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The accessible promoter-mediated supplementary effect of host factors provides new insight into the tropism of SARS-CoV-2

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In the past year, the rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in the worldwide coronavirus disease 2019 (COVID-19) pandemic. Yet our understanding of the SARS-CoV-2 tropism mechanism is still insufficient. In this study, we examined the chromatin accessibility at the promoters of host factor genes (*ACE2*, *TMPRSS2*, *NRP1*, *BSG*, *CTSL*, and *FURIN*) in 14 tissue types, 23 tumor types, and 189 cell lines. We showed that the promoters of *ACE2* and *TMPRSS2* were accessible in a tissue- and cell-specific pattern, which is accordant with previous clinical research on SARS-CoV-2 tropism. We were able to further verify that type I interferon (IFN) could induce angiotensin-converting enzyme 2 (ACE2) expression in Caco-2 cells by enhancing the binding of HNF1A, the transcription factor of ACE2, to ACE2 promoter without changing chromatin accessibility. We then performed transcription factor (TF)-gene interactions network and pathway analyses and discovered that the TFs regulating host factor genes are enriched in pathways associated with viral infection. Finally, we established a novel model that suggests that open chromatin at the promoter mediates the host factors' supplementary effect and ensures SARS-CoV-2 entry. Our work uncovers the relationship between epigenetic regulation and SARS-CoV-2 tropism and provides clues for further investigation of COVID-19 pathogenesis.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had major incidences of outbreaks in many parts of the world and threatens overall public health.[1] The World Health Organization has declared that the 2019 novel coronavirus disease (COVID-19), caused by SARS-CoV-2, has created a public health emergency of international concern. Tissue and cell tropism, defined as the tissues and cell types that can be infected by a virus, are key determinants of viral pathogenesis. SARS-CoV-2 is primarily transmitted between people through respiratory droplets and contact routes. In addition to affecting the respiratory system, SARS-CoV-2 could also cause injuries to various other tissue types, such as nerve, adrenal, thymus, esophagus, pancreas, cervix, breast, skin, and lymph node.[2,3] Host cell factors involved in the viral entry steps are the major determinants of coronavirus tropism and efficiency of cellular entry.

Angiotensin-converting enzyme 2 (ACE2) was identified as the cellular receptor for SARS-CoV-2 entry,[4] similarly to SARS-CoV.[5] However, previous studies have documented generally low ACE2 expression in multiple tissue types, including lung and airway epithelium.[6–8] The expression pattern of ACE2 does not correlate with the tissue tropism of SARS-CoV-2. We confirmed this observation by analyzing RNA sequencing (RNA-seq) data from Genotype-Tissue Expression (GTEx) project[9] (Figure S1; Table S1). Wang et al.[10] investigated the expression and associated *cis*-regulatory landscape of host factor genes at single-cell resolution in individual lung cell types across age. Their study provides insight into the regulatory logic underlying genes implicated in COVID-19 in individual lung cell types across ages. Despite the minor expression difference among cell subtypes, the mechanism of lower ACE2 mRNA expression in lungs than that in other non-susceptible tissues remains unknown. The discordance between ACE2 mRNA expression and SARS-CoV-2 tropism complicates the current understanding of the infection, spread, and clearance of this virus. We also checked the protein expression in multiple tissues from the Human Protein Atlas (https://www.proteinatlas.org). ACE2 protein expression was not detected in lung tissue (Figure S2). Type II transmembrane serine protease (TMPRSS2)[6] has been shown to be also essential for

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coronavirus viral entry, as this protease could cleave the viral spike protein, and promote the fusion of SARS-CoV-2 with cells.[6] In the same way, cathepsin L (CTSL) has also been proven to be a crucial factor for SARS-CoV-2 to enter the host cell.[7] Wang et al. indicated that basigin (BSG) could be another receptor as a binding partner for spike protein with functional significance in SARS-CoV-2 infection.[11] SARS-CoV-2 evolves a multi-base site at the S1-S2 boundary, which is supposed to be cleaved by furin. So furin could also promote SARS-CoV-2 infectivity.[12,13] Neuropilin-1 (NRP1), known to bind furin-cleaved substrates, has also been proven to significantly facilitate the invasion of SARS-CoV-2.[14] These studies provide important evidence to understand the pathogenesis of COVID-19, reveal the law of SARS-CoV-2 tropism, and further help identify novel host-directed therapies that may have efficacy against SARS-CoV-2.

Chromatin state dictates fundamental cellular processes, including gene expression.[15] Accessible chromatin has long been known to mark regulatory sequences and to interact with transcription factors to execute transcriptional programs instructing cell-fate determination and development.[16,17] Generally, the activation of genes positively correlates with increased promoter accessibility.[18] Thus, examination of chromatin accessibility will help to reveal the transcriptional activity and cell state. However, limited study has focused on learning the relationship between chromatin accessibility and SARS-CoV-2 infection. Here, by investigating the chromatin accessibility at the promoters of the host factor genes (*ACE2*, *TMPRSS2*, *NRP1*, *BSG*, *CTSL*, and *FURIN*) from different tissues and cell lines, we show that the *ACE2* and *TMPRSS2* promoter is accessible in limited tissues and cell types and that this tissue- and cell-specific pattern is quite accordant with clinical findings. We propose that open chromatin at the promoter mediates the *ACE2* and *TMPRSS2* supplementary effect and further induces SARS-CoV-2 tropism.

RESULTS

The tissue specificity of chromatin accessibility at host factor promoters is consistent with tissue tropism of SARS-CoV-2

As previously reported, assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) and DNase-seq have been recognized as some of the best methods for determining chromatin accessibility.[19] We first examined chromatin accessibility at the promoters of six host factor genes (*ACE2*, *TMPRSS2*, *NRP1*, *BSG*, *CTSL*, and *FURIN*) in 14 human tissue types (Figures 1A and 1B; Table S2). We observed that the promoters of *NRP1*, *BSG*, *CTSL*, and *FURIN* were accessible in almost all tissue types (Figure 1A). In contrast, the promoter of *ACE2* was only accessible in the lungs, intestines, and placentas (Figures 1C and 1E), while the promoter of *TMPRSS2* was only accessible in lung, eye, kidney, intestine, and testis (Figures 1D and 1F). These observations are consistent with those of tissue tropism commonly observed in the following. First, as a respiratory virus, SARS-CoV-2 primarily targets the lungs. Secondly, the unexpected symptom of diarrhea was reported in patients with COVID-19,[20] most testing positive from their stool samples, which persisted in some patients even after the viral RNA load had decreased to an undetectable level in the respiratory tract.[21] Third, ACE2 expression was noticeable in certain placental and decidual cell types without transmembrane serine protease 2 (TMPRSS2).[22] Furthermore, clinical reports suggested that SARS-CoV-2 could infect the placentas;[23–25] however, that remains a point of contention, since some researchers have also demonstrated that direct placental infection by SARS-CoV-2 is a rare event.[26]

Currently, cancer has been identified as an individual risk factor for COVID-19.[27–29] Thus, we next investigated the chromatin accessibility at the *ACE2* promoter in cancer cells. We collected ATAC-seq data for 23 types of tumors from The Cancer Genome Atlas project.[30] *ACE2* promoter was accessible in five tumor samples (lung adenocarcinoma, colon adenocarcinoma, adrenocortical carcinoma, breast invasive carcinoma, and thyroid carcinoma; Figure S3A; Table S3).

Appropriate activity levels of gene promoters are essential for coordinated transcription within a cell.[19] Chromatin accessibility at the promoter represents potency in terms of the ability to execute transcriptional programs. Thus, this specificity of chromatin accessibility at the promoters of host factor genes, especially *ACE2* and *TMPRSS2*, provides important hints for explaining the tissue tropism of SARS-CoV-2 infection.

The chromatin accessibility of host factors promoter is consistent with cell-type-specific tropism of SARS-CoV-2 within human lung tissue

Since it is evident that COVID-19 could cause severe respiratory symptoms, the lungs are the main target of SARS-CoV-2. We then performed an analysis on a set of lung single-nucleus ATAC-seq data from Wang et al.[10] The results demonstrate that the promoter regions of *NRP1*, *BSG*, *CTSL*, and *FURIN* were accessible in almost all cell types (Figure 2A). In contrast, *ACE2* promoter was accessible only in alveolar type 1 (AT1) cells, alveolar type 2 (AT2) cells, basal cells, ciliated cells, and club cells (Figures 2A and 2B), and the *TMPRSS2* promoter was accessible only in AT1 cells, AT2 cells, and club cells (Figures 2A and 2C). These observations were almost consistent with the cell type tropism exhibited by SARS-CoV-2 in COVID-19 lung autopsies by Hou et al.,[31] as they reported that AT1 cells, AT2 cells, and ciliated cells were infected by SARS-CoV-2. Robert et al. proved that the expression levels of *ACE2* increased significantly in a subset of epithelial cells (including AT1 and AT2), but not in immune cells.[32] Thus, our results indicate that the cell-type-specific up-regulation of *ACE2* expression may be driven by the chromatin accessibility at the promoter.

The cell lines with accessible host factors promoters could be infected by the virus

We also collected DNase-seq data and examined DNase I hypersensitivity sites (DHSs) in 63 cancer and 126 normal cell lines from the Encyclopedia of DNA Elements project (ENCODE)[33] (Table S4). Similarly, the percentage of cancer cell lines (10/63 [16%]; Figure S3B) with accessible *ACE2* promoter was much higher than that of normal cell lines (11/126 [9%]; Figure S3C; Table S5). Furthermore, we selected two

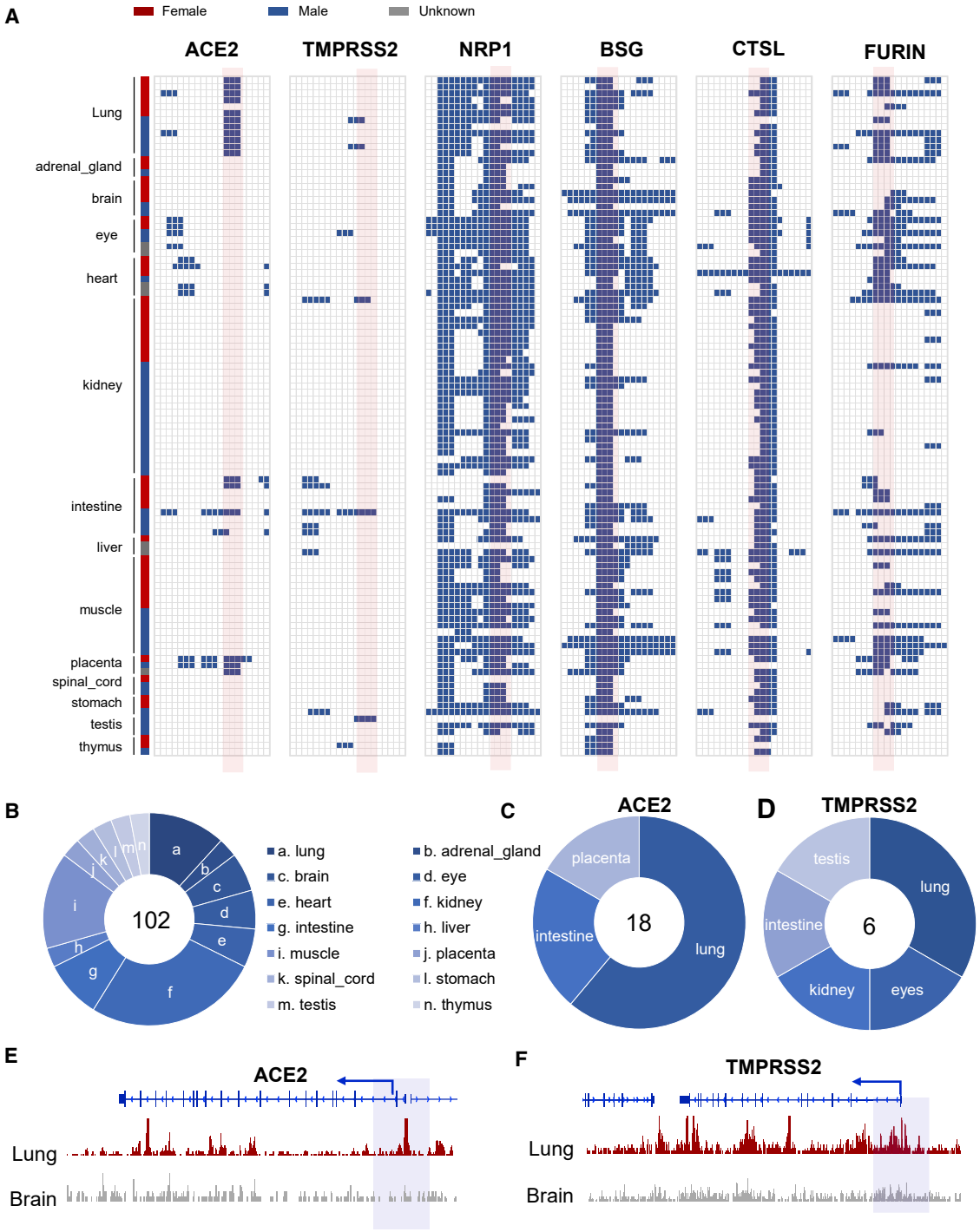


Figure 1. The chromatin accessibility of host factors promoter in human tissues

(A) Heatmaps are showing the distribution of ATAC-seq peaks and DHSs in the binned genome regions, which cover the promoters of *ACE2*, *TMPRSS2*, *NRP1*, *BSG*, *CTSL*, and *FURIN*. Promoter regions of these host factors are highlighted within red box. (B) Data of ATAC-seq peaks and DHSs from the ENCODE project are shown. Donut pie chart shows the proportions of samples in each tissue type group. (C and D) Donut pie chart shows the proportions of tissue samples in which the promoter region of *ACE2* (*TMPRSS2*) was accessible. (E and F) Schematic representation of the chromatin accessibility of *ACE2* (*TMPRSS2*) loci in the human lung and brain tissues.

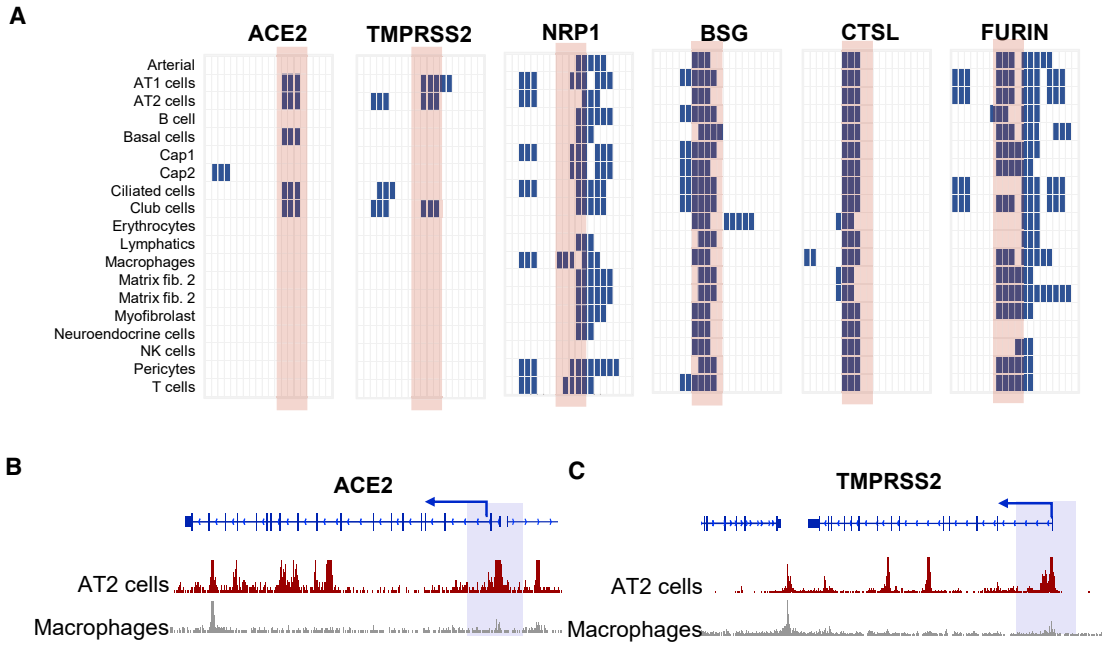


Figure 2. The chromatin accessibility of host factors promoter in different cell types within human lung tissue

(A) Heatmaps show the distribution of ATAC-seq peaks and DHSs in the binned genome regions, which cover the promoters of *ACE2*, *TMPRSS2*, *NRP1*, *BSG*, *CTSL*, and *FURIN*. Promoter regions of these host factors are highlighted within a red box. (B and C) Schematic representation of the chromatin accessibility of *ACE2* (*TMPRSS2*) loci in the AT2 and macrophages cells of human lungs is shown.

cell lines (Caco-2 and A549) to validate the relationship of host factors' promoter accessibility and susceptibility to SARS-CoV-2. First, we examined DHSs at the promoters and found that the promoters of *ACE2* and *TMPRSS2* were accessible in Caco-2 (Figures 3A and 3B) but inaccessible in the A549 cell line (Figures 3A and 3B). Then, we conducted a SARS-CoV-2 pseudovirus infection experiment in these two cell lines by infecting cells using HIV-based pseudovirus bearing the SARS-CoV-2 Spike protein. As expected, we observed that the Caco-2 cell line was susceptible to the SARS-CoV-2 pseudovirus and A549 was unsusceptible (Figure 3C). It is reported that ACE2 can be induced by type I interferons (IFNs) during SARS-CoV-2 infection.[34] Thus, we performed a 50 ng/mL type I IFN induction in both Caco-2 and A549 cells. Interestingly, we observed that type I IFN can only induce ACE2 mRNA and protein expression in Caco-2 cells, but not in A549 cells (Figures 3D and 3E). However, the mechanism of type I IFN inducing ACE2 expression was still unknown. Thus, we performed ATAC-qPCR of ACE2 promoter in Caco-2 and A549 with or without IFN-α treatment (Figures 3F and 3H). The accessibility of the ACE2 promoter in the two cell lines was not significantly changed. Moreover, we noticed the motif of HNF1A,[35] a transcription factor of ACE2, located in the ACE2 promoter region (Figure 3G). Thus, we performed CUT&Tag-qPCR of HNF1A in ACE2 loci (Figures 3F–3I). We found that type I IFN enhanced the binding of HNF1A to ACE2 promoter in Caco-2 cells.

We inferred that the promoter of ACE2 in Caco-2 cells remains in a permissive chromatin state. In this way, the ACE2 promoter in Caco-2 cells has the potential to bind transcription factors. Type I IFN induced the transcription factors binding to ACE2 promoter without changing chromatin accessibility and promoted the ACE2 expression in Caco-2 cells. As a result, the type I IFN induced ACE2 expression and SARS-CoV-2 infection in the cell-specific pattern.

Transcription factors regulating host factor genes are enriched in pathways associated with viral infection

Although ACE2[4] and TMPRSS2[6] are known to be associated with SARS-CoV-2 infection, few studies have been conducted to explore the transcription factors that regulate them. Thus, we analyzed the transcription factor (TF)-gene interaction networks of host factor genes. We found the TFs that regulate host factor genes generate complex TF-gene interaction networks. For instance, *GATA2* mediates gene regulation of *ACE2*, *CTSL*, and *BSG*. And *FOXC1* mediates gene regulation of *BSG*, *TMPRSS2*, and *ACE2* (Figure 4A). Furthermore, we analyzed the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of TFs that regulate host factor genes. The pathways are associated with viral infection (Figure 4B), such as HILV-I infection, hepatitis C, and Kaposi sarcoma-associated herpesvirus (KSHV) infection. Since the accessibility of *ACE2* and *TMPRSS2* promoters is consistent with cell-type-specific tropism of SARS-CoV-2, we analyzed the generic protein-protein interactions (PPIs) networks of TFs regulating *ACE2* and *TMPRSS2*, respectively (Figures 4C and 4D). We also analyzed the KEGG pathways of the PPI network. As expected, the pathways are highly associated with viral infection (Figures 4E and 4F).

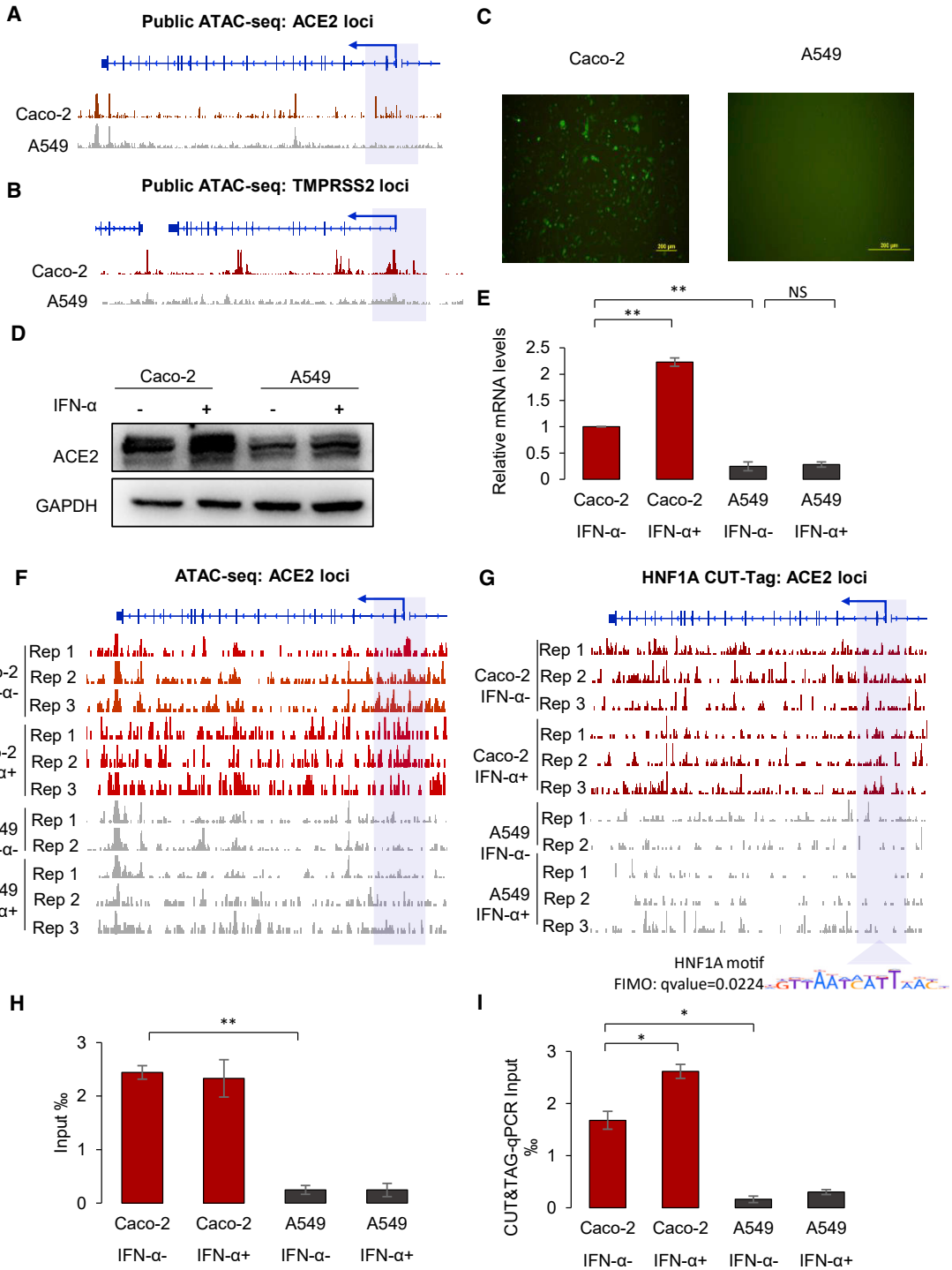
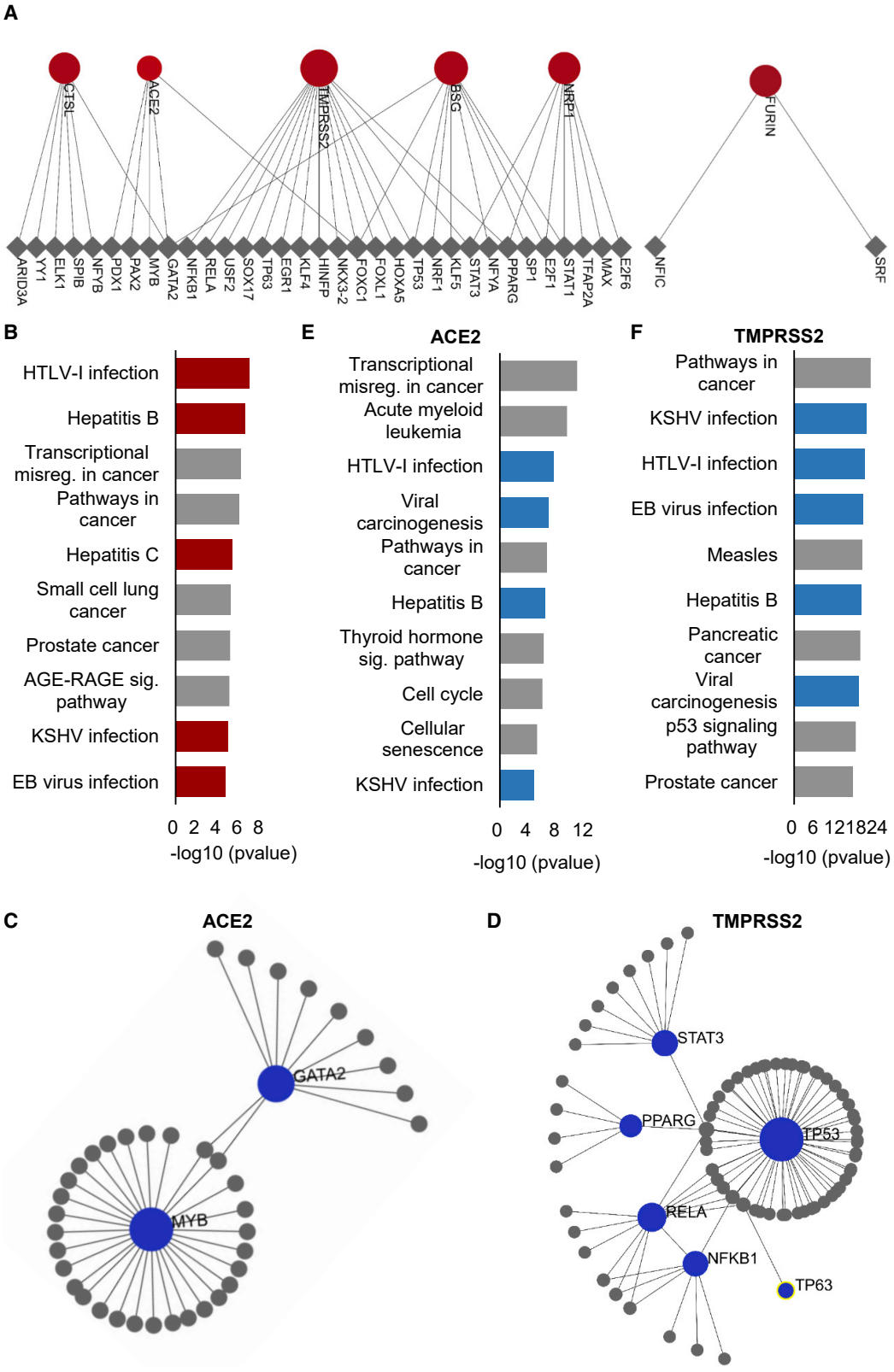


Figure 3. The accessibility and transcription factors binding in ACE2/TMPRSS2 promoter and SARS-CoV-2 tropism in Caco-2 and A549 cells

(A and B) Schematic chromatin accessibility of *ACE2* (*TMPRSS2*) loci in the Caco-2 and A549 cell lines. (C) Representative images of SARS-CoV-2 pseudovirus infectivity in Caco-2 and A549 cell lines are shown. Scale bars, 200 μm. (D) Western blot of ACE2 and GAPDH in Caco-2 and A549 cells with or without IFN-α treatment is shown. (E) mRNA level of ACE2 in Caco-2 and A549 with or without IFN-α treatment is shown. **p<0.01, NS, not significant, t test. (F) Schematic chromatin accessibility of ACE2 loci in the Caco-2 and A549 cell lines with or without IFN-α treatment is shown. (G) Schematic HNF1A binding signal of ACE2 loci in the Caco-2 and A549 cell lines with or without IFN-α treatment is shown. (H) ATAC-qPCR of ACE2 promoter in Caco-2 and A549 with or without IFN-α treatment is shown. **p<0.01, t test. (I) CUT&Tag-qPCR of HNF1A in ACE2 promoter loci in Caco-2 and A549 with or without IFN-α treatment is shown. *p<0.05, t test.



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Previous report[36] based on multi-omics (interactome,[37] proteome,[38] transcriptome,[39,40] and bibliomic) data and subsequent integrated analysis identified SARS-CoV-2 infection shared pathways with influenza A, Epstein-Barr virus, human T-lymphotropic virus type 1 (HTLV-I), measles, and hepatitis virus, which is highly consistent with our results.

A model of the accessible promoter-mediated supplementary effect of host factors

The host factors, *ACE2*, *TMPRSS2*, *NRP1*, *BSG*, *CTSL*, and *FURIN*, are essential for SARS-CoV-2 infection.[13,41,42] However, these host factors need transcriptional supplementation to maintain protein content on cell surface for virus entry. For instance, as endocytosis is essential for the establishment of virus entry, ACE2 is internalized together with SARS-CoV-2 upon infection.[42–45] In addition, ACE2 undergoes ectodomain shedding, making it subject to juxtamembrane cleavage events.[46,47] As such, ACE2 expression on cell surfaces is dynamic, with new ACE2 supplementing diminished ACE2 protein on cell surface. Similarly, the TMPRSS2 and other host factors are also used up while performing their functions, and they also need to be expressed and supplemented.

Here, we proposed an accessible promoter-based model to show the potential supplementary mechanism of the expression host factors. Taking the lungs as an example (Figure 5), the accessible chromatin at the promoters of host genes provides templates for TF binding, and the TFs then recruit cofactors and RNA polymerase II (RNA Pol II) for transcription initiation. Thus, promoter accessibility of host genes is required for continuous transcription in order to compensate for protein expression, which is then consumed by the internalization and shedding processes. Due to the tissue and cell-specific promoter accessibility of host factors, especially *ACE2* and *TMPRSS2*, different tissues and cell types would have different susceptibility to SARS-CoV-2.

DISCUSSION

Here, we report a systematic survey of accessible chromatin of host factor genes (*ACE2*, *TMPRSS2*, *NRP1*, *BSG*, *CTSL*, and *FURIN*) in 14 tissue types, 23 tumor types, and 189 cell lines. Our results revealed that the promoter of *ACE2* and *TMPRSS2* had limited accessibility, restricted to SARS-CoV-2-susceptible tissues and cell lines, including those of human lungs. We confirmed this pattern by performing SARS-CoV-2 pseudovirus infections in cell lines. We further verified that type I IFN could induce the TFs binding to ACE2 promoter without changing chromatin accessibility and promotes the ACE2 expression in Caco-2 cells but could not induce ACE2 expression and SARS-CoV-2 in A549 cells. We performed TF-gene interaction network and pathways analysis and found that the TFs regulating host factor genes are enriched in pathways associated with viral infection. Based on our results, we propose that open chromatin at the promoter mediates the host factors through a supplementary effect and ensures the intracellular entry of SARS-CoV-2. For instance, although human lungs express lower ACE2 levels, owing to the accessibility of *ACE2* promoter, the lungs may have already acquired the regulatory potential for higher ACE2 expression. This mechanism is likely to be the underlying cause of persistent lung invasion and pathological changes. These results provide new insights into the tissue tropism of SARS-CoV-2. Further investigations with additional experiments, especially acquisition and integration analysis of RNA-seq and ATAC-seq data of tissues with or without SARS-CoV-2 infection, are warranted to fully confirm this hypothesis.

Moreover, our findings highlighted that the host epigenetic machinery could contribute to the SARS-CoV-2 infection. In addition, lots of studies have proven that the epigenetic mechanisms could also affect COVID-19 progressions by regulating immune-related signaling.[48–50] Epigenetics, as a new area of life science, has been proven to play a critical role in the occurrence and progression of many other common diseases,[51] with epigenetic research providing evidence that replication of DNA and RNA of viruses are closely related to the host epigenome.[52] Thus, epigenetic studies could reveal new mechanisms of virus-host interaction and its function in the progression of diseases. Evaluating specific epigenetic regulators as targets may contribute to the development of antiviral drugs and exploring new epigenetics-based therapies for different virus families, including coronaviruses. Overall, our study serves as a catalyst for seeking the major reasons for the continuous invasion of cells by SARS-CoV-2 from the perspective of epigenetics, which would be beneficial in developing specific novel strategies for preventing and treating infections.

MATERIALS AND METHODS

Datasets

ATAC-seq peaks and DHSs data of 14 human tissue types were downloaded from the ENCODE project (https://www.encodeproject.org/). ATAC-seq peaks of tumors were from the Cancer Genome Atlas (TCGA) project. Processed single-cell ATAC-seq data of the human lung were from Allen et al. (https://www.lungepigenome.org/).[10] RNA-seq processed data of tissues were from the GTEx project (https://www.gtexportal.org).

Mapping ATAC-seq peaks and DHSs to bin-based genomic regions

To investigate the chromatin accessibility of host factors, we developed a pipeline by simply mapping ATAC-seq peaks and DHSs to

Figure 4. Transcription factors regulating host factor genes are enriched in pathways associated with viral infection

(A) TF-gene interaction network of host factor genes. Host factors are shown as red nodes, and transcription factors are shown as gray nodes. (B) KEGG pathways of transcription factors regulating host factor genes are shown. Pathways related to viral infection are shown as red bars. (C and D) Protein-protein interaction network of transcription factors regulating ACE2 (TMPRSS2) is shown. Transcription factors regulating host factors are shown as blue nodes, and protein interactions with transcription factors are shown as gray nodes. (E and F) KEGG pathways of PPI network in (C) and (D) are shown. Pathways related to viral infection are shown as blue bars.

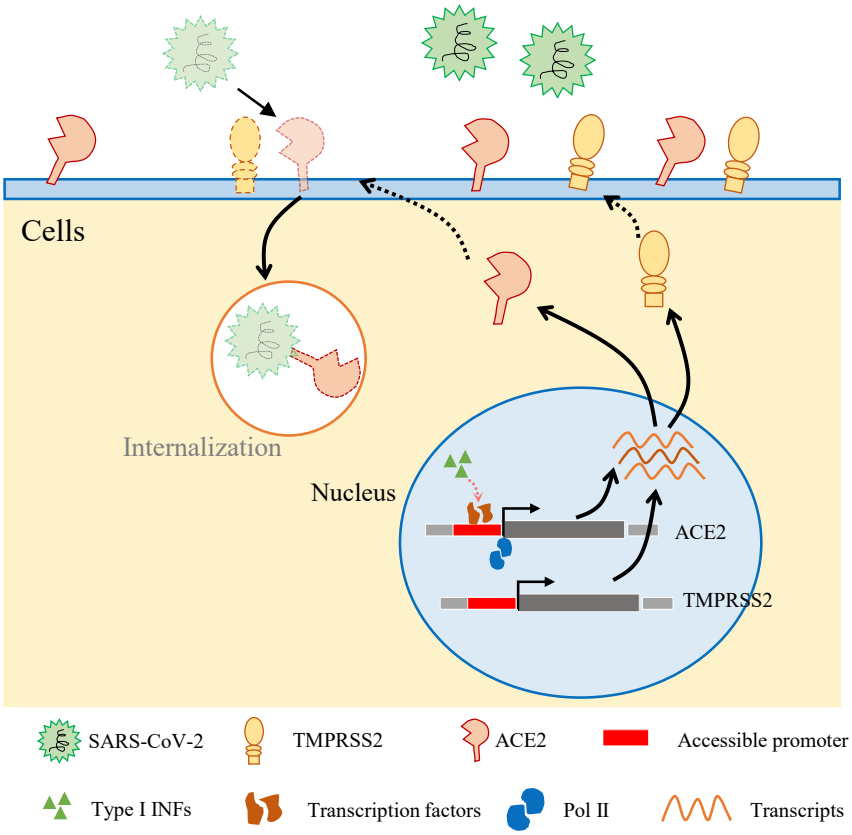

Figure 5. Hypothetical model in which open chromatin at the promoter mediates entry genes supplementation and ensures the entry of SARS-CoV-2

Type I IFNs induce the transcription factors binding to ACE2 promoter without changing chromatin accessibility and promote the ACE2 expression in susceptible cells.

bin-based genomic regions. Genomic regions of covering *ACE2*, *TMPRSS2*, *NRP1*, *BSG*, *CTSL*, and *FURIN* (chrX:15,577,500–15,623,500, chr21:42,833,500–42,906,000, chr10:33,463,500–33,628,000, chr19:568,500–585,500, chr9:90,337,500–90,351,500, and chr15:91,409,000–91,429,500, respectively) were split by 500 bp/bin. Accessible bins were defined as those overlapping with ATAC-seq peaks and DHSs by at least 1 bp.

SARS-CoV-2 pseudovirus infection assays

Caco-2 and A549 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco). All growth media were supplemented with 10% fetal bovine serum (FBS). Cells were seeded at a density of 1×10^4/well in 96-well plates 16 h before infection. Cells in each well were then infected with 150 μL SARS-CoV-2 pseudovirus bearing dual-reported genes (EGFP and luciferase) at an MOI of 10 and incubated at 37°C for 12 h. The virus was then removed, and 200 μL of fresh culture medium was added for further incubation. Three days post-infection, images of EGFP expression were captured under a fluorescent microscope (IX73; Olympus).

RNA extraction and qRT-PCR

Total RNA was isolated using TRIzol reagent (Invitrogen). For qRT-PCR of mRNAs, cDNA synthesis was performed with 1 μg of total RNA using ReverTra Ace qPCR RT Master Mix (TOYOBO; FSQ-201). Quantitative PCR detecting ACE2 and TMPRSS2 was carried out using SYBR green master mix (TOYOBO; QPK-201). Expression was measured using the ΔΔCt method. PCR primers are as follows: ACE2 forward 5′-CGA AGCCGAAGACCTGTTCTA-3′, reverse 5′-GG GCAAGTGTGGACTGTTCC-3′; β-actin forward 5′-ATCGTCCACCGCAAATGCTTCTA-3′, reverse 5′-AGCCATGCCAATCTCATCTT GTT-3′.

Western blot assay

Cells were lysed in Radio Immunoprecipitation Assay (RIPA) buffer supplemented with protease inhibitors and sonicated to shear DNA. Cleared lysates were resolved by SDS-PAGE, transferred to polyvinylidene fluoride (PVDF) membrane and probed with specified antibodies. The following primary antibodies were used: anti-ACE2 (Proteintech; 21115-1-AP), anti-TMPRSS2 (Proteintech; 14437-1-AP), and GAPDH (Cell Signaling Technology; no. 4970).

CUT&Tag library preparation and CUT&Tag-qPCR

Anti-HNF1A (Proteintech; 22426-1-AP) primary antibody and a rabbit secondary antibody (Abcam; 31,238) were utilized for CUT&Tag analysis. CUT&Tag libraries were prepared using CUT&Tag 3.0 High-Sensitivity Kit (Novoprotein; N259-YH01). In brief, 50,000 cells were prepared and immobilized on concanavalin A beads. Beads are incubated with a primary antibody, followed by incubation with a secondary antibody. Beads were washed and incubated with pA-Tn5. Tn5 was activated by addition of Mg^{2+} and incubated at 37°C for 1 h. Reactions were stopped by the addition of 10 μL 0.5M EDTA, 3 μL 10% SDS, and 2.5 μL 20 mg/mL Proteinase K to each sample. DNA was extracted with phenol-chloroform and constructed CUT&Tag library according to the manufacturer's instructions.

ATAC-seq library preparation and ATAC-qPCR

ATAC libraries were prepared using ATAC-seq Kit (Novoprotein; N248). In short, 50,000 cells per sample were lysed in 50 μL of lysis buffer. Immediately after lysis, nuclei were collected and incubated with Tn5 transposase and tag mentation buffer at 37°C for 30 min. After tag mentation, the transposed DNA was purified with Tagment DNA extract beads. A polymerase chain reaction was performed to amplify the library. The final library was purified by DNA clean beads.

The chromatin accessibility of the ACE2 promoter region was also measured using the qRT-PCR. PCR primers are as follows: ACE2 forward 5′-AGCTCAGTGTCCTCATTGCC-3′, reverse 5′-AATCATCG TCAGGTAGGCCC-3′.

CUT&Tag data analysis

CUT&TAG libraries were paired-end sequenced on an Illumina Xten (Illumina, San Diego, CA) with 150 paired reads. Paired-end reads were trimmed with TrimGalore (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/): -phred33 -q 20. The clean fastq files were aligned to human genome using Bowtie2[53] with the following parameters: -p 20 –very-sensitive-local –no-unal –no-mixed –no-discordant –phred33 -I 10 -X 700. The sam files were transformed into sorted bam files with samtools.[54] PCR duplication was removed, and bam index was built using samtools. Bam files were converged to bigwig files with deepTools[55] using the bamCoverage command.

ATAC-seq data analysis

ATAC-seq libraries were paired-end sequenced on an Illumina Xten (Illumina, San Diego, CA) with 150 paired reads. Reads then were trimmed with TrimGalore. The trimmed fastq files were aligned to the human genome with Bowtie2 followed by the removal of PCR duplicates and mitochondrial reads. Bam files were converged to bigwig files with deepTools[55] using bamCoverage command.

Network and pathway analysis

TF-gene interaction networks and pathways analyses were performed and visualized with the NetworkAnalyst tool.[56] Host factor genes (*ACE2*, *TMPRSS2*, *NRP1*, *BSG*, *CTSL*, and *FURIN*) were used as the input to generate TF-gene interaction network (parameters: TF-gene interaction and JASPAR). KEGG pathway analysis was performed, and the ten pathways with the lowest p value were shown. Then, *ACE2* was used as the TF-gene interaction networks input in order to find TFs regulating it. The TFs regulating *ACE2* were used as input to generate generic PPI networks (parameters: STRING Interactome, confidence score cutoff: 950, and require experimental evidence). Furthermore, the PPI networks were taken to analyze the KEGG pathways. The *TMPRSS2* was analyzed the same as *ACE2*.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtn.2022.03.010.

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AUTHOR CONTRIBUTIONS

X.B., H.C., and H.L. conceived the project. X.X., G.D., J.W., X.W., Y.D., F.L., Y.S., H.T., and Y.L. conducted the investigations and data mining. X.W. conducted the pseudovirus infection experiment. X.X., G.D., J.W., and Y.L. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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