Electronic Supplementary Information (ESI) for Analyst

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Electronic Supplementary Information

Large-scale investigation of single cell activities and response dynamics in a microarray chip with microfluidics-fabricated microporous membrane

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Abstract. This supplementary information provides all the additional information as mentioned in the text.



Fig. S1 Optical images of actual microfluidic devices (top), membrance peeling from the the fluidic layer of the device (middle), and membrane obtaining (bottom) for the fabrication of PDMS microporous membranes with different arrangements (left: array; right: chinese character) of through-holes using a PDMS mixture with 8 : 1 mixing ratio.



Fig. S2 Optical imaging of actual PDMS microporous membranes. (A) Optical images of membranes with various hole sizes (diameter from top to bottom: 35, 75, and 100 μ m) and spaces (from left to right: 50, 75, and 100 μ m). (B) Optical images of microporous membranes with different through-hole arrangements (i.e., chinese character "zhong", chinese character "nan", smiling face, flower, fan, five-pointed star, bluetooth, honeycomb, and circle).

	Diamond		Heart		Rectangle	
SEM	000000000000 00000000000 00000000000	<u>500 µ</u> т		5 <u>00 μ</u> m		1111111111111111111111111111111111111
Optical		00000000000000000000000000000000000000	00000000000000000000000000000000000000	00000000000000000000000000000000000000		

Fig. S3 SEM (top) and Optical (bottom) images of membranes with various hole shapes and sizes.



Fig. S4 Optical and fluorescent images of cell trapping in chips using different seeding densities $(5.0 \text{ to } 10.0 \times 10^5 \text{ cells mL}^{-1})$.



Fig. S5 Quantification of cell trapping with different periods of loading time (10 to 60 min) in chips. Four types of cell trapping states, namely, no cell, 1 cell, 2 cells, and \geq 3 cells were included.



Fig. S6 Time of the first cell division of single cells (n=118) after the trapping in chips. The start time point of the first cell division for these mother cells was uncertain.



Fig. S7 Caspase-3 activation of single Hela cells treated with 10 μ g mL⁻¹ VLBT at various treatment times (0, 6, 13, 18, 21, and 24 h) in the microwell array chip. Caspase-3⁺ cell distribution were visualized based on fluorescence labeling. The pseudo color images (B) correspond to the fluorescence images (A).



Fig. S8 Intracellular fluorescence accumulation in single cells after VLBT stimulation with various concentrations for 6 h. The control was set here.