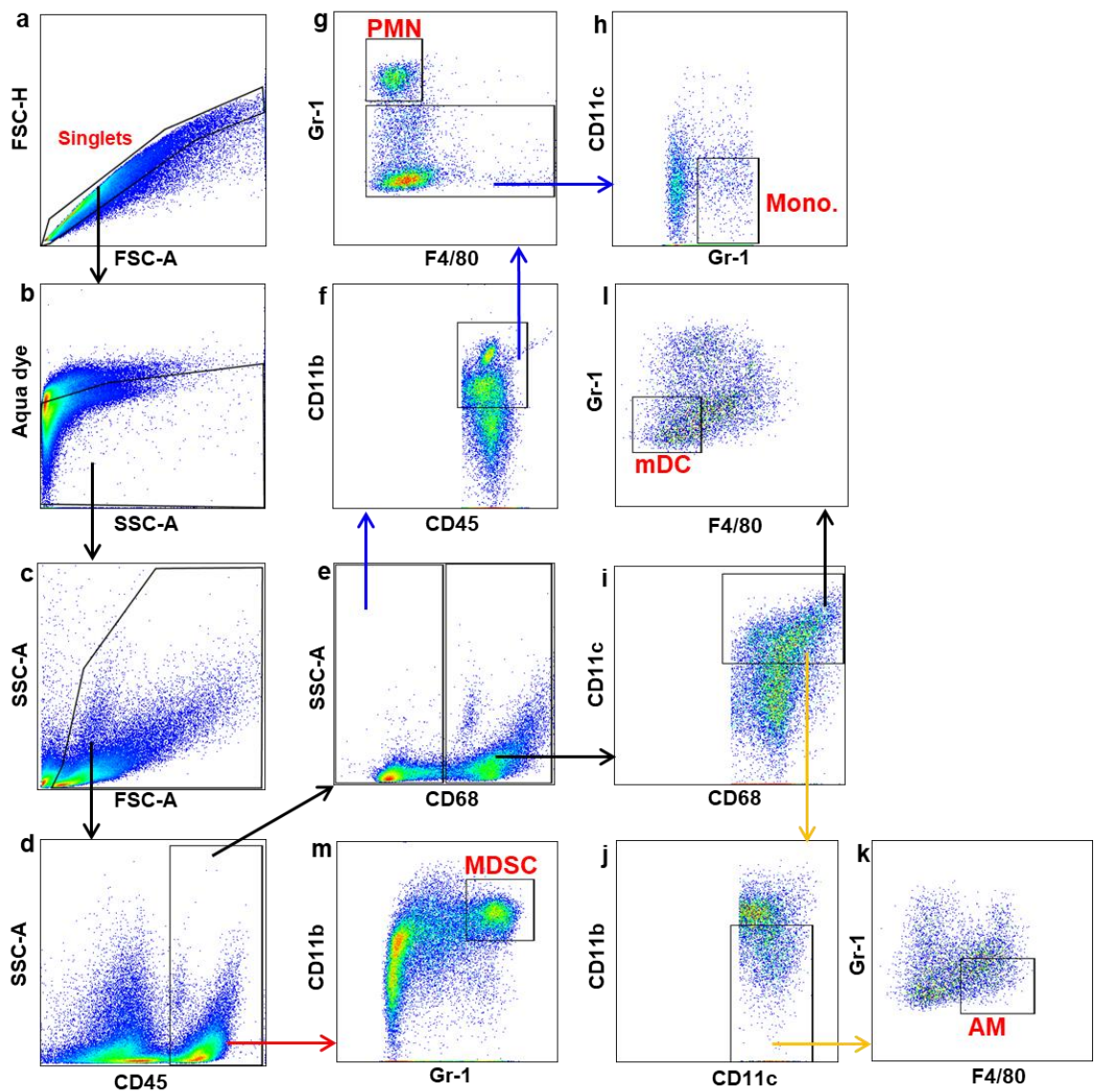


Supplementary Figure 1: Gating strategy used to identify major lymphocyte populations, NK, B, CD3⁺ T, CD4⁺ T, CD8⁺ T, and T_{reg} cells. Tumor-containing lung-derived single cells were stained with Aqua fluorescent reactive dye (dead cell staining) together with either fluorophore-labeled anti-CD45, CD3, CD4, CD8, CD19, and NK1.1 antibodies (Abs) or fluorophore-labeled anti-CD45, CD3, CD4, CD8, CD19, and Fcpx3 Abs to identify various cell subsets. (a) Doublets deviating from the diagonal in the plot of forward scatter area (FSC-A) versus forward scatter height (FSC-H) were excluded. (b) Following, dead cells were excluded by their staining with Aqua fluorescent reactive dye. (c) Live cells, which were higher up on the FSC-A and side scatter area (SSC-A) profile, were further selected. (d) CD45⁺ hematopoietic cells were then selected. (e) Subsequently, NK cells (NK1.1⁺SSC-A^{low}) and (f) B cells (CD19⁺SSC-A^{low}) were identified. (g) T cells (CD3⁺SSC^{low}) were identified and gated. (h) CD4⁺ T cells (CD3⁺CD4⁺CD8⁻) and CD8⁺ T cells (CD3⁺CD4⁻CD8⁺) were identified and (i) T_{reg} (CD3⁺CD4⁺CD8⁻Fcpx3⁺) were identified among CD4⁺ T cells. Isotype-matched controls for all Abs were used to determine positive populations. Identified populations are marked in red text.



Supplementary Figure 2: Gating strategy used to identify major pulmonary myeloid populations PMN, Monocyte (Mono), AM, mDC, and MDSC from tumor-containing mouse lung tissues. This strategy is based on the report by Rinat Zaynagetdinov group at Vanderbilt University (26). Lung-derived single cells were stained with fluorophore-labeled anti-CD45, CD68, CD11b, F4/80, CD11c, and Gr-1 Abs together with Aqua fluorescent reactive dye (dead cell staining). (a) Doublets deviating from the diagonal in the plot of FSC-A versus FSC-H were excluded. (b) Viable Aqua dye-negative cells were then selected, and (c) live cells with higher FSC/SSC profile were further selected. (d) CD45⁺ cells were selected, and (e) CD68^{hi} and CD68^{low} cells were separately gated. (f) CD68^{low}CD11b⁺ cells were selected. (g) PMN (Polymorphonuclear cell, CD68^{low}CD11b⁺F4/80⁺Gr-1^{hi}) was identified, and CD68^{low}CD11b⁺Gr-1^{low} cells were gated. (h) Monocyte (Mono, CD68^{low}CD11b⁺Gr-1^{low}CD11c⁻) were identified. (i) CD68^{hi}CD11c⁺ cells were gated, and (j) CD68^{hi}CD11c⁺CD11b⁻ cells were selected. (k) AM (Alveolar macrophage, CD68^{hi}CD11c⁺CD11b⁻F4/80⁺Gr-1⁻) were identified. (l) mDC (CD68^{hi}CD11c⁺F4/80⁺Gr-1⁻) were identified, and (m) broadly defined MDSC cells (CD11b⁺Gr-1⁺) were identified. Isotype-matched controls for all Abs were used to determine positive populations. Identified populations are marked in red text.