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# **Supporting Information**

# Cell pairing and polyethylene glycol (PEG)-mediated cell fusion using two-step centrifugation-assisted single-cell trapping (CAScT)

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**Figure S1.** Representative fluorescence images of cell pairs in TCMA using two-step CAScT under conditions of (a) centrifugation rate, 1,250 rpm and 2,000 rpm for the first- and second-step trapping, respectively; cell concentration, 800,000 cells/mL (the green and red spots represent DiO- and DiD-stained HeLa cells) and (b) centrifugation rate, 1,250 rpm and 2,000 rpm for the first- and second-step trapping, respectively; cell concentration, 200,000 cells/mL (the green and red spots represent DiO- and DiD-stained HeLa cells) and (b) centrifugation rate, 1,250 rpm and 2,000 rpm for the first- and second-step trapping, respectively; cell concentration, 200,000 cells/mL (the green and red spots represent DiO-stained C2C12 cells and DiD-stained HeLa cells). Dual-positive spots in merged images indicated successful cell pairing events. The code numbers were marked in corresponding colors at the original position located from the bright-field images. (scale bars, 500 µm)



**Figure S2.** Viability test after two-step CAScT. Representative fluorescent images from (a) live cells (stained by FDA, in green color) and (b) cell nuclei (stained by Hoechst 33342, in blue color) (c) Bright-field image. (scale bar, 500 μm).

#### C. Long-term culture of C2C12 cells on addressable TCMA after two-step CAScT.



**Figure S3.** Long-term culture of C2C12 cells on addressable TCMA chip after two-step CAScT. (a) Bright-field images of trapped C2C12 cells that were cultured for indicated timescales (0 h, 24 h and 48 h). (b) Viability test after 48-hours culture. Representative fluorescent images came from FDA (left, in green color) and Hoechst 33342 (middle, in blue color) staining, which represent for live and total cells, respectively. Merged image (right) was obtained from overlaid FDA and Hoechst fluorescent images. (scale bar, 500 μm)



**Figure S4.** Long-term culture of C2C12 and HeLa cells on addressable TCMA chip after two-step CAScT. (a) Bright-field images of trapped C2C12 and HeLa cells that were cultured for indicated timescales (0 h, 24 h and 48 h). (b) Viability test after 48-hours culture. Representative fluorescent images came from FDA (left, in green color) and Hoechst 33342 (middle, in blue color) staining, which represent for live and total cells, respectively. Merged image (right) was obtained from overlaid FDA and Hoechst fluorescent images. (scale bar, 500 μm)

### E. Retrieval of HeLa cells cultured for 48 hours on an addressable TCMA.



**Figure S5.** Retrieval of HeLa cells cultured for 48 hours on an addressable TCMA. Phase-contrast images of (a) HeLa cells cultured on TCMA for 48 h before trypsinization; (b) HeLa cells trypsinized for 5 min; (c) TCMA after retrieval of cells (scale bar, 200 μm).