SARS-CoV-2 infection paralyzes cytotoxic and metabolic functions of immune cells

Yogesh Singh[1,2,6*,#], Christoph Trautwein[3*], Rolf Fendel[4*], Naomi Krickeberg[4], Jana Held[4], Andrea Kreidenweiss[4], Georgy Berezhnoy[3], Rosi Bissinger[5], Stephan Ossowski[1,2], Madhuri S. Salker[6], Nicolas Casadei[1,2], Olaf Riess[1,2#] and the Deutsche COVID-19 OMICS Initiative (DeCOI)

[1]Institute of Medical Genetics and Applied Genomics, University of Tübingen, Calwerstrasse 7, 72076, Tübingen, Germany

[2]NGS Competence Center Tübingen (NCCT), University of Tübingen, Calwerstrasse 7, 72076 Tübingen, Germany

[3]Werner Siemens Imaging Center, University of Tübingen, Röntgenweg 13, 72076, Tübingen, Germany

[4]Institute of Tropical Medicine, University Hospital of Tübingen, Tübingen, Wilhelmstrasse 27, 72076, Tübingen, Germany

[5]Department of Internal Medicine, Division of Endocrinology, Diabetology and Nephrology, University Hospital of Tübingen, Germany

[6]Research Institute of Women's Health, University of Tübingen, Calwerstrasse 7/6, 72076, Tübingen, Germany

*Equal contributions

Correspondence
Dr Yogesh Singh or Prof Olaf Riess
Institute of Medical Genetics and Applied Genomics, Tübingen University
Calwerstraße 7, 72076, Tübingen, Germany
Phone: 0049 7071 29 72257/78264 Fax: 0049 7071 29 25355
Email: yogesh.singh@med.uni-tuebingen.de or olaf.riess@med.uni.tuebingen.de

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Short title: Defective immune-metabolic functions in COVID-19 patient

Abstract:

The SARS-CoV-2 virus is the causative agent of the global COVID-19 infectious disease outbreak, which can lead to acute respiratory distress syndrome (ARDS). However, it is still unclear how the virus interferes with immune cell and metabolic functions in the human body. In this study, we investigated the immune response in 10 acute or convalescent COVID19 patients. We characterized the peripheral blood mononuclear cells (PBMCs) using flow cytometry and found that $CD8_+$ T cells were significantly subsided in moderate COVID-19 and convalescent patients. Furthermore, characterization of $CD8_+$ T cells suggested that patients with a mild and moderate course of the COVID-19 disease and convalescent patients have significantly diminished expression of both perforin and granzyme B in $CD8_+$ T cells. Using $_1$H-NMR spectroscopy, we characterized the metabolic status of their autologous PBMCs. We found that fructose, lactate and taurine levels were elevated in infected (mild and moderate) patients compared with control and convalescent patients. Glucose, glutamate, formate and acetate levels were attenuated in COVID-19 (mild and moderate) patients. Our findings reveal patients who suffer from an over activation of the immune system, a change of composition in infusion/intravenous fluids during infection with the aim to lower blood levels of glucose, glutamate, acetate and formate could avoid a life-threatening cytokine storm. In summary, our report suggests that SARS-CoV-2 infection leads to disrupted $CD8_+$ T cytotoxic functions and changes the overall metabolic functions of immune cells.

Introduction:

The first cases of severe acute respiratory coronavirus-2 (SARS-CoV-2) infection appeared in December 2019, in Wuhan, China[1]. This zoonotic virus has infected by now more than 26.1 million people (03.09.2020) and killed more than 0.86 million[2,3] worldwide. The containment of the pandemic is challenging and is still continuing with roughly 200,000 or more new infections being reported daily since July 2020[2,3]. There is an urgent need for a better understanding of the immunopathology, as SARS-CoV-2 has become the leading cause of morbidity and mortality in many countries.

Coronaviruses (CoV) are a large family of viruses that can cause illnesses such as the common cold and seasonal influenza[4]. Pathologically, SARS-CoV-2 infects angiotensin-converting enzyme 2 (ACE2)-expressing nasal epithelial cells in the upper respiratory tract and type II alveolar epithelial cells in patients exhibiting pneumonitis[1,5]. The most severe disease courses led to death frequently but not exclusively in older patients with and without risk conditions. The primary symptoms of SARS-CoV-2 infections are fatigue, fever, sore throat, dry cough, loss of smell and taste within 5-21 days of incubation of the virus[6-9]. COVID-19 symptoms are heterogeneous and range from asymptomatic to mild, moderate, and severe pathological symptoms, presenting with or without pneumonia[10,11], however, most infected people develop mild to moderate illness and recover without hospitalization[12,13]. Primarily the older COVID-19 patients can develop acute severe respiratory distress syndrome (ARDS) due to a cytokine storm which is a life-threatening situation, requiring ventilation and intensive care support[14-18]. High serum levels of IL-6, IL-8, IL-10, TNF-α cytokines and an immune hyper-responsiveness referred to as a 'cytokine storm' is connected with poor clinical outcome[19,20].

Several break-through discoveries have extended our understanding how the virus takes advantage of the host and modulates immunity[12,17,21-25]. Recovered COVID-19 patients have an increased number of antibody-secreting cells, activated $CD4_+$ and $CD8_+$ T cells, and immunoglobulin M (IgM) and SARS-CoV-2 reactive IgG antibodies were detected in blood before full symptomatic recovery[26-28]. Most severely affected COVID-19 patients had a lower T cell but elevated B cell counts[13,19,29,30]. Interestingly, patients with mild symptoms were shown to have increased T and B cells compared with severely affected patients[26,29-31]. There could be several reasons for different disease outcomes including over-activated innate or hyper-activated adaptive immune responses leading to cytokine storms and resulting in severe injury to the lungs[10,13,25,32]. Despite of several ongoing efforts, the immunological mechanisms of the host-pathogen interaction are not well understood[33].

There is an intricate balance between the metabolic state of immune cells and generation of immune response[17,34-37]. $CD8_+$ T cells require energy to proliferate and accomplish their effective functions[38]. Most propagating cells such as lymphocytes utilize the most abundant energy substrates including, glucose, lipids, and amino acids[39]. In response to SARS-CoV-2 and other virus infections, $CD8_+$ T cells play a pivotal role in profound growth and proliferation to generate their effective functional cells which can produce copious amounts of effector molecules such as cytokines and cytotoxic granules[30,38-40]. An activated immune system is coupled with a change in metabolic reprogramming to produce enough energy needed during (viral) infection[38,39]. Proliferating T cells ferment glucose to lactate even in the presence of oxygen to meet high energy demands[34,37-39]. Furthermore, glucose and glutamine are involved in the hexosamine biosynthetic pathway, which regulates the production of uridine

diphosphate N-acetyl glucosamine necessary for T cell clonal expansion and function[41]. The synthesis of lactate intracellularly is crucial for T cells to have an increased glycolytic flux[38].

Peripheral blood mononuclear cells (PBMCs) can be analyzed to measure the health status of an individual and can serve as a health biomarkers[42]. Therefore, the metabolic status of lymphocytes could help to predict disease severity or to select the optimal therapeutic intervention to boost the immune function during infection. Generally, most of the metabolism-related functions in PBMCs during SARS-CoV-2 infections were inferred based on transcriptomics analysis[34,43] and no functional data (biochemical level) have been presented. Thereof, understanding the kinetics of adaptive immune response as well as the metabolic functions during SARS-CoV-2 infections will help to elucidate the host immune response to SARS-CoV-2 infection. In this study, using flowcytometry and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, we characterized the PBMCs from SARS-CoV-2 infected and convalescent patients for the their immunophenotypic and metabolic functions.

Results:

Characteristics of study participants

PBMCs were isolated and cryopreserved from blood samples obtained from COVID-19 patients suffering from mild ('Mild (outpatient)') or moderate/severe ('Moderate (inpatient)') disease or were already recovered ('Convalescent') and from healthy controls ('HC'). Classification of disease severity for this analysis was based on the requirement of hospitalization. Patients with mild COVID-19 were recruited within three days after confirmation of infection by RT-qPCR. From moderate to severe COVID-19 patient blood samples were collected one week after their admittance. The moderate patients were admitted to the hospital requiring medical care, however, they did not need ventilation or O_2 supply. Recovered patients were included based on a positive SARS-CoV-2 antibody testing. Study participant characteristics are described in Table 2.

Immunophenotyping of COVID-19 mild, moderate and convalescent COVID-19 patients

To compare the number of lymphocytes and monocytes amongst the four study groups, PBMCs were stained and analysed by flow cytometry. Both, lymphocytes ($p=0.005$) and monocytes ($p=0.04$), were significantly decreased in moderate COVID-19 patients compared with HC (Suppl. Fig. 1a, b). However, mild and convalescent patients also had a reduced, but not significantly reduced, count of lymphocytes/monocytes compared to HC.

Increased inflammatory monocytes and reduced NK cells in moderate COVID-19 patients

Monocytes were further classified into classical, non-classical and intermediate based on expression of CD14 and/or CD16 and we used the same gating strategies as described earlier[44] (Suppl. Fig. 1c). We found that $CD16^{++}CD14^{+}$ patrolling (non-classical) monocytes were significantly increased ($p=0.0008$) in numbers in moderate patients compared to HC, whereas this number is decreased again significantly compared with convalescent patients ($p=0.01$) (Fig. 1a). The percentage of $CD16^{++}CD14^{+}$ monocytes was also significantly increased ($p=0.006$) in mild patients (outpatients) compared with moderate patients (Fig. 1a Panel I). Interestingly, $CD16^{++}CD14^{++}$ pro-inflammatory monocytes (intermediate) were again

significantly increased in moderate (p=0.003) compared with HC as well as between mild and HC (p=0.02) (Fig. 1a panel II). Furthermore, we observed a significantly reduced percentage of $CD14^{++}CD16^{-}$ phagocytic monocytes (classical) in moderate compared with mild (p=0.0009), HC (p<0.0001) and convalescent (p=0.0003) patients (Fig. 1a Panel III). Finally, we explored the lymphoid cells compartment for NK cells ($CD56^{+}CD3^{-}CD19^{-}$). We found that both mild (p=0.0003) and moderate (p=0.0002) patients were significantly different from convalescent and HC (p<0.0001; HC *vs* mild or moderate) patients (Fig. 1b).

Dynamics of B and T cells in mild, moderate and convalescent patients

Both T and B cells are indispensable for the immune response against viral infections such as SARS-CoV-2. Firstly, we compared the number of B cells amongst the study groups, which give rise to virus-specific antibodies (see gating strategy in Suppl. Fig. 1c). The $CD19^{+}CD3^{-}$ cells (B cells) were significantly increased in mild (p=0.008; 1.7x times) and moderate (p=0.0008; 1.9x times) patients compared with HC (Fig. 2a). Whilst, B cells were significantly decreased in moderate compared to convalescent (p=0.03) patients (Fig. 2a). Comparing $CD3^{+}CD19^{-}$ lymphocytes among the different patient groups we observed no significant difference. However, there was an increased trend of $CD3^{+}$ cells in the outpatients, inpatients and convalescent groups compared with HC.

$CD3^{+}$ cells were analysed for the $CD4^{+}$ and $CD8^{+}$ T cell compartment. There was a tendency of increased $CD4^{+}$ T cells for outpatients, inpatients and convalescent patients compared to HC, but no significant difference was observed among any of the groups. $CD8^{+}$ T cells were significantly different between HC compared to moderate (p=0.04) or convalescent (p=0.04) patients (Fig. 2b). Finally, we characterized $CD4^{+}Foxp3^{+}CD45R^{-}$ regulatory T cells (Tregs), however, no significant difference was observed among the different groups (Suppl. Fig. 2).

Impaired activation and defective cytotoxic functions of $CD8^{+}$ T cells

We found that the percentage of $CD8^{+}$ T cells was decreased in mild and convalescent patients compared to HC. Thus, we explored the activation status of $CD8^{+}$ T cells based on HLA-DR expression. We found that $CD8^{+}$ T cell activation status in all three groups of infected patients were significantly different from HC (mild p=0.01, moderate p=0.009, and convalescent p=0.008, Fig. 3a). We characterized the cytotoxic potential of $CD8^{+}$ T cells based on granzyme B and perforin levels and found that there was a tendency of decreased granzyme B expression in mild, moderate and convalescent patients compared with HC (Fig. 3b), however it did not reach significance. Perforin was significantly decreased in convalescent (p=0.03) patients compared with HC (Fig. 3b), although mild patients also had borderline significantly reduced levels (p=0.06). Furthermore, we studied the expression of CD38, a marker of cell activation, which was significantly upregulated in convalescent patients compared with HC (p=0.01), mild (p=0.03) and moderate (p=0.02) patients (Fig. 4a). Similarly, convalescent patients had significantly increased numbers of $CD38^{+}PD\text{-}1^{+}$ cytotoxic $CD8^{+}$ T cells compared with HC (p=0.005), moderate (p=0.002) and mild (p=0.002), which reflects the exhaustion and non-responsiveness (anergy) of $CD8^{+}$ T cells (Fig. 4b). Overall, our data suggested that $CD8^{+}$ T cells have reduced activation, diminished expression of cytotoxic molecules such as perforin and granzyme B and severely exhausted phenotype.

Dynamics of metabolites production in mild, moderate and convalescent patient

PBMCs from all patient groups were subjected to $_1$H-NMR spectroscopy analysis. We identified and quantified a total of 18 metabolites (Fig. 5a). Hereby, unsupervised PCA showed that spectral data from mild and moderate patients formed overlapping clusters clearly distinct from a cluster formed by HC and convalescent patients (Fig. 5b), indicating a strong difference in metabolite levels between infectious state compared to healthy or recovered state. Statistical analysis of the four different groups, revealed that 15 metabolites showed p-values < 0.05, with highest significance for metabolites from the energy metabolism (Fig. 5c, Suppl. Fig. 3 & Table 1). The data indicate a strong consumption of glucose, acetate, formate during infection, while with lactate levels are increased. Furthermore, we also found very high levels of fructose in PBMCs from mild patients, medium concentrations in moderate and, low levels in HC and convalescent patients (Fig. 5c). Furthermore, glutamate was almost abolished in mild and moderate patients, potentially as a consequence of enhanced production of α-ketoglutarate in the TCA cycle in PBMCs *via* glutamate dehydrogenase (Fig. 5c).

To find an association between different metabolites, we applied the variable importance of projection (VIP) score. We found that formate and glucose had the highest score compared to another other metabolites (Fig. 6A). In order to determine if additional metabolites are positively associated with changes in glucose, lactate and fructose, we performed a pattern hunter analysis for all metabolites. We found that high glucose levels correlated with high formate, acetate and glutamate and low lactate and fructose (Fig. 6b), indicating enhanced glycolysis and TCA cycle in PBMCs. Similarly, fructose, that is entered *via* fructose-1-phosphate and dihydroxy acetone phosphate (DAP) into the glycolysis, is correlated positively with lactate and citrate and a decrease in acetate and formate, respectively (Fig. 6b). Interestingly, levels of the ROS scavenger taurine are only positively correlated with lactate and fructose, but not glucose (Fig. 6).

Table 1: Summary of metabolites dysregulated in PBMCs

No	Metabolites	HC	Mild	Moderate	Convalescent
1	Glucose	↑↑	↓↓	↓↓	↑↑
2	Formate	↑↑	↓↓↓	↓↓	↑↑
3	Acetate	↑	↓↓	↓	↑
4	Lactate	↓↓	↑↑	↑↑	↓↓
5	Fructose	↓	↑↑	↑	↓
6	Glutamate	↑	↓	↓	↑
7	Citrate	↓	↑↑	↑	↓
8	Taurine	↓↓	-	↑↑	↓
9	Creatine	↓	↑	-	-
10	Alanine	↓	-	↑↑	↓
11	Glycine	↑	↓	↓	-
12	Isoleucine	-	↓↓	↓	↑

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acetate and glutamate and low lactate and fructose (Fig. 6b), indicating enhanced glycolysis and TCA cycle in PBMCs. Similarly, fructose, that is entered *via* fructose-1-phosphate and dihydroxy acetone phosphate (DAP) into the glycolysis, is correlated positively with lactate and citrate and a decrease in acetate and formate, respectively (Fig. 6b). Interestingly, levels of the ROS scavenger taurine are only positively correlated with lactate and fructose, but not glucose (Fig. 6).

Discussion:

SARS-CoV-2 infections are an intense and rapidly evolving area of research due to the ongoing global pandemic[19,25]. In this study, we used flow cytometry and $_1$H-NMR to decipher the cell proportions and functional state of immune cells (PBMCs) in mild, moderate and convalescent COVID-19 patients compared to HC. Recent reports from COVID-19 patients suggested that mild and severe patient had lymphopenia[11,45-47]. Here, we found that mild patients have reduced lymphocyte numbers whereas convalescent patients have recovered the total lymphocyte counts. Similarly, monocytes were also reduced in mild patients, which is in agreement with other recent studies[29,48,49]. Importantly, our characterization of myeloid cell compartment based on CD16 and CD14 markers suggested that non-classical and intermediate monocytes were increased during an active mild or moderate SARS-CoV-2 infection, once infections are cleared the monocyte numbers return to normal. These results are in accordance with some of the recent published studies[48,50,51], while another study suggested the opposite[52].

In our cohort, specifically CD56$^+$NK cells were dramatically decreased during the course of active SARS-CoV-2 viral infections (mild and moderate), while during recovery the numbers were comparable to HC as report by others[53]. Similarly another recent study suggested the decrease in number of NK cell subsets in COVID-19 patients, with no change in CD56^{bright} or CD56^{dim} cells[54]. Thus, these data point to a crucial role of CD56$^+$NK cells in eliminating SARS-CoV-2 infections[47]. CD19$^+$ B lymphocytes were increased during the course of infection and remain slightly higher than HC, thus reflecting the antibody response against the COVID-19 virus. Thus, this data implicated that these patients were able to generate the SARS-CoV-2 specific B cells.

A major difference was found in the T lymphocytes compartment. On the one hand, CD4$^+$ T cells were increased during infection, but not dramatically. On the other hand, CD8$^+$ T cells were significantly decreased in moderate and convalescent patients as reported earlier[53]. Thus, it appears that during viral infection non-virus specific CD8$^+$ T cells are dead, while the viral-specific surviving CD8$^+$ T cells are clonally expanded but appeared to lost their effector functions[55]. To confirm this, we first measured the activation status of CD8$^+$ T cells and found that CD8$^+$ T appeared to be less activated based on their HLA-DR activation marker[26]. Further, CD8$^+$ T cells were examined for another activation marker CD38 which is involved in cell adhesion, signal transduction and calcium signalling[56] and was found to be upregulated in convalescent patients but not during active infection. These CD38$^+$CD8$^+$ T cells, were also expressing higher levels of PD-1, which is an immune checkpoint and marker of exhaustion[24,30,49,57,58]. It guards against autoimmunity, promotes apoptosis of antigen-specific T cells and promotes self-tolerance by suppressing T cell inflammatory activity. Thus, viral infection leaves convalescent patients with exhausted phenotypes. We found that although there was not a significant change in the numbers of Tregs in COVID-19 patients, there was

a trend towards elevated levels of Tregs in COVID-19 patients and rescued Tregs in convalescent patients, in agreement with previous studies[57].

A key finding of our study was the surprising observation that granzyme B and perforin secreting $CD8_+$ T cells were significantly reduced in convalescent patients. The possible implication of our finding is that convalescent patients, specifically including cancer patients under treatment, could be susceptible to future opportunistic infections with other viruses including different strains of SARS-CoV-2.

To date, the general metabolic physiology of PBMCs is not well defined in literature. However, it is clear that PBMCs are dependent on circulating nutrients and hormones in the blood system[59]. The defective immune response in COVID-19 patients prompted us to investigate the metabolic functions of these immune cells. Our metabolomics data indeed shows that PBMCs from actively infected patients have a distinct metabolic profile from convalescent or healthy individuals. The most notable difference we observed were for metabolites from the glycolysis and oxidative phosphorylation (TCA cycle) pathway, which is in accordance with recently published transcriptome data for PBMCs[39,43]. Metabolites such as glucose, formate, acetate and choline were also reduced in PBMCs in infected patients whereas, HC and convalescent patients had a normal profile. Accordingly, the glycolytic pathway end products such as lactate were higher in active mild and moderate COVID-19 patients compared with HC and convalescent individuals. Therefore, our data suggests that PBMCs (which constitute a major fraction of T lymphoid cells: 70- 80%) may have changed their metabolic functions, particularly favouring the oxidative phosphorylation pathway over the glycolytic pathway, to meet the high demands of energy needed to combat the ongoing viral infection.

A recent report suggested that elevated glucose levels enhance SARS-CoV-2 replication and cytokine expression in monocytes and glycolysis sustains the viral-induced monocyte response[60]. Recently, it was emphasized that glucose consumption in PBMCs during COVID-19 disease could be also a read-out of cytokine storms[34]. Further, a higher abundance of citrate in PBMCs suggested that perhaps T cells could use the oxidative phosphorylation pathway for energy consumption to endure the infection, as recent transcriptomic data also suggested that higher expression of genes related to oxidative phosphorylation both in peripheral mononuclear leukocytes and bronchoalveolar lavage fluid (BALF) could play a crucial role in increased mitochondrial activity during SARS-CoV-2 infection[34].

Another interesting finding of our study was the increase of fructose levels in PBMCs during the course of infection. Previous findings suggested that fructose is involved in the inflammatory pathways for the production of IL-1β and IL-6 production[61]. Thus, it is possible that the immune cells (most probably monocytes) could be triggered by higher fructose and simultaneously induce inflammation and IFN-γ production by T cells[61]. These findings are correlating with recent transcriptomic studies on the BALF from infected COVID-19 patients and plasma of COVID-19 patients that also identified changes in fructose metabolism[34,62].

We finally observed a reduction of granzyme B and perforin in $CD8_+$ T cells and detected the antioxidant amino acid taurine, which could be involved in the cytotoxic functions of $CD8_+$ T cells. Both granzyme B and perforin are involved in ROS production and taurine serves as ROS scavenger[63,64]. Thus, decreased granzyme B and perforin could be implicated in reduced ROS production for the impaired effectiveness of $CD8_+$ T cells in convalescent or COVID-19

patients. This should be the case, as taurine levels that are generally increased during an active infection in mild patients compared to healthy controls are not specifically decreasing due to granzyme B and perforin lacking ROS activity in COVID-19 patients. However, this finding needs further investigation to validate this hypothesis. In summary, the metabolomics data generated in this study provides first and crucial insights into the complex metabolic changes of PBMCs during SARS-CoV-2 infections, warranting further investigation.

Conclusions:

Using immunophenotyping and metabolomics approaches we detected significant changes in PBMC samples of mildly and moderately affected COVID-19 as well as convalescent patients compared to healthy controls. The significantly reduced amount of NK cells in both mild and moderate patient groups corresponded with the clustering of PBMCs metabolite levels in the principal component analysis distinct from the cluster formed by healthy and convalescent individuals. The dramatic changed metabolic activity and pathways, such as glycolysis and TCA cycle, might not only lead to a vulnerability of COVID-19 patients to subsequent infections, but can also offer insights into how PBMCs could be manipulated towards a better survival and personalized treatment of moderate and severe COVID-19 patients.

Materials and Methods:

Ethics statement

The study protocols were approved by the University of Tübingen, Germany Human Research Ethics Committee (TÜCOV: 256/2020BO2 (convalescent study), COMIHY: (225/2020AMG1) (outpatient study)-COMIHY, EUDRA-CT: 2020-001512-26, ClinicalTrials.gov ID: NCT04340544, and COV-HCQ: (190//2020AMG1) (inpatient study)-COV-HCQ, EUDRA-CT: 2020-001224-33, ClinicalTrials.gov ID: NCT04342221, 556/2018BO2) and all associated procedures were carried out in accordance with approval guidelines. All participants provided written informed consent in accordance with the Declaration of Helsinki.

Study participants

SARS-CoV-2 positive patients were used for this study and no other virus species were analysed in this study (COMIHY and COV-HCQ). Blood was collected from COVID-19 patients enrolled into two different prospective randomized, placebo-controlled, double blind clinical trials evaluating safety and efficacy of hydroxychloroquine in COVID-19 outpatients (COMIHIY) and hospitalized patients (COV-HCQ). We analysed subsets of these study cohort and used outpatient (n=3; COMIHY) which came to a specified outpatient ward at in the Institute of Tropical Medicine with mild symptoms and blood was taken and usually defined as D1 outpatients. Inpatients (n=3; COV-HCQ), blood was taken after 7-9 days after study inclusion defined as D7. These patients had moderate symptoms needing hospital care, however not being transferred to the intensive care unit in the hospital. Furthermore, convalescent COVID-19 patients (n=4) were defined as positive for serum antibody reactive to SARS-CoV-2 and blood was taken when they visited the Institute of Tropical Medicine for testing of antibody levels. Amongst this cohort, 3 persons reported mild fever for 10-11 days and 1 individual reported no fever but found positive for SARS-CoV-2 antibodies. Blood from healthy controls (n=5) was obtained from the hospital blood bank.

Table 2: Overview of study participants

No	COVID-19 status	Blood sampling	COVID-19 severity	Sex	Age
1	Outpatient (mild)	Day1	mild	F	21
2	Outpatient (mild)	Day1	mild	M	59
3	Outpatient (mild)	Day1	mild	F	40
4	Inpatient (moderate)	Day7	Moderate	M	57
5	Inpatient (moderate)	Day7	Moderate	M	47
6	Inpatient (moderate)	Day7	Moderate	F	78
7	Convalescent (Sero +ve)	Convalescent	Recovered, healthy	F	50
8	Convalescent (Sero +ve)	Convalescent	Recovered, healthy	F	24
9	Convalescent (Sero +ve)	Convalescent	Recovered, healthy	M	50
10	Convalescent (Sero +ve)	Convalescent	Recovered, healthy	F	51
11	HC1	-	None	F	36
12	HC2	-	None	M	60
13	HC3	-	None	M	40
14	HC4	-	None	M	37
15	HC5	-	None	M	47

Flow cytometry

PBMCs were isolated by standard Ficoll method[65]. A total of 1-2 x10^6 PBMCs per participants were used for three FACS panels (Table 2). In brief, cells were stained with surface markers in DPBS (Sigma) with Super Bright stain Buffer (ThermoFisher) for 30 minutes at room temperature (RT). To distinguish between live from dead, the cells were also incubated with LIVE/DEAD Fixable Infra-Red Dead stain (ThermoFisher). After surface staining cells were also stained for intracellular (IC) markers. Before IC staining, cells were fixed for 30-45 minutes and permeabilized for 5 minutes followed by IC antibody incubation for additional 30 minutes at RT. Cells were washed and resuspended in DPBS containing 2%FBS. Fixing of cells was performed irrespective of whether panel was used for IC staining or not to prevent the possible contamination during acquisition of the samples. For each sample 200,000 cells were acquired using BD LSRFortessa (core facility) equipped with 4 lasers (violet, blue and yellow-green and Red). Data were analysed using Flow Jo (Tree Star) and fluorescence minus one controls (FMO) were used for setting up the arbitrary gates for the major cell markers.

441 **Table 2: Antibodies and other reagents used for Flow cytometry**
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No.	Product Name	Clone	Fluorochrome	Product ID	Company
		NK cells and Monocytes (Panel 1)			
1	CD3	UCHT1	eFluor 450	48-0038-42	Thermofisher
2	CD4	SK3	SuperBright 600	63-0047-42	Thermofisher
3	CD8a	SK1	PerCP-eFluor 710	46-0087-42	Thermofisher
4	CD19	HIB19	eFluor 506	69-0199-42	Thermofisher
5	CD45-RA	HI100	PE-Cy7	25-0458-42	Thermofisher
6	HLA-DR	L243	Alexa Fluor647	A51010	Thermofisher
7	CD38	HIT2	PE-eFluor610	61-0389-42	Thermofisher
8	CD56	MEM188	PE	MA119638	Thermofisher
9	CD16	3G8	Super Bright702	67-0166-42	Thermofisher
10	CD14	61D3	Alexa Fluor700	56-0149-42	Thermofisher
11	Foxp3 (IC)	PCH101	FITC	11-4776-42	Thermofisher
		CD8 exhaustion, T helper follicular cells (Tfh) and antibody secreting cell (ASC) (Panel 2)			
1	CD3	UCHT1	eFluor 450	48-0038-42	Thermofisher
2	CD19	HIB19	eFluor 506	69-0199-42	Thermofisher
3	CD4	SK3	Super Bright 600	63-0047-42	Thermofisher
4	CD8a	SK1	PerCP-eFluor 710	46-0087-42	Thermofisher
5	CD38	HIT2	PE-eFluor 610	61-0389-42	Thermofisher
6	CD27	O323	Alexa Fluor700	56-0279-42	Thermofisher
7	CXCR5 (CD185)	MU5UBEE	FITC	11-9185-42	Thermofisher
8	ICOS (CD278)	C398.4A	PE	12-9949-81	Thermofisher
9	PD-1 (CD279)	eBioJ105 (J105)	PE-Cy7	25-2799-42	Thermofisher
10	HLA-DR	L243	Alexa Fluor647	A51010	Thermofisher
		Cytotoxic potential			
1	CD4	SK3	SuperBright600	63-0047-42	Thermofisher
2	CD8	SK1	PerCP-eFluor710	46-0087-42	Thermofisher
3	CD19	HIB19	eFluor 506	69-0199-42	Thermofisher
4	CD38	HIT2	PE-eFluor 610	61-0389-42	Thermofisher
5	HLA-DR	L243	Alexa Fluor 647	A51010	Thermofisher

6	GZMA (IC)	CB9	Alexa Fluor 488		Thermofisher
7	GZMB (IC)	GB11	PE	MA523639	Thermofisher
8	Perforin (IC)	dG9	PE-Cy7	12-9177-42	Thermofisher
		Other Flow reagents			
1	Ultracompensation bead			01-2222-42	Thermofisher
2	FOXP3/TRN FACTOR STAIN BUFFER SET			00-5523-00	Thermofisher
3	FLOW STAIN BUFFER SOLN			00-4222-57	Thermofisher
4	SB COMPLETE STAINING BUFFER			SB-4401-42	Thermofisher
5	DPBS			D8537	Sigma
6	Pancoll human			P04-601000	Pan Biotech

$_1$H-NMR metabolomics

To obtain PBMCs metabolites, PBMCs were suspended in an optimized solvent extraction mixture of 9:1 (methanol:chloroform) as described elsewhere in detail[66] and extracted with a focused ultrasound system (Covaris E220, Woburn, USA). The extraction solutions were evaporated to dryness for 4 hours in a vacuum concentrator and afterwards pellets resuspended with 45 µL in a 1 mM TSP containing deuterated phosphate buffer. After centrifugation at 20,000 x g for 10 min to remove residual macromolecules, 40 µL of the clear supernatant were transferred to 1.7 mm NMR tubes. Spectra were recorded on an ultrashielded 600 MHz spectrometer (Bruker AVANCE III HD, Karlsruhe, Germany) with a triple resonance 1.7 mm room temperature probe. Spectra used for analysis were acquired with a 2h 55min lasting CPMG pulse program. Metabolite annotation and quantification was done with ChenomX NMR Suite 8.3.

Statistical analysis

Bar diagrams were prepared using GraphPad Prism 6.0. FACS data were analysed using one-way ANOVA for multiple group comparisons (mild, moderate, convalescent and HC) in GraphPad Prism software. No matching or pairing was used. Assumed Gaussian distribution with equal standard deviations (SDs) for experimental design. Mean of each group was compared with the mean of every other group and Tukey's post-hoc tests for multiple comparisons. P value considered significant less than 0.05. Metabolites data were analysed with MetaboAnalyst 4.0 software.

Figure legends:

Fig. 1: Comparison of monocytes and NK cell percentage amongst study groups.

A. The stained PBMCs were gated on the monocyte population and $CD3^{+}CD19^{+}$ cells were excluded. Cell populations are displayed for CD16 and CD14 expression (upper FACS panel). One exemplary dot plot is shown per study group. The bar diagrams (lower panel) show the non-classical ($CD16^{++}CD14^{+}$), intermediate ($CD16^{++}CD14^{++}$) and classical ($CD16^{-}CD14^{++}$) monocytes. *P value <0.05, **P value <0.01 and ***P value <0.001.
B. The stained PBMCs were gated on lymphocyte population and further excluded the $CD3^{+}CD19^{+}$ cells and examined for the CD56 and CD16 expression in HC, mild, moderate and convalescent (upper FACS panel). One exemplary dot plot is shown per study group. The bar diagram shows the $CD56^{+}CD3^{-}CD19^{-}$ NK cells. **P value <0.01 and ****P value <0.0001.

Fig. 2: Increased B cells in mild and moderate patients and reduced $CD8^{+}$ cytotoxic T cells in mild and convalescent patients

A. The stained PBMCs were gated on lymphocyte population and examined for the CD19 and CD3 expression in HC, mild, moderate and convalescent (upper FACS panel). One exemplary dot plot is shown per study group. The bar diagram shows $CD3^{-}CD19^{+}$ B cells. *P value <0.05, **P value <0.01 and ***P value <0.001.
B. The $CD19^{-}CD3^{+}$ lymphocytes were examined for $CD4^{+}$ and $CD8^{+}$ T marker expression. One exemplary dot plot is shown per study group. There was statistically significant difference among HC, mild, moderate and convalescent (upper FACS panel). However, $CD8^{+}$ T cells were significantly reduced in outpatient and convalescent patients. *P value <0.05.

Fig. 3: Decreased activation and cytotoxic functional protein expression of $CD8^{+}$ T cells in convalescent patients

A. $CD8^{+}$ T cells were examined for the expression of activation marker HLA-DR (upper FACS panel). One exemplary dot plot is shown per study group. The bar diagram (lower panel) shows that HLA-DR was significantly lower on $CD8^{+}$ T cells in mild, moderate and convalescent COVID-19^{+} patients compared with HC.
B. $CD8^{+}$ T cells were examined for the expression of their cytotoxic potential using granzyme B and perforin expression using IC staining (upper FACS panel). One exemplary dot plot is shown per study group. There was statistically significant difference among HC, mild, moderate and convalescent (upper FACS panel) for granzyme B. The bar diagram (lower panel) shows that perforin expression was significantly lower on $CD8^{+}$ T cells in convalescent COVID-19^{+} patients compared with HC, though mild and moderate represent lower expression of perforin, but it did not to a significant level. *P value <0.05.

Fig. 4: Increased exhausted $CD8^{+}$ T cells in convalescent patients

A. Expression of activation marker CD38 on $CD8_+$ T cells (upper FACS panel). One exemplary dot plot is shown per study group. The bar diagram (lower panel) shows that CD38 expression was significantly higher on $CD8_+$ T cells in convalescent COVID-19_+ patients compared with HC. *P value <0.05.

B. Expression of activation marker CD38 and PD-1 on $CD8_+$ T cells (upper FACS panel). One exemplary dot plot is shown per study group. The bar diagram (lower panel) shows that PD-1_+CD38_+ expression on was significantly higher on $CD8_+$ T cells in convalescent COVID-19_+ patients compared with HC. *P value <0.05, **P value <0.01.

Fig. 5: ^{1}H-NMR spectroscopy of PBMC extracts

A. Heatmap of featured metabolites' concentrations plotted with SARS-CoV-2 progression group clustering.
B. Principle component analysis (PCA) was performed to identify the clustering of two different groups. HC and convalescent COVID-19 patient samples cluster together while SARS-Co-2 infected mild and moderate patients cluster in a separate cluster with PC1: 90.7% and PC2: 2.6%.
C. Box plots for differentially abundantly present metabolites in different group including HC, mild, moderate, and convalescent COVID-19 patient. *P value <0.05, **P value <0.01 and ***P value <0.001.

Fig. 6: Pattern hunter plots provide an insight of close correlations with other metabolites during COVID-19 infection.

A. Variable Importance in Projection (VIP) scores for all metabolites in the four studied groups.
B. Pattern hunter plot for glucose.
C. Pattern hunter plot for lactate and fructose.

Suppl. Fig. 1: Total % counts of monocytes and lymphocytes from PBMCs of COVID-19 patients.

A. Fixed PBMCs samples were acquired on flow cytometry on 2-3 different days for the entire experiments. Total 200,000 cells were acquired by flow cytometry and gating was performed based on FSC and SSC parameters for lymphocytes, monocytes and dead cells as described earlier[67-69].
B. The bar graphs represent the % of lymphocytes and monocytes.
C. Gating strategy for T lymphocytes (CD3, CD4 and CD8) monocytes (CD14 and CD16)[44], NK cells (CD56) using FMO controls.

Suppl. Fig. 2: Kinetics of regulatory T cells is not affected significantly in mild, moderate and convalescent patients.

$Foxp3_+$ expression on $CD19^-CD3_+CD4_+CD45RA^-$ T cells to identify the regulatory T cells in HC, outpatient, outpatient and convalescent (upper FACS panel). There was statistically significant difference among HC, mild, moderate and convalescent (upper FACS panel).

Suppl. Fig. 3: Metabolite analysis in COVID-19 patients

A. Analysis of Variance (ANOVA) for multi-group comparisons
B. Partial Least Squares Discriminant Analysis (PLSDA) scores plot

C. Hierarchical clustering of metabolites (distance measured with Pearson r correlation coefficient)
D. Boxplots for branched chain amino acids valine and leucine

Authors contributions:

YS: Overall study design and project coordination, flow cytometry experiments, data analysis and interpretation and initial metabolic sample preparation, funding generation, and writing the manuscript.
CT: Metabolites sample preparation, Processing of the sample on $_1$H-NMR, data analysis, data interpretation and writing
MO: Metabolites sample preparation, Processing of the sample on $_1$H-NMR
RF, NK: Provided patient materials, Isolation of PBMCs, Performed the experiments for flowcytometry in BSL-2 facility, flow data interpretations.
JH, AK: Coordination of the clinical trials, revision of the manuscript.
RB: Isolation of PBMCs from HC and sample preparation for flow cytometry.
SO, MS, NC, OR: Study design, providing research tools, funding generation, data interpretation and writing and amending the manuscript.

All the authors have seen the manuscript, substantially contributed and agreed to be co-author.

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Competing interests:

Authors declare no financial competing interests.

Consortia

The members of the Deutsche COVID-19 Omics Initiative (DeCOI) are Angel Angelov, Robert Bals, Alexander Bartholomäus, Anke Becker, Daniela Bezdan, Ezio Bonifacio, Peer Bork, Nicolas Casadei, Thomas Clavel, Maria Colome-Tatche, Andreas Diefenbach, Alexander Dilthey, Nicole Fischer, Konrad Förstner, Julia-Stefanie Frick, Julien Gagneur, Alexander Goesmann, Torsten Hain, Michael Hummel, Stefan Janssen, Jörn Kalinowski, René Kallies, Birte Kehr, Andreas Keller, Sarah Kim-Hellmuth, Christoph Klein, Oliver Kohlbacher, Jan O.

Korbel, Ingo Kurth, Markus Landthaler, Yang Li, Kerstin Ludwig, Oliwia Makarewicz, Manja Marz, Alice McHardy, Christian Mertes, Markus Nöthen, Peter Nürnberg, Uwe Ohler, Stephan Ossowski, Jörg Overmann, Silke Peter, Klaus Pfeffer, Anna R. Poetsch, Alfred Pühler, Nikolaus Rajewsky, Markus Ralser, Olaf Rieß, Stephan Ripke, Ulisses Nunes da Rocha, Philip Rosenstiel, Antoine-Emmanuel Saliba, Leif Erik Sander, Birgit Sawitzki, Philipp Schiffer, Eva-Christina Schulte, Joachim L. Schultze, Alexander Sczyrba, Yogesh Singh, Oliver Stegle, Jens Stoye, Fabian Theis, Janne Vehreschild, Jörg Vogel, Max von Kleist, Andreas Walker, Jörn Walter, Dagmar Wieczorek, and John Ziebuhr.

References:

1 Zhu, N. et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 382, 727-733, doi:10.1056/NEJMoa2001017 (2020).

2 Dashboard, J. C.-L. https://coronavirus.jhu.edu/map.html. (2020).

3 Dashboard, W. L. https://covid19.who.int. (2020).

4 Chang, L., Yan, Y. & Wang, L. Coronavirus Disease 2019: Coronaviruses and Blood Safety. Transfus Med Rev, doi:10.1016/j.tmrv.2020.02.003 (2020).

5 Lehner, N. J. M. P. J. How does SARS-CoV-2 cause COVID-19? Science, 510-511 (2020).

6 Liu, J. et al. Community Transmission of Severe Acute Respiratory Syndrome Coronavirus 2, Shenzhen, China, 2020. Emerg Infect Dis 26, doi:10.3201/eid2606.200239 (2020).

7 Singhal, T. A Review of Coronavirus Disease-2019 (COVID-19). Indian J Pediatr 87, 281-286, doi:10.1007/s12098-020-03263-6 (2020).

8 WHO. <WHO-2019-nCoV-Sci_Brief-Transmission_modes-2020.3-eng.pdf>. WHO Report (2020).

9 Rothe, C. et al. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. N Engl J Med 382, 970-971, doi:10.1056/NEJMc2001468 (2020).

10 Xu, Z. et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet Respiratory Medicine 8, 420-422, doi:10.1016/s2213-2600(20)30076-x (2020).

11 Wei Huang, J. B., Michelle McNamara, Suraj Saksena, Marsha Hartman, Tariq Arshad, Scott J. Bornheimerand Maurice O'Gorman. Lymphocyte Subset Counts in COVID-19 Patients: A Meta-Analysis. doi:10.1002/cytoa.24172 (2020).

12 Chen, G. et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 130, 2620-2629, doi:10.1172/JCI137244 (2020).

13 Shi, Y. et al. COVID-19 infection: the perspectives on immune responses. Cell Death Differ, doi:10.1038/s41418-020-0530-3 (2020).

14 NcA OANA dOcEA, A. T., DANA ALBULEScU, OANA CRISTEA, & OVIdIU ZLATIAN, M. V., STERGHIOS A. MOScHOS, dIMITRIS TSOUKALAS, MARINA GOUMENOU, NIKOLAOS dRAKOULIS, JOSEF M. dUMANOV, VIcTOR A. TUTELYAN, GENNAdII G. ONIScHENKO, MIcHAEL AScHNER, dEMETRIOS A. SPANdIdOS and dANIELA cALINA. A new threat from an old enemy: Re-emergence of coronavirus (Review). INTERNATIONAL JOURNAL OF MOLEcULAR MEdIcINE 45, 1631-1643 (2020).

15 Ong, E. Z. et al. A Dynamic Immune Response Shapes COVID-19 Progression. Cell Host Microbe 27, 879-882 e872, doi:10.1016/j.chom.2020.03.021 (2020).

16 Picchianti Diamanti, A., Rosado, M. M., Pioli, C., Sesti, G. & Lagana, B. Cytokine Release Syndrome in COVID-19 Patients, A New Scenario for an Old Concern: The Fragile Balance between Infections and Autoimmunity. Int J Mol Sci 21, doi:10.3390/ijms21093330 (2020).

17 Vabret, N. et al. Immunology of COVID-19: Current State of the Science. Immunity 52, 910-941, doi:10.1016/j.immuni.2020.05.002 (2020).

18 Velavan, T. P. & Meyer, C. G. Mild versus severe COVID-19: Laboratory markers. Int J Infect Dis 95, 304-307, doi:10.1016/j.ijid.2020.04.061 (2020).

19 Daniela Weiskopf1, K. S. S., Matthijs P. Raadsen, Alba Grifoni, Nisreen M.A. Okba, Henrik Endeman, Johannes P.C. van den Akker, Richard Molenkamp, Marion P.G. Koopmans, Eric C.M. van Gorp, Bart L. Haagmans, Rik L. de Swart, Alessandro Sette, Rory D. de Vries. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Science Immunology, doi:10.1126/sciimmunol.abd2071 (2020). (2020).

20 Del Valle, D. M. et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nature Medicine, doi:10.1038/s41591-020-1051-9 (2020).

21 Wu, H. et al. Clinical and Immune Features of Hospitalized Pediatric Patients With Coronavirus Disease 2019 (COVID-19) in Wuhan, China. JAMA Netw Open 3, e2010895, doi:10.1001/jamanetworkopen.2020.10895 (2020).

22 Du, S. Q. & Yuan, W. Mathematical modeling of interaction between innate and adaptive immune responses in COVID-19 and implications for viral pathogenesis. J Med Virol, doi:10.1002/jmv.25866 (2020).

23 Giamarellos-Bourboulis, E. J. et al. Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. Cell Host Microbe 27, 992-1000 e1003, doi:10.1016/j.chom.2020.04.009 (2020).

24 Braciale, T. J. & Hahn, Y. S. Immunity to viruses. Immunol Rev 255, 5-12, doi:10.1111/imr.12109 (2013).

25 McKechnie, J. L. & Blish, C. A. The Innate Immune System: Fighting on the Front Lines or Fanning the Flames of COVID-19? Cell Host Microbe 27, 863-869, doi:10.1016/j.chom.2020.05.009 (2020).

26 Thevarajan, I. et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nature Medicine, doi:10.1038/s41591-020-0819-2 (2020).

27 Assis, R. R. d. et al. Analysis of SARS-CoV-2 Antibodies in COVID-19 Convalescent Plasma using a Coronavirus Antigen Microarray . bioRxiv preprint doi: https://doi.org/10.1101/2020.04.15.043364., doi:10.1101/2020.04.15.043364 (2020).

28 Cao, Y. et al. Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients' B Cells. Cell 182, 73-84 e16, doi:10.1016/j.cell.2020.05.025 (2020).

29 Qin, C. et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis, doi:10.1093/cid/ciaa248 (2020).

30 Song, J. W. et al. Immunological and inflammatory profiles in mild and severe cases of COVID-19. Nat Commun 11, 3410, doi:10.1038/s41467-020-17240-2 (2020).

31 Channappanavar, R., Fett, C., Zhao, J., Meyerholz, D. K. & Perlman, S. Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome coronavirus infection. J Virol 88, 11034-11044, doi:10.1128/JVI.01505-14 (2014).

32 Janice Oh, H. L., Ken-En Gan, S., Bertoletti, A. & Tan, Y. J. Understanding the T cell immune response in SARS coronavirus infection. Emerg Microbes Infect 1, e23, doi:10.1038/emi.2012.26 (2012).

33 Bost, P. et al. Host-Viral Infection Maps Reveal Signatures of Severe COVID-19 Patients. Cell, doi:10.1016/j.cell.2020.05.006 (2020).

34 Gardinassi, L. G., Souza, C. O. S., Sales-Campos, H. & Fonseca, S. G. Immune and Metabolic Signatures of COVID-19 Revealed by Transcriptomics Data Reuse. Frontiers in Immunology 11, doi:10.3389/fimmu.2020.01636 (2020).

35 Ayres, J. S. Immunometabolism of infections. Nat Rev Immunol 20, 79-80, doi:10.1038/s41577-019-0266-9 (2020).

36 Thaker, S. K., Ch'ng, J. & Christofk, H. R. Viral hijacking of cellular metabolism. BMC Biol 17, 59, doi:10.1186/s12915-019-0678-9 (2019).

37 Moreno-Altamirano, M. M. B., Kolstoe, S. E. & Sanchez-Garcia, F. J. Virus Control of Cell Metabolism for Replication and Evasion of Host Immune Responses. Front Cell Infect Microbiol 9, 95, doi:10.3389/fcimb.2019.00095 (2019).

38 Gupta, S. S., Wang, J. & Chen, M. Metabolic Reprogramming in CD8+ T Cells During Acute Viral Infections. Frontiers in Immunology 11, doi:10.3389/fimmu.2020.01013 (2020).

39 Shehata, H. M. et al. Sugar or Fat?-Metabolic Requirements for Immunity to Viral Infections. Front Immunol 8, 1311, doi:10.3389/fimmu.2017.01311 (2017).

40 Pallett, L. J., Schmidt, N. & Schurich, A. T cell metabolism in chronic viral infection. Clin Exp Immunol 197, 143-152, doi:10.1111/cei.13308 (2019).

41 Swamy, M. et al. Glucose and glutamine fuel protein O-GlcNAcylation to control T cell self-renewal and malignancy. Nat Immunol 17, 712-720, doi:10.1038/ni.3439 (2016).

42 Liew, C. C., Ma, J., Tang, H. C., Zheng, R. & Dempsey, A. A. The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. J Lab Clin Med 147, 126-132, doi:10.1016/j.lab.2005.10.005 (2006).

43 Xiong, Y. et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect 9, 761-770, doi:10.1080/22221751.2020.1747363 (2020).

44 Wong, K. L. et al. The three human monocyte subsets: implications for health and disease. Immunol Res 53, 41-57, doi:10.1007/s12026-012-8297-3 (2012).

45 Liu, J. et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine 55, 102763, doi:10.1016/j.ebiom.2020.102763 (2020).

46 Wang, F. et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. J Infect Dis, doi:10.1093/infdis/jiaa150 (2020).

47 Wen, W. et al. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell Discov 6, 31, doi:10.1038/s41421-020-0168-9 (2020).

48 Zhang, D. et al. COVID-19 infection induces readily detectable morphological and inflammation-related phenotypic changes in peripheral blood monocytes, the severity of which correlate with patient outcome. medRxiv preprint doi: https://doi.org/10.1101/2020.03.24.20042655. , doi:10.1101/2020.03.24.20042655 (2020).

49 Zhou, R. et al. Acute SARS-CoV-2 Infection Impairs Dendritic Cell and T Cell Responses. Immunity, doi:10.1016/j.immuni.2020.07.026 (2020).

50 Silvin, A. et al. Elevated Calprotectin and Abnormal Myeloid Cell Subsets Discriminate Severe from Mild COVID-19. Cell, doi:10.1016/j.cell.2020.08.002 (2020).

51 Takahashi, T. et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. Nature, doi:10.1038/s41586-020-2700-3 (2020).

52 Schulte-Schrepping, J. et al. Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. Cell, doi:10.1016/j.cell.2020.08.001 (2020).

53 Jiang, Y. et al. COVID-19 pneumonia: CD8(+) T and NK cells are decreased in number but compensatory increased in cytotoxic potential. Clin Immunol 218, 108516, doi:10.1016/j.clim.2020.108516 (2020).

54 Christopher Maucourant, I. F., Andrea Ponzetta, Soo Aleman, Martin Cornillet, Laura Hertwig Benedikt Strunz, Antonio Lentini, Björn Reinius, Demi Brownlie, Angelica Cuapio Gomez, Eivind Heggernes Ask, Ryan M. Hull, Alvaro Haroun-Izquierdo, Marie Schaffer, Jonas Klingström, Elin Folkesson, Marcus Buggert, Johan K. Sandberg, Lars I. Eriksson, Olav Rooyackers, Hans-Gustaf Ljunggren, Karl-Johan Malmberg, Jakob Michaëlsson, Nicole Marquardt, Quirin Hammer, Kristoffer Strålin, Niklas K. Björkström; and the Karolinska COVID-19 Study Group. Natural killer cell immunotypes related to COVID-19 disease severity. Science Immunology, doi:10.1126/sciimmunol.abd6832 (2020). (2020).

55 Diao, B. et al. Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). Front Immunol 11, 827, doi:10.3389/fimmu.2020.00827 (2020).

56 Katsuyama, E. et al. The CD38/NAD/SIRTUIN1/EZH2 Axis Mitigates Cytotoxic CD8 T Cell Function and Identifies Patients with SLE Prone to Infections. Cell Rep 30, 112-123 e114, doi:10.1016/j.celrep.2019.12.014 (2020).

57 Zheng, H. Y. et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. Cell Mol Immunol, doi:10.1038/s41423-020-0401-3 (2020).

58 De Biasi, S. et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. Nat Commun 11, 3434, doi:10.1038/s41467-020-17292-4 (2020).

59 Zeng, Y. et al. Peripheral Blood Mononuclear Cell Metabolism Acutely Adapted to Postprandial Transition and Mainly Reflected Metabolic Adipose Tissue Adaptations to a High-Fat Diet in Minipigs. Nutrients 10, doi:10.3390/nu10111816 (2018).

60 Codo, A. C. et al. Elevated Glucose Levels Favor SARS-CoV-2 Infection and Monocyte Response through a HIF-1alpha/Glycolysis-Dependent Axis. Cell Metab, doi:10.1016/j.cmet.2020.07.007 (2020).

61 Jaiswal, N., Agrawal, S. & Agrawal, A. High fructose-induced metabolic changes enhance inflammation in human dendritic cells. Clin Exp Immunol 197, 237-249, doi:10.1111/cei.13299 (2019).

62 Di Wu, T. S., Xiaobo Yang, Jian-Xin Song Mingliang Zhang Chengye Yao Wen Liu, Muhan Huang, Yuan Yu, Qingyu Yang Tingju Zhu, Jiqian Xu, Jingfang Mu, Yaxin Wang, Hong Wang, Tang Tang, Yujie Ren Yongran Wu, Shu-Hai Lin, Yang Qiu, Ding-Yu Zhang, You Shang, Xi Zhou. Plasma Metabolomic and Lipidomic Alterations Associated with COVID-19. Natl Sci Rev, doi:10.1093/nsr/nwaa086 (2020).

63 Max W. S. Oliveira, J. B. M., Marcos R. de Oliveira, Alfeu Zanotto-Filho, Guilherme A. Behr, Ricardo F. Rocha, José C. F. Moreira, Fábio Klamt. Scavenging and antioxidant potential of physiological taurine concentrations against different reactive oxygen/nitrogen species. Pharmaceutical Reports 62, 185-193 (2010).

64 Cunningham, L., Simmonds, P., Kimber, I., Basketter, D. A. & McFadden, J. P. Perforin and resistance to SARS coronavirus 2. J Allergy Clin Immunol 146, 52-53, doi:10.1016/j.jaci.2020.05.007 (2020).

65 Maskus, D. J. et al. Characterization of a novel inhibitory human monoclonal antibody directed against Plasmodium falciparum Apical Membrane Antigen 1. Sci Rep 6, 39462, doi:10.1038/srep39462 (2016).

66 Lorenz, M. A., Burant, C. F. & Kennedy, R. T. Reducing time and increasing sensitivity in sample preparation for adherent mammalian cell metabolomics. Anal Chem 83, 3406-3414, doi:10.1021/ac103313x (2011).

67 Marimuthu, R. et al. Characterization of Human Monocyte Subsets by Whole Blood Flow Cytometry Analysis. J Vis Exp, doi:10.3791/57941 (2018).

68 Autissier, P., Soulas, C., Burdo, T. H. & Williams, K. C. Evaluation of a 12-color flow cytometry panel to study lymphocyte, monocyte, and dendritic cell subsets in humans. Cytometry A 77, 410-419, doi:10.1002/cyto.a.20859 (2010).

69 Davies, R., Vogelsang, P., Jonsson, R. & Appel, S. An optimized multiplex flow cytometry protocol for the analysis of intracellular signaling in peripheral blood mononuclear cells. J Immunol Methods 436, 58-63, doi:10.1016/j.jim.2016.06.007 (2016).

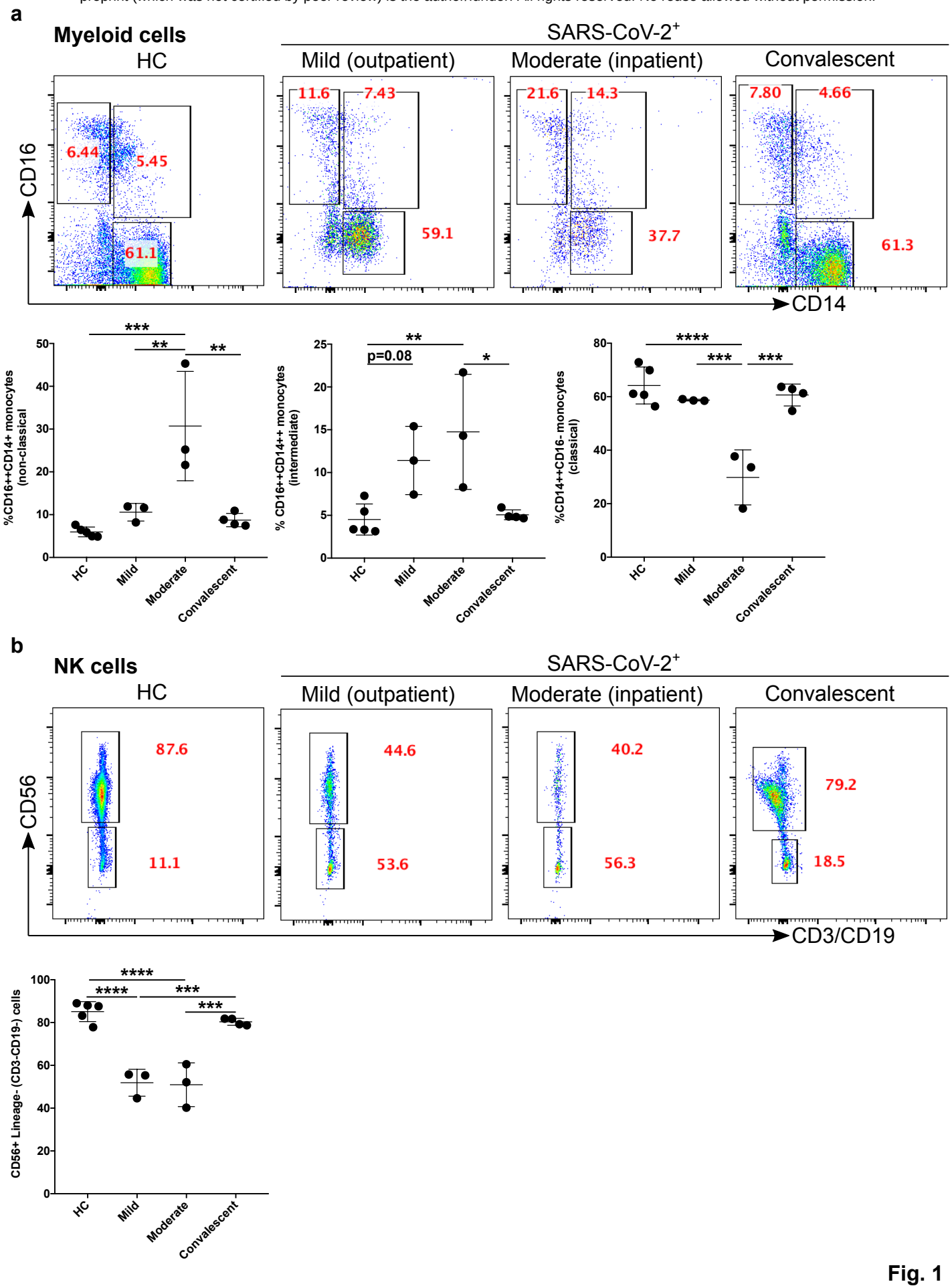
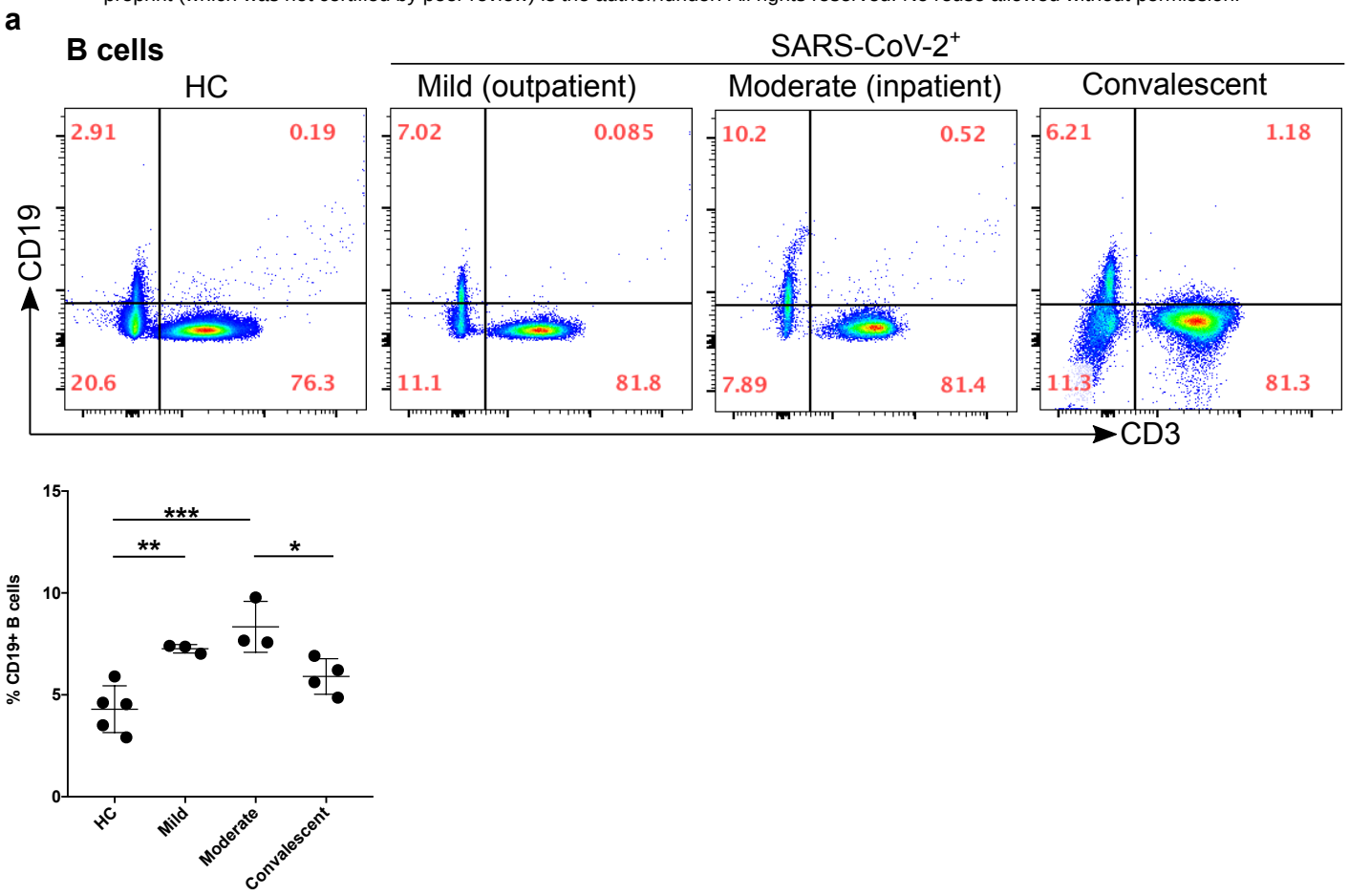
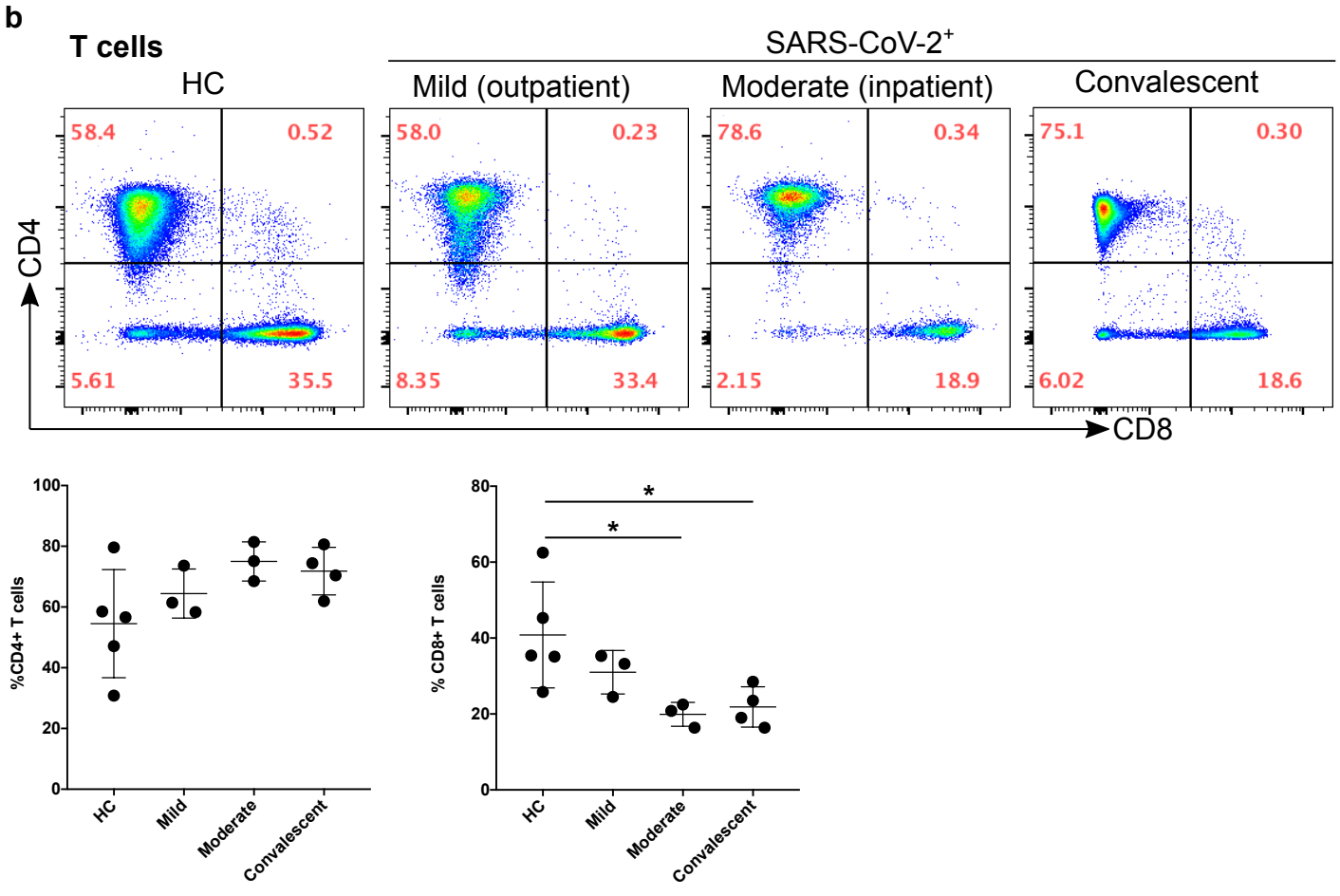


Fig. 1





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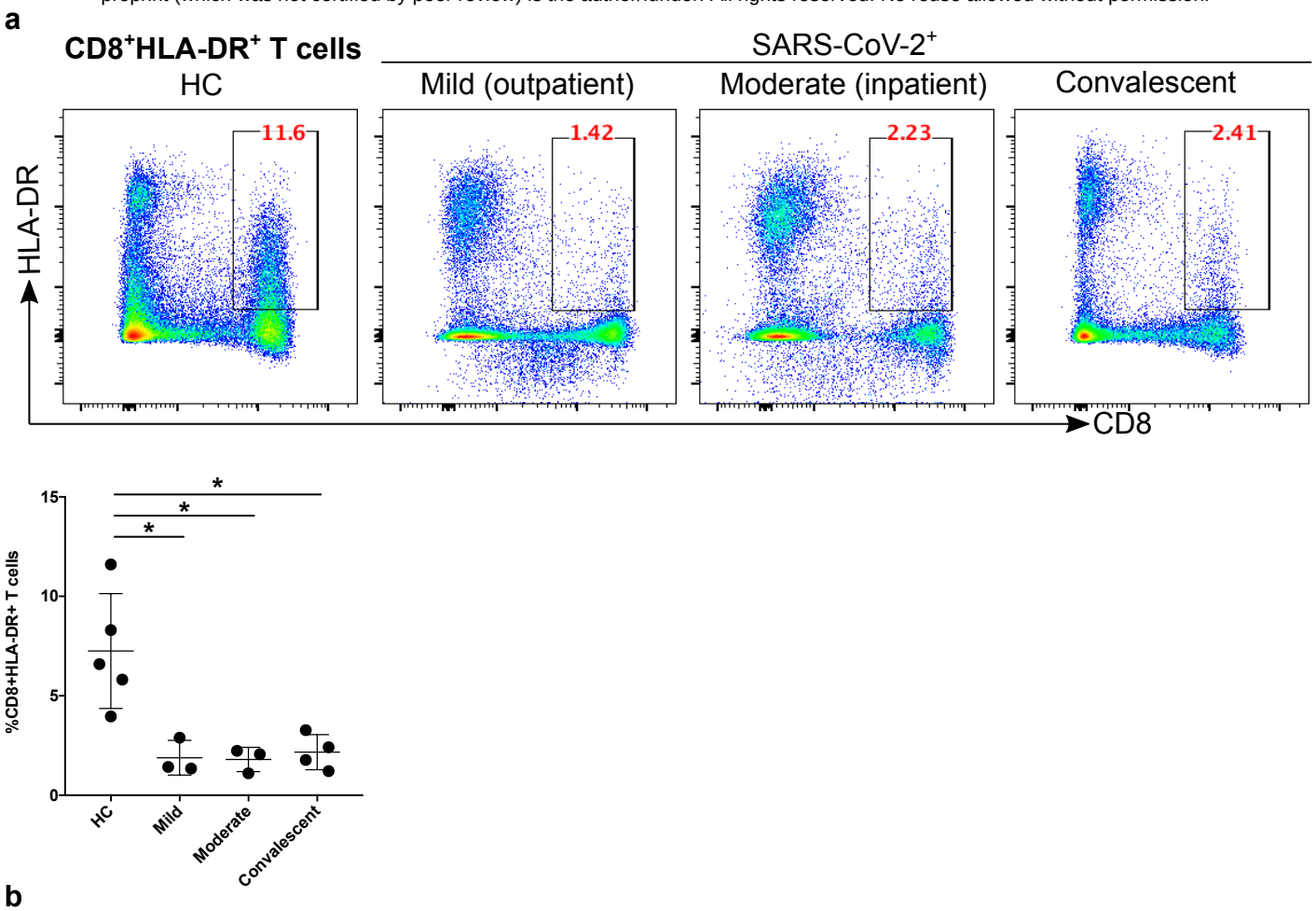


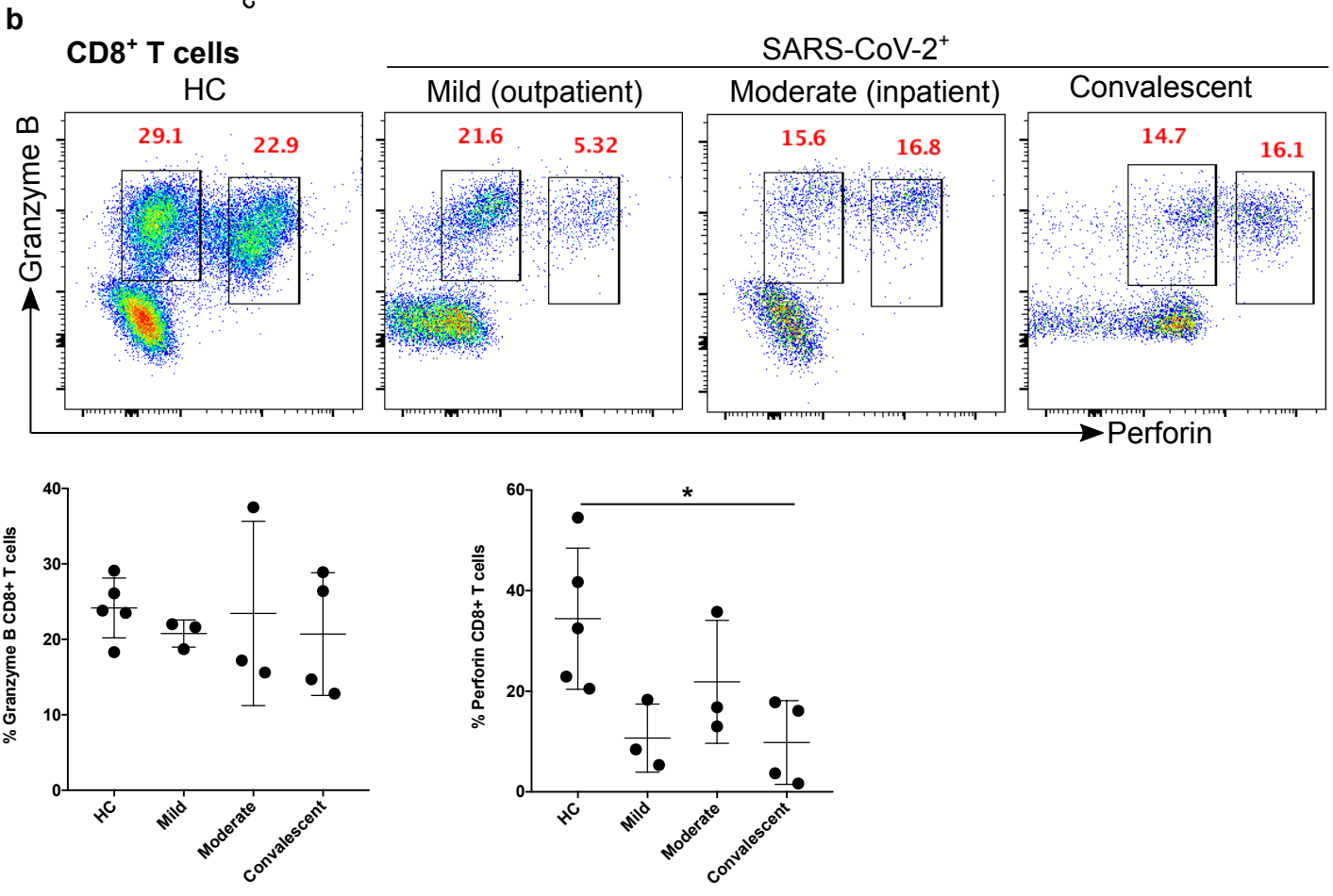
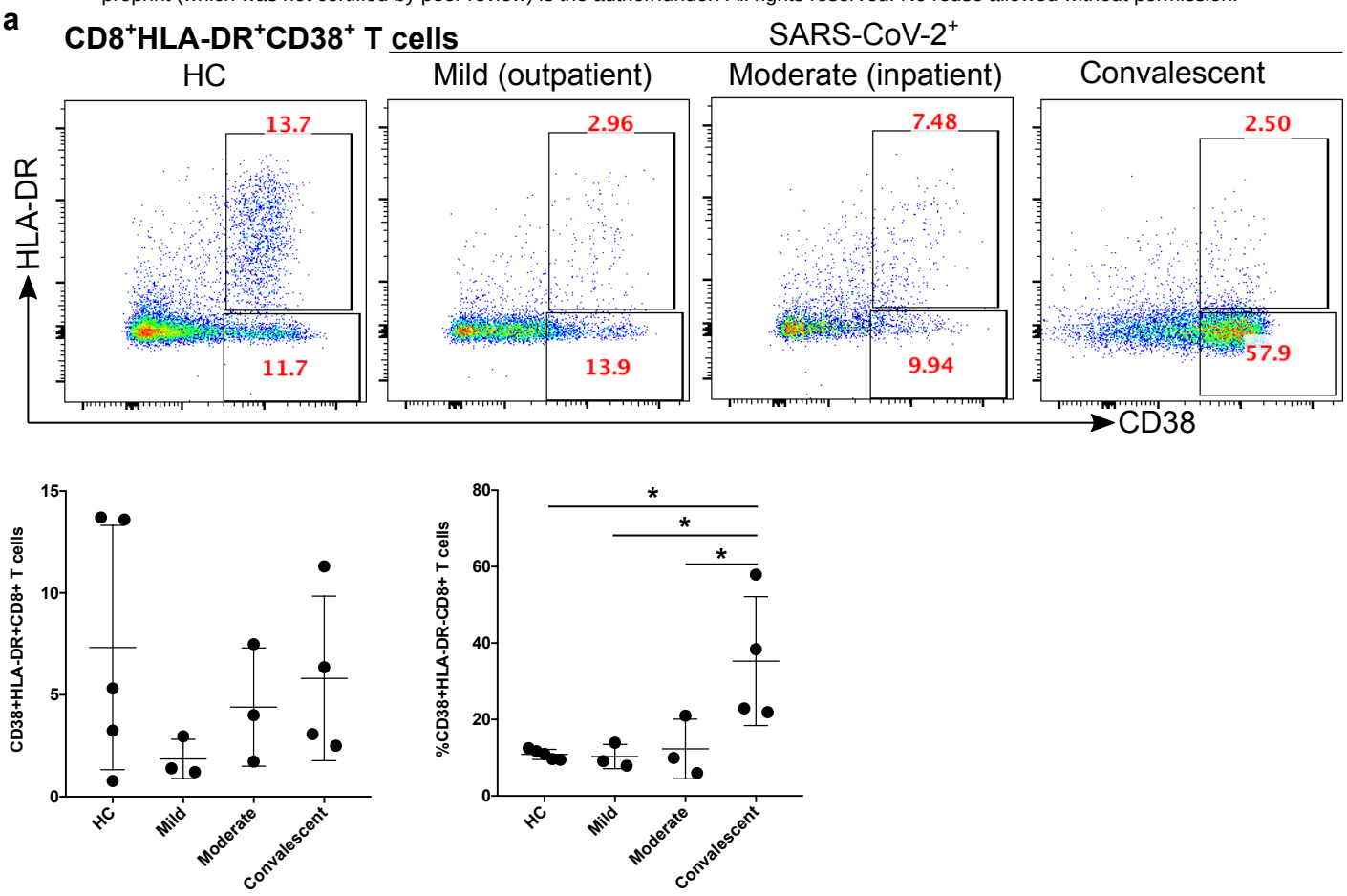


Fig. 3



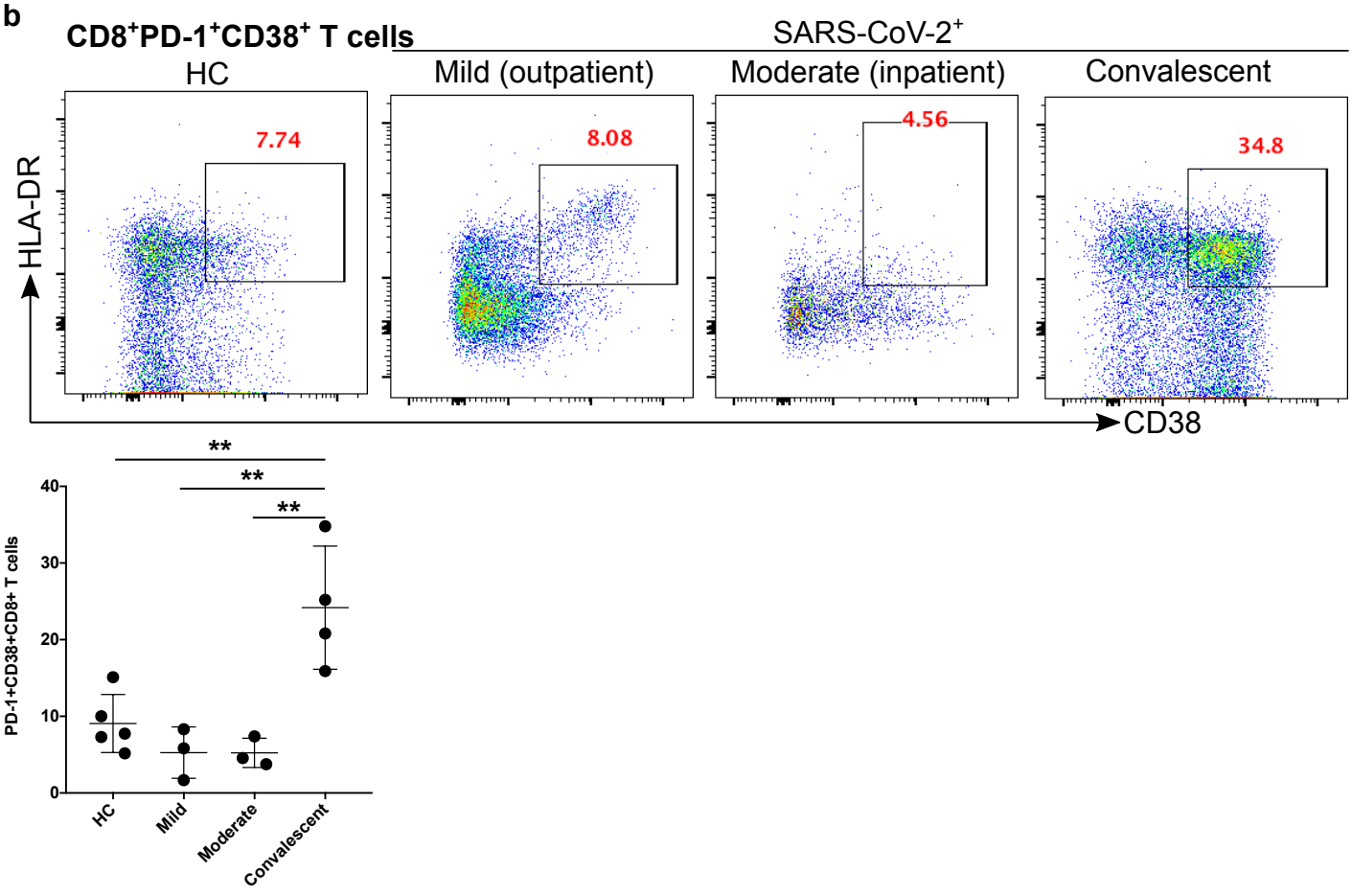


Fig. 4

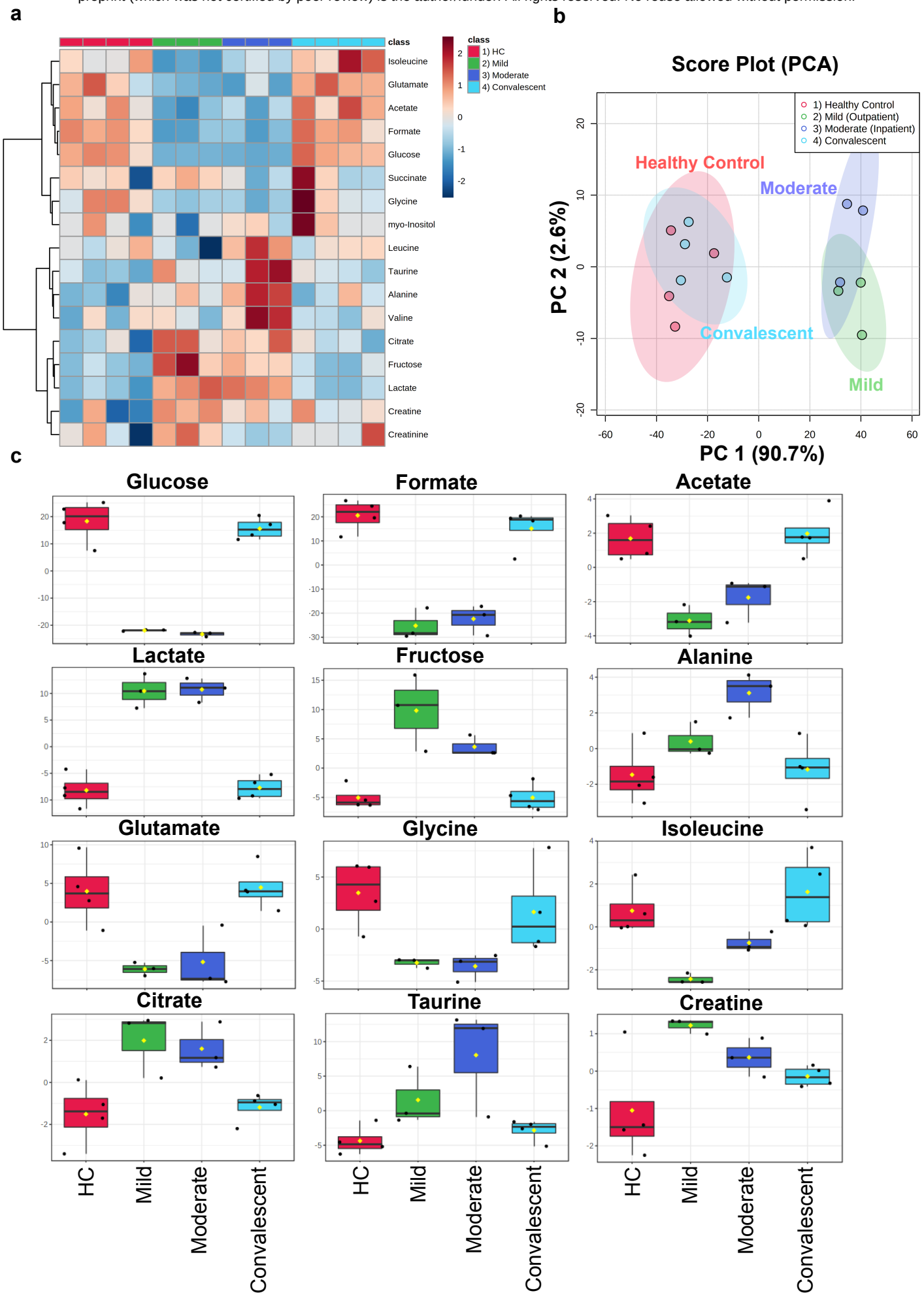


Fig. 5

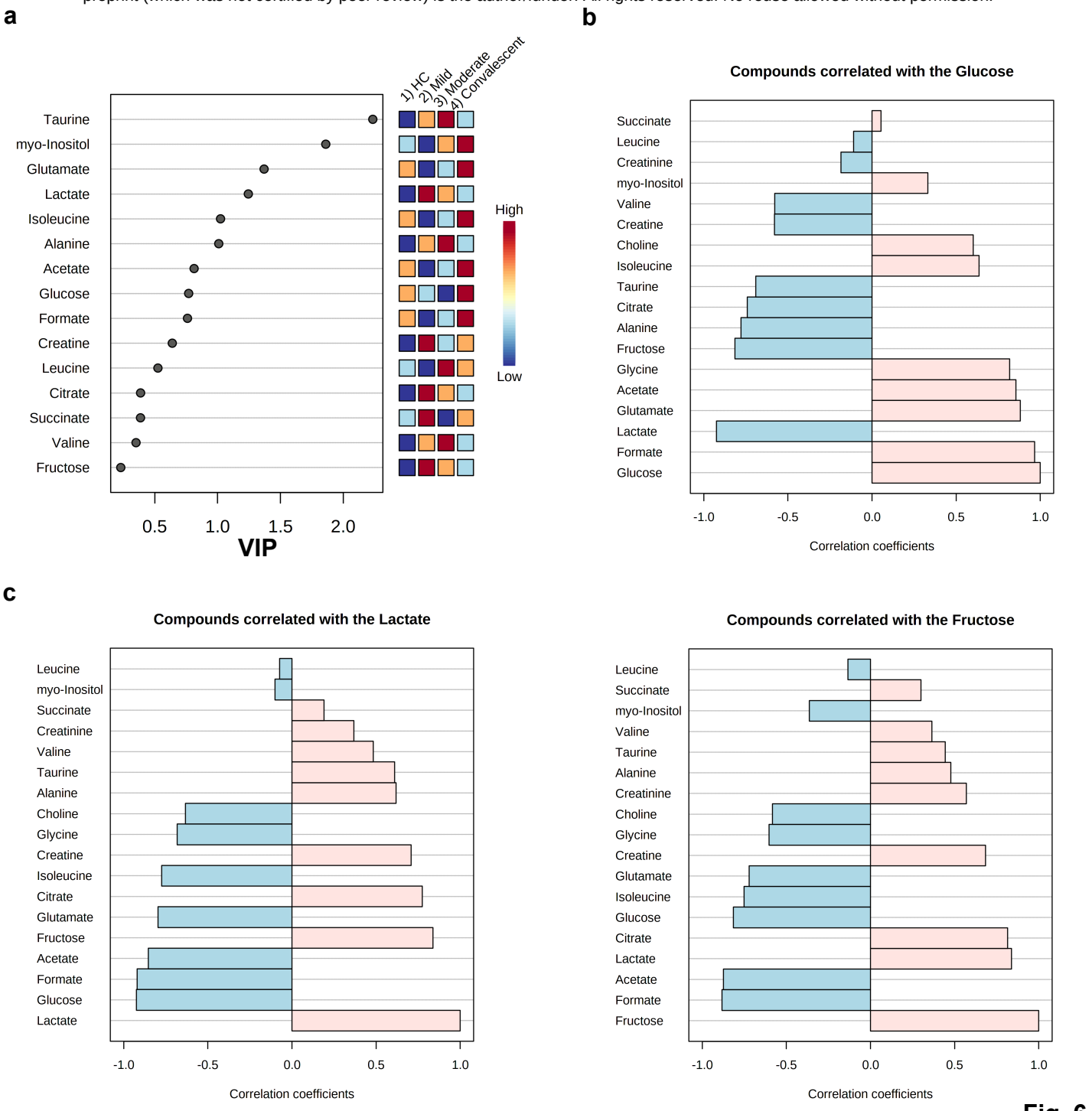
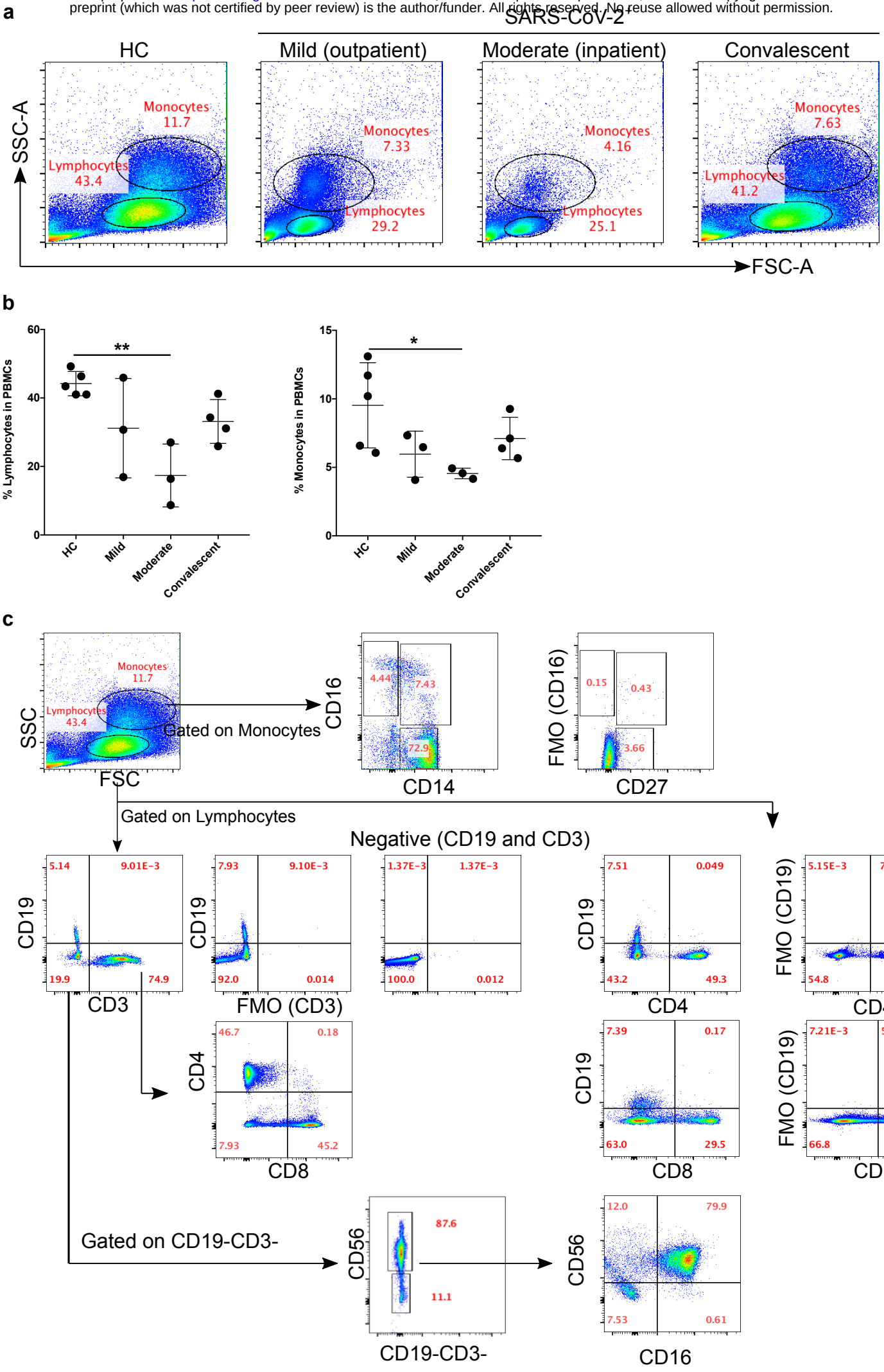
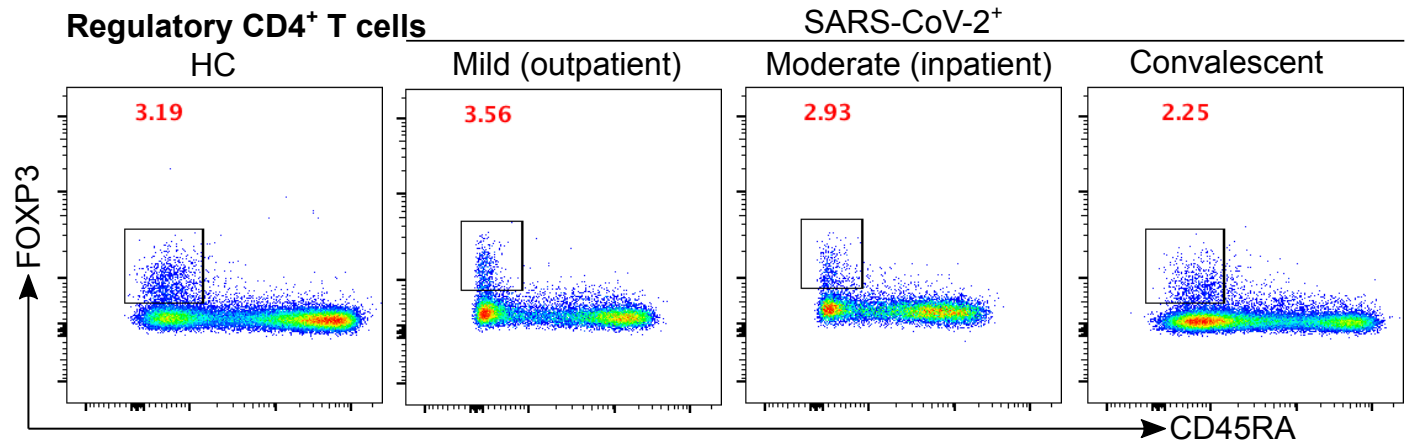
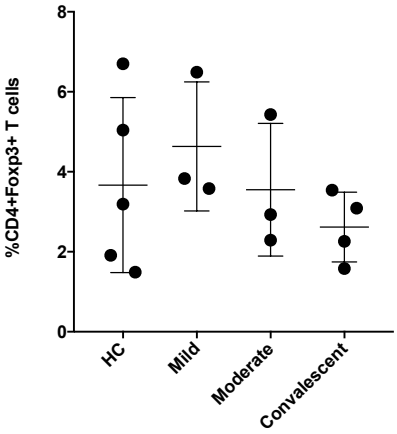


Fig. 6

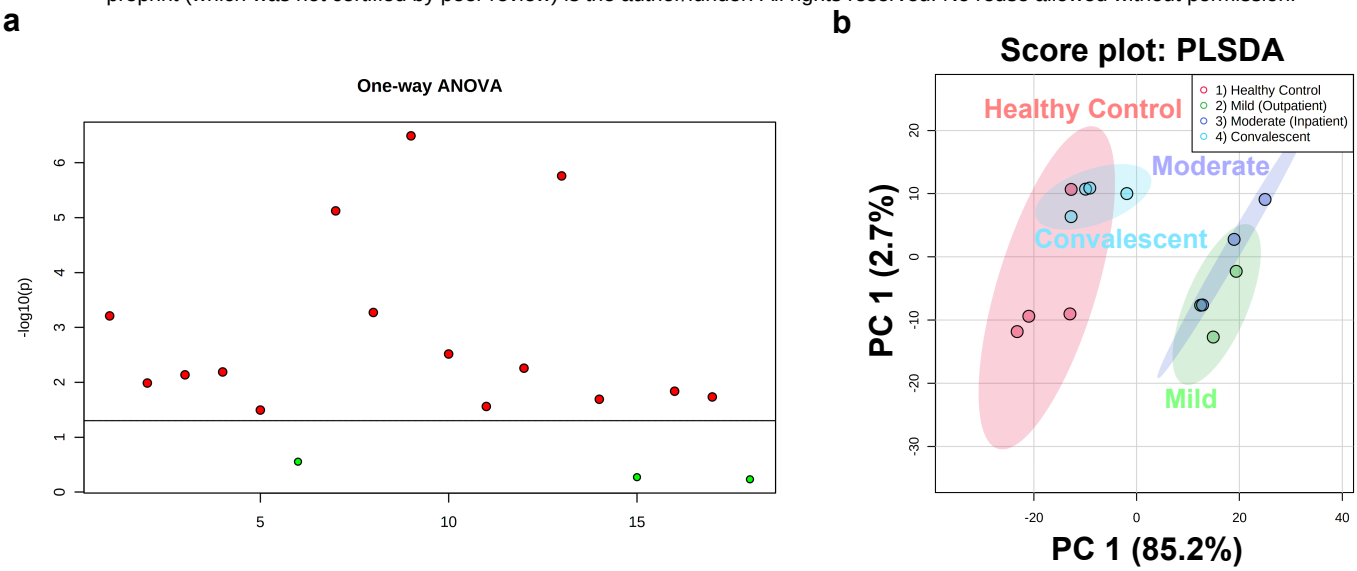


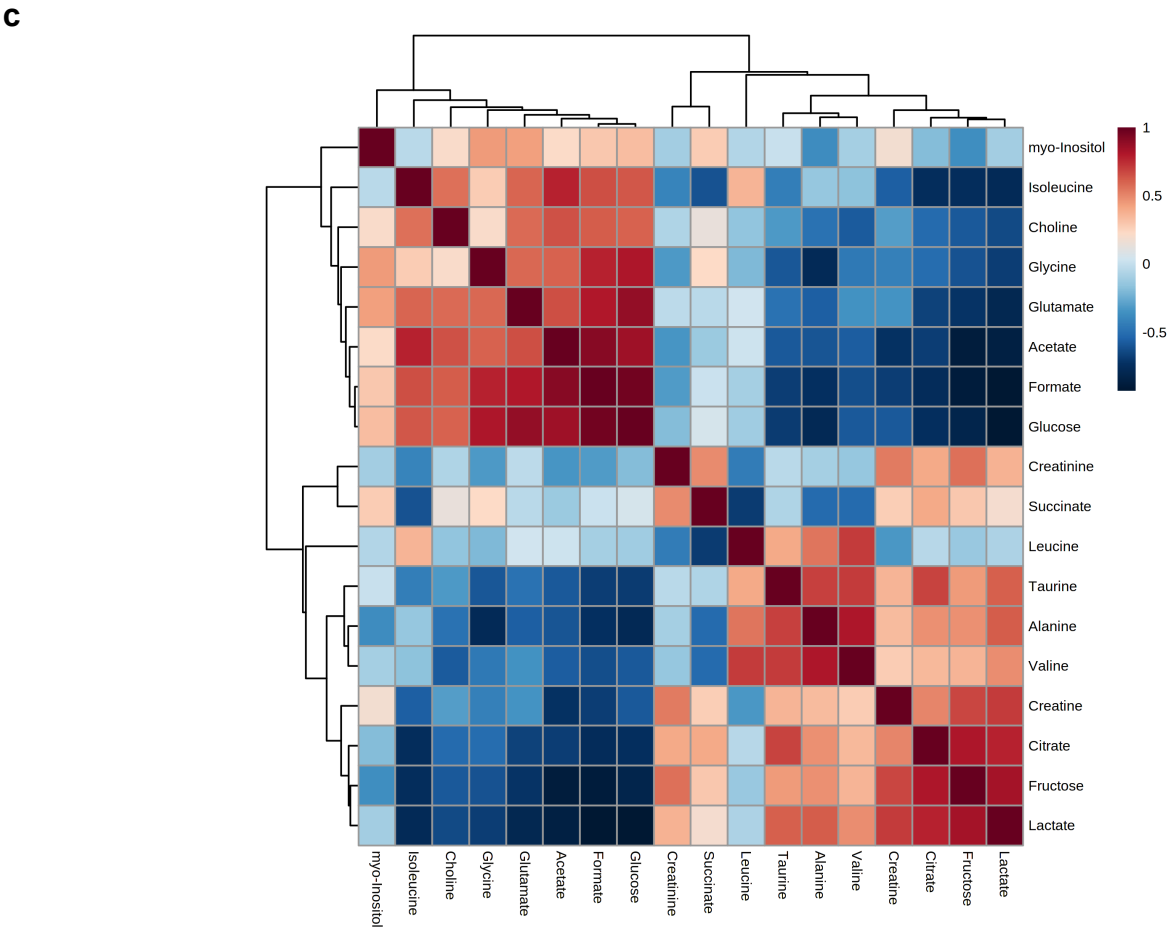
Suppl. Fig. 1

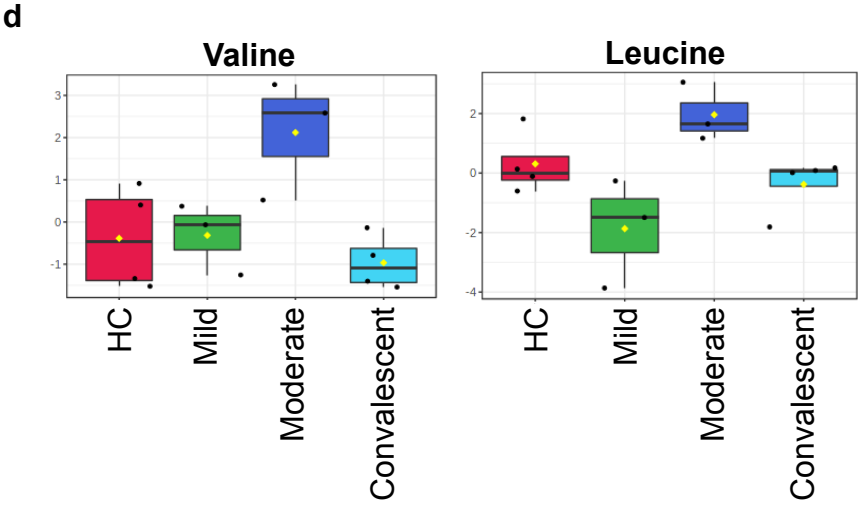




Suppl. Fig. 2







Suppl. Fig. 3