Science Advances

Supplementary Materials for

Waning immunity and IgG4 responses following bivalent mRNA boosting

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Supplementary Methods

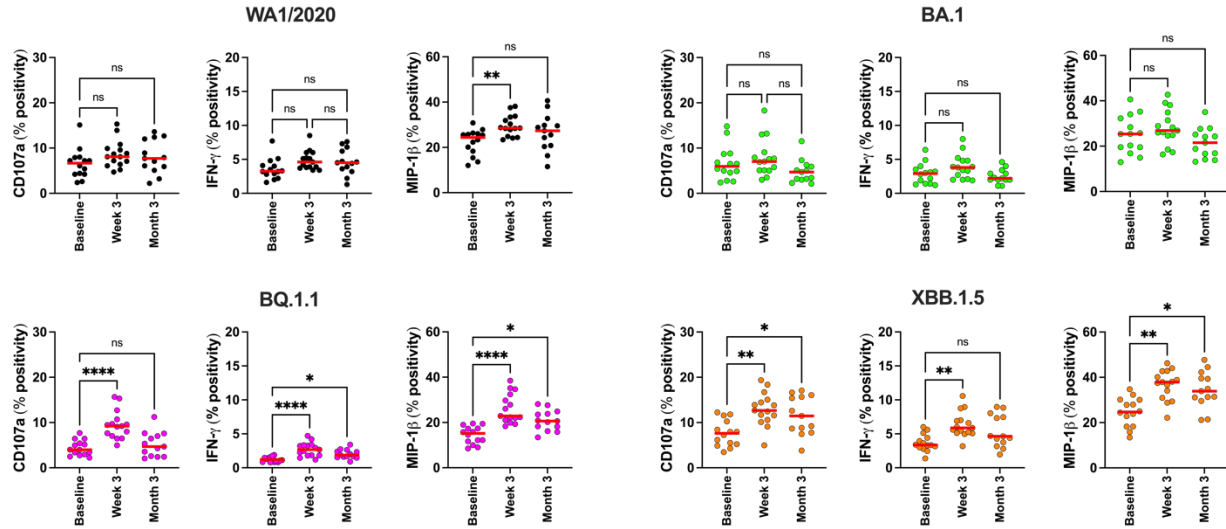### *Antibody-dependent NK Cell activation (ADNKA)*

Antibody-dependent NK Cell activation (ADNKA) assessed the ability of antigen-specific antibodies to activate human NK cells to up-regulate the production of CD107a, IFN-γ, and MIP-1β/CCL4. NK cells were isolated from healthy donor buffy coats (Massachusetts General Hospital Blood Donor Center) using the RosetteSep NK cell enrichment kit (StemCell). In brief, 96-well ELISA plates were coated with SARS-CoV-2 Spike antigens (strains WA1/2020, BA.1, BQ.1.1, XBB.1.5) and an Ebolavirus glycoprotein as a negative control. The plates were then blocked using 5% bovine serum albumin (Sigma) in PBS (Corning). Antigen-coated ELISA plates were then incubated with serum samples (dilution 1:25) for 2 hours at 37°C, followed by washing and incubation with purified NK cells from donors for 5 hours at 37°C. NK cells were then removed from the spike antigen coated plates, fixed, permeabilized, and stained for both cell surface and intracellular markers (CD3, (Clone UCHT1), CD16 (Clone 3G8), CD56 (Clone B159), CD107a (Clone H4A3), MIP-1β (Clone D21-1351), IFN-γ (Clone 25723.11). Cells were analyzed with an iQue Screener PLUS with Forecyt software (Intellicyt), and the results are reported as the percent of NK cells (CD3−, CD56/CD16+) positive for each activation marker (CD107a, IFN-γ, and MIP-1β). Statistical analysis for ADNKA was performed using GraphPad Prism v10.0.3. A Two-way ANOVA with Tukey's multiple comparison test was applied to evaluate the significance of % expression of CD107a, IFN-γ, and MIP-1β in NK cells.
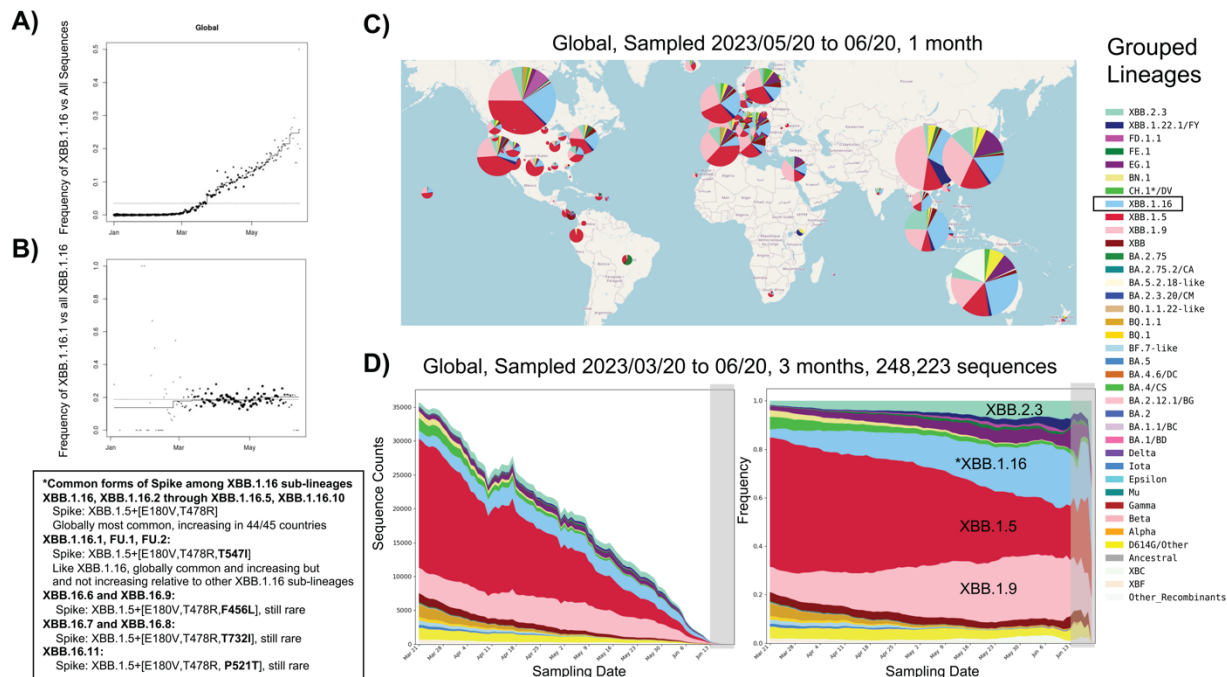
Fig S1. Increasing prevalence of the XBB.1.5 and XBB.1.16 variants. A. The increase in the frequency of sampling of XBB.1.16 variants globally in 2023, between January 1 and June 20. In less than 4 months since it began its expansion, XBB.1.16 has reached 25% of global sampling. **B.** The consensus form of XBB.1.16 we used for the experiments in this study (**Fig. 1**) is the most commonly circulating form of this lineage. The second most common XBB.1.16 Spike, assigned to Pango sublineages XBB.1.16.1 and FU, adds the mutation S T547I. XBB.1.16.1, however, is not increasingly sampled relative to other XBB.1.16 lineage members, suggesting it does not confer a selective advantage over the baseline XBB.1.16. **C.** The global distribution of common Pango lineages in the last thirty days. The most common spike variants in the rapidly expanding XBB.1.16 lineage are shown in the key on the left. **D.** XBB.1.16 lineages (light blue), and XBB.1.9 lineages (pink), are rapidly overtaking XBB.1.5 (red) which was the globally dominant form of the virus in early 2023. XBB.1.9 and XBB.1.16 lineages are both expanding more rapidly than XBB.1.5, although XBB.1.16 is increasing most rapidly. XBB.1.9 major forms share the same Spike as XBB.1.5, so if it has a selective advantage over XBB.1.5 it must be due to mutations outside of Spike. Other variants are newly emergent and that may come to prominence soon are noted.
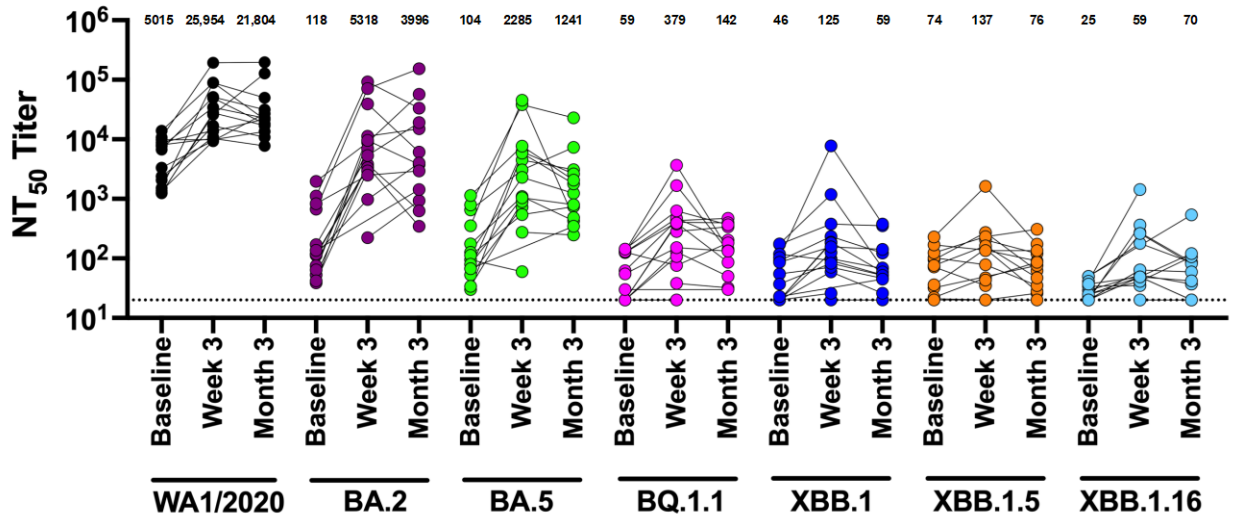
Fig S2. Paired analysis of neutralizing antibody responses to SARS-CoV-2 Omicron and XBB subvariants. Neutralizing antibody (NAb) titers against the WA1/2020, BA.2, BA.5, BQ.1.1, XBB.1, XBB.1.5, and XBB.1.16 variants by luciferase-based pseudovirus neutralization assays at baseline prior to boosting, at week 3 following boosting, and at month 3 following boosting in nucleocapsid seronegative participants. Median NAb titers are shown numerically. LOD indicated by dotted lines represents the starting dilution used for the NAb assay, 1:20.

Fig S3. Antibody-dependent NK Cell activation (ADNKA) profiling by systems serology following bivalent mRNA immunization. WA1/2020, BA.1, BQ.1.1, and XBB.1.5 spike-specific NK cells measured for percent expression of CD107a, IFN-γ, and MIP-1β in bivalent mRNA vaccinated subjects at baseline prior to boosting, week 3 following boosting, and at month 3 following boosting in nucleocapsid seronegative participants is shown. Two-way ANOVA with Tukey's multiple comparison test was applied to determine % expression significance between time points. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, and ****$p \leq 0.0001$. ns-not significant.

Table S1. Bivalent mRNA booster study cohort characteristics

	Bivalent mRNA Booster N=30
Age (years), median (range)	42 (24-77)
Sex at birth, Female	21 (70)
Race	
White	27 (90)
Asian	3 (10)
Black	0
More than one race	0
Ethnicity	
Hispanic or Latino	0
Non-Hispanic	30 (100)
Medical condition	
Obesity (BMI $\geq$30 kg/m2)	6 (20)
Hypertension	3 (10)
Diabetes	1 (3)
Pregnant	1 (3)
Asthma	2 (7)
Living with HIV	2 (7)
Most recent COVID-19 vaccine	
Pfizer bivalent booster	11 (37)
Moderna bivalent booster	19 (63)
Prior COVID-19 vaccines	
BNT (4 doses)	3 (10)
BNT (3 doses)	10 (33)
BNT (2 doses) / 1273	2 (7)
BNT (2 doses) / Ad26 / BNT	1 (3)
BNT (2 doses) / Ad26 / 1273	1 (3)
1273 (4 doses)	2 (7)
1273 (3 doses)	9 (30)
Ad26 / BNT (2 dose) / 1273	1 (3)
Ad26 / 1273 (1 dose)	1 (3)
Interval between last two vaccine doses (days)	326 (250-364)
Days from bivalent vaccine dose to initial sampling	21 (16-24)
Days from bivalent vaccine dose to 3 mo sampling	92 (86-96)
Known COVID-19 positive*	13 (43)

BNT=BNT162b2; 1273=mRNA-1273; Ad26=Ad26.COV2.S
Data displayed as median (range or interquartile range, IQR) and n (%); BMI, body mass index; pregnant designation reflects time of last vaccine dose and/or time of sampling.

* Two participants had infections between day 7-40 following the bivalent booster.

Table S2. Individual participant level data for bivalent mRNA booster cohort

	Age	Sex	Race	Ethnicity	Medical History	Bivalent Booster	Vaccine History (prior to Bivalent Booster)	Bivalent Booster to Peak (d)	Bivalent Booster to 3-4 Month Sample (d)	# Prior Infections	Last Infection to Most Recent Sample (d)
1	63	M	White	Not H/L	HTN, Obesity	Pfizer	BNT/BNT/BNT	13	119	0	N/A
2	55	M	White	Not H/L	Asthma	Moderna	1273/1273/1273	22	N/A	0	N/A
3	40	M	White	Not H/L	Obesity	Moderna	Ad.26/1273	20	93	1	73
4	43	M	White	Not H/L	Asthma	Pfizer	BNT/BNT/1273	14	84	1	73
5	28	F	White	Not H/L		Moderna	BNT/BNT/BNT	14	91	1	77
6	56	M	White	Not H/L	Obesity	Pfizer	BNT/BNT/BNT/BNT	21	91	1	205
7	45	F	White	Not H/L		Moderna	Ad.26/BNT/BNT/1273	24	92	0	N/A
8	39	F	White	Not H/L		Moderna	BNT/BNT/1273	22	95	1	215
9	25	M	Asian	Not H/L		Pfizer	BNT/BNT/BNT	15	82	0	N/A
10	50	F	White	Not H/L	HTN	Pfizer	BNT/BNT/BNT/BNT	32	87	1	186
11	77	F	White	Not H/L	Obesity	Pfizer	BNT/BNT/BNT	15	101	0	N/A
12	68	M	White	Not H/L		Moderna	BNT/BNT/Ad.26/BNT	23	N/A	0	N/A
13	39	F	Asian	Not H/L		Pfizer	BNT/BNT/BNT	16	91	0	N/A
14	31	F	White	Not H/L		Pfizer	BNT/BNT/BNT	16	N/A	0	N/A
15	32	F	White	Not H/L		Moderna	BNT/BNT/BNT	23	96	0	N/A
16	37	F	Asian	Not H/L		Pfizer	BNT/BNT/Ad.26/1273	34	96	0	N/A
17	42	F	White	Not H/L		Moderna	BNT/BNT/BNT	28	91	0	N/A
18	58	F	White	Not H/L		Moderna	1273/1273/1273	N/A	84	0	N/A
19	60	M	White	Not H/L		Moderna	BNT/BNT/BNT	20	91	1	71
20	43	F	White	Not H/L		Pfizer	1273/1273/1273	15	112	0	N/A
21	39	F	White	Not H/L		Moderna	1273/1273/1273	24	96	1	72
22	43	F	White	Not H/L	Obesity	Moderna	1273/1273/1273	28	97	1	69
23	35	F	White	Not H/L	HTN, Obesity, Pregnant	Pfizer	BNT/BNT/BNT	26	89	0	N/A
24	26	F	White	Not H/L		Moderna	1273/1273/1273	21	98	1	77
25	68	F	White	Not H/L		Moderna	1273/1273/1273/1273	N/A	80	0	N/A
26	27	F	White	Not H/L		Moderna	1273/1273/1273	N/A	N/A	2	168
27	64	F	White	Not H/L		Moderna	1273/1273/1273/1273	N/A	N/A	1	127

28	42	M	White	Not H/L		Moderna	1273/1273/1273	N/A	N/A	1	683
29	24	F	White	Not H/L		Moderna	1273/1273/1273	N/A	N/A	1	228
30	63	F	White	Not H/L		Pfizer	BNT/BNT/BNT	N/A	N/A	1	200

Table S3. 3rd mRNA vaccination recipient study cohort characteristics.

	Monovalent Booster N=33
Age (years), median (range)	35 (23-62)
Sex at birth, Female	28 (85)
Race	
White	28 (85)
Asian	2 (6)
Black	2 (6)
More than one race	1 (3)
Ethnicity	
Hispanic or Latino	2 (6)
Non-Hispanic	31 (94)
Medical condition	
Obesity (BMI $\geq$30 kg/m2)	3 (9)
Hypertension	8 (24)
Diabetes	1 (3)
Lactating	7 (21)
Asthma	2 (6)
Most recent COVID-19 vaccine	
BNT162b2 booster	32 (97)
mRNA-1273 booster	1 (3)
Prior COVID-19 vaccines	
BNT (3 doses)	32 (97)
1273 (3 doses)	1 (3)
Interval between last two vaccine doses (days)	255 (249-263)
Days from last vaccine dose to initial sampling	21 (16-28)
Days from last vaccine dose to 6 month sampling	176 (171-181)
Known COVID-19 positive prior to 6 month sampling	10 (30)

BNT=BNT162b2; 1273=mRNA-1273
Data displayed as median (range or interquartile range, IQR) and n (%); BMI, body mass index;