



Supplemental Figure S5. IL-18BP blockade alters the immune infiltrate composition of E0771 tumors. E0771 tumor cells were inoculated in C57Bl/6 mice and treated either with anti-IL-18BP Ab or isotype control (n=6 per group, 15mg/kg) twice a week for a total of 3 treatments. TME modulation was assessed by flow cytometry, scRNA-seq and cytokines profiling. Cytokine intracellular staining was done following *ex vivo* stimulation with phorbol myristate acetate and ionomycin. **(A)** Number of CD3⁺, CD8⁺ and CD4⁺ T cells per mg tumor were analyzed using flow cytometry. **(B)** Heatmap showing markers for different T/NK subpopulations. **(C)** Visualization of the average cell density within the anti-IL-18BP (bottom) and Isotype control (top) group, using

embedding density estimation on T/NK UMAP. Darker colors correspond to denser regions. **(D-F)** Numbers of functional CD8⁺ **(D)** and CD4⁺ **(E)** T cells and activated NK cells **(F)** were analyzed using flow cytometry. **(G)** Stacked bar plot for IL-18BP Ab treatment group showing the fraction of T cells that belong to an expanded clonotype separated for T cell subtypes. **(H)** Heatmap showing markers for different monocyte and macrophage subpopulations. **(I)** UMAP projection of DC subpopulations in E0771 tumors. **(J)** Heatmap showing markers for different DC subpopulations. **(K)** Enrichment of DC subpopulations frequencies in anti-IL-18BP Ab treatment compared to the control group. Depicted is the log₂ fold change of the mean frequency. The size of the dots indicates the average fraction of the cell population between treatments, while the color of the dots represents the P values of two-tailed t test. **(L)** CXCL9, MIP-1a and IL-1b levels in tumor derived supernatant were analyzed using CBA inflammation kit. Bar graphs show the mean \pm SEM; P<0.05*, P<0.01** by two-tailed t test or Mann-Whitney test.