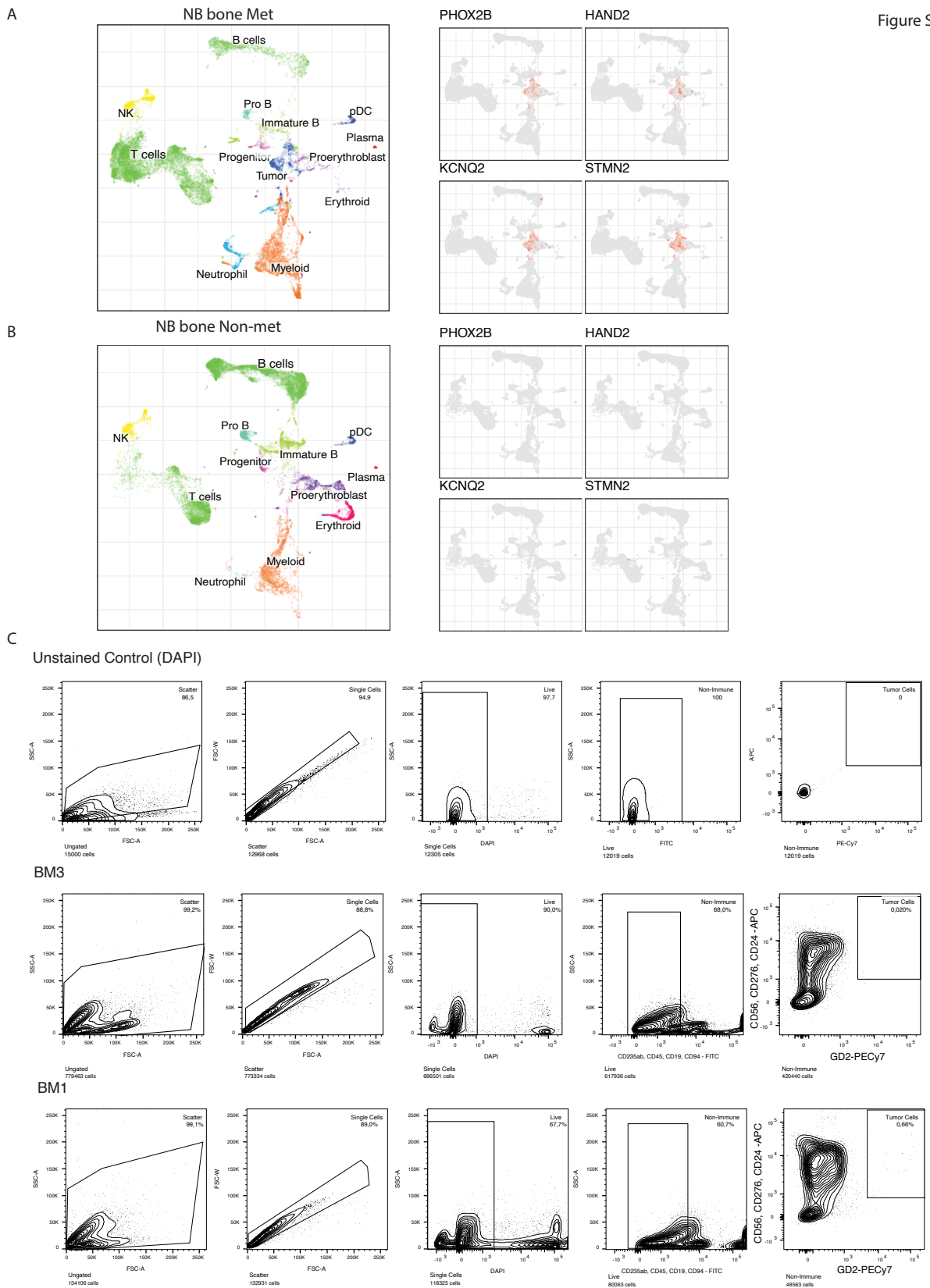




Supplementary Figure 1. Overview of tumor microenvironment of neuroblastoma bone metastatic and non-metastatic tumors

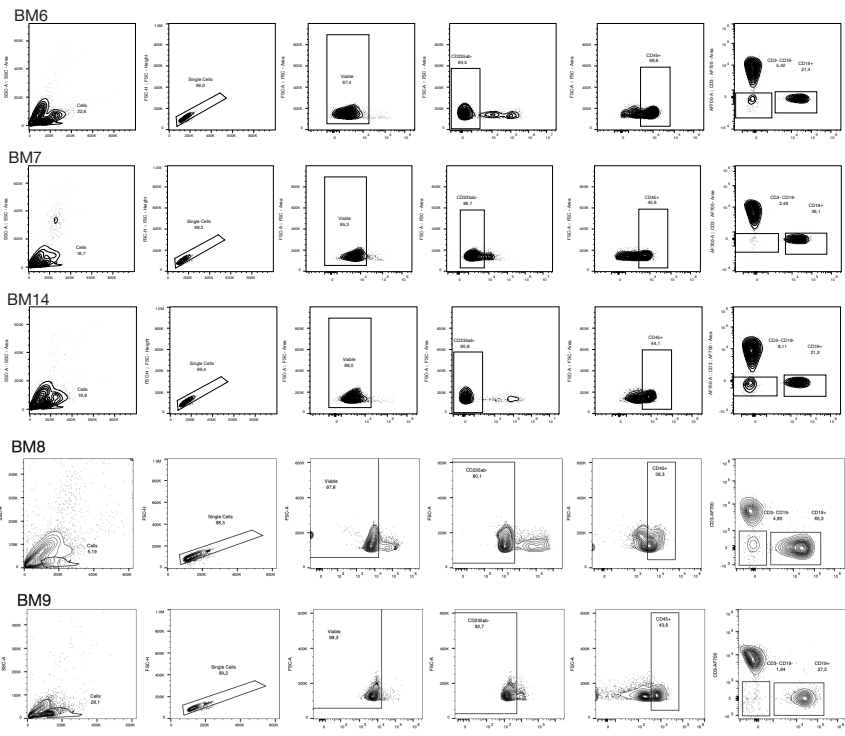
- A.** UMAP visualization of joint alignment of NB bone Met samples, color-coded by the cell subtypes and representative tumor marker genes.
- B.** UMAP visualization of joint alignment of NB bone Non-met samples, color-coded by the cell subtypes and representative tumor marker genes.
- C.** Gating strategy for enrichment of *CD2*⁺ tumor cells in NB bone met samples BM1 and BM3.

Figure S2

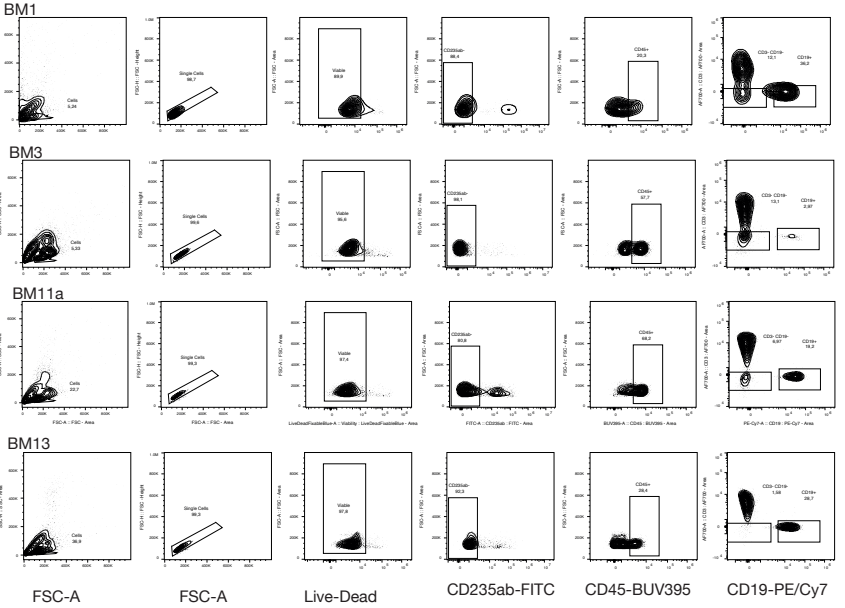
A

Gating strategy CD19+ B cells

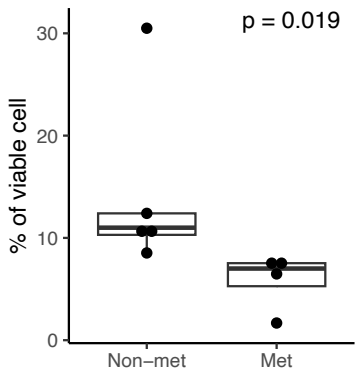
Non-metastatic



Metastatic



B

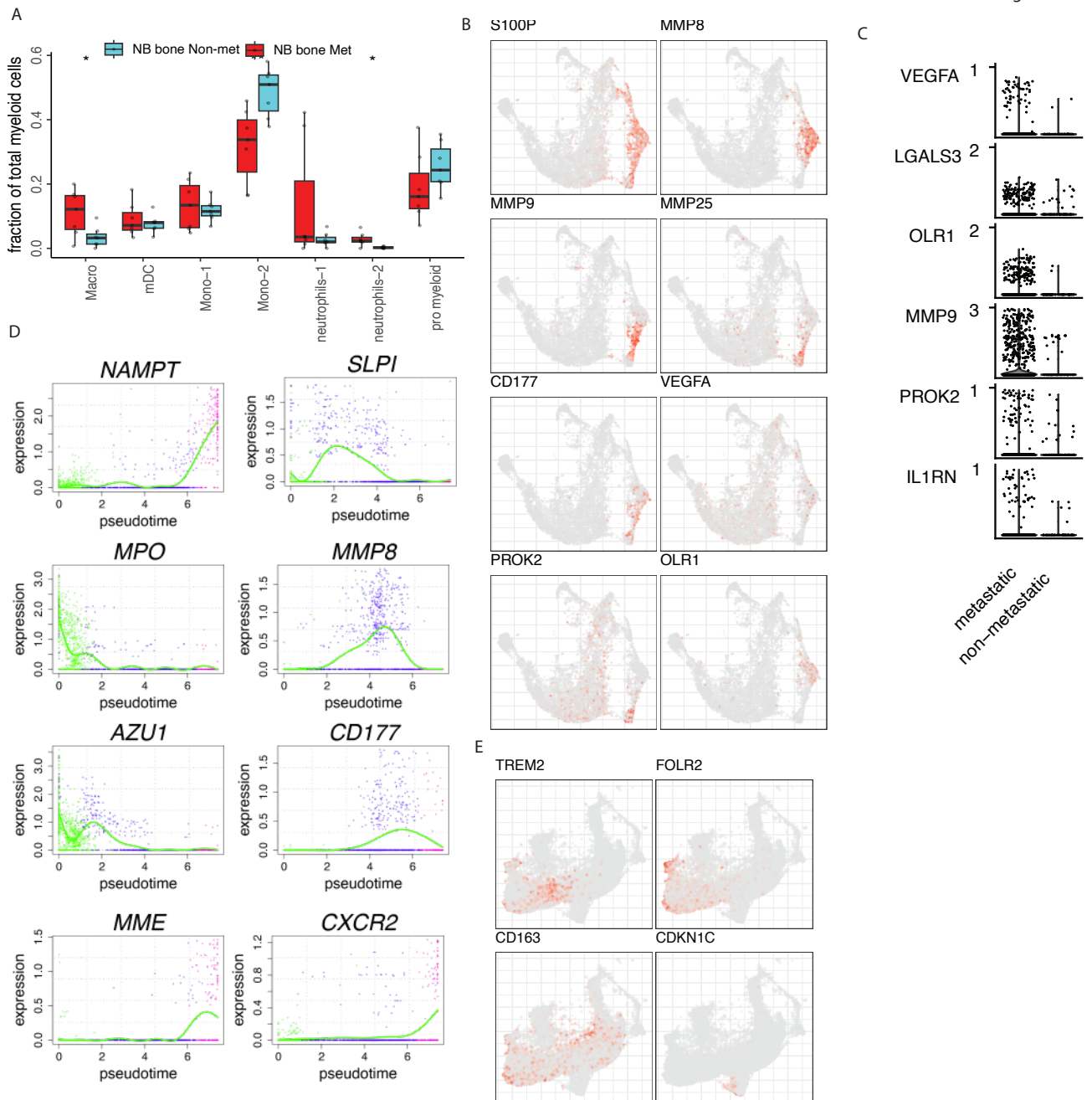


Supplementary Figure 2. Gating strategy for B cells

A. Gating strategy for enrichment of *CD19*⁺ B cells. Labels above the flow plots refer to the parent population in the percentages are of the parent gate.

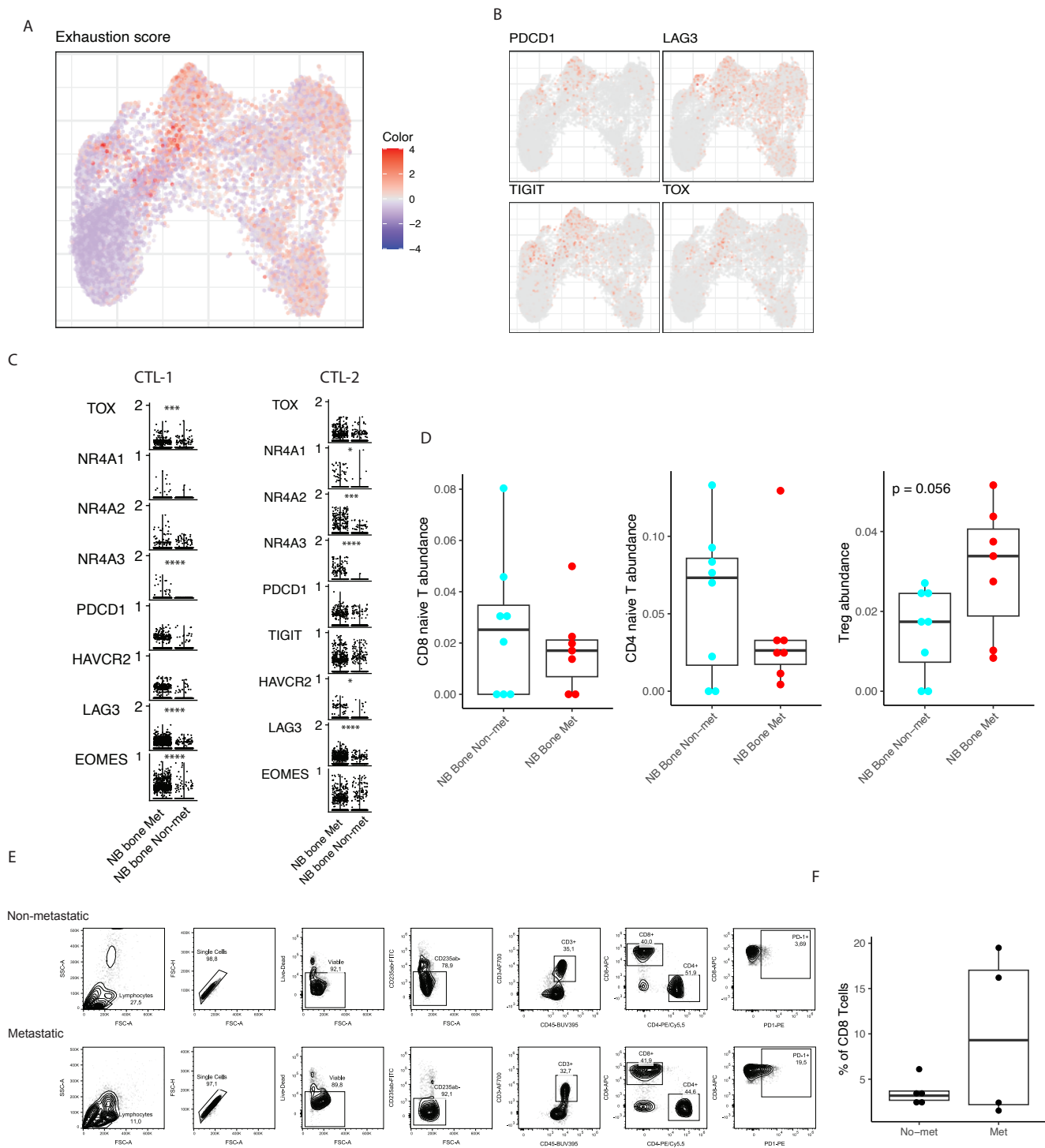
B. Boxplot showing the percent of *CD19*⁺ B cells in NB bone Met and NB bone Non-met samples (n=3). Statistical significance determined using two-sided t-test. Boxplots include center line, median; box limits, upper and lower quartiles; whiskers are highest and lowest values no greater than $1.5 \times$ IQR.

Figure S3



Supplementary Figure 3. Myeloid cells characterization, enrichment and differentiation

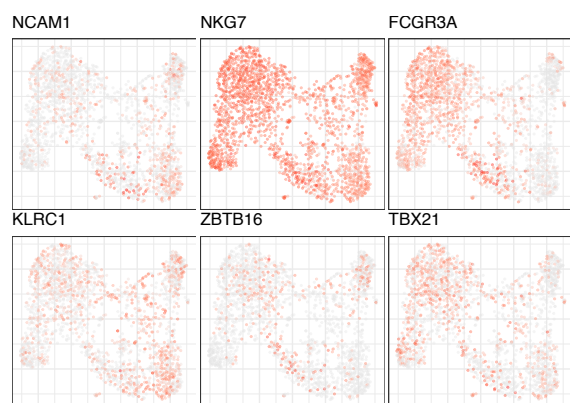
- A.** Comparison of relative cell abundance of myeloid cell subpopulations between NB bone Met (n=7) and NB bone Non-met (n=6) samples. Statistics are accessed with Wilcoxon rank sum test (*p<0.05, ***p<0.001). Boxplots include center line, median; box limits, upper and lower quartiles; whiskers are highest and lowest values no greater than 1.5× IQR.
- B.** Expression of selected neutrophil marker genes, visualized in myeloid UMAP embedding.
- C.** Violin plot showing representative marker gene expression in neutrophils.
- D.** Similar to Figure 2F, showing the representative gene expression along with pseudo-time moving from progenitor myeloid cells to neutrophils-1 and neutrophils-2.
- E.** UMAP embedding showing selected gene expression in tissue resident macrophage and bone marrow derived macrophage.



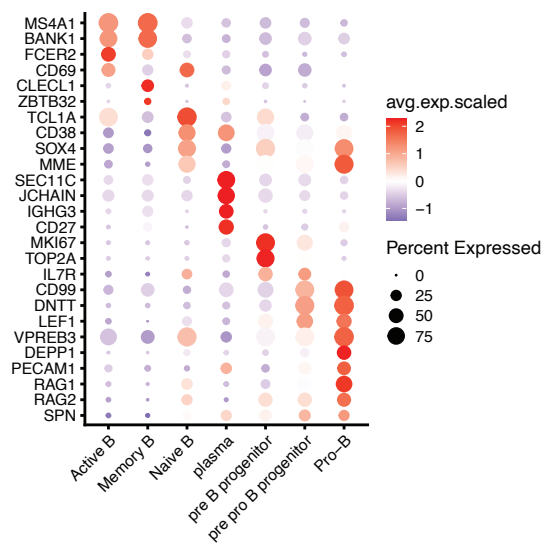
Supplementary Figure 4. Increased abundance of tumor-infiltrating CTLs and Tregs in NB bone metastasis tumor

- A.** UMAP visualization of average gene expression of T cell exhaustion signatures.
- B.** UMAP visualization showing *PDCD1*, *LAG3*, *TIGIT* and *TOX* expression.
- C.** Violin plot showing representative T cell exhaustion signature genes expression in CTL-1 and CTL-2, stratifying cells by non-metastatic and metastatic samples.
- D.** Comparison of CD4 naïve T, CD8 naïve T and Tregs proportion in NB bone Met (n=7) and NB bone Non-met (n=7) samples. Statistics are accessed with Wilcoxon rank sum test(*p<0.05). Data are expressed as mean $\pm$ SEM.
- E.** Gating strategy for PDCD1+ CD8+ T cells. Labels above the flow plots refer to the parent population in the percentages are of the parent gate.
- F.** Boxplot showing the percent of PDCD1+ CD8+ T cells in NB bone Met and NB bone Non-met samples (n=3).

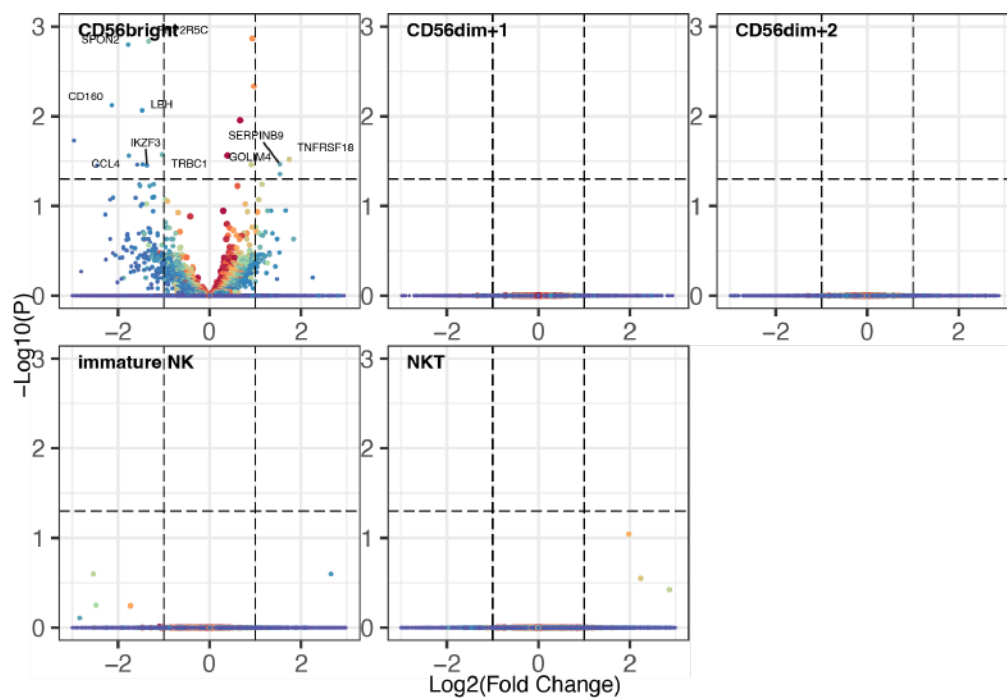
A



C



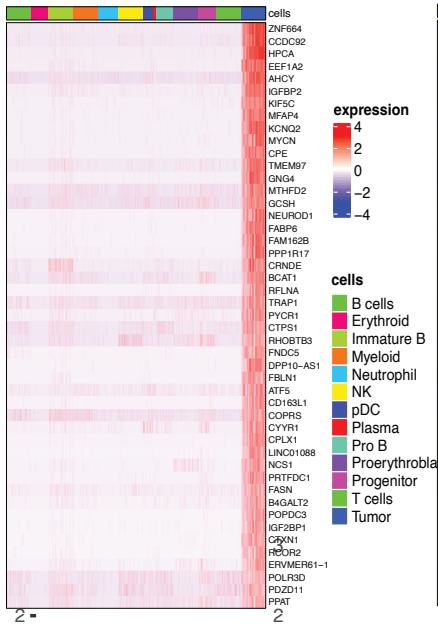
B



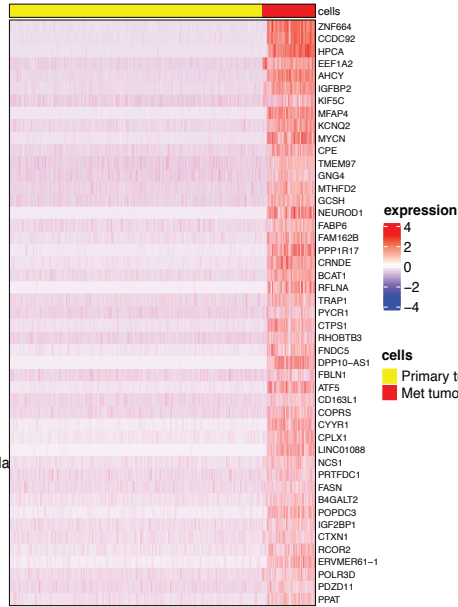
Supplementary Figure 5. Characterization of B and NK subpopulations

- A.** Representative NK cell marker gene expression on joint UMAP embedding.
- B.** Volcano plot of differential expressed genes for each NK cell subpopulations comparing metastatic samples with non-metastatic samples.
- C.** Dot plot demonstrating marker gene expression across different B cell subpopulations. The color represents scaled average expression of marker genes in each cell type, and the size indicates the proportion of cells expressing marker genes.

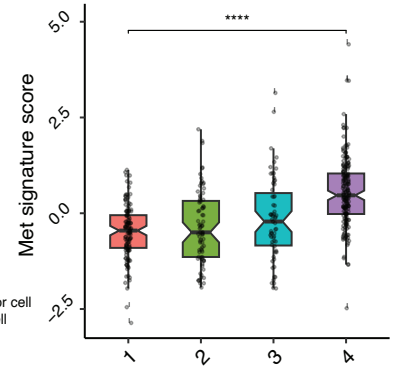
A



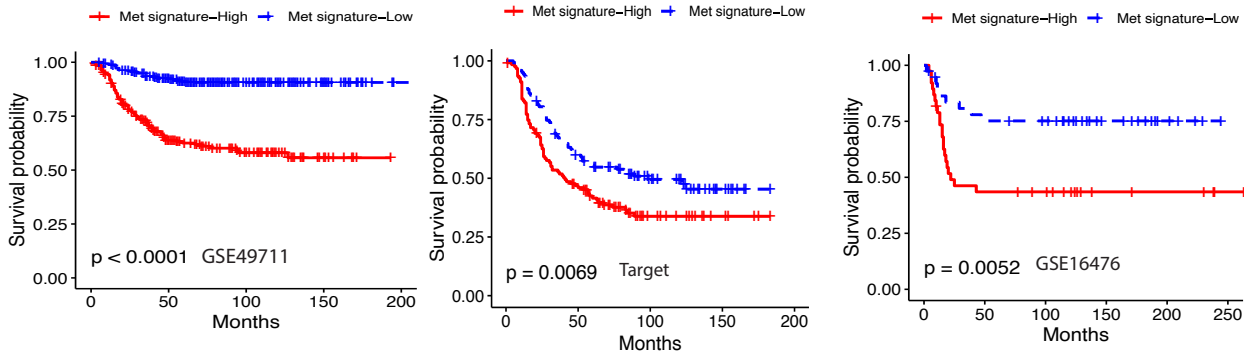
B



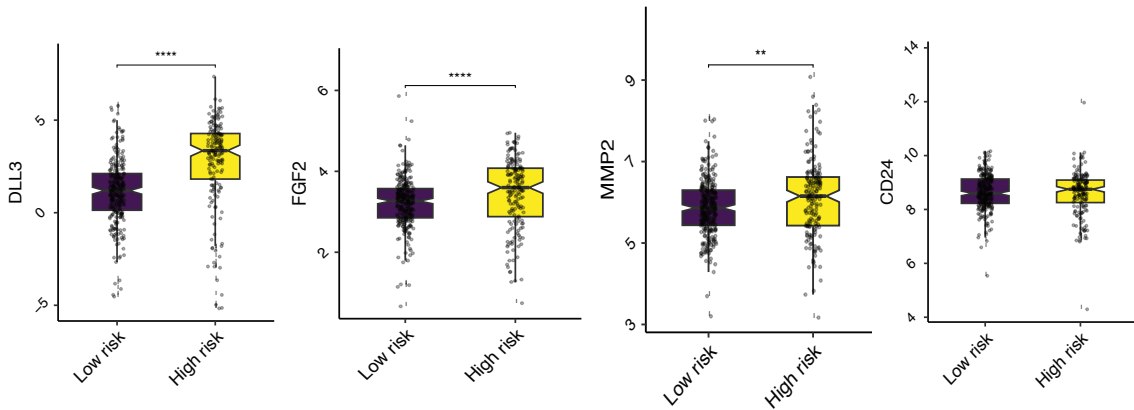
C



D



E



Supplementary Figure 6: Metastatic signature predicts neuroblastoma patient survival

A. Heatmap showing metastatic signature gene expression in major cell types in NB bone metastatic scRNAseq data.

B. Similar to Figure 5C, showing metastatic signature gene expression in NB bone metastatic and NB primary tumor cells.

C. Boxplot representing metastatic signature score in bulk RNA-seq of NB patients (GSE49711), stratifying patients by INSS stage (stage1=121, stage2=78, stage1=63, stage1=183). Significance was assessed using Wilcoxon rank sum test and BH multiple testing correction (** $p < 0.001$).

D. Kaplan –Meier survival curves showing NB patients with higher metastatic signature gene expression have worse overall survival in three independent NB datasets (GSE49711 $n=488$, Target $n=247$, GSE16476 $n=76$). Patients were stratified into two groups based on the average expression (binary: top 25% versus bottom 25%) of metastatic signatures as annotated by key marker genes in panel. Statistics are assessed by two-side log-rank test.

E. Boxplot representing *DLL3*, *FGF2*, *MMP2* and *CD24* expression in low-risk ($n=273$) and high-risk ($n=172$) NB patients (GSE49711). Significance was assessed using two-sided Wilcoxon rank sum test (** $p < 0.01$, *** $p < 0.001$). Boxplots include center line, median; box limits, upper and lower quartiles; whiskers are highest and lowest values no greater than $1.5 \times$ IQR.