

1 **TITLE**

2 Severe COVID-19 is associated with fungal colonization of the nasopharynx and potent induction of IL-17  
3 responses in the nasal epithelium

4  
5 **AUTHORS**

6 Carly G. K. Ziegler<sup>1,2,3,4,5,\*</sup>, Anna H. Owings<sup>6,\*</sup>, Vincent N. Miao<sup>1,2,3,5</sup>, Andrew W. Navia<sup>2,3,5,7</sup>, Ying Tang<sup>8</sup>,  
7 Joshua D. Bromley<sup>2,3,5,9</sup>, Peter Lotfy<sup>3,8</sup>, Meredith Sloan<sup>6</sup>, Hannah Laird<sup>10</sup>, Haley B. Williams<sup>10</sup>, Micayla  
8 George<sup>2,3,5</sup>, Riley S. Drake<sup>2,3,5</sup>, Yilianys Pride<sup>10</sup>, George E. Abraham III<sup>11</sup>, Michal Senitko<sup>11</sup>, Tanya O.  
9 Robinson<sup>10</sup>, Michail S. Lionakis<sup>12</sup>, Alex K. Shalek<sup>1,2,3,4,5,7,13,14,15,^</sup>, Jose Ordovas-Montanes<sup>2,3,8,14,15,^</sup>, Bruce  
10 H. Horwitz<sup>8,14,16,^</sup>, Sarah C. Glover<sup>10,17,^</sup>

11  
12 **AFFILIATIONS**

13 <sup>1</sup> Program in Health Sciences & Technology, Harvard Medical School & MIT, Boston, MA, USA

14 <sup>2</sup> Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA, USA

15 <sup>3</sup> Broad Institute of MIT and Harvard, Cambridge, MA, USA

16 <sup>4</sup> Harvard Graduate Program in Biophysics, Harvard University, Cambridge, MA, USA

17 <sup>5</sup> Institute for Medical Engineering & Science, Massachusetts Institute of Technology, Cambridge, MA, USA

18 <sup>6</sup> Department of Medicine, University of Mississippi Medical Center, Jackson, MS, USA

19 <sup>7</sup> Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, USA

20 <sup>8</sup> Division of Gastroenterology, Hepatology, and Nutrition, Boston Children's Hospital, Boston, MA, USA

21 <sup>9</sup> Department of Microbiology, Massachusetts Institute of Technology, Cambridge, MA, USA

22 <sup>10</sup> Division of Digestive Diseases, University of Mississippi Medical Center, Jackson, MS, USA

23 <sup>11</sup> Division of Pulmonary, Critical Care, and Sleep Medicine, University of Mississippi Medical Center, Jackson, MS, USA

24 <sup>12</sup> Fungal Pathogenesis Section, Laboratory of Clinical Immunology and Microbiology (LCIM), National Institute of Allergy and  
25 Infectious Diseases (NIAID), Bethesda, MD, USA

26 <sup>13</sup> Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA

27 <sup>14</sup> Program in Immunology, Harvard Medical School, Boston, MA, USA

28 <sup>15</sup> Harvard Stem Cell Institute, Cambridge, MA, USA

29 <sup>16</sup> Division of Emergency Medicine, Boston Children's Hospital, Boston, MA, USA

30 <sup>17</sup> Center for Immunology and Microbial Research, Department of Cell & Molecular Biology, University of Mississippi Medical Center,  
31 Jackson, MS, USA

32 \* these authors contributed equally

33 ^ these senior authors contributed equally

34  
35 Correspondence to: Jose Ordovas-Montanes ([jose.ordovas-montanes@childrens.harvard.edu](mailto:jose.ordovas-montanes@childrens.harvard.edu)), Bruce H. Horwitz

36 ([bruce.horwitz@childrens.harvard.edu](mailto:bruce.horwitz@childrens.harvard.edu)), Sarah C. Glover ([scglover@umc.edu](mailto:scglover@umc.edu)), and Alex K. Shalek ([shalek@mit.edu](mailto:shalek@mit.edu))

37 **ABSTRACT**

38 Recent case reports and epidemiological data suggest fungal infections represent an under-appreciated  
39 complication among people with severe COVID-19. However, the frequency of fungal colonization in  
40 patients with COVID-19 and associations with specific immune responses in the airways remain  
41 incompletely defined. We previously generated a single-cell RNA-sequencing (scRNA-seq) dataset  
42 characterizing the upper respiratory microenvironment during COVID-19, and mapped the relationship  
43 between disease severity and the local behavior of nasal epithelial cells and infiltrating immune cells. Our  
44 study, in agreement with findings from related human cohorts, demonstrated that a profound deficiency in  
45 host immunity, particularly in type I and type III interferon signaling in the upper respiratory tract, is  
46 associated with rapid progression to severe disease and worse clinical outcomes. We have now performed  
47 further analysis of this cohort and identified a subset of participants with severe COVID-19 and concurrent  
48 detection of *Candida* species-derived transcripts within samples collected from the nasopharynx and  
49 trachea. Here, we present the clinical characteristics of these individuals, including confirmatory diagnostic  
50 testing demonstrating elevated serum (1, 3)- $\beta$ -D-glucan and/or confirmed fungal culture of the predicted  
51 pathogen. Using matched single-cell transcriptomic profiles of these individuals' respiratory mucosa, we  
52 identify epithelial immune signatures suggestive of IL-17 stimulation and anti-fungal immunity. Further, we  
53 observe significant expression of anti-fungal inflammatory cascades in the nasal and tracheal epithelium of  
54 all participants who went on to develop severe COVID-19, even among participants without detectable  
55 genetic material from fungal pathogens. Together, our data suggests that IL-17 stimulation – in part driven  
56 by *Candida* colonization – and blunted type I/III interferon signaling represents a common feature of severe  
57 COVID-19 infection.

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72 **KEYWORDS:** SARS-CoV-2, COVID-19, human, nasal mucosa, epithelial immunity, *Candida*, fungal  
73 infection, IL-17, cytokine, interferon, anti-viral, scRNA-seq

## 74 INTRODUCTION

75 Infection with SARS-CoV-2, the virus that causes COVID-19, can lead to severe viral pneumonitis and the  
76 development of acute respiratory distress syndrome.<sup>1,2</sup> Severe COVID-19 is characterized by peripheral  
77 immune dysregulation, and we and others, have previously demonstrated blunted interferon responses  
78 within the nasal mucosa of patients with severe COVID-19.<sup>3–5</sup> Recent case reports and retrospective cohort  
79 studies suggest secondary infection with fungal pathogens may be a significant contributor to morbidity and  
80 mortality in patients with severe COVID-19.<sup>6–11</sup> The frequency of fungal colonization of the airways in  
81 patients with severe COVID-19 and potential impact on local mucosal immunity remains unknown.<sup>9,12–15</sup> IL-  
82 17, released by CD4 T cells and innate lymphocytes, is a key effector cytokine that coordinates mucosal  
83 anti-fungal immunity among other adaptive and innate leukocytes, granulocytes, and mucosal stroma.<sup>16–19</sup>  
84 Recent work has uncovered complex interactions between IL17-driven inflammation, type 1 interferon  
85 responses, and susceptibility to fungal pathogens, however the effect of fungal colonization and anti-fungal  
86 immune responses during co-occurrent SARS-CoV-2 infection have yet to be explored<sup>20</sup>. Here, using a  
87 previously-published dataset derived from a cohort of individuals acutely infected with SARS-CoV-2, we  
88 directly assessed co-occurrent fungal colonization in the airways of patients with severe COVID-19 and  
89 examine pathways associated with anti-fungal immunity.<sup>3</sup>

## 91 RESULTS

92 We had previously described a cohort of 58 individuals – 56 of which are further characterized here –  
93 including 15 healthy participants, 35 individuals diagnosed with acute COVID-19, and 6 intubated patients  
94 that were negative for SARS-CoV-2.<sup>3</sup> Nasopharyngeal (NP) swabs obtained from these patients were  
95 employed in a cross-sectional study of the nasal respiratory cellular composition using single-cell RNA  
96 sequencing (scRNA-seq) (**Figure 1A, 1B**). Patients in this cohort with COVID-19 were sampled within 9  
97 days of hospital admission (median: hospital day 2), which we estimated was within 2 weeks of initial  
98 respiratory symptoms. Full cohort demographic data and findings relating to the cellular composition,  
99 behaviors, and response to SARS-CoV-2 infection between disease groups can be found in our prior  
100 manuscript. We had previously applied a meta-transcriptomic taxonomic classification analysis to each  
101 sample to assign both cell-associated and ambient/extracellular sequencing reads to a reference database  
102 of human and microbial genomes (generated on 5/5/2020 from the NCBI Reference Sequence Database  
103 including archaeal, bacterial, viral, protozoan, and fungal genomes).<sup>21,22</sup> This approach, in addition to direct  
104 reference-based alignment, enabled us to quantify respiratory abundances of SARS-CoV-2, and connect  
105 viral abundances to the cellular sources of viral replication, as well as concurrent epithelial host responses  
106 (**Figure 1B**). Here, we describe further analysis of the data generated from these nasopharyngeal samples,  
107 as well as additional data generated from matched endotracheal aspirates (ETA) obtained from 4 of the  
108 individuals in the original cohort with severe COVID-19.

109

110 Using meta-transcriptomic alignment of scRNA-seq data, we identified additional high-abundance microbial  
111 taxa across healthy participants and those with COVID-19 including common commensal microbes such  
112 as *Cutibacterium acnes*, *Malassezia restricta*, and *Staphylococcus aureus* (**Supplemental Figure 1A**,  
113 **Supplemental Table 1**). After SARS-CoV-2, the second-most abundant microbe detected was *Candida*  
114 *albicans*, which was detected on six NP samples obtained from patients with severe COVID-19 (**Figures**  
115 **1C, 1D, Supplemental Figure 1A**). We also identified high levels of *Candida glabrata* in NP samples from  
116 2 patients with severe COVID-19, and *Candida dubliniensis* in 4 samples. All samples that were positive  
117 for *C. glabrata* or *C. dubliniensis* were also positive for *C. albicans* with the exception of the NP sample  
118 obtained from COVID-19 participant 12. *Candida albicans* was also detected in 3 of 4 ETA samples  
119 obtained from patients with severe COVID-19. For one patient (COVID-19 participant 32) *C. albicans* was  
120 detected via ETA, but was not detected on their matched NP swab. All 3 ETA samples that were positive  
121 for *C. albicans* were also positive for *C. dubliniensis*, and one of these was also positive for *C. glabrata*  
122 (**Figures 1C, 1D, and Supplemental Figures 1A, 1B**). Notably, no *Candida spp.* or other fungal pathogens  
123 were detected within samples obtained from healthy individuals, those obtained from individuals with  
124 mild/moderate COVID-19, or those obtained from SARS-CoV-2 negative intubated patients in the intensive  
125 care unit with severe respiratory failure due to alternative causes. Thus, all *Candida spp.* reads were  
126 detected among patients who developed severe COVID-19 requiring intubation and mechanical ventilation  
127 (WHO severity score of 6-8).

128  
129 Nearly all NP or ETA samples that were positive for *Candida spp.* were collected within one week of hospital  
130 admission (**Supplemental Figure 2A**). The majority (6/8) had been intubated for at least 1 day and 5/8 had  
131 received at least 1 day of corticosteroid treatment prior to sample collection. Clinical evaluations during  
132 hospitalization were performed to search for possible fungal infection for 3 of the 8 patients with high  
133 abundances of *Candida spp.* by meta-transcriptomic classification. COVID-19 participant 12, who had high  
134 abundance of *Candida glabrata* RNA sampled by NP swab on hospital day 2, was intubated on hospital  
135 day 1 and ETA fungal cultures sampled on hospital day 2 revealed growth of *Candida glabrata* (**Figure 1D**).  
136 For two participants, detection of *Candida spp.*-derived RNA via NP/ETA sampling significantly preceded  
137 clinical diagnostic testing for fungal pathogens. COVID-19 participant 38, whose NP swab revealed both  
138 *Candida albicans* and *Candida dubliniensis* RNA on hospital day 8, tested positive for serum (1,3)- $\beta$ -D-  
139 glucan on hospital day 14 and had *Candida albicans* growth from ETA culture on hospital day 16. Both the  
140 NP and ETA samples from COVID-19 participant 18 obtained on hospital day 6 contained high abundances  
141 of reads classified as *C. albicans*, and *C. dubliniensis* was detected in the ETA sample. On hospital day 12,  
142 (1,3)- $\beta$ -D-glucan was detected in this individual's serum, prompting treatment with micafungin (an  
143 echinocandin antifungal).<sup>23</sup> Together, this demonstrates that for a subset of patients, there was significant  
144 clinical concern during their hospitalization to prompt additional testing to evaluate for *Candida* respiratory  
145 infection or fungemia. Among those individuals, we find concordance between detection of *Candida* derived  
146 RNA from scRNA-seq libraries and clinical assays during their hospitalization.

147

148 We did not detect a difference in demographics or clinical characteristics – apart from severity of COVID-  
149 19 – between patients whose samples did or did not contain *Candida*-specific reads (**Table 1,**  
150 **Supplemental Figure 2B-2G**). 28-day mortality rates among individuals with severe COVID-19 were  
151 similar between *Candida spp.* positive vs. negative groups: 62.5% (5/8) among participants with *Candida*  
152 *spp.* detected, compared to 84.6% (11/13) among participants without *Candida spp.* detected. Nearly all  
153 (7/8) participants with COVID-19 whose samples contained *Candida spp.*-aligning RNA were previously  
154 diagnosed with type 2 diabetes mellitus (T2DM), and 8/8 were diagnosed with chronic hypertension.  
155 Notably, although T2DM represents an independent risk factor for mucosal *Candida* colonization, we did  
156 not find *Candida* or other fungal species among individuals with T2DM within the Healthy, non-COVID-19  
157 intubated, or COVID-19 mild/moderate groups.<sup>24</sup> Additionally, for individuals with recently measured  
158 HbA1c, we did not identify significant differences in the degree of glycemic control between individuals with  
159 different COVID-19 severity or by detection of *Candida*-specific reads (**Supplemental Figure 2G**).<sup>25</sup>  
160 Critically, this analysis is based on a limited sample size, and merits further investigation with adequately-  
161 powered cohorts.

162

163 Given the high frequency of *Candida spp.* colonization and clinically-relevant infection among individuals  
164 who developed severe COVID-19, we wondered whether the nasal mucosa of these individuals exhibits  
165 evidence for reactive or aberrant IL-17 responses. To better define the response of the human nasal  
166 epithelium to IL-17 we reanalyzed our previously-published population RNA-seq data which reflects gene  
167 expression in human nasal epithelial cells following *in vitro* exposure to a range of doses of IL17A  
168 (**Supplemental Figure 3**).<sup>26,27</sup> Across multiple human donors, IL17A exposure led to upregulation of genes  
169 involved in keratinization (*SPRR2E*, *SPRR2F*, and *SPRR2G*), chemoattractant cytokines for lymphocytes,  
170 monocytes, and neutrophils (*CCL20*, *CXCL1*, *CXCL2*, and *CXCL3*), and pro-inflammatory factors such as  
171 *S100A7* and *S100A8* (**Figure 2A, Supplemental Figure 3A**).<sup>28–30</sup> IL17A additionally resulted in dose-  
172 dependent induction of serum amyloid A genes *SAA1* and *SAA2* from nasal epithelial cells, which has  
173 previously been linked to pathogenic Th17 responses at barrier tissues.<sup>31</sup>

174

175 Using this RNA-seq data, we generated consensus gene sets for each tested cytokine that were robust  
176 across distinct donors, thereby giving us cell-type specific gene expression modules for IL17A, IFN $\alpha$ , IFN $\gamma$ ,  
177 IL1 $\beta$ , and IL4 (**Supplemental Figure 3, Supplemental Table 2**). Next, we returned to the scRNA-seq data  
178 from our human COVID-19 cohort and evaluated epithelial cells for transcriptomic signatures consistent  
179 with exposure to each cytokine. Compared to epithelial cells isolated from healthy controls, epithelial cells  
180 isolated from individuals with severe COVID-19 expressed significantly higher levels of genes that were  
181 also induced by IL17A exposure *in vitro* (**Figures 2B-D, Supplemental Figure 3B**). In particular, IL17A-  
182 induced genes *SAA1*, *SAA2*, *SAT1*, *LCN2*, *S100A8*, and *GLUL* were repeatedly significantly upregulated  
183 among diverse epithelial cell subsets in severe COVID-19, but were not found to be significantly induced

184 within the nasal epithelia of patients with milder COVID-19. We confirmed that treatment of nasal epithelial  
185 cells with other inflammatory signals potentially found within the respiratory epithelium, including IL4 and  
186 IL1 $\beta$ , do not appreciably induce these factors, suggesting that induction of this gene module is a specific  
187 downstream effect of IL17 sensing (**Supplemental Figure 3C**).

188  
189 Next, we directly scored epithelial cells for expression of gene signatures indicative of IL17A, IFN $\alpha$ , or IFN $\gamma$ .  
190 (**Figures 2E-G**). Individuals who developed severe COVID-19 expressed consistently higher abundances  
191 of IL17A-induced genes compared to healthy participants or those with mild/moderate COVID-19 (**Figure**  
192 **2E**). Interestingly, we did not detect higher abundances of genes in the IL17A gene module among  
193 participants with severe COVID-19 and detectable *Candida spp.* compared to participants with severe  
194 COVID-19 without detectable fungal pathogens. Likewise, we did not detect significant differences in levels  
195 of interferon-induced signatures between individuals with and without *Candida spp.* detected (**Figures 2F,**  
196 **2G**). On an individual level, participants whose nasal epithelial cells expressed higher abundances of IFN $\alpha$ -  
197 induced genes did not express IL17A-induced genes, and vice versa (**Figure 2H**). Together, this suggests  
198 that induction of IL17A-stimulated genes in the nasal epithelium represents a shared feature of individuals  
199 who develop severe COVID-19, and is correlated with the absence of robust interferon-induced anti-viral  
200 responses.

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202

## 203 **DISCUSSION**

204 Our data demonstrate *Candida spp.* colonization of the upper respiratory tract in a significant proportion  
205 (38%) of individuals hospitalized with severe COVID-19 in a cohort from the University of Mississippi  
206 Medical Center sampled during the summer 2020 COVID-19 peak. Notably, no fungal pathogens were  
207 identified among individuals with mild or moderate COVID-19, non-COVID-19 ICU patients, or healthy  
208 controls. Fungal reads were detected by NP swab at early timepoints following hospital admission and  
209 within the acute phase of patients COVID-19 disease trajectory, suggesting that in some patients, fungal  
210 colonization and infection likely occurred prior to hospital admission and in advance of nosocomial  
211 exposures. *Candida* is not typically present in the nares of healthy people, being more readily detected in  
212 the oropharynx, thus identification of *Candida* from NP swabs of a subset of patients with severe COVID-  
213 19 would suggest either that severe COVID-19 predisposes to ectopic colonization in some hosts, or  
214 alternatively that our methodology is detecting increased fungal abundances derived from the oral  
215 mucosa<sup>32</sup>. Further direct comparisons of colonization in the mouth and nose of patients with severe COVID-  
216 19 will be necessary to clarify this issue.<sup>33</sup>

217

218 Our analysis additionally unites the use of single-cell transcriptomic technologies in human clinical cohorts  
219 with emerging computational approaches for meta-genomic pathogen classification of human samples, all  
220 derived from limited cellular material captured on a single NP swab<sup>21,22</sup>. By linking unbiased pathogen

221 detection with single-cell nasal epithelial and immune transcriptional profiles, we have identified specific  
222 host behaviors indicative of response to a fungal pathogen and IL-17 signaling. For a subset of patients,  
223 early detection of *Candida spp.* in the upper respiratory mucosa corresponded to development of more  
224 extensive colonization and clinical concern for secondary fungal pneumonia and/or candidemia. Mounting  
225 evidence across various clinical cohorts, including our prior work, suggests that severe COVID-19 arises in  
226 individuals with impaired anti-viral immunity.<sup>3,34-41</sup> While there is a paucity of evidence to suggest that type  
227 I/III interferon signaling directly restricts fungal colonization, prior *in vitro* studies indicate IL-17 signaling  
228 among airway epithelial cells may attenuate cellular responses to type I/III interferon<sup>20,42-45</sup>. Additionally,  
229 enhanced virally-induced epithelial injury resulting from impaired IFN signaling could facilitate *Candida*  
230 colonization.<sup>46,47</sup> Surprisingly, we observe that IL17A-induced gene sets are elevated among epithelial cells  
231 from *all* individuals with severe COVID-19 in our cohort, even those patients without genomic or clinical  
232 evidence for co-incident fungal infection. Crucially, our data does not allow us to determine whether our  
233 observation of elevated IL-17 responses in patients with severe COVID-19 *without* overt evidence for fungal  
234 colonization is 1) the result of colonization below levels of detection in these assays, or 2) suggest that IL17  
235 elevation represents a general phenotype of epithelial cells of patients with severe COVID-19, independent  
236 of fungal colonization. Future experiments encompassing longitudinal sampling of patients with COVID-19  
237 could shed additional light on whether variability in the dynamics of IL-17 and interferon signaling may  
238 underlie *Candida* colonization in the upper airways.

239  
240 Together, our data suggests upper respiratory *Candida* colonization and infection represents an  
241 underappreciated phenomenon among patients with severe COVID-19. Further research with larger  
242 cohorts is warranted to understand the frequency and timing of co-occurring infection with *Candida spp.*  
243 and other fungal pathogens following SARS-CoV-2 infection. Further, our data suggests that dedicated,  
244 multi-institutional studies are required to disentangle how clinical and subclinical fungal infections impact  
245 patient outcomes during hospitalization for COVID-19, and may hold key insights into determinants of  
246 severe respiratory failure for these patients and new strategies for diagnostic or therapeutic intervention.

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265

266 **AUTHOR CONTRIBUTIONS**

267 Conceptualization: J.O.-M., A.K.S., S.C.G., B.H.H.  
268 Methodology: C.G.K.Z., A.H.O., S.C.G., J.O.-M., A.K.S., B.H.H.  
269 Formal analysis: C.G.K.Z., A.H.O.  
270 Investigation: C.G.K.Z., A.H.O.  
271 Resources: S.C.G., A.H.O., M.SI., H.L., A.P., C.B.S., M.W.B., M.Se., M.H., G.E.A.  
272 Data Curation: T.O.R., Y.T., M.SI. A.H.O., H.L., H.B.W., T.C., C.G.K.Z.  
273 Writing – Original Draft: C.G.K.Z., A.H.O., J.O.-M., A.K.S., S.C.G., B.H.H.  
274 Writing – Review & Editing: C.G.K.Z., V.N.M., A.H.O., A.W.N., Y.T., J.D.B., P.L., M.SI., H.L., H.B.W., M.G.,  
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276 Visualization: C.G.K.Z.  
277 Supervision: J.O.-M., A.K.S., S.C.G., B.H.H., T.O.R.  
278 Project Administration: Y.P., T.O.R.  
279 Funding Acquisition: J.O.-M., A.K.S., S.C.G., B.H.H.

280

281 **COMPETING INTERESTS**

282 A.K.S. reports compensation for consulting and/or SAB membership from Merck, Honeycomb  
283 Biotechnologies, Cellarity, Repertoire Immune Medicines, Hovione, Ochre Bio, Third Rock Ventures, FL82,



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## 286 METHODS

287

### 288 Participant Recruitment and Respiratory Sampling

289 Full participant characteristics are provided in previously published study<sup>3</sup>. The UMMC Institutional Review  
290 Board approved the study under IRB#2020-0065. All participants or their legally authorized representative  
291 provided written informed consent. Briefly, participants were eligible for inclusion in the COVID-19 group if  
292 they were at least 18 years old, had a positive nasopharyngeal swab for SARS-CoV-2 by PCR, had COVID-  
293 19 related symptoms including fever, chills, cough, shortness of breath, and sore throat, and weighed more  
294 than 110 lb. Participants were eligible for inclusion in the Healthy group if they were at least 18 years old,  
295 had a current negative SARS-CoV-2 test (PCR or rapid antigen test), and weighed more than 110 lb.  
296 COVID-19 participants were classified according to the 8-level ordinal scale proposed by the WHO  
297 representing their peak clinical severity and level of respiratory support required. Nasopharyngeal samples  
298 and endotracheal aspirate samples were collected by a trained healthcare provider, all processing and  
299 handling was carried out as previously described.<sup>3,26</sup>

300

### 301 scRNA-seq Data Generation and Alignment

302 Annotated scRNA-seq data was recovered from Single Cell Portal (see **Data Availability** below) and single  
303 cell annotations were used as described in Ziegler et al. *Cell* 2021. Briefly, data represent aligned scRNA-  
304 seq libraries generated using Seq-Well S<sup>3</sup>, libraries were generated using Illumina Nextera XT Library Prep  
305 Kits and sequenced on NextSeq 500/550 High Output 75 cycle v2.5 kits to an average depth of 180 million  
306 aligned reads per array: read 1: 21 (cell barcode, UMI), read 2: 50 (digital gene expression), index 1: 8  
307 (N700 barcode).<sup>48</sup> Libraries were aligned using STAR within the Drop-Seq Computational Protocol  
308 (<https://github.com/broadinstitute/Drop-seq>) and implemented on Cumulus  
309 ([https://cumulus.readthedocs.io/en/latest/drop\\_seq.html](https://cumulus.readthedocs.io/en/latest/drop_seq.html), snapshot 9, default parameters).<sup>49,50</sup> As  
310 previously described, data were aligned using a custom reference which combined human GRCh38 (from  
311 Cell Ranger version 3.0.0, Ensembl 93) and SARS-CoV-2 RNA genomes.<sup>3,51</sup>

312

### 313 Meta-Transcriptomic Pathogen Classification

314 To identify co-detected microbial taxa present in the cell-associated or ambient RNA of nasopharyngeal  
315 swabs, we used the Kraken2 software implemented using the Broad Institute viral-ngs pipelines on Terra  
316 (<https://github.com/broadinstitute/viral-pipelines/tree/master>).<sup>21,22</sup> A previously-published reference  
317 database included human, archaea, bacteria, plasmid, viral, fungi, and protozoa species and was  
318 constructed on May 5, 2020, therefore included sequences belonging to the novel SARS-CoV-2 virus.  
319 Inputs to Kraken2 were: `kraken2_db_tgz = "gs://pathogen-public-dbs/v1/kraken2-broad-20200505.tar.zst"`,  
320 `krona_taxonomy_db_kraken2_tgz = "gs://pathogen-public-dbs/v1/krona.taxonomy-20200505.tab.zst"`,  
321 `ncbi_taxdump_tgz = "gs://pathogen-public-dbs/v1/taxdump-20200505.tar.gz"`, `trim_clip_db =`  
322 `"gs://pathogen-public-dbs/v0/contaminants.clip_db.fasta"` and `spikein_db = "gs://pathogen-public-`

323 dbs/v0/ERCC\_96\_nopolyA.fasta". Results were collected using the merge\_metagenomics tool  
324 ([https://viral-pipelines.readthedocs.io/en/latest/merge\\_metagenomics.html](https://viral-pipelines.readthedocs.io/en/latest/merge_metagenomics.html)), and analysis and visualization  
325 of each samples' metagenomic alignments was implemented in Prism (v6) or R (v4.0.2; packages ggplot2  
326 (v3.3.2), Seurat (v3.2.2), ComplexHeatmap (v2.7.3)). All classification data is included as **Supplemental**  
327 **Table 1**.

328

### 329 **Human Nasal Epithelial Cell Response to *In Vitro* Cytokine Exposure**

330 Gene lists representing human nasal epithelial cell responses to various exogenous cytokines *in vitro* are  
331 derived from a previously-published population RNA-seq data.<sup>26,27</sup> Briefly, human nasal epithelial basal  
332 cells from 2 donors were stimulated *in vitro* with 0.1-10 ng/mL IFN $\alpha$ , IL17A, IFN $\gamma$ , IL1 $\beta$ , or IL4 for 12 hours.  
333 Following stimulation, cells were lysed and bulk RNA-seq libraries were generated using the SMART-Seq2  
334 protocol.<sup>52</sup> We identified epithelial gene sets induced by each cytokine independently by testing for  
335 differentially expressed genes compared to matched, untreated nasal epithelial samples (n=10). Differential  
336 expression testing was carried out using a likelihood ratio test assuming a negative binomial distribution,  
337 implemented with the Seurat (v3.1.5) FindAllMarkers function (test.use = "negbinom"). We considered  
338 genes as differentially expressed with an FDR-adjusted p value < 0.05.<sup>53</sup>

339

340 To score for cytokine-specific gene expression among COVID-19 or Healthy scRNA-seq samples, we first  
341 subsetted our scRNA-seq data to only epithelial cells using "Coarse" cell types, as defined by cell typing  
342 procedure carried out in prior publication (Ziegler et al. *Cell* 2021). Coarse cell type groups that were  
343 included in the analysis: "Ciliated Cells", "Developing Ciliated Cells", "Secretory Cells", "Goblet Cells",  
344 "Ionocytes", "Deuterosomal Cells", "Squamous Cells", "Basal Cells", "Mitotic Basal Cells", and "Developing  
345 Secretory and Goblet Cells". We calculated module scores over all epithelial cells using the Seurat function  
346 AddModuleScore with default inputs. The average module score for each NP or ETA sample was utilized  
347 as a representative measure of epithelial behavior for each participant, as represented in **Figures 2E-2H**.

348

### 349 **scRNA-seq Analysis of Differential Expression**

350 To compare gene expression between cells from distinct disease groups (e.g. Healthy vs. Severe COVID-  
351 19, *Candida spp.*), we employed a likelihood ratio test assuming a negative binomial distribution as  
352 described above (using Seurat FindAllMarkers function (test.use = "negbinom")).<sup>53</sup> We considered genes  
353 as differentially expressed with an FDR-adjusted p value < 0.001 and log fold change > 0.25. Results from  
354 select "Detailed" cell types, as defined and previously reported by Ziegler et al. are represented in **Figures**  
355 **2B-D**.<sup>3</sup> Full results of differential expression as represented in **Figures 2B-D** can be found in Supplementary  
356 Tables accompanying the published dataset.

357

### 358 **Statistical Testing**

359 All statistical tests were implemented in R (v4.0.2) or Prism (v6) software. Specific statistical tests, p-values,

360 n, and all summary statistics are provided in the results section, figure legends, and/or figure panels.

361

### 362 **Data Availability**

363 All scRNA-seq and RNA-seq data analyzed in this study is publicly available from prior manuscripts. Aligned  
364 and annotated scRNA-seq data can be downloaded via the Single Cell Portal, study SCP1289  
365 ([https://singlecell.broadinstitute.org/single\\_cell/study/SCP1289](https://singlecell.broadinstitute.org/single_cell/study/SCP1289)). Aligned TPM-normalized RNA-seq data  
366 can be downloaded via the Single Cell Portal, study SCP822  
367 ([https://singlecell.broadinstitute.org/single\\_cell/study/SCP822](https://singlecell.broadinstitute.org/single_cell/study/SCP822)). Results from Kraken2 meta-genomic  
368 classification are reported in **Supplemental Table 1**.

369 **MAIN FIGURE TITLES AND LEGENDS**

370

371 **Figure 1. Co-detection of host single-cell transcriptome with intracellular and microenvironmental**  
372 **pathogen-derived genomic material**

373 **A.** Schematic of biological sample processing pipeline

374 **B.** Schematic of computational pipeline for each sample

375 **C.** Abundance of human, SARS-CoV-2, and *Candida spp.* by participant and disease group as defined  
376 by meta-transcriptomic classification. N=56 participants. Lines represent median +/- interquartile  
377 range.

378 **D.** Summary of results from sequencing, fungal culture, and serum (1,3)-  $\beta$ -D-glucan assays from 8  
379 participants with detectable *Candida* species reads by nasopharyngeal swab or endotracheal  
380 aspirate.

381 **Figure 2. Respiratory epithelial transcriptional signatures following *in vitro* IL17A stimulation and**  
382 ***in vivo* fungal colonization in a severe COVID-19 cohort**

383 **A.** Heatmap of population RNA-seq data comparing untreated nasal epithelial cells to those treated  
384 with increasing concentrations of IL17A as indicated across columns. Genes (rows) with significant  
385 differential expression between untreated and IL17A-treated conditions (FDR-corrected  $p < 0.05$ ).

386 **B.-D.** Volcano plots of differentially expressed genes between select epithelial cell types from healthy  
387 participants vs. those with severe COVID-19: developing ciliated cells (**B**),  $CCL5^{\text{high}}$  squamous cells  
388 (**C**), and early response  $FOXP1^{\text{high}}$  ciliated cells (**D**). Grey points: all genes. Black points: genes  
389 induced in human nasal epithelial cells following IL17A treatment (as in **Figure 2A**).

390 **E.-G.** Average gene module scores calculated for each participant, separated by disease group.  
391 Module score expression was computed over all epithelial cells. Input module genes derived from  
392 *in vitro* stimulation with each labeled cytokine: IL17A (**E**),  $IFN\alpha$  (**F**),  $IFN\gamma$  (**G**). Statistical testing by  
393 Kruskal-Wallis test with Dunn's post-hoc testing. \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$ . Lines  
394 represent mean  $\pm$  SEM.

395 **H.** Average gene module scores by participant,  $IFN\alpha$  module on x-axis, IL17A module on y-axis. Points  
396 only reflecting NP samples. Point shapes and colors by disease group: Healthy: dark blue circles;  
397 Mild/Moderate COVID-19: red circles; Severe COVID-19, *Candida spp.* neg.: pink filled squares;  
398 Severe COVID-19, *Candida spp.* pos.: pink outlined squares. Statistical testing by Spearman's  
399 correlation over all points:  $\rho = 0.43$ , \*\* $p = 0.0018$ .

400 **MAIN TABLE TITLE AND LEGEND**

401

402 **Table 1. Demographics and medical comorbidities of patients with severe COVID-19**

403 **SUPPLEMENTAL FIGURE TITLES AND LEGENDS**

404

405 **Supplemental Figure 1. Meta-transcriptomic classification of reads from nasopharyngeal swabs and**  
406 **endotracheal aspirates**

407 **A.** Heatmap of top detected microbes (rows) across all samples (columns). Bar plot on left: total  
408 classified reads per million (M) for each microbe listed along heatmap rows. Heatmap colors  
409 represent total classified reads per M, dark red: higher abundance, yellow: lower abundance, white:  
410 < 10 reads per M.

411 **B.** SARS-CoV-2 and *Candida spp.* reads detected from matched nasopharyngeal (NP, black bars)  
412 and endotracheal aspirate (ETA, grey bars) samples from the same participants.



413 **Supplemental Figure 2. Participant characteristics and clinical course**

414 **A.** Hospital timelines for 8 participants with *Candida spp.* detected from scRNA-seq NP or ETA  
415 samples.

416 **B.-D.** Select categorical demographic and clinical information from study participants by disease cohort:  
417 28-day mortality (**B**), type 2 diabetes mellitus (T2DM) (**C**), and chronic hypertension (**D**). Statistical  
418 testing by multi-group chi-square test, significance result is reported above each plot.

419 **E.-F.** Select continuous demographic and clinical information among participants with severe COVID-  
420 19, separated by detection of *Candida spp.*: age (**E**) and BMI (**F**). Statistical testing using student's  
421 t-test. Lines represent median +/- interquartile range.

422 **G.** Hemoglobin A1c (HbA1c) for each study participant by disease cohort. Dashed line at HbA1c 6.5%.  
423 Group differences non-significant by Kruskal-Wallis test. Numbers in parenthesis reflect patients  
424 per group with available data in medical record.

425 **Supplemental Figure 3. Transcriptional responses of respiratory epithelial cells following *in vitro***  
426 **exposure to various cytokines**

427 **A.** Expression of select genes following 12-hour stimulation with increasing doses of IL17A. Each  
428 gene significantly upregulated following IL17A expression by likelihood ratio test, FDR-adjusted p-  
429 value < 0.001. Lines represent mean +/- SEM.

430 **B.** Heatmap of IL17A-induced genes among developing ciliated cells from NP swabs. Heatmap colors  
431 reflect scaled gene expression: red: higher expression, blue: lower expression. Left columns (blue  
432 bar): developing ciliated cells from n=15 Healthy participants; right (pink bar): developing ciliated  
433 cells from n=8 participants with severe COVID-19 and *Candida spp.* detected. Genes (rows)  
434 selected represent genes significantly upregulated among cultured human nasal epithelia cells  
435 following *in vitro* exposure to IL17A. Statistical significance comparing Healthy-derived vs. Severe  
436 COVID-19, *Candida spp.* positive-derived single cells by likelihood ratio test assuming an  
437 underlying negative binomial distribution. \*\*\* FDR-corrected p < 0.001, \*\* FDR < 0.01, \* FDR <  
438 0.05.

439 **C.** Expression of select genes among human nasal basal cells by stimulation condition.

440 **SUPPLEMENTAL TABLES TITLES**

441

442 **Supplemental Table 1. Raw meta-transcriptomic classification data from Kraken2**

443

444 **Supplemental Table 2. Gene lists for scoring epithelial responses to cytokines**

445 **REFERENCES**

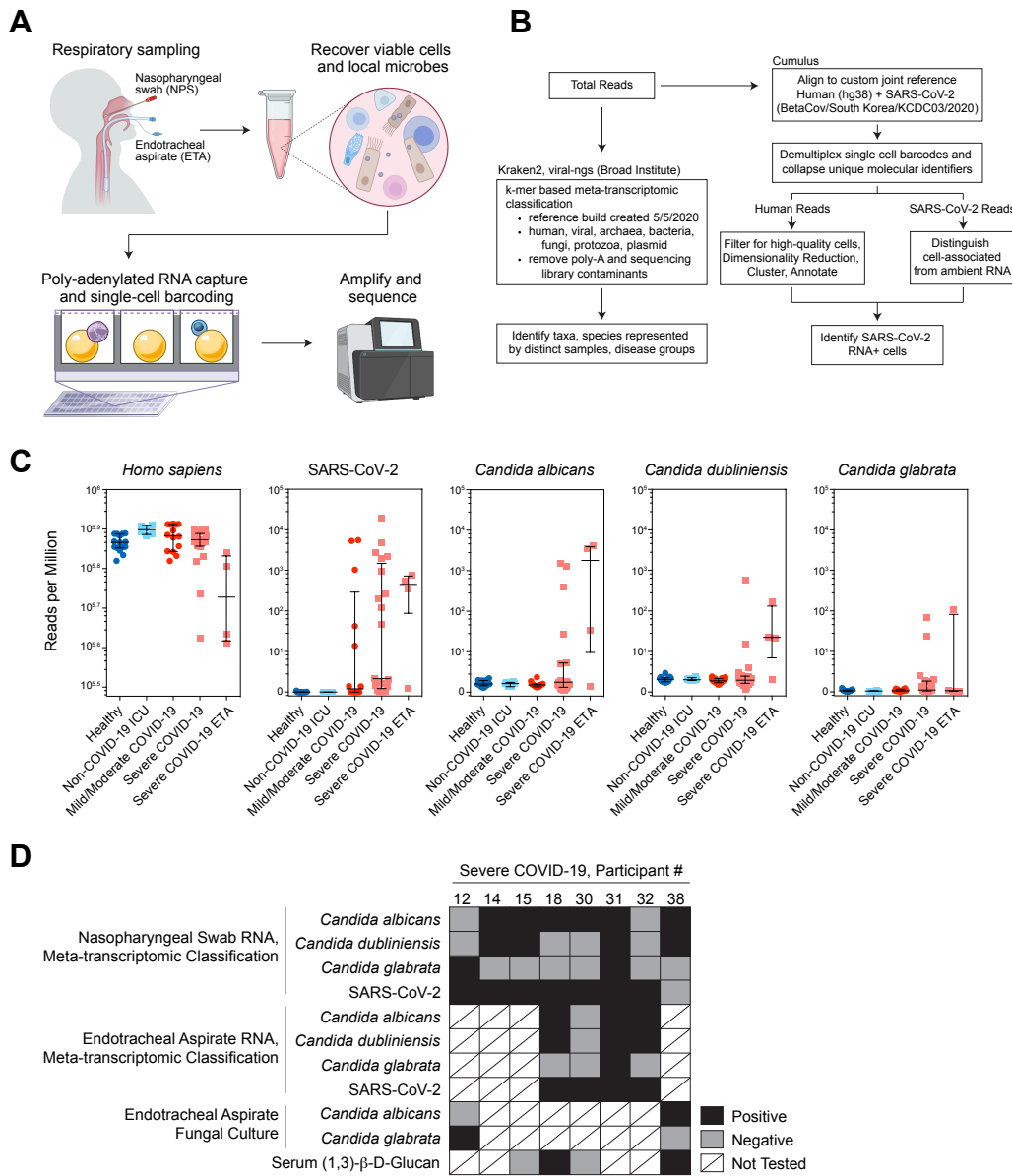
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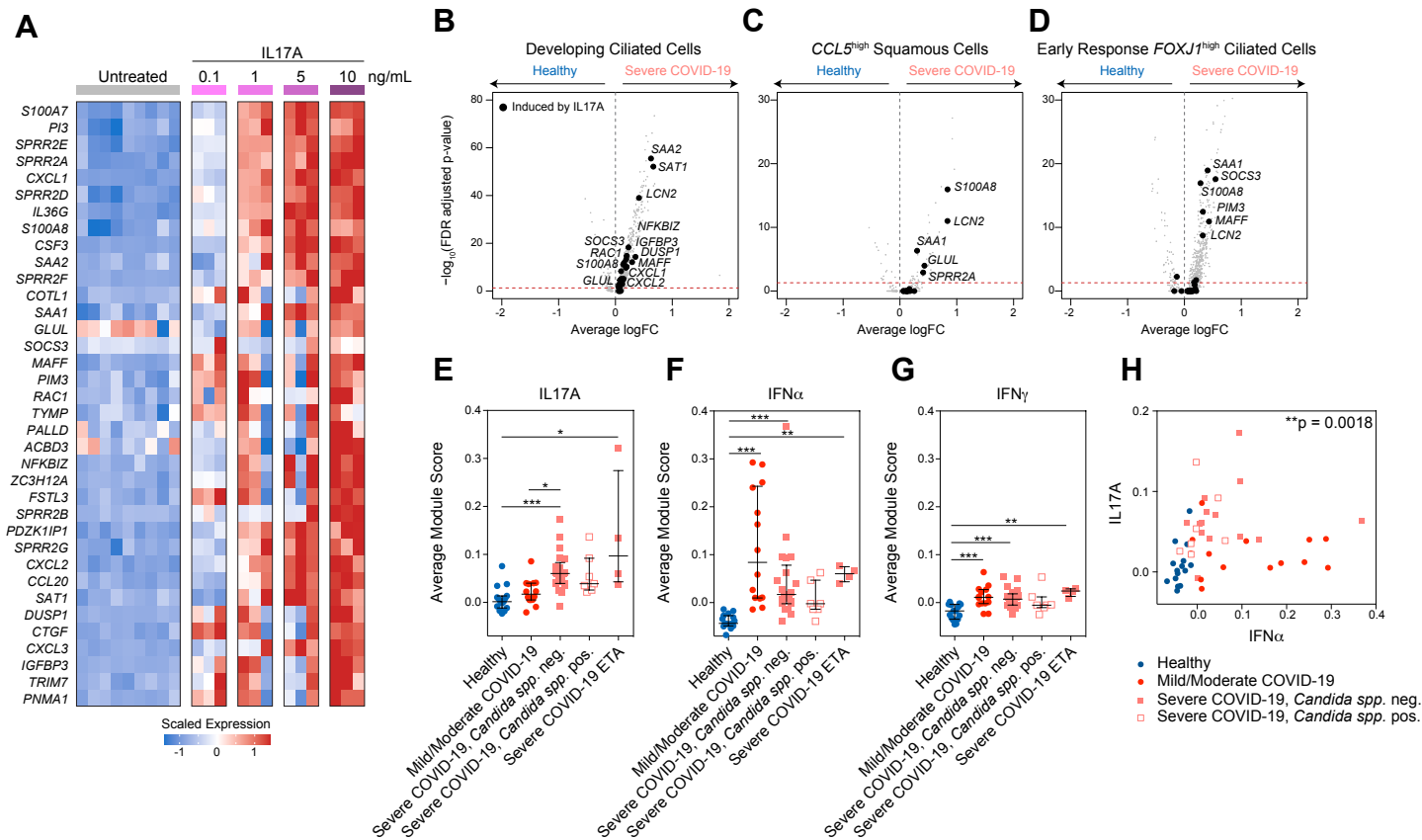
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622

## Figure 1





## Figure 2



**Table 1**

	COVID-19 severe (WHO score 6-8) <i>Candida spp.</i> negative	COVID-19 severe (WHO score 6-8) <i>Candida spp.</i> positive
Case number	23.2% (13/56)	14.3% (8/56)
Age (years)		
Minimum	28	38
Median (IQR)	63 (49)	57 (54.3)
Maximum	79	84
Sex		
Female	38.5% (5/13)	62.5% (5/8)
Male	61.5% 8/13	37.5% (3/8)
Ethnicity		
Hispanic	7.7% (1/13)	0% (0/13)
Not Hispanic	92.3% (12/13)	100% (13/13)
Race		
Black/African American	53.8% (7/13)	75% (6/8)
White	23.1% (3/13)	25% (2/8)
American Indian	23.1% (3/13)	0% (0/8)
BMI		
Median (IQR)	29.9 (27.8)	37.0 (33.6)
Pre-existing conditions		
Diabetes	61.5% (8/13)	87.5% (7/8)
Chronic kidney disease	15.4% (2/13)	25% (2/8)
Congestive heart failure	7.7% (1/13)	0% (0/8)
Lung disorder	30.1% (4/13)	50% (4/8)
Hypertension *	69.2% (9/13)	100% (8/8)
IBD	0% (0/13)	0% (0/8)
Treatment		
Corticosteroids	61.5% (8/13)	75% (6/8)
Remdesivir	7.7% (1/13)	0% (0/8)
28-day mortality ***	84.6% (11/13)	62.5% (5/8)

IQR: inter-quartile range; BMI: body mass index; IBD: inflammatory bowel disease