Early temporal dynamics of cellular responses to SARS-CoV-2

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Abstract

Two highly pathogenic human coronaviruses that cause severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) have evolved proteins that can inhibit host antiviral responses, likely contributing to disease progression and high case-fatality rates. SARS-CoV-2 emerged in December 2019 resulting in a global pandemic. Recent studies have shown that SARS-CoV-2 is unable to induce a robust type I interferon (IFN) response in human cells, leading to speculation about the ability of SARS-CoV-2 to inhibit innate antiviral responses. However, innate antiviral responses are dynamic in nature and gene expression levels rapidly change within minutes to hours. In this study, we have performed a time series RNA-seq and selective immunoblot analysis of SARS-CoV-2 infected lung (Calu-3) cells to characterize early virus-host processes. SARS-CoV-2 infection upregulated transcripts for type I IFNs and interferon stimulated genes (ISGs) after 12 hours. Furthermore, we analyzed the ability of SARS-CoV-2 to inhibit type I IFN production and downstream antiviral signaling in human cells. Using exogenous stimuli, we discovered that SARS-CoV-2 is unable to modulate *IFNβ* production and downstream expression of ISGs, such as *IRF7* and *IFIT1*. Thus, data from our study indicate that SARS-CoV-2 may have evolved additional mechanisms, such as masking of viral nucleic acid sensing by host cells to mount a dampened innate antiviral response. Further studies are required to fully identify the range of immune-modulatory strategies of SARS-CoV-2.

Significance

Highly pathogenic coronaviruses that cause SARS and MERS have evolved proteins to shutdown antiviral responses. The emergence and rapid spread of SARS-CoV-2, along with its relatively low case-fatality rate have led to speculation about its ability to modulate antiviral

69 responses. We show that SARS-CoV-2 is unable to block antiviral responses that are mounted by
70 exogenous stimuli. Data from our study provide promising support for the use of recombinant
71 type I IFN as combination therapy to treat COVID-19 patients. Furthermore, our data also
72 suggest that the inability of SARS-CoV-2 to efficiently modulate antiviral responses may be
73 associated with its low case-fatality rate compared to other pathogenic CoVs that cause SARS
74 and MERS.

75 **Main Text**

76 **Introduction**

77 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December
78 2019 to cause a global pandemic of coronavirus disease 2019 (COVID-19) (1). SARS-CoV-2
79 causes a respiratory infection with acute respiratory distress syndrome (ARDS) in severe cases.
80 Innate antiviral responses, which include type I interferons (IFNs) are the first line of defense
81 after a virus enters a cell (2). Cellular pattern recognition receptors (PRRs) recognize viral
82 nucleic acids and activate key cellular kinases, such as inhibitor of nuclear factor kappa-B kinase
83 subunit epsilon (IKKε) and TANK-binding kinase 1 (TBK1). These kinases activate
84 transcription factors, such as interferon regulatory factor 3 (IRF3) to stimulate downstream
85 production of type I IFNs (3).

86 To counteract host antiviral responses, viruses encode proteins that can modulate type I
87 IFN production and signaling (4, 5). Emerging pathogenic human coronaviruses, such as SARS-
88 CoV and Middle East respiratory syndrome (MERS)-CoV have evolved multiple proteins that
89 can inhibit type I IFN responses in human cells (6-10). Thus, to better understand SARS-CoV-2
90 pathogenesis, it is critical to identify the early dynamic interactions of SARS-CoV-2 and the type
91 I IFN response.

92 Data from *in vitro* and *in vivo* work have demonstrated the lack of induction of type I IFN
93 responses following SARS-CoV-2 infection (11). Interestingly, on the contrary, emerging data
94 from patients with mild and moderate cases of COVID-19 have demonstrated the presence of
95 type I IFN (12, 13). Thus, the inability to mount an effective IFN response to SARS-CoV-2 may
96 also be associated with underlying host factors, along with the duration and extent of viral
97 infection. Furthermore, it is unclear if SARS-CoV-2 is unable to stimulate a type I IFN response
98 or actively suppresses the response after initiating it in infected cells.

99 In this study, we have identified global early transcriptional responses that are initiated
100 during SARS-CoV-2 infection of human lung epithelial (Calu-3) cells at 0, 1, 2, 3, 6, and 12
101 hours post infection. SARS-CoV-2 infected cells mounted a type I IFN response between 6 and
102 12 hours post infection (hpi) and the degree of this response correlated with virus replication and
103 transcription. However, a high dose infection of SARS-CoV-2 is unable to modulate poly (I:C)-
104 induced IFNβ production and signaling. Furthermore, SARS-CoV-2 is unable to modulate
105 interferon stimulated gene (ISG) expression in response to exogenous IFNβ. Our study provides
106 insights into early host responses that are generated on infection with SARS-CoV-2 and the
107 inability of the virus to efficiently modulate these responses, which may explain the low case-
108 fatality rate of COVID-19. Furthermore, it is likely that comorbidities and deficiencies in type I
109 IFN responses are associated with severe outcomes in COVID-19 patients. In summary, our data
110 indicate that SARS-CoV-2 is inefficient in modulating type I IFN production and signaling when
111 cells are exogenously stimulated. Further investigations into the ability of SARS-CoV-2 to mask
112 its nucleic acid pathogen associated molecular pattern (PAMP) from cellular PRRs to generate a
113 dampened innate antiviral response is warranted.

114 **Results**

115 **SARS-CoV-2 replication proceeds in a directional manner.** The replication cycle of CoVs is
116 complex and involves the generation of sub-genomic RNA molecules, which in turn code for
117 mRNA that are translated into proteins (14, 15). To determine SARS-CoV-2 replication kinetics
118 in human cells using RNA-seq, we infected human lung epithelial cells (Calu-3) at a multiplicity
119 of infection (MOI) of 2. One hour post incubation, virus inoculum was replaced with cell growth
120 media and the clock was set to zero hours. We extracted and sequenced poly-A enriched RNA at
121 0, 1, 2, 3, 6 and 12 hours post infection (hpi). SARS-CoV-2 genome, sub-genomic RNA and
122 transcripts were detected in infected samples; viral transcript expression clustered based on post-
123 infection time using PCA (*SI Appendix,* Fig. S1). From our RNA-seq analysis, we were able to
124 detect high levels of expression of SARS-CoV-2 structural and accessory genes at the 3' end of
125 the genome as early as 0 hpi (Fig. 1A). Significant expression of *ORF1ab*, relative to 0 hpi was
126 detected at 6 hpi (Fig. 1A). SARS-CoV-2 nucleocapsid (*N*) gene was highly expressed relative to
127 other genes as early as 0 hpi (Fig 1B), with relative expression significantly increasing over time
128 (p = 1.4e-16). The absolute expression of other genes increased over time with levels of *N* > *M* >
129 *ORF10* > *S* > *ORF1ab* > *ORF7a* > *ORF8* > *ORF3a* > *ORF6* > *E* > *ORF7b* > *ORF1a* at 12 hpi
130 (Fig. 1B; *SI Appendix*, Table S1).
131
132 **SARS-CoV-2 induces a mild type I IFN response.** We analyzed the early host response
133 mounted by Calu-3 cells that were infected with SARS-CoV-2. Gene expression levels in these
134 cells clustered based on time-points via PCA (*SI Appendix*, Fig. S2). One hundred and twenty-
135 four genes were significantly (FDR-adjusted $p < 0.05$) differentially expressed in infected cells,
136 relative to mock infected cells in at least one time point post infection (absolute $\log_2$ fold-change
137 > 1) (Fig. 1D; *SI Appendix*, Table S2 and Fig. S3). The extent of antiviral gene expression at 12

138 hpi correlated with an increase in viral transcripts (*SI Appendix*, Fig. S3). Interestingly, at early
139 time points of 2 and 3 hpi, pathway enrichment analysis revealed numerous cellular processes
140 that were significantly downregulated in SARS-CoV-2 infected cells, relative to mock infected
141 cells (FDR-adjusted $p < 0.05$). These processes included RNA splicing, apoptosis, ATP
142 synthesis, and viral and host translation, while genes associated with viral processes, cell
143 adhesion and double-stranded RNA binding were upregulated in infected cells relative to mock
144 infected cells (Fig. 1C and *SI Appendix*, Figs. S4 and S5, Table S3). Cellular pathways associated
145 with type I IFN production and signaling, along with OAS/TRAF-mediated antiviral responses
146 were upregulated at 12 hpi (Figs. 1C and 1D). Consistent with other reports, transcript levels for
147 *IFNβ1* and *IFNλ1* were significantly upregulated at 12 hpi with SARS-CoV-2 at a high MOI of 2
148 (Fig. 1E) (11). Transcript levels of *IFNλ2* and *IFNλ3* also increased at 6 and 12 hpi, but the
149 levels did not reach significance relative to mock infected cells at these time points (Fig. 1E). At
150 least 19 well-studied antiviral ISGs were upregulated in infected cells, relative to mock infected
151 cells at 12 hpi, including interferon induced protein with tetratricopeptide repeats 1 (*IFIT1*),
152 interferon regulatory factor 7 (*IRF7*), 2'-5-oligoadenylate synthetase 2 (*OAS2*) and MX dynamin
153 GTPase 1 (*MX1*) (Fig. 1F and *SI Appendix*, Fig. S6 and Table S2). Genes associated with
154 structural molecule activity, cell adhesion and exocytosis were downregulated in SARS-CoV-2
155 infected cells, relative to uninfected cells at 12 hpi (*SI Appendix*, Fig. S5).
156
157 **SARS-CoV-2 is unable to modulate type I IFN gene expression induced by an exogenous**
158 **stimulus.** Coronaviruses, such as those that cause SARS and MERS have evolved multiple
159 proteins that can modulate type I IFN expression (7-10, 16, 17). To confirm that SARS-CoV-2
160 infection is sufficient to induce type I IFN and ISG responses in Calu-3 cells, we infected the

161 cells with SARS-CoV-2 and assessed transcript levels of *IFNβ*, *IRF7* and *IFIT1* by quantitative
162 polymerase chain reaction (qPCR). *IFNβ* induction was observed 12 hpi in SARS-CoV-2
163 infected cells, relative to mock-infected cells (Fig. 2A). Consistent with the upregulation of *IFNβ*
164 transcripts in SARS-CoV-2 infected cells, transcript levels for ISGs, such as *IRF7* and *IFIT1*
165 were also upregulated 12 hpi (Figs. 2B and 2C).

166 Next, to identify if SARS-CoV-2 is able to modulate type I IFN responses mounted
167 against an exogenous stimulus, we infected Calu-3 cells with SARS-CoV-2 for 12 hours at a
168 MOI of 2 and stimulated these cells with exogenous double-stranded RNA [poly(I:C)] for 6
169 hours. We measured the levels of *IFNβ* transcripts in these cells by qPCR. Poly(I:C) transfection
170 alone induced significantly higher levels of *IFNβ* transcripts relative to mock transfected cells
171 (Fig. 2D). Similar to that shown in Fig. 2A, SARS-CoV-2 infection alone also induced high
172 levels of *IFNβ* transcripts relative to mock infected cells (Fig. 2D). However, SARS-CoV-2
173 infection-induced levels of *IFNβ* transcripts were significantly lower compared to both poly(I:C)
174 transfected cells and SARS-CoV-2 infected + poly(I:C) transfected cells. Interestingly, there was
175 no significant difference in *IFNβ* transcript levels between poly(I:C) transfected and SARS-CoV-
176 2 infected + poly(I:C) transfected cells (Fig. 2D). In fact, there was an increasing trend in *IFNβ*
177 transcript levels in SARS-CoV-2 infected + poly(I:C) transfected cells relative to cells that were
178 transfected with poly(I:C) alone; however, the data were not significant at this time point.

179 To support our observations with *IFNβ* transcripts in SARS-CoV-2 infected and/or
180 poly(I:C) transfected cells, we also quantified the levels of ISG transcripts, such as *IRF7* and
181 *IFIT1* in these cells. Poly(I:C) transfection alone induced significantly higher levels of *IRF7* and
182 *IFIT1* transcripts relative to mock transfected cells (Figs. 2E and 2F). Similar to that shown in
183 Figs. 2B and 2C, SARS-CoV-2 alone also induced high levels of *IRF7* and *IFIT1* transcripts

184 relative to mock infected cells (Figs. 2E and 2F). However, SARS-CoV-2 infection-induced
185 levels of *IRF7* and *IFIT1* transcripts were significantly lower compared to both poly(I:C)
186 transfected cells and SARS-CoV-2 infected + poly(I:C) transfected cells. Notably, *IRF7* and
187 *IFIT1* transcript levels in SARS-CoV-2 infected + poly(I:C) transfected cells were significantly
188 higher than levels in cells that were transfected with poly(I:C) alone (Figs. 2E and 2F).
189 To corroborate our gene expression studies, we repeated our experiments and performed
190 immunoblots for SARS-CoV-2 N, IFIT1 and glyceraldehyde 3-phosphate dehydrogenase
191 (GAPDH). Poly(I:C) transfection induced low levels of IFIT1 in Calu-3 cells, while SARS-CoV-
192 2 infection alone was unable to induce detectable levels of IFIT1 in our immunoblots (Fig. 2G).
193 SARS-CoV-2 infection + poly(I:C) transfection also induced low, but detectable levels of IFIT1
194 (Fig. 2G). We confirmed SARS-CoV-2 infection in these cells by detecting N protein in the
195 samples (Fig. 2G).
196
197 **SARS-CoV-2 is unable to modulate type I IFN signaling.** SARS-CoV and MERS-CoV
198 proteins can also modulate downstream IFN signaling to restrict the production of ISGs (6). To
199 determine if SARS-CoV-2 can modulate type I IFN signaling in response to exogenous IFNβ
200 treatment, we infected Calu-3 cells for 12 hours at a MOI of 2 and stimulated these cells with
201 recombinant human IFNβ for 6 hours. We monitored gene expression levels of *IRF7* and *IFIT1*
202 in these cells by qPCR. For this assay, we developed and utilized recombinant human IFNβ1. To
203 demonstrate the antiviral efficacy of our recombinant IFN, we pre-treated human fibroblast
204 (THF) cells with IFNβ1, followed by RNA and DNA virus infections. Pre-treatment of THF
205 cells with IFNβ1 inhibited the replication of herpes simplex virus (HSV), vesicular stomatitis
206 virus (VSV) and H1N1 in a dose-dependent manner (*SI Appendix*, Fig. S7).

207 Exogenous IFNβ treatment alone significantly upregulated transcript levels of *IRF7* and
208 *IFIT1* relative to mock treated Calu-3 cells (Figs. 2H and 2I). Consistent with our RNA-seq data,
209 SARS-CoV-2 infection alone induced significant levels of *IRF7* and *IFIT1* transcripts (Figs. 2H
210 and 2I). However, SARS-CoV-2 induced *IRF7* and *IFIT1* transcript levels were significantly
211 lower compared to levels in both IFNβ treated cells and SARS-CoV-2 infected + IFNβ treated
212 cells (Figs. 2H and 2I). Transcript levels of *IRF7* and *IFIT1* in IFNβ treated cells and SARS-
213 CoV-2 infected + IFNβ treated cells were not significantly different (Figs. 2H and 2I).
214 Finally, we repeated the experiments with exogenous IFNβ treatment and performed
215 immunoblots to determine if SARS-CoV-2 can modulate type I IFN-mediated upregulation of
216 IFIT1. Exogenous IFNβ treatment alone induced a robust expression of IFIT1 (Fig. 2J). SARS-
217 CoV-2 infection alone was not sufficient for a visible increase in IFIT1 expression in our
218 immunoblots (Fig. 2J). Interestingly, IFNβ treatment after 12 hours of high dose infection (MOI
219 = 2) of SARS-CoV-2 also induced a robust expression of IFIT1 (Fig. 2J). We confirmed SARS-
220 CoV-2 infection in these cells by detecting N protein (Fig. 2J).

221 **Discussion**

222 SARS-CoV-2 emerged in December 2019 and has since caused a global pandemic of COVID-19
223 (1, 18). Clinical observations and emerging data from *in vitro* and *in vivo* studies have
224 demonstrated the limited ability of SARS-CoV-2 to induce type I IFNs (11). However, the ability
225 of SARS-CoV-2 to modulate IFN production and signaling remains unknown. Furthermore, gene
226 expression kinetics of SARS-CoV-2, along with time-associated host responses have not been
227 described. In this study, we have identified early virus-host interactions using a time-series
228 RNA-seq experiment. Consistent with other studies (11), we demonstrate that a high dose of
229 SARS-CoV-2 induces a type I IFN response; however, our data show that SARS-CoV-2 is

230 unable to modulate cellular type I IFN production and signaling that are mounted in response to
231 exogenous stimuli.

232 RNA-seq analysis of poly(A)-enriched RNA allowed us to map the progression of SARS-
233 CoV-2 replication and transcription in Calu-3 cells. As observed with other coronaviruses (19-
234 21), SARS-CoV-2 replicated and transcribed sub-genomic RNA and mRNA in a directional
235 manner (Figs. 1A and 1B). SARS-CoV-2 *N* gene was highly expressed as early as 0 hpi. High
236 MOI of SARS-CoV-2 produced cytopathic effects (CPE) in Calu-3 cells at later time points,
237 which made it difficult to reliably assess host gene expression relative to unstable levels of
238 house-keeping genes.

239 Coronaviruses, including highly pathogenic SARS-CoV, MERS-CoV and porcine
240 epidemic diarrhea virus (PEDV) have evolved proteins that can efficiently modulate type I IFN
241 responses (7-10, 16, 17, 22, 23). The recently demonstrated inability of SARS-CoV-2 to
242 stimulate the expression of robust amounts of type I IFNs (11) may be associated with its ability
243 to mask the detection of viral RNA by cellular PRRs and/or its ability to inactivate cellular
244 mechanisms involved in type I IFN upregulation. Data from our studies show that SARS-CoV-2
245 is indeed unable to stimulate high levels of *IFNβ* transcripts relative to poly(I:C) (Fig. 2D).
246 However, SARS-CoV-2 is unable to efficiently shutdown poly(I:C)-mediated upregulation of
247 *IFNβ* transcripts and downstream ISGs (Figs. 2D-F). In fact, poly(I:C) + SARS-CoV-2 induced
248 higher levels of ISG transcripts relative to poly(I:C) alone. Thus, our data hint at additional
249 mechanisms that SARS-CoV-2 may have evolved to mitigate the recognition of viral PAMPs by
250 cellular PRRs. MERS-CoV protein 4a interferes with RIGI and MDA5-mediated sensing of viral
251 RNA (7). Murine hepatitis virus (MHV) encodes an endoribonuclease that cleaves poly-uridine
252 residues in the viral genome, thus limiting the activation of cellular PRRs (24). Endoribonuclease

253 deficient mouse CoVs induce a robust type I IFN response and can only replicate in cells that are
254 IFN deficient (25, 26). It is possible that SARS-CoV-2 uses a similar strategy to limit the
255 detection of its nucleic acid by cellular PRRs, thus leading to a dampened antiviral IFN response
256 in these cells. Future studies will identify the full breadth of strategies deployed by SARS-CoV-2
257 to modulate innate antiviral responses.

258 A recent study has identified the ability of SARS-CoV-2 to replicate to higher titers in the
259 upper respiratory tract, including nasal cells (27). Hou *et al.* have shown that high levels of virus
260 replication in nasal cells is associated with high levels of angiotensin-converting enzyme 2
261 (ACE2) receptor expression in these cells, relative to cells in the lower respiratory tract (27).
262 Studies have also shown that rhinovirus (common cold virus) replicates to higher titers in nasal
263 cells due to diminished temperature-dependent innate antiviral responses in these cells (28).
264 Thus, the inability of SARS-CoV-2 to induce a robust type I IFN response, coupled with the
265 dampened ability of nasal cells to potentiate an innate immune response may lead to high levels
266 of virus replication in the upper respiratory tract, as observed in COVID-19 patients (29).

267 In conclusion, our study demonstrates that SARS-CoV-2 is a weak stimulator of type I
268 IFN responses in infected human cells, relative to the more potent form of PAMP, poly(I:C).
269 However, our data suggest that the lack of a robust type I IFN response in SARS-CoV-2 infected
270 cells is likely due to the inability of the cells to recognize viral PAMPs, such as double-stranded
271 RNA. The inability of SARS-CoV-2 to modulate downstream IFN responses is promising for the
272 development of IFNβ as a treatment or post-exposure prophylactic. Clinical trials for
273 combination IFNβ therapy against MERS-CoV are currently ongoing (30). IFNβ, in combination
274 with lopinavir-ritonavir and ribavirin has been used with promising results in COVID-19 patients
275 (31). While it is possible that over-expressing viral proteins may identify interactions that can

276 modulate type I IFN production in human cells, we did not observe these effects when cells were
277 infected with a high MOI of SARS-CoV-2 and stimulated exogenously. Future studies will shed
278 more light on the full breadth of immune modulatory capabilities of SARS-CoV-2.

279 Materials and Methods

280 **Cells and viruses.** Vero E6 cells (African green monkey cells; ATCC, Manassas, VA, USA)
281 were maintained in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% fetal
282 bovine serum (FBS; Sigma-Aldrich), 1x L-Glutamine and Penicillin/Streptomycin (Pen/Strep;
283 Corning, VWR, Mississauga, ON, Canada). Calu-3 cells (human lung adenocarcinoma derived;
284 ATCC) were cultured as previously mentioned (32). THF cells (human telomerase life-extended
285 cells; from Dr. Victor DeFilippis' lab) were cultured as previously mentioned (33). *Drosophila*
286 S2 cells (ThermoFisher Scientific, Waltham, MA, USA) were cultured in Schneider's
287 *Drosophila* medium supplemented with 10% FBS (Sigma-Aldrich) as recommended by the
288 manufacturer and cells were incubated at 28°C. Stocks of genetically engineered vesicular
289 stomatitis virus (VSV-GFP) carrying a green fluorescent protein (GFP) cassette (34) were stored
290 at -80°C. H1N1 (A/Puerto Rico/8/1934 mNeon – 2A-HA) stocks were obtained from Dr.
291 Matthew Miller's laboratory. HSV-GFP stocks were generated and maintained as mentioned
292 previously (35). SARS-CoV-2/SB3 virus stocks were propagated on Vero E6 cells and validated
293 by next generation sequencing (36). Virus stocks were thawed once and used for an experiment.
294 A fresh vial was used for each experiment to avoid repeated freeze-thaws. VSV-GFP, HSV-GFP
295 and H1N1 infections were performed at an MOI of 1. SARS-CoV-2 infections were performed at
296 an MOI of 2. Experiments with SARS-CoV-2 were performed in a BSL3 laboratory and all
297 procedures were approved by institutional biosafety committees at McMaster University and the
298 University of Toronto.

299 **RNA-Seq**

300 RNA was isolated from cells using RNeasy Mini kit (Qiagen, Hilden, Germany). Sequencing
301 was conducted at the McMaster Genomics Facility, Farncombe Institute at McMaster University.
302 Sample quality was first assessed using a Bioanalyzer (Agilent 2100 Bioanalyzer G2938C,
303 Aligent RNA 6000 Nano Kit, Agilent; Santa Clara, CA, USA), then enriched (NEBNext
304 Poly(A) mRNA Magnetic Isolation Module; NEB, Ipswich, MA, USA). Library preparations
305 were conducted (NEBNext Ultra II Directional RNA Library Prep Kit; NEB, Ipswich, MA,
306 USA) and library fragment size distribution was verified (Agilent TapeSection D1000; Agilent,
307 Santa Clara, CA, USA). Libraries were quantified by qPCR, pooled in equimolar amounts, and
308 qPCR and fragment size distribution verification was conducted again. Libraries were then
309 sequenced on an Illumina HiSeq 1500 across 3 HiSeq Rapid v2 flow cells in 6 lanes (Illumina;
310 San Diego, CA, USA) using a paired-end, 2x50 bp configuration, with onboard cluster
311 generation averaging 30.8M clusters per replicate (minimum 21.9M, maximum 46.0M).

312 **Transcript quantification and differential expression analysis**

313 Sequence read quality was checked with FastQC
314 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), with reads subsequently aligned to
315 the human reference transcriptome (GRCh37.67) obtained from the ENSEMBL database (37) ,
316 indexed using the 'index' function of Salmon (version 0.14.0) (38) with a k-mer size of 31.
317 Alignment was performed using the Salmon 'quant' function with the following parameters: "-l
318 A --numBootstraps 100 --gcBias --validateMappings". All other parameters were left to defaults.
319 Salmon quantification files were imported into R (version 3.6.1) (39) using the tximport library
320 (version 1.14.0) (40) with the 'type' option set to 'salmon'. Transcript counts were summarized
321 at the gene-level using the corresponding transcriptome GTF file mappings obtained from

322 ENSEMBL. Count data was subsequently loaded into DESeq2 (version 1.26.0) (41) using the
323 'DESeqDataSetFromTximport' function. In order to determine time/treatment dependent
324 expression of genes, count data was normalized using the 'estimateSizeFactors' function using
325 the default 'median ratio method' and output using the 'counts' function with the 'normalized'
326 option.

327 For subsequent differential-expression analysis, a low-count filter was applied prior to
328 normalization, wherein a gene must have had a count greater than 5 in at least three samples in
329 order to be retained. Using all samples, this resulted in the removal of 12,980 genes for a final set
330 of 15,760 used. Principal Component Analysis (PCA) of samples across genes was performed
331 using the 'vst' function in DESeq2 (default settings) and was subsequently plotted with the
332 ggplot2 package in R (42). Differential expression analyses were carried out with three designs:
333 (a) the difference between infection/control status across all timepoints, (b) considering the
334 effects of post-infection time (i.e. the interaction term between time and infection status) and (c)
335 the difference between infection/control status at individual timepoints. (a) and (b) were
336 performed using the 'DESeq' function of DESeq2 using all samples, with results subsequently
337 summarized using the 'results' function with the 'alpha' parameter set to 0.05; *p*-values were
338 adjusted using the Benjamini-Hochberg FDR method (43), with differentially expressed genes
339 filtered for those falling below an adjusted *p*-value of 0.05. For (c), infected/mock samples were
340 subset to individual timepoints, with differential expression calculated using DESeq as described
341 above. Additionally, given the smaller number of samples at individual time-points, differential-
342 expression analysis was also performed with relaxation of the low-count filter described above,
343 with results and p-value adjustments performed as above.

344 **Viral transcript quantification**

345 Paired-end sequencing reads were mapped to CDS regions of the SARS-CoV-2 genomic
346 sequence (Assembly ASM985889v3 - GCF_009858895.2) obtained from NCBI, indexed using
347 the 'index' function of Salmon (version 0.14.0) (38) with a k-mer size of 31. Subsequently, reads
348 were aligned using the Salmon 'quant' function with the following parameters: "-l A --
349 numBootstraps 100 --gcBias --validateMappings". All other parameters were left to defaults.
350 Salmon quantification files were imported into R (version 3.6.1) (39) using the tximport library
351 (version 1.14.0) (40) with the 'type' option set to 'salmon'. All other parameters were set to
352 default. Transcripts were mapped to their corresponding gene products via GTF files obtained
353 from NCBI. Count data was subsequently loaded into DESeq2 (version 1.26.0) (41) using the
354 'DESeqDataSetFromTximport' function. Principal Component Analysis (PCA) of samples
355 across viral genes was performed using the 'vst' function in DESeq2 (default settings) and was
356 subsequently plotted with the ggplot2 package in R (42). As viral transcript levels increased over
357 time post-infection, we first converted non-normalized transcript counts to a $\log_2$ scale, and
358 subsequently compared these across time-points (Fig. 1B; *SI Appendix*, Table S1). To look at the
359 changes in the expression of viral transcripts relative to total viral expression as a function of
360 post-infection time, normalized transcript counts were used to perform differential-expression
361 analysis with DESeq2. Results and p-value adjustments were performed as described above.
362 In order to compare host/viral expression patterns, normalized transcript counts from
363 infected samples were compared with either normalized or non-normalized viral transcript
364 counts (from the same sample) across time-points. For each viral transcript (n = 12), all host
365 genes (n = 15,760, after filtering described above) were tested for correlated expression changes
366 across matched infected samples (n = 18, across 5 time-points) using Pearson's correlation
367 coefficient (via the cor.test function in R). Correlation test *p*-values were adjusted across all-by-

368 all comparisons using the Benjamini-Hochberg FDR method, and gene-transcript pairs at
369 adjusted *p*-value < 0.05 were retained. To account for possible effects of cellular response to
370 plate incubation, viral transcript abundance was averaged at each time-point and compared to
371 host transcript abundance similarly averaged at each time-point for non-infected samples;
372 correlation testing was done all-by-all for n = 5 data-points. Host genes that correlated with viral
373 transcription in mock samples across time were removed from subsequent analyses; to increase
374 stringency, mock correlation was defined using un-adjusted *p*-value < 0.05. Host genes were
375 sorted by correlation coefficient (with any given viral transcript), with the top 100 unique genes
376 retained for visualization. Normalized host transcript counts were z-score transformed per-gene
377 using the 'scale' function in R, with normalized/un-normalized viral transcript counts similarly
378 transformed per-transcript. Resulting z-score expression heatmaps were generated using the
379 ComplexHeatmap library in R (version 2.2.0) (44). Heatmaps were generated for normalized/un-
380 normalized viral transcript counts, given the different information revealed by absolute and
381 relative viral expression patterns.

382 **Viral genome mapping**

383 Paired-end RNA-seq reads were filtered for quality control with Trim Galore! (version
384 0.6.4_dev) (45) and mapped to the SARS-CoV-2 reference sequence (NC_045512.2) with the
385 Burrow-Wheeler Aligner (46), using the BWA-MEM algorithm (47). Output SAM files were
386 sorted and compressed into BAM files using Samtools (version 1.10) (48). Read coverage
387 visualization was performed from within the R statistical environment (version 4.0.0) (39) using
388 the "scanBam" function from the Rsamtools R package (version 1.32.0) to extract read coverage
389 data and the ggplot2 R package (version 3.3.0) (42) to plot read coverage histograms (using 300
390 bins across the SARS-CoV-2 sequence).

391 **Cellular pathway enrichment analysis**

392 To determine cellular pathways that were associated with differentially expressed genes (DEGs),
393 the ActivePathways R (version 1.0.1) (49) package was utilized to perform gene-set based
394 pathway enrichment analysis. DEGs at each time point were treated as an independent set for
395 enrichment analysis. Fisher's combined probability test was used to enrich pathways after *p*-
396 value adjustment using Holm-Bonferroni correction. Pathways of gene-set size less than 5 and
397 greater than 1000 were excluded. Only pathways enriched at individual time-points were
398 considered for downstream analysis; pathways enriched across combined timepoints as
399 determined by ActivePathways Brown's *p*-value merging method were filtered out. Visualization
400 of enriched pathways across timepoints was done using Cytoscape (version 3.8.0) (50) and the
401 EnrichmentMap plugin (version 3.2.1) (51), as outlined by Reimand *et al*. (52). Up-to-date Gene-
402 Matrix-Transposed (GMT) files containing information on pathways for the Gene Ontology
403 (GO) Molecular Function (MF), GO Biological Process (BP) (53) and REACTOME (54)
404 pathway databases were utilized with ActivePathways. Only pathways that were enriched at
405 specific time points were considered. Bar plots displaying top ActivePathway GO terms and
406 REACTOME enrichments for infection versus mock were plotted using the ggplot2 R package
407 (version 3.2.1) for 1, 2, 3, and 12 hour time points. Zero and 6 hour time points were omitted due
408 to a lack of sufficient numbers of differentially expressed genes required for functional
409 enrichment analysis.

410 **Poly(I:C) transfection and IFNβ treatment.** Calu-3 cells were mock transfected with 4 µl of
411 lipofectamine 3000 (ThermoFisher Scientific) only or transfected with 100 ng of poly(I:C)
412 (InvivoGen, San Diego, CA, USA). Recombinant human IFNβ1 was generated using *Drosophila*
413 Schneider 2 (S2) cells following manufacturer's recommendation and by using ThermoFisher

414 Scientific's *Drosophila* Expression system. As a control, recombinant GFP was also generated
415 using the same protocol and used for mock treated cells. For VSV-GFP, HSV-GFP and H1N1-
416 mNeon infections, cells were treated with increasing concentrations of IFNβ1 or GFP (control).
417 SARS-CoV-2 infected cells were treated with 200 μg/ml of IFNβ1 or GFP.
418 **Quantitative PCR.** Calu-3 cells were seeded at a density of 3 x 10^5 cells/well in 12-well plates.
419 Cells were infected with SARS-CoV-2 and RNA extraction was performed using RNeasy® Mini
420 Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. 200 ng of purified RNA
421 was reverse transcribed using iScript™ gDNA Clear cDNA Synthesis Kit (Bio-Rad, Hercules,
422 CA, USA). Quantitative PCR reactions were performed with TaqMan™ Universal PCR Master
423 Mix (Applied Biosystems, Foster City, CA, USA) using pre-designed Taqman gene expression
424 assays (ThermoFisher Scientific) for *IFNβ1* (catalog #4331182), *IRF7* (catalog #4331182), *IFIT1*
425 (catalog #4331182) and *GAPDH* (catalog #4331182) according to manufacturer's protocol.
426 Relative mRNA expression was normalized to *GAPDH* and presented as 1/ΔCt.
427 **Immunoblots.** Calu-3 cells were seeded at a density of 3 x 10^5 cells/well in 12-well plates. Cells
428 were infected with SARS-CoV-2 at an MOI of 2. Control cells were sham infected. Twelve
429 hours post infection, cells were transfected or treated with poly(I:C) or IFNβ, respectively. Cell
430 lysates were harvested for immunoblots and analyzed on reducing gels as mentioned previously
431 (33). Briefly, samples were denatured in a reducing sample buffer and analyzed on a reducing
432 gel. Proteins were blotted from the gel onto polyvinylidene difluoride (PVDF) membranes
433 (Immobilon, EMD Millipore, Burlington, MA, USA) and detected using primary and secondary
434 antibodies. Primary antibodies used were: 1:1000 mouse anti-GAPDH (EMD Millipore;
435 Catalogue number: AB2302; RRID: AB_10615768), 1:1000 mouse anti-SARS-CoV-2 N
436 (ThermoFisher Scientific; Catalogue number: MA5-29981; RRID: AB_2785780 and 1:1000

437 rabbit anti-IFIT1 (ThermoFisher Scientific; Catalogue number: PA3-848; RRID: AB_1958733).
438 Secondary antibodies used were: 1:5000 donkey anti-rabbit 800 (LI-COR Biosciences, Lincoln,
439 NE, USA; Catalogue number: 926-32213; RRID: 621848) and 1:5000 goat anti-mouse 680 (LI-
440 COR Biosciences; Catalogue number: 925-68070; RRID: AB_2651128). Blots were observed
441 and imaged using Image Studio (LI-COR Biosciences) on the Odyssey CLx imaging system (LI-
442 COR Biosciences).
443 **Antiviral bioassay.** THF cells were pre-treated or mock treated with recombinant human IFNβ,
444 followed by VSV-GFP, HSV-GFP or H1N1-mNeon infection at an MOI of 1. Infected cells were
445 incubated at 37°C for 1 hour with gentle rocking every 15 minutes. After 1 hr, virus inoculum
446 was aspirated and Minimum Essential Medium (MEM) with Earle's salts (Sigma) containing 2%
447 FBS and 1% carboxymethyl cellulose (CMC; Sigma) was added on the cells. Cells were
448 incubated for 19 hours at 37°C and green fluorescent protein (GFP) or mNeon levels were
449 measured using a typhoon scanner (Amersham, GE Healthcare, Chicago, IL, USA).
450 **Statistics.** Statistical analyses for RNA-seq data were performed in R and are mentioned under
451 the respective RNA-seq analyses sections. All other statistical calculations were performed in
452 GraphPad Prism (version 8.4.2; www.graphpad.com) using two-tailed paired t-test. Significance
453 values are indicated in the figures and figure legends. p*<0.05, **<0.01, ***<0.001 and
454 ****<0.0001.

455 ## Data Availability

456 The DESeq2 normalized transcript counts for all genes with RNA-Seq data, significant or
457 otherwise, plus the raw sequencing FASTQ reads have been deposited into the Gene Expression
458 Omnibus (GEO) database with NCBI GEO accession number GSE151513.

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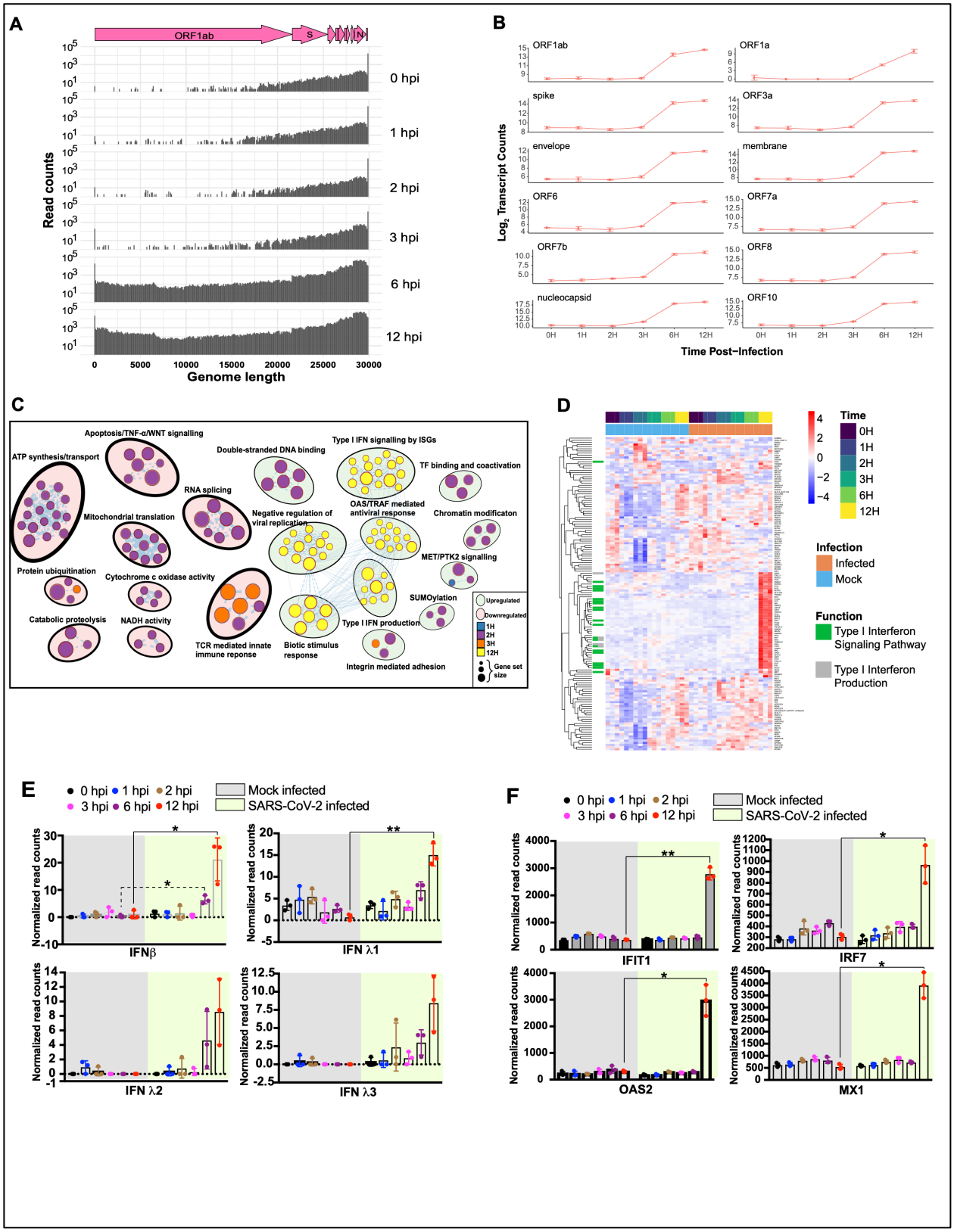
Figures and Figure Legends



595 **Figure 1. SARS-CoV-2 and cellular gene expression.** Calu-3 cells were infected with SARS-
596 CoV-2 at an MOI of 2. Virus and host gene expression were determined using time-series RNA-
597 seq analyses. **(A)** SARS-CoV-2 gene expression over 12 hours (n = 3/time point). The genome
598 organization of SARS-CoV-2 is indicated above in pink. **(B)** Major SARS-CoV-2 gene
599 expression levels at different times post infection (n = 3/time point). **(C)** Cellular processes that
600 are down or upregulated at different times post infection. The size of the circles represents the
601 number of genes that are down or upregulated at different times after infection (n = 3/time point).
602 **(D)** Cellular genes (n = 124) that are significantly up or downregulated (FDR-adjusted $p<0.05$;
603 $|\log_2 FC| > 1$) in SARS-CoV-2 infected cells, relative to mock infected cells at different times
604 post infection. Transcript levels are shown as z-score normalized expression (scaled by gene).
605 **(E)** Transcript abundance of type I interferon (IFN) genes (*IFNβ* and *IFNλ1-3*) in mock infected
606 and SARS-CoV-2 infected Calu-3 cells at different times (Mean ± SD; n = 3). **(F)** Transcript
607 abundance of representative interferon stimulated genes (ISGs) in mock infected and SARS-
608 CoV-2 infected Calu-3 cells at different times (Mean ± SD; n = 3). For Figs. A-D, statistical
609 analysis was performed in R (see methods). For Figs. E and F, statistical significance was
610 calculated using two-tailed paired t-test. hpi, hours post infection. $p^{*}<0.05$, $^{**}<0.01$.
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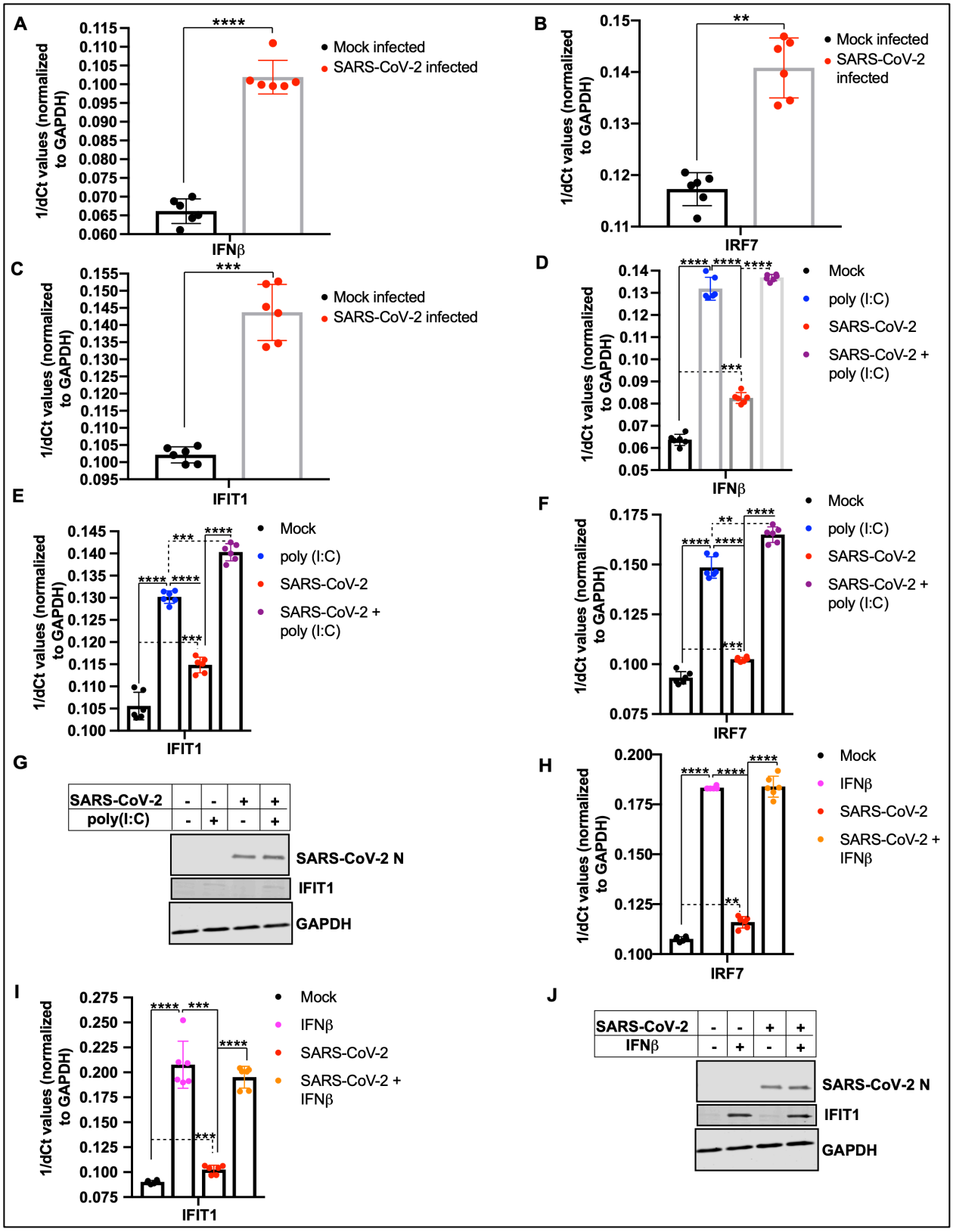
A
1/dCt values (normalized to GAPDH)
0.115
0.110
0.105
0.100
0.095
0.090
0.085
0.080
0.075
0.070
0.065
0.060
****
IFNβ
Mock infected
SARS-CoV-2 infected
B
1/dCt values (normalized to GAPDH)
0.15
0.14
0.13
0.12
0.11
**
IRF7
Mock infected
SARS-CoV-2 infected
C
1/dCt values (normalized to GAPDH)
0.155
0.150
0.145
0.140
0.135
0.130
0.125
0.120
0.115
0.110
0.105
0.100
0.095
***
IFIT1
Mock infected
SARS-CoV-2 infected
D
1/dCt values (normalized to GAPDH)
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
****
***
IFNβ
Mock
poly (I:C)
SARS-CoV-2
SARS-CoV-2 + poly (I:C)
E
1/dCt values (normalized to GAPDH)
0.145
0.140
0.135
0.130
0.125
0.120
0.115
0.110
0.105
0.100
***
****
IFIT1
Mock
poly (I:C)
SARS-CoV-2
SARS-CoV-2 + poly (I:C)
F
1/dCt values (normalized to GAPDH)
0.175
0.150
0.125
0.100
0.075
**
****
***
IRF7
Mock
poly (I:C)
SARS-CoV-2
SARS-CoV-2 + poly (I:C)
G
SARS-CoV-2 - - + +
poly(I:C) - + - +
SARS-CoV-2 N
IFIT1
GAPDH
H
1/dCt values (normalized to GAPDH)
0.200
0.175
0.150
0.125
0.100
****
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IRF7
Mock
IFNβ
SARS-CoV-2
SARS-CoV-2 + IFNβ
I
1/dCt values (normalized to GAPDH)
0.275
0.250
0.225
0.200
0.175
0.150
0.125
0.100
0.075
****
***
IFIT1
Mock
IFNβ
SARS-CoV-2
SARS-CoV-2 + IFNβ
J
SARS-CoV-2 - - + +
IFNβ - + - +
SARS-CoV-2 N
IFIT1
GAPDH

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613 **Figure 2. SARS-CoV-2 is unable to modulate type I IFN gene expression and downstream**
614 **ISG production.** To determine if SARS-CoV-2 is able to induce type I IFN production, Calu-3
615 cells were infected at an MOI of 2 for 12 hours. Transcript levels for *IFNβ* were quantified using
616 qPCR. To assess if SARS-CoV-2 can modulate *IFNβ* gene expression and downstream
617 stimulation of ISGs, Calu-3 cells were infected with SARS-CoV-2 at an MOI of 2 for 12 hours,
618 following which cells were treated or transfected with recombinant IFNβ or poly(I:C),
619 respectively for 6 hours. Mock infected and mock treated cells served as controls. **(A)** *IFNβ*
620 transcript levels in SARS-CoV-2 infected or mock infected Calu-3 cells, normalized to *GAPDH*
621 (Mean ± SD; n = 6). **(B)** *IRF7* transcript levels in SARS-CoV-2 infected or mock infected Calu-3
622 cells, normalized to *GAPDH* (Mean ± SD; n = 6). **(C)** *IFIT1* transcript levels in SARS-CoV-2
623 infected or mock infected Calu-3 cells, normalized to *GAPDH* (Mean ± SD; n = 6). **(D)** *IFNβ*
624 transcript levels in SARS-CoV-2 infected or mock infected, and poly(I:C) transfected or mock
625 transfected Calu-3 cells, normalized to *GAPDH* (Mean ± SD; n = 6). **(E)** *IFIT1* transcript levels
626 in SARS-CoV-2 infected or mock infected, and poly(I:C) transfected or mock transfected Calu-3
627 cells, normalized to *GAPDH* (Mean ± SD; n = 6). **(F)** *IRF7* transcript levels in SARS-CoV-2
628 infected or mock infected, and poly(I:C) transfected or mock transfected Calu-3 cells,
629 normalized to *GAPDH* (Mean ± SD; n = 6). **(G)** SARS-CoV-2 N, IFIT1 and GAPDH protein
630 expression in SARS-CoV-2 infected or mock infected, and poly(I:C) transfected or mock
631 transfected Calu-3 cells (Mean ± SD; n = 3). **(H)** *IRF7* transcript levels in SARS-CoV-2 infected
632 or mock infected, and recombinant IFNβ treated or mock treated Calu-3 cells, normalized to
633 *GAPDH* (Mean ± SD; n = 6). **(I)** *IFIT1* transcript levels in SARS-CoV-2 infected or mock
634 infected, and recombinant IFNβ treated or mock treated Calu-3 cells, normalized to *GAPDH*
635 (Mean ± SD; n = 6). (J) SARS-CoV-2 N, IFIT1 and GAPDH protein expression in SARS-CoV-2

636 infected or mock infected, and recombinant IFNβ treated or mock treated Calu-3 cells (Mean ±
637 SD; n = 3). Statistical significance was calculated using two-tailed paired t-test. Ct, cycle
638 threshold. p*<0.05, **<0.01, ***<0.001 and ****<0.0001.
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SI Appendix

Tables

Table S1. Mean raw read counts for SARS-CoV-2 genes. INF, SARS-CoV-2 infected; H, hours; SD, standard deviation.

Mean INF 0H	SD INF 0H	Mean INF 1H	SD INF 1H	Mean INF 2H	SD INF 2H	Mean INF 3H	SD INF 3H	Mean INF 6H	SD INF 6H	Mean INF 12H	SD INF 12H	SARS-CoV-2 gene	Transcript
257.67	38.59	285.33	56.13	243.67	39.25	278.00	23.00	12173.33	3006.93	25827.33	2054.93	ORF1ab	lcl|NC_045512.2_cds_YP_009724389.1_1
1.00	1.73	0.00	0.00	0.33	0.58	0.00	0.00	33.67	6.81	1061.00	468.03	ORF1a	lcl|NC_045512.2_cds_YP_009725295.1_2
500.67	94.52	491.33	86.19	378.00	61.39	521.67	49.69	19232.33	3952.46	26903.33	3860.82	spike	lcl|NC_045512.2_cds_YP_009724390.1_3
173.67	24.99	172.33	43.68	127.33	17.16	203.33	26.50	9995.00	1736.00	13976.33	2233.55	ORF3a	lcl|NC_045512.2_cds_YP_009724391.1_4
43.67	5.51	44.67	13.65	39.00	2.65	63.00	11.53	2903.33	485.15	4086.33	627.70	envelope	lcl|NC_045512.2_cds_YP_009724392.1_5
199.67	27.02	196.00	37.32	162.33	28.87	298.67	19.60	22344.33	3354.18	31200.33	4915.23	membrane	lcl|NC_045512.2_cds_YP_009724393.1_6
34.67	2.08	32.33	10.50	25.00	7.81	45.33	1.53	3508.00	509.12	4704.67	886.56	ORF6	lcl|NC_045512.2_cds_YP_009724394.1_7
107.33	19.50	102.33	23.35	94.00	22.61	173.67	34.00	14834.00	2357.53	21920.67	3441.71	ORF7a	lcl|NC_045512.2_cds_YP_009724395.1_8
10.33	2.52	11.67	2.31	15.33	1.53	20.67	1.15	1516.33	241.00	2191.33	526.17	ORF7b	lcl|NC_045512.2_cds_YP_009725318.1_9
109.33	22.19	107.00	27.22	98.00	21.70	189.00	14.00	14651.33	2136.80	21518.67	3992.04	ORF8	lcl|NC_045512.2_cds_YP_009724396.1_10
1251.00	230.97	1157.33	247.52	1067.67	144.58	2945.67	402.61	258553.00	34843.96	393221.67	62159.07	nucleocapsid	lcl|NC_045512.2_cds_YP_009724397.2_11
112.33	27.57	97.00	22.52	94.67	10.69	250.00	19.00	18385.33	2239.71	27679.00	5406.01	ORF10	lcl|NC_045512.2_cds_YP_009725255.1_12

Table S2. Mean normalized read counts for differentially expressed IFN and ISG transcripts. H, hour; INF, SARS-CoV-2 infected; MOCK, mock infected; IFN, interferon; ISG, interferon stimulated genes.

		0H INF (N=3)	**0H MOCK (N=3)**	**1H INF (N=3)**	**1H MOCK (N=3)**	**2H INF (N=3)**	**2H MOCK (N=3)**	**3H INF (N=3)**	**3H MOCK (N=3)**	**6H INF (N=3)**	**6H MOCK (N=3)**	**12H INF (N=3)**	**12H MOCK (N=3)**
IFNs	IFNB1	1.35	0.00	1.21	0.41	1.48	0.97	0.57	1.93	6.40	0.30	21.23	0.89
	IFNL1	3.49	3.45	2.20	4.80	4.93	5.46	3.17	1.90	7.00	2.66	15.07	0.73
	IFNL2	0.00	0.00	0.36	0.96	4.11	0.35	0.28	0.00	4.66	0.00	8.61	0.00
	IFNL3	0.35	0.00	0.58	0.44	2.38	0.31	0.88	0.00	3.02	0.00	8.46	0.00
ISGs	IFIT1	388.42	358.77	370.80	487.33	447.59	590.32	425.31	498.05	463.17	408.65	2790.57	367.50
	IRF7	278.50	283.73	320.43	284.00	339.99	383.89	399.07	363.67	399.93	432.29	966.54	305.31
	OAS2	172.67	236.24	178.18	222.85	287.61	208.20	252.85	296.10	292.36	378.90	2979.22	303.60
	MX1	588.48	620.75	624.79	647.52	758.95	800.13	839.47	867.29	728.29	811.68	3922.41	546.94
	RSAD2	204.76	216.53	228.73	272.67	313.84	348.31	365.12	393.68	274.53	269.56	948.75	210.54
	SLC44A4	1247.82	1171.72	1218.77	1046.17	1138.09	1128.19	1129.60	1106.06	1010.30	1142.19	1032.09	1298.09
	IFIH1	1052.81	1100.39	1134.78	1163.76	1235.36	1164.31	1223.66	1371.55	1189.70	1191.00	2492.69	1087.88
	GBP1	506.79	512.73	503.57	608.29	496.74	485.28	458.14	509.15	530.04	509.53	1151.35	488.92
	IFI44	689.16	741.40	789.19	803.61	963.68	1113.99	997.06	1052.67	785.42	782.39	1889.54	671.51
	IFI27	311.49	318.74	302.63	399.59	343.37	472.30	328.28	361.48	333.63	351.85	921.55	342.54
	IFI6	592.82	612.04	599.90	697.80	673.06	1010.20	692.26	752.25	729.19	775.17	2066.30	709.85
	ISG15	430.95	447.57	443.60	533.02	465.88	704.43	490.49	554.07	473.88	502.97	1260.48	435.91
	IFIT2	657.23	698.02	676.46	795.49	645.57	732.08	455.75	504.29	493.48	422.04	1465.16	413.27
	USP18	212.27	217.53	218.01	257.03	253.55	301.50	266.17	297.44	243.57	232.66	873.18	218.27
	IFIT3	648.15	656.89	747.61	858.17	810.13	1069.67	567.26	668.13	458.25	428.90	1900.07	420.64
	CMPK2	163.89	179.41	169.11	182.05	219.35	244.03	235.97	265.54	172.78	201.60	906.22	153.23
	XAF1	58.53	82.76	73.40	53.61	69.79	60.14	79.67	55.09	86.30	91.97	513.01	90.51
	IFITM1	27.68	34.25	21.94	27.89	28.53	53.49	26.88	34.91	34.59	35.75	182.01	34.33
	MX2	82.11	87.24	69.22	81.96	100.75	83.43	87.84	87.48	108.05	78.88	547.98	64.92

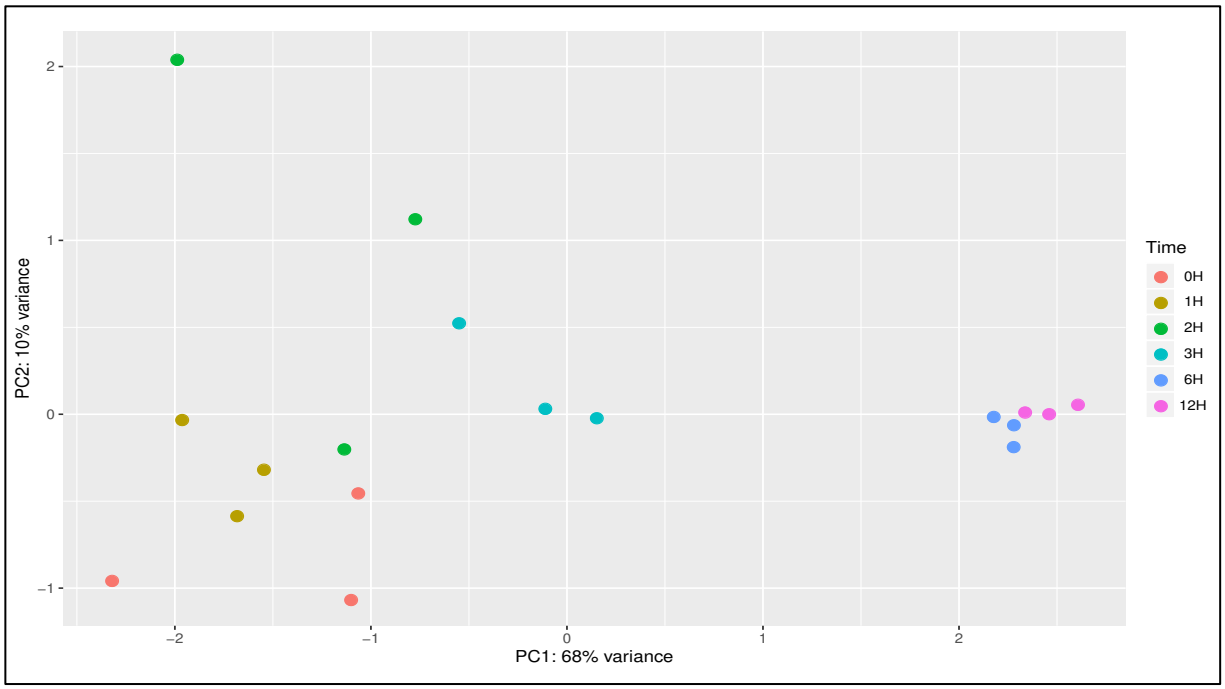
683 **Table S3.** Pathway enrichment analysis. Significance was determined after FDR correction. H,
684 hour; 0, non-significant; 1, significant.
685

term.id	term.name	adjusted.p.val	1H	2H	3H	12H
GO:0000976	transcription regulatory region sequence-specific DNA binding	0.004824255	0	1	0	0
GO:0001067	regulatory region nucleic acid binding	0.004203707	0	1	0	0
GO:0001816	cytokine production	0.005529472	0	0	0	1
GO:0001817	regulation of cytokine production	0.001829233	0	0	0	1
GO:0002230	positive regulation of defense response to virus by host	0.002197834	0	0	0	1
GO:0002831	regulation of response to biotic stimulus	8.60E-08	0	0	0	1
GO:0002833	positive regulation of response to biotic stimulus	0.008687053	0	0	0	1
GO:0003690	double-stranded DNA binding	0.000112873	0	1	0	0
GO:0003712	transcription coregulator activity	1.30E-06	0	1	0	0
GO:0003713	transcription coactivator activity	2.39E-05	0	1	0	0
GO:0005178	integrin binding	0.013874905	0	0	1	0
GO:0008270	zinc ion binding	0.000103938	0	1	0	0
GO:0009615	response to virus	1.39E-35	0	0	0	1
GO:0010810	regulation of cell-substrate adhesion	0.008350323	0	1	0	0
GO:0016482	cytosolic transport	0.011086056	0	1	0	0
GO:0019058	viral life cycle	3.92E-11	0	0	0	1
GO:0019079	viral genome replication	3.87E-15	0	0	0	1
GO:0019221	cytokine-mediated signaling pathway	8.45E-16	0	0	0	1
GO:0019900	kinase binding	0.003539788	0	1	0	0
GO:0019901	protein kinase binding	0.012867428	0	1	0	0
GO:0030099	myeloid cell differentiation	0.011382292	0	1	0	0
GO:0031347	regulation of defense response	2.16E-05	0	0	0	1
GO:0031589	cell-substrate adhesion	0.002867293	0	1	0	0
GO:0032020	ISG15-protein conjugation	0.008627708	0	0	0	1
GO:0032069	regulation of nuclease activity	1.26E-06	0	0	0	1
GO:0032479	regulation of type I interferon production	4.92E-06	0	0	0	1
GO:0032480	negative regulation of type I interferon production	0.005210998	0	0	0	1
GO:0032481	positive regulation of type I interferon production	0.00531473	0	0	0	1
GO:0032606	type I interferon production	6.14E-06	0	0	0	1
GO:0032607	interferon-alpha production	0.005237546	0	0	0	1
GO:0032647	regulation of interferon-alpha production	0.00400414	0	0	0	1
GO:0032727	positive regulation of interferon-alpha production	0.001567461	0	0	0	1
GO:0034340	response to type I interferon	9.21E-31	0	0	0	1
GO:0034341	response to interferon-gamma	1.44E-10	0	0	0	1
GO:0034504	protein localization to nucleus	0.00295333	0	1	0	0
GO:0035455	response to interferon-alpha	3.29E-10	0	0	0	1
GO:0035456	response to interferon-beta	2.08E-07	0	0	0	1

GO:0042393	histone binding	0.002987285	0	1	0	0
GO:0043900	regulation of multi-organism process	2.03E-17	0	0	0	1
GO:0043901	negative regulation of multi-organism process	3.86E-17	0	0	0	1
GO:0043902	positive regulation of multi-organism process	0.008274484	0	0	0	1
GO:0043903	regulation of symbiosis encompassing mutualism through parasitism	6.66E-20	0	0	0	1
GO:0044212	transcription regulatory region DNA binding	0.004047416	0	1	0	0
GO:0045069	regulation of viral genome replication	1.01E-16	0	0	0	1
GO:0045071	negative regulation of viral genome replication	3.61E-17	0	0	0	1
GO:0045088	regulation of innate immune response	5.98E-06	0	0	0	1
GO:0045089	positive regulation of innate immune response	0.005979802	0	0	0	1
GO:0046596	regulation of viral entry into host cell	0.048025337	0	0	0	1
GO:0048525	negative regulation of viral process	3.26E-20	0	0	0	1
GO:0050657	nucleic acid transport	0.048485615	0	0	1	0
GO:0050658	RNA transport	0.048485615	0	0	1	0
GO:0050688	regulation of defense response to virus	0.002163216	0	0	0	1
GO:0050691	regulation of defense response to virus by host	0.009541892	0	0	0	1
GO:0050792	regulation of viral process	1.31E-20	0	0	0	1
GO:0051056	regulation of small GTPase mediated signal transduction	0.026048495	0	1	0	0
GO:0051607	defense response to virus	1.25E-37	0	0	0	1
GO:0060333	interferon-gamma-mediated signaling pathway	1.36E-13	0	0	0	1
GO:0060337	type I interferon signaling pathway	3.69E-31	0	0	0	1
GO:0060700	regulation of ribonuclease activity	6.89E-07	0	0	0	1
GO:0060759	regulation of response to cytokine stimulus	0.000740173	0	0	0	1
GO:0060760	positive regulation of response to cytokine stimulus	0.007105564	0	0	0	1
GO:0061629	RNA polymerase II-specific DNA-binding transcription factor binding	0.011126656	0	1	0	0
GO:0070566	adenylyltransferase activity	0.006545402	0	0	0	1
GO:0071346	cellular response to interferon-gamma	1.05E-09	0	0	0	1
GO:0071357	cellular response to type I interferon	3.69E-31	0	0	0	1
GO:0098586	cellular response to virus	0.0037813	0	0	0	1
GO:1903900	regulation of viral life cycle	1.50E-18	0	0	0	1
GO:1903901	negative regulation of viral life cycle	1.15E-18	0	0	0	1
GO:1990837	sequence-specific double-stranded DNA binding	0.002945526	0	1	0	0
GO:2001251	negative regulation of chromosome organization	0.039979672	0	1	0	0
REAC:R-HSA-1169408	ISG15 antiviral mechanism	5.61E-12	0	0	0	1
REAC:R-HSA-1169410	Antiviral mechanism by IFN-stimulated genes	5.77E-19	0	0	0	1
REAC:R-HSA-1280215	Cytokine Signaling in Immune system	1.52E-19	0	0	0	1
REAC:R-HSA-168928	DDX58/IFIH1-mediated induction of interferon-alpha/beta	0.001851135	0	0	0	1
REAC:R-HSA-2990846	SUMOylation	0.000289223	0	1	0	0
REAC:R-HSA-3108214	SUMOylation of DNA damage response and repair proteins	0.023406467	0	1	0	0
REAC:R-HSA-	SUMO E3 ligases SUMOylate target proteins	0.000850049	0	1	0	0

3108232						
REAC:R-HSA-3247509	Chromatin modifying enzymes	0.016088428	0	1	0	0
REAC:R-HSA-4839726	Chromatin organization	0.016088428	0	1	0	0
REAC:R-HSA-6806834	Signaling by MET	2.89E-05	0	1	0	0
REAC:R-HSA-877300	Interferon gamma signaling	2.97E-09	0	0	0	1
REAC:R-HSA-8874081	MET activates PTK2 signaling	0.000994797	1	0	0	0
REAC:R-HSA-8934593	Regulation of RUNX1 Expression and Activity	0.000745328	0	1	0	0
REAC:R-HSA-8983711	OAS antiviral response	3.29E-08	0	0	0	1
REAC:R-HSA-9006934	Signaling by Receptor Tyrosine Kinases	0.017643755	0	1	0	0
REAC:R-HSA-909733	Interferon alpha/beta signaling	2.97E-31	0	0	0	1
REAC:R-HSA-913531	Interferon Signaling	4.75E-36	0	0	0	1
REAC:R-HSA-918233	TRAF3-dependent IRF activation pathway	0.000139967	0	0	0	1
REAC:R-HSA-933541	TRAF6 mediated IRF7 activation	0.018776243	0	0	0	1
REAC:R-HSA-936440	Negative regulators of DDX58/IFIH1 signaling	0.000931238	0	0	0	1

710 # Figures
711



712
713 **Figure S1. SARS-CoV-2 transcripts clustering.** To determine SARS-CoV-2 replication
714 kinetics in human cells using RNA-seq, we infected human lung epithelial cells (Calu-3) at a
715 multiplicity of infection (MOI) of 2. One hour post incubation, virus inoculum was replaced with
716 cell growth media and the clock was set to zero hours. We extracted and sequenced poly-A
717 enriched RNA at 0, 1, 2, 3, 6 and 12 hours post infection (hpi). SARS-CoV-2 genome, sub-
718 genomic RNA and transcripts were detected in infected samples. PCA clustering was performed
719 on quantified SARS-CoV-2 transcript levels in infected samples across time-points. Axes labels
720 indicate the proportion of between-samples variance explained by the first two principal
721 components. H, hours post infection.
722
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726

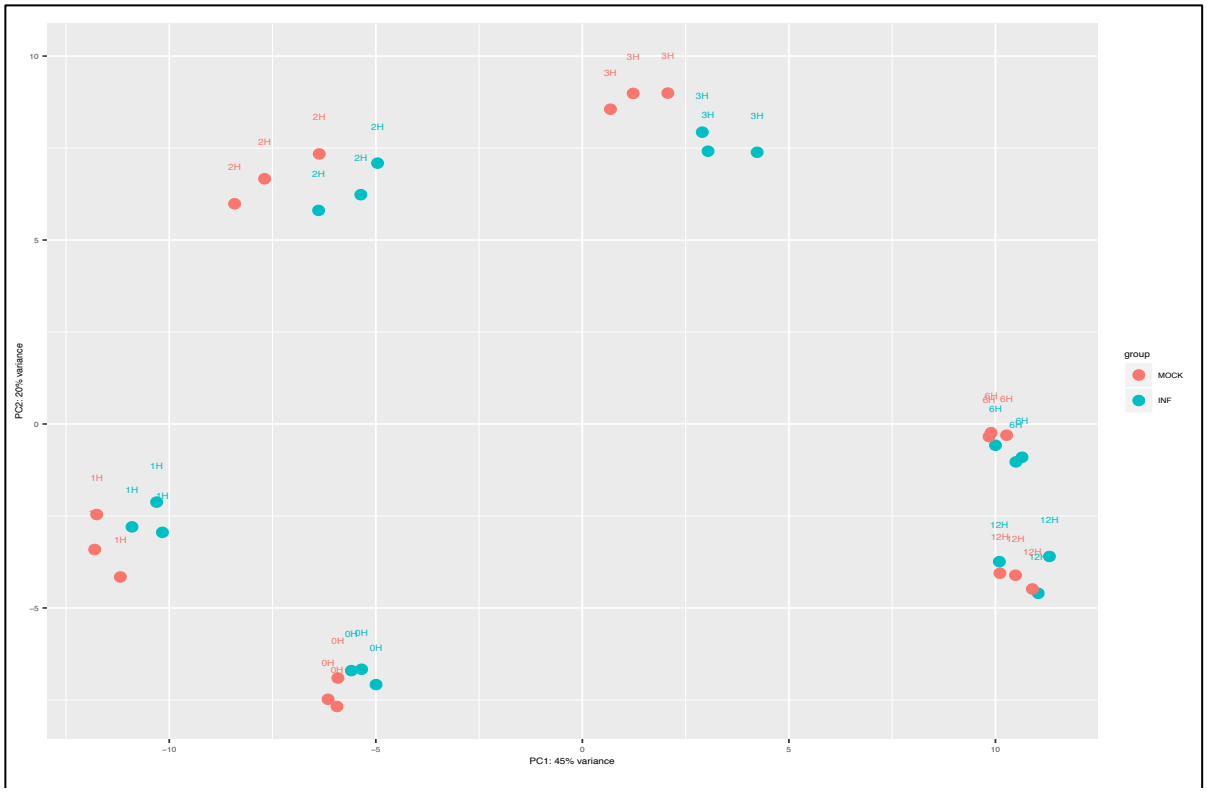

Figure S2. Host-cell transcripts clustering. To determine gene expression in human cells using RNA-seq, we infected human lung epithelial cells (Calu-3) at a multiplicity of infection (MOI) of 2. One hour post incubation, virus inoculum was replaced with cell growth media and the clock was set to zero hours. We extracted and sequenced poly-A enriched RNA at 0, 1, 2, 3, 6 and 12 hours post infection (hpi). PCA clustering was performed on quantified and filtered host gene transcripts in both SARS-CoV-2 infected (blue) and mock infected (red) samples across time-points (indicated in text for each data-point). Axes labels indicate the proportion of between-samples variance explained by the first two principal components. H, hours post infection; Mock, mock infected; INF, SARS-CoV-2 infected.

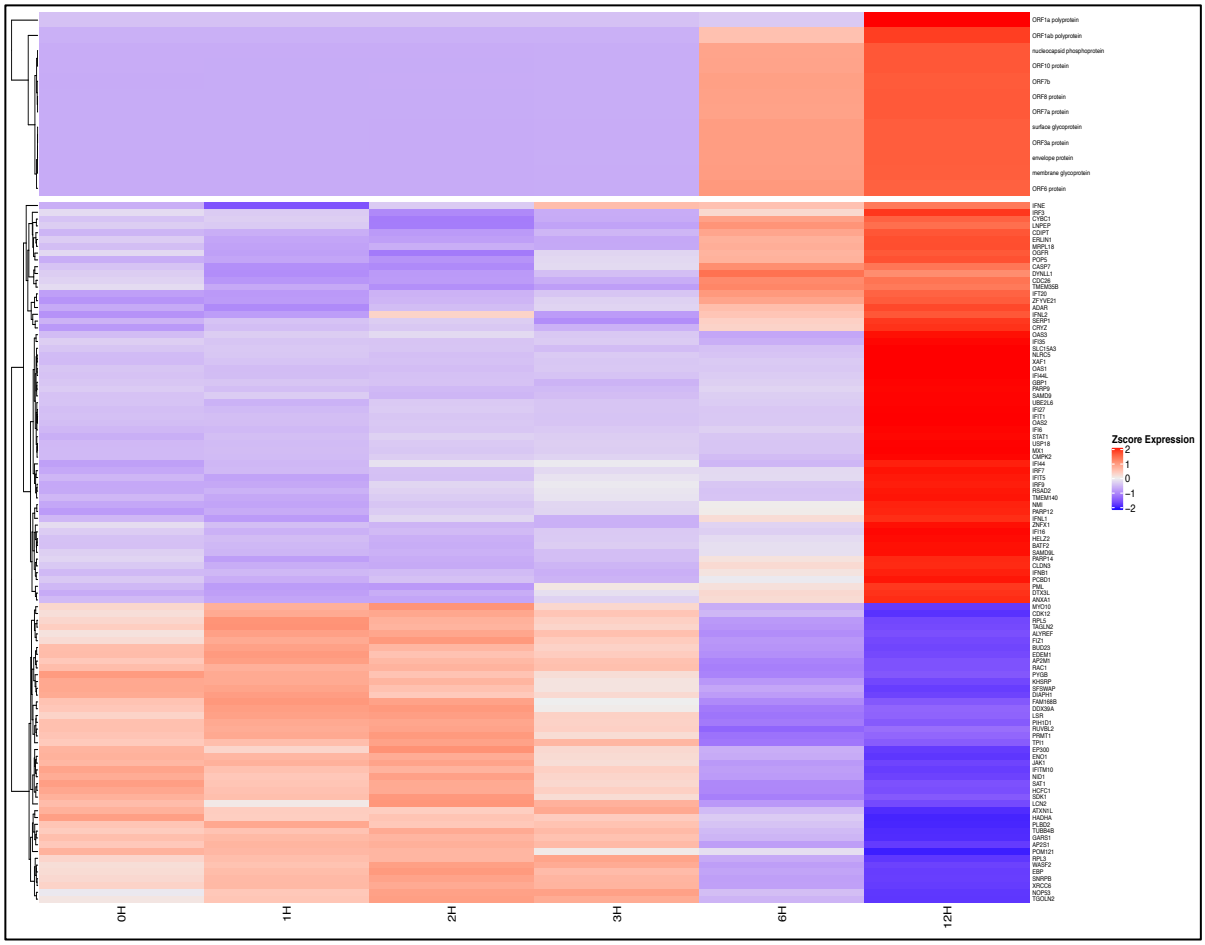

Figure S3. Virus-host correlated transcriptional profiles. Host gene expression that correlated with one or more viral transcripts over the course of infection are shown as z-score normalized expression (bottom), along with viral transcripts (top). Top 100 strongly-correlated genes are represented here. H, hour.

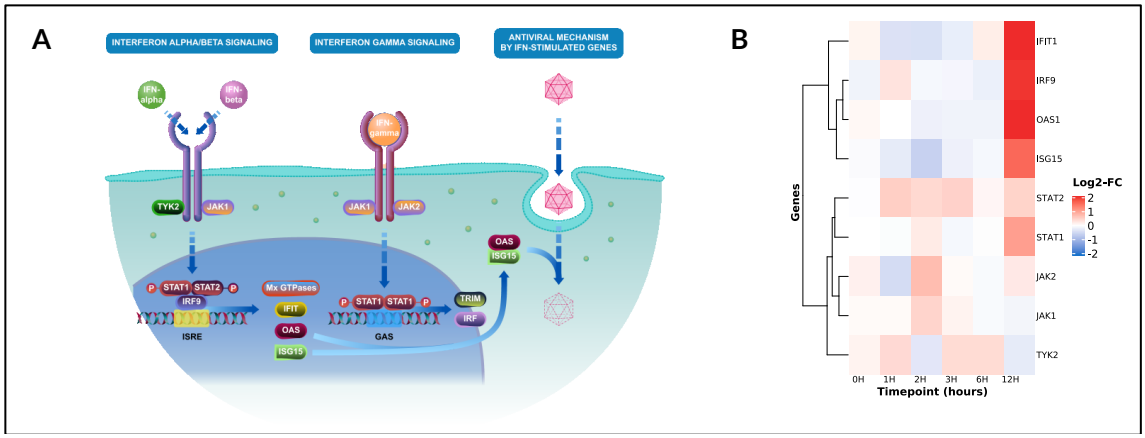

Figure S4. Cytokine signaling in immune system (REAC:R-HSA-1280215). **(A)** Pathway schematic of REACTOME cytokine signalling pathway involving interferon alpha/beta/gamma signalling, and OAS antiviral response mediated by interferon stimulated genes. **(B)** Heatmap of genes within REACTOME cytokine signalling pathway and their $\log_2$ transformed fold-change (FC) between SARS-CoV-2 infected and mock infected samples across all timepoints (0, 1, 2, 3, 6, 12 hrs). H, hours.

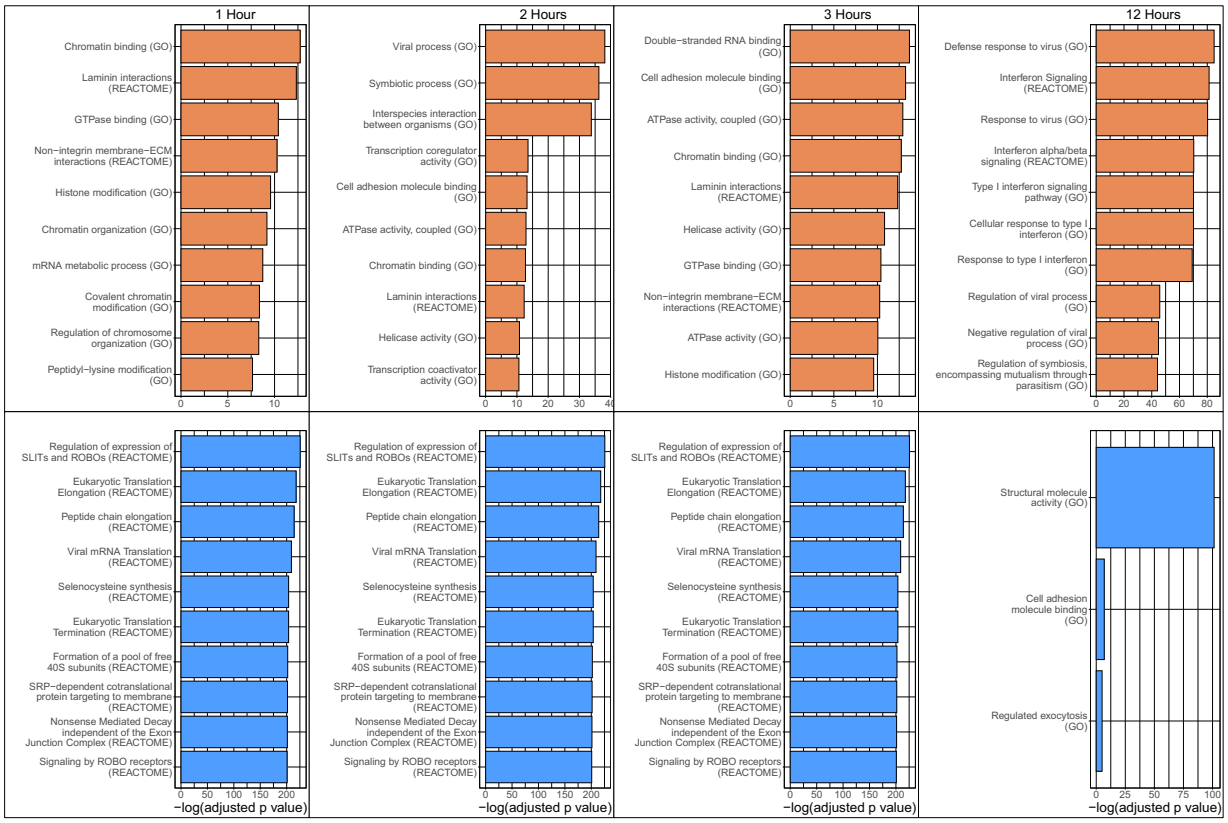

Figure S5. Top functional enrichments over time. Top significantly (adjusted p<0.05) enriched ActivePathway GO terms and REACTOME enrichments for infection vs. mock at 1, 2, 3 and 12 hrs post infection with SARS-CoV-2. Orange bars represent enriched terms associated with genes upregulated in infection vs. mock. Blue bars represent enriched terms associated with genes downregulated in infection vs. mock. 0 and 6 hr time points were omitted due to lack of sufficient numbers of differentially expressed genes.

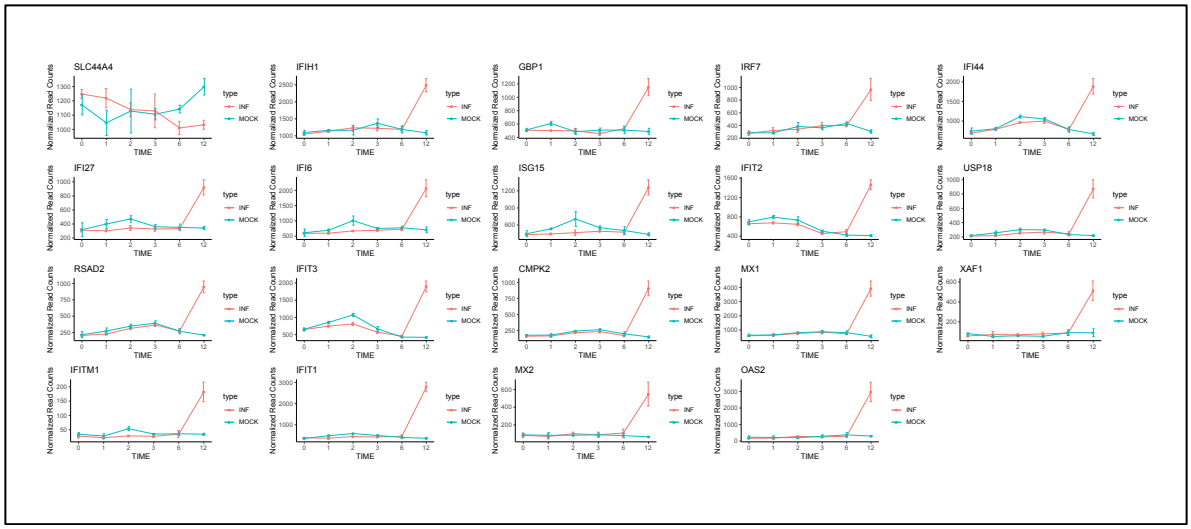

Figure S6. Infection-responsive gene expression profiles for ISGs. ISGs with significantly different levels of transcript expression between mock (blue) and SARS-CoV-2 infected (red) samples at 12 hpi are shown. Normalized read counts per gene, across six time-points are represented here. Time indicated is in hours. Mock, mock infected; INF, SARS-CoV-2 infected.

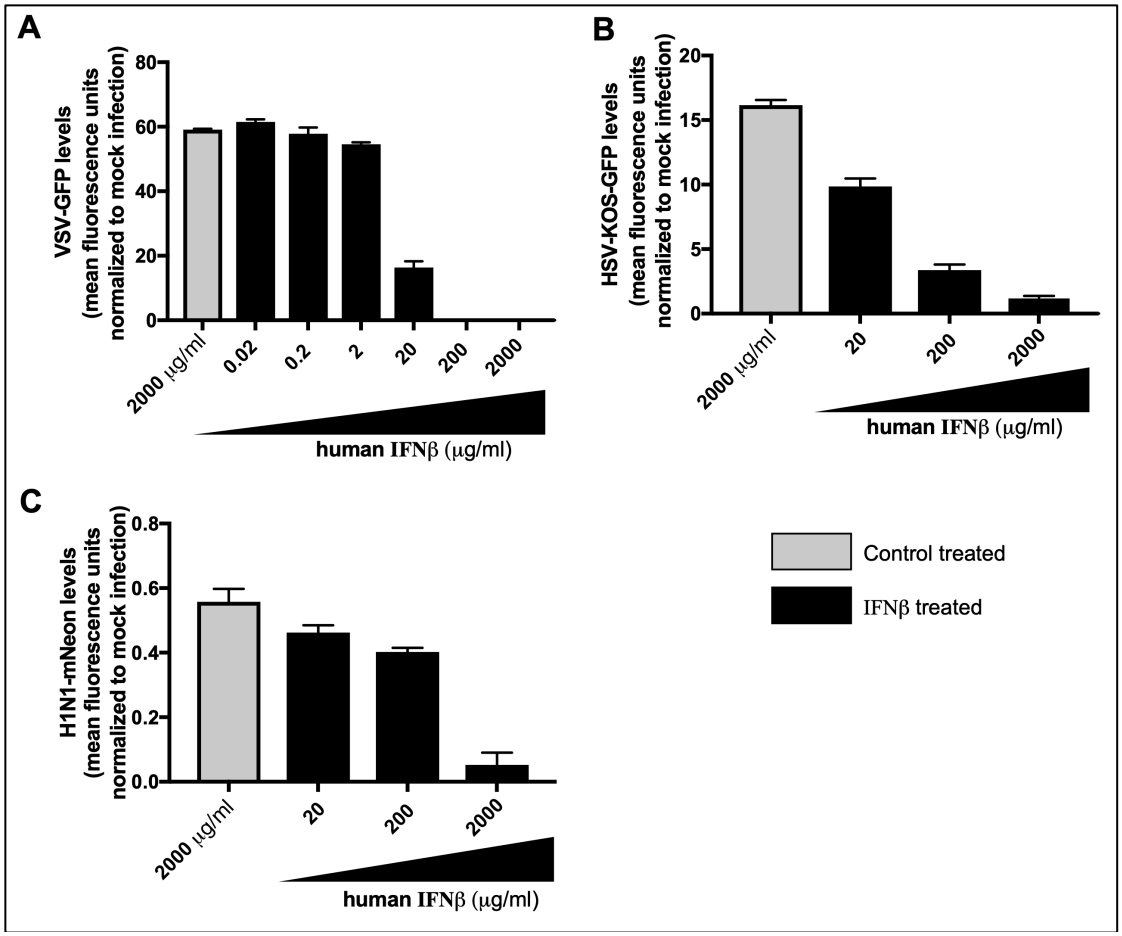

Figure S7. Recombinant human IFNβ1 inhibits VSV, HSV and H1N1 replication. Human fibroblast (THF) cells were treated with increasing concentrations of recombinant human IFNβ1 or mock treated with GFP containing media (control) for 6 hrs. Cells were then infected with vesicular stomatitis virus (VSV-GFP), herpes simplex virus (HSV-KOS-GFP) or H1N1 influenza virus (H1N1-mNeon). VSV and HSV were engineered to express green fluorescent protein (GFP). H1N1 expressed mNeon that is detectable in the same wavelength as GFP. Nineteen hours post infection, GFP/mNeon levels were measured in mock infected and virus infected cells as a surrogate for virus replication. **(A)** VSV-GFP replication in THF cells treated or mock treated with IFNβ1, normalized to mock infection (Mean±SD; n=3). **(B)** HSV-KOS-GFP replication in THF cells treated or mock treated with IFNβ1, normalized to mock infection (Mean±SD; n=3). **(C)** H1N1-mNeon replication in THF cells treated or mock treated with IFNβ1, normalized to mock infection (Mean±SD; n=3).