

Supplementary Appendix

Supplement to: Miller J, Hachmann NP, Collier AY, et al. Substantial neutralization escape by SARS-CoV-2 omicron variants BQ.1.1 and XBB.1. *N Engl J Med*. DOI: 10.1056/NEJMc2214314

This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

CONTENTS

Supplementary Methods	2
Table S1. Study Population	6
Figure S1. SARS-CoV2 Variant Dynamics	7
Figure S2. BF.7 and Related Forms	8
Figure S3. BQ.1/BQ.1.1 and Related Forms	9
Figure S4. BA.2.75/BA.2.75.2 and Related Forms	10
Figure S5. XBB/XBB.1 and Related Forms	11
Spike Sequences and Relationships to Pango Lineage Designations in GISAID	12

Supplementary Methods

Study Population

A specimen biorepository at Beth Israel Deaconess Medical Center (BIDMC) obtained samples from individuals who received SARS-CoV-2 vaccines as well as monovalent or bivalent mRNA boosters. The BIDMC institutional review board approved this study (2020P000361). All participants provided informed consent. This study included 16 uninfected individuals who received the original monovalent mRNA booster BNT162b2 in 2021. Participants were excluded from this group if they had a history of SARS-CoV-2 infection or a positive nucleocapsid (N) serology by electrochemiluminescence assays (ECLA), or if they received other COVID-19 vaccines or immunosuppressive medications. This study also included 15 individuals who received the original monovalent mRNA boosters BNT162b2 or mRNA-1273 predominantly in late summer or early fall 2022 and 18 individuals who received the bivalent mRNA boosters in September 2022 with various vaccination and infection history backgrounds. In these cohorts, 33% had documented SARS-CoV-2 Omicron infection during the BA.1/BA.2 or BA.5 surges, but we suspect that the majority of participants in these groups were likely infected due to the high prevalence of Omicron infection during 2022 as well as their immunologic profiles showing higher WA1/2020 and BA.5 NAb titers compared with the 2021 cohort. Participants were excluded if they utilized immunosuppressive medications.

Pseudovirus Neutralizing Antibody Assay

Neutralizing antibody (NAb) titers against SARS-CoV-2 variants utilized pseudoviruses expressing a luciferase reporter gene. In brief, the packaging construct psPAX2 (AIDS Resource

and Reagent Program), luciferase reporter plasmid pLenti-CMV Puro-Luc (Addgene), and Spike protein expressing pcDNA3.1-SARS-CoV-2 S Δ CT were co-transfected into HEK293T cells (ATCC CRL_3216) with lipofectamine 2000 (ThermoFisher Scientific). Pseudoviruses of SARS-CoV-2 variants were generated using the Spike protein from WA1/2020 (Wuhan/WIV04/2019, GISAID ID: EPI_ISL_402124), Omicron BA.5 (GISAID ID: EPI_ISL_14026118), BF.7 (GISAID ID: EPI_ISL_15379594), BA.2.75.2 (GISAID ID: EPI_ISL_14913457), and BQ.1.1 (GISAID ID: EPI_ISL_14752457, and XBB.1 (GISAID ID: EPI_ISL_15232105). The supernatants containing the pseudotype viruses were collected 48h after transfection, and pseudotype viruses were purified by filtration with 0.45- μ m filter. To determine NAb titers in human serum, HEK293T-hACE2 cells were seeded in 96-well tissue culture plates at a density of 2×10^4 cells per well overnight. Three-fold serial dilutions of heat-inactivated serum samples were prepared and mixed with 50 μ l of pseudovirus. The mixture was incubated at 37 °C for 1 h before adding to HEK293T-hACE2 cells. After 48 h, cells were lysed in Steady-Glo Luciferase Assay (Promega) according to the manufacturer's instructions. SARS-CoV-2 neutralization titers were defined as the sample dilution at which a 50% reduction (NT50) in relative light units was observed relative to the average of the virus control wells.

Variant Evolution and Dynamics

All variant sequence data are available through GISAID (<https://gisaid.org/>) using sequences and metadata sampled between August 1, 2022 and November 11, 2022. We gratefully acknowledge all data contributors, including the authors and originating laboratories responsible for obtaining the specimens, and the submitting laboratories for generating the genetic sequence and metadata and sharing via the GISAID Initiative, on which this research is based. Pango

lineage designations were adopted from the Pango designation lineage notes at cov-lineages, maintained by Cornelius Roemer and dated November 11, 2022 (https://github.com/cov-lineages/pango-designation/blob/master/lineage_notes.txt). The analysis was performed via tools provided at the Los Alamos COVID-19 viral genome analyses pipeline (<https://cov.lanl.gov/content/index>). In particular we used *Isotonic Regression*, *Embers*, and *Common forms of Spike with a given Pango Lineage designation*. For the isotonic regression analysis, all SARS-CoV-2 sequences sampled between August 1, 2022 and November 11, 2002 were considered, and isotonic regression was used to see if one form was increasing in sampling relative to another over time. The p-value is based on a one-sided resampling statistic to evaluate whether the test strain was changing in frequency relative to the background. If the sample was decreasing in frequency, it is noted, otherwise it is assumed to be increasing.

Data Availability

All sequences in this dataset are compared relative to hCoV-19/Wuhan/WIV04/2019 (WIV04), the official reference sequence employed by GISAID (EPI_ISL_402124; <https://gisaid.org/WIV04>). All genome sequences and associated metadata in this dataset are published in GISAID's EpiCoV database. To view the contributors of each individual sequence with details such as Accession Number, Virus name, Collection Date, Originating Lab, Submitting Lab, and List of Authors, doi links are provided below for the two relevant data sets.

GISAID data set 1 involves all the reference sequences referred to in this study (GISAID ID EPI_SET_221117zc). EPI_SET_221117zc is composed of 7 individual genome sequences that have a Spike gene that encodes the Spike proteins relevant to this study. The collection

dates range from February 27, 2022 to October 4, 2022. Data were collected in 5 countries and territories. doi: [10.55876/gis8.221117zc](https://doi.org/10.55876/gis8.221117zc)

GISAID data set 2 involves all the sequences used for the graphics in **Figs. S2-S5**, from Belgium, France, Singapore and India (GISAID ID EPI_SET_221114vn). EPI_SET_221114vn is composed of 64,113 individual genome sequences. The collection dates range from August 1, 2022 to November 8, 2022. Data were collected in 4 countries and territories. doi: [10.55876/gis8.221114vn](https://doi.org/10.55876/gis8.221114vn)

Table S1. Study Population

	Monovalent mRNA Booster (2021) N=16	Bivalent mRNA Booster (2022) N=18	Monovalent mRNA Booster (2022) N=15
Age (years), median (range)	34 (23-62)	42 (37-57)	50 (33-64)
Sex at birth , Female	15 (94)	11 (61)	13 (87)
Race			
White	12 (75)	16 (89)	10 (67)
Asian	2 (13)	2 (11)	2 (13)
Black	1 (6)	0	2 (13)
More than one race	1 (6)	0	0
Other	0	0	1 (7)*
Ethnicity			
Hispanic or Latino	2 (13)	0	1 (7)
Non-Hispanic	14 (88)	17 (94)	14 (93)
Unknown	0	1 (6)	0
Medical condition			
Hypertension	3 (19)	2 (11)	3 (20)
Diabetes	0	1 (6)	1 (7)
Pregnant	2 (13)	1 (6)	2 (13)
Asthma	0	2 (11)	0
Most recent COVID-19 vaccine booster			
Pfizer monovalent booster	16 (100)	N/A	5 (33)
Moderna monovalent booster	N/A	N/A	10 (67)
Pfizer bivalent booster	N/A	8 (44)	N/A
Moderna bivalent booster	N/A	10 (53)	N/A
Prior COVID-19 vaccine history			
BNT (4 doses)	0	2 (11)	0
BNT (3 doses)	0	8 (44)	2 (13)
BNT (2 doses)	16 (100)	0	0
BNT / BNT / 1273 (3 doses)	0	2 (11)	1 (7)
BNT / BNT / Ad26 (3 doses)	0	1 (6)	4 (27)
1273 (3 doses)	0	3 (17)	6 (40)
1273 / BNT (2 doses)	0	0	1 (7)
Ad26 / BNT / 1273 (3 doses)	0	1 (6)	0
Ad26 / 1273 (2 doses)	0	1 (6)	1 (7)
Days from last vaccine dose to sampling	20 (15-28)	21 (16-23)	32 (17-64)
Known COVID-19 positive test	0	6 (33)	5 (33)
Days from positive test to last vaccine	N/A	166 (149-257)**	143 (103-183)**

BNT=BNT162b2 (Pfizer); 1273=mRNA-1273 (Moderna); Ad26=Ad26.COV2.S (Janssen)

Data displayed as median (range or interquartile range, IQR) and n (%);PCR, polymerase chain reaction; pregnant designation reflects time of last vaccine dose. All individuals with known prior infection had mild disease.

*Self-reported race as Latina

**Reported for only those with known prior infection

Figure S1. SARS-CoV2 Variant Dynamics. BF.7 is the most sampled BA.5 sublineage among many that carry the R346T mutation on a BA.5 backbone. BQ.1/BQ.1.1 are BA.5 sublineages that include K444T and N460K on a BA.5 backbone, with BQ.1.1 also carrying the R346T mutation. The BQ.1/BQ.1.1 lineages have displayed a particularly rapidly increase in frequency, suggesting that K444T and N460K may confer further selective advantage in addition to R346T. BA.2.75 has been gradually increasing globally but with only a modest increase in the BA.2.75.2 sublineage regionally in Asia, although most BA.2.75 sublineages now contain the R346T mutation. XBB/XBB.1 represent recombinants between BA.2 and BA.2.75 sublineages and carry additional mutations relative to the BA.2.75 backbone, including R346T.

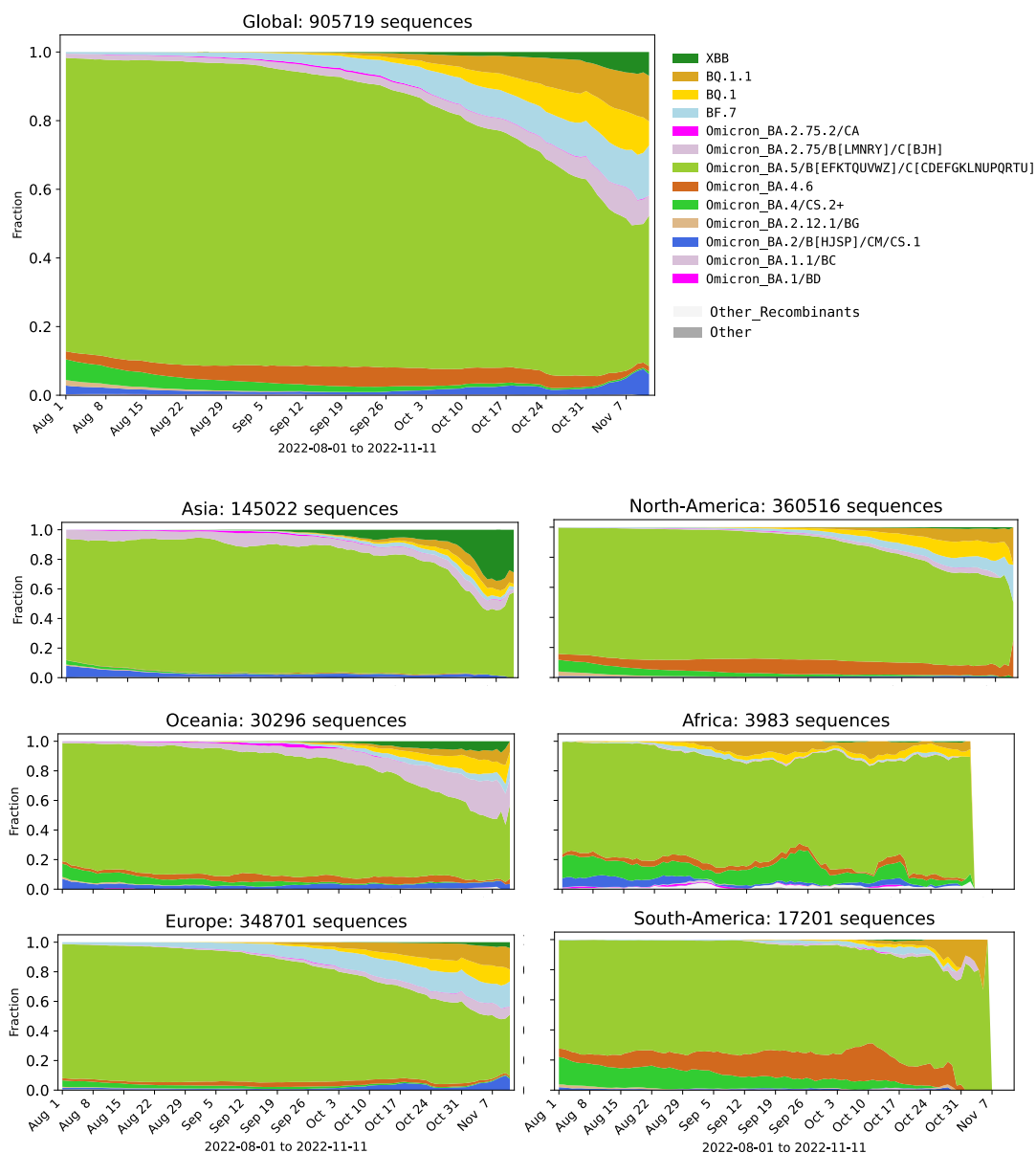
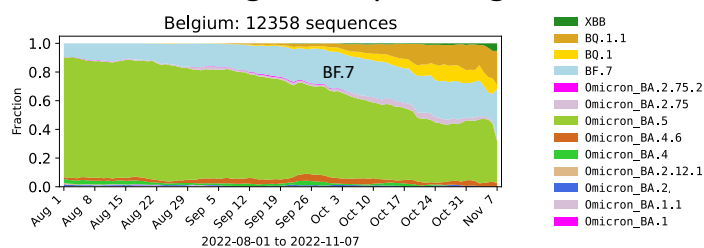
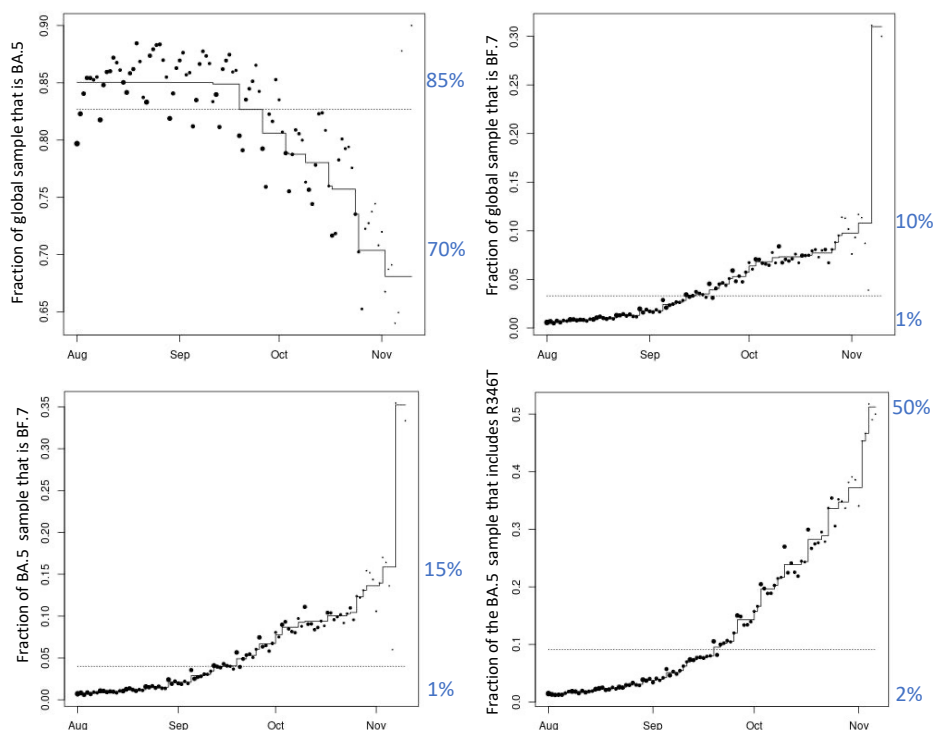


Figure S2. BF.7 and Related Forms. BF.7 is the most sampled BA.5 sublineage among many that carry the R346T mutation on a BA.5 backbone. **A**, Relative variant changes in sampling frequency in Belgium, a country where BA.7 was frequently sampled. BQ.1 lineages are currently increasing in frequency at a higher pace. **B**, Global trends showing that while BA.5 lineages have been declining in general (top left), the BF.7 sublineage of BA.5 has been slowly increasing globally (top right), and accounts for a rising fraction of the BA.5 sublineage globally (bottom left). This is part of a larger pattern within BA.5, where variants that carry the R346T mutation currently comprise ~50% of the BA.5 sample (bottom right).

A. BF.7, BA.5 sublineage example: Belgium



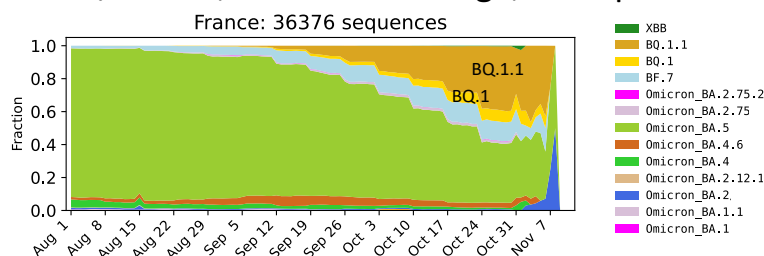
B. Global fractions



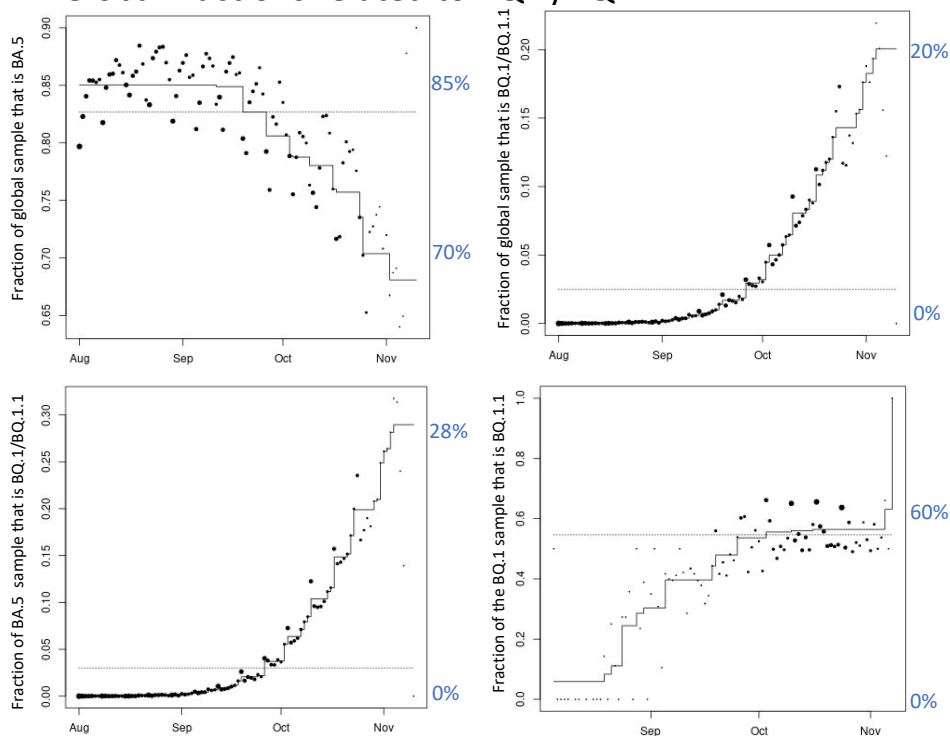
Test	Background	#Test	#Others	Total	T/Total (%)	p-val
BA.5	All	728357	152487	880844	82.69	0.00249 decreasing
BF.7	All	29173	851671	880844	3.31	0.00249
BF.7	BA.5	29173	699184	728357	4.01	0.00249
BA.5+R346T	BA.5	48151	481970	530121	9.08	0.00249

Figure S3. BQ.1/BQ.1.1 and Related Forms. BQ.1 and BQ.1.1 lineages are rapidly increasing in frequency, and combined they are approaching 20% of global viruses. **A**, Relative variant changes in sampling frequency in France, a country where both BQ.1 and BQ.1.1 are frequently sampled. BQ.1.1 lineages are increasing in frequency at a higher pace than BQ.1. **B**, Global trends showing that while BA.5 lineages have been declining in general (top left), the BQ.1+BQ.1.1 sublineages have been rapidly increasing globally (top right) and account for a rising fraction of the BA.5 sublineage globally (bottom left). BQ.1.1 is increasing rapidly within the BQ.1 sublineage, with a key difference being the addition of the R346T mutation in BQ.1.1, and it is currently about 60% of the global sample of BQ.1 lineages members (bottom right).

A. BQ.1/BQ.1.1, a BA.5 sublineage, example: France



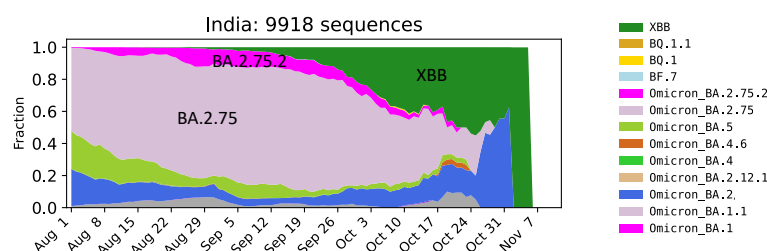
B. Global fractions related to BQ.1/BQ.1.1



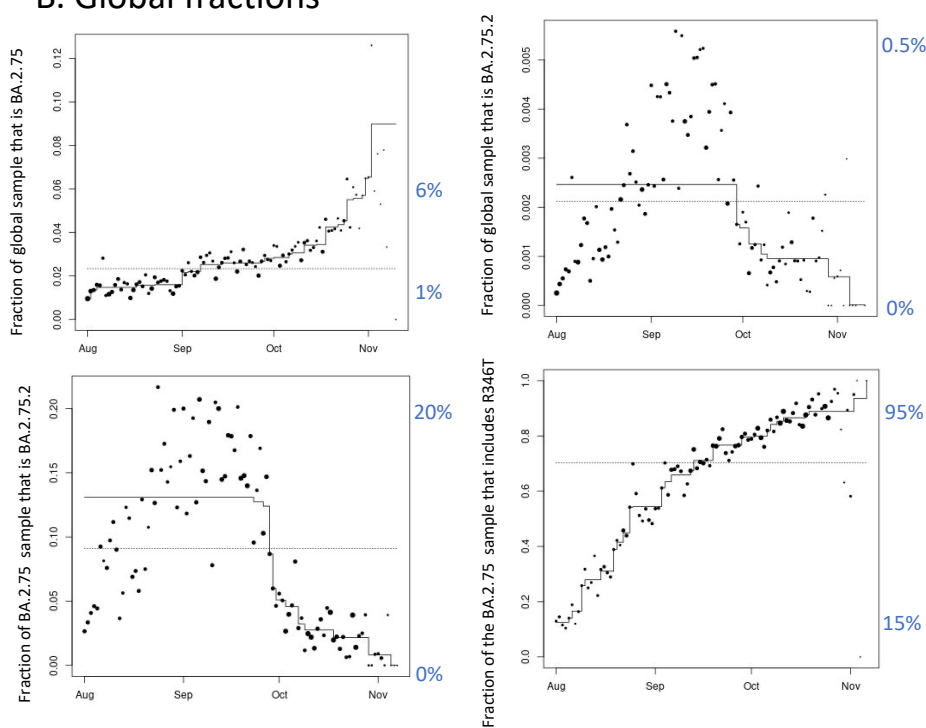
Test	Background	#Test	#Others	Total	T/Total (%)	p-val
BA.5	All	728357	152487	880844	82.69	0.00249 decreasing
BQ.1.1/BQ.1	All	21928	858916	880844	2.49	0.00249
BQ.1.1/BQ.1	BA.5	21928	706429	728357	3.01	0.00249
BQ.1.1	BQ.1	11981	9947	21928	54.64	0.00249

Figure S4. BA.2.75/BA.2.75.2 and Related Forms. BA.2.75 first became prominent in India, and the subvariant BA.2.75.2 increased relative to BA.2.75. **A**, Relative variant changes in sampling frequency in India, a country where both forms were found, but are now being replaced with XBB lineage recombinants. **B**, Global trends showing that while BA.2.75 lineages have been increasing globally (top left), they are increasing at a slower pace than BQ.1 and XBB lineages. BA.2.75.2 had a brief period of increased sampling globally in September 2022 (top right) and within the BA.2.75 lineage (bottom left). The BA.2.75.2 variant has three mutations: R346T, F486S and D1199N. The R346T mutation, a part of the BA.2.75.2 mutational signature, has come to dominate the entire BA.2.75 lineage (bottom right).

A. BA.2.75/BA.2.75.2 example: India



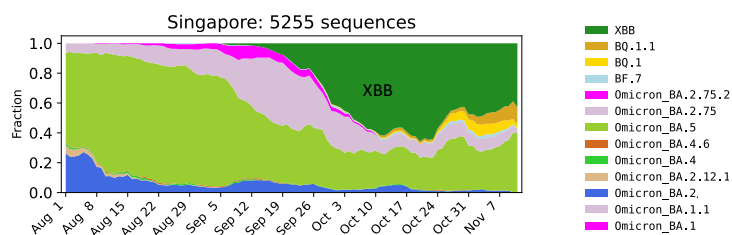
B. Global fractions



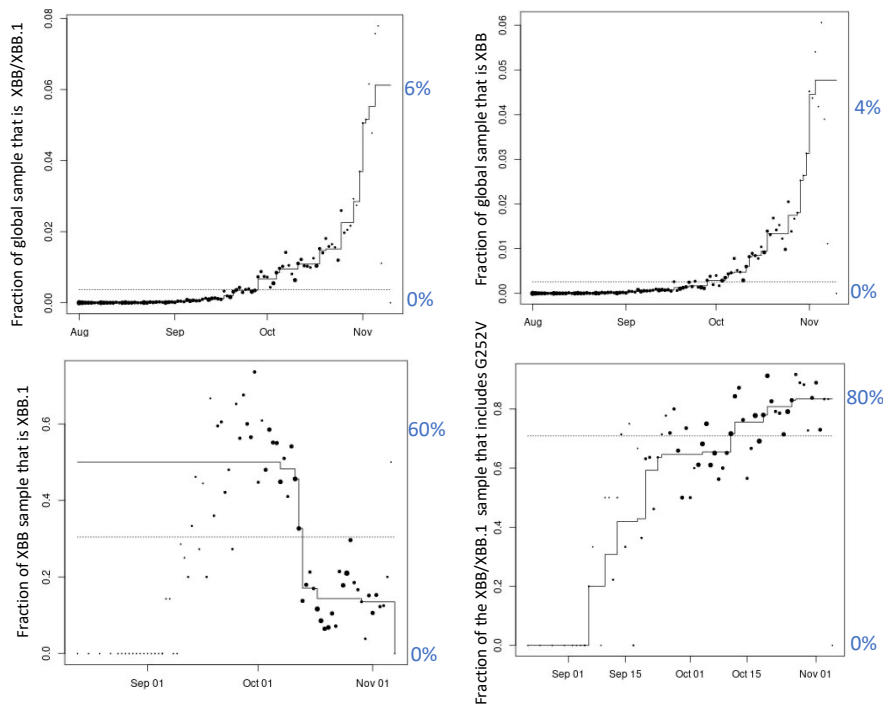
Test	Background	#Test	#Others	Total	T/Total (%)	p-val
BA.2.75	All	20561	860283	880844	2.33	0.00249
BA.2.75.2	All	1870	878974	880844	0.21	0.00249 decreasing
BA.2.75.2	BA.2.75	1870	18691	20561	9.09	0.00249 decreasing
BA.2.75+R346T	BA.2.75	7553	3200	10753	70.24	0.00249

Figure S5. XBB/XBB.1 and Related Forms. The XBB recombinant lineage is rapidly expanding globally. **A**, Relative variant changes in sampling frequency in Singapore, where XBB first became prevalent. **B**, Global trends showing that XBB lineages have been rapidly increasing globally to over 5% (top left). This recombinant lineage carries multiple changes relative to a BA.2.75 backbone, as well as several positions that reverted to ancestral form. The most common forms of XBB also carry the Spike G252V mutation, which arose after the recombination event. This mutation is found in just over half of the viruses with the XBB designation, and most of those viruses with the XBB.1 designation. XBB accounts for most of the global increase (top right versus top left). While XBB.1 is declining in frequency relative to other XBB designated sequences (bottom left), sublineages that carry G252V are increasingly sampled among both XBB and XBB.1 sequences.

A. XBB/XBB.1 example: Singapore



B. Global fractions



Test	Background	#Test	#Others	Total	T/Total (%)	p-val		
XBB/XBB.1	All	3250	877594	880844	0.37	0.00249		
XBB	All	2262	878582	880844	0.26	0.00249		
XBB.1	XBB/XBB.1	988	2262	3250	30.40	86	0.00249 decreasing	
XBB/XBB.1+ G252V	XBB/XBB.1	1230	505	1735	70.89	68	75	0.00249

Spike Sequences and Relationships to Pango Lineage Designations in GISAID

BF.7

The representative form of BF.7 used in this study represents a commonly circulating form of BA.5, found in many BA.5 sublineage. We chose to track BF.7 here as it is the most common designation, but this underestimates the global levels of the form of Spike BA.5+[R346T].

BA.5 baseline mutations with BF.7 addition noted in bold font: BA.5+[**R346T**]

T19I,L24del,P25del,P26del,A27S,H69del,V70del,G142D,V213G,G339D,**R346T**,S371F,S373P,S375F,T376A,D405N,R408S,K417N,N440K,L452R,S477N,T478K,E484A,F486V,Q498R,N501Y,Y505H,D614G,H655Y,N679K,P681H,N764K,D796Y,Q954H,N969K

Pango lineages that share the BF.7 representative Spike as their most common form. Counts are based on sequences with the exact form of Spike among high quality SARS-CoV-2 Spike sequences sampled in GISAID during 60 days ending November 11, 2022:

Lineage	Lineage Count	Form Count	Percent of Lineage of Form
BF.7	16282	11402	70.0%
BA.5.2.6	4443	2992	67.3%
BF.11	2001	1237	61.8%
BA.5.2.13	749	585	78.1%
BA.5.1.18	486	406	83.5%
BE.1.2	382	230	60.2%
BA.5.1.27	269	207	77.0%
BA.5.1.28	72	53	73.6%
BA.5.1.20	67	50	74.6%
BA.5.1.26	86	39	45.3%

Pango lineages in which BA.5+[R346T] is present as minor form within the lineage. Lineages are listed that contained this form of Spike sampled at least 10 times in GISAID during 60 days ending November 11, 2022:

Lineage	Lineage Count	Form Count	Percent of Lineage of Form
BA.5.2	42893	446	1.0%
BA.5.2.1	38822	866	2.2%
BA.5	3742	217	5.8%
BF.10	3452	191	5.5%
BA.5.2.20	3315	63	1.9%
BA.5.6	3204	56	1.7%
BE.1	2292	58	2.5%
BA.5.1.22	1805	217	12.0%
BF.26	1795	183	10.2%
BA.5.3.1	1407	150	10.7%
BA.5.1.1	1122	19	1.7%
BF.28	724	20	2.8%
BA.5.2.28	664	15	2.3%
BA.5.2.12	644	29	4.5%
BF.8	539	12	2.2%
BA.5.1.25	470	26	5.5%
BA.5.1.6	342	34	9.9%

BA.5.3.3	209	10	4.8%
BA.5.3	39	10	25.6%

BQ.1/BQ.1.1

The most common form of BQ.1 adds two mutations to a BA.5 backbone BA.5+[K444T,N460K], but often also adds R346T. In contrast, the BQ.1.1 sublineage generally includes all three mutations: BA.5+[K444T,N460K,R346T]. In this study we tested BQ.1.1 with R346T:

T19I,L24del,P25del,P26del,A27S,H69del,V70del,G142D,V213G,G339D,**R346T**,S371F,S373P,S375F,T376A,D405N,R408S,K417N,N440K,**K444T**,L452R,**N460K**,S477N,T478K,E484A,F486V,Q498R,N501Y,Y505H,D614G,H655Y,N679K,P681H,N764K,D796Y,Q954H,N969K

BQ-related Pango lineages sampled at least 100 times in GISAID during the 60 days ending November 11, 2022. The representative form used in this study is in bold.

Lineage	Lineage Count	Form Count	Percent of Lineage of Form	Mutations relative to BA.5
BQ.1.1	8470	6390	75.4%	+[R346T,K444T,N460K]
BQ.1.1	8470	563	6.6%	+[Y144-,R346T,K444T,N460K]
BQ.1	6927	4216	60.9%	+[K444T,N460K]
BQ.1	6927	1063	15.3%	+[Y144-,K444T,N460K]
BQ.1	6927	243	3.5%	+[R346T,K444T,N460K]
BQ.1.2	776	605	78.0%	+[K444T,N460K,I666V]
BQ.1.3	433	339	78.3%	+[K444T,N460K,E619Q]
BE.1.1	4541	117	2.6%	+[R346T,K444T,N460K]

BA.2.75/BA.2.72.2

BA.2.75 has evolved to include diverse forms, and while the originally expanding form of BA.2.75.2 eventually declined, it shares the R346T addition with many different sublineages that are continuing to circulate.

BA.2.75 baseline mutations with BA.2.75.2 additional mutations noted in bold font: BA.2.75+[**R346T,F486S,D1199N**]:

T19I,L24S,P25del,P26del,A27del,G142D,K147E,W152R,F157L,I210V,V213G,G257S,G339H,**R346T**,S371F,S373P,S375F,T376A,D405N,R408S,K417N,N440K,G446S,N460K,S477N,T478K,E484A,**F486S**,Q498R,N501Y,Y505H,D614G,H655Y,N679K,P681H,N764K,D796Y,Q954H,N969K,**D1199N**

XBB/XBB.1

XBB is a recombinant between BJ.1, a BA.2 sublineage of BA.2.10.1, and a BA.2.75 lineage virus. What follows is a comparison of two Spike sequences that match the XBB sequence on either side of its breakpoint, and the XBB.1 lineage shows how the recombination event impacted Spike. Amino acid changes in bold are where the two parental lineages differ.

BJ.1 parental lineage (a BA.2 sublineage) of Spike mutations relative to the ancestral form. An example of the sequence is GISAID ID EPI_ISL_15157654:

T19I,L24S,P25del,P26del,A27del,**V83A**,G142D,**Y145Q**,**H146del**,**Q183E**,**V213E**,G339H,R346T,**L368I**,S371F,S373P,S375F,T376A,D405N,R408S,K417N,N440K,**V445P**,G446S,S477N,T478K,**V483A**,E484A,**F490V**,**Q493R**,Q498R,N501Y,Y505H,D614G,H655Y,N679K,P681H,N764K,D796Y,**G798D**,Q954H,N969K,**S1003I** (BJ.1 consensus)

BM.1.1.1 parental lineage example (a BA.2.75 sublineage) of Spike mutations relative to the ancestral form. An example of the sequence GISAID ID EPI_ISL_14733813:

T19I,L24S,P25del,P26del,A27del,G142D,K147E,W152R,F157L,**I210V**,**V213G**,**G257S**,G339H,R346T,S371F,S373P,S375F,T376A,D405N,R408S,K417N,N440K,G446S,**N460K**,S477N,T478K,E484A,**F486S**,**F490S**,Q498R,N501Y,Y505H,D614G,H655Y,N679K,P681H,N764K,D796Y,Q954H,N969K

Below is the XBB.1 representative form we used in this study. The recombination breakpoint was between Spike 445 and 460, and the highlighted **G252V** arose after the initial recombination event that gave rise to the XBB variant lineage. An example of this sequence is GISAID ID EPI_ISL_15232105:

T19I,L24S,P25del,P26del,A27del,**V83A**,G142D,**Y145Q**,**H146del**,**Q183E**,**V213E**,**G252V**,G339H,R346T,**L368I**,S371F,S373P,S375F,T376A,D405N,R408S,K417N,N440K,**V445P**,G446S,**N460K**,S477N,T478K,E484A,**F486S**,**F490S**,Q498R,N501Y,Y505H,D614G,H655Y,N679K,P681H,N764K,D796Y,Q954H,N969K

The G252V mutation is present in most sequences in both the XBB and XBB.1 designated lineages. Frequencies of the exact form of Spike are provided. Other rarer forms carry additional substitutions.

Lineage	Lineage Count	Form Count	Percent of Form in Lineage	Mutations relative to the XBB recombinant
XBB	1259	669	53.1%	XBB + [G252V]
XBB	1259	186	14.8%	XBB
XBB.1	448	369	82.4%	XBB + [G252V]