

# SARS-CoV-2 infection and persistence throughout the human body and brain

**Daniel Chertow** (✉ [chertowd@cc.nih.gov](mailto:chertowd@cc.nih.gov))

National Institutes of Health <https://orcid.org/0000-0002-1675-1728>

**Sydney Stein**

National Institutes of Health <https://orcid.org/0000-0002-0259-4485>

**Sabrina Ramelli**

National Institutes of Health

**Alison Grazioli**

National Institutes of Health

**Joon-Yong Chung**

NCI <https://orcid.org/0000-0001-5041-5982>

**Manmeet Singh**

National Institutes of Health

**Claude Kwe Yinda**

National Institute of Allergy and Infectious Diseases

**Clayton Winkler**

National Institutes of Health

**James Dickey**

National Institutes of Health

**Kris Ylaya**

National Cancer Institute

**Sung Hee Ko**

National Institutes of Health

**Andrew Platt**

National Institutes of Health

**Peter Burbelo**

8Dental Clinical Research Core, NIDCR, NIH <https://orcid.org/0000-0003-1717-048X>

**Martha Quezado**

National Institutes of Health

**Stefania Pittaluga**

NIH

**Madeleine Purcell**

University of Maryland School of Medicine

**Vincent Munster**

National Institute of Allergy and Infectious Diseases <https://orcid.org/0000-0002-2288-3196>

**Frida Belinky**

NIH

**Marcos Ramos-Benitez**

National Institutes of Health

**Eli Boritz**

National Institutes of Health

**Daniel Herr**

University of Maryland School of Medicine

**Joseph Rabin**

University of Maryland School of Medicine

**Kapil Saharia**

University of Maryland School of Medicine

**Ronson Madathil**

University of Maryland School of Medicine

**Ali Tabatabai**

University of Maryland School of Medicine

**Shahabuddin Soherwardi**

TidalHealth Peninsula Regional

**Michael McCurdy**

University of Maryland St. Joseph Medical Center <https://orcid.org/0000-0001-5319-0475>

**Karin Peterson**

National Institutes of Health

**Jeffrey Cohen**

National Institutes of Health

**Emmie de Wit**

National Institute of Allergy and Infectious Diseases <https://orcid.org/0000-0002-9763-7758>

**Kevin Vannella**

National Institutes of Health

**Stephen Hewitt**

National Cancer Institute, National Institutes of Health <https://orcid.org/0000-0001-8283-1788>

**David Kleiner**

National Cancer Institute <https://orcid.org/0000-0003-3442-4453>

---

**Biological Sciences - Article**

**Keywords:**

**Posted Date:** December 20th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-1139035/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

1 SARS-CoV-2 infection and persistence throughout the human body and brain

2  
3 Authors:

4 Sydney R. Stein<sup>1,2</sup>, Sabrina C. Ramelli<sup>3</sup>, Alison Grazioli<sup>4</sup>, Joon-Yong Chung<sup>5</sup>, Manmeet Singh<sup>6</sup>,  
5 Claude Kwe Yinda<sup>6</sup>, Clayton W. Winkler<sup>7</sup>, James M. Dickey<sup>1,2</sup>, Kris Ylaya<sup>5</sup>, Sung Hee Ko<sup>8</sup>,  
6 Andrew Platt<sup>1,2</sup>, Peter D. Burbelo<sup>9</sup>, Martha Quezado<sup>5</sup>, Stefania Pittaluga<sup>5</sup>, Madeleine Purcell<sup>10</sup>,  
7 Vincent J. Munster<sup>6</sup>, Frida Belinky<sup>8</sup>, Marcos J. Ramos-Benitez<sup>1,2,11</sup>, Eli A. Boritz<sup>8</sup>, Daniel L.  
8 Herr<sup>12</sup>, Joseph Rabin<sup>13</sup>, Kapil K. Saharia<sup>14,15</sup>, Ronson J. Madathil<sup>16</sup>, Ali Tabatabai<sup>17</sup>,  
9 Shahabuddin Soherwardi<sup>18</sup>, Michael T. McCurdy<sup>19,17</sup>, NIH COVID-19 Autopsy Consortium<sup>^</sup>,  
10 Karin E. Peterson<sup>7</sup>, Jeffrey I. Cohen<sup>19</sup>, Emmie de Wit<sup>6</sup>, Kevin M. Vannella<sup>1,2</sup>, Stephen M.  
11 Hewitt<sup>5</sup>, David E. Kleiner<sup>5</sup>, Daniel S. Chertow<sup>1,2\*</sup>

12 Affiliations:

- 13 1. Emerging Pathogens Section, Critical Care Medicine Department, Clinical Center,  
14 National Institutes of Health, Bethesda, MD, USA
- 15 2. Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases,  
16 National Institutes of Health, Bethesda, MD, USA
- 17 3. Critical Care Medicine Department, Clinical Center, National Institutes of Health,  
18 Bethesda, MD, USA
- 19 4. Kidney Disease Section, Kidney Diseases Branch, National Institute of Diabetes and  
20 Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA
- 21 5. Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National  
22 Institutes of Health, Bethesda, MD, USA

- 23 6. Laboratory of Virology, Division of Intramural Research, National Institute of Allergy  
24 and Infectious Diseases, National Institute of Health, Hamilton, MT, USA
- 25 7. Laboratory of Persistence Viral Diseases, Rocky Mountain Laboratories, National  
26 Institute of Allergy and Infectious Diseases, National Institute of Health, Hamilton, MT,  
27 USA
- 28 8. Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National  
29 Institutes of Health, Bethesda, MD, USA
- 30 9. National Institute of Dental and Craniofacial Research, National Institutes of Health,  
31 Bethesda, MD, USA
- 32 10. University of Maryland School of Medicine, Baltimore, MD, USA
- 33 11. Postdoctoral Research Associate Training Program, National Institute of General Medical  
34 Sciences, National Institutes of Health, Bethesda, MD, USA
- 35 12. R Adams Cowley Shock Trauma Center, Department of Medicine and Program in  
36 Trauma, University of Maryland School of Medicine, Baltimore, MD, USA
- 37 13. R Adams Cowley Shock Trauma Center, Department of Surgery and Program in Trauma,  
38 University of Maryland School of Medicine, Baltimore, MD, USA
- 39 14. Department of Medicine, Division of Infectious Disease, University of Maryland School  
40 of Medicine, Baltimore, MD, USA
- 41 15. Institute of Human Virology, University of Maryland School of Medicine, Baltimore,  
42 MD, USA
- 43 16. Department of Surgery, Division of Cardiac Surgery, University of Maryland School of  
44 Medicine, Baltimore, MD, USA

45 17. Department of Medicine, Division of Pulmonary and Critical Care Medicine, University  
46 of Maryland School of Medicine, Baltimore, MD, USA

47 18. Hospitalist Department, TidalHealth Peninsula Regional, Salisbury, MD, USA

48 19. Division of Critical Care Medicine, Department of Medicine, University of Maryland St.  
49 Joseph Medical Center, Towson, MD, USA

50 20. Medical Virology Section, Laboratory of Infectious Diseases, National Institute of  
51 Allergy and Infectious Diseases, National Institutes of Health, Bethesda MD

52 ^See Acknowledgements

53 \*Corresponding author. Email: chertowd@cc.nih.gov

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68           **COVID-19 is known to cause multi-organ dysfunction<sup>1-3</sup> in acute infection, with**  
69 **prolonged symptoms experienced by some patients, termed Post-Acute Sequelae of SARS-**  
70 **CoV-2 (PASC)<sup>4-5</sup>. However, the burden of infection outside the respiratory tract and time**  
71 **to viral clearance is not well characterized, particularly in the brain<sup>3,6-14</sup>. We performed**  
72 **complete autopsies on 44 patients with COVID-19 to map and quantify SARS-CoV-2**  
73 **distribution, replication, and cell-type specificity across the human body, including brain,**  
74 **from acute infection through over seven months following symptom onset. We show that**  
75 **SARS-CoV-2 is widely distributed, even among patients who died with asymptomatic to**  
76 **mild COVID-19, and that virus replication is present in multiple pulmonary and**  
77 **extrapulmonary tissues early in infection. Further, we detected persistent SARS-CoV-2**  
78 **RNA in multiple anatomic sites, including regions throughout the brain, for up to 230 days**  
79 **following symptom onset. Despite extensive distribution of SARS-CoV-2 in the body, we**  
80 **observed a paucity of inflammation or direct viral cytopathology outside of the lungs. Our**  
81 **data prove that SARS-CoV-2 causes systemic infection and can persist in the body for**  
82 **months.**

83 **Main text:**

84           Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the  
85 causative agent of coronavirus disease 2019 (COVID-19), has well described pulmonary and  
86 extrapulmonary manifestations<sup>1-3</sup>, including multiorgan failure and shock among severe and fatal  
87 cases. Some survivors experience Post-Acute Sequelae of SARS-CoV-2 (PASC) – also known as  
88 Long COVID—with cardiovascular, pulmonary, and neurological manifestations with or without  
89 functional impairment<sup>4-5</sup>. While autopsy studies of fatal COVID-19 cases support the ability of  
90 SARS-CoV-2 to infect multiple organs<sup>3,7-12</sup>, extra-pulmonary organs often lack histopathological

91 evidence of direct virally-mediated injury or inflammation<sup>10-14</sup>. The paradox of extra-pulmonary  
92 infection without injury or inflammation raises many pathogen- and host-related questions.  
93 These questions include, but are not limited to: What is the burden of infection within versus  
94 outside of the respiratory tract? What cell types are infected across extra-pulmonary tissues, and  
95 do they support SARS-CoV-2 infection and replication? In the absence of cellular injury and  
96 inflammation in extra-pulmonary tissues, does SARS-CoV-2 persist, and if so, over what  
97 interval? Does SARS-CoV-2 evolve as it spreads to and persists in different anatomical  
98 compartments?

99         To inform these pathogen-focused questions and to evaluate for the presence or absence  
100 of associated histopathology in matched tissue specimens, we performed extensive autopsies on  
101 a diverse population of 44 individuals who died from or with COVID-19 up to 230 days  
102 following initial symptom onset. Our approach focused on timely, systematic, and  
103 comprehensive tissue sampling and preservation of adjacent tissue samples for complementary  
104 analyses. We performed droplet digital polymerase chain reaction (ddPCR) for sensitive  
105 detection and quantification of SARS-CoV-2 gene targets in all tissue samples collected. To  
106 elucidate SARS-CoV-2 cell-type specificity and validate ddPCR findings, we performed *in situ*  
107 hybridization (ISH) broadly across sampled tissues. Immunohistochemistry (IHC) was used to  
108 further validate cell-type specificity in the brain where controversy remains on the regional  
109 distribution and cellular tropism of SARS-CoV-2 infection. In all samples where SARS-CoV-2  
110 RNA was detected by ddPCR, we performed qRT-PCR to detect subgenomic (sg)RNA, an assay  
111 suggestive of recent virus replication<sup>15</sup>. We confirmed the presence of replication-competent  
112 SARS-CoV-2 in extrapulmonary tissues by virus isolation in cell culture. Lastly, in six



113 individuals, we measured the diversity and anatomic distribution of intra-individual SARS-CoV-  
114 2 variants using high-throughput, single-genome amplification and sequencing (HT-SGS).

115 We categorized autopsy cases of SARS-CoV-2 infection as “early” (n=17), “mid”  
116 (n=13), or “late” (n=14) by illness day (D) at the time of death, being  $\leq$ D14, D15-D30, or  $\geq$ D31,  
117 respectively. We defined persistence as presence of SARS-CoV-2 RNA among late cases. Due to  
118 the extensive tissue collection, we analyzed and described the results in terms of grouped tissue  
119 categories as the following: respiratory tract; cardiovascular; lymphoid; gastrointestinal; renal  
120 and endocrine; reproductive; muscle, skin, adipose, & peripheral nerves; and brain.

121

## 122 **Autopsy cohort overview**

123 Between April 26, 2020 and March 2, 2021, we performed autopsies on 44 PCR-  
124 confirmed cases (Extended Data Fig. 1). SARS-CoV-2 seroconversion was detected in 38 of  
125 these cases (Supplementary Data 1); three early cases (P27, P36, P37) had not seroconverted and  
126 perimortem plasma was unavailable for the other three cases (P3, P4, P15). Extensive sampling  
127 of the brain was accomplished in 11 of the 44 cases (Fig. 1). The cohort was 29.5% female with  
128 a mean age of 59.2 years and was diverse across race and ethnicity (Extended Data Table 1).  
129 95.5% of patients had at least one comorbidity, with hypertension (54.5%), obesity (52.3%), and  
130 chronic respiratory disease (34.1%) being most common. Patients presented to the hospital a  
131 mean of 9.4 days following symptom onset and were hospitalized a mean of 26.4 days. Overall,  
132 the mean interval from symptom onset to death was 35.2 days and the mean postmortem interval  
133 was 26.2 hours. 81.8% of patients required intubation with invasive mechanical ventilation,  
134 22.7% received extracorporeal membrane oxygenation (ECMO) support, and 40.9% required  
135 renal replacement therapy. Vasopressors, systemic steroids, systemic anticoagulation, and

136 antibiotics were commonly administered (Extended Data Table 1). Individual patient-level  
137 demographic and clinical information can be found in Extended Data Table 2.

138

### 139 **Widespread infection and persistence**

140 SARS-CoV-2 RNA was detected in all 44 cases and across 79 of 85 anatomical locations  
141 and body fluids sampled (Extended Data Fig. 2, Supplementary Data 1). The highest burden of  
142 SARS-CoV-2 RNA (i.e., >100,000 N gene copies/ng RNA input) was detected in the respiratory  
143 tract of early cases (Figure 1), but we detected at least 100 N gene copies/ng RNA input from  
144 every tissue group besides reproductive tissues from multiple individuals among early cases. The  
145 mean SARS-CoV-2 N gene copies/ng RNA detected from tissues in each grouping among early  
146 cases are as follows: 9,210.10 across respiratory tissues; 38.75 across cardiovascular tissues;  
147 30.01 across lymphoid tissues; 24.68 across gastrointestinal tissues; 12.76 across renal and  
148 endocrine tissues; 0.36 across reproductive tissues; 27.50 across muscle, peripheral nerve,  
149 adipose, and skin tissues; 57.40 across ocular tissues; and 32.93 across brain tissues (Extended  
150 Data Table 3).

151 With a few exceptions, the overall burden of SARS-CoV-2 RNA decreased by a log or  
152 more across tissue categories among mid cases, and further decreased among late cases.  
153 However, several mid and late cases had high levels ( $\geq 5$  N gene copies/ng RNA input) detected  
154 among multiple tissues (Extended Data Fig. 2). Further, persistence of low-level SARS-CoV-2  
155 RNA (0.0004 to <0.5 N gene copies/ng RNA input) was frequently detected across multiple  
156 tissue categories among all late cases, despite being undetectable in plasma (Extended Data Fig.  
157 2, Supplementary Data 1). Notably, SARS-CoV-2 RNA was detected in the brains of all six late

158 cases and across most locations evaluated in the brain in five of these six, including P42 who  
159 died at D230 (Fig. 1).

160 Overall, SARS-CoV-2 RNA was detected in respiratory tissue of 43/44 cases (97.7%);  
161 cardiovascular tissue of 35/44 cases (79.5%); lymphoid tissue of 38/44 cases (86.4%);  
162 gastrointestinal tissue of 32/44 (72.7%); renal and endocrine tissue of 28/44 cases (63.6%);  
163 reproductive tissue in 17/40 cases (42.5%); muscle, skin, adipose, and peripheral nervous tissue  
164 in 30/44 cases (68.2%); ocular tissue and humors of 22/28 cases (57.9%); and brain tissue in  
165 10/11 cases (90.9%) (Extended Data Table 3).

166 We additionally detected SARS-CoV-2 sgRNA across all tissue categories,  
167 predominately among early cases (14/17, 82.4%), as well as in plasma, pleural fluid, and vitreous  
168 humor (Fig. 1, Extended Data Fig. 2, Supplementary Data 1). sgRNA was also detected in at  
169 least one tissue of 61.5% of mid cases and 42.9% of late cases, including across three tissue  
170 categories in a case at D99 (P20).

171 We isolated SARS-CoV-2 in cell culture from multiple pulmonary and extrapulmonary  
172 tissues, including lung, bronchus, sinus turbinate, heart, mediastinal lymph node, small intestine,  
173 and adrenal gland from early cases up to D7 (P19, P27, P32, P37; Supplementary Data 1).

174

### 175 **Intra-individual viral variant diversity**

176 We used HT-SGS to analyze SARS-CoV-2 spike gene variant sequences from a total of  
177 46 tissues in six individuals. In five individuals from the early group, predominant spike  
178 sequences were largely identical across tissues. In P27, P19, and P18, no non-synonymous virus  
179 genetic diversity was detected in pulmonary and extrapulmonary sites despite a high depth of  
180 single-molecule sampling (Extended Data Fig. 3). Thus, virus populations that were relatively

181 homogeneous had disseminated in these individuals without coding changes in spike. However,  
182 we also noted important patterns of intra-individual virus diversity in several patients from the  
183 early group. In P27, although all 4,525 inferred spike amino acid sequences were identical, two  
184 virus haplotypes, each with a single synonymous substitution, were preferentially detected in  
185 extrapulmonary sites including right and left ventricles and mediastinal LN. In P38, we observed  
186 clear virus genetic differences between the lung lobes and the brain, with a D80F residue found  
187 in 31/31 pulmonary but 0/490 brain sequences and a G1219V residue that was restricted to brain  
188 minor variants. A similar distinction was observed between sequences from dura mater and other  
189 sites in P36, albeit at very low sampling depth (n = 2 sequences) from dura mater. Overall, these  
190 findings suggested no need for alterations in receptor utilization to permit extrapulmonary  
191 dissemination of SARS-CoV-2, while also revealing genetic compartmentalization between  
192 viruses in the lung lobes and those in extrapulmonary sites, including the brain.

193

#### 194 **ISH reveals SARS-CoV-2 cellular tropism**

195 We validated our ddPCR results across all tissue categories via ISH for SARS-CoV-2  
196 spike RNA across selected early, mid, and late cases (Supplementary Data 3). Overall, we  
197 detected SARS-CoV-2 RNA via ISH in 36 distinct cell types across all sampled organs  
198 (Extended Data Table 4, Supplementary Data 3). Spike RNA was detected throughout the  
199 respiratory tract in early cases, as well as within the sinus turbinate, trachea, lungs, from late  
200 cases (i.e., P33, P20, P42).

201 The heart contained spike RNA within myocytes, endothelium, and smooth muscle of  
202 vessels of both early (P18, P19) and late (P3 & P42) cases. The pericardium demonstrated a  
203 positive signal for spike RNA within fibroblasts of the stroma. Intimal cells of the aorta were

204 additionally found to contain spike RNA. Mononuclear leukocytes within the lymph node,  
205 spleen, and appendix of an early case (P19) contained spike RNA, as did colonic epithelium (Fig  
206 2).

207         Epithelial cells along the intestinal tract in early cases (P16, P18, P19) contained viral  
208 RNA, as well as stratified squamous epithelium of the esophagus. Mononuclear leukocytes were  
209 again visualized with SARS-CoV-2 RNA in lymphoid aggregates and the interstitium of the  
210 small and large intestine, with infected cells still present in the colon of late cases (P33, P42).  
211 Kupffer cells, hepatocytes, and bile duct epithelium within the liver were additionally found to  
212 contain spike RNA.

213         Within the kidney, spike RNA could be visualized within parietal epithelium of  
214 Bowman's capsule, collecting duct cells, distal tubule cells, and glomerular endothelium. The  
215 adrenal glands contained spike RNA within endocrine cells. Endocrine follicular cells of the  
216 thyroid and glandular cells of the pancreas were also positive for spike RNA (Fig. 2). Among  
217 reproductive organs, spike RNA was visualized within Leydig and Sertoli cells of the testis,  
218 germ cells within the testicular tubules, endometrial gland epithelium, endometrial stromal cells,  
219 uterine smooth muscle cells, and stromal cells of the post-menopause ovary (Fig. 2).

220         Myocytes within skeletal muscle contained spike RNA in both early (P18) and late (P20)  
221 cases. In addition to the organ-specific cell type infection of SARS-CoV-2, endothelium,  
222 muscularis of atrial vessels, and Schwann cells were identified as infected throughout the body,  
223 and were similarly positive across early and late cases.

224         Spike RNA was found in neurons, glia and ependyma, as well as endothelium of vessels  
225 across all lobes of the brain of early, mid, and late cases. Within the cerebellum specifically,

226 neurons, Purkinje cells, and endothelium of vasculature also contained spike protein via IHC  
227 (Fig. 3).

228

### 229 **COVID-19 histological findings**

230 The histopathology findings from our cohort were similar to those reported in other case  
231 series (Extended Data Fig. 4). All but five cases were considered to have died from COVID-19  
232 (Extended Data Table 5), and, of these, 37 (94.5%) had either acute pneumonia or diffuse  
233 alveolar damage at the time of death (Supplementary Data 2). Phases of diffuse alveolar damage  
234 showed clear temporal associations, with the exudative phase seen mainly within the first three  
235 weeks of infection and the fibrosing phase not seen until after a month of infection (Extended  
236 Data Fig. 5). Pulmonary thromboembolic complications, which were also likely related to  
237 SARS-CoV-2 infection, with or without infarction, were noted in 10 (23%) cases. Another  
238 finding likely related to SARS-CoV-2 infection included myocardial infiltrates in four cases,  
239 including one case of significant myocarditis<sup>16</sup> (P3). Some of the cases of microscopic ischemia  
240 appeared to be associated with fibrin-platelet microthrombi, and may therefore be related to  
241 COVID-19 thrombotic complications. Within the lymph nodes and spleen, we observed  
242 lymphodepletion and both follicular and paracortical hyperplasia.

243 Outside the lungs, histological changes were mainly related to complications of therapy  
244 or preexisting co-morbidities: mainly obesity, diabetes, and hypertension. Five cases had old  
245 ischemic myocardial scars and three had coronary artery bypass grafts in place. Given the  
246 prevalence of diabetes and obesity in our cohort, it was not surprising to find diabetic  
247 nephropathy (10 cases, 23%) or steatohepatitis (5 cases, 12%). One case was known to have  
248 chronic hepatitis C with cirrhosis, but the other cases of advanced hepatic fibrosis were likely

249 related to fatty liver disease, even if diagnostic features of steatohepatitis were not present.  
250 Hepatic necrosis (13 cases, 30%) and changes consistent with acute kidney injury (17 cases,  
251 39%) were likely related to hypoxic-ischemic injury in these very ill patients.

252 In the examination of the 11 brains, we found few histopathologic changes, despite the  
253 evidence of substantial viral burden. Vascular congestion was an unusual finding that had an  
254 unclear etiology and could be related to the hemodynamic changes incurred with infection.  
255 Global hypoxic/ischemic change was seen in two cases, one of which was a juvenile (P36) with a  
256 seizure disorder who was found to be SARS-CoV-2 positive on hospital admission, but who  
257 likely died of seizure complications unrelated to viral infection.

258

## 259 **Discussion**

260 Here we provide the most comprehensive analysis to date of SARS-CoV-2 cellular  
261 tropism, quantification, and persistence across the body and brain, in a diverse autopsy cohort  
262 collected throughout the first year of the pandemic in the United States. Our focus on short post-  
263 mortem intervals, comprehensive approach to tissue collection, and preservation techniques –  
264 *RNAlater* and flash freezing of fresh tissue – allowed us to detect and quantify viral levels with  
265 high sensitivity by ddPCR and ISH, as well as culture virus, which are notable differences  
266 compared to other studies.

267 We show SARS-CoV-2 disseminates across the human body and brain early in infection  
268 at high levels, and provide evidence of virus replication at multiple extrapulmonary sites during  
269 the first week following symptom onset. We detected sgRNA in at least one tissue in over half of  
270 cases (14/27) beyond D14, suggesting that prolonged viral replication may occur in extra-  
271 pulmonary tissues as late as D99. While others have questioned if extrapulmonary viral presence

272 is due to either residual blood within the tissue<sup>8,17</sup> or cross-contamination from the lungs during  
273 tissue procurement<sup>8</sup>, our data rule out both theories. Only 12 cases had detectable SARS-CoV-2  
274 RNA in a perimortem plasma sample, and of these only two early cases also had SARS-CoV-2  
275 sgRNA in the plasma, which occurred at Ct levels higher than nearly all of their tissues with  
276 sgRNA. Therefore, residual blood contamination cannot account for RNA levels within tissues.  
277 Furthermore, blood contamination would not account for the SARS-CoV-2 sgRNA or virus  
278 isolated from tissues. Contamination of additional tissues during procurement, is likewise ruled  
279 out by ISH demonstrating widespread SARS-CoV-2 cellular tropism across the sampled organs,  
280 by IHC detecting viral protein in the brain, and by several cases of virus genetic  
281 compartmentalization in which spike variant sequences that were abundant in extrapulmonary  
282 tissues were rare or undetected in lung samples.

283         Using both ddPCR and sgRNA analysis to inform our selection of tissue for virus  
284 isolation and ISH staining allow us to describe a number of novel findings. Others<sup>6,8-12,17</sup> have  
285 previously reported SARS-CoV-2 RNA within the heart, lymph node, small intestine, and  
286 adrenal gland. We demonstrate conclusively that SARS-CoV-2 is capable of infecting and  
287 replicating within these tissues. Current literature has also reported absent or controversial  
288 expression of ACE2 and/or TMPRSS2 in several extrapulmonary tissues, such as the colon,  
289 lymphoid tissues, and ocular tissues, calling into question if these tissues can become infected by  
290 SARS-CoV-2<sup>1-3</sup>. However, we observed high levels of SARS-CoV-2 RNA and evidence of  
291 replication within these organs, as well as SARS-CoV-2 RNA via ISH in colonic mucosal  
292 epithelium and mononuclear leukocytes within the spleen, thoracic cavity lymph nodes, and GI  
293 lymphoid aggregates. We believe these ISH positive cells represent either infection or



294 phagocytized virus in resident macrophages. Further, we isolated virus from a mediastinal lymph  
295 node and ocular tissue from two early cases (P19, P32).

296 Our use of a single-copy sequencing approach for the SARS-CoV-2 spike allowed us to  
297 demonstrate homogeneous virus populations in many tissues, while also revealing informative  
298 virus variants in others. Low intra-individual diversity of SARS-CoV-2 sequences has been  
299 observed frequently in previous studies<sup>18-20</sup>, and likely relates to the intrinsic mutation rate of the  
300 virus as well as lack of early immune pressure to drive virus evolution in new infections. It is  
301 important to note that our HT-SGS approach has both a high accuracy and a high sensitivity for  
302 minor variants within each sample, making findings of low virus diversity highly reliable<sup>21</sup>. The  
303 virus genetic compartmentalization that we observed between pulmonary and extrapulmonary  
304 sites in several individuals supports independent replication of the virus at these sites, rather than  
305 spillover from one site to another. Importantly, lack of compartmentalization between these sites  
306 in other individuals does not rule out independent virus replication, as independently replicating  
307 populations may share identical sequences if overall diversity is very low. It was also interesting  
308 to note several cases where brain-derived virus spike sequences showed non-synonymous  
309 differences relative to sequences from other tissues. These differences may indicate differential  
310 selective pressure on spike by antiviral antibodies in brain versus other sites, though further  
311 studies will be needed to confirm this speculation.

312 Our results collectively show while that the highest burden of SARS-CoV-2 is in the  
313 airways and lung, the virus can disseminate early during infection and infect cells throughout the  
314 entire body, including widely throughout the brain. While others have posited this viral  
315 dissemination occurs through cell trafficking<sup>11</sup> due to a reported failure to culture virus from  
316 blood<sup>3,22</sup>, our data support an early viremic phase, which seeds the virus throughout the body

317 following pulmonary infection. Recent work by Jacobs et al.<sup>22</sup> in which SARS-CoV-2 virions  
318 were pelleted and imaged from COVID-19 patient plasma, supports this mechanism of viral  
319 dissemination. Although our cohort is primarily made up of severe cases of COVID-19, two  
320 early cases had mild respiratory symptoms (P28; fatal pulmonary embolism occurred at home) or  
321 no symptoms (P36; diagnosed upon hospitalization for ultimately fatal complications of a  
322 comorbidity), yet still had SARS-CoV-2 RNA widely detected across the body, including brain,  
323 with detection of sgRNA in multiple compartments. Our findings, therefore, suggest viremia  
324 leading to body-wide dissemination, including across the blood-brain barrier, and viral  
325 replication can occur early in COVID-19, even in asymptomatic or mild cases. Further, P36 was  
326 a juvenile with no evidence of multisystem inflammatory syndrome in children, suggesting  
327 infected children without severe COVID-19 can also experience systemic infection with SARS-  
328 CoV-2.

329         Finally, a major contribution of our work is a greater understanding of the duration and  
330 locations at which SARS-CoV-2 can persist. While the respiratory tract was the most common  
331 location in which SARS-CoV-2 RNA tends to linger,  $\geq 50\%$  of late cases also had persistence in  
332 the myocardium, thoracic cavity lymph nodes, tongue, peripheral nerves, ocular tissue, and in all  
333 sampled areas of the brain, except the dura mater. Interestingly, despite having much lower  
334 levels of SARS-CoV-2 in early cases compared to respiratory tissues, we found similar levels  
335 between pulmonary and the extrapulmonary tissue categories in late cases. This less efficient  
336 viral clearance in extrapulmonary tissues is perhaps related to a less robust innate and adaptive  
337 immune response outside the respiratory tract.

338         We detected sgRNA in tissue of over 60% of the cohort. While less definitive than viral  
339 culture<sup>23,24</sup>, multiple studies have shown that sgRNA levels correlate with acute infection and can

340 be detected in respiratory samples of immunocompromised patients experiencing prolonged  
341 infection<sup>24</sup>. These data coupled with ISH suggest that SARS-CoV-2 can replicate within tissue  
342 for over 3 months after infection in some individuals, with RNA failing to clear from multiple  
343 compartments for up to D230. This persistence of viral RNA and sgRNA may represent infection  
344 with defective virus, which has been described in persistent infection with measles virus –  
345 another single-strand enveloped RNA virus—in cases of subacute sclerosing panencephalitis<sup>25</sup>.

346         The mechanisms contributing to PASC are still being investigated; however, ongoing  
347 systemic and local inflammatory responses have been proposed to play a role<sup>5</sup>. Our data provide  
348 evidence for delayed viral clearance, but do not support significant inflammation outside of the  
349 respiratory tract even among patients who died months after symptom onset. Understanding the  
350 mechanisms by which SARS-CoV-2 persists and the cellular and subcellular host responses to  
351 viral persistence promises to improve the understanding and clinical management of PASC.

352

353

354

355

356

357

358

359

360

361

362

363 **Main References:**

- 364 1. Bourgonje, A. R. *et al.* Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the  
365 pathophysiology of coronavirus disease 2019 (COVID-19). *J Pathol.* **251**(3), 228-248  
366 (2020). <https://doi.org/10.1002/path.5471>.
- 367 2. Salamanna, F., Maglio, M., Landini, M. P., & Fini, M. Body Localization of ACE-2: On  
368 the Trail of the Keyhole of SARS-CoV-2. *Front Med (Lausanne)*. **7**, 594495 (2021).  
369 <https://doi.org/10.3389/fmed.2020.594495>.
- 370 3. Sridhar, S., & Nicholls, J. Pathophysiology of infection with SARS-CoV-2-What is  
371 known and what remains a mystery. *Respirology*. **26**(7), 652-665 (2021).  
372 <https://doi.org/10.1111/resp.14091>.
- 373 4. Al-Aly, Z., Xie, Y., & Bowe, B. High-dimensional characterization of post-acute  
374 sequelae of COVID-19. *Nature*. **594**(7862), 259-264 (2021).  
375 <https://doi.org/10.1038/s41586-021-03553-9>.
- 376 5. Crook, H., Raza, S., Nowell, J., Young, M., & Edison, P. Long covid-mechanisms, risk  
377 factors, and management. *BMJ*. **374**, n1648 (2021). <https://doi.org/10.1136/bmj.n1648>.
- 378 6. Puelles, V. G., *et al.* Multiorgan and Renal Tropism of SARS-CoV-2. *N Engl J Med*.  
379 **383**(6), 590-592 (2020). <https://doi.org/10.1056/NEJMc2011400>.
- 380 7. Martines, R. B., *et al.* Pathology and Pathogenesis of SARS-CoV-2 Associated with Fatal  
381 Coronavirus Disease, United States. *Emerg Infect Dis*. **26**(9), 2005-2015 (2020).  
382 <https://doi.org/10.3201/eid2609.202095>.
- 383 8. Bhatnagar, J., *et al.* Evidence of Severe Acute Respiratory Syndrome Coronavirus 2  
384 Replication and Tropism in the Lungs, Airways, and Vascular Endothelium of Patients

- 385 With Fatal Coronavirus Disease 2019: An Autopsy Case Series. *J Infect Dis.* **223**(5), 752-  
386 764 (2021). <https://doi.org/10.1093/infdis/jiab039>.
- 387 9. Dorward, D. A., *et al.* Tissue-Specific Immunopathology in Fatal COVID-19. *Am J*  
388 *Respir Crit Care Med.* **203**(2),192-201 (2021). <https://doi.org/10.1164/rccm.202008->  
389 3265OC.
- 390 10. Schurink, B., *et al.* Viral presence and immunopathology in patients with lethal COVID-  
391 19: a prospective autopsy cohort study. *Lancet Microbe.* **1**(7), e290-e299 (2020).  
392 [https://doi.org/10.1016/S2666-5247\(20\)30144-0](https://doi.org/10.1016/S2666-5247(20)30144-0).
- 393 11. Yao, X.H., *et al.* A cohort autopsy study defines COVID-19 systemic pathogenesis. *Cell*  
394 *Res.* **31**(8), 836-846 (2021). <https://doi.org/10.1038/s41422-021-00523-8>.
- 395 12. Remmelink, M., *et al.* Unspecific post-mortem findings despite multiorgan viral spread in  
396 COVID-19 patients. *Crit Care.* **24**(10), 495 (2020). <https://doi.org/10.1186/s13054-020->  
397 03218-5.
- 398 13. Mukerji, S. S., & Solomon, I. H. What can we learn from brain autopsies in COVID-19?  
399 *Neurosci Lett.* **742**, 135528 (2021). <https://doi.org/10.1016/j.neulet.2020.135528>.
- 400 14. Matschke, J., *et al.* Neuropathology of patients with COVID-19 in Germany: a post-  
401 mortem case series. *Lancet Neurol.* **19**(11), 919-929 (2020).  
402 [https://doi.org/10.1016/S1474-4422\(20\)30308-2](https://doi.org/10.1016/S1474-4422(20)30308-2).
- 403 15. Speranza, E., *et al.* Single-cell RNA sequencing reveals SARS-CoV-2 infection dynamics  
404 in lungs of African green monkeys. *Sci Transl Med.* **13**(578), eabe8146 (2021).  
405 <https://doi.org/10.1126/scitranslmed.abe8146>
- 406 16. Vannella, K. M., *et al.* Evidence of SARS-CoV-2-specific T-cell-mediated myocarditis in  
407 a MIS-A case. *Front Immunol.* Accepted (manuscript ID: 779026).

- 408 17. Desai, N., et al. Temporal and spatial heterogeneity of host response to SARS-CoV-2  
409 pulmonary infection. *Nat Commun.* **11**(1), 6319 (2020). [https://doi.org/10.1038/s41467-](https://doi.org/10.1038/s41467-020-20139-7)  
410 [020-20139-7](https://doi.org/10.1038/s41467-020-20139-7).
- 411 18. Tonkin-Hill, G., et al. Patterns of within-host genetic diversity in SARS-CoV-2. *Elife.* **10**,  
412 [e66857](https://doi.org/10.7554/eLife.66857) (2021). <https://doi.org/10.7554/eLife.66857>.
- 413 19. Lythgoe, K. A., et al. SARS-CoV-2 within-host diversity and transmission. *Science.*  
414 **372**(6539), eabg0821 (2021). <https://doi.org/10.1126/science.abg0821>.
- 415 20. Valesano, A. L., et al. Temporal dynamics of SARS-CoV-2 mutation accumulation  
416 within and across infected hosts. *PLoS Pathog.* **17**(4), e1009499 (2021).  
417 <https://doi.org/10.1371/journal.ppat.1009499>.
- 418 21. Ko, S. H., et al. High-throughput, single-copy sequencing reveals SARS-CoV-2 spike  
419 variants coincident with mounting humoral immunity during acute COVID-19. *PLoS*  
420 *Pathog.* **17**(4), e1009431 (2021). <https://doi.org/10.1371/journal.ppat.1009431>.
- 421 22. Jacobs, J. L., et al. SARS-CoV-2 Viremia is Associated with COVID-19 Severity and  
422 Predicts Clinical Outcomes. *Clin Infect Dis.* **10**, ciab686 (2021).  
423 <https://doi.org/10.1093/cid/ciab686>.
- 424 23. Alexandersen, S., Chamings, A., & Bhatta, T. R. SARS-CoV-2 genomic and subgenomic  
425 RNAs in diagnostic samples are not an indicator of active replication. *Nat Commun.*  
426 **11**(1), 6059 (2020). <https://doi.org/10.1038/s41467-020-19883-7>.
- 427 24. Binnicker, M. J. Can Testing Predict SARS-CoV-2 Infectivity? The Potential for Certain  
428 Methods To Be Surrogates for Replication-Competent Virus. *J Clin Microbiol.* **59**(11),  
429 [e0046921](https://doi.org/10.1128/JCM.00469-21) (2021). <https://doi.org/10.1128/JCM.00469-21>.

430 25. Sidhu, M. S., et al. Defective measles virus in human subacute sclerosing panencephalitis  
431 brain. *Virology*. **202**(20), 631-641 (1994). <https://doi.org/10.1006/viro.1994.1384>.

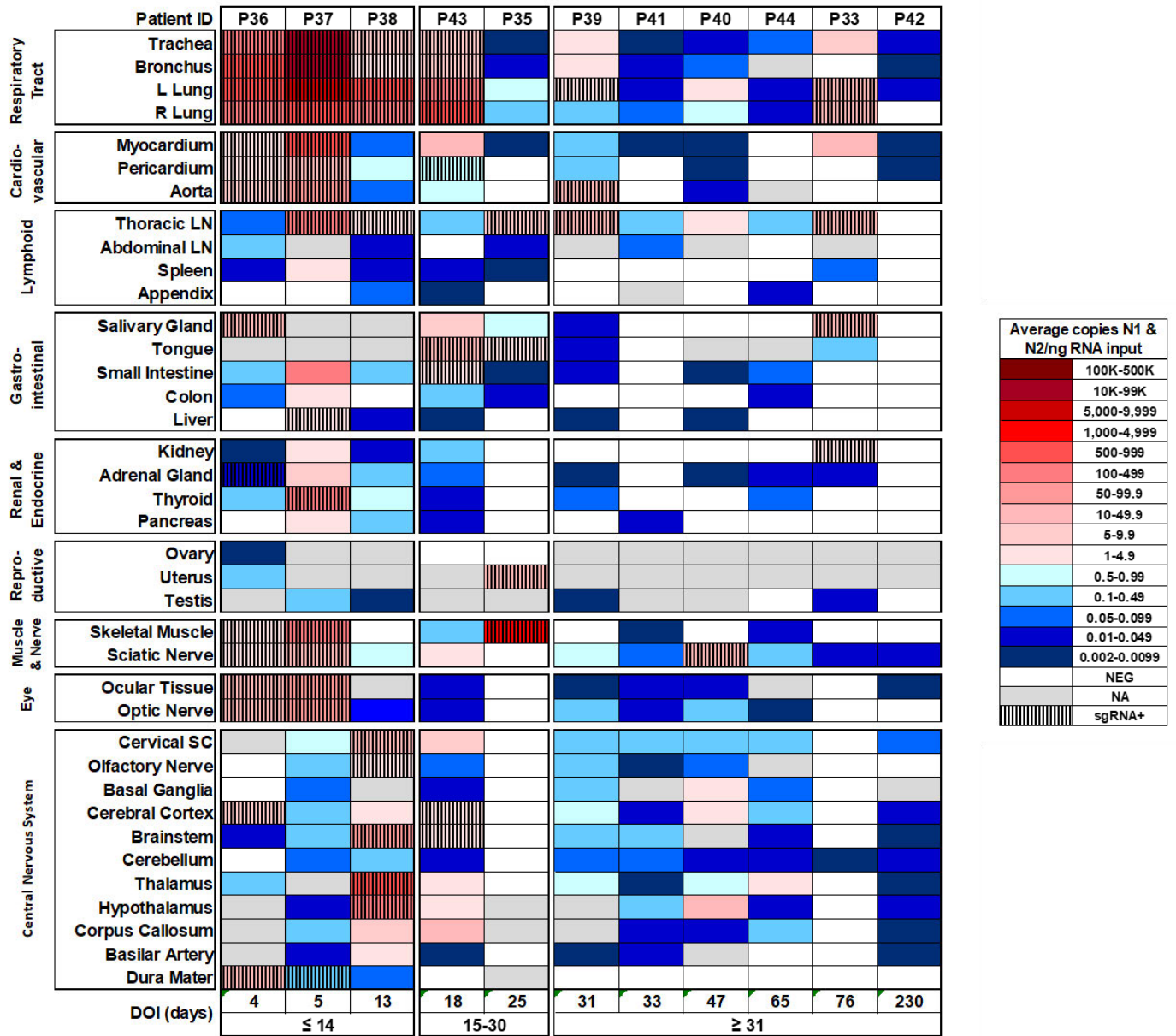
432

433

434

435

436



437

438 **Fig. 1 Distribution, quantification, and replication of SARS-Cov-2 across the human body**

439 **and brain.** The heat map depicts the highest mean quantification of SARS-CoV-2 RNA (N) via

440 ddPCR present within the tissues of eleven COVID-19 autopsy patients who underwent whole

441 body and brain sampling. Patients are aligned from shortest to longest duration of illness (DOI)

442 prior to death, listed at the bottom of the figure, and grouped into early ( $\leq 14$  days), mid (15-30

443 days), and late ( $\geq 31$  days) DOI. Tissues are grouped by tissue category beginning with the



444 respiratory tract at the top and central nervous system at the bottom. Viral RNA levels range  
445 from 0.002 to 500,000 N gene copies per ng of RNA input, depicted as a gradient from dark blue  
446 at the lowest level to dark red at the highest level. Tissues that were also positive for sgRNA via  
447 real-time RT-PCR are shaded with black vertical bars. L/left, LN/lymph node, NA/not acquired,  
448 R/right, SC/spinal cord.

449

450

451

452

453

454

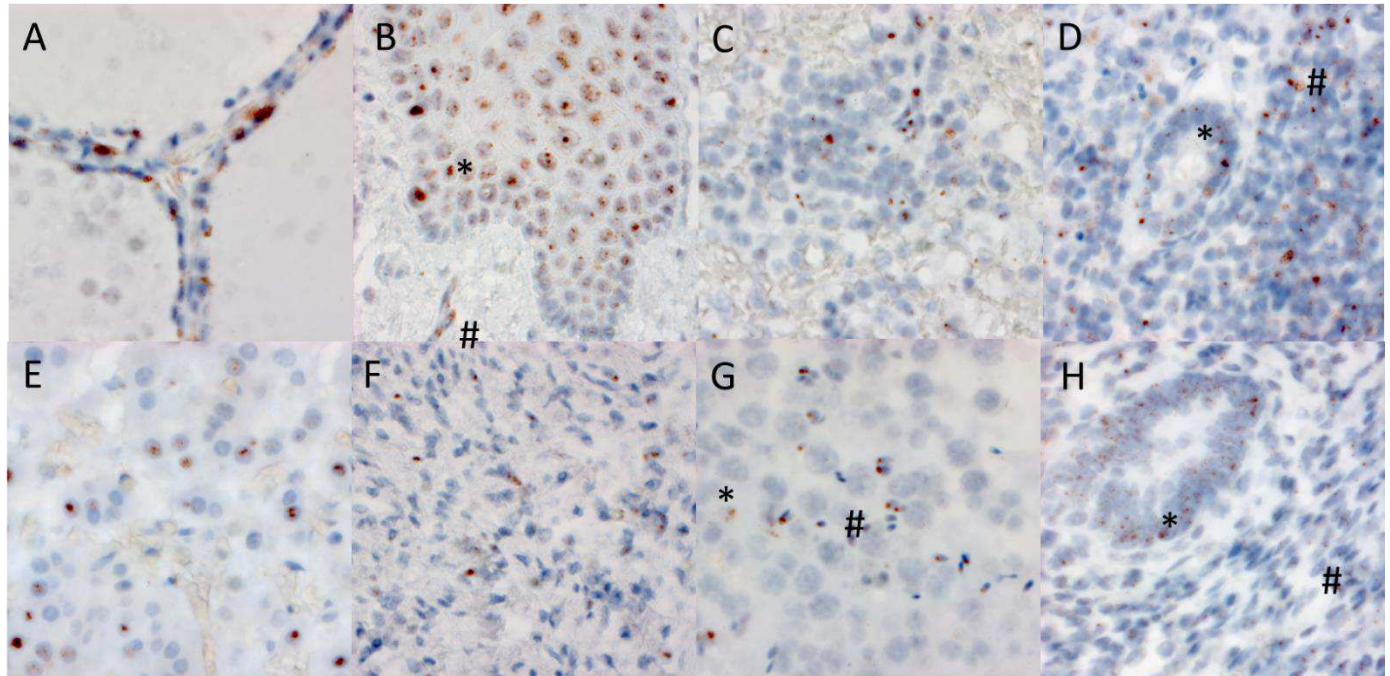
455

456

457

458

459

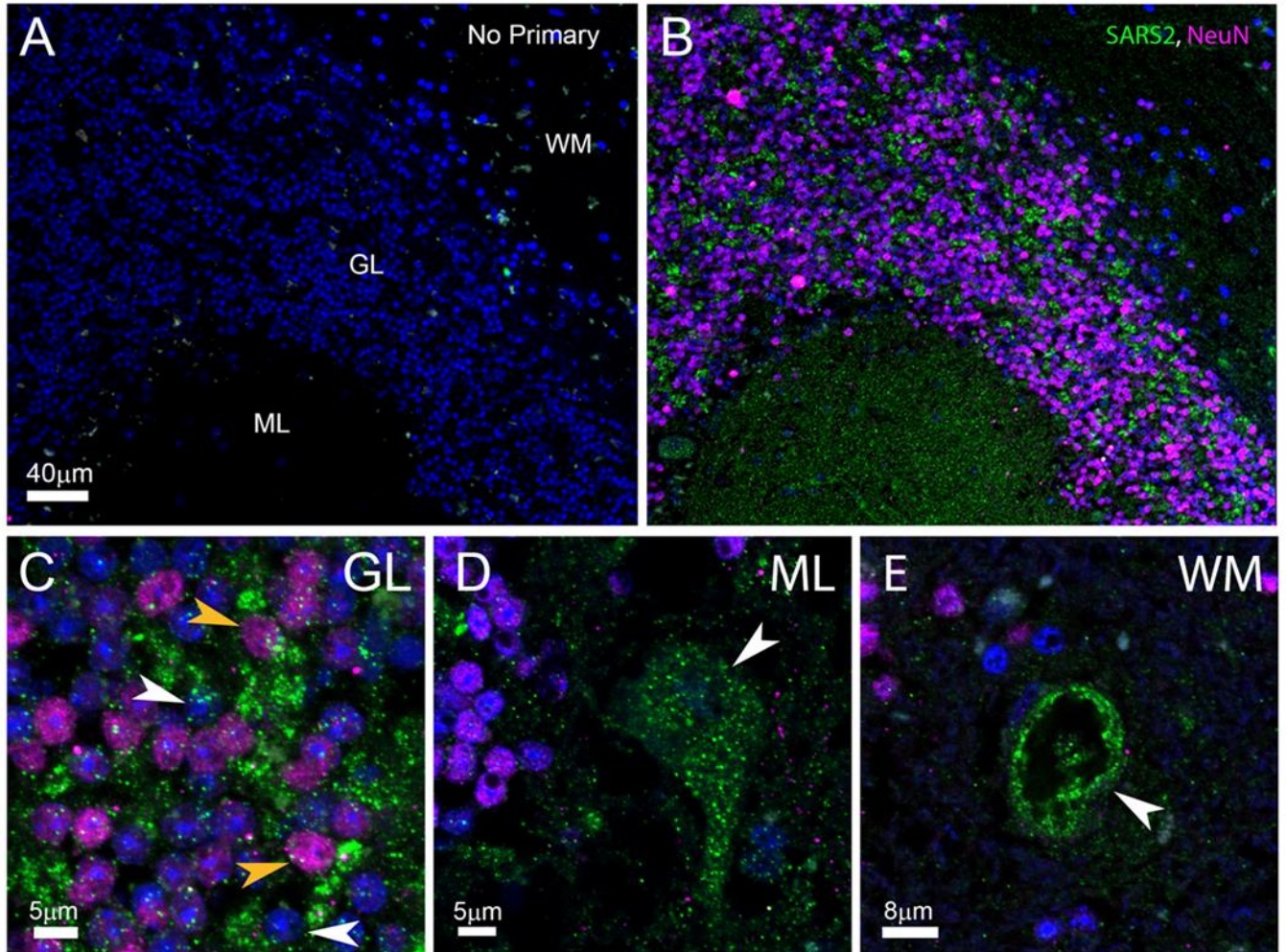


460

461 **Fig. 2 RNA *in situ* (RNAscope) detection of SARS-CoV-2 in extrapulmonary tissues.**

462 SARS-CoV-2 virus is localized to the Golgi and endoplasmic, peri-nuclear in appearance, in the  
 463 following organs and cell types (500 X magnifications): A) Thyroid, demonstrating presence of  
 464 virus within follicular cells. B) Esophagus, demonstrating the presence of virus within the  
 465 stratified squamous epithelium (\*), as well as signal in capillaries within the stroma (#). C.  
 466 Spleen, demonstrating the presence of mononuclear lymphoid cells within the white pulp. D)  
 467 Appendix, demonstrating the presence of virus in both colonic epithelium (\*) and mononuclear  
 468 lymphoid cells in the stroma (#). E) Adrenal demonstrates virus within endocrine secretory cells  
 469 of the adrenal gland. F) Ovary demonstrates the presence of virus in stromal cells of the ovary in  
 470 a post-menopausal ovary. G) Testis demonstrates the presence of virus in both Sertoli cells (\*)  
 471 and maturing germ cells within the seminiferous tubules of the testis (#). H) Endometrium  
 472 demonstrates the presence of virus within endometrial gland epithelium (\*) and stromal cells (#),  
 473 in a pre-menopausal endometrial sample.

474



475

476 **Fig. 3 SARS-CoV-2 protein expression in human cerebellum.** Low magnification  
 477 visualization of no-primary control (A) and primary-added adjacent (B) cerebellar sections  
 478 labeled for SARS-CoV-2 (green) and NeuN (magenta) demonstrate viral-specific protein  
 479 expression within the tissue. The locations of the molecular layer (ML), granular layer (GL), and  
 480 white matter (WM) are indicated in (A) and also correspond to (B). Higher magnification images  
 481 demonstrate cell type-specific infection (C-E). Both NeuN positive neurons (yellow arrows) and  
 482 other unidentified cells (white arrows) are associated with viral protein in the GL (C). Purkinje  
 483 cells adjacent to the ML are infected (D, white arrow). In rare instances, blood vessels adjacent

484 to the GL and WM were associated with viral protein (E, white arrow). The scale bars in A is  
485 also associated with B. All immunofluorescent images were obtained by confocal microscopy.

486

487

488

489

490

491

492

493

494

495

496

497

498

499

500

501

502

503

504

505

506

507 **Methods:**

508 **Autopsies**

509 Autopsies were performed and tissues were collected as previously described<sup>26</sup> in the National  
510 Cancer Institute's Laboratory of Pathology at the National Institutes of Health Clinical Center  
511 following consent of the legal next of kin.

512

513 **Measurement of IgG and IgM antibodies against Nucleocapsid and Spike protein of SARS-**  
514 **CoV-2**

515 Fluid-phase luciferase immunoprecipitation systems (LIPS) assays were used to study IgG and  
516 IgM antibody response to SARS-CoV-2. For IgG LIPS measurements, *Renilla* luciferase-  
517 nucleocapsid and *Gaussia* luciferase-spike protein extracts were employed with protein A/G  
518 beads (Protein A/G UltraLink Resin, Thermo Fisher Scientific) as the IgG capture reagent as  
519 previously described with microtiter filter plates<sup>27</sup>. For IgM measurements, anti-human IgM goat  
520 agarose beads (Sigma) were substituted as the capture reagent using both the microfilter plate  
521 and microtube format<sup>28</sup>. The IgM immunoprecipitation assays performed in 1.5 ml microfuge  
522 tube format containing 1 µl sera or plasma, *Renilla* luciferase-nucleocapsid (10 million light unit  
523 input per tube) or *Gaussia* luciferase-spike protein (40 million light input per tube) and buffer A  
524 (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM MgCl<sub>2</sub>, 0.1% Triton X-100) to a total volume of 100  
525 µl. After mixing, the tubes were incubated at room temp for 1 hour. Next 10 µl of the anti-human  
526 IgM agarose bead suspension was added to each tube for additional 60 minutes and tubes were  
527 placed on a rotating wheel at 4° C. The samples were then washed by brief centrifugation to  
528 collect the bead pellet at room temperature 3 times with 1.5 ml Buffer A and once with 1.5 ml of  
529 PBS. After the final wash, the beads were mixed with coelenterazine substrate (100 µl) and light

530 units measured in a tube luminometer. Known seronegative and seropositive samples for IgG and  
531 IgM antibodies against nucleocapsid and spike proteins were used for assigning seropositive cut-  
532 off values and for standardization.

533

#### 534 **SARS-CoV-2 RNA quantification of tissues and body fluids**

535 Total RNA was extracted from RNAlater (Invitrogen)-preserved tissues and body fluids  
536 collected at autopsy using the RNeasy Mini, RNeasy Fibrous Tissue Mini, RNeasy Lipid Tissue  
537 Mini Kit, and QIAamp Viral RNA Mini Kits (Qiagen) according to the manufacturer's protocols.  
538 Upstream tissue processing and subsequent RNA quantification have been previously  
539 described<sup>26</sup>. The QX200 AutoDG Droplet Digital PCR System (Bio-Rad) was used to detect and  
540 quantify SARS-CoV-2 RNA in technical replicates of 5.5 uL RNA for fluids and up to 550 ng  
541 RNA for tissues as previously described<sup>26</sup>. Results were then normalized to copies of N1, N2,  
542 and RP per mL of sample input for fluids and per ng of RNA concentration input for tissues. For  
543 samples to be considered positive for SARS-CoV-2 N1 or N2 genes, they needed to mean the  
544 manufacturer's limit of detection of  $\geq 0.1$  copies/ $\mu$ L and  $\geq 2$  positive droplets per well. Over 60  
545 control autopsy tissues from uninfected patients, representing all organs collected for COVID-19  
546 autopsy cases, were used to validate the manufacturer's EUA published LOD for nasopharyngeal  
547 swabs for tissues (Extended Data Table 8). ddPCR data for P3<sup>16</sup> as well as a portion of tissues  
548 from the oral cavity<sup>26</sup> have been previously reported.

549

#### 550 **sgRNA analysis of ddPCR positive tissues**

551 Tissues that tested positive for one or both SARS-CoV-2 N gene targets via ddPCR had RNA  
552 submitted for sgRNA analysis. Briefly, five  $\mu$ l RNA was used in a one-step real-time RT-PCR

553 assay to sgRNA (forward primer 5'- CGATCTCTTGTAGATCTGTTCTC-3'; reverse primer 5'-  
554 ATATTGCAGCAGTACGCACACA-3'; probe 5'-FAM-  
555 ACACTAGCCATCCTTACTGCGCTTCG-ZEN-IBHQ-3')<sup>29</sup> using the Rotor-Gene probe kit  
556 (Qiagen) according to instructions of the manufacturer. In each run, standard dilutions of counted  
557 RNA standards were run in parallel to calculate copy numbers in the samples. The limit of  
558 detection for this assay was determined to be <40 Cq (Supplemental Data 1) using 40 control  
559 autopsy tissues from uninfected patients, representing all organs collected for COVID-19  
560 autopsy cases.

561

#### 562 **Viral isolation from select postmortem tissues**

563 Select tissues with high viral RNA levels via ddPCR and sgRNA PCR measuring at or below a  
564 30 Cq underwent virus isolation to prove the presence of infectious virus. Virus isolation was  
565 performed on tissues by homogenizing the tissue in 1ml DMEM and inoculating Vero E6 cells in  
566 a 24-well plate with 250 µl of cleared homogenate and a 1:10 dilution thereof. Plates were  
567 centrifuged for 30 minutes at 1000 rpm and incubated for 30 minutes at 37°C and 5% CO<sub>2</sub>. The  
568 inoculum was then removed and replaced with 500 µl DMEM containing 2% FBS, 50 U/ml  
569 penicillin and 50 µg/ml streptomycin. Six days after inoculation, cytopathic effect (CPE) was  
570 scored. A blind passage of samples where no CPE was present, was performed according to the  
571 same method. Supernatants from plates with CPE present were analyzed via PCR for SARS-  
572 CoV-2 to rule out other causes of CPE.

573

#### 574 **Virus Sequencing Methods**

575 Patients with duration of illness  $\leq 7$  d (P27, P19) and 8-14 d (P18) with multiple body site  
576 tissues containing sgRNA levels  $\leq 31$  Cq value were selected for high throughput, single-genome  
577 amplification and sequencing (HT-SGS) as previously described<sup>21</sup>. Presence of variants of  
578 SARS-CoV-2 were analyzed within and between tissues.

579

### 580 **SARS-CoV-2 RNA *in situ* hybridization**

581 Chromogenic *in situ* detection was performed using the manual RNAScope 2.5 HD assay (Cat#  
582 322310, Advanced Cell Diagnostics, Hayward, CA) with a modified pretreatment protocol.  
583 Briefly, formalin-fixed and paraffin-embedded (FFPE) tissue sections were cut at 7  $\mu\text{m}$ , air dried  
584 overnight, and baked for 2 hrs at 60°C. The FFPE tissue sections were deparaffinized,  
585 dehydrated, and then treated with pretreat 1 for 10 min at room temperature. The slides were  
586 boiled with pretreatment reagent for 15 min, digested with protease at 40°C for 10 min, then  
587 hybridized for 2 hours at 40°C with probe-*V-nCov2019-S* (Cat# 848561, Advanced Cell  
588 Diagnostics). In addition, probe-*Hs-PP1B* (Cat# 313901, Advanced Cell Diagnostics) and probe-  
589 *dapB* (Cat# 310043, Advanced Cell Diagnostics) were used as a positive and negative control,  
590 respectively. Subsequent amplification was done according to the original protocol. Detection of  
591 specific probe binding sites were visualized with RNAScope 2.5 HD Reagent kit-brown  
592 chromogenic labels (Advanced Cell Diagnostics). The slides were counterstained with  
593 hematoxylin and cover-slipped.

594

### 595 **SARS-CoV-2 immunohistochemistry**

596 FFPE cerebellar sections were deparaffinized, rehydrated and subject to 0.01M Citrate buffer  
597 antigen retrieval for 20min at 120°C. Slides were incubated in 0.1% TritonX100 in PBS for



598 30min, washed extensively with PBS and fresh True Black Plus® solution (1:40, Cat#23014,  
599 Biotium) applied for 7min. Following PBS wash, blocking serum (5% normal donkey  
600 serum/0.3M glycine) was applied for 30min. Primary antibodies against SARS-CoV-2 NP1  
601 (1:250, custom made) and NeuN (1:200, Cat#MAB377, Chemicon) were diluted in blocking  
602 serum and applied to slides overnight at 4°C. Species-specific secondary conjugates (1:500,  
603 Cat#A32790 and #A32744, ThermoFisher) were applied for 1hr at RT. Hoescht 33342 applied  
604 for 10min (1:2000, Cat#H3570, ThermoFisher) labeled nuclei. Slides were cover-slipped with  
605 Prolong Gold (Cat#P36930, ThermoFisher).

606

#### 607 **Data Availability**

608 The datasets that support the findings of this study are available in Supplementary Data 1, 2 and  
609 3. Sequence data described in this manuscript have been deposited (database accession numbers  
610 XXXX). The bioinformatic pipeline for HT-SGS data analysis has been deposited  
611 (<https://github.com/niaid/UMI-pacbio-pipeline>). ISH images from our cohort as well as positive  
612 and negative controls are available in Supplementary Data 3, which is available at  
613 <https://halo.cancer.gov>, Authentication method: NIH, username: [halocancernci@gmail.com](mailto:halocancernci@gmail.com),  
614 password: covid19N!H.

615

#### 616 **Methods References:**

- 617 26. Huang, N., et al. SARS-CoV-2 infection of the oral cavity and saliva. *Nat Med.* **27**, 892–  
618 903 (2021). <https://doi.org/10.1038/s41591-021-01296-8>.
- 619 27. Burbelo, P. D., et al. Sensitivity in Detection of Antibodies to Nucleocapsid and Spike  
620 Proteins of Severe Acute Respiratory Syndrome Coronavirus 2 in Patients With

621 Coronavirus Disease 2019. *J Infect Dis.* **222**(2), 206-213 (2020).

622 <https://doi.org/10.1093/infdis/jiaa273>.

623 28. Burbelo, P. D., Goldman, R., & Mattson, T. L. A simplified immunoprecipitation method  
624 for quantitatively measuring antibody responses in clinical sera samples by using  
625 mammalian-produced Renilla luciferase-antigen fusion proteins. *BMC Biotechnol.* **5**, 22  
626 (2005). <https://doi.org/10.1186/1472-6750-5-22>.

627 29. Wölfel R., et al. Virological assessment of hospitalized patients with COVID-19. *Nature.*  
628 **581**(7809), 465-469 (2020). <https://doi.org/10.1038/s41586-020-2196-x>.

629

### 630 **Acknowledgements:**

631 This study was funded and supported by the Intramural Research Program of the National  
632 Institutes of Health, Clinical Center, National Institute of Dental and Craniofacial Research, and  
633 National Institute of Allergy and Infectious Diseases.

634 This research was made possible through the NIH Medical Research Scholars Program, a  
635 public-private partnership supported jointly by the NIH and contributions to the Foundation for  
636 the NIH from the Doris Duke Charitable Foundation, Genentech, the American Association for  
637 Dental Research, and the Colgate-Palmolive Company.

638

### 639 **NIH COVID-19 Autopsy Consortium**

640 Daniel S. Chertow<sup>1,2</sup>, Kevin M. Vannella<sup>1,2</sup>, Sydney R. Stein<sup>1,2</sup>, Marcos J. Ramos-Benitez<sup>1,2,4</sup>,

641 Andrew P. Platt<sup>1,2</sup>, James M. Dickey<sup>1,2</sup>, Ashley L. Babyak<sup>1,2</sup>, Luis J. Perez Valencia<sup>1,2</sup>, Sabrina

642 C. Ramelli<sup>3</sup>, Shelly J. Curran<sup>3</sup>, Mary E. Richert<sup>3</sup>, David E. Kleiner<sup>5</sup>, Stephen M. Hewitt<sup>5</sup>, Martha

643 Quezado<sup>5</sup>, Willie J. Young<sup>5</sup>, Sarah P. Young<sup>5</sup>, Billel Gasmi<sup>5</sup>, Michelly Sampaio De Melo<sup>5</sup>,

644 Sabina Desar<sup>5</sup>, Saber Tadros<sup>5</sup>, Nadia Nasir<sup>5</sup>, Xueting Jin<sup>5</sup>, Sharika Rajan<sup>5</sup>, Esra Dikoglu<sup>5</sup>, Neval  
645 Ozkaya<sup>5</sup>, Kris Ylaya<sup>5</sup>, Joon-Yong Chung<sup>5</sup>, Stefania Pittaluga<sup>5</sup>, Grace Smith<sup>5</sup>, Elizabeth R.  
646 Emanuel<sup>6</sup>, Brian L. Kelsall<sup>6</sup>, Justin A. Olivera<sup>7</sup>, Megan Blawas<sup>7</sup>, Robert A. Star<sup>7</sup>, Alison  
647 Grazioli<sup>8</sup>, Nicole Hays<sup>9</sup>, Madeleine Purcell<sup>9</sup>, Shreya Singireddy<sup>9</sup>, Jocelyn Wu<sup>9</sup>, Katherine Raja<sup>9</sup>,  
648 Ryan Curto<sup>9</sup>, Jean E. Chung<sup>10</sup>, Amy J. Borth<sup>10</sup>, Kimberly A. Bowers<sup>10</sup>, Anne M. Weichold<sup>10</sup>,  
649 Paula A. Minor<sup>10</sup>, Mir Ahmad N. Moshref<sup>10</sup>, Emily E. Kelly<sup>10</sup>, Mohammad M. Sajadi<sup>11,12</sup>, Kapil  
650 K. Saharia<sup>11,12</sup>, Daniel L. Herr<sup>13</sup>, Thomas M. Scalea<sup>14</sup>, Douglas Tran<sup>15</sup>, Ronson J. Madathil<sup>15</sup>,  
651 Siamak Dahi<sup>15</sup>, Kristopher B. Deatruck<sup>15</sup>, Eric M. Krause<sup>16</sup>, Joseph Rabin<sup>17</sup>, Joseph A. Herrold<sup>18</sup>,  
652 Ali Tabatabai<sup>18</sup>, Eric S. Hochberg<sup>18</sup>, Christopher R. Cornachione<sup>18</sup>, Andrea R. Levine<sup>18</sup>, Justin E.  
653 Richards<sup>19</sup>, John Elder<sup>20</sup>, Allen P. Burke<sup>20</sup>, Michael A. Mazzeffi<sup>21</sup>, Robert H. Christenson<sup>22</sup>,  
654 Zackary A. Chancer<sup>23</sup>, Mustafa Abdulmahdi<sup>24</sup>, Sabrina Sopha<sup>24</sup>, Tyler Goldberg<sup>24</sup>, Shahabuddin  
655 Soherwardi<sup>25</sup>, Yashvir Sangwan<sup>26</sup>, Michael T. McCurdy<sup>27,12</sup>, Kristen Sudano<sup>27</sup>, Diane Blume<sup>27</sup>,  
656 Bethany Radin<sup>27</sup>, Madhat Arnouk<sup>27</sup>, James W. Eagan Jr<sup>28</sup>, Robert Palermo<sup>29</sup>, Anthony D.  
657 Harris<sup>30</sup>

658

659 Affiliations:

- 660 1. Emerging Pathogens Section, Department of Critical Care Medicine, Clinical Center,  
661 National Institutes of Health, Bethesda, MD, USA
- 662 2. Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases,  
663 Bethesda, MD, USA
- 664 3. Critical Care Medicine Department, Clinical Center, National Institutes of Health,  
665 Bethesda, MD, USA

- 666 4. Postdoctoral Research Associate Training Program, National Institute of General Medical  
667 Sciences, National Institutes of Health, Bethesda, MD, USA
- 668 5. Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National  
669 Institutes of Health, Bethesda, MD, USA
- 670 6. Mucosal Immunobiology Section, Laboratory of Molecular Immunology, National  
671 Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD,  
672 USA
- 673 7. Renal Diagnostics and Therapeutics Unit, Kidney Diseases Branch, National Institute of  
674 Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda,  
675 MD, USA
- 676 8. Kidney Disease Section, Kidney Diseases Branch, National Institute of Diabetes and  
677 Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA
- 678 9. University of Maryland School of Medicine, Baltimore, MD, USA
- 679 10. University of Maryland Medical Center, Baltimore, MD, USA
- 680 11. Institute of Human Virology, University of Maryland School of Medicine, Baltimore,  
681 MD, USA
- 682 12. Department of Medicine, Division of Pulmonary and Critical Care Medicine, University  
683 of Maryland School of Medicine, Baltimore, MD, USA
- 684 13. R Adams Cowley Shock Trauma Center, Department of Medicine and Program in  
685 Trauma, University of Maryland School of Medicine, Baltimore, MD, USA
- 686 14. Department of Shock Trauma Critical Care, University of Maryland School of Medicine,  
687 Baltimore, MD, USA

- 688 15. Department of Surgery, Division of Cardiac Surgery, University of Maryland School of  
689 Medicine, Baltimore, MD, USA
- 690 16. Department of Surgery, Division of Thoracic Surgery, University of Maryland School of  
691 Medicine, Baltimore, MD, USA
- 692 17. R Adams Cowley Shock Trauma Center, Department of Surgery and Program in Trauma,  
693 University of Maryland School of Medicine, Baltimore, MD, USA
- 694 18. Department of Medicine, Division of Infectious Disease, University of Maryland School  
695 of Medicine, Baltimore, MD, USA
- 696 19. Department of Anesthesiology, Division of Critical Care Medicine, University of  
697 Maryland School of Medicine, Baltimore, MD, USA
- 698 20. Department of Autopsy and Thoracic Pathology, University of Maryland School of  
699 Medicine, Baltimore, MD, USA
- 700 21. Department of Anesthesiology and Critical Care Medicine, George Washington School  
701 of Medicine and Health Sciences, Washington, DC USA
- 702 22. Department of Laboratory Science, University of Maryland School of Medicine,  
703 Baltimore, MD, USA
- 704 23. Department of Anesthesiology, University of Southern California Keck School of  
705 Medicine, Los Angeles, CA, USA
- 706 24. Critical Care Medicine, University of Maryland Baltimore Washington Medical Center,  
707 Glen Burnie, MD, USA
- 708 25. Hospitalist Department, TidalHealth Peninsula Regional, Salisbury, MD, USA
- 709 26. Department of Interventional Pulmonology, TidalHealth Peninsula Regional, Salisbury,  
710 MD, USA

711 27. Division of Critical Care Medicine, Department of Medicine, University of Maryland St.  
712 Joseph Medical Center, Towson, MD, USA

713 28. Department of Pathology, University of Maryland, St. Joseph Medical Center, Towson,  
714 MD, USA

715 29. Department of Pathology, Greater Baltimore Medical Center, Towson, MD, USA

716 30. Department of Epidemiology and Public Health, University of Maryland School of  
717 Medicine, Baltimore, MD, USA

718

719 **Author Contributions**

720 DSC, KMV, SRS, MJRB, ALB, LJPV, AG, DLH, SMH & DEK contributed to the study design  
721 and protocols for autopsy procurement. APP, JMD, MER, AG, NH, MP, SS, JW, KR, RC, JEC,  
722 AJB, KAB, AMW, PAM, MANM, EEK, MMS, KKS, DLH, TMS, DT, RJM, SD, KBD, EMK,  
723 JR, JAH, AT, ESH, CRC, ARL, JER, JE, APB, MAM, RHC, ZAC, MA, SS, TG, SS, YS, MTM,  
724 KS, DB, BR, MA, JWE Jr, RP, and ADH provided care for, recruited, collected samples from,  
725 and/or procured medical records for the patients in this study. DEK, SMH, MQ, WJY, SPY, BG,  
726 MSDM, SD, ST, NN, XJ, SR, ED, NO, KY, JYC, SP, and GS conducted the autopsies and/or  
727 histological and ISH analysis. SRS, MJRB, APP, JMD, ALB, LJPV, SCR, SJC, ERE, BLK,  
728 JAO, MB, and RAS assisted with procurement and preservation of autopsy specimens. SRS with  
729 assistance from SCR and JMD performed RNA extraction, ddPCR, and data analysis. MS, CKY,  
730 VJM, and EDW performed and analyzed data for sgRNA RT-PCR. CWW and KEP conducted  
731 IHC on cerebellum. PDB and JIC measured antibody responses to SARS-CoV-2 in perimortem  
732 plasma samples. SHK, FB, and EAB performed viral sequencing. SRS drafted the manuscript  
733 with critical input from DSC, KMV, SMH, DEK, SCR, APP, MJRB, EDW, VJM, AG, DLH,

734 KKS, MMS MTM, PDB, JIC, CWW, KEP, and SJC. All authors approved the submitted version  
735 of the manuscript.

736 **Competing Interests:**

737 The authors declare no competing or conflict of interest.

738 **Additional Information:**

739 Supplementary information is available for this paper.

740 Correspondence and requests for materials should be addressed to DSC.

741

742

743

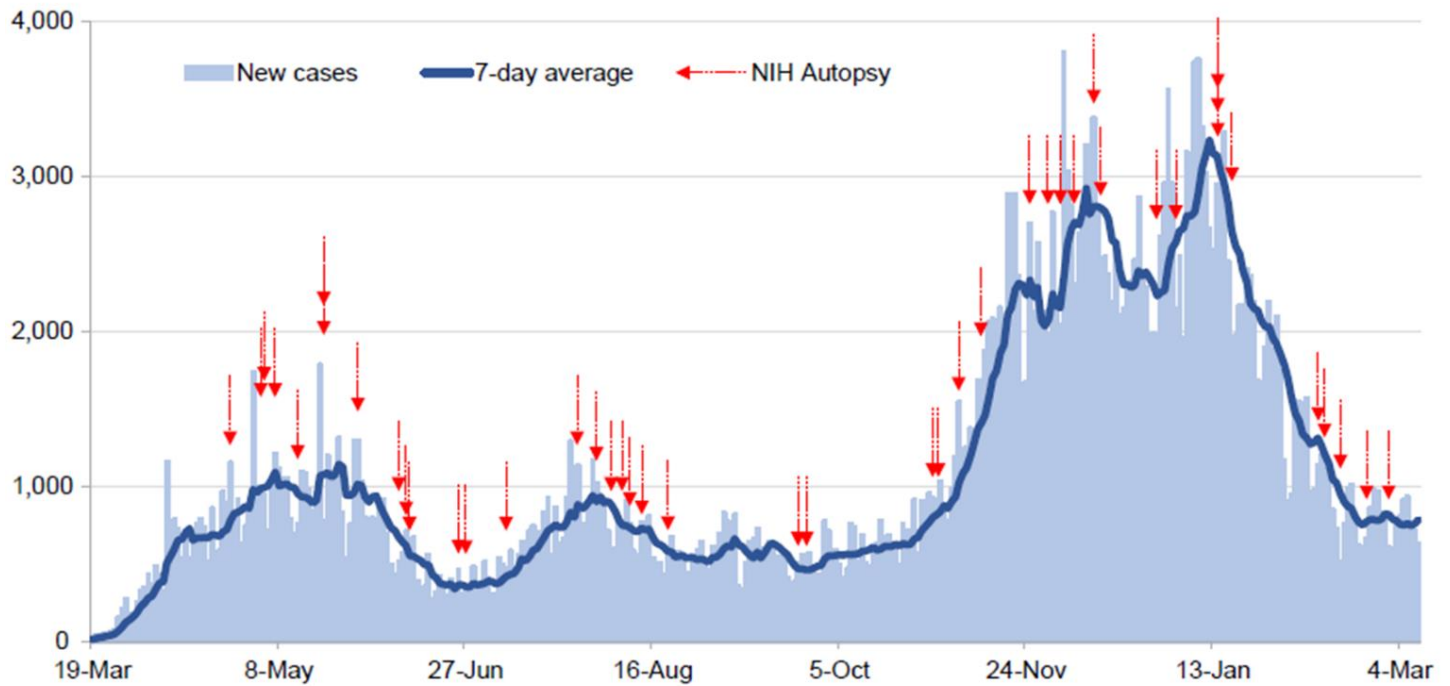
744

745

746

747

748



749

750 Extended Data Fig. 1 **Autopsy procurement relative to Maryland COVID-19 cases, March**  
 751 **19th, 2020 to March 9th, 2021.** Daily COVID-19 reported cases for Maryland (light blue bars)  
 752 with 7-day average (dark blue line) with timing of autopsies (red arrows).

753

754

755

756

757

758

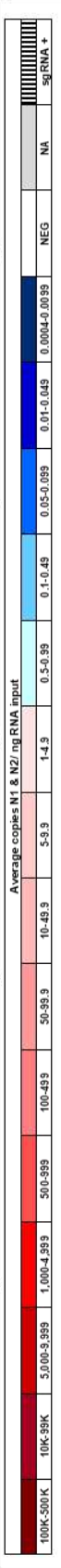
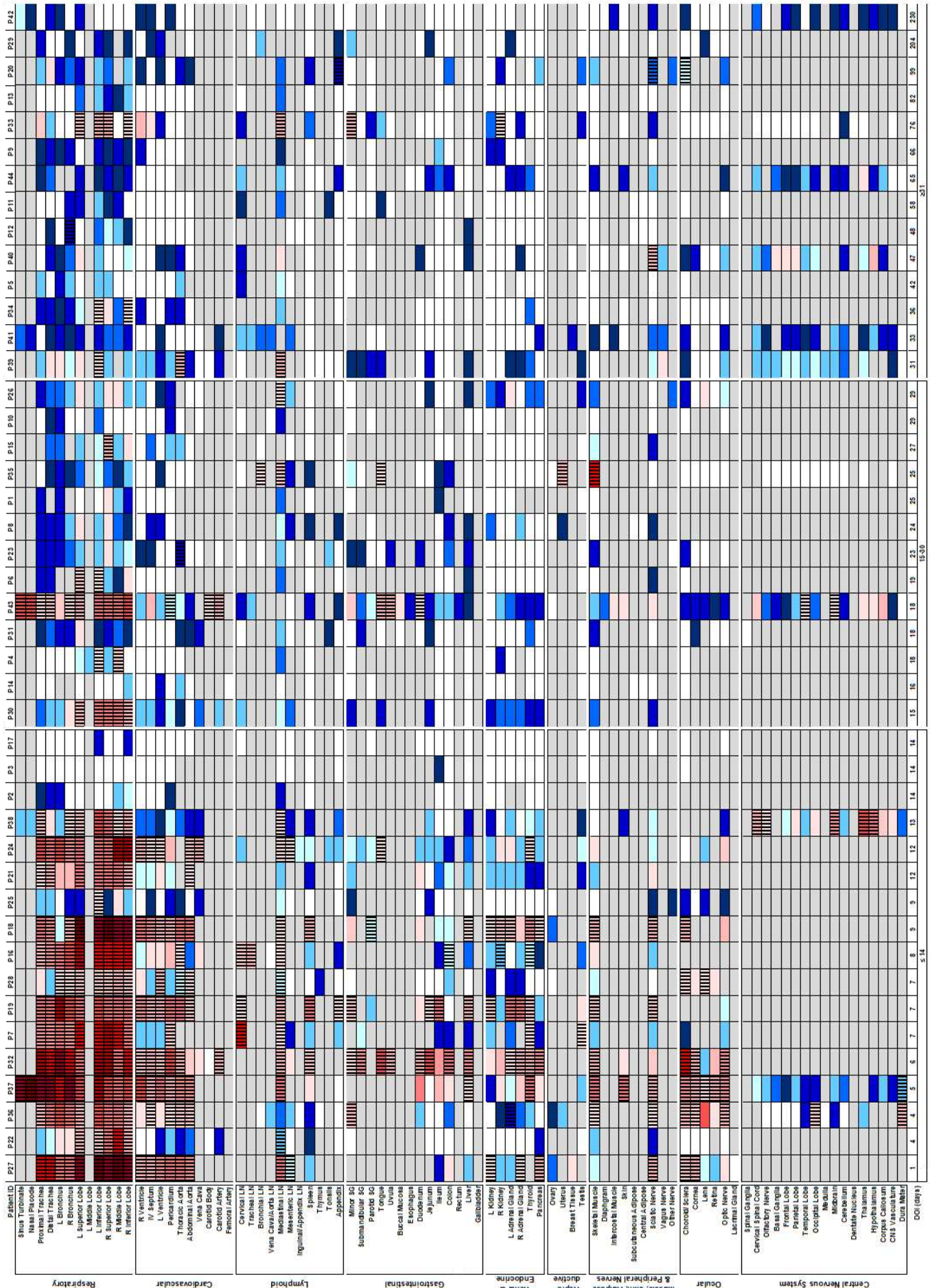
759

760

761

762





DOI (dpi)

764 Extended Data Fig. 2 **Distribution, quantification, and replication of SARS-CoV-2 across the**  
765 **body and brain over time.** The heat map depicts the highest average quantification of SARS-  
766 CoV-2 RNA (N) via ddPCR present within all sampled tissues of 44 autopsy cases. Patients are  
767 aligned from shortest to longest duration of illness (DOI) prior to death, listed at the bottom of  
768 the figure, and grouped into early (0-14 d), mid (15-30 d), and late ( $\geq 31$  d) DOI. Tissues are  
769 grouped by body system beginning with the respiratory tract at the top and CNS at the bottom.  
770 Viral RNA levels range from 0.0004 to 500,000 copies per ng of RNA input, depicted as a  
771 gradient from dark blue at the lowest level to dark red at the highest level. Tissues that were also  
772 positive for sgRNA via real-time RT-PCR are shaded with black vertical bars.

773

774

775

776

777

778

779

780

781

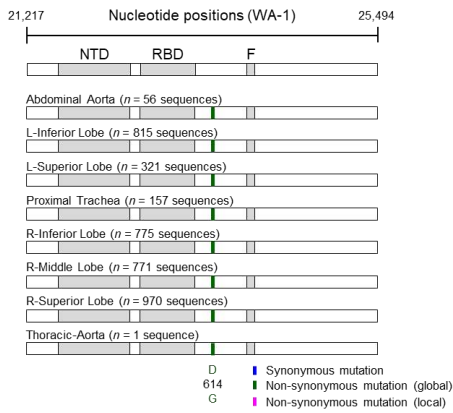
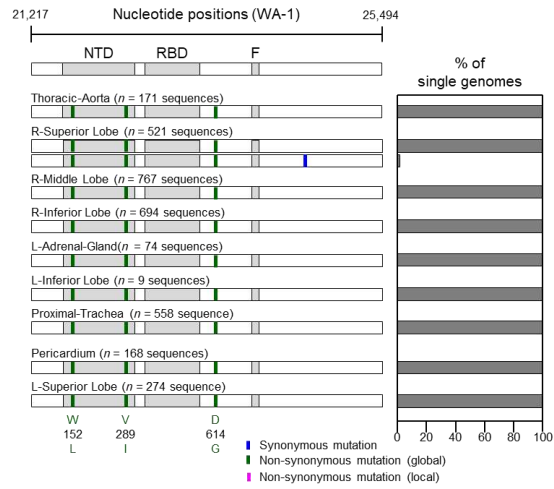
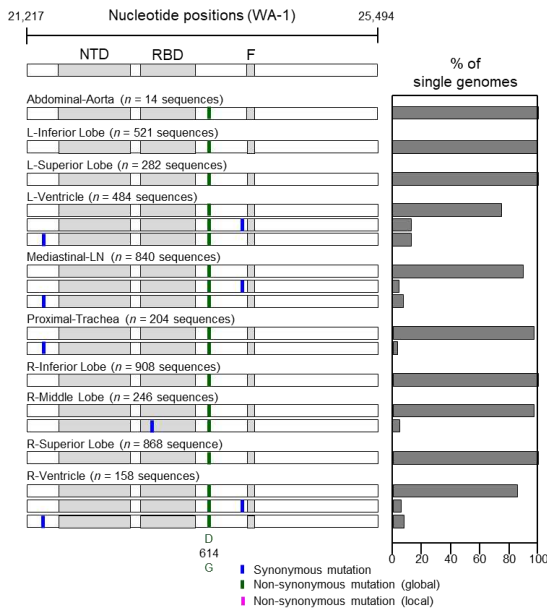
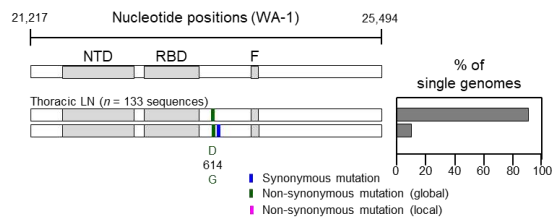
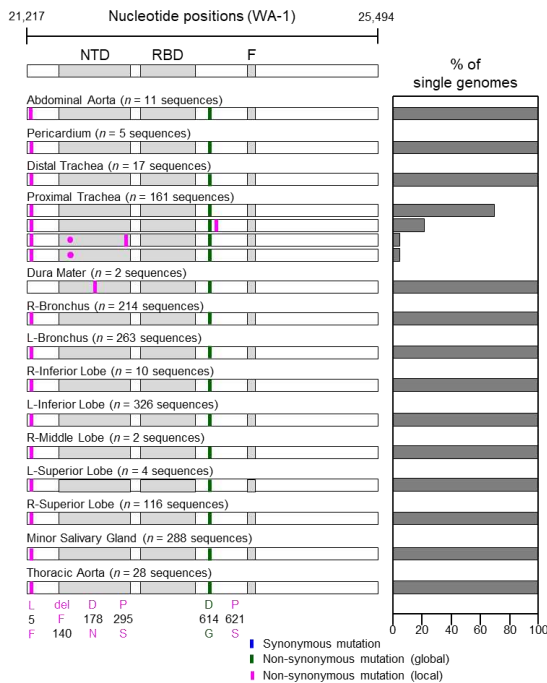
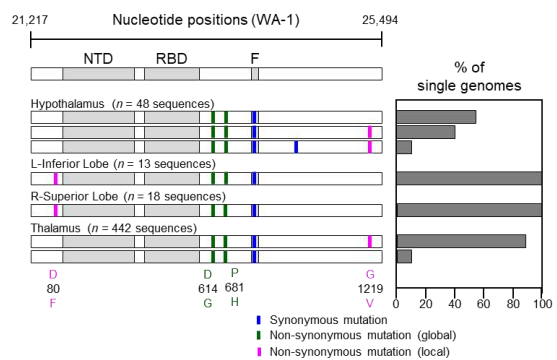
782

783

784

785

786

**a****b****c****d****e****f**

788 Extended Data Figure 3: **Analysis of SARS-CoV-2 genetic diversity across body**  
789 **compartments in patients.** (a) P18, (b) P19, (c) P27, (d) P33, (e) P36, (f) P38. Haplotype  
790 diagrams (left) show SARS-CoV-2 spike single genome sequences detected in multiple organs.  
791 Spike NH2-terminal domain (NTD), receptor-binding domain (RBD), and furin cleavage site (F)  
792 regions are shaded grey, and remaining regions of the spike are shaded white. Ticks with  
793 different colors indicate mutations relative to the WA-1 reference sequence; green indicates non-  
794 synonymous differences from WA-1 detected in all sequences in the individual; blue indicates  
795 synonymous mutations detected variably within the individual, and pink indicates non-  
796 synonymous mutations detected variably within the individual. Bar graphs (right) show the  
797 percentage of all single genome sequences in the sample matching each haplotype.

798

799

800

801

802

803

804

805

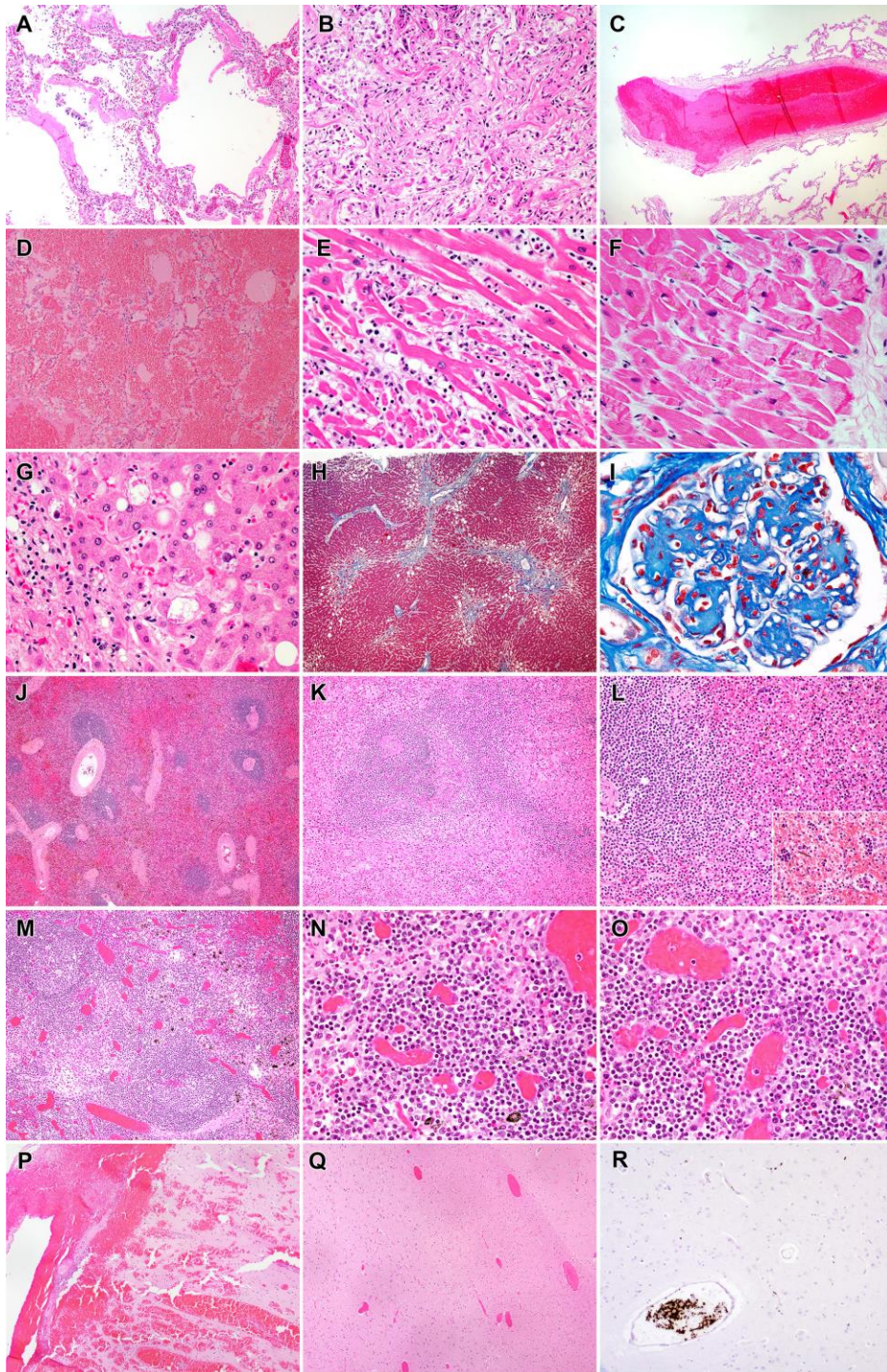
806

807

808

809

810



811

812 Extended Data Fig. 4 **Representative findings in patients in the COVID-19 cohort.** A. Lung,

813 Subject P22. Exudative phase diffuse alveolar damage with hyaline membranes and mild

814 interstitial inflammation (H&E, 100x). B. Lung, Subject P26. Proliferative phase diffuse alveolar

815 damage and sparse inflammation. (H&E, 200x). C. Lung, Subject P22. Organizing thrombus in  
816 medium sized pulmonary artery. (H&E, 40x). D. Lung, Subject P28. Diffuse pulmonary  
817 hemorrhage. (H&E, 100x). E. Heart, Subject P3. Active lymphocytic myocarditis with  
818 cardiomyocyte necrosis. (H&E, 400x). F. Heart, Subject P38. Microscopic focus of bland  
819 myocardial contraction band necrosis. (H&E, 400x). G. Liver, Subject P41. Steatohepatitis with  
820 mild steatosis and scattered ballooned hepatocytes. (H&E, 400x), H. Liver, Subject P41. Focal  
821 bridging fibrosis involving central hepatic veins. (Masson trichrome, 40x). I. Kidney, Subject  
822 P16. Nodular glomerulosclerosis. (Masson trichrome, 600x). J. Spleen, Subject P16. Preservation  
823 of white pulp and congestion (H&E, 40x) K. Spleen, Subject P14. Lymphoid depletion of white  
824 pulp with proteinaceous material and red pulp congestion. (H&E, 100x) L. Spleen, Subject P34.  
825 Relative preservation of white pulp with extramedullary hematopoiesis (inset) in red pulp (H&E,  
826 200x) M. Lymph node, Subject P25. Follicular hyperplasia with well-defined follicles. (H&E, )  
827 N. Lymph node, Subject P25. Marked plasmacytosis in the medullary cord. (H&E, 400x) O.  
828 Lymph node, Subject P25. Marked plasmacytosis and sinus histiocytosis. (H&E, 400x) P. Brain,  
829 Subject P35, Focal subarachnoid and intraparenchymal hemorrhage. (H&E, 40x) Q. Brain,  
830 Subject P44, Vascular congestion. (H&E, 40x) R. Brain, Subject P43, Intravascular platelet  
831 aggregates. (anti-CD61 stain, 100x)

832

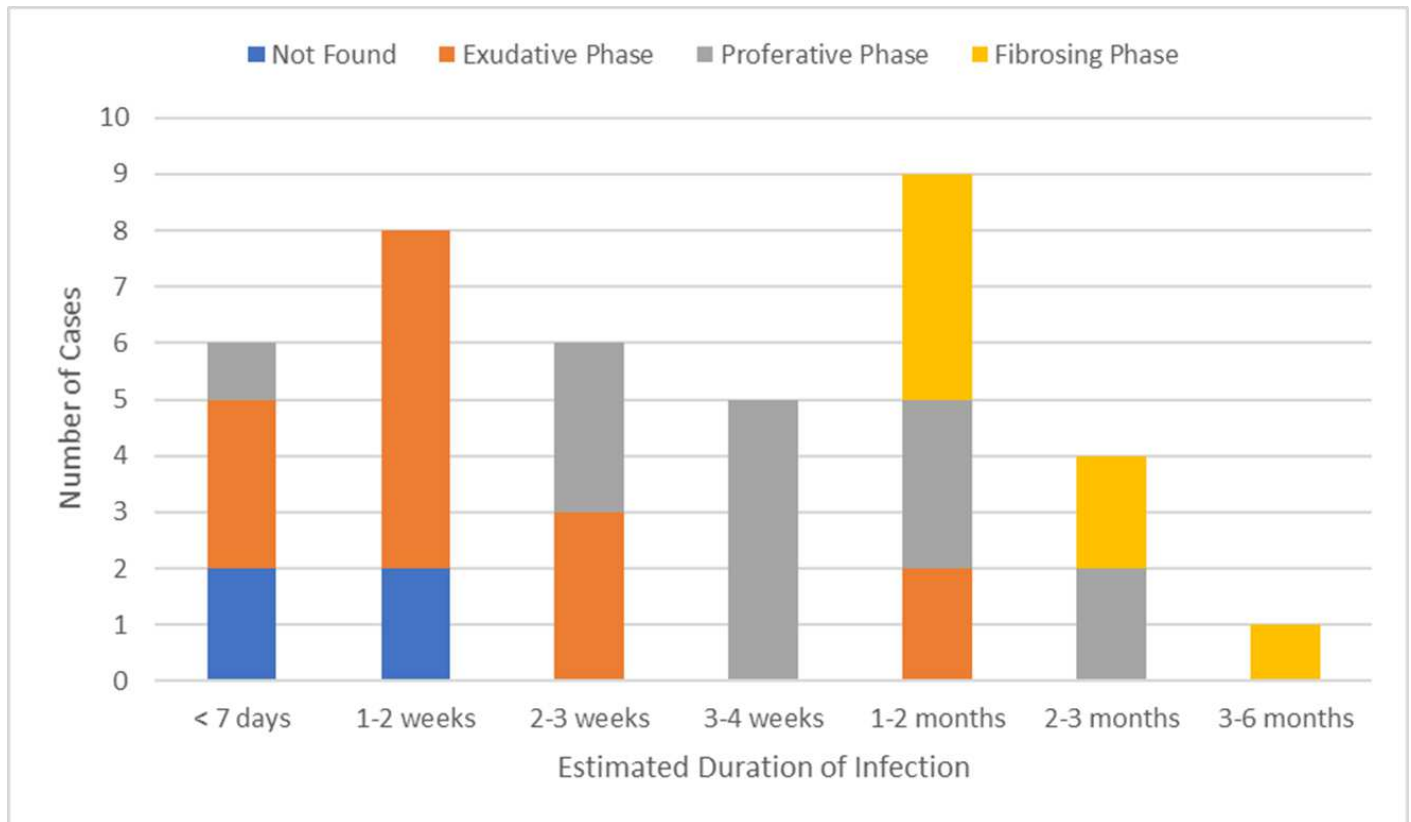
833

834

835

836

837



838

839 Extended Data Fig. 5 **Temporal association of diffuse alveolar damage in patients dying**

840 **from COVID-19.** Number of autopsy cases with stages of diffuse alveolar damage via

841 histopathologic analysis by duration of illness. Early time points mainly show the initial

842 exudative phase of diffuse alveolar damage, while patients dying after prolonged illness are more

843 likely to show organizing or fibrosing stages.

844

845

<b>a</b>		<b>(n=44)</b>		<b>b</b>		<b>Mean (Min, Max)</b>	
<b>Age (years)</b>				<b>Disease Course Intervals</b>			
Mean (Min, Max)		59.2 (6, 91)		Symptom onset to hospital admission, days		9.4 (-4, 108)	
<b>Age by group (years)</b>		<b>n (%)</b>		Symptom onset to death, days		35.2 (1, 230)	
0-17		1 (2.3)		Days hospitalized		26.4 (0, 188)	
18-24		1 (2.3)		Postmortem Interval (hours)		26.2 (10, 67)	
25-34		2 (4.5)		<b>Pharmacologic Interventions</b>		<b>n (%)</b>	
35-44		6 (13.6)		Vasopressors		38 (86.4)	
45-54		4 (9.1)		Antibiotics		41 (93.2)	
55-64		11 (25.0)		Systemic Steroids		39 (88.6)	
65-74		11 (25.0)		Systemic Anticoagulation		34 (77.3)	
75-84		5 (11.4)		Paralytics		25 (56.8)	
≥85		3 (6.8)		Inhaled Vasodilators		10 (22.7)	
<b>Sex</b>				Remdesivir		16 (36.4)	
Male		30 (68.2)		Tocilizumab		4 (9.1)	
Female		13 (29.5)		Convalescent Plasma		6 (13.6)	
Intersex		1 (2.3)		<b>Nonpharmacologic Interventions</b>			
<b>Race/Ethnicity</b>				ECMO		10 (22.7)	
Non-Hispanic Asian		1 (2.3)		Renal Replacement Therapy		18 (40.9)	
Non-Hispanic Black or African American		18 (40.9)		Intubated		36 (81.8)	
Non-Hispanic White		18 (40.9)		Tracheostomy		9 (20.5)	
Hispanic or Latino		7 (15.9)		Chest Tube(s)		11 (25.0)	
<b>BMI</b>							
<18.5		2 (4.5)					
18.5-24.9		9 (20.5)					
25-29.9		10 (22.7)					
30-34.9		9 (20.5)					
35.0-39.9		6 (13.6)					
≥40		8 (18.1)					
<b>Comorbidities</b>							
Autoimmune Disease		5 (11.4)					
Cancer		7 (15.9)					
Cardiovascular Disease		14 (31.8)					
Cerebrovascular Disease		4 (9.1)					
Chronic Immunosuppression		6 (13.6)					
Chronic Respiratory Disease		15 (34.1)					
Diabetes Mellitus		14 (31.8)					
History of Thromboembolic Event(s)		3 (6.8)					
Hypertension		24 (54.5)					
Hyperlipidemia		14 (31.8)					
Liver Disease		4 (9.1)					
Obesity (body mass index ≥30)		23 (52.3)					
Renal Disease		8 (18.2)					
1+		42 (95.5)					
2+		33 (75.0)					
3+		29 (65.9)					

846

847 Extended Data Table 1 **Autopsy cohort demographics, comorbidities, and clinical**  
848 **intervention summary.** (a) Summary of demographics and known comorbidities for autopsy  
849 cases. (b) Summary of illness course and clinical care for autopsy cases. Data compiled from  
850 available patient medical records. ECMO/extracorporeal membrane oxygenation.

851

852

853



Patient ID	Sex	Age, years	Duration of illness, days	BMI	Comorbidities	Immediate Cause of death	Highest level of respiratory support	COVID-19 treatment(s)
Patient 1	M	61	25	31.80	DM, HTN, obesity	Bacterial sepsis and fungal pneumonia	Intubation	Systemic steroids, systemic anticoagulation
Patient 2	F	71	14	39.60	HTN, HLD, COPD, breast cancer, cerebrovascular event, Hx DVT/PE, CHF, AF, dementia, obesity, hypothyroidism, anemia, seizure disorder	Acute pyelonephritis with abscess and likely sepsis	Intubation	Systemic steroids
Patient 3	M	26	14	25.80	Asthma	Lymphocytic myocarditis	Intubation, ECMO	Systemic anticoagulation
Patient 4	M	68	18	31.40	HTN, HLD, obesity	DAD	Intubation	Systemic steroids, remdesivir, tocilizumab, convalescent plasma
Patient 5	M	41	42	50.60	Obesity	Fungal pneumonia	Intubation	Systemic steroids, Systemic anticoagulation
Patient 6	M	62	19	45.00	HTN, obesity	Acute bronchopneumonia	Intubation	Systemic steroids, systemic anticoagulation, inhaled vasodilators
Patient 7	M	60	7	24.30	DM, CML, HTN, AF, CHF, CAD s/p bypass, PVD, CKD, Hx kidney transplant, chronic immunosuppression, HLD, hyperparathyroidism, hypothyroidism, anemia	Acute polymicrobial bronchopneumonia superimposed on DAD	Intubation	Systemic anticoagulation
Patient 8	F	68	24	58.10	HTN, asthma, COPD, cerebrovascular disease, obesity, anemia, chronic fatigue, fibromyalgia	Acute polymicrobial bronchopneumonia superimposed on DAD	Intubation	Systemic steroids, systemic anticoagulation
Patient 9	M	43	66	34.00	Obesity	Pneumonia and sepsis	Intubation, Tracheostomy, ECMO	Systemic steroids, systemic anticoagulation, paralytics, remdesivir, convalescent plasma
Patient 10	F	70	29	35.43	DM, HTN, HLD, CHF, COPD, obesity	Sepsis	Nasal Canula	Remdesivir
Patient 11	M	50	58	36.70	Obesity	Acute pneumonia	Intubation, Tracheostomy, ECMO	Systemic steroids, systemic anticoagulation, tocilizumab
Patient 12	M	61	48	41.80	DM, HTN, HLD, CHF, LV dysfunction, asthma, obesity	DAD, exudative phase	Intubation	Systemic anticoagulation
Patient 13	M	48	82	41.00	Obesity	DAD, organizing phase	Intubation, Tracheostomy, ECMO	Systemic steroids, systemic anticoagulation, tocilizumab, convalescent plasma
Patient 14	M	64	16	30.60	HTN, COPD, obesity	Acute bacterial bronchopneumonia	Intubation	Systemic steroids, systemic anticoagulation
Patient 15	M	65	27	19.70	HLD, sarcoidosis, chronic immunosuppression	Fungal pneumonia and sepsis	Intubation	Systemic steroids, systemic anticoagulation, remdesivir, convalescent plasma
Patient 16	M	87	8	26.90	DM, HTN, HLD, CAD, CHF, ESRD	DAD, exudative phase	AVAPS	Systemic steroids, systemic anticoagulation, convalescent plasma
Patient 17	M	36	14	27.17	Drug abuse	Bilateral bronchopneumonia	Intubation, ECMO	Systemic steroids, systemic anticoagulation
Patient 18	F	79	9	32.60	DM, HTN, COPD, Hx DVT, CAD, cirrhosis, CKD, obesity, anemia, seizure disorder	DAD, exudative phase	Intubation	Systemic steroids
Patient 19	M	43	7	21.90	DM	Sudden cardiac death	Intubation	Systemic steroids, systemic anticoagulation
Patient 20	M	42	99	23.50	DM, HLD	DAD, proliferative and fibrosing phase	Intubation, Tracheostomy, ECMO	Systemic steroids, systemic anticoagulation, convalescent plasma
Patient 21	M	77	12	25.20	DM, HTN, COPD, pulmonary fibrosis, CAD, CHF, CKD, Hx prostate cancer, cerebrovascular disease	DAD, exudative phase	High flow nasal canula, BiPAP	Systemic steroids, systemic anticoagulation
Patient 22	M	64	4	29.90	HTN, HLD	DAD/ARDS	Intubation	Systemic steroids, systemic anticoagulation
Patient 23	M	79	23	28.00		Pulmonary hemorrhage	Intubation	Systemic steroids, systemic anticoagulation
Patient 24	M	59	12	34.20	Hx recurrent aspiration pneumonia, MS, chronic immunosuppression, obesity	Acute pneumonia	BiPAP	Systemic steroids, systemic anticoagulation
Patient 25	F	91	9	16.50	Cardiomyopathy, arrhythmia, dementia, inflammatory polyneuropathy, anemia	DAD, acute phase	None	Systemic steroids, remdesivir
Patient 26	M	48	29	28.20		Cerebral hemorrhage	Intubation, ECMO	Systemic steroids, systemic anticoagulation, remdesivir
Patient 27	Inter-sex	76	1	20.90	Turner Syndrome, aortic stenosis, sick sinus syndrome s/p pacemaker, dementia, hypothyroidism	Bronchopneumonia and DAD, exudative phase	Intubation	Systemic steroids, systemic anticoagulation, remdesivir
Patient 28	F	44	7	30.9	HTN, obesity	Pulmonary thromboembolic disease in the setting of DAD, exudative phase of	None	
Patient 29	M	60	204	24.91	HTN, ILD, cerebrovascular disease, CAD, RA, Hx lung transplant, chronic immunosuppression	Herpetic tracheobronchitis and DAD, s/p bilateral lung transplantation	Intubation, Tracheostomy, ECMO	Systemic steroids, systemic anticoagulation
Patient 30	F	70	15	26.00	HTN, ILD, PH, CHF, CAD, PAD, CKD, ESRD, congenital heart malformation, calciphylaxis	Bacterial pneumonia, SARS-CoV-2 infection	Intubation	Systemic steroids, systemic anticoagulation, remdesivir
Patient 31	M	59	18	26.50	DM, HTN, HLD	Bacterial pneumonia	Intubation, Tracheostomy	Systemic steroids, systemic anticoagulation, remdesivir
Patient 32	F	71	6	31.50	Asthma, COPD, sarcoidosis, cirrhosis, ESRD, Hx endocarditis, obesity, hypothyroidism, seizure disorder, anemia	Right heart failure	BiPAP	Systemic steroids
Patient 33	M	71	76	29.1	HTN, CKD, Hx Lyme disease	Bacterial pneumonia	High flow nasal canula	Systemic steroids, systemic anticoagulation
Patient 34	M	87	36	22.20	HLD, MM, COPD, CKD, seizure disorder, chronic immunosuppression, hypothyroidism	DAD, organizing to fibrosing phase	Intubation	Systemic steroids, remdesivir
Patient 35	F	45	25	63.00	DM, HTN, HLD, COPD, obesity, chronic lower extremity lymphedema	DAD, organizing phase	Intubation	Systemic steroids, systemic anticoagulation, remdesivir
Patient 36	F	6	4	17.40	Dravet syndrome, SCN1A gene mutation, seizure disorder	Acute cerebral ischemia with tonsillar herniation	Intubation	Systemic steroids, remdesivir
Patient 37	M	63	5	19.80	DM, HTN, Hx femoral artery thrombosis, CHF, CAD, PAD, AF, cardiomyopathy, Hx cardiac tamponade, hepatitis C, abnormal liver function, drug abuse	Bronchopneumonia	Intubation	Systemic steroids, systemic anticoagulation
Patient 38	M	71	13	40.20	HTN, HLD, COPD, prostate cancer, obesity	Bronchopneumonia	Intubation	Systemic steroids, systemic anticoagulation, remdesivir
Patient 39	M	27	31	39.20	Obesity	DAD, organizing to fibrotic phase and multiple pulmonary infarcts	Intubation, Tracheostomy, ECMO	Systemic steroids, systemic anticoagulation, remdesivir
Patient 40	F	68	47	35.11	HTN, uterine cancer, obesity	Sepsis with signs of cardiac dysfunction in the setting of DAD, proliferative and fibrotic phase	Intubation, Tracheostomy	Systemic steroids, systemic anticoagulation, remdesivir
Patient 41	F	75	33	24.94	DM, HTN, HLD, hypothyroidism	DAD, proliferative and fibrotic phase	Intubation	Systemic steroids
Patient 42	M	68	230	36.87	CAD, hepatitis A, liver failure, Hx liver transplant, chronic immunosuppression, obesity	Massive hepatic necrosis, status-post liver transplant	Intubation, Tracheostomy	Systemic steroids, systemic anticoagulation
Patient 43	F	61	18	32.22	DM, HTN, breast cancer, CAD, obesity	DAD, exudative and proliferative phase	Intubation	Systemic steroids, systemic anticoagulation
Patient 44	M	21	65	58.00	Obesity	Bacterial pneumonia superimposed on DAD, fibrosing stage	Intubation, ECMO	Systemic steroids, systemic anticoagulation, remdesivir, tocilizumab

855 Extended Data Table 2 **Individual case demographics and clinical summary.** Data obtained  
856 from available medical records. AF/atrial fibrillation, AVAPS/average volume-assured pressure  
857 support, BiPAP/bilevel positive airway pressure, CAD/coronary artery disease, CHF/congestive  
858 heart failure, CKD/chronic kidney disease, CML/chronic myeloid leukemia, COPD/chronic  
859 obstructive pulmonary disease, DAD/diffuse alveolar damage, DM/diabetes mellitus, DVT/deep  
860 vein thrombosis, ECMO/extracorporeal membrane oxygenation, ESRD/end-stage renal disease,  
861 HLD/hyperlipidemia, HTN/hypertension, Hx/historical, ILD/interstitial lung disease, LV/left  
862 ventricular, MS/multiple sclerosis, PE/pulmonary embolism, PVD/peripheral vascular disease,  
863 PH/pulmonary hypertension, s/p/status post.

864

a

Tissue Category	DOI (days)	Avg. N gene copies/ng RNA (SD)
Respiratory Tract	≤14	9,210.10 (43,179.20)
	15-30	19.67 (77.98)
	≥31	0.65 (2.61)
Cardiovascular	≤14	38.75 (106.08)
	15-30	0.59 (3.43)
	≥31	0.42 (2.51)
Lymphoid	≤14	30.01 (157.86)
	15-30	0.35 (1.28)
	≥31	0.73 (3.83)
Gastrointestinal	≤14	24.68 (99.37)
	15-30	0.87 (4.38)
	≥31	0.24 (2.17)
Renal & Endocrine	≤14	12.76 (59.01)
	15-30	0.03 (0.16)
	≥31	0.04 (0.33)
Reproductive	≤14	0.36 (0.58)
	15-30	1.87 (6.72)
	≥31	0.01 (0.02)
Muscle, Nerve, Adipose, & Skin	≤14	27.50 (101.13)
	15-30	50.65 (284.46)
	≥31	0.54 (3.03)
Ocular	≤14	57.40 (242.40)
	15-30	0.07 (0.24)
	≥31	0.03 (0.12)
Central Nervous System	≤14	32.93 (121.69)
	15-30	2.37 (7.34)
	≥31	0.39 (1.40)

b

Tissue	DOI (days)	ddPCR+ (n, %)	sgRNA+ (n, %)
All Respiratory		43/44, 97.7	23/43, 53.5
Trachea	≤14	15/17, 88.2	11/15, 73.3
	15-30	11/13, 84.6	1/11, 9.1
	≥31	12/14, 85.7	0/12, 0
Total		38/44, 86.4	12/38, 31.6
Bronchus	≤14	15/17, 88.2	11/15, 73.3
	15-30	10/11, 90.9	1/10, 10.0
	≥31	11/13, 84.6	1/11, 9.1
Total		36/41, 87.8	13/36, 36.1
Lung	≤14	16/17, 94.1	14/16, 87.5
	15-30	13/13, 100	5/13, 38.5
	≥31	14/14, 100	3/14, 21.4
Total		43/44, 97.7	22/43, 51.2
All Cardiovascular		35/44, 79.5	14/35, 40.0
Myocardium	≤14	14/17, 82.4	8/14, 57.1
	15-30	8/13, 61.5	0/8, 0
	≥31	9/14, 64.3	0/9, 0
Total		31/44, 70.5	8/31, 25.8
Pericardium	≤14	15/17, 88.2	7/15, 46.7
	15-30	5/13, 38.5	1/5, 20.0
	≥31	4/13, 30.8	0/4, 0
Total		24/43, 55.8	8/24, 33.3
Aorta	≤14	13/14, 92.9	10/13, 76.9
	15-30	6/10, 60.0	1/5*, 20.0
	≥31	5/13, 38.5	1/5, 20.0
Total		24/37, 64.9	12/23, 52.2
Vena Cava	≤14	5/5, 100	1/5, 20.0
	15-30	2/5, 40.0	0/2, 0
	≥31	0/2, 0.0	NA
Total		7/12, 58.3	1/7, 14.3
All Lymphoid		38/44, 86.4	16/38, 42.1
LN from Thorax	≤14	15/17, 88.2	11/15, 73.3
	15-30	11/13, 84.6	2/11, 18.2
	≥31	12/13, 92.3	2/12, 16.7
Total		38/43, 88.4	15/38, 39.5
LN from Abdomen	≤14	6/11, 54.5	2/6, 33.3
	15-30	4/8, 50	0/4, 0
	≥31	1/5, 20	0/1, 0
Total		11/24, 45.8	2/11, 18.2
Spleen	≤14	12/17, 70.6	3/12, 25.0
	15-30	3/13, 23.1	0/3, 0
	≥31	2/14, 14.3	0/2, 0
Total		17/44, 38.6	3/17, 17.6
Appendix	≤14	5/13, 38.5	1/5, 20.0
	15-30	2/9, 22.2	0/2, 0
	≥31	3/13, 23.1	1/3, 33.3
Total		10/35, 28.6	2/10, 20.0
All Gastrointestinal		32/44, 72.7	10/32, 31.3
Salivary Glands	≤14	8/11, 72.7	4/8, 50.0
	15-30	5/11, 45.5	0/5, 0
	≥31	3/13, 23.1	1/3, 33.3
Total		16/35, 45.7	5/16, 31.3
Tongue	≤14	2/2, 100	2/2, 100
	15-30	3/5, 60.0	2/3, 66.7
	≥31	3/5, 60.0	0/3, 0
Total		8/12, 66.7	4/8, 50.0
Small Intestine	≤14	13/17, 76.5	2/13, 15.4
	15-30	7/13, 53.8	1/7, 14.3
	≥31	5/14, 35.7	0/5, 0
Total		25/44, 56.8	3/25, 12.0
Colon	≤14	10/17, 58.8	2/10, 20.0
	15-30	4/11, 36.4	0/4, 0
	≥31	2/14, 14.3	0/2, 0
Total		16/42, 38.1	2/16, 12.5
Liver	≤14	10/17, 58.8	4/10, 40.0
	15-30	5/13, 38.5	0/5, 0
	≥31	3/14, 21.4	0/2*, 0
Total		18/44, 40.9	4/17, 23.5
All Renal & Endocrine		28/44, 63.6	10/28, 35.7
Kidney	≤14	12/17, 70.6	4/12, 33.3
	15-30	5/13, 38.5	0/5, 0
	≥31	3/14, 21.4	1/3, 33.3
Total		20/44, 45.5	5/20, 25.0
Adrenal Gland	≤14	12/16, 75.0	5/12, 41.7
	15-30	4/13, 30.8	0/4, 0
	≥31	5/14, 35.7	0/5, 0
Total		21/43, 48.8	5/21, 23.8
Thyroid	≤14	10/16, 62.5	7/10, 70.0
	15-30	4/12, 33.3	0/4, 0
	≥31	3/13, 23.1	0/3, 0
Total		17/41, 41.5	7/17, 41.2
Pancreas	≤14	11/17, 64.7	3/11, 27.3
	15-30	3/12, 25.0	0/3, 0
	≥31	2/14, 14.3	0/2, 0
Total		16/43, 37.2	3/16, 18.8

Tissue	DOI (days)	ddPCR+ (n, %)	sgRNA+ (n, %)
All Reproductive		17/40, 42.5	2/17, 11.8
Ovary	≤14	3/5, 60.0	0/3, 0
	15-30	0/3, 0	NA
	≥31	NA	NA
Total		3/8, 37.5	0/3, 0
Uterus	≤14	1/1, 100	0/1, 0
	15-30	2/2, 100	1/2, 50.0
	≥31	NA	NA
Total		3/3, 100	1/3, 33.3
Testis	≤14	7/10, 70.0	1/7, 14.3
	15-30	1/8, 12.5	0/1, 0
	≥31	3/12, 25.0	0/3, 0
Total		11/30, 36.7	1/11, 9.1
Muscle, Skin, & Peripheral Nerves		30/44, 68.2	9/30, 30.0
Skeletal Muscle	≤14	12/17, 70.6	5/12, 41.7
	15-30	7/13, 53.8	1/7, 14.3
	≥31	3/14, 21.4	0/3, 0
Total		22/44, 50	6/22, 27.3
Skin	≤14	3/3, 100	1/3, 33.3
	15-30	1/1, 100	0/1, 0
	≥31	1/7, 14.3	0/1, 0
Total		5/11, 45.5	1/5, 20.0
Peripheral Nervous System	≤14	13/15, 86.7	5/13, 38.5
	15-30	6/11, 54.5	0/6, 0
	≥31	7/14, 50.0	2/7, 28.6
Total		26/40, 65.0	8/26, 30.8
All Ocular		22/38, 57.9	7/21*, 33.3
Ocular Tissue	≤14	9/12, 75.0	6/9, 67.7
	15-30	4/9, 44.4	0/4, 0
	≥31	6/11, 54.5	1/5*, 20.0
Total		19/32, 59.4	7/18, 38.9
Ocular Humor	≤14	6/13, 46.2	1/6, 16.7
	15-30	3/11, 27.3	0/3, 0
	≥31	2/10, 20.0	0/1*, 0
Total		11/34, 32.4	1/10, 10.0
Optic Nerve	≤14	10/12, 83.3	3/10, 30.0
	15-30	2/6, 33.3	0/2, 0
	≥31	5/11, 45.5	0/5, 0
Total		17/29, 58.6	3/17, 17.6
All Central Nervous System		10/11, 90.9	4/10, 40.0
Cervical Spinal Cord	≤14	2/2, 100	1/2, 50.0
	15-30	1/1, 100	0/1, 0
	≥31	5/6, 83.3	0/5, 0
Total		8/9, 88.9	1/8, 12.5
Olfactory Nerve	≤14	2/3, 66.7	1/2, 50.0
	15-30	1/2, 50.0	0/1, 0
	≥31	3/5, 60.0	0/3, 0
Total		6/10, 60.0	1/6, 16.7
Basal Ganglia	≤14	1/2, 50.0	0/1, 0
	15-30	1/2, 50.0	0/1, 0
	≥31	3/4, 75.0	0/3, 0
Total		5/8, 62.5	0/5, 0
Cerebral Cortex	≤14	3/3, 100	1/3, 33.3
	15-30	1/2, 50.0	1/1, 100
	≥31	5/6, 83.3	0/5, 0
Total		9/11, 81.8	2/9, 22.2
Brainstem	≤14	3/3, 100	1/3, 33.3
	15-30	1/2, 50.0	1/1, 100
	≥31	4/5, 80.0	0/4, 0
Total		8/10, 80.0	2/8, 25.0
Cerebellum	≤14	2/3, 66.7	0/2, 0
	15-30	1/2, 50.0	0/1, 0
	≥31	6/6, 100	0/6, 0
Total		9/11, 81.8	0/9, 0
Thalamus	≤14	2/2, 100	1/2, 50.0
	15-30	1/2, 50.0	0/1, 0
	≥31	5/6, 83.3	0/5, 0
Total		8/10, 80.0	1/8, 12.5
Hypothalamus	≤14	2/2, 100	1/2, 50.0
	15-30	1/1, 100	0/1, 0
	≥31	4/4, 100	0/4, 0
Total		7/7, 100	1/7, 14.3
Corpus Callosum	≤14	2/2, 100	0/2, 0
	15-30	1/1, 100	0/1, 0
	≥31	4/4, 100	0/4, 0
Total		7/7, 100	0/7, 0
CNS Vasculature	≤14	2/2, 100	0/2, 0
	15-30	1/2, 50	0/1, 0
	≥31	3/5, 60	0/3, 0
Total		6/9, 66.7	0/6, 0
Dura Mater	≤14	3/3, 100	2/3, 66.7
	15-30	0/1, 0	NA
	≥31	0/5, 0	NA
Total		3/9, 33.3	2/9, 66.7

866 Extended Data Table 3 **Summary of SARS-CoV-2 RNA and sgRNA by tissue category over**  
867 **time.** (a) Summary of the average nucleocapsid gene copies/ng RNA across cases by tissue  
868 category and duration of illness (days). (b) Summary of the number and percentage of cases with  
869 SARS-CoV-2 RNA detected via droplet digital (dd)PCR by tissue category for all cases and by  
870 tissue and duration of illness (days). The number and percentage of tissues positive for ddPCR  
871 that were additionally positive for subgenomic (sg)RNA PCR is listed in the right most column.  
872 \*A tissue positive via ddPCR was not tested via sgRNA PCR. CNS/central nervous system,  
873 LN/lymph node.

874

Cell Type	Locations
Bile duct epithelium	Liver
Chondrocytes	Bronchial cartilage rings
Collecting duct epithelium	Kidney
Distal tubule epithelium	Kidney
Endocrine cells of adrenal	Adrenal gland
Endocrine cells of thyroid	Thyroid
Endothelium	Vasculature, all
Ependyma	Brain
Exocrine cells of pancreas	Pancreas
Fibroblast-like cells	Pericardium, heart, trachea, bronchus
Germ cells	Testis
Glandular epithelium	Uterus
Glia	Brain, all locations
Hepatocytes	Liver
Hyaline Membrane	Lung
Interstitial cells of endometrium	Uterus
Intimal cells	Aorta
Kupffer cells	Liver
Leydig cells	Testis
Mononuclear leukocytes	Lung, spleen, lymph nodes, lymphoid aggregates of GI
Mucosal epithelium	Small intestine, colon
Mucus secreting epithelium, salivary type	Salivary glands, trachea, bronchus
Myocytes, Cardiac	Heart
Myocytes, Striated	Psoas muscle
Myocytes, Smooth	Uterus, GI
Neurons	Brain, all locations
Parietal cells	Kidney, Bowman's capsule
Pneumocytes, type I & II	Lung
Purkinje cell	Cerebellum
Schwann cells	Nerves, all
Sertoli cells	Testis
Stratified epithelium (& basal layer)	Trachea, esophagus
Stromal cells	Pericardium, uterus, ovary
Vascular smooth muscle	Arteries, all

875

876 Extended Data Table 4 **SARS-CoV-2 cellular tropism**. Summary of cell types that were  
877 identified as SARS-CoV-2 positive by ISH, and the corresponding anatomic sites in which this  
878 was observed.

<b>Cause of Death</b>	<b>N = 44</b>
Death with (but not from) COVID-19	5 (11%)
Death from COVID-19 or complications	39 (89%)
<b>Pulmonary Findings<sup>1</sup></b>	<b>N (%) or Median (IQR)</b>
Left Lung Weight (g) <sup>2</sup>	795 (327)
Right Lung Weight (g) <sup>2</sup>	820 (365)
Combined Lung Weight (g)	1600 (528)
Diffuse Alveolar Damage	
Exudative	14 (32%)
Proliferate	15 (34%)
Fibrosing	7 (16%)
Not Found	8 (18%)
Acute Pneumonia	27 (61%)
Pulmonary Edema	30 (68%)
Pulmonary Hemorrhage (at least focal)	14 (32%)
Pulmonary Thromboembolism, Infarction	10 (23%)
Emphysematous changes (underlying COPD)	12 (27%)
<b>Cardiac Findings</b>	
Heart Weight (g)	500 (175)
Myocardial Infiltrate	4 (9%)
Focal infiltrate without myocyte necrosis	3 (7%)
Diffuse lymphocytic myocarditis	1 (2%)
Myocardial Ischemic Necrosis	
Remote, fibrotic	5 (11%)
Acute microscopic ischemia	4 (9%)
Coronary Artery Disease with ≥ 50% in at least 1 artery	16 (36%)
<b>Renal Findings</b>	
Left Kidney Weight (g) <sup>4</sup>	180 (107)
Right Kidney Weight (g) <sup>4</sup>	168 (79)
Changes consistent with Acute Kidney Injury	17 (39%)
Changes consistent with Diabetic glomerulopathy	10 (23%)
<b>Splenic Findings</b>	
Splenic Weight (g)	235 (215)
Follicular hyperplasia	15 (34%)
Lymphodepletion	
Present	8 (18%)
Some, Partial Preservation	34 (77%)
No Lymphodepletion	2 (5%)
Red Pulp Congestion	35 (80%)
Infarction	2 (5%)

<b>Lymph Node Findings<sup>5</sup></b>	<b>N (%) or Median (IQR)</b>
Lymphodepletion	
Present	5 (12%)
Some, Partial Preservation	4 (10%)
No Lymphodepletion	31 (78%)
Follicular Hyperplasia	
Present	22 (55%)
Present, regressed	2 (5%)
Paracortical Hyperplasia	32 (80%)
Plasmacytosis	19 (48%)
Plasmablasts noted	4 (10%)
<b>Hepatic Findings<sup>3</sup></b>	
Liver Weight (g) <sup>4</sup>	1670 (900)
Hepatic necrosis	
None	30 (70%)
Zonal	12 (28%)
% Zonal Necrosis	30% (40%)
Massive	1 (2%)
Steatosis	
None to Minimal	24 (56%)
Mild	14 (33%)
Moderate	5 (12%)
Steatohepatitis	5 (12%)
Portal Inflammation	
None to Minimal	16 (37%)
Mild	23 (53%)
Moderate	4 (9%)
Fibrosis	
None	27 (63%)
Periportal or perisinusoidal	6 (14%)
Periportal and perisinusoidal	1 (2%)
Bridging fibrosis	6 (14%)
Cirrhosis	3 (7%)
<b>Central Nervous System Findings (N=11)</b>	
Brain Weight (g)	1350 (230)
Hypoxic/Ischemic Injury (focal or diffuse)	5 (45%)
Vascular congestion	5 (45%)
Focal (microscopic) hemorrhage	2 (18%)
No pathological findings	3 (27%)

879

880 Extended Data Table 5 **Histopathologic findings of COVID-19 autopsy cases.** Summary of  
881 histopathologic findings across organ system across 44 autopsy cases. Central nervous system  
882 findings are reported for the 11 cases in which consent for sampling was obtained. <sup>1</sup>Includes one

883 case in which the COVID lungs were transplanted and data from explanted lungs used in table.

884 <sup>2</sup>Individual lung weights were missing in 4 cases. <sup>3</sup>Findings missing on 1 case due to extreme

885 autolysis. <sup>4</sup>Weight missing on one case. <sup>5</sup>Lymph node findings missing in 4 cases

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryData1.xlsx](#)
- [SupplementaryData2.xlsx](#)