

1 **The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor**  
2 **ACE2 and the cellular protease TMPRSS2 for entry into target cells**

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24 **Abstract: The emergence of a novel, highly pathogenic coronavirus, 2019-nCoV, in China,**  
25 **and its rapid national and international spread pose a global health emergency.**  
26 **Coronaviruses use their spike proteins to select and enter target cells and insights into**  
27 **nCoV-2019 spike (S)-driven entry might facilitate assessment of pandemic potential and**  
28 **reveal therapeutic targets. Here, we demonstrate that 2019-nCoV-S uses the SARS-**  
29 **coronavirus receptor, ACE2, for entry and the cellular protease TMPRSS2 for 2019-nCoV-**  
30 **S priming. A TMPRSS2 inhibitor blocked entry and might constitute a treatment option.**  
31 **Finally, we show that the serum from a convalescent SARS patient neutralized 2019-nCoV-**  
32 **S-driven entry. Our results reveal important commonalities between 2019-nCoV and**  
33 **SARS-coronavirus infection, which might translate into similar transmissibility and disease**  
34 **pathogenesis. Moreover, they identify a target for antiviral intervention.**

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36 **One sentence summary:** The novel 2019 coronavirus and the SARS-coronavirus share central  
37 biological properties which can guide risk assessment and intervention.

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47 Several members of the family *Coronaviridae* constantly circulate in the human population and  
48 usually cause mild respiratory disease (1). In contrast, the severe acute respiratory syndrome-  
49 associated coronavirus (SARS-CoV) and the Middle East respiratory syndrome-associated  
50 coronavirus (MERS-CoV) are transmitted from animals to humans and cause severe respiratory  
51 diseases in afflicted human patients, SARS and MERS, respectively (2). SARS emerged in 2002  
52 in Guangdong province, China, and its subsequent global spread was associated with 8096 cases  
53 and 774 deaths (3, 4). The virus uses Chinese horseshoe bats as natural reservoir (5, 6) and is  
54 transmitted via intermediate hosts to humans. Thus, SARS-CoV was identified in Civet cats and  
55 raccoon dogs, which are sold as food sources in Chinese wet markets (7). No specific antivirals  
56 or approved vaccines are available to combat SARS and the SARS pandemic in 2002/2003 was  
57 stopped by conventional control measures, including travel restrictions and patient isolation.

58 In December 2019 a new infectious respiratory disease emerged in Wuhan, Hubei  
59 province, China (8-10). Initial infections occurred at Huanan seafood market, potentially due to  
60 animal contact. Subsequently, human-to-human transmission occurred (11)  
61 and the disease rapidly spread within China. A novel coronavirus, 2019-nCoV, which is closely  
62 related to SARS-CoV, was detected in patients and is believed to be the etiologic agent of the  
63 new lung disease (10). On January 28, 2020, at total of 4593 laboratory confirmed infections  
64 were reported, including 976 severe cases and 106 deaths (12). Infections were also detected in  
65 14 countries outside China and were associated with international travel. At present, it is  
66 unknown whether the sequence similarities between 2019-nCoV and SARS-CoV translate into  
67 similar biological properties, including pandemic potential (13).

68 The spike (S) protein of coronaviruses facilitates viral entry into target cells. Entry  
69 depends on S protein binding to a cellular receptor and on S protein priming by a cellular  
70 protease. SARS-S engages angiotensin-converting enzyme 2 (ACE2) as entry receptor (14) and

71 employs the cellular serine protease TMPRSS2 for S protein priming (15-17). The SARS-  
72 S/ACE2 interface has been elucidated and the efficiency of ACE2 usage was found to be a key  
73 determinant of SARS-CoV transmissibility (6, 18). SARS-S and 2019-nCoV-S share ~76%  
74 amino acid identity. However, it is unknown whether 2019-nCoV-S like SARS-S employs ACE2  
75 and TMPRSS2 for host cell entry.

76 Replication-defective vesicular stomatitis virus (VSV) particles bearing coronavirus S  
77 proteins faithfully reflect key aspects of coronavirus host cell entry (19). We employed VSV  
78 pseudotypes bearing 2019-nCoV-S to study cell entry of 2019-nCoV. Both 2019-nCoV-S and  
79 SARS-S were comparably expressed (Fig. 1A) and incorporated into VSV particles (Fig. 1B),  
80 allowing a meaningful side-by-side comparison. We first focused on 2019-nCoV cell tropism.  
81 Transduction of cell lines of animal and human origin revealed that all cell lines were readily  
82 susceptible to entry driven by the pantropic VSV glycoprotein (VSV-G) (Fig. 1C), as expected.  
83 (Fig. 1C). Notably, 2019-nCoV-S facilitated entry into an identical spectrum of cell lines as  
84 SARS-S (Fig. 1C), suggesting similarities in receptor choice.

85 Sequence analysis revealed that 2019-nCoV clusters with SARS-CoV-related viruses  
86 from bats (SARSr-CoV), of which some but not all can use ACE2 for host cell entry (Fig. 2A and  
87 fig. S1). Analysis of the receptor binding motif (RBM), a portion of the receptor binding domain  
88 (RBD) that makes contact with ACE2, revealed that most amino acid residues essential for ACE2  
89 binding were conserved in 2019-nCoV-S but not in the S proteins of SARSr-CoV previously  
90 found not to use ACE2 for entry (Fig. 2B). In agreement with these findings, directed expression  
91 of human and bat ACE2 but not human DPP4, the entry receptor used by MERS-CoV (20), or  
92 human APN, the entry receptor used by HCoV-229E (21), allowed 2019-nCoV-S- and SARS-S-  
93 driven entry into otherwise non-susceptible BHK-21 cells (Fig. 2C), indicating that 2019-nCoV-S  
94 like SARS-S uses ACE2 for cellular entry.

95 We next investigated protease dependence of 2019-nCoV entry. SARS-CoV can use the  
96 endosomal cysteine proteases cathepsin B and L (CatB/L) for S protein priming in TMPRSS2<sup>-</sup>  
97 cell lines (22). However, TMPRSS2 is expressed in viral target cells in the lung (23) and entry  
98 into TMPRSS2<sup>+</sup> cell lines is promoted by TMPRSS2 (15-17) and is partially CatB/L  
99 independent, although blockade of both proteases is required for efficient entry inhibition (24).  
100 Moreover, TMPRSS2 but not CatB/L activity is essential for spread of SARS-CoV and other  
101 coronaviruses in the infected host (25, 26). For initial insights into 2019-nCoV-S protease choice,  
102 we employed ammonium chloride, which elevates endosomal pH and thereby blocks CatB/L  
103 activity. Ammonium chloride treatment blocked VSV-G-driven entry into both cell lines studied  
104 while entry driven by the Nipah virus F and G proteins was not affected (Fig. 3A), in keeping  
105 with expectations. Moreover, ammonium chloride treatment strongly inhibited 2019-nCoV-S-  
106 and SARS-S-driven entry into TMPRSS2<sup>-</sup> 293T cells while inhibition of entry into TMPRSS2<sup>+</sup>  
107 Caco-2 cells was less efficient, which would be compatible with 2019-nCoV-S priming by  
108 TMPRSS2 in Caco-2 cells. Indeed, the serine protease inhibitor camostat mesylate, which is  
109 active against TMPRSS2 (24), efficiently blocked 2019-nCoV-S-driven entry into Caco-2  
110 (TMPRSS2<sup>+</sup>) but not 293T (TMPRSS2<sup>-</sup>) cells while the CatB/L inhibitor E64d had the opposite  
111 effect (Fig. 3B). Moreover, directed expression of TMPRSS2 rescued 2019-nCoV-S-driven entry  
112 from inhibition by E64d (Fig. 3C), demonstrating that 2019-nCoV-S uses TMPRSS2 for priming.

113 Convalescent SARS patients exhibit a neutralizing antibody response directed against the  
114 viral S protein (27). We investigated whether such antibodies block 2019-nCoV-S-driven entry.  
115 Serum from a convalescent SARS patient inhibited SARS-S- but not VSV-G-driven entry in a  
116 concentration dependent manner (Fig. 4). In addition, the serum reduced 2019-nCoV-S-driven  
117 entry, although with somewhat lower efficiency as compared to SARS-S (Fig. 4). Thus, antibody

118 responses raised against SARS-S during infection or vaccination might offer some protection  
119 against 2019-nCoV infection.

120 The finding that 2019-nCoV-S and SARS-S use the same receptor, ACE2, for entry into  
121 target cells has important implications for our understanding of 2019-nCoV transmissibility and  
122 pathogenesis. Thus, one can expect that 2019-nCoV targets the same cells as SARS-CoV and that  
123 the previously documented modest ACE2 expression in the upper respiratory tract (23, 28) might  
124 limit 2019-nCoV transmissibility. Moreover, it is noteworthy that ACE2 expression is not limited  
125 to the lung and that extrapulmonary spread of SARS-CoV in ACE2<sup>+</sup> tissues was observed (29,  
126 30). The same can be expected for 2019-nCoV, although affinity of SARS-S and 2019-nCoV-S  
127 for ACE2 remains to be compared.

128 Priming of coronavirus S proteins by host cell proteases is essential for viral entry into  
129 cells and protease choice can determine zoonotic potential (31). The S proteins of SARS-CoV  
130 can use the endosomal cysteine proteases for S protein priming in TMPRSS2<sup>-</sup> cells (22).  
131 However, S protein priming by TMPRSS2 but not CatB/L is essential for viral entry into primary  
132 target cells and for viral spread in the infected host (24-26). The present study suggests that 2019-  
133 nCoV spread might also depend on TMPRSS2 activity and it is noteworthy that the serine  
134 protease inhibitor camostat mesylate blocks TMPRSS2 activity (24, 26) and has been approved in  
135 Japan for human use, although for an unrelated indication. This compound or related ones should  
136 be considered for treatment of 2019-nCoV infected patients.

137 Convalescent SARS patients exhibit a neutralizing antibody response that can be detected  
138 even 24 months after infection (27) and this is largely directed against the S protein. Moreover,  
139 experimental SARS vaccines, including recombinant S protein (32) and inactivated virus (33)  
140 induce neutralizing antibody responses. Our results, although limited in significance due to a  
141 single patient serum being available for testing, indicate that neutralizing antibody responses

142 raised against SARS-S should also offer some protection against 2019-nCoV infection, which  
143 may have implications for outbreak control.

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268 provided upon signature of an appropriate material transfer agreement. All data are available in  
269 the main text or the supplementary materials

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285 **Supplementary Materials**

286 Figure S1

287

288 **Figure legends**

289

290 **Fig. 1. 2019-nCoV-S and SARS-S facilitates entry into a similar panel of mammalian cell**

291 **lines.** Analysis of 2019-nCoV-S expression (A) and pseudotype incorporation (B) by Western

292 blot. Representative blots from three experiments are shown.  $\beta$ -Actin (cell lysates) and VSV-M

293 (particles) served as loading controls. (C) Cell lines of human and animal origin were inoculated

294 with pseudotyped VSV harboring VSV-G, SARS-S or 2019-nCoV-S. At 16 h postinoculation,

295 pseudotype entry was analyzed. Shown are the combined data of three experiments. Error bars

296 indicate SEM.

297

298 **Fig. 2. 2019-nCoV-S utilizes ACE2 as cellular receptor.** (A) The S protein of 2019-nCoV

299 clusters phylogenetically with S proteins of known bat-associated betacoronaviruses (see also SI

300 Figure 1 for more details). (B) Alignment of the receptor binding motif of SARS-S with

301 corresponding sequences of bat-associated betacoronavirus S proteins that are able or unable to

302 use ACE2 as cellular receptor reveals that 2019-nCoV possesses amino acid residues crucial for

303 ACE2 binding. (C) 293T cells transiently expressing ACE2 of human (dark blue) or bat (light

304 blue) origin, human APN (purple) or hDPP4 (green) were inoculated with pseudotyped VSV

305 harboring VSV-G, SARS-S, 2019-nCoV-S, MERS-S or 229E-S. At 16 h postinoculation,

306 pseudotype entry was analyzed. The average of three independent experiments is shown. Error

307 bars indicate SEM.

308

309 **Fig. 3. 2019-nCoV-S employs TMPRSS2 for S protein priming.** Ammonium chloride (**A**),  
310 E64d (CatB/L inhibitor) (**B**) and/or camostat (TMPRSS2 inhibitor) (**B**) were added to the  
311 indicated target cells before transduction with pseudotypes bearing the indicated glycoproteins.  
312 (**C**) 293T cells transiently expressing ACE2 alone or in combination with TMPRSS2 were  
313 incubated with CatB/L inhibitor E64d or PBS as control and inoculated with pseudotypes bearing  
314 the indicated viral surface proteins. The average of three independent experiments is shown in  
315 panels A-C. Error bars indicate SEM. Statistical significance was tested by two-way ANOVA  
316 with Dunnett posttest.

317  
318 **Fig. 4. Serum from a convalescent SARS patient cross-neutralizes 2019-nCoV-S-driven**  
319 **entry.** Pseudotypes harboring the indicated viral surface proteins were incubated with different  
320 dilutions of serum from a convalescent SARS patient and subsequently inoculated onto 293T  
321 cells that transiently express ACE2 in order to evaluate cross-neutralization. The results from a  
322 representative experiment with triplicate samples are shown and were confirmed in a separate  
323 experiment. Error bars indicate SD. Statistical significance was tested by two-way ANOVA with  
324 Dunnett posttest.

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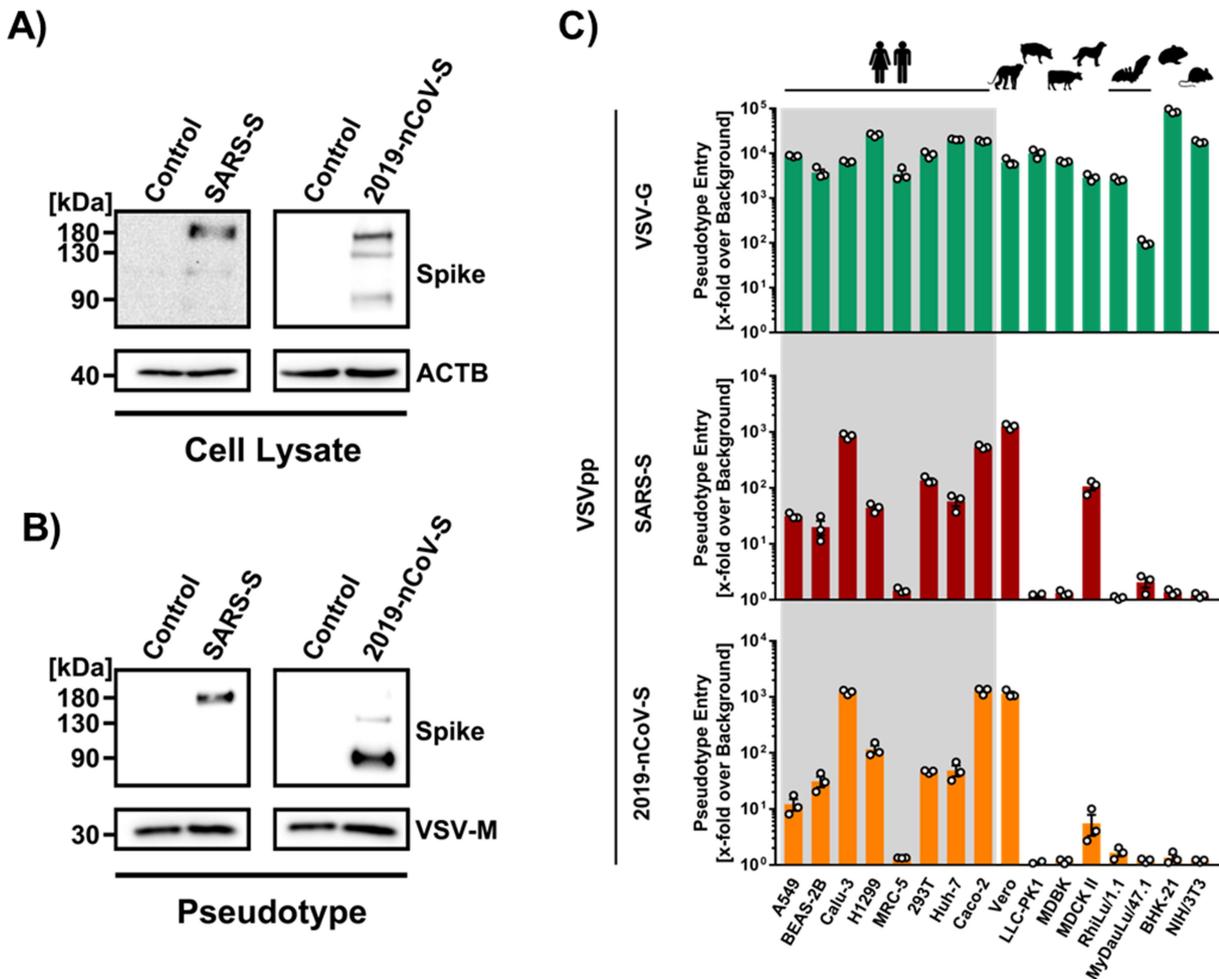
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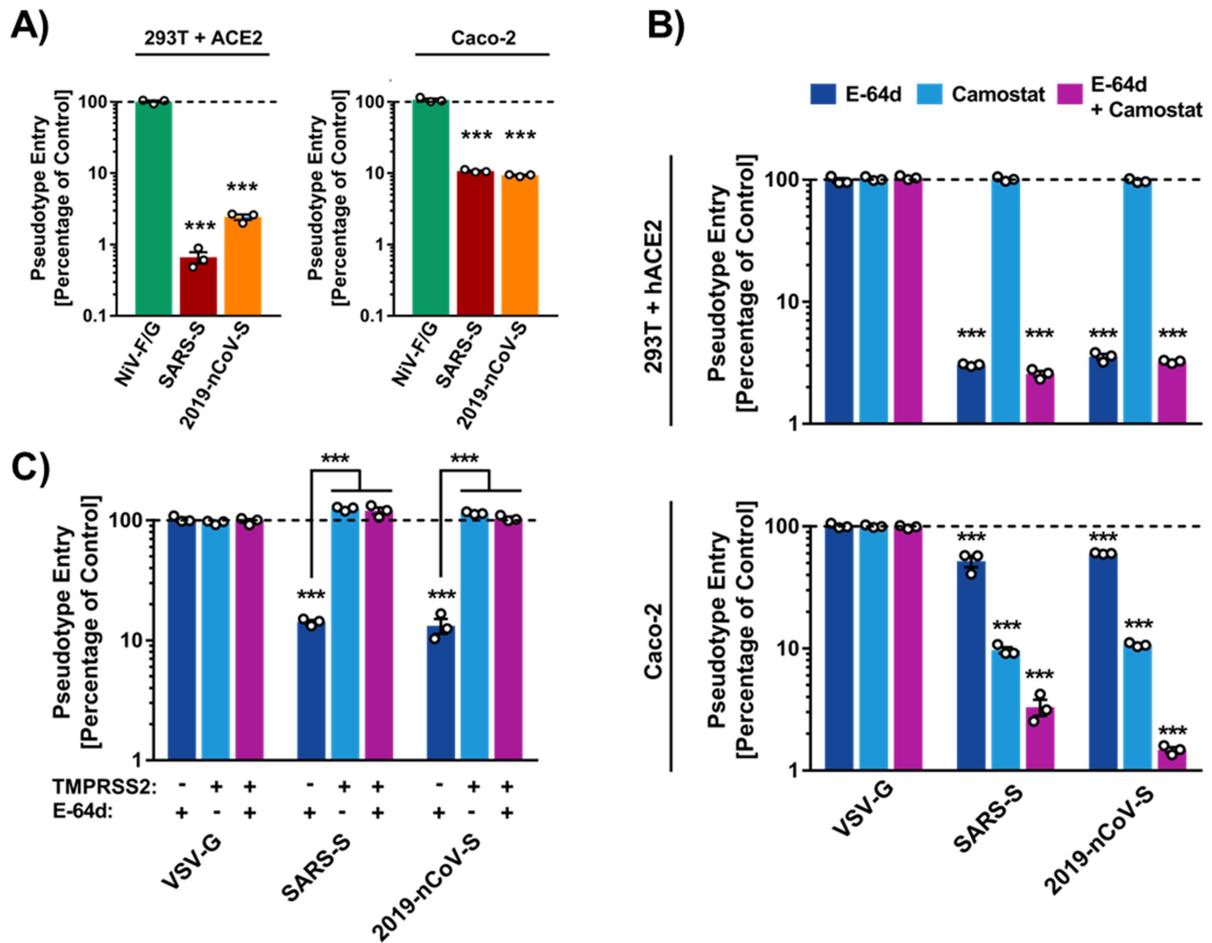
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 338 pseudotype entry was analyzed. Shown are the combined data of three experiments. Error bars  
 339 indicate SEM.

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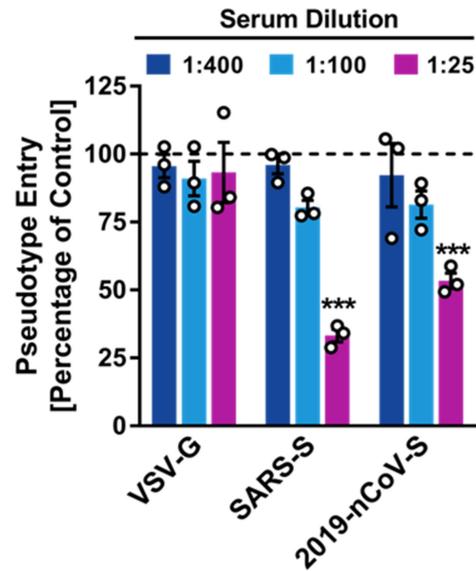
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 361 the indicated viral surface proteins. The average of three independent experiments is shown in  
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373 representative experiment with triplicate samples are shown and were confirmed in a separate  
374 experiment. Error bars indicate SD. Statistical significance was tested by two-way ANOVA with  
375 Dunnett posttest.  
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## 1 **Supplementary Materials**

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### 3 **Cell Culture**

4 All cell lines were incubated at 37 °C and 5 % CO<sub>2</sub> in a humidified atmosphere. 293T (human,  
5 kidney), BHK-21 (Syrian hamster, kidney cells), Huh-7 (human, liver), LLC-PK1 (pig, kidney),  
6 MRC-5 (human, lung), MyDauLu/47.1 (Daubenton's bat [*Myotis daubentonii*], lung), NIH/3T3  
7 (Mouse, embryo), RhiLu/1.1 (Halcyon horseshoe bat [*Rhinolophus alcyone*], lung), Vero  
8 (African green monkey, kidney) cells were incubated in Dulbecco's' modified Eagle medium  
9 (PAN-Biotech). Calu-3 (human, lung), Caco-2 (human, colon), MDBK (cattle, kidney) and  
10 MDCK II (Dog, kidney) cells were incubated in Minimum Essential Medium (ThermoFisher  
11 Scientific). A549 (human, lung), BEAS-2B (human, bronchus) and NCI-H1299 (human, lung)  
12 cells were incubated in DMEM/F-12 Medium with Nutrient Mix (ThermoFisher Scientific). All  
13 media were supplemented with 10 % fetal bovine serum (Biochrom), 100 U/ml of penicillin and  
14 0.1 mg/ml of streptomycin (PAN-Biotech), 1x non-essential amino acid solution (10x stock,  
15 PAA) and 10 mM sodium pyruvate solution (ThermoFisher Scientific). For seeding and  
16 subcultivation, cells were first washed with phosphate buffered saline (PBS) and then incubated  
17 in the presence of trypsin/EDTA solution (PAN-Biotech) until cells detached. Transfection was  
18 carried out by calcium-phosphate precipitation.

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### 20 **Plasmids**

21 Expression plasmids for vesicular stomatitis virus (VSV, serotype Indiana) glycoprotein (VSV-  
22 G), SARS-S (derived from the Frankfurt-1 isolate) with or without a C-terminal HA epitope tag,  
23 HCoV-229E-S, MERS-S, human and bat angiotensin converting enzyme 2, human  
24 aminopeptidase N, human dipeptidyl-peptidase 4 and human TMPRSS2 have been described  
25 elsewhere (1-6). For generation of the expression plasmids for 2019-nCoV-S with or without a C-  
26 terminal HA epitope tag we PCR-amplified the coding sequence of a synthetic, codon-optimized  
27 (for human cells) 2019-nCoV-S DNA (GeneArt Gene Synthesis, ThermoFisher Scientific) based  
28 on the publicly available protein sequence in the National Center for Biotechnology Information  
29 database (NCBI Reference Sequence: YP\_009724390.1) and cloned in into the pCG1 expression  
30 vector via BamHI and XbaI restriction sites.

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### 32 **Pseudotyping of VSV and transduction experiments**

33 *Pseudotyping*: VSV pseudotypes were generated according to a published protocol (7). In brief,  
34 293T transfected to express the viral surface glycoprotein under study were inoculated with a  
35 replication-deficient VSV vector that contains expression cassettes for eGFP (enhanced green

36 fluorescent protein) and firefly luciferase instead of VSV-G the open reading frame, VSV\*ΔG-  
37 fLuc (kindly provided by Gert Zimmer, Institute of Virology and Immunology,  
38 Mittelhäusern/Switzerland). After an incubation period of 1 h at 37 °C, the inoculum was  
39 removed and cells were washed with PBS before medium supplemented with anti-VSV-G  
40 antibody (I1, mouse hybridoma supernatant from CRL-2700; ATCC) was added (no antibody  
41 was added to cells expressing VSV-G). Pseudotype particles were harvested 16 h postinoculation,  
42 clarified from cellular debris by centrifugation and used for experimentations.

43 *Transduction of target cells:* Target cells were grown in 96-well plates until they reached 50-75  
44 % confluency before they were inoculated with the respective pseudotype vectors. For  
45 experiments addressing the search for the 2019-nCoV receptor cells were transfected with  
46 expression plasmids 24 h in advance. For experiments involving ammonium chloride (final  
47 concentration 50 mM) and protease inhibitors (E-64d, 25 μM; camostat mesylate, 100 μM),  
48 target cells were treated with the respective chemical 2 h in advance. For neutralization  
49 experiments, pseudotypes were pre-incubated for 30 min at 37 °C with different serum dilutions.  
50 Transduction efficiency was quantified 16 h posttransduction by measuring the activity firefly  
51 luciferase in cell lysates.

52  
53 **Analysis of 2019-nCoV-S expression and particle incorporation by SDS-PAGE and**  
54 **immunoblot**

55 *Preparation of whole cell lysates:* 293T cells were transfected with expression vectors for HA-  
56 tagged 2019-nCoV-S or SARS-S , or empty expression vector (negative control). The culture  
57 medium was replaced at 16 h posttransfection and the cells were incubated for an additional 24 h.  
58 Then, the culture medium was removed and cells were washed once with PBS before 2x SDS-  
59 sample buffer (0.03 M Tris-HCl, 10% glycerol, 2% SDS, 0.2% bromophenol blue, 1 mM EDTA)  
60 was added and cells were incubated for 10 min at room temperature. Next, the samples were  
61 heated for 15 min at 96 °C and subjected to SDS-PAGE and immunoblotting. *Preparation of*  
62 *pseudotype particle lysates:* 1 ml of the respective VSV pseudotype were loaded on a 20 % (w/v)  
63 sucrose cushion (volume 40 μl) and subjected to high-speed centrifugation (25.000 g for 120 min  
64 at 4 °C). Thereafter, 1 ml of supernatant was removed and the residual volume was mixed with  
65 40 μl of 2x SDS-sample buffer, heated for 15 min at 96 °C and subjected to SDS-PAGE and  
66 immunoblotting

67 After protein transfer, nitrocellulose membranes were blocked in 5 % skim milk (in PBS  
68 containing 0.05 % Tween-20, PBS-T) for 1 h at room temperature and then incubated over night  
69 at 4 °C with the primary antibody (diluted in PBS-T). Following three washing intervals of 10  
70 min in PBS-T the membranes were incubated for 1 h at room temperature with the secondary  
71 antibody (diluted in PBS-T), before the membranes were washed and imaged using an in in  
72 house-prepared enhanced chemiluminescent solution (0.1 M Tris-HCl [pH 8.6], 250 μg/ml  
73 luminol, 1 mg/ml para-hydroxycoumaric acid, 0.3 % H<sub>2</sub>O<sub>2</sub>) and the ChemoCam imaging system

74 along with the ChemoStar Professional software (Intas Science Imaging Instruments GmbH). The  
75 following primary antibodies were used: Mouse anti-HA tag (Sigma-Aldrich, H3663, 1:2,500),  
76 mouse anti- $\beta$ -actin (Sigma-Aldrich, A5441, 1:2,000), mouse anti-VSV matrix protein (Kerafast,  
77 EB0011, 1:2,500). As secondary antibody we used a peroxidase-coupled goat anti-mouse  
78 antibody (Dianova, 115-035-003, 1:10000).

79

## 80 **Phylogenetic analysis**

81 Phylogenetic analysis (neighbor-joining trees) was performed using the MEGA7.0.26 software.  
82 Reference sequences were obtained from the National Center for Biotechnology Information and  
83 GISAID (Global Initiative on Sharing All Influenza Data) databases. Reference numbers are  
84 indicated in the figures.

85

## 86 **Statistical analysis**

87 One-way or two-way analysis of variance (ANOVA) with Dunnett's or Sidaks' posttest was used  
88 to test for statistical significance. Only p values of 0.05 or lower were considered statistically  
89 significant ( $p > 0.05$  [ns, not significant],  $p \leq 0.05$  [\*],  $p \leq 0.01$  [\*\*],  $p \leq 0.001$  [\*\*\*]). For all  
90 statistical analyses, the GraphPad Prism 7 software package was used (GraphPad Software).

91

## 92 **Supplementary references**

93

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113

114 **Supplementary figure S1**



115

116 **Legend to supplementary figure S1.** Phylogenetic analysis (neighbor-joining tree) of spike  
117 protein sequences. Small numbers indicate bottstrap values (only values higher that 75 are  
118 shown).

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