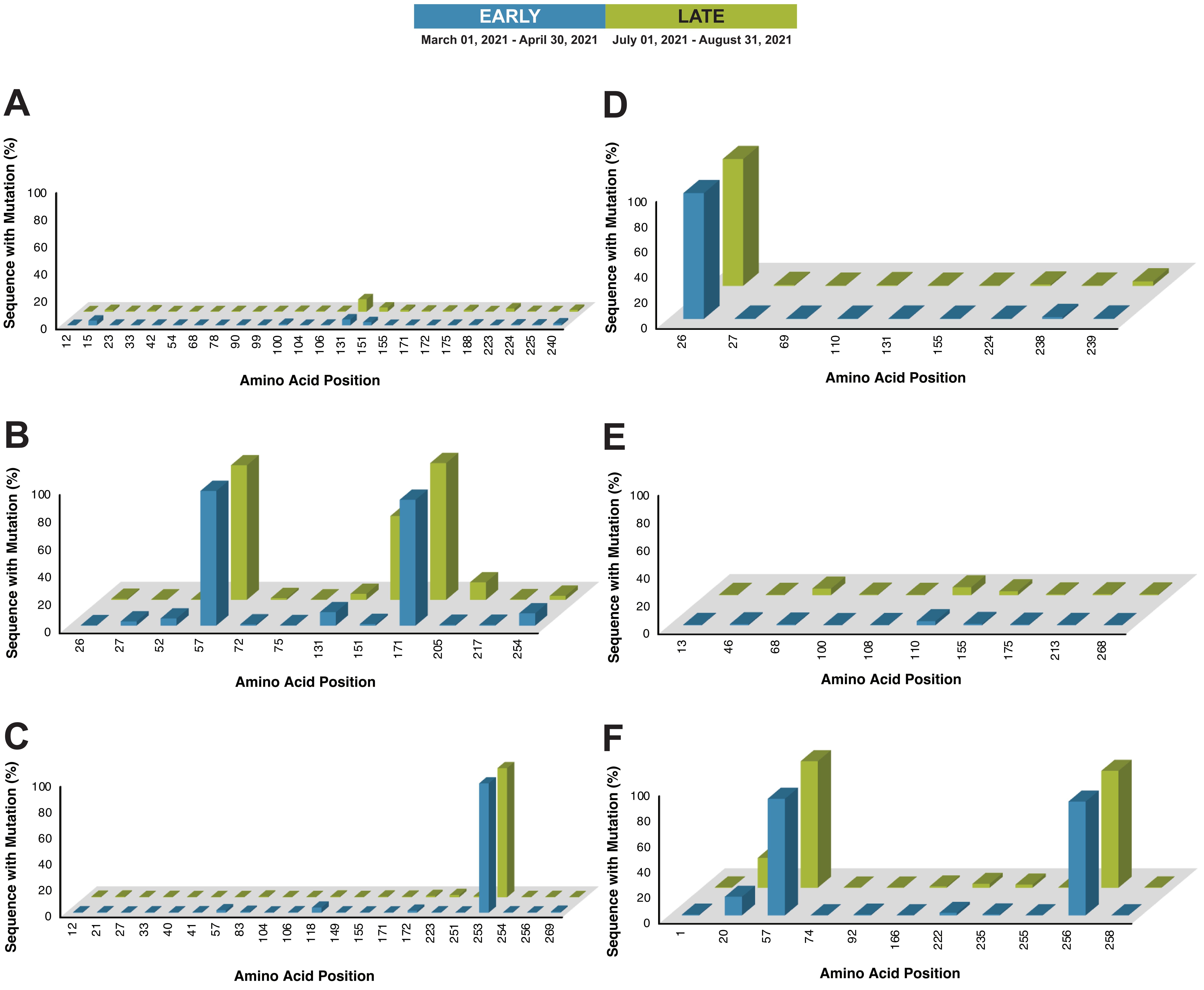
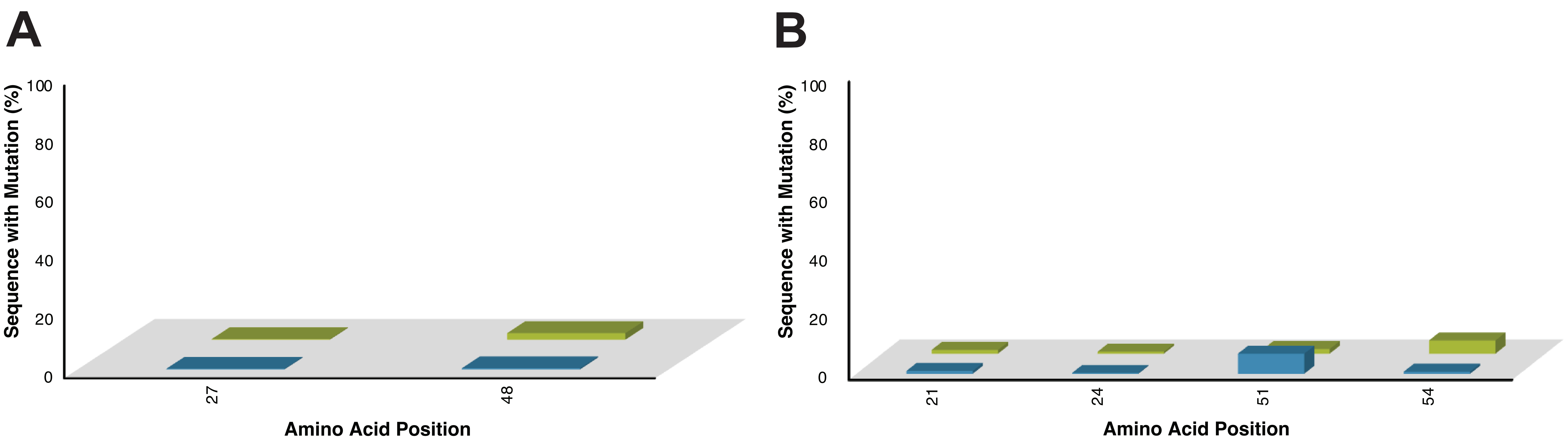


G

Variant	Mutation Frequency (%)			
	Early		Late	
Alpha	W131C	4	W131C	8
	L15F	3	T151I	3
	T151I	2	T223I	2
	P240S	1	L85F	1
	G100C	1	L15F	1
Beta	Q57H	100	S171L	100
	S171L	90	Q57H	100
	W131L	10	T151I	60
	G254*	9	V90I	60
	L52F	5	S205T	10
Gamma	S253P	100	S253P	100
	I118M	4	T223I	2
	Q57H	2	G251C	1
	G172C	1	S216L	1
	T269M	1	W193R	1
Delta	S26L	100	S26L	100
	Q57R	2	E239Q	3
	D238Y	2	P104S	1
	G172R	0.4	G224C	1
	G172C	0.3	D27Y	1
Lambda	A110S	3	A110S	6
	D155Y	1	R68K	4
	R68K	0.6	E242V	4
	T175I	0.5	D155Y	3
	I118V	0.5	G172R	2
Mu	Q57H	90	Q57H	100
	V256I	90	V256I	90
	I20M	20	I20M	20
	D222Y	2	D222Y	2
	K235R	1	K235R	2

Supplementary Figure 1 Frequency of ORF3a Mutations. Point mutations with frequencies greater than or equal to 0.2% and less than or equal to 100% were plotted based on the location of each mutation across the ORF3a amino acid sequences of the Alpha (A), Beta (B), Gamma (C), Delta (D), Lambda (E), and Mu (F) SARS-CoV-2 variants. The most frequent mutations were selected for each variant (G). Data shown are both from the early (🟦, March 01, 2021 – April 30, 2021) and late (🟩, July 01, 2021 – August 31, 2021) time points.

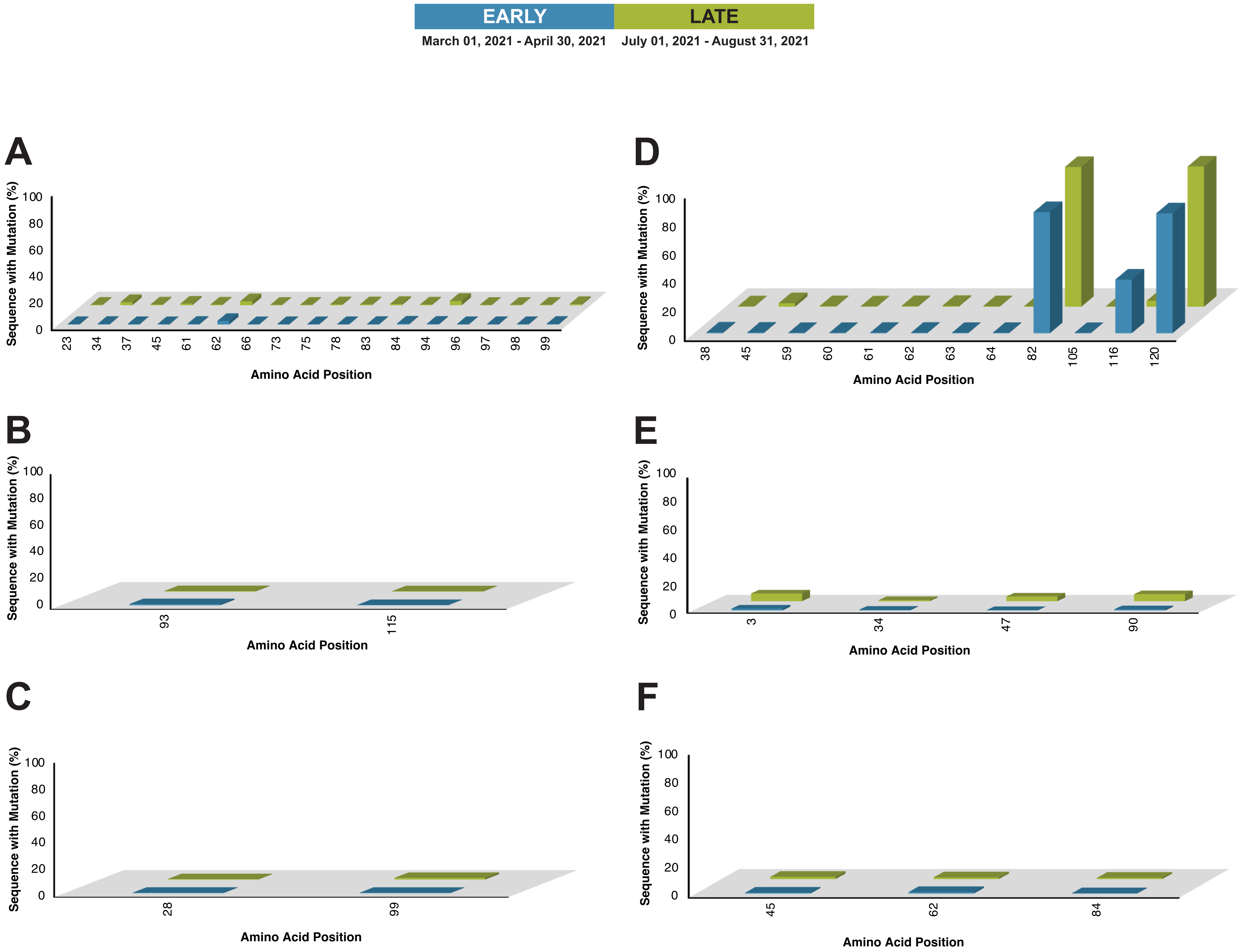
EARLY	LATE
March 01, 2021 - April 30, 2021	July 01, 2021 - August 31, 2021



C

Variant	Mutation Frequency (%)			
	Early		Late	
Alpha	T21I	0.2	L52V	0.2
	R20S	0.2	T21I	0.2
	Y31H	0.1	R20S	0.2
	W27L	0.1	W27L	0.2
	F7L	0.09	F7Y	0.1
Beta	K48E	0.1	P57L	2
	P57L	0.03	K48E	1
	T21I	0.01	T21I	0.4
	E54D	0.01	E54D	0.2
			I36T	0.1
Gamma	P57L	0.8	T21I	0.2
	F2S	0.2	P57L	0.1
	W27L	0.1	W27L	0.1
	K42N	0.04	I36T	0.1
	R20S	0.03	S25-	0.1
Delta	K48N	0.4	K48N	2
	E54*	0.3	E46V	1
	W27L	0.2	Q56R	0.2
	R20K	0.1	W27L	0.2
	E13D	0.08	P57L	0.1
Lambda	A51S	7	E54*	4
	T21I	1	A51S	2
	E54*	1	T21I	1
	L127F	0.4	V24I	1
	V24I	0.3	K48N	0.5
Mu			Y31F	0.2
			L4H	0.1
			V5I	0.1

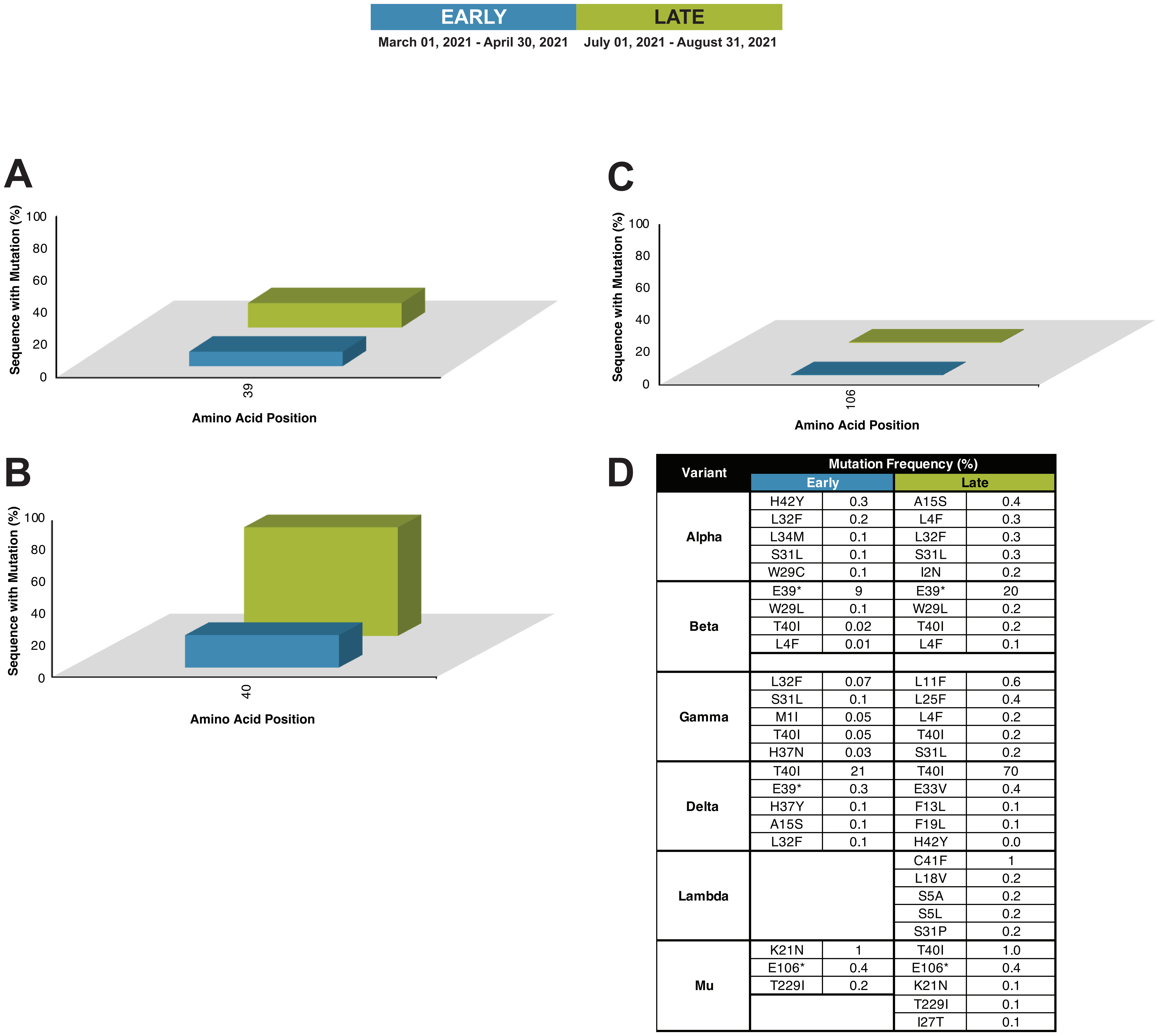
Supplementary Figure 2 Frequency of ORF6 Mutations. Point mutations with frequencies greater than or equal to 0.2% and less than or equal to 100% were plotted based on the location of each mutation across the ORF6 amino acid sequences of the Delta (A) and Lambda (B) SARS-CoV-2 variants. The most frequent mutations were selected for each variant (C). Data shown are both from the early (🟦, March 01, 2021 – April 30, 2021) and late (🟩, July 01, 2021 – August 31, 2021) time points.



G

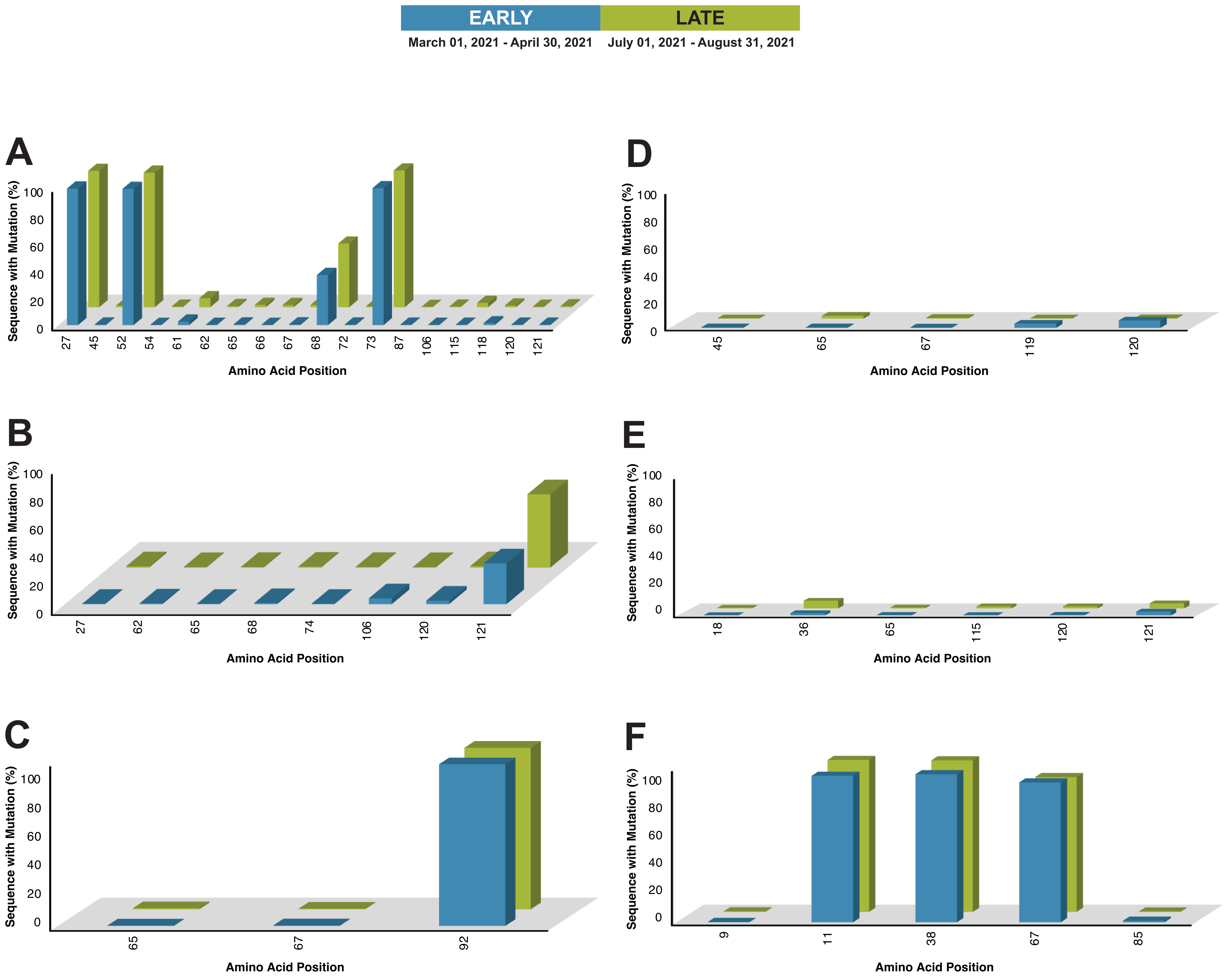
Variant	Mutation Frequency (%)			
	Early		Late	
Alpha	Q62*	2	Q94L	3
	P84S	0.4	Q62*	3
	P84L	0.3	K119N	1
	S83L	0.3	P34S	1
	P34S	0.3	P34L	1
Beta	F63-	1.0	V93F	0.4
	V93F	0.9	T115I	0.3
	T115I	0.2	F63-	0.2
	Q62H	0.1	T61I	0.2
	L96F	0.1	H73Y	0.2
Gamma	V93F	5	P99L	0.9
	L96F	0.5	T11I	0.4
	T28I	0.3	T28I	0.4
	P99S	0.3	P99S	0.4
	I103-	0.2	S83L	0.3
Delta	V82A	90	T120I	100
	T120I	80	V82A	100
	L116F	40	L116F	4
	G38V	0.4	P45L	2
	P45L	0.4	V71I	1
Lambda	I3T	1	I3T	6
	Q90*	0.8	Q90*	5
	P34S	0.4	H47Y	3
	H47Y	0.2	T115I	3
	V24F	0.2	G38V	1
Mu	V62L	1	P45L	1
	L18F	0.8	L12F	1
	P45L	0.6	P84S	0.9
	P84S	0.2	G38V	0.8
			T120I	0.7

Supplementary Figure 3 Frequency of ORF7a Mutations. Point mutations with frequencies greater than or equal to 0.2% and less than or equal to 100% were plotted based on the location of each mutation across the ORF7a amino acid sequences of the Alpha (A), Beta (B), Gamma (C), Delta (D), Lambda (E), and Mu (F) SARS-CoV-2 variants. The most frequent mutations were selected for each variant (G). Data shown are both from the early (🟦, March 01, 2021 – April 30, 2021) and late (🟩, July 01, 2021 – August 31, 2021) time points.



Variant	Mutation Frequency (%)			
	Early		Late	
Alpha	H42Y	0.3	A15S	0.4
	L32F	0.2	L4F	0.3
	L34M	0.1	L32F	0.3
	S31L	0.1	S31L	0.3
	W29C	0.1	I2N	0.2
Beta	E39*	9	E39*	20
	W29L	0.1	W29L	0.2
	T40I	0.02	T40I	0.2
	L4F	0.01	L4F	0.1
Gamma	L32F	0.07	L11F	0.6
	S31L	0.1	L25F	0.4
	M1I	0.05	L4F	0.2
	T40I	0.05	T40I	0.2
	H37N	0.03	S31L	0.2
Delta	T40I	21	T40I	70
	E39*	0.3	E33V	0.4
	H37Y	0.1	F13L	0.1
	A15S	0.1	F19L	0.1
	L32F	0.1	H42Y	0.0
Lambda			C41F	1
			L18V	0.2
			S5A	0.2
			S5L	0.2
			S31P	0.2
Mu	K21N	1	T40I	1.0
	E106*	0.4	E106*	0.4
	T229I	0.2	K21N	0.1
			T229I	0.1
			I27T	0.1

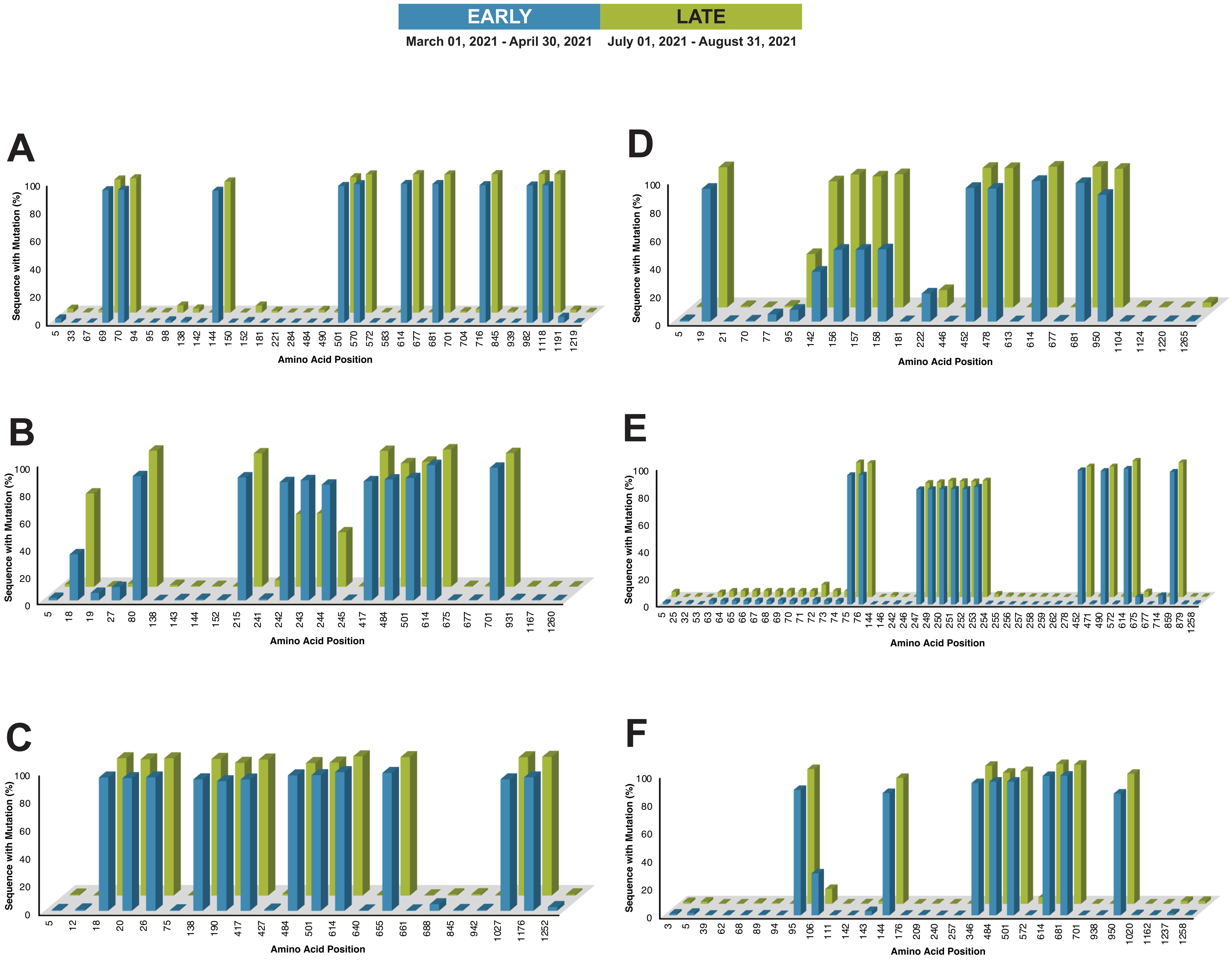
Supplementary Figure 4 Frequency of ORF7b Mutations. Point mutations with frequencies greater than or equal to 0.2% and less than or equal to 100% were plotted based on the location of each mutation across the ORF6 amino acid sequences of the Beta (A), Delta (B), and Mu (C) SARS-CoV-2 variants. The most frequent mutations were selected for each variant (D). Data shown are both from the early (🟦, March 01, 2021 – April 30, 2021) and late (🟩, July 01, 2021 – August 31, 2021) time points.



G

Variant	Mutation Frequency (%)			
	Early		Late	
Alpha	Y73C	100	Y73C	100
	Q27*	100	Q27*	100
	R52I	100	R52I	100
	K68*	40	K68*	43
	C61F	3	C61F	7
Beta	I121L	30	I121L	50
	E106*	4	P93H	3
	F120V	2	W45L	3
	P38S	0.8	F120V	1.0
	K68E	0.8	Q27*	0.9
Gamma	E92K	100	E92K	100
	R52I	0.4	G8*	0.6
	S67F	0.3	A65V	0.4
	A65V	0.2	S67F	0.4
	Y73C	0.1	Q72H	0.3
Delta	F120L	5	A65S	2
	D119V	3	L60F	2
	G8V	2	F86-	0.5
	I121L	0.3	T87S	0.5
	E106*	0.2	S67F	0.5
Lambda	I121L	3	P36L	5
	P36L	2	I121L	4
	L242F	1	H17Y	2
	F120V	0.4	G66D	2
	A65V	0.3	F120V	1
Mu	P38S	100	T11K	100
	T11K	100	P38S	100
	S67F	90	S67F	90
	L85F	1	R52I	4
	I9T	0.2	E106Q	1

Supplementary Figure 5 Frequency of ORF8 Mutations. Point mutations with frequencies greater than or equal to 0.2% and less than or equal to 100% were plotted based on the location of each mutation across the ORF8 amino acid sequences of the Alpha (A), Beta (B), Gamma (C), Delta (D), Lambda (E), and Mu (F) SARS-CoV-2 variants. The most frequent mutations were selected for each variant (G). Data shown are both from the early (🟦, March 01, 2021 – April 30, 2021) and late (🟩, July 01, 2021 – August 31, 2021) time points.



G

Variant	Mutation Frequency (%)			
	Early		Late	
Alpha	D614G	100	S982A	100
	A570D	100	D614G	100
	P681H	100	D1118H	100
	T716I	100	A570D	100
	S982A	100	T716I	100
Beta	D614G	100	D614G	100
	A701V	100	D80A	100
	D80A	90	K417N	100
	D215G	90	A701V	100
	N501Y	90	D215G	100
Gamma	D614G	100	D614G	100
	H655Y	100	V1176F	100
	N501Y	100	H655Y	100
	E484K	100	T1027I	100
	V1176F	100	L18F	100
Delta	D614G	100	D614G	100
	P681R	100	P681R	100
	L452R	90	T19R	100
	T478K	90	L452R	100
	T19R	90	T478K	100
Lambda	D614G	100	D614G	100
	L452Q	100	T859N	100
	F490S	100	L452Q	100
	T859N	100	F490S	100
	T76I	90	G75V	90
Mu	P681H	100	D614G	100
	D614G	100	P681H	100
	E484K	100	R346K	100
	N501Y	100	T95I	100
	R346K	90	N501Y	90

Supplementary Figure 6 Frequency of Spike Protein Mutations. Point mutations with frequencies greater than or equal to 0.2% and less than or equal to 100% were plotted based on the location of each mutation across the Spike protein amino acid sequences of the Alpha (A), Beta (B), Gamma (C), Delta (D), Lambda (E), and Mu (F) SARS-CoV-2 variants. The most frequent mutations were selected for each variant (G). Data shown are both from the early (🟦, March 01, 2021 – April 30, 2021) and late (🟩, July 01, 2021 – August 31, 2021) time points.

A

BA.1			
Protein	**Mutation**	**Protein**	**Mutation**
Spike	**A67V**	N	**P13L**
Spike	**H69-**	N	**E31-**
Spike	**V70-**	N	**R32-**
Spike	**T95I**	N	**S33-**
Spike	**G142-**	N	**R203K**
Spike	**V143-**	N	**G204R**
Spike	**Y144-**	ORF1a	**K856R**
Spike	**Y145D**	ORF1a	**S2083-**
Spike	**N211-**	ORF1a	**L2084I**
Spike	**L212I**	ORF1a	**A2710T**
Spike	**G339D**	ORF1a	**T3255I**
Spike	**S371L**	ORF1a	**P3395H**
Spike	**S373P**	ORF1a	**L3674-**
Spike	**S375F**	ORF1a	**S3675-**
Spike	**K417N**	ORF1a	**G3676-**
Spike	**N440K**	ORF1a	**I3758V**
Spike	**G446S**	ORF1b	**P314L**
Spike	**S477N**	ORF1b	**I1566V**
Spike	**T478K**	ORF9b	**P10S**
Spike	**E484A**	ORF9b	**E27-**
Spike	**Q493R**	ORF9b	**N28-**
Spike	**G496S**	ORF9b	**A29-**
Spike	**Q498R**	E	**T9I**
Spike	**N501Y**	M	**D3G**
Spike	**Y505H**	M	**Q19E**
Spike	**T547K**	M	**A63T**
Spike	**G614G**		
Spike	**H655Y**		
Spike	**N679K**		
Spike	**P681H**		
Spike	**N764K**		
Spike	**D796Y**		
Spike	**N856K**		
Spike	**Q954H**		
Spike	**N969K**		
Spike	**L981F**		

B

BA.2			
Protein	**Mutation**	**Protein**	**Mutation**
Spike	**T19II**	N	**P13L**
Spike	**L24-**	N	**E31-**
Spike	**P25-**	N	**R32-**
Spike	**P26-**	N	**S33-**
Spike	**G142D**	N	**R203K**
Spike	**V213G**	N	**G204R**
Spike	**G339D**	N	**S413R**
Spike	**S371F**	ORF1a	**S135R**
Spike	**S373P**	ORF1a	**T842I**
Spike	**S375F**	ORF1a	**G1307S**
Spike	**T376A**	ORF1a	**L3027F**
Spike	**D405N**	ORF1a	**T3090I**
Spike	**R408S**	ORF1a	**L3201F**
Spike	**K417N**	ORF1a	**T3255I**
Spike	**N440K**	ORF1a	**P3395H**
Spike	**S477N**	ORF1a	**S3675-**
Spike	**T478K**	ORF1a	**G3676-**
Spike	**E484A**	ORF1a	**F3677-**
Spike	**Q493R**	ORF1b	**P314L**
Spike	**Q498R**	ORF1b	**R1315C**
Spike	**N501Y**	ORF1b	**I1566V**
Spike	**Y505H**	ORF1b	**T2163I**
Spike	**D614G**	ORF3a	**T233I**
Spike	**H655Y**	ORF6	**D61L**
Spike	**N679K**	E	**T9I**
Spike	**P681H**	M	**Q19E**
Spike	**N764K**	M	**A63T**
Spike	**D796Y**		
Spike	**Q954H**		
Spike	**N969K**		

Supplementary Figure 7 Defining mutations of the Omicron variant. As of writing, the Omicron variant has rapidly taken over the Delta variant all over the world. This variant has already split into two sublineages. The tables show the defining mutations for sublineage BA.1 (A) and BA.2 (B), which were derived from the CoVariants database.

A

ORF3a	
Mutation	**Effect on Secondary Structure**
T223I	No structural damage

B

ORF6	
Mutation	**Effect on Secondary Structure**
D61L	-

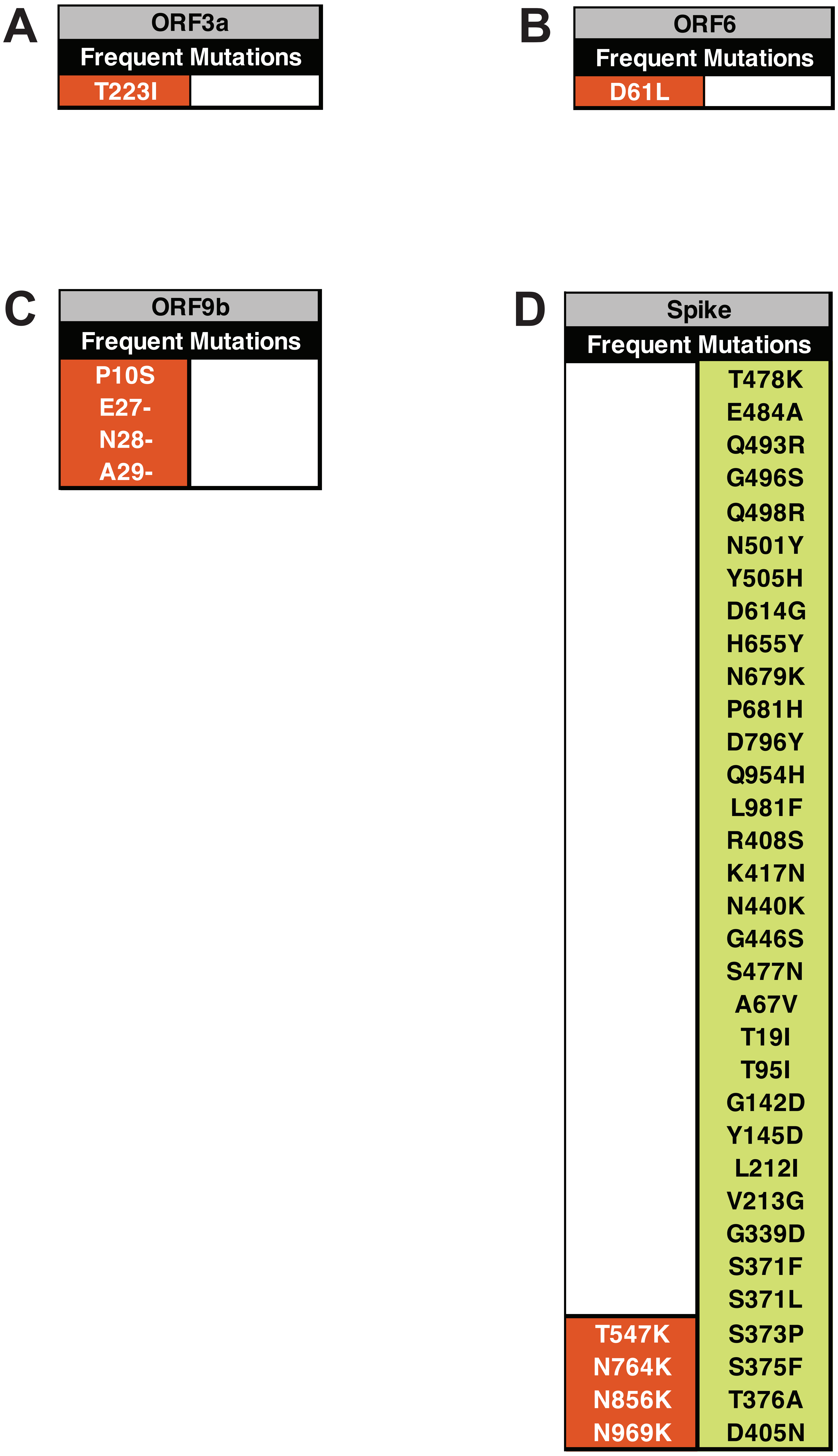
C

ORF9b	
Mutation	**Effect on Secondary Structure**
P10S	-
E27-	-
N28-	-
A29-	-

D

Spike			
Mutation	**Effect on Secondary Structure**	**Mutation**	**Effect on Secondary Structure**
T19I	No structural damage	**K417N**	No structural damage
L24-	-	**N440K**	No structural damage
P25-	-	**G446S**	No structural damage
P26-	-	**S477N**	No structural damage
A67V	No structural damage	**T478K**	No structural damage
T95I	Buried H-bond breakage	**E484A**	No structural damage
G142D	Buried charge introduced, Buried glycine replaced	**Q493R**	No structural damage
G142-	-	**G496S**	No structural damage
V143-	-	**Q498R**	No structural damage
Y144-	-	**N501Y**	No structural damage
Y145D	No structural damage	**Y505H**	No structural damage
N211-	-	**T547K**	No structural damage
L212I	No structural damage	**D614G**	No structural damage
V213G	No structural damage	**H655Y**	No structural damage
G339D	No structural damage	**N679K**	No structural damage
S371F	No structural damage	**P681H**	No structural damage
S371L	No structural damage	**N764K**	No structural damage
S373P	Disallowed phi/psi	**D796Y**	No structural damage
S375F	No structural damage	**N856K**	No structural damage
T376A	No structural damage	**Q954H**	No structural damage
D405N	No structural damage	**N969K**	No structural damage
R408S	No structural damage	**L981F**	No structural damage

Supplementary Figure 8 Effects of Accessory and Spike Protein Mutations of the Omicron variant on Secondary Structure. The structural consequences of each point mutation of the corresponding accessory and Spike protein were determined using the Missense3D software. Each table represents an accessory or Spike protein and the selected point mutations for the Omicron variant.



A

ORF3a	
Frequent Mutations	
T223I	

B

ORF6	
Frequent Mutations	
D61L	

C

ORF9b	
Frequent Mutations	
P10S	
E27-	
N28-	
A29-	

D

Spike	
Frequent Mutations	
	T478K
	E484A
	Q493R
	G496S
	Q498R
	N501Y
	Y505H
	D614G
	H655Y
	N679K
	P681H
	D796Y
	Q954H
	L981F
	R408S
	K417N
	N440K
	G446S
	S477N
	A67V
	T19I
	T95I
	G142D
	Y145D
	L212I
	V213G
	G339D
	S371F
	S371L
T547K	S373P
N764K	S375F
N856K	T376A
N969K	D405N

Supplementary Figure 9 Effects of Accessory and Spike Protein Mutations of the Omicron variant on Biologic Function. The functional consequences of each point mutation in the corresponding accessory and Spike protein were determined using the PROVEAN software. Each table (A-D) represents an accessory protein or Spike protein and the selected point mutations for the Omicron variant. The effect on biologic function was classified into loss-of-function (🟥) and neutral (🟩) mutations.