Supplementary Figure 1. Increasing cell number to antibody ratio does not significantly impact phospho antibody staining.  

a) U937 cells were left unstimulated or stimulated with 10ng/ml of IFNγ for 10 minutes. The samples were fixed with formaldehyde and permeabilized with methanol. At this stage, the samples were placed in a 96 well plate, and either left untreated, or were labeled with 6 concentrations of PacBlu. Non-PacBlu treated samples were stained separately to generate 6 stained samples. The PacBlu barcoded samples were combined at two different cell concentrations and stained. These samples contained either the same amount of total cells as each of the separately stained samples (1x = one million cells), or five times the amount of cells (5x = five million cells). All samples were stained with the same amount of pStat1 Ax647.

b) One separately stained sample compared to the barcoded samples (all six populations are shown as one histogram) for the unstimulated and stimulated states. Note the nearly identical staining intensities.

c) Plot of CV versus sample or population. The CV was measured on the Ax647 channel for each of the separately stained samples or for the 6 barcoded populations in the FCB samples.