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Supplemental information

Dissecting the cellular landscape and transcriptome network in viral myocarditis by single-cell RNA sequencing

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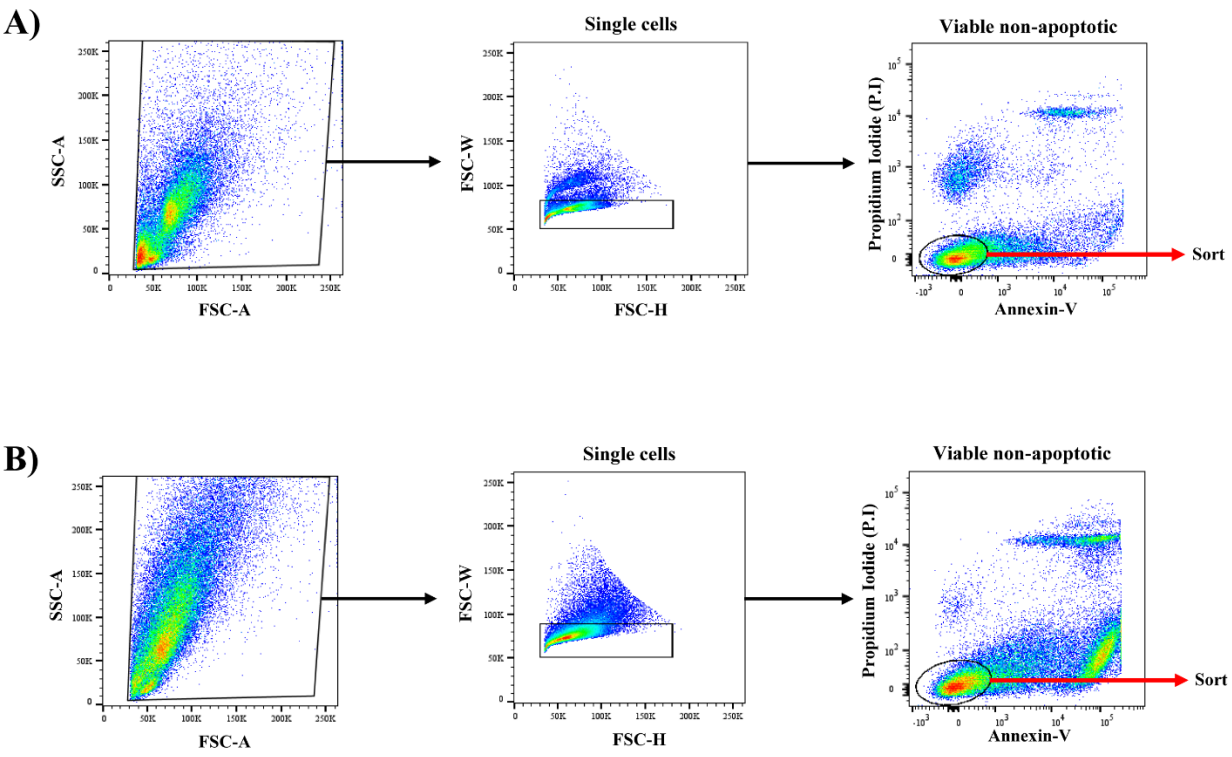


Fig S1. Schematic representation for sorting single cells from heart infiltrates, Related to Fig 1. Groups of mice were infected with or without CVB3, and after 21 days, hearts were collected at euthanasia following perfusion. Hearts were enzymatically digested to obtain single-cell suspensions, and cells were then stained with annexin-V and PI for sorting by flow cytometry, where viable (annexin-V-, PI-) cells were sorted by gating the singlets.

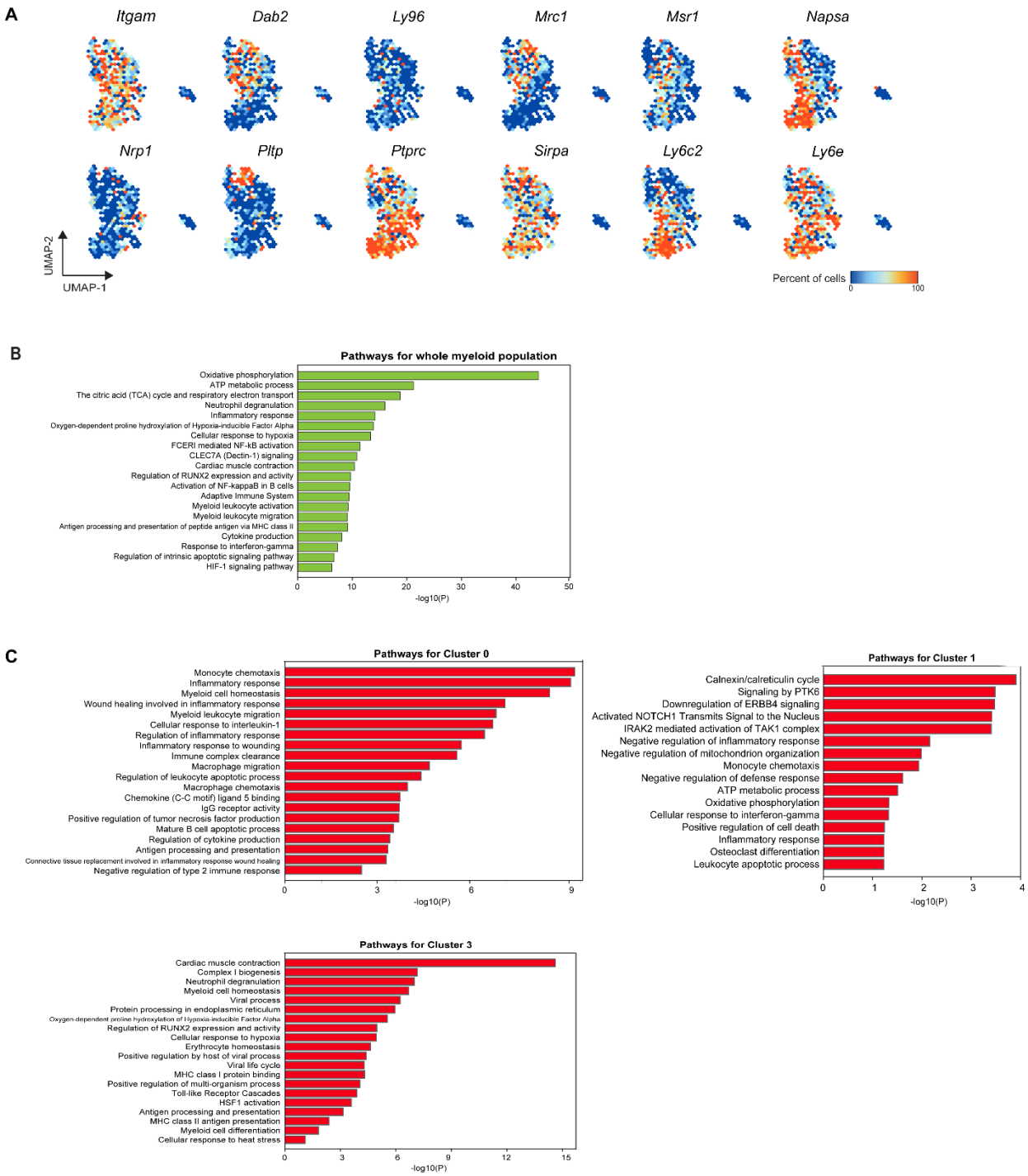


Fig S2. Canonical markers and GSEA in myeloid cells, Related to Fig 2. A. UMAPs showing percentage expression of indicated markers in myeloid cell clusters. **B.** Enriched GO terms with respect to pathways upregulated in myeloid cells of myocarditic mice. **C.** Enriched pathways upregulated in myeloid cell clusters 0, 1, 3 of myocarditic mice.

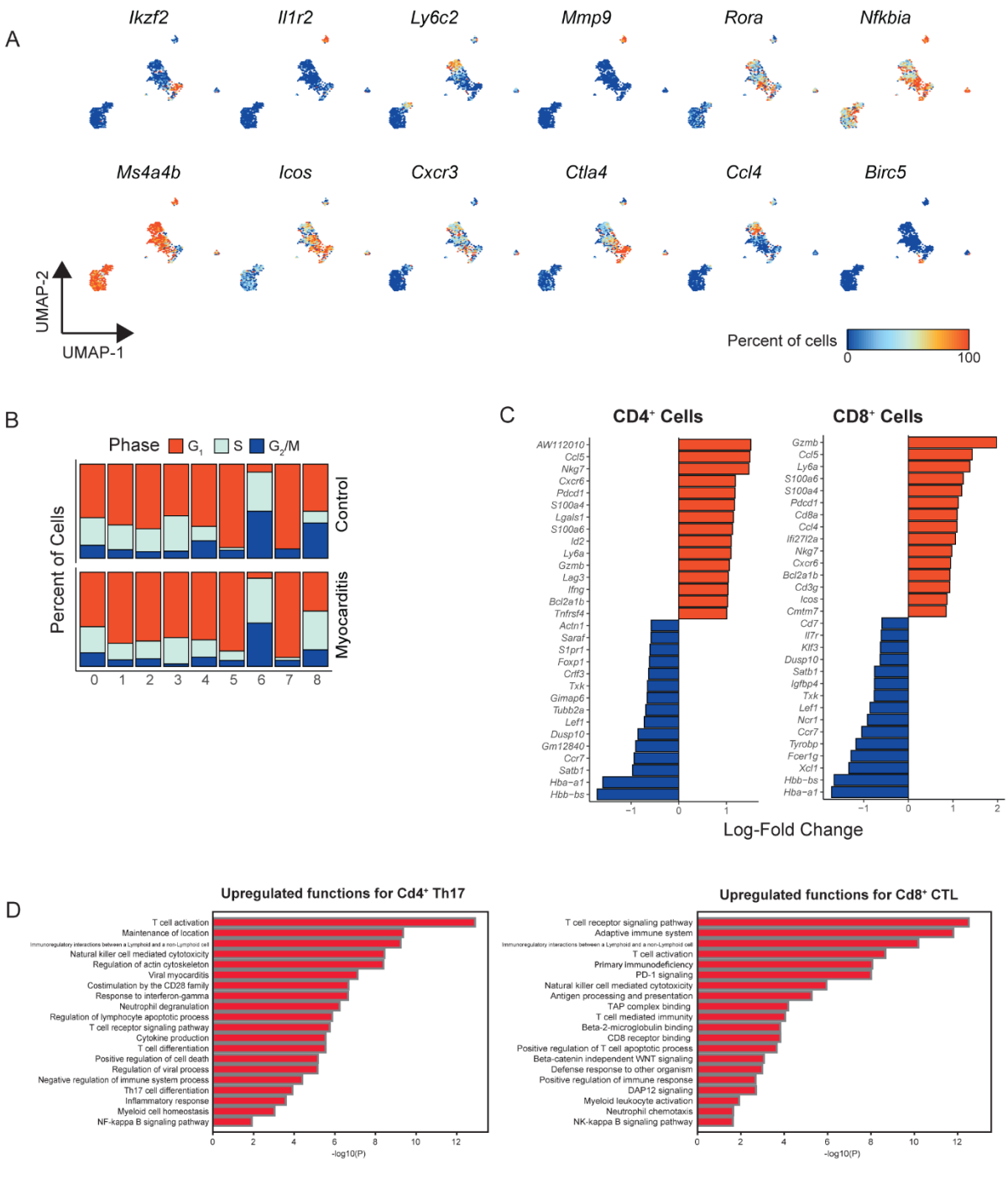


Fig S3. Differentially expressed genes and their pathway analysis in T cells, Related to Fig 3. A. UMAPs showing the percentage expression of canonical T cell markers. **B.** Cell cycle phases by clusters in myocarditic mice in relation to healthy group. **C.** Top 15 genes upregulated and downregulated in myocarditis versus healthy controls by log-fold change in myocarditic mice. **D.** Pathway analysis corresponding to Th17 subset (cluster 1) and CTLs (cluster 2).

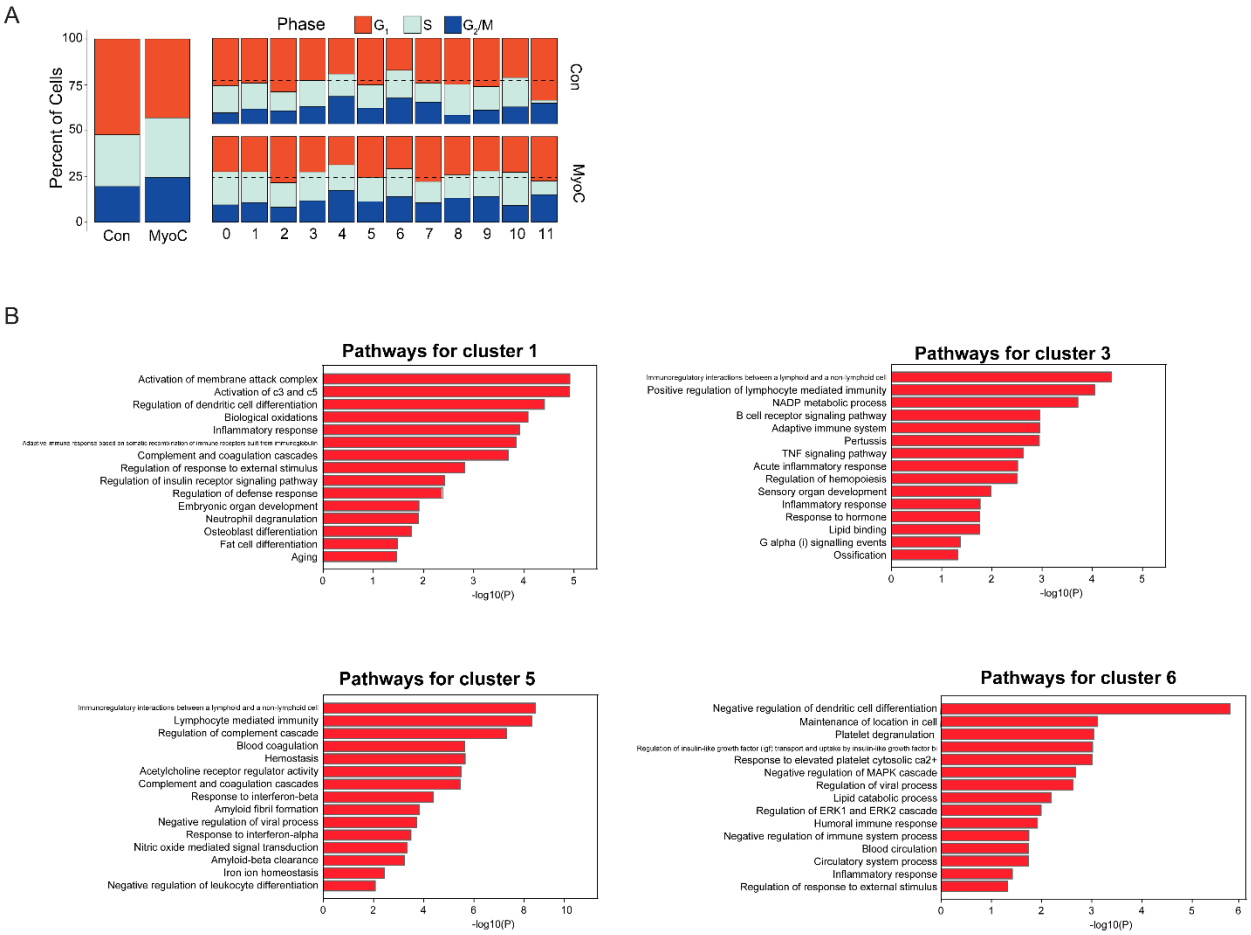


Fig S4. Cell cycle phases and GSEA of fibroblast cell clusters in heart infiltrates, Related to Fig 4. A. Cell cycle phases by clusters between groups. **B**. Pathway analysis is shown for clusters 1, 3, 5, and 6.

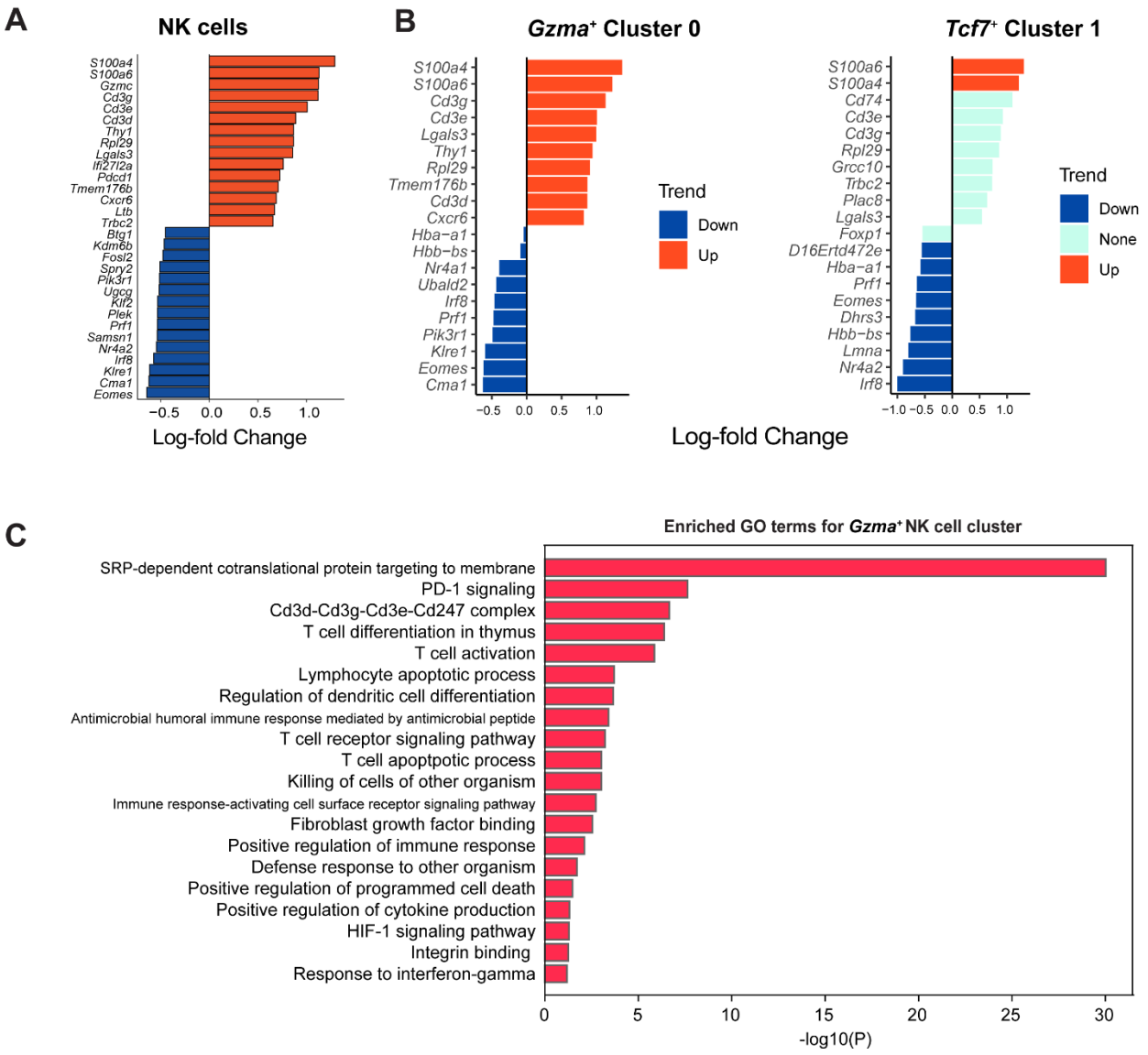


Fig S5. Differentially expressed genes and their pathway analysis in NK cells, Related to Fig 5. A. Top 15 upregulated and downregulated genes in myocarditic mice versus healthy controls by log-fold change in NK cells. **B**. Differential expression of the top 10 genes in *Gzma*$^{+}$ cluster 0, *Tcf7*$^{+}$ cluster 1 of myocarditic mice relative to healthy group. **C.** Pathway analysis for *Gzma*$^{+}$ cluster 0.

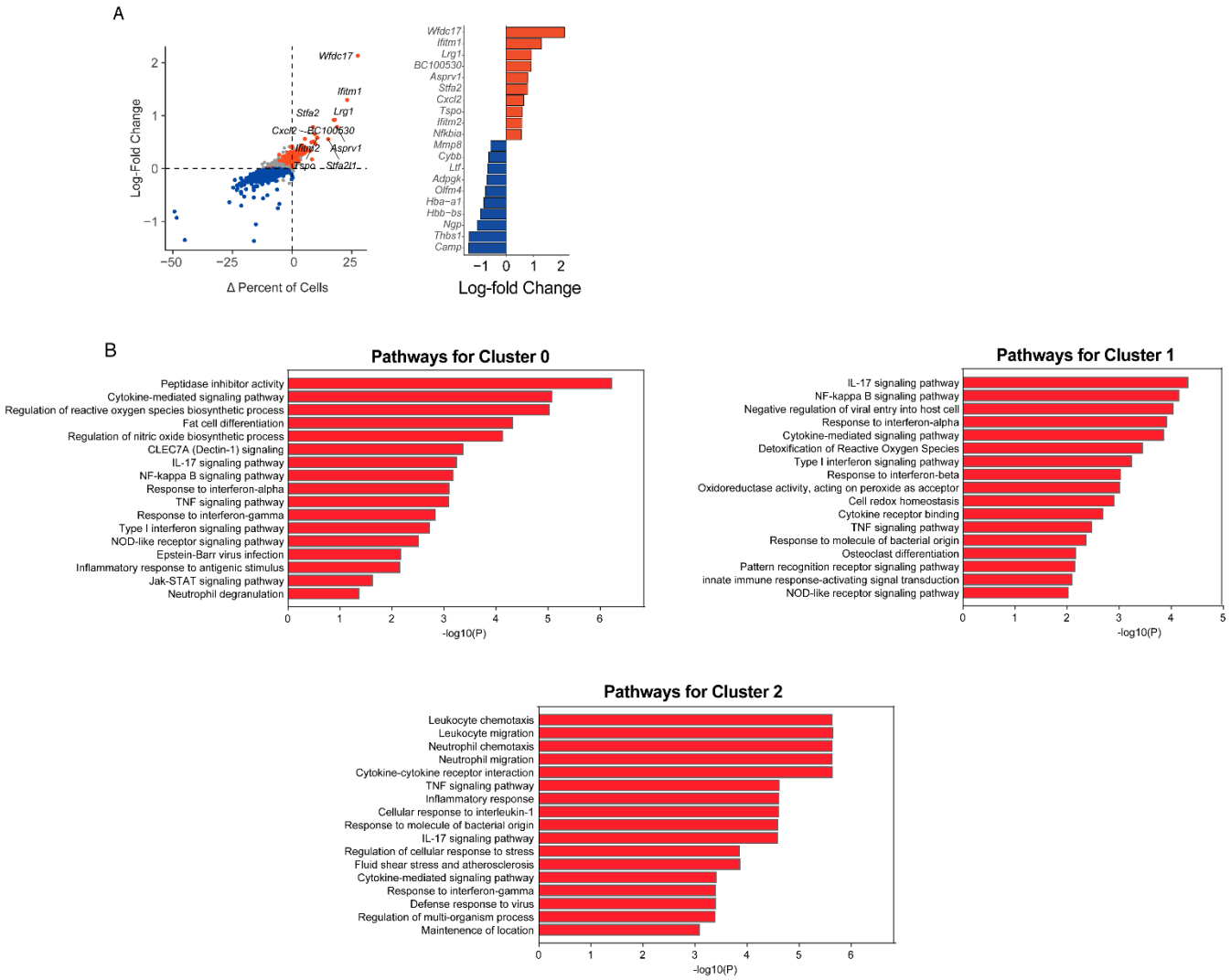


Fig S6. Differentially expressed genes and their pathway analysis in neutrophils, Related to Fig 6. A. Overall differential expression of genes in neutrophils is shown in the volcano plot (left), including the top 10 genes in the bar plot (right panel). **B.** Pathway analysis for clusters 0, 1, and 2.

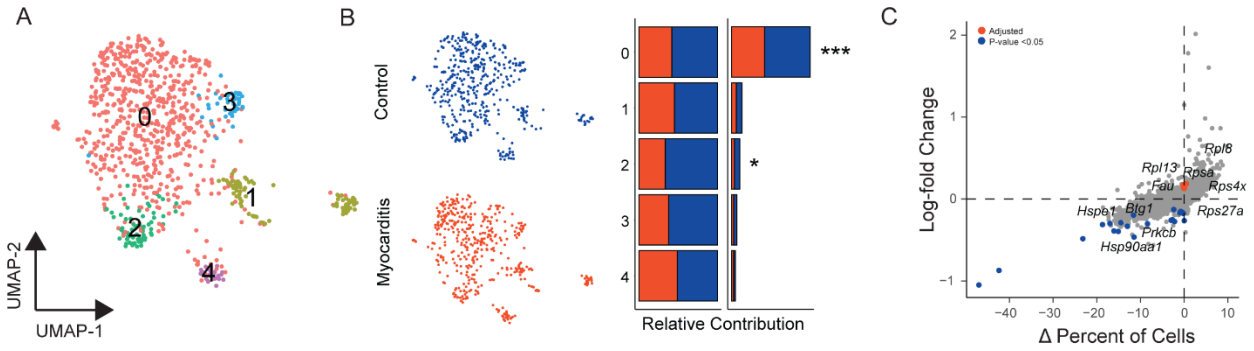


Fig S7. Analysis of B cell clusters in myocarditic hearts A, Related to Fig 1. UMAP showing different clusters of B cells in control and myocarditic mice. **B.** Distribution of B cells and their relative proportions in control and myocarditic mice. **C.** Volcano plot showing the differentially expressed genes.

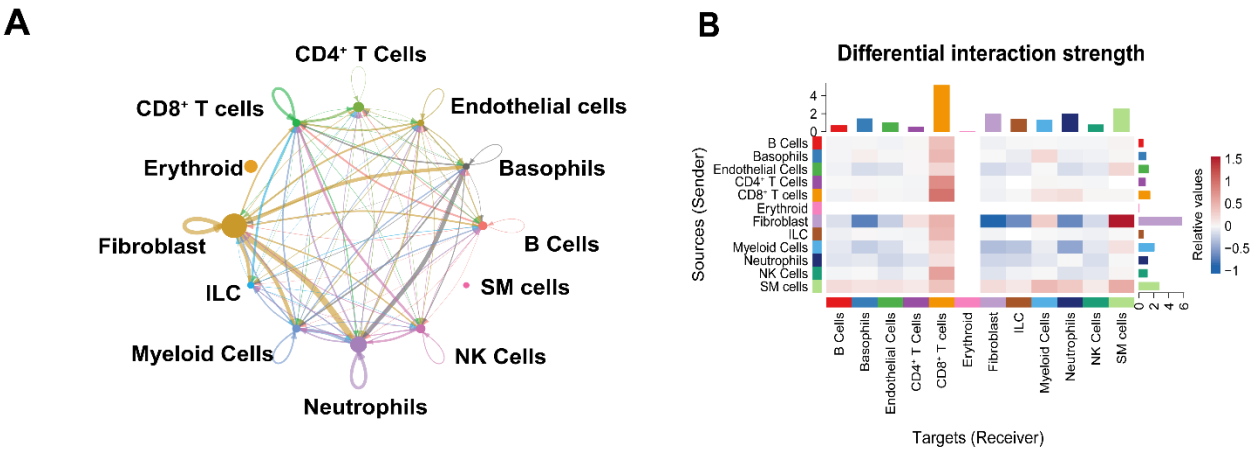
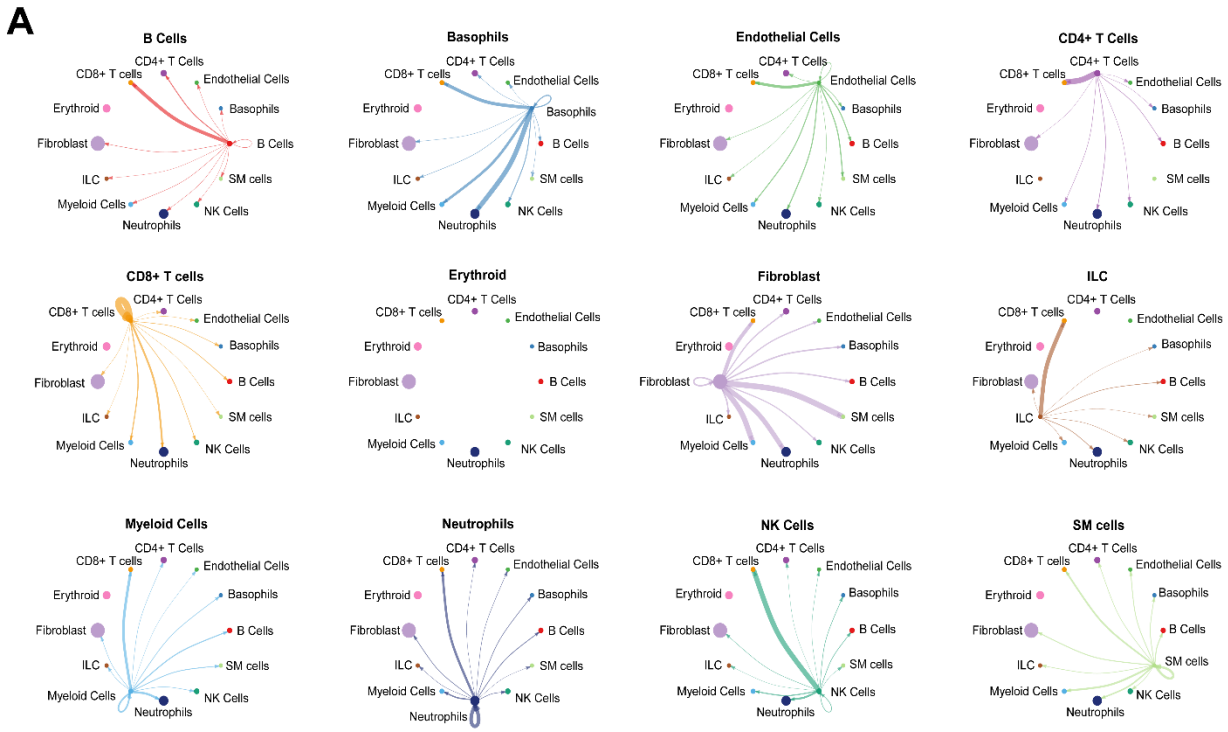


Fig S8. Intercellular communication in the healthy and myocarditic cardiac cellulome, Related to Fig 7. A. Circle plot showing intercellular communication between major cardiac cell types for control hearts using CellChat R workflow. The lines originating from a cell type indicate ligands being broadcast, with these lines connecting to the cell type where the receptors are expressed. Thickness of the line is proportional to the number of unique ligand-receptor interactions, with loops representing autocrine circuits. **B**. Heatmap of differential interaction strength between myocarditic and healthy mice in the cell-cell communication network. The top-colored bar plot indicates the sum of column values (incoming signaling), and the right bar plot indicates the sum of row values (outgoing signaling). Red indicates increased signaling in myocarditis, and blue indicates decreased signaling.


A
B Cells
Basophils
Endothelial Cells
CD4+ T Cells
CD8+ T cells
Erythroid
Fibroblast
ILC
Myeloid Cells
Neutrophils
NK Cells
SM cells

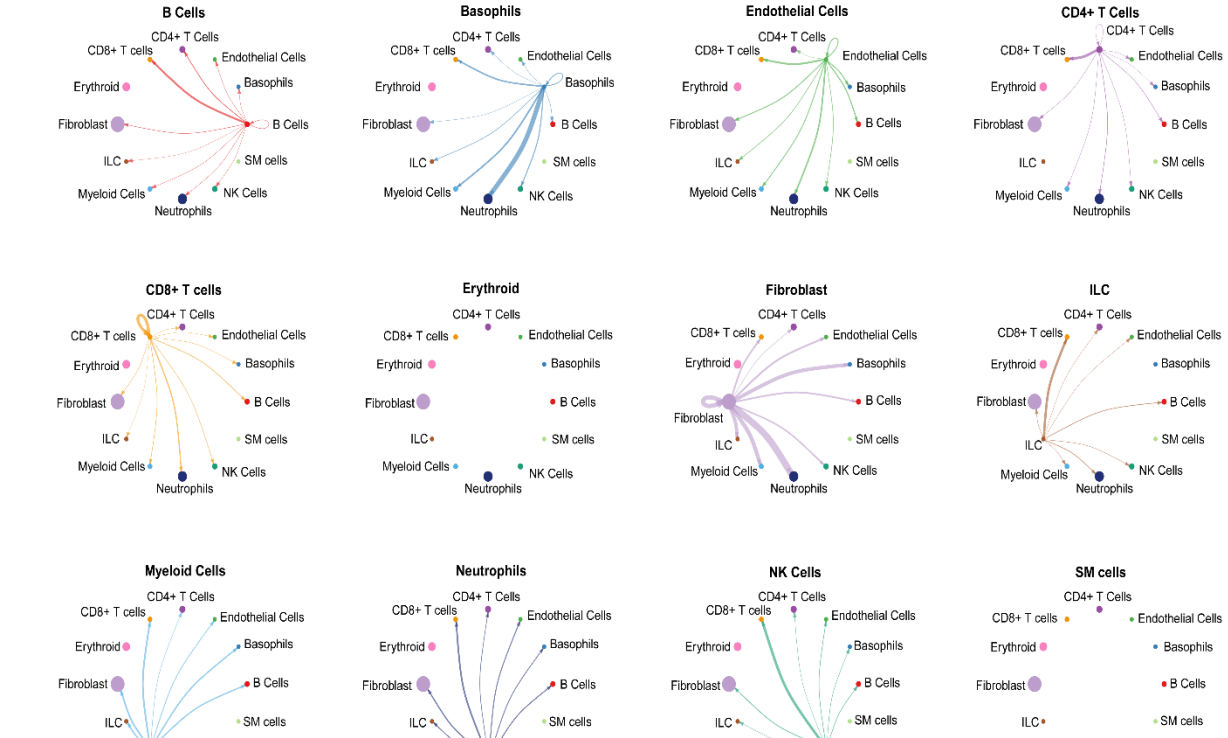

B
B Cells
Basophils
Endothelial Cells
CD4+ T Cells
CD8+ T cells
Erythroid
Fibroblast
ILC
Myeloid Cells
Neutrophils
NK Cells
SM cells

Fig S9. Intercellular interactions between cell types in control and myocarditic hearts, Related to Fig 7. A detailed view of ligand and cognate receptor interaction for each cell type in myocarditic (**A**), and control (**B**) hearts.

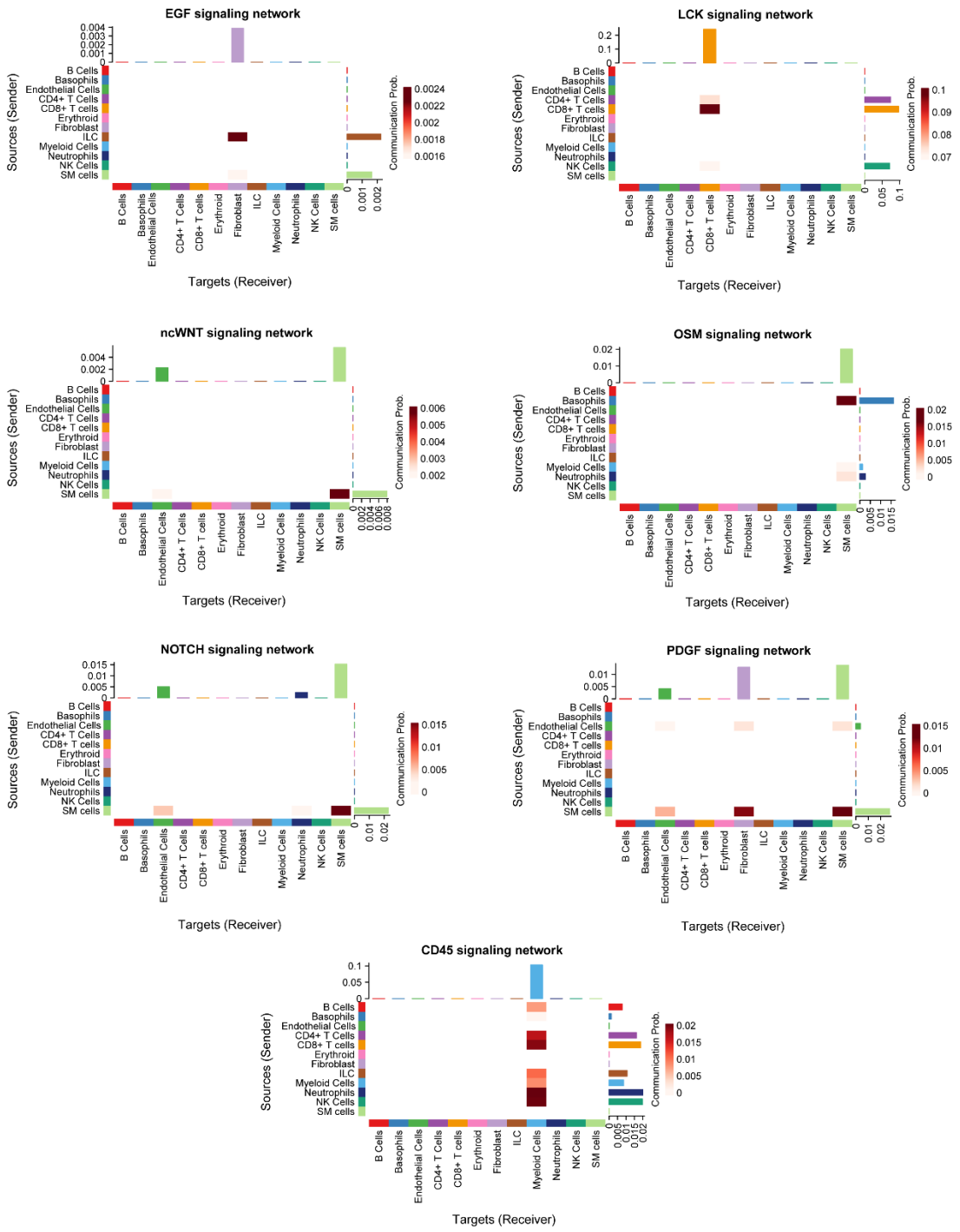


Fig S10. Major signaling pathways inferred from intercellular communication in the myocarditic hearts, Related to Fig 7. Signaling interactions for specific pathways between cells in the cardiac cellulome in myocarditic mice. Signals are being sent from the source (senders) along the *y* axis to the targets (receivers) along the *x* axis.

Fig S11. GO functions of TF-regulated target genes, Related to Fig 8. The upregulated GO functions of various TF-regulated target genes found enriched in **(A)** T cells, (**B**) myeloid cells, (**C**) neutrophils, and (**D**) NK cells.

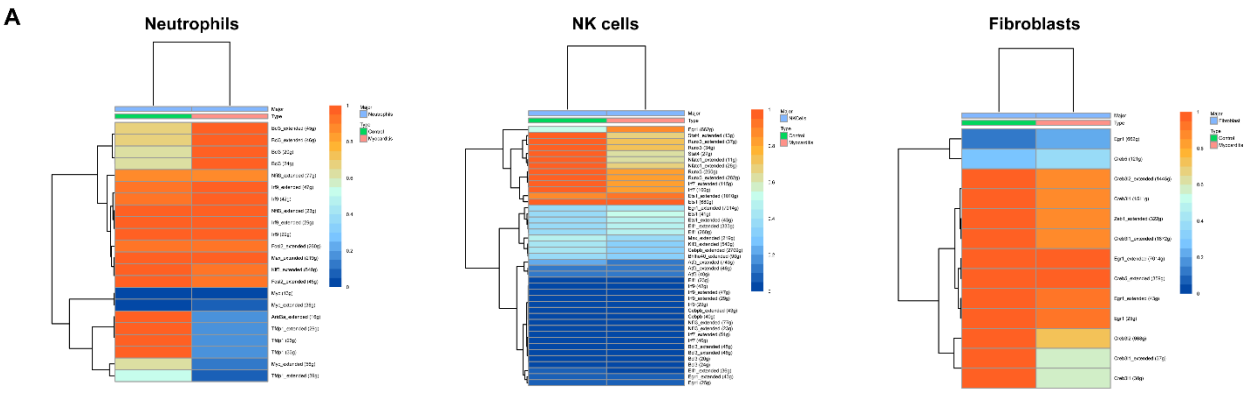


Fig S12. Analysis of transcription factors in neutrophils, NK cells and fibroblasts, Related to Fig 8. Enrichment of TFs in neutrophils, NK cells and fibroblasts from control and myocarditic mice as analyzed by SCENIC.

Table S1: Marker genes used for cell assignments (related to Fig 1)

Cell Types	Marker genes
B cells	*Cd19, Ighm, Bank1, Cd79b, Fcmr, H2-Ob, Ms4a1, Tnfrsf13c*
Basophils	*Mcpt4, Mcpt8, Ifitm1, Cyp11a1*
Endothelial Cells	*Cdh5, Egfl7, Apold1, Arhgef15, Emcn, Gpihbp1, Rasgrp3, Rbp7, Tie1*
T Cells	*Cd3e, Cd3g, Cd4, Cd8a, Cd8b1, Lat, Tcf7, Trbc1, Trbc2, Ctla4, Tnfrsf4*
Erythroid cells	*Hba-a1, Hba-a2, Hbb-bt, Alas2, Bpgm, Snca*
Fibroblast	*Serpinh1, Pcsk6, Col1a2, Col5a1, Lamb1, Tcf21, Lpl*
Innate lymphoid cells	*Gata3, Rorc, Tbx21*
Myeloid Cells	*Ccr2, Cd209a, Adreg1, Msr1, Chil3, Arg1, Ms4a6d, Ms4a6c*
Neutrophils	*Hp, Ccrl2, Lcn2, Pglyrp1, Cxcl2, Ngp*
Natural killer Cells	*Ncr1, Gzma, Gzmb, Eomes, Klre1, Klrk1, Ncr1, Prf1*
Smooth muscle cells	*Myh11, Mylk, Lmod1, Mustn1, Nrip2, Cnn1, Des, Grip2, Kcnmb1*

Table S2: Total cell numbers in different cell types after QC in myocarditis and control samples (related to Fig 1)

Cell Types	Control	Myocarditis
B cells	432	420
Basophils	24	26
Endothelial Cells	13	47
$CD4^+$ T Cells	808	1369
$CD8^+$ T cells	266	586
Erythroid cells	1448	647
Fibroblast	3828	6258
Innate lymphoid cells	55	42
Myeloid Cells	305	1061
Neutrophils	2030	2471
Natural killer Cells	524	295
Smooth muscle cells	1	29