A comprehensive SARS-CoV-2-human protein-protein interactome network reveals novel pathobiology and host-targeting therapies for COVID-19

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A comprehensive SARS-CoV-2-human protein-protein interactome network reveals novel pathobiology and host-targeting therapies for COVID-19

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Abstract
Physical interactions between viral and host proteins are responsible for almost all aspects of the viral life cycle and the host’s immune response. Studying viral-host protein-protein interactions is thus crucial for identifying strategies for treatment and prevention of viral infection. Here, we use high-throughput yeast two-hybrid and affinity purification followed by mass spectrometry to generate a comprehensive SARS-CoV-2-human protein-protein interactome network consisting of both binary and co-complex interactions. We report a total of 739 high-confidence interactions, showing the highest overlap of interaction partners among published datasets as well as the highest enrichment for genes differentially expressed in samples (such as upper airway and bronchial epithelial cells) from patients with SARS-CoV-2 infection. Showcasing the utility of our network, we describe a novel interaction between the viral accessory protein ORF3a and the host zinc finger transcription factor ZNF579 to illustrate one of the first examples of a viral factor mediating a direct impact on host transcription. Leveraging our interactome, we performed network-based drug screens for over 2,900 FDA-approved/investigational drugs and obtained a curated list of 21 drugs that had significant network proximities to SARS-CoV-2 host factors, one of which, carvedilol, showed promising antiviral properties. We performed electronic health record-based validation using two independent large-scale, longitudinal COVID-19 patient databases and found that carvedilol usage was associated with a significantly lowered probability (17%-20%, \(P < 0.001\)) of obtaining a SARS-CoV-2 positive test after adjusting various confounding factors. Carvedilol additionally showed anti-viral activity against SARS-CoV-2 in a human lung epithelial cell line (EC\(_{50}\) value of 4.1 µM), suggesting a mechanism for its beneficial effect in COVID-19. Our study demonstrates the value of large-scale network systems biology approaches for extracting biological insight from complex biological processes.
The global coronavirus disease 2019 (COVID-19) pandemic caused by the highly transmissible and pathogenic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains a persistent part of everyday life for much of the world. Epidemiological models predict, in line with virological theory, that the pandemic is unlikely to end with the complete eradication of the virus\textsuperscript{1,2}. Indeed, SARS-CoV-2 becoming endemic in pockets of the world’s population is hypothesized to be a natural consequence of the virus’s widespread propagation\textsuperscript{2}. Recent studies are now exploring the waning of immunity with time from point of natural infection or immunization\textsuperscript{3-5}, and the emergence of numerous viral variants raise concern of a perennial selection for more infectious or virulent mutants to again sweep through the globe. More recently, engineering of SARS-CoV-2 spike (S) protein chimeras containing several mutations of concern found a variant that completely evaded the immune response of all except those that were afforded protection with recovery from natural infection followed by vaccination\textsuperscript{6,7}. This, along with other elusive phenomena, highlight the gaps in our understanding of the interplay between this virus and its host upon natural infection and immunization, and thus, much work is still to be done to elucidate the pathobiology of SARS-CoV-2, especially as the maintenance of immunity against this pathogen remains at utmost interest to global public health.

Viruses interface with host cell surfaces to gain entry, wherein they interact with intracellular proteins to hijack host mechanisms that facilitate viral replication and evasion of an immune response\textsuperscript{8}. Studying viral-host protein-protein interactions (PPIs) is therefore pivotal for understanding the mechanisms by which viral infection progresses and how the host responds to said infection and thus, for identifying strategies for treatment and prevention\textsuperscript{9-12}. These networks of interactions are especially important as proteins rarely act in isolation and their roles should be evaluated in conjunction with their neighborhood of interacting partners. Such interactomes can thus reveal biological pathways and processes impacted by the viral proteome, allowing for the discovery of novel drug targets that directly or indirectly affect the viral-host point of contact.
To that effect, here, we leverage high-throughput yeast two-hybrid (Y2H) and tandem mass tag affinity purification followed by mass spectrometry (TMT-AP/MS) to generate the first binary and co-complex SARS-CoV-2-human protein-protein interactome network, which we propose to be a more complete resource for exploration of the viral-host interactome (Fig. 1a). We adopted this approach for several reasons. To date, yeast two-hybrid (Y2H) and affinity purification-mass spectrometry (AP/MS) are the only two methodologies available for mapping protein-protein interactome networks on a proteomic scale\textsuperscript{13,14}. While both Y2H and AP/MS alone produce high-quality interactome datasets, they fundamentally capture different yet complementary aspects of the full network and thus together can provide a more comprehensive view of the topological and biological properties of the interactome\textsuperscript{15}. Moreover, labelled (e.g., TMT-based) AP/MS has been shown to provide more precise, accurate, and reproducible quantification of proteins compared to label-free AP/MS-based approaches, which is an important criterion when trying to identify true protein interactions and generate high-quality interactome networks\textsuperscript{16-26}.

Together, Y2H and AP/MS generated a total of 739 high confident interactions among 579 human proteins and 28 SARS-CoV-2 proteins. Our interactome had an unprecedented scale and coverage compared with existing ones. Using our interactome, we identified important pathways such as protein translation, mRNA splicing, Golgi transportation, neutrophil mediated immunity, and glucose metabolism. Moreover, we prioritized host-targeting therapies by searching U.S. FDA-approved/investigational drugs for their potential anti-SARS-CoV-2 effect using state-of-the-art network proximity methods. Using two large independent COVID-19 patient databases, we found that usage of one of the top candidates, carvedilol, was associated with a lowered risk (17%-20%) of a positive COVID-19 test. Experimental validation shows that carvedilol inhibits SARS-CoV-2 infection with an EC\textsubscript{50} of 4.1 µM. Altogether, these results suggest that our comprehensive SARS-CoV-2-human protein interactome offers great opportunities for
understanding the pathobiological process of SARS-CoV-2 in human and identifying host-targeting therapies for COVID-19.

Results

A comprehensive SARS-CoV-2-human protein-protein interactome network

To generate a binary SARS-CoV-2-human protein-protein interactome, we systematically tested all pairwise combinations of 28 SARS-CoV-2 proteins (GenBank entry - MN908947) against ~16,000 human proteins (hORFeome V8.1) using high-throughput Y2H screens (Fig. 1a). We treated each protein as both a bait and a prey, yielding over 896,000 (28 × ~16,000 × 2) total tested pair combinations. Prior to screening, all autoactivating DB ORF clones were removed from further tests (see Methods). To increase experimental throughput, viral ORF AD and DB clones were mated against pools of 24 human ORF DB or AD clones, respectively. Following nutrition selection, AD:DB pairs were identified via PLATE-seq to generate a list of candidate interactions (see Methods). Interaction candidates were then subsequently re-tested using Y2H to ascertain high reproducibility. In all, we report a total of 299 high-quality binary SARS-CoV-2-human PPIs via our high-throughput Y2H screen, 267 of which were unique to this assay (Table S1).

To complement our binary SARS-CoV-2-human protein-protein interactome, we independently expressed each of the 28 SARS-CoV-2 proteins in the human epithelial cell line Caco-2 (HTB-37; ATCC) to identify viral-host co-complex interactions using TMT-AP/MS proteomics (Fig. 1a). We used Caco-2 as our cell line model due to its endogenous expression of TMPRSS2 required for SARS-CoV-2 S protein priming and subsequent cell entry, the line’s extensive use in SARS-CoV and SARS-CoV-2 infection studies, supported by known in vivo replication of SARS-CoV-2 in gastrointestinal cells, and desirable cell culture characteristics including robust transfectability and rapid propagation. All Strep-, Myc-, or FLAG-tagged SARS-CoV-2 baits and their corresponding empty vector controls were transfected in biological duplicates, followed by subsequent affinity purification, TMT10 labeling, and SPS-MS3-based
quantification. We filtered for interactions that met stringent fold change and p-value based cutoffs (see Methods). In all, we report a total of 472 high-confidence co-complex SARS-CoV-2-human PPIs via AP/MS, 440 of which were unique to this assay (Table S1). Altogether, our orthogonal approaches generated a network composed of 739 interactions among 28 viral and 579 host proteins (Table S1).

We visualized the SARS-CoV-2-human protein-protein interactome through a network shown in Fig. 1b. The colors of the edges between the viral proteins (represented as diamond nodes) and the host proteins (represented as circle nodes) indicate the methods that detected the interaction. Host proteins that interact with a single viral protein are shown in boxes connected to their interacting partner. Several human proteins interact with multiple SARS-CoV-2 proteins, such as ACTN4, ITGB1BP2, TRIM27, and ACTN1 (n=7, 6, 5, and 5, respectively), while the majority of human proteins (469, 81%) interact with only one SARS-CoV-2 protein (Fig. S1a).

Among the viral proteins, N, ORF7b, and ORF9b achieved the highest network degrees (n=56, 56, and 54, respectively), whereas E, NSP7, and NSP1 have the lowest network degrees (n=9, 11, and 11, respectively) (Fig. S1b). In terms of the shared interacting partners, overall, the viral proteins showed significant overlaps (Fisher’s exact test, \( P < 0.05 \)) (Fig. S1c). For example, the S protein overlaps significantly with 14 of the other 27 viral proteins in their host factors. Even the viral protein with the lowest number of significant overlaps, NSP4, significantly overlaps with NSP13 and ORF7b. These observations suggest potential functional overlap or redundancy of the viral proteins.

Compared to the published SARS-CoV-2-human protein-protein interactome networks\(^9,11,12\), our interactome validated 218 (38%) human host factors previously reported. Proteins including ALG5, G3BP1, CLCC1, VPS39, SIGMAR1, G3BP2, and RAP1GDS1 are identified in all four interactomes (Fig. 2a). Importantly, our interactome offers 361 (62%) newly discovered human host factors which in total interact with SARS-CoV-2 proteins in 493 novel interactions. For S protein, which plays a key role in the entry of SARS-CoV-2 into host cells\(^37\),
we identified 24 novel interacting partners. Among these interacting partners of S protein, we found that CORO1C\textsuperscript{38} and STON2\textsuperscript{39} express on the cell membrane, suggesting potential cell entry of SARS-CoV-2 through these human proteins in addition to known mechanisms.

For the entire interactome, functional enrichment analysis revealed significantly overrepresented biological processes (Fig. S2a), including protein translation, transcription, and neutrophil-mediated immunity (highlighted with yellow background in Fig. 1b). Pathway enrichment analysis show top enriched pathways including protein processing in the endoplasmic reticulum, tight junction, glycolysis, ribosome, and protein export (Fig. S2b). For individual SARS-CoV-2 proteins, many pathways and biological processes are shared in these viral proteins (Fig. S3). For example, NSP12, NSP13, and NSP16 share biological processes such as regulation of cellular component movement, negative regulation of cell morphogenesis involved in differentiation, and negative regulation of substrate adhesion-dependent cell spreading (Fig. S3a).

Overall, our interactome is comprised of abundant information that can be utilized for the identification of novel pathobiology and host-targeting therapies. We also developed an interactive visualization tool for our interactome which can be accessed from https://github.com/ChengF-Lab/COVID-19_PPI.

This SARS-CoV-2-human interactome is of high coverage and quality

To ensure the authenticity when applying our interactome for downstream studies, we first evaluated the quality through several means. We examined three previously published SARS-CoV-2-human protein-protein interactome networks\textsuperscript{9,11,12}. Importantly, all three of these interactomes were generated using AP/MS-based methods alone. We found that only a few interactions overlapped among these datasets (Fig. 2a and Table S2), which could be explained by differences in the cell line models used (Gordon et al. and Li et al. transfected HEK-293T/17; Stukalave et al. infected A549-ACE2) as well as distinct computational and/or experimental
methodologies implemented in their respective study. Still, we found that our interactome had the highest overlap of interaction partners among published SARS-CoV-2-human protein-protein interactome networks (Fig. 2a and Table S2), suggesting that our interactome had a high level of coverage.

We next performed several comparisons using these interactomes and our own. First, we examined whether these datasets contained interaction partners that coincided with genes that had expression changes in response to SARS-CoV-2 infection. To this end, we performed differential expression analysis for several bulk and single-cell RNA-seq datasets (see Methods). For the single-cell dataset which we compared the gene expression in SARS-CoV-2+ and SARS-CoV-2- cells, we found that our interactome showed significant overlap (Fisher’s exact test, \( P < 0.01 \)) with the differentially expressed genes (DEGs) in more cell types than that of other interactomes (Fig. 2b and Table S2). Using four bulk RNA-seq datasets that contained samples such as upper airway and bronchial epithelial cells, we found that our interactome had a comparable number of significant overlaps to other datasets and showed the highest overall Jaccard index with the bulk RNA-seq datasets (Fig. 2c and Table S2). These results suggest that our interactome is highly enriched in genes differentially expressed in response to SARS-CoV-2 infection.

Gene expression patterns in disease-related tissues carry important information for revealing the pathogenesis of the disease and identifying potential treatments. We therefore examined the expression of the human host factors in different tissues (Fig. S4 and Table S3) using the GTEx data. By normalizing the expression of each gene across different tissues (tissue specificity, see Methods), we found that lung ranked the 7th out of 33 tissues in terms of the number of host factors with positive tissue specificity (Fig. 2d), suggesting that lung is one of the tissues where these host factors have high expression.

We next inspected the evolutionary factors of the SARS-CoV-2 human host factors (Fig. 2e-f and Table S4). Our SARS-CoV-2-human interactome showed more purifying selection
(quantified by lower non-synonymous versus synonymous substitution rate ratio \([dN/dS \text{ ratio}]\),
as well as a lower evolutionary rate ratio, compared to the random background from the human
protein interactome. These bioinformatics observations further suggested high evolutionary
conservation of host factors of SARS-CoV-2 identified by our Y2H and TMT-AP/MS proteomics
platforms, consistent with previous studies\(^ {50,51} \).

Altogether, these results show the high quality of the SARS-CoV-2-human interactome
identified in this study and strongly encouraged us to further look into the pathobiology of COVID-
19 and potential treatment using our interactome.

**This SARS-CoV-2-human interactome reveals novel pathobiology of COVID-19**

ORF3a is a SARS-CoV-2 accessory protein that has been reported to induce apoptosis in 293T
cells\(^ {52} \) and to suppress the innate immune response\(^ {53-55} \) via unclear molecular mechanisms. Our
interactome revealed that ORF3a physically interacts with ZNF579, a previously uncharacterized
human protein likely to be a transcription factor. We were able to validate this interaction using
coa-immunoprecipitation (co-IP) western blotting (Fig. 3a). Furthermore, we found that the level of
ZNF579 protein is decreased after overexpression of ORF3a in 293T cells (Fig. 3b). As a result,
we hypothesized that the presence of ORF3a in cells might trigger changes in the transcriptional
state of human genes that are normally regulated by ZNF579. Using ENCODE ChIP-seq data\(^ {56,57} \),
we identified that ZNF579 is bound strongly to the promoter of \( \text{HSPA6} \) (Fig. 3c). We found that
overexpression of ORF3a in 293T cells causes massive induction of \( \text{HSPA6} \) using qPCR (Fig.
3d). These results indicate that the multifunctional SARS-CoV-2 accessory protein ORF3a can
induce expression of \( \text{HSPA6} \), presumably by disrupting ZNF579, which is likely to normally exert
repressive activity at the \( \text{HSPA6} \) promoter. This represents an additional previously unknown
activity of this multifunctional viral accessory protein.

The oligosaccharyltransferase (OST) complex catalyzes the N-glycosylation of nascent
polypeptides in the endoplasmic reticulum\(^ {59} \). Glycoproteins are critical for normal cell-cell
interactions, RNA replication and pathogenesis\textsuperscript{59-61}. Interestingly, OST inhibitor has been shown to have activity against Dengue virus, Zika virus, West Nile virus, yellow fever viruses, and HSV1\textsuperscript{62-64} by affecting the viral replication. The OST complex was also found to be crucial for innate immune responses triggered by lipopolysaccharide\textsuperscript{65}. Notably, the OST complex subunits STT3A/B, RPN1/2, and DDOST\textsuperscript{66} were all shown to be present in our Y2H and AP/MS interactome datasets, which we further validated using co-IP (Fig. 3e). Additionally, we also found Sec61 (Fig. 3f-g), which is a major component of the ER translocon that facilitates the entry of nascent polypeptides into the ER lumen for protein processing\textsuperscript{67}. Evidence suggests that Sec61 may participate in the replication and transcription of several viruses like Ebola virus, Influenza virus, HIV, and Dengue virus\textsuperscript{67-69}. Thus, we hypothesize that OST and Sec61 may also participate in SARS-COV-2 replication and/or the host immune response, offering potential targets for host-targeting therapy development.

The SARS-CoV-2 nucleocapsid (N) protein binds to the viral RNA genome and is multifunctional in viral RNA transcription, replication, and genome condensation\textsuperscript{70-72}. N protein is conserved and stable with \textasciitilde90\% amino acid homology to the SARS-COV N protein\textsuperscript{73}. From our dataset, we confirmed known interactions, including the stress granule core protein G3BP1/2 also found in three other interactome datasets. In addition to these known interactions, we identified a novel interaction between histone H1.4 and N protein. To validate this histone H1.4 and N protein interaction, we overexpressed both N protein and histone H1.4 to perform co-IP, confirming their interaction (Fig. 3h). Histone H1, also known as linker histone, mainly functions in chromatin condensation and transcriptional repression\textsuperscript{74,75}. Accumulating evidence suggests that linker histone is essential in the pathogenesis of several diseases, particularly for viral infection\textsuperscript{75}. There is also evidence that Histone H1 could regulate IFN and inhibit influenza replication\textsuperscript{76}, in addition to playing a role in the regulation of viral gene expression\textsuperscript{77}. Thus, we hypothesize that this novel viral-host interaction could also be involved in mediation viral replication and/or gene expression.
Discovery of interactome-based host-targeting therapies for COVID-19

In our previous studies, we demonstrated that by using the SARS-CoV-2 host factors and the human protein-protein interactome, we could prioritize existing drugs for their anti-SARS-CoV-2 potential\(^{78,79}\). Using our newly discovered SARS-CoV-2-human protein-protein interactome network, we performed network-based drug screening for more than 2,900 FDA-approved/investigational drugs (Fig. 4a). We obtained a list of 189 drugs with significantly small network proximities to the SARS-CoV-2 host factors, among which 27 had clinical trials for SARS-CoV-2 (Table S5). To refine this list, we obtained the antiviral profiles of the top 189 drugs from NCATS (https://opendata.ncats.nih.gov/covid19/assays, National Center for Advancing Translational Sciences) and evaluated each drug for their desired antiviral properties (see Methods). From this, we obtained a curated list of 21 drugs with significant network proximities to the SARS-CoV-2 host factors as well as desired anti-SARS-CoV-2 activities in at least two NCATS assays (Fig. 4b, Fig. S5, Table S6).

Overall, these top drugs fall into several major categories, including anti-infective (amodiaquine, azithromycin, tetracycline, adefovir dipivoxil, tipranavir), anti-inflammatory (apremilast, mefenamic acid, balsalazide, fenoprofen), antihypertensive (carvedilol, hydrochlorothiazide, nilvadipine), and antineoplastic (toremifene, decitabine, venetoclax). Among these drugs, apremilast, toremifene, decitabine, amodiaquine, and azithromycin are currently being or have been tested in clinical trials for SARS-CoV-2. These top 21 drugs offer candidate treatments for SARS-CoV-2 infections across diverse mechanism-of-actions identified from our human-SARS-CoV-2 interactome. For example, balsalazide, toremifene, tetracycline, venetoclax, tipranavir, and brimonidine may inhibit viral replication by inhibiting papain-like protease 3CL (Fig. S5 and Table S6). Other drugs, such as carvedilol and hydrochlorothiazide, may directly inhibit viral entry by disrupting the Spike-ACE2 PPI (Fig. S5 and Table S6).

In our previous efforts using existing SARS-CoV-2 interactomes and literature evidence, we identified toremifene, a selective estrogen receptor modulator, as a one of the top candidates
for SARS-CoV-2 treatment\textsuperscript{78,79}. Previous studies show that toremifene blocks various viral infections efficiently, including SARS-CoV-2\textsuperscript{80} (IC\textsubscript{50} = 3.58 \(\mu\)M), SARS-CoV\textsuperscript{1} (EC\textsubscript{50} = 11.97 \(\mu\)M), MERS-CoV\textsuperscript{82} (EC\textsubscript{50} = 12.9 \(\mu\)M), and Ebola virus\textsuperscript{83} (IC\textsubscript{50} = \sim 1 \(\mu\)M). Indeed, NCATS data show that toremifene is active in four assays, including Spike-ACE2 protein-protein interaction (AC\textsubscript{50} = 11.92 \(\mu\)M), SARS-CoV pseudotyped particle entry (AC\textsubscript{50} = 15.85 \(\mu\)M), MERS-CoV pseudotyped particle entry (AC\textsubscript{50} = 31.62 \(\mu\)M), and 3CL enzymatic activity (AC\textsubscript{50} = 5.01 \(\mu\)M) (Fig. S5). Mechanistically, a previous study that toremifene may inhibit SARS-CoV-2 cell entry by blocking the S and NSP14 proteins\textsuperscript{84}. These comprehensive validations show potential implications of SARS-CoV-2 interactome-predicted drug candidates (i.e., toremifene) offer promising drug candidates to be tested further in COVID-19 patients.

**Population-based and experimental validation of interactome-predicted drugs**

We further selected candidate drugs for patient-level data validation and experimental validation using subject matter expertise based on a combination of factors: (1) strength of the interactome network-based prediction associations (a stronger network proximity score in Table S5); (2) novelty of predicted drugs; (3) availability of sufficient patient data for meaningful evaluation (exclusion of infrequently used medications); and (4) ideal pharmacokinetics properties in lung of interactome-predicted drugs. Applying these criteria resulted in 2 top candidate drugs, carvedilol (\(Z = -2.195, \textit{FDR} = 0.03\)) and hydrochlorothiazide (\(Z = -2.428, \textit{FDR} = 0.005\)), which are originally approved for treatment of hypertension.

To identify the drug-outcome relationships of these drugs, we used a state-of-the-art active user-design approach\textsuperscript{46,79} based on large-scale electronic health record (EHR) data. Using the Northwestern Medicine Enterprise Data Warehouse (NMEDW) COVID-19 dataset (481,526 total patients, 66,541 COVID-19 positive cases, Table 1), we found that both carvedilol (odds ratio [OR] = 0.8, 95% confidence interval [CI] 0.68-0.94, \(P = 0.008\)) and hydrochlorothiazide (OR = 0.62, 95% CI 0.47-0.82, \(P < 0.001\)) were associated with a significantly lowered risk of positive
COVID-19 test after confounding adjustment (age, sex, race, and comorbidities) using a propensity score (PS) matching approach\textsuperscript{46,79} (Fig. 4c-d and Table S7). The effect of carvedilol was consistent for different race and sex subgroups (Fig. 4c and Table S7). To validate these observations, we used a second EHR database as an external validation set (168,712 total individuals, 83,340 SARS-CoV-2 positive cases, Table S8). We found that carvedilol had a sufficient number of usage cases for the drug-outcome evaluation. By comparing individuals with and without carvedilol usages (PS-matched by age, sex, race, and/or comorbidities), we found that carvedilol usage was associated with a 17% (OR = 0.83, 95% CI 0.78-0.88, \( P < 0.001 \)) significantly lowered risk of COVID-19 positive test (Fig. 4e). This effect was also consistent when we examined subgroups from the registry in terms of race and sex (Fig. 4e).

We found that carvedilol not only showed favorable results in the EHR-based validation, but also has a promising antiviral profile from NCATS, showing high potencies for multiple desired activities (Fig. 4f). The NCATS profile of carvedilol is comparable to that of remdesivir, whose profile was deemed highly desirable\textsuperscript{85} (Fig. 4f). We then investigated carvedilol’s anti-SARS-CoV-2 activity experimentally. We treated A549-ACE2 cells with 0.3-20 \( \mu \)M of carvedilol for 2 hours followed by infection with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.5 and incubation for 2 days. Cells were subsequently fixed and immunostained to detect for S protein, which was used as a marker for infection. We found that carvedilol inhibited SARS-CoV-2 infection with an EC\textsubscript{50} value of 4.1 \( \mu \)M (Fig. 4g), mechanistically supporting our SARS-CoV-2-human interactome-based prediction and EHR-based findings. Lastly, we conducted drug-target network analysis of carvedilol’s targets and SARS-CoV-2 host factors (Fig. S6 and Table S9). We found that carvedilol could potentially affect the SARS-CoV-2 host factors through PPIs with its targets (Fig. S6).

**Discussion**

In this study, we leveraged high-throughput Y2H and TMT-AP/MS to generate the first binary and co-complex SARS-CoV-2-human protein-protein interactome network that overcomes the...
shortcomings of using AP/MS as the sole approach for PPI identification. This interactome validated 218 previously published SARS-CoV-2 host factors, and more importantly, revealed 361 novel ones. By comparing with previous interactomes, this interactome is the most comprehensive in terms of overlaps among the interactomes and differentially expressed genes captured by bulk and single-cell RNA-seq of SARS-CoV-2 infection. These host factors identified in this interactome, particularly those altered in response to SARS-CoV-2 infection, present an invaluable opportunity for understanding the disease pathobiology of COVID-19 and prioritizing potential drug targets for treatment development.

Among the novel interacting partners for S protein, we identified several human proteins which may play important roles in SARS-CoV-2 infection. CORO1C\textsuperscript{38} and STON2\textsuperscript{39} are expressed on the cell membrane. CORO1C is highly expressed in lung (\textbf{Table S3}). STON2 is ubiquitously expressed and involved in endocytic machinery\textsuperscript{39}. It is possible that SARS-CoV-2 can enter host cells through binding of S protein not only to ACE2, NRP1\textsuperscript{86,87} and BSG\textsuperscript{88}, but also other (unknown) factors such as CORO1C and STON2. We also noticed two proteins, EPPK1\textsuperscript{89} and SPECC1L\textsuperscript{90}, that both express on the cell junctions. It has been suggested that SARS-CoV-2 could spread through cell-to-cell transmission\textsuperscript{91}. These cell junction proteins that can be targeted by SARS-CoV-2 S protein may facilitate its cell-to-cell transmission.

We noticed an overall high functional overlap of the SARS-CoV-2 proteins in terms of both shared interacting partners (\textbf{Fig. S1c}) and shared enriched pathways and biological processes (\textbf{Fig. S3}). This observation shows a high redundancy of SARS-CoV-2 proteins, which further suggests that SARS-CoV-2 therapies that targets only specific viral protein or its host factors may not be sufficient. This observation also justified the advantage and necessity of using network-based approach of drug discovery for SARS-CoV-2. As shown by the drug repurposing results, the top drugs can potentially affect multiple SARS-CoV-2 host factors (\textbf{Fig. 4b}).

We identified a previously uncharacterized human transcription factor, ZNF579, that interacts with SARS-CoV-2 accessory protein ORF3a, and report that this interaction leads to the
de-repression of *HSPA6*. HSPA6 is a HSP70 family molecular chaperone, which are known to be involved in the entry, replication, assembly, and release of various viral pathogens.\(^{92}\) We speculate that SARS-CoV-2 has evolved this activity to ensure sufficient levels of molecular chaperones are available to assist with the production of viral proteins in cells.

Next, using this newly discovered SARS-CoV-2-human protein-protein interactome, we performed drug repurposing and identified a list of top 21 drugs. We found that although some of these drugs can directly target the host factors, most of them indirectly affect the host factors through PPIs with their targets (Fig. 4b). Further, we have identified carvedilol and hydrochlorothiazide as potential host-targeting treatments for COVID-19 supported by multiple lines of evidence (strong network proximities to SARS-CoV-2 host factors, significantly reduced SARS-CoV-2 positive test risks in patients using these drugs based on large-scale EHR data, strong anti-SARS-CoV-2 profiles based on high-throughput drug screening, and experimental validation of anti-SARS-CoV-2 activity). To understand the potential mechanisms of carvedilol’s anti-SARS-CoV-2 activity, we examined the carvedilol’s mechanism-of-action impacted by SARS-CoV-2 host factors using network analysis (Fig. S6 and Table S9). Among the 579 unique host factors, 237 (41%) have PPIs with carvedilol targets. A large portion of the human proteins in the enriched pathways (protein translation [26/37, 70%], mRNA splicing [14/21, 67%], glucose metabolism [9/15, 60%], and neutrophil mediated immunity [14/27, 52%]) have PPIs with carvedilol targets, suggesting a potential mechanism in which carvedilol inhibits SARS-CoV-2 replication through multiple important pathways such as protein translation and mRNA splicing.

At individual target level, several targets showed significant proximities, including VEGFA, NDUFC2, GJA1, ABCB1, HIF1A, VCAM1, and KCNH2 (Fig. S6). These targets may play potential roles in carvedilol’s anti-SARS-CoV-2 activity, which warrants future in-depth mechanistic investigations.

We acknowledge several limitations. The network-based SARS-CoV-2 treatment discovery may be affected by the incompleteness of the human protein-protein interactome and
drug-target network. Therefore, we relied not only on the network discoveries, but also incorporated other types of evidence, such as EHR-based validation and experimental validation. Our EHR-based validation is retrospective and can only be applied to commonly used drugs due to data availability. Although we adjusted for several confounding factors, other unknown factors may still have effect on the results of EHR-based validation. Therefore, the drugs identified in this study must be validated using randomized clinical trials before they can be used in patients with COVID-19.
Materials and Methods

SARS-CoV-2 ORF clones

ORF3b (plasmid no. 141384; Addgene), NSP4 (plasmid no. 141369; Addgene), NSP12 (plasmid no. 141378; Addgene), NSP13 (plasmid no. 141379; Addgene), and NSP14 (plasmid no. 141380; Addgene) were a gift from Nevan Krogan, University of California, San Francisco. NSP6 (plasmid no. 149309; Addgene) and NSP16 (plasmid no. 141269; Addgene) were a gift from Fritz Roth, University of Toronto, which we cloned into our pHAGE-CMV-GAW-3xMyc-IRES-PURO construct using Gateway. E, M, N, NSP1, NSP2, NSP3, NSP5, NSP7, NSP8, NSP9, NSP10, NSP15, ORF3a, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, ORF10, and S, cloned into pCAG-FLAG and pcDNA6B-FLAG constructs, were a gift from Pei-Hui Wang, Shandong University. All SARS-CoV-2 ORFs were codon-optimized and expressed in either pLVX-EF1alpha-eGFP-2xStrep-IRES-Puro (plasmid no. 141395; Addgene), pHAGE-CMV-GAW-3xMyc-IRES-PURO, pCAG-FLAG, or pcDNA6B-FLAG mammalian expression vectors.

Y2H

Y2H screens were carried out as previously described\textsuperscript{15,28-31}. In brief, viral ORFs were cloned into pDEST-AD and pDEST-DB vectors using Gateway LR to generate N-terminal ORF fusions. Similarly, human ORFeome 8.1\textsuperscript{27} was cloned into pDEST-AD and pDEST-DB vectors. All AD and DB expression clones were transformed into Y2H Saccharomyces cerevisiae strains MATa Y8800 and MATa Y8930 (genotype: leu2-3, 112 trp1-901 his3Δ200 ura3-52 gal4Δ gal80Δ GAL2::ADE2 GAL1::HIS3@LYS2 GAL7::lacZ@MET2 cyh2R), respectively. To screen out autoactivating DB-ORFs, all DB-ORF MATa Y8930 transformants were mated pairwise against empty pDEST-AD MATa Y8800 transformants and scored for growth on SC-Leu-Trp+3AT and SC-Leu-Trp-Ade plates, where DB-ORFs that triggered reporter activity were removed from further experiments. To increase screening throughput, 24 human ORF AD or DB clones were pooled into single human ORF AD or DB wells, respectively. Viral ORF AD and DB clones were then mated pairwise against pools of human ORF DB and AD clones,
respectively. Mated transformants were incubated overnight at 30 °C before being plated onto SC-Leu-Trp to select for mated diploid yeast. After another overnight incubation at 30 °C, diploid yeast was plated onto SC-Leu-Trp-His+3AT and SC-Leu-Trp-Ade selection plates. After another overnight incubation at 30 °C, plates were replica-cleaned and incubated again for three days at 30 °C for final interaction calling.

**PLATE-seq**

Each colony was picked 6 times into 96-well plates containing 15 μL of 2.5 mg/mL Zymolyase (catalog no. E1004; Zymo Research) and incubated for 45 min at 37 °C followed by 10 min at 95 °C to prepare yeast cell lysate used as PLATE-seq DNA template. PLATE-seq was carried out as previously described\(^3\). In brief, plasmid(s) from individual wells of 96-well plates were PCR amplified using a plasmid-specific forward primer and a reverse primer consisting of a well-position-specific barcode and TruSeq 3′ sequencing adapter. Amplicons derived from the same 96-well plate were pooled and purified using QIAquick PCR Purification Kit (catalog no. 28104, Qiagen). Each amplicon pool was subject to Tn5 tagmentation to fragment the amplicons and append adapters consisting of a plate-specific barcode and TruSeq 5′ sequencing adapter. Tagmented DNA was purified using QIAquick PCR Purification Kit (catalog no. 28104, Qiagen) and pooled across all 96-well plates. These pools were then subjected to low-cycle PCR both to extend the TruSeq end adapters with sequences compatible for binding to the Illumina flow cell and to enrich for only DNA fragments consisting of TruSeq adapter sequences on both ends of the plate specific and well-position-specific barcodes. PLATE-seq libraries were paired-end sequenced on an Illumina MiSeq.

**Affinity purification**

Caco-2 (HTB-37; ATCC) cells were cultured in EMEM (catalog no. 30-2003; ATCC) with 15% FBS (catalog no. 30-2020; ATCC) at 37 °C with 5% CO\(_2\). All 28 SARS-CoV-2 ORFs were codon-optimized and cloned into mammalian expression vectors that contained Strep, Myc, or FLAG
SARS-CoV-2 ORF plasmids and corresponding empty vectors were individually transfected in biological duplicates into Caco-2 cells using Lipofectamine 3000 Transfection Reagent (catalog no. L3000001; Invitrogen) following manufacturer’s instructions. Cells were harvested 72 hr post-transfection and lysed using RIPALysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1% (v/v) Nonidet P 40 Substitute, 5 mM EDTA, phosphatase inhibitor (catalog no. 4906845001; Roche), and protease inhibitor cocktail (catalog no. 11873580001; Roche)). Samples were incubated for 30 min at 4 °C and then centrifuged at 13,000 × g for 15 min at 4 °C. Supernatants were collected and incubate with either MagStrep "type3" XT beads (catalog no. 2-4090-002; IBA Lifesciences), Myc-Trap Agarose (catalog no. yta-10; ChromoTek) or Anti-FLAG M2 Affinity Gel (catalog no. A2220; Millipore) overnight at 4 °C. Strep-tagged samples were washed with 10x Buffer W (catalog no. 2-1003-100; IBA Lifesciences) three times at 4°C. Myc- and FLAG-tagged samples were washed with RIPALysis buffer. Strep-tagged samples were eluted using 10x Buffer BXT (catalog no. 2-1042-025; IBA Lifesciences). Myc- and FLAG-tagged samples were eluted using IP elution buffer (100 mM Tris-HCl [pH 7.5], 1% (v/v) SDS) and incubated for 15 min at 65 °C. Other primary antibodies used in this study include c-Myc Monoclonal Antibody (catalog no. 13-2500; Invitrogen) and Monoclonal Anti-FLAG M2 Antibody (catalog no. F3165; Sigma-Aldrich).

**Proteomic sample preparation**

IP eluates were reduced using 200 mM TCEP for 1 hr at 55 °C. Samples were then alkylated using 375 mM iodoacetamide for 30 min at room temperature in the absence of light. Samples were digested using Trypsin Gold, Mass Spectrometry Grade (catalog no. V5280; Promega) at an enzyme-to-substrate ratio of 1:100 and incubated overnight with nutation at 37 °C. Peptide concentrations were measured using Pierce Quantitative Colorimetric Peptide Assay (catalog no. 23275; Thermo Scientific). Samples were normalized and resuspended using 1M Triethylammonium bicarbonate (TEAB) for TMT experiments (catalog no. 90114; Thermo...
Samples were labeled using TMT10plex Isobaric Mass Tagging Kit (catalog no. 90113; Thermo Scientific) at a (w/w) label-to-peptide ratio of 10:1 for 1 hr at room temperature. Labeling reactions were quenched by the addition of 5% hydroxylamine and immediately pooled and dried using a SpeedVac. Labeled peptides were enriched and fractionated using Pierce High pH Reversed-Phase Peptide Fractionation Kit according to the manufacturer’s protocol (catalog no. 84868; Thermo Scientific).

**LC-MS/MS**

Fractions were analyzed using an EASY-nLC 1200 System (catalog no. LC140; Thermo Scientific) equipped with an in-house 3 µm C18 resin- (Michrom BioResources) packed capillary column (75 µm × 25 cm) coupled to an Orbitrap Fusion Lumos Tribrid Mass Spectrometer (catalog no. IQLAAEAPFADMBHQ; Thermo Scientific). The mobile phase and elution gradient used for peptide separation were as follows: 0.1% formic acid in water as buffer A and 0.1% formic acid in 80% acetonitrile as buffer B; 0-5 min, 5%-10% B; 5-65 min, 10-55% B; 66-67 min, 55%-95% B; 67-68 min, 2% B; 68-72 min, 95% B; 72-80 min, 5% B; with a flow rate set to 200 nL/min. MS1 precursors were detected at m/z = 375-1500 and resolution = 120,000. A CID-MS2-HCD-MS3 method was used for MS^n data acquisition. Precursor ions with charge of 2+ to 7+ were selected for MS2 analysis at resolution = 30,000, isolation width = 0.4 m/z, maximum injection time = 50 ms, and CID collision energy at 35%. 6 SPS precursors were selected for MS3 analysis and ions were fragmented using HCD collision energy at 65%. Spectra were recorded using Thermo Xcalibur Software Version 4.1 (catalog no. OPTON-30965; Thermo Scientific) and Tune application version 3.0 (Thermo Scientific). Raw data were searched using Proteome Discoverer Software 2.3 (Thermo Scientific) against an UniProtKB human database containing all SARS-COV-2 proteins. Search parameters specified precursor mass and fragment mass tolerance of 15 ppm. Peptide-spectrum matches (PSMs) were searched with SEQUEST HT and Percolator and filtered at FDR < 1 were considered significantly.
**Downstream proteomic analysis**

We developed a novel pipeline using a customized linear model (inspired by MSstatsTMT\textsuperscript{93}) to identify high-confidence viral-host interactions from TMT-AP/MS datasets. Briefly, PSMs filtered at 1% FDR were selected for quantification by (1) the number of reporter intensity values per fraction, (2) percent isolation interference, and (3) precursor intensity values to select for one instance of a peptide peak. If more than one PSM passed these criteria, then the average of the reporter ion intensities per channel of these PSMs were taken to represent the quantification of the peptide peak. The reporter intensity values of selected PSMs were log transformed, weighed with their respective precursor intensities, and averaged to obtain protein level quantification values. Our pipeline’s novelty lies in its ability to retain useful information separated across fractions at the PSM level while ensuring no violation of the assumption of independence, such that our linear fixed-effects model with conditions (e.g., sample vs. control), as a fixed effect, can be utilized. An improved \textit{p}-value calculation was used through Empirical Bayes estimation of prior variance as implemented in R limma package\textsuperscript{94}.

The fold change (FC) and \textit{p}-values obtained from this linear model-based approach are used to generate volcano plots for each viral bait protein compared to control and a hyperbolic curve is optimized using the distribution of the log-transformed FCs of all identified proteins to identify high-confidence interactors. A PSM cutoff, along with a peptide-coverage percent cutoff (i.e., the percentage of all possible trypsin digested peptides, accounting for up to two missed cleavages that can be found), based on the number of the viral protein’s PSMs and peptide-coverage percentage, is also implemented prior to the optimization of this hyperbolic curve.

**Co-immunoprecipitation**

HEK 293T (CRL-3216; ATCC) cells were cultured in DMEM (catalog no. 30-2002; ATCC) supplemented with 10% FBS (catalog no. 30-2020; ATCC) and incubated at 37 °C with 5% CO\textsubscript{2}. Cells were seeded onto six-well plates and grown until reaching 70-80% confluency. SARS-CoV-
2 N, ORF3a, ORF7b, histone H1.4 or N+histone H1.4, Sec61 or ORF7b+Sec61, STT3A or ORF7b+STT3A, and empty vector controls were transfected into cells by combining 2 μg of DNA with 10 μL of 1 mg/mL PEI (catalog no. 23966; Polysciences Inc.) and 150 μL Opti-MEM (catalog no. 31985062; Gibco). After 24 hr incubation, cells were gently washed three times with DPBS (1X) (catalog no. 14040117; Gibco), resuspended with 200 μL cell lysis buffer (10 mM Tris-HCl [pH 8.0], 137 mM NaCl, 1% (v/v) Triton X-100, 10% (v/v) glycerol, 2 mM EDTA, and protease inhibitor cocktail (catalog no. 11873580001; Roche)) and incubated on ice for 30 min. Extracts were then cleared by centrifugation at 16,000 ×g for 10 min at 4 °C. To perform co-immunoprecipitation (co-IP), 100 μL cell lysate was incubated with 5 μL Red Anti-FLAG M2 Affinity Gel (catalog no. F2426; Millipore) overnight at 4 °C under gentle rotation. Bound proteins were then washed three times with cell lysis buffer, eluted with 50 μl elution buffer (10 mM Tris-HCl [pH 8.0], 1% (v/v) SDS) and incubated for 10 min at 65 °C. Cell lysates and co-IP samples were then treated with 6X SDS protein loading buffer (1 M Tris-HCl [pH 6.8], 10% (v/v) SDS, 50% (v/v) glycerol, 0.03% (v/v) bromophenol blue, and 10% (v/v) β-mercaptoethanol), subjected to SDS-PAGE, and transferred onto PVDF membranes (catalog no. GE10600023; Amersham). For immunoblotting analysis, V5 Tag Monoclonal Antibody (catalog no. R960-25; Invitrogen), c-Myc Monoclonal Antibody (catalog no. 13-2500; Invitrogen), Monoclonal Anti-FLAG M2 Antibody (catalog no. F1804; Sigma-Aldrich), or ZNF579 Polyclonal Antibody (catalog no. A303-275A; Bethyl Laboratories) were used at 1:1,000 dilutions.

qPCR

293T cells were cultured as above, and ORFa-FLAG or empty vector were introduced with Lipofectamine 2000 Transfection Reagent (catalog no. 11668030; Invitrogen) using manufacturer instructions. Transfection experiments were performed in duplicate. Media was replaced 6 hours after transfection, and RNA was harvested using TRIzol Reagent (catalog no. 15596018; Invitrogen). Reverse transcription was performed with the Maxima First Strand cDNA Synthesis
Kit for RT-qPCR, with dsDNase (catalog no. K1671; Thermo Scientific). qPCR was performed on a LightCycler 480 System using LightCycler FastStart DNA Master SYBR Green I (catalog no. 03003230001; Roche Diagnostics). We used two primer sets for HSPA6 (FWD1: CAAGGTGCGGTATGCTAC, REV1: GCTCATTGATGATCCGCAAC, FWD2: CATCGCCTATGGGCTGGAC, REV2: GGAGAGAACCGACACATCGAA), and performed 3 technical replicates of 3 concentrations of cDNA (1:10, 1:100, 1:1000) for each replicate, and then compared expression levels normalized to GAPDH using the double-delta Cp method.

**Interactome comparative analysis**

We compared our SARS-CoV-2-human interactome to a collection of three previously reported interactomes\(^\text{10-12}\), and compared with ours in terms of the overlap (Fisher’s exact test) with the differentially expressed genes in SARS-CoV-2 from several SARS-CoV-2 RNA-seq/proteomics datasets. These datasets include: (1) a single-cell dataset that contains CD8, Epithelial (Epi) - Ciliated, Epi-Secretory, Epi-Squamous, Macro, Mono, and NK cells from BALF\(^\text{40}\). We performed comparisons of virus\(^+\) vs. virus\(^-\) cells for each cell type; (2) bulk RNA-seq of human bronchial epithelial cells infected with SARS-CoV-2\(^\text{41}\) (GSE147507), denoted as SARS2-DEG; (3) proteomic dataset of human Caco-2 cells infected with SARS-CoV-2\(^\text{42}\), denoted as SARS2-DEP. (4) bulk RNA-seq of upper airway from COVID-19 patients vs. non-COVID-19 patients (GSE156063) \(^\text{43}\), denoted as DE-NS; (5) bulk RNA-seq of peripheral blood mononuclear cell (PBMC) isolated from COVID-19 patients vs. non-COVID-19 patients (GSE157103) \(^\text{44}\), denoted as DE-PBMC. For differential expression analysis, a cutoff of \(|\log_2 FC| > 0.5\) and FDR < 0.05 was considered significant.

**Functional enrichment analysis**

Functional enrichment of our SARS-CoV-2 host factors were analyzed using Enrichr\(^\text{95}\) against the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) biological process data sets. Pathways and GO terms with FDR < 0.05 were considered significantly enriched.
Selective pressure and evolutionary rates

The nonsynonymous and synonymous substitution rate ratio \( (dN/dS) \) ratio and the evolutionary rate ratio of our SARS-CoV-2 host factors were evaluated as described in a previous study. For \( dN/dS \) ratio, \( dN/dS < 1 \) was considered purifying selection; \( dN/dS = 1 \) was considered neutral evolution; and \( dN/dS > 1 \) was considered positive Darwinian selection. The evolutionary rate ratio >1 was regarded as a fast rate and <1 as a slow rate.

Tissue gene expression specificity

We evaluated the gene expression specificity of the SARS-CoV-2 host factors in 33 tissues using the RNA-Seq data from GTEx V8 (https://www.gtexportal.org/home/). The expression specificity of gene \( i \) in tissue \( t \) was defined as

\[
z_{it} = \frac{E_{it} - E_i}{\sigma_i}
\]

where \( E_i \) was the mean and \( \sigma_i \) was the standard deviation of gene \( i \)'s expression across all considered tissues, and \( E_{it} \) was the mean expression of gene \( i \) in tissue \( t \).

Construction of human protein-protein interactome and drug-target network

The human protein-protein interactome and the drug-target network were used to screen for drugs against the SARS-CoV-2 host factors. The human protein-protein interactome, composed of 17,706 protein nodes and 351,444 unique PPI edges was constructed in our previous studies. Briefly, several types of high-quality PPI evidence gathered from public databases and datasets were considered, including: binary PPIs identified by high-throughput yeast two-hybrid in three datasets; low- or high-throughput experimentally discovered kinase-substrate interactions from KinomeNetworkX, PhosphoNetworks, Human Protein Resource Database (HPRD), DbPTM 3.0, Phospho.ELM, and PhosphositePlus; signaling networks identified using low-throughput experiments in SignaLink2.0; protein...
complexes revealed by robust affinity purification-mass spectrometry in BioPlex V2.016\textsuperscript{10}; and curated PPIs from Instruct\textsuperscript{111}, IntAct\textsuperscript{112}, BioGRID\textsuperscript{113}, MINT\textsuperscript{114}, PINA\textsuperscript{115}, and InnateDB\textsuperscript{116} that were identified by yeast two-hybrid studies, affinity purification-mass spectrometry, protein three-dimensional structures, or low-throughput experiments.

The drug-target network was constructed using several data sources as described in our recent studies\textsuperscript{45,46,99}: DrugBank database (v4.3)\textsuperscript{117}, BindingDB\textsuperscript{118}, ChEMBL (v20)\textsuperscript{119}, Therapeutic Target Database (TTD)\textsuperscript{120}, PharmGKB database\textsuperscript{121}, and IUPHAR/BPS Guide to PHARMACOLOGY\textsuperscript{122}. Binding affinities $K_i$, $K_d$, IC$_{50}$ or EC$_{50}$ ≤ 10 μM were used as cutoff for the drug-target interactions. All networks were visualized using Cytoscape 3.8.0\textsuperscript{123}.

**Network proximity-based drug and drug combination screening**

The “closest” network proximity measure was used to screen for 2,938 FDA approved or investigational drugs. The “closest” distance $d_{AB}$ for two gene/protein sets $A$ (e.g., drug targets) and $B$ (e.g., SARS-CoV-2 host factors) was calculated as:

$$\langle d_{AB} \rangle = \frac{1}{\|A\| + \|B\|} \left( \sum_{a \in A} \min_{b \in B} d(a, b) + \sum_{b \in B} \min_{a \in A} d(a, b) \right)$$

where $d(a, b)$ is the shortest path length of $a$ and $b$ in the human protein-protein interactome.

Network proximity $d_{AB}$ was further normalized to obtain a Z score using a permutation test with randomly selected proteins from the interactome with similar degree distributions to $A$ and $B$. Permutation tests were repeated 1,000 times. We prioritized drugs by $Z < -2$ and FDR < 0.05.

The antiviral profiles of the prioritized drugs were retrieved from NCATS (https://opendata.ncats.nih.gov/covid19/assays). NCATS contains experimental high-throughput screening results for drugs from a series of screenings (some accompanied by counterscreens) to evaluate their anti-SARS-CoV-2 potential. We included the following screening results: SARS-CoV-2 cytopathic effect (CPE) and its counterscreen SARS-CoV-2 cytopathic effect (host tox Counter) / Cytotoxicity; human fibroblast toxicity (hCYTOX); spike-ACE2 protein-protein...
interaction (AlphaLISA) and its counterscreen spike-ACE2 protein-protein interaction (TruHit Counter); ACE2 enzymatic activity; SARS-CoV pseudotyped particle entry (CoV-PPE) and its counterscreen SARS-CoV pseudotyped particle entry counter screen (CoV-PPE_cs); MERS-CoV pseudotyped particle entry (MERS-PPE) and its counterscreen MERS-CoV pseudotyped particle entry counter screen (MERS-PPE_cs); and 3CL enzymatic activity. Based on the NCATS SARS-CoV-2 data, we further selected a list of top drugs from the network proximity-based prioritization that show ideal activities in at least two of these screenings.

**COVID-19 patient data observations**

Two independent datasets revealed corroborating evidence for the drug carvedilol which was identified by our interactome prioritization framework. The first dataset (discovery dataset) was from the Northwestern Medicine Enterprise Data Warehouse (NMEDW). We first identified 512,198 patients who had SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) test results recorded in NMEDW. Patients with a positive RT-PCR test were considered COVID-19 positive, where the earliest time of the test was recorded as the effective time. Patients that did not have any positive or presumptive positive RT-PCR tests and the latest PCR test was negative (excluding pending and undetermined results) were considered COVID-19 negative, where the latest time of the test was recorded as the effective time. By these metrics, 29,224 patients with pending or undetermined results were removed, yielding 482,974 patients of interest. We excluded patients without age or sex information yielding a cohort of 481,526 patients, 66,541 of which were COVID-19 positive. We then extracted the carvedilol (and other drugs) administration information for all patients in the final cohort. If a patient had a carvedilol administration record with an administration date in the 6-month time window leading to the effective RT-PCR result date and an administered dose > 0, the patient was considered carvedilol+. We also extracted comorbidity information of the cohort for propensity score (PS) matching, for which we used the Charlson Comorbidity Index (CCI). All comorbidities and
corresponding patient numbers are listed in Table 1.

The second dataset (external validation dataset) was an institutional review board-approved COVID-19 registry dataset that included 168,712 individuals tested for SARS-CoV-2 infection (83,340 of which were positive cases) from March 8th to May 26th, 2021, at the Cleveland Clinic in Ohio and Florida, United States (Table S8). Pooled oropharyngeal and nasopharyngeal swab specimens were used to test for SARS-CoV-2 by RT-PCR assay in the Cleveland Clinic Pathology and Laboratory Medicine Institute. All SARS-CoV-2 testing followed the guidelines established by the Centers for Disease Control and Prevention of United States. The dataset included baseline demographic information, medications, and COVID-19 test results. We used REDCap electronic data capture tools to extract the patient data from the electronic health records (EPIC Systems), and the data were manually checked by a professional team trained on uniform sources for the study variables. A carvedilol exposure group (carvedilol+) included patients that were actively taking carvedilol at the time of SARS-CoV-2 testing. Positive laboratory test results for COVID-19 were used as the primary outcome. PS was used to match age, sex, and race to reduce various confounding factors. Odds ratio was used to evaluate the carvedilol benefit to primary outcome. All analyses were conducted by matchit package in the R v4.1.0 platform.

**Anti-SARS-CoV-2 activity assay for carvedilol**

A549 (CCL-185; ATCC) cells exogenously expressing angiotensin-converting enzyme 2 (ACE2) (A549-ACE2) were a gift from Benjamin R. Tenoever, Icahn School of Medicine at Mount Sinai. A549-ACE2 cells were cultured in DMEM (catalog no. 11965092; ThermoFisher) with 10% FBS (catalog no. 100-106; GeminiBio) and used for SARS-CoV-2 infection. SARS-CoV-2 virus (nCoV/Washington/1/2020) was provided by the Biocontainment Laboratory–University of Texas Medical Branch Galveston National Laboratory, Texas, United States. Vero E6 (CRL-1586; ATCC) cells were used to propagate and titer SARS-CoV-2. SARS-CoV-2 infections were
performed under biosafety level 3 conditions at the Biocontainment Laboratory—University of Chicago Howard T. Ricketts Laboratory, Illinois, United States. A549-ACE2 cells cultured in DMEM with 2% FBS were treated with carvedilol for 2 hours at the indicated concentrations. Cells were infected with an MOI of 0.5 in media containing the appropriate concentration of drug. 48 hr post-infection, cells were fixed with 10% formalin (catalog no. 305-510; Fisherbrand), blocked, and probed with mouse anti-SARS-CoV-2-spike antibody (catalog no. GTX632604; GeneTex) diluted 1:1,000 for 4 hr, rinsed, and probed with anti-mouse-HRP (catalog no. MP7401; Vector Laboratories) for 1 hr, washed, and then developed with DAB substrate (catalog no. 34065; ThermoScientific) for 10 min. Spike positive cells (n>40) were quantified by light microscopy as blinded samples. A sigmoid fit was used to extract EC50 values using MATLAB.

**Data availability**
We downloaded the GTEx v8 dataset from https://gtexportal.org/home/. The human protein-protein interactome and drug-target network are found in https://github.com/ChengF-Lab/COVID-19_Map. An interactive version of Fig. 1b can be found in https://github.com/ChengF-Lab/COVID-19_PPI. All other data can be found in the supplementary tables. We accessed ZNF579 ChIP-seq data from the ENCODE portal under accession ENCSR018MQH.

**Code availability**
The network proximity framework can be found in https://github.com/ChengF-Lab/COVID-19_Map.

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**Competing interests**
The authors declare that there are no competing interests.
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Table 1. Patient Characteristics of NMEDW dataset.

|                         | All patients | SARS-CoV-2 positive patients |
|-------------------------|--------------|------------------------------|
|                         | Carvedilol-  | Carvedilol+                  | Carvedilol-  | Carvedilol+                  |
| Total                   | 478,536      | 2,990                        | 66,289       | 252                          |
| Age                     | 44.67 ± 21.74| 67.37 ± 15.08                | 41.86 ± 21.09| 63.52 ± 17.20                |
| Sex, male               | 202,994 (42.4)| 1,674 (56.0)                | 30,550 (46.1)| 146 (57.9)                  |
| Race                    |              |                              |              |                              |
| Black                   | 41,858 (8.7) | 725 (24.2)                   | 6,136 (9.3)  | 73 (29.0)                    |
| White                   | 343,549 (71.8)| 1,927 (64.4)               | 46,493 (70.1)| 143 (56.7)                  |
| Other                   | 60,273 (12.6)| 274 (9.2)                    | 9,407 (14.2) | 27 (10.7)                    |
| Comorbidity             |              |                              |              |                              |
| AIDS HIV                | 1,843 (0.4)  | 36 (1.2)                     | 248 (0.4)    | 5 (2.0)                      |
| CD                      | 32,555 (6.8) | 1,336 (44.7)                 | 3,498 (5.3)  | 103 (40.9)                   |
| CPD                     | 87,868 (18.4)| 1,249 (41.8)                 | 12,078 (18.2)| 123 (48.8)                  |
| CHF                     | 26,973 (5.6) | 2,042 (68.3)                 | 3,331 (5.0)  | 172 (68.3)                   |
| Dementia                | 8,567 (1.8)  | 358 (12.0)                   | 1,297 (2.0)  | 42 (16.7)                    |
| Diabetes w cc           | 20,670 (4.3) | 1,359 (45.5)                 | 3,031 (4.6)  | 139 (55.2)                   |
| Diabetes wo cc          | 54,322 (11.4)| 1,629 (54.5)                 | 8,301 (12.5) | 150 (59.5)                   |
| HP                      | 5,380 (1.1)  | 298 (10.0)                   | 568 (0.9)    | 27 (10.7)                    |
| Malignancy              | 47,660 (10.0)| 805 (26.9)                   | 4,606 (6.9)  | 67 (26.6)                    |
| MST                     | 23,690 (5.0) | 413 (13.8)                   | 2,385 (3.6)  | 36 (14.3)                    |
| MLD                     | 29,730 (6.2) | 583 (19.5)                   | 3,711 (5.6)  | 53 (21.0)                    |
| MSLD                    | 2,906 (0.6)  | 119 (4.0)                    | 307 (0.5)    | 11 (4.4)                     |
| MI                      | 7,913 (1.7)  | 630 (21.1)                   | 1,017 (1.5)  | 54 (21.4)                    |
| PUD                     | 11,785 (2.5) | 309 (10.3)                   | 1,289 (1.9)  | 32 (12.7)                    |
| PVD                     | 23,925 (5.0) | 1,158 (38.7)                 | 2,626 (4.0)  | 99 (39.3)                    |
| RD                      | 26,068 (5.4) | 1,820 (60.9)                 | 3,479 (5.2)  | 181 (71.8)                   |
| RhD                     | 13,887 (2.9) | 232 (7.8)                    | 1,607 (2.4)  | 18 (7.1)                     |

Age is shown as mean ± standard deviation. All other characteristics are shown as number of cases (percentage). P values were calculated by two-sided t test for age and Fisher’s exact test for other variables. AIDS HIV, acquired immunodeficiency syndrome and human immunodeficiency virus; CD, cerebrovascular disease; CPD, chronic pulmonary disease; CHF, congestive heart failure; Diabetes w/wo cc, diabetes with/without chronic complications; HP, hemiplegia or paraplegia; MST, metastatic solid tumor; MLD, mild liver disease; MSLD, moderate or severe liver disease; MI, myocardial infarction; PUD, peptic ulcer disease; PVD, peripheral vascular disease; RD, renal disease; RhD, rheumatic disease.
Figure 1

Pairwise yeast two-hybrid screen

Y2H

ORF α ---- ORF A
ORF β ---- ORF B
ORF γ ---- ORF D

Identify AD:DB pairs via PLATE-Seq

28 viral baits × ~16,000 host preys × 2 way tests = 896,000 tested pairs

299 final binary interactions

Express in Caco-2 immunoprecipitate

TMT10 labeling and SPS-MS3-based quantification

472 final co-complex interactions

Viral proteins

Human proteins

1 Degree

Y2H

Y2H & AP/MS
Figure 1. SARS-CoV-2-human protein interactome. (a) Pipelines using Y2H and AP/MS for detecting SARS-CoV-2-human protein-protein interactions. (b) Edges between viral proteins (diamonds) and human proteins (circles) represent protein-protein interactions. Edge colors indicate the methods used to detect the protein-protein interaction. Several biological processes that are significantly enriched in these human proteins are highlighted with yellow background. Human proteins that interact with only one SARS-CoV-2 protein are shown in the box connected to that specific protein. The interactome can be found in Table S1.
**Figure 2**

(a) Cumulative evolutionary ratio and dN/dS ratio for genes identified in the study (red) compared to previous studies (green, blue, purple).

(b) Jaccard index for the intersection size between the current study and previous studies.

(c) Jaccard index for the cumulative evolutionary ratio and dN/dS ratio.

(d) Intersection size for host factors with positive tissue specificity across different tissues.

(e) Cumulative evolutionary ratio across different genes.

(f) Cumulative dN/dS ratio across different genes.
Figure 2. Characteristics of the interactome. (a) Overlap of the host factors identified in several published interactomes (Table S2). (b) Overlap of the host factors with differentially expressed genes in SARS-CoV-2+ vs SARS-CoV-2- cells in seven cell types. (c) Overlap of the host factors with differentially expressed genes from several bulk RNA-seq datasets. (d) Expression of the human host factors in different tissues (Table S3). (e and f) Biological characteristics of the SARS-CoV-2 host factors. The host factors have lower non-synonymous to synonymous substitutions (dN/dS) ratios (e) and lower evolutionary ratios (f) compared to random background (grey, mean ± standard deviation of 100 repeats) (Table S4).
Figure 3

a) Input and IP experiment results for ORF3a-FLAG and anti-ZNF579 antibodies.

b) Western blot analysis showing ORF3a-FLAG and anti-ZNF579 antibodies.

(c) Chromatin Immunoprecipitation-sequencing (ChIP-seq) data for ZNF579.

(d) qPCR analysis of HSPA6 expression.

(e) Flag pull down experiment results for ORF7b-FLAG and anti-ZNF579 antibodies.

(f) Western blot analysis showing Sec61-V5 and anti-V5 antibodies.

(g) Network diagram of viral proteins, host factors, and PPI neighbors.

(h) Flag pull down experiment results for N-Flag and anti-GFP antibodies.
Figure 3. Validation of novel SARS-CoV-2-human interactions. (a) Co-immunoprecipitation confirming ORF3a-ZNF579 interaction in HEK 293T cells following transfection with ORF3a-FLAG or empty vector. (b) Western blot showing levels of ZNF579 along with GAPDH as a loading control in 293T cells following transfection with ORF3a-FLAG or empty vector. (c) ChIP-seq for ZNF579 in MCF7 cells from the ENCODE consortium at the HSPA6 locus. Signal is log2 fold change over input. (d) Expression of HSPA6 after transfection with ORF3a-FLAG or empty vector. Two transfection replicates were probed with two primer pairs to HSPA6 at three different template dilutions in technical triplicate (18 total reactions for each condition). Expression is normalized to GAPDH and then to the empty vector average using the double-delta Ct method. (e-f) Co-immunoprecipitation confirming ORF7b-STT3A and ORF7b-Sec61 interactions in HEK293T cells following transfection with ORF7b-FLAG or empty vector and STT3A-MYC or Sec61-V5, respectively. (g) Interactions among Sec61, their neighbors, and SARS-CoV-2 viral proteins in our interactome. (h) Co-immunoprecipitation confirming N-histone H1.4 interaction in HEK293T cells following transfection with N or empty vector and histone H1.4.
**Figure 4**

a) Diagram showing the flow of 189 top drugs significantly proximal to host factors.

b) Drug-target interactions.

- Nilvadipine
- Probuloc
- Venetoclax
- Xylometazoline
- Balsalazide
- Brimonidine
- Carvedilol
- Amitriptyline
- Fenoprofen
- Mefenamic acid
- Tipranavir
- Toremifene
- Amodiaquine
- Azithromycin
- Apremilast
- Tetracycline
- Repaglinide
- Decitabine
- Hydrochlorothiazide
- Adefovir dipivoxil
- Calcipotriol

- SARS-CoV-2 cytopathic effect
- SARS-CoV-2 cytopathic effect / Cytotoxicity
- Human fibroblast toxicity
- Spike-ACE2 protein-protein interaction
- Spike-ACE2 protein-protein interaction
- ACE2 enzymatic activity
- SARS-CoV pseudotyped particle entry
- SARS-CoV pseudotyped particle entry counter screen
- MERS-CoV pseudotyped particle entry
- MERS-CoV pseudotyped particle entry counter screen
- 3CL enzymatic activity

| Drug | Potency (µM) |
|------|-------------|
| Carvedilol | 12.59 |
| Remdesivir | 7.52 |
| Balsalazide | 11.07 |
| Toremifene | 14.13 |

- Has desired property

- Inactive

- Raw

- Adjusted

**c)** NMEDW cohort, carvedilol use vs. non-use

| Group      | COVID-19 Positive Odds Ratio (95% CI) | P value |
|------------|--------------------------------------|---------|
| All        | 0.57 (0.50-0.65)                     | <0.001  |
| Black      | 0.57 (0.44-1.08)                     | 0.114   |
| White      | 0.65 (0.45-0.94)                     | 0.022   |
| Female     | 0.63 (0.44-0.91)                     | 0.013   |
| Male       | 0.41 (0.28-0.59)                     | <0.001  |

**d)** NMEDW cohort, hydrochlorothiazide use vs. non-use

| Group      | COVID-19 Positive Odds Ratio (95% CI) | P value |
|------------|--------------------------------------|---------|
| All        | 0.51 (0.40-0.64)                     | <0.001  |
| Black      | 0.73 (0.49-1.08)                     | 0.114   |
| White      | 0.46 (0.34-0.62)                     | <0.001  |
| Female     | 0.63 (0.44-0.91)                     | 0.013   |
| Male       | 0.41 (0.28-0.59)                     | <0.001  |

**e)** CCF cohort, carvedilol use vs. non-use

| Group      | COVID-19 Positive Odds Ratio (95% CI) | P value |
|------------|--------------------------------------|---------|
| All        | 0.80 (0.76-0.86)                     | 0.001   |
| Black      | 0.83 (0.78-0.88)                     | 0.001   |
| White      | 0.83 (0.76-0.90)                     | 0.001   |
| Female     | 0.82 (0.75-0.91)                     | 0.001   |
| Male       | 0.78 (0.72-0.85)                     | 0.001   |

**g)** Graph showing the percentage of SARS-CoV-2 infection vs. Carvedilol (µM)

**Table:**

| Drug     | Remdesivir | Has desired property |
|----------|------------|----------------------|
| Carvedilol | Yes        | Yes                  |
| Remdesivir | Yes        | Yes                  |
| Balsalazide | Yes        | Yes                  |
| Toremifene | Yes        | Yes                  |

**Legend:**

- Anti-infective
- Anti-inflammatory
- Antineoplastic
- Antihypertensive
- Other
Figure 4. Discovery of interactome-based host-targeting therapies for COVID-19. (a) Workflow of drug repurposing for COVID-19 using our interactome. We ranked the drugs by their proximity to the SARS-CoV-2 host factors (Table S5), filtered the top drugs by their NCATS anti-SARS-CoV-2 profiles (Table S6), and finally analyzed their drug-outcome relationship using electronic health records (EHR) data (Table 1, Table S7-S8). (b) The top 21 drugs can target the SARS-CoV-2 host factors directly or through protein-protein interactions with their targets. (c-e) Drug-outcome evaluation using the Northwestern Medicine Enterprise Data Warehouse (NMEDW) and Cleveland Clinic Foundation (CCF) COVID-19 databases. (f) Carvedilol has several desired activities against SARS-CoV-2 infections. (g) Experimental validation of the anti-SARS-CoV-2 activity of carvedilol showed an EC$_{50}$ value of 4.1 µM.
Figure S1. Summary statistics of the SARS-CoV-2-human protein-protein interactome. (a) Degree distribution of the human proteins. (b) Degrees of the viral proteins. (c) Overlap of the interaction partners of the viral proteins. Heatmap color shows Jaccard index. Dot indicates $P < 0.05$ by Fisher's exact test.
Figure S2. Pathway and gene ontology enrichment analyses for SARS-CoV-2 host factors. Enrichment analyses were performed with Enrichr (https://maayanlab.cloud/Enrichr/).
Figure S3. Pathway and gene ontology enrichment analyses for host factors of individual SARS-CoV-2 viral proteins. Black dot indicates FDR < 0.05. Color shows overlap. Enrichment analyses were performed with Enrichr (https://maayanlab.cloud/Enrichr/). Pathways and gene ontology terms that are significant in at least three viral proteins are shown.
Figure S4. Tissue-specificity of the host factors. RNA-Seq data in transcript per million were downloaded from GTEx V8 (https://www.gtexportal.org/home/). Data were z-score scaled for each gene across 33 tissues.
Figure S5. Anti-SARS-CoV-2 profiles for the top 21 drugs. Data were retrieved from NCATS (https://opendata.ncats.nih.gov/covid19/assays)
Figure S5. continued

Balsalazide

Azithromycin

Toremifene

Spike-ACE2 protein–protein interaction (AlphaLISA)
Spike-ACE2 protein–protein interaction (TruHit Counterscreen)
ACE2 enzymatic activity
TMPRSS2 enzymatic activity
3CL enzymatic activity
SARS-CoV Pseudotyped particle entry
SARS-CoV Pseudotyped particle entry (VeroE6 tox countscreen)
MERS Pseudotyped particle entry
MERS Pseudotyped particle entry (Huh7 tox countscreen)
SARS-CoV-2 cytopathic effect (CPE)
SARS-CoV-2 cytopathic effect (host tox countscreen)
HEK293 cell line toxicity
Human fibroblast toxicity
Figure S5. continued

**Hydrochlorothiazide**

- 31.84µM: Spike-ACE2 protein–protein interaction (AlphaLISA)
- Inactive: Spike-ACE2 protein–protein interaction (TruHit Counterscreen)
- Inactive: ACE2 enzymatic activity
- Inactive: TMPRSS2 enzymatic activity
- Inactive: 3CL enzymatic activity
- Inactive: SARS–CoV Pseudotyped particle entry
- Inactive: SARS–CoV Pseudotyped particle entry (VeroE6 tox counterscreen)
- Inactive: MERS Pseudotyped particle entry
- Inactive: MERS Pseudotyped particle entry (Huh7 tox counterscreen)
- Inactive: SARS–CoV–2 cytopathic effect (CPE)
- Inactive: SARS–CoV–2 cytopathic effect (host tox counterscreen)
- Inactive: HEK293 cell line toxicity
- Inactive: Human fibroblast toxicity

**Nilvadipine**

- 11.92µM: Spike-ACE2 protein–protein interaction (AlphaLISA)
- Inactive: Spike-ACE2 protein–protein interaction (TruHit Counterscreen)
- Inactive: ACE2 enzymatic activity
- Inactive: TMPRSS2 enzymatic activity
- Inactive: 3CL enzymatic activity
- Inactive: SARS–CoV Pseudotyped particle entry
- Inactive: SARS–CoV Pseudotyped particle entry (VeroE6 tox counterscreen)
- Inactive: MERS Pseudotyped particle entry
- Inactive: MERS Pseudotyped particle entry (Huh7 tox counterscreen)
- Inactive: SARS–CoV–2 cytopathic effect (CPE)
- Inactive: SARS–CoV–2 cytopathic effect (host tox counterscreen)

**Amodiaquin**

- 8.79µM: Spike-ACE2 protein–protein interaction (AlphaLISA)
- Inactive: Spike-ACE2 protein–protein interaction (TruHit Counterscreen)
- Inactive: ACE2 enzymatic activity
- Inactive: TMPRSS2 enzymatic activity
- Inactive: 3CL enzymatic activity
- Inactive: SARS–CoV Pseudotyped particle entry
- Inactive: SARS–CoV Pseudotyped particle entry (VeroE6 tox counterscreen)
- Inactive: MERS Pseudotyped particle entry
- Inactive: MERS Pseudotyped particle entry (Huh7 tox counterscreen)
- Inactive: SARS–CoV–2 cytopathic effect (CPE)
- Inactive: SARS–CoV–2 cytopathic effect (host tox counterscreen)
Figure S5. continued

Tetracycline

- Spike-ACE2 protein–protein interaction (AlphaLISA)
- Spike-ACE2 protein–protein interaction (TruHit Counterscreen)
- ACE2 enzymatic activity
- TMPRSS2 enzymatic activity
- 3CL enzymatic activity
- SARS–CoV Pseudotyped particle entry
- SARS–CoV Pseudotyped particle entry (VeroE6 tox counterscreen)
- MERS Pseudotyped particle entry
- MERS Pseudotyped particle entry (Huh7 tox counterscreen)
- SARS–CoV–2 cytopathic effect (CPE)
- SARS–CoV–2 cytopathic effect (host tox counterscreen)
- HEK293 cell line toxicity
- Human fibroblast toxicity

Xylometazoline

- Spike-ACE2 protein–protein interaction (AlphaLISA)
- Spike-ACE2 protein–protein interaction (TruHit Counterscreen)
- ACE2 enzymatic activity
- TMPRSS2 enzymatic activity
- 3CL enzymatic activity
- SARS–CoV Pseudotyped particle entry
- SARS–CoV Pseudotyped particle entry (VeroE6 tox counterscreen)
- MERS Pseudotyped particle entry
- MERS Pseudotyped particle entry (Huh7 tox counterscreen)
- SARS–CoV–2 cytopathic effect (CPE)
- SARS–CoV–2 cytopathic effect (host tox counterscreen)
- HEK293 cell line toxicity
- Human fibroblast toxicity

Decitabine

- Spike-ACE2 protein–protein interaction (AlphaLISA)
- Spike-ACE2 protein–protein interaction (TruHit Counterscreen)
- ACE2 enzymatic activity
- TMPRSS2 enzymatic activity
- 3CL enzymatic activity
- SARS–CoV Pseudotyped particle entry
- SARS–CoV Pseudotyped particle entry (VeroE6 tox counterscreen)
- MERS Pseudotyped particle entry
- MERS Pseudotyped particle entry (Huh7 tox counterscreen)
- SARS–CoV–2 cytopathic effect (CPE)
- SARS–CoV–2 cytopathic effect (host tox counterscreen)
- HEK293 cell line toxicity
- Human fibroblast toxicity
Figure S5. continued

**Adefovir dipivoxil**

| Potency (µM) | 1µM | 3µM | 5µM |
|-------------|-----|-----|-----|
| **Inactive** |     |     |     |
| Spike-ACE2 protein–protein interaction (AlphaLISA) |     |     |     |
| Spike-ACE2 protein–protein interaction (TruHit Counterscreen) |     |     |     |
| ACE2 enzymatic activity |     |     |     |
| TMPRSS2 enzymatic activity |     |     |     |
| 3CL enzymatic activity |     |     |     |
| SARS-CoV Pseudotyped particle entry |     |     |     |
| SARS-CoV Pseudotyped particle entry (VeroE6 tox countsscreen) |     |     |     |
| MERS Pseudotyped particle entry |     |     |     |
| MERS Pseudotyped particle entry (Huh7 tox countsscreen) |     |     |     |
| SARS-CoV-2 cytopathic effect (CPE) |     |     |     |
| SARS-CoV-2 cytopathic effect (host tox countsscreen) |     |     |     |
| HEK293 cell line toxicity |     |     |     |
| Human fibroblast toxicity |     |     |     |

**Venetoclax**

| Potency (µM) | 1µM | 3µM | 5µM |
|-------------|-----|-----|-----|
| **Inactive** |     |     |     |
| Spike-ACE2 protein–protein interaction (AlphaLISA) |     |     |     |
| Spike-ACE2 protein–protein interaction (TruHit Counterscreen) |     |     |     |
| ACE2 enzymatic activity |     |     |     |
| TMPRSS2 enzymatic activity |     |     |     |
| 3CL enzymatic activity |     |     |     |
| SARS-CoV Pseudotyped particle entry |     |     |     |
| SARS-CoV Pseudotyped particle entry (VeroE6 tox countsscreen) |     |     |     |
| MERS Pseudotyped particle entry |     |     |     |
| MERS Pseudotyped particle entry (Huh7 tox countsscreen) |     |     |     |
| SARS-CoV-2 cytopathic effect (CPE) |     |     |     |
| SARS-CoV-2 cytopathic effect (host tox countsscreen) |     |     |     |
| HEK293 cell line toxicity |     |     |     |
| Human fibroblast toxicity |     |     |     |

**Calcipotriol**

| Potency (µM) | 1µM | 3µM | 5µM |
|-------------|-----|-----|-----|
| **Inactive** |     |     |     |
| Spike-ACE2 protein–protein interaction (AlphaLISA) |     |     |     |
| Spike-ACE2 protein–protein interaction (TruHit Counterscreen) |     |     |     |
| ACE2 enzymatic activity |     |     |     |
| TMPRSS2 enzymatic activity |     |     |     |
| 3CL enzymatic activity |     |     |     |
| SARS-CoV Pseudotyped particle entry |     |     |     |
| SARS-CoV Pseudotyped particle entry (VeroE6 tox countsscreen) |     |     |     |
| MERS Pseudotyped particle entry |     |     |     |
| MERS Pseudotyped particle entry (Huh7 tox countsscreen) |     |     |     |
| SARS-CoV-2 cytopathic effect (CPE) |     |     |     |
| SARS-CoV-2 cytopathic effect (host tox countsscreen) |     |     |     |
| HEK293 cell line toxicity |     |     |     |
| Human fibroblast toxicity |     |     |     |
Figure S5. continued

**Amitriptyline**

- At 19.95 μM: Inactive
- At 35.48 μM: Inactive
- At 12.59 μM: Inactive
- At 29.80 μM: Inactive

**Fenoprofen**

- At 37.69 μM: Inactive
- At 3.08 μM: Inactive

**Tipranavir**

- At 12.67 μM: Inactive
- At 9.85 μM: Inactive
- At 5.93 μM: Inactive
- At 2.82 μM: Inactive
- At 7.94 μM: Inactive
- At 25.93 μM: Inactive
**Figure S5. continued**

**Probucol**

- 25.29 μM: Spike-ACE2 protein–protein interaction (AlphaLISA)
- 29.81 μM: SARS-CoV Pseudotyped particle entry
- 5.01 μM: Inactive

**Brimonidine**

- 37.69 μM: Spike-ACE2 protein–protein interaction (AlphaLISA)
- 39.81 μM: SARS-CoV Pseudotyped particle entry
- Inactive

**Repaglinide**

- 13.37 μM: Spike-ACE2 protein–protein interaction (AlphaLISA)
- 44.67 μM: SARS-CoV Pseudotyped particle entry
- Inactive
Figure S6. Carvedilol can indirectly target the SARS-COV-2 host factors through protein-protein interactions with its targets.
Supplementary Files

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

- SupplementaryTablesS1S8.xlsx