

**Supplemental information**

**Metabolic modeling of single  
bronchoalveolar macrophages reveals  
regulators of hyperinflammation in COVID-19**

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## Supplementary Figures and Legends

### **Figure S1. Metabolic heterogeneity of immune subpopulations from BALF, related to Figure 1.**

**(A-B)** A schematic diagram shows principal component analysis (A) and t-SNE visualization of subpopulations (B) in BALF from one healthy control based on all gene expression profiles.

**(C-E)** A schematic diagram shows principal component analysis (C) and t-SNE visualization of subpopulations (D and E) in BALF from one healthy control based on expression levels of 1526 metabolic genes.

**(F)** Bar plot shows the relative contributions of cell subpopulations in each cluster in figure D.

### **Figure S2. Metabolic reprogramming of macrophages in patients with mild or severe COVID-19, related to Figure 2.**

**(A)** Metabolic pathway activities in macrophages from each mild or severe COVID-19 patient. Statistically non-significant values (random permutation test  $p > 0.01$ ) were shown as blank.

**(B)** Distribution of pathway activities in macrophages and key clinical data for each COVID-19 patient.

**(C)** Expression levels of 1526 metabolic gene expression in macrophages from different groups.

**(D)** Metabolic gene set enrichment analysis of macrophages from severe versus mild patients.

### **Figure S3. Metabolic reprogramming of macrophages in patients with 2 mild and 20 severe COVID-19 patients in another cohort, related to Figure 2 and 5.**

**(A)** Metabolic pathway activity analysis in macrophages from BALFs of 2 mild and 20 severe COVID-19 patients. Statistically non-significant values (random permutation test  $p > 0.05$ ) were shown as blank.

**(B)** Distribution of pathway activities in macrophages from different groups.

**(C)** Metabolic gene set enrichment analysis of macrophage from BALF of severe patients versus mild patients.

**(D-E)** Gene ontology analysis of upregulated pathways (D) and downregulated pathways (E) in macrophage from severe patients versus mild patients.

**Figure S4. Metabolic pathway and flux balance analysis comparing macrophages with or without SARS-CoV-2 transcripts from severe patients to macrophages from HC and mild patients, related to Figure 2 and 3.**

**(A)** Feature plots show macrophages from HC, mild and severe patients (left panel) and macrophages with or without SARS-CoV-2 transcripts (right panel).

**(B)** Distribution of pathway activities in macrophages from HC and mild patients, and macrophages from severe patients with or without SARS-CoV-2 transcripts.

**(C)** Metabolic pathway activity analysis of macrophages from HC and mild patients, and macrophages from severe patients with or without SARS-CoV-2 transcripts. Statistically non-significant values (random permutation test  $p > 0.01$ ) were shown as blank.

**(D)** Single-cell flux balance analysis (scFBA) analysis of macrophages from HC and mild patients, and macrophages from severe patients with or without SARS-CoV-2 transcripts.

**Figure S5. Metadata analysis of plasma metabolites in mild and severe COVID-19 patients, related to Figure 3.**

Metadata (by Su et al. 2020) analysis of plasma metabolites. Each dot corresponds to an individual patient.

**(A)** Violin plots show levels of indicated amino acids in plasma of COVID-19 patients.

**(B)** Violin plots show levels of indicated lipids in plasma of COVID-19 patients.

**(C)** Violin plots show levels of ornithine, urea and kynurenine in plasma of COVID-19 patients.

**Figure S6. t-SNE visualization of cells from paired PBMCs, related to Figure 4.**

**(A-C)** A schematic diagram shows principal component analysis (A) and t-SNE visualization of subpopulations (B and C) in PBMCs of mild ( $n=2$ ) and severe ( $n=5$ ) COVID-19 patients based on all gene expression profiles. Different colors indicate distinct cell types (B) and patient groups (C). **(D-F)** A schematic diagram shows principal component analysis (D) and t-SNE visualization of subpopulations (E and F) in PBMCs of mild ( $n=2$ ) and severe ( $n=5$ ) COVID-19 patients based on 1420 metabolic genes expression profiles. Different colors indicate distinct cell types (E) and patient groups (F). The dash circles indicate position of monocytes on each plot.

**Figure S7. Distinct metabolic reprogramming during monocyte to macrophage transition in mild versus severe COVID-19, related to Figure 4.**

**(A)** Metabolic pathway activities of monocytes (PBMC-Monocyte) and macrophages (BALF-macrophage) from PBMCs and BALF of paired mild (n=2) and severe (n=5) COVID-19 patients. Statistically non-significant values (random permutation test  $p > 0.01$ ) were shown as blank.

**(B)** Distribution of pathway activities in monocytes and macrophages from different groups.

**(C)** Heatmap shows expression of 1441 metabolic genes in PBMC-Monocyte and BALF-Macrophage from different groups.

**(D-E)** Trajectory (D) and inference (E) of pseudo time ordering of PBMC-Monocyte and paired BALF-Macrophage from mild patients visualized in the DDRTree based reduced dimensional space.

**(F)** Pearson correlation plot of metabolic scores and pseudo time in PBMC-Monocyte and paired BALF- Macrophage from mild COVID-19 patients.

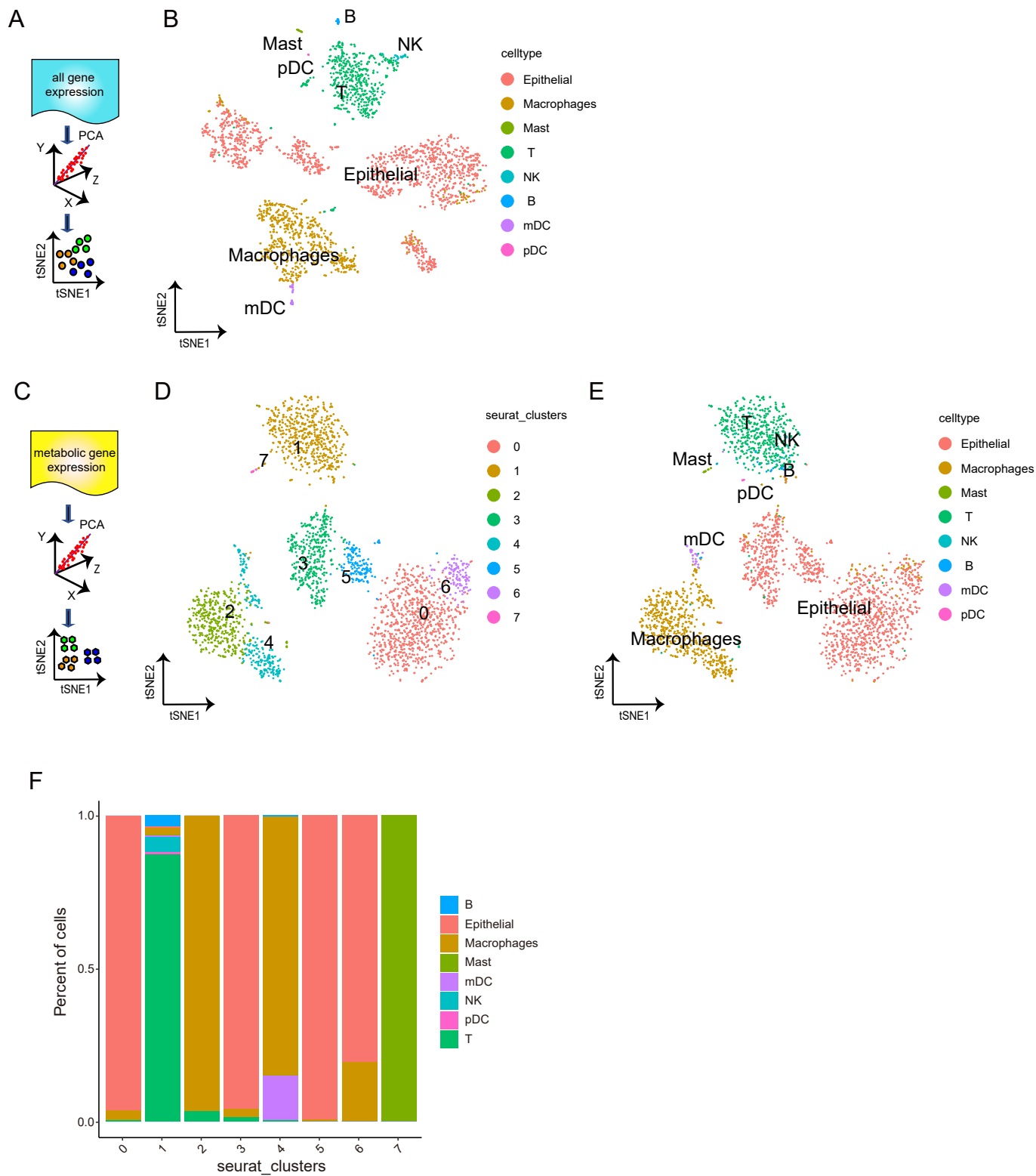
**(G-I)** Trajectory(G), inference of pseudo time ordering (H) and Pearson correlation analysis (I) of PBMC-Monocyte and paired BALF-Macrophage from severe COVID-19 patients.

**Figure S8. Inhibition of glycolysis by 2-DG reduces pro-inflammatory cytokines and chemokines production in macrophages, related to Figure 6.**

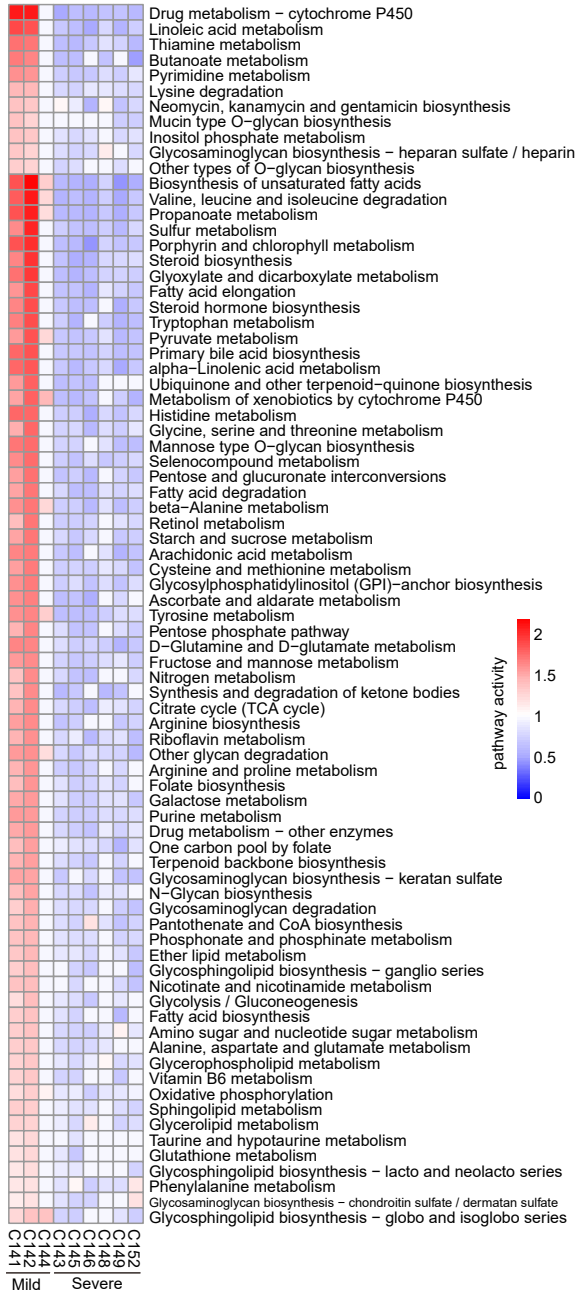
**(A-B)** Human primary macrophages cultured in complete medium or medium without glutamine and pyruvate were stimulated with R848 (A). IL-6, CCL2, CCL3 and IL-1 $\beta$  levels in culture supernatant were detected by ELISA (B).

**(C-D)** Human primary macrophages cultured with or without 2-DG were stimulated with R848 (C). IL-6, CCL2, CCL3 and TNF- $\alpha$  levels in culture supernatant were detected by ELISA 24 hours after stimulation(D).

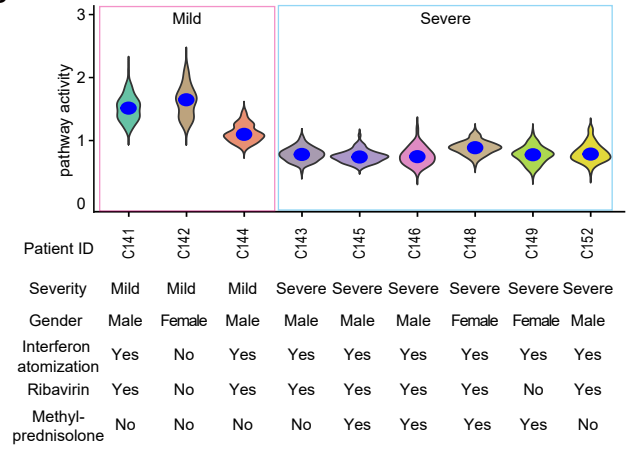
Shown are representative data (n=6 for each group) from 2 independent donors with mean values. \*\*\* $p < 0.001$ . One-way analysis of variance (ANOVA) and Bonferroni's post hoc test were performed to compare between groups.



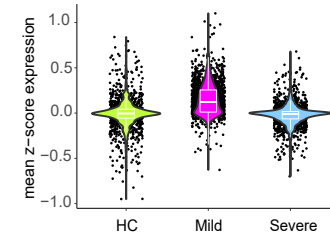
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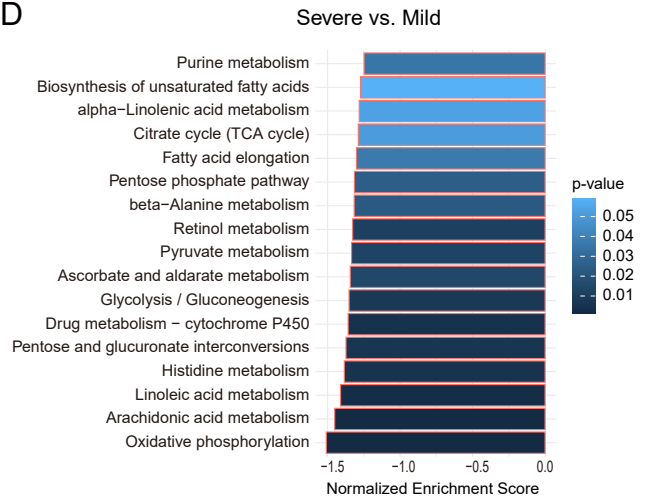
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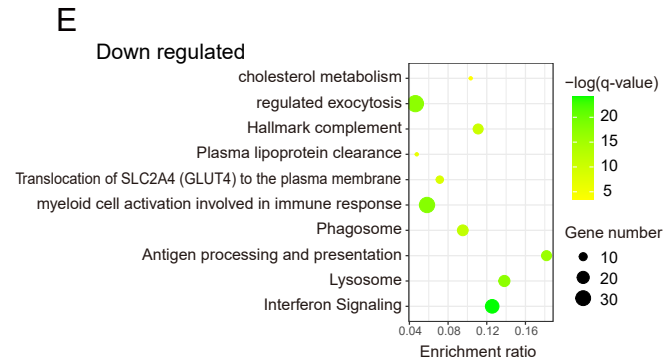
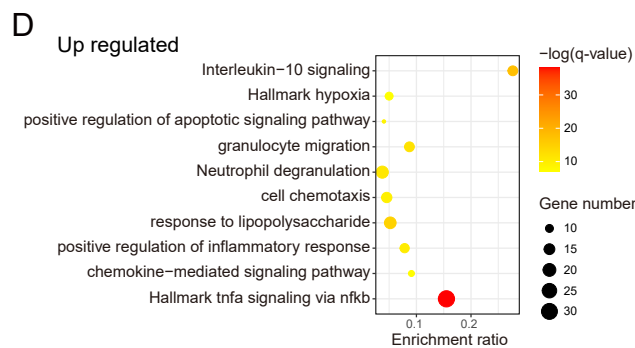
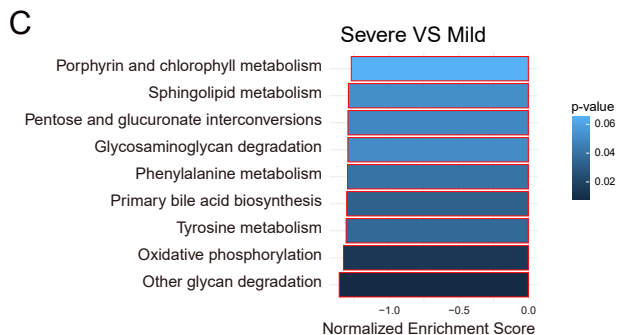
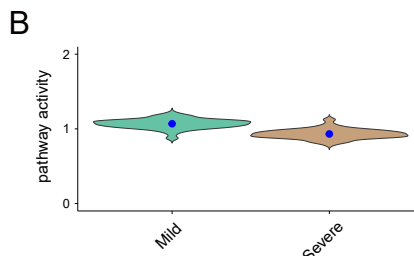
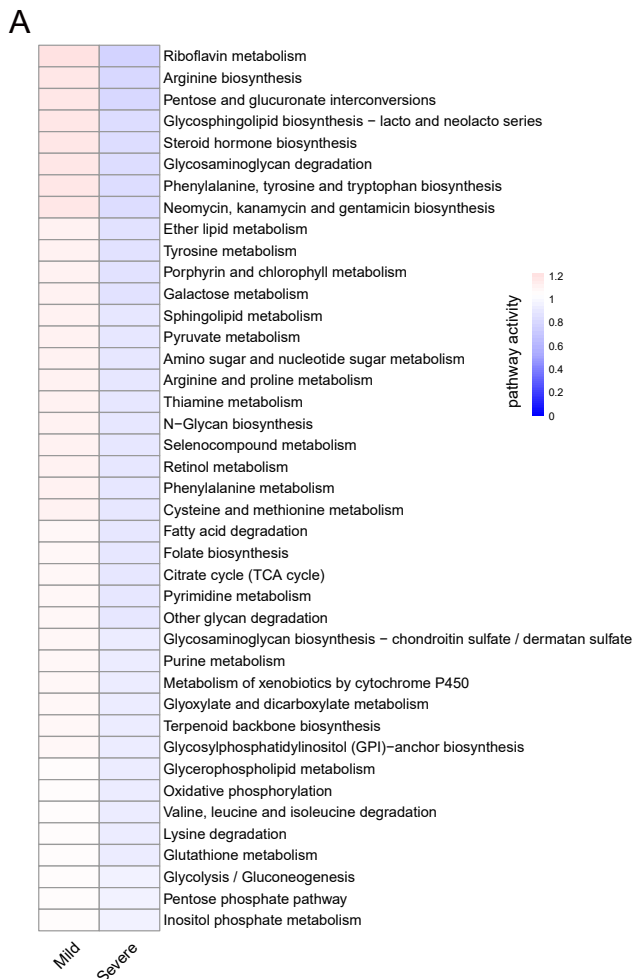


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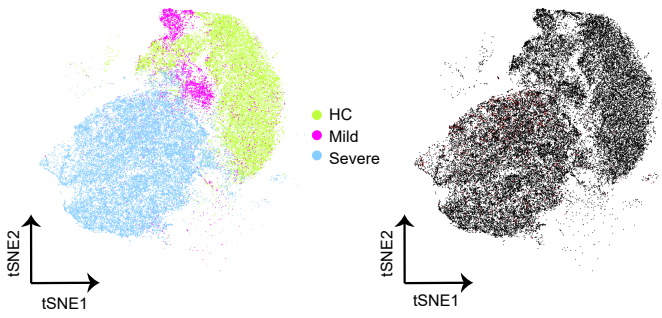


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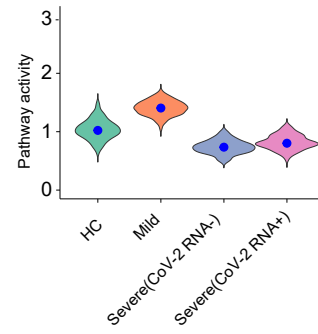




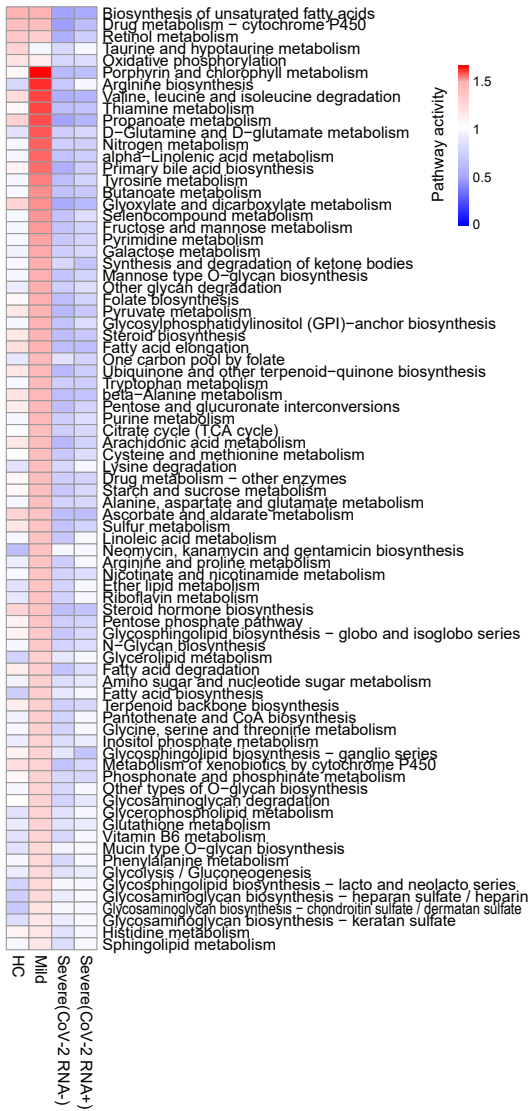
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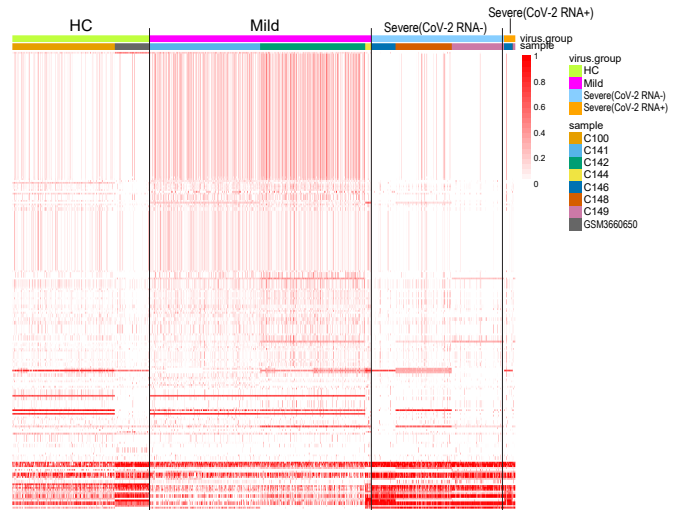
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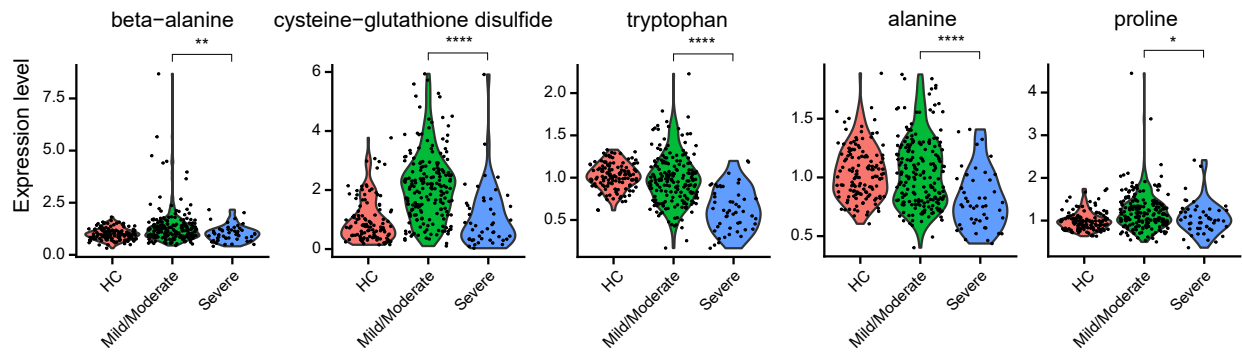


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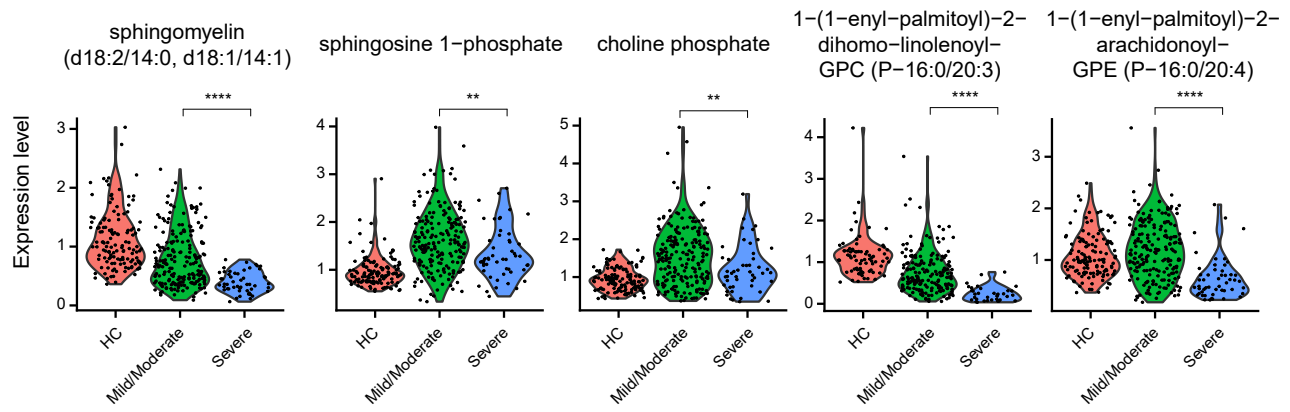




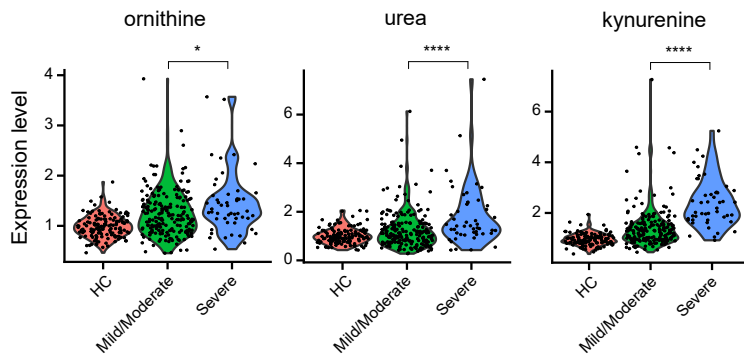
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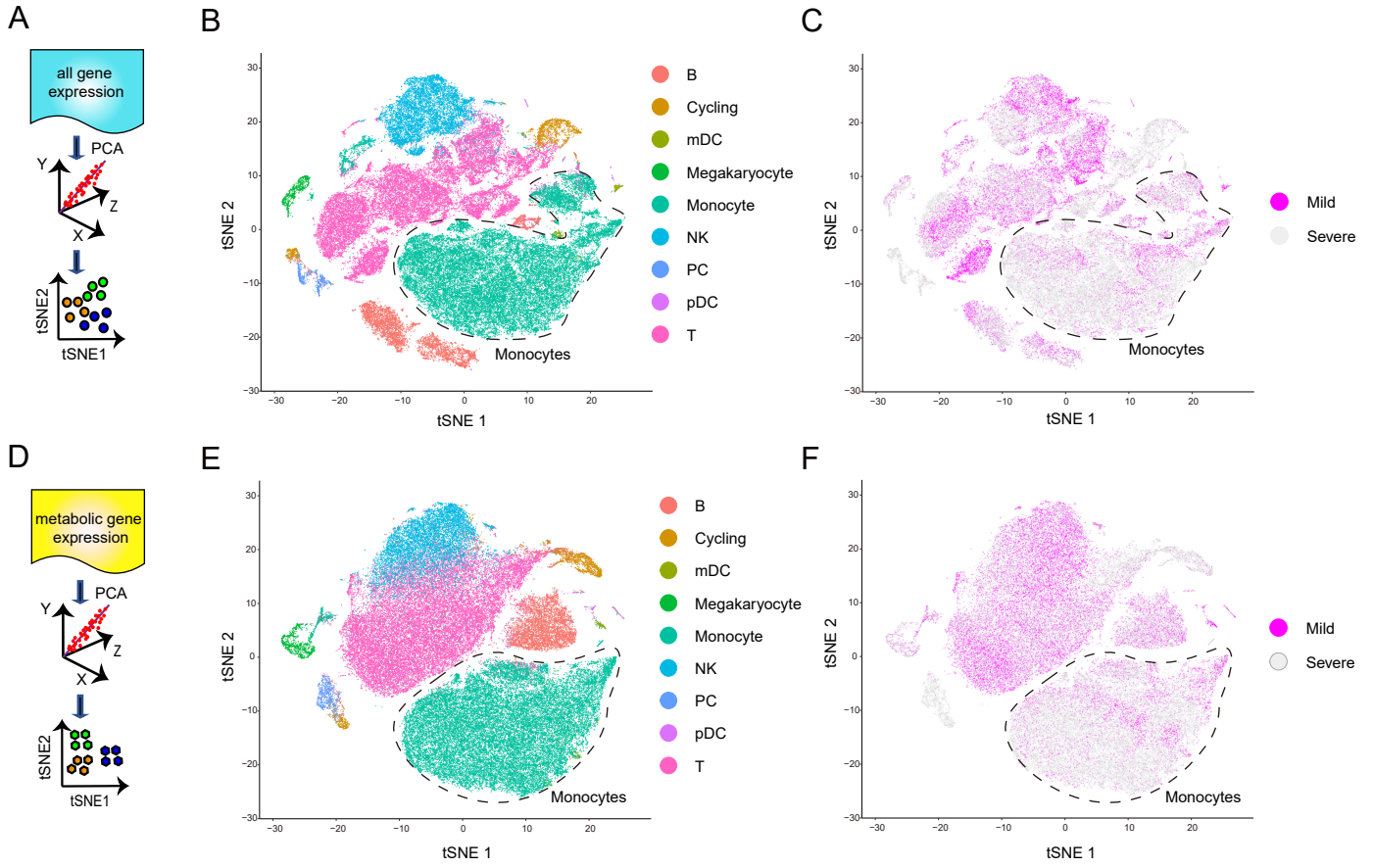


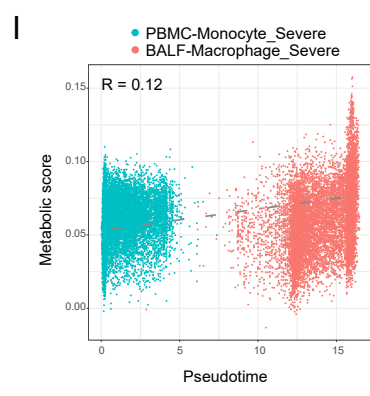
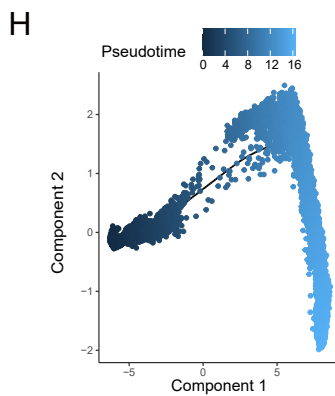
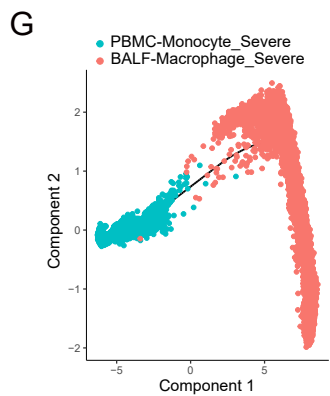
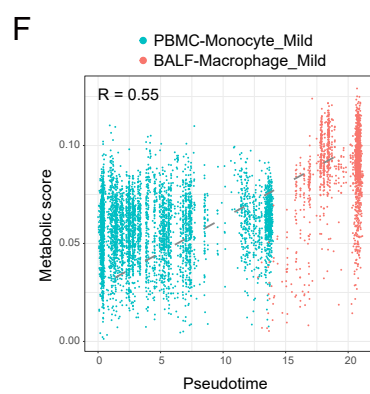
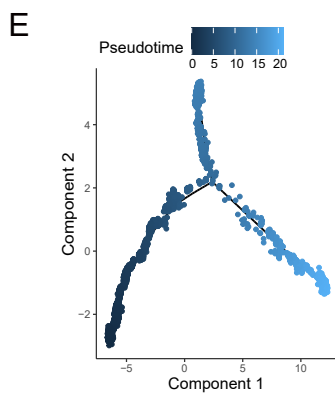
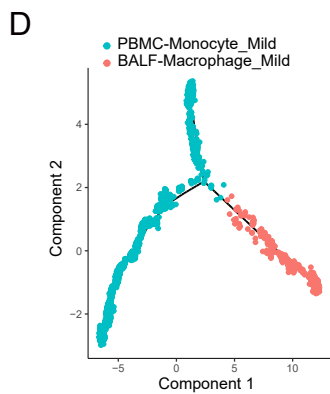
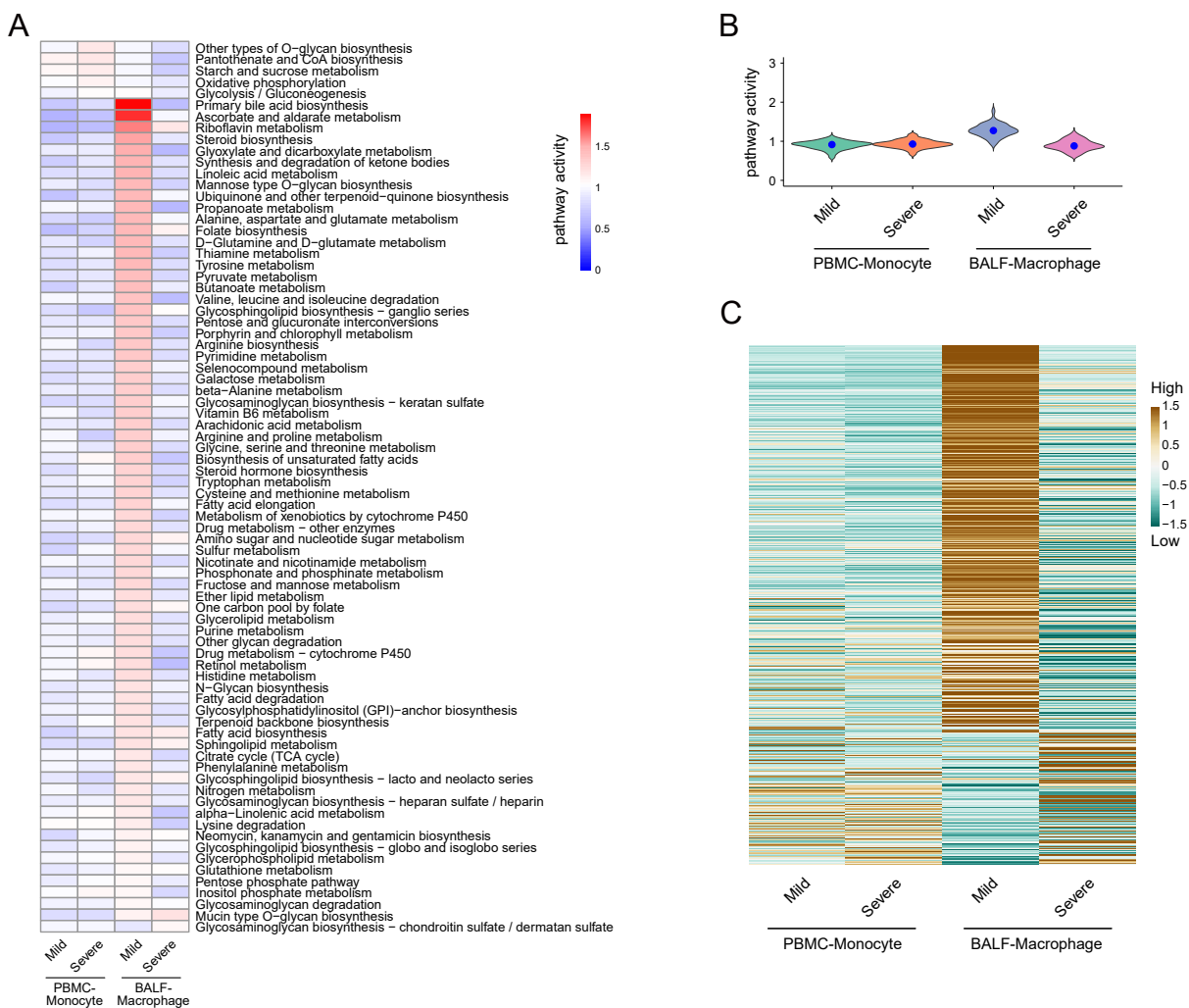
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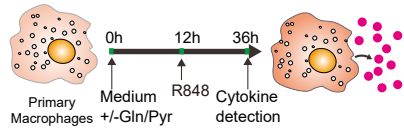
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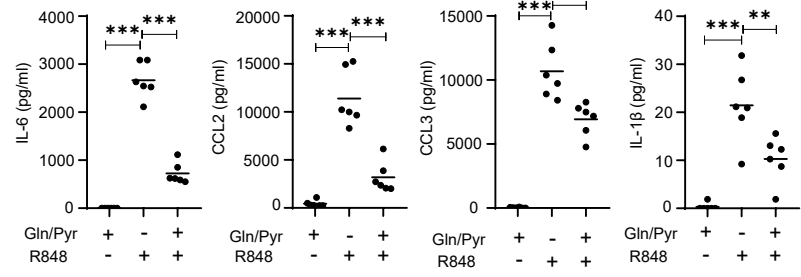




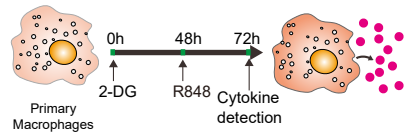
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