Nanofountain Probe Electroporation of Single Cells

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Supporting Information

S1. Packaged probe and experimental setup

The experimental setup used for single cell NFP electroporation (NFP-E) is shown in Figure S1a. The setup includes (1) a micromanipulator that can precisely control the position of the probes with respect to cells, (2) a signal generator that is able to apply different pulse types with different frequencies, amplitudes, and train timing, (3) a microinjector or other fluid flow controller that provides the required pressure to deliver the solution containing the molecules to be transfected to the reservoir of the chips, and (4) an inverted microscope to obtain images of the cells during electroporation experiments. The packaged NFP chip that is mounted on this system is shown in Figure S1b. As explained in the main text, the packaging is designed to seal the microreservoirs on the chips to enable use under cell culture media. Moreover, the filling tubes and the electrode are connected to the probes using the packaging. The molecular solution to be delivered is stored in the microreservoir and the solution is loaded into the microchannels, by externally applied pressure and capillary forces, to the ends of the apertured dispensing tips. This is shown by the optical microscope image in Figure S3a of the NFP microchannels filled to the probe tips with dextran/Alexa Flour 488.

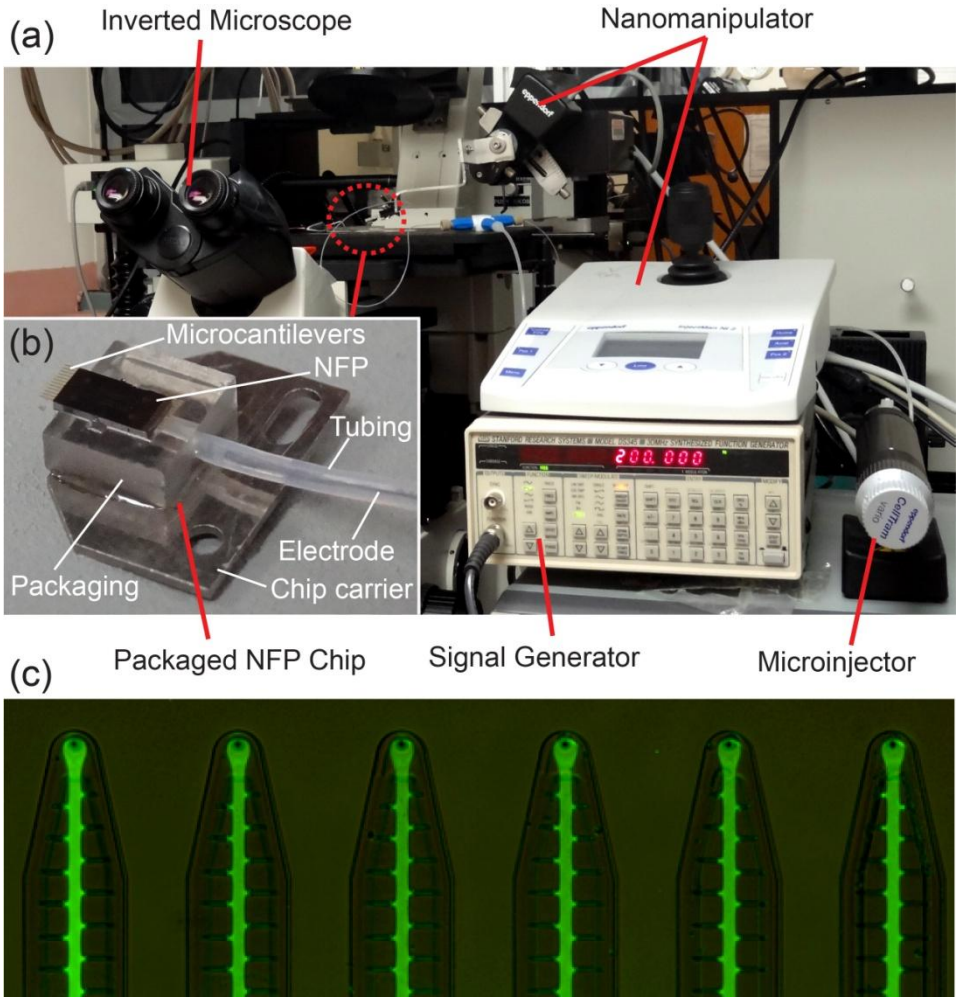

Figure S1 NFP-E system: (a,b) Experimental setup including the packaged NFP chip mounted on the nanomanipulator on top of an inverted optical microscope, (c) Bright field optical microscope image merged with a fluorescence image of the NFP probes during wetting of the NFP microchannels with a solution containing Alexa Flour 488.

S2. Parametric study of input voltage

To increase electroporation viability, it is desirable to minimize cell stress during electroporation, e.g., application of mechanical force or toxicity due to the magnitude of the input voltage. The optimal range of operating parameters will depend on the cell type due to their inherently different cytoskeleton. To optimize electroporation, while using an applied voltage that is as low as possible, a parametric study of the input voltage was performed. A summary is given in Table 2. For this study, the frequency of the input square waves was kept constant at 200 Hz with 1 sec duration. At voltages in the range of 0-7 V, no fluorescence was detected after electroporation despite the probe tip being placed in contact with a target cell. This is definitively shown in Figure S2 where images of target cell A are shown before (a1) and after (a2) electric

pulse application. There was a slight indentation mark on the cell after the electroporation attempt, but there was no fluorescence detected to indicate transfer of the fluorophore into the cell (Figure S2a3). Using an intermediate input voltage between 15-30 V, we successfully transfected the cells with dextran/Alexa Fluor without damaging them, as shown in Figure 5 in the main text. Higher input voltages of 40-60 V damaged the cells, as can be observed by the considerable change in cell morphology after electroporation shown in Figure S2b2 and Figure S2c2 for 40 and 60 V, respectively. Note that the cell nuclei, Figure S2b1 and Figure S2c1, were stained by Hoechst 33342 (Invitrogen) for better single cell selection. *Note that we report on a control experiment on cell A in Figure S2a2 where we intentionally applied force such that an indentation mark on the cell was clearly observed but the applied voltage was low (7V). The experiment resulted in no intracellular delivery. This confirms that when using probes with a radius of approximately 250 nm, a critical transmembrane potential drop is essential for successful electroporation*.

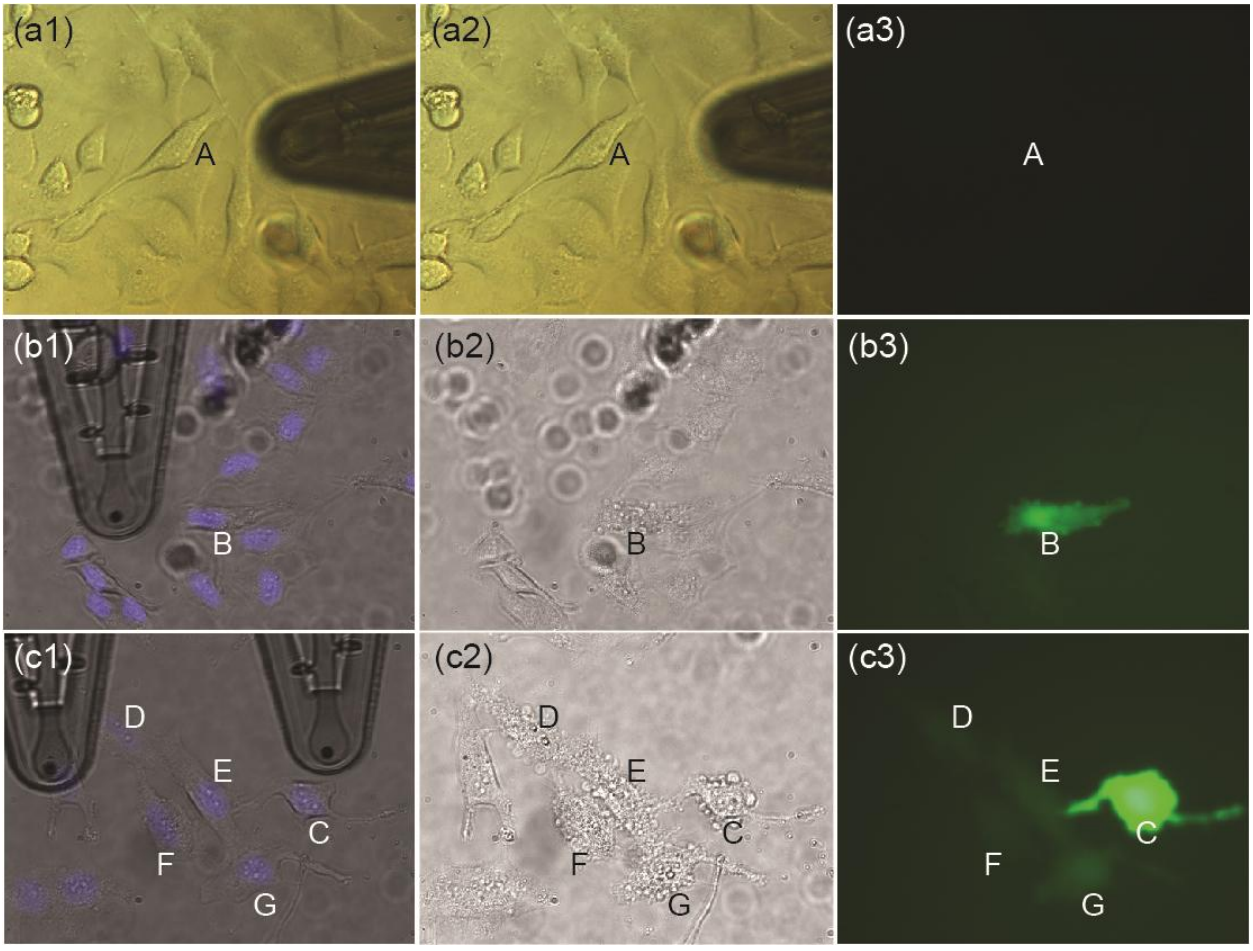

Figure S2 Parametric study for input voltage: (a) at 7 V, (b) at 40 V, and (c) at 60 V. Images in a1, b1, and c1 are before electroporation while a2, b2, and c2 are those after electroporation. The corresponding fluorescence images are shown in a3, b3, and c3, respectively. At 7 V in a3, no fluorescence was detected in cell A after electroporation. Note the indentation mark in a2 due to contact between the NFP tip and

cell. For voltages of 40 V and 60 V, strong fluorescence was observed as shown in b3 and c3; however, considerable change in cell morphology in b2 and c2 suggests some cell damage. The cell nuclei in (b1) and (c1) are stained by Hoechst 33342 (Invitrogen) for better single cell selection.

S3. Control experiment

Control experiments were performed with propidium iodide (PI) to demonstrate that PI staining worked as expected for dead cell discrimination.

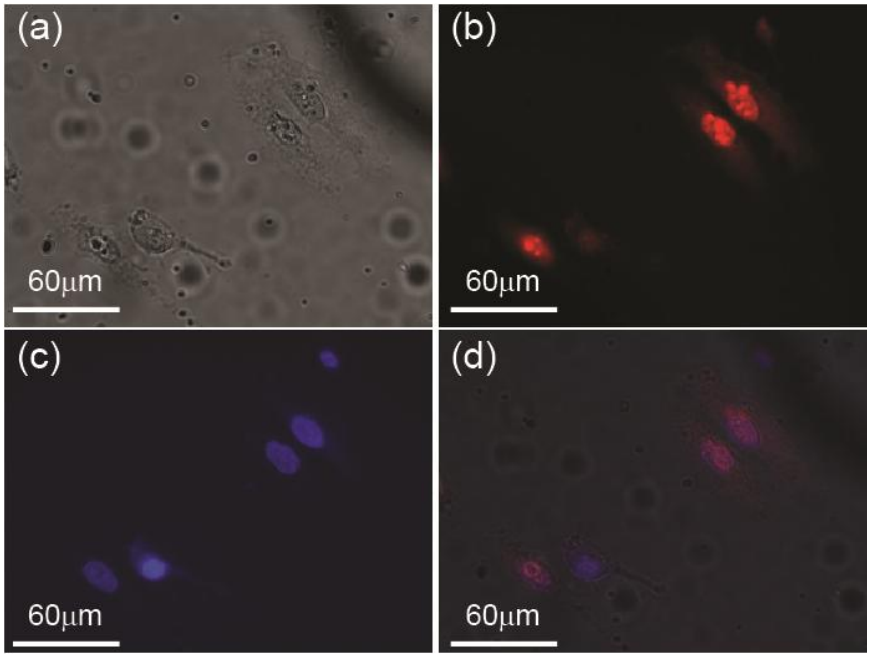

Figure S3 Control experiment for propidium iodide (PI) staining: (a) bright field image of dead cells on a coverslip, (b) red fluorescence from PI, (c) blue fluorescence from Hoechst, and (d) merged image of (a)-(c).

S4. Viability assay

The viability of HeLa cells after electroporation was tested. First, target cells shown in Figure S4 were electroporated with dextran/Alexa Fluor. Fluorescence images in Figure S4 indicate successful electroporation. Then, the HeLa cells were kept in an incubator in complete DMEM media for 4 hours. After incubation, PI was used to stain dead cells to discriminate dead cells from live cells. Red fluorescence in the electroporated cells, which would indicate dead cells, was not observed in multiple viability tests. Figure S4 illustrates results from five such tests. Note that dark shadows in Figure S4-1 and 4-2 are part of tracking marks on bottom of the coverslip to monitor the position of electroporated cells during viability testing.

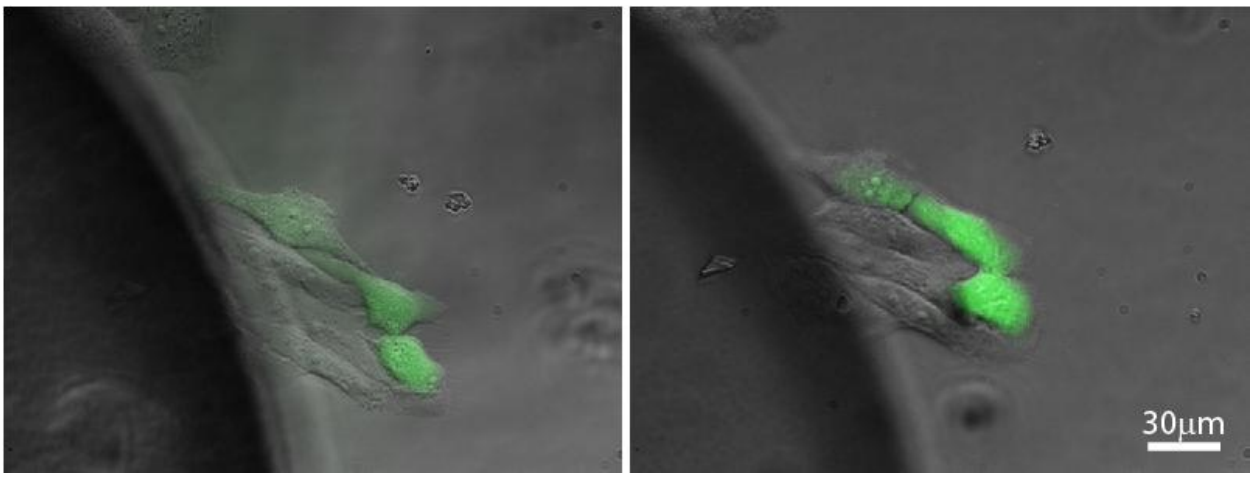

Figure S4-1 Viability test 1 before (left) and after (right) 4 hr incubation.

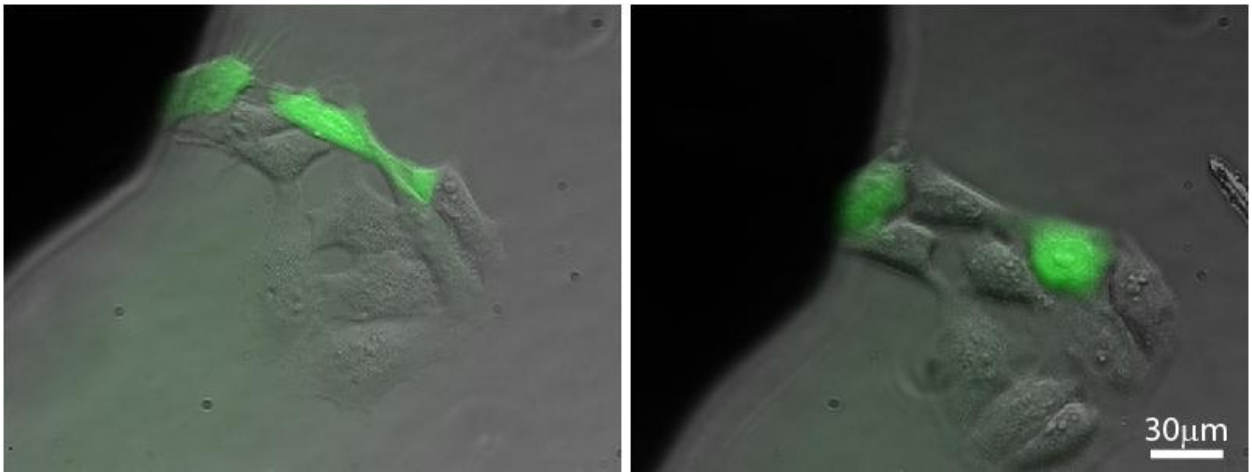

Figure S4-2 Viability test 2 before (left) and after (right) 4 hr incubation.

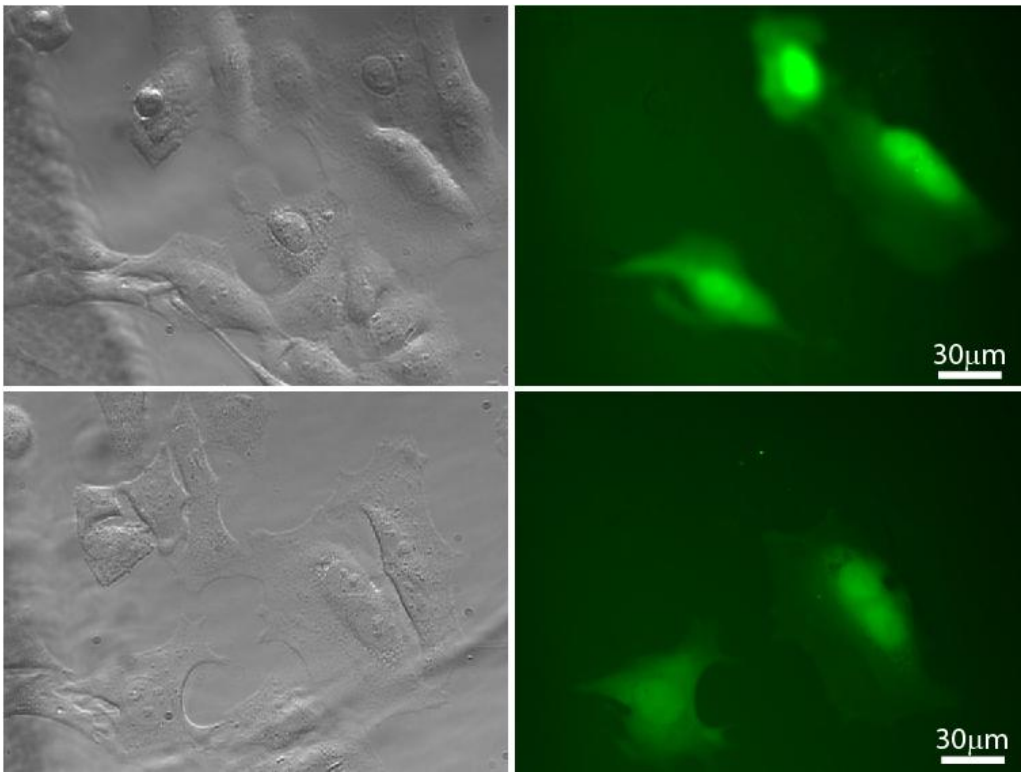

Figure S4-3 Viability test 3 before (upper) and after (lower) 4 hr incubation

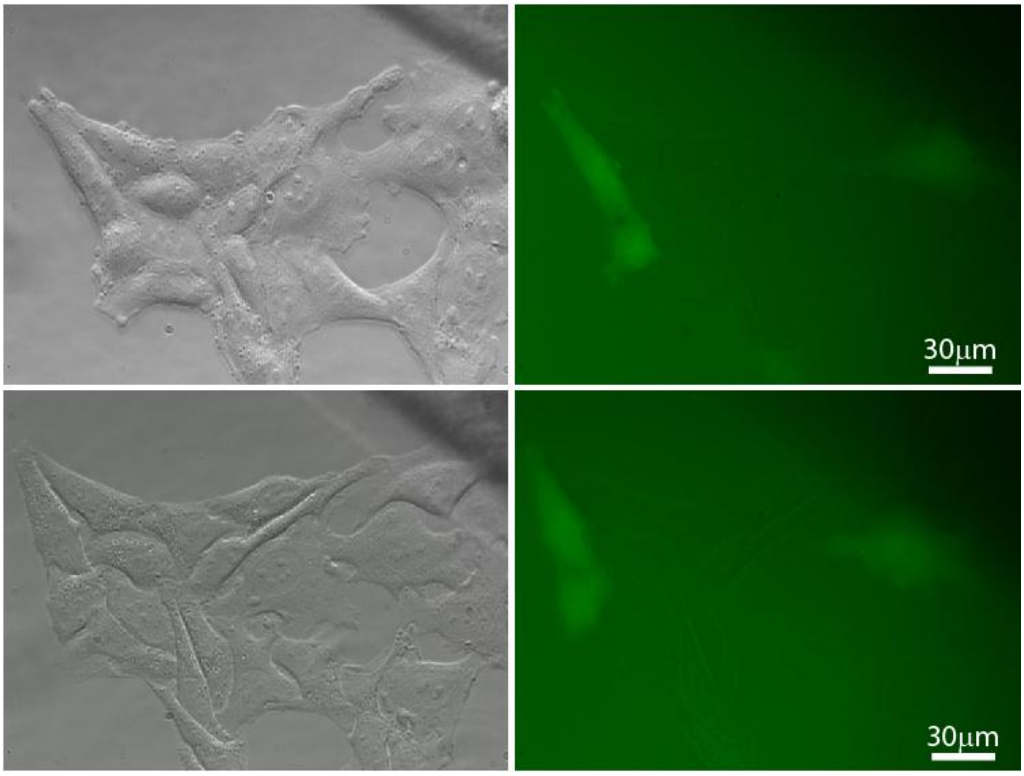

Figure S4-4 Viability test 4 before (upper) and after (lower) 4 hr incubation

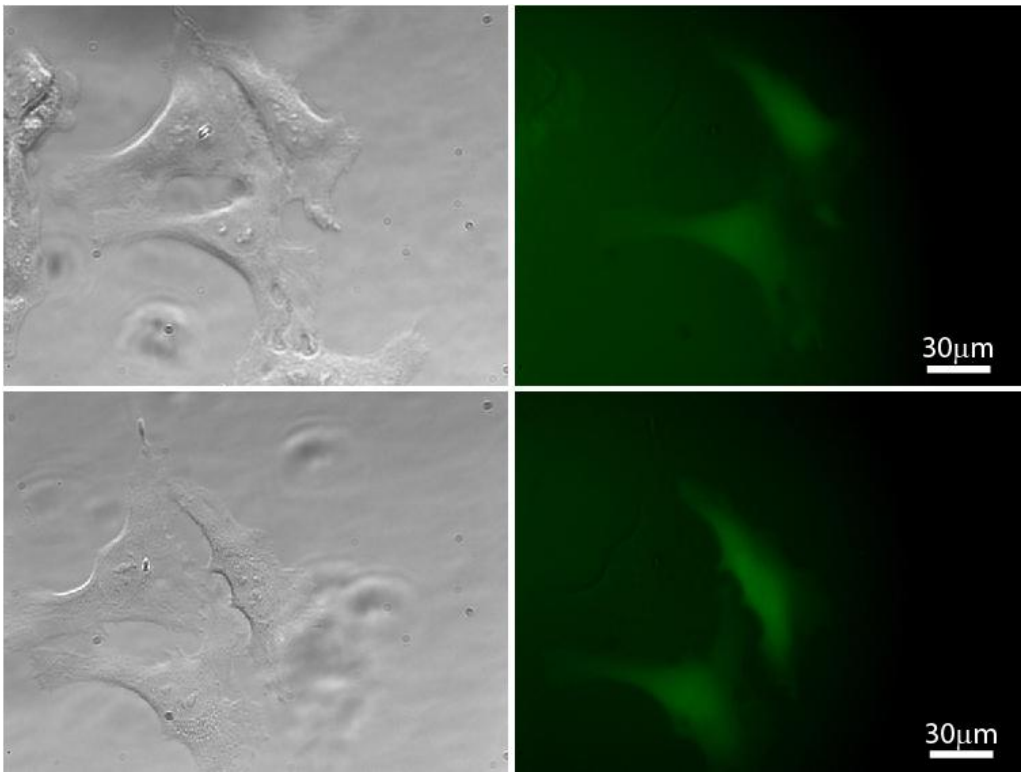

Figure S4-5 Viability test 5 before (upper) and after (lower) 4 hr incubation

S5. Reliability of NFP-E

Using NFP-E, many cells in Figure S3a were electroporated at 30 V with constant signal duration (two 0.5 sec input square-wave signals with 1 sec time interval at 200Hz). The results demonstrate that NFP-E can be used to transfect many target cells with single cell selectivity at a high success rate.

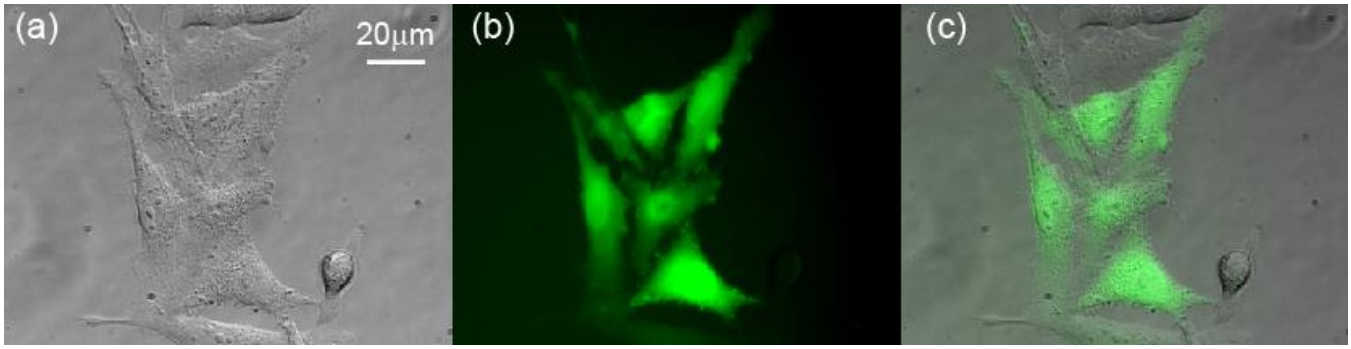

Figure S5 Reliability of NFP-E: (a) bright field, (b) fluorescence, and (c) merged image of HeLa cells transfected with Alexa Fluor 488 using the NFP-E system.