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Supplemental Information

Tumor-Promoting Ly-6G^{+} SiglecF^{high} Cells Are Mature and Long-Lived Neutrophils

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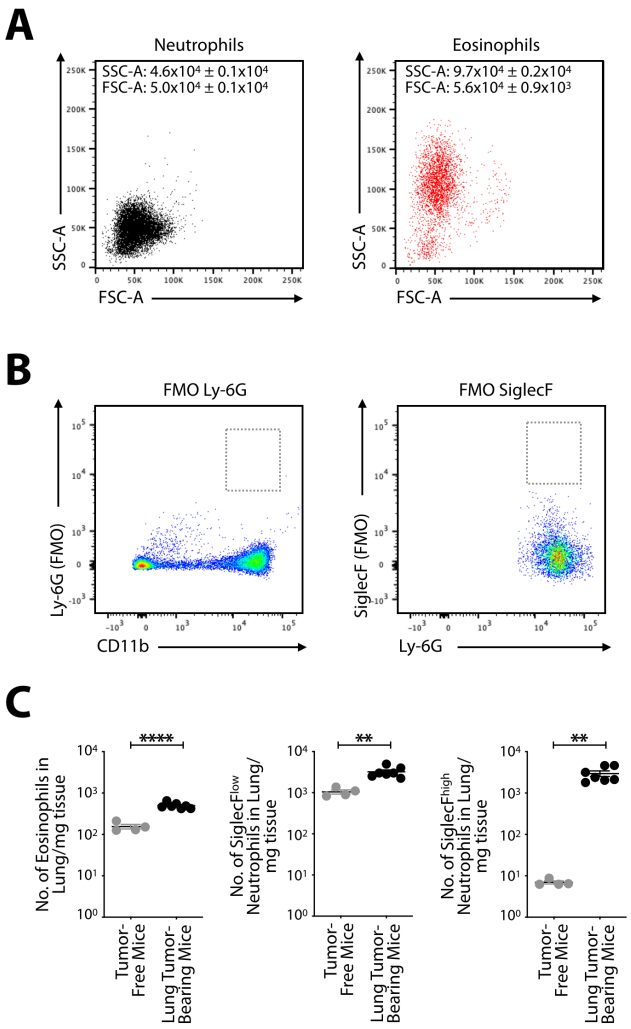

Figure S1. Related to Figure 1. Flow cytometry-based cell numbers and cellular properties as well as control staining for the analysis of neutrophils and eosinophils

(A) Representative flow cytometry dot plots showing forward (FSC) and side scatter (SSC) profiles of CD11b$^+$ Ly-6G$^+$ total neutrophils and CD11b$^+$ Ly-6G$^-$ SiglecF$^+$ eosinophils obtained from KP1.9 tumor-bearing lungs (d29 after intravenous tumor cell injection). Mean ± SEM values are presented of n = 4 mice.

(B) Representative flow cytometry dot plots of fluorescence minus one (FMO) controls for Ly-6G and SiglecF stainings. The highlighted quadrant shows the area were positive staining is expected.

(C) Eosinophil (left), SiglecF^{low} (middle) and SiglecF^{high} (right) neutrophil cell numbers per mg lung tissue of tumor-free or KP1.9 lung tumor-bearing mice (d29 after intravenous tumor cell injection) were investigated by flow cytometry (n = 4-7 mice/group). Cell types were gated based on live endogenous CD45.2$^+$ cells using the following cell marker combinations to identify SiglecF^{low} neutrophils (CD11b$^+$ Ly-6G$^+$ SiglecF^{low}), SiglecF^{high} neutrophils (CD11b$^+$ Ly-6G$^+$ SiglecF^{high}) and eosinophils (CD11b$^+$ Ly-6G$^-$ SiglecF^{high}).

Data are represented as mean ± SEM. For comparisons between two groups, Student's two-tailed t test was used. **p < 0.01, ****p < 0.0001.

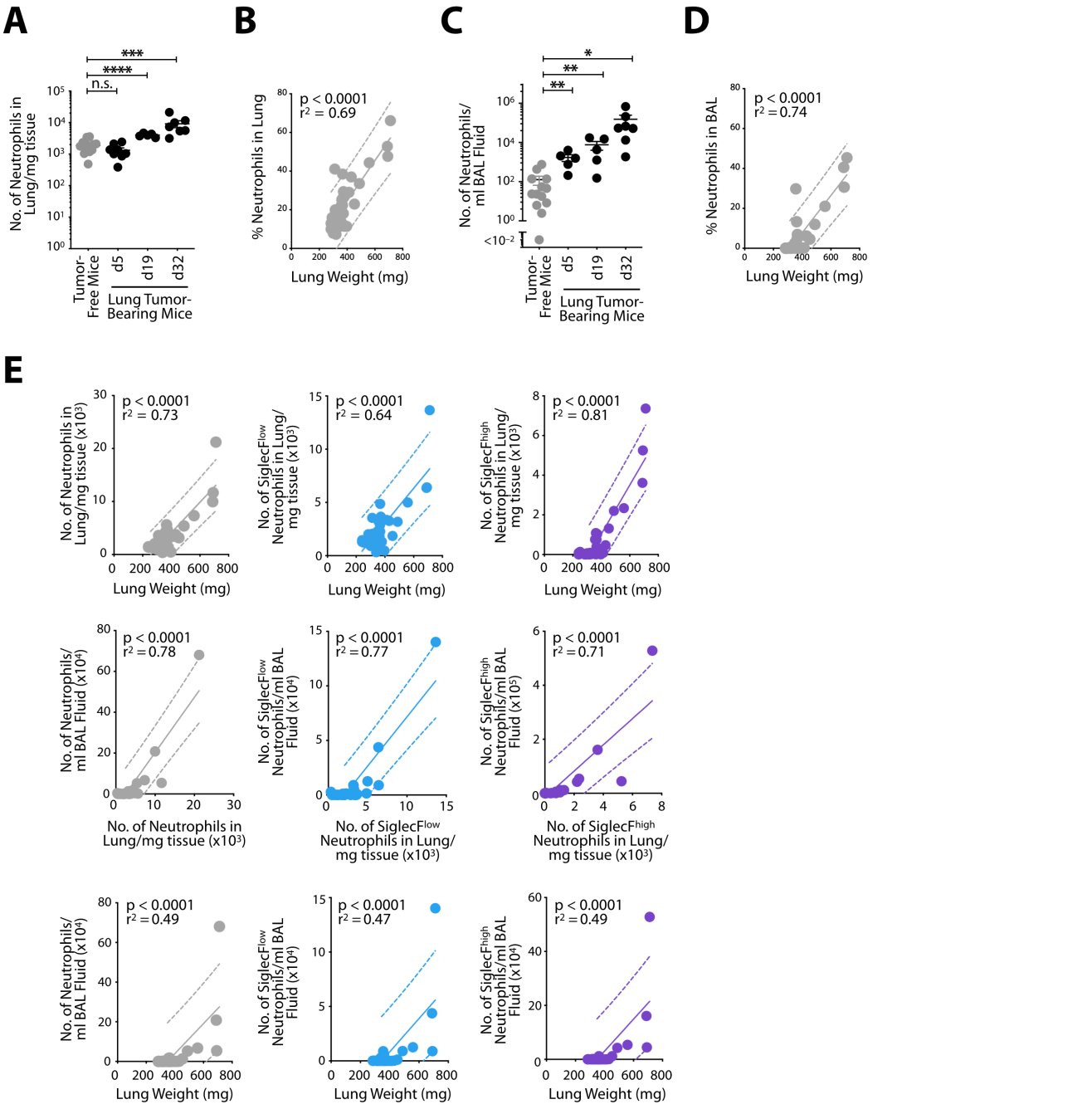

Figure S2. Related to Figure 2. Analysis of neutrophils in lung tissue and bronchoalveolar lavage fluid on day 5, 19 and 32 after intravenous KP1.9 tumor cell injection or in tumor-free mice

(A) Quantification of total lung CD11b⁺ Ly-6G⁺ neutrophils in tumor-free and tumor-bearing mice detected by flow cytometry (n = 5-14 mice/group).

(B) Total lung CD11b⁺ Ly-6G⁺ neutrophils (percent of live cells measured by flow cytometry) plotted against lung weight (proxy of tumor burden) of KP1.9 lung tumor-bearing or tumor-free mice (n = 34 mice) and linear regression was performed.

(C) Quantification of total CD11b⁺ Ly-6G⁺ neutrophils in bronchoalveolar lavage (BAL) fluid of tumor-free and tumor-bearing mice detected by flow cytometry (n = 5-13 mice/group).

(D) Flow cytometry-based detection of total CD11b^{+} Ly-6G^{+} neutrophils in BAL fluid plotted against lung weight of KP1.9 lung tumor-bearing or tumor-free mice (n = 30 mice) and linear regression was performed. Percent of live cells are shown.

(E) Linear regression analysis of neutrophil numbers (Left: total CD11b^{+} Ly-6G^{+} neutrophils; Middle: SiglecF^{low} neutrophils; Right: SiglecF^{high} neutrophils) in lung tissue or BAL fluid of KP1.9 lung tumor-bearing or tumor-free mice measured by flow cytometry. Top row: Lung neutrophils plotted against lung weight (n = 34 mice). Middle row: Neutrophils in BAL fluid plotted against neutrophils in lung tissue (n = 30 mice). Bottom row: Neutrophils in BAL fluid plotted against lung weight (n = 30 mice).

Data are represented as mean ± SEM. For comparisons between two groups, Student's two-tailed t test was used. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

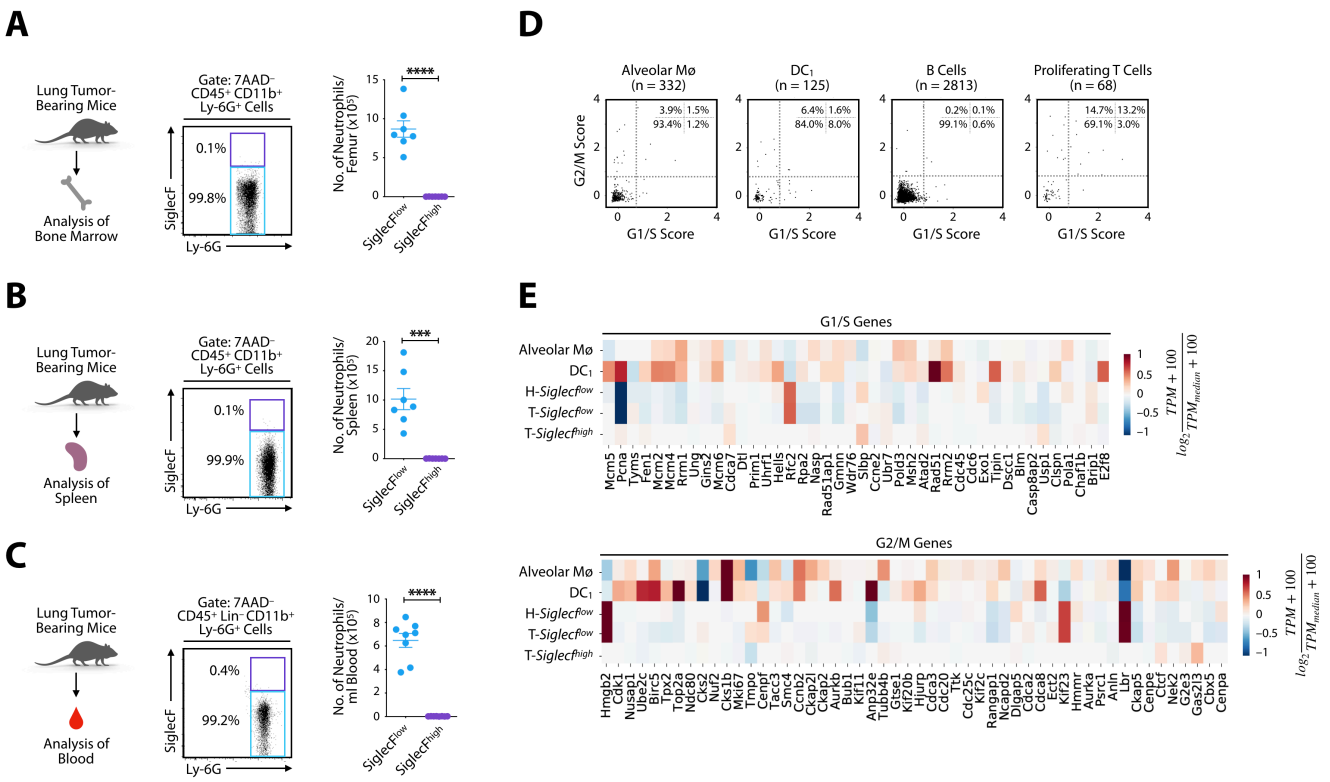

Figure S3. Related to Figure 3. Analysis of neutrophils in bone marrow, spleen and blood as well as expression of cell cycle related genes in immune cell populations

(A) Neutrophils (SiglecF^{high} and SiglecF^{low}) were analyzed in the bone marrow of KP1.9 lung tumor-bearing mice (d29 after intravenous tumor cell injection) by flow cytometry (n = 7 mice/group). A representative dot plot is shown (pre-gated on live CD45$^+$ CD11b$^+$ Ly-6G$^+$ cells).

(B) SiglecF^{high} and SiglecF^{low} neutrophil cell numbers per murine spleen of KP1.9 lung tumor-bearing mice (d29 after intravenous tumor cell injection) were investigated by flow cytometry (n = 7 mice/group). A representative dot plot is shown (pre-gated on live CD45$^+$ CD11b$^+$ Ly-6G$^+$ cells).

(C) Flow cytometry-based analysis of SiglecF^{high} and SiglecF^{low} neutrophils in blood of KP1.9 tumor-bearing mice (d20 after intravenous tumor cell injection; n = 8 mice/group). A representative dot plot is shown (pre-gated on live CD45$^+$ Lineage (Lin)$^-$ CD11b$^+$ Ly-6G$^+$ cells). The lineage master mix contained antibodies specific for CD90.2 and B220.

(D) Relative expression of G1/S or G2/M gene signatures from (Kowalczyk et al., 2015; Tirosh et al., 2016) in resident murine alveolar macrophages (Mø4), which are known to locally self-renew in the lung (Hashimoto et al., 2013), and murine DC$_1$ cells, B cells and proliferating T cells (T$_3$) from (Zilionis et al., 2019). Two biological replicates are presented. Expression values were obtained from (Zilionis et al., 2019). Dashed lines indicate a relative expression of 0.8 that was set by visual inspection at the boundary of the population of G1/S and G2/M negative cells.

(E) Expression of genes from the G1/S (top) and G2/M (bottom) gene signatures from (Kowalczyk et al., 2015; Tirosh et al., 2016) in neutrophil subsets derived from lungs of KP1.9 tumor-bearing (T-Siglecf^{low}, T-Siglecf^{high}) or tumor-free mice (H-Siglecf^{low}) as well as murine alveolar macrophages (Mø4) and murine DC$_1$ cells from (Zilionis et al., 2019). Two biological replicates are pooled for each cell type presented in the rows.

Data are represented as mean ± SEM. For comparisons between two groups, Student's two-tailed t test was used. ***p value < 0.001, ****p < 0.0001.