SARS-CoV-2 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 coronaviruses 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 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 coronavirus is much more sensitive to type I interferon pretreatment. We subsequently 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 response between SARS-CoV and SARS-CoV-2 that could help inform disease progression, treatment options, and animal model development.

Introduction

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

In the wake of the outbreak, major research efforts have sought to rapidly characterize the novel CoV to aid in treatment and control. Initial modeling studies predicted [6] and subsequent cell culture studies confirmed that spike protein of SARS-CoV-2 utilizes human angiotensin converting enzyme 2 (ACE2) for entry, the same receptor as SARS-CoV [7,8]. Extensive case studies indicated a similar range of disease onset and severe symptoms seen with SARS-CoV [5]. Notably, less severe SARS-CoV-2 cases have also been observed and were not captured in the original SARS-CoV outbreak. Importantly, screening and treatment guidance has relied on previous CoV data generated with SARS-CoV and MERS-CoV. Treatments with both protease inhibitors and type I interferon have been employed [4]; similarly, remdesivir, a drug targeting viral polymerases, has been reported to have efficacy against SARS-CoV-2 similar to findings with both SARS- and MERS-CoV [9-12]. Importantly, several vaccine efforts have been initiated with a focus on the SARS-CoV-2 spike protein as the major antigenic determinate [13].

41 Together, the similarities with SARS-CoV have been useful in responding to the newest CoV
42 outbreak.

43 In this study, we further characterize SARS-CoV-2 and compare it to the original SARS-
44 CoV. Using Vero E6 cells, we demonstrate that SARS-CoV-2 maintains similar viral replication
45 kinetics as SARS-CoV following a low dose infection. In contrast, we find that SARS-CoV-2 is
46 much more sensitive to type I interferon (IFN) pretreatment as compared to SARS-CoV. These
47 results suggest distinct changes between the CoVs in terms of IFN antagonism and we
48 subsequently examined sequence homology between the SARS-CoV and SARS-CoV-2 viral
49 proteins that may be responsible for these differences. Together, the results suggest SARS-
50 CoV-2 lacks the same capacity to control the type I IFN response as SARS-CoV.

51 **Results**

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

61 We next evaluated the susceptibility of SARS-CoV-2 to type I interferon (IFN)
62 pretreatment. Type I IFN treatment has been a standard approach for a wide variety of
63 pathogens including hepatitis B and C viruses as well as HIV [14]. During both the SARS and
64 MERS-CoV outbreaks, type I IFN has been employed with limited effect [15,16]. In this study, we
65 pretreated Vero E6 cells with 1000 units of recombinant type I IFN 18 hours prior to infection.

66 Vero E6 lack the capacity to produce type I IFN, but are able to respond to exogenous forms [17].
67 Following pretreatment with type I IFN, SARS-CoV infection has a modest reduction in viral titer
68 (1.5 log plaque forming units (PFU)) as compared to untreated control 24 hours post infection
69 (**Fig. 1B**). However, by 48 hours, SARS-CoV has nearly equivalent viral yields as the untreated
70 conditions (7.2 log PFU versus 7.5 log PFU). In contrast, SARS-CoV-2 shows a significant
71 reduction in viral replication following type I IFN treatment. At both 24 and 48 hours post
72 infection, SARS-CoV-2 had massive 3-log (24 HPI) and 4-log (48 HPI) drops in viral titer as
73 compared to control untreated cells. Together, the results demonstrate clear type I IFN
74 sensitivity in SARS-CoV-2 not observed with SARS-CoV.

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

76 Considering the sensitivity to type I IFN, we next sought to evaluate changes between SARS-
77 CoV and SARS-CoV-2 viral proteins. Previous work has established several key IFN antagonist
78 in the SARS-CoV genome including NSP1, NSP3, ORF3b, ORF6, and others [18]. Therefore, we
79 compared the sequence homology across viral proteins from SARS-CoV, SARS-CoV-2, and
80 several bat SARS-like viruses including WIV16-CoV [19], SHC014-CoV [20], and HKU3.1-CoV [21].
81 Using sequence analysis, we found several changes to SARS-CoV-2 that potentially contribute
82 to its type I IFN sensitivity (**Fig. 2**). For SARS-CoV structural proteins including the nucleocapsid
83 (N) and matrix (M) protein, a high degree of sequence homology (>90%AA identity) suggests
84 that their reported IFN antagonism is likely maintained in SARS-CoV-2 and other SARS-like
85 viruses. Similarly, the ORF1ab poly-protein retains high sequence identity in SARS-CoV-2 and
86 several known antagonists contained within the poly-protein (NSP1, NSP7, NSP14-16) are
87 highly conserved relative to SARS-CoV. One notable exception is the large papain-like
88 proteases, NSP3, which only 76% conserved between SARS-CoV and SARS-CoV-2. However,
89 SARS-CoV-2 does maintain a deubiquitinating domain thought to confer IFN resistance [22]. For
90 SARS-CoV ORF3b, a 154 amino acid (AA) protein known to antagonize the type I IFN

91 responses by blocking IRF3 phosphorylation [23], sequence analysis indicates that SARS-CoV-2
92 ORF3b contains a premature stop codon resulting in a truncated 20 AA protein. Similarly,
93 HKU3.1-CoV also has a premature termination resulting in a predicted 39 AA protein. Both
94 WIV16-CoV and SHC014-CoV, the most closely related bat viruses to SARS-CoV, encode
95 longer 114 AA truncated protein with >99% homology with SARS-CoV ORF3b suggesting that
96 IFN antagonism might be maintained in these specific group 2B CoV strains. Similarly, SARS-
97 CoV ORF6 has been shown to be an IFN antagonist that disrupts karyopherin transportation of
98 transcriptions factors like STAT1 [23,24]. In contrast to ORF3b, all five surveyed group 2B CoVs
99 maintain ORF6; however, SARS-CoV-2 had only 69% homology with SARS-CoV while the
100 other three group 2B bat CoVs had >90% conservation. Importantly, SARS-CoV-2 has a two
101 amino acid truncation in its ORF6; previous work has found that alanine substitution in this C-
102 terminal of SARS-CoV ORF6 resulted in ablated antagonism [24]. Together, the sequence
103 homology analysis suggests that differences in NSP3, ORF3b, and/or ORF6 may be key drivers
104 of SARS-CoV-2 type I IFN susceptibility.

105 **Discussion**

106 With the ongoing outbreak of COVID-19 caused by SARS-CoV-2, viral characterization remains
107 a key factor in responding to the emergent novel virus. In this report, we describe differences in
108 the type I IFN sensitivity between SARS-CoV-2 and the original SARS-CoV. While both viruses
109 maintain similar replication in untreated Vero E6 cells, SARS-CoV-2 has a significant decrease
110 in viral replication following type I IFN pretreatment. This sensitivity to type I IFN is distinct from
111 the original SARS-CoV and suggests that the novel CoV has distinct host interactions driving
112 disease outcomes. Analysis of viral proteins finds SARS-CoV-2 has several changes that
113 potentially impact its capacity to modulate the type I IFN response, including loss of ORF3b and
114 a short truncation of ORF6, both known as type I IFN antagonists for SARS-CoV [23]. Together,
115 our results suggest SARS-CoV and SARS-CoV-2 have differences in their ability to control the

116 type I IFN response once initiated and that they may have major implication for COVID-19
117 disease and treatment.

118 With a similar genome organization and disease symptoms in humans, the SARS-CoV-2
119 outbreak has drawn insights from the closely related SARS-CoV. However, the differences in
120 sensitivity to type I IFN pretreatment illustrates a clear distinction between the two CoVs.
121 Coupled with a novel furin cleavage site [25], robust upper airway infection [8], and potential
122 transmission prior to symptomatic disease [26], the differences between SARS-CoV and SARS-
123 CoV-2 could prove important in disrupting the ongoing spread of COVID-19. For SARS-CoV, *in*
124 *vitro* studies have consistently found that wild-type SARS-CoV is indifferent to type I IFN
125 pretreatment [27,28]. Similarly, *in vivo* SARS-CoV studies have found that the loss of type I IFN
126 signaling had no significant impact on disease suggesting the virus controlled this pathway [29].
127 However, more recent reports suggest that host genetic background may majorly influence this
128 finding [30]. For SARS-CoV-2, our results suggest that type I IFN pretreatment produces a 3 - 4
129 log drop in viral titer. This level of sensitivity is similar to MERS-CoV and suggests the novel
130 CoV lacks the same capacity to modulate a primed type I IFN response as SARS-CoV [31,32].
131 Notably, the sensitivity to type I IFN does not completely ablate viral replication; unlike SARS-
132 CoV 2'O methyl-transferase mutants [27], SARS-CoV-2 is able to replicate to low, detectable
133 levels even in the presence of type I IFN. This finding could help explain positive test in patients
134 with minimal symptoms and the range of disease observed. In addition, while SARS-CoV-2 is
135 sensitive to type I IFN pretreatment, both SARS-CoV and MERS-CoV employ effective means
136 to disrupt recognition until late during infection [33]; a similar mechanism may also be employed
137 by SARS-CoV, diminishing the overall effect of type I IFN during infection.

138 For SARS-CoV-2, the sensitivity to type I IFN indicates a distinction from SARS-CoV and
139 suggests differential host immune modulation between the viruses. The loss of ORF3b and
140 truncation/changes in ORF6 could signal a reduced capacity of SARS-CoV-2 to modulate type I

141 IFN responses. For SARS-CoV ORF6, the N-terminal domain has been shown to have a clear
142 role in its ability to disrupt karyopherin transport [24]; in turn, the loss of ORF6 function for SARS-
143 CoV-2 would likely render it much more susceptible to type I IFN pretreatment as activated
144 STAT1 and other transcriptional factors would now have the capacity to enter the nucleus and
145 induce an interferon stimulated gene response. For SARS-CoV ORF3b, the viral protein has
146 been shown to disrupt phosphorylation of IRF3, a key transcriptional factor in the induction of an
147 antiviral state [23]. While its mechanism of action is not clear, the ORF3b absence in SARS-CoV-2
148 infection likely impacts its ability to control the type I IFN response. Similarly, while NSP3
149 deubiquitinating domain remains intact, SARS-CoV-2 has a 24 AA insertion upstream of this
150 deubiquitinating domain that could potentially alter that function [22]. Similarly, while other
151 antagonists are maintained with high levels of conservation (>90%), single point mutations in
152 key locations could modify function and contribute to increased IFN sensitivity. Overall, the
153 sequence analysis suggests that differences between SARS-CoV and SARS-CoV-2 viral
154 proteins may drive attenuation in the context of type I IFN pretreatment.

155 The increased sensitivity of SARS-CoV-2 suggests utility in treatment using type I IFN.
156 While type I IFN has been used in response to chronic viral infection [34], previous examination of
157 SARS-CoV cases found inconclusive effect for type I IFN treatment [35]. However, the findings
158 from the SARS-CoV outbreak were complicated by combination therapy of type I IFN with other
159 treatments including ribavirin/steroids and lack of a regimented protocol. While type I IFN has
160 been utilized to treat MERS-CoV infected patients, no conclusive data yet exists to determine
161 efficacy [36]. Yet, *in vivo* studies with MERS-CoV has found that early induction with type I IFN
162 can be protective in mice [37]; importantly, the same study found that late type I IFN induction can
163 be detrimental for MERS-CoV disease [37]. Similarly, early reports have described treatments
164 using type I IFN in combination for SARS-CoV-2 infection; yet the efficacy of these treatments
165 and the parameters of their use is not known [38]. Overall, sensitivity data suggest that type I IFN

166 treatment may have utility for treating SARS-CoV-2 if the appropriate parameters can be
167 determined. In addition, use of type III IFN, which is predicted to have utility in the respiratory
168 tract, could offer another means for effective treatment for SARS-CoV-2.

169 In addition to treatment, the sensitivity to type I IFN may also have implications for
170 animal model development. For SARS-CoV, mouse models that recapitulate human disease
171 were developed through virus passage in immune competent mice [39]. Similarly, mouse models
172 for MERS-CV required adaptation in mice that had genetic modifications of their dipeptidyl-
173 peptidase 4 (DPP4), the receptor for MERS-CoV [40,41]. However, each of these MERS-CoV
174 mouse models still retained full immune capacity. In contrast, SARS-CoV-2 sensitivity to type I
175 IFN may signal the need to use an immune deficient model to develop relevant disease. While
176 initial work has suggested incompatibility to SARS-CoV-2 infection in mice based on receptor
177 usage [8], the type I IFN response may be a second major barrier that needs to be overcome.
178 Similar to the emergent Zika virus outbreak, the use of type I IFN receptor knockout mice or
179 type I IFN receptor blocking antibody may be necessary to develop auseful SARS-CoV-2 animal
180 models for therapeutic testing [42].

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

188

189 **Methods**

190 **Viruses and cells.** SARS-CoV-2 USA-WA1/2020, provided by the World Reference Center for
191 Emerging Viruses and Arboviruses (WRCEVA) and was originally obtained from the USA
192 Centers of Disease Control as described [43]. SARS-CoV-2 and mouse-adapted recombinant
193 SARS-CoV (MA15) [39] were titrated and propagated on VeroE6 cells, grown in DMEM with 5%
194 fetal bovine serum and 1% antibiotic/antimytotic (Gibco). Standard plaque assays were used for
195 SARS-CoV and SARS-CoV-2 [44,45]. All experiments involving infectious virus were conducted at
196 the University of Texas Medical Branch (Galveston, TX) in approved biosafety level 3 (BSL)
197 laboratories with routine medical monitoring of staff.

198 **Infection and type I IFN pretreatment.** Viral replication in Vero E6 were performed as
199 previously described [27,46]. Briefly, cells were washed with two times with PBS and inoculated
200 with SARS-CoV or SARS-CoV-2 at an multiplicity of infection (MOI) 0.01 for 60 minutes at 37
201 °C. Following inoculation, cells were washed 3 times, and fresh media was added to signify time
202 0. Three or more biological replicates were harvested at each described time point and results
203 are from combination of two experiments. No blinding was used in any sample collections, nor
204 were samples randomized. For type I IFN pretreatment, experiments were completed as
205 previously described [27]. Briefly, Vero E6 cells were incubated with 1000 units/mL of
206 recombinant type I IFN alpha (PBL Assay Sciences) 18 hours prior to infection [27]. Cells were
207 infected as described above and type I IFN was not added back after infection.

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

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

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References

1. Gralinski, L.E. & Menachery, V.D. Return of the Coronavirus: 2019-nCoV. *Viruses* **12**(2020).
2. Gorbalenya, A.E.*, et al.* The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology* (2020).
3. Zhu, N.*, et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* **382**, 727-733 (2020).
4. Huang, C.*, et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497-506 (2020).
5. Wu, Z. & McGoogan, J.M. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* (2020).
6. Xu, X.*, et al.* Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci* **63**, 457-460 (2020).
7. Letko, M., Marzi, A. & Munster, V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol* (2020).
8. Zhou, P.*, et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* (2020).
9. de Wit, E.*, et al.* Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. *Proceedings of the National Academy of Sciences of the United States of America* (2020).
10. Wang, M.*, et al.* Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* (2020).
11. Sheahan, T.P.*, et al.* Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nature communications* **11**, 222 (2020).
12. Sheahan, T.P.*, et al.* Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci Transl Med* **9**(2017).
13. Ahmed, S.F., Quadeer, A.A. & McKay, M.R. Preliminary Identification of Potential Vaccine Targets for the COVID-19 Coronavirus (SARS-CoV-2) Based on SARS-CoV Immunological Studies. *Viruses* **12**(2020).
14. Lin, F.C. & Young, H.A. Interferons: Success in anti-viral immunotherapy. *Cytokine Growth Factor Rev* **25**, 369-376 (2014).
15. Zumla, A., Hui, D.S. & Perlman, S. Middle East respiratory syndrome. *Lancet* **386**, 995-1007 (2015).
16. Song, Z.*, et al.* From SARS to MERS, Thrusting Coronaviruses into the Spotlight. *Viruses* **11**(2019).
17. Diaz, M.O.*, et al.* Homozygous deletion of the alpha- and beta 1-interferon genes in human leukemia and derived cell lines. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 5259-5263 (1988).
18. Totura, A.L. & Baric, R.S. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. *Current opinion in virology* **2**, 264-275 (2012).
19. Yang, X.L.*, et al.* Isolation and Characterization of a Novel Bat Coronavirus Closely Related to the Direct Progenitor of Severe Acute Respiratory Syndrome Coronavirus. *Journal of virology* **90**, 3253-3256 (2015).
20. Ge, X.Y.*, et al.* Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538 (2013).

271 21. Lau, S.K., *et al.* Severe acute respiratory syndrome coronavirus-like virus in Chinese
272 horseshoe bats. *Proceedings of the National Academy of Sciences of the United States of*
273 *America* **102**, 14040-14045 (2005).
274 22. Clementz, M.A., *et al.* Deubiquitinating and interferon antagonism activities of
275 coronavirus papain-like proteases. *Journal of virology* **84**, 4619-4629 (2010).
276 23. Kopecky-Bromberg, S.A., Martinez-Sobrido, L., Frieman, M., Baric, R.A. & Palese, P.
277 Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and
278 nucleocapsid proteins function as interferon antagonists. *Journal of virology* **81**, 548-557 (2007).
279 24. Frieman, M., *et al.* Severe acute respiratory syndrome coronavirus ORF6 antagonizes
280 STAT1 function by sequestering nuclear import factors on the rough endoplasmic
281 reticulum/Golgi membrane. *Journal of virology* **81**, 9812-9824 (2007).
282 25. Coutard, B., *et al.* The spike glycoprotein of the new coronavirus 2019-nCoV contains a
283 furin-like cleavage site absent in CoV of the same clade. *Antiviral Res* **176**, 104742 (2020).
284 26. Tong, Z.D., *et al.* Potential Presymptomatic Transmission of SARS-CoV-2, Zhejiang
285 Province, China, 2020. *Emerg Infect Dis* **26**(2020).
286 27. Menachery, V.D., *et al.* Attenuation and restoration of severe acute respiratory syndrome
287 coronavirus mutant lacking 2'-o-methyltransferase activity. *Journal of virology* **88**, 4251-4264
288 (2014).
289 28. Thiel, V. & Weber, F. Interferon and cytokine responses to SARS-coronavirus infection.
290 *Cytokine Growth Factor Rev* **19**, 121-132 (2008).
291 29. Frieman, M.B., *et al.* SARS-CoV pathogenesis is regulated by a STAT1 dependent but a
292 type I, II and III interferon receptor independent mechanism. *PLoS pathogens* **6**, e1000849
293 (2010).
294 30. Channappanavar, R., *et al.* Dysregulated Type I Interferon and Inflammatory Monocyte-
295 Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice. *Cell host &*
296 *microbe* **19**, 181-193 (2016).
297 31. Menachery, V.D., *et al.* Middle East Respiratory Syndrome Coronavirus Nonstructural
298 Protein 16 Is Necessary for Interferon Resistance and Viral Pathogenesis. *mSphere* **2**(2017).
299 32. Falzarano, D., *et al.* Inhibition of novel beta coronavirus replication by a combination of
300 interferon-alpha2b and ribavirin. *Scientific reports* **3**, 1686 (2013).
301 33. Menachery, V.D., *et al.* Pathogenic influenza viruses and coronaviruses utilize similar
302 and contrasting approaches to control interferon-stimulated gene responses. *mBio* **5**, e01174-
303 01114 (2014).
304 34. Finter, N.B., *et al.* The use of interferon-alpha in virus infections. *Drugs* **42**, 749-765
305 (1991).
306 35. Stockman, L.J., Bellamy, R. & Garner, P. SARS: systematic review of treatment effects.
307 *PLoS Med* **3**, e343 (2006).
308 36. de Wit, E., van Doremalen, N., Falzarano, D. & Munster, V.J. SARS and MERS: recent
309 insights into emerging coronaviruses. *Nat Rev Microbiol* **14**, 523-534 (2016).
310 37. Channappanavar, R., *et al.* IFN-I response timing relative to virus replication determines
311 MERS coronavirus infection outcomes. *The Journal of clinical investigation* **130**, 3625-3639
312 (2019).
313 38. Pang, J., *et al.* Potential Rapid Diagnostics, Vaccine and Therapeutics for 2019 Novel
314 Coronavirus (2019-nCoV): A Systematic Review. *J Clin Med* **9**(2020).
315 39. Roberts, A., *et al.* A mouse-adapted SARS-coronavirus causes disease and mortality in
316 BALB/c mice. *PLoS pathogens* **3**, e5 (2007).
317 40. Cockrell A, Y.B., Scobey T, Jensen K, Douglas M, Beall A, Tang X-C, Marasco WA,
318 Heise MT, Baric RS A Mouse Model for MERS Coronavirus Induced Acute Respiratory Distress
319 Syndrome. . *Nature Microbiology* **In Press**(2016).

320 41. Li, K., *et al.* Mouse-adapted MERS coronavirus causes lethal lung disease in human
321 DPP4 knockin mice. *Proceedings of the National Academy of Sciences of the United States of*
322 *America* **114**, E3119-E3128 (2017).
323 42. Lazear, H.M., *et al.* A Mouse Model of Zika Virus Pathogenesis. *Cell host & microbe* **19**,
324 720-730 (2016).
325 43. Harcourt, J., *et al.* Isolation and characterization of SARS-CoV-2 from the first US
326 COVID-19 patient. *bioRxiv*, 2020.2003.2002.972935 (2020).
327 44. Sims, A.C., *et al.* Release of severe acute respiratory syndrome coronavirus nuclear
328 import block enhances host transcription in human lung cells. *J Virol* **87**, 3885-3902 (2013).
329 45. Josset, L., *et al.* Cell host response to infection with novel human coronavirus EMC
330 predicts potential antivirals and important differences with SARS coronavirus. *MBio* **4**, e00165-
331 00113 (2013).
332 46. Sheahan, T., Rockx, B., Donaldson, E., Corti, D. & Baric, R. Pathways of cross-species
333 transmission of synthetically reconstructed zoonotic severe acute respiratory syndrome
334 coronavirus. *Journal of virology* **82**, 8721-8732 (2008).

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336

337 **Figure Legends**

338 **Figure 1. SARS-CoV-2 sensitive to type I IFN pretreatment.** A) Vero E6 cells infected with
339 either SARS-CoV WT (black) or SARS-CoV-2 (blue) at an MOI of 1. Media harvested at 4, 24,
340 and 48 hours post infection. B) Vero E6 cells were treated with 1000 units recombinant type I
341 IFN or mock for 18 hours prior to infection. Cells were subsequently infected at with either
342 SARS-CoV WT (black) or SARS-CoV-2 (blue) at an MOI of 1 as described above. Each point
343 on the line graph represents the group mean, N=6 for 24 and 48HPI, N=3 for 3HPI. All error
344 bars represent SD. The two tailed students t-test was used to determine P-values: *** P <
345 0.001**.**

346 **Figure 2, Conservation of SARS-CoV IFN antagonists.** Viral protein sequences of the
347 indicated viruses were aligned according to the bounds of the SARS-CoV open reading frames
348 for each viral protein. Sequence identities were extracted from the alignments for each viral
349 protein, and a heat map of percent sequence identity was constructed using EvolView
350 (www.evolgenius.info/evolview) with SARS-CoV as the reference sequence. TR = truncated
351 protein.

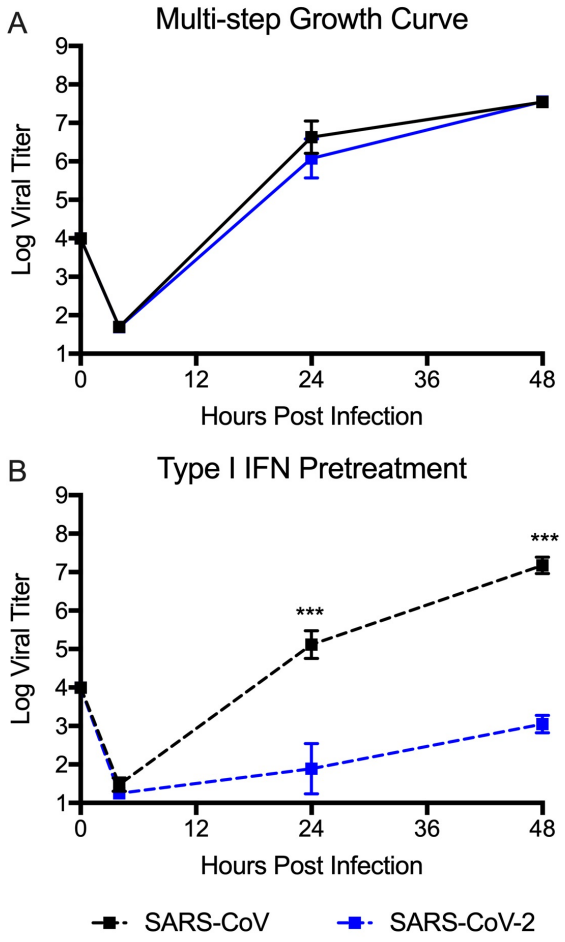
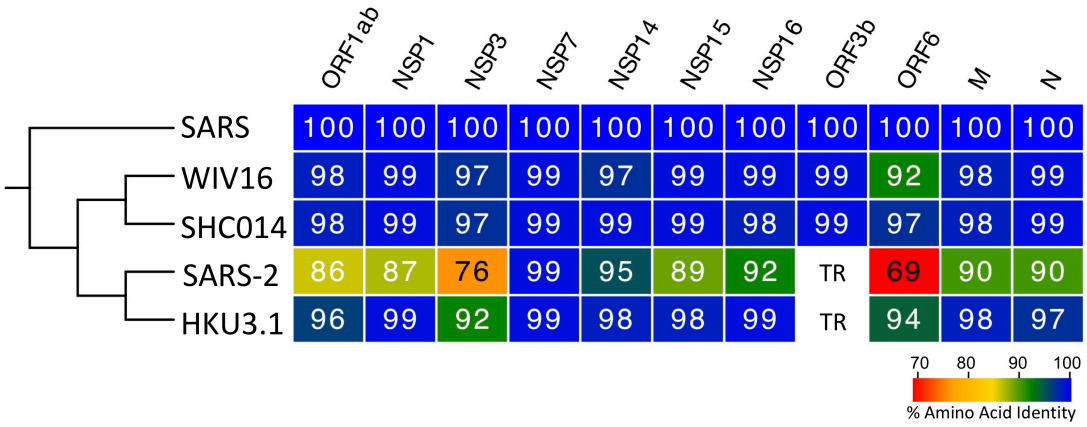


Figure 1. SARS-CoV-2 sensitive to type I IFN pretreatment. A) Vero E6 cells infected with either SARS-CoV WT (black) or SARS-CoV-2 (blue) at an MOI of 1. Media harvested at 4, 24, and 48 hours post infection. B) Vero E6 cells were treated with 1000 units recombinant type I IFN or mock for 18 hours prior to infection. Cells were subsequently infected at with either SARS-CoV WT (black) or SARS-CoV-2 (blue) at an MOI of 1 as described above. Each point on the line graph represents the group mean, N=6 for 24 and 48HPI, N=3 for 3HPI. All error bars represent SD. The two tailed students t-test was used to determine P-values: *** $P < 0.001$.



2. Figure 2, Conservation of SARS-CoV IFN antagonists. Viral protein sequences of the indicated viruses were aligned according to the bounds of the SARS-CoV open reading frames for each viral protein. Sequence identities were extracted from the alignments for each viral protein, and a heat map of percent sequence identity was constructed using EvolView (www.evolgenius.info/evolview) with SARS-CoV as the reference sequence. TR = truncated protein.