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► **To cite this version:**

Laure Perrin-Coccon, Olivier Diaz, Clémence Jacquemin, Valentine Barthel, Eva Ogire, et al.. The current landscape of coronavirus-host protein-protein interactions. 2020. hal-02874650v2

HAL Id: hal-02874650

<https://hal.science/hal-02874650v2>

Preprint submitted on 28 Jul 2020 (v2), last revised 16 Nov 2020 (v3)

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The current landscape of coronavirus-host protein-protein interactions

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Abstract

In less than twenty years, three deadly coronaviruses, SARS-CoV, MERS-CoV and SARS-CoV-2, have emerged in human population causing hundreds to hundreds of thousands of deaths. Other coronaviruses are causing epizootic representing a significant threat for both domestic and wild animals. Members of this viral family have the longest genome of all RNA viruses, and express up to 29 proteins establishing complex interactions with the host proteome. Deciphering these interactions is essential to identify cellular pathways hijacked by these viruses to replicate and escape innate immunity. Virus-host

1 interactions also provide key information to select targets for antiviral drug development.
2 Here, we have manually curated the literature to assemble a unique dataset of 1311
3 coronavirus-host protein-protein interactions. Functional enrichment and network-based
4 analyses showed coronavirus connections to RNA processing and translation, DNA damage
5 and pathogen sensing, interferon production, and metabolic pathways. In particular, this
6 global analysis pinpointed overlooked interactions with translation modulators (GIGYF2-
7 EIF4E2), components of the nuclear pore, proteins involved in mitochondria homeostasis
8 (PHB, PHB2, STOML2), and methylation pathways (MAT2A/B). Finally, interactome data
9 provided a rational for the antiviral activity of some drugs inhibiting coronaviruses
10 replication. Altogether, this work describing the current landscape of coronavirus-host
11 interactions provides valuable hints for understanding the pathophysiology of coronavirus
12 infections and developing effective antiviral therapies.

13

14 **Keywords:** SARS-CoV-2, coronavirus, interactome, virus-host interactions, protein-protein
15 interactions

16

17 **Background**

18 Like other viruses, coronaviruses are obligate parasites and have evolved a swarm of
19 molecular interactions for hijacking the cellular machinery to replicate. Among those, the
20 subset of physical interactions between viral and cellular proteins — usually referred to as
21 the virus-host interactome — is playing a key role [1]. Mapping these protein-protein
22 interactions (PPIs) has proven extremely useful for an intimate comprehension of viral
23 replication cycles, shedding light on the molecular modules used by viruses to replicate.
24 Virus-host interactomics also helps to understand how viruses are detected by the immune
25 system but also escape immune defense through the evolution of countermeasures. Finally,
26 interactome data can be used to identify and prioritize valuable cellular targets for
27 developing antiviral drugs as previously exemplified [2][3].

28 Along with genomic sequences and viral protein structures, interactome data are
29 now considered as basic pieces of information for characterizing a virus at the molecular
30 level. Tremendous efforts have been made to characterize the emerging SARS-CoV-2 (Severe
31 acute respiratory syndrome coronavirus 2), a positive-strand RNA virus of the *Coronaviridae*
32 family, identified as the etiological agent of the ongoing COVID-19 respiratory disease

1 pandemic. In a recent report, viral proteins from the SARS-CoV-2 were individually expressed
2 in human cells and targeted host proteins were identified by affinity purification and mass
3 spectrometry [3]. This first virus-host interactome of the SARS-CoV-2 provided essential
4 information on the pathways targeted by this emerging pathogen, and allowed the authors
5 to propose a list of antiviral drug candidates to be tested. Although this represents an
6 important step in our understanding of this virus, it is known that a single interactomic study
7 cannot offer a comprehensive picture of a virus-host interactome [4]. Indeed, despite the
8 use of top-notch technologies by skilled operators, each dataset contains a substantial level
9 of unidentified interactions and artifacts that is inherent to the technology and bias the
10 results. For instance, protein complex analysis by mass spectrometry does not distinguish
11 direct and indirect virus-host interactions and is usually well-complemented by other
12 technological approaches for detecting binary PPIs such as yeast two-hybrid or protein
13 complementation assays. For these different reasons, mapping the interactome of SARS-
14 CoV-2 can be considered as a work in progress.

15 As a contribution to this effort, we used an orthogonal approach by looking at virus-
16 host interactions already reported for other coronaviruses. This compendium of data
17 gathered from literature was used to identify both overlapping and complementary
18 interactions to build the framework of a generic coronavirus-host interactome. Although
19 each coronavirus is expected to have evolved specific interactions accounting for host range
20 specificity and pathogenesis, a majority of coronavirus-host PPIs are most likely shared
21 across multiple species considering the high level of conservation of the coronavirus
22 replication machinery [5]. Gathering interactomic data from several related viruses is an
23 efficient way to fill in the blanks from literature and identify cellular pathways and
24 complexes that are common coronavirus targets. Although a proof of concept of this
25 approach was recently established [5], we were able to retrieve 10 times more interactions
26 from literature to assemble an unmatched collection of coronavirus-host interactions. In
27 addition, we identified PPIs that could explain the antiviral activity of approved drugs
28 previously characterized as coronaviruses inhibitors, thus strengthening their interest
29 against SARS-CoV-2.

30

31 **Methods**

32 ***Virus-host interaction data collection***

1 PubMed database was interrogated to collect virus-host interaction data for the following
2 coronaviruses: HCoV-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43, MERS-CoV, SARS-CoV,
3 SARS-CoV-2, TGEV, PRCV, PEDV, MHV, IBV, and PDCoV. The following query sentence was
4 used: "virus name"[Title/Abstract] AND (bind*[Title/Abstract] OR interact*[Title/Abstract]).
5 After careful analysis of the retrieved abstracts, 112 publications explicitly reporting physical
6 interactions between viral and host proteins were selected. These publications were
7 analyzed by at least two curators to determine, according to the EMBL-EBI ontology
8 nomenclature for molecular interactions, which methods were used to characterize the
9 reported interactions. Collected information were gathered in a single data file
10 (Supplementary Table 1). All human interactors were identified by their UniProt gene name.

11

12 ***KEGG pathway enrichment analysis***

13 The list of host proteins interacting with coronavirus proteins was submitted by their
14 UniProt identifier to the Functional Annotation Tool of the online knowledge base DAVID
15 Bioinformatics Resources 6.8, NIAID/NIH [6]. Statistical enrichments in KEGG pathway
16 annotations [7] were calculated by the Functional Annotation Tool, using *Homo sapiens* as
17 background, with EASE score threshold (Fisher Exact Statistics, referring to one-tail Fisher
18 Exact Probability Value used for gene-enrichment analysis) set to 0.01 and a count threshold
19 of 5 genes/pathway for short lists of proteins (below 500) or 15 for longer lists. The protein
20 list was considered to be significantly associated (enriched) with a pathway when Benjamini-
21 Hochberg adjusted p-value was above 0.05.

22

23 ***Interactions with metabolic pathways***

24 The complete list of human genes associated to "Metabolic pathways" in KEGG was
25 retrieved through DAVID Bioinformatics Resources 6.8, NIAID/NIH
26 ([https://david.ncifcrf.gov/kegg.jsp?path=hsa01100\\$Metabolic%20pathways&termId=55002](https://david.ncifcrf.gov/kegg.jsp?path=hsa01100$Metabolic%20pathways&termId=550028675&source=kegg)
27 [8675&source=kegg](https://david.ncifcrf.gov/kegg.jsp?path=hsa01100$Metabolic%20pathways&termId=550028675&source=kegg)). This list was compared to the list of host proteins interacting with
28 coronaviruses to identify overlaps. In total, 62 host genes were present in the two lists and
29 were manually clustered according to the specific pathways they belong to using KEGG
30 hierarchical annotation.

31

32 ***Metascape analysis***

1 Mestascope is a web tool designed to integrate multi-platform OMICs data [8], and was used
2 to interrogate the human interactome with the list of host proteins interacting with
3 coronaviruses. Analysis parameters set by default on Metascope website were applied
4 (“Express analysis” settings). PPIs from the human interactome were retrieved from three
5 databases: BioGrid, InWeb_IM and Omni-Path (Min Network Size=3, Max Network
6 Size=500). Densely connected regions were extracted by Metascope using the MCODE
7 algorithm. Finally, GO Enrichment analysis (“GO Biological Process”; integrated to
8 Metascope) was applied to each MCODE component independently, and the best-scoring
9 term by p-value has been retained as the functional description of the corresponding
10 component.

11

12 ***Host proteins interacting with antiviral drugs***

13 To identify host proteins interacting with drugs showing some antiviral activity against
14 coronaviruses, we used the Drug Repurposing Hub database [9]. The database was
15 downloaded (v03/24/2020), and filtered for drugs reported to inhibit coronaviruses in four
16 large-scale screenings [10][11][12][13]. Host proteins interacting with these drugs were
17 compared to the list of host proteins interacting with coronaviruses to identify overlaps.

18

19

20 **Results and discussion**

21 ***Gathering coronavirus-host interactions from literature***

22 Several members of the *Coronaviridae* family are pathogenic in human and animals,
23 and represent a threat for public health and livestock. To date, seven coronaviruses have
24 been reported to infect human (Human coronaviruses; HCoV). HCoV-NL63 and HCoV-229E
25 (from the α genus) and HCoV-HKU1 and HCoV-OC43 (from the β genus) are responsible for
26 common cold. In addition, three β coronaviruses, MERS-CoV (Middle East respiratory
27 syndrome-related coronavirus), SARS-CoV and SARS-CoV-2, are associated to life-
28 threatening respiratory diseases in human. Important animal coronaviruses include but are
29 not limited to TGEV (Transmissible Gastroenteritis Virus, α genus), PRCV (Porcine
30 Respiratory Coronavirus, α genus), PEDV (Porcine Epidemic Diarrhea Virus, α genus), MHV
31 (Murine Hepatitis Virus, β genus), IBV (Infectious Bronchitis Virus, γ genus), and PDCoV
32 (Porcine Deltacoronavirus, δ genus). The genome of coronaviruses is a positive-strand RNA

1 with two third at the 5' end occupied by the overlapping open reading frames ORF1a and b
2 that encode non-structural proteins (nsPs). The genes encoding structural proteins and a
3 variable number of accessory factors are nested at the 3' end of the genome. Viral genomes
4 are directly translated by host cell's ribosomes into a large polyprotein encoded by the
5 ORF1a/b gene. This polyprotein is cleaved into 16 non-structural proteins (nsP1-16), except
6 for IBV and PDCoV where nsP1 is missing. Most of these nsPs assemble into a complex that
7 replicates viral genome and synthesizes subgenomic mRNA from the other genes to express
8 structural proteins S, E, M and N and the additional accessory factors.

9 We collected virus-host interaction data from literature for the 13 coronaviruses
10 mentioned above (*i.e.* HCoV-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43, MERS-CoV, SARS-
11 CoV, SARS-CoV-2, TGEV, PRCV, PEDV, MHV, IBV, and PDCoV), and 112 publications explicitly
12 reporting physical interactions between viral and host proteins were identified (Figure 1a).
13 Their analysis allowed us to collect 1,544 entries for virus-host PPIs that were gathered in a
14 single data file (Supplementary Table 1). As 133 interactions were characterized by more
15 than one method or were detected across multiple host species, and that 23 interactions
16 were reported in two or more independent publications, this corresponds to 1,311 distinct
17 virus-host interactions involving 1,140 different host proteins (orthologous proteins from
18 different host species were collapsed; Figure 1a and Supplementary Table 2). A majority of
19 the reported interactions (92%) were from four viruses for which high-throughput
20 interactomic methods have been applied: MHV, SARS-CoV-2, SARS-CoV, and IBV (Figure 1b,
21 Supplementary Table 3) [3][14][15][16][17][18][19][20].

22

23 ***Shared interactions and host protein targets across multiple coronaviruses***

24 We then looked for interactions that are conserved across two or more viruses. To
25 perform this analysis, orthologous proteins from different host species were collapsed as
26 above. In total, we identified 51 orthologous interactions in the dataset, providing a robust
27 network of shared PPIs between several coronaviruses (Figure 1c; Supplementary Table 4).
28 Membrane receptors shared by several coronaviruses (ACE2, ANPEP) as well as cellular
29 proteases involved in the processing of the spike glycoproteins S (TMPRSS2, TMPRSS11D,
30 CTSB, CTSL, FURIN) were highlighted. Multiple interactions between the nucleoprotein N and
31 host factors involved in mRNA synthesis, maturation, nuclear export, translation and stability
32 were also identified (EEF1A1, PABPC1, PABPC4, RNRNPA1, HNRNPU, YBX1, LARP1), including

1 ribosomal components (RPS9, RPS13, RPS19, RPL26), multiple helicases (UPF1, MOV10,
2 DHX9, DDX21, DDX1) and key components of the innate antiviral response (G3BP1, G3BP2,
3 PRKRA). GSK3A/B were previously reported to phosphorylate the viral nucleocapsid of MHV
4 to recruit DDX1 [21]. In the assembled interactome dataset, GSK3A/B were also found to
5 interact with N of IBV and SARS-CoV, and interaction of DDX1 with both N and nsP14 of IBV
6 and TGEV as well as nsP14 of SARS-CoV were also reported [20][22][23][24]. This analysis
7 also highlights the well-known function of nsP3 in coronavirus escape from the innate
8 immune response through interactions with both ubiquitination/ISGylation factors (RCHY1,
9 ISG15) and key antiviral factors (STING1, TBK1, IRF3)[19][25][26][27][28][29]. Other
10 remarkable interactions are between nsP2, EIF4E2 (4EHP) and GIGYF2, which are two
11 components of a complex repressing mRNA translation [30]. Although EIF4E2 and GIGYF2
12 were identified by high-throughput interactomic applied to SARS-CoV, SARS-CoV-2 and MHV
13 [17][3][14], they were never investigated in details. These highly conserved interactions
14 suggest a crucial role of nsP2 in regulating viral and/or cellular mRNA translation and
15 degradation.

16 We also looked for host proteins interacting with several coronaviruses, but not
17 necessarily targeted by the same viral proteins. Because coronavirus proteins assemble into
18 large molecular complexes, the same host protein can be captured, either directly or
19 indirectly, by distinct viral proteins used as bait. In total, 105 of the 1,140 host proteins from
20 the dataset (9.2%) were shared by at least two coronaviruses, including 50 host proteins
21 involved in the conserved interactions displayed in Figure 1c. Shared targets of different
22 viruses are illustrated in Figure 1d and details are provided in Supplementary Table 5.
23 Interestingly, several host factors involved in viral RNA sensing (DDX58, IFIH1, PRKRA), DNA
24 sensing (STING1) or downstream signalling to activate the innate immune response (TRAF3,
25 TBK1, IKBKE, IKBKB, ISG15, IRF3) were targeted by unrelated viral proteins (Figure 1e)
26 [3][28][29][31][32][33][34][35][36][37][38][39][40][41][42][43][44]. This highlights the
27 importance of these interactions for coronaviruses, but is puzzling from an evolutionary
28 perspective. Indeed, this suggests that coronaviruses have evolved different but convergent
29 strategies to target these innate immunity factors. An alternative explanation is that
30 multiple proteins of a virus target the same host factor in redundant ways, although this
31 information is not present in the current interaction datasets yet. The two hypotheses are
32 not mutually exclusive and this will require further investigation to be addressed.

1

2 ***Enrichment analysis for specific cellular pathways.***

3 To identify cellular complexes or pathways that are enriched in the list of host factors
4 interacting with coronavirus proteins, we retrieved associated KEGG annotations and
5 calculated statistical enrichments using the DAVID functional annotation tool [7][6]. To
6 evaluate the interest of combining interactome data from several coronaviruses, we
7 analyzed host targets of SARS-CoV-2 alone (Table 1a), of human highly-pathogenic
8 coronaviruses (Table 1b) and of all coronaviruses (Table 1c). Although SARS-CoV-2 restricted
9 analysis only identified “Protein processing in endoplasmic reticulum” and “RNA transport”
10 as significantly enriched KEGG pathways, expanding the dataset doubled the number of host
11 factors falling in these two categories and unraveled enrichments for “Ribosome”, “RIG-I-like
12 receptor signaling”, “Endocytosis”, “Spliceosome” and “Phagosome” pathways (Table 1c and
13 Supplementary Table 6). Up to 68% of the KEGG-annotated eukaryotic ribosomal proteins
14 and 18% of the spliceosome components are targeted by coronavirus proteins, thus
15 demonstrating the importance for these viruses to control the mRNA processing and
16 translation machinery. We used KEGG Mapper to highlight targeted host proteins on
17 schematic representation of the most enriched cellular pathways (Figure 2a-d)[45]. As
18 shown in Figure 2a, several factors involved in protein export were targeted together with
19 components of the unfolded protein response/endoplasmic-reticulum-associated protein
20 degradation (ERAD) pathway. This observation is in line with numerous reports showing that
21 coronavirus replication induces a strong ER stress response, and that virulence factors
22 expressed by these viruses aim at controlling and even subvert this cellular response to
23 assemble convoluted and double-membrane vesicles [46]. Several regulators of intracellular
24 trafficking and vesicular transport associated to endocytic compartments, especially late
25 endosomes and multivesicular bodies, are also hijacked (Figure 2b). These interactions most
26 likely contribute to the entry, assembly and/or secretion of viral particles [47]. A statistical
27 enrichment of the KEGG annotation “RNA transport” was also observed because
28 components of the nuclear pore complex (NPC) and translation initiation factors, which take
29 in charge mRNA after their transport through the nuclear pore, are also overrepresented in
30 the list of host proteins targeted by coronaviruses (Figure 2c). The number of Interactions
31 with translation initiation factors, together with ribosomal components, reflects the intense
32 hijacking of translational machinery by coronaviruses [14]. Some of these interactions could

1 also prevent the translational shut-off associated to antiviral and unfolded protein responses
2 as demonstrated for the ORF7 of TGEV [48], while others could help the virus to control host
3 protein expression [49]. Consequences of viral factors interacting with components of the
4 NPC are poorly documented [50], but interference mechanisms with the innate antiviral
5 response have been suggested [51]. Whether these interactions prevent the nuclear import
6 of transcription factors or the nuclear export of cellular mRNA to blunt the immune response
7 as shown for other viruses should be explored [52]. Finally, this analysis highlighted multiple
8 interactions with components of the RIG-like receptor pathways (Figure 2d), that is essential
9 for viral RNA detection and the induction of interferon secretion. This includes the multi-
10 targeted host factors already presented in Figure 1e but also additional proteins such as the
11 adaptors MAVS (IPS1), TBKBP1 (SINTBAD), TRAF2 and TRAF6, the negative (NLRX1) or
12 positive (DDX3X) regulators, and kinases such as RIPK1 (RIP1) or IKBKG (IKK γ)[3][20][53][54].
13 Some of these interactions probably reflect the sensing of coronavirus components by
14 immune receptors which trigger the antiviral response, whereas others correspond to
15 countermeasures that coronaviruses evolved to escape this response [55]. Altogether, this
16 provides an overview of cellular pathways that are major targets of coronaviruses.

17 Despite several reports showing that viruses have evolved strategies to finely tune
18 cellular metabolism for promoting their replication [56], no statistical enrichment for
19 components of a specific metabolic pathway was identified. We thus conducted an
20 orientated analysis by looking at potential overlaps between the list of host proteins
21 targeted by coronaviruses and the proteins annotated in the “Metabolic pathways” of the
22 KEGG database. In total, 62 proteins were identified at the intersection of the two lists, and
23 were clustered according to their specific metabolic pathways using KEGG annotation
24 (Figure 3). Multiple interactions were identified with factors involved in nucleotide
25 metabolism, including enzymes of the nucleotide/-side biosynthesis and degradation
26 pathways (IMPDH2, RRM2, ADK, DCTPP1, NT5C2, XDH, ADA) and more surprisingly, several
27 components of cellular DNA and RNA polymerases (POLA1, POLA2, POLD1, PRIM1, PRIM2
28 and POLR2B). These interactions could contribute to the DNA replication stress and cell cycle
29 arrest induced by coronaviruses as previously suggested for the nsP13-POLD1 interaction,
30 but their role is still poorly defined [57]. Several components of complex I of the
31 mitochondrial respiratory chain were also targeted (NDUF9/10/13), as well as V-type ATPase
32 subunits involved in the acidification of cellular compartments, especially during

1 phagocytosis (Supplementary Table 6). Lipid and glycan biosynthesis enzymes were highly
2 represented, in line with previous reports showing the dependence of coronaviruses to
3 these two pathways [58][59]. In the “amino acid metabolism” cluster, both methionine
4 adenosyltransferase 2A (MAT2A) and the regulatory subunit MAT2B were present (captured
5 by ORF3 from PEDV and nsP9 of SARS-CoV-2, respectively). MAT2A catalyzes the synthesis of
6 S-adenosyl-L-methionine, the major biological methyl donor. This may indicate that
7 coronaviruses critically need S-adenosyl methionine (SAM) to methylate the viral RNA cap
8 structures to allow transcription and prevent their recognition by cellular innate immunity
9 receptors [60]. In the same cluster were also found two methyltransferases (COMT,
10 DNMT1). Although the significance of targeting COMT (Catechol O-Methyl Transferase), an
11 enzyme involved in catecholamine synthesis, remains elusive, interactions with DNMT1
12 could be a mechanism used by coronaviruses to alter the epigenetic landscape of infected
13 cells as observed for other viruses [61]. Finally, coronavirus proteins also interacted with
14 several host factors flagged as “central carbon metabolism” enzymes. This cluster includes
15 multiple enzymes contributing to protein sialylation (GNE, NANS) and glycosylation (UGP2),
16 inositol synthesis (MTMR3, ISYNA1), and two enolases (ENO1, ENO3) whose interactions
17 with viral proteins could modulate glycolysis and energy supply for promoting viral growth.
18 Altogether, this analysis suggests that, although the completeness of the available dataset
19 does not allow statistical validation, coronaviruses extensively interact with metabolic
20 enzymes.

21

22 ***A network-based identification of host protein complexes targeted by coronaviruses***

23 To identify densely connected regions in the list of targeted host proteins, we used
24 the web-based application Metascape [8]. As no result could be retrieved when the full list
25 of 1,140 host proteins was submitted to Metascape, we limited this analysis to the core list
26 of 178 host proteins for which multiple experimental evidences exist to support an
27 interaction with coronavirus proteins. We also excluded the interactors of the spike
28 glycoproteins that are already well-characterized and skewed the analysis of the other virus-
29 host PPIs (Supplementary Table 7). From the list of 156 host proteins, this identified 7
30 protein clusters whose biological role was determined by Metascape using GO enrichment
31 analysis (Figure 4)[62][63] and largely confirmed our analyses discussed above. The
32 functional annotations enriched in the four largest clusters were “mRNA catabolic process”,

1 “Regulation of type I interferon production”, “Response to virus”, and “Translation”. These
2 interaction modules partially overlap and well-complement two of the KEGG pathways
3 pinpointed by the enrichment analysis described above, *i.e.* “RNA transport” and “RIG-I-Like
4 Receptor Signaling Pathway”, respectively (Fig. 2c and d; Supplementary Table 6). The
5 “mRNA catabolic process” cluster contains four proteins involved in KEGG’s “RNA transport
6 pathway”. This includes UPF1 that is involved in nonsense-mediated decay of mRNAs
7 containing premature stop codons, EIF4A2 and EIF4E2 that repress mRNA translation, and
8 the importin subunit KPNB1. Interestingly, this analysis also highlighted the link between
9 XRCC5/6 dimers and components of the RIG-like receptor pathway. XRCC5/6 binds to DNA
10 double-strand break ends and activates the catalytic activity of DNA-PK to promote DNA
11 repair. In the cytosolic compartment, it also participates to type I interferon induction in
12 response to foreign DNA [64]. Furthermore, it has been shown that DNA-PK is activated and
13 participates to interferon induction in dengue virus-infected cells [65], and that NS5A from
14 Hepatitis C virus (HCV) is phosphorylated by this kinase [66]. The targeting of XRCC5 and 6 by
15 the N protein of IBV and OC43 [20][67] suggests that these proteins also play a role in the
16 induction of innate immunity and/or the replication of coronaviruses. Three clusters of only
17 three components were also identified. The first one includes PHB, PHB2 and STOML2 that
18 are all members of the stomatin-prohibitin-flotillin-HflC/K (SPFH) superfamily. These
19 proteins colocalize at the inner mitochondrial membrane where they assemble into ring-like
20 structures [68]. Cornillez-Ty CT. & al. previously highlighted interactions of SARS-CoV nsP2
21 with prohibitins PHB and PHB2 [17], and STOML2 was also present in their dataset.
22 Interestingly, the recent report of Gordon & al. also identified an interaction of ORF3B from
23 SARS-CoV-2 with STOML2 [3]. These cellular proteins regulate mitochondrial homeostasis,
24 and are involved in processes such as mitophagy and mitochondrial fusion. By targeting
25 these proteins, coronavirus proteins may also impact key mitochondrial functions such as
26 respiration but also lipid homeostasis and innate immunity. Indeed, mitochondria are
27 involved in both lipogenesis and lipolysis, and prohibitin expression has been shown to
28 impact lipid accumulation and degradation [69][70]. It should be determined if such
29 interactions with prohibitins contribute to the massive remodeling of intracellular lipid
30 membranes induced by coronavirus infections. As prohibitins also regulate mitochondrial
31 fusion and fission, for which the impact on antiviral-signaling has been well documented
32 [71], this could have indirect consequences on the antiviral response. In addition, recent

1 studies have established physical interactions between components of the prohibitin
2 complex and MAVS, a pivotal adaptor in viral RNA sensing and interferon induction [72]. The
3 second small cluster is composed of MARK1, 2, and 3, three Ser/Thr protein kinases involved
4 in the control of cell polarity, microtubule stability and cancer. MARK proteins have been
5 reported to interact with nsP2 of MHV, nsP13 of SARS-CoV, and ORF9B of both SARS-CoV
6 and SARS-CoV-2 [3][14][15][16]. Although their role in coronavirus replication is unknown,
7 they could contribute to viral trafficking as they control microtubule dynamics and vesicular
8 transport. Interestingly, several approved drugs inhibiting MARK kinases were recently
9 proposed as potential antivirals against coronaviruses and especially SARS-CoV-2 [3]. The
10 last cluster is composed of the cytoskeleton and microtubule-associated proteins DCTN2,
11 FBF1 and CKAP5 that could participate to the transport of viral complexes within infected
12 cells.

13

14 ***Overlaps with functional in vitro screenings of chemical drug libraries***

15 To provide a rationale for the antiviral activity of some drugs targeting cellular factors,
16 we determined to what extent the coronavirus-host interactome intersects the large-scale
17 chemical screenings of antivirals against coronaviruses. We selected four publications
18 reporting *in vitro* screening of chemical libraries against SARS-CoV, MERS-CoV and HCoV-
19 OC43 [10][11][12][13]. In total, 77 molecules were identified for their antiviral activity
20 against at least one coronavirus. Chloroquine was identified in all four publications,
21 amodiaquine and loperamide were identified in three of them, and chlorpromazine,
22 cycloheximide, emetine, and hydroxychloroquine were cited in two publications. Then we
23 searched for cellular targets of these 77 molecules using the Drug Repurposing Hub
24 database [9], and looked for overlaps with the list of 1,140 host proteins interacting with
25 coronaviruses. We identified 9 drugs and 13 host proteins matching these criteria (Figure 5).
26 Anisomycine, cycloheximide and homoarringtonine are translational inhibitors interacting
27 with ribosomal components. As coronaviruses exhibit tight interactions with translation
28 initiation factors and recruit multiple ribosomal components (Table 1c and Figure 4), this
29 could explain the antiviral effect of these three drugs. Three molecules, fluphenazine,
30 loperamide and chlorpromazine, target calmodulin (CALM1) which interacts with ORF3 from
31 PEDV [42]. CALM1 is a Ca²⁺-binding messenger protein that regulates the function of
32 numerous proteins. In particular, it controls the activity of the multifunctional CAMKII

1 proteins (Ca²⁺/calmodulin-dependent protein kinase II), which are targeted by
2 coronaviruses as well. Indeed, CAMK2D and CAMK2G interact with nsP3 of SARS-CoV and
3 nsP2 of MHV, respectively (Supplementary Table 1)[14][19]. Furthermore, ACE2 receptor of
4 SARS-CoV-2, SARS-CoV, HCoV-NL63 and HCoV-229E, and CEACAM1 receptor of MHV are also
5 calmodulin-binding proteins [73][74][75]. Although fluphenazine, loperamide and
6 chlorpromazine have multiple targets in host cells, interactions with CALM1 could account
7 for the inhibition of coronaviruses by these drugs. Clomipramine, which is structurally and
8 functionally closely related to chlorpromazine, was also selected in the analysis as a
9 consequence of its interaction with GSTP1, one of the glutathione S-transferase isoenzymes.
10 This analysis also highlighted interactions between coronavirus proteins and host factors
11 involved in nucleotide biosynthesis. First, nsP14 interacts with inosine-5'-monophosphate
12 dehydrogenase 2 (IMPDH2), a key enzyme of *de novo* purine biosynthesis pathway. The
13 inhibitor mycophenolic acid, that is depleting cells in purine, is well-known for its broad-
14 spectrum antiviral activity and is in a list of drug candidates proposed against SARS-CoV-2
15 [3]. In addition, the analysis pinpointed to the interaction of nsP2 from MHV with the host
16 enzyme RRM2, one of the two subunits of ribonucleotide reductase that is inhibited by
17 gemcitabine. This interaction between an enzyme involved in deoxyribonucleotide synthesis
18 and an RNA virus protein was unexpected. However, RRM2 was previously reported to
19 interact with NS5B from HCV, preventing its degradation and promoting the replication of
20 this RNA virus [76]. Furthermore, gemcitabine has been shown to inhibit the replication of
21 many different RNA viruses by blocking cellular DNA replication. Indeed, this induces a
22 genomic stress that triggers the innate antiviral response [77]. Thus, RNA viruses could have
23 evolved interactions with cellular enzymes involved in nucleoside/nucleotide synthesis such
24 as IMPDH and RRM2 to sustain DNA replication and prevent genotoxic stress.

25

26 **Concluding remarks**

27 We have assembled a large coronavirus-host interactome built upon 1311 PPIs
28 retrieved from literature. Functional annotation and network-based analyses highlighted
29 targeted cellular pathways and modules. Overall, mRNA processing and transport,
30 translation initiation and protein translation, endosomal trafficking, and innate immunity
31 were the most enriched pathways. This conclusion is consistent with previous reports
32 identifying these biological processes as targets of specific coronaviruses. The global analysis

1 suggest that these interactions are highly conserved across most if not all coronaviruses. We
2 also pinpointed to a couple of small protein complexes that appear particularly relevant to
3 coronavirus infection but were not previously investigated. This includes in particular the
4 EIF4E2-GIGYF2 dimer involved in the repression of protein translation, the MAT2A-MAT2B
5 complex controlling SAM synthesis, the DNA-PK kinase that contributes to interferon
6 induction, and the mitochondrial proteins PHB, PHB2 and STOML2 regulating mitophagy.
7 Multiple evidences also support a key role of the MARK kinases. Finally, we identified a
8 dozen of host factors that are bound by coronavirus proteins and functionally modulated by
9 compounds selected from antiviral screens, giving hints to explain the inhibition of
10 coronavirus replication by these drugs. Among these molecules is chlorpromazine, an
11 antipsychotic drug that is currently evaluated in a clinical trial against SARS-CoV-2 [78]. In
12 conclusion, this work has highlighted the importance of several neglected coronavirus-host
13 interactions that deserve to be further investigated. It also illustrates the interest of
14 combining virus-host interactome datasets from different laboratories, obtained by different
15 approaches and generated in various cell types to increase coverage and get closer to
16 completeness.

17

18 **Abbreviations:** SARS-CoV: Severe acute respiratory syndrome coronavirus, MERS-CoV:
19 Middle East respiratory syndrome-related coronavirus, PPIs: Protein-Protein Interactions,
20 HCoV: Human coronavirus, TGEV: Transmissible Gastroenteritis Virus, PRCV: Porcine
21 Respiratory Coronavirus, PEDV: Porcine Epidemic Diarrhea Virus, MHV: Murine Hepatitis
22 Virus, IBV: Infectious Bronchitis Virus, PDCoV: Porcine Deltacoronavirus, nsPs: non-structural
23 proteins, ERAD: endoplasmic-reticulum-associated protein degradation, ER: endoplasmic
24 reticulum.

25

26 **Declarations**

27 ***Ethics approval and consent to participate***

28 *Not applicable*

29 ***Consent for publication***

30 *Not applicable*

1 ***Availability of data and materials***

2 All data generated or analyzed during this study are included in this published article and its
3 supplementary information file.

4 ***Competing interests***

5 The authors declare that they have no competing interests.

6 ***Funding***

7 This work was supported by the Institut National de la Santé et de la Recherche Médicale
8 (INSERM) and the Centre National de Recherche Scientifique (CNRS) and Université de Lyon.
9 EO is supported by the Conseil Régional de l'île de La Réunion DIREC 20181189.

10 ***Authors' contributions***

11 LPC, OD, CJ, VB, EO, and POV collected data from literature. LPC, OD and POV analyzed the
12 data and generated the figures. LPC, POV, OD and VL wrote the manuscript. PA and CR
13 provided advises to analyze data and build the figures, and corrected the manuscript. All
14 authors read and approved the final manuscript.

15 ***Acknowledgements***

16 *Not applicable*

17

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- 22
- 23

1 **Figure Legends**

2 **Figure 1 Quantitative analysis of collected virus host-interactions.** (A) Key numbers
3 describing the database that has been assembled. (B) Numbers of distinct interactions that
4 have been collected for each virus. (C) Orthologous interactions conserved between several
5 viruses. The thickness of the lines is proportional to the number of viruses for which the
6 interaction was reported. Displayed graph was generated using Cytoscape [79]. (D) Circular
7 diagram showing the proportion of shared host protein targets between analyzed
8 coronaviruses. Display was obtained using the Circos table viewer [80]. (E) Innate immunity
9 factors interacting with several coronavirus proteins.

10

11 **Figure 2 KEGG pathways enriched in the list of coronavirus-interacting proteins.** (A) Protein
12 processing in endoplasmic reticulum (KEGG map ID: 04141). (B) Endocytosis (KEGG map ID:
13 04144). (C) RNA transport (KEGG map ID: 03013). (D) RIG-I-like receptor signaling (KEGG
14 map ID: 04622). Host proteins interacting with coronavirus proteins are marked with red
15 stars.

16

17 **Figure 3 Interactions of coronavirus proteins with metabolic pathways.** Coronavirus-
18 interacting proteins that are involved in cellular metabolism are displayed (i.e. with the KEGG
19 tag “metabolic pathway”). Host factors were clustered and colored according to the specific
20 pathways they belong to using KEGG annotation.

21

22 **Figure 4 Interactions of coronavirus proteins with clusters of tightly connected proteins in**
23 **the human interactome.** We established a core list of host proteins for which multiple
24 experimental evidences exist to support an interaction with coronavirus proteins. This
25 includes virus-host interactions validated by different technics in one report or confirmed
26 across multiple publications. Host proteins captured independently by different viral proteins
27 were also included. Metascope was used to identify the seven clusters that are presented
28 (blue lines indicate PPIs from the human interactome). Supporting virus-host interactions are
29 detailed in the left table. Functional annotation tool integrated to Metascope was used to
30 determine most statistically enriched GO terms (“Biological Process”) and annotate the
31 clusters (p-values are indicated).

32

1 **Figure 5 Intersection between the coronavirus-host interactome and cellular targets of**
2 **drugs inhibiting coronavirus replication.** Viral proteins are in red, host proteins in blue and
3 small molecules in green. Virus-host PPIs are from the coronavirus-host interactome.
4 Compound-target interactions were retrieved from the Drug Repurposing Hub database. For
5 each molecule, stars indicate how many screenings out of the four compiled for this analysis
6 identified their anti-coronavirus activity.
7
8

1 **Table 1**

2 **a- Kegg pathways enrichment in SARS-CoV-2 host protein targets (n=333)**

Kegg pathway (Term)	Count	%	p-Value	Fold Enrichment	Benjamini p-value
Protein processing in endoplasmic reticulum	13	4	8,80E-05	4	1,70E-02
RNA transport	12	3,7	4,40E-04	3,6	4,10E-02

3

4 **b- Kegg pathways enrichment in highly pathogenic hCoVs host protein targets (n=483)**

Kegg pathway (Term)	Count	%	p-Value	Fold Enrichment	Benjamini p-value
RIG-I-like receptor signaling pathway	14	3	1,30E-07	6,6	3,00E-05
NF-kappa B signaling pathway	13	2,8	1,10E-05	4,9	8,00E-04
RNA transport	19	4,1	4,10E-06	3,6	4,50E-04
Protein processing in endoplasmic reticulum	18	3,9	1,30E-05	3,5	7,20E-04
Epstein-Barr virus infection	13	2,8	3,10E-04	3,5	1,10E-02
Influenza A	18	3,9	1,90E-05	3,4	8,40E-04
Measles	13	2,8	6,80E-04	3,2	2,10E-02

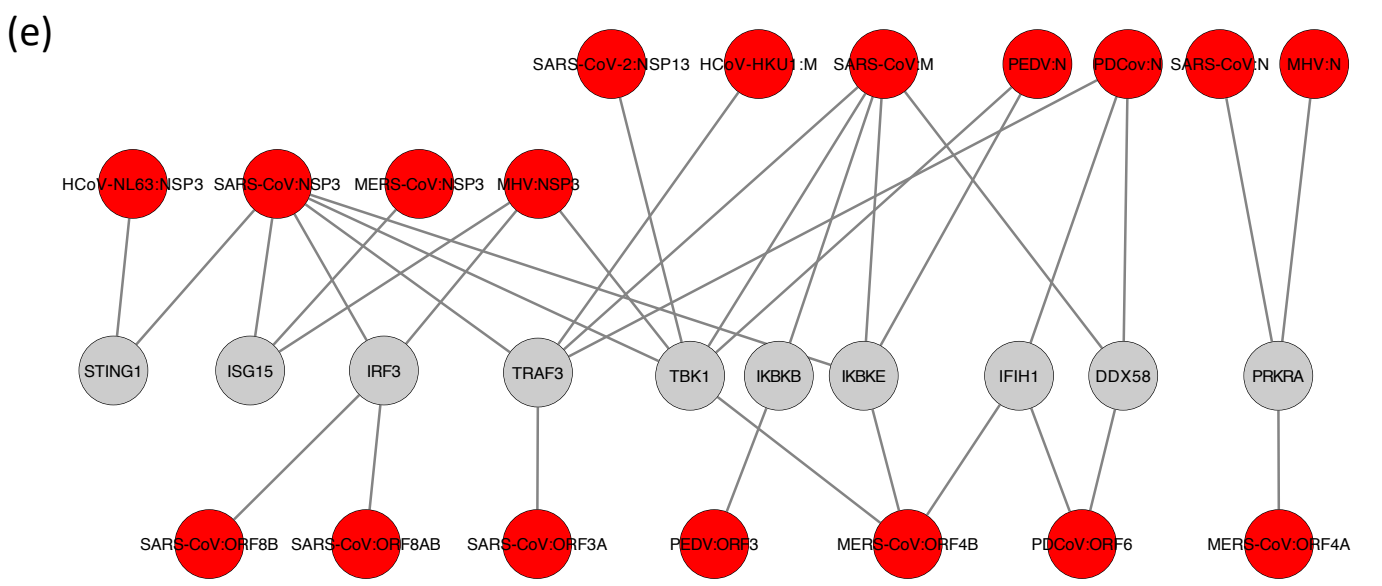
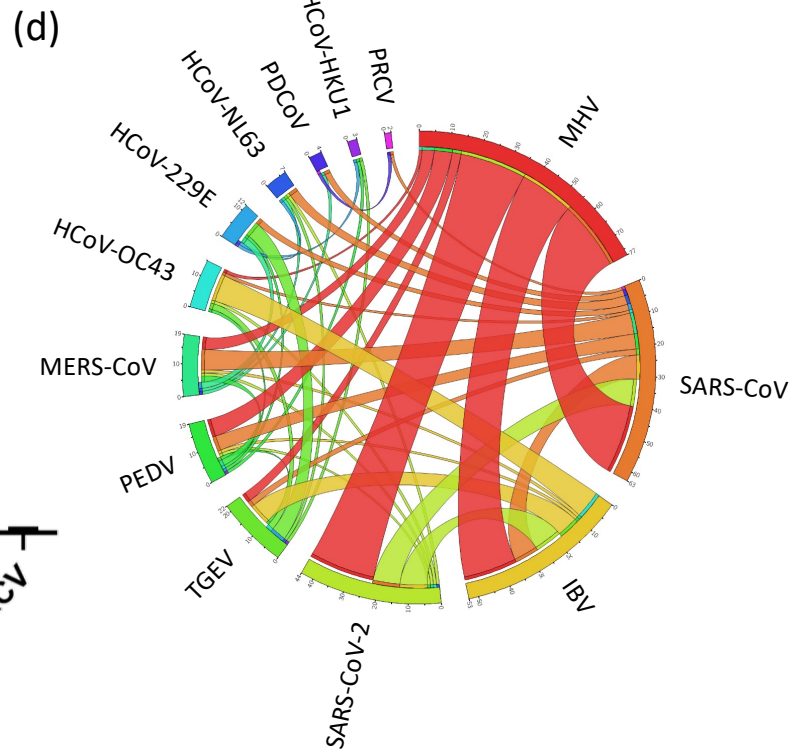
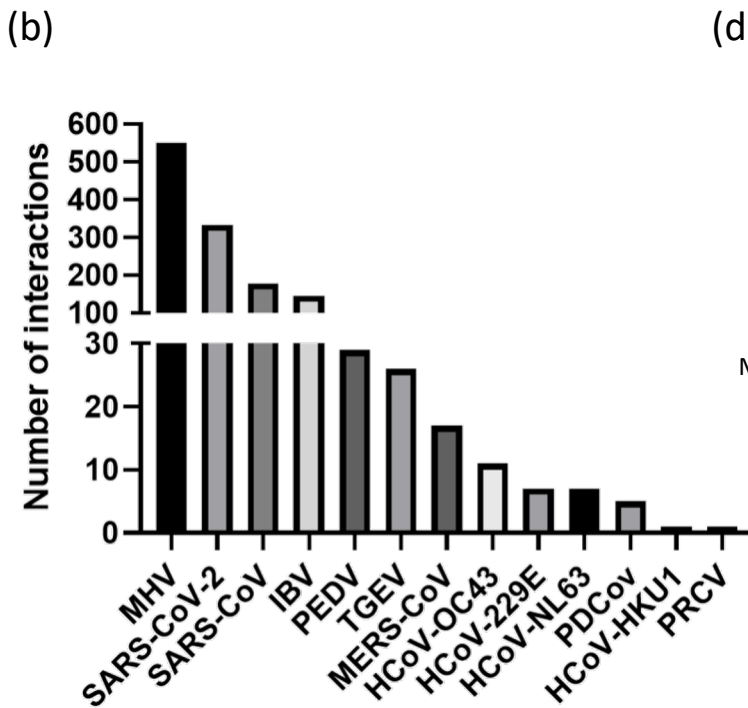
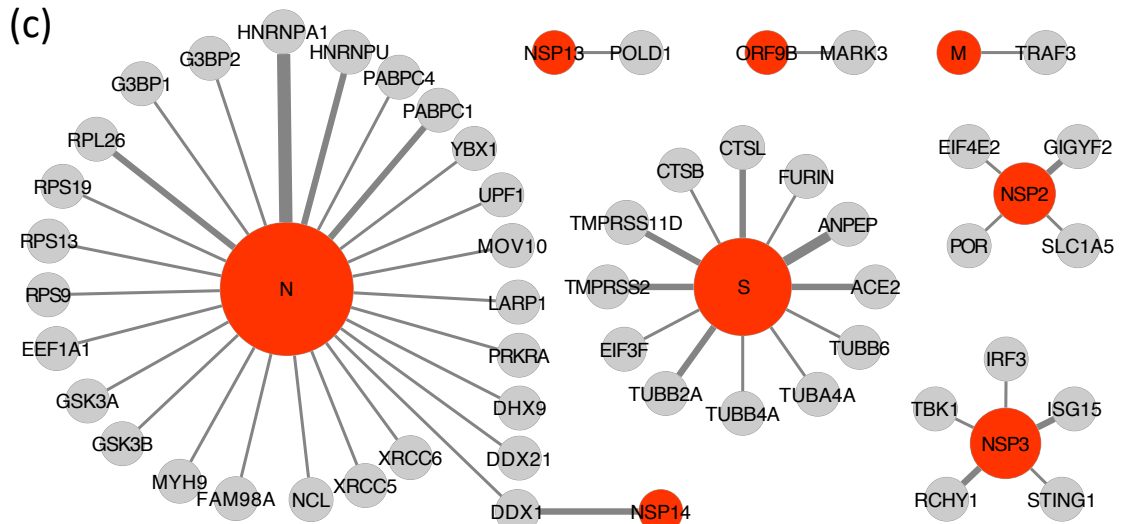
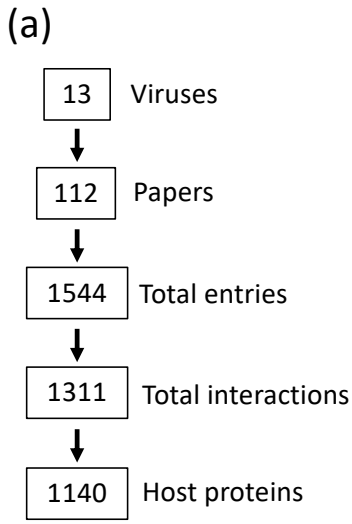
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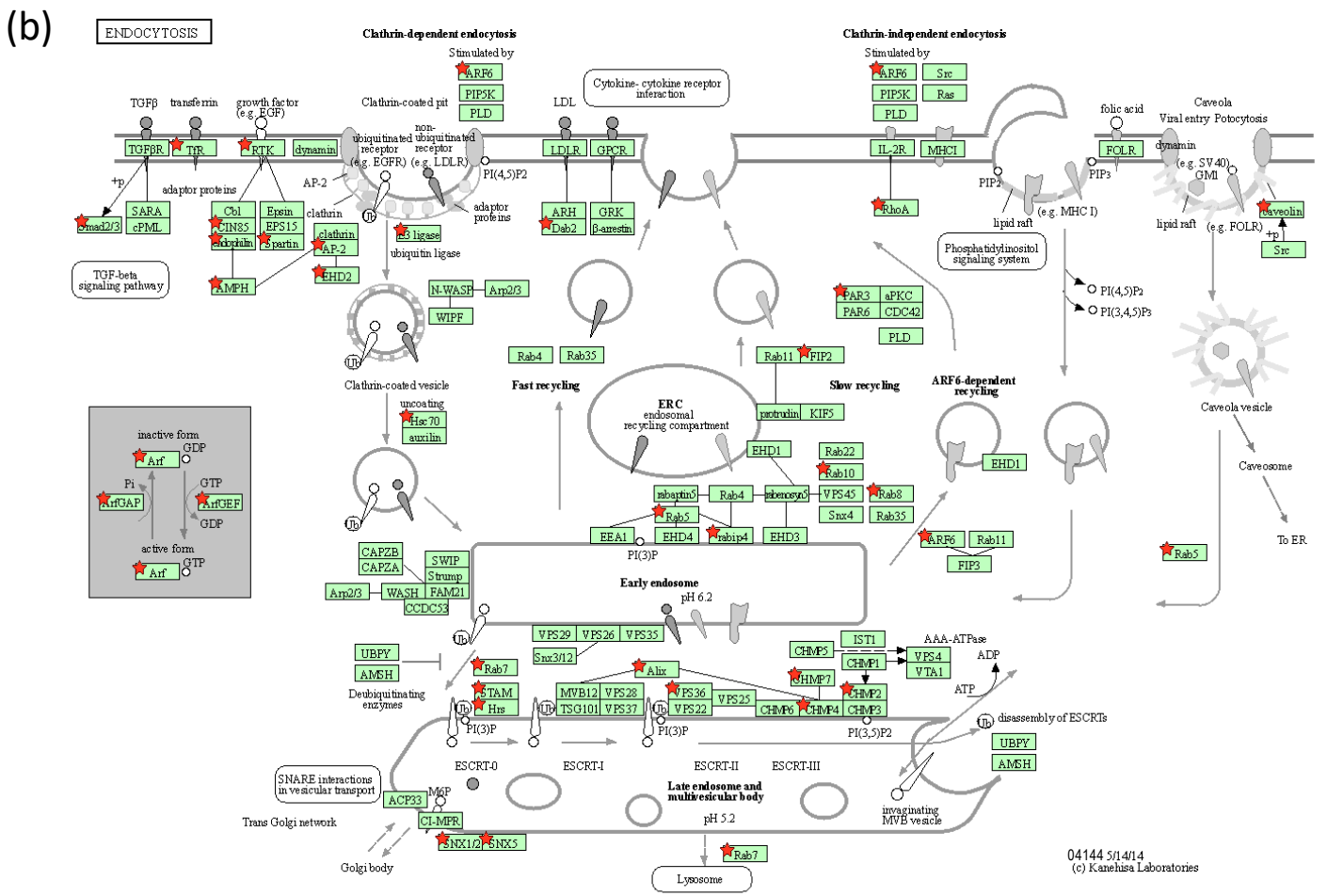
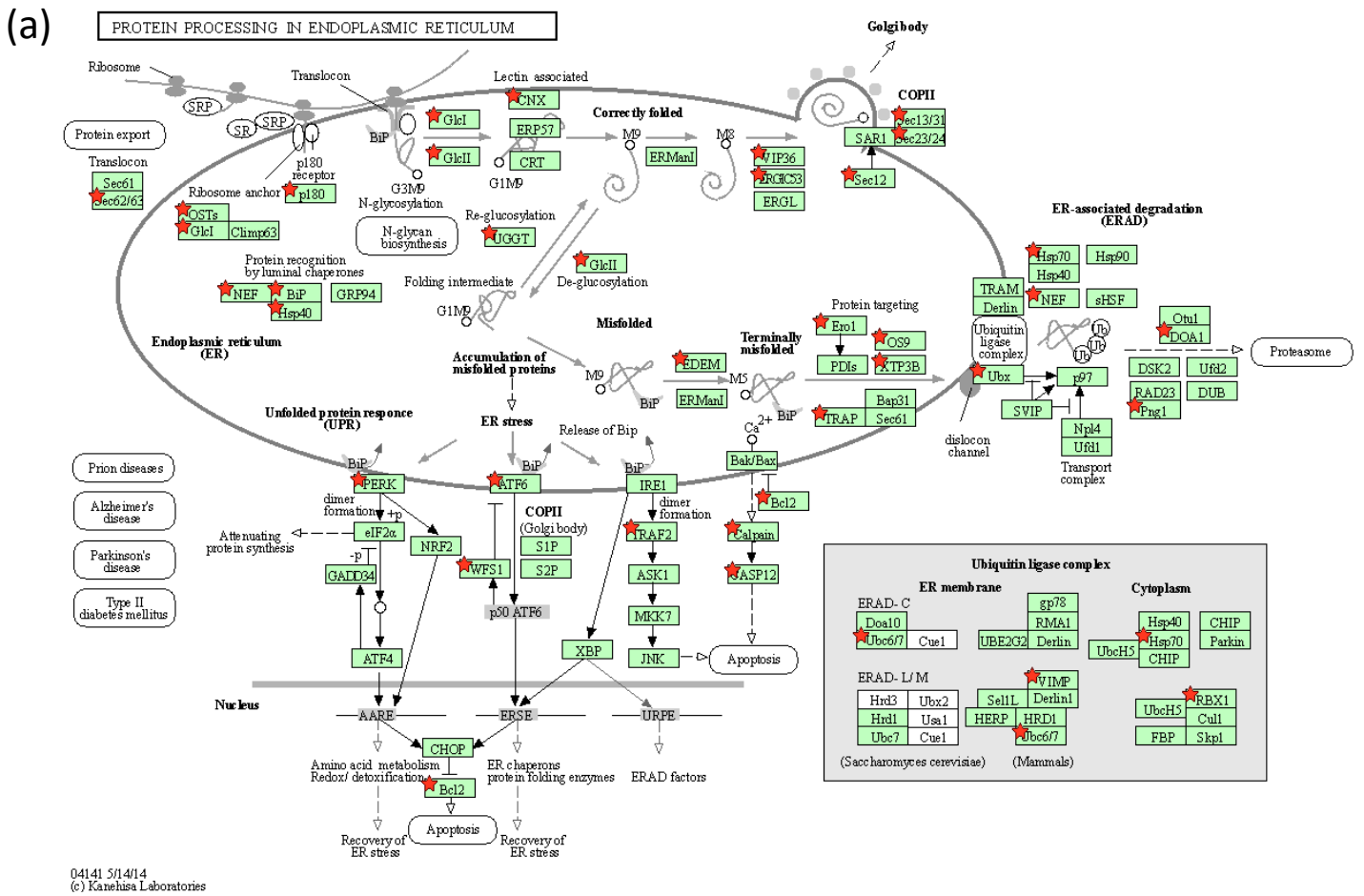
6 **c- Kegg pathways enrichment in Coronaviruses host protein targets (n=1,140)**

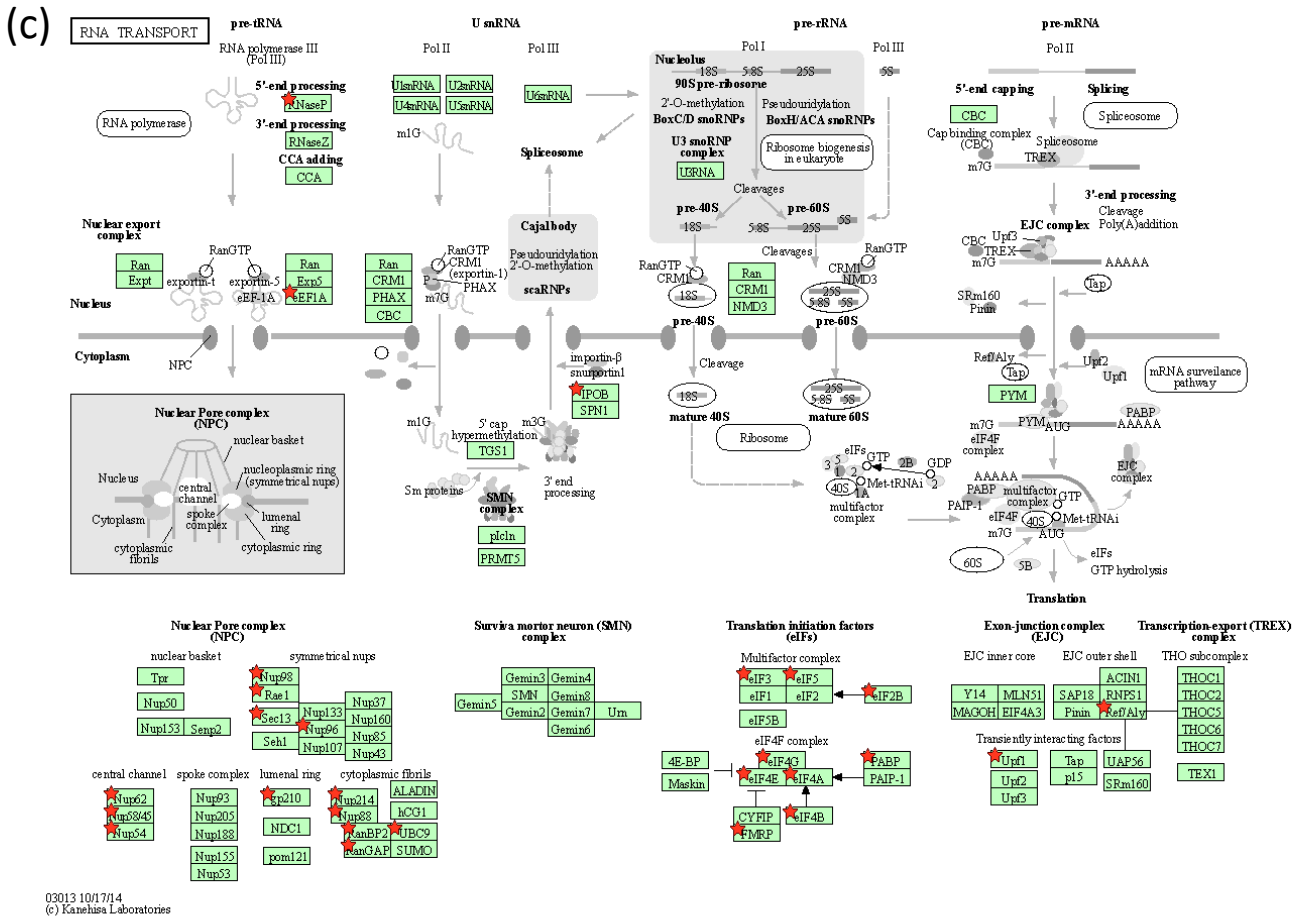
Kegg pathway (Term)	Count	%	p-Value	Fold Enrichment	Benjamini p-value
Ribosome	59	5,4	9,0E-31	5,8	2,3E-28
RIG-I-like receptor signaling pathway	16	1,5	1,60E-04	3,1	5,60E-03
Protein processing in endoplasmic reticulum	37	3,4	5,20E-09	2,9	6,60E-07
RNA transport	33	3	1,00E-06	2,6	6,30E-05
Endocytosis	43	3,9	1,30E-07	2,4	1,10E-05
Spliceosome	24	2,2	1,10E-04	2,4	4,70E-03
Phagosome	26	2,4	1,00E-04	2,3	5,30E-03

7

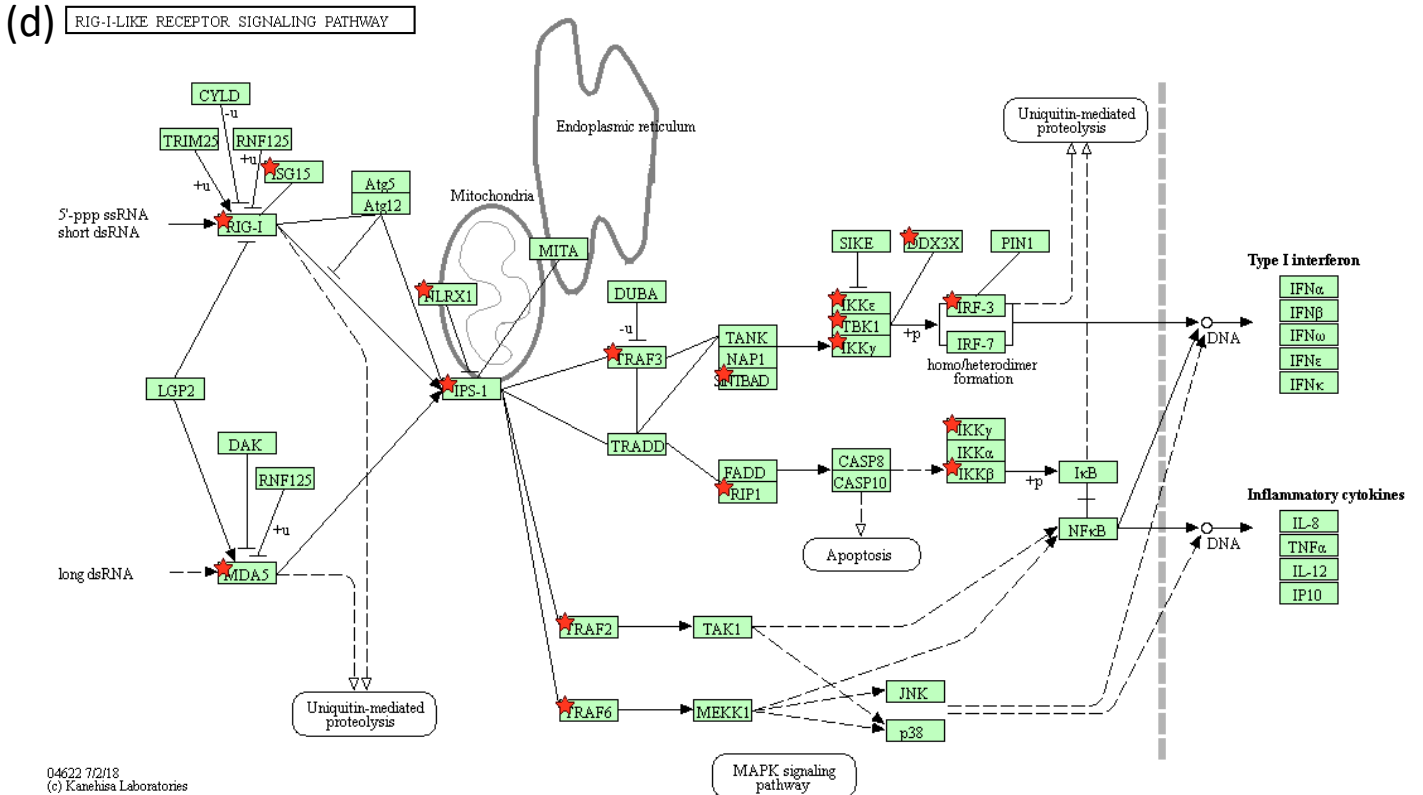
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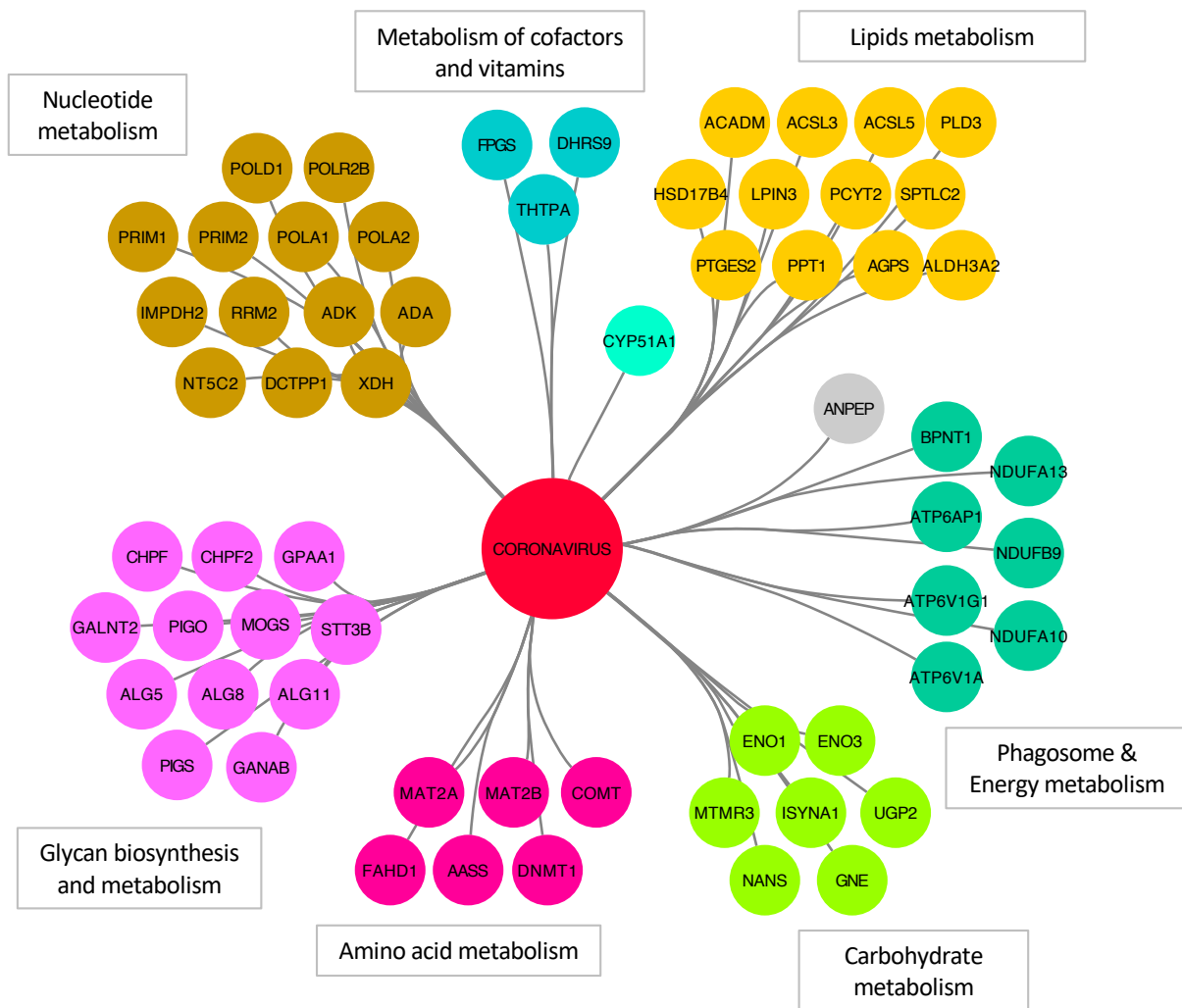




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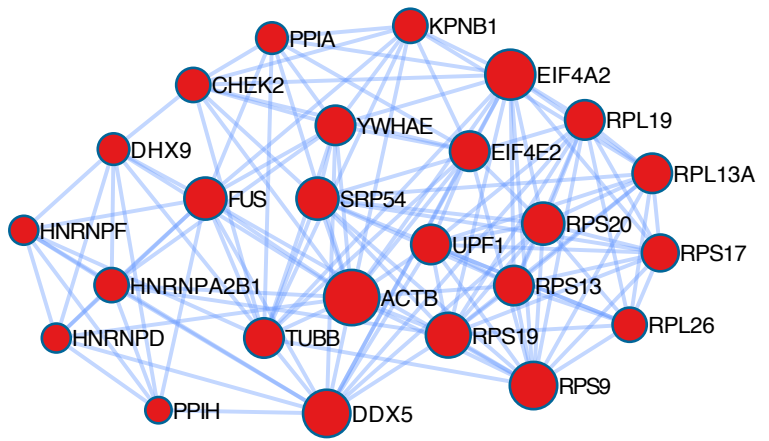


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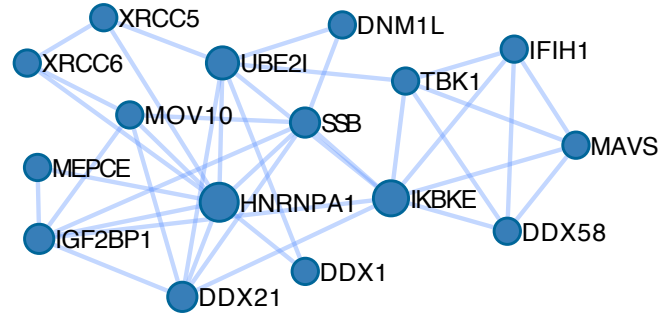


HOST PROTEIN	VIRAL PROTEIN
ACTB	IBV:M
CHEK2	SARS-CoV:NSP13
DDX5	IBV:N SARS-CoV:NSP13
DHX9	IBV:N TGEV:N
EIF4A2	TGEV:M
EIF4E2	SARS-CoV-2:NSP2 SARS-CoV:NSP2
FUS	IBV:N MHV:NSP2
HNRNPA2B1	IBV:N
HNRNPD	IBV:N MHV:NSP2
HNRNPF	IBV:N MHV:NSP2
KPNB1	MHV:NSP2 SARS-CoV:ORF6
PPIA	SARS-CoV:N SARS-CoV:NSP1 SARS-CoV:NSP15
PPIH	SARS-CoV:NSP1
RPL13A	IBV:N MHV:NSP2
RPL19	IBV:N
RPL26	HCoV-OC43:N IBV:N TGEV:N
RPS13	IBV:N TGEV:N
RPS17	IBV:N SARS-CoV:ORF9B
RPS19	HCoV-OC43:N IBV:N
RPS20	SARS-CoV:ORF9B
RPS9	IBV:N TGEV:N
SRP54	MHV:NSP2 SARS-CoV-2:NSP8
TUBB	MHV:N
UPF1	IBV:N SARS-CoV-2:N
YWHAE	SARS-CoV:NSP7 SARS-CoV:ORF3A
DDX1	IBV:N IBV:NSP14 MHV:NSP2 SARS-CoV:NSP14 TGEV:N TGEV:NSP14
DDX21	IBV:N SARS-CoV-2:N
DDX58	PDCoV:N PDCoV:ORF6 SARS-CoV:M
DNM1L	MHV:NSP2 SARS-CoV:ORF9B
HNRNPA1	HCoV-OC43:N IBV:N MHV:N PEDV:N SARS-CoV:N
IFIH1	MERS-CoV:ORF4B PDCoV:N PDCoV:ORF6
IGF2BP1	IBV:N MHV:NSP2
IKBKE	MERS-CoV:ORF4B PEDV:N SARS-CoV:M SARS-CoV:NSP3
MAVS	SARS-CoV:ORF9B
MEPCE	MHV:NSP2 SARS-CoV-2:NSP8
MOV10	IBV:N SARS-CoV-2:N
SSB	IBV:N MHV:NSP2
TBK1	MERS-CoV:ORF4B MHV:NSP3 PEDV:N SARS-CoV-2:NSP13 SARS-CoV:M SARS-CoV:NSP3
UBE2I	SARS-CoV:N
XRCC5	HCoV-OC43:N IBV:N
XRCC6	HCoV-OC43:N IBV:N
G3BP1	IBV:N MHV:NSP2 SARS-CoV-2:N
GSK3B	IBV:N SARS-CoV:N
HNRNPU	HCoV-OC43:N IBV:N TGEV:N
IKBKB	PEDV:ORF3 SARS-CoV:M
IKBKG	PEDV:NSP5
IRF3	MHV:NSP3 SARS-CoV:NSP3 SARS-CoV:ORF8AB SARS-CoV:ORF8B
ISG15	MERS-CoV:NSP3 MHV:NSP3 SARS-CoV:NSP3
NCL	HCoV-OC43:N IBV:N
NPM1	IBV:N
STING1	HCoV-NL63:NSP3 SARS-CoV:NSP3
TRAF3	HCoV-HKU1:M PDCoV:N SARS-CoV:M SARS-CoV:NSP3 SARS-CoV:ORF3A
YBX1	HCoV-OC43:N IBV:N
BTF3	MHV:NSP2 SARS-CoV:NSP10
EEF1A1	SARS-CoV:N TGEV:N
PABPC1	HCoV-OC43:N IBV:N SARS-CoV-2:N
PABPC4	IBV:N SARS-CoV-2:N
PPP1CA	TGEV:ORF7
PRKAR2B	MHV:NSP2 SARS-CoV-2:NSP13
RAB5C	MHV:NSP2 SARS-CoV-2:NSP7
USP10	IBV:N MHV:NSP2
CKAP5	MHV:NSP2 SARS-CoV:NSP1
DCTN2	SARS-CoV:NSP14 SARS-CoV:ORF14 SARS-CoV:ORF3A SARS-CoV:ORF3B SARS-CoV:ORF6
FBF1	MHV:NSP14 MHV:NSP16
MARK1	MHV:NSP2 SARS-CoV-2:ORF9B
MARK2	MHV:NSP2 SARS-CoV-2:ORF9B SARS-CoV:NSP13
MARK3	MHV:NSP2 SARS-CoV-2:ORF9B SARS-CoV:NSP13 SARS-CoV:ORF9B
PHB	SARS-CoV:NSP2
PHB2	SARS-CoV:NSP2
STOML2	SARS-CoV-2:ORF3B SARS-CoV:NSP2

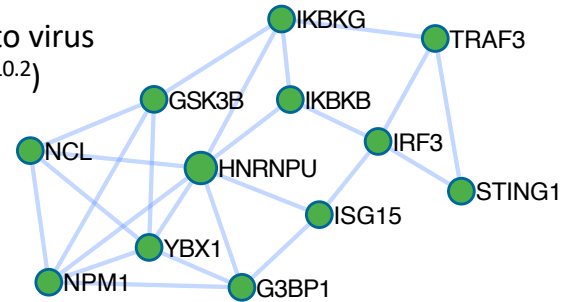
mRNA catabolic process ($p=10^{-17}$)



Regulation of type I interferon production ($p=10^{-12}$)



Response to virus ($p=10^{-10.2}$)



Translation ($p=10^{-4.3}$)

