Supplemental Figures for, "Impact of the canine osteosarcoma tumor microenvironment on immune cell composition and gene expression profiles"

Running title: Immune modulation by the osteosarcoma TME

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Supplemental table file descriptions:

All tables are presented in one .xslx file with each table on a separate sheet.

Supplemental Table 1: List of genes included in the 46 gene tumor tissue signature.

Supplemental Table 2: List of genes included in the 108 gene platelet signature.

Supplemental Table 3-9: Summary statistics for differential abundance within the full dataset and each major cell type.

Column descriptions:
- Cell.Type: cell type being tested statistically
- MEAN_Blood/TILs: mean cell type percentage of cells derived from blood/TILs
- MEDIAN_Blood/TILs: median cell type percentage of cells derived from blood/TILs
- SD_Blood/TILs: standard deviation of cell type percentage for cells derived from blood/TILs
- MIN_Blood/TILs: minimum cell type percentage of cells derived from blood/TILs
- MAX_Blood/TILs: maximum cell type percentage of cells derived from blood /TILs
- p: unadjusted P value
- p.adj: Holm–Bonferroni corrected P value
- p.signif: significance stars based on “p.adj”
- method: statistical test used
- lfc: log2FoldChange calculated using the mean values
- lab_col: classification of the statistical results, see methods for description
- contrast: direction of contrast used to calculate log2FoldChange (TILs versus Blood)

Supplemental data file descriptions:

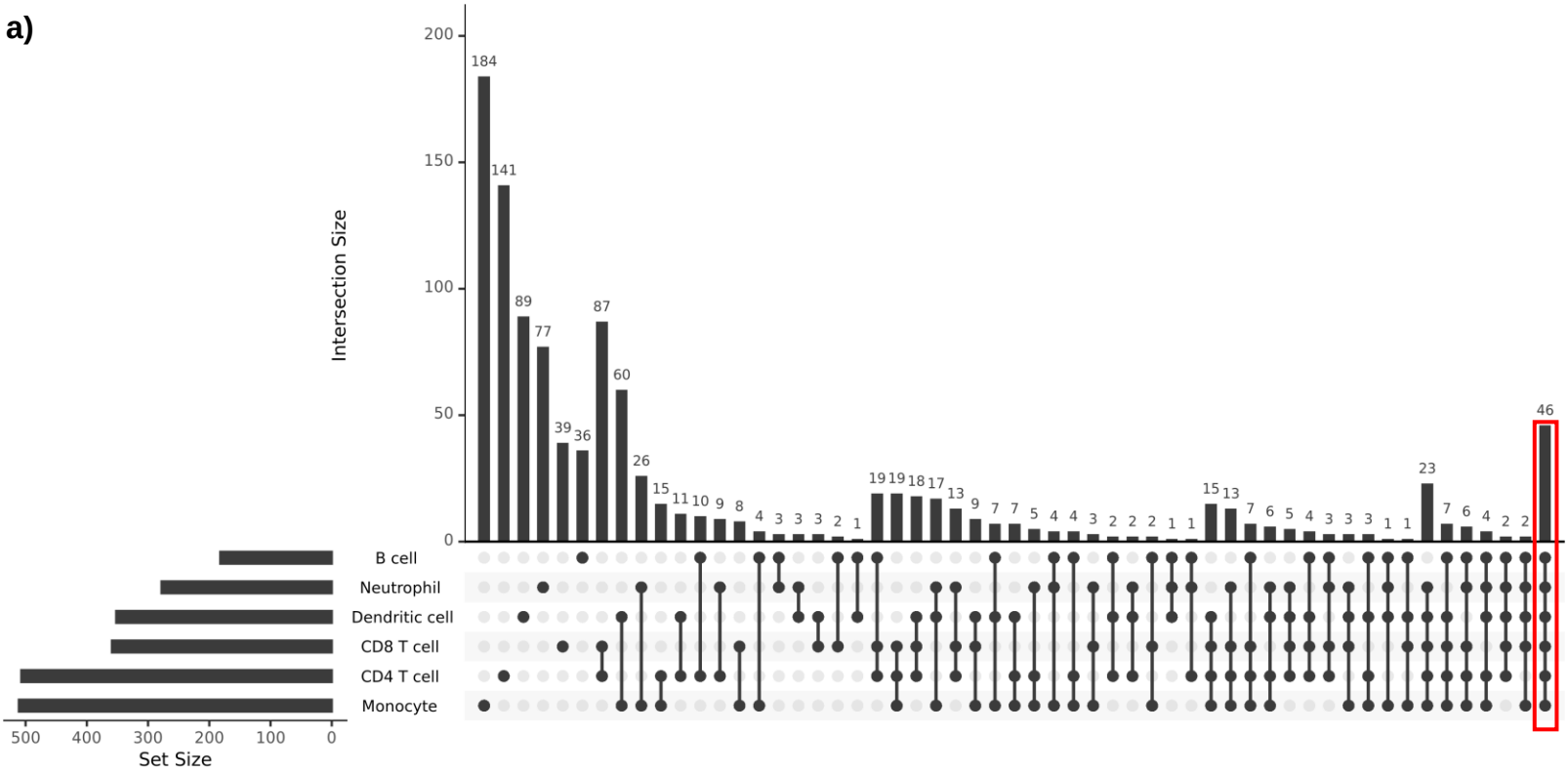
Supplemental Data 1, 4-8: Results of FindAllMarkers() for every cluster identified in each major cell type subset.

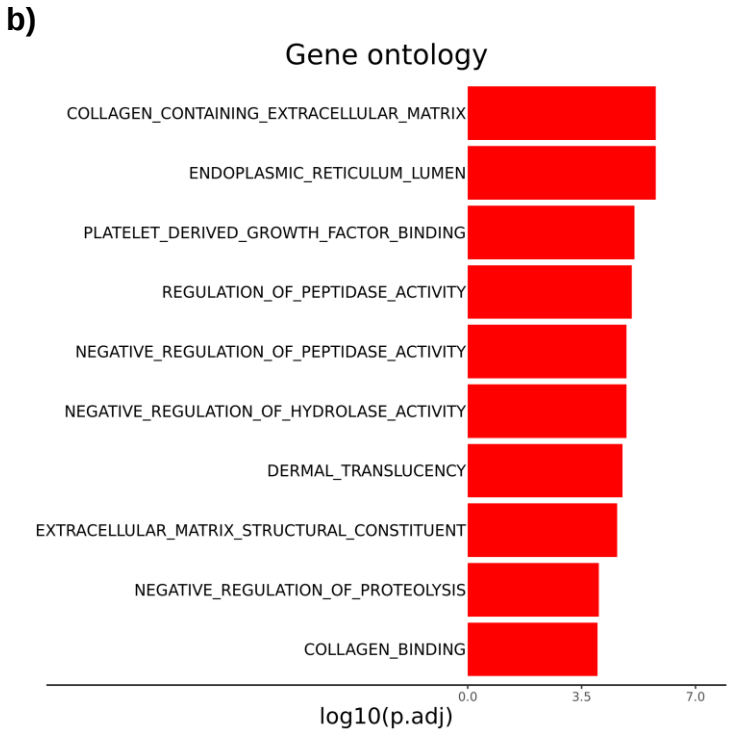
Supplemental Data 2: Results of differential gene expression analysis using DESeq2 within each major cell type subset. The first 7 columns are standard DESeq2 output values.

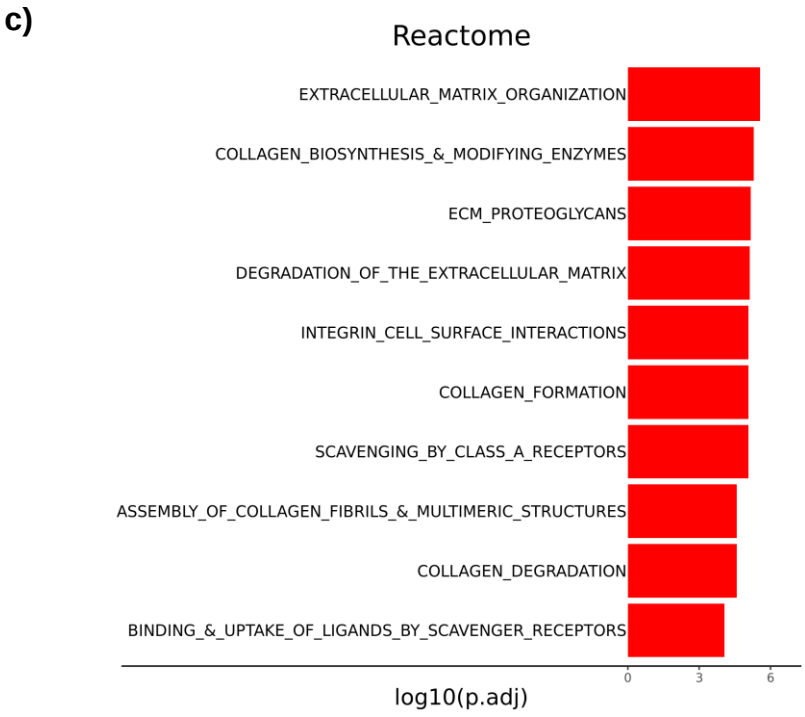
Additional columns include:
- gs_base: direction of contrast used to calculate log2FoldChange (TILs versus Blood)
- subset: major cell type subset that the DE analysis was completed in

Supplemental Data 3: Results of gene set enrichment analysis using clusterProfiler of significantly differentially expressed gene identified within each major cell type subset. The first 9 columns are standard clusterProfiler output values.

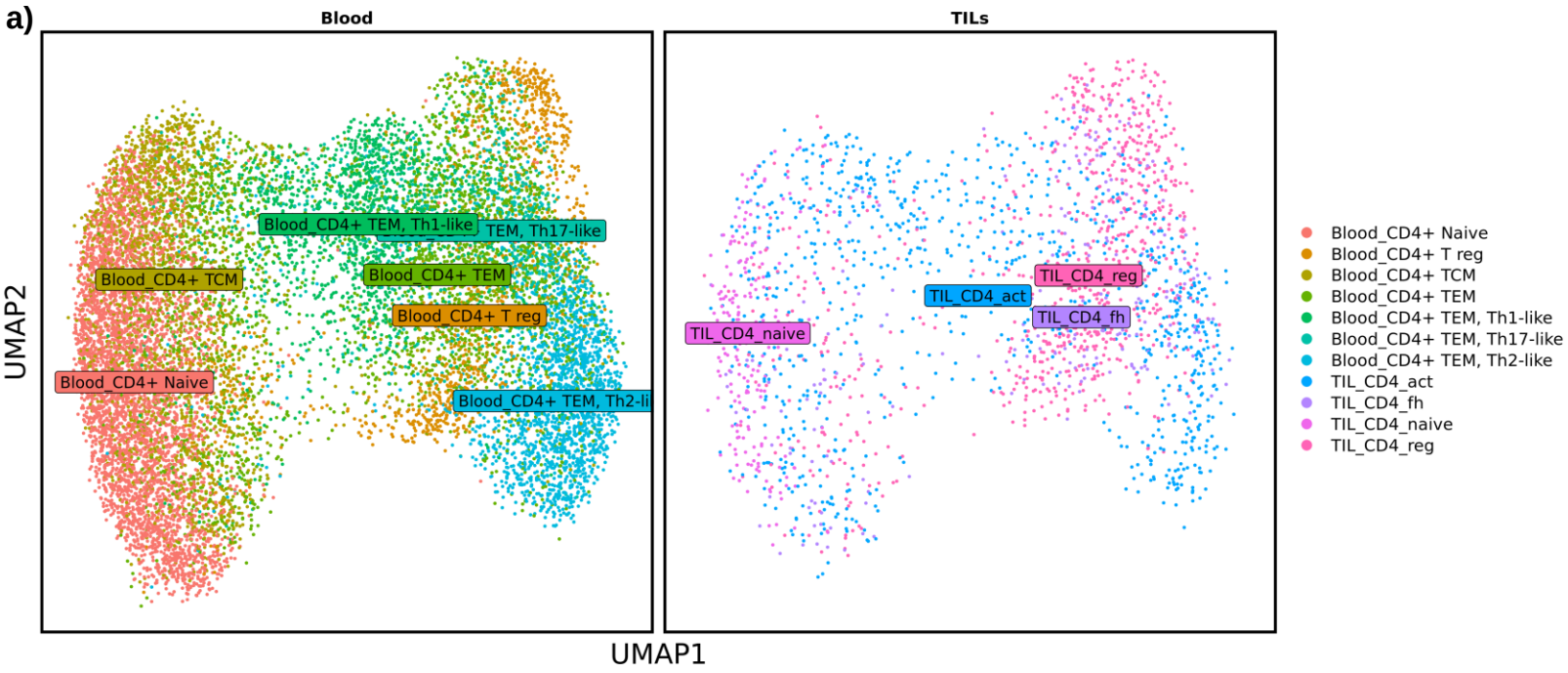
Additional columns include:
- x_axis: signed log10 adjusted P value (p.adjust column)
- direction: indicator of which condition the term was enriched in (“UP” = enriched in TILs)
- msigdb_reference: GSEA term database used for analysis (C2 = Reactome, C5 = Gene ontology)
- subset: major cell type subset that the GSEA was completed in

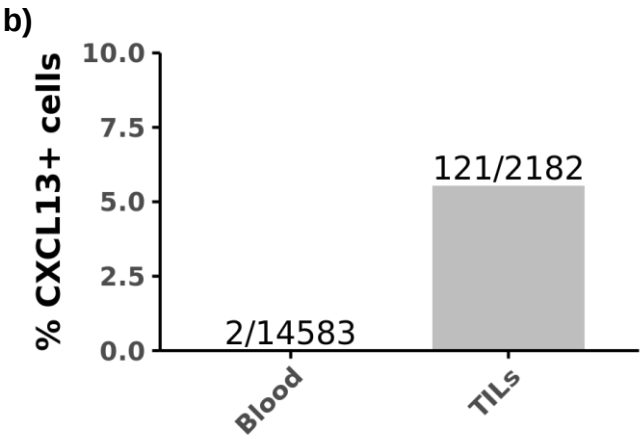




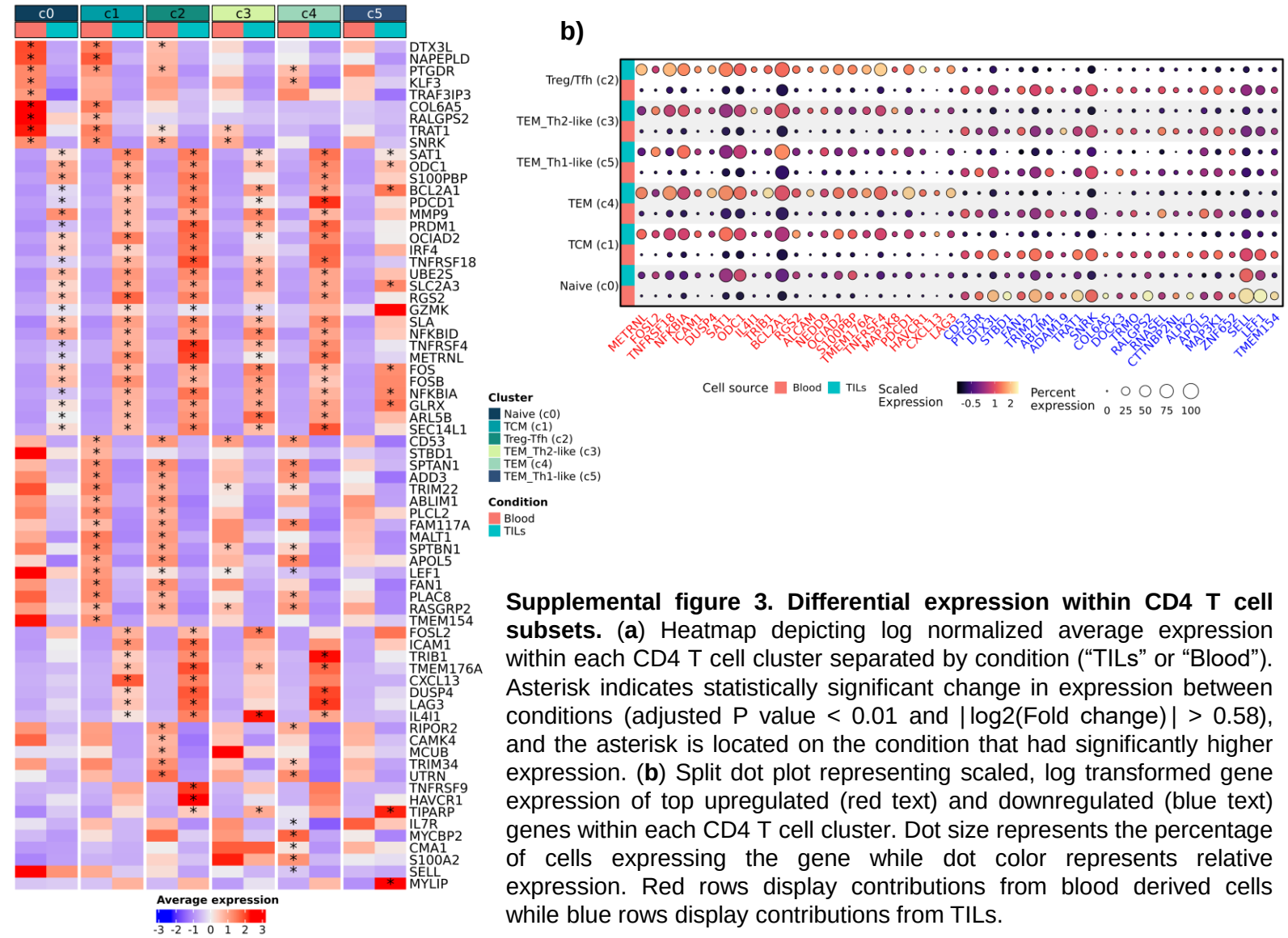


Supplemental figure 1. Differential gene expression analysis reveals a tumor dissociation associated gene signature. (**a**) Upset plot depicting the intersection of differential gene expression analysis in the unfiltered analysis used to establish the 38-feature tumor tissue signature (boxed in red). Bar charts depicting P value from gene set enrichment analysis using genes in the tumor dissociation signature against gene ontology (**b**) and Reactome (**c**) gene sets.

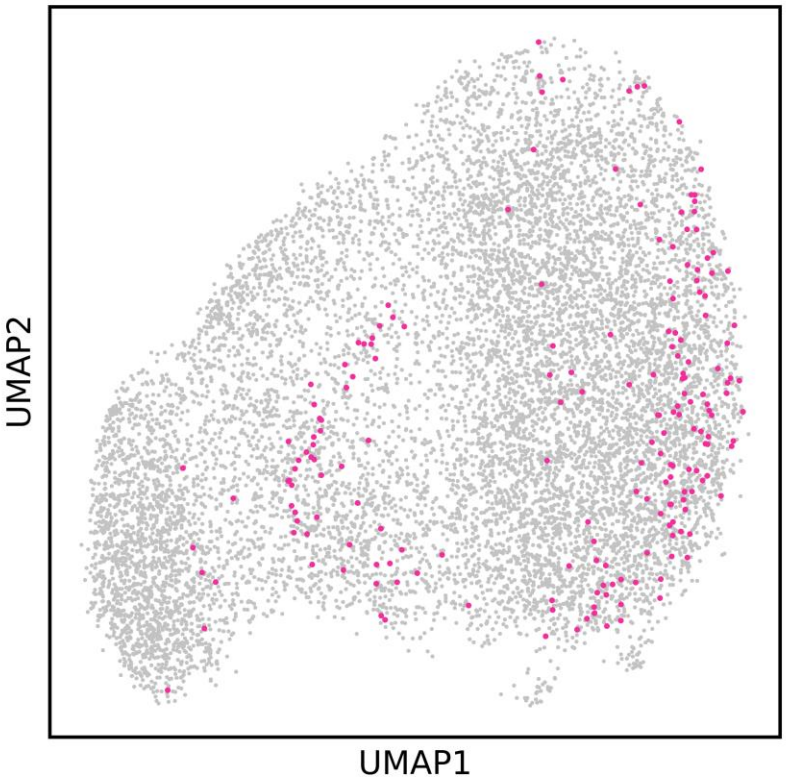




Supplemental figure 2. Additional CD4 T cell data. (**a**) Original annotation as defined in the blood (left) and the tumor (right). (**b**) Bar chart depicting the percentage of CD4 T cells with normalized CXCL13 expression greater than 0. Fraction above bar indicated number of positive cells out of the total cells sampled.

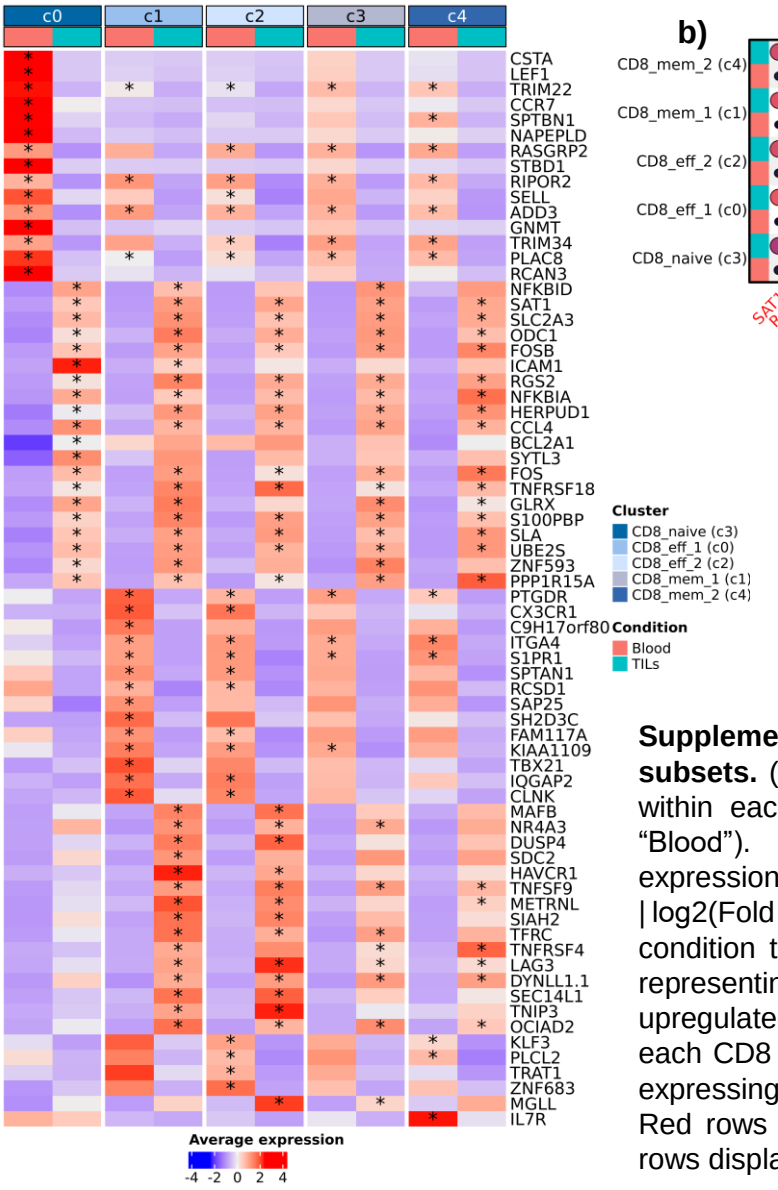


Supplemental figure 3. Differential expression within CD4 T cell subsets. (**a**) Heatmap depicting log normalized average expression within each CD4 T cell cluster separated by condition ("TILs" or "Blood"). Asterisk indicates statistically significant change in expression between conditions (adjusted P value < 0.01 and |log2(Fold change)| > 0.58), and the asterisk is located on the condition that had significantly higher expression. (**b**) Split dot plot representing scaled, log transformed gene expression of top upregulated (red text) and downregulated (blue text) genes within each CD4 T cell cluster. Dot size represents the percentage of cells expressing the gene while dot color represents relative expression. Red rows display contributions from blood derived cells while blue rows display contributions from TILs.

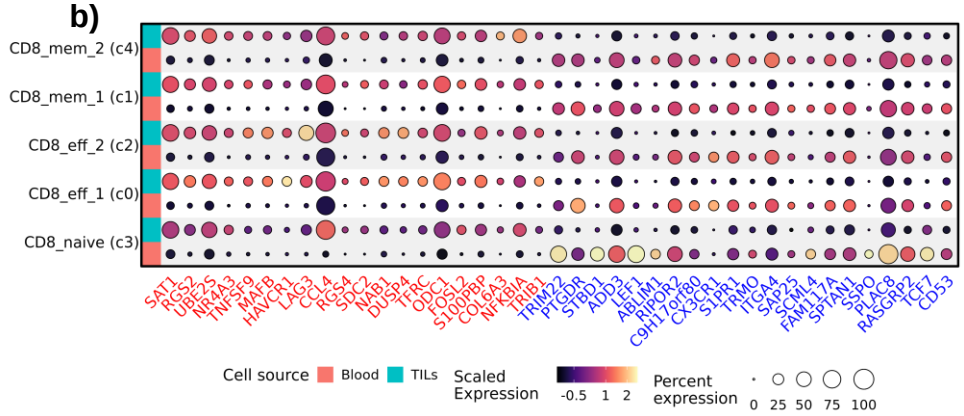


Supplemental figure 4. UMAP highlighting the location of NK cells as annotated in original datasets. Pink dots indicate cells that were originally annotated as NK cells.

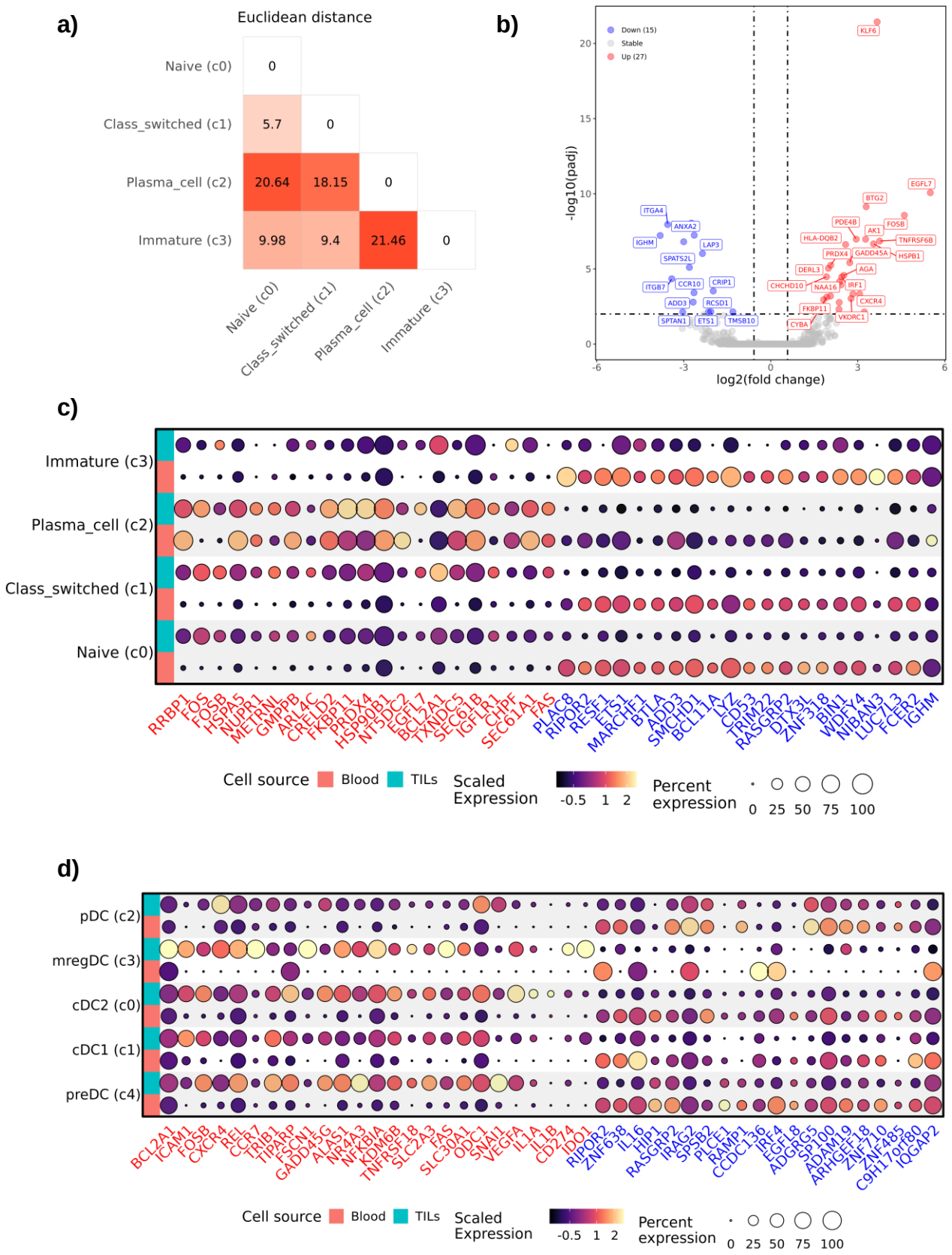
a)



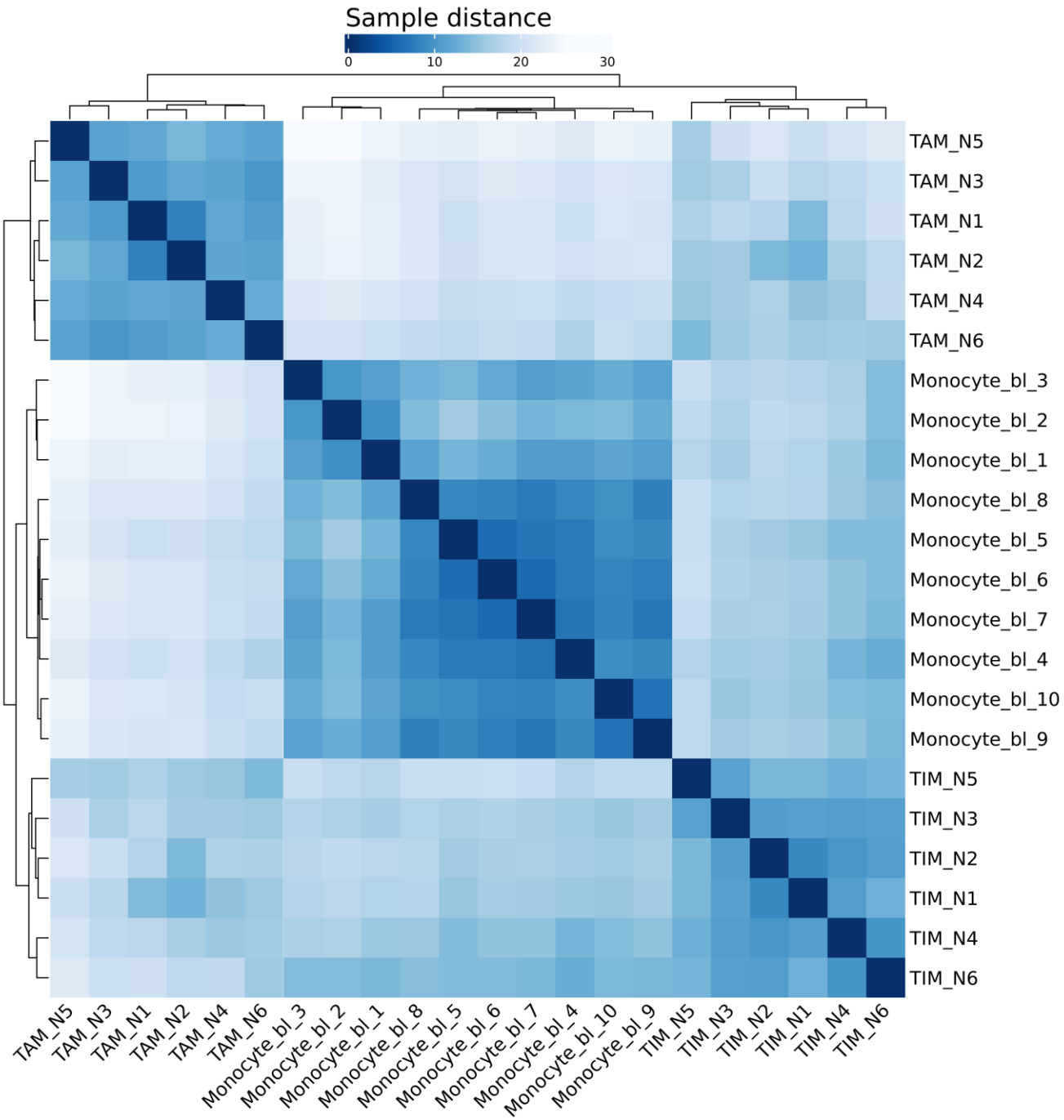
b)



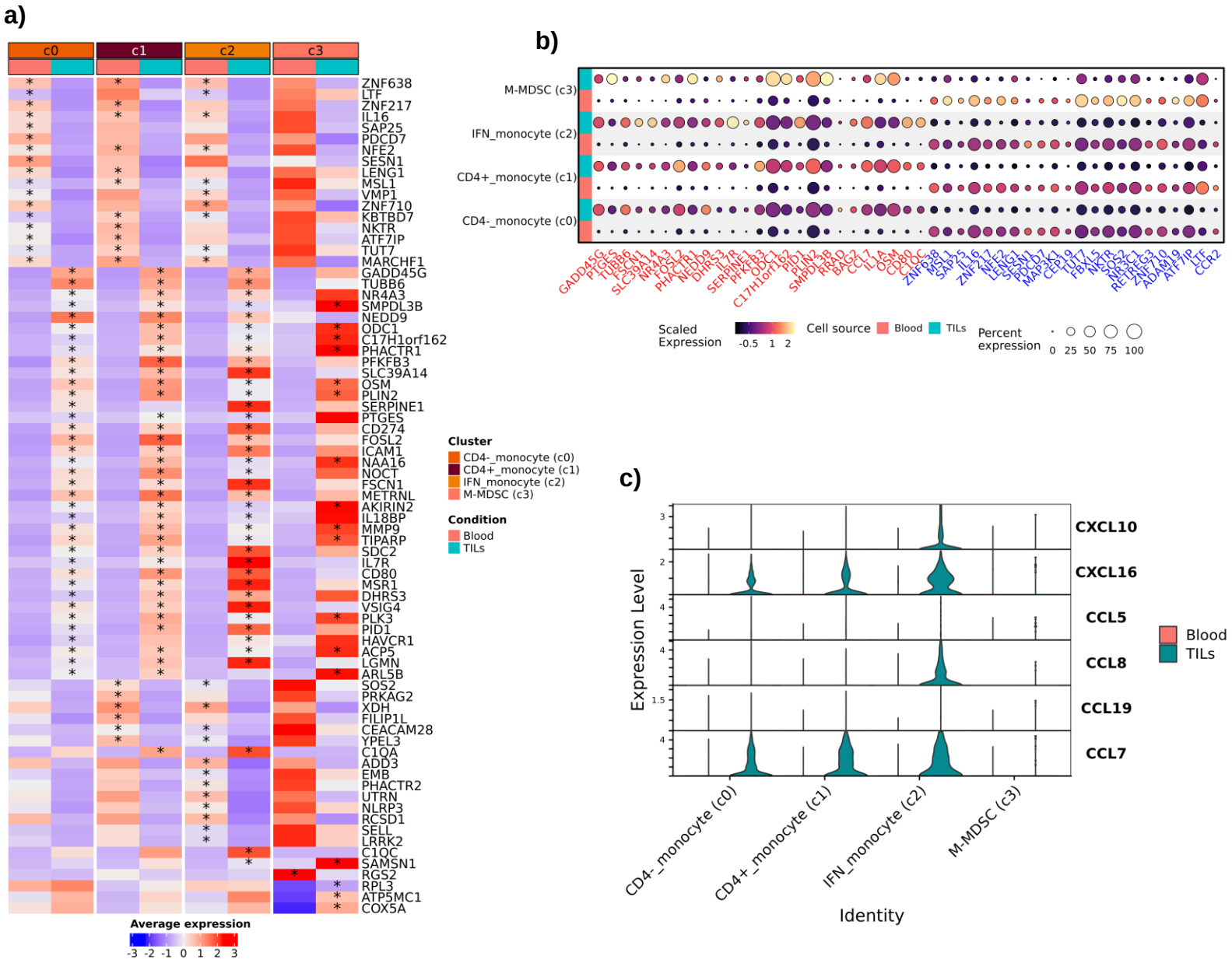
Supplemental figure 5. Differential expression within CD8 T cell subsets. (**a**) Heatmap depicting log normalized average expression within each CD8 T cell cluster separated by condition ("TILs" or "Blood"). Asterisk indicates statistically significant change in expression between conditions (adjusted P value < 0.01 and |log2(Fold change)| > 0.58), and the asterisk is located on the condition that had significantly higher expression. (**b**) Split dot plot representing scaled, log transformed gene expression of top upregulated (red text) and downregulated (blue text) genes within each CD8 T cell cluster. Dot size represents the percentage of cells expressing the gene while dot color represents relative expression. Red rows display contributions from blood derived cells while blue rows display contributions from TILs.



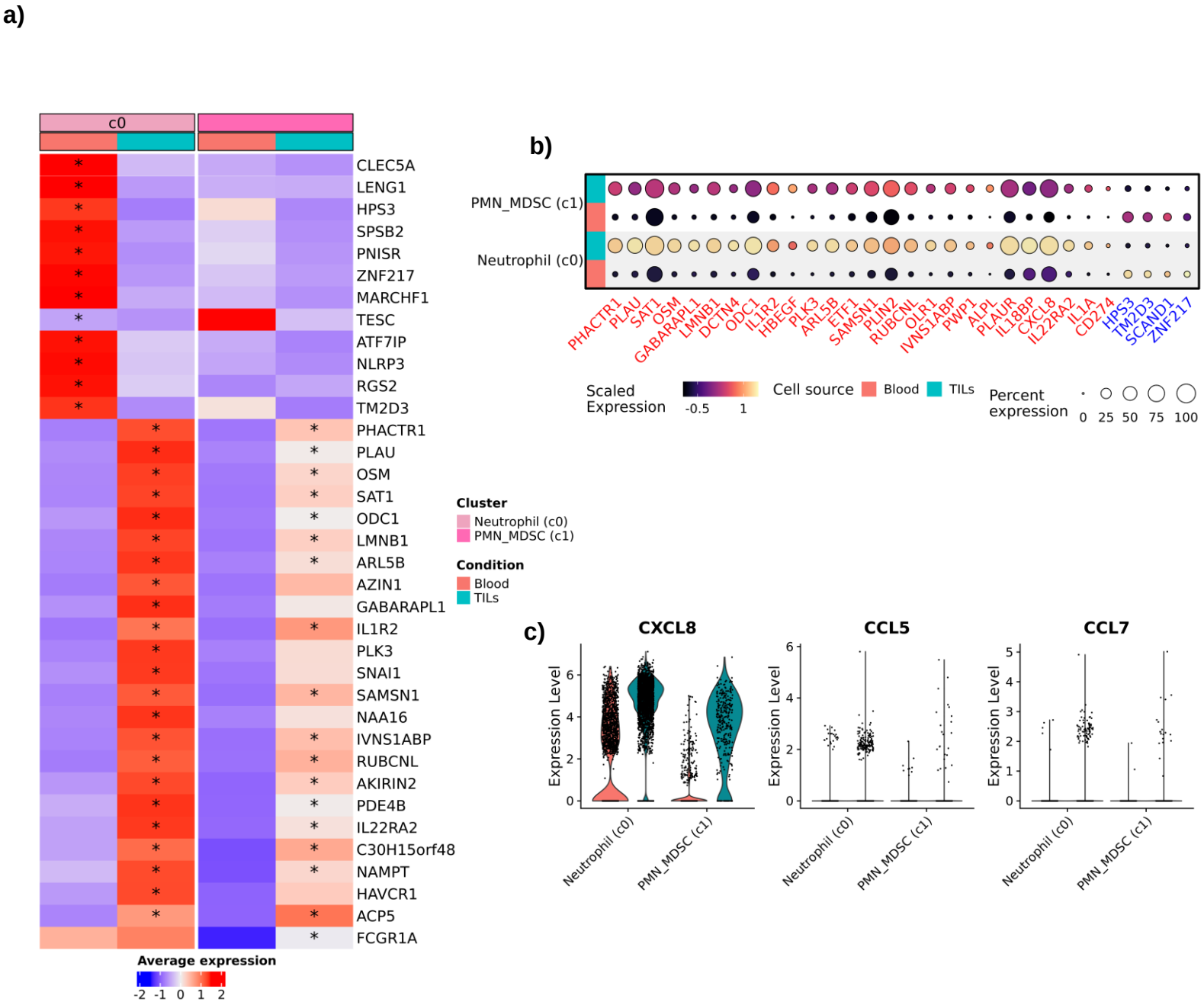
Supplemental figure 6. Differential expression within B cell and dendritic cell subsets. (**a**) Diagonal Euclidean distance matrix of the B cell subtypes and plasma cells. (**b**) Volcano plot depicting results of differential gene expression analysis contrasting all tumor infiltrating dendritic cells versus all circulating dendritic cells. Split dot plot representing scaled, log transformed gene expression of top upregulated (red text) and downregulated (blue text) genes within each (**a**) B cell cluster and **(b)** dendritic cell cluster**.** Dot size represents the percentage of cells expressing the gene while dot color represents relative expression. Red rows display contributions from blood derived cells while blue rows display contributions from TILs.



Supplemental figure 7. Tumor infiltrating monocytes more closely resemble blood monocytes than tumor associated macrophage. Heatmap depicting distance between samples using blood monocytes (Monocyte_bl_), tumor infiltrating monocytes (TIM_), and tumor associated macrophage (TAM_). Row and columns are clustered by Euclidean distance.



Supplemental figure 8. Differential expression within monocyte subsets. (**a**) Heatmap depicting log normalized average expression within each monocyte cluster separated by condition ("TILs" or "Blood"). Asterisk indicates statistically significant change in expression between conditions (adjusted P value < 0.01 and | log2(Fold change) | > 0.58), and the asterisk is located on the condition that had significantly higher expression. (**b**) Split dot plot representing scaled, log transformed gene expression of top upregulated (red text) and downregulated (blue text) genes within each monocyte cluster. Dot size represents the percentage of cells expressing the gene while dot color represents relative expression. Red rows display contributions from blood derived cells while blue rows display contributions from TILs. (**c**) Violin plots depicting log transformed gene expression of differentially expressed chemokines within monocyte clusters.



Supplemental figure 9. Differential expression within neutrophil subsets. (**a**) Heatmap depicting log normalized average expression within each neutrophil cluster separated by condition ("TILs" or "Blood"). Asterisk indicates statistically significant change in expression between conditions (adjusted P value < 0.01 and |log2(Fold change)| > 0.58), and the asterisk is located on the condition that had significantly higher expression. (**b**) Split dot plot representing scaled, log transformed gene expression of top upregulated (red text) and downregulated (blue text) genes within each neutrophil cluster. Dot size represents the percentage of cells expressing the gene while dot color represents relative expression. Red rows display contributions from blood derived cells while blue rows display contributions from TILs. (**c**) Violin plots depicting log transformed gene expression of differentially expressed chemokines within neutrophil clusters.