Two distinct immunopathological profiles in lungs of lethal COVID-19

Short title: Profiles of COVID-19 lungs

Ronny Nienhold1§, Yari Ciani2§, Viktor H. Koelzer3,4§, Alexandar Tzankov5, Jasmin D. Haslbauer5, Thomas Menter5, Nathalie Schwab1, Maurice Henkel1, Angela Frank1, Veronika Zsikla1, Niels Willi1, Werner Kempf6, Thomas Hoyler7, Mattia Barbareschi8, Holger Moch3, Markus Tolnay5, Gieri Cathomas1, Francesca Demichelis2,9*, Tobias Junt7*, Kirsten D. Mertz1*

Affiliations:

1 Institute of Pathology, Cantonal Hospital Baselland, Liestal, Switzerland
2 Laboratory of Computational and Functional Oncology, Department for Cellular, Computational and Integrative Biology – CIBIO, University of Trento, Trento, Italy
3 Department of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, Switzerland
4 Department of Oncology and Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom
5 Pathology, Institute of Medical Genetics and Pathology, University Hospital Basel, Basel, Switzerland
6 Kempf und Pfaltz Histologische Diagnostik, Zurich, Switzerland
7 Novartis Institutes for BioMedical Research (NIBR), Basel, Switzerland
8 Anatomia ed Istologia Patologica, Ospedale S. Chiara di Trento, Trento, Italy
9 Caryl and Israel Englander Institute for Precision Medicine, Institute for Computational Biomedicine, New York Presbyterian Hospital, Weill Cornell Medicine, New York, New York, USA

§ These authors jointly contributed to this work.
* These authors jointly directed this work.

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Correspondence:

PD Dr. med. Kirsten D. Mertz, MD PhD
Institute of Pathology Liestal
Cantonal Hospital Baselland
Mühlemattstrasse 11
CH-4410 Liestal, Switzerland
Phone: + 41 (0) 61 925 2635
Email: kirsten.mertz@ksbl.ch
Abstract

Immune responses in lungs of Coronavirus Disease 2019 (COVID-19) are poorly characterized. We conducted transcriptomic, histologic and cellular profiling of post mortem COVID-19 and normal lung tissues. Two distinct immunopathological reaction patterns were identified. One pattern showed high expression of interferon stimulated genes (ISGs) and cytokines, high viral loads and limited pulmonary damage, the other pattern showed severely damaged lungs, low ISGs, low viral loads and abundant immune infiltrates. Distinct patterns of pulmonary COVID-19 immune responses correlated to hospitalization time and may guide treatment and vaccination approaches.

COVID-19 is a pandemic respiratory disease with 2-3% lethality\(^1\). While a coronavirus (SARS-CoV-2) is the causative agent, the antiviral immune response- may also contribute to devastating end organ damage\(^2\). Therefore analysis of post mortem tissues is critical for the mechanistic understanding of COVID-19 pathophysiology\(^3\).

Here we analyzed 34 post mortem lung samples from 16 deceased COVID-19 patients and 9 post mortem lung samples from 6 patients who died from non-infectious causes (Extended Data Table 1). Of note, we did not find bacterial superinfections in COVID-19 lungs except in three samples (Extended Data Figure 1). Differential gene expression analysis based on a commercial assay (OIRRA) designed for quantification of immune cell and inflammatory transcripts (Extended Data Table 2) identified 68 up-regulated and 30 down-regulated genes in COVID-19 infected lungs compared to normal tissue (Extended Data Table 3). Using a consensus of 30 different indices\(^4\) we identified by K-means clustering three groups of samples defined by the expression levels of the deregulated genes (Figure 1a, Extended Data Figure 2). Clusters 1 (50% of samples) and 2 (41%) contained COVID-19 samples and cluster 3 contained all normal lung samples and 3 COVID-19 samples (9%). To understand why the majority of COVID-19 lung tissues segregated into defined clusters 1 or 2, we undertook a gene ontology analysis. We identified interferon stimulated genes (ISGs) as a key upregulated pathway in COVID-19 autopsy lungs (Figure 1b), which was differentially represented in cluster 1 and 2, respectively (Figure 1c). Identification of an ISG high cluster (Cluster 1, ISG\(^{\text{high}}\)) was surprising, since SARS-CoV-2 was proposed to lead to limited ISG induction, yet only in comparison to other respiratory viruses\(^5\). Next, we analyzed frequencies of specific immune cells in lungs by computational image analysis. T cells (CD3\(^+\)), particularly CD8\(^+\) T cells, and macrophages (CD68\(^+\), CD163\(^+\)) were selectively enriched in samples from the ISG\(^{\text{low}}\) cluster 2 (Figure 1d), as were CD4\(^+\) T cells, while CD20\(^+\) B cells and CD123\(^+\) plasmacytoid dendritic cells (pDCs) did not show differential frequencies between ISG\(^{\text{high}}\) and ISG\(^{\text{low}}\) lungs (Extended Data Figure 3a,b). Since T cell exhaustion was observed in COVID-19\(^6\), we compared frequencies of CD8+PD1+ cells in ISG\(^{\text{high}}\) and
ISG^{low} samples, and observed a higher frequency of CD8+PD1+ T-cells in the ISG^{low} subgroup, potentially indicative of advanced disease (Extended Data Figure 3a,b). Histological analysis of COVID-19 lung tissues revealed striking pulmonary damage exclusively in ISG^{low} samples, with distinct peri-alveolar foci of infiltrating CD68+ macrophages and CD8+ T cells (Figure 1e). Expression of ISGs was tightly correlated with pulmonary viral load (Figure 2a), and immunohistochemical staining showed SARS-CoV-2 nucleocapsid protein mostly in pneumocytes of ISG^{high} lungs (Figure 2b). Since a cytokine storm has been proposed to cause adverse outcome of COVID-19\(^7\), and cytokines were highly expressed in bronchoalveolar lavages (BALs) of COVID-19 patients\(^8\), we investigated expression of a pro-inflammatory cytokine signature (TNF, IL6, IL1b, CCL2, IFNA17, IFNB1, CXCL9, CXCL10, CXCL11) in lung samples from lethal COVID-19. The proinflammatory gene signature was significantly enriched in the ISG^{high} subset (Figure 2c), but was not associated with alveolar hemorrhage (Figure 2d). Within this cytokine signature, co-regulated subgroups (IL1B/IL6/TNF, IFNB1/IFNA17, CCL2/CXCL9/CXCL19/CXCL11) were identified (Figure 2e). Importantly, only the CXCL9/10/11 sub-signature was positively associated with alveolar hemorrhage (Figure 2f, Extended Data Figure 4). This is in line with observations that these chemokines compromise endothelial integrity via CXCR3\(^9\), and that CXCL10 is a key determinant of severe COVID-19\(^10\). Interestingly, basal levels of CXCL9 or CXCL10 are elevated in elderly, hypertensive and obese individuals, which were strongly represented in our autopsy cohort and are predisposed to severe COVID-19\(^11,12\). Of note, our study could not take extrapulmonary cytokine sources or effects into account.

Since none of the above pulmonary cytokine sub-signatures was positively associated with diffuse alveolar damage (DAD, Extended Data Figure 5), we investigated which other local immune signature showed this association. We found a strong association of DAD with low expression of ISGs (Figure 2g), and an activated CD8+ T cell signature (CD38, GZMA, GZMB, CCR5, Figure 2h), yet not with pulmonary CD8+ T-cell infiltration (Figure 2i). In addition, the activated CD8+ signature was inversely correlated to viral counts, particularly in ISG^{low} cases (Figure 2j). Therefore we speculate that activated CD8+ T cells are essential for virus elimination, similar to murine models of coronavirus infection\(^13\), yet it is possible that they contribute to pulmonary damage as well. Of note, ISG^{low} samples also expressed elevated p53 and Ki67 (Figure 2k), i.e. reactive markers of DAD which indicate lung remodeling\(^14\). Since the ISG^{low} pattern showed lower viral counts, higher accumulation and activation of CD8+ T cells in tissues and accrual of pulmonary damage and remodeling, the ISG^{low} phase may follow an earlier ISG^{high} phase during the course of infection. This was supported by significantly longer hospitalization in COVID-19 patients from whom ISG^{low} lung samples were obtained (Figure 2l). This is in line with epidemiologic data showing that one defined group of critical COVID-19 patients quickly succumbs to disease after hospitalization, while others die after longer intensive care\(^15\).

Discovery of two patterns of immunopathology in COVID19 post mortem lungs suggests that only patients with an ISG^{high} pattern show high viral loads and may benefit from antivirals. ISG^{low} patients
instead show low viral loads yet strong complement activation in lungs (Figure 2m) and thus may potentially benefit from complement inhibition\textsuperscript{16}. In addition, the ISG\textsuperscript{low} pattern suggests that CD8\textsuperscript{+} T cells are involved in antiviral protection and should be considered for vaccination efforts.
1. Hopkins, J. Corona virus resource center. Latest update: 05/22/2020: https://coronavirus.jhu.edu/data (2020).
2. Chen, G., et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. The Journal of clinical investigation 130(2020).
3. Menter, T., et al. Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction. Histopathology (2020).
4. Charrad, M., Ghazzali, N., Boiteau, V. & Niknafs, A. NbClust Package: finding the relevant number of clusters in a dataset. J. Stat. Softw (2012).
5. Blanco-Melo, D., et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell (2020).
6. Diao, B., et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). Frontiers in Immunology 11, 827 (2020).
7. Huang, C., et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet 395, 497-506 (2020).
8. Liao, M., et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nature Medicine, 1-3 (2020).
9. Bodnar, R.J., Yates, C.C., Rodgers, M.E., Du, X. & Wells, A. IP-10 induces dissociation of newly formed blood vessels. Journal of cell science 122, 2064-2077 (2009).
10. Yang, Y., et al. Exuberant elevation of IP-10. MCP-3 and IL-1ra during SARS-CoV-2 infection is associated with disease severity and fatal outcome. medRxiv 2002, 2020 (2020).
11. Hueso, L., et al. Upregulation of angiostatic chemokines IP-10/CXCL10 and I-TAC/CXCL11 in human obesity and their implication for adipose tissue angiogenesis. Int J Obes (Lond) 42, 1406-1417 (2018).
12. Bonfante, H.L., et al. CCL2, CXCL8, CXCL9 and CXCL10 serum levels increase with age but are not altered by treatment with hydroxychloroquine in patients with osteoarthritis of the knees. Int J Rheum Dis 20, 1958-1964 (2017).
13. Cupovic, J., et al. Central Nervous System Stromal Cells Control Local CD8(+) T Cell Responses during Virus-Induced Neuroinflammation. Immunity 44, 622-633 (2016).
14. Shetty, S.K., et al. p53 and miR-34a Feedback Promotes Lung Epithelial Injury and Pulmonary Fibrosis. Am J Pathol 187, 1016-1034 (2017).
15. Salje, H., et al. Estimating the burden of SARS-CoV-2 in France. Science (2020).
16. Magro, C., et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res (2020).
Methods

Ethics statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. Ethics approval was obtained from the Ethics Committee of Northwestern and Central Switzerland (Project-ID 2020-00629). For all patients, either personal and/or family consent was obtained for autopsy and sample collection.

Patients and sample collection

The study is based on the analysis of 16 out of 21 consecutive COVID-19 autopsies performed between March 9th and April 14th 2020 at the Institute of Pathology Liestal and Institute of Medical Genetics and Pathology Basel, Switzerland. Clinical features including symptoms, course of disease, comorbidities, laboratory results and therapy are listed in Extended Data Table 1a. Detailed autopsy findings for each patient were recently published, and the identifiers (with the prefix “C”) for each COVID-19 patient are consistent with the description of this Swiss COVID-19 autopsy cohort3. In this study, we analysed formalin fixed and paraffin embedded (FFPE) lung tissue of distinct areas of the lungs of 16 of these COVID-19 patients. All 16 COVID-19 patients had positive nasopharyngeal swabs collected while alive. In all COVID-19 patients, diagnosis was confirmed by detection of SARS-CoV-2 in postmortal lung tissues. 5/16 patients were additionally tested by postmortal nasopharyngeal swabs which were positive for SARS-CoV-2 in all 5 cases.

As a control cohort, we selected 6 autopsies performed between January 2019 and March 2020 at the Institute of Pathology Liestal (“normal” patients N1 – N6). These control patients died of other, non-infectious causes and had a similar age, gender and cardiovascular risk profile. Patients with infections were excluded from this control cohort. Another control cohort consisted of 4 autopsies of patients suffering from various infections mainly with bacteria affecting the lung (patients with lung pathology, P1 – P4). Details for both control cohorts are listed in Extended Data Table 1b,c. SARS-CoV-2 was ruled out for each control patient by PCR-examination of lung tissue samples.

Nucleic acid extraction

RNA was extracted from up to six sections of FFPE tissue blocks using RecoverAll Total Nucleic Acid Isolation Kit (Cat No. AM1975, Thermo Fisher Scientific, Waltham, MA, USA). Extraction of DNA from up to 10 sections of FFPE tissue samples was automated by EZ1 Advanced XL (Qiagen, Hilden,
Germany) using the EZ1 DNA Tissue Kit (Cat No. 953034, Qiagen, Hilden, Germany). Concentration of DNA and RNA were measured with Qubit 2.0 Fluorometer and Qubit dsDNA HS Assay or Qubit RNA HS Assay (Cat No. Q33230 & Q32852, Thermo Fisher Scientific, Waltham, MA, USA), respectively.

Quantification of SARS-CoV-2 in FFPE tissue samples

Post mortem viral load was individually measured in all lung tissue blocks from all patients included in this study. SARS-CoV-2 was detected in 15ng of human total RNA using the TaqMan 2019-nCoV Assay Kit v1 (Cat No. A47532, Thermo Fisher Scientific, Waltham, MA, USA), which targets three genomic regions (ORFab1, S Protein, N Protein) specific for SARS-CoV-2 and the human RNase P gene (RPPH1). The copy numbers of the SARS-CoV-2 viral genome was determined by utilizing the TaqMan 2019-nCoV Control Kit v1 (Cat No. A47533, Thermo Fisher Scientific, Waltham, MA, USA) and a comparative "ΔΔCт" method. The control kit contains a synthetic sample with a defined amount of target molecules for the human RPPH1 and the three SARS-CoV-2 assays, and was re-analyzed in parallel with patient samples. For each patient sample, this method resulted in individual copy numbers of the human RPPH1 and the three SARS-CoV-2 targets. Finally, the mean copy number of the SARS-CoV-2 targets was normalized to 1 x 10^6 RPPH1 transcripts.

Profiling of immune response by targeted RNAseq

The expression levels of 398 genes, including genes relevant in innate and adaptive immune response and housekeeping genes for normalization, were analyzed with the Oncomine Immune Response Research Assay (OIRRA, Cat No. A32881, Thermo Fisher Scientific, Waltham, MA, USA). The OIRRA is a targeted gene expression assay designed for the Ion™ next-generation sequencing (NGS) platform. This gene expression assay was originally designed to interrogate the tumor microenvironment to enable mechanistic studies and identification of predictive biomarkers for immunotherapy in cancer. The assay is optimized to measure the expression of genes involved in immune cell interactions and signaling, including genes expressed at low levels and involved in inflammatory signaling. The 398 genes covered by this assay are listed in Extended Data Table 2.

The NGS libraries were prepared as recommended by the supplier. In brief, 30ng of total RNA were used for reverse transcription (SuperScript VILO, Cat No. 11754250, Thermo Fisher Scientific, Waltham, MA, USA) and subsequent library preparation. The libraries were quantified (Ion Library TaqMan Quantitation Kit, Cat No. 4468802, Thermo Fisher Scientific, Waltham, MA, USA), equimolarly pooled and sequenced utilizing the Ion GeneStudio S5xl (Thermo Fisher Scientific,
Detection of co-infections by whole genome sequencing

To identify potential pathogens accompanying an infection with SARS-CoV-2, we analyzed the DNA of the same tissue samples used for detection and profiling of the SARS-CoV-2-specific immune response. First, 250ng of genomic DNA was enzymatically sheared (15 minutes at 37°C) and barcoded using the Ion Xpress Plus Fragment Library Kit (Cat No. 4471269, Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the libraries were quantified (Ion Library TaqMan Quantitation Kit, Cat No. 4468802, Thermo Fisher Scientific, Waltham, MA, USA) and up to three libraries were pooled at equimolar levels for analysis with Ion GeneStudio S5xl (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing data for each sample was analysed using the CLC genomics workbench (version 20.0.3, Qiagen, Hilden, Germany) in combination with the microbial genomics module (version 20.0.1, Qiagen, Hilden, Germany): The raw reads were trimmed by quality (Mott algorithm with limit 0.05 and a maximum of 2 ambiguous bases per read) and mapped to the human genome (GRCh37 hg19, match score: 1, mismatch cost: 2, indel opening cost: 6, indel extension cost: 1). Unmapped reads were analysed by taxonomic profiling to identify reads of viral or bacterial origin. The profiling utilized an index of 11'540 viral genomes with a minimum length of 1'000 bp and 2'715 bacterial reference genomes with a minimum length of 500'000 bp, retrieved from the NCBI Reference Sequence Database (date of download: 2020-04-02).

Immunohistochemistry

Immunohistochemical analyses for CD3, CD4, CD8, CD15, CD20, CD68, CD123, CD163, PD-1, MPO, p53, Ki67, C3d and C5b-9 were performed on all lung tissue blocks used in this study. Antibodies, staining protocols and conditions are detailed in Extended Data Table 4.

Qualitative and semiquantitative assessment of histopathological lung damage and neutrophilic infiltration

Hematoxylin and eosin (H&E) and Elastica van Gieson (EvG) stained sections of all lung tissues used in this study were independently evaluated by two experienced and board certified pathologists (VZ and KDM) (Extended Data Table 5). Both pathologists evaluated the presence of diffuse alveolar damage (DAD), and if present, its stage, intra-alveolar edema and hemorrhage. In addition, both
pathologists evaluated the severity of histopathological changes in COVID-19 lungs (1 = mild / discrete alterations, 2 = moderate, 3 = severe changes) based on resemblance between normal and pathologically altered lung tissues. Parameters that were taken into account included reduction of alveolar air-filled spaces, typical histologic features of DAD with hyaline membrane formation, infiltration of lymphocytes, monocytes and neutrophils into interstitial and alveolar spaces, type 2 pneumocyte hyperplasia, desquamation of pneumocytes, histologic features of organizing pneumonia including intra-alveolar fibrin deposition and fibrosis (acute fibrinous and organizing pneumonia, AFOP)\textsuperscript{17,18}. The number of neutrophils per lung tissue section was estimated on H&E stained sections and by immunohistochemical stains for CD15 and MPO using a three tiered system (1 = few or no neutrophils, 2 = moderate number of neutrophils, 3 = high number of neutrophils). Assessment of the two pathologists was concordant in the vast majority of cases. Discrepant cases were reviewed by a third pathologist (NW) to reach consent.

Digital image analysis

Slides were digitalized on a 3DHistech™ P1000 slide scanner at 400x magnification (3DHISTECH Ltd. Budapest, Hungary). Digital slide review and quality control was performed by a board-certified pathologist (VHK). Tissue regions with staining artefacts, folds or other technical artefacts were excluded from analysis. A deep neural network (DNN) algorithm (Simoyan and Zisserman VGG, HALO AI™ on HALO™ 3.0.311.167, Indica Labs, Corrales, NM) was trained using pathologist annotations to automatically localize and measure the area of each lung tissue sample on the digital slides. Background regions and glass were excluded from analysis. Mark-up images for tissue classification were generated and classification accuracy was confirmed through pathology review. For cell-level analysis, color deconvolution for DAB, AP and hematoxylin channels was performed and nuclear segmentation was optimized using cell-morphometric parameters. Marker-positive cells in stromal and epithelial regions were quantified. For CD3, CD4, CD8, CD20, CD68, CD123, CD163 and PD1, staining detection was optimized for the cytoplasmic / membranous compartment and marker expression was measured on a continuous scale at single cell resolution. For assessment of CD8/PD1 double stains, color deconvolution was optimized for separation of DAB (PD1) and AP (CD8) staining products. Internal controls (non-immune cells) and external controls (tonsil) were used to calibrate the detection limits and cross-validated by visual review. For each tissue sample, the total area of lung tissue in mm\(^2\), the absolute number of marker-positive cells, cell morphometric parameters and staining intensity were recorded.
Identification of SARS-CoV-2 immune response pattern

**Gene expression analysis**

Samples were included in the study based on quality of libraries and alignment performance. Applied inclusion criteria are: >1 million of mapped reads, good concentration of libraries, average read length >100bp, > 300 target genes with more than 10 reads. One sample with > 1 Mio reads was excluded from the study because of shorter read length and a low library concentration. Notably this sample had the longest time between death and autopsy (72h) before analysis. Differential expression analysis was performed using the edgeR package comparing normal lung samples, COVID-19 samples and samples from patients with other infections. Genes were selected for downstream analyses by fdr <0.05 and |logFC| >1 for clustering analysis. Clustering analysis was performed using k-means algorithm and complete linkage. Ideal number of clusters (n=3) was chosen based on 30 different algorithms and the final clustering derives from the consensus of 2000 iterations. Expression of gene signatures was calculated as median of log2(cpm + 1) of selected genes.

**Functional enrichment analysis**

Biological processes enrichment was performed using the enrichGO function of the package clusterProfiler setting all the genes included in the assay as universe.

**Statistical analysis**

All the analyses and graphical representations were performed using the R statistical environment software and the following packages: ggplot2, circlize, ComplexHeatmap, ggfortify, reshape2 and factoextra. Correlation between transcripts and viral counts was performed using Pearson’s correlation. Association between continuous and categorical data were tested using Wilcoxon rank sum test.

Box-plots elements indicate the median (center line), upper and lower quartiles (box limits) and show all the data points. Whiskers extend to the most extreme value included in 1.5x interquartile range.
Figure Legends

Figure 1. Transcriptomic and immunologic profile of COVID-19 autopsy lungs.
(a) Heatmap showing K-means clustering of COVID-19 and normal lung samples based on expression levels of deregulated genes in COVID-19 versus normal lungs. (b) Gene ontology enrichment analysis of genes upregulated in COVID-19 samples. (c) ISG signature expression in clusters 1 and 2 of COVID-19 lungs. (d) Frequencies of immune cells on ISG<sup>high</sup> and ISG<sup>low</sup> COVID-19 lung sections and controls. (e) Representative H&E stains and immunohistochemistry (CD3, CD8, CD68, CD163) of ISG<sup>high</sup> and ISG<sup>low</sup> COVID-19 lungs and controls, size bar 500 μm.

Figure 2. Relationship between cytokine signature and histopathological changes.
(a) Correlation of viral load and ISG expression in COVID-19 lungs. Solid lines, sample data from the same patient. Dotted line, regression for all samples and 95% CI (Pearson’s correlation=0.83, adjusted R-squared=0.68, p-value=1.66e-08). (b) Representative immunohistochemistry for SARS-CoV-2 on ISG<sup>high</sup> and ISG<sup>low</sup> COVID-19 lung samples and controls. Size bar 100 μm. (c) Expression of a cytokine signature (IL-1b, IL6, TNF, IFNa17, IFNb1, CCL2, CXCL9, CXCL10, CXCL11) in ISG<sup>high</sup> and ISG<sup>low</sup> COVID-19 lung samples. (d) Association of the cytokine signature with intraalveolar hemorrhage. (e) Pearson’s correlation of pro-inflammatory cytokines in the cytokine signature indicates presence of co-regulated cytokines. (f) Association of CXCL9/10/11 expression in lung samples with intraalveolar hemorrhage. Association of DAD stage with (g) ISG expression, (h) activated CD8+ T cell signature, (i) CD8 T cell counts. (j) Inverse correlation of viral load and activated CD8+ T cell signature. Solid line, sample data from the same patient. Dotted line, regression for all the samples and 95% CI (Pearson’s correlation=-0.5 adjusted R-squared=0.22, p-value=0.005). (k) Representative immunohistochemistry for p53 and Ki67. Size bar 100 μm. (l) Hospitalization time in ISG<sup>low</sup> patients versus ISG<sup>high</sup> patients. (m) Representative IHC staining for complement activation products C5b-9 and C3d in ISG<sup>high</sup>, ISG<sup>low</sup> COVID-19 and normal control lungs. Size bar 100 μm. ISG<sup>high</sup> samples, red; ISG<sup>low</sup> samples, blue.

Extended Data Figure 1. Co-infections in COVID-19 lungs identified by WGS metagenomics.
(a) Total number of reads generated for each sample. (b) Percentage of reads and (c) absolute numbers of reads not mapping to the human genome (GRCh37 hg19). (d) Bacterial and (e) viral co-infections across lung samples, WGS metagenomic analysis. Purple dots, numbers of reads sufficient for identification of non-human species. Samples are ordered by increasing SARS-CoV-2 viral load in each group. Stacked bars, relative abundance of the most common species. Grey bars represent frequent species, colored bars show pathogenic species. *One COVID-19 patient (C3) clustered in the normal control group.
Extended Data Figure 2: Transcriptomics of COVID-19 and non-COVID-19 lung samples.
(a) Heatmap showing K-means clustering of COVID-19 and normal lung samples; version of Figure 1a with annotated gene names. (b) Comparison of up- and down-regulated genes in lung samples from COVID-19 patients, normal lung samples and other bacterial / viral pneumonia samples (Extended Data Figure 1). (c) Principal component analysis (PCA) of all lung samples reveals segregation based on diagnosis and viral load.

Extended Data Figure 3. Immune cell infiltrate on COVID-19 lung sections.
(a) Frequencies of immune cells on ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung sections and controls. (b) Representative immunohistochemistry (CD4, CD20, CD123, CD8/PD1) of ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lungs and controls, size bar 500 μm.

Extended Data Figure 4. Association of cytokine signatures in ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung samples with intraalveolar hemorrhage.
Association of: (a) Median IL6, TNF, IL1B expression in ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung tissue with presence of intraalveolar hemorrhage. (b) Median IFNA17, IFNB1 expression in ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung tissue with presence of intraalveolar hemorrhage. (c) Median CCL2 expression in ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung tissue with presence of intraalveolar hemorrhage.

Extended Data Figure 5. Association of cytokine signatures in ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung samples with DAD stage.
Association of: (a) Median IL6, TNF, IL1B expression in ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung tissue with DAD stage. (b) Median IFNA17, IFNB1 expression in ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung tissue with DAD stage. (c) Median CXCL9/10/11 expression in ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung tissue with DAD stage. (d) Median CCL2 expression in ISG\textsuperscript{high} and ISG\textsuperscript{low} COVID-19 lung tissue with DAD stage.
Data availability

The datasets generated and analysed during this study can be accessed in GEO (GSE151764) and are available from the corresponding author upon request.

References

17. Copin, M.-C., Parmentier, E., Duburcq, T., Poissy, J. & Mathieu, D. Time to consider histologic pattern of lung injury to treat critically ill patients with COVID-19 infection. Intensive Care Medicine, 1-3 (2020).
18. Xu, Z., et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 8, 420-422 (2020).
19. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823 (2014).
20. Chikina, M., Robinson, J.D. & Clark, N.L. Hundreds of genes experienced convergent shifts in selective pressure in marine mammals. Molecular biology and evolution 33, 2182-2192 (2016).
21. Team, R.C. R: A language and environment for statistical computing. (2013).
22. Wickham, H. ggplot2: elegant graphics for data analysis, (Springer, 2016).
23. Gu, Z., Gu, L., Eils, R., Schlesner, M. & Brors, B. circlize implements and enhances circular visualization in R. Bioinformatics 30, 2811-2812 (2014).
24. Gu, Z., Eils, R. & Schlesner, M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics 32, 2847-2849 (2016).
25. Horikoshi, M. & Tang, Y. ggfortify: Data visualization tools for statistical analysis results. v0.1.0. URL http://CRAN.R-project.org/package=ggfortify. R package version 0.4.1, 28 (2018).
26. Wickham, H. Reshaping data with the reshape package. Journal of statistical software 21, 1-20 (2007).
27. Kassambara, A. & Mundt, F. Package ‘factoextra’. Extract and visualize the results of multivariate data analyses 76(2017).

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Author contributions

RN, YC, VHK, FD, TJ and KDM jointly conceived the study, performed data interpretation and prepared the manuscript. AT, MB, HM, MT and CA provided intellectual input, provided critical resources and critically reviewed the manuscript. RN, YC, VHK, MH, TH and TJ performed bioinformatic and statistical analysis. AT, JDH, TM, NS, AF collected autopsy specimens and patient data. VZ, NW, WK and CA performed histomorphological evaluation. All authors approved the final manuscript.

Competing interests

VHK has served as an invited speaker on behalf of Indica Labs. TH and TJ are employees of Novartis. The other authors declare no competing interests.
## Extended Data Table 1a. Clinical Data, COVID-19 cohort (16 patients)

| Characteristics                          | N or x | % or range          |
|------------------------------------------|--------|---------------------|
| Time between symptoms and death          | 7.4 d  | 0 - 20 d            |
| Hospitalisation                          | 5.6 d  | 0 - 13 d            |
| Postmortem interval                      | 28.4 h | 11 - 67 h           |
| Age                                      | 75 y   | 53 - 96 y           |
| Sex                                      | M 13 : F 3 |
| Comorbidities                            |        |                     |
| Hypertension                             | 16     | 100 %               |
| Cardiovascular disease                   | 11     | 68 %                |
| (Pre-)Adipositas                         | 12     | 75 %                |
| Diabetes                                 | 6      | 37.5 %              |
| Initial clinical presentation            |        |                     |
| Cough                                    | 13     | 81.25 %             |
| Fever                                    | 12     | 75 %                |
| Dyspnea                                  | 6      | 37.5 %              |
| Renal failure                            | 6      | 37.5 %              |
| Laboratory results                       |        |                     |
| Interleukin-6 (IL6)                      | 5774.72 ng/l | 159.00 - 35.152.00 ng/l |
| C-reactive protein (CRP)                 | 216.36 mg/l | 36.00 - 512.00 mg/l |
| Ferritin                                 | 18.037.71 µg/l | 1.025.00 - 228.225.00 µg/l |
| Procalcitonin (PCT)                      | 1.996.92 µg/l | 0.47 - 5.300.00 µg/l |
| Lactate dehydrogenase (LDH)              | 870.42 U/l | 256.00 - 5.267.00 U/l |
| Troponin T (cTnT)                        | 54.65 ng/l | 2.07 - 126.00 ng/l |
| Treatment                                |        |                     |
| Hydroxychloroquine                       | 10     | 62.5 %              |
| Lopinavir/Ritonavir                      | 5      | 31.25 %             |
| Antibiotics                              | 12     | 75 %                |
Listed laboratory results correspond to the highest (LDH, cTnT), latest (IL6, ferritin, PCT) or last value before administration of ACTEMRA/Tocilizumab (CRP).

Extended Data Table 1b. Clinical Data, Control cohort (6 patients)

| Characteristics   | N or x | % or range       |
|-------------------|--------|------------------|
| Postmortem interval | 31.4 h | 25 - 46 h        |
| Age               | 81.3 y | 63 - 104 y       |
| Sex               | M 5 : F 1 |

Extended Data Table 1c. Clinical Data, Cohort of patients with other infections (4 patients)

| Characteristics   | N or x | % or range       |
|-------------------|--------|------------------|
| Postmortem interval | 31.5 h | 12 - 79 h        |
| Age               | 79.5 y | 58 - 81 y        |
| Sex               | M 2 : F 2 |

Patients were suffering from other infections of the lung (bacterial or viral pneumonia). Detailed analysis of individual pathogens is shown in Extended Data Figure 1.
## Extended Data Table 2. OIRRA gene list

| Gene      | Gene      | Gene      | Gene      | Gene      | Gene      | Gene      
|-----------|-----------|-----------|-----------|-----------|-----------|-----------
| ABCF1     | CCL5      | CD40LG    | CTAG2     | FASLG     | HIF1A     | IFIT1     | IL3RA     |
| ADGRE5    | CCNB2     | CD44      | CTLA4     | FCER1G    | HLA-A     | IFIT2     | IL4       |
| ADORA2A   | CCR1      | CD47      | CTSS      | FCGR1A    | HLA-B     | IFIT3     | IL6       |
| AIF1      | CCR2      | CD48      | CX3CL1    | FCGR2B    | HLA-C     | IFITM1    | IL7       |
| AKT1      | CCR4      | CD52      | CX3CR1    | FCGR3A    | HLA-DMA   | IFITM2    | IL7R      |
| ALOX15B   | CCR5      | CD53      | CX3CR1    | FCGR3B    | HLA-DMB   | IFNA17    | IRF1      |
| ARG1      | CCR6      | CD6       | CX3CR1    | FCRLA     | HLA-DOA   | IFNB1     | IRF4      |
| AXL       | CCR7      | CD63      | CX3CR1    | FOXM1     | HLA-DOB   | IFNG      | IRF9      |
| B3GAT1    | CD14      | CD68      | CXCL1     | FOXO1     | HLA-DPA1  | IGF1R     | IRS1      |
| BAGE      | CD160     | CD69      | CXCL10    | FOXP3     | HLA-DPB1  | IGSF6     | ISG15     |
| BATF      | CD163     | CD70      | CXCL11    | FUT4      | HLA-DQA1  | IKZF1     | ISG20     |
| BCL2      | CD19      | CD74      | CXCL13    | FYB       | HLA-DQA2  | IKZF2     | ITGA1     |
| BCL2L11   | CD1C      | CD79A     | CXCL8     | G6PD      | HLA-DQB2  | IKZF3     | ITGAE     |
| BCL6      | CD1D      | CD79B     | CXCL9     | GADD45GIP1| HLA-DRA   | IKZF4     | ITGAL     |
| BRCA1     | CD2       | CD80      | CXCR2     | GAGE1     | HLA-DRB1  | IL10      | ITGAM     |
| BRCA2     | CD209     | CD83      | CXCR3     | GAGE10    | HLA-E     | IL10RA    | ITGAX     |
| BST2      | CD22      | CD86      | CXCR4     | GAGE12J   | HLA-F     | IL12A     | ITGB1     |
| BTLA      | CD226     | CD8A      | CXCR5     | GAGE13    | HLA-F-AS1 | IL12B     | ITGB2     |
| BUB1      | CD244     | CD8B      | CXCR6     | GAGE2C    | HLA-G     | IL13      | ITGB7     |
| C10orf54  | CD247     | CDK1      | CYBB      | GATA3     | HMBS      | IL15      | ITK       |
| C1QA      | CD27      | CDKN2A    | DDX58     | GBP1      | ICAM1     | IL17A     | JAML      |
| C1QB      | CD274     | CDKN3     | DGAT2     | GNLY      | ICOS      | IL17F     | JCHAIN    |
| CA4       | CD276     | CEACAM1   | DMBT1     | GPR18     | ICOSL1    | IL18      | KIAA0101  |
| CBLB      | CD28      | CEACAM8   | EBI3      | GRAP2     | ID2       | IL1A      | KIR2DL1   |
| CCL17     | CD33      | CIITA     | EFNA4     | GUSB      | ID3       | IL1B      | KIR2DL2   |
| CCL18     | CD37      | CLEC4C    | EGFR      | GZMA      | IDO1      | IL2       | KIR2DL3   |
| CCL2      | CD38      | CMKL1R1   | EGR2      | GZMB      | IDO2      | IL21      | KLF2      |
| CCL20     | CD3D      | CORO1A    | EGR3      | GZMH      | IFI27     | IL22      | KLRB1     |
| CCL21     | CD3E      | CRTAM     | EIF2AK2   | GZMK      | IFI35     | IL23A     | KLRD1     |
| CCL22     | CD3G      | CSF1R     | ENTPD1    | HAVCR2    | IFI44L    | IL2RA     | KLRF1     |
| CCL3      | CD4       | CSF2RB    | EOMES     | HERC6     | IFI6      | IL2RB     | KLRG1     |
| CCL4      | CD40      | CTAG1B    | FAS       | HGF       | IFIH1     | IL2RG     | KLRK1     |
| Gene       | Gene     | Gene   | Gene   | Gene  |
|------------|----------|--------|--------|-------|
| KREMEN1    | MLANA    | POLR2A | SSX2   | TNFSF9|
| KRT5       | MMP2     | POU2F1 | STAT1  | TOP2A |
| KRT7       | MMP9     | PRDM1  | STAT3  | TP63  |
| LAG3       | MPO      | PRF1   | STAT4  | TRIM29|
| LAMP1      | MRC1     | PSMB9  | STAT5A | TUBB  |
| LAMP3      | MS4A1    | PTEN   | STAT6  | TWIST1|
| LAPTM5     | MTO1     | PTGS2  | TAGAP  | TYROBP|
| LCK        | MX1      | PTK7   | TAP1   | VCAM1 |
| LCN2       | MYC      | PTPN11 | TARP   | VEGFA |
| LEXM       | NCAM1    | PTPN6  | TBP    | VTCN1 |
| LILRB1     | NCF1     | PTPN7  | TBX21  | XAGE1B|
| LILRB2     | NCR1     | PTPRC  | TCF7   | ZAP70 |
| LMNA       | NCR3     | PTPRC  | TDO2   | ZBTB46|
| LRG1       | NECTIN2  | PVR    | TFRC   | ZEB1  |
| LRP1       | NFATC1   | PYGL   | TGFB1  |       |
| LST1       | NFKBIA   | RB1    | TIGIT  |       |
| LY9        | NKG7     | RORC   | TLR3   |       |
| LYZ        | NOS2     | RPS6   | TLR7   |       |
| M6PR       | NOTCH3   | S100A8 | TLR8   |       |
| MAD2L1     | NRP1     | S100A9 | TLR9   |       |
| MADCAM1    | NT5E     | SAMHD1 | TNF    |       |
| MAGEA1     | NTN3     | SDHA   | TNFAIP8|       |
| MAGEA10    | OAS1     | SELL   | TNFRSF14|      |
| MAGEA12    | OAS2     | SH2D1A | TNFRSF17|     |
| MAGEA3     | OAS3     | SH2D1B | TNFRSF18|     |
| MAGEA4     | PDCD1    | SIT1   | TNFRSF4|       |
| MAGEC2     | PDCD1LG2 | SKAP2  | TNFRSF9|       |
| MAPK1      | PECAM1   | SLAMF7 | TNFSF10|       |
| MAPK14     | PGF      | SLAMF8 | TNFSF13B|      |
| MELK       | PIK3CA   | SNAI1  | TNFSF14|       |
| MIF        | PIK3CD   | SNAI2  | TNFSF18|       |
| MKI67      | PMEL     | SRGN   | TNFSF4 |       |
Extended Data Table 3. Differentially expressed genes, COVID-19 versus controls

| ID              | logFC  | logCPM      | PValue       | FDR        |
|-----------------|--------|-------------|--------------|------------|
| DMBT1_64696575  | 5.0864| 9.507465672 | 8.27E-12     | 3.29E-09   |
| TDO2_55162      | 3.6066| 9.53520997  | 3.39E-11     | 6.12E-09   |
| IFI6_47156      | 3.7786| 14.24783272 | 4.61E-11     | 6.12E-09   |
| KIAA0101_319426 | 3.1469| 8.549063739 | 8.30E-11     | 8.26E-09   |
| IGF1R_12291338  | -1.3469| 10.50777116 | 1.63E-10     | 1.29E-08   |
| MELK_300401     | 2.8994| 7.833266318 | 9.68E-10     | 6.29E-08   |
| BUB1_701803     | 2.4292| 8.360010662 | 1.11E-09     | 6.29E-08   |
| ISG15_66173     | 4.2766| 13.15626013 | 1.97E-09     | 9.78E-08   |
| IFI27_37143     | 1.9113| 12.5471898  | 3.00E-09     | 1.33E-07   |
| PSMB9_384491    | 1.3857| 10.9106894  | 6.49E-09     | 2.58E-07   |
| OAS3_667776     | 2.7546| 9.890613941 | 9.75E-09     | 3.53E-07   |
| CDK1_837939     | 2.2629| 9.211945164 | 1.08E-08     | 3.59E-07   |
| HLA-G_483585    | 4.0308| 6.375748944 | 1.47E-08     | 4.35E-07   |
| SLAMF8_9071016  | 2.2934| 9.725983998 | 1.53E-08     | 4.35E-07   |
| MLANA_159265    | -5.7577| 0.738276085 | 1.83E-08     | 4.86E-07   |
| CDKN3_434534    | 1.9398| 8.894315977 | 2.32E-08     | 5.46E-07   |
| OAS1_757865     | 2.6074| 11.38534225 | 2.33E-08     | 5.46E-07   |
| JAML_78188      | -1.7597| 7.103911839 | 6.73E-08     | 1.49E-06   |
| IRS1_37283828   | -1.3544| 10.24560172 | 7.98E-08     | 1.62E-06   |
| OAS2_15981707   | 2.0217| 10.70108718 | 8.12E-08     | 1.62E-06   |
| CXCL11_261361   | 4.7127| 12.17691208 | 8.78E-08     | 1.66E-06   |
| IDO1_268369     | 2.7774| 10.21297062 | 1.32E-07     | 2.38E-06   |
| FOXM1_10551166  | 3.5110| 7.302016812 | 3.85E-07     | 6.66E-06   |
| LAMP3_12361344  | 2.2184| 9.184121563 | 4.93E-07     | 8.17E-06   |
| IFIT3_72174     | 2.8265| 12.01665835 | 6.87E-07     | 1.07E-05   |
| MAD2L1_115221   | 1.7490| 8.034765012 | 7.01E-07     | 1.07E-05   |
| CD38_519628     | 1.9408| 9.824900589 | 7.36E-07     | 1.08E-05   |
| Gene     | Log2 Fold Change | T-Statistics (T) | P-Value (P) |
|----------|-----------------|-----------------|-------------|
| CCR1_54149 | 1.473037432     | 10.11920316     | 8.34E-07    | 1.19E-05    |
| GZMB_581688 | 1.898001877     | 10.41173908     | 8.96E-07    | 1.23E-05    |
| LAG3_13111419 | 2.386185632    | 7.994487875     | 1.01E-06    | 1.34E-05    |
| TCF7_677799 | -1.154491364    | 6.98289475      | 1.15E-06    | 1.48E-05    |
| IFI44L_12771376 | 2.551619364 | 11.04872147     | 1.20E-06    | 1.49E-05    |
| TOP2A_27522855 | 2.412862908    | 7.994487875     | 1.01E-06    | 1.34E-05    |
| KLF2_9111017 | -1.14655278     | 10.32922348     | 1.82E-06    | 2.13E-05    |
| MX1_232336 | 2.598359407     | 11.58716187     | 2.53E-06    | 2.88E-05    |
| CXCL9_149250 | 2.475393657     | 11.64602422     | 3.36E-06    | 3.71E-05    |
| CD276_1201310 | 1.404468545    | 6.909158714     | 3.83E-06    | 4.12E-05    |
| STAT1_18871996 | 1.341291438   | 12.54396256     | 4.11E-06    | 4.31E-05    |
| IFI35_419526 | 1.590766479     | 9.999010988     | 4.93E-06    | 5.03E-05    |
| IFIH1_20172123 | 1.978276916    | 9.64184768      | 5.53E-06    | 5.50E-05    |
| CCL18_198296 | 2.559340347     | 11.50058854     | 6.63E-06    | 6.43E-05    |
| KLKB1_177284 | -1.356445562    | 10.32648685     | 7.21E-06    | 6.84E-05    |
| CXCR4_100208 | -1.262015044    | 12.69186025     | 8.67E-06    | 8.03E-05    |
| GBP1_771872 | 1.624043959     | 12.95069926     | 1.21E-05    | 0.000109887 |
| CEACAM8_745847 | -2.38182276    | 6.835412175     | 1.51E-05    | 0.000133479 |
| IFIT2_124224 | 2.601872644     | 12.64896588     | 1.80E-05    | 0.000155644 |
| PDCD1LG2_315423 | 1.435337704    | 9.765019917     | 1.94E-05    | 0.000164473 |
| CXCL10_354459 | 2.921361689    | 12.20854003     | 2.10E-05    | 0.000173779 |
| IFIT1_158259 | 3.138880653     | 12.7962216      | 2.30E-05    | 0.000186992 |
| BCL6_21502257 | -1.12692675     | 11.38395943     | 2.37E-05    | 0.000188627 |
| CD69_195303 | -1.167440855    | 10.29551815     | 2.49E-05    | 0.000194401 |
| PTGS2_14761583 | -1.787690656    | 9.487046641     | 3.11E-05    | 0.000229188 |
| CD226_9021006 | -1.055303117    | 8.20462834      | 3.51E-05    | 0.000249595 |
| C1QB_111199 | 1.318021235     | 14.14106331     | 5.40E-05    | 0.000370819 |
| CXCL13_202307 | 2.634779914     | 10.17220128     | 5.53E-05    | 0.000372978 |
| Gene          | DLog2FoldChange | pValue  | FDR       |
|--------------|----------------|---------|-----------|
| CCNB2_9861095| 2.451586323    | 6.34E-05| 0.000420281|
| BRCA2_98179922 | 1.408177984 | 6.59E-05| 0.000429787|
| MKI67_581686 | 1.830582251    | 8.31E-05| 0.000533209|
| BCL2_10401144 | -1.054459851  | 9.27E-05| 0.000576492|
| DDX58_540643 | 1.637092216    | 0.000122508| 0.000750128|
| KLRG1_410518 | -1.049817337   | 6.871878081| 0.000780569|
| SNAI2_722828 | 1.272531289    | 9.779931728| 0.000839096|
| POU2AF1_230337| 1.981866553 | 9.738343726| 0.000967573|
| XAGE1B_469547 | 2.99835029    | 0.000177305| 0.000980102|
| C1QA_67171   | 1.197339812    | 12.61310409 | 0.000989574|
| PTPRC_710817 | -1.011649066   | 9.527123762| 0.000989574|
| CCL17_288394 | -1.834523807   | 6.992710035| 0.001220359|
| CD1C_12531357| -2.519073627   | 6.111702351| 0.001541811|
| MPO_15121620 | -1.908031327   | 8.211592408| 0.001598665|
| CCR5_85193   | 1.040859769    | 9.653446555| 0.001851239|
| CD83_480580  | -1.053864486   | 8.523628945| 0.001889203|
| CRTAM_312417 | -1.623243078   | 5.490123515| 0.001916303|
| HERC6_17021806| 1.846150693  | 8.504611085| 0.001930837|
| BST2_218322  | 1.11377782     | 14.61484839| 0.002148274|
| HLA-DQB2_142243| -2.208546554  | 2.6772892 | 0.00275705|
| CMKLR1_36143 | 3.089416269    | 4.095184302| 0.003860634|
| ISG20_642750 | 1.288727823    | 10.51232195| 0.004203845|
| TNFSF18_128228| 2.048786407  | 5.115450907| 0.004854718|
| CD163_21422245| 1.190748679  | 13.00811752| 0.005033238|
| CYBB_14221529| 1.034200312    | 11.76129988| 0.005525454|
| IFITM1_359459| 1.094499983    | 14.73592207| 0.005845111|
| RORC_12011307| -1.232621407   | 6.368975553| 0.00625033|
| TNFRSF9_894998| -1.557976838  | 6.277732459| 0.007593748|
| Gene       | Expression | Log2 Fold Change | p-value  | False Discovery Rate |
|------------|------------|------------------|----------|----------------------|
| IL2_366451 | -1.787299469 | 2.137968085 | 0.002118414 | 0.007735127          |
| IRF4_786895 | 1.2991475   | 7.589669323 | 0.002433884 | 0.008497246          |
| IL21_368450 | 2.713025334 | 3.179107785 | 0.002892483 | 0.009924208          |
| FCGR1A_547652 | 1.358837352 | 8.984718333 | 0.003056301 | 0.010396649          |
| SLAMF7_9161020 | 1.178583741 | 10.97955754 | 0.00366687  | 0.012263986          |
| GZMA_165265 | 1.05539306  | 10.51173829 | 0.004314939 | 0.014311214          |
| CCR6_271363 | -1.108756703 | 5.421780719 | 0.004727498 | 0.015422493          |
| ARG1_174278 | -2.016630732 | 7.856291723 | 0.005246505 | 0.016976497          |
| IL10_491598 | 1.240451265 | 6.779053894 | 0.005949936 | 0.018944598          |
| TNFRSF17_254359 | 1.607351136 | 7.694337923 | 0.006172838 | 0.019498329          |
| MAGEC2_249358 | -2.203350303 | -1.572033969 | 0.007805425 | 0.024081854          |
| CXCR5_153252 | -1.570791614 | 3.754056017 | 0.009131323 | 0.027555897          |
| KRT5_10631165 | 2.093456369 | 10.18852794 | 0.009780171 | 0.029488696          |
| KRT7_440543 | 1.017265847 | 12.56930876 | 0.012411449 | 0.035795337          |
| GAGE10_137250 | -2.028791387 | 1.82618068 | 0.018144659 | 0.049462838          |
### Extended Data Table 4. Antibodies and staining conditions

| Antibody  | Supplier       | Product Number     | Clone     | Dilution | Pretreatment | Staining platform | Detection system                      |
|-----------|----------------|--------------------|-----------|----------|--------------|-------------------|---------------------------------------|
| CD3       | Novocastra     | NCL-L-CD3-565      | LN10      | 1:100    | H2(20)100    | Bond III          | Bond Polymer Refine Red Detection     |
| CD4       | Novocastra     | CD4-368-L-CE       | 4B12      | 1:80     | 30min ER2    | Bond III          | Bond Polymer Refine Red Detection     |
| CD8       | Novocastra     | NCL-L-CD8-4B11     | 4B11      | 1:40     | H2(30)95     | Bond III          | Bond Polymer Refine Red Detection     |
| CD15      | Cellmarque     | 115M               | MMA       | 1:25     | H1(20)100    | Bond III          | Bond Polymer Refine Red Detection     |
| CD20      | Agilent        | M0755              | L26       | 1:600    | H2(20)95     | Bond III          | Bond Polymer Refine Red Detection     |
| CD68      | Agilent        | M0876              | PG-M1     | 1:100    | E1(5)        | Bond III          | Bond Polymer Refine Red Detection     |
| CD123     | Novocastra     | CD123-L-CE         | BR4MS     | 1:20     | 20min ER2    | Bond III          | Bond Polymer Refine Red Detection     |
| CD163     | Novocastra     | NCL-L-CD163        | 10D6      | 1:200    | H1(20)100    | Bond III          | Bond Polymer Refine Red Detection     |
| C3d       | Dako           | A0063              | polyclonal| 1:700    | E1(10)       | Bond III          | Bond Polymer Refine Red Detection     |
| C5b-9     | Lifespan Biosciences | aE11     | 1:50     | Enzyme 1 | Benchmark GX | OptiView DAB       | Bond Polymer Refine Red Detection     |
| Ki67      | Agilent        | M7240              | MIB-1     | 1:50     | H2(20)95     | Bond III          | Bond Polymer Refine Red Detection     |
| MPO       | Agilent        | A0398              | polyclonal| 1:8000   | H2(20)95     | Bond III          | Bond Polymer Refine Red Detection     |
| p53       | Agilent        | M7001              | D07       | 1:1200   | H1(20)100    | Bond III          | Bond Polymer Refine Red Detection     |
| PD1       | Roche Ventana  | 760-4895           | NAT105    | no dilution | CC1 40min | Benchmark GX     | Bond Polymer Refine Red Detection     |
| SARS-CoV-2| BioConcept     | 200-401-A50        | Anti-SARS-CoV Nucleocapsid | 1:6400 | H2(20)95 | Bond III | Bond Polymer Refine Red Detection     |
### Extended Data Table 5. Histopathology

| Case No. | Severity of histological changes in lungs | DAD stage | Intraalveolar edema | Intraalveolar hemorrhage | Neutrophils | SARS-CoV-2 genomes / 10^6 RNaseP copies | SARS-CoV-2 IHC |
|----------|------------------------------------------|-----------|---------------------|-------------------------|-------------|-----------------------------------------|---------------|
| C12      | 1                                        | -         | 1                   | 1                       | 1           | 673                                     | 1             |
|          | 2                                        | 1         | 1                   | 1                       | 2           | 2'871                                    | 2             |
|          | 2                                        | 1         | 1                   | 1                       | 2           | 116'310                                  | 3             |
| C13      | 3                                        | 1 and 2   | 1                   | 1                       | 2           | 38                                      | 0             |
|          | 3                                        | 1 and 2   | 1                   | 1                       | 2           | 310                                     | 0             |
|          | 3                                        | 1 and 2   | 1                   | 1                       | 2           | 225                                     | 0             |
| C16      | 1                                        | 1         | 1                   | 0                       | 1           | 91                                      | 0             |
|          | 1                                        | 1 and 2   | 1                   | 0                       | 1           | 20                                      | 0             |
| C17      | 1                                        | 2         | 1                   | 1                       | 1           | 12                                      | 0             |
|          | 2                                        | 1 and 2   | 1                   | 1                       | 2           | 25                                      | 0             |
| C19      | 1                                        | 1         | 1                   | 1                       | 1           | 7'603                                    | 2             |
|          | 1                                        | 1         | 0                   | 1                       | 1           | 18'867                                   | 2             |
| C20      | 3                                        | 1 and 2   | 1                   | 1                       | 2           | 145                                     | 0             |
|          | 2                                        | 1 and 2   | 1                   | 1                       | 2           | 3'205                                    | 1             |
|          | 3                                        | 1 and 2   | 1                   | 1                       | 2           | 32                                      | 0             |
| C21      | 3                                        | 2         | 0                   | 0                       | 2           | 119                                     | 0             |
|          | 3                                        | 2         | 0                   | 0                       | 1           | 53                                      | 0             |
|          | 2                                        | 2         | 0                   | 0                       | 1           | 119                                     | 0             |
| C15      | 2                                        | 1         | 1                   | 0                       | 2           | 91'186                                   | 3             |
|          | 2                                        | 1         | 1                   | 0                       | 1           | 2'993                                    | 2             |
| C1       | 1                                        | -         | 1                   | 0                       | 1           | 0.4                                     | 0             |
|          | 1                                        | -         | 1                   | 0                       | 1           | 0.0                                     | 0             |
|          | 1                                        | -         | 1                   | 0                       | 1           | 0.2                                     | 0             |
| C3       | 2                                        | -         | 0                   | 0                       | 3           | 14'311                                   | n.d.          |
|          | 2                                        | -         | 1                   | 1                       | 3           | 249'937                                  | 3             |
|          | 2                                        | -         | 0                   | 0                       | 3           | 157'042                                  | 2             |
| C4       | 1                                        | -         | 0                   | 1                       | 1           | 314'728                                  | 3             |
|          | 2                                        | -         | 1                   | 1                       | 1           | 245'331                                  | 3             |
|          | 2                                        | 1         | 1                   | 1                       | 1           | 187'540                                  | 3             |
| C5       | 2                                        | -         | 0                   | 0                       | 1           | 8                                       | 0             |
|          | 2                                        | 1 and 2   | 0                   | 0                       | 1           | 180                                     | 0             |
|          | 2                                        | 1 and 2   | 0                   | 1                       | 2           | 82                                      | 0             |
| C6       | 1                                        | -         | 0                   | 0                       | 1           | 10'451                                   | 1             |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 1 | - | 0 | 0 | 1 | 118'620 | 3 |
| 1 | - | 1 | 0 | 1 | 114'407 | 3 |
| C7 | 1 | 1 and 2 | 0 | 0 | 1 | 12'939 | 3 |
| 1 | 1 and 2 | 0 | 0 | 1 | 1'443 | 1 |
| 1 | 1 and 2 | 0 | 1 | 2 | 16'534 | 2 |
| C8 | 1 | - | 0 | 0 | 1 | 17'598 | n.d. |
| 3 | - | 1 | 0 | 3 | 316 | n.d. |
| 1 | - | 0 | 0 | 1 | 139 | n.d. |
| C9 | 1 | - | 0 | 0 | 1 | 64'242 | n.d. |
| 1 | - | 0 | 0 | 1 | 85'137 | n.d. |
| 1 | - | 1 | 0 | 1 | 29'487 | n.d. |

At least two different tissue blocks from different areas of the lungs were evaluated for each case.

1 = slight to moderate changes; 2 = moderate changes; 3 = severe changes

2 = exudative; 2 = proliferating/organizing; 3 = fibrotic

3 = yes; 0 = no

4 = very few or few; 2 = moderate; 3 = numerous
Extended Data Figure 3

(a) Graphs showing the distribution of CD4+, CD20+, CD123+, and CD8+ PD1+ cells/mm² across ISG^high, ISG^low, and control groups. The graphs include box plots and statistical significance p-values (0.002, 0.039, 0.001).

(b) Images of CD4, CD20, CD123, and CD8/PD1 staining across ISG^high, ISG^low, and control groups. The images show distinct patterns and cell distributions.
Extended Data Figure 4

(a) IL6, TNF & IL1B log2(cpm+1) vs. Intraalveolar hemorrhage

(b) IFNA17 & IFNB1 log2(cpm+1) vs. Intraalveolar hemorrhage

(c) CCL2 log2(cpm+1) vs. Intraalveolar hemorrhage
Extended Data Figure 5

(a) IL6, TNF & IL1B log2(cpm+1)

- ISG^high
- ISG^low

(b) IFNA17 & IFNB1 log2(2^cpm+1)

(c) CXCL9, CXCL10 & CXCL11 log2(2^cpm+1)

- none 1 1 to 2 2

(d) CCL2 log2(2^cpm+1)

0.02

- none 1 1 to 2 2