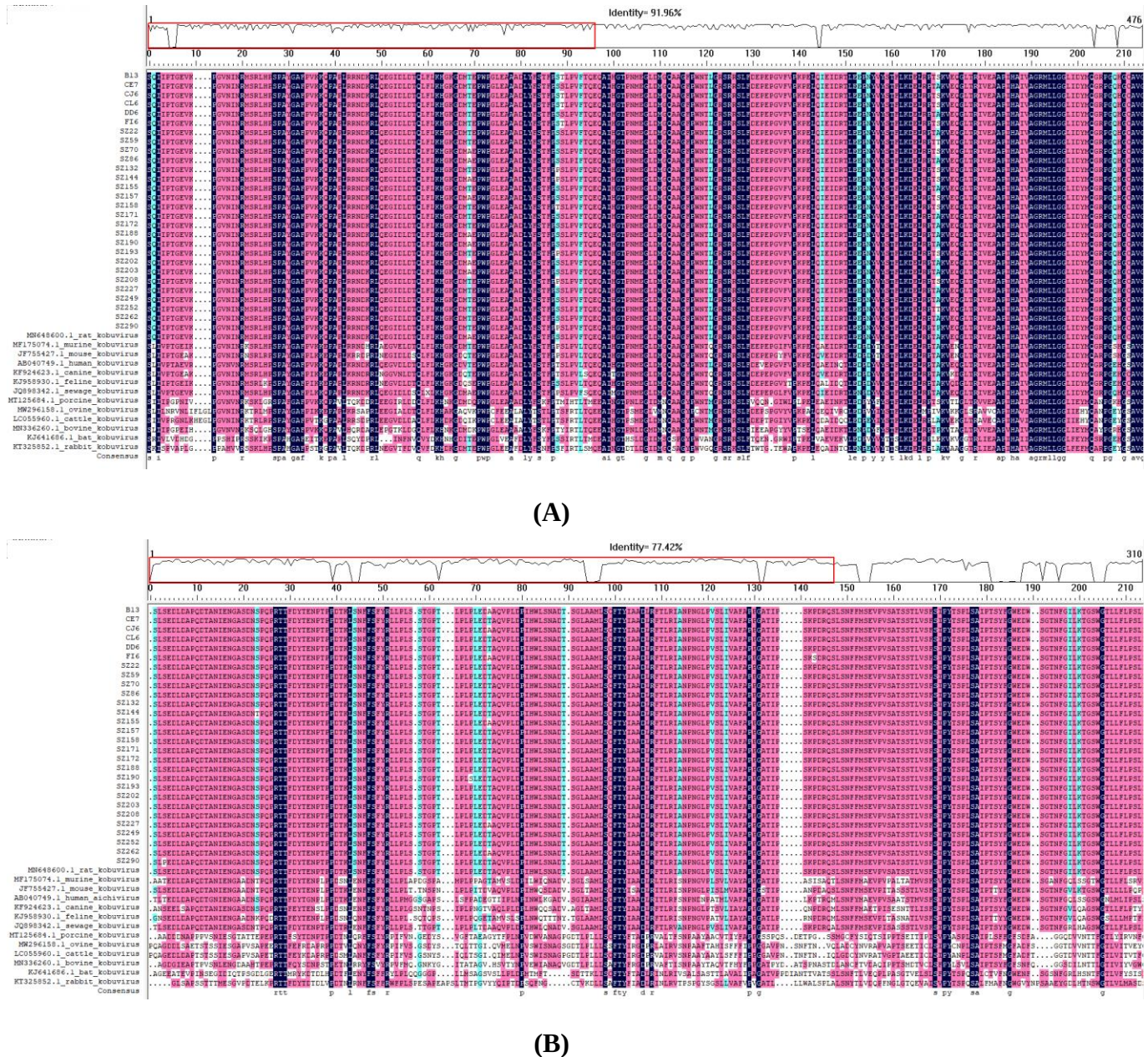


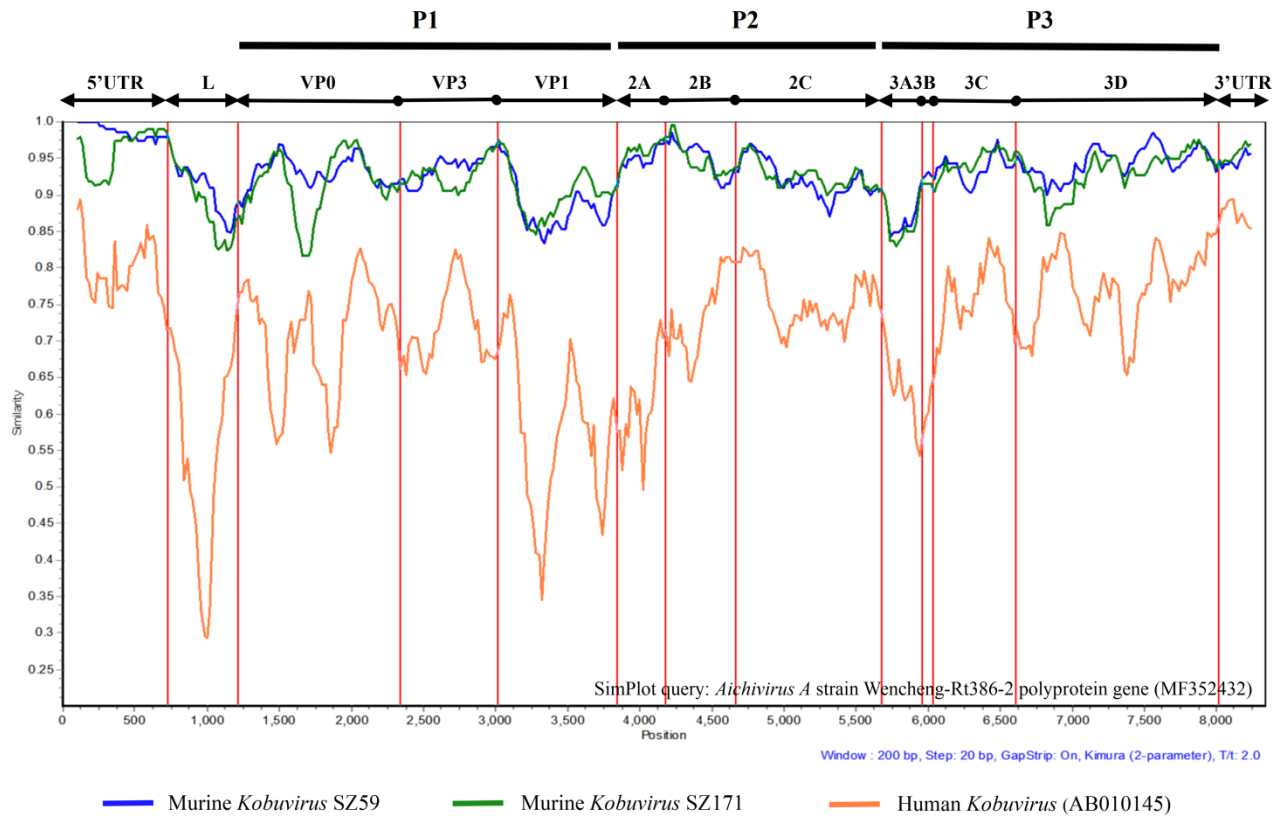
Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures



Supplementary Figure 1. Comparison of the deduced protein amino acid sequences of the study strains and the reference strains. (A) Comparison of the deduced 3D protein amino acid sequences (468 aa). (B) Comparison of the deduced VP1 amino acid sequences (277 aa). Color marks amino acid substitution; dot (.) indicates amino acid deletion.



Supplementary Figure 2. Similarity plot analysis of the complete genomes of SZ59 (blue line) and SZ171 (green line) identified in this study. The human *Kobuvirus* AB010145 (orange line) was used as an out-group sequence, and *Aichivirus A* strain Wencheng-Rt386-2 (MF352432) as a query sequence, using a Kimura (2-parameter) model with a sliding window of 200 nt and a moving step size of 20 nt in Simplot 3.5.1 software.

1.2 Supplementary Tables

Supplementary Table 1. Information for primer sequences.

Number	Primer Name	Sequences (5'-3')	Target fragment(bp)
1	UNIV-kobu-F	TGGAYTACAARTGTTTTGATGC	216
	UNIV-kobu-R	ATGTTGTTRATGATGGTGTTGA	
2	Kobu-F14	TCGGCCCCCTCACCTCTTTTC	597
	Kobu-R610	GTCATCCCTTCCAGGGCGTTGG	
3	Kobu-F262	CAGCCTGACGTGTCACAGGCTG	514
	Kobu-R775	GCAGCAACAGCAGAGCGCACAG	
4	Kobu-F658	GCGTAAGGTTCAAGTGC	1000
	Kobu-R1657	CGGTTGCCTGAGGAGAC	
5	Kobu-F1536	GGCATCGAAGGTGCTGGTAAGG	790
	Kobu-R2325	GTCACGTAGATGGAGCAGGA	
6	Kobu-F2238	ACCACCAGTGGGCTCCATGC	841
	Kobu-R3078	GCAGTRTCCTGCGGAGCATC	
7	Kobu-F2556	TCCCTCAACTGGACTATGGC	727
	Kobu-R3282	AGCCAATGGATRGGGTCTAG	
	Kobu-VP1-os	GTCCAYTGGAARACAGTGGACAT	
8	Kobu-VP1-oa	ACCGACTCCRAGRGCCARTG	831
	Kobu-VP1-is	TCCTTYAACGTYCGYYTCATGC	
	Kobu-VP1-ia	CARTGAGAAGCCGGTGTTGGG	
9	Kobu-F3929	TGATGGTAGCCTGAAGCAGATC	963
	Kobu-R4891	GCCAATATGGAATCYGAGTAGT	
10	Kobu-F4742	CAATGACGGTGCACTCGCCATG	792
	Kobu-R5533	TTGGACTGGGTTCCRGGGATGG	
11	Kobu-F5316	CCCAACCTGGTTTCCTCAGC	885
	Kobu-R6200	GTGGGGACAATGAGGTAGCG	
12	Kobu-F5748	GAGGCTGAGCCCCGGGAAAT	781
	Kobu-R6528	TCCGTGACAAGCGGGGAACC	
13	Kobu-F6426	GTCCGCATCTCTGACAACAG	808
	Kobu-R7233	AGACCACCGAGGAGCATCCG	
14	Kobu-F7085	GTCGTTCGCTCTTTGAT	851
	Kobu-R7935	CACTGATTGCTCGTAGGT	
15	Kobu-F7818	CCTGATGACAAGCGTCCATTC	472
	Kobu-R8289	AAGCTCAAAGACWACCCTGGC	

Supplementary Table 2.

Protease-cleavage sites of Murine *Kobuvirus* (MuKV) sequences obtained in this study and several *Kobuvirus* reference sequences from other species.

		Cleavage between									
		L/VP0	VP0/VP3	VP3/VP1	VP1/2A	2A/2B	2B/2C	2C/3A	3A/3B	3B/3C	3C/3D
Rat	SZ-B13	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GN	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ-CE7	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GN	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ-CJ6	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GN	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ-CL6	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GN	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ-DD6	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GN	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ-FI6	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ22	QLQRQ/G	RYVTP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ59	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSTQ/AA	HIQRQ/GI	ATTHQ/S
	SZ70	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PTSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ86	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ132	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSTQ/AA	HIQRQ/GI	ATTHQ/S
	SZ144	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ155	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ157	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PTSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ158	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ171	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ172	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRE/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ188	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S

	SZ190	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ193	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSTQ/AA	HIQRQ/GI	ATTHQ/S
	SZ202	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ203	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ208	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ227	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ249	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSAQ/AA	HIQRQ/GI	ATTHQ/S
	SZ252	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GN	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ262	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	SZ290	QLQRQ/G	RYVAP/QH	ALSSQ/SL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GR	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
	MN116647	QLQRQ/G	RYVAP/QH	ALSSQ/TL	QRPTY/VH	GIRRRQ/GL	TLEHQ/GL	LIKRRQ/GN	PSSSQ/AA	HIQRQ/GI	ATTHQ/S
Human	AB010145	NLQRQ/G	RYLAP/QH	ALTSQ/TL	QRPTY/VH	GIRRRQ/GL	TLEPQ/GL	LIRRRQ/GN	PQEPQ/AA	HIQRQ/GI	ATTQQ/SL
Feline	KJ958930	QIKPQ/GN	YVAPQ/HW	ALSSQ/GN	VIKRRQ/GA	GIKRRQ/GL	TLEPQ/GL	LIKRRQ/GR	QSAAQ/A	HIQRQ/GL	ATTQQ/SL
Canine	MH747478	QIKPQ/GN	YVAPQ/HW	ALTSQ/AN	AVVKQ/G	GIKRRQ/GL	TLEPQ/GL	LIKRRQ/GR	KSETQ/AA	HIQRQ/GL	ATTHQ/SL
Ovine	MW29615	PVERQ/GT	MVARQ/H	SLALQ/PQ	LARRQ/CQ	VAERQ/G	GVESQ/GL	VRQGA/T	EGVEQ/GA	PVQRQ/GC	ASQHQ/SL
Porcine	KC204684	QIVRQ/GN	QVQKQ/HW	GFQIQ/AA	RIVRQ/CQ	LVVRQ/GL	TVEHQ/G	LVKRQ/G	PDKTQ/GA	SVVRQ/SL	AIDQQ/SI
Bovine	MN336260	NIVTQ/GS	HVTKQ/HW	TLALQ/A	MVRRQ/C	RITRRQ/GL	AVEPQ/GV	LIKRRQ/GA	PDEPQ/AA	GVVRQ/A	ATECQ/SL
Untreated	JQ898342	-	RYVAP/QH	ALSSQ/TL	AVVRQ/GA	GIRRRQ/GL	VLKHQ/G L	LIKRRQ/GN	PESSQ/AA	HIQRQ/GI	ALTQQ/SL