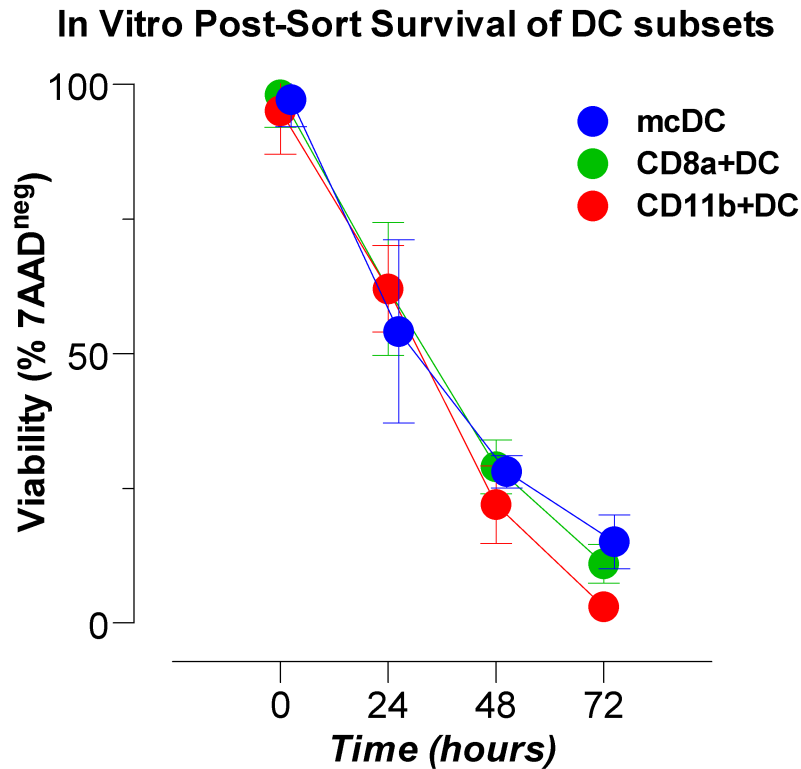


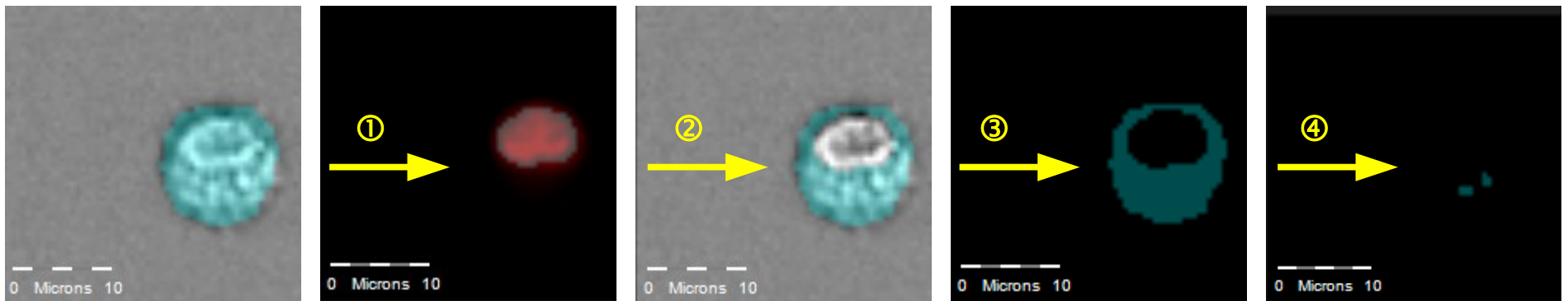
**Supplemental Figure 1: Expansion of mcDC by Flt3L in vivo has no effect on cell surface phenotype of DC.** DC were harvested and analyzed from B16-Flt3L-treated (FLT3L) or age-matched wild type (WT) NOD mice, 7-10 days post tumor transplant. The in vivo expansion of cDC subsets by Flt3L had no effect on maturation state or activation markers compared to WT mice. Both Flt3L and WT DC subsets were immature and not activated (mature) as judged by CD40, CD54, CD80, CD86, and MHC class I and class II expression, which showed DC as resting immature cells. Background fluorescence in dotted lines, WT in solid black lines, Flt3L-treated in solid gray lines. Histograms are representative of 4 Flt3L-treated and 2 WT NOD mice.



**Supplemental Figure 2: Viability of flow-sort purified DC subsets.**

DC subsets were sort-purified and incubated in culture for the indicated time and then reanalyzed for viability using 7AAD. Viable cells are expressed as % of 7AAD<sup>neg</sup> cells  $\pm$  SD. No subset specific differences were observed.

## Supplemental Figure 3: Spot Counting Mask for Amnis ImageStream Analysis



① Erode in 2px from edge of cell (Internal)

② Subtract a tight Morphology mask of nucleus to make the Cytoplasm Mask (Internal but Not nucleus)

③ Apply the Cytoplasm Mask to Channel 3 (CFSE)

④ Apply Spot Mask (1.75x above background) to collect data on particles