A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug-Repurposing

Supplementary Information

Supplementary Discussion

In this study we capture and identify 332 high confidence host-pathogen protein-protein interactions (PPIs) between SARS-CoV-2 and human proteins. Below in the Supplemental Discussion we provide what we hope is a thorough, well-sourced, review of the principal interactions for each bait. We have focused on prey proteins involved in comorbidities (e.g. cancer; lung and heart function and disease) and those that are connected to other viral infections or viral protein interactions. When applicable, we have indicated those prey proteins that exhibit host and restriction factor activities. Along these lines, we have characterized evolutionary properties of all prey proteins and identified 40 that evolved under recurrent positive selection in simian primates, thus highlighting a subset of prey proteins that are potentially hijacked or targetted by different pathogens (**Supplementary Table 3**).

All SARS-CoV-2 protein and gene functions described in the subnetwork appendices provided here, including the text below and the text found in the individual bait subnetwork figures, are based on the functions of homologous genes from other coronavirus species. These are mainly from SARS-CoV and MERS-CoV, but when available and relevant other related viruses were used to provide insight into function. The SARS-CoV-2 proteins in this study were designed and researched based on the gene alignments provided by Chan et. al. 2020[1] and Wu et. al. 2020[2]. Though we are reasonably sure the genes here are well annotated, we want to note that not every protein has been verified to be expressed or functional during SARS-CoV-2 infections, either *in vitro* or *in vivo*. In an effort to be as comprehensive and transparent as possible, we are reporting the subnetworks of these functionally unverified proteins along with the other SARS-CoV-2 proteins. In such cases, we have made notes within the text and on the corresponding subnetwork figures, and would advise that more caution be taken when examining these proteins and their molecular interactions. Due to practical limits in our sample preparation and data collection process, we were unable to generate data for proteins corresponding to Nsp3, Orf7b, and Nsp16. Therefore these three genes have been left out of the following literature review of the SARS-CoV-2 proteins and PPIs identified in this study.

To facilitate visualization and understanding we have provided zoomed views of individual bait subnetworks. An interactive version of these networks, including relevant drug and functional information, can be found at the following website: http://kroganlab.ucsf.edu/network-maps. Along with our partners at Zoic Labs, we are providing this fully interactive network and the corresponding data tables as a free service to the public. Given the urgent and unprecedented nature of the current crisis and global pandemic, we hope that this will be of significant use to both the scientific and global communities.

STRUCTURAL PROTEINS (S, E, M, N)

S

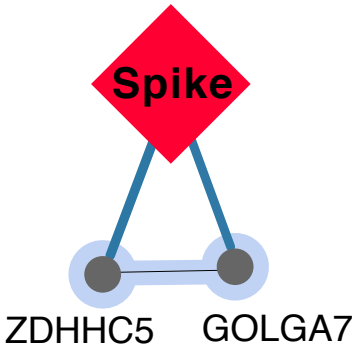
Function*: Spike (S) is a surface glycoprotein that is responsible for binding and fusion with the host membrane.

- Classified as a class I fusion protein.

- Has 2 subunits that need to be processed by cellular protease TMPRSS2. S1 mediates receptor (ACE2) binding whereas S2 mediates fusion.

Similarity to SARS-CoV

Identity: 76.3%	**Similarity:** 87.0%



Protein Palmitoylation: GOLGA7-ZDHHC5 is a protein acyl-transferase (PAT) complex that may play a role in Spike palmitoylation.

E

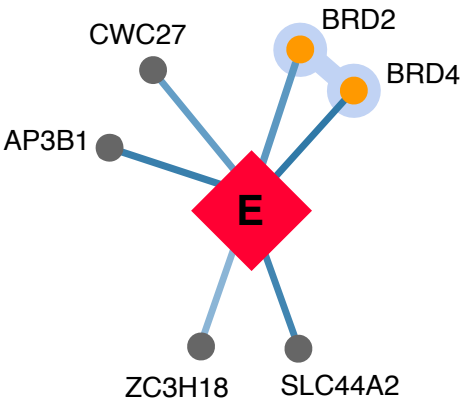
Function*: Envelope (E) protein plays a central role in virus morphogenesis and assembly.

- Acts as a viroporin, assembling in host membranes and forming pentameric protein-lipid pores that allow ion transport.

- Binds to protein M. Co-expression of M and E is sufficient for VLP formation and release. Lack of E reduces viral titers about 20-fold.

Similarity to SARS-CoV

Identity: 94.7%	**Similarity:** 96.1%



BRD2/BRD4: Bromodomain extra terminal (BET) proteins are implicated as epigenetic factors that regulate genes crucial for cell cycle progression, inflammation and immune response.

M

Function*: Membrane (M) protein is the major driver for virus assembly and budding.

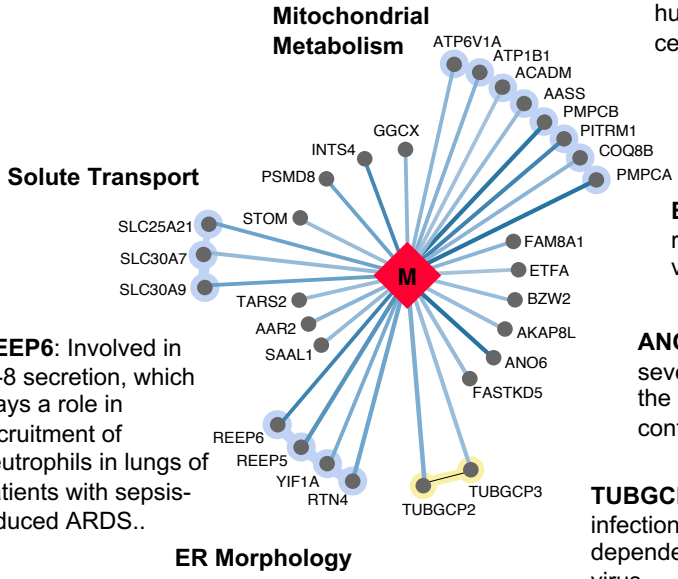
- Exists as a dimer in two major conformations, long and compact, which together determine the membrane curvature and spike density.

- M-M, M-S and M-N protein interactions contribute to virus assembly.

Similarity to SARS-CoV

Identity: 90.5%	**Similarity:** 96.4%

ATPBV1A: Affects Dengue, West Nile and Influenza A H1N1 virus infections.



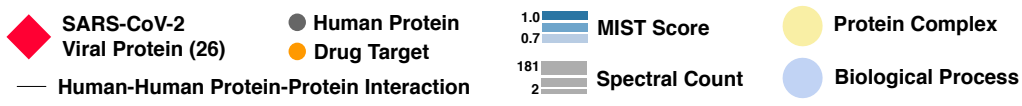
REEP6: Involved in IL-8 secretion, which plays a role in recruitment of neutrophils in lungs of patients with sepsis-induced ARDS..

PITRM1: Differentially expressed in RSV-infected human small airway epithelial cells.

BZW2:: A known host restriction factor for Dengue virus infection.

ANO6: Deficiency leads to severe T cell exhaustion and the inability of the host to control viral burden

TUBGCP2:: Induced by EBV infection and is a known host dependency factor for Influenza A virus.



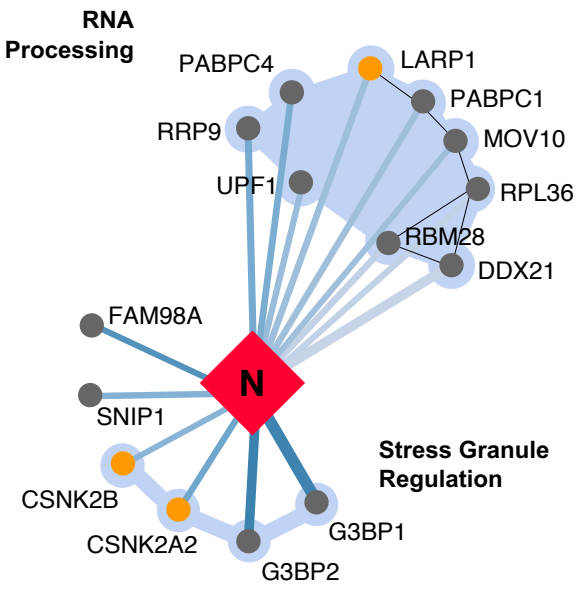
STRUCTURAL PROTEINS (S, E, M, N)

N

Function*: Nucleocapsid (N) protein binds to the RNA genome.

G3BP1 and G3BP2: G3BP1 and G3BP2 are core structural components of stress granules (SGs) which are broadly refractory to replication of viruses. Viruses have evolved diverse mechanisms, such as direct cleavage of G3BPs (poliovirus, FMDV) or sequestration away from other granule components (SmFV, VV, TMCV, SAFV-2, Mengovirus, DENV, JEV, Ebola virus) to prevent granule formation. Coronaviridae like MERS and IBV also possess specific mechanisms to abrogate SG assembly. Certain viruses utilize G3BPs to promote their replication cycle, a function that is almost exclusively extra-granular.

CK2 (CSNK2A2 and CSNK2B): CSNK2A2 and CSNK2B are subunits of the tetrameric Casein Kinase 2. CK2 phosphorylates G3BPs and disassembles and/or inhibits the formation of stress granules. The activity of CK2 is thus presumptively proviral. CK2 is inhibited at sub-nanomolar concentrations by an orally bioavailable molecule, Silmitasertib.



LARP1: LARP1 is a major effector of the mTOR pathway, suppressing translation of terminal oligopyrimidine mRNAs. LARP1 binds the N protein in a variety of viruses (e.g. IBV, IAV). LARP1 knockdown decreases DENV viral titers, while inhibition of mTOR (e.g. with rapamycin) impairs MERS-CoV replication and exerts immunosuppressant functions.

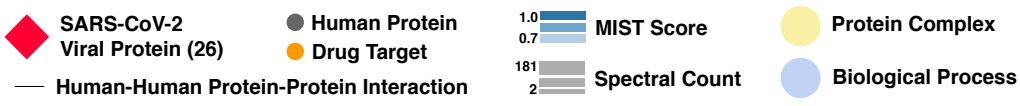
MOV10: MOV10 is a 5' to 3' RNA helicase that interacts with UPF1 and binds 3'UTRs. Its antiviral functions are independent of the helicase activity, and often through IFN stimulation. MOV10 exhibits P-body dependent antiviral activity by binding the N protein and preventing its nuclear localization.

PABPC1/4: Poly-A binding proteins are involved in many steps of mRNA processing. Viral factors cause PABPC1/4 to shuttle into the nucleus, causing mRNA hyperadenylation and nuclear retention. In a complex with LARP1 and RyDEN, PABPC1/4 can promote virulence.

UPF1: UPF1 is a RNA helicase that functions in the nonsense-mediated mRNA decay (NMD) pathway. Murine Hepatitis Virus (MHV) mRNAs are subjected to NMD, while the MHV N protein has been shown to have NMD inhibitory functions.

Similarity to SARS-CoV

Identity: 90.5%	**Similarity:** 94.3%



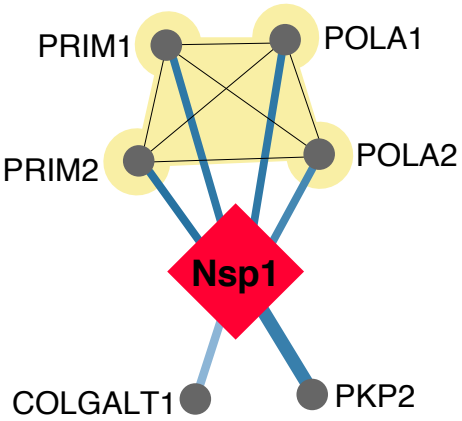
NON-STRUCTURAL PROTEINS

Nsp1

Function*: Nsp1 antagonizes interferon induction to suppress host antiviral response.

- Overexpression of Nsp1 in A549 cells increases production of pro-inflammatory chemokines CCL5, CXCL10, and CCL3.

- Can also inhibit host gene expression by binding to ribosomes and modifying host mRNAs.



DNA Polymerase α Complex: Regulates the activation of type I interferons through cytosolic RNA-DNA synthesis and primes DNA replication in the nucleus.

PKP2 (Plakophilin): Binds cadherins and intermediate fibers, crucial for desmosome formation.

COLGALT1:: Required for galactosylation of collagen IV and VI to form the collagen triple helix.

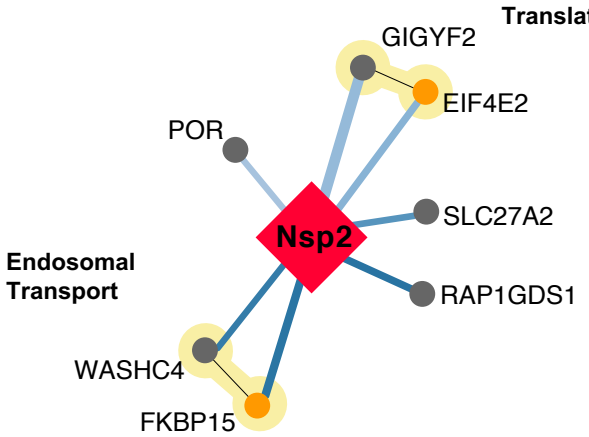
Similarity to SARS-CoV

Identity: 84.4%	**Similarity:** 91.1%

Nsp2

Function*: Nsp2 is translated as part of a single protein along with Nsp3 and may serve as an adaptor for Nsp3.

-While not essential for viral replication, deletion of Nsp2 diminishes viral growth and RNA synthesis.



SLC27A2: Previously described as a Dengue virus restriction factor.

Similarity to SARS-CoV

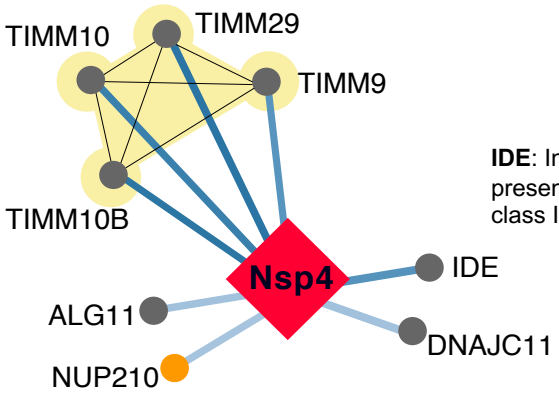
Identity: 68.3%	**Similarity:** 82.9%

Nsp4

Function*: Nsp4 forms a complex with Nsp3 and Nsp6.

- Together, these proteins are predicted to nucleate and anchor viral replication complexes on double-membrane vesicles in the cytoplasm.

TIM Complex: Involved in the import and insertion of hydrophobic membrane proteins into the mitochondrial inner membrane. Regulates import of transmembrane proteins into the inner mitochondrial membrane.



IDE: Involved in antigen presentation via MHC class I.

ALG11: Mannosyltransferase involved in the synthesis of core oligosaccharide.

Similarity to SARS-CoV

Identity: 80.0%	**Similarity:** 90.8%

SARS-CoV-2 Viral Protein (26) | **Human Protein** | **Drug Target** | **Human-Human Protein-Protein Interaction** | **MIST Score** (1.0 / 0.7) | **Spectral Count** (181 / 2) | **Protein Complex** | **Biological Process**

NON-STRUCTURAL PROTEINS

Nsp5

Function*: Nsp5 is the 3C-like protease.

-Cleaves the viral polyprotein.

HDAC2: Deacetylates lysines at the N-terminus of histones. Reduced levels of HDAC2 leads to increased transcription of inflammatory genes. Low HDAC2 expression contributes to disease severity in COPD patients.

Similarity to SARS-CoV

Identity: 96.1%	**Similarity:** 98.7%

Nsp5_C145A

Function*: Nsp5_C145A is a catalytically dead mutant of the Nsp5 3C-like protease.

-The catalytic residues of SARS-CoV Nsp5 align to H41 and C145 of SARS-CoV2 Nsp5.

GPX1: Glutathione peroxidase 1 is an antioxidant enzyme helps regulate the redox state of the cell, mitochondrial function, and apoptosis. It has been linked to a number of diseases that increase the risk of severe SARS-CoV-2 comorbidity including cancer, diabetes, and cardiovascular disease.

Similarity to SARS-CoV

Identity: 96.1%	**Similarity:** 98.7%

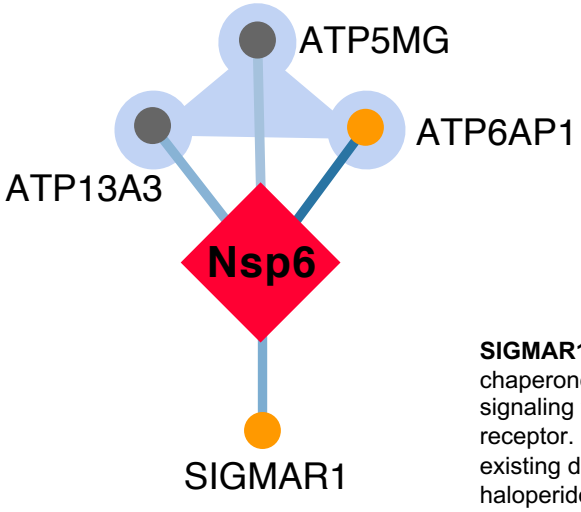
Nsp6

Function*: Nsp6 limits autophagasome expansion.

- Nsp6 may favor SARS-CoV infection by compromising the ability of autophagosomes to deliver lysosomes for degradation

-Complexes with Nsp3 and Nsp4 to form double-membrane vesicles that anchor viral replication complexes.

Ion Transport: Components of the Mitochondrial Complex V co-purify with Nsp6. This complex regenerates ATP from ADP.

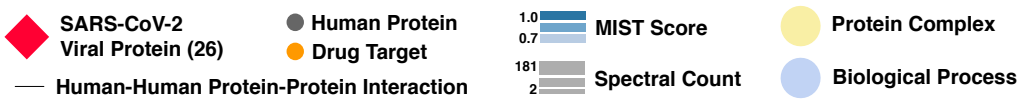


ATP6AP1: Subunit of the vacuolar ATP synthase protein pump. Dysregulation results in impaired vesicle acidification and intracytoplasmic granules, resulting in a range of pathologies including an immunodeficiency syndrome and granular cell tumors. Identified as a potential host factor for IAV, WNV, and DENV.

SIGMAR1: Sigma Receptor 1 is an ER chaperone protein that modulates calcium signaling through its interaction with the IP3 receptor. It is targeted by a number of existing drugs including Chloroquine and haloperidol.

Similarity to SARS-CoV

Identity: 87.2%	**Similarity:** 94.8%



REPLICATION COMPLEX (Nsp7, Nsp8, Nsp12)

Nsp7

Function*: The Nsp7-Nsp8 complex is part of a unique multimeric RNA-dependent RNA replicase capable of both de novo initiation and primer extension.

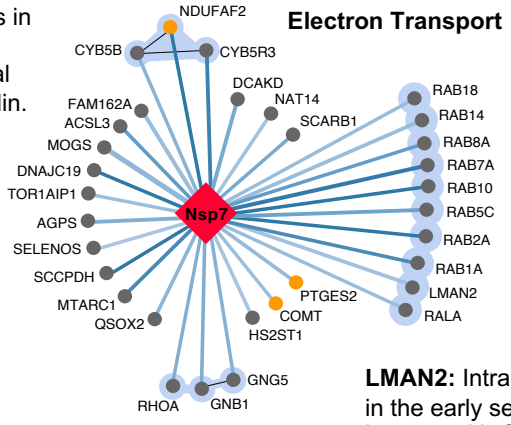
- Forms the primase in complex with Nsp8.

Similarity to SARS-CoV

Identity: 98.8%	**Similarity:** 100.0%

ACSL3: Induction results in increased acyl-CoA synthesis that is essential for providing prostaglandin.

MOGS: Cleaves the distal alpha 1,2-linked glucose residue from the N-linked oligosaccharide precursor in a highly specific manner.



LMAN2: Intracellular lectin involved in the early secretory pathway. May interact with O-linked glycans and N-acetyl-D-galactosamine.

Nsp8

Function*: Nsp5_C145A is a catalytically dead mutant of the Nsp5 3C-like protease.

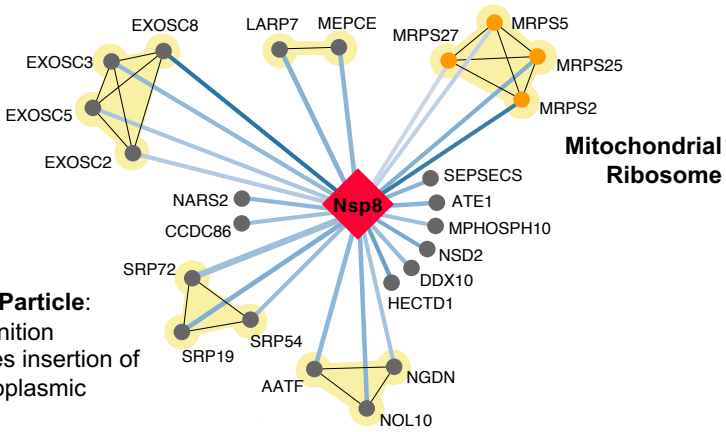
-The catalytic residues of SARS-CoV Nsp5 align to H41 and C145 of SARS-CoV2 Nsp5.

Similarity to SARS-CoV

Identity: 97.5%	**Similarity:** 99.0%

Exosome: Degrades ssRNA in a 3' to 5' direction and is involved in homeostatic degradation of host RNA as well as antiviral immunity.

Signal Recognition Particle: Binds to signal recognition peptides and mediates insertion of proteins into the endoplasmic reticulum.



Nsp12

Function*: Nsp12 is the RNA-dependent RNA polymerase (RdRp).

- Nsp12 contains a large two-domain N-terminus of little known function and a canonical RdRp domain in the C-terminus.

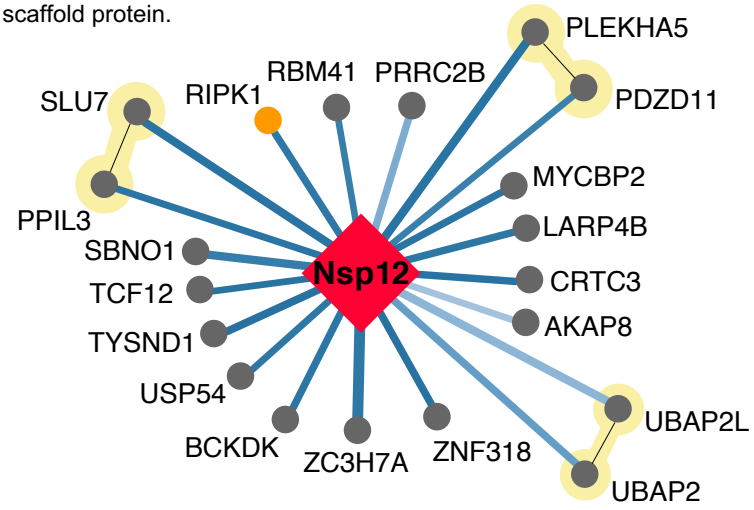
- Nsp7-Nsp8 heterodimer binds the RdRp domain of Nsp12.

Similarity to SARS-CoV

Identity: 96.4%	**Similarity:** 98.3%

RIPK1: Triggers cell death by apoptosis or necrosis as an active regulatory kinase. Regulates inflammatory signaling and inhibits cell death as a scaffold protein.

Spliceosome: Removes introns from pre-mRNA. SLU7 is essential for the second catalytic step of pre-mRNA splicing.



SARS-CoV-2 Viral Protein (26)
Human Protein
Drug Target
Human-Human Protein-Protein Interaction
1.0
0.7
MIST Score
181
2
Spectral Count
Protein Complex
Biological Process

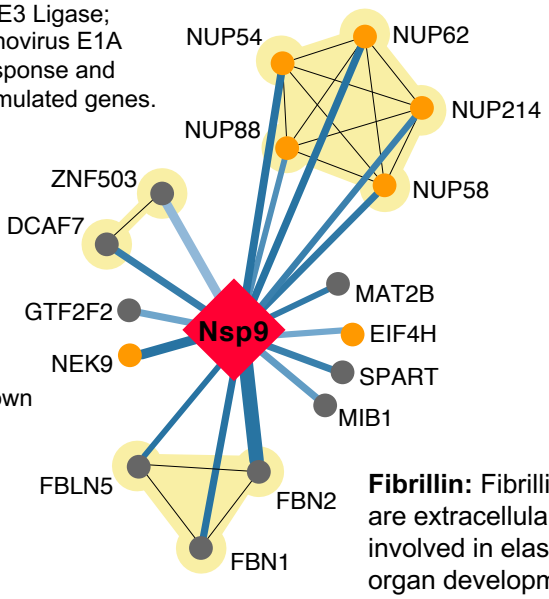
NON-STRUCTURAL PROTEINS

Nsp9

Function*: Nsp9 is an essential single-stranded RNA binding protein.

- Shown to interact with the replication complex (Nsp7, Nsp8, and Nsp12).
- Binds to both DNA and RNA but preferentially binds single-stranded RNA.

DCAF7: Potential substrate adaptor for CUL4 E3 Ligase; interacts with adenovirus E1A innate immune response and depresses IFN stimulated genes.



Nuclear Pore Complex: Several components of the NPC are targeted by viruses in an effort to block or promote nuclear transport beneficial to virus replication.

NEK9: Serine/threonine kinase shown as an adenovirus dependency factor.

Fibrillin: Fibrillin-1 and -2 (FBN1/2) are extracellular matrix glycoproteins involved in elastin fiber and respiratory organ development.

Similarity to SARS-CoV

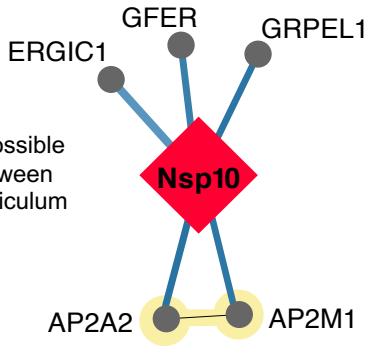
Identity: 97.3%	**Similarity:** 98.2%

Nsp10

Function*: Nsp10 is a zinc-finger protein essential for replication.

- Has been implicated in negative-strand RNA synthesis.
- Acts as a stimulatory factor for Nsp16 to execute its methyltransferase activity.

GFER: FAD-dependent sulfhydryl oxidase that regenerates the redox-active disulfide bonds in CHCHD4/MIA40.



ERGIC1: Plays a possible role in transport between the endoplasmic reticulum and Golgi.

GRPEL1: Essential component of the PAM complex, required for the translocation of transit peptide-containing proteins from the inner membrane into the mitochondrial matrix in an ATP-dependent manner.

AP2 Clathrin: Nps10 may hijack the clathrin machinery and endocytose host proteins.

Similarity to SARS-CoV

Identity: 97.1%	**Similarity:** 99.3%

Nsp11

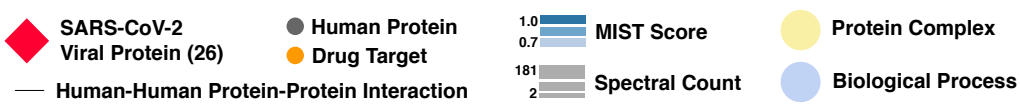
Function*: It is unclear if Nsp11 encodes a functional viral protein.

- Short peptide (13 amino acids) at the end of Orf1a.

TBCA: Involved in the early steps of the tubulin folding pathway

Similarity to SARS-CoV

Identity: 84.6%	**Similarity:** 92.3%

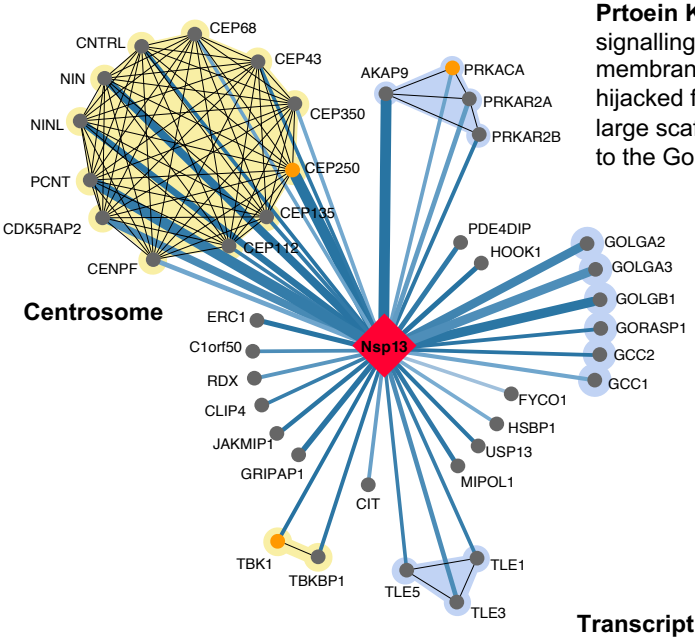


CAPPING ENZYMES (Nsp13, Nsp14)

Nsp13

Function*: Nsp13 is a helicase and triphosphatase that initiates the first step in viral mRNA capping.

- Nsp13, along with Nsp14 and Nsp16, installs the cap structure onto viral mRNA in the cytoplasm instead of the nucleus where host mRNA is capped.



Prtoein Kinase A Signaling: PKA signalling coordinates multiple steps of membrane transport and may be hijacked for viral benefit. AKAP9 is a large scaffolding protein that localizes to the Golgi and centrosomes.

Similarity to SARS-CoV

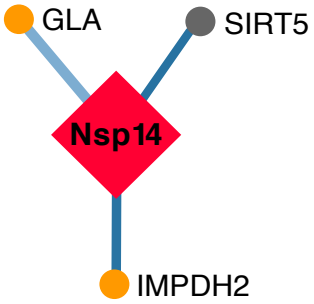
Identity: 99.8%	**Similarity:** 100.0%

Nsp14

Function*: Nsp14 is a bifunctional enzyme encoding both an exonuclease and a SAM dependent methyltransferase domain.

- The exonuclease domain corrects mutations that arise during genome replication.

- The SAM dependent methyltransferase domain facilitates capping of viral mRNA.



IPDH2: Catalyzes the conversion of isosine 5' phosphate (IMP) ultimately to guanine 5' monophosphate for de novo synthesis of guanine nucleotides. Nsp14's interaction with IMPDH2 may reflect an interplay with purine nucleotide metabolism.

Similarity to SARS-CoV

Identity: 95.1%	**Similarity:** 98.7%

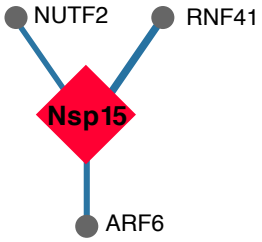
Nsp15

Function*: Nsp15 has uridine-specific endoribonuclease (endoU) activity and is essential for viral RNA synthesis.

- endoUs shown to: (i) have endonucleolytic activity; (ii) cleave 3' of pyrimidines, preferring uridine > cytidine; and (iii) release reaction products with 2'-3'-cyclic phosphate and 5'-OH ends.

- Shown to form homohexamers composed of a dimer of trimers.

NUTF2: Mediates the import of GDP-bound RAN from the cytoplasm into the nucleus, thus indirectly plays a more general role in cargo receptor-mediated nucleocytoplasmic transport.

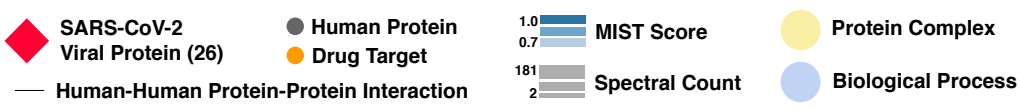


RNF41: E3 ubiquitin-protein ligase that promotes TRIF-dependent production of type I interferon and inhibits infection with vesicular stomatitis virus.

ARF6: GTP-binding protein involved in protein trafficking. Regulates endocytic recycling and cytoskeleton remodeling.

Similarity to SARS-CoV

Identity: 88.7%	**Similarity:** 95.7%



OPEN READING FRAMES

Orf3a

Function*: Orf3a is not essential for replication but contributes to pathogenesis. It is packaged into virions.

- Mediates trafficking of Spike (S protein) by providing an ER/Golgi retention signal.

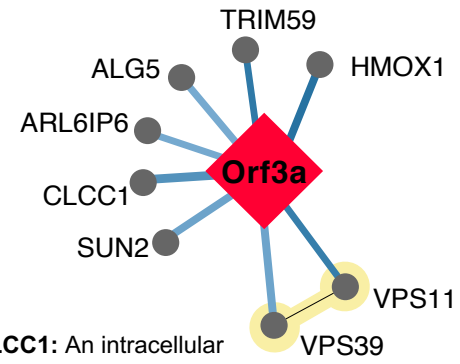
- Induces elevation of IL-1β secretion and activates NF-κB and the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of p105 and ASC.

- Expression of Orf3a induces apoptosis in viral infection and cell line models.

Similarity to SARS-CoV

Identity: 72.4%	**Similarity:** 85.1%

ALG5: Involved in N-linked protein glycosylation. Identified as a host factor for IAV replication.



CLCC1: An intracellular chloride channel implicated in ER stress. Interacts physically with RSV and druggable protein SLC5A13.

HMOX1: Key enzyme in heme catabolism. Has shown a cytoprotective and anti-inflammatory effect both in pulmonary pathologies and viral pathogenesis.

HOPS Complex: VPS11 and VPS39 are members of the HOPS and CORVET complexes, respectively, which coordinate fusion of the lysosome with the endosome and autophagosome.

Orf3b

Function*: Orf3b is shown to be an interferon antagonist and is involved in pathogenesis.

STOML2: Stimulates cardiolipin biosynthesis and binds and stabilizes prohibitin. Both STOML2 and prohibitin have been shown to be host dependency factors for Enterovirus (EV)71.

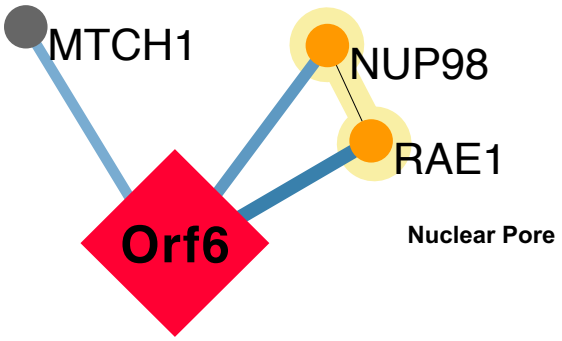
Similarity to SARS-CoV

Identity: 7.1%	**Similarity:** 9.5%

Orf6

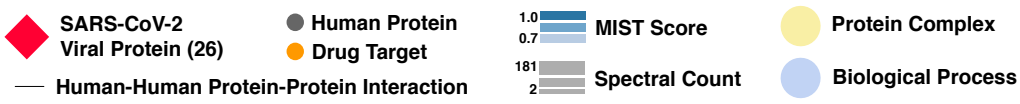
Function*: Orf6 is a type 1 interferon antagonist. Expression of Orf6 suppresses the induction of interferon and interferon signaling pathways.

- C-terminal region of SARS-CoV Orf6 interacts with the nuclear import protein, karyopherin alpha-2, sequestering it in the cytoplasm. This prevents import of STAT1, activator of interferon response genes, into the nucleus.



Similarity to SARS-CoV

Identity: 66.7%	**Similarity:** 85.7%



OPEN READING FRAMES

Orf7a

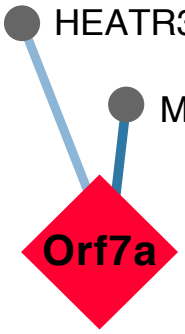
Function*: Orf7a may play a role in pathogenesis via its role in virus-induced apoptosis.

- ΔOrf7a SARS-CoV is still able to release virions at similar levels as wild-type virus.

Similarity to SARS-CoV

Identity: 85.2%	**Similarity:** 90.2%

HEATR3: Involved in ribosomal protein transport. Implicated in mediating NF-kB signaling.



MDN1: Nuclear chaperone that is critical for the export of pre-60s ribosome subunits.

Orf8

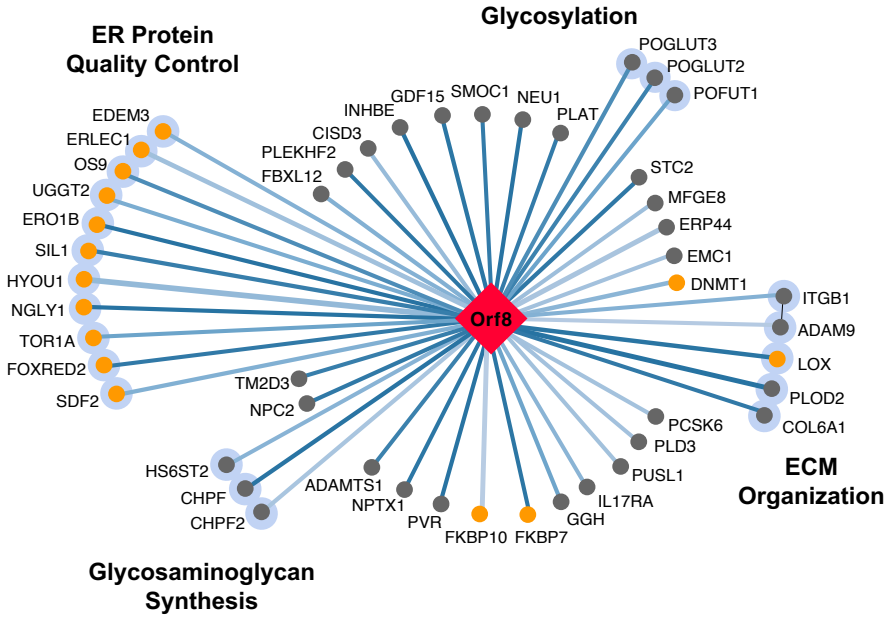
Function*: Orf8 is an accessory protein not essential for virus replication in vitro and in vivo.

- Previously shown to be a recombination hotspot, one of the most rapidly evolving regions among SARS-CoV genomes.

- SARS-CoV Orf8b was shown to induce ER stress and activate NLRP3 inflammasomes.

Similarity to SARS-CoV

Identity: 28.5%	**Similarity:** 45.3%



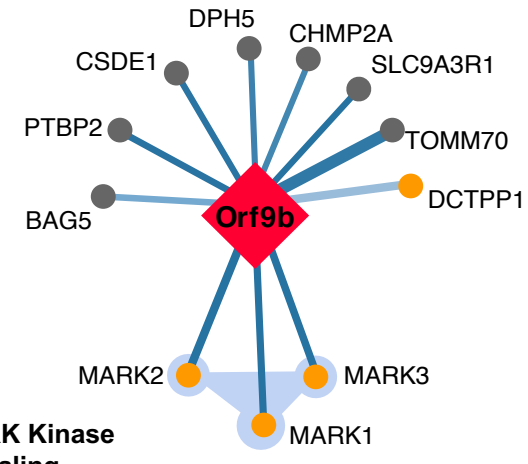
Orf9b

Function*: Orf9b is an accessory protein synthesized from an alternative complete reading frame within the viral N gene.

- Targets the mitochondrial-associated adaptor molecule MAVS signalosome by utilizing PCBP2 and E3 ligase AIP4, resulting in the degradation of MAVS and therefore limiting host cell interferon responses.

Similarity to SARS-CoV

Identity: 72.4%	**Similarity:** 84.7%



TOMM70: Receptor that accelerates the import of all mitochondrial precursor proteins. TOMM70 interacts with MAVS protein upon virus infection.

SARS-CoV-2 Viral Protein (26)
Human Protein
Drug Target
Human-Human Protein-Protein Interaction
1.0
0.7
MIST Score
181
2
Spectral Count
Protein Complex
Biological Process

OPEN READING FRAMES

Orf9c

Function*: Orf9c is a short polypeptide (70 amino acids) dispensable for viral replication. There is no data yet providing evidence that the protein is expressed during SARS-CoV-2 infection.

ACC1: ABCC1 (also MRP1) is a multifunctional ATP-binding cassette protein involved in controlling the efflux of drugs in cells. MRP1 is a well-known viral host factor and physical interactor of both IAV and WNV proteins. Further, it has been implicated in disease progression of pneumonia and COPD, as well as drug resistance in the lung.

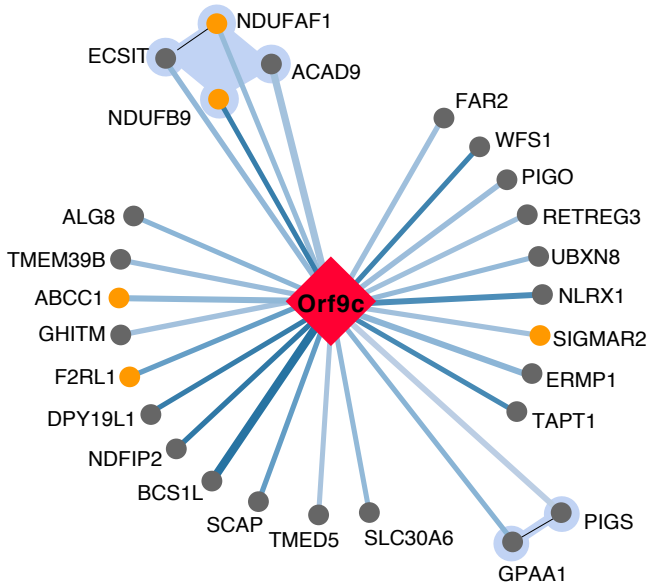
F2LR1: The protein product of F2LR1, PAR2, is a protease-activated receptor that has a cytoprotective and inflammatory role in the pathogenesis of viral infection and progression of pulmonary disease.

BCS1L: BCS1L is a mitochondrial chaperone located in the inner mitochondrial membrane. Loss of BCS1L is associated with severe clinical disorders such as GRACILE and Bjornstad Syndrome.

Similarity to SARS-CoV

Identity: 74.0%	**Similarity:** 78.1%

Electron Transport: Orf9c interacts with four proteins in the mitochondrial respiratory electron transport chain complex I, which has demonstrated roles in TLR/IL-1 signaling and mediating inflammation.



GPI-Anchor Biosynthesis: GPAA1 is essential for GPI-anchoring of precursor proteins, while PIGS and PIGO are involved in GPI-synthesis.

Orf10

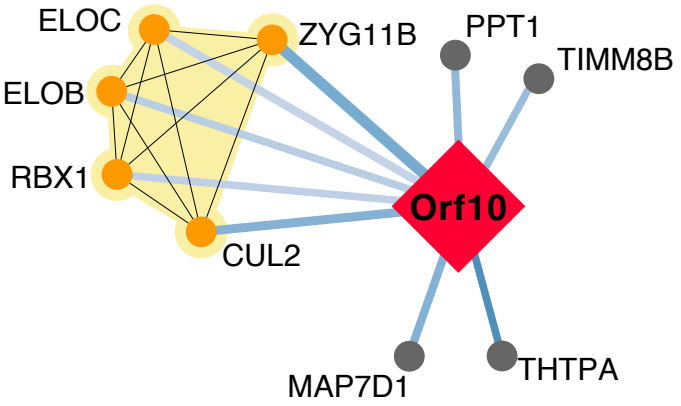
Function*: Orf10 codes for a peptide only 38 amino acids long. There is no data yet providing evidence that the protein is expressed during SARS-CoV-2 infection.

- Does not have a homolog in SARS-CoV.

Similarity to SARS-CoV

Identity: NA	**Similarity:** NA

Cul2 Complex: Commonly hijacked by viral proteins to ubiquitinate and degrade viral restriction factors. The CUL2ZYG11B E3 ligase targets substrates with exposed N-terminal glycines for degradation



SARS-CoV-2 Viral Protein (26)
Human Protein
Drug Target
Human-Human Protein-Protein Interaction
1.0
0.7
MIST Score
181
2
Spectral Count
Protein Complex
Biological Process

SARS-CoV Structural Proteins

SARS-CoV-2 Spike/S Protein

Spike (S protein) is the viral surface protein that mediates viral entry through its interaction with cellular host factor angiotensin-converting enzyme 2 (ACE2)[3]. The full-length S protein is 141 kDa (1273 amino acids (aa)) and encodes a N-terminal signal peptide, a transmembrane region (aa 1214-1237), and a patch of cysteine residues (aa 1235-1254) which are predicted to be palmitoylated (protein S-acylation of cysteine residues)[4,5]. In previous studies, mutation of these cytosolic cysteine residues inhibited fusogenicity of S, and suggest that targeting the s-acylation modifications via acyl-transferase inhibitors could potentially be a therapeutic strategy for inhibiting coronavirus infection[6].

In our interactome, we demonstrate high-confidence binding of SARS-CoV-2 Spike to the GOLGA7-ZDHHC5 complex, a protein acyl-transferase (PAT) complex. GOLGA7 characteristically localizes to the Golgi apparatus[7] but the GOLGA7-ZDHHC5 complex has been found at the plasma membrane[8]. While this interaction is exciting and could potentially point to a targetable enzymatic activity responsible for Spike palmitoylation, further experiments need to be done to fully clarify the functional role of this complex and the consequence of its inhibition. ZDHHC5 is highly expressed in neuronal tissue and is reported to have a role in hippocampal function[9]. In addition, ZDHHC5 is shown to palmitoylate G protein-coupled receptors[10] and activate nucleotide oligomerization domain (NOD)-like receptors 1 and 2 (NOD1/2)[11] which recognize pathogenic peptidoglycans and activate immune signaling[11]. GOLGA7 is also a general adaptor for ZDHHC proteins and, in complex with these acyl-transferase proteins, can regulate additional processes including the palmitoylation of Ras[12]. It should also be noted that the ZDHHC5-GOLGA7 complex is required for the non-apoptotic cell death phenotype attributed to the synthetic oxime CIL56, indicating a role for this complex in the cell death pathway[8]. Thus, we would caution against long-term inhibition of this enzymatic complex as a general treatment strategy.

Though identified just below our cutoffs, one of the lower confidence hits for Spike was ATP1A1, a protein at the plasma membrane that promotes viral entry and replication of related coronaviruses (M-CoV, FCoV, MERS-CoV), and a number of other viruses including Ebola, Lassa, live-attenuated Junín virus, and respiratory syncytial virus (RSV)[13–16]. Interestingly, this protein is from the same ATPase family as ATP1B1, a high-confidence interactor of M protein.

SARS-CoV-2 E Protein

The SARS-CoV Envelope (E) protein, a small integral membrane protein, is important for virus production, assembly, intracellular trafficking, morphogenesis and virulence[17,18]. The transmembrane domain of E has amphipathic properties and can oligomerize to form cation-permeant ion channels[19]. The C-terminal cytoplasmic domain contains a targeting signal for protein localization to the Golgi complex[20,21] and a PDZ binding motif important for virulence[22]. Interestingly, E protein is expressed at very high levels inside the infected cells, but only a small portion is incorporated in virions and the rest is largely distributed in intracellular membranes between ER and Golgi compartments indicating an important role of E protein in manipulating the cellular environment[23]. Increased cellular stress, unfolded protein response, apoptosis, and heightened host immune response were all observed in infected cells lacking E protein[18]. In addition, E has also been implicated in inflammasome activation and consequential inflammation in the lung parenchyma[18].

In our study, we identify six high-confidence host protein interactions with E protein: AP31B, BRD2, BRD4, CWC27, SLC44A2, and ZC3H18. BRD2 and BRD4 are bromodomain extra terminal (BET) family

proteins that bind acetylated chromatin and activate transcription[24]. BRD4 is a co-activator of NF-kB and facilitates the transcription of NF-kB-dependent inflammatory response[25]. Sequestration of BRD4 by E protein may represent a means for SARS-CoV-2 to protect against the host immune response. Previously it's been shown that inhibition of BRD4 activity in primary lung epithelial cells results in diminished innate immune response after challenge with poly(I:C), a viral pattern that simulates acute RNA virus infections[26]. In addition, the interaction of BRD4 with Bovine Papillomavirus E2 protein tethers viral DNA to host mitotic chromosomes ensuring viral persistence in infected cells[27]. BRD2 is a transcriptional regulator that binds hyperacetylated histones and is implicated in a variety of cellular processes[28,29]. Though functionally similar, BRD2 and BRD4 are shown to regulate different transcriptional programs[30]. Interestingly, the α3 helix at the N-terminus of histone 2A shares local sequence similarity (~15 non-contiguous aa residues) with an α-helix of E protein (see main text **Fig. 4d**). This aligned sequence spans many acetylated lysine residues shown to bind BRD2[31]. This information suggests a potential role for E protein to act as a sort of molecular mimic, disrupting the H2A-BRD2 interaction in order to disrupt BRD2 regulated transcription programs in a manner beneficial to the virus. Given that bromodomain proteins and their ability to regulate transcription are implicated in the life cycle of several viruses[27,28,32,33], the observed interactions of E protein with BRD2 and BRD4 present exciting avenues to pursue. However, future research is still needed to fully establish the structural basis of these interactions and the resulting functional implication for SARS-CoV-2 infection.

E protein also interacts with host factors involved in protein and mRNA trafficking, important for potentially carrying out many of E protein's functions. AP3B1 encodes the beta-1 subunit of adaptor protein complex 3 (AP-3), that is involved in signal-mediated protein sorting from Golgi membranes to endosomal-lysosomal organelles[34,35]. Mutations in AP3B1 affect protease activity in endosomes and produce pathology-associated defects with aberrant transmembrane lysosomal protein trafficking[36]. AP-3 depletion resulted in decreased localization of the human immunodeficiency virus (HIV)-1 major structural protein Gag from plasma membrane and late endosome, suggesting that intact AP-3 is required for HIV-1 particle production and release[35]. Also, we have previously shown that AP-3 is targeted by the NS5 protein of West Nile virus[37]. Thus it is possible that SARS-CoV-2 may also utilize AP-3 for particle assembly, transport, and release.

Other notable high-confidence host protein interactions with E protein include CWC27 and SLC44A2. Both SLC44A2 and CWC27 are proteins implicated in other diseases, but have yet to be characterized in relation to viral infections.

SARS-CoV-2 M Protein

Membrane (M) protein, the most abundant protein in coronavirus particles, is a type III transmembrane glycoprotein located in the virus envelope. M protein manipulates cellular membranes to bring viral and host factors together and therefore is the main driver for virus assembly and budding processes. M-M, M-S and M-N protein interactions all contribute to this assembly process[38]. In the virion, M exists as a dimer in two major conformations (long and compact), which together determine the membrane curvature and spike density[39,40]. SARS-CoV M protein is known to affect various host processes in a manner beneficial to viral replication and infectivity[41]. M suppresses key inflammatory molecules (e.g. NF-kB and Cox-2)[42] and counteracts host viral defenses by inhibiting type I interferon production[43]. SARS-CoV M protein modulates apoptosis by interfering with PDK1–PKB/Akt signaling. This interaction between M protein and PDK1 could be targeted as a plausible therapeutic approach to modulate the pro-apoptotic properties attributed to coronavirus infection[44–46].

In this study, we identified 30 high-confidence physical interactors of SARS-CoV-2 M protein. Transmembrane domain-mediated oligomerization of M protein at ER-Golgi intermediate compartment (ERGIC) membranes drives the assembly of coronavirus which buds into the lumen of the ERGIC[47]. Therefore,

it was not surprising to find many SARS-CoV-2 M protein interactors were transmembrane proteins including mitochondrial proteases (PITRM1, PMPCA, PMPCB), ATPases (ATP6V1A, ATP1B1) and kinases (AKAP8L, COQ8B, FASTKD5). In addition, we identify known host dependency (ATP6V1A, ATP1B1, TUBGCP2, RTN4) and restriction (BZW2) factors of other viruses. ATP6V1A is a dependency factor for Dengue virus[48], West Nile virus[48] and Influenza A H1N1 virus[49], ATP1B1 is involved in viral autophagy[50] and has a role in Sindbis virus (SINV) infection[51], and TUBGCP2 is a host dependency factor for Influenza A virus[52,53]. RTN4 is a reticulon protein involved in the formation and stabilization of endoplasmic reticulum (ER) tubules[54,55]. Reticulon proteins play crucial roles in viral RNA replication, compartment formation, and function[56] and are known to protect ER membrane integrity during polyomavirus SV40 infection[57]. RTN4 is also involved in the formation of the *Legionella pneumophila* containing vacuoles[58]. In a functional RNAi screen, BWZ2 was identified as a host restriction factor for dengue virus (DENV) infection[59]. BZW2 is a translation repressor known to regulate the PI3K/AKT/mTOR signaling pathway[60–62]. Recently, BZW2 was identified as an oncogene implicated in a number of cancers including lung adenocarcinoma, where overexpression was negatively correlated with overall disease free survival[61–65]. Studies suggest that BWZ2 overexpression could also contribute to drug resistance in cancer cells, including resistance to rapamycin[62].

The mechanism behind acute respiratory distress in severe SARS-CoV-2 infections is not understood but the host immune response is likely to determine the pathogenesis to some extent. We found that two related host factors REEP5 and REEP6 copurify with SARS-CoV-2 M protein. REEP5 and REEP6 can refine CXCR1-mediated cellular responses and lung cancer progression[66]. REEP6 is also involved in IL-8 secretion[67]. IL-8 is the major chemoattractant for neutrophils and is implicated to have a major role in the recruitment of neutrophils to the lungs in patients with sepsis-induced acute respiratory distress syndrome[68]. Another high-confidence M protein interactor ANO6 is also very exciting from the perspective of viral pathogenesis. ANO6 is required to curb excessive T cell responses in chronic viral infections. ANO6-deficient T cells are hyperactivated during the early phase of infection, exhibiting increased proliferation and cytokine production. This overactivation ultimately leads to severe T cell exhaustion and the inability of the host to control viral burden[69]. Interestingly, ANO6 is localized in late endosomes and SARS-CoV is also targeted to endosomes[70]. Mice deficient for a related protein ANO1 suffer from tracheomalacia and die shortly after birth because of respiratory failure; ANO6 is highly expressed in the respiratory system and its involvement in respiratory functions is likely[71] making it an interesting candidate for future mechanistic studies in the context of SARS-CoV-2 infection.

SARS-CoV-2 N Protein

SARS-CoV nucleocapsid (N) protein is an essential structural protein that binds to viral genomic RNA (gRNA) in virions. N protein dimerization and its association with viral gRNA are crucial for viral assembly[72,73]. It has been shown that the N protein binds to both intracellular gRNA and subgenomic RNA (sgRNA), suggesting functions in viral transcription and translation[74]. N protein also has implications in a variety of cellular responses, including stress granule formation and host translation shutoff[75], inhibition of nonsense mediated mRNA decay (NMD)[76] and cell cycle regulation[77].

The SARS-CoV-2 N interactome includes 15 high-confidence host protein interactions, many of which are host mRNA binding proteins, including stress granule (SG)-related factors (G3BP1/2, MOV10, CK2 subunits, and PABP proteins), mRNA decay factors (MOV10 and UPF1), mTOR translational repressors (LARP1), and protein kinases (CK2 subunits). Several N protein interactors including G3BP1, G3BP2, LARP1, MOV10, PABPC1 and PABPC4 have also been detected in a mouse hepatitis virus interactome[78] and in two previous nucleocapsid protein interactomes from infectious bronchitis virus[79] and Influenza A virus[80]. Numerous viruses have evolved diverse mechanisms to abrogate SG assembly. Poliovirus specifically cleaves G3BP to

facilitate translation of viral mRNAs[81]. Foot and Mouth Disease virus cleaves G3BP and inhibits SG formation[82]. Several other viruses including Semliki Forest virus, vaccinia virus, cardioviruses, dengue virus, japanese encephalitis virus, and Ebola virus sequester G3BP to inhibit SG formation and to promote virus replication[83,84,85,86,87,88,89]. Amongst *Coronaviridae*, MERS virus employs its dsRNA-binding 4a protein to inhibit SG formation and promote viral replication[90] whereas avian infectious bronchitis virus (IBV) infection prevents the formation of SGs via yet-unknown mechanisms[91].

N protein also interacts with subunits of the broad-spectrum Casein Kinase 2 (CK2) complex (CSNK2A2 and CSNK2B). The N protein of other *Coronaviridae* has been shown to be phosphorylated at sites predicted to be substrates for CK2[92], and there is a NetPhos predicted CK2 phosphorylation site in N. CK2 has previously been shown to inhibit granule formation or promote granule disassembly in a G3BP phosphorylation-dependent manner[93]. CK2 inhibition sequesters SARS-CoV N protein in the nucleus, away from the G3BP subunits of SGs[77]. It is possible that the SARS-CoV-2 N protein inhibits the formation of cellular SGs, potentially by mediating the phosphorylation of G3BP by CK2. siRNA-mediated knockdown of either CSKN2A2 or CSNK2B reduced viral replication of SARS-CoV, indicating that CK2 may have a proviral function[94]. These findings suggest that the CK2 inhibitor Silmitasertib (also known as CX-4945) could be effective in slowing SARS-CoV-2 replication. Silmitasertib is a sub-nanomolar inhibitor of CK2 currently in phase II trials for indications such as multiple myeloma or metastatic basal cell carcinoma[95]. Potential antiviral effects of Silmitasertib via inhibition of CK2 and subsequent increase in the formation of antiviral SGs merits further investigation.

UPF1 is an RNA helicase functioning in the nonsense-mediated mRNA decay (NMD) pathway. In common with other virus species in the order *Nidovirales*, SARS-CoV-2 produces a nested set of sgRNAs sharing the same 3'UTR but are different in the number of ORFs contained[96]. In principle, only the first ORF in the 5' end is translated. Thus, many of the subgenomic mRNAs have long 3' UTRs and are potentially targeted for NMD. A previous study showed that Murine Hepatitis Virus (MHV) mRNAs were subjected to NMD[76]. Transfection of plasmids containing MHV N protein had an NMD inhibitory function and prevented MHV mRNA from rapid decay. Based on the observation that SARS-CoV-2 shares a similar feature of nested sgRNAs, we suspect it is also subjected to NMD and that its N protein may have similar NMD inhibitory function. It is also possible that UPF1 is involved in the programmed ribosomal frameshifting during SARS-CoV-2 translation given its discovered function in suppressing nonsense mutations[97].

LARP1 is an RNA binding protein, which is known to regulate protein synthesis as well as modulate mTOR pathway[98]. LARP interacts with actively translating ribosomes via another N protein interactor, PABPC1, also a regulator of mTOR pathway[99]. Activation of the mTOR pathway is advantageous for a broad spectrum of virus species, as it counteracts the host cell response by inhibiting autophagy and apoptosis[100] Therefore inhibition of mTOR activity has been proven useful to counteract viral infection and replication. For instance, Sirolimus (rapamycin) has been used to reduce MERS-CoV infection[101,102] and alleviate H1N1 pneumonia and acute respiratory failure[103] Therefore, mTOR pathway represents a potential therapeutic target for SARS-CoV-2 as well.

PABPC1 and PABPC4 are poly-A binding proteins, involved in both nuclear and cytoplasmic mRNA processing[104]. They are known to shuttle to the nucleus via interaction with Nsp1 proteins from different viruses (including SARS-CoV), down-regulating gene expression via hyperadenylation and nuclear retention of mRNAs[105]. In a complex with LARP1 and RyDEN, PABPC1/4 have been shown to promote DENV replication[106]. PABPC4 has been described as a potential biomarker for primary lung adenocarcinoma[107].

Moloney Leukemia Virus 10 Protein (MOV10) is a host cytoplasmic 5' to 3' RNA helicase that interacts with UPF1 and binds 3'UTRs[108]. It exhibits antiviral functions independent of its helicase activity towards PRRSV, DENV and Influenza A viruses, through IFN stimulation[109,110]. Additionally, MOV10 exhibits P-body

dependent antiviral activity by binding to nucleocapsid proteins of other viruses and preventing their nuclear localization[111,112].

SARS-CoV Non-Structural Proteins

SARS-CoV-2 Nsp1

In SARS-CoV, Nsp1 is likely dispensable for CoV RNA synthesis[113], but may play specific roles in the interaction of the virus with the innate immune response via directly antagonizing IFN induction[114]. In addition, overexpression of Nsp1 in lung epithelial cells (A549) increases the production of the chemokines CCL5, CXCL10 and CCL3 30-200-fold compared with mock-transfected cells or cells expressing Nsp5, suggesting that Nsp1 may contribute to the inflammatory phenotype of SARS-CoV and SARS-CoV-2 pathology[115].

In our interactome, SARS-CoV-2 Nsp1 interacts with six host proteins. Four of these host proteins form the DNA polymerase alpha complex (POLA1, POLA2, PRIM1, and PRIM2). The DNA polymerase alpha complex was recently shown to modulate the type I interferons through cytosolic RNA:DNA synthesis[116], raising a possibility that SARS-CoV-2 Nsp1 may bind to the DNA polymerase alpha complex in the cytosol and modulate its activity to antagonize the innate immune response. Alternatively but not exclusively, Nsp1 may also interfere with the canonical DNA replication function of the complex, causing DNA replication stress and ATR activation[117]. Along this line, treatment with ATR inhibitors significantly reduced viral RNA replication for avian infectious bronchitis virus (IBV) coronavirus, but it remains elusive how ATR activation promotes viral replication[117]. One otherprotein interacting with Nsp1 is PKP2, an Influenza A virus PB1 protein interactor that restricts Influenza A virus replication[118]; and therefore may also act as a restriction factor for SARS-CoV-2.

SARS-CoV-2 Nsp2

Nsp2 is highly variable among *Coronaviridae*, and while not essential for viral replication, deletion of Nsp2 in SARS-CoV diminishes viral growth and RNA synthesis[119,120]. Nsp2 is translated as part of a single protein along with Nsp3 and may serve as an adaptor for Nsp3[120]. The SARS-CoV-2 Nsp2 protein has a stabilizing mutation in the endosome-associated-protein-like domain, and is thought to have implication in virus pathogenesis[121].

In this study, we identified seven high-confidence host protein interactions of Nsp2. Among these are two endosomal proteins, FKBP15 and WASHC4, that are known to regulate endosome transport[122–124]. It is known that SARS-CoV is translocated to endosomes after cell entry[3] and interaction with endosomal transport proteins may reflect a mechanism by which SARS-CoV-2 regulates this process. Nsp2 also interacts with translational repressors EIF4E2 and GIGYF2, and disruption of the EIF4E2-GIGYF2 complex leads to increased translation[125]. Nsp2 may bind to EIF4E2 and GIGYF2 to modulate translation of host and viral mRNAs. Nsp2 was also shown to interact with the acyl-CoA synthetase SLC27A2, a previously described Dengue virus restriction factor[126]. Nsp2 interacts with chaperone protein and guanine nucleotide exchange factor RAP1GDS1/SmgGDS, which is known to promote the malignant phenotype in non-small cell lung carcinoma (NSCLC) by regulating cell proliferation, migration, and NF-κB transcriptional activity[127]. Activation of NF-κB in pulmonary epithelial cells induces transcription of a variety of proteins that protect the cells from the inflammatory process, suggesting that RAP1GDS1/SmgGDS could be implicated in lung inflammation during SARS-CoV-2 infection[128].

SARS-CoV-2 Nsp4

Nsp4 is a non-structural, transmembrane protein that forms a complex with Nsp3 and Nsp6, and is involved in double membrane vesicle (DMV) formation[129]. Together, Nsp3, Nsp4, and Nsp6 are predicted to nucleate and anchor viral replication complexes on DMVs in the cytoplasm[130]. Nsp4 is likely essential, as loss of the Nsp3-Nsp4 interaction eliminated viral replication using an infectious cDNA clone and replicon system of SARS-CoV[131].

We identified eight high-confidence host protein interactions with Nsp4. One such interactor NUP210, is a nuclear pore membrane glycoprotein involved in nuclear pore assembly, fusion, spacing and structural integrity[132,133]. Although RNA viruses replicate in the cytoplasm, they still target the nuclear pore complex (NPC) to improve viral replication and transmission. Certain RNA viruses such rhinoviruses and polioviruses inhibit active nuclear import by the proteolytic degradation of NUPs, such as NUP62, NUP153, and NUP98, and thereby weaken the host cell's immune response against the virus [134]. SARS-CoV-1 protein nsp1 is known to disrupt the localization of NUP93 from the NPC deregulating host cell functions. SARS-CoV-2 Nsp4 interactor Nup210 identified in this study forms the ring structure of the NPC along with NUP93[135], and it is therefore likely that Nsp4-NUP210 interaction also affects SARS-CoV-2 pathology by NPC deregulation.

Nsp4 also interacts with Insulin degrading enzyme (IDE), which is involved in intercellular signaling through the cellular breakdown of diverse signaling peptides[136,137]. IDE is also involved in antigen processing through the production of an antigenic peptide that is presented to cytotoxic T lymphocytes by MHC class I[138]. Interestingly, IDE acts as an entry receptor for varicella-zoster virus (VZV), where VZV glycoprotein E interacts with IDE through its extracellular domain[139,140].

Viruses are known to target components of mitochondrial proteins to enhance their own replication[141]. Nsp4 interacts with various mitochondrial proteins linked to transport, including TIMM10, TIMM10B, TIMM9 and TIMM29, members of the TIMM22 complex. TIMM22 facilitates the import and insertion of multi-pass transmembrane proteins into the mitochondrial inner membrane, with TIMM9 additionally involved in protein homodimerization and chaperone binding[142]. TIMM10 and TIMM29 are known host dependency factors in Zika virus infection[143,144]. Another Nsp4 interactor DNAJC11 is also involved in mitochondrial function. DNAJC11 is required for mitochondrial inner membrane assembly and functions through involvement with the MICOS complex and the MOM sorting assembly machinery complex[145]. Other Nsp4 interactor ALG11, a mannosyltransferase catalyzing oligosaccharide linkage[146,147] has no known links to viral infections.

SARS-CoV-2 Nsp5/Nsp5_C145

Nsp5 encodes the coronavirus main protease (M^{pro}) responsible for cleaving itself and the other subunits from the polyproteins Orf1/1ab[148]. As these proteins include the replicase machinery, Nsp5 M^{pro} is essential for all coronaviruses, and indeed is functionally and structurally conserved throughout the order *Nidovirales*[149]. Many protease PPIs are transient as substrates are further cleaved and processed, making it harder to capture and identify interacting proteins by native affinity purification mass spectrometry (AP-MS) experiments. To address this issue, we carry out pull-down experiments on both wild-type and catalytically inactive protease proteins. Here, we have used both the wild-type and C145A mutant Nsp5 proteins as baits. We have called C145A catalytically inactive based on sequence alignments, as the catalytic residues of SARS-CoV align to H41 and C145 in our SARS-CoV-2 construct. However, we have not yet tested its activity *in vitro* or *in vivo*, and suggest researchers keep this in mind when examining the functional significance of high confidence host interactors.

Catalytically active Nsp5, but not the C145A mutant, interacts with Histone Deacetylase 2 (HDAC2). HDACs deacetylate lysines on the N-termini of histones creating epigenetic markers for transcriptionally silent genes (HDACs involved in disease reviewed in [150]). HDAC2 is a class I deacetylase that regulates gene expression important for several processes including hormone signaling, cytokine signaling, embryonic development, as well as heart, muscle, and neuronal function[150]. Specifically, HDAC2 is known to regulate genes involved in inflammatory response, especially in the pulmonary context where low HDAC2 expression contributes to increased disease severity and glucocorticoid resistance in patients with asthma[151] and chronic obstructive pulmonary disease[152]. Cigarette smoke is also shown to down-regulate HDAC2 expression levels through ubiquitylation and proteasomal degradation, with overexpression of the deubiquitinase USP17 restoring HDAC2 levels[153]. In addition to its connection to lung diseases, HDAC2 can act as either a restriction or dependency factor for various viruses. For example, during infection with Influenza A virus, HDAC2 is targeted for proteasomal degradation, and it was shown that siRNA knockdown of HDAC2 increases IAV replication and growth[154]. In contrast, during human respiratory syncytial virus (RSV) infection HDAC2 expression is increased and treatment with HDAC inhibitors limited RSV replication and alleviated airway inflammation in mouse models of the disease, indicating HDAC2 as a dependency factor for RSV[155]. General HDAC inhibitors have also shown antiviral activity against human adenovirus where treatment repressed viral gene expression and viral replication[156]. While HDAC inhibitors represent a potential strategy for treatment of viral infection, more research is required to determine HDAC2 role in SARS-CoV-2 infection. It is clear that HDAC2 has different roles during infection with different pathogens, and its role in inflammation response can be modulated on an individual patient level depending on their history and genetic background.

Catalytically inactive Nsp5_C145A interacts with two host proteins: TRMT1 and GPX1. TRMT1 is a tRNA methylase that has recently been linked to intellectual disorders[157] but has no other known functions or links to viral infections. The strongest interactor of the C145A Nsp5 mutant is GPX1, a glutathione peroxidase antioxidant enzyme that catalyzes the reduction of peroxides like H_2O_2 to less harmful substances like water[158]. GPX1 is a complex regulator that not only helps control the redox state of the cell, but also mediates a number of cellular processes including mitochondrial function and apoptosis[158]. The enzyme is transcriptionally regulated by a variety of factors including NFkB and has been linked to inflammation[159,158]. In addition, GPX1 has been linked to a number of human diseases that increase the risk of comorbidity with SARS-CoV-2 infection including cancer, diabetes, and cardiovascular disease (reviewed in detail in [158]). Interestingly, there is evidence suggesting GPX1 is regulated by oestrogens contributing to a higher expression of GPX1 in females over males[160]. Taken together, it's possible that GPX1 could play a role in the severity of disease in different individuals, though more research is needed to clarify its interaction with Nsp5 as a substrate or interactor, and determine its role during infection. As we learn more about COVID-19 and individual risk factors, we can more accurately assess the clinical relevance of specific interactors.

Given we typically utilize catalytically dead mutants to stabilize protease interactions with substrates, it is not surprising that we identify more interactors for the C145A Nsp5 mutant. The fact that HDAC2 specifically interacts with wild-type Nsp5 may suggest that this interaction is unusually stable, and perhaps that the C145A mutation may interfere with this interaction.

SARS-CoV-2 Nsp6

Nsp6 complexes with two other transmembrane proteins, Nsp3 and Nsp4, to form double membrane vesicles (DMVs), anchoring viral replication complexes inside[161–163]. Chemical inhibition and targeted mutagenesis studies have shown that these DMVs are crucial to viral replication and are formed early after viral entry into the cell[164–167]. The most well-characterized function of Nsp6 is limiting autophagosome expansion, which likely benefits the virus by preventing its components from being sent to the lysosome for

degradation[168]. In the context of IBV it was shown that the Nsp6-mediated generation of autophagosomes can be induced by chemical inhibition of the mTOR pathway[168]. While this behavior has been shown for SARS-CoV Nsp6 and related viruses[168], its exact mechanism and whether SARS-CoV-2 Nsp6 will function similarly remains uncertain.

In this study, we identified four high confidence interactors of SARS-CoV-2 Nsp6 (ATP5MG, ATP6AP1, ATP13A3, and SIGMAR1). Notably, three out of four of these prey proteins are subunits of different ATP synthases, suggesting that Nsp6 may be manipulating the metabolic program of the cell through ATP synthases of different organelles. As viral infection requires a new surplus of energy, it is unsurprising that viruses hijack host metabolic machinery to carry out a wide variety of cellular functions[169–172]. Specifically, ATPases are crucial for budding by multiple viruses, including HIV-1, Influenza A virus (IAV), and Ebola[173–175]. One such target is vacuolar ATPases (vATPases), which are responsible for modulating endo-lysosomal acidification and thus a number of processes like membrane trafficking and protein degradation[176]. In the context of IAV infection, vATPase ATP6V1A was identified as a key host dependency factor for viral replication across multiple RNAi knockdown screens[49,177]. Influenza A virus is believed to leverage this interaction to lower the pH of the lysosomal environment to accelerate the process of viral uncoating in the early stages of viral infection[178,179]. ATP6AP1, a subunit of the vATPase protein pump and high-confidence physical interactor of Nps6 in this study, has also been described as a host factor for a number of viruses, including IAV, West Nile virus (WNV), and DENV[48,49,177,180,181]. Further, dysregulation of AT6AP1 leads to impaired vesicle acidification and intracytoplasmic granules, resulting in multiple clinical pathologies including an immunodeficiency syndrome and granular cell tumors[182,183]. Given the known role of ATP6V1A as an IAV host factor, the mechanism of SARS-CoV-2 Nsp6 could leverage its interaction with ATP6AP1 similarly to speed replication in DMV complexes.

ATP13A3 is a poorly understood cation-transporting P-type subunit of the mitochondrial ATPase that has recently been implicated in both host innate immunity to pathogens and pulmonary pathologies. During Herpes simplex virus (HSV)-1 infection, ATP13A3 was shown to be down regulated at the plasma membrane of infected cells, but its expression could be rescued by the deletion of a specific HSV-1 protein pUL56, suggesting that it may be specifically targeted as a host factor in the context of some viral infections[184]. Furthermore, ATP13A3 was identified in the plasma membrane of primary CD4+ T cells infected with Vpu-deficient and Nef-deficient HIV-1, and was differentially expressed in CD8+ T cells during IAV infection[185]. More broadly, ATP13A3 expression was found to be a significant variable in determining interindividual response to lipopolysaccharide sensing in human dendritic cells[186]. It was recently reported that loss of function of *ATP13A3* results in disruption of polyamine homeostasis in pulmonary arterial endothelial cells, leading to endothelial dysfunction and ultimately pulmonary arterial hypertension[187,188]. Notably, ATP13A3 is also amenable to direct drug targeting.

Sigma Receptor 1 (SIGMAR1) is an ER chaperone protein that modulates calcium signaling through its interaction with the IP3 receptor (IP3R-3) and adaptor protein ankyrin B (ANK2)[189]. While SIGMAR1 has no known role in viral pathogenesis or pulmonary pathologies, it is targeted by a number of potential SARS-CoV-2 drug candidates highlighted in this study, including approved drugs chloroquine and haloperidol, as well as other preclinical drugs such as RS-PPCC and PB28.

SARS-CoV RNA polymerase: Nsp7, Nsp8 and Nsp12

SARS-CoV Nsp7, Nsp8 and Nsp12 proteins form the RNA-dependent RNA polymerase (RdRp)[190,191]. Eight Nsp7 proteins bind stoichiometrically to eight Nsp8 proteins to form a hexadecameric barrel-shaped structure thought to surround double-stranded RNA[192]. The catalytic activity of the Nsp7-Nsp8 complex is much

weaker than that of Nsp12, though this macromolecular complex is primer-independent and RNA products of Nsp7-Nsp8 complex are typically ≤6 bases. Thus it is believed that the Nsp7-Nsp8 complex functions as a primase, generating short RNA primers for Nsp12's more processive, primer-dependent RNA polymerase activity[193,194].

Nsp12 encodes a canonical, primer-dependent RdRp domain in its C-terminus[193,195], and is essential for viral RNA synthesis. A Nsp7-Nsp8 heterodimer binds to and stabilizes loops in the polymerase domain of Nsp12, and is thought to facilitate interactions between Nsp12 and RNA synthesis/processing machinery[191]. Nsp12 contains an unusually large N-terminal extension with a conserved, essential nucleotidyltransferase domain[196] and protein interface domain that binds a second Nsp8[191]. The full functions of these domains in virus replication or fitness and host biology are not known. Nsp12 may have additional roles outside of the RdRp complex mediated through interactions with the uncharacterized N-terminus[191,193,196] and potentially other virus proteins including Nsp5, Nsp8 and Nsp9[197].

The structure of SARS-CoV-2 Nsp7/Nsp8/Nsp12 complex closely mimics that of SARS-CoV[198], and the complex members bear 98.8%, 97.5%, and 96.4% genetic similarity at the codon level respectively, suggesting that function will be conserved.

SARS-CoV-2 Nsp7

We identified 32 high-confidence host protein interactions with Nsp7. These interactors include multiple Rab GTPases with various roles in exocytic and endocytic membrane trafficking, mitochondrial proteins such as cytochromes, and several other factors that were previously identified as host interactors of other viruses.

Rab GTPases act as molecular switches that regulate dynamic networks and complexes involved in vesicle trafficking[199]. Among the Rab GTPases identified as Nsp7 interactors are Rab14, Rab1a, Rab7a, Rab5c and Rab8a. Rab14 regulates the membrane recycling pathway from endosomes to the plasma membrane which is required for ADAM protease trafficking and regulation of cell-cell junctions[200]. Rab14 was previously characterized to be required for HIV-1 envelope glycoprotein particle incorporation[201]. Rab1a regulates vesicular protein transport from the ER to the Golgi and was identified to be required for production of extracellular enveloped virions of vaccinia virus (VACV)[202], as well as for particle assembly of classical swine fever virus (CSFV)[203]. Rab7a and Rab5c are essential for Hepatitis B virus (HBV) infection[204] with Rab7a implicated in promoting virus entry in early stages[204] and restricting exocytic virion release in late stages[205]. Rab8a was shown to be an important regulator of HIV-1 trafficking to the virological synapses[206] which enables delivery of the virus to CD4+ T-cells.

The ACSL3-LPIAT1 fusion protein is a known cancer gene and ACLS3 is highly expressed in human lung cancer[207]. ACSL3 induction results in prostaglandin production by increasing acyl-CoA synthesis, which isrequired for the maintenance of non-small cell lung cancer[208] and mutant KRAS lung cancer tumorigenesis *in vivo*[207]. Besides the function of ACSL3 in lung cancer, it is known to interact with several viral proteins and is required for poliovirus replication[209–211].

MOGS is a Nsp7 interactor that has previously been shown to interact with various pathogen proteins, including HIV Vpr and gp120, and *Mycobacterium tuberculosis* (Mtb) Ppe11[210,212,213]. This gene is well-characterized in the context of a congenital disorder of glycosylation. Interestingly, patients with genetic defects in MOGS manifest decreased susceptibility to viral infections[214], providing a potential target for broad spectrum therapy of viral infections.

SARS-CoV-2 Nsp8

We find that SARS-CoV-2 Nsp8 interacts with 23 host proteins that are enriched for a number of functions and complexes, including the exosome complex, ribosome biogenesis, and regulation of protein translation. In our dataset, we identify four (EXOSC2, EXOSC3, EXOSC5, and EXOSC8) members of the exosome complex as high confidence Nsp8 interactors, with an additional six subunits just narrowly missing our stringent threshold (EXOSC1, EXOSC4, EXOSC6, EXOSC7, EXOSC9, and EXOSC10). The eukaryotic RNA exosome complex plays a critical role in RNA processing, degradation, and quality control. It consists of 10-11 subunits and functions to remove unstable transcripts including cryptic unstable transcripts, antisense RNA, and unstable bidirectional transcripts/enhancer RNAs,[215],[216217]. Its barrel-shaped catalytically inactive core is made up of nine subunits that form the 'cap' and 'ring' subcomplexes, all of which are identified in our Nsp8 pull-down to varying degrees of confidence. In addition, we identify (below our threshold) one of the putative catalytic subunits, EXOSC10,[215217]. In drosophila and human cells the RNA exosome complex broadly restricts a number of viral infections including vesicular stomatitis virus (VSV), Sindbis virus (SINV), and Rift Valley fever virus (RVFV)[215]. However, other studies have shown that the RNA exosome can act as a dependency factor. For instance, H1N1 influenza a virus (IAV) polymerase PA subunit binds to subunits of the exosome, which were then shown to promote viral ribogenesis and IAV growth[216]. Given that IAV PA and SARS-CoV-2 Nsp8 are part of viral polymerase complexes, it is tempting to speculate host cell RNA exosome complexes function as dependency factors in a similar manner during both IAV and SARS-CoV-2 infection.

LARP7, a member of the 7SK snRNP RNA complex, interacts with WNV, ZIKV capsid, and HIV-1 Tat in such a way as to compete with viral proteins for the transcriptional elongator pTEFb. The interaction of LARP7 and coronavirus primase points to a mechanism by which the virus promotes elongation of its vRNA.

Interestingly, we find that Nsp8 interacts with proteins just under our scoring threshold found to be differentially regulated or expressed during acute lung disease secondary to pathogen infection, including CEBPZ, DAP3, NKRF and ZN512. NFKR for example is upregulated in the circulating monocytes and alveolar macrophages of patients with active pulmonary TB, and NKRF serves as an endogenous repressor for IP-10 and IL-8 synthesis to hinder host from robust response to Mtb infection[217,218].

SARS-CoV-2 Nsp12

In our study, SARS-CoV-2 Nsp12 interacts with 20 high confidence host proteins. Consistent with Nsp12 RdRp activity, eight host protein interactors are RNA binding factors involved at multiple steps of RNA processing and regulation. These host proteins could facilitate long-range RNA interactions that occur during genome replication and discontinuous transcription[219], or mediate viral protein translation. Of note, Nsp12 interacts with three proteins involved in pre-mRNA splicing: A-kinase anchor protein 8 (AKAP8)[220]; and spliceosome components pre-mRNA-splicing factor SLU7 (SLU7)[221] and peptidyl-prolyl cis-trans isomerase-like 3 (PPIL3). Notably, siRNA knockdown of SLU7 inhibits early stages of HIV-1 replication[222]. Nsp12 also interacts with La-related protein 4B (LARP4B), a cytoplasmic RNA binding protein that promotes mRNA translation and interacts with PABPC1 and RACK1 kinase, potentially connecting 3' mRNA factors with translation machinery[223]. PABP, a 3' poly(A) tail binding protein, is a cis acting element on coronavirus RNA that is essential for bovine coronavirus replication[224,225]. Nsp12 could be targeting LARP4B as a bridge to recruit members of the PABP family for efficient RNA replication and translation. Nsp12 interacts with two RNA binding proteins that localize to and nucleate formation of stress granules: ubiquitin-associated protein 2 (UBAP2) and ubiquitin-associated protein 2-like (UBAP2L)[226]. Interestingly, UBAP2L interaction is also identified in SARS-CoV-2 Nsp9 pull-downs, although it falls below our scoring threshold. Coronavirus species

mouse hepatitis virus (MHV) Nsp9 has been shown to interact with Nsp12[197]. This may suggest a potential role for SARS-CoV-2 Nsp12 and Nsp9 in coordinating host and/or viral RNA regulation (see also Nsp9), as stress granules are sites of RNA storage and intermediate stages between translation and mRNA decay[227]. Additionally, since MERS-CoV has been shown to inhibit stress granule formation to promote viral replication[90], SARS-CoV-2 Nsp12 may interact with and sequester UBAP2L as a potential mechanism to inhibit stress granule nucleation and promote virus replication (see also N protein for role of stress granules).

Although coronavirus genome replication and transcription occurs in the cytoplasm, SARS-CoV-2 Nsp12 interacts with five nuclear DNA-related factors: transcription factors CREB-regulated transcription coactivator 3 (CRTC3), transcription factor 12 (TCF12) and zinc finger protein 318 (ZNF318); chromatin factor SBNO1; and AKAP8 which regulates histone methylation and gene expression[228]. Nsp12 shows DNA-dependent activity and can synthesize nucleotides from a DNA template *in vitro*[193,194]. Nsp12 may have novel roles in chromatin and transcription regulation, although the advantage to the virus is unclear.

Nsp12 also interacts with host proteins that regulate inflammatory signaling and apoptotic pathways. One of the top Nsp12 interactors is receptor-interacting serine/threonine-protein kinase 1 (RIPK1). As an active regulatory kinase, RIPK1 triggers cell death by apoptosis or necroptosis; as a scaffold independent of its kinase activity, RIPK1 regulates inflammatory signaling and inhibits cell death[229–231]. Many diverse viral and bacterial proteins interact with and modify RIPK1 to modulate host defense pathways, cytokine signaling and host cell death[232–234]. For example, HIV-1 protease cleaves and inactivates RIPK1, which then impairs host defense pathways[234]. Importantly, RIPK1 is a druggable target, and many inhibitors are being tested as anti-inflammatory treatments for neurodegenerative diseases and cancer[235]. However, given the two faces of RIPK1 regulating apoptosis for pathogen clearance[233] or stimulating cytokine production as part of the inflammatory response[231], the usefulness of drugs targeting RIPK1 as a treatment for SARS-CoV-2 will depend on future research identifying which pathway is engaged by Nsp12. In addition, Nsp12 interactor AKAP8 binds and shuttles caspase 3 to the nucleus as part of caspase-mediated proteolysis and apoptosis[236]. These interactions suggest additional novel roles for Nsp12 outside of canonical RdRp activity in regulating host inflammation and cell death, perhaps mediated through the large uncharacterized N-terminal domain of Nsp12.

SARS-CoV-2 Nsp9

In SARS-CoV and related coronaviruses, Nsp9 is an essential non-structural protein that binds to RNA and DNA, with a preference for single-stranded RNA[237–240]. The function of Nsp9 is not well annotated, though in SARS-CoV it is believed to interact with Nsp8 and the viral replication complex (Nsp7, Nsp8, and Nsp12)[240,241]. In our hands, SARS-CoV-2 Nsp9 interacts with 16 high confidence host proteins, with many having been shown in previous studies to regulate nuclear transport, transcription, and mRNA degradation in response to various viral infections. Unexpectedly, Nsp9 also demonstrates some strong interactions with extracellular matrix proteins involved in elastin formation, lung development, and lung injury and repair.

Nsp9 interacts with Nup62, Nup58, and Nup54, the three components of the Nup62 subcomplex which forms the channel of the nuclear pore complex (NPC)[242–246]. In addition, Nsp9 interacts with nucleoporins on the cytoplasmic side of the nuclear pore complex (i.e. NUP214 and NUP88) but none from the nucleoplasmic side[247] indicating a cytoplasmic role of Nsp9 at the NPC[247]. Many viruses have been shown to exploit cellular nuclear transport machinery to block host-related transport or promote viral transport in a manner beneficial to the virus[248–261]. Nup62 in particular interacts with a number of viral proteins including Epstein Barr Virus (EBV) BGLF4 protein kinase[262], human papillomavirus (HPV)16 and HPV8 E7 proteins[263,264], and HIV-1 IN[265] . Several positive-sense single strand RNA viruses (e.g. poliovirus, enterovirus (EV)71, rhinovirus, and cardioviruses) target Nup62 either through viral protease-directed cleavage[251–257] or induced hyperphosphorylation[258–260] in order to limit nuclear transport. Vaccinia virus, a DNA poxvirus, replicates in

cytoplasmic virus factories that recruit G3BP1 and Nup62[261]. Nup54 interacts with Influenza A virus polymerase and was shown to be important for virus replication and transcriptional activity[266]. In addition to the nucleoporins, Nsp9 interacts with MIB1, a RING-type E3 ubiquitin ligase shown to act as a dependency factor during adenovirus infection[267]. Adenoviruses are non-enveloped DNA viruses that utilize the NPC to dock and deliver genomic cargo into the nucleus, and it has been shown that MIB1 mediates the delivery of viral DNA through the NPC[267].

In addition to nuclear transport machinery, Nsp9 interacts with three transcription regulators, Nek9, DCAF7 and eIF4H. Nek9 is a serine/threonine kinase that regulates mitotic progression[268]. It has been shown as an adenovirus dependency factor promoting viral growth, interacting with E1A protein to silence the expression of certain host genes, and was demonstrated to colocalize at adenovirus replication centers[269]. DCAF7 is a potential substrate adaptor for CUL4-DDB1 E3 ligase[270] that has also been shown to interact with adenovirus E1A protein to suppress innate immune response and depresses IFN stimulated genes (ISGs)[271]. In combination with eIF4A, eIF4H was shown to interact with HSV virus host shut off proteins, with eIF4A shown to help degrade mRNA and switch from host to viral gene expression[272–275]. Taken together, these interactors suggest a potential function of Nsp9 in the inhibition of host mRNA expression potentially to limit the express of ISGs.

Somewhat unexpectedly, Nsp9 interacts with several proteins involved in lung development, lung injury and repair, and lung cancers. The most abundant interactor in the Nsp9 interactome is Fibrillin-2 (FBN2), an extracellular matrix glycoprotein involved in elastin fiber and respiratory organ development[276]. Additional related interactors were identified, albeit with lower abundance. Fibrillin-1 (FBN1) and Fibulin-5 (FBLN5) are both extracellular matrix proteins, also involved in elastin fibre development. Mutations in FBN1 cause Marfan syndrome (MFS), a connective tissue disease that can result in early morbidity and mortality mainly caused by aortic aneurysm and rupture, though additional clinical manifestations include lung complications[277]. FBLN5 is indicated to serve a role during lung injury and repair, is frequently silenced in lung cancer, and has been shown to suppress cancer cell invasion[278–283].

The Nsp9 interactome reveals a potential function for the protein not only in viral replication, but potentially in regulating nuclear transport, though it is unclear if this regulation would block host cell transport or promote viral transport. In addition, the interaction with transcription regulators indicates a potential role in inhibition of host cell transcription and potentially host shut off. And finally, the unexpected interaction with fibrillins-1 and -2, and fibulin-5 could implicate Nsp9 as a potential complicating factor during disease pathogenesis, and may point to additional molecular reasons underlying SARS-CoV-2 complications in the lungs.

SARS-CoV-2 Nsp10

Nsp10 contains two Zn-finger motifs, binds nucleic acids non-specifically, and has been implicated in minus-strand RNA synthesis, thus performing an essential role in viral replication[284,285]. A unique feature for SARS-CoV is that Nsp16 requires Nsp10 as a stimulatory factor to execute its methyltransferase activity[286]. Nsp10 on its own forms a dodecameric homomeric complex[287] which excludes its interaction interfaces with Nsp14 and Nsp16, meaning that Nsp10 could have at least two different functional quaternary structures.

In our map, we identify five high-confidence host protein interactions with Nsp10, including two subunits of the clathrin adaptor protein complex 2 (AP-2), AP2A2 and AP2M1. This interaction is reminiscent of Nef proteins from Human and Simian Immunodeficiency Viruses (HIV and SIV) which are shown to bind the AP-2 complex through a canonical AP-2 recognition acidic dileucine motif ([RQED]XXXL[LIV])[288]. HIV and SIV are thought to use the interaction between Nef and AP-2 to hijack the clathrin machinery and endocytose host proteins such as CD4 and MHC-I[289]. Interestingly, coronavirus Nsp10 proteins do not appear to contain the

canonical AP-2 binding motifs (either the acidic dileucine motif [RQED]XXXL[LIV] for binding AP2A2 nor YXXΦ for binding AP2M1).

In addition to the AP-2 complex, Nsp10 interacts with GFER, ERGIC1, and GRPEL1. GFER was identified as a host dependency factor for West Nile and Dengue virus infections[48]. ERGIC1 and GRPEL do not have known roles in viral pathogenesis. ERGIC1 plays a possible role in transport between endoplasmic reticulum and Golgi[290]; and GRPEL1 participates in the translocation of transit peptide-containing proteins from the inner membrane into the mitochondrial matrix[291].

SARS-CoV-2 Nsp11

SARS-CoV Nsp11 encodes a short peptide that is 13 aa in length. It is not clear whether Nsp11 encodes a functional protein. In our map, we identify only one high-confidence host protein as an interactor of Nsp11. Tubulin-specific chaperone A (TBCA) is a tubulin-folding protein and is known to be involved in the early step of the tubulin folding pathway[292].

SARS-CoV Capping Enzymes: Nsp13, Nsp14 and Nsp16

The m7GpppN (N = any nucleotide) cap of mRNA promotes translation. SARS-CoV Nsp13, Nsp14, and Nsp16 encode enzymes that install cap structure onto mRNA[293,294]. The pathway of cap synthesis on nascent viral mRNA is thought to be similar to capping of cellular mRNA, but occurs in the cytoplasm instead of the nucleus[293,295,296].

SARS-CoV-2 Nsp13

Nsp13 is essential for SARS-CoV viral RNA synthesis[297], and SARS-CoV-2 Nsp13 shares 100% amino acid similarity with SARS-CoV. Nsp13 is a helicase/triphosphatase, and triphosphate cleavage initiates the first step in mRNA capping[298]. Nsp13 hydrolyses the gamma phosphate of nascent mRNA and the resulting diphosphate is then converted to a GpppN RNA by a yet-to-be identified guanylyl transferase.

SARS-CoV-2 Nsp13 was found to interact with 40 host proteins with a broad range of cellular processes and complexes from Protein Kinase A (PKA) signalling, to the Golgi apparatus, to multiple members of microtubules and centrosomes. SARS-CoV-2 Nsp13 showed a strong interaction with Giantin (GOLGB1), which has previously been shown to interact with HSV-1 UL37[299] and the Tick-borne encephalitis virus (TBEV) replicon[300]. Interestingly, in macaque experiments, expression of the HIV protein Nef resulted in increased GOLGB1 levels and led to Golgi disruption and specific pulmonary vasculopathies[301].

Unexpectedly, we found that SARS-CoV-2 Nsp13 pulled down both the regulatory (PRKAR2A and PRKAR2B) and the catalytic (PRKACA) subunits of PKA as well as the A-kinase anchoring protein AKAP9 and the phosphodiesterase interacting protein PDE4DIP. AKAP9 (also called AKAP450) is a large scaffolding protein that localizes to the Golgi apparatus and centrosomes[302,303] where it assembles multiple signaling proteins (e.g., PKA and PDE4D) that control microtubule organization[304], polarized secretion[305], Golgi morphology[305,306], ciliogenesis[307], directional cell migration[308] and cell cycle progression[303]. Importantly, the activities of PKA signaling complexes have been implicated in multiple membrane transport steps[309–312], suggestive of a role for SARS-CoV-2 Nsp13 in hijacking the host secretory pathway for viral benefit. Also notably, a pool of AKAP9 relocalizes to RNA stress granules upon treatment with arsenite where it forms a complex with G3BP and CCAR1 and regulates stress granule size and composition[313]. PKA, being a kinase, is also targetable by small molecule inhibitors or peptides that target the AKAP-PKA binding interface.

An additional SARS-CoV-2 Nsp13 interaction partner was the endosomal transport protein ERC1. Knockout of ERC1 causes a significant decrease in dengue virus replication, and interestingly a similar phenotype was observed with additional SARS-CoV-2 Nsp13 pulldown target GOLGA2[314]. The NS3 protein of hepatitis C virus binds to ERC1 and may mediate the pathogenesis of HCV[315], and knockdown of ERC1 significantly decreases human cytomegalovirus (HCMV) viral production[316]. Of particular interest, ERC1 has previously been identified as a potential drug target in dengue virus infection[317].

Lastly, Nsp13 interacts with transducin-like enhancer of split (TLE) family members TLE1, 3 and 5.

SARS-CoV-2 Nsp14

Nsp14 is a bifunctional enzyme. It encodes an exonuclease (exo) domain that corrects mutations that arise during genome replication[318]. In addition, a separate domain of Nsp14 functions as a SAM dependent methyltransferase (MTase) that generates the N-7 Guanosine of the m7GpppN cap on viral mRNA. High-resolution crystal structures of SARS-CoV Nsp10/Nsp14 suggest exo and MTase activity is stimulated by Nsp10 through an allosteric mechanism[319].

There are three host factors that copurify with Nsp14 with all three being druggable targets. GLA is an alpha galactosidase implicated in Fabry Disease[320]. Migalastat, a pharmacological chaperone, targets GLA through the inhibition of alpha-glucosidase and glycosylation, increasing its lysosomal activity[321]. SIRT5 is a mitochondrial protein linked to metabolism and aging that removes malonyl, succinyl, acetyl, and glutaryl groups on lysines of target proteins[322–325] Numerous compounds target SIRT5, including HDAC inhibitors[326]. IMPDH2 catalyzes the conversion of isosine 5’ phosphate (IMP) to xanthine 5’-phosphate (XMP) which is then converted into guanine 5’ monophosphate for *de novo* synthesis of guanine nucleotides[327]. It is tempting to speculate that the copurification of Nsp14 and IMPDH2 reflects an interplay between Nsp14 activities and purine nucleotide metabolism, though more experiments need to be done to verify this. Merimepodib, a nucleoside analog and broad spectrum antiviral, is among the compounds that target IMPDH2[328].

SARS-CoV-2 Nsp15

In SARS-CoV, Nsp15 has uridine-specific endoribonuclease (endoU) activity, and is essential for viral RNA synthesis[329]. The Nsp15-associated endoU domain is one of the most conserved proteins among CoVs and related viruses, suggesting important functions in the viral replicative cycle. The endoUs were shown (i) to have endonucleolytic activity, (ii) to cleave 3' of pyrimidines, preferring uridine over cytidine, and (iii) to release reaction products with 2'-3'-cyclic phosphate and 5'-OH ends. Deletion of a conserved domain for this enzymatic activity led to loss of viral RNA generation measured by RT-PCR[329]. Nsp15 is also known to be critical for evasion of host dsRNA sensors in macrophages[330]. Nsp15 proteins were shown to form homohexamers composed of a dimer of trimers[331].

In our map, Nsp15 interacts with three host proteins. NUFT2 mediates the import of GDP-bound RAN from the cytoplasm to the nucleus and indirectly plays a general role in cargo receptor-mediated nucleocytoplasmic transport[332,333]. ARF6 is a GTP-binding protein involved in protein trafficking that regulates endocytic recycling and cytoskeleton remodeling[334–338]. ARF6 has been shown to play a role in the activation of cholera toxin[339]. RNF41 acts as E3 ubiquitin-protein ligase and promotes TRIF-dependent production of type I interferon; it has been shown to inhibit vesicular stomatitis virus infection[340].

SARS-CoV Open Reading Frames

SARS-CoV-2 Orf3a

Orf3a is the largest (274 aa) group-specific Orf in the SARS-CoV-2 genome and encodes a type IIIa integral membrane protein. It is thought to be non-essential but has multiple key functions in viral pathogenesis from mediating the trafficking of SARS-CoV Spike (S protein), to inducing apoptosis and inflammation during infection[341–346]. Specifically, SARS-CoV Orf3a induces pro-IL-1β expression and protein maturation through the TRAF3-dependent ubiquitination of ASC and p105, ultimately activating NF-κB and the NLRP3 inflammasome[342]. In addition, Orf3a has been been shown to upregulate the secretion of fibrinogen in lung epithelial cells, a process responsible for the induction of cytokine storm, particularly in the respiratory tract[347] Within the cell, Orf3a localizes to the perinuclear region and plasma membrane, forming punctae throughout the cytoplasm as it complexes with cellular factors [341,348,349] . In the rER and Golgi, Orf3a has been shown to cause substantial ER stress during infection[350]. A YxxΦ motif present in the C-terminal cytoplasmic domain of Orf3a is required for delivery to the plasma membrane, from which Orf3a is internalized and traffics through the endocytic pathway to lysosomes[351]. Orf3a can form tetramers that are proposed to act as cation-permeant ion channels[352]. In this study, Orf3a pulled down eight high-confidence host protein interactors (ALG5, ARL6IP6, CLCC1, HMOX1, SUN2, TRIM59, VPS11, VPS39) with functional enrichments in autophagy (HMOX1, VPS11, VPS39) and organelle localization (HMOX1, VPS11, SUN2). VPS11 and VPS39 serve as members of the HOPS and CORVET complexes, respectively, which coordinate fusion of the lysosome with the endosome and autophagosome[353,354].

HMOX1 is a key enzyme in heme catabolism. This oxygenase cleaves free heme to produce iron, biliverdin and carbon monoxide, providing a cytoprotective effect to cells as excess free heme is shown to induceapoptosis[355]. In turn, heme cleavage elicits a cascade of physiological events, most notably the induction of anti-inflammatory cytokines IL-10 and IL-1RA[356,357]. Given these features of HMOX1 and its high expression in the lungs, it has been implicated in a broad range of disease states, including diabetes, heart failure, lung carcinoma and chronic obstructive pulmonary disease (COPD)[358,359]. In the context of infection, upregulation of HMOX1 has been shown to have a protective effect against the oxidative stress produced during various pathogen infections, including viruses like HIV, DENV, HCV, and IAV, as well as parasites and *Mycobacterium* species[360,361]. As severe inflammatory response in the respiratory tract is a key clinical feature of SARS-CoV-2 infection, further exploration of HMOX1 could elucidate mechanisms of SARS-CoV-2 pathogenesis and a path forward for treatments.

Orf3a also strongly interacts with, CLCC1, an intracellular chloride channel that is localized to the ER and Golgi apparatus and is ubiquitously expressed in numerous tissues including the lung[362]. Loss of *CLCC1* results in ER stress and disruption of the protein folding capacity of the ER, leading to misfolded protein accumulation and well-characterized retinal cell dysfunction in the clinic[363,364]. Notably, both CLCC1 and SARS-CoV Orf3a are localized to the ER membrane [362,341]. In addition, expression of *CLCC1* has been correlated with volume of adipose tissue in HIV-infected men[365]. RSV NS1 protein also interacts with CLCC1, though the function of the interaction is unknown[366]. Interestingly, CLCC1 also interacts with solute carrier superfamily member SLC15A3, a druggable peptide/histidine transporter and known interferon-stimulated gene that is regulated by TLR-activation and contributes to TLR4-mediated inflammation in macrophages and epithelial cells[367,368]. While SLC15A3 was not identified as an interactor of Orf3a, we identified interactions between SARS-CoV-2 proteins and other members of the solute carrier superfamily including SLC25A17 (SARS-CoV-2 Orf9b and Orf9c) and SLC6A15 (SARS-CoV-2 Orf9c, M and Nsp6), although these interactions

fall below our scoring thresholds. This may suggest that Orf3a may play a role in modulating host innate immunity via an indirect interaction with CLCC1 as part of a larger protein complex or pathway.

In addition to HMOX1 and CLCC1, Orf3a also interacts with known viral host factors and interactors. ARL6IP6, a transmembrane protein responsible for ADP ribosylation, is known to physically interact with both HPV-31 E5 and WNV NS4b, and was differentially expressed in CD8+ T cells from HIV+ progressors on HAART[37,369,370]. ALG5, an ER-localized glycosyltransferase involved in N-glycan biosynthesis, has been characterized as a cellular host factor for IAV replication in multiple genome-wide CRISPR-Cas9 knockout screening efforts, along with other ALG family proteins[52,5337,369,370].

SARS-CoV-2 Orf3b

SARS-CoV-2 Orf3b encodes a 168 aa protein that is not well conserved (9.5% codon similarity as compared to SARS-CoV Orf3b). While Orf3b is thought to be non-essential[1,343], it has been shown to be an IFN antagonist in both SARS-CoV and SL-CoV (bat)[371,372] and to subsequently be involved in pathogenesis[1]. The only confident interaction partner detected for Orf3b was the mitochondrial protein STML2/STOML2. STOML2 stimulates cardiolipin biosynthesis and recruits and stabilizes prohibitin. Both STOML2 and its interactor prohibitin have been shown to be host dependency factors for EV71 neuropathogenesis[373]. STOML2 forms large complexes with the i-AAA protease YME1L and the rhomboid protease PARL at the inner mitochondrial membrane, which regulate key proteins of the mitochondrial stress response such as PGAM5 and PINK1[374]. STOML2 may directly be involved in regulating T-cell-mediated immune responses by modulating T-cell receptor activation[375].

SARS-CoV-2 Orf6

SARS-CoV-2 Orf6 encodes a 7.3kDa protein that is dispensable for virus replication, but affects viral production[376]. Orf6 functions as a type I IFN antagonist and suppresses IFN induction and IFN signalling pathways. It is localized to the ER/Golgi membrane in infected cells, where it binds to and disrupts nuclear import complex formation by tethering karyopherin alpha 2 and karyopherin beta 1 to the membrane. This results in the loss of nuclear import of the interferon signalling responsive transcription factor STAT1[372,377] and therefore blocks the expression of STAT1-activated genes establishing an antiviral state.In SARS-CoV infected cells, Orf6 complexes with Orf9b[378]. SARS-CoV-2 Orf6 was found to interact with three host proteins: NUP98, RAE1, and MTCH1. As described in the main text, NUP98-RAE1 is an interferon-inducible mRNA nuclear export complex that is targeted by multiple viruses including VSV, IAV, KSHV, and Polio[379,380]. Orf6 also interacts with mitochondrial carrier homologs 1 (MTCH), which is known to regulate apoptosis by modulating activity of the mitochondrial permeability transition pore[381] and therefore could be involved in virus-induced apoptosis.

SARS-CoV-2 Orf7a

SARS-CoV Orf7 is divided into two open reading frames, designated Orf7a and Orf7b. Orf7a (also known as U122) encodes a 122-amino-acid protein and contains a compact seven-stranded β-stack similar in structure to members of the immunoglobulin superfamily[382]. The role of Orf7a in apoptosis was highlighted by an increase in caspase-3 protease activity that resulted in a significant induction of apoptosis[383]. Orf7a expression has also been shown to downregulate cyclin D3, resulting in the accumulation of retinoblastoma protein (Rb) and ultimately cell cycle arrest in G0/G1 phase[384]. Orf7a traffics to different locations throughout the cell, including the perinuclear region, the cytoplasm, and the plasma membrane through it's type-1

transmembrane domain[383–385]. Further, Orf7a has been shown to colocalize with ER and ER-Golgi intermediate compartment (ERGIC) markers during infection[385]. Interestingly, this cellular localization coincides with that of high-confidence protein interactors MDN1 and HEATR3, as well as a factor that was just below our MIST threshold, TNPO1. While it did not satisfy our stringent scoring criteria, TNPO1, or transportin-1, is worth mentioning due to its demonstrated role in other viral infections such as Influenza A virus, HIV-1 and Hepatitis C virus, where it plays a crucial role in mediating nuclear transport of viral proteins and protein complexes[386–389] HEATR3 has also been shown to activate the NF-kB pathway via NOD-2, which has been implicated in a pro-inflammatory response during Crohn's disease[390,391] and therefore Orf7a may target HEATR3 to modulate inflammatory response upon SARS-CoV-2 infection.

SARS-CoV-2 Orf8

Orf8 is an accessory protein and is not essential for virus replication *in vitro* and *in vivo*[343,392]. It is one of the most rapidly evolving regions among SARS-CoV genomes and was previously shown to be a recombination hotspot[393–395]. Pairwise comparison of amino acid sequences showed that SARS-CoV-2 Orf8 exhibited 45.3% sequence similarity with SARS-CoV. Orf8 in human isolates from the 2003 epidemic contained a signature 29-nucleotide deletion compared to all civet and bat SARS-related CoVs, which causes the split of full-length Orf8 into two small proteins: 8a and 8b[396]. Orf8 from SARS-CoV-2 encodes a single polypeptide and lacks the aggregation motif VLVVL present in SARS-CoV Orf8b, which was shown to induce ER stress and activate NLRP3 inflammasomes[397]. Further, Orf8b protein has been shown to be modified by N-linked glycosylation on N81 residue, which protects Orf8ab protein from proteasomal degradation[398]. This novel Orf8 likely encodes a secreted protein and has an N-glycosylation site at N78, within the consensus sequence NYT.

We identified 47 high-confidence host protein interactions with Orf8. Several Orf8 interactors are involved in ER stress and ER-associated degradation (ERAD) pathway, including UDP-glucose/glycoprotein glucosyltransferase 2 (UGGT2), ER degradation enhancing alpha-mannosidase like protein 3 (EDEM3), OS9[399], N-glycanase 1 (NGLY1), and FAD-dependent oxidoreductase domain-containing protein 2 (FOXRED2)[400–403]. The ERAD pathway targets unassembled glycoproteins for ubiquitylation and proteasomal degradation. EDEM3 has been shown to increase ubiquitylation of HCV envelope proteins via direct physical interaction and consequently reduce viral production[404]. OS9 and ERLEC1 proteins are also known to be targeted by other virus-encoding proteins from Dengue virus[211], HIV[210], WNV[37], HPV and KSHV[405], suggesting common molecular mechanisms of infection and proliferation used by these different pathogens.

Infection with the SARS virus results in severe inflammation in the lungs, which can lead to respiratory distress and fibrosis during the late stages of infection[406]. Fibroblast activation and overexpression of collagen are two important aspects of the pathogenesis of lung fibrosis. Interestingly, we identified a number of Orf8 interactors implicated in pulmonary fibrogenesis including FKBP10[407], GDF15[408], NEU1[409] and IL17RA[410]. In addition, the expression levels of ADAMTS1[411] and HS6ST2[412] are modulated during lung inflammation and fibrosis and are identified as Orf9b interactors. Growth differentiation factor 15 (GDF15) is a fibroblast-inhibiting cytokine that inhibits the growth and activation of lung fibroblasts by inactivating the TGF–Smad pathway, suggesting this cytokine could be a potential therapeutic for ameliorating interstitial lung fibrosis during severe SARS infection. FK506-binding protein 10 (FKBP10) is a collagen chaperone and inhibition attenuates expression of profibrotic mediators and effectors[407], suggesting that this protein can be targeted to reduce virus-induced lung fibrosis. Activation of the pro-inflammatory cytokine receptor IL17RA in lung tissues is an important host defense mechanism upon fungal, bacterial and viral infections, but its overactivation increases collagen secretion and exacerbates pulmonary fibrosis[410]. Inhibition of IL17RA signaling promotes resolution of

pulmonary inflammation and fibrosis in *in vivo* models[413] and may therefore serve as a therapeutic strategy to reduce lung fibrosis during SARS infection.

Though below our stringent scoring threshold, one additional interesting protein identified in Orf8 pull-downs was the cellular guanyl transferase RNGTT/Mce1. This interaction was significant (>0.05 BFDR) but just below our MIST threshold (MIST score = 0.649). Previous studies suggest RNGTT can exist in the cytoplasm[414,415], it is possible that Orf8 recruits RNGTT to viral mRNA to add G to nascent mRNAs after they are acted on by Nsp13 to make GpppN mRNA that is subsequently acted on by Nsp14 and Nsp16 (see section on SARS-CoV Capping Enzymes).

SARS-CoV-2 Orf9b

Orf9b is an accessory protein synthesized from an alternative complete reading frame within the viral N gene, which encodes for a 98-aa long protein. Orf9b has been shown to be expressed in SARS-CoV-infected cells and antibodies against Orf9b were detected in the sera from convalescent-phase SARS patients[416,417], however the function of Orf9b is largely unknown. It is known that Orf9b can passively diffuse into the nucleus and is actively exported via Crm1-mediated nucleocytoplasmic export[418]. In addition, Orf9b localizes to mitochondria and causes mitochondrial elongation by inducing ubiquitination-mediated proteasomal degradation of the main pro-fission factor dynamin-like protein 1 (DRP1)[419]. Orf9b targets the mitochondrial-associated adaptor molecule MAVS signalosome by utilizing PCBP2 and the HECT domain-containing E3 ligase AIP4, resulting in the degradation of MAVS and therefore limiting host cell interferon responses[419].

We found that SARS-CoV-2 Orf9b interacts with 11 human proteins, including with a mitochondrial import receptor, translocase of outer membrane 70 (TOM70). TOM70 is known to interact with MAVS protein upon RNA virus infection and it acts as a critical adaptor linking MAVS to TBK1/IRF3, resulting in the activation of IRF-3[420]. TOM70 also makes a dynamic protein complex with HSP90/IRF3/BAX and mediates virus-induced apoptosis[421]. Though more studies need to be done to fully flesh out this interaction, it is possible SARS-CoV-2 Orf9b may target TOM70 to modulate IRF3-mediated gene expression or apoptosis upon virus infection. Another mitochondrial protein identified as interacting with Orf9b is BCL2-associated athanogene 5 (BAG5). BAG5 inhibits mitophagy of damaged mitochondria by suppressing recruitment of Parkin to the sites of damage[422]. Several viruses trigger Parkin-dependent mitophagy to promote persistent infection and impair the innate immune response[423]. SARS-CoV-2 Orf9b might act similarly by antagonizing the function of BAG5.

In addition to mitochondrial proteins, SARS-CoV-2 Orf9b was also found to interact with CHMP2A, a member of the endosomal sorting complex required for transport (ESCRT)-III machinery[424]. CHMP2A was shown to contribute to the budding of a variety of viruses, including HIV[425], equine infectious anemia virus (EIAV)[426], and murine leukemia virus[426], suggesting a critical role for virus release. Other Orf9b interactors of interest include microtubule affinity-regulating kinases MARK1, MARK2, and MARK3. These proteins are involved in regulating microtubule dynamics and phosphorylation of tau[427], and MARK2 was also shown to regulate HIV trafficking through phosphorylation of FEZ1[428].

SARS-CoV-2 Orf9c

SARS-CoV-2 Orf9c (referred to as Orf9B in Wu et. al.) encodes a short polypeptide that is 70 aa in length[2]. There is some debate over whether Orf9c encodes a functional protein, or what it's function would be, as Orf9c is thought to be dispensable for virus replication[429–431]. Therefore it is unclear how clinically relevant molecular interactions for this bait would be in the context of coronavirus infection. Keeping that in mind, we are able to express and purify Orf9c in HEK293T/17 cells, identifying 26 high confidence human protein

interactors of diverse functional enrichments. These functions include mitochondrial respiratory chain complex assembly (NDUFB9, NDUFAF1, ACAD9, ECSIT, BCS1L)[432–434], GPI-anchor biosynthesis (PIGO, PIGS, GPAA1)[435], and regulation of I-kappaβ kinase and NF-kappaβ signaling (NLRX1, F2RL1, NDFIP2).

F2RL1 is implicated in a variety of cellular processes related to the pathogenesis of respiratory viruses and pulmonary disease, including NFκB activation, cooperativity with Toll-like receptors, innate immune recruitment and activation, and acute lung inflammation[436–440]. In the context of Influenza A virus (IAV) pathogenesis of monocytes and macrophages, F2RL1 activation protects against viral infection through an IFN-gamma-mediated mechanism[441,442]. Importantly, F2RL1 is the target of four known pharmacologic agents AC-55541, AZ8838, GB110 and Z3451. Another interactor linked to pulmonary function is NLRX1, an attenuator of IAV-induced inflammation[443]. During IAV infection, NLRX1 promotes type I IFN signaling and macrophage survival[443]. It is an essential moderator of macrophage immunity, as it senses the extent of viral replication and maintains a protective balance between antiviral immunity and excessive inflammation within the lungs[443].

In our study, Orf9c is also shown to interact with MRP1 (encoded by *ABCC1*), a multifunctional ATP-binding cassette protein that, among other diverse functions, controls the ATP-dependent efflux of drugs from the cell. It has been implicated in multidrug resistance, viral pathogenesis, and pulmonary disease, and is directly targetable by FDA-approved pharmacologic agents daunorubicin and mitoxantrone[444]. MRP1 has a demonstrated role in both HIV and CMV biology, is found to be differentially expressed in a polarized subset of macrophages during HIV-1 infection, and is associated with CMV latency[445–447]. Interestingly, HIV-1 protease inhibitors saquinavir, rotinavir, and nelfinavir are substrates of MRP1, though it was found that MRP1 did not affect the antiviral activity of these drugs in cell lines[448]. The role of MRP1 in determining the severity of diseases (e.g., COPD, pneumonia, and lung carcinoma) and multidrug resistance in the lung has been well-characterized, which could be particularly relevant given the clinical manifestation and treatment of ARDS and pneumonia during SARS-CoV-2 infection[449–452].

SARS-CoV-2 Orf10

SARS-CoV-2 Orf10 codes for a peptide only 38 aa long and does not have a homolog in SARS-CoV. There is no data yet providing evidence that the protein is expressed during SARS-CoV-2 infection, however we found that upon expression in HEK293T/17 cells Orf10 interacts with nine host proteins. Among these are multiple members of the Cullin RING E3 ligase 2 (CRL2) complex, including CUL2, ELOB, ELOC, RBX1 and ZYG11B. Cullin RING E3 ligases play a central role in viral infections, since they are commonly hijacked by viral proteins to ubiquitinate and degrade viral restriction factors. CRL2 has been previously found to be targeted by poxviral ANK/BC via a C-terminal BC box domain resulting in potent suppression of inflammatory cytokines production, including interferon[453]. Similarly, HPV16 E7 protein binds to an active CRL2 complex, and this association correlates with the ability of HPV16 E7 to transform cells[454]. ZYG11B, a substrate adapter of CUL2, is the highest scoring hit in the Orf10 interactome indicating that Orf10 might bind to the assembled $CUL2^{ZYG11B}$ complex. Interestingly, ZYG11B targets substrates with exposed N-terminal glycines for degradation[455]. Orf10 contains an N-terminal glycine but does not have lysine residues, suggesting a few possible models: (1) Orf10 hijacks the $CUL2^{ZYG11B}$ complex for ubiquitination and degradation of restriction factors, or (2) Orf10 blocks $CUL2^{ZYG11B}$ and prevents the ubiquitination of its targets, or (3) Orf10 is targeted by $CUL2^{ZYG11B}$ for degradation through N-terminal ubiquitination (see main text Figure 4B).

FUTURE DIRECTIONS AND AVAILABLE RESOURCES

As we've described above, a number of SARS-CoV-2 proteins are known to assemble and function in multi-viral subunit complexes. It is likely that co-expression of specific proteins forming these complexes could identify and implicate additional clinically relevant interactions within the host. At the time of this publication, we are in the process of generating a library of untagged transient expression constructs suitable for co-transfection with the tagged library generated and used in this study. In parallel we are also integrating endogenous affinity tags into SARS-CoV-2 replicons and full-length recombinant SARS-CoV-2 genomes to study interactions in the context of infection. Performing affinity purifications in the context of infection will also address concerns regarding protein overexpression, which often allows the identification of weaker or lower abundance interactions, but can lead to false positive or less clinically relevant identifications. Replication of our HEK293T overexpression AP-MS experiments is currently ongoing in other cell types, including cultured and primary lung epithelial cells. Comparison and integration of AP-MS overexpression data from multiple cell lines under different cellular contexts (e.g. co-expression with SARS-CoV-2 proteins or replicons) will help clarify and prioritize high-confidence host interactions for further follow-up studies. Given the urgent and acute global need for SARS-CoV-2 research, we will make these resources available to the scientific community upon completion, as we have done in this study.

Supplementary Methods

Sequence Analysis of Overlapping Coding Regions (Extended Data Figure 1a). The alignment of 2,784 SARS-CoV-2 sequences (obtained from GISAID on April 4, 2020) was performed with MAFFT v7.450 with default settings using Geneious Prime® 2019.1.1 software.

Western Blotting (Extended Data Figure 1b). Transfected cell lysate (IP input samples) was mixed with SDS sample buffer and beta-mercaptoethanol, heated to 95°C for 5 minutes, then run on a 26-well Criterion TGX gel (BioRad) until the dye front was 1.5 cm from the bottom of the gel. Protein was transferred to PVDF membrane, and all western blotting steps were performed in PBS containing 5% milk and 1% Tween-20. Anti-strep antibody (Qiagen 34850) was used at a concentration of 1:2,500, and goat anti-mouse HRP conjugate (BioRad) was used at a concentration of 1:20,000. Membranes were washed with PBS prior to addition of HRP substrate and signal detection on film.

Pearson correlation and clustering analysis of SARS-CoV-2 affinity purification-mass spectrometry (AP-MS) Data (Extended Data Figure 2). All MS runs were compared and clustered using standard artMS (https://github.com/biodavidjm/artMS) procedures on observed feature intensities computed by MaxQuant. Pearson's pairwise correlations between MS runs are clustered according to similar correlation patterns.

PFAM domain enrichment analysis (Extended Data Figure 4). The enrichment of individual PFAM domains (or PFAM clans)[456] was calculated with a hypergeometric test where success is defined as number of domains, and the number of trials is the number of individual preys pulled-down with each viral bait. The population values were the numbers of individual PFAM domains and clans in the human proteome. To make sure that the p-values that signify enrichment were meaningful, we only considered PFAM domains that have been pulled-down at least three times with any SARS-CoV-2 protein, and which occur in the human proteome at least five times.

Expression analysis of interacting genes (Extended Data Figure 5). We used GTEx (version 8, median gene-level transcripts per million (TPM) by tissue), which consisted of 17382 samples (578 lung samples)[457] to examine the mRNA expression of all interacting proteins (n=323). The comparison gene group was all RefSeq genes (n=24,491). The lung expression values represent the median expression of each gene across the GTEx lung samples. The lung enrichment values are calculated by dividing the median expression of each gene in lung tissue by the median expression of each gene across all tissues (including lung). A value of greater than one indicates that the gene expression is enriched in lung tissue. Values were plotted on a log10 scale. All figures and statistics were produced in Python3 and code and reference tables can be found at: (https://github.com/stephaniewanko/Fraser_Lab/tree/master/QCRG_COVID19).

Nsp5 main protease (3Clpro) cleavage prediction (Extended Data Figure 6). We used sequence specificity data for SARS Nsp5[458] (98.7% identical to SARS-CoV-2 nsp5) and NetCorona[459] to predict cleavage sites within interacting factors. PDB ID: 1UJ1 served as template for peptide docking which was performed using the predicted P4-P1 residues (BioLuminate, Schrödinger, LLC). Illustration of the docked model was generated in PyMol (Schrödinger, LLC).

Orf6 consensus sequence analysis (Extended Data Figure 7). Orf6 sequence homologs were identified using the BLAST tool[460] (accession number YP_009724394.1), run with the default settings: gap opening and extension costs of 11 and 1, respectively, BLOSUM62 as the scoring matrix, and an e-value threshold of 10. The search yielded 34 homologous sequences. The multiple sequence alignment was visualized using the

MView web server: https://www.ebi.ac.uk/Tools/msa/mview/[461] and the WebLogo server (https://weblogo.berkeley.edu/logo.cgi)[462].

Analysis of off-target activities for characteristic Sigma receptor ligands (Extended Data Figure 11). Radioligand binding assays were performed as previously described[463]. Detailed experimental protocols are available on the NIMH PDSP website (https://pdspdb.unc.edu/html/tutorials/UNC-CH%20Protocol%20Book.pdf).

Evolutionary analysis (Supplementary Table 3). For each gene, we obtained a human ORF sequence, choosing the splice isoform with the longest ORF. We used this ORF as query in a blastn search[464] of NCBI's NR database and for each non-human primate species we collected the blast hit with the highest bit score, filtering out matches of <60% identity or <100bp alignment length, and ignoring database sequences that are >20kb long or have no annotated ORF. We also blasted each primate hit to a collection of all human genes, to ensure all sequences are reciprocal best hits (a proxy for true orthology, albeit imperfect). We extracted ORFs from each primate match, and aligned orthologous sequences using MACSE[465], treating the human sequence as 'reliable' and the other primate sequences as 'less reliable' (parameters: -fs_lr 10 and -stop_lr 10). We then manually inspected and, if necessary, edited all alignments to remove unreliable sequence segments, because gene predictions found in NR sometimes contain erroneous exons. In a few cases, manual inspection led us to select a different splice isoform, because the longest ORF occasionally included one very poorly-alignable exon. We used phyml[466] to estimate a phylogeny for each alignment (parameters: -m GTR --pinv e --alpha e -f e). The alignment and phylogeny were then used as input for PAML's codeml algorithm[467] comparing the neutral/purifying model 8a (where dN/dS for codons follows a beta distribution with values between 0 and 1, with an extra class of sites with dN/dS fixed at 1) with model 8 that allows a subset of codons to have dN/dS > 1 (parameters: codon frequency F3x4, estimate kappa, initial kappa 2, initial omega 0.4, ncatG 10, cleandata 0). We performed a likelihood ratio test[467] to obtain a p-value, by comparing twice the difference in log-likelihoods with the chi-squared distribution with 1 degree of freedom. After running all 332 analyses, we used the Benjamini-Hochberg procedure[468] to control the false-discovery rate.

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