Implications of central carbon metabolism in SARS-CoV-2 replication and disease severity — Source link ↗

Shuba Krishnan, Hampus Nordqvist, Anoop T. Ambikan, Soham Gupta ...+19 more authors

Institutions: Karolinska Institutet, Science for Life Laboratory, Indian Institute of Science, Karolinska University Hospital ...+1 more institutions

Related papers:

- Metabolic Perturbation Associated With COVID-19 Disease Severity and SARS-CoV-2 Replication.

- Mitochondrial metabolic manipulation by SARS-CoV-2 in peripheral blood mononuclear cells of patients with COVID-19.

- Host metabolic reprogramming in response to SARS-CoV-2 infection: A systems biology approach.

- Host metabolic reprogramming in response to SARS-Cov-2 infection

- An untargeted metabolomic approach to identify antiviral defense mechanisms in memory leukocytes secreting in vitro IgG anti-SARS-Cov-2

Share this paper: f 𝕏 in ✉

View more about this paper here: https://typeset.io/papers/implications-of-central-carbon-metabolism-in-sars-cov-2-t89xlw1xab

1   **Implications of central carbon metabolism in SARS-CoV-2 replication and**

2   **disease severity**

3

4   **Authors:** Shuba Krishnan[1], Hampus Nordqvist[2], Anoop T. Ambikan[1], Soham Gupta[1], Maike

5   Sperk[1], Sara Svensson-Akusjärvi[1], Flora Mikaeloff[1], Rui Benfeitas[3], Elisa Saccon[1], Sivasankaran

6   Munusamy Ponnan[4], Jimmy Esneider Rodriguez[5], Negin Nikouyan[1], Amani Odeh[6], Gustaf

7   Ahlén[1], Muhammad Asghar [6], Matti Sällberg[1], Jan Vesterbacka[7], Piotr Nowak[7,8], Ákos Végvári[5],

8   Anders Sönnerborg[1,6], Carl Johan Treutiger[2,7], Ujjwal Neogi[1*]

9

10   **Affiliations:**

11   [1]Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institute,

12   ANA Futura, Campus Flemingsberg, S-14152 Stockholm, Sweden.

13   [2]Södersjukhuset (The South General Hospital), Stockholm, Sweden.

14   [3]National Bioinformatics Infrastructure Sweden (NBIS), Science for Life Laboratory, Department

15   of Biochemistry and Biophysics, Stockholm University, S-10691 Stockholm, Sweden.

16   [4]Centre for Infectious Disease Research, Indian Institute of Science (IISc), CV Raman Avenue,

17   Bangalore, India.

18   [5]Division of Chemistry I, Department of Medical Biochemistry and Biophysics, Karolinska

19   Institutet, SE-171 65 Stockholm, Sweden.

20   [6]Division of Infectious Diseases, Department of Medicine Solna, Karolinska Institutet,

21   Stockholm, Sweden

22   [7]Department of Medicine Huddinge, Division of Infectious Diseases, Karolinska Institute, I73,

23   Karolinska University Hospital, Huddinge, 141 86 Stockholm, Sweden.

24   [8]The Laboratory for Molecular Infection Medicine Sweden MIMS, Umeå University, Sweden.

25

26   *To whom correspondence should be addressed: Ujjwal Neogi (ujjwal.neogi@ki.se)

27

28

29   **Short Title:** COVID-19 and central carbon metabolism

30

Abstract

Viruses hijack host metabolic pathways for their replicative advantage. Several observational trans-omics analyses associated carbon and amino acid metabolism in coronavirus disease 2019 (COVID-19) severity in patients but lacked mechanistic insights. In this study, using patient-derived multi-omics data and *in vitro* infection assays, we aimed to understand i) role of key metabolic pathways in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) reproduction and ii) its association with disease severity. Our data suggests that monocytes are key to the altered immune response during COVID-19. COVID-19 infection was associated with increased plasma glutamate levels, while glucose and mannose levels were determinants of the disease severity. Monocytes showed altered expression pattern of carbohydrate and amino acid transporters, GLUT1 and xCT respectively in severe COVID-19. Furthermore, lung epithelial cells (Calu-3) showed a strong acute metabolic adaptation following infection *in vitro* by modulating central carbon metabolism. We found that glycolysis and glutaminolysis are essential for virus replication and blocking these metabolic pathways caused significant reduction in virus production. Taken together, our study highlights that the virus utilizes and re-wires pathways governing central carbon metabolism leading to metabolic toxicity. Thus, the host metabolic perturbation could be an attractive strategy to limit the viral replication and disease severity.

Introduction

48

49   The global pandemic of coronavirus disease 2019 (COVID-19) caused by the severe acute

50   respiratory syndrome coronavirus-2 (SARS-CoV-2) created a severe public health crisis

51   worldwide. Although most patients presented with mild to moderate or no symptoms, patients

52   having pre-existing metabolic disorders like diabetes, cardiovascular diseases, and obesity are at

53   risk for severe and critical cases of infection. Some recent observational studies indicate that

54   disease severity in COVID-19 patients are associated with plasma metabolic abnormalities that

55   include a shift towards amino acid and fatty acid synthesis, altered energy and lipid metabolism

56   [1-3] and metabolic profile can identify the disease severity [4]. However, metabolic regulation

57   of an individual always depends on several factors including age, gender, environmental factors,

58   dietary intake and lifestyle. Such alterations in metabolic regulation can change rapidly or adapt

59   to an altered situation and sometimes have sustained effects over an extended period. The initial

60   phase of characterization of the metabolic landscape of COVID-19 and its association with

61   disease severity has urged the need to understand how metabolic reprogramming occurs during

62   the acute SARS-CoV-2 infection with the ultimate goal towards therapeutic intervention.

63   Viruses are known to exploit the host metabolic machinery to meet their biosynthetic demands

64   for optimal replication capacity. This cellular exploration is highly connected with the initial

65   host-viral response, thereby determining the disease pathogenesis. Viral replication is dependent

66   on extracellular carbon sources such as glucose and glutamine. It induces a plethora of metabolic

67   alterations in host-cell including host central carbon metabolism, nucleotide, fatty acids and lipid

68   synthesis that modulate viral pathogenesis and host-response [5, 6]. Recent *in vitro* multi-omics

69   studies have shown that the SARS-CoV-2 dysregulates PI3K/Akt/mTOR and HIF-1 signaling in

70   infected cells. These pathways regulate glycolysis by altering glucose transporters (GLUT) across

4

71   cell membranes. Targeting these pathways with inhibitors such as MK2206 (Akt inhibitor) or 2-

72   deoxy-D-glucose (2-DG, glycolysis inhibitor) can lower the viral burden in the cells *in vitro* [7,

73   8]. This opens the area for host-based metabolic strategies to inhibit viruses as an additional way

74   other than direct-acting antivirals to weaken the viral replication by metabolic intervention.

75   In this study, we performed plasma proteomics targeting 92 plasma proteins related to

76   inflammation and plasma untargeted metabolomics followed by immune phenotyping of the

77   lymphocyte and monocyte cell population towards the metabolite transporters. We also

78   performed quantitative untargeted proteomics studies *in vitro* by infecting lung, liver, kidney, and

79   colon cells with the SARS-CoV-2 virus to understand the viral-induced metabolic rewiring. We

80   also modulate the key metabolic pathways identified in the patient-based metabolomics data and

81   cell-model based quantitative proteomics data to regulate the viral reproduction. Our clinical and

82   experimental studies thus provided an account of metabolic control during SARS-CoV-2

83   infection that can aid antiviral therapeutics in COVID-19 through metabolic perturbation.

84

Results

Patient characteristics

87   The study population included healthy controls (HC, n=31), SARS-CoV-2 PCR positive

88   hospitalized-mild (mild, O_2 consumption<4lit/min, n=29) and hospitalized-severe (severe, O_2

89   consumption≥4lit/min, n=12) patients. The mild and severe groups were matched by gender

90   (male: 79% vs 91%, p=0.6514), BMI [median (IQR): 29 (25-31) vs 28 (25-34); p=0.8622] and

91   age [median (IQR): 57 (44-63) vs 57 (52-69); p=0.2831]. The HC has significantly lower age

92   [median (IQR): 48 (46-55)], lower BMI [median (IQR): 24 (21-25)] (Table S1). The IgG CoV-2

93   antibody test showed 10 of the HC were CoV-2 antibody positive (HC-CoV-2 Ab+, Fig S1).

94 Among the COVID-19 patients, the classical co-morbidities were observed at 45% (13/29) in

95 mild and at 66% (8/12) in severe (p=0.3058). The samples were collected within median (IQR) 2

96 (2-3.5) days of hospitalization [median (IQR) mild: 2 (1-3) and severe 3 (2-4); p=0.1170].

97 **Plasma proteomics identified distinct clusters of HC and COVID-19 individuals**

98 We performed targeted proteomics analyses (secretome) looking at 92 plasma proteins involved

99 in inflammatory responses. As expected, several cytokines and chemokines were significantly

100 elevated in COVID-19 patients (mild and severe) compared to HCs (HC and HC-CoV-2 Ab+),

101 including IL-6 (Fig 1a). Pathway enrichment analyses of the proteins that were significantly

102 changed between HCs and COVID-19 patients revealed that the majority of altered proteins were

103 involved in cytokine-cytokine receptor interaction and chemokine signaling, followed by

104 intestinal network for IgA production, IL-17 signaling pathway, and Toll-like receptor signaling

105 pathway; to name the top five pathways (Fig 1b). Interestingly, 11 proteins were altered between

106 the mild and severe COVID-19 patients (Fig 1a and 1c): hepatocyte growth factor (HGF),

107 pleiotrophin (PTN), the chemokines CXCL12, CXCL13, and CCL23 (also known as macrophage

108 inflammatory protein 3 or MIP-3), monocyte-chemotactic protein (MCP-3, also known as

109 CCL7), interleukin 12 (IL-12), tumor necrosis factor-like weak inducer of apoptosis (TWEAK),

110 vascular endothelial growth factor A (VEGFA), angiopoietin 2 (ANGPT2), and Fas ligand

111 (FASLG) (adj p<0.05). The majority of these proteins were elevated in COVID-19 patients with

112 highest levels in the severe group, except for IL-12 which was increased in the mild group

113 compared to both severe group and HCs (Fig 1a and 1b). Further, FASLG followed the opposite

114 trend of being lower in COVID-19 patients compared to HC and lowest in severe COVID-19

115 patients. This data showed that although IL-12 levels were increased in COVID-19 patients

116   compared to HC, the COVID-19 severe patients had reduced IL-12 compared to the mild

117   patients.

Distinct amino acid and carbohydrate profile in COVID-19 patients

119   The plasma metabolomic profile followed a pattern similar to the plasma proteomics. However,

120   no metabolites were significantly different between HC and HC-CoV-2 Ab+ (adj p>0.05).

121   Therefore we combined the two groups as HC for further analysis. The distribution of all samples

122   for metabolite enrichment showed a fair separation between samples of HC and COVID-19

123   patients (Fig 2a). Differential analysis between COVID-19 patients and healthy individuals after

124   adjusting for age, gender, and BMI identified 444 significantly regulated metabolites (adj

125   p<0.05), many of which are lipids followed by amino acids (Fig 2b). Metabolite set enrichment

126   analysis of the significant metabolites (adj p<0.05) identified amino acid-related pathways were

127   most predominantly affected during infection, as shown in Fig 2c. Hierarchical clustering of the

128   metabolites showed two clusters that had distinct enrichment patterns in COVID-19 infected

129   patients compared to HCs (Fig 2d). Among these, amino acids such as glycine, proline,

130   tryptophan, alanine, histidine, glutamine and arginine had lower levels in COVID-19 patients,

131   while glutamate, aspartate and phenylalanine had higher levels (Fig 2d and S2) as also observed

132   in earlier studies [1, 3, 9]. Interestingly, metabolites of the central carbon metabolism including

133   glycolysis (glucose, 3-phosphoglycerate, pyruvate, lactate) and tricarboxylic acid (TCA) cycle

134   (citrate, aconitate, α-ketoglutarate) showed distinct changes when comparing HCs and COVID-

135   19 patients and the different COVID-19 disease states (Fig S3).

Severe COVID-19 patients show a distinct metabolic profile with mannose as a biomarker

137   Next, we aimed to identify the metabolic signature in COVID-19 severe patients. Statistical

138   analysis found 88 metabolites that significantly differed in the severe group compared to mild

139   samples. Hierarchical clustering of the metabolites showed two clusters with moderately distinct

140   enrichment patterns in severe samples compared to mild samples (Fig 3a). Metabolic pathway

141   enrichment analysis of the significant metabolites by IPA showed that several amino acid-related

142   pathways, IL-12 signaling and production in macrophages and insulin signaling pathways were

143   mainly dysregulated in the severe patient samples compared to mild ones (Fig 3b). Next, we

144   sought to identify biomarkers that differentiate the severe and mild samples. Using R package

145   MUVR that is suitable for small sample size, a total of eight metabolites were identified as

146   biomarkers (Fig S4). After adjusting for age, gender and BMI, seven remained significant of

147   which four had higher abundance and three had lower abundance in COVID-19 severe patients

148   compared to the mild ones (Fig 3c and S4). Mannose was identified as one of the biomarkers of

149   disease severity, being upregulated in COVID-19 infection and also in severe patients compared

150   to mild ones. COVID-19 infection was associated with increased glutamate levels while glucose

151   and mannose were determinants of the severity of the disease (Fig 3d and 3e). This data suggests

152   alterations in the glycolysis/gluconeogenesis, glutaminolysis and mannose metabolism in

153   COVID-19 patients irrespective of severity.

154   **Increased mannose-binding lectin (MBL) in COVID-19 patients without any correlation**

155   **with mannose levels**

156   Plasma mannose can bind to several C-type lectins (*e.g.* MBL) and play an important role in viral

157   pathogenesis by recognizing glycans present in the viral envelope and subsequently activating

158   antiviral immune response and T-cells [10, 11]. Our targeted secretome data identified the C-type

159   lectin receptor signaling pathway as one of the top 28 ranked protein pathways that were

160   significantly changed between HCs and COVID-19 patients (Fig 1b). We, therefore, measured

161   plasma levels of soluble MBL using ELISA and observed an increase in COVID-19 patients

162   compared to HC. Strikingly, there was no significant difference between the mild and severe

163   COVID-19 patients, but the HC-CoV-2 Ab+ individuals showed increased MBL levels compared

164   to HC (Fig 3f). This data shows a prominent elevation of MBL during COVID-19 infection that

165   can persist over a prolonged duration of time after recovery. We did not observe any correlation

166   between MBL and mannose in COVID-19 patients [Spearman correlation: 0.1437 (95%CI: -

167   0.1806 - 0.4399)]. We, therefore, speculate that elevated MBL might not directly be the

168   consequence of the higher plasma mannose levels but regulated by SARS-CoV-2.

169   **Immune phenotyping of glucose, glutamate, and mannose transporters**

170   Metabolite transporters are known to dictate immune cell activity by controlling access to

171   nutrients, thereby maintaining cellular homeostasis [12]. Therefore, we next measured the

172   expression of glucose/mannose and glutamate transporters that were associated with

173   SARS-CoV-2 infection or disease severity, GLUT1 (SLC2A1) and xCT (SLC7A11) respectively,

174   in PBMCs of HC (n=19), HC-CoV-2 Ab+ (n=9) and COVID-19 patients: mild (n=21) and severe

175   (n=11) using flow cytometry. The relative frequency of lymphocytes significantly decreased in

176   COVID-19 patients than HCs, which was more prominent in severe patients (Fig 4a). In total

177   lymphocytic populations the CD3$^+$ T-cells were significantly reduced in COVID-19 severe

178   patients compared to mild patients and HCs (Fig 4b). Although there was no difference in total

179   monocyte frequencies, we observed a mild increase in the frequency of intermediate monocytes

180   and a significant decrease in non-classical monocytes in the COVID-19 patients than HCs (Fig 4a

181   and 4b). This highlights the potential role of monocytes in COVID19 as was also recently

182   reported in single-cell transcriptomics data [4] and functional analysis on COVID-19 patient

183   monocytes [13].

184   More than 98% CD8$^+$ T-cells expressed GLUT1, and the surface expression of GLUT1 was

185   significantly higher in COVID-19 severe patients than mild and HCs (Fig 4c and 4d). We also

186   found increased frequencies of intermediate and non-classical monocytes expressing GLUT1 and

187   significantly higher surface expression of GLUT1 on the intermediate monocytes of COVID-19

188   severe patients compared to HCs (Fig 4c and 4d). While all classical and intermediate monocytes

189   expressed xCT, we observed decreased frequency of non-classical monocytes expressing xCT in

190   COVID-19 patients compared to HCs (Fig 4e). The surface expression of xCT was higher in

191   classical and intermediate subpopulations of COVID-19 patients than HCs (Fig 4f), emphasizing

192   the role of metabolite transporters in monocytes in COVID-19 infection and disease severity.

193   **SARS-CoV-2 regulates central carbon metabolic pathways in a cell-type-specific manner**

194   Our previous study, together with other observational studies, indicate that SARS-CoV-2

195   infection causes dysregulation of PI3K/Akt/mTOR and HIF-1 signaling pathway [7, 14, 15], and

196   affect mitochondrial functions [13, 16]. Based on these findings, we hypothesized that the altered

197   extracellular glucose, mannose and glutamate levels are due to dysregulated carbohydrate

198   metabolism and mitochondrial function. Therefore, to identify the acute host cellular response to

199   the SARS-CoV-2 infection we infected different human cell lines including Calu-3 (lung), Caco-

200   2 (colon), 293FT (kidney) and Huh7 (liver) followed by untargeted quantitative proteomics 24

201   hours post-infection (hpi) at multiplicity of infection (moi) of 1 [17]. Differential protein

202   abundance analysis identified 6462 proteins differentially expressed in Calu-3, 177 in Caco-2,

203   four in Huh7 and none in 293FT following 24 hpi. This data indicated an acute response

204   predominantly in the lung cells that are the primary site of SARS-CoV-2 infection. The protein

205   set enrichment analysis restricted to metabolic pathways identified that most of the highly

206   abundant proteins in infected Calu-3 cells belonged to pentose phosphate pathway (PPP), fructose

207 and mannose metabolism, as well as amino acid biosynthesis (Fig 5a). Proteins detected at a

208 lower level in the infected cells mainly belonged to TCA cycle, oxidative phosphorylation and

209 N-glycan biosynthesis (Fig 5a). Parallelly, in patients' metabolomic analysis we observed

210 unbalanced levels of glycolysis, fructose and mannose metabolism and TCA cycle intermediates

211 (Fig 2d and 3d). We, therefore, focused our analysis on the proteins (n=78) that are a part of

212 glycolysis/gluconeogenesis, fructose and mannose metabolism and TCA cycle (KEGG Human

213 2019) (Fig 5b). A clear change in metabolic poise was observed upon SARS-CoV-2 infection in

214 Calu-3 cells where a majority of the significantly upregulated proteins belonged to

215 glycolysis/gluconeogenesis and fructose and mannose metabolism while most of the proteins of

216 the TCA cycle were significantly downregulated (Fig 5b and S5). However this phenomenon was

217 not observed in the other three cell culture models (Fig S6). Only two out of 177 proteins

218 identified were significantly different in Caco-2 cells (ACSS1 and PFKFB4) and no differences

219 were observed in Huh7 cells out of the four identified proteins in the three pathways mentioned

220 above (Fig S6). This shows that Calu-3 cells, which are lung epithelial cells, have a distinct

221 metabolic modulation caused by SARS-CoV-2 infection. Interestingly, although all the

222 mitochondrial TCA cycle enzymes were downregulated, cytosolic enzymes such as MDH1,

223 IDH1, ACO1 and ACLY that convert TCA cycle intermediates outside the mitochondria were

224 upregulated in infected Calu-3 cells (Fig 5c). This points towards dysfunctional mitochondria

225 caused by COVID-19 infection. Alterations in mitochondrial DNA (mtDNA) copy number in

226 circulating blood cells can serve as a surrogate for mitochondrial dysfunction [18]. Indeed in our

227 patient cohort we observed a decreasing trend of the mtDNA copy numbers with the disease

228 severity (Fig 5d). In addition to changes in glucose and glutamate (Fig 3d), we also observed a

229 significant increase in metabolites such as pyruvate, lactate and α-ketoglutarate (more

230 pronounced in mild patients) and decrease in citrate and aconitate in COVID-19 patients

11

231   compared to HCs (Fig S6). This indicated an impact of SARS-CoV-2 infection on glycolysis and

232   glutaminolysis to meet biosynthetic and bioenergetic demands. In order to determine the

233   requirement of glycolysis and glutaminolysis for optimal replication of SARS-CoV-2 in Calu-3

234   cells, we blocked these pathways using 2-DG and 6-diazo-5-oxo-L-norleucine (DON)

235   respectively (Fig 5e) following infection. Infectivity of SARS-CoV-2, quantified as relative *E-*

236   *gene* levels in cells lysates, showed ~50-fold decrease in 2-DG treated cells and >100-fold

237   decrease in DON treated cells (Fig 5f). This was also corroborated with virus production in the

238   cell culture supernatant, quantified by viral *E-gene* levels that decreased by more than 2log10

239   RNA copies/ml in both 2-DG and DON treated cells compared to untreated cells (Fig 5g). While

240   several studies have shown the role of glycolysis on SARS-CoV-2 infection [14, 15], so far there

241   is no direct evidence linking the role of glutaminolysis to replication and spread of SARS-CoV-2

242   and here we show for the first time that both glutaminolysis and glycolysis are essential for

243   SARS-CoV-2 infection.

244   **Role of increased sugars in SARS-CoV-2 infection and the complement system in vitro**

245   To understand the role of sugars like glucose and mannose in SARS-CoV-2 infection, we

246   performed *in vitro* infection assays in Calu-3 cells with varying media concentrations of glucose

247   (11.1, 22.2, 44.4mM) and mannose (0, 11.1, 22.2, 44.4mM) with 0.001moi. We did not observe

248   any statistically significant difference in virus production in the supernatant in the

249   glucose/mannose concentrations tested (p>0.05, Fig 6a), while we found a significant reduction

250   in viral *E-gene* expression at cellular level with high glucose concentration of 44.4 mM (Fig 6b,

251   p<0.05). Supplementation with high mannose did not cause any significant change in expression

252   of the *E-gene*. Overall, our data indicate that increased glucose levels but not mannose levels has

253   an effect on viral replication *in vitro*.

254   Several studies, including ours, have shown an effect of SARS-CoV-2 infection on complement

255   and coagulation cascades and thrombosis as unique features of COVID-19 infection [7, 19, 20].

256   Temporal transcriptomic and proteomic profiling of SARS-CoV-2 infected Huh7 cells have also

257   revealed an upregulation of proteins involved in complement activation at a later stage of

258   infection [20]. Since complement component C4 is part of the cascade that leads to C3 activation

259   in the classical and MBL pathways, we also studied whether increased glucose/mannose in the

260   media during infection affected upstream events of C3 activation (Fig 6c). This was performed by

261   determining gene expression levels of MBL associated serine protease 2 (*MASP2*) and

262   complement *C4* in Calu-3 cells infected with SARS-CoV-2 (moi 0.001) at different

263   concentrations of glucose and mannose. Mannose availability in the culture medium slightly

264   reduced gene expression of *MASP2* in mock-infected that increased upon infection. However no

265   significant changes in *MASP2* expression were observed compared to the basal condition (Fig

266   6d). An interesting trend was observed with *C4* gene expression (Fig 6e). In mock-infected cells,

267   we observed an increase in *C4* expression with increasing glucose concentrations that decreased

268   dose-dependently following addition of mannose. In the infected cells, a general increase was

269   detected in *C4* expression compared to mock that also followed a similar trend in different

270   glucose and mannose concentrations as was observed in mock-infected cells (Fig 6e). Since

271   COVID-19 patients in this study had high plasma concentrations of glucose and mannose, we

272   also measured *MASP2* and *C4* gene expression in whole blood originating from these patients.

273   No change was observed in *MASP2* expression while a significant decrease in C4 expression was

274   observed in COVID-19 severe patients compared to HCs (Fig 6f and 6g). Combined, *in vitro* and

275   *in vivo* data indicate a potential role of extracellular glucose and mannose concentrations in

276   complement   activation.   Further,   our   data   suggest   that   SARS-CoV-2   infection   can

277   transcriptionally repress the C4 system in the presence of high mannose, but this needs further

278   mechanistic investigations.

279

280   **Discussion**

281   In this study, we used metabolomics, proteomics and immunophenotyping to observe the effect

282   of SARS-CoV-2 infection on metabolic dysregulation in COVID-19 patients and performed *in*

283   *vitro* infection in four different cell models to find out potential host metabolic regulation during

284   acute SARS-CoV-2 infection. Our study has shed light on the role of monocytes, especially in

285   severe COVID-19 disease. As expected, COVID-19 patients presented a cytokine storm.

286   Interestingly IL-12 plasma levels were decreased in severe compared to mild COVID-19 patients.

287   Among the carbohydrates, plasma mannose emerged as a biomarker for disease severity, but *in*

288   *vitro* assays showed no effect of mannose on viral replication. Furthermore, host cellular response

289   following SARS-CoV-2 infection identified a strong acute metabolic adaptation in the lung

290   epithelial cells (Calu-3) by modulating central carbon metabolism and indicative of mitochondrial

291   dysfunction that is also observed in severe COVID-19 patients. Glycolysis and glutaminolysis are

292   essential for virus replication and metabolic perturbations of these processes can impede SARS-

293   CoV-2 and could be an attractive antiviral strategy. Finally, SARS-CoV-2 potentially regulates

294   the *C4* system transcriptionally in the presence of carbohydrates.

295   As reported, the cytokine storm syndrome is evident in COVID-19 patients [21, 22]. Several

296   plasma pro-inflammatory cytokines including IL-6 were elevated in both mild and severe

297   COVID-19 patients. In our study, though we observed higher levels of soluble IL-12 in mild

298   patients, the severe patients showed IL-12 levels similar to HCs. Elevated plasma levels of IL-12

299   have also been described in hospitalized patients infected with other coronaviruses, such as

300   SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) [23, 24]. However,

301   to our knowledge, no associations of IL-12 levels with disease severity were reported in these

302   infections. In a recent study on COVID-19 patients on ventilators, the reported low level of IL-12

303   could be caused by impaired monocytes and affect natural killer (NK) cell functions [25, 26]. *In*

304   *vitro* studies on IL-12 administration have shown enhanced host cellular responses that generally

305   promote virus clearance and host recovery from infection [27]. IL-12 also plays a critical role in

306   viral immunity by activating the NK cells and promoting differentiation of Th1 CD4$^+$ T-cells.

307   Based on the different levels of IL-12 in COVID-19 patients at varying disease stages, its role in

308   disease severity needs further attention.

309   Both DNA and RNA viruses rewire host cell metabolism by altering central carbon metabolic

310   pathways   such   as   glycolysis,   gluconeogenesis,   PPP,   TCA   cycle,   amino   acid

311   synthesis/degradation, and lipid synthesis. In our metabolomics data, we observed increased

312   glucose, pyruvate and lactate levels in the plasma of COVID-19 patients compared to HCs

313   indicative of toxic metabolic dysregulation during acute phase of infection. Under aerobic,

314   standard growth conditions, primary mammalian cells use glucose for generation of ATP.

315   Glucose is enzymatically broken down to pyruvate that can feed into the TCA cycle in the

316   mitochondria ultimately driving electron transport chain to generate ATP with the help of

317   oxygen.

318   Viral infections including SARS-CoV-2 are known to enhance the glycolytic flux and increase

319   the production of lactate from pyruvate [6, 28, 29]. Viruses can target glycolysis by regulating

320   glucose transporters' expression, which is also vital for immune cell activation during host

321   cellular response [30, 31]. Increased GLUT1 does not only result in higher glucose uptake but

322   also gives rise to increased PPP intermediates that enhance nucleotide pool required for viral

323   replication [5]. GLUT1 transports glucose, mannose, glucosamine and docosahexaenoic acid

15

324   across the cell membrane [32]. We observed a significant increase in surface expression (MFI) of

325   GLUT1 in CD8^{+} T-cells and intermediate monocytes in COVID-19 severe patients. We also

326   measured surface expression of xCT, a cystine/glutamate antiporter that exchanges glutamate for

327   cystine essential for maintenance of redox balance. While there was a decrease in frequency of

328   only non-classical monocytes expressing xCT in COVID-19 patients, a significant increase in

329   surface expression of xCT in classical and intermediate monocytes COVID-19 patients was

330   noted. To the best of our knowledge, the association of xCT expression in respiratory viral

331   diseases has not been studied before and our data for the first time highlights the crucial role of

332   expression of xCT in monocytes. A growing body of evidence highlights the potential role and

333   metabolic status of monocytes in COVID-19 disease severity [4, 13, 33]. Combining all the data,

334   metabolite transporters, xCT and GLUT1, play an essential role in disease severity that could be

335   linked to higher plasma glucose and mannose levels. The specific changes of metabolic

336   transporters were more prominent in monocytes, indicating that metabolic profile of the different

337   monocytic sub-populations could contribute to mediating the severity of the disease.

338   Plasma mannose emerges as a robust biomarker of disease severity and can also lead to activation

339   of the lectin complement system at a later stage of infection [20]. In addition to mannose, we also

340   observed increased levels of MBL in both COVID-19 patients and convalescent patients

341   compared to the COVID-19 negative individuals. However, no correlation between plasma MBL

342   and mannose were observed in COVID-19 and these two can very well be independent factors.

343   Monomeric mannose is a crucial element of N-linked glycosylation of proteins. Recent studies

344   reported that plasma mannose levels were an indicator of glycogenolysis as well as glucose

345   tolerance and associated with the future risk of developing chronic diseases such as type 2

346   diabetes, cardiovascular diseases, and albuminuria [34, 35]. In our study cohort, the incidence of

347   type 2 diabetes was low in all study groups and can therefore not explain the high plasma

348   mannose levels in COVID-19 patients. This further strongly suggests that increased mannose is

349   an effect of SARS-CoV-2 infection. However, in light of recent research indicating a possibility

350   of a bidirectional link between SARS-CoV-2 and diabetes, it is tempting to speculate that

351   increased mannose has a role to play in new-onset diabetes after SARS-CoV-2 infection [36-39].

352   Mannose can bind to several sugar-binding proteins, called lectins, including C-type lectins, such

353   as MBL, mannose receptor, and DC-SIGN. These receptors recognize carbohydrates, particularly

354   on the surface of microorganisms leading to activation of the complements cascade and

355   phagocytosis [10]. Although N-linked mannose residues have been identified on SARS-CoV-2

356   Spike protein, it is improbable that the elevated plasma mannose levels in the patients would be

357   derived from the virus itself [40]. The processing of endogenous glycoconjugates and their

358   subsequent efflux from the cells are currently thought to be responsible for mannose levels in the

359   blood and steady-state maintenance [41]. Interestingly, a recent study by Heindel et al. describes

360   endogenous high mannose levels as a key mediator of influenza virus-induced pathogenesis and

361   disease severity [42]. High mannose is induced through unfolded protein response pathway and

362   the influenza virus-infected cells are recognized in a high mannose-dependent manner by MBL.

363   Finally, authors state that MBL signaling contributes to disease severity through complement

364   cascade activation and inflammatory response. High mannose and/or high MBL could thus

365   dysregulate the immune system and lead to severe damage associated with disease severity [42].

366   Activation of the complement is one of the features seen in COVID-19, as described in earlier

367   studies [7, 43]. In concordance with that and with Heindel *et al.*, we also noticed increased

368   plasma MBL in COVID-19 and even in healthy convalescent controls compared to HC. This

369   increase in MBL could potentially regulate the complement cascade.

370   In the *in vitro* Calu-3 infection model, most of the proteins from carbohydrate metabolism and

371   PPP were upregulated while most of the proteins of TCA cycle, oxidative phosphorylation, and

372   fatty acid metabolism were downregulated in infected cells compared to the mock-infected

373   controls (Fig 5). Further delineation of the pathways indicated an inefficient mitochondrial

374   metabolism as majority of the TCA cycle enzymes were downregulated in the infected cells. This

375   was in line with the decreased mtDNA copy numbers in severe COVID-19 patients (Fig 5f)

376   indicating a possible mitochondrial dysfunction as reported previously [13, 44-46].

377   The metabolism and concentration of sugars and amino acids such as glucose, mannose,

378   glutamine and glutamate among others, play an important role in cellular metabolic homeostasis

379   and are targeted by viruses for their replication. Recent studies have shown that elevated

380   glycolysis favors SARS-CoV-2 infection and replication [14, 15]. While glutaminolysis has been

381   implicated as a carbon source for other human DNA and RNA viruses [5]. Our data shows for the

382   first time that glutaminolysis is also crucial for SARS-CoV-2 infection and replication. The

383   inhibition of glutaminolysis has a larger effect on viral replication and production compared to

384   the inhibition of the glycolysis in lung cell model (Fig 5f and 5g). Glutaminolysis is a process of

385   converting glutamine to TCA cycle intermediates and also essential for biosynthesis of proteins,

386   lipids and nucleic acids. Some viruses (e.g. Herpes simplex virus 1, human cytomegalovirus,

387   Hepatitis C virus etc) use glutamine as an anaplerotic substrate to replenish TCA cycle via

388   generation of α-ketoglutarate [29, 47]. Recently, researchers have proposed that the metabolic

389   reprogramming of glutamine in SARS-CoV-2 can trigger pathogenesis. They further

390   hypothesized that metabolic intervention of glutaminolysis could be an antiviral strategy for

391   COVID-19 [47-49]. Although the exact underlying mechanism is unknown, our *in vitro* study

392   shows that SARS-CoV-2 replication depends on both glycolysis and glutaminolysis.

393   Finally, to elucidate the effects of extracellular glucose and mannose in both infection of

394   SARS-CoV-2 and its impact on the complement cascade, we established an *in vitro* infection set

395   up with varying media concentrations of glucose and mannose post-infection. Virus production in

18

396 the cell culture supernatant was unaffected by both glucose and mannose concentrations.

397 However, high extracellular glucose decreased viral infectivity, measured as relative expression

398 of viral *E-gene* in cells. This contradicts the earlier finding that an increase in glucose

399 concentration aids in virus replication [14]. However, it is to be noted, the observations by Codo

400 et al. were made in peripheral monocytes while we performed our experiments in Calu-3 cells.

401 Despite this fact, several studies attempted to isolate infectious virus particles from blood but

402 failed [50, 51] and we were not able to find any residual viral RNA in patients' whole blood cells.

403 Also, blood cell populations do not express the ACE2 or TMPRSS2 (Blood Atlas in the Human

404 Protein Atlas [52]). Thus the infectivity of the blood cell population including lymphocytes and

405 monocytes needs careful consideration [53].

406 In conclusion, our patient based multi-omics studies and *in vitro* analysis emphasizes the need to

407 understand the host metabolic reprogramming due to acute SARS-CoV-2 infection. Among other

408 factors, the role of carbohydrate and amino acid transporters, mainly in the monocytic-

409 macrophage lineages, under the altered central carbon metabolism regulated by

410 AKT/mTOR/HIF-1 signaling may potentially define disease severity. The metabolic alteration in

411 glucose, mannose, lactate, pyruvate, and glutamate in severe COVID-19 cases need further

412 clinical considerations. Changes in these metabolites might have a sustained effect on insulin

413 resistance, type 2 diabetes, neurocognitive impairments, and multiorgan failure which is already

414 reported in COVID-19 infection.

415

416 **Methods**

417 **Experimental Model and Subject Details**

418 **Study designing, patients.** The COVID-19 patients (n=41) who were PCR positive and

419 hospitalized in May 2020, were recruited from the South Hospital, Stockholm. Based on the

420 oxygen requirements the patients were categorized into, 1) Hospitalized-mild (O_2 consumption

421 <4lit/min) and 2) Hospitalized-severe (O_2 consumption ≥4lit/min). The exclusion criteria

422 included known liver cirrhosis, severe renal insufficiency (estimated eGFR≤30mL/min/1.73m^2),

423 chronic obstructive pulmonary disease, and chronic lung disease leading to habitual SpO$_2$≤92%.

424 Additionally, COVID-19 PCR negative samples (HC herein, n=31) were also collected. IgG

425 antibody was tested on the HC samples as described previously [51] and ten samples turned out

426 to be SARS-CoV-2 Ab positive further defined as HC-CoV-2 Ab+. The study was approved by

427 regional ethics committees of Stockholm (dnr 2020-01865). All participants gave informed

428 consent. The patient identity was anonymized and delinked before analysis.

429 **Cell lines and viruses.** Human colon adenocarcinoma cell line, Caco-2, and lung

430 adenocarcinoma cell line, Calu-3 (ATCC$^®$ HTB-55^{TM}), were purchased from CLS Cell Lines Service

431 GmbH, Germany and LGC Standards, UK, respectively. Hepatocyte-derived carcinoma cell line,

432 Huh7, was obtained from Marburg Virology Lab, Philipps-Universität Marburg, Marburg,

433 Germany matching the STR reference profile of Huh7 [54], and human embryonic kidney cell

434 line, 293FT (Invitrogen). SARS-CoV-2 virus used in this study was the first virus isolated from a

435 Swedish patient[55].

436 **Method Details**

437 **Materials.** All information regarding reagents, antibodies, and critical commercial kits are listed

438 in Table S2.

439 **IgG Antibody detection against SARS-CoV-2.** In brief, 96-well ELISA plates (Nunc

440 MaxiSorp, ThermoFisher Scientific) were coated with SARS-CoV-2 N protein, diluted 1:1000 in

441   50 mM sodium carbonate pH 9.6 (1 ug/ml final concentration), at 4°C overnight. The plates were

442   then blocked for 1h using PBS containing 1% BSA and 2% goat serum (dilution buffer) at 37°C.

443   Serum samples were serially diluted from 1/200 to 1/6400 in dilution buffer and incubated on the

444   plates at 37°C for 1h. Antibodies to the N protein was detected by incubation for 1h at 37°C with

445   anti-human IgG peroxidase (1:30,000, Sigma). The plates were washed three times with PBS

446   with 0,05% Tween-20 between each step. The bound conjugate was visualized using

447   Tetramethylbenzidine (Sigma, USA) substrate. The OD was measured at 450 nm with subtraction

448   of background at 650 nm using a TECAN Infinite M200 plate reader (Tecan, USA).

449   **Plasma inflammation profiling and metabolomics.** The plasma inflammation profiling was

450   performed using proximity extension assay technology targeting 96 inflammation markers by

451   Olink Immuno-Oncology panel (Olink, Sweden). Plasma untargeted metabolomics was

452   performed by Global Metabolomics (HD4) in Metabolon, NC, US as described by us recently

453   [56]. The metabolomics method is ISO 9001:2015 certified and the lab is accredited by the

454   College of American Pathologist (CAP), USA.

455   **Statistical and bioinformatics analysis.** For targeted proteomics data analysis, we used Mann-

456   Whitney U through the R package stats v3.6.1 for pair-wise analysis as the data was not normally

457   distributed. For metabolomics data, dimensionality reduction of all samples were performed with

458   Uniform Manifold Approximation and Projection (UMAP) using R package umap v0.2.6.0 [57].

459   Reduced dimensions of the data were plotted in 2D space using R package ggplot v3.3.2[58]. The

460   metabolite measurements were log2 scaled before differential analysis. Differential analysis was

461   done using R/Bioconductor package limma v3.42.2 [59]. R package MUVR v0.0.973 [60] was

462   used for biomarker discovery. It is a software package that performs predictive multivariate

463   modeling by integrating minimally biased variable selection procedure into repeated double

464 cross-validation architecture. Random forest core modeling was selected from the package for

465 biomarker identification. Minimal-optimal variables selected by the model were considered as

466 biomarkers. Correlation analysis was performed using corr.test function from the package psych

467 v1.9.12.31 based on Spearman rank correlations. Untargeted protein raw data abundance was first

468 filtered for empty rows and quantile normalized. Differential expression analysis was performed

469 with R package limma v3.42.2 2 [59] to determine proteins with differential abundance.

470 Functional analysis of the proteins was performed using enrichr module of python package

471 GSEAPY v 0.9.16 (https://pypi.org/project/gseapy/) [61, 62], where all the quantified proteins

472 were considered as background. KEGG 2019 Human gene-set library downloaded from Enrichr

473 web resources was used for the enrichment test for molecular pathway analysis. Functional

474 analysis of the metabolites was carried out using Ingenuity Pathway Analysis (IPA) software

475 package. All reported p-values were corrected (*Benjamini-Hochberg) throughout* and considered

476 statistically significant if <0.05 unless otherwise stated.

477 **Data visualization.** Heatmaps were generated using R/Bioconductor package ComplexHeatmap

478 v2.2.0 [63]. Violin plots, box plots, bubble plots and volcano plots were made using geom_violin,

479 geom_boxplots, geom_point objects from R package ggplot2 v3.3.2 respectively. A density plot

480 was created using the function stat_density_ridges function from the R package ggridges v0.5.2.

481 Alluvial plot was made with geom_alluvium function from ggalluvial v0.11.3 R package.

482 Correlation pairs plot was made using ggpairs function from GGally v2.0.0 R package. Network

483 input files were made using R 3.6.3. The network was represented using Cytoscape ver 3.6.1

484 (https://cytoscape.org/). Protein-protein interactions were retrieved from STRING Db (v5.0)

485 (https://string-db.org/). Only interactions with high confidence (interaction score>0.7) from

486 databases and experiences were kept.

487 **Flow Cytometry.** Peripheral blood mononuclear cells (PBMCs) were subjected to flow

488 cytometry analysis. Samples were thawed in 37°C water bath and washed with flow cytometry

489 buffer (PBS+2% FBS+2mM EDTA). All samples were stained with Live/Dead fixable near IR

490 dye (Invitrogen), and cell surface markers were detected by incubating cells with relevant

491 antibodies for 20min on ice in flow cytometry buffer (antibodies listed in Table S2). All cells

492 were fixed with 2% paraformaldehyde before acquiring a BD FACS Symphony flow cytometer

493 (BD Bioscience). Compensation setup was performed using single-stained controls prepared with

494 antibody-capture beads: Anti-Mouse Ig, κ/Negative Control Compensation Particles Set (BD

495 Biosciences) for mouse antibodies, AbC™ Total Antibody Compensation Bead Kit (Invitrogen)

496 for rabbit antibodies and ArC™ Amine Reactive Compensation Bead Kit (Invitrogen) for use

497 with LIVE/DEAD™ Fixable dead cell stain kits. Flow cytometry data were analyzed and

498 compensated with FlowJo 10.6.2 (TreeStar Inc), Prism 8 (GraphPad Software Inc).

499 **Measurement of mtDNA copy number.** Mitochondrial DNA (mtDNA) copy number was

500 measured using an Absolute Human Telomere Length and Mitochondrial DNA Copy Number

501 Dual Quantification qPCR Assay Kit (ScienCell Research Laboratories, USA). Each 15µl qPCR

502 reaction contained 7.5µl QuantiNova SYBR green (Qiagen, Sweden), 1µl single copy reference

503 (SCR) and mitochondrial primers, 0.1µl ROX (passive reference dye), 1.9µl DNA/RNA free

504 water and 5µl (1ng/µl) template DNA. Thermal cycle profile comprised incubation at 50°C for

505 2min and 95°C for 10min before running 40 thermal cycles (95°C for 15s, 54°C for 45 s and

506 72°C for 45s). mtDNA and SCR primers were run on separate plates and each plate contained a

507 serially diluted DNA sample to calculate PCR efficiency. A reference genomic DNA was added

508 on each plate with known mtDNA copy number (925 copies). Each sample was run in duplicates,

509 and relative mtDNA copy number and SCR to reference was calculated by ΔCT (CT target

23

510   sample - CT reference sample), after adjusting PCR efficiency using the Pfaffl method [64].

511   Finally mtDNA copy number per diploid cell of target sample to reference sample was calculated

512   by (2^- ΔΔCT x 925), where ΔΔCT is ΔCT mtDNA/ΔCT SCR.

513   **SARS-CoV-2 infection and proteomics.** In 6-well plate, Calu-3 cells were grown for 72h, and

514   Huh7, 293FT, and Caco-2 cells were grown for 24h in DMEM-high glucose (Sigma-Aldrich,

515   USA) supplemented with 10% FBS (Gibco, USA). The cells were either mock-infected in

516   medium only or infected with SARS-CoV-2 at a multiplicity of infection (moi) of 1 in DMEM-

517   high glucose supplemented with 5% FBS. After 1h the inoculum was removed and was

518   replenished with 2mL of fresh DMEM-high glucose containing 5% FBS. Twenty-four hours

519   post-infection (hpi) the supernatant was removed, and the cells were scrapped in 1mL PBS,

520   centrifuged and the washed cell pellets were lysed in 100μL of 2% SDS-lysis buffer (50mM Tris-

521   Cl, 150mM NaCl, 2% SDS and 1mM EDTA) freshly supplemented with protease inhibitor

522   cocktail, phosphatase inhibitor and 1mM DTT. The lysates were heated at 92°C for 10min to

523   deactivate the virus, followed by sonication in a water-bath sonicator for 2min to clear the lysate.

524   Lysates were centrifuged at 13,000rpm for 15min and supernatants were collected. Protein

525   estimation was performed by Bio-rad DC protein assay kit (Bio-Rad Laboratories, USA). The

526   supernatant proteins were used for in-solution digestion and TMT-pro labeled proteomics as

527   described by us previously [7].

528   **Metabolic perturbation and virus infection.** Calu-3 cells were seeded in 24-well plate, and

529   after 72h of seeding, the cells were infected with SARS-CoV-2 at moi of 0.001 for 1h. Following

530   infection, the cells were treated with DMEM (Gibco, USA) which contained pyruvate (1mM) and

531   glutamine (4mM) as the basal carbon source and were supplemented with 5% FBS and different

532   concentrations of glucose (11.1mM, 22.2mM and 44.4mM) (Gibco, USA) and keeping basal

24

533 glucose concentration at 11.1mM, different concentrations of mannose (11.1mM, 22.2mM and

534 44.4mM) (Sigma-Aldrich, USA). To inhibit glycolysis and glutaminolysis, following 1hpi (moi

535 0.001) the cells were treated with 2-deoxy-D-glucose (2-DG, 10mM) and diazo-5-oxo-L-

536 norleucine (DON, 200µM) respectively. The supernatants were collected after 24hpi and the cells

537 were lysed in TRI reagent (Zymo Research, USA) and stored in -70°C for RNA extraction.

538 **RT-qPCR Analysis.** The virus production and infectivity were determined by qRT-PCR

539 targeting the viral *E-gene* in the supernatant and RNA extracted from the cells. RNA was

540 extracted using Direct-zol™ RNA Miniprep kit according to manufacturer's instructions (Zymo

541 Research, USA). The supernatant or RNA were directly used for one-step RT-qPCR using

542 PrimeDirect™ Probe RT-qPCR Mix (TaKaRa, Japan) according to manufacturer's instructions.

543 Primer and probe sequences for the viral *E-gene* and human *RNaseP* gene are listed in Table S3.

544 To measure gene expression of *MASP2* and *C4* in patient blood, whole blood was collected in

545 Tempus™ Blood RNA Tubes (Applied Biosystems, USA) and RNA was extracted using

546 Tempus™ Spin RNA Isolation Kit (Invitrogen, USA). Quality and concentration of extracted

547 RNA was measured using Nanodrop ND-2000 (Thermo Scientific, USA).

548 The RNA purified from cells and whole blood was reverse transcribed using a High-Capacity

549 cDNA reverse transcription kit (Applied Biosystems, USA) according to manufacturer's

550 instructions. qPCR reactions were performed using KAPA SYBR Fast qPCR kit (KAPA

551 Biosystems, USA) on an Applied Biosystems™ 7500 Fast qPCR machine. Detailed information

552 on primers is included in Table S3.

553 **Plasma MBL Measurement.** MBL levels in patient plasma were determined using Human MBL

554 Quantikine ELISA Kit (R&D Systems) according to manufacturer's instructions. The optical

25

555   density of each well was determined using NanoQuant Infinite M200 plate reader (Tecan, USA)

556   microplate reader at 450nm with background subtraction at 570nm.

557   **Data and Code Availability.** The scaled normalised metabolomics data can be obtained from the

558   dx.doi.org/ 10.6084/m9.figshare.13336862

559   Proteomics data can be obtained from the ProteomeXchange Consortium via the PRIDE partner

560   repository with the dataset identifier PXD022847.

561   All the codes are available at github: https://github.com/neogilab/COVIDOMICS

562

563

564   **Acknowledgments**

569

570   **References**

571   1.   Thomas T, Stefanoni D, Reisz JA, Nemkov T, Bertolone L, Francis RO, et al. COVID-19

572   infection alters kynurenine and fatty acid metabolism, correlating with IL-6 levels and renal

573   status. JCI Insight. 2020;5(14). Epub 2020/06/20. doi: 10.1172/jci.insight.140327. PubMed

574   PMID: 32559180; PubMed Central PMCID: PMCPMC7453907.

575   2.   Wu D, Shu T, Yang X, Song J-X, Zhang M, Yao C, et al. Plasma metabolomic and

576   lipidomic alterations associated with COVID-19. National Science Review. 2020;7(7):1157-68.

577   doi: 10.1093/nsr/nwaa086.

578   3.   Shen B, Yi X, Sun Y, Bi X, Du J, Zhang C, et al. Proteomic and Metabolomic

579   Characterization of COVID-19 Patient Sera. Cell. 2020;182(1):59-72.e15. Epub 2020/06/04. doi:

580   10.1016/j.cell.2020.05.032. PubMed PMID: 32492406; PubMed Central PMCID:

581   PMCPMC7254001.

582   4.   Su Y, Chen D, Yuan D, Lausted C, Choi J, Dai CL, et al. Multi-omics resolves a sharp

583   disease-state shift between mild and moderate COVID-19. Cell. 2020. doi:

584   10.1016/j.cell.2020.10.037.

585   5.   Mayer KA, Stockl J, Zlabinger GJ, Gualdoni GA. Hijacking the Supplies: Metabolism as a

586   Novel Facet of Virus-Host Interaction. Front Immunol. 2019;10:1533. Epub 2019/07/25. doi:

587   10.3389/fimmu.2019.01533. PubMed PMID: 31333664; PubMed Central PMCID:

588   PMCPMC6617997.

589   6.   Thaker SK, Ch'ng J, Christofk HR. Viral hijacking of cellular metabolism. BMC Biol.

590   2019;17(1):59. Epub 2019/07/20. doi: 10.1186/s12915-019-0678-9. PubMed PMID: 31319842;

591   PubMed Central PMCID: PMCPMC6637495.

592   7.   Appelberg S, Gupta S, Svensson Akusjarvi S, Ambikan AT, Mikaeloff F, Saccon E, et al.

593   Dysregulation in Akt/mTOR/HIF-1 signaling identified by proteo-transcriptomics of SARS-CoV-2

594   infected cells. Emerg Microbes Infect. 2020;9(1):1748-60. Epub 2020/07/22. doi:

595   10.1080/22221751.2020.1799723. PubMed PMID: 32691695; PubMed Central PMCID:

596   PMCPMC7473213.

597   8.   Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, et al. A SARS-CoV-2

598   protein interaction map reveals targets for drug repurposing. Nature. 2020. Epub 2020/05/01.

599   doi: 10.1038/s41586-020-2286-9. PubMed PMID: 32353859.

600   9.   Barberis E, Timo S, Amede E, Vanella VV, Puricelli C, Cappellano G, et al. Large-Scale

601   Plasma Analysis Revealed New Mechanisms and Molecules Associated with the Host Response

602   to SARS-CoV-2. Int J Mol Sci. 2020;21(22). Epub 2020/11/20. doi: 10.3390/ijms21228623.

603   PubMed PMID: 33207699.

604   10.   Kerrigan AM, Brown GD. C-type lectins and phagocytosis. Immunobiology.

605   2009;214(7):562-75. Epub 2009/03/06. doi: 10.1016/j.imbio.2008.11.003. PubMed PMID:

606   19261355; PubMed Central PMCID: PMCPMC2702671.

607   11.   Monteiro JT, Lepenies B. Myeloid C-Type Lectin Receptors in Viral Recognition and

608   Antiviral Immunity. Viruses. 2017;9(3). Epub 2017/03/23. doi: 10.3390/v9030059. PubMed

609   PMID: 28327518; PubMed Central PMCID: PMCPMC5371814.

610   12.   Weiss HJ, Angiari S. Metabolite Transporters as Regulators of Immunity. Metabolites.

611   2020;10(10). Epub 2020/10/23. doi: 10.3390/metabo10100418. PubMed PMID: 33086598;

612   PubMed Central PMCID: PMCPMC7603148.

613   13.   Gibellini L, De Biasi S, Paolini A, Borella R, Boraldi F, Mattioli M, et al. Altered

614   bioenergetics and mitochondrial dysfunction of monocytes in patients with COVID-19

615   pneumonia. EMBO Mol Med. 2020:e13001. Epub 2020/10/21. doi: 10.15252/emmm.202013001.

616   PubMed PMID: 33078545; PubMed Central PMCID: PMCPMC7645870.

617   14.   Codo AC, Davanzo GG, Monteiro LB, de Souza GF, Muraro SP, Virgilio-da-Silva JV, et

618   al. Elevated Glucose Levels Favor SARS-CoV-2 Infection and Monocyte Response through a

619   HIF-1alpha/Glycolysis-Dependent Axis. Cell Metab. 2020;32(3):498-9. Epub 2020/09/03. doi:

620   10.1016/j.cmet.2020.07.015. PubMed PMID: 32877692; PubMed Central PMCID:

621   PMCPMC7462530.

622   15.   Bojkova D, Klann K, Koch B, Widera M, Krause D, Ciesek S, et al. Proteomics of SARS-

623   CoV-2-infected host cells reveals therapy targets. Nature. 2020;583(7816):469-72. Epub

624   2020/05/15. doi: 10.1038/s41586-020-2332-7. PubMed PMID: 32408336.

625   16.   Nunn AVW, Guy GW, Brysch W, Botchway SW, Frasch W, Calabrese EJ, et al. SARS-

626   CoV-2 and mitochondrial health: implications of lifestyle and ageing. Immunity & Ageing.

627   2020;17(1):33. doi: 10.1186/s12979-020-00204-x.

628   17.   Saccon E, Krishnan S, Vinhas BS, Byrareddy SN, Mirazimi A, Neogi U, et al. Replication

629   dynamics and cytotoxicity of SARS-CoV-2 Swedish isolate in commonly used laboratory cell

630   lines. 2020:2020.08.28.271684. doi: 10.1101/2020.08.28.271684 %J bioRxiv.

631   18.   Fazzini F, Lamina C, Fendt L, Schultheiss UT, Kotsis F, Hicks AA, et al. Mitochondrial

632   DNA copy number is associated with mortality and infections in a large cohort of patients with

633   chronic kidney disease. Kidney Int. 2019;96(2):480-8. Epub 2019/06/30. doi:

634   10.1016/j.kint.2019.04.021. PubMed PMID: 31248648.

635   19.   Eriksson O, Hultstrom M, Persson B, Lipcsey M, Ekdahl KN, Nilsson B, et al. Mannose-

636   Binding Lectin is Associated with Thrombosis and Coagulopathy in Critically Ill COVID-19

637   Patients. Thromb Haemost. 2020. Epub 2020/09/02. doi: 10.1055/s-0040-1715835. PubMed

638   PMID: 32871607.

639   20.   Tiwari R, Mishra AR, Mikaeloff F, Gupta S, Mirazimi A, Byrareddy SN, et al. In silico and

640   In vitro Studies Reveal Complement System Drives Coagulation Cascade in SARS-CoV-2

641   Pathogenesis. Comput Struct Biotechnol J. 2020. Epub 2020/11/18. doi:

642   10.1016/j.csbj.2020.11.005. PubMed PMID: 33200027; PubMed Central PMCID:

643   PMCPMC7657020.

644   21.   Gao YM, Xu G, Wang B, Liu BC. Cytokine storm syndrome in coronavirus disease 2019:

645   A narrative review. J Intern Med. 2020. Epub 2020/07/23. doi: 10.1111/joim.13144. PubMed

646   PMID: 32696489; PubMed Central PMCID: PMCPMC7404514.

647   22.   Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine

648   Storm; What We Know So Far. Front Immunol. 2020;11:1446. Epub 2020/07/03. doi:

649   10.3389/fimmu.2020.01446. PubMed PMID: 32612617; PubMed Central PMCID:

650   PMCPMC7308649.

651   23.   Min CK, Cheon S, Ha NY, Sohn KM, Kim Y, Aigerim A, et al. Comparative and kinetic

652   analysis of viral shedding and immunological responses in MERS patients representing a broad

653   spectrum of disease severity. Sci Rep. 2016;6:25359. Epub 2016/05/06. doi:

654   10.1038/srep25359. PubMed PMID: 27146253; PubMed Central PMCID: PMCPMC4857172.

655   24.   Wong CK, Lam CW, Wu AK, Ip WK, Lee NL, Chan IH, et al. Plasma inflammatory

656   cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol.

657   2004;136(1):95-103. Epub 2004/03/20. doi: 10.1111/j.1365-2249.2004.02415.x. PubMed PMID:

658   15030519; PubMed Central PMCID: PMCPMC1808997.

659   25.   Schulte-Schrepping J, Reusch N, Paclik D, Bassler K, Schlickeiser S, Zhang B, et al.

660   Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. Cell.

661   2020;182(6):1419-40 e23. Epub 2020/08/19. doi: 10.1016/j.cell.2020.08.001. PubMed PMID:

662   32810438; PubMed Central PMCID: PMCPMC7405822.

663   26.   Osman M, Faridi RM, Sligl W, Shabani-Rad MT, Dharmani-Khan P, Parker A, et al.

664   Impaired natural killer cell counts and cytolytic activity in patients with severe COVID-19. Blood

665   Adv. 2020;4(20):5035-9. Epub 2020/10/20. doi: 10.1182/bloodadvances.2020002650. PubMed

666   PMID: 33075136; PubMed Central PMCID: PMCPMC7594380 interests.

667   27.   Komastu T, Ireland DD, Reiss CS. IL-12 and viral infections. Cytokine Growth Factor

668   Rev. 1998;9(3-4):277-85. Epub 1999/01/26. doi: 10.1016/s1359-6101(98)00017-3. PubMed

669   PMID: 9918125; PubMed Central PMCID: PMCPMC7129962.

670   28.   Shi J, Li Y, Zhou X, Zhang Q, Ye X, Wu Z, et al. Lactate dehydrogenase and

671   susceptibility to deterioration of mild COVID-19 patients: a multicenter nested case-control study.

672   BMC Med. 2020;18(1):168. Epub 2020/06/05. doi: 10.1186/s12916-020-01633-7. PubMed

673   PMID: 32493370; PubMed Central PMCID: PMCPMC7268591.

674   29.   Sanchez EL, Lagunoff M. Viral activation of cellular metabolism. Virology. 2015;479-

675   480:609-18. Epub 2015/03/31. doi: 10.1016/j.virol.2015.02.038. PubMed PMID: 25812764;

676   PubMed Central PMCID: PMCPMC4424078.

677   30.   Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al.

678   The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector

679   function. Cell Metab. 2014;20(1):61-72. Epub 2014/06/17. doi: 10.1016/j.cmet.2014.05.004.

680   PubMed PMID: 24930970; PubMed Central PMCID: PMCPMC4079750.

681   31.   Palmer CS, Cherry CL, Sada-Ovalle I, Singh A, Crowe SM. Glucose Metabolism in T

682   Cells and Monocytes: New Perspectives in HIV Pathogenesis. EBioMedicine. 2016;6:31-41.

683   Epub 2016/05/24. doi: 10.1016/j.ebiom.2016.02.012. PubMed PMID: 27211546; PubMed

684   Central PMCID: PMCPMC4856752.

685   32.   Song W, Li D, Tao L, Luo Q, Chen L. Solute carrier transporters: the metabolic

686   gatekeepers of immune cells. Acta Pharm Sin B. 2020;10(1):61-78. Epub 2020/01/30. doi:

687   10.1016/j.apsb.2019.12.006. PubMed PMID: 31993307; PubMed Central PMCID:

688   PMCPMC6977534.

689   33.   Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for

690   monocytes and macrophages. Nat Rev Immunol. 2020;20(6):355-62. Epub 2020/05/08. doi:

691   10.1038/s41577-020-0331-4. PubMed PMID: 32376901; PubMed Central PMCID:

692   PMCPMC7201395.

693   34.   Mardinoglu A, Stancakova A, Lotta LA, Kuusisto J, Boren J, Bluher M, et al. Plasma

694   Mannose Levels Are Associated with Incident Type 2 Diabetes and Cardiovascular Disease. Cell

695   Metab. 2017;26(2):281-3. Epub 2017/08/03. doi: 10.1016/j.cmet.2017.07.006. PubMed PMID:

696   28768165.

697   35.   Yoshimura K, Hirano S, Takata H, Funakoshi S, Ohmi S, Amano E, et al. Plasma

698   mannose level, a putative indicator of glycogenolysis, and glucose tolerance in Japanese

699   individuals. J Diabetes Investig. 2017;8(4):489-95. Epub 2017/02/24. doi: 10.1111/jdi.12622.

700   PubMed PMID: 28084015.

701   36.   Chee YJ, Ng SJH, Yeoh E. Diabetic ketoacidosis precipitated by Covid-19 in a patient

702   with newly diagnosed diabetes mellitus. Diabetes Res Clin Pract. 2020;164:108166. Epub

703   2020/04/28. doi: 10.1016/j.diabres.2020.108166. PubMed PMID: 32339533; PubMed Central

704   PMCID: PMCPMC7194589 competing financial interests or personal relationships that could

705   have appeared to influence the work reported in this paper.

706   37.   Fadini GP, Morieri ML, Boscari F, Fioretto P, Maran A, Busetto L, et al. Newly-diagnosed

707   diabetes and admission hyperglycemia predict COVID-19 severity by aggravating respiratory

708   deterioration. Diabetes Res Clin Pract. 2020;168:108374. Epub 2020/08/18. doi:

709   10.1016/j.diabres.2020.108374. PubMed PMID: 32805345; PubMed Central PMCID:

710   PMCPMC7428425.

711   38.   Hollstein T, Schulte DM, Schulz J, Gluck A, Ziegler AG, Bonifacio E, et al. Autoantibody-

712   negative insulin-dependent diabetes mellitus after SARS-CoV-2 infection: a case report. Nat

713   Metab. 2020;2(10):1021-4. Epub 2020/09/04. doi: 10.1038/s42255-020-00281-8. PubMed PMID:

714   32879473.

715   39.   Li J, Wang X, Chen J, Zuo X, Zhang H, Deng A. COVID-19 infection may cause ketosis

716   and ketoacidosis. Diabetes Obes Metab. 2020. Epub 2020/04/22. doi: 10.1111/dom.14057.

717   PubMed PMID: 32314455; PubMed Central PMCID: PMCPMC7264681.

718   40.   Shajahan A, Supekar NT, Gleinich AS, Azadi P. Deducing the N- and O- glycosylation

719   profile of the spike protein of novel coronavirus SARS-CoV-2. Glycobiology. 2020. doi:

720   10.1093/glycob/cwaa042. PubMed PMID: 32363391; PubMed Central PMCID:

721   PMCPMC7239183.

722   41.   Sharma V, Freeze HH. Mannose efflux from the cells: a potential source of mannose in

723   blood. J Biol Chem. 2011;286(12):10193-200. Epub 2011/01/29. doi: 10.1074/jbc.M110.194241.

724   PubMed PMID: 21273394; PubMed Central PMCID: PMCPMC3060472.

725   42.   Heindel DW, Koppolu S, Zhang Y, Kasper B, Meche L, Vaiana CA, et al. Glycomic

726   analysis of host response reveals high mannose as a key mediator of influenza severity.

727   Proceedings of the National Academy of Sciences. 2020:202008203. doi:

728   10.1073/pnas.2008203117.

729   43.   Java A, Apicelli AJ, Liszewski MK, Coler-Reilly A, Atkinson JP, Kim AH, et al. The

730   complement system in COVID-19: friend and foe? JCI insight. 2020;5(15):e140711. doi:

731   10.1172/jci.insight.140711. PubMed PMID: 32554923.

33

732   44.   Thompson E, Cascino K, Ordonez A, Zhou W, Vaghasia A, Hamacher-Brady A, et al.

733   Mitochondrial induced T cell apoptosis and aberrant myeloid metabolic programs define distinct

734   immune cell subsets during acute and recovered SARS-CoV-2 infection. medRxiv. 2020. Epub

735   2020/09/17. doi: 10.1101/2020.09.10.20186064. PubMed PMID: 32935120; PubMed Central

736   PMCID: PMCPMC7491535.

737   45.   Singh KK, Chaubey G, Chen JY, Suravajhala P. Decoding SARS-CoV-2 hijacking of host

738   mitochondria in COVID-19 pathogenesis. Am J Physiol Cell Physiol. 2020;319(2):C258-C67.

739   Epub 2020/06/09. doi: 10.1152/ajpcell.00224.2020. PubMed PMID: 32510973; PubMed Central

740   PMCID: PMCPMC7381712.

741   46.   Scozzi D, Cano M, Ma L, Zhou D, Zhu JH, O'Halloran JA, et al. Circulating mitochondrial

742   DNA is an early indicator of severe illness and mortality from COVID-19. JCI Insight. 2021. Epub

743   2021/01/15. doi: 10.1172/jci.insight.143299. PubMed PMID: 33444289.

744   47.   Hirabara SM, Gorjao R, Levada-Pires AC, Masi LN, Hatanaka E, Cury-Boaventura MF,

745   et al. Host cell glutamine metabolism as a potential antiviral target. Clin Sci (Lond).

746   2021;135(2):305-25. Epub 2021/01/23. doi: 10.1042/CS20201042. PubMed PMID: 33480424.

747   48.   Mansouri K, Rastegari-Pouyani M, Ghanbri-Movahed M, Safarzadeh M, Kiani S,

748   Ghanbari-Movahed Z. Can a metabolism-targeted therapeutic intervention successfully

749   subjugate SARS-COV-2? A scientific rational. Biomed Pharmacother. 2020;131:110694. Epub

750   2020/09/14. doi: 10.1016/j.biopha.2020.110694. PubMed PMID: 32920511; PubMed Central

751   PMCID: PMCPMC7451059.

752   49.   Icard P, Lincet H, Wu Z, Coquerel A, Forgez P, Alifano M, et al. The key role of Warburg

753   effect in SARS-CoV-2 replication and associated inflammatory response. Biochimie.

754   2021;180:169-77. Epub 2020/11/16. doi: 10.1016/j.biochi.2020.11.010. PubMed PMID:

755   33189832; PubMed Central PMCID: PMCPMC7659517.

756   50.   Kim JM, Kim HM, Lee EJ, Jo HJ, Yoon Y, Lee NJ, et al. Detection and Isolation of SARS-

757   CoV-2 in Serum, Urine, and Stool Specimens of COVID-19 Patients from the Republic of Korea.

758   Osong Public Health Res Perspect. 2020;11(3):112-7. Epub 2020/06/13. doi:

759   10.24171/j.phrp.2020.11.3.02. PubMed PMID: 32528816; PubMed Central PMCID:

760   PMCPMC7282421.

761   51.   Varnaitė R, García M, Glans H, Maleki KT, Sandberg JT, Tynell J, et al. Expansion of

762   SARS-CoV-2-Specific Antibody-Secreting Cells and Generation of Neutralizing Antibodies in

763   Hospitalized COVID-19 Patients. J Immunol. 2020;205(9):2437-46. Epub 2020/09/04. doi:

764   10.4049/jimmunol.2000717. PubMed PMID: 32878912; PubMed Central PMCID:

765   PMCPMC7576114.

766   52.   Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al.

767   Proteomics. Tissue-based map of the human proteome. Science. 2015;347(6220):1260419.

768   Epub 2015/01/24. doi: 10.1126/science.1260419. PubMed PMID: 25613900.

769   53.   Borsa M, Mazet JM. Attacking the defence: SARS-CoV-2 can infect immune cells. Nat

770   Rev Immunol. 2020;20(10):592. Epub 2020/08/23. doi: 10.1038/s41577-020-00439-1. PubMed

771   PMID: 32826975; PubMed Central PMCID: PMCPMC7441839.

772   54.   Rohde C, Becker S, Krahling V. Marburg virus regulates the IRE1/XBP1-dependent

773   unfolded protein response to ensure efficient viral replication. Emerg Microbes Infect.

774   2019;8(1):1300-13. Epub 2019/09/10. doi: 10.1080/22221751.2019.1659552. PubMed PMID:

775   31495285; PubMed Central PMCID: PMCPMC6746283.

776   55.   Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, et al. Inhibition of

777   SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2.

778   Cell. 2020.

779   56.   Babu H, Sperk M, Ambikan AT, Rachel G, Viswanathan VK, Tripathy SP, et al. Plasma

780   Metabolic Signature and Abnormalities in HIV-Infected Individuals on Long-Term Successful

781   Antiretroviral Therapy. Metabolites. 2019;9(10). Epub 2019/10/03. doi: 10.3390/metabo9100210.

782   PubMed PMID: 31574898.

783   57.   Leland M, John, H., Nathaniel, S. & Lukas, G. UMAP: uniform manifold approximation

784   and projection. Journal of Open Source Software. 2018;3:861.

785   58.   Wickham H. Elegant Graphics for Data Analysis - ggplot2. New York: New York:

786   Springer-Verlag; 2009.

787   59.   Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential

788   expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res.

789   2015;43(7):e47. Epub 2015/01/22. doi: 10.1093/nar/gkv007. PubMed PMID: 25605792; PubMed

790   Central PMCID: PMCPMC4402510.

791   60.   Shi L, Westerhuis JA, Rosen J, Landberg R, Brunius C. Variable selection and validation

792   in multivariate modelling. Bioinformatics. 2019;35(6):972-80. Epub 2018/08/31. doi:

793   10.1093/bioinformatics/bty710. PubMed PMID: 30165467; PubMed Central PMCID:

794   PMCPMC6419897.

795   61.   Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a

796   comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res.

797   2016;44(W1):W90-7. Epub 2016/05/05. doi: 10.1093/nar/gkw377. PubMed PMID: 27141961;

798   PubMed Central PMCID: PMCPMC4987924.

799   62.   Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene

800   set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression

801   profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545-50. Epub 2005/10/04. doi:

802   10.1073/pnas.0506580102. PubMed PMID: 16199517; PubMed Central PMCID:

803   PMCPMC1239896.

804   63.   Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in

805   multidimensional genomic data. Bioinformatics. 2016;32(18):2847-9. Epub 2016/05/22. doi:

806   10.1093/bioinformatics/btw313. PubMed PMID: 27207943.

807   64.   Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR.

808   Nucleic Acids Res. 2001;29(9):e45. Epub 2001/05/09. doi: 10.1093/nar/29.9.e45. PubMed

809   PMID: 11328886; PubMed Central PMCID: PMCPMC55695.

810

Figure Legends

Fig 1. Targeted plasma proteomics in COVID-19 patients. (a) Heatmap of Z-score

transformed quantitative measurements of all proteins detected by the immuno-oncology panel.

Column annotation represents each patient sample and their corresponding groups and pairs of

statistical analysis. Rows are proteins hierarchically clustered based on the Euclidean distance

and complete linkage method. Names of proteins which are identified as significant in any of the

statistical analysis printed. **(b)** KEGG pathway enrichment analysis results of significantly

changed proteins between healthy control (HC + HC-CoV-2 Ab+) and COVID-19 (hospitalized

mild+hospitalized severe) groups. **(c)** Violin plot of significantly regulated (Mann-Whitney U

test) proteins between hospitalized mild and hospitalized severe, *adj p<0.05, **adj p<0.01.

Fig 2. Untargeted global plasma metabolomics in COVID-19 patients (a) Sample distribution

for quantitative metabolite measurements plotted in 2-dimensional space after performing

dimensionality reduction using UMAP. **(b)** Stacked bar plots visualizing percentage of

metabolites significantly changed between healthy control (HC + HC-CoV-2 Ab+) and COVID-

19 (hospitalized mild + hospitalized severe) group concerning their corresponding super-

pathways and sub-pathways. **(c)** Metabolic set enrichment analysis using the significantly

enriched metabolites between HCs and COVID-19 patients. The size of the bubble indicates

adjusted p-values. **(d)** Heatmap of log2 scaled and Z-score transformed measurements of

metabolites significantly changed between healthy control (HC + HC-CoV-2 Ab+) and COVID-

19 (hospitalized mild + hospitalized severe) groups. Column annotation represents each patient

sample and the corresponding groups. Row annotation represents super pathways of the

metabolites. Rows are metabolites hierarchically clustered based on Euclidean distance and

complete linkage method.

834 **Fig 3. Untargeted plasma metabolites differing between the mild and severe hospitalized**

835 **COVID19 patients. (a)** Heatmap of log2 scaled, and Z-score transformed significantly changed

836 metabolites between hospitalized mild and hospitalized severe groups. Column annotation

837 represents each patient sample and the corresponding groups. Rows are metabolites hierarchically

838 clustered based on Euclidean distance and complete linkage method. **(b)** Alluvial plot

839 representing pathways resulted from IPA pathway enrichment analysis using all metabolites that

840 differs significantly between hospitalized mild and severe groups. **(c)** Volcano plot showing all

841 the metabolites that differ significantly between hospitalized mild and hospitalized severe groups.

842 **(d)** Box plots of key metabolites glutamate, glucose and mannose **(e)** Schematic representation of

843 the key steps of glycolysis, mannose and glutamate metabolism and TCA cycle. **(f)** Box plots of

844 soluble mannose-binding lectin levels in patients' plasma. P-values determined by Mann-

845 Whitney U test.

846 **Fig 4. Glucose, mannose and glutamate transporters in COVID-19 severity: (a)** Percentage

847 of total lymphocytes and monocytes in all 4 patient groups; **(b)** Percentage of PBMC

848 subpopulations, $CD3^+$ T-cells of lymphocytes, $CD4^+$ T-cells (of $CD3^+$ cells) $CD8^+$ T-cells (of

849 $CD3^+$ cells), classical monocytes (CM, $CD14^+CD16^-$ of monocytes), intermediate monocytes

850 (IM, $CD14^+CD16^+$ of monocytes) and non-classical monocytes (NCM, $CD14^-CD16^+$ of

851 monocytes). Median values are indicated by lines. **(c)** Density plot of percentage of $CD8^+$ T-cells,

852 IM and NCM expressing GLUT1. Histograms show percentage of cells expressing GLUT1

853 (x-axis) and GLUT1 read density of each sample (y-axis). The median percentage of cells

854 expressing GLUT1 is shown for each patient group. **(d)** MFI of GLUT1 in $CD8^+$ T-cells, IM and

855 NCM in all four patient groups. **(e)** Density plot of percentage of CM, IM and NCM expressing

856 xCT. Histograms show percentage of cells expressing xCT (x-axis) and xCT read density of each

857   sample (y-axis). The median percentage of cells expressing xCT is shown for each patient group.

858   **(f)** MFI of xCT in CM, IM and NCM in all four patient groups. In all the panels the median

859   values are indicated by lines, p-values are determined by Mann-Whitney U test, *p<0.05,

860   **p<0.01, and ***p<0.001.

861   **Fig 5. Cell-specific regulation of central carbon metabolic pathways by SARS-CoV-2: (a)**

862   Bubble plots of protein set enrichment analysis (adj. p<0.05) restricted to metabolic pathways

863   showing highly upregulated (red) and downregulated (green) proteins in SARS-CoV-2 infected

864   Calu-3 cells compared to mock-infected cells. Bubble size is relative to number of proteins. **(b)**

865   Network analysis of proteins from glycolysis/gluconeogenesis, fructose and mannose metabolism

866   and TCA cycle that were significantly different in SARS-CoV-2 infected and mock-infected

867   Calu-3 cells. Rectangular shapes represent the three pathways. Circular shapes show each protein

868   that is either upregulated (red) or downregulated (green) in infected cells compared to mock-

869   infected cells. The size of the circle indicates fold-change. Lines denote connection of each

870   protein to its respective pathway and connection between each protein-protein (STRING,

871   confidence>0.7). **(c)** Schematic map of the glycolysis/gluconeogenesis, fructose and mannose

872   metabolism and TCA cycle. Red indicates significantly upregulated proteins and green indicates

873   significantly downregulated proteins in SARS-CoV-2 infected Calu-3 cells. **(d)** mtDNA copy

874   number in whole blood cells in all 4 patient groups. Median values are indicated by lines, p-

875   values are determined by Mann-Whitney U test, *p<0.05. **(e)** Schematic of inhibitors of

876   metabolic pathways, 2-DG inhibits glycolysis and DON inhibits glutaminolysis. **(f, g)** Viral load

877   of SARS-CoV-2 determined by RT-qPCR targeting the viral *E-gene* is measured in **(f)** cells

878   lysates and **(g)** cell culture supernatants, at moi 0.001 in Calu-3 cells treated with 2-DG or DON

40

879   as indicated. The data is represented as mean±SEM of two individual experiments, triplicates in

880   each experiment. P-values are determined by student T-test, *p<0.05, ***p<0.001.

881   **Fig 6. Role of glucose and mannose in SARS-CoV-2 infectivity and complement pathways:**

882   Viral load of SARS-CoV-2 determined by RT-qPCR targeting the viral *E-gene* is measured in **(a)**

883   cell culture supernatants, and **(b)** cell lysates at moi 0.001 infection of Calu-3 cells grown in

884   different glucose and mannose concentrations (mM) as indicated. The data is represented as

885   mean±SEM of two independent experiments, duplicates in each experiment. P-values are

886   determined by student T-test, *p<0.05. **(c)** Schematic of complement cascade showing pathway

887   upstream of C3 and C5 activation. Gene expressions of **(d)** *MASP2*, and **(e)** *C4* in SARS-CoV-2

888   infected (moi 0.001) and mock-infected Calu-3 cells at different glucose and mannose

889   concentrations (mM) as indicated. All gene expression data in Calu-3 cells (viral *E-gene*, *MASP2*

890   and *C4*) at each glucose/mannose concentration were compared with baseline expression at

891   11.1mM glucose, 0mM mannose and data represented as mean±SEM. Gene expressions of **(f)**

892   *MASP2*, and **(g)** *C4* in whole blood of HC, HC-CoV-2 Ab+, COVID-19 hospitalized mild and

893   hospitalized severe patients determined by RT-qPCR. Median values are indicated by line, p-

894   values are determined by Mann-Whitney-U test, *p<0.05 and **p<0.01.

895 **Supplementary Files:**

896   **Table S1.** Clinical Features of the study population

897   **Table S2**: List of reagents, kits and antibodies

898   **Table S3**: List of primer and probe sequences

899   **Fig S1:** The IgG Ab showed 10 of the HC were CoV-2 Ab-positive.

900   **Fig S2:** Metabolite profile of amino acids altered in COVID-19 patients. Line within box plots

901   represents median values, $*p<0.05$, $**p<0.01$, $***p<0.001$.

902   **Fig S3:** Levels of metabolites related to glycolysis/gluconeogenesis and fructose and mannose

903   metabolism and the TCA cycle

904   **Fig S4:** Biomarker of the COVID-19 severity identified by MVUR. The size of the bubble

905   indicates rank. Box plot of the biomarkers indicating the level in HCs and COVID-19 patients.:

906   update

907   **Fig S5:** Differential protein abundance in Calu-3 cells following SARS-CoV-2 infection after

908   24h. The analysis was restricted to glycolysis/gluconeogenesis, fructose and mannose metabolism

909   and the TCA cycle.

910   **Fig S6:** Differential protein abundance in Caco-2, Huh7 and 293FT cells following SARS-CoV-

911   2 infection after 24h. The analysis was restricted to glycolysis/gluconeogenesis, fructose and

912   mannose metabolism and the TCA cycle.

913   **Fig S7:** Gating strategy of flow cytometry data.

a.
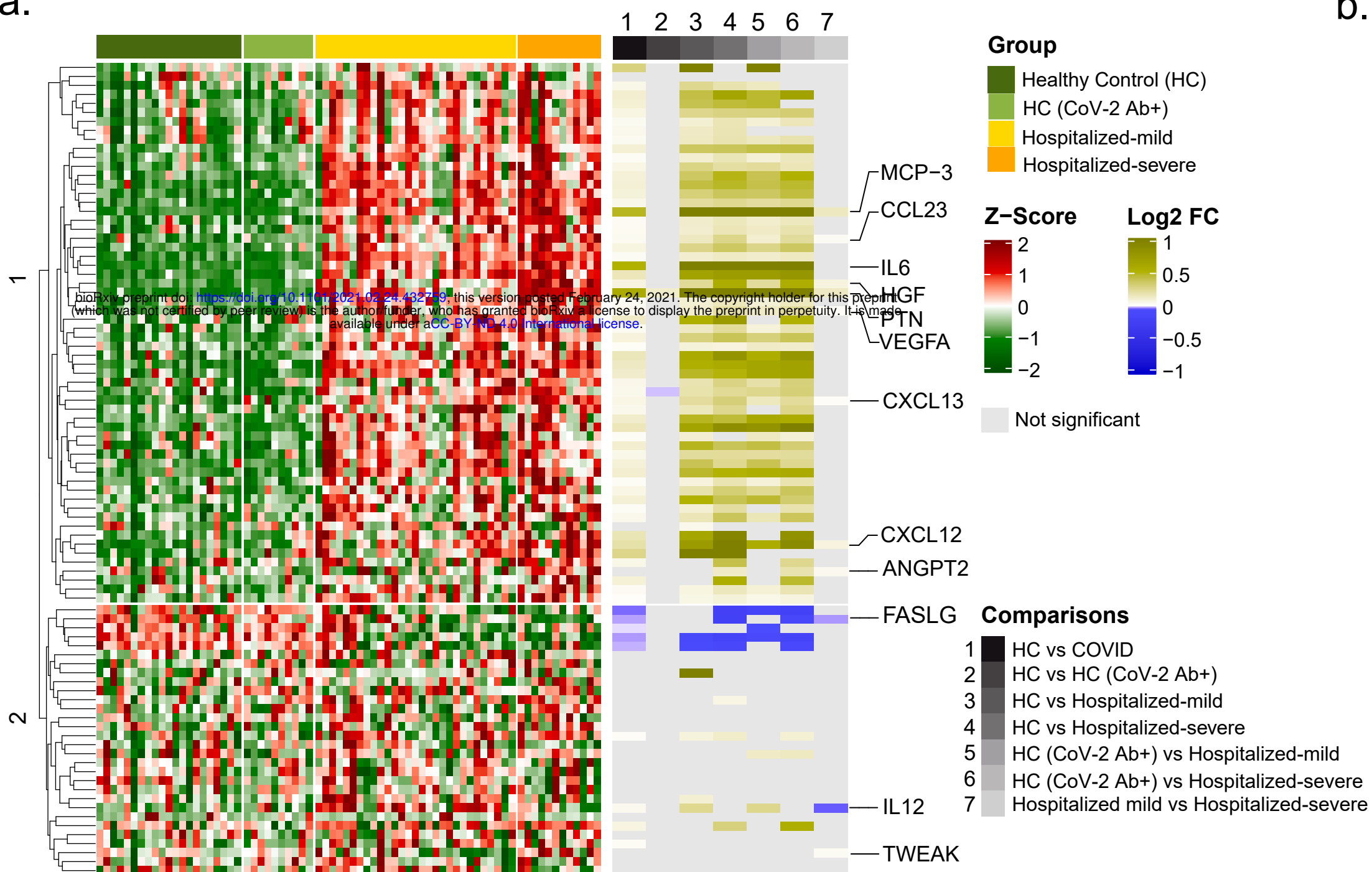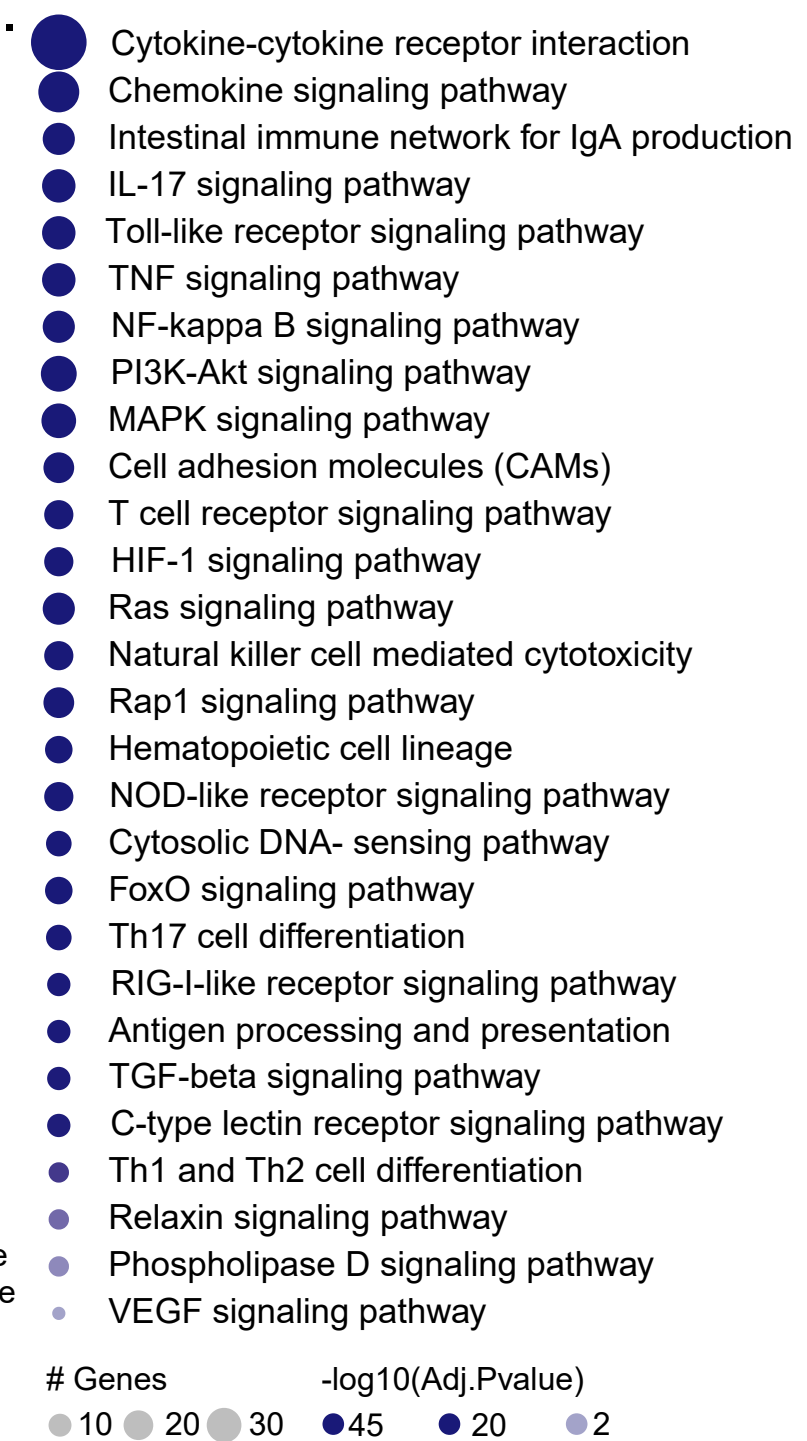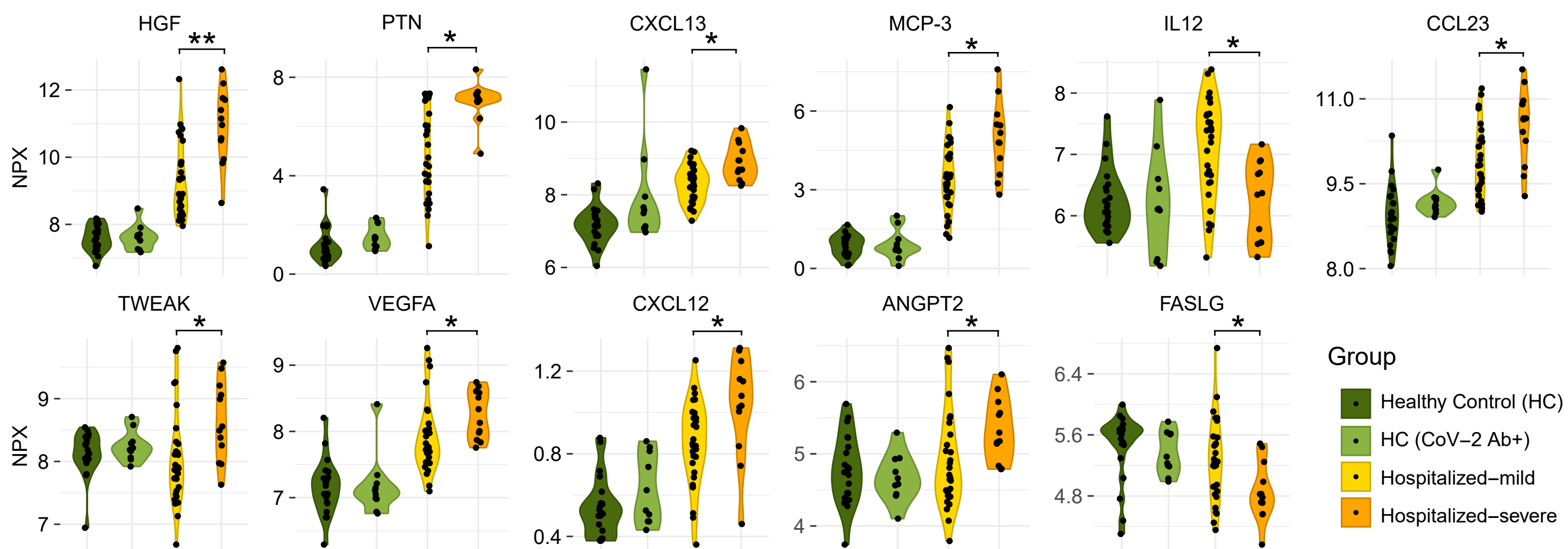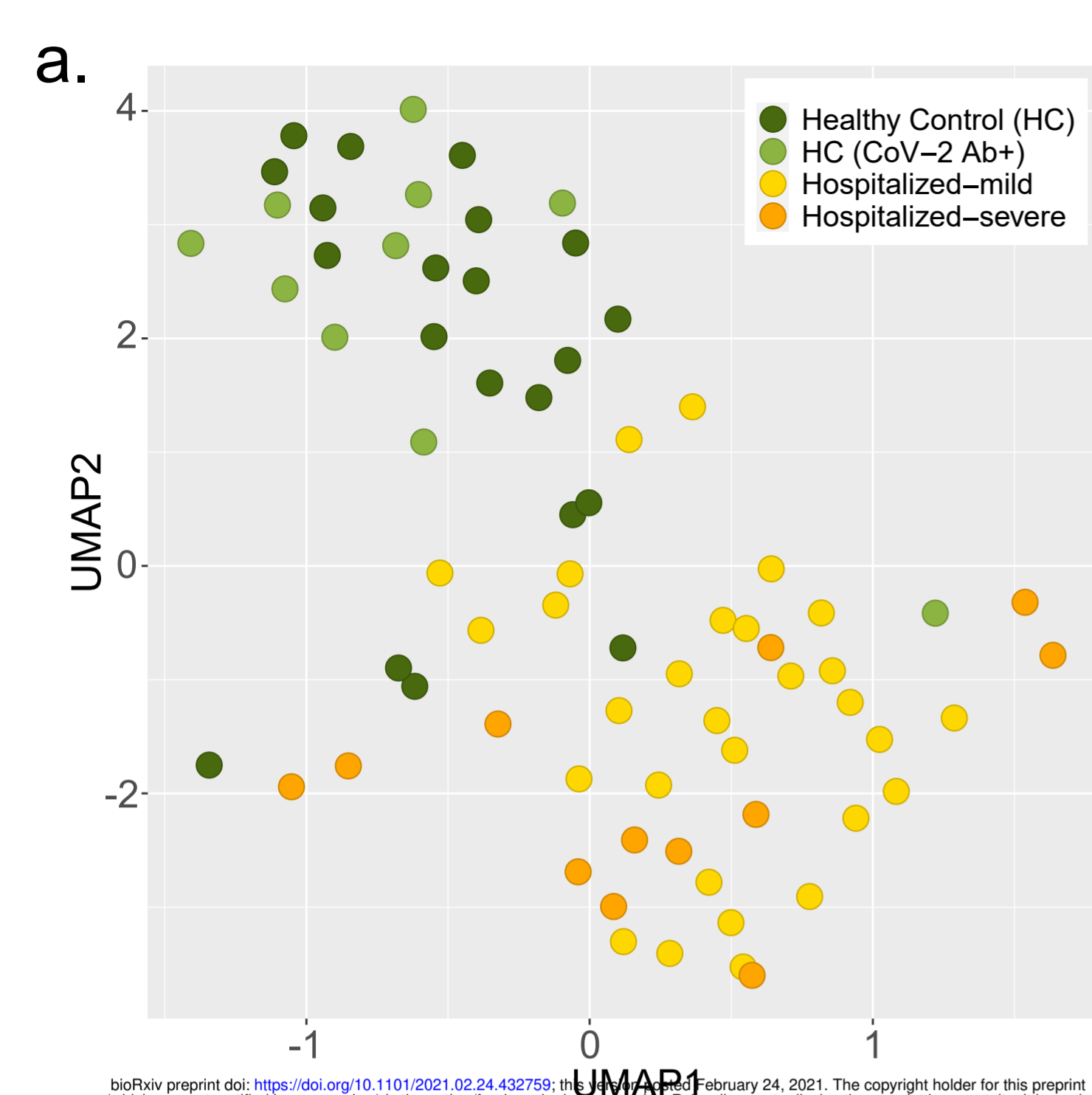
% Lymphocytes — *** *** *** *

% Monocytes

b.

% CD3+ of Lymphocytes — *

% CD4+ of CD3+ — *

% CD8+ of CD3+

% Classical Monocytes CD14+CD16− of Monocytes

% Intermediate Monocytes CD14+CD16+ of Monocytes

% Non-Classical Monocytes CD14−CD16+ of Monocytes — * ** * *

c.

GLUT1-CD8 — 99, 98, 98, 97

GLUT1-IM — 1.60, 0.92, 0.53, 0.57

GLUT1-NCM — 1.90, 1.80, 0.64, 0.76

d.

MFI GLUT1 in CD8+ — ** **

MFI GLUT1 in IM — ** * *

MFI GLUT1 in NCM

e.

xCT-CM — 100, 100, 100, 100

xCT-IM — 100, 100, 100, 100

xCT-NCM — 85, 77, 92, 92

f.

MFI xCT in CM — ** * **

MFI xCT in IM — *** *** * **

MFI xCT in NCM — *
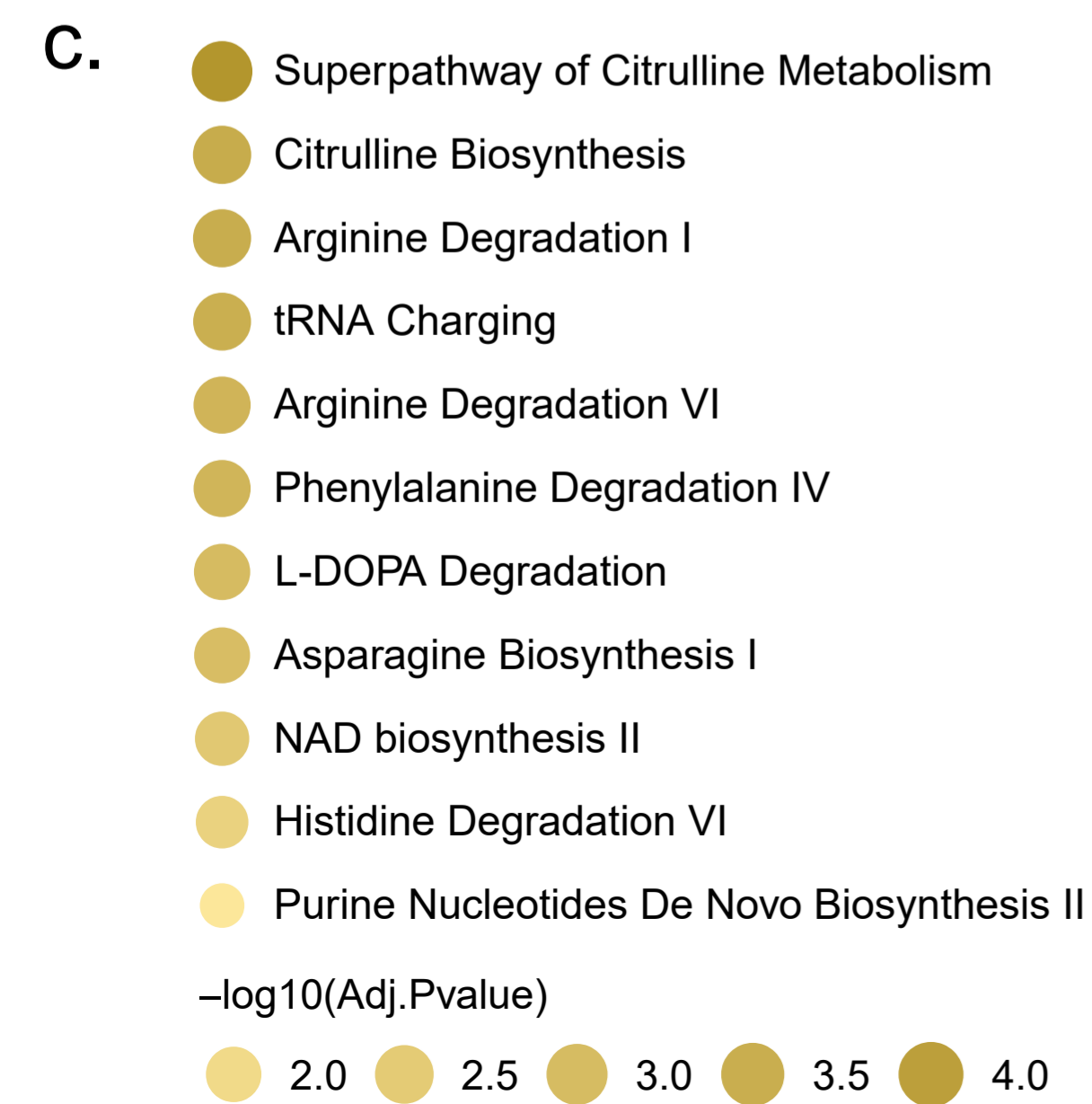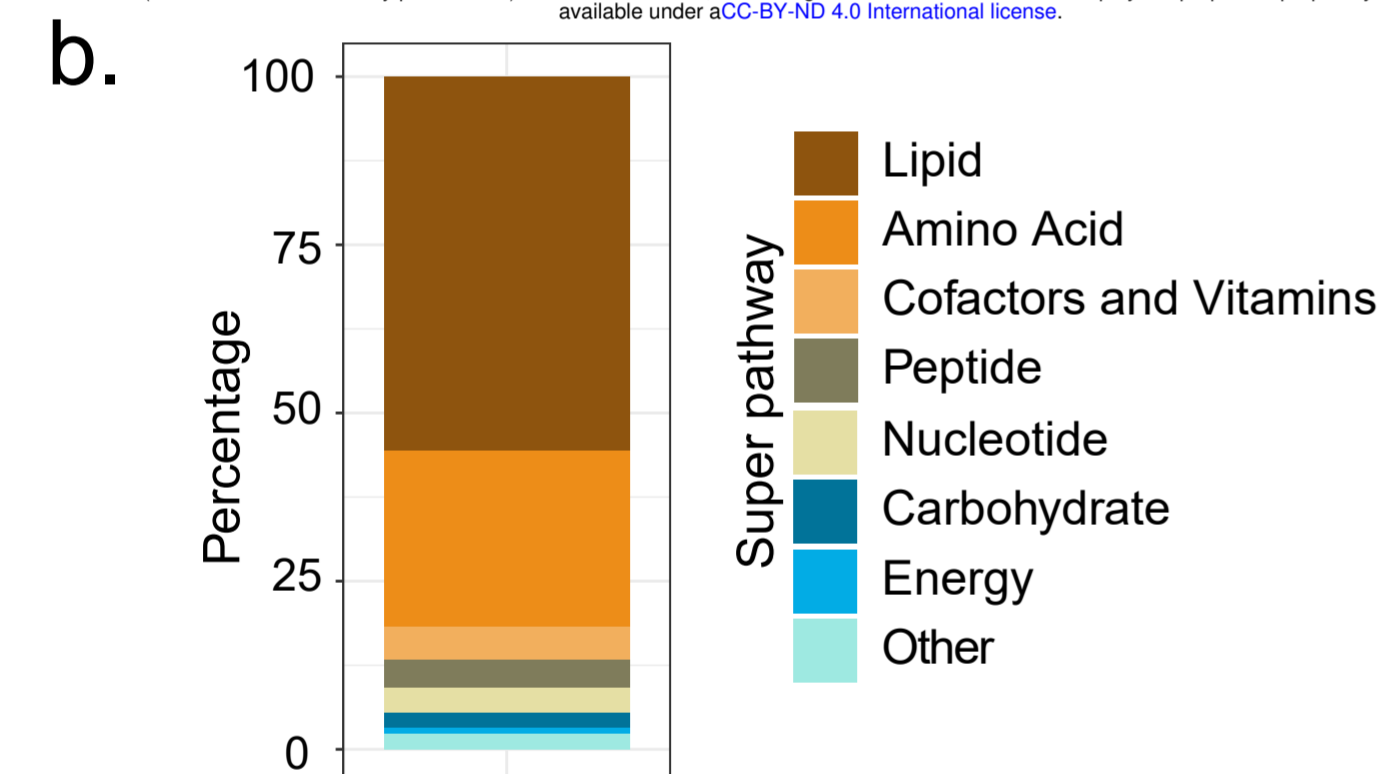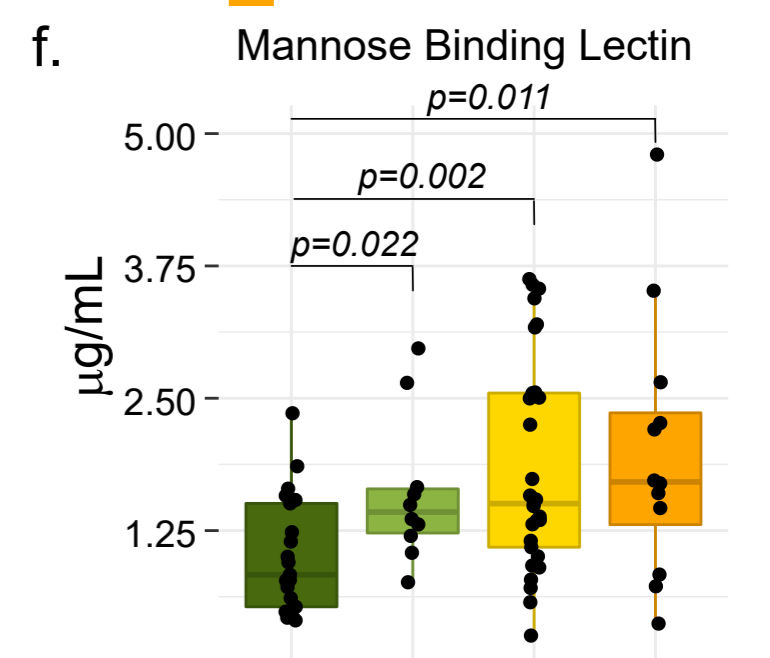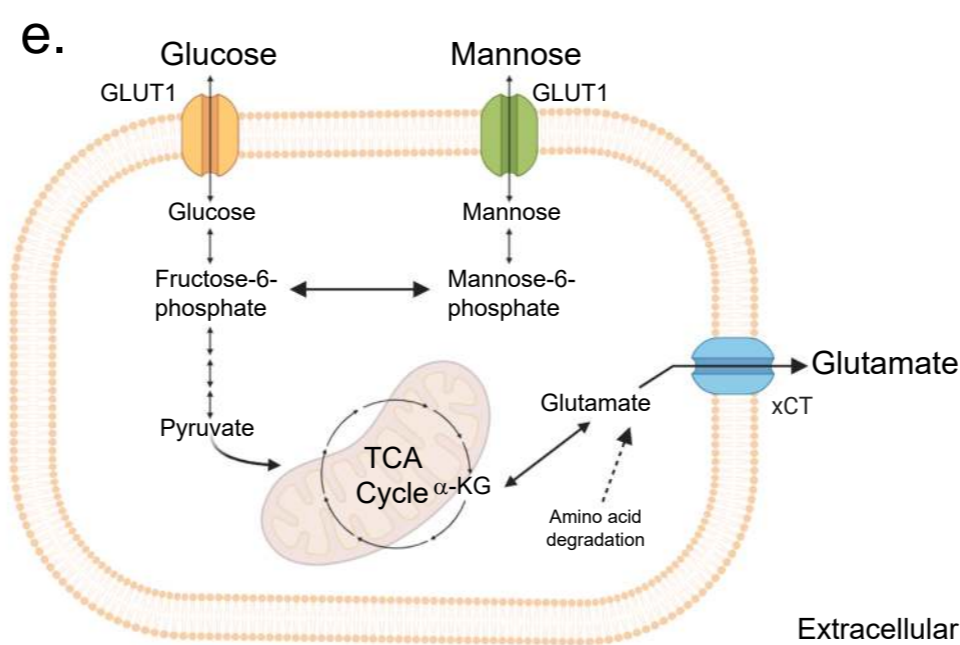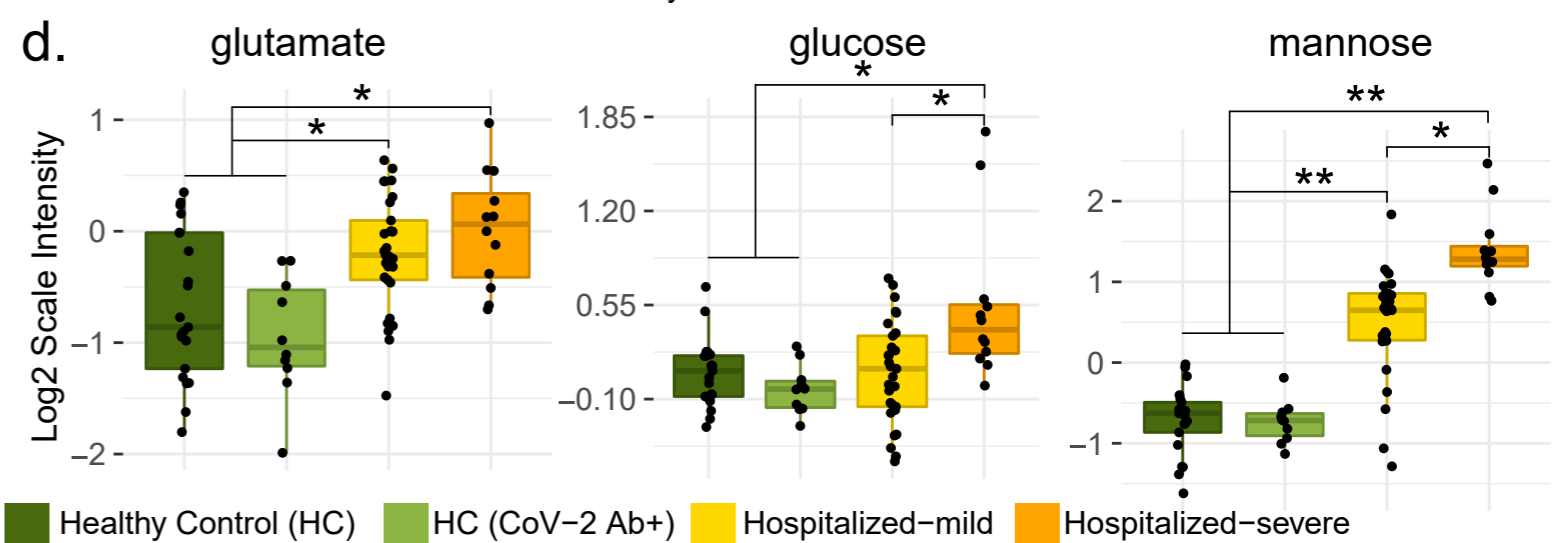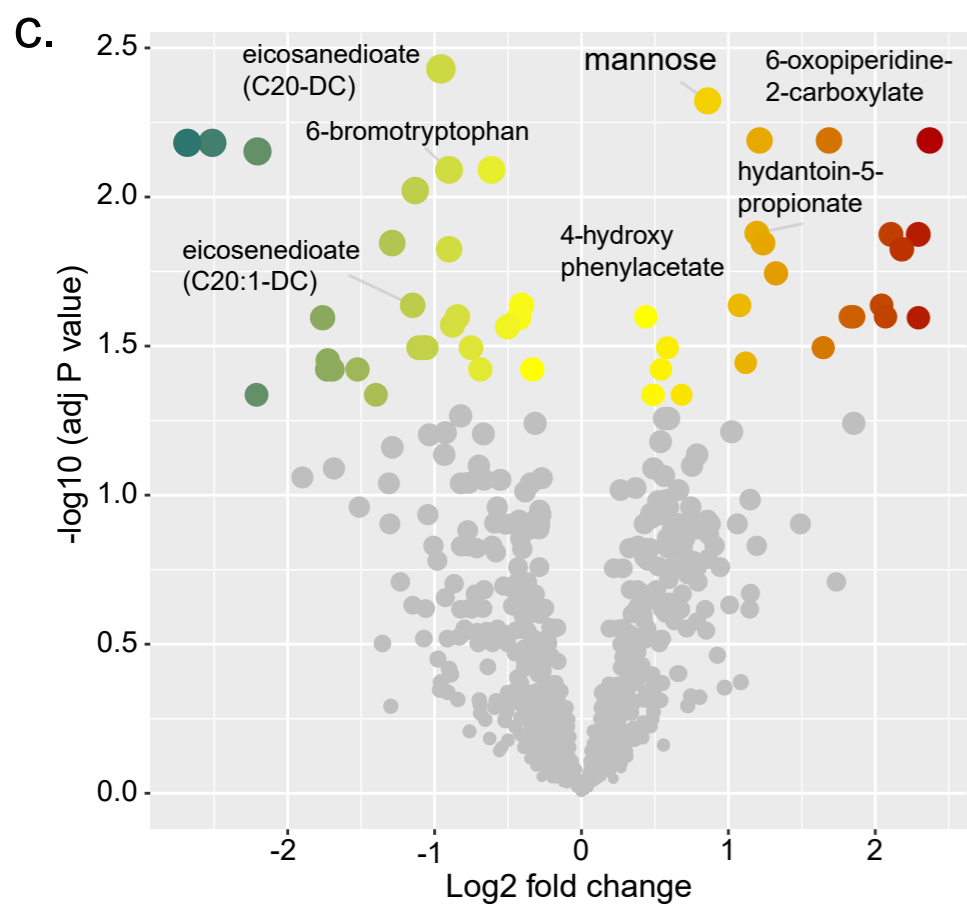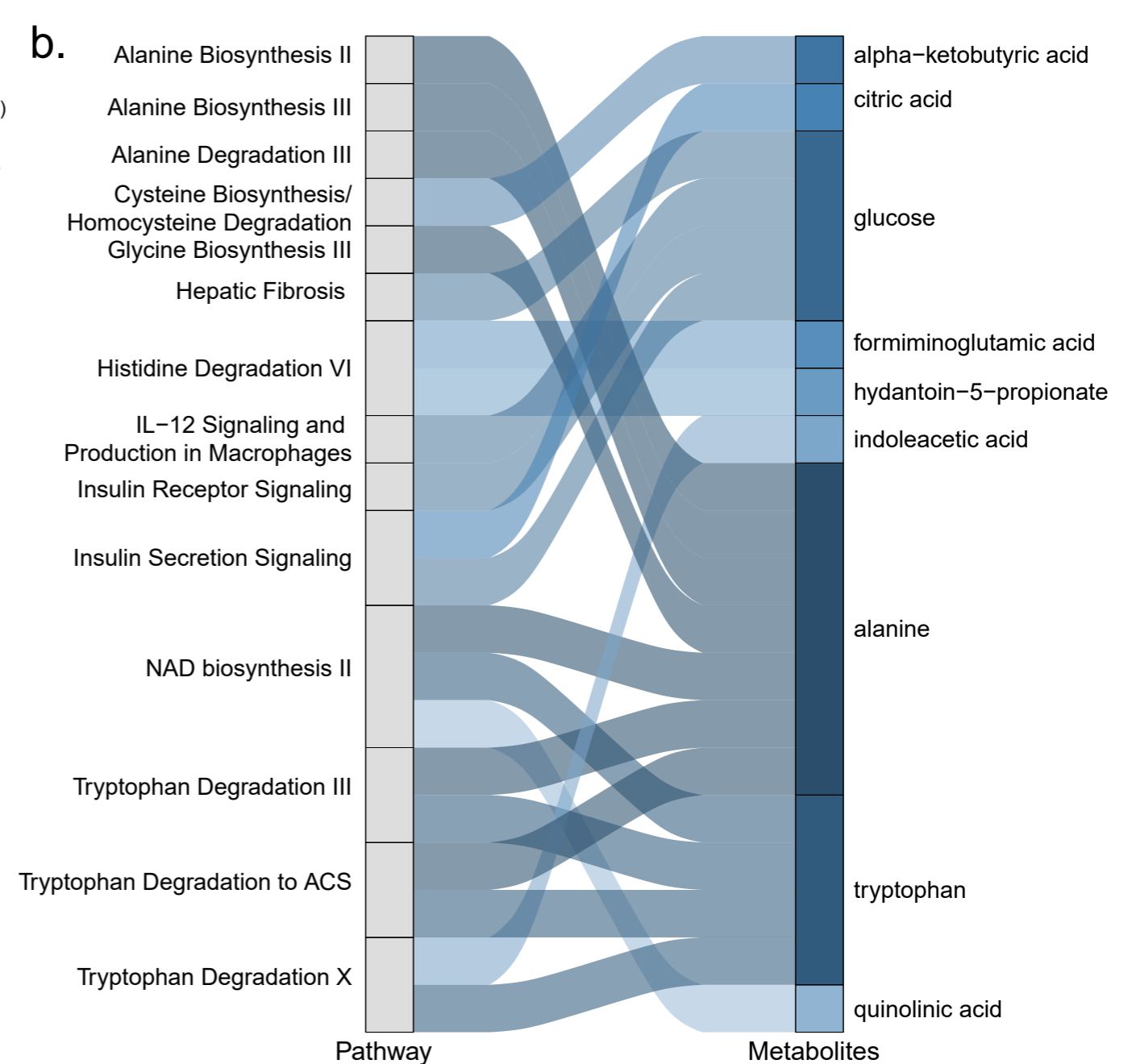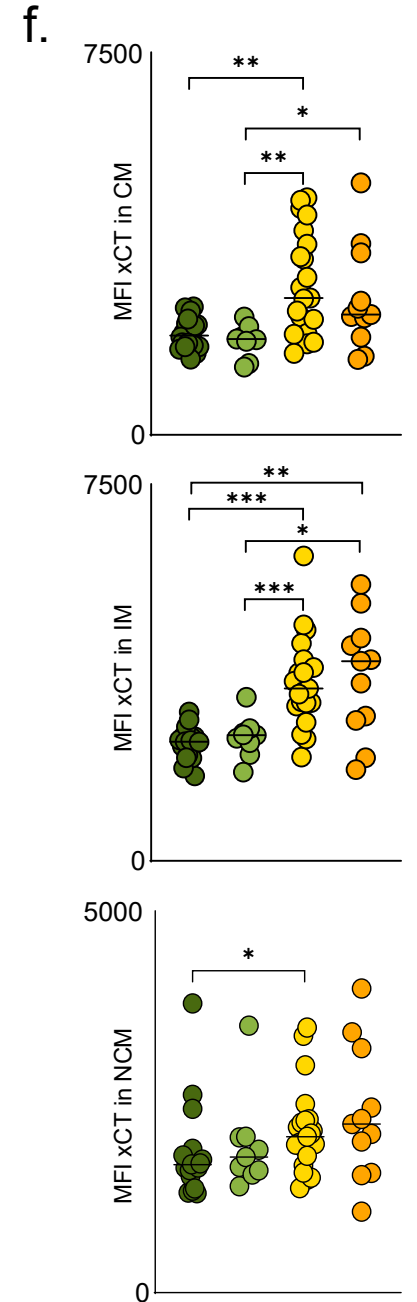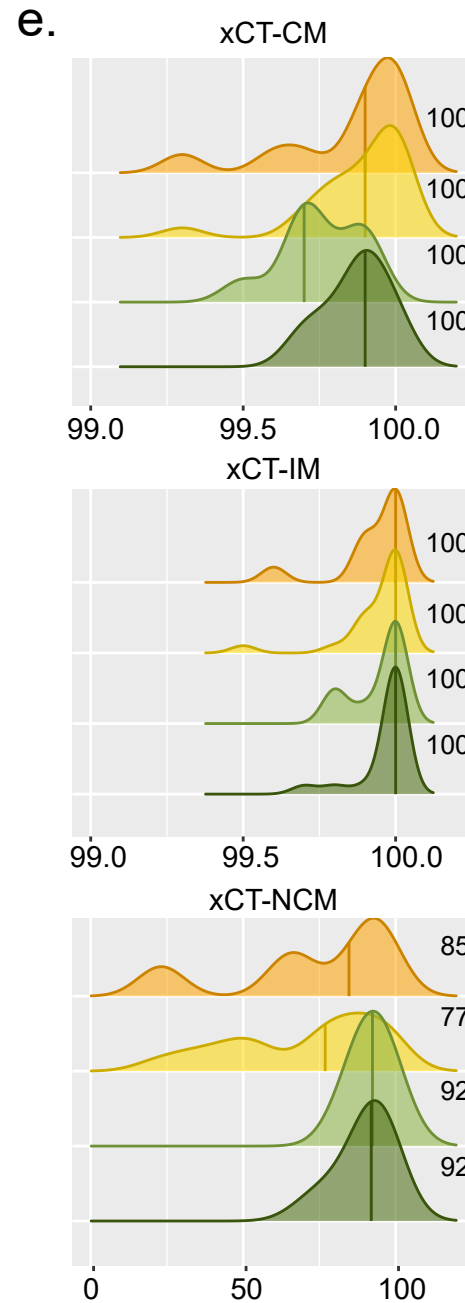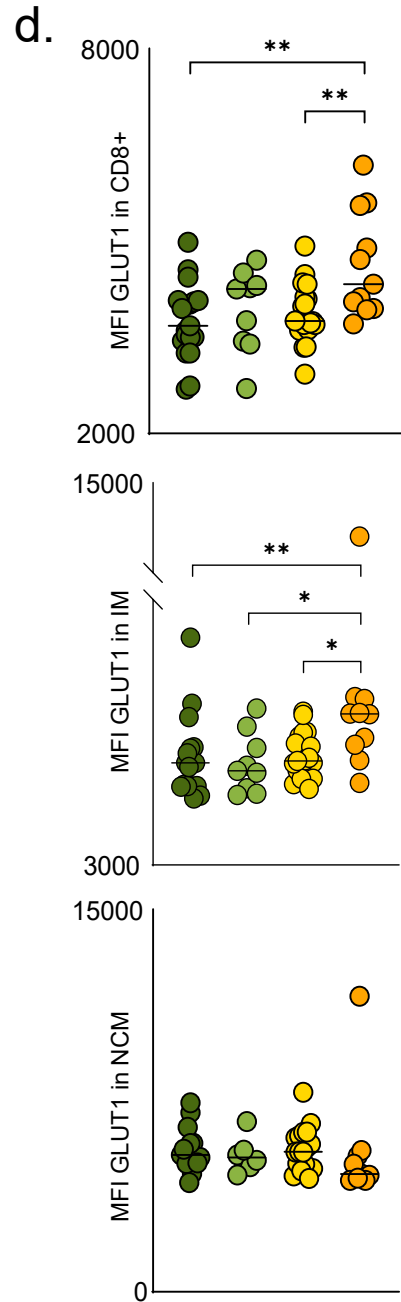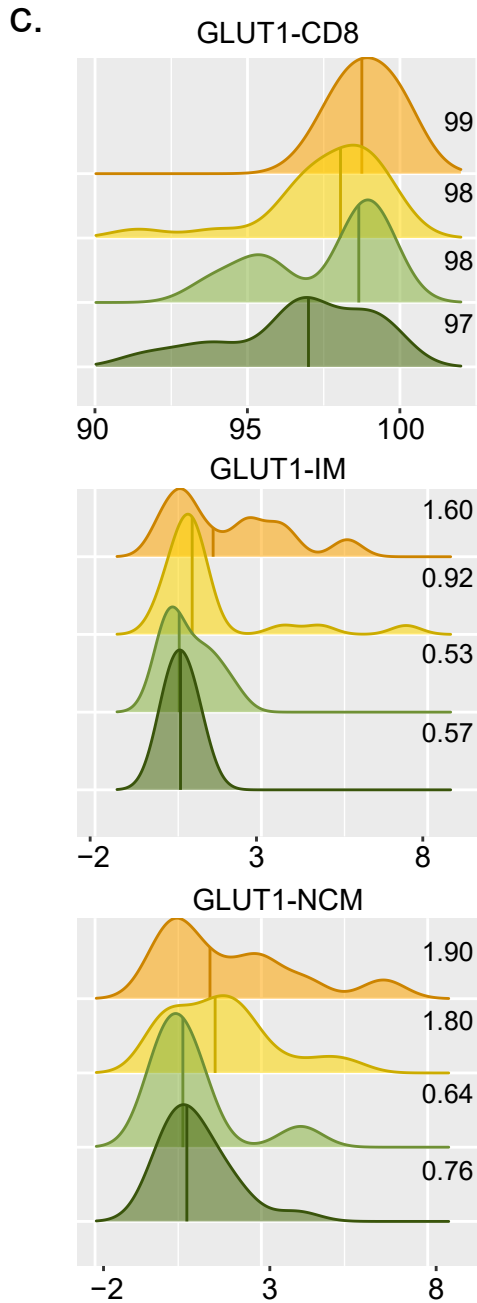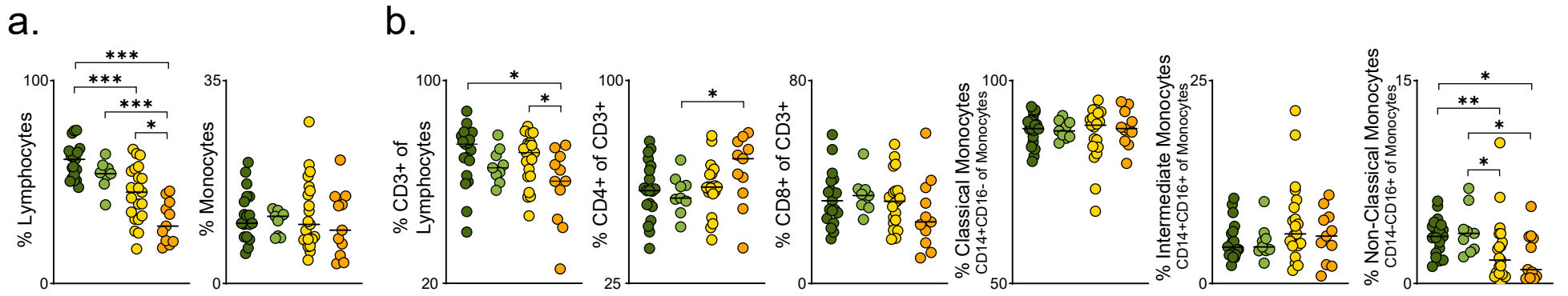
Legend: Healthy Control (HC), HC (CoV−2 Ab+), Hospitalized−mild, Hospitalized−severe