

Supplementary table 1. Significant recombination events.

Recombinant Sequence(s)	In Recombinant Sequence		Gene	Minor Parental Sequence(s)	Major Parental Sequence(s)	Detection Methods						
	Begin	End				RDP	GENEC ONV	Bootscan	Maxchi	Chimaera	SiScan	3Seq
^OQ175260.1	326	20853	ORF1ab	MW924112.1	Unknown (EfONCAN22.320328)	1.23 E-140	2.57E-195	3.35E-46	1.05E-45	4.34E-08	NS	1.62E-12
				MZ293738.1								
^OL410608.1	19790	23130	ORF1ab; Spike	OL410607.1	OL415261.1	4.74 E-99	1.19E-91	2.41E-100	8.93E-40	1.26E-30	3.06E-65	2.57E-22
^OL415262.1	20885	23133	Spike	OL410607.1	OL410610.1	1.56 E-86	2.97E-92	2.33E-82	4.29E-40	9.18E-31	4.86E-61	4.06E-13
					OL415261.1							
KX900407.1	20318	21514	Spike	Unknown (NC_002306.3)	JQ404409.1	4.68 E-83	1.06E-73	5.58E-59	1.19E-11	6.70E-15	1.00E-32	NS
^EfONCAN22.320328	19259	20500	ORF1ab	MW924112.1	Unknown (OL415261.1)	4.16 E-66	1.92E-80	5.25E-55	1.42E-08	1.77E-31	2.95E-39	4.06E-13
				MZ293738.1	Unknown (OL410610.1)							
^KF430219.1	20615	20927	Spike	Unknown (DQ648858.1)	MIONCAN23.320388	5.01 E-54	NS	1.48E-18	3.16E-08	7.88E-03	1.44E-03	1.01E-11
					KY799179.1							
OL410609.1	17787	26370	ORF1ab; Spike; ORF3, Envelope, Membrane	Unknown (OL415261.1)	OL410610.1	1.68 E-36	5.88E-51	1.60E-42	5.70E-16	3.14E-19	5.59E-91	2.88E-11
^KF430219.1	1238	18377	ORF1ab	KY799179.1	Unknown (MIONCAN23.320388)	6.15 E-09	NS	NS	2.61E-16	6.44E-05	NS	3.13E-22
^OL410607.1	8818	10268	ORF1ab	OL415262.1	OL410609.1	2.02 E-11	1.92E-17	5.99E-20	9.56E-07	1.59E-06	8.29E-16	4.07E-04
~^NC_032730.1	23413	24638	Spike	Unknown (NC_002306.3)	MZ293738.1	4.26 E-15	NS	3.03E-08	1.19E-02	4.92E-11	1.98E-31	NS
				Unknown (JQ404409.1)	OL415262.1							
				Unknown (KX900407.1)	OL410607.1							
					OL415261.1							
					OL410610.1							
					EfONCAN22.320328							
					MW924112.1							

^OL415261.1	9323	10578	ORF1ab	MW924112.1	OL410610.1	6.33 E-14	NS	5.22E-15	1.84E-02	2.93E-03	5.09E-08	6.90E -12
				MZ293738.1								
~^OK078018.1	20861	21383	Spike	Unknown (HM245925.1)	OL410610.1	2.39 E-11	NS	1.20E-06	NS	4.78E-02	3.94E-07	1.03E -07
^OQ175339.1	22276	24164	Spike	Unknown (JQ404409.1)	EfONCAN22.320328	NS	NS	NS	2.09E-05	6.90E-03	NS	8.46E -10
				Unknown (KX900407.1)	OL410610.1							
^OL410608.1	2313	4406	ORF1ab	OL410607.1	OL415261.1	1.70 E-09	2.59E-07	3.19E-04	8.57E-05	2.54E-04	8.27E-05	2.07E -07
~^EU420137.1	23114	26004	Spike; ORF3	HM245925.1	DQ648858.1	NS	NS	NS	5.31E-09	2.50E-09	NS	2.22E -09
NC_010437.1					NC_003436.1							
~^MW924112.1	25799	27932	Envelope; Membrane, Nucleocapsid	Unknown (EfONCAN22. 320328)	OL415262.1	6.49 E-06	NS	3.12E-05	4.54E-02	3.47E-02	6.38E-09	2.31E -02
MZ293738.1												
~^OL410610.1	2606	3427	ORF1ab	OL410607.1	OL410609.1	4.26 E-04	1.64E-04	8.79E-07	3.34E-02	2.17E-05	1.26E-09	NS
~^OQ175138.1	20631	21015	Spike	Unknown (MIONCAN23. 320388)	DQ648858.1	NS	NS	NS	1.58E-02	NS	1.75E-06	7.46E -03
~^OQ175138.1	20617	21260	Spike	Unknown (DQ648858.1)	KJ645702.1	3.54 E-04	NS	2.92E-02	NS	NS	1.70E-10	NS
					NC_003436.1							
~^OL410609.1	8159	10903	ORF1ab	Unknown (OL415262.1)	MZ293738.1	8.21 E-06	2.57E-02	5.62E-06	1.48E-02	3.16E-03	5.94E-04	5.57E -05
					MW924112.1							
~^OL410608.1	11849	12654	ORF1ab	OL415262.1	Unknown (OL415261.1)	2.70 E-02	5.59E-04	1.99E-05	NS	NS	7.54E-04	NS
~OL410607.1	15343	16315	ORF1ab	MW924112.1	Unknown (OL410608.1)	1.19 E-04	1.51E-02	9.42E-05	2.63E-03	5.28E-04	8.21E-06	NS
				MZ293738.1	Unknown (OL415261.1)							
~^KY369913.1	20650	24008	Spike	Unknown (HM245925.1)	MW924112.1	NS	NS	NS	5.49E-07	1.52E-05	NS	3.21E -05
KF530105.1					MZ293738.1							
~^KY369913.1	10646	11081	ORF1ab	MIONCAN23.320388	KF530105.1	NS	NS	NS	2.75E-03	3.84E-05	7.18E-10	NS
				KY799179.1								
~^KY369913.1	4393	5342	ORF1ab	HM245925.1	MIONCAN23.320388	3.65 E-02	NS	NS	3.45E-02	2.28E-04	NS	NS

					KY799179.1							
~^OL415262.1	9197	11198	ORF1ab	Unknown (OL410610.1)	MZ293738.1	3.99 E-02	NS	4.22E-03	NS	NS	2.40E-04	NS
					MW924112.1							
~OL410607.1	4567	4980	ORF1ab	Unknown (OL410610.1)	OL410609.1	NS	NS	NS	NS	1.13E-02	9.96E-04	NS
				Unknown (OL415261.1)								
~^NC_003436.1	14062	15206	ORF1ab	Unknown (NC_032730.1)	OK078018.1	NS	NS	NS	NS	1.99E-02	NS	1.99E-03
KJ645702.1					EF203065.1							

~ It is possible that this apparent recombination signal could have been caused by an evolutionary process other than recombination.

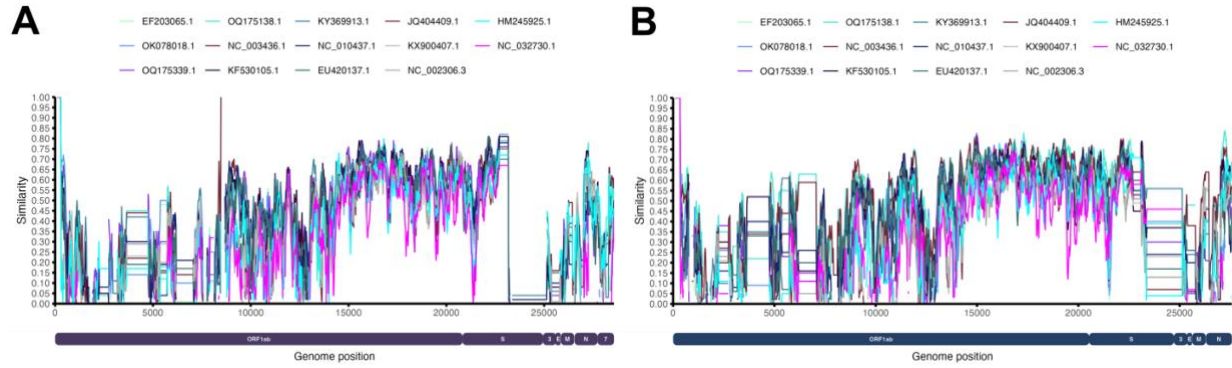
^ The recombinant sequence may have been misidentified (one of the identified parents might be the recombinant).

Minor Parent = Parent contributing the smaller fraction of sequence.

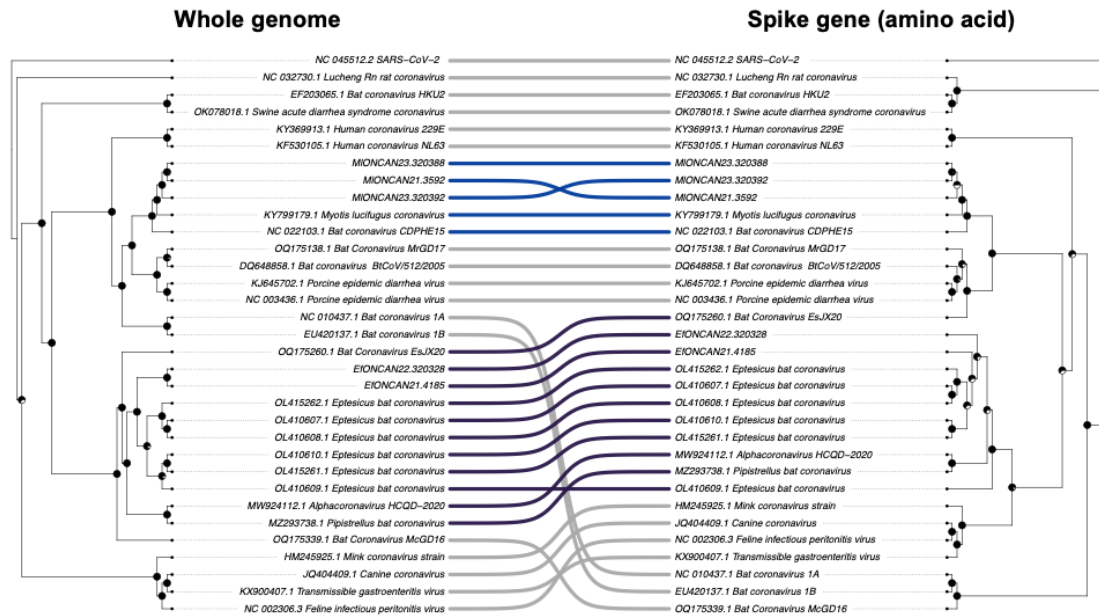
Major Parent = Parent contributing the larger fraction of sequence.

Unknown = Only one parent and a recombinant need be in the alignment for a recombination event to be detectable. The sequence listed as unknown was used to infer the existence of a missing parental sequence..

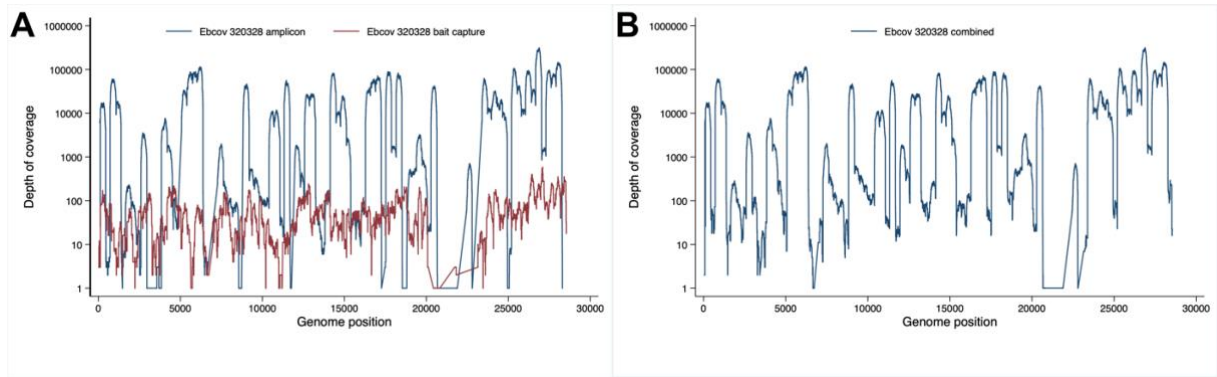
NS = No significant P-value was recorded for this recombination event using this method.



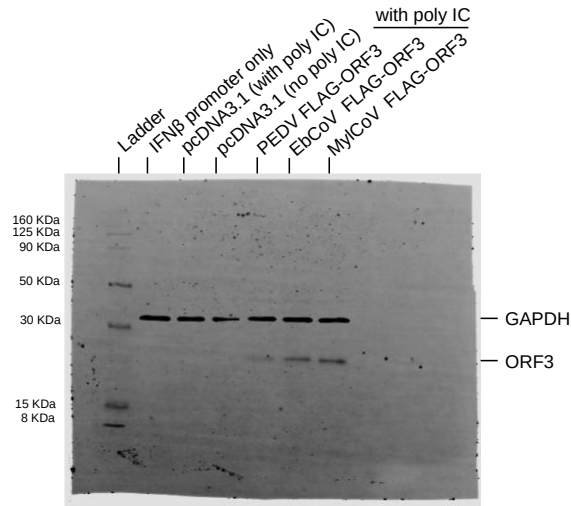
Supplementary figure 1. Similarity analysis of (A) EfONCAN22.320328 and (B) MIONCAN23.320388 with bat coronavirus HKU2 (EF203065.1), swine acute diarrhea syndrome coronavirus (OK078018.1), bat coronavirus McGB16 (OQ175339.1), bat coronavirus MrGD17 (OQ175138.1), porcine epidemic diarrhea virus (NC_003436.1), HCoV-NL63 (KF530105.1), HCoV-229e (KY369913.1), bat coronavirus 1A (NC_010437.1), bat coronavirus 1B (EU420137.1), canine coronavirus (JQ404409.1), transmissible gastroenteritis virus (KX900407.1), feline infectious peritonitis virus (NC_002306.3), mink coronavirus (HM245925.1), and Lucheng Rn rat coronavirus (NC_032730.1). Genome organization for MylCoV (KY799179.1) and EbCoV (OL415262.1) are on the bottom of each plot.



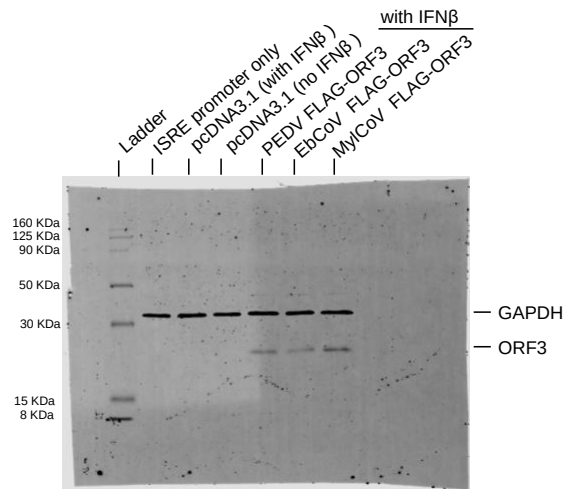
Supplementary figure 2. Tanglegram showing maximum-likelihood trees estimated with RAxML from select alpha-CoV between whole genomes and spike gene amino acid alignment with SARS-CoV-2 as an outgroup. Support calculated with bootstrap analysis (500 replicates) are indicated with pie charts (black). Links between the phylogenies link tips belonging to the same sequence to compare tree topologies. Blue and purple linkages represent *Myotis* bat coronaviruses and *Eptesicus* bat coronaviruses, respectively. Sequences from this study are highlighted in grey.



Supplementary figure 3. (A) Comparison of the depth of coverage for EfONCAN22.320328 (EbCoV 320328) whole genome sequencing using the amplicon based sequencing approach and Illumina pan-coronavirus enrichment panel and (B) the depth of coverage for the EfONCAN22.320328 whole genome with both methods combined.



A. Lysates from IFN β promoter assay



B. Lysates from ISRE promoter assay

Supplementary figure 4. Immunoblot analysis of FLAG-ORF3 and GAPDH expression. A. Lysates from IFN β promoter assay. B. Lysates from ISRE promoter assay. Cells were harvested in Passive Lysis 5X Buffer. The membrane was probed with anti-FLAG (mouse, 1:2000 dilution) and anti-GAPDH (rabbit, 1:2000 dilution) primary antibodies, followed by incubation with respective secondary antibodies (1:10,000 dilution). Protein bands were visualized using an Odyssey CLx imager. Chameleon® Duo Pre-Stained Protein Ladder was used.