SARS-CoV-2 causes severe alveolar inflammation and barrier dysfunction

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Short Running Title: SARS-CoV-2 damage alveolar barrier function

46   **ABSTRACT (172):**

47   Infections with SARS-CoV-2 lead to mild to severe coronavirus disease-19 (COVID-19)

48   with systemic symptoms. Although the viral infection originates in the respiratory

49   system, it is unclear how the virus can overcome the alveolar barrier, which is

50   observed in severe COVID-19 disease courses.

51   To elucidate the viral effects on the barrier integrity and immune reactions, we used

52   mono-cell culture systems and a complex human alveolus-on-a-chip model composed

53   of epithelial, endothelial, and mononuclear cells.

54   Our data show that SARS-CoV-2 efficiently infected epithelial cells with high viral

55   loads and inflammatory response, including the interferon expression. By contrast, the

56   adjacent endothelial layer was no infected and did neither show productive virus

57   replication or interferon release. With prolonged infection, both cell types are

58   damaged, and the barrier function is deteriorated, allowing the viral particles to

59   overbear.

60   In our study, we demonstrate that although SARS-CoV-2 is dependent on the

61   epithelium for efficient replication, the neighboring endothelial cells are affected, e.g.,

62   by the epithelial cytokine release, which results in the damage of the alveolar barrier

63   function and viral dissemination.

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65

66 **INTRODUCTION**

67 The novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) is a highly

68 pathogenic virus causing severe respiratory infections, described as coronavirus

69 disease-19 (COVID-19) (Bar-On et al., 2020). Patients suffer from various symptoms as

70 fever, cough, breath shortness, headache, muscle aches, and gastrointestinal

71 symptoms. Hallmarks of severe COVID-19 courses are pneumonia, pulmonary

72 edema, acute respiratory distress syndrome (ARDS), and multiple organ failure. In

73 most patients, the disease has a mild course, but in some cases, e.g., elderly with

74 comorbidities, the infection can develop into a life-threatening condition. In particular

75 preexisting lung pathologies and systemic diseases such as diabetes predispose to

76 severe infection courses described by George et al. (2020).

77 Clinical studies revealed that the virus primarily replicates in the lung, which can

78 cause severe lung damage up to necrotic destruction of large areas of the lung tissue

79 (Carsana et al., 2020). In autopsies of deceased COVID-19 patients, it has been

80 observed that particularly in severe cases viral particles can disseminate throughout

81 the body (Deinhardt-Emmer et al., 2020b; Wichmann et al., 2020). Additionally,

82 systemic complications have been reported, such as the massive release of

83 proinflammatory cytokines and thromboembolic events in various organs (Becker,

84 2020). Consequently, SARS-CoV-2 is regarded as a pneumotropic virus that infects the

85 patient via the lung but can also cause a systemic infection that affects different organs

86 with a high mortality rate.

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87   Little is known about the initial infection process in the alveolar lung tissue,

88   particularly about mechanisms that destroy the lung and mechanisms that allow the

89   virus to affect different organs in the body. Infection models that closely reflect the

90   patient's situation are mainly lacking in part due to the challenges to infect mice and

91   the difficulty of accessing and analyzing infected human alveolar lung cells. Up to

92   now, SARS-CoV-2 infection models have been mainly performed with human airway

93   (non-alveolar) cells or non-human cell lines that naturally express the ACE2 viral

94   receptor, such as the African Green Monkey Vero 76 cell line (Hoffmann et al., 2020).

95   These cells lack organ- and species-specific characteristics of human lung epithelial

96   cells. For this purpose, cancerous lung epithelial cells (Calu-3 cells), can at least to some

97   extent, reflect the response of the lung epithelium to viral infection (Bestle et al., 2020).

98   In our study, we present a human-specific *in vitro*, alveolus-on-a-chip model

99   composed of cells of human origin susceptible for a SARS-CoV-2 infection. This model

100   was only recently developed in our lab (Deinhardt-Emmer et al., 2020a). Within the

101   present study, it was modified by using SARS-CoV-2 permissive epithelial cells (Calu-

102   3 cells). The epithelial and vascular (primarily isolated human umbilical vascular

103   endothelial cells; HUVECs) cells were co-cultured with macrophages (primarily

104   isolated peripheral blood mononuclear cells; PBMCs) resembling the human alveolus

105   architecture and function. This composition is not only relevant for the gas exchange

106   but also for an adequate immune response.

107   We were able to show that SARS-CoV-2 replicates in the epithelial layer while

108   inducing an acute and robust inflammatory response followed by the destruction of

4

109   the epithelial layer. Interestingly, in this infection scenario, the endothelial cells were

110   not invaded by SARS-CoV-2 and did not propagate the virus, but nevertheless the

111   epithelial/endothelial barrier integrity was disrupted.

112

113   **RESULTS**
114

115   **Efficient SARS-CoV-2 isolation from patients and propagation in cell-culture**

116   To gain fully infectious viral particles for our studies, we collected three respiratory

117   specimens from qRT-PCR-proven COVID-19 patients and performed SARS-CoV-2

118   propagation in cell culture systems (Vero-76 cells). By repeated infection of host cells

119   and viral replication, we were able to isolate high viral titers originating from three

120   different patients. Within our studies, the SARS-CoV-2 isolates SARS-CoV-

121   2/hu/Germany/Jena-vi005159/2020   (5159),   SARS-CoV-2/hu/Germany/Jena-

122   vi005187/2020 (5587) and SARS-CoV-2/hu/Germany/Jena-vi005588/2020 (5588) were

123   employed. Sequencing of virus isolates verified that all three viral strains belong to

124   SARS-CoV-2 (species *Severe acute respiratory syndrome-related coronavirus*, genus

125   *Betacoronavirus*) (Gorbalenya et al., 2020). Phylogenetic analysis revealed a close

126   relationship of SARS-CoV-2 to the SARS-related coronaviruses RaTG13, bat-SL-

127   CoVZXC21 and bat-SL-CoVZC45 (Figure 1A). Within the SARS-CoV-2 clade, the

128   sequences of strains 5587 and 5588 exhibit two base substitutions T8,782C (**nsp1ab:**

129   synonymous) and C28,144T (**nsp8:** S84L), which are characteristic of the all strains of

130   lineage L ((Tang et al., 2020) nomenclature) or lineage B ((Rambaut et al., 2020)

131   nomenclature). Accordingly, 5587 and 5588 clustered with lineage L/lineage B strains

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132 in the phylogenetic analysis (Figure 1B). Furthermore, both strains exhibit deletion of

133 **nsp1ab** D448 and two synonymous substitutions (T514C, C5512T). Beside the **nsp8**

134 S84L substitution, strain 5159 has accumulated three additional amino acid

135 substitutions (**S:** D614G, **nsp1ab:** P4715L and **N:** R203K/G204R) which place this virus

136 in lineage B.1.1 according to the proposed SARS-CoV-2 nomenclature of Rambaut et

137 al. (2020) (Figure 1B).

138

139 **Mono-culture cells can be infected by SARS-CoV-2 and produce replication**

140 **complexes at ER-derived membranes.**

141 At first, we infected mono-cell culture systems with SARS-CoV-2 and compared the

142 infection rate between Vero-76 cells and Calu-3 cells. It is already well known that

143 Vero-76 cells can be efficiently infected by SARS-CoV-2 (Hoffmann et al., 2020; Shang

144 et al., 2020). Using transmission electron microscopy (TEM), we could demonstrate

145 that Vero-76 cells host and efficiently propagate the virus (Figure 2A). Figure 2A

146 (upper panel) illustrates infected Vero-76 cell containing viral replication organelles.

147 In the lower-left panel, protein accumulation and generation of double-membrane

148 vesicles are visible. In the middle panel, virion assembly in the ER–Golgi-intermediate

149 compartment (ERGIC) and a Golgi complex are imaged containing morphologically

150 complete viral particles. Here, the particles are packed to be transported to the cellular

151 surface for virus release, which is demonstrated in the right panel. Some particles are

152 still attached to the host cell membrane, whereas some viral particles are already fully

153 released. These results indicate that SARS-CoV-2 induces replication complexes at ER-

154   derived membranes, which were already shown for other types of coronaviruses

155   (Stertz et al., 2007) and also confirm the findings for Vero E6 cells (Ogando et al., 2020).

156   To better mimic the situation in the human pulmonary alveoli, we performed the

157   infection in Calu-3 cells. In Figure 2B, immunofluorescence measurements compare

158   infected Vero-76 cells with infected Calu-3 cells. In both cell types, viral particles can

159   be visualized to a similar extent using specific antibodies against SARS-CoV-2 spike

160   proteins. These results are confirmed by western blot analysis, demonstrating

161   increased levels of SARS-CoV-2 spike protein in the cell lysates after 8h and 24h

162   (Figure 2C).

163   Additionally, we could verify progeny virus particles by performing plaque assays

164   from supernatants of both cell types indicating increased replication during ongoing

165   infection (Figure 2D). Measuring viral RNA-loads in different infected host cell types,

166   we found high RNA levels in Vero-76 and Calu-3 cells (Figure S1A). By contrast,

167   HUVEC mono-cell cultures could not be infected by SARS-CoV-2 (Figure S1B).

168   We further analyzed the host response to the infection by measuring the cytokine

169   mRNA expression of Calu-3 cells. 24h post-infection, many inflammatory cytokines

170   were significantly increased compared to control cells (Figure 3). These results reflect

171   the high cytokine levels found in COVID-19 patients (Costela-Ruiz et al., 2020),

172   indicating that infected epithelial cells contribute to the "cytokine storm" in severe

173   COVID-19 cases. Since different cytokines and chemokines are involved in the

174   infection process, a robust immune response has been associated with a severe clinical

175   course (Coperchini et al., 2020). Our results clearly show that the epithelial cell line

176   Calu-3 can be efficiently infected by SARS-CoV-2, propagates the virus and answer to

177   the viral infection with a strong cytokine release.

178

179   **SARS-CoV-2 infects epithelial cells within the alveolus-on-a-chip model causing a**

180   **strong IFN-response**

181   In the next step, we modified our alveolar-on-a-chip model (Deinhardt-Emmer et al.,

182   2020a) by seeding Calu-3 cells on the epithelial side, primarily isolated HUVECs on

183   the endothelial side and integrated PBMCs to represent the immune response.

184   However, macrophages not show an productive viral replication (Yip et al., 2014), they

185   are mainly involved in inflammatory response (Kumar et al., 2020).

186   The applied system is ventilated and perfused and can be infected with SARS-CoV-2

187   via the epithelial side. Using the viral particles isolated from the three COVID-19

188   patients' specimens, an infection by SARS-CoV-2 on the epithelial cells was proven

189   and the viral particles were propagated (Figure 4A). This effect was still visible after

190   40h post-infection (Figure S2A). In response to the infection the epithelial cells reacted

191   with a robust cytokine response, demonstrated by elevated IFN-levels in the cell

192   culture supernatants of the epithelial side (Figure 4C).

193   In general, the production of IFN is the most efficient way of fighting viral infections;

194   e.g. secretion of type I IFN (IFN-α/β) exhibits direct antiviral effects by inhibiting viral

195   replication (Thiel and Weber, 2008) among many other interferon effects that promote

196   the immune response to infection (Kindler 2016). Yet, evasion strategies for different

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197   types of coronaviruses have been described. The viruses express factors and posses

198   strategies to inhibit IFN induction/expression (Thoms et al., 2020) or IFN signaling or

199   to increase IFN resistance, which is reviewed by E. Kindler et al. (2016). Consequently,

200   SARS-CoV-2 is apparently able to cope with the interferon response of epithelial cells,

201   which is reflected by our measurements, demonstrating efficient viral replication and

202   persistence for up to 40h despite a strong epithelial interferon response (Figure 3 and

203   4).

204   By contrast, we did not detect viral propagation and did not measure an interferon

205   response at the endothelial side of the biochip (Figure 4C). We could not visualize viral

206   components within endothelial cells neither at 28h (Figure 4B) nor at 40h post-infection

207   (Figure S2B) demonstrating that the viral particles do not productively infect

208   endothelial cells in the human alveolus-on-a-chip model. Additionally, endothelial

209   mono-cell culture systems could not be infected by SARS-CoV-2 (Figure S1A, B)

210   confirming the cell-type specificity of the viral pathogens for lung epithelial cells. This

211   is in line with *in vivo* studies that describe only a weak IFN-response in the serum of

212   COVID-patients (Hu et al., 2020). In animal models with mouse-adapted SARS virus a

213   delayed onset of the IFN-response resulting in immune dysregulation was described

214   (Channappanavar et al., 2016). The weak and delayed IFN-levels in the serum are

215   probably due to the host cell specificity of SARS-CoV-2, as lung epithelium represents

216   the main infection focus and endothelial cells are only hardly/not infected.

217   Taken together, these results indicate that endothelial cells of the lung model are not

218   the primary target cells of SARS-CoV-2 which is in agreement with previous studies

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219   (Bar-On et al., 2020). Further, it is in line with the observation that endothelial layer of

220   the alveolar capillaries of deceased COVID-19 patients were still intact but epithelial

221   tissue was found seriously damaged (Deinhardt-Emmer et al., 2020b). Although the

222   mechanism is not clear the increased proinflammatory cytokine release might cause

223   an endothelial dysfunction. In addition to the origin of endothelial cells from different

224   organs, comorbidities like obesity and diabetes might render endothelial cells

225   susceptible to be infected as recently described (Huertas et al., 2020; Pons et al., 2020).

226   There is clinical evidence for severe courses of COVID-19 in particular, when

227   preexisting endothelial damage can be suspected (Varga et al., 2020).

228   Further studies are required to elaborate the impact of the endothelial phenotype and

229   the infection conditions at which these cells are targeted by SARS-CoV-2. In the model

230   used in this study, the pneumotropic features of SARS-CoV-2 could be confirmed

231   based on viral uptake and replication in epithelial cells accompanied with an

232   interferon response described in previous studies. The viral particles were not

233   transferred to the neighboring endothelial layer, although the cells were co-cultivated

234   in a bioinspired manner to recreate the alveolar structure.

235

236   **SARS-CoV-2 disrupts the alveolar barrier within the alveolus-on-a-chip model**

237   Next, we analyzed the barrier function of the epithelial-endothelial cell layers in our

238   biochip model. Many clinical case reports and studies describe that critical ill COVID-

239   patients develop severe lung destructions and a systemic sepsis-like syndrome

240   (Ackermann et al., 2020; Gao et al., 2020) that could be partly explained by a disrupted

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241 barrier function in the lung. From the immunofluorescence image (Figure 4), we could

242 observe some destruction of the epithelial or endothelial layer, in particular 40h post-

243 infection (Figure S2). To better visualize the cell-layers during the infection, we

244 performed scanning electron microscopy (SEM) analysis of the surface structures

245 following 28h post-infection. On the epithelial side we found dead cells and remnants

246 attached to the cell layer. Dying cells are identified by shrinking, balling, disruption of

247 the plasma membrane, and the loss of microvilli, which can be observed in the upper

248 and middle panel of the infected cells, but to a much lesser extent in mock-treated cells

249 (Figure 5A, upper panel). Interestingly, at a higher magnification we could display the

250 viral particles on the surface of the dead cells (Figure 5A, lower panel).

251 It is well known that respiratory viral pathogens induce cell death (including

252 apoptosis) in the respiratory epithelium, such as the influenza virus (Atkin-Smith et

253 al., 2018). Already the 2003 SARS-CoV led to apoptotic cell death induced by

254 membrane proteins via modulation of the Akt-pathway (Chan et al., 2007).

255 Additionally, prolonged stress of the endoplasmic reticulum (ER) was identified as a

256 trigger for apoptosis (Fung and Liu, 2014). Within the recent pandemic, it has been

257 shown that the largest unique open reading frame (ORF) of the SARS-CoV-2 genome

258 ORF3a is associated with a pro-apoptotic activity (Ren et al., 2020). These studies

259 indicate that the induction of an apoptotic process in the course of SARS-CoV2

260 infection is highly probable.

261 By contrast, on the endothelial side, we could not observe differences in the

262 morphological appearance between SARS-CoV-2-infected and mock-treated cells.

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263   Here, the cell integrity of the cell layers appeared intact apart from shrinking artefacts

264   due to the drying procedure (Figure 5B). At the high magnification (lower panel) dead

265   cell remnants show granular residues of the cytoplasm indicating the loss of the

266   plasma membrane, but no viral particles could be visualized.

267   To measure the epithelial and endothelial cell viability we performed LDH-assays on

268   cells grown in the biochip. 28h post-infection we observed in the infected epithelial

269   layer a significantly enhanced release of LDH, whereas SARS-CoV-2 did not induce an

270   enhanced LDH release in endothelial cells (Figure 5D). However, after more extended

271   infection periods (40h; Figure 5D), the barrier function on the endothelial side was also

272   affected. These results suggest that even if the endothelial layer is not infected the cell

273   integrity gets disturbed, most likely by cytokines released by macrophages. In this

274   respect, it is known that high cytokine/interferon levels can induce the disruption of

275   the alveolar barrier function (Broggi et al., 2020; Gustafson et al., 2020; Pelaia et al.,

276   2020).

277   To further analyze the barrier integrity of the biochip system on a functional level, we

278   performed permeability assays using FITC-dextran to investigate the endothelial and

279   epithelial cell barrier integrity. We were able to show that SARS-CoV-2 significantly

280   increased the tissue permeability with its barrier function severely impaired (Figure

281   5C). As a consequence of the disrupted barrier, we detected viral particles by

282   performing plaque assays of the cell supernatants in the endothelial chamber at the

283   late time point of 40h (Figure 5E). These results show that endothelial cells are affected

284   by the viral infection at late time points and that the disturbed cell integrity results in

285   translocation of the viral particles over the alveolar barrier.

286

287   **DISCUSSION**

288   In this manuscript, we present an *in vitro* human alveolus-on-a-chip model based on

289   human cells that closely mimics alveolar structures and can be efficiently infected by

290   SARS-CoV-2. The epithelial of the alveolar models was demonstrated to be prone to

291   SARS-CoV2 infection and to propagate viral replication with high viral loads. These

292   findings are in line with clinical observations that in lung tissue by far, the highest viral

293   burdens are measured (Carsana et al., 2020). This phenomenon can be explained by

294   the cell tropism of SARS-CoV-2 to airway cells that contribute to the high shedding of

295   viral particles in the respiratory system and the high infectivity of patients via aerosols.

296   Nevertheless, due to the systemic symptoms in severe COVID-patients, it has been

297   discussed, whether other cell types besides the airway epithelium, are targeted by

298   SARS-COV-2, as well. In particular, vascular complications, such as thrombotic events

299   (Helms et al., 2020), could result from the dissemination and propagation of viral

300   particles in the endothelial system. In our model system, we could not confirm viral

301   invasion into endothelial cells, although the cells were cultured in close proximity to

302   the infected epithelium.

303   Yet, with increased time of infection (40h), the endothelial cells become damaged

304   resulting in a decline in tissue barrier function. This effect is most likely mediated by

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305   the cytokine release of the infected neighboring epithelium. Cytokine release is known

306   to disturb various cellular functions, such as protein biosynthesis and barrier integrity.

307   Many studies reveal that most severe cases of SARS-CoV-2 infections are not only due

308   to enhanced viral burden, but to a large extent due to aberrant immune responses

309   (Broggi et al., 2020).

310   In our manuscript, we present an infection model that could be further used to study

311   several aspects during the SARS-CoV-2 infection: (i) At first, the cellular interaction

312   can be analyzed in detail with increasing complexity. Here, the interaction between

313   endothelial and epithelial cells, and the role of different immune cells that can be

314   integrated into the biochip could be elaborated. (ii) Another crucial aspect is

315   preexisting damage, such as diabetic vascular changes or inflammatory foci that may

316   promote a COVID-19 infection. These factors can be mimicked in the biochip model to

317   investigate their impact on infection development. (iii) A third important issue are

318   novel therapeutic agents. Antiviral and anti-inflammatory therapies can be tested in

319   the biochip model to obtain initial results on their mode of action.

320   Consequently, our biochip model represents a valuable tool to study many aspects

321   during COVID-19 infections.

322

323   **MATERIAL AND METHODS**

324   **Virus isolation, propagation and standard plaque-assays.** SARS-CoV-2 was isolated

325   from the respiratory specimen of three different patients and named (SARS-CoV-

326   2/hu/Germany/Jena-vi005159/2020   (5159),   SARS-CoV-2/hu/Germany/Jena-

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327 vi005187/2020 (5587) and SARS-CoV-2/hu/Germany/Jena-vi005188/2020) (5588) (ethic

328 approvement of the Jena University Hospital, no.: 2018-1263) by using Vero-76 cells.

329 For this, cells were washed 12 h after seeding and infected with 200 μl filtered patient

330 sample (sterilized syringe filter, pore size 0,2 μm) under the addition of Panserin 401

331 (PanBiotech, Germany). After five days, the cytopathic effect was notable. Then, cells

332 were frozen, centrifuged, and clear supernatants were obtained.

333 To generate well-defined viral stocks, plaque purification procedures were performed.

334 For this, confluent Vero-76 cell cultures were infected with serial dilutions of virus

335 isolates diluted in EMEM for 60 min at 37°C and 5% CO_2. Thereafter, inoculum was

336 exchanged with 2 ml MEM/BA (medium with 0.2 % BSA) supplemented with 0.9 %

337 agar (Oxoid, Wesel, Germany), 0.01 % DEAE-Dextran (Pharmacia Biotech, Germany)

338 and 0.2% $NaHCO_3$ until plaque formation was observed. Single plaques were marked

339 using inverse microscopy. Contents of these plaques were used to infect confluent

340 Vero-76 cell monolayers in T25 flasks. Cells were incubated at 37°C and 5% CO_2 until

341 pronounced cytopathic effects were visible. Then, cell cultures were frozen again and

342 clear supernatants were obtained. This plaque purification procedure was repeated

343 again. Finally, virus stocks were generated and titrated using plaque assays. For this,

344 Vero-76 cells were seeded in 6-well plates until a 90 % confluency and infected with

345 serial dilutions of the supernatants in PBS/BA (1 mM MgCl2, 0,9 mM CaCl, 0,2 % BSA,

346 100 U/ml Pen/Strep) for 90 min at 37°C. After aspiration of the inoculum, cells were

347 incubated with 2 ml MEM/BA (medium with 0.2 % BSA) supplemented with 0.9 %

348 agar (Oxoid, Wesel, Germany), 0.01 % DEAE-Dextran (Pharmacia Biotech, Germany)

15

349 and 0,2% NaHCO3 at 37°C and 5 % CO_2 for four days. The visualization was

350 performed by the staining with crystal violet solution (0.2 % crystal violet, 20 %

351 ethanol, 3.5 % formaldehyde in water) and the number of infectious particles (plaque-

352 forming units (PFU) ml^{-1}) was determined.

353

354 **Sequencing and genome reconstruction.** Library preparation was performed

355 according   to   the   "nCoV-2019   sequencing   protocol"

356 (dx.doi.org/10.17504/protocols.io.bdp7i5rn)   from   the   ARTICnetwork

357 (https://artic.network/ncov-2019). Briefly, viral RNA was isolated for SARS-CoV-2

358 virus strains 5159, 5587, and 5588 via the QIAmp viral RNA Kit (Qiagen, Hilden,

359 Germany) according to the manufacturers' guide. The cDNA preparation was

360 performed using the SuperScript IV (Thermofisher), followed by a multiplex PCR to

361 generate overlapping 400 nt amplicons using version 3 of the primer set

362 (https://github.com/artic-network/artic-ncov2019/tree/master/primer_schemes/nCoV-

363 2019/V3). After PCR cleanup, library preparation was performed using the Ligation

364 Sequencing Kit (LSK-109, Oxford Nanopore Technologies) and the Native Barcoding

365 Expansion (EXP-NBD104, native Barcoding Kit (Oxford Nanopore Technologies)).

366 Sequencing was performed on a MinION device using an R.9.4.1 flow cell (Oxford

367 Nanopore Technologies). Basecalling and genome reconstruction was performed

368 using poreCov v.0.2 with the default settings (https://github.com/replikation/poreCov).

369

370   **Cell culture and virus infection.** Vero-76 cells were cultured in EMEM with HEPES

371   modification, and 5 mM L-Glutamine. Calu-3 cells were cultured in RPMI-1640

372   supplemented with 10 % fetal calves' serum (FCS). M199 was purchased from Lonza

373   (Verviers, Belgium), fetal calf serum (FCS), human serum and endothelial growth

374   supplement were from Sigma (Taufkirchen, Germany).

375   PBMCs were isolated and cultivated as previously described (Deinhardt-Emmer et al.,

376   2020a). Human umbilical vein endothelial cells (HUVEC) were isolated from

377   anonymously acquired human umbilical cords according to the Declaration of

378   Helsinki, "Ethical principles for Medical Research Involving Human Subjects" (1964).

379   After rinsing the cord veins with 0.9 % NaCl, endothelial cells were detached with

380   collagenase (0.01 %, 3 min at 37 °C), suspended in M199/10 % FCS, washed once (500

381   x g, 6 min) and seeded on a cell culture flask coated with 0.2 % gelatin. 24 h later, full

382   growth medium was added (M199, 17.5 % FCS, 2.5 % human serum, 7.5 µg/ml

383   endothelial mitogen, 7.5 U/ml heparin, 680 µM glutamine, 100 µM vitamin C, 100 U/ml

384   penicillin, 100 µg/ml streptomycin). HUVEC from the second passage were seeded on

385   30-mm dishes or on 90-mm dishes at a density of 27,500 cells/cm². Experiments were

386   performed 72 h after seeding. For the cultivation of the human alveolus-on-a-chip

387   model we used Calu-3 cells and macrophages at the epithelial side, and HUVECs at

388   the endothelial side. The Multiorgan tissue flow (MOTiF) biochips were manufactured

389   and obtained from microfluid ChipShop GmbH (Jena, Germany), as explained

390   previously (Deinhardt-Emmer et al., 2020a).

391   For infection of Vero-76 or Calu-3 cells, cells were washed with PBS and either left

392   uninfected (mock) or infected with SARS-CoV-2 with a multiplicity of infection (MOI)

393   of 1 for 120 min in medium (EMEM with HEPES modification and 5 mM L-Glutamine

394   for Vero-76 cells and RPMI 1640 for Calu-3 cells) supplemented with 10 % FCS.

395   Subsequently, supernatants were removed, and cells were supplemented with fresh

396   medium supplemented with 10 % FCS and further incubated for the times indicated

397   at 37°C, 5 % CO_2.

398   For the infection of the human alveolus-on-a-chip, cells were washed with PBS once,

399   followed by treatment with PBS (mock) at 37°C and RPMI (0.2 % autologous human

400   serum, 1 mM $MgCl_2$, 0.9 mM $CaCl_2$) or infection with SARS-CoV-2 virus (1 MOI). After

401   90 min incubation cells were washed and supplemented with medium. Afterwards,

402   cells were incubated for the indicated times at 37°C, 5 % CO_2.

403

404   **Transmission electron microscopy.** Confluent monolayers of Vero-76 cells (9 cm petri

405   dishes) were infected with SARS-CoV-2 (isolate 5159) using an MOI of 1. After 24 h,

406   supernatants were removed, and samples were fixed with freshly prepared modified

407   Karnovsky fixative consisting of 4 % w/v paraformaldehyde and 2.5 % v/v

408   glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 for 1h at room temperature.

409   After washing 3 times for 15 min each with 0.1 M sodium cacodylate buffer (pH 7.4)

410   the cells were post-fixed with 2 % w/v osmium tetroxide for 1h at room temperature.

411   Subsequently, the cells were rewashed with 0.1 M sodium cacodylate buffer (pH 7.4),

412   thoroughly scraped off the petri dishes, and pelleted by centrifuging at 600 x g for 10

413 min. During the following dehydration in ascending ethanol series, post-staining with

414 1 % w/v uranyl acetate was performed. Afterwards, the pellets were embedded in

415 epoxy resin (Araldite) and ultrathin sectioned (70 nm) using a Leica Ultracut S (Leica,

416 Wetzlar, Germany). Finally, the sections were mounted on filmed Cu grids, post-

417 stained with lead citrate, and studied in a transmission electron microscope (EM 900,

418 Zeiss, Oberkochen, Germany) at 80 kV and magnifications of 3,000x to 85,000x. For

419 image recording, a 2K slow-scan CCD camera (TRS, Moorenweis, Germany) was used.

420

421 **Immunofluorescence microscopy.** Membranes of the human alveolus-on-a-chip were

422 fixed for at least 30 min with 4 % paraformaldehyde at 37°C and permeabilized with

423 0.1 % saponin buffer for one hour at room temperature. For the alveolus-on-a-chip

424 model the membrane was removed from the chip after fixation and before

425 permeabilization and cut in two halves to analyze either the epithelial or the

426 endothelial side. Infection by SARS-CoV-2 was visualized using mouse anti-SARS-

427 CoV-2 spike (GeneTex; #GTX632604) IgG monoclonal, primary antibodies and

428 AlexaFluor® goat anti-mouse IgG polyclonal antibodies (Dianova; # 115-545-146). The

429 nuclei were stained with bisBenzimide H 33342 trihydrochloride (Hoechst 33342)

430 (Merck; #14533). Rabbit anti-E-cadherin IgG monoclonal (CellSignaling; 3195S) or

431 rabbit anti-VE-cadherin polyclonal, primary antibodies (CellSignaling; 2158S) and Cy5

432 goat anti-mouse IgG polyclonal antibodies (Dianova; #111-175-144) were used to

433 detect cell borders of Calu-3 or HUVEC cells on the membrane of the alveolus-on-a-

434 chip model, respectively. Primary antibodies were added 1:100, overnight at 4°C.

19

435   Afterwards, secondary antibodies and Hoechst 33342 were added 1:100 and 1:1000 for

436   1 h, at room temperature and in the dark. Cells and membranes were mounted with

437   fluorescence mounting media (Dako; #S3023).

438   Images were acquired using an Axio Observer.Z1 microscope (Zeiss) with Plan

439   Apochromat 20x/0.8 objective (Zeiss), ApoTome.2 (Zeiss) and Axiocam 503 mono

440   (Zeiss) and the software Zen 2.6 (blue edition; Zeiss). Apotome defolding with phase

441   error correction and deconvolution was done by the software Zen 2.6 as well. Fiji V

442   1.52b (ImageJ) was used for further image processing, including Z-stack merging with

443   maximum intensity projection and gamma correction. Parameters were kept the same

444   for all pictures which were compared with each other.

445

446   **Scanning electron microscopy.** The fixation of the cells was performed inside the

447   human alveolus-on-a-chip model by using the same fixative as for TEM for 60 min at

448   room temperature as described previously (Deinhardt-Emmer et al., 2020a; Maurer et

449   al., 2019; Rennert et al., 2015). Afterwards, the chips were rinsed three times with fresh

450   cacodylate buffer for 10 min each and the membranes were cut out. After post-fixation

451   with 2 % w/v osmiumtetroxide for 1h the samples were dehydrated in ascending

452   ethanol concentrations (30, 50, 70, 90 and 100 %) for 15 min each. Subsequently, the

453   samples were critical-point dried using liquid CO_2 and sputter coated with gold

454   (thickness approx. 2 nm) using a CCU-010 sputter coater (safematic GmbH, Zizers,

455   Switzerland). The specimens were investigated with a field emission SEM LEO-1530

456   Gemini (Carl Zeiss NTS GmbH, Oberkochen, Germany).

457

Western-Blot Analysis. For western blotting, cells were lysed with Triton lysis buffer (TLB; 20 mM Tris-HCl, pH 7.4; 137 mM NaCl; 10% Glycerol; 1% Triton X-100; 2 mM EDTA; 50 mM sodium glycerophosphate, 20 mM sodium pyrophosphate; 5 μg ml^{-1} aprotinin; 5 μg ml^{-1} leupeptin; 1 mM sodium vanadate and 5 mM benzamidine) for 30 min. Cell lysates were cleared by centrifugation, supplemented with 5x Lämmli buffer (10% SDS, 50% glycerol, 25% 2-mercaptoethanol, 0.02% bromophenol blue, 312 mM Tris 6.8 pH) (diluted 1:5), boiled for 10 min (95°C), and subjected to SDS-PAGE and subsequent blotting. For the detection of SARS-CoV-2 spike protein a rabbit polyclonal anti-SARS-CoV-2 spike S2 antibody (Sino Biological #40590-T62) was used.

468

Lactate Dehydrogenase Cytotoxicity Assay. Cell cytotoxicity was determined with CyQUANT Lactate Dehydrogenase (LDH) Cytotoxicity Assay Kit (Invitrogen/Thermo Fisher Scientific,Waltham, USA) according to the manufacturer´s instructions. Cells were infected as previously described. After infection 25 μl of the supernatant was transferred in technical duplicates to a 96-well plate and mixed with 25 μl of the LDH cytotoxicity assay reagent. The plate was incubated at 37°C for 30 min. Stop-Solution (25 μl) was added, and OD_{492nm} was directly measured using a TECAN Spectra fluor plate reader (Tecan Group Ltd, Maennedorf, Switzerland). The OD_{620nm} was subtracted to correct for background signal.

478

21

479 **Permeability Assay.** To test the permeability of the epithelial and endothelial barrier,

480 1 mg ml^{-1} of 3–5 kDa fluorescein isothio-cyanate (FITC)-dextran (Sigma-Aldrich,

481 Germany) in phenol-red free DMEM/F12 medium (Sigma-Aldrich, Germany) was

482 injected into the upper chamber of the chip. The lower chamber contained only phenol

483 red free DMEM/F12. The alveolus model was incubated for 60 min under static

484 conditions. Afterwards, the media from the lower and upper chambers were collected,

485 and fluorescence intensity (exc. 488nm; em. 518 nm) was measured in a 96-well μClear

486 black plate (Greiner BioOne, Frickenhausen, Germany) by a BMG Labtech FLUOStar

487 Omega microplate reader (BMG Labtech GmbH, Ortenberg, Germany). The

488 permeability coefficient (P_{app}) was calculated according to P_{app} (cm s^{-1}) = (dQ/dt)

489 (1/AC$_0$). For this, dQ/dt represent the steady-state flux (g s^{-1}), A the culture surface area

490 (cm^2) and C$_0$ the initial concentration (mg ml^{-1}) (Thomas et al., 2017).

491

492 **Detection of mRNA-expression by using qRT-PCR.** For RNA isolation cells were

493 lysed with 350 μl RLT lysis buffer and detached from the plate using a rubber cell

494 scraper. RNA isolation was performed using the RNeasy Mini Kit (QIAGEN, Hilden,

495 Germany) according to the manufacturer's protocol. RNA concentration was

496 measured using the Nano Drop Spectrophotometer ND-1000 (preqlab/vwr, Radnor,

497 USA).

498 For cDNA synthesis, the QuantiNova Reverse Transcription Kit (QIAGEN, Venlo,

499 Netherlands) was used. RNA was thawed on ice. 400 nanogram (ng) RNA were

500 diluted in RNase free water to a volume of 13 μl. 2 μl gDNA removal mix were added

501   to the diluted RNA; followed by incubation at 45°C for 2 min. After incubating the

502   samples for at least 1 min on ice, 5 µl of RT master mix (containing 4 µl Reverse

503   Transcription Mix and 1 µl Reverse Transcription enzyme per sample) were added.

504   The resulting mixture was incubated for 3 min at 25°C, followed by incubation at 45°C

505   for 10 min and an inactivation step at 85°C for 5 min. The cDNA was either directly

506   used for the subsequent experiments or stored at -20°C.

507   qRT-PCRs were performed using the QuantiNova SYBR Green PCR Kit (QIAGEN,

508   Venlo, Netherlands). 1 µl cDNA was added to 19 µl master mix (containing 10 µl SYBR

509   Green, 1.5 µl Forward Primer (10 µM), 1.5 µl Reverse Primer (10 µM) and 6 µL RNase

510   free ddH$_2$O per sample; for primer sequences see table 1), and the real time PCR

511   reaction was started using the following cycle conditions: 95l°C for 2 min, followed by

512   40 cycles of 95°C for 5 sec and 60°C for 10 sec. The qPCR cycle was ended by a stepwise

513   temperature-increase from 60°C to 95°C (1°C every 5 sec).

514

515   **Detection of SARS-CoV-2 by using qRT-PCR.** For the determination of SARS-CoV-

516   2, we used the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to

517   manufacturer's guide. A qRT-PCR from RIDAgene (r-biopharm, Darmstadt,

518   Germany) followed on Rotor-Gene Q (Qiagen, Hilden, Germany) to detect the E-gene

519   of SARS-CoV-2. The RNA standard curve, prepared from the positive control of the

520   RIDAgene (r-biopharm, Darmstadt, Germany) kit, Cycle conditions were set as

521   follows: 10 min at 58°C, 1 min at 95°C and 45 cycles of 95°C for 15 sec and 60°C for 30

522   sec.

23

523

524   **Statistical analysis.** Statistical analyses were performed using Prism 8 (GraphPad

525   Software). Statistical methods are described in the figure legends.

526

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535

536   **Author contributions.** SDE, BL and CE conceived and designed the experiments, SDE,

537   SB, CH, LG, AH, RZ, FH, CB, MM, MS, and SN performed the experiments, SDE, SB,

538   CH, LG, RZ, ASM, CB, SN, BL and CE analyzed the data, SDE, BL and CE wrote the

539   manuscript, SDE, ASM, MWP, RH, SN, BL and CE provided resources. All authors

540   critically read and commented on the manuscript.

541

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Figure legends

Figure 1: Phylogenetic tree of SARS-CoV-2.

(A) Phylogenetic analysis revealed a close relationship of SARS-CoV-2 to the SARS-related coronaviruses RaTG13, bat-SL-CoVZXC21 and bat-SL-CoVZC45. Sequences of strains 5587 and 5588 exhibit two base substitutions T8,782C (**nsp1ab:** synonymous) and C28,144T (**nsp8:** S84L). (B) Accordingly, 5587 and 5588 clustered with lineage L/lineage B strains in the phylogenetic analysis. Both strains exhibit deletion of **nsp1ab** D448 and two synonymous substitutions (T514C, C5512T). Beside the **nsp8** S84L substitution, strain 5159 has accumulated three additional amino acid substitutions (**S:** D614G, **nsp1ab:** P4715L and **N:** R203K/G204R) which place this virus in lineage B.1.1.

Figure 2: SARS-CoV-2 replicates in Vero-76 and Calu-3 cells.

Vero-76 (A-D) and Calu-3 (B-D) cells were left uninfected (mock) (B-D) or were infected (A-D) with a SARS-CoV-2 patient isolate (5159) (MOI=1).

(A) Transmission electron microscopy was performed 24h post infection (p.i.): (upper panel, scale bar: 5 µm) overview of 3 SARS-CoV-2-infected Vero-76 cells; (lower left panel, scale bar: 200 nm) generation of double membrane vesicles; (lower middle panel, scale bar: 200 nm) virion assembly in the ER–Golgi-intermediate compartment

709   (ERGIC); (lower right panel, scale bar: 200 nm) viral release. (B) SARS-CoV-2 was

710   visualized by detection of the spike protein via a spike-specific antibody and an Alexa

711   Fluor™ 488-conjugated goat anti-mouse IgG (green). The nuclei were stained with

712   Hoechst 33342 (blue). Immunofluorescence (IF) microscopy was acquired by use of the

713   Axio Observer.Z1 (Zeiss) with a 200×magnification. (C) Total cell lysates were

714   harvested at the times indicated and expression of the spike protein was analyzed by

715   western-blot assay. ERK2 served as loading control. (D) Progeny virus particles were

716   measured in the supernatant by standard plaque assay at the indicated times post

717   infection. Shown are means (±SD) of plaque forming units (PFU) ml^{-1} of three

718   independent experiments including two biological samples. Statistical significance

719   was analyzed by unpaired, two-tailed t-test (***$p < 0.001$).

720

721   **Figure 3: SARS-CoV-2 infection results in induction of antiviral and**

722   **proinflammatory mRNA synthesis.**

723   Calu-3 cells were left uninfected (mock) or were infected with a SARS-CoV-2 patient

724   isolate (5159, 5587, 5588) (MOI=1). RNA-lysates were performed 24h p.i. Levels of

725   IFNα, IFNβ, IFNλ1, IFNλ2,3, IL6, IL8, IP10, TNFα, cIAP2, TRAIL, and RIPK1 mRNA

726   were measured of three patient isolate (5159, 5587, 5588) and two technical samples in

727   3 independent experiments. Means ± SD of three independent experiments are shown.

728   Levels of mock-treated samples were arbitrarily set as 1. After normalization, two-

729   tailed unpaired t-tests were performed for comparison of mock-treated and SARS-

730   CoV-2-infected and samples. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

29

731

Figure 4: SARS-CoV-2 efficiently infects epithelial cells of the human-alveolus-on-

a chip model and provokes type I and III interferon production.

(A-C) The epithelial chamber of the alveolus-on-a-chip model was left uninfected

(mock) or infected with three different SARS-CoV-2 patient isolates (5159, 5587, 5588)

(MOI=1). (A, B) Immunofluorescence staining was performed 28h p.i. and analyzed by

immunofluorescence microscopy (Axio Observer.Z1 (Zeiss)). (A) The E-cadherin of

the epithelial layer and the (B) VE-cadherin of the endothelial layer were visualized by

an anti-E-Cadherin-specific antibody or an anti-VE-Cadherin antiserum, respectively,

and a Cy5 goat anti-rabbit IgG (red). (A, B) The SARS-CoV-2 was visualized by

detection of the spike protein via a spike-specific antibody and an Alexa Fluor™ 488-

conjugated goat anti-mouse IgG (green). The nuclei were stained with Hoechst 33342

(blue). Scale bars represent 100 μm. (C) Production of antiviral cytokines derived from

the epithelial side was determined by use of Legendplex Panel (Biolegend, CA, USA).

SARS-CoV-2 induced IFNβ, IFNλ1 and IFNλ2,3 release (pg/ml) was measured. Means

± SD of three independent experiments each infected with another patient isolate

(5159, 5587, 5588) are shown. Levels of mock-treated samples were arbitrarily set as 1.

After normalization, two-tailed unpaired t-tests were performed for comparison of

mock-treated and SARS-CoV-2-infected and samples. (**$p < 0.01$).

750

Figure 5: SARS-CoV-2 infection results in the disruption of barrier integrity in the

human-alveolus-on-a chip model.

753 The epithelial side of the alveolus-on-a-chip model was left uninfected (mock) or

754 infected with the SARS-CoV-2 patient isolate (5159) (MOI=1) for 28h. An overview

755 (upper panel) of the (A) epithelial layer and (B) endothelial layer are depicted. Dead

756 cells (middle panel) are focused. The surface of dead cells (lower panel) shows

757 particles (arrows) attached to the plasma membranes of the epithelial cells only. Scale

758 bars represent 50 μm (200×magnification), 5 μm (2.000×magnification) and 200 nm

759 (60.000×magnification).

760 (C) Barrier function of the human alveolus-on-a-chip model was analyzed by a

761 permeability assay of mock-infected and SARS-CoV-2-infected human alveolus-on-a-

762 chip model using FITC-dextran at 28h p.i., FITC-dextran was measured via the

763 fluorescence intensity (exc. 488nm; em. 518 nm) and depicted as the permeability

764 coefficient (P_{app}), calculated according to P_{app} (cm s^{-1}) = (dQ/dt) (1/AC$_{o}$). Results show

765 significant higher barrier permeability after SARS-CoV-2 infection. (D) Supernatants

766 of the epithelial- and endothelial side of SARS-CoV-2 infected human alveolus-on-a-

767 chip models were used to perform LDH-assays indicating cell membrane rupture at

768 28h and 40h p.i.. (E) Progeny virus titers were analyzed in the supernatants of the

769 epithelial- and endothelial layer by standard plaque assay.

770 Shown are means (±SD) of (C) three independent experiments each infected with

771 another patient isolate (5159, 5587, 5588), (D) LDH release, and (E) plaque forming

772 units (PFU/ml). Statistical significance was analyzed by unpaired, two-tailed t-test (*p

773 < 0.05, **p < 0.01).

774

775 **Figure S1: SARS-CoV-2 infects epithelial cells productively.**

776 (A) Vero-76, Calu-3, and HUVECs were infected with a SARS-CoV-2 patient isolate

777 (5159, 5587, 5588) (MOI=1). RNA-lysates were performed 24h p.i. and copies of viral

778 RNA (E-gene) were determined by r-biopharm qRT-PCR. Means ± SD of three
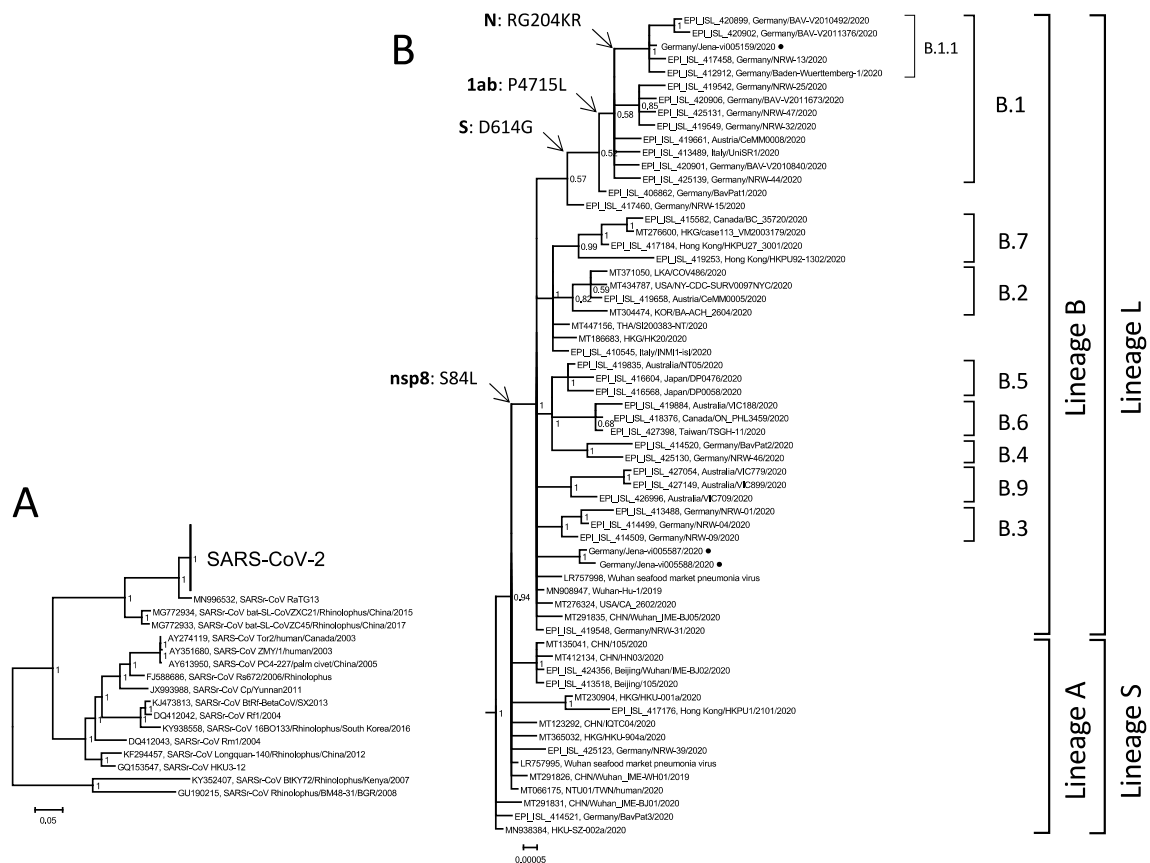
779 independent experiments are shown.

780 (B) HUVECs were infected with a SARS-CoV-2 patient isolate (5159) (MOI=1) for 4h,

781 8h, and 24h. SARS-CoV-2 was visualized by detection of the spike protein via a spike-

782 specific antibody and an Alexa Fluor™ 488-conjugated goat anti-mouse IgG (green).

783 The nuclei were stained with Hoechst 33342 (blue). Immunofluorescence (IF)

784 microscopy was acquired by use of the Axio Observer.Z1 (Zeiss) with a

785 200×magnification.

786

787 **Figure S2: Infection with SARS-CoV-2 results in the disruption of the epithelial-**

788 **and endothelial barrier.**

789 The epithelial side of the alveolus-on-a-chip model was left uninfected (mock) or

790 infected with three different SARS-CoV-2 patient isolates (5159, 5587, 5588) (MOI=1).

791 Immunofluorescence staining was performed 40h p.i., (A) The E-cadherin of the

792 epithelial layer and the (B) VE-cadherin of the endothelial layer were visualized by an

793 anti-E-Cadherin-specific antibody or an anti-VE-Cadherin antiserum, respectively,

794 and a Cy5 goat anti-rabbit IgG (red). (A, B) The SARS-CoV-2 was visualized by

795 detection of the spike protein via a spike-specific antibody and an Alexa Fluor™ 488-

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796   conjugated goat anti-mouse IgG (green). The nuclei were stained with Hoechst 33342
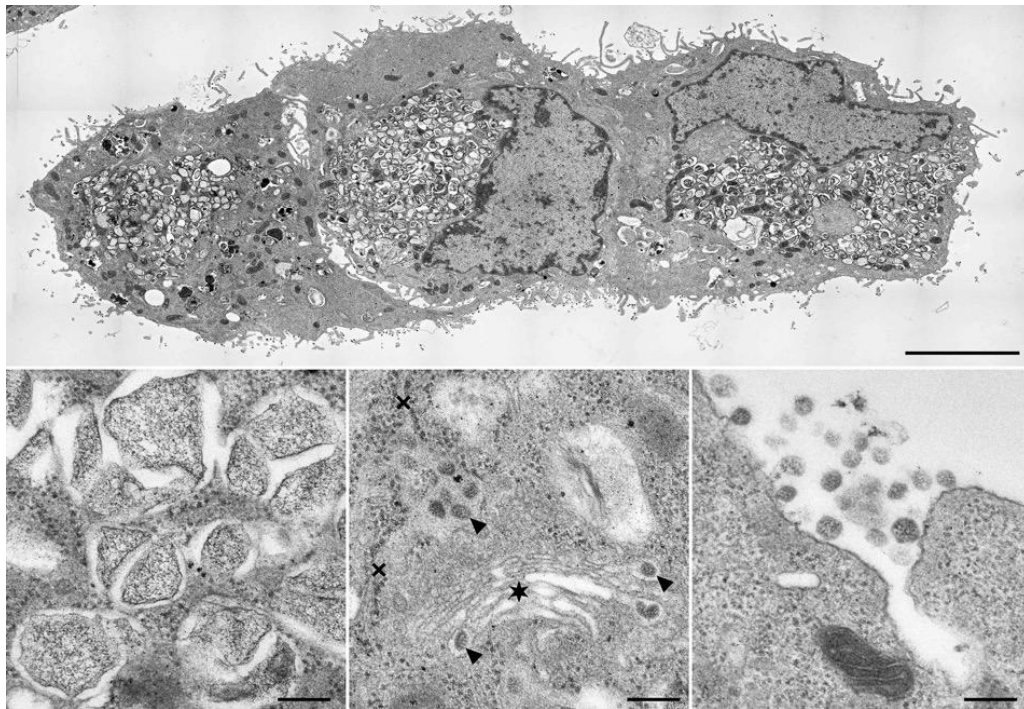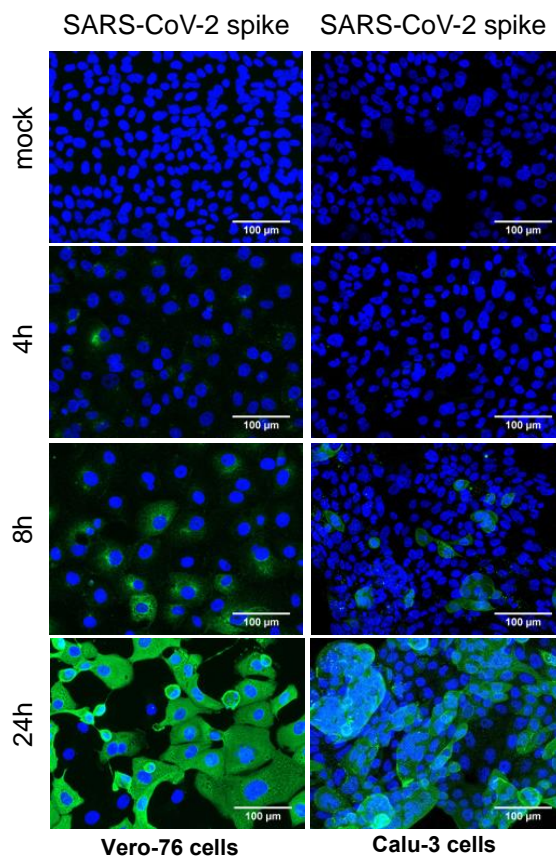
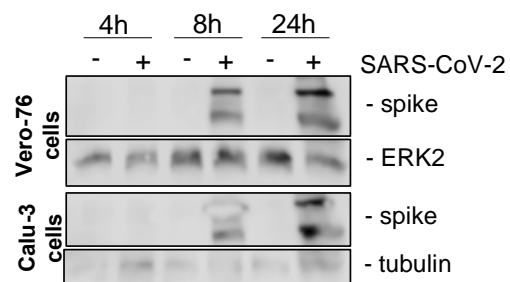797   (blue). Scale bars represent 100 μm.

Figure 1

Figure 2
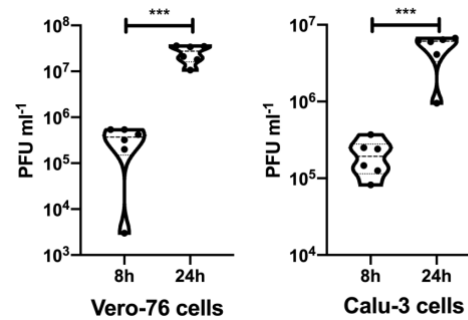
A

B

C

D

800

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Figure 3

A

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803

Figure 4

Epithelial chamber (28h)

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805

Figure 4

Endothelial chamber (28h)
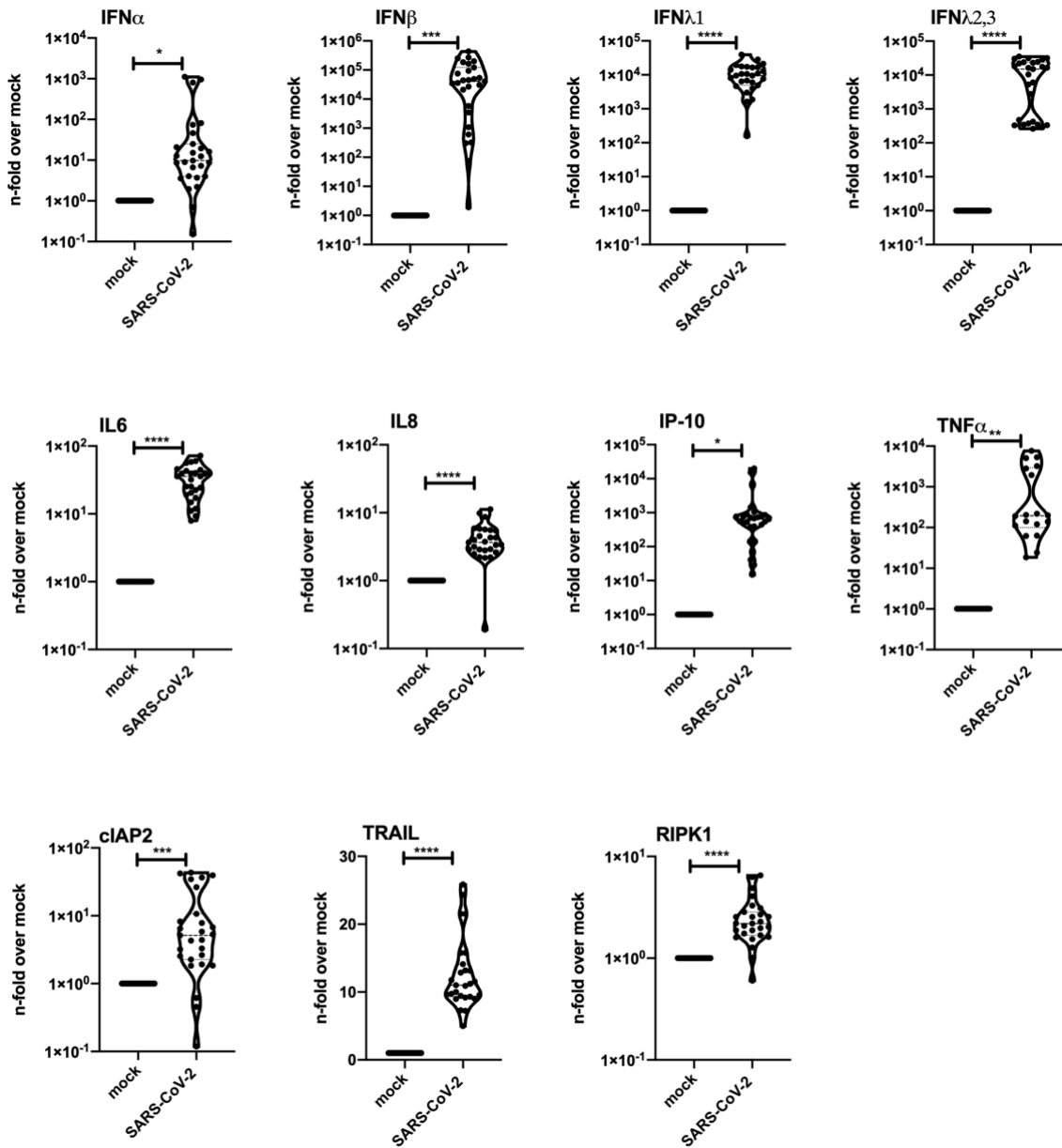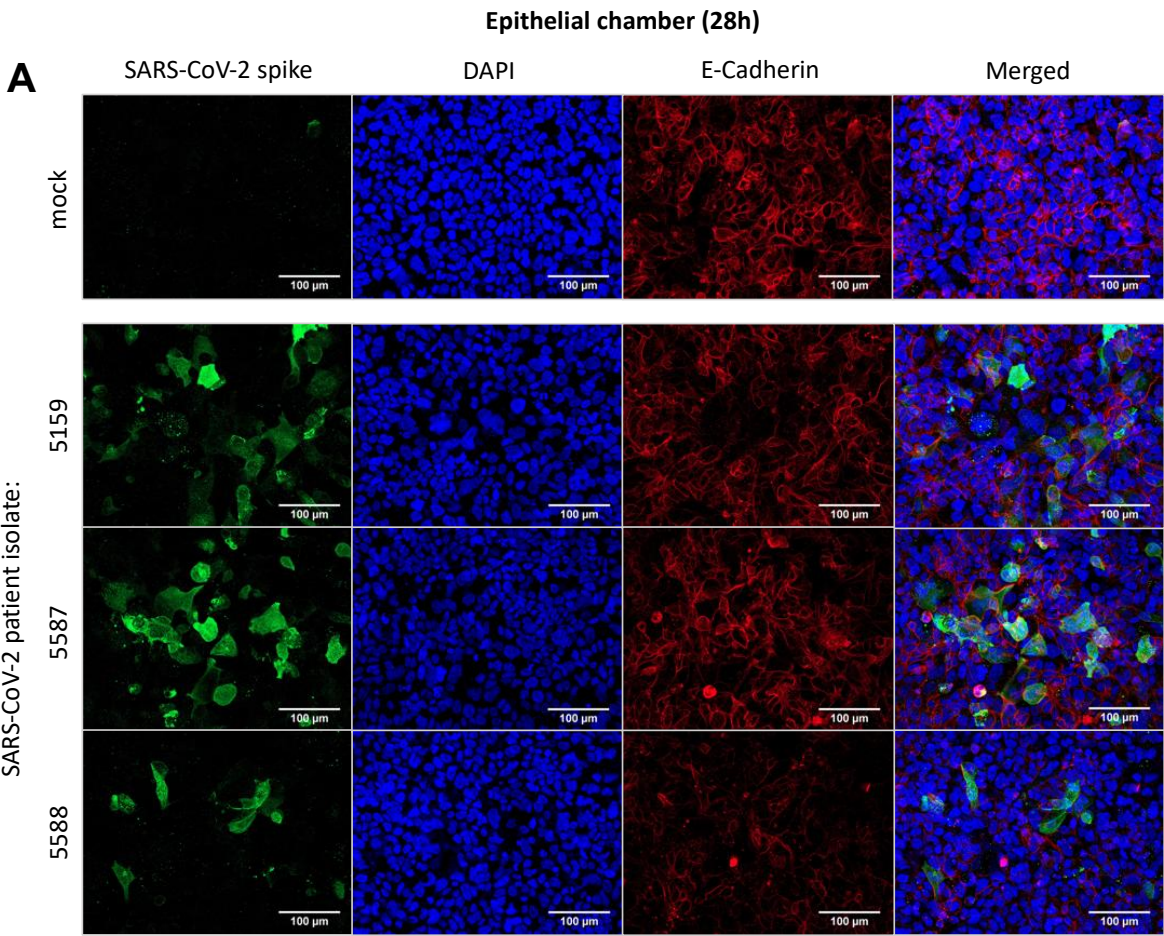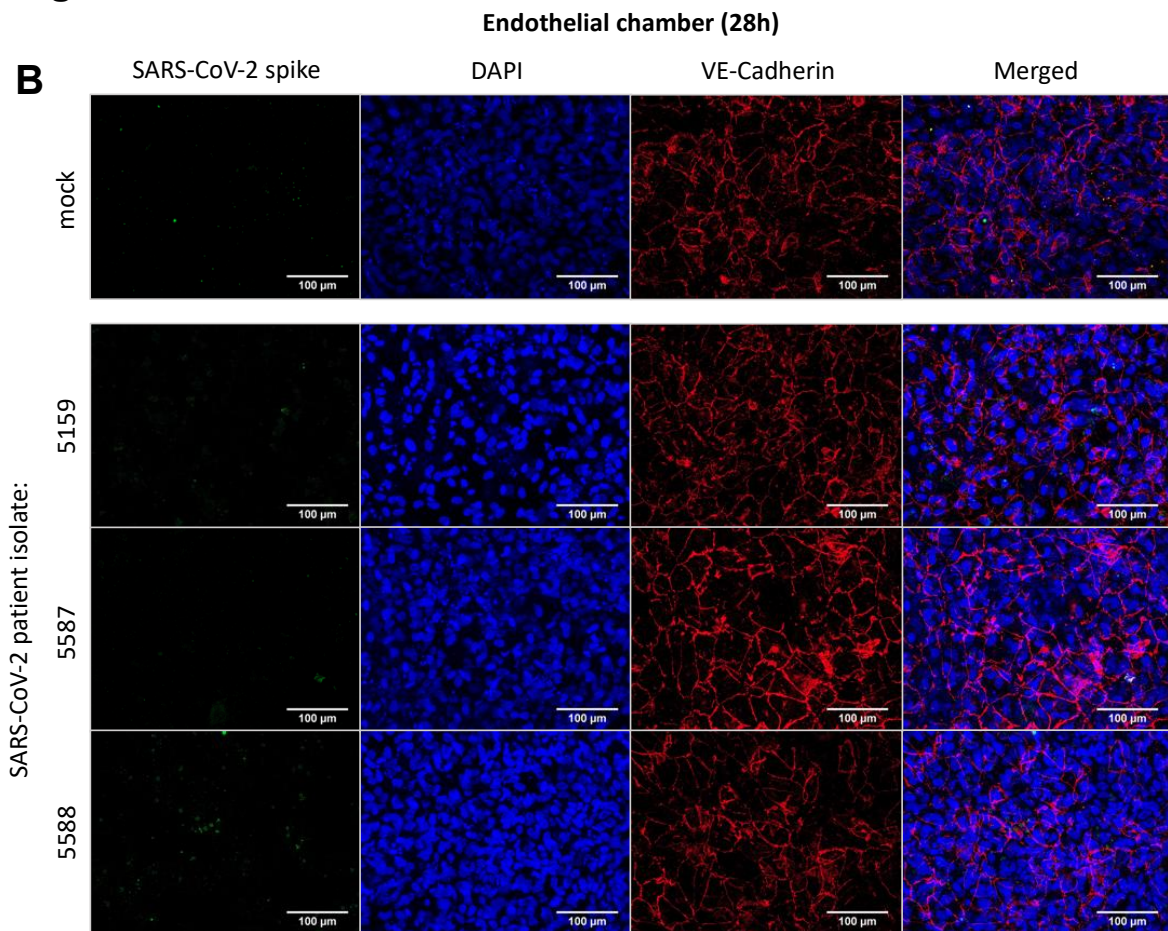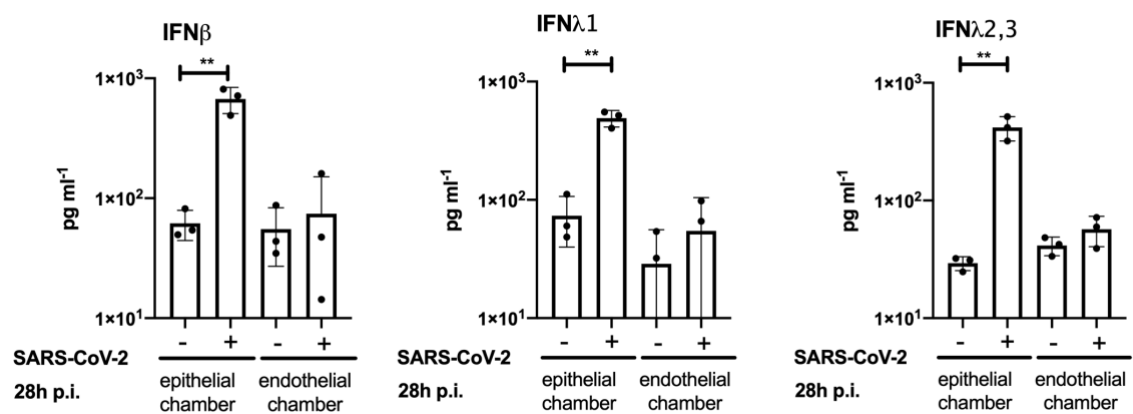
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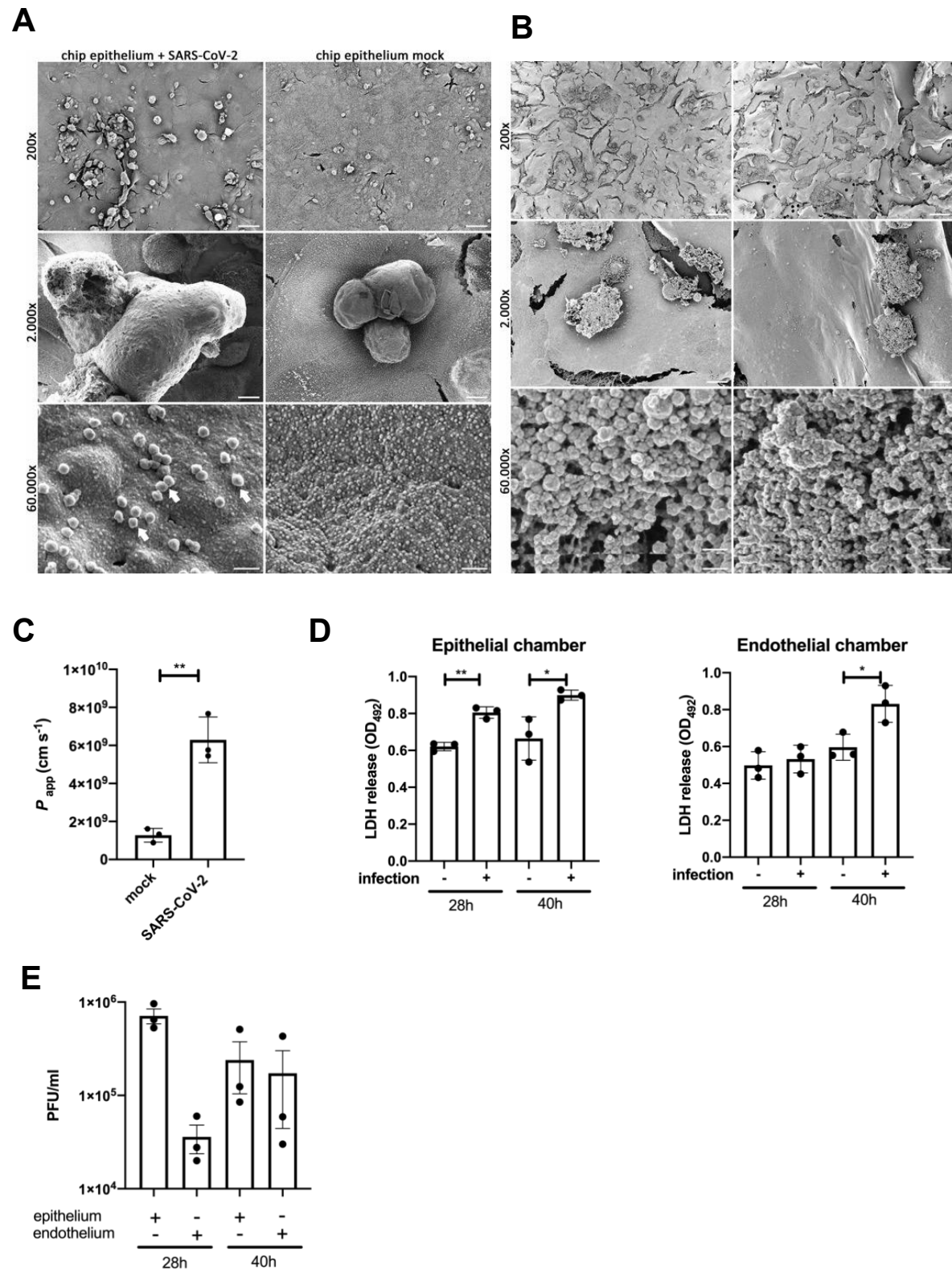
Figure 5

808

809

Figure S1

A

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811

B

Spike (GeneTex)

SARS-CoV-2 spike (green)
Nucleus (DAPI, blue)

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Figure S2

Epithelial chamber (40h)

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Figure S2

B

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818 STAR ★ METHODS

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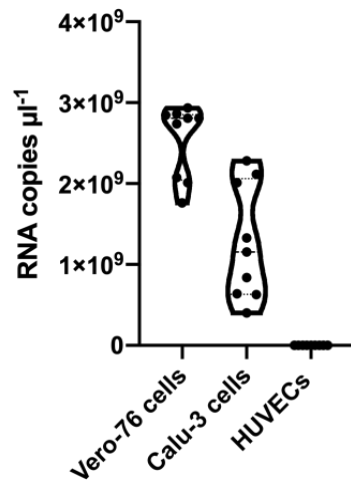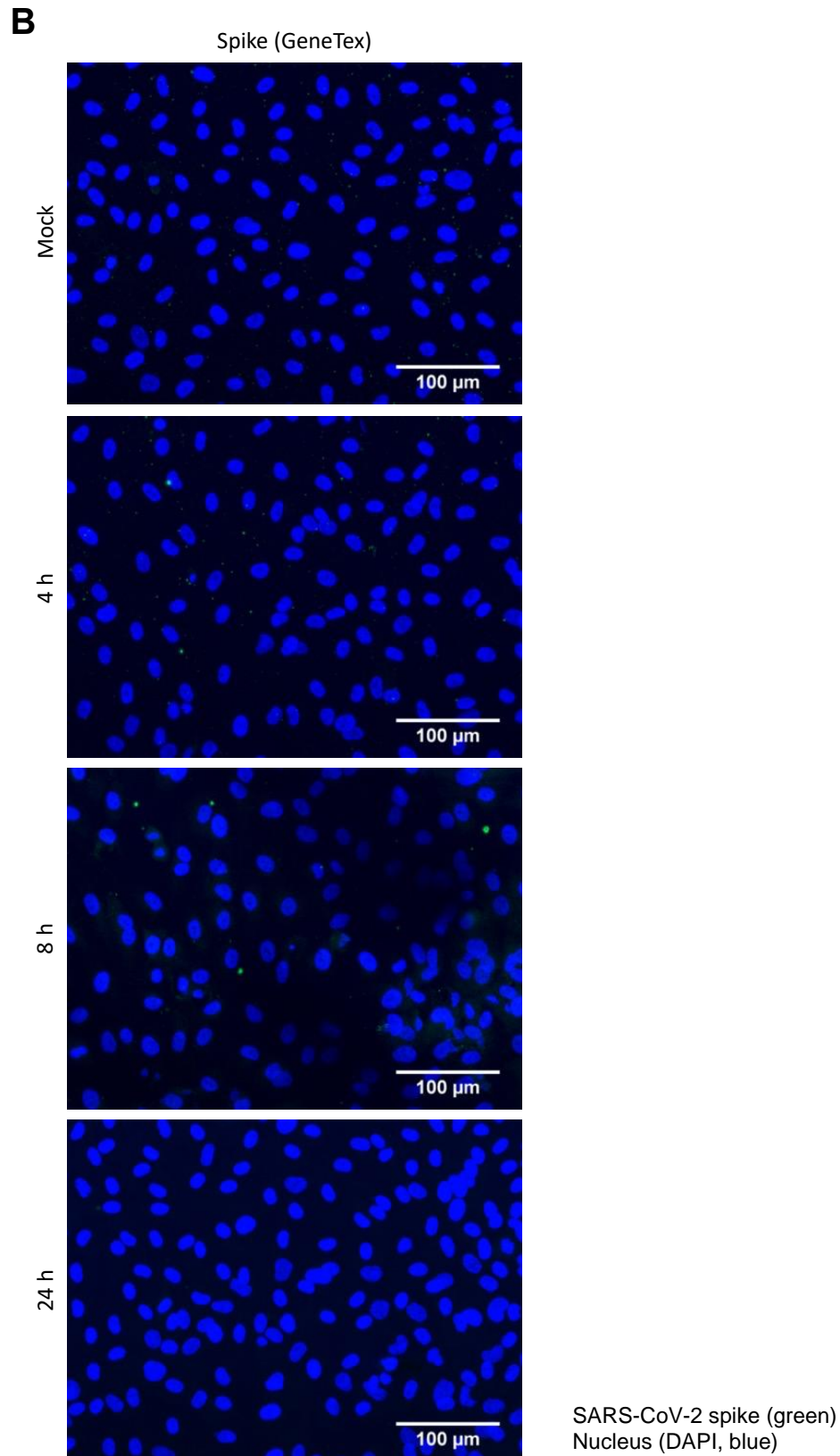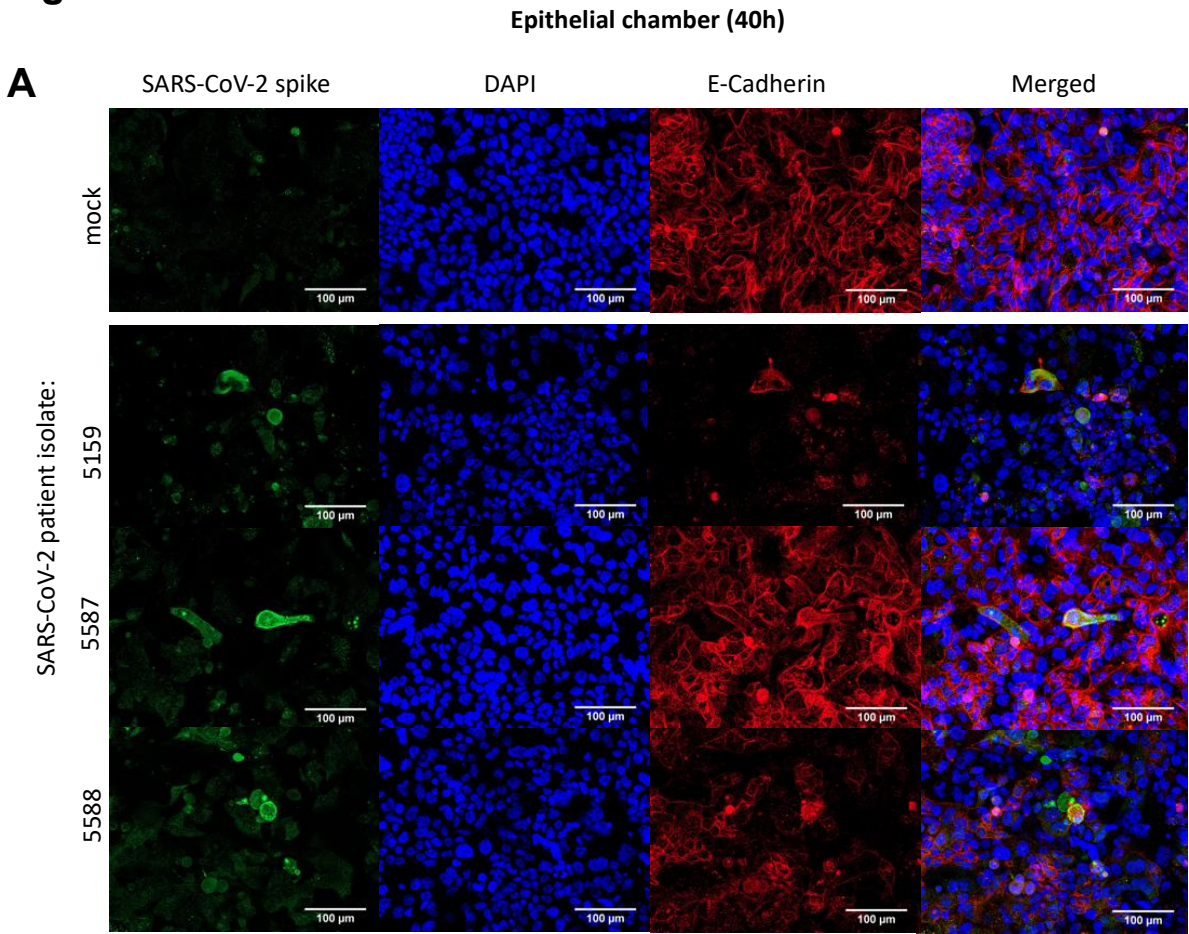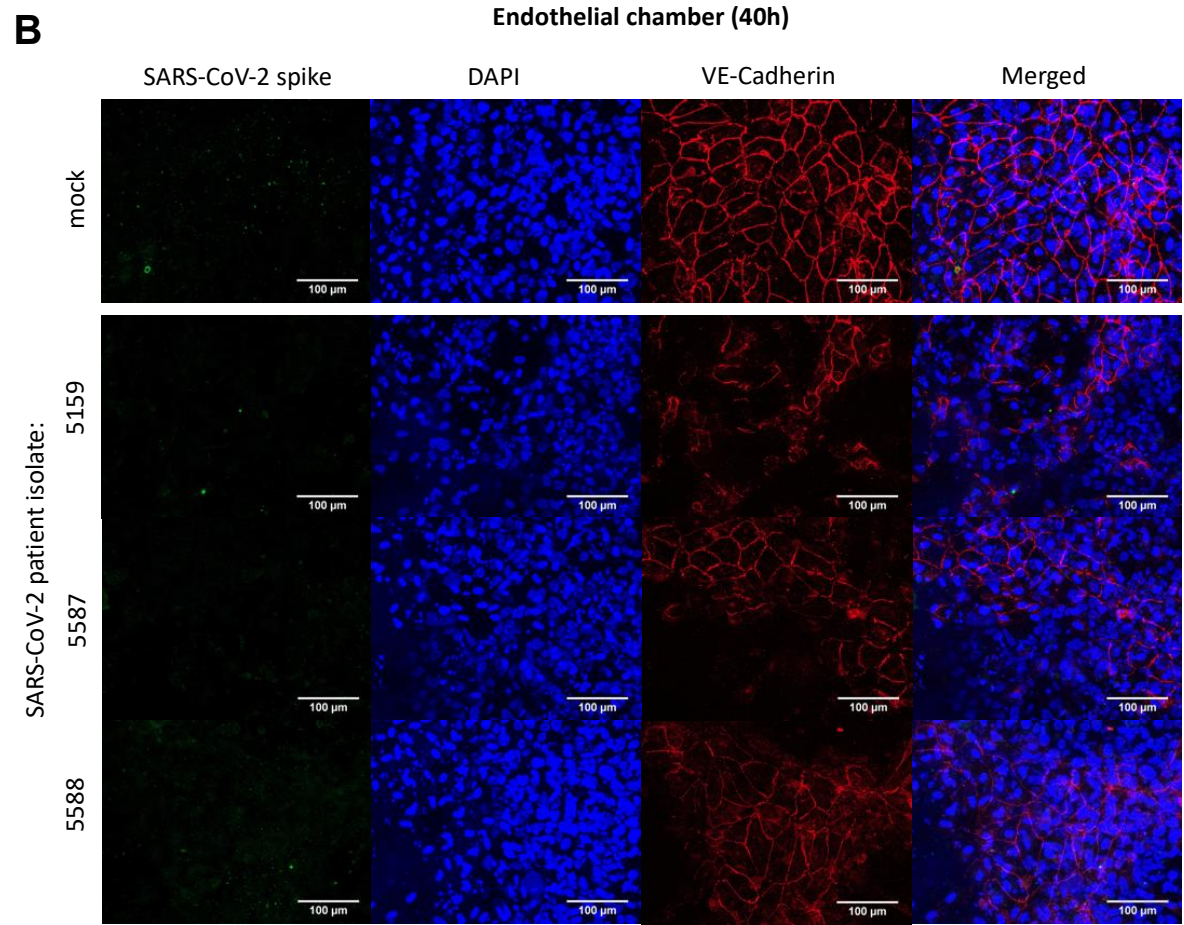
820 KEY RESOURCES TABLES

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
SARS-CoV-2 spike, mouse	GeneTex	GTX632604
E-Cadherin, rabbit	Cell Signaling	3195S
VE-Cadherin, rabbit	SinoBiological	2158S
ERK2, mouse	Santa Cruz	sc-1647
SARS-CoV-2/2019-nCoV Spike/S2 Rabbit Antibody	Sino Biological	40590-T62
Goat anti-Mouse IgG (H+L) Secondary Antibody, HRP	BIORad	31430
goat-anti-rabbit-igg-h-l-hrp-conjugate	BIORad	1706515
AF488, secondary antibody	Dianova	115-545-146
Cy5, secondary antibody	Dianova	111-175-144
Alpha-Tubulin, Rabbit	Cell signaling Technology	2144S
Cell lines		
Vero-76	ATCC	CRL 1587
Calu-3	ATCC	HTB-55
Chemicals		
RPMI 1640	Lonza	BE12-115F
EMEM	Sigma Aldrich	M7278
M199	Lonza	BE12-117F
Fetal serum	Sigma Aldrich	F7524
Human serum	Sigma Aldrich	H4522
10x MEM for plaque assay medium	Gibco	21430
Endothelial growth supplement (ECGS)	Sigma	02-102
Hoechst 33342	Merck	14533
Commercial Kits		
CyQUANT LDH cytotoxicity assay kit	Thermo Fisher Scientific	C20301
RNeasy Mini Kit	Qiagen	74106
QIAmp viral RNA Mini Kit	Qiagen	52906
RIDA Gene SARS-CoV-2 real-time PCR kit	R-Biopharm	PG6815
Legendplex Human anti-virus response panel kit	Biolegend	740349
Oligonucleotides		
human_IP-10_fw 5'-CCAGAATCGAAGGCCATCAA-3'	metabion	N/A
human_IP-10_rev 5'-TTTCCTTGCTAACTGCTTTCAG-3'	metabion	N/A
human_TNF-alpha_fw 5'-GGAGAAGGGTGACCGACTCA-3'	metabion	N/A
human_TNF-alpha_rev 5'-CTGCCCAGACTCGGCAA-3'	metabion	N/A
human_IFNβ_fwd 5'-TCTGGCACAACAGGTAGTAGGC-3'	metabion	N/A

human_IFNβ_rev 5'-GAGAAGCACAACAGGAGAGCAA-3'	metabion	N/A
human_GAPDH_fw 5'-CTCTGCTCCTCCTGTTCGAC-3'	metabion	N/A
human_GAPDH_rev 5'-CAATACGACCAAATCCGTTGAC-3'	metabion	N/A
C_GAPDH_fwd 5'-ACACCCACTCTTCCACCTTC-3'	metabion	N/A
C_ GAPDH_rev 5'-CTCTCTCTTCCTCTTGTGCTC-3'	metabion	N/A
human_IP-10_fw 5'-CCAGAATCGAAGGCCATCAA-3'	metabion	N/A
Software		
Zen, 2.6 (blue edition)	Carl Zeiss AG	N/A
MrBayes	v3.2	N/A
Systems for cDNA Synthesis and qRT-PCR		
Nano-Drop	Nano-Drop 1000 (PEQLAB Biotechnology GmbH)	N/A
Thermo cycler (for cDNA synthesis)	Peqstar (PEQLAB Biotechnology GmbH)	N/A
Realtime PCR cycler	Rotor Gene Q (QIAGEN)	N/A
Acquisition software for RNA concentration	NanoDrop 1000 V3.8.1 (ThermoFisher Scientific)	N/A
Acquisition software for qRT-PCR	Q-Rex V1.1.0.4 (QIAGEN)	N/A
Software used for subsequent data analysis	Microsoft Office 2010 (Microsoft) GraphPad Prism V8.3.0.538 (Graphpad Software, Inc.)	N/A
Systems for microscopy		N/A
Model	Carl Zeiss Observer.Z1	N/A
Type of objective lenses	Plan-Apochromat	N/A
Magnification of objective lenses	20x	N/A
Numercial aperture of objective lenses	0.8	N/A
Imaging medium	Dako Fluorescence Mounting Medium	N/A

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camera	Zeiss Axioxam 503 mono	N/A
ApoTome	ApoTome.2 Carl Zeiss	N/A
Aquisition software	Zeiss Zen 2.6 (blue edition)	N/A
Operations involved (deconvolution, Fourier-Filter, gamma)	Deconvolution, gamma correction, phase error correction	N/A
File formats	16-bit	N/A
Sample Preparation, Sequencing and Analysis		
MinION - Sequencer	Oxford Nanopore Technologies	N/A
Ligation Sequencing Kit (SQK-LSK109)	Oxford Nanopore Technologies	N/A
R9.4.1 flow cells Nanopore FLO-MIN106	Oxford Nanopore Technologies	N/A
Native Barcoding Expansion (EXP-NBD104)	Oxford Nanopore Technologies	N/A
Workflow: poreCov via nextflow using docker	For genome reconstruction (version 0.2)	https://github.com/replikation/poreCov
Primer Scheme V3 was used for amplicon-based sequencing of SARS-CoV-2	V3	https://github.com/artic-network/artic-ncov2019/blob/master/primer_schemes/nCoV-2019/V3/nCoV-2019.tsv
NEBNext Ultra II End-prep	NEB	M0493S
NEBNext Quick Ligation Module	NEB	E6056S
Q5 Hot Start HF Polymerase	NEB	M0493S
RNase OUT (125 rxn)	Thermofischer	10777019
Random Hexamers (50 μM)	Thermofischer	N8080127
Zymo Quick-RNA Viral Kit	Zymo	R1034
SuperScript IV (50 rxn)	Thermofischer	18090050
dNTP mix (10 mM each)	Thermofischer	R0192

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