

Neutralization of omicron subvariants and antigenic cartography following multiple COVID 19 vaccinations and repeated omicron non JN.1 or JN.1 infections

Nungruthai Suntronwong, Sitthichai Kanokudom, Thaneeya Duangchinda, Warangkana Chantima, Pattarakul Pakchotanon, Sirapa Klinfueng, Jiratchaya Puenpa, Thaksaporn Thatsanathorn, Nasamon Wanlapakorn, Yong Poovorawan

Supplementary information

Table S1 Summarizes participant information across all cohorts. Demographic, vaccine and blood sampling details are summarized for each cohort. Sera samples from vaccinated individuals, with or without prior infection, were collected between August 2021 and October 2022. Sera samples from individuals with non-JN.1 PVI were collected between April and May 2023, while those with JN.1 PVI were collected in May 2024.

	Total (n=209)	Vaccinated participants with anti-N Negative (n=55)	Vaccinated participants with anti-N Positive (n=57)	Non-JN.1 PVI (n=60)	JN.1 PVI^a (n=34)
Age, year Mean (SD)	41.7 (15)	49.7 (17.9)	42.1 (13.5)	38 (12.5)	34.7 (9.85)
Sex, n (%)					
Male	55 (26.7%)	22 (40%)	20 (35.1%)	9 (15%)	4 (11.8%)
Female	151 (73.3%)	33 (50%)	37 (64.9%)	51 (85%)	30 (88.2%)
Number of vaccinations					
2 doses	2 (1%)	-	-	-	2 (5.9%)
3 doses	53 (25.7%)	20 (36.4%)	18 (31.6%)	8 (13.3%)	7 (20.6%)
4 doses	82 (39.8%)	21 (38.2%)	20 (35.1%)	27 (45%)	14 (41.1%)
5 doses	65 (31.5%)	14 (25.4%)	19 (33.3%)	25 (41.7%)	7 (20.6%)
6 doses	2 (1%)	-	-	-	2 (5.9%)
7 doses	2(1%)	-	-	-	2 (5.9%)

Days since last vaccination, Median, IQR					
2 doses	920 (859–981)	-	-	-	920 (859–981)
3 doses	151 (116–230)	146 (107–188)	125 (114–140)	468 (357–559)	944 (859–1035)
4 doses	237 (155–469)	143 (127–159)	217 (170–256)	388 (269–470)	860 (859–863)
5 doses	177 (121–289)	98 (89–105)	222 (174–289)	176 (136–288)	678 (480–701)
6 doses	390 (375–405)		-	-	390 (375–405)
7 doses	529 (378–680)			-	529 (378–680)
Number of infections					
0	55 (26.7%)	55 (100%)	-	-	-
1	80 (38.8%)	-	26 (45.6%)	48 (80%)	6 (17.7%)
2	32 (15.5%)	-	-	12 (20%)	20 (58.8%)
3	8 (3.9%)	-	-	-	8 (23.5%)
N/A	31 (15.1%)	-	31 (54.4%)	-	-
Days since last infection, median (IQR)					
1	160 (118–227)	-	134 (84–207)	163 (154–287)	34 (27–82)
2	105 (55–133)	-	-	144 (124–168)	67 (27–93)
3	28 (20–34)	-	-	-	28 (20–34)
Days since last antigen exposure, median (IQR)	137 (107–169)	132 (105–159)	182 (128–254)	150 (134–163)	35 (25–76)
Number of participants with anti-N Ig seropositive results	151/206	0/55	57/57	60/60	34/34

^a PVI with JN.1 exposures was determined based on the date of infection reported by participants corresponds to the period of JN.1 variants predominance analyzed through available sequencing data. Abbreviations: IQR; interquartile range, PVI; post-vaccination infection.

Table S2 Summarizes information on vaccinated participants with or without prior infection. Individuals were grouped by their history of infection and seropositivity for anti-SARS-CoV-2 N Ig. Demographic, vaccine regimens and blood sampling information are summarized for each group.

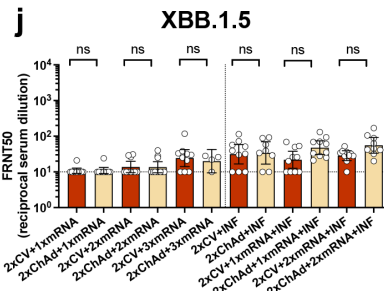
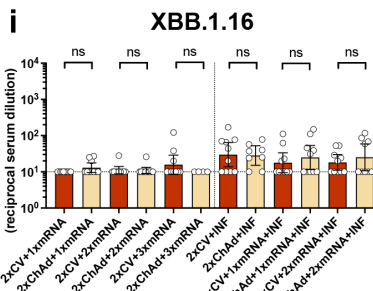
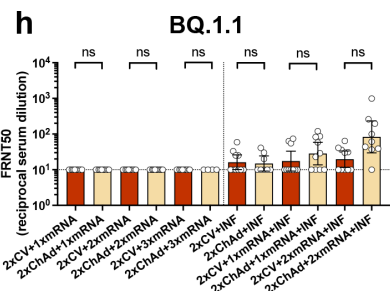
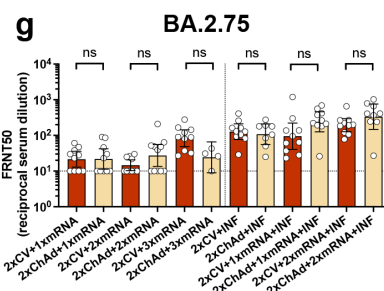
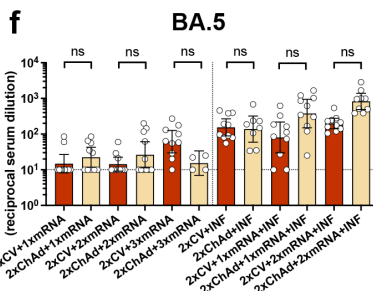
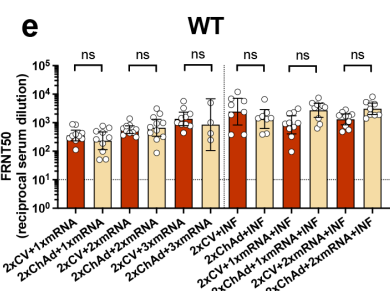
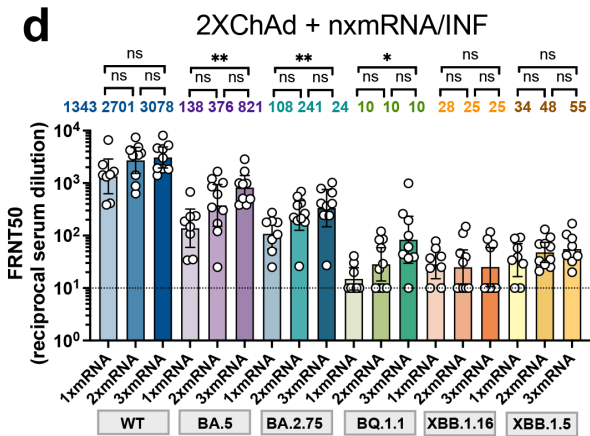
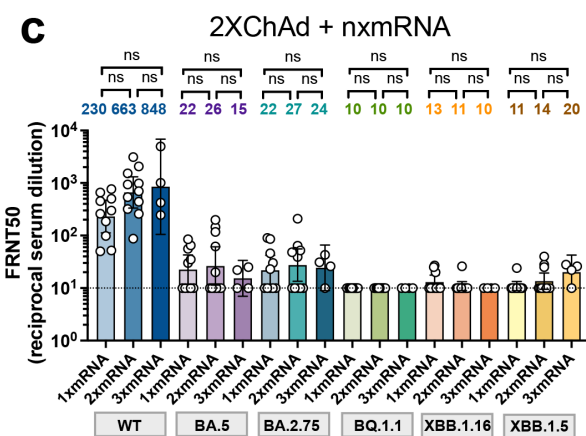
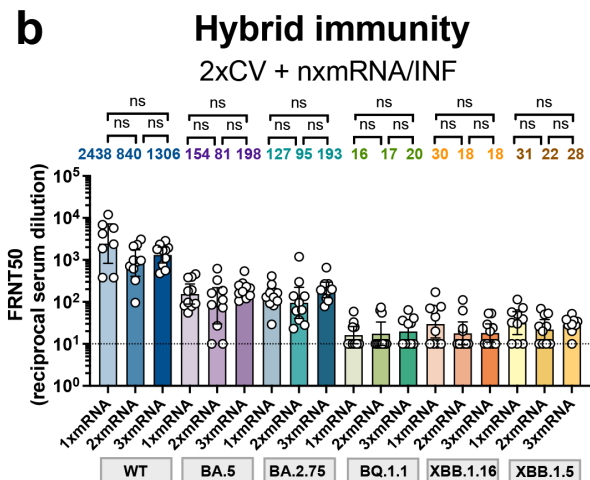
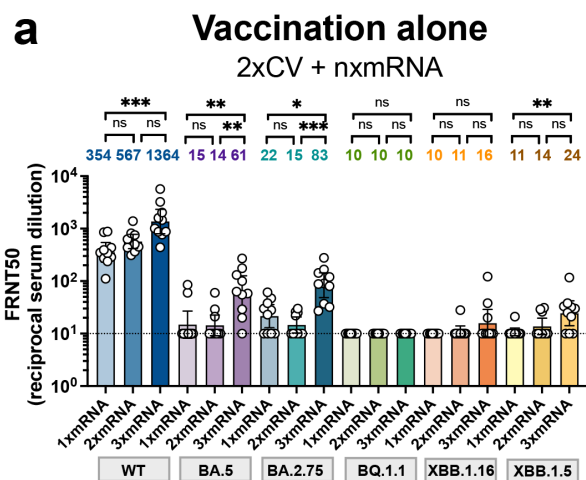
	Vaccination alone			Hybrid immunity		
	3xV (n=20)	4xV (n=21)	5xV (n=14)	2xV+INF (n=18)	3xV+INF (n=20)	4xV+INF (n=19)
Age, year Mean (SD)	52.7 (17.1)	51.4 (18.3)	42.9 (17.9)	42.8 (12.3)	45.1 (12.1)	38.4 (15.7)
Sex, n (%)						
Male	10 (50%)	9 (42.9%)	3 (21.4%)	8 (44.4%)	7 (35%)	5 (26.3%)
Female	10 (50%)	12 (57.1%)	11 (78.6%)	10 (55.6%)	13 (65%)	14 (73.7%)
Vaccine regimens	2xCV +1xmRNA (n=10) 2xChAd +1xmRNA (n=10)	2xCV +2xmRNA (n=10) 2xChAd +2xmRNA (n=11)	2xCV +3xmRNA (n=10) 2xChAd +3xmRNAT (n=4)	2xCV +INF* (n=10) 2xChAd +INF* (n=8)	2xCV +1xmRNA+INF* (n=10) 2xChAd +1xmRNA+INF* (n=10)	2xCV +2xmRNA+INF* (n=10) 2xChAd +2xmRNA+INF* (n=9)
Days since last vaccination median (IQR)	146 (106–188)	143 (126–162)	98 (84–107)	125 (114–142)	217 (169–265)	222 (165–289)
Number of participants with anti-N IgG seropositive results	0/20	0/21	0/14	18/18	20/20	19/19

*INF defined as individuals experienced SARS-CoV-2 infection which identified based on the history of infection reported by participants and/or seropositivity of anti-N Ig. 1xmRNA: a single-dose booster; 2xmRNA: two dose booster; 3xmRNA: three dose booster; mRNA referred to BNT162b2 or mRNA-1273; CV: CoronaVac; ChAd: ChAdOx-1 nCoV-19.

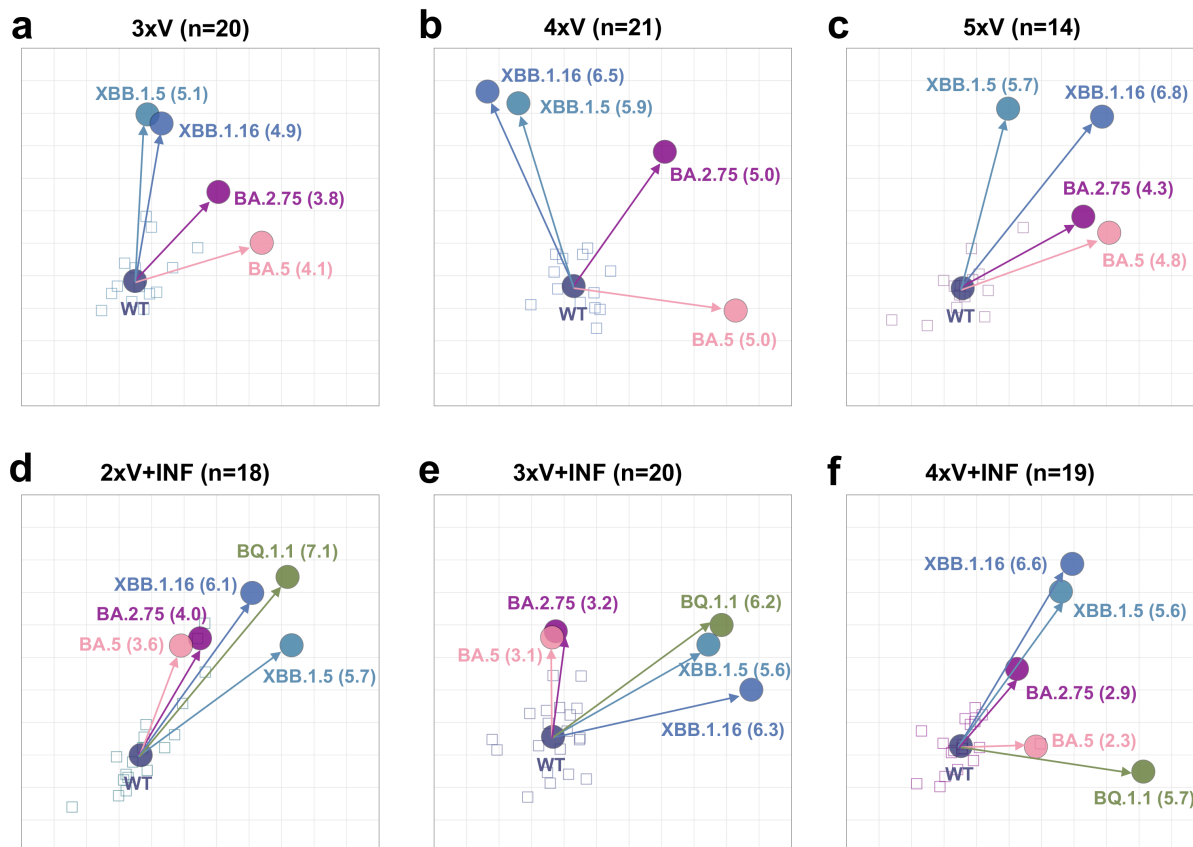
Table S3 Summarized information on individuals with post-vaccination infections (PVIs).

Individuals were grouped by the number of SARS-CoV-2 exposures and the presumed variants causing infection. Demographic, vaccine and blood sampling data are summarized for each group.

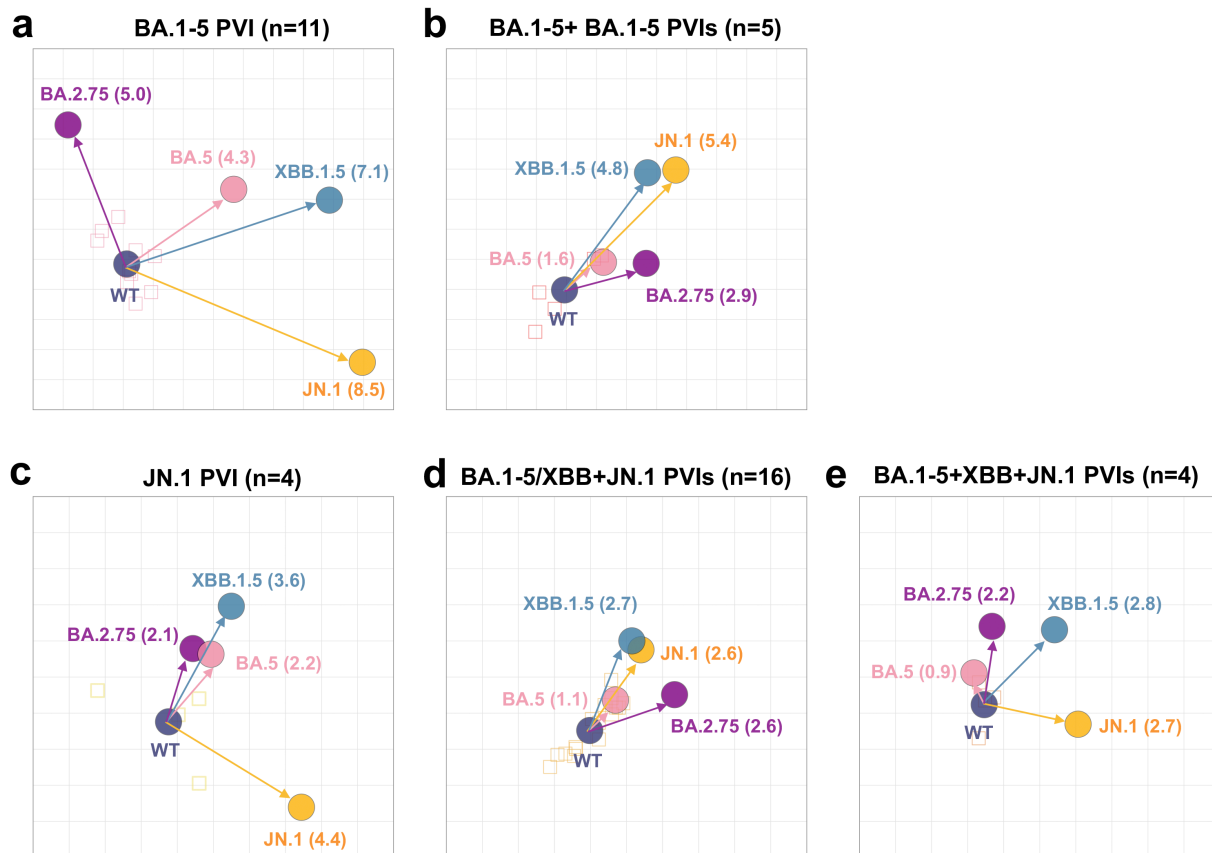
	Non-JN.1 PVI		JN.1 PVI		
	BA.1-5 single infection (n=51)	BA.1-5+BA.1-5 reinfection (n=9)	JN.1 single infection (n=6)	BA.1-5/XBB+JN.1 reinfection (n=24)	BA.1-5+XBB+JN.1 reinfection (n=4)
Age, year Mean (SD)	38.3 (12.4)	36.8 (13.6)	38.5 (11.5)	33.7 (10.2)	34.8 (3.9)
Sex, n (%)					
Male	6 (11.8%)	6 (66.7%)	1 (16.7%)	3 (12.5%)	0
Female	45 (88.2%)	3 (33.3%)	5 (83.3%)	21 (87.5%)	4 (100%)
Days since last antigen exposure median (IQR)	153 (136–166)	124 (121–150)	34 (26–90)	55 (21–80)	30 (25–34)
Number of participants with anti-N IgG seropositive results	51/51	9/9	6/6	24/24	4/4



Supplementary Fig. S1 Neutralization against WT, BA.5, BA.2.75, BQ.1.1, XBB.1.16, and XBB.1.5 was assessed using sera from individuals who were primed with two doses of CoronaVac and subsequently received one to three doses of the ancestral monovalent mRNA vaccine, either (a) without prior SARS-CoV-2 infection (2xCV+nxmRNA) or (b) with prior infection (2xCV+nxmRNA/INF). Another group was primed with two doses of ChAdOx1 nCoV-19, followed by one to three doses of the ancestral monovalent mRNA vaccine, either (c) without prior infection (2xChAd+nxmRNA) or (d) with prior infection (2xChAd+nxmRNA/INF). The numbers above represent geometric mean titers (GMT), with bars indicating GMT and 95% confidence intervals. Neutralizing antibody titers were compared between 2xCV+nxmRNA and 2xChAd+nxmRNA, as well as between 2xCV+nxmRNA/INF and 2xChAd+nxmRNA/INF, against (e) WT, (f) BA.5, (g) BA.2.75, (h) BQ.1.1, (i) XBB.1.16, and (j) XBB.1.5. The dashed line represents the threshold for detectable neutralizing antibody titers (FRNT50=10). Differences between groups were assessed using the Kruskal–Wallis test in panels a-d and the Mann-Whitney test in panels e-j, with $p < 0.05$ considered statistically significant. ns, not significant; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Abbreviations: CV, CoronaVac; ChAd, ChAdOx1 nCoV-19; INF, prior infection.



Supplementary Fig. S2 Antigenic maps generated from the neutralizing data against WT, BA.2.75, BA.5, BQ.1.1, XBB.1.16 and XBB.1.5 analyzed using serum from vaccinated-only individuals who received (a) three doses (3xV), four doses (4xV) and five doses (5xV) of ancestral monovalent vaccine and serum from individuals with hybrid immunity (without presumed variants causing infection who experienced SARS-CoV-2 infection and received two doses (2xV+INF), three doses (3xV+INF) and four doses (4xV+INF) of ancestral monovalent vaccine. Viruses are shown as colored circles. Sera from vaccinated-only individuals and hybrid immunity are shown as colored squares. The distances between virus antigen and sera positioned in the map are inversely related to the neutralizing antibody titers, with minimized error. A spacing line of each square in the antigenic map refer to the antigenic unit which corresponds to two-fold dilution change in neutralizing FRNT50 titers. Number in parenthesis shows an approximately antigenic distance from WT to each virus antigen.



Supplementary Fig. S3 Antigenic maps generated using sera from (a) a single omicron BA.1-5 PVI, (b) repeated omicron BA.1-5+BA.1-5 PVIs, (c) a single JN.1 PVI, (d) repeated omicron BA.1-5/XBB+JN.1 PVIs and (e) repeated omicron BA.1-5+XBB+JN.1 PVIs tested neutralization against WT, BA.2.75, BA.5, XBB.1.5 and JN.1. Viruses are shown as colored circles. Sera from PVIs are shown as colored squares. The distances between virus antigen and sera positioned in the map are inversely related to the neutralizing antibody titers, with minimized error. A spacing line of each square in the antigenic map refer to the antigenic unit which corresponds to two-fold dilution change in neutralizing FRNT50 titers. Number in parenthesis shows an approximately antigenic distance from WT to each virus antigen.