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Supplementary appendix

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Humoral immune evasion of the Omicron Subvariants BQ.1.1 and XBB.

Supplementary Appendix

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Supplementary Materials and Methods

Cells.

Vero E6-TMPRSS2-T2A-ACE2 cells (provided by Dr. Barney Graham, NIAID Vaccine Research Center) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% Fetal Calf Serum (FCS), 100 U/mL penicillin–streptomycin, and 10 μg/mL puromycin. VeroE6/TMPRSS2 (JCRB 1819) cells were propagated in the presence of 1 mg/ml geneticin (G418; Invivogen) and 5 μg/ml plasmocin prophylactic (Invivogen) in DMEM containing 10% FCS. Vero E6-TMPRSS2-T2A-ACE2 and VeroE6/TMPRSS2 cells were maintained at 37 °C with 5% CO_2. The cells were regularly tested for mycoplasma contamination by using PCR, and confirmed to be mycoplasma-free.

Viruses.

hCoV-19/Japan/TY41-796/2022 (Omicron BQ.1.1)[1], hCoV-19/Japan/TY41-795/2022 (Omicron XBB), hCoV-19/Japan/UT-NCD1288-2N/2022 (Omicron BA.2)[2], hCoV-19/Japan/TY41-702/2022 (Omicron BA.5)[3], and SARS-CoV-2/UT-NC002-1T/Human/2020/Tokyo were propagated in VeroE6/TMPRSS2 cells.

All experiments with SARS-CoV-2 were performed in enhanced biosafety level 3 (BSL3) containment laboratories at the University of Tokyo and the National Institute of Infectious Diseases, Japan, which are approved for such use by the Ministry of Agriculture, Forestry, and Fisheries, Japan.

Clinical specimens.

After informed consent was obtained, plasma specimens were collected from COVID-19 convalescent individuals and vaccinees. The research protocol was approved by the Research Ethics Review Committee of the Institute of Medical Science of the University of Tokyo (approval numbers: 2019–71–0201 and 2020-74-0226).

Focus reduction neutralisation test.

Neutralisation activities of plasma were determined by using a focus reduction neutralisation test as previously described.[4] After the plasma samples were incubated at 56 °C for 1 h, the samples were serially diluted five-fold with DMEM containing 2% FCS in 96-well plates and mixed with 100–400 focus-forming units (FFU) of virus/well, followed by incubation at room temperature for 1 h. The plasma-virus mixture (50 μl) was then inoculated onto Vero E6-TMPRSS2-T2A-ACE2 cells in 96-well plates in duplicate and incubated for 1 h at 37 °C. An equal volume of 1.5% Methyl Cellulose 400 (FUJIFILM Wako Pure Chemical Corporation) in culture medium was then added to each well. The cells were incubated for 14–16 h at 37 °C and then fixed with formalin. After the formalin was removed, the cells were immunostained with a mouse monoclonal antibody against SARS-CoV1/2 nucleoprotein [clone 1C7C7 (Sigma-Aldrich)], followed by a horseradish peroxidase-labeled goat anti-mouse immunoglobulin (SeraCare Life Sciences or Jackson ImmunoResearch Laboratories Inc.). The infected cells were stained with TrueBlue Substrate (SeraCare Life Sciences) and then washed with distilled water. After cell drying, the focus numbers were quantified by using an ImmunoSpot S6 Analyzer, ImmunoCapture software, and BioSpot software (Cellular Technology). The results are expressed as the 50% focus reduction neutralisation titre ($FRNT_{50}$). The $FRNT_{50}$ values were calculated by using GraphPad Prism (GraphPad Software).

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Author Contributions
R.U.: conceptualization, formal analysis, validation, visualization, and writing the first draft. M. Ito.: data curation, formal analysis, and methodology. Y.F.: data curation. S. Yamayoshi: conceptualization and methodology. K.I-H.: resources and validation. E.A., M.S., M. Koga., T.T, S. Yamamoto, A.O, M. Kiso, Y.S-T., H.U., H.Y. : resources. M. Imai: conceptualization, supervision, formal analysis, validation, visualization, and writing the first draft. Y.K.: conceptualization, supervision, writing (review and editing), and funding acquisition. R.U., M. Ito, and Y.F. contributed equally.

Declaration of Interests
Y.K. has received unrelated funding support from Daiichi Sankyo Pharmaceutical, Toyama Chemical, Tauns Laboratories, Inc., Shionogi & Co. LTD, Otsuka Pharmaceutical, KM Biologics, Kyoritsu Seiyaku, Shinya Corporation, and Fuji Rebio. The remaining authors declare that they have no competing interests.

Table S1. Amino acid Substitutions in the clinical isolates of BA.2, BA.5, BQ.1.1, and XBB used in this study

Protein			Position	Virus (Pango lineage)				
				Early SARS-CoV-2 (A)	hCoV-19/Japan/IT-NCD1288-2N-2022 (BA.2)	hCoV-19/Japan/TY41-702/2022 (BA.5)	hCoV-19/Japan/TY41-796/2022 (BQ.1.1)	hCoV-19/Japan/TY41-795/2022 (XBB)
ORF1a			47	Lys	Lys	Lys	Lys	Arg
			135	Ser	Arg	Arg	Arg	Arg
			556	Gln	Gln	Lys	Lys	Gln
			842	Thr	Ile	Ile	Ile	Ile
			1307	Gly	Ser	Ser	Ser	Ser
			3027	Leu	Phe	Phe	Phe	Phe
			3090	Thr	Ile	Ile	Ile	Ile
			3201	Leu	Phe	Leu	Leu	Phe
			3255	Thr	Ile	Ile	Ile	Ile
			3395	Pro	His	His	His	His
			3650	Phe	Phe	Leu (85%)	Phe	Phe
			3675	Ser	del	del	del	del
			3676	Gly	del	del	del	del
			3677	Phe	del	del	del	del
			3730	Ala	Ala	Ala	Ala	Val(22%)
			3753	Phe	Val(20%)	Phe	Phe	Phe
			3829	Leu	Leu	Leu	Phe	Leu
			4391	Leu	Phe(19%)	Leu	Leu	Leu
ORF1b			264	Tyr	Tyr	Tyr	His	Tyr
			314	Pro	Leu	Leu	Leu	Leu
			659	Met	Met	Met	Met	Ile
			662	Gly	Val	Gly	Gly	Ser
			959	Ser	Pro	Ser	Ser	Pro
			1156	Met	Met	Ile	Ile	Met
			1161	Pro	Ser(27%)	Pro	Pro	Pro
			1191	Asn	Asn	Asn	Ser	Asn
			1315	Arg	Cys	Cys	Cys	Cys
			1566	Ile	Val	Val	Val	Val
			2163	Thr	Ile	Ile	Ile	Ile
			2513	Ala	Val(18%)	Ala	Ala	Ala
		NTD (13-304)	19	Thr	Ile	Ile	Ile	Ile
			24	Leu	del	del	del	del
			25	Pro	del	del	del	del
			26	Pro	del	del	del	del
			27	Ala	Ser	Ser	Ser	Ser
			69	His	His	del	del	His
			70	Val	Val	del	del	Val
			83	Val	Val	Val	Val	Ala
			142	Gly	Asp	Asp	Asp	Asp
			145	Tyr	Tyr	Tyr	Tyr	del
			146	His	His	His	His	Gln
			183	Glu	Glu	Glu	Glu	Glu
			213	Val	Gly	Gly	Gly	Glu
			252	Gly	Gly	Gly	Gly	Val
		RBD (331-527)	339	Gly	Asp	Asp	Asp	His
			346	Arg	Arg	Arg	Thr	Thr
			368	Leu	Leu	Leu	Leu	Ile
			371	Ser	Phe	Phe	Phe	Phe
			373	Ser	Pro	Pro	Pro	Pro
			375	Ser	Phe	Phe	Phe	Phe
			376	Thr	Ala	Ala	Ala	Ala
			405	Asp	Asn	Asn	Asn	Asn
			408	Arg	Ser	Ser	Ser	Ser
			417	Lys	Asn	Asn	Asn	Asn
			440	Asn	Lys	Lys	Lys	Lys
			444	Lys	Lys	Lys	Thr	Lys
			445	Val	Val	Val	Val	Pro
			446	Gly	Gly	Gly	Gly	Ser
			452	Leu	Leu	Arg	Arg	Leu
			460	Asn	Asn	Asn	Lys	Lys
			477	Ser	Asn	Asn	Asn	Asn
			478	Thr	Lys	Lys	Lys	Lys
			484	Glu	Ala	Ala	Ala	Ala
			486	Phe	Phe	Val	Val	Ser
			490	Phe	Phe	Phe	Phe	Ser
			493	Gln	Arg	Gln	Gln	Gln
			498	Gln	Arg	Arg	Arg	Arg
			501	Asn	Tyr	Tyr	Tyr	Tyr
			505	Tyr	His	His	His	His
		SD1/2 (528-684)	614	Asp	Gly	Gly	Gly	Gly
			655	His	Tyr	Tyr	Tyr	Tyr
			679	Asn	Lys	Lys	Lys	Lys
			681	Pro	His	His	His	His
		S2 (685-1210)	764	Asn	Lys	Lys	Lys	Lys
			796	Asp	Tyr	Tyr	Tyr	Tyr
			954	Gln	His	His	His	His
			969	Asn	Lys	Lys	Lys	Lys
ORF3a			223	Thr	Ile	Ile	Ile	Ile
E			9	Thr	Ile	Ile	Ile	Ile
			11	Thr	Thr	Thr	Thr	Ala
M			3	Asp	Asp	Asn	Asn	Asp
			19	Gln	Glu	Glu	Glu	Glu
			63	Ala	Thr	Thr	Thr	Thr
ORF6			61	Asp	Leu	Asp	Asp	Leu
ORF7b			10	Pro	Ser	Pro	Pro	Pro
			27	Glu	del	Glu	Glu	Glu
			28	Asn	del	Asn	Asn	Asn
			29	Ala	del	Ala	Ala	Ala
N			13	Pro	Leu	Leu	Leu	Leu
			31	Glu	del	del	del	del
			32	Arg	del	del	del	del
			33	Ser	del	del	del	del
			136	Glu	Glu	Asp	Asp	Glu
			203	Arg	Lys	Lys	Lys	Lys
			204	Gly	Arg	Arg	Arg	Arg
			413	Ser	Arg	Arg	Arg	Arg

Substitutions are shown relative to the reference strain Wuhan/Hu-1/2019. The spike protein is composed of two subunits, S1 and S2. ORF, open reading frame; SP, signal peptide; NTD, N-terminal domain; RBD, receptor-binding domain; SD1/2, subdomain 1 and 2; E, Envelope; M, Membrane; and N, Nucleocapsid.
del; deletion

Table S2. Neutralising antibody titres of human plasma obtained from individuals immunized with three doses of BNT162b2 or mRNA-1273 vaccine

Sample ID	Gender	Age	Documented SARS-CoV-2 infection	Vaccine type	Days post-vaccination	FRNT50: 50% focus reduction neutralisation titre				
						(A)	Omicron/BA.2	Omicron/BA.5	Omicron/BQ.1.1	Omicron/XBB
						SARS-CoV-2/UT-NC002-1T/Human/2020/Tokyo	hCoV-19/Japan/UT-NCD1288-2N-2022	hCoV-19/Japan/TY41-702/2022	hCoV-19/Japan/TY41-796/2022	hCoV-19/Japan/TY41-795/2022
HP(H)- 004	F	50	No	BNT162b2x 3	183	426.5	32.8	28.7	<10	<10
HP(H)- 019	F	57	No	BNT162b2x 3	182	147.7	32	10.2	<10	<10
HP(H)- 026	F	25	No	BNT162b2x 3	181	335.9	30.5	15.1	<10	<10
HP(H)- 065	F	40	No	BNT162b2x 3	181	166.9	11	<10	<10	<10
HP(H)- 106	F	29	No	BNT162b2x 3	182	620.1	156.3	49.3	<10	<10
HP(H)- 115	F	48	No	BNT162b2x 3	182	162.1	17.2	<10	<10	<10
HP(H)- 131	M	61	No	BNT162b2x 3	183	172.2	19.4	<10	<10	<10
HP(H)- 168	M	49	No	BNT162b2x 3	182	86.6	<10	<10	<10	<10
HP(H)- 173	M	39	No	BNT162b2x 3	180	74.3	<10	<10	<10	<10
HP(H)- 181	M	54	No	BNT162b2x 3	182	607.9	15.4	<10	<10	<10
HP(H)- 182	F	51	No	BNT162b2x 3	176	411.9	64.9	13.5	<10	<10
HP(H)- 194	M	49	No	BNT162b2x 3	182	86.6	30.7	14.4	<10	<10
HP(H)- 221	F	55	No	BNT162b2x 3	181	66	<10	<10	<10	<10
HP(H)- 303	M	50	No	BNT162b2x 3	188	135.4	15.8	<10	<10	<10
AG- 001	F	54	No	mRNA-1273x 3	189	843.2	284.8	235.2	33.7	73.7
AG- 002	F	48	No	mRNA-1273x 3	187	353.1	<10	<10	<10	<10
AG- 007	F	53	No	mRNA-1273x 3	183	721.6	60.9	38.7	<10	<10
AG- 008	F	57	No	mRNA-1273x 3	183	250.7	22.6	27.7	<10	<10
AG- 010	M	61	No	mRNA-1273x 3	184	585.2	297.8	278.6	122.3	42.2
AG- 011	F	55	No	mRNA-1273x 3	185	647.5	74.7	48.1	13.1	<10

Table S3. Neutralising antibody titres of human plasma obtained from individuals immunized with four doses of BNT162b2 or mRNA-1273 vaccine

Sample ID	Gender	Age	Documented SARS-CoV-2 infection	Vaccine type	Days post-vaccination	FRNT50: 50% focus reduction neutralisation titre				
						(A)	Omicron/BA.2	Omicron/BA.5	Omicron/BQ.1.1	Omicron/XBB
						SARS-CoV-2/UT-NC002-1T/Human/2020/Tokyo	hCoV-19/Japan/UT-NCD1288-2N-2022	hCoV-19/Japan/TY41-702/2022	hCoV-19/Japan/TY41-796/2022	hCoV-19/Japan/TY41-795/2022
HP(H)-032	M	39	No	BNT162b2x 3, mRNA-1273 x 1	53	629	58.9	47.2	10	<10
HP(H)-058	F	58	No	BNT162b2x 3, mRNA-1273 x 1	48	4108.2	836.7	632.9	158.9	97.5
HP(H)-088	F	46	No	BNT162b2x 3, mRNA-1273 x 1	43	844.2	125.8	59.2	24.7	21.6
HP(H)-113	M	62	No	BNT162b2x 3, mRNA-1273 x 1	41	972.2	63	64.9	<10	<10
HP(H)-158	F	29	No	BNT162b2x 3, mRNA-1273 x 1	41	356.3	19.9	<10	<10	<10
HP(H)-182	F	52	No	BNT162b2x 4	54	1581.7	191.7	86.6	29.8	14.9
HP(H)-183	F	47	No	BNT162b2x 3, mRNA-1273 x 1	44	728.2	108.4	72	<10	<10
HP(H)-185	F	48	No	BNT162b2x 3, mRNA-1273 x 1	50	1275.5	203.8	129.4	35.8	13.7
HP(H)-189	F	57	No	BNT162b2x 4	33	598.8	83.7	60	12.7	<10
HP(H)-198	F	33	No	BNT162b2x 3, mRNA-1273 x 1	45	590.3	39.5	21.6	<10	<10
HP(H)-220	M	34	No	BNT162b2x 3, mRNA-1273 x 1	44	360.4	41.7	13	<10	<10
HP(H)-228	F	49	No	BNT162b2x 3, mRNA-1273 x 1	51	595.6	178.2	102.6	21.8	<10
HP(H)-241	F	62	No	BNT162b2x 4	57	659.2	116.2	91	<10	10
HP(H)-250	F	35	No	BNT162b2x 3, mRNA-1273 x 1	49	1741.7	300.5	335.6	42.9	32.7
HP(H)-255	F	51	No	BNT162b2x 3, mRNA-1273 x 1	42	892.1	78.1	39.9	<10	<10
HP(H)-264	M	43	No	BNT162b2x 3, mRNA-1273 x 1	41	901	34.7	65.7	<10	<10
HP(H)-282	M	43	No	BNT162b2x 3, mRNA-1273 x 1	48	256.6	36.5	35.5	<10	<10
HP(H)-297	M	56	No	BNT162b2x 3, mRNA-1273 x 1	45	1184.8	253.9	154.7	64.9	72.8
HP(H)-299	F	53	No	BNT162b2x 4	53	202.9	21	25.4	<10	<10
HP(H)-303	M	51	No	BNT162b2x 4	49	349.6	52.6	41.4	<10	<10

Table S4. Neutralising antibody titres of human plasma obtained from individuals who were infected with Omicron BA.2 after three doses of COVID-19 vaccines

Sample ID	Gender	Age	Documented SARS-CoV-2 infection	Vaccine type	Days post-onset	FRNT50: 50% focus reduction neutralisation titre				
						(A)	Omicron/BA.2	Omicron/BA.5	Omicron/BQ.1.1	Omicron/XBB
						SARS-CoV-2/UT-NC002-1T/Human/2020/Tokyo	hCoV-19/Japan/UT-NCD1288-2N-2022	hCoV-19/Japan/TY41-702/2022	hCoV-19/Japan/TY41-796/2022	hCoV-19/Japan/TY41-795/2022
HPCo-383	F	56	Yes (BA.2)	mRNA-1273 x 2, BNT162b2 x 1	29	425.6	46.4	51.8	<10	<10
HP-S(H)0377	M	29	Yes (BA.2)	BNT162b2x 3	44	499.4	227.7	103.2	30.2	13.6
HP-S(H)0380	M	22	Yes (BA.2)	BNT162b2x 3	42	1452.2	262.9	194.2	16.9	13.9
HP-S(H)0381	M	22	Yes (BA.2)	BNT162b2x 3	44	650.4	218.6	161.5	<10	10
HP-S(H)0382	M	21	Yes (BA.2)	BNT162b2x 3	44	4222.7	656.6	399.2	94.4	25.1
HP-S(H)0383	M	18	Yes (BA.2)	BNT162b2x 3	42	648.9	377.7	258.4	76.9	14.9
HP-S(H)0882	M	23	Yes (BA.2)	BNT162b2x 3	89	601.7	156.5	92.7	<10	<10
HP-S(H)0883	M	24	Yes (BA.2)	BNT162b2x 3	84	3532.7	595.1	219.5	66.4	28.6
HP-S(H)0888	M	21	Yes (BA.2)	BNT162b2x 3	89	3813.5	734.8	300	72.5	36.7
HP-S(H)1056	M	22	Yes (BA.2)	BNT162b2x 3	42	3448.3	1141.6	417.2	288.1	252.7

Supplementary References

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