

1 **The type 2 asthma mediator IL-13 inhibits SARS-CoV-2 infection of bronchial epithelium**

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15

16 **Contributions:**

17 LRB, WLE, LR, and DJE contributed to study conception and design. LRB, KDK, LTZ, and LR
18 performed experiments. LRB, WLE, JS, KDK, SC, PGW, and DJE analyzed and interpreted
19 data. WEF and DJE supervised study execution. LRB and DJE drafted the manuscript. All
20 authors reviewed the manuscript.

21

22 **Support:** NIH awards U19 AI 077439 and R35 HL145235 (to DJE) and R00 HL135403 (to
23 WLE), University of California, San Francisco Program for Breakthrough Biomedical Research
24 (PBBR; to LRB).

25

26 **Running title:** IL-13 inhibits SARS-CoV-2 infection

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28 **Subject Category:** 7.18 Mucosal Immunity of the Respiratory Tract

29

30 **Total Word Count:** 3427

31 **Abstract**

32

33 **Rationale:** Asthma is associated with chronic changes in the airway epithelium, a key target of
34 SARS-CoV-2. Many epithelial changes are driven by the type 2 cytokine IL-13, but the effects of
35 IL-13 on SARS-CoV-2 infection are unknown.

36 **Objectives:** We sought to discover how IL-13 and other cytokines affect expression of genes
37 encoding SARS-CoV-2-associated host proteins in human bronchial epithelial cells (HBECs)
38 and determine whether IL-13 stimulation alters susceptibility to SARS-CoV-2 infection.

39 **Methods:** We used bulk and single cell RNA-seq to identify cytokine-induced changes in SARS-
40 CoV-2-associated gene expression in HBECs. We related these to gene expression changes in
41 airway epithelium from individuals with mild-moderate asthma and chronic obstructive
42 pulmonary disease (COPD). We analyzed effects of IL-13 on SARS-CoV-2 infection of HBECs.

43 **Measurements and Main Results:** Transcripts encoding 332 of 342 (97%) SARS-CoV-2-
44 associated proteins were detected in HBECs (≥ 1 RPM in 50% samples). 41 (12%) of these
45 mRNAs were regulated by IL-13 (>1.5 -fold change, FDR < 0.05). Many IL-13-regulated SARS-
46 CoV-2-associated genes were also altered in type 2 high asthma and COPD. IL-13 pretreatment
47 reduced viral RNA recovered from SARS-CoV-2 infected cells and decreased dsRNA, a marker
48 of viral replication, to below the limit of detection in our assay. Mucus also inhibited viral
49 infection.

50 **Conclusions:** IL-13 markedly reduces susceptibility of HBECs to SARS-CoV-2 infection
51 through mechanisms that likely differ from those activated by type I interferons. Our findings
52 may help explain reports of relatively low prevalence of asthma in patients diagnosed with
53 COVID-19 and could lead to new strategies for reducing SARS-CoV-2 infection.

54

55 **Abstract Word Count:** 250 (max: 250)

56

57 **Keywords:** COVID-19, SARS-CoV-2, asthma, IL-13, airway epithelium

58 **Introduction**

59 One remarkable feature of the COVID-19 pandemic is the wide range of disease severity seen
60 following SARS-CoV-2 infection. Host factors, including age and male sex (1), inborn or
61 acquired disorders of type I interferon-mediated antiviral immunity (2–4), and various pre-
62 existing medical conditions (5) influence the risk of severe disease. There have been concerns
63 that COVID-19 risks would also be increased in persons with asthma, which affects
64 ~339,000,000 individuals worldwide (6). These concerns arose from experience with other
65 respiratory viruses which trigger asthma exacerbations and can be associated with worse
66 outcomes in individuals with pre-existing asthma (7). However, asthma was underrepresented in
67 early studies of patients with COVID-19 as well as prior studies of the severe acute respiratory
68 syndrome (SARS) (8). Subsequent studies have failed to find consistent evidence of increased
69 risk of COVID-19 diagnosis, hospitalization, or mortality due to asthma (1, 9, 10), and some
70 studies concluded that risk of acquiring and being hospitalized for COVID-19 are lower in those
71 with asthma (10, 11). Factors that might provide protection against COVID-19 in individuals with
72 asthma (12–15) include increased attention to limiting viral exposure, younger age, absence of
73 co-morbidities, use of inhaled corticosteroids, and a variety of biological features of asthma,
74 including chronic airway inflammation, mucus hypersecretion, and altered expression of SARS-
75 CoV-2 receptor (12). However, direct evidence that these features alter SARS-CoV-2 infection
76 has been lacking.

77 Asthma is associated with changes in the structure and function of the airway epithelium,
78 a critical site for SARS-CoV-2 infection (16, 17). Airway epithelial gene expression changes
79 attributable to the type 2 cytokine IL-13 are seen in approximately half of individuals with asthma
80 (18, 19). IL-13 stimulation of airway epithelial cells decreases expression of *ACE2*, which
81 encodes the SARS-CoV-2 receptor, and increases expression of *TMPRSS2*, which encodes a
82 transmembrane protease that primes the viral spike protein (20–22). Similar changes are seen

83 in airways of individuals with type 2 high asthma (21, 22). IL-13 has also been reported to
84 protect against other RNA viruses, including respiratory syncytial virus (RSV) (23) and
85 rhinovirus (24), that do not rely on ACE2 and TMPRSS2 for entry, indicating that other IL-13-
86 regulated genes can also protect against viral infection. We therefore hypothesized that IL-13
87 induces changes in expression of airway epithelial cell genes important in SARS-CoV-2
88 infection in a large subset of individuals with asthma and that IL-13 reduces susceptibility of
89 these cells to SARS-CoV-2 infection.

90

91 **Methods**

92 Additional details are provided in the Online Data Supplement.

93

94 **Human bronchial epithelial cell (HBEC) culture**

95 Primary HBECs from 13 individuals listed in Table E1 were cultured at air-liquid interface (ALI)
96 as previously described (25, 26). For cytokine stimulation, cultures were stimulated by addition
97 of cytokines (10 ng/ml) to the basolateral medium (IFN- α : 24 hours, IFN- γ : 24 hours, IL-13: 7
98 days, IL-17: 7 days). The UCSF Committee on Human Research approved these studies.

99

100 **RNA-seq**

101 RNA was isolated from cytokine-stimulated HBECs derived from six individuals and bulk RNA-
102 seq was performed as previously described (27, 28). We previously reported other analyses
103 based upon data from unstimulated cells and cells stimulated with individual cytokines (27, 28);
104 data from cells treated with a combination of IL-13 and IFN- α have not been previously
105 reported. For scRNA-seq, single cell suspensions were generated from HBECs from four of the
106 donors used for bulk RNA-seq and analyzed using the 10X Genomics platform.

107

108 **Analysis of gene expression in asthma and COPD**

109 Using gene expression data from studies of asthma (27) and COPD (29) we correlated
110 measures of type 2 activity with IL-13-induced SARS-CoV-2-associated genes in HBECs using
111 Pearson's correlation coefficient and a linear regression model that adjusted for age and sex.

112

113 **SARS-CoV-2 infection**

114 SARS-CoV-2 virus (USA-WA1/2020 strain) was provided by Dr. Melanie Ott and propagated in
115 Vero E6 cells. HBECs were cultured in the absence or presence of IL-13 (10 ng/ml). Mucus was
116 allowed to accumulate for 3 d or was removed immediately prior to viral infection by washing the
117 apical surface with a prewarmed solution of 10 mM dithiothreitol (DTT; Thermo) in PBS for 10
118 minutes (26) and then washing twice with PBS without DTT. Cells were inoculated by adding
119 virus to the apical surface. After 2 h, the apical surface was washed with twice with PBS and
120 cells were returned to the incubator. Cells were harvested 48 h post-infection for analysis of viral
121 RNA (by qRT-PCR) and dsRNA-staining (by immunofluorescence).

122

123 **Results**

124

125 **SARS-CoV-2-associated host genes are highly expressed in HBECs**

126 We compiled a list of 342 SARS-CoV-2-associated genes belonging to one of two gene sets.
127 One set of 11 genes encode proteins implicated in viral entry: *ACE2* (30), *TMPRSS2* (30), the
128 cathepsins *CTSB* and *CTSL*, *FURIN(PCSK3)*, and the furin-like proteases *PCSK1*, *PCSK2*, and
129 *PCSK4-7* (31). The second set of 332 genes encode host cell proteins shown to interact with
130 high confidence with 26 of the 29 SARS-CoV-2 proteins in HEK293T cells (32). One gene,
131 *PCSK6*, belonged to both sets. Using bulk RNA-seq, we detected 332 of the 342 SARS-CoV-2-
132 associated genes (97%; ≥ 1 read per million mapped reads [RPM] in $\geq 50\%$ of samples) in

133 differentiated HBECs cultured without cytokine (Table E2). SARS-CoV-2-associated genes were
134 substantially overrepresented among genes with high read counts (Fig. 1A).

135

136 **IL-13 and other cytokines regulate expression of many SARS-CoV-2-associated genes**

137 We examined the effect of IL-13 stimulation on SARS-CoV-2-associated gene expression. We
138 also analyzed the effects of IFN- α , which plays a central role in defense against SARS-CoV-2
139 infection (3, 4) and has been shown to inhibit SARS-CoV-2 infection of a human lung epithelial
140 cell line (33), and IFN- γ (27) and IL-17 (34), each of which have been implicated in subsets of
141 individuals with asthma. Each cytokine had the expected effects on expression of known
142 cytokine-responsive genes (Figure 1B). Of the 332 SARS-CoV-2-associated genes detected in
143 HBECs, IL-13 regulated 41 genes, IFN- α regulated 19 genes, the combination of IL-13 and IFN-
144 α regulated 63 genes, IFN- γ regulated 14 genes, and IL-17 regulated 21 genes (false discovery
145 rate [FDR] $q \leq 0.05$ and absolute fold change ≥ 1.5 ; Figure 1C; Table E2). *ACE2* was reduced
146 by IL-13 (29% decrease, $q = 0.003$), consistent with a prior report (24). In contrast, *ACE2* was
147 the most highly upregulated gene following stimulation with IFN- α (451% increase, $q = 3 \times 10^{-74}$)
148 or IFN- γ (185% increase, $q = 9 \times 10^{-29}$) and was less strongly upregulated following IL-17
149 stimulation (31% increase, $q = 0.02$). Analysis of *ACE2* splicing confirmed prior reports that
150 interferon stimulation increased expression of the decoy isoform (35), but we found no
151 significant effect of IL-13 on levels of this isoform (mean decoy *ACE2* normalized reads:
152 unstimulated, 62; IFN- α -stimulated: 588, IL-13-stimulated, 61). *TMPRSS2* was increased by IL-
153 13 (61% increase, $q = 7 \times 10^{-15}$) and IL-17 (22% increase, $q = 0.005$) but modestly decreased
154 by IFN- α (17% decrease, $q = 0.01$) and IFN- γ (16% decrease, $q = 0.03$). No significant
155 correlation between IL-13- and IFN- α -induced changes in the 342 SARS-CoV-2-associated
156 genes was observed, and combined stimulation with both cytokines resulted in additive effects

157 with no evidence of synergy or antagonism (Figure E1). This suggests that these two cytokines
158 affect SARS-CoV-2-associated genes by different and independent mechanisms.

159

160 **Expression of many SARS-CoV-2-associated genes differs between airway epithelial cell** 161 **types**

162 We used single cell RNA sequencing (scRNA-seq) to assess cell type-specific expression of
163 SARS-CoV-2-associated genes in unstimulated HBEC cultures from four individuals (Figure
164 E2). Of the 332 SARS-CoV-2-associated genes detected in HBECs by bulk RNA-seq, 322
165 (97%) were detected in at least 10 cells. We detected *ACE2* in 1.7% of basal cells, 3.1% of
166 secretory cells, and 1.6% of ciliated cells from unstimulated HBEC cultures. 113 of the 322
167 SARS-CoV-2 genes detected in our scRNA-seq dataset were differentially expressed between
168 cell types (FDR $q < 0.05$; Table E3; selected genes shown in Fig. 2A). We found similar patterns
169 of epithelial cell subset-specific expression of these SARS-CoV-2 associated genes in a scRNA-
170 seq dataset from human bronchial tissue (36) (Figure E3), confirming that our cell culture model
171 recapitulated cell type-specific gene expression seen *in vivo* and that the expression of host cell
172 proteins that interact with SARS-CoV-2 proteins differs between cell types.

173

174 **IL-13 affects expression of some SARS-CoV-2-associated genes in a cell type-specific** 175 **manner**

176 We explored the effect of cytokine stimulation on SARS-CoV-2 associated gene expression in
177 each cell type. Whereas many IL-13-regulated SARS-CoV-2 associated genes were affected
178 similarly in each cell type, some IL-13 effects were cell type-specific, with 11 cases in which IL-
179 13 had opposite effects (increased in one cell type and decreased in another cell type, FDR $q <$
180 0.1 for both; Figure 2B and Table E3). Notably, IL-13 upregulated *TMPRSS2* expression in
181 secretory cells, but decreased expression in ciliated cells. Cell type-specific effects of IL-13
182 could have implications for the outcome of infection in different airway epithelial subsets.

183

184 **Type 2 signatures are associated with expression of many SARS-CoV-2-associated**
185 **genes in individuals with asthma and COPD**

186 We examined whether IL-13-induced SARS-CoV-2-associated genes identified in HBECs in
187 culture were altered in asthma using a transcriptomic profiling dataset derived from
188 endobronchial brush biopsies from individuals with mild to moderate asthma and healthy
189 individuals (Mechanisms of Asthma STudy [MAST]) (27, 37). We used the three gene metric
190 (TGM), an established measure of IL-13-induced airway inflammation in individuals with asthma
191 (18, 19, 38), for this analysis. 24 of 27 SARS-CoV-2-associated genes induced by IL-13 in
192 HBECs were positively correlated with the TGM (Pearson's $R > 0$); in 13 cases this correlation
193 was statistically significant after adjustment for multiple comparisons (adjusted $p < 0.05$; Figure
194 3; Table E4). 16 of 27 IL-13-induced genes were significantly associated with the TGM using a
195 linear model that included age and sex (adjusted $p < 0.05$; Table E4).

196 A study of former smokers with and without COPD found that subsets of individuals with
197 COPD also have airway epithelial gene expression changes indicative of type 2 inflammation
198 (39). 26 SARS-CoV-2-associated genes induced by IL-13 in HBECs were measured in that
199 study, and 21 of those positively correlated with the type 2 gene expression score developed for
200 that study (Pearson's $R > 0$). In 16 cases this association was statistically significant (adjusted p
201 < 0.05) and remained so in a model that included age and sex (Figure E4; Table E4). Taken
202 together, our data indicate that many IL-13-induced SARS-CoV-2-associated gene changes
203 seen in the HBEC culture model recapitulate alterations seen in epithelial cells from individuals
204 with asthma or COPD and type 2 inflammation.

205

206 **IL-13 protects HBECs from SARS-CoV-2 infection**

207 We next investigated whether stimulation with IL-13 protected HBECs from SARS-CoV-2
208 infection by quantifying viral RNA 48 h after viral inoculation. Mucus produced by HBECs was

209 left in place or was removed by washing the apical surface immediately prior to inoculation. In
210 the first experiment, we found that the presence of mucus decreased the amount of SARS-CoV-
211 2 RNA detected after infection of unstimulated cells by 74% compared with cells infected after
212 removal of mucus (Fig. 4A). Pre-stimulation with IL-13 markedly reduced levels of SARS-CoV-2
213 RNA when infections were performed after removal of mucus (95% reduction) and when cells
214 were infected in the presence of mucus (97% reduction). In a second experiment with a different
215 HBEC donor (Fig. 4B), mucus was more effective in inhibiting infection (90-97% reduction for
216 three different viral inocula). In cells infected after removal of mucus, IL-13 pre-stimulation
217 reduced viral RNA by 82-92%. Since both mucus and IL-13 pre-stimulation had large effects in
218 this donor, it was not clear whether mucus and IL-13 had additive effects in this experiment.

219 We also assessed the effects of mucus and IL-13 on double-stranded RNA (dsRNA),
220 which is produced during SARS-CoV-2 viral replication. dsRNA was not detected in uninfected
221 cells but was detected within isolated cells or clusters of cells in unstimulated SARS-CoV-2-
222 infected cultures. Analysis of cultures inoculated with varying amounts of virus after removal of
223 mucus revealed a total of 27 dsRNA-stained foci with a mean volume of $6958 \mu\text{m}^3$ (Fig. 5). In
224 contrast, only 3 foci (mean $1246 \mu\text{m}^3$) were seen in paired cultures inoculated without removing
225 mucus (Fig. E5). In cultures pre-stimulated with IL-13, no foci were observed whether or not
226 mucus was removed. The observation that relatively small amounts of viral RNA were
227 detectable in IL-13-stimulated cultures (Fig. 4) but dsRNA staining was not evident under these
228 conditions (Fig. 5 and Fig. E5) might indicate that dsRNA staining is less sensitive than qRT-
229 PCR for viral RNA. Alternatively, it is possible that IL-13 completely prevented viral replication,
230 and that viral RNA detected in IL-13-stimulated cells was residual RNA from the viral inoculum.
231 Most dsRNA-containing infected cells co-stained for the ciliated cell marker acetylated- α -tubulin,
232 although dsRNA was occasionally seen in non-ciliated cells that stained for mucins. Since PCR
233 analysis of viral RNA indicated that the protective effects of mucus were greater in donor 2, it is
234 noteworthy that mucin expression differed between the two donors. In the absence of IL-13,

235 donor 1 cultures had MUC5B-containing cells but no detectable MUC5AC-containing cells (Fig.
236 5A and Fig. E5A), whereas donor 2 cultures had both MUC5AC- and MUC5B-containing cells
237 (Fig. 5B and Fig. E5B). IL-13 stimulation increased MUC5AC in cells from both donors and
238 caused an obvious decrease in MUC5B in donor 1; these effects of IL-13 are consistent with
239 those observed in our previous studies (25) and people with type 2 asthma (19). Based on viral
240 RNA measurements and dsRNA staining, we conclude that both the presence of a mucus gel
241 and IL-13 stimulation reduced viral infection, and that the effects of IL-13 were seen even after
242 removal of the mucus layer prior to infection.

243

244 **Discussion**

245 Our studies reveal that IL-13 stimulation of HBECs affects expression of many SARS-CoV-2-
246 associated genes and substantially inhibits SARS-CoV-2 infection of these cells. Genes
247 encoding the large majority of SARS-CoV-2-interacting proteins identified in a previous study of
248 HEK293T cells were expressed in HBECs. Expression of many SARS-CoV-2-associated genes
249 differed between basal, ciliated, and secretory cells, potentially affecting how these cell types
250 respond to SARS-CoV-2 infection. Many IL-13-induced SARS-CoV-2-associated gene
251 expression changes we detected in culture were also seen in bronchial epithelium obtained
252 directly from individuals with type 2 high asthma. This provides a plausible mechanism for
253 protection against COVID-19, although the impact of asthma on COVID-19 risk is still
254 incompletely understood and other factors may also influence COVID-19 risk in individuals with
255 asthma (13–16). We also found significant associations of many IL-13-induced SARS-CoV-2-
256 associated genes with type 2 inflammation in a large group of smokers with and without COPD,
257 suggesting that the effects of IL-13 on SARS-CoV-2 risk may also be relevant in some
258 individuals without asthma. The effects of IL-13 on SARS-CoV-2-associated genes were clearly
259 different than the effects of IFN- α , suggesting that these two cytokines induce different antiviral

260 mechanisms. In experiments that established the inhibitory effect of IL-13 on SARS-CoV-2
261 infection of epithelial cells, we found evidence that another barrier component, the mucus gel,
262 also provides protection against infection. Taken together, these studies provide insights into
263 airway epithelial responses that can protect against SARS-CoV-2 and might influence COVID-
264 19 susceptibility and severity in individuals with asthma or other airway diseases.

265 IL-13 had a substantial effect on SARS-CoV-2 infection of HBECs as demonstrated by
266 measurements of viral RNA and dsRNA following viral inoculation. Prior studies report a variety
267 of effects of asthma and IL-13 on development of illnesses caused by other viruses. IL-13 can
268 increase susceptibility of HBECs to rhinovirus infection by suppressing induction of interferons
269 (40–42), although another study reported that prolonged pre-treatment with IL-13 of HBECs
270 reduced rhinovirus infection (24). Mice with acute allergic airway inflammation (43) and people
271 with pre-existing asthma (44) are reportedly protected from H1N1 influenza. Studies in IL-13-
272 overexpressing transgenic mice and IL-13-deficient mice showed that IL-13 reduced respiratory
273 syncytial virus replication and severity of illness (23). While effects of IL-13 on the airway
274 epithelium are an important contributor to asthma pathogenesis, it is intriguing to speculate that
275 IL-13 responses may have evolved at least in part to provide protection against viral infections.
276 The finding that levels of IL-13 and the related type 2 cytokine IL-4 were higher in patients with
277 moderate COVID-19 compared with severe COVID-19 or healthy controls is also consistent with
278 an antiviral role for these cytokines (45).

279 Many mechanisms might account for IL-13-driven inhibition of SARS-CoV-2 infection. A
280 recent study identified 65 interferon-stimulated genes that mediate restriction of SARS-CoV-2
281 infection (46), illustrating how a single cytokine can activate a large set of antiviral pathways.
282 We found that gene expression changes induced by IL-13 were quite distinct from those
283 induced by IFN- α , suggesting that these cytokines activate different antiviral pathways. We
284 confirmed prior studies (20, 21) showing that IL-13 induced a decrease in expression of the

285 SARS-CoV-2 receptor *ACE2*, which could contribute to decreased infection. However, the
286 reduction in *ACE2* expression was modest compared with the effects of IL-13 on infection,
287 suggesting that other IL-13 effects should also be considered. As in the previous reports, we
288 found that IL-13 increased expression of *TMPRSS2*, a protease that is important for viral entry.
289 However, we found that the IL-13 effects were cell type-dependent: *TMPRSS2* expression was
290 increased in secretory cells but decreased in ciliated cells. Since ciliated cells were the primary
291 cell type infected in our experiments, it is possible that decreased *TMPRSS2* in ciliated cells
292 contributed to an overall reduction in infection. Many other host cell factors influence viral entry,
293 RNA synthesis and translation, and egress, and further studies will be required to determine
294 which of these contribute to the antiviral effects of IL-13.

295 Our studies provided clear evidence that the mucus barrier produced by HBECs in cell
296 culture inhibits SARS-CoV-2 infection. Airway mucus is a complex hydrogel that derives its
297 characteristic viscoelastic properties from the mucin glycoproteins MUC5B and MUC5AC (47).
298 Prior studies establish that mucins play important roles as restriction factors for other viruses,
299 including influenza (48, 49). We found that SARS-CoV-2 infection was decreased when mucus
300 gels were left in place at the time of viral inoculation. We studied two subjects with distinct
301 patterns of mucin expression and found somewhat different levels of protection from the gel.
302 While further studies are clearly required to investigate this further, this result suggests that
303 differences in mucus gels are also likely to be important in SARS-CoV-2 infection. Changes in
304 airway mucus volume, composition, and organization are prominent features of many airway
305 diseases, including asthma (47). IL-13 is an important regulator of mucins, and we speculate
306 that IL-13-driven increases in MUC5AC, which results in tethering of the mucus gel to the
307 epithelium (25), might contribute to IL-13-induced inhibition of SARS-CoV-2 infection. However,
308 this is unlikely to completely account for the antiviral effect since IL-13 inhibited viral infection
309 even when the mucus gel was removed immediately prior to inoculation.

310 Our study has some important limitations. While we focused on a set of SARS-CoV-2-
311 associated genes that have been defined in previous studies, other IL-13-regulated genes are
312 also likely to be important for anti-viral effects. Some IL-13-regulated genes we identified in cell
313 culture were not associated with a type 2 signature in cells from individuals with asthma or
314 COPD, reflecting the influence of other factors, including other asthma mediators, or differences
315 in IL-13 responses in cell culture versus *in vivo*. As individual genes that contribute to inhibition
316 of viral infection in HBECs are identified, it will be important to specifically examine the
317 expression of those genes in asthma and COPD. Our HBEC infection studies used only one
318 strain of SARS-CoV-2 and cells from only two donors, and further experiments with additional
319 strains and more donors (including donors with asthma), will be required to better understand
320 the interactions between virus, epithelial cells, and IL-13. Finally, our infection model focuses
321 solely on the role of epithelial cells, but the effects of IL-13 on other cell types found in the lung
322 are also deserving of further study.

323 In conclusion, we found that the central asthma mediator IL-13 has a strong inhibitory
324 effect on SARS-CoV-2 infection of HBECs. The mechanisms that account for this effect are
325 unknown, but widespread effects of IL-13 on expression of SARS-CoV-2 associated genes that
326 are distinct from those induced by interferons suggest that some of these mechanisms may be
327 novel. While the use of IL-13 itself as a therapeutic may well be prevented by the pro-asthmatic
328 effects of this cytokine, identification of IL-13-induced antiviral pathways could help address the
329 urgent need for development of novel targeted treatments for COVID-19.

330

331 **Acknowledgements**

332 We thank Paul Wolters (UCSF) for providing HBECs from transplant recipients, Khadija Ghias
333 and Dingyuan Sun for assistance with HBEC culture, Melanie Ott (Gladstone Institute of

334 Virology), Semil Choksi (UCSF), and Jeremy Reiter (UCSF) for providing advice and the staff of
335 the UCSF Center for Advanced Light Microscopy for technical assistance.

336

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- 525
526

527 **Figure legends**

528

529 **Figure 1. SARS-CoV-2-associated genes are highly expressed in HBECs and many are**

530 **regulated by cytokines.** HBECs from six donors were cultured without cytokine (–), or with IL-

531 13, IFN- α , a combination of IL-13 and IFN- α , IFN- γ , or IL-17 and analyzed by RNA-seq. **(A)**

532 Comparison of read counts between SARS-CoV-2 associated genes, including *ACE2* and

533 *TMPRSS2*, and all detected genes (≥ 1 read per million mapped reads in $\geq 50\%$ of samples) in

534 unstimulated HBECs. **(B, C)** Heatmap illustrating canonical cytokine-regulated genes (B), and

535 cytokine regulated SARS-CoV-2-associated genes (C; FDR $q \leq 0.05$; absolute fold change ≥ 1.5

536 for any cytokine).

537

538 **Figure 2. scRNA-seq reveals cell type-specific expression of many SARS-CoV-2-**

539 **associated genes and cell type-specific effects of IL-13. (A)** Cell type-specific expression in

540 unstimulated HBECs. Genes were selected from a set of 113 differentially expressed SARS-

541 CoV-2-associated genes listed in Table E3. **(B)** Cell type-specific differences in IL-13

542 responses. For 11 SARS-CoV-2-associated genes, IL-13 increased expression in at least one

543 cell type and decreased expression in at least one other cell type (FDR $q < 0.1$ for both). Gene

544 expression was determined by aggregating data from all cells from experiments with four

545 donors. Coloring of each dot indicates expression level relative to other cell types (A) or in IL-

546 13-stimulated cells compared with unstimulated cells (B). The size of each dot is proportional to

547 the percentage of cells with at least one read mapped to the gene, and black circles at the

548 perimeter of each dot indicate that expression levels are significantly different ($q < 0.1$)

549 compared to other cell types (A) or in IL-13-stimulated compared to unstimulated cells of the

550 same type (B).

551

552 **Figure 3. Expression of many IL-13-regulated SARS-CoV-2-associated genes correlates**
553 **with an IL-13 signature in asthma.** Correlation of IL-13-induced, SARS-CoV-2 associated
554 genes with a type 2/IL-13 signature (the three gene metric, TGM) in endobronchial brushing
555 samples from participants with asthma (red) and healthy controls (cyan). Values for gene
556 expression represent \log_2 of normalized read counts from bulk RNA-seq. The eight SARS-CoV-
557 2 genes with the highest Pearson's correlations (R) are shown and associated P values are
558 adjusted for multiple comparisons. Correlations for the full set of IL-13-induced SARS-CoV-2-
559 associated genes are shown in Table E4.

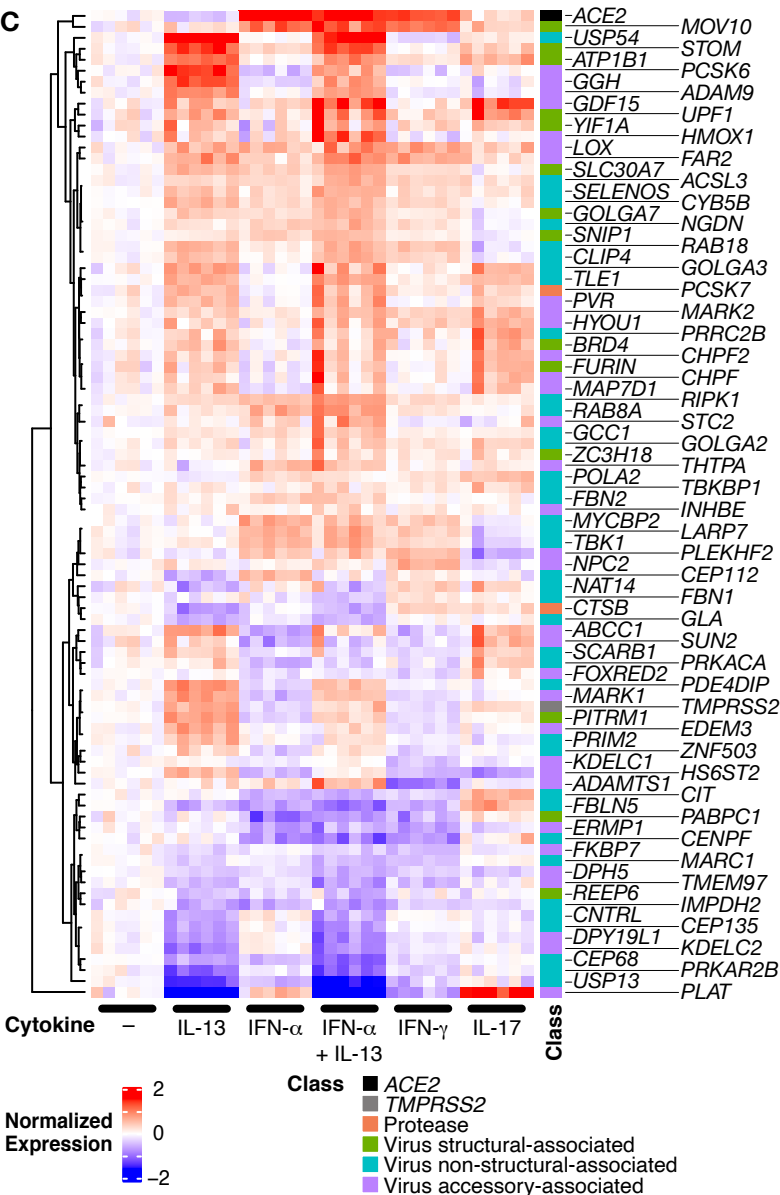
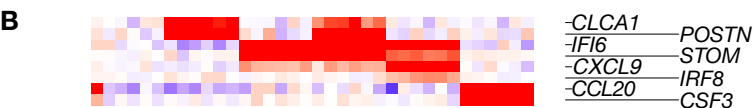
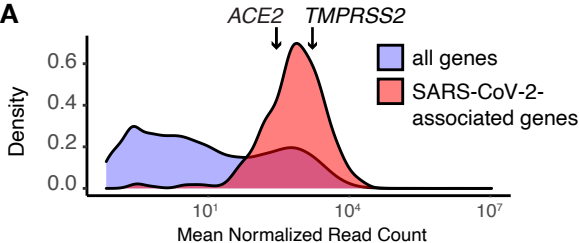
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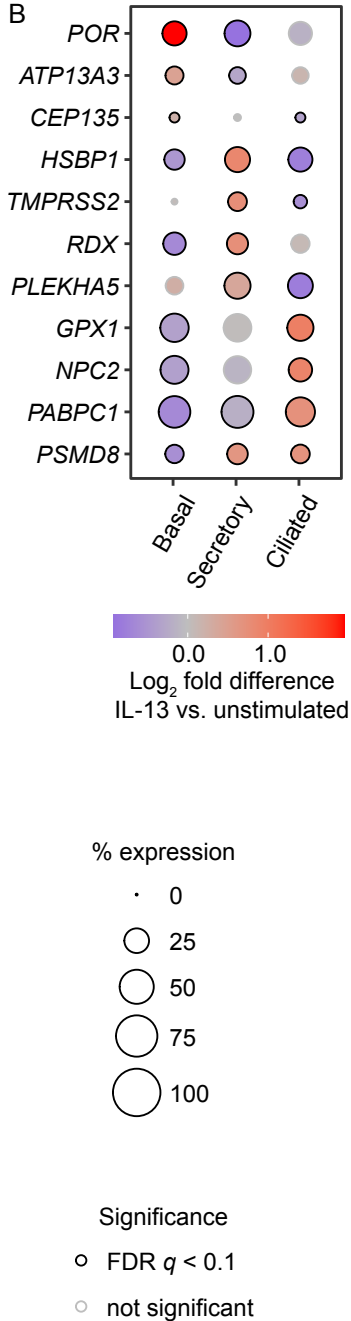
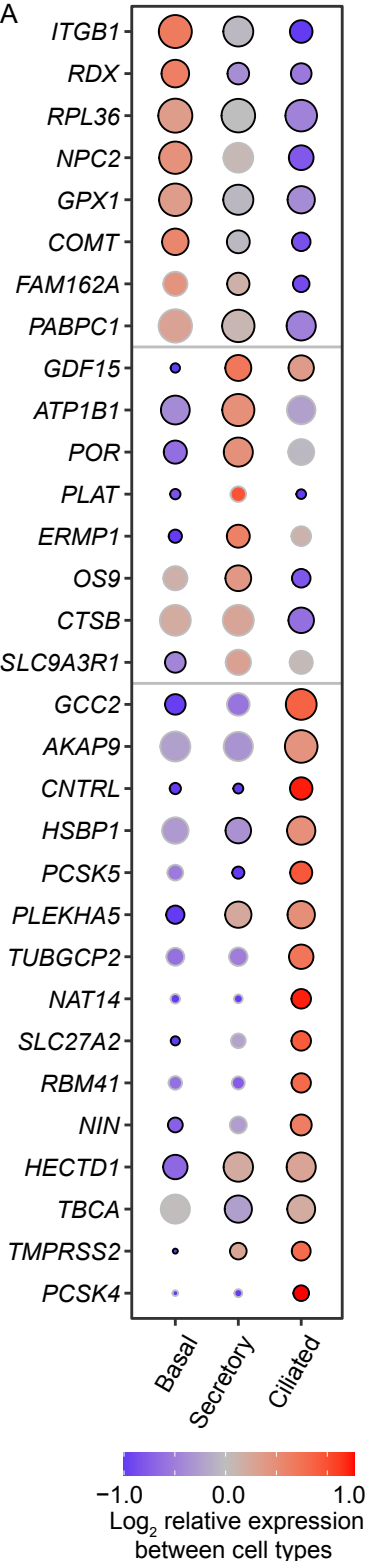
561 **Figure 4. IL-13 stimulation and mucus reduce SARS-CoV-2 virus RNA levels in infected**
562 **HBECs. (A)** HBECs from a single donor were left unstimulated (–) or stimulated with IL-13 (+),
563 washed with a DTT-containing solution (Mucus Removed) or left unwashed (Mucus Intact), and
564 inoculated with SARS-CoV-2 (0.3 plaque forming units [pfu] based on titration in Vero E6 cells).
565 SARS-CoV-2 mRNA was measured 48 h after infection. **(b)** In a second experiment, cells from
566 a different donor were studied using the same protocol, except that three different inocula (0.3,
567 1.0, and 1.3 pfu) from another virus preparation were used. Each point represents a separate
568 Transwell culture ($n = 3$ per condition except as shown). **, $p < 0.01$; *** $p < 0.0001$ for the
569 effects of IL-13 by ANOVA with Tukey-Kramer post-tests. For cells not stimulated with IL-13,
570 viral RNA load was lower in infections performed with mucus intact compared with infections
571 performed with mucus removed ($p < 0.0001$ for all viral inocula in both experiments, except for p
572 = 0.01 for the 0.3 pfu inoculum in the second experiment, by ANOVA with Tukey-Kramer post-
573 tests). For viral RNA, 1 unit represents the amount of viral RNA present in 1 pfu from the viral
574 stock, based on titration in Vero E6 cells.

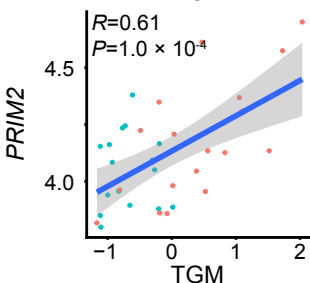
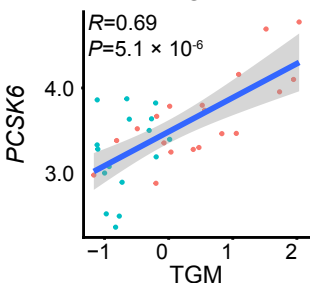
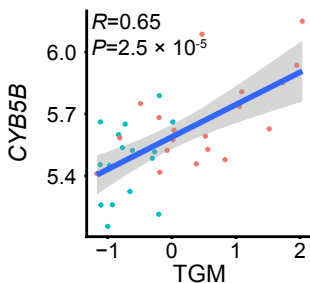
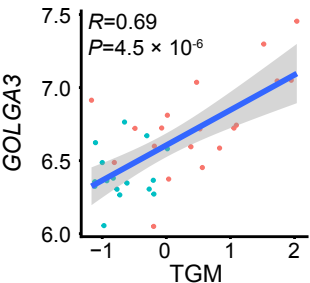
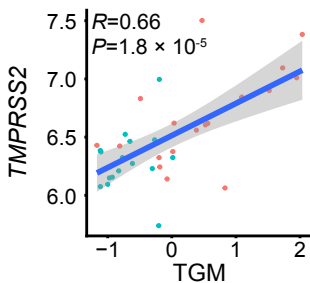
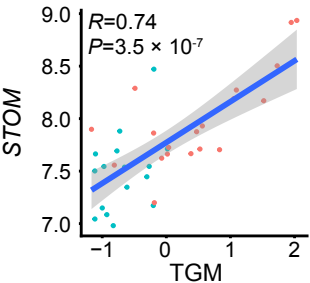
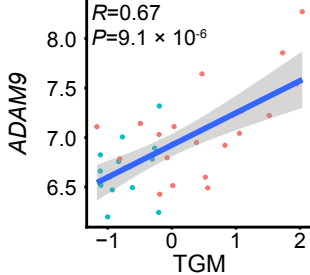
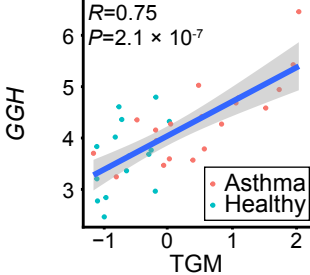
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576 **Figure 5. IL-13 stimulation reduces SARS-CoV-2 replication in HBECs.** Additional HBEC
577 cultures derived from cells from donor 1 **(A)** and donor 2 **(B)** were inoculated with virus after

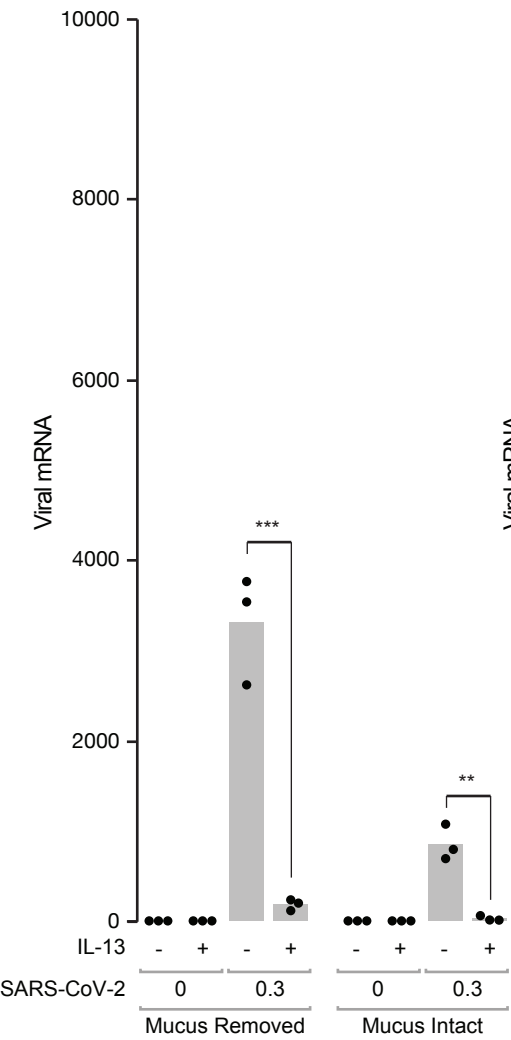
578 removal of mucus as part of the same experiments shown in Fig. 4. After 48 h, cells were
579 stained with antibodies against dsRNA (yellow); the ciliated cell marker acetylated alpha tubulin
580 (Ac- α -tubulin) and DAPI (both imaged in the same channel, purple); MUC5B (red); and
581 MUC5AC (cyan). We surveyed the entire sample (16.6 μm^2) for dsRNA staining and acquired
582 stacks encompassing each dsRNA-stained focus. Numbers of dsRNA-stained foci and total
583 volumes of dsRNA staining are shown below representative images for each condition. Scale
584 bar = 20 μM .







A



B

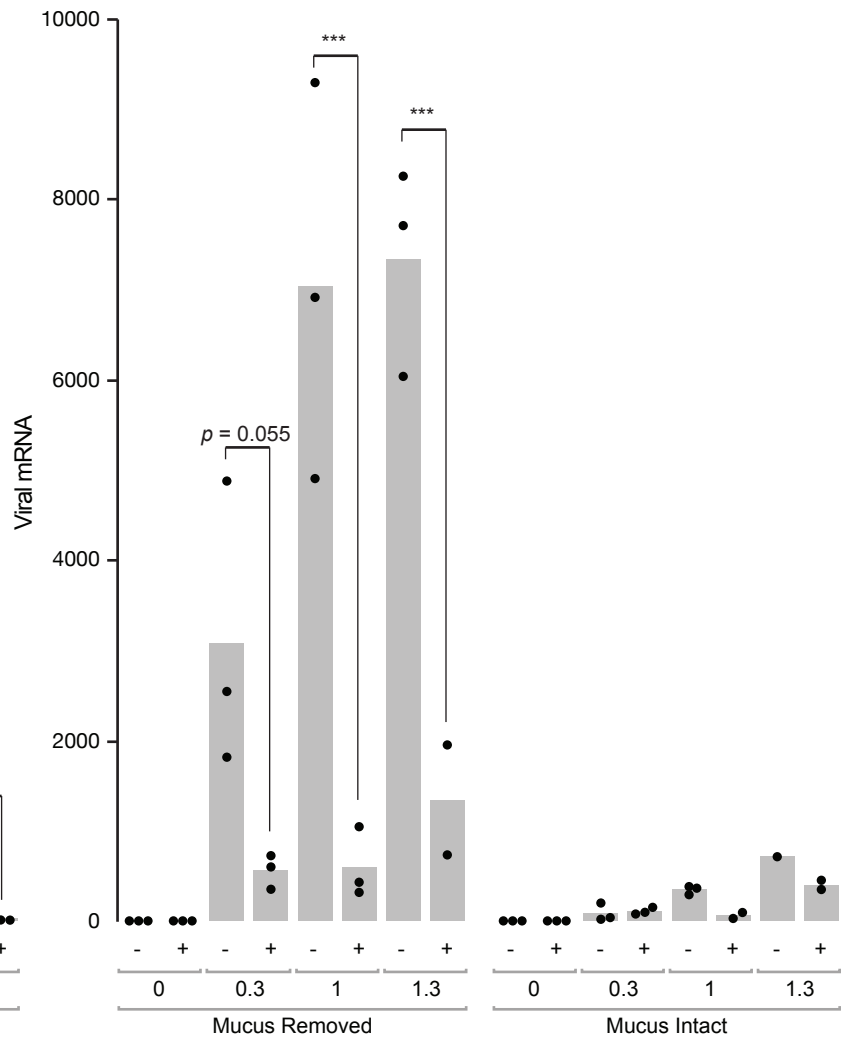


Figure 5

