Electronic Supplementary Information (ESI) for Lab on a Chip

Electronic Supplementary Information

Heterotypic 3D tumor culture in a reusable platform using pneumatic microfluidics

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Abstract. This supplementary information provides all the additional information and a more detailed discussion of the current study.

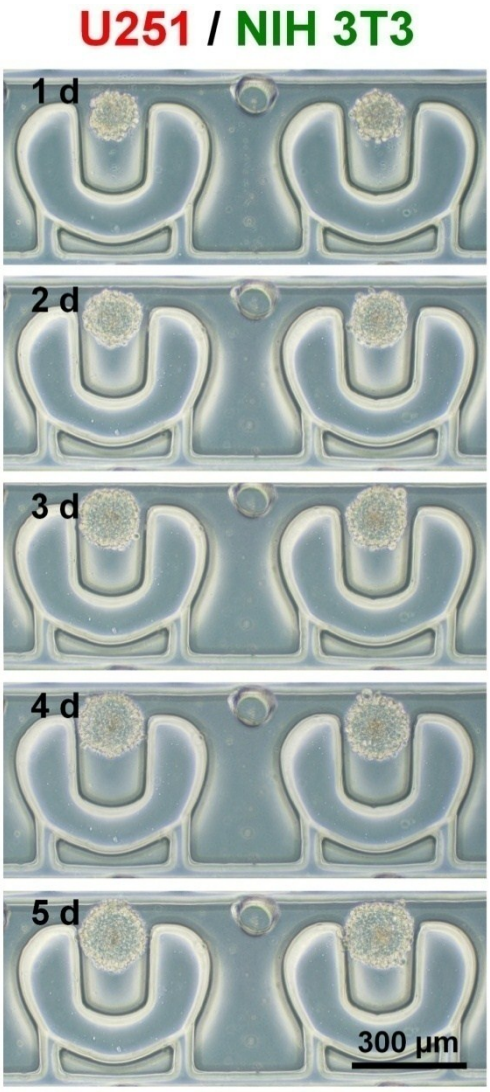

Fig. S1. Optical images of U251/NIH 3T3 tumors at different times of 5 days coculture in the microfluidic device at a flow rate of 2 μL min^{-1}. The original mixing ratio of U251 cells and NIH 3T3 cells was of 1:1.

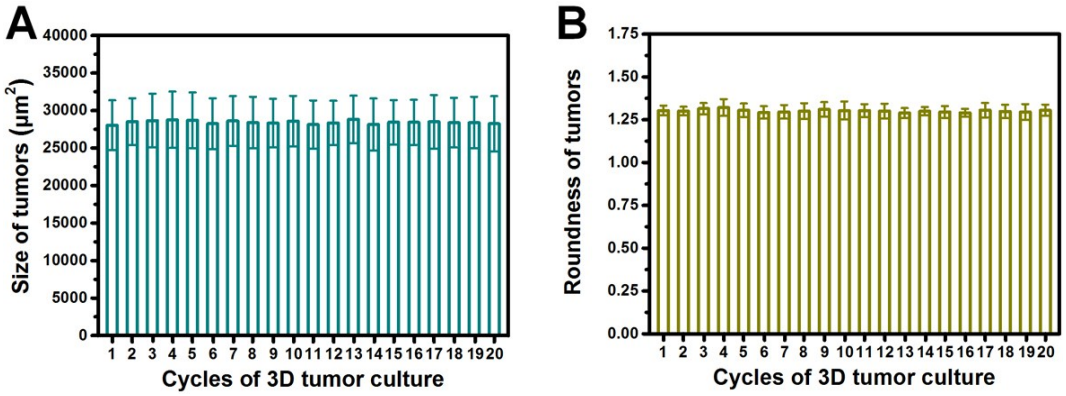

Fig. S2. Reproducibility of tumor production in different culture cycles in single devices. (A) Tumor size in different repeated cultures. (B) Tumor roundness in different repeated cultures.

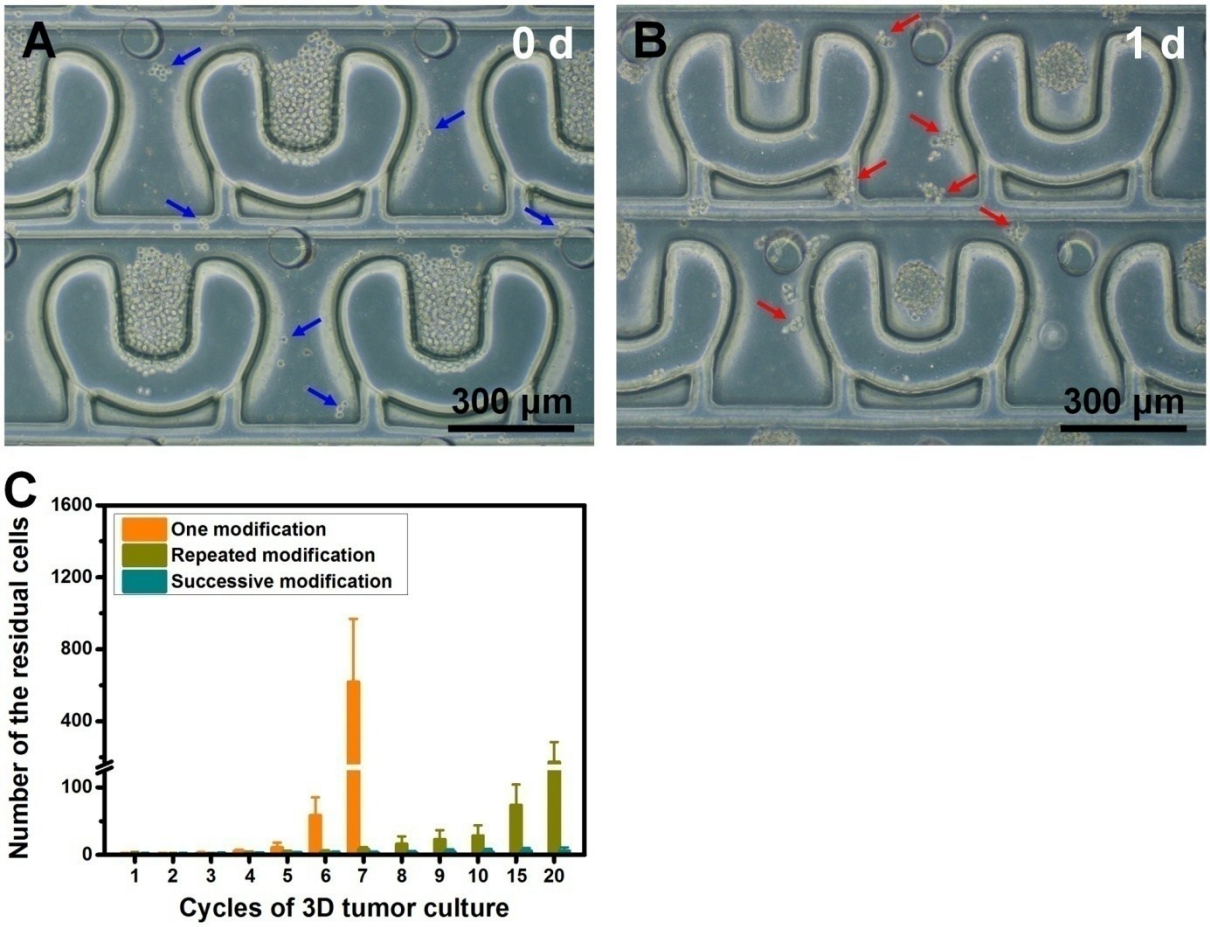

Fig. S3. Precise cell localization in the microfluidic device. (A) Cell residue (blue arrows) in the non-trapping regions after the seventh trapping process in the device. The chambers were treated with Pluronic F127 solution (5 mg mL^{-1} in water) only before the cell trapping in the first culture. (B) Cell aggregations (red arrows) at the non-trapping regions after 1 day in culture. The results (A and B) indicate that Pluronic F127 pretreatment for one time was unable to maintain a long-term inhibitory effect of cell adhesion, which significantly influences precise cell localization in the trapping regions of the chamber using PμSs. (C) Number of the residual cells in the non-trapping regions of the chamber after cell trapping in different culture cycles. Three anti-adhesive treatments including one modification (i.e., anti-adhesive treatment only before the first culture cycle using 5 mg mL^{-1} Pluronic F127 in water), repeated modification (i.e., anti-adhesive treatment before each culture cycle using 5 mg mL^{-1} Pluronic F127 in water), and successive modification (i.e., persistent anti-adhesive treatment during each culture cycle using 1 mg mL^{-1} Pluronic F127 in DMEM) were experimentally compared to optimize the inhibitory effect of cell adhesion.

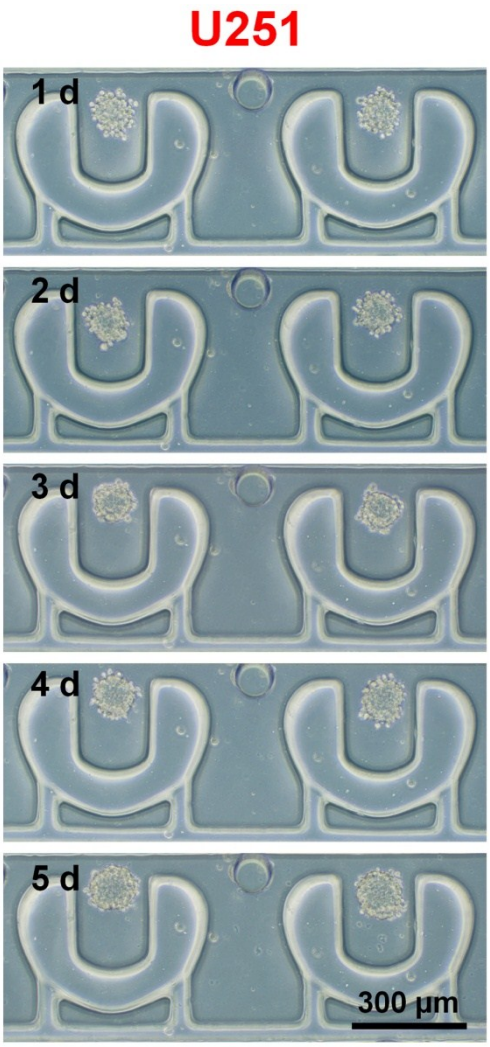

Fig. S4. Optical images of U251 tumors at different times of 5 days monoculture in the microfluidic device at a flow rate of 2 µL min^{-1}.

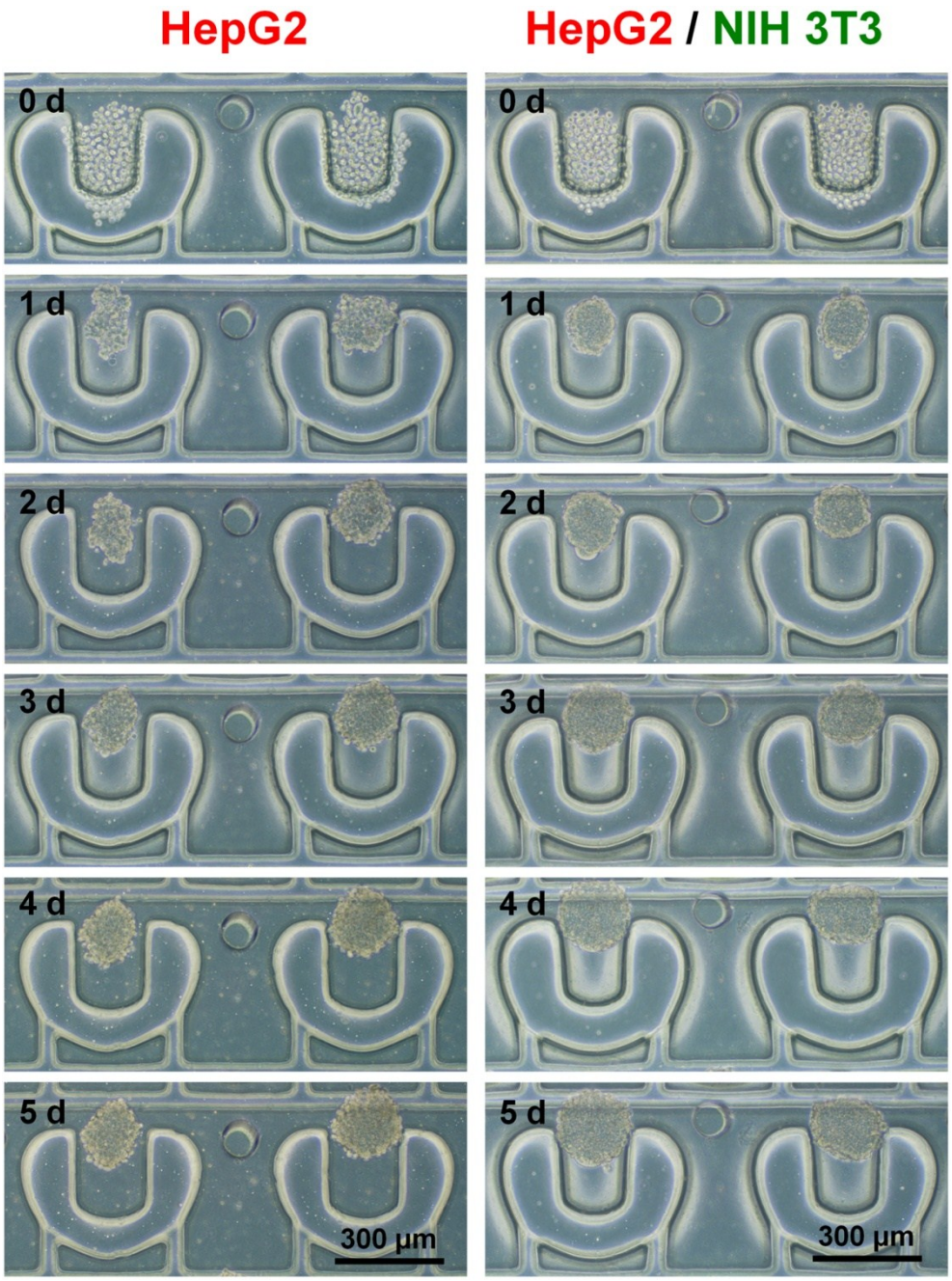

Fig. S5. Optical images of HepG2 and HepG2/NIH 3T3 tumors at different times of 5 days monoculture and coculture respectively in the microfluidic device at a flow rate of 2 μL min^{-1}. The original mixing ratio of HepG2 cells and NIH 3T3 cells was of 1:1 in coculture.

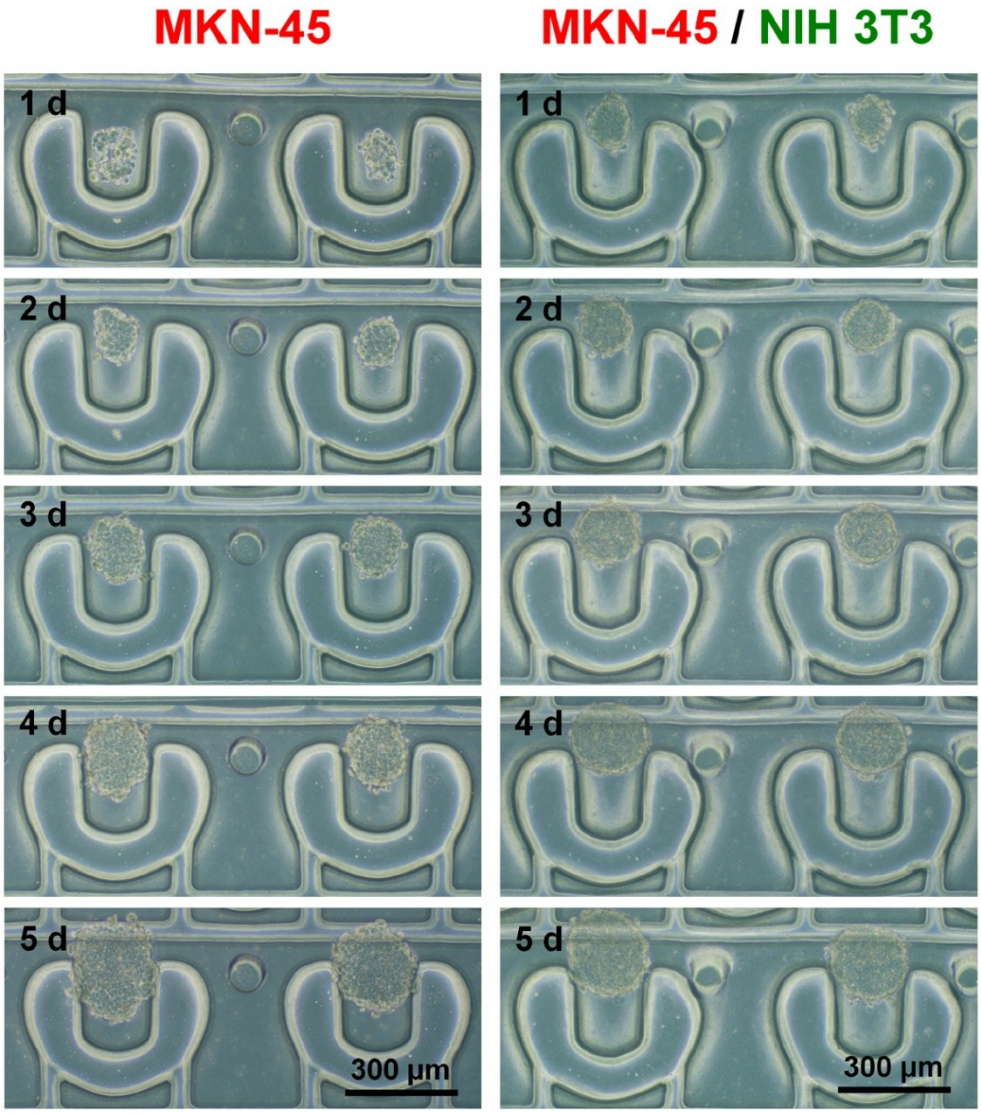

Fig. S6. Optical images of MKN-45 and MKN-45/NIH 3T3 tumors at different times of 5 days monoculture and coculture respectively in the microfluidic device at a flow rate of 2 μL min^{-1}. The original mixing ratio of MKN-45 cells and NIH 3T3 cells was of 1:1 in coculture.

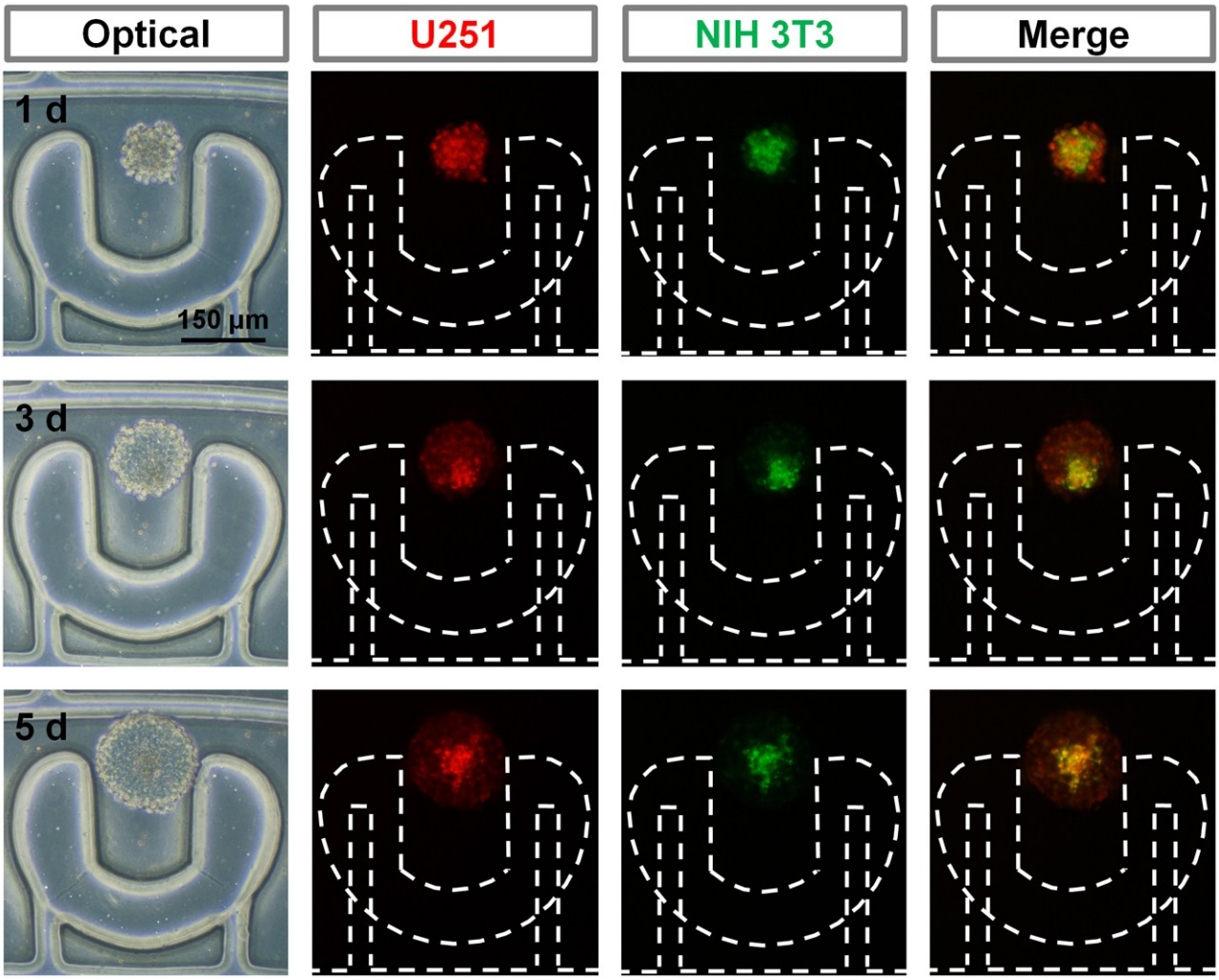

Fig. S7. Spatial distributions of U251 cells and NIH 3T3 cells at different time points of microfluidic coculture. U251 cells and NIH 3T3 cells were prestained with DiI (red) and DiO (green) for specific cell tracking.

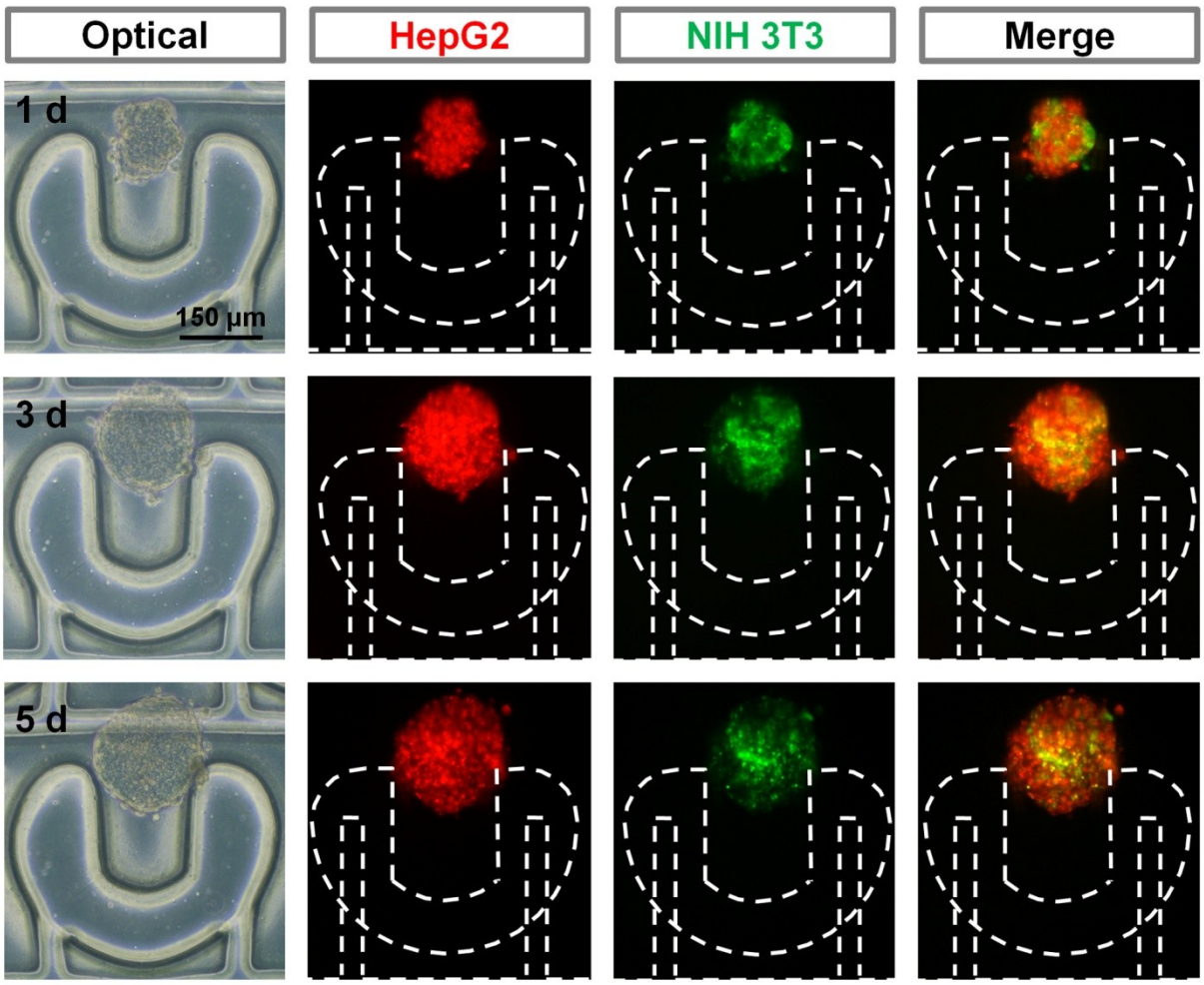

Fig. S8. Spatial distributions of HepG2 cells and NIH 3T3 cells at different time points of microfluidic coculture. HepG2 cells and NIH 3T3 cells were prestained with DiI (red) and DiO (green) for specific cell tracking.

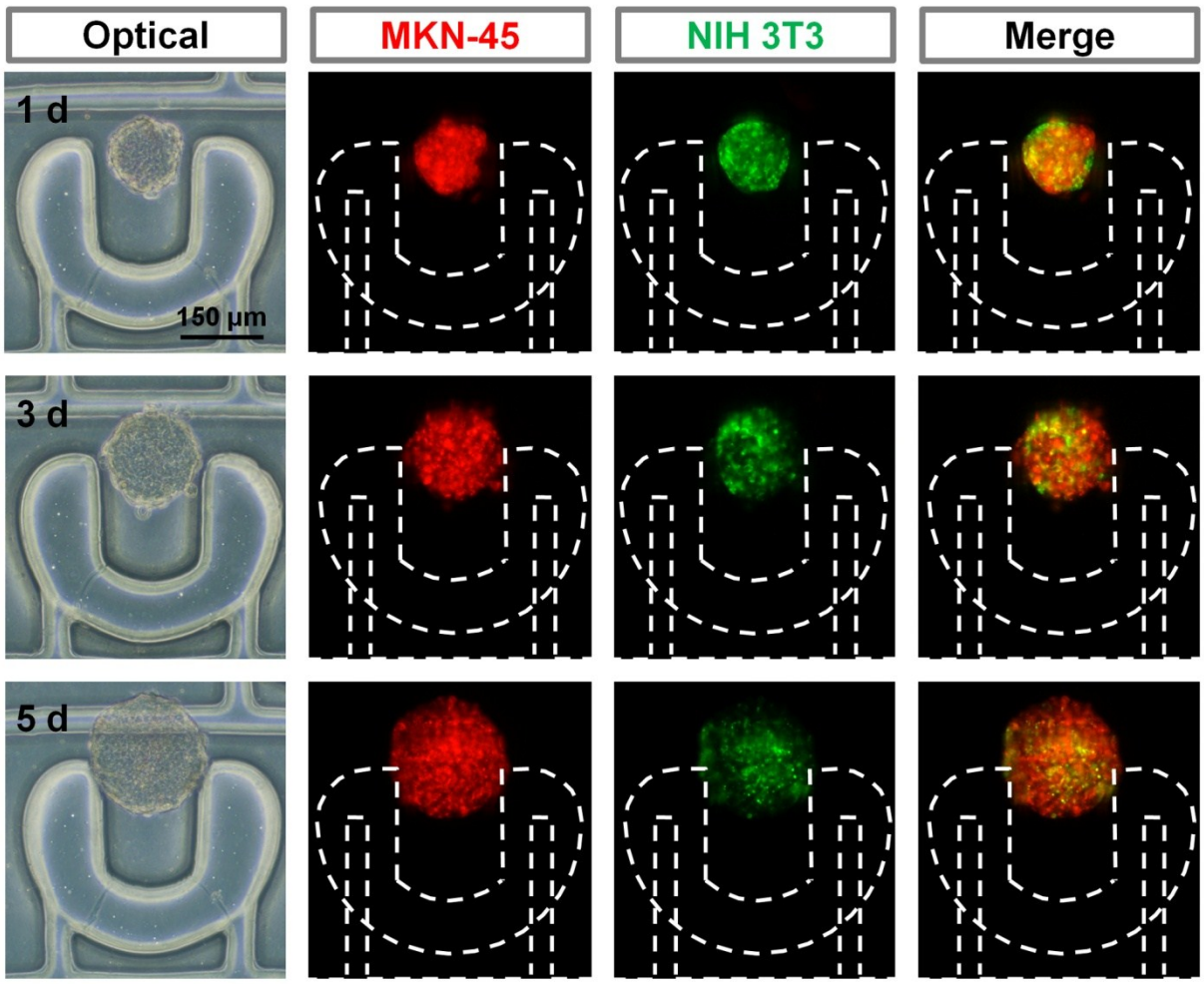

Fig. S9. Spatial distributions of MKN-45 cells and NIH 3T3 cells at different time points of microfluidic coculture. MKN-45 cells and NIH 3T3 cells were prestained with DiI (red) and DiO (green) for specific cell tracking.

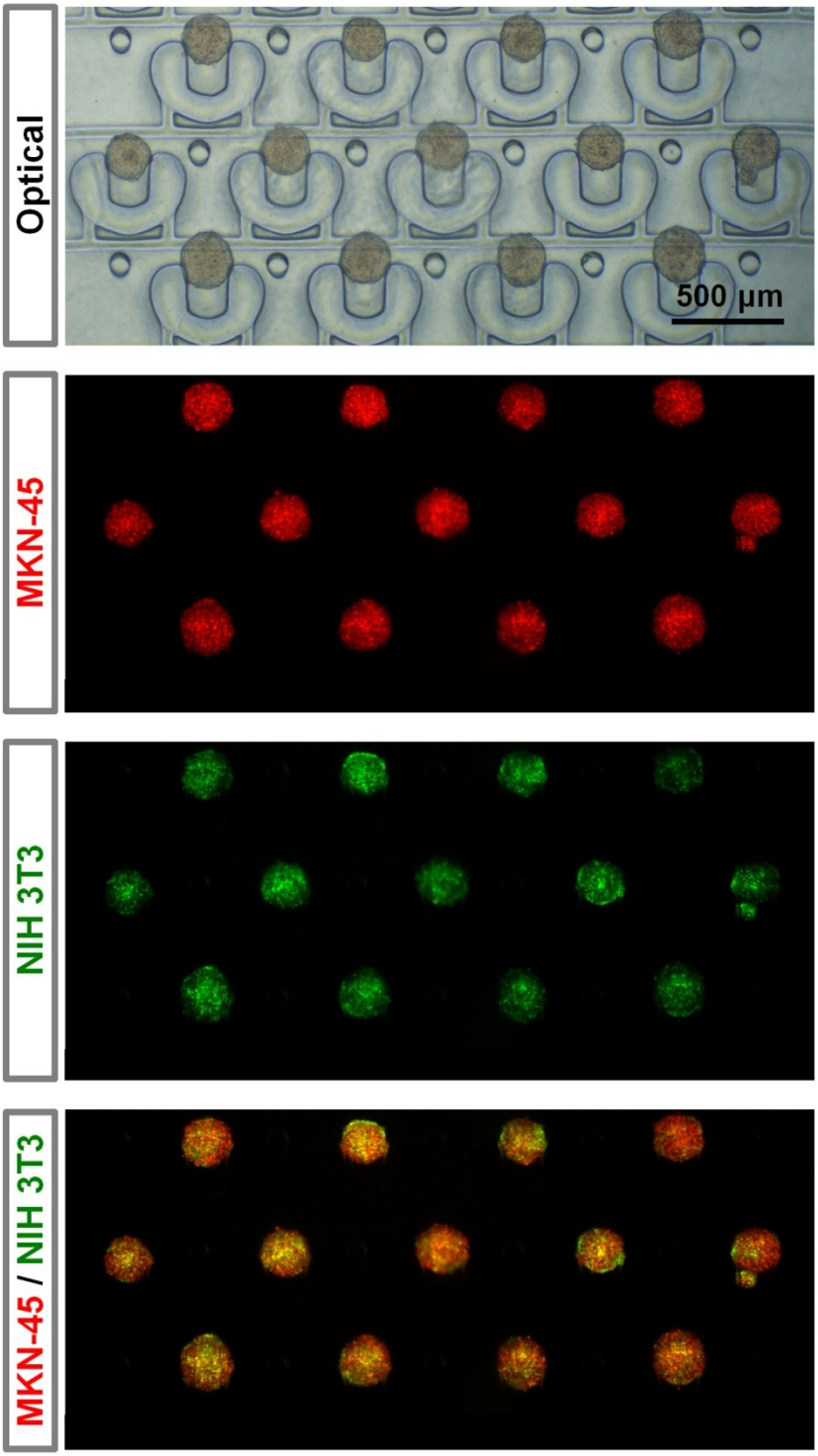

Fig. S10. Cocultured MKN-45/NIH 3T3 tumor array for throughput analysis of MKN-45 (red) and NIH 3T3 (green) cell arrangements.

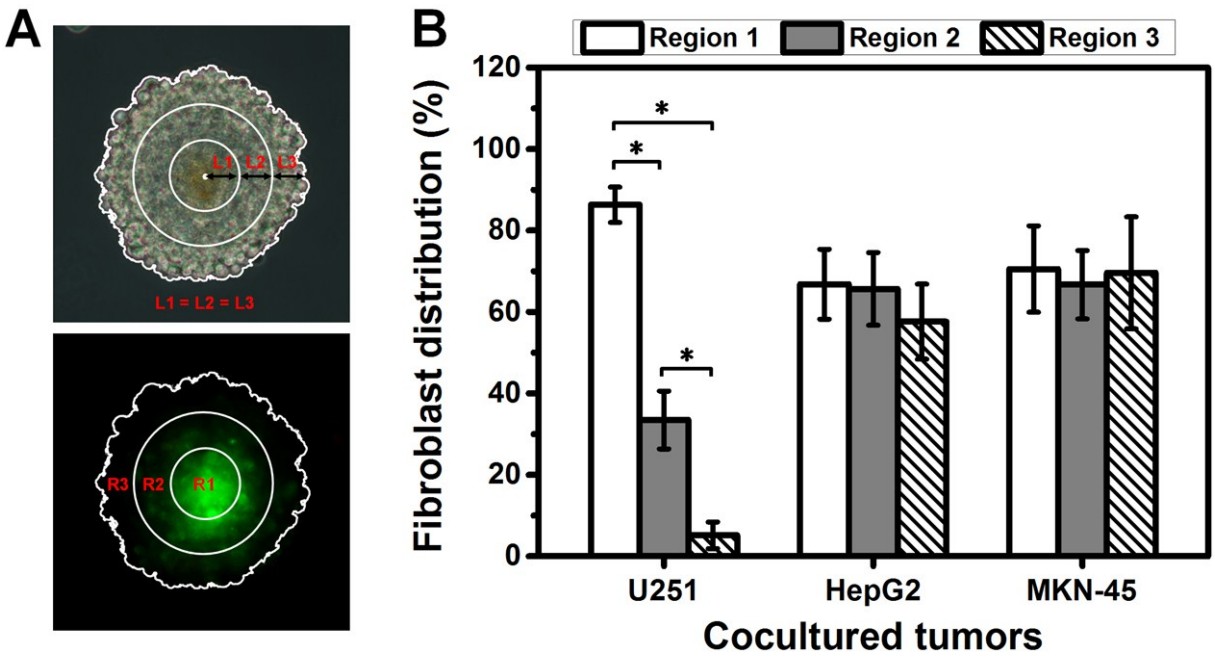

Fig. S11. Quantitative analysis of fibroblast distribution in different regions of the tumors. (A) The principle of quantitative analysis of NIH 3T3 cell distribution. Tumor was divided into three regions including a circular region (R1 for region 1) and two annular regions (R2 for region 2 and R3 for region 3). The radius of R1 (L1) equals the width of R2 (L2) and R3 (L3) respectively. Fibroblast distribution in each region of tumor was calculated as the area of NIH 3T3 cell distribution in the specific region divided by the area of the same region. (B) NIH 3T3 cell distribution in different regions of the cocultured U251, HepG2, and MKN-45 tumors. *P < 0.05.

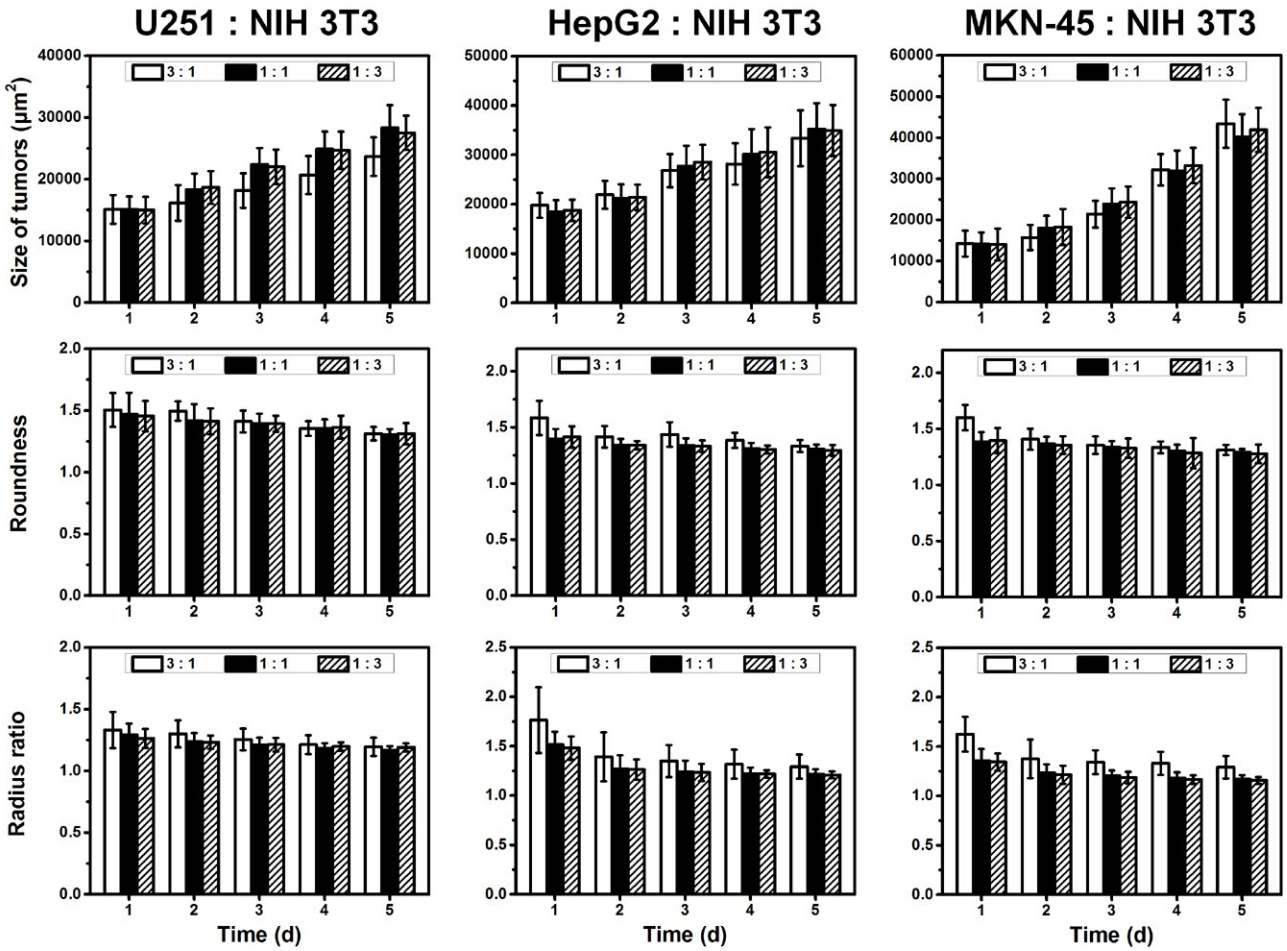

Fig. S12. Investigation of growth and formation of different heterotypic tumors with 5 days in coculture after the loading and trapping processes using different mixing ratios (3:1, 1:1, and 1:3) of tumor cells (U251, HepG2, and MKN-45) and fibroblasts (NIH 3T3).

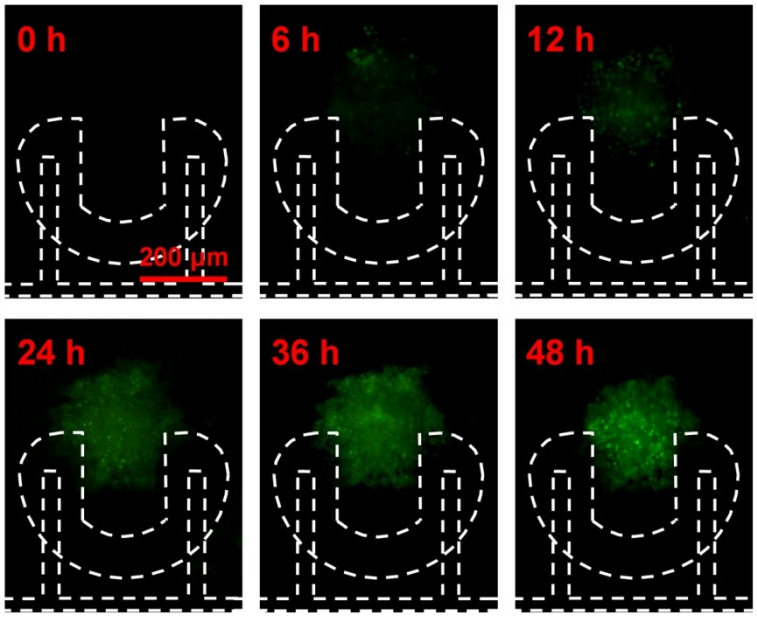

Fig. S13. Fluorescent images of caspase-3$^+$ cells in cocultured MKN-45 tumor treated with 100 μg mL^{-1} VNR at various treating times (0, 6, 12, 24, 36, and 48 h).

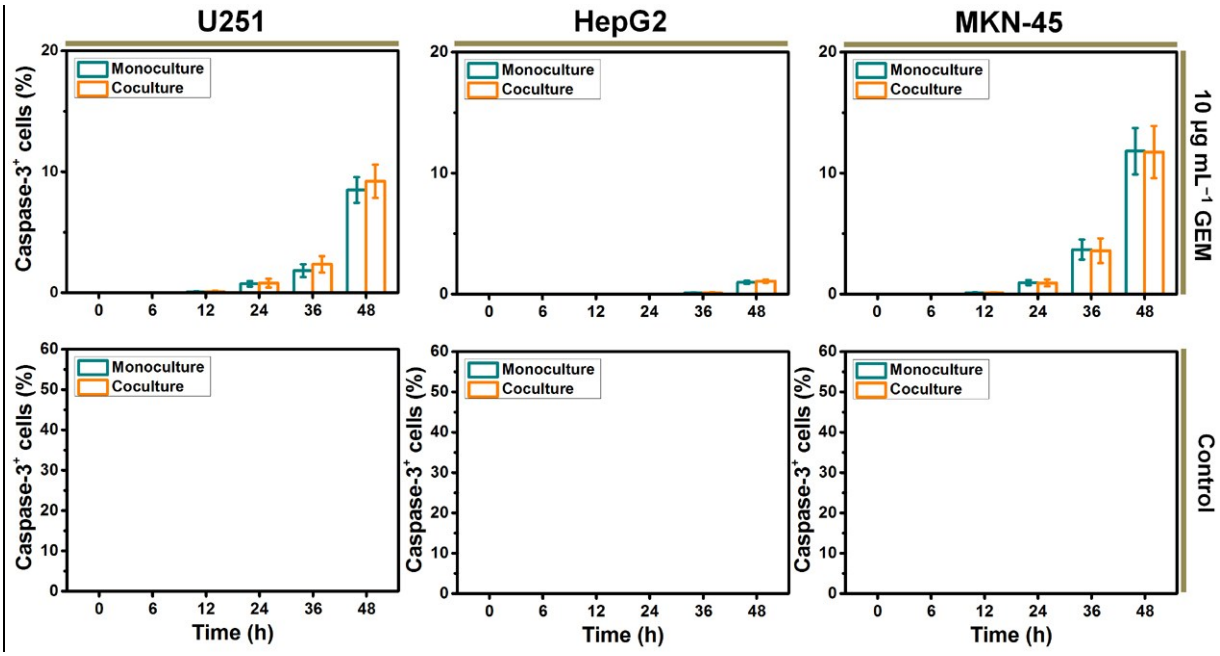

Fig. S14. Percentage of caspase-3$^+$ cells in cocultured and monocultured tumors with GEM (10 μg mL^{-1}) treatment or no drug treatment at different time points.

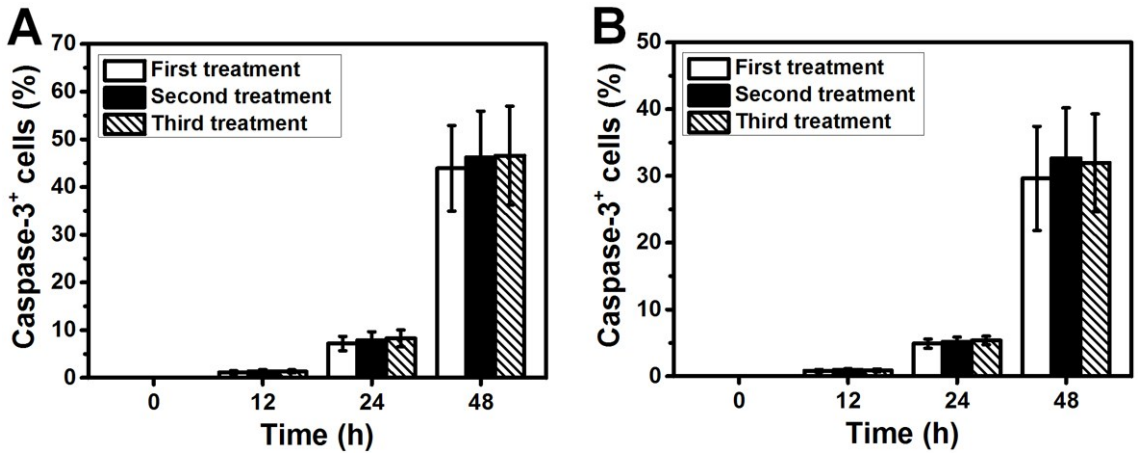

Fig. S15. Percentage of caspase-3$^+$ cells in U251/NIH 3T3 (A) and HepG2/NIH 3T3 (B) tumors treated with 100 μg mL^{-1} VNR at different treating times (0, 12, 24, and 48 h). Drug treatments of either U251/NIH 3T3 or HepG2/NIH 3T3 tumors was repeated three times based on three culture cycles respectively in the single device. Similar caspase-3 activation happened in heterotypic U251 or HepG2 tumors from three VNR treatments.

ESI Movies

Movie S1. Repeated activation of PμSs in the microfluidic device

Movie S2. Persistent activation of PμSs in the microfluidic device.