

## SUPPLEMENTARY INFORMATION FOR

### Sequential intrahost evolution and onward transmission of SARS-CoV-2 variants

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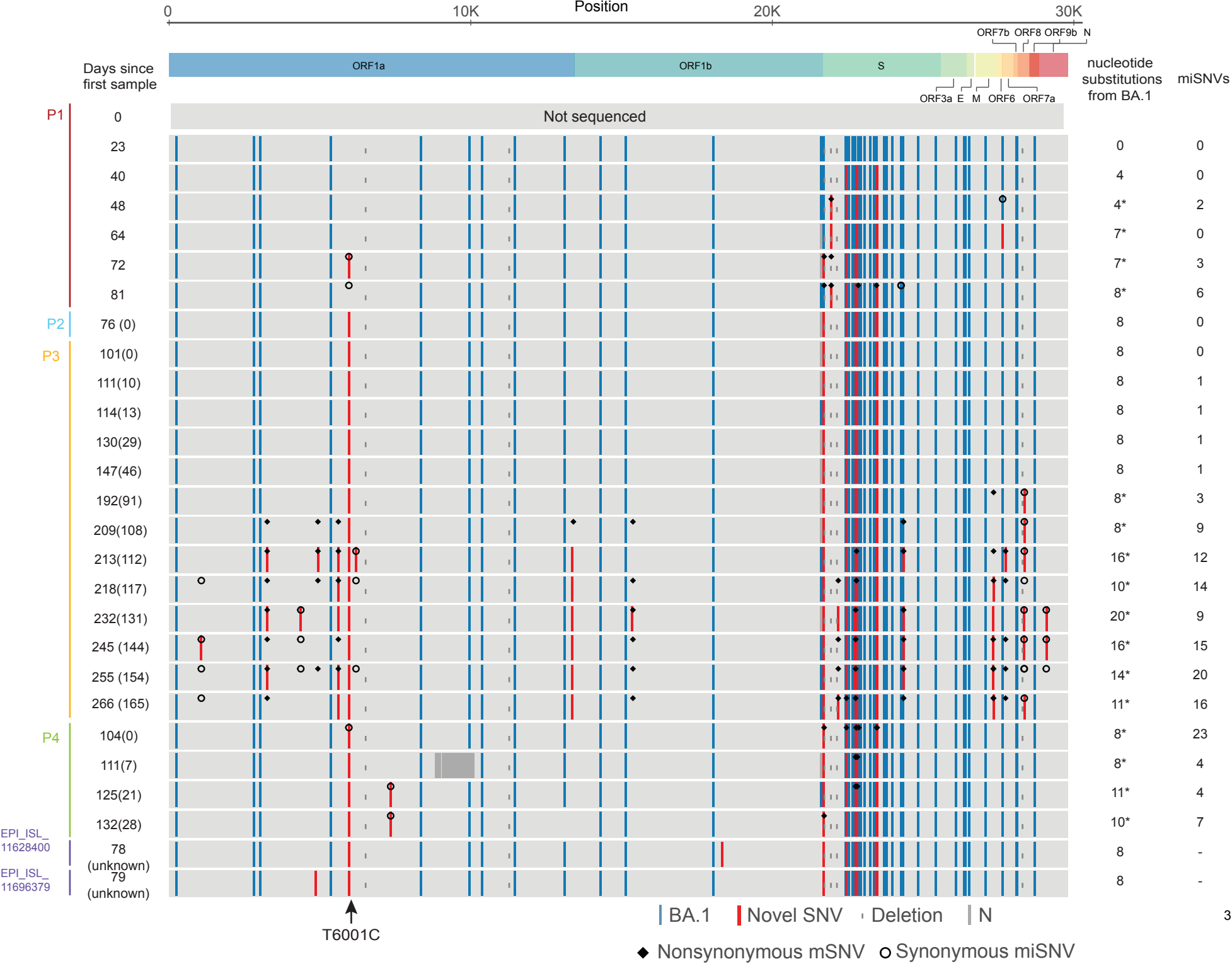
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#### **This PDF contains:**

Supplementary Figures 1 to 4.  
Supplementary Tables 1 to 3.

**Supplementary Figure 1: Overview of genome-wide SARS-CoV-2 mutations found in diagnostic nasal biospecimen.** The mutations present across the complete SARS-CoV-2 genome are shown. Nucleotide substitutions observed in sequential specimens obtained from patient 1 (P1) with prolonged infection with BA.1 and forward transmission cases (P2, P3 and P4). There is accumulation and fixation of new SNVs in the spike region in P1. The same constellation of mutations was subsequently detected in three documented transmission cases (P2, P3 and P4) and in two sequences from GISAID (EPI\_ISL\_11628400 and EPI\_ISL\_11696379). The only shared synonymous SNV outside of the spike (ORF1a:T6001C) is indicated with an arrow. The number of days since the first positive test in P1 is shown on the left, with the number of days after the first positive test for each patient between brackets. The BA.1 substitutions are shown in blue and novel substitutions are shown in red, relative to the reference genome sequence NC\_045512.2. “N” represents ambiguous bases with coverage below the base-calling threshold of 10X. The minimum required sequence read coverage for minor variant calling is 100X.

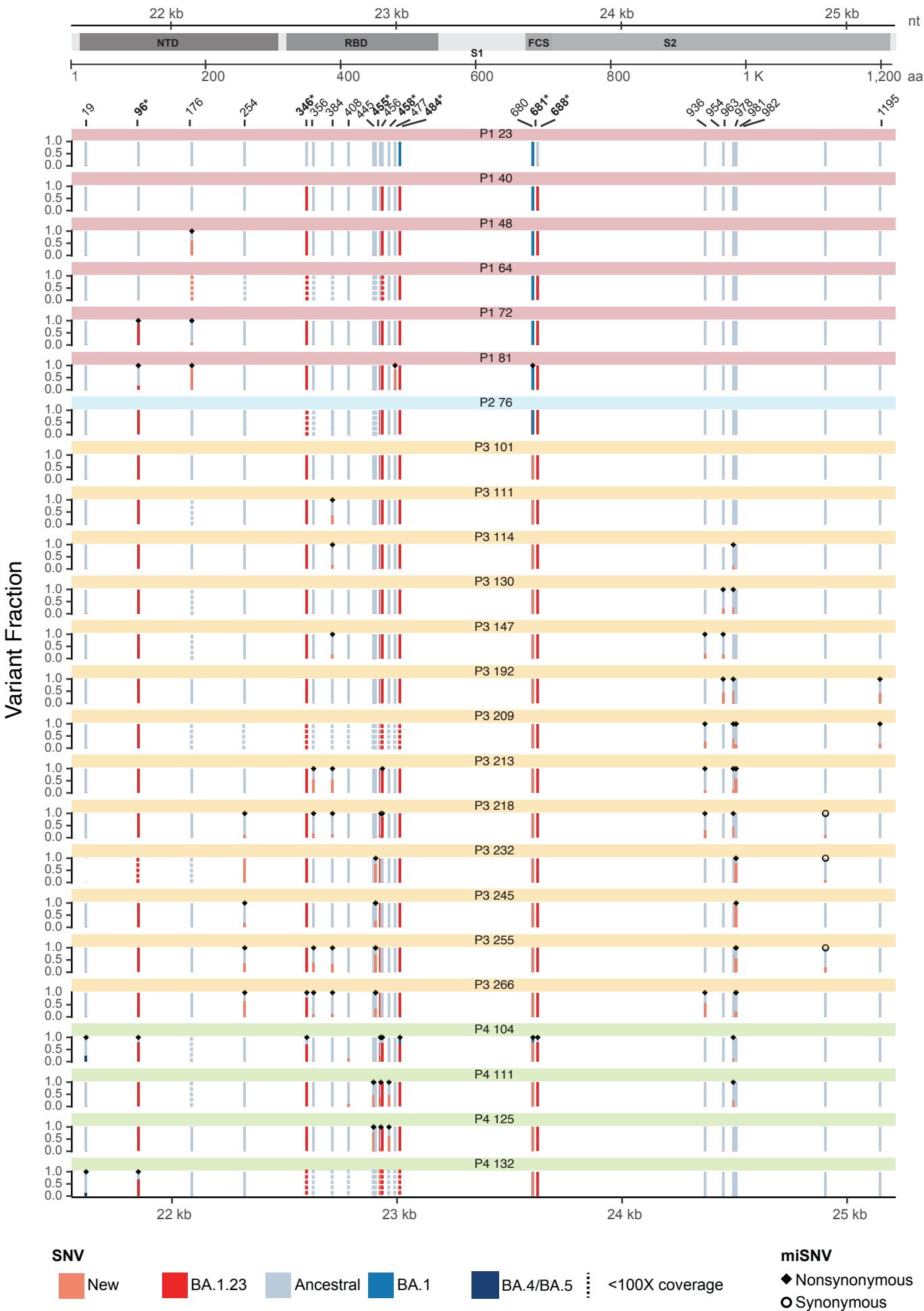
Supplementary Figure 1



**Supplementary Figure 2: Summary of SARS-CoV-2 mutations within the spike gene in minority viral populations.**

The distribution of minority intrahost single nucleotide variants (miSNVs) for positions that were fixed over the course of infection in the index and transmission cases are shown indicated with asterisks. Single nucleotide variants are colored by strain. The dotted bars indicate positions with coverage below the threshold of 100X coverage for calling miSNVs. The positions with minority variants present at frequencies  $> 0.1$  are indicated with diamonds for their respective samples.

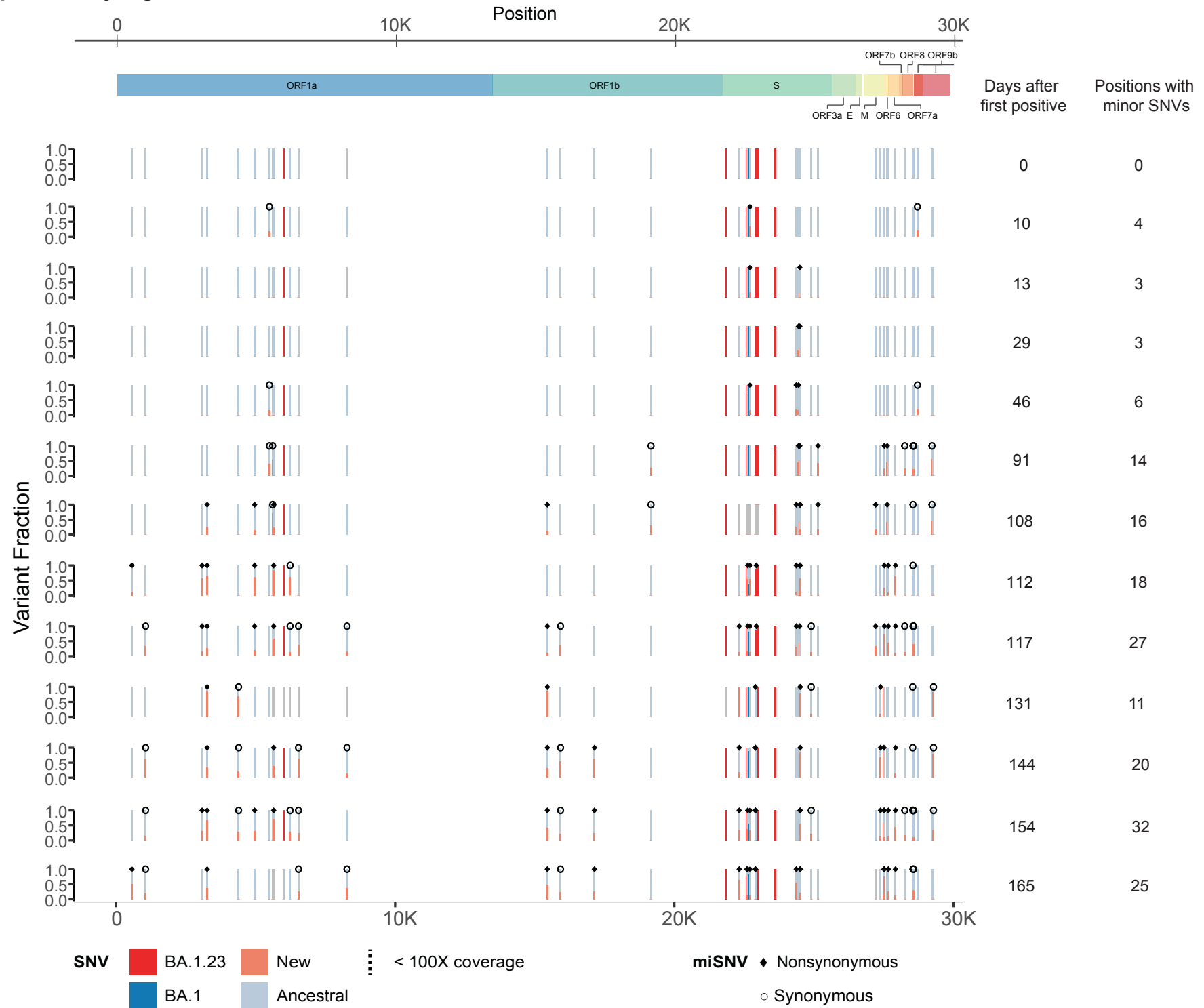
Supplementary Figure 2



### **Supplementary Figure 3: Summary of genome-wide SARS-CoV-2 mutations in minority viral populations for patient P3**

Similar to Supplementary Fig. 2 but showing the distribution of nucleotide variants below the consensus level observed in one or more specimens from P3. Single nucleotide variants (SNVs) are colored by strain. The dotted bars indicate positions with coverage below the threshold of 100X coverage for calling miSNVs. The positions with minority variants at frequencies  $> 0.1\%$  are indicated with triangles (nonsynonymous) or circles (synonymous) for their respective samples.

Supplementary Figure 3

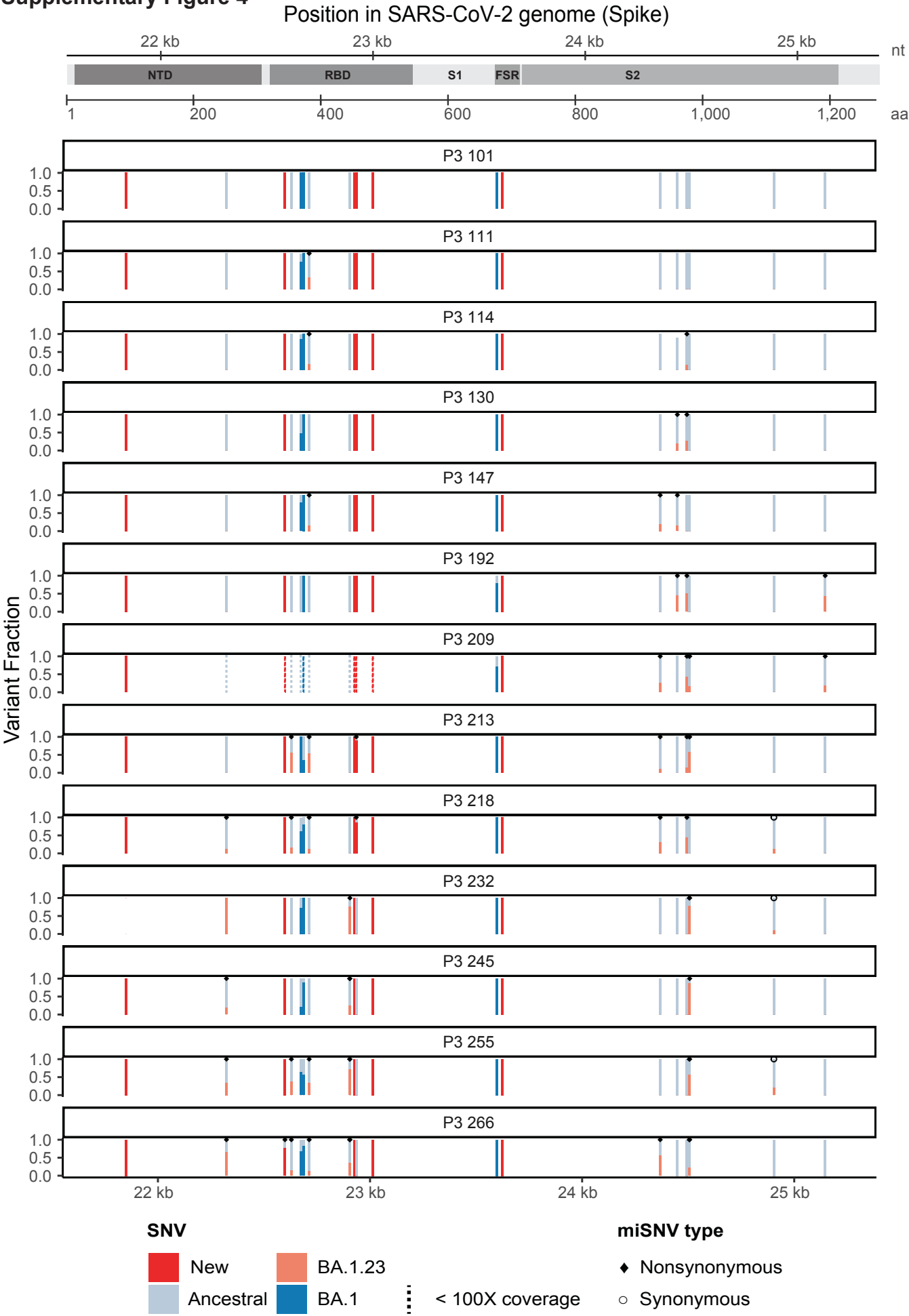


**Supplementary Figure 4: Summary of SARS-CoV-2 mutations within the Spike gene in minority viral populations for patient P3**

Similar to Supplementary Fig. 3 but showing minority mutations in the Spike gene region. Single nucleotide variants (SNVs) are colored by strain. The dotted bars indicate positions with coverage below the threshold of 100X coverage for calling minor SNVs (miSNVs). The positions with minority variants at frequencies  $> 0.1$  are indicated with triangles for their respective samples.



Supplementary Figure 4



**Supplementary Table 1.** Overview of the two serum panels used in this study

Panel 1 (Three mRNA vaccine doses)	Total # of participants selected		9	
	Age average (min-max)		59.7 (50-69 years)	
	Sex	Female	8	
		Male	1	
	Total # Sera		18	
	Days between sample collection and vaccine dose	Before	Average Range	19 0-38
		After	Average Range	31 14-59
Panel 2 (Omicron BA.1 Breakthroughs)	Total # of participants selected		11	
	Age average (min-max)		43.6 (18-69 years)	
	Sex	Female	7	
		Male	4	
	Total # Sera		22	
	Days between sample collection and infection	Before	Average Range	11 0-27
		After	Average Range	27 15-37

**Supplementary Table 2.** Detailed characteristics of the two serum panels used in this study

Participant ID	Age Bracket	Sex	Time points included in this study
BO-1	60-69	Male	Pre-Booster Post Booster
BO-2	50-59	Female	Pre-Booster Post Booster
BO-3	50-59	Female	Pre-Booster Post Booster
BO-4	60-69	Female	Pre-Booster Post Booster
BO-5	60-69	Female	Pre-Booster Post Booster
BO-6	50-59	Female	Pre-Booster Post Booster
BO-7	50-59	Female	Pre-Booster Post Booster
BO-8	60-69	Female	Pre-Booster Post Booster
BO-9	60-69	Female	Pre-Booster Post Booster
BABI-1	30-39	Female	Pre-infection, Boosted Post-infection, Boosted
BABI-2	40-49	Male	Pre-infection, Boosted Post-infection, Boosted
BABI-3	40-49	Male	Pre-infection, Unboosted Post-infection, Unboosted
BABI-4	40-49	Female	Pre-infection, Unboosted Post-infection, Unboosted
BABI-5	30-39	Female	Pre-infection, Unboosted Post-infection, Unboosted
BABI-6	30-39	Female	Pre-infection, Boosted Post-infection, Boosted
BABI-7	60-69	Female	Pre-infection, Boosted Post-infection, Boosted
BABI-8	18-29	Male	Pre-infection, Boosted Post-infection, Boosted
BABI-9	60-69	Male	Pre-infection, Boosted Post-infection, Boosted
BABI-10	40-49	Female	Pre-infection, Boosted Post-infection, Boosted
BABI-11	40-49	Female	Pre-infection, Boosted Post-infection, Boosted Pre-infection, Unboosted
Patient 2 described in this paper	60-69	Female	Pre-infection, Boosted Post-infection, Boosted Post-infection, Boosted