

1                   **Heterogeneous expression of the SARS-Coronavirus-2 receptor ACE2**  
2                   **in the human respiratory tract**  
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15  
16 **Running title:** Expression of ACE2 in the human respiratory tract

17  
18 **Competing interest declaration:**

19 The authors declare no competing interests.

20 **Abstract:**

21           Zoonotically transmitted coronaviruses are responsible for three disease outbreaks since  
22 2002, including the current COVID-19 pandemic, caused by SARS-CoV-2. Its efficient  
23 transmission and range of disease severity raise questions regarding the contributions of virus-  
24 receptor interactions. ACE2 is a host ectopeptidase and the receptor for SARS-CoV-2. Despite  
25 numerous reports describing ACE2 mRNA abundance and tissue distribution, there remains a  
26 paucity of data evaluating ACE2 protein and its correlation with other SARS-CoV-2  
27 susceptibility factors. Here, we systematically examined the human upper and lower respiratory  
28 tract using single-cell RNA sequencing and immunohistochemistry to determine receptor  
29 expression and evaluated its association with risk factors for severe COVID-19. Our results  
30 reveal that ACE2 protein is highest within the sinonasal cavity and pulmonary alveoli, sites of  
31 presumptive viral transmission and severe disease development, respectively. In the lung  
32 parenchyma, ACE2 protein was found on the apical surface of a small subset of alveolar type II  
33 cells and colocalized with TMPRSS2, a cofactor for SARS-CoV2 entry. ACE2 protein was not  
34 increased by pulmonary risk factors for severe COVID-19. However, ACE2 protein was  
35 increased in children, a demographic with a reduced incidence of severe COVID-19. These  
36 results offer new insights into ACE2 localization and function in susceptibility to COVID-19.

37

38           Key words: Lung, expression, alveolar type II cells, ciliated cells, immunohistochemistry

39

40 **Introduction:**

41           Angiotensin-converting enzyme 2 (ACE2) is the cellular receptor for both severe acute  
42 respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 (1, 2). SARS-CoV caused a  
43 pneumonia outbreak in 2002-2003 with a mortality rate of 9.6% and over 800 deaths worldwide  
44 (3). SARS-CoV-2 is the etiologic agent of coronavirus disease 2019 (COVID-19) which was first  
45 recognized in December 2019 and has now reached pandemic proportions (2, 4). SARS-CoV-2  
46 infection can be fatal, with the risk for increased disease severity correlating with advanced age  
47 and underlying comorbidities, while children and younger individuals generally have milder  
48 disease (5-8). These trends in disease severity could reflect differences in ACE2 distribution and  
49 expression in the respiratory tract.

50           Previous studies have evaluated ACE2 expression in the respiratory tract. Studies of  
51 ACE2 mRNA transcript abundance have provided conflicting interpretations, as well as a lack of  
52 protein validation (9-16). ACE2 protein studies have been limited by a paucity of lung tissue  
53 substrates and reports that have yielded contradictory results (17-20) ([Supplemental Table 1](#)). It  
54 is reported that some clinical factors (sex, age, or presence of comorbidities) could influence  
55 ACE2 expression in the human lower respiratory tract. The ACE2 gene resides on the X  
56 chromosome and therefore could be differentially regulated between males and females due to  
57 variable X-inactivation (21). Increased abundance of circulating ACE2 protein is reported to  
58 correlate with male sex, advanced age, and chronic comorbidities such as diabetes,  
59 cardiovascular disease, and renal disease (reviewed in (22)). Recent single-cell mRNA  
60 sequencing (scRNA-seq) studies of respiratory tract cells have reported contradictory evidence  
61 regarding the correlation between ACE2 transcript abundance and age, sex, smoking status, and  
62 other comorbidities (9-14).

63           We investigated the hypothesis that ACE2 drives disease severity in susceptible patient  
64   populations through enhanced abundance or distribution in different locations or cell types of the  
65   respiratory tract. We reanalyzed publicly available scRNA-seq data from distal lung biopsies  
66   (23), nasal brushings and nasal turbinate samples (24) to evaluate ACE2 transcript abundance in  
67   specific cell types. We complemented these analyses with optimized and validated ACE2  
68   immunostaining protocols, to corroborate single cell analyses as well as to screen for differences  
69   in cellular ACE2 protein in lung tissues derived from a cohort of control and chronic diseased  
70   patients.

71 **Results:**

72 In the alveoli, ACE2 transcripts were detected mostly in alveolar type II (AT2) cells  
73 (89.5% of all ACE2<sup>+</sup> cells) (Figure 1a), but specifically within a subset of these cells (1.2% of  
74 AT2 cells) (Figure 1b, Supplemental Figure 1a-b). These data indicate ACE2 transcripts are  
75 uncommon in most alveolar cell types. Alveoli had apical ACE2 protein only in a small number  
76 (usually ~1% or less) of AT2 cells (Figure 1c), consistent with the scRNA-seq results. The  
77 identity of these cells was confirmed by co-staining for surfactant protein-C. These ACE2<sup>+</sup> AT2  
78 cells were observed within areas of alveolar collapse (Figures 1d-f) and had morphologic  
79 features of hyperplastic AT2 cells, being more plump and larger than ACE2<sup>-</sup> AT2 cells in the  
80 same tissue section (Figure 1g). Interestingly, alveolar macrophages were negative for ACE2  
81 protein staining by immunohistochemistry, despite previous reports of ACE2 protein in these  
82 cells (Supplemental Table 1). The lack of ACE2 transcripts in macrophages was also confirmed  
83 by scRNA-seq data that revealed ACE2 mRNA in only 0.1% of macrophages, monocytes, or  
84 dendritic cells (Supplemental Figure 1a-b). The concordance between scRNA-seq and  
85 immunohistochemistry results provides compelling evidence that ACE2 is primarily present in a  
86 subset of AT2 cells and that alveolar macrophages lack ACE2.

87

88 Recent evidence indicates that proteases such as TMPRSS2 facilitate entry of SARS-  
89 CoV-2 into ACE2<sup>+</sup> cells (25). We evaluated scRNA-seq data and observed that TMPRSS2  
90 mRNA was present in 35.5% of all AT2 cells (Figure 2a) but was more prevalent (50.0%) in  
91 ACE2<sup>+</sup> AT2 cells (Figure 2b). Additionally, we observed colocalization of ACE2 and TMPRSS2  
92 on the apical membrane of these AT2 cells (Figure 2c). These findings suggest that AT2 cells

93 with apical ACE2 and TMPRSS2 could readily facilitate SARS-CoV-2 cellular infection and  
94 disease as seen in COVID-19 patients.

95

96 We next evaluated ACE2 in the conducting airways (trachea, bronchi, bronchioles). In  
97 the trachea and bronchi, apical ACE2 was rare and limited to ciliated cells ([Figure 3a](#)), similar to  
98 localization results in primary cultures of well-differentiated human airway epithelial cells (26).  
99 In the submucosal glands of large airways, occasional serous cells and vessels near the acini  
100 were positive for ACE2 ([Supplemental Figures 2a-b](#)). In bronchioles, ACE2 was regionally  
101 localized ([Figures 3b-d](#)). These findings show nominal detection of ACE2, corresponding with  
102 the lack of primary airway disease (e.g., bronchitis, etc.) seen in COVID-19 patients.

103

104 Detection of ACE2 protein has been variably reported in several small studies (18, 20). In  
105 this larger study, we saw that the regional distribution of ACE2 protein varied between donors.  
106 In the surface epithelium of trachea and bronchi, we detected ACE2 in only 12% and 27% of  
107 donors, respectively ([Figure 4a](#)). In the distal areas of the lung, ACE2 detection was more  
108 common, with bronchiolar and alveolar protein detection in 36% and 59% of donors,  
109 respectively ([Figure 4a](#)). A similar pattern of variable alveolar ACE2 was seen for mRNA  
110 transcripts in the scRNA-seq data, where 50% of donors showed low abundance in AT2 cells, and  
111 the other 50% of donors showed high abundance in the same cell type ([Figure 4b](#)). These  
112 findings suggest that ACE2 expression can vary between different lung regions and between  
113 individuals. Importantly, this low level of cellular protein provided us with an opportunity to  
114 investigate the potential for various clinical factors to increase ACE2 expression.

115

116 Independent risk factors associated with severe COVID-19 include male sex, increased  
117 age, and presence of comorbidities (6, 7, 27, 28). To evaluate whether the spatial distribution and  
118 abundance of ACE2 protein in the lower respiratory tract differed by these risk factors, we  
119 scored tissues for ACE2 protein detection ([Supplemental Table 2](#)). In the cohort, neither age nor  
120 sex were associated with ACE2 protein detection (using the median age as cut-off) ([Figures 4c-](#)  
121 [d](#)). Since recent studies of COVID-19 infections suggested that young children have reduced  
122 disease severity when infected by SARS-CoV-2 (6, 8), we compared lung tissue samples from  
123 children <10 years of age to those from the remaining older subjects (19-71 years of age) and  
124 found that ACE2 protein detection was higher in this subset of young children ([Figure 4e](#)). To  
125 test whether ACE2 distribution was affected by the presence of underlying diseases, we assessed  
126 the ACE2 localization pattern using tissues from subjects with chronic comorbidities (asthma,  
127 cardiovascular disease, chronic obstructive pulmonary disease, cystic fibrosis, diabetes, and  
128 smoking) and compared them to controls ([Supplemental Table 2](#)). The control group was similar  
129 in age to the chronic disease group ([Figure 4f](#)). We observed no significant differences between  
130 the two groups in ACE2 distribution, except for bronchioles, where ACE2 protein was reduced  
131 in the chronic disease group ([Figure 4g, Supplemental Figure 2c](#)). These results show that ACE2  
132 levels in the respiratory tract were not increased in association with risk factors for severe  
133 COVID-19, such as male sex, advanced age, and underlying chronic comorbidities. Instead, we  
134 saw increased ACE2 detection in children <10 years of age and in the small airways  
135 (bronchioles) of individuals without chronic comorbidities in our cohort.

136

137 Given the unexpected heterogeneity in the lower respiratory tract, we also investigated  
138 ACE2 in the upper respiratory tract. scRNA-seq data from nasal brushing and nasal turbinate

139 samples (24) show ACE2 mRNA transcripts in 2-6% of epithelial cells ([Supplemental Figure 3a-](#)  
140 [d](#)). We then studied nasal biopsy tissues and found that ACE2 protein was detected in all tissue  
141 samples and, when present, was seen exclusively on the apical surface of ciliated cells.  
142 Distribution varied regionally based on the characteristics of the epithelium, with rare detection  
143 in thicker ciliated pseudostratified epithelium, and more abundant protein in thinner epithelium  
144 ([Figures 5a-g](#)). Thinner epithelial height is expected in specific regions including the floor of the  
145 nasal cavity, meatuses, and paranasal sinuses (29). The sinonasal cavity is an interface between  
146 the respiratory tract and the environment, and high SARS-CoV-2 viral loads can be detected in  
147 nasal swabs from infected patients (30), consistent with our ACE2 expression data. This  
148 reservoir of ACE2<sup>+</sup> cells may facilitate the reported transmission from individuals who have very  
149 mild or asymptomatic disease (31).



150 **Discussion:**

151 A critical aspect of this study was to evaluate ACE2 protein expression and distribution  
152 by immunohistochemistry to more accurately corroborate single cell transcript studies and better  
153 evaluate clinical groups for COVID-19 disease susceptibility. Previously, limited reports have  
154 variably shown ACE2 protein in the upper and lower respiratory tract, but cellular localization  
155 and distribution in human lung tissues have been inconsistent and contradictory (17-20)  
156 ([Supplemental Table 1](#)). *In vitro* studies demonstrate that ACE2 protein is found at the apical  
157 membrane of polarized airway epithelia, where it permits virus binding and cell entry (18, 26). In  
158 our study, ACE2 was consistently localized to the apical membranes of cells. ACE2 was more  
159 commonly found in the sinonasal cavity where transmission likely occurs and on AT2 cells of  
160 the lung parenchyma where severe disease develops. Expression of ACE2 in the sinonasal cavity  
161 could explain the high transmissibility of SARS-CoV-2 and HCoV-NL63, a cold-related  
162 coronavirus, which also uses ACE2 as a receptor. One mystery is why SARS-CoV, which also  
163 uses ACE2, was apparently less efficient at human-to-human transmission (32). Whether this  
164 represents differences in the interactions of SARS-CoV and SARS-CoV-2 with co-receptors (33)  
165 or other factors in the nasal cavity remains to be investigated.

166  
167 SARS-CoV and SARS-CoV-2 both replicate in the lungs (34, 35), consistent with the  
168 ACE2 protein distribution defined in this study and suggested by previous studies (17, 18). We  
169 show that ACE2 and TMPRSS2 coexpress in AT2 cells at the mRNA and protein levels,  
170 suggesting susceptibility to infection. Additionally, it may also be possible that TMPRSS2<sup>-</sup>  
171 ACE2<sup>+</sup> AT2 cells can become infected through the use of other airway proteases (36). AT2 cells  
172 are critical for surfactant protein production and serve as progenitor cells for the AT1 cells, thus

173 damage to these AT2 cells could contribute to acute lung injury (37), which is a common feature  
174 of severe COVID-19 (5). Additionally, the larger morphology of ACE2<sup>+</sup> AT2 cells is consistent  
175 with a type of hyperplastic AT2 population that, if damaged, could affect the repair mechanisms  
176 of the alveoli. Infection of AT2 cells could disrupt epithelial integrity leading to alveolar edema,  
177 and facilitate viral spread to ACE2<sup>+</sup> interstitial cells/vessels for systemic virus dissemination,  
178 given that SARS-CoV-2 has been detected in pulmonary endothelium (38) and blood (39).  
179 Furthermore, cell-to-cell spread of coronaviruses to other epithelial cells after initial infection  
180 could also occur via receptor-independent mechanisms related to the fusogenic properties of the  
181 S protein (40). It is interesting that computerized tomography studies of early disease in people  
182 with COVID-19 demonstrate patchy ground glass opacities in the peripheral and posterior lungs,  
183 regions that are more susceptible to alveolar collapse (41).

184

185 ACE2 protein detection in the lower respiratory tract was heterogeneous. The relatively  
186 small number of ACE2<sup>+</sup> cells found in our study proved advantageous in evaluating whether  
187 conditions that predispose to severe disease also increased cellular ACE2 expression, but this  
188 was not observed. Rather we saw elevated ACE2 protein in demographic pools with expected  
189 low risk for severe COVID-19 (young children and in bronchioles of the control group) and  
190 these results suggest alternative explanations. First, the potential relationship between ACE2  
191 abundance in the respiratory tract and severe COVID-19 is likely complex. On one hand, more  
192 receptor availability could enhance viral entry into cells and worsen disease outcomes;  
193 alternatively, ACE2 may play a protective role in acute lung injury through its enzymatic activity  
194 (42-44) and therefore could improve disease outcomes. Our data would support the latter and  
195 implicate a dualistic role for ACE2 as both a viral receptor and a protective agent in acute lung

196 injury. Additionally, ACE2 exists in cell-associated and soluble forms (45). It is possible that  
197 greater ACE2 expression could result in increased soluble ACE2 in respiratory secretions where  
198 it might act as a decoy receptor and reduce virus entry (1, 46). Second, other factors such as  
199 TMPRSS2 expression might be more important in regulating disease severity. TMPRSS2 on the  
200 apical membrane of AT2 cells might facilitate SARS-CoV-2 entry when ACE2 is rare or even  
201 below the limit of detection in this study. Third, low levels of the receptor could be sufficient for  
202 the virus to infect and cause severe disease. Importantly, unlike SARS or HCoV-NL63, the  
203 SARS-CoV-2 spike glycoproteins undergo proteolytic processing at a multibasic S1/S2 site by  
204 furin intracellularly, prior to virion release (25, 47). Additionally, compared to SARS-CoV, the  
205 SARS-CoV-2 receptor binding motif has a higher affinity for ACE2 (48, 49). These features may  
206 enhance the ability of SARS-CoV-2 to bind to cells, undergo S2' cleavage by TMPRSS2 or other  
207 surface proteases, fuse to the host cell membrane, and release its genome. It is important to  
208 mention that the lack of correlation between SARS-CoV-2 receptor expression and disease  
209 severity contrasts with another severe coronavirus disease, MERS, where comorbidities were  
210 observed to increase its receptor detection in respiratory tissues (50, 51).

211  
212 mRNA transcript abundance is not always representative of protein levels (52), and  
213 therefore both should be evaluated in conjunction before making conclusions about gene  
214 expression. Some of the factors that account for these differences include post-transcriptional  
215 regulation or rapid protein turnover. Additionally, other factors limit direct comparisons between  
216 scRNA-seq results and protein staining, including sample size, tissue heterogeneity, and  
217 undefined biopsy sites. In the alveoli, we show ACE2 protein in a small subset of AT2 cells,  
218 which correlates with the scRNA-seq data and with other RNA sequencing publications (11, 15,

219 16). In the lower airways and sinonasal cavity, RNA sequencing data indicate ACE2 transcripts  
220 in both ciliated and secretory cells (11, 15, 16), but we show ACE2 protein is only found in  
221 ciliated cells. Likewise, some authors have reported lower ACE2 transcript abundance in  
222 children (9, 12) and suggested this finding as an explanation for the lower disease severity in this  
223 age group. In contrast, we show that children have more cellular ACE2 protein than older adults,  
224 and while children are likely at similar risk for infection (8, 53) they appear protected from  
225 severe lung disease, possibly through enhanced ACE2 protein.

226

227         In summary, we find that ACE2 protein has heterogeneous expression in the respiratory  
228 tract with more frequent ACE2 detection in the sinonasal epithelium and AT2 cells that  
229 correlates with putative sites for transmission and severe disease, respectively. The small subset  
230 of ACE2<sup>+</sup> AT2 cells in the lung could be further studied to reveal factors regulating ACE2  
231 expression and clarify potential targets for antiviral therapies. Contrary to our initial hypothesis,  
232 we saw no increase of ACE2 protein in the chronic disease group. Interestingly, we observed  
233 increased ACE2 in young children and control group bronchioles, suggesting a possible  
234 protective effect by ACE2 expression. These results suggest that features driving disease  
235 susceptibility and severity are complex. Factors other than ACE2 protein abundance, including  
236 viral load, host innate and adaptive immune responses, and the activities of the pulmonary renin-  
237 angiotensin system may also be important determinants of outcomes.

238 **Materials and methods:**

239

240 **Tissues:**

241 Studies on human tissues were approved by the institutional review board of the University of  
242 Iowa. Tissues included nasal biopsies (n=3, deidentified and lacked evidence of significant  
243 disease or cancer), lung donors, primary cell cultures (54), and autopsy tissues (control tissues)  
244 that were selected from archival repositories as formalin-fixed paraffin-embedded blocks. Lung  
245 cases were selected to comprise two case study groups: 1) Chronic disease group was defined as  
246 having chronic comorbidities including: asthma, cardiovascular disease, chronic obstructive  
247 pulmonary disease, cystic fibrosis, diabetes, and smoking. 2) Control group was defined as  
248 lacking these chronic comorbidities and lacking clinical lung disease. The definition of chronic  
249 comorbidities was informed by reported independent risk factors for mortality in COVID-19  
250 (7). The cumulative cohort included 29 cases (15 chronic comorbidities and 14 controls) with a  
251 broad range of ages (0.5 – 71 years) and both sexes were represented (13 female and 16 male).  
252 For these lungs, if a trachea or bronchus tissue block was available from the same case – these  
253 were included as well ([Supplemental Table 2](#)). Bronchioles were observed in most lung sections  
254 and were defined as intrapulmonary airways lacking evidence of cartilage or submucosal glands  
255 (55).

256

257 **Immunohistochemistry and immunofluorescence:**

258 All formalin-fixed paraffin-embedded tissues were sectioned (~4  $\mu$ m) and hydrated through a  
259 series of xylene and alcohol baths to water. Immunohistochemical techniques were used for the  
260 following markers: angiotensin-converting enzyme 2 (ACE2) (26), allograft inflammatory factor

261 1 (AIF1, also known as IBA1) (56), surfactant protein C (SP-C) (57), and mucin 5B (MUC5B)  
262 (56). For more specifics about the reagents please see [Supplemental Table 3](#).

263 The immunostaining protocols for ACE2 were rigorously optimized and validated to  
264 avoid nonspecific staining that is commonplace and give confidence in the sensitivity of the  
265 protocol and quality of the tissues ([Supplemental Figure 4](#), [Supplemental Table 1](#) and  
266 [Supplemental Table 3](#)). We analyzed ACE2 protein expression in human upper and lower  
267 respiratory tract by immunohistochemistry ([Supplemental Table 2](#)). Human respiratory tract  
268 tissues were scored for ACE2 expression by a masked pathologist, following principles for  
269 reproducible tissue scores (17).

270

271 For immunofluorescence, formalin-fixed and paraffin-embedded human lung blocks were  
272 sectioned (~4  $\mu\text{m}$ ). Slides were baked (55°C x 15 min) and then deparaffinized (hydrated) in a  
273 series of xylene and progressive alcohol baths. Antigen retrieval was performed using Antigen  
274 Unmasking Solution (1:100, #H-3300) in citrate buffer (pH 6.0) solution to induce epitope  
275 retrieval (5 min x 3 times) in the microwave. Slides were washed (PBS, 3 times, 5 min each) and  
276 a PAP pen used to encircle the tissue. Slides were blocked with background blocking solution  
277 (2% BSA in Superblock 1 hr in humid chamber). Primary antibodies anti-ACE2 (1:100, Mouse  
278 monoclonal, MAB933, R&D Systems, Minneapolis, MN USA) and anti-TMPRSS2 (1:200,  
279 Rabbit monoclonal, #ab92323, Abcam, Cambridge, MA USA) were diluted in blocking solution  
280 (2% BSA in Superblock overnight 4°C). Secondary antibodies anti-mouse Alexa568 (for ACE2)  
281 and anti-rabbit Alexa488 (for TMPRSS2) were applied at a concentration of 1:600 for 1 hour at  
282 room temperature. Slides were washed and mounted with Vectashield containing DAPI.

283

284 **Tissue scoring:**

285 Stained tissue sections were examined for ACE2 localization using a post-examination method  
286 for masking and scored by a masked pathologist following principles for reproducible tissue  
287 scores (58). The initial examination showed a low heterogenous incidence of ACE2 staining for  
288 various tissues, so the following ordinal scoring system was employed to quantify number of  
289 staining-positive cells: 0 = below the limit of detection; 1 = <1%; 2 = 1-33%; 3 = 34-66%; and 4  
290 = >66% of cells. For these anatomic regions (e.g. airway or alveoli), cell counts for each tissue  
291 were made to know the population density per microscopic field to make reproducible  
292 interpretations. For determination of AT2 cell size, ACE2 and SP-C protein immunostaining  
293 were evaluated on the same lung tissue section for each case. A region of minimally diseased  
294 lung was examined and SP-C<sup>+</sup> AT2 cells were measured for diameter in the plane perpendicular  
295 to the basement membrane. Similar measurements were then made for ACE2<sup>+</sup>/SP-C<sup>+</sup> cells.

296

297 **Analysis of single cell RNA sequencing data:**

298 Single cell RNA sequencing data sets were accessed from Gene Expression Omnibus (GEO)  
299 series GSE121600 (24) and GSE122960 (23). For GSE121600, raw H5 files for bronchial biopsy  
300 (GSM3439925), nasal brushing (GSM3439926), and turbinate (GSM3439927) samples were  
301 downloaded, and barcodes with less than 1000 unique molecular identifiers (UMIs) were  
302 discarded. For GSE122960, filtered H5 files for eight lung transplant donor samples from lung  
303 parenchyma (GSM3489182, GSM3489185, GSM3489187, GSM3489189, GSM3489191,  
304 GSM3489193, GSM3489195, GSM3489197) were downloaded, and all barcodes were retained.  
305 The eight donors varied from 21-63 years of age (median age = 48) and were composed of five

306 African American, one Asian, and two white donors, and 2 active, 1 former, and 5 never  
307 smokers. Gene count matrices from the eight donors were aggregated for analysis.

308

309 For the bronchial biopsy sample, 82.4% of cells had less than 3,000 UMIs, so we lacked  
310 confidence in assigned cell types, and thus results were not reported. The other three data sets  
311 (nasal brushing, turbinate, and lung parenchyma) were processed in a similar manner. Gene-by-  
312 barcode count matrices were normalized, log-transformed, and scaled followed by dimension  
313 reduction using principal components analysis (PCA). Principal components were used to obtain  
314 uniform manifold approximation and projection (UMAP) visualizations, and cells were clustered  
315 using a shared nearest neighbor (SNN) approach with resolution parameter 0.4, giving 14  
316 clusters for nasal brushing, 15 clusters for turbinate, and 28 clusters for lung parenchyma. Cell  
317 types associated with each cluster were identified by determining marker genes for each cluster  
318 and comparing the list of marker genes to known cell type markers ([Supplemental Figure 5](#)). All  
319 analyses were performed using R package Seurat version 3.1.1 (59). In the nasal brushing  
320 sample, we were unable to associate a cell type with one cluster containing 776 cells (16.5%) due  
321 to low UMIs, so these cells were discarded.

322

323 For the lung parenchyma data, gene expression in alveolar type II cells for a single donor was  
324 quantified by summing up gene counts for all alveolar type II cells and dividing by total UMIs  
325 for all alveolar type II cells to get normalized counts, followed by rescaling the normalized  
326 counts to obtain counts per million (CPM).

327

328 **Statistical analyses:**



329 Statistical analyses for group comparisons and tissue scoring data were performed using  
330 GraphPad Prism version 8 (GraphPad Software, La Jolla, CA USA). Mann Whitney U tests or T-  
331 tests were used for group comparisons as appropriate. ACE2 protein detection in different tissues  
332 was analyzed using the ordinal scoring system (0-4) and Cochran-Armitage test for trend.  
333

334 **Author contributions:**

335 Conceptualization and writing – original draft, M.O.B., P.B.M. and D.K.M.; Data curation, A.T.;

336 Formal analysis, M.O.B., A.T., A.P. and D.K.M.; Investigation, A.T., A.P., M.R.L., C.W.-L. and

337 D.K.M.; Visualization, M.O.B., A.T., D.K.M.; Resources, A.P., J.A.K.-T., P.H.K., P.T., P.B.M.

338 and D.K.M.; Writing – review and editing, M.O.B, A.T., A.P., M.R.L., J.A.K.-T., P.H.K., P.T.,

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349 **References:**

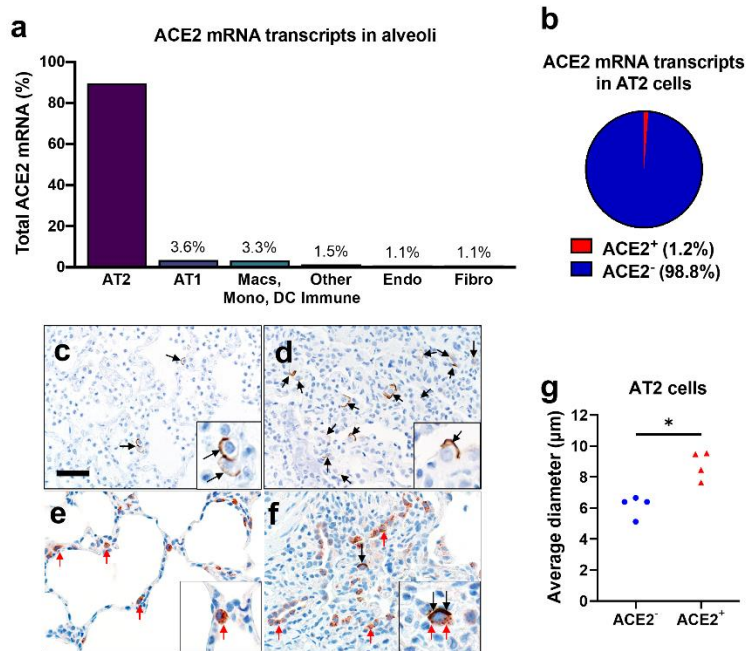
- 350 1. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2  
351 is a functional receptor for the SARS coronavirus. *Nature*. 2003;426(6965):450-4.
- 352 2. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with  
353 a new coronavirus of probable bat origin. *Nature*. 2020.
- 354 3. Lam CW, Chan MH, and Wong CK. Severe acute respiratory syndrome: clinical and laboratory  
355 manifestations. *Clin Biochem Rev*. 2004;25(2):121-32.
- 356 4. Wilder-Smith A, Chiew CJ, and Lee VJ. Can we contain the COVID-19 outbreak with the same  
357 measures as for SARS? *Lancet Infect Dis*. 2020.
- 358 5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult  
359 inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020.
- 360 6. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus  
361 Disease 2019 in China. *N Engl J Med*. 2020.
- 362 7. Mehra MR, Desai SS, Ruschitzka F, and Patel AN. Hydroxychloroquine or chloroquine with or  
363 without a macrolide for treatment of COVID-19: a multinational registry analysis. *The Lancet*.  
364 2020.
- 365 8. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiology of COVID-19 Among Children in  
366 China. *Pediatrics*. 2020.
- 367 9. Bunyavanich S, Do A, and Vicencio A. Nasal Gene Expression of Angiotensin-Converting Enzyme  
368 2 in Children and Adults. *JAMA*. 2020.
- 369 10. Li G, He X, Zhang L, Ran Q, Wang J, Xiong A, et al. Assessing ACE2 expression patterns in lung  
370 tissues in the pathogenesis of COVID-19. *J Autoimmun*. 2020:102463.
- 371 11. Lukassen S, Chua RL, Trefzer T, Kahn NC, Schneider MA, Muley T, et al. SARS-CoV-2 receptor  
372 ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. *EMBO J*.  
373 2020;39(10):e105114.
- 374 12. Muus C, Luecken MD, Eraslan G, Waghray A, Heimberg G, Sikkema L, et al. Integrated analyses  
375 of single-cell atlases reveal age, gender, and smoking status associations with cell type-specific  
376 expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in  
377 putative target cells. *bioRxiv*. 2020.
- 378 13. Pinto BG, Oliveira AE, Singh Y, Jimenez L, Goncalves AN, Ogava RL, et al. ACE2 Expression is  
379 Increased in the Lungs of Patients with Comorbidities Associated with Severe COVID-19.  
380 *medRxiv*. 2020.
- 381 14. Smith JC, Sausville EL, Girish V, Yuan ML, Vasudevan A, John KM, et al. Cigarette smoke exposure  
382 and inflammatory signaling increase the expression of the SARS-CoV-2 receptor ACE2 in the  
383 respiratory tract. *Dev Cell*. 2020.
- 384 15. Sungnak W, Huang N, Becavin C, Berg M, Queen R, Litvinukova M, et al. SARS-CoV-2 entry  
385 factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat*  
386 *Med*. 2020;26(5):681-7.
- 387 16. Ziegler CGK, Allon SJ, Nyquist SK, Mbanjo IM, Miao VN, Tzouanas CN, et al. SARS-CoV-2 Receptor  
388 ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in  
389 Specific Cell Subsets across Tissues. *Cell*. 2020.
- 390 17. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, and van Goor H. Tissue distribution of ACE2  
391 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS  
392 pathogenesis. *J Pathol*. 2004;203(2):631-7.

- 393 18. Ren X, Glende J, Al-Falah M, de Vries V, Schwegmann-Wessels C, Qu X, et al. Analysis of ACE2 in  
394 polarized epithelial cells: surface expression and function as receptor for severe acute  
395 respiratory syndrome-associated coronavirus. *J Gen Virol.* 2006;87(Pt 6):1691-5.
- 396 19. Bertram S, Glowacka I, Muller MA, Lavender H, Gnirss K, Nehlmeier I, et al. Cleavage and  
397 activation of the severe acute respiratory syndrome coronavirus spike protein by human airway  
398 trypsin-like protease. *J Virol.* 2011;85(24):13363-72.
- 399 20. Bertram S, Heurich A, Lavender H, Gierer S, Danisch S, Perin P, et al. Influenza and SARS-  
400 coronavirus activating proteases TMPRSS2 and HAT are expressed at multiple sites in human  
401 respiratory and gastrointestinal tracts. *PLoS One.* 2012;7(4):e35876.
- 402 21. Carrel L, and Willard HF. X-inactivation profile reveals extensive variability in X-linked gene  
403 expression in females. *Nature.* 2005;434(7031):400-4.
- 404 22. Anguiano L, Riera M, Pascual J, and Soler MJ. Circulating ACE2 in Cardiovascular and Kidney  
405 Diseases. *Curr Med Chem.* 2017;24(30):3231-41.
- 406 23. Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, Chiu S, et al. Single-Cell  
407 Transcriptomic Analysis of Human Lung Provides Insights into the Pathobiology of Pulmonary  
408 Fibrosis. *Am J Respir Crit Care Med.* 2019;199(12):1517-36.
- 409 24. Ruiz Garcia S, Deprez M, Lebrigand K, Cavard A, Paquet A, Arguel MJ, et al. Novel dynamics of  
410 human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial  
411 cultures. *Development.* 2019;146(20).
- 412 25. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2  
413 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease  
414 Inhibitor. *Cell.* 2020.
- 415 26. Jia HP, Look DC, Shi L, Hickey M, Pewe L, Netland J, et al. ACE2 receptor expression and severe  
416 acute respiratory syndrome coronavirus infection depend on differentiation of human airway  
417 epithelia. *J Virol.* 2005;79(23):14614-21.
- 418 27. Lu Q, and Shi Y. Coronavirus disease (COVID-19) and neonate: What neonatologist need to  
419 know. *J Med Virol.* 2020.
- 420 28. Mo P, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, et al. Clinical characteristics of refractory COVID-  
421 19 pneumonia in Wuhan, China. *Clin Infect Dis.* 2020.
- 422 29. Sternberg SS. *Histology for Pathologists.* Philadelphia, PA USA: Lippincott-Raven Publishers;  
423 1997.
- 424 30. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in Different Types of  
425 Clinical Specimens. *JAMA.* 2020.
- 426 31. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, et al. Presumed Asymptomatic Carrier Transmission of  
427 COVID-19. *JAMA.* 2020.
- 428 32. Wang Y, Wang Y, Chen Y, and Qin Q. Unique epidemiological and clinical features of the  
429 emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. *J*  
430 *Med Virol.* 2020.
- 431 33. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the  
432 2019-nCoV spike in the prefusion conformation. *Science.* 2020;367(6483):1260-3.
- 433 34. Yen YT, Liao F, Hsiao CH, Kao CL, Chen YC, and Wu-Hsieh BA. Modeling the early events of severe  
434 acute respiratory syndrome coronavirus infection in vitro. *J Virol.* 2006;80(6):2684-93.
- 435 35. Zhang H, Zhou P, Wei Y, Yue H, Wang Y, Hu M, et al. Histopathologic Changes and SARS-CoV-2  
436 Immunostaining in the Lung of a Patient With COVID-19. *Ann Intern Med.* 2020.
- 437 36. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARS-CoV-2 on  
438 virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun.* 2020;11(1):1620.

- 439 37. Ware LB, and Matthay MA. The acute respiratory distress syndrome. *N Engl J Med*.  
440 2000;342(18):1334-49.
- 441 38. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary  
442 Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med*. 2020.
- 443 39. Chen W, Lan Y, Yuan X, Deng X, Li Y, Cai X, et al. Detectable 2019-nCoV viral RNA in blood is a  
444 strong indicator for the further clinical severity. *Emerg Microbes Infect*. 2020;9(1):469-73.
- 445 40. Gallagher TM, Buchmeier MJ, and Perlman S. Dissemination of MHV4 (strain JHM) infection  
446 does not require specific coronavirus receptors. *Adv Exp Med Biol*. 1993;342:279-84.
- 447 41. Song F, Shi N, Shan F, Zhang Z, Shen J, Lu H, et al. Emerging 2019 Novel Coronavirus (2019-nCoV)  
448 Pneumonia. *Radiology*. 2020;295(1):210-7.
- 449 42. Gu H, Xie Z, Li T, Zhang S, Lai C, Zhu P, et al. Angiotensin-converting enzyme 2 inhibits lung injury  
450 induced by respiratory syncytial virus. *Sci Rep*. 2016;6:19840.
- 451 43. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects  
452 from severe acute lung failure. *Nature*. 2005;436(7047):112-6.
- 453 44. Zou Z, Yan Y, Shu Y, Gao R, Sun Y, Li X, et al. Angiotensin-converting enzyme 2 protects from  
454 lethal avian influenza A H5N1 infections. *Nat Commun*. 2014;5:3594.
- 455 45. Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, et al. Tumor necrosis factor-  
456 alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute  
457 respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2  
458 (ACE2). *J Biol Chem*. 2005;280(34):30113-9.
- 459 46. Hofmann H, Geier M, Marzi A, Krumbiegel M, Peipp M, Fey GH, et al. Susceptibility to SARS  
460 coronavirus S protein-driven infection correlates with expression of angiotensin converting  
461 enzyme 2 and infection can be blocked by soluble receptor. *Biochem Biophys Res Commun*.  
462 2004;319(4):1216-21.
- 463 47. Hoffmann M, Kleine-Weber H, and Pohlmann S. A Multibasic Cleavage Site in the Spike Protein  
464 of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. *Mol Cell*. 2020;78(4):779-84 e5.
- 465 48. Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, et al. Structural basis of receptor recognition by  
466 SARS-CoV-2. *Nature*. 2020;581(7807):221-4.
- 467 49. Wan Y, Shang J, Graham R, Baric RS, and Li F. Receptor Recognition by the Novel Coronavirus  
468 from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol*.  
469 2020;94(7).
- 470 50. Meyerholz DK, Lambertz AM, and McCray PB, Jr. Dipeptidyl Peptidase 4 Distribution in the  
471 Human Respiratory Tract: Implications for the Middle East Respiratory Syndrome. *Am J Pathol*.  
472 2016;186(1):78-86.
- 473 51. Seys LJM, Widagdo W, Verhamme FM, Kleinjan A, Janssens W, Joos GF, et al. DPP4, the Middle  
474 East Respiratory Syndrome Coronavirus Receptor, is Upregulated in Lungs of Smokers and  
475 Chronic Obstructive Pulmonary Disease Patients. *Clin Infect Dis*. 2018;66(1):45-53.
- 476 52. Liu Y, Beyer A, and Aebersold R. On the Dependency of Cellular Protein Levels on mRNA  
477 Abundance. *Cell*. 2016;165(3):535-50.
- 478 53. Bi Q, Wu Y, Mei S, Ye C, Zou X, Zhang Z, et al. Epidemiology and transmission of COVID-19 in 391  
479 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. *Lancet*  
480 *Infect Dis*. 2020.
- 481 54. Itani OA, Chen JH, Karp PH, Ernst S, Keshavjee S, Parekh K, et al. Human cystic fibrosis airway  
482 epithelia have reduced Cl<sup>-</sup> conductance but not increased Na<sup>+</sup> conductance. *Proc Natl Acad Sci*  
483 *U S A*. 2011;108(25):10260-5.
- 484 55. Meyerholz DK, Suarez CJ, Dintzis SM, and Frevert CW. *Comparative Anatomy and Histology: A*  
485 *Mouse, Rat and Human Atlas*. Academic Press - Elsevier; 2018.

- 486 56. Meyerholz DK, Lambertz AM, Reznikov LR, Ofori-Amanfo GK, Karp PH, McCray PB, Jr., et al.  
487 Immunohistochemical Detection of Markers for Translational Studies of Lung Disease in Pigs and  
488 Humans. *Toxicol Pathol.* 2016;44(3):434-41.
- 489 57. Krishnamurthy S, Wohlford-Lenane C, Kandimalla S, Sartre G, Meyerholz DK, Theberge V, et al.  
490 Engineered amphiphilic peptides enable delivery of proteins and CRISPR-associated nucleases to  
491 airway epithelia. *Nat Commun.* 2019;10(1):4906.
- 492 58. Meyerholz DK, and Beck AP. Principles and approaches for reproducible scoring of tissue stains  
493 in research. *Lab Invest.* 2018;98(7):844-55.
- 494 59. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM, 3rd, et al. Comprehensive  
495 Integration of Single-Cell Data. *Cell.* 2019;177(7):1888-902 e21.
- 496

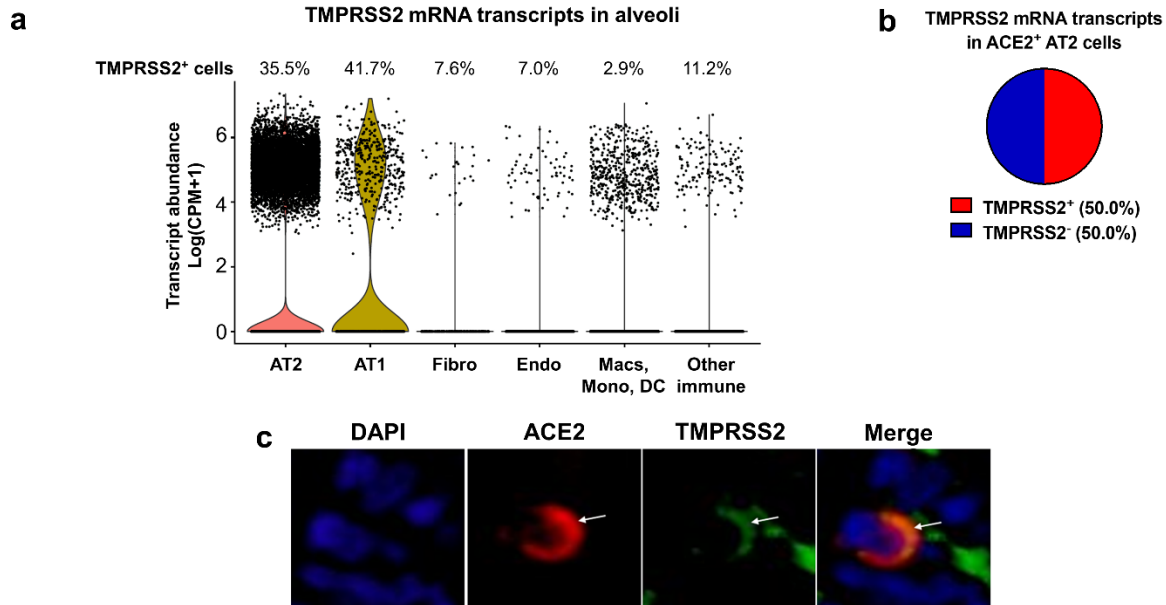
**Figure 1**



497  
 498 **Figure 1.** ACE2 expression in human lung. **a, b)** Single-cell RNA sequencing reanalysis of  
 499 ACE2 transcript abundance in alveoli from lung parenchyma samples (23). Summative  
 500 observations from all donors. Airway cells (basal, mitotic, ciliated, club) are not shown. **a)**  
 501 89.5% of the cells with detectable ACE2 mRNA in the alveoli are alveolar type II cells. **b)** Only  
 502 1.2% of alveolar type II cells have ACE2 mRNA transcripts. **c-f)** Detection of ACE2 protein  
 503 (brown color, black arrows and insets) in representative sections of lower respiratory tract  
 504 regions and tissue scoring (see [Supplemental Table 2](#)) (**g**). **c, d)** Alveolar regions had uncommon  
 505 to regional polarized apical staining of solitary epithelial cells (**c**) that (when present) were more  
 506 readily detected in collapsed regions of lung (**d**). **e, f)** SP-C (red arrows, inset) and ACE2 (black  
 507 arrows, inset) dual immunohistochemistry on the same tissue sections. **e)** Non-collapsed regions  
 508 had normal SP-C<sup>+</sup> AT2 cells lacking ACE2. **f)** Focal section of peri-airway remodeling and  
 509 collapse with several SP-C<sup>+</sup> (red arrows) AT2 cells, but only a small subset of AT2 cells had  
 510 prominent apical ACE2 protein (black arrows, inset). **g)** SP-C<sup>+</sup>/ACE2<sup>+</sup> AT2 cells were often  
 511 larger than SP-C<sup>+</sup>/ACE2<sup>-</sup> AT2 cells from same lung sections (see also **d** and **e** insets) indicative  
 512 of AT2 hypertrophy, each data point represents the average value for each case from 5-10 cell  
 513 measurements per group, P=0.0014, paired T-test. AT2: alveolar type II. AT1: alveolar type I.  
 514 Macs: Macrophages. Mono: Monocytes. DC: dendritic cells. Other immune cells: B cells, mast  
 515 cells, natural killer/T cells. Endo: Endothelial. Fibro: Fibroblasts/myofibroblasts. Bar = 35 μm.



## Figure 2



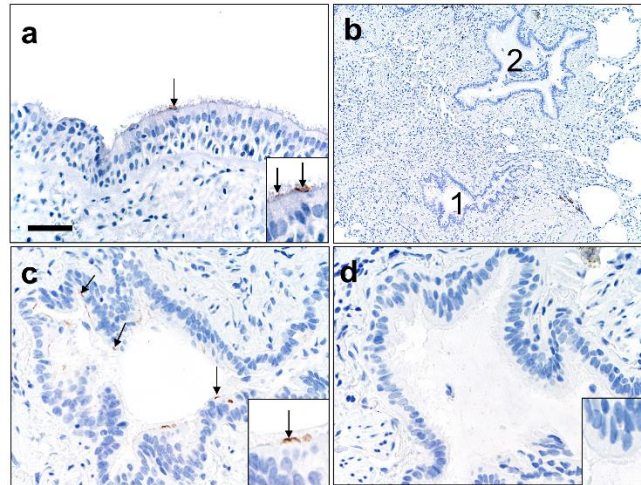
516

517 **Figure 2.** TMPRSS2 expression in the alveoli. **a, b**) Single-cell RNA sequencing reanalyses of  
518 TMPRSS2 transcript abundance in alveoli from lung parenchyma (23). Summative observations  
519 from all donors. **a)** Percentage of TMPRSS2<sup>+</sup> cells within each cell type shows TMPRSS2  
520 transcripts in 35.5% of alveolar type II cells. Airway cells (basal, mitotic, ciliated, club) are not  
521 shown. Violin plots represent expression, each data point denotes a cell. **b)** TMPRSS2 transcripts  
522 in ACE2<sup>+</sup> alveolar type II cells. **c)** Immunofluorescence of alveoli shows apical colocalization of  
523 ACE2 and TMPRSS2 (white arrows). AT2: alveolar type II. AT1: alveolar type I. Macs:  
524 Macrophages. Mono: Monocytes. DC: dendritic cells. Other immune cells: B cells, mast cells,  
525 natural killer/T cells. Endo: Endothelial. Fibro: Fibroblasts/myofibroblasts. CPM: counts per  
526 million.

527



**Figure 3**



528

529 **Figure 3.** ACE2 protein in human lower airways. **a)** Large airways (trachea and bronchi)

530 exhibited rare ACE2 protein on the apical surface of ciliated cells. **b-d)** Small airways

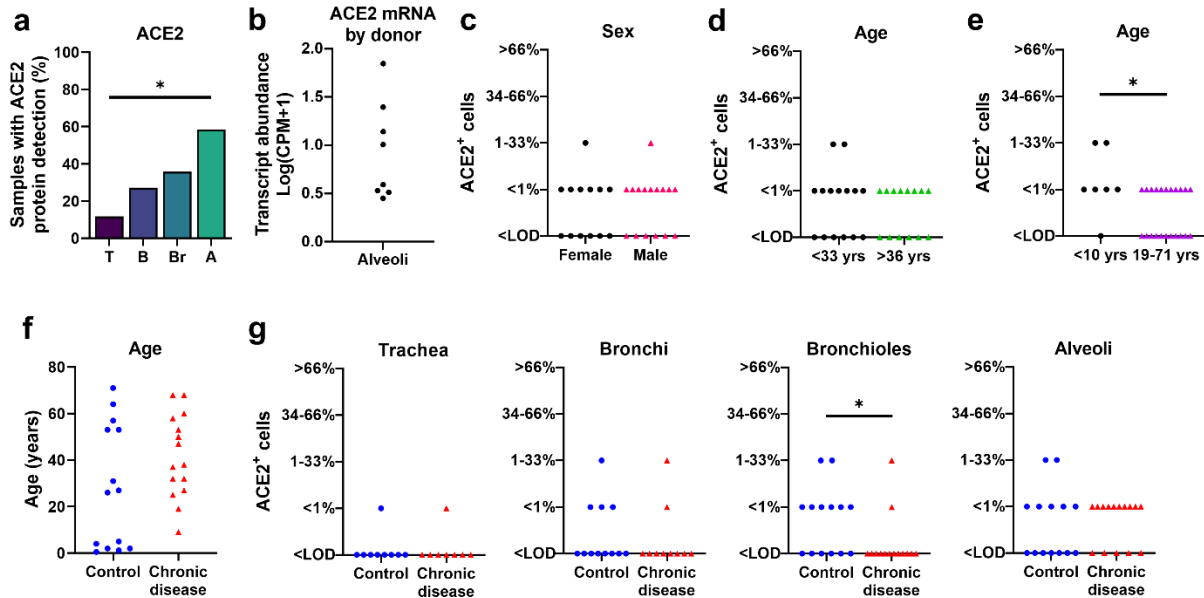
531 (bronchioles) exhibited uncommon to localized apical ACE2 protein in ciliated cells (**c**, #1 in **b**)

532 while the adjacent bronchioles (**d**, #2 in **b**) lacked protein. Bar = 35 (a), 140 (b), and 70  $\mu$ m (c,

533 d).

534

**Figure 4**



535

536 **Figure 4.** ACE2 distribution and scores in respiratory tissues. **a)** ACE2 protein had progressively

537 increased detection between donors in tissues from trachea (T), bronchi (B), bronchioles (Br), to

538 alveoli (A), ( $P=0.0009$ , Cochran-Armitage test for trend). **b)** Single-cell RNA sequencing

539 reanalyses of ACE2 mRNA transcript abundance in the alveoli shows variation between donors

540 (23). **c-d)** ACE2 protein scores from lung donors showed no differences based on sex or lower

541 vs. upper ages (using median age as a cut-off) (A,  $P=0.7338$  and B,  $P=0.7053$ , Mann-Whitney U

542 test). **e)** ACE2 protein scores were elevated in young children (<10 yrs) compared to the

543 remaining subjects (19-71 yrs) ( $P=0.0282$  Mann-Whitney U test). **f)** Control and chronic disease

544 groups did not have any significant differences in age ( $P=0.1362$  Mann-Whitney U test). **g)**

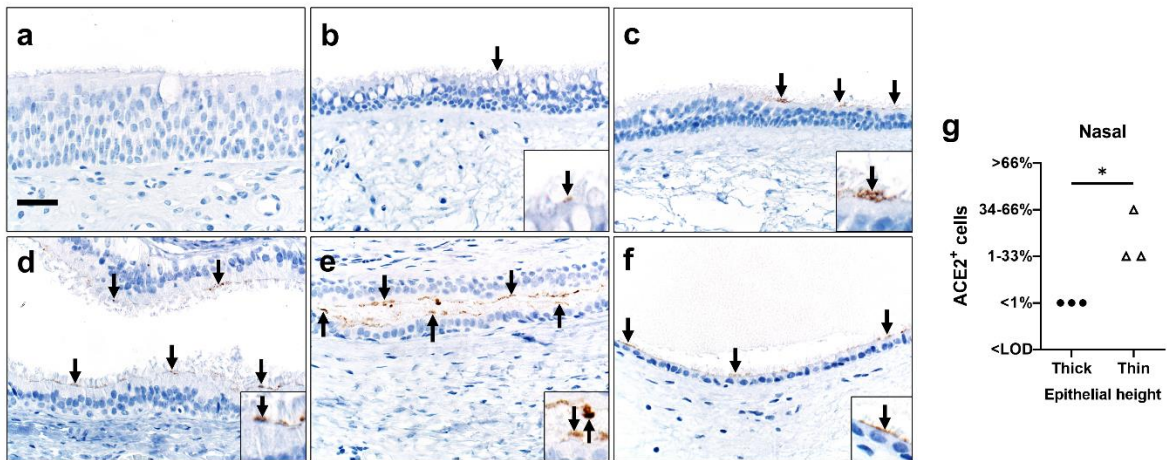
545 ACE2 protein scores for trachea, bronchi, bronchiole, and alveoli in control versus chronic

546 disease groups ( $P= >0.9999$ ,  $0.6263$ ,  $0.0433$ , and  $0.7359$ , respectively, Mann-Whitney U test).

547 Each symbol represents one donor. CPM: counts per million. LOD: Limit of detection.

548

**Figure 5**



549

550 **Figure 5.** Detection of ACE2 protein (brown color, arrows and insets, **a-f**) and tissue scoring (**g**)

551 in representative sections of nasal tissues. **a, b**) In thick pseudostratified epithelium (PSE) ACE2

552 protein was absent (**a**) to rare (**b**) and apically located on ciliated cells. **c**) Tissue section shows a

553 transition zone from thick (left side, > ~4 nuclei) to thin (right side, ≤ ~4 nuclei) PSE and ACE2

554 protein was restricted to the apical surface of the thin PSE. **d-f**) ACE2 protein was detected

555 multifocally on the apical surface of ciliated cells in varying types of thin PSE, even to simple

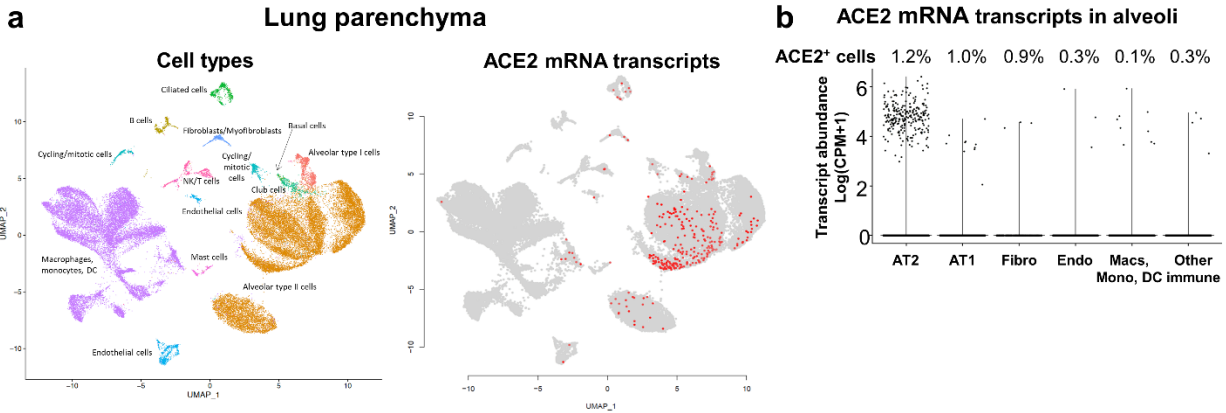
556 cuboidal epithelium (**f**). Bar = 30 μm. **g**) ACE2 protein detection scores for each subject were

557 higher in thin than thick epithelium, (P=0.05, Mann-Whitney U test). LOD: Limit of detection.

558

559 **Supplemental information:**

**Supplemental Figure 1**



560

561 **Supplemental Figure 1.** Single-cell RNA sequencing reanalyses of ACE2 transcript abundance

562 in lung parenchyma (23). Summative observations from all donors. **a)** Uniform manifold

563 approximation and projection (UMAP) visualizations. Cells were clustered using a shared

564 nearest neighbor (SNN) approach. Cell types associated with each cluster were identified by

565 determining marker genes for each cluster. Each data point denotes a cell. On the right panel,

566 cells with ACE2 transcripts are shown in red. **b)** Violin plots representing ACE2 expression in

567 the alveoli. Airway cells (basal, mitotic, ciliated, club) are not shown. Percentage of ACE2<sup>+</sup> cells

568 within each cell type shows ACE2 transcripts in 1.2% of alveolar type II cells and in 0.1% of

569 macrophages, monocytes, or dendritic cells. Each data point denotes a cell, most cells have no

570 expression (0). AT2: alveolar type II. AT1: alveolar type I. Macs: Macrophages. Mono:

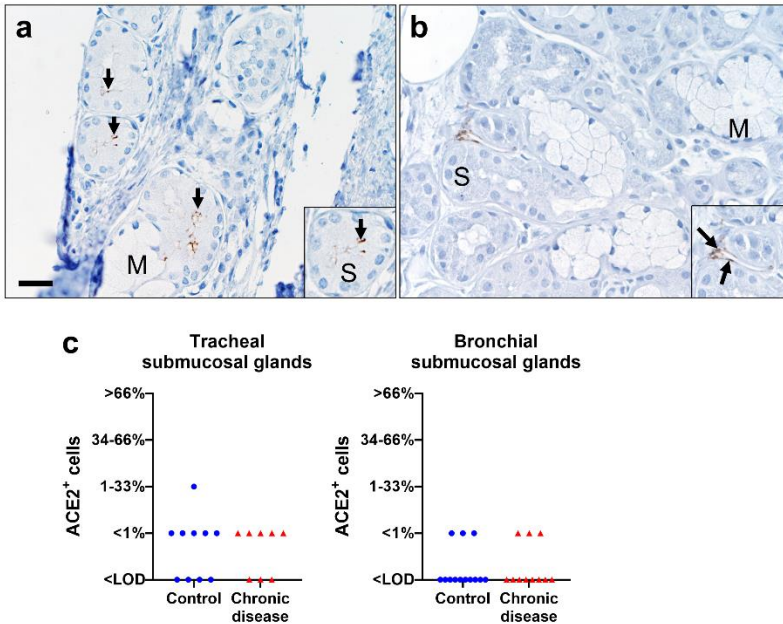
571 Monocytes. DC: dendritic cells. Other immune cells: B cells, mast cells, natural killer/T cells.

572 Endo: Endothelial. Fibro: Fibroblasts/myofibroblasts. NK: Natural killer. CPM: Counts per

573 million.

574

## Supplemental Figure 2



575

576 **Supplemental Figure 2.** Representative tissue section from submucosa of large airways

577 (trachea/bronchi) showing ACE2 protein localization (brown color, black arrows) (**a, b**) and

578 scores (**c**). **a**) Submucosal glands had uncommon to localized apical ACE2 protein (arrows) in

579 serous (S) cells, but not mucous (M) cells. **b**) Submucosal glands also had absent to uncommon

580 ACE2 protein (arrows) in the interstitium that centered on vascular walls and endothelium. This

581 vascular staining was uncommonly seen in lung too and corresponded to the low levels seen in

582 transcripts for these endothelial cells ([Supplemental Figure 1a-b](#)). Note the absence of ACE2

583 staining in serous (S) or mucous (M) cells of the gland (**b**). **c**) ACE2 protein scores for each

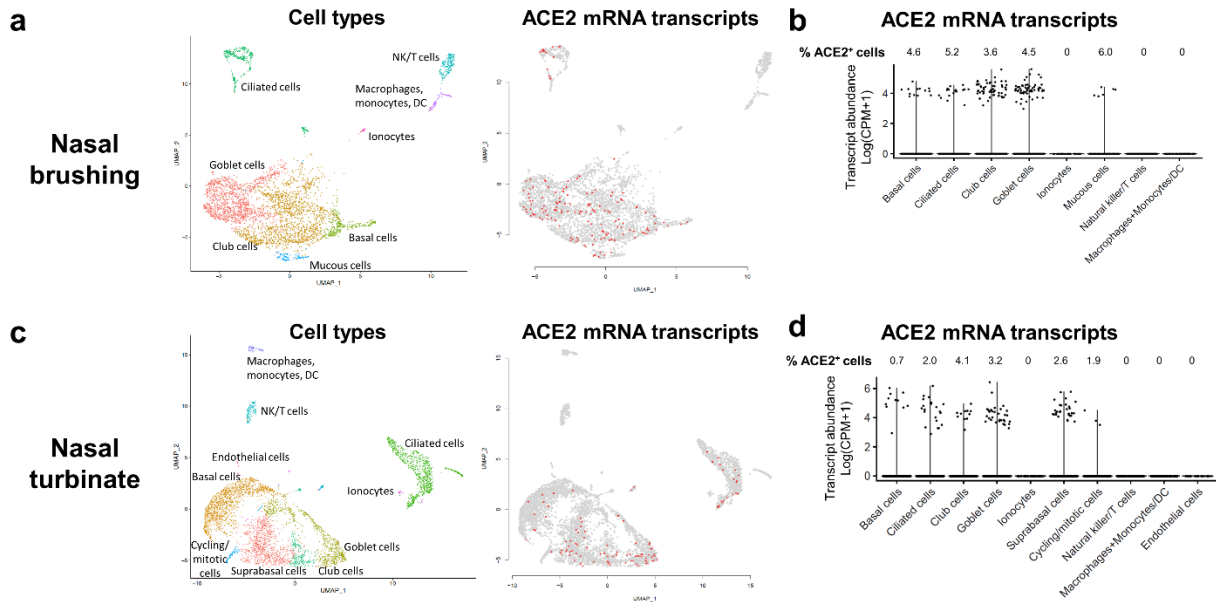
584 subject for serous cells in submucosal glands from trachea and bronchi, in control versus chronic

585 disease groups ( $P > 0.9999$ ,  $0.9999$ , respectively, Mann-Whitney U test). Bar = 25  $\mu\text{m}$ . LOD:

586 Limit of detection.

587

### Supplemental Figure 3



588

589 **Supplemental Figure 3.** Single-cell RNA sequencing reanalyses of ACE2 transcript abundance

590 in nasal brushing (**a, b**) and nasal turbinate (**c, d**) (24). **a, c**) Uniform manifold approximation

591 and projection (UMAP) visualizations. Cells were clustered using a shared nearest neighbor

592 (SNN) approach. Cell types associated with each cluster were identified by determining marker

593 genes for each cluster. Each data point denotes a cell. On the right panels, cells with ACE2

594 transcripts are shown in red. **b, d**) Violin plots representing ACE2 expression. In nasal turbinate

595 and nasal brushing, percentage of ACE2<sup>+</sup> cells within each cell type shows ACE2 expression on

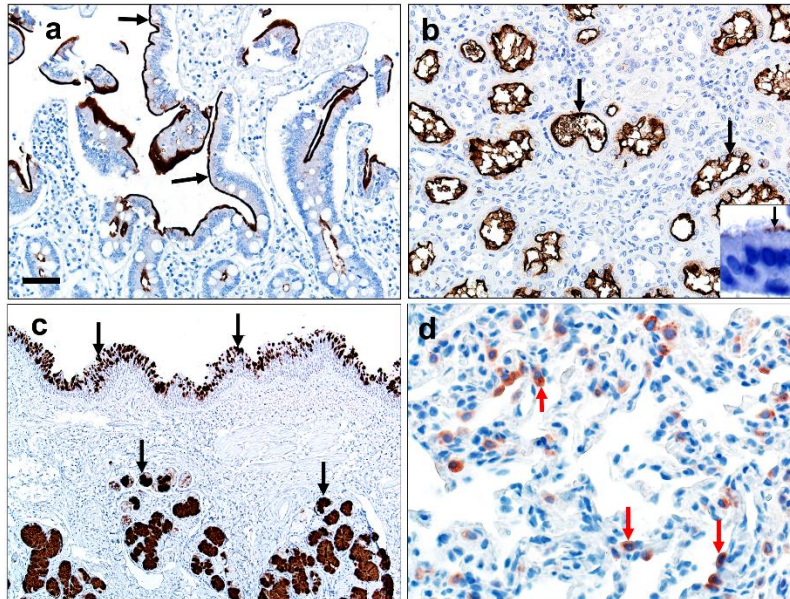
596 epithelial cells. Each data point denotes a cell, most cells have no expression (0). DC: dendritic

597 cells. NK: Natural killer. CPM: Counts per million.

598



## Supplemental Figure 4

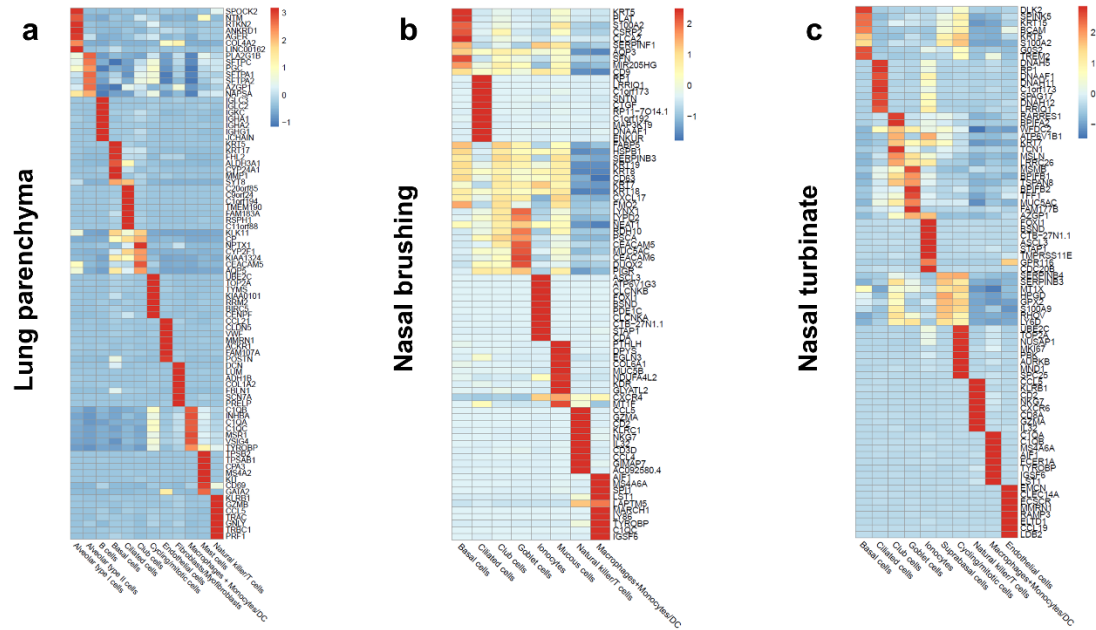


599

600 **Supplemental Figure 4.** Quality controls for ACE2 immunohistochemistry technique (**a, b**) and  
601 tissue quality (**c, d**). **a, b**) ACE2 protein (brown color, black arrows) was detected along the  
602 apical surface of small intestine enterocytes (**a**), renal tubule epithelium (**b**), and ciliated cells (**b**,  
603 **inset**) of primary airway cell cultures. These findings demonstrate specific detection of ACE2  
604 protein in cells/tissues consistent with known ACE2 expression. **c**) Representative  
605 immunostaining of bronchus detected abundant MUC5B protein (brown color, black arrows) in  
606 mucous cells of surface epithelium (top) and submucosal glands (bottom). **d**) Representative  
607 sections of alveoli had SP-C<sup>+</sup> alveolar type II cells (red color, red arrows). These results (**c, d**)  
608 demonstrate the tissues were intact and that immunostaining can be used to detect native airway  
609 (**c**) and lung (**d**) proteins. Bar = 40 (**a, b**), 80 (**c**), and 20  $\mu$ m (**d**).

610

### Supplemental Figure 5



611

612 **Supplemental Figure 5.** Single-cell RNA sequencing reanalyses of lung parenchyma (a) (23),

613 nasal brushing (b) and nasal turbinate (c) (24). Heatmaps depicting the marker genes for each

614 cluster that were used to assign cell types.



615 **Supplemental Table 1. ACE2 protein reported in surface epithelium (SE) of human**  
 616 **respiratory tract surface epithelium.**

Reported Cases [n]	Primary Ab	SN	T	B	Br	Al	Summary comments
Non-diseased lungs / nasal [5 each]; diseased lungs [5] (17)	Polyclonal	SE (C++, basal cells in squamous epithelium)	n.d.	SE (C+)	n.d.	AT1 (C++); AT2 (C++)	Abundant ACE2 protein in lung epithelia
Non-diseased lungs [5] (18)	Undefined	n.d.	SE (C+, A+)	SE (C+, A+)	n.d.	"Alveoli" (A+) Mac (A+)	ACE2 is present on epithelia in several parts of the respiratory tract and macrophages
Lung [undefined] (19)	Polyclonal	n.d.	n.d.	SE (C+, N+, M+)	n.d.	AT1- AT2 (N+)	ACE2 is present in bronchial epithelium, AT2 cells and macrophages
Sinus [undefined] and Lung [undefined, same tissues as above] (20)	Polyclonal	SE (N++)	SE (-)	SE (C+, N++)	n.d.	AT1- AT2 (N++)	ACE2 is present in sinus and bronchial epithelium, AT2 cells and macrophages

617

618 Non-diseased: The cause of death was not directly related to lung disease

619 n.d.: Not described

620 Tissues: Sinonasal (SN), trachea (T), bronchi (B), bronchioles (Br), and alveoli (Al)

621 Cellular localization: cytoplasmic (C), nuclear (N), apical membrane (A)

622 Cells: Surface epithelium (SE), alveolar type I cells (AT1), alveolar type II cells (AT2), alveolar

623 macrophages (Mac)

624 ACE2 protein (based on published reports/figures): negative (-), weak (+), moderate to abundant

625 (++)

626 **Supplemental Table 2. Donor demographics and ACE2 distribution scores for each tissue**  
 627 **region.**

Case #	Group	Age (yrs)	Sex	Comorbidities	Trachea	Bronchi	Bronchioles	Alveoli
1	Control	5	F	Trauma	NA	2	2	1
2	Control	57	M	Arrhythmia	0	0	0	1
3	Control	31	M	Stroke (Joubert syndrome)	1	1	0	0
4	Control	53	F	Trauma	NA	0	0	1
5	Control	2	M	Brain hemorrhage	0	0	0	1
6	Control	2	M	Trauma	0	0	1	2
7	Control	0.5	M	Spinomuscular atrophy	NA	0	1	0
8	Control	71	M	Stroke, Parkinson's disease, nonsmoker	0	1	1	0
9	Control	4	F	Trauma	0	0	0	2
10	Control	1.2	M	Trauma	0	NA	1	1
11	Control	53	F	Trauma, nonsmoker	0	0	2	0
12	Control	26	F	NA	0	NA	0	0
13	Control	27	F	NA	NA	0	1	0
14	Control	64	M	NA	NA	1	1	0
15	Chronic disease	53	F	Smoker	0	NA	0	1
16	Chronic disease	60	M	COPD, smoker	NA	NA	0	1
17	Chronic disease	32	M	COPD, smoker	0	0	0	1
18	Chronic disease	68	M	COPD	NA	1	0	1
19	Chronic disease	68	F	COPD	NA	NA	1	1
20	Chronic disease	9	M	Asthma	0	0	0	1
21	Chronic disease	25	F	Cystic fibrosis	NA	0	0	0
22	Chronic disease	47	F	Cardiovascular disease	1	2	2	1
23	Chronic disease	27	M	Cystic fibrosis	0	NA	NA	1
24	Chronic disease	50	F	Cardiovascular disease, diabetes, asthma	NA	0	0	0
25	Chronic disease	37	M	Drug use, smoker	0	0	0	0
26	Chronic disease	38	M	Asthma (status asthmaticus)	0	0	0	0
27	Chronic disease	32	M	Cystic fibrosis	NA	NA	0	1
28	Chronic disease	58	F	Cardiovascular disease, diabetes, NASH	0	0	0	1
29	Chronic disease	19	F	Cystic fibrosis	NA	0	0	0

628

629 NA: Not available for analyses / COPD: Chronic obstructive pulmonary disease / NASH: Non-  
 630 alcoholic steatohepatitis.

631 Scoring: 0 = below limit of immunohistochemical detection; 1 = rare (<1%); 2 = 1-33%; 3 = 34-  
 632 66%; 4 = >66% of cells.

633

634 **Supplemental Table 3. Parameters for immunohistochemistry on fixed tissues.**

Target	Primary Antibody	Antigen Retrieval	Secondary Reagents
Allograft Inflammatory Factor 1 (AIF1)	Anti-AIF1 polyclonal (#019-19741, Wako Pure Chemical Industries, Ltd., Richmond, VA USA) in diluent 1:1000 x 1 hour	HIER, Citrate buffer pH 6.0, 110°C for 15 min; 20 min cool down (Decloaking Chamber Plus, Biocare Medical, Concord, CA USA)	Dako EnVision+ System-HRP Labeled Polymer Anti-rabbit, 30 min (Dako North America, Inc., Carpinteria, CA USA) AEC chromogen, counterstain.
Angiotensin-Converting Enzyme 2 (ACE2)	Anti-ACE2, monoclonal (MAB933, R&D Systems, Minneapolis, MN USA) in diluent at 1:100 x 1 hour.	HIER, Citrate Buffer, pH 6.0, 110°C for 15 minutes; 20 min cool down (Decloaking Chamber Plus, Biocare Medical, Concord, CA USA)	Dako EnVision+ System-HRP Labeled Polymer Anti-mouse, 60 min (Dako North America, Inc., Carpinteria, CA USA), DAB Chromogen, counterstain.
MUC5B	Rabbit anti-MUC5B polyclonal, (LSBio #LS-B8121, LifeSpan BioSciences, Inc., Seattle, WA) in Dako Antibody Diluent (Dako North America, Inc., Carpinteria, CA); 1:60,000/30 min	HIER, Citrate buffer pH 6.0, 110°C for 15min; 20 min cool down	Step 1: Biotinylated anti-Rabbit IgG (H+L) (Vector Laboratories, Inc., Burlingame, CA) in Dako Wash Buffer (Dako North America, Inc., Carpinteria, CA); 1:500, 30 min Step 2: Vectastain ABC Kit (Vector Laboratories, Inc., Burlingame, CA), 30min. DAB Chromogen, counterstain.
Surfactant Protein – C (SP-C)	Anti-SP-C, polyclonal (PA5-71680, Thermo Fisher Scientific, Waltham, MA USA) in diluent 1:100 x 1 hour	HIER, Citrate Buffer, pH 6.0, 110°C for 15 minutes; 20 min cool down (Decloaking Chamber Plus, Biocare Medical, Concord, CA USA)	Dako EnVision+ System-HRP Labeled Polymer Anti-rabbit, 60 min (Dako North America, Inc., Carpinteria, CA USA), AEC chromogen, counterstain.

635

636 HIER – Heat-induced epitope retrieval

637 DAB – 3,3'-Diaminobenzidine (produces brown stain)

638 AEC - aminoethyl carbazole (produces red stain)

639 Counterstain – Harris hematoxylin (blue color)