

Supplementary data

In order to select the optimal stimulation time for IgD induced NF- κ B nuclear translocation in Jurkat cells, we set 2h, 4h, 8h and 12h respectively. NF- κ B nuclear translocation was monitored and quantitated using the similarity score by Image Stream technology described in the Material and Methods. Stimulation of Jurkat cells with 3 μ g/mL of IgD clearly resulted in nuclear translocation of NF- κ B within 4h which as obviously as measured by the G-mean.

Image Stream fluorescence imaging of different hours of IgD induced NF- κ B nuclear translocation in Jurkat cells. **(A)** Quantification of nuclear translocation using the similarity algorithm. **(A₁)** Control un-stimulated Jurkat cells, **(A₂)** Jurkat cells treated with 3 μ g/mL IgD for 2h, **(A₃)** Jurkat cells treated with 3 μ g/mL IgD for 4h, **(A₄)** Jurkat cells treated with 3 μ g/mL IgD for 8h, **(A₅)** Jurkat cells treated with 3 μ g/mL IgD for 12h. The region High Sim drawn on the dark field/DAPI similarity plot represents the region for positive image correlation for the similarity algorithm. This region is applied to the NF- κ B/DAPI similarity plot. The percentages of cells that fall within the High Sim region are displayed on the right of each histogram. **(B)** The multispectral imaging system acquires up to 6 images per cell in three different imaging modes: bright field (morphology) (1), and fluorescence; Ch07-DAPI (Purple) (2), Ch03-NF- κ B (yellow-PE) (3), and NF- κ B/DAPI composite images (4) for five representative (of 10000 images) cells and are shown for each treatment group. Pixel intensities from the NF- κ B images are plotted against the corresponding pixel intensities from the DAPI or dark-field images.