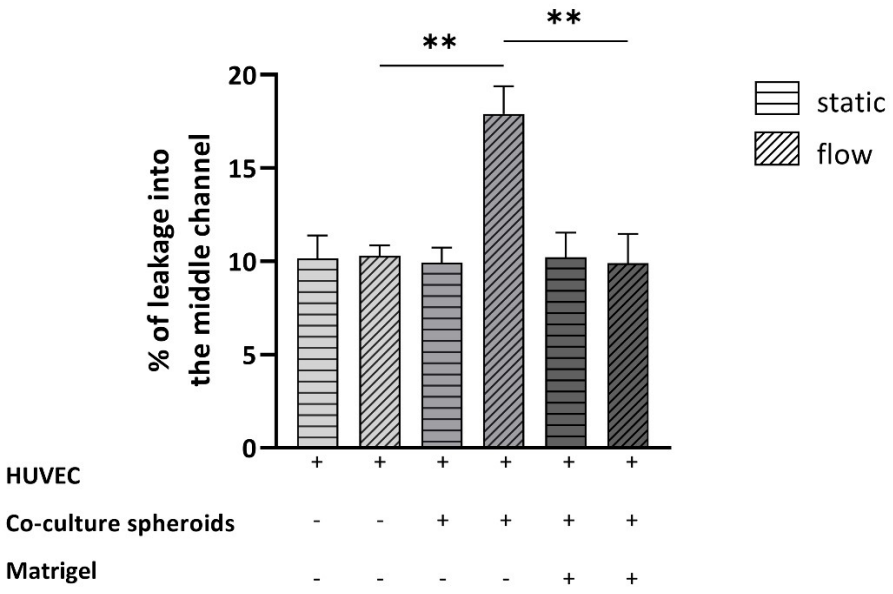
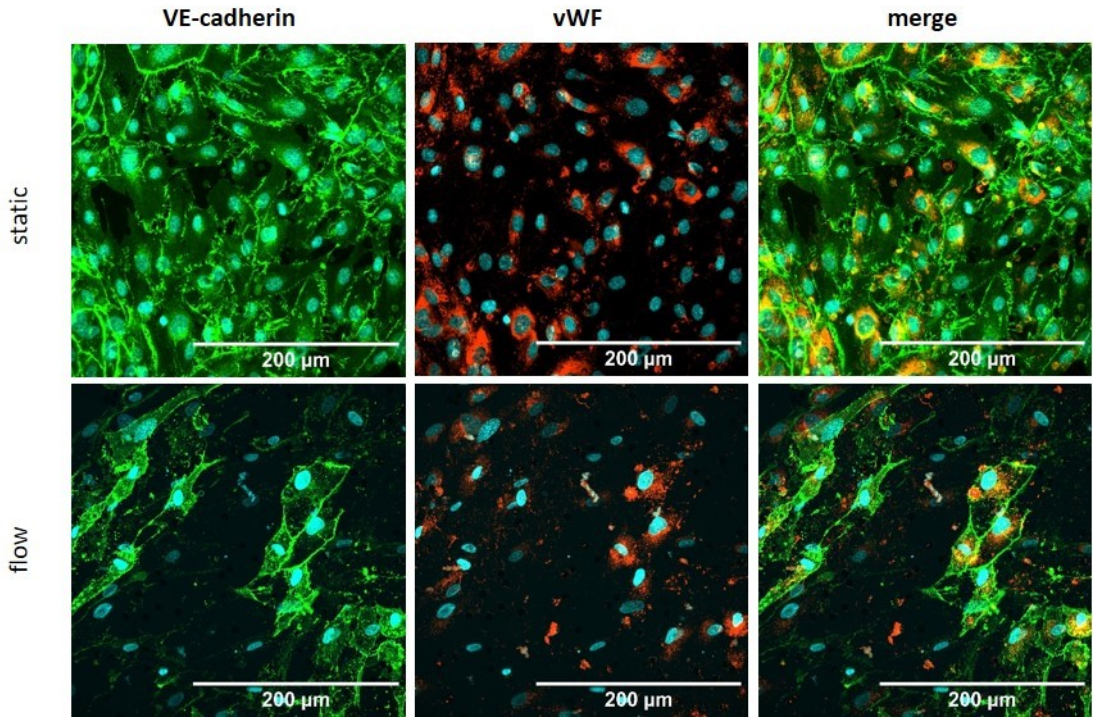
Supplements

Supplementary Table 1. Gradient for UHPLC / HRMS measurement.

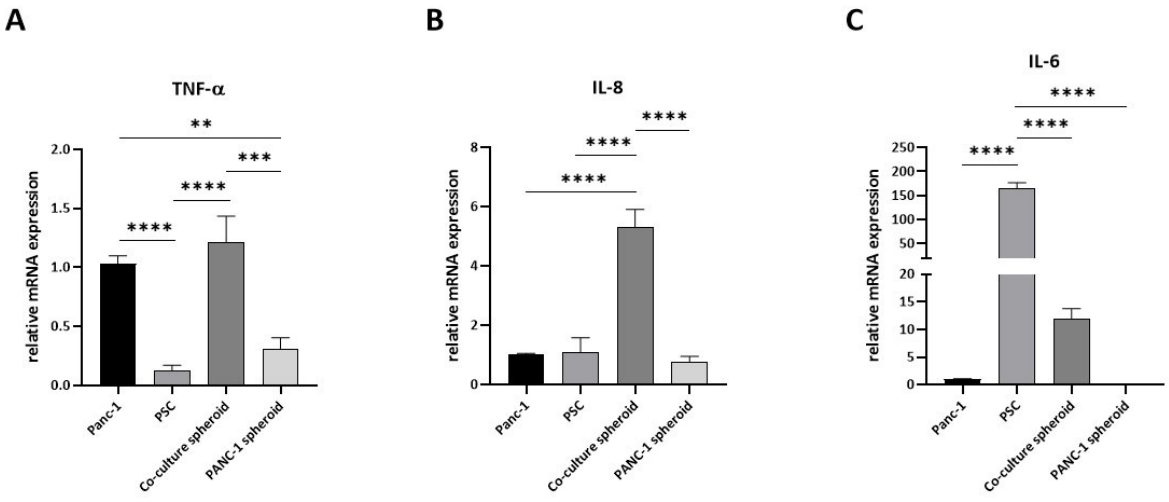
time [min]	solvent B [%]
0	0
0.2	0
8.0	100
11.0	100
11.1	0
12.0	0



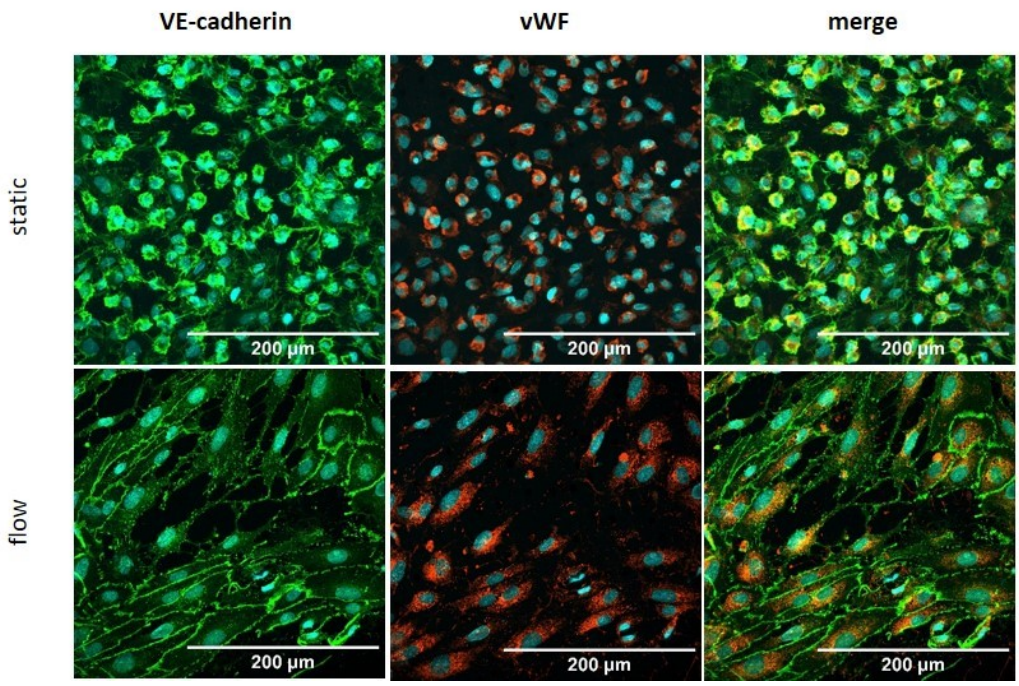
Supplementary Figure 1. Quantitative permeability assessment of the HUVEC layer. FITC dextran assay was performed to measure the barrier integrity of the HUVEC layer after 72 h under static or flow conditions and in the presence or absence of co-culture spheroids, either with or without the addition of Matrigel. FITC dextran solution was added into the top channel. After 1 h the leakage into the middle channel was determined via fluorescence measurement. Error bars indicate the standard errors of the mean of three independent experiments, with **= $p \leq 0.01$.



Supplementary Figure 2. Integrity of the vasculature consisting of HUVEC in the presence of co-culture spheroids in the biochip after 72 h. The vasculature remains stable in the presence of the co-culture spheroids under static conditions (top row). In the presence of the spheroids and under flow conditions, the endothelial cells do not show a stable vasculature (bottom row). The endothelial markers VE-cadherin (green), von Willebrand factor (vWF; red) and the nuclear dye DAPI (blue) were stained. Representative images are shown. Scale bar is 200 µm.



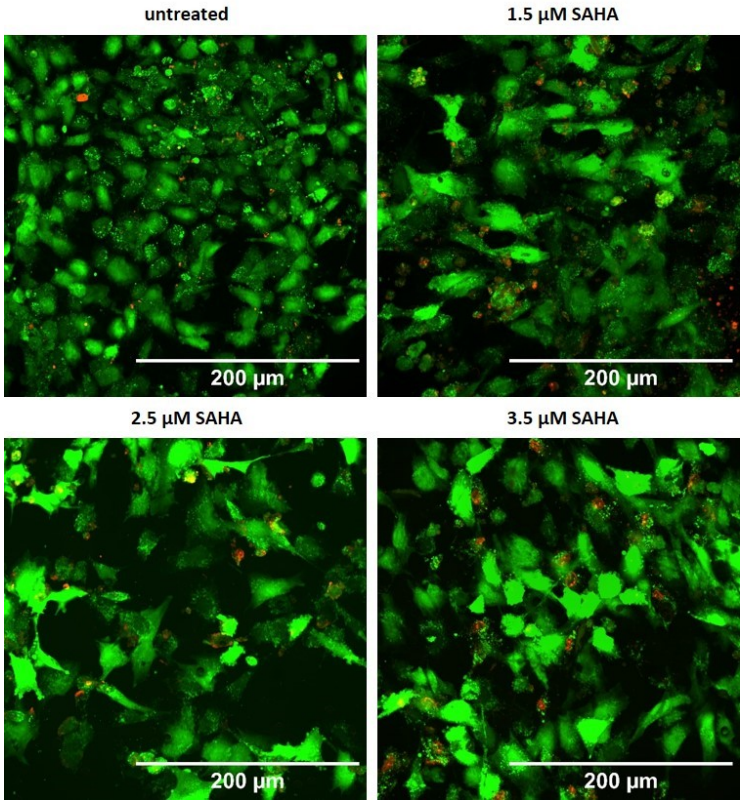
Supplementary Figure 3. Cytokine expression in PANC-1 cells, PSC cells, co-culture spheroids and PANC-1 spheroids. PANC-1 cells, PSC cells, co-culture spheroids and PANC-1 spheroids were cultivated for 72h in 3D medium. mRNA levels of TNF-α (A), IL-8 (B), and IL-6 (C) were analyzed by qRT-PCR. Error bars indicate standard errors of mean (of at least three independent experiments, with; ** = $p \leq 0.01$; *** = $p \leq 0.001$; and **** = $p \leq 0.0001$).



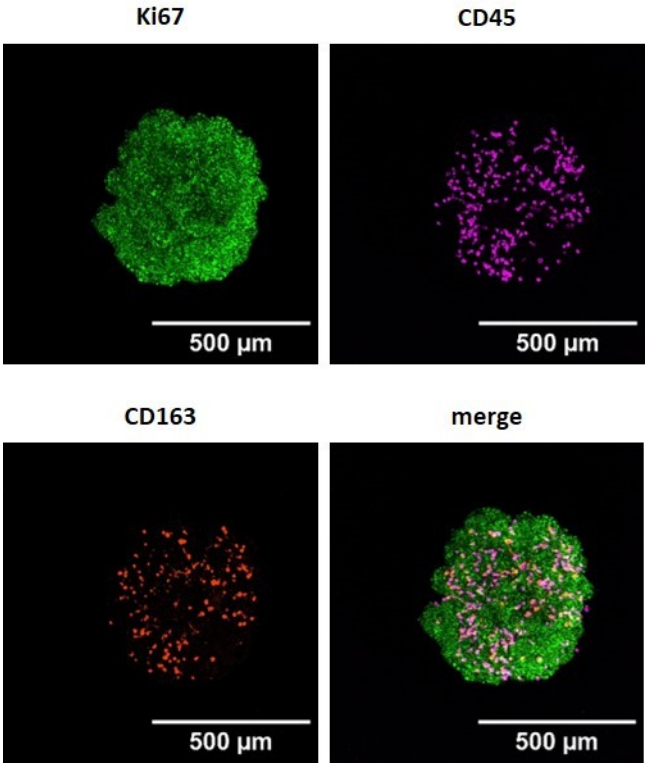
Supplementary Figure 4. Cultivation of HUVEC in the biochip. HUVEC were cultivated in the presence of conditioned media from co-culture spheroids for 72 h under static (top row) or flow (bottom row) conditions. The endothelial markers vascular endothelial (VE-) cadherin (green), von Willebrand factor (vWF; red) and the nuclear dye DAPI (blue) were stained. Representative images are shown. Scale bar is 200 µm.

Supplementary Table 2. The cytotoxic efficacy of the HDAC inhibitor vorinostat represented by the respective IC_{50} values in 2D HUVEC cultures as well as in 3D PANC-1 spheroids and in co-culture spheroids after 72 h incubation are shown, respectively. The arithmetic means and their respective standard deviations of three independent experiments are depicted.

Cell type	IC_{50} [µM]
HUVEC	5.70 ± 0.49
PANC-1 spheroid	2.08 ± 0.32
Co-culture spheroid	0.85 ± 0.25

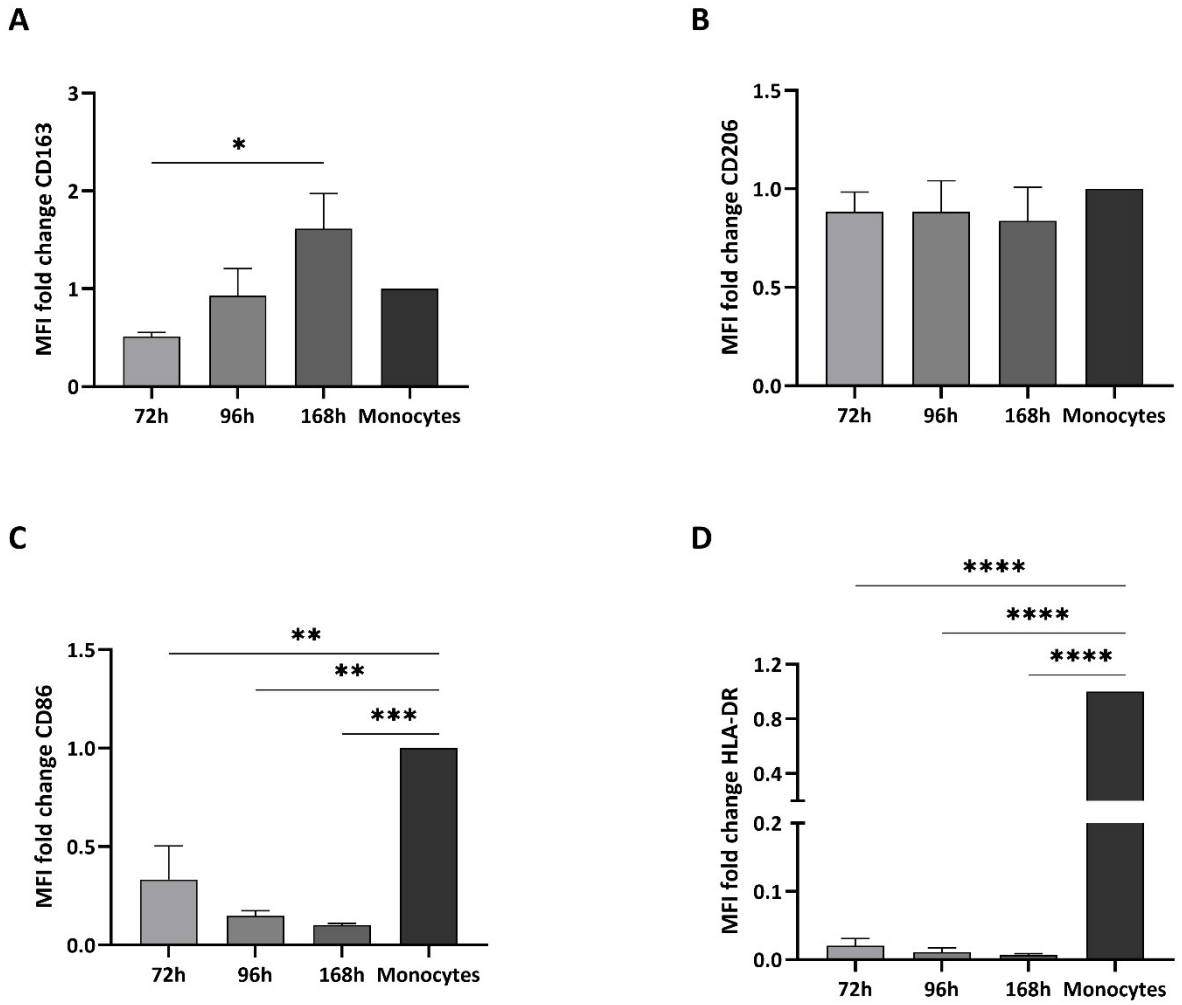


Supplementary Figure 5. Live / Dead staining of HUVEC layer after treatment with SAHA. HUVECs were stained with Calcein (green) and Ethidium homodimer-1 (red) after 72 h treatment with 1.5, 2.5 or 3.5 µM SAHA, untreated cells served as control. Green staining shows viable cells, red staining indicates dead cells. Representative images are shown. Scale bar is 200 µm.



Supplementary Figure 6. IF staining of PANC-1 spheroids after addition of primary monocytes. PANC-1 spheroids were seeded into 96-well plates and primary monocytes were added at day 4 of the cultivation. After 72 h of co-cultivation the PANC-1

spheroids were stained for the tumor cell marker Ki67 (green), the immune cell marker CD45 (purple), and the M2 marker CD163 (red); merging of all channels. Shown as maximum intensity projection MIP of a z-stack. Scale is 500 µm.



Supplementary Figure 7. Time course of macrophage polarization after infiltration of the co-culture spheroids. $CD14^+$ monocytes were added to 4-day-old spheroids and were analyzed by flow cytometry 72 h, 96 h, and 168 h after infiltration. Macrophages identified as $CD11b^+$ and $CD45^+$ were analyzed for surface expression of (A) CD163, (B) CD206, (C) CD86, and (D) HLA-DR. Infiltrated cells were compared to unstimulated monocytes as controls. Error bars indicate the standard errors of the mean of three independent experiments, with * = $p \leq 0.05$; ** = $p \leq 0.01$; $p \leq 0.005$; **** = $p \leq 0.0001$.

Supplementary Video 1. Spatial distribution of infiltrated monocytes. 3D-rendered confocal Z-stack image of a monocyte-infiltrated spheroid, consisting of 300 slices each 1 µm per step, visualized using Image Software Fiji with the 3D Viewer plugin. For enhanced clarity, the spheroid was spatially cut in a slice, allowing better visualization of the fibrotic shell, the core of PANC-1 cells and the spatial distribution of infiltrating macrophages. The spatial orientation of the section is indicated. IF staining for the tumor cell marker pan-cytokeratin (purple), the fibroblast marker α-SMA (blue), the and the M2 marker CD163 (green) merged in one image. White arrows indicate $CD163^+$ signals.