Electronic Supplementary Information

One-step extraction of subcellular proteins from eukaryotic cells

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Experimental

Microchip fabrication Microfluidic devices were fabricated based on polydimethylsiloxane (PDMS) using a standard soft lithography method as described in our previous work [1,2].

Cell cultures Syk- and Lyn-deficient (Syk/Lyn-deficient) chicken DT40 B cells were described previously [3]. DT40 cells were grown at 37 ºC with 5% CO_2 in complete media (RPMI 1640 supplemented with 10% heat inactivated fetal calf serum, 1% chicken serum, 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate, 100 IU/mL penicillin G, and 100 μg/ml streptomycin). Cells were subcultured every two days to maintain exponential growth. Harvested cells were centrifuged at 260 × *g* for 5 min and resuspended in an electroporation buffer (8 mM Na_2HPO_4, 2 mM KH_2PO_4, and 250 mM sucrose, pH = 7.2) at 1.5×10^7 cells/ml for electroporation.

The CHO/GFP-NFκBp65 cell line (Panomics) was generated by co-transfection of an expression vector for a fusion protein of turboGFP (Evrogen) and human NFκBp65, as well as pHyg into Chinese hamster ovary (CHO) cells. CHO cells were grown at 37 ºC with 5% CO_2 in Hams F12K medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin G, 100 μg/ml streptomycin and 100 μg/ml hygromycin. For stimulations, cells were incubated in serum free medium for 4 h and then placed in complete Hams F12K media supplemented with IL-1 β (Cell Science) at 40 ng/ml for indicated periods. Cells were resuspended in the electroporation buffer at a concentration of 1.5×10^7 cells/ml before electroporation.

Cell electroporation and protein sample preparation The inlet of the microfluidic channels was connected to a syringe pump (PHD infusion pump, Harvard Apparatus) to establish steady flow rates for the electroporation of incoming cell suspension. Different electric fields were established across the channel by applying various voltages generated by a high-voltage power supply (PS350, Stanford Research Systems). A magnetic bar was stirring inside the syringe to prevent cells from settling to the bottom. The solution from the outlet of the channel (~40 µl) was transferred to a microcentrifuge tube. After centrifugation at 4,500 × *g* for 3 min, one half of the supernatant (~20 µl) was collected for analysis. The pellet was washed twice (each time with 40 µl electroporation buffer) before further processing.

Western blotting analysis Protein samples (the supernatant or the pellets) were separated by standard SDS-PAGE and analyzed by Western blotting. To analyze the pellets, 40 µl of SDS lysis buffer was added to solubilize the pellet. Chromosomal DNA was sheared by repeated passages through a 1-ml syringe with a 26G × ½ needle. One half of the sheared solution (~20 µl) was used for the analysis of pellet fraction. For SDS-PAGE, 20 µl of SDS sample buffer was added to the sample (the supernatant or the pellet fraction). The sample was then heated in a boiling water bath for 5 min. For DT40 cells, anti-p38 and anti-Sp1 (Santa Cruz Biotechnology) were used to detect p38 and Sp1, respectively. For CHO/GFP-NFκBp65 cells, anti-p65 (Santa Cruz Biotechnology) was used. The intensity of each band was quantified using ImageJ software.

Supplementary Figures

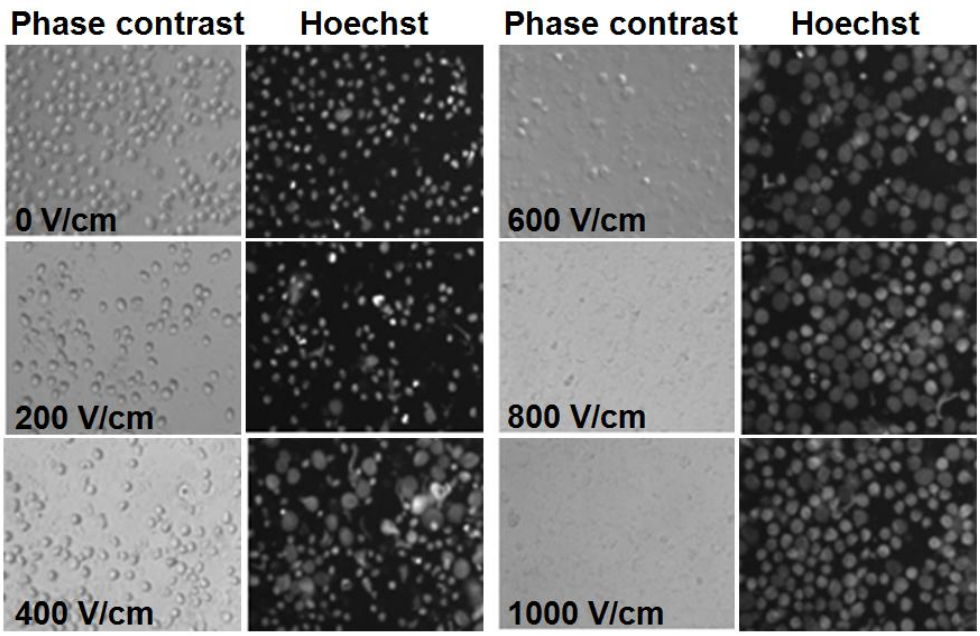

Figure S1. Electroporation of Hoechst 33342 (Invitrogen) stained DT40 cells at different field strengths. The field duration is 100 ms. *The images indicate that the nuclei stay in the cells with field intensity up to 1000 V/cm.*

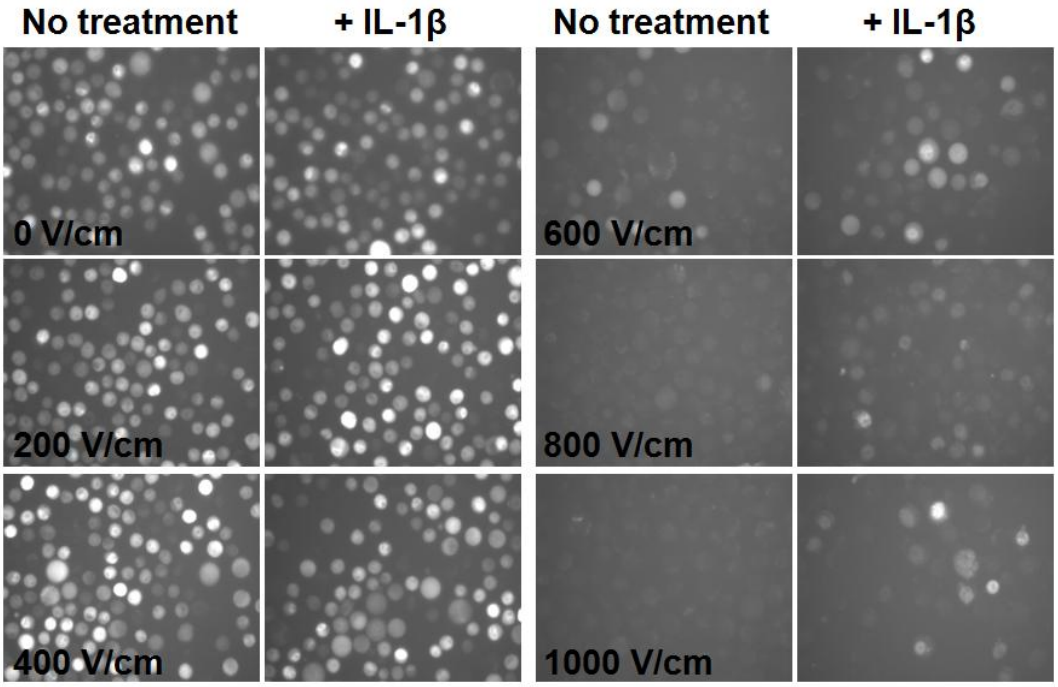

Figure S2. Phase contrast and fluorescence images of GFP-NFκBp65 CHO cells after electroporation with different field strengths (0, 200, 400, 600, 800, 1000 V/cm) for a duration of 50 ms. *The images indicate that cells stimulated by IL-1β (with translocation) retain more NF-κB after electroporation.*

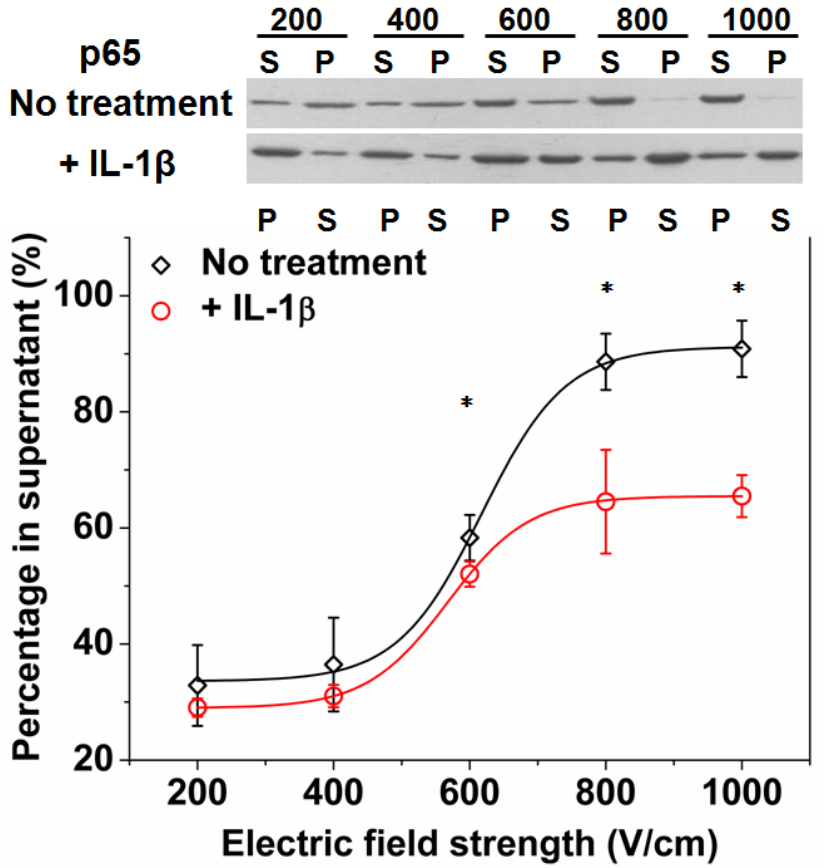

Figure S3. The release of NF-κB into the supernatant by electroporation. The levels of p65 in supernatant (S) and pellet (P) fractions from CHO/GFP-NFκBp65 cells treated with or without IL-1β (incubation for 0.5 h) and then electroporated at different field strengths (200, 400, 600, 800, 1000 V/cm) for 50 ms was analyzed by Western blotting (upper panel). The percentage of p65 in the supernatant fractions at different field strengths for CHO/GFP-NFκBp65 cells with and without stimulation with IL-1β is shown in the lower panel (each data point is derived from three trials). The difference between the two data points is statistically significant with P values less than 0.05 (*). *The data indicate that the release by electroporation is substantially affected by the subcellular location of the NF-κB especially at the field intensities of 800 and 1000 V/cm.*

References

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(3) Ma, H.; Yankee, T. M.; Hu, J. J.; Asai, D. J.; Harrison, M. L.; Geahlen, R. L. *J. Immunol.* **2001**, *166*, 1507.