ACE2 Protein-protein Interaction Networks Reveal Potential Druggable Targets for SARS-CoV-2

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Research

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Abstract

Background The novel coronavirus SARS-CoV-2 pandemic has infected more than 10 million people, killed over 500,000 so far. Currently, no effective drugs are available to treat this infectious disease, due to limited knowledge of the molecular mechanisms of SARS-CoV-2 infection. ACE2 (angiotensin I converting enzyme 2) has long been identified as the major receptor for coronavirus entry the host cells.

Methods In this study, we constructed the protein-protein interaction networks (PPIN) based on ACE2 and its interacting proteins, combined with the expression change and co-expression relationship. The potential drugs targeting the proteins in the PPIN were explored.

Results ACE2 and its interacting proteins AAMP and HRAS are obviously increased, and their PPIN show distinguishing expression patterns during the COVID-19 progression. At least six pathways are activated for the host cell in the response to the virus. Moreover, drug-target networks were built to provide important clues to block ACE2 and its interacting proteins. Except the reported four drugs for ACE2, its interacting protein CALM1 and HRAS are great potentially druggable. We also considered the path initiated from ACE2 to nucleus by cascades of interaction, especially for the transcription factors in the PPIN which are also druggable.

Conclusion In summary, this study provides new insight into the disruption of the biological response to virus mediated by ACE2, but also its cascade interacting proteins when considering of PPIN.

Introduction

The outbreak of global SARS-CoV-2 pandemic has profoundly threatened the health of billions of individuals, strained national health care systems and millions lives have lost by the end of July 2020 (https://coronavirus.jhu.edu/map.html). The most distinguishing clinic character of COVID-19 patients is a dysfunction of breath primarily due to acute respiratory distress syndrome, causing a greater risk of mortality [1]. The first step of viral infections is the entry of the virus of the host cell, following the replication multiple viral copies using the host cellular machinery. It has been widely acknowledge that angiotensin-converting enzyme-2 (ACE2, EC 3.4.17.23) acts as a major receptor for COVID-19 to gain intracellular entry into the host cells[2]. ACE2 is a membrane-bound protein, with a NH_2-terminal domain comprises the catalytic site oriented extracellularly[3]. ACE2 is normally expressed in many human tissues, including lung, small intestine, heart, brain stem, nasal and oral mucosa[4].

The mechanism for COVID-19 causes lung dysfunction has been proposed. ACE2 acts as mediator of coronavirus entry into the cells, but also contributes to diffuse alveolar damage through imbalances in the renin–angiotensin system. These immunologic reactions in severe COVID-19 may characterize the cytokine storm phenomenon: a massive release of macrophage migration inhibitory factor (MIF), tumour necrosis factor (TNF)-α, and interleukin (IL)-1, IL-2R, IL-6, IL-8, and IL-10 from host cells and immune cells[5, 6]. These inflammatory cells and factors will lead to the typical ARDS (Acute respiratory distress

syndrome) pathology with acute diffuse alveolar damage, pulmonary oedema, and formation of hyaline membranes[7].

However, the detailed of molecular mechanism of how ACE2 is stimulated by virus and the change of phenotype in host cell remain unclear. Since proteins perform different functions depending on the time-space interaction with other proteins or substrates, their functions maybe predicted through the interactions with neighboring proteins. Analyses based on protein-protein interaction networks (PPINs) have become prevalent and important after the generation of high-throughput data[8]. Network-based analyses aim to systematically integrate measurements from high-throughput data to gain a global understanding of cellular function under changing conditions, e.g. different disease sub-types, progressive stages of cancer and drug[9, 10].

In this study, we constructed the protein-protein interaction network (PPIN) for ACE2 and its interacting proteins, and analyzed the expressed change and expression pattern during the COVID-19 progression. Moreover, the drug-target network was built to provide the clues to block the path initiated from ACE2.

Materials And Methods

Search of proteins that interacts with ACE2

The known interacting proteins of ACE2, confirmed and reported by previous publications, were obtained from two databases of BioGRID (https://thebiogrid.org/) and MINT (https://mint.bio.uniroma2.it/). They were curated to obtain the unique interacting protein list.

Construction of the protein-protein interaction network (PPIN)

The latest known human protein-protein interaction data were obtained from several databases, including BioGRID (http://thebiogrid.org/), HPRD (http://www.hprd.org/), MINT (https://mint.bio.uniroma2.it/), and IntAct (http://www.ebi.ac.uk/intact/). These protein interaction dataset were integrated manually to reduce redundancy and obtain a unique dataset, containing all known published human protein-protein interactions so far. This unique PPI dataset has 24,046 unique proteins and 438,656 interactions, and is considered as the parental PPI network from which new or sub-PPI networks were constructed by Cytoscape[11]. First, ACE2 and its interacting proteins were mapped to the parental PPIN as the seed proteins, a sub-network was built by the extraction of their first class directly interacting proteins. This subnetwork is named “ACE2 Full-PPIN”. Second, ACE2 and its interacting proteins link through one or more partner proteins was illustrated in a smaller PPI sub-network, which is named “ACE2 Core-PPIN”.

Analyses of the PPIN topological parameters

Topological parameters were analyzed by NetworkAnalyzer, which would provide insight into the organization and structure of the complex network[12]. The power law of distribution of node degrees, one of most important network topological characteristics, was analyzed as we described previously[13]. Briefly, the degree distribution *P(k)* of a network was then defined to be the fraction of nodes in the network with degree k. Thus, if there were *n* nodes in total in a network and n_k of them have degree *k*, we have $P(k) = n_k / n$. Moreover, several other important topological parameters, including shortest path length, topological coefficients, and closeness centrality, were also analyzed and shown.

Subcellular distribution of the PPIN

Subcellular locations for the proteins in the “ACE2 Full-PPIN” were obtained from the HPRD database and were imported into the network as a node attribute as we previously described[14]. If some proteins are annotated with multiple locations, e.g. if a protein translocated from the cytoplasm into the nucleus, these locations were merged as cytoplasm/nucleus. Cerebral plugin was applied to distribute the protein nodes in the “ACE2 Full-PPIN” into different layers according to their subcellular localization, generating a pathway-like graph without changing their interactions[15].

Functional enrichment and pathway enrichment analyses

Functional enrichment analysis of the Full-network was performed in DAVID database (https://david.ncifcrf.gov/) to identify the enriched Gene Ontology (GO) “Biological Process” term. Only statistically significant GO terms with a *P*-value less than 0.05 were remained for visualization using ggplot2 R package.

Expression pattern for the Core-PPIN in COVID infected lung cells

GSE147507, an expression dataset containing SARS-CoV-2 infected normal lung cells, were obtained from GEO database (https://www.ncbi.nlm.nih.gov/geo), which was submitted by Blanco-Melo et al[16]. In this dataset, primary human lung epithelium (NHBE) was mock treated or infected with SARS-CoV-2 by biological triplicates. To mimic the effect of inflammatory factor, NHBE cell was treated with human interferon-beta (INFB) with the timepoint was set as 4h, 6h and 12h. The RNA-seq data was normalized and the differentially expression were analyzed by SangerBox (http://soft.sangerbox.com/). The Pearson expression correlation for each pair proteins in the “ACE2 Core-PPIN” were analyzed by an R script, according to the expression data. The expression fold changes and correlations were integrated into the “ACE2 Core-PPIN” as the node attribute, to illustrate the dynamic changes of the Core-PPIN under different conditions. The correlation matrixs and their expression profile were clustered by Cluster 3.0 and illustrated by TreeView[17].

Activated pathways analyses

The expression matrix of proteins in the “ACE2 Core-PPIN” was submitted to the Pathview (https://pathview.uncc.edu/), which provides easy interactive access, and generates high quality pathway graphs[18].

Drug-target network construction

The latest release of DrugBank (version 5.1.6, https://www.drugbank.ca/) was downloaded, which contains 13,580 drug entries, including FDA approved small molecule drugs, biologics, 131 nutraceuticals, 6,376 experimental drugs, as well as 5,223 non-redundant target proteins. An intersection was obtained between the proteins in “ACE2 Full-PPIN” and target proteins from DrugBank, to construct a drug-target network.

Shortest path between ACE2 and transcription factor

The shortest path problem is any possible but the minimized path(s) from one node to another given node in the network. The proteins in the “ACE2 Full-PPIN” was compared the human transcription factors (http://humantfs.ccbr.utoronto.ca/) to identify the transcription factors in this network. The path from ACE2 to these transcription factors were analyzed by R “igraph” package as we previously described[19]. The visualization for the path from ACE2 to transcription factors, as well as their targeted drug were performed by Cytoscape.

Results

Construction of the PPIN for ACE2 and its interacting proteins

The reported interacting proteins of ACE2 were obtained from the BioGRID and other databases. At the beginning of this study, there are 12 ACE2 interacting proteins (detailed information provided in Table 1) (Figure 1A). First, a PPIN was constructed, using ACE2 and its interacting proteins as the seed nodes to extract all their known interacting proteins from the parental PPIN, which was called as the “ACE2 Full-PPIN” (Figure 1B). The Full-PPIN contained 1,318 nodes (proteins) and 1,292 edges (interactions), suggesting that ACE2 and its interacting proteins could be linked to more than a thousand other proteins by cascaded interactions to expand their biological effects. The top three genes with the highest number of interacting proteins were HRAS (620 edges), CALM1 (472 edges) and CAT (119 edges).

In the Full-PPIN, we found multiple ACE2-interacting protein were linked by many other partner proteins. For a better illustration, ACE2 and its interacting proteins linked through one or more partner proteins were shown in another smaller PPI sub-network, referred as the “ACE2 Core-PPIN”, whereby the nodes with only

one link in the Full-PPIN were removed (Figure 1C). This Core-PPIN contained 80 nodes and 154 edges, in which ACE2 and its interacting proteins were indicated in light pink, while the linker proteins were shown in light blue. Interestingly, we found that HRAS and CALM1 have the highest dozens of common interacting proteins. This suggests that HRAS and CALM1 would have the greatest potential to transduce the stimulus from ACE2 (Figure 1C). Moreover, We consider these linker proteins between ACE2 and its interacting proteins might serve as switch proteins, then determine the trend or the direction of cellular signal transduction by their co-expression correlation strength.

Table 1
ACE2 interacting proteins from database

Gene ID	Official Symbol	Full name	Experimental System	Pubmed ID
801	CALM1	calmodulin 1 (phosphorylase kinase, delta)	Affinity Capture-Western	18070603
183	AGT	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	Biochemical Activity	10969042
14	AAMP	angio-associated, migratory cell protein	Affinity Capture-MS	26186194
847	CAT	catalase	Co-fractionation	26344197
51477	ISYNA1	inositol-3-phosphate synthase 1	Co-fractionation	26344197
8847	DLEU2	deleted in lymphocytic leukemia 2 (non-protein coding)	Affinity Capture-RNA	28977802
3265	HRAS	Harvey rat sarcoma viral oncogene homolog	Proximity Label-MS	30639242
43740568	S	Spike glycoprotein	Co-crystal Structure	32132184
340024	SLC6A19	solute carrier family 6 (neutral amino acid transporter), member 19	Co-crystal Structure	32132184
7431	VIM	vimentin	Affinity Capture-Western	26801988
1670	DEFA5	defensin, alpha 5, Paneth cell-specific	Reconstituted Complex	DOI:10.1101 /2020.03.29.013490
51738	GHRL	ghrelin/obestatin prepropeptide		11815627

The topology parameters of the “ACE2 Full-PPIN”

The true biological networks, including PPIN, are distinguishable from random or other chaos networks by their distinguishing topological parameters. Many networks have been shown to be scale-free with a degree distribution following a power law[20]. For “ACE2 Full-PPIN”, the distributions of node degree followed an approximate power law, with the equation $y = 288.18\ x^{-1.07}$ and an $R^2 = 0.833$ (Supplementary Figure S1A). The shortest path length (number of edges from one node to another) of the Full-PPIN was shown ss mainly arranged from 2 and 3 step lengths (Supplementary Figure S1B). It also suggests that one protein can contact another protein by only a few steps, enabling the formation of different protein complexes or/and component switching. Topological coefficient is a measure for the extent to which a node shares neighbors with other nodes. A topological coefficient of 0 is assigned to nodes which have one or no neighbors (Supplementary Figure S1C). Closeness centrality measures how fast the flow of information would be through a given node to other nodes in the network, considering the efficiency of information spreading in the network. Some nodes have high closeness centrality when links <10, but it gradually increases with the number of links while links >10 (Supplementary Figure S1D).

Subcellular layers of the PPIN indicate stimulus from the extracellular to the nucleus

A given protein may have diverse functions not only dependents on interaction with other proteins, as well as its cellular location[21, 22]. So the subcellular location and/or translocation of proteins is critical for its function. In this study, the “ACE2 Full-PPIN” was divided into 11 layers with their percentage as follows: secreted (2.43%), membrane (22.53%), cytoskeleton (0.15%), cytoskeleton/cytoplasm (0.23%), cytoplasm (19.35%), secreted/nucleus (0.46%), membrane/nucleus (0.23%), cytoskeleton/nucleus (0.61%), cytoplasm/nucleus (18.06%), nucleus (8.19%) and uncertain (27.77%, distribute near the interacting proteins) (Figure 2). These results suggest that near 20% ACE2-interacting proteins and their partners have multiple subcellular locations. These results also indicate that the ACE2 could transfer stimulus from the extracellular/membrane into the intracellular environment, eventually to the nucleus, forming non-canonical pathways by cascades of interactions.

Functional enrichment of the Full-PPIN

Many proteins have multiple functions, we presumed that ACE2 and its interacting proteins are involved in diverse biological functions, especially in the pathology of COVID-19, through cascading protein-protein interactions to expand their biological effects. To examine this the possibility, GO “Biological Process” enrichment analysis of the Full-PPIN was performed, resulting in more than a hundred of significantly enriched GO terms (data not shown). Two big groups GO terms caused us a great interest (Figure 3). One group relates to virus process, including “GO:0046718~viral entry into host cell”, “GO:0039694~viral RNA genome replication”, “GO:0019083~viral transcription” and “GO:0016032~viral process”. The other

interested group is about immunity, includes “GO:0050900~leukocyte migration”, “GO:0031295~T cell costimulation”, “GO:0050690~regulation of defense response to virus by virus” and “GO:0042110~T cell activation”. These detailed significant GO terms with their enriched genes are listed in Supplementary file 1.

Dynamic expression pattern for the Core-PPIN in COVID infected lung cells

PPINs in living cells are not static, but instead dynamically vary in different tissues, or different types of diseases, even in the different stages of the same tissue. In this study, the expression profile of GSE147507 containing different conditions of lung cells was obtained. The expression log2(fold-change) (logFC) of the proteins in the Core-PPIN, as well as the co-expression correlation coefficient of each pair of proteins were analyzed. Next, the logFC and correlation coefficient were integrated into the Core-PPIN as a node attribute and an edge attribute, respectively, to illustrate the dynamic changes in different treatments. In the Series1 of GSE147507, the primary human lung epithelium (NHBE) were infected with SARS-CoV-2 (USA-WA1/2020) with mock treated as a control (Figure 4A). ACE2 is significantly increased, followed by its interacting protein AAMP and HRAS. On the other hand, MYC in the network is also significantly upregulated (Figure 4B). For expression coefficient, there are near 70-80% of positive co-expression in mock NHBE, while it increases to more than 90% positive in SARS-CoV-2 treated cells. The co-expression patters between normal and treated are rather different, as indicated by the heatmap (Figure 4C-D). In the Series9 of GSE147507, NHBE were treated with human interferon-beta at the time point of 4h, 6h and 12h to mimic the stimulus of inflammation. After the treatment, ACE2 and several interacting proteins, such as CALM1, DLEU2, ISYNA1 and NTS are obviously increased in the time series. While GHRL is consistently decrease. HRAS is upregulated gradually from 4h to 12h, though not reach a very high expression level. For the co-expression relationship, it is interesting to point out that both CALM1 and HRAS have positive correlation with their interacting proteins in the control, but only CALM1 remains most positive correlation, while HRAS mostly turns to negative correlation with its interacting proteins (Figure 5A-D, left panel). For global co-expression pattern as shown by the heatmaps, the total positive correlations are reduced while the total negative correlations are increased (Figure 5A-D, right panel). On the other hand, the expression pattern of “ACE2 Core-PPIN” could distinguish the control group from the three treatment groups (Supplementary Figure S2).

Activated pathways in IFN stimulated lung cells

To exam what kinds of pathway are active during the mimic of COVID-19 stimulus, the expression matrix of NHBE treated with IFN was analyzed by Pathview, which maps omics data to the KEGG pathway. There are six significant enriched pathways, including “PI3K-Akt signaling pathway”, “Focal adhesion”, “ECM-receptor interaction”, “Cell adhesion molecules”, “Antigen processing and presentation”, and “Regulation of actin cytoskeleton” (Supplementary Figure S3). To our great interesting, the genes mapped to the

pathways are mostly increased, suggesting these pathways are activated during the mimic of COVID-19 inflammation environment.

The proteins in Full-network are potential drug targets

Though many scientists and doctors are working hand to find ways to cure the COVID-19, including the drugs targeting ACE2 itself, or the replication of SARS-CoV-2 virus. We consider it would be great helpful to find drugs that target the ACE2 PPIN, to restrict the biological activity stimulated by the virus, thus reduce the replication and spread of the SARS-CoV-2. To achieve this, we search the DrugBank database and constructed drug-protein target networks. Currently, there are four registered drugs targeting ACE2, there are DB01611 (Hydroxychloroquine), DB05203 (SPP1148), DB15643 (N-(2-Aminoethyl)-1-aziridineethanamine), DB00608 (Chloroquine) (though some drugs are now in great controversy) (Figure 6A). On the other hand, five ACE2 interacting proteins (CALM1, HRAS, AGT, ISYNA1 and CAT) are reported druggable. Among them, CALM1 (calmodulin 1) has the largest number of 29 drugs. The famous signal molecular HRAS (HRas proto-oncogene, GTPase) has five tested drugs (Figure 6A). By targeting the “ACE2 Full-PPIN” , there are 2075 nodes (1728 drugs and 347 proteins) and 2396 edges (targeting relations) in the drug-protein network (Figure 6B). At least 26% of proteins in “Full-PPIN” are druggable, suggesting a great potential for treatment. The top 10 proteins have highest number of drugs, and the top 10 drugs have the highest number of target proteins are shown in the list (Figure 6C). ESR1 (estrogen receptor 1) has the highest of 118 drugs, while DB12010 (Fostamatinib) targets the highest number (61) of proteins. Some drugs have more than one targets. The detailed information about the drugs in these networks (Figure 6) is provided in the Supplementary file 2.

The shortest paths from ACE2 to transcription factors

Usually an external stimulus, or the overexpression or knockdown of one gene, could cause a wide range alternation of expression profile. We assumed the involved transcription factors play critical roles in the alternation of mRNA expression profile. We applied the shortest path algorithm to illustrate how ACE2 reaches a specific transcription factor by the cascades of interaction in the PPIN. 37 transcription factors are present in the “ACE2 Full-PPIN”. Consistent with the shortest path distribution described above, there are only two steps from ACE2 to these transcription factors (Figure 7A), suggesting a quick response is exist from extracellular stimulus into nucleus, triggering the change of expression profile, then the change of cellular activities. These transcription factors are also ideal targets for treatment. So we construct a small PPIN, in which the drugs target the ACE2-TF. There 278 drugs in the small PPIN, targeting the 37 transcription factors (Figure 7B). It is interesting to understand why a gene overexpression or knockdown could cause a wide range alternation of expression profile. In this study, we applied shortest path algorithm to identify possible paths from ACE2 to the transcription factor in the protein-protein interaction (PPI) network.

The verification cohort from public data

During the preparation of this manuscript, Gordon *et al*. reported a comprehensive SARS-CoV-2 protein interaction map[23]. They expressed 26 of the 29 SARS-CoV-2 proteins to identify their interacting human proteins. They reported 332 high-confidence SARS-CoV-2-human protein-protein interactions, in which 66 druggable human proteins or host factors are targeted by 69 compounds[23]. To test the reliable of ACE2 based PPIN in this study, We compared the “ACE2 Full-PPIN” with the 332 SARS-CoV-2 interacting proteins, and found 44 intersection proteins which were used to construct a co-network containing 510 nodes and 1264 edges (Supplementary Figure S4A). Among these nodes, at least six proteins (CALM1, HRAS, DEFA5, CAT, S and ISYNA1,6/12 ACE2 interacting proteins) from “ACE2 Full-PPIN”, and fifteen SARS-CoV-2 coding proteins are presented. After reduce the nodes with single connection, a core smaller PPIN was obtained to show a clear relationship between ACE2 interacting proteins and SARS-CoV-2 coding proteins (Supplementary Figure S4B). It also show that HRAS and CALM1 are still the nodes with a large number of interaction, suggesting the consistent between the networks in this study and networks from Gordon, D. E. et al.

Discussion

COVID-19 is a global pandemic that has already caused more than 500,000 deaths worldwide so far. Biological activities, includes stimulus response and signal transduction, are built on protein physically interactions. In this study, several high confident PPINs were constructed based on the ACE2 and its interacting proteins. These protein might connect or cross-talk through at least one partner protein, which might serve as switch protein to determine the trend or the orientation signal transduction.

Many previous reported biological networks are static networks, which merely reflect the activities of cellular proteins under one specific condition or at one time point.

It has been recognized that cellular system, including the PPIN, is highly flexible in the response to environmental stimulus, which enables the cells adapt to different physiological conditions or pathological stages[24]. Pearson correlation coefficient is a popular correlation method to measure the co-expression of each pair of interacting proteins in expression profiles[25]. In this study, we constructed not only the static PPI network for ACE2 and its interacting proteins, but also the dynamic networks by considering their expression profiles in different kinds of conditions mimic COVID-19 progress. Several proteins are consistent change during the progress, showing a dynamic co-expression pattern.

Since there is no single effective drug for COVID-19 by now, we consider whether there are multiple drugs available for the ACE2 based PPIN to disturb the biological activities caused by the virus. Inspire by this hypothesis, we found hundreds of drugs targeting ACE2 and its cascaded interacting proteins. With the development of network pharmacology, the drug discovery paradigm has changed from the traditional model “one drug → one target → one disease"' to the network mode “multi-drugs → multi-targets → multi-diseases"[26, 27]. One successful application of drug cocktails is the clinical treatment of

HIV/AIDS patients[28]. The cocktail treatment strategy has been also applied in other diseases, such as breast cancer and leukemia[29-31]. To overcome this combinatorial explosion problem, Zimmer et al. have developed a model that predicted the effects of cocktails at all doses based on measuring pairs of drugs[32]. So we believe that cocktails drug from the ACE2 drug-target network might provide important clues to treat the virus in the clinic trial. Some of ACE2 interacting proteins are reported as drug targets in different diseases. HRAS is a proto-oncogene which overexpresses in many tumors. Tipifarnib is a potent and highly selective inhibitor of farnesyltransferase, a critical enzyme requisite for HRAS activation and now a phase II trial in urothelial carcinoma is carried on[33]. CALM1 is one of kind of the EF-hand calcium-binding protein, which modulates the function of ion channels by calmodulin regulation. CBP501, a drug currently in phase II clinical trial for lung cancer, may sensitize tumors to the chemotherapeutic agents bleomycin and cisplatin by inhibiting CALM1[34].

It is usually causes a wide range alternation of expression profile when the cells are stimulated. We assumed the transcription factors or transcriptional regulators in the PPIN play critical roles in this expression profile alternation. We also presumed the information transmitted from one protein to another would also adopt the most economic ways, through the cascade of PPI activities. So we identified there are only two steps for ACE2 to transcription factors, to cause the alternation of gene expression profile to make the cell adapt to the stimulus. These transcription factors are considered as drug targets. The transcription factor MYC, obviously upregulates both in the COVID-19 and IFN treated lung cells, is a promising therapy target for multiple human cancers. Small molecules are found capable of repression the transcriptional regulation of c-Myc, including IIA6B17, NY2267, and 28RH-NCN-1, which inhibit MYC binds to DNA[35]. Based the above analyses, we propose a working model for ACE2 and its interacting proteins, showing the cascaded interaction and their potential drugs for treatment (Figure 8).

Conclusion

In summary, this study provides new insight into the disruption of the biological response to SARS-CoV-2 mediated by ACE2, but also its cascade interacting proteins when considering of PPIN.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

We agree to publish the manuscript in Journal of Translational Medicine.

Availability of data and materials

Supplementary Figure S1 describes the topology parameters of the “ACE2 Full-PPIN”. Supplementary dataset 2 contains gel source data (original western blots) with cropping and exposure strength strategies. Supplementary Figure S2 describes the expression pattern of “ACE2 Core-PPIN” could distinguish the control group from the three treatment groups. Supplementary Figure S3 describes six significant enriched pathways are activated pathways in IFN stimulated lung cells. Supplementary Figure S4 contains the verification cohort from public data. Supplementary file 1 contains detailed the significant enriched GO terms of “ACE2 Full-PPIN”. Supplementary file 2 contains the detailed information about the drugs in these networks.

Competing interests

All the authors declare no conflict of interest.

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Authors' contributions

Qiaoxi Xia and Mantong Chen completed data analysis, interpretation and manuscript writing.

Xiao Zhou and Ling Lin completed the drawing of figures and tables in the manuscript.

Yan Zhao and Xiaoqi Zheng completed data collection.

Meihui Huang and Shaohong Wang completed the compilation of references.

Zepeng Du completed the design and proofreading of the manuscript.

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Figures

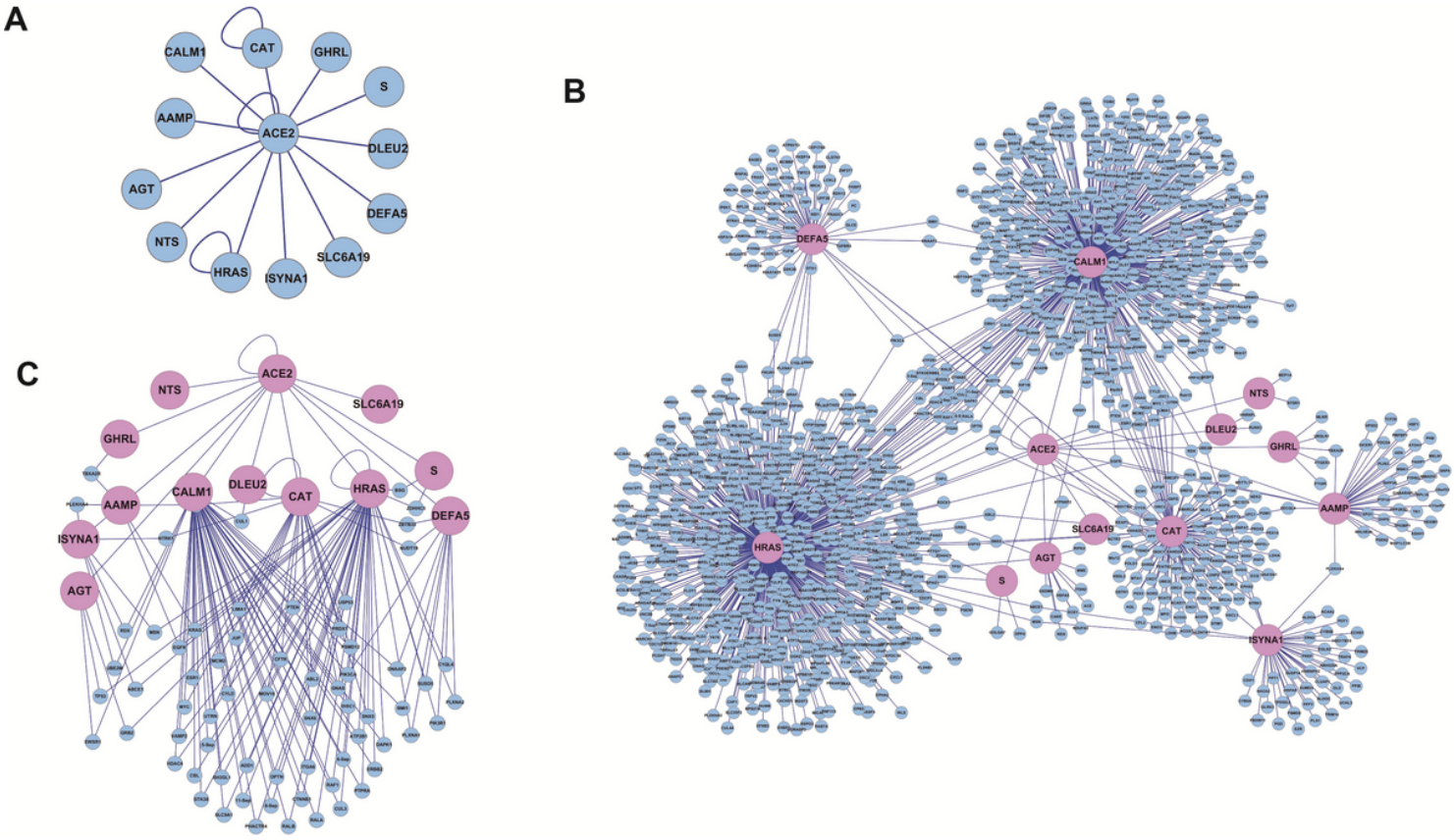


Figure 1

Protein-protein interaction network (PPIN) for ACE2 and its interacting proteins. A. the reported ACE2 interacting proteins. B. The Full-PPIN contains ACE2 and its interacting proteins with all their known interacting proteins. C. The “ACE2 Core-PPIN” was generated from the Full-PPIN by reducing the nodes had only one link, illustrating ACE2 and its interacting proteins are linked through many partner proteins.

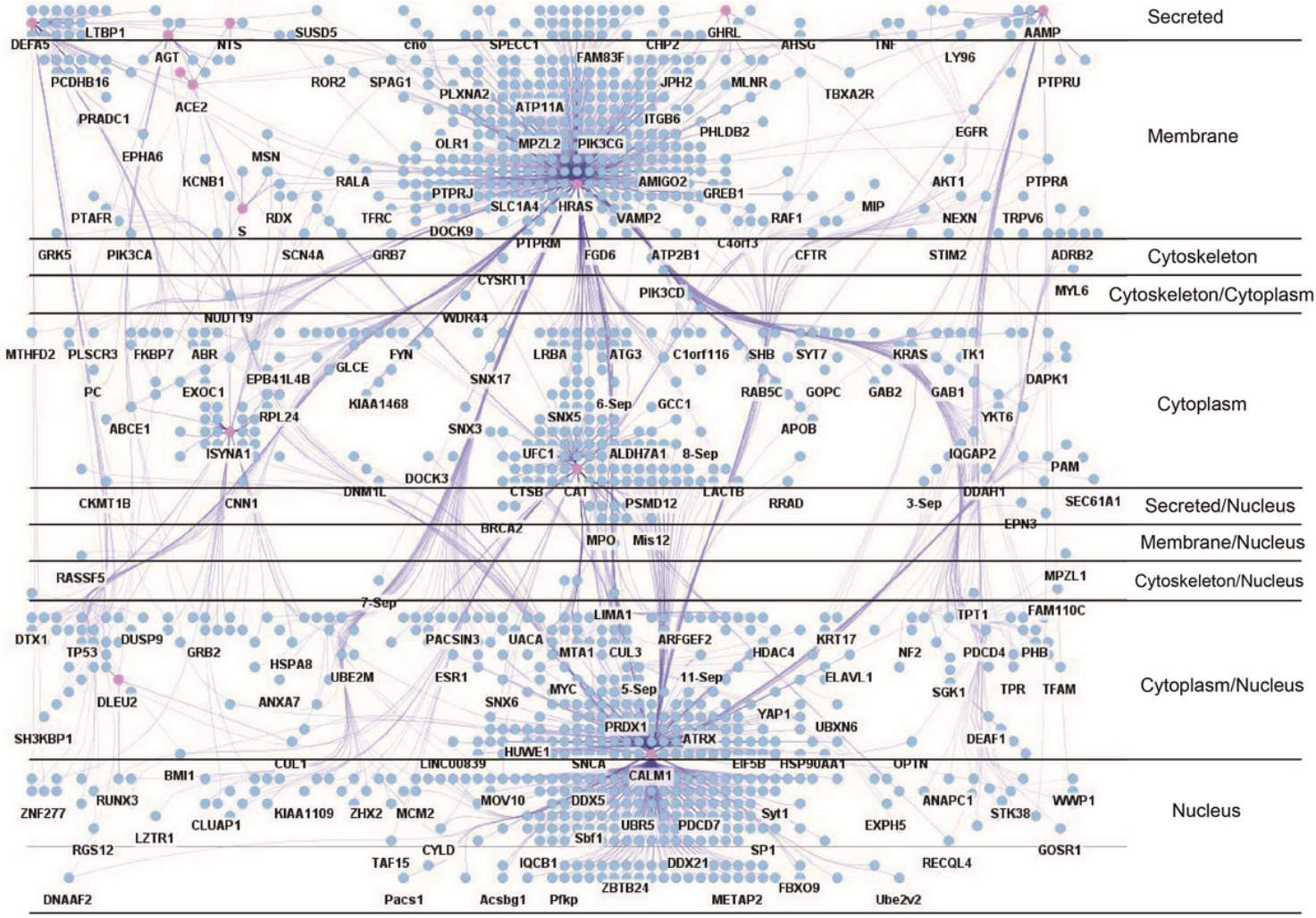


Figure 2

Subcellular layers for the “ACE2 Full-PPIN”. The network is separated into 11 layers retaining their interactions. ACE22 and its interacting proteins are indicated in pink.

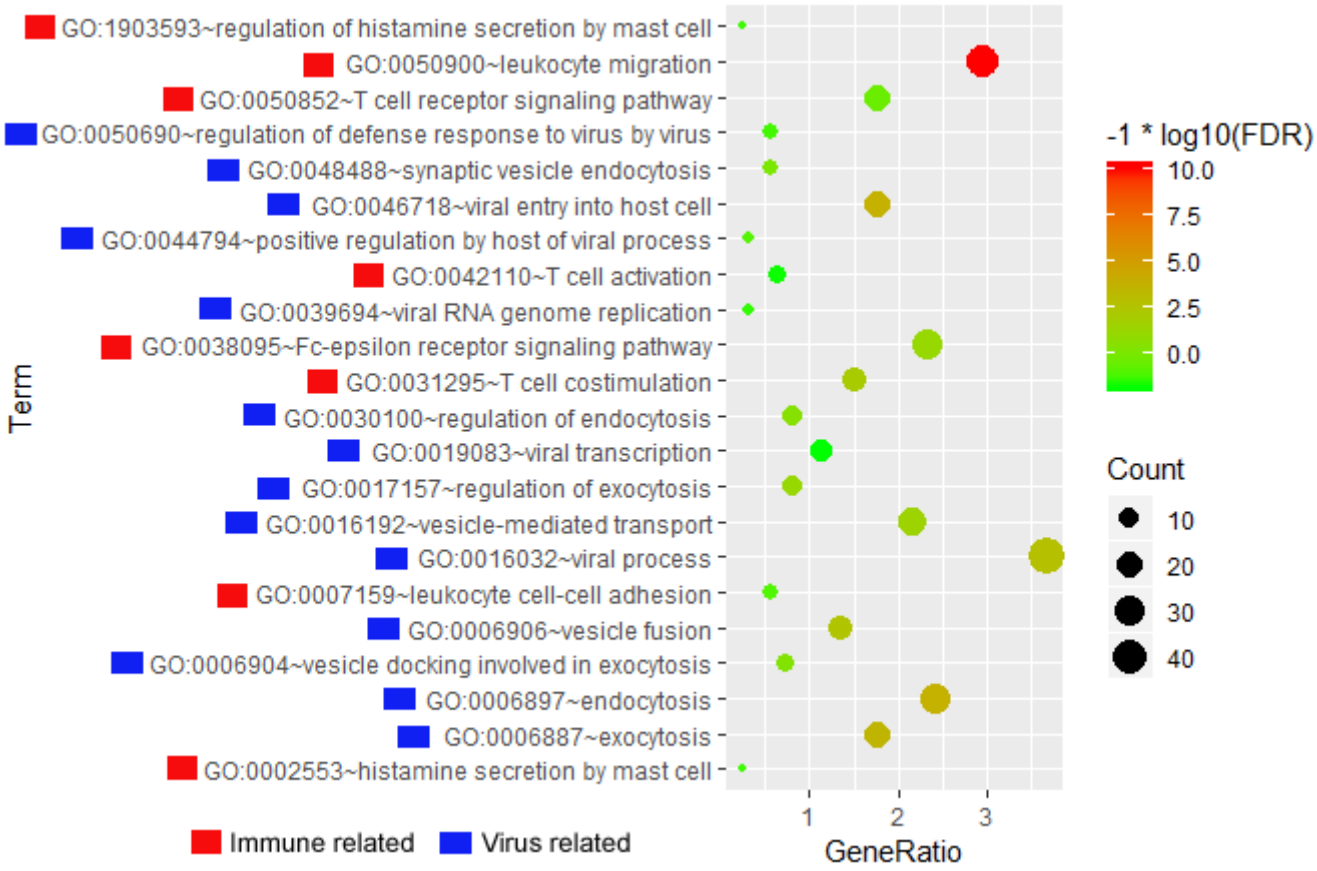


Figure 3

Gene Ontology “Biological Process” enrichment of the Full-PPIN shows significant virus activity and immune related terms.

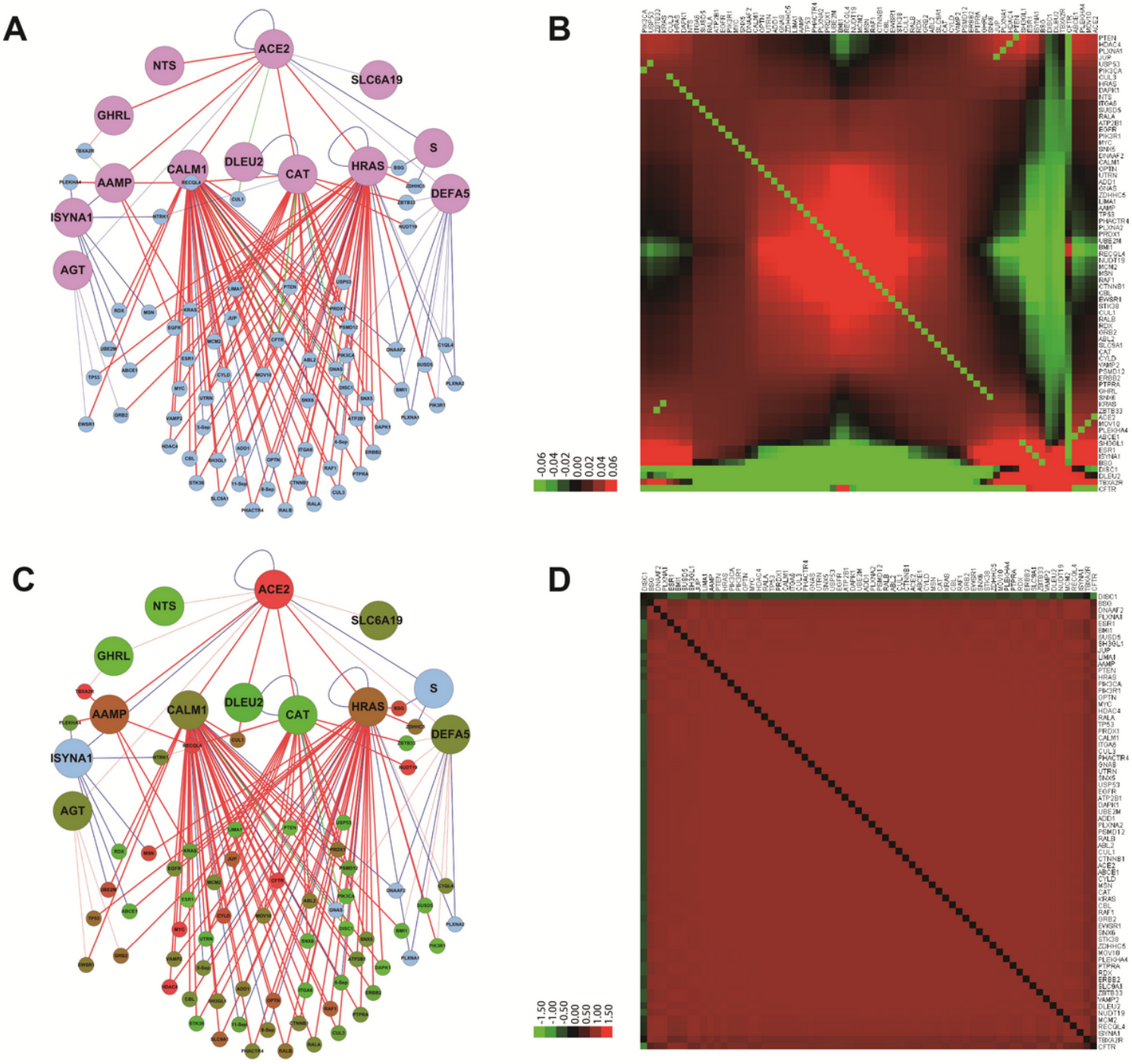


Figure 4

Expression fold-change and co-expression correlation of proteins in the “ACE2 Core-PPIN” for normal lung and that treated with SARS-CoV-2. A. The co-expression relationship for “ACE2 Core-PPIN” in normal lung cells. B. The heatmap of co-expression relationship for proteins in “ACE2 Core-PPIN” in normal lung cells. C. The expression fold-change for the Core-PPIN in lung cells treated with SARS-CoV-2. D. The heatmap of co-expression relationship for “ACE2 Core-PPIN” in lung cells treated with SARS-CoV-2.

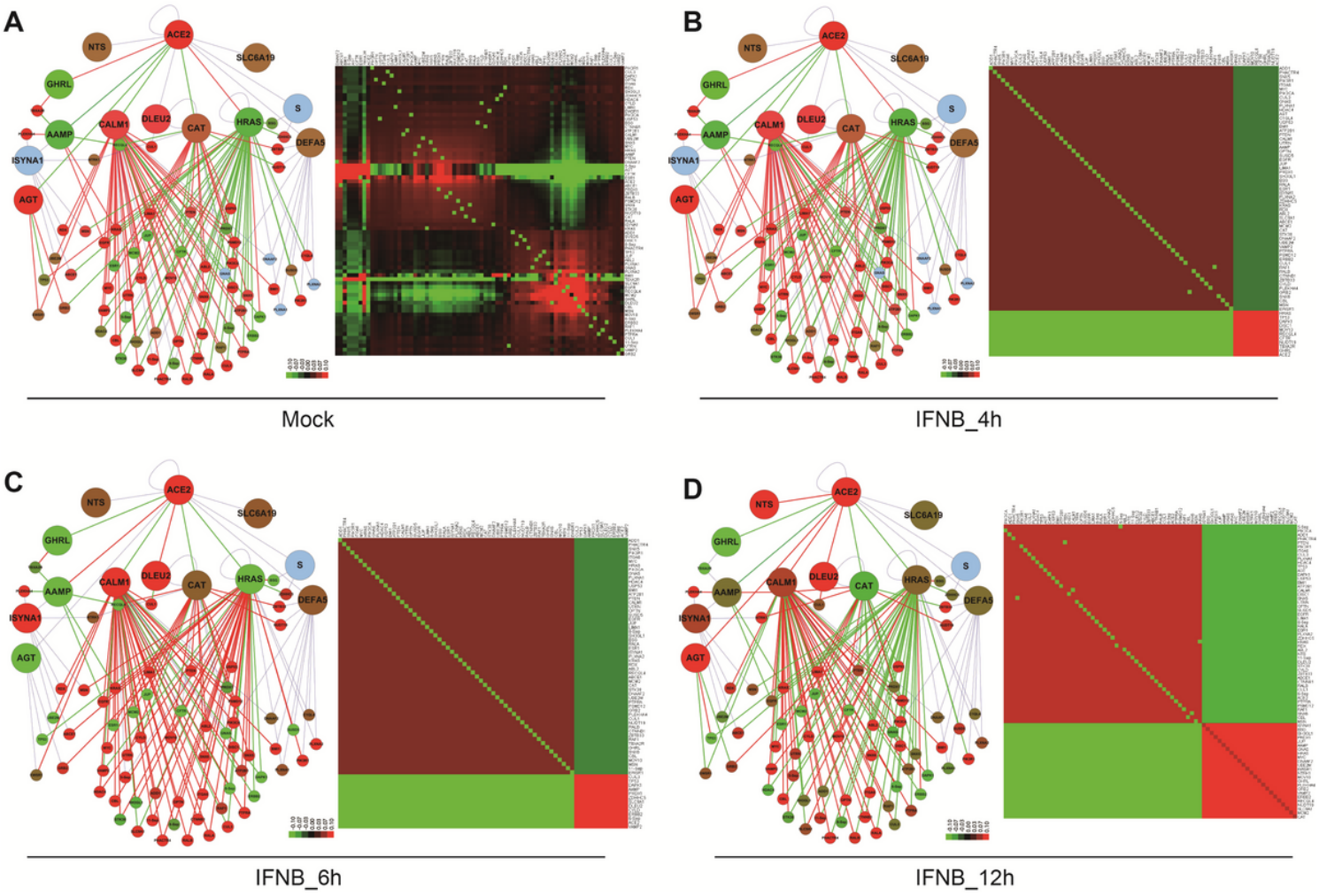
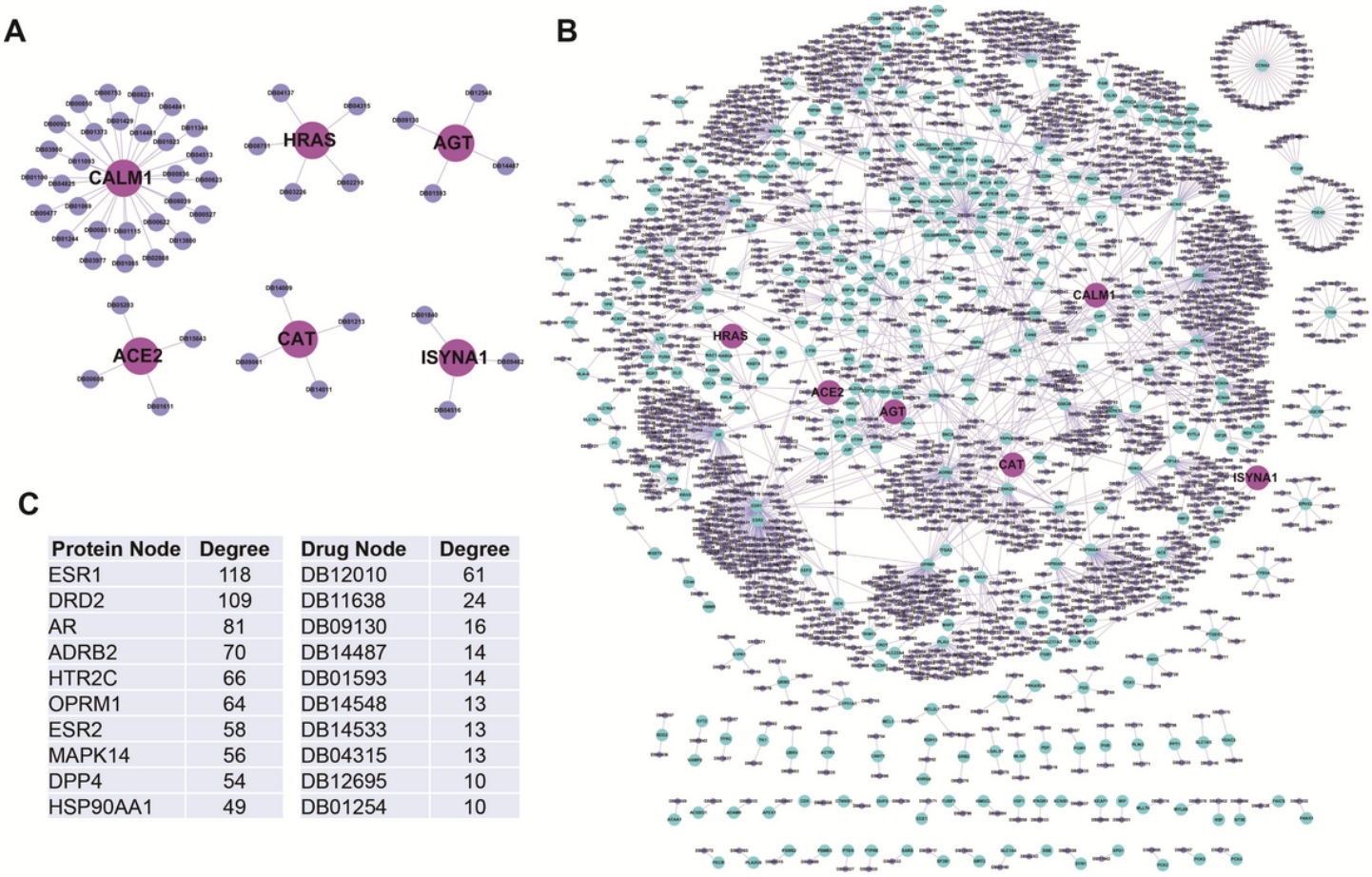


Figure 5

Expression fold-change and co-expression correlations of proteins in the “ACE2 Core-PPIN” for normal lung cells treated with INFN at different time points. (A-D, left panel)The expression fold-change for “ACE2 Core-PPIN” in normal lung cells with mock treatment or with INFN at 4h, 6h and 12h, with the mock as the control. (A-D, right panel)The heatmap of co-expression relationship for “ACE2 Core-PPIN” in normal lung cells.



C

Protein Node	Degree	Drug Node	Degree
ESR1	118	DB12010	61
DRD2	109	DB11638	24
AR	81	DB09130	16
ADRB2	70	DB14487	14
HTR2C	66	DB01593	14
OPRM1	64	DB14548	13
ESR2	58	DB14533	13
MAPK14	56	DB04315	13
DPP4	54	DB12695	10
HSP90AA1	49	DB01254	10

Figure 6

Drug-protein network for ACE2 and its interacting proteins. A. Reported drugs for ACE2 and its interacting proteins, respectively. B. Drugs target the proteins in “ACE2 Full-PPIN”. ACE2 and its interacting proteins are indicated in light pink, their secondary interacting proteins are shown in light blue. Drugs are illustrated in light purple. C. The top 10 proteins and drugs have the highest number of edges.

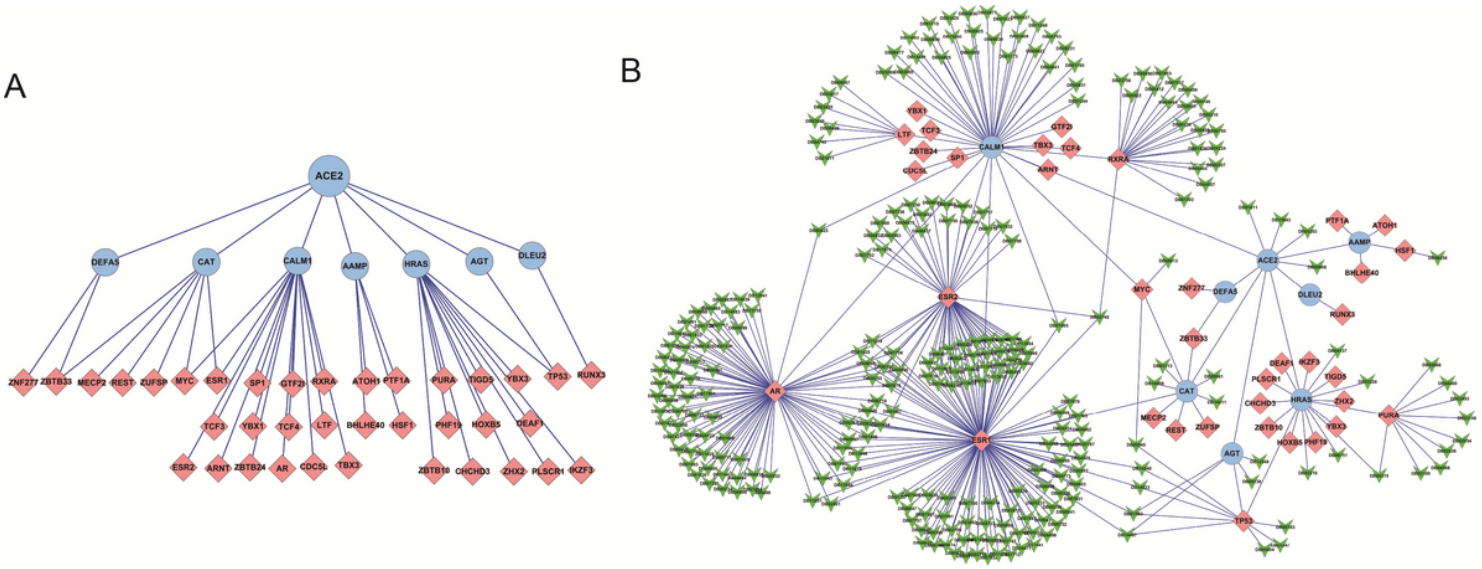


Figure 7

The shortest paths for ACE2 and transcription factors and their drugs. A. All the possible shortest paths from ACE2 to transcription factors. The transcription factors are indicated in light pink. B. The drug network targets the shortest paths from ACE2 to transcription factors, suggesting the possible disturbing their signal transduction.

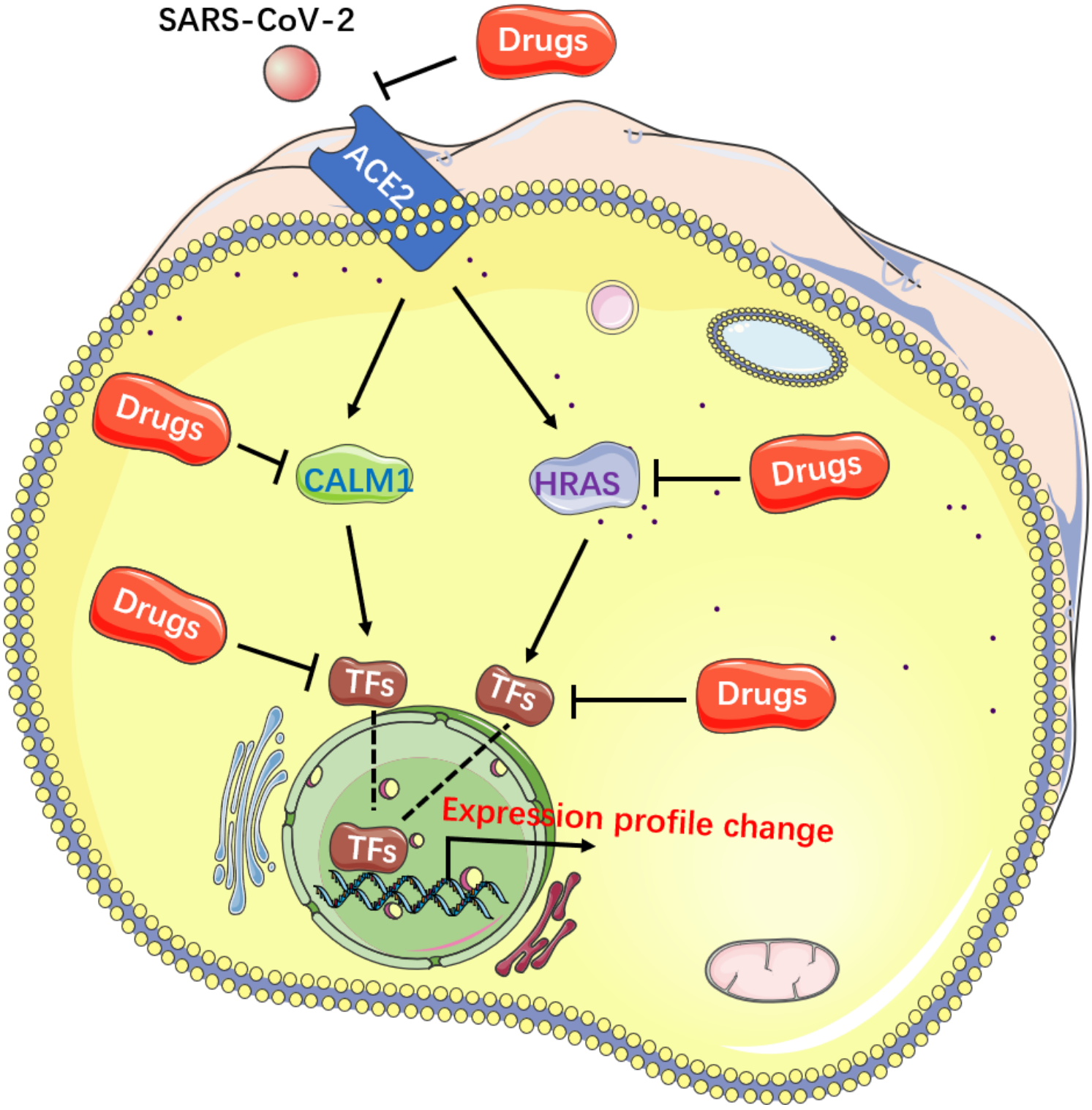


Figure 8

A propose model for drugs disrupt the biological activity caused by SARC-CoV-2 mediated by ACE2 and its cascaded interacting proteins.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile2uniquedrugcommonname.csv
- Supplementaryfile1GOterm.xlsx
- SupplementaryFigures.docx