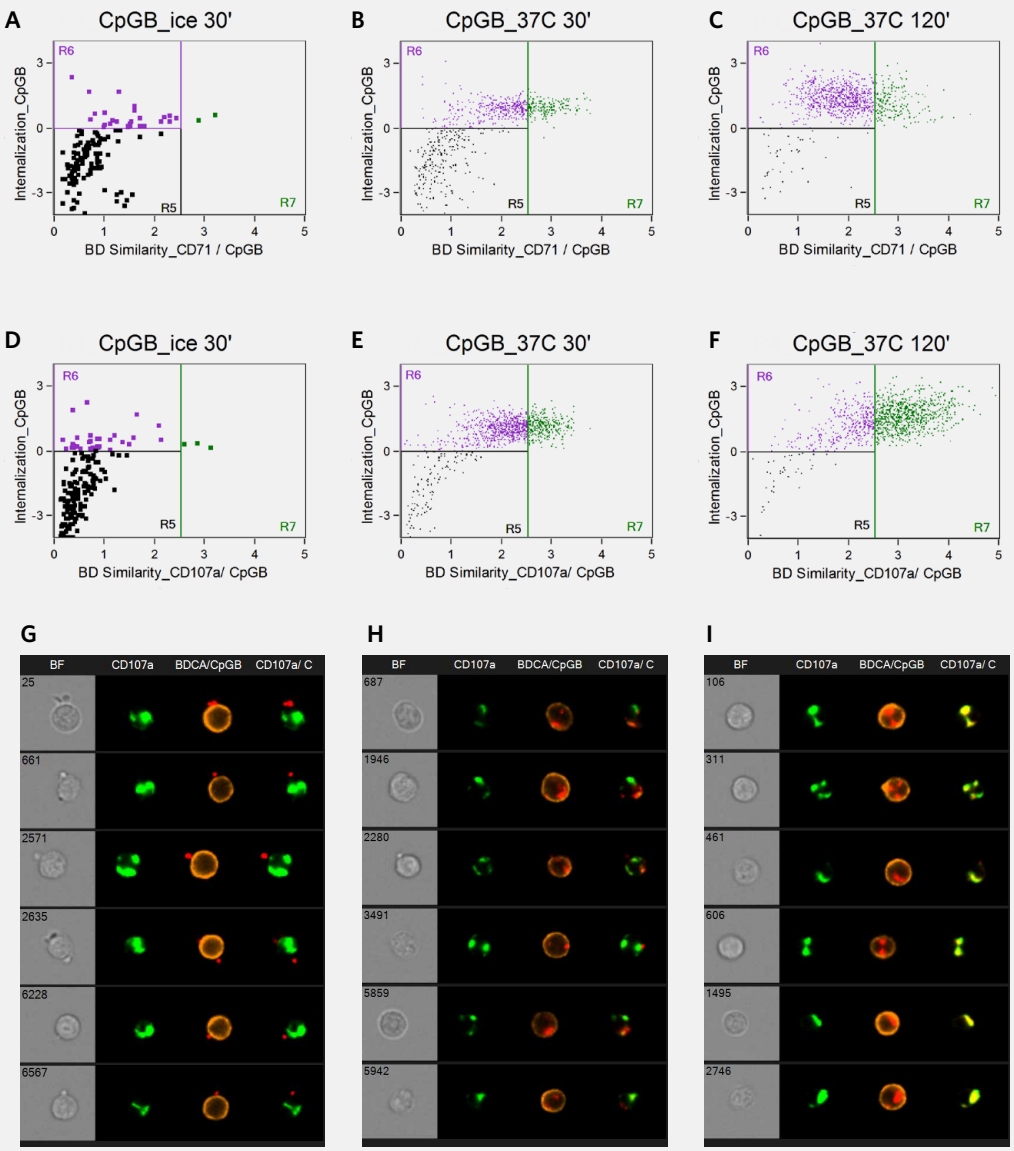
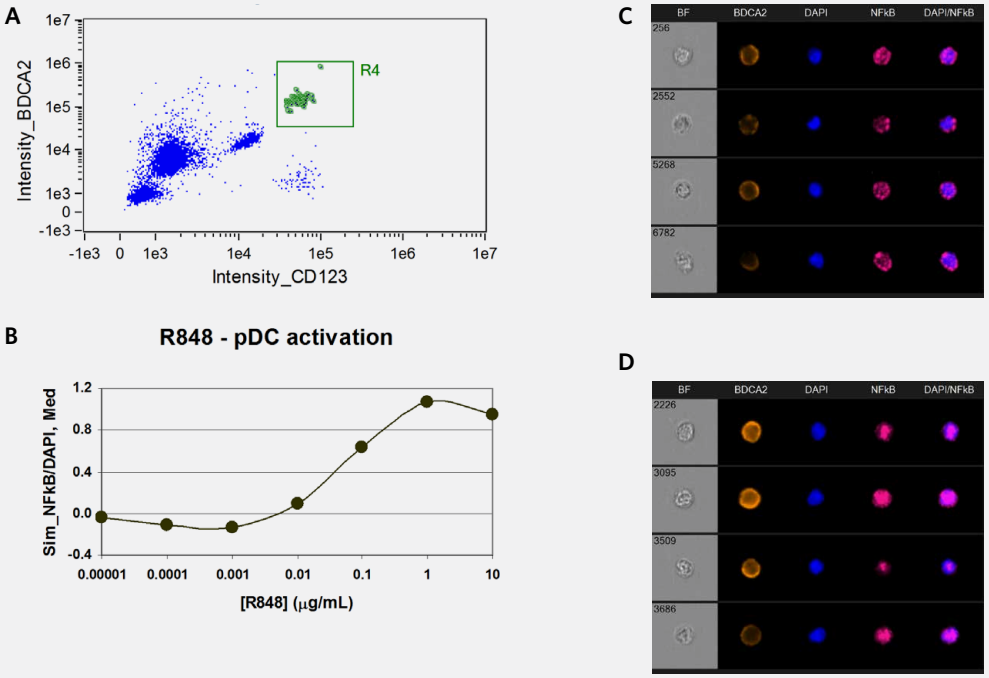
Plasmacytoid dendritic cell (pDC) binding of double-stranded viral DNA results in a potent antiviral response that triggers the secretion of copious amounts of IFN-α. The internalization (y-axis) and trafficking (x-axis) of Cy5-labeled CpGB in human pDCs was measured using the ImageStream System (**Figure 2**). BDCA+ gated events were analyzed. With time, CpGB accumulated inside the pDCs (as indicated by increasing internalization scores), trafficking to the endosome first (as indicated by high Similarity Bright Detail scores; **Figures 2A-2C**), and then to the lysosome (**Figures 2D-2F**). Representative images of cells from the indicated regions are shown (**Figures 2G-2I**).

Figure 2. Internalization and trafficking of CpGB within human plasmacytoid dendritic cells



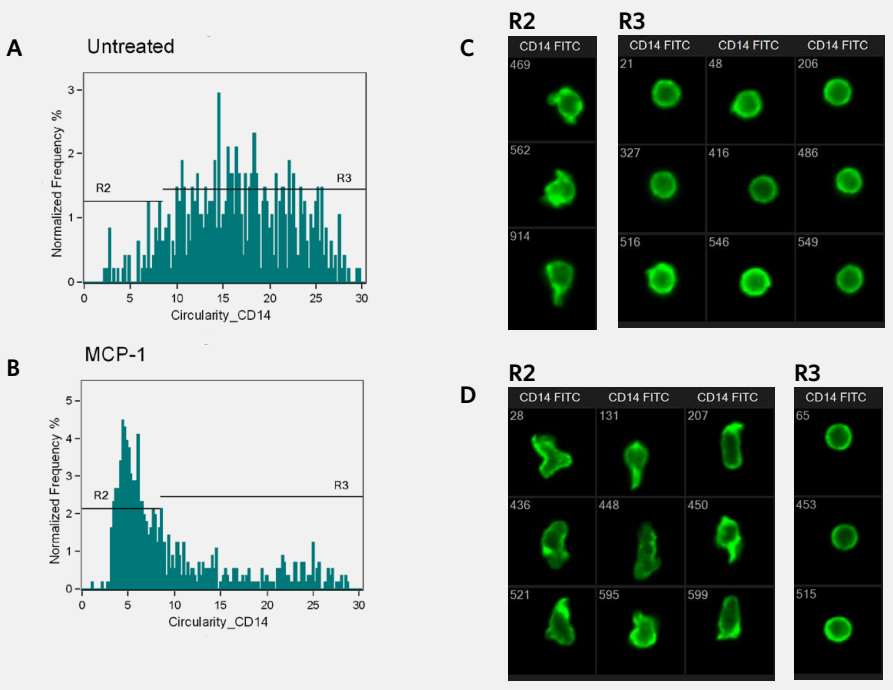
Plasmacytoid dendritic cells (pDCs) express pattern recognition receptors that transmit activation signals upon ligand binding. Translocation of NFκB was measured as a marker for TLR7-induced activation in whole blood pDCs. NFκB translocation was measured using the Similarity score for the gated pDCs from whole blood samples exposed to a range of R848 doses (**Figure 3B**). Images of representative cells treated with 1 ng/ml (**Figure 3C**) and 1,000 ng/ml (**Figure 3D**) are shown.

Figure 3. Measurement of NFκB activation in whole blood plasmacytoid dendritic cells



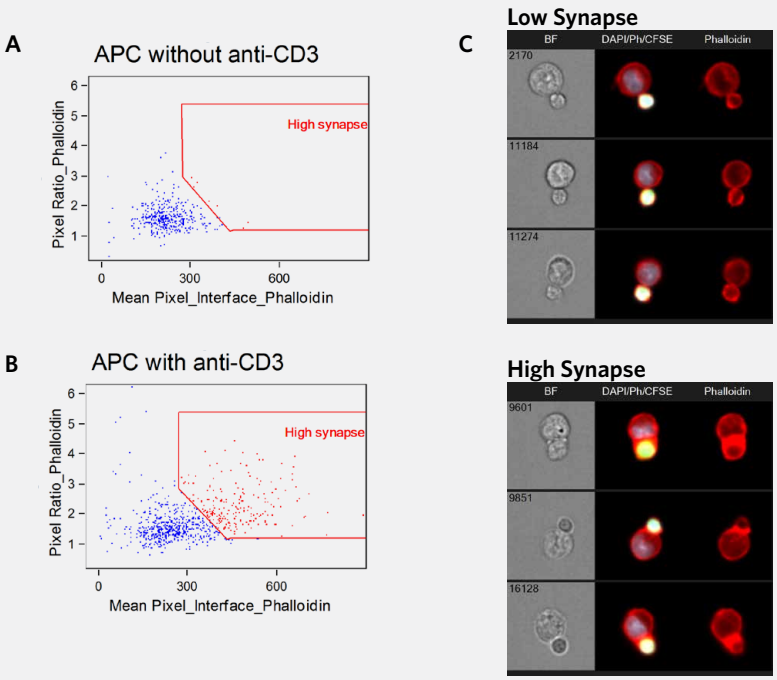
As shown in **Figure 3**, pDC express pattern recognition receptors that transmit activating signals upon ligand binding. Translocation of NF-κB was measured as a marker for TLR7-induced activation in whole blood pDC. NF-κB translocation was measured using the Similarity score[1] for the gated pDC from whole blood samples exposed to a range of R848 doses (**Figure 3B**). Images of representative cells from the 1 ng/ml (**Figure 3C**) and the 1,000 ng/ml (**Figure 3D**) samples are shown.

Figure 4. Measurement of chemokine-induced monocyte shape change



Circulating monocytes rapidly change shape when exposed to chemokine gradients. To measure chemokine-induced shape change, human PBMCs were incubated with MCP-1 for 30 minutes, stained with FITC anti-CD14, and RBC-lysed samples were analyzed for circularity (**Figure 4**). Representative images of CD14+ monocytes from regions R2 and R3 are shown (**Figure 4C and 4D**).

Figure 5. Immune synapse formation



Murine lymph node cells (LNCs) were incubated with control- (**Figure 5A**) or anti-CD3- (**Figure 5B**) coated, artificial antigen presenting cells (APCs), and conjugates were analyzed for actin accumulation at the immune synapse. Representative images are shown for the indicated regions of the anti-CD3 plots (**Figure 5C**).

REFERENCES:

1. George T, Fanning S, Fitzgerald-Bocarsly P, et al. Quantitative measurement of nuclear translocation events using similarity analysis of multispectral cellular images obtained in flow. J Immunol Methods. 2006 Apr 20; 311(1-2): 117–129. 2006 Mar. doi: 10.1016/j.jim.2006.01.018.