Molecular consequences of SARS-CoV-2 liver tropism

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Extrapulmonary manifestations of COVID-19 have gained attention due to their links to clinical outcomes and their potential long-term sequelae. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) displays tropism towards several organs, including the heart and kidney. Whether it also directly affects the liver has been debated. Here we provide several organs, including the heart and kidney. Whether it also directly affects the liver has been debated. Here we provide evidence for the hepatic tropism of SARS-CoV-2. We find that liver injury, indicated by a high frequency of abnormal liver function tests, is a common clinical feature of COVID-19 in two independent cohorts of patients with COVID-19 requiring hospitalization. Using autopsy samples obtained from a third patient cohort, we provide multiple levels of evidence for SARS-CoV-2 liver tropism, including viral RNA detection in 69% of autopsy liver specimens, and successful isolation of infectious SARS-CoV-2 from liver tissue postmortem. Furthermore, we identify transcription-, proteomic- and transcription factor-based activity profiles in hepatic autopsy samples, revealing similarities to the signatures associated with multiple other viral infections of the human liver. Together, we provide a comprehensive multimodal analysis of SARS-CoV-2 liver tropism, which increases our understanding of the molecular consequences of severe COVID-19 and could be useful for the identification of organ-specific pharmacological targets.

In this study, we examined three patient cohorts to characterize severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-associated liver injury and infection. First, a clinical cohort (Hamburg; cohort 1, n=99) was used to examine changes in liver function tests (LFTs) in patients admitted to hospital due to coronavirus disease 2019 (COVID-19) and those who were diagnosed with COVID-19 during hospitalization (Fig. 1a); 42% of these patients were at least 65 years old, 70% were males, only 23% had at least 3 coexisting conditions (Source Data Fig. 1). Among patients admitted due to COVID-19 (n=72), raised aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were shown in 63 and 16% of cases (Fig. 1b), respectively despite the low frequency of liver dysfunction in 33% versus 59%, the percentage of patients with elevated AST almost tripled (22 versus 67%) (Fig. 1d). Next, we evaluated a large validation cohort of patients admitted to hospital due to COVID-19 (Michigan; cohort 2, n=1,219) (Fig. 1e); 44% of patients were at least 65 years old, 57% were males and only 16% had at least 3 coexisting conditions (Supplementary Table 1). Importantly, 57% (n=699) of patients showed elevations of AST and 37% (n=452) of ALT at admission (Fig. 1e). Furthermore, LFT elevations at admission and during the second week of hospitalization were associated with mortality (Fig. 1f), raising questions about their role in disease severity. Together, these observations clearly highlight hepatic injury as an important clinical feature of patients with COVID-19 requiring hospitalization.

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It has been postulated that LFT elevations in hospitalized patients with COVID-19 may result from systemic inflammation or severe cellular stress (for example, hypoxia), as generally observed in critically ill patients. However, an autopsy study reported ultrastructural evidence of SARS-CoV-2 (ref. 12), and a second study reported histopathological findings in a liver biopsy of a patient with abnormal liver enzymes, including no obvious inflammation in the portal area, with normal description of the interlobular bile duct, interlobar vein, interlobular artery and hepatocytes with minimal inflammatory cell infiltration. Given that liver biopsies are not routinely performed in patients with COVID-19 with altered LFTs, we evaluated a third cohort (cohort 3, n = 45 autopsy cases) in search of direct evidence of liver infection (Supplementary Table 2). 73% (n = 33) were older than 65, 69% (n = 31) had at least 3 coexisting conditions and 62% (n = 28) were males, matching the demographic characteristics linked to severe COVID-19. SARS-CoV-2 RNA was detected using quantitative PCR with reverse transcription (RT-qPCR) targeting the E gene in 69% of cases (n = 31) (Fig. 2a), which was more frequently associated with older age, male sex and multiple coexisting conditions, as shown in previous reports (13,14).

Pathology assessment only revealed signs of shock, probably due to severe systemic disease, and adiopathic changes without classical signs of hepatitis or significant immune infiltration (n = 18; Extended Data Fig. 1). However, a combination of indirect immunofluorescence and high-resolution confocal microscopy identified hepatic expression of angiotensin-converting enzyme 2 (ACE2), the main cellular receptor of SARS-CoV-2 (Fig. 2b and Extended Data Fig. 2; n = 5 liver samples), and SARS-CoV-2 spike protein (Fig. 2c and Extended Data Fig. 3, n = 3 examples). In addition, SARS-CoV-2 RNA was also found in hepatic cells with in situ hybridization (Supplementary Fig. 1; n = 4, that is, n = 2 SARS-CoV-2 PCR+ and n = 2 controls (Ctrls)), confirming the spatial location of SARS-CoV-2 in the human liver using multiple techniques that complement the findings of previous studies (12,13). To provide additional context, we first compared the levels of SARS-CoV-2 RNA copies per cell between airway samples (previously reported in Puellar et al.) and a subset of autopsy livers, showing a similar range but a lower median viral RNA load in hepatic specimens (Supplementary Fig. 2a; n = 18 lung, n = 16 pharynx and n = 18 liver), and we quantified the number of SARS-CoV-2 spike-positive cells in a small subset of our autopsy samples (n = 6 liver samples, that is, n = 3 SARS-CoV-2 PCR+ and n = 3 Ctrl with quantification provided in Supplementary Fig. 2b), both of which may explain the lack of overt cytopathic changes (that is, hepatitis). Notably, we isolated infectious SARS-CoV-2 from 2 out of 3 autopsy livers and corresponding lungs, confirming the infectivity of postmortem liver

Fig. 1 | Elevated LFTs among patients with COVID-19. a, Overview of cohort 1, n = 99 patients required hospitalization due to COVID-19 (Germany). n = 72 were admitted due to moderate/severe COVID-19 and n = 27 acquired COVID-19 during their hospital stay. b, Only 1.4% of patients admitted due to COVID-19 from cohort 1 had a history of liver disease, yet LFTs at admission showed elevated AST in 63% and ALT in 39% of patients. c, d, AST and ALT levels in patients with COVID-19 acquired during hospitalization worsened after COVID-19 diagnosis in 81% and 67% of patients, respectively. e, Demographic overview of cohort 2. n = 1,219 patients required hospitalization due to COVID-19 (Michigan). Only 2.4% of admitted patients from cohort 2 had a history of liver disease, yet LFTs at admission showed elevated AST in 57% and ALT in 37% of patients with COVID-19. f, Variation of mean AST and ALT over time in patients with COVID-19, showing elevations in LFTs associated with mortality.

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of SARS-CoV-2 hepatic tropism, at least in cases of fatal COVID-19. Thus, liver tissues from a subset of COVID-19 autopsies were selected for transcriptomic and proteomic analyses ($n = 11$; Fig. 2e). Briefly, 3 comparison groups were defined a priori: (1) patients with COVID-19 and SARS-CoV-2-positive livers ($n = 5$ for transcriptomics and $n = 6$ for proteomics); (2) patients with COVID-19 and SARS-CoV-2-negative livers ($n = 5$ for transcriptomics and proteomics) to account for the systemic effect of COVID-19; and (3) patients with non-COVID-19-related deaths ($n = 5$ for proteomics and $n = 7$ for proteomics).
SARS-CoV-2 liver tropism is associated with transcriptional regulation of IFN responses. a. Clinical heatmap of 45 patients indicating age ≥65 years, sex, 3 or more coexisting conditions and SARS-CoV-2 liver tropism (PCR+ liver). b. Immunofluorescence images show the presence of the SARS-CoV-2 receptor ACE2 in hepatic cells (that is, Kupffer cells). Staining was performed in samples from five different patients (data shown in Extended Data Fig. 2). c. SARS-CoV-2 spike protein detection in autopsy liver tissues (that is, Kupffer cells and hepatocytes). d. Successful SARS-CoV-2 isolation in postmortem livers and respective lungs from two of three autopsy cases, showing increases in SARS-CoV-2 RNA levels in the supernatants of infected cells (43-45 refers to the number sequence in a). e. Schematic of autopsy tissue selection for molecular profiling. f. Generally applicable gene set enrichment (GAGE) analysis of gene sets significantly regulated between liver PCR-positive, PCR-negative and Ctrl conditions. g. Single-sample jitter plot for gene sets significant (P_adj < 0.05) in at least 1 sample and significant difference between the PCR-positive and PCR-negative groups (Wilcoxon signed-rank test, P < 0.05). Box plot (box extending from the 25th to the 75th percentile with the median shown as a line in the middle and the whiskers indicating the smallest and largest values) showing the most relevant pathways by set enrichment (GAGE) analysis of gene sets significantly regulated between liver PCR-positive, PCR-negative and Ctrl conditions. h. Single-sample enrichment barcode illustrating the distribution of the hallmark gene set ‘IFN-α response’ in every single sample. Genes were ranked according to their normalized TPM value. The colour coding represents the enrichment score of the gene set in a random walk over the ranked list of genes. Samples were divided into PCR-positive and PCR-negative and then ordered from left to right based on their enrichment score, from high to low.

Global proteomic profiling in hepatic tissues of the same autopsy cohort showed substantial regulation of IFN, viral and in particular SARS-CoV-2 responses (Fig. 3a and Source Data Fig. 3). Protein ontology analysis revealed upregulation of IFN signalling and downregulation of basic biological processes, including oxidation reduction, oxidative phosphorylation and cellular respiration (Fig. 3b). Type I IFN responses were confirmed at the single patient level (Fig. 3c). Gene/protein sets for hallmark ‘interferon-α (IFN-α) response’ (R = 0.65, P < 0.05; Fig. 3d) and the GO Biological Process ‘defence response to virus’ (R = 0.71, P < 0.05; Fig. 3e), as well as single gene/protein analysis for IFN-inducible genes (R = 0.74–0.87, P < 0.05; Fig. 3f), showed strong associations between transcriptional and proteomic profiles, highlighting a coherent modulation of SARS-CoV-2-related pathways at the transcriptional and translational level. Furthermore, transcription factor activity profiling (Fig. 3g) provided individual (Fig. 3h) and global (Fig. 3i) signatures that combined transcription factor upregulation (for example, signal transducer and activator of transcription 2 (STAT2), a signal transducer and activator of transcription that mediates signalling by type I IFNs) and downregulation (that is, nuclear receptor coressor 2 (N-CoR2), which aids histone deacetylases to modify chromatin structure, thus preventing basal transcriptional activity). Canonical pathway analysis confirmed upregulation of immune-mediated, unregulated IFN responses in tissues with traces of SARS-CoV-2 RNA and without classical cytopathic changes. In addition, we also identified that components of IFN-related JAK-STAT signalling (Supplementary Fig. 3), and other central cellular processes involved in lipid/cholesterol synthesis, including terpenoid and steroid biosynthesis were all modulated in patients with SARS-CoV-2 liver tropism. Altogether, these data provide a comprehensive framework to understand the molecular consequences of SARS-CoV-2-mediated liver injury.
IFN and JAK-STAT-related responses (Extended Data Fig. 8a), as well as downregulation of glucose and carbohydrate metabolism in association with liver tropism (Extended Data Fig. 8b). Interestingly, modulation of JAK-STAT signalling has been proposed to target SARS-CoV-2 viral entry and replication\textsuperscript{12} in a study that dissected SARS-CoV-2 infectivity using organotypic three-dimensional cultures of primary human liver cells.

The clinical and molecular data from our study suggest that SARS-CoV-2-mediated liver injury is comparable to previously characterized hepatotropic viruses. For this reason, we compared
HBV-positive and HCV high ISG samples. Mean transcription factor activity showed similar transcription factor use in COVID-19 liver positive samples, HIV-positive and HCV low ISG, followed by Process downregulated for COVID-19 liver positive versus negative samples and HCV ISG high versus Ctrl showing overlap in 8 out of 20 pathways. GO Biological upregulated for COVID-19 liver positive versus negative samples and HCV ISG high versus Ctrl showing overlap in 13 out of 20 pathways. Hallmark pathways HBV, HCV and HIV datasets with our downregulated gene signatures showing a small overlap with no significant gene signature. Were classified using www.interferome.org (v2.01) as type I or type II IFN-specific. Overlapping genes with the leading edge of the GSEA of the HBV, HCV and HIV datasets with our upregulated gene signatures showing most overlap all four datasets belonging to the gene sets, IFN-γ, Overlapping genes with the leading edge of the GSEA of the HBV, HCV and HIV datasets with our upregulated gene signatures showing most overlap all four datasets belonging to the gene sets, IFN-γ, Overlapping genes with the leading edge of the GSEA of the HBV, HCV and HIV datasets with our upregulated gene signatures showing most overlap all four datasets belonging to the gene sets, IFN-γ, Overlapping genes with the leading edge of the GSEA of the HBV, HCV and HIV datasets with our upregulated gene signatures showing most overlap all four datasets belonging to the gene sets, IFN-γ.

Mean transcription factor activity (HBV, HCV and HIV modelling) showed similar transcription factor activity in COVID-19 liver positive samples, HIV-positive and HCV low ISG, followed by HBV-positive and HCV high ISG samples.
publicly available datasets containing bulk RNA sequencing (RNA-seq) from liver tissue samples from patients with hepatitis C virus (HCV)\(^\text{23}\), human immunodeficiency virus (HIV)\(^\text{24}\) and hepatitis B virus (HBV)\(^\text{25}\) via gene set enrichment analysis (GSEA) (Fig. 4a). While our literature search was carefully performed, it was not possible to find studies that could be perfectly matched our study demographics (for example, our population was notably older than the other three studies as per the demographic trends of fatal COVID-19; Supplementary Table 4). Interestingly, and despite of multiple methodological limitations, all three infections affecting the liver, including HBV (Fig. 4b), HCV (Fig. 4c) and HIV (Fig. 4d), showed overlapping gene expression patterns with our proposed SARS-CoV-2 signature. Specific examples include upregulation of IFN signalling gene sets (Fig. 4e); especially types I, II and III IFN responses (Fig. 4f), and downregulation of viral gene expression and related cellular functions (that is, translational initiation and peptide metabolic processes) (Fig. 4g). Pathway analysis also revealed remarkable overlap between SARS-CoV-2 and HCV (13 of 20 pathways for upregulated; Fig. 4h; 8 of 20 pathways for downregulated; Fig. 4i). In addition, we identified similar transcription factor use in livers from patients with severe COVID-19 and patients with liver steatosis and HIV, inactive carriers of HBV and chronic HCV (Fig. 4j). Taken together, this comparative analysis highlights a shared molecular signature between SARS-CoV-2 and multiple viruses with well-characterized patterns of liver injury.

Since the topic of SARS-CoV-2 hepatic tropism has been highly debated\(^\text{6}\), we aimed to tackle this question from multiple perspectives, including clinical (two large independent patient cohorts that showcase COVID-19-associated liver injury), histopathological (identification of SARS-CoV-2 at the RNA and protein levels with high spatial resolution), virological (RT-qPCR in human autopsy samples and isolation of SARS-CoV-2 from human autopsy liver specimens), molecular (transcriptomic, proteomic and transcription factor activity use profiling, indicating the presence of SARS-CoV-2 in association with significant tissue responses) and bioinformatic (detection of subgenomic RNA and data validation with external public datasets of virus-mediated hepatic injury). Despite all these layers of evidence supporting SARS-CoV-2 hepatic tropism, the exact mechanism of infection is unclear. Our findings show that the human liver is susceptible to SARS-CoV-2 infection since cell entry receptors and facilitators are clearly expressed in hepatic cells. However, we observed a mismatch between the expression of the ACE2 protein, mostly observed in Kupffer cells, and the location of the SARS-CoV-2 spike protein, which was observed in Kupffer and parenchymal cells (for example, hepatocytes). In this context, it is worth considering a recent study that proposed high-density lipoprotein scavenger receptor class B member 1 (SRB1) as a cell entry facilitator for SARS-CoV-2 and multiple viruses with well-characterized patterns of liver injury.

In summary, our findings provide multimodal evidence of SARS-CoV-2 human liver tropism and a molecular signature, featuring a strong upregulation of IFN responses, JAK-STAT signalling and liver-specific metabolic modulation. This unique human data set can be used as a rational framework for future studies aiming to characterize the potential consequences of SARS-CoV-2 extra-pulmonary manifestations and identify personalized therapeutic strategies.

**Methods**

**Tissue and data collection.** Autopsies were performed at the Institute of Legal Medicine of the University Medical Center Hamburg-Eppendorf. From every liver specimen collected by the Institute of Legal Medicine, multiple randomly chosen small samples were available for different analyses. The ethics committee of the Hamburg Chamber of Physicians was informed about the study (nos. 2020-10353-B0-Iv and PV7311). CorS included cases of sudden, non-infectious deaths. The postmortem interval was on average 6 d. Informed consent was obtained from a next of kin or legal representative for autopsy and tissue sampling. No compensation was paid.

The study protocol for clinical data collection (patient cohorts) was approved by the institutional review board (IRB) of the University of Michigan (nos. HUM00178971) and Hamburg (no. WF-052/20). The IRB approved a waiver of informed consent for this observational study.

**Molecular detection of SARS-CoV-2.** Tissue samples were systematically sampled during the autopsy procedure in 45 individuals. Automated nucleic acid extraction was performed according to the manufacturer’s recommendations with whole process control (control kit; Roche), with a final elution volume of 100 μl. For virus quantification, a previously published assay was adopted with modifications using chimieric 2′-O-methyl RNA bases at the penultimate base of both primers (me and mc) to reduce primer dimer formation\(^\text{7}\). The forward primer 5′-ACACCTAGTATTTAAATAGCmGT-3′ (400 nM end concentration), and probe 5′-TATTGCAGACTTACGCAACGACTAAANCA-3′ (400 nM end concentration) and probe 5′-Fam-AACACTAGCC/ZEN/ATCCTTACGCGCTTCGG-Iowa Black FQ-3′ (100 nM end concentration) were used. Primer and probes were obtained from Integrated DNA Technologies. One-step RT-qPCR (25 μl volume) was run on the LightCycler 480 system (Roche) using the one-step RNA control kit as master mix (Roche) and 5 μl of eluate. The Ct value for the target SARS-CoV-2 RNA (FAM) was determined using the second derivative maximum method. To quantify the standard in vitro-transcribed RNA, the E gene of SARS-CoV-2 was used. The standard was obtained via the European Virus Archive\(^\text{8}\). The linear range of the assay was between 1 × 10\(^3\) and 1 × 10\(^7\) copies μl\(^{-1}\). β-Globin qPCR was performed with the commercial TaqMan primer set (catalogue no. 401846; Thermo Fisher Scientific) and Roche DNA control kit. The PCR was run on the LightCycler 480 system. The amount of DNA was normalized using a human DNA standard (KR0545). SARS-CoV-2 RNA levels in tissues were normalized to β-globin DNA.

**Histology, immunolabelling and quantification.** Liver tissue was fixed in formaldehyde. Then, 2-μm slides were cut and haematoxylin and eosin, periodic acid–Schiff and Masson–Goldner stainings were performed. Expert pathology review was conducted by two independent and experienced pathologists. ACE2 and SARS-CoV-2 spike protein were detected in formalin-fixed paraffin-embedded sections using a protocol for indirect immunofluorescence and confocal microscopy\(^\text{9}\). We used primary antibodies against ACE2 (dilution: 1:200, catalogue no. AF933; R&D Systems) and SARS-CoV/SARS-CoV-2 (COVID-19) (dilution 1:200, catalogue no. GTX632604; GeneTex), which were...
validated in previous studies\(^1\),\(^2\). We also used a primary antibody against SRB1 (dilution 1:200, catalogue no. ab217318; Abcam).

For quantification, and considering the size and degree of autolysis, we performed targeted sequencing of five fields of view based on the presence of at least one SARS-CoV-2 spike\(^+\) cell with a random location within the field; for Controls, five random fields were chosen from non-autolytic sites. Then, we quantified the number of SARS-CoV-2 spike\(^+\) cells and the total number of complete nuclei per field, which allowed us to calculate a percentage of positive cells per field. Statistics were performed with Prism v.9.2.0 (GraphPad Software).

RNA in situ hybridization (RNAscope). In situ hybridization was carried out to detect virus RNA of SARS-CoV-2 on paraffin sections utilizing the RNAscope 2.5 HD detection kit (catalogue no. 322310; Advanced Cell Diagnostics) according to the manufacturer’s instructions\(^3\). Briefly, tissue sections were deparaffinized in xylene followed by target retrieval at 95°C for 10 min. Subsequently, internal peroxidase activity was quenched by hydrogen peroxide incubation for 10 min followed by permeabilization using protease plus treatment at 40°C for 30 min. The SARS-CoV-2-specific RNA probe V-nCoV2019-S (catalogue no. 848561) was hybridized at 40°C for 2 h. RNAscope probes specific for either the human ubiquitin C mRNA (catalogue no. 310041) or the bacterial dithydricipolicoteline reductase mRNA (catalogue no. 310043) were used as positive or negative Ctrl, respectively. The RNAscope signal was developed with 3,3’-diaminobenzidine and nuclei were counterstained with haematoxylin.

Cell culture and virus isolation. Liver tissues were homogenized\(^4\) and 250μl of the homogenized tissue solution were used to infect Vero cells (CRL-1586, ATCC). Growth was confirmed by RT-qPCR of cell culture supernatants\(^5\).

RNA-seq. Sample selection was based on collection date and RNA quality. RNA was extracted with the QIAGEN RNeasy Micro Kit according to the manufacturer’s instructions followed by Agilent Bioanalyzer sample quality control, library preparation with Lexogen CORALL Total RNA and ribosomal RNA depletion. Single-end RNA-seq was done using 75 base pair pair NextSeq v.2.5 with >30 million reads. Raw reads were trimmed with Trim Galore! v.0.4.3 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and aligned with RNA STAR: Galaxy Version 2.7.2h\(^6\). Mapped reads were counted with HTSeq: Galaxy Version 0.9.1\(^7\).

GTX data. Raw counts from several healthy tissues were downloaded directly from the GTX portal (www.gtxexportal.org) on 4 March 2020\(^8\).

Differential analysis. Genes with a non-null count in at least two samples were selected. Raw counts were processed with the limma R package v.3.42.2 (ref. \(^9\)) and differentially regulated genes between PCR-positive and PCR-negative groups were identified after library size normalization. Both groups were compared to GTEX differential regulated genes between PCR-positive and PCR-negative groups were identified after library size normalization. Both groups were compared to GTEX data. Genes with a non-null count in at least two samples were selected for analysis.

Dietary intake. The average diet consumed per patient was calculated from dietary intake assessment and metabolic parameters, including age, sex, and body mass index. The dietary intake assessment was performed using an electronic food-frequency questionnaire. The metabolic parameters were estimated from daily energy expenditure and energy intake. Both values were adjusted for gender and body fat percentage. The dietary intake assessment was performed using a computerized program (DoRothEA; see Methods). The energy intake was calculated using the factor of 1.2, which reflects the difference between energy expenditure and energy intake. The energy expenditure was estimated using the factor of 1.1, which is based on body weight and metabolic rate. The energy intake was calculated using the factor of 1.1, which is based on body weight and metabolic rate.

Detection of sgRNA. The relative fraction of single-guide RNAs (sgRNAs) was determined by identifying the leader sgRNA fusion containing sequencing reads normalized to read counts containing the genomic RNA leader sequence\(^1\). Pharyngeal swab data served as a positive control for the detection of sgRNA due to the high amount of replicating virus in this specimen. Swab sample data were reproduced from our own data\(^2\).

Proteomic analysis. For sample preparation, paraffin-embedded liver samples were deparaffinized, resuspended in 100μl lysis buffer (50% trifluoroethanol (TFE), 300 mM Tris-HCl, pH 8.0) and processed\(^3\). Briefly, samples were sonicated at 4°C for 15 min (high ‘settings on a Bioruptor; Drainogene), boiled at 95°C for 90 min and sonicated again as described above. Cleared lysates were reduced/ alkylated with 5 mM DTT and 5 mM iodoacetamide, 25 mM iodoacetamide in the dark and concentrated on a vacuum centrifuge (45 min, 60°C). A total of 50μg proteins were digested overnight in digestion buffer (10% TFE containing trypsin/LysC (1:50 protein/protein ratio). Digestion was stopped with 1% trifluoroacetic acid (TFA) and peptides were purified on stage tips with two SDB-RRS Empore filter discs (3M) and resuspended in 2% acetonitrile (ACN)/0.1% TFA to a final concentration of 250 ng/μl\(^4\).

Ultra-high-performance liquid chromatography and trapped ion mobility spectrometry quadrupole time of flight (HRTOF Pro) was operated in parallel accumulation-serial fragmentation (PASEF) mode using Compass Hystar v.5.30.0. Settings were as follows: mass range 100–1700 m/z, 1/0 start 0.6 s/ cm² End 1.6 s/cm²; ramp time 110.1 ms; lock duty cycle to 100%; capillary voltage 1,600 V; dry gas 31 min⁻¹; dry temperature 180°C. The PASEF settings were: 10 random mass spectrometry (MS) scans (total cycle time, 1.27 s); charge range 0–5; active exclusion for 0.4 min; scheduling target intensity 10,000; intensity threshold 2,500; collision-induced dissociation energy 42 eV.

Data integration. The mRNA and protein datasets were integrated and the correlation analysis was performed based on two different approaches: gene-wise and sample-wise.

Gene-wise. Spearman’s correlation between mRNA and protein across all samples was calculated for every single gene separately. For each gene, lower- and upper-tail P values were calculated from the empirical cumulative distribution function (ECDF). Significantly highly correlated genes (upper-tail P < 0.05) were selected to perform GSEA using Fisher’s exact test on the MSigDB. A Benjamini–Hochberg-adjusted P < 0.05 was considered significant.

Sample-wise. For every single gene, we calculated the average fold change between positive and negative. The gene set-specific correlation was assessed by first selecting the genes that belonged to a given gene set, then by calculating the mRNA fold change versus the protein fold change Spearman’s correlation of these genes. To estimate the significance of the correlation for one gene set, correlation values were also calculated from 10,000 random sets of genes, using the same number of genes as the given gene set. Finally, the ECDF was used to return lower- and upper-tail P values.

Transcription factor analysis. Functional analysis was performed with the DoRothEA package (1.4.1)\(^1\) for transcription factor activity and the PROGENy package (1.1.4.0)\(^1\) for pathway activity. To test the difference in activities, a factorial experiment was designed for each modality using the limma package. In both functional types, the first factor was the infection status of the patient (COVID-19/COVID-19+) and the second was if there were traces of the virus in their lungs (PCR+; PCR−). Thus, there were three factor combinations: a healthy patient (COVID-19/PCR−; Ctrl); an early-stage infection (COVID-19+/PCR−; negative); and a late-stage infection (COVID-19+/PCR+; positive). A linear model
was fitted for each transcription factor and pathway activity to obtain coefficients for each factorial combination. Then, these coefficients were used to compute three contrastic effects of COVID-19/PCR: based on COVID-19/PCR samples (negative versus Ctrl effects of COVID-19/PCR – based on COVID-19/PCR samples (positive versus Ctrl); and the difference between these two comparisons (positive versus negative). The first and second contrasts were aimed at detecting systematic changes in activity when an early or late-stage COVID-19 infection was taking place. However, the third contrast was aimed at detecting the specific difference in activity between late-stage and early-stage infection. The sign of the obtained coefficients can be interpreted as an increase or decrease of the mean activity for a given comparison, each with an associated probability.

**Reporting Summary** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability** The RNA-seq raw and processed data have been deposited at the University Hamburg Research Data Repository (https://www.fdr.uni-hamburg.de/) with the following identifiers: https://doi.org/10.25592/uhhfdm.8538 and https://doi.org/10.25592/uhhfdm.8572. The mass spectrometry-based proteomics data have been deposited at the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with the following dataset identifier: PXD027289. Source data are provided with this paper.

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Author contributions
V.G.P. and T.B.H. conceptualized the study. N.W., G.A. and V.G.P. prepared the manuscript. N.W., G.A., P.B.M., T.G., P.S. and V.G.P. analysed, visualized and curated the data. C.E., S.P., M.T.L., C.S.-L., J.R., M.N.W., Y.O., F.B., E.M., S.L., M.L.M.N., A.G., D.L., F.H., K.R. and S.S.H. performed the investigations. M.L., A.G., N.F., D.W., S.K., M.M.A., S.H., A.W.L., J.R., B.O., J.P.S., J.S.-R., M.B. and M.A. provided advice and expertise. P.S., V.G.P. and T.B.H. supervised the study.

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Extended Data Fig. 1 | Histopathology in autopsy samples. a, b, Histological sections from COVID-19 autopsy tissue without signs of overt cytopathic changes. Examples of other pathological alterations. c, Overview of a case with focal fatty changes and centrilobular necrosis, likely as a consequence of shock. d, Zoom in of a case with moderate fatty liver and marked centrilobular necrosis, also likely due to shock. These images represent the main findings after careful examination of 18 cases. Scale bar represents 350um.
Extended Data Fig. 2 | ACE2 expression. ACE expression (orange) and DNA (grey) in the human liver. Based on anatomical location, the staining pattern suggests expression by Kupffer cells. All images were taken at the same magnification. Scale bar represents 10um. This experiment was repeated 3 times in 3 samples with identical results.
Extended Data Fig. 3 | SARS-CoV-2 spike protein in autopsy samples. a, Expression of SARS-CoV-2 spike protein (orange) and DNA (grey) in autopsy tissue from 3 patients with confirmed RT-qPCR+ for SARS-CoV-2 in the respiratory tract (during clinical course) and in the liver (at autopsy). Based on anatomical locations, these findings suggest expression of SARS-CoV-2 spike protein in Kupffer cells, immune cells, and hepatocytes. Scale bar represents 10um.
Extended Data Fig. 4 | Expression of known SARS-CoV-2 entry receptors and facilitators in liver autopsy samples. 

a, Gene expression of ACE2 in different GTEX tissues shows log2 transcript per million (TPM) expression levels in the liver after intestine, kidney and lung. ACE2 expression levels in the liver samples of COVID-19 patients and controls are comparable with liver and lung expression in GTEX data set. 
b, Gene expression of CTSL and c, TMPRSS2 and d, RAB7A in different GTEX tissues shows log2 TPM expression levels in the liver after intestine, kidney and lung. Box plots showing boxes from the 25th to the 75th percentile with the median shown as a line in the middle and whiskers indicating 1.5 times the interquartile range.
Extended Data Fig. 5 | Detection of SARS-CoV-2 subgenomic RNA. a, Relative fraction of sgRNA in SARS-CoV-2-positive liver samples is comparable to pharyngeal swab sample data (medRxiv 2020.06.11.20127332; doi: 10.1101/2020.06.11.20127332). b, Total number of SARS-CoV-2-positive reads divided by total number of human reads (×1000) shows a higher number of reads aligning to the SARS-CoV-2 genome in SARS-CoV-2 liver POS samples than in SARS-CoV-2 liver NEG samples and controls. *, p-value = 0.0122 (Mann-Whitney test, two-tailed). Box plot: box extending from the 25th to the 75th percentile with the median shown as a line in the middle and whiskers indicating smallest and largest values.
Extended Data Fig. 6 | Transcriptional changes associated with SARS-CoV-2 hepatic tropism. a, Principal Component Analysis performed on the normalized intensity showing PCR positive (blue), PCR negative (red) Covid-19 liver samples and Control liver (green) samples. PCR+ samples are well embedded with healthy liver tissue. b, Volcano plot shows the differentially regulated genes (red dots, adjusted p-value < 0.05) in SARS-CoV-2 PCR positive vs. negative liver samples. Top 25 regulated genes, based on p-value, are labeled. c, Row-wise scaled intensity (mat, z-score) heatmap showing genes from Notch signaling pathway and d, Interferon gamma response gene-set. Genes are ranked according to the log2 fold change, indicated on the right side. Adjusted p-value < 0.05 is indicated with ‘*’. Within each group, samples are clustered based on Euclidean distance.
Extended Data Fig. 7 | Gene ontology analysis. Barplots showing the top 10 UP- and DOWN-regulated gene-sets from Gene Ontology Biological processes between PCR positive and negative samples. Color code represent the number of genes within each gene set.
Extended Data Fig. 8 | Canonical pathway analysis. **a**, Transcription factor analysis reveals differential transcription factor usage in CTL, COVID-19 liver NEG and liver POS samples. Summary of mean pathway activity in each infection status. **b**, Canonical pathways up (+) or down (−) regulated.
Extended Data Fig. 9 | Gene expression of SCARB1. Gene expression of SCARB1 in different GTEx tissues shows log2 transcript per million (TPM). Our own datasets are also used to provide context, including Liver (Controls) and Liver (COVID-19). Box plots showing boxes from the 25th to the 75th percentile with the median shown as a line in the middle and whiskers indicating 1.5 times the interquartile range.
Extended Data Fig. 10 | Protein expression of SR-B1 and SARS-CoV-2 spike in autopsy tissue. Protein expression of SR-B1 (cyan) in one post-mortem liver sample – and co-expression with SARS-CoV-2 spike protein (orange) within the same cell. In A, we show an overview of a large liver region, showcasing widespread expression of SR-B1 among hepatocytes with a zoom-in to a cell expressing also SARS-CoV-2 spike. In B, we show two additional regions with hepatocytes showing co-expression of SR-B1 and SARS-CoV-2 spike in the same cell. All images were performed in one specimen as proof-of-principle. Scale bars represent 20um.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection | all softwares used to perform data collection are described in the method section of the manuscript.

Data analysis | R package v3.4.2.2, GAGE R package v2.36.0, fgsa R package v1.12.0, PathView, MaxQuant 1.6.17, DoRothEA, PRG, GraphPad Prism v9.2.0

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RNA-sequencing raw and processed data has been deposited at the University Hamburg Research Data Repository (https://www.fdr.uni-hamburg.de/) with the following identifiers: 10.25592/uhhdfm.858 and 10.25592/uhhdfm.8372

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**

No statistical method were used to determine sample size as they were chosen based on availability (unique autopsy material) and previous experience for omics analyses, enabling us to analyse at least n=5 for each experimental group.

**Data exclusions**

No data was excluded.

**Replication**

Experiments were replicated as indicated in each figure legend.

**Randomization**

Not relevant to this study, as this is an observational and not an interventional study.

**Blinding**

Blinding was used whenever possible (i.e. validation of IF/ISH vs RT-qPCR in tissues, pathology examination). However, in most experiments blinding was not possible as analytical groups were defined a priori (i.e. omics, where samples were selected into specific categories).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a                              | n/a     |
| ☒ ☒ Antibodies                   | ☒ ☒ Involved in the study |
| ☒ Eukaryotic cell lines          | ☒ ChiP-seq |
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| ☒ Human research participants    |         |
| ☒ Clinical data                  |         |
| ☒ Dual use research of concern   |         |

**Antibodies**

Antibodies used

- ACE2 [R&D systems, AF933, dilution 1:200]
- SARS-CoV SA10 within S2 domain protein (Genetex, GTX632604, dilution 1:200)
- SR-B1 [Abscam, ab217318, dilution 1:200]

Validation

ACE2 was validated using human kidney tissues, showing strong expression in the proximal tubuli. SARS-CoV SA10 within S2 domain protein (Genetex, GTX632604) was validated in previous experiments (Puelles et al NEJM 2020 and Braun et al Lancet 2020) using infected Vero cells as positive controls and normal human tissue as negative controls.

SR-B1 was validated comparing the expression pattern to the report of Wei, C., et al. HDL scavenger receptor B type 1 facilitates SARS-CoV-2 entry. Nat. Metab. 2(12):1391-1400. doi: 10.1038/s42255-020-00324-0 (2020).

**Human research participants**

Policy information about: studies involving human research participants

**Population characteristics**

De-identified clinical data from two independent clinical cohorts was included - detailed information can be obtained from Fig. 1, Table S1, Table S2, and Data Source File 1.

The rest of the study was performed on autopsy tissue samples. Details can be obtained in Fig. 2 and Table S3

**Recruitment**

For clinical cohorts, patient data was acquired using an institutional board waiver of informed consent, meaning there was no active recruitment, rather clinical data collection of all hospitalized patients due to COVID-19 or diagnosis of COVID-19 during hospitalization. All available data was used, minimizing bias introduction.

For autopsy cohorts, we included tissues of patients with confirmed COVID-19 diagnosis.
Ethics oversight

Data collection from clinical patient cohorts were approved by the Institutional Review Board (IRB) of the University of Michigan (HUM00178971) and Hamburg (WF-052/20). The IRB approved a waiver of informed consent for this observational study. No compensation was provided.

For the autopsy study, the Ethics Committee of the Hamburg Chamber of Physicians was informed about the study (2020-10534-BO-H and PV7311). Informed consent was obtained from a next of kin or legal representatives for autopsy and tissue sampling. No compensation was paid.

Note that full information on the approval of the study protocol must also be provided in the manuscript.