SARS-CoV-2 is sensitive to type I interferon pretreatment.

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Article Summary: SARS-CoV-2 has similar replication kinetics to SARS-CoV, but demonstrates significant sensitivity to type I interferon treatment.

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Abstract

SARS-CoV-2, a novel coronavirus (CoV), has recently emerged causing an ongoing outbreak of viral pneumonia around the world. While genetically distinct from the original SARS-CoV, both group 2B CoVs share similar genome organization and origins to coronaviruses harbored in bats. Importantly, initial guidance has used insights from SARS-CoV infection to inform treatment and public health strategies. In this report, we evaluate type-I Interferon (IFN-I) sensitivity of SARS-CoV-2 relative to the original SARS-CoV. Our results indicate that while SARS-CoV-2 maintains similar viral replication kinetics to SARS-CoV in Vero cell, the novel CoV is much more sensitive to IFN-I pretreatment. Examining transcriptional factor activation and interferon stimulated gene (ISG) induction, SARS-CoV-2 in the context of type I IFN induces phosphorylation of STAT1 and increased ISG proteins. In contrast, the original SARS-CoV has no evidence for STAT1 phosphorylation or ISG protein increases even in the presence of type I IFN pretreatment. Finally, we examined homology between SARS-CoV and SARS-CoV-2 in viral proteins shown to be interferon antagonist. The absence of open reading frame (ORF) 3b and significant changes to ORF6 suggest the two key IFN antagonists may not maintain equivalent function in SARS-CoV-2. Together, the results identify key differences in susceptibility to the IFN-I response between SARS-CoV and SARS-CoV-2. that could help inform disease progression, treatment options, and animal model development.

Importance

With the ongoing outbreak of COVID-19 disease, differences between the SARS-CoV-2 and the original SARS-CoV could be leveraged to inform disease progression and eventual treatment options. In addition, these findings could have key implications for animal model development as well as further research into how SARS-CoV-2 modulates the type I IFN response early during infection.

26 **Introduction**

27 At the end of 2019, a cluster of patients in Hubei Province, China was diagnosed with a
28 viral pneumonia of unknown origins. With community links to the Hunnan seafood market in
29 Wuhan, the disease cluster had echoes of the severe acute respiratory syndrome coronavirus
30 (SARS-CoV) outbreak that emerged at the beginning of the century [1]. The 2019 etiologic agent
31 was identified as a novel coronavirus, 2019-nCoV, and subsequently renamed SARS-CoV-2 [2].
32 The new virus has nearly 80% nucleotide identity to the original SARS-CoV and the
33 corresponding CoV disease, COVID-19, has many of the hallmarks of SARS-CoV disease
34 including fever, breathing difficulty, bilateral lung infiltration, and death in the most extreme
35 cases [3,4]. In addition, the most severe SARS-CoV-2 disease corresponded to old age (>50
36 years old), health status, and health care workers, similar to both SARS and MERS-CoV [5].
37 Together, the results indicate SARS-CoV-2 infection and disease have strong similarity to the
38 original SARS-CoV epidemic occurring nearly two decades earlier.

39 In the wake of the outbreak, major research efforts have sought to rapidly characterize
40 the novel CoV to aid in treatment and control. Initial modeling studies predicted [6] and
41 subsequent cell culture studies confirmed that spike protein of SARS-CoV-2 utilizes human
42 angiotensin converting enzyme 2 (ACE2) for entry, the same receptor as SARS-CoV [7,8].
43 Extensive case studies indicated a similar range of disease onset and severe symptoms seen
44 with SARS-CoV [5]. Notably, less severe SARS-CoV-2 cases have also been observed and were
45 not captured in the original SARS-CoV outbreak. Importantly, screening and treatment guidance
46 has relied on previous CoV data generated with SARS-CoV and MERS-CoV. Treatments with
47 both protease inhibitors and type I interferon (IFN-I) have been employed [4]; similarly, remdesivir,
48 a drug targeting viral polymerases, has been reported to have efficacy against SARS-CoV-2
49 similar to findings with both SARS- and MERS-CoV [9-12]. Importantly, several vaccine efforts
50 have been initiated with a focus on the SARS-CoV-2 spike protein as the major antigenic

51 determinate [13]. Together, the similarities with SARS-CoV have been useful in responding to the
52 newest CoV outbreak.

53 The host Innate immune response is initiated when viral products are recognized by host
54 cell pattern recognition receptors, including Toll-like receptors (TLRs) and RIG-I-like receptors
55 (RLRs) [14,15]. This response ultimately results in production of IFN-I and other cytokines, which
56 together are essential for an effective antiviral response [16]. IFN-I then triggers its own signaling
57 cascade via its receptor, in autocrine or paracrine manner, which induces phosphorylation of
58 signal transducers and activators of transcription 1 (STAT1) and STAT2. Together, STAT1,
59 STAT2, and a third transcription factor, IRF9, form the Interferon Stimulated Gene Factor 3
60 (ISGF3) complex, which is essential for induction of many IFN-stimulated genes (ISGs), and
61 ultimately and effective antiviral response [17,18]. To establish productive replication, viruses have
62 developed different mechanisms to escape this antiviral response targeting different parts of the
63 IFN-I response machinery [19].

64 In this study, we further characterize SARS-CoV-2 and compare it to the original SARS-
65 CoV. Using Vero E6 cells, we demonstrate that SARS-CoV-2 maintains similar viral replication
66 kinetics as SARS-CoV following a low dose infection. In contrast, we find that SARS-CoV-2 is
67 much more sensitive to IFN-I pretreatment as compared to SARS-CoV. Examining further, we
68 determined that SARS-CoV-2 induces STAT1 phosphorylation and ISG expression, which is
69 absent in SARS-CoV. These results suggest distinct changes between the CoVs in terms of IFN
70 antagonism and we subsequently examined sequence homology between the SARS-CoV and
71 SARS-CoV-2 viral proteins that may be responsible for these differences. Together, the results
72 suggest SARS-CoV-2 lacks the same capacity to control the IFN-I response as SARS-CoV.

73

74 **Results**

75 Our initial studies infected Vero E6 cells using a low multiplicity of infection (MOI) to
76 explore the viral replication kinetics of SARS-CoV-2 relative to SARS-CoV. Following infection,
77 we find that both SARS-CoV and SARS-CoV-2 replicate with similar kinetics, peaking 48 hours
78 post infection (**Fig. 1A**). While SARS-CoV-2 titer had slightly lower viral titers at 24 hours post
79 infection, the results were statistically different between the novel CoV and the original epidemic
80 strain. By 48 hours, replication of both viruses had plateaued and significant cytopathic effect
81 (CPE) was observed for both SARS-CoV and SARS-CoV-2 infections. Together, the results
82 indicated that SARS-CoV and SARS-CoV-2 replicate with similar replication kinetics in Vero E6
83 cells.

84 We next evaluated the susceptibility of SARS-CoV-2 to IFN-I pretreatment. Treatment
85 with IFN-I (recombinant IFNα) has been attempted as an antiviral approach for a wide variety of
86 pathogens including hepatitis B and C viruses as well as HIV [20]. During both the SARS and
87 MERS-CoV outbreaks, IFN-I has been employed with limited effect [21,22]. In this study, we
88 pretreated Vero E6 cells with 1000 units of recombinant IFN-I (IFN-α) 18 hours prior to infection.
89 Vero E6 lack the capacity to produce IFN-I, but are able to respond to exogenous treatment [23].
90 Following pretreatment with IFN-I, SARS-CoV infection has a modest reduction in viral titer (1.5
91 log plaque forming units (PFU) as compared to untreated control 24 hours post infection (**Fig.
92 1B**). However, by 48 hours, SARS-CoV has nearly equivalent viral yields as the untreated
93 conditions (7.2 log PFU versus 7.5 log PFU). In contrast, SARS-CoV-2 shows a significant
94 reduction in viral replication following IFN-I treatment. At both 24 and 48 hours post infection,
95 SARS-CoV-2 had massive 3-log (24 HPI) and 4-log (48 HPI) drops in viral titer as compared to
96 control untreated cells. Finally, we examined viral protein production finding a major deficit in
97 nucleocapsid protein production in IFN-I treated cells following SARS-CoV-2 infection (**Fig. 1C**).
98 In contrast, viral proteins were robustly expressed for SARS-CoV-2 in untreated cells and for

99 SARS-CoV in both conditions. Together, the results demonstrate a clear sensitivity to a primed
100 IFN-I response in SARS-CoV-2, which is not observed with SARS-CoV.

101 **SARS-CoV-2 fails to attenuate STAT1 phosphorylation and ISG production.**

102 To explore differences in IFN-I antagonism between SARS-CoV and SARS-CoV, we examined
103 both STAT1 activation and IFN stimulated gene (ISG) expression following IFN pretreatment
104 and infection. Examining Vero cell protein lysates, we found that IFN-I treated cells infected with
105 SARS-CoV-2 induced phosphorylated STAT-1 by 48 hours post infection (**Fig. 2**). STAT1
106 phosphorylation was absent in untreated cells infected with SARS-CoV-2 and suggest the novel
107 CoV is unable to inhibit a IFN-I preprimed response. In contrast, SARS-CoV had no evidence
108 for STAT1 phosphorylation in either IFN-I treated or untreated cells, illustrating robust control
109 over IFN-I induction pathways. Examining further, STAT1, IFIT2, and TRIM25, known ISGs [17],
110 had increased protein expression in the context of SARS-CoV-2 infection following IFN
111 pretreatment (**Fig. 2**). Basal STAT1 and TRIM25 levels are reduced during SARS-CoV and
112 SARS-CoV-2 infection relative to control likely due to the mRNA targeting activity of non-
113 structural protein 1 (NSP1) [24]. However, IFN-I treatment results in augmented protein levels for
114 both ISGs following SARS-CoV-2 infection as compared to untreated control. In contrast, IFN
115 treated SARS-CoV had no significant increase in ISG protein relative to control infection.
116 Together, the STAT1 phosphorylation, ISG production, and viral protein levels indicate that
117 SARS-CoV-2 lacks the same capacity to modulate a primed type I IFN response as the original
118 SARS-CoV.

119 **Conservation of IFN antagonists across SARS-CoV and SARS-CoV-2**

120 Considering the sensitivity to IFN-I, we next sought to evaluate changes between SARS-CoV
121 and SARS-CoV-2 viral proteins. Previous work has established several key IFN antagonist in
122 the SARS-CoV genome including NSP1, NSP3, ORF3b, ORF6, and others [25]. Therefore, we

123 compared the sequence homology across viral proteins from SARS-CoV, SARS-CoV-2, and
124 several bat SARS-like viruses including WIV16-CoV [26], SHC014-CoV [27], and HKU3.1-CoV [28].
125 Using sequence analysis, we found several changes to SARS-CoV-2 that potentially contribute
126 to its type I IFN sensitivity (**Fig. 3**). For SARS-CoV structural proteins including the nucleocapsid
127 (N) and matrix (M) protein, a high degree of sequence homology (>90%AA identity) suggests
128 that their reported IFN antagonism is likely maintained in SARS-CoV-2 and other SARS-like
129 viruses. Similarly, the ORF1ab poly-protein retains high sequence identity in SARS-CoV-2 and
130 several known antagonists contained within the poly-protein (NSP1, NSP7, NSP14-16) are
131 highly conserved relative to SARS-CoV. One notable exception is the large papain-like
132 proteases, NSP3, which only 76% conserved between SARS-CoV and SARS-CoV-2. However,
133 SARS-CoV-2 does maintain a deubiquitinating domain thought to confer IFN resistance [29]. For
134 SARS-CoV ORF3b, a 154 amino acid (AA) protein known to antagonize the type I IFN
135 responses by blocking IRF3 phosphorylation [30], sequence alignments indicates that the SARS-
136 CoV-2 equivalent ORF3b contains a premature stop codon resulting in a truncated 24 AA
137 protein. Similarly, HKU3.1-CoV also has a premature termination resulting in a predicted 39 AA
138 protein. Both WIV16-CoV and SHC014-CoV, the most closely related bat viruses to SARS-
139 CoV, encode longer 114 AA truncated protein with >99% homology with SARS-CoV ORF3b
140 suggesting that IFN antagonism might be maintained in these specific group 2B CoV strains. In
141 addition, SARS-CoV ORF6 has been shown to be an IFN antagonist that disrupts karyopherin
142 transportation of transcriptions factors like STAT1 [30,31]. In contrast to ORF3b, all five surveyed
143 group 2B CoVs maintain ORF6; however, SARS-CoV-2 had only 69% homology with SARS-
144 CoV while the other three group 2B bat CoVs had >90% conservation. Importantly, SARS-CoV-
145 2 has a two amino acid truncation in its ORF6; previous work has found that alanine substitution
146 in this C-terminal of SARS-CoV ORF6 resulted in ablated antagonism [31]. Together, the
147 sequence homology analysis suggests that differences in NSP3, ORF3b, and/or ORF6 may be
148 key drivers of SARS-CoV-2 type I IFN susceptibility.

149 **Discussion**

150 With the ongoing outbreak of COVID-19 caused by SARS-CoV-2, viral characterization remains
151 a key factor in responding to the emergent novel virus. In this report, we describe differences in
152 the IFN-I sensitivity between SARS-CoV-2 and the original SARS-CoV. While both viruses
153 maintain similar replication in untreated Vero E6 cells, SARS-CoV-2 has a significant decrease
154 in viral protein and replication following IFN-I pretreatment. The decreased SARS-CoV-2
155 replication correlates with phosphorylation of STAT1 and augmented ISG expression largely
156 absent following SARS-CoV infection. This sensitivity to IFN-I is distinct from the original SARS-
157 CoV and suggests that the novel CoV has distinct host interactions driving disease outcomes.
158 Analysis of viral proteins finds SARS-CoV-2 has several changes that potentially impact its
159 capacity to modulate the type I IFN response, including loss of ORF3b and a short truncation of
160 ORF6, both known as IFN-I antagonists for SARS-CoV [30]. Together, our results suggest SARS-
161 CoV and SARS-CoV-2 have differences in their ability to antagonize the IFN-I response once
162 initiated and that they may have major implication for COVID-19 disease and treatment.

163 With a similar genome organization and disease symptoms in humans, the SARS-CoV-2
164 outbreak has drawn insights from the closely related SARS-CoV. However, the differences in
165 sensitivity to IFN-I pretreatment illustrate a clear distinction between the two CoVs. Coupled with
166 a novel furin cleavage site [32], robust upper airway infection [8], and potential transmission prior to
167 symptomatic disease [33], the differences between SARS-CoV and SARS-CoV-2 could prove
168 important in disrupting the ongoing spread of COVID-19. For SARS-CoV, *in vitro* studies have
169 consistently found that wild-type SARS-CoV is indifferent to IFN-I pretreatment [34,35]. Similarly, *in*
170 *vivo* SARS-CoV studies have found that the loss of IFN-I signaling had no significant impact on
171 disease [36], suggesting that this virus is not sensitive to the antiviral effects of IFN-I. However,
172 more recent reports suggest that host genetic background may majorly influence this finding [37].
173 For SARS-CoV-2, our results suggest that IFN-I pretreatment produces a 3 - 4 log drop in viral

174 titer and correlates to STAT1 phosphorylation. This level of sensitivity is similar to MERS-CoV
175 and suggests that the novel CoV lacks the same capacity to escape a primed IFN-I response as
176 SARS-CoV [38,39]. Notably, the sensitivity to IFN-I does not completely ablate viral replication;
177 unlike SARS-CoV 2'O methyl-transferase mutants [34], SARS-CoV-2 is able to replicate to low,
178 detectable levels even in the presence of IFN-I. This finding could help explain positive test in
179 patients with minimal symptoms and the range of disease observed. In addition, while SARS-
180 CoV-2 is sensitive to IFN-I pretreatment, both SARS-CoV and MERS-CoV employ effective
181 means to disrupt virus recognition and downstream signaling until late during infection [40]; a
182 similar mechanism may also be employed by SARS-CoV, diminishing the overall effect of IFN-I
183 during infection.

184 For SARS-CoV-2, the sensitivity to IFN-I indicates a distinction from SARS-CoV and
185 suggests differential host innate immune modulation between the viruses. The loss of ORF3b
186 and truncation/changes in ORF6 could signal a reduced capacity of SARS-CoV-2 to interfere
187 with type I IFN responses. For SARS-CoV ORF6, the N-terminal domain has been shown to
188 have a clear role in its ability to disrupt karyopherin transport [31]; in turn, the loss of ORF6
189 function for SARS-CoV-2 would likely render it much more susceptible to IFN-I pretreatment as
190 activated STAT1 has the capacity to enter the nucleus and induce ISGs and the antiviral
191 response. In these studies, we have found that following IFN-I pretreatment, STAT1
192 phosphorylation is induced following SARS-CoV-2 infection. The increase in ISG proteins
193 (STAT1, IFIT2, TRIM25) suggests that SARS-CoV-2 ORF6 does not effectively block nuclear
194 transport as well as SARS ORF6. For SARS-CoV ORF3b, the viral protein has been shown to
195 disrupt phosphorylation of IRF3, a key transcriptional factor in the induction of IFN-I and the
196 antiviral state [30]. While its mechanism of action is not clear, the ORF3b absence in SARS-CoV-2
197 infection likely impacts its ability to inhibit the IFN-I response and eventual STAT1 activation.
198 Similarly, while NSP3 deubiquitinating domain remains intact, SARS-CoV-2 has a 24 AA

199 insertion upstream of this deubiquitinating domain that could potentially alter that function [29].
200 While other antagonists are maintained with high levels of conservation (>90%), single point
201 mutations in key locations could modify function and contribute to increased IFN sensitivity.
202 Overall, the sequence analysis suggests that differences between SARS-CoV and SARS-CoV-2
203 viral proteins may drive attenuation in the context of type I IFN pretreatment.

204 The increased sensitivity of SARS-CoV-2 suggests utility in treatment using type I IFN.
205 While IFN-I has been used in response to chronic viral infection [41], previous examination of
206 SARS-CoV cases found inconclusive effect for type I IFN treatment [42]. However, the findings
207 from the SARS-CoV outbreak were complicated by combination therapy of type I IFN with other
208 treatments including ribavirin/steroids and lack of a regimented protocol. While type I IFN has
209 been utilized to treat MERS-CoV infected patients, no conclusive data yet exists to determine
210 efficacy [43]. Yet, *in vivo* studies with MERS-CoV has found that early induction with type I IFN
211 can be protective in mice [44]; importantly, the same study found that late type I IFN induction can
212 be detrimental for MERS-CoV disease [44]. Similarly, early reports have described treatments
213 using type I IFN in combination for SARS-CoV-2 infection; yet the efficacy of these treatments
214 and the parameters of their use are not known [45]. Overall, sensitivity data suggest that type I
215 IFN treatment may have utility for treating SARS-CoV-2 if the appropriate parameters can be
216 determined. In addition, use of type III IFN, which is predicted to have utility in the respiratory
217 tract, could offer another means for effective treatment for SARS-CoV-2.

218 In addition to treatment, the sensitivity to type I IFN may also have implications for
219 animal model development. For SARS-CoV, mouse models that recapitulate human disease
220 were developed through virus passage in immune competent mice [46]. Similarly, mouse models
221 for MERS-CoV required adaptation in mice that had genetic modifications of their dipeptidyl-
222 peptidase 4 (DPP4), the receptor for MERS-CoV [47,48]. However, each of these MERS-CoV
223 mouse models still retained full immune capacity. In contrast, SARS-CoV-2 sensitivity to type I

224 IFN may signal the need to use an immune deficient model to develop relevant disease. While
225 initial work has suggested incompatibility to SARS-CoV-2 infection in mice based on receptor
226 usage [8], the type I IFN response may be a second major barrier that needs to be overcome.
227 Similar to the emergent Zika virus outbreak, the use of type I IFN receptor knockout mice or
228 type I IFN receptor blocking antibody may be necessary to develop a useful SARS-CoV-2
229 animal models for therapeutic testing [49].

230 Overall, our results indicate that SARS-CoV-2 has a much higher sensitivity to type I IFN
231 than the previously emergent SARS-CoV. This augmented type I IFN sensitivity is likely due to
232 changes in viral proteins between the two epidemic CoV strains. Moving forward, these data
233 could provide important insights for both the treatment of SARS-CoV-2 as well as developing
234 novel animal models of disease. In this ongoing outbreak, the results also highlight a distinction
235 between the highly related viruses and suggest insights from SARS-CoV must be verified for
236 SARS-CoV-2 infection and disease.

237

238 **Methods**

239 **Viruses and cells.** SARS-CoV-2 USA-WA1/2020, provided by the World Reference Center for
240 Emerging Viruses and Arboviruses (WRCEVA) and was originally obtained from the USA
241 Centers of Disease Control as described[50]. SARS-CoV-2 and mouse-adapted recombinant
242 SARS-CoV (MA15) [46] were titrated and propagated on VeroE6 cells, grown in DMEM with 5%
243 fetal bovine serum and 1% antibiotic/antimytotic (Gibco). Standard plaque assays were used for
244 SARS-CoV and SARS-CoV-2 [51,52]. All experiments involving infectious virus were conducted at
245 the University of Texas Medical Branch (Galveston, TX) in approved biosafety level 3 (BSL)
246 laboratories with routine medical monitoring of staff.

247 **Infection and type I IFN pretreatment.** Viral replication in Vero E6 were performed as
248 previously described [34,53]. Briefly, cells were washed with two times with PBS and inoculated
249 with SARS-CoV or SARS-CoV-2 at an multiplicity of infection (MOI) 0.01 for 60 minutes at 37
250 °C. Following inoculation, cells were washed 3 times, and fresh media was added to signify time
251 0. Three or more biological replicates were harvested at each described time point and results
252 are from combination of two experiments. No blinding was used in any sample collections, nor
253 were samples randomized. For type I IFN pretreatment, experiments were completed as
254 previously described [34]. Briefly, Vero E6 cells were incubated with 1000 units/mL of
255 recombinant type I IFN alpha (PBL Assay Sciences) 18 hours prior to infection [34]. Cells were
256 infected as described above and type I IFN was not added back after infection.

257 **Phylogenetic Tree and Sequence Identity Heat Map**. Heat maps were constructed from a set
258 of representative group 2B coronaviruses by using alignment data paired with neighbor-joining
259 phylogenetic trees built in Geneious (v.9.1.5). Sequence identity was visualized using EvolView
260 (http://evolgenius.info/) and utilized SARS-CoV Urbani as the reference sequence. Tree shows
261 the degree of genetic similarity of SARS-CoV-2 and SARS-CoV across a selected group 2B
262 coronaviruses

263 **Immunoblot Analysis and Antibodies:**

264 Viral and host protein analysis were evaluated as previously described [50,54]. Briefly, cell lysates
265 were resolved on 7.5% Mini-PROTEAN TGX SDS-PAGE gels and then transferred to
266 polyvinylidene difluoride (PVDF) membranes using a Trans-Blot Turbo transfer system (Bio-
267 Rad). Membranes were blocked with 5% (w/v) non-fat dry milk in TBST (TBS with 0.1% (v/v)
268 Tween-20) for 1 hr, and then probed with the indicated primary antibody in 3% (w/v) BSA in
269 TBST at 4°C overnight. Following overnight incubation, membranes were probed with the
270 following secondary antibodies in 5% (w/v) non-fat dry milk in TBST for 1 hr at room
271 temperature: anti-rabbit or anti-mouse IgG-HRP conjugated antibody from sheep (both 1:10,000
272 GE Healthcare). Proteins were visualized using ECL or SuperSignal West Femto
273 chemiluminescence reagents (Pierce) and detected by autoradiography. The following primary
274 antibodies were used: anti-pSTAT1 (Y701) (1:1000 9171L Cell Signaling Technologies), anti-
275 STAT1 D1K9Y (1:1000 14994P Cell Signaling Technologies), anti-IFIT2 (1:2000 PA3-845
276 Invitrogen), anti-TRIM25 (1:1000 610570 BD Biosciences), anti-SARS-CoV Nucleocapsid
277 (1:1000), and anti-β-Actin (1:1000 ab8227 Abcam).

278 **Statistical analysis.** All statistical comparisons in this manuscript involved the comparison
279 between 2 groups, SARS-CoV or SARS-CoV-2 infected groups under equivalent conditions.
280 Thus, significant differences in viral titer were determined by the unpaired two-tailed students T-
281 Test.

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287

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417

418 **Figure Legends**

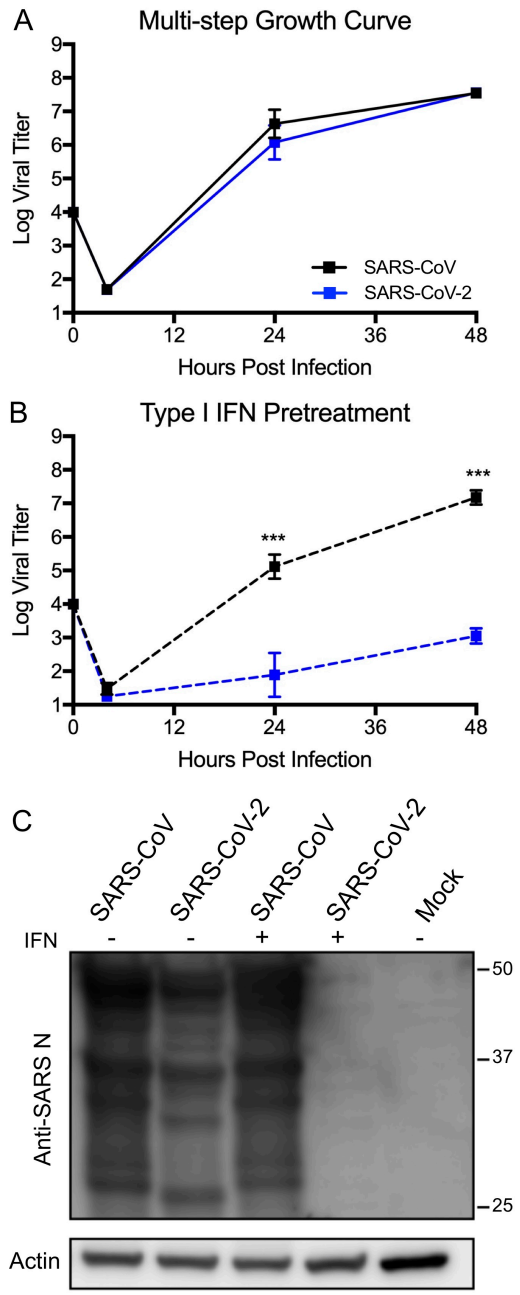
419 **Figure 1. SARS-CoV-2 sensitive to type I IFN pretreatment.** A) Vero E6 cells infected with
420 either SARS-CoV WT (black) or SARS-CoV-2 (blue) at an MOI of 0.01. Media harvested at 4,
421 24, and 48 hours post infection. B) Vero E6 cells were treated with 1000 units recombinant type
422 I IFN or mock for 18 hours prior to infection. Cells were subsequently infected at with either
423 SARS-CoV WT (black) or SARS-CoV-2 (blue) at an MOI of 0.01 as described above. Each
424 point on the line graph represents the group mean, N=6 for 24 and 48HPI, N=3 for 3HPI. All
425 error bars represent SD. The two tailed students t-test was used to determine P-values: *** P <
426 0.001. C) Cell protein lysates from IFN treated and untreated cells were probed 48 hours post
427 infection by using Western blotting with rabbit polyclonal anti-SARS N antibody or actin.

428

429 **Figure 2. SARS-CoV-2 infection induces STAT1 phosphorylation and ISG production.**
430 Vero cell protein lysates from IFN-I treated and untreated cells were probed 48 hours post
431 infection by Western blotting for phosphorylated STAT1 (Y701), STAT1, IFIT2, TRIM25, and
432 Actin.

433

434 **Figure 3, Conservation of SARS-CoV IFN antagonists.** Viral protein sequences of the
435 indicated viruses were aligned according to the bounds of the SARS-CoV open reading frames
436 for each viral protein. Sequence identities were extracted from the alignments for each viral
437 protein, and a heat map of percent sequence identity was constructed using EvolView
438 (www.evolgenius.info/evolview) with SARS-CoV as the reference sequence. TR = truncated
439 protein.



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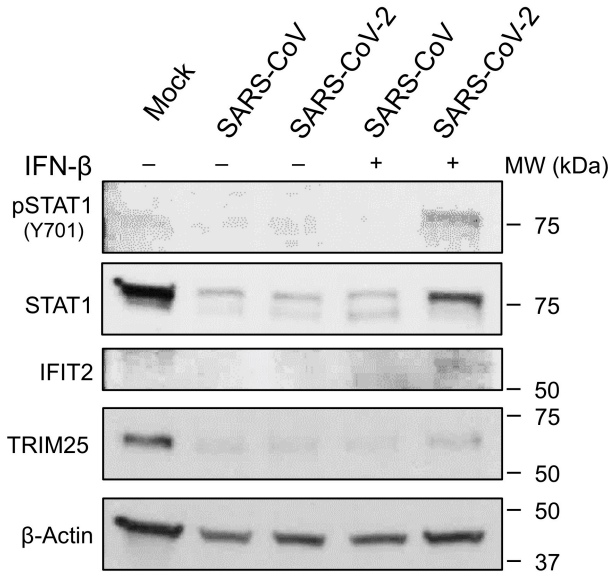

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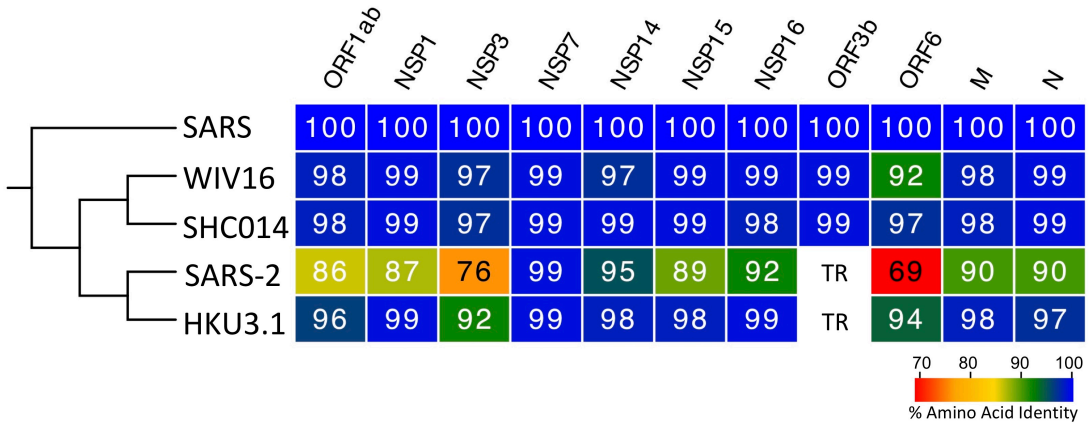

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