Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information for:

Controllable electrofusion of lipid vesicles: initiation and analysis of reactions within biomimetic containers

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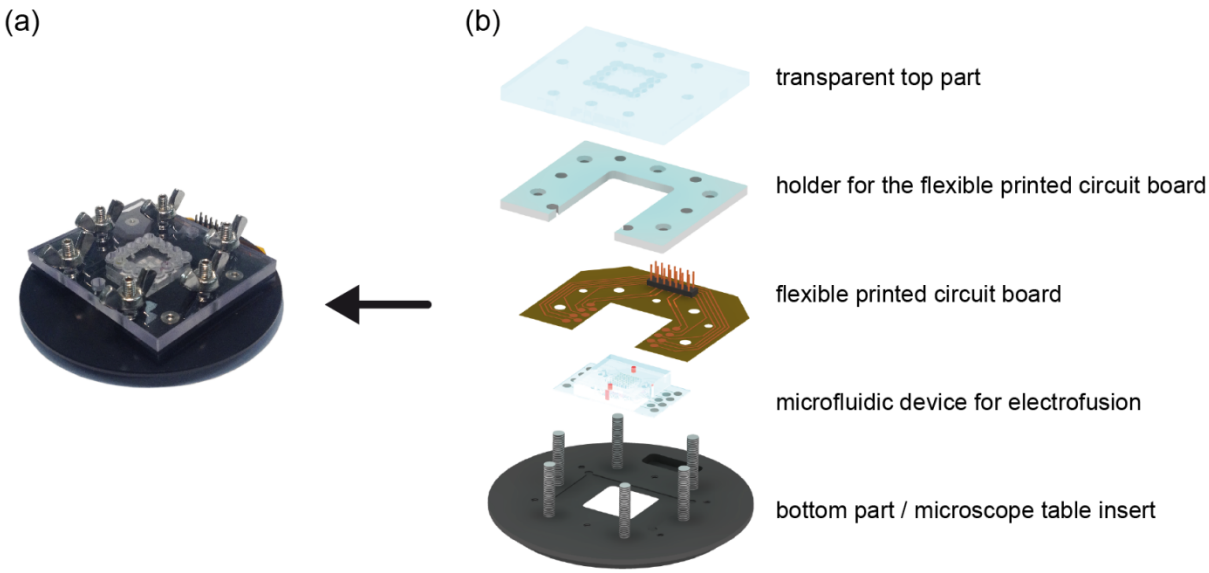

Figure S1: (a) Photograph of the custom build clamping holder. (b) Exploded view drawing of the holder (screws and nuts not shown). The microfluidic device is placed in a grove in the bottom part, which also serves as microscope insert. A flexible printed circuit board is clamped on the exposed part of patterned microelectrodes on the glass slide. It serves as interface between the microelectrodes and the programmable pulse generator. To seal the microfluidic channels a transparent top part clamps the PDMS slab to the glass slide.

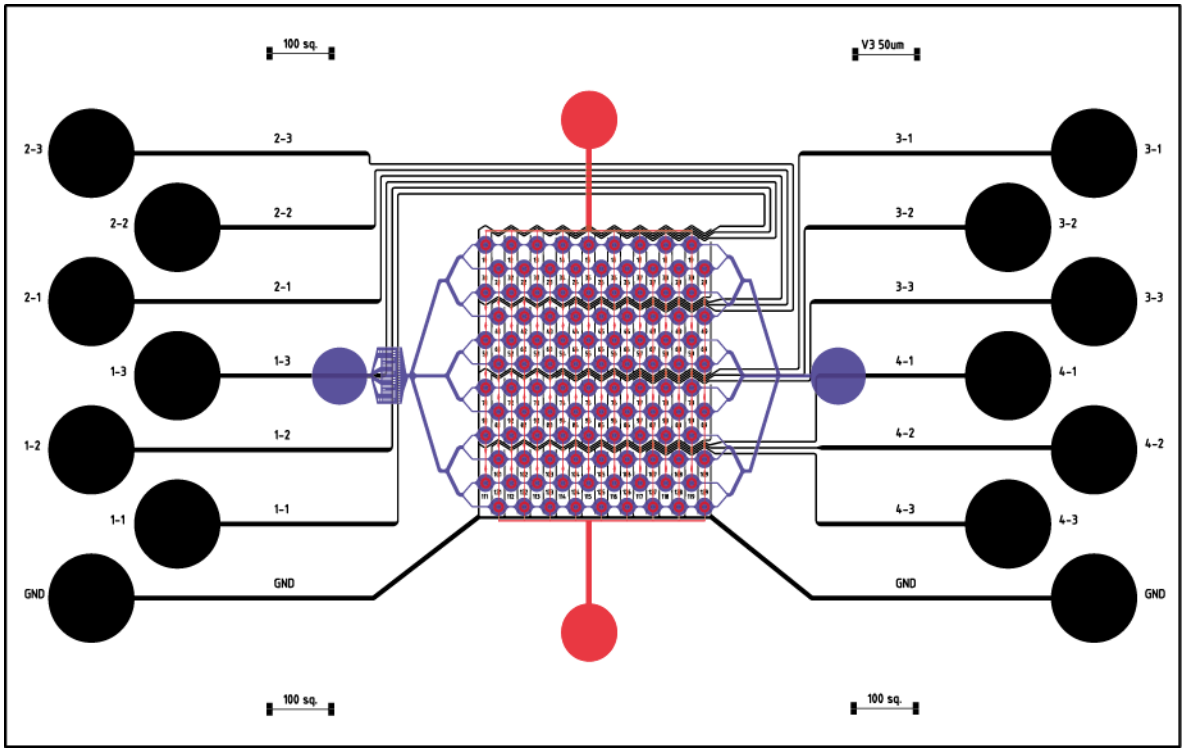

Figure S2: Scheme showing the designs of the electrodes (black), of the fluid layer (blue), and of the pressure layer (red).

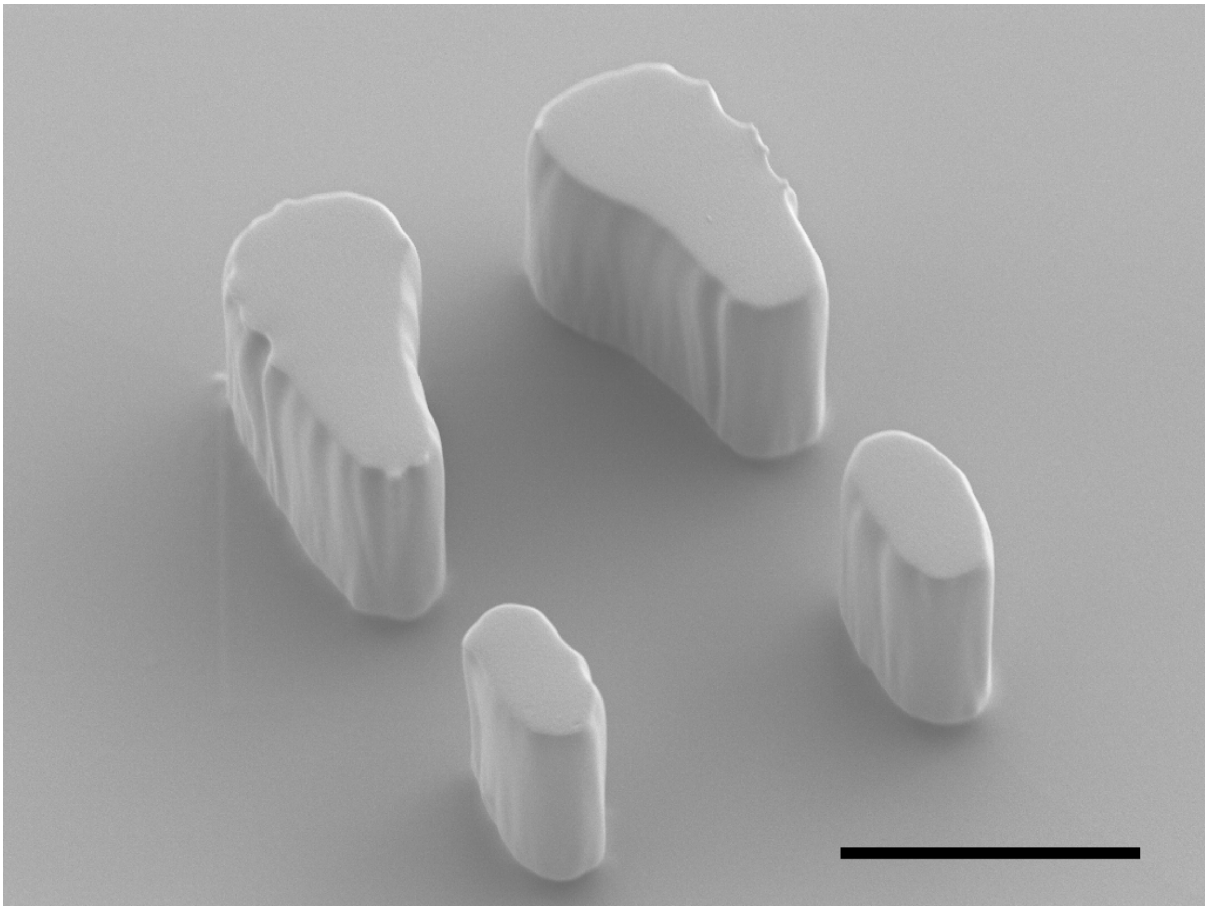

Figure S3: Scanning electron microscope image of a PDMS trap Scale bar 20 µm.

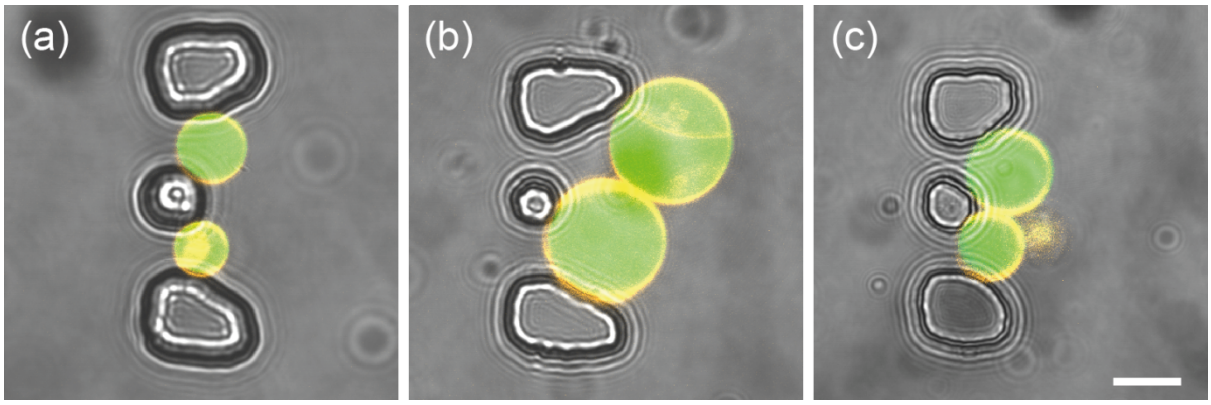

Figure S4: Alternative design to trap multiple GUVs adjacently. Confocal fluorescence images (overlaid with transmitted light images) of trapping (a) two small GUVs, b) two large GUVs, and c) the ideal. The membrane of the GUVs contained Liss-Rhod-PE (orange) and lumen contained calcein (green). Scale bar: 10 µm.

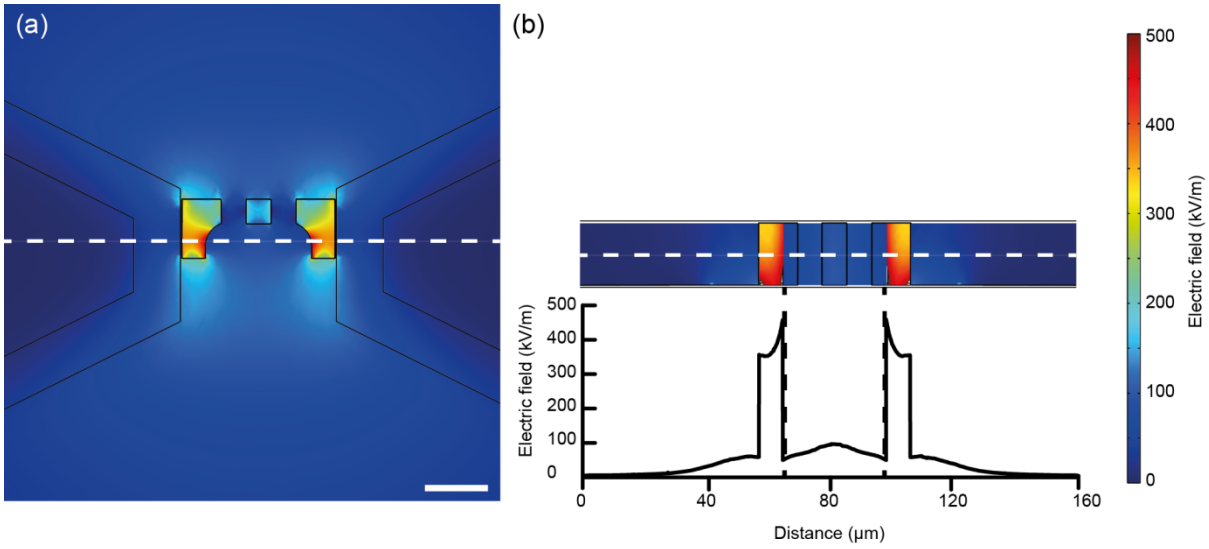

Figure S5: Simulation of the electrical field in alternative design performed using COMSOL. The potential difference between the electrodes is 10 V and the chamber is filled with water. (a) View from above. (b) View from the side, including a plot of the electric field strength along the white dashed line. The black dashed lines on the plot indicate the region where the GUVs are typically located. Scale bar 20 µm.

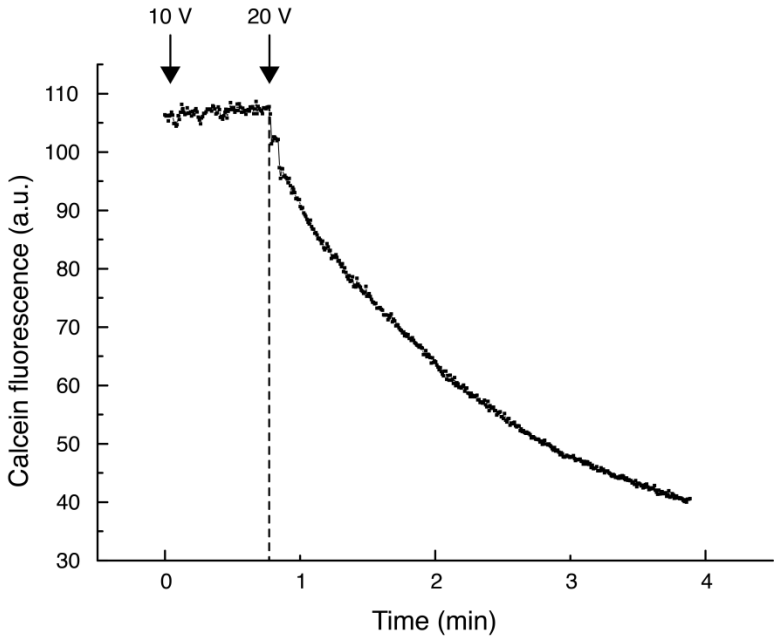

Figure S6: Calcein leakage with high voltages. First a 10 V pulse was applied without loss of calcein. Then a second pulse of 20 V was applied causing leakage of calcein and subsequent loss of fluorescence.

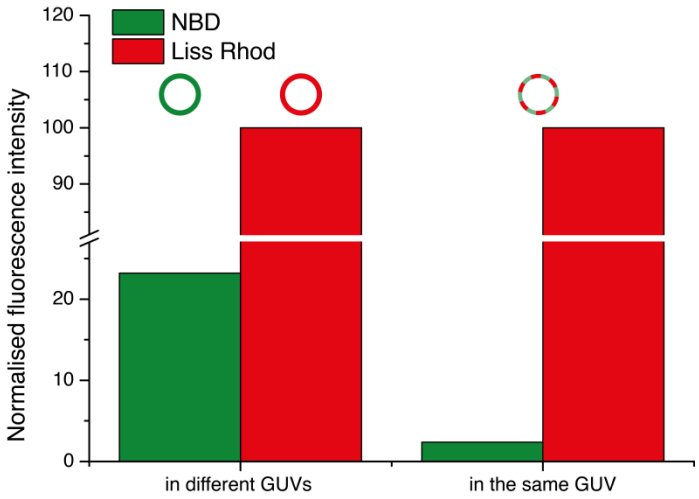

Figure S7: The fluorescence intensity of NBD and Liss-Rhod when in separate membranes and in the same membrane. In the same membrane the fluorescent labels are in the close proximity causing FRET and therefore a reduction in the fluorescence intensity of NBD.

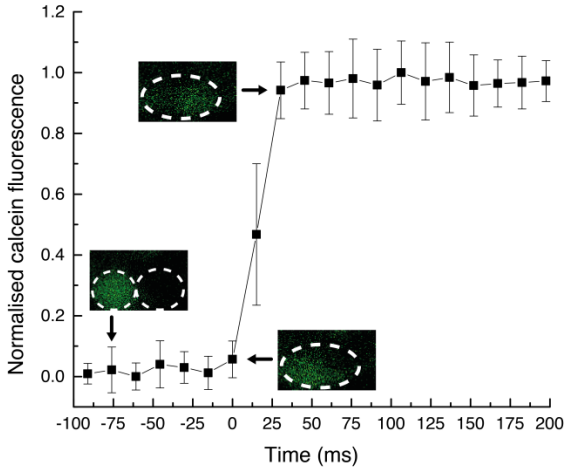

Figure S8: High frame-rate analysis of content mixing. GUVs with and without 100 µM calcein were loaded into the traps. Electrofusion was performed and fluorescence images were recorded at 65.8 fps. Data was averaged from 4 experiments and error bars are taken from the standard deviations.

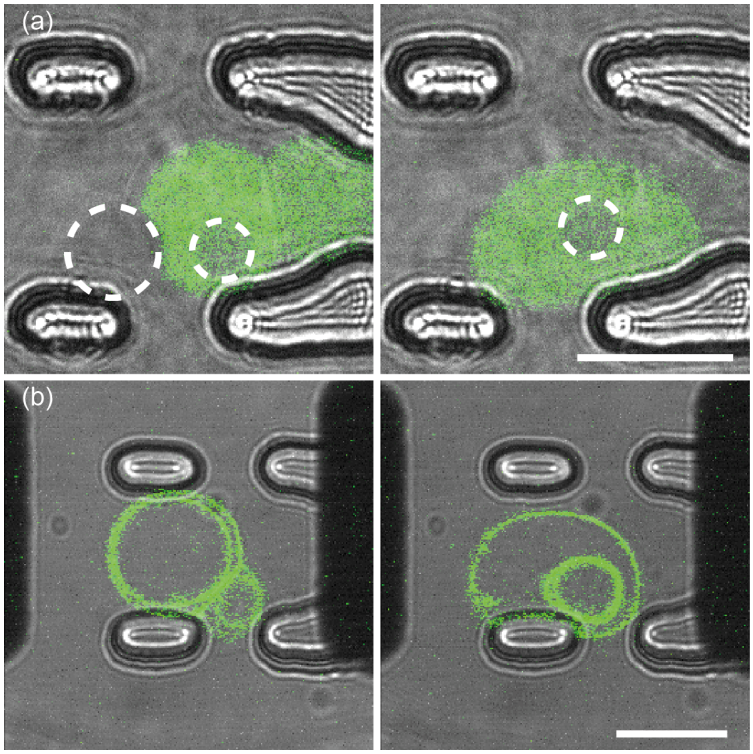

Figure S9: Fusion of the outer membrane of multivesicular vesicles (MVVs). a) Fusion of three GUVs (left), two with and one without calcein inside (indicated with a large dashed circle). The central vesicle contains a smaller vesicle (small dashed circle), which remains inside the final fused vesicle (right). b) Fusion of two GUVs with a third vesicle inside (left). After fusion, the smaller vesicle is engulfed by the large GUVs, which have also fused together (right). Scale bar: 20 µm.

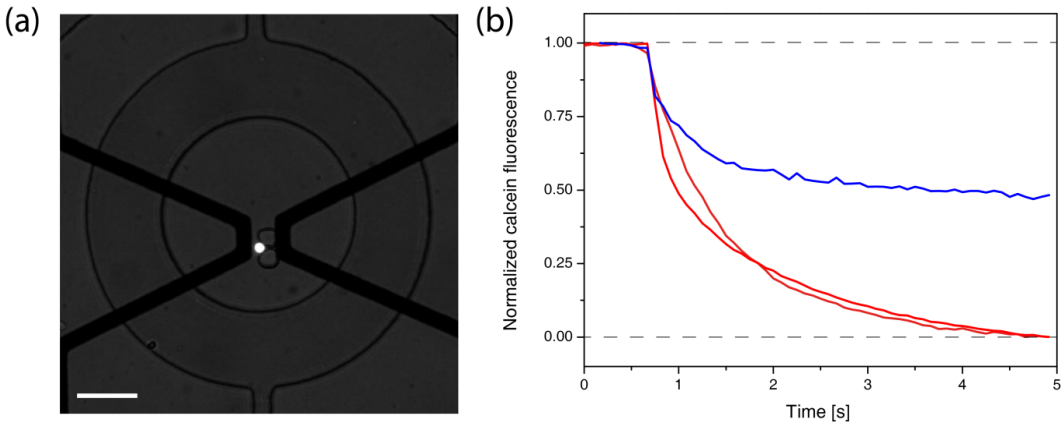

Figure S10: Cell lysis and electroporation. a) Micrograph from the final assembled device using the traps designed for hosting one cell or vesicle. The traps in this example were optimized to trap single mammalian cells. Here, U937 suspension cells in PBS buffer were introduced and trapped using a flow rate of 5 µl/min. The image is an overlay of a bright field image of the device, and a fluorescence image of the cell stained with calcein AM. The cell is confined between the electrodes so that lysis or electroporation can be achieved efficiently. Scale bar: 50 µm. b) Example curves from cells subjected to pulses from the electrodes. Upon switching on the electrical field, the fluorescence signal is reduced, i.e. the cellular integrity is compromised. Depending on the strength, length and repeats of the pulses, complete loss of the calcein AM signal (red, 500 v, 10 pulses, 2 µs, 100 µs delay) or electroporation (blue, 500 v, 5 pulses, 2 µs, 100 µs delay) can be achieved.