Electronic Supplementary Material (ESI) for Lab on a Chip.

Electronic Supplementary Information (ESI)

Article title: A multilayered blood vessel/tumor tissue chip to investigate T cell infiltration into solid tumor tissues

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Supplementary figures

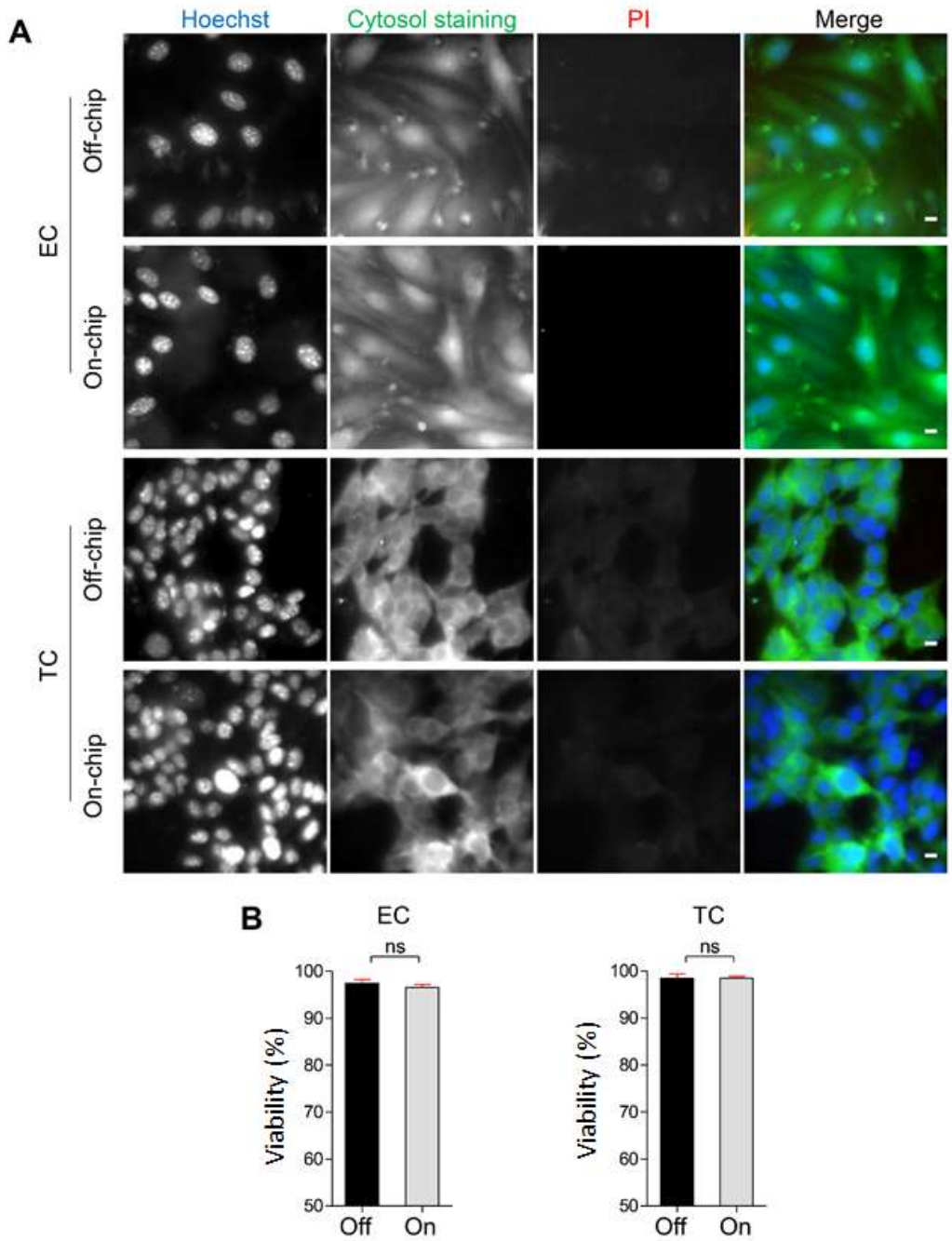


Figure S1. Viability of endothelial cells (ECs) and tumor cells (TCs) in multilayered blood vessel/tumor tissue chips (MBTCs). ECs and TCs stained with cytosolic fluorescence dyes were used. ECs on porous membranes and TCs in collagen gel blocks were cultured 2 days and assembled to produce MBTCs. To assess viability, Hoechst 33342, which stains nuclei of both live and dead cells, and propidium iodide (PI), which stains dead cells, were added to cells, and fluorescence images were acquired and analysed. Viability of cells prior to MBTC assembly (off-chip) and 1 day culture in MBTC (on-chip) was measured. (A) Representative fluorescence images for viability measurement. (B) Quantification of viability. For image analysis, 12 images were acquired in randomly selected positions for each case and analysed. More than 350 cells were analysed for each case. Mann–Whitney U-test, ns: not significant.

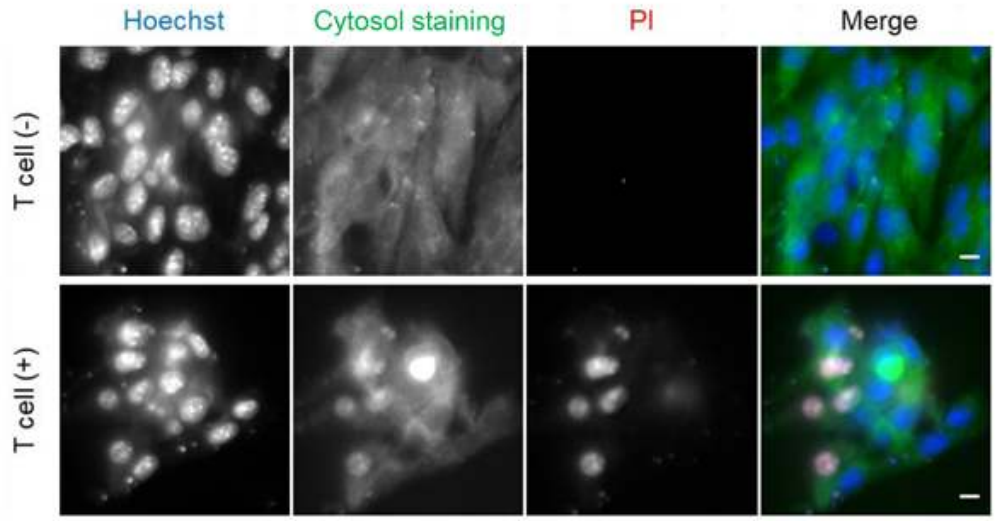


Figure S2. Representative images showing T cell-mediated tumor cell (TC) death in MBTCs. TCs stained with cytosolic fluorescence dyes were used. Hoechst 33342 and PI staining was performed 16 h after T cell infusion.

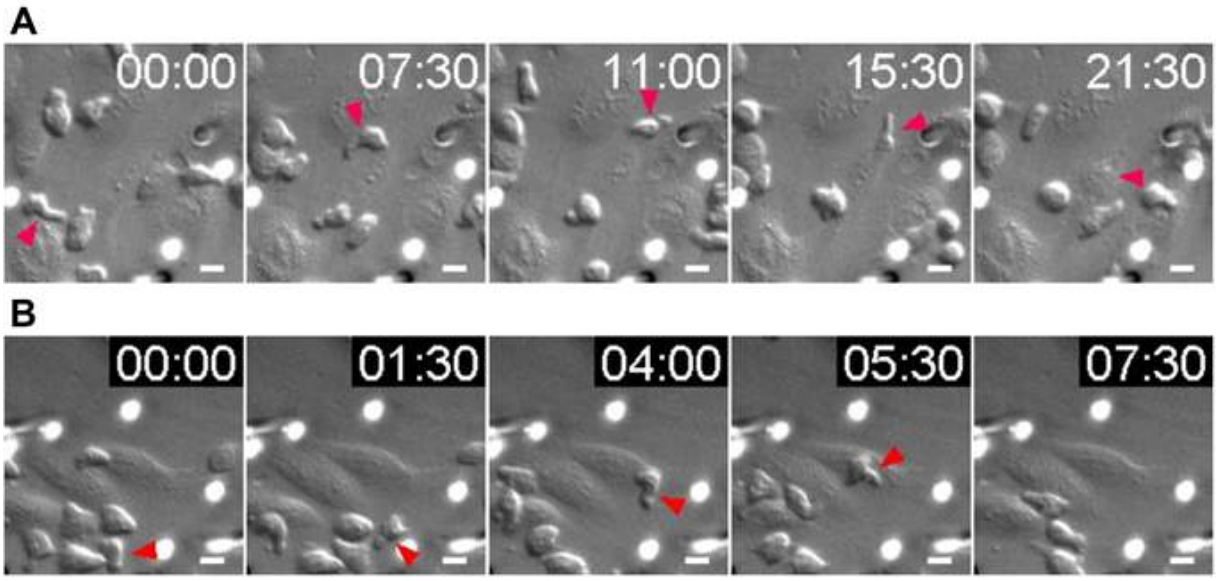
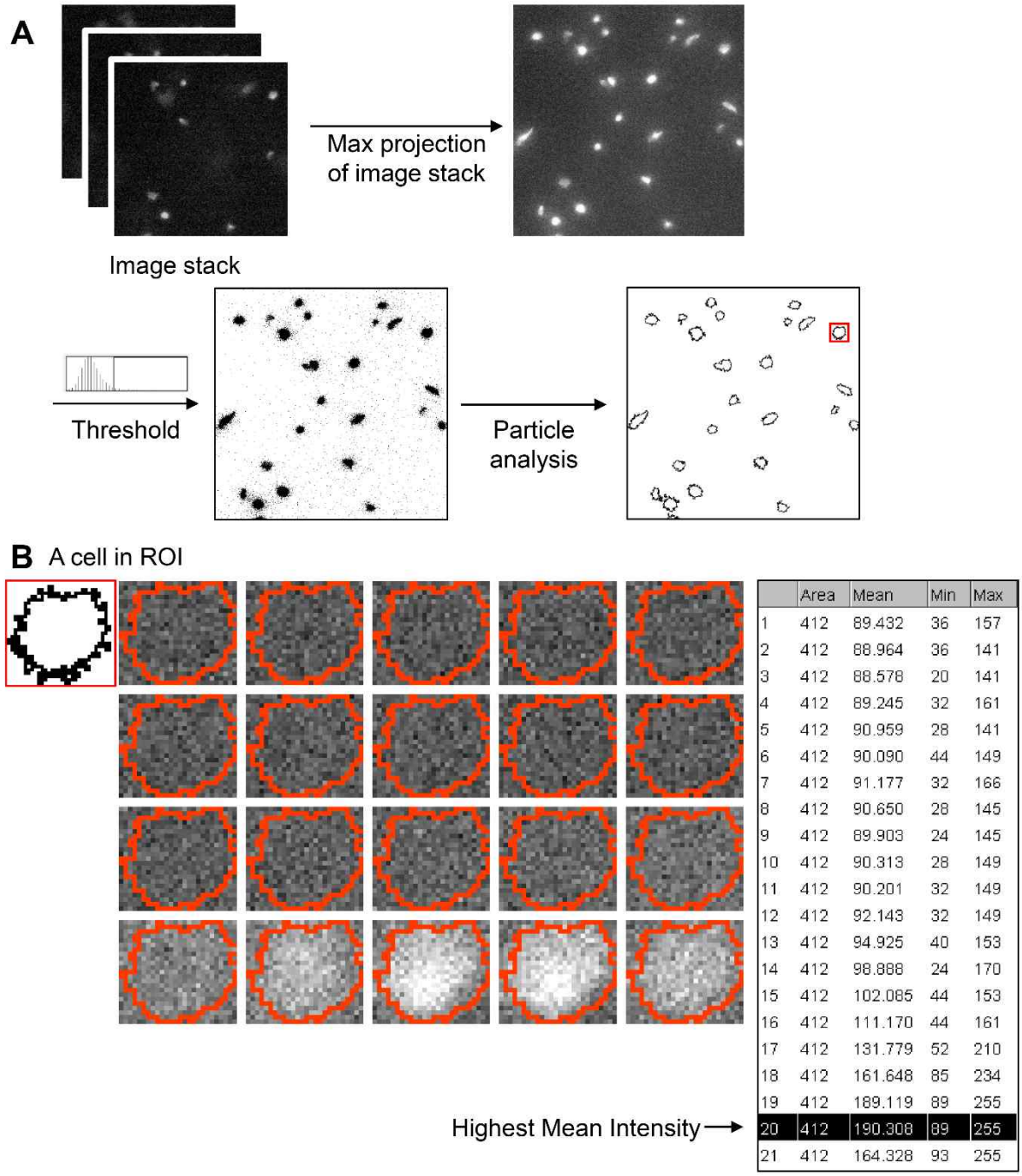


Figure S3. Representative time-lapse images showing a T cell undergoing transendothelial migration (TEM, A: red arrowed; TEM was performed in between 15:30 and 21:30) or being detached by flow (B: red arrowed; detachment occurred in between 5:30 and 7:30). Scale bar : 10 μm, Elapsed time: mm:ss.



	Area	Mean	Min	Max
1	412	89.432	36	157
2	412	88.964	36	141
3	412	88.578	20	141
4	412	89.245	32	161
5	412	90.959	28	141
6	412	90.090	44	149
7	412	91.177	32	166
8	412	90.650	28	145
9	412	89.903	24	145
10	412	90.313	28	149
11	412	90.201	32	149
12	412	92.143	32	149
13	412	94.925	40	153
14	412	98.888	24	170
15	412	102.085	44	153
16	412	111.170	44	161
17	412	131.779	52	210
18	412	161.648	85	234
19	412	189.119	89	255
20	412	190.308	89	255
21	412	164.328	93	255

Figure S4. Image processing procedures to obtain T cell distribution from z-section images of MBTCs. (A) A schematic procedure to identify xy-position of each T cell. Z-section images were projected to a single 'Max projection' image by using 'Image>Stacks>Z projection; Max Intensity' function of ImageJ. Then, threshold was applied to select T cells, and outline of each T cell was extracted as a region of interest (ROI) by using 'Analyze>Analyze Particles; size 10 μm^2~infinity' function of ImageJ. (B) A schematic procedure to identify z-position of each T cell in the ROI. Z-plane with the highest mean intensity was identified. This method may undercount T cell numbers if more than two T cells are located in an identical xy-position. However, in our experiments, ~ 5 % of T cells among ~ 3000 T cells analyzed were in the same xy-position, thus errors would be minimal.

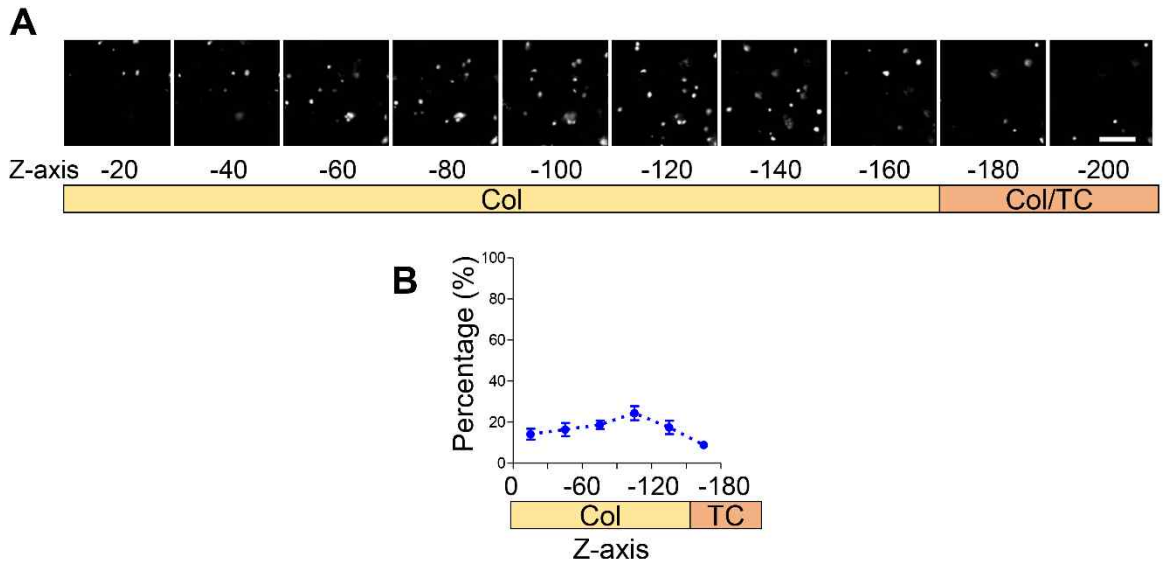


Figure S5. T cell interstitial migration in the absence of EC layers. (A) Representative fluorescence images of T cells in each plane. White: T cells, Scale bar: 100 μm (B) The distribution of T cells over z-axis. 3 chips were analyzed. Each chip, 4 randomly selected areas were examined by z-sectioning (typically, total T cell number in each area over z-sections > 30).

Supplementary movie legends

Movie S1. A representative movie of a T cell (red arrows) undergoing extravasation. Scale bar: 10 μm. Elapsed time: hh:mm:ss.

Movie S2. A representative movie of a T cell (red arrows) undergoing interstitial migration through collagen gel to encounter tumor cells. Scale bar: 10 μm. Elapsed time: hh:mm:ss.

Movie S3. A representative movie of a T cell (red arrows) killing a tumor cells (yellow arrows). Scale bar: 10 μm. Elapsed time: hh:mm:ss.