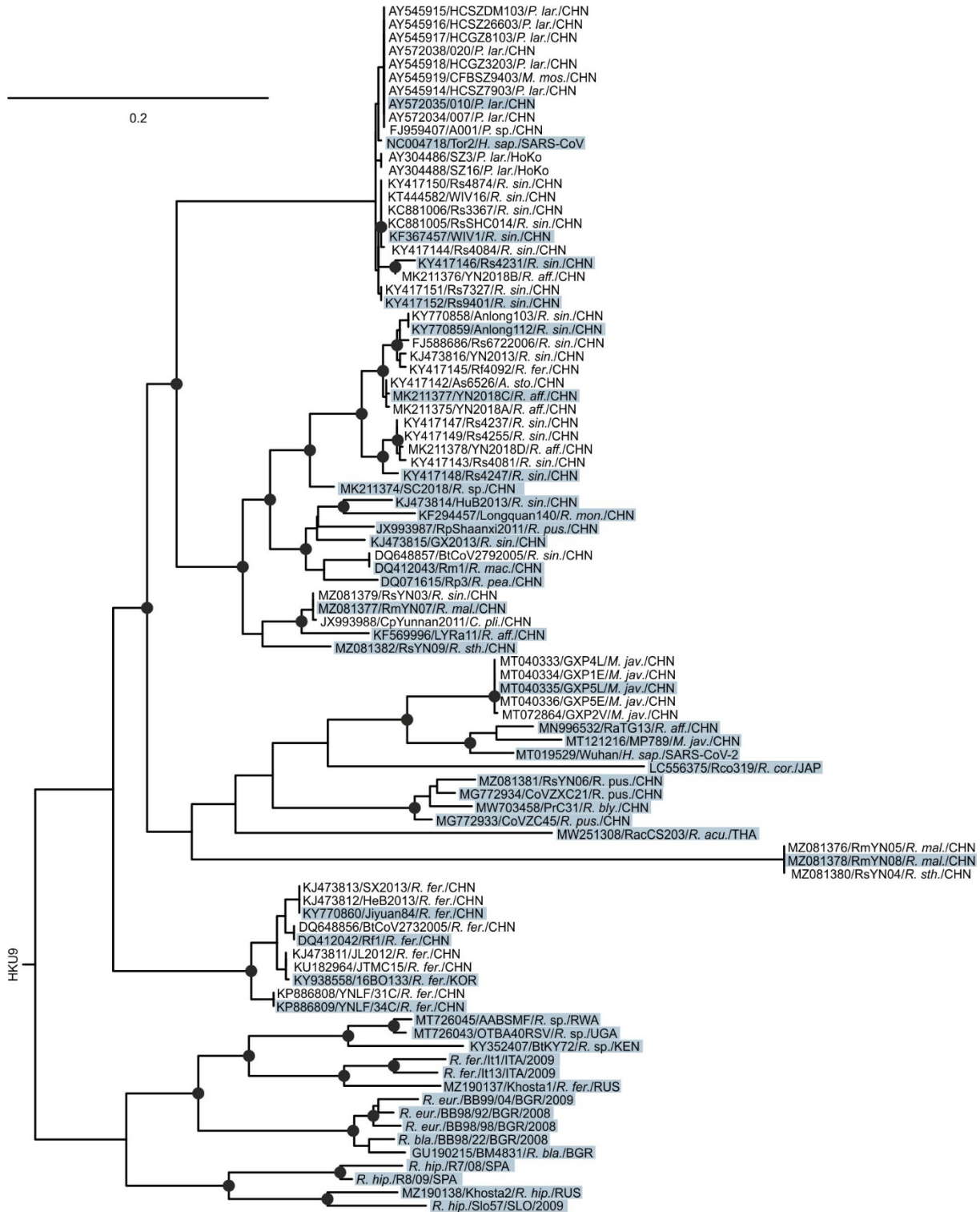
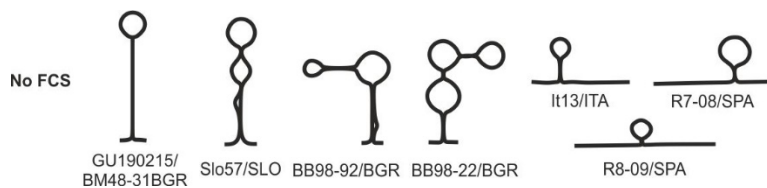


Genomic determinants of Furin cleavage in diverse European SARS-related bat coronaviruses



Supplementary Figure 1. ML phylogeny showing the complete diversity of members of the species SARS-related Coronavirus (SrC). The final dataset comprised 91 sequences (2 human, 12 civet, 6

pangolin and 71 bat-associated *SrC*). Sequences are named as followed: GenBank Acc. number/strain name/host species/country of detection. Circles at nodes indicate support of grouping in $\geq 90\%$ of 1,000 bootstrap replicates. Scale bar represents nucleotide substitutions per site. Sequences highlighted in blue were chosen for phylogenetic analysis shown in Figure panel A, representing the complete diversity of *SrC*. *A. sto.*, *Aselliscus stoliczkanus*; *C. pli.*, *Chaerephon plicatus*; *H. sap.*, *Homo sapiens*; *M. jav.*, *Manis javanica*; *M. mos.*, *Melogale moschata*; *P. lar.*, *Paguma larvata*; *P. sp.*; *Paguma* species; *R. acu.*, *Rhinolophus acuminatus*; *R. aff.*, *Rhinolophus affinus*; *R. bla.*, *Rhinolophus blasii*; *R. bly.*, *Rhinolophus blythi*; *R. cor.*, *Rhinolophus cornutus*; *R. eur.*, *Rhinolophus euryale*; *R. fer.*, *Rhinolophus ferrumequinum*; *R. hip.*, *Rhinolophus hipposideros*; *R. mac.*, *Rhinolophus macrotis*; *R. mal.*, *Rhinolophus malayanus*; *R. mon.*, *Rhinolophus monoceros*; *R. pea.*, *Rhinolophus pearsonii*; *R. pus.*, *Rhinolophus pusillus*; *R. sin.*, *Rhinolophus sinicus*; *R. sp.*, *Rhinolophus* species; *R. sth.*, *Rhinolophus stheno*; BGR, Bulgaria; CHN, China; ITA, Italy; JPN, Japan; KEN, Kenya; KOR, Korea; RUS, Russia; RWA, Ruanda; SLO, Slovenia; SPA, Spain; THA, Thailand; UGA, Uganda.



Supplementary Figure 2. Predicted RNA secondary structures of the polybasic cleavage site regions of *SrC* that are not predicted to acquire FCS through nucleotide substitutions or insertions.

Supplementary table 1. Amino acid identities of a partial *RdRp* fragment of European bat coronaviruses and prototype betacoronaviruses for species delineation.

% amino acid sequence identity (range):			
Within European bat CoVs	European bat CoVs compared to		
	SARS-CoV (AY274119)	SARS-CoV-2 (MT019529)	MERS-CoV (NC019843)
97.8-100%	97.4-99.3	96.7-97.8	76.4-76.5

Supplementary table 2. FCS prediction in human coronaviruses and their ancestors

Related HCoV	FCS region	Accession number	Prototype	Host category	Order	FCS	ProP score
229E	S2	KT253272		Evolutionary origin	Chiroptera	GSGRTGR SV	0.675
HKU1	S1S2	AC000192		Evolutionary origin	Rodentia	KSRRARR SV	0.884
HKU1	S1S2	FJ647219		Evolutionary origin	Rodentia	KSRRARR SV	0.884
HKU1	S1S2	FJ647220		Evolutionary origin	Rodentia	KSRRARR SV	0.884
HKU1	S1S2	FJ647221		Evolutionary origin	Rodentia	KSRRARR SV	0.884
HKU1	S1S2	FJ647224		Evolutionary origin	Rodentia	KSRRARR SV	0.884
HKU1	S1S2	FJ647226		Evolutionary origin	Rodentia	KSRRARR SV	0.884

HKU1	S1S2	JX169866		Evolutionary origin	Rodentia	KSRRARR SV	0.884
HKU1	S1S2	JX169867		Evolutionary origin	Rodentia	KSRRARR SV	0.884
HKU1	S1S2	MW620427		Evolutionary origin	Rodentia	KSRRARR SV	0.884
HKU1	S1S2	FJ938068		Evolutionary origin	Rodentia	TAHRARR SV	0.881
HKU1	S1S2	JF792617		Evolutionary origin	Rodentia	TAHRARR SV	0.881
HKU1	S1S2	NC012936		Evolutionary origin	Rodentia	TAHRARR SV	0.881
HKU1	S1S2	AB551247		Evolutionary origin	Rodentia	KARRARR SV	0.875
HKU1	S1S2	MF416379		Evolutionary origin	Rodentia	XAHRARR SV	0.871
HKU1	S1S2	JF792616		Evolutionary origin	Rodentia	IAHRARR SV	0.867
HKU1	S1S2	KF850449		Evolutionary origin	Rodentia	IAHRARR SV	0.867
HKU1	S1S2	FJ647223		Evolutionary origin	Rodentia	TSHRARR SI	0.865
HKU1	S1S2	MW773844		Evolutionary origin	Rodentia	TSHRARR SI	0.865
HKU1	S1S2	FJ647218		Evolutionary origin	Rodentia	KSRRADR SV	0.808
HKU1	S1S2	FJ647222		Evolutionary origin	Rodentia	KSRRADR SV	0.808
HKU1	S1S2	FJ647225		Evolutionary origin	Rodentia	KSRRADR SV	0.808
HKU1	S1S2	FJ647227		Evolutionary origin	Rodentia	KSRRADR SV	0.808
HKU1	S1S2	MF618252		Evolutionary origin	Rodentia	KSRRADR SV	0.808
HKU1	S1S2	FJ884687		Evolutionary origin	Rodentia	KSRRADR SV	0.798
HKU1	S1S2	NC001846		Evolutionary origin	Rodentia	KSRRADR SV	0.798
HKU1	S1S2	NC048217		Evolutionary origin	Rodentia	KSRRADR SV	0.808
HKU1	S1S2	NC006577	HCoV-HKU1		Primates	SSRRKRR SI	0.877
HKU1	S1S2	NC006577	HCoV-HKU1		Primates	SSRRKRR RS	0.654
MERS	S1S2	MH002341		Evolutionary origin	Chiroptera	TSSRVRR AT	0.853
MERS	S1S2	EF065509		Evolutionary origin	Chiroptera	TSSRVRR AT	0.822
MERS	S1S2	NC_009020		Evolutionary origin	Chiroptera	TSSRVRR AT	0.822
MERS	S1S2	KJ473820		Evolutionary origin	Chiroptera	TSSLRRR AT	0.811
MERS	S1S2	MH002340		Evolutionary origin	Chiroptera	TPSRVLR AA	0.746
MERS	S1S2	MH002342		Evolutionary origin	Chiroptera	PSARLAR SA	0.701
MERS	S1S2	EF065510		Evolutionary origin	Chiroptera	TSTRFRR AT	0.588
MERS	S1S2	EF065511		Evolutionary origin	Chiroptera	TSTRFRR AT	0.588
MERS	S1S2	EF065512		Evolutionary origin	Chiroptera	TSTRFRR AT	0.588
MERS	S1S2	KC869678		Evolutionary origin	Chiroptera	TNLRSGR ST	0.572
MERS	S1S2	MF593268		Evolutionary origin	Chiroptera	TNLRSGR ST	0.572
MERS	S1S2	MH002342		Evolutionary origin	Chiroptera	RLARSAR SG	0.509
MERS	S1S2	MK967708		Intermediate host	Artiodactyla	LTPRSVR SV	0.607
MERS	S1S2	KJ477103		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	KU740200		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	KY581695		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	KY581696		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	KY581697		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	KY581698		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	KY581699		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	KY581700		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	KY673149		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598594		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
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MERS	S1S2	MF598597		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598598		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598599		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598600		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
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MERS	S1S2	MF598602		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598603		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598604		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598605		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598606		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598607		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598608		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598609		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598610		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598611		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598612		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598613		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
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MERS	S1S2	MF598617		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598618		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598619		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598620		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598621		Intermediate host	Artiodactyla	LTPRSVR SV	0.563
MERS	S1S2	MF598622		Intermediate host	Artiodactyla	LTPRSVR SV	0.563

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MERS	S2	MT226607		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MW086527		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MW086528		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MW086529		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MW086530		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MW086531		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MW086532		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MW086533		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MW086534		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
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MERS	S2	MW545527		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MW545528		Intermediate host	Artiodactyla	TGSR SAR SA	0.707
MERS	S2	MH371127	MERS-CoV		Primates	TGSR SAR SA	0.707
MERS	S2	NC019843	MERS-CoV		Primates	TGSR SAR SA	0.707
MERS	S2	NC038294	MERS-CoV		Primates	TGSR SAR SA	0.707
NL63r	S2	NC005831	HCoV-NL63		Primates	LPQR NIR SS	0.519
OC43	S1S2	KM349742		Evolutionary origin	Rodentia	STWR AKR DL	0.749
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OC43	S1S2	KM349744		Evolutionary origin	Rodentia	STWR AKR DL	0.749
OC43	S1S2	MT820628		Evolutionary origin	Rodentia	ATRR AKR DL	0.761
OC43	S1S2	MT820629		Evolutionary origin	Rodentia	ATRR AKR DL	0.761
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OC43	S1S2	MT820631		Evolutionary origin	Rodentia	ATRR AKR DL	0.761
OC43	S1S2	NC026011		Evolutionary origin	Rodentia	STWR AKR DL	0.749
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OC43	S1S2	AF391541		Intermediate host	Artiodactyla	TKRR SRR SI	0.851
OC43	S1S2	AF391542		Intermediate host	Artiodactyla	TKRR SRR SI	0.851
OC43	S1S2	DQ011855		Intermediate host	Artiodactyla	TALRSRR SF	0.715
OC43	S1S2	DQ811784		Intermediate host	Artiodactyla	TKRR SRR SI	0.851
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OC43	S1S2	KU558922		Intermediate host	Artiodactyla	IKRR SRR SI	0.829
OC43	S1S2	KU558923		Intermediate host	Artiodactyla	IKRR SRR SI	0.829
OC43	S1S2	KU886219		Intermediate host	Artiodactyla	TKRR SRR SI	0.851
OC43	S1S2	KX982264		Intermediate host	Artiodactyla	TKRR SRR AI	0.776
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OC43	S1S2	KY419105		Intermediate host	Artiodactyla	TALRSRR SF	0.715
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OC43	S1S2	KY419112		Intermediate host	Artiodactyla	TALRSRR SF	0.715
OC43	S1S2	KY419113		Intermediate host	Artiodactyla	TSLSRR SL	0.759
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OC43	S1S2	LC494126		Intermediate host	Artiodactyla	TKRR SRR SI	0.851
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OC43	S1S2	MN514963		Intermediate host	Artiodactyla	TDRRSRR AV	0.812
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OC43	S1S2	U00735		Intermediate host	Artiodactyla	TKRRSRR AI	0.776
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OC43	S1S2	NC017083		Intermediate host	Lagomorpha	TQLRSRR AI	0.627
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OC43	S1S2	FJ415324	Human enteric CoV 4408		Primates	TKRRSRR AI	0.776
OC43	S1S2	FJ938067	Human enteric CoV 4408		Primates	TKRRSRR AI	0.776
OC43	S1S2	MH685718	HCoV-OC43		Primates	KNRRSRR AI	0.751
OC43	S1S2	NC006213	HCoV-OC43		Primates	SKNRRSR GA	0.529
SARS-related	S1S2	MT019529	SARS-CoV-2		Primates	NSPRRAR SV	0.620

Accession numbers of viruses tested in ProP:

HCoV-229E: 41 Sequences (Hosts: 1 Primate, 6 Chiroptera, 34 Artiodactyla); JQ410000, KT253269, KT253270, KT253271, KT253272, KT253324, KT253325, KT253326, KT253327, KT253328, KT368892, KT368893, KT368894, KT368895, KT368896, KT368897, KT368898, KT368899, KT368900, KT368901, KT368902, KT368903, KT368904, KT368905, KT368906, KT368907, KT368908, KT368909, KT368910, KT368911, KT368912, KT368913, KT368914, KT368915, KT368916, KU291449, KY073747, KY073748, MF593473, NC002645, NC028752

MERS-CoV: 276 Sequences (Hosts: 3 Primates, 241 Artiodactyla, 29 Chiroptera, 3 Eulipotyphla); DQ648794, EF065505, EF065506, EF065507, EF065508, EF065509, EF065510, EF065511, EF065512, KC545386, KC869678, KJ473820, KJ473821, KJ473822, KJ477103, KU740200, KY581695, KY581696, KY581697, KY581698, KY581699, KY581700, KY673149, MF593268, MF598594, MF598595, MF598596, MF598597, MF598598, MF598599, MF598600, MF598601, MF598602, MF598603, MF598604, MF598605, MF598606, MF598607, MF598608, MF598609, MF598610, MF598611, MF598612, MF598613, MF598614, MF598615, MF598616, MF598617,

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MG923477, MG923478, MG923479, MG923480, MG923481, MG987420, MG987421, MH002337,
MH002338, MH002339, MH002340, MH002341, MH002342, MH259485, MH259486, MH371127,
MH734114, MH734115, MK357908, MK357909, MK564474, MK564475, MK679660, MK967708,
MN654970, MN654971, MN654972, MN654973, MN654974, MN654975, MN654976, MN654977,
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MN655002, MN655003, MN655004, MN655005, MN655006, MN655007, MN655008, MN655009,
MN655010, MN655011, MN655012, MN655013, MN655014, MN655015, MN655016, MN655017,
MN758606, MN758607, MN758608, MN758609, MN758610, MN758611, MN758612, MN758613,
MN758614, MT226600, MT226601, MT226602, MT226603, MT226604, MT226605, MT226606,
MT226607, MW086527, MW086528, MW086529, MW086530, MW086531, MW086532,

MW086533, MW086534, MW086535, MW086538, MW086539, MW218395, MW545527, MW545528, NC009019, NC009020, NC019843, NC038294, NC039207

HCoV-NL63: 6 Sequences (Hosts: 1 Primate, 5 Chiroptera); KY073744, KY073745, KY073746, NC32107, NC48216, NC5831

HCoV-OC43: 151 sequences (Hosts: 4 Primates, 131 Artiodactyla, 8 Rodentia, 5 Perissodactyla, 2 Carnivora, 1 Lagomorpha); AB354579, AF220295, AF391541, AF391542, DQ011855, DQ811784, DQ915164, EF424615, EF424617, EF424619, EF424620, EF424621, EF424622, EF424623, EF424624, EF446615, FJ415324, FJ425184, FJ425185, FJ425186, FJ425187, FJ425188, FJ425189, FJ938063, FJ938064, FJ938065, FJ938066, FJ938067, JX860640, KF906249, KF906250, KM349742, KM349743, KM349744, KU558922, KU558923, KU886219, KX432213, KX982264, KY419103, KY419104, KY419105, KY419106, KY419107, KY419109, KY419110, KY419112, KY419113, KY994645, LC061272, LC061273, LC061274, LC494126, LC494127, LC494128, LC494129, LC494130, LC494131, LC494132, LC494133, LC494134, LC494135, LC494136, LC494137, LC494138, LC494139, LC494140, LC494141, LC494142, LC494143, LC494144, LC494145, LC494147, LC494148, LC494149, LC494150, LC494151, LC494152, LC494153, LC494154, LC494155, LC494156, LC494157, LC494158, LC494159, LC494160, LC494161, LC494162, LC494163, LC494164, LC494165, LC494166, LC494167, LC494168, LC494169, LC494170, LC494171, LC494172, LC494173, LC494174, LC494175, LC494176, LC494177, LC494178, LC494179, LC494180, LC494181, LC494182, LC494183, LC494184, LC494185, LC494186, LC494187, LC494188, LC494189, LC494190, LC494191, LC494192, LC592689, MF083115, MG518518, MG757138, MG757139, MG757140, MG757141, MG757142, MH043952, MH043953, MH043954, MH043955, MH685718, MH810163, MN514962, MN514963, MN514964, MN514965, MN514966, MN514967, MN982198, MN982199, MT820628, MT820629, MT820630, MT820631, MW165134, MW711287, NC026011, NC0003045, NC006213, NC017083, U00735

HCoV-HKU1: 31 sequences (Hosts: 1 Primate, 30 Rodentia); AB551247, AC000192, AF201929, FJ647218, FJ647219, FJ647220, FJ647221, FJ647222, FJ647223, FJ647224, FJ647225, FJ647226, FJ647227, FJ884687, FJ938068, GU593319, JF792616, JF792617, JQ173883, JX169866, JX169867,

KF850449, MF416379, MF618252, MF618253, MW620427, MW773844, NC001846, NC006577, NC012936, NC048217

SARS-related CoV: 82 sequences (Hosts: 2 Primates, 62 Chiroptera, 12 Carnivora, 6 Pholidota);
AY304486, AY304488, AY545914, AY545915, AY545916, AY545917, AY545918, AY545919, AY572034, AY572035, AY572038, DQ071615, DQ412042, DQ412043, DQ648856, DQ648857, FJ588686, FJ959407, GU190215, JX993987, JX993988, KC881005, KC881006, KF294457, KF367457, KF569996, KJ473811, KJ473812, KJ473813, KJ473814, KJ473815, KJ473816, KP886808, KP886809, KT444582, KU182964, KY352407, KY417142, KY417143, KY417144, KY417145, KY417146, KY417147, KY417148, KY417149, KY417150, KY417151, KY417152, KY770858, KY770859, KY770860, KY938558, LC556375, MG772933, MG772934, MK211374, MK211375, MK211376, MK211377, MK211378, MN996532, MT019529, MT040333, MT040334, MT040335, MT040336, MT072864, MT121216, MT726043, MT726045, MW251308, MW703458, MZ081376, MZ081377, MZ081378, MZ081379, MZ081380, MZ081381, MZ081382, MZ190137, MZ190138, NC004718