Immunomodulatory LncRNA on antisense strand of ICAM-1 augments SARS-CoV-2 infection-associated airway mucoinflammatory phenotype

**Highlights**

- COVID19 airway mucoinflammatory response strongly correlates with LASI lncRNA level
- Silencing LASI lncRNA suppresses SARS-CoV-2 viral load and associated inflammation
- LASI lncRNA shows a potential direct interaction with SARS-CoV-2 spike viral RNA
- Hosts of airway epithelial miRNAs are modulated by LASI to regulate inflammation
IMMUNOMODULATORY LNCRNA ON ANTISENSE STRAND OF ICAM-1 AUGMENTS SARS-COV-2 INFECTION-ASSOCIATED AIRWAY MUCOINFLAMMATORY PHENOTYPE

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SUMMARY
Noncoding RNAs are important regulators of mucoinflammatory response, but little is known about the contribution of airway long noncoding RNAs (lncRNAs) in COVID-19. RNA-seq analysis showed a more than 4-fold increased expression of IL-6, ICAM-1, CXCL-8, and SCGB1A1 inflammatory factors; MUC5AC and MUC5B mucins; and SPDEF, FOXA3, and FOXJ1 transcription factors in COVID-19 patient nasal samples compared with uninfected controls. A lncRNA on antisense strand to ICAM-1 or LASI was induced 2-fold in COVID-19 patients, and its expression was directly correlated with viral loads. A SARS-CoV-2-infected 3D-airway model largely recapitulated these clinical findings. RNA microscopy and molecular modeling indicated a possible interaction between viral RNA and LASI lncRNA. Notably, blocking LASI lncRNA reduced the SARS-CoV-2 replication and suppressed MUC5AC mucin levels and associated inflammation, and select LASI-dependent miRNAs (e.g., let-7b-5p and miR-200a-5p) were implicated. Thus, LASI lncRNA represents an essential facilitator of SARS-CoV-2 infection and associated airway mucoinflammatory response.

INTRODUCTION
Vaccination efforts have significantly reduced the spread of COVID-19, although variants arising from genomic mutations pose a significant risk even among vaccinated people (Fageeh et al., 2021; Kannan et al., 2022; Vitale et al., 2021). SARS-CoV-2 gains entry via the upper respiratory mucosa, and any host factor dysregulations occurring during this interaction can result in pulmonary and/or extrapulmonary complications (Hoffmann et al., 2020; Hou et al., 2020; Olwenyi et al., 2020; Rothan and Byrareddy, 2020). In the first week of infection, viral shedding and replication are relatively predominant in upper respiratory tract (Ravindra et al., 2021). During these events, virus evades host’s mucosal and innate immune responses, allowing it to move toward the lower respiratory tract and eventually to systemic invasion (He et al., 2020). As such, limiting shedding and viral replication in the upper respiratory tract blocks disease progression, and various immunomodulators regulate the expression of viral receptors and entry factors. That said, besides being the protective barrier and a competent airway lumen clearance mechanism, airway mucins typically moderate the mucosal immune responses (Hewitt and Lloyd, 2021). During SARS-CoV-2 infection, however, induced inflammatory factors drive airway tissue remodeling, and severe inflammation can cause mucus hyperexpression potentially leading to acute respiratory distress syndrome (ARDS).

Analyzing host-viral interactions is vital for understanding viral pathogenesis. Several notable protein interactions necessary for SARS-CoV-2 infection and/or progression are now well documented; specific roles for the host lncRNAs during SARS-CoV-2 infection remain elusive. Roles for lncRNAs in regulating virtually every cellular function have now been reported including moderating the immune response of infected host cells as well as regulating the genomic packing and replication of several viruses (Devadoss et al., 2019; Statello et al., 2021; Yao et al., 2019). lncRNAs play a vital role in regulating the innate immune responses, and recent studies have shown that specific lncRNAs are differentially expressed during host-pathogen interactions following viral infection (Blanco-Melo et al., 2020; Natarelli et al., 2021; Turjya et al., 2020; Vishnubalaji et al., 2020). Although a few reports have examined lncRNA expression profiles...
for stratifying the COVID-19 disease severity and associated immune dysfunction (Cheng et al., 2021; Meydan et al., 2020; Morenikeji et al., 2020; Mukherjee et al., 2021), studies examining roles of respiratory epithelial specific lncRNAs in SARS-CoV-2 infection are far more limited (Blanco-Melo et al., 2020; Vishnubalaji et al., 2020). Our recent studies identified a novel epithelial lncRNA LASI differentially expressed during the mucus hypersecretory response (Devadoss et al., 2021a). In this study, we have assessed the acute mucoinflammatory response, i.e., the excessive mucus expression, inflammation, other immunomodulatory factors, and lncRNAs in a 3D airway tissue model of acute SARS-CoV-2 infection and COVID-19 patient nasopharyngeal samples to gain novel insights into early innate responses of human respiratory cells.

RESULTS

SARS-CoV-2-infected individuals show induced airway mucoinflammatory responses and increased LASI lncRNA expression

The mucosal immune response of the upper respiratory tract plays a critical role in viral entry, replication and dissemination of systemic infection, and expiratory shedding. Several innate immunoregulatory transcripts of both protein-coding and noncoding RNAs are implicated in the SARS-CoV-2 infection and host antiviral response, yet very few validated roles of lncRNAs have been reported. Therefore, we performed an analysis of the airway immune response in publicly available RNA sequencing datasets obtained from SARS-CoV-2-positive (CoV-2+) patients and uninfected (CoV-2−) controls (Table S1, GeneSet Accession: PRJNA730941) (Cheemarla et al., 2021). Our initial analysis examined RNA-seq files obtained from nasal swab samples of 26 CoV-2+ and eight CoV-2− control individuals. We specifically analyzed for the lung mucoinflammatory response genes and associated lncRNAs, and the expression levels of IL-6, ICAM-1, and CXCL-8 mRNAs were around 10-, 6-, and 5-fold higher in CoV-2+ individuals, respectively, compared with CoV-2− controls (Figure 1A). Among the intracellular viral RNA sensor genes analyzed, there was a 3-fold increased expression of IFIH1 (MDA-5) mRNA in CoV-2+ individuals with no significant change in DDX58 (RIG-1) and the downstream mediator IRF3 mRNA expression compared with healthy CoV-2− controls (Figure S1A).

Although roles for airway mucins in innate antiviral immunity are well documented (Ridley and Thornton, 2018; Weitnauer et al., 2016), SARS-CoV-2-infection-associated mucin signatures have not been thoroughly evaluated to date (Lu et al., 2021). Notably, the airway secretory mucin MUC5AC mRNA levels in the present cohort were 4-fold higher in CoV-2+ individuals than in CoV-2− controls (Figure 1B), and there was no significant change in MUC5B mRNA levels. The mRNA levels for mucin transcriptional regulators (Chen et al., 2014; Rajavelu et al., 2015), SPDEF and FOXA3, as well as the ciliogenesis transcriptional factor, FOXJ1, were 2- to 3-fold higher among CoV-2+ individuals (Figure 1C). There was no change in FOXA2 and FOXM1 transcription factors (Figure S1B) but the mRNA levels of airway secretoglobulin SCGB1A1 was 3-fold lower among CoV-2+ individuals (Figure S1B). The expression levels of host factors facilitating the viral entry, such as ACE2, NRP1, DPP4, IFITM, and DDX1 as well as the Spike protein processing cellular
proteases such as TMRPSS2, Furin, CTSB, CTSL, and TMPRSS11D showed no significant change between CoV-2 positive versus negative individuals (data not shown).

We have recently characterized airway lncRNAs that play essential roles in innate immune responses of respiratory mucosa (Devadoss et al., 2019, 2021a) and propose that these immunomodulatory lncRNAs may be dysregulated during SARS-CoV-2 infection and thus drive the COVID-19-associated respiratory comorbidities. Among the immunomodulatory lncRNAs analyzed (Figure 1D), we found a lncRNA antisense to intercellular adhesion molecule 1 (ICAM-1) or LASI, described recently (Devadoss et al., 2021a), showed 2-fold higher expression in CoV-2+ individuals compared with CoV-2/C0 controls. In contrast, expression levels of NEAT1 or nuclear enriched abundant transcript-1 (Li et al., 2020; Maltezou et al., 2021; Vishnubalaji et al., 2020) and MALAT1 or metastasis associated lung adenocarcinoma transcript-1 (Cui et al., 2019) lncRNAs exhibited no significant change (Figure 1D). Thus, our analyses indicate that compared with CoV-2- controls, the airway mucoinflammatory gene signature is strongly correlated with induced LASI lncRNA expression among CoV-2+ individuals.

COVID-19-positive individuals with higher viral load show increased mucoinflammatory response

Recent epidemiological and clinical studies suggest that there is no difference in SARS-CoV-2 viral load between symptomatic and asymptomatic CoV-2+ individuals; however, disease severity and mortality rate among symptomatic individuals is directly correlated with airway viral load (Fajnzylber et al., 2020; Lee et al., 2020; Maltezou et al., 2021). Therefore, to assess the correlation of viral load with mucoinflammatory responses and associated lncRNAs, we examined the nasopharyngeal swab samples procured locally from twenty CoV-2+ individuals (Table 1). Viral load in each subject was determined by qRT-PCR for SARS-CoV-2 N1 nucleocapsid viral RNA (vRNA) levels and were assigned as low-viral load (Lo-VL) or high-viral load (Hi-VL) samples based on the cycle threshold (Ct) values of >30 or <30, respectively (Figure S2A). Applying this threshold, 10 subjects were characterized as Hi-VL (Ct = 25.9 ± 1.3; 7M/3F, 63.1 ± 3.8 years) and 10 as Lo-VL (Ct = 35.5 ± 0.7; 6M/4F, 61.5 ± 4.0 years), with Hi-VL subjects exhibiting 100-fold higher CoV-2 vRNA levels than Lo-VL subjects (Figure 2A). Interestingly, no significant differences were observed between the two groups in ACE2 (Figure S2B) and TMPRSS2 (Figure S2C) mRNA levels. Among antiviral interferon responsive genes, we failed to detect DDX58, IFIH1, IRF3, and IFNG transcripts, and there was no difference in IFNB1 mRNA levels between Hi-VL and Lo-VL samples (Figure S2D). In contrast, Hi-VL samples exhibited ~1.5-fold higher IL-6 (Figure 2B) and ICAM-1 (Figure 2C) mRNAs compared with Lo-VL samples. The expression levels analyzed in individual samples showed that IL-6 (Figure S3A) and ICAM-1 (Figure S3B) mRNAs significantly correlated with CoV-2 N1 viral RNA levels, showing distinct clustering of Hi-VL and Lo-VL subjects.

Table 1. Demographics of COVID-19 patients whose nasopharyngeal swab samples were analyzed

|                      | Lo-VL | Hi-VL | p-value |
|----------------------|-------|-------|---------|
| Subjects             | 10 (7) | 10 (5) | —       |
| Sex (M/F)            | 6M/4F | 7M/3F | —       |
| Age (Y)              | 61.5 ± 4.0 | 63.1 ± 3.8 | 0.8163 |
| SARS-CoV-2 N1 vRNA (Ct) | 35.5 ± 0.7 | 25.9 ± 1.3 | <0.0001 |
| Hospitalization      | 7     | 5     | —       |
| Hypertension         | 5     | 5     | —       |
| Diabetes             | 4     | 0     | —       |
| Infectious Disease   | 1     | 2     | —       |
| Pneumonia            | 5     | 5     | —       |
| Oxygen Supplementation (>6L O2) | 1 | 5 | — |
| ICU                  | 3     | 5     | —       |
| ARDS                 | 3     | 5     | —       |
| Obesity              | 5     | 3     | —       |

Medical history was available for twelve patients.
In agreement with RNA-seq database analyses (Figure 1), all of the nasal swab samples in our study cohort showed upregulated mucin expression with Hi-VL samples exhibiting robust 10-fold higher MUC5AC (Figure 2D) and 4-fold higher MUC5B (Figure 2E) mucin mRNA levels. Notably, although Hi-VL subjects trended toward lower MUC2 mRNA expression (Figure S2E), they exhibited 10-fold higher MUC4 mRNA expression. Data shown as box and whisker plots with minimum to maximum range; n = 10/gp; *p < 0.05; **p < 0.01; ***p < 0.001 by Student’s t test). Increased expression of MUC4 and SCGB1A1 mRNAs was also observed in Hi-VL subjects with no changes in select viral entry facilitating host factor mRNAs and IFN1B mRNA expression (Figure S2) and select host mucoinflammatory genes and IncRNAs strongly correlated with CoV-2 viral load (Figure S3).

Innate immunomodulatory IncRNAs are associated with SARS-CoV-2 viral load

Next, we analyzed the expression levels of select airway IncRNAs in our cohort. We found that compared with the Lo-VL samples, the expression levels of IncRNA LASI (Figure 3A), NEAT1 (Figure 3B), and a wound and keratinocyte-migration-associated IncRNA 2 (WAKMAR2) (Figure 3D) were all significantly upregulated in Hi-VL samples, whereas IncRNA MALAT1 levels were not altered (Figure 3C). When analyzed individually, the expression levels of IncRNA LASI (Figure S3E), NEAT1 (Figure S3F), and WAKMAR2 (Figure S3G) significantly correlated with CoV-2 viral RNA levels, with IncRNA LASI showing the highest correlation ($r^2 = 0.6381$; $p < 0.0001$). These data suggest that LASI IncRNA, which plays pivotal role in airway innate responses (Devadoss et al., 2021a), may be involved in SARS-CoV-2-associated airway inflammation.

Next, we performed an RNA-FISH analysis to examine LASI IncRNA expression in airway epithelial cells of nasal swab samples and cells labeled for SARS-CoV-2 N1 vRNA. Compared with Lo-VL samples, SARS-CoV-2 N1 vRNA and LASI transcripts were enriched in perinuclear and cytosolic regions with increased expression of LASI in Hi-VL subjects (Figure 3E). In agreement with qRT-PCR analyses (Figure 3A), the H-score

![Figure 2. COVID-19 positive individuals with high nasopharyngeal viral load show increased mucoinflammatory phenotype compared with low viral load individuals](image-url)
values that denote RNA expression per cell, as described recently (Devadoss et al., 2021a), were 2.7- and 2.4-fold higher for vRNA and LASI lncRNA, respectively, in Hi-VL compared with Lo-VL samples (Figure 3G).

The protein expression of MUC5AC mucin as determined by immunopositivity was 16-fold higher in Hi-VL versus Lo-VL samples (Figures 3Fa and 3H), with no noticeable change in MUC5B expression (Figures S4A and S4B). These datasets indicate that the immunomodulatory LASI lncRNA expression is significantly induced in Hi-VL than Lo-VL patient cells, and its expression strongly correlates with MUC5AC mucin expression in airway cells corroborating the RNA-seq data (Figure 1).

3D airway tissue model of SARS-CoV-2 infection demonstrates an immediate-early hypermucoinflammatory response

To better understand the association of CoV-2-induced airway inflammatory response and LASI lncRNA, a 3D airway tissue culture model of SARS-CoV-2 infection was employed. Briefly, the primary human airway epithelial cells differentiated on air-liquid interface to mimic the conducting airway epithelium were infected with one MOI of SARS-CoV-2 primary clinical isolate (USA-WI1/2020). CoV-2 vRNA load was followed in apical washes, in the basal culture supernatant, and in cells at 0, 1, 4, 24, and 48 h postinfection (hpi). In apical washes, $0.89 \times 10^5$ viral genomic equivalents per mL were observed at 1 hpi, increasing to 20.2, 31.2, and $14.7 \times 10^5$ at 4, 24, and 48 hpi, respectively (Figure 4A). Viral load in basal media supernatant was $0.3 \times 10^5$ per mL at 1 hpi then increased to 3.6, 4.3, and $5.0 \times 10^5$ at 4, 24, and 48 hpi, respectively (Figure S5A). The cellular level of CoV-2 vRNA increased by 2.0-, 7.0-, and 2.6-fold at 4, 24, and 48 hpi, respectively, compared with 1 hpi (Figure 4B). Thus, 3D airway cells were productively infected with
SARS-CoV-2 clinical isolate, and the virions were shed at a 10-fold higher level from the apical surface than the basal region.

The expression of ACE2 mRNA was more than 3-fold suppressed in infected at all time-points analyzed (Figures S5B), and TMPRSS2 mRNA levels showed no change at 1, 4, 24, and 48 hpi but was significantly reduced at 48 hpi (Figures S5C). Thus, SARS-CoV-2 infection modulates ACE2 and TMPRSS2 expression, as reported earlier (Fliesser et al., 2021; Matusiak and Schurch, 2020). Expression of IL-6 mRNA was 8.0-fold induced at 1 hpi with no change at 4, 24, and 48 hpi, although there was a trend toward induced expression (Figure 4C). ICAM-1 mRNA expression was 2.0-, 5.4-, and 2.4-fold higher at 1, 4, and 24 hpi, respectively, with no change at 48 hpi (Figure 4D). Expression of neither NRP1 (data not shown) nor CXCL-8 (Figure S5D) mRNA was affected following infection. However, the expression of SCGB1A1 was induced by 4- to 5-fold at 1, 4, and 24 hpi (Figure S5E).

Interestingly, SARS-CoV-2 infection robustly upregulated MUC5AC mRNA expression by 53-, 46-, 37-, and 51-fold at 1, 4, 24, and 48 hpi, respectively (Figure 4E). MUC5B mRNA levels was also highly upregulated following infection by 13-, 11-, 16-, and 33-fold at 1, 4, 24, and 48 hpi, respectively (Figure S5F). Similarly, MUC2 mRNA levels were 8-, 9-, 10-, and 13-fold at 1, 4, 24, and 48 hpi, respectively (Figure S5G), whereas MUC4 mRNA levels were induced by 5-fold at 24 hpi (Figure S5H). In addition, there was a 3- to 5-fold induction in SPDEF mRNA levels (Figure 4F) and more than a 25-fold induction in FOXA3 mRNA levels following CoV-2 infection (Figure S5I).

MUC5AC protein expression was assessed by immunofluorescence and quantified by ELISA to corroborate these results. In agreement with qRT-PCR analyses, CoV-2-infected (CoV-2+) cells showed robust MUC5AC immunoreactivity (Figure 4G) with as much as ~40% MUC5AC+ cells observed at 48 hpi compared with
6.0% in uninfected (CoV-2−) controls (Figure 4H). In addition, apical wash MUC5AC protein levels were ~10-fold higher in CoV-2+ cells with ~120 ng/mL compared with 18 ng/mL observed in uninfected cells (Figure 4I). The secreted interleukin-6 (IL-6) levels were at 691 pg/mL in uninfected cells compared with 1400 pg/mL in the CoV-2+ cells at 48 hpi (Figure 4J), and ICAM-1 protein levels showed a trend toward increased expression from 823 pg/mL in uninfected cells to 1600 pg/mL in CoV-2+ cells (Figure 4K). Remarkably, there was no change in the expression of DDX58, IFIH1, IRF3, and IFNB1 in CoV-2 infected cells at 0, 1, and 48 hpi (Figure 6A–6D).

SARS-CoV-2 infection induces LASI IncRNA expression that could potentially interact with CoV-2 spike viral RNA

In agreement with the increased LASI IncRNA observed in COVID-19 individuals, there was induced expression of LASI IncRNA at 0, 1, 4, 24, and 48 hpi in our 3D model of CoV-2 infection (Figure 5A). Dual-RNA FISH analyses showed CoV-2 N1 vRNA and LASI transcripts co-expression around the nuclear/perinuclear region (Figure 5B) with nearly 2-fold more IncRNAs in CoV-2+ cells as assessed by H-score analysis (Figures 5C and 5D). We also analyzed the expression of other IncRNAs, and in contrast to recent reports (Li et al., 2020; Maltezou et al., 2021; Vishnubalaji et al., 2020), the expression of IncRNA NEAT1 (Figure 5E) and MALAT1 (Figure 5F) was significantly reduced in CoV-2-infected cells at 0, 1, and 48 hpi, whereas WAKMAR2 IncRNA levels were induced by more than 7-fold at 48 hpi (Figure 5G).

As FISH analyses strongly suggest LASI IncRNA and SARS-CoV-2 vRNA may colocalize, the potential for direct interaction was assessed informatically. Notably, our sequence analyses identified a single, high-scoring potential base-pairing between SARS-CoV-2 Spike vRNA (CoV-2 vRNA) and a specific LASI IncRNA sequence. The segment of the modeled CoV-2 vRNA (sequence 1198-1268 nts) after minimization and equilibration is shown in Figure 5E. This region forms a hairpin where 1227-1237 nts are intra-sequence base-paired with 1244-1254 nts (Figure 5F). 3D modeling predicts a structure where this CoV-2 vRNA region can duplex with LASI IncRNA bases 635-646 nts with a stable base-paired model obtained upon relaxing the structure for 100 ns of MD simulation (Figure 5G and see Video S1 online at supplemental data). This structure shows a partially unfolded CoV-2 vRNA sequence with excellent base-pairing with the LASI IncRNA sequence (Figure 5H). Compared with the intra-sequence base-pairing in CoV-2 vRNA in Figure 5F, the inter-sequence base-pairing with LASI IncRNA is energetically more favorable (−9.6 kcal/mol versus −15.0 kcal/mol) at the local interacting region. Moreover, this LASI IncRNA interacting region is conserved among CoV-2 variants of concern (VOCs) such as delta and omicron (Figure S7). Taken together, our experimental and molecular docking simulation studies suggest that LASI IncRNA may serve as the molecular scaffold for CoV-2 vRNA to assist in viral infection and replication.

Blocking LASI IncRNA expression attenuates CoV-2-altered antiviral interferon response effects and suppresses infection-dependent MUC5AC induction

To determine the role of LASI IncRNA in CoV-2 infection and associated mucoinflammatory response, we depleted LASI levels using RNAi (siRNA) technology. Briefly, 3D cultured cells transfected with either LASI-targeting siRNA (siLASI) or a control siRNA (siCTRL) were infected with one MOI of SARS-CoV-2 clinical isolate and then analyzed at 48 hpi. Compared with siCTRL-transfected cells, siLASI-transfected cells exhibited a 7-fold reduction in LASI IncRNA expression (Figure 6A). Surprisingly, we observed a marked 27-fold reduction in CoV-2 vRNA load in siLASI-transfected cells (Figure 6B). Furthermore, although ICAM-1 (Figure 5A), IL-6 (Figure 5B), IFNB1 (Figure 5C), and IFIH1 (Figure 5D) mRNA expressions were unaffected, mRNA levels of the antiviral interferon response gene, IRF3, were significantly reduced in siLASI-transfected cells (Figure 6C). In contrast, levels of DDX58 mRNA were conversely induced compared with the siCTRL-transfected cells (Figure 6D). Among mucin genes, MUC5AC mRNA levels in siLASI-transfected cells showed a trend toward lower expression (Figure 6E) with no change in MUC5B mRNA levels (Figure 5E); however, MUC2 (Figure 5F) and MUC4 (Figure 5G) mRNAs were reduced following CoV-2 infection in siLASI-transfected cells compared with siCTRL-transfected cells. Notably, expressions of the SPDEF (Figure 5H) and the IncRNA MALAT1 (Figure 5I) and WAKMAR2 (Figure 5J) were not altered by siLASI treatment during CoV-2 infection.

To further explore potential effects on mucin genes, we next immunoprobed for MUC5AC protein expression in siCTRL and siLASI transfected cells infected with CoV-2 at 48 hpi (Figure 6F). We found siLASI treatment resulted in a >2-fold reduction of MUC5AC+ cells, where siCTRL-transfected cells had around 17.7% cells showing MUC5AC immunopositivity compared with 7.4% among siLASI-transfected cells (Figure 6G).
In agreement, MUC5AC-specific ELISA of apical washes from siCTRL-transfected cells averaged 121 ng/mL of MUC5AC content, whereas siLASI-transfected cells averaged 47.5 ng/mL (Figure 6H), a more than 2-fold reduction. In contrast, we found ICAM-1 (Figure S8K) and IL-6 (Figure S8L) levels were not significantly changed in basal media supernatant following CoV-2 infection of siLASI-transfected cells as compared with siCTRL-transfected cells. Taken together, these data strongly suggest that LASI IncRNA represents an essential regulatory mediator in CoV-2 infection and the ensuing mucoinflammatory response of the respiratory airway epithelium.

Figure 5. SARS-CoV-2 infection induces LASI IncRNA expression in human respiratory epithelial cells that potentially show direct interaction with CoV-2 spike RNA

(A) Relative expression levels of LASI IncRNA in SARS-CoV-2-infected cells at 0, 1, 4, 24, and 48 hpi. (n = 4/gp; *p < 0.05; **p < 0.01; ***p < 0.001 by ANOVA).

(B) Colocalization of SARS-CoV-2 vRNA and LASI transcripts in CoV-2-infected (CoV-2+) cells as determined by dual-FISH staining and the structured-illumination imaging analysis. Representative micrographs of dual-FISH-stained cells showing SARS-CoV-2 N1 vRNA (in red) and LASI IncRNAs (in green) along with DAPI-stained nuclei (in blue); scale bar: 2 μm.

(C and D) H-score quantitation of (C) CoV-2 vRNA and (D) LASI IncRNAs per cell in CoV-2+ and control cells. (n = 9–10 cells/gp; **p < 0.01; ***p < 0.001 by Student’s t test).

(E) Modeled 3D structure of SARS-CoV-2 spike vRNA nucleotide sequence from 1198 to 1268, and the LASI IncRNA interacting region (1227-1237) is highlighted in blue.

(F) The intra-sequence base-pairing of spike nucleotides forms the hairpin stem structure.

(G) Modeled 3D structure of the CoV-2 Spike vRNA duplexed with LASI IncRNA sequence 646-635 (highlighted in orange) at the end of 100 ns simulation (see Video S1 at online supplemental data).

(H) Inter-sequence base-pairing of CoV-2 vRNA with LASI IncRNA sequence (shown in orange). There was no significant change in interferon-related gene expression; however, expression of other immunomodulatory IncRNAs were differentially regulated following CoV-2 infection (Figure S6). LASI-interacting sequence is conserved in Spike viral RNAs of CoV-2 Delta and Omicron variants (Figure S7).
LASI lncRNA differentially modulates the host miRNAs associated with SARS-CoV-2 infection

LncRNAs also act as the molecular scaffold or sponge for miRNAs. Thus, they could indirectly modulate host cellular immune responses, for example, by miRNA-mediated target mRNA-decay or by competing for the same miRNA binding region, like ceRNA (Abu-Izneid et al., 2021; Siniscalchi et al., 2021; Yousefi et al., 2020; Zhang et al., 2019). In addition, host miRNAs can also directly bind to viral RNAs and/or modify the associated cell signaling mechanisms. So, to identify the miRNAs that LASI lncRNA may regulate, we performed a small RNA-sequencing of our 3D ALI model and analyzed for the expression of miRNAs in SARS-CoV-2-infected cells that are siLASI transfected compared with control cells. From the total of 721 miRNAs sequenced, 155 miRNAs showed significant change. Further data mining revealed a total of 38 miRNAs differentially expressed in SARS-CoV-2 infection, of which seven miRNAs (miR-4488, let-7b-5p, miR-1301-3p, miR-181b-5p, miR-2110, miR-320b, and miR-744-5p) were expressed >2 log-fold higher, whereas twenty-one miRNAs showed lower expression than uninfected cells including miR-23a-3p, miR-30d-5p, miR-200a-5p, miR-200b-3p, and miR-1246. Interestingly, knocking down the LASI lncRNA reverted the expression levels of several of these miRNAs; for example, some of the miRNAs induced by CoV-2 infection were suppressed by silencing LASI lncRNA (Figure 7A), of which miR-4488, let-7b-5p, miR-584-5p, miR125-1-3p, and miR-181b-5p showed very high expression in siLASI-transfected and CoV-2-infected cells. At the same time, there were miRNAs suppressed by CoV-2 infection that were highly induced in siLASI-transfected cells, e.g., miR-151a-3p, miR-6510-3p, miR-200a-5p, miR-197-3p, and miR-4644-3p (Figure 7B). The changes in the expression levels of select miRNAs were confirmed by qPCR analysis of siCTRL and siLASI cells where levels of miR-4488 (Figure 7C), let-7b-5p (Figure 7D), miR-150-5p (Figure 7E), miR-6510-3p (Figure 7F), miR-200a-5p (Figure 7G), and miR-197-3p (Figure 7H) were confirmed to be reverted in siLASI-transfected cells compared with siCTRL-transfected cells. We also confirmed the expression levels of these lncRNA LASI-regulated miRNAs in nasal swab samples of our cohort and of these miRNAs (Figure S9). Expression levels of let-7b-5p, miR-150-5p, and miR-200a-5p showed a viral-load dependent increased expression. Thus, these data suggest that let-7b-5p, miR-150-5p, and miR-200a-5p may be targets of LASI lncRNA.
important mediators of LASI-lncRNA-mediated modulation of CoV-2-induced airway epithelial mucoinflammatory responses.

**DISCUSSION**

The pathogen-specific inflammatory response and subsequent successful resolution of inflammation and associated tissue remodeling in the upper respiratory tract determine the outcome of airborne infections. The SARS-CoV-2 infection continues to be a significant healthcare problem. Many studies suggest that dysregulated inflammation and impaired ability to resolve inflammation and adjoining tissue remodeling are primary causes of increased morbidity and mortality among COVID-19 patients. Airway mucoinflammatory response, being the primary host defense system, is severely altered by SARS-CoV-2 infection. Not much is known about the immediate-early innate responses to SARS-CoV-2 infection. Thus, in this study, we focused on the effects of SARS-CoV-2 infection on airway mucus and inflammatory responses. We analyzed available RNA-seq data from COVID-19 patient nasal swab samples. We found that the expression of immunomodulatory IncRNAs, particularly LASI IncRNA, were altered in infected patients with high nasal swab viral load compared with patients with low viral load. Expression levels of select IncRNAs were also elevated in high viral load nasal swab samples of our study cohort (Figure S9).

To understand the immediate-early response of AECs to SARS-CoV-2 infection, we examined a 3D human airway tissue model infected with high MOI of SARS-CoV-2 isolate USA-W1/2020 (B.1.1.7). We observed elevated secretory mucus and inflammatory factors expression as early as 1 h postinfection. Among the
CoV-2 infection on airway mucin expression linked to mucins were significantly upregulated during CoV-2 infection and that depleting the effective mucociliary clearance of inhaled viral particles. We find the expressions of membrane-bound separate computational analysis (Vishnubalaji et al., 2020). Further, we find that an acute effect of SARS-CoV-2 2021; Smet et al., 2021; Yin et al., 2021). If not treated, these conditions can cause a decline in lung function these mucoinflammatory responses in agreement with other seminal studies (Desai et al., 2020; Lu et al., 2014; Ma et al., 2018). Excess mucus expression can lead to airway mucous obstruction due to mucin dysregulation of these processes leads to mucoinflammatory comorbidities and respiratory viral diseases (Holtzman et al., 2019). Although little is known about specific IncRNA contributions to COVID-19 pathophysiology, several IncRNAs have now been suggested to potentially interact with SARS-CoV-2. Using the experimentally determined secondary structure of HOTAIR IncRNA (Somarowthu et al., 2015), a study recently predicted its interacting regions with spike RNA (Natarelli et al., 2021). LINC01505 has also been reported to bear a binding propensity for SARS-CoV-2 3’UTR. In addition, IncRNA H19 has been predicted to carry a significant binding potential for SARS-CoV-2 5’UTR and spike RNA (Natarelli et al., 2021). That said, logistic predictions suggest that most of these IncRNAs interactions, if true, would constitute negative modulators of CoV-2 infection.

In contrast, our data suggest that LASI IncRNA may be a positive modulator of CoV-2 infection. Our computational analyses identified a putative interaction between LASI IncRNA and SARS-CoV-2 Spike RNA, suggesting that LASI may act as a molecular scaffold or sponge for viral RNA, potentially protecting from degradation and/or other host factors. Regardless, we find inhibiting LASI IncRNA attenuates the SARS-CoV-2 viral load and alleviates the expression of inflammatory factors, mucus, and type-1 interferon (IFN) responsive genes in airway epithelial cells. Thus, targeting LASI may not only suppress the mucoinflammatory pathways but could also decrease the number of molecular scaffolds responsible for harboring the viral RNA and other components. Functional-RNA-based therapeutics are currently being actively pursued as potential treatment strategies due to minimal off-target effects to a high-efficiency presentation (Civenni, 2017), and IncRNA-targeted interventions can possibly mimic the mechanisms of many host immune response modulating RNAs (Winkle et al., 2021).

Acute inflammatory response to SARS-CoV-2 infection alters innate epithelial and vascular remodeling factors (Froberg and Diavatopoulos, 2021; Smith et al., 2021) with distinct inflammatory signatures reported among COVID-19 patients with low versus higher viral load (Cervia et al., 2021). Notably, the localized expression and secretion of ICAM-1 and IL-6 by respiratory epithelial cells may regulate airway responses and mucosal tissue remodeling (Manevski et al., 2020). In previous immunohistological analyses, IL-6 and ICAM-1 expression were reportedly higher in the alveolar epithelium of COVID-19 patients compared with H1N1-influenza-virus-infected patients or uninfected control subjects (Malaquias et al., 2021). We likewise find that ICAM-1 and IL-6 expressions are significantly higher in COVID-19 patients compared with controls, and patients with Hi-VL show increased expression of these inflammatory factors in our cohort.

Also of note, IncRNAs-mediated regulation of immune responses is potentially central to establishing airway mucosal immunity (Agirre et al., 2019; Devadoss et al., 2019; Ouyang et al., 2016) and could be dysregulated in COVID-19 patients. Numerous IncRNAs have been experimentally characterized and were shown to affect the immune responses of AEC, exert epigenetic changes, and induce the premature aging of the lung epithelium (Devadoss et al., 2019). However, among the IncRNAs analyzed, we found only LASI IncRNA expression to be consistently elevated in COVID-19 patients, although there were significant downregulations of NEAT1 and MALAT1 IncRNAs (Blanco-Melo et al., 2020), as recently confirmed in a separate computational analysis (Vishnubalaji et al., 2020). Further, we find that an acute effect of SARS-CoV-2 infection on airway mucin expression linked to LASI IncRNA expression could potentially hinder the effective mucociliary clearance of inhaled viral particles. We find the expressions of membrane-bound mucins were significantly upregulated during CoV-2 infection and that depleting LASI IncRNA could suppress the CoV-2-induced expression of these mucin genes. Airway mucins trap inhaled viral particles in the lumens and direct them toward the oropharynx via mucociliary clearance mechanisms. Any dysregulation of these processes leads to mucoinflammatory comorbidities and respiratory viral diseases (Holtzman et al., 2014; Ma et al., 2018). Excess mucus expression can lead to airway mucous obstruction due to mucin hyperexpression, hypersecretion, and goblet cell hyperplasia, and as reported here, SARS-CoV-2 alters these mucoinflammatory responses in agreement with other seminal studies (Desai et al., 2020; Lu et al., 2021; Smet et al., 2021; Yin et al., 2021). If not treated, these conditions can cause a decline in lung function and significantly higher morbidity and mortality. That said, our findings strongly suggest that LASI IncRNA constitutes an important master mucin regulator.
In addition, blocking LASI IncRNA expression in the 3D airway model led to a suppressed expression of MUC5AC protein levels following CoV-2 infection, implicating a direct role of LASI in viral-induced mucoinflammatory response. However, there was no change in any of the mucin regulating transcription factors’ expression among CoV-2-infected cells, unlike our previous observation in the allergic asthma model (Devaldoss et al., 2021a). This discrepancy observed between the effects of silencing LASI IncRNA in this and our previous study is unexpected and needs further investigation. Interestingly, among IFN response genes analyzed, blocking LASI expression suppressed the IRF3 mRNA expression, with the expression of IFNB1 mRNA also trending lower. In contrast, the upstream mediator DDX58 mRNA expression was significantly induced in cells with suppressed LASI IncRNA levels and IFIH1 mRNA trending toward a higher expression; this noted incongruity between type 1 IFN and its mediators needs more careful analysis, as it could potentially involve the RIG-1-like receptors engaged by siRNAs used for silencing LASI expression in our experimental set-up (Song and Rossi, 2017; van der Veen et al., 2018). Recent studies have also highlighted the differential role of DDX58 and IFIH1 in innate immune responses to CoV-2 infection and the role of the cellular IncRNAs therein (Brisse and Ly, 2019; Ji et al., 2022; Yang et al., 2021). However, the changes in IRF3 and DDX58 expression can also be a cause or consequence of lower infectivity in our experimental set-up as well as in the nasal samples analyzed.

There are various other small ncRNAs called miRNAs that interact with IncRNAs and modulate the gene expression and functions (Statello et al., 2021; Yao et al., 2019). We have also identified several putative miRNA candidates that could assist LASI IncRNA in modulating the mucoinflammatory response genes that contribute to airway inflammation and mucin expression. Our targeted small RNA-seq data implicated many host miRNAs that could possibly mediate the LASI IncRNA-based modulation of SARS-CoV-2 infection and associated inflammation. For example, following CoV-2 infection, a set of miRNAs including miR-4488, miR-584-5p, miR-125-1-3p, miR-181b-5p, and let-7b-5p were highly upregulated but blocking the LASI IncRNA resulted in their downregulation compared with controls. These miRNAs might be facilitating the CoV-2 infection in a LASI-dependent manner, where LASI IncRNA may be acting as a molecular sponge or scaffold for these “inflammatory” miRNAs. There is very limited information about the role of these miRNAs. A computational prediction analysis performed by Pierce et al. identified that miR-181-5p binds to the mRNAs of both TMPRSS2 and IFN-γ in human lung tissue (Pierce et al., 2020). Another RNA-seq data analysis from SARS-CoV-2-infected lung tissues identified that miRNA let-7b-5p might be involved in COVID-19-associated lung pathologies (Chow and Salmena, 2020; Jafarinejad-Farsangi et al., 2020).

In contrast, specific miRNAs suppressed by CoV-2 infection were highly induced in siLASI-transfected cells, such as miR-151a-3p, miR-6510-3p, miR-200a-5p, miR-25-3p, miR-3158-3p, miR-3158-5p, and miR-4644-3p. These miRNAs play an important role in regulating innate cellular responses and signaling pathways (Banaganapalli et al., 2021; Hosseini Rad Sm and McLellan, 2020), suggesting that these miRNAs are an important component of host immune response and are thus suppressed by CoV-2 infection in a mechanism dependent on LASI IncRNA levels. Based on the recent report on the interaction of miR-150-5p with CoV-2 nsp10 gene (Akula et al., 2022), we also found that CoV-2 infection modulates miR-150-5p expression in a LASI-dependent manner. It is noteworthy that nasal airway miR-150-5p expression was positively correlated with CoV-2 viral load in our cohort compared with the lower miR-150-5p levels reported in COVID-19 plasma samples (Akula et al., 2022). Further investigations are needed to determine the role of miR-150-5p in systemic versus pulmonary inflammation by examining the levels of cellular and plasma miR-150-5p collected from the same subjects. Thus, there is a large number of miRNAs that are directly impacted by LASI IncRNA and could be involved in CoV-2 infection and airway inflammatory response. We also found that there are a number of miRNAs whose expression was not significantly impacted by LASI IncRNA levels, such as miR-1301-3p, which is known to interact with CoV-2 genes/RNAs (Chow and Salmena, 2020; Sacar Demirci and Adan, 2020) and miR-320b, which interacts with viral NSP8 gene and is associated with transcription and replication of viral RNAs (Pierce et al., 2020).

Due to the combined colossal efforts of the continuous global surveillance and the large-scale vaccination drives, COVID-19 has been mitigated to a manageable endemic threat. Nonetheless, with a more significant proportion of the population still infected, the undetected and asymptomatic SARS-CoV-2 infections and invading vaccine/natural antibodies in vaccinated or infected individuals are resulting in the emergence of variants of concerns or VOCs (Kannan et al., 2021; Spratt et al., 2021), and thus, continuous efforts are needed to improve the current understanding on the host-pathogen interactions for these VOCs and to better prepare for any possible future viral outbreaks. The work presented here strongly suggests that
IncRNAs constitute a significant component of our innate immunity that directly interacts with viral RNA and describing these interactions may significantly improve our understanding of host factors involved in the viral response. Specifically, we report that LASI lncRNA in airway epithelial cells is not only a putative scaffold for SARS-CoV-2 RNA but also modulates respiratory mucoinflammatory responses. Thus, modulating specific functional lncRNA expression may aptly equip the host with innate immunity to combat the COVID-19 infection and associated mucoinflammatory comorbidities.

Limitations of the study
The nonavailability of a more significant number of patient nasal swab samples from COVID-19 subjects and the low yield of airway epithelial cells per sample may underscore the broader implications of the reported findings. The inadequate amount and quality of samples could have also contributed to the undiscernible and/or undetected levels of the genes analyzed that are expressed in low copy numbers such as genes regulating the antiviral and interferon response pathways. Further, our in-vitro studies primarily focused on the acute infection model, and we used high MOI of the original SARS-CoV-2 clinical isolate (B.1.1.7), and only the early time points were analyzed to understand the immediate-early changes in host innate response factors. The miRNAs identified to be responsible for LASI lncRNAs role in CoV-2 infection and associated inflammation need to be further validated by using the targeted molecular editing techniques as well as by longitudinally analyzing a larger study cohort.

STAR METHODS
Detailed methods are provided in the online version of this paper and include the following:

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Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104685.

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AUTHOR CONTRIBUTIONS

Conception and study design: HSC; Formalizing hypothesis: HSC, GMB, SNB; Patient samples and BSL3 experiments: MM2, AA, KP, SNB; Data acquisition: DD, MM1, AA, DH, MDC, KP; Data analysis: DD, MM1, AA, DH, MDC, KP, PC; Data interpretation: DD, MM1, AA, KP, PC, GMB, MN, MM2, SNB, HSC; Drafting the manuscript for important intellectual content: DD, SNB, HSC; Reviewing manuscript: all authors; Approving final version of the manuscript for submission: SNB and HSC. All authors read and approved the final version of the manuscript. All figures are original creations for this manuscript, and no additional permissions are required for inclusion into the manuscript.

DECLARATION OF INTERESTS

Dr. Hitendra S. Chand and Dr. Madhavan Nair are coinventor on a US utility patent #10,851,376 for long noncoding RNAs in pulmonary airway inflammation. The authors have no competing financial interests to declare.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENTS or RESOURCE | SOURCE | IDENTIFIER |
|-----------------------|--------|------------|
| **Antibodies**        |        |            |
| Anti-Mucin MUC5AC, clone CLH2 antibody | Millipore | Cat# MAB2011; RRID: AB_2146983 |
| MUC5B antibody [19.4E] | Abcam | Cat# ab77995; RRID: AB_2146987 |
| Donkey Anti-Mouse IgG (H+L) Antibody, Alexa Fluor 488 Conjugated | Invitrogen | Cat# A-21202; RRID: AB_141607 |
| **Bacterial and virus strains**       |        |            |
| SARS-CoV-2 isolate USA-WI1/2020 | BEI Resources | Cat# NR-52384 |
| **Biological samples**                |        |            |
| Human Nasal Swabs | University of Miami | N/A |
| Human Nasal Swabs | iSpecimen | N/A |
| **Chemicals, peptides, and recombinant proteins** | | |
| Tyramide Signal Amplification (TSA™ Plus Cyanine 5 System) | PerkinElmer | Cat# K1051 |
| Cyanin 3 Tyramine Signal Amplification (TSA) Fluorescence System Kit | APEXBio | Cat# NEL745001KT |
| MISSION® siRNA Transfection Reagent | Sigma-Aldrich | Cat#1452 |
| TaqMan™ Universal PCR Master Mix | ThermoFisher | Cat# 4304437 |
| iTaq Universal SYBR Green Supermix | BIORAD | Cat# 1725121 |
| TaqMan™ Advanced miRNA cDNA Synthesis Kit | ThermoFisher | Cat# A28007 |
| TaqMan™ Fast Advanced Master Mix | ThermoFisher | Cat# 4445557 |
| DAPI Fluoromount-G® | SouthernBiotec | Cat# 0100-20 |
| Collagen IV | Sigma-Aldrich | Cat# C5533 |
| **Critical commercial assays**        |        |            |
| mucin-5AC (MUC5AC), ELISA Kit | MyBiosource | Cat# MBS701926 |
| LEGEND MAX™ Human IL-6 ELISA Kit | BioLegend | Cat#430507 |
| Human ICAM-1/CD54 (Sandwich ELISA) ELISA Kit | LifeSpan Biosciences | Cat# LS-F4019 |
| RNAscope® Multiplex Fluorescent Detection Kit v2 | Advanced Cell Diagnostics | Cat# 323110 |
| RNAscope Probe-V-SARS-CoV-2-N-O1-C2 | Advanced Cell Diagnostics | Cat# 863831-C2 |
| RNAscope Probe-Hs-AC011511.2 | Advanced Cell Diagnostics | N/A |
| iScript™ cDNA Synthesis Kit | BIORAD | Cat#1708891 |
| RNeasy Mini Kit | Qiagen | Cat# 74106 |
| **Deposited data** | | |
| Sequence read archive (SRA) database | N/A | PRJNA730941 |
| **Experimental models: Cell lines** | | |
| Primary human airway epithelial cells | MATTEK | AIR-100 |
| Primary human airway epithelial cells | Marsico Lung Institute Tissue Core, University of North Carolina | Normal HBE |
| Vero-STAT-1 knockout cells | ATCC | Cat# CCL-81-VHG; RRID: CVCL_YZ45 |

(Continued on next page)
### REAGENT or RESOURCE SOURCE IDENTIFIER

**Experimental models: Organisms/strains**  
A 3D mucociliary tissue model called EpiAirway that consists of normal, human-derived tracheal/bronchial epithelial cells were used in this study.  
MatTek Corp #AIR-100

**Oligonucleotides**

| Description                                      | Source                          | Identifier            |
|--------------------------------------------------|--------------------------------|-----------------------|
| SARS-CoV-2 nCoV_N1 Forward primer                | Integrated DNA Technologies     | Cat#10006821          |
| SARS-CoV-2 nCoV_N1 Reverse primer               | Integrated DNA Technologies     | Cat#10006822          |
| WAKMAR2 (AL35_X3 Set 1) PrimerTime® Std qPCR Assay | Integrated DNA Technologies     | Cat# 423484306        |
| TaqMan gene expression assay-LASI                | ThermoFisher                    | Cat# Hs00273907_s1    |
| TaqMan gene expression assay-MALAT1             | ThermoFisher                    | Cat# Hs00873651_mH    |
| TaqMan gene expression assay-MUC5AC             | ThermoFisher                    | Cat# Hs00861595_m1    |
| TaqMan gene expression assay-MUC5B              | ThermoFisher                    | Cat# Hs00171092_m1    |
| TaqMan gene expression assay-FOXA3              | ThermoFisher                    | Cat# Hs002070130_m1   |
| TaqMan gene expression assay-GAPDH              | ThermoFisher                    | Cat# Hs02786624_g1    |
| PrimePCR™ SYBR® Green Assay-GAPDH               | BIORAD                           | Cat# qHsaCED0038674   |
| PrimePCR™ SYBR® Green Assay-MUC2                | BIORAD                           | Cat# qHsaCID0011696   |
| PrimePCR™ SYBR® Green Assay-MUC4                | BIORAD                           | Cat# qHsaCID0021392   |
| PrimePCR™ SYBR® Green Assay-MUC16               | BIORAD                           | Cat# qHsaCID0018430   |
| PrimePCR™ SYBR® Green Assay-MUC19               | BIORAD                           | Cat# qHsaCID0036986   |
| PrimePCR™ SYBR® Green Assay-ICAM-1              | BIORAD                           | Cat# qHsaCED0004281   |
| PrimePCR™ SYBR® Green Assay-IL-6                | BIORAD                           | Cat# qHsaCED0046677   |
| PrimePCR™ SYBR® Green Assay-NEAT1               | BIORAD                           | Cat# qHsaLED0134812   |
| PrimePCR™ SYBR® Green Assay-SPDEF               | BIORAD                           | Cat# qHsaCID0021209   |
| PrimePCR™ SYBR® Green Assay-ACE2                | BIORAD                           | Cat# qHsaCID0009100   |
| PrimePCR™ SYBR® Green Assay-TMPRSS              | BIORAD                           | Cat# qHsaCID0013558   |
| PrimePCR™ SYBR® Green Assay-NRP1                | BIORAD                           | Cat# qHsaCID0036359   |
| PrimePCR™ SYBR® Green Assay-IFIH1               | BIORAD                           | Cat# qHsaCID0006794   |
| PrimePCR™ SYBR® Green Assay-DDX58               | BIORAD                           | Cat# qHsaCED0043180   |
| PrimePCR™ SYBR® Green Assay-iFN1                | BIORAD                           | Cat# qHsaCED0046851   |
| PrimePCR™ SYBR® Green Assay-iRF3                | BIORAD                           | Cat# qHsaCID0013112   |
| TaqMan™ microRNA Control Assays- U6 snRNA       | ThermoFisher                     | Cat# 001973           |
| TaqMan™ Advanced miRNA Assay- hsa-miR-150-5p    | ThermoFisher                     | Cat# 477918_mir       |
| TaqMan™ Advanced miRNA Assay- hsa-miR-let-7b-5p | ThermoFisher                     | Cat# 478576_mir       |
| TaqMan™ Advanced miRNA Assay- hsa-miR-197-3p    | ThermoFisher                     | Cat# 477959_mir       |
| TaqMan™ Advanced miRNA Assay- hsa-miR-4488      | ThermoFisher                     | Cat# 478906_mir       |
| TaqMan™ Advanced miRNA Assay- hsa-miR-6510-3p   | ThermoFisher                     | Cat# 480744_mir       |
| TaqMan™ Advanced miRNA Assay- hsa-miR-125b-1-3p | ThermoFisher                     | Cat# 478665_mir       |
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Hitendra S Chand.

Materials availability
This study did not generate new unique reagents.

Data and code availability
- Data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Datasets and bioinformatics
Raw RNA sequencing datasets were retrieved from the sequence read archive (SRA) database under accession no. PRJNA730941. Single-end FASTQ files for all COVID-19 patients and healthy controls (Table S1) were obtained using the SRA toolkit version 2.11.2 that was reported recently (Cheemarla et al., 2021). FASTQ files were converted to FASTA using seqtk (https://github.com/lh3/seqtk). Reads were aligned to a database consisting of select cDNA sequences (obtained from Ensembl Biomart) using BLAST+ (version 2.11.0). Alignments were required to uniquely map to a single cDNA and bear >94% identity.
over 100 base pairs. Reads aligning to individual genes were enumerated and gene expression calculated in Reads Per Million (RPM).

COVID-19 patient samples
COVID-19 patient nasal swab samples (see Table 1 for demographics) were either obtained from the University of Miami Biobank facility or iSpecimen Inc. (Lexington, MA). There were 13 males and 7 females with the mean age of 62.5 ± 3.51 y. Frozen cell pellets were processed for total RNA isolation and formalin-fixed cells were spread onto the slides for downstream analysis.

Airway epithelial cell model of SARS-CoV-2 infection
Studies involving SARS-CoV-2 virus infection were conducted at the University of Nebraska Medical Center BSL-3 high containment core facility. SARS-CoV-2 isolate USA-W11/2020 (BEI; cat# NR-52384, B.1.1.7) was passaged in Vero-STAT-1 knockout cells and viral titer was determined using plaque assay (Abdelmoaty et al., 2021; Acharya et al., 2021). Primary human airway epithelial cells were purchased either from the MarSico Lung Institute Tissue Core (University of North Carolina) or from MatTek Incorp (EpiAirway™, MA). Airway epithelial cells were infected with 1 MOI of SARS-CoV-2 inoculum prepared in culture media. For 3D ALI cultured cells, 200 μL was added to the apical surface and 1 mL was added to the basal side. Infected transwells were incubated for 1 h at 37°C in 5% CO2 incubator and the plates were gently mixed every 15 min. After 30 m, apical inoculum was removed and added to the basal media and after additional 30 m total virus inoculum was removed. Transwells were washed thoroughly with PBS and fresh media was added basally. Apical washes and basolateral medium culture supernatants were collected at 1, 4, 24, and 48 h post-infection (hpi) and stored at -80°C for downstream analysis, viral titration, and cytokine analysis. Cells were either lyzed in RLT buffer (RNeasy, Qiagen Inc) with 143 mM 2-ME for total RNA isolation or were fixed with 4% PFA (paraformaldehyde).

METHOD DETAILS

Immunostaining and fluorescent imaging analysis
Formalin-fixed cells were washed in 0.05% Brij-35 in PBS (pH 7.4) and immunostaining was performed as described previously (Chand et al., 2018). Cells were stained with antibodies to MUC5AC (Cat# MAB2011, Millipore Inc., Burlington, MA), and MUC5B (Cat# ab77995, Abcam, Boston, MA). Immunolabelled cells were detected using respective secondary antibodies conjugated fluorescent dyes (Jackson ImmunoResearch, PA) and images were captured using the BZX700 Microscopy system (Keyence Corp) and analyzed using NIH ImageJ software.

Real-Time quantitative PCR for mRNAs, lncRNAs and miRNAs
Total RNA was isolated from swabs or cells using the RNeasy kit (Qiagen, MD) as per the manufacturer's instructions. Primer/probe sets for MUC5AC, MUC5B, SCGB1A1, FOXA3, LASI, WAKMAR2, NEAT1, and MALAT1 transcripts were obtained from Applied Biosystems (ThermoFisher Inc.), and the expression levels were quantified by qRT-PCR using TaqMan Gene expression kit (ThermoFisher Inc.). SYBR green-based primer sets for SARS-CoV-2 N1 viral RNA, ACE2, TMPRSS2, NRP1, ICAM-1, IL-6, MUC2, MUC4, MUC16, MUC19, SPDEF, NEAT1, IFIH1, DDX58, IFNB1, and IRF3 mRNA were obtained from BioRad and quantified by qRT-PCR using the iTag SYBR-green Master Mix (BioRad) in the BioRad CFX96 Real-Time PCR System. Relative quantities were calculated by normalizing data to GAPDH as described previously (Devadoss et al., 2021b). To detect the miRNA levels, cDNA was synthesized from isolated total RNA with a TaqMan® Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, ThermoFisher Inc.). TaqMan® Advanced miRNA assays for let-7b-5p, hsa-miR-150-5p, hsa-miR-197-3p, hsa-miR-200a-5p, hsa-miR-125b-1-3p and hsa-miR-4488 were obtained from Applied Biosystems (ThermoFisher Inc.), and the expression levels were quantified by qRT-PCR using TaqMan® Fast Advanced Master Mix (ThermoFisher Inc.). Relative quantities were calculated by normalizing data to U6 snRNA (Applied Biosystems, ThermoFisher Inc.).

Dual RNA fluorescent in-situ hybridization (FISH) and quantification
Dual-RNA FISH for LASI and SARS-CoV-2 N1 transcripts was performed using the RNAscope® Multiplex reagent kit (Advanced Cell Diagnostics, CA) per the manufacturer’s protocol, the custom-made probe sets and as described recently (Devadoss et al., 2021b). Signals were amplified using Tyramide signal amplification (TSA) reaction using TSA-Plus Cy5 or Cy3 kit (PerkinElmer Bioscience) for LASI or SARS-CoV-2 N1 vRNA, respectively. Images were captured using a structured-illumination module of the
BZX700 Microscopy system and analyzed by NIH ImageJ software and RNA FISH expression was quantified as reported recently (Devadoss et al., 2021a). Briefly, RNA FISH expression was quantified by RNAscope® data analysis suite (Advanced Cell Diagnostics). Probe signals (dots)/cell were counted and allotted to separate bins as follows: Bin 0 (0 Dots/Cell); Bin 1 (1–3 Dots/Cell); Bin 2 (4–9 Dots/Cell); Bin 3 (10–15 Dots/Cell); Bin 4 (>15 Dots/Cell). Then, the Histo-score (H-Score) was calculated as follows: H-Score = sum of each (bin number × percentage of cells per bin) i.e., H-score = (0 × % cells in Bin 0) + (1 × % cells in Bin 1) + (2 × % cells in Bin 2) + (3 × % cells in Bin 3) + (4 × % cells in Bin 4). Final scores derived by this metric have a range between 0 and 400.

Enzyme-linked immunosorbent assays
Apical washes and basal culture media supernatants from SARS-CoV-2 infected cells were analyzed for MUC5AC mucin and the inflammatory factors, interleukin-6 (IL-6) and intercellular adhesion molecule-1 (ICAM-1), protein levels. Apical washes were analyzed for MUC5AC mucin protein levels using specific ELISA (MB8701926, MyBioSource Inc., CA) as per manufacturer’s instructions. Levels of IL-6 (#430507, BioLegend Inc., CA) and soluble ICAM-1 (#LS-F4019, LifeSpan Biosciences Inc., WA) were measured in the basal culture media supernatants in separate ELISAs following manufacturers’ instructions.

Modeling of potential interaction site prediction and system preparation
The IntaRNA 2.0 webserver (Mann et al., 2017) was used to predict potential RNA-RNA binding sites between the SARS-CoV-2 spike viral RNA and LASI lncRNA. The SARS-CoV-2 vRNA sequence 1227-1238 nucleotide (nt) was predicted to bind LASI lncRNA sequence at 635-646 nt. To generate a 3D structure of SARS-CoV-2 vRNA duplexed with the interacting sequence of LASI lncRNA, SARS-CoV-2 sequence from 1198-1268 nt was used. To allow LASI lncRNA nucleotides to base-pair with SARS-CoV-2 vRNA, we concatenated LASI lncRNA sequence 635-646 nt next to the SARS-CoV-2 vRNA 1227-1238. This tandem sequence was then used to predict the 3D structure using RNAComposer (Antczak et al., 2016) webserver, with the secondary structure information provided by RNAfold (Gruber et al., 2015). Finally, LASI lncRNA segment was renamed as a different chain (chain B), with remaining SARS-CoV-2 S vRNA sequence renumbered (chain A). Resulting complex is energy minimized, equilibrated, and further relaxed with molecular dynamics (MD) simulations.

System setup and molecular dynamics simulations
The CHARMM-GUI (Lee et al., 2016) Solution Builder tool was used to prepare the system for MD simulations. The solvated system with a physiological salt concentration of 0.15 M KCl contained a total of 87,000 atoms. All-atom MD simulations were performed using the CUDA version of NAMD 2.14 (Philips et al., 2020) with the Charmm36m force field (Baral et al., 2021). Briefly, the structure was minimized for 10,000 steps with the steepest descent method and equilibrated for 125,000 steps with 2 fs timestep under NVT (constant volume and temperature) conditions. The production run was performed under NPT (constant pressure and temperature) conditions at a temperature of 303.15 K and a pressure of 1 bar. The production run consisted of 2 fs timesteps and a total of 50M steps collecting a 100-ns trajectory. Visualization and analysis of the trajectories were performed with visual molecular dynamics (VMD) (Humphrey et al., 1996).

RNA interference-based lncRNA silencing
The 3D cultured cells were transfected with siRNA specific to LASI (siLASI) or control siRNAs (siCTRL) using X2 (Mirus biotechnologies) transfection reagent (custom made by IDT technologies Inc.) as per manufacturer’s instructions and as described recently (Devadoss et al., 2021a). After 48 h, cells were infected with 1 MOI of SARS-CoV-2 clinical isolate, and cells, apical wash and the basal media supernatant were analyzed at 48 hpi.

Small RNA sequencing analysis
Small RNA-seq was performed by Novogene inc. using an Illumina HiSeq v4 genome sequencer with 20M SE50 Reads. RNA was isolated from the 3D cultured airway cells infected with SARS-CoV-2 and transfected with siLASI to block LASI lncRNA expression. Briefly, Novogene employed the NEB-Next Multiplex Small RNA Library Prep Set for Illumina (NEB) coupled with automated agarose gel size selection (30 to 200 nt) using the Pipin Prep Instrument (Sage Science) for small RNA library preparation. Raw RNA sequencing datasets were retrieved, and FASTQ files were converted to FASTA using seqtk.
Adapter sequences were removed with cutadapt, and reads were aligned to the GRCh38.P10 reference genome using BLAST+ (version 2.11.0) (Camacho et al., 2009). Alignments were required to uniquely map to a single position and bear 100% identity over at least 17 base pairs. Reads aligning to individual microRNAs were enumerated and gene expression calculated in Reads Per Million (RPM). Heat maps and unsupervised hierarchical clustering were generated using Heatmapper (Babicki et al., 2016).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Mean and standard error for replicates of the experiments were calculated, plotted, and analyzed using GraphPad Prism v9.0 (GraphPad Software Inc., San Diego, CA). Grouped results were analyzed using a one-way analysis of variance (ANOVA) with multiple comparisons, and student’s t-test was used for data analysis between two groups. Data with FDR (false discovery rate) adjusted p-value threshold of ≤0.05 are considered as significantly relevant using Benjamini-Hochberg correction.