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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-02874650v1

**HAL Id: hal-02874650**

**<https://hal.science/hal-02874650v1>**

Preprint submitted on 19 Jun 2020 (v1), 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 interactions also provide key information to select targets for antiviral drug development. Here, we have manually

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

12

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

15

## 16 **Background**

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

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

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

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

28

## 29 **Results and discussion**

### 30 ***Gathering coronavirus-host interactions from literature***

31 Several members of the *Coronaviridae* family are pathogenic in human and animals,  
32 and represent a threat for public health and livestock. To date, seven coronaviruses have been

1 reported to infect human (Human coronaviruses; HCoVs). HCoV-NL63 and HCoV-229E (from  
2 the  $\alpha$  genus) and HCoV-HKU1 and HCoV-OC43 (from the  $\beta$  genus) are responsible for common  
3 cold. In addition, three  $\beta$  coronaviruses, MERS-CoV (Middle East respiratory syndrome-related  
4 coronavirus), SARS-CoV and SARS-CoV-2, are associated to life-threatening respiratory  
5 diseases in human. Important animal coronaviruses include but are not limited to TGEV  
6 (Transmissible Gastroenteritis Virus,  $\alpha$  genus), PRCV (Porcine Respiratory Coronavirus,  $\alpha$   
7 genus), PEDV (Porcine Epidemic Diarrhea Virus,  $\alpha$  genus), MHV (Murine Hepatitis Virus,  $\beta$   
8 genus), IBV (Infectious Bronchitis Virus,  $\gamma$  genus), and PDCoV (Porcine Deltacoronavirus,  $\delta$   
9 genus). The genome of coronaviruses is a positive-strand RNA with two third at the 5' end  
10 occupied by the overlapping open reading frames ORF1a and b that encode non-structural  
11 proteins (nsPs). The genes encoding structural proteins and a variable number of accessory  
12 factors are nested at the 3' end of the genome. Viral genomes are directly translated by host  
13 cell's ribosomes into a large polyprotein encoded by the ORF1a/b gene. This polyprotein is  
14 cleaved into 16 non-structural proteins (nsP1-16), except for PDCoV where nsP1 is missing.  
15 Most of these nsPs assemble into a complex that replicates viral genome and synthesizes  
16 subgenomic mRNA from the other genes to express structural proteins S, E, M and N and the  
17 additional accessory factors.

18 To collect virus-host interaction data for the 13 coronaviruses mentioned above (*i.e.*  
19 HCoV-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43, MERS-CoV, SARS-CoV, SARS-CoV-2, TGEV,  
20 PRCV, PEDV, MHV, IBV, and PDCoV), we interrogated the PubMed database using the  
21 following query sentence: "virus name"[Title/Abstract] AND (bind\*[Title/Abstract] OR  
22 interact\*[Title/Abstract]). After careful analysis of the retrieved abstracts, 112 publications  
23 explicitly reporting physical interactions between viral and host proteins were selected (Figure  
24 1a). These publications were analyzed by at least two curators to determine which methods  
25 were used to characterize the reported interactions, and collected information was gathered  
26 in a single data file (Supplementary Table 1). In total, this file contains 1,544 entries. As 133  
27 interactions were characterized by more than one method or were detected across multiple  
28 host species, and that 23 interactions were reported in two or more independent publications,  
29 this corresponds to 1,311 distinct virus-host interactions involving 1,139 different host  
30 proteins (orthologous proteins from different host species were collapsed; Figure 1a and  
31 Supplementary Table 2). A majority of the reported interactions (92%) were from four viruses

1 for which high-throughput interactomic methods have been applied: MHV, SARS-CoV-2, SARS-  
2 CoV, and IBV (Figure 1b, Supplementary Table 3)[6][7][3][8][9][10][11][12].

3

#### 4 ***Shared interactions and host protein targets across multiple coronaviruses***

5 We then looked for interactions that are conserved across two or more viruses. To  
6 perform this analysis, orthologous proteins from different host species were collapsed as  
7 above. In total, we identified 51 orthologous interactions in the dataset, providing a robust  
8 network of shared PPIs between several coronaviruses (Figure 1c; Supplementary Table 4).  
9 Membrane receptors shared by several coronaviruses (ACE2, ANPEP) as well as cellular  
10 proteases involved in the processing of the spike glycoproteins S (TMPRSS2, TMPRSS11D,  
11 CTSB, CTSL, FURIN) were highlighted. Multiple interactions between the nucleoprotein N and  
12 host factors involved in mRNA synthesis, maturation, nuclear export, translation and stability  
13 were also identified (EEF1A1, PABPC1, PABPC4, RNRNPA1, HNRNPU, YBX1, LARP1), including  
14 ribosomal components (RPS9, RPS13, RPS19, RPL26), multiple helicases (UPF1, MOV10, DHX9,  
15 DDX21, DDX1) and key components of the innate antiviral response (G3BP1, G3BP2, PRKRA).  
16 GSK3A/B were previously reported to phosphorylate the viral nucleocapsid of MHV to recruit  
17 DDX1 [13]. In the assembled interactome dataset, GSK3A/B were also found to interact with  
18 N of IBV and SARS-CoV, and interaction of DDX1 with both N and nsP14 of IBV and TGEV as  
19 well as nsP14 of SARS-CoV were also reported [12][14][15][16]. This analysis also highlights  
20 the well-known function of nsP3 in coronavirus escape from the innate immune response  
21 through interactions with both ubiquitination/ISGylation factors (RCHY1, ISG15) and key  
22 antiviral factors (STING1, TBK1, IRF3)[11][17][18][19][20][21]. Other remarkable interactions  
23 are between nsP2, EIF4E2 (4EHP) and GIGYF2, which are two components of a complex  
24 repressing mRNA translation [22]. Although EIF4E2 and GIGYF2 were identified by high-  
25 throughput interactomic applied to SARS-CoV, SARS-CoV-2 and MHV [3][9][6], they were  
26 never investigated in details. These highly conserved interactions suggest a crucial role of nsP2  
27 in regulating viral and/or cellular mRNA translation and degradation.

28 We also looked for host proteins interacting with several coronaviruses, but not  
29 necessarily targeted by the same viral proteins. Because coronavirus proteins assemble into  
30 large molecular complexes, the same host protein can be captured, either directly or  
31 indirectly, by distinct viral proteins used as bait. In total, 106 of the 1,139 host proteins from  
32 the dataset (9.3%) were shared by at least two coronaviruses, including 50 host proteins

1 involved in the conserved interactions displayed in Figure 1c. Shared targets of different  
2 viruses are illustrated in Figure 1d and details are provided in Supplementary Table 5.  
3 Interestingly, several host factors involved in viral RNA sensing (DDX58, IFIH1, PRKRA), DNA  
4 sensing (STING1) or downstream signalling to activate the innate immune response (TRAF3,  
5 TBK1, IKKKE, IKKKB, ISG15, IRF3) were targeted by unrelated viral proteins (Figure  
6 1e)[3][20][21][23][24][25][26][27][28][29][30][31][32][33][34][35][36]. This highlights the  
7 importance of these interactions for coronaviruses, but is puzzling from an evolutionary  
8 perspective. Indeed, this suggests that coronaviruses have evolved different but convergent  
9 strategies to target these innate immunity factors. An alternative explanation is that multiple  
10 proteins of a virus target the same host factor in redundant ways, although this information  
11 is not present in the current interaction datasets yet. The two hypotheses are not mutually  
12 exclusive and this will require further investigation to be addressed.

13

#### 14 ***Enrichment analysis for specific cellular pathways.***

15 To identify cellular complexes or pathways that are enriched in the list of host factors  
16 interacting with coronavirus proteins, we retrieved associated KEGG annotations and  
17 calculated statistical enrichments using the DAVID functional annotation tool [37][38]. To  
18 evaluate the interest of combining interactome data from several coronaviruses, we analyzed  
19 host targets of SARS-CoV-2 alone (Table 1a), of human highly-pathogenic coronaviruses (Table  
20 1b) and of all coronaviruses (Table 1c). Although SARS-CoV-2 restricted analysis only identified  
21 “Protein processing in endoplasmic reticulum” and “RNA transport” as significantly enriched  
22 KEGG pathways, expanding the dataset doubled the number of host factors falling in these  
23 two categories and unraveled enrichments for “Ribosome”, “RIG-I-like receptor signaling”,  
24 “Endocytosis”, “Spliceosome” and “Phagosome” pathways (Table 1c and Supplementary  
25 Table 6). Up to 69% of the KEGG-annotated eukaryotic ribosomal proteins and 18% of the  
26 spliceosome components are targeted by coronavirus proteins, thus demonstrating the  
27 importance for these viruses to control the mRNA processing and translation machinery. We  
28 used KEGG Mapper to highlight targeted host proteins on schematic representation of the  
29 most enriched cellular pathways (Figure 2a-d)[39]. As shown in Figure 2a, several factors  
30 involved in protein export were targeted together with components of the unfolded protein  
31 response/endoplasmic-reticulum-associated protein degradation (ERAD) pathway. This  
32 observation is in line with numerous reports showing that coronavirus replication induces a

1 strong ER stress response, and that virulence factors expressed by these viruses aim at  
2 controlling and even subvert this cellular response to assemble convoluted and double-  
3 membrane vesicles [40]. Several regulators of intracellular trafficking and vesicular transport  
4 associated to endocytic compartments, especially late endosomes and multivesicular bodies,  
5 are also hijacked (Figure 2b). These interactions most likely contribute to the entry, assembly  
6 and/or secretion of viral particles [41]. The nuclear pore complex (NPC) and translation  
7 initiation factors are also overrepresented in the list of host proteins targeted by  
8 coronaviruses, which accounts for the statistical enrichment of KEGG annotation “RNA  
9 transport” (Figure 2c). The number of Interactions with translation initiation factors, together  
10 with ribosomal components, reflects the intense hijacking of translational machinery by  
11 coronaviruses [6]. Some of these interactions could also prevent the translational shut-off  
12 associated to antiviral and unfolded protein responses as demonstrated for the ORF7 of TGEV  
13 [42], while others could help the virus to control host protein expression [43]. On the contrary,  
14 viral factors interacting with components of the NPC to interfere with the nuclear export of  
15 cellular mRNA and with the antiviral response are poorly documented [44][45]. Finally, this  
16 analysis highlighted multiple interactions with components of the RIG-like receptor pathways  
17 (Figure 2d), that is essential for viral RNA detection and the induction of interferon secretion.  
18 This includes the multi-targeted host factors already presented in Figure 1e but also additional  
19 proteins such as the adaptors MAVS (IPS1), TBKBP1 (SINTBAD), TRAF2 and TRAF6, the negative  
20 (NLRX1) or positive (DDX3X) regulators, and kinases such as RIPK1 (RIP1) or IKBKG  
21 (IKK $\gamma$ )[3][12][46][47]. Altogether, this provides an overview of cellular pathways that are  
22 major targets of coronaviruses.

23         Despite several reports showing that viruses have evolved strategies to finely tune  
24 cellular metabolism for promoting their replication [48], no statistical enrichment for  
25 components of a specific metabolic pathway was identified. We thus conducted an orientated  
26 analysis by looking at potential overlaps between the list of host proteins targeted by  
27 coronaviruses and the 1226 proteins annotated in the “Metabolism pathways” of the KEGG  
28 database. In total, 62 proteins were identified at the intersection of the two lists, and were  
29 clustered according to their specific metabolic pathways using KEGG annotation (Figure 3).  
30 Multiple interactions were identified with factors involved in nucleotide metabolism,  
31 including enzymes of the nucleo-tide/-side biosynthesis and degradation pathways (IMPDH2,  
32 RRM2, ADK, DCTPP1, NT5C2, XDH, ADA) and more surprisingly, several components of cellular



1 DNA and RNA polymerases (POLA1, POLA2, POLD1, PRIM1, PRIM2 and POLR2B). These  
2 interactions could contribute to the DNA replication stress and cell cycle arrest induced by  
3 coronaviruses as previously suggested for the nsP13-POLD1 interaction, but their role is still  
4 poorly defined [49]. Several components of complex I of the mitochondrial respiratory chain  
5 were also targeted (NDUF9/10/13), as well as V-type ATPase subunits involved in the  
6 acidification of cellular compartments, especially during phagocytosis (Supplementary Table  
7 6). Lipid and glycan biosynthesis enzymes were highly represented, in line with previous  
8 reports showing the dependence of coronaviruses to these two pathways [50][51]. In the  
9 “amino acid metabolism” cluster, both methionine adenosyltransferase 2A (MAT) and the  
10 regulatory subunit MAT2B were present (captured by ORF3 from PEDV and nsP9 of SARS-CoV-  
11 2, respectively). MAT2A catalyzes the synthesis of S-adenosyl-L-methionine, the major  
12 biological methyl donor. This may indicate that coronaviruses critically need S-adenosyl  
13 methionine (SAM) to methylate the viral RNA cap structures to allow transcription and  
14 prevent their recognition by cellular innate immunity receptors [52]. In the same cluster were  
15 also found two methyltransferases (COMT, DNMT1). Although the significance of targeting  
16 COMT (Catechol O-Methyl Transferase), an enzyme involved in catecholamine synthesis,  
17 remains elusive, interactions with DNMT1 could be a mechanism used by coronaviruses to  
18 alter the epigenetic landscape of infected cells as observed for other viruses [53]. Finally,  
19 coronavirus proteins also interacted with several host factors flagged as “central carbon  
20 metabolism” enzymes. This cluster includes multiple enzymes contributing to protein  
21 sialylation (GNE, NANS) and glycosylation (UGP2), inositol synthesis (MTMR3, ISYNA1), and  
22 two enolases (ENO1, ENO3) whose interactions with viral proteins could modulate glycolysis  
23 and energy supply for promoting viral growth. Altogether, this analysis suggests that, although  
24 the completeness of the available dataset does not allow statistical validation, coronaviruses  
25 extensively interact with metabolic enzymes.

26

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

28 To identify densely connected regions in the list of targeted host proteins, we used the  
29 web-based application Metascape [54]. PPIs from the human interactome were retrieved  
30 from the BioGrid knowledgebase, InWeb\_IM and Omni-Path, and densely connected regions  
31 were extracted using the MCODE algorithm. As no result could be retrieved when the full list  
32 of 1139 host proteins was submitted to Metascape, we limited this analysis to the core list of

1 179 host proteins for which multiple experimental evidences exist to support an interaction  
2 with coronavirus proteins. We also excluded the interactors of the spike glycoproteins that  
3 are already well-characterized and skewed the analysis of the other virus-host PPIs  
4 (Supplementary Table 7). From the list of 157 host proteins, this identified 7 protein clusters  
5 whose biological role was determined by Metascape using GO enrichment analysis (Figure  
6 4)[55][56] and largely confirmed our analyses discussed above. The functional annotations  
7 enriched in the four largest clusters were “mRNA catabolic process”, “Regulation of type I  
8 interferon production”, “Response to virus”, and “Translation”. Interestingly, this analysis  
9 highlighted the link between XRCC5/6 dimers and components of the RIG-like receptor  
10 pathway. XRCC5/6 binds to DNA double-strand break ends and activates the catalytic activity  
11 of DNA-PK to promote DNA repair. In the cytosolic compartment, it also participates to type I  
12 interferon induction in response to foreign DNA [57]. Furthermore, it has been shown that  
13 DNA-PK is activated and participates to interferon induction in dengue virus-infected cells  
14 [58], and that NS5A from Hepatitis C virus (HCV) is phosphorylated by this kinase [59]. The  
15 targeting of XRCC5 and 6 by the N protein of IBV and OC43 [12][60] suggests that these  
16 proteins also play a role in the induction of innate immunity and/or the replication of  
17 coronaviruses. Three clusters of only three components were also identified. The first one  
18 includes PHB, PHB2 and STOML2 that are all members of the stomatin-prohibitin-flotillin-  
19 HflC/K (SPFH) superfamily. These proteins colocalize at the inner mitochondrial membrane  
20 where they assemble into ring-like structures [61]. They regulate mitochondrial homeostasis,  
21 and are involved in processes such as mitophagy and mitochondrial fusion. By targeting these  
22 proteins, coronavirus proteins may also impact key mitochondrial functions such as  
23 respiration, lipid biosynthesis and innate immunity. The second small cluster is composed of  
24 MARK1, 2, and 3, three Ser/Thr protein kinases involved in the control of cell polarity,  
25 microtubule stability and cancer. MARK proteins have been reported to interact with nsP2 of  
26 MHV, nsP13 of SARS-CoV, and ORF9B of both SARS-CoV and SARS-CoV-2 [3][6][7][8]. Although  
27 their role in coronavirus replication is unknown, they could contribute to viral trafficking as  
28 they control microtubule dynamics and vesicular transport. Interestingly, several approved  
29 drugs inhibiting MARK kinases were recently proposed as potential antivirals against  
30 coronaviruses and especially SARS-CoV-2 [3]. The last cluster is composed of the cytoskeleton  
31 and microtubule-associated proteins DCTN2, FBF1 and CKAP5 that could participate to the  
32 transport of viral complexes within infected cells.

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

To provide a rationale for the antiviral activity of some drugs targeting cellular factors, we determined to what extent the coronavirus-host interactome intersects the large-scale chemical screenings of antivirals against coronaviruses. We selected four publications reporting *in vitro* screening of chemical libraries against SARS-CoV, MERS-CoV and HCoV-OC43 [62][63][64][65]. In total, 77 molecules were identified for their antiviral activity against at least one coronavirus. Chloroquine was identified in all four publications, amodiaquine and loperamide were identified in three of them, and chlorpromazine, cycloheximide, emetine, and hydroxychloroquine were cited in two publications. Then we searched for cellular targets of these 77 molecules using the Drug Repurposing Hub database [66], and looked for overlaps with the list of 1139 host proteins interacting with coronaviruses. We identified 10 drugs and 14 host proteins matching these criteria (Figure 5). Anisomycine, cycloheximide, emetine and homoarringtonine are translational inhibitors interacting with ribosomal components. As coronaviruses exhibit tight interactions with translation initiation factors and recruit multiple ribosomal components (Table 1c and Figure 4), this could explain the antiviral effect of these three drugs. Three molecules, fluphenazine, loperamide and chlorpromazine, target calmodulin (CALM1) which interacts with ORF3 from PEDV [34]. CALM1 is a Ca<sup>2+</sup>-binding messenger protein that regulates the function of numerous proteins. In particular, it controls the activity of the multifunctional CAMKII proteins (Ca<sup>2+</sup>/calmodulin-dependent protein kinase II), which are targeted by coronaviruses as well. Indeed, CAMK2D and CAMK2G interact with nsP3 of SARS-CoV and nsP2 of MHV, respectively (Supplementary Table 1)[6][11]. Furthermore, ACE2 receptor of SARS-CoV-2, SARS-CoV, HCoV-NL63 and HCoV-229E, and CEACAM1 receptor of MHV are also calmodulin-binding proteins [67][68][69]. Although fluphenazine, loperamide and chlorpromazine have multiple targets in host cells, interactions with CALM1 could account for the inhibition of coronaviruses by these drugs. Clomipramine, which is structurally and functionally closely related to chlorpromazine, was also selected in the analysis as a consequence of its interaction with GSTP1, one of the glutathione S-transferase isoenzymes. This analysis also highlighted interactions between coronavirus proteins and host factors involved in nucleotide biosynthesis. First, nsP14 interacts with inosine-5'-monophosphate dehydrogenase 2 (IMPDH2), a key enzyme of *de novo* purine biosynthesis pathway. The inhibitor mycophenolic acid, that is depleting cells in purine, is well-

1 known for its broad-spectrum antiviral activity and is in a list of drug candidates proposed  
2 against SARS-CoV-2 [3]. In addition, the analysis pinpointed to the interaction of nsP2 from  
3 MHV with the host enzyme RRM2, one of the two subunits of ribonucleotide reductase that  
4 is inhibited by gemcitabine. This interaction between an enzyme involved in  
5 deoxyribonucleotide synthesis and an RNA virus protein was unexpected. However, RRM2  
6 was previously reported to interact with NS5B from HCV, preventing its degradation and  
7 promoting the replication of this RNA virus [70]. Furthermore, gemcitabine has been shown  
8 to inhibit the replication of many different RNA viruses by blocking cellular DNA replication.  
9 Indeed, this induces a genomic stress that triggers the innate antiviral response [71]. Thus,  
10 RNA viruses could have evolved interactions with cellular enzymes involved in  
11 nucleoside/nucleotide synthesis such as IMPDH and RRM2 to sustain DNA replication and  
12 prevent genotoxic stress.

13

#### 14 **Concluding remarks**

15 We have assembled a large coronavirus-host interactome built upon 1311 PPIs  
16 retrieved from literature. Functional annotation and network-based analyses highlighted  
17 targeted cellular pathways and modules. Overall, mRNA processing and transport, translation  
18 initiation and protein translation, endosomal trafficking, and innate immunity were the most  
19 enriched pathways. This conclusion is consistent with previous reports identifying these  
20 biological processes as targets of specific coronaviruses. The global analysis suggest that these  
21 interactions are highly conserved across most if not all coronaviruses. We also pinpointed to  
22 a couple of small protein complexes that appear particularly relevant to coronavirus infection  
23 but were not previously investigated. This includes in particular the EIF4E2-GIGYF2 dimer  
24 involved in the repression of protein translation, the MAT2A-MAT2B complex controlling SAM  
25 synthesis, the DNA-PK kinase that contributes to interferon induction, and the mitochondrial  
26 proteins PHB, PHB2 and STOML2 regulating mitophagy. Multiple evidences also support a key  
27 role of the MARK kinases. Finally, we identified a dozen of host factors that are bound by  
28 coronavirus proteins and functionally modulated by compounds selected from antiviral  
29 screens, giving hints to explain the inhibition of coronavirus replication by these drugs. Among  
30 these molecules is chlorpromazine, an antipsychotic drug that is currently evaluated in a  
31 clinical trial against SARS-CoV-2. In conclusion, this work has highlighted the importance of  
32 several neglected coronavirus-host interactions that deserve to be further investigated. It also

1 illustrates the interest of combining virus-host interactome datasets from different  
2 laboratories, obtained by different approaches and generated in various cell types to increase  
3 coverage and get closer to completeness.

4

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

11

## 12 **Declarations**

### 13 ***Ethics approval and consent to participate***

14 *Not applicable*

### 15 ***Consent for publication***

16 *Not applicable*

### 17 ***Availability of data and materials***

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

### 20 ***Competing interests***

21 The authors declare that they have no competing interests.

### 22 ***Funding***

23 This work was supported by the Institut National de la Santé et de la Recherche Médicale  
24 (INSERM) and the Centre National de Recherche Scientifique (CNRS) and Université de Lyon.

25 EO is supported by the Conseil Régional de l'île de La Réunion DIREC 20181189.

### 26 ***Authors' contributions***

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

5 ***Acknowledgements***

6 *Not applicable*

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6

7

1 **Figure Legends**

2 **Figure 1 Quantitative analysis of collected virus host-interactions.** (A) Keynumbers describing  
3 the database that has been assembled. (B) Numbers of distinct interactions that have been  
4 collected for each virus. (C) Orthologous interaction conserved between several viruses. The  
5 thickness of the lines is proportional to the number of viruses for which the interaction was  
6 reported. Displayed graph was generated using Cytoscape [72]. (D) Circular diagram showing  
7 the proportion of shared host protein targets between analyzed coronaviruses. Display was  
8 obtained using the Circos table viewer [73]. (E) Innate immunity factors interacting with  
9 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 map  
14 ID: 04622). Host proteins interacting with coronavirus proteins are highlighted in red.

15

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

20

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

30

31 **Figure 5 Intersection between the coronavirus-host interactome and cellular targets of drugs**  
32 **inhibiting coronavirus replication.** Viral proteins are in red, host proteins in blue and small

1 *molecules in green. Virus-host PPIs are from the coronavirus-host interactome. Compound-*  
2 *target interactions were retrieved from the Drug Repurposing Hub database. For each*  
3 *molecule, stars indicate how many screenings out of the four compiled for this analysis*  
4 *identified their anti-coronavirus activity.*

5

6

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
<a href="#">Protein processing in endoplasmic reticulum</a>	13	4	8,80E-05	4	1,70E-02
<a href="#">RNA transport</a>	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
<a href="#">RIG-I-like receptor signaling pathway</a>	14	3	1,30E-07	6,6	3,00E-05
<a href="#">NF-kappa B signaling pathway</a>	13	2,8	1,10E-05	4,9	8,00E-04
<a href="#">RNA transport</a>	19	4,1	4,10E-06	3,6	4,50E-04
<a href="#">Protein processing in endoplasmic reticulum</a>	18	3,9	1,30E-05	3,5	7,20E-04
<a href="#">Epstein-Barr virus infection</a>	13	2,8	3,10E-04	3,5	1,10E-02
<a href="#">Influenza A</a>	18	3,9	1,90E-05	3,4	8,40E-04
<a href="#">Measles</a>	13	2,8	6,80E-04	3,2	2,10E-02

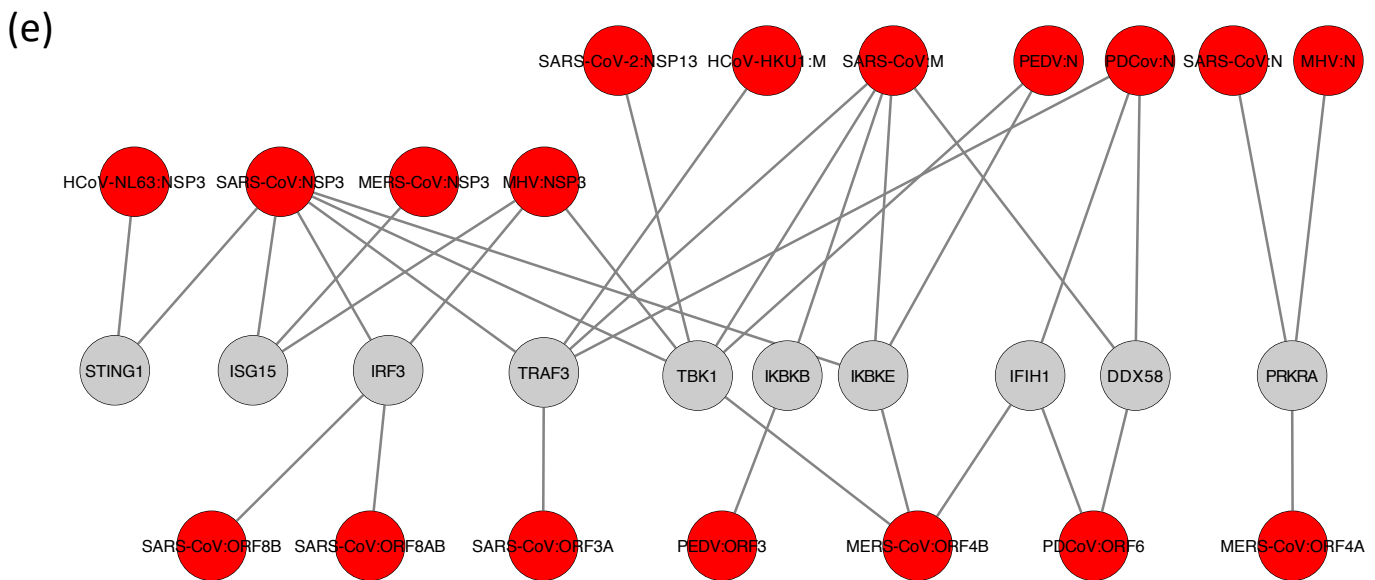
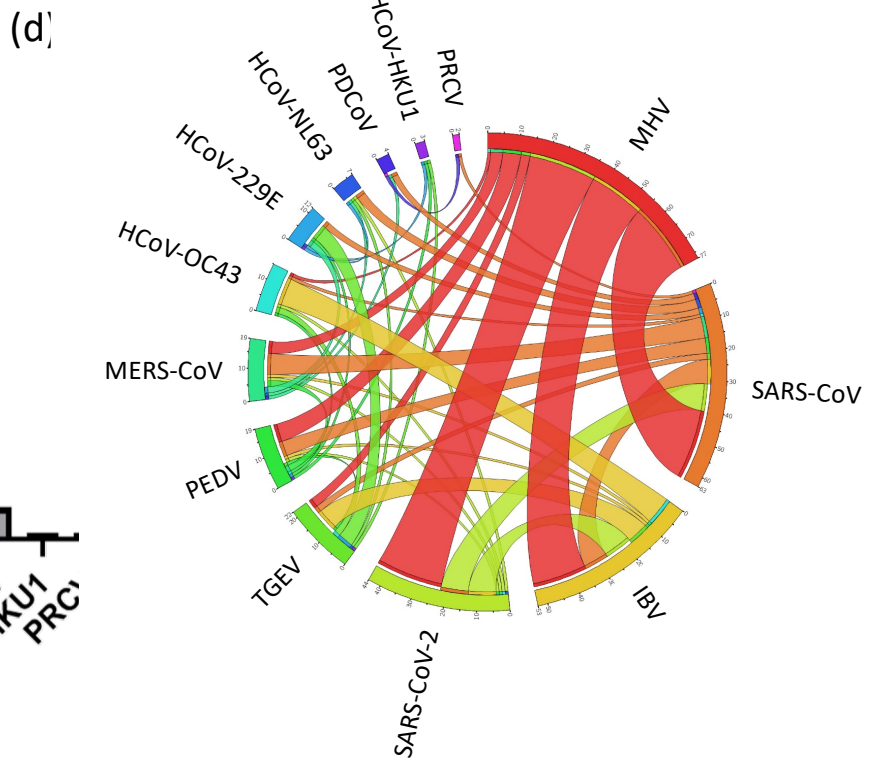
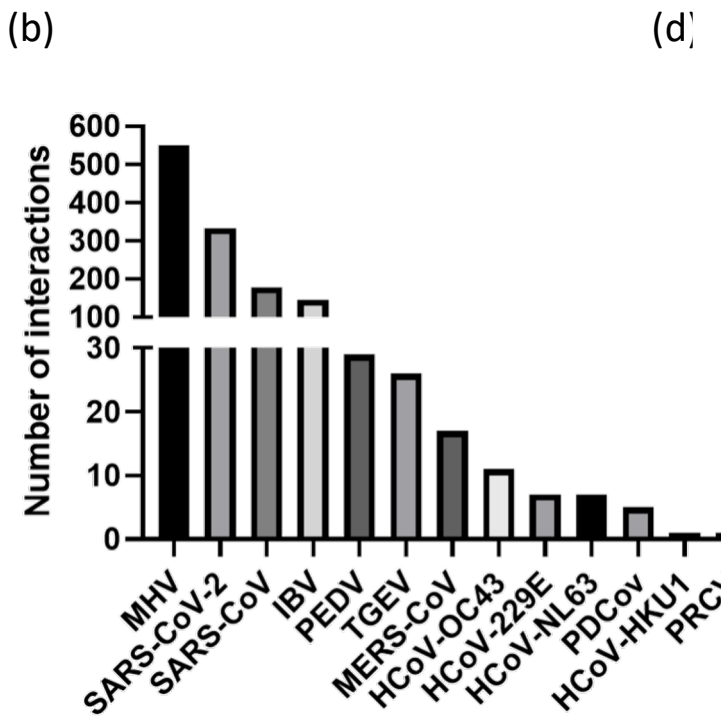
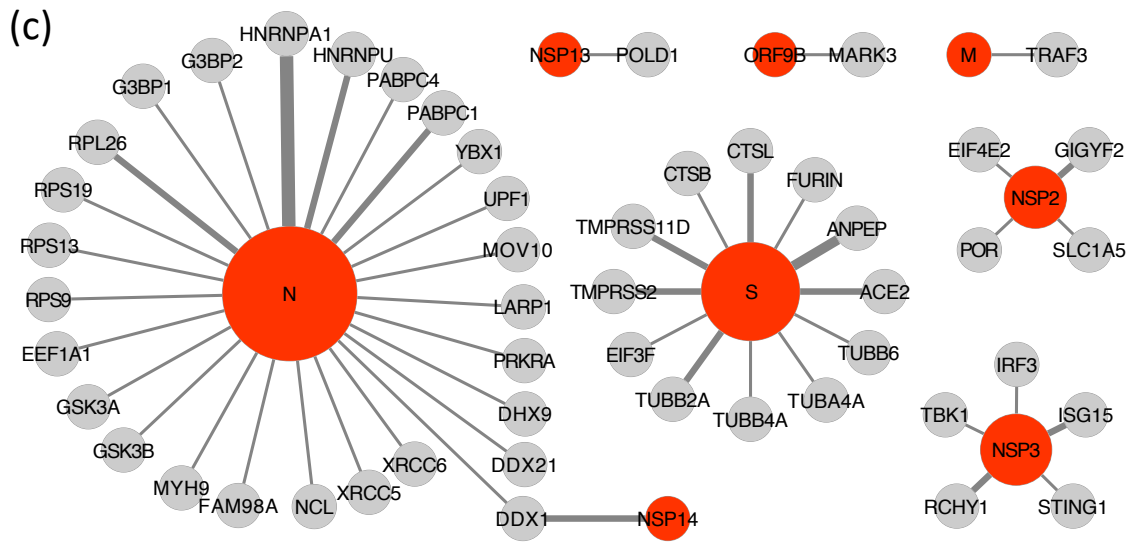
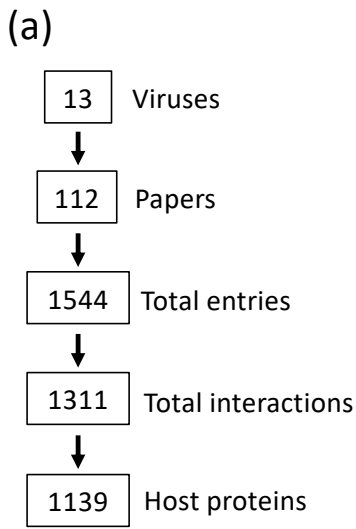
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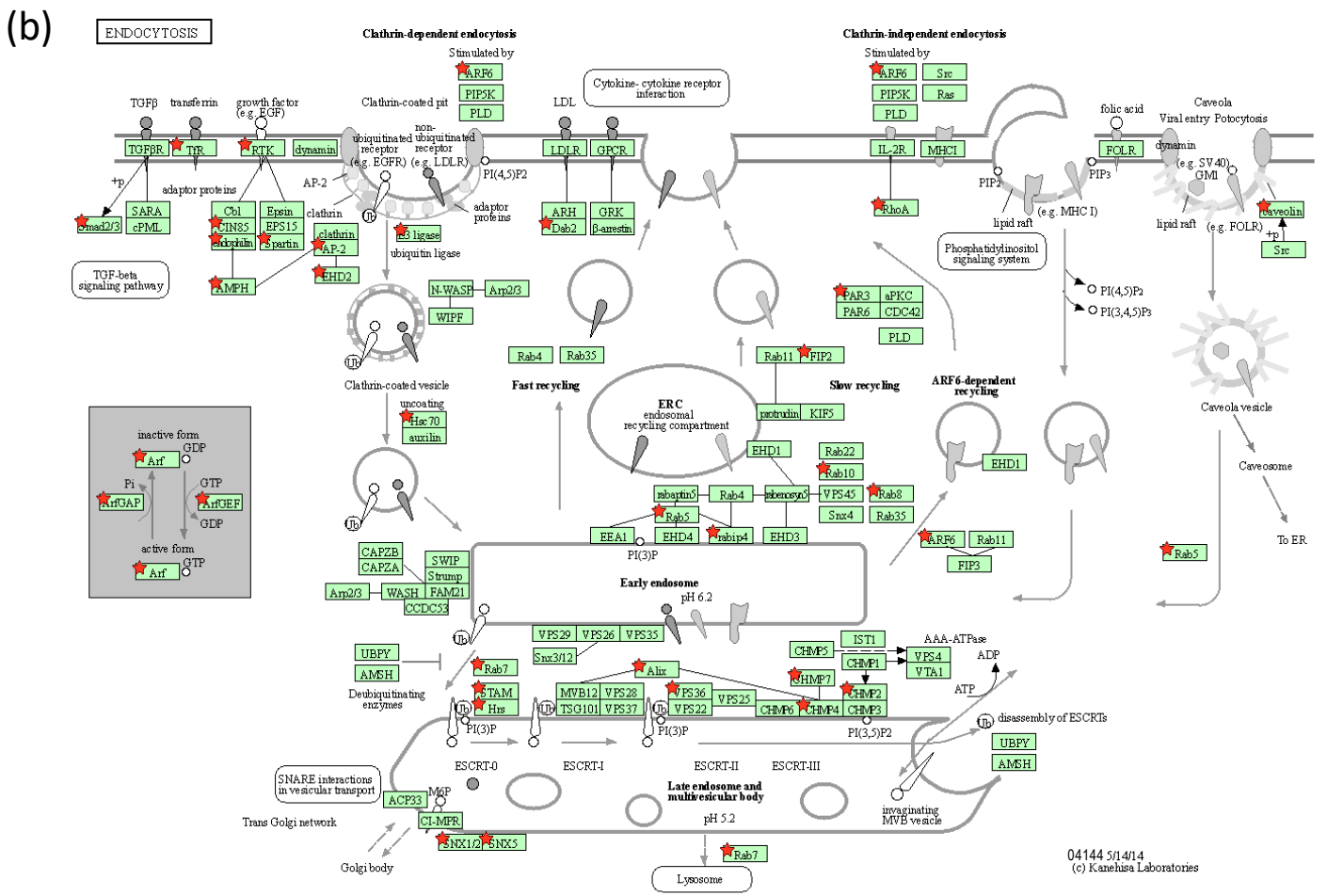
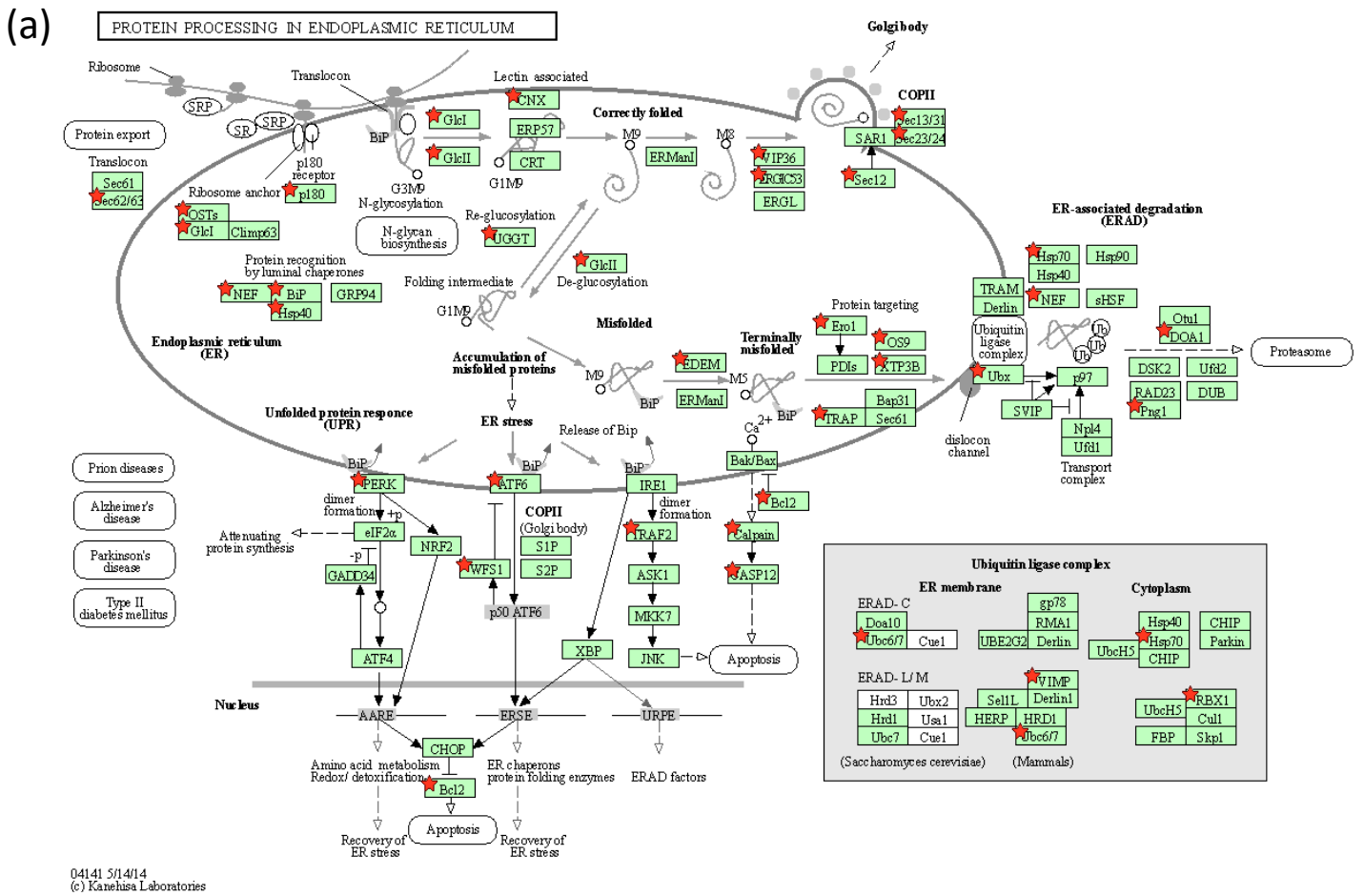
6 **c- Kegg pathways enrichment in Coronaviruses host protein targets (n=1139)**

Kegg pathway (Term)	Count	%	p-Value	Fold Enrichment	Benjamini p-value
<a href="#">Ribosome</a>	60	5,5	1,10E-31	5,9	2,70E-29
<a href="#">RIG-I-like receptor signaling pathway</a>	16	1,5	1,60E-04	3,1	5,60E-03
<a href="#">Protein processing in endoplasmic reticulum</a>	37	3,4	5,20E-09	2,9	6,60E-07
<a href="#">RNA transport</a>	33	3	1,00E-06	2,6	6,30E-05
<a href="#">Endocytosis</a>	43	3,9	1,30E-07	2,4	1,10E-05
<a href="#">Spliceosome</a>	24	2,2	1,10E-04	2,4	4,70E-03
<a href="#">Phagosome</a>	26	2,4	1,00E-04	2,3	5,30E-03

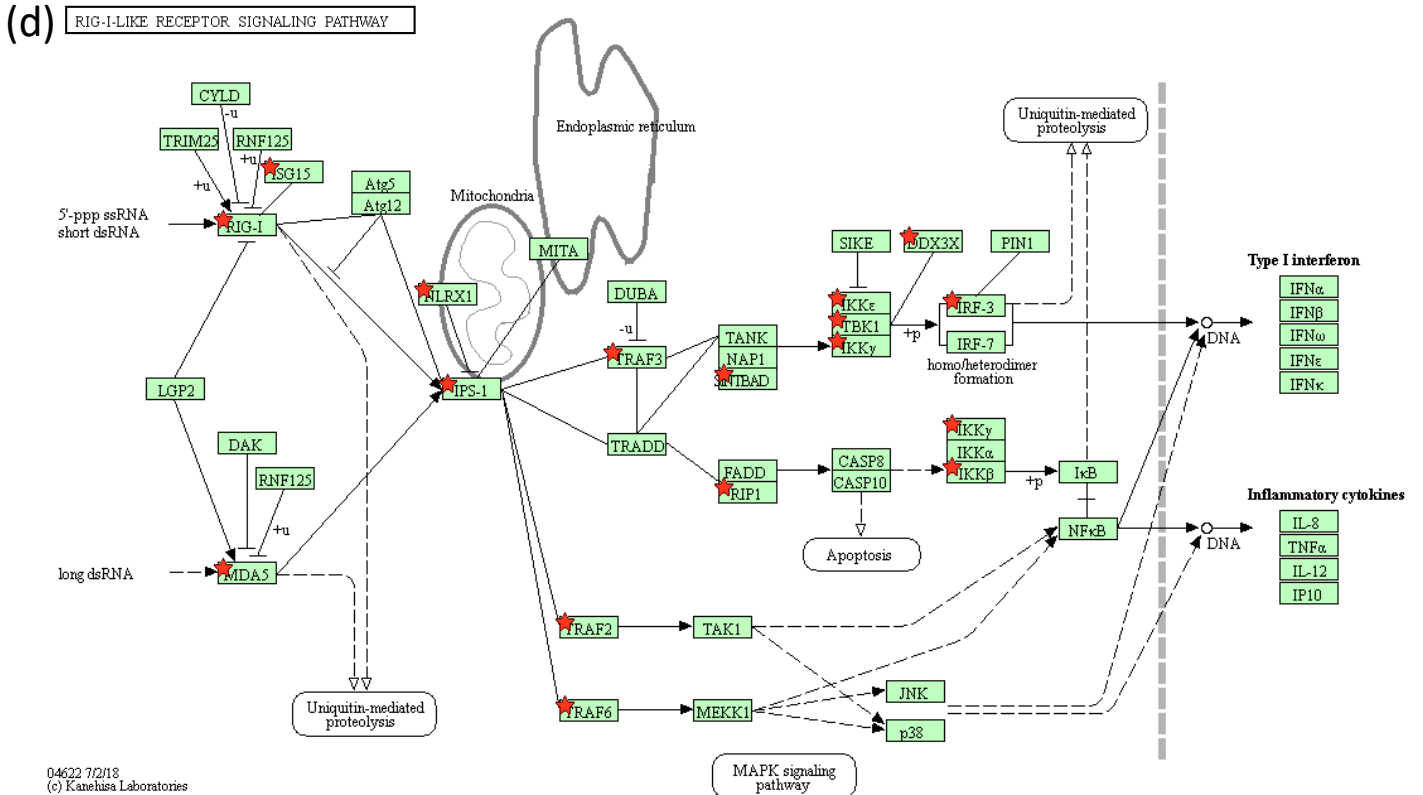
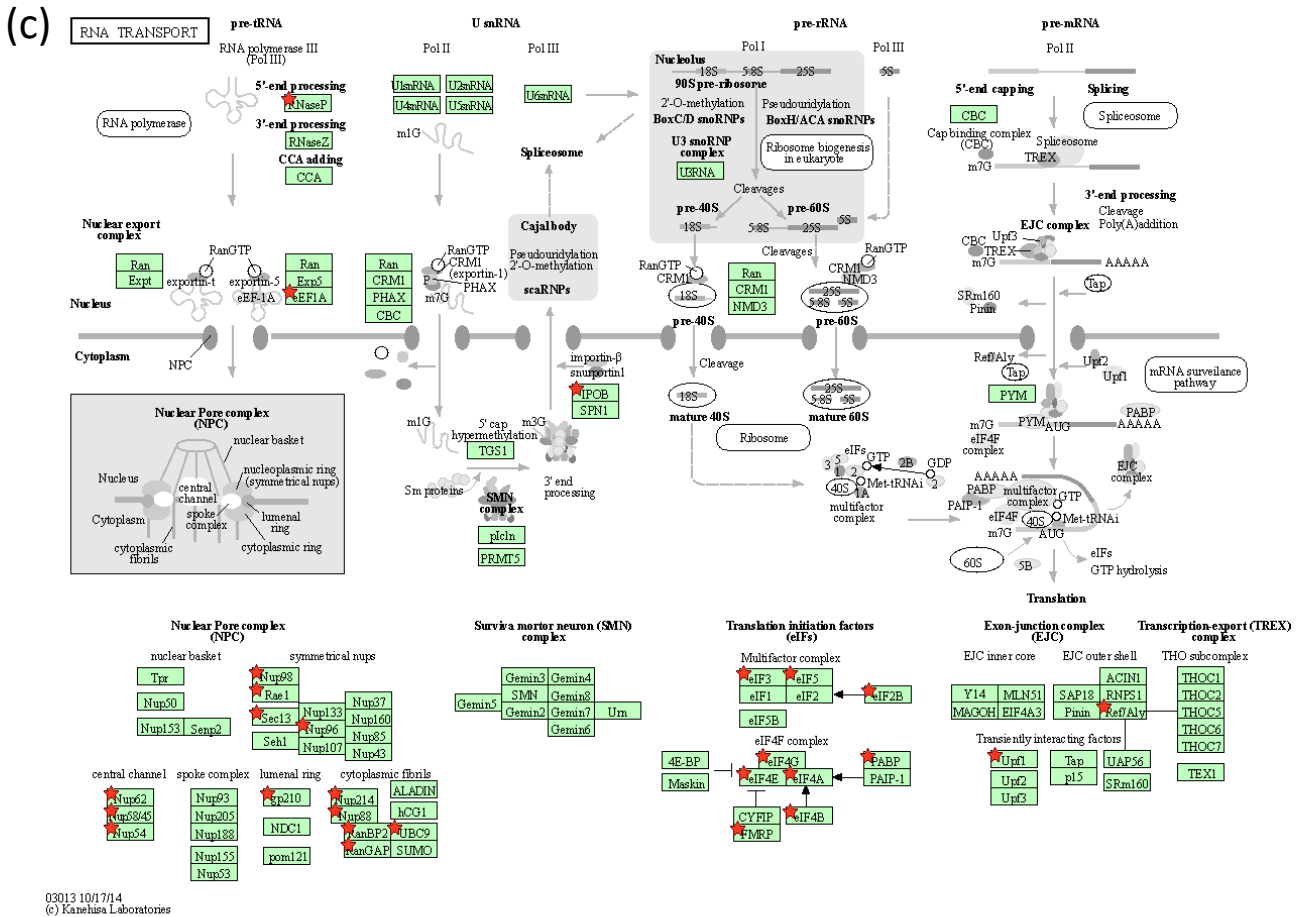
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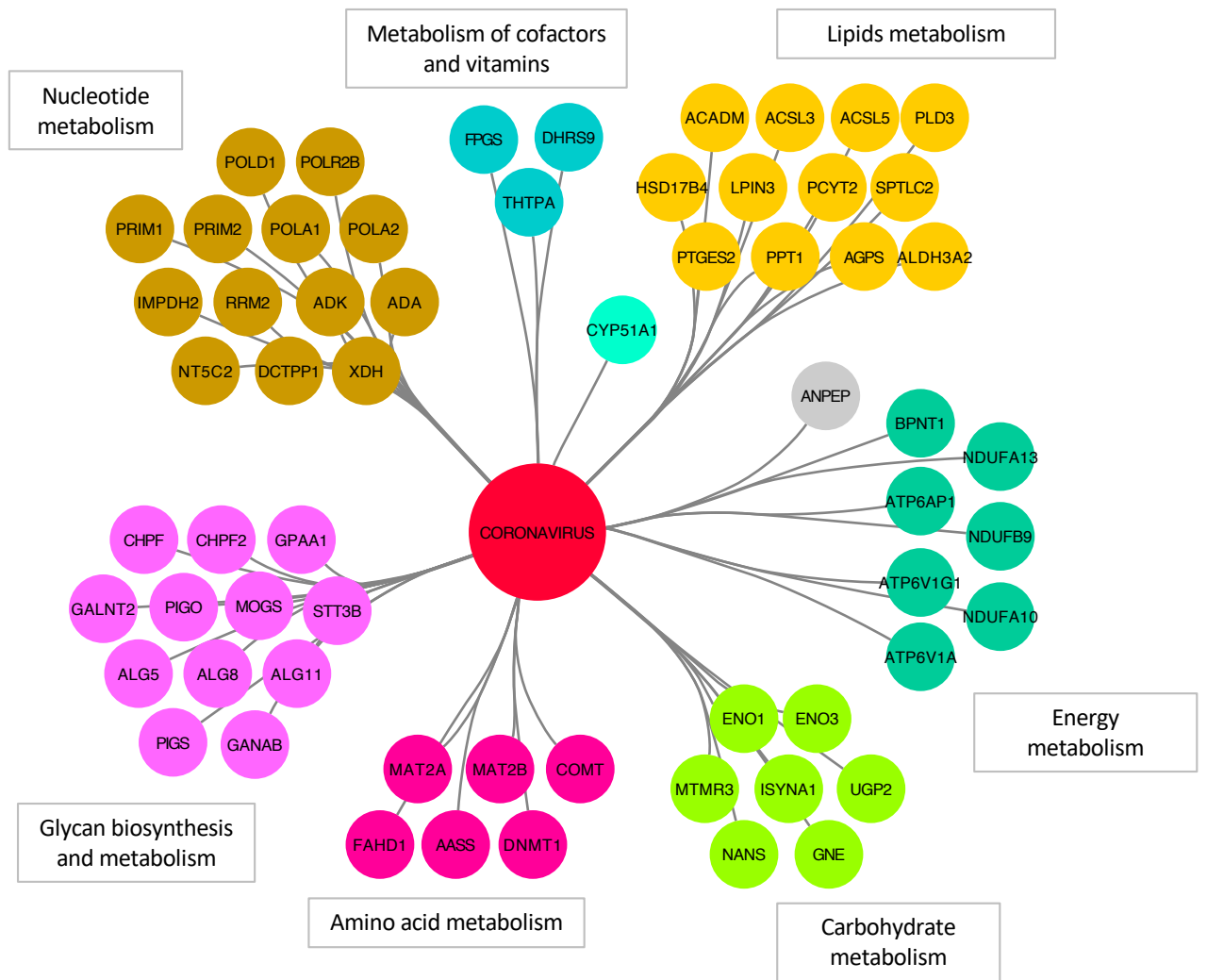
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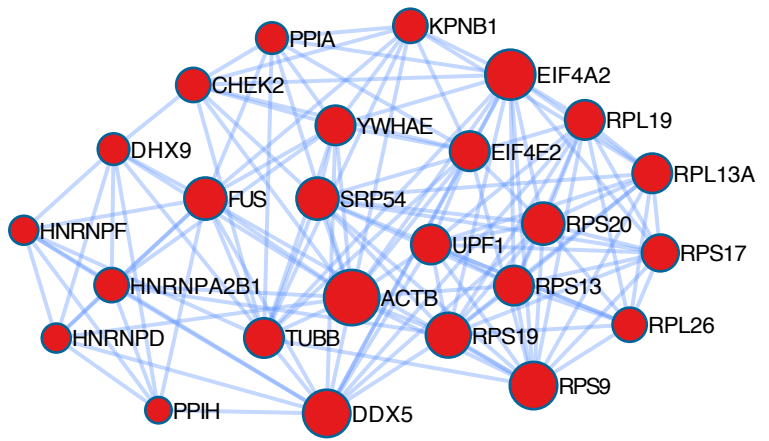




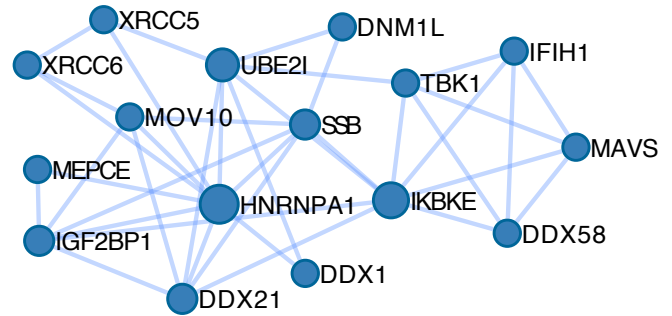


HOST PROTEIN	VIRAL PROTEIN
ACTB	IBV:M
CHEK2	SARS-CoV:NSP13
DDX5	IBV:N SARS-CoV:NSP13
DHX9	IBV:N TGEV:N
EIF4A2	TGEV:M
EIF4E2	SARS-CoV-2:NSP2 SARS-CoV:NSP2
FUS	IBV:N MHV:NSP2
HNRNPA2B1	IBV:N
HNRNPD	IBV:N MHV:NSP2
HNRNPF	IBV:N MHV:NSP2
KPNB1	MHV:NSP2 SARS-CoV:ORF6
PPIA	SARS-CoV:N SARS-CoV:NSP1 SARS-CoV:NSP15
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

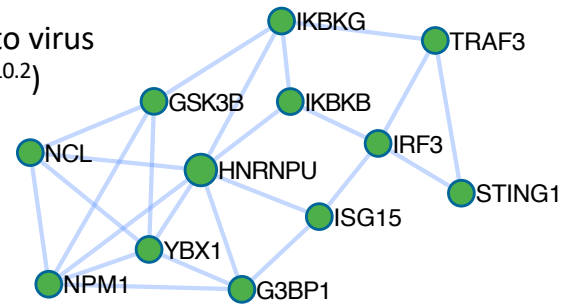
### mRNA catabolic process (p=10<sup>-17</sup>)



### Regulation of type I interferon production (p=10<sup>-12</sup>)



### Response to virus (p=10<sup>-10.2</sup>)



### Translation (p=10<sup>-4.3</sup>)

