Functional assessment of cell entry and receptor usage for lineage B β-coronaviruses, including 2019-nCoV

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SUPPLEMENTAL INFORMATION

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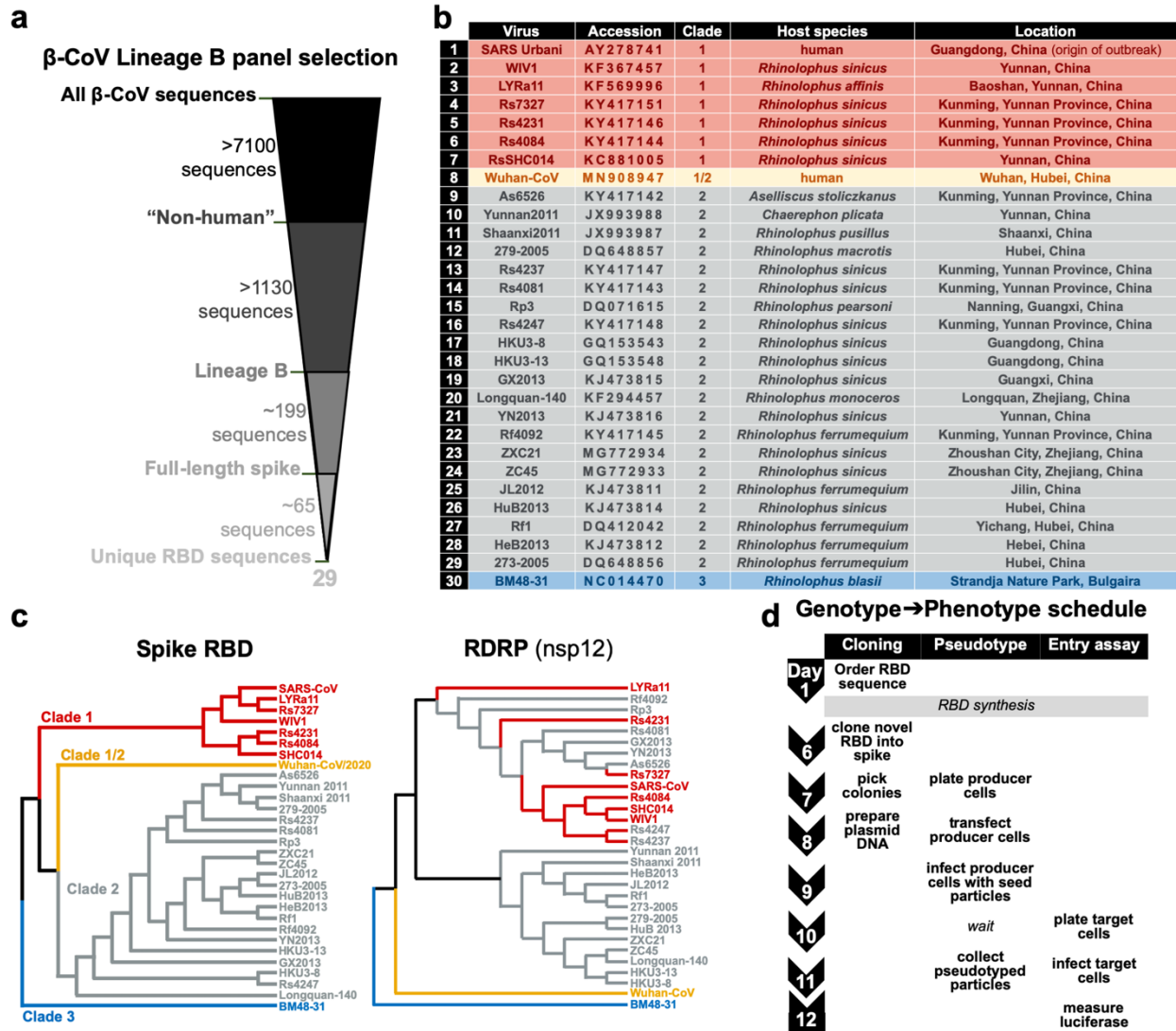Rocky Mountain Laboratories

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a

β-CoV Lineage B panel selection

All β-CoV sequences

>7100 sequences

"Non-human"

>1130 sequences

Lineage B

~199 sequences

Full-length spike

~65 sequences

Unique RBD sequences

29

b

	Virus	Accession	Clade	Host species	Location
1	SARS Urbani	AY278741	1	human	Guangdong, China (origin of outbreak)
2	WIV1	KF367457	1	*Rhinolophus sinicus*	Yunnan, China
3	LYRa11	KF569996	1	*Rhinolophus affinis*	Baoshan, Yunnan, China
4	Rs7327	KY417151	1	*Rhinolophus sinicus*	Kunming, Yunnan Province, China
5	Rs4231	KY417146	1	*Rhinolophus sinicus*	Kunming, Yunnan Province, China
6	Rs4084	KY417144	1	*Rhinolophus sinicus*	Kunming, Yunnan Province, China
7	RsSHC014	KC881005	1	*Rhinolophus sinicus*	Yunnan, China
8	Wuhan-CoV	MN908947	1/2	human	Wuhan, Hubei, China
9	As6526	KY417142	2	*Aselliscus stoliczkanus*	Kunming, Yunnan Province, China
10	Yunnan2011	JX993988	2	*Chaerephon plicata*	Yunnan, China
11	Shaanxi2011	JX993987	2	*Rhinolophus pusillus*	Shaanxi, China
12	279-2005	DQ648857	2	*Rhinolophus macrotis*	Hubei, China
13	Rs4237	KY417147	2	*Rhinolophus sinicus*	Kunming, Yunnan Province, China
14	Rs4081	KY417143	2	*Rhinolophus sinicus*	Kunming, Yunnan Province, China
15	Rp3	DQ071615	2	*Rhinolophus pearsoni*	Nanning, Guangxi, China
16	Rs4247	KY417148	2	*Rhinolophus sinicus*	Kunming, Yunnan Province, China
17	HKU3-8	GQ153543	2	*Rhinolophus sinicus*	Guangdong, China
18	HKU3-13	GQ153548	2	*Rhinolophus sinicus*	Guangdong, China
19	GX2013	KJ473815	2	*Rhinolophus sinicus*	Guangxi, China
20	Longquan-140	KF294457	2	*Rhinolophus monoceros*	Longquan, Zhejiang, China
21	YN2013	KJ473816	2	*Rhinolophus sinicus*	Yunnan, China
22	Rf4092	KY417145	2	*Rhinolophus ferrumequium*	Kunming, Yunnan Province, China
23	ZXC21	MG772934	2	*Rhinolophus sinicus*	Zhoushan City, Zhejiang, China
24	ZC45	MG772933	2	*Rhinolophus sinicus*	Zhoushan City, Zhejiang, China
25	JL2012	KJ473811	2	*Rhinolophus ferrumequium*	Jilin, China
26	HuB2013	KJ473814	2	*Rhinolophus sinicus*	Hubei, China
27	Rf1	DQ412042	2	*Rhinolophus ferrumequium*	Yichang, Hubei, China
28	HeB2013	KJ473812	2	*Rhinolophus ferrumequium*	Hebei, China
29	273-2005	DQ648856	2	*Rhinolophus ferrumequium*	Hubei, China
30	BM48-31	NC014470	3	*Rhinolophus blasii*	Strandja Nature Park, Bulgaria

c

Spike RBD

Clade 1
Clade 1/2
Clade 2
Clade 3

RDRP (nsp12)

d **Genotype→Phenotype schedule**

	Cloning	Pseudotype	Entry assay
Day 1	Order RBD sequence		
	RBD synthesis		
6	clone novel RBD into spike		
7	pick colonies	plate producer cells	
8	prepare plasmid DNA	transfect producer cells	
9		infect producer cells with seed particles	
10		*wait*	plate target cells
11		collect pseudotyped particles	infect target cells
12			measure luciferase

Supplemental figure 1: Lineage B RBD panel assembly and phylogenetic analysis

a, Coronavirus sequences were downloaded from NCBI and further parsed to 29 unique RBD variants. **b,** Virus isolate name, accession number, clade, host species and location of identification listed for the 29 unique lineage B RBDs used in this study. **c,** Cladograms for the spike RBD and coronavirus RNA-dependent RNA polymerase (nsp12). **d,** Overview of experimental timeline.
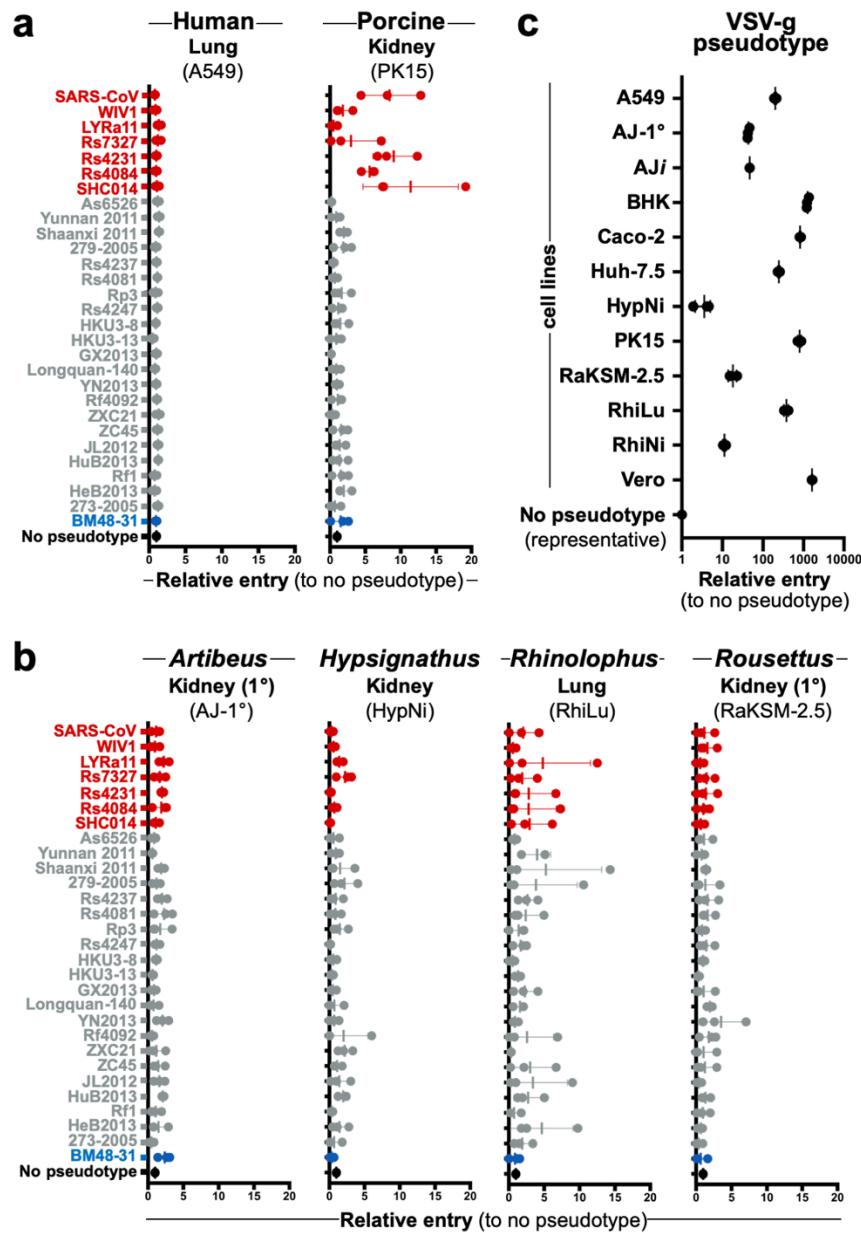
Supplemental figure 2: additional cell lines tested without protease

a, Additional human and cell lines or **b,** bat-derived cell lines derived from other species were infected with VSV-reporter particles pseudotyped with chimeric spikes and luciferase was measured a readout for cell entry. Trypsin was not used in these infections. **c,** All cell lines tested in this study supported entry and reporter expression of VSV-g pseudotyped particles. Shown are the data for 3 replicates.
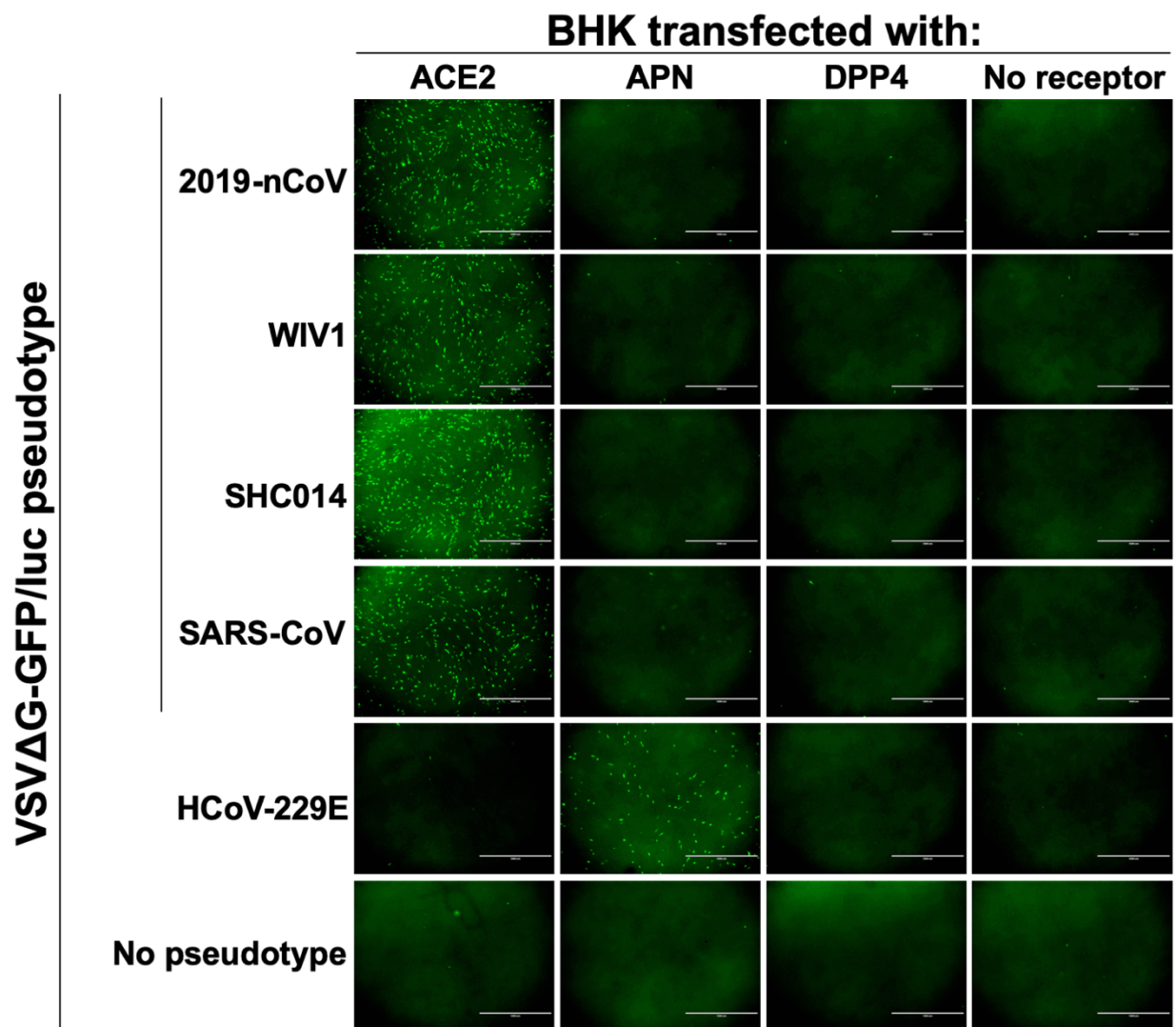
Supplemental figure 3: 2019-nCoV uses human ACE2 to enter cells

VSVΔG-GFP/luciferase particles were pseudotyped with the indicated spikes and used to infect BHKs transfected with known coronavirus receptors. Microscopy images were taken 12-20 hours-post infection.
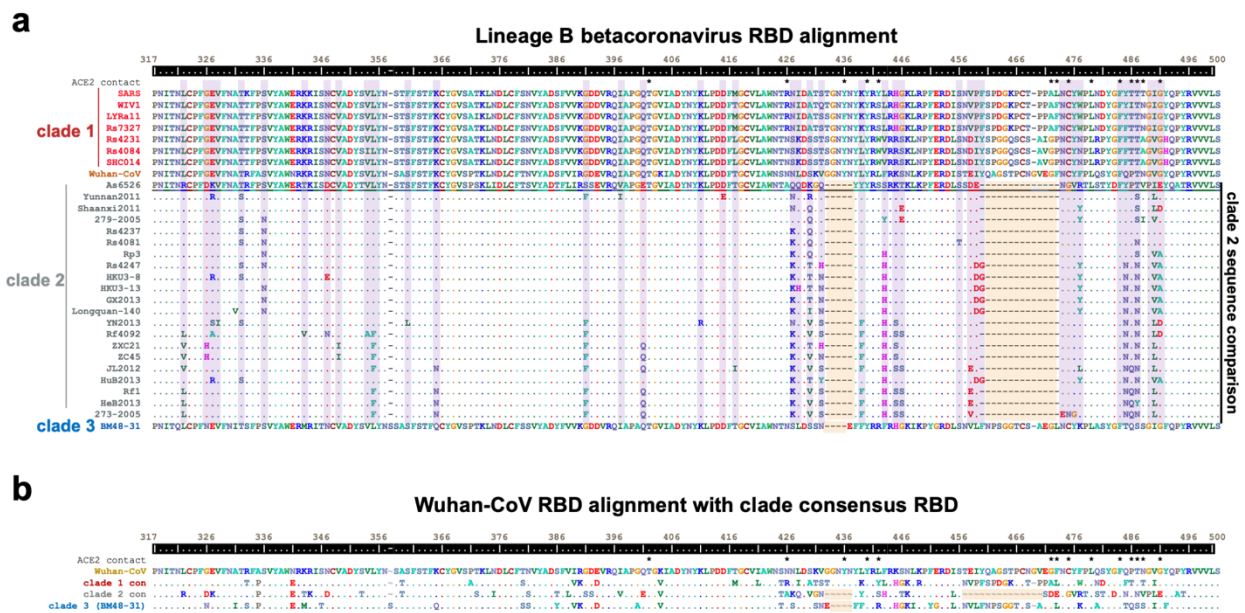
Supplemental figure 4: Lineage B panel RBD sequence features

a, Amino acid sequences corresponding to SARS-spike residues 317 through 500 were aligned with ClustalW. Contact points between SARS-spike and human ACE2 are indicated with an (*). Clade 2 sequences are shown as compared to clade 2 As6526, with identical residues indicated with a (.) and sites that vary between clade 2 viruses highlighted in purple. Loop deletions are highlighted in orange. **b,** Amino acid alignment of 2019-nCoV RBD and consensus RBD sequences for clade 1 and 2 and BM48-31 (clade 3). Loop deletions are highlighted in orange.
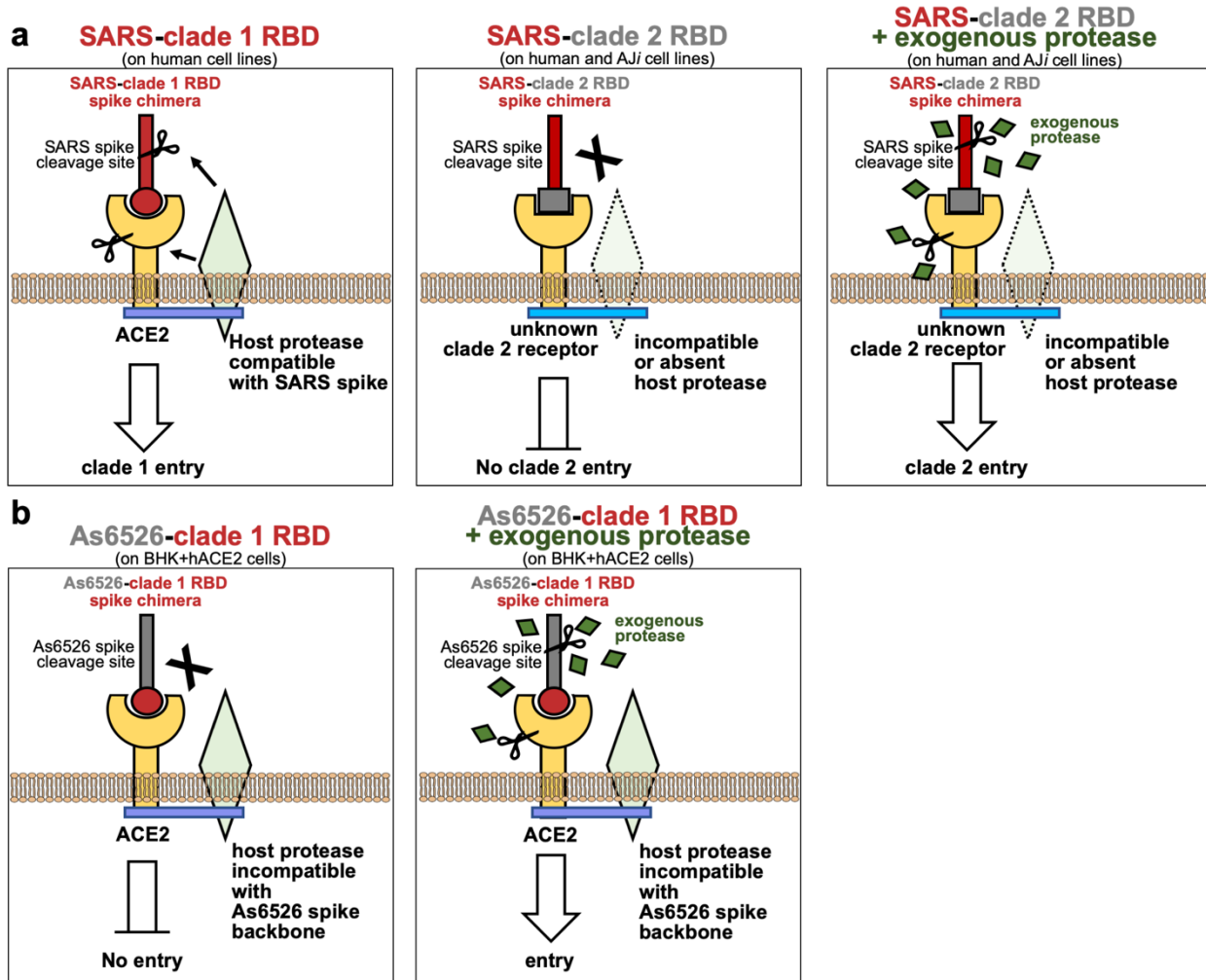
Supplemental figure 5: model of lineage B entry

a, SARS spike with a clade 1 RBD enters cells expressing ACE2 and a host protease capable of cleaving SARS spike (left panel). While the clade 2 RBD can bind an unknown host receptor, the SARS spike backbone is incompatible with the receptor-associated protease, resulting in a lack of cleavage and entry (middle panel). The addition of exogenous protease may overcome the lack of endogenous protease cleavage of spike, resulting in receptor-dependent entry. Alternatively, the addition of exogenous protease may activate the receptor to facilitate entry. **b,** Replacing the RBD of As6526 spike with the clade 1 RBD allows for ACE2 interaction, but the As6526 spike backbone is incompatible with the ACE2-associated protease (left panel). Addition of exogenous protease overcomes protease incompatibility, allowing for ACE2-mediate entry (right panel).