stored in a three-dimensional array. For tumor *i* on Day 2 and tumor *k* on Day *j+1*, the distance is given by:

$$Dist_{i,j+1,k} = \sqrt{(X_{2,i} - X_{j+1,k})^2 + (Y_{2,i} - Y_{j+1,k})^2}$$

1. **Tumor Identification:**
   - For each tumor *i* on Day 2, the tumor on Day *j+1* with the minimum distance is recognized as the same tumor.
   - Its identity is reassigned as tumor I on Day *j+1*

1. **Handling Tumor Merging:**
   - In cases where a single tumor on Day *j+1* is traced back to two different tumors from day 2, the areas of those earlier tumors are combined, and they are treated as a single tumor across all affected days.
   - A random selection of merged tumors was also manually confirmed.