Accession Number	Virus	Accession Number	Virus
MK581202	IBV/Poland/80/1989	MN996532	BtCoV/RaTG13
KF294380	RnCoV/Lucheng-19	MN908947	SARS-CoV-2
MF577027	PEDV/Belgorod/2008	KJ473811	BtCoV/RfJL2012
AY567487	HCoV-NL63	MG772934	BtCoV/ZXC21
MN611517	229Er-CoV/Ra5425	MG772933	BtCoV/ZC45
AF304460	HCoV-229E	KY417145	BtCoV/Rf4092
KJ473808	BtCoV/RfYN2012	KF367457	BtCoV/WIV1
MN611512	HKU2r-BtCoV/160660	AY278741	SARS-CoV/Urbani
NC_009988	BtCoV/HKU2	MT350598	HKU9r-COV/GCCDC1
MF094685	SADSr-CoV/8462	MG762674	BtCoV/HKU9
MF094687	SADSr-CoV/141388	AY700211	MHV-A59
MF094681	SADS-CoV/FarmA	KF686346	HCoV-HKU1
MF094688	SADSr-CoV/162140	NC_046954	RfMfCoV-2/JL2014
MF094686	SADSr-CoV/8495	MH687976	VZ-BetaCoV/22084-1
KC869678	NeoCoV/PML-PHE- 1/RSA2011	NC_026011	RnCoV/HKU24
JX869059	MERS_CoV	KY370052	RtMmCoV-1/IM2014
EF065505	BtCoV/HKU4	KY370044	RtAsCoV/IM2014
MH002342	BtCoV/HKU5	JN874559	RbCoV/HKU14
KY352407	BtCoV/KY72	DQ011855	PHEV
NC_014470	BtCoV/BM48- 31/BGR/2008	AY391777	HCoV-OC43
EPI_ISL_410538	PangolinCoV/GX17-P4L	EF446615	ECoV-NC99
EPI_ISL_410721	PangolinCoV/GD19	MH043952	BCoV
EPI_ISL_412977	BtCoV/RmYN02		

## Figure S1. Accession numbers of viral sequences analyzed

# Α

	BCoV	HCoV-OC43	PHEV	ECoV-NC99	RbCoV/HK
BCoV		97.5%	97.3%	97.8%	90.9%
HCoV-OC43	97.5%		97.7%	96.8%	90.5%
PHEV	97.3%	97.7%		96.6%	90.7%
ECoV-NC99	97.8%	96.8%	96.6%		90.3%
RbCoV/HKU14	90.9%	90.5%	90.7%	90.3%	

	162140	141388	HKU2	FarmA	8495	8462	160660	RfYN2012
162140		99.3%	97.6%	99.5%	99.3%	97.6%	95.3%	94.4%
141388	99.3%		97.8%	99.2%	98.8%	98.0%	95.3%	94.5%
HKU2	97.6%	97.8%		97.6%	97.4%	98.4%	95.1%	94.8%
FarmA	99.5%	99.2%	97.6%		99.1%	97.7%	95.1%	94.4%
8495	99.3%	98.8%	97.4%	99.1%		97.4%	95.2%	94.3%
8462	97.6%	98.0%	98.4%	97.7%	97.4%		94.9%	94.2%
160660	95.3%	95.3%	95.1%	95.1%	95.2%	94.9%		94.6%
RfYN2012	94.4%	94.5%	94.8%	94.4%	94.3%	94.2%	94.6%	

# С

	SARS-CoV-2	RmYN02	RaTG13	PangolinC
SARS-CoV-2		98.4%	97.8%	91.3%
RmYN02	98.4%		97.4%	91.4%
RaTG13	97.8%	97.4%		90.7%
PangolinCoV/GD19	91.3%	91.4%	90.7%	

**Figure S2. Distance tables of full-length RdRp coding region of Orf1ab.** A) Distance table for *Betacoronavirus-1* RdRp sequences, as well as RbCoV/HKU14. B) Distance table for SADSr-CoV RdRp.C) Distance table for RdRp coding region of SARS-CoV-2-like viruses.

В



**Figure S3. 3CIPro and Helicase phylogenetic trees.** A) Schematic showing position of Orf1ab regions encoding 3CIPro, RdRp, and Helicase B) Maximum-likelihood phylogenetic tree of 3CIPro-encoding region. C) ML tree of helicase-encoding region of Orf1ab.

#### 3CIPro

	BCoV	HCoV-OC43	PHEV	ECoV-NC99	RbCoV/HK
BCoV		96.6%	95.3%	99.2%	87.9%
HCoV-OC43	96.6%		94.7%	96.7%	87.8%
PHEV	95.3%	94.7%		95.2%	86.7%
ECoV-NC99	99.2%	96.7%	95.2%		88.1%
RbCoV/HKU14	87.9%	87.8%	86.7%	88.1%	

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## Helicase

	BCoV	HCoV-OC43	PHEV	ECoV-NC99	RbCoV/HK
BCoV		97.4%	95.6%	92.3%	88.6%
HCoV-OC43	97.4%		96.0%	91.5%	88.5%
PHEV	95.6%	96.0%		90.3%	87.7%
ECoV-NC99	92.3%	91.5%	90.3%		92.5%
RbCoV/HKU14	88.6%	88.5%	87.7%	92.5%	

### 3CIPro

	SARS-CoV-2	RmYN02	RaTG13	PangolinC
SARS-CoV-2		96.9%	95.9%	93.8%
RmYN02	96.9%		95.8%	93.1%
RaTG13	95.9%	95.8%		92.9%
PangolinCoV/GD19	93.8%	93.1%	92.9%	

#### D

С

## Helicase

	SARS-CoV-2	RmYN02	RaTG13	PangolinC
SARS-CoV-2		97.5%	98.1%	92.2%
RmYN02	97.5%		97.8%	92.1%
RaTG13	98.1%	97.8%		92.2%
PangolinCoV/GD19	92.2%	92.1%	92.2%	

#### 3CIPro

	162140	141388	HKU2	FarmA	8495	8462	BtCoV/16	RfYN2012
162140		99.8%	98.8%	99.3%	99.9%	99.3%	97.0%	95.6%
141388	99.8%		98.7%	99.1%	99.9%	99.1%	97.0%	95.4%
HKU2	98.8%	98.7%		98.6%	98.8%	98.6%	96.6%	95.3%
FarmA	99.3%	99.1%	98.6%		99.2%	99.8%	97.2%	95.5%
8495	99.9%	99.9%	98.8%	99.2%		99.2%	97.1%	95.5%
8462	99.3%	99.1%	98.6%	99.8%	99.2%		97.2%	95.5%
BtCoV/160660	97.0%	97.0%	96.6%	97.2%	97.1%	97.2%		95.3%
RfYN2012	95.6%	95.4%	95.3%	95.5%	95.5%	95.5%	95.3%	

## F

## Helicase

	162140	141388	FarmA	HKU2	8495	8462	BtCoV/16	RfYN2012
162140		99.0%	99.1%	98.7%	95.6%	96.2%	95.1%	93.9%
141388	99.0%		99.8%	98.4%	96.3%	96.3%	95.1%	93.5%
FarmA	99.1%	99.8%		98.4%	96.2%	96.4%	95.1%	93.4%
HKU2	98.7%	98.4%	98.4%		95.9%	96.3%	95.6%	94.3%
8495	95.6%	96.3%	96.2%	95.9%		96.4%	94.1%	93.1%
8462	96.2%	96.3%	96.4%	96.3%	96.4%		94.1%	93.1%
BtCoV/160660	95.1%	95.1%	95.1%	95.6%	94.1%	94.1%		94.5%
RfYN2012	93.9%	93.5%	93.4%	94.3%	93.1%	93.1%	94.5%	

**Figure S4. 3CIPro and Helicase genetic distance tables.** A and B) Distance tables for BetaCoV1 viruses. C and D) Distance tables for SARS-CoV-2-like viruses. E and F) Distance tables for SADSr-CoVs.

Region	Position (in native sequence)	% identity to reference	Top BLAST hit (% identity)
PangolinCoV/GD19 RR1	21,431-22,321	64.2%	MG772934.1 – BtCoV/ZXC21 (80.4%)
BtCoV/RmYN02 RR1	21,424-24,102	62.5%	<u>KY417145.1</u> – BtCoV/Rf4092 (73.8%)
BtCoV/RmYN02 RR2	27,700-28,078	51.9%	AY515512.1 – civetSARS-CoV (81%)



BtCoV/RmYN02	94.6%		95.6%	92.8%	
BtCoV/RaTG13	95.0%	95.6%		92.8%	
PangolinCoV/GD19	93.0%	92.8%	92.8%		



	SARS-CoV-2	BtCoV/Rm	BtCoV/Ra	PangolinC
SARS-CoV-2		65.6%	88.4%	87.4%
BtCoV/RmYN02	65.6%		64.2%	63.6%
BtCoV/RaTG13	88.4%	64.2%		84.6%
PangolinCoV/GD19	87.4%	63.6%	84.6%	



	SAKS-COV-2	BICOV/Rm	BICOV/Ra	Pangolinc	
ARS-CoV-2		62.5%	91.9%	81.3%	
tCoV/RmYN02	62.5%		62.2%	62.5%	
tCoV/RaTG13	91.9%	62.2%		80.2%	
angolinCoV/GD19	81.3%	62.5%	80.2%		



93.4%

92.2%

PangolinCoV/GD19

93.4%



	SARS-CoV-2	BtCoV/Rm	BtCoV/Ra	PangolinC
SARS-CoV-2		95.8%	98.0%	97.0%
BtCoV/RmYN02	95.8%		95.8%	94.8%
BtCoV/RaTG13	98.0%	95.8%		97.2%
PangolinCoV/GD19	97.0%	94.8%	97.2%	







Figure S5. Breakpoint and phylogenetic analysis of SARSr-CoV. A. Table showing boundaries of divergent recombinant regions in PangolinCoV/GD19 and BtCoV/RmYN02 with nucleotide identity to SARS-CoV-2 and the top BLAST hit in GenBank. B-H) Maximum-likelihood phylogenetic trees of breakpoint-defined regions in PangolinCoV/GD19 and BtCoV/RmYN02, demonstrating changes in topology due to recombination. "Up" and "Dn" regions reflect the 500 nt upstream and downstream of 5' and 3' breakpoints, respectively.



Figure S6. Breakpoint and phylogenetic analysis of Betacoronavirus-1. A. Table showing boundaries of divergent recombinant regions in ECoV and PHEV with nucleotide identity to BCoV and the top BLAST hit in GenBank. B-H) Maximum-likelihood phylogenetic trees of breakpoint-defined regions in ECoV. I-K) MLphylogenetic trees of PHEV-RR and 500 nucleotide stretches flanking the 5' and 3' breakpoints. ECoV and PHEV regions in black type are conserved among BetaCoV1 (not of recombinant origin) and demonstrate changes in tree topology and/or branch length due to recombination.

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**Figure S7. BetaCoV-1 IDPlot results with alternative reference sequences.** A) The IDPlot output for *Betacoronavirus-1* displayed in Figure 4 of the main text. B) The IDPlot output from a run using the same sequences but with OC43 as the reference rather than BCoV.















	0.05 ECoV-NC99	BCoV	HCoV-OC43	PHEV	RbCoV/HK
CoV-NC99		81%	81%	81%	67%
CoV	81%		96%	97%	69%
CoV-OC43	81%	96%		97%	68%
HEV	81%	97%	97%		68%
bCoV/HKU14	67%	69%	68%	68%	



	ECoV-NC99	BCoV	HCoV-OC43	PHEV	RbCoV/HK
ECoV-NC99		86%	85%	86%	86%
BCoV	86%		98%	94%	82%
HCoV-OC43	85%	98%		94%	82%
PHEV	86%	94%	94%		82%
RbCoV/HKU14	86%	82%	82%	82%	

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Region	Top BLAST hit (% identity)
ECoV R2.1	<u>MN514962.1</u> – DrCoV/HKU23 (69.8)
ECoV R2.2	<u>LC494130.1</u> – BCOV (81.7)
ECoV R2.3	<u>JN874562.1</u> – RbCoV/HKU14 (80)
ECoV R2.4	<u>JN874560.1</u> – RbCoV/HKU14 (92.9)
ECoV R2.5	<u>MG518518.1</u> – Water Deer CoV (87)
ECoV R2.6	<u>JN874561.1</u> – RbCoV/HKU14 (87.9)
ECoV R2.7	<u>MF083115.1</u> – PHEV (91.4)



	0.05				
	ECoV-NC99	BCoV	HCoV-OC43	PHEV	RbCoV/HK
ECoV-NC99		62%	63%	62%	80%
BCoV	62%		97%	96%	56%
HCoV-OC43	63%	97%		95%	56%
PHEV	62%	96%	95%		56%
RbCoV/HKU14	80%	56%	56%	56%	



	0.05				
	ECoV-NC99	BCoV	HCoV-OC43	PHEV	RbCoV/HK
CoV-NC99		57%	57%	56%	88%
SCoV	57%		96%	88%	57%
ICoV-OC43	57%	96%		90%	57%
PHEV	56%	88%	90%		56%
RbCoV/HKU14	88%	57%	57%	56%	

**Figure S8. Recombination analysis of ECoV R2.** A) IDPlot of ECoV Region 2 B-H) ML phylogenetic trees and distance tables for each putative sub-region of ECoV R2. I) BLAST analysis of ECoV R2 sub-regions.





Region	Position (in native sequence)	% identity to reference	BLAST hits (% identity)
RfYN2012 RR1	2,854-3,657	67.5%	MF094687.1 - SADSr-CoV/141388 (69%)
RfYN2012 RR2	3,658-8,017	85.6%	MK994935.1 - SADS-CoV/GDWT-P18 (85.9%)
RfYN2012 RR3	20,287-22,002	67%	MF769424.1 – SADS-CoV/182 (69.1%)
RfYN2012 RR4	22,003-23,800	80.5%	EF203065.1 – BtCoV/HKU2-HK (81.3%)
RfYN2012 RR5	25,318-26,693	82.7%	MF094685.1 – SADSr-CoV/8462 (83.7%)
BtCoV/160660 RR1	19,807-24,631	69.5%	EF203065.1 – BtCoV/HKU2-HK (71.5%)
BtCoV/160660 RR2	25,547-26,738	71.9%	MF094688.1 – SADSr-CoV/162140 (71.9%
BtCoV/HKU2 RR1	20,489-22,496	73.9%	MF769407.1* - SADSr-CoV/141194 (99.8%) EF203065.1 – BtCoV/HKU2-HK (98.8%) MF769415.1* – SADSr-CoV/NL140388 (98.6%) MF769414.1* – SADSr-CoV/140344 (96.4%) MF094686.1 – SADSr-CoV/8495 (75.6%)
BtCoV/HKU2 RR2	22,497-23,252	86.2%	EF203067.1 – BtCoV/HKU2-HK (89.1%) MF094688.1 – SADSr-CoV/162140 (86.2%)
SADSr-CoV/8462 RR1	20,530-22,044	77.3%	MF094700.1* - SADSr-CoV/HD13590 (99.9%) MF094697.1* - SADSr-CoV/8355 (98.6%) MF094691.1* - SADSr-CoV/8462 (96.8%) MK977618.1 - SADS-CoV/p10 (78%)
SADSr-CoV/8495 RR1	20,501-22,167	71.4%	MF094689.1* – SADSr-CoV/8495 (84.5%) EF203065.1 – BtCoV/HKU2-HK (75.2%)
SADSr-CoV/8495 RR2	26,653-26,942	70.7%	MF094687.1 – SADSr-CoV/141388 (98.6%) MK994935.1 – SADS-CoV/GWDT-p18 (75.7%)
SADSr-CoV/141388 RR1	26,629-26,918	71.4%	MF094686.1 - SADSr-CoV/8495 (98.6%) MK994935.1 – SADS-CoV/GWDT-p18 (74.8%)

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# **MTase NTD**

	162140	141388	FarmA	HKU2	8495	8462	BtCoV/16	RfYN2012
162140		100%	97.7%	99.2%	96.7%	99.0%	93.6%	96.2%
141388	100%		97.7%	99.2%	96.7%	99.0%	93.6%	96.2%
FarmA	97.7%	97.7%		98.5%	95.7%	98.2%	93.6%	96.2%
HKU2	99.2%	99.2%	98.5%		96.9%	99.7%	94.4%	96.4%
8495	96.7%	96.7%	95.7%	96.9%		96.7%	93.1%	95.2%
8462	99.0%	99.0%	98.2%	99.7%	96.7%		94.1%	96.2%
BtCoV/160660	93.6%	93.6%	93.6%	94.4%	93.1%	94.1%		92.3%
RfYN2012	96.2%	96.2%	96.2%	96.4%	95.2%	96.2%	92.3%	

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	162140	141388	FarmA	HKU2	8495	8462	BtCoV/16	RfYN2012
162140		97.7%	97.7%	98.3%	98.1%	99.0%	89.7%	91.4%
141388	97.7%		98.8%	97.2%	97.4%	96.7%	90.1%	91.6%
FarmA	97.7%	98.8%		96.7%	97.4%	97.0%	90.1%	91.8%
HKU2	98.3%	97.2%	96.7%		97.7%	97.5%	89.1%	91.9%
8495	98.1%	97.4%	97.4%	97.7%		97.4%	89.0%	91.4%
8462	99.0%	96.7%	97.0%	97.5%	97.4%		89.4%	91.9%
BtCoV/160660	89.7%	90.1%	90.1%	89.1%	89.0%	89.4%		87.0%
RfYN2012	91.4%	91.6%	91.8%	91.9%	91.4%	91.9%	87.0%	

Figure S10. Breakpoint and nucleotide identity analysis of SADSr-CoV recombination. A) Breakpoint analysis of SADSr-CoVs including nucleotide identity to reference sequence and top BLAST hits. BLAST result accession numbers with an asterisk indicate the entry is a partial spike sequence only. B -C) Distance tables for MTase NTD, and M genes shows high nucleotide identity across SADSr-CoVs upstream and downstream of spike-encompassing recombinant regions.



**Figure S11. GARD Simulations.** A-C) ANI plots showing sequence divergence at 3%, 6%, or 9% randomly introduced error rates D) Histograms showing even breakpoint distribution for each simulation.