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2 SARS-CoV-2 utilizes a multipronged strategy to suppress host
3 protein synthesis

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18 **Abstract:**

19 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the ongoing
20 coronavirus disease 19 (COVID-19) pandemic. Despite the urgent need, we still do not fully
21 understand the molecular basis of SARS-CoV-2 pathogenesis and its ability to antagonize innate
22 immune responses. Here, we use RNA-sequencing and ribosome profiling along SARS-CoV-2
23 infection and comprehensively define the mechanisms that are utilized by SARS-CoV-2 to
24 shutoff cellular protein synthesis. We show SARS-CoV-2 infection leads to a global reduction in
25 translation but that viral transcripts are not preferentially translated. Instead, we reveal that
26 infection leads to accelerated degradation of cytosolic cellular mRNAs which facilitates viral
27 takeover of the mRNA pool in infected cells. Moreover, we show that the translation of
28 transcripts whose expression is induced in response to infection, including innate immune genes,
29 is impaired, implying infection prevents newly transcribed cellular mRNAs from accessing the
30 ribosomes. Overall, our results uncover the multipronged strategy employed by SARS-CoV-2 to
31 commandeer the translation machinery and to suppress host defenses.

32

33 **Introduction:**

34 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the ongoing
35 coronavirus disease 19 (COVID-19) pandemic ^{1,2}. SARS-CoV-2 is an enveloped virus consisting
36 of a positive-sense, single-stranded RNA genome of ~30 kb. From the positive strand genomic
37 RNA, two overlapping open reading frames (ORFs) are translated, ORF1a (pp1a) and ORF1b
38 (pp1ab). The translation of ORF1b is mediated by a -1 frameshift that allows translation to
39 continue beyond the stop codon of ORF1a. These generate continuous polypeptides which are
40 cleaved into a total of 16 nonstructural proteins (NSP 1-16, Snijder et al., 2016; Sola et al., 2015;
41 Stadler et al., 2003; V'kovski et al., 2020). Among them are the subunits that constitute the
42 RNA-dependent RNA polymerase (RdRP), which in turn transcribes subgenomic RNAs that
43 contain a common 5' leader fused to different segments from the 3' end of the viral genome ³⁻⁵.
44 The different subgenomic RNAs encode 4 conserved structural proteins- spike protein (S),
45 envelope protein (E), membrane protein (M), nucleocapsid protein (N)- and several accessory
46 proteins and non-canonical protein products ⁷⁻⁹.

47 Translation of viral proteins relies solely on the cellular translation machinery; therefore, viruses
48 must commandeer this machinery to translate their own mRNAs. In addition, viruses need to
49 counteract the antiviral response cells mount upon infection ¹⁰. The first line of defense applied
50 by infected cells engages the interferon (IFN) pathway, which amplifies signals resulting from
51 detection of intracellular viral components to induce a systemic anti-viral response. Specifically,
52 cells contain various sensors that detect the presence of viral RNAs and promote nuclear
53 translocation of transcription factors, leading to transcription and secretion of type I IFNs ¹¹.
54 Binding of IFN to its cognate receptor in autocrine and paracrine manners leads to the
55 propagation of the signal and to the transcription and translation of hundreds of interferon
56 stimulated genes that act to hamper viral replication at various stages of the viral life cycle ¹². In
57 the case of SARS-CoV-2, interferon response seems to play a critical role in pathogenesis ¹³⁻¹⁷.
58 In addition, the extent to which SARS-CoV-2 suppresses the IFN response is a key characteristic
59 that distinguishes it from other respiratory viruses ^{18,19}.

60 Viruses utilize various strategies to cause shutoff of host mRNA translation ¹⁰, including
61 hampering mRNA processing steps and export, inducing degradation of mRNAs and inhibiting

62 translation. Coronaviruses (CoVs) are known to cause host shutoff^{10,20} and several strategies
63 have been proposed for how the beta CoVs may shut-off host protein synthesis and evade
64 immune detection. These include degradation of host mRNA in the nucleus or the cytosol and
65 inhibition of host translation^{21,22}. Nonetheless, the extent to which SARS-CoV-2 uses these or
66 other strategies remains unclear.

67 NSP1 is the best characterized and most prominent coronavirus host shutoff factor²³. Several
68 recent studies showed SARS-CoV-2 NSP1 binds the 40s ribosome and inhibits translation²⁴⁻²⁸.
69 In addition, other SARS-CoV-2 proteins were shown to interfere with cellular gene expression.
70 For example, one of the SARS-CoV-2 accessory proteins, ORF6, was suggested to disrupt
71 nucleocytoplasmic transport leading to inhibition of gene expression²⁹ and several viral proteins
72 have been demonstrated to antagonize IFN-I production and signaling^{24,30}. Although, different
73 SARS-CoV-2 proteins are implicated in host expression shutoff, a comprehensive picture of the
74 effect of SARS-CoV-2 infection on cellular gene expression is still lacking.

75 Here we employ RNA-sequencing and ribosome profiling along SARS-CoV-2 infection to
76 explore the mechanisms which the virus utilizes to interfere with host protein synthesis. We
77 reveal SARS-CoV-2 uses a multi-faceted approach to shutoff cellular protein production. SARS-
78 CoV-2 infection induces global translation inhibition but surprisingly the translation of viral
79 transcripts is not preferred over their cellular counterparts. Instead we reveal that infection leads
80 to accelerated cellular mRNAs degradation, likely conferred by NSP1. Viral transcripts are
81 refractory to these effects, an evasion potentiated by their 5'UTRs, enabling viral dominance
82 over the mRNA pool in infected cell. Finally, we show that the translation of transcripts whose
83 expression is induced in response to infection, including innate immune genes, is severely
84 impaired. Overall these findings reveal the key mechanisms SARS-CoV-2 is applying to
85 suppress cellular protein synthesis and host defenses.

86

87 **Results:**

88 **Simultaneous monitoring of RNA levels and translation during SARS-CoV-2 infection**

89 To gain a detailed view of the changes that occur in viral and host translation over the course of
90 SARS-CoV-2 infection, we infected Calu3 cells with SARS-CoV-2 at multiplicity of infection
91 (MOI) of 3 and harvested infected cells at 3, 5, and 8 hours post infection (hpi) as well as
92 uninfected cells. This high MOI infection results in infection of the majority of the cells and thus
93 a synchronous cell population, allowing for molecular dissection of the events along infection.
94 We designed our experiment to simultaneously monitor both RNA levels and translation (Figure
95 1A). Deep sequencing of mRNA (RNA-seq) generates a detailed depiction of transcript levels
96 during infection and this was paired with ribosome profiling, which allows accurate
97 measurement of protein synthesis by capturing the overall *in-vivo* distribution of ribosomes on a
98 given message^{31,32}. In order to assess the reproducibility of our experiments we prepared two
99 independent biological replicates for the uninfected and 8hpi time points and both the mRNA
100 and footprint measurements were reproducible (Figure S1A and S1B). Metagene analysis, in
101 which gene profiles are aligned and then averaged, revealed the expected profiles of footprints
102 along mRNAs; ribosome density accumulates from the start codon along the gene body ending at
103 the first in-frame stop codon with pronounced accumulation of ribosomes at the initiation sites
104 and 3 bp periodicity along the gene body (Figure S1C). Using this data, we quantitatively
105 assessed the expression pattern of 8627 cellular transcripts and 12 canonical viral ORFs that are
106 expressed from the genomic and sub-genomic RNAs along SARS-CoV-2 infection.

107 Analysis of the mRNAs and footprints originating from cellular and viral transcripts illustrates
108 SARS-CoV-2's unprecedented dominance over the mRNA pool. At 8 hpi viral mRNAs comprise
109 almost 90% of the mRNAs in infected cells (Figure 1B). Surprisingly, however, at the same time
110 point, viral mRNAs account for only ~34% of the RNA fragments engaged with ribosomes in the
111 cells (Figure 1C). In order to quantitatively evaluate the ability of SARS-CoV-2 to co-opt the
112 host ribosomes we calculated the relative translation efficiency (TE) of viral and cellular RNA
113 along infection. TE is defined as the ratio of footprints to mRNA reads for a given gene and
114 reflects how well a gene is being translated. We then compared the TE of human genes to that of
115 viral genes at each of the time points along infection. The analysis shows that at 3 hpi viral gene

116 translation efficiencies fall within the general range of cellular gene translation. This indicates
117 that when infection initiates, viral transcripts are translated with efficiencies similar to those of
118 host transcripts. As infection progresses, viral gene translation efficiency relative to cellular
119 genes is significantly reduced (Figure 1D). Since it seems improbable that the virus will promote
120 conditions that are unfavorable to the translation of its mRNA, this relative reduction in
121 translation of viral genes at 5 and 8 hpi may indicate that not all viral RNAs are accessible for
122 translation. Since double-membrane sealed replication compartments are formed to
123 accommodate viral genome replication³³, an appealing possibility is that these compartments
124 sequester a sizable fraction of the viral RNA molecules and thus prevent them from being a part
125 of the translated mRNA pool.

126 By their nature, deep sequencing measurements provide relative values but not absolute
127 quantification of RNA and translation levels. Since SARS-CoV-2 encoded protein, NSP1, was
128 recently shown to interfere with translation by blocking the mRNA entry channel of ribosomes
129²⁵⁻²⁸, and since the extent to which SARS-CoV-2 interferes with the overall levels of cellular
130 mRNA was not assessed, we next examined if SARS-CoV-2 infection affects global translation
131 and RNA levels. To quantify absolute translation levels, we measured nascent protein synthesis
132 levels using an analogue of puromycin, O-Propargyl Puromycin (OPP), which incorporates into
133 elongating polypeptide chains³⁴. The nascent polypeptides with incorporated OPP are
134 fluorescently-labeled via a Click reaction and the levels of OPP incorporation into elongating
135 polypeptides is quantified by flow cytometry (Figure S2A and S2B). We infected Calu3 cells
136 with SARS-CoV-2 at an MOI of 3 and measured nascent protein synthesis levels in uninfected
137 cells and at 3, 5 and 8 hpi. We observed a significant reduction in global translation levels
138 already at 3 hpi which was augmented with time, and at 8 hpi translation activity was reduced by
139 50% (Figure 1E). In parallel we measured the total RNA levels and rRNA levels extracted from
140 uninfected cells and at 3, 5, and 8 hpi. This analysis illustrated there are no major changes in
141 total RNA or in rRNA levels along SARS-CoV-2 infection (Figure S3A and S3B). Since the vast
142 majority of RNA in cells originates from rRNA and this dominance of rRNA may mask changes
143 in mRNA levels we sequenced total RNA, without rRNA depletion, to assess the relative
144 abundance of cellular and viral mRNAs in uninfected cells and at 3, 5, and 8 hpi. This analysis
145 demonstrates that the pool of mRNA molecules relative to rRNA is growing during infection,

146 due to the massive production of viral transcripts but at the same time the relative fraction of
147 cellular mRNA is reduced by approximately 2-fold (Figure 1F). This suggests that during
148 infection there is both massive production of viral transcripts and a concomitant substantial
149 reduction in the levels of cellular transcripts.

150 Next, we quantitatively assessed the expression pattern of cellular genes along SARS-CoV-2
151 infection. We clustered the mRNA levels of genes that showed the most significant changes
152 along infection using partitioning clustering, allowing grouping of cellular transcripts into three
153 distinct classes based on similarities in temporal expression profiles in the RNA-seq. Overall, we
154 found that changes in ribosome footprints tracked the changes in transcript abundance (Figure
155 1G), with some exceptions that will be discussed in detail below. This shows that part of the
156 reduction in cellular protein synthesis is driven by the reduction in cellular RNA levels.
157 Interestingly, although the levels of the majority of host transcripts were reduced during SARS-
158 CoV-2 infection, we identified numerous transcripts that were significantly elevated (Figure 1G,
159 cluster 3). We carried out pathway enrichment analysis for each of these three clusters. As
160 expected, the group of upregulated mRNAs (cluster 3) was significantly enriched with genes
161 related to immune response, including Toll receptor Signaling, chemokine and cytokine
162 signaling (Figure 1H and Table S1) and these upregulated genes include IL6 and IL8 which play
163 a significant role in the pathogenesis of SARS-CoV-2³⁵ and several IFN stimulated genes like,
164 IFIT1, 2 and 3, IRF1, ISG15 and TNF alpha induced proteins. These measurements and
165 analyses reveal that the shutoff in host protein synthesis is driven by several mechanisms
166 including; general reduction in the translation capacity of infected cells and reduction in the
167 levels of most cellular mRNAs.

168

169 **Viral gene expression dynamics along infection**

170 We next analyzed viral gene expression dynamics along SARS-CoV-2 infection. Viral ORFs are
171 translated from the 30kb genomic RNA or from a nested series of subgenomic RNAs that
172 contain a common 5' leader fused to different segments from the 3' end of the viral genome
173 (Figure 2A and V'kovski et al., 2020). Viral transcripts and translation levels significantly

174 increased from 3 to 5 hpi but the relative abundance of some viral transcripts and their translation
175 rates were reduced from 5 to 8 hpi (Figure 2B and 2C). This relative reduction in translation rates
176 of some viral transcripts prompted us to assess the relative translation efficiency of viral genes
177 along infection. Since, as indicated above, the translation efficiency of viral genes compared to
178 their cellular counterparts is relatively reduced along infection (Figure 1D), here we examined
179 how the translation of viral genes is distributed between different viral transcripts at different
180 times post infection. This focused analysis revealed an interesting connection between changes
181 in translation efficiency of viral ORFs and their relative location on the viral genome. ORFs that
182 are located at the 5' end of the genome showed relative increase in their translation efficiency
183 along infection whereas ORFs that are encoded towards the 3' end of the genome showed
184 relative reduction in their translation efficiencies and ORFs located in the middle of genome
185 showed no major changes in their relative translation efficiency (Figure 2D). Since all viral
186 subgenomic RNAs share the same 5'UTR these differences in translation capacity according to
187 the location in the genome point to a potential unappreciated role for the 3'UTR (the 3' portion
188 of which is shared between all transcripts) or for the length of viral transcripts, which varies
189 greatly between viral transcripts (Figure 2A). To examine if this phenomenon reflects a general
190 beta CoVs feature, we analyzed the expression and translation of Mouse Hepatitis Virus strain
191 A59 (MHV) ORFs along MHV infection using published RNA-seq and ribosome profiling data
192 along MHV infection³⁶. MHV has a similar genomic organization (Figure 2E) and the increase
193 in viral mRNA and translation between 5 and 8 hpi varied between viral ORFs (Figure 2F and
194 2G). Although this phenomenon was not as strong, also in the case of MHV, ORFs that are
195 located at the 5' end of the genome showed relative increase or no change in their translation
196 efficiency along infection whereas ORFs that are encoded towards the 3' end of the genome
197 showed relative reduction in their translation efficiency (Figure 2H). Together these results
198 indicate that the 3'UTR or viral gene length are likely regulating the translation efficacy of viral
199 mRNAs along infection.

200 Most SARS-CoV-2 ORFs are 3'-proximal and translated from dedicated subgenomic mRNA
201 (Figure 2A). However several subgenomic RNAs encode for additional out-of-frame ORFs,
202 likely via a leaky scanning mechanism^{9,37-39}. These include ORFs 7b and ORF9b, which are
203 translated from the ORF7a and N subgenomic RNAs, and two ORFs we recently identified by

204 ribosome profiling⁹, that are supported by additional evidence^{37,39}, ORF3c and ORFS.iORF
205 that are translated from ORF3a and ORF-S subgenomic RNAs. Since scanning efficiency can be
206 regulated by stress conditions⁴⁰ we examined if the ratio between the translation of a 3'-proximal
207 ORF and its corresponding out-of-frame ORF (encoded by the same subgenomic RNA) changes
208 during infection. Since ORF9b expression was low in our measurements it was excluded from
209 this analysis. The translation of ORF7b, ORF3c and ORFS.iORF correlated with the expression
210 of the 3'-proximal main ORF, indicating there are no major changes in the efficiency of ribosome
211 scanning of viral transcripts along infection (Figure 2I).

212

213 **Cellular mRNAs are degraded during SARS-CoV-2 infection**

214 Our results indicate that the levels of the majority of cellular RNA are reduced during SARS-
215 CoV-2 infection and this reduction contributes to the shutoff of cellular protein synthesis.
216 Reduction in cellular RNA levels could be due to interference with RNA production or
217 accelerated RNA degradation. To explore the molecular mechanism, we analyzed if the
218 reduction in cellular transcripts is associated with their subcellular localization. We used
219 measurements of the subcellular localization of transcripts by cytoplasmic and nuclear
220 fractionation⁴¹ to assess the importance of subcellular localization. The levels of transcripts that
221 mostly localize to the cytoplasm were more reduced in infected cells compared to transcripts that
222 are mostly nuclear (Figure 3A) and there was a clear correlation between subcellular localization
223 and the extent of reduction in transcript levels following SARS-CoV-2 infection (Figure S4A).
224 Furthermore, compared to transcripts encoded in the nuclear genome, mitochondrial encoded
225 transcripts were refractory to the effects of SARS-CoV-2 infection (Figure 3B). The specific
226 sensitivity of cytosolic transcripts implies these transcripts may be specifically targeted during
227 SARS-CoV-2 infection. In CoVs, the most prominent and well characterized cellular shutoff
228 protein is NSP1²³. So far, studies on SARS-CoV-2 NSP1 demonstrated it restricts translation by
229 directly binding to the ribosome 40S subunit²⁵⁻²⁸, thereby globally inhibiting translation
230 initiation. For SARS-CoV, on top of this translation effect, NSP1 interactions with the 40S was
231 also shown to induce cleavage of translated cellular mRNAs, thereby accelerating their turnover
232²¹. To examine if the reduction in cellular transcripts in SARS-CoV-2 infected cells is coupled to

233 their translation we compiled a list of 14 long non-coding RNAs (lncRNAs) that localize to the
234 cytoplasm and are well expressed in our data but, as expected, poorly translated (Figure S4B).
235 Relatively to cellular mRNAs, cytoplasmic lncRNAs were less affected by SARS-CoV-2
236 infection (Figure 3C), indicating accelerated turnover of cellular transcripts in infected cells may
237 be related to their translation. Recently, ribosome profiling and RNA-seq were conducted on
238 cells transfected with NSP1⁴². Analysis of the RNA expression from this data revealed that
239 ectopic NSP1 expression leads to weaker but similar signatures to the ones we identified in
240 infected cells; stronger reduction of cytosolic transcripts compared to nuclear transcripts,
241 stronger sensitivity of nuclear encoded transcripts, and stronger reduction of translated mRNA
242 compared to cytosolic lncRNAs (Figure S5A-C).

243 We further noticed SARS-CoV-2 infection leads to increased levels of intronic reads in many
244 cellular transcripts (Figures 3D and 3E) indicating SARS-CoV-2 may interfere with cellular
245 mRNA splicing, as was recently suggested²⁴. However, massive degradation of mature cytosolic
246 mRNAs may also generate a relative increase in intronic reads. We therefore analyzed the ratio
247 of intronic and exonic reads relative to rRNA. Whereas relative to rRNA levels, exonic reads
248 showed drastic reduction along SARS-CoV-2 infection, the intronic reads levels showed a more
249 subtle change (Figure 3F). Furthermore, the increase in the ratio of intronic to exonic reads was
250 greater in genes whose expression was reduced along infection compared to genes whose
251 expression was induced (Figure 3G and Figure S6A), illustrating the relative increase in intronic
252 reads is mostly independent of newly transcribed RNAs. Finally, we also detected more intronic
253 reads in cells that exogenously expressed NSP1⁴² (Figure S6B). Together these results indicate
254 that the increase in intronic reads compared to exonic reads during SARS-CoV-2 infection is
255 largely driven by accelerated degradation of mature cellular transcripts that leads to relative
256 reduction in exonic reads. It is likely that SARS-CoV-2 also directly regulates splicing
257 efficiency, as was recently proposed²⁴, but this effect seems more subtle. Together these
258 findings indicate SARS-CoV-2 infection leads to accelerated degradation of cytosolic cellular
259 mRNAs and that this degradation significantly affects the production of cellular proteins.

260 **SARS-CoV-2 5'UTR protects viral RNA from NSP1 induced RNA degradation**

261 An important aspect of host shutoff during infection is the ability of the virus to hamper the
262 translation of cellular transcripts while recruiting the ribosome to its own transcripts. Although it
263 has been suggested that SARS-CoV-2 mRNAs are refractory to the translation inhibition induced
264 by NSP1^{24,43} our measurements indicate that SARS-CoV-2 transcripts are not preferentially
265 translated in infected cells (Figure 1D and ⁹), that RNA degradation plays a prominent role in
266 remodeling the mRNA pool in infected cells and that SARS-CoV-2 dominates the mRNA pool.
267 All SARS-CoV-2-encoded subgenomic RNAs contain a common 5' leader sequence that is
268 added during negative-strand synthesis⁴⁴. We therefore explored whether the 5'UTR sequence
269 protects viral mRNAs from NSP1 induced degradation. We fused the viral 5'UTR sequence or a
270 short control 5'UTR to the 5' end of a GFP reporter (Figure 4A) and transfected these constructs
271 together with expression vectors encoding NSP1 or NSP2 (that was used as a control) into 293T
272 cells. We found that NSP1 expression suppresses the production of the control-GFP but not of
273 the 5'UTR-containing GFP (Figure 4B and 4C). We extracted RNA from these cells and
274 observed that the NSP1 induced reduction in control-GFP expression was associated with 15-
275 fold reduction in the RNA GFP levels whereas the levels of the GFP RNA with the SARS-CoV-
276 2 5'UTR were not reduced, and were even slightly increased, by NSP1 expression (Figure 4D).
277 The plasmid we used also contains an mCherry reporter expressed from an independent
278 promoter. Reassuringly NSP1 also induces a reduction in both mCherry protein (Figure S7A and
279 S7B) and RNA levels (Figure S7C) when compared to NSP2. These results indicate that the 5'
280 UTR of viral RNAs provides them protection from NSP1 induced degradation and that this
281 protection contributes to the ability of the virus to dominate the mRNA pool in infected cells.

282 **The translation efficiency of transcriptionally induced genes is impaired during infection**

283 Our results so far exemplify how SARS-CoV-2 remodels the transcript pool in infected cells. To
284 quantitatively evaluate the role of translational control along SARS-CoV-2 infection, we
285 calculated relative translation efficiency (TE, ratio of footprints to mRNAs for a given gene)
286 along infection. We then centered on genes that showed the strongest change in their relative TE
287 along infection. We clustered these genes into four clusters, based on similarity in their temporal
288 TE profiles, which largely reflect genes whose relative TE is reduced along infection and genes
289 whose relative TE is increased. The mRNA and footprint temporal profiles of these genes reveal
290 a clear signature; the genes whose relative TE along infection was reduced were genes whose

291 mRNA increased during infection but did not show a corresponding increase in footprints
292 (Figure 5A and Figure S8). These genes were enriched in immune response genes ($FDR < 10^{-4}$)
293 like IRF1, IL-6 and CXCL3. Comparing changes in mRNA and TE levels of cellular genes along
294 infection demonstrates that generally transcripts which are transcriptionally induced following
295 infection show a reduction in their relative TE and vice versa (Figure 5B). This inability of RNA
296 that is elevated in response to infection to reach the ribosomes, may explain why infected cells
297 fail to launch a robust IFN response^{13,14}. These data demonstrate that the overall capacity of
298 infected cells to generate new proteins is severely impaired.

299

300 **Discussion:**

301 Many viruses developed varied and sophisticated mechanisms to repress host mRNA translation
302 in order to block the cellular innate immune response and concomitantly allow the translation of
303 viral mRNAs. Specifically, CoVs have evolved specialized mechanisms to hijack the host gene
304 expression machineries, including inhibition of host protein synthesis and induction of
305 endonucleolytic cleavage of host mRNAs^{23,45}. In the case of SARS-CoV-2 several viral ORFs
306 have been suggested to interfere with viral gene expression^{24-28,30}, but what occurs during viral
307 infection remained an open question.

308 Using unbiased measurements of translation and RNA expression along SARS-CoV-2 infection,
309 we identified three major courses by which SARS-CoV-2 interferes with cellular gene
310 expression in infected cells; 1. global inhibition of protein translation, 2. degradation of cytosolic
311 cellular transcripts and 3. specific translation inhibition of newly transcribed mRNAs.

312 Disruption of cellular protein production using these three components may represent a multi-
313 pronged mechanism that synergistically acts to suppress the host antiviral response (Figure 5C).
314 These mechanisms may explain the molecular basis of the potent suppression of IFN response
315 observed in animal models and in severe COVID-19 patients^{15,18}.

316 Despite differences in protein size and mode of action, NSP1 proteins from alpha- and beta-
317 CoVs suppress host gene expression^{21,22,46}, making Nsp1 a central host shutoff factor of CoVs.
318 Indeed, several studies focused on SARS-CoV-2 encoded NSP1, showing it binds to the 40S

319 ribosomal subunit, leading to reduction in mRNA translation both in vitro and in cells^{24–28}. Still,
320 how the virus can overcome the NSP1-mediated block of translation and how NSP1 effects come
321 into play during infection remained unclear. We reveal here that in resemblance to what had been
322 described for SARS-CoV NSP1, SARS-CoV-2 NSP1 induces shutdown of host protein
323 translation by two mechanisms: first, it stalls canonical mRNA translation as was reported by
324 others^{24–28,41} leading to general reduction in the translation capacity of infected cells. Second,
325 NSP1 leads to accelerated cellular mRNA degradation. SARS-CoV NSP1 induces
326 endonucleolytic cleavage and subsequent degradation of host mRNAs and this activity depends
327 on its binding to the 40S ribosome subunit^{21,47}. Our results indicate a similar mechanism
328 operates in SARS-CoV-2 infected cells as we show cytosolic RNAs, specifically ones that are
329 being translated, are more susceptible to SARS-CoV-2-mediated degradation. several studies
330 have shown that mRNAs with viral 5' UTR are translated more efficiently compared to control
331 5'UTR in the presence of NSP1^{24,27,43 26} but it was further demonstrated that NSP1 inhibits
332 translation of both cellular and a viral 5' UTR-containing reporter mRNA^{27 26} implying viral
333 mRNAs do not simply evade translation inhibition in the context of the 5' UTR sequence. Our
334 measurements from infected cells support these findings as we show viral RNA are not
335 preferentially translated in infected cells. Instead, using a reporter, we show that the viral 5'UTR
336 renders mRNAs refractory to RNA degradation mediated by NSP1. Together these results
337 support a model in which NSP1 acts as a strong inhibitor of translation, tightly binding
338 ribosomes and reducing the pool of available ribosomes that can engage in translation. At the
339 same time ribosome bound NSP1 leads to accelerated degradation of cellular but not of viral
340 mRNAs providing means for viral mRNA to quickly take over and dominate the mRNA pool.
341 This accumulation of SARS-CoV-2 mRNAs explains how infected cells switch their translation
342 towards viral mRNAs. In support of this model, we show that within 8 hours 88% of the mRNAs
343 in infected cells is viral. Notably, interactions between SARS-CoV NSP1 and the viral 5' UTR
344 prevent NSP1-induced RNA cleavage⁴⁷, indicating that likely similar mechanisms are applied
345 by SARS-CoV and SARS-CoV-2 viruses.

346 On top of the general translation reduction and cellular mRNA degradation which are likely
347 mediated by NSP1, we also observed that in mRNAs that are being induced in response to
348 infection there is no corresponding increase in footprints, indicating these newly generated

349 transcripts are less likely to engage with ribosomes. Since mRNAs that are induced in infection
350 are enriched in cytokines and IFN induced genes, this distinctive mechanism may provide
351 additional explanation why infected cells fail to mount an efficient anti-viral response. Although
352 we do not know the underlying molecular mechanism, one appealing hypothesis is viral
353 inhibition of cellular mRNA nuclear export. Since SARS-CoV-2 replicates in the cytoplasm,
354 inhibiting the nuclear export of mRNAs can provide unique advantages as it will specifically
355 inhibit cellular mRNA translation and more explicitly it will lead to suppression of the host's
356 antiviral response genes which are transcriptionally induced and therefore fully depend on
357 efficient nuclear export. ORF6 was recently shown to co-purify with host mRNA export factors
358 ⁴⁸ and by over expression it disrupts nucleocytoplasmic export ²⁹, providing a candidate that may
359 explain the phenotype we observe in infection. Another possibility is that NSP16, which was
360 recently shown to inhibit cellular RNA splicing ²⁴, is driving this phenotype as interference with
361 splicing will prevent efficient nuclear export. Indeed, we show here that infection leads to
362 increased levels of intronic reads in many cellular transcripts. Although our analysis reveals
363 some of this signature is attributed to accelerated degradation of mature cellular transcripts it is
364 likely there is also a processing and splicing defect that leads to more intronic reads and
365 eventually aberrant export.

366 Overall, our study provides an in-depth picture of how SARS-CoV-2 efficiently interferes with
367 cellular gene expression, leading to shutdown of host protein production using a multipronged
368 strategy.

369

370 **Figure legends:**

371 **Figure 1:** Global reduction of translation and in cellular mRNA levels along SARS-CoV-2
372 Infection

373 (A) Calu3 cells were left uninfected or were infected with SARS-CoV-2 (MOI=3) for 3, 5 or 8
374 hours and harvested for RNA-seq and for Ribo-seq. (B and C) Percentage of reads that aligned
375 to human or viral transcripts from the sum of aligned reads shown for mRNAs (B) and footprints
376 (C) in uninfected cells and in cells harvested at 3, 5 and 8 hpi. (D) Cumulative TE distribution
377 among well-expressed human (black points) and viral (colored points) genes at 3, 5 and 8 hpi.
378 (E) Protein synthesis measurement by flow cytometry of Calu3 cells infected with SARS-CoV-2
379 (MOI = 3) for 3, 5 and 8 hpi or an uninfected control following O-Propargyl Puromycin (OPP)
380 incorporation and fluorescent labelling. (F) Percent of reads that aligned to the human or viral
381 transcripts from the sum of total RNA reads in uninfected cells and in cells harvested at, 3, 5 and
382 8 hpi. (G) Heat map presenting relative mRNA and footprints expression of well-expressed
383 human transcripts that showed the most significant changes in their mRNA levels at 8 hpi
384 relative to uninfected, across time points during SARS-CoV-2 infection. Shown are expression
385 levels scaled by gene after partitioning clustering. Three main clusters are marked on the right.
386 (H) Summary of pathway enrichment analysis of genes enriched in cluster 3 (upregulated genes).
387 Dot size reflects the number of genes from each pathway included in the tested set, and dot color
388 reflects the false discovery rate (FDR) of the pathway enrichment.

389

390 **Figure 2:** Changes in viral gene expression along SARS-CoV-2 and MHV infection

391 (A) Schematic presentation of the SARS-CoV-2 genome organization, the subgenomic mRNAs
392 and the main ORFs. (B and C) RNA (B) and translation levels (C) of each of the SARS-CoV-2
393 canonical ORFs at 3, 5 and 8 hpi. (D) Relative translation efficiency of each canonical viral ORF
394 along infection. Genes are divided to three groups based on their physical location along the
395 genome. (E) Schematic presentation of the MHV subgenomic mRNAs, and the main ORFs.
396 ORFs that are not expressed in the strain that was used for ribosome profiling (MHV-A59) are

397 marked by empty rectangles. **(F and G)** RNA **(F)** and translation levels **(G)** of each of the MHV
398 ORFs at 2.5, 5 and 8 hpi. Expression was calculated from ³⁶. **(H)** Relative translation efficiency
399 of each canonical viral ORF along infection. Genes are divided to two groups based on their
400 location on the genome. **(I)** Relative translation efficiency of the main ORFs (ORF7a, S and
401 ORF3a, labeled by circle) and the out of frame ORFs (ORF7b, S.iORF and ORF3c, labeled by
402 diamond) of the S, ORF3a and ORF7a subgenomic transcripts, respectively, along SARS-CoV-
403 2 infection. Translation levels were calculated from ribosome densities using ORF-RATER ⁴⁹.

404

405 **Figure 3: Cytosolic cellular RNAs are degraded during SARS-CoV-2 infection**

406 **(A)** RNA level fold change of cellular RNAs at different time points after infection relative to
407 uninfected cells. RNAs were grouped to ten bins based on their cytosol to nucleus localization
408 ratio ⁴¹. **(B)** The change in RNA levels of nuclear encoded or mitochondrial encoded RNAs at
409 different time points after infection relative to uninfected cells. **(C)** The change in RNA
410 expression of cytosolic lncRNAs and protein coding mRNAs at different time points after
411 infection relative to uninfected cells. **(D)** RNA reads on exons and introns of the end of IL-32
412 gene from uninfected cells and at 8 hpi. **(E)** Box plots presenting the ratio of intronic to exonic
413 reads for each gene in uninfected cells and at the different time points along SARS-CoV-2
414 infection. **(F)** The % of reads that align to exonic or intronic regions relative to rRNA abundance
415 along SARS-CoV-2 infection. **(H)** Histograms showing the change in the ratio of intronic to
416 exonic reads of cellular genes at 8hpi relative to uninfected cells. Genes are divided according to
417 the three clusters shown in figure 1G (representing different expression pattern along infection).

418

419 **Figure 4: The viral 5' UTR protects mRNA from NSP1-mediated degradation**

420 **(A)** 293T cells were transfected with expression vectors encoding either NSP1 or NSP2 (as a
421 control) and with a GFP reporter (GFP (-) 5'UTR) or a GFP reporter that includes the viral
422 5'UTR (GFP (+) 5'UTR). **(B)** Microscopy images of cells co-transfected with NSP2 (top) or
423 NSP1 (bottom) together with either GFP (-) 5'UTR or GFP (+) 5'UTR. Scale bars are 100µm.

424 (C) Flow cytometry analysis of cells co-transfected with NSP1 or NSP2 together with either GFP
425 (-) 5'UTR or GFP (+) 5'UTR. (D) Relative GFP RNA levels from GFP (-) 5'UTR or GFP (+)
426 5'UTR in cells expressing NSP1 or NSP2 as measured by quantitative RT-PCR. Data points
427 show measurement of biological replicates. Shown is one representative experiment out of two
428 performed.

429

430 **Figure 5:** The translation of induced transcripts is impaired during infection

431 (A) Heat map presenting relative TE, mRNA and footprints (FP) of human genes that showed the
432 most significant changes in their relative TE along SARS-CoV-2 infection. Shown are relative
433 expression ratios after partitioning clustering based on changes in relative TE values. (B) Scatter
434 plot presenting cellular transcript levels in uninfected cells compared to 8hpi. Genes are colored
435 based on the relative change in their TE between uninfected and 8hpi. Central cytokines and IFN
436 stimulated genes are labeled. (C) A model of how SARS-CoV-2 suppresses host gene expression
437 through multi-pronged approach: 1. Global translation reduction; 2. Degradation of cytosolic
438 cellular mRNAs; 3. Specific translation inhibition of newly synthesized cellular mRNAs.

439

440

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450 **Methods**

451 Cells and viruses

452 Calu3 cells (ATCC HTB-55) were cultured in 6-well or 10cm plates with RPMI supplemented
453 with 10% fetal bovine serum (FBS), MEM non-essential amino acids, 2mM L-Glutamine,
454 100Units/ml Penicillin and 1% Na-pyruvate. Monolayers were washed once with RPMI without
455 FBS and infected with SARS-CoV-2 virus, at a multiplicity of infection (MOI) of 3, in the
456 presence of 20 µg per ml TPCK trypsin (Thermo scientific). Plates were incubated for 1 hour at
457 37°C to allow viral adsorption. Then RPMI medium supplemented with 2% fetal bovine serum,
458 MEM non-essential amino acids, L glutamine and penicillin-streptomycin-Nystatin at 37°C, 5%
459 CO₂ was added to each well. SARS-CoV-2 BavPat1/2020 Ref-SKU: 026V-03883 was kindly
460 provided by C. Drosten, Charité–Universitätsmedizin Berlin, Germany. It was propagated (5
461 passages) and tittered on Vero E6 cells and then sequenced⁹ before it was used. Handling and
462 working with SARS-CoV-2 virus was conducted in a BSL3 facility in accordance with the
463 biosafety guidelines of the Israel Institute for Biological Research. The Institutional Biosafety
464 Committee of Weizmann Institute approved the protocol used in these studies.

465

466 Preparation of ribosome profiling and RNA sequencing samples

467 For RNA-seq, cells were washed with PBS and then harvested with Tri-Reagent (Sigma-
468 Aldrich), total RNA was extracted, and poly-A selection was performed using Dynabeads
469 mRNA DIRECT Purification Kit (Invitrogen) mRNA sample was subjected to DNaseI
470 treatment and 3' dephosphorylation using FastAP Thermosensitive Alkaline Phosphatase
471 (Thermo Scientific) and T4 PNK (NEB) followed by 3' adaptor ligation using T4 ligase (NEB).
472 The ligated products used for reverse transcription with SSIII (Invitrogen) for first strand cDNA
473 synthesis. The cDNA products were 3' ligated with a second adaptor using T4 ligase and
474 amplified for 8 cycles in a PCR for final library products of 200-300bp. For Ribo-seq libraries,
475 cells were treated with 100µg/mL CHX for 1 minute. Cells were then placed on ice, washed
476 twice with PBS containing 100µg/mL CHX, scraped from 10cm plates, pelleted and lysed with

477 lysis buffer (1% triton in 20mM Tris 7.5, 150mM NaCl, 5mM MgCl₂, 1mM dithiothreitol
478 supplemented with 10 U/ml Turbo DNase and 100µg/ml cycloheximide). After lysis samples
479 stood on ice for 2h and subsequent Ribo-seq library generation was performed as previously
480 described⁵⁰. Briefly, cell lysate was treated with RNaseI for 45min at room temperature
481 followed by SUPERase-In quenching. Sample was loaded on sucrose solution (34% sucrose,
482 20mM Tris 7.5, 150mM NaCl, 5mM MgCl₂, 1mM dithiothreitol and 100µg/ml cycloheximide)
483 and spun for 1h at 100K RPM using TLA-110 rotor (Beckman) at 4c. Pellet was harvested using
484 TRI reagent and the RNA was collected using chloroform phase separation. For size selection,
485 15uG of total RNA was loaded into 15% TBE-UREA gel for 65min, and 28-34 footprints were
486 excised using 28 and 34 flanking RNA oligos, followed by RNA extraction and ribo-seq
487 protocol⁵⁰

488

489 Sequence alignment, metagene analysis

490 Sequencing reads were aligned as previously described⁵¹. Briefly, linker
491 (CTGTAGGCACCATCAAT) and poly-A sequences were removed and the remaining reads
492 were aligned to the Hg19 and to the SARS-Cov-2 genome (Genebank NC_045512.2) with 3
493 changes to match the used strain (BetaCoV/Germany/BavPat1/2020 EPI_ISL_406862),
494 241:C→T, 3037:C→T, 23403:A→G]. Alignment was performed using Bowtie v1.1.2⁵² with
495 maximum two mismatches per read. Reads that were not aligned to the genome were aligned to
496 the transcriptome and to SARS-CoV-2 junctions that were recently annotated⁴⁴. The aligned
497 position on the genome was determined as the 5' position of RNA-seq reads, and for Ribo-seq
498 reads the p-site of the ribosome was calculated according to read length using the off-set from
499 the 5' end of the reads that was calculated from canonical cellular ORFs. The offsets used are
500 +12 for reads that were 28-29 bp and +13 for reads that were 30-33 bp. Reads that were in
501 different length were discarded. In all figures presenting ribosome densities data, all footprint
502 lengths (28-33bp) are presented.

503 Junctions spanning reads were quantified using STAR 2.5.3a aligner⁵³, with running flags as
504 suggested by⁴⁴, to overcome filtering of non-canonical junctions. Reads aligned to multiple
505 locations were discarded⁴⁴.

506 For the metagene analysis only genes with more than 10 reads were used. For each gene,
507 normalization was done to its maximum signal and each position was normalized to the number
508 of genes contributing to the position.

509 Filtering of genes, quantification and RPKM normalization

510 For analysis of cellular genes, the genes were filtered according to the number of reads as
511 follows. The number of reads on a gene in each replicate, from at least one of the conditions
512 (uninfected or 8hr) had to be greater than 50 for the mRNA and greater than 25 for the FP. For
513 heatmaps and clustering, gene filtering was done according to mRNA as described above, and
514 genes with zero reads in any of the samples (mRNA and FP of all time points) were excluded.
515 Histone genes were excluded. Read coverage on genes was normalized to units of RPKM in
516 order to normalize for gene length and for sequencing depth. For analysis comparing host and
517 viral genes, the RPKM was calculated based on the total number of reads of both the host and the
518 virus. For analysis concentrating on cellular genes alone, the RPKM was calculated based on the
519 total number of host reads, and the RPKM values were further scaled to keep the number of total
520 reads equal across samples. The scale factors for RNA-seq were calculated from the ratio of the
521 host mRNA reads to total reads, including viral, rRNA and tRNA reads. in the total RNA
522 sequencing (without polyA selection), and the scale factors for footprints were calculated from
523 the fraction of host reads from the total aligned ribosome profiling reads, including viral and host
524 reads.

525 Since the viral RNAs are widely overlapping, RNA-seq RPKM levels of viral genes were
526 computed with deconvolution as detailed here. First, values for each gene were calculated by
527 subtracting the RPKM of an ORF from the RPKM of the ORF located just upstream of it in the
528 genome. Then, for subgenomic RNAs, leader-body junction counts were counted based on
529 STAR alignment number of uniquely mapped reads crossing the junction. Finally, based on the
530 correlation between the deconvoluted RPKM and junction abundance of the subgenomic RNAs,

531 the RPKM levels of all viral RNAs was estimated. To quantify the translation levels of non-
532 canonical viral ORFs ORF-RATER was used ⁴⁹.

533 Quantification of intronic reads

534 To avoid biases from intron read count, genes without introns, or where at least one of the
535 introns is overlapping with an exon of another gene were excluded. In addition, genes with low
536 number of reads (< 20 on the exons, < 2 on the introns) were ignored. The number of reads on
537 exons and introns was normalized by the total length of the exons and introns respectively to get
538 quantification proportional to the number of molecules. Finally, the normalized number of reads
539 on the introns was calculated as percentage of the normalized number of reads on the exons.
540 Statistical significance (in figure 3E) was tested using a paired t-test on the log values of the
541 percentage (with offset of 0.1 to overcome zero values).

542 Comparison of cytosolic lncRNAs and mitochondrial mRNAs

543 The list of cytosolic lncRNAs is based on ⁴¹. The list of 14 lncRNAs (with number of reads >=
544 50) that were used in the analysis are given in supplementary table 2. The lncRNAs were
545 compared to protein coding genes, and mitochondrial encoded mRNAs were compared to
546 nuclear encoded mRNAs. Statistical significance was tested using Wilcoxon rank test on the log
547 fold change (compared to uninfected) of the RPKM values.

548 Protein synthesis measurement using O-Propargyl Puromycin (OPP)

549 OPP assay (OPP, Thermo Fisher Scientific) was carried out following the manufacturer's
550 instructions. Briefly, cells were collected following treatment with 10 μ M O-Propargyl
551 Puromycin for 30 minutes at 37 °C. The cells were then fixed for 15 minutes in 3.7%
552 formaldehyde, and permeabilized in 0.1% Triton X-100 for 15 minutes. OPP was then
553 fluorescently labeled by a 30 minute incubation in Click-iT® Plus OPP reaction cocktail with
554 Alexa Fluor®594 picolyl azide (Thermo Fisher Scientific). Cells were analyzed using BD LSRII
555 flow cytometer.

556

557 Pathway enrichment analysis

558 Enrichment analysis of cellular pathways in specific gene clusters was done with PANTHER
559 version 15.0, with default settings and the PANTHER pathways data set ^{54,55}.

560 Reporter assay

561 pLVX-EF1alpha-SARS-CoV-2-nsp1-2XStrep-IRES-Puro and pLVX-EF1alpha-SARS-CoV-2-
562 nsp2-2XStrep-IRES-Puro were kindly provided by Nevan Krogan, University of California, San
563 Francisco.

564 The viral 5'UTR was constructed based on nucleotides 4-265 of the reported sequence of SARS-
565 CoV-2 isolate Wuhan-Hu-1 (NC_045512.2) by sequential annealing of DNA oligonucleotides
566 (IDT, 5'UTR oligo_1-5 listed in the table below). The coding sequence for the first 12 amino
567 acids (aa) of ORF1a as well as the AcGFP homology region were added to the 5'UTR by two
568 PCR amplifications. For the 5'UTR-less control plasmid, the 12 aa region with AcGFP
569 homology was ordered from Sigma-Aldrich as oligonucleotides. The viral 5'UTR with the 12 aa
570 region and the 12 aa region on its own were cloned into pAcGFP1-C1 (Takara Biotech) using
571 restriction-free cloning. The entire expression cassette from the promoter to the poly-A site was
572 amplified and cloned into pDecko-mCherry (Addgene plasmid #78534) using restriction-free
573 cloning. Primers for PCR amplification of fragments were ordered from Sigma-Aldrich. All
574 primers and oligonucleotides used for cloning are listed in the table below.

Primer	Sequence
5'UTR oligo_1	caaGGTACCaaaggtttataccttcccaggttaacaaaccaaccaacttgcgatctct tgtagatctgttctctaaacgaa
5'UTR oligo_2	Tgcgtgagtgcactaagcatgcagccgagtgacagccacacagattttaagttcgttt agagaacagatctacaagaga

pDecko cloning Rev	gaccacctccaaccccgaggggaccagtaagatacattgatgagtttgacaaac cacaac
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575

576

577 293T cells were transfected using JetPEI (Polyplus-transfection) following the manufacturer's
578 instructions. Cells were assayed 24 hours post transfection by flow cytometry on a BD Accuri C6
579 flow cytometer and imaged on a Zeiss AxioObserver Z1 wide-field microscope using a X20
580 objective and AxioCam 506 mono camera.

581

582 Quantitative real-time PCR analysis

583 Total RNA was extracted using Tri-Reagent (Sigma) and polyA selected using the Dynabeads
584 mRNA DIRECT purification kit (Thermo Fisher Scientific) following the manufacturer's
585 instructions. cDNA was prepared using qScript cDNA Synthesis Kit (Quanta Biosciences)
586 following the manufacturer's instructions. Real time PCR was performed using the SYBR Green
587 PCR master-mix (ABI) on the QuantStudio 12K Flex (ABI) with the following primers (forward,
588 reverse):

589 ActinB (GTCATTCCAAATATGAGATGCGT, GCTATCACCTCCCCTGTGTG)

590 AcGFP (TGACCCTGAAGTTCATCTGC, GAAGTCGTGCTGCTTCATGT)

591 mCherry (ACCGCCAAGCTGAAGGTGAC, GACCTCAGCGTCGTAGTGGC)

592 GFP and mCherry mRNA levels were calculated relative to the human ActinB transcript.

593

594

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724

Figure 1

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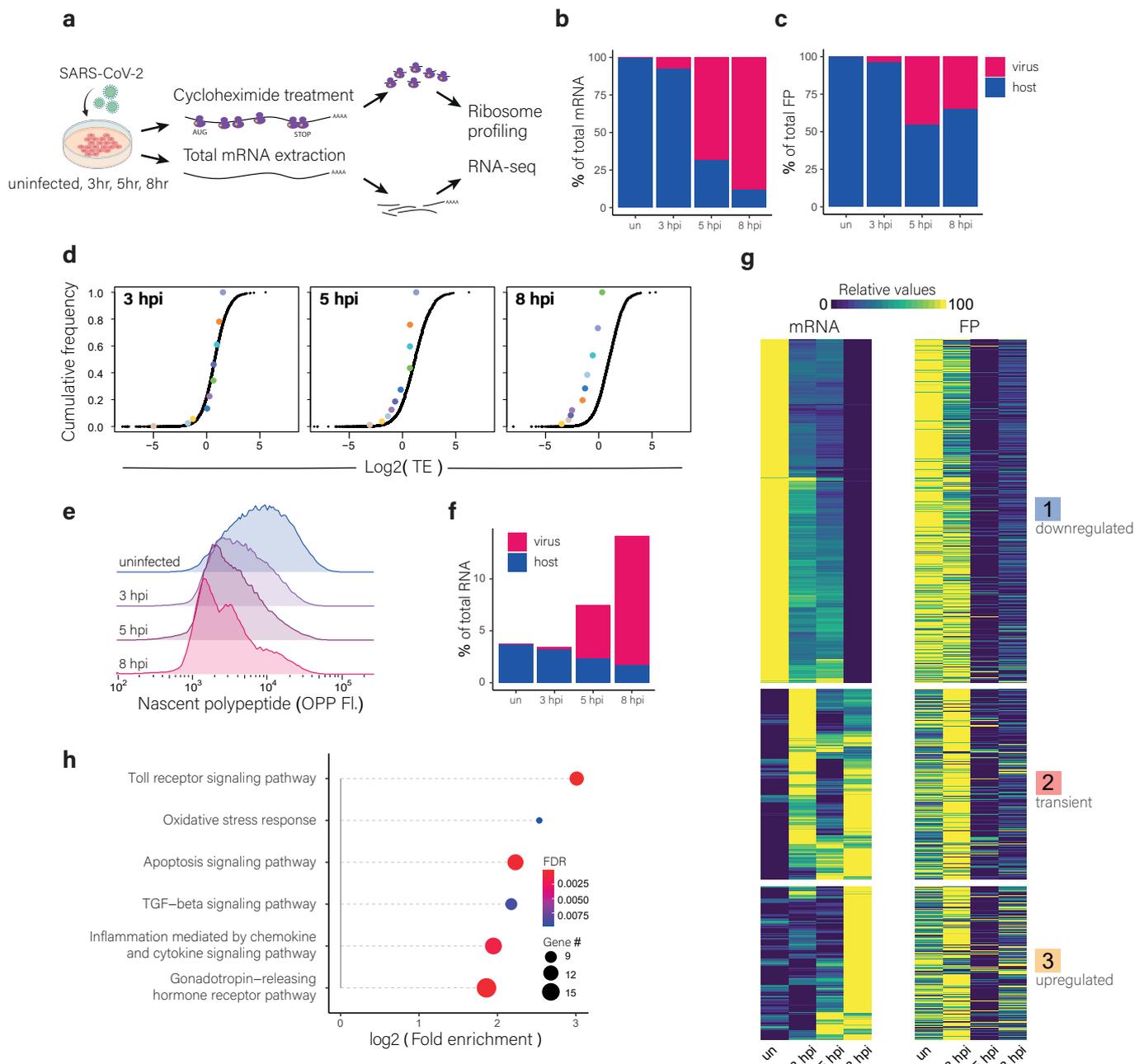


Figure 1: Global reduction of translation and in cellular mRNA levels along SARS-CoV-2 infection

(A) Calu3 cells were left uninfected or were infected with SARS-CoV-2 (MOI=3) for 3, 5 or 8 hours and harvested for RNA-seq and for Ribo-seq. (B and C) Percentage of reads that aligned to human or viral transcripts from the sum of aligned reads shown for mRNAs (B) and footprints (C) in uninfected cells and in cells harvested at 3, 5 and 8 hpi. (D) Cumulative TE distribution among well-expressed human (black points) and viral (colored points) genes at 3, 5 and 8 hpi. (E) Protein synthesis measurement by flow cytometry of Calu3 cells infected with SARS-CoV-2 (MOI = 3) for 3, 5 and 8 hpi or an uninfected control following O-Propargyl Puromycin (OPP) incorporation and fluorescent labelling. (F) Percent of reads that aligned to the human or viral transcripts from the sum of total RNA reads in uninfected cells and in cells harvested at 3, 5 and 8 hpi. (G) Heat map presenting relative mRNA and footprints expression of well-expressed human transcripts that showed the most significant changes in their mRNA levels at 8 hpi relative to uninfected, across time points during SARS-CoV-2 infection. Shown are expression levels scaled by gene after partitioning clustering. Three main clusters are marked on the right. (H) Summary of pathway enrichment analysis of genes enriched in cluster 3 (upregulated genes). Dot size reflects the number of genes from each pathway included in the tested set, and dot color reflects the false discovery rate (FDR) of the pathway enrichment.

Figure 2

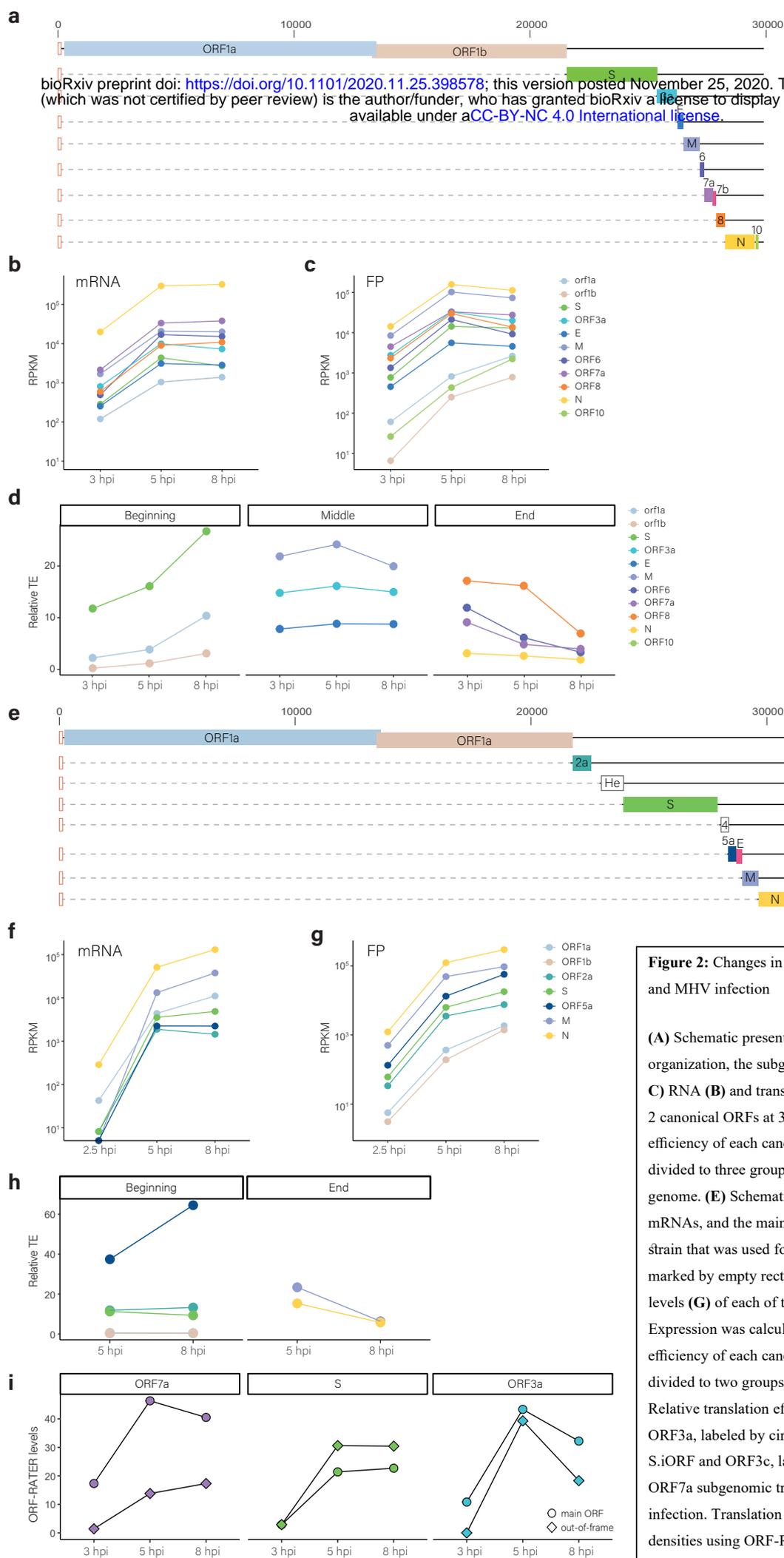


Figure 2: Changes in viral gene expression along SARS-CoV-2 and MHV infection

(A) Schematic presentation of the SARS-CoV-2 genome organization, the subgenomic mRNAs and the main ORFs. **(B and C)** RNA **(B)** and translation levels **(C)** of each of the SARS-CoV-2 canonical ORFs at 3, 5 and 8 hpi. **(D)** Relative translation efficiency of each canonical viral ORF along infection. Genes are divided to three groups based on their physical location along the genome. **(E)** Schematic presentation of the MHV subgenomic mRNAs, and the main ORFs. ORFs that are not expressed in the strain that was used for ribosome profiling (MHV-A59) are marked by empty rectangles. **(F and G)** RNA **(F)** and translation levels **(G)** of each of the MHV ORFs at 2.5, 5 and 8 hpi. Expression was calculated from ³⁶. **(H)** Relative translation efficiency of each canonical viral ORF along infection. Genes are divided to two groups based on their location on the genome. **(I)** Relative translation efficiency of the main ORFs (ORF7a, S and ORF3a, labeled by circle) and the out of frame ORFs (ORF7b, S.iORF and ORF3c, labeled by diamond) of the S, ORF3a and ORF7a subgenomic transcripts, respectively, along SARS-CoV-2 infection. Translation levels were calculated from ribosome densities using ORF-RATER ⁴⁹.

Figure 3

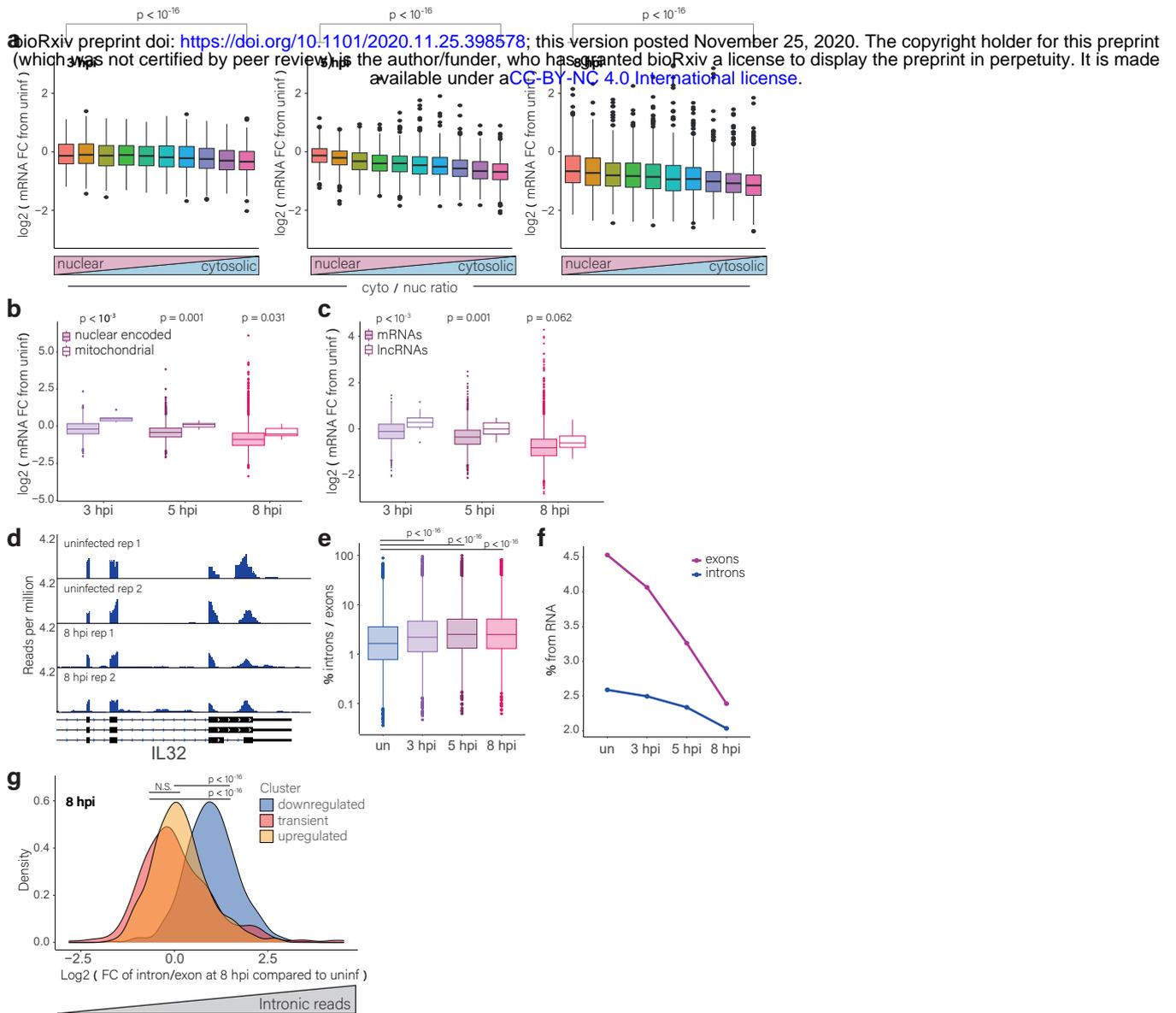


Figure 3: Cytosolic cellular RNAs are degraded during SARS-CoV-2 infection

(A) RNA level fold change of cellular RNAs at different time points after infection relative to uninfected cells. RNAs were grouped to ten bins based on their cytosol to nucleus localization ratio⁴¹. **(B)** The change in RNA levels of nuclear encoded or mitochondrial encoded RNAs at different time points after infection relative to uninfected cells. **(C)** The change in RNA expression of cytosolic lncRNAs and protein coding mRNAs at different time points after infection relative to uninfected cells. **(D)** RNA reads on exons and introns of the end of IL-32 gene from uninfected cells and at 8 hpi. **(E)** Box plots presenting the ratio of intronic to exonic reads for each gene in uninfected cells and at the different time points along SARS-CoV-2 infection. **(F)** The % of reads that align to exonic or intronic regions relative to rRNA abundance along SARS-CoV-2 infection. **(H)** Histograms showing the change in the ratio of intronic to exonic reads of cellular genes at 8hpi relative to uninfected cells. Genes are divided according to the three clusters shown in figure 1G (representing different expression pattern along infection).

Figure 4

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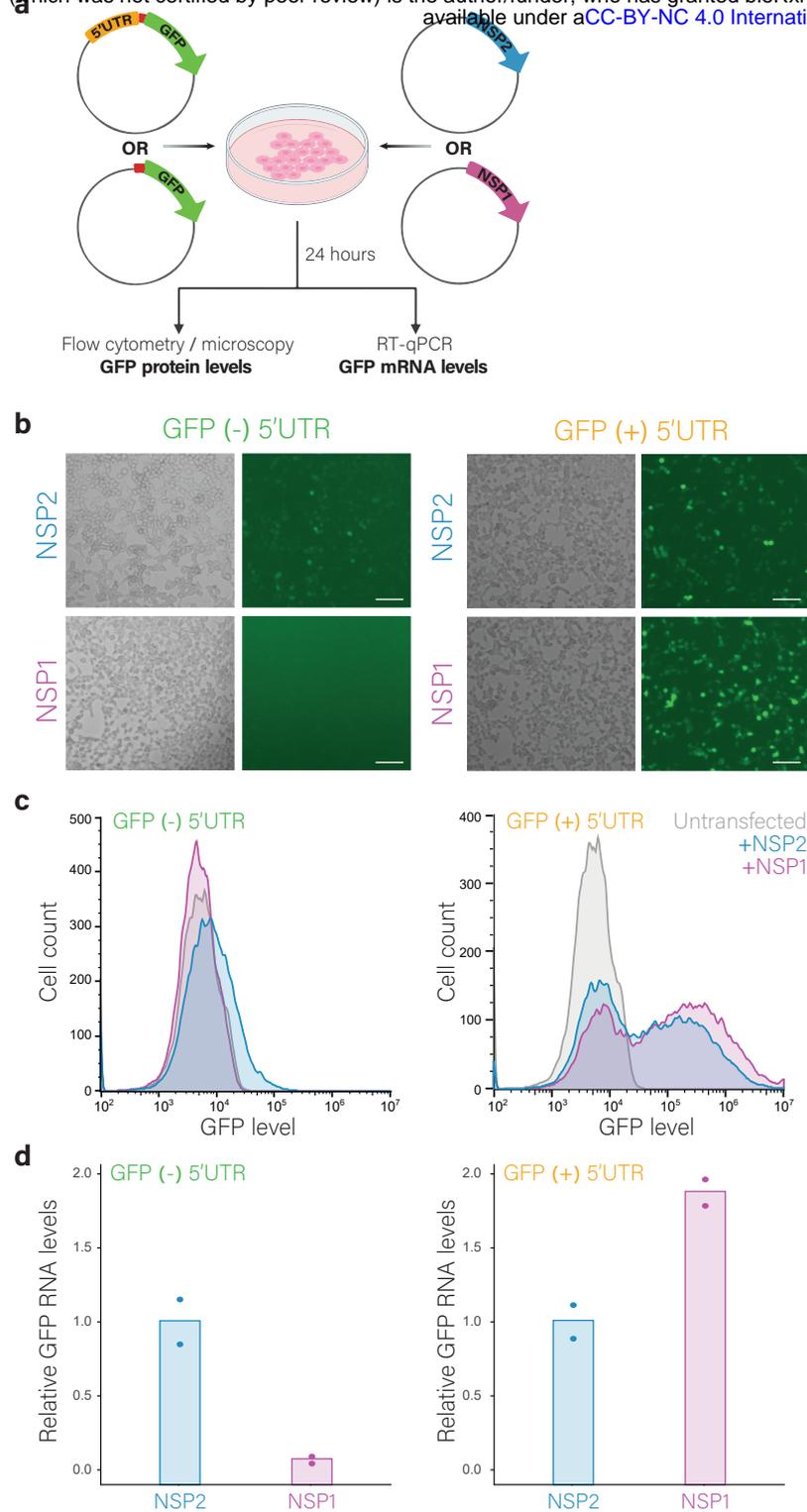


Figure 4: The viral 5' UTR protects mRNA from NSP1-mediated degradation

(A) 293T cells were transfected with expression vectors encoding either NSP1 or NSP2 (as a control) and with a GFP reporter (GFP (-) 5'UTR) or a GFP reporter that includes the viral 5'UTR (GFP (+) 5'UTR). **(B)** Microscopy images of cells co-transfected with NSP2 (top) or NSP1 (bottom) together with either GFP (-) 5'UTR or GFP (+) 5'UTR. Scale bars are 100µm. **(C)** Flow cytometry analysis of cells co-transfected with NSP1 or NSP2 together with either GFP (-) 5'UTR or GFP (+) 5'UTR. **(D)** Relative GFP RNA levels from GFP (-) 5'UTR or GFP (+) 5'UTR in cells expressing NSP1 or NSP2 as measured by quantitative RT-PCR. Data points show measurement of biological replicates. Shown is one representative experiment out of two performed.

Figure 5

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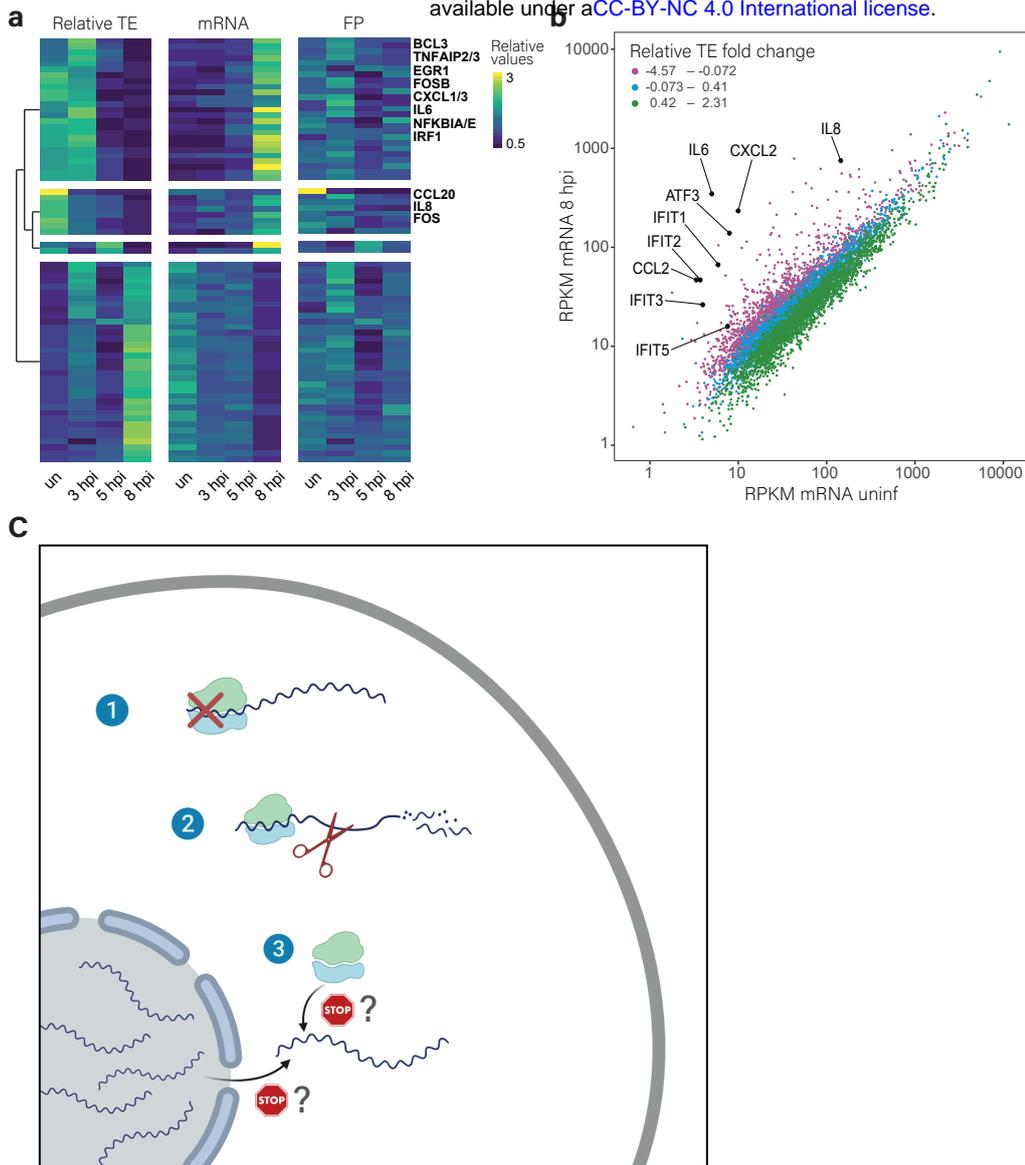


Figure 5: The translation of induced transcripts is impaired during infection

(A) Heat map presenting relative TE, mRNA and footprints (FP) of human genes that showed the most significant changes in their relative TE along SARS-CoV-2 infection. Shown are relative expression ratios after partitioning clustering based on changes in relative TE values. (B) Scatter plot presenting cellular transcript levels in uninfected cells compared to 8hpi. Genes are colored based on the relative change in their TE between uninfected and 8hpi. Central cytokines and IFN stimulated genes are labeled. (C) A model of how SARS-CoV-2 suppresses host gene expression through multi-pronged approach: 1. Global translation reduction; 2. Degradation of cytosolic cellular mRNAs; 3. Specific translation inhibition of newly synthesized cellular mRNAs.