Supporting Information

A 3D PANCREATIC TUMOR MODEL TO STUDY T-CELL INFILTRATION

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Fluorescent microscope acquisition of FITC-Dextran in the vascular and gel channels

ROIs to quantify the fluorescent intensity with ImageJ to calculate the permeability coefficient P as:

$$P = \frac{(I_f - I_i)w}{(I_v - I_i)\Delta t}$$

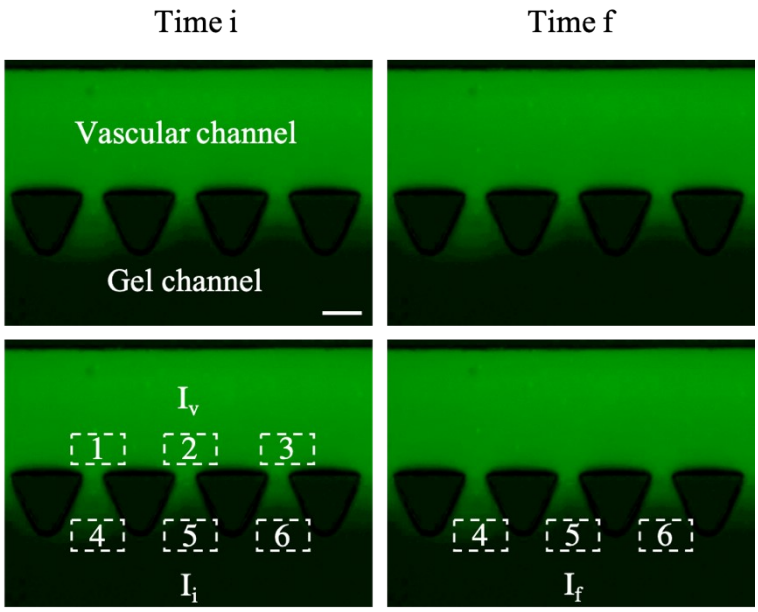

Figure S1. Permeability assay and intensity measurements. Representative images to quantify the fluorescent intensity of 70 kDa FITC-Dextran diffusing into the vascular channel to measure the permeability coefficient P. Fluorescent images were acquired with a FITC filter for a total of 30 minutes. Three ROIs for each channel were defined to calculate P of the 70 kDa FITC-Dextran. If is the mean fluorescent intensity of the extravascular ROIs at the final time, Ii is the mean fluorescent intensity of the extravascular ROIs at the initial time. IV is the mean fluorescent intensity in the vascular ROIs. Δt is the time difference between the analyzed frames, and w is the width of the ROI. The scale bar is 150 μm.

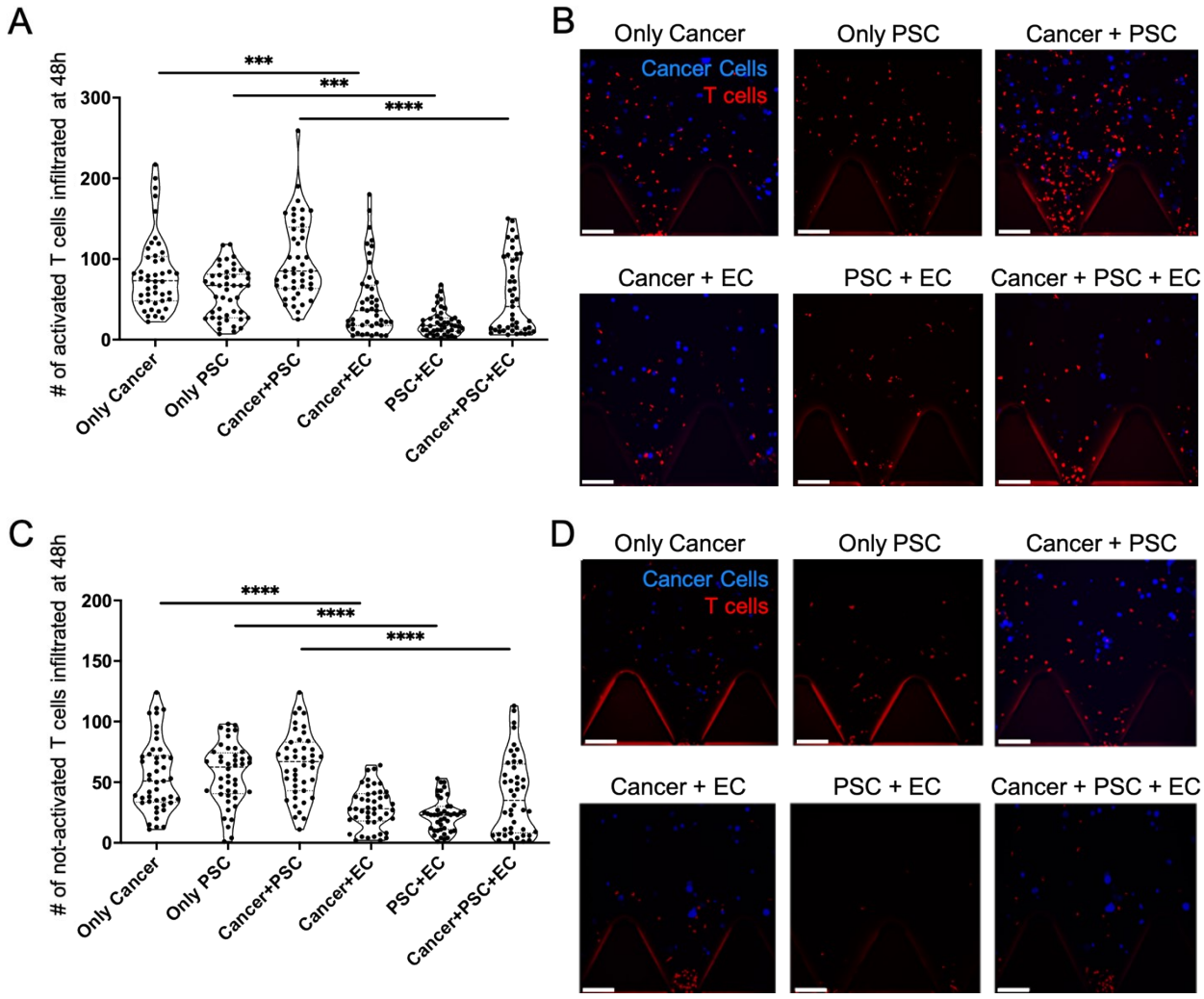

Figure S2. Effects of the endothelial barrier on T cells infiltration in the PDAC-TME model at 48 h. (A) Violin plot of the number of activated T cells infiltrated within the central region after 48 h from their injection into the device. T cells were stimulated with anti-CD3/CD28 Dynabeads for 5 days to promote activation. (B) Representative confocal images of activated T cells infiltrating into the central hydrogel channel of the microfluidic device in the different experimental conditions. (C) Violin plot of the number of not-activated T cells infiltrated within the central region T cells after 48 h from their injection into the device. (D) Representative confocal images of not-activated T cells infiltrating in the central hydrogel channel in the different experimental conditions. T cells were labelled with Draq5 and showed in red in B and D. PDAC cells were labelled with DAPI and showed in blue in B and D. The red trapezoidal shapes in B and D are the posts of the microfluidic device that allow to identify the gel interface during imaging and data analysis. Data in A and C are plotted with violin plots showing the probability density for each value, n = 5. Statistical analysis is done

with one-way ANOVA with multiple comparisons. **** $p < 0.0001$. *** $p < 0.001$. Scale bars are 100 μm.

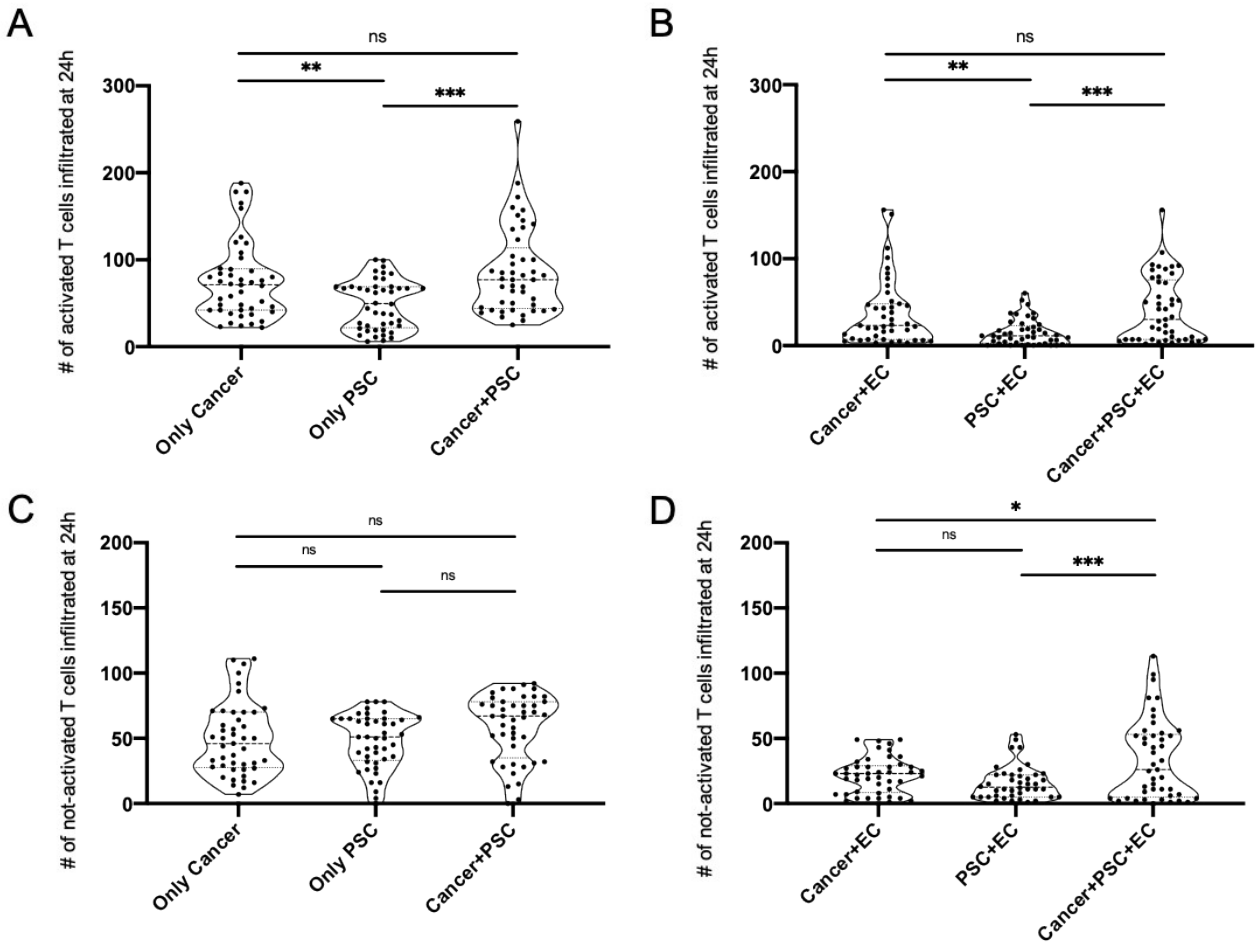

Figure S3. T cell infiltration of activated and not-activated T cells at 24 h. (A) Absolute number of activated infiltrating T cells. (B) Absolute number of activated infiltrating T cells in the presence of endothelial barrier. (C) Absolute number of not-activated infiltrating T cells. (D) Absolute number of not-activated infiltrating T cells in the presence of endothelial barrier. Data are plotted with violin plots showing the probability density for each value, n = 5. Statistical analysis done with one-way ANOVA with multiple comparisons*** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. ns: not significant.

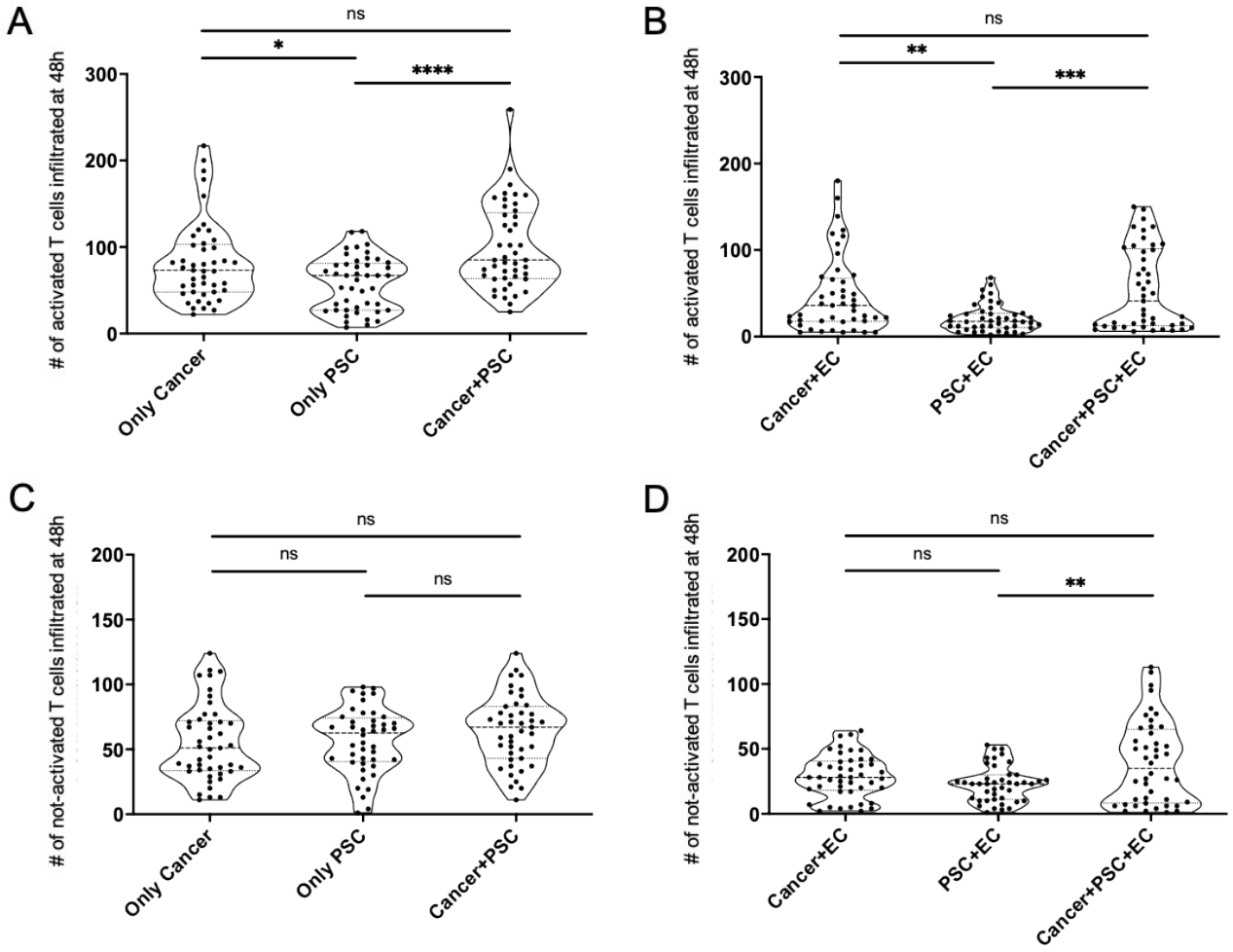

Figure S4. T cell infiltration of activated and not-activated T cells at 48 h. (A) Absolute number of activated infiltrating T cells. (B) Absolute number of activated infiltrating T cells in the presence of endothelial barrier. (C) Absolute number of not-activated infiltrating T cells. (D) Absolute number of not-activated infiltrating T cells in the presence of the endothelial barrier. Data are plotted with violin plots showing the probability density for each value, n = 5. Statistical analysis done with one-way ANOVA with multiple comparisons. **** $p < 0.0001$. *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. ns: not significant.

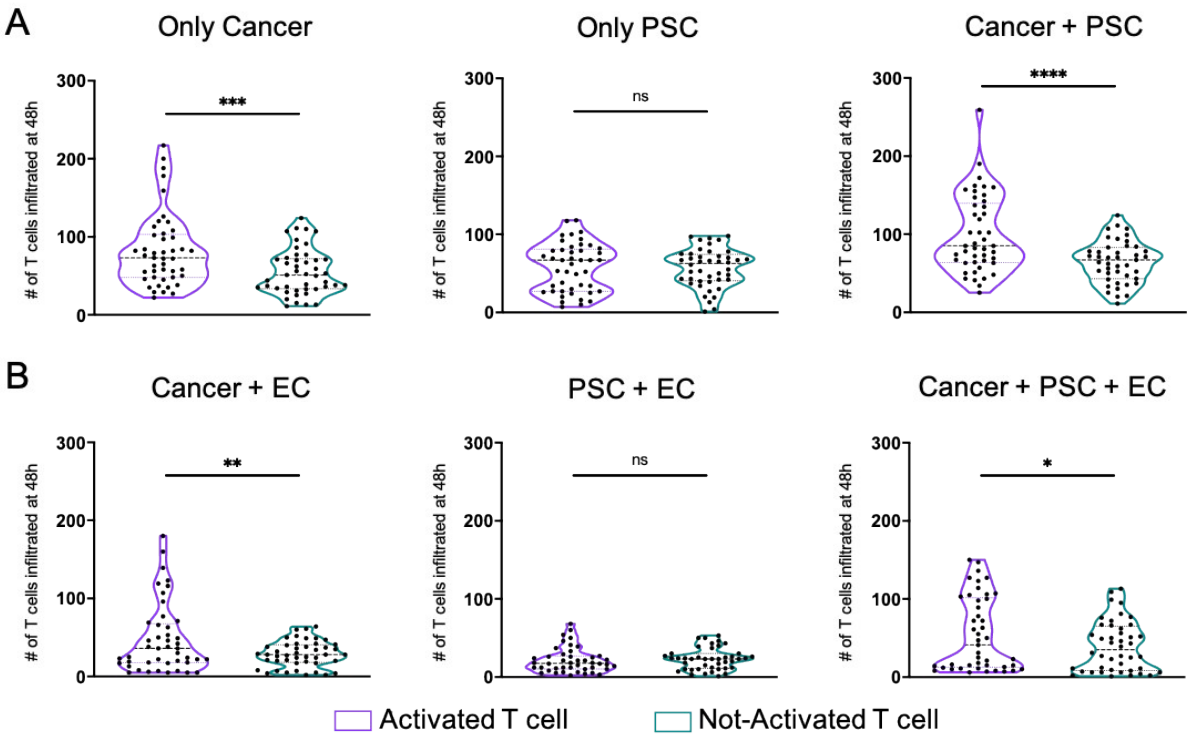

Figure S5. Effect of the activation on T cells infiltration in PDAC model at 48 h. (A) Absolute number of infiltrating T cells (48 h from their injection) in all tested conditions without endothelial cells (Only cancer; Only PSCs; Cancer + PSCs). (B) Absolute number of infiltrating T cells (48 h from their injection) in all tested conditions in presence of endothelial barrier (Cancer + ECs; PSCs + ECs; Cancer + PSCs + ECs). Activated T cells are depicted in purple, not-activated T cells in green. Data are plotted with violin plots showing the probability density for each value, n = 5. Statistical analysis ANOVA. **** $p < 0.0001$. *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. ns: not significant.

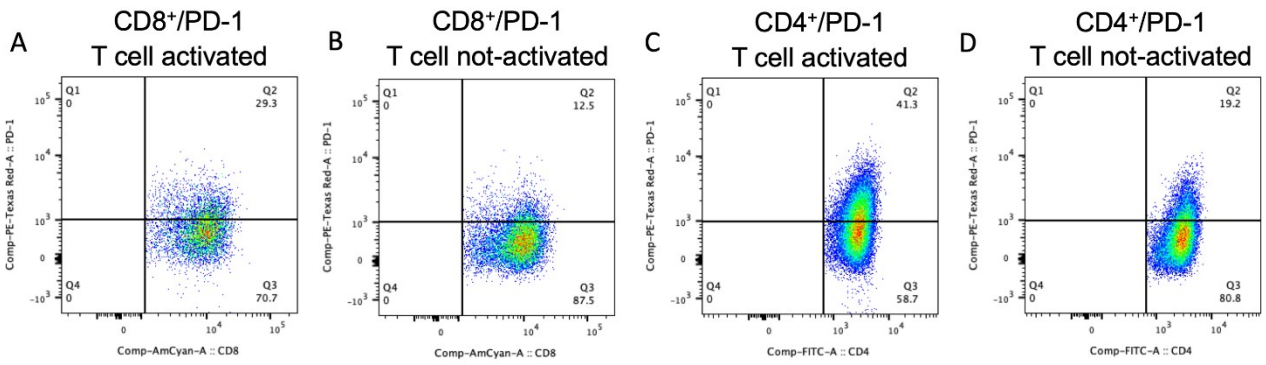

Figure S6. Flow cytometry analysis of PD-1 on $CD8^+$/$CD4^+$ T cells subsets. $CD3^+$ T cells gated for $CD8^+$ either activated (A) or not-activated (B) T cells. Cells were stained with AmCyan labeled anti-CD8 and Texas Red labeled anti-PD-1. $CD3^+$ T cells gated for $CD4^+$ and either activated (C) or not-activated (D). Cells were stained with FITC-labeled anti-$CD4^+$ and Texas Red labeled anti-PD-1. $CD3^+$ T cells were stimulated with anti-CD3/CD28 Dynabeads for 5 days in the presence of IL-2. At least 50000 events were acquired.

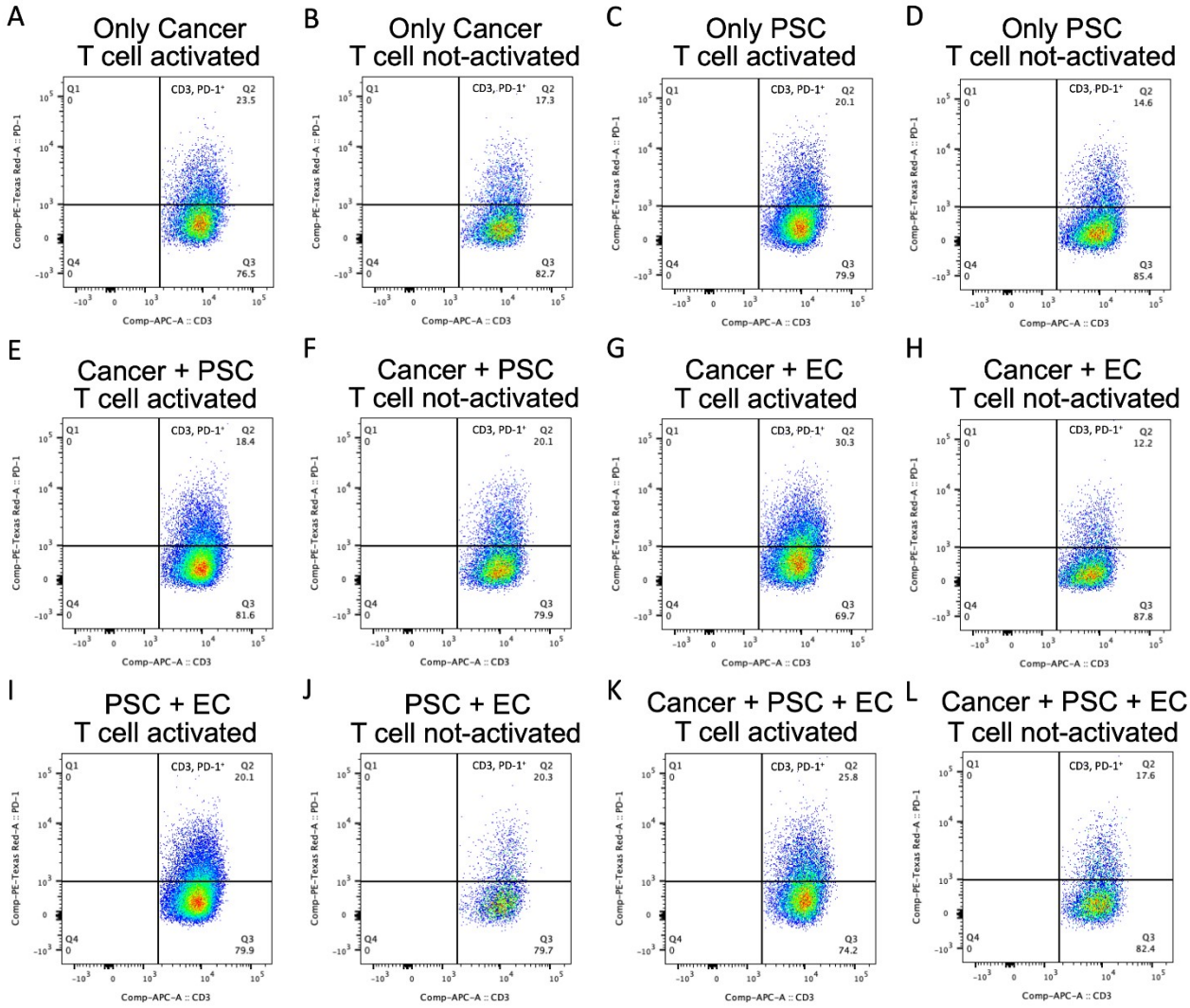

Figure S7. Flow cytometry analysis of PD-1 on $CD3^+$ T cells. (A-L) $CD3^+$ T cells gated for PD-1 either activated and not-activated with Dynabeads in all tested conditions (Only cancer; Only PSC; Cancer + PSC; Cancer + EC; PSC + EC; Cancer + PSC + EC). Cells were stained with APC-labeled anti-CD3 and Texas Red labeled anti-PD-1 T cells were stimulated with anti-CD3/CD28 Dynabeads for 5 days in the presence of IL-2. At least 50000 events were acquired.

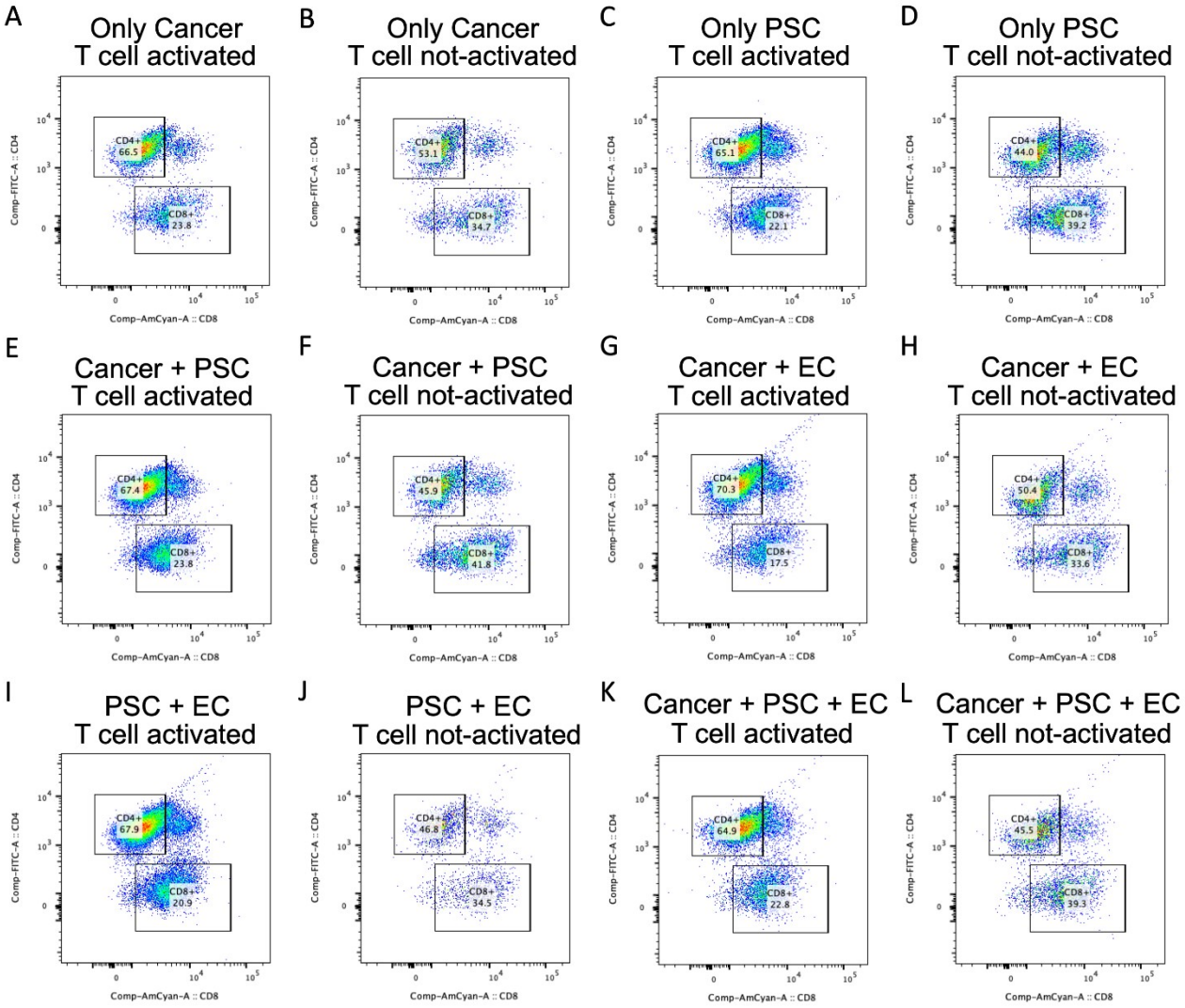

Figure S8. Flow cytometry analysis on $CD8^+/CD4^+$ T cells. (A-L) $CD3^+$ T cells gated for $CD4^+$ and $CD8^+$ either activated and not-activated with Dynabeads in all tested conditions (Only cancer; Only PSC; Cancer + PSC; Cancer + EC; PSC + EC; Cancer + PSC + EC). Cells were stained with AmCyan labeled anti-$CD8^+$ FITC-labeled anti-$CD4^+$. $CD3^+$ T cells were stimulated with anti-CD3/CD28 Dynabeads for 5 days in the presence of IL-2. At least 50000 events were acquired.

SUPPORTING MOVIE

Movie S1. Three-dimensional reconstruction of the endothelial monolayer by confocal microscopy. The movie shows the 3D reconstruction of confocal Z stack images of endothelial cells

immunostained with VE-cadherin (red) and DAPI (blue). The movie is made by IMARIS image analysis software.