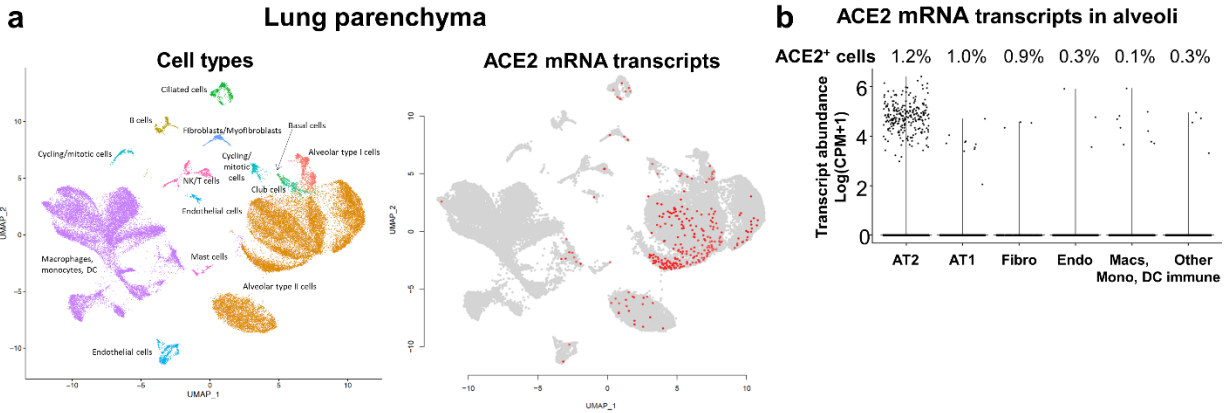


611 **Supplemental information:**

Supplemental Figure 1



612

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

614 in lung parenchyma (26). Summative observations from all donors. **a)** Uniform manifold

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

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

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

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

619 the alveoli. Airway cells (basal, mitotic, ciliated, club) are not shown. Percentage of ACE2⁺ cells

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

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

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

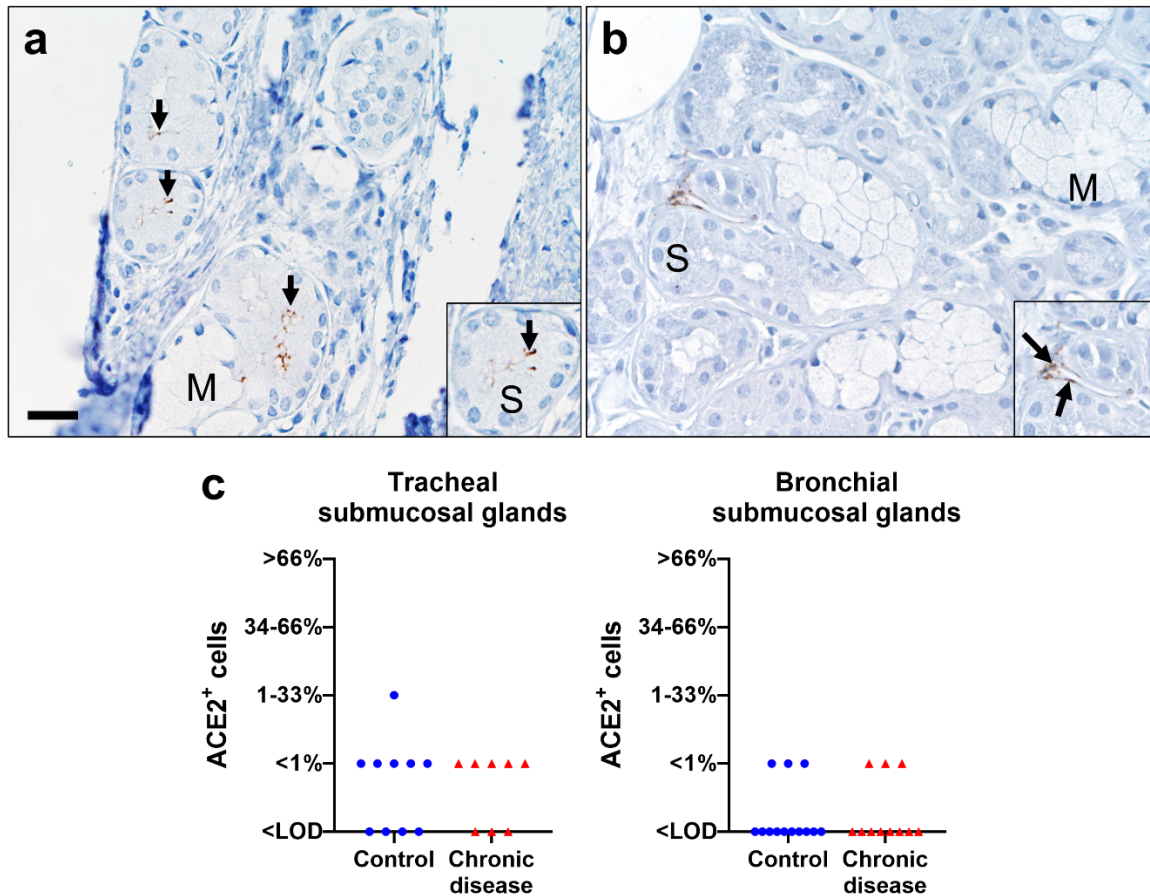
623 Monocytes. DC: dendritic cells. Other immune cells: B cells, mast cells, natural killer/T cells.

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

625 million.

626

Supplemental Figure 2



627

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

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

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

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

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

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

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

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

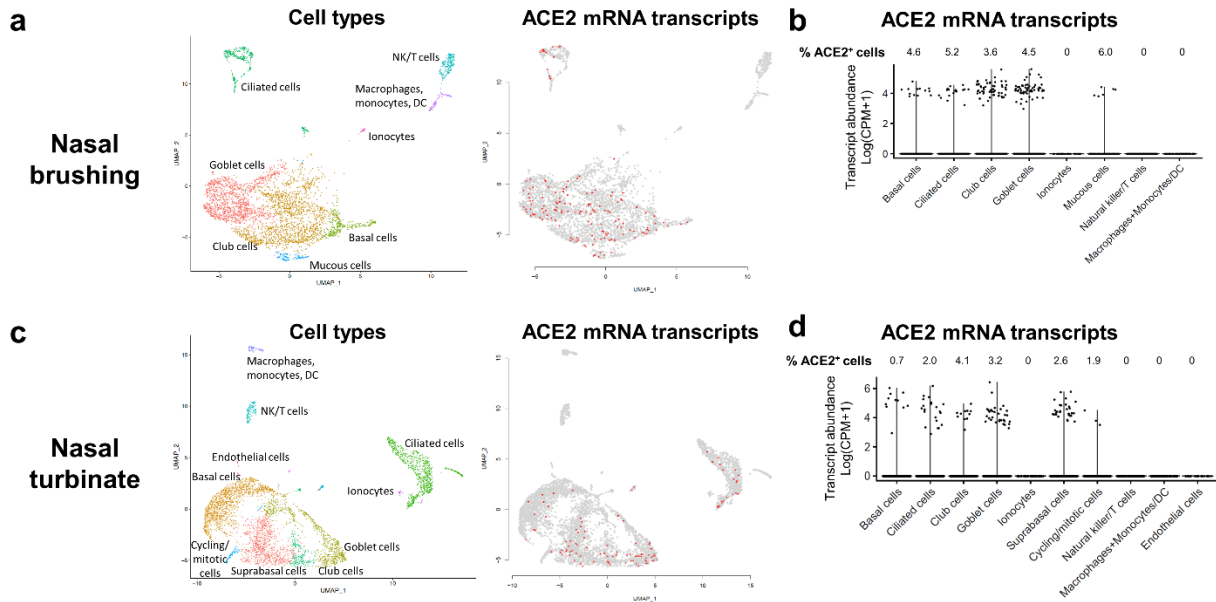
636 subject for serous cells in submucosal glands from trachea and bronchi, in control versus chronic

637 disease groups ($P > 0.9999$, 0.9999 , respectively, Mann-Whitney U test). Bar = 25 μm . LOD:

638 Limit of detection.

639

Supplemental Figure 3

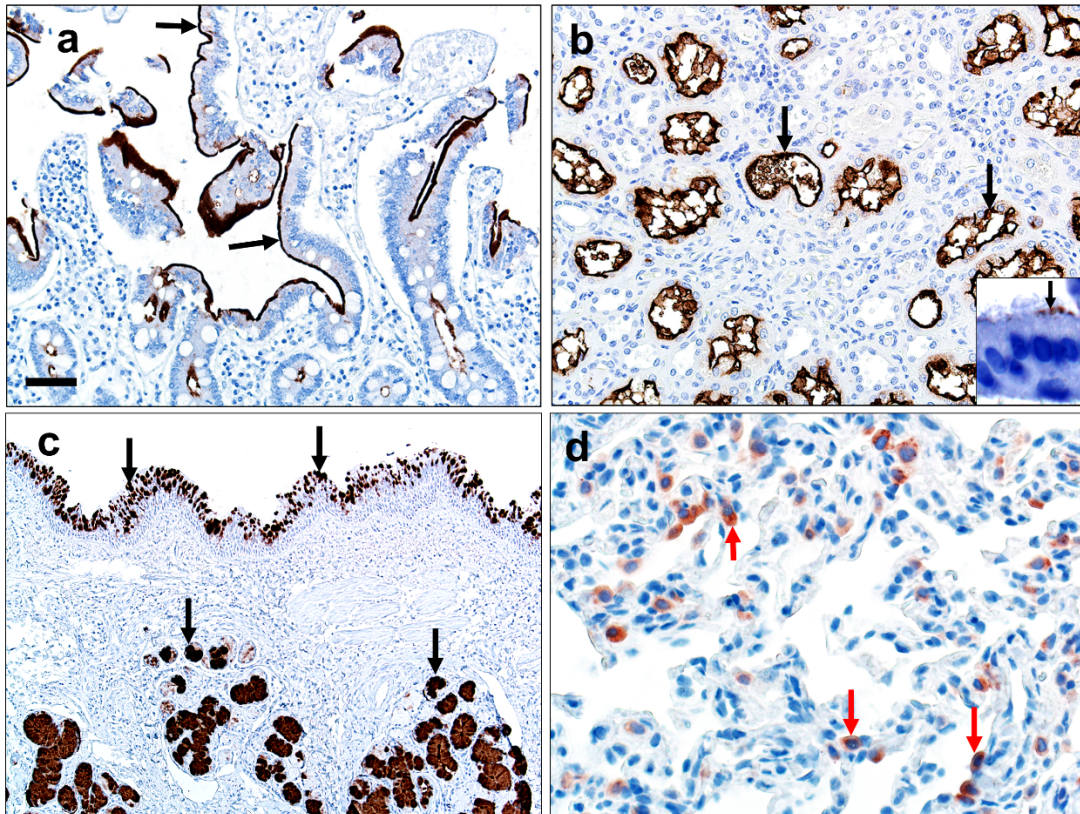


640

641 **Supplemental Figure 3.** Single-cell RNA sequencing reanalyses of ACE2 transcript abundance
 642 in nasal brushing (**a, b**) and nasal turbinate (**c, d**) (27). **a, c**) Uniform manifold approximation
 643 and projection (UMAP) visualizations. Cells were clustered using a shared nearest neighbor
 644 (SNN) approach. Cell types associated with each cluster were identified by determining marker
 645 genes for each cluster. Each data point denotes a cell. On the right panels, cells with ACE2
 646 transcripts are shown in red. **b, d**) Violin plots representing ACE2 expression. In nasal turbinate
 647 and nasal brushing, percentage of ACE2⁺ cells within each cell type shows ACE2 expression on
 648 epithelial cells. Each data point denotes a cell, most cells have no expression (0). DC: dendritic
 649 cells. NK: Natural killer. CPM: Counts per million.

650

Supplemental Figure 4



651

652 **Supplemental Figure 4.** Quality controls for ACE2 immunohistochemistry technique (**a, b**) and
653 tissue quality (**c, d**). **a, b**) ACE2 protein (brown color, black arrows) was detected along the
654 apical surface of small intestine enterocytes (**a**), renal tubule epithelium (**b**), and ciliated cells (**b**,
655 **inset**) of primary airway cell cultures. These findings demonstrate specific detection of ACE2
656 protein in cells/tissues consistent with known ACE2 expression. **c**) Representative
657 immunostaining of bronchus detected abundant MUC5B protein (brown color, black arrows) in
658 mucous cells of surface epithelium (top) and submucosal glands (bottom). **d**) Representative
659 sections of alveoli had SP-C⁺ alveolar type II cells (red color, red arrows). These results (**c, d**)
660 demonstrate the tissues were intact and that immunostaining can be used to detect native airway
661 (**c**) and lung (**d**) proteins. Bar = 40 (a, b), 80 (c), and 20 μ m (d).

666 **Supplemental Table 1. ACE2 protein reported in surface epithelium (SE) of human**
 667 **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] (20)	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] (21)	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] (22)	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] (23)	Polyclonal	SE (N++)	SE (-)	SE (C+, N++)	n.d.	AT1- AT2 (N++)	ACE2 is present in sinus and bronchial epithelium, AT2 cells, and macrophages

668

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

670 n.d.: Not described

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

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

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

674 macrophages (Mac)

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

676 (++)

677 **Supplemental Table 2. Donor demographics and ACE2 distribution scores for each tissue**
 678 **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

679

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

682 Scoring: 0 = below limit of immunohistochemical detection; 1 = rare (<1%); 2 = 1-33%; 3 = 34-
 683 66%; 4 = >66% of cells.

684

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

Target	Primary Antibody	Antigen Retrieval	Secondary Reagents
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.

686

687 HIER – Heat-induced epitope retrieval

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

689 AEC - aminoethyl carbazole (produces red stain)

690 Counterstain – Harris hematoxylin (blue color)