Supporting Information

Li et al. 10.1073/pnas.0912807107

Table S1. Highly replicative reassortant viruses between SK06 (H5N1) and Tok07 (H3N2) viruses

Virus No.	Virus name	Virus segments								CPE 48h	Aliquot hour	Titer (pfu/mL)	Plaque size
		PB2	PB1	PA	HA	NP	NA	M	NS				
1	r1									+++	48	1.8×10^{8}	L
2	r2									++	72	4×10^{8}	M,L
4	r4									++	72	3.5×10^{7}	L
6	r6									+	72	6.7×10^{7}	S
7	r7									+++	48	2.2×10^{8}	L
8	r8									++++	48	1.4×10^{8}	L
9	r1,2									++++	48	6.7×10^{7}	M,L
10	r1,3									++/+++	48	1.1×10^{7}	L
11	r1,4									+++	48	6.3×10^{7}	L
13	r1,6									+++	48	3.5×10^{8}	S
14	r1,7									++++	48	7.4×10^{8}	L
15	r1,8									++++	48	3.1×10^{8}	L
16	r2,3									++	72	1.3×10^{7}	S
17	r2,4									++	72	2.8×10^{7}	L
19	r2,6									+++	48	6.8×10^{7}	S
20	r2,7									+++	48	3.1×10^{8}	M,L
21	r2,8									++++	48	4.9×10^{8}	L
28	r4,6									+/++	72	4.1×10^{7}	S,M
29	r4,7									+	72	3.8×10^{7}	S
34	r6,7									+	72	5.7×10^{7}	S
35	r6,8									++++	48	2×10^{8}	S
36	r7,8									++++	48	1.3×10^{8}	L
37	r1,2,3									++	72	1.1×10^{8}	L
38	r1,2,4									+++	48	5.6×10^{7}	L
40	r1,2,6									++	72	1.1×10^{8}	S
41	r1,2,7									++	72	4.1×10^{8}	L
42	r1,2,8									++++	48	7.8×10^{8}	L
43	r1,3,4									+++	48	9×10^{7}	L
45	r1,3,6									+++	48	1.6×10^{8}	S
46	r1,3,7									++++	48	5×10^{8}	L
47	r1,3,8									++++	48	1.1×10^{8}	M
49	r1,4,6									++/+++	48	4.2×10^{7}	L
50	r1,4,7									++	72	5.1×10^{7}	S,M,L
51	r1,4,8									++++	48	1.2×10^{7}	M,L
55	r1,6,7									++++	48	4.9×10^{8}	S
56	r1,6,8									++++	48	4.6×10^{8}	S
57	r1,7,8									++++	48	6×10^{8}	L
66	r2,4,8									+	72	1.4×10^{7}	M
70	r2,6,7									+	72	2.6×10^{8}	S
71	r2,6,8									++++	48	2.7×10^{8}	S
72	r2,7,8									++++	48	2.3×10^{8}	L
87	r4,6,8									++	72	5.7×10^{7}	S,M
91	r5,7,8									–/+	72	7×10^{7}	M,L
92	r6,7,8									++++	48	2.1×10^{7}	S
95	r1,2,3,6									+++	48	1.8×10^{8}	S
96	r1,2,3,7									++++	48	1.3×10^{8}	L
97	r1,2,3,8									++++	48	7×10^{7}	L
105	r1,2,6,7									++/+++	48	1.6×10^{8}	S
106	r1,2,6,8									++++	48	1.9×10^{8}	S
107	r1,2,7,8									++++	48	3.8×10^{8}	L
109	r1,3,4,6									++	72	3.2×10^{7}	L
110	r1,3,4,7									+/++	72	1.4×10^{8}	L
111	r1,3,4,8									+++/++++	48	4.2×10^{7}	M,L
113	r1,3,5,7									+	72	1.7×10^{7}	S

Table S1. Cont.

115	r1,3,6,7								++++	48	1.4×10^{8}	M
116	r1,3,6,8								++++	48	5.8×10^{7}	S
117	r1,3,7,8								++++	48	1.3×10^{8}	L
122	r1,4,6,8								+++/++++	48	3.9×10^{7}	L
123	r1,4,7,8								++++	48	2×10^{7}	L
127	r1,6,7,8								++++	48	8.3×10^{7}	S
146	r2,5,7,8								+	72	2×10^{8}	L
147	r2,6,7,8								++++	48	4.3×10^{7}	S
164	r1,2,3,4,6								+	72	4×10^{7}	L
165	r1,2,3,4,7								+	72	2.9×10^{7}	L
166	r1,2,3,4,8								++++	48	2.7×10^{7}	L
168	r1,2,3,5,7								+	72	3.9×10^{7}	S,M,L
170	r1,2,3,6,7								+++/++++	48	2.2×10^{8}	S
171	r1,2,3,6,8								++++	48	2.5×10^{7}	S
172	r1,2,3,7,8								++++	24	6×10^{8}	L
177	r1,2,4,6,8								+++	48	2.8×10^{7}	L
178	r1,2,4,7,8								++++	48	7×10^{7}	M,L
179	r1,2,5,6,7								–/+	72	1×10^{7}	S
180	r1,2,5,6,8								++++	48	7×10^{7}	S
181	r1,2,5,7,8								++++	48	1.9×10^{7}	M,L
182	r1,2,6,7,8								+++/++++	24	2.1×10^{7}	S
186	r1,3,4,6,7								+	72	2.8×10^{7}	L
187	r1,3,4,6,8								+++	48	1.2×10^{8}	M,L
188	r1,3,4,7,8								++++	48	8.4×10^{7}	L
191	r1,3,5,7,8								+++	48	2×10^{7}	S,M
192	r1,3,6,7,8								++++	48	2.1×10^{8}	S
197	r1,5,6,7,8								++++	48	1×10^{7}	S
221	r1,2,3,4,5,8								++	72	1.2×10^{7}	S,M
223	r1,2,3,4,6,8								+/++	72	2×10^{7}	L
224	r1,2,3,4,7,8								++	72	5.1×10^{7}	L
226	r1,2,3,5,6,8								+++	48	2.4×10^{8}	S
227	r1,2,3,5,7,8								+++	48	3.4×10^{8}	L
228	r1,2,3,6,7,8								++++	48	3×10^{8}	M
233	r1,2,5,6,7,8								++++	48	2.2×10^{8}	S,M
236	r1,3,4,5,7,8								+	72	1.5×10^{7}	M
237	r1,3,4,6,7,8								++	72	2.6×10^{7}	M
238	r1,3,5,6,7,8								++++	48	7×10^{7}	S
248	r1,2,3,4,5,6,8								++	72	9×10^{7}	L
249	r1,2,3,4,5,7,8								++	72	1.2×10^{8}	L
250	r1,2,3,4,6,7,8								+/++	72	3.4×10^{7}	L
251	r1,2,3,5,6,7,8								++++	48	3.2×10^{8}	M
253	r1,3,4,5,6,7,8								+	72	1.1×10^{8}	S,M
255	Tok07								++/+++	48	4.2×10^{7}	L
256	SK06								+++	48	1.8×10^{8}	L

The parental or reassortant viruses were generated by reverse genetics as described in *Materials and Methods*. The transfection supernatant from 293T cells was collected at 48 h posttransfection and used to infect Madin-Darby canine kidney (MDCK) cells. The cytopathic effects (CPE) of MDCK cells were checked at 48 h postinfection (with two exceptions, r1,2,3,7,8 and r1,2,6,7,8) and recorded on a scale ranging from "–" (no CPE) to "++++" (obvious CPE; all cells died and detached). "r'" in the virus names denotes reassortant. The numbers in the virus names indicate segments derived from the Tok07 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 SK06 virus were not assigned numbers. Gene segments derived from Tok07 virus are shown in gray, while those derived from SK06 virus are shown in rose. The virus titers were determined by plaque assay on MDCK cells, and the plaque sizes were roughly determined by eye as small (S), medium (M), or large (L).

Table S2. Moderately replicative reassortant viruses between SK06 (H5N1) and Tok07 (H3N2) viruses

Virus No.	Virus name	Virus segments								CPE 48h	Aliquot hour	Titer (pfu/mL)	Plaque size
		PB2	PB1	PA	HA	NP	NA	M	NS				
3	r3									+++	48	2×10^6	S
22	r3,4									+	72	9×10^6	L
24	r3,6									–	72	3.2×10^4	S
25	r3,7									+	72	1.9×10^6	M
30	r4,8									++	72	8.7×10^6	S
33	r5,8									+	72	3.3×10^6	S,M
44	r1,3,5									–/+	72	7×10^4	S
52	r1,5,6									+	72	1.3×10^6	S
53	r1,5,7									+	72	1×10^5	S
54	r1,5,8									++++	48	3.6×10^4	S,M
64	r2,4,6									–/+	72	1.6×10^5	M
65	r2,4,7									+	72	4.1×10^6	S
69	r2,5,8									+	72	3.9×10^6	S
74	r3,4,6									–/+	72	3×10^4	M
75	r3,4,7									+	72	7.8×10^5	S,M
81	r3,6,8									–/+	72	1×10^4	S
85	r4,5,8									+	72	6.6×10^5	S
86	r4,6,7									+	72	2.8×10^5	S
88	r4,7,8									+	72	7.7×10^4	S
90	r5,6,8									+	72	1.5×10^5	S
93	r1,2,3,4									+	72	7×10^6	M
94	r1,2,3,5									+	72	2.8×10^4	S
99	r1,2,4,6									+	72	2.3×10^6	L
100	r1,2,4,7									+	72	2.1×10^6	M,L
101	r1,2,4,8									+++	48	4.2×10^6	L
102	r1,2,5,6									–/+	72	6.6×10^5	S
103	r1,2,5,7									–/+	72	2.2×10^5	S
104	r1,2,5,8									++++	48	3.2×10^6	M,L
112	r1,3,5,6									–/+	72	1.7×10^4	S
114	r1,3,5,8									+/++	72	1.4×10^5	M
120	r1,4,5,8									+	72	8.4×10^4	S,M
121	r1,4,6,7									++	72	7×10^6	M
124	r1,5,6,7									+	72	2.8×10^6	S
125	r1,5,6,8									++++	48	1.1×10^6	S
126	r1,5,7,8									++++	48	7×10^6	M,L
136	r2,3,6,8									+	72	6.1×10^4	S
140	r2,4,5,8									+/++	72	2.5×10^4	S
141	r2,4,6,7									+	72	2.1×10^6	S,M
142	r2,4,6,8									+	72	6×10^6	M
143	r2,4,7,8									++/+++	48	5.7×10^6	S
161	r4,6,7,8									–/+	72	3.1×10^6	S
163	r1,2,3,4,5									+	72	1.1×10^5	M
167	r1,2,3,5,6									+	72	8.1×10^6	S
169	r1,2,3,5,8									+++/++++	48	1.5×10^6	M,L
173	r1,2,4,5,6									–/+	72	3.4×10^4	M,L
175	r1,2,4,5,8									+	72	6.1×10^4	M
176	r1,2,4,6,7									+	72	3.7×10^5	M
184	r1,3,4,5,7									+	72	1.9×10^5	M,L
185	r1,3,4,5,8									++	72	1.4×10^5	S
189	r1,3,5,6,7									+	72	5.9×10^6	S,M
190	r1,3,5,6,8									+++	48	3.5×10^5	S
194	r1,4,5,6,8									+	72	2.8×10^5	S,M
195	r1,4,5,7,8									++++	48	7.2×10^5	S,M
196	r1,4,6,7,8									++	72	7×10^6	M,L
209	r2,4,5,6,8									++	72	2.2×10^4	S
211	r2,4,6,7,8									+++	48	2.3×10^6	S,M
219	r1,2,3,4,5,6									+	72	1.1×10^6	M
220	r1,2,3,4,5,7									+	72	4×10^6	M
222	r1,2,3,4,6,7									–/+	72	3.9×10^6	M
225	r1,2,3,5,6,7									+	72	7×10^6	S
230	r1,2,4,5,6,8									++	72	1.3×10^6	S,M
231	r1,2,4,5,7,8									++++	48	1×10^6	S
232	r1,2,4,6,7,8									+/++	72	3.6×10^6	M
234	r1,3,4,5,6,7									+	72	1.2×10^5	M
235	r1,3,4,5,6,8									+/++	72	1.6×10^5	S,M
239	r1,4,5,6,7,8									+	72	1.4×10^6	S,M
247	r1,2,3,4,5,6,7									+	72	3.5×10^6	M,L
252	r1,2,4,5,6,7,8									+	72	7.7×10^5	S,M

Symbols and nomenclature of reassortant viruses are as described in Table S1.

Table S3. Low replicative reassortant viruses between SK06 (H5N1) and Tok07 (H3N2) viruses

Virus No.	Virus name	Virus segments								CPE 48h	Aliquot hour	Titer (pfu/mL)	Plaque size
		PB2	PB1	PA	HA	NP	NA	M	NS				
12	r1,5									+	72	1.7×10^{3}	S
26	r3,8									++	48	5.7×10^{3}	S,M
39	r1,2,5									+	72	1.2×10^{3}	S
61	r2,3,7									–	72	1.3×10^{2}	S
62	r2,3,8									++	72	2×10^{3}	M
80	r3,6,7									–	72	5×10^{3}	S
82	r3,7,8									+/++	72	2.4×10^{2}	M
98	r1,2,4,5									+	72	2×10^{1}	S
108	r1,3,4,5									+/–	72	2.8×10^{3}	S
118	r1,4,5,6									–/+	72	1.7×10^{2}	S,M
119	r1,4,5,7									–/+	72	7×10^{1}	S,M,L
131	r2,3,4,8									–/+	72	1×10^{1}	S
137	r2,3,7,8									+	72	3×10^{2}	S,M
145	r2,5,6,8									–	72	9.7×10^{3}	S
157	r3,6,7,8									–	72	1.3×10^{2}	S
162	r5,6,7,8									–/+	72	1.5×10^{3}	S
210	r2,4,5,7,8									–/+	72	6×10^{1}	S
212	r2,5,6,7,8									–	72	3.3×10^{3}	S
229	r1,2,4,5,6,7									+	72	3.1×10^{2}	S
245	r2,4,5,6,7,8									–/+	72	2.8×10^{2}	M

Symbols and nomenclature of reassortant viruses are as described in Table S1.

Table S4. Nonviable reassortant viruses between SK06 (H5N1) and Tok07 (H3N2) viruses

Virus No.	Virus name	Virus segments								CPE 48h	Aliquot hour	Titer (pfu/mL)
		PB2	PB1	PA	HA	NP	NA	M	NS			
5	r5									–	72	0
18	r2,5									–	72	0
23	r3,5									–	72	0
27	r4,5									–	72	0
31	r5,6									–	72	0
32	r5,7									–	72	0
48	r1,4,5									–	72	0
58	r2,3,4									–	72	0
59	r2,3,5									–	72	0
60	r2,3,6									–	72	0
63	r2,4,5									–	72	0
67	r2,5,6									–	72	0
68	r2,5,7									–	72	0
73	r3,4,5									–	72	0
76	r3,4,8									–	72	0
77	r3,5,6									–	72	0
78	r3,5,7									–	72	0
79	r3,5,8									–	72	0
83	r4,5,6									–	72	0
84	r4,5,7									–	72	0
89	r5,6,7									–	72	0
128	r2,3,4,5									–	72	0
129	r2,3,4,6									–	72	0
130	r2,3,4,7									–	72	0
132	r2,3,5,6									–	72	0
133	r2,3,5,7									–	72	0
134	r2,3,5,8									–	72	0
135	r2,3,6,7									–	72	0
138	r2,4,5,6									–	72	0
139	r2,4,5,7									–	72	0
144	r2,5,6,7									–	72	0
148	r3,4,5,6									–	72	0
149	r3,4,5,7									–	72	0
150	r3,4,5,8									–	72	0
151	r3,4,6,7									–	72	0
152	r3,4,6,8									–	72	0
153	r3,4,7,8									–	72	0
154	r3,5,6,7									–	72	0
155	r3,5,6,8									–	72	0
156	r3,5,7,8									–	72	0
158	r4,5,6,7									–	72	0
159	r4,5,6,8									–	72	0
160	r4,5,7,8									–	72	0
174	r1,2,4,5,7									–	72	0
183	r1,3,4,5,6									–	72	0
193	r1,4,5,6,7									–	72	0
198	r2,3,4,5,6									–	72	0
199	r2,3,4,5,7									–	72	0
200	r2,3,4,5,8									–	72	0
201	r2,3,4,6,7									–	72	0
202	r2,3,4,6,8									–	72	0
203	r2,3,4,7,8									–	72	0
204	r2,3,5,6,7									–	72	0
205	r2,3,5,6,8									–	72	0
206	r2,3,5,7,8									–	72	0
207	r2,3,6,7,8									–	72	0
208	r2,4,5,6,7									–	72	0
213	r3,4,5,6,7									–	72	0
214	r3,4,5,6,8									–	72	0
215	r3,4,5,7,8									–	72	0
216	r3,4,6,7,8									–	72	0
217	r3,5,6,7,8									–	72	0
218	r4,5,6,7,8									–	72	0
240	r2,3,4,5,6,7									–	72	0
241	r2,3,4,5,6,8									–	72	0
242	r2,3,4,5,7,8									–	72	0
243	r2,3,4,6,7,8									–	72	0
244	r2,3,5,6,7,8									–	72	0
246	r3,4,5,6,7,8									–	72	0
254	r2,3,4,5,6,7,8									–	72	0

Symbols and nomenclature of reassortant viruses are as described in Table S1. The nonviability (determined by the absence of CPE and pfu titer) of these reassortant viruses was validated by two independent rescue experiments. The reassortant viruses highlighted in gray indicate the 32 combinations of reassortant viruses that contained the two combinations of the ribonucleoprotein complex, $S_{PB2}S_{PB1}T_{PA}T_{NP}$ and $S_{PB2}T_{PB1}T_{PA}T_{NP}$, that had the lowest activity in the minigenome assay.

Table S5. Contribution of Tok07 *PB1* to the pathogenicity of the reassortant viruses

Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days	Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days
2	r2	$\geq 10^{4.5}$	+0.1	> 14	57	r1,7,8	$10^{3.3}$	–(> 20.0)	9
16	r2,3	$\geq 10^{4.5}$	–10.5	> 14	107	r1,2,7,8	56.2	–(> 20.0)	7
19	r2,6	$\geq 10^{4.5}$	+14.9	> 14					
20	r2,7	$\geq 10^{4.5}$	+12.5	> 14	113	r1,3,5,7	$10^{3.5}$	–(> 20.0)	11.5
21	r2,8	$\geq 10^{4.5}$	+21.3	> 14	168	r1,2,3,5,7	$10^{3.3}$	–(> 20.0)	9.3
69	r2,5,8	$\geq 10^{4.5}$	+21.3	> 14					
70	r2,6,7	$\geq 10^{4.5}$	+12.7	> 14	115	r1,3,6,7	$\geq 10^{4.5}$	–11.4	> 14
71	r2,6,8	$\geq 10^{4.5}$	+12.1	> 14	170	r1,2,3,6,7	$10^{4.3}$	–17.8	13
72	r2,7,8	$\geq 10^{4.5}$	+18.9	> 14					
146	r2,5,7,8	$\geq 10^{4.5}$	+7.1	> 14	116	r1,3,6,8	$\geq 10^{4.5}$	+12.1	> 14
147	r2,6,7,8	$\geq 10^{4.5}$	+14.1	> 14	171	r1,2,3,6,8	$\geq 10^{4.5}$	+7.0	> 14
1	r1	3.2	–(> 20.0)	7.3	117	r1,3,7,8	$10^{2.5}$	–(> 20.0)	11.8
9	r1,2	1.8	–(> 20.0)	7	172	r1,2,3,7,8	$10^{2.5}$	–(> 20.0)	8.8
10	r1,3	$10^{4.3}$	–19.4	13.5	124	r1,5,6,7	$\geq 10^{4.5}$	+13.9	> 14
37	r1,2,3	$10^{3.3}$	–(> 20.0)	7.8	179	r1,2,5,6,7	$\geq 10^{4.5}$	+12.2	> 14
13	r1,6	17.8	–(> 20.0)	7.8	125	r1,5,6,8	$\geq 10^{4.5}$	+10.4	> 14
40	r1,2,6	17.8	–(> 20.0)	6.8	180	r1,2,5,6,8	$10^{2.8}$	–(> 20.0)	9.3
14	r1,7	$10^{2.0}$	–(> 20.0)	7.3	126	r1,5,7,8	$\geq 10^{4.5}$	+16.0	> 14
41	r1,2,7	56.2	–(> 20.0)	8	181	r1,2,5,7,8	$10^{2.3}$	–(> 20.0)	7
15	r1,8	$\geq 10^{4.5}$	–15.0	> 14	127	r1,6,7,8	$\geq 10^{4.5}$	+15.7	> 14
42	r1,2,8	10	–(> 20.0)	7.3	182	r1,2,6,7,8	$\geq 10^{4.5}$	+12.8	> 14
45	r1,3,6	$10^{4.0}$	–16.5	13.3	189	r1,3,5,6,7	$\geq 10^{4.5}$	+13.0	> 14
95	r1,2,3,6	$10^{3.0}$	–(> 20.0)	11.8	225	r1,2,3,5,6,7	$\geq 10^{4.5}$	+4.1	> 14
46	r1,3,7	$10^{2.5}$	–(> 20.0)	7	191	r1,3,5,7,8	$10^{4.3}$	–15.4	12.8
96	r1,2,3,7	56.2	–(> 20.0)	6	227	r1,2,3,5,7,8	$10^{2.5}$	–(> 20.0)	8.8
47	r1,3,8	$\geq 10^{4.5}$	+11.8	> 14	192	r1,3,6,7,8	$\geq 10^{4.5}$	+11.5	> 14
97	r1,2,3,8	$\geq 10^{4.5}$	+13.0	> 14	228	r1,2,3,6,7,8	$\geq 10^{4.5}$	+21.0	> 14
55	r1,6,7	$10^{4.0}$	–18.9	12.3	197	r1,5,6,7,8	$\geq 10^{4.5}$	+16.0	> 14
105	r1,2,6,7	$10^{3.5}$	–16.7	8.5	233	r1,2,5,6,7,8	$\geq 10^{4.5}$	+9.6	> 14
56	r1,6,8	$10^{3.8}$	–15.9	13	238	r1,3,5,6,7,8	$\geq 10^{4.5}$	+12.8	> 14
106	r1,2,6,8	$10^{2.0}$	–(> 20.0)	7.5	251	r1,2,3,5,6,7,8	$\geq 10^{4.5}$	+9.9	> 14

This table was derived from Tables 1, 2, and 3 and was used to evaluate the contribution of Tok07 *PB1* to the pathogenicity of the reassortant viruses. The first 11 viruses, from r2 to r2,6,7,8, contained Tok07 *PB1* with SK06 *PB2* and were all of low pathogenicity in mice. The viruses from r1 to r1,2,3,5,6,7,8 all contained Tok07 *PB2* and are listed as pairs of viruses with or without Tok07 *PB1*. The pathogenicity of the two viruses in each pair was compared. MLD_{50}, mouse LD_{50}; MST, mean survival time of the mice infected with 10^4 pfu.

Table S6. Contribution of Tok07 *NP* to the pathogenicity of the reassortant viruses

Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days	Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days
33	r5,8	$\geq 10^{4.5}$	+9.7	> 14	46	r1,3,7	$10^{2.5}$	–(> 20.0)	7
52	r1,5,6	$\geq 10^{4.5}$	+3.9	> 14	113	r1,3,5,7	$10^{3.5}$	–(> 20.0)	11.5
69	r2,5,8	$\geq 10^{4.5}$	+21.3	> 14					
91	r5,7,8	$\geq 10^{4.5}$	+14.8	> 14	96	r1,2,3,7	56.2	–(> 20.0)	6
104	r1,2,5,8	$\geq 10^{4.5}$	+10.4	> 14	168	r1,2,3,5,7	$10^{3.3}$	–(> 20.0)	9.3
124	r1,5,6,7	$\geq 10^{4.5}$	+13.9	> 14					
125	r1,5,6,8	$\geq 10^{4.5}$	+10.4	> 14	106	r1,2,6,8	$10^{2.0}$	–(> 20.0)	7.5
126	r1,5,7,8	$\geq 10^{4.5}$	+16.0	> 14	180	r1,2,5,6,8	$10^{2.8}$	–(> 20.0)	9.3
146	r2,5,7,8	$\geq 10^{4.5}$	+7.1	> 14					
167	r1,2,3,5,6	$\geq 10^{4.5}$	+10.7	> 14	107	r1,2,7,8	56.2	–(> 20.0)	7
169	r1,2,3,5,8	$\geq 10^{4.5}$	+16.1	> 14	181	r1,2,5,7,8	$10^{2.3}$	–(> 20.0)	7
179	r1,2,5,6,7	$\geq 10^{4.5}$	+12.2	> 14					
189	r1,3,5,6,7	$\geq 10^{4.5}$	+13.0	> 14	117	r1,3,7,8	$10^{2.5}$	–(> 20.0)	11.8
197	r1,5,6,7,8	$\geq 10^{4.5}$	+16.0	> 14	191	r1,3,5,7,8	$10^{4.3}$	–15.4	12.8
225	r1,2,3,5,6,7	$\geq 10^{4.5}$	+4.1	> 14					
226	r1,2,3,5,6,8	$\geq 10^{4.5}$	+15.4	> 14	172	r1,2,3,7,8	$10^{2.5}$	–(> 20.0)	8.8
233	r1,2,5,6,7,8	$\geq 10^{4.5}$	+9.6	> 14	227	r1,2,3,5,7,8	$10^{2.5}$	–(> 20.0)	8.8
238	r1,3,5,6,7,8	$\geq 10^{4.5}$	+12.8	> 14					
251	r1,2,3,5,6,7,8	$\geq 10^{4.5}$	+9.9	> 14					

This table was derived from Tables 1, 2, and 3 and was used to evaluate the contribution of Tok07 *NP* to the pathogenicity of the reassortant viruses. The viruses from r5,8 to r1,2,3,5,6,7,8 contained Tok07 *NP* and were all of low pathogenicity in mice. The viruses from r1,3,7 to r1,2,3,5,7,8 are listed as pairs of viruses with or without Tok07 *NP*. The pathogenicity of the two viruses in each pair was compared.

Table S7. Contribution of Tok07 *PA* to the pathogenicity of the reassortant viruses

Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST,days	Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days
3	r3	$\geq 10^{4.5}$	−6.2	> 14					
16	r2,3	$\geq 10^{4.5}$	−10.5	> 14	105	r1,2,6,7	$10^{3.5}$	−16.7	8.5
25	r3,7	$\geq 10^{4.5}$	−4.5	> 14	170	r1,2,3,6,7	$10^{4.3}$	−17.8	13
1	r1	3.2	−(> 20.0)	7.3	106	r1,2,6,8	$10^{2.0}$	−(> 20.0)	7.5
10	r1,3	$10^{4.3}$	−19.4	13.5	171	r1,2,3,6,8	$\geq 10^{4.5}$	+7.0	> 14
9	r1,2	1.8	−(> 20.0)	7	107	r1,2,7,8	56.2	−(> 20.0)	7
37	r1,2,3	$10^{3.3}$	−(> 20.0)	7.8	172	r1,2,3,7,8	$10^{2.5}$	−(> 20.0)	8.8
13	r1,6	17.8	−(> 20.0)	7.8	124	r1,5,6,7	$\geq 10^{4.5}$	+13.9	> 14
45	r1,3,6	$10^{4.0}$	−16.5	13.3	189	r1,3,5,6,7	$\geq 10^{4.5}$	+13.0	> 14
14	r1,7	$10^{2.0}$	−(> 20.0)	7.3	126	r1,5,7,8	$\geq 10^{4.5}$	+16.0	> 14
46	r1,3,7	$10^{2.5}$	−(> 20.0)	7	191	r1,3,5,7,8	$10^{4.3}$	−15.4	12.8
15	r1,8	$\geq 10^{4.5}$	−15.0	> 14	127	r1,6,7,8	$\geq 10^{4.5}$	+15.7	> 14
47	r1,3,8	$\geq 10^{4.5}$	+11.8	> 14	192	r1,3,6,7,8	$\geq 10^{4.5}$	+11.5	> 14
40	r1,2,6	17.8	−(> 20.0)	6.8	179	r1,2,5,6,7	$\geq 10^{4.5}$	+12.2	> 14
95	r1,2,3,6	$10^{3.0}$	−(> 20.0)	11.8	225	r1,2,3,5,6,7	$\geq 10^{4.5}$	+4.1	> 14
41	r1,2,7	56.2	−(> 20.0)	8	180	r1,2,5,6,8	$10^{2.8}$	−(> 20.0)	9.3
96	r1,2,3,7	56.2	−(> 20.0)	6	226	r1,2,3,5,6,8	$\geq 10^{4.5}$	+15.4	> 14
42	r1,2,8	10	−(> 20.0)	7.3	181	r1,2,5,7,8	$10^{2.3}$	−(> 20.0)	7
97	r1,2,3,8	$\geq 10^{4.5}$	+13.0	> 14	227	r1,2,3,5,7,8	$10^{2.5}$	−(> 20.0)	8.8
55	r1,6,7	$10^{4.0}$	−18.9	12.3	182	r1,2,6,7,8	$\geq 10^{4.5}$	+12.8	> 14
115	r1,3,6,7	$\geq 10^{4.5}$	−11.4	> 14	228	r1,2,3,6,7,8	$\geq 10^{4.5}$	+21.0	> 14
56	r1,6,8	$10^{3.8}$	−15.9	13	197	r1,5,6,7,8	$\geq 10^{4.5}$	+16.0	> 14
116	r1,3,6,8	$\geq 10^{4.5}$	+12.1	> 14	238	r1,3,5,6,7,8	$\geq 10^{4.5}$	+12.8	> 14
57	r1,7,8	$10^{3.3}$	−(> 20.0)	9	233	r1,2,5,6,7,8	$\geq 10^{4.5}$	+9.6	> 14
117	r1,3,7,8	$10^{2.5}$	−(> 20.0)	11.8	251	r1,2,3,5,6,7,8	$\geq 10^{4.5}$	+9.9	> 14
104	r1,2,5,8	$\geq 10^{4.5}$	+10.4	> 14					
169	r1,2,3,5,8	$\geq 10^{4.5}$	+16.1	> 14					

This table was derived from Tables 1, 2, and 3 and was used to evaluate the contribution of Tok07 *PA* to the pathogenicity of the reassortant viruses. The first three viruses, from r3 to r3,7, contained Tok07 *PA* with SK06 *PB2* and were all of low pathogenicity in mice. The viruses from r1 to r1,2,3,5,6,7,8, which all contained Tok07 *PB2*, are listed as pairs of viruses with or without Tok07 *PA*. The pathogenicity of the two viruses in each pair was compared.

Table S8. Contribution of Tok07 NA to the pathogenicity of the reassortant viruses

Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days	Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST,days
6	r6	$10^{4.3}$	−13.6	12.8	57	r1,7,8	$10^{3.3}$	−(> 20.0)	9
19	r2,6	≥ $10^{4.5}$	+14.9	> 14	127	r1,6,7,8	≥ $10^{4.5}$	+15.7	> 14
34	r6,7	≥ $10^{4.5}$	+11.6	> 14					
35	r6,8	≥ $10^{4.5}$	+7.8	> 14	96	r1,2,3,7	56.2	−(> 20.0)	6
70	r2,6,7	≥ $10^{4.5}$	+12.7	> 14	170	r1,2,3,6,7	$10^{4.3}$	−17.8	13
71	r2,6,8	≥ $10^{4.5}$	+12.1	> 14					
92	r6,7,8	≥ $10^{4.5}$	+12.4	> 14	97	r1,2,3,8	≥ $10^{4.5}$	+13.0	> 14
147	r2,6,7,8	≥ $10^{4.5}$	+14.1	> 14	171	r1,2,3,6,8	≥ $10^{4.5}$	+7.0	> 14
1	r1	3.2	−(> 20.0)	7.3	104	r1,2,5,8	≥ $10^{4.5}$	+10.4	> 14
13	r1,6	17.8	−(> 20.0)	7.8	180	r1,2,5,6,8	$10^{2.8}$	−(> 20.0)	9.3
9	r1,2	1.8	−(> 20.0)	7	107	r1,2,7,8	56.2	−(> 20.0)	7
40	r1,2,6	17.8	−(> 20.0)	6.8	182	r1,2,6,7,8	≥ $10^{4.5}$	+12.8	> 14
10	r1,3	$10^{4.3}$	−19.4	13.5	113	r1,3,5,7	$10^{3.5}$	−(> 20.0)	11.5
45	r1,3,6	$10^{4.0}$	−16.5	13.3	189	r1,3,5,6,7	≥ $10^{4.5}$	+13.0	> 14
14	r1,7	$10^{2.0}$	−(> 20.0)	7.3	117	r1,3,7,8	$10^{2.5}$	−(> 20.0)	11.8
55	r1,6,7	$10^{4.0}$	−18.9	12.3	192	r1,3,6,7,8	≥ $10^{4.5}$	+11.5	> 14
15	r1,8	≥ $10^{4.5}$	−15.0	> 14	168	r1,2,3,5,7	$10^{3.3}$	−(> 20.0)	9.3
56	r1,6,8	$10^{3.8}$	−15.9	13	225	r1,2,3,5,6,7	≥ $10^{4.5}$	+4.1	> 14
37	r1,2,3	$10^{3.3}$	−(> 20.0)	7.8	169	r1,2,3,5,8	≥ $10^{4.5}$	+16.1	> 14
95	r1,2,3,6	$10^{3.0}$	−(> 20.0)	11.8	226	r1,2,3,5,6,8	≥ $10^{4.5}$	+15.4	> 14
41	r1,2,7	56.2	−(> 20.0)	8	172	r1,2,3,7,8	$10^{2.5}$	−(> 20.0)	8.8
105	r1,2,6,7	$10^{3.5}$	−16.7	8.5	228	r1,2,3,6,7,8	≥ $10^{4.5}$	+21.0	> 14
42	r1,2,8	10	−(> 20.0)	7.3	181	r1,2,5,7,8	$10^{2.3}$	−(> 20.0)	7
106	r1,2,6,8	$10^{2.0}$	−(> 20.0)	7.5	233	r1,2,5,6,7,8	≥ $10^{4.5}$	+9.6	> 14
46	r1,3,7	$10^{2.5}$	−(> 20.0)	7	191	r1,3,5,7,8	$10^{4.3}$	−15.4	12.8
115	r1,3,6,7	≥ $10^{4.5}$	−11.4	> 14	238	r1,3,5,6,7,8	≥ $10^{4.5}$	+12.8	> 14
47	r1,3,8	≥ $10^{4.5}$	+11.8	> 14	227	r1,2,3,5,7,8	$10^{2.5}$	−(> 20.0)	8.8
116	r1,3,6,8	≥ $10^{4.5}$	+12.1	> 14	251	r1,2,3,5,6,7,8	≥ $10^{4.5}$	+9.9	> 14

This table was derived from Tables 1, 2 , and 3 and was used to evaluate the contribution of Tok07 *NA* to the pathogenicity of the reassortant viruses. The first eight viruses from r6 to r2,6,7,8 contained Tok07 *NA* with SK06 *PB2* and were all of low pathogenicity in mice, except for r6, which showed a level of pathogenicity similar to that of the parental SK06 virus. The viruses from r1 to r1,2,3,5,6,7,8, which all contained Tok07 *PB2*, are listed as pairs of viruses with or without Tok07 *NA*. The pathogenicity of the two viruses in each pair was compared.

Table S9. Contribution of Tok07 *NS* to the pathogenicity of the reassortant viruses

Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days	Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days
8	r8	$\geq 10^{4.5}$	+14.1	> 14					
21	r2,8	$\geq 10^{4.5}$	+21.3	> 14	52	r1,5,6	$\geq 10^{4.5}$	+3.9	> 14
33	r5,8	$\geq 10^{4.5}$	+9.7	> 14	125	r1,5,6,8	$\geq 10^{4.5}$	+10.4	> 14
35	r6,8	$\geq 10^{4.5}$	+7.8	> 14					
36	r7,8	$10^{4.3}$	−18.5	13.5	55	r1,6,7	$10^{4.0}$	−18.9	12.3
69	r2,5,8	$\geq 10^{4.5}$	+21.3	> 14	127	r1,6,7,8	$\geq 10^{4.5}$	+15.7	> 14
71	r2,6,8	$\geq 10^{4.5}$	+12.1	> 14					
72	r2,7,8	$\geq 10^{4.5}$	+18.9	> 14	95	r1,2,3,6	$10^{3.0}$	−(> 20.0)	11.8
91	r5,7,8	$\geq 10^{4.5}$	+14.8	> 14	171	r1,2,3,6,8	$\geq 10^{4.5}$	+7.0	> 14
92	r6,7,8	$\geq 10^{4.5}$	+12.4	> 14					
146	r2,5,7,8	$\geq 10^{4.5}$	+7.1	> 14	96	r1,2,3,7	56.2	−(> 20.0)	6
147	r2,6,7,8	$\geq 10^{4.5}$	+14.1	> 14	172	r1,2,3,7,8	$10^{2.5}$	−(> 20.0)	8.8
1	r1	3.2	−(> 20.0)	7.3	105	r1,2,6,7	$10^{3.5}$	−16.7	8.5
15	r1,8	$\geq 10^{4.5}$	−15.0	> 14	182	r1,2,6,7,8	$\geq 10^{4.5}$	+12.8	> 14
9	r1,2	1.8	−(> 20.0)	7	113	r1,3,5,7	$10^{3.5}$	−(> 20.0)	11.5
42	r1,2,8	10	−(> 20.0)	7.3	191	r1,3,5,7,8	$10^{4.3}$	−15.4	12.8
10	r1,3	$10^{4.3}$	−19.4	13.5	115	r1,3,6,7	$\geq 10^{4.5}$	−11.4	> 14
47	r1,3,8	$\geq 10^{4.5}$	+11.8	> 14	192	r1,3,6,7,8	$\geq 10^{4.5}$	+11.5	> 14
13	r1,6	17.8	−(> 20.0)	7.8	124	r1,5,6,7	$\geq 10^{4.5}$	+13.9	> 14
56	r1,6,8	$10^{3.8}$	−15.9	13	197	r1,5,6,7,8	$\geq 10^{4.5}$	+16.0	> 14
14	r1,7	$10^{2.0}$	−(> 20.0)	7.3	167	r1,2,3,5,6	$\geq 10^{4.5}$	+10.7	> 14
57	r1,7,8	$10^{3.3}$	−(> 20.0)	9	226	r1,2,3,5,6,8	$\geq 10^{4.5}$	+15.4	> 14
37	r1,2,3	$10^{3.3}$	−(> 20.0)	7.8	168	r1,2,3,5,7	$10^{3.3}$	−(> 20.0)	9.3
97	r1,2,3,8	$\geq 10^{4.5}$	+13.0	> 14	227	r1,2,3,5,7,8	$10^{2.5}$	−(> 20.0)	8.8
40	r1,2,6	17.8	−(> 20.0)	6.8	170	r1,2,3,6,7	$10^{4.3}$	−17.8	13
106	r1,2,6,8	$10^{2.0}$	−(> 20.0)	7.5	228	r1,2,3,6,7,8	$\geq 10^{4.5}$	+21.0	> 14
41	r1,2,7	56.2	−(> 20.0)	8	179	r1,2,5,6,7	$\geq 10^{4.5}$	+12.2	> 14
107	r1,2,7,8	56.2	−(> 20.0)	7	233	r1,2,5,6,7,8	$\geq 10^{4.5}$	+9.6	> 14
45	r1,3,6	$10^{4.0}$	−16.5	13.3	189	r1,3,5,6,7	$\geq 10^{4.5}$	+13.0	> 14
116	r1,3,6,8	$\geq 10^{4.5}$	+12.1	> 14	238	r1,3,5,6,7,8	$\geq 10^{4.5}$	+12.8	> 14
46	r1,3,7	$10^{2.5}$	−(> 20.0)	7	225	r1,2,3,5,6,7	$\geq 10^{4.5}$	+4.1	> 14
117	r1,3,7,8	$10^{2.5}$	−(> 20.0)	11.8	251	r1,2,3,5,6,7,8	$\geq 10^{4.5}$	+9.9	> 14

This table was derived from Tables 1, 2, and 3 and was used to evaluate the contribution of Tok07 *NS* to the pathogenicity of the reassortant viruses. The first 12 viruses, from r8 to r2,6,7,8, contained Tok07 *NS* with SK06 *PB2* and were all of low pathogenicity in mice, except for r7,8, which showed a level of pathogenicity similar to that of the parental SK06 virus. The viruses from r1 to r1,2,3,5,6,7,8, which all contained Tok07 *PB2*, are listed as pairs of viruses with or without Tok07 *NS*. The pathogenicity of the two viruses in each pair was compared.

Table S10. Contribution of Tok07 *M* to the pathogenicity of the reassortant viruses

Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days	Virus no.	Virus name	MLD_{50}, pfu	Weight change, %	MST, days
7	r7	$\geq 10^{4.5}$	−16.1	> 14	52	r1,5,6	$\geq 10^{4.5}$	+3.9	> 14
20	r2,7	$\geq 10^{4.5}$	+12.5	> 14	124	r1,5,6,7	$\geq 10^{4.5}$	+13.9	> 14
25	r3,7	$\geq 10^{4.5}$	−4.5	> 14					
34	r6,7	$\geq 10^{4.5}$	+11.6	> 14	56	r1,6,8	$10^{3.8}$	−15.9	13
36	r7,8	$10^{4.3}$	−18.5	13.5	127	r1,6,7,8	$\geq 10^{4.5}$	+15.7	> 14
70	r2,6,7	$\geq 10^{4.5}$	+12.7	> 14					
72	r2,7,8	$\geq 10^{4.5}$	+18.9	> 14	95	r1,2,3,6	$10^{3.0}$	−(> 20.0)	11.8
91	r5,7,8	$\geq 10^{4.5}$	+14.8	> 14	170	r1,2,3,6,7	$10^{4.3}$	−17.8	13
92	r6,7,8	$\geq 10^{4.5}$	+12.4	> 14					
146	r2,5,7,8	$\geq 10^{4.5}$	+7.1	> 14	97	r1,2,3,8	$\geq 10^{4.5}$	+13.0	> 14
147	r2,6,7,8	$\geq 10^{4.5}$	+14.1	> 14	172	r1,2,3,7,8	$10^{2.5}$	−(> 20.0)	8.8
1	r1	3.2	−(> 20.0)	7.3	104	r1,2,5,8	$\geq 10^{4.5}$	+10.4	> 14
14	r1,7	$10^{2.0}$	−(> 20.0)	7.3	181	r1,2,5,7,8	$10^{2.3}$	−(> 20.0)	7
9	r1,2	1.8	−(> 20.0)	7	106	r1,2,6,8	$10^{2.0}$	−(> 20.0)	7.5
41	r1,2,7	56.2	−(> 20.0)	8	182	r1,2,6,7,8	$\geq 10^{4.5}$	+12.8	> 14
10	r1,3	$10^{4.3}$	−19.4	13.5	116	r1,3,6,8	$\geq 10^{4.5}$	+12.1	> 14
46	r1,3,7	$10^{2.5}$	−(> 20.0)	7	192	r1,3,6,7,8	$\geq 10^{4.5}$	+11.5	> 14
13	r1,6	17.8	−(> 20.0)	7.8	125	r1,5,6,8	$\geq 10^{4.5}$	+10.4	> 14
55	r1,6,7	$10^{4.0}$	−18.9	12.3	197	r1,5,6,7,8	$\geq 10^{4.5}$	+16.0	> 14
15	r1,8	$\geq 10^{4.5}$	−15.0	> 14	167	r1,2,3,5,6	$\geq 10^{4.5}$	+10.7	> 14
57	r1,7,8	$10^{3.3}$	−(> 20.0)	9	225	r1,2,3,5,6,7	$\geq 10^{4.5}$	+4.1	> 14
37	r1,2,3	$10^{3.3}$	−(> 20.0)	7.8	169	r1,2,3,5,8	$\geq 10^{4.5}$	+16.1	> 14
96	r1,2,3,7	56.2	−(> 20.0)	6	227	r1,2,3,5,7,8	$10^{2.5}$	−(> 20.0)	8.8
40	r1,2,6	17.8	−(> 20.0)	6.8	171	r1,2,3,6,8	$\geq 10^{4.5}$	+7.0	> 14
105	r1,2,6,7	$10^{3.5}$	−16.7	8.5	228	r1,2,3,6,7,8	$\geq 10^{4.5}$	+21.0	> 14
42	r1,2,8	10	−(> 20.0)	7.3	180	r1,2,5,6,8	$10^{2.8}$	−(> 20.0)	9.3
107	r1,2,7,8	56.2	−(> 20.0)	7	233	r1,2,5,6,7,8	$\geq 10^{4.5}$	+9.6	> 14
45	r1,3,6	$10^{4.0}$	−16.5	13.3	226	r1,2,3,5,6,8	$\geq 10^{4.5}$	+15.4	> 14
115	r1,3,6,7	$\geq 10^{4.5}$	−11.4	> 14	251	r1,2,3,5,6,7,8	$\geq 10^{4.5}$	+9.9	> 14
47	r1,3,8	$\geq 10^{4.5}$	+11.8	> 14					
117	r1,3,7,8	$10^{2.5}$	−(> 20.0)	11.8					

This table was derived from Tables 1, 2, and 3 and was used to evaluate the contribution of Tok07 *M* to the pathogenicity of the reassortant viruses. The first 11 viruses, from r7 to r2,6,7,8, contained Tok07 *M* with SK06 *PB2* and were all of low pathogenicity in mice, except for r7,8, which showed a level of pathogenicity similar to that of the parental SK06 virus. The viruses from r1 to r1,2,3,5,6,7,8, which all contained Tok07 *PB2*, are listed as pairs of viruses with or without Tok07 *M*. The pathogenicity of the two viruses in each pair was compared.