Goblet Cell Hyperplasia Increases SARS-CoV-2 Infection in COPD

Jaspreet Osan[a,*], Sattya N. Talukdar[a], Friederike Feldmann[b], Beth Ann DeMontigny[a], Kailey Jerome[a], Kristina L. Bailey[c], Heinz Feldmann[b], and Masfique Mehedi[1,#]

Supplemental Material:

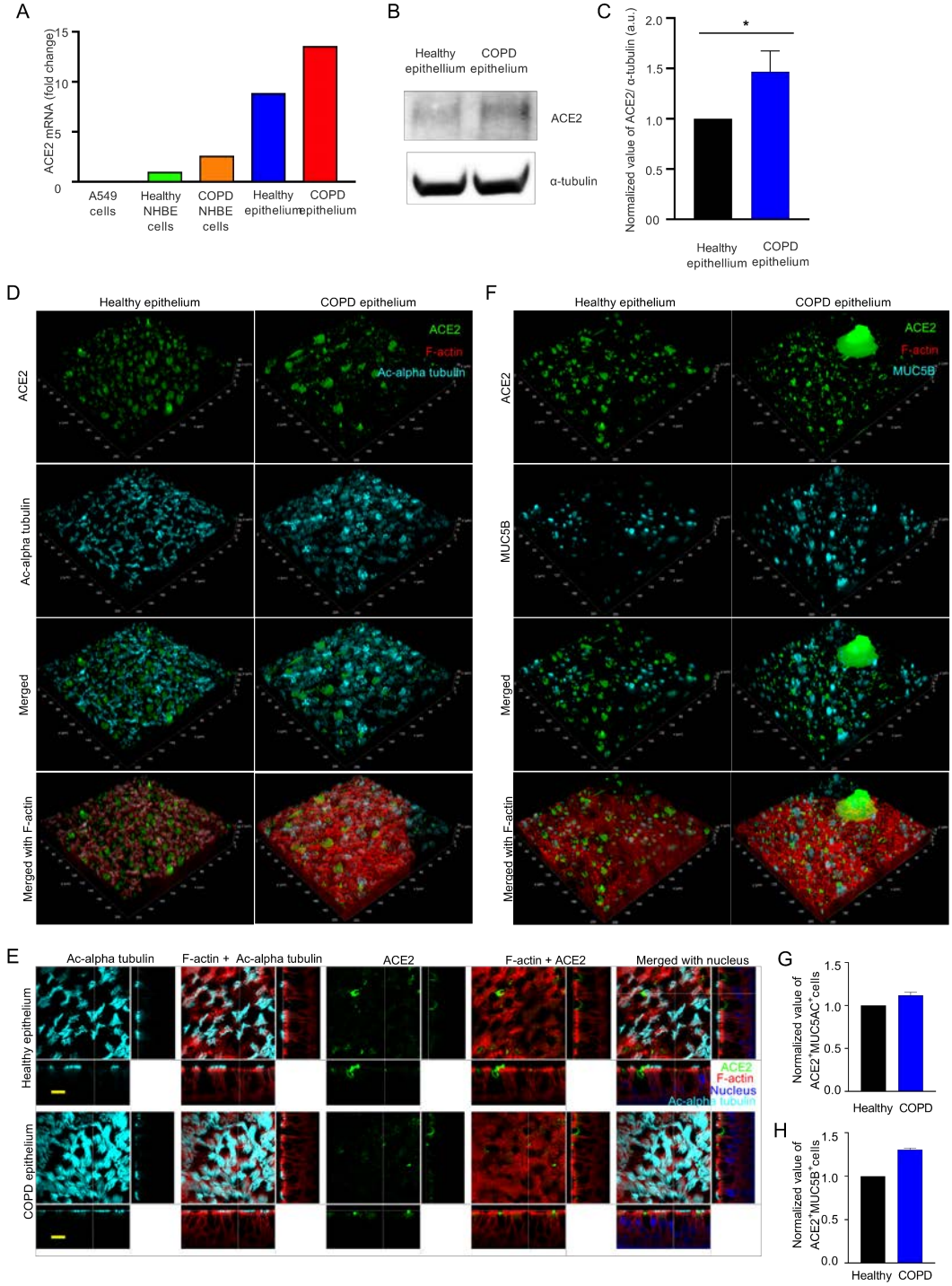

Fig S1. ACE2 expression is higher in the COPD airway epithelium. (A) ACE2 mRNA expression in total RNA extracted from the lung epithelial cell line (A549 cells) and primary NHBE cells from healthy adults and patients with COPD in monolayers or differentiated into airway epithelia was quantified by real-time PCR. The data were plotted as expression levels normalized to those in the NHBE healthy monolayer. The data were obtained by combining the quadruplet technical replicates of each sample. The graph represents the results from two independent real-time PCR runs. (B). ACE2 was detected in the airway epithelium (obtained after 4 weeks of differentiation of NHBE cells) by Western blotting. (C) The ACE2 signal (shown in B) was quantified (normalized to the alpha-tubulin level) and plotted relative to that in the healthy epithelium. The graph represents the results from four independent experiments. The error bar represents the SEMs. (D) The apical sites of the airway epithelia were fixed and stained for ACE2 (anti-ACE2, green), cilia (anti-acetyl-alpha tubulin, cyan) and F-actin (rhodamine phalloidin, red). Deconvoluted Z-stack images are presented in the 3D view. (E) The apical sites of the airway epithelia were fixed and stained for cilia (anti-acetyl-alpha tubulin, cyan), ACE2 (anti-ACE2, green), F-actin (rhodamine phalloidin, red) and nucleus (DAPI, blue). Bar = 15 μm. (F) The airway epithelia (described in D) were stained for ACE2 (anti-ACE2, green), MUC5B (anti-MUC5B, cyan) and F-actin (rhodamine phalloidin, red). Deconvoluted Z-stack images are presented in the 3D view. The fluorescence signal of ACE2 in $MUC5AC^+$ cells (G) and in $MUC5B^+$ cells (H) were measured on the images taken under the fluorescence microscope by IMARIS software from healthy and COPD epithelium. The error bars represent the SDs. Data was obtained from one independent experiment.

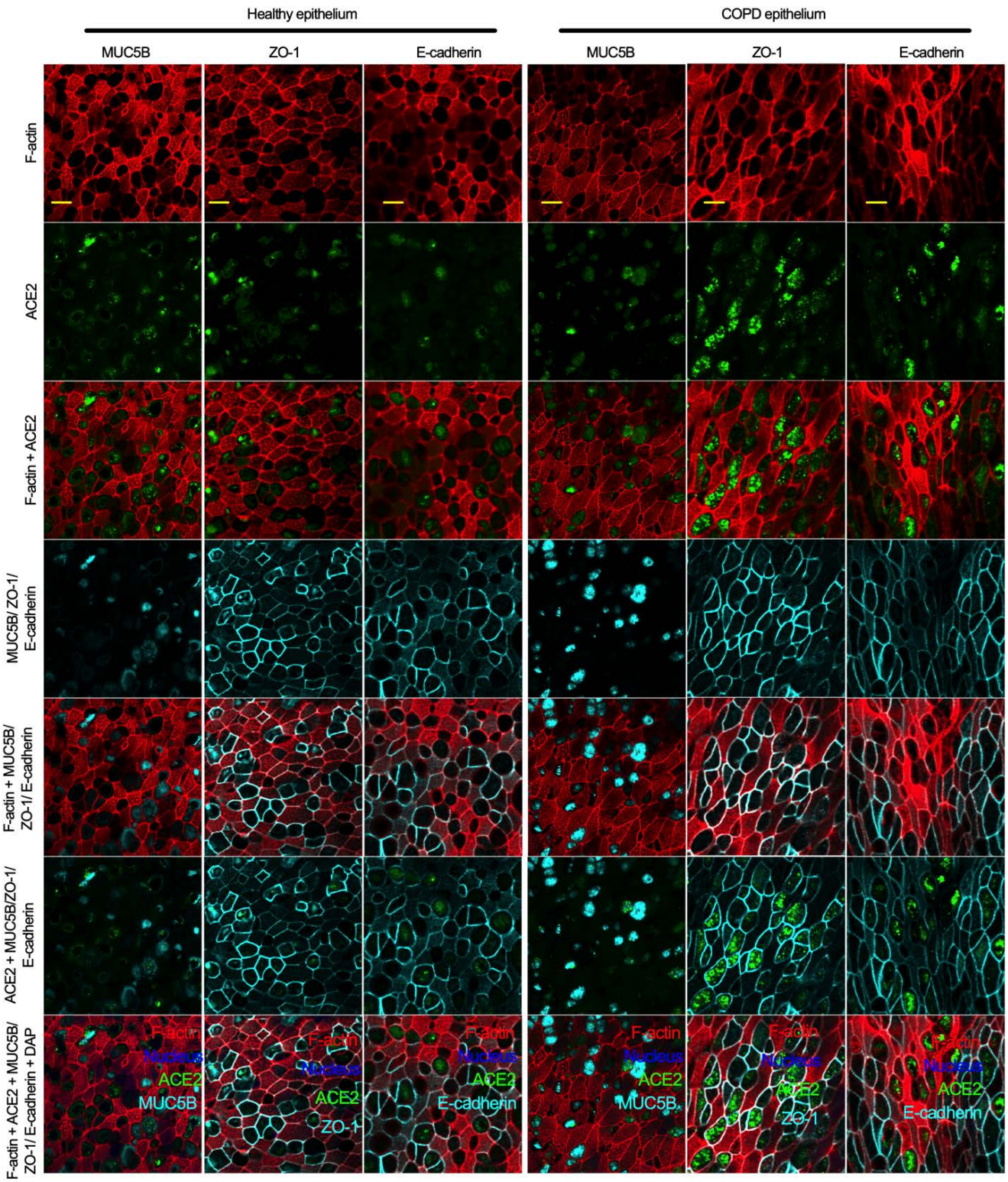
Healthy epithelium
COPD epithelium
MUC5B
ZO-1
E-cadherin
MUC5B
ZO-1
E-cadherin
F-actin
ACE2
F-actin + ACE2
MUC5B/ ZO-1/ E-cadherin
F-actin + MUC5B/ ZO-1/ E-cadherin
ACE2 + MUC5B/ZO-1/ E-cadherin
F-actin + ACE2 + MUC5B/ ZO-1/ E-cadherin + DAP
F-actin
Nucleus
ACE2
MUC5B
F-actin
Nucleus
ACE2
ZO-1
F-actin
Nucleus
ACE2
E-cadherin
F-actin
Nucleus
ACE2
MUC5B
F-actin
Nucleus
ACE2
ZO-1
F-actin
Nucleus
ACE2
E-cadherin

Fig S2. ACE2 expression within the goblet cell boundary. The airway epithelium (obtained after 4 weeks of differentiation of NHBE cells) was fixed and stained for ACE (anti-ACE2 antibody, green) and MUC5B (anti-MUC5B, cyan) or ZO-1 (anti-ZO-1 antibody, cyan) or E-cadherin (anti-e-cadherin antibody, cyan). F-actin (red) and nuclei (blue) were stained with rhodamine phalloidin and DAPI, respectively. The images represent multiple random areas obtained from an independent experiment. Bar = 10 μm.

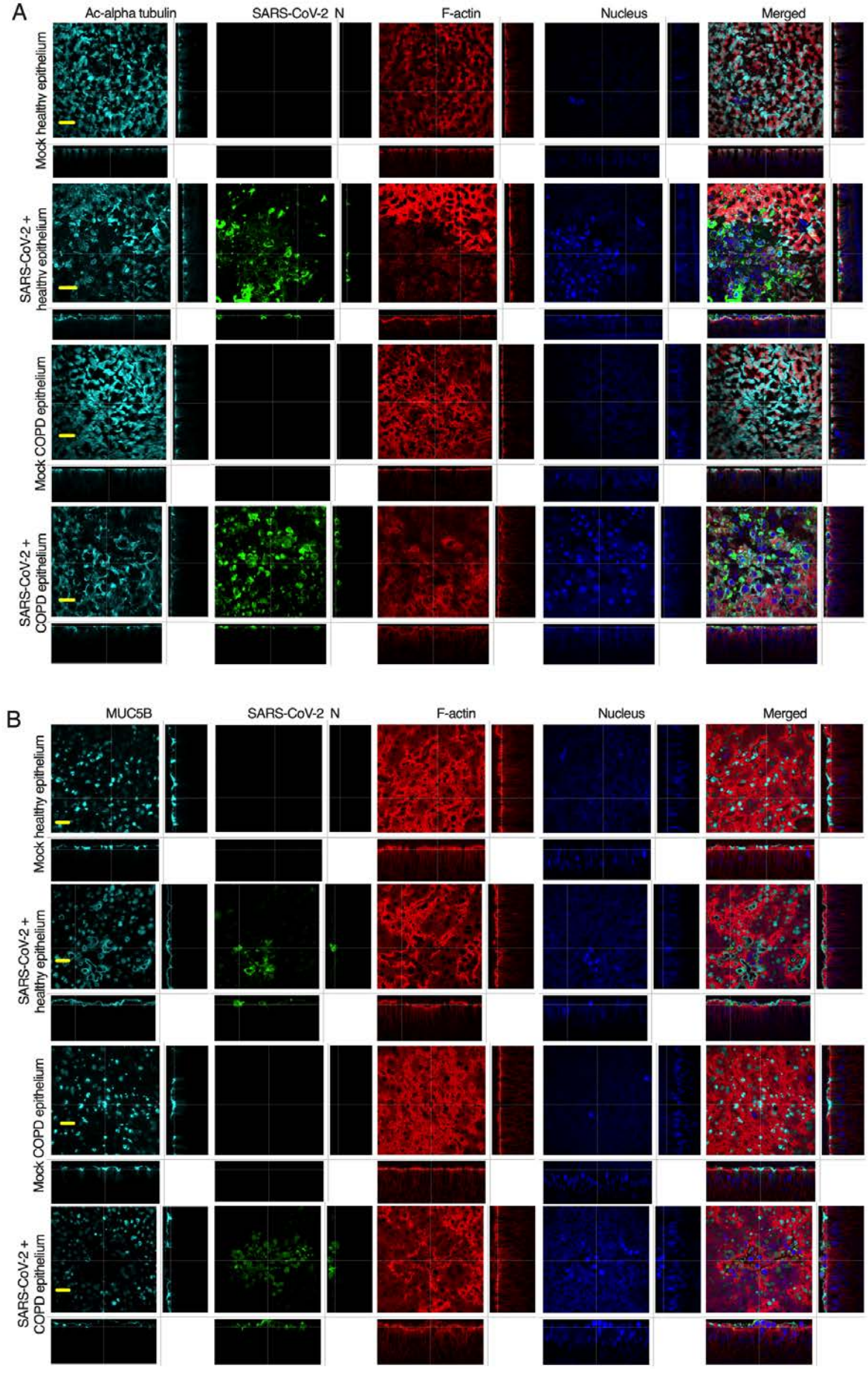
A
Ac-alpha tubulin
SARS-CoV-2 N
F-actin
Nucleus
Merged
Mock healthy epithelium
SARS-CoV-2 + healthy epithelium
Mock COPD epithelium
SARS-CoV-2 + COPD epithelium
B
MUC5B
SARS-CoV-2 N
F-actin
Nucleus
Merged
Mock healthy epithelium
SARS-CoV-2 + healthy epithelium
Mock COPD epithelium
SARS-CoV-2 + COPD epithelium

Fig S3. SARS-CoV-2 induces a cytopathic effect in the airway epithelium. The airway epithelium was mock-infected or infected with SARS-CoV-2 at an MOI of 0.1. At 4 days post infection (DPI), the epithelium was fixed, permeabilized, and stained for cilia (anti-acetyl-alpha tubulin, cyan) (A) or stained for goblet cells (anti-MUC5B, cyan) (B), SARS-CoV-2 N (anti-N, green), and F-actin (rhodamine phalloidin, red); the nuclei were stained with DAPI (blue). The images represent multiple independent random areas from an independent experiment. Bar = 30 μm.

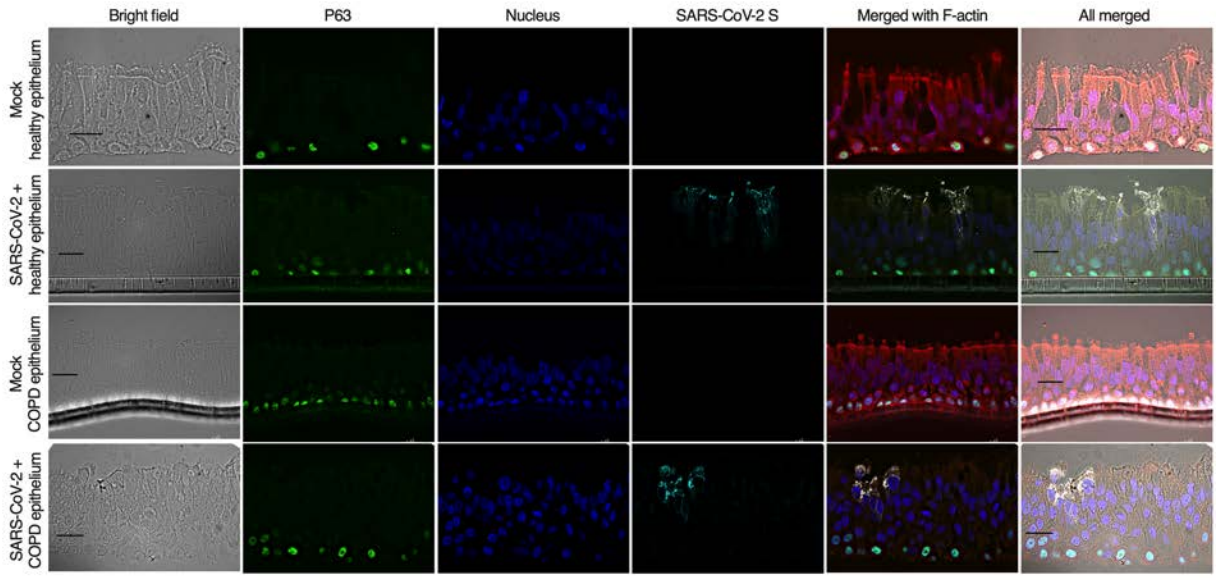

Fig S4. SARS-CoV-2 does not infect basal cells in the airway epithelium. The airway epithelium was mock-infected or infected with SARS-CoV-2 at an MOI of 0.1. At 4 days post infection (DPI), the epithelium was fixed, embedded in paraffin, sectioned, and stained for the basal cell marker P63 (anti-P63, green), SARS-CoV-2 spike (S) (anti-S, cyan), and F-actin (rhodamine phalloidin, red); the nuclei were stained with DAPI (blue). Bar = 20 μm.

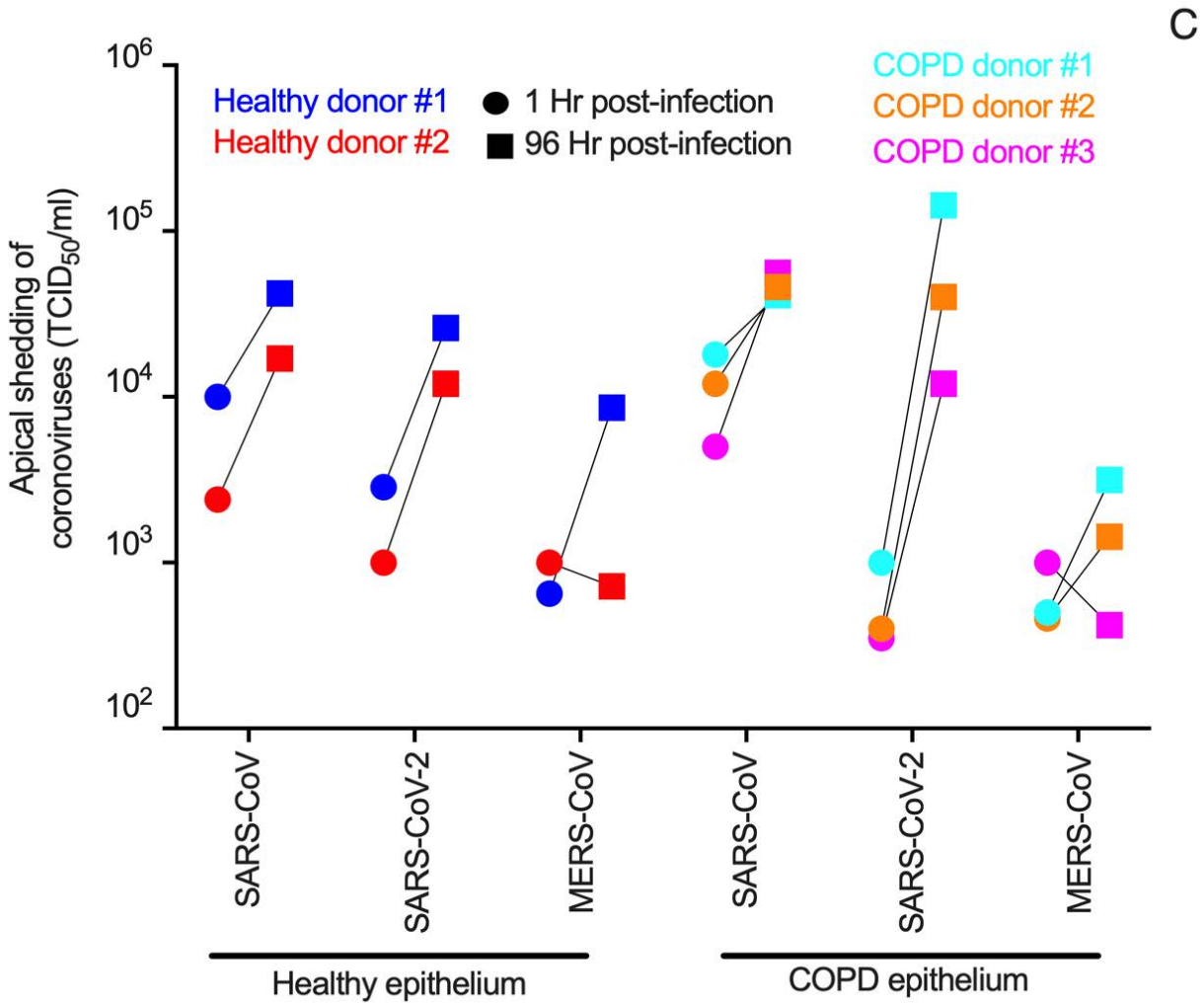

Fig S5. SARS-CoV-2 replicates better in COPD airway epithelium. At 1 hr post-infection (HPI) or 96 HPI, the apical wash of the SARS-CoV-2, SARS-CoV, or MERS-CoV-infected airway epithelium (MOI = 0.1) was collected, and SARS-CoV-2, SARS-CoV, or MERS-CoV titration was performed based on the tissue culture infective dose 50 ($TCID_{50}$). The results represent viral infections in the airway epithelium models developed from primary NHBE cells of different donors (healthy adult donors, n=2 and COPD donors, n=3) with triplicate samples are shown.

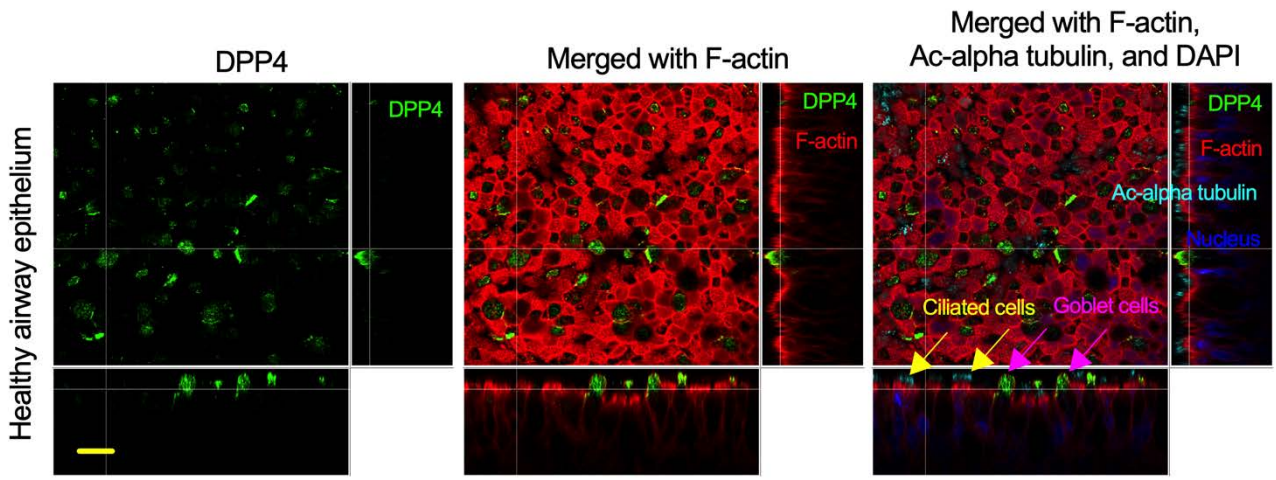

Fig S6. MERS-CoV receptor DPP4 expression is higher in goblet cells. The airway epithelium (obtained after 4 weeks of differentiation of healthy adult NHBE cells) was fixed and stained for DPP4 (anti-DPP4 antibody, green) and Acetyl-alpha tubulin (anti-Ac-alpha tubulin, cyan). F-actin and the nucleus were stained with rhodamine phalloidin (red) and DAPI (blue), respectively. Bar = 20 μm.

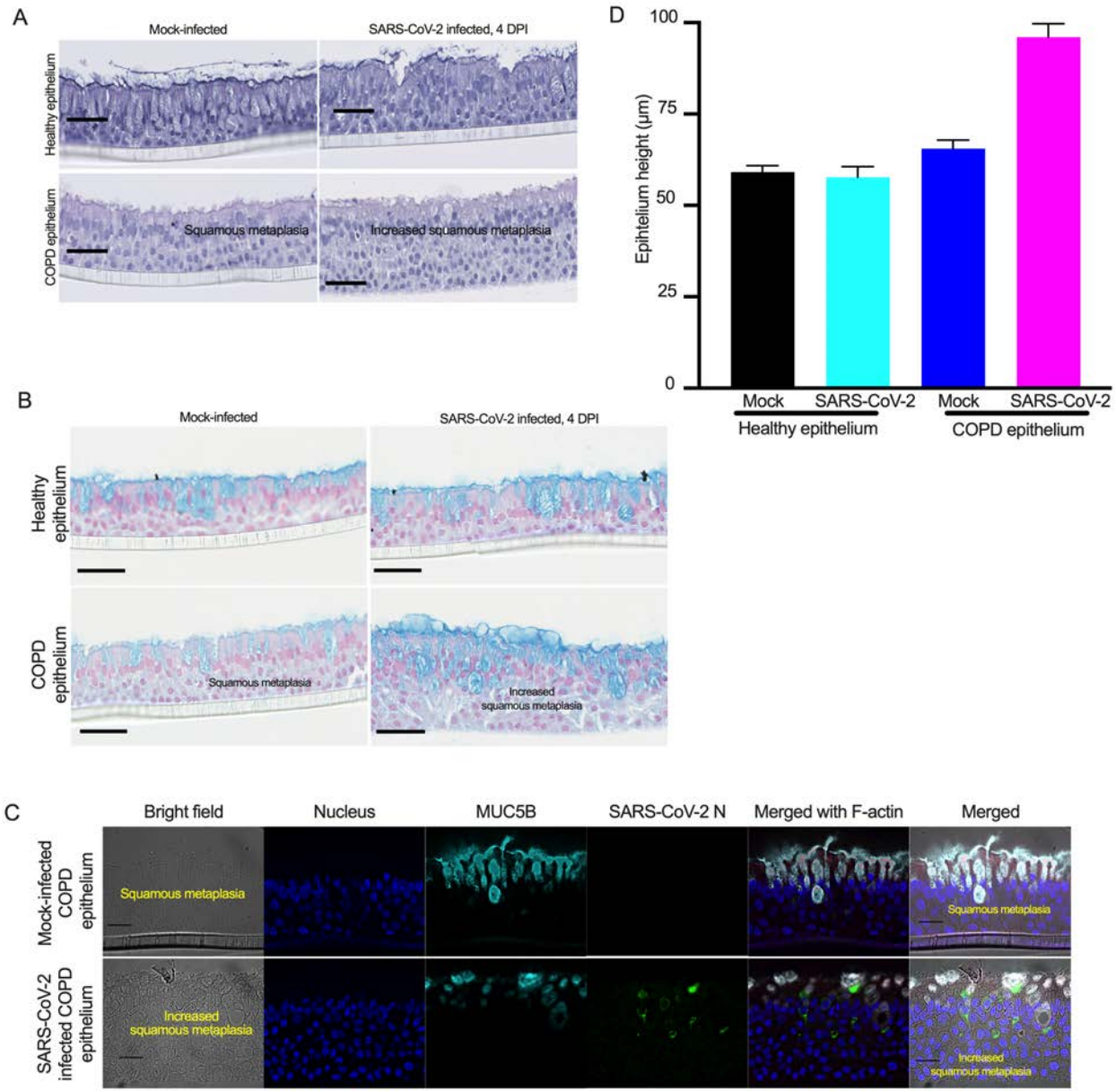

Fig S7. SARS-CoV-2 replicates at a higher rate and increases squamous metaplasia in the COPD epithelium. (A) The apical wash of the SARS-CoV-2-infected airway epithelium (described in Figure 4) was collected, and SARS-CoV-2 titration was performed based on the tissue culture infective dose 50 (TCID50). The results were obtained from independent experiment done in triplicate (n=3) (healthy donors, n=2; COPD donors, n =3) are shown. (B) The sectioned epithelium (described in Figure 4) was stained with H&E staining. Bar = 50 µm. (C) The sectioned

epithelium (described in Figure 4) was stained with Alcian blue. Bar = 50 μm. (D) At 4 DPI, the sectioned epithelium (COPD epithelium after mock or SARS-CoV-2 infection) were stained for the goblet cell marker MUC5B (anti-MUC5B, cyan) and SARS-CoV-2 N (anti-N, green), and the nuclei were stained with DAPI (blue). Bar = 20 μm. (E) The height of the airway epithelium (described in C) was measured from the images (n=5), the average of 12 points was plotted.