Supporting Information

Droplet-based Microfluidic Platform for High Spatiotemporal Resolved Single-Cell Signalling Profiling

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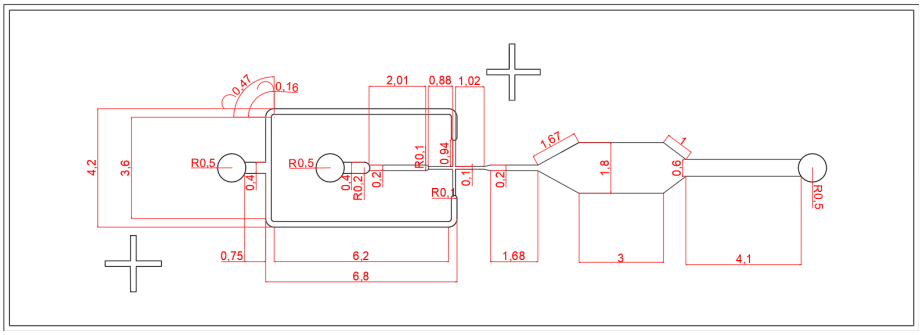

Figure S1. Schematic view with dimensions of the whole chip (a), the junction (b) and the anchors (c).

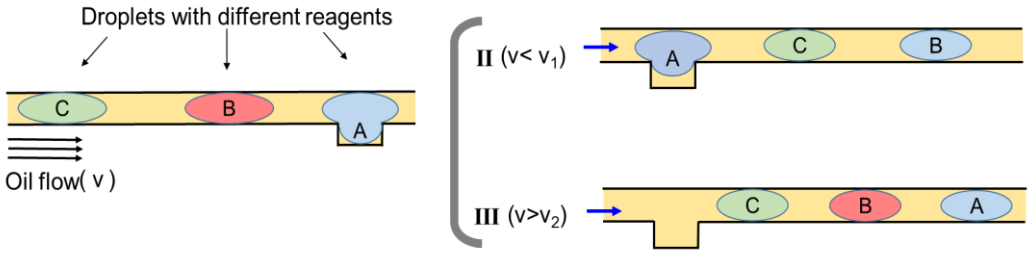

Figure S2. (II) The parking mode ($v<v_1$) or (III) the passing mode ($v>v_2$) would happen to the droplets on SEWs depending on the oil flow rate.

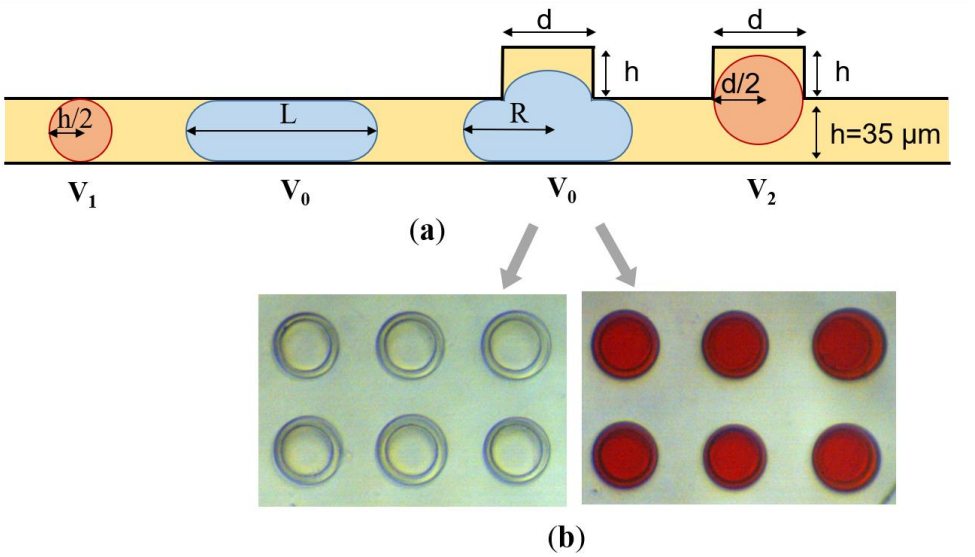

Figure S3. (**a**) V_1 is equal to 0.5 nL for a 35 μm high channel ($V_1 = \pi h^3/6$); V_2 is equal to 0.5 nL for a 50 μm SEW diameter ($V_2 = \pi d^3/6$); (**b**) Typical droplet volumes used here are around 0.6 nL, larger than V_1 and V_2, squashed into a flattened pancake shape.

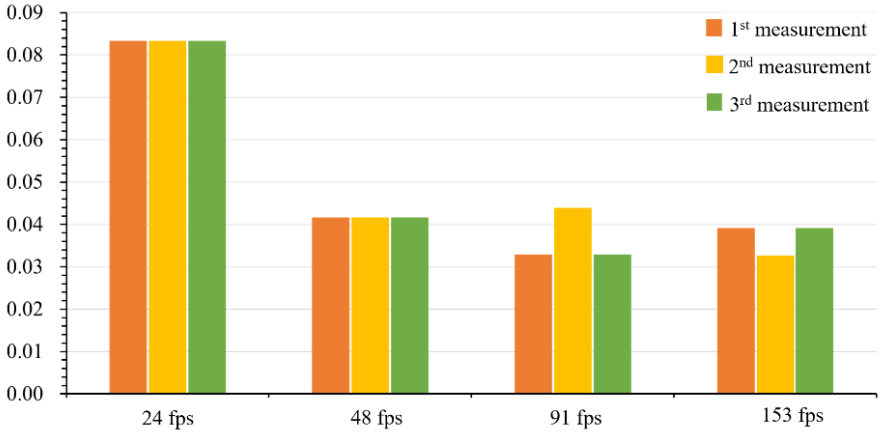

Figure S4. In the stable droplets generation stage under the combination of oil low rate (5.3 μL/min) and liquid flow rate (4.8 μL/min), the time resolution of the replacement process was calculated respectively under different camera frames set by the high-speed camera.

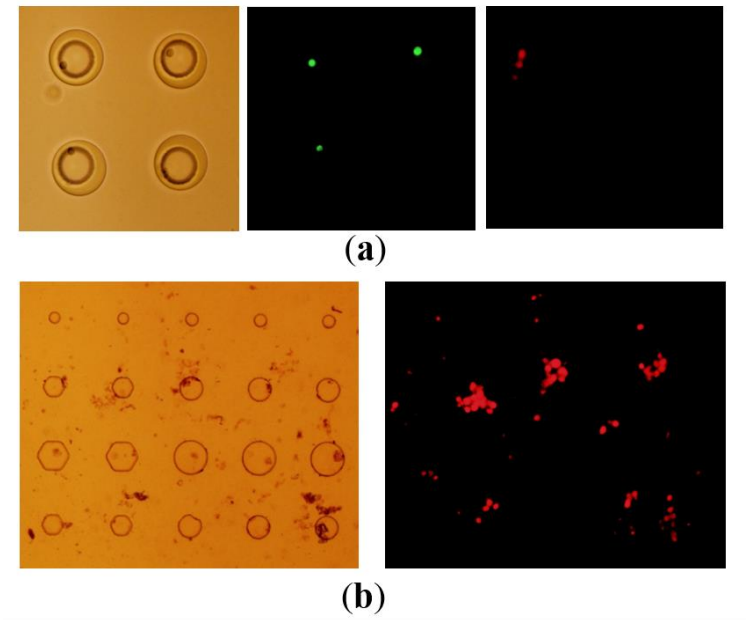

Figure S5. (**a**) Cells were loaded into a 100 μL syringe, while HFE oil containing surfactant is loaded into 1 mL syringes. Droplets of single cells were generated at the flow focusing junction, by controlling the flow rates. After delivering, the single-cell droplets were trapped on the SEWs array, at which point the green fluorescence observed by FDA staining indicated living cells. After culture for 1 h in the CO_2 incubator, the results showed that most of the cells in the droplet did not adhere, although a few cells did adhere but their PI staining was red indicating dead cells; (**b**) After injection of the cell suspension into the chamber, the cells were cultured in the incubator under static conditions for 2 hours, no continuous flow of culture medium and the PI staining results showed that the cells could adhere to the bottom channel but almost all died.

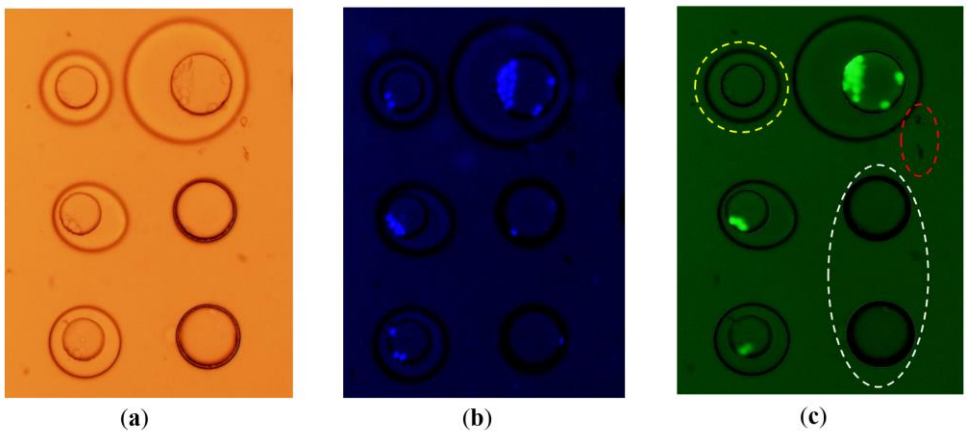

Figure S6. After single cells were patterned in the chamber, six Hoechst droplets were first trapped under the SEWs array for 5 min. Then, four FDA droplets were trapped under the SEWs array for 10 min (a). Finally, the chip was placed on a fluorescence microscope to observe the fluorescence images under UV and blue excitation light. The results showed that all the covered cells presented blue fluorescence (**b**) and all the covered and living cells had red fluorescence (**c**). In particular, yellow circle indicated that cells are adherent but dead, white circle indicated that cells uncovered by FDA droplets are not stained, and red circle indicated that cells far from the SEW structure cannot be stained by Hoechst droplets nor FDA droplets.

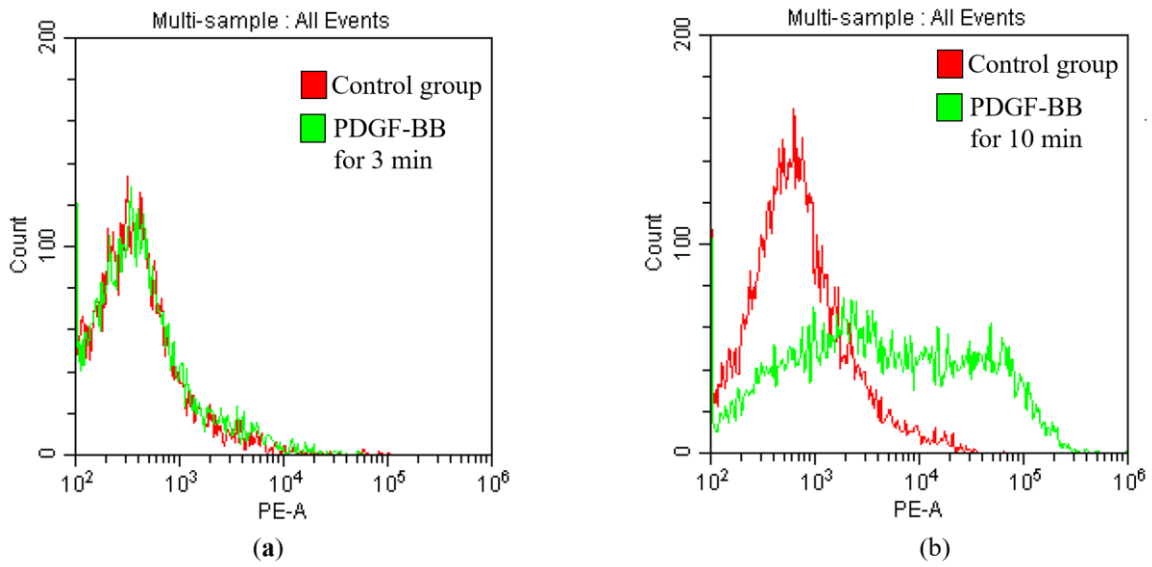

Figure S7. MCF-7 cells in a petri dish were subjected to immunohistochemical assay using the same procedure, and then digested for flow cytometry. (**a**) The average level of Akt phosphorylation after 3 min stimulus was basically similar to the control group without ligand stimulus; (**b**) The level of Akt phosphorylation after 5 min stimulus was significantly different from that of the control group.

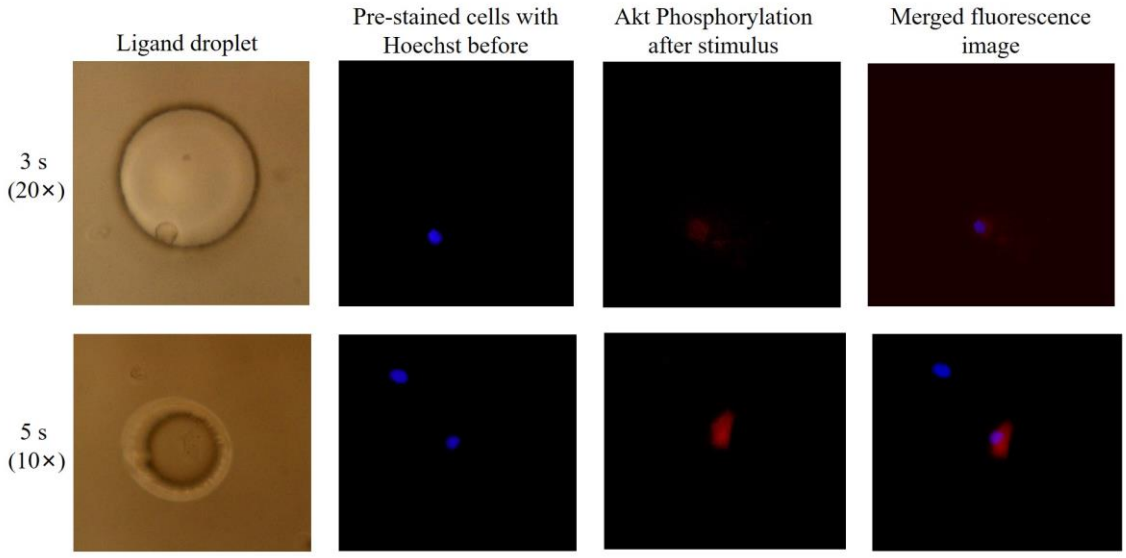

Figure S8. The inhibited 4T1 cells were first continuously performed in the replacement mode with medium droplets for 1 min, comprising about 240 droplets, and then stimulated with PDGF-BB droplets. The final fluorescence of Akt phosphorylation induced by PDGF-BB stimulus for 3 s and 5 s, respectively, showing that the phosphorylation response for cells treated in this manner is not significantly different from undisturbed cells.