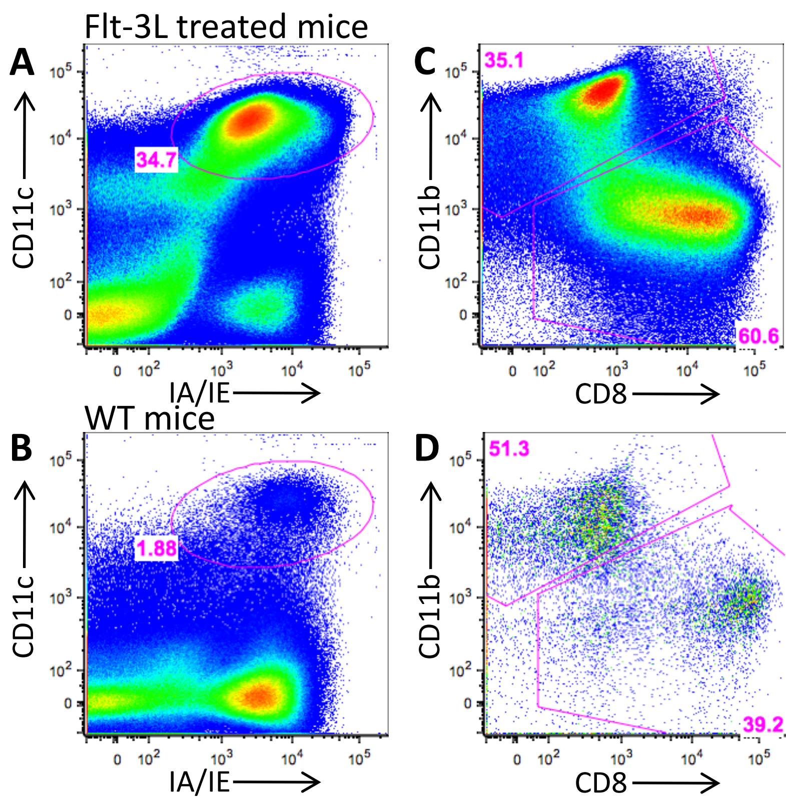

Figure I. Splenic cells from mice treated with flt-3 ligand show enrichment in flt-3 derived DCs. Flow cytometry analysis of surface expression of CD11c and IA/IE (MHCII) in (A) Mice treated with flt-3 ligand or (B) WT mice. $CD11c^{+}$ IA/IE^{+} cells were gated and further analyzed for surface expression of CD11b and CD8 (C, D). Numbers given are percentages of cells contained within each gate.

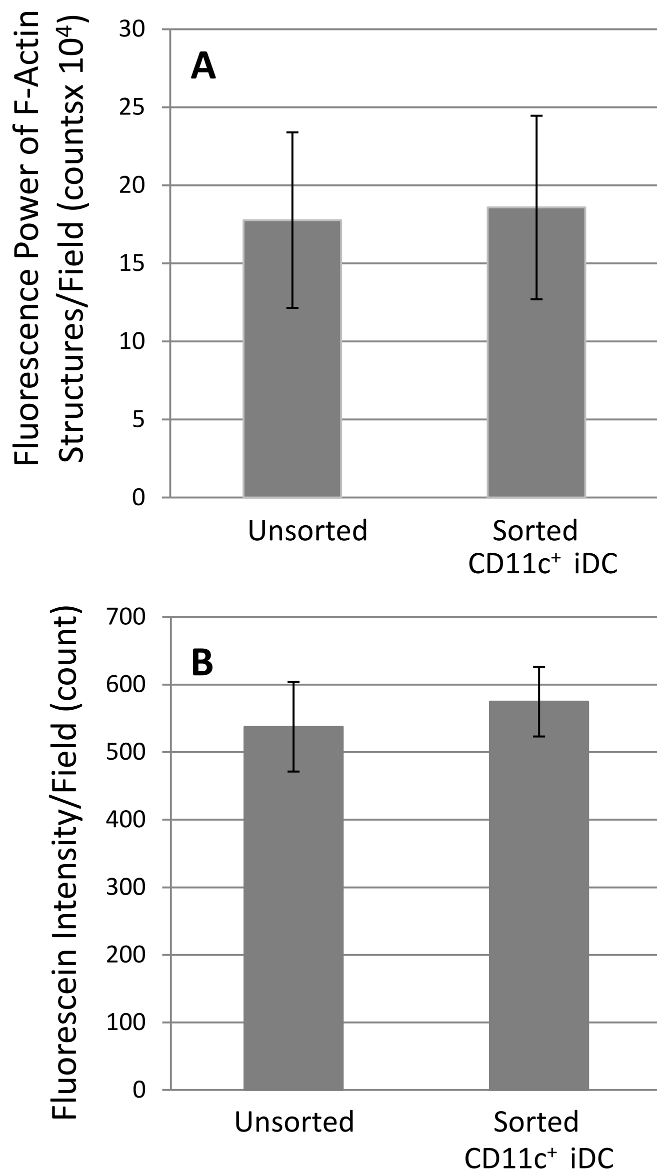

Figure II. Cells used in microscopy experiments represent a homogenous population of CD11c^{+} iDCs. iDC generated via GM-CSF represent a heterogeneous population of cells. However, the main contaminating cells, macrophages and B cells, are not plated for microscopy experiments as macrophage adhere strongly to the culture dish and B cells do not adhere to the microscopy dishes. To confirm that a homogenous population of iDC were used for microscopy experiments, we isolated iDC with flow cytometry using Alexa647-CD11c. (A) Unsorted and CD11c^{+} cells were incubated with Alexa546-agLDL for 60 min, fixed and stained with Alexa488-phalloidin to show F-actin. No quantitative difference in the amount of local F-actin polymerization at sites of contact with the aggregate was seen between the unsorted culture and the isolated CD11c^{+} iDC cells. (B) Unsorted and CD11c^{+} cell lysosomes were loaded with biotin-fluorescein-dextran via overnight incubation. Cells were subsequently incubated with streptavidin and Alexa546 dual-labeled agLDL for 90 min, fixed and permeabilized. Colocalization of dextran with the aggregate indicates areas of exocytosis. No quantitative difference was seen in the amount of exocytosis to areas of contact with the aggregate between the unsorted culture and the isolated CD11c^{+} iDC cells.

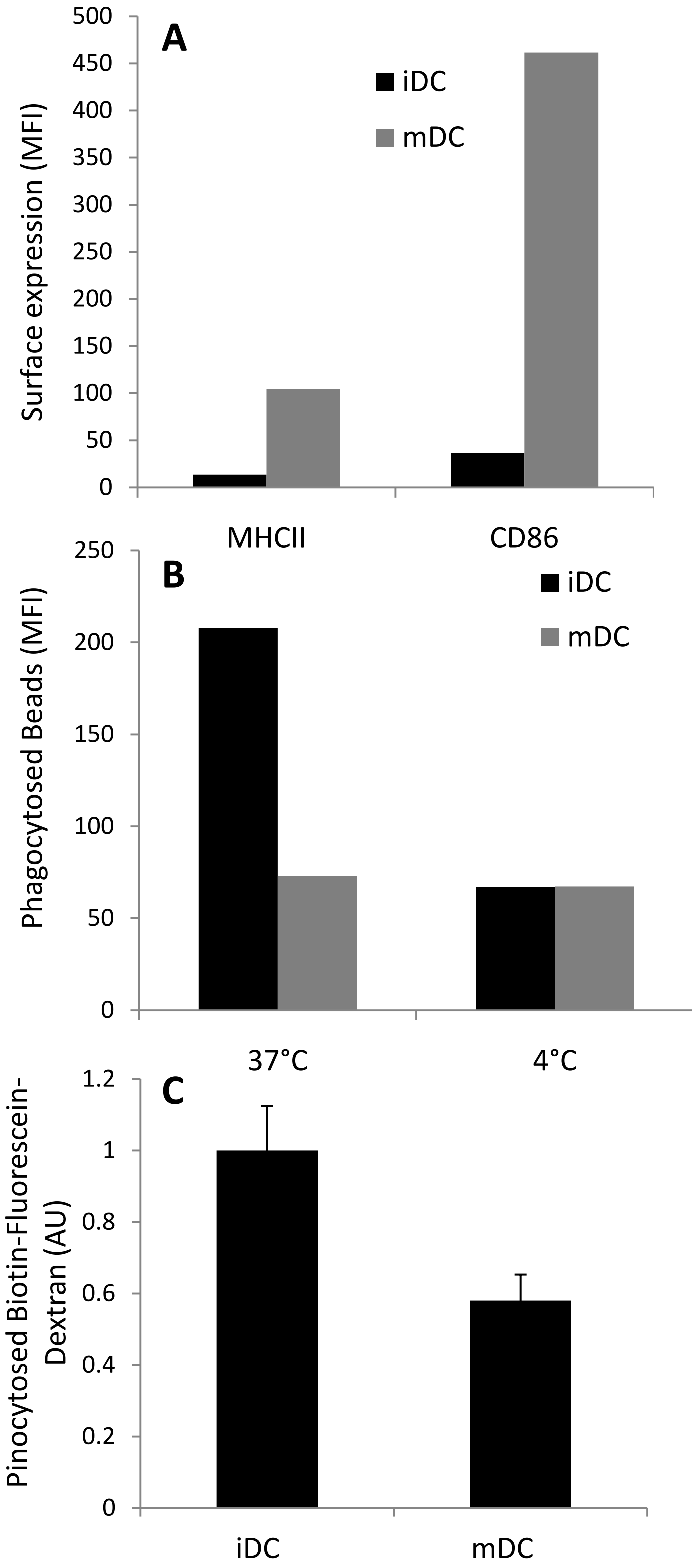

Figure III. DCs upregulate MHC II and CD86 and down regulate phagocytosis and pinocytosis upon maturation. (A) Surface expression of MHC II and CD86, markers of DC maturation, were examined in iDC and mDC using flow cytometry. (B) Quantification of the phagocytosis of Fluoresbrite YG latex beads after 60 min in iDC and mDC at 37°C or 4°C to assess non-internalized surface bound beads. (C) The amount of biotin-fluorescein-dextran delivered to lysosomes via pinocytosis was quantified using confocal imaging in iDC and mDC. a.u. arbitrary units. MFI – Mean Fluorescence Intensity.