

Table S3. Non-engineered mutations identified in S-ExoN1 viruses.

P3 viral clone(s) ^a	Mutation ^b	Codon change	Amino acid change ^d	Location ⁱ
All 10	T 5588 C	<u>G</u> <u>T</u> <u>A</u> → <u>G</u> <u>C</u> <u>A</u>	Val 957 Ala	nsp 3
	G 7125 A	<u>A</u> <u>C</u> <u>G</u> → <u>A</u> <u>C</u> <u>A</u>	none	nsp 3
	G 8872 A	<u>G</u> <u>G</u> <u>C</u> → <u>A</u> <u>G</u> <u>C</u>	Gly 130 Ser	nsp 4
	A 12439 T	<u>A</u> <u>A</u> <u>C</u> → <u>I</u> <u>A</u> <u>C</u>	Asn 140 Tyr	nsp 8
	T 20258 C	<u>G</u> <u>T</u> <u>I</u> → <u>G</u> <u>T</u> <u>C</u>	none	nsp 15
	G 23631 A	<u>G</u> <u>C</u> <u>T</u> → <u>A</u> <u>C</u> <u>T</u>	Ala 714 Thr	Spike
	T 27148 C	<u>G</u> <u>C</u> <u>T</u> → <u>G</u> <u>C</u> <u>C</u>	none	ORF 6
46, 60	T 4731 A	<u>G</u> <u>A</u> <u>T</u> → <u>G</u> <u>A</u> <u>A</u>	Asp 671 Glu	nsp 3
37, 53	T 5412 C	<u>G</u> <u>C</u> <u>T</u> → <u>G</u> <u>C</u> <u>C</u>	none	nsp 3
8, 34	T 7142 C	<u>C</u> <u>T</u> <u>A</u> → <u>C</u> <u>C</u> <u>A</u>	Leu 1475 Pro	nsp 3
12, 23, 46, 60	T 8922 C	<u>G</u> <u>C</u> <u>T</u> → <u>G</u> <u>C</u> <u>C</u>	none	nsp 4
1, 12, 23, 37, 46, 53, 60	T 14876 A	<u>G</u> <u>C</u> <u>T</u> → <u>G</u> <u>C</u> <u>A</u>	none	nsp 12
12, 23, 46, 60	T 18860 C	<u>C</u> <u>C</u> <u>T</u> → <u>C</u> <u>C</u> <u>C</u>	none	nsp 14
12, 23, 46, 60	T 23243 C	<u>A</u> <u>T</u> <u>I</u> → <u>A</u> <u>T</u> <u>C</u>	none	Spike
8, 34	T 24536 C	<u>G</u> <u>T</u> <u>I</u> → <u>G</u> <u>T</u> <u>C</u>	none	Spike
12, 19	T 26335 C	<u>G</u> <u>A</u> <u>T</u> → <u>G</u> <u>A</u> <u>C</u>	none	E
1	T 5061 C	<u>C</u> <u>C</u> <u>T</u> → <u>C</u> <u>C</u> <u>C</u>	none	nsp 3
	T 7484 G	<u>A</u> <u>T</u> <u>G</u> → <u>A</u> <u>G</u> <u>G</u>	Met 1589 Arg	nsp 3
	T 12411 C	<u>G</u> <u>T</u> <u>I</u> → <u>G</u> <u>T</u> <u>C</u>	none	nsp 8
	A 13925 G	<u>T</u> <u>C</u> <u>A</u> → <u>T</u> <u>C</u> <u>G</u>	none	nsp 12
	A 15497 G	<u>T</u> <u>C</u> <u>A</u> → <u>T</u> <u>C</u> <u>G</u>	none	nsp 12
	A 15821 T	<u>A</u> <u>C</u> <u>A</u> → <u>A</u> <u>C</u> <u>T</u>	none	nsp 12
	C 17269 T	<u>G</u> <u>C</u> <u>T</u> → <u>G</u> <u>T</u> <u>T</u>	Ala 368 Val	nsp 13
	T 20789 C	<u>G</u> <u>T</u> <u>I</u> → <u>G</u> <u>T</u> <u>C</u>	none	nsp 16
	G 22423 T	<u>G</u> <u>G</u> <u>A</u> → <u>G</u> <u>T</u> <u>A</u>	Gly 311 Val	Spike (RBD)
	T 23374 C	<u>G</u> <u>T</u> <u>A</u> → <u>G</u> <u>C</u> <u>A</u>	Val 628 Ala	Spike
	G 24957 A	<u>G</u> <u>C</u> <u>T</u> → <u>A</u> <u>C</u> <u>T</u>	Ala 1156 Thr	Spike
	C 26049 T	<u>C</u> <u>C</u> <u>A</u> → <u>C</u> <u>T</u> <u>A</u>	Pro 261 Leu	ORF 3a
		<u>C</u> <u>A</u> <u>A</u> → <u>T</u> <u>A</u> <u>A</u>	Gln 121 Stop ^e	ORF 3b
	G 29271 A	<u>C</u> <u>C</u> <u>G</u> → <u>C</u> <u>C</u> <u>A</u>	none	N
8	G 3166 A	<u>G</u> <u>C</u> <u>T</u> → <u>A</u> <u>C</u> <u>T</u>	Ala 150 Thr	nsp 3
	T 3558 C	<u>C</u> <u>C</u> <u>T</u> → <u>C</u> <u>C</u> <u>C</u>	none	nsp 3
	G 5466 A	<u>C</u> <u>A</u> <u>G</u> → <u>C</u> <u>A</u> <u>A</u>	none	nsp 3
	A 10602 G	<u>G</u> <u>C</u> <u>A</u> → <u>G</u> <u>C</u> <u>G</u>	none	nsp 5
	A 11050 G	<u>A</u> <u>T</u> <u>G</u> → <u>G</u> <u>T</u> <u>G</u>	Met 50 Val	nsp 6
	A 11530 G	<u>A</u> <u>T</u> <u>C</u> → <u>G</u> <u>T</u> <u>C</u>	Ile 210 Val	nsp 6
	T 12339 C	<u>A</u> <u>T</u> <u>I</u> → <u>A</u> <u>T</u> <u>C</u>	none	nsp 8
	T 15333 C	<u>I</u> <u>T</u> <u>A</u> → <u>C</u> <u>T</u> <u>A</u>	none	nsp 12
	A 16232 G	<u>A</u> <u>G</u> <u>A</u> → <u>A</u> <u>G</u> <u>G</u>	none	nsp 13
	G 25002 A	<u>G</u> <u>T</u> <u>C</u> → <u>A</u> <u>T</u> <u>C</u>	Val 1171 Ile	Spike
	T 26354 C	n.a.	n.a.	IGR
	A 26899 T	<u>A</u> <u>C</u> <u>T</u> → <u>I</u> <u>C</u> <u>T</u>	Thr 168 Ser	M
	A 28736 T	<u>A</u> <u>A</u> <u>T</u> → <u>A</u> <u>I</u> <u>T</u>	Asn 206 Ile	N
	A 29628 G	n.a.	n.a.	3' UTR
12	T 3603 C	<u>T</u> <u>A</u> <u>T</u> → <u>T</u> <u>A</u> <u>C</u>	none	nsp 3
	T 13874 C	<u>A</u> <u>A</u> <u>T</u> → <u>A</u> <u>A</u> <u>C</u>	none	nsp 12
	T 18749 C	<u>C</u> <u>A</u> <u>T</u> → <u>C</u> <u>A</u> <u>C</u>	none	nsp 14
	A 27167 G	<u>A</u> <u>T</u> <u>A</u> → <u>G</u> <u>T</u> <u>A</u>	Ile 32 Val	ORF 6
	T 27648 C	<u>C</u> <u>I</u> <u>C</u> → <u>C</u> <u>C</u> <u>C</u>	Leu 4 Pro	ORF 7b
19	A 654 T	<u>G</u> <u>G</u> <u>A</u> → <u>G</u> <u>G</u> <u>T</u>	none	nsp 1
	T 5236 A	<u>I</u> <u>T</u> <u>A</u> → <u>A</u> <u>T</u> <u>A</u>	Leu 840 Ile	nsp 3
	A 6476 G	<u>G</u> <u>A</u> <u>T</u> → <u>G</u> <u>G</u> <u>T</u>	Asp 1253 Gly	nsp 3
	A 10119 G	<u>A</u> <u>C</u> <u>A</u> → <u>A</u> <u>C</u> <u>G</u>	none	nsp 5
	T 14630 C	<u>T</u> <u>A</u> <u>T</u> → <u>T</u> <u>A</u> <u>C</u>	none	nsp 12
	C 27290 T	<u>T</u> <u>T</u> <u>C</u> → <u>T</u> <u>T</u> <u>I</u>	none	ORF 7a
	C 29412 T	n.a.	n.a.	3' UTR
23	G 1771 A	<u>G</u> <u>T</u> <u>T</u> → <u>A</u> <u>T</u> <u>T</u>	Val 323 Ile	nsp 2
	T 1905 C	<u>A</u> <u>T</u> <u>I</u> → <u>A</u> <u>T</u> <u>C</u>	none	nsp 2
	T 2976 C	<u>G</u> <u>C</u> <u>T</u> → <u>G</u> <u>C</u> <u>C</u>	none	nsp 3
	C 3491 T	<u>A</u> <u>C</u> <u>A</u> → <u>A</u> <u>T</u> <u>A</u>	Thr 258 Ile	nsp 3
	A 3845 G	<u>G</u> <u>A</u> <u>G</u> → <u>G</u> <u>G</u> <u>G</u>	Glu 376 Gly	nsp 3

	T 6265 C	<u>I</u> CA→ <u>C</u> CA	Ser 1183 Pro	nsp 3
	C 21038 T	<u>T</u> TC→ <u>T</u> TI	none	nsp 16
	T 22206 C	<u>I</u> CA→ <u>C</u> CA	Ser 239 Pro	Spike
	T 23468 C	<u>AG</u> T→ <u>AG</u> C	none	Spike
	A 26121 T	<u>T</u> AC→ <u>T</u> IC	Tyr 2 Phe	E
		<u>ACT</u> → <u>TCT</u>	Thr 145 Ser	ORF 3b
34	A 707 G	<u>G</u> AG→ <u>G</u> GG	Glu 148 Gly	nsp 1
	T 5015 C	<u>A</u> TT→ <u>A</u> CT	Ile 766 Thr	nsp 3
	T 17602 C	<u>G</u> TT→ <u>G</u> CT	Val 479 Ala	nsp 13
	T 19917 C	<u>I</u> TT→ <u>C</u> TT	Phe 123 Leu	nsp 15
	A 22352 G	<u>AAA</u> → <u>AAG</u>	none	Spike
	A 24910 T	<u>AA</u> T→ <u>A</u> TT	Asn 1140 Ile	Spike
	T 25783 A	<u>GG</u> T→ <u>G</u> GA	none	ORF 3a
		<u>G</u> TG→ <u>G</u> AG	Val 32 Glu	ORF 3b
	A 26241 G	<u>T</u> AC→ <u>T</u> GC	Tyr 42 Cys	E
	T 27917 A	<u>ACT</u> → <u>ACA</u>	none	ORF 8b
37	T 19082 C	<u>G</u> CT→ <u>G</u> CC	none	nsp 14
	C 24249 T	<u>C</u> TT→ <u>I</u> TT	Leu 920 Phe	Spike
	G 24873 A	<u>G</u> AC→ <u>A</u> AC	Asp 1128 Asn	Spike
46	T 141 C	n.a.	n.a.	5' UTR
	G 159 A	n.a.	n.a.	5' UTR
	A 13149 G	<u>CAA</u> → <u>CAG</u>	none	nsp 10
	T 15605 C	<u>TT</u> I→ <u>TT</u> C	none	nsp 12
	T 16356 C	<u>I</u> AT→ <u>C</u> AT	Tyr 64 His	nsp 13
	C 25800 T	<u>CCA</u> → <u>CTA</u>	Pro 178 Leu	ORF 3a
		<u>CAA</u> → <u>TAA</u>	Gln 38 Stop ^f	ORF 3b
	Δ_{26340} TGG $_{26342}$ ^c	<u>C</u> (<u>TG</u> . <u>G</u>)TC →CTC	Δ Val 76	E
53	T 3229 C	<u>I</u> CA→ <u>C</u> CA	Ser 171 Pro	nsp 3
	T 4808 C	<u>C</u> IG→ <u>C</u> CG	Leu 697 Pro	nsp 3
	T 8004 C	<u>AG</u> T→ <u>AG</u> C	none	nsp 3
	C 10658 T	<u>ACT</u> → <u>ATT</u>	Thr 225 Ile	nsp 5
	T 15905 C	<u>GAT</u> → <u>GAC</u>	none	nsp 12
	T 16386 C	<u>I</u> CA→ <u>C</u> CA	Ser 74 Pro	nsp 13
	T 26226 A	<u>CTT</u> → <u>CAT</u>	Leu 37 His	E
	C 28557 T	<u>CAC</u> → <u>CAT</u>	none	N
	G 29417 A	n.a.	n.a.	3' UTR
60	T 21860 A	<u>G</u> TT→ <u>G</u> TA	none	Spike
	C 23518 T	<u>G</u> CT→ <u>G</u> TT	Ala 676 Val	Spike
	T 25550 C	<u>I</u> TG→ <u>C</u> TG	none	ORF 3a
	C 25626 T	<u>G</u> CA→ <u>G</u> TA	Ala 120 Val	ORF 3a
	G 27248 A	<u>G</u> AG→ <u>A</u> AG	Glu 59 Lys	ORF 6
	T 27639 C	<u>T</u> GA→ <u>C</u> GA	Stop→Arg ^g	ORF 7a
		<u>A</u> TG→ <u>A</u> CG	Met 1 Thr ^h	ORF 7b
	A 29324 G	<u>GAT</u> → <u>GGT</u>	Asp 402 Gly	N

^a P3 clones analyzed were c1, c8, c12, c19, c23, c34, c37, c46, c53, and c60. Entire genomes were sequenced except for terminal nucleotides as indicated in Table S1.

^b **Red**, mutations in all clones; **green**, mutations in a subset of clones, **blue**, mutations unique to single clones.

^c We cannot differentiate between Δ_{26340} TGG $_{26342}$ [C(TG.G)TC→CTC] or Δ_{26341} GGT $_{26343}$ [CT(G.GT)C→CTC], but both have the same effect of deleting the C-terminal Val76 residue of E protein.

^d Amino acid positions in nsps refer to location within the respective mature nsp. 'None' indicates synonymous changes. Note that the Δ_{26340} TGG $_{26342}$ mutation in c46 removes nucleotides from adjacent codons.

^e Early termination removes the carboxy-terminal 34 residues of the ORF 3b protein.

^f Early termination removes the carboxy-terminal 117 residues of the ORF 3b protein.

^g Effectively adds five residues (RMSSL) to the C-terminus of the ORF 7a protein.

^h Effectively removes the 23 amino-terminal residues from the ORF 7b protein.

ⁱ IGR, intergenic region; UTR, untranslated region at either terminus of the genome; RBD, receptor-binding domain.