

1 ABSTRACT

2 The outbreak of the severe acute respiratory syndrome coronavirus 2
3 (SARS-CoV-2) poses a huge threat to many countries around the world. However,
4 where is it origin and which animals are sensitive to cross-species transmission is
5 unclear. The interaction of virus and cell receptor is a key determinant of host range
6 for the novel coronavirus. Angiotensin-converting enzyme 2 (ACE2) is demonstrated
7 as the primary entry receptor for SARS-CoV-2. In this study, we evaluated the
8 SARS-CoV-2 entry mediated by ACE2 of 11 different species of animals, and
9 discovered that ACE2 of *Rhinolophus sinicus* (Chinese horseshoe bat), *Felis catus*
10 (domestic cat), *Canis lupus familiaris* (dog), *Sus scrofa* (pig), *Capra hircus* (goat) and
11 especially *Manis javanica* (Malayan pangolin) were able to render SARS-CoV-2 entry
12 in non-susceptible cells. This is the first report that ACE2 of Pangolin could mediate
13 SARS-CoV-2 entry which increases the presume that SARS-CoV-2 may have a
14 pangolin origin. However, none of the ACE2 proteins from *Rhinolophus*
15 *ferrumequinum* (greater horseshoe bat), *Gallus gallus* (chicken), *Notechis scutatus*
16 (mainland tiger snake), *Mus musculus* (house mouse) rendered SARS-CoV-2 entry.
17 Specifically, a natural isoform of *Macaca mulatta* (Rhesus monkey) ACE2 with a
18 mutation of Y217N was resistance to infection, which rises the possible impact of this
19 type of ACE2 during monkey studies of SARS-CoV-2. Overall, these results clarify
20 that SARS-CoV-2 could engage receptors of multiple species of animals and it is a
21 perplexed work to track SARS-CoV-2 origin and its intermediate hosts.

22

1 **IMPORTANCE**

2 In this study, we illustrated that SARS-CoV-2 is able to engage receptors of
3 multiple species of animals. This indicated that it may be a perplexed work to track
4 SARS-CoV-2 origin and discover its intermediate hosts. This feature of virus is
5 considered to potentiate its diverse cross-species transmissibility. Of note, here is the
6 first report that ACE2 of Pangolin could mediate SARS-CoV-2 entry which increases
7 the possibility that SARS-CoV-2 may have a pangolin origin. And we also
8 demonstrated that not all species of bat were sensitive to SARS-CoV-2 infection. At
9 last, it is also important to detect the expression ratio of the Y217N ACE2 to the
10 prototype in Rhesus monkeys to be recruited for studies on SARS-CoV-2 infection.

11 **Keyword:** SARS-CoV-2, ACE2, susceptibility, cross-species, pangolin

12

1 INTRODUCTION

2 In December 2019, a novel pneumonia, termed as COVID-19 by World Health
3 Organization (WHO) thereafter, emerged in Wuhan, China, and the causative agent
4 was soon identified as a novel coronavirus, which is termed as severe acute
5 respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee
6 on Taxonomy of Viruses, ICTV (1, 2). The SARS-CoV-2 outbreak has been
7 speculatively associated with a seafood market where sales also various land wild
8 animals (3). Bats are recognized as a potential natural reservoir for SARS-CoV-2 (1,
9 3). However, recently studies indicated that pangolins were also considered as
10 possible natural hosts of this coronavirus (4, 5). Discovering the potential
11 intermediate animal hosts of SARS-CoV-2 and evaluating their possible cross-species
12 transmissibility will be scientifically very important. Unfortunately, we know little
13 about this. Currently, there are no suitable animal models for SARS-CoV-2 infection.
14 A recent study revealed that ferrets and cats were sensitive to SARS-CoV-2 infection,
15 however, these animals showed no clinical symptoms (6). Whether there exist other
16 animal(s) as a candidate SARS-CoV-2 infection model should be further explored.

17 The interaction between receptor and virus is a key determinant of the host range.
18 It has been demonstrated that SARS-CoV-2 resembles SARS-CoV, which uses
19 angiotensin-converting enzyme 2 (ACE2) as the primary cell entry receptor (1, 7-9).
20 When we retrace the origin of coronavirus, the cell susceptibility to viruses conferred
21 by receptors of speculated animals is a preferential consideration (10, 11). Before
22 clarifying that *Rhinolophus sinicus* is the natural reservoir of SARS-CoV, scientists

1 first evaluated the susceptibility provided by ACE2 from different bat species to
2 SARS-CoV. They found that the ACE2 of *Rhinolophus sinicus* was responsible for the
3 susceptibility to SARS-CoV and subsequently confirmed that *Rhinolophus sinicus*
4 was the natural reservoir of SARS-CoV (10, 12, 13). The Middle East respiratory
5 syndrome coronavirus (MERS-CoV) was also recognized has a bat origin due to that
6 both the MERS-CoV and two MERS-CoV-related viruses from bats could utilize
7 human or bat dipeptidyl peptidase 4 (DPP4) for cell entry (14-16).

8 Therefore, in this study, we systemically evaluated the ability of SARS-CoV-2 to
9 infect two types of non-susceptible cells utilizing ACE2 proteins from nine different
10 species of animals and the human being to determine its possible origin and further to
11 explore its potentiate cross-species transmission. Our findings provide evidence that
12 SARS-CoV-2 was able to engage broad receptors of different species, which poses a
13 huge challenge to search the animal origin of SARS-CoV-2 for the control and
14 prevention in future.

15 **RESULT**

16 To investigate which animal's ACE2 could render SARS-CoV-2 entry, we
17 synthesized the full-length cDNA fragments of ACE2 from 11 species of animals, as
18 well as the human being. These species were *Rhinolophus sinicus* (Chinese horseshoe
19 bat), *Rhinolophus ferrumequinum* (greater horseshoe bat), *Felis catus* (domestic cat),
20 *Capra hircus* (goat), *Canis lupus familiaris* (dog), *Sus scrofa* (pig), *Manis javanica*
21 (Malayan pangolin), *Gallus gallus* (chicken), *Notechis scutatus* (mainland tiger snake),
22 *Mus musculus* (house mouse) and *Macaca mulatta* (Rhesus monkey) and *Homo*

1 *sapiens* (human). Synthesized DNA fragments were then sub-cloned into the
2 pCAGGS-HA vector for the expression in eukaryotic cells. The origins and GenBank
3 accession numbers of these ACE2 molecules were listed in the Table. We firstly
4 compared the nucleotide sequences of ACE2 coding regions of these animals to that
5 of human. The sequence similarities of these ACE2 cDNAs were exhibited in the
6 Table. Among these sequences, the ACE2 of Rhesus monkey was most close to
7 human, and in contrast, the ACE2 of snake was the farthest. It has been reported that
8 two virus-binding hotspots, Lys31 and Lys353 in hACE2, were critical for
9 SARS-CoV infection (17, 18). In this study, we found that the Lys31 was not
10 conserved in ACE2 of all the 11 animal species observed in this study. However, the
11 Lys353 was conserved in all the 10 animal species except mouse (Table).

12 Next, we tested whether ACE2 of the nine animal species were able to render
13 SARS-CoV-2 entry to non-susceptible HEK293T cell lines. Different ACE2s could be
14 expressed and presented in the surface of HEK293T cells by IFA (Figure 1).
15 HEK293T Plasmids expressing ACE2 of human and mice were applied as the positive
16 and negative control of the entry assay, respectively. No attempt was made to quantify
17 infection efficiency in this study due to difficulties encountered in conducting
18 experiments under BSL-3 conditions. As expected, the human ACE2 supported
19 SARS-CoV-2 entry whereas mouse ACE2 did not (Figure 1). One previous study
20 indicated that the SARS-CoV outbreak in 17 years ago was originated from
21 *Rhinolophus affinis* (Intermediate horseshoe bat) (12). A recent study further
22 demonstrated that ACE2 of *Rhinolophus sinicus* (Chinese horseshoe bat) also

1 rendered SARS-CoV-2 entry besides SARS-CoV (1). In this study, we was not able to
2 synthesize the ACE2 cDNA of *Rhinolophus affinis* due to the absent of its sequence.
3 Therefore, we synthesized the ACE2 cDNA of *Rhinolophus sinicus* and *Rhinolophus*
4 *ferrumequinum* (Greater horseshoe bat) to test whether ACE2 of other bat species was
5 responsible for the susceptibility to SARS-CoV-2. Interestingly, we found that the
6 ACE2 of *Rhinolophus ferrumequinum* did not support the SARS-CoV-2 entry as
7 *Rhinolophus sinicus* (Figure 2), suggesting that not all species of bat were sensitive to
8 SARS-CoV-2 infection.

9 A recent study indicated that SARS-CoV-2 did not replicate and shed in dogs,
10 pigs, chickens and ducks, but fairly good in ferrets and effectively in cats (6).
11 Consistently, our study demonstrated that the cat ACE2 supported viral entry (Figure
12 1). Although pigs, dogs and chickens were non-sensitive to SARS-CoV-2 infection,
13 we know little about its molecular mechanisms and the role of receptor avidity for the
14 resistance. Our data demonstrated that ACE2 proteins of dog and pig supported
15 SARS-CoV-2 entry as that of cat.

16 Old world monkeys (*Macaca mulatta* and *Macaca fascicularis*) were used as
17 animal models of experimental SARS-CoV-2 infection (19). Surprisingly, we found
18 that the ACE2 of *Macaca mulatta* in our study did not support the SARS-CoV-2 entry
19 as expected (Figure 1). By investigating the monkey ACE2 sequence, we found that
20 an ACE2 isoform of *Macaca mulatta*, which contained two natural variations (R192G
21 and Y217N) comparing with the wild type ACE2, was cloned (Figure 2A). Our data
22 revealed that the ACE2 of *Macaca mulatta* with the Y217N mutation also fault to

1 support SARS-CoV-2 infection. When we restored Y217N mutation as wild type
2 ACE2 of *Macaca mulatta*, N217Y recovery the ability to support SARS-CoV-2
3 infection (Figure 2B). We noticed that the prototype 217Y was conserved in other
4 species of animals investigated in this study excluding *Macaca mulatta* (data not
5 shown), which suggests that the 217 position is a key residual for SARS-CoV-2
6 infection.

7 There is a dispute on if SARS-CoV-2 originated from bats or pangolins (1, 4, 5).
8 It has been demonstrated that a bat ACE2 mediated the SARS-CoV-2 entry (1).
9 However, whether ACE2 of pangolins support the virus entry was unclear. Therefore,
10 we expressed the ACE2 of Malayan pangolins (*Manis javanica*) and tested its role in
11 conferring the susceptibility to SARS-CoV-2. For the first time, we demonstrated that
12 SARS-CoV-2 could engaged the ACE2 of pangolins to entry (Figure 1).

13 At last, we demonstrated ACE2 of *Notechis scutatus* (mainland tiger snake) could
14 not support SARS-CoV-2 entry as previously predicted (20). And snakes may be not
15 the source of SARS-CoV-2.

16 **DISSCUSSION**

17 Spike features of coronaviruses and lysosomal proteases of hosts determine the
18 tropism of coronavirus (21). A bat coronavirus RaTG13 in *Rhinolophus affinis*
19 (Intermediate horseshoe bat) from Yunnan exhibits the highest sequence similarity to
20 SARS-CoV-2 until now (1). In this study, we found that the ACE2 of *Rhinolophus*
21 *ferrumequinum* (Greater horseshoe bat) failed to mediate SARS-CoV-2 entry, whereas
22 ACE2 of *Rhinolophus sinicus* (Chinese horseshoe bat) rendered SARS-CoV-2 entry to

1 non-susceptible cells. In fact, in contrast to the genetically homogenous human ACE2,
2 bat ACE2 proteins have great genetic diversity (13). A number of ACE2 molecules
3 isolated from different bat species failed to mediate efficient SARS-CoV entry (13). A
4 study has reported that *Rhinolophus sinicus* serves as natural reservoirs of SARS-CoV
5 and an isolated bat-origin SARS-CoV-like virus is able to employ ACE2 proteins
6 from humans and civets for cell entry (12). These results suggest that analysis of the
7 receptor-conferred susceptibility to the virus entry is important before investigating
8 for the bat-origin of SARS-CoV-2.

9 Recently, pangolins are also considered as a possible natural host of SARS-CoV-2
10 (4, 5). Coronaviruses with high sequence homology were identified in Malayan
11 pangolins (*Manis javanica*) suggesting to be a possible source for the emergence of
12 SARS-CoV-2 (4). We demonstrated that the ACE2 of Malayan pangolin supported
13 SARS-CoV-2 entry in non-susceptible cells (Figure 1). SARS-CoV and MERS-CoV
14 engage receptors of both human and the natural animal hosts (12, 16). Similarly, the
15 ability of pangolin ACE2 to confer the susceptibility to SARS-CoV-2 entry increases
16 the possibility that SARS-CoV-2 originated from pangolins.

17 In this study, we demonstrated that ACE2 of pig and dog rendered SARS-CoV-2
18 entry. However, a recent study reported that SARS-CoV-2 replicated poorly in dogs
19 and pigs (6). We speculate that there exist other factors determining the host tropism
20 besides receptor interaction. A recent study demonstrated that pigs and dogs exhibit
21 relatively low levels of ACE2 in the respiratory tract, this may be the reason that
22 SARS-CoV-2 replicated poorly in dogs and pigs (22). Although dogs and pigs may

1 not sensitive for SARS-CoV-2 infection, we do not know whether these animals are
2 appeared as asymptomatic carriers of SARS-CoV-2 in certain environments. In
3 humans asymptotically infected with SARS-CoV-2, the viral loads were reported
4 similar to that in the symptomatic patients, which implicates the similar risk of viral
5 transmission from asymptomatic carriers (23, 24).

6 Based on structure analysis of human ACE2 and spikes of SARS-CoV-2, the
7 receptor binding domain (RBD) of the spike takes a more compact conformation than
8 SARS-CoV, implicating a relation to the higher transmission of SARS-CoV-2 than
9 SARS-CoV (25, 26). However, in mouse ACE2, the Lys at site 353 is substituted by
10 His, which does not fit into the virus-receptor binding interface as tightly as the lysine
11 at the same site of human ACE2. Consequently, this may result in the failure of mice
12 ACE2 confer the susceptibility to SARS-CoV-2 entry. A recent publication also
13 demonstrated that the substitution of Lys353 of human ACE2 (hACE2) by Ala was
14 sufficient to abolish the interaction between hACE2 and the S protein of SARS-CoV-2
15 (27). In addition, although the residue at site 217 of ACE2 is not directly contact to
16 the RBD, this site in Rhesus monkey ACE2 is still critical for SARS-CoV-2 entry. It
17 was observed that the natural variation of Y217N at this site of the monkey ACE-2
18 significantly reduced the susceptibility to SARS-CoV entry, which demonstrated that
19 this residual variation is responsible for the down regulation of ACE2 expression (28).
20 However, our results showed that this Y217N-isoform of ACE2 expressed at a similar
21 level as the human ACE2 in transfected cells (data not shown). Therefore, the failure
22 of monkey ACE2 isoform to convert the cell susceptibility to SARS-CoV-2 entry is

1 not due to the poor expression of the receptor as previously speculated (28). The
2 detailed mechanism needs further investigation. It is also important to detect the
3 expression ratio of the Y217N ACE2 to the prototype in Rhesus monkeys to be
4 recruited for studies on SARS-CoV-2 infection.

5 **MATERIALS AND METHODS**

6 **Cells and SARS-CoV-2**

7 HEK293T cells cells were maintained in DMEM (Gibco, USA) with 10% fetal bovine
8 serum (HyClone, USA). The SARS-CoV-2 used in this study (GenBank: MT123290)
9 was isolated from a patient's throat swab and stored at the Biosafety Level 3
10 Laboratory of Guangzhou Customs Technical Center.

11 **Plasmids**

12 The full-length cDNA fragments of different species of ACE2 were synthesized at
13 either the Sangon Biotech (Shanghai, China) or TsingKe Biotech (Nanjing, China).
14 The species and GenBank accession numbers of ACE2 sequences were listed in the
15 Table. Synthesized DNA fragments were then sub-cloned into a eukaryotic expression
16 vector pCAGGS-HA for the expression in human cell lines.

17 **Sequence analysis**

18 ACE2 sequences of 12 different species were acquired from NCBI and their
19 alignment were assessed using the ClustalW method in Lasergene software (Version
20 7.1) (DNASTAR Inc., USA).

21 **Entry assay**

1 HEK293T cells were plated in 48-well plates, and were transfected with indicated
2 plasmids by the X-tremeGENE HP DNA Transfection Reagent (Roche, USA) when
3 the cell confluence was up to 90%. Cells were infected with 0.5 MOI of SARS-CoV-2
4 24 h after being transfected. The detection of infected cells were performed 12 h late
5 by using an immunofluorescence assay as described previously (29). An HA-Alexa
6 Fluor 488 monoclonal antibody (Thermo Fisher Scientific, USA) was used to stain
7 ACE2 with an HA tag. The nucleoprotein (N) of SARS-CoV was detected for
8 infection and replication of the virus using an N-specific polyclonal antibody
9 (Sinobiological, China), and a donkey anti-rabbit IgG (H+L) labeled with Cy3
10 (Jackson, USA) was used as the secondary antibody. All the cells were stained with
11 DAPI (Sigma, USA) for nuclear visualization.

12 **Compliance with ethical standards**

13 **Funding:** This study was funded by grants from The National Key Research and
14 Development Program of China (2018YFC1200100, 2018ZX10301403), the
15 emergency grants for prevention and control of SARS-CoV-2 of Ministry of Science
16 and Technology (2020YFC0841400) and Guangdong province (2020B111108001,
17 2018B020207013).

18 **Conflicts of interest:** Authors declare no conflict of interest.

19 **Ethical approval:** This article does not contain any studies with human participants

20 **or Acknowledgments**

21 The authors are grateful to Prof. Jian-Hua Zhou for kindly revising the manuscript.

22

1 Reference

- 2 1. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL,
3 Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X,
4 Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF,
5 Shi ZL. 2020. A pneumonia outbreak associated with a new coronavirus of probable
6 bat origin. *Nature* 579:270-273.
- 7 2. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY,
8 Yuan ML, Zhang YL, Dai FH, Liu Y, Wang QM, Zheng JJ, Xu L, Holmes EC, Zhang YZ.
9 2020. A new coronavirus associated with human respiratory disease in China. *Nature*
10 579:265-269.
- 11 3. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma
12 X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J,
13 Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen
14 W, Shi W, Tan W. 2020. Genomic characterisation and epidemiology of 2019 novel
15 coronavirus: implications for virus origins and receptor binding. *Lancet* 395:565-574.
- 16 4. Lam TT, Shum MH, Zhu HC, Tong YG, Ni XB, Liao YS, Wei W, Cheung WY, Li WJ, Li
17 LF, Leung GM, Holmes EC, Hu YL, Guan Y. 2020. Identifying SARS-CoV-2 related
18 coronaviruses in Malayan pangolins. *Nature* doi:10.1038/s41586-020-2169-0.
- 19 5. Zhang T, Wu Q, Zhang Z. 2020. Probable Pangolin Origin of SARS-CoV-2 Associated
20 with the COVID-19 Outbreak. *Curr Biol* doi:10.1016/j.cub.2020.03.022.
- 21 6. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, Liu R, He X, Shuai L, Sun Z, Zhao
22 Y, Liu P, Liang L, Cui P, Wang J, Zhang X, Guan Y, Tan W, Wu G, Chen H, Bu Z.

- 1 2020. Susceptibility of ferrets, cats, dogs, and other domesticated animals to
2 SARS-coronavirus 2. Science doi:10.1126/science.abb7015.
- 3 7. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan
4 JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. 2003. Angiotensin-converting
5 enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426:450-4.
- 6 8. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S,
7 Schiergens TS, Herrler G, Wu NH, Nitsche A, Muller MA, Drosten C, Pohlmann S.
8 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a
9 Clinically Proven Protease Inhibitor. Cell doi:10.1016/j.cell.2020.02.052.
- 10 9. Wan Y, Shang J, Graham R, Baric RS, Li F. 2020. Receptor Recognition by the Novel
11 Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of
12 SARS Coronavirus. J Virol 94.
- 13 10. Li W, Wong SK, Li F, Kuhn JH, Huang IC, Choe H, Farzan M. 2006. Animal origins of
14 the severe acute respiratory syndrome coronavirus: insight from ACE2-S-protein
15 interactions. J Virol 80:4211-9.
- 16 11. Ren W, Qu X, Li W, Han Z, Yu M, Zhou P, Zhang SY, Wang LF, Deng H, Shi Z. 2008.
17 Difference in receptor usage between severe acute respiratory syndrome (SARS)
18 coronavirus and SARS-like coronavirus of bat origin. J Virol 82:1899-907.
- 19 12. Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, Epstein JH, Mazet JK, Hu B, Zhang W,
20 Peng C, Zhang YJ, Luo CM, Tan B, Wang N, Zhu Y, Crameri G, Zhang SY, Wang LF,
21 Daszak P, Shi ZL. 2013. Isolation and characterization of a bat SARS-like coronavirus
22 that uses the ACE2 receptor. Nature 503:535-8.

- 1 13. Hou Y, Peng C, Yu M, Li Y, Han Z, Li F, Wang LF, Shi Z. 2010. Angiotensin-converting
2 enzyme 2 (ACE2) proteins of different bat species confer variable susceptibility to
3 SARS-CoV entry. *Arch Virol* 155:1563-9.
- 4 14. Raj VS, Mou H, Smits SL, Dekkers DH, Muller MA, Dijkman R, Muth D, Demmers JA,
5 Zaki A, Fouchier RA, Thiel V, Drosten C, Rottier PJ, Osterhaus AD, Bosch BJ,
6 Haagmans BL. 2013. Dipeptidyl peptidase 4 is a functional receptor for the emerging
7 human coronavirus-EMC. *Nature* 495:251-4.
- 8 15. Lau SKP, Fan RYY, Luk HKH, Zhu L, Fung J, Li KSM, Wong EYM, Ahmed SS, Chan
9 JFW, Kok RKH, Chan KH, Wernery U, Yuen KY, Woo PCY. 2018. Replication of
10 MERS and SARS coronaviruses in bat cells offers insights to their ancestral origins.
11 *Emerg Microbes Infect* 7:209.
- 12 16. Yang Y, Du L, Liu C, Wang L, Ma C, Tang J, Baric RS, Jiang S, Li F. 2014. Receptor
13 usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human
14 transmission of MERS coronavirus. *Proc Natl Acad Sci U S A* 111:12516-21.
- 15 17. Li F. 2008. Structural analysis of major species barriers between humans and palm
16 civets for severe acute respiratory syndrome coronavirus infections. *J Virol*
17 82:6984-91.
- 18 18. Wu K, Peng G, Wilken M, Geraghty RJ, Li F. 2012. Mechanisms of host receptor
19 adaptation by severe acute respiratory syndrome coronavirus. *J Biol Chem*
20 287:8904-11.
- 21 19. Lu S, Zhao, Y., Yu, W., Yang, Y., Gao, J., Wang, J., Kuang, D., Yang, M., Yang, J., Ma,
22 C., et, al. 2020. Comparison of SARS-CoV-2 infections among 3 species of

- 1 non-human primates. bioRxiv.
- 2 20. Ji W, Wang W, Zhao X, Zai J, Li X. 2020. Cross-species transmission of the newly
3 identified coronavirus 2019-nCoV. *J Med Virol* 92:433-440.
- 4 21. Zheng Y, Shang J, Yang Y, Liu C, Wan Y, Geng Q, Wang M, Baric R, Li F. 2018.
5 Lysosomal Proteases Are a Determinant of Coronavirus Tropism. *J Virol* 92.
- 6 22. Zhai X, Sun J, Yan Z, Zhang J, Zhao J, Zhao Z, Gao Q, He WT, Veit M, Su S. 2020.
7 Comparison of SARS-CoV-2 spike protein binding to ACE2 receptors from human,
8 pets, farm animals, and putative intermediate hosts. *J Virol* doi:10.1128/JVI.00831-20.
- 9 23. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J,
10 Guo Q, Song T, He J, Yen HL, Peiris M, Wu J. 2020. SARS-CoV-2 Viral Load in Upper
11 Respiratory Specimens of Infected Patients. *N Engl J Med* 382:1177-1179.
- 12 24. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T,
13 Thiel V, Janke C, Guggemos W, Seilmaier M, Drosten C, Vollmar P, Zwirgmaier K,
14 Zange S, Wolfel R, Hoelscher M. 2020. Transmission of 2019-nCoV Infection from an
15 Asymptomatic Contact in Germany. *N Engl J Med* 382:970-971.
- 16 25. Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, Geng Q, Auerbach A, Li F. 2020.
17 Structural basis of receptor recognition by SARS-CoV-2. *Nature*
18 doi:10.1038/s41586-020-2179-y.
- 19 26. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. 2020. Structural basis for the recognition
20 of the SARS-CoV-2 by full-length human ACE2. *Science*
21 doi:10.1126/science.abb2762.
- 22 27. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuen KY,

- 1 Zhou H, Yan J, Qi J. 2020. Structural and Functional Basis of SARS-CoV-2 Entry by
2 Using Human ACE2. *Cell* doi:10.1016/j.cell.2020.03.045.
- 3 28. Chen Y, Liu L, Wei Q, Zhu H, Jiang H, Tu X, Qin C, Chen Z. 2008. Rhesus angiotensin
4 converting enzyme 2 supports entry of severe acute respiratory syndrome coronavirus
5 in Chinese macaques. *Virology* 381:89-97.
- 6 29. Yang YL, Meng F, Qin P, Herrler G, Huang YW, Tang YD. 2020. Trypsin promotes
7 porcine deltacoronavirus mediating cell-to-cell fusion in a cell type-dependent manner.
8 *Emerg Microbes Infect* 9:457-468.
- 9
- 10
- 11

1 **Figure 1. Susceptibility to SARS-CoV-2 of HEK293T cells conferred by different**
2 **species of ACE2.** HEK293T cells were transfected with plasmids expressing
3 indicated ACE2. Cells were infected with 0.5 MOI of SARS-CoV-2 24 h after the
4 transfection, and were detected for the replication of SARS-CoV-2 by IFA.

5 **Figure 2. Sequence composition of the Rhesus monkey ACE2 cloned in this study**
6 **with that of the prototype monkey ACE2 and susceptibility to SARS-CoV-2 of**
7 **217 restoration.** (A) Two sites of natural variation (R192G and Y217N) were
8 identified in the cDNA of Rhesus monkey ACE cloned in this study were compared
9 with the monkey prototype ACE and the human ACE. (B) HEK293T cells were
10 transfected with plasmids expressing indicated ACE2. Cells were infected with 0.5
11 MOI of SARS-CoV-2 24 h after the transfection, and were detected for the replication
12 of SARS-CoV-2 by IFA.

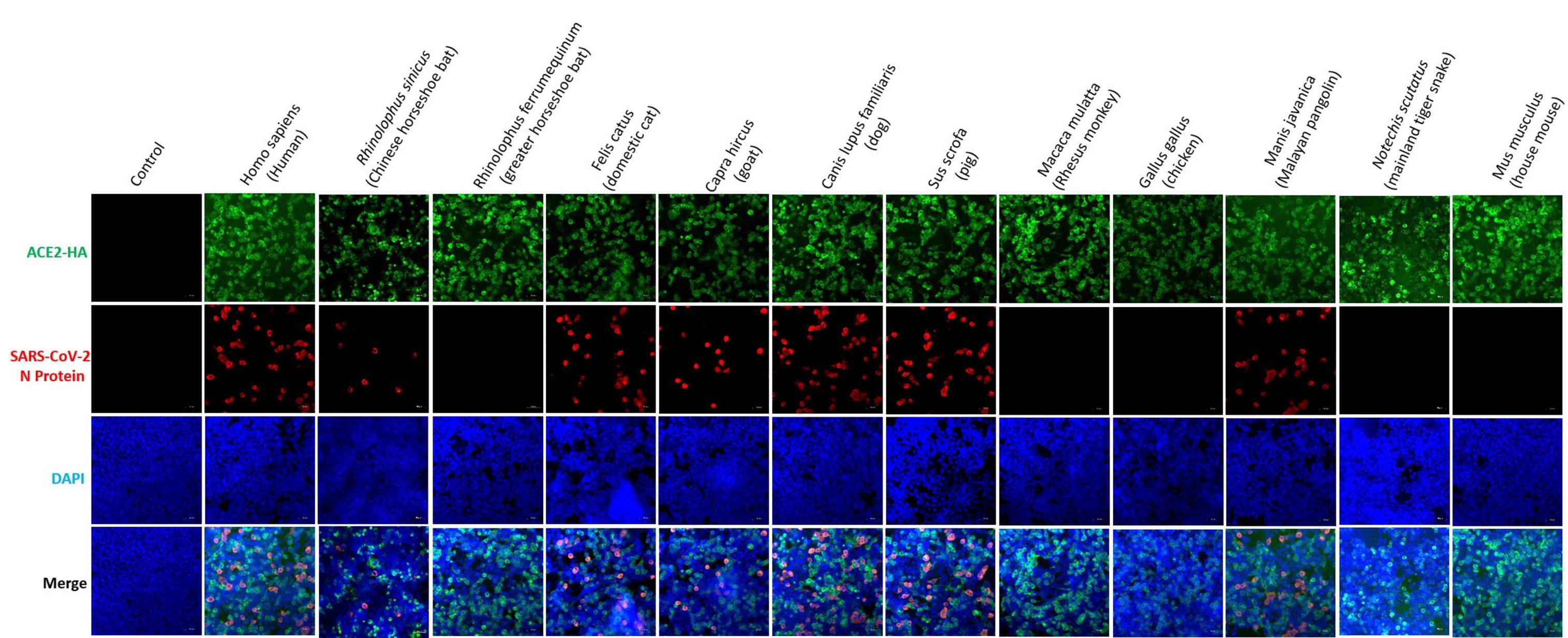
13

14

Table 1. Nucleotide sequence similarity of various animal ACE-2 to human ACE-2.

ACE-2 origin	Length of coding sequence (bp)	Similarity to human ACE-2 (%)	Position 31	Position 353	GenBank accession number
<i>Homo sapiens</i> (Human)	2418	100	K	K	AB046569.1
<i>Rhinolophus ferrumequinum</i> (Greater horseshoe bat)	2418	86.2	D	K	AB297479.1
<i>Rhinolophus sinicus</i>	2418	85.5	E	K	GQ262791.1
<i>Macaca mulatta</i> (Rhesus monkey)*	2418	96.6	K	K	NM_001135696.1
<i>Sus scrofa</i> (Pig)	2418	84.5	K	K	NM_001123070.1
<i>Canis lupus familiaris</i> (Dog)	2415	87	K	K	NM_001165260.1
<i>Capra hircus</i> (Goat)	2415	85.5	K	K	KF921008.1
<i>Felis catus</i> (Cat)	2418	86.8	K	K	AY957464.1
<i>Gallus gallus</i> (Chicken)	2427	68.1	E	K	MK560199.1
<i>Manis javanica</i> (Malayan pangolin)	2418	86.5	K	K	XM_017650257.1
<i>Notechis scutatus</i> (mainland tiger snake)	2487	66.5	Q	K	XM_026674969
<i>Mus musculus</i> (Mouse)	2418	85.2	N	H	NM_001130513.1

*NOTE: The sequence of *Macaca mulatta* (Rhesus monkey) here is with a natural mutation Y217N.



A

192 217

Homo sapiens M A R A N H Y E D Y G D Y W R G D Y E V N G V D G Y D Y S R G

Macaca mulatta M A R A N H Y K D Y G D Y W R G D Y E V N G V D G Y D Y N R D

Macaca mulatta
isoform M A G A N H Y K D Y G D Y W R G D Y E V N G V D G Y D N R D

B