

# Supporting Information

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**Table S1. Reassortants with high replication ability (replication group I)**  
Virus segments

Virus no.	Virus name	PB2	PB1	PA	HA	NP	NA	M	NS	Titer (log <sub>10</sub> pfu/mL)
	HB08 WT									8.9
	BJ09 WT									7.5
15	r2/8									8.9
6	r8									8.7
18	r3/8									8.7
48	r1/3/5/7									8.7
22	r1/2/3									8.6
124	r1/2/3/6/7/8									8.6
5	r7									8.5
20	r5/8									8.5
8	r1/3									8.4
25	r1/2/8									8.4
47	r1/2/7/8									8.4
19	r5/7									8.3
31	r1/7/8									8.3
125	r1/2/3/5/6/8									8.3
2	r2									8.2
7	r1/2									8.2
28	r1/3/8									8.2
30	r1/5/8									8.2
21	r7/8									8.1
33	r2/3/7									8.1
46	r1/2/5/8									8.1
72	r1/3/6									8.1
3	r3									8
23	r1/2/5									8
26	r1/3/5									8
27	r1/3/7									8
49	r1/3/5/8									8
70	r6/8									8
71	r1/2/6									8
127	r1/2/3/5/6/7/8									8
64	r6									7.9
69	r6/7									7.9
1	r1									7.8
11	r1/8									7.8
34	r2/3/8									7.8
44	r1/2/3/8									7.8
59	r1/2/5/7/8									7.8
62	r1/2/3/5/7									7.8
4	r5									7.7
40	r3/7/8									7.7
41	r5/7/8									7.7
55	r2/5/7/8									7.7
63	r1/2/3/5/7/8									7.7
65	r1/6									7.7
66	r2/6									7.7
73	r1/5/6									7.7
68	r5/6									7.6
29	r1/5/7									7.5
43	r1/2/3/7									7.5
45	r1/2/5/7									7.5
60	r1/2/3/7/8									7.5
61	r1/2/3/5/8									7.5

Table S1. Cont.

Virus no.	Virus name	Virus segments								Titer (log <sub>10</sub> pfu/mL)
		PB2	PB1	PA	HA	NP	NA	M	NS	
75	r1/6/8	Yellow	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.5
115	r1/2/6/7/8	Yellow	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.5
57	r2/3/5/7/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.4
67	r3/6	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.4
37	r2/7/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.3
39	r3/5/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.3
50	r1/3/7/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.3
51	r1/5/7/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.3
54	r2/3/7/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.3
104	r3/6/7/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.3
126	r1/2/3/5/6/7	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.3
38	r3/5/7	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.2
80	r3/5/6	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.2
85	r6/7/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.2
86	r1/2/3/6	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.2
103	r3/5/6/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.2
118	r1/2/3/6/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.2
58	r1/3/5/7/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.1
78	r2/6/7	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.1
88	r1/2/6/7	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7.1
56	r3/5/7/8	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7
81	r3/6/7	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	7

"r" in virus names denotes reassortant. The numbers in virus names indicate gene segments derived from the BJ09 virus as follows: 1, PB2; 2, PB1; 3, PA; 4, HA; 5, NP; 6, NA; 7, M; and 8, NS. The virus segments derived from the HB08 virus were not assigned numbers. Segments derived from BJ09 virus are in yellow, and those derived from HB08 virus are in blue. The virus titers were determined by plaque assay on Madin–Darby canine kidney (MDCK) cells.

**Table S2. Reassortants with moderate replication ability (replication group II)**

Virus no.	Virus name	Virus segments								Titer (log <sub>10</sub> pfu/mL)
		PB2	PB1	PA	HA	NP	NA	M	NS	
42	r1/2/3/5									6.9
76	r2/3/6									6.7
87	r1/2/5/6									6.7
106	r3/5/6/7/8									6.7
108	r2/3/6/7/8									6.7
111	r1/5/6/7/8									6.6
9	r1/5									6.4
82	r3/6/8									6.4
84	r5/6/8									6.4
119	r1/2/3/6/7									6.3
90	r1/3/5/6									6.2
17	r3/7									6.1
53	r2/3/5/8									6
83	r5/6/7									6
89	r1/2/6/8									6
36	r2/5/8									5.9
91	r1/3/6/7									5.8
92	r1/3/6/8									5.7
122	r1/3/5/6/7/8									5.6
77	r2/5/6									5.5
123	r1/2/5/6/7/8									5.5
114	r1/3/5/6/7									5.4
79	r2/6/8									5.3
117	r1/2/5/6/7									5.2
105	r5/6/7/8									5.1
74	r1/6/7									4.9
112	r1/3/6/7/8									4.9
101	r2/6/7/8									4.7
120	r1/2/3/5/6									4.7
113	r1/3/5/6/8									4.6
109	r2/3/5/6/8									4.5
116	r1/2/5/6/8									4.5
16	r3/5									4.2
24	r1/2/7									4.2
98	r2/3/6/8									4

Symbols and nomenclature of reassortant viruses are as described in the legend in [Table S1](#).

**Table S3. Reassortants with low replication ability (replication group III)**

Virus no.	Virus name	Virus segments								Titer (log <sub>10</sub> pfu/mL)
		PB2	PB1	PA	HA	NP	NA	M	NS	
95	r1/6/7/8									3.7
12	r2/3									3.6
94	r1/5/6/8									3.6
121	r2/3/5/6/7/8									3.6
100	r2/5/6/8									3.2

Symbols and nomenclature of reassortant viruses are as described in the legend in [Table S1](#).

**Table S4. Nonviable reassortants (replication group IV)**

Virus no.	Virus name	Virus segments							Titer (log <sub>10</sub> pfu/mL)
		PB2	PB1	PA	HA	NP	NA	M	
10	r1/7								0
13	r2/5								0
14	r2/7								0
32	r2/3/5								0
35	r2/5/7								0
52	r2/3/5/7								0
93	r1/5/6/7								0
96	r2/3/5/6								0
97	r2/3/6/7								0
99	r2/5/6/7								0
102	r3/5/6/7								0
107	r2/5/6/7/8								0
110	r2/3/5/6/7								0

Symbols and nomenclature of reassortant viruses are as described in the legend in [Table S1](#).

**Table S5. Effect of a single genomic segment from BJ09 virus on the replication of reassortants in vitro**

Segment of BJ09 origin	Significance	Effect on the replication of reassortants
PB2	$P < 0.01$	+
PB1	$P < 0.01$	-
PA	$P > 0.05$	None
NP	$P < 0.01$	-
NA	$P < 0.01$	-
M	$P < 0.01$	-
NS	$P < 0.05$	+

"+" indicates that the corresponding segment of BJ09 origin enhances the replication ability of the reassortants in MDCK cells; "-" indicates that the corresponding segment of BJ09 origin reduces the replication ability of the reassortants; "None" indicates that the corresponding segment of BJ09 origin had no significant effect on the replication ability of the reassortants.

**Table S6. The interaction between two segments for the replication of reassortants in vitro**

Segment that was affected	BJ09 segment that was affected on the corresponding segment	Significance	Effect on the replication of reassortants
HB08 PB2	BJ09 PB1	$P < 0.01$	–
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P < 0.01$	–
	BJ09 NA	$P > 0.05$	None
	BJ09 M	$P > 0.05$	None
	BJ09 NS	$P < 0.05$	+
HB08 PB1	BJ09 PB2	$P > 0.05$	None
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P > 0.05$	None
	BJ09 NA	$P < 0.01$	–
	BJ09 M	$P < 0.05$	–
	BJ09 NS	$P > 0.05$	None
HB08 PA	BJ09 PB2	$P > 0.05$	None
	BJ09 PB1	$P > 0.05$	None
	BJ09 NP	$P < 0.05$	–
	BJ09 NA	$P < 0.05$	–
	BJ09 M	$P < 0.01$	–
	BJ09 NS	$P > 0.05$	None
HB08 NP	BJ09 PB2	$P > 0.05$	None
	BJ09 PB1	$P > 0.05$	None
	BJ09 PA	$P > 0.05$	None
	BJ09 NA	$P > 0.05$	None
	BJ09 M	$P < 0.05$	–
	BJ09 NS	$P > 0.05$	None
HB08 NA	BJ09 PB2	$P > 0.05$	None
	BJ09 PB1	$P < 0.05$	–
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P > 0.05$	None
	BJ09 M	$P > 0.05$	None
	BJ09 NS	$P < 0.01$	+
HB08 M	BJ09 PB2	$P < 0.05$	+
	BJ09 PB1	$P < 0.01$	–
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P < 0.01$	–
	BJ09 NA	$P < 0.05$	–
	BJ09 NS	$P > 0.05$	None
HB08 NS	BJ09 PB2	$P > 0.05$	None
	BJ09 PB1	$P < 0.05$	–
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P < 0.01$	–
	BJ09 NA	$P > 0.05$	None
	BJ09 M	$P < 0.05$	–
BJ09 PB2	BJ09 PB1	$P < 0.05$	+
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P > 0.05$	None
	BJ09 NA	$P < 0.01$	–
	BJ09 M	$P > 0.05$	None
	BJ09 NS	$P > 0.05$	None
BJ09 PB1	BJ09 PB2	$P < 0.01$	+
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P < 0.01$	–
	BJ09 PB1A6	$P > 0.05$	None
	BJ09 M	$P > 0.05$	None
	BJ09 NS	$P < 0.01$	+

Table S6. Cont.

Segment that was affected	BJ09 segment that was affected on the corresponding segment	Significance	Effect on the replication of reassortants
BJ09 PA	BJ09 PB2	$P < 0.01$	+
	BJ09 PB1	$P > 0.05$	None
	BJ09 NP	$P < 0.01$	-
	BJ09 NA	$P < 0.01$	-
	BJ09 M	$P > 0.05$	None
	BJ09 NS	$P < 0.05$	+
BJ09 NP	BJ09 PB2	$P < 0.01$	+
	BJ09 PB1	$P < 0.05$	-
	BJ09 PA	$P > 0.05$	None
	BJ09 NA	$P < 0.01$	-
	BJ09 M	$P > 0.05$	None
	BJ09 NS	$P < 0.05$	+
BJ09 NA	BJ09 PB2	$P > 0.05$	None
	BJ09 PB1	$P > 0.05$	None
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P < 0.01$	-
	BJ09 M	$P < 0.05$	-
	BJ09 NS	$P > 0.05$	None
BJ09 M	BJ09 PB2	$P > 0.05$	None
	BJ09 PB1	$P > 0.05$	None
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P > 0.05$	None
	BJ09 NA	$P < 0.05$	-
	BJ09 NS	$P < 0.01$	+
BJ09 NS	BJ09 PB2	$P > 0.05$	None
	BJ09 PB1	$P > 0.05$	None
	BJ09 PA	$P > 0.05$	None
	BJ09 NP	$P < 0.01$	-
	BJ09 NA	$P < 0.01$	-
	BJ09 M	$P > 0.05$	None

"+" indicates that the corresponding segment of BJ09 origin enhances the replication ability of the reassortants in MDCK cells; "-" indicates that the corresponding segment of BJ09 origin reduces the replication ability of the reassortants; "None" indicates that the corresponding segment of BJ09 origin had no significant effect on the replication ability of the reassortants.

**Table S7. Infectivity and replication ability of reassortants in mice**

Virus no.	Virus name	Virus segments								MID <sub>50</sub> (log <sub>10</sub> pfu)	Virus titer (pfu/mL ± SD)	
		PB2	PB1	PA	HA	NP	NA	M	NS		Lung	Nasal turbinate
	HB08 WT									<1.0	7.8 ± 0.1	6.9 ± 0.5
	BJ09 WT									<1.0	7.2 ± 0.4	6.7 ± 0.7
1	r1									<1.0	6.8 ± 0.5	6.1 ± 0.5
2	r2									<1.0	7.9 ± 0.4	6.5 ± 0.4
3	r3									<1.0	7.7 ± 0.4	6.7 ± 0.3
4	r5									<1.0	7.8 ± 0.2	6.6 ± 0.4
5	r7									<1.0	5.2 ± 0.1	4.0 ± 0.4
6	r8									<1.0	5.3 ± 0.2	4.1 ± 0.1
7	r1/2									<1.0	5.7 ± 0.2	4.8 ± 0.5
8	r1/3									<1.0	5.1 ± 0.9	5.1 ± 0.3
11	r1/8									<1.0	4.5 ± 0.3	3.6 ± 0.1
15	r2/8									<1.0	4.7 ± 0.4	4.2 ± 0.5
18	r3/8									<1.0	5.9 ± 0.1	4.7 ± 0.1
19	r5/7									<1.0	6.8 ± 0.5	5.9 ± 0.7
20	r5/8									<1.0	6.9 ± 0.1	6.2 ± 0.9
21	r7/8									<1.0	5.8 ± 0.4	4.6 ± 0.1
22	r1/2/3									<1.0	6.0 ± 1.2	4.8 ± 0.4
23	r1/2/5									<1.0	5.6 ± 0.3	5.4 ± 0.3
25	r1/2/8									<1.0	5.4 ± 0.4	5.2 ± 0.2
26	r1/3/5									<1.0	4.7 ± 0.6	3.8 ± 0.1
27	r1/3/7									<1.0	5.2 ± 0.1	5.0 ± 0.1
28	r1/3/8									<1.0	3.9 ± 0.1	4.2 ± 0.2
29	r1/5/7									<1.0	5.7 ± 0.4	5.3 ± 0.3
30	r1/5/8									<1.0	5.6 ± 0.3	5.4 ± 0.4
31	r1/7/8									<1.0	5.4 ± 0.3	5.2 ± 0.6
33	r2/3/7									<1.0	5.7 ± 0.5	5.0 ± 0.1
34	r2/3/8									<1.0	5.8 ± 0.2	3.9 ± 0.7
37	r2/7/8									<1.0	6.0 ± 0.3	5.9 ± 0.4
38	r3/5/7									<1.0	7.1 ± 0.4	6.1 ± 0.3
39	r3/5/8									1.3	6.2 ± 0.2	4.0 ± 0.3
40	r3/7/8									<1.0	6.3 ± 0.1	5.2 ± 0.1
41	r5/7/8									<1.0	4.9 ± 0.1	4.2 ± 0.2
43	r1/2/3/7									<1.0	6.3 ± 0.7	6.2 ± 0.1
44	r1/2/3/8									<1.0	5.5 ± 0.1	3.8 ± 0.9
45	r1/2/5/7									<1.0	4.5 ± 0.5	4.6 ± 0.3
46	r1/2/5/8									<1.0	4.4 ± 0.1	3.2 ± 0.4
47	r1/2/7/8									<1.0	5.2 ± 0.1	4.3 ± 0.1
48	r1/3/5/7									<1.0	6.6 ± 1.2	5.7 ± 0.9
49	r1/3/5/8									<1.0	4.5 ± 0.4	4.3 ± 0.1
50	r1/3/7/8									<1.0	4.0 ± 0.5	4.2 ± 0.2
51	r1/5/7/8									1.8	4.2 ± 0.1	2.9 ± 0.1
54	r2/3/7/8									<1.0	5.4 ± 0.5	4.0 ± 0.1
55	r2/5/7/8									1.3	4.3 ± 0.6	3.7 ± 0.2
56	r3/5/7/8									<1.0	5.6 ± 0.1	5.0 ± 0.4
57	r2/3/5/7/8									<1.0	4.9 ± 0.3	3.6 ± 0.1
58	r1/3/5/7/8									<1.0	4.1 ± 1.2	3.3 ± 0.1
59	r1/2/5/7/8									<1.0	4.6 ± 0.3	4.2 ± 0.1
60	r1/2/3/7/8									<1.0	4.9 ± 0.4	4.3 ± 0.3
61	r1/2/3/5/8									<1.0	4.5 ± 0.1	3.1 ± 0.1
62	r1/2/3/5/7									<1.0	5.1 ± 0.1	3.5 ± 0.4
63	r1/2/3/5/7/8									<1.0	5.8 ± 1.0	4.5 ± 0.1
64	r6									<1.0	5.3 ± 0.4	5.3 ± 0.7
65	r1/6									<1.0	4.1 ± 0.5	3.4 ± 0.4
66	r2/6									1.3	5.2 ± 1.0	3.2 ± 0.3
67	r3/6									<1.0	7.0 ± 0.3	4.9 ± 0.5
68	r5/6									<1.0	5.2 ± 0.3	3.6 ± 0.5
69	r6/7									<1.0	4.4 ± 0.3	4.2 ± 0.5
70	r6/8									<1.0	5.5 ± 0.6	4.0 ± 0.7
71	r1/2/6									<1.0	4.7 ± 0.4	4.2 ± 0.3

**Table S7. Cont.**

Virus no.	Virus name	Virus segments								Virus titer (pfu/mL ± SD)		
		PB2	PB1	PA	HA	NP	NA	M	NS	MID <sub>50</sub> (log <sub>10</sub> pfu)	Lung	Nasal turbinate
72	r1/3/6									<1.0	4.5 ± 0.5	4.1 ± 0.4
73	r1/5/6									<1.0	5.2 ± 0.3	4.9 ± 0.7
75	r1/6/8									<1.0	4.1 ± 0.1	3.8 ± 0.4
78	r2/6/7									<1.0	4.4 ± 0.1	4.0 ± 0.1
80	r3/5/6									<1.0	6.6 ± 1.0	5.9 ± 0.9
85	r6/7/8									<1.0	7.2 ± 0.5	4.3 ± 0.5
86	r1/2/3/6									<1.0	6.3 ± 0.3	6.2 ± 0.6
88	r1/2/6/7									<1.0	3.7 ± 0.9	3.2 ± 0.4
103	r3/5/6/8									<1.0	7.0 ± 0.4	5.0 ± 0.2
104	r3/6/7/8									<1.0	7.1 ± 0.8	4.8 ± 0.4
115	r1/2/6/7/8									<1.0	5.8 ± 0.8	4.2 ± 0.5
118	r1/2/3/6/8									<1.0	6.1 ± 0.3	3.2 ± 0.1
124	r1/2/3/6/7/8									<1.0	6.7 ± 0.3	4.5 ± 0.3
125	r1/2/3/5/6/8									<1.0	5.5 ± 0.8	4.5 ± 0.7
126	r1/2/3/5/6/7									<1.0	7.5 ± 0.3	4.2 ± 0.1
127	r1/2/3/5/6/7/8									<1.0	6.5 ± 0.5	6.3 ± 0.2

Virus titers in lung and nasal turbinate are means ± SDs of the log<sub>10</sub> pfu/mL on day 4 postinfection of three mice infected with 10<sup>4</sup> pfu.

**Table S8. Contribution of BJ09 PA to the pathogenicity of reassortants**

Effect of BJ09 PA on pathogenicity	Viruses without BJ09 PA					Viruses with BJ09 PA				
	Virus no.	Virus name	LD <sub>50</sub> (log <sub>10</sub> pfu)	Weight loss (%)	MST (days)	Virus no.	Virus name	LD <sub>50</sub> (log <sub>10</sub> pfu)	Weight loss (%)	MST (days)
Enhanced		HB08 WT	≥5.5	8.1	>14	3	r3	4.3	>20	6
	19	r5/7	≥5.5	0	>14	38	r3/5/7	2.8	>20	6
	64	r6	4.8	13.2	9	67	r3/6	3.3	>20	5
	85	r6/7/8	4.8	11.9	8.7	104	r3/6/7/8	2.8	>20	6.7
	1	r1	≥5.5	4	>14	8	r1/3	5.3	9.5	12
	21	r7/8	≥5.5	0	>14	40	r3/7/8	4.8	17.9	8
	29	r1/5/7	≥5.5	7.9	>14	48	r1/3/5/7	5.3	8.7	12
	51	r1/5/7/8	≥5.5	3.8	>14	58	r1/3/5/7/8	4.8	18.8	9.7
	115	r1/2/6/7/8	4.8	13.9	10	124	r1/2/3/6/7/8	4.8	15.8	9.7
	30	r1/5/8	≥5.5	0	>14	49	r1/3/5/8	≥5.5	6.1	>14
31	r1/7/8	≥5.5	0	>14	50	r1/3/7/8	≥5.5	6.5	>14	
Attenuated	11	r1/8	4.8	19.7	7.7	28	r1/3/8	≥5.5	0	>14
	15	r2/8	≥5.5	6.6	>14	34	r2/3/8	≥5.5	0	>14

This table was derived from Tables 1–4 and was used to evaluate the contribution of BJ09 PA to the pathogenicity of the reassortant viruses. Pairs of reassortants with or without BJ09 PA are listed. The pathogenicity of the two viruses in each pair was compared. “Enhanced” indicates that the pathogenicity of viruses was enhanced after introduction of BJ09 PA; “Attenuated” indicates that the pathogenicity of viruses was attenuated after introduction of BJ09 PA. LD<sub>50</sub>, 50% mouse lethal dose. MST, mean survival time.



**Table S9. Contribution of BJ09 PB1 to the pathogenicity of reassortants**

Effect of BJ09 PB1 on pathogenicity	Viruses without BJ09 PB1					Viruses with BJ09 PB1					
	Virus no.	Virus name	LD <sub>50</sub> (log <sub>10</sub> pfu)	Weight loss (%)	MST (days)	Virus no.	Virus name	LD <sub>50</sub> (log <sub>10</sub> pfu)	Weight loss (%)	MST (days)	
Attenuated	8	r1/3	5.3	9.5	12	22	r1/2/3	≥5.5	0	>14	
	11	r1/8	4.8	19.7	7.7	25	r1/2/8	≥5.5	0	>14	
	40	r3/7/8	4.8	17.9	8	54	r2/3/7/8	≥5.5	0	>14	
	48	r1/3/5/7	5.3	8.7	12	62	r1/2/3/5/7	≥5.5	0	>14	
	58	r1/3/5/7/8	4.8	18.8	9.7	63	r1/2/3/5/7/8	≥5.5	2.9	>14	
	64	r6	4.8	13.2	9	66	r2/6	≥5.5	0	>14	
	29	r1/5/7	≥5.5	7.9	>14	45	r1/2/5/7	≥5.5	0	>14	
	41	r5/7/8	≥5.5	5.6	>14	55	r2/5/7/8	≥5.5	2.2	>14	
	49	r1/3/5/8	≥5.5	6.1	>14	61	r1/2/3/5/8	≥5.5	0	>14	
	50	r1/3/7/8	≥5.5	6.5	>14	60	r1/2/3/7/8	≥5.5	0	>14	
	69	r6/7	≥5.5	10.4	>14	78	r2/6/7	≥5.5	0.8	>14	
			HB08 WT	≥5.5	8.1	>14	2	r2	≥5.5	4.1	>14
	Enhanced	6	r8	≥5.5	2.1	>14	15	r2/8	≥5.5	6.6	>14

This table was derived from Tables 1–4 and was used to evaluate the contribution of BJ09 PB1 to the pathogenicity of the reassortant viruses. Pairs of reassortants with or without BJ09 PB1 are listed. The pathogenicity of the two viruses in each pair was compared. Enhanced, means the pathogenicity of viruses were enhanced after introduction of BJ09 PB1; Attenuate, means the pathogenicity of viruses were attenuated after introduction of BJ09 PB1. LD<sub>50</sub>, 50% mouse lethal dose. MST, mean survival time.

**Table S10. Contribution of BJ09 NA to the pathogenicity of reassortants**

Effect of BJ09 NA on pathogenicity	Viruses without BJ09 NA					Viruses contained with BJ09 NA					
	Virus no.	Virus name	LD <sub>50</sub> (log <sub>10</sub> pfu)	Weight loss (%)	MST (days)	Virus no.	Virus name	LD <sub>50</sub> (log <sub>10</sub> pfu)	Weight loss (%)	MST (days)	
Enhanced	3	r3	4.3	>20	6	67	r3/6	3.3	>20	5	
	39	r3/5/8	≥5.5	4.7	>14	103	r3/5/6/8	4.3	>20	6	
	40	r3/7/8	4.8	17.9	8	104	r3/6/7/8	2.8	>20	6.7	
	62	r1/2/3/5/7	≥5.5	0	>14	126	r1/2/3/5/6/7	2.8	>20	7	
			HB08 WT	≥5.5	8.1	>14	64	r6	4.8	13.2	9
	6	r8	≥5.5	2.1	>14	70	r6/8	4.8	19.4	10	
	21	r7/8	≥5.5	0	>14	85	r6/7/8	4.8	11.9	8.7	
	47	r1/2/7/8	≥5.5	0	>14	115	r1/2/6/7/8	4.8	13.9	10	
	60	r1/2/3/7/8	≥5.5	0	>14	124	r1/2/3/6/7/8	4.8	15.8	9.7	
	61	r1/2/3/5/8	≥5.5	0	>14	125	r1/2/3/5/6/8	5.3	8.3	12.3	
	63	r1/2/3/5/7/8	≥5.5	2.9	>14	127	r1/2/3/5/6/7/8	4.5	>20	6.7	
	4	r5	≥5.5	0	>14	68	r5/6	≥5.5	5.7	>14	
	5	r7	≥5.5	0	>14	69	r6/7	≥5.5	10.4	>14	
	44	r1/2/3/8	≥5.5	4.3	>14	118	r1/2/3/6/8	≥5.5	8.3	>14	
Attenuated	8	r1/3	5.3	9.5	12	72	r1/3/6	≥5.5	0	>14	
	11	r1/8	4.8	19.7	7.7	75	r1/6/8	≥5.5	0	>14	

This table was derived from Tables 1–4 and was used to evaluate the contribution of BJ09 NA to the pathogenicity of the reassortant viruses. Pairs of reassortants with or without BJ09 NA are listed. The pathogenicity of the two viruses in each pair was compared. “Enhanced” indicates that the pathogenicity of viruses was enhanced after introduction of BJ09 NA; “Attenuated” indicates that the pathogenicity of viruses was attenuated after introduction of BJ09 NA. LD<sub>50</sub>, 50% mouse lethal dose. MST, mean survival time.