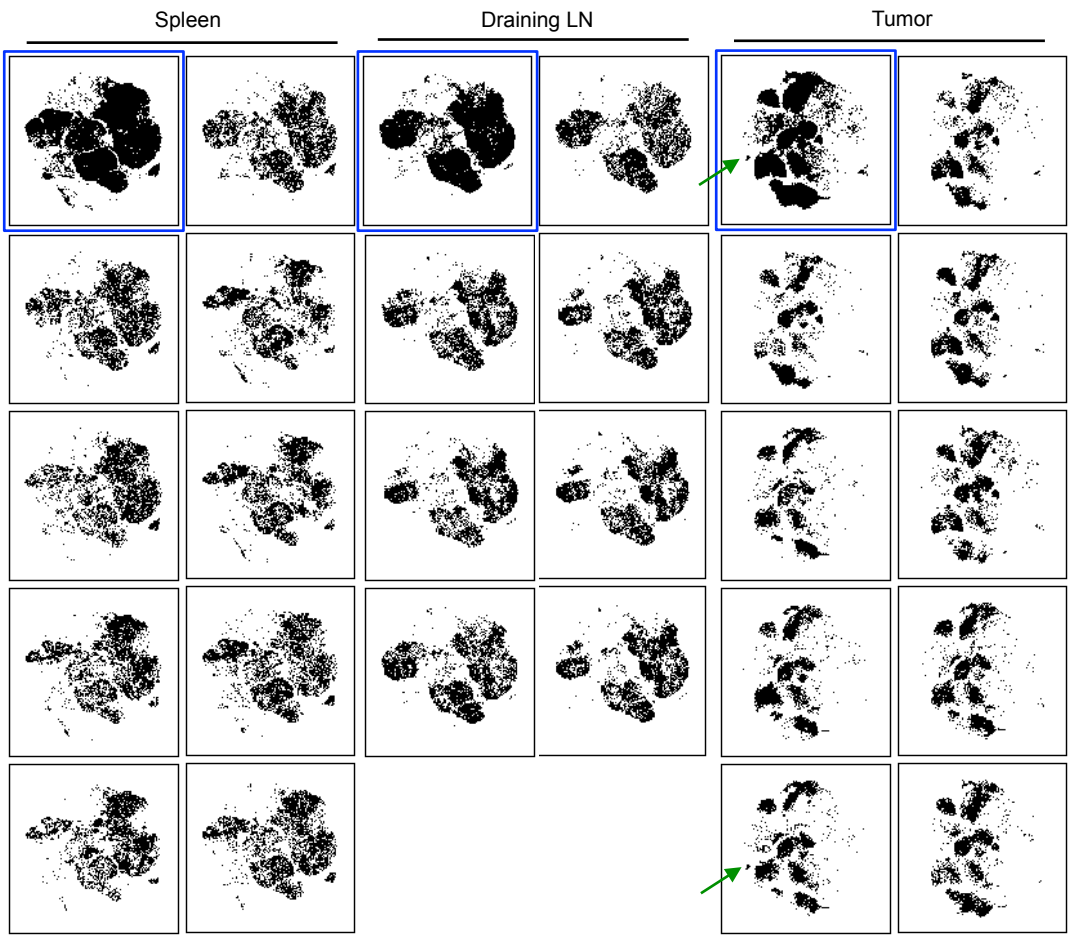
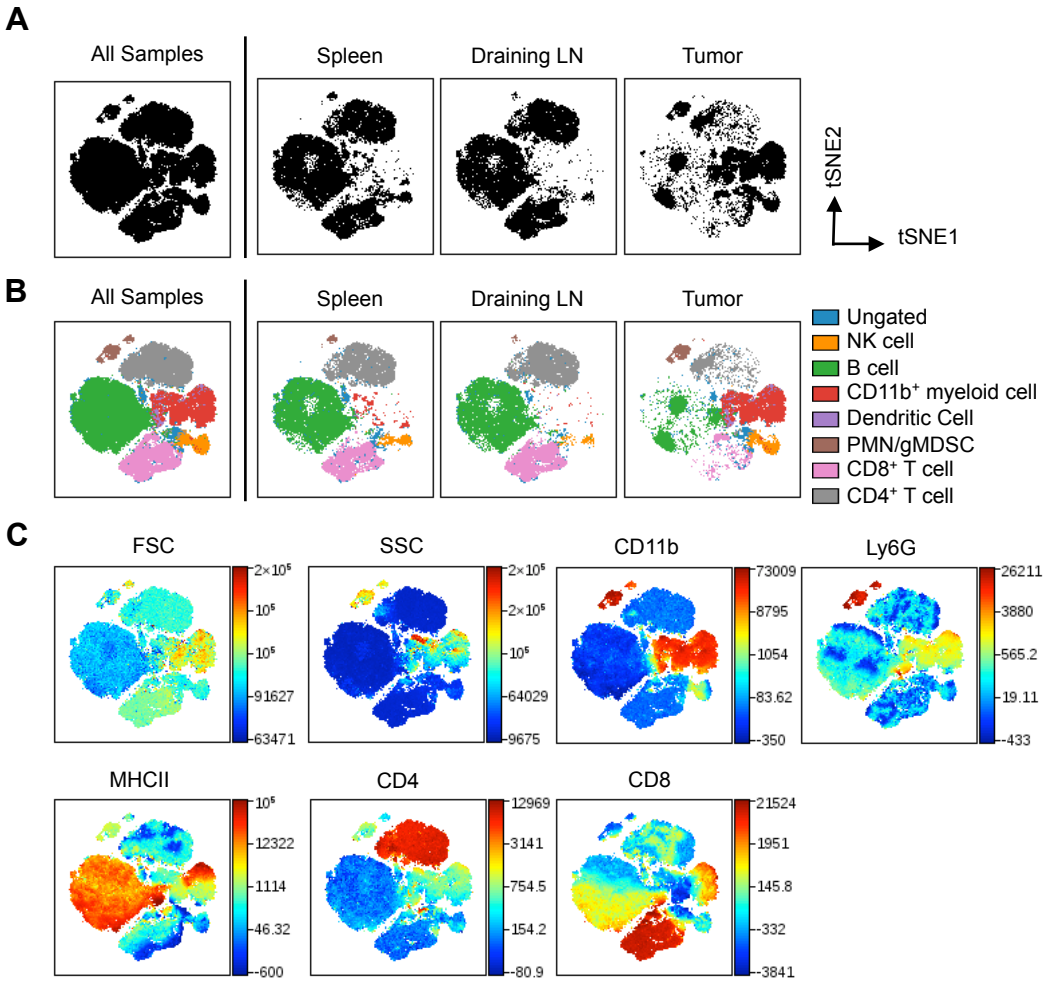
Supplemental Figure 1



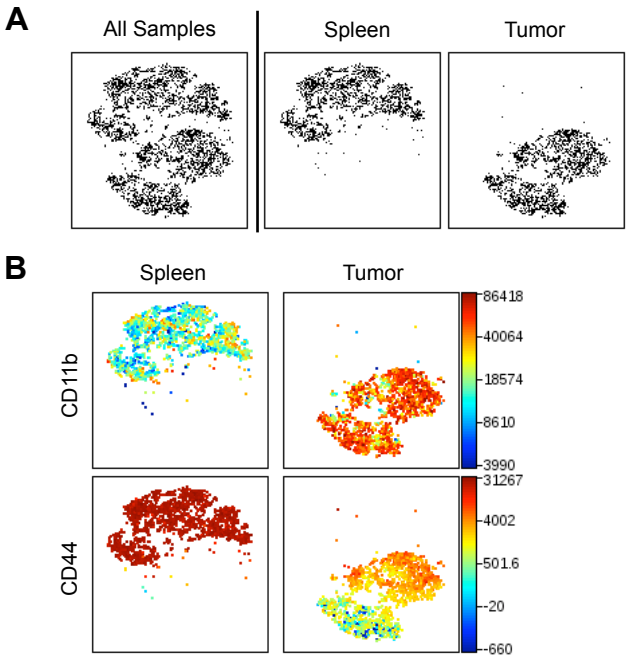
Supplemental Figure 1- Individual viSNE FCS files concatenanted into a single FCS file. viSNE analysis of immune cells in MB49 tumors, spleens, and draining lymph nodes were stained with 16 markers and measured by flow cytometry. viSNE analysis was run on 6,000 live CD45+ single cells per sample using all surface markers. viSNE maps show individual cells from the spleen, draining lymph node or tumor separated into spatially resolved populations. Using the FCS file concatenation tool, individual FCS files were combined into a single FCS file (blue borders). Green arrows indicate a tumor sample with cell population 3 as depicted in Figure 1B. Spleens and tumors = 54,000 events in the concatenated file, dLN = 42,000 events in the concatenated file.

Supplemental Figure 2



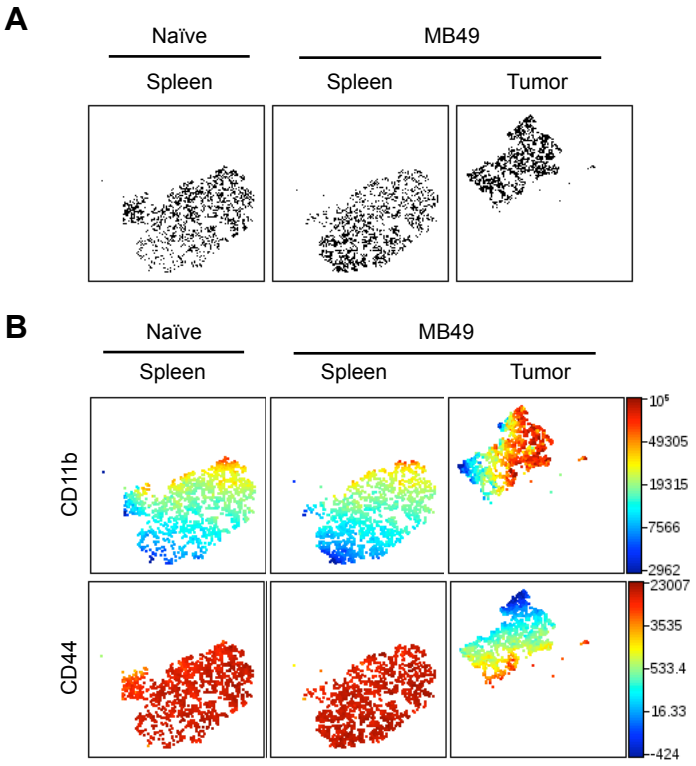
Supplemental Figure 2- viSNE defines immune cell populations in B16F10 melanoma. viSNE analysis of murine immune cells in B16F10 tumors, spleens, and draining lymph nodes. Cells were stained with 16 markers and measured by flow cytometry. viSNE analysis was run on 6,000 live CD45+ single cells per sample using all surface markers. (A) Concatenated FCS files for all samples, spleen, draining lymph node and tumor. (B) Overlay of manually gated cell populations onto concatenated viSNE plots. (C) Heat maps of markers in all samples overlaid on viSNE maps, N=4. All samples = 72,000 events, spleen, dLN and tumor = 24,000 events.

Supplemental Figure 3



Supplemental Figure 3- viSNE defines CD44+, CD44lo and CD44- granulocytes in B16F10 melanoma. viSNE analysis of murine immune cells in B16F10 tumors, spleens, and draining lymph nodes. Cells were stained with 16 markers and measured by flow cytometry. viSNE analysis was run on 500 live CD45+Ly6C+Ly6G+ single cells per sample using all surface markers. (A) Concatenated FCS files for all samples, spleen, and tumor. (B) Heat maps of markers in all samples overlaid on viSNE maps, N=4. All samples = 4,000 events, spleen and tumor = 2,000 events.

Supplemental Figure 4



Supplemental Figure 4- Ly6C+Ly6G+ granulocytes isolated from naïve splenocytes express CD44. viSNE analysis of murine immune cells in MB49 tumors and spleens from naïve and tumor bearing mice. Cells were stained with 6 markers (Table II) and measured by flow cytometry. viSNE analysis was run on 500 live CD45+Ly6C+Ly6G+ single cells per sample using all surface markers. (A) Concatenated FCS files from all samples, naïve splenocytes, and splenocytes and MB49 tumors from tumor bearing mice. (B) Heat maps of markers in all samples overlaid on viSNE maps. N=3. Concatenated files = 1,500 events.