PPARγ Cistrome Repression during Activation of Lung Monocyte-Macrophages in Severe COVID-19

HIGHLIGHTS
PPARγ expression is repressed in inflammatory lungs of patients with severe COVID-19
PPARγ trajectory is disrupted in bronchoalveolar CD14+/CD16+ cells of patients with COVID-19
We report here the epigenetics repression of PPARγ-NR3R1-RXRA cistrome in this setting
SUMO1, as repressed PPARγ partner, interacts with nucleoprotein of the human SARS virus
PPARγ Cistrome Repression during Activation of Lung Monocyte-Macrophages in Severe COVID-19

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SUMMARY
The molecular mechanisms of cytokine storm in patients with severe COVID-19 infections are poorly understood. To uncover these events, we performed transcriptome analyses of lung biopsies from patients with COVID-19, revealing a gene enrichment pattern similar to that of PPARγ-knockout macrophages. Single-cell gene expression analysis of bronchoalveolar lavage fluids revealed a characteristic trajectory of PPARγ-related disturbance in the CD14+/CD16+ cells. We identified a correlation with the disease severity and the reduced expression of several members of the PPARγ complex such as EP300, RXRA, RARA, SUMO1, NR3C1, and CCDC88A. ChIP-seq analyses confirmed repression of the PPARγ-RXRA-NR3C1 cistrome in COVID-19 lung samples. Further analysis of protein-protein networks highlighted an interaction between the PPARγ-associated protein SUMO1 and a nucleoprotein of the SARS virus. Overall, these results demonstrate for the first time the involvement of the PPARγ complex in severe COVID-19 lung disease and suggest strongly its role in the major monocyte/macrophage-mediated inflammatory storm.

INTRODUCTION
The pandemic caused by the novel SARS-CoV-2 coronavirus has rapidly become the chief public health challenge for many countries around the world. Respiratory complications have been well documented in patients with this disease (COVID-19). This coronavirus harbors a viral S-protein, which, during infection, binds to the human protein receptor ACE2 (Zhou et al., 2020b). ACE2 is abundant in lung, heart, kidney, and adipose tissue and, thus, the binding of the S-protein to ACE2 enables membrane fusion and the introduction of SARS-CoV-2 RNA into these cells (Turner et al., 2004). The mean incubation period of the disease is about 3–9 days (Li et al., 2020) and about 18% of cases remain asymptomatic (Nishiura et al., 2020). About 41.8% of patients develop acute respiratory distress syndrome (ARDS), for which diabetes mellitus can be a contributing factor. Other comorbidities associated with ARDS include hypertension, cardiovascular disease, and chronic kidney disease (Wu et al., 2020a). The severity of the disease is also age associated, with the risk of the mortality increasing in patients over 60 years old (Wu and McGoogan, 2020). One of the potential consequences of SARS-CoV-2 infection is an uncontrolled immune response in the lungs, which can require treatment in an intensive care unit. This immune response, associated with a cytokine storm, is heterogeneous and variable among individuals and is still not well understood (McGonagle et al., 2020). It also remains unclear why the heterogenous immune response to COVID-19 worsens in certain patients; as yet, we lack adequate knowledge to predict which cases will evolve from mild to severe infection.

In this work, we investigated the immune infiltration in biopsied lung tissues from patients with COVID-19 using different bioinformatics approaches, including whole transcriptome experiments, single-cell transcriptome characterization, and investigations of the epigenetic landscape. Mechanistically, we observed major deregulation of the innate immune response in these lung samples. In particular, we identified notable dysregulation of the PPARγ-dependent genetic program in macrophages, which is associated with an increase in the inflammation-promoting program in monocyte-macrophage cells. PPARγ repression was found in different independent cohorts and was strongly correlated in multiple patients with disease severity at the single-cell level in the CD14–CD16 myeloid population. PPARγ is a member of peroxisome proliferator-activated receptor family with PPARα and PPARβ. They have common structure comprising an amino terminal activation function-1 domain, a DNA binding domain, a hinge domain, and a conserved activation function-2 domain which allow ligand binding (Nolte et al., 1998). PPARγ was
first described as an important regulator of adipocyte differentiation and could have some unsaturated fatty acids, eicosapentaenoic acids, and oxidized lipids as natural ligands (Bell-Parikh et al., 2003). In immune system and lung, PPARγ is expressed in various cell types such as monocytes and macrophages (Standiford et al., 2005), dendritic cells (Gosset et al., 2001), and epithelial airway cells (Hetzel et al., 2003). During lung inflammation, PPARγ could represses transcription factors that regulated expression of pro-inflammatory molecules like NFKB1, STAT1, and AP1 (Honda et al., 2004; Straus and Glass, 2007). In our work, PPARγ repression in lung was associated with the induction of several inhibitory immune checkpoints, the lipopolysaccharide-sensitive TLR2 receptor, and HLA class I molecules, and these relationships were all associated with disease severity. In an epigenetic analysis of patients with severe COVID-19, we detected the repression of components of PPARγ-associated cistrome, including NR3C1 and RXRA, which had RGS12 as target in its second promoter. Finally, we also observed the repression of SUMO1, a partner of PPARγ, which is able to interact with nucleoprotein of human SARS-CoV virus and could potentially affect sumoylation.

RESULTS

Extensive Innate Response and Interferon Gamma Activation in COVID-19 Lung Biopsies

Transcriptome data of human lung biopsy samples from the dataset GSE147507 (Blanco-Melo et al., 2020) were subjected to gene set enrichment analysis using the “hallmarks” gene set from the MSigDB database. As compared with healthy donor samples, the majority of samples from COVID-19 lungs were significantly enriched in genes associated with innate immunity, such as the interferon alpha response (with a normalized enrichment score [NES] of +7.72, p value<0.001; Figure S1A), TNFA signaling via NF-kB (NES of +4.88, p value<0.001; Figure 1), complement cascade (NES of +3.51, p value<0.001; Figure S1A), and reactive oxygen species pathway (NES of +2.78, p value<0.001; Figure S1A). Likewise, these tissues were also enriched in genes linked with IL-6/Jak signaling, which plays a role in both innate and adaptive immunity (NES = +2.49, p value<0.001; Figure S1B). Furthermore, we observed dramatic enrichment in the interferon gamma response, which is involved in the adaptive immune response (NES = +8.11, p value<0.001; Figure S1C). Taken together, changes in the expression patterns of these immune-related genes enabled us to clearly differentiate, via unsupervised classification (Euclidean distances), between lung samples from patients with COVID-19 and those from healthy donors (Figure S1D).

These results suggested that in COVID-19 lung samples there was a dramatic upregulation of the interferon gamma response, which was accompanied by a large innate immune response. Indeed, a network analysis of this immune-activated program revealed centralized connections around the interferon gamma response (Figure 1A), with some targets shared with the interferon alpha response. A large part of the network was organized around aspects of the innate response such as interferon alpha, TNFA, the complement cascade, and reactive oxygen species (ROS). The majority of the immune network (Figure 1A) was composed of connections between enzymes, followed by signaling molecules, receptors, and transcription factors. We detected expression changes for a few ligands including chemokines and interleukins (Figure 1A), which were mainly induced by the IFNα and IFNγ response. To validate these results, we obtained single-cell transcriptome data from the bronchoalveolar lavage fluid of healthy donors (n = 6, green) and patients with mild COVID-19 (n = 3, blue) or severe COVID-19 (n = 3, red) from the dataset GSE145926. These data were merged together for the purpose of UMAP dimensionality reduction (Figure 1B) (Liao et al., 2020); after canonical correlation and filtration, the merged transcriptome analysis comprised 90,696 cells, with a representative proportion of each subgroup (Figure 1C). In this analysis, some of the main subpopulations identified were T lymphocytes that expressed CD3E and/or CD8A (Figure S2, at the top of the UMAP in Figure 1B), with a reduction in the CD8A cluster size in severe COVID-19 samples (Figure S2); natural killer cells that expressed NKG7, for which the cluster size increased in severe disease (Figure 1D, Figure S2, at the top right of the UMAP in Figure 1B); some epithelial cells that expressed KRT8 (Figure S3, on the right of the UMAP in Figure 1B); and some B lymphocytes expressing MS4A1 alias CD20 (Figure S3, on the top of the UMAP Figure 1B). This analysis also confirmed a major increase in the monocyte-macrophage markers CD14 and CD16 (alias FCGR3A; Italiani and Borschi, 2014) with increased disease severity (Figures 1D–1F). These results suggest a major role for the innate immune response in the lungs of patients with COVID-19, which intensifies with disease severity.
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Figure 1. Immune Network and Immune Checkpoint Regulation in COVID-19 Lung Samples

(A) Functional network of immune genes that were upregulated in lung samples from patients with COVID-19 compared with those from healthy donors; functions are represented as octagons, and their size represents the number of direct edge connections; (B) UMAP dimensionality reduction performed on merged single-cell transcriptome data from bronchoalveolar lavage fluid of healthy donors (green), patients with mild COVID-19 (blue), and patients with severe COVID-19 (red); major markers of cell subpopulations are indicated in black; (C) Pie chart representing the proportions of different cell types in the merged single-cell transcriptome analysis; (D) Dot plot of single-cell expression patterns (bronchoalveolar lavage fluid) of the main markers of each cluster, by patient of origin (HD, mild COVID-19, and severe COVID-19) (percent: percent of cells expressing each marker, expression level: color intensity); (E) Dimensionally reduced (UMAP) single-cell transcriptome (bronchoalveolar lavage fluid) expression patterns for the monocyte markers FCGR3A (CD16) and CD14 (F), by patient of origin (HD, mild COVID-19, and severe COVID-19).

COVID-19 Lung Transcriptome Reveals Defects in Cell Proliferation, Stem Cell Signaling, and Metabolism, with Major Myeloid Infiltration

A differentially expressed gene (DEG) analysis was performed on lung biopsies from patients with COVID-19 and healthy donors; this revealed widespread repression of many gene pathways in COVID-19 lungs (Figures S4A and S4B), which could affect major functionalities of the cells in this organ. These results were consistent with those of a gene set enrichment analysis (Figure S3). Specifically, the gene set enrichment analysis (performed using the “hallmarks” gene set of the MSigDB database) highlighted repression of the mitosis spindle and p53 pathway (cell cycle gatekeeper) in samples of COVID-19 lungs compared with those of healthy donors (NES = −3.45 and −2.77, respectively, with p value < 0.001, Figure S3A). We also detected the repression of signaling pathways that are implicated in stem cell functionality, such as the NOTCH pathway (NES = −1.70, p value < 0.001, Figure S3B) and the beta-catenin signaling pathway (NES = −2.17, p value < 0.001, Figure S3B). Furthermore, heme metabolism was also repressed in these samples (NES = −1.70, p value < 0.001, Figure S3C). These results suggest that in COVID-19 lung pathology there are major defects in important functionalities linked with tissue homeostasis, such as the regulation of cell proliferation, stem cell signaling, and metabolism. Of the 108 genes that were found to be upregulated in COVID-19 lung samples (Table S1), the majority were implicated in immune functionalities. In particular, the DEG analysis highlighted IFI6 as the most upregulated molecule in COVID-19 samples, and it was expressed in individual cells at progressively higher levels in more severe cases (Figure S4C). A landscape immune score analysis, performed on lung biopsies with the xcell algorithm and downstream limma analysis (Table S2), revealed a significant increase in immune score in COVID-19 lung samples, representing increases in neutrophil, monocyte, M1 macrophage, and adaptive dendritic cell infiltrations (Figure S4D). At the single-cell level, CD68 macrophage M1 marker was confirmed to be increased in bronchoalveolar lavage fluid of mild COVID-19 as compared with healthy donor samples but it was not case in severe COVID-19. In contrast, expression of CD163 macrophage M2 marker seems to be constant between different groups of samples (Figure S6). In order to characterize the immune infiltration of COVID-19 lungs, transcriptome data from lung biopsies were cross-integrated with transcriptome data from human hematopoietic and non-hematopoietic tissues (GSE76340) (Pont et al., 2016), which were taken to be representative of the micro-environment of the lung samples. The resulting cross-matrix comprised 170 whole transcriptome analyses and was submitted to the cross-batch normalization algorithm Combat to correct batch error (Figure S7 and Table S3). After mathematical correction, the normalized matrix was reduced to the 108 genes that were found to be upregulated in COVID-19 lung samples (Table S1). An unsupervised principal component analysis revealed major differentiation between healthy donor lungs and COVID-19 lungs along the first principal axis (p value = 3.215239 × 10⁻²⁹, Figure S4E). Mononuclear cells, monocytes, and macrophages were found in positions similar to the COVID-19 lung samples, suggesting major infiltrations in this tissue (Figure S4E) and confirming the results of the “xcell” immune score analysis (Figure S4C). In this principal component analysis, lung samples from healthy donors clustered together with normal primary bronchial epithelial cells, as well as endothelial cells and fibroblasts, suggesting that these cell populations are not implicated in the immune infiltration of COVID-19 lungs. Hematopoietic stem cells, B cells, T cells, and dendritic cells were found in intermediary positions between healthy donor and COVID-19 lung samples, which suggested that these cells played only a minor role in the immune infiltration (Figure S4E). We also confirmed that the microarray batches coming from different datasets used in this analysis did not influence the results, as they were found to be well distributed across the first principal axis (Figure S8).

Induction of Inhibitory Immune Checkpoints and CD48 in COVID-19 Lung Samples

Immune checkpoints have been widely discussed as new therapeutic targets for the regulation of the immune system in cancer therapy (Pardoll, 2012). In the case of COVID-19, a patient’s prognosis has been found to depend, at least in part, on the immune response (Xu et al., 2020). For this reason, we next investigated the expression of immune checkpoints in COVID-19 lung biopsies compared with healthy donor.
samples and constructed an expression heatmap with unsupervised classification that enabled us to differentiate COVID19 samples (COVA and COVB, COVID-19 lung biopsy replicate A and B) from those of healthy donors (HD1 and HD2, lung biopsies from healthy donors 1 and 2) (Figure S9A). The majority of inhibitory immune checkpoints, including CEACAM1, IDO1, LGALS9, CYBB, PDL1 (alias CD274), and CD47, were found to be overexpressed in COVID-19 lung samples (Figure S9A). Some of these, such as CD47, LGALS9, HAVCR2, IDO1, and CD274, were confirmed to be induced during severe disease progression (Figure S9B) at a single-cell level in the CD14-CD16 subpopulation (Figures S9C, 9D and S10). These results suggest a potential modification of communication among immune cells in COVID-19 lungs. Instead, our investigation of stimulatory immune checkpoints did not differentiate among COVID-19 and healthy donor lung samples (Figure S9E). In bronchoalveolar lavage fluid at the single-cell level, CD48 and CD40 were found to be expressed in COVID-19 lung tissue; CD48 was associated with increased disease severity (Figure S9F) but CD40 was not (Figure S11). In cases of severe COVID-19, CD48 was found to be expressed in the CD14-CD16 subpopulation (Figure S9G). These results mainly suggest that immune cell communication, which is regulated by immune checkpoints, may be modified during infection by COVID-19, particularly by the upregulation of inhibitory checkpoints.

### Activation of HLA Class I Molecules and Pathogen-Associated Molecular Patterns of Lipopolysaccharide-Sensitive TLRs in COVID-19 Lung Samples

When we examined the transcriptomes of the COVID-19 lung biopsies, we observed that, compared with data from healthy donors, there was a notable increase in antigen-presenting molecules from the HLA class I subcategory, including HLA-A, HLA-B, and HLA-C. We also detected an increase, although lower in magnitude, in antigen-presenting molecules from the class II subcategory: HLA-DPA1, HLA-DQA1, and HLA-DRB5 (Figure 2A). The strong expression of HLA class I molecules was confirmed with the single-cell transcriptome data obtained from bronchoalveolar lavage fluid, especially in the case of severe COVID-19 (Figure 2B). Specifically, HLA-B was highly expressed in all lymphoid and myeloid cell subpopulations in the lungs of the patient with severe COVID-19 (Figure 2C). When we examined the expression of Toll-like receptors (TLRs), we found no evidence for the induction of the TLR7 sensor in COVID-19 lung samples but did observe upregulation of TLR4, which is sensitive to lipopolysaccharides (Figure 2D). In bronchoalveolar lavage fluid at the single-cell level, expression of the lipopolysaccharide (LPS) sensors TLR2 and TLR4 was found to be induced in COVID-19 lungs (Figure 2E) but expression of TLR7 was unchanged. TLR2 expression appeared to increase with disease severity, especially in the CD14-CD16 cell populations (Figure 2F), whereas TLR4 induction was less dramatic (Figure 2G). These results provide evidence for CD14-CD16 myeloid infiltration in COVID-19 lung samples, with a substantial induction of HLA class I-presenting molecules and the TLR2 LPS sensor. To further characterize the transcriptome of COVID-19 lung biopsies, specifically with respect to the lympho-myeloid lineages, we performed transcriptome immune cell deconvolution on this subset of the bulk transcriptome data (Figure 3A). We observed that COVID-19 samples were segregated from those of healthy donors by a notable differentiation in lymphoid and myeloid components along the first principal axis (p value = 2.003047 × 10^-17, Figure 3A). COVID-19 samples were distinguished by positive correlations with myeloid markers (blue bar plot, Figure 3B) and a single negative correlation with one lymphoid IFITM1 (pink bar plot, Figure 3B). These results confirmed the strong influence of the myeloid signature, particularly when compared with that of the lymphoid signature, in COVID-19 lung samples. We observed a similar pattern at the single-cell level in the bronchoalveolar lavage liquid of patients with severe COVID-19 (Figure 3C), especially with the markers CCL7, FCERG1, and S100A11. In patients with severe COVID-19, S100A11 was found to be mainly induced in the CD14-CD16 subpopulations (Figure 3D). FCERG1 (Fc Fragment of IgE Receptor Ig) is known to be expressed in monocytes and macrophages (Bourazos et al., 2016), and here its induction was also found to be more restricted to the CD14-CD16 cell subpopulations (Figure 3E). These results suggested that, along with HLA and TLRs, some myeloid markers are also induced in the CD14-CD16 cell subpopulation during severe COVID-19.

### Monocyte-Macrophage Dysregulation Characterized by PPARγ Repression in COVID-19 Lung Samples

A gene set enrichment analysis performed using the MSigDB immune gene subset revealed that, compared with samples from healthy donors, COVID-19 samples were enriched in genes that had previously been found to be upregulated in macrophages following knockout of PPARγ (NES = +3.20, p value<0.001, Figure 4A). Consistent with this, we determined that PPARγ expression was in fact lower in COVID-19 lung samples compared with those from healthy donors (Figure 4B). By unsupervised
as well as superoxide dismutase (SOD2), were overexpressed in severe COVID-19 samples (Figure 5F). In this cell compartment, inflammatory transcription factors such as NFKBIA and MAFB, spanin CD9, the transferrin receptor TFRC, the cell junction molecule JAML, and the free fatty acid receptor FFAR4 (Figure 5E). In this cell compartment, PPARγ-dependent induction trajectory of the inhibitory immune checkpoint IDO1 (Figure 6G). These results suggest that deregulation of this PPARγ-dependent program plays a central role in the macrophage response during severe COVID-19 lung infection. In particular, the CD14+/CD16+ cell compartment of bronchoalveolar lavage fluids demonstrated a characteristic immune disturbance in samples from patients with severe COVID-19.

Deregulation of PPARγ Interactome in COVID-19 Bronchoalveolar Lavage Fluid

To further investigate the dysregulation of the PPARγ-dependent macrophage program in the lungs of patients with COVID-19, we constructed an interactome of the PPARγ network and examined the changes in expression among groups. To do this, PPARγ interactions were collected from the innateDB database of immune network interactions (Breuer et al., 2013) (Figure 7A). Of the gene partners associated with PPARγ, we found evidence in the single-cell transcriptomes (bronchoalveolar lavage fluid) for changes in expression associated with COVID-19 infection (Figure 7B), specifically in SUMO1, NR3C1, KLF6, RXRA, CEBPD, and PLAGL1. At the single-cell level, a strong correlation was found between PPARγ and SUMO1 (r = 0.47, Figure 7C), PPARγ and NR3C1 (r = 0.44, Figure 7C), and PPARγ and RXRA (r = 0.35, Figure 7C). A weaker correlation was detected between PPARγ and KLF6 (r = 0.13, Figure 7D) in patients with COVID-19, and indeed, we did observe a strong activation of KLF6 associated with the severe cases of the disease.
Figure 3. Main Monocyte-Macrophage Infiltration in COVID-19 Lung Samples

(A) Transcriptome deconvolution of lymphoid-myeloid populations from biopsies of COVID-19 and healthy lungs (p value: differentiation of groups by Pearson correlation along the first principal axis). Abbreviations: HD_lung, lung biopsy of healthy donor; Lung_COVID-19, lung biopsy of patient with COVID-19; MO, monocyte; M1, M1 macrophage; M2, M2 macrophage; IDC, immature dendritic cell; MDC, mature dendritic cells; LYB, B lymphocyte; LYT, T lymphocyte; BEC, primary bronchial epithelial cells.

B

| Gene       | Score |
|------------|-------|
| FCER1G     | 0.94  |
| SERPIN A1  | 0.86  |
| LILR A6    | 0.83  |
| P2RY13     | 0.82  |
| CXCL16     | 0.79  |
| HCK        | 0.76  |
| TREM1      | 0.75  |
| CCL7       | 0.72  |
| SH3BGRL3   | 0.69  |
| S100A11    | 0.69  |
| FAM49A     | 0.69  |
| GPR84      | 0.68  |
| TRIM38     | 0.68  |
| ALOX5AP    | 0.67  |
| IFITM1     | -0.76 |

C

COVID19 severe

COVID19 mild

Healthy donors

D

Healthy donors

COVID19 mild

COVID19 severe

S100A11

E

Healthy donors

COVID19 mild

COVID19 severe

FCER1G
was performed with the BETA cistrome application +/ the project database and processed the dataset using version HG38 of the human genome. Promoter mapping database (Figure 8 A). When we integrated the PPAR
tified (Table S5). By integrating these interactions with the PPAR
et al., 2013). This analysis confirmed the presence of a well-conserved signal in the mammalian promoter
coronavirus. This result suggests that, during lung infection, repression of the PPAR
SUMO1 is capable of interacting with a SARS virus, through the nucleoprotein NCAP_CVHSA of the human
host network (Figure 8 F). Using this, we determined that, within the PPAR
mediated via the interaction of SUMO1 with the virus and that sumoylation function may also be affected.

(Figure 7E) in all lympho-myeloid cell compartments. We constructed another PPARγ interactome using the IntAct protein database (Figure 7F) and, again, found evidence of relationships between PPARγ and some of its partners at the single-cell level in the bronchoalveolar lavage fluid of patients with COVID-19 (Figure 7 G). For example, PPARγ expression was strongly correlated with that of EP300 (r = 0.29, Figure 7H), CCDC88A (r = 0.60, Figure 7H), and RXRA (r = 0.45, Figure 7H) in COVID-19 cases. The expression of OPTN did not demonstrate a strong correlation to PPARγ (r = 0.15, Figure 7I); however, its expression did progressively increase in the CD14-CD16 myeloid compartment with increased disease severity (Figure 7J). These results suggest that, along with PPARγ, some of its associated proteins are also affected by COVID-19, particularly in the CD14-CD16 cell population in severe cases of the disease. The corepression of PPARγ-associated DNA-binding partners such as NR3C1, RARA, RXRA, and EP300 could be evidence for coregulation of the entire PPARγ cistrome in the CD14-CD16 cell population during severe COVID-19.

Co-repression of PPARγ-NR3C1-RXRA Cistrome during COVID-19 Disease in the Lungs
We retrieved data from a PPARγ ChIP-sequencing (ChIP-seq) experiment performed on the THP-1 mono-
cyte cell line (Gene Expression Omnibus [GEO] sample GSM624141) (Pott et al., 2012) from the Cistrome project database and processed the dataset using version HG38 of the human genome. Promoter mapping was performed with the BETA cistrome application +/ 100 kb around transcription starting sites (Wang et al., 2013). This analysis confirmed the presence of a well-conserved signal in the mammalian promoter database (Figure 8A). When we integrated the PPARγ ChIP-seq data with the PPARγ-repressed signature found in the lungs of patients with COVID-19 (Table S2), we were able to identify a genomic program with a landscape of both distal and proximal promoters (Figure 8B and Table S4). To confirm the relationships between the PPARγ-NR3C1-RXRA cistrome and the PPARγ repression signature of the COVID-19 transcriptionomes, ChIP-seq data obtained from the THP-1 monocyte cell line were retrieved for both NR3C1 (GEO sample GSM2661793) (Rollins et al., 2017) and RXRA (GEO sample GSM624142) (Pott et al., 2012) from the Cistrome project database. We also collected THP-1 CHIP-seq data for POLR2A (GEO sample GSM1905827) (Yu et al., 2015) to highlight transcriptional activity in this program. Using the “deeptools” pipeline (Ramirez et al., 2014), all of these CHIP-seq experiments were integrated with the PPARγ-repressed signature from COVID-19 lungs in order to identify common promoter signals +/- 5 kb around transcription starting sites. As shown in Figure 8C, the proximal PPARγ-repressed epigenetic program in monocytes shared its normal transcriptionally active promoters with RXRA and NR3C1. To characterize the active promoters in monocytes, we retrieved THP-1 CHIP-seq experiments for histone H3 lysine 27 acetylation (GEO sample GSM2544237) (Phanstiel et al., 2017) from the Cistrome project database. From the PPARγ-repressed program (Table S4 and Figure 8B), RGS12 was confirmed to present a PPARγ signal in its second promoter; its activity was demonstrated with the H3K27Ac histone mark and binding of RNA polymerase II (Figure 8D), suggesting that this target belongs to the PPARγ-repressed program during COVID-19 in the lungs. At the single-cell level, RGS12 was confirmed to be repressed in the CD14-CD16 cell compartment in COVID-19 lungs, especially in the severe form of the disease (Figure 8E).

SUMO1, from the COVID-19 PPARγ-Repressed Complex, Interacts with Nucleoprotein in Human SARS Virus
In order to investigate the interactions of the PPARγ complex with the human SARS virus, we explored the VirusHostNet 2.0 database (Guinmand et al., 2015) using the deregulated partners of PPARγ that we identified (Table S5). By integrating these interactions with the PPARγ network, we were able to create a virus-host network (Figure 8F). Using this, we determined that, within the PPARγ-repressed complex, only SUMO1 is capable of interacting with a SARS virus, through the nucleoprotein NCAP_CVHSA of the human coronavirus. This result suggests that, during lung infection, repression of the PPARγ complex may be mediated via the interaction of SUMO1 with the virus and that sumoylation function may also be affected.
Figure 4. Deregulation of PPARγ Program in COVID-19 Lung Samples
(A) Gene set enrichment analysis revealed that COVID-19 lung samples are enriched in genes that are upregulated in macrophages following PPARγ knockout (NES, normalized enrichment score; FDR, false discovery rate); (B) Boxplot of PPARγ expression in COVID-19 lung biopsies compared with samples from healthy donors, data from two independent datasets (p value from two-sided test with Welch correction); (C) Expression heatmap of PPARγ-knockout-dependent macrophage expression program in the transcriptome of COVID-19 lung biopsies compared with that of healthy donors (Euclidean distances); (D) Violinplot of PPARγ count expression in single-cell transcriptome of bronchoalveolar lavage fluid; (E) UMAP representation of single-cell transcriptome (bronchoalveolar lavage fluid) expression of PPARγ in HDs and patients with mild or severe COVID-19.
Severe COVID19 (CD14+/CD16+)
Healthy donors (CD14+/CD16+)

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C

D

E

F

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Figure 5. Single-Cell Expression Signature of CD14+/CD16+ Cell Compartment, in Which PPARγ was Repressed in the Lungs of Patients with Severe COVID-19
(A) Dot plot of PPARγ expression in CD14+/CD16+ (FCGR3A) cells in bronchoalveolar lavage fluid from patients with severe COVID-19 as compared with samples from healthy donors; (B) UMAP dimensionality reduction for CD14, FCGR3A, PPARγ and groups (sc: severe COVID-19, control: healthy donors); (C) Principal component analysis performed on CD14+/CD16+ PPARγ+/− cell compartment (sc: severe COVID-19, control: healthy donors); (D) Expression heatmap of the predictive signature for the first principal axis of the analysis in (C); (E) UMAP representation of the expression of membrane molecules that are repressed in the CD14+/CD16+ cell compartment of bronchoalveolar lavage fluid from severe COVID-19 cases; (F) UMAP representation of the expression of molecules that are upregulated in the CD14+/CD16+ cell compartment of bronchoalveolar lavage fluid from severe COVID-19 cases.

DISCUSSION
The COVID-19 pandemic has already affected hundreds of thousands of people and has become the greatest health challenge worldwide. The range of clinical presentations varies from asymptomatic and mild clinical symptoms to acute respiratory-distress syndrome (ARDS) and death. Respiratory complications have been well described in this disease, which is caused by infection with the virus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Diabetes mellitus has been identified as a factor that contributes to the development of ARDS, whereas other associated comorbidities include hypertension, cardiovascular disease, and chronic kidney disease (Wu et al., 2020a). In addition, a link has been demonstrated between obesity and COVID-19 cases that require invasive mechanical ventilation (Simonnet et al., 2020).

In this work, by comparing the transcriptomes of COVID-19 lung biopsies with those of healthy donors, we were able to characterize the immune infiltration in this tissue using different bioinformatics approaches. The major finding of our work is the discovery of a link between the severity of COVID-19 and repression of the PPARγ complex. In general, we observed that the adaptive immune response to the disease appeared to be driven by the induction of interferon gamma, whereas the innate response was largely the result of the induction of interferon alpha, TNF alpha, the complement cascade, and reactive oxygen species. Immune scoring of COVID-19 lung biopsies revealed major myeloid infiltration, specifically by monocytes, M1 macrophages, and neutrophils. Instead, the lymphoid scores for this sample were not significant, which suggests that the role of the adaptive immune response is less important than that of the innate response. This finding was confirmed by immune deconvolution analysis of the transcriptome data from COVID-19 lung samples. The SARS-CoV-2 coronavirus is a single-, positive-stranded RNA virus enveloped in a lipid bilayer (Wu et al., 2020b) (Lu et al., 2020). The lipid bilayer fuses with the host cell membrane, releasing RNA into the cytoplasm and resulting in the translation of various viral proteins. The replicated RNA genome and synthesized viral proteins reassemble into new viruses, which burst out of the cell (Qinfen et al., 2004). When we analyzed the induction of TLRs in COVID-19 lung samples, only the lipopolysaccharide sensor TLR2 was found to be upregulated with respect to controls, which is consistent with detection of the coronavirus envelope. The TLR2 sensor did not seem to be affected by COVID-19 infection in our samples, which suggests a lack of presentation of genetic material from the virus in the lungs; this is consistent with our other data demonstrating inefficiency in the adaptive immune response to this disease.

Compared with the virus that caused the SARS outbreak in 2003, the virus that causes COVID-19 uses the same mechanism to enter host cells, but at a slower speed. However, SARS-CoV-2 accumulates to higher concentrations in the body compared with SARS-CoV. This explains why COVID-19 has a longer incubation period and is more contagious, whereas SARS instead presents with more symptoms and increased disease severity (Guo et al., 2020). Immune scoring of COVID-19 lung biopsies revealed the infiltration of monocytes/macrophages in this tissue, and gene set enrichment analysis confirmed a major role for this type of cells in the immune program described in COVID-19 lungs. Several types of cytokines and chemokines belong to this dysregulated macrophage program. As in macrophage activation syndrome (MAS), macrophages in the lungs of patients with COVID-19 could play a central role in the non-adapted lung immune response through their contributions to cytokine storm. In COVID-19 lung samples, we detected an increase in interleukin 1 (IL1A and IL1B) and its receptor IL1R2. IL-1 is a pro-inflammatory cytokine produced primarily by monocytes and macrophages. It is present in the inactive form, pro-IL-1β, but, upon monocyte/macrophage activation it is cleaved by caspase-1 and becomes biologically active. Via signaling through its receptor, IL-1β causes lymphocyte and endothelial activation as well as the production of other inflammatory cytokines including IL-6 (Pascual et al., 2005b). IL-6 signaling, through the JAK cascade, was found to be enriched in COVID-19 lung samples compared with healthy donor tissues and expression of IL-6ST was likewise upregulated. IL-6 is a pleotropic cytokine produced in the early stages of inflammation and is central in driving the acute-phase response. One study of patients with MAS demonstrated that IL-6 produced activated macrophages in tissue obtained from liver biopsies (Billiau et al., 2005). For this reason, treatment
A. Component 1 vs. Component 2 plot showing COVID-19 severe (cyan) and healthy donors (red).

B. Pie chart indicating the distribution of PPARγ high, low, and medium across the samples.

C. PPARγ trajectory (pseudotime) plot with COVID-19 severe (cyan) and healthy donors (red).

D. PPARγ trajectory (pseudotime) plot for PPARγ high, low, and medium.

E. Heatmap representing the expression levels of various genes.

F. Scatter plots for RETN, FABP4, MRC1, PPARγ, and IDO1 showing the distribution across Component 1 and Component 2.

G. Scatter plot for IDO1 showing the distribution across Component 1 and Component 2.
with tocilizumab monoclonal antibody, which blocks IL-6 receptors, has been proposed to neutralize the cytokine storm that can occur during severe cases of COVID-19 (Fu et al., 2020). Within the IL-6 pathway, we found that the SCOC3 (suppressor of cytokine signaling 3) protein was upregulated in COVID-19 lung samples and, at the same time, we detected an induction of inhibitory immune checkpoints such as PDL1. IL-6 was reported to signal, via SCOS3, for the induction of its receptor PD1 during a cytokine storm, which was then found to neutralize the cell-mediated antiviral response (Vélazquez-Salinas et al., 2019). Among the inhibitory immune checkpoints that were upregulated in COVID-19 lungs, there is an FDA-approved targeted therapy (consisting of an antagonistic monoclonal antibody) for only one, CD274. However, a potential therapeutic strategy of targeting PDL1/PD1 in order to manipulate the adaptive immune response could be dangerous in COVID-19, because a variety of fatal adverse events related to the respiratory system have been recorded, including but not limited to pneumonia and respiratory failure (Wang et al., 2020).

In the lungs of patients with COVID-19, we detected a strong induction of the TNF-alpha response using gene set enrichment analysis. TNF-alpha is a pleomorphic cytokine that has been implicated in the pathogenesis of several inflammatory diseases. It is produced largely by monocytes and macrophages that are activated by TLR ligands such as endotoxins as well as cytokines such as IL-18 and stimulates local endothelial cells as well as lymphocytes (Shenoi and Wallace, 2010). IL-18 was also found to be upregulated in COVID-19 lung biopsies compared with controls; this is a unique cytokine in the IL-1 family because it is constitutively present in keratinocytes, epithelial cells, and blood monocytes (Puren et al., 1999). In addition to promoting secretion of TNF-alpha and chemokines by macrophages, IL-18 also induces production of the pro-inflammatory cytokine IFN by NK cells and T cells (Dinarello, 2007). Indeed, using gene set enrichment analysis, we observed substantial activation of the IFN-gamma response in COVID-19 lung samples, the primary function of which is to strongly activate monocytes and macrophages (Schröder et al., 2004). Activated macrophages are divided into several general classes based upon their respective stimuli and their resulting polarization, with M1 macrophages driven by IFN-gamma into a classical pro-inflammatory phenotype that is characterized by increased microbicidal ability, heightened responses to TLR ligands, and upregulated antigen processing and presentation. Consistent with this, we found that the immune scores of COVID-19 lung biopsies highlighted significant infiltration of macrophages of the M1 type. These cells are potent producers of pro-inflammatory cytokines, including IL-6, IL-12, and IL-23, as well as the chemokines IP-10, MIG, and ITAC, which recruit polarized Th1 cells in addition to NK cells (Mosser, 2003) (Mantovani et al., 2004). There is also evidence that IFN-gamma may be a critical driver of hemophagocytosis by these activated macrophages (Zoller et al., 2011). This could be connected to the reduction in heme metabolism-related activity we observed in the transcriptome of COVID-19 lung samples.

This dysregulation in secreted effectors in the lungs of patients with COVID-19 places monocytes/macrophages at the center of the cytokine storm that has been observed in severe cases of this disease. This was further corroborated by our finding that a gene set enrichment analysis performed on COVID-19 lung samples highlighted upregulation in a transcriptional program that was characteristic of macrophage cells in which PPARγ has been knocked out. Specifically, this program involved the activation of interferon-related genes, including several cytokines and chemokines. We determined that the expression of PPARγ was indeed downregulated in COVID-19 lung samples, which confirmed the origin of this macrophage dysregulation. Previous research has shown that PPARγ in macrophages limits pulmonary inflammation and promotes host recovery following respiratory viral infection (Huang et al., 2019b). In a murine influenza infection model with genetic-induced obesity (db/db), it was shown that the transcription factor PPARγ was downregulated in the lung macrophages of db/db mice after influenza infection. The treatment of 15-deoxy-A12, 14-prostaglandin J2 (15d-PGJ2), a PPAR-γ agonist, largely rescued the survival of db/db mice after influenza infection (Huang et al., 2019a). As was demonstrated in H1N1 infection, there could be a link between PPARγ and macrophages that drives inflammation in patients with COVID-19 (Josset...
with diabetes are over-represented (Zhou et al., 2020a) and this disorder has been defined as a major co-
et al., 2019) and PPAR
2018). PPAR
could suggest changes in the sensitivity of macrophages to glucocorticoids via their anti-inflammatory ac-
classical retinoic acid pathway, such as RXRA, and also to the glucocorticoid receptor NR3C1; this result
study (GWAS) of Finns (Scott et al., 2007, p. 2). Within the hospitalized COVID-19 population, individuals
during severe COVID-19; this molecule has been characterized as an interleukin-1 receptor-associated ki-
g
the severe immune response that has been linked with these innate cells. Several PPARγ agonists exist on
market (in phases II/III) for this purpose, and they have already been proposed as an alternative therapy
to target the cytokine storm (Ciavarella et al., 2020). In an animal model, it has been shown that loss of
PPARγ can delay monocyte differentiation into macrophages and increase a pro-inflammatory phenotype
with long-term LPS stimulation, characterized by an elevated production of the pro-inflammatory cytokines
TNF-α, IL-1β, IL-6, and IL-12 and reduced production of the anti-inflammatory cytokine IL-10 (Heming et al.,
PPARγ has also been implicated in the homeostasis of lipid metabolism in macrophages (Li et al.,
), and PPAR receptors have been characterized as a crossroad between lipid metabolism and inflam-
mation (Chinetti et al., 2000). In mice, systemic PPARγ deletion provokes severe type-2 diabetes (Gilardi
et al., 2019) and PPARγ has been associated with type-2 diabetes in humans via a genome-wide association
study (GWAS) of Finns (Scott et al., 2007, p. 2). Within the hospitalized COVID-19 population, individuals
with diabetes are over-represented (Zhou et al., 2020a) and this disorder has been defined as a major co-
morbidity of COVID-19 (Zhu et al., 2020). It is possible that the link between COVID-19 and type-2 diabetes
could be PPARγ. Furthermore, genetic polymorphism in PPARγ has also been linked to body mass index
(BMI) (Fornage et al., 2005) via GWAS analysis by the Framingham Heart Study 100K project (Fox et al.,
). PPARγ could also represent a link with the obesity comorbidity found with COVID-19 that could
reach 44% (Richardson et al., 2020).

The epigenetically repressed cistrome program identified in COVID-19 lungs extended to proteins of the
classical retinoic acid pathway, such as RXRA, and also to the glucocorticoid receptor NR3C1; this result
could suggest changes in the sensitivity of macrophages to glucocorticoids via their anti-inflammatory ac-
tion on this cell type (Mylka et al., 2018). Inside the PPARγ interactome, optineurin was found to be induced
during severe COVID-19; this molecule has been characterized as an interleukin-1 receptor-associated ki-
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within the PPARγ-associated repressed protein network, we identified SUMO1 as capable of interacting
with a nucleoprotein of the human SARS virus. PPARγ sumoylation is important for the LPS response
and transregulation of proinflammatory cytokines, via NCOR occupancy of kappaB-binding sites (Jenne-
wein et al., 2008). Sumoylation could act as a brake on PPARγ-associated repression of the transcriptional
activation of inflammatory response genes in macrophages (Pascual et al., 2005a).

In this work, the immune response to COVID-19 infection in the lungs was investigated; using different ap-
proaches, we identified dysfunction in M1 monocytes/macrophages in the innate response process. This
dysfunction was characterized by repression of PPARγ, which may play a key role in the cytokine storm
of inflammatory monocytes/macrophages in the SARS-CoV-2-infected lung.

Limitations of the Study

Integrative analysis through different bioinformatics methods on lung transcriptomes of patients with
COVID-19 enabled the characterization of the lung innate response as major in this pathological tissue.
A fundamental deregulation of PPARγ disruption was found in the innate immune response through mono-
cyte-macrophage cells. Repression of PPARγ was confirmed at the single-cell level in the severity of the dis-
ease. Network analysis on the PPARγ interacting complex revealed also the disruption of some of its part-
ers. PPARγ is an essential molecule implicated in macrophage activation and glucose, fatty acid, and
cholesterol metabolism; these mechanisms could link to the severity of the disease highly correlated to di-
betes and obesity. Interestingly, PPARγ is a druggable target. This bioinformatics study on disturbed im-
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cholesterol metabolism; these mechanisms could link to the severity of the disease highly correlated to dia-
betes and obesity. Interestingly, PPARγ is a druggable target. This bioinformatics study on disturbed im-
mune markers and immune cell deconvolution was validated at the single-cell level in an independent
cohort of samples. Epigenetic integration in the analysis allowed one to confirm the genome landscape
Human SARS virus proteins in VHN database

Human interactome

PPARγ complex

PPARγ
NR3C1
RXRA
POLR2A
RGS12 (HG38)

PPARγ - repression in COVID-19 lung

GUCD1
SLC25A4
ULK3
EXOSC7
RGS12RGS12 MYZAP
COG1 ING5
SNX25
GUSBP11
RAPGEF1
SSTR2FAM189B
TTT2RPL23AP82
WDR53
RAD9A CEP85
CEP85
PHACTR3
SLC19A1
B3GNT8
ZSWIM3

A

Average Phastcons around the Center of Sites

Distance from the Center [bp]

B

PPARγ - repression in COVID-19 lung

H3K27Ac
POLR2A
PPARγ

RGS12 (HG38)

C

PPARγ
NR3C1
RXRA
POLR2A

D

Healthy donors COVID19 mild COVID19 severe

E

RGS12

F

PPARγ complex

Human SARS virus proteins in VHN database

Human interactome
implication of PPARγ-NR3C1-RXRA cistrome repression in severe lung COVID-19 diseases. SUMO1 that represses the PPARγ partner in COVID-19 diseased lungs was also found to interact with the SARS virus. The discovery of this original mechanism could help to better understand the huge innate inflammatory processes in COVID-19 diseased lungs as well as the associated risk factors found in this disease.

Resource Availability

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Frank Griscelli (franck.griscelli@gustaveroussy.fr).

Materials Availability
This study did not generate specific reagents.

Data and Code Availability
We obtained transcriptomes from COVID-19 lung biopsies from dataset GSE147507 in the Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE147507) and now publish in Cell on 28th May 2020 (https://www.cell.com/cell/fulltext/S0092-8674(20)30489-X) (Blanco-Melo et al., 2020). "Volcanoplot" software was developed to analyze the significance of immune scores; the corresponding R functions are available at the web address: https://github.com/cdesterke/volcanoplot. Chip2Heat.sh software was developed in SHELL BASH (Bourne-again SHELL) and implemented with the deepTools pipeline, specifically computeMatrix and plotHeatmap, with the promoter plot saved in different formats: PNG, PDF, SVG. This software can be downloaded at https://github.com/cdesterke/chip2heat. The R code for the single-cell analyses for marker validation is detailed in the Transparent Methods section of the Supplemental Information and also provided at the following address: https://github.com/cdesterke/covid19sc. Additional resources with interactive Web interface of the single cell analysis: to facilitate data exploration of disturb CD14+/CD16+ double population in bronchoalveolar lavage fluid of patients with severe COVID-19, we developed an interactive web interface available at https://cdesterke.shinyapps.io/COVID19LUNG/. This website was built with flexdashboard and shiny application inclusion and with graphical interactivity display by R plotly. This data dashboard allows exploring expression of biomarkers found on PPARγ trajectory in lung monocyte-macrophage disrupted cell population in severe COVID-19. The user needs to select gene ID on the left sidebar, and the application will display expression of this selected marker with interactivity on TSNE graph, number of positive cells for this marker will be displayed in the value box at the top right of the dashboard, also expression by group will be displayed on violinplot, finally statistical summary (mean and standard deviation, SD) will be displayed by group of samples.

Mendeley Dataset associated to this manuscript: 10.17632/3xnypzvcf7.1.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101611.
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AUTHOR CONTRIBUTIONS
C.D., A.B.-G., F.G.: Conceptualization. C.D., F.G., A.G.T.: Methodology. C.D., F.G., A.B.-G., A.G.T.: Validation. C.D., F.G., A.B.-G., A.G.T.: Data analysis. C.D., F.G.: Writing – original draft preparation. F.G., A.B.-G.: Supervision. C.D., A.B.-G., A.G.T., F.G.: Funding acquisition.

DECLARATION OF INTERESTS
The authors do not have any conflict of interest to declare.

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Supplemental Information

PPAR$_{\gamma}$ Cistrome Repression during Activation of Lung Monocyte-Macrophages in Severe COVID-19

Christophe Desterke, Ali G. Turhan, Annelise Bennaceur-Griscelli, and Frank Griscelli
Transparent Methods

Transcriptomes from public datasets
We obtained transcriptomes from COVID-19 lung biopsies from dataset GSE147507 in the Gene Expression Omnibus database (https://www.biorxiv.org/content/10.1101/2020.03.24.004655v1) and now published in Cell on 28th May 2020 (https://www.sciencedirect.com/science/article/pii/S009286742030489X?via%3Dihub) (Blanco-Melo et al., 2020, p.).

This dataset comprised human biological samples: lung biopsies from one male (age 72) and one female (age 60), used as biological replicates. Additionally, lung samples from a single male (COVID-19 deceased, age 74) patient were processed in technical replicates. These experiments had been performed with the approval of the institutional review board at the Icahn School of Medicine at Mount Sinai under protocol HS#12-00145. Samples were sequenced on NextSeq 500 technology (Illumina, CA) after the selection of a polyA RNA library using the TruSeq RNA Library Prep Kit v2 (Illumina, CA); the library was prepared from total RNA extracted using the RNeasy Mini Kit (Qiagen). Raw sequencing reads were aligned to the human genome (hg19) using the RNA-Seq Alignment App (v2.0.1). We then used the matrix of raw count data to perform the bioinformatics analyses described below.

The GSE150316 dataset had been prepared from distinct tissues from 5 COVID-19 patients and 5 healthy donors via RNAseq sequencing; samples from COVID-19 patients were processed in triplicate. Sequencing was performed on an Illumina NextSeq500 instrument after library preparation with the Smarter Stranded Total RNA-Seq kit v2 (634413, Illumina) starting from 10 ng of RNA (FFPE slides) extracted with the FormaPure Total nucleic acid extraction kit (C16675, Beckman Coulter).

Transcriptome of human tissues with immune cell sub-populations
Normalized transcriptomes from different human tissues were retrieved from the dataset GSE76340 on the GEO website. Samples in this dataset had been processed with different versions of the HumanHT-12 beadchip (Illumina, CA): versions V3 & V4 were annotated with the transcriptome platforms gpl6947 & gpl10558. These experiments comprised 166 human samples which were representative of hematopoietic and non-hematopoietic tissues present in the human body and were compatible with immune deconvolution analysis (Pont et al., 2016).

CHIP-sequencing from THP-1 cell line
Data from CHIP-sequencing experiments conducted using the THP-1 cell line were downloaded from the Cistrome Project website, in Bed and Bigwig format and aligned on version HG38 of the human genome. Promoter mapping was performed with BETA cistrome with a prediction +/-100 kb around transcription starting sites; promoter heatmaps were drawn with the deepTools application +/- 5 kb around transcription starting sites. CHIP-seq signals were visualized in the Integrative Genomics Viewer (IGV) standalone software after uploading the corresponding BigWig files.
Bioinformatics analyses

Bioinformatics analyses were performed in R software environment version 3.5.3. Functional network analysis was carried out using an immune gene-set enrichment analysis with the standalone software Cytoscape version 3.4.0. Raw counts of next-generation sequencing data were normalized with the algorithm ‘variance stabilization transformation’ (VST) from EdgeR. The cross-matrix between datasets, which comprised 170 transcriptome samples (Supplemental Table 3), was constructed by merging experiment sets based on their unique gene symbol identifiers. Cross-batch normalization was applied to the resulting matrix with the algorithm ‘Combat’ of the SVA R-package. Transcriptome expression heatmaps were created with the Made4 and pheatmap R-packages, with default Pearson distances for small heatmaps and Euclidean distances for large ones. Immune landscape transcriptome analysis was performed with the xcell R-package and multi-testing linear model fit correction was applied to immune scores with the limma R-package. Gene-set enrichment analysis was performed on human lung biopsy samples with the standalone software GSEA version 4.0.3 using the MSigDB database, version 7.1.

Single-cell analysis of lung samples from healthy donors and patients with mild and severe COVID-19

In order to validate, at the single-cell level, the disruption observed in immune molecules in the transcriptome analysis of lung cells from COVID-19 patients, single-cell transcriptome (10x Genomics) data from bronchoalveolar lavage fluid samples were downloaded in H5 format from the dataset GSE145926. From this dataset, we created a merged matrix by aggregating a total of 90696 transcriptomes. This included six healthy donor samples—GSM4475048, GSM4475049, GSM4475050, GSM4475051, GSM4475052, GSM4475053—comprising 39900 transcriptomes, three mild COVID-19 samples—GSM4339769, GSM4339770, GSM4339772—comprising 9710 transcriptomes, and three severe COVID-19 samples—GSM4339771, GSM4339773, GSM4339774—comprising 41086 transcriptomes.

After canonical correlation and scaling, a total of 23742 features were analyzed, with 38738 anchors identified between samples. After variable feature selection with the VST algorithm, dimensionality reduction was carried out by principal component analysis on 2000 variable features (30 components) and UMAP dimensionality reduction of the 20 best components of the PCA. Single-cell analyses on the CD14+/CD16+ subset of cells were performed in Seurat and PPARγ gamma-dependent trajectories were constructed for the CD14+/CD16+ subset with the monocle2 R-package. The transparent bioinformatics code for all single-cell analyses in R software is provided in the supplemental data (supplemental bioinformatics code).

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Supplemental figure legends

Supplemental Figure 1. Transcriptomic view of the immune response in lungs of COVID-19 patients compared to those of healthy donors, related to figure 1:

A. Genes highlighted by the gene-set enrichment analysis that are associated with the innate immune response; B. Genes highlighted by the gene-set enrichment analysis that are implicated both in the innate and adaptive immune responses; C. Genes highlighted by the gene-set enrichment analysis that are implicated in the adaptive immune response (for A to C: NES: normalized enrichment score, FDR: false discovery rate); D. Expression heatmap of immune-related genes that were upregulated in COVID-19 lung biopsy samples (Euclidean distances).

Supplemental Figure 2: Single cell expression of T and NK lymphocyte markers in lung of COVID-19 patients as compared to Healthy donors, related to figure 1:

UMAP projection of single cell expression split on lung from Healthy donors (control), from patient with COVID-19 mild and from patient with COVID-19 severe: respective expression of CD3E, CD8A (T lymphocyte) and NKG7 (Natural Killers)

Supplemental Figure 3. Single cell expression of B lymphocyte and epithelial markers in lung of COVID-19 patients as compared to Healthy donors, related to figure 1:

UMAP projection of single cell expression split on lung from Healthy donors (control), from patient with COVID-19 mild and from patient with COVID-19 severe: respective expression of MS4A1 alias CD20 (B lymphocyte) and KRT8 (epithelial cells)

Supplemental Figure 4. Immune score and immune characterization of COVID-19 lung samples, related to figure 2:

A. Volcanoplot of differentially expressed genes (DEG) in COVID-19 lung biopsies compared to healthy donor tissues; B. Expression heatmap of DEGs between COVID-19 lung biopsies and healthy tissues; C. Expression of the most upregulated marker, IFI6, in single cells from healthy donors, patients with mild COVID-19, and patients with severe COVID-19; D. Volcanoplot and heatmap of significant immune scores found in the immune infiltration of COVID-19 lung biopsies compared to those healthy donors; E. Immune deconvolution of the transcriptomes of COVID-19 and healthy donor lung biopsies via unsupervised principal component analysis (p-value: group stratification by Pearson correlation along the first principal axis)

Supplemental Figure 5: Repression of mitosis/cell cycle, stem cell and heme metabolism in COVID-19 lung biopsy as compared to healthy donor ones, related to figure 1:

(NES: normalized enrichment score, FDR: false discovery rate)
Supplemental Figure 6: Single cell expression of macrophages M1 and M2 markers in lung of COVID-19 patients as compared to Healthy donors, related to figure 3:

UMAP projection of single cell expression split on lung from Healthy donors (control), from patient with COVID-19 mild and from patient with COVID-19 severe: respective expression of CD68 for M1 macrophages and CD163 for M2 macrophages.

Supplemental Figure 7: Workflow procedure of Batch cross normalization between transcriptome datasets used for immune deconvolution of COVID-19 lung biopsy, related to figure 3:

Cross transcriptome matrix from two datasets with three platforms was built on unique gene symbol, ComBat batch normalization was applied to the matrix to correct batch error.

Supplemental Figure 8: Unsupervised principal component analysis performed with immune induced signature according to the batches used to build the transcriptome cross dataset matrix, related to figure 3

(respective batches merged during microarray analysis B1: batch1, B2: batch2, B3: batch3)

Supplemental Figure 9: Deregulation of inhibitory immune checkpoints in COVID-19 lung samples, related to figure 1:

A. Transcriptome expression heatmap of inhibitory immune checkpoints expressed in COVID-19 lung samples compared to those from healthy donors; B-D. Single-cell expression (bronchoalveolar lavage fluid) dotplots of inhibitory immune checkpoint markers by patient of origin: HD, mild COVID-19, and severe COVID-19 (percent: percent of cells expressing each marker, expression level: color intensity); C. UMAP representation of single-cell transcriptome (bronchoalveolar lavage fluid) expression data from HDs and patients with mild or severe COVID-19 for CD47 and D. LGALS9; E. Expression heatmap of stimulatory immune checkpoints expressed in COVID-19 lung samples compared to healthy donors; F. Single-cell expression (bronchoalveolar lavage fluid) dotplots of stimulatory immune checkpoints markers by patient of origin: HD, mild COVID-19, and severe COVID-19 (percent: percent of cells expressing each marker, expression level: color intensity); G. UMAP representation of single-cell transcriptome (bronchoalveolar lavage fluid) expression data for CD48 from HDs and patients with mild or severe COVID-19.

Supplemental Figure 10: Single cell expression of inhibitory immune checkpoints in lung of COVID-19 patients as compared to Healthy donors, related to figure 1:

UMAP projection of single cell expression split on lung from Healthy donors (control), from patient with COVID-19 mild and from patient with COVID-19 severe: respective expression of HAVCR2, IDO1, CD274.
Supplemental Figure 11. Single cell expression of stimulatory immune checkpoints in lung of COVID-19 patients as compared to Healthy donors, related to figure 1:

UMAP projection of single cell expression split on lung from Healthy donors (control), from patient with COVID-19 mild and from patient with COVID-19 severe: respective expression of CD40.
Cell cycle and mitosis

Enrichment plot: HALLMARK_MITOTIC_SPINDLE
NES = -3.45
P-value < 0.001
FDR < 0.001

Enrichment plot: HALLMARK_PS3_PATHWAY
NES = -2.77
P-value < 0.001
FDR < 0.001

Stem cell

Enrichment plot: HALLMARK_NOTCH_SIGNALING
NES = -1.70
P-value < 0.001
FDR = 0.02

Enrichment plot: HALLMARK_WNT_BETA_Catenin_SIGNALING
NES = -2.17
P-value < 0.001
FDR = 0.02

C

Enrichment plot: HALLMARKHEME_METABOLISM
NES = -1.70
P-value < 0.001
FDR = 0.02

Supplemental Figure 5
Supplemental Figure 6
Three dataset expression matrix before batch normalization

Combat Cross Normalization

Three dataset expression matrix after batch normalization
Inhibitory CP

Stimulatory CP

Supplemental Figure 9
Supplemental Figure 10

Healthy donors | COVID19 mild | COVID19 severe
---|---|---
HAVCR2 | | |
IDO1 | | |
CD274 (alias PDL1) | | |
Supplemental Figure 11

CD40
Supplemental Table 1: list of differential expressed genes found to be up regulated in COVID-19 lung biopsy transcriptome as compared to healthy donor ones, related to figure 1

| gene symbol | logFC  | AveExpr | t    | P.Value | adj.P.Val | B    |
|-------------|--------|---------|------|---------|----------|------|
| IFI6        | 7.1776 | 13.6855 | 62.6608 | 0.0000 | 0.0416 | 2.8234 |
| DAPP1       | 5.3198 | 9.5271  | 40.7539 | 0.0001 | 0.0416 | 2.4574 |
| MX1         | 4.8482 | 12.2353 | 38.6539 | 0.0001 | 0.0416 | 2.3942 |
| CLEC4E      | 7.4028 | 11.3749 | 37.9561 | 0.0001 | 0.0416 | 2.3713 |
| CASP5       | 5.0590 | 8.6360  | 37.1177 | 0.0001 | 0.0416 | 2.3426 |
| FFAR2       | 6.4971 | 10.1686 | 35.2980 | 0.0001 | 0.0416 | 2.2748 |
| RAC2        | 3.8602 | 11.8497 | 31.1533 | 0.0001 | 0.0416 | 2.0869 |
| TREM1       | 4.5248 | 11.1755 | 28.9204 | 0.0002 | 0.0416 | 1.9616 |
| TNFSF10     | 4.5746 | 12.1425 | 28.7743 | 0.0002 | 0.0416 | 1.9527 |
| TREML3P     | 3.7333 | 7.7467  | 27.8337 | 0.0002 | 0.0416 | 1.8931 |
| LGALS9      | 3.5443 | 11.3215 | 26.9698 | 0.0002 | 0.0416 | 1.8346 |
| CCL16       | 3.2593 | 11.6221 | 26.7020 | 0.0002 | 0.0416 | 1.8157 |
| CYP19A1     | 3.9088 | 7.3177  | 26.6198 | 0.0002 | 0.0416 | 1.7650 |
| ALOX5AP     | 6.2144 | 12.7068 | 26.0076 | 0.0002 | 0.0416 | 1.7500 |
| PTK2B       | 3.8377 | 11.0414 | 25.2922 | 0.0002 | 0.0416 | 1.7098 |
| HCK         | 2.7985 | 10.7928 | 24.9444 | 0.0002 | 0.0416 | 1.6819 |
| ZC3HAV1     | 3.4884 | 11.5295 | 24.8897 | 0.0002 | 0.0416 | 1.6775 |
| PLAC8       | 3.8467 | 9.6419  | 24.1251 | 0.0002 | 0.0416 | 1.6132 |
| ST20        | 6.8379 | 9.7565  | 23.9457 | 0.0003 | 0.0416 | 1.5975 |
| FDCSP       | 5.4337 | 8.0010  | 21.9213 | 0.0003 | 0.0416 | 1.4047 |
| TRIM21      | 3.0902 | 10.4027 | 21.6234 | 0.0004 | 0.0416 | 1.3736 |
| TLE4        | 2.6830 | 9.6468  | 21.1623 | 0.0004 | 0.0416 | 1.3240 |
| BASP1       | 4.0718 | 11.5338 | 20.1227 | 0.0004 | 0.0416 | 1.2048 |
| ZBP1        | 3.4269 | 7.9723  | 20.0985 | 0.0004 | 0.0416 | 1.2019 |
| KLK10       | 3.9032 | 8.8989  | 20.0770 | 0.0004 | 0.0416 | 1.1993 |
| WIPF1       | 2.3939 | 10.4371 | 19.7917 | 0.0005 | 0.0416 | 1.1646 |
| METTL7B     | 2.3545 | 6.6711  | 19.6145 | 0.0005 | 0.0416 | 1.1426 |
| CERS6       | 2.4488 | 8.0154  | 19.2357 | 0.0005 | 0.0416 | 1.0945 |
| HSH2D       | 4.2234 | 8.9230  | 19.0770 | 0.0005 | 0.0416 | 1.0739 |
| S100A11     | 4.4486 | 14.6746 | 18.8674 | 0.0005 | 0.0416 | 1.0462 |
| STAT2       | 2.3554 | 10.8830 | 18.7197 | 0.0005 | 0.0416 | 1.0264 |
| TAOK3       | 3.1629 | 10.2652 | 18.5327 | 0.0005 | 0.0416 | 1.0010 |
| TRIM38      | 4.2132 | 10.8972 | 18.4762 | 0.0005 | 0.0416 | 0.9932 |
| PLP2        | 2.4331 | 11.3802 | 18.4719 | 0.0005 | 0.0416 | 0.9926 |
| MSMO1       | 2.1889 | 9.7341  | 18.1669 | 0.0006 | 0.0416 | 0.9500 |
| C19ORF66    | 3.8901 | 10.8223 | 17.9042 | 0.0006 | 0.0416 | 0.9123 |
| MXD1        | 4.9895 | 11.6460 | 17.9034 | 0.0006 | 0.0416 | 0.9122 |
| PAD2        | 4.2443 | 8.9215  | 17.8949 | 0.0006 | 0.0416 | 0.9109 |
| CD38        | 2.2730 | 7.4678  | 17.7960 | 0.0006 | 0.0416 | 0.8965 |
| Gene  | Value1 | Value2 | Value3 | Value4 | Value5 | Value6 | Value7 | Value8 | Value9 | Value10 | Value11 | Value12 | Value13 |
|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|----------|----------|----------|----------|
| TET2  | 2.3199 | 9.0819 | 17.6588| 0.0006 | 0.0416 | 0.8763 |
| P2RY13 | 6.5491 | 9.5865 | 17.5438| 0.0006 | 0.0416 | 0.8592 |
| TEMEM71| 3.8052 | 8.8363 | 17.1109| 0.0007 | 0.0416 | 0.7931 |
| FCER1G | 7.1125 | 14.1999| 16.9234| 0.0007 | 0.0416 | 0.7636 |
| SH3BGR1| 2.9494 | 13.5637| 16.7161| 0.0007 | 0.0416 | 0.7305 |
| LCP1  | 4.1459 | 12.8424| 16.2003| 0.0008 | 0.0416 | 0.6452 |
| YIPF1 | 1.8240 | 8.9549 | 16.0496| 0.0008 | 0.0416 | 0.6195 |
| HP    | 1.8822 | 7.6909 | 15.8171| 0.0008 | 0.0416 | 0.5792 |
| LILRA6| 5.4992 | 9.7715 | 15.7799| 0.0008 | 0.0416 | 0.5726 |
| EIF2AK2| 5.8701 | 10.5396| 15.7027| 0.0009 | 0.0416 | 0.5590 |
| TNFSF8| 2.2703 | 7.9982 | 15.5407| 0.0009 | 0.0416 | 0.5300 |
| STAP1 | 5.0370 | 8.4880 | 15.3400| 0.0009 | 0.0416 | 0.4936 |
| HPGDS | 1.9920 | 8.2100 | 15.2378| 0.0009 | 0.0416 | 0.4747 |
| BCL2A1| 6.3646 | 12.8711| 15.1775| 0.0009 | 0.0416 | 0.4635 |
| SP140 | 5.1011 | 9.3682 | 15.1710| 0.0009 | 0.0416 | 0.4623 |
| GSK3A | 2.0767 | 9.9251 | 15.0107| 0.0010 | 0.0416 | 0.4321 |
| OAS1  | 6.2611 | 11.2966| 14.7159| 0.0010 | 0.0416 | 0.3755 |
| SELL  | 8.5898 | 12.3057| 14.6870| 0.0010 | 0.0416 | 0.3699 |
| SLC9B2| 2.1402 | 9.2398 | 14.6463| 0.0010 | 0.0416 | 0.3619 |
| PLEK  | 6.5675 | 12.6105| 14.5396| 0.0011 | 0.0418 | 0.3408 |
| DHR59 | 2.0806 | 7.6688 | 14.5022| 0.0011 | 0.0418 | 0.3334 |
| S100P | 3.9222 | 9.9067 | 14.5012| 0.0011 | 0.0418 | 0.3332 |
| MMP8  | 4.8508 | 8.4352 | 14.4707| 0.0011 | 0.0419 | 0.3271 |
| SERPINA1| 3.6621| 13.6439| 14.3424| 0.0011 | 0.0420 | 0.3014 |
| RTP4  | 3.4262 | 8.7756 | 14.3371| 0.0011 | 0.0420 | 0.3003 |
| KCNH7 | 3.8933 | 7.7884 | 14.2806| 0.0011 | 0.0421 | 0.2888 |
| CCL8  | 7.0072 | 11.9994| 14.0218| 0.0012 | 0.0428 | 0.2355 |
| UBE2L6| 3.2563 | 11.7618| 13.9081| 0.0012 | 0.0430 | 0.2117 |
| CASP4 | 3.4312 | 12.2297| 13.8508| 0.0012 | 0.0430 | 0.1995 |
| GBP1P1| 3.0089 | 8.5281 | 13.6575| 0.0013 | 0.0433 | 0.1581 |
| IFITM1| 5.0111 | 13.7490| 13.6058| 0.0013 | 0.0433 | 0.1469 |
| ANXA3 | 1.6882 | 10.8230| 13.4706| 0.0013 | 0.0436 | 0.1173 |
| SERF2 | 2.8845 | 12.6592| 13.3953| 0.0013 | 0.0437 | 0.1006 |
| IFIT1 | 6.4460 | 11.5361| 13.3266| 0.0013 | 0.0437 | 0.0853 |
| RNASE2| 4.8415 | 7.6583 | 13.3042| 0.0014 | 0.0438 | 0.0803 |
| CXORF21| 3.7354| 7.9206 | 13.1232| 0.0014 | 0.0442 | 0.0939 |
| PIFN1 | 2.5686 | 14.7434| 13.1121| 0.0014 | 0.0442 | 0.0367 |
| IFIT3 | 6.0584 | 12.4936| 12.9875| 0.0014 | 0.0445 | 0.0080 |
| FAM49A| 1.8188 | 9.4999 | 12.9724| 0.0014 | 0.0445 | 0.0045 |
| POGlut1| 1.5187| 8.4467 | 12.9673| 0.0014 | 0.0445 | 0.0034 |
| DOK3  | 4.0627 | 9.5715 | 12.8563| 0.0015 | 0.0451 | -0.0226 |
| CCL11 | 6.0886 | 10.9454| 12.8467| 0.0015 | 0.0451 | -0.0248 |
| ISG15 | 5.3753 | 12.6734| 12.8325| 0.0015 | 0.0451 | -0.0282 |
| C1orf194| 2.0250| 6.9651 | 12.7017| 0.0015 | 0.0457 | -0.0592 |
| LYRM1 | 2.0250 | 9.9753 | 12.5245| 0.0016 | 0.0457 | -0.1019 |
| Gene       | Expression | MAF   | NAF   | P-Value | FDR   | Log2FC |
|------------|------------|-------|-------|---------|-------|--------|
| CPNE8      | 1.5333     | 9.2754| 12.5214| 0.0016  | 0.0457| -0.1026|
| DDX60L     | 5.1716     | 10.7959 | 12.5189 | 0.0016  | 0.0457| -0.1032|
| WAS        | 4.7416     | 9.4159 | 12.5157 | 0.0016  | 0.0457| -0.1040|
| BCL2L14    | 3.4702     | 7.6795 | 12.4910 | 0.0016  | 0.0457| -0.1100|
| PSME3      | 2.5692     | 11.4849 | 12.4163 | 0.0016  | 0.0460| -0.1283|
| CCL19      | 4.1790     | 9.5531 | 12.4143 | 0.0016  | 0.0460| -0.1288|
| TIMM23     | 2.8022     | 10.1641 | 12.3870 | 0.0016  | 0.0461| -0.1355|
| CISD2      | 1.9337     | 8.6465 | 12.3203 | 0.0017  | 0.0464| -0.1521|
| NFKBID     | 3.0303     | 9.3245 | 12.0344 | 0.0018  | 0.0479| -0.2242|
| CCL7       | 4.5305     | 8.3294 | 12.0065 | 0.0018  | 0.0479| -0.2313|
| GPR84      | 5.7629     | 10.1132 | 11.7846 | 0.0019  | 0.0486| -0.2890|
| CACNA1A    | 4.3593     | 8.1171 | 11.7675 | 0.0019  | 0.0486| -0.2936|
| EIF1B      | 2.5061     | 11.4329 | 11.5612 | 0.0020  | 0.0495| -0.3485|
| C2CD4B     | 1.6818     | 9.4010 | 11.5384 | 0.0020  | 0.0495| -0.3547|
| NME1       | 2.4402     | 9.5301 | 11.5345 | 0.0020  | 0.0495| -0.3557|
| GAPT       | 5.0651     | 8.6691 | 11.4180 | 0.0021  | 0.0496| -0.3875|
| MX2        | 5.8224     | 11.3090 | 11.4094 | 0.0021  | 0.0496| -0.3998|
| CYSTM1     | 3.2616     | 12.3467 | 11.4046 | 0.0021  | 0.0496| -0.3911|
| HESX1      | 3.3577     | 7.3091 | 11.3742 | 0.0021  | 0.0496| -0.3995|
| ABHD16A    | 1.2973     | 9.4706 | 11.3154 | 0.0021  | 0.0496| -0.4157|
| NBN        | 4.5922     | 10.9019 | 11.2639 | 0.0021  | 0.0497| -0.4300|
| RGL4       | 3.2771     | 8.8751 | 11.2201 | 0.0022  | 0.0498| -0.4423|
| U2AF2      | 1.3388     | 10.9096 | 11.2121 | 0.0022  | 0.0498| -0.4445|
| PAK2       | 1.4921     | 10.7463 | 11.1938 | 0.0022  | 0.0498| -0.4496|
Supplemental Table 2: limma score performed on immune score cell subpopulations of COVID-19 lung biopsy as compared to healthy donor ones, related to figure 3

| cell populations         | logFC  | AveExpr | t         | P.Value | adj.P.Val | B      |
|--------------------------|--------|---------|-----------|---------|-----------|--------|
| Neutrophils              | 0.1488 | 0.0744  | 41.9348   | 0.0002  | 0.0121    | 1.1275 |
| Monocytes                | 0.2360 | 0.1180  | 24.2226   | 0.0007  | 0.0223    | -0.7054|
| Osteoblast               | -0.1505| 0.0865  | -13.2767  | 0.0028  | 0.0475    | -2.7148|
| aDC                      | 0.1128 | 0.0599  | 13.1170   | 0.0028  | 0.0475    | -2.7550|
| ImmuneScore              | 0.2760 | 0.1617  | 9.7578    | 0.0057  | 0.0761    | -3.7348|
| DC                       | 0.0064 | 0.0032  | 8.1319    | 0.0087  | 0.0969    | -4.3321|
| Macrophages              | 0.0378 | 0.0213  | 6.9778    | 0.0123  | 0.1181    | -4.8279|
| MicroenvironmentScore    | 0.2182 | 0.2119  | 5.2521    | 0.0235  | 0.1966    | -5.7282|
| MacrophagesM1            | 0.0243 | 0.0146  | 4.1635    | 0.0391  | 0.2911    | -6.4352|
| Pericytes                | -0.1096| 0.0603  | -2.7570   | 0.0912  | 0.6108    | -7.5848|
| Melanocytes              | -0.0004| 0.0002  | -2.0301   | 0.1588  | 0.7562    | -8.3090|
| mv Endothelial cells     | -0.0837| 0.0491  | -1.7636   | 0.1994  | 0.7562    | -8.5945|
| MSC                      | -0.0613| 0.0307  | -1.5870   | 0.2336  | 0.7562    | -8.7872|
| Endothelial cells        | -0.0548| 0.0383  | -1.4402   | 0.2675  | 0.7562    | -8.9479|
| Plasma cells             | 0.0209 | 0.0152  | 1.3946    | 0.2791  | 0.7562    | -8.9976|
| Neurons                  | -0.0016| 0.0012  | -1.2885   | 0.3086  | 0.7562    | -9.1127|
| Smooth muscle            | 0.0283 | 0.0142  | 1.0903    | 0.3736  | 0.7562    | -9.3223|
| CD8+ T-cells             | -0.0193| 0.0097  | -1.0903   | 0.3736  | 0.7562    | -9.3224|
| CD4+ T-cells             | -0.0133| 0.0067  | -1.0903   | 0.3736  | 0.7562    | -9.3224|
| CD4+ Tcm                 | -0.0112| 0.0056  | -1.0902   | 0.3737  | 0.7562    | -9.3225|
| Basophils                | 0.0080 | 0.0040  | 1.0900    | 0.3737  | 0.7562    | -9.3227|
| Megakaryocytes           | -0.0061| 0.0030  | -1.0898   | 0.3738  | 0.7562    | -9.3229|
| MacrophagesM2            | -0.0047| 0.0024  | -1.0895   | 0.3739  | 0.7562    | -9.3233|
| naive B-cells            | -0.0033| 0.0017  | -1.0885   | 0.3743  | 0.7562    | -9.3242|
| Tgd cells                | 0.0027 | 0.0014  | 1.0877    | 0.3746  | 0.7562    | -9.3251|
| Keratinocytes            | -0.0022| 0.0011  | -1.0863   | 0.3751  | 0.7562    | -9.3265|
| CD4+ memory T-cells      | -0.0022| 0.0011  | -1.0860   | 0.3752  | 0.7562    | -9.3269|
| CD8+ Tem                 | -0.0016| 0.0008  | -1.0821   | 0.3766  | 0.7562    | -9.3308|
| Adipocytes               | 0.0015 | 0.0007  | 1.0812    | 0.3770  | 0.7562    | -9.3318|
| B-cells                  | 0.0114 | 0.0059  | 1.0679    | 0.3819  | 0.7562    | -9.3455|
| Mesangial cells          | 0.0009 | 0.0005  | 1.0677    | 0.3819  | 0.7562    | -9.3456|
| Hepatocytes              | 0.0007 | 0.0004  | 1.0540    | 0.3871  | 0.7562    | -9.3597|
| CD8+ naive T-cells       | -0.0187| 0.0172  | -1.0419   | 0.3916  | 0.7562    | -9.3720|
| Memory B-cells           | 0.0006 | 0.0003  | 1.0350    | 0.3943  | 0.7562    | -9.3790|
| Mast cells               | 0.0063 | 0.0035  | 1.0331    | 0.3950  | 0.7562    | -9.3809|
| Th2 cells                | -0.0206| 0.0120  | -1.0030   | 0.4068  | 0.7571    | -9.4112|
| Stromascore              | -0.0578| 0.0502  | -0.9484   | 0.4291  | 0.7770    | -9.4653|
| Ly endothelial cells     | -0.0113| 0.0089  | -0.8289   | 0.4821  | 0.8295    | -9.5784|
| Cell Type                | EG001 | EG002 | EG004 | EG005 | EG006 | EG007 |
|-------------------------|-------|-------|-------|-------|-------|-------|
| Chondrocytes            | -0.0177 | 0.0142 | -0.8273 | 0.4828 | 0.8295 | -9.5799 |
| iDC                     | 0.0420 | 0.0531 | 0.7703 | 0.5103 | 0.8442 | -9.6308 |
| Myocytes                | -0.0098 | 0.0088 | -0.7577 | 0.5166 | 0.8442 | -9.6418 |
| Fibroblasts             | -0.0622 | 0.0614 | -0.7328 | 0.5292 | 0.8442 | -9.6632 |
| Platelets               | -0.0002 | 0.0001 | -0.6848 | 0.5542 | 0.8583 | -9.7031 |
| Epithelial cells        | -0.0144 | 0.0157 | -0.6670 | 0.5637 | 0.8583 | -9.7174 |
| Sebocytes               | -0.0044 | 0.0053 | -0.6383 | 0.5793 | 0.8625 | -9.7400 |
| HSC                     | -0.1026 | 0.1514 | -0.5509 | 0.6291 | 0.8848 | -9.8042 |
| CD8+ Tcm                | 0.0032 | 0.0079 | 0.5413 | 0.6348 | 0.8848 | -9.8108 |
| Tregs                   | -0.0114 | 0.0172 | -0.5206 | 0.6471 | 0.8848 | -9.8248 |
| CLP                     | -0.0023 | 0.0036 | -0.5048 | 0.6567 | 0.8848 | -9.8352 |
| GMP                     | 0.0198 | 0.0289 | 0.4988 | 0.6603 | 0.8848 | -9.8390 |
| Th1 cells               | -0.0026 | 0.0091 | -0.3123 | 0.7801 | 1.0000 | -9.9392 |
| pDC                     | 0.0001 | 0.0003 | 0.2239 | 0.8406 | 1.0000 | -9.9719 |
| Class-switched memory B-cells | 0.0002 | 0.0009 | 0.2108 | 0.8498 | 1.0000 | -9.9758 |
| MEP                     | 0.0003 | 0.0027 | 0.0719 | 0.9483 | 1.0000 | -10.0034 |
| pro B-cells             | 0.0000 | 0.0000 | -0.0107 | 0.9923 | 1.0000 | -10.0070 |
| Astrocytes              | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| CD4+ naive T-cells      | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| CD4+ Tem                | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| cDC                     | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| CMP                     | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| Eosinophils             | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| Erythrocytes            | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| MPP                     | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| NK cells                | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| NK T                    | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| Preadipocytes           | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |
| Skeletal muscle         | 0.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | -10.0071 |

**Supplemental Table 3:** list of Gene expression omnibus (GEO) transcriptome samples included to performed integrative immune deconvolution of COVID-19 lung biopsy, related to figure 3: DOI: [http://dx.doi.org/10.17632/3xnypzvcf7.1#file-39267130-458c-41d8-8bf7-26ae18b717d1](http://dx.doi.org/10.17632/3xnypzvcf7.1#file-39267130-458c-41d8-8bf7-26ae18b717d1)

**Supplemental Table 4:** PPARγ integrative repressed program in COVID-19 lung transcriptome, related to the figure 8: DOI: [http://dx.doi.org/10.17632/3xnypzvcf7.1#file-03b71cecf317-439c-9176-170edd6ac7e0](http://dx.doi.org/10.17632/3xnypzvcf7.1#file-03b71cecf317-439c-9176-170edd6ac7e0)

**Supplemental Table 5:** Human and SARS virus interactome connections in VirusHostNet database, related to figure 8: DOI: [http://dx.doi.org/10.17632/3xnypzvcf7.1#file-71c195a0-6834-4a0a-8719-826bae172b31](http://dx.doi.org/10.17632/3xnypzvcf7.1#file-71c195a0-6834-4a0a-8719-826bae172b31)