Supplemental Information

SARS-CoV-2 infects the human kidney and drives fibrosis in kidney organoids

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Figure S1, related to Figure 1. Additional COVID-19 patient and control kidney SARS-CoV-2 nucleocapsid and KIM1 stainings. (A-D) SARS-CoV-2 nucleocapsid (red, arrow), ACE2 (green) and LTL (blue) expression in human autopsy kidney tissue derived from four different patients. Scale bar A, D 20 µm. Scale bar B, C, 10 µm. (E) SARS-CoV-2 nucleocapsid (red) and ACE2 (green) expression in human autopsy lung tissue. (F) Human non-COVID-19 nephrectomy kidney tissue and (G) autopsy kidney tissue control stained against SARS-CoV-2 nucleocapsid (red), ACE2 (green) and LTL (blue) (H) second antibody controls (SAB). (I-M) Kidney injury molecule-1 (KIM1), LTL and NKCC2 expression in human autopsy kidney tissue derived from 5 different COVID-19 patients. (N-O) Human non-COVID-19 autopsy kidney tissue controls stained against KIM1, LTL and NKCC2. (P) secondary antibody controls (SAB). Scale bars 50 µm.
Figure S2, related to Figure 2. COVID-19 snRNA-seq dataset characteristics and SARS-CoV-2 entry factor expression in COVID-19 kidney tissue. (A-D) snRNA-seq data quality plots. (E) Heatmap depicting the top 5 DE genes per cluster in COVID-19 patient snRNA-seq data. The top 20 DE genes are listed in Table S2. (F) Violin plots showing SARS-CoV-2 cell entry factors ACE2, TMPRSS2, Furin, and BSG, as well as SARS-CoV-2-related genes PLCG2 and AFDN per cluster in snRNA-seq data of COVID-19 patient kidney tissue. (G) Percentage of SARS-CoV-2-infected cells per cluster in the COVID-19 patient kidney tissue. Data are derived from one COVID-19 patient kidney biopsy specimen.
Figure S3, related to Figure 3. Viral transcript detection over time and SARS-CoV-2 nucleocapsid protein quantification in organoids and Immuno-based correlative light microscopy and electron microscopy pipeline of a SARS-CoV-2 infected cell. (A) Viral transcripts were detected 24, 48, 72, and 96 hours post infection (hpi) in kidney organoids. Data are mean and SD from 2 independent experiments each performed with 2 biological replicates. (B) Scoring of SARS-CoV-2 nucleocapsid protein expression in podocytes and proximal tubules. At least 80 podocyte clusters and proximal tubules per sample were manually scored. Data are mean and SD from 3 independent experiments each performed with 2 biological replicates. ** = p<0.01, *** = p<0.001. (C-D) Representative images of (C) one infected podocyte (NPHS1, red) cluster (white solid line) and (D) one proximal tubule (LTL, white solid line) as shown by SARS-CoV-2 nucleocapsid protein expression (green, white dashed line). Scale bar 20 µm. (E-F) SARS-CoV-2 nucleocapsid protein fluorescent signal detection (green) in a mesenchymal cell using confocal microscopy (E) prior to and (F) after high-pressure freezing followed by freeze substitution. (G) Fluorescent imaging of the organoid section containing the infected mesenchymal cell on the transmission electron microscopy (TEM) grid. The fluorescent image and the TEM image are from the same cell, but a different focal plane. (H) The SARS-CoV-2 nucleocapsid protein fluorescent signal matches the (I) corresponding ultrastructure analysis, as shown in the (J) overlay. (K-L) Viral particles are shown in vacuoles (arrows).
Figure S4, related to Figure 4. Supplementary data for scRNA seq of iPSC-derived kidney organoids. 

(A-D) Quality control data of scRNA seq data from organoids: (A) Number of genes detected in each cell per sample. (B) Total number of RNA detected within a cell per sample. Percentage of (C) mitochondrial and (D) ribosomal genes per sample. 

(E-G) Endothelial cell progenitors and PDGFRa/b+ cell population in organoids: 

(E) Endothelial cell progenitors (CD34+ETS1+EMCN+(Uhlén et al., 2015)) are present in mesenchyme cell cluster 1. 

(F) PDGFR alpha and (G) beta expression in kidney organoids. PDGFRα/b+ fibroblast population is most pronounced in mesenchyme cell cluster 1. 

(H) PDGFRα (green, interstitial compartment) and Nephrin 1 (NPHS1, purple, podocytes) protein expression in iPSC-derived kidney organoids. 

(I) PDGFRb (green, interstitial compartment) and LTL (blue, proximal tubules) protein expression in iPSC-derived kidney organoids.
(J) Violin plots illustrating the expression of ACE2, TMPRSS2, Furin, BSG, PLCG2, and AFDN per cluster in control and SARS-CoV-2-infected kidney organoids. (K) Virus genome coverage plot in SARS-CoV-2 sample 1. (L) Percentage of SARS-CoV-2 infected cells per cluster and samples SARS-CoV-2 1 and SARS-CoV-2 2. The respective percentages per cluster represent proportions of virus mapped cells. Data are derived from one scRNA sequencing experiment. A total of eight separate kidney organoids were pooled in one sample. Two mock-infected control samples and two SARS-CoV-2-infected samples were used as input for scRNAseq.
Figure S5, related to Figure 5. Additional GO terms, PROGENy, MSigDB Hallmark pathway activity and Ligand-receptor interaction profiling of SARS-CoV-2-infected human iPSC-derived kidney organoids. (A) GO terms analysis showing up- and downregulation of virus infection and processing related pathways upon SARS-CoV-2 infection of kidney organoids. (B) PROGENy analysis showing Wnt, TGFβ, Hypoxia, VEGF,
Androgen, and Estrogen pathway activity in Mesenchyme clusters (Mes 1, Mes 2) and kidney epithelial cells. * = p<0.05, ** = p<0.01, *** = p<0.001. (C-E) MSigDB Hallmark analysis providing evidence of upregulation of virus infection-related cellular target upregulation in (C) proximal tubular cells, (D) podocytes, and (E) mesenchyme 1. (F-H) GO terms analysis comparing infected vs. non-infected cells per cluster in (F) proximal tubular cells, (G) podocytes, and (H) mesenchyme 1. (I) Barplot showing all up and downregulated ligand-receptor pairs in the kidney - and mesenchyme clusters. (J) Cell-cell interaction bar plot showing the total number of up and downregulated ligand-receptor pairs in the kidney - and mesenchyme clusters. Data are derived from one scRNA sequencing experiment. A total of eight separate kidney organoids were pooled in one sample. Two mock-infected control samples and two SARS-CoV-2-infected samples were used as input for scRNAseq.
Supplementary Tables

Table S1, related to Figure 1. Characteristics and corresponding statistics for the COVID-19 and control patients’ cohorts.

| Parameter        | Cohorts           | Statistical analysis                             | test applied  |
|------------------|-------------------|-------------------------------------------------|---------------|
|                  | control | ARDS    | COVID-19 | control vs. COVID-19 | ARDS vs. COVID-19 | control vs. ARDS |               |
| Age [years]*     | 79.0 (±16) | 54.5 (±15.5) | 73.0 (±17.5) | ns | p<0.001 | p<0.001 | Mann-Whitney U test |
| male [count]     | 29      | 6       | 47       | ns | ns      | ns      | χ² test |
| female [count]   | 14      | 8       | 15       | p<0.001 | ns | p<0.001 | χ² test |
| CKD (%)          | 55.8    | 14.2    | 30.6     | p=0.003 | ns | ns      | χ² test |
| Hypertension (%) | 44.2    | 57.1    | 51.6     | p=0.004 | p=0.047 | p=0.007 | χ² test |
| Diabetes (%)     | 18.6    | 0       | 27.4     | p=0.004 | p=0.047 | p=0.007 | χ² test |

*Median ± interquartile range
Table S2, related to Figure 2. Top 20 differentially expressed genes per cluster in the COVID-19 tissue specimen.

| Rank | Principal cells | PTEC | LOH-TAL | DCT | IC-A | IC-B | PEC | CNT | EC | Mesengial cell | Podocytes | Leucocytes | Fibroblasts |
|------|----------------|------|---------|-----|-----|-----|-----|-----|----|----------------|-----------|------------|------------|
| 1    | ATP1B3         | PDK4 | AC012593.1 | CALCR | UNC5D | AL136962.1 | PDK4 | RGS6 | PDE3A | AC096577.1 | AC096577.1 | LEF1 | IGF1 |
| 2    | NR4A1          | LINGO2 | EGF | TRPM7 | CA8 | AC5M2B | NR4A1 | DEFB1 | AC096577.1 | DHFR | EGR1 | NR4A1 | ADAMTS3 |
| 3    | GDF15          | AFF2 | TIPARP | ADGR3 | PPM1E | AC096577.1 | ZFP36 | AC092691.1 | AC5M2B | SLCA4A4 | MSR3 | IL7R | SYT1 |
| 4    | PDE10A         | LRRCC7 | PCK1 | SLC12A3 | MSRA | CCBE1 | KLHL13 | AC004784.1 | MT2A | NRXN3 | WDR49 | RNF212B | PLPP1 |
| 5    | AC087379.2     | MID1 | VTCN1 | LINC01828 | AC013472.2 | ACM2A | ATPB3 | SNTG1 | ACM2A | MT2E | PAMR1 | RGS2 | GREB1L |
| 6    | RALYL          | FRY | FGL2 | AC004870.4 | FTH1 | LRP2 | SNAP25 | BOX1 | SULF1 | SLC12A1 | KALRN | TSPAN5 | LINC02388 |
| 7    | AC004784.1     | DGKG | RIPOR1 | FAM20C | RUNX1 | FYN | FGF14 | CLSN2 | COL8A1 | RCAN2 | AC009975.1 | SLC22A6 | CLIC5 |
| 8    | PNM5           | CYP4A11 | LINC01482 | LINC01482 | AC092650.1 | CFTR | NAMPT | PTCHD4 | EMP1 | LGR6 | MID1 | ACM2A | PCDH11X |
| 9    | ZFP36          | NECAB1 | SCNN1G | KSR2 | AC098829.1 | PTPRO | VCAM1 | HSP9A | FTCD | ACSMA2 | ANX1A | ATP10A | NFATC2 |
| 10   | PACRG          | CREB5 | ATP6V0A4 | UBE2D2 | SLC12A3 | SDK2 | NXN | STGAL1 | NAV3 | CLKN | PCDH9 | ADAMTS1L | LINC00924 |
| 11   | MAG1           | HSPO9A1A | BOX1 | ABTB2 | HSPO9A1A | LAMA2 | ACSS2 | KCD8 | SIPA1L2 | RBFOX1 | ESRPG | NAV2 | HGF |
| 12   | FGF13          | PLCG2 | CCDC178 | DEPTOR | EFNA5 | CPEB4 | SLT2 | LINC0278 | ADAMTS1L | CPE | TMEM117 | GPAM | PWRN1 |
| 13   | GALNT17        | AKAP12 | LRRK2 | SLC8A1 | TOX | MTRNR2L12 | COL4A3 | MT-ATP8 | NCO7 | ACER2 | ADAMTS19-AS1 | TTN | LINC00989 |
| 14   | ARHGP18        | SAMD4A | UBE2D2 | MOY1E | GALTNL6 | MT-ATP8 | AL020268.1 | SOX5 | ADGR8B3 | KClPN4 | COBL1 | CD36 | PDE1C |
| 15   | ITGA6          | ITGB8 | DEPTOR | MIR4435-2HG | MAGIC2 | SGCD | HMGS1 | LTBP1 | MTRNR2L8 | NRG3 | MECOM | ZFPM2 | ABCA8 |
| 16   | ATP6V0D2       | NRG1 | AC019197.1 | NRP1 | LINC01320 | ATP6V1C2 | KClPN4 | PRKAG2 | STGAL1 | SEMA5A | PCDH7 | MIR99AHG | ACSS2 |
| 17   | RCAN2          | DCC | AC024022.1 | ARIL5 | CCSSER1 | AST2 | PTPRD | LINC01482 | ETS1 | P4HA1 | KClPN4 | IGFBP7 | ARHGP15 |
| 18   | SLC26A7        | DLGAP1 | PLCG2 | HSPO9A1A | SLC8A1 | AC138340.1 | FMN2 | LINC01320 | FLRT2 | SLC2A3 | LRP1B | KALRN | GPAM |
| 19   | CLNK           | COL4A1 | SLC8A1 | PRKG1 | KClPN4 | KClNB2 | PLCG2 | NLGN1 | HSPO9A1A | HSPO9A1A | SLC8A1 | PLPP1 | NEGR1 |
| rank | Mesenchyme 1 | Mesenchyme 2 | PT Cells | Podocytes | LP Cells | Neural 1 | Neural 2 | Neural 3 | Neural 4 | Neural 5 | NC Cells | Muscle 1 | Muscle 2 | Muscle 3 | Muscle 4 |
|------|-------------|-------------|----------|-----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| 1    | COL3A1      | PAX7        | SPP1     | MAFB      | TOP2A    | RSP03    | ZIC2     | STMN2    | STMN2    | PTN      | NHLH1    | USP18    | ACTC1    | TTN      | ACTC1   |
| 2    | COL1A1      | TCEAL9      | GSTP1    | ITM2B     | CENPF    | MSX1     | NFIA     | STMN4    | DUSP8    | SOX2     | STMN2    | MYOG     | MYH3     | MYH3     | TNNC2   |
| 3    | DGN         | PITX2       | KRT18    | MAGi2-AS3 | HMBG2    | ZIC2     | RMST     | CRABP1   | JUN      | FABP7    | NEUROD1  | CDKN1C   | ACTA1    | NEB      | MYL1    |
| 4    | LGALS1      | FRMD4A      | EPCAM    | NPHS2     | UBE2C    | SOX2     | GRID2    | TUBB3    | ANK3     | CCN1D    | TUBB3    | KLHL41   | TNNC2    | TNNC2    | MYLPF   |
| 5    | COL1A2      | RRBP1       | FXYD2    | PODXL     | MKS7     | ID4      | MSX1     | TUBB3B   | STMN4    | CALR     | INSM1    | HSPA1A   | TTN      | MEF2C    | MYH3    |
| 6    | PRRX1       | CLCN5       | DCDC2    | ANXA1     | ASPM     | LYPD1    | AEBP1    | TUBA1A   | NRTK2    | ACAN1A   | MAP1B    | ZNF106   | IL17B    | MYLPF    | KLHL41  |
| 7    | SFRP2       | PDGFA       | APOE     | PTPRO     | PRC1     | ID2      | NEAT1    | JUN      | GRIA2    | TTYH1    | CACNA1A  | RBM24    | MYL1     | ANKRD3C  | ACTA1   |
| 8    | COL5A2      | TBX1        | MT−CO3   | S100A6    | TPX2     | RFX4     | PREX1    | RTN1     | RAPGEF6  | EDNRB    | ENC1     | PDLIM3   | MYLFP    | TNNT2    | TNNT2   |
| 9    | COL1A2      | FGFR4       | S100A9   | WT1       | HistH4C  | ZNF503   | EFNB2    | GAP43    | TUBA1A   | IGFBP5   | PHLD1A   | ALPK2    | TNNT1    | NEXN     | TNNP    |
| 10   | NUPR1       | PDGFC       | FTL      | MME       | UBE2S    | CCN2     | ZNF503   | MLTL1    | ACAN1A   | NRTK2    | MAP1B    | ZNF106   | IL17B    | MYLPF    | KLHL41  |
| 11   | VCAN        | SPATS2L     | KRT19    | RASSF8    | CKS2     | TMEM47   | CCN2     | KIF5C    | CLASP2   | IFITM3   | BASP1    | RGS2     | MYBPH    | TRDN     | IL17B   |
| 12   | AKAP12      | SIX1        | MT−ND3   | GADD45A   | CDK1     | WLS      | SLC5A    | CD24     | KIF5C    | HSP90B1  | SOX4     | RASSF4   | TNNT2    | ACTA1    | TNNT2   |
| 13   | COL6A3      | TAF1D       | S100A6   | CLIC5     | NUSAP1   | HOXD4    | RFX4     | BCL2L1   | NCAM1    | SPARC1   | TUBA1A   | SOX8     | TPM2     | MYL1     | TTN     |
| 14   | MGP          | HSFA1B      | ALDH1A1  | BST2      | SMC4     | EFNB2    | MED13L   | GPM6A    | MIA      | HSPA5    | TGLN3    | PRDX6    | COL1A1   | RYR1     | DES     |
| 15   | TM2A        | NRK         | MT−CO2   | SQSTM1    | PTTG1    | PTPN     | MIR99AHG | BASP1    | PLCG2    | IGFBP2   | SSTR2    | EFHD2    | NEXN     | MYBPH    | COL1A1  |
| 16   | LUM         | COL1A2      | TAGLN    | SERPINE2  | CDC20    | ZFP36L1  | LINC00472| MAP1B    | NOVA1    | PPB      | TUBB2B   | HSPB1    | DES      | TNNI     | ZNF106   |
| 17   | PDGFA      | PLCG2       | MT−ND6   | TGFB3     | ARL6L1   | ZIC5     | MT−CO2   | NSG2     | ACAP3    | ITPR2D   | NRXN1    | PL3      | TNNT1    | TNNT3    | MYOG    |
| 18   | COL6A2      | ADAT5       | EMX2     | FTL       | CCNB1    | AS1      | MALAT1   | DUSP8    | RTN1     | MKD      | TOX3     | FND5C    | SMPX     | MYH8     | PRDX6   |
| 19   | CPE         | SOX8        | LAMA1    | VEGFA     | KPN2A    | ID1      | ADGRV1   | NRR1     | CRABP1   | SAT1     | POU4F1   | VGLL2    | CRYAB    | MACF1    | MYBPH   |
| 20   | COL4A2      | IGFBP5      | MPC2     | MXRA8     | PLK1     | SLC3A2   | XPO1     | GNG3     | TUBB3    | PDI3     | RGS10    | CHRN1B   | TNN3     | TNNI2    | HSPB2   |
Table S4, related to Figure 4. Viral reads and percentage of infected cells per cell type per organoid sample.

| Cluster     | viral reads SARS-CoV-2 s1 | viral reads SARS-CoV-2 s2 | total cell numbers per cluster SARS-CoV-2 s1 | % of infected cells per cluster s1 | total cell numbers per cluster SARS-CoV-2 s2 | % of infected cells per cluster s2 |
|-------------|---------------------------|---------------------------|---------------------------------------------|----------------------------------|---------------------------------------------|----------------------------------|
| 1 Mesenchyme 1 | 21                        | 12                        | 199                                         | 6.03                             | 26                                          | 10                               | 4.50                             |
| 2 Mesenchyme 2 | 4                         | 4                         | 107                                         | 3.74                             | 3                                           | 3                                | 118                              |
| 3 PT Cells    | 3                         | 2                         | 93                                          | 2.15                             | 2                                           | 2                                | 92                               |
| 4 Podocytes   | 7                         | 4                         | 137                                         | 2.92                             | 2                                           | 1                                | 131                              |
| 5 LP Cells    | 0                         | 0                         | 81                                          | 0.00                             | 8                                           | 3                                | 65                               |
| 6 Neural Cells 1 | 35                       | 15                        | 256                                         | 5.86                             | 144                                         | 13                               | 220                              |
| 7 Neural Cells 2 | 19                       | 5                         | 219                                         | 2.28                             | 22                                          | 5                                | 220                              |
| 8 Neural Cells 3 | 1                        | 1                         | 85                                          | 1.18                             | 22                                          | 1                                | 102                              |
| 9 Neural Cells 4 | 0                        | 0                         | 44                                          | 0.00                             | 0                                           | 0                                | 34                               |
| 10 Neural Cells 5 | 18                       | 11                        | 92                                          | 11.96                            | 31                                          | 7                                | 96                               |
| 11 NP Cells   | 8                         | 3                         | 80                                          | 3.75                             | 2                                           | 2                                | 87                               |
| 12 Muscle Cells 1 | 0                        | 0                         | 29                                          | 0.00                             | 2                                           | 1                                | 30                               |
| 13 Muscle Cells 2 | 0                        | 0                         | 8                                           | 0.00                             | 1                                           | 1                                | 33                               |
| 14 Muscle Cells 3 | 0                        | 0                         | 22                                          | 0.00                             | 0                                           | 0                                | 24                               |
| 15 Muscle Cells 4 | 0                        | 0                         | 2                                           | 0.00                             | 0                                           | 0                                | 1                                |

S1: Organoid sample 1, S2: organoid sample 2.
Table S5, related to STAR Methods section CLEM. Freeze substitution protocol used during the CLEM sample preparation.

| Step | Tstart (°C) | Tend (°C) | Slope | Time | Reagent  | Conc.  | Transfer | Pause | UV |
|------|-------------|-----------|-------|------|----------|--------|----------|-------|----|
| 1    | -90         | -90       | 0     | 4:00 | Fs cocktail | 100%   | Stay     |       |    |
| 2    | -90         | -45       | 5     | 9:00 | Fs cocktail | 100%   | Stay     |       |    |
| 3    | -45         | -45       | 0     | 5:00 | Fs cocktail | 100%   | Stay     |       |    |
| 4    | -45         | -45       | 0     | 0:10 | Acetone    | 100%   | Exchange |       |    |
| 5    | -45         | -45       | 0     | 0:10 | Acetone    | 100%   | Exchange |       |    |
| 6    | -45         | -45       | 0     | 0:10 | Acetone    | 100%   | Exchange |       |    |
| 7    | -45         | -45       | 0     | 2:00 | R221/acetone | 25%   | Exchange |       |    |
| 8    | -45         | -45       | 0     | 2:00 | R221/acetone | 50%   | Exchange |       |    |
| 9    | -45         | -30       | 7.5   | 2:00 | R221/acetone | 75%   | Exchange |       |    |
| 10   | -30         | -30       | 0     | 12:00| R221      | 100%   | Exchange | X     |    |
| 11   | -30         | -30       | 0     | 2:00 | R221      | 100%   | Exchange | X     |    |
| 12   | -30         | -30       | 0     | 48:00:00 | R221 | 100%   | Stay     | X     |    |
| 13   | -30         | 20        | 5     | 10:00| R221      | 100%   | Stay     | X     |    |
| 14   | 20          | 20        | 0     | 48:00:00| R221  | 100%   | Stay     | X     |    |