Electronic Supplementary Material (ESI) for Lab on a Chip.
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A Microfluidic Chip for Screening High-Producing Hybridomas at Single Cell Level

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Supplementary Data S1

SHS-Chip

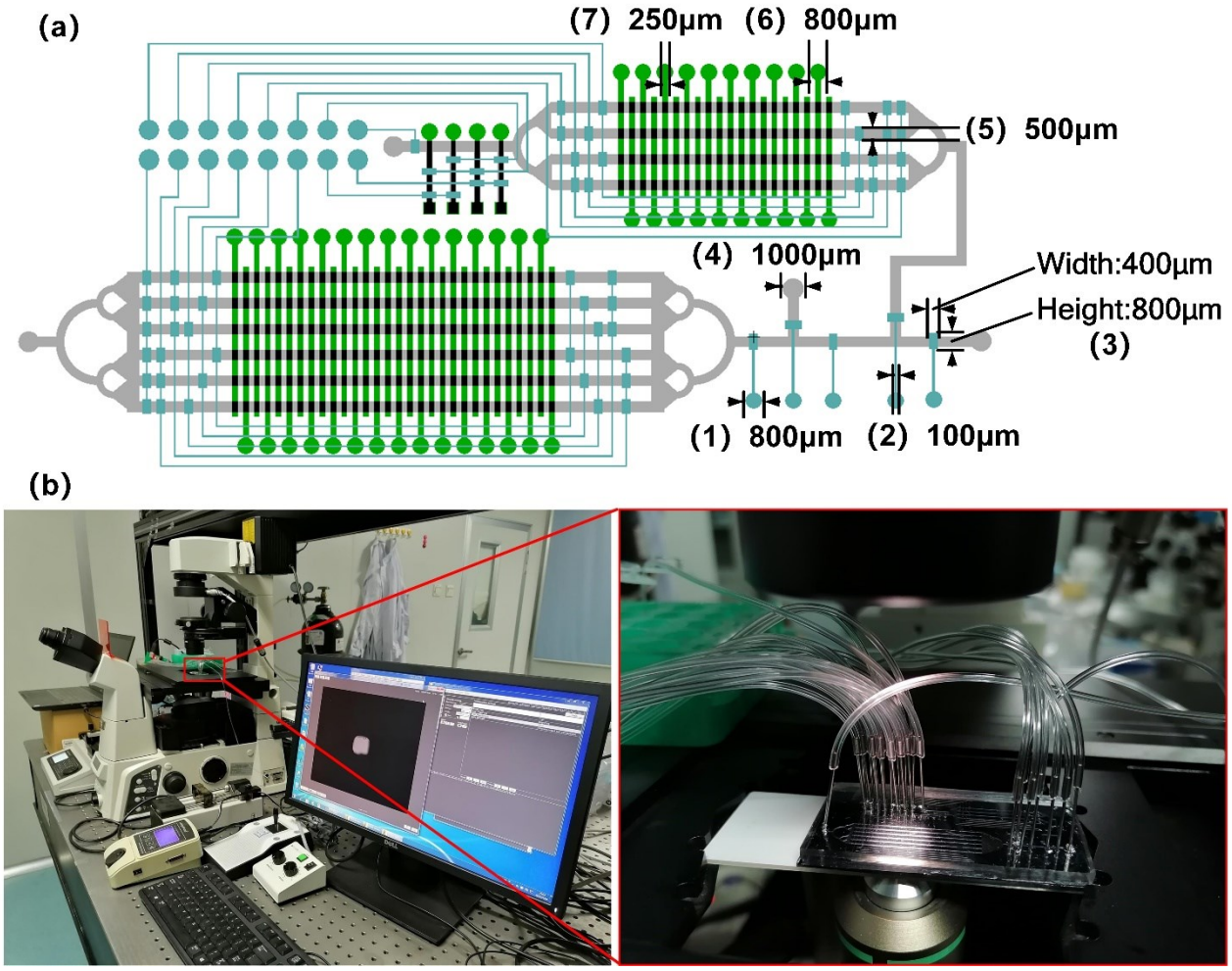

Figure S1 The dimensional parameters of SHS-Chip are listed in Figure (a): (1), (2) and (3) illustrate the dimensions of valve control channel (colored cyan). (4) and (5) describe the dimensions of hybridoma processing channels (colored gray). The dimensions of hybridoma retrieving channel (colored green) are labelled by (6) and (7). Figure (b) is SHS-Chip platform: microchip is connected to micro-pump for injecting cell suspension and reagents, and to microvalves which controlled by computer. The photo of SHS-Chip platform (left) includes a florescent microscopy (TiE, Nikon) combined with CCD, micro-pump, two computers for controlling valves and imaging. The red box (right) shows an enlarged image of SHS-Chip.

Supplementary Data S2

The setup of valves in single cells transferring process

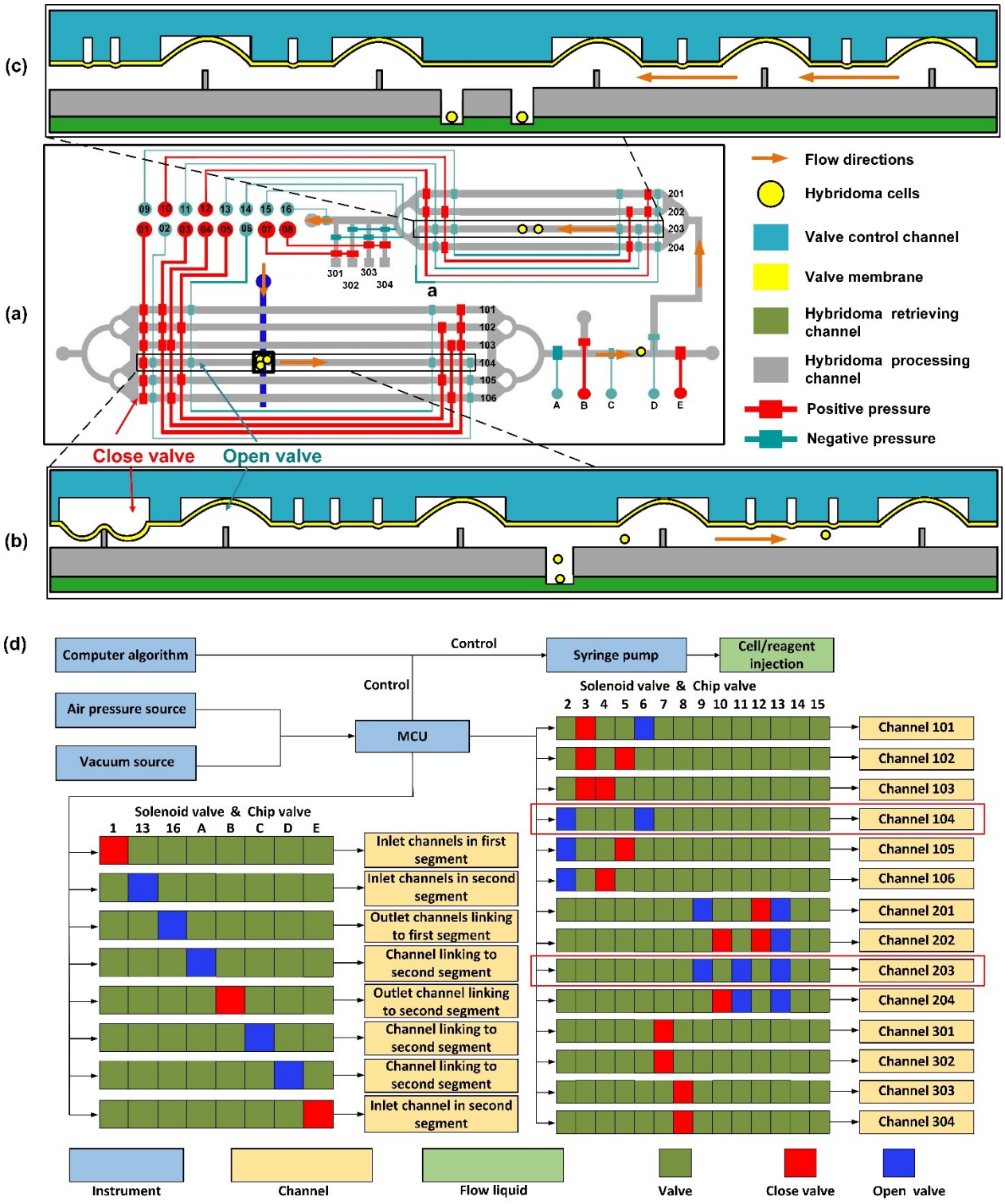

Figure S2 Valve operation. Figure S2a shows the channel/valve setup while transferring cells in a given micro-well. The first screening segment has 6 channels (labelled 101,102,103,104,105 and 106); the second screening segment contains 4 channels (labelled 201, 202, 203 and 204); 4 channels (labelled 301, 302, 303 and 304) is linked to

expanding microwells. If a valve membrane is exposed positive pressure, it will be transfer to concave to block the flow in cell processing channel (Figure S2b). On the contrary, negative pressure deform the valve membrane to convex to pass flow liquid (Figure S2c). For example, desired hybridomas in a given microwell (labelled black box) from channel 104 of first screening segment are transferred from first screening segment to channel 203 of second screening segment. Figure S2b and S2c are the enlarged picture of horizontal section view of channels. Figure S2d shows the connection diagram of controlling devices to SHS-Chip. A syringe pump (LSP04-1A, LongerPump) is used to inject cell suspension/reagents, which is controlled by computer. All the 21 solenoid valves (SMC S070M-5BC-32), which linked to chip valves in correspondence, are controlled by an Intel 8051 MCU (Microcontroller Unit) connected with computer to switch the connection between air pressure/Vacuum and solenoid valve. Related information of valve controlling is described in detailed as following. Each of the channels in the two screening segments (from 101 to 304) can be controlled by more than two valves. For example, channel 104 can be blocked by vale 02 or valve 04 or both them; valve 02 is used for simultaneously blocking channel 104, 105 and 106. Other channels linked to inlet/outlet and two screening segments is controlled by one or two valves (among 01, 13, 16 and A to E). In detail, the channel 104 is opened by valve 02 and 06 under negative pressure, while other five channels of first screening segment are closed by four valves (01, 03, 04, 05) under positive pressure. Culture medium are injected from the cell retrieving channel (colored blue) linking to channel 104 to rinse hybridoma cells to next segment. For the status of second screening segment (Figure S2c), only channel 203 is opened by controlling valve 10 and 12, while the channels (from 301 to 304) linked to expansion microwells are blocked by valve 07 and 08.

Supplementary Data S3

Hybridoma trapping in micro-wells

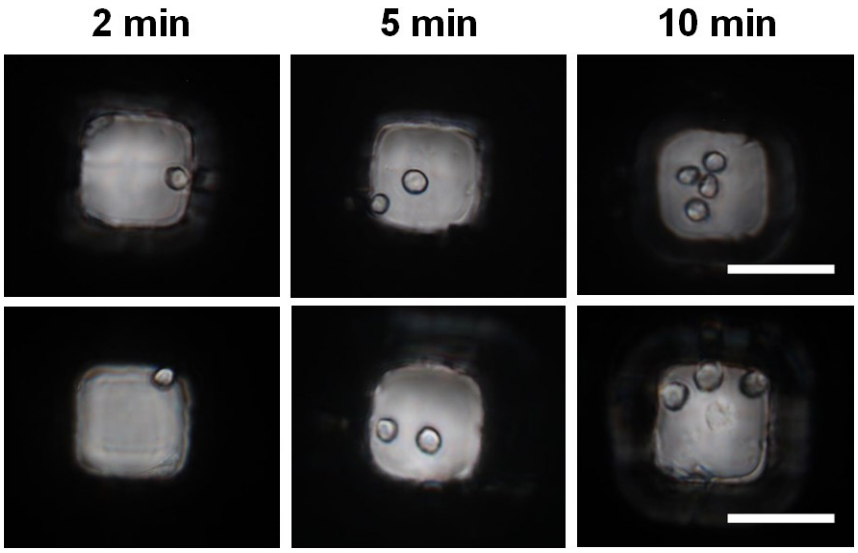

Figure S3 Different flow pausing times of hybridoma suspension result in different trapping statuses. The density of hybridomas was 12 ×10^5 cells/mL. Scale bar: 50 μm.

Supplementary Data S4

Coating micro-wells with protein

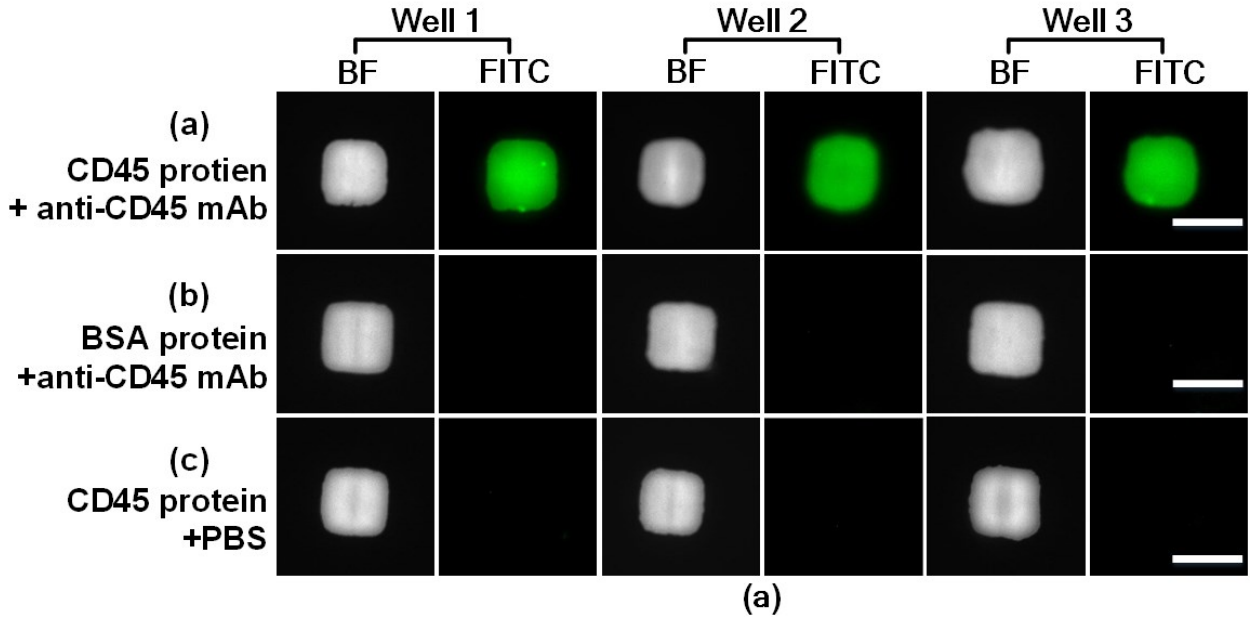

Figure S4 The protein coating of micro-wells was evaluated by fluorescent detection. (a) The micro-wells were coated by CD45 protein; anti-CD45 mAb was used to bind CD45 protein. (b) The micro-wells were coated by BSA protein, anti-CD45 mAb was also injected into micro-wells. (c) The micro-wells were coated by CD45 protein, PBS was used to replace anti-CD45 protein. (a), (b) and (c), FITC labelled secondary antibody was used to bind immobilized anti-CD45 mAb. Even fluorescence was observed in (a), while no fluorescence was observed in (b) and (c), demonstrating that the coated protein was evenly distributed in micro-wells. BF: Bright field. Scale bar: 50μm.

Supplementary Data S5

Single hybridoma culture in micro-well

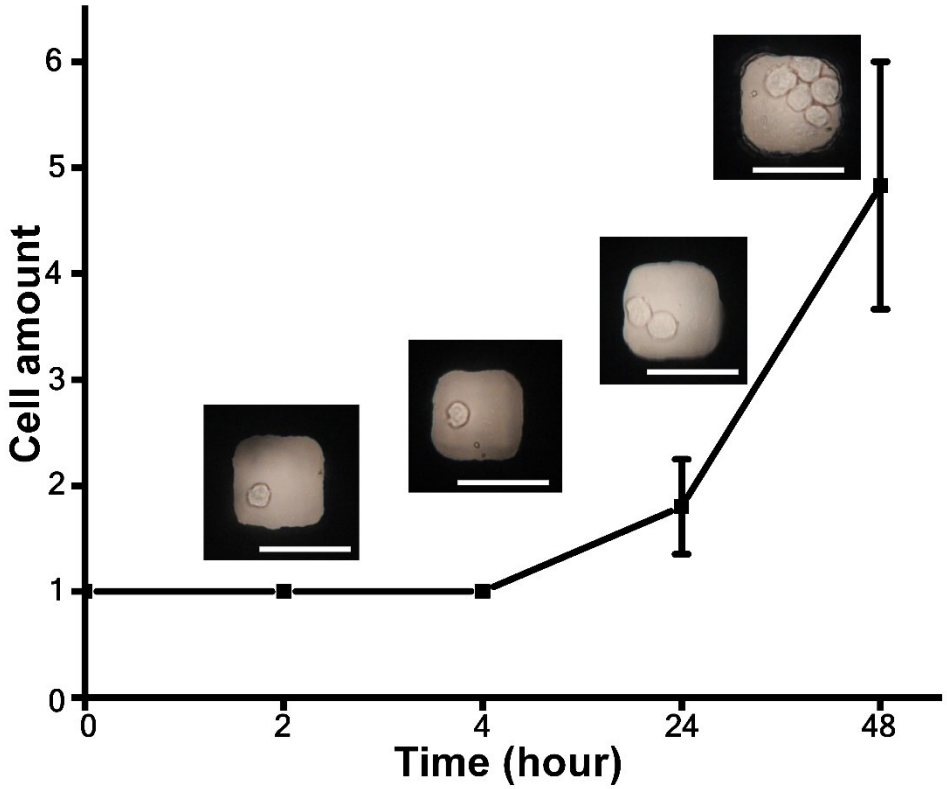

Figure S5 Monitoring single hybridoma proliferation in a micro-well. Screened hybridoma was continuously cultured in a micro-well, and imaged at different culture periods. From 4 to 48 hours, cultured single hybridomas successfully proliferated in micro-wells. 4 microwells were imaged at each time (4, 12, 24, 48 hours) to monitor the changes in cell amount. It demonstrates that the single cell remained well viability after detecting assay. Scale bar: 50μm.