Cell Systems Transparent Peer Review Record

# Multiomics personalized network analyses highlight progressive disruption of central metabolism associated with COVID-19 severity

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## Summary

Initial Submission: Received Dec 8, 2021
Preprint: dx.doi.org/10.2139/ssrn.3988390
Scientific editor: Ernesto Andrianantoandro, Ph.D.

First round of review: Number of reviewers: 3
*3 confidential, 0 signed*
Revision invited Feb 23, 2022
*Major changes anticipated*
Revision received April 18, 2022

Second round of review: Number of reviewers: 3
*3 original, 0 new*
*3 confidential, 0 signed*
Accepted June 27, 2022

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## Editorial decision letter with reviewers' comments, first round of review

Dear Dr. Neogi,

I'm enclosing the comments that reviewers made on your paper, which I hope you will find useful and constructive. As you'll see, they express interest in the study, but they also have a number of criticisms and suggestions. Based on these comments, it seems premature to proceed with the paper in its current form; however, if it's possible to address the concerns raised with additional experiments and/or analysis, we'd be interested in considering a revised version of the manuscript.

As a matter of principle, I usually only invite a revision when I'm reasonably certain that the authors' work will align with the reviewers' concerns and produce a publishable manuscript. In the case of this manuscript, the reviewers and I have make-or-break concerns that can be addressed by:

1. Improved contextualization of this study within previous literature.
2. Clearer presentation of the rationale and structure of the study, and clearer articulation of the main advance.
3. Reorganization of the Results to better reflect the scientific argument.
4. Clearer description of the methodology.
5. Addressing technical concerns and additional experiments or analyses to substantiate the main claims.

Given the pre-existence of the Zhang 2020 scRNAseq dataset, this should be mentioned in the introduction along with the Krishnan 2021 paper as the baseline and context for the rationale and motivation of the current study.

It's not clear if the main advance is intended to be methodological (e.g. the patient re-stratification and personalized GSMMs) or biological/clinical (e.g. the SLC transporter results). In my experience, papers with both methodological development and biological insights need to stick to a single message and prioritize one over the other -- having more than one message will confuse the reader. In the case of a methodological advance, any resulting biological insights function as great applications of the method. In the case of biological advance, the method functions to deliver the biological insights.

I agree with Reviewer #2 that the different experiments are disjointed from each other. It seems they are presented in order of discovery rather than as a scientific argument. Please reorganize the manuscript to reflect your scientific argument. Each experiment should support each other and the whole argument. Laying out the structure of the study beforehand and ensuring it reflects the structure of your argument and the organization of the paper itself will help elucidate any gaps that may need to be filled with further experiments or analyses. Essential to this process will be Reviewer #1's comments (especially those regarding Fig 5).

To help guide revision, I've highlighted portions of the reviews that strike me as particularly critical. I'd also like to be explicitly clear about an almost philosophical stance that we take at Cell Systems…

- We believe that understanding how approaches fail is fundamentally interesting: it provides critical insight into understanding how they work. We also believe that all approaches do fail and that it's unreasonable, even misleading, to expect otherwise. Accordingly, when papers are transparent and forthright about the limitations and crucial contingencies of their approaches, we consider that to be a great strength, not a weakness.
- We believe that the figures are the scientific backbone of the paper. Currently, it's not possible to understand the manuscript's conceptual advance from figures presented. Similarly, it's not possible to understand where your approach gets its analytical power. These things need to be demonstrated with data and analysis, in the form of figures with their legends or mathematical argumentation, and then supported with explanatory text.

Please keep these in mind when addressing the reviewer and editorial concerns.

As you address these concerns, it's important that you and I stay on the same page. I'm always happy to talk, either over email or by Zoom, if you'd like feedback about whether your efforts are moving the manuscript in a productive direction. Do note that we generally consider papers through only one major round of revision, so the revised manuscript would be either accepted or rejected based on the next round of comments we receive from the reviewers. If you have any questions or concerns, please let me know. More technical information and advice about resubmission can be found below my signature. Please read it carefully, as it can save substantial time and effort later.

I look forward to seeing your revised manuscript.

All the best,

Ernesto Andrianantoandro, Ph.D.
Scientific Editor, Cell Systems

## Reviewers' comments:

Reviewer #1: The manuscript "Multiomics personalized network analyses highlight progressive immune disruption of central metabolism associated with COVID-19 severity" by Ambikan et al describes a comprehensive systems-level characterization of patient data in order to gain insights in biomarkers associated with SARS-CoV-2. Portions of this patient set have been previously analyzed by untargeted bulk plasma metabolomics and interleukin levels; here, additional whole blood transcriptomics and single cell type metabolomics is performed on the same samples. The authors analyzed the multi-omics features associated with COVID-19 severity by digital cell quantification, a variety of network tools, and integrative genome-scale flux balance analysis. They identify specific metabolic transporters and TCA intermediates associated with disease. Overall, I found the repertoire of analytical methods applied to the data interesting and somewhat insightful, however falling short of pinpointing how the disparate patient class

features of metabolism are specific to targetable cell types.

For a large data mining exercise like that performed here, visualization of results and communication of the data processing steps is of utmost importance and there were places in which clarity in how the results were generated was lacking:

Study design: "Health controls" is used to describe the n=31 patients that are PCR-negative. 10 of these patients are identified as convalescent. Throughout the paper, the 21 patients that are CoV-2-Ab- should be clearly and consistently identified distinctly from the 10 patients that are CoV-2-Ab+. For example "health control" is used in Fig 1A for CoV-2-Ab-. Given the recent publications that suggest residual viral latency and/or tissue damage in asymptomatic and/or recovered patients, the separation of these groups should be maintained when possible in subsequent analyses.

Figure 1A: the rest of the paper is predicated on the separation between healthy and convalescent versus mild & severe hospitalized patients on the UMAP plot of whole blood transcriptomics. Has an analysis been performed on the duration of SARS-CoV-2 infection? While severity is associated with length of time post-infection, some patients will progress to this stage/category faster than others. Transcriptomics and subsequent FBA will reflect a remodeling of expression levels that will occur over time. This may influence membership in SNF clusters in Figure 4 as well.

Figure 1E/F: Please provide titles on top of the heatmaps as was done for S2B/C. It is striking that the membership of altered KEGG pathways between 1E and 1F is quite low (I counted 11 common ones?) 1E does not support the GSEA result of top biological processes being mitochondrial. Please discuss.

Figure 2: Is "healthy control" defined here as all PCR-negative individuals? This analysis has the opportunity to characterize any T cell exhaustion or residual effects of infection in immune cell populations of formerly infected individuals.

Figure S3A: How is significance determined in this figure?

Figure 4F. Please provide methodology for data processing/scaling of interleukin levels for generating this figure.

Figure 5: At this point in the paper, I found it very challenging to follow how the patient-specific FBA models were generated. The details in the methods section are inadequate. The Github link is inactive. Please provide a table of which metabolites were used to define a closed form model with boundary levels. "The exchange reactions in the model were constrained using plasma metabolomics data as reference. " Is this performed independently using each patient's plasma values?

For the personalized GSMMs in Fig 5D, it appears that the bulk whole-blood transcriptomic data is used to populate a generic blood cell model of metabolism to elucidate flux features that distinguish the SNF clusters. It is unfortunate that the study design did not allow for single cell RNAseq on these patients, however I am puzzled as to why the authors did not take advantage of the digital cell quantification via EPIC to estimate proportions of transcript counts originating from each cell type. After all of the detailed

immunotyping performed earlier in the paper demonstrating pronounced differences in immune cell populations, why is the FBA lumped together, including cell types that require de novo nucleoside synthesis (and thus the importance of SLC29A1) and those that don't? Can a monocyte-derived DC specific metabolic flux model, for example, provide more insight on TCA-related changes than this generic model?

Discussion: No mention of limitations of this study. The use of an independent scRNAseq data set from Zhang et al instead of internally consistent transcriptomics is a very large drawback for interpretation of the current study.

Reviewer #2: In their work Ambikan et al. investigate the molecular response to COVID-19 using a variety of OMICs data sets from blood from COVID-19 patients and data analysis techniques. They identify molecular signatures of disease severity in COVID-19 and relate them to potential pathomechanisms that could cause the high heterogeneity in the course of the disease. To this end, the authors use RNA-Seq data from whole blood to identify differentially expressed genes between cohorts of patients with different disease severity. Subsequently, they use digital cell quantification to explore the frequency of different immune cell subtypes and find a loss of correlation between marker gene expression and subtype frequency for several immune cell subsets. In consequence, they use FACS to determine the frequency of different immune cell subsets. Subsequently, they use these data sets to stratify patients into novel clusters and use this stratification as basis for the analysis of sub-group specific changes in predicted metabolic activities through reconstruction of context-specific metabolic networks. While the research question being asked in the manuscript is important and the underlying data sets as well as insights gained quite relevant, it seems more like a patchwork of several independent stories woven together. Also the GitHub repository in which the code of the study is reported was not accessible (https://github.com/neogilab/COVID_GSMM). I cannot provide line numbers or page numbers in my review since those were missing from the submitted manuscript.

Major points:
Overall, the manuscript appears like a patchwork of several different stories woven together. Thus, the analysis of the bulk sequencing data are somehow disconnected from the FACS analysis of immune cell subsets and this is again disconnected from the modelling-driven part. Moreover, some of the questions being addressed could have been more easily answered with already available data sets. For instance, the authors use digital cell quantification to investigate the frequency of different immune cell subsets in the blood of COVID-19 patients. However, there is a large number of scRNA-Seq studies already available (e.g. PMID 32810438) that are much better able to elucidate changes in cell frequency in different stages as well as severity of COVID-19. Moreover, while this prompted a quantification of immune cell subsets in their samples, this information probably could also have been obtained from published studies. Such datasets could also have been easily used to investigate the observed loss of correlation between marker gene expression and abundance of specific immune cell subsets. Thus, the FACS-based analysis as well as the digital cell quantification somehow stand out as not really contributing to the storyline of the paper.

I like the approach of mapping transcriptomic data from bulk sequencing data to metabolic models that

have been conditioned with the specific metabolomics data. However, the authors include a viral replication reaction in the model for SARS-CoV-2 infected patients. While this could be justified if this was data from nasopharyngal swabs or lung lavage, a conclusive proof of productive viral replication in blood is still lacking. Moreover, the inclusion of the viral replication reaction only for SARS-CoV-2 infected patients while reasonable, might also affect the identified reactions. Thus, the authors should clarify which of the patient-cohort specific reactions are due to the addition of the viral replication reaction and which arise from differences in gene expression. Ideally, since there is no viral replication in blood, the viral replication reaction should not be used in the models.

Several key results are reported in a somewhat unreflected manner. For instance, the authors discuss the transporter SLC16A6 extensively but fail to point out that most of its functions are only inferred from homology. There is one report that this transporter is involved in beta-hydroxybutyrate transport but the remaining transport functions are just inferred. Thus, this result seems somewhat questionable or at least needs to be put in a better context. Moreover, they report changes in butyrate concentrations between patient groups while this metabolite is of microbial origin and mostly consumed by enterocytes. This is also potentially interesting and could be discussed in more detail.

The authors report glycolysis and glutaminolysis as potential targets to inhibit viral replication. While blocking these pathways would certainly prevent viral replication they are also essential for normal cellular function. This should be mentioned and it should be checked whether the approach also returns reactions/pathways that are less central for normal cellular function. Moreover, the model is based on blood data (see my previous comment) and hence it is questionable to which extent it reflects metabolic needs in virally infected cells.

While there are different views on the relevance of a clear storyline for a manuscript, this is clearly missing from the manuscript. Thus, it is difficult to judge what are the most important and central findings of the study. While this is probably difficult to assess given the ongoing high frequency of COVID-19-related publications, a lot of streamlining would clearly benefit the manuscript.

The abstract contains a lot of grammatical errors, which are not as prevalent in the main text (e.g. "However, the clinical outcome and disease severity are heterogenous and cannot explain by a single factor."). The manuscript should be checked by a native English speaker.

Minor points:
Please provide a citation for the statement "Though the primary site of infection is the upper respiratory tract, SARS-CoV-2 can invade several organs, tissues, and cells of the body."

The figures are in low quality but that's probably due to some hickup in the submission system.

Reviewer #3: This study presented network-based integrative analysis of metabolic and transcriptomic data across COVID-19 phenotypes, including COVID-19 negative, hospitalized-mild, and -severe. The authors identified a variety of metabolic pathways related to COVID-19 severity. Overall, this is an interesting study, which may offer metabolic pathobiology of COVID-19 severity and identify potential

metabolic pathways for future intervention approach development. However, several below major concerns should be considered further.

It is not clear why the authors define hospitalized-mild (n=26, O2 requirement<4lit/min) and hospitalize-severe (n=11, O2 requirement>4lit/min).

Differential gene expression (DGE) analysis (Adj. p<0.05) identified 8492 genes that were differentially regulated between the HC and COVID-19 patients based on this small sample size. It is very hard to understand why 40% of human genome are differentially expressed. A more strict cutoff, including fold change, must be used to define the DEG.

During DEG and GSEA analysis, disease comorbidities, such as diabetes and obesity, should be adjusted.

Immune cell differences, including neutrophils and classical monocytes, are well described in recent studies, which are related to COVID-19 disease severities, such as doi: 10.1111/acel.13544 and doi: 10.1038/s41392-021-00709-x. The authors are suggested to highlight novelty of the current findings. These COVID-19 disease severity-specific immune cell changes are sex- or age-dependent or not.

Topological network analysis using Adj. p<10-5 (Spearman rank correlation). Yet, Adj. p<0.05 was used for differential expression analysis. Different adj. p-values were used without any rationale.

More details for single-cell type metabolomics should be provided.

Network-based integrative analysis of metabolomics and transcriptomics have pointed out multiple metabolic pathways related to COVID-19 severity; yet, most metabolic pathways have been widely reported in COVID-19 studies. The authors should highlight which metabolic pathways are novel pathways compared to previously published COVID-19 metabolic studies. In addition, which metabolic pathways cannot be funded by metabolomics or transcriptomics analysis alone.

The resolution of figures is very poor. The reviewers cannot see all images and figure's text across all main figures.

## Authors' response to the reviewers' first round comments

Attached.

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## Editorial decision letter with reviewers' comments, second round of review

Dear Dr. Neogi,

I hope this email finds you well. The reviews are back on your manuscript and I've appended them below. You'll see that the reviewers find the revisions satisfactory, for the most part. However, Reviewer #1 still has concerns that need to be addressed through revision of the text and figures and some additional analysis to resolve whether the metabolic signatures you find are unique to COVID-19 (see highlighted portion of Reviewer #1's comments).

If you have any questions or concerns about the revision, I'd be happy to talk about them, either over email or by Zoom. More technical information and advice about resubmission can be found below my signature. Please read it carefully, as it can save substantial time and effort later.

I look forward to seeing your revised manuscript.

All the best,

Ernesto Andrianantoandro, Ph.D.
Scientific Editor, *Cell Systems*

### Reviewer comments:

Reviewer #1: The revision submitted by Ambikan et al is much improved over the previous version; specifically, the clear distinction between healthy controls and convalescent patients improves the paper and addition of a discussion paragraph on limitations is a welcome addition that acknowledges many of the shortcomings of the paper. The major scientific contribution of this work over previous efforts of applying network analysis and flux balance analysis to understanding COVID-19 is the use of blood-based immune cells on an individual patient basis. The authors report distinct metabolic signature associated with COVID-19 infection that differs from prior efforts in lung alveolar cells or culture, however there are lingering questions in this work regarding the cause of these metabolic signatures - are these due to direct infection of the cells under this analysis, or simply changes in enzymatic expression in response to the high levels of circulating cytokines associated with severity of the disease?

The authors do not consider this question sufficiently in the response to Reviewer 2, despite the mention in the rebuttal of qPCR performed on the samples for vRNA. Where is this information provided in the STAR Methods and elsewhere in the text? I would like details on the genome alignment methods, primers used, etc. The comparison of the metabolic phenotypes to data from non-SARS-CoV-2 infections, for example, could address the alternative hypothesis. Are these signatures unique to COVID-19?

Fig 3E: impossible to view gradations in coloring between -log10 Padj values. I do not follow why the "distinctly down" regulation category is null for both sets of comparison
Figure 4C: why is this listed as "used transcriptomic and metabolic data"? What is tossed out?
In text: "…the integrative characterization of the samples pointed to 4 clusters (Table S1)." Should refer to Figure 4C in this statement.

"While using the single-cell transcriptomics data published by Zhang et al. 2020 (Zhang et al., 2020) who had previously characterized patient groups similar to ours, indicated that the members of the mitochondrial carrier family (SLC25) (SLC25A1, and SLC25A11) were highly expressed (log expression>2) in more cells in the monocyte populations (Fig 5B and Fig S7)" incomplete sentence

Fig 5D: impossible to view gradations in coloring between 0 and 1000 or 0 and -1000. Those that are highlighted in red are low flux, what is the interpretation of this? If they are near equilibrium do they matter much?
Fig 6A: Again, I can't tell differences in color scales for gene and metabolite betweenness

Reviewer #2: The authors have addressed most of my concerns. The last sentence of the abstract is quite incoherent and needs to be corrected. Moreover, a brief discussion about a potential role of the microbiome due to the observation of butyrate as a metabolite associated with disease pathology would be appreciated.

Reviewer #3: The authors have addressed my previous concerns.

## Authors' response to the reviewers' second round comments

Attached.

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## Editorial decision letter

Dear Dr. Neogi,

I'm very pleased to let you know that the peer-review process is complete, and only a few minor, editorially-guided changes are needed to move forward towards publication.

In addition to the final comments from the reviewers, I've made some suggestions about your manuscript within the "Editorial Notes" section, below. Please consider my editorial suggestions carefully, ask any questions of me that you need, make all warranted changes, and then upload your final files into Editorial Manager.

I'm looking forward to going through these last steps with you. Although we ask that our editorially-guided changes be your primary focus for the moment, you may wish to consult our FAQ (final formatting checks tab) to make the final steps to publication go more smoothly. More technical information can be found below my signature, and please let me know if you have any questions.

All the best,

Ernesto Andrianantoandro, Ph.D.
Scientific Editor, Cell Systems

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## Editorial Notes

*Transparent Peer Review:* Thank you for electing to make your manuscript's peer review process transparent. As part of our approach to Transparent Peer Review, we ask that you add the following sentence to the end of your abstract: "A record of this paper's Transparent Peer Review process is included in the Supplemental Information." Note that this ***doesn't*** count towards your 150 word total!

Also, if you've deposited your work on a preprint server, that's great! Please drop me a quick email with your preprint's DOI and I'll make sure it's properly credited within your Transparent Peer Review record.

*Abstract:* The abstract reads nicely, but could do with some slight modification to prevent reader confusion:

- "…identify the mechanism…" is a strong claim and the term "mechanism" could be confusing. Please change this to "…identify potential determinants…"
- I do appreciate the motivation to address Reviewer #2's remaining concerns, but he last sentence is too speculative to be part of the Abstract. It is more appropriate for a Discussion point in the main manuscript. Please remove it from the Abstract.

*Manuscript Text:*

- Although the last paragraph of the Introduction provides a nice brief overview of the paper, how everything fits together needs additional explanation in greater depth in the Results section. Please flesh out the section "Study design and patient cohorts" at the beginning of the Results section to also include a more detailed description of the structure of the entire study (i.e. including all analyses in the paper). How exactly the analyses are connected needs to be mapped out before presenting the rest of the results, to give the reader a chance to see how each of the results fits in. For example, it needs to be clear whether the DCQ and immune phenotyping is based on the original patient stratifications. You need to be able to see which outputs of each

analysis feed into other analyses, and whether some run in parallel. A new Figure 1 with the overall study structure will help (see the Figures section below for my suggestions). Walking through this figure in the text and explaining the rationale will provide the reader with a better framework for interpreting the rest of the paper.

- Please be more consistent with terminology, e.g. use either GSMM or GEM, but not both throughout all the text and figures.
- In the Introduction, please better distinguish between the current work and previous work. You can use present tense to indicate what is presented in this paper and past tense to indicate previous work.
- There can only be one reference list – please combine the reference lists and place the combined version in the main text.

Also:

- House style disallows editorializing within the text (e.g. strikingly, surprisingly, importantly, etc.), especially the Results section. These terms are a distraction and they aren't needed—your excellent observations are certainly impactful enough to stand on their own. Please remove these words and others like them. “Notably” is suitably neutral to use once or twice if absolutely necessary.
- We don’t allow “priority claims” (e.g. new, novel, etc.). For a discussion of why, read: http://crosstalk.cell.com/blog/getting-priorities-right-with-novelty-claims, http://crosstalk.cell.com/blog/novel-insights-into-priority-claims.
- Please make sure to only use the word "significantly" in the statistical sense.

*Figures and Legends:*

- Figure S1 only depicts the personalized GSMM workflow, and does not provide the structure of the entire study. Please either create a new Figure 1 that depicts the entire structure of the study, or move Figure S1 into the main figures as the new figure 1 and modify/revise to add information about the DCQ, Immune phenotyping, and sctMetabolomics and display graphically how they are connected to the rest of the analyses.
- Reviewer #1’s point about the difficulty of seeing the gradations in figures 3E, 5D, and 6A is well taken. I recommend trying some different color combinations to improve legibility (e.g. red and blue instead of purple and green for 3E). For 5D, it is difficult to tell the difference between 0 to 500 and 500 to 1000 with the chosen colors. For 6A, having the betweenness scale going between two different colors (e.g. purple to teal) is too confusing, better would be saturation of just one color (e.g. light to dark blue).
- Please revise the titles of the figure legends for Figures 2, 3, 5, and 6 to be more concrete and better describe the purpose of the figure. The titles for Figures 1 and 4 are good examples of what to aim for.

Also, please look over your figures keeping the following in mind:

- When data visualization tools are used (e.g. UMAP, tSNE), please ensure that the dataset being visualized is named in the figure legend and, when applicable, its accession number is included.
- When color scales are used, please define them, noting units or indicating "arbitrary units," and specify whether the scale is linear or log.
- Please ensure that every time you have used a graph, you have defined "n's" specifically and listed statistical tests within your figure legend.

*STAR Methods:*

- Please include an entry for your original code and the github doi for it in the Key Resources Table under “Software and Algorithms.”
- For “Identifier” in the Key Resources table, please list how the resource can be found/obtained (i.e. a product number for material reagents, kits, etc. or a web page or doi for accessing an electronic resource). If this is not applicable, please write “N/A” in this column.

**Thank you!**

**Response to Reviewer:**

Reviewers' comments:

**Reviewer #1: The manuscript "Multiomics personalized network analyses highlight progressive immune disruption of central metabolism associated with COVID-19 severity" by Ambikan et al describes a comprehensive systems-level characterization of patient data in order to gain insights in biomarkers associated with SARS-CoV-2. Portions of this patient set have been previously analyzed by untargeted bulk plasma metabolomics and interleukin levels; here, additional whole blood transcriptomics and single cell type metabolomics is performed on the same samples. The authors analyzed the multi-omics features associated with COVID-19 severity by digital cell quantification, a variety of network tools, and integrative genome-scale flux balance analysis. They identify specific metabolic transporters and TCA intermediates associated with disease. Overall, I found the repertoire of analytical methods applied to the data interesting and somewhat insightful, however falling short of pinpointing how the disparate patient class features of metabolism are specific to targetable cell types.**

**For a large data mining exercise like that performed here, visualization of results and communication of the data processing steps is of utmost importance and there were places in which clarity in how the results were generated was lacking:**

**Study design: "Health controls" is used to describe the n=31 patients that are PCR-negative. 10 of these patients are identified as convalescent. Throughout the paper, the 21 patients that are CoV-2-Ab- should be clearly and consistently identified distinctly from the 10 patients that are CoV-2-Ab+. For example "health control" is used in Fig 1A for CoV-2-Ab-. Given the recent publications that suggest residual viral latency and/or tissue damage in asymptomatic and/or recovered patients, the separation of these groups should be maintained when possible in subsequent analyses.**

**Reply:** We are thankful for the insightful suggestions. We have now separated CoV-2-Ab+ (n=10) and CoV-2-Ab-(n=21) and executed all the subsequent analysis. The CoV-2-Ab- (n=21) samples are now termed as healthy controls throughout the manuscripts. Fig 1 (digital cell quantification; previously Fig 2) and Fig 3 (differential expression analysis; previously Fig1) are changed accordingly. We have also adjusted for cell type proportions, BMI and other hidden factors in the data while performing differential expression analysis for all pair of comparisons. We have used the R package RUVSeq (Remove Unwanted Variations from RNASeq data) to remove unwanted and unknown confounding factors from the transcriptomics data. The method computes factors of unwanted variation originate from covariates of interests and other hidden sources and those factors can be added in the design matrix for differential expression analysis. (Method section is updated). Differential expression analysis between CoV-2-Ab+ and CoV-2-Ab- identified two significantly regulated genes (adj.p <0.05 and |LFC| > 1.5) and CoV-2-Ab- (n=21) showed higher number of co-expression (n=61960) while CoV-2-Ab+ (n=10) showed 2800 positive correlations among markers genes of cell types (Fig 1).

**Figure 1A: the rest of the paper is predicated on the separation between healthy and convalescent versus mild & severe hospitalized patients on the UMAP plot of whole blood transcriptomics. Has an analysis been performed on the duration of SARS-CoV-2 infection? While severity is associated with length of time post-infection, some patients will progress to this stage/category faster than others. Transcriptomics and subsequent FBA will reflect a remodeling of expression levels that will occur over time. This may influence membership in SNF clusters in Figure 4 as well.**

**Reply:** Thank you for your critical comment. We agree that any source of variations in the data can affect further transcriptomics analysis to find differentially regulated genes. So that, we have now

adjusted for cell type proportions, BMI and other hidden factors in the data while performing differential expression analysis for all pair of comparisons as mentioned in response to the previous comment. Also, the Flux Balance Analysis (FBA) mentioned in the manuscript were performed for each patient group as well as for each sample (personalized GSMM). While group-specific FBA analysis may be affected by sample heterogeneity, for which we thus performed sample-specific FBA. We generated context specific genome scale metabolic models for each sample by inputting the corresponding gene expression table and identified the metabolic reactions showed flux through FBA. Then we looked for reactions common for each patient group and by this way the heterogeneity was handled. The Similarity Network Fusion (SNF) was performed by integrating transcriptomics and metabolomics data. The resulting clusters (Fig 4) are equally influenced by both transcriptomics and metabolomics signatures in each of the sample data. So, the variation in transcriptomics data may have very little effect in the results.

**Figure 1E/F: Please provide titles on top of the heatmaps as was done for S2B/C. It is striking that the membership of altered KEGG pathways between 1E and 1F is quite low (I counted 11 common ones?) 1E does not support the GSEA result of top biological processes being mitochondrial. Please discuss.**

**Reply:** Thank you for the comments. Titles are now added to the respective figures. The KEGG pathway GSEA were executed for COVID19-vs-HC and Hosp-Mild-vs-Hosp-Severe differential analysis. Biological process analysis was performed only for genes which are uniquely expressed in Hospitalized-severe identified from the Venn-diagram. As both functional analyses were performed on different sets of genes, the results show certain level of difference.

**Figure 2: Is "healthy control" defined here as all PCR-negative individuals? This analysis has the opportunity to characterize any T cell exhaustion or residual effects of infection in immune cell populations of formerly infected individuals.**

**Reply:** Thank you for your suggestion. The digital cell quantification analysis is now re-executed for Cov-2-Ab- (n=21; Healthy controls) and Cov-2-Ab+ (n=10) separately. The new analysis has identified higher number of positive correlations between the cell type marker genes among Cov-2-Ab- samples (n=61960) compared to Cov-2-Ab+ samples (n=2800). The results sections and corresponding figures are updated.

**Figure S3A: How is significance determined in this figure?**

**Reply:** The nominal p values computed from the correlation analysis are adjusted using Benjamini-Hochberg method.

**Figure 4F. Please provide methodology for data processing/scaling of interleukin levels for generating this figure.**

**Reply:** Normalized values of interlukins are scaled down and mean values of each sample are computed. It is now mentioned in method section.

**Figure 5: At this point in the paper, I found it very challenging to follow how the patient-specific FBA models were generated. The details in the methods section are inadequate. The Github link is inactive. Please provide a table of which metabolites were used to define a closed form model with boundary levels. "The exchange reactions in the model were constrained using plasma metabolomics data as reference. " Is this performed independently using each patient's plasma values?**

**Reply:** Thank you for your comment. We have performed genome scale metabolic modelling and flux balance analysis (FBA) at patient group level, and at personalized level of each sample (patient specific / personalized FBA). Patient specific modeling and FBA was performed to account for the possible sample heterogeneity. Briefly, we have re-constructed genome scale metabolic model for each sample by inputting corresponding gene expression table as input through tINIT (PMID: 24646661, 22615553). Further, metabolic reactions show significant flux are identified through FBA from each sample-wise model and common reactions for each cohort are found (Fig 5D). The resultant list of reactions identified from FBA were used for the metabolic network generation and topology analysis to rank metabolites and enzymatic genes important for the coordinated metabolic system (Fig 6A). While performing FBA, the exchange reactions were constrained to control the nutrient uptake by the system using plasma metabolomics data independently using each patient's plasma metabolic measurements. The list of exchange reactions constrained for each model are now uploaded to Github repository (68 files). We already provided the detailed description of the packages and inputs used for the analysis in the Star Method Section.

**For the personalized GSMMs in Fig 5D, it appears that the bulk whole-blood transcriptomic data is used to populate a generic blood cell model of metabolism to elucidate flux features that distinguish the SNF clusters. It is unfortunate that the study design did not allow for single cell RNAseq on these patients, however I am puzzled as to why the authors did not take advantage of the digital cell quantification via EPIC to estimate proportions of transcript counts originating from each cell type. After all of the detailed immunotyping performed earlier in the paper demonstrating pronounced differences in immune cell populations, why is the FBA lumped together, including cell types that require de novo nucleoside synthesis (and thus the importance of SLC29A1) and those that don't? Can a monocyte-derived DC specific metabolic flux model, for example, provide more insight on TCA-related changes than this generic model?**

**Reply:** Thank you for your suggestion. It is indeed better to generate metabolic models specific for cell types and analyse the metabolic changes. But the analysis requires certain cell type specific information to feed the models. One such information is cell type specific metabolic measurements to constrain the exchange reactions to adjust the nutrients uptakes by the single cell system. This is important to accurately compute the metabolic flux in response to the disease. Currently, we lack the cell type specific metabolic measurements. Further, while we fully agree with the reviewer that a single-cell level metabolic model flux prediction would be ideal, to the best of our knowledge no robust metabolic modelling framework can currently satisfactory do so. Due to the large sparsity of scRNAseq data, such models can neither 1. fulfil minimum biological requirements for biological feasibility by the metabolic flux distributions; 2. sufficiently cover a substantial fraction of the model enzymatic genes such that metabolic flux distributions can be more accurately quantified. As such, existing approaches still substantially rely on bulk quantifications for flux prediction. We have now mentioned these in the limitation section.

**Discussion: No mention of limitations of this study. The use of an independent scRNAseq data set from Zhang et al instead of internally consistent transcriptomics is a very large drawback for interpretation of the current study.**

**Reply:** We are thankful for the suggestion. This limitation is now acknowledged in the discussion. We should note that the focus for this study was the GSMM and personalised modelling insights. As stated above the single cell metabolomics is not yet developed and integration of scRNAseq data for accurate model flux prediction is still not currently possible, preventing the construction of robust single-cell level GSMMs. This prompted us to developed single cell type metabolomics (sctMetabolomics). We use the Zhang et al data to rationalise the cell type for sctMetabolomics base on the transporter reactions. We have now clarified this. We are developing the scMetabolomics and hopefully will be available in a couple year time given the technological limitations of the metabolomics profiling (our present method need atleast 200,000 cells). Given that our focus was not transcriptomics we didn't performed the scRNAseq in the same population.

**Reviewer #2: In their work Ambikan et al. investigate the molecular response to COVID-19 using a variety of OMICs data sets from blood from COVID-19 patients and data analysis techniques. They identify molecular signatures of disease severity in COVID-19 and relate them to potential pathomechanisms that could cause the high heterogeneity in the course of the disease. To this end, the authors use RNA-Seq data from whole blood to identify differentially expressed genes between cohorts of patients with different disease severity. Subsequently, they use digital cellquantification to explore the frequency of different immune cell subtypes and find a loss of correlation between marker gene expression and subtype frequency for several immune cell subsets. In consequence, they use FACS to determine the frequency of different immune cell subsets. Subsequently, they use these data sets to stratify patients into novel clusters and use this stratification as basis for the analysis of sub-group specific changes in predicted metabolic activities through reconstruction of context-specific metabolic networks. While the research question being asked in the manuscript is important and the underlying data sets as well as insights gained quite relevant, it seems more like a patchwork of several independent stories woven together. Also the GitHub repository in which the code of the study is reported was not accessible (https://github.com/neogilab/COVID_GSMM). I cannot provide line numbers or page numbers in my review since those were missing from the submitted manuscript.**

**Major points:**

**Overall, the manuscript appears like a patchwork of several different stories woven together. Thus, the analysis of the bulk sequencing data are somehow disconnected from the FACS analysis of immune cell subsets and this is again disconnected from the modelling-driven part.**

**Reply:** We are thankful for the critical comment. We have used transcriptomics data generated from whole blood samples so the gene expression variations can come from various cell types. In order to examine the level of difference in cell type proportions among the samples, we have used a deconvolution algorithm adapted from the package EPIC (Estimating the Proportions of Immune and Cancer cells) to compute the proportions of 18 different cell types. For this purpose, we have used reference gene expression profile for 18 different cell types obtained from Human Protein Atlas and a list of signature genes of the 18 cell types obtained from resources such as CellMarker and PangloDB and estimated the cell type proportions using the sample-wise gene expression table. We have mentioned the entire procedure as digital cell quantification (DCQ) in the manuscript. The results from DCQ are reported in Fig 1 (previously Fig 2). The information is now used in the differential expression analysis to adjust for the variation due to the cell type proportions (Fig 2; previously Fig 1). We have noticed that the digital cell count is highly dependent upon the depth and quality of the RNAseq data. We have recently published a study (doi: 10.7554/eLife.76071) where the same algorithm didn't work because of the low depth of the data. We therefore used the FACS to validate the DCQ.

**Moreover, some of the questions being addressed could have been more easily answered with already available data sets. For instance, the authors use digital cell quantification to investigate the frequency of different immune cell subsets in the blood of COVID-19 patients. However, there is a large number of scRNA-Seq studies already available (e.g. PMID 32810438) that are much better able to elucidate changes in cell frequency in different stages as well as severity of COVID-19. Moreover, while this prompted a quantification of immune cell subsets in their samples, this information probably could also have been obtained from published studies. Such datasets could also have been easily used to investigate the observed loss of correlation between marker gene expression and abundance of specific immune cell subsets. Thus, the FACS-based analysis as well as the digital cell quantification somehow stand out as not really contributing to the storyline of the paper.**

**Reply:** Thank you for your critical comments. We agree that there already exists many single cell data belong to COVID19 research. As we mentioned in the response to the previous comment, since the transcriptomics data is from whole blood samples, we have performed DCQ analysis to compute the cell type proportion and we have now used this information to adjust for any variation that can affect the differential expression and updated the results. The DCQ results is further validated using FACS based method. The focus of the study was not transcriptomics that several great reports were there but the GSMM. We are sorry that the first version of the manuscript, we didn't precisely mention this. We have now clearly mentioned this. We can't use the published data as we need paired transcriptomics and metabolomics in the same matrix (here blood).

**I like the approach of mapping transcriptomic data from bulk sequencing data to metabolic models that have been conditioned with the specific metabolomics data. However, the authors include a viral replication reaction in the model for SARS-CoV-2 infected patients. While this could be justified if this was data from nasopharyngal swabs or lung lavage, a conclusive proof of productive viral replication in blood is still lacking.Moreover, the inclusion of the viral replication reaction only for SARS-CoV-2 infected patients while reasonable, might also affect the identified reactions. Thus, the authors should clarify which of the patient-cohort specific reactions are due to the addition of the viral replication reaction and which arise from differences in gene expression. Ideally, since there is no viral replication in blood, the viral replication reaction should not be used in the models.**

**Reply:** Thank you for your suggestion. Though the replication of the virus in the blood cell is matter of debate, we strongly align with the reviewer's view. Now we also aligned the data with the SARS-CoV-2 ref genome and performed qPCR. We did not find any evidence virus in our patient population. We also have now performed the flux balance analysis without viral biomass objective function (VBOF) for all samples. The new analysis provided same results as before, i.e. with addition of VBOF. This further support that the VBOF has no significant influence on the metabolic models generated from blood samples where there is no presence of the virus. We now mentioned this in the manuscript.

**Several key results are reported in a somewhat unreflected manner. For instance, the authors discuss the transporter SLC16A6 extensively but fail to point out that most of its functions are only inferred from homology. There is one report that this transporter is involved in beta-hydroxybutyrate transport but the remaining transport functions are just inferred. Thus, this result seems somewhat questionable or at least needs to be put in a better context. Moreover, they report changes in butyrate concentrations between patient groups while this metabolite is of microbial origin and mostly consumed by enterocytes. This is also potentially interesting and could be discussed in more detail.**

**Reply:** Here we have taken advantage of GSMMs as frameworks to characterize which specific metabolic reactions were active, prioritize disrupted metabolic systems, prioritize enzymatic genes/metabolites based on their metabolic importance, and to mechanistically characterize the flux flow differences between patient groups and at personalized levels. By constraining the models with patient gene expression and metabolomic profiles, we could thus identify how metabolic activities differed with respect to disease severity. While we are fully aware that metabolic models, like other models, represent approximations to the *in vivo* profiles, we have used the latest and most curated reference model for humans to date (DOI: 10.1126/scisignal.aaz1482) updated since the original publication). Thus, while it is true that some of the genes present in the model are inferred, they nevertheless represent the closest and best curated approximation to model human metabolism that we are currently aware.

We agree with the reviewer that SLC16A6 transport functions are inferred. Our study is not designed to infer the function rather we reported what is known for SLC16A6 and partly speculative (the last sentence). Potential role of SLC16A6 is already reported in cancer and other respiratory viral diseases. As suggested, we have now revised the sections as follows

*"The SLC16 gene family comprised of 14 members of the MCT family plays an important role in energy metabolism as it catalyzes the rapid transport of lactate and pyruvate across the cell membrane that are essential components for glycolysis [reviewed in (Halestrap, 2013)]. Though no study reported the role of SLC transporter earlier, a co-expression analysis performed in microarray analysis data of influenza-infected pediatric patients reported upregulated expression of SLC16A6 (Zarei Ghobadi et al., 2019), suggesting its potential role in respiratory viral infections. The SL16A6 has been reported to act as a transporter of ketone bodies like β-hydroxybutyrate out of liver (Newman and Verdin, 2017) that is again internalized by tissues other than liver and is utilized in TCA-cycle in mitochondria or fatty acid synthesis in cytoplasm (Sheraj et al., 2021). A more recent study also reported SL16A6 as taurine transporter (Higuchi et al., 2022) indicate its role in energy metabolism. It is also possible that transporters such as SLC16A6, which was differentially upregulated in COVID-19, can also regulate the intracellular and extra-cellular levels of α-keto acids, like α-ketoglutarate, which are essential components of TCA-cycle."*

**The authors report glycolysis and glutaminolysis as potential targets to inhibit viral replication. While blocking these pathways would certainly prevent viral replication they are also essential for normal cellular function. This should be mentioned and it should be checked whether the approach also returns reactions/pathways that are less central for normal cellular function. Moreover, the model is based on blood data (see my previous comment) and hence it is questionable to which extent it reflects metabolic needs in virally infected cells.**

**Reply:** Thank you for the suggestion. We have previously reported that blocking of glutaminolysis and glycolysis inhibit viral replication (Krishnan et al 2021) and discussed in context to our own findings (Krishnan et al 2021 and Appelberg et al 2020). Moreover 2-DG is approved in India for emergency use in SARS-CoV-2 severe patients. However, no data was released. It may not be ideal choice in mild or moderate cases. Rather more for the severe lifesaving cases as pointed out our study too. Though no scientific literature available, the press release of the Indian Govt (https://pib.gov.in/PressReleasePage.aspx?PRID=1717007), reported that the Phase 2 trials of the 2-DG arm showed faster symptomatic cure than Standard of Care (SoC) arm on various endpoints (vital parameters) with a significantly favorable trend (2.5 days difference). In the 2-DG arm, 42% of the patients improved symptomatically and became free from supplemental oxygen by Day-3 compared to 31% in the SoC arm, indicating an early relief from oxygen therapy/dependence. A higher proportion of patients treated with 2-DG showed RT-PCR negative conversion in COVID patients.

We have recently shown that blocking glycolysis and glutaminolysis can also block the replication of CCHF Viruses (doi:10.7554/eLife.76071). A recent study in HIV-1 showed that DON reversed cognitive impairment in EcoHIV-infected mice in HIV-associated neurocognitive disorders (HAND). We also recently shown that what are the other pathways can alter in HIV-1 latent cell model while treated with DON (Mikealoff et al 2022, Communication Biology). Given that metabolic reprograming is plastic and reversible, we noticed (in case of HIV) that after removal of the pressure the cells behave normally (unpublished data).

So, we believe there is high feasibility of targeting host metabolism as a host-directed therapy during severe cases if we undertsnd the metabolic reprogramming during acute viral infection but more research is warranted to take it to the clinical trials.

**While there are different views on the relevance of a clear storyline for a manuscript, this is clearly missing from the manuscript. Thus, it is difficult to judge what are the most important and central findings of the study. While this is probably difficult to assess given the ongoing high frequency of COVID-19-related publications, a lot of streamlining would clearly benefit the manuscript.**

**Reply:** Thank you for the suggestion. We have now changed the flow of the manuscript following the addition of other experiments and analysis and rationalised all the experiments. The novelty of the study is personalised GSMM, FBA and DCQ. However, both the methods were dependent upon the quality of the data. Therefore, we provided fig 1-3 to validate what we observed in the computational prediction is correct. For example, suppose we found one metabolic reaction is

significant it could also possible that the metabolic pathways are not dysregulated (please see below one example). Similarly DCQ by our methods do not work with low depth samples. We have now mentioned this in the manuscript.

**The abstract contains a lot of grammatical errors, which are not as prevalent in the main text (e.g. "However, the clinical outcome and disease severity are heterogenous and cannot explain by a single factor."). The manuscript should be checked by a native English speaker.**

**Reply:** We have now corrected, and the manuscript is read by a native speaker.

**Minor points:**

**Please provide a citation for the statement "Though the primary site of infection is the upper respiratory tract, SARS-CoV-2 can invade several organs, tissues, and cells of the body."**
**Reply:** Reference provided.

**The figures are in low quality but that's probably due to some hickup in the submission system.**
**Reply:** We don't know what happened. All the figures were 300dpi. We have now re-uploaded as single files.

Also the GitHub repository in which the code of the study is reported was not accessible (https://github.com/neogilab/COVID_GSMM).

**Reply:** The github link was embargoed. Now we have made it public.

**Reviewer #3: This study presented network-based integrative analysis of metabolic and transcriptomic data across COVID-19 phenotypes, including COVID-19 negative, hospitalized-mild, and -severe. The authors identified a variety of metabolic pathways related to COVID-19 severity. Overall, this is an interesting study, which may offer metabolic pathobiology of COVID-19 severity and identify potential metabolic pathways for future intervention approach development. However, several below major concerns should be considered further.**

**It is not clear why the authors define hospitalized-mild (n=26, O2 requirement<4lit/min) and hospitalize-severe (n=11, O2 requirement>4lit/min).**

**Reply:** We are thankful for the comment. When we collected the samples there were no guideline from WHO. The definitions were based on the "clinicians' decision". We have now clarified this. Moreover as we stated earlier *"Clinical categorization of COVID-19 patients relies on their oxygen requirement. However, our earlier study identified that $O_2$ need at hospitalization did not predict mortality (Saccon et al., 2021)."* It rationalized SNF and personalised GSMM. We also added this in the limitation section.

**Differential gene expression (DGE) analysis (Adj. p<0.05) identified 8492 genes that were differentially regulated between the HC and COVID-19 patients based on this small sample size. It is very hard to understand why 40% of human genome are differentially expressed. A more strict cutoff, including fold change, must be used to define the DEG.**

**Reply:** Thank you for the suggestion. The cut-off is now updated. Fold change cut-off of 1.5 is also added along with the adj.pvalue cut-off of 0.05.

**During and GSEA analysis, disease comorbidities, such as diabetes and obesity, should be adjusted.**

**Reply:** Thank you for the suggestion. We agree the various confounding factors can affect the differential expression analysis. Therefore, we have now re-performed the analysis with adjustment for the corresponding confounding factors. We have used the R package RUVSeq (Remove Unwanted Variations from RNASeq data) to remove any unwanted noise from the transcriptomics data. The method computes factors of unwanted variation originate from covariates of interests and other hidden sources and those factors can be added in the design matrix for differential expression analysis. (Method section is updated).

**Immune cell differences, including neutrophils and classical monocytes, are well described in recent studies, which are related to COVID-19 disease severities, such as doi: 10.1111/acel.13544 and doi: 10.1038/s41392-021-00709-x. The authors are suggested to highlight novelty of the current findings. These COVID-19 disease severity-specific immune cell changes are sex- or age-dependent or not.**

**Reply:** We are thankful for the comment. The novelty of the study is the SNF and personalized GSMM and cellular metabolic environment. We are sorry it was not explicitly mentioned in the first version of the manuscript. We have now RUVseq to adjust the variations coming from the co-variates and a new Fig 3 is added.

**Topological network analysis using Adj. p<10-5 (Spearman rank correlation). Yet, Adj. p<0.05 was used for differential expression analysis. Different adj. p-values were used without any rationale.**

**Reply:** Thank you for the comment. In differential expression analysis, the multiple hypothesis correction was performed for all protein-coding genes (~19999). Based on the number of detected differentially expressed genes (adj.P < 0.05) we estimate a maximum 405 (adj.p <0.05) of potential false positives in HC-vs-COVID19 and a maximum 232 (adj.p<0.05) false positives in Hospitalized-Mild-vs Hospitalized-Severe analysis. In turn, the topological network analysis tests ~85 Million hypotheses (correlations), under which a traditional cut-off of Adj.P<0.05 would lead to a maximum of ~1.9 Million potential false positives. For this reason, we imposed a stringent cut-off of $10^{-5}$ in network analysis (~333 potential false positives).

**More details for single-cell type metabolomics should be provided**

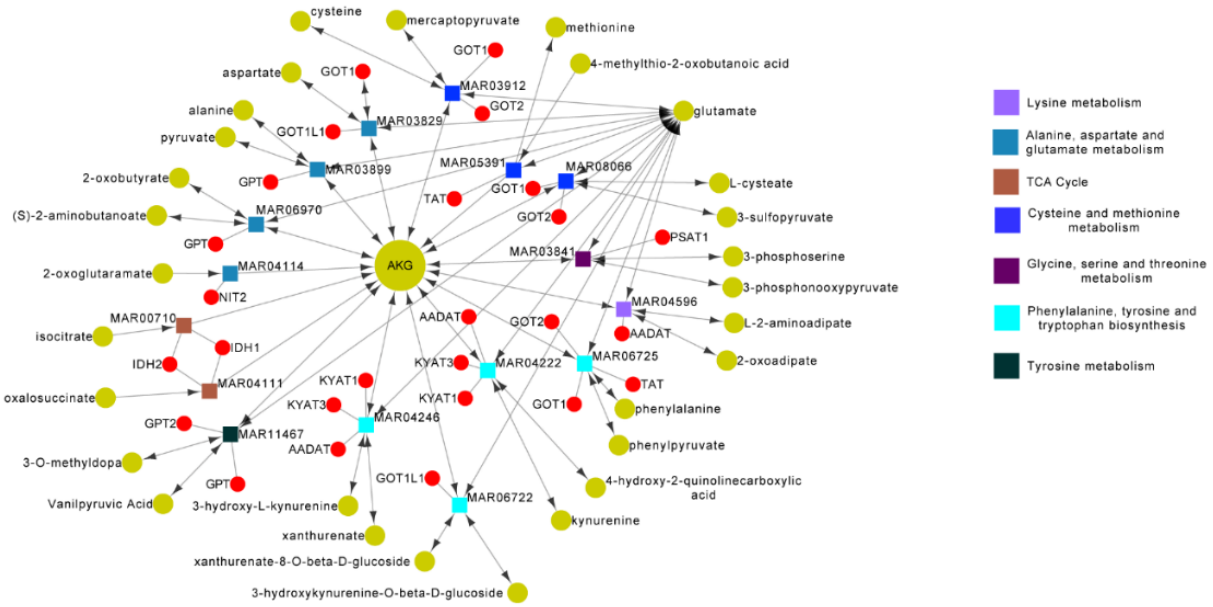
**Reply:** The detailed method is already provided in the Star Method section.

**Network-based integrative analysis of metabolomics and transcriptomics have pointed out multiple metabolic pathways related to COVID-19 severity; yet, most metabolic pathways have been widely reported in COVID-19 studies.**

**Reply:** Thank you for the comments. We agree that there are several great publications that discuss about metabolic pathways in the context of COVID19. But in the current study our main objective is to study the specific metabolic reactions (not pathway as whole) that alter in COVID19 and COVID19 disease severity and to rank the metabolites and enzymatic genes important for existence of the metabolic network. To answer the objectives, we have used context specific genome scale metabolic modelling and flux balance analysis to identify the metabolic reactions specific for COVID19 (Fig 5A). Further we have generated metabolic networks from the results obtained from FBA and did topological analysis to rank the metabolites and enzymatic genes important for exitance COVID19 metabolic network (Fig 6A)

The metabolic reprograming is plastic and reversible. Under certain metabolic environment or stress the metabolic pathways changes it's source of metabolites, essentials for the cell to survive. Therefore metabolic pathways based on the RNAseq data can provide valuable information about the regulation of the pathways but can't infer the metabolic reprogramming.

As an example, the production of the alpha-ketoglutarate is through several reactions and part of several metabolic pathways as you see in the below figure. We give the Fig 3 just to show the pathways that are regulated based on the gene expression data while GSMM provide the metabolic reactions and essentiality analysis to point out specific metabolites or metabolic reprograming.




**The resolution of figures is very poor. The reviewers cannot see all images and figure's text across all main figures.**

**Reply:** We don't know what happened. All the figures were 300dpi. We have now re-uploaded.

**Response to Reviewer:**

Reviewers' comments:

**Reviewers' comments:**

Reviewer #1: The revision submitted by Ambikan et al is much improved over the previous version; specifically, the clear distinction between healthy controls and convalescent patients improves the paper and addition of a discussion paragraph on limitations is a welcome addition that acknowledges many of the shortcomings of the paper. The major scientific contribution of this work over previous efforts of applying network analysis and flux balance analysis to understanding COVID-19 is the use of blood-based immune cells on an individual patient basis. The authors report distinct metabolic signature associated with COVID-19 infection that differs from prior efforts in lung alveolar cells or culture, however there are lingering questions in this work regarding the cause of these metabolic signatures - are these due to direct infection of the cells under this analysis, or simply changes in enzymatic expression in response to the high levels of circulating cytokines associated with severity of the disease?

**Response:** We are thankful for the suggestion. This is an exciting question. We have now added the hypothesis to the discussion as follows::

*"As the SARS-CoV-2 was not dectected in the blood cell populations in our cohort, we therefore hypothesised that the systemic metabolic alterations are most likely because of the bystander effect of the infection due to the inflammatory conditions and the specific metabolic environment that may differ from the metabolic alteration during productive replication in the infecting cells."*

The authors do not consider this question sufficiently in the response to Reviewer 2, despite the mention in the rebuttal of qPCR performed on the samples for vRNA. Where is this information provided in the STAR Methods and elsewhere in the text? I would like details on the genome alignment methods, primers used, etc.

**Response:** We already published the method in our earlier paper, both the primers details and the alignment method (Appelberg et al. 2020, Emerg Microb Infection). We have now quoted that papers in the Star Method section are instead repeating.

The comparison of the metabolic phenotypes to data from non-SARS-CoV-2 infections, for example, could address the alternative hypothesis. Are these signatures unique to COVID-19?

**Response:** Thank you for the suggestion. Systemic GSMM was not performed earlier. We recently constructed GSMM for HIV-1. We are in the process of developing an Atlas for emerging and re-emerging viruses like CCHF, Ebola, and Dengue. As suggested, we have added the below text in the discussion:

*"Though systemic GSMM was not reported in other respiratory diseases caused by viruses, in our recent study to understand the natural control of HIV-1 infection, we observed the regulation of the similar metabolic pathways but difference in the metabolic reactions, potentiate a disease specific contextualisation of the metabolic flux (Ambikan et al., 2022). A viral disease-specific systemic GSMM atlas for other emerging and re-emerging viruses is currently in progress."*

Fig 3E: impossible to view gradations in coloring between -log10 Padj values. I do not follow why the "distinctly down" regulation category is null for both sets of comparison

**Response:** We are thankful for the comment. Few of the pathways were found to have the same adjusted p values, which is the reason for the color gradient to be not clearly visible. We have used the R package Piano (PMID: 23444143) for pathway enrichment analysis. The tool takes gene-level

statistics (p values) and direction of expression (log2 fold change) as input to find down-regulated and upregulated pathways. The tool reports three classes of significance values for each pathway: distinct-directional, mixed-directional, and nondirectional. The nondirectional class uses the absolute values of the log2 fold change, thereby not considering the direction of change to computing the pathway statistics. The distinct-directional class takes the direction of regulation into account. If a gene set has an identical pattern of significant up-regulation and down-regulation, it will cancel out and thus won't come substantial. The mixed-directional class takes a subset of upregulated and downregulated genes separately to calculate the pathway statistics. In the study we have considered a distinct-directional class to define down-regulated and upregulated pathways where majority of genes are down-regulated and upregulated, respectively. Distinct-directional significant pathways show a distinct pattern of change in the pathways. In the present analysis, no pathways were found to be significantly ($p.adj < 0.05$) down-regulated in distinct directional classes. This means that there are no pathways with a distinct pattern of down-regulation of the genes. We can further observe that some pathways such as B cell receptor signaling pathway, FoxO signaling pathway and Phospholipase D signaling pathway etc. came significant in mixed directional class which implies. However, these pathways are distinctly upregulated; there exist the specific number of genes which are down-regulated as well.

Figure 4C: why is this listed as "used transcriptomic and metabolic data"? What is tossed out? In text: "…the integrative characterization of the samples pointed to 4 clusters (Table S1)." Should refer to Figure 4C in this statement.

**Response:** Thank you for pointing out this. We have used the similarity network fusion method to integrate transcriptomics and metabolomics data to classify the patients solely based on omics data signatures. We have re-written the figure legends for better clarity. Also Figure 4C is referred in the pointed out text in the manuscript.

"While using the single-cell transcriptomics data published by Zhang et al. 2020 (Zhang et al., 2020) who had previously characterized patient groups similar to ours, indicated that the members of the mitochondrial carrier family (SLC25) (SLC25A1, and SLC25A11) were highly expressed (log expression>2) in more cells in the monocyte populations (Fig 5B and Fig S7)" incomplete sentence

**Response:** We have corrected it now.

Fig 5D: impossible to view gradations in coloring between 0 and 1000 or 0 and -1000. Those that are highlighted in red are low flux, what is the interpretation of this? If they are near equilibrium do they matter much?
Fig 6A: Again, I can't tell differences in color scales for gene and metabolite betweenness

**Response:** The color scale shows a gradient corresponding to the values between 0 – 1000 and 0 - -1000 (1000 splits in each direction) for 5D and 0 to 600 in 6A. Generally, the heatmaps were drawn using Z-score (typically -4 to 4), where very few color input is required. To get a proper gradient, we have to input several hundred colors. Also, the flux value does not show a drastic difference among the patients. This can be the reason for the gradient to be not clear. However this is not impacted by the result or interpretation. We did not analyze the data quantitatively rather, we analyzed it qualitatively. We checked for reactions that alter in forward (positive flux) or revere direction (negative flux) or completely absent (zero flux). The explanation for responses highlighted in red is mentioned in the main text and the figure legend.

Reviewer #2: The authors have addressed most of my concerns. The last sentence of the abstract is quite incoherent and needs to be corrected. Moreover, a brief discussion about a potential role of the microbiome due to the observation of butyrate as a metabolite associated with disease pathology would be appreciated.

**Response:** We are thankful for the suggestion. We have now changed the last sentence of the abstract and added a brief discussion about the microbiome..

"Further, metabolite essentiality analysis identified butyrate, a microbiome-derived four-carbon short-chain fatty acid, as an essential metabolite in severe COVID-19, which plays an essential role in energy metabolism and intestinal homeostasis (Liu et al., 2018). Recently our integrative analysis of cytokines, metabolites, and microbiome features suggested a potential role of microbial-derived immunoregulatory processes in fatal outcomes in COVID-19 due to the failure of the negative feedback mechanism that should confine the cytokine storm (Albrich et al., 2022). Interestingly, MCTs are also involved in butyrate transportation (Chang et al., 2014)."

Reviewer #3: The authors have addressed my previous concerns.

**Response:** Thank you