Supporting Information

Detection of Kinase Translocation Using Microfluidic Electroporative Flow Cytometry

Jun Wang, Ning Bao, Leela L. Paris, Hsiang-Yu Wang, Robert L. Geahlen and Chang Lu

Departments of Agricultural and Biological Engineering, Department of Medicinal Chemistry and Molecular Pharmacology, School of Chemical Engineering, Birck Nanotechnology Center and Bindley Bioscience Center, Purdue University, West Lafayette, Indiana 47907, USA.

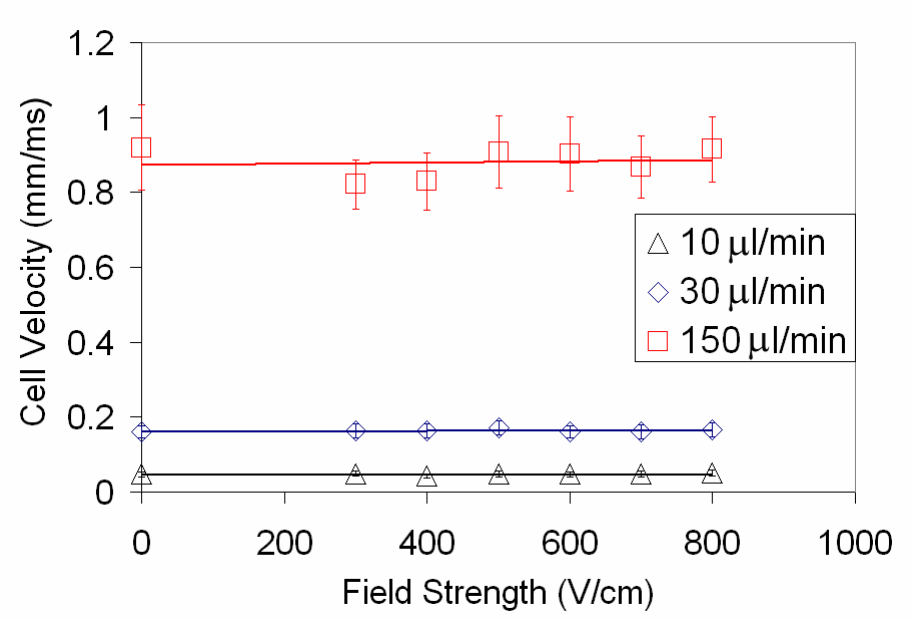

Figure S1 The effect of the field strength on the cell velocity (measured in a straight channel with a cross section of 62 µm × 48 µm). Cell velocities at different infusion rates do not substantially change with the field intensity. To record cell movement under the microscope, harvested CHO-K1 cells were re-suspended in the electroporation buffer containing 1 µg/ml calcein AM (Invitrogen, Carlsbad, CA). The mixture was incubated on ice for 15 minutes and then dispensed into the device for electroporation. In live cells the nonfluorescent calcein AM is converted to green-fluorescent calcein, after acetoxymethyl ester hydrolysis by intracellular esterases. The images of flowing fluorescent cells were taken at different infusion rates and field intensities. The cell velocity was then obtained by dividing the length of the fluorescent trail (left by individual cells) by the exposure time. A small program written in MATLAB was used to analyze the images. Each data point was based on a cell population of ~150 cells.

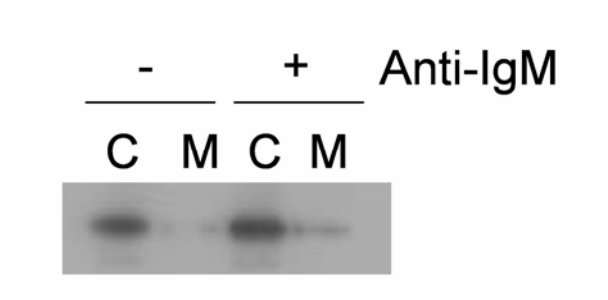

Figure S2 Western blotting analysis of the SykEGFP fraction in the cytosol (C) and on the membrane (M) with and without stimulation by anti-IgM antibody using the conditions in the main text. The experiment was repeated twice. Based on the analysis of the images using ImageJ 1.36b, the percentage of the kinase moving to the plasma membrane was 9.3±1.1% of the entire kinase amount in the cells.

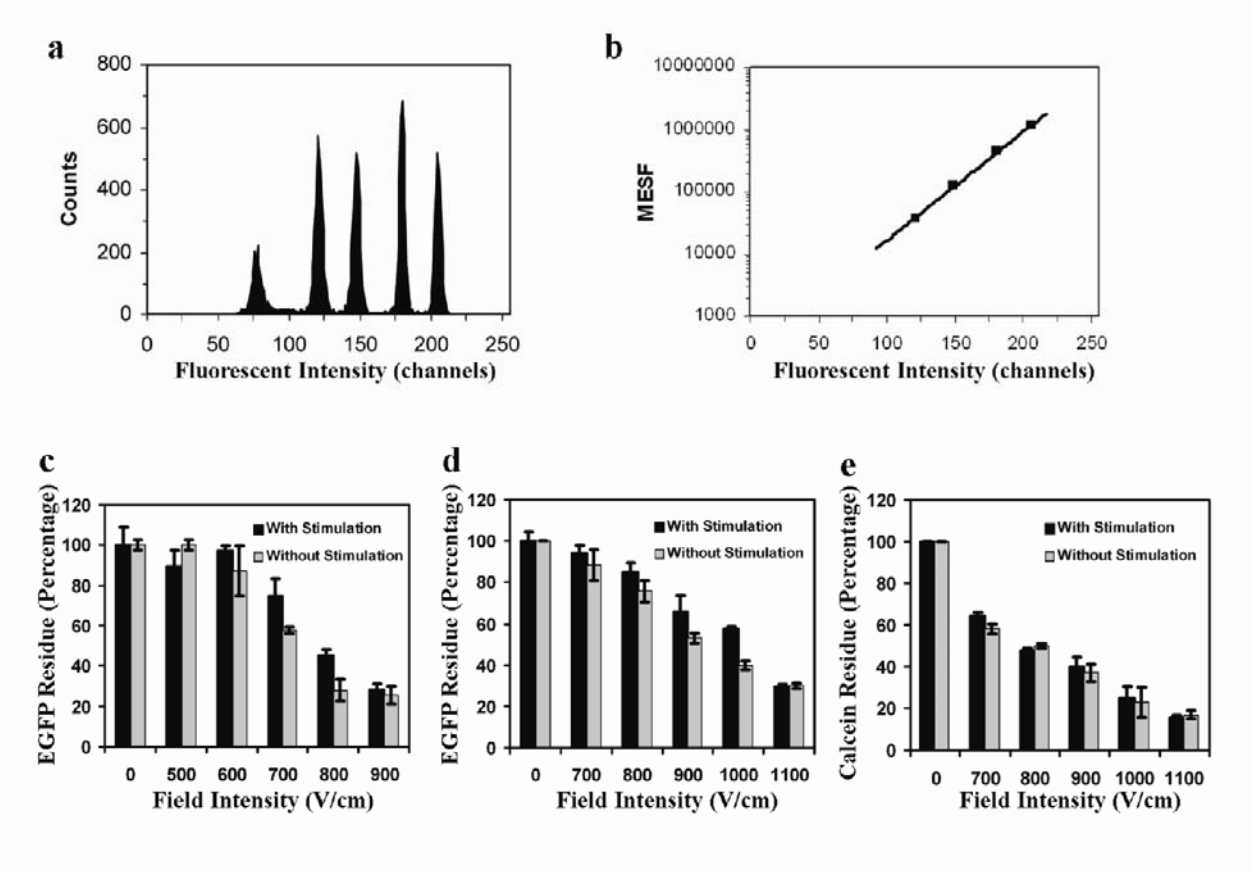

Figure S3 The quantitative analysis of the fluorescence intensity of DT40 cells. (**a**) The histogram of the fluorescence intensity from a mixture of beads with varying predefined fluorescence intensity levels. The data were generated by the microfluidic EFC system and the population with the lowest intensity is the reference blank. (**b**) The calibration curve with the MESF values of the beads plotted against the peak channel numbers (the fluorescent intensities) of the beads obtained by our microfluidic system (R^2=0.9978, solid square). The mean MESF value of the cells, $EGFP_{mean}$ in equation (1), is determined by finding the corresponding MESF value for a mean channel value known from the experiments. We assumed that the quantity of the fluorescent protein/calcein of the cell population detected by the system without the field (E = 0) was 100%. (**c**) The relative EGFP content in SykEGFP-DT40-Syk^--Lyn^- cells under different electric field intensities with a electroporation duration of 120 ms. (**d**) The relative EGFP content in SykEGFP-DT40-Syk^--Lyn^- cells under different electric field intensities with a electroporation duration of 60 ms. (**e**) The relative calcein content in calcein AM stained DT40-Syk^--Lyn^- under different electric field intensities with a electroporation duration of 60 ms.

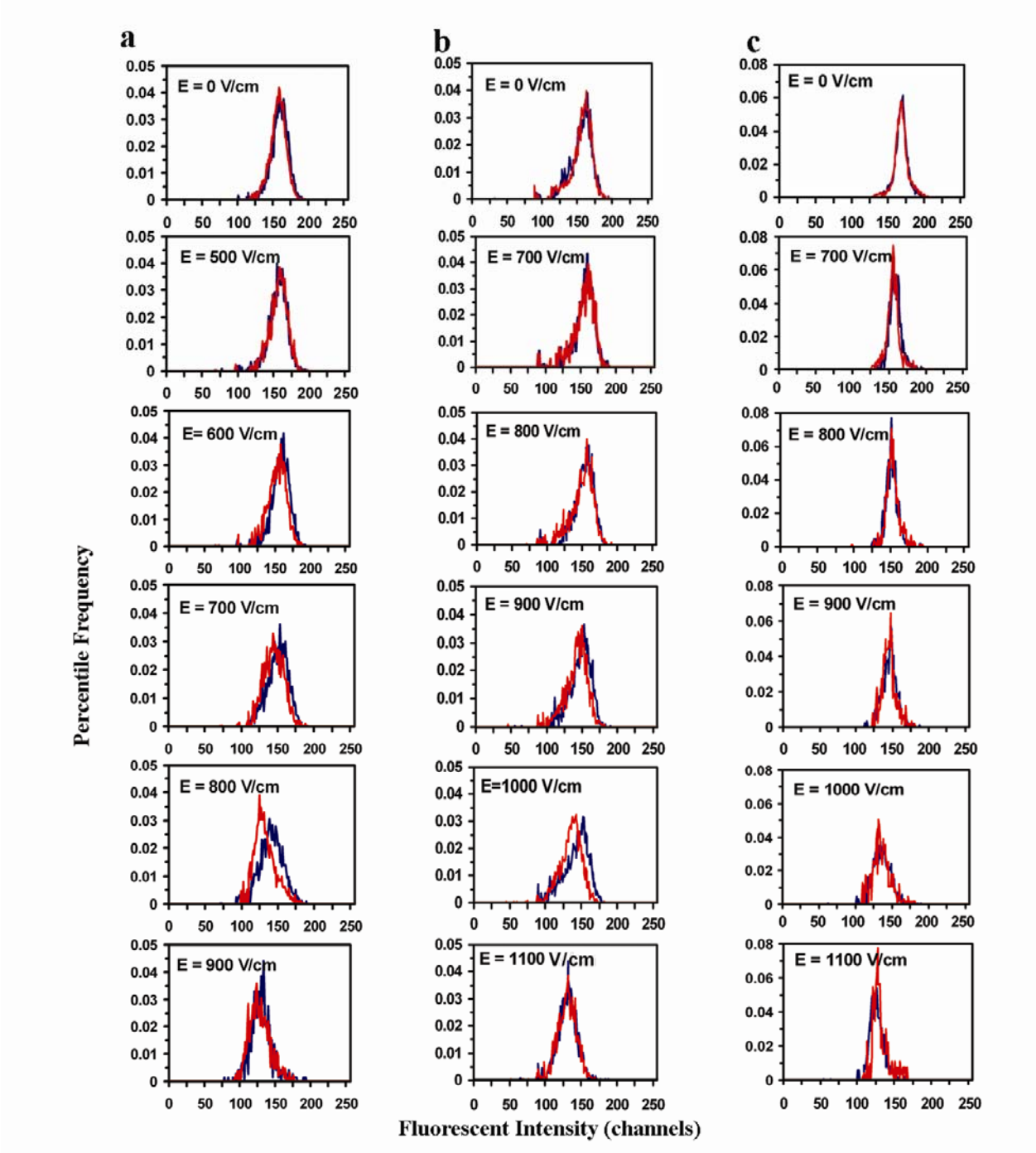

Figure S4 The histograms of the fluorescent intensity of DT40 cells detected by the microfluidic EFC under different electric field intensities and durations. SykEGFP-DT40-Syk$^-$-Lyn$^-$ cells were applied in (**a**) and (**b**), while calcein AM stained DT40-Syk$^-$-Lyn$^-$ cells were used in (**c**). The blue curves were generated by cells stimulated by anti-IgM and the red curves were obtained from cells without stimulation. The data in (**a**) and (**b**) were obtained with different electroporation durations of 120 and 60 ms, respectively. The duration in (**c**) was 60 ms. The field intensity in the narrow section is indicated for each histogram.

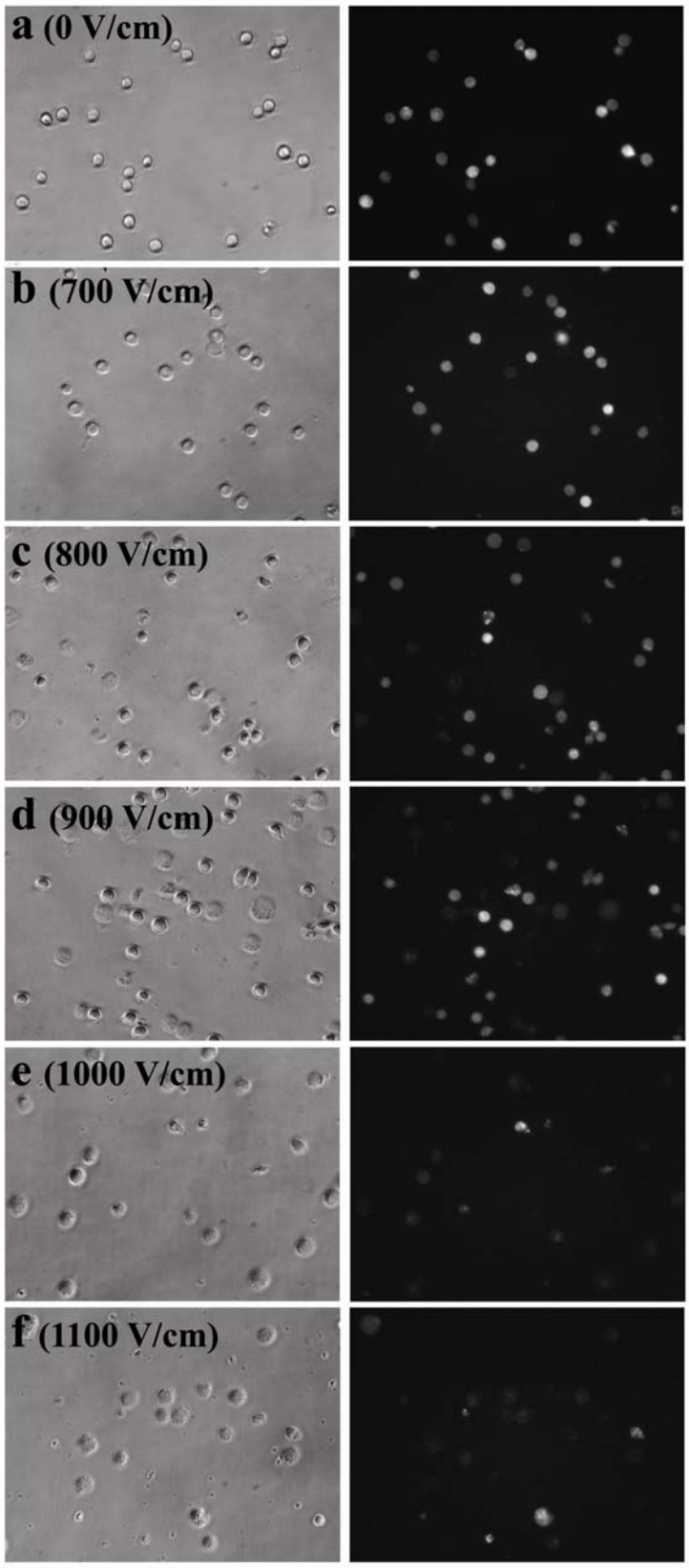

Figure S5 The images of SykEGFP-DT40-Syk$^-$-Lyn$^-$ cells after the microfluidic EFC screening with different field intensities and field duration of 60 ms. The phase contrast images are at the left and the fluorescent images are at the right. All images were taken with the same magnification. The cells were not stimulated.

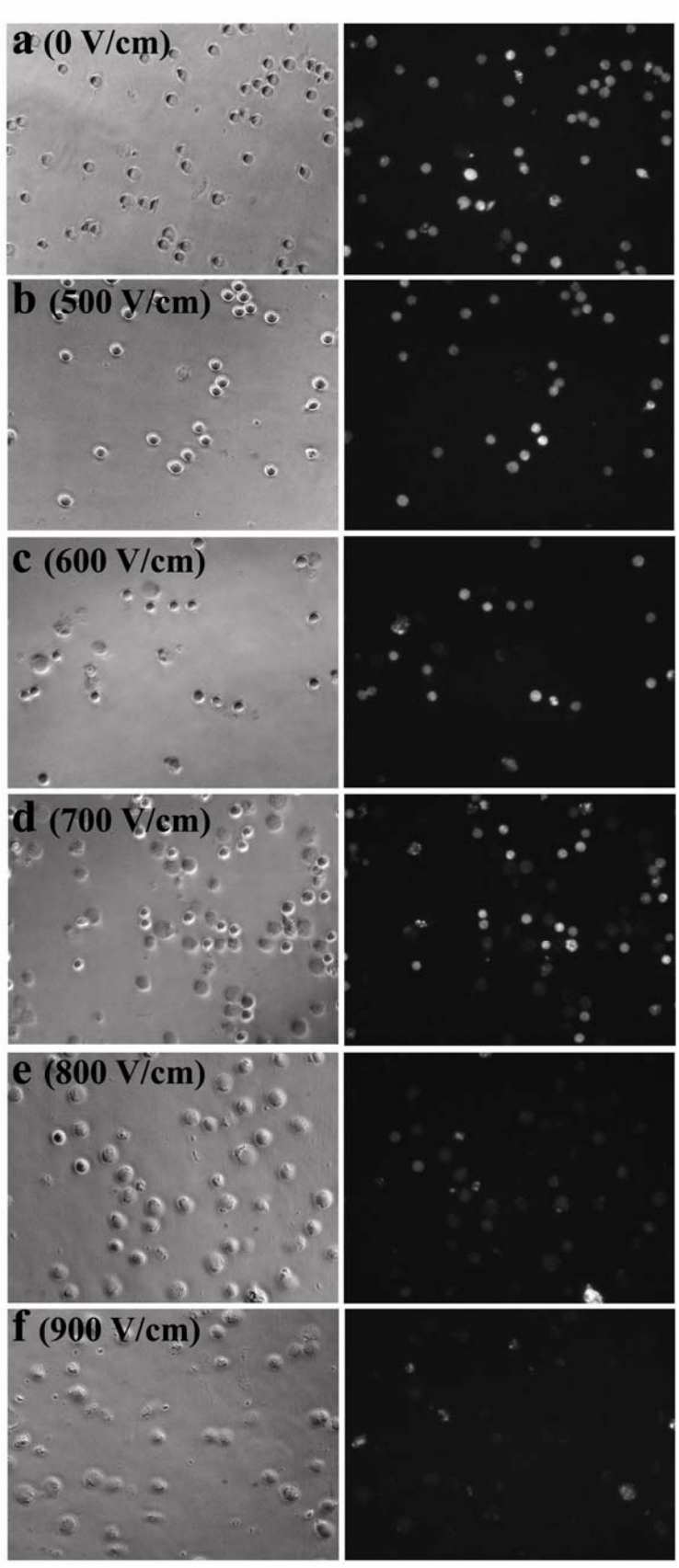

Figure S6 The images of SykEGFP-DT40-Syk$^-$-Lyn$^-$ cells after the microfluidic EFC screening with different field intensities and field duration of 120 ms. The phase contrast images are at the left and the fluorescent images are at the right. All images were taken with the same magnification. The cells were not stimulated.