

Supplementary Figures S1a-S1af: Sequence alignments of the conserved coronaviral aminoacid sequences with attenuation targets identified in this review.

The WT sequence of the SARS-CoV-2 protein (top) is compared against the sequence of another WT coronavirus(es) (bottom) and the amino acid residues known to be altered in the attenuated non-SARS-CoV-2 virus are indicated. The exact mutation position is presented for both SARS-CoV-2 (top) and other coronaviruses (bottom).



Figure S1a. A sequence alignment of the Envelope protein sequences with attenuating mutations indicated – Part 1.

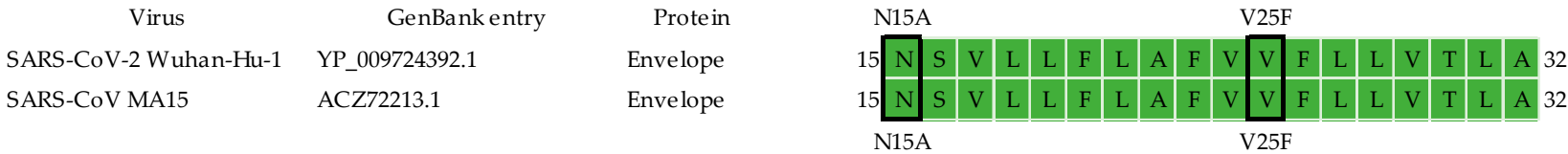


Figure S1b. A sequence alignment of the Envelope protein sequences with attenuating mutations indicated – Part 2.

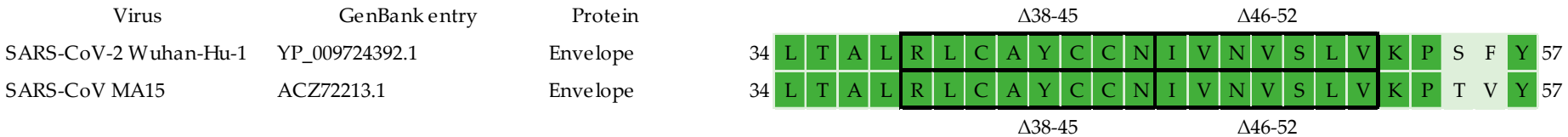


Figure S1c. A sequence alignment of the Envelope protein sequences with attenuating mutations indicated – Part 3.

Virus	GenBank entry	Protein	DLLV72-75GGGG														
SARS-CoV-2 Wuhan-Hu-1	YP_009724392.1	Envelope	64	N	L	N	S	S	R	-	V	P	D	L	L	V	75
SARS-CoV MA15	ACZ72213.1	Envelope	64	N	L	N	S	S	E	G	V	P	D	L	L	V	76
			DLLV73-76GGGG														

Figure S1d. A sequence alignment of the Envelope protein sequences with attenuating mutations indicated – Part 4.

Virus	GenBank entry	Protein	Δ 122-130																
SARS-CoV-2 Wuhan-Hu-1	YP_009725297.1	NSP1	120	V	L	L	R	K	N	G	N	K	G	A	G	G	H	S	135
SARS-CoV MA15	FJ882957.1	NSP1	120	V	L	L	R	K	N	G	N	K	G	A	G	G	H	S	135
			Δ 122-130																

Figure S1e. A sequence alignment of the NSP1 sequences with attenuating mutations indicated – Part 1.

Virus	GenBank entry	Protein	R124S K125R/K125E																		
SARS-CoV-2 Wuhan-Hu-1	YP_009725297.1	NSP1	120	V	L	L	R	K	N	G	N	K	G	A	-	-	G	G	H	S	135
MHV-A59	YP_009915673.1	NSP1	190	I	L	L	R	K	G	G	N	K	G	S	V	T	S	G	H	F	206
MHV-JHM.WU	AFO11514.1	NSP1	190	I	L	L	R	K	G	G	N	K	G	S	V	T	S	G	H	F	206
			R193S K194R/K194E																		

Figure S1f. A sequence alignment of the NSP1 sequences with attenuating mutations indicated – Part 2.

Virus	GenBank entry	Protein	F480L																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725307.1	NSP12	470	L	F	V	V	E	V	V	D	K	Y	F	D	C	Y	D	G	G	C	I	N	A	490
SARS-CoV MA15 NanoLuc	ACZ72209.1	NSP12	470	L	F	V	V	E	V	V	D	K	Y	F	D	C	Y	D	G	G	C	I	N	A	490
MHV-A59	YP_009924352.1	NSP12	466	L	F	V	L	E	V	V	N	K	Y	F	E	I	Y	E	G	G	C	I	P	A	486
			F480L/F476L																						

Figure S1g. A sequence alignment of the NSP12 sequences with attenuating mutations indicated – Part 1.

Virus	GenBank entry	Protein	V557L																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725307.1	NSP12	547	A	I	S	A	K	N	R	A	R	T	V	A	G	V	S	I	C	S	T	M	T	567
SARS-CoV MA15 NanoLuc	ACZ72209.1	NSP12	547	A	I	S	A	K	N	R	A	R	T	V	A	G	V	S	I	C	S	T	M	T	567
MHV-A59	YP_009924352.1	NSP12	543	A	I	S	A	K	N	R	A	R	T	V	A	G	V	S	I	L	S	T	M	T	563
			V557L/V553L																						

Figure S1h. A sequence alignment of the NSP12 sequences with attenuating mutations indicated – Part 2.

Virus	GenBank entry	Protein	A336V																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725307.1	NSP13	325	P	I	D	K	C	S	R	I	I	P	A	R	A	R	V	E	C	F	D	K	F	346
MHV-JHM.WU	AFO11514.1	NSP13	324	N	I	N	D	C	T	R	I	V	P	A	K	V	R	V	D	C	Y	D	K	F	345
			A335V																						

Figure S1i. A sequence alignment of the NSP13 sequences with attenuating mutations indicated.

Virus	GenBank entry	Protein	D331A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725309.1	NSP14	321	L	L	A	D	K	F	P	V	L	H	D	I	G	N	P	K	A	I	K	C	V	341
MHV-A59	YP_009924354.1	NSP14	320	M	L	C	N	R	Y	D	V	C	Y	D	I	G	N	P	K	G	L	A	C	V	340
			D330A																						

Figure S1j. A sequence alignment of the NSP14 sequences with attenuating mutations indicated – Part 1.

Virus	GenBank entry	Protein	N386A																		V398L									
SARS-CoV-2 Wuhan-Hu-1	YP_009725309.1	NSP14	380	G	V	C	L	F	W	N	C	N	V	D	R	Y	P	A	N	S	I	V	C	R	F	D	T	R	V	405
MHV-A59	YP_009924354.1	NSP14	374	G	L	C	M	F	W	N	C	N	V	D	K	Y	P	A	N	A	V	V	C	R	F	D	T	R	V	399
M41R-nsp10.14rep	AAW33784.1	NSP14	375	G	L	C	M	F	W	N	C	N	V	D	C	Y	P	D	N	S	L	V	C	R	Y	D	T	R	N	400
			N380A																		V393L									

Figure S1k. A sequence alignment of the NSP14 sequences with attenuating mutations indicated – Part 2.

Virus	GenBank entry	Protein	Y420H/Y420A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725309.1	NSP14	410	N	L	P	G	C	N	G	G	S	L	Y	V	N	K	H	A	F	H	T	S	P	430
MHV-A59	YP_009924354.1	NSP14	404	N	L	P	G	C	D	G	G	S	L	Y	V	N	K	H	A	F	H	T	P	A	424
			Y414H/Y414A																						

Figure S1l. A sequence alignment of the NSP14 sequences with attenuating mutations indicated – Part 3.

Virus	GenBank entry	Protein	H234A												H249A															
SARS-CoV-2 Wuhan-Hu-1	YP_009725310.1	NSP15	230	Y	A	F	E	H	I	V	Y	G	D	F	S	H	S	Q	L	G	G	L	H	L	L	I	G	L	A	255
MHV-A59	AAX23975.1	NSP15	258	Y	A	F	E	H	V	V	Y	G	S	F	N	Q	K	I	I	G	G	L	H	L	L	I	G	L	A	283
IBV strain YN	QQL06463.1	NSP15	219	L	G	L	Q	H	I	L	Y	G	E	V	E	K	P	Q	L	G	G	L	H	T	V	I	G	M	Y	244
PEDV strain Colorado	AGO58929.1	NSP15	217	Y	G	F	E	H	V	V	Y	G	D	V	S	K	T	T	L	G	G	L	H	L	L	I	S	Q	V	247
HCoV-229E	AAG48591.1	NSP15	231	F	N	F	E	H	V	V	Y	G	D	V	S	K	T	T	L	G	G	L	H	L	L	I	S	Q	V	256
				H262A/H238A/H226A												H277A/H238A/H250A														

Figure S1m. A sequence alignment of the NSP15 sequences with attenuating mutations indicated – Part 1.

Virus	GenBank entry	Protein	K289A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725310.1	NSP15	279	F	I	T	D	A	Q	T	G	S	S	K	C	V	C	S	V	I	D	L	L	L	299
IBV strain YN	QQL06463.1	NSP15	269	F	V	L	-	A	D	N	G	S	Y	K	Q	V	C	T	V	V	D	L	L	L	288
				K278A																					

Figure S1n. A sequence alignment of the NSP15 sequences with attenuating mutations indicated – Part 2.

Virus	GenBank entry	Protein	Y342A																
SARS-CoV-2 Wuhan-Hu-1	YP_009725310.1	NSP15	332	W	C	K	D	G	H	V	E	T	F	Y	P	K	L	Q	346
IBV strain YN	QQL06463.1	NSP15	324	W	F	E	D	G	S	I	K	T	C	Y	P	Q	L	Q	338
			Y334A																

Figure S1o. A sequence alignment of the NSP15 sequences with attenuating mutations indicated – Part 3.

Virus	GenBank entry	Protein	Y15A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725311.1	NSP16	5	W	Q	P	G	V	A	M	P	N	L	Y	K	M	Q	R	M	L	L	E	K	C	25
MHV-A59	YP_009924356.1	NSP16	5	W	K	P	G	Y	V	M	P	V	L	Y	K	Y	L	E	S	P	L	E	R	V	25
			Y15A																						

Figure S1p. A sequence alignment of the NSP16 sequences with attenuating mutations indicated – Part 1.

Virus	GenBank entry	Protein	D130A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725311.1	NSP16	120	T	A	N	K	W	D	L	I	I	S	D	M	Y	D	P	K	T	K	N	V	T	140
SARS-CoV Urbani	AAP13442.1	NSP16	120	T	A	N	K	W	D	L	I	I	S	D	M	Y	D	P	R	T	K	H	V	T	140
SARS-CoV MA15	ACZ72209.1	NSP16	120	T	A	N	K	W	D	L	I	I	S	D	M	Y	D	P	R	T	K	H	V	T	140
HCoV-229E	AAG48591.1	NSP16	119	L	E	D	K	F	D	L	L	I	S	D	M	Y	D	G	R	T	K	A	I	D	139
PEDV icPC22A	ARM65495.1	NSP16	119	L	S	D	K	F	D	L	V	I	S	D	M	Y	D	G	K	I	K	S	C	D	139
MERS-CoV EMC	YP_009047227.1	NSP16	120	V	G	Q	Q	V	D	L	V	I	S	D	M	Y	D	P	T	T	K	N	V	T	140
MERS-CoV MA1	JX869059.2	NSP16	120	V	G	Q	Q	V	D	L	V	I	S	D	M	Y	D	P	T	T	K	N	V	T	140
			D130A/D129A																						

Figure S1r. A sequence alignment of the NSP16 sequences with attenuating mutations indicated – Part 2.

Virus	GenBank entry	Protein	K170A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725311.1	NSP16	160	K	L	A	L	G	G	S	V	A	I	K	I	T	E	H	S	W	N	A	D	L	180
SARS-CoV Urbani	AAP13442.1	NSP16	160	K	L	A	L	G	G	S	I	A	V	K	I	T	E	H	S	W	N	A	D	L	180
PEDV icPC22A	ARM65495.1	NSP16	159	K	L	A	L	G	G	T	V	A	I	K	V	T	E	F	S	W	N	K	K	L	179
			K170A/K169A																						

Figure S1s. A sequence alignment of the NSP16 sequences with attenuating mutations indicated – Part 3.

Virus	GenBank entry	Protein	K46A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725311.1	NSP16	36	L	P	K	G	I	M	M	N	V	A	K	Y	T	Q	L	C	Q	Y	L	N	T	56
SARS-CoV Urbani	AAP13442.1	NSP16	36	I	P	K	G	I	M	M	N	V	A	K	Y	T	Q	L	C	Q	Y	L	N	T	56
PEDV icPC22A	ARM65495.1	NSP16	35	L	P	D	G	I	M	F	N	V	V	K	Y	T	Q	L	C	Q	Y	L	N	S	55
			K46A/K45A																						

Figure S1t. A sequence alignment of the NSP16 sequences with attenuating mutations indicated – Part 4.

Virus	GenBank entry	Protein	E203A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725311.1	NSP16	193	F	V	T	N	V	N	A	S	S	S	E	A	F	L	I	G	C	N	Y	L	G	213
PEDV icPC22A	ARM65495.1	NSP16	192	F	C	T	S	V	N	T	S	S	S	E	A	F	L	I	G	V	H	Y	L	G	212
			E202A																						

Figure S1u. A sequence alignment of the NSP16 sequences with attenuating mutations indicated – Part 5.

Virus	GenBank entry	Protein	D226A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009725299.1	NSP3	216	L	T	D	N	V	Y	I	K	N	A	D	I	V	E	E	A	K	K	V	K	P	236
SARS-CoV MA15	ACZ72208.1	NSP3	194	L	T	D	N	V	A	I	K	C	V	D	I	V	K	E	A	Q	S	A	N	P	214
rJIA-GFP _{rev} N1347	YP_209246.1	NSP3	487	I	T	P	N	V	C	F	V	K	G	D	V	I	K	V	L	R	R	V	G	A	507
			D204A/D497A																						

Figure S1v. A sequence alignment of the NSP3 sequences with attenuating mutations indicated – Part 1.

Virus	GenBank entry	Protein	N244A																H249A											
SARS-CoV-2 Wuhan-Hu-1	YP_009725299.1	NSP3	234	V	K	P	T	V	V	V	N	A	A	N	V	Y	L	K	H	G	G	G	V	A	G	A	L	N	K	259
SARS-CoV MA15	ACZ72208.1	NSP3	212	A	N	P	M	V	I	V	N	A	A	N	I	H	L	K	H	G	G	G	V	A	G	A	L	N	K	237
rJIA-GFP _{prev} N1347	YP_209246.1	NSP3	505	V	G	A	E	V	I	V	N	P	A	N	G	R	M	A	H	G	A	G	V	A	G	A	I	A	K	530
MHV-A59	AAX23975.1	NSP3	506	V	N	A	E	V	I	V	N	P	A	N	G	R	M	A	H	G	A	G	V	A	G	A	I	A	E	531
			N222A/N515A/N516A																H227A											

Figure S1w. A sequence alignment of the NSP3 sequences with attenuating mutations indicated – Part 2.

Virus	GenBank entry	Protein	A333G G334V																										
SARS-CoV-2 Wuhan-Hu-1	YP_009725299.1	NSP3	323	H	E	V	L	L	A	P	L	L	S	A	G	I	F	G	A	D	P	I	H	S	L	344			
SARS-CoV MA15	ACZ72208.1	NSP3	301	Q	D	I	L	L	A	P	L	L	S	A	G	I	F	G	A	K	P	L	Q	S	L	322			
rJIA-GFPprevN1347	YP_209246.1	NSP3	596	C	D	N	V	V	T	T	L	I	S	A	G	I	F	S	V	P	T	D	V	S	L	617			
			A606G G312V/G607V																										

Figure S1x. A sequence alignment of the NSP3 sequences with attenuating mutations indicated – Part 3.

Virus	GenBank entry	Protein	S40A																										
SARS-CoV-2 Wuhan-Hu-1	YP_009724391.1	ORF3a	1	R	A	T	A	T	I	P	I	Q	A	S	L	P	F	G	W	L	I	V	G	V		32			
SARS-CoV MA15	ACZ72211.1	ORF3a	1	H	A	T	A	T	I	P	L	Q	A	S	L	P	F	G	W	L	V	I	G	V		32			
			S40A																										

Figure S1y. A sequence alignment of the ORF3a protein sequences with attenuating mutations indicated – Part 1.

Virus	GenBank entry	Protein	S58A																						
SARS-CoV-2 Wuhan-Hu-1	YP_009724391.1	ORF3a	48	I	G	V	A	F	L	A	V	F	Q	S	A	T	K	I	I	A	L	N	K	R	68
SARS-CoV MA15	ACZ72211.1	ORF3a	48	V	G	V	A	L	L	A	V	F	Q	S	A	S	K	I	I	T	L	K	K	R	68

Figure S1z. A sequence alignment of the ORF3a protein sequences with attenuating mutations indicated – Part 2.

Virus	GenBank entry	Protein	Y109A Y113A Q116A																			
SARS-CoV-2 Wuhan-Hu-1	YP_009724391.1	ORF3a	104	P	F	L	Y	L	Y	A	L	V	Y	F	L	Q	S	I	N	F	V	121
SARS-CoV MA15	ACZ72211.1	ORF3a	104	Q	F	L	Y	L	Y	A	L	I	Y	F	L	Q	C	I	N	A	C	121

Figure S1aa. A sequence alignment of the ORF3a protein sequences with attenuating mutations indicated – Part 3.

Virus	GenBank entry	Protein	SVPL272-275GMSM															
SARS-CoV-2 Wuhan-Hu-1	YP_009724391.1	ORF3a	262	P	I	Y	D	E	P	T	T	T	T	S	V	P	L	275
SARS-CoV MA15	ACZ72211.1	ORF3a	261	P	I	Y	D	E	P	T	T	T	T	S	V	P	L	274

Figure S1ab. A sequence alignment of the ORF3a protein sequences with attenuating mutations indicated – Part 3.

Virus	GenBank entry	Protein	T345I																						
SARS-CoV-2 Wuhan-Hu-1	YP_009724390.1	Spike	335	L	C	P	F	G	E	V	F	N	A	T	R	F	A	S	V	Y	A	W	N	R	355
SARS-CoV Urbani	AAP13441.1	Spike	332	L	C	P	F	G	E	V	F	N	A	T	K	F	P	S	V	Y	A	W	E	R	342
				T332I																					

Figure S1ac. A sequence alignment of the Spike protein sequences with attenuating mutations indicated – Part 1.

Virus	GenBank entry	Protein	Q965H
SARS-CoV-2 Wuhan-Hu-1	YP_009724390.1	Spike	955 N A Q A L N T L V K Q L S S N F G A I S S 975
SJHM-RA59/S4R29	YP_209233.1	Spike	1057 N A E A L N N L L N Q L S N R F G A I S A 1077
			Q1067H

Figure S1ad. A sequence alignment of the Spike protein sequences with attenuating mutations indicated – Part 2.

Virus	GenBank entry	Protein	Q992H
SARS-CoV-2 Wuhan-Hu-1	YP_009724390.1	Spike	982 S R L D K V E A E V Q I D R L I T G R L Q 1002
SJHM-RA59/S4R29	YP_209233.1	Spike	1084 T R L D A V E A K A Q I D R L I N G R L T 1104
			Q1094H

Figure S1ae. A sequence alignment of the Spike protein sequences with attenuating mutations indicated – Part 3.

Virus	GenBank entry	Protein		L1012R
SARS-CoV-2 Wuhan-Hu-1	YP_009724390.1	Spike	982	Q S L Q T Y V T Q Q L I R A A E I R A S A 1002
SJHM-RA59/S4R29	YP_209233.1	Spike	1084	T A L N A Y I S K Q L S D S T L I K F S A 1104
				L1114R

Figure S1af. A sequence alignment of the Spike protein sequences with attenuating mutations indicated – Part 4.