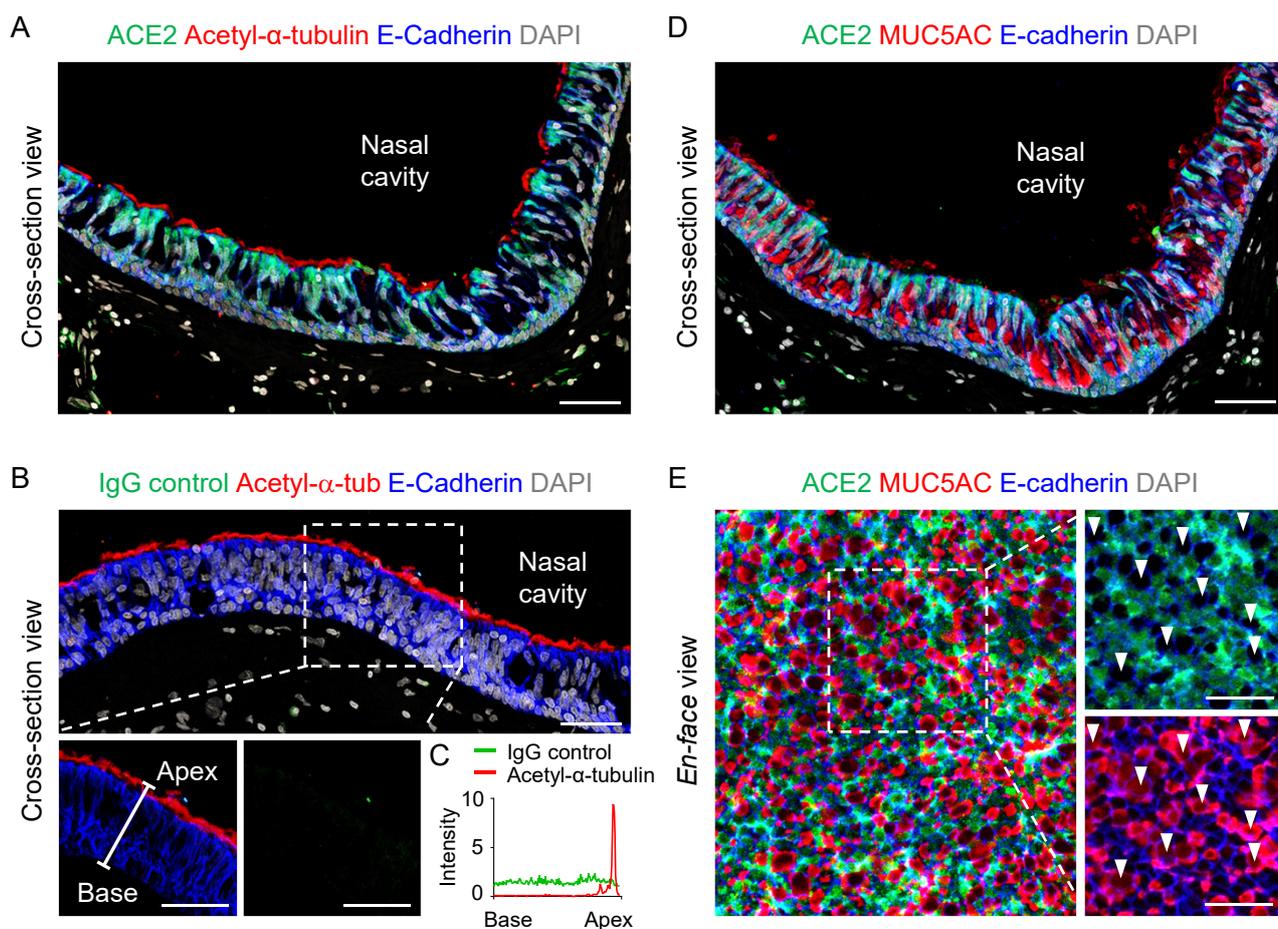


[Supplemental Data](#)

Supplemental Figures and Legends (1-9)

Nasal Ciliated Cells Are Primary Targets for SARS-CoV-2 Replication in Early Stage of COVID-19

Ji Hoon Ahn, JungMo Kim, Seon Pyo Hong, Sung Yong Choi, Myung Jin Yang, Young Seok Ju, Young Tae Kim, Ho Min Kim, MD Tazikur Rahman, Man Ki Chung, Sang Duk Hong, Hosung Bae, Chang-Seop Lee and Gou Young Koh



Supplemental Figure 1. ACE2 protein is highly present in apical side of ciliated, but not in goblet, cells of human nasal epithelium

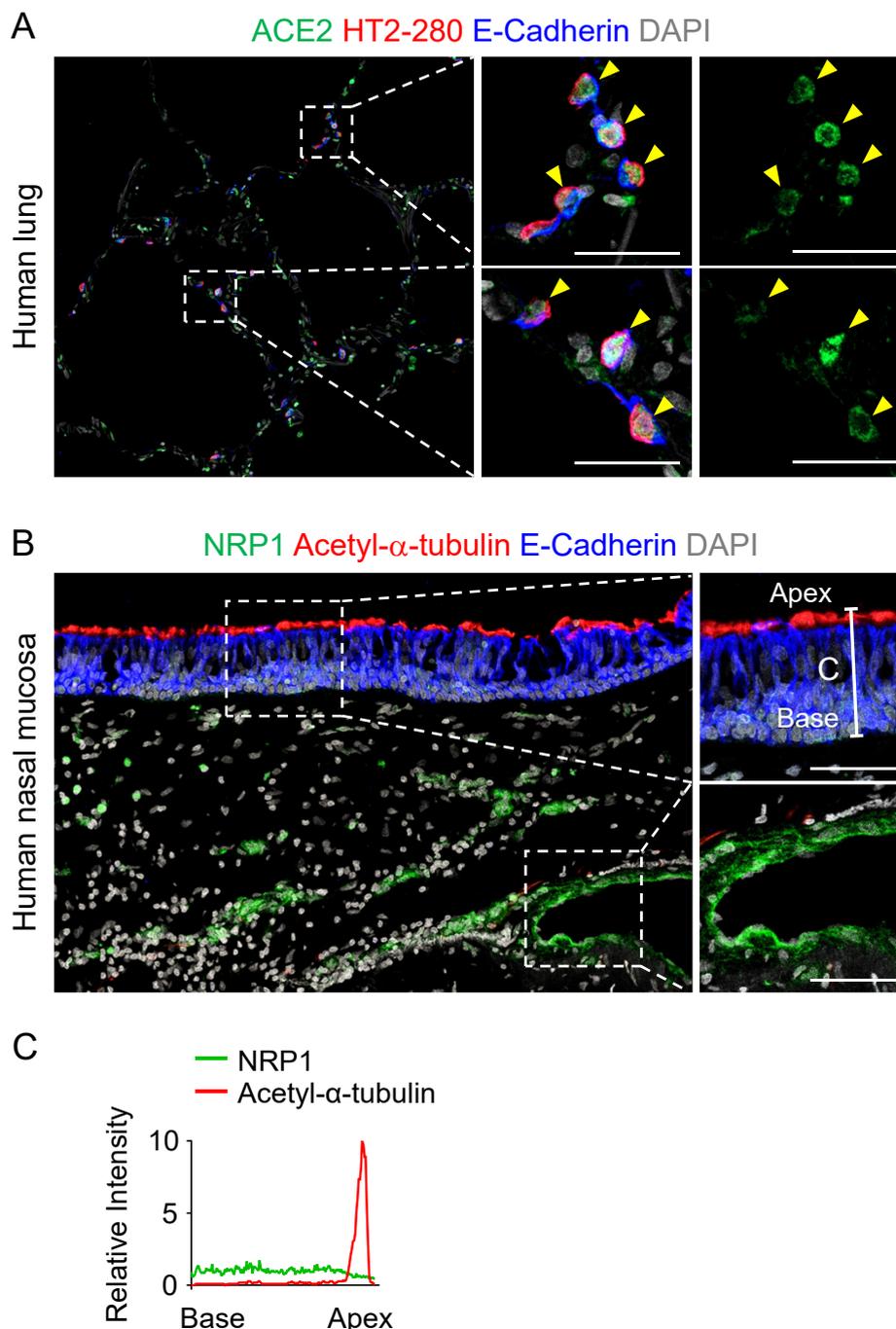
(A) Representative image of cross-sectional view of human nasal epithelium showing robust ACE2 protein in acetylated- α -tubulin⁺ ciliated epithelium. Scale bar, 50 μ m.

(B and C) Representative images of cross-sectional view of human nasal epithelium showing no signal of IgG isotype control antibody. Box region of upper panel is highlighted in lower panels. Scale bars, 50 μ m. (C) Profile analysis of relative IgG isotype signal intensity along the white line in (B).

(D) Representative image of cross-sectional view of human nasal epithelium showing ACE2 is not distinctly detected in MUC5AC⁺ goblet cells. Scale bar, 50 μ m.

(E) *En-face* view of nasal epithelium showing ACE2 protein signal is not detected in MUC5AC⁺ goblet cells (white arrowheads). Box region is magnified in right panels. Scale bars, 25 μ m.

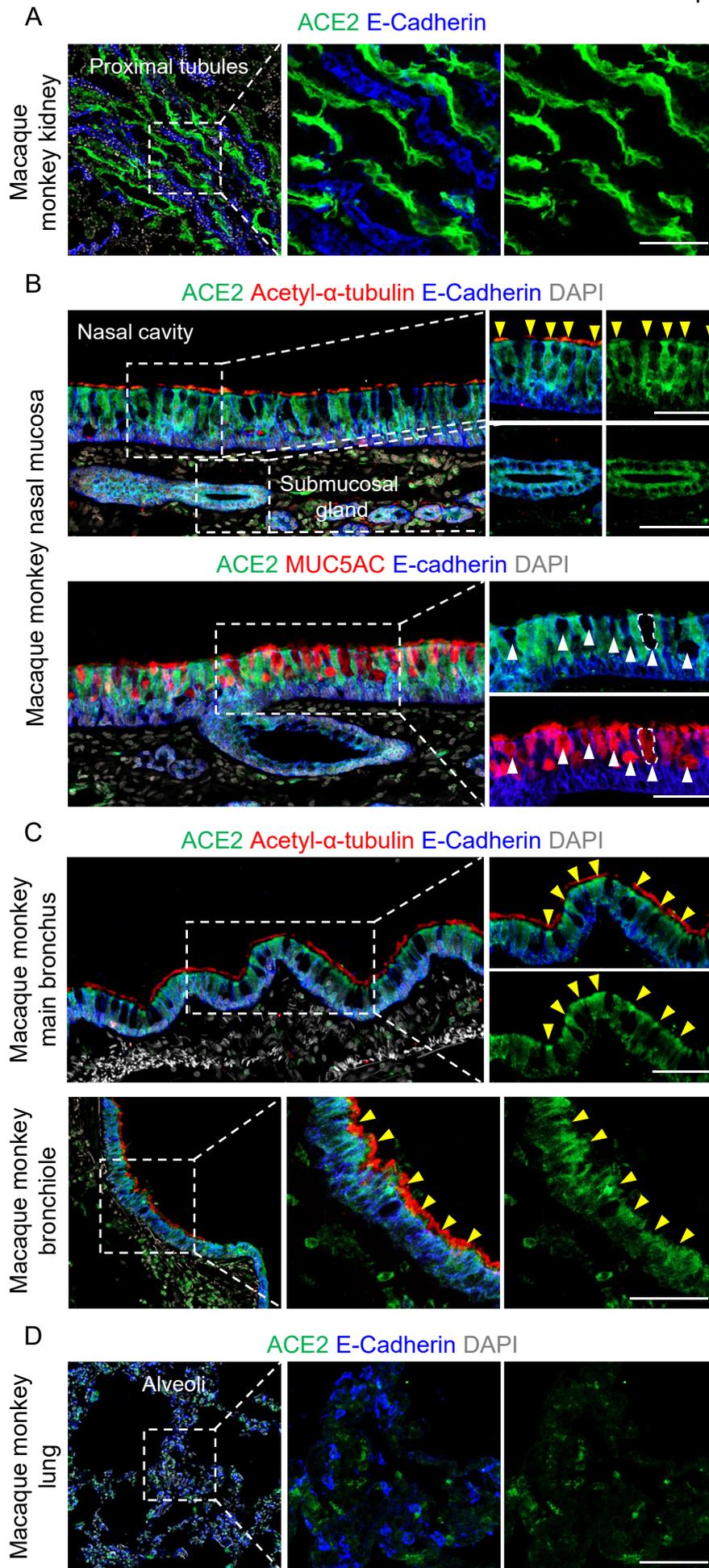
(A–E) Similar findings were observed in $n = 3$ human normal tissues from three independent experiments.



Supplemental Figure 2. Localization of ACE2 protein in human lung and NRP1 in human nasal mucosa epithelium

(A) Representative images of ACE2 protein in the HT2-280⁺/E-cadherin⁺ AT2 cells (yellow arrowheads) of human lung. Each box region is magnified and displayed as right two panels. All scale bars, 50 μ m. Similar findings were observed in $n = 2$ normal portions of human lungs from two independent experiments.

(B and C) Representative images of cross-sectional view of human nasal epithelium showing that NRP1 protein is not detected in ciliated epithelium (upper box region) but detected in vascular endothelium (lower box region). Box regions are magnified as right two panels. Scale bars, 50 μ m. Profile analysis of NRP1 protein signal intensity along the white line in right upper panel of (B). Similar findings were observed in $n = 3$ human normal tissues from three independent experiments.



Supplemental Figure 3. Conserved expression and localization of ACE2 protein in ciliated epithelium across respiratory tract of normal macaque monkey

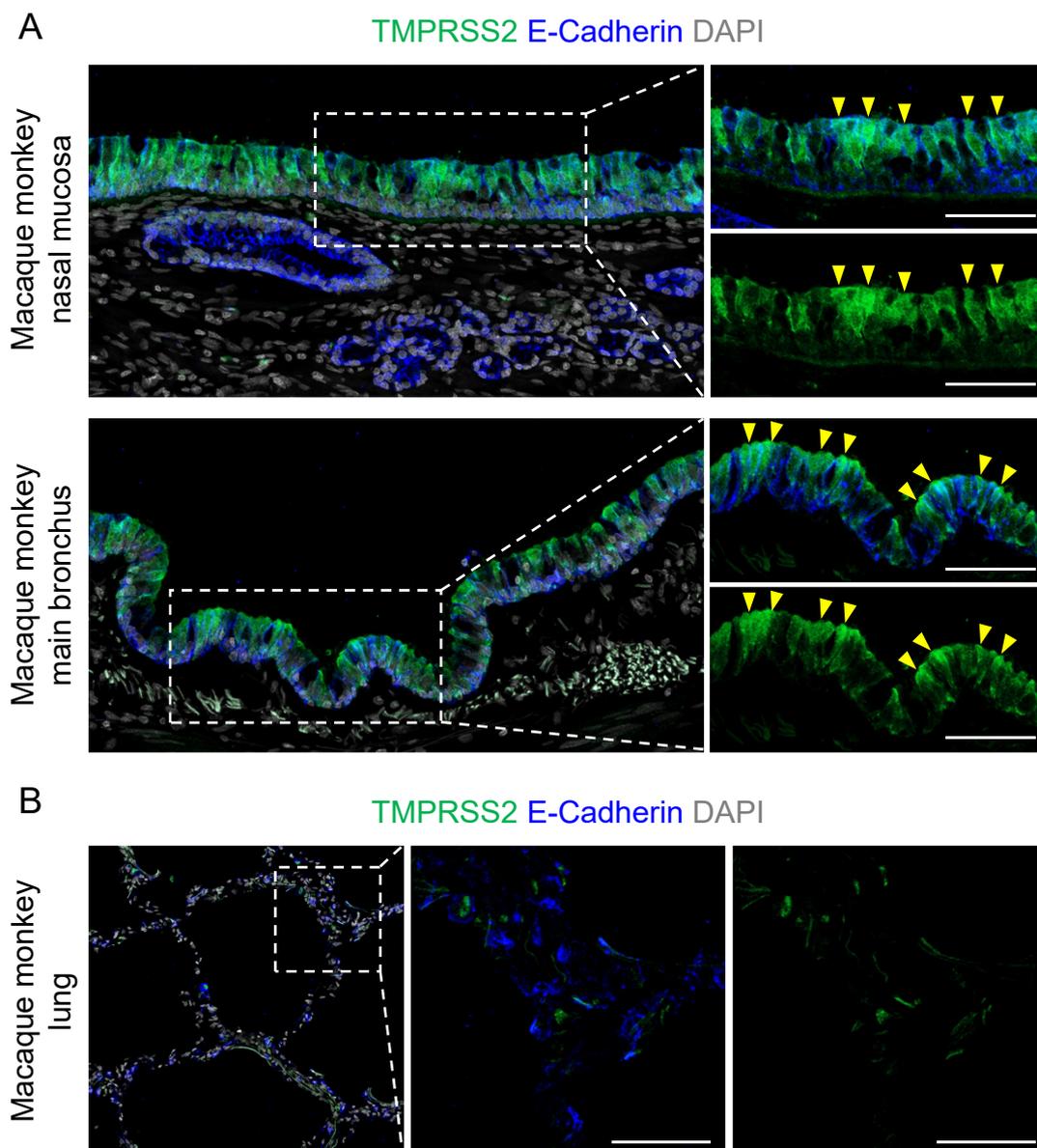
(A) Representative images showing robust ACE2 protein in proximal tubules of kidney.

(B) Representative images of nasal mucosal epithelium showing high ACE2 protein in acetylated- α -tubulin⁺ ciliated cells (yellow arrowheads) and submucosal glandular cells, but not in goblet cells (white arrowheads and dashed-lined circle).

(C) Representative images showing high ACE2 protein in ciliated epithelium of lower respiratory tract including bronchus and bronchiole (yellow arrowheads).

(D) Representative images showing relatively low ACE2 protein in lung.

(A-D) Each box region is magnified and displayed as right panels. All scale bars, 50 μ m. Similar findings were observed in $n = 2$ macaque monkeys from two independent experiments.



Supplemental Figure 4. Localization of TMPRSS2 protein in respiratory epithelium in macaque monkey

(A) Representative images showing high TMPRSS2 in nasal respiratory epithelium and main bronchus (yellow arrowheads). Scale bars, 50 μm .

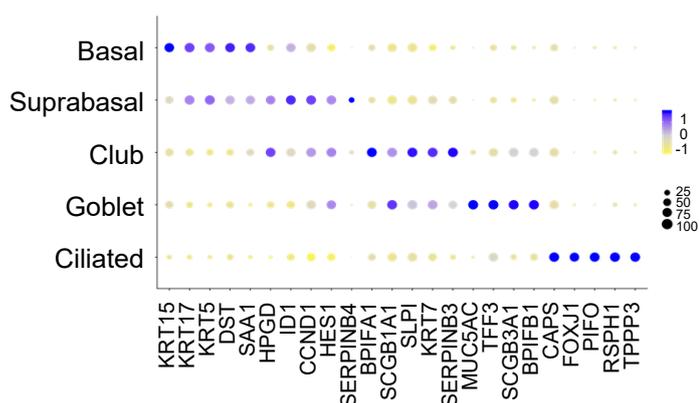
(B) Representative images showing relatively low TMPRSS2 in lung. Scale bars, 50 μm .

(A and B) Each box region is magnified and displayed as right sided panels. Similar findings were observed in $n = 2$ macaque monkeys from two independent experiments.

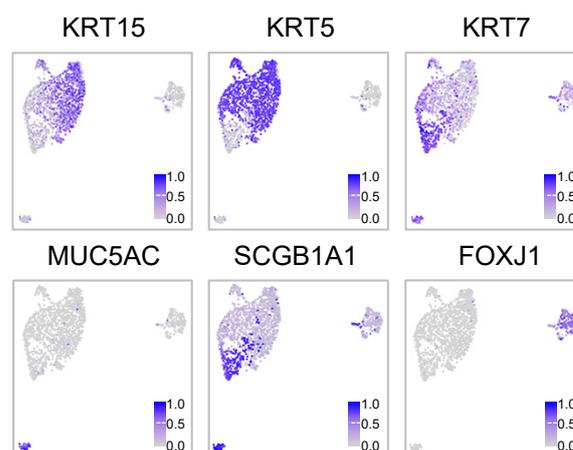
A

<i>Types of epithelial cell</i>	<i>Markers</i>
Basal cell	KRT15 (Keratin 15) KRT5 (Keratin 5)
Suprabasal cell	SERPINB4 (Serpin Family B Member 4) KRT5 (Keratin 5)
Club cell	KRT7 (Keratin 7) SCGB1A1 (Secretoglobin family 1A member 1)
Goblet cell	MUC5AC (Mucin 5AC) KRT7 (Keratin 7) SCGB1A1 (Secretoglobin family 1A member 1)
Multiciliated cell	FOXJ1 (Forkhead Box J1) Acetylated- α -tubulin

B



C

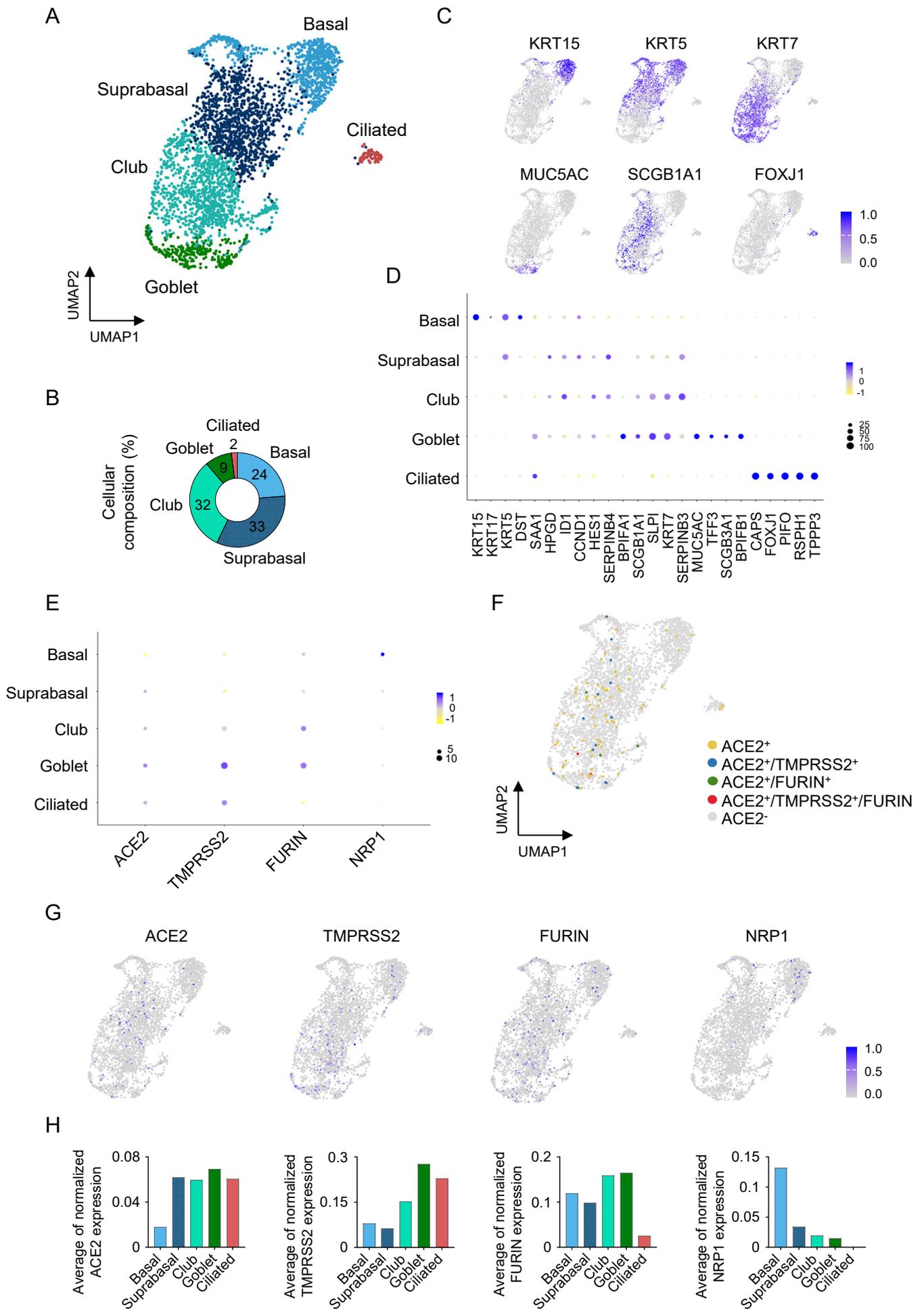


Supplemental Figure 5. Differentially expressed genes and annotation markers for human nasal epithelial cell clusters.

(A) Representative each specific markers for nasal epithelial cells.

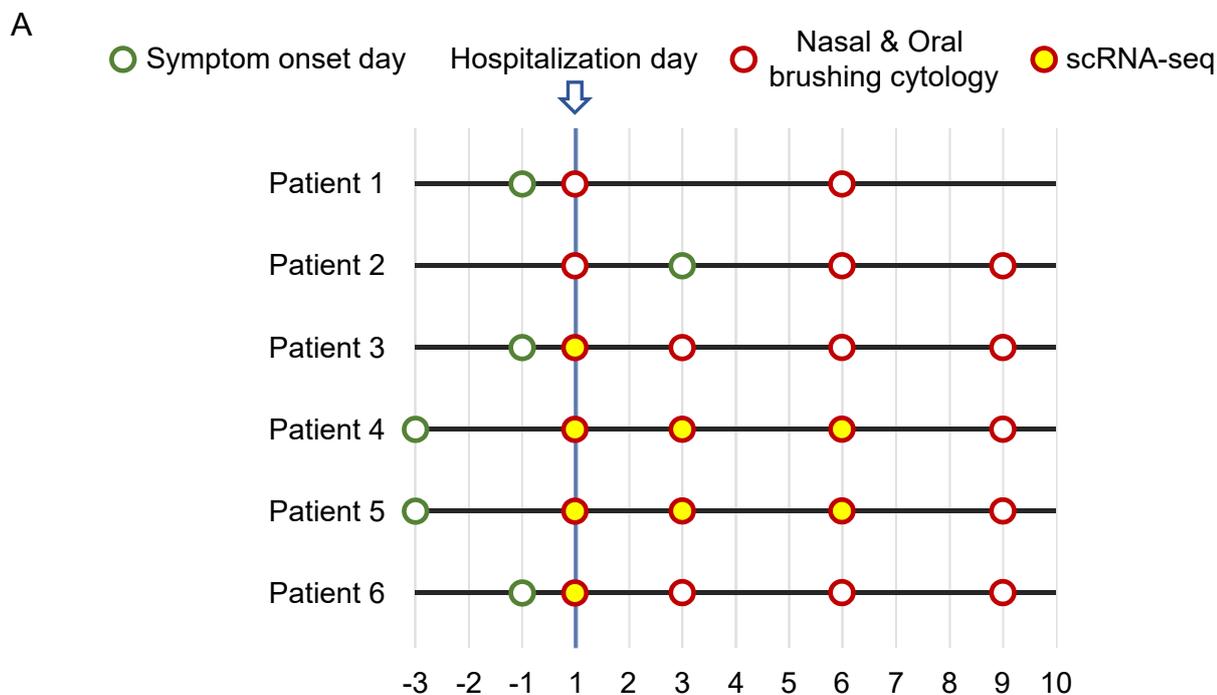
(B) Dot plot heatmap displaying differentially expressed genes per cluster in human nasal epithelial cells.

(C) Feature plots delineating normalized mRNA expression levels of key annotation markers.



Supplemental Figure 6. Low and sparse mRNA expressions of SARS-CoV-2 entry-related host molecule genes revealed by scRNA-seq of public human nasal epithelial tissue data

- (A) Unsupervised clustering projected on 2D UMAP plot using public scRNA-seq dataset of human nasal mucosal epithelial cells.
- (B) Donut plot exhibiting cellular composition of pooled and clustered ~3,415 epithelial cells.
- (C) Feature plots delineating normalized mRNA expression levels of key annotation markers.
- (D) Dot plot heatmap displaying differentially expressed genes per cluster.
- (E) Dot plot heatmap displaying expression of SARS-CoV-2 entry molecule genes per cluster.
- (F) UMAP plot showing distribution of ACE2⁺, ACE2⁺/TMPRSS2⁺, ACE2⁺/FURIN⁺, ACE2⁺/TMPRSS2⁺/FURIN⁺ and ACE2⁻ cells across all clustered epithelial cells.
- (G) Feature plots delineating normalized mRNA expression levels of indicated SARS-CoV-2 entry molecule genes.
- (H) Comparison of average of normalized expression levels of SARS-CoV-2 entry molecule genes per cluster.



B

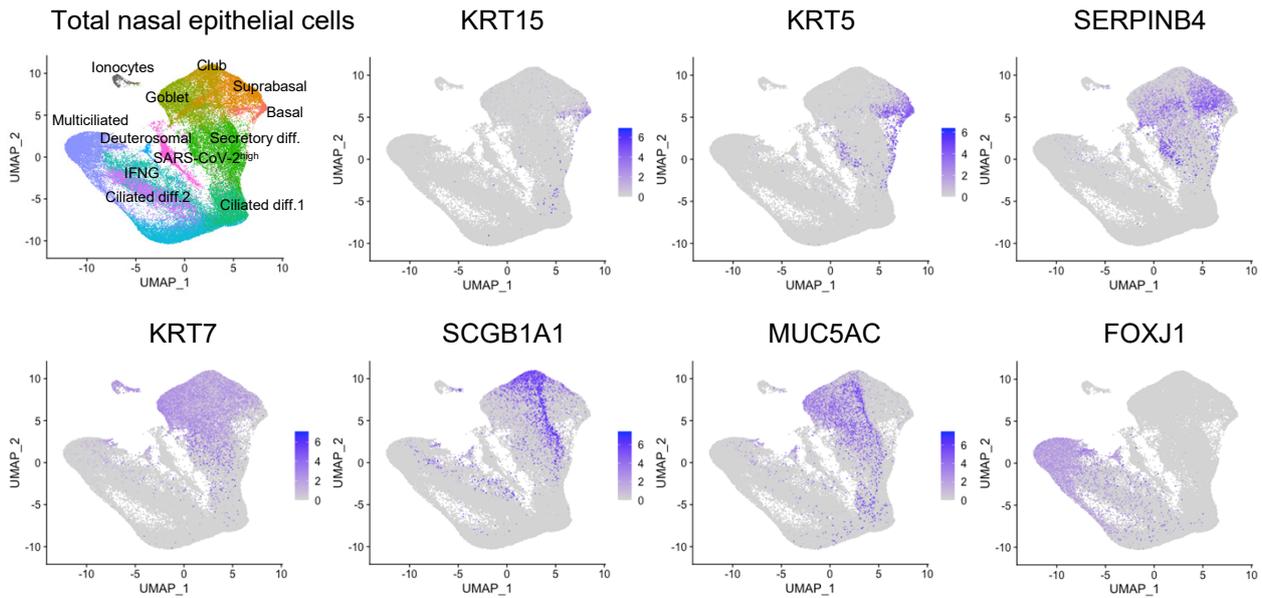
Patient No.	1	2	3	4	5	6
Age	43	38	55	55	38	31
Sex	M	M	M	F	F	M
Symptoms						
Fever	+	-	+	-	-	-
Myalgia	-	-	+	+	+	-
Anosmia	-	-	-	-	-	-
Headache	-	-	-	-	+	+
Sore throat	-	+	-	-	+	-
Rhinorrhea	-	+	-	-	-	-
Cough	-	+	-	+	-	-
Underlying disease						
Hypertension	-	-	-	-	-	-
Diabetes mellitus	-	-	+	-	-	-
Pneumonia	-	-	+	-	-	-
Outcome	Recovered	Recovered	Recovered	Recovered	Recovered	Recovered

Supplemental Figure 7. Hospitalization, procedures and general characteristics of COVID-19 patients for this study

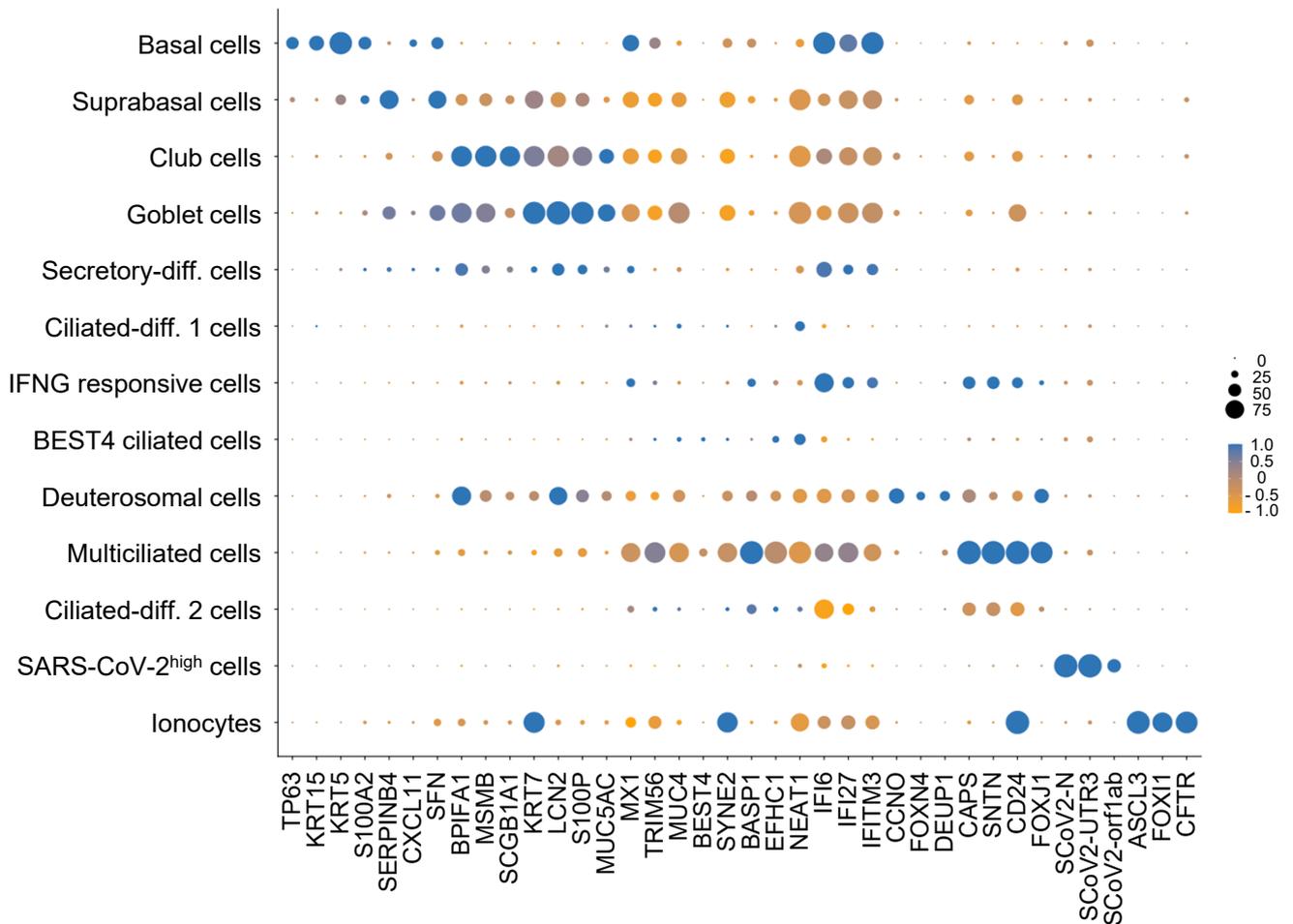
(A) Diagram depicting days of hospitalization, symptom onset, and sampling in each COVID-19 patient, and their performed analyses. COVID-19 was confirmed by the RT-PCR diagnostic method on the first day of hospitalization.

(B) General characteristics of COVID-19 patients. All patients had a mild to moderate course of COVID-19 without intubation or ICU admission.

A



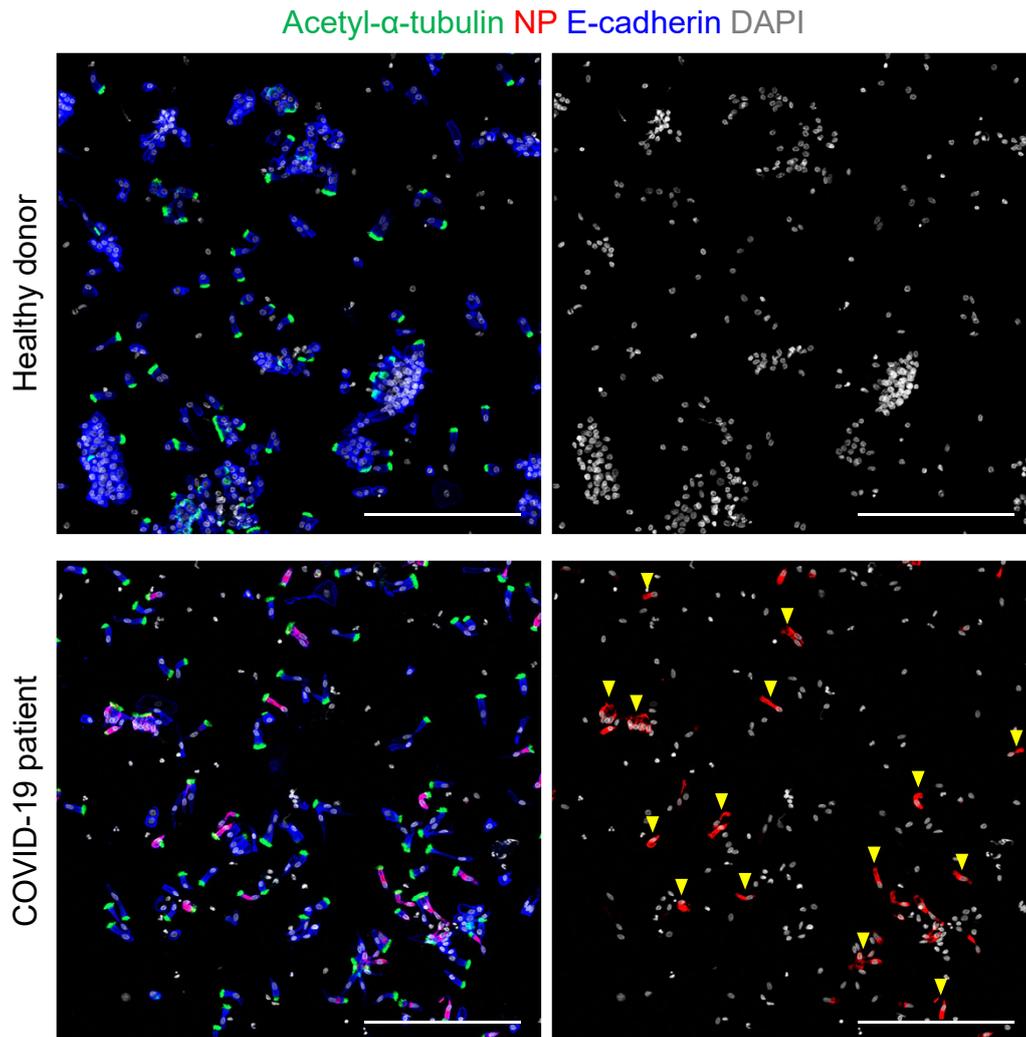
B



Supplemental Figure 8. Differentially expressed genes and annotation markers in total nasal epithelial cell clusters from healthy controls and COVID-19 patients

(A) Feature plots delineating normalized mRNA expression levels of key annotation markers to define major epithelial cell types.

(B) Dot plot heatmap displaying differentially expressed genes and annotation markers in each cell cluster.



Supplemental Figure 9. SARS-CoV-2 NP is detected in nasal epithelial cells of COVID-19 patients (1st day of hospitalization) but is not detected in those of healthy donors

Representative images of smeared nasal cells showing that SARS-CoV-2 NP is detected in acetylated- α -tubulin⁺ multiciliated epithelial cells of COVID-19 patients (yellow arrowheads) but is undetected in those of healthy donor. Scale bars, 200 μ m. Identical finding was observed in $n = 6$ COVID-19 patients and $n = 5$ healthy donors.