Supplemental information

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

Qiuchen Zhao, Zhenyang Yu, Shengyuan Zhang, Xu-Rui Shen, Hao Yang, Yangyang Xu, Yang Liu, Lin Yang, Qing Zhang, Jiaqi Chen, Mengmeng Lu, Fei Luo, Mingming Hu, Yan Gong, Conghua Xie, Peng Zhou, Li Wang, Lishan Su, Zheng Zhang, and Liang Cheng
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.
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-1β 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-α 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.
**A**

| Metabolism Pathways |
|---------------------|
| Riboflavin metabolism |
| Arginine biosynthesis |
| Pentose and glucuronate interconversions |
| Glycosphingolipid biosynthesis – lacto and neolacto series |
| Steroid hormone biosynthesis |
| Glycoamino-sugar biosynthesis |
| Phenylalanine, tyrosine and tryptophan biosynthesis |
| Neomycin, kanamycin and gentamicin biosynthesis |
| Other lipid metabolism |
| Tyrosine metabolism |
| Porphyrin and chlorophyll metabolism |
| Galactose metabolism |
| Sphingolipid metabolism |
| Pyruvate metabolism |
| Amino sugar and nucleotide sugar metabolism |
| Arginine and proline metabolism |
| Thiamine metabolism |
| N-Glycan biosynthesis |
| Selenocompound metabolism |
| Retinol metabolism |
| Phenylalanine metabolism |
| Cysteine and methionine metabolism |
| Fatty acid degradation |
| Folate biosynthesis |
| Citrate cycle (TCA cycle) |
| Pyrimidine metabolism |
| Other glycan degradation |
| Glycoamino-sugar biosynthesis – chondroitin sulfate / dermatan sulfate |
| Purine metabolism |
| Metabolism of xenobiotics by cytochrome P450 |
| Glyoxylate and dicarboxylate metabolism |
| Terpenoid backbone biosynthesis |
| Glycosphingolipid (GPI)-anchor biosynthesis |
| Glycosphingolipid metabolism |
| Oxidative phosphorylation |
| Valine, leucine and isoleucine degradation |
| Lysine degradation |
| Glutathione metabolism |
| Glycolysis / Gluconeogenesis |
| Pentose phosphate pathway |
| Neolactosulfate phosphoribosyltransferase metabolism |

**B**

Comparison of pathway activity in mild vs severe. The graph shows the normalized enrichment score for different metabolisms in mild and severe conditions.

**C**

Comparison of pathway activity in severe vs mild. The graph shows the normalized enrichment score for different metabolisms in severe and mild conditions.

**D**

**Up regulated**

- Interleukin-10 signaling
- Hallmark hypoxia
- Positive regulation of apoptotic signaling pathway
- Granulocyte migration
- Neutrophil degranulation
- Cell chemotaxis
- Response to lipopolysaccharide
- Positive regulation of inflammatory response
- Chemokine-mediated signaling pathway
- Hallmark Inflammation via ROR2

**E**

**Down regulated**

- Cholesterol metabolism
- Regulated exocytosis
- Hallmark complement
- Plasma lipoprotein clearance
- Translocation of SLC2A4 (GLUT4) to the plasma membrane
- Myeloid cell activation involved in immune response
- Phagosome
- Antigen processing and presentation
- Lysosome
- Interferon Signaling

**Normalized Enrichment Score**

- **Mild**
  - Severe
  - **Normalized Enrichment Score**
  - 0.02
  - 0.04
  - 0.06

**Gene number**

- **0**
  - 10
  - 15
  - 20
  - 25
  - 30
  - Enrichment ratio

**Gene number**

- **0**
  - 10
  - 15
  - 20
  - 25
  - 30
  - Enrichment ratio
### Table 1: Metabolic Pathways and Activities

| Pathway Description                                                                 | Activity Level   |
|------------------------------------------------------------------------------------|-----------------|
| Other types of C-glycan biosynthesis                                               | High            |
| Pentofuranose and UDP-glycosylation                                                | Mild            |
| Sphingosine and sphingolipid metabolism                                            | Severe          |
| Primary bile acid biosynthesis                                                     | Mild            |
| Steroid and sterol biosynthesis                                                    | Severe          |
| Serine biosynthesis                                                                | Mild            |
| Arginine biosynthesis                                                              | Severe          |
| Arginine and proline biosynthesis                                                  | Mild            |
| Glycine, serine and threonine biosynthesis                                         | Severe          |
| Urea cycle                                                                         | Mild            |
| Glutamate metabolism                                                               | Severe          |
| Alanine, aspartate and glutamate metabolism                                        | Mild            |
| Folate biosynthesis                                                                | Severe          |
| D-Glutamine and D-glutamate metabolism                                            | Mild            |
| Thiamine biosynthesis                                                             | Severe          |
| Pyruvate metabolism                                                               | Mild            |
| Butanoate metabolism                                                              | Severe          |
| Valine, leucine and isoleucine degradation                                         | Mild            |
| Glycosphingolipid biosynthesis − gangliosides                                       | Severe          |
| Pentose and glucuronate interconversions                                           | Mild            |
| Porphyrin and chlorophyll metabolism                                              | Severe          |
| Arginine biosynthesis                                                              | Mild            |
| Pyrimidine metabolism                                                              | Severe          |
| Selenocompound metabolism                                                          | Mild            |
| Galactose metabolism                                                               | Severe          |
| beta-Alanine metabolism                                                            | Mild            |
| Glycosaminoglycan biosynthesis − keratan sulfate                                   | Severe          |
| Vitamin B6 metabolism                                                              | Mild            |
| Arachidonic acid metabolism                                                       | Severe          |
| Arginine and proline biosynthesis                                                  | Mild            |
| Glycine, serine and threonine biosynthesis                                         | Severe          |
| Citrate cycle (TCA cycle)                                                          | Mild            |
| Pheynylalanine metabolism                                                         | Severe          |
| Glycosphingolipid biosynthesis − lacto and neolacto series                         | Mild            |
| Nitrogen metabolism                                                                | Severe          |
| Fatty acid degradation                                                             | Mild            |
| Glycan, asparagine and glutamine metabolism                                        | Severe          |
| Fatty acid elongation                                                              | Mild            |
| Metabolism of xenobiotics by cytochrome P450                                       | Severe          |
| Drug metabolism − cytochrome P450                                                  | Mild            |
| Retinol metabolism                                                                 | Severe          |
| Histidine metabolism                                                               | Mild            |
| N-Glycan biosynthesis                                                              | Severe          |
| Fatty acid degradation                                                             | Mild            |
| Glycosylphosphatidylinositol (GPI)-anchor biosynthesis                             | Severe          |
| Terpenoid backbone biosynthesis                                                    | Mild            |
| Fatty acid biosynthesis                                                            | Severe          |
| Sphingolipid metabolism                                                            | Mild            |
| Glycerolipid metabolism                                                            | Severe          |
| Purine metabolism                                                                  | Mild            |
| Other glycan degradation                                                            | Severe          |
| Drug metabolism − other enzymes                                                   | Mild            |
| Amino sugar and nucleotide sugar metabolism                                        | Severe          |
| Sulfur metabolism                                                                  | Mild            |
| Nicotinate and nicotinamide metabolism                                            | Severe          |
| Phosphonate and phosphinate metabolism                                            | Mild            |
| Fructose and mannose metabolism                                                   | Severe          |
| Ether lipid metabolism                                                             | Mild            |
| One carbon pool by folate                                                          | Severe          |
| Glycine, serine and threonine biosynthesis                                         | Mild            |
| Glycosaminoglycan synthetase − keratan sulfate                                     | Severe          |
| Glycosaminoglycan synthetase − chondroitin sulfate / dermatan sulfate               | Mild            |

### Figure S7: Pseudotime Trajectories and Metabolic Scores

- **A**: Heatmap showing metabolic pathway activity across different conditions.
- **B**: Heatmap comparing metabolic scores between PBMC-Monocyte and BALF-Macrophage.
- **C**: Heatmap showing the distribution of metabolic scores across different conditions.
- **D-E**: Scatter plots depicting pseudotime trajectories for different conditions.
- **F**: Scatter plot showing metabolic scores with R = 0.55.
- **G-H**: Scatter plots depicting pseudotime trajectories for different conditions.
- **I**: Scatter plot showing metabolic scores with R = 0.12.
