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].

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

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

Results

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

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

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

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

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

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

Discussion

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

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

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

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

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

The increased sensitivity of SARS-CoV-2 suggests utility in treatment using type I IFN. While type I IFN has been used in response to chronic viral infection [34], previous examination of SARS-CoV cases found inconclusive effect for type I IFN treatment [35]. However, the findings from the SARS-CoV outbreak were complicated by combination therapy of type I IFN with other treatments including ribavirin/steroids and lack of a regimented protocol. While type I IFN has been utilized to treat MERS-CoV infected patients, no conclusive data yet exists to determine efficacy [36]. Yet, *in vivo* studies with MERS-CoV has found that early induction with type I IFN can be protective in mice [37]; importantly, the same study found that late type I IFN induction can be detrimental for MERS-CoV disease [37]. Similarly, early reports have described treatments using type I IFN in combination for SARS-CoV-2 infection; yet the efficacy of these treatments 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

Methods

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

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

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

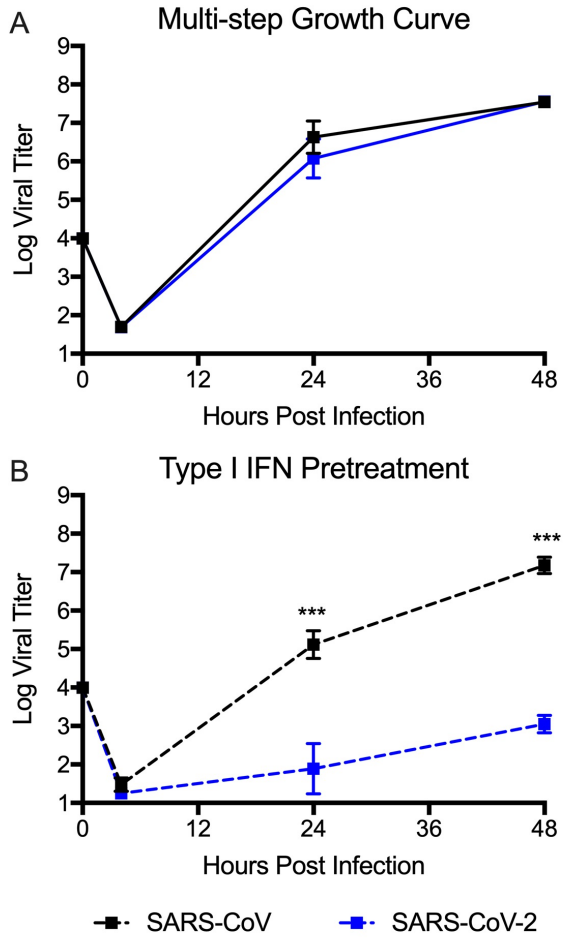
21. Lau, S.K., *et al.* Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 14040-14045 (2005).
22. Clementz, M.A., *et al.* Deubiquitinating and interferon antagonism activities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

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

Figure Legends

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.

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.



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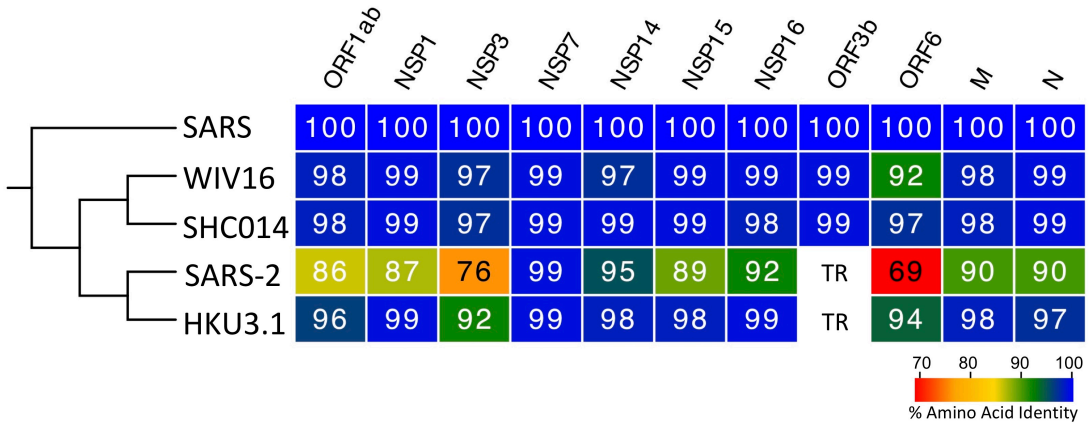

2. Figure 2, Conservation of SARS-CoV IFN antagonists. Viral protein sequences of the indicated viruses were aligned according to the bounds of the SARS-CoV open reading frames for each viral protein. Sequence identities were extracted from the alignments for each viral protein, and a heat map of percent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