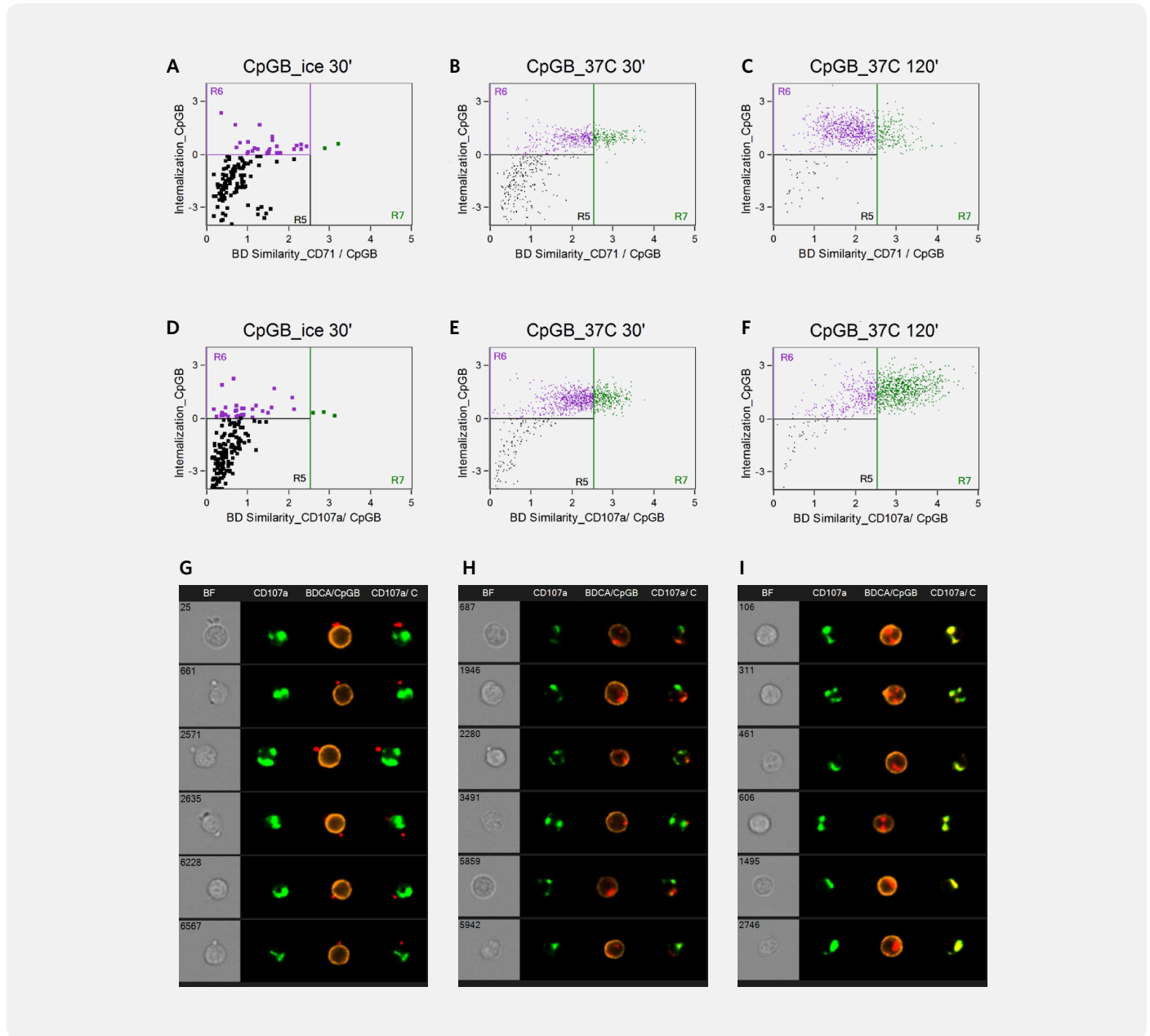


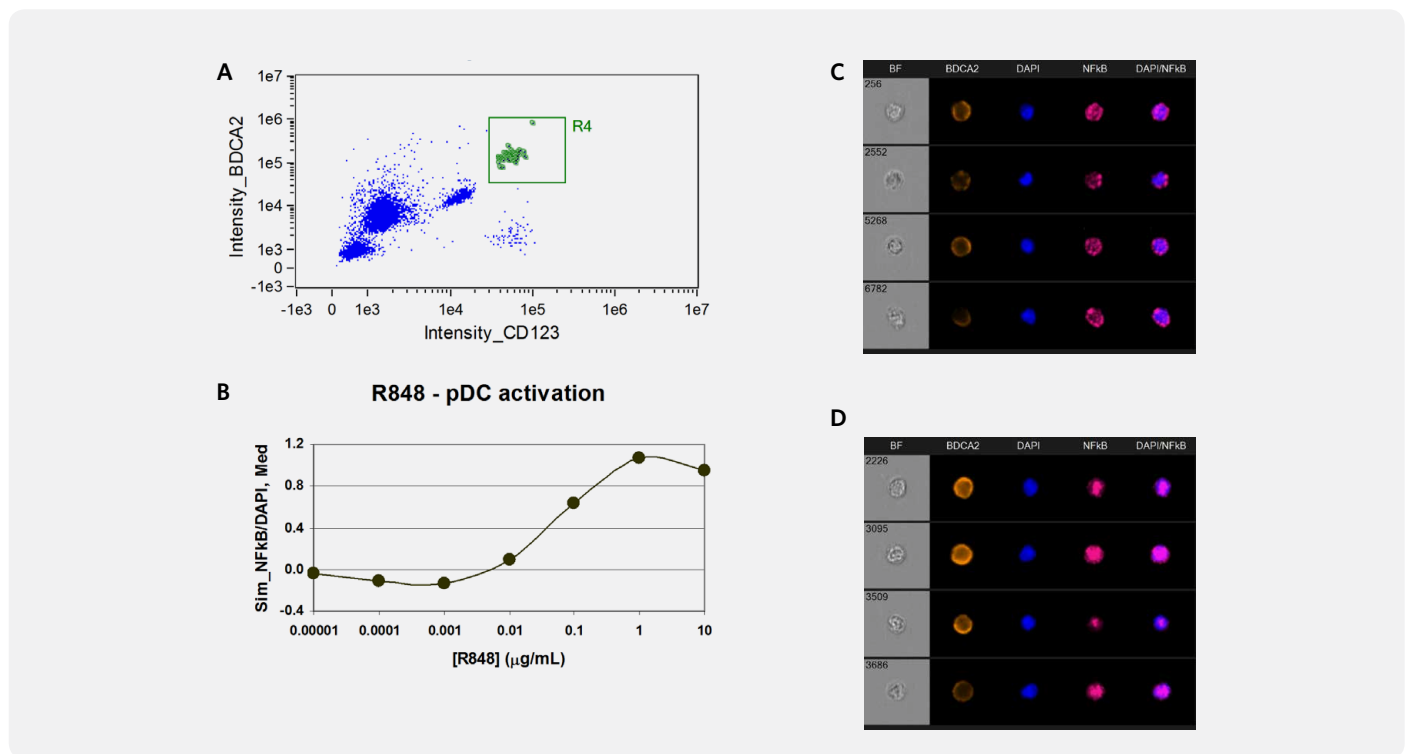
Plasmacytoid dendritic cell (pDC) binding of double-stranded viral DNA results in a potent antiviral response that triggers the secretion of copious amounts of IFN- α . The internalization (y-axis) and trafficking (x-axis) of Cy5-labeled CpGB in human pDCs was measured using the ImageStream System (**Figure 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



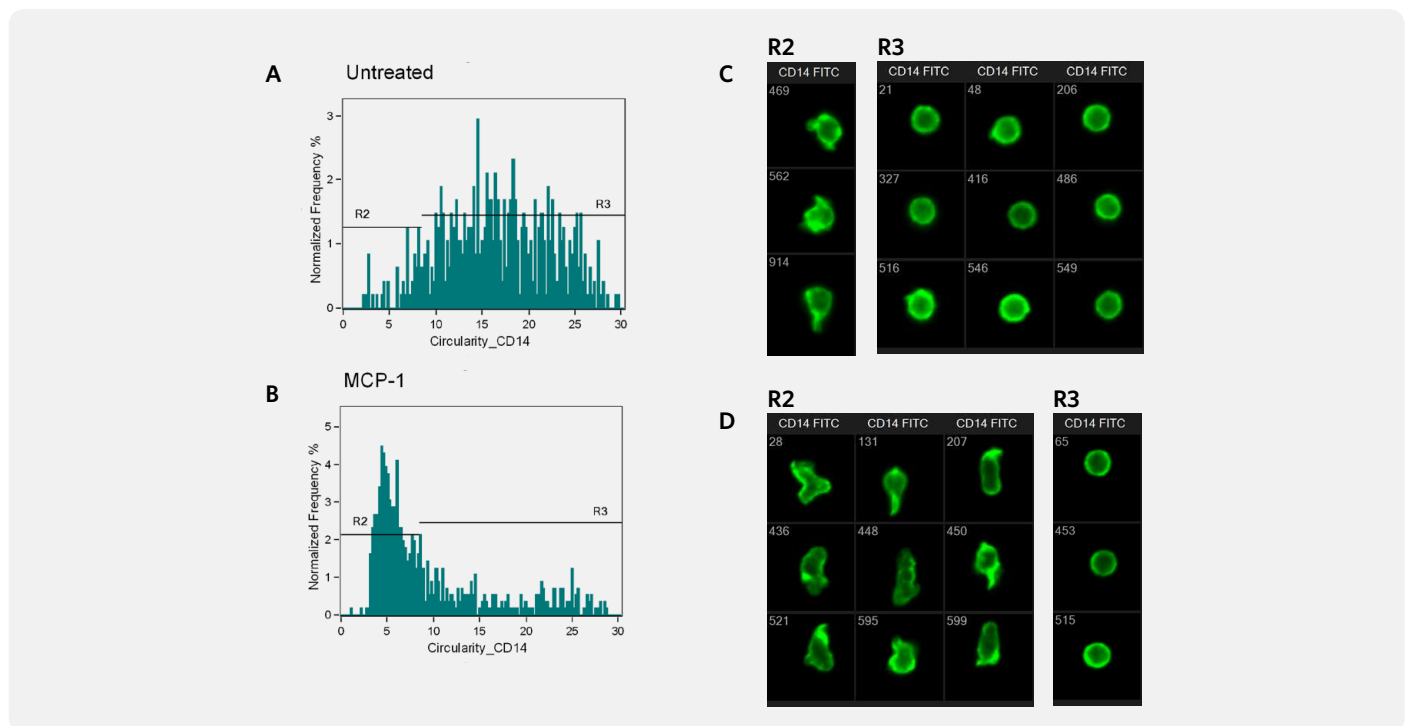
Plasmacytoid dendritic cells (pDCs) express pattern recognition receptors that transmit activation signals upon ligand binding. Translocation of NF κ B was measured as a marker for TLR7-induced activation in whole blood pDCs. NF κ B translocation was measured using the Similarity score for the gated 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



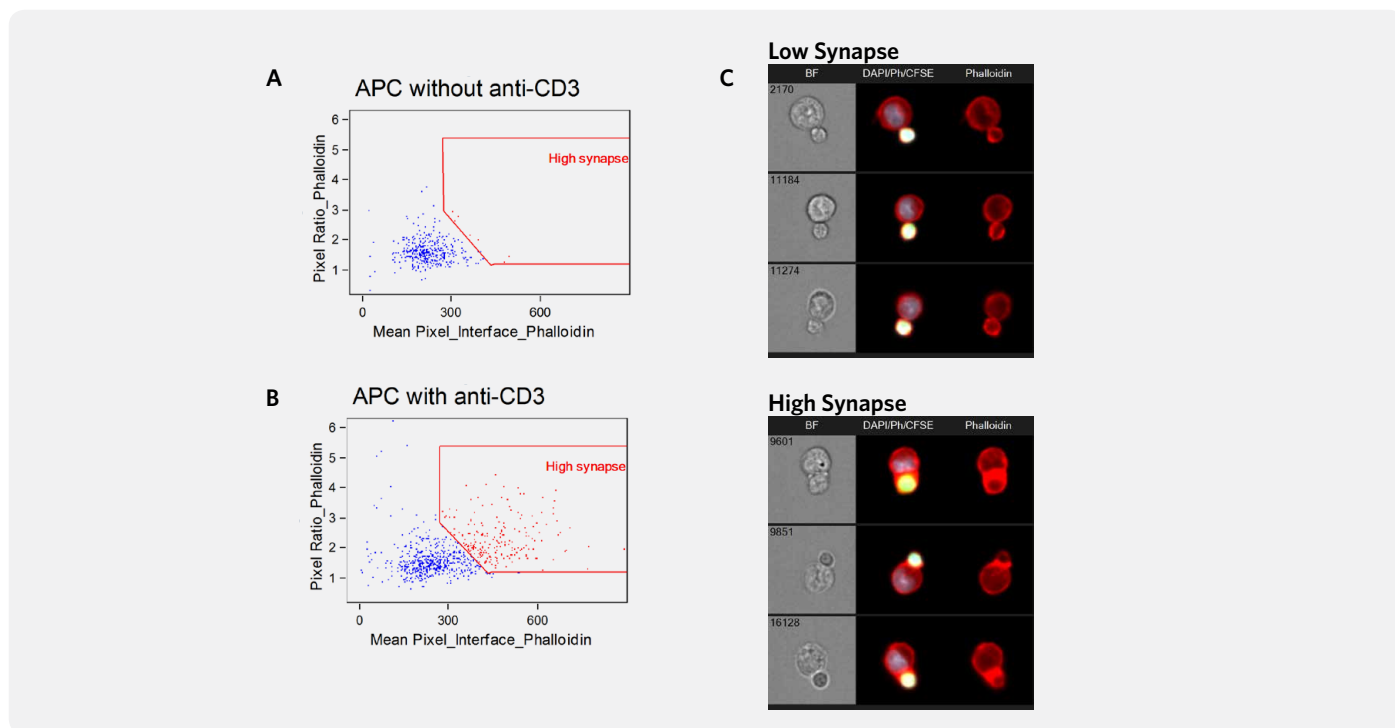
As shown in **Figure 3**, pDC express pattern recognition receptors that transmit activating signals upon ligand binding. Translocation of NF-κB was measured as a marker for TLR7-induced activation in whole blood pDC. NF-κB translocation was measured using the Similarity score¹ for the gated pDC from 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.

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