

## Supplemental information

### **Determinants of SARS-CoV-2 entry and replication in airway mucosal tissue and susceptibility in smokers**

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**Cell Reports Medicine, Volume 2**

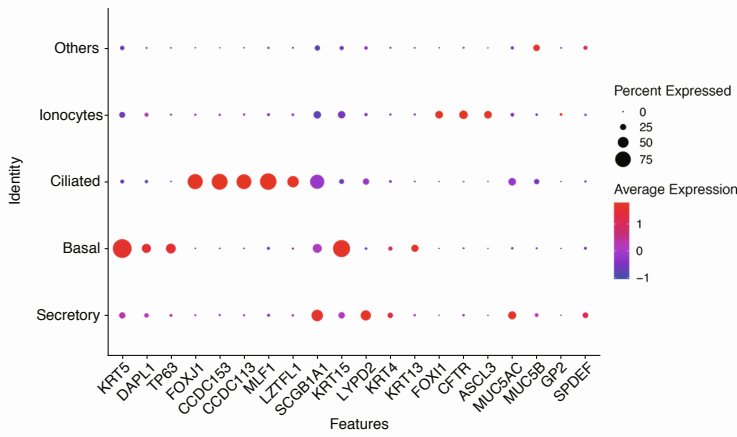
**Supplemental information**

**Determinants of SARS-CoV-2 entry  
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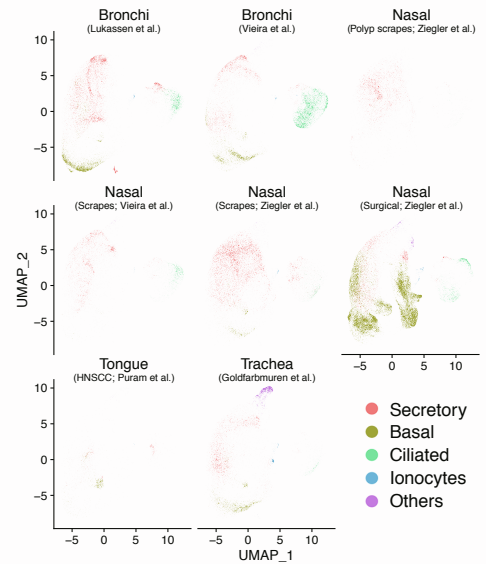
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**Figure S1**

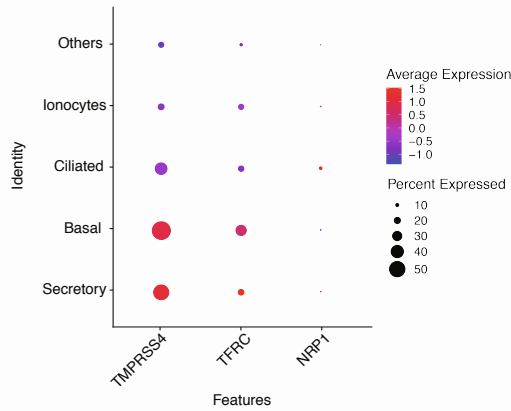
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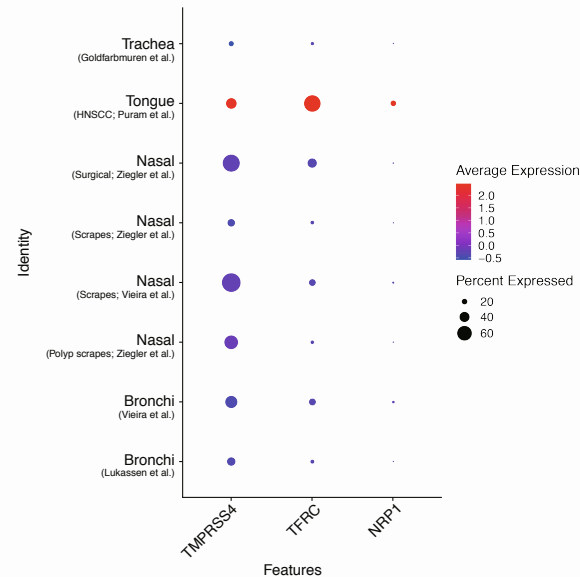
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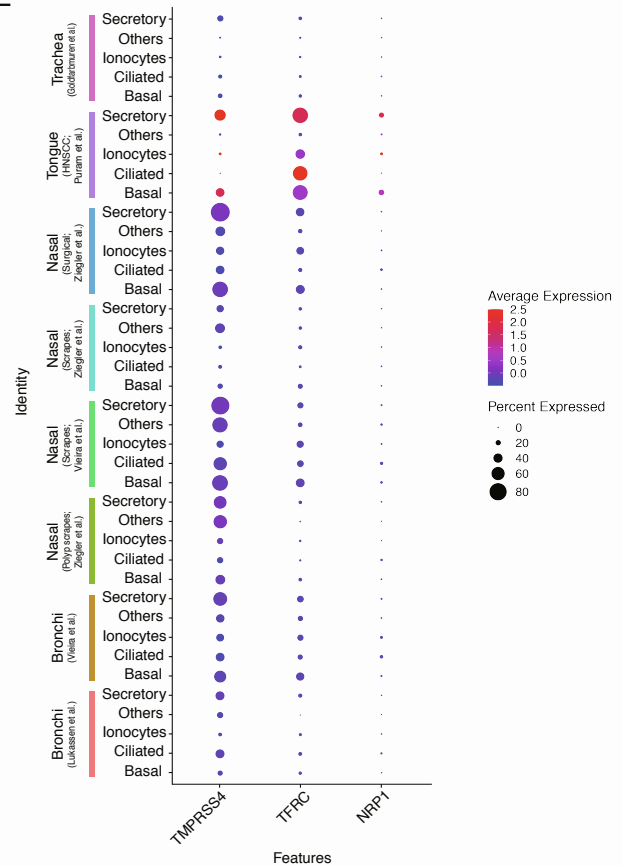
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**D**



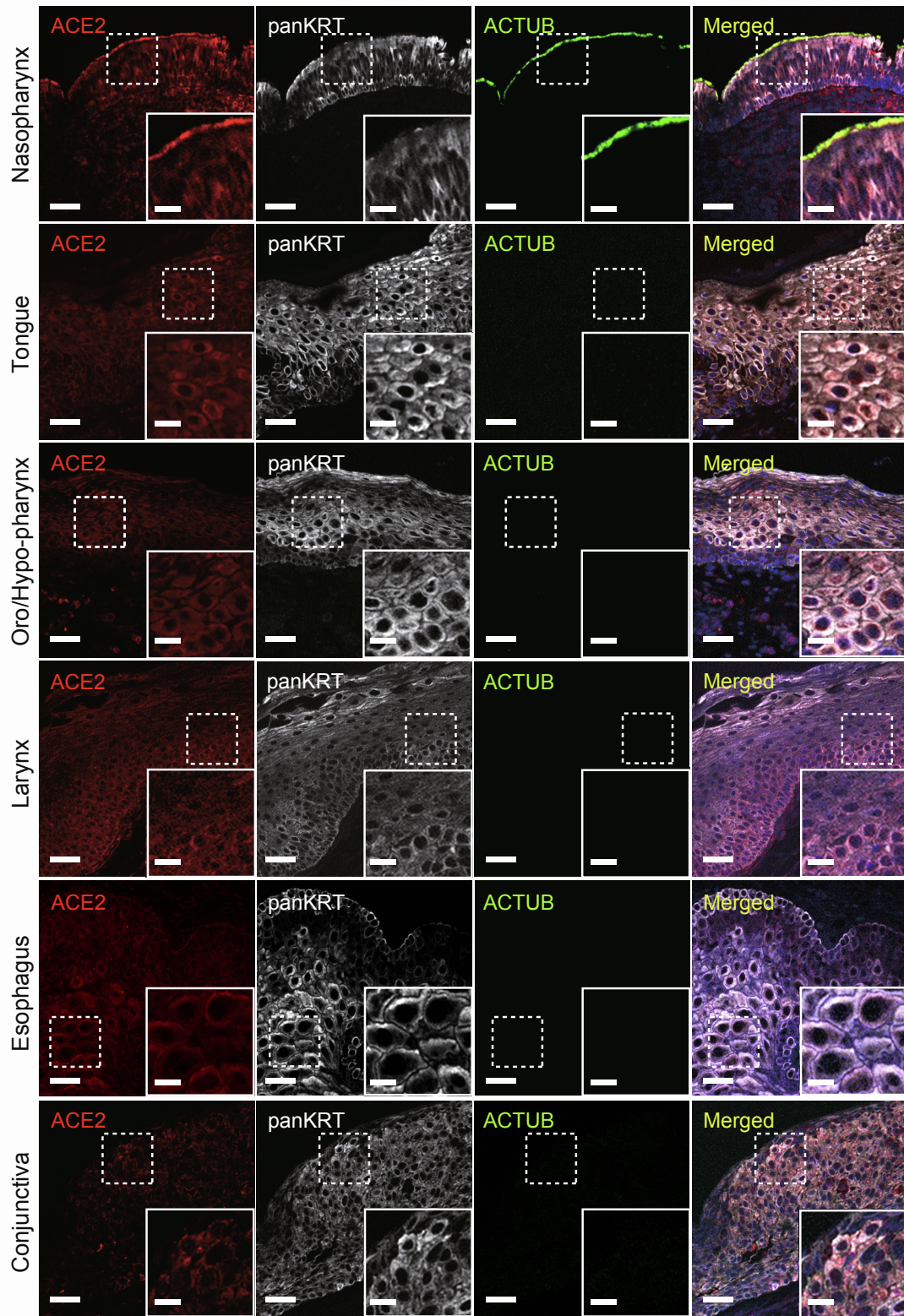
**E**



**Figure S1. Integration of 8 head & neck tissue sources for single-cell gene expression analysis. Related to Figure 1. (A)** Dot plot of the gene markers used to identify epithelial cell types of interest based on Ravindra et al. 2020. **(B)** An integrated UMAP representation of the combined data (Figure 1B) as divided by the annotated cell type. **(C)** RNA expression levels of *TMPRSS4*, *TFRC*, and *NRPI* in each cell type in epithelia. **(D)** RNA expression levels of *TMPRSS4*, *TFRC*, and *NRPI* in the 8 tissue sources. **(E)** RNA expression levels of *TMPRSS4*, *TFRC*, and *NRPI* in each cell type across the 8 tissue sources.



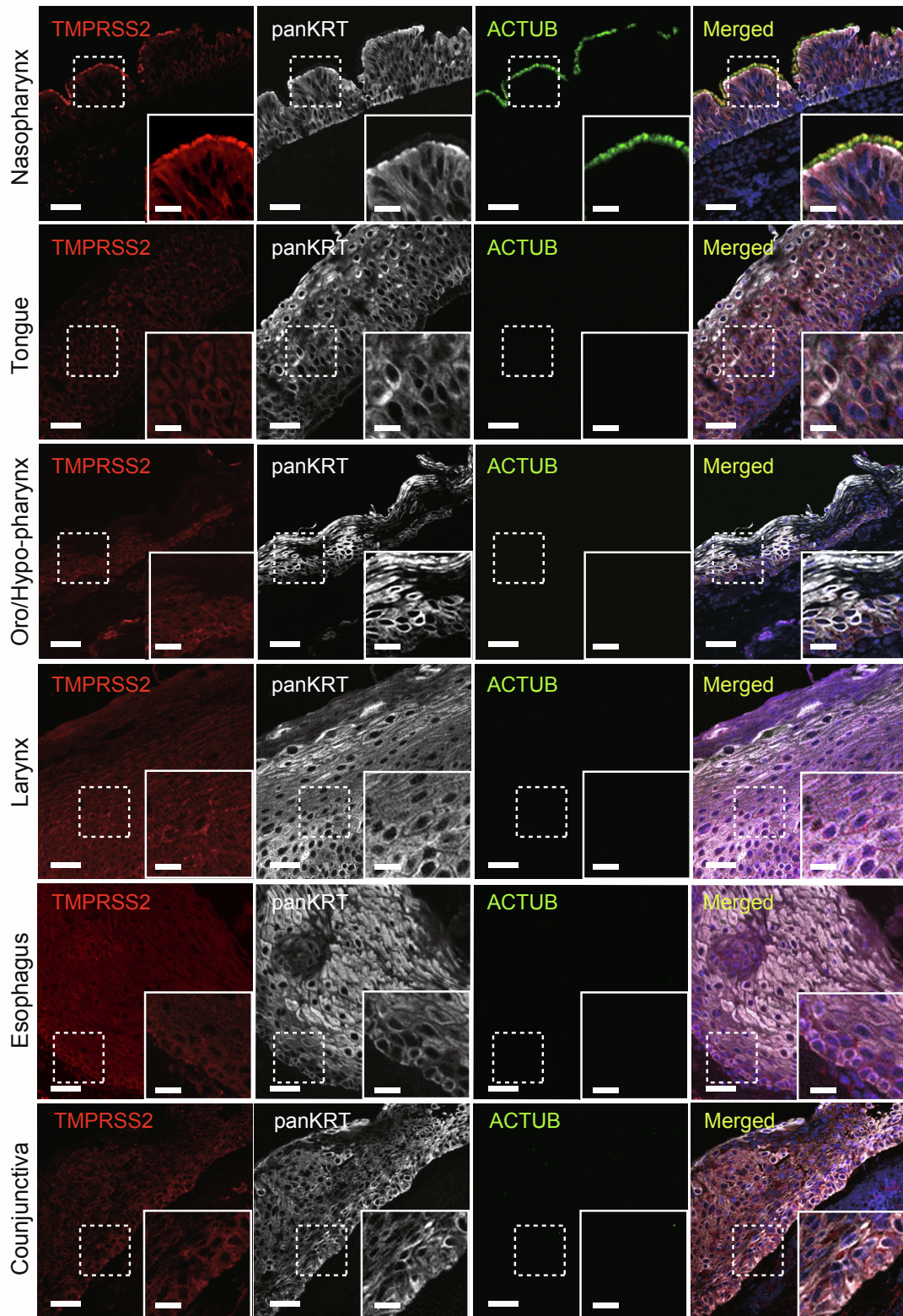
Figure S2



**Figure S2. ACE2 protein expression in normal human head & neck tissues. Related to Figure 3.** Representative immunofluorescence staining of ACE2, pan-cytokeratin (panKRT), and acetylated  $\alpha$ -tubulin (ACTUB) in normal human nasopharynx, tongue, oro/hypo-pharynx, larynx, esophagus, and conjunctiva. The nuclei were stained using Hoechst (blue) as a counterstain. ACTUB signal (green) is only present in tissues shown here that physiologically contain motile cilia (i.e. nasopharynx). Scale bars: 50  $\mu$ m and 20  $\mu$ m (inset images).



Figure S3

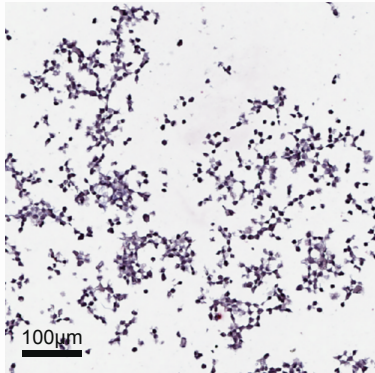


**Figure S3. TMPRSS2 protein expression in normal human head & neck tissues. Related to Figure 3.** Representative immunofluorescence staining of TMPRSS2, pan-cytokeratin (panKRT), and acetylated  $\alpha$ -tubulin (ACTUB) in normal human nasopharynx, tongue, oro/hypo-pharynx, larynx, esophagus, and conjunctiva. The nuclei were stained using Hoechst (blue) as a counterstain. ACTUB signal (green) is only present in tissues shown here that physiologically contain motile cilia (i.e. nasopharynx). Scale bars: 50  $\mu$ m and 20  $\mu$ m (insets).

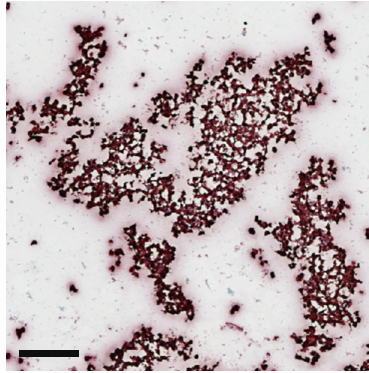
**Figure S4**

**A**

Vero E6  
Mock infected

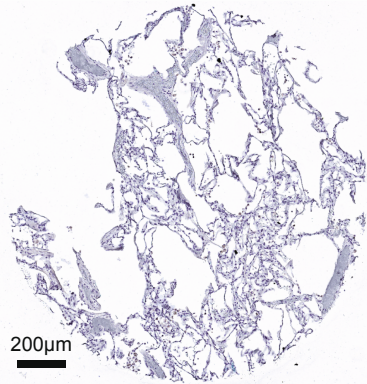


Vero E6  
SARS-CoV-2 infected

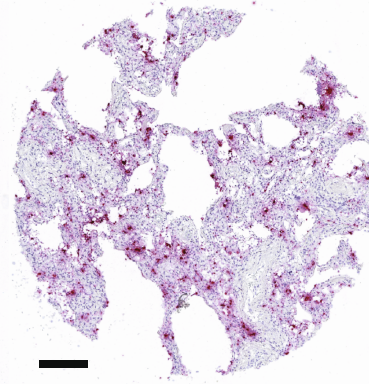


**B**

Lung  
Non-COVID-19

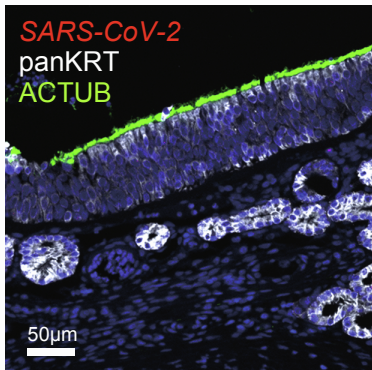


Lung  
COVID-19

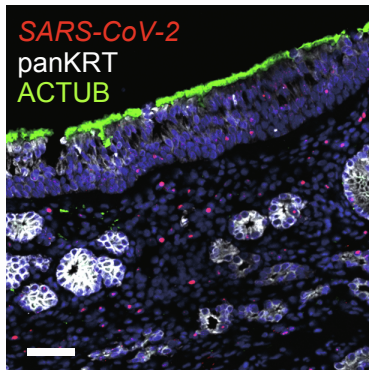


**C**

Nasal  
Non-COVID-19



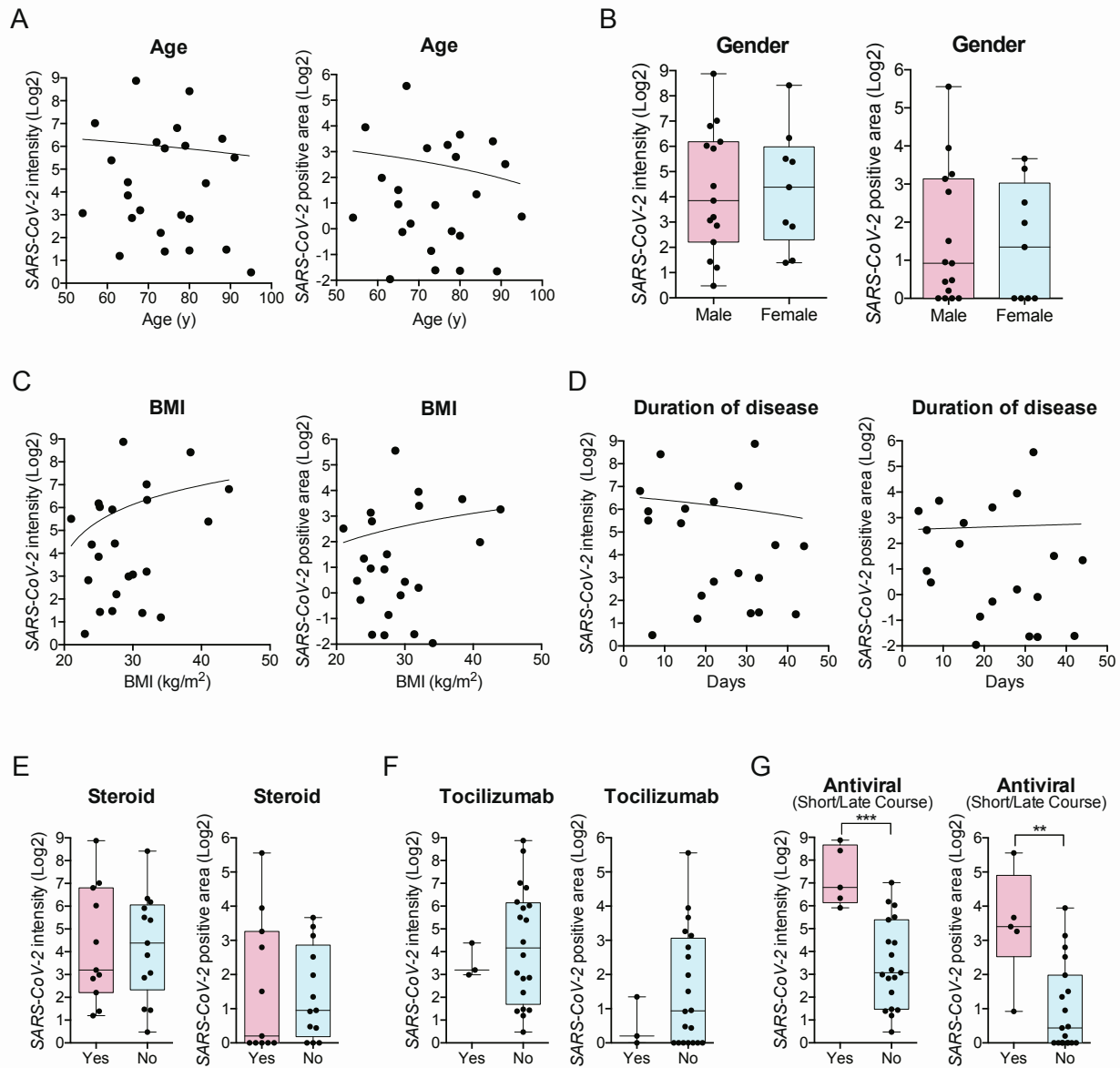
Nasal  
COVID-19



**Figure S4: SARS-CoV-2 Spike mRNA probe and assay validation. Related to Figure 4.** (A) SARS-CoV-2 Spike mRNA signal was detected in Vero E6 Formalin Fixed Paraffin Embedded (FFPE) cell pellets that were infected with SARS-CoV-2 (right). No signal was noted in the mock-treated pellets (left). (B) SARS-CoV-2 Spike mRNA probe detected signal only in lung autopsy tissue from patients with COVID-19 (right) and not in lung parenchyma from non-COVID controls (left). (C) SARS-CoV-2 Spike mRNA probe detected signal only in nasal autopsy tissues from patients with COVID-19 (right) and not in nasal tissues from non-COVID controls (left).



**Figure S5**



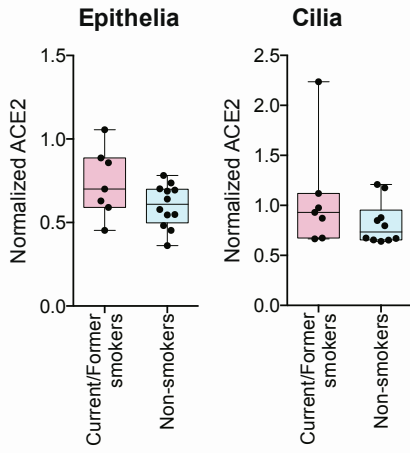
**Figure S5. Comparison of *SARS-CoV-2 Spike* mRNA epithelial expression by patient demographics, comorbidities, and therapeutic medications. Related to Figure 6. (A)** No statistically significant correlation was observed between age and *SARS-CoV-2 Spike* mRNA intensity (left)/positive area (right). The solid lines represent results from simple linear regression (n=24) (Left panel:  $y = 121.2 - 0.7731x$ ;  $R^2 = 0.0055$ ,  $p = 0.7304$ , F test. Right panel:  $y = 14.48 - 0.1174x$ ;  $R^2 = 0.0167$ ,  $p = 0.5476$ , F test). **(B)** No statistically significant differences were seen in *SARS-CoV-2 Spike* mRNA intensity (left)/positive area (right) between males and females (n=15 and n=9, respectively) (Two-tailed Mann-Whitney test,  $p = 0.9534$  and  $0.9070$ , respectively). **(C)** No statistically significant correlation was observed between body mass index (BMI) and *SARS-CoV-2 Spike* mRNA intensity (left)/positive area (right). The solid line represents results from simple linear regression (n=23) (Left panel:  $y = -96.88 + 5.568x$ ,  $R^2 = 0.0797$ ,  $p = 0.1920$ , F test. Right panel:  $y = -1.292 + 0.2482x$ ;  $R^2 = 0.0208$ ,  $p = 0.5118$ , F test). **(D)** No statistically significant correlation was observed between duration of disease (days from initial symptom to death) and *SARS-CoV-2 Spike* mRNA intensity (left)/positive area (right). The solid line represents results from simple linear regression

(n=20) (Left panel:  $y = 95.59 - 1.074x$ ,  $R^2 = 0.0121$ ,  $p = 0.6446$ , F test. Right panel:  $y = 5.763 + 0.0226x$ ;  $R^2 = 0.0007$ ,  $p = 0.9116$ , F test). (E) No statistically significant differences were seen in *SARS-CoV-2 Spike* mRNA intensity (left) or positive area (right) among patients who received or did not receive systemic steroids during hospitalization for COVID-19 (n=11 and n=13, respectively) (Two-tailed Mann-Whitney test,  $p > 0.9999$  and  $p = 0.8201$ , respectively). (F) *SARS-CoV-2 Spike* mRNA intensity (left) and positive area (right) are not statistically significantly different among patients who received tocilizumab compared to patients who did not receive IL-6 blockade during hospitalization for COVID-19 (n=3 and n=20, respectively) (Two-tailed Mann-Whitney test,  $***p = 0.8300$  and  $p = 0.6979$ , respectively). (G) *SARS-CoV-2 Spike* mRNA intensity (left) and positive area (right) are statistically significantly higher in patients who received incomplete and/or compassionate late courses of intravenous antivirals (remdesivir or lopinavir-ritonavir) compared to patients who did not receive antivirals during hospitalization for COVID-19 (n=5 and n=19, respectively) (Two-tailed Mann-Whitney test,  $***p = 0.0009$  and  $**p = 0.0071$ , respectively). The bands within the box plot show the median value. The bottom and top of the box plots represent the 25th and 75th percentiles, respectively. The whiskers extending from both ends of the boxes are minimum and maximum values. Each dot represents one patient.

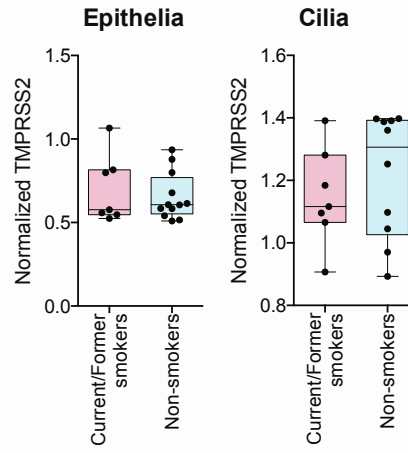


Figure S6

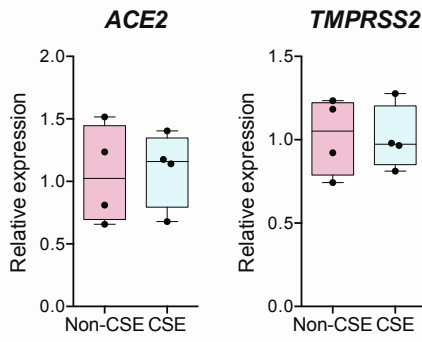
A



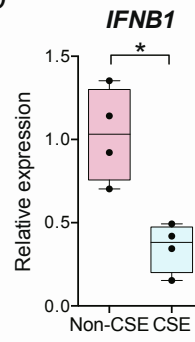
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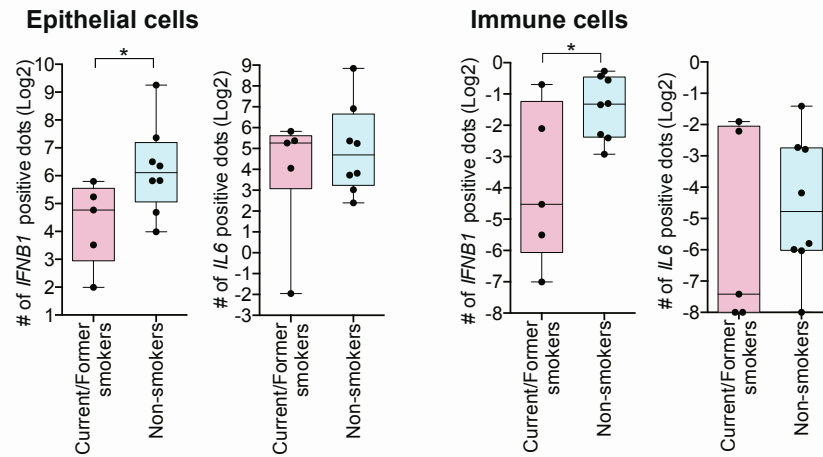
C



D



E



**Figure S6: Smoking does not affect SARS-CoV-2 entry factors, but reduces interferon- $\beta$ 1 response in SARS-CoV-2 infection. Related to Figure 7.** (A) Quantification of ACE2 in the epithelia and cilia in airway mucosal tissues from patients with COVID-19. ACE2 protein expression was not statistically significantly different in the epithelia and cilia between smokers (current/former) and non-smokers. (Two-tailed Mann-Whitney test,  $p = 0.1956$  and  $p = 0.2295$ , respectively). (B) Quantification of TMPRSS2 in the epithelia and cilia in airway mucosal tissues from COVID-19 patients. TMPRSS2 protein expression was not statistically significantly different in the epithelia and cilia between smokers (current/former) and non-smokers. (Two-tailed Mann-Whitney test,  $p = 0.5362$  and  $p > 0.9999$ , respectively). (C) Cigarette smoke extract (CSE) exposure does not affect expression of *ACE2* and *TMPRSS2* in SARS-CoV-2 infected air-liquid interface cultured human nasal epithelial cells by qPCR ( $n=4$ ) (One-tailed Mann-Whitney test,  $p = 0.5000$  and  $p = 0.4429$ , respectively). (D) Cigarette smoke extract (CSE) exposure suppresses *IFNB1* production in air-liquid interface cultured human nasal epithelial cells by qPCR ( $n=4$ ) (One-tailed Mann-Whitney test,  $p = 0.0143$ ). (E) The number of *IFNB1* dots in the epithelial cells and CD45<sup>+</sup> cells are significantly higher in non-smokers compared to smokers (current/former) ( $n=8$  and  $n=5$ , respectively) (One-tailed Mann-Whitney test,  $*p = 0.0225$  and  $*p = 0.0466$ , respectively). No statistically significant differences were seen in *IL6* in the epithelial cells and CD45<sup>+</sup> cells between both groups (One-tailed Mann-Whitney test,  $p = 0.3092$  and  $p = 0.4716$ , respectively). Each dot represents one individual sample.

**Table S1. COVID-19 patient cohort demographics, comorbidities, and therapeutic medication use. Related to Fig. 6 and S5.**

	Age (yrs)	Sex	Body mass index (kg/m <sup>2</sup> )	Smoking	Diabetes (pre-existing)	Hypertension (pre-existing)	Days from symptom onset	Systemic steroids	Tocilizumab	Antiviral
1	88	Female	32	Unknown	No	Yes	22	No	Unknown	Remdesivir (2 doses starting on day 21 of symptoms)
2	65	Male	27	Current	No	No	37	Yes	No	No
3	80	Female	38	Never	No	Yes	9	No	No	Remdesivir (2 doses starting on day 8 of symptoms)
4	57	Male	32	Current	Yes	Yes	28	Yes	No	No
5	91	Female	21	Never	No	No	6	No	No	No
6	65	Male	25	Never	Yes	No	Unknown	No	No	No
7	67	Male	29	Former	No	No	32	Yes	No	Remdesivir (5 doses starting on day 9 of symptoms)
8	54	Male	30	Never	No	No	Unknown	No	No	No
9*	53	Male	49	Unknown	No	No	Unknown	Unknown	Unknown	Unknown
10	61	Female	41	Unknown	Yes	No	14	No	No	No
11	72	Male	25	Former	No	Yes	Unknown	No	No	No
12*	96	Male	25	Unknown	No	Yes	18	Unknown	Unknown	Unknown
13	89	Female	27	Unknown	Yes	Yes	33	No	No	No
14	84	Female	24	Unknown	Yes	Yes	44	No	Yes	No
15	95	Male	23	Never	No	Yes	7	No	No	No
16	77	Male	44	Former	Yes	Yes	4	Yes	No	Lopinavir/Ritonavir (2 doses starting on day 4 of symptoms)
17	74	Male	27	Former	Yes	Yes	6	No	No	Lopinavir/Ritonavir (2 doses starting on day 4 of symptoms)
18*	88	Male	28	Never	No	No	16	Unknown	Yes	Unknown
19	79	Male	25	Never	Yes	Yes	15	Yes	No	No
20	66	Male	Unknown	Never	No	Unknown	Unknown	No	No	No
21	73	Male	28	Never	Yes	Yes	19	Yes	No	No
22	78	Female	29	Never	Yes	Yes	33	Yes	Yes	No
23	80	Female	24	Never	No	Yes	22	Yes	No	No
24	80	Male	25	Never	No	Yes	31	No	No	No
25	63	Male	34	Never	No	No	18	Yes	No	No
26	68	Male	32	Never	No	Yes	28	Yes	Yes	No
27	74	Female	31	Never	No	No	42	Yes	No	No

\*Patients not included in analyses of association between viral replication and clinical characteristics (Fig. 6 and S5) because nasal/tracheal samples were not available from these patients. See Results section for more details.