

S3 Table. Summary of the results from various assays in this study.

Serial No.	Type of mutation	Gene (No. of mutations)	Mutation	Inter-segment combination with mutation	In vitro polymerase activity in: †				In vitro virus growth in: ‡			In vivo virus titers in: *						
					293T cells transfected with		QT-6 cells transfected with		SAE cells at		CEF cells at	Mouse lungs at						
					vRNA at 37 °C	vRNA at 33 °C	cRNA at 37 °C	cRNA at 33 °C	vRNA at 37 °C	cRNA at 37 °C	37°C	33°C	37°C	3 d post-infection	6 d post-infection			
1	Single mutations	PB2 (14)	K80R	Inter-5	=	=	=	+	=	=	ND	ND	ND	ND	ND			
2			T129N		=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
3			D195N		=	+	+	+	=	=	ND	ND	ND	ND	ND	ND	ND	
4			Q257L		=	=	=	=	=	=	ND	ND	ND	ND	ND	ND	ND	
5			I292M		=	=	=	=	=	=	ND	ND	ND	ND	ND	ND	ND	
6			R299K		=	=	=	=	=	=	=	=	=	=	=	=	=	
7			R389K		=	+	+	+	+	+	+	+	=	=	=	=	=	
8			N456D		=	+	=	+	+	+	=	=	ND	ND	ND	ND	ND	
9			I463M		=	=	=	=	=	=	=	=	=	=	=	=	=	
10			M473V		=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	
11			M570I		=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	
12			K617E		=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	
13			T637I		=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	
14			T683I		=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	
15		K54R	=	=	=	+	+	=	=	=	ND	ND	ND	ND	ND			
16		D175N	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND			
17		T182I	=	=	=	+++	++	+	=	+	+	+	+	+	+	+		
18		K198R	=	=	=	+++	++	+	=	+	+	+	+	+	+	+		
19		K214R	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
20		A374T	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
21		L384S	=	=	=	+	=	=	=	+	ND	ND	ND	ND	ND	ND		
22		K386R	=	=	=	+	=	=	=	=	ND	ND	ND	ND	ND	ND		
23		M616R	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
24		E618D	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
25		P627L	=	=	=	+++	=	+++	=	=	=	++	=	=	=	=		
26		N642K	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
27		P756S	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
28		D55N	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
29		N321S	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
30		N321I	=	=	=	=	=	=	=	=	=	=	=	=	=	=		
31		E327K	=	=	=	+	+	++	=	=	=	=	=	=	=	=		
32		V341L	=	=	=	=	=	+	=	=	=	=	=	=	=	=		
33		E382D	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
34		S400T	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
35		T608S	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
36		K615R	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
37		E629H	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
38		G631C	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
39		L641P	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
40		S644F	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
41		L649I	=	=	=	+	=	=	=	=	ND	ND	ND	ND	ND	ND		
42		A669V	=	=	=	+	+	=	=	=	ND	ND	ND	ND	ND	ND		
43		F681L	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
44		K716N	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
45		G287S	=	=	=	=	=	=	=	=	=	=	=	=	=	=		
46		T371M	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
47	Intra-segment combinations	PB2(8)	D195N/R299K	Inter-2,7	=	+	+	+	=	=	ND	ND	ND	ND	ND			
48			D195N/R299K/N456D		=	+	+	++	++	=	=	=	=	=	=	=	=	
49			M473V/D195N		=	=	=	=	+	=	=	=	+	+	=	=	=	
50			M473V/D195N/T683I		=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	
51		M473V/N456D	=	=	=	+	+	=	=	=	ND	ND	ND	ND	ND	ND		
52		M570I/K80R	=	=	=	+	+	=	=	=	=	=	=	=	=	=		
53		M570I/K80R/T129N	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
54		M570I/K80R/T129N/I292M	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
55		T182I/K214R	=	=	=	+	=	++	+	=	=	=	=	=	=	=		
56		T182I/K214R/L384S	=	=	=	+	=	+++	++	+	+	+	+	+	+	+		
57		K198R/D175N	=	=	=	+	=	+++	++	+	+	+	+	+	+	+		
58		A374T/K54R	=	=	=	+	=	+	++	=	ND	ND	ND	ND	ND	ND		
59		P756S/P627L	=	=	=	+	+++	+	++	=	ND	ND	ND	ND	ND	ND		
60		P756S/P627L/E618D	=	=	=	+	+++	+	+++	=	ND	ND	ND	ND	ND	ND		
61		P756S/P627L/E618D/K386R	=	=	=	+++	+++	++	++	=	=	=	=	=	=	=		
62		N321S/K716N	=	=	=	=	=	=	=	=	=	=	=	=	=	=		
63		N321S/K716N/S400T	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
64		N321S/K716N/S400T/K615R	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND		
65	G631C/E629H	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND			
66	G631C/E629H/A669V	=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND			
67	A669V/L649I	=	=	=	=	=	+	=	=	=	=	=	=	=	=			
68	A669V/E382D	=	=	=	=	=	=	=	=	=	=	=	=	=	=			
69	G287S/T371M	=	=	=	=	=	=	=	=	=	=	=	=	=	=			
70	Inter-segment combination of mutations	Intersegment(16)	Inter-1	Inter-2,7	+	+	+++	+++	+	+	++	+++	+	+	+	+		
71			Inter-2		+	+	+++	+++	+	+	+	+	++	+++	+	+	+	+
72			Inter-3		+	+	+++	+++	+	+	+	+	+	++	+	+	+	+
73			Inter-4		+	+	+++	+++	+	+	+	+	++	+++	+	+	+	+
74			Inter-5		+	+	+++	++	+	+	+	+	+	++	+	+	+	+
75			Inter-6		+	+	+++	+++	+	+	++	+	+	+	+	+	+	+
76			Inter-7		+	+	+++	+++	+	+	+	+	+	+	+	+	+	+
77			Inter-8		+	+	+++	++	+	+	++	+	+	+	+	+	+	+
78			Inter-9		=	=	=	=	=	=	=	=	=	=	=	=	=	=
79			Inter-10		+	+	++	++	+	+	+	+	ND	ND	ND	ND	ND	ND
80			Inter-11		+	+	++	++	+	+	+	+	ND	ND	ND	ND	ND	ND
81			Inter-12		=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND
82			Inter-13		+	+	++	++	+	+	+	+	ND	ND	ND	ND	ND	ND
83			Inter-14		+	+	+	+	+	+	+	+	ND	ND	ND	ND	ND	ND
84			Inter-15		=	=	=	=	=	=	=	=	ND	ND	ND	ND	ND	ND
85			Inter-16		=	=	=	=	=	+	=	=	ND	ND	ND	ND	ND	ND

†Relative comparison with wild-type polymerase activity: +, 1.5-3-fold increase; ++, 3-5-fold increase; +++, >5-fold increase; =, similar to wild-type virus (<1.5-fold change); -, lower than wild-type.

‡Relative comparison with wild-type virus yield: +, 2-5-fold increase; ++, >5-10-fold increase; +++, >10-fold increase; =, similar to wild-type virus (<2-fold change); -, lower than wild-type.

*Relative comparison with wild-type virus titer: +, 2-10-fold increase; ++, >10-100-fold increase; +++, >100-fold increase; =, similar to wild-type virus (<2-fold change); -, lower than wild-type.

† ND: not determined.