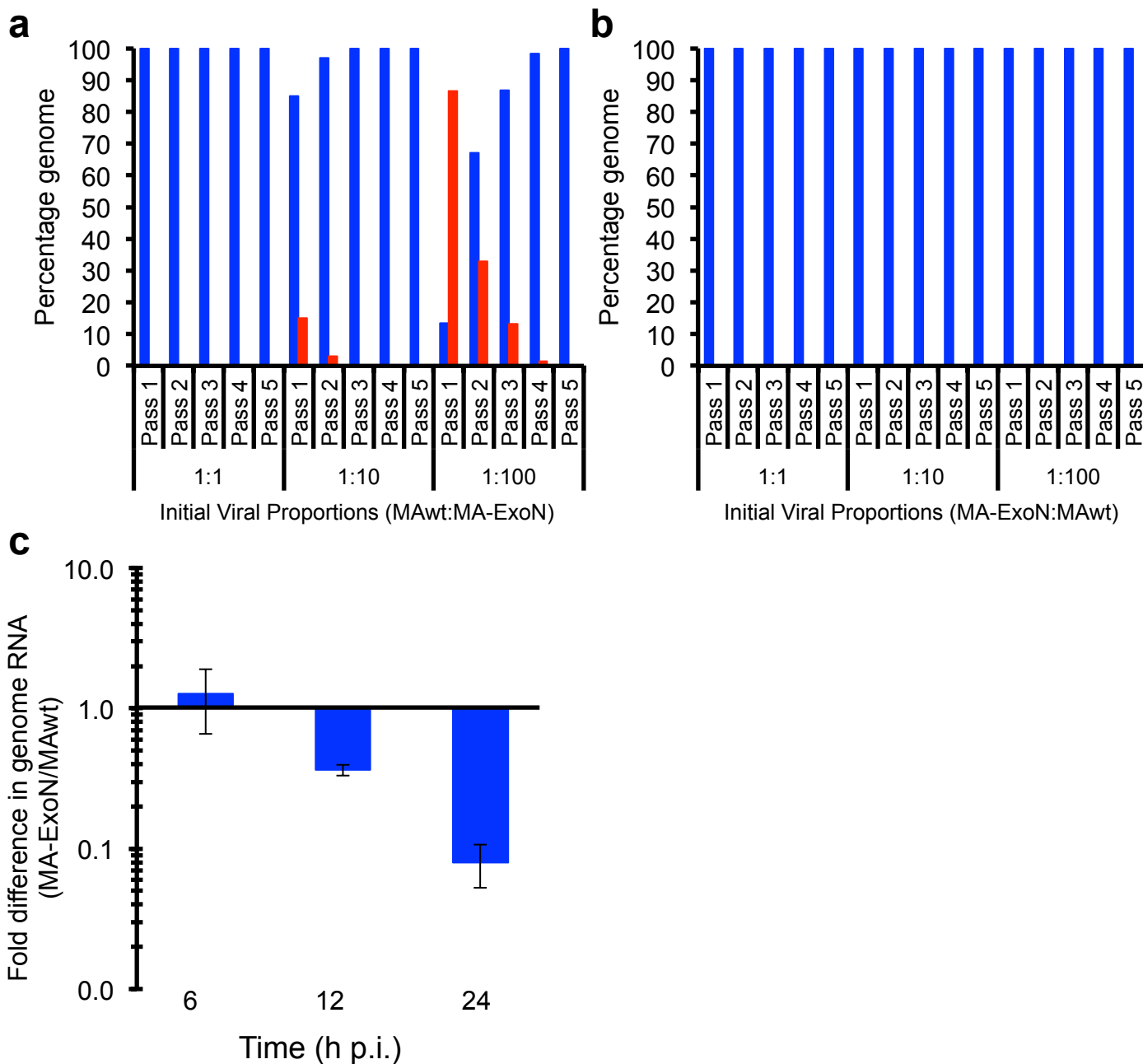
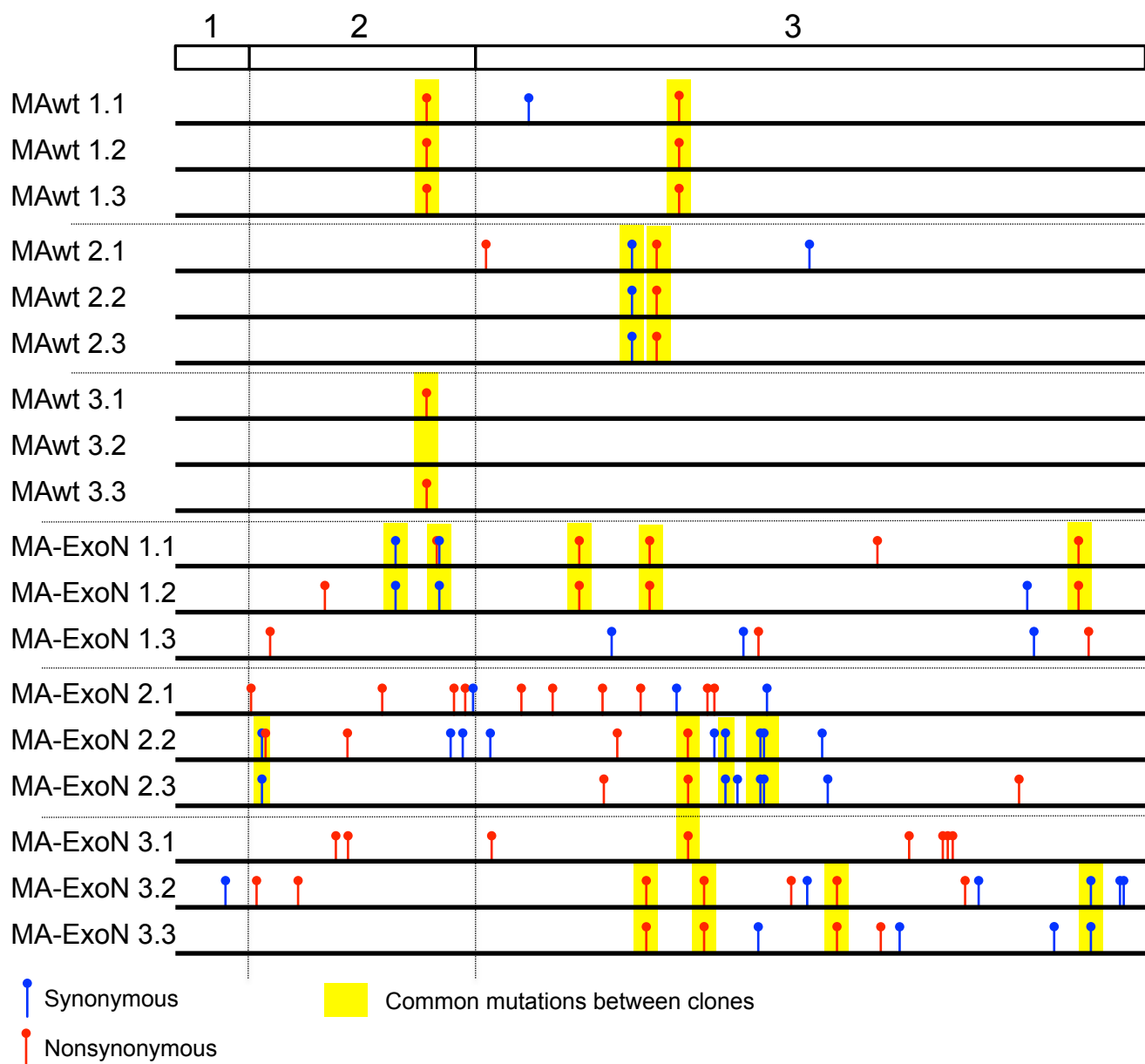


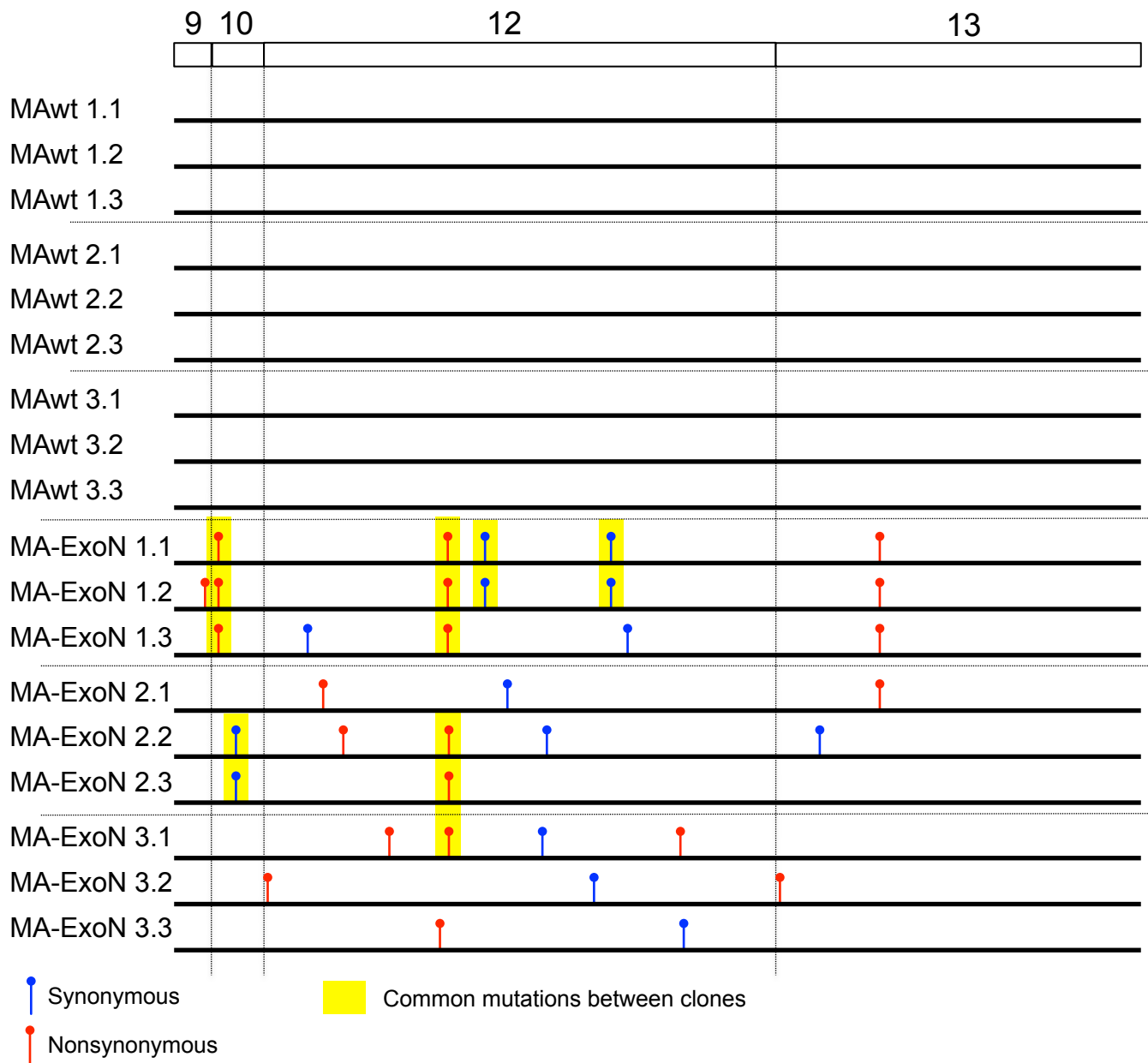
Supplementary Information for “A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model” by R.L. Graham, M.M. Becker, L.D. Eckerle, M. Bolles, M.R. Denison, and R.S. Baric



Supplementary Figure 1. Replication advantage of MAwt vs. MA-ExoN. Results from a competition assay using initial proportions of 1:1, 1:10, and 1:100 of MAwt and MA-ExoN favoring either MA-ExoN (a) or MAwt (b) are shown. Graphs show percentages of MAwt genomes vs. MA-ExoN genomes over five serial passages. Blue bars: MAwt; red bars: MA-ExoN. (c) Real-time RT-PCR was used to quantify viral genome RNA from MA-ExoN and MAwt infections. Results are expressed as GAPDH-normalized ratios of MA-ExoN/MAwt.



Supplementary Figure 2. Mutation accumulation in ORF1a in infected SCID mice at 30 d p.i. Multiple plaque isolates from each mouse lung (see Fig. 4) were sequenced from nts 493–8603. Individual SCID mouse genome sequences are represented by black horizontal lines. Dashed lines separate the nsp sequences in ORF1a. Mutations are indicated by lollipop shapes. Colors are as follows: blue: synonymous; red: nonsynonymous. Common mutations identified between clones are shaded in yellow.



Supplementary Figure 3. Mutation accumulation in ORF1b in infected SCID mice at 30 d p.i. Multiple plaque isolates from each mouse lung (see Fig. 4) were sequenced from nts 12,915–16,520. Individual SCID mouse genome sequences are represented by black horizontal lines. Dashed lines separate the nsp sequences in ORF1b. Mutations are indicated by lollipop shapes. Colors are as follows: blue: synonymous; red: nonsynonymous. Common mutations identified between clones are shaded in yellow.

Supplementary Table 1. *In Vivo* Mann-Whitney *U* Test Results for Weight Loss Differences, Days 1–4 Post-Infection

Mouse/Virus Combination	Day 1	Day 2	Day 3	Day 4
STAT ExoN/MAwt	0.1774892	0.01731602	0.004329	0.004329
ExoN STAT/129	0.930736	0.1255412	0.536796	0.662338
MAwt STAT/129	0.082251	0.1774892	0.008658	0.004329
129 ExoN/MAwt	0.1255412	0.082251	0.01731602	0.030303
RAG ExoN/MAwt	0.0079365	0.420634	0.031746	1
ExoN RAG/B6	0.030303	0.930736	0.246754	0.246754
MAwt RAG/B6	0.082251	0.1774892	1	0.536796
B6 ExoN/MAwt	0.588744	0.01515152	0.0021645	0.0930736
SCID ExoN/MAwt	0.181018	0.986466	0.000996702	5.05 × 10⁻⁵
ExoN SCID/BALB	0.581148	0.00262108	1.04 × 10⁻⁵	0.0755026
MAwt SCID/BALB	0.068171	1.16 × 10⁻⁷	4.08 × 10⁻⁶	4.08 × 10⁻⁶
BALB ExoN/MAwt	0.846998	8.51 × 10⁻⁵	1.55 × 10⁻⁴	1.55 × 10⁻⁴

Significant values ($P < 0.05$) are displayed in bold font.

Supplementary Table 2. SCID 30-Day Mutations: Whole-Genome, ORF1a, and ORF1b

SCID Clone	Genome Position	Nucleotide Change	Region	Synonymous (S) or Nonsynonymous (N)	AA Change	Present in Clones*
Whole genome						
MA-ExoN 1.1	1980	T-C	nsp2	S		1.1, 1.2
	2300	T-C	nsp2	N	I499T	1.1
	2304	T-C	nsp2	S		1.1, 1.2
	3634	G-A	nsp3	N	A306T	1.1, 1.2
	4127	T-C	nsp3	N	M470T	1.1, 1.2
	6211	A-G	nsp3	N	N1165D	1.1
	7951	G-A	nsp3	N	V1745I	1.1, 1.2
	11231	T-C	nsp6	N	L110P	
	11405	T-C	nsp6	N	V168A	
	13042	T-C	nsp10	N	Y30H	1.1, 1.2, 1.3
	14469	A-T	nsp12	N	S367C	1.1, 1.2, 1.3
	14738	T-C	nsp12	S		1.1, 1.2
	15308	T-C	nsp12	S		1.1, 1.2
	16999	C-A	nsp13	N	S278Y	1.1, 1.2, 1.3, 2.1
	18238-18239	AT-CC	nsp14	N (ExoN inactivation)	D90A	
	18244-18245	AG-CC	nsp14	N (ExoN inactivation)	E92A	
	18734	T-C	nsp14	S		
	20431	T-C	nsp15	N	V294A	
	23053	A-C	Spike	N	K521T	
	23638	C-T	Spike	N	T716I	
	23678	T-C	Spike	S		
	27304-27323	deletion	ORF7a	Truncates to 17 aa		
	27334	A-G	ORF7a	Part of truncated portion		
	27724	G-A	ORF7b	Creates stop after 28 aa		
	28584	T-C	Nucleocapsid	S		
	28949	G-A	Nucleocapsid	N	R277K	
	MA-ExoN 2.1	321	T-C	nsp1	S	
465		C-T	nsp1	S		
809		T-C	nsp2	N	V2A	2.1
1924		G-C	nsp2	N	A374P	2.1
2506		G-A	nsp2	N	D568N	2.1
2599		G-A	nsp2	N	V599I	2.1
2673		T-C	nsp2	S		2.1
3054		G-T	nsp3	N	E112D	2.1
3235		A-G	nsp3	N	I173V	2.1
3704		T-C	nsp3	N	V329A	2.1
4076		T-C	nsp3	N	D453V	2.1
4314		T-C	nsp3	S		2.1
4542		T-G (het)	nsp3	N	I608M	2.1
4610		C-T	nsp3	N	A631V	2.1
5094		G-A	nsp3	S		2.1
9499		C-T	nsp4	N	P339S	
11405		T-C	nsp6	N	V168A	
11550		C-T	nsp6	S		
13621		A-G	nsp12	N	E84G	2.1
14842		A-G	nsp12	N	N491S	2.1
16999		C-A	nsp13	N	S278Y	1.1, 1.2, 1.3, 2.1
17185		T-C	nsp13	N	V340A	
17693		T-C	nsp13	S		

Supplementary Table 2, page 2

	18083	A-G	nsp14	S		
	18238-18239	AT-CC	nsp14	N (ExoN inactivation)	D90A	
	18244-18245	AG-CC	nsp14	N (ExoN inactivation)	E92A	
	19755	A-G	nsp15	N	I69V	
	21579	T-C	Spike	N	Y30H	
	22778	T-C (het)	Spike RBD	S		
	22793	T-G (het)	Spike RBD	S		
	23321	T-C	Spike	S		
	25983	A-G	ORF3a	N	D239G	
	27519	T-C	ORF7a	N	S83P	
	28696	G-A	Nucleocapsid	N	G193S	
MA-ExoN 3.1	98	G-A	5' UTR	n/a		
	1516	A-G	nsp2	N	N238D	3.1
	1711	T-C	nsp2	N	S303P	3.1
	2848	A-T	nsp3	N	T44S	3.1
	4444	T-C	nsp3	N	Y576H	2.2, 2.3, 3.1
	6559	A-G	nsp3	N	I1281V	3.1
	6803	T-C	nsp3	N	V1362A	3.1
	6887	T-C	nsp3	N	M1390T	3.1
	6971	C-T	nsp3	N	A1419V	3.1
	8640	T-A	nsp4	S		
	9800	C-T	nsp4	N	T439I	
	10154	T-G	nsp5	N	L57R	
	10437	C-T	nsp5	S		
	10548	A-G	nsp5	S		
	12831	T-C	nsp9	S		
	14046	G-A	nsp12	N	A226T	3.1
	14471	T-A	nsp12	N	S367R	2.2, 2.3, 3.1
	14891	T-C	nsp12	S		3.1
	15685	C-T	nsp12	N	A772V	3.1
	16710	T-C	nsp13	N	F182L	
	18238-18239	AT-CC	nsp14	N (ExoN inactivation)	D90A	
	18244-18245	AG-CC	nsp14	N (ExoN inactivation)	E92A	
	18533	T-C	nsp14	S		
	19881	C-T	nsp15	N	P111S	
	20918	T-A	nsp16	S		
	21620	T-A	Spike	S		
	21925	G-A	Spike	N	G145D	
	22569	T-C	Spike RBD	N	F360L	
	22913	T-C	Spike RBD	S		
	26341	G-T	Envelope	S		
	26648	C-T	Membrane	N	A84V	
	27084	T-C	ORF6	N	L4P	
	27107	G-A	ORF6	N	A12T	
	27355	C-T	ORF7a	N	T28I	
	27453	A-G	ORF7a	N	T61A	
	28097	T-C	ORF8b	S		
	28266	T-C	Nucleocapsid	S		
MAwt 1.1	2274	C-A	nsp2	N	N490K	1.1, 1.2, 1.3, 3.1, 3.3
	3138	A-G (het)	nsp3	S		1.1
	4394	C-T	nsp3	N	T559I	1.1, 1.2, 1.3
	9690	C-A	nsp4	S		
	18718	C-T	nsp14	N	T250M	

Supplementary Table 2, page 3

	20528	A-G	nsp15	S		
MAwt 2.1	2819	A-G	nsp3	N	K34R	2.1
	3921	G-A	nsp3	S		2.1, 2.2, 2.3
	4192	T-A	nsp3	N	C492S	2.1, 2.2, 2.3
	5584	C-T	nsp3	S		2.1
	9866	C-T	nsp4	N	T461I	
	20528	A-G	nsp15	S		
MAwt 3.1	2274	C-A	nsp2	N	N490K	1.1, 1.2, 1.3, 3.1, 3.3
	20528	A-G	nsp15	S		
ORF1a (nts 493–8603)						
MA-ExoN 1.1	1980	T-C	nsp2	S		1.1, 1.2
	2300	T-C	nsp2	N	I499T	1.1
	2304	T-C	nsp2	S		1.1, 1.2
	3634	G-A	nsp3	N	A306T	1.1, 1.2
	4127	T-C	nsp3	N	M470T	1.1, 1.2
	6211	A-G	nsp3	N	N1165D	1.1
	7951	G-A	nsp3	N	V1745I	1.1, 1.2
MA-ExoN 1.2	1402	C-T	nsp2	N	P200S	1.2
	1980	T-C	nsp2	S		1.1, 1.2
	2304	T-C	nsp2	S		1.1, 1.2
	3634	G-A	nsp3	N	M470T	1.1, 1.2
	4127	T-C	nsp3	N	A306T	1.1, 1.2
	7404	T-A	nsp3	S		1.2
	7951	G-A	nsp3	N	V1745I	1.1, 1.2
MA-ExoN 1.3	872	A-G	nsp2	N	D23G	1.3
	3759	C-T	nsp3	S		1.3
	4896	T-C	nsp3	S		1.3
	5006	T-C	nsp3	N	V763A	1.3
	7452	A-G	nsp3	S		1.3
	8114	G-A	nsp3	N	G1799D	1.3
MA-ExoN 2.1	809	T-C	nsp2	N	V2A	2.1
	1924	G-C	nsp2	N	A374P	2.1
	2506	G-A	nsp2	N	D568N	2.1
	2599	G-A	nsp2	N	V599I	2.1
	2673	T-C	nsp2	S		2.1
	3054	G-T	nsp3	N	E112D	2.1
	3235	A-G	nsp3	N	I173V	2.1
	3704	T-C	nsp3	N	V329A	2.1
	4076	T-C	nsp3	N	D453V	2.1
	4314	T-C	nsp3	S		2.1
	4542	T-G (het)	nsp3	N	I608M	2.1
	4610	C-T	nsp3	N	A631V	2.1
	5094	G-A	nsp3	S		2.1
MA-ExoN 2.2	855	T-C	nsp2	S		2.2, 2.3
	865	A-G (het)	nsp2	N	I21V	2.2
	1699	T-C (het)	nsp2	N	S299P	2.2
	2487	T-C (het)	nsp2	S		2.2
	2595	T-C (het)	nsp2	S		2.2
	2841	T-C (het)	nsp3	S		2.2
	3857	T-C (het)	nsp3	N	V380A	2.2
	4444	T-C	nsp3	N	Y576H	2.2, 2.3, 3.1
	4614	A-G (het)	nsp3	S		2.2
	4704	A-het	nsp3	S		2.2, 2.3

Supplementary Table 2, page 4

	5034	T-C	nsp3	S		2.2, 2.3
	5067	T-C	nsp3	S		2.2, 2.3
	5631	T-C (het)	nsp3	S		2.2
MA-ExoN 2.3	855	T-C	nsp2	S		2.2, 2.3
	3731	A-G	nsp3	N	N338S	2.3
	4444	T-C	nsp3	N	Y576H	2.2, 2.3, 3.1
	4704	A-G	nsp3	S		2.2, 2.3
	4839	T-A	nsp3	S		2.3
	5034	T-C	nsp3	S		2.2, 2.3
	5067	T-C	nsp3	S		2.2, 2.3
	5697	T-C	nsp3	S		2.3
	7379	A-T	nsp3	S		2.3
MA-ExoN 3.1	1516	A-G	nsp2	N	N238D	3.1
	1711	T-C	nsp2	N	S303P	3.1
	2848	A-T	nsp3	N	T44S	3.1
	4444	T-C	nsp3	N	Y576H	2.2, 2.3, 3.1
	6559	A-G	nsp3	N	I1281V	3.1
	6803	T-C	nsp3	N	V1362A	3.1
	6887	T-C	nsp3	N	M1390T	3.1
	6971	C-T	nsp3	N	A1419V	3.1
MA-ExoN 3.2	648	T-C (het)	nsp1	S		3.2
	832	T-C (het)	nsp2	N	F10L	3.2
	1108	A-G (het)	nsp2	N	K102E	3.2
	4106	A-C	nsp3	N	K463T	3.2, 3.3
	4536	G-A	nsp3	N	M606I	3.2, 3.3
	5353	A-G (het)	nsp3	N	K879E	3.2
	5550	T-C (het)	nsp3	S		3.2
	5818	A-G (het)	nsp3	N	T1034A	3.2, 3.3
	7001	T-C (het)	nsp3	N	L1428S	3.2
	7101	A-T	nsp3	S		3.2
	8166	T-A	nsp3	S		3.2, 3.3
	8343	A-G (het)	nsp3	S		3.2
	8361	T-C (het)	nsp3	S		3.2
MA-ExoN 3.3	4106	A-C	nsp3	N	K463T	3.2, 3.3
	4536	G-A	nsp3	N	M606I	3.2, 3.3
	5025	T-C (het)	nsp3	S		3.3
	5818	A-G (het)	nsp3	N	T1034A	3.2, 3.3
	6254	A-G	nsp3	N	D1179G	3.3
	6462	G-A	nsp3	S		3.3
	7602	T-C	nsp3	S		3.3
	8166	T-A	nsp3	S		3.2, 3.3
MAwt 1.1	2274	C-A	nsp2	N	N490K	1.1, 1.2, 1.3, 3.1, 3.3
	3138	A-G (het)	nsp3	S		1.1
	4394	C-T	nsp3	N	T559I	1.1, 1.2, 1.3
MAwt 1.2	2274	C-A	nsp2	N	N490K	1.1, 1.2, 1.3, 3.1, 3.3
	4394	C-T	nsp3	N	T559I	1.1, 1.2, 1.3
MAwt 1.3	2274	C-A	nsp2	N	N490K	1.1, 1.2, 1.3, 3.1, 3.3
	4394	C-T	nsp3	N	T559I	1.1, 1.2, 1.3
MAwt 2.1	2819	A-G	nsp3	N	K34R	2.1
	3921	G-A	nsp3	S		2.1, 2.2, 2.3
	4192	T-A	nsp3	N	C492S	2.1, 2.2, 2.3

	5584	C-T	nsp3	S		2.1
MAwt 2.2	3921	G-A	nsp3	S		2.1, 2.2, 2.3
	4192	T-A	nsp3	N	C492S	2.1, 2.2, 2.3
MAwt 2.3	3921	G-A	nsp3	S		2.1, 2.2, 2.3
	4192	T-A	nsp3	N	C492S	2.1, 2.2, 2.3
MAwt 3.1	2274	C-A	nsp2	N	N490K	1.1, 1.2, 1.3, 3.1, 3.3
MAwt 3.2	None					
MAwt 3.3	2274	C-A	nsp2	N	N490K	1.1, 1.2, 1.3, 3.1, 3.3
ORF1b (nts 12,915–16,520; nt 16999 was verified separately)						
MA-ExoN 1.1	13042	T-C	nsp10	N	Y30H	1.1, 1.2, 1.3
	14469	A-T	nsp12	N	S367C	1.1, 1.2, 1.3
	14738	T-C	nsp12	S		1.1, 1.2
	15308	T-C	nsp12	S		1.1, 1.2
	16999	C-A	nsp13	N	S278Y	1.1, 1.2, 1.3, 2.1
MA-ExoN 1.2	12935	C-T (het)	nsp9	N	A107V	1.2
	13042	T-C	nsp10	N	Y30H	1.1, 1.2, 1.3
	14469	A-T	nsp12	N	S367C	1.1, 1.2, 1.3
	14738	T-C	nsp12	S		1.1, 1.2
	15308	T-C	nsp12	S		1.1, 1.2
	16999	C-A	nsp13	N	S278Y	1.1, 1.2, 1.3, 2.1
MA-ExoN 1.3	13042	T-C	nsp10	N	Y30H	1.1, 1.2, 1.3
	13541	G-A	nsp12	S		1.3
	14469	A-T	nsp12	N	S367C	1.1, 1.2, 1.3
	15428	A-G	nsp12	S		1.3
	16999	C-A	nsp13	N	S278Y	1.1, 1.2, 1.3, 2.1
MA-ExoN 2.1	13621	A-G	nsp12	N	E84G	2.1
	14842	A-G	nsp12	N	N491S	2.1
	16999	C-A	nsp13	N	S278Y	1.1, 1.2, 1.3, 2.1
MA-ExoN 2.2	13104	T-C	nsp10	S		2.2, 2.3
	13798	A-G (het)	nsp12	N	K143R	2.2
	14471	T-A	nsp12	N	S367R	2.2, 2.3, 3.1
	14915	T-C (het)	nsp12	S		2.2
	16334	T-C (het)	nsp13	S		2.2
MA-ExoN 2.3	13104	T-C	nsp10	S		2.2, 2.3
	14471	T-A	nsp12	N	S367R	2.2, 2.3, 3.1
MA-ExoN 3.1	14046	G-A	nsp12	N	A226T	3.1
	14471	T-A	nsp12	N	S367R	2.2, 2.3, 3.1
	14891	T-C	nsp12	S		3.1
	15685	C-T	nsp12	N	A772V	3.1
MA-ExoN 3.2	13380	T-A (het)	nsp12	N	D3E	3.2
	15236	T-C (het)	nsp12	S		3.2
	16177	T-C	nsp13	N	V4A	3.2
MA-ExoN 3.3	14452	T-C	nsp12	N	L361S	3.3
	15698	A-T	nsp12	S		3.3
MAwt 1.1	None					

MAwt 1.2	None					
MAwt 1.3	None					
MAwt 2.1	None					
MAwt 2.2	None					
MAwt 2.3	None					
MAwt 3.1	None					
MAwt 3.2	None					
MAwt 3.3	None					

*Data entered in "Present in Clones" column only if more than one plaque isolate per mouse was sequenced at that nucleotide position.

Supplementary Table 3. SNP Results from Short-Term BALB/c Infections

Virus	SNP	Region	AA Change	Young BALB/c	Old BALB/c	SCID Clones
MA-ExoN	C1142Y	nsp2	T113I	1/10	0/10	
	A3053R	nsp3	E112G	0/10	1/10	
	T11405Y	nsp6	V168A	0/10	1/10	2/9
	C16999M*	nsp13	S278Y	10/10	10/10	4/9
	G22822R	Spike	R444K	1/10	2/10	
	C23638Y	Spike	T716I	2/10	6/10	1/9
MAwt	T4192A	nsp3	C492S	10/10	10/10	3/9
	T11006C	nsp6	F35S	10/10	10/10	2/9
	C29536Y	3'UTR	n/a	1/10	0/10	

*Upon Sanger sequencing of this allele, mutation frequency was consistently maintained at approximately 40% both *in vitro* and *in vivo*.

Supplementary Table 4. PCR and Sequencing Primers and Amplicon Sets

Primer	Nt position	Sequence*
SURB1F ¹	1–24	ATATTAGTTTTTACCTACCCAGG
S1F ^A	493–513	TTAAGCACCAATCACGGCCAC
S2aF	1056–1075	CACTTTCAAAGGGGAATGCC
S3F	1577–1596	TTACTGGTGACAATGTGGAG
S4F ^{2,B}	2149–2171	GAATGGATTGAGGCGAACTTAG
S5F	2743–2764	GGAGAAGATACTGTTTGGGAAG
S6F	3324–3344	TGTTGACATCGTTAAGGAGGC
S7F ^C	3861–3880	GAAGCCTGTCGATGTGAAGC
S8F ³	4431–4450	GGGCATCGTTGACTATGGTG
S9F	4989–5009	CTTGGATGGTGCTGATGTTAC
S10aF ^D	5608–5627	GTTATGATGTCTGCACCACC
S11aF	6166–6185	GTTTGGCACATTAACCAGGC
S12F ^{4,E}	6662–6682	GCGCTAAGAGATTAGCACAAAC
S13F	7219–7240	GGTCTTTCAGCTATAATGCAGG
S14F	7824–7843	TGATGGCAAGTCCAAATGCC
S15F	8381–8400	TTCGTAGTGCTGCCAAGAAG
S16F ⁵	8935–8954	GTTCTTGCTGCTGAGTGAC
S17aF	9552–9571	TGTTTCATTCTTGGCTCACC
S18F	10084–10103	GACACAGTATACTGTCCAAG
S19F ^X	10611–10630	TGCTGCTGTTATCAATGGTG
S20F ⁶	11168–11187	GCTGGGTGATGCGTATCATG
S21F	11721–11741	TAAGTTGTTGGGTATTGGAGG
S22aF	12389–12408	CTACAGCAGCCAAACTCATG
S23F ^F	12915–12934	TATGGTGCTGGGCAGTTTAG
S24F ⁷	13450–13469	GTACTGATGTCGTCTACAGG
S25F	14021–14040	CGATTTCCGGTGATTTCTGAC
S26F	14548–14569	CTACATGCTTTTCAGTAGCTGC
S27F ^G	15101–15120	GTCAATAGCCGCCACTAGAG
S28F ⁸	15646–15665	CTGATGATGCCGTTGTGTGC
S29aF ^Y	16243–16262	GTTGCAAGTGCTGCTATGAC
S30F	16775–16794	TGACTATGGTGATGCTGTTG
S31aF	17399–17418	GCTGACTAAAGGCACACTAG
S32F ⁹	17931–17951	AGTCTAGAAATACCACGTCGC
S33aF	18569–18588	GATTGGACCTGAAAGAACGT
S34F	19132–19151	ACGTTGATCGTTACCCAGCC
S35F	19706–19726	TGTTGCATTTGAGCTTTGGGC
S36F ¹⁰	20261–20280	TGGAGATTTCAATCATGGAC
S37F	20801–20820	TGCTGGCTCTGATAAAGGAG
S38aF	21420–21439	GGTAGGCTTATCATTAGAGA
S39F	21924–21944	GGTACACAGACACATACTATG
S40F ¹¹	22491–22510	TTCCCTTCTGTCTATGCATG
S41F	23081–23100	TGGACTCACTGGTACTGGTG
S42aF	23724–23743	CTAAATCGTGCCTCTCAGG
S43F	24263–24282	AACTGCATTGGGCAAGCTGC
S44F ¹²	24851–24870	TGATCCTCTGCAACCTGAGC
S45F	25411–25430	TGGCGTTGCATTTCTTGCTG
S46F	25978–25997	TAAAGACCCACCGAATGTGC
S47F	26590–26609	GTGCTTGCTGCTGTCTACAG
S48F ¹³	27132–27153	GGACTTTCAGGATTGCTATTTG
S49F	27729–27749	CACTCGAAATCCAGGATCTAG
S50F	28273–28292	TCTTGTTACAGCTCTCAC
S51F	28840–28860	CAACAACAAGGCCAAACTGTC
S52F	29410–29429	GGCAGATGGGCTATGTAAAC
S1R	411–430	GCTCTACTAGACCACAAGTG
S2R	911–931	CGATGTAATCAAGTTGTTCCGG
S3R	1318–1337	CCACATGTAGTAGGTCCTTC

S4R	1877–1896	GATAACACCAGCAGCCTGTG
S5R ^{1,A}	2421–2440	GCTCCTTGCCACGTATACAC
S6R	3063–3082	CCTCACACTCTGCATCGTCC
S7R	3619–3638	GGTGCAAGTAAGATGTCCTG
S8R	4111–4131	GAGCATCTCAGTAGTGCCACC
S9R ^{2,B}	4654–4673	GATGACGAAGTGAGGTATCC
S10R	5301–5322	GTTAGCAGCATCACCAGCACGG
S11R ^C	5819–5838	CGACACAGGCTTGATGGTTG
S12R	6401–6420	GACATTGCCTACAACCTTCGG
S13R ^{3,D}	6983–7002	CAATTCTCTAACGCCATTAC
S14R	7514–7533	CTTGCGAAGCCACGGCCTC
S15aR	8061–8080	CACCATCTAAAGCTACACCC
S16aR ^E	8584–8603	GTGTAACCATCATGGATTGA
S17aR ⁴	9144–9163	CGCATGTACCATGTCTACAG
S18R	9781–9800	GTAAGTGGCAACAGTGTCTC
S19aR	10265–10284	CTTGGGTGTCTTAGGGTTAG
S20R	10884–10903	CTTGAAGGTAACACCAGAG
S21R ^{5,X}	11512–11532	GATACACTGTAAGGTGTTGCC
S22R	12076–12095	GCCTGCTCATAGGCCCTCCTG
S23R	12654–12673	GTGTGGTACCAGCCGCACAG
S24R	13184–13204	GATGGTCAATGTGGCATCTAC
S25R ⁶	13704–13723	CGCTGACGTGATATATGTGG
S26R	14276–14295	GAAGGATACACCTATCATCC
S27R	14811–14830	CAGCCACCATCGTAACAATC
S28aR ^F	15326–15345	ACTCGTTAGCTAACCTGTAG
S29R ⁷	15947–15966	CATCAATAGCCAGTGACACG
S30R ^G	16501–16520	GCCAGCATTAGTCCAATCAC
S31R	17068–17087	CGTATACACTATGCGAGCAG
S32R ^Y	17664–17683	CAAGCAGGATTGCGTGTAA
S33R ⁸	18248–18268	GCATCTCTAGTTGCATGACAG
S34R	18833–18852	CAACAGACCAATCAACGCGC
S35R	19439–19458	CATCCAAGTACTGTGGTAC
S36aR	19862–19881	GTTTCTTGGCAATGTCAGTC
S37aR ⁹	20420–20439	GATCAATCACAGAACACACA
S38R	21103–21123	CAGCATTCCAAGAATGCTCTG
S39R	21812–21833	CACCGACTGTGACTTGTTGTTT
S40R	22341–22360	ACAGAGCATTGAGTTCAGC
S41aR ¹⁰	22765–22784	AGTAGCATCAATGTTCTAG
S42aR	23343–23362	GTAGAATATATGCGCCAAGC
S43R	23912–23931	CAGCATCAGCGAGTGTCCACC
S44aR	24432–24451	TGAAGGCTTTGAAGTCTGCC
S45R ¹¹	24987–25006	GCGACCTCATTGAGGCGGTC
S46R	25552–25571	CCATACCTGCAGCGACAAGC
S47aR	26054–26073	GTCGTCGTCGGCTCATCATA
S48R	26590–26609	CTGTAGACAGCAGCAAGCAC
S49R ¹²	27285–27304	ACAATCAATGTCAGGAAGAG
S50aR	27739–27758	AAGTTCTTCTAGATCCTGG
S51aR	28330–28349	GTGTTGATTGGAACGCCCTG
S52aR	28869–28888	TAGATGCCTCAGCAGCAGAT
S53aR	29553–29572	CTTTCAAGTCCTCCCTAATG
S54aR ¹³	29682–29701	TCACATGGGGATAGCACTAC

Amplicon-generating primers (1–13, A–G, X, Y) are indicated in superscript next to the relevant primers.

Amplicons were sequenced with all primers that fall between the amplicon-generating primers.

*All sequences are listed 5'–3'.