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# The proximal proteome of 17 SARS-CoV-2 proteins links to disrupted antiviral signaling and host translation

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23 **Abstract**

24 **Viral proteins localize within subcellular compartments to subvert host machinery and**  
25 **promote pathogenesis. To study SARS-CoV-2 biology, we generated an atlas of 2422**  
26 **human proteins vicinal to 17 SARS-CoV-2 viral proteins using proximity proteomics. This**  
27 **identified viral proteins at specific intracellular locations, such as association of accessory**  
28 **proteins with intracellular membranes, and projected SARS-CoV-2 impacts on innate**  
29 **immune signaling, ER-Golgi transport, and protein translation. It identified viral protein**  
30 **adjacency to specific host proteins whose regulatory variants are linked to COVID-19**  
31 **severity, including the TRIM4 interferon signaling regulator which was found proximal to**  
32 **the SARS-CoV-2 M protein. Viral NSP1 protein adjacency to the EIF3 complex was**  
33 **associated with inhibited host protein translation whereas ORF6 localization with MAVS**  
34 **was associated with inhibited RIG-I 2CARD-mediated *IFNB1* promoter activation.**  
35 **Quantitative proteomics identified candidate host targets for the NSP5 protease, with**  
36 **specific functional cleavage sequences in host proteins CWC22 and FANCD2. This data**  
37 **resource identifies host factors proximal to viral proteins in living human cells and**  
38 **nominates pathogenic mechanisms employed by SARS-CoV-2.**

39

40 **Author Summary**

41 **SARS-CoV-2 is the latest pathogenic coronavirus to emerge as a public health threat. We**  
42 **create a database of proximal host proteins to 17 SARS-CoV-2 viral proteins. We validate**  
43 **that NSP1 is proximal to the EIF3 translation initiation complex and is a potent inhibitor**  
44 **of translation. We also identify ORF6 antagonism of RNA-mediate innate immune**  
45 **signaling. We produce a database of potential host targets of the viral protease NSP5,**  
46 **and create a fluorescence-based assay to screen cleavage of peptide sequences. We**  
47 **believe that this data will be useful for identifying roles for many of the uncharacterized**

- 48 **SARS-CoV-2 proteins and provide insights into the pathogenicity of new or emerging**  
49 **coronaviruses.**

## 50 INTRODUCTION

51 Coronaviruses comprise a diverse family of large positive-sense single stranded (+ss)RNA  
52 enveloped viruses that cause respiratory and gastrointestinal disease. In addition to common  
53 seasonal coronaviruses, a number of strains can cause severe disease, as seen in the Severe  
54 Acute Respiratory Syndrome (SARS-CoV-1) virus outbreak in 2003 (1), the Middle Eastern  
55 Respiratory Syndrome (MERS) virus outbreak in 2012 (2) and the 2019 outbreak of SARS-CoV-  
56 2 (3). This viral family has large (26 to 32kb) genomes that encode tens of viral proteins. All  
57 coronaviruses have a similar organization consisting of a large open reading frame encoding two  
58 overlapping polyproteins, ORF1A and ORF1B. These polyproteins are cleaved by one of two  
59 viral proteases, NSP3 and NSP5, with the resulting protein products sequentially numbered  
60 NSP1-NSPX. ORF1AB is invariably followed by structural genes, including the Spike protein (S),  
61 Envelope protein (E), Membrane protein (M), and Nucleocapsid protein (N). SARS-CoV-2  
62 encodes NSP1-16 as well as the accessory proteins ORF3a, ORF3b, ORF6, ORF7a, ORF7b,  
63 ORF8, ORF9b, ORF9c, ORF10, and ORF14, though it is not known if each open reading frame  
64 encodes for a functional protein product. The function of many SARS-CoV-2 accessory proteins  
65 is either unknown or highly variable across differing coronaviruses, underscoring the need to  
66 begin mapping their putative localizations and functions.

67 Proximity proteomics (BioID) uses enzymes, such as the modified bacterial biotin ligase, BirA, to  
68 biotinylate nearby proteins on lysine residue-containing proteins within a radius of 10-20nm (4).  
69 When fused to a protein of interest it labels not only proteins that directly bind the fused protein  
70 but also those adjacent to it, enabling rapid isolation of biotinylated proteins whose identity can  
71 provide clues about the localization and function of the protein studied. When coupled to mass  
72 spectrometry it provides an alternative to traditional tandem affinity purification and mass  
73 spectrometry (TAP-MS) (5). Whereas, TAP-MS can isolate protein complexes that stably bind  
74 the protein of interest in a manner robust enough to survive protein extraction, BioID-MS labels  
75 both transient and stable interactors in living cells, particularly those stabilized by cellular

76 membranes that can be destroyed in traditional TAP-MS experiments. In this way, BioID may  
77 localize the cellular “neighborhoods” of a given fusion protein. We recently generated a biotin  
78 ligase derived from *Bacillus subtilis*, which has 50 times greater activity than the original *E. coli*  
79 BirA (4, 6), allowing decreased labeling times and increased signal-to-noise ratios. Applying  
80 proximity proteomics to SARS-CoV-2 viral proteins in human cells may facilitate insight into their  
81 localization and putative functions.

82 The actions of specific SARS-CoV-2-encoded proteins are only partially understood at present.  
83 The replication transcription complex, which includes the RNA-dependent RNA polymerase and  
84 other factors, and the structural proteins, which are necessary for protecting the newly  
85 synthesized genomes and assembling the viral particles, comprise the core viral replication  
86 machinery. Other viral gene products, generally termed accessory factors, are believed to be  
87 dedicated to manipulating the host environment to foster viral replication (7). One of the main  
88 functions of accessory factors is to block host antiviral response (8). Non-SARS-CoV-2  
89 coronaviruses have also been shown to block host translation (9, 10), inhibit interferon signaling  
90 (11, 12), antagonize viral RNA sensing (13, 14), and degrade host mRNAs (15). The degree of  
91 homology between SARS-CoV-2 and other coronaviruses, suggests the existence of both shared  
92 and divergent host protein interactions between its viral proteins and those of the other members  
93 of the coronavirus family.

94 Here we used proximity proteomics to identify the human proteins vicinal to 17 major SARS-CoV-  
95 2 proteins and, from that data and validation studies, to predict their likely location and function.  
96 We examined the intersection of the resulting atlas of human factors adjacent to SARS-CoV-2  
97 viral proteins with risk loci associated with severe COVID-19 by genome wide association studies  
98 (GWAS). This nominated specific, viral protein-adjacent host candidates whose natural variation  
99 in expression may contribute to differences in COVID-19 susceptibility in the population. We also  
100 demonstrated that multiple SARS-CoV-2 products can affect host translation and host innate

101 immune signaling and define a list of potential host targets and pathways for the NPS5 protease.  
102 Taken together, these resource data plot the location of the 17 major SARS-CoV-2 within the cell,  
103 define an atlas of human host proteins adjacent to them, and offer insight into potential pathogenic  
104 mechanisms engaged by SARS-CoV-2.

## 105 **RESULTS**

### 106 **Host proteins proximal to viral proteins and their subcellular localization**

107 To identify the human host proteins vicinal to the 17 major SARS-CoV-2 encoded viral proteins,  
108 HA epitope tagged fusions of BASU-BirA (6) were generated with each of these 17 viral ORFs  
109 (**Fig. 1A**). BASU was introduced at the N and C terminus to minimize disruption as previously  
110 described (16). Samples were prepared from plasmid-transfected 293T cells after 2 hours of  
111 biotin labeling and the biotinylated proteins were then isolated using streptavidin. Samples were  
112 divided for LC-MS/MS and immunoblotting (**Fig. S1**). MS data search was performed and protein  
113 lists were analyzed and scored using the Significance Analysis of Interactome (SAINT) method  
114 (17). Using a cutoff of a SAINT score of 0.9 generated a list of 2422 host proteins (**Fig. 1B, Fig.**  
115 **S2, Table S1**) across the 17 viral proteins studied, 514 of which were unique to a specific viral  
116 protein. These data (**Table S2**) comprise a compendium of candidate human proteins adjacent to  
117 SARS-CoV-2-encoded proteins.

118 The identity of these 2422 human proteins provided clues to SARS-CoV-2 biology. Molecular  
119 function analysis (**Fig. 1B-D**) identified processes associated with SARS-CoV-2 viral protein  
120 impacts. This included translation initiation, RNA binding, the 26S proteasome, signaling, and  
121 SNARE-associated intracellular transport. It also identified adjacencies to major histocompatibility  
122 (MHC) proteins and components of the nuclear pore complex (NPC). A number of these  
123 processes, such as protein translation, are known processes affected by coronaviruses, while  
124 others, such as RNA-binding, are less well characterized.

125 To begin to map putative localizations for the 17 studied SARS-CoV-2 proteins within the cell,  
126 cellular component GO-term enrichment analysis was performed (**Fig. 2A**), which pointed to  
127 possible intracellular localizations for each viral protein based on curated knowledge of the host  
128 proteins identified adjacent to each viral protein. To validate and extend this, protein fractions  
129 were prepared from cells expressing each SARS-CoV-2 protein studied. These included four  
130 overlapping fractions: a) cytoplasm b) cytoplasm/membrane c) nucleus/membrane, and d)  
131 nucleus (**Fig. 2B**). Integrating GO-term analysis with immunoblotting of these fractions enabled  
132 predictions of the likely intracellular localization of each viral protein (**Fig. 2C**). We further  
133 confirmed NSP5 diffuse expression and ORF3a membrane localization through immunostaining.  
134 Many SARS-CoV-2 accessory proteins concentrate in the ER or in ER-proximal membranes (M,  
135 ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, and ORF10). A number, however, appear to be  
136 predominantly cytoplasmic (NSP1, NSP2, NSP5, NSP9, NSP15, ORF9b) and, interestingly,  
137 several appear to localize in part to the nucleus (NSP14, ORF6, ORF9c). The localization  
138 predicted from these data is consistent with observations from other recent work (16, 18). Of the  
139 membrane localized proteins, subtle differences in location could be inferred. In the case of M  
140 protein, association with membranes in the endocytic pathway as well as lysosomal membranes  
141 was predicted. ORF8 and ORF10 clustered similarly with enrichment for ER interactions in the  
142 lumen. These data indicate that specific SARS-CoV-2 may display increased localization to a  
143 variety of intracellular sites, including the cytoplasm, nucleus and distinct endomembranes.

#### 144 **Viral proximal interactors include drug targetable host genes.**

145 There is a lack of SARS-CoV-2 specific antiviral therapies or against coronaviruses generally.  
146 Many current and experimental therapeutics were developed for activity against other viruses and  
147 are being tested for cross efficacy against SARS-CoV-2. Others are therapies known to have  
148 broad antiviral effects. There is significant interest in developing drugs that directly target SARS-  
149 CoV-2 viral proteins, but research and development may take years before use in patients.  
150 Another approach is using drugs against host genes critical to virus infection and replication. For

151 example, drugs targeting ACE-2, the main receptor for SARS-CoV-2, or ACE-2 expression and  
152 function have been pursued. To expand the list of possible drugs beyond entry inhibitors, we  
153 compared the viral proximal proteome generated in this study against the “druggable” genome,  
154 which include databases of the gene targets of available drugs. This generated a list of 47 host  
155 genes (**Fig. S3, Table S3**) and highlights, as previously reported (16), a group of cellular kinases  
156 associated with N protein. The viral nucleocapsid has been shown to be phosphorylated and  
157 phosphorylation is suggested to be important for its function (19, 20). This highlights cellular  
158 kinase inhibitors as drugs with possible activity against SARS-CoV-2.

### 159 **GWAS-linked host proteins in the viral proximal proteome**

160 The genetic basis for the wide spectrum of COVID-19 severity in different individuals within the  
161 human population is not fully understood. A number of recent genome wide association studies  
162 (GWAS) studies have endeavored to map genetic risk loci associated with SARS-CoV-2 infection  
163 and COVID-19 clinical severity (21, 22). These studies leverage large numbers of patients to  
164 identify SNPs that are correlated with outcomes such as infection and severity of disease,  
165 including hospitalization and mortality. Such linkage studies have identified a number of non-  
166 coding variants that may perform a regulatory function, for example, by altering expression of  
167 effect genes (eGenes) important in host susceptibility to SARS-CoV-2.

168 To determine if any putative COVID-19 risk-linked regulatory variants might control the expression  
169 of host proteins proximal to SARS-CoV-2 viral proteins, the following analysis was performed.  
170 Using publicly available data from GWAS studies (21, 22), all single nucleotide polymorphisms  
171 (SNPs) associated with increased risk of COVID disease that reside in noncoding DNA were  
172 identified. These were filtered for variants localized to open chromatin, characteristic of regulatory  
173 DNA, in cell types relevant to COVID-19 pathogenesis, including immune and pulmonary cells.  
174 The resulting disease risk-linked variants were further distilled to those identified as expression  
175 quantitative trait loci (eQTLs) for specific putative eGene targets (**Fig. 3A**). These eGenes, which



176 represent a set of genes whose expression may be controlled by natural variants in the human  
177 population linked to COVID-19 risk, were then intersected with the atlas of host factors identified  
178 as adjacent to SARS-CoV-2 viral proteins by proximity proteomics. Publicly available protein  
179 interaction data was then integrated to project the connectedness of resulting gene set (**Fig. 3B**).  
180 The resulting network was notable for host proteins implicated in cytokine signaling, cell cycle  
181 control, transcription, and translation, suggesting that genetic susceptibility to COVID-19 may link  
182 to variations in the expression of proteins that mediate these processes.

183 Among proteins identified by this analysis was TRIM4, a RING E3 ligase, that activates type I  
184 interferon signaling through activation of the cytosolic RNA sensor RIG-I. TRIM4 was significantly  
185 associated with SARS-CoV-2 M protein in proximity proteomics data (**Table S1**) and, using  
186 eQTLgen (23), a regulatory SNP (rs1569055) approximately 230kb downstream of the TRIM4  
187 promoter was recently associated with increased COVID severity in patients (22). High-C  
188 chromatin immunoprecipitation (HiChIP) data from the immortalized B-cell line GM12878 as well  
189 as primary T-cell populations demonstrated chromatin looping from the SNP to the TRIM4  
190 promoter (**Fig. 3C**). Looping of this SNP increased contact strength in naïve, T-regs and Th-17  
191 T-cells. TRIM4 is one of a group of ubiquitin ligases (24-27) that can activate RIG-I during RNA-  
192 sensing and subsequent antiviral signaling. Altered expression coupled with disruption by  
193 potential association with the SARS-CoV-2 M protein supports a model with the following features;  
194 a) individuals with this regulatory variant may express less TRIM4 b) physical association with  
195 SARS-CoV-2 M protein further reduces functional TRIM4 c) a relative reduction in biologically  
196 active TRIM4 leads to reduced innate immune signaling d) this reduction leads to increased  
197 susceptibility to SARS-CoV-2 pathogenesis. Integrating proximity proteomics data with genetic  
198 risk eQTL variants may help identify such candidate susceptibility mechanisms for natural  
199 variations in disease outcomes within the population.

200 **Predicted viral antagonism of host protein translation and antiviral response**

201 NSP1 is a part of the viral polyprotein ORF1 and during normal viral replication is cleaved and  
202 liberated by the viral protease NSP3. Earlier work has identified NSP1 of SARS-CoV-1 as a  
203 potent inhibitor of translation in a mechanism that involves interactions with the host ribosomes  
204 (9, 12). Recently other groups have shown that NSP1 of SARS-CoV-2 similarly blocks translation  
205 through interaction with the 40s ribosome (28, 29). High confidence proteins proximal to NSP1  
206 included EIF3A, EIF3B, EIF2G, and EIF4G2 (**Fig. 4A**) of which the first 3 are components of the  
207 EIF3 translation initiation complex. Interestingly, members of the EIF3 complex were not  
208 identified as high-confidence interactors by traditional TAP-MS studies(16, 18). To test if SARS-  
209 CoV-2 NSP1 inhibits host translation, NSP1 was expressed in HEK293T cells followed 24 hours  
210 later by transfection of in-vitro transcribed capped and polyadenylated mRNA expressing  
211 luciferase. NSP1 reduced luciferase signal by (49.7%) as compared to GFP control (**Fig. 4B**),  
212 demonstrating that NSP1 can inhibit host cap-dependent translation, consistent with data reported  
213 by others (28, 29). To determine if NSP1 could inhibit translation of host-derived 5' UTRs and  
214 host IRES elements, two host UTRs (IFIT1 and ISG15) were subcloned separately upstream of  
215 luciferase along with two host IRES sequences (XIAP1 and APAF1) and luciferase measured in  
216 cells transfected with or without NSP1 construct. NSP1 reduced luciferase signal of both 5' UTRs  
217 (IFIT1 = 55.2%, ISG15 = 53.1%) and IRES elements (XIAP1 = 55.0%, APAF1 = 40.0), indicating  
218 a block in translation of these elements (**Fig. 4B**). Lastly, NSP1 effects were tested on the SARS-  
219 CoV-2 5' UTR and the Cricket Paralysis Virus (CRPV) IRES. CRPV IRES is a minimal viral-  
220 derived IRES that initiates translation completely independent of EIF3. Surprisingly, NSP1  
221 blocked both viral elements (SARS-CoV-2 = 59.1%, CRPV = 52.2%) compared to GFP control.  
222 NSP1 therefore exhibits broad translation inhibition of mRNAs containing various regulatory  
223 elements, suggesting NSP1 action on the initiating ribosome, however, additional actions, such  
224 as mRNA cleavage (9, 15), may also be operative.

225 Host innate immune detection and signaling pathways are heavily targeted by viral proteins,  
226 especially accessory proteins (30). Mitochondrial Activation of Viral Signaling (MAVS) is a critical  
227 signaling adaptor for RIG-I like receptors (RLR) cytosolic sensing pathway (31-34). It recruits  
228 activated RLR sensors RIG-I and MDA-5 at mitochondrial and mitochondrial-proximal membranes  
229 and leads to the activation of both IRF3 and NF- $\kappa$ B and expression of type-I interferons (35). RIG-  
230 I and MDA-5 recognize various types of non-host or aberrant RNA species and are critical for host  
231 defense against RNA viruses (36). MAVS was found as a high confidence protein proximal to  
232 two SARS-CoV-2 proteins: ORF6 and ORF9b (**Fig. 4C-D**). ORF6 has been found to inhibit type-  
233 I interferon SARS-CoV-2 (37, 38) and the closely related SARS-CoV-1 (11, 39). One study  
234 demonstrated that ORF6 inhibition of type-1 interferon expression was linked to ORF6 binding to  
235 nuclear import complex Rael/Nup98 (38), both of which were also captured as proximal  
236 interactors of ORF6, but not ORF9B. We tested the ability of our SARS-CoV-2 ORF6 and ORF9b  
237 constructs to inhibit RLR signaling by co-transfecting constructs expressing ORF6 or ORF9B  
238 along with a reporter expressing nanoluciferase under the control of the *IFNB1* promoter for  
239 interferon  $\beta$ 1 along with a second reporter constitutively expressing firefly luciferase. To activate  
240 RLR signaling we transfected in a plasmid expressing a truncated version of RIG-I only containing  
241 the 2 CARD domains. This truncation is constitutively recruited to MAVS and initiates signaling  
242 in absence of any RNA stimulus and will test the viral proteins ability to block any signaling  
243 downstream of sensing. ORF6 significantly inhibited RIG-I 2CARD activation of *IFNB1* promoter  
244 activity by 96 percent (**Fig. 4E**) while ORF9b showed no effect on inhibiting *IFNB1* promoter  
245 activity. These data demonstrating ORF6 proximity to MAVS, along with ORF6 inhibition of *IFNB1*  
246 promoter induction, implicate ORF6 impairment of MAVS in the RLR innate immune signaling  
247 pathway.

#### 248 **NSP5 proteomics prediction of potential host cleavage targets**

249 NSP5 is one of two critical proteases encoded by SARS-CoV-2 and is also known as SARS-CoV-  
250 2 3CLpro due to its similarity to picornavirus 3C proteases and a number of other +ssRNA viruses.

251 These proteases all contain chymotrypsin-like folds and a triad of residues harboring the critical  
252 cysteine residue (40). 3CLpro-like proteases are considered important therapeutically since they  
253 are essential for cleaving large polyprotein products produced by +ssRNA viruses and chemical  
254 protease inhibitors may act broadly across members of a given virus family (41, 42). In addition  
255 to their necessity in the virus life cycle, many viral proteases can target host proteins and  
256 specifically affect antiviral responses or other cellular processes (43-45). Complementing  
257 previous efforts to infer targets of the NSP5 protease, we identified 34 host proteins in the NSP5  
258 proximal proteome (**Fig S2C**). To nominate possible host targets of NSP5 whose levels are  
259 decreased upon protease expression, we performed SILAC mass spectrometry comparing wild  
260 type SARS-CoV-2 NSP5 to the catalytically-inactive NSP5<sup>C145A</sup> mutant (16, 46). Residue 145 is  
261 the critical catalytic cysteine and mutation to alanine prevents protease activity (47). A number  
262 of host proteins showed significant depletion in cells expressing wild type NSP5, but not protease-  
263 inactive NSP5<sup>C145A</sup> (**Fig. 5A**). Combining both data generated identified an additional 26  
264 candidates resulting in a pool of 60 potential host protein targets for NSP5 (**Fig 5B**).

265 To begin to examine potential cleavage of these candidate proteins by NSP5, we searched their  
266 peptide sequences for potential cleavage sites using a published a cleavage prediction algorithm  
267 (48). We then took these peptide sequences and tested them for cleavage by NSP5 using a loss  
268 of fluorescence resonance energy transfer (FRET) fluorescence assay. In brief, potential  
269 cleavage sites were inserted between a FRET pair and then this construct was co-transfected  
270 along with plasmids expressing either NSP5 or the NSP5<sup>C145A</sup>, with loss of FRET signal only after  
271 wild type NSP5 expression as indicative of cleavage. Four sequences taken from SARS-CoV-2-  
272 ORF1AB polyprotein, which is normally cleaved by NSP5, were cleaved as expected and as  
273 demonstrated by loss of FRET signal (**Fig. 5C-D**). Testing of sequences from human CDKN2AIP,  
274 CWC22, FANCD2, and P53 proteins indicated NSP5 cleavage of one CWC22 and two FANCD2  
275 peptide sequences (**Fig. 5C-D**). Neither CDKN2AIP nor P53 sequences tested were cleavable  
276 by NSP5 in our assay and their depletion in the SILAC data may represent indirect effects of

277 NSP5 activity. CWC22 is a component of the RNA spliceosome required for pre-mRNA splicing  
278 via promotion of exon-junction complex assembly (49, 50). FANCD2 is activated by ATM and  
279 localizes at BRCA1 foci during DNA damage (51). These data suggest that SARS-CoV-2 may  
280 target host RNA splicing and DNA damage pathways via NSP5-mediated reduction in key  
281 proteins, namely CWC22 and FANCD2, that are involved in these processes.

282 As noted, viral protease inhibitors are a powerful class of drugs that potently block viral replication  
283 by preventing processing of viral polyproteins into functional subunits. Inhibition of viral proteases  
284 should also prevent cleavage of host proteins which may serve to blunt toxic effects on infected  
285 cells. GC376 is a NSP5 protease inhibitor developed against feline coronavirus, the causative  
286 agent of fatal feline infectious peritonitis. Recent reports showed GC376 to be effective against  
287 SARS-CoV-2 NSP5. We tested the effect of GC376 on the cleavage of the ORF1ab-2 and  
288 FANCD2-2 peptide sequences. Using a range of concentrations up to 80  $\mu$ M, ORF1ab-2 showed  
289 a modest inhibition of NSP5 as compared to NSP5<sup>C145A</sup> in the FRET assay. FANCD2-2 showed  
290 a dramatic reduction in cleavage by NSP5 even at concentrations of 20  $\mu$ M (**Fig. 5D-E**). These  
291 data support GC376 inhibition of SARS-CoV-2 NSP5 action on viral and human host protein  
292 sequences cleavable by the viral protease.

## 293 **DISCUSSION**

294 Here we present a compendium of human host proteins adjacent to 17 SARS-CoV-2 viral  
295 proteins, with a goal to offer insight into potential mechanisms that these viral proteins may  
296 engage during pathogenesis. These data encompass the less well understood SARS-CoV-2  
297 accessory factors and predict the localization of each these viral proteins as well as identify  
298 significant adjacencies to proteins that mediate core cellular processes, including translation,  
299 signaling, RNA interactions, and intracellular transport. For translation, SARS-CoV-2 NSP1 was  
300 found to be adjacent to subunits of the EIF3 translation initiation complex and proved a broad  
301 inhibitor of translation. For innate immune signaling, viral ORF6 was found proximal to the RLR

302 pathway component, MAVS, with ORF6 potently inhibiting induction of the RLR downstream  
303 *IFNB1* promoter. Integration of GWAS data in COVID-19 identified SNPs associated with natural  
304 variation in the expression of specific genes, including the viral M protein-proximal TRIM4  
305 activator of type I interferon, that may contribute to disease susceptibility differences in the human  
306 population. Comparing wild type NSP5 with its catalytically inactive point mutant helped identify  
307 proteins whose levels were decreased by this viral protease and nominated cleavage sequences  
308 in human CWC22 and FANCD2, implicating specific candidates for viral disruption of normal pre-  
309 mRNA splicing and DNA damage pathways, respectively. We also observed a number of SARS-  
310 CoV-2 proteins (M, NSP2, NSP9, NSP15, ORF6, ORF7a, ORF7b, ORF8, ORF9c, and ORF10)  
311 vicinal to nuclear pore proteins. Given that coronavirus replication takes place exclusively in the  
312 cytosol of cells, these interactions, if functional, might point to a viral role in disrupting nuclear  
313 import/export. This is further supported by several lines of genetic evidence. GWAS data suggest  
314 the importance of nuclear pore component NUP43 and overlap of our proteomics data with whole  
315 genome CRISPR screen hits from recently studies(52-54) suggests that the mRNA export factors  
316 MCM3AP and NXF1 are necessary for viral replication. Taken together, these data indicate  
317 potential intracellular locations and candidate functions of the SARS-CoV-2 viral proteins studied  
318 and provide a resource for future studies of pandemic coronaviruses.

319 SARS-CoV-2, as the etiological agent of COVID-19, joins SARS-CoV-1 and MERS as an  
320 important coronavirus pathogen. Very minor mutations in the viral spike protein (55-58) along  
321 with a number of animal reservoirs in endemic regions represent a significant risk for new  
322 pandemic coronavirus strains to emerge (59), underscoring the need to understand coronaviral  
323 accessory protein functions and virus-host interactions. Comparative studies that analyze  
324 multiple coronaviruses (18, 60), including both highly pathogenic and nonpathogenic, will be very  
325 beneficial to understanding what can identify new possibly pandemic virus strains. Such  
326 resources may allow the research community to not only address current concerns and also  
327 provide insight to address with newly emerging coronaviruses in the future. Currently, the ability

328 of S proteins capable of binding to human ACE2 receptor, such as in SARS-CoV-1, SARS-Cov-  
329 2, and MERS, has been used as an indicator of human pathogenicity. But there are  
330 coronaviruses, such as HCoV-NL63, that also use ACE2 as a receptor but only cause mild  
331 disease (61). Thus, comparing the actual molecular interactions and effects of viral proteins on  
332 the host between pathogenic and non-pathogenic virus strains may provide actual insight on what  
333 makes certain coronaviruses more medically dangerous and highlight critical virus-host  
334 interactions that may be targeted to reduce disease.

335 The viral envelope of SARS-CoV-2 must contain the proper structural components comprised of  
336 S, E, M, and N with a completed viral genome (62) and transcription of both subgenomic and  
337 genomic RNA occurs in membranous compartments (63). Accordingly, coronaviruses devote  
338 substantial portions of its large genome to manipulating host processes involved in ER-Golgi  
339 transport and endocytic and exocytic activity, which was captured in the proximal interactome.  
340 We also found evidence of the interaction of SARS-CoV-2 with MHC class I molecules with M,  
341 ORF7a, ORF7b, ORF8, and ORF10. Down-regulation of surface expressed proteins has been  
342 reported for SARS (64). It is still an open question to how SARS-CoV-2 affects surface expression  
343 of important host receptors, which viral proteins affect this process, and the effects on virus  
344 replication and disease.

345 Translation inhibition is a general strategy utilized by many virus families including other RNA  
346 viruses like orthomyxoviruses (65), picornaviruses (66, 67), rhabdoviruses (68), and togaviruses  
347 (69). Host translational blockade may broadly block antiviral responses and can also cause affect  
348 the viability of the infected cell. Some but not all viruses have strategies to overcome translational  
349 shutoff, biasing translation of viral mRNA, including the use of IRES elements (70). Lung tissue  
350 from COVID patients, in particular, displayed proteomic changes associated with translation  
351 inhibition. NSP1 from both SARS-CoV-1 (9) and SARS-CoV-2 (28, 29) have been shown to be  
352 potent inhibitors of host translation and are thought to do so using at least two mechanisms:

353 binding to and inhibition of EIF3 translation initiation complex and direct cleavage of host mRNAs.  
354 Cryo-EM studies place a domain of NSP1 as sitting in the mRNA channel of the 40S ribosome.  
355 Our proximity proteomics data shows NSP1 of SARS-CoV-2 binding to a significant number of  
356 EIF3 complex subunits and we demonstrate that NSP1 is able to block translation of capped  
357 transcripts as well as transcripts containing host and viral IRES elements. We also observe, as  
358 another study has shown (28), that NSP1-induced translational shutoff affects host and viral  
359 transcripts containing the viral 5' UTR. This element exists on all genomic and subgenomic viral  
360 RNAs (71). Whether other SARS-CoV-2 factors are necessary to overcome NSP1 translational  
361 inhibition or if, during viral replication, the large number of viral transcripts simply outcompetes  
362 host transcripts, as seen in vesicular stomatitis virus (72), remains to be determined. A recent  
363 study(73) from autopsies of COVID patients characterized whole proteome changes in multiple  
364 organs.

365 Innate immune signaling is a central mechanism of host cell response to viral infection. ORF6 of  
366 SARS-CoV-1 (11) and SARS-CoV-2 (38) were shown to be potent inhibitors of such antiviral  
367 signaling. One proposed mechanism is that ORF6, through association with specific NPCs  
368 (RAE1-Nup98), blocks import of activated transcription factors needed to induce *IFNB1*  
369 transcripts and other primary interferon-stimulated genes. In this regard, we identified MAVS  
370 proximal to ORF6 and ORF9b. We observed that ORF6, but not ORF9b, inhibited RLR signaling  
371 downstream of RIG-I RNA-binding. Taken with the observed adjacencies to nuclear pore proteins  
372 noted above, it is likely that the model suggested for ORF6 from SARS-CoV-1 (39) may also be  
373 operative for SARS-CoV-2 and that disruption of nuclear import may not be specific only to  
374 immune-specific transcription factors but may affect a wider variety of imported proteins.

375 Viral proteases, such as SARS-CoV-2 NSP5 studied here, have been shown to be potent antiviral  
376 targets (74). These proteases are essential for viral replication and escape has proven difficult in  
377 resistance studies (75). Coronaviruses encode two proteases NSP3 and NSP5, with NSP5



378 classified as the main protease. They are both necessary for the processing of the ORF1ab  
379 polyprotein containing the viral replicase proteins. NSP5 shows similarity to proteases found in  
380 picornaviruses and noroviruses (76). Beyond their importance in viral replication, these viral  
381 proteases can target host proteins containing their target residues (77). NSP5 recognizes certain  
382 glutamine-serine/alanine/glycine residues, with added specificity being determined by two to three  
383 flanking residues (48). Picornavirus virulence has been shown to be mediated in part by 3C  
384 protease cleavage of host proteins (44). Using both BioID and SILAC metabolic labeling followed  
385 by mass spectrometry, we sought to identify candidate host proteins and use a modified FRET-  
386 based cleavage assay to determine if these candidates contained sequences cleavable by NSP5.  
387 We identified human CWC22 and FANCD2 as candidates; both proteins contained sequences  
388 that could be cleaved by NSP5 in an assay used here which can be used to rapidly assess other  
389 potential host targets. The proteomic studies also identified clusters of host factors involved in  
390 DNA damage and repair and RNA splicing. Furthermore, we show the effects of GC376 (78), a  
391 protease inhibitor of feline coronavirus, displays evidence of inhibition of NSP5 cleavage activity.  
392 Consistent with this, GC376 has been shown to block viral replication of SARS-CoV-2 in early  
393 studies (79) and we observe that this protease inhibitor blocks NSP5 cleavage of both host and  
394 viral target peptide sequences.

395 The global impacts of the SARS-CoV-2 pandemic have focused attention on identifying new  
396 treatments and interventions. Given both the newness of the virus and the relative dearth of  
397 research into human coronaviruses, it is important that many resources are generated to better  
398 understand aspects of the virus-host interaction. The present work contains a proximal proteomic  
399 resource for 17 SARS-CoV-2 viral proteins and combining such proximal proteomics with TAP-  
400 based proteomics may be helpful in leveraging the strengths associated with each technique.  
401 While proximity proteomics can identify transient, indirect, and weak binding events, including  
402 those dependent on intact membranes, TAP-based approaches can focus attention on complexes  
403 of proteins that stably associated with each other. We validate the quality of the present proximity

404 data set by corroborating spatial insights with biochemical fractionation experiments. Taken  
405 together with other efforts to generate high-quality resources, these data should prove helpful in  
406 both generating hypotheses and better understanding dynamics of virus-host interactions in  
407 regards to human disease.

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#### 412 **Author Contributions**

413 Conceptualization, J.M.M., M.R., P.A.K.; Methodology J.M.M., M.R., R.L.S.; Formal Analysis,  
414 L.D., I.F., M.G.G., X.Y., Y.Z.; Investigation, J.M.M., W.M., M.R., D.S.R., D.R., R.L.S., X.Y., Y.Y.;  
415 Writing – Original Draft, J.M.M.; Writing – Review & Editing, J.M.M., M.R., R.L.S., Y.W., P.A.K.;  
416 Visualization, L.D., I.F., M.G.G., R.L.S.; Project Administration, J.M.M.; Supervision, Y.W., P.A.K.

#### 417 **Declaration of Interests**

418 The authors declare no competing interests.

419

420 **FIG. LEGENDS**

421 **Fig. 1. Proximal Interactome of 17 SARS-CoV-2 proteins.**

422 **A)** Schematic of BioID workflow. **B)** Curated network of SARS-CoV2 virus-host protein  
423 associations (SAINT  $\geq 0.9$ ) obtained from BASU BioID. Coronavirus proteins are labeled in light  
424 blue and virus-host interactions are connected by red edges, while host-host protein interactions  
425 obtained from high confidence STRING interactions are labeled in grey. Highlighted node clusters  
426 of similar function, including 26S proteasome components (black), MHC Class I (red), nuclear  
427 pore (dark blue), RNA-binding (maroon), SNARE complex (purple), translation initiation complex  
428 (green) proteins were selected based on GO term analysis. **C)** Selected biological process GO  
429 term enrichment; enrichment scores are given as  $-\text{Log}_{10}$  p-values. Selected GO terms are  
430 nuclear pore organization, translational initiation, endosomal transport, and RNA splicing. **D)**  
431 Heatmap of molecular function GO term enrichment of SARS-CoV-2 proteins. All presented GO  
432 terms have a  $-\text{Log}_{10}$  p-value  $>3$  for the Nucleoprotein, the listed non-structural proteins, or the  
433 listed open reading frames or a  $-\text{Log}_{10}$  p-Value  $>5$  for the M membrane protein.

434 **Fig. 2. Localization of SARS-CoV-2 proteins.**

435 **A)** Heatmap of cell component GO term enrichment of SARS-CoV-2 proteins. **B)** Western blots  
436 of SARS-CoV-2 viral protein-expressing HEK293T cell fractions; whole cell lysate (WCL), cytosol,  
437 cytosol/membrane, nucleus/membrane and nucleus fractions. Alpha-tubulin, calnexin, and  
438 histone H3 were used as fractionation controls for cytosol, membrane, and nucleus  
439 respectively. Schematic **C)** and table **D)** depicting the predicted location of all SARS-CoV-2  
440 proteins surveyed in this study based on both the BioID and fractionation analysis.

441 **Fig. 3. COVID disease risk eGenes proximal to viral proteins.**

442 **A)** Table of GWAS risk SNPs which also scored as BioID hit. **B)** Map of connectedness of eGenes  
443 (Mauve) with BioID interactors (Gray) and the corresponding viral proteins (Purple). eGenes also  
444 identified by BioID are outlined in black. GWAS-identified eGenes-associated with antiviral

445 response, cell-cycle, transcription, and translation are also highlighted. **C)** Virtual 4-C plot  
446 showing chromatin contact between TRIM4 promoter and linked COVID disease risk SNP  
447 (rs1569055). Genome tracks showing ATAC peaks and contact loops of GM12878, Naïve T, Th-  
448 17, and T-reg cells.

449 **Fig. 4. NSP1 and ORF6 disruption of host translation and innate immune signaling.**

450 **A)** Curated map of NSP1 proximal interactors. Highlighted are host proteins involved in  
451 translation initiation. **B)** Effect of NSP1 on translation of in vitro transcribed, capped  
452 polyadenylated transcripts containing 5' UTRs from SARS CoV-2, *IFIT1*, and *ISG15* as well as  
453 IRES elements from *XIAP1*, *APAF1*, and CRPV. Data shown is the average of three independent  
454 experiments and significance was calculated using Student's T Test where \* indicates p  
455 value<0.005. Curated maps of ORF6 **C)** and ORF9b **D)** proximal proteins showing nuclear pore  
456 protein complex association with ORF6 and MAVS association with both ORF6 and ORF9b. **E)**  
457 Effect of ORF6 and ORF9b on *IFNB1* promoter activity after RIG-I 2-CARD induction. Normalized  
458 luciferase shown is the ratio of nano luciferase/firefly luciferase normalized to empty vector  
459 control. Data shown is the average of three independent experiments.

460 **Fig. 5. BioID and SILAC MS identify candidate targets for the viral protease NSP5.**

461 **A)** Comparison of two biological replicates of protein abundance in HEK293T cells expressing  
462 either NSP5 wild type (WT) or the catalytically inactive NSP5<sup>C145A</sup> mutant by log<sub>2</sub> fold change. **B)**  
463 Map of NSP5 proximal interactome (Gray) overlaid with host proteins decreased in abundance in  
464 SILAC (Red). CDNK2AIP was detected as both a BioID hit and decreased in abundance in  
465 SILAC. **C)** Peptide cleavage assay of four sequences from SARS-CoV-2 polyprotein ORF1AB  
466 (PP1ab) and the indicated host genes: CWC22, CDNK2AIP, FANCD2, P53. Normalized FRET  
467 signal is shown comparing HEK293T cells expressing either wild type NSP5 or NSP5<sup>C145A</sup>.  
468 ORF1ab and CWC22 mutant (mut) sequences contain QS→AS mutation in the peptide  
469 sequence. Data shown is representative of three independent experiments and significance was

470 calculated using Student's T Test. \* indicates p value <0.05, NS not significant. Dose-dependent  
471 effect of coronavirus protease inhibitor G376 on cleavage of ORF1ab-2 **D)** and FANCD2-2 **E)**  
472 peptide sequences.

473 **Fig. 6. SARS-CoV-2 proximal proteins in translation and interferon activation.**

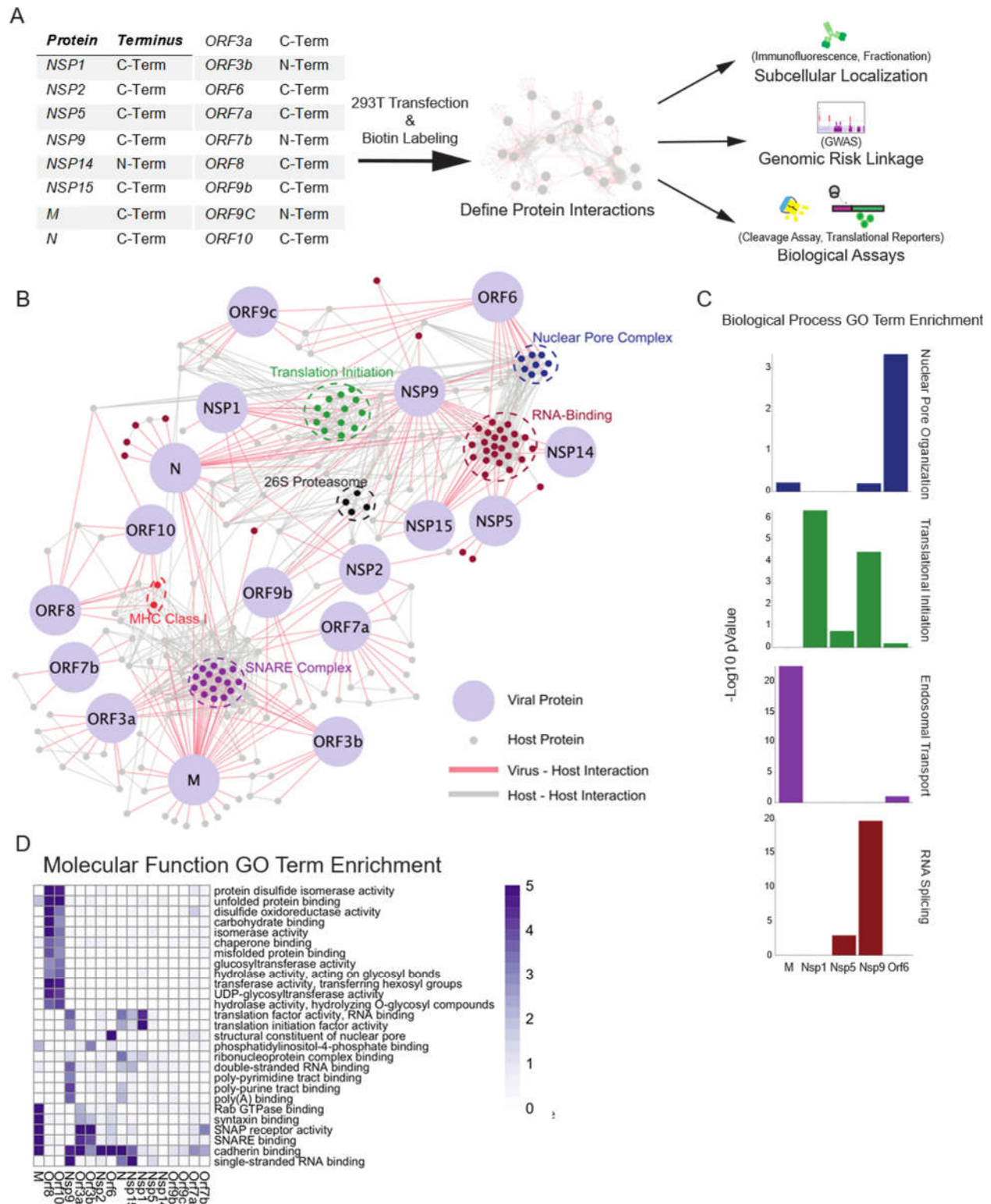
474 Model of SARS-CoV-2 antagonism of host antiviral response. ORF6 protein inhibits RLR  
475 signaling leading to decreased type I interferon and ISG transcription, M protein through TRIM4  
476 interactions may also alter host response. NSP1 disrupts host translation of transcripts containing  
477 both ISG 5' UTR and stress responsive IRES elements.

478

479

480 **Figures**

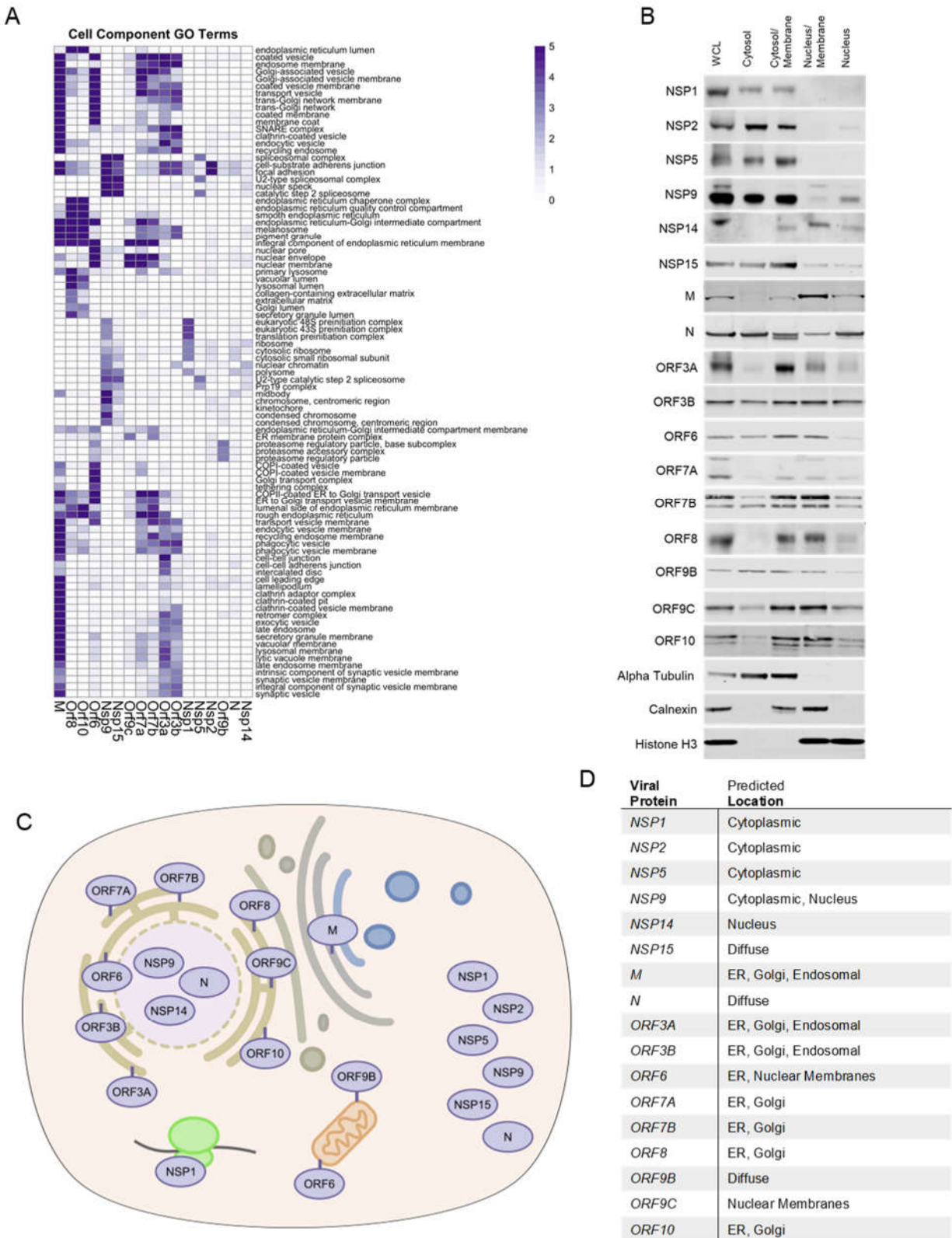
481 **Figure 1. Proximal Interactome of 17 SARS-CoV-2 proteins.**



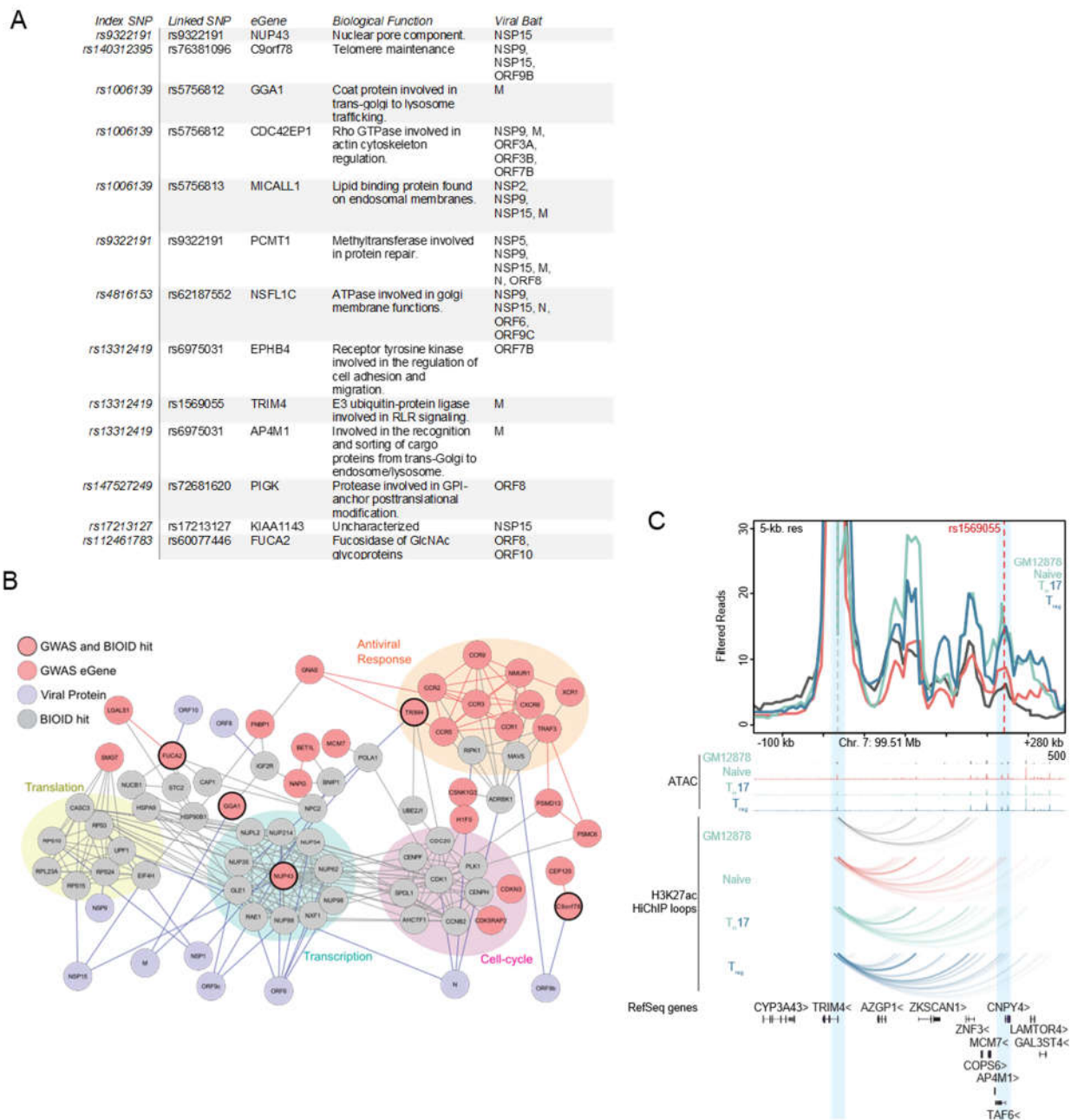
482

483

484 **Figure 2. Localization of SARS-CoV-2 proteins.**



486 **Figure 3. COVID disease risk eGenes proximal to viral proteins.**

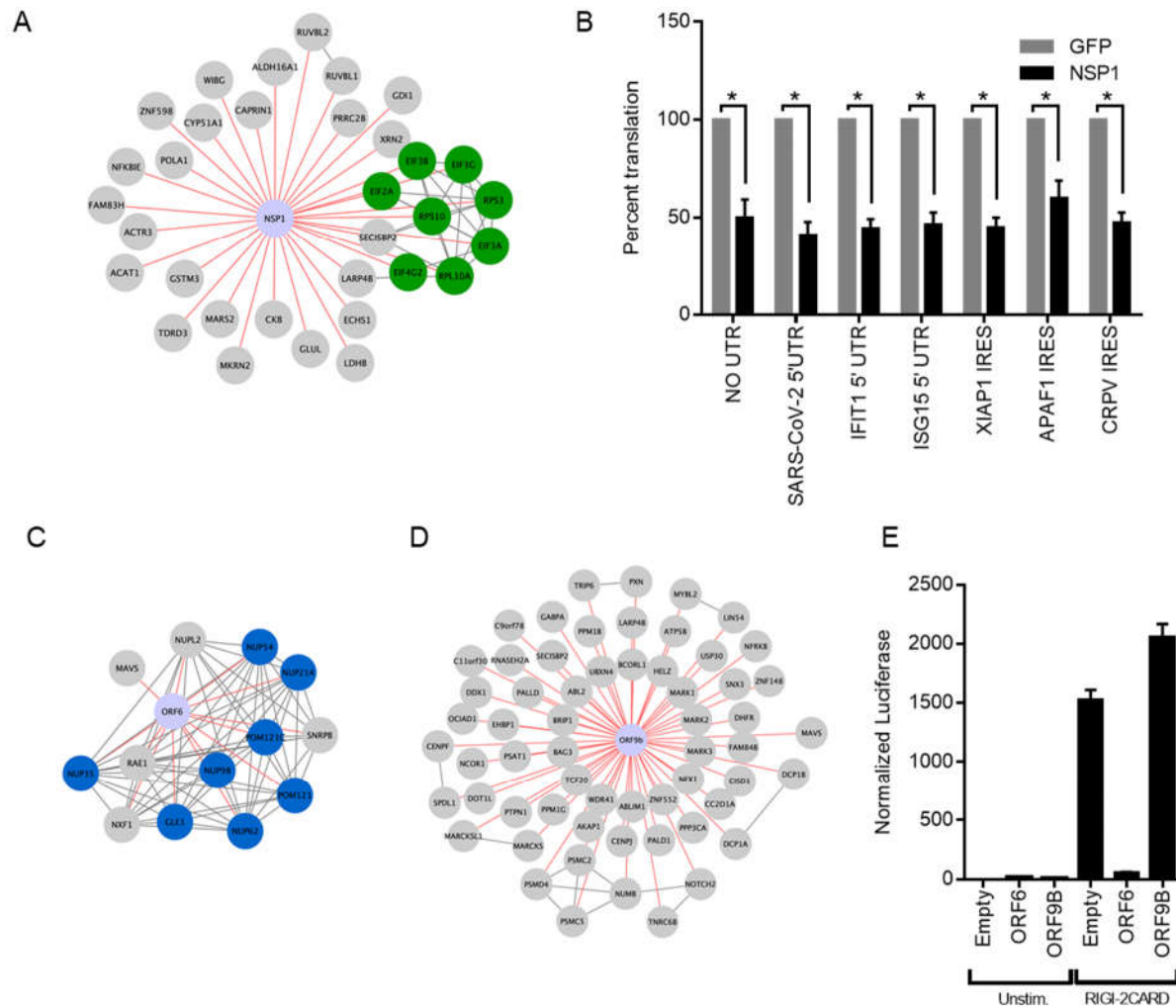


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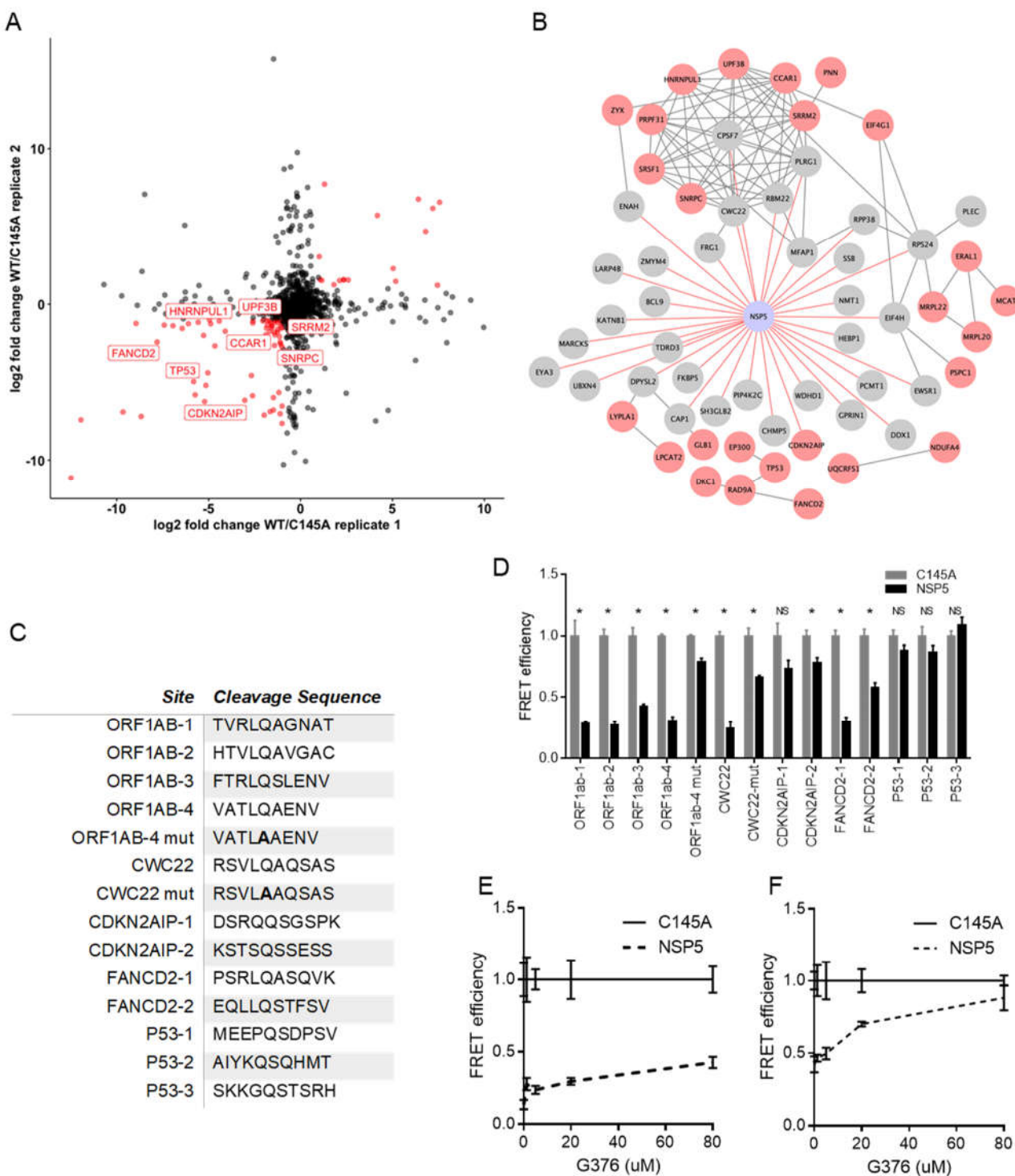
489 **Figure 4. NSP1 and ORF6 disruption of host translation and innate immune signaling.**



490

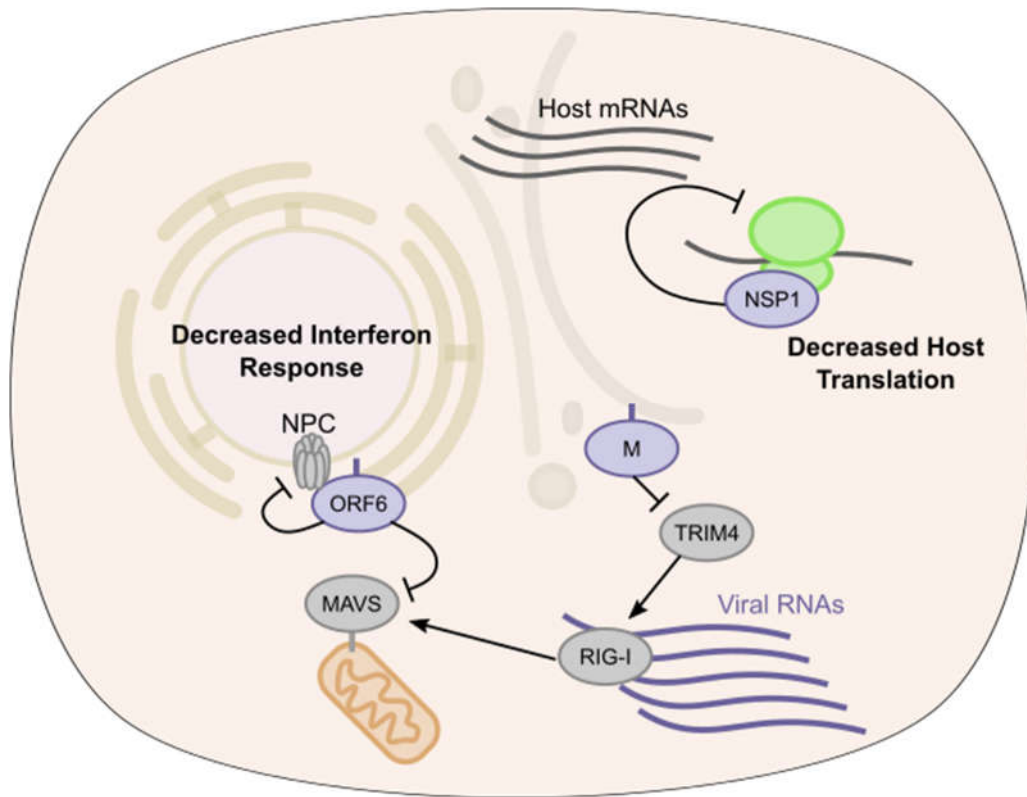
491

492 **Figure 5. BIOD and SILAC mass spectrometry identify candidate targets for the viral protease**  
 493 **NSP5.**



494

495 **Figure 6. SARS-CoV-2 proximal proteins in translation and interferon activation.**



496

497

## 498 **Materials and Methods**

### 499 *Cell Culture*

500 HEK293T were obtained from Takara Bio and were cultured on DMEM 10% FBS, 1%  
501 Penicillin/Streptomycin and grown at 37C, 5% CO<sub>2</sub>. For SILAC experiments,(80) the cells were  
502 cultured in a medium containing [<sup>13</sup>C<sub>6</sub>,<sup>15</sup>N<sub>2</sub>]-lysine and [<sup>13</sup>C<sub>6</sub>]-arginine for at least 2 weeks to  
503 promote complete incorporation of the stable isotope-labeled amino acids. Cells were tested for  
504 mycoplasma prior to experiments using MycoAlert Mycoplasma Detection kit (Lonza).

### 505 *Transfection, Biotin Labeling, and Streptavidin Pulldown.*

506 All viral expression constructs were obtained from Addgene (16). HA-BASU was cloned in frame  
507 with either an N-terminal or C-terminal linker as indicated. For BioID experiments 5x10<sup>6</sup> HEK293T  
508 were plated and transfected with 5ug of each viral expression plasmid. 24 hours post transfection,  
509 biotin was added (50 uM final concentration) for 4 hours, then media was exchanged twice with  
510 DPBS and the cells harvested and lysed in RIPA buffer (Thermo Scientific) supplemented with  
511 protease inhibitors (. Lysates were sonicated and then, using the Kingfisher Flex automated  
512 Purification, incubated for six hours with 100 uL of ReSYN (ReSYN Biosciences) streptavidin  
513 microparticles and then washed sequentially with 2% LDS buffer, Triton X-100 buffer (1% Triton  
514 X-100 0.1%, Sodium Deoxycholate 500mM, 1mM EDTA, 50mM HEPES pH 7.5), Igepal Wash  
515 Buffer (0.5% Igepal, 0.5% Sodium Deoxycholate, 10mM TRIS pH 7.5, 333.3mM LiCL, 20mM  
516 EDTA), and deposited into 50mM TRIS pH 7.4. Samples were washed with automated mixing  
517 for 30 minutes for each step. A portion of the whole cell lysate was saved and ran on SDS-PAGE  
518 gel, transferred to PVDF, and then probed with anti-HA antibody with 800CW anti-rabbit (LICOR)  
519 secondary along with 800CW streptavidin dye (LICOR) to confirm viral protein expression and  
520 total biotinylation. For SILAC experiments with NSP5 and C145A, approximately 2x10<sup>6</sup> cells were  
521 harvested, washed with ice-cold PBS for three times, and lysed by incubating on ice for 30 min  
522 with CellLytic M (Sigma) cell lysis reagent containing 1% protease inhibitor cocktail. The cell  
523 lysates were centrifuged at 7,000g and at 4°C for 15 min, and the resulting supernatants collected.

524 *Sample Preparation for Mass Spectrometry*

525 After wash and purification samples contained bound proteins on beads in TRIS buffer. The  
526 protein on the beads were reduced with dithiothreitol, and alkylated with iodoacetamide. The  
527 processed proteins were subsequently digested with Trypsin/Lys-C (Promega) at an  
528 enzyme/substrate ratio of 1:100 in 50 mM  $\text{NH}_4\text{HCO}_3$  (pH 8.5) at 37 °C for overnight.

529 For SILAC samples, the protein lysates prepared from cells with WT or mutant NSP5 were  
530 combined at 1:1 ratio (by mass), and 30  $\mu\text{g}$  of the mixed protein lysate was loaded onto a 10%  
531 SDS-PAGE gel. After electrophoresis, the gel lanes were cut into 11 slices according to apparent  
532 molecular weight ranges of proteins (< 20, 20-25, 25-30, 30-37, 37-42, 42-50, 50-62, 62-75, 75-  
533 100, 100-150, >150 kDa), reduced in-gel with dithiothreitol, and alkylated with iodoacetamide.  
534 The processed proteins were subsequently digested in-gel with Trypsin/Lys-C (Promega) at an  
535 enzyme/substrate ratio of 1:100 in 50 mM  $\text{NH}_4\text{HCO}_3$  (pH 8.5) at 37 °C for overnight. Subsequently,  
536 peptides were recovered from gels with a solution containing 5% acetic acid in  $\text{H}_2\text{O}$  and then with  
537 a solution containing 2.5% acetic acid in an equi-volume mixture of  $\text{CH}_3\text{CN}$  and  $\text{H}_2\text{O}$ .

538 All the resulting peptide mixture was subsequently dried in a Speed-vac, and desalted by  
539 employing OMIX C18 pipet tips (Agilent Technologies, Santa Clara, CA). LC-MS/MS experiments  
540 were conducted on a Q Exactive Plus mass spectrometer equipped with an UltiMate 3000 UPLC  
541 system (Thermo Fisher Scientific).

542 *LC-MS/MS Analysis*

543 Samples were automatically loaded at 3  $\mu\text{L}/\text{min}$  onto a precolumn (150  $\mu\text{m}$  i.d. and 3.5 cm in  
544 length) packed with ReproSil-Pur 120 C18-AQ stationary-phase material (5  $\mu\text{m}$  in particle size,  
545 120 Å in pore size, Dr. Maisch). The precolumn was connected to a 20-cm fused-silica analytical  
546 column (PicoTip Emitter, New Objective, 75  $\mu\text{m}$  i.d.) packed with 3  $\mu\text{m}$  C18 beads (ReproSil-Pur  
547 120 C18-AQ, Dr. Maisch). The peptides were then resolved using a 180-min gradient of 2-45%  
548 acetonitrile in 0.1% formic acid, and the flow rate was maintained at 300 nL/min.

549 The mass spectrometer was operated in a data-dependent acquisition mode. Full-scan mass  
550 spectra were acquired in the range of  $m/z$  350-1500 using the Orbitrap analyzer at a resolution of  
551 70,000 at  $m/z$  200. Up to 25 most abundant ions found in MS with a charge state of 2 or above  
552 were sequentially isolated and collisionally activated in the HCD cell with a normalized collision  
553 energy of 28 to yield MS/MS.

#### 554 *Database Search*

555 Maxquant, Version 1.5.2.8, was used to analyze the LC-MS and MS/MS data for protein  
556 identification and quantification.(81) The database we used for search was human IPI database,  
557 version 3.68, which contained 87,061 protein entries. The maximum number of miss-cleavages  
558 for trypsin was two per peptide. Cysteine carbamidomethylation and methionine oxidation were  
559 set as fixed and variable modifications, respectively. The tolerances in mass accuracy were 20  
560 ppm for both MS and MS/MS. The maximum false discovery rates (FDRs) were set at 0.01 at  
561 both peptide and protein levels, and the minimum required peptide length was 6 amino acids.  
562 Spectral match assignment files were collapsed to the gene level and false positive matches and  
563 contaminants were removed. SAINT analysis (Choi et al., 2011) (crapome.org) was run with the  
564 following parameters: 10,000 iterations, LowMode ON, Normalize ON and the union of MinFold  
565 ON and OFF. Minimum interactome inclusion criteria were SAINT $\geq$  0.9, fold change over matched  
566 cell type control  $\geq$  4. Low normalized spectral count proteins were removed.

#### 567 *Gene Ontology*

568 Gene Ontology (GO) term analyses were produced using the clusterProfiler (Yu G. et al., 2012.)  
569 R package. Proteins with SAINT score  $\geq$ 0.9 were classified as likely interactions and used to  
570 identify enriched GO terms for the individual SARS-CoV2 protein interactomes. Highly redundant  
571 GO terms were removed for readability. Bar plots and heatmaps were produced with the ggplot2  
572 (Wickham H et al. 2016) and heatmap (Kolde R., 2018) packages respectively in R.

573 *Host-Virus Interaction Network*

574 Host-virus interaction network produced from BASU BioID interactions with a SAINT score  $\geq 0.9$   
575 in Cytoscape (Shannon et al. 2003). The network was further curated to emphasize the  
576 significantly enriched GO terms for each SARS-CoV-2 protein. Edges denoting Host-virus protein  
577 interactions are indicated in red. Host-host interactions were determined from high confidence  
578 ( $>0.700$ ) STRING database interactions obtained from experimental evidence and database  
579 interactions for all of the curated proteins. Cell endogenous protein interactions are denoted by  
580 grey edges. Clusters were highlighted based on highly enriched GO terms for SARS-CoV-2  
581 proteins.

582 *Cellular Fractionation*

583 Cellular fractionations were generated using a previous protocol(82) with minimal modification.  
584 Cells were transfected as described previously. Cell pellets were split into three separate  
585 samples. The first was lysed using RIPA buffer (Thermo Scientific) and was labeled whole cell  
586 lysate (WCL). The second sample was resuspended in buffer containing 0.3% Igepal, 10mM  
587 HEPES, 10mM KCl, 1.5mM MgCl<sub>2</sub>. Sample was pelleted at 1500G and supernatant was collected  
588 and labeled cytoplasm/membrane fraction. The remaining pellet was washed once and then  
589 lysed in RIPA and labeled nuclear fraction. The third sample was lysed in buffer containing  
590 100ug/mL Digitonin, 50mM HEPES, and 150mM NaCl. Sample was pelleted at 2400G and  
591 supernatant was collected and labeled cytoplasm fraction. The remaining pellet was washed once  
592 and lysed in RIPA and labeled nuclear/membrane fraction. Equal volumes of each fraction along  
593 with 20ug of WCL were loaded and ran in a 4-12% Tris-Bis Polyacrylamide Gel (Invitrogen).  
594 Samples were transferred to PVDF and blotted for HA (Viral Proteins), Alpha tubulin (cytoplasm  
595 control), calnexin (Membrane control), Histone H3 (nuclear control).

596 *GWAS COVID Risk SNP Analysis*

597 COVID GWAS datasets were sourced from COVID-19 Host Genome Initiative  
598 (<https://www.covid19hg.org/>), the Ellinghaus, Degenhardt, et al study (21), and from the UK  
599 Biobank (<https://grasp.nhlbi.nih.gov/Covid19GWASResults.aspx>). GWAS hits were converted to  
600 hg19 coordinates and phenotypes for each GWAS study were noted. Gene locations are sourced  
601 from gencode v19 exon coordinates. The GWAS SNPs were then expanded by LD  $r^2 > 0.8$  with  
602 phase 1000 Genomes LD information using LDlinkR (83), and phase 1 1000 Genome LD  
603 information using HaploReg (84). The expanded SNP list was then overlapped with GTEx lung,  
604 spleen, blood, cis-eQTL data, DICE cis-QTL data, and eQTLGen cis-eQTL data (23, 85, 86).

605 *HiChIP Data Processing and Virtual 4C Visualization*

606 HiChIP all valid pair matrices for GM12878, Naïve T cells, Th17 cells and Treg were  
607 downloaded from GEO (GSE101498, (87)). v4C plots were generated from HiChIP valid pair  
608 matrices. The interaction profile of a specific 5-kb bin containing the TRIM4 anchor was then  
609 plotted in R. H3K27ac ChIP-seq peaks for GM12878, Naïve T cells, Tregs and T helper cells  
610 were downloaded from ENCODE as 1d peak sets. FitHiChIP pipeline was used to call loops  
611 with 5kb bin, peak-to-all interaction type, loose background, and FDR < 0.01 (88). The merged  
612 significant interaction files from FitHiChIP pipeline along with corresponding ATAC-seq profiles  
613 were visualized in WashU Epigenome web browser. Browser shots from WashU track sessions  
614 were then included in the v4C and interaction map anecdote.

615

616 *Luciferase Assays*

617 For NSP1 translation assays, in-vitro transcribed transcripts were generated by first PCR  
618 amplifying DNA containing T7 promoter followed by UTR or IRES elements and firefly or renilla  
619 luciferase. Second, using HiScribe™ T7 ARCA mRNA Kit (with tailing) (NEB) capped and  
620 polyadenylated transcripts were synthesized.  $5 \times 10^5$  293T cells were transfected with 2ug of



621 plasmids expressing either GFP or NSP1 and then incubated overnight. The next day the cells  
622 were transfected with 2ug of the corresponding IVT transcripts and were harvested 8 hours post  
623 second transfection. Cells were harvested with 400ul of Passive Lysis Buffer (Promega) and  
624 quadruplicate samples were plated on an opaque 96 well plate. 50ul of LARII firefly luciferase  
625 substrate (Promega) was added and the plate was read on the luminescence setting of the  
626 Spectramax i5 plate reader. 50ul of Stop & Glo renilla luciferase substrate was then added and  
627 the plates reread. For *IFNB1* promoter activity assays,  $2.5 \times 10^5$  293T cells were transfected with  
628 2ug of plasmids expressing either ORF6 or ORF9b along with 1ug of plasmid containing  
629 nanoluciferase under the control of a the human *ifnb1* promoter and 50ng of a plasmid containing  
630 firefly luciferase under the control of the constitutive TK promoter and either 1ug of empty vector  
631 or 1ug of a plasmid expressing the 2-CARD domain of RIG-I. 24 hours post transfection cells  
632 were harvested in 200ul of Passive Lysis Buffer and triplicate samples were plated on an opaque  
633 96 well plate. Nano-Glo Dual-Luciferase Reporter Assay System (Promega) was used to obtain  
634 a firefly luciferase reading for IFN-beta promoter activity normalized to the firefly luciferase  
635 transfection control.

### 636 *NSP5 Cleavage Site Prediction*

637 Protein sequences for hits from SILAC and BASU-BioID proteomics experiments were run  
638 through the NetCorona algorithm (48) using the web application:  
639 (<https://services.healthtech.dtu.dk/service.php?NetCorona-1.0>). For Coronavirus Polyprotein  
640 controls, the SARS-COV-2 ORF1ab protein sequence (from Uniprot Fasta UP000464024) was  
641 run through the NetCorona web application. A previously tested SARS-COV-1 sequence(48),  
642 VATLQAENV, was found to be shared in the SARS-COV-2 protein sequence and was also used  
643 as a control.

644 *FRET-based NSP5 Cleavage Assay*

645 Predicted NSP5 cleavage sites were cloned into ECFP-TevS-YPET (Addgene Plasmid  
646 #100097)(89) Briefly, the plasmid was re-cloned to put the Tev Protease Site between an XbaI  
647 site and a BsiWI site. Cleavage sequences were cloned in between XbaI and BsiWI sites,  
648 restoring the XbaI and BsiWI sites, with one Glycine on each side of the predicted NSP5 cleavage  
649 sequences. This approach was based on a cloning strategy used previously to study norovirus  
650 protease cleavage sites(90). For protease cleavage assay, 3e4 HEK 293T cells were plated in  
651 DMEM + 10% FBS into 96 well black, clear bottom plates (Greiner). 24 hours later, cells were  
652 transfected in quadruplicate with 0.1ug of FRET plasmid containing the NSP5 cut-site and 0.1ug  
653 of either WT-NSP5 or mutant NSP5<sup>C145A</sup> expression plasmids (16) with Lipofectamine 3000  
654 following manufacturer protocol for 96-well plates. 24 hours later, media was removed and PBS  
655 was added to wells, and wells were imaged on a Spectramax i5 instrument (Molecular Devices)  
656 with the following wavelengths: 420/485 nm for ECFP, 485/535 nm for YPET and 420/535 nm for  
657 FRET as previously described(89). After background subtraction of un-transfected wells, FRET  
658 efficiency was calculated as FRET/ECFP.

659 *Quantification and Statistical Analysis*

660 Gene ontology adjusted p-values were produced using the Benjamini-Hochberg method. For  
661 heatmaps, a threshold was set whereby at least one protein had a significant score for the  
662 presented GO terms. A -Log<sub>10</sub> p-Value threshold of >5 for M proteins and >1.3 for all others  
663 was used for 'non-stringent' heatmaps (with the exception of molecular function heatmaps,  
664 which uses an M protein threshold of 3) and -Log<sub>10</sub> p-Value > 5 for M proteins and >3 for all  
665 others was used for more stringent heat maps presented in figures 1D and 2A

666 Graphed data are expressed as mean ± SEM and sample size (N) represents independent  
667 experiments as noted. Statistical analysis was performed in GraphPad Prism 7 and described  
668 in the Fig. legends.

669 Student's t test was performed comparing the means between GFP controls and the  
670 experimental conditions where N is three independent experiments. For IFN $\beta$ 1 reporter assays,  
671 empty vector without RIG-I 2-CARD was set to one and all other conditions are relative to that  
672 empty control and is the average of three independent experiments.

673 NSP5 FRET-cleavage was calculated as described previously and Student's t test was  
674 performed comparing the means between NSP5<sup>C145A</sup> mutant and NSP5 WT where N is three  
675 independent experiments.

676 *Plasmids used in this study*

Plasmid	Origin	Source
eGFP-HA_BASU	This study	Addgene 153996
SARS_COV2_nsp1_HA_BASU	This study	Addgene 154071
SARS_COV2_nsp2_HA_BASU	This study	Addgene 153988
SARS_COV2_nsp5_HA_BASU	This study	Addgene 153987
SARS_COV2_nsp9_HA_BASU	This study	Addgene 153986
SARS_COV2_nsp14_HA_BASU	This study	Addgene 153993
SARS_COV2_nsp15_HA_BASU	This study	Addgene 153985
SARS_COV2_M_HA_BASU	This study	Addgene 153990
SARS_COV2_N_HA_BASU	This study	Addgene 153989
SARS_COV2_ORF3a_HA_BASU	This study	Addgene 153994
SARS_COV2_ORF3b_HA_BASU	This study	Addgene 153992
SARS_COV2_ORF6_HA_BASU	This study	Addgene 153984
SARS_COV2_ORF7a_HA_BASU	This study	Addgene 153983
SARS_COV2_ORF7b_HA_BASU	This study	Addgene 153995
SARS_COV2_ORF8_HA_BASU	This study	Addgene 153982
SARS_COV2_ORF9b_HA_BASU	This study	Addgene 153981
SARS_COV2_ORF9c_HA_BASU	This study	Addgene 153991

SARS_COV2_ORF10_HA_BASU	This study	Addgene 153980
pRF-CRPV-IGR-IRES		
pRF-ISG15-CRPV-IGR-IRES	This study	
pRF-IFIT1-CRPV-IGR-IRES	This study	
pRF-APAF1-CRPV-IGR-IRES	This study	
pRF-XIAP1-CRPV-IGR-IRES	This study	
pRF-5'SARS2-CRPV-IGR-IRES	This study	
ECFP-TevS-YPET		Addgene 100097
pMODnano-IFNBpro	This study	
pGL4.13[luc2/TK]	Promega	Cat# E5061

677

678 *Antibodies Used in This Study*

<b>Antibody</b>	<b>Origin</b>	<b>Catalog Number</b>
HA-tag (C29F4) (Rabbit)	Cell Signaling Technologies	Cat# 3724; RRID:AB_1549585
HA-tag (Mouse)	Abcam	Cat# ab130275; RRID:AB_11156884
HA-tag (Mouse)	Cell Signaling Technologies	Cat # 2367
$\beta$ -Actin	Sigma-Aldrich	Cat# A1978; RRID:AB_476692
Calnexin	Cell Signaling Technologies	Cat# 2679
Lamin A/C	Cell Signaling Technologies	Cat# 4777
800CW Goat anti-Mouse IgG (H + L)	LI-COR	Cat# 926-32210; RRID:AB_621842

800CW Goat anti-Rabbit IgG (H + L)	LI-COR	Cat# 926-32211; RRID:AB_621843
680RD Goat anti-Mouse IgG (H + L)	LI-COR	Cat# 926-68070; RRID:AB_10956588
800CW Streptavidin	LI-COR	Cat# 926-32230; RRID:AB_2877131
680RD Streptavidin	LI-COR	Cat# 926-68079; RRID:AB_2877132
680RD Goat anti-Rabbit IgG (H + L)	LI-COR	Cat# 926-68071; RRID: AB_10956166

679

680 *Data Availability*

681 The mass spectrometry proteomics data for SARS-CoV-2 BIOID and NSP5 SILAC experiments  
682 have been deposited to the ProteomeXchange Consortium via the PRIDE(91) partner repository  
683 with the dataset identifier PXD023239 and PXD023277 respectively. Raw data has been  
684 deposited to Mendeley Data and can be accessed through DOI: 10.17632/mj7jnmvx95.1.

685

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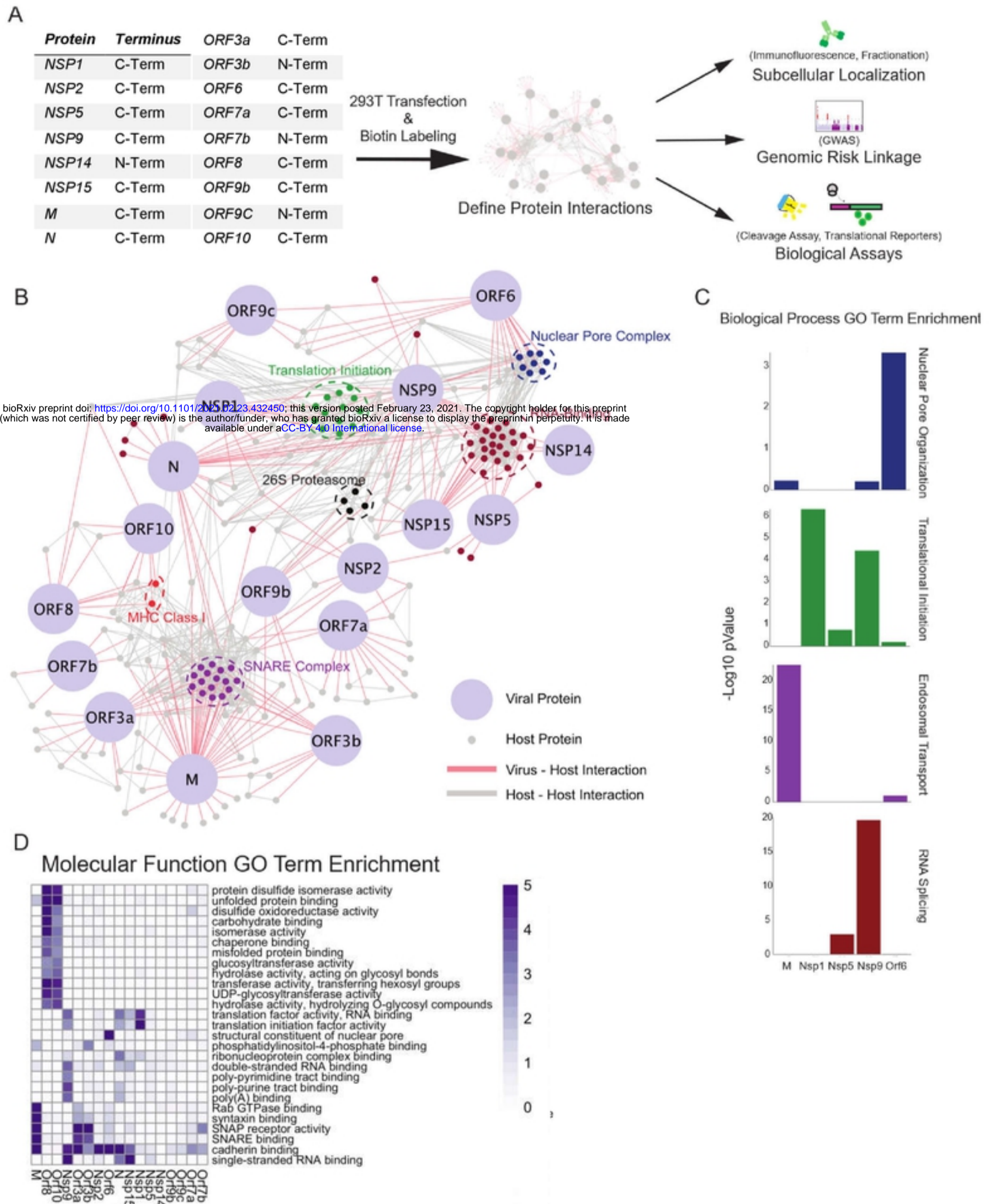
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911

Figure 1. Proximal Interactome of 17 SARS-CoV-2 proteins.

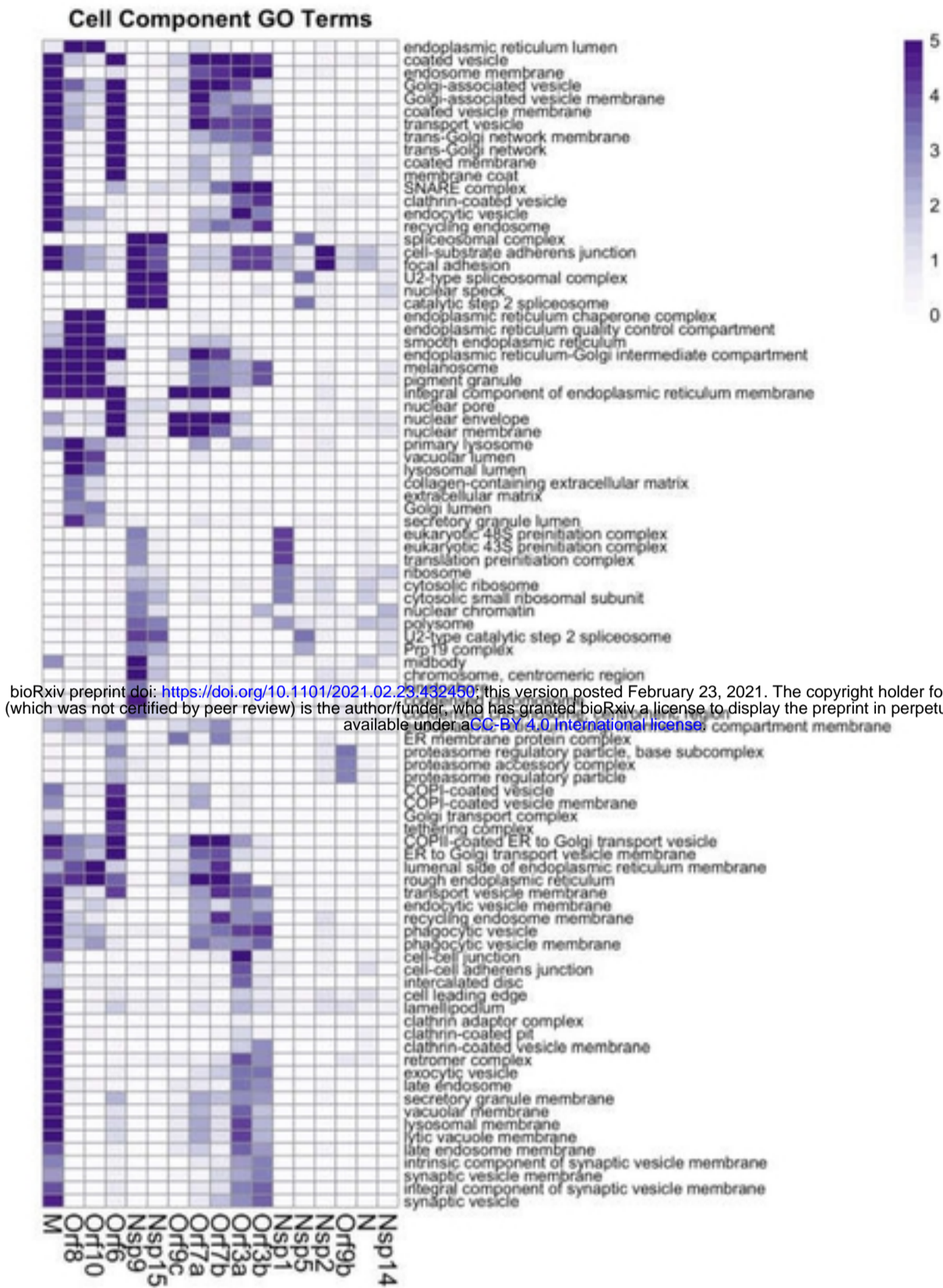


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Figure 1

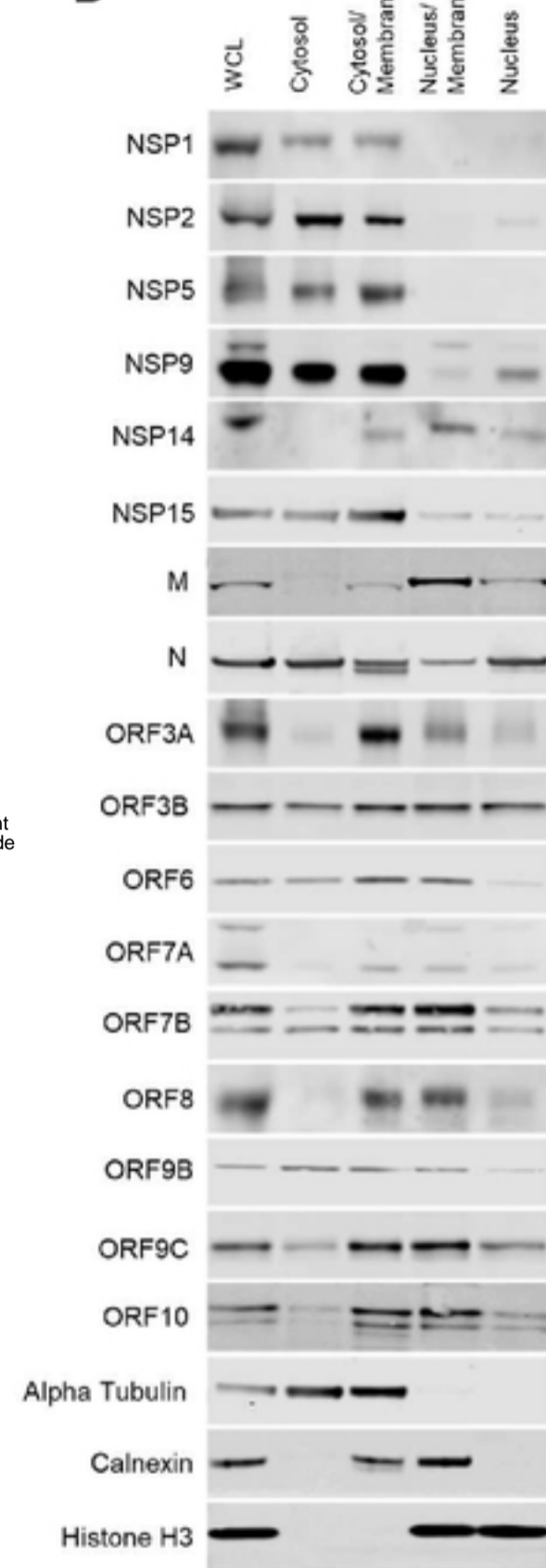
Figure 2. Localization of SARS-CoV-2 proteins.

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B



C

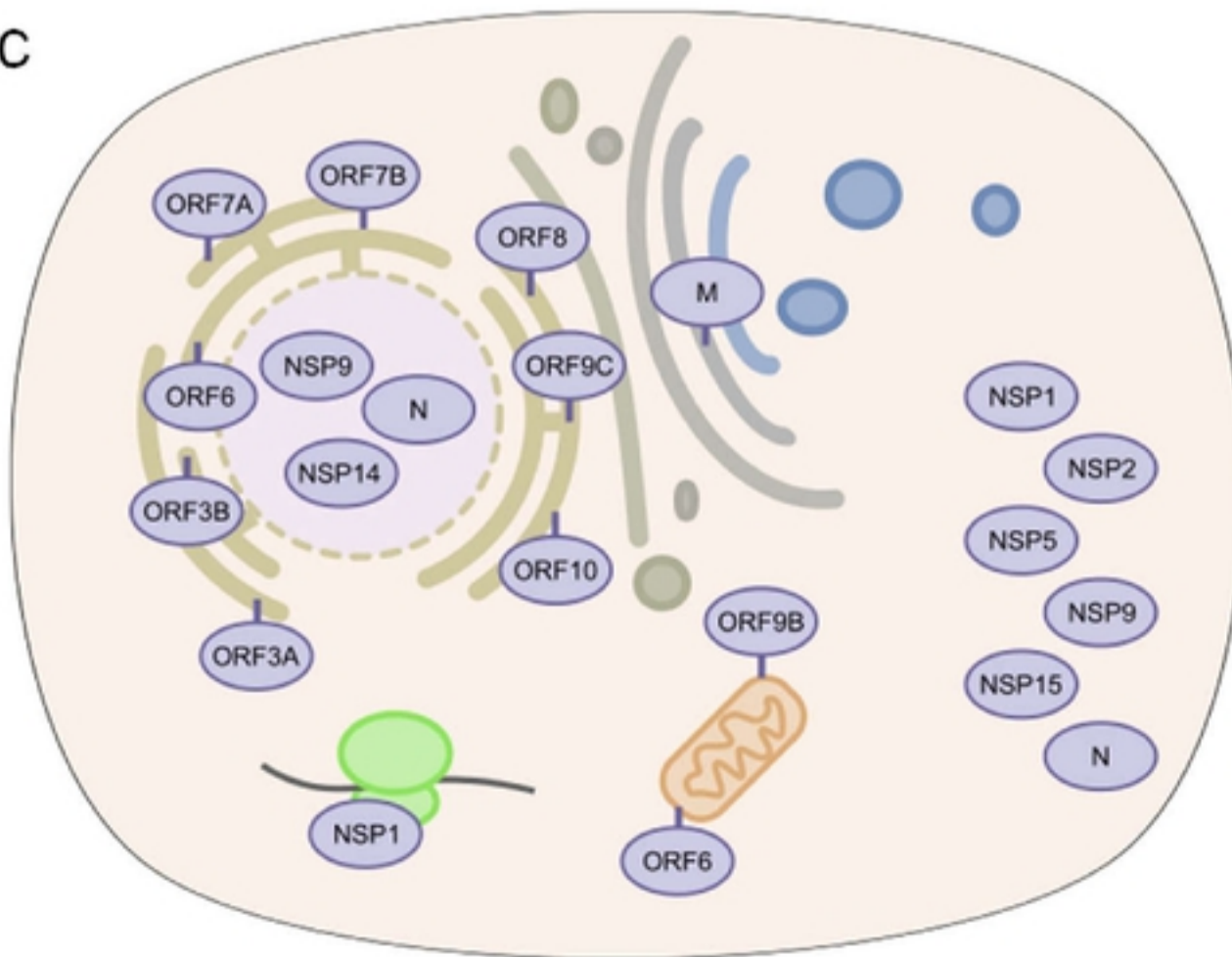


Figure 3. COVID disease risk eGenes proximal to viral proteins.

**A**

Index SNP	Linked SNP	eGene	Biological Function	Viral Bait
rs9322191	rs9322191	NUP43	Nuclear pore component.	NSP15
rs140312395	rs76381096	C9orf78	Telomere maintenance	NSP9, NSP15, ORF9B
rs1006139	rs5756812	GGA1	Coat protein involved in trans-golgi to lysosome trafficking.	M
rs1006139	rs5756812	CDC42EP1	Rho GTPase involved in actin cytoskeleton regulation.	NSP9, M, ORF3A, ORF3B, ORF7B
rs1006139	rs5756813	MICALL1	Lipid binding protein found on endosomal membranes.	NSP2, NSP9, NSP15, M
rs9322191	rs9322191	PCMT1	Methyltransferase involved in protein repair.	NSP5, NSP9, NSP15, M, N, ORF8
rs4816153	rs62187552	NSFL1C	ATPase involved in golgi membrane functions.	NSP9, NSP15, N, ORF6, ORF9C
rs13312419	rs6975031	EPHB4	Receptor tyrosine kinase involved in the regulation of cell adhesion and migration.	ORF7B
rs13312419	rs1569055	TRIM4	E3 ubiquitin-protein ligase involved in cargo and sorting of cargo proteins from trans-Golgi to endosome/lysosome.	M
rs13312419	rs6975031	available	involved in cell adhesion and sorting of cargo proteins from trans-Golgi to endosome/lysosome.	M
rs147527249	rs72681620	PIGK	Protease involved in GPI-anchor posttranslational modification.	ORF8
rs17213127	rs17213127	KIAA1143	Uncharacterized	NSP15
rs112461783	rs60077446	FUCA2	Fucosidase of GlcNAc glycoproteins	ORF8, ORF10

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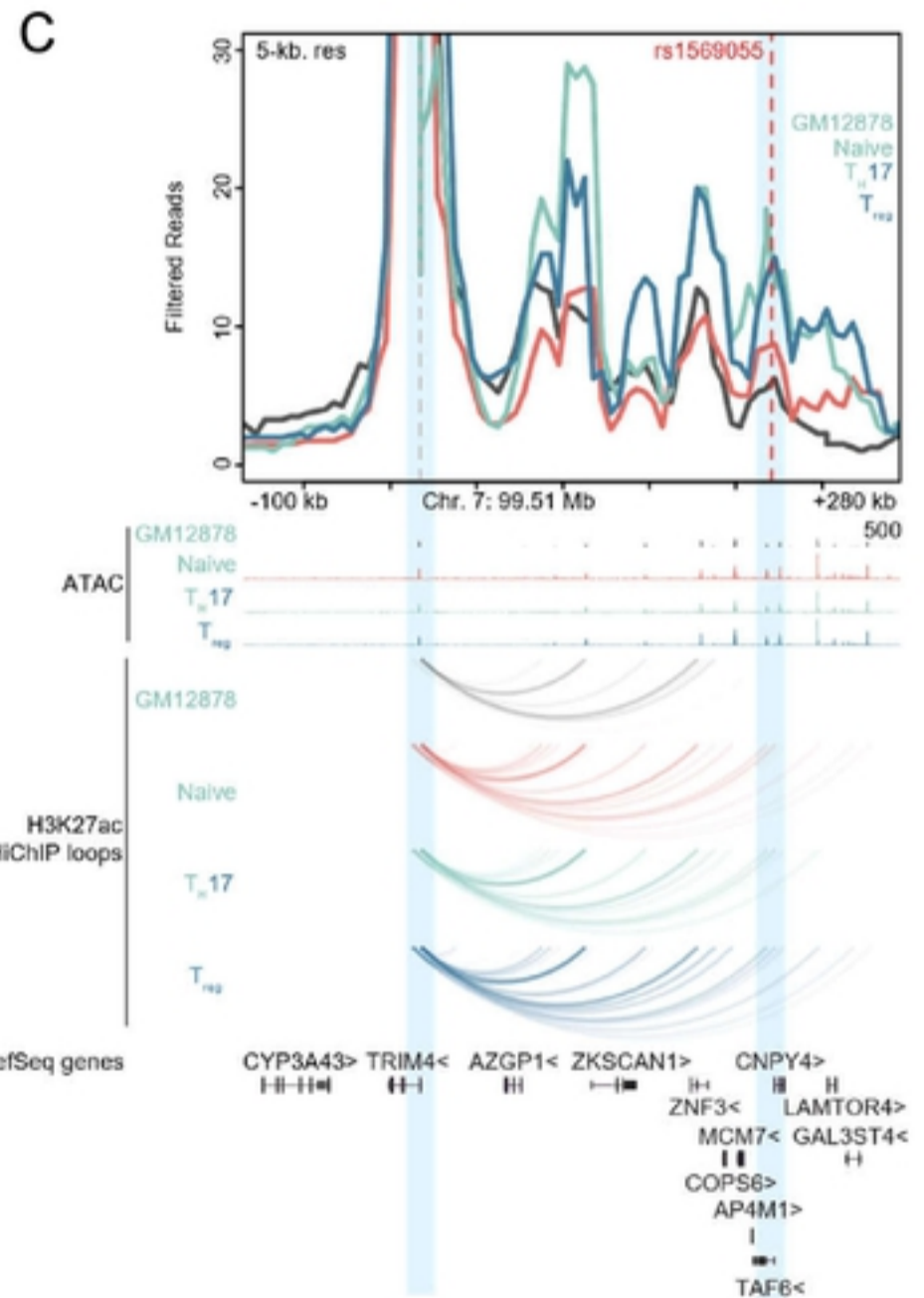
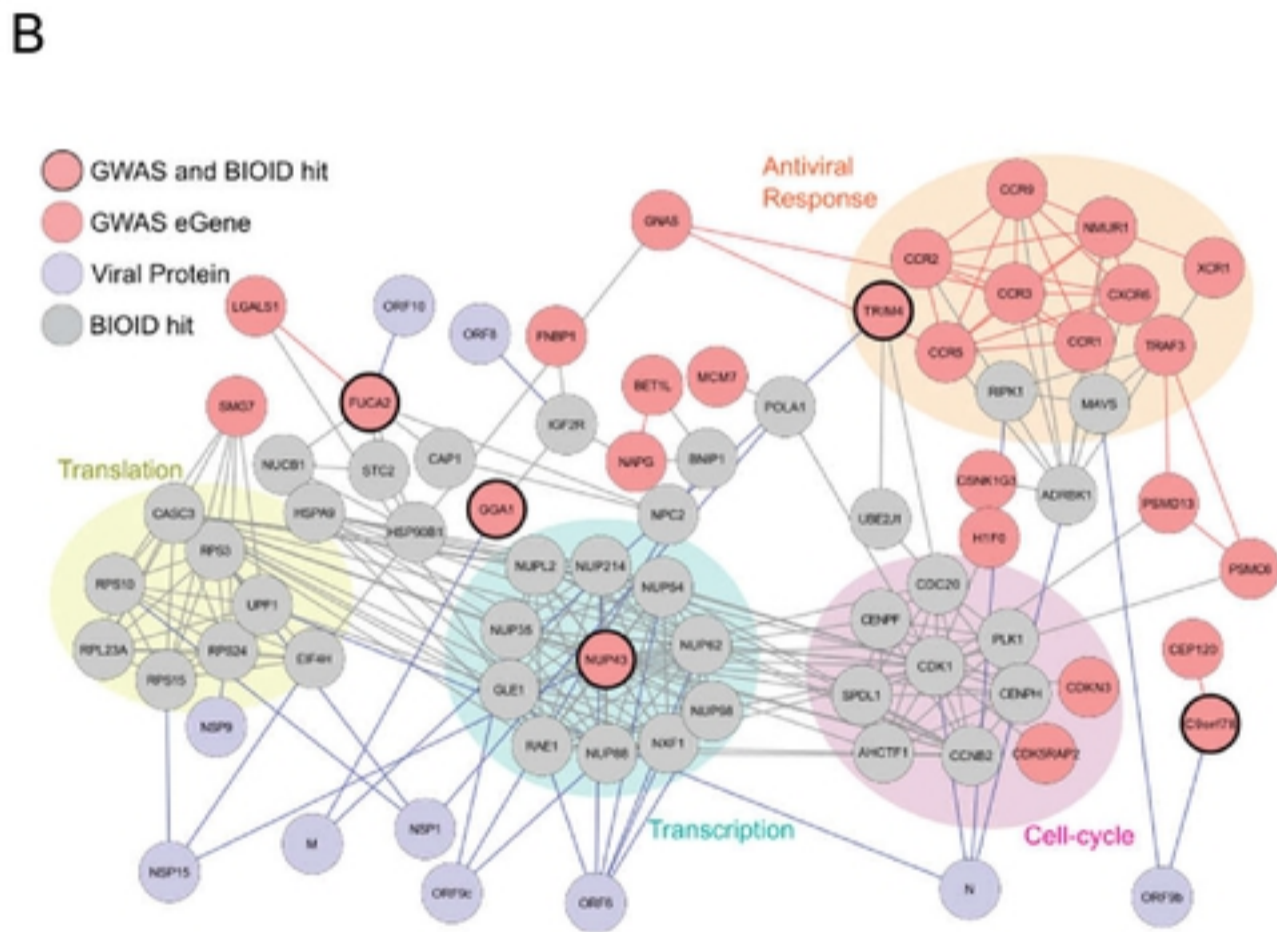
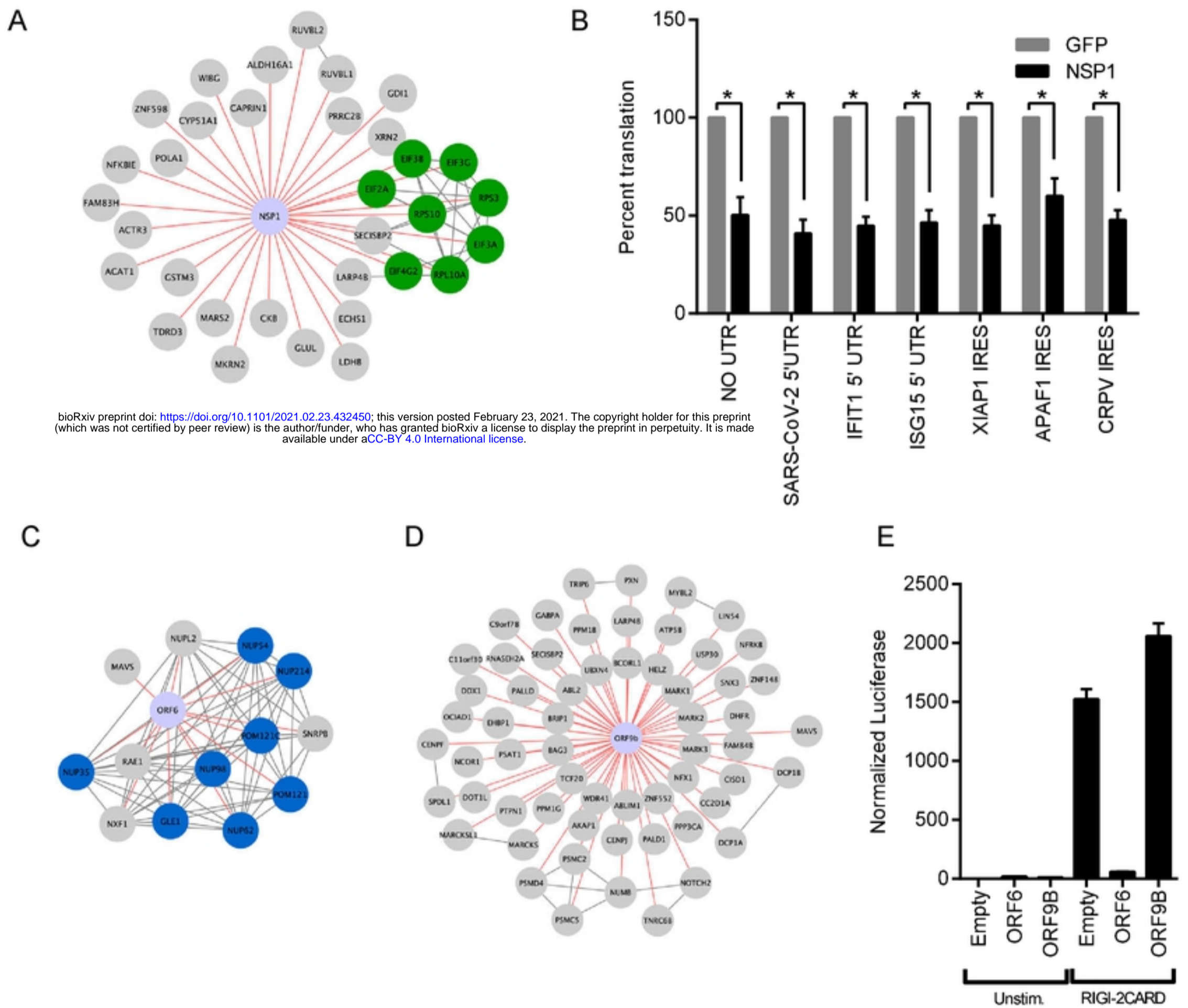


Figure 3

Figure 4. NSP1 and ORF6 disruption of host translation and innate immune signaling.



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Figure 4



Figure 5. BIOID and SILAC mass spectrometry identify candidate targets for the viral protease NSP5.

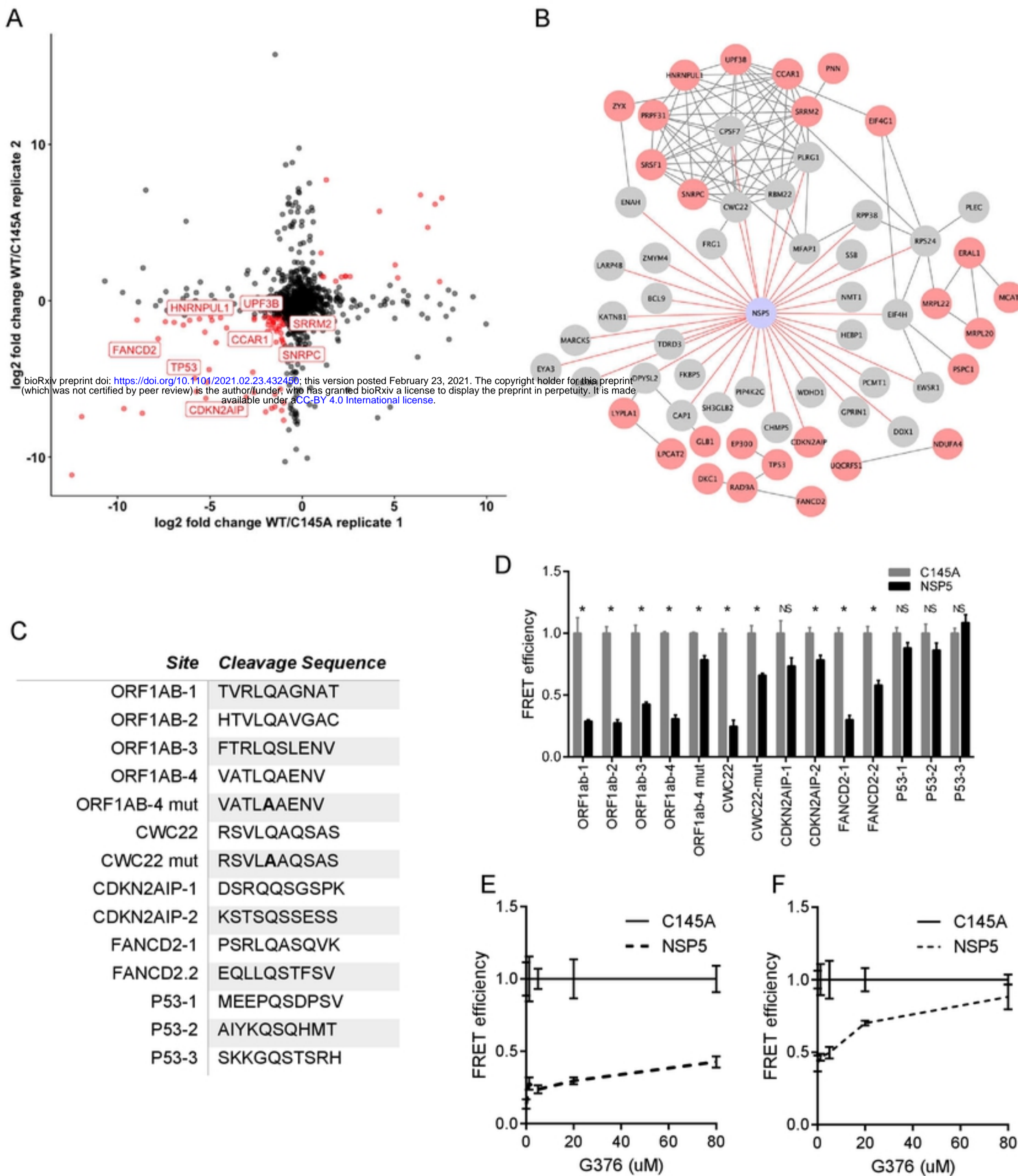
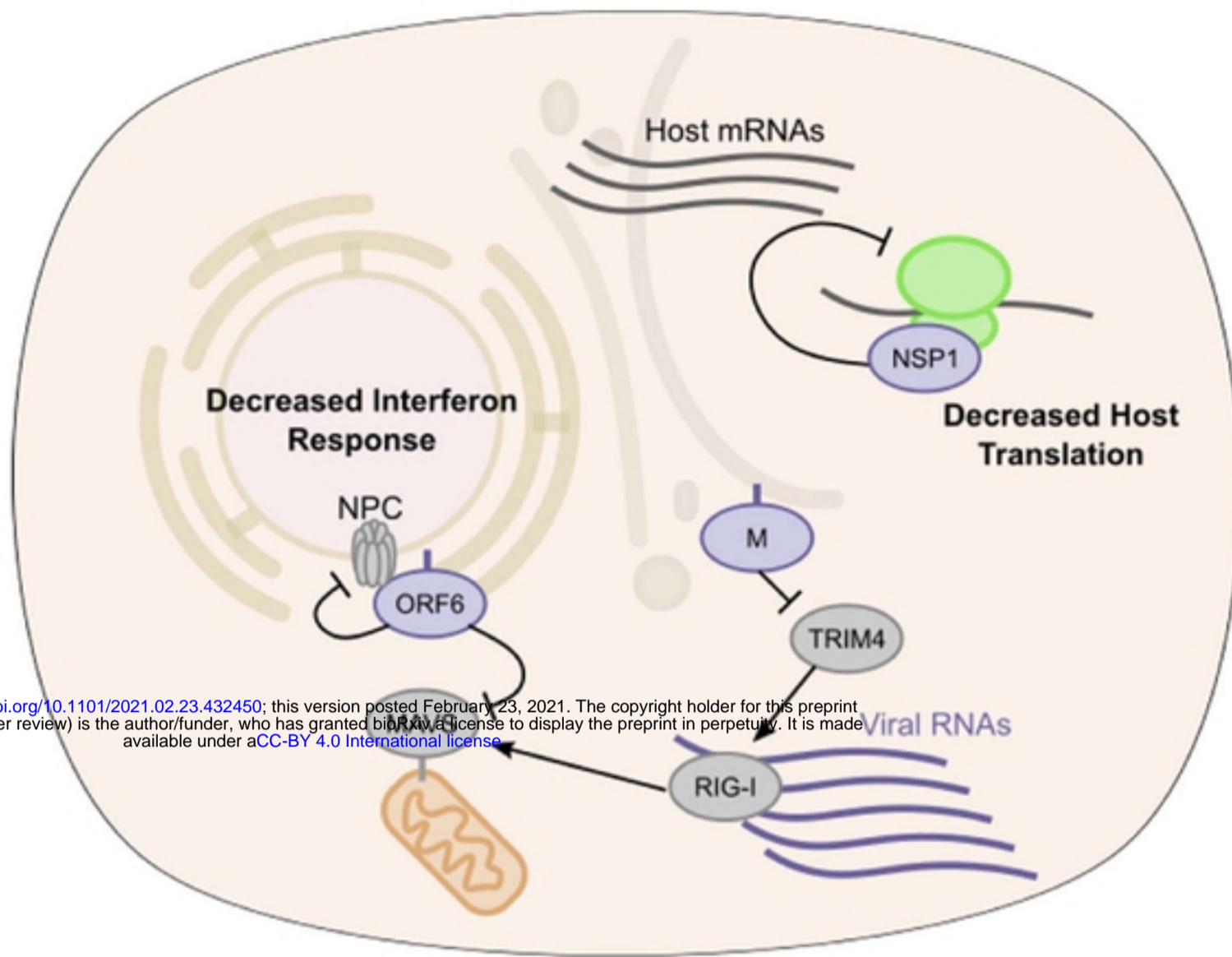


Figure 5

Figure 6. SARS-CoV-2 proximal proteins in translation and interferon activation.



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