Supporting Information

Materials and methods

Microfluidic chip fabrication: The microfluidic channels for electroporation were fabricated using standard soft lithography, as described in our previous paper[1]. Briefly, photomasks with microscale patterns designed by computer software (FreeHand MX) were printed out on transparencies at high-resolution (5080 dpi). The designed micro-patterns were then transferred to 3 inch silicon wafers (University Wafer) to form masters using a negative photoresist SU8 2025 (Microchem Corp.) after exposure and development. A layer (~5 mm thick) of polydimethylsiloxane (PDMS, General Electric Silicones RTV 615, MG chemicals) prepolymer mixture with a mass ratio of A:B=10:1 was then poured onto the master and baked in an 80 ºC oven for 1 h for curing. PDMS replicas were peeled off from the master and punched for inlet and outlet holes. PDMS and a pre-cleaned glass slide were then oxidized by plasma treatment and put in contact for bonding. Finally, the bonded PDMS and glass pieces were baked at 80 ºC for 1 h to increase the bonding strength between them.

Cell culture: Wild type mouse embryonic fibroblasts (MEF) or Src/Yes/Fyn triple-knockout (SYF-/-) version of MEF cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Life technologies) supplemented with 10% fetal bovine serum (Atlanta Biologicals) and 100 mg/mL streptomycin (Sigma) in a humidified cell incubator containing 5% CO_2 at 37 ºC. Cells were subcultured every 2 d at a ratio of 1:10 to maintain their exponential growth phase. They were collected in culture medium after detaching from the flask bottom by Trypsin-EDTA (Sigma).

Src FRET biosensor (the protein form) production: The construct of the Src FRET biosensor has been described previously[2, 3]. N-terminal 6x His tag fusion biosensor protein was produced in Escherichia coli and purified by nickel chelation chromatography as described in previous publications[2, 3]. Briefly, pRSETb vector containing the Src biosensor was transformed into BL21 (DE3) (Promega). A bright colony was picked under the excitation light with wavelength of 480 nm and grown in 50 mL LB broth supplemented with 100 μM ampicillin at 37 ℃ with 250 rpm shake for 4-8 h till OD_{600} of 0.2-0.4. Following the initial growth, the culture of the bacterial cells was continued in 200 mL LB broth supplemented with 100 μM ampicillin and 0.4 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) with shaking (250 rpm) at 25℃ or room temperature for overnight to induce protein expression. After harvesting cells by centrifugation, the Src protein biosensor was extracted following lysis of bacteria in 8mL B-PER (Pierce) with ¼ protease cocktail tablet (Sigma) and 100 μM phenylmethylsulfonyl fluoride (PMSF), and purified by 0.5 mL Ni-NTA agarose beads (Qiagen) in a flow through column. The bound protein was then eluted with an elution buffer (50mM Tris HCl pH=7.4, 300mM NaCl, 100mM imidazole) and dialyzed twice at 4 ℃ for overnight.

Delivery of the Src biosensor (the protein form) into cells by microfluidic electroporation: MEF or SYF-/- cells were resuspended in an electroporation buffer (8 mM Na_2HPO_4, 2mM KH_2PO_4, 1 mM $MgSO_4 \cdot 7H_2O$ and 250 mM sucrose, pH=7.4) at a concentration of $1x10^7$cells/mL. Purified Src biosensor protein was added into the electroporation buffer to generate a final concentration of 2 μM. Fibronectin (Sigma) in PBS with a concentration of 100 mg/ml was used to pretreat the microfluidic channel for 1 h at 37 ℃ to facilitate cell adhesion. The cell suspension (containing the Src biosensor) was then loaded into the microfluidic channel from one end by gravity. We conducted electroporation after incubating the cells in the microfluidic channel for 15 min at room temperature, so that cells had attachment to the channel bottom but remained round without full adhesion. Electroporation was carried out via two platinum wires placed in the two reservoirs at the ends of the microfluidic channel (W150 μm × D40 μm× L3.8 mm).

Millisecond pulses were generated by the setup shown in Fig. 1B. The voltage V was set by the power supply and the pulse intensity was calculated by V/L. Pulse durations were controlled by a relay (5501, Coto technology, North Kingstown, RI) that was operated by a data acquisition card (PCI-6254, National Instruments, Austin, TX) via LabVIEW software. The generated square pulses were verified by an oscilloscope. At 0.5 h after electroporation, fresh 0.5% FBS cell culture medium was flowed through the device slowly to wash away excessive Src biosensor and supply nutrients to the cells. We incubated the cells (inside the microfluidic channel) in a humidified, 5% CO_2, 37 ºC incubator for 36 h (while attaching a cut pipette tip that held the culture medium for slow perfusion over the period)[4, 5], before PVD stimulation and observation of the Src activity using fluorescence microscopy.

Pervanadate (PVD) stimulation: serum-starved cells (by incubation with 0.5% FBS for 36 h) were washed by flowing through the channel with PBS slowly. Fresh 100X pervernadate (PVD, 10 mM) was prepared by mixing 10 µl of 0.25 M sodium orthovanadate (Sigma) solution in PBS with 28 µl of 30% hydrogen peroxide (H_2O_2) (Alfa Aesar) and 212 µl PBS and incubating at room temperature for 10 min. The 100X PVD was diluted by 100 times by PBS to get a working concentration of 100 μM (1X) for cell stimulation. Freshly-made 1X PVD solution was then flowed into the microfluidic channel at 2.5 µl/min for 2 min. Then a cut pipette tip filled with 1X PVD solution was inserted into one end of the channel to provide a continuous supply of PVD for cell stimulation for 18 min.

Live cell imaging: An inverted fluorescence microscope (IX-71, Olympus) equipped with 20X and 40X dry objectives, a mercury lamp, and a CCD camera (ORCA-285, Hamamatsu) was used for cell imaging in the microfluidic channel. Phase contrast images of cells were taken before electroporation and at 36 h after electroporation to determine the cell number and thus the cell viability in percentage. For fluorescent imaging of the Src biosensor in cells, the excitation light (from the mercury) was filtered by a D420/20 filter. To reduce photobleaching, a neutral density (ND) filter (30%) was placed in front of the light source. The emission light was filtered by a D480/40 filter for ECFP signal collection and a D535/25 filter for YPet signal. The ECFP and YPet images were taken at a frequency of one per 2 min after PVD stimulation for 20 min.

References

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