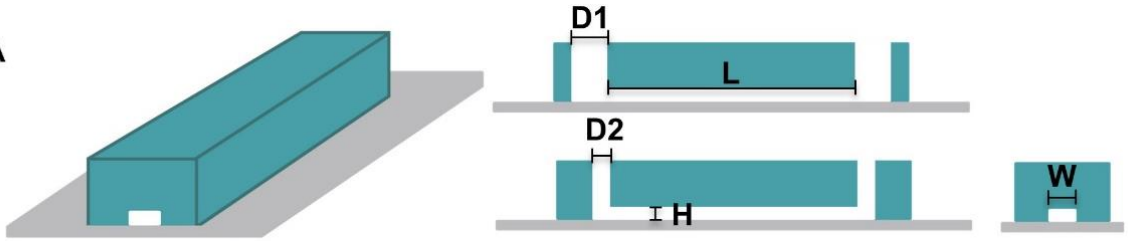
Supporting information

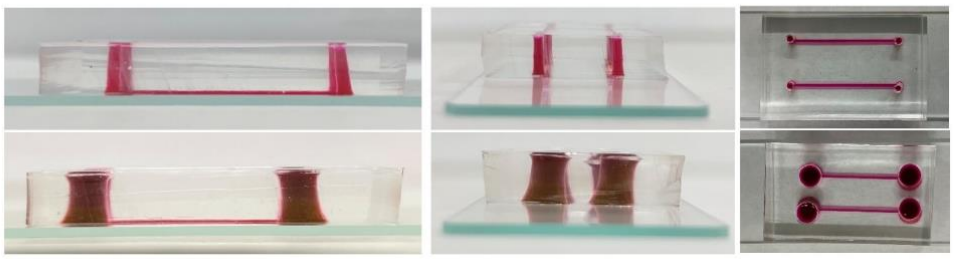
Title: 3D spheroid-microvasculature-on-a-chip for tumor-endothelium mechanobiology interplay

A

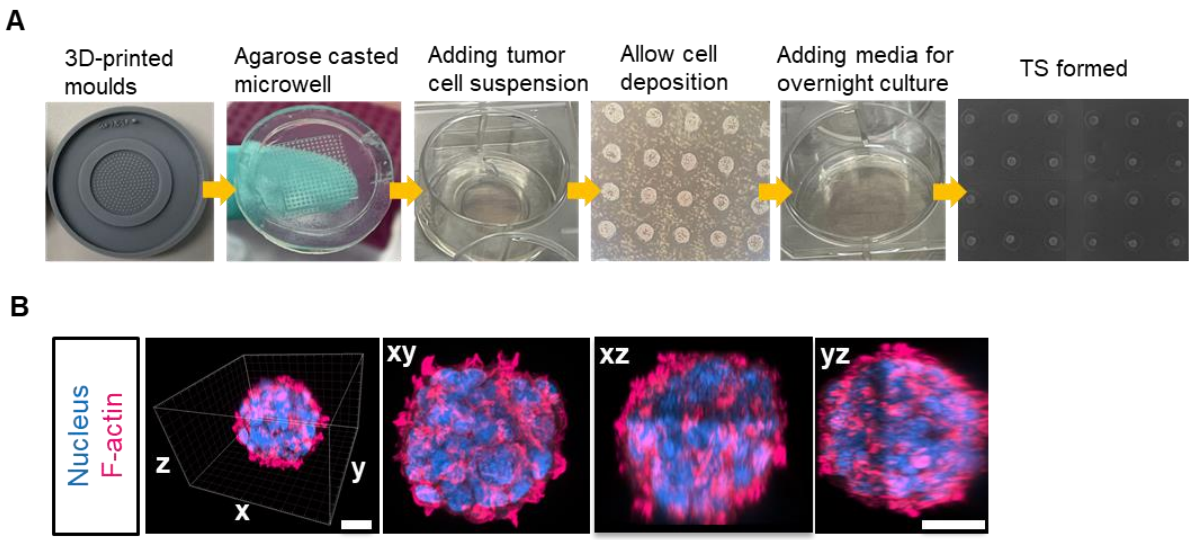


L	Total length of channel	25 mm
W	Width of channel	600 µm
H	Channel height	300 µm
D1	Diameter of channel reservoir for static culture	6 mm
D2	Diameter of channel reservoir for dynamic culture	2mm

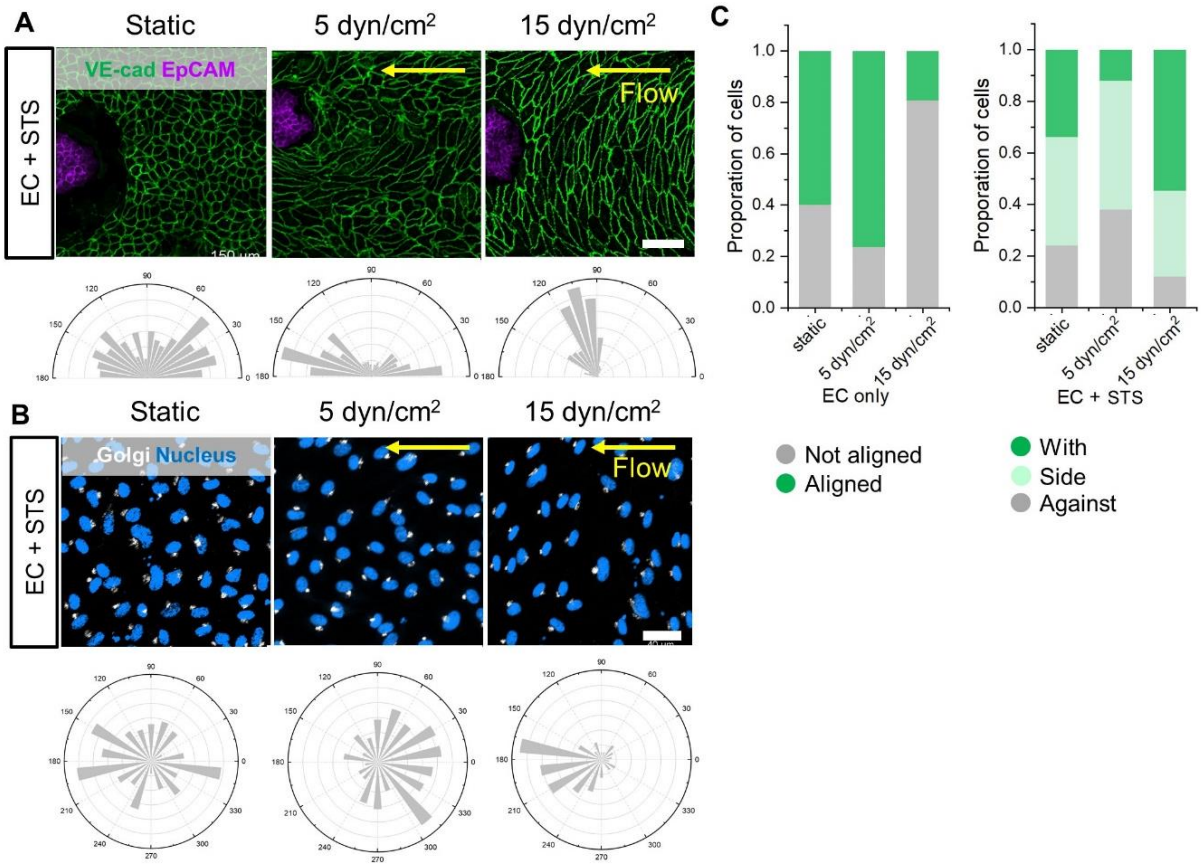
B



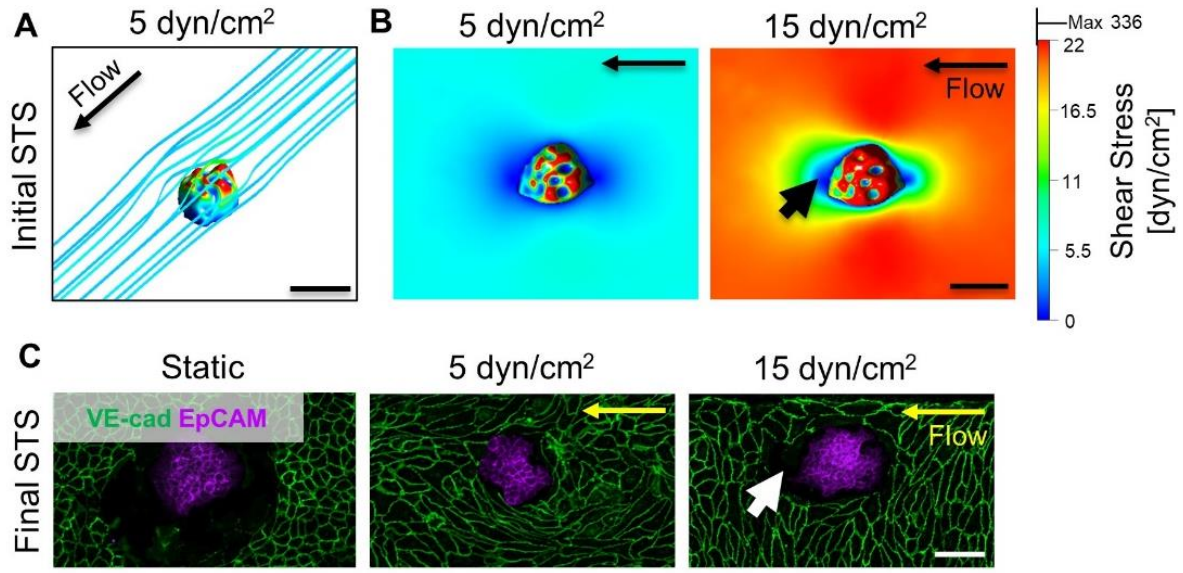
Supplementary figure 1. (A) Schematics of the microfluidic channel and its dimension. (B) Images of the SMAC device from different views.



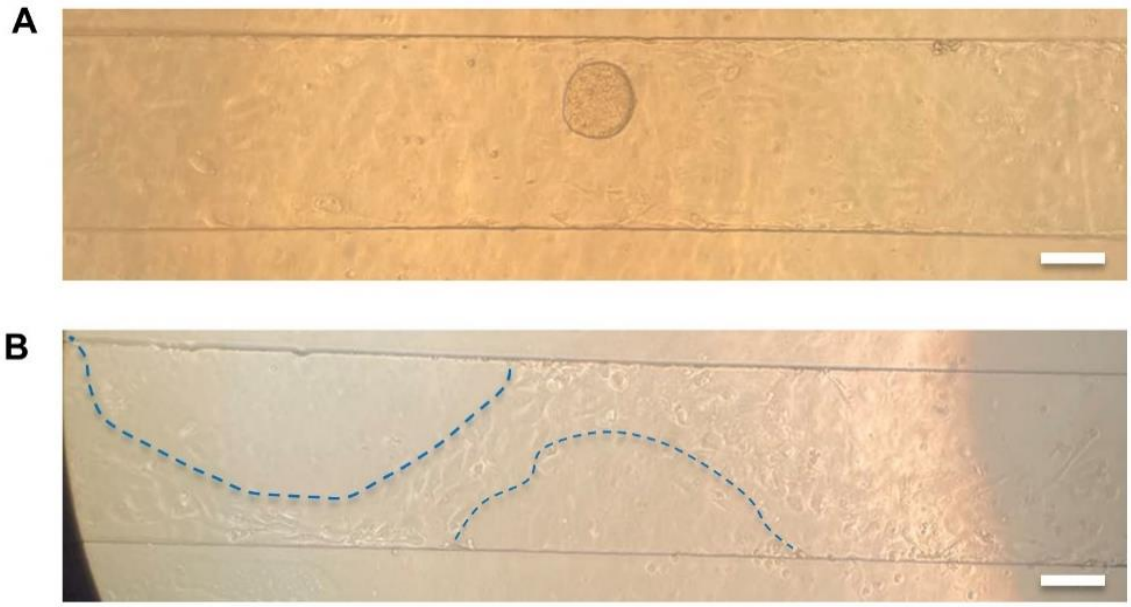
Supplementary figure 2. (A) Workflow for tumor spheroid formation. (B) Representative confocal images of the nucleus (*blue*) and F-actin (*magenta*) of MCF-7 spheroids. Scale bar = 30µm.



Supplementary figure 3. (A) Representative confocal images of ECs with STS exposed to shear stress for 40 hours (static: 0 dyn/cm^2; 5 dyn/cm^2; 15 dyn/cm^2) and associated orientation quantification shown as circular plots. Scale bar = 100µm. (B) Representative confocal images ECs with STS exposed to shear stress for 40 h (static: 0 dyn/cm^2; 5 dyn/cm^2; 15 dyn/cm^2) and associated polarization quantification shown as rose graphs. Scale bars = 40µm. (C) Left: Quantification of the percentage of ECs aligned with the flow direction (in between 45° around the flow axis,) in the presence of STS; Right: Quantification of ECs polarization relative to the flow direction (n = 3; with: in between 135 and 225° around the flow axis, side: 45–135° and 225–315° against 0–45° and 315–360°; approximately 600-700 cells were analyzed).



Supplementary figure 4. (A) 3D computational fluid dynamic (CFD) simulation of shear rate distribution and flow line around STS. Scale bar = 50μm. (B) The top view of CFD simulation of shear rate distribution of the channel with STS. Scale bar = 50μm. (C) Representative confocal images of STS after 40 hours of static culture (left), exposure to 5 dyn/cm^2 (middle) and 15 dyn/cm^2 (right). Scale bar = 100μm.



Supplementary figure 5. (A) Bright field image of tumor spheroid immediately after being seeded in a microfluidic channel with 300 × 100µm (width × height). Scale bar = 100µm. (B) Bright field image of endothelialized channel (300 × 100µm, width × height) showing that after exposure to shear overnight, the tumour spheroids were detached. The blue dashed lines outline the original positions of the tumor spheroids. Scale bar = 100µm.