Supplementary information

Device Fabrication



Figure S1: Fabrication process step for NL-SCNEP chip



Fig. S2: Back side of the NL-SCNEP chip



Figure S3: CL1-0 cells seeded on chip surface and some of them are targeted for nanoelectroporation experiment (those cells seeded in-between nanoelectrode gap)



Figure S4: Electric field applied only in single nano-electrode pair of CL1-0 cell with applied voltage 5Vpp, 10 ms single square wave pulse. Here PI dye only diffuse through single nano-localized area of single cell.



Figure S5: QDs delivery into single AGS cell with applied voltage 6V, 40 ms, two square pulses. QD successfully deliver within 40~50 minutes.



Figure S6: Plasmid (Pmax E_2F_1) delivery into CL1-0 cell with applied voltage 6Vpp ,40ms square wave pulse (3 pulses). Plasmid successfully deliver and protein express into single cell within 20 hrs.



Figure S7: Plasmid delivery (Pmax E_2F_1) into AGS cell with applied voltage 6V, 40ms square wave pulse (3 pulses). Plasmid successfully deliver and protein express into cell within 18~22 hours.



Figure S8: Viability of CL1-0 cell after single and multiple position nano-electroporation experiment with different applied voltages and fixed pulses (30ms, 3 pulses, i-vi). The result shows approximate 8Vpp to 10Vpp, with single electrode confinement, cells are dead. The cells are live at 6Vpp, 50 ms, 3 pulses (Figure vii) and 7Vpp, 50 ms, 5 pulses (Figure viii) with two nano-electrode pair.

Bright field Cell image	Live cell (Calcein)	Dead cell (PI dye)
i 5Vpp		•
ii 6Vpp		-
iii 7Vpp	Je)	
iv 8Vpp	<u></u>	6
v 9Vpp		
vi 10Vpp		2
vii 6Vpp		
viii 7Vpp		

HCT-8 Cell

Figure S9: Viability of HCT-8 cell after single and multiple position nano-electroporation experiment with different applied voltages and fixed pulses (30 ms, 3 pulses, i-vi). The result shows approximate 8Vpp to 10Vpp, with single electrode confinement, cells are dead. The cells are live at 6Vpp, 50 ms, 3 pulses (Figure vii) and 7Vpp, 50 ms, 5 pulses (Figure viii) with two nano-electrodes pair.



Hela Cell

Figure S10: Viability of HeLa cell after single and multiple position nano-electroporation experiment with different applied voltages and fixed pulses (30ms, 3 pulses, i-vi). The result shows approximate 8Vpp to 10Vpp, with single electrode confinement, cells are dead. The cells are live at 6Vpp, 50 ms, 3 pulses (Figure vii) and dead at 7Vpp, 50 ms, 5 pulses (Figure viii) with two nano-electrodes pair.



Figure S11: Viability of AGS cell after single and multiple position nano-electroporation experiment with different applied voltages and fixed pulses (15ms, 3 pulses, i-vi). The result shows approximate 9Vpp to 10Vpp, with single electrode confinement, cells are dead. The cells are live at 6Vpp, 50 ms, 3 pulses (Figure vii) and dead at 7Vpp, 50 ms, 5 pulses (Figure viii) with two nano-electrodes pair.