

# Exhibit 579

Unnatural evolutionary processes of SARS-CoV-2 variants and possibility of deliberate natural selection

<https://zenodo.org/records/8361577>

# Unnatural evolutionary processes of SARS-CoV-2 variants and possibility of deliberate natural selection

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15 **Keywords:** SARS-CoV-2, evolution, Omicron BA.1, Omicron BA.2, SARS-CoV-2 Omicron, Puerto Rico.

## 16 **Abstract**

17 Over the past three years, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has repeatedly  
18 caused pandemics, generating various mutated variants ranging from Alpha to Omicron. In this study, we  
19 aimed to clarify the evolutionary processes leading to the formation of SARS-CoV-2 Omicron variants,  
20 focusing on Omicron variants with many amino acid mutations in the spike protein among SARS-CoV-2  
21 isolates. To determine the order of mutations leading to the formation of the SARS-CoV-2 Omicron variants,  
22 we compared the sequences of 129 Omicron BA.1-related, 141 BA.1.1-related, and 122 BA.2-related isolates,  
23 and attempted to clarify the evolutionary processes of SARS-CoV-2 Omicron variants, including the order of  
24 mutations leading to their formation and the occurrence of homologous recombination. As a result, we  
25 concluded that the formation of a part of Omicron isolates BA.1, BA.1.1, and BA.2 was not the product of  
26 genome evolution, as is commonly observed in nature, such as the accumulation of mutations and homologous  
27 recombinations. Furthermore, the study of 35 recombinant isolates of Omicron variants BA.1 and BA.2  
28 confirmed that Omicron variants were already present in 2020. The analysis showed that Omicron variants  
29 were formed by an entirely new mechanism that cannot be explained by previous biology, and knowing how  
30 the SARS-CoV-2 variants were formed prompts a reconsideration of the SARS-CoV-2 pandemic.

## 31 **1 Introduction**

32  
33 COVID-19, the coronavirus disease 2019, caused by severe acute respiratory syndrome coronavirus 2 (SARS-  
34 CoV-2), was first reported in December 2019 in Wuhan, China (1). This emerging infectious disease was  
35 unprecedentedly fast, spreading worldwide, leading the World Health Organization (WHO) to declare a global  
36 pandemic of COVID-19 on March 11, 2020 (<https://www.who.int/>). SARS-CoV-2, belonging to  
37 betacoronavirus subgroup B, has a single-stranded positive-sense RNA genome that codes for ten genes,  
38 ultimately producing 26 proteins according to an annotation of NCBI Reference Sequence: NC\_045512.2. Its  
39 genome size varies from 29.8 to 29.9 kb. It consists of four structural proteins: spike (S), envelope (E),  
40 membrane (M), and nucleocapsid (N) proteins (2, 3). In the structural proteins, the S protein as the surface  
41 glycoprotein is the largest protein, being approximately 180 kDa, and consisting of two subunits, S1 and S2. It  
42 mediates membrane fusion and ultimately facilitates virus entry. The receptor-binding domain (RBD) (amino

43 acid residues 319-541) of the S1 subunit interacts with angiotensin - converting enzyme 2 (ACE2), binding to  
44 its peptidase domain (4, 5).

45

46 Over the three years from 2019 to 2022, SARS-CoV-2 was re-accelerated by new variants that emerged over  
47 several months in various geographical regions and disseminated throughout the world, to induce the pandemic  
48 repeatedly.

49

50 In the early stage of the first pandemic, the most impactful mutation of SARS-CoV-2 was the non-synonymous  
51 mutation D614G in the S protein. This mutation, which was not present in the ancestral lineage that caused the  
52 Wuhan outbreak, quickly became dominant worldwide (6). Soon after, the variant of concern, B.1.1.7 : 20I  
53 (Alpha, V1), the lineage B.1.1.7 (clade 501.YV1), or Alpha, characterized by 17 unique mutations containing  
54 ten amino acid differences in the S protein, was first detected in southeastern England in late September 2020  
55 (7) and expanded rapidly across the United Kingdom to become predominant during early 2021, spreading to  
56 most European countries with similar success. By November 2021, local transmission of this lineage had been  
57 reported in 175 countries (8). Shortly after, the emergence of variant strains of SARS-CoV-2 Alpha, variants  
58 B.1.351 : 20H (Beta, V2), was identified in October 2020, which was first detected in the Eastern Cape province  
59 of South Africa from specimens collected in early August. This Beta variant spread within South Africa and was  
60 considered to have displaced the other SARS-CoV-2 lineages circulating there (9). Then, the variant P.1: 20J  
61 (Gamma, V3) was identified in Brazil in December 2020, thought to have evolved in Brazil. Health officials in  
62 Japan first reported it publicly on January 10, 2021, after identifying the Gamma variant in four Brazilian  
63 travelers at Haneda Airport in Tokyo, Japan (10).

64 At about the same time, the Delta variant (Pango lineage designation B.1.617.2), which was first detected in  
65 India in February 2021, and the Mu variant, also known as lineage B.1.621 first detected in Colombia in January  
66 2021, were reported (11, 12). While the lambda variant (Pango lineage designation C.37), was detected in Peru  
67 in August 2020, but designated in June 15, 2021 by WHO (13, 14).

68

69 Almost one year later, regarding these emergences of variants of concern, Omicron (phylogenetic assignment  
70 of named global outbreak (Pango) lineage designation B.1.1.529; BA.1, Nextstrain clade 21K) was a variant of  
71 SARS-CoV-2 first reported to WHO by the Network for Genomics Surveillance in South Africa on November  
72 24, 2021 (15, 16) with more than 50 amino acids changes when compared with the first reported strain Wuhan-  
73 Hu-H1 (NCBI Reference Sequence: NC\_045512.2.), and 39 of these amino acids difference were observed in  
74 the S protein. This variant was first detected in Botswana and became the predominant circulating variant  
75 worldwide (17).

76 In the United States, the San Francisco Department of Public Health confirmed that a case of COVID-19 among  
77 individuals in California was caused by Omicron variant BA.1, carried by a traveler who returned from South  
78 Africa on November 22, 2021 (<https://www.cdc.gov/media/releases/2021/s1201-Omicron-variant.html>). Then,  
79 the first Omicron sub-lineage BA.1 expanded rapidly and replaced the Delta variant (18).

80 Less than two weeks later, the Omicron variant BA.1, the new Omicron variant, BA.2 lineage, showing 31  
81 amino acids changes in the S protein when compared with the Wuhan-Hu-H1, was initially identified in  
82 Denmark on December 5, 2021 (19). On February 22, 2022, WHO mentioned the Omicron sublineage BA.2  
83 (<https://www.who.int/news/item/22-02-2022-statement-on-Omicron-sublineage-ba.2>), whereby the Omicron  
84 variant of concern was currently the dominant variant circulating globally, replacing the Delta variant (Pango  
85 lineage designation B.1.617.2) ([https://www.who.int/docs/default-source/coronaviruse/2022-01-07-global-technical-brief-and-priority-action-on-Omicron---corr2.pdf?sfvrsn=918b09d\\_20](https://www.who.int/docs/default-source/coronaviruse/2022-01-07-global-technical-brief-and-priority-action-on-Omicron---corr2.pdf?sfvrsn=918b09d_20)), accounting for nearly all  
86 sequences reported to GISAID. Then, as of March 16, 2023, WHO stated that the Omicron variants accounted  
87 for over 98% of the publicly available viral sequences after February 2022 (<https://www.who.int/news/item/16-03-2023-statement-on-the-update-of-who-s-working-definitions-and-tracking-system-for-SARS-CoV-2-variants-of-concern-and-variants-of-interest>).

91 Omicron variants BA.1 and BA.2 were suggested to have expanded and diverged around October to December  
92 2021, respectively. These mutants were estimated to have diverged from a common ancestor around February  
93 to March 2021 (20). Since Omicron variants BA.1 and BA.2 share a common 14-amino acid mutation in the S

94 protein, the common ancestor of Omicron variants BA.1 and BA.2 may have already acquired the 14-amino  
95 acid mutation in the S protein region around February to March 2021; however, no common ancestral strain has  
96 been found in the international databases, and the strains may have acquired their mutations through different  
97 pathways.

98 In this study, we attempted to clarify the evolutionary processes of the Omicron variant, which has two-times  
99 more amino acid mutations in the S protein than other variants, by examining the rank order of introduced amino  
100 acid mutations in the S protein.

## 101 2 Results

102 Each variant is considered to have arisen through an independent evolutionary pathway from isolates with the  
103 D614G mutation in the S protein. Concerning the genetic variation in the S protein of these variants, most of the  
104 mutations were non-synonymous (Fig. 1). There were no synonymous mutations in the Alpha, Beta, Gamma,  
105 Delta, or Mu variants, but only one each in the Lambda and Omicron variants. Among these variants, the  
106 Omicron variant (BA.1 lineage), which shows the greatest accumulation of mutations in the S protein, is  
107 primarily non-synonymous in the S protein and has only one synonymous mutation, at c25000u. The  
108 synonymous/non-synonymous ratio is abnormal, considering how human coronaviruses have mutated (See  
109 Supplemental Figure 1).

110 At first, we considered the existence of the isolate of SARS-CoV-2, whose amino acid sequence in the S protein  
111 contains the Omicron-BA.1-type mutation subsets, but one mutation position was not mutated and comprised  
112 the original Wuhan-type amino acid sequence. We designated these isolates as BA.1-01. Each amino acid  
113 sequence of the S protein region was named BA.1-0.1\_S: amino acids of the Omicron-BA.1 type (Oaa) and  
114 Wuhan type (Waa) and its position number (XXX) (Ex., BA.1-0.1\_S:OaaXXXWaa), as described in Methods.  
115 Then, the putative isolates bearing BA.1-0.1\_S:OaaXXXWaa were searched for using the BLAST program  
116 based on their amino acid sequences. In this search, we obtained the isolates whose identities showed 100%  
117 matches with the query amino acid sequence except for SARS-CoV-2\_human\_USA\_NY-  
118 PV55373\_2022(GenBank: ON246090.1), whose identity was 99.92%.

119 Surprisingly, we found that Omicron BA.1-0.1 isolates were detected at all mutation sites except N501Y (Fig.  
120 2A). In the BA.1 lineage of the Omicron variant, there are Omicron isolates (BA.1.1) with the R346K mutation  
121 seen in the Mu(m) variant (termed B.1.621), *i.e.*, BA.1\_S can be defined as BA.1.1\_S:K346R. We also  
122 performed a BLAST search for isolates with amino acid sequences of BA.1-0.1.1\_S:OaaXXXWaa, as described  
123 in Methods. As a result, Omicron BA.1.1-subset-0.1 isolates were detected at all mutation sites except S373P  
124 (Fig. 2B). Similar to the BA.1 lineage of the Omicron variant, in the BA.2 lineage of the Omicron variant,  
125 isolates of BA.2-0.1 were found at all mutant sites except T478K and P681H in the S protein (Supplemental  
126 Figure 2). The presence of these isolates refutes the establishment of Omicron strains through a continuous  
127 evolutionary process by accumulating mutations. So, we could not determine which mutation occurred first or  
128 last to form the Omicron variants. As shown in Fig. 2B, over half of the BA.1.1-0.1 isolates have the synonymous  
129 mutation c21595u detected in the S protein. However, this does not help explain the order in which the c21595u  
130 mutation arose. Curiously, in BA.1 strain isolates, this c21595u mutation was only detected in SARS-CoV-  
131 2\_human\_USA\_ID-CDC-LC0481844\_2022 (GenBank: OM409228.1) and SARS-CoV-2\_human\_USA\_MI-  
132 CDC-ASC210597972\_2022 (GenBank: OM396816.1). These isolates commonly lack the G339D mutation.  
133 This synonymous mutation is in a mutation-prone hotspot location. Still, if the same mutation occurred  
134 independently in different isolates, it is highly unnatural for the proportion of c21595u occurrences to be  
135 significantly biased in the Omicron variants BA.1.1-0.1.

136 It has been reported that two different variants were infected in a single cell while establishing various SARS-  
137 CoV-2 variants, causing homologous recombination in the process of viral RNA synthesis, resulting in multiple  
138 variants. On considering that homologous recombination caused the isolates shown in Fig. 2, some of the  
139 breakpoints at which strand changes occur by homologous recombination are too short (1nt, 2nt, 3nt, etc.) (Fig.  
140 3 and Supplemental Figure 3). Therefore, it is unreasonable to employ homologous recombination as the basis  
141 for establishing these isolates. Also, most of these isolates were found in the USA between 2021 and 2022;

142 however, considering that the most prevalent variant in the USA in August 2021 was the Delta variant, it is most  
143 unlikely that it did not cause mutations such as T478K and D614G, which were already prevalent. It is  
144 inconceivable that the oldest variants (such as T478K and D614G), which were no longer prevalent, were  
145 sufficiently present to cause superinfection and be involved in homologous recombination. Also, most of these  
146 isolates were isolated in the USA between 2021 and 2022. Still, given that the isolates primarily prevalent in the  
147 USA in August 2021 were Delta variants, it is unlikely that an older type of variant without the T478K or D614G  
148 mutation was present to cause superinfection and be involved in homologous recombination. This consideration  
149 is supported by the fact that all of these BA.1-0.1 and BA.1.1-0.1 isolates retained the sequence of the BA.1  
150 lineage in all regions except the S protein (Fig. 4). In addition, the fact that all of these BA.1-0.1 and BA.1.1-  
151 0.1 strains retained the sequence of Omicron strain BA.1 except for the S protein also makes it unreasonable to  
152 consider that these isolates arose by homologous recombination with an older type of mutant without the T478K  
153 or D614G mutations (Fig. 4).

154 Furthermore, some of the BA.1 and BA.1-0.1 isolates have mutation subsets (synonymous: u10135c, ORF3:  
155 L106F, N: D343G) up- and downstream of the S gene, and the u10135c and L106F (ORF3) mutations  
156 correspond perfectly. Therefore, it is considered that homologous recombination between the BA.1 variant and  
157 variants without these mutations did not occur during the mutants' formation processes (Fig. 4). The synonymous  
158 mutation c2470u occurred in BA.1.1 compared with BA.1, and this c2470u mutation was retained by most,  
159 excluding a few of the BA.1.1-0.1 isolates (SARS-CoV-2\_human\_USA\_IL-CDC-ASC210695497\_2022 :  
160 GenBank: OM770362.1; SARS-CoV-2\_human\_USA\_NY-CDC-LC0450936\_2021: GenBank: OM228453.1) .  
161 The synonymous mutation c2470u has also only been observed in a minimal number of BA.1-0.1 isolates  
162 ( SARS-CoV-2\_human\_USA\_OR-CDC-LC0470830\_2022: GenBank: OM367679.1; SARS-CoV-  
163 2\_human\_USA\_ID-CDC-LC0481844\_2022: GenBank: OM409228.1; SARS-CoV-2\_human\_USA\_MI-CDC-  
164 ASC210597972\_2022: GenBank:OM396816.1; SARS-CoV-2\_human\_USA\_WI-CDC-LC0494047\_2022:  
165 GenBank: OM500517.1) . These results suggest that the establishment of BA.1-0.1 and BA.1.1-0.1 isolates  
166 occurred independently. On the other hand, if reversion mutations caused each of these isolates with one amino  
167 acid different to the Wuhan-type, these isolates could be detected by examining an astronomical number of  
168 isolates. However, these virus strains were detected in the number of sequenced whole genomes (a limited  
169 number), rather than in astronomical numbers examined. The fact that most of these mutations occurred without  
170 synonymous mutations (Fig. 2) suggests that none of them arose as a result of trial-and-error random mutations  
171 in nature. Few synonymous mutations are detected in some BA.1-0.1, BA.1.1-0.1, and BA.2-0.1 isolates (Fig.  
172 2 and Supplemental Figure 2), as seen in other viruses (Supplemental Figure 1). The c25000u is the only  
173 synonymous mutation that did not occur until BA.1, BA.1.1, BA.2, BA.1-0.1 BA.1.1-0.1, and BA.2-0.1 isolates  
174 were formed and was not observed in previous variants such as alpha, beta, gamma, delta, etc. Nevertheless, it  
175 is curious to find the occurrence of mutants with synonymous mutations such as c22120u, c24034u, c23635u,  
176 c24448u, c21811u, a23884g, c22987u, c23609a, c23413u, c23896u, c22879u, u24097a, c23893u, c24442u,  
177 u24847c, c24382u, c22264u, c22879u, c22480u, u21976c, c22480u, g24577a, and u23101c in BA.1.1, BA.1-  
178 0.1, and BA.1.1-0.1 isolates (Fig. 2 Synonymous Others), and a22948g, c23635u, c21859u, c22945u, c23701u,  
179 c22987u, a24433g, c23347u, u24640c, a24619g, c24865u, a23989g, u23047c, u24346c, c21811u, c21952u,  
180 a22753u, c23635u, c24023u, c24382u, and c22572u in BA.2-0.1 isolates (Supplemental Figure 2 Synonymous  
181 Others) after the formation of mutants with these subsets.

182 Although the only bias in our isolates collection, was only selection of isolates whose identities showed 100%  
183 matches with the query amino acid sequence in the BLAST search, these curious tendencies were observed is  
184 very interesting.

185 If two different viral variants infect a single cell simultaneously in the process of establishing various SARS-  
186 CoV-2 variants, and if homologous recombination occurs during viral RNA synthesis between the Omicron  
187 variant BA.1 lineage and BA.2 lineage, it is expected that there are variants caused by homologous  
188 recombination between the BA.1 and BA.2 lineages.

189 Therefore, we also performed BLAST searches for isolates with mutant amino acid subsets of both the Omicron  
190 variant BA.1 and BA.2 strains. We detected recombinant isolates of Omicron BA.1 and BA.2 lineages.

191 Surprisingly, the recombinant Omicron BA.1 and BA.2 lineages, SARS-CoV-2/human/PRI/PR-PR-UPRRP-  
192 582/2020 (GenBank: ON928946.1), were already present in Puerto Rico in 2020. Omicron (B.1.1.529) is a  
193 variant of SARS-CoV-2 first reported to WHO by the Network for Genomics Surveillance in South Africa on  
194 November 24, 2021 (15, 16). It was first detected in Botswana and spread to become the predominant variant in  
195 circulation worldwide (17). Following the appearance of the original B.1.1.529 variant, several subvariants of  
196 Omicron emerged, including BA.1, BA.2, BA.3, BA.4, and BA.5 (21). After October 2022, two subvariants of  
197 BA.5 called BQ.1 and BQ.1.1 emerged.

198 The question then arose about why a recombinant strain, SARS-CoV-2/human/PRI/PR-UPRRP-582/2020  
199 (GenBank: ON928946.1), already existed in 2020. We searched for SARS-CoV-2 isolates prevalent in Puerto  
200 Rico using the keywords "PRI", "PR-UPRRP", and "2020" in the NCBI search; nucleotide  
201 (<https://www.ncbi.nlm.nih.gov/>). Consequently, we found 29 Omicron-associated sequences in the 127 hits  
202 obtained (Fig. 5B). These results suggest that the SARS-CoV-2 variants bearing the amino acid sequences of  
203 the S protein are identical to Omicron BA.1 and Omicron BA.2 variants, which were already prevalent in Puerto  
204 Rico in 2020, with 15 isolates showing the complete Omicron BA.1+ R346K\_mut-subset (BA1.1) , and 14  
205 isolates showing a synonymous substitution of c21595u. Four isolates had an amino acid sequence of the S  
206 protein that perfectly matched that of Omicron BA2 (BA.2\_S), four isolates were Omicron BA.2-0.1 (BA.2-  
207 S:K440N) and four isolates were Omicron BA.2-0.1 (BA.2-S:K440N)+F79S, BA.2-0.1 (BA.2-  
208 S:K440N)+Q613H, BA.2-0.1 (BA.2-S:K440N)+212S+D215E and BA.2-0.1 (BA.2-S:K440N)+212S (Fig. 5B).

209

### 210 3 Discussion

211 Several hypotheses have been proposed in which the original SARS-CoV-2 virus resulted from an accidental  
212 laboratory spill. With recent developments in biotechnology, many viruses, including coronaviruses, have been  
213 artificially synthesized and used in various experiments (22-24). The artificial generation of mutant viruses in  
214 laboratories and study of viral phenotypes by introducing mutations is called "reverse genetics", being a common  
215 technique in virology. It has been claimed that SARS-CoV-2 must have been artificially generated because of  
216 the unnatural presence of a codon (CGG) encoding a contiguous arginine at the furin cleavage site of SARS-  
217 CoV-2. This claim has been refuted based on the following facts: 1) there is no logical reason for a genetically  
218 engineered virus to utilize such a suboptimal furin cleavage site; 2) The only previous study on artificial insertion  
219 of furin cleavage sites at the S1/S2 boundary of the S protein of SARS-CoV-1 using the pseudotype virus  
220 experimental system utilized the optimal "RRSRR" sequence, which is different from the furin cleavage site's  
221 sequence present in SARS-CoV-2; 3) There is no evidence of previous studies at the Wuhan Institute of Virology  
222 that artificially inserted a complete furin cleavage site in coronaviruses; 4) Unnatural CGG codons adjacent to  
223 arginine at the furin cleavage site are rare in coronaviruses but are observed at a particular frequency in SARS-  
224 CoV-1, SARS-CoV-2, and other human coronaviruses. However, these are only declarations and are not logical.  
225 No one has offered an explanation why a naturally occurring virus would utilize a suboptimal furin cleavage  
226 site. There has been no mention of the technical possibility of inserting this furin cleavage site or a CGG codon  
227 artificially. The insertion of a polybasic furin cleavage site into the S protein makes it impossible to conclude  
228 whether SARS-CoV-2 is a naturally occurring or an artificial virus.

229 Despite the accumulation of many mutations in the S protein of Omicron mutants, most of the mutations are  
230 non-synonymous, with only one synonymous mutation of c25000u, which is highly unnatural, leading to the  
231 hypothesis that the Omicron mutants were artificially synthesized. The following results presented in this study  
232 may support the hypothesis that the Omicron variants were artificially synthesized rather than naturally  
233 occurring: 1) the presence of Omicron variant-associated isolates with one mutation site being the Wuhan-type;  
234 2) the almost complete absence of synonymous mutations in the S protein in these isolates; 3) the Omicron  
235 variant, which should have been first reported to WHO from South Africa on November 24, 2021, was already  
236 endemic in Puerto Rico in 2020, and there were isolates that were recombinants between Omicron strains BA1  
237 and BA2. In addition, the Omicron mutant-related isolates (BA.1-0.1, BA.1.1-0.1, and BA.2-0.1 isolates) with  
238 a Wuhan-type mutation at one of the mutation sites were established. Some had synonymous mutations after

239 establishing the Omicron mutant-related isolates (Fig. 2 and Supplemental Figure 2 Synonymous Others). It is  
240 reasonable to assume that viruses with the reversion amino acid mutations caused by non-synonymous mutations  
241 in the S protein were artificially synthesized and then acquired further synonymous mutations in the natural  
242 environment.

243 Assuming that artificially synthesized mutants with only non-synonymous mutations are spread globally, this  
244 would explain how mutants with non-synonymous mutations without previous synonymous mutations develop  
245 synonymous mutations under natural circumstances. Considering the current epidemic situation of SARS-CoV-  
246 2, it is unlikely that these viruses arose spontaneously. Based on our efforts to explain the formation of the  
247 SARS-CoV-2 isolates, they were formed by a completely new mechanism that cannot be explained by previous  
248 biology.

249 One idea, the hypothesis that these viruses were artificially generated, is more reasonable than proposing a novel  
250 mutation acquisition mechanism. However, is there any reason to artificially create these mutants, which are  
251 unlikely to have occurred naturally, given the current SARS-CoV-2 epidemic?

252 It is known that the pathogenicity, host specificity, cell tropism, and immunogenicity of numerous viruses can  
253 be altered by mutation of a single (or several) amino acid(s) of a viral protein on the viral envelope (envelope  
254 protein, HA protein, spike protein, etc.). A single-amino-acid substitution in the HA protein of the 2009  
255 pandemic A (H1N1) influenza viruses changed their replication and pathogenicity (25). In the Chikungunya  
256 virus, single amino acid changes in the E2 glycoprotein influenced glycosaminoglycan utilization for target-cell  
257 binding (26), and a single amino acid change in the E1 glycoprotein affected mosquito vector specificity and  
258 epidemic potential (27). In previous coronaviruses such as MERS-CoV and SARS-CoV-1, point mutations have  
259 been demonstrated to confer resistance to neutralizing antibodies (28-30).

260 Consider that the SARS-CoV-2 Omicron variant and its one-amino-acid reversion mutants were artificially and  
261 systematically generated. In that case, we should suspect that the other variants (Alpha to Delta) were also  
262 artificially generated viruses. Indeed, the lack of findings to date that many of the various mutations seen,  
263 especially in the early variants, are indeed associated with increased viral infection (31) supports the hypothesis  
264 that each variant was artificially synthesized to identify the amino acids of the S protein responsible for  
265 infectivity and pathogenicity. The possibility that the set of mutants was artificially generated to identify amino  
266 acids of the S protein involved in the infectivity and virulence is supported.

267 Reverse genetics experiments are an essential part of virus research, and it is inimical to virus research to  
268 consider that artificially synthesized viruses were deliberately spread throughout the world. However, now that  
269 reverse genetics has become common in virus research, we believe it is not scientific to discuss the mutation  
270 process of SARS-CoV-2 without excluding the possibility of artificially synthesized viruses.

271 Finally, we would like to add that while artificially synthesized viruses may have spread, we are not criticizing  
272 reverse genetics technology, as such technology has led to marked advances in virology. In addition, our analysis  
273 employed databases with a limited number of viral sequences, and we cannot deny the possibility that unreliable  
274 data may have been registered due to technical problems in sequencing or some other issues. Furthermore, we  
275 do not conclude that these viruses were artificially synthesized and distributed based on malicious intent. This  
276 study aims to point out that SARS-CoV-2 has undergone unthinkable mutations based on conventional  
277 coronavirus mutation mechanisms, and we hope that the possibility of artificial creation is included in serious  
278 discussions on the formation of SARS-CoV-2 variants.

279 Nonetheless, the analysis we have shown here concludes that the Omicron variants were formed by a completely  
280 new mechanism that cannot be explained by previous biology. The process of how SARS-CoV-2 mutations  
281 occurred should prompt a reconsideration of the SARS-CoV-2 pandemic. If the SARS-CoV-2 epidemic strain  
282 is an artificially mutated virus and if the corona disaster (corona hoopla) was a well-designed global experiment  
283 in human inoculation and a social experiment, then the design of this experiment and the nature of the virus used  
284 make it likely that this experiment (corona hoopla) is a preliminary experiment.

285 4 **Methods**

286 4. 1 **Data acquisition**

287 The SARS-CoV-2 RNA genome, genes, and proteins according to an annotation of SARS-CoV-2 Wuhan-Hu-  
288 H1 (COVID-19/Wuhan-Hu-1CHN/2019/First Isolate) NCBI Reference Sequence: NC\_045512.2 were used as  
289 references for the definition of mutations, and provided a basis for the numbering of nucleotides and amino acids  
290 of each protein. Genome data of SARS-CoV-2 isolates described in this article were obtained from the NCBI  
291 Nucleotide database (<https://www.ncbi.nlm.nih.gov/>) on 25/11/2022 to 17/03/2023.

292 4. 2 **Query of representative SARS-CoV-2 variant genome**

293 Amino acid sequences of spike protein of SARS-CoV-2 variants (Alpha:B.1.1.7, Beta:B.1.351, Gamma:P1,  
294 Delta:B.1.617.2.63, Lambda:C.37, Mu:B.1.621, Omicron:BA.1, BA.1.1, and BA.2) were obtained from an  
295 Internet site, Stanford Coronavirus Antiviral & Resistance Database (<https://covdb.stanford.edu/>) or Covariant  
296 (<https://covariants.org/>), and used as a query sequence for an NCBI protein BLAST search (blastp: protein-  
297 protein BLAST,  
298 [https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)). Then, the whole genome sequence of each isolate bearing the query spike sequence was derived from the  
299 BLAST search result, identified with query amino acid sequences of 100%. The nucleotide sequences of the  
300 detected SARS-CoV-2 genome were as follows: GenBank Accession No.: GenBank: MW423686.2;  
301 MW430966.1; MW430967.1; MW422256.1; MW598419.1; MW667552.1; MW667553.1; MW721502.1;  
302 MW721504.1; MW520923.1; MW642248.1; MW642249.1; MW642250.1; MZ182066.1; MZ155303.1;  
303 MZ155230.1; MZ170364.1; MZ179869.1; MW666666.1; MW696612.1; MW699217.1; MW644498.1;  
304 MZ727706.1; MZ620161.1; MZ637380.1; MZ637401.1; MZ780550.1; OL672836.1; OL677199.1;  
305 OP769083.1; OL764360.1; OL815447.1; ON762438.1; OL849989.1; OL897126.1; OL896945.1;  
306 OL896936.1; OL896931.1; OM233931.1; OM072551.1; OM072822.1; OM296922.1.  
307  
308

309 4. 3 **Query of SARS-CoV-2 Omicron variant genome bearing an S protein amino acid sequence in**  
310 **which one of the Omicron-type nucleotide mutation subsets was not mutated and retains the original**  
311 **SARS-CoV-2 Wuhan-Hu-H1-type arrangement.**

312 For each of the Omicron variants, BA.1, BA.1.1, and BA.2, the isolate series bearing an S protein amino acid  
313 sequence in which one of the Omicron-type nucleotide mutation subsets is not mutated and retains the original  
314 SARS-CoV-2 Wuhan-Hu-H1-type arrangement were named BA.1-0.1, BA.1.1-0.1 and BA.2-0.1, respectively.  
315 In addition, in this article, we named the amino acid sequences of spike protein of BA.1, BA.1.1, and BA.2 as  
316 BA.1\_S, BA.1.1\_S, and BA.2\_S, respectively, and then the series of amino acid sequences of spike protein of  
317 BA.1-0.1, BA.1.1-0.1, and BA.2-0.1 were named, respectively, as follows: Omicron BA.1-0.1 spike series  
318 (BA.1-0.1\_Ss) were named as BA.1\_S:V67A; BA.1\_S:69H\_70V; BA.1\_S:I95T;  
319 BA.1\_S:D142G\_143V\_144Y\_145Y; BA.1\_S:I211N\_212L; BA.1\_S:ΔEPE; BA.1\_S:D339G; BA.1\_S:L371S;  
320 BA.1\_S:P373S; BA.1\_S:F375S; BA.1\_S:N417K; BA.1\_S:K440N; BA.1\_S:S446G; BA.1\_S:N477S;  
321 BA.1\_S:K478T; BA.1\_S:A484E; BA.1\_S:R493Q; BA.1\_S:S496G; BA.1\_S:R498Q; BA.1\_S:Y501N;  
322 BA.1\_S:H505Y; BA.1\_S:K547T; BA.1\_S:G614D; BA.1\_S:Y655H; BA.1\_S:K679N; BA.1\_S:H681P;  
323 BA.1\_S:K764N; BA.1\_S:Y796D; BA.1\_S:K856N; BA.1\_S:H954Q; BA.1\_S:K969N and BA.1\_S:F981L /  
324 Omicron BA.1.1-0.1 spike series (BA.1.1-0.1\_Ss) were named as BA.1.1\_S:V67A; BA.1.1\_S:69H\_70V;  
325 BA.1.1\_S:I95T; BA.1.1\_S:D142G\_143V\_144Y\_145Y; BA.1.1\_S:I211N\_212L; BA.1.1\_S:ΔEPE;  
326 BA.1.1\_S:D339G; BA.1.1\_S:L371S; BA.1.1\_S:P373S; BA.1.1\_S:F375S; BA.1.1\_S:N417K;  
327 BA.1.1\_S:K440N; BA.1.1\_S:S446G; BA.1.1\_S:N477S; BA.1.1\_S:K478T; BA.1.1\_S:A484E;  
328 BA.1.1\_S:R493Q; BA.1.1\_S:S496G; BA.1.1\_S:R498Q; BA.1.1\_S:Y501N; BA.1.1\_S:H505Y;  
329 BA.1.1\_S:K547T; BA.1.1\_S:G614D; BA.1.1\_S:Y655H; BA.1.1\_S:K679N; BA.1.1\_S:H681P;  
330 BA.1.1\_S:K764N; BA.1.1\_S:Y796D; BA.1.1\_S:K856N; BA.1.1\_S:H954Q; BA.1.1\_S:K969N;  
331 BA.1.1\_S:F981L / Omicron BA.2-0.1 spike series (BA.2-0.1\_Ss) were named as BA.2\_S:I19T;

332 BA.2\_S:24L\_25P\_26P\_S27A; BA.2\_S:D142G; BA.2\_S:V213G; BA.2\_S:D339G; BA.2\_S:F371S;  
333 BA.2\_S:P373S; BA.2\_S:F375S; BA.2\_S:A376T; BA.2\_S:N405D; BA.2\_S:S408R; BA.2\_S:N417K;  
334 BA.2\_S:K440N; BA.2\_S:N477S; BA.2\_S:K478T; BA.2\_S:A484E; BA.2\_S:R493Q; BA.2\_S:R498Q;  
335 BA.2\_S:Y501N; BA.2\_S:H505Y; BA.2\_S:G614D; BA.2\_S:Y655H; BA.2\_S:K679N; BA.2\_S:H681P;  
336 BA.2\_S:K764N; BA.2\_S:Y796D; BA.2\_S:H954Q; BA.2\_S:K969N, and these constructs are shown in Figs. 2,  
337 4 and supplemental Figure 1. These amino acids sequences of spike protein of SARS-CoV-2 Omicron variants,  
338 BA.1-0.1, BA.1.1-0.1, and BA.2-0.1, were used as query sequences for an NCBI protein BLAST search. Then,  
339 the whole genome sequences of BA.1-0.1, BA.1.1-0.1, and BA.2-0.1 isolates bearing the query spike sequence  
340 were derived from the BLAST search results, identified with a query amino acid sequence of 100%. The  
341 nucleotide sequences of the detected SARS-CoV-2 genome were as follows: GenBank Accession No.:  
342 OM117411.1; OP797378.1; OM789835.1; OP928789.1; OP928803.1; OP929381.1; OP929396.1;  
343 OP929417.1; OM173977.1; OM518459.1; OM566981.1; ON019560.1; OM097227.1; OM096937.1;  
344 OM099902.1; OM117114.1; OM096685.1; OM354436.1; OM646886.1; OM472901.1; OM364511.1;  
345 OM131858.1; OL815451.1; OL896986.1; OL897116.1; OL897118.1; OL896964.1; OM367679.1;  
346 OM343778.1; OM409228.1; OM396816.1; OM134162.1; OM075886.1; OM123427.1; OM122677.1;  
347 OM121681.1; OM224850.1; ON246090.1; OM931599.1; OM864873.1; OM906519.1; OM906587.1;  
348 OM464776.1; OM015999.1; OM015958.1; OM015597.1; OM016329.1; OL898806.1; OL898861.1;  
349 OM016937.1; OM016186.1; OM036549.1; OM051171.1; OM126493.1; OM079115.1; OM099199.1;  
350 OM134489.1; OM098796.1; ON618279.1; ON618009.1; OM627701.1; OM356511.1; OM295457.1;  
351 ON700063.1; OM033824.1; ON368355.1; OM084700.1; ON208126.1; OM566593.1; OM945690.2;  
352 ON030252.1; ON019844.1; OM890075.1; ON020044.1; OM833954.1; ON376082.1; OM084604.1;  
353 OP795273.1; ON066609.1; OM352882.1; OM290510.1; OM369978.1; OM199342.1; OM011974.1;  
354 OM090274.1; OM043984.1; OM121683.1; OM121624.1; OM175506.1; OM360429.1; OM360221.1;  
355 OM358058.1; OM500517.1; OM135027.1; OM742858.1; OM521685.1; OM896558.1; ON694155.1;  
356 OM686755.1; OM484260.1; OM332056.1; OM156397.1; OM079447.1; OM134645.1; OM173298.1;  
357 OM123082.1; OM116023.1; OM652943.1; OL994299.1; OL994920.1; OM122027.1; OM121015.1;  
358 OL898817.1; OM527504.1; OM225320.1; OM931491.1; OM931575.1; OM931587.1; OM034409.1;  
359 OM036283.1; OL996129.1; OM035680.1; OM096996.1; ON065532.1; OM968098.1; OM816604.1;  
360 ON235452.1; ON334146.1; OP024162.1; OP209732.1; OM354578.1; OM099080.1; OM297301.1;  
361 OM297438.1; OM365368.1; OM449159.1; OM078863.1; OM096959.1; OM117155.1; OM133880.1;  
362 OM077358.1; OM372005.1; OM770362.1; OM897488.1; OM918459.1; OM918478.1; OL897115.1;  
363 OL897114.1; OL986779.1; OL986696.1; OL987046.1; ON831866.1; OM864099.1; OM863888.1;  
364 OP745925.1; ON831672.1; OM043643.1; OM176192.1; OM226685.1; OM343689.1; OM295527.1;  
365 OM894975.1; OM846676.1; OM822024.1; OM846844.1; OM906550.1; OM015933.1; OM016323.1;  
366 OM016331.1; OM035685.1; OM022498.1; OM156115.1; OM036875.1; OM099560.1; OM199246.1;  
367 OM067048.1; OM079299.1; OM099911.1; OM116588.1; OM097010.1; OM173300.1; OM805961.1;  
368 OM983266.1; OM983325.1; ON618010.1; OM084691.1; ON021265.1; ON039239.1; ON056981.1;  
369 ON144127.1; OM770527.1; OM156164.1; OM155119.1; OM199353.1; OM084630.1; OM084605.1;  
370 OM084621.1; OM359369.1; OM411574.1; OM584789.1; OM720486.1; OM429777.1; ON047062.1;  
371 ON065416.1; OP415118.1; OM954373.1; ON042406.1; OM335528.1; OM332335.1; OM353626.1;  
372 OM332813.1; OM197398.1; OM226919.1; OM228399.1; OM225859.1; OM271353.1; OM159454.1;  
373 OM224473.1; OM358278.1; OM361030.1; OM412141.1; OM496298.1; OM044048.1; OM121864.1;  
374 OM224477.1; OM227379.1; OM228453.1; OM622156.1; OM906370.1; OM970683.1; ON117965.1;  
375 OM198667.1; OM357800.1; OM357161.1; OM335230.1; OM261124.1; OM077578.1; OM497172.1;  
376 OM625194.1; OM907131.1; ON047464.1; OM911851.1; OM042846.1; OM155337.1; OM097339.1;  
377 OM116805.1; OM134409.1; OM686782.1; OM695863.1; OM724725.1; OM174366.1; OM822132.1;  
378 OM822106.1; OM822105.1; OM822485.1; OM135143.1; OM125829.1; OM098855.1; OM156118.1;  
379 OM155114.1; OM863926.1; OP359104.1; ON209298.1; ON232806.1; ON421981.1; ON811217.1;  
380 OM698275.1; ON052769.1; ON060006.1; ON060007.1; ON060009.1; OM843171.1; OM843276.1;  
381 OM843550.1; OM843316.1; OM843340.1; ON049267.1; ON450720.1; ON250163.1; ON256603.1;  
382 ON480422.1; OM888844.1; OM890089.1; ON009425.2; ON082904.1; OM901275.1; OM877094.2;  
383 OM877095.2; OM877096.2; OM877097.2; ON378542.1; ON389858.1; ON389889.1; ON390359.1;  
384 OM936703.1; ON352711.1; ON378000.1; ON177702.1; ON205494.1; ON378633.1; ON617689.1;

385 ON619375.1; OM567618.1; OM659585.1; OM770913.1; OM781641.1; OM533441.1; OM533458.1;  
386 OM570235.1; OM570252.1; OM570249.1; OM283361.1; OM283362.1; OM283320.1; OM283343.1;  
387 ON618014.1; ON618018.1; ON618019.1; ON618363.1; ON311615.1; ON383919.1; OP579158.1;  
388 OP054411.1; ON633107.1; ON414693.1; ON422887.1; OP364296.1; OP629673.1; ON363097.1;  
389 OP633561.1; ON458445.1; ON592247.1; ON549687.1; ON067040.1; ON321116.1; ON199452.1;  
390 ON200331.1; OM861064.1; OM969592.1; ON019120.1; ON221861.1; OM861619.1; ON091288.1;  
391 ON151370.1; ON233850.1; ON236456.1; ON296711.1; ON535443.1; ON624524.1; ON377450.1;  
392 ON397268.1; ON239032.1; ON373649.1; ON481637.1; ON701163.1; ON312677.1; ON349263.1;  
393 ON377487.1; ON377609.1; OM638574.1; OM911616.1; OM988767.1; ON019770.1; OM988769.1;  
394 ON468158.1; ON608924.1; ON604965.1; ON535763.1; ON378227.1; ON378238.1; ON728470.1.

#### 395 4. 4 Query of recombinant SARS-CoV-2 Omicron variant between BA.1 and BA.2 genome

396 Deduced recombinant spike protein between Omicron variants, BA.1 and BA.2 shown as BA.1\_S:T19I\_L24-  
397 \_P25-\_P26-\_A27S\_V213G\_S371F\_T376A\_D405N\_R408S was used as a query sequence for an NCBI  
398 protein BLAST search. The whole genome sequence of BA.1 and BA.2 recombinant-Omicron isolates showed  
399 some of the specific amino acid mutations observed in variant BA.1 and BA.2 in the S protein. The nucleotide  
400 sequences of the detected SARS-CoV-2 genome were as follows: GenBank Accession No.: OM360636.1;  
401 OM410816.1; OM429902.1; OM497964.1; OM565587.1; OM628132.1; ON549899.1; ON449685.1;  
402 ON176765.1; OM628094.1; ON099844.1; OM942313.1; ON395480.1; ON171854.1; ON172005.1;  
403 ON076710.1; ON928946.1; OM932113.1; OM942438.1; OM989528.1; OM499181.1; ON414822.1;  
404 OM878325.1; ON103067.1; ON103153.1; ON419036.1; ON928719.1; ON337887.1; ON420444.1;  
405 ON146520.1; OM469541.1; OM904085.1; ON254531.1; OM881098.1; ON373310.1.

#### 406 4. 5 Query of SARS-CoV-2 Omicron variant genome detected in Puerto Rico in 2020

407 Nucleotide sequences were searched using the keywords PRI PR-UPRRP 2020 (Search details: PRI[All  
408 Fields] AND (PR[All Fields] AND UPRRP[All Fields]) AND 2020[All Fields]). The search results were all  
409 SARS-CoV-2 isolate genome sequences. Among these sequences, SARS-CoV-2 Omicron variant-related  
410 sequences were picked up as follows: GenBank Accession No.: ON928761.1; ON928660.1; ON928794.1;  
411 ON928762.1; ON928848.1; ON928741.1; ON928918.1; ON928680.1; ON928975.1; ON928949.1;  
412 ON928673.1; ON928865.1; ON928716.1; ON928663.1; ON928779.1; ON928896.1; ON928946.1;  
413 ON928912.1; ON928704.1; ON928873.1; ON928813.1; ON928898.1; ON928765.1; ON928912.1;  
414 ON928883.1; ON928957.1; ON928880.1; ON928699.1; ON928724.1; ON928941.1.

415 Genomes were aligned using SnapGene software or GENETYX software. Numbering of nucleotides and  
416 amino acids of each protein was determined using Wuhan-Hu-1 (NC\_045512.2; COVID-19/Wuhan-Hu-  
417 1CHN/2019/First Isolate) as a reference strain for the definition of mutations.

418 5. References

- 419 1. F. Wu, S. Zhao, B. Yu, Y. M. Chen, W. Wang, Z. G. Song, Y. Hu, Z. W. Tao, J. H. Tian, Y. Y. Pei, M.  
420 L. Yuan, Y. L. Zhang, F. H. Dai, Y. Liu, Q. M. Wang, J. J. Zheng, L. Xu, E. C. Holmes and Y. Z. Zhang: A new  
421 coronavirus associated with human respiratory disease in China. *Nature*, 579(7798), 265-269 (2020)  
422 doi:10.1038/s41586-020-2008-3
- 423 2. R. A. Khailany, M. Safdar and M. Ozaslan: Genomic characterization of a novel SARS-CoV-2. *Gene*  
424 *Rep*, 19, 100682 (2020) doi:10.1016/j.genrep.2020.100682
- 425 3. Y. M. Bar-On, A. Flamholz, R. Phillips and R. Milo: SARS-CoV-2 (COVID-19) by the numbers. *Elife*,  
426 9 (2020) doi:10.7554/eLife.57309
- 427 4. J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang and X. Wang:  
428 Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, 581(7807),  
429 215-220 (2020) doi:10.1038/s41586-020-2180-5
- 430 5. D. Wrapp, N. Wang, K. S. Corbett, J. A. Goldsmith, C. L. Hsieh, O. Abiona, B. S. Graham and J. S.  
431 McLellan: Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, 367(6483), 1260-  
432 1263 (2020) doi:10.1126/science.abb2507
- 433 6. B. Korber, W. M. Fischer, S. Gnanakaran, H. Yoon, J. Theiler, W. Abfalterer, N. Hengartner, E. E.  
434 Giorgi, T. Bhattacharya, B. Foley, K. M. Hastie, M. D. Parker, D. G. Partridge, C. M. Evans, T. M. Freeman, T.  
435 I. de Silva, C.-G. G. Sheffield, C. McDanal, L. G. Perez, H. Tang, A. Moon-Walker, S. P. Whelan, C. C.  
436 LaBranche, E. O. Saphire and D. C. Montefiori: Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G  
437 Increases Infectivity of the COVID-19 Virus. *Cell*, 182(4), 812-827 e19 (2020) doi:10.1016/j.cell.2020.06.043
- 438 7. A. Carrasco-Montalvo, A. Bruno, D. de Mora, M. Olmedo, J. Garces, M. Paez, M. Regato-Arrata, M.  
439 Gonzalez, J. Romero, O. Mestanza, B. Freire-Paspuel, A. Gaviria, S. A. Orlando, M. A. Garcia-Bereguain and  
440 L. Patino: First Report of SARS-CoV-2 Lineage B.1.1.7 (Alpha Variant) in Ecuador, January 2021. *Infect Drug*  
441 *Resist*, 14, 5183-5188 (2021) doi:10.2147/IDR.S319439
- 442 8. S. Zarate, B. Taboada, J. E. Munoz-Medina, P. Isa, A. Sanchez-Flores, C. Boukadida, A. Herrera-  
443 Estrella, N. Selem Mojica, M. Rosales-Rivera, B. Gomez-Gil, A. G. Salas-Lais, C. E. Santacruz-Tinoco, H.  
444 Montoya-Fuentes, J. E. Alvarado-Yaah, G. M. Molina-Salinas, G. E. Espinoza-Ayala, J. A. Enciso-Moreno, R.  
445 M. Gutierrez-Rios, A. Loza, J. Moreno-Contreras, R. Garcia-Lopez, X. Rivera-Gutierrez, A. Comas-Garcia, R.  
446 M. Wong-Chew, M. E. Jimenez-Corona, R. M. Del Angel, J. A. Vazquez-Perez, M. Matias-Florentino, M.  
447 Perez-Garcia, S. Avila-Rios, H. G. Castelan-Sanchez, L. Delaye, L. P. Martinez-Castilla, M. Escalera-Zamudio,  
448 S. Lopez and C. F. Arias: The Alpha Variant (B.1.1.7) of SARS-CoV-2 Failed to Become Dominant in Mexico.  
449 *Microbiol Spectr*, 10(2), e0224021 (2022) doi:10.1128/spectrum.02240-21
- 450 9. H. Tegally, E. Wilkinson, M. Giovanetti, A. Iranzadeh, V. Fonseca, J. Giandhari, D. Doolabh, S. Pillay,  
451 E. J. San, N. Msomi, K. Mlisana, A. von Gottberg, S. Walaza, M. Allam, A. Ismail, T. Mohale, A. J. Glass, S.  
452 Engelbrecht, G. Van Zyl, W. Preiser, F. Petruccione, A. Sigal, D. Hardie, G. Marais, N.-y. Hsiao, S. Korsman,  
453 M.-A. Davies, L. Tyers, I. Mudau, D. York, C. Maslo, D. Goedhals, S. Abrahams, O. Laguda-Akingba, A.  
454 Alisoltani-Dehkordi, A. Godzik, C. K. Wibmer, B. T. Sewell, J. Lourenço, L. C. J. Alcantara, S. L. Kosakovsky  
455 Pond, S. Weaver, D. Martin, R. J. Lessells, J. N. Bhiman, C. Williamson and T. de Oliveira: Detection of a  
456 SARS-CoV-2 variant of concern in South Africa. *Nature*, 592(7854), 438-443 (2021) doi:10.1038/s41586-021-  
457 03402-9
- 458 10. T. Fujino, H. Nomoto, S. Kutsuna, M. Ujiie, T. Suzuki, R. Sato, T. Fujimoto, M. Kuroda, T. Wakita and  
459 N. Ohmagari: Novel SARS-CoV-2 Variant in Travelers from Brazil to Japan. *Emerg Infect Dis*, 27(4), 1243-5  
460 (2021) doi:10.3201/eid2704.210138
- 461 11. M. Joshi, M. Kumar, V. Srivastava, D. Kumar, D. S. Rathore, R. Pandit, D. W. Graham and C. G. Joshi:  
462 Genetic sequencing detected the SARS-CoV-2 delta variant in wastewater a month prior to the first COVID-19  
463 case in Ahmedabad (India). *Environ Pollut*, 310, 119757 (2022) doi:10.1016/j.envpol.2022.119757
- 464 12. K. Laiton-Donato, C. Franco-Munoz, D. A. Alvarez-Diaz, H. A. Ruiz-Moreno, J. A. Usme-Ciro, D. A.  
465 Prada, J. Reales-Gonzalez, S. Corchuelo, M. T. Herrera-Sepulveda, J. Naizaque, G. Santamaria, J. Rivera, P.  
466 Rojas, J. H. Ortiz, A. Cardona, D. Malo, F. Prieto-Alvarado, F. R. Gomez, M. Wiesner, M. L. O. Martinez and  
467 M. Mercado-Reyes: Characterization of the emerging B.1.621 variant of interest of SARS-CoV-2. *Infect Genet*  
468 *Evol*, 95, 105038 (2021) doi:10.1016/j.meegid.2021.105038

- 469 13. P. L. Wink, F. C. Z. Volpato, F. L. Monteiro, J. B. Willig, A. P. Zavascki, A. L. Barth and A. F. Martins:  
470 First identification of SARS-CoV-2 lambda (C.37) variant in Southern Brazil. *Infect Control Hosp Epidemiol*,  
471 43(12), 1996-1997 (2022) doi:10.1017/ice.2021.390
- 472 14. COVID-19 Weekly Epidemiological Update Edition 44, published 15 June 2021. *World Health*  
473 *Organization* (2021)
- 474 15. J. Quarleri, V. Galvan and M. V. Delpino: Omicron variant of the SARS-CoV-2: a quest to define the  
475 consequences of its high mutational load. *Geroscience*, 44(1), 53-56 (2022) doi:10.1007/s11357-021-00500-4
- 476 16. A. Gowrisankar, T. M. C. Priyanka and S. Banerjee: Omicron: a mysterious variant of concern. *Eur*  
477 *Phys J Plus*, 137(1), 100 (2022) doi:10.1140/epjp/s13360-021-02321-y
- 478 17. A. Vitiello, F. Ferrara, A. M. Auti, M. Di Domenico and M. Boccellino: Advances in the Omicron  
479 variant development. *J Intern Med*, 292(1), 81-90 (2022) doi:10.1111/joim.13478
- 480 18. Y. Fan, X. Li, L. Zhang, S. Wan, L. Zhang and F. Zhou: SARS-CoV-2 Omicron variant: recent progress  
481 and future perspectives. *Signal Transduct Target Ther*, 7(1), 141 (2022) doi:10.1038/s41392-022-00997-x
- 482 19. J. Fonager, M. Bennedbaek, P. Bager, J. Wohlfahrt, K. M. Ellegaard, A. C. Ingham, S. M. Edslev, M.  
483 Stegger, R. N. Sieber, R. Lassauniere, A. Fomsgaard, T. Lillebaek, C. W. Svarrer, F. T. Moller, C. H. Moller,  
484 R. Legarth, T. V. Sydenham, K. Steinke, S. J. Paulsen, J. A. S. Castruita, U. V. Schneider, C. H. Schouw, X. C.  
485 Nielsen, M. Overvad, R. T. Nielsen, R. L. Marvig, M. S. Pedersen, L. Nielsen, L. L. Nilsson, J. Bybjerg-  
486 Grauholm, I. H. Tarpgaard, T. S. Ebsen, J. U. H. Lam, V. Gunalan and M. Rasmussen: Molecular epidemiology  
487 of the SARS-CoV-2 variant Omicron BA.2 sub-lineage in Denmark, 29 November 2021 to 2 January 2022.  
488 *Euro Surveill*, 27(10) (2022) doi:10.2807/1560-7917.ES.2022.27.10.2200181
- 489 20. L. B. Shrestha, C. Foster, W. Rawlinson, N. Tedla and R. A. Bull: Evolution of the SARS-CoV-2  
490 omicron variants BA.1 to BA.5: Implications for immune escape and transmission. *Rev Med Virol*, 32(5), e2381  
491 (2022) doi:10.1002/rmv.2381
- 492 21. L. Yao, K. L. Zhu, X. L. Jiang, X. J. Wang, B. D. Zhan, H. X. Gao, X. Y. Geng, L. J. Duan, E. H. Dai  
493 and M. J. Ma: Omicron subvariants escape antibodies elicited by vaccination and BA.2.2 infection. *Lancet Infect*  
494 *Dis*, 22(8), 1116-1117 (2022) doi:10.1016/S1473-3099(22)00410-8
- 495 22. S. Torii, C. Ono, R. Suzuki, Y. Morioka, I. Anzai, Y. Fauzyah, Y. Maeda, W. Kamitani, T. Fukuhara  
496 and Y. Matsuura: Establishment of a reverse genetics system for SARS-CoV-2 using circular polymerase  
497 extension reaction. *Cell Rep*, 35(3), 109014 (2021) doi:10.1016/j.celrep.2021.109014
- 498 23. T. Y. Taha, I. P. Chen, J. M. Hayashi, T. Tabata, K. Walcott, G. R. Kimmerly, A. M. Syed, A. Ciling,  
499 R. K. Suryawanshi, H. S. Martin, B. H. Bach, C. L. Tsou, M. Montano, M. M. Khalid, B. K. Sreekumar, G.  
500 Renuka Kumar, S. Wyman, J. A. Doudna and M. Ott: Rapid assembly of SARS-CoV-2 genomes reveals  
501 attenuation of the Omicron BA.1 variant through NSP6. *Nat Commun*, 14(1), 2308 (2023) doi:10.1038/s41467-  
502 023-37787-0
- 503 24. W. Wang, X. Peng, Y. Jin, J. A. Pan and D. Guo: Reverse genetics systems for SARS-CoV-2. *J Med*  
504 *Virol*, 94(7), 3017-3031 (2022) doi:10.1002/jmv.27738
- 505 25. L. Xu, L. Bao, Q. Lv, W. Deng, Y. Ma, F. Li, L. Zhan, H. Zhu, C. Ma and C. Qin: A single-amino-acid  
506 substitution in the HA protein changes the replication and pathogenicity of the 2009 pandemic A (H1N1)  
507 influenza viruses in vitro and in vivo. *Virol J*, 7, 325 (2010) doi:10.1186/1743-422X-7-325
- 508 26. L. A. Silva, S. Khomandiak, A. W. Ashbrook, R. Weller, M. T. Heise, T. E. Morrison and T. S.  
509 Dermody: A single-amino-acid polymorphism in Chikungunya virus E2 glycoprotein influences  
510 glycosaminoglycan utilization. *J Virol*, 88(5), 2385-97 (2014) doi:10.1128/JVI.03116-13
- 511 27. K. A. Tsetsarkin, D. L. Vanlandingham, C. E. McGee and S. Higgs: A single mutation in chikungunya  
512 virus affects vector specificity and epidemic potential. *PLoS Pathog*, 3(12), e201 (2007)  
513 doi:10.1371/journal.ppat.0030201
- 514 28. X. C. Tang, S. S. Agnihothram, Y. Jiao, J. Stanhope, R. L. Graham, E. C. Peterson, Y. Avnir, A. S.  
515 Tallarico, J. Sheehan, Q. Zhu, R. S. Baric and W. A. Marasco: Identification of human neutralizing antibodies  
516 against MERS-CoV and their role in virus adaptive evolution. *Proc Natl Acad Sci U S A*, 111(19), E2018-26  
517 (2014) doi:10.1073/pnas.1402074111
- 518 29. J. Sui, D. R. Aird, A. Tamin, A. Murakami, M. Yan, A. Yammanuru, H. Jing, B. Kan, X. Liu, Q. Zhu,  
519 Q. A. Yuan, G. P. Adams, W. J. Bellini, J. Xu, L. J. Anderson and W. A. Marasco: Broadening of neutralization  
520 activity to directly block a dominant antibody-driven SARS-coronavirus evolution pathway. *PLoS Pathog*, 4(11),  
521 e1000197 (2008) doi:10.1371/journal.ppat.1000197

- 522 30. J. ter Meulen, E. N. van den Brink, L. L. Poon, W. E. Marissen, C. S. Leung, F. Cox, C. Y. Cheung, A.  
523 Q. Bakker, J. A. Bogaards, E. van Deventer, W. Preiser, H. W. Doerr, V. T. Chow, J. de Kruif, J. S. Peiris and  
524 J. Goudsmit: Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of  
525 escape mutants. *PLoS Med*, 3(7), e237 (2006) doi:10.1371/journal.pmed.0030237  
526 31. L. van Dorp, D. Richard, C. C. S. Tan, L. P. Shaw, M. Acman and F. Balloux: No evidence for increased  
527 transmissibility from recurrent mutations in SARS-CoV-2. *Nat Commun*, 11(1), 5986 (2020)  
528 doi:10.1038/s41467-020-19818-2

529

530 **Conflict of Interest**

531 The authors declare that the research was conducted in the absence of any commercial or financial  
532 relationships that could be construed as a potential conflict of interest.

533 **Figure legends**

534 **Fig. 1. Mutation subsets of S protein of SARS-CoV-2 variants.**

535 Sequences of S protein of SARS-CoV-2 variants (variants of concern, VOCs: Alpha:B.1.1.7, Beta:B.1.351,  
536 Gamma:P1, Delta:B.1.617.2.63, and Omicron:BA.1; BA.2 and variants of interest, VOIs: Lambda:C.37,  
537 Mu:B.1.621) are compared with the SARS-CoV-2 Wuhan-Hu-H1 reference sequence, and different amino acids  
538 (amino acid change, deletion, and insertion) and synonymous changes of nucleotides are shown. Non-  
539 synonymous changes are shown by amino acid changes (capital letters), and synonymous changes are shown by  
540 nucleotide changes (small letters). Amino acids different from Wuhan-Hu-H1 found in each variant: Alpha:  
541 B.1.1.7, Beta: B.1.351, Gamma: P1, Delta: B.1.617.2.63, Lambda: C.37, Mu: B.1.621, and Omicron: BA.1,  
542 BA.2 are highlighted with red, orange, green, yellow, aquamarine, lime, deep sky blue, and blue violet,  
543 respectively. Amino acid changes common to Omicron: BA.1 and BA.2 are shown in purple.

544

545 **Fig. 2. Mutations of S proteins of SARS-CoV-2 Omicron isolates.**

546 **(A)** Different amino acids and synonymous changes of nucleotides in S proteins of SARS-CoV-2 Omicron BA.1,  
547 BA.1.1 isolates, and BA.1-0.1s compared with SARS-CoV-2 Wuhan-Hu-H1. Nucleotide deletions and  
548 insertions were "deletion<sup>1</sup>" (deletion: nt 21,766-21,771), "deletion<sup>2</sup>" (deletion: nt 21,987-21,995), "deletion<sup>3</sup>"  
549 (deletion: nt 22,194-22,196), and "insertion<sup>4</sup>" (insertion between 22,206-22,196), and introduced amino acid  
550 changes were H69\_V70-, G142D\_V143\_Y144\_Y145-, N211I\_L212-, and 215ins.EPE, respectively. **(B)**  
551 Different amino acids and synonymous nucleotide changes in S proteins of SARS-CoV-2 Omicron BA.1.1-0.1  
552 isolates. Amino acids different from Wuhan-Hu-H1 found in each variant: Alpha: B.1.1.7, Beta: B.1.351,  
553 Gamma: P1, Delta: B.1.617.2.63, Mu: B.1.621, and Omicron: BA.1, BA.2 are highlighted with red, orange,  
554 green, yellow, lime, deep sky blue, and blue violet, respectively. Amino acid changes common to Omicron:BA.1  
555 and BA.2 are highlighted with purple.

556

557 **Fig. 3. Estimated homologous recombination breakpoints of the SARS-CoV-2 S gene of Omicron BA.1-**  
558 **0.1 or BA.1.1-0.1.**

559 Sequence alignment of amino acids and their coding nucleotides (nt.21,746-21,787; nt.22,658-22,702;  
560 nt.22,976-23,011, and nt.23,582-23,620) containing the mutation point of the SARS-CoV-2 S gene of the  
561 Omicron BA.1 variant compared with SARS-CoV-2 Wuhan-Hu-H1. Changed nucleotides and amino acids of  
562 Omicron BA.1 are shown in red letters. Estimated homologous recombination breakpoints of the SARS-CoV-  
563 2 S gene of Omicron BA.1-0.1 or BA.1.1-0.1 are shown by asterisks.

564

565 **Fig. 4. Representative mutations of SARS-CoV-2 Omicron isolates other than S protein.**

566 **(A)** Representative amino acids and synonymous changes of nucleotides of SARS-CoV-2 Omicron BA.1,  
567 BA.1.1 isolates, and BA.1-0.1 compared with SARS-CoV-2 Wuhan-Hu-H1. **(B)** Representative amino acids  
568 and synonymous changes of nucleotides of SARS-CoV-2 Omicron BA.1.1-0.1 compared with SARS-CoV-2  
569 Wuhan-Hu-H1. Amino acids different from Wuhan-Hu-H1 found in each variant: Alpha: B.1.1.7, Lambda: C.37,  
570 Mu: B.1.621, and Omicron: BA.1 are highlighted with red, aquamarine, deep sky blue, and blue violet,  
571 respectively.

572 Amino acid changes common to Omicron: BA.1 and BA.2 are shown in purple. Synonymous nucleotide  
573 changes: c2470u observed in Omicron:BA.1.1 mainly shown with blue. Synonymous and non-synonymous  
574 changes: u10135c of nsp5, L106F in ORF3, and D343G in N protein subset observed in ~40% of Omicron;

575 BA.1-0.1 are highlighted with emerald-green. Undetermined nucleotides or amino acids are shown as UD or X,  
576 respectively.

577

578 **Fig. 5. Mutations of S proteins of SARS-CoV-2 Omicron BA.1-BA.2 recombinant isolates and SARS-CoV-2 Omicron BA.1 and BA.2 isolates detected in Puerto Rico in 2020.**

580 (A) Different amino acids and synonymous change of nucleotides in S proteins of SARS-CoV-2 Omicron BA.1-  
581 BA.2 recombinant isolates compared with SARS-CoV-2 Wuhan-Hu-H1. Nucleotide deletions, "deletion<sup>5</sup>"  
582 (deletion: nt 21,633-21,641), introduced the amino acids changes L24- P25- P26- A27S. (B) Different amino  
583 acids and synonymous change of nucleotides in S proteins of SARS-CoV-2 Omicron BA.1.1 and Omicron  
584 BA.1-BA.2 recombinant isolate, highlighted with magenta (GenBank: ON928946.1), Omicron BA.2, and  
585 Omicron 2-0.1(K440N), detected in Puerto Rico in 2020. Amino acids different from Wuhan-Hu-H1 found in  
586 each variant: Alpha: B.1.1.7, Beta: B.1.351, Gamma: P1, Delta: B.1.617.2.63, Mu: B.1.621, and Omicron: BA.1,  
587 BA.2 are highlighted with red, orange, green, yellow, lime, deep sky blue, and blue-violet, respectively. Amino  
588 acid changes common to Omicron: BA.1 and BA.2 are highlighted with purple.

589

590 **Supplemental Figure 1**

591 **Human coronavirus 229E strains detected in Seattle, USA, in 2015 and 2019.**

592 Alignment of nucleotide (A) and amino acid (B) sequences of the S protein of Human coronavirus 229E strains,  
593 HCoV\_229E/Seattle/USA/SC3112/2015 (GenBank: KY983587.1), and CoV\_229E/Seattle/USA/SC0865/2019  
594 (GenBank: MN306046.1). The number of nucleotide substitutions observed between them was 32, amino acid  
595 substitutions numbered 18 between them, and the synonymous (14: 32-18)-non-synonymous mutation (18) rate  
596 between them was 1.285

597

598 **Supplemental Figure 2**

599 **Different amino acids and synonymous changes of nucleotides in S proteins of SARS-CoV-2 Omicron**  
600 **BA.2 isolates and BA.2-0.1s compared with SARS-CoV-2 Wuhan-Hu-H1.**

601 Nucleotide deletions, "deletion<sup>5</sup>" (deletion: nt 21,633-21,641), introduced the amino acid changes L24- P25-  
602 P26- A27S. Amino acids different from Wuhan-Hu-H1 found in each variant: Alpha: B.1.1.7, Beta: B.1.351,  
603 Gamma: P1, Delta: B.1.617.2.63, Mu: B.1.621, and Omicron: BA.1, BA.2 are highlighted with red, orange,  
604 green, yellow, lime, deep sky blue, and blue-violet, respectively. Amino acid changes common to Omicron:  
605 BA.1 and BA.2 are highlighted with purple.

606

607 **Supplemental Figure 3**

608 **Estimated homologous recombination breakpoints of the SARS-CoV-2 S gene of the Omicron BA.2-0.1**  
609 **or BA.1-BA.2 recombinant.**

610 (A) Sequence alignment of the amino acids and coding nucleotides (nt. 22,658-22,702) containing the mutation  
611 point of the SARS-CoV-2 S gene of Omicron BA.2 variants compared with SARS-CoV-2 Wuhan-Hu-H1. (B)  
612 Sequence alignment of the amino acids and coding nucleotides (nt. 22,178-22,222) containing the mutation point  
613 of the SARS-CoV-2 S gene of Omicron BA.1, BA.2 variant and BA.1-BA.2 recombinant isolate compared with  
614 SARS-CoV-2 Wuhan-Hu-H1. Changed nucleotides and amino acids of Omicron variants BA.1, BA.2, and

615 BA.1-BA.2 recombinant isolates compared with SARS-CoV-2 Wuhan-Hu-H1 sequences are shown in red  
616 letters. Asterisks show an estimated homologous recombination breakpoint of the SARS-CoV-2 S gene of  
617 Omicron BA.2-0.1.







Fig. 3

```

SARS-CoV-2_Wuhan-Hu-1      21,750  21,760  21,770  21,780
GUUACUUGGUUCCAUGCUAUACAUGUCUCUGGGACCAUUGGU
SARS-CoV-2_Omicron_BA.1    21,750  21,760  21,770  21,780
GUUACUUGGUUCCAUGUUAU-----CUCUGGGACCAUUGGU
break point                ****
V U W F H A I H V S G U N G
V U W F H V I - - S G U N G
A67V  H69- V70-

22,660  22,670  22,680  22,690  22,700
UCUGUCUAUAUAUUUCCGCAUCAUUUCCACUUUUAAGUGUUAU
UCUGUCUAUAUAUUUCCGCAUCAUUUUCAGUUUUUAAGUGUUAU
**** *****
S V L Y N S A S F S T F K C Y
S V L Y N L A P F F T F K C Y
S371L S373P S375F

22,980  22,990  23,000  23,010
AUCUAUCAGGCCGGUAGCACACCCUUGUAUUGGUGU
AUCUAUCAGGCCGGUAACAACCCUUGUAUUGGUGU
**
I Y Q A G S T P C N G V
I Y Q A G N K P C N G V
S477N  T478K

23,590  23,600  23,610  23,620
UAUCAGACUCAGACUAUAUUCUCCUGCGCGGGCAGCUAGU
UAUCAGACUCAGACUAAGUCUCAUCGGCGGGCAGCUAGU
****
Y Q T Q T N S P R R A R S
Y Q T Q T K S H R R A R S
N679K  P681H

```









# Supplemental Figure 2

Variant	Definition	GenBank	deletion										Non-synonymous																												Synonymous																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
			T19	L24	P25	P26	A275	G1420	V2136	G3900	S371F	S373F	S375F	S376F	D406N	R408S	K411N	N404K	S471N	T478K	E484A	Q493R	Q498R	N501Y	I505H	D614G	H659Y	N679K	F681H	N764K	D796Y	Q854H	N989K	Δ27792A*	Δ29000u	Others*																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																		
Ba.2	OMC_S2123 Human USM CA CDC-2019-01-2021	GenBank: OM231111.1	131	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000

Ba.2  
-01

# Supplemental Figure 3

## A

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22,660      22,670      22,680      22,690      22,700
SARS-CoV-2_Wuhan-Hu-1.    UUGUGUCUAUAUAUAUUUCGCAUGCAUUUUUCAGCUUUUAAGUGUUAU
SARS-CoV-2_Omicron_BA. 2  UUGUGUCUAUAUAUAUUUCGCACCGAUUUUUUCGGCUUUUAAGUGUUAU
Omicron_BA.2-0.1 break point.
                                     **** *
S   V   L   Y   N   S   A   S   F   S   T   F   K   C   Y
S   V   L   Y   N   F   A   P   F   F   A   F   K   C   Y
                                     S371F S373P S375F T376A

```

## B

```

22,180      22,190      22,200      22,210      22,220
SARS-CoV-2_Wuhan-Hu-1    AAGCAGACGGCCUAUAUAUUUAGUGCGUGA-----UCUCCUCAGGGUUU
SARS-CoV-2_Omicron_BA. 1  AAGCAGACGGCCUAUAU---AGUGCGUGAGCCCAGAAGAGAUCUCCUCAGGGUUU
SARS-CoV-2_Omicron_BA. 2  AAGCAGACGGCCUAUAUAUUUAGGGCGUGA-----UCUCCUCAGGGUUU
Omicron_BA.1-BA.2_rec     AAGCAGACGGCCUAUAU---AGGGCGUGAGCCCAGAAGAGAUCUCCUCAGGGUUU
Omicron_BA.1-BA.2_rec break point
                                     ** *****
K   H   U   P   I   N   L   V   R   -   -   D   L   P   Q   G   F
K   H   U   P   I   -   I   V   R   E   P   E   D   L   P   Q   G   F
K   H   U   P   I   N   L   G   R   -   -   D   L   P   Q   G   F
K   H   U   P   I   -   I   G   R   E   P   E   D   L   P   Q   G   F
N211- L212I V213G insertion

```