

Supplemental information

A delicate balance between antibody evasion and ACE2 affinity for Omicron BA.2.75

Jiandong Huo, Aiste Dijokaite-Guraliuc, Chang Liu, Daming Zhou, Helen M. Ginn, Raksha Das, Piyada Supasa, Muneeswaran Selvaraj, Rungtiwa Nutalai, Aekkachai Tuekprakhon, Helen M.E. Duyvesteyn, Alexander J. Mentzer, Donal Skelly, Thomas G. Ritter, Ali Amini, Sagida Bibi, Sandra Adele, Sile Ann Johnson, Neil G. Paterson, Mark A. Williams, David R. Hall, Megan Plowright, Thomas A.H. Newman, Hailey Hornsby, Thushan I. de Silva, Nigel Temperton, Paul Klenerman, Eleanor Barnes, Susanna J. Dunachie, Andrew J. Pollard, Teresa Lambe, Philip Goulder, OPTIC consortium, ISARIC4C consortium, Elizabeth E. Fry, Juthathip Mongkolsapaya, Jingshan Ren, David I. Stuart, and Gavin R. Screaton

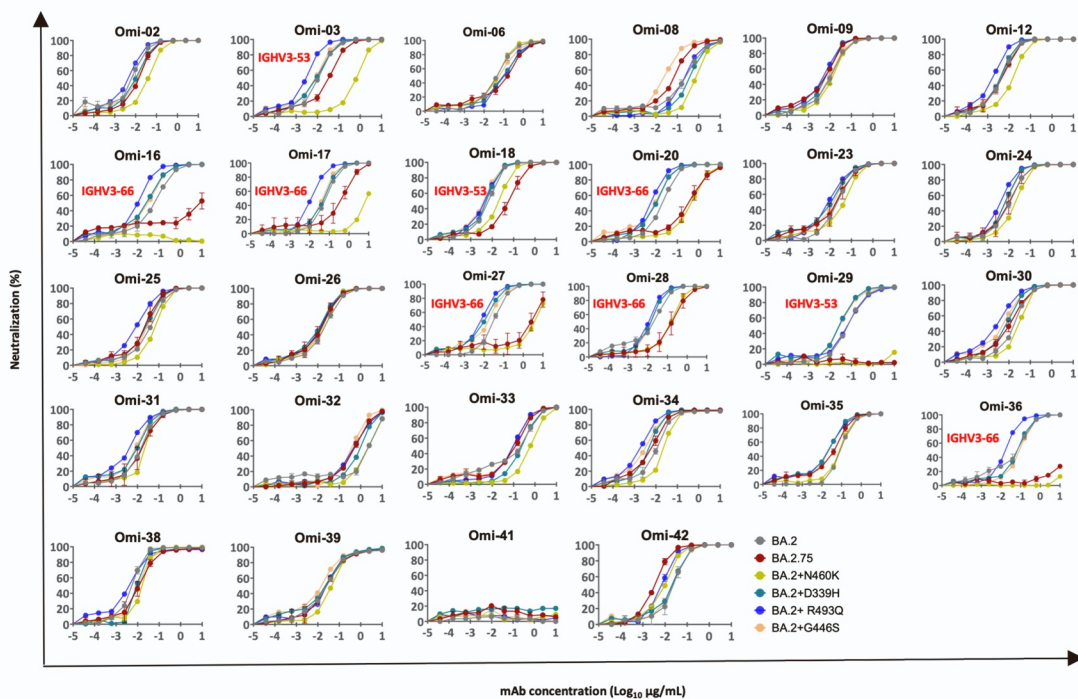


Figure S1 Pseudoviral neutralization assays against monoclonal antibodies. (A) Neutralization curves for a panel of 28 monoclonal antibodies made from samples taken from vaccinees infected with BA.1. Titration curves for single mutations of BA.2.27 in the BA.2 background are compared with BA.2 and BA.2.75. IC50 titres are shown in Table S2. Related to Figure 5. All assays have been done at least twice.

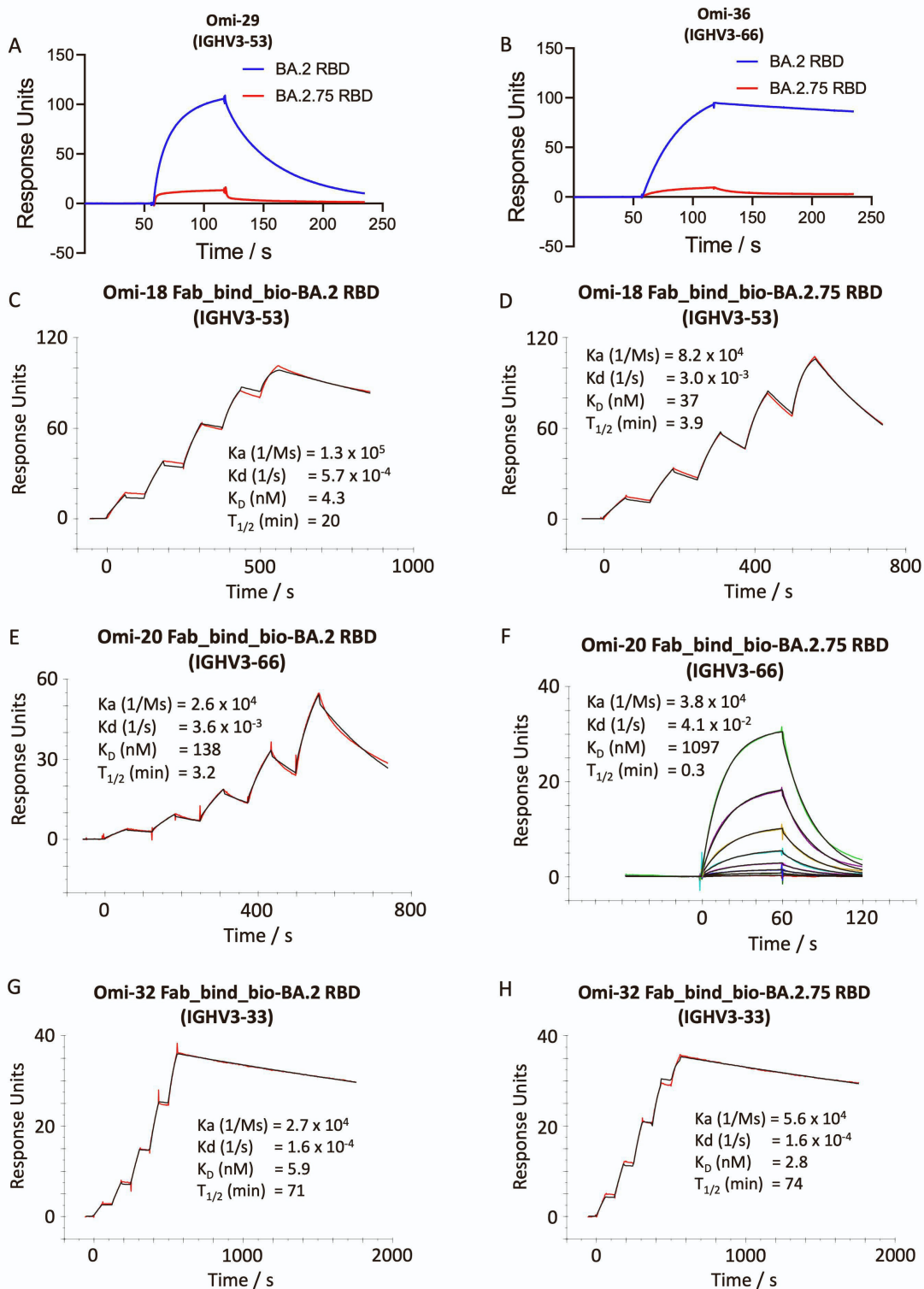


Figure S2 Surface plasmon resonance (SPR) analysis of interaction between BA.2 or BA.2.75 RBD and selected mAbs. (A) Binding of Omi-29 (IGHV3-53) to BA.2.75 RBD is severely reduced compared to that of BA.2, as shown by a single-injection of 1 μ M Omi-29 Fab over sample flow cells containing biotinylated BA.2 or BA.2.75 RBD. (B) Binding of Omi-36 (IGHV3-66) to BA.2.75 RBD is severely reduced compared to that of BA.2, as shown by a single-injection of 0.2 μ M BA.2 or BA.2.75 RBD over sample flow cells containing Omi-36 in the IgG form. (C-H) Sensorgrams (Red / Coloured: original binding curve; black: fitted curve) showing the interactions between BA.2 or BA.4/5 RBD and selected mAbs, with kinetics data shown. Related to Figure 5.

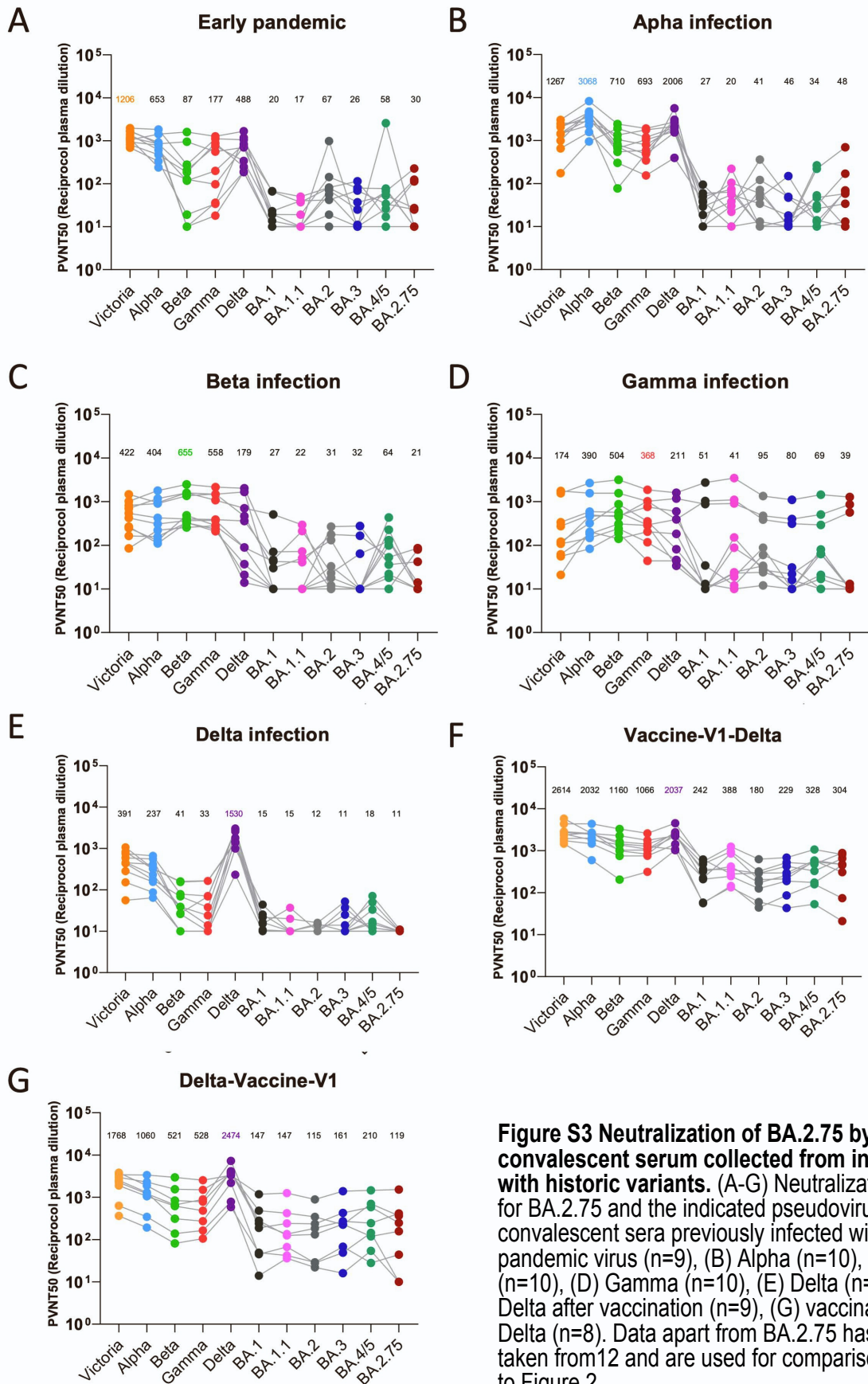


Figure S3 Neutralization of BA.2.75 by panels of convalescent serum collected from infection with historic variants. (A-G) Neutralization titres for BA.2.75 and the indicated pseudoviruses using convalescent sera previously infected with (A) Early pandemic virus (n=9), (B) Alpha (n=10), (C) Beta (n=10), (D) Gamma (n=10), (E) Delta (n=10), (F) Delta after vaccination (n=9), (G) vaccination after Delta (n=8). Data apart from BA.2.75 has been taken from 12 and are used for comparison. Related to Figure 2.

Figure S3

Table S1. (A) IC50 of BA.1 mAbs against Victoria and Omicron variant PV including BA.2.75 and BA.2+N460K (related to Figure 5A)

mAbs	Victoria	BA.1	BA.1.1	BA.2	BA.3	BA.4/5	BA.2.75	BA.2+N460K
Omi-02	0.002 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.003 ± 0.001	0.019 ± 0.007	>10	0.009 ± 0.002	0.025 ± 0.003
Omi-03 (3-53)	0.003 ± 0.000	0.005 ± 0.002	0.003 ± 0.001	0.008 ± 0.001	0.022 ± 0.003	0.017 ± 0.005	0.017 ± 0.000	0.401 ± 0.026
Omi-06	0.007 ± 0.000	0.017 ± 0.003	0.139 ± 0.033	0.039 ± 0.008	0.696 ± 0.106	>10	0.063 ± 0.005	0.026 ± 0.002
Omi-08	0.008 ± 0.004	0.003 ± 0.000	0.002 ± 0.000	0.114 ± 0.045	0.032 ± 0.001	0.086 ± 0.005	0.036 ± 0.002	0.552 ± 0.090
Omi-09	0.006 ± 0.002	0.005 ± 0.000	0.005 ± 0.002	0.008 ± 0.002	0.017 ± 0.002	0.166 ± 0.007	0.003 ± 0.000	0.010 ± 0.002
Omi-12	0.006 ± 0.002	0.002 ± 0.000	0.002 ± 0.001	0.003 ± 0.001	0.006 ± 0.001	0.429 ± 0.060	0.003 ± 0.001	0.011 ± 0.002
Omi-16 (3-66)	0.014 ± 0.003	0.012 ± 0.002	0.011 ± 0.003	0.034 ± 0.012	0.111 ± 0.008	0.029 ± 0.007	>10	>10
Omi-17 (3-66)	0.023 ± 0.011	0.018 ± 0.012	0.022 ± 0.009	0.060 ± 0.004	0.123 ± 0.002	0.028 ± 0.001	0.255 ± 0.169	>10
Omi-18 (3-53)	0.008 ± 0.003	0.002 ± 0.000	0.002 ± 0.000	0.005 ± 0.000	0.006 ± 0.002	0.005 ± 0.001	0.035 ± 0.007	0.014 ± 0.002
Omi-20 (3-66)	0.009 ± 0.002	0.006 ± 0.001	0.005 ± 0.001	0.015 ± 0.003	0.020 ± 0.004	0.014 ± 0.006	0.178 ± 0.075	0.315 ± 0.142
Omi-23	0.005 ± 0.002	0.029 ± 0.006	0.023 ± 0.12	0.019 ± 0.005	0.011 ± 0.000	>10	0.011 ± 0.006	0.022 ± 0.005
Omi-24	0.005 ± 0.000	0.006 ± 0.002	0.054 ± 0.015	0.007 ± 0.001	0.009 ± 0.002	>10	0.008 ± 0.004	0.014 ± 0.000
Omi-25	0.005 ± 0.001	0.023 ± 0.005	0.027 ± 0.005	0.024 ± 0.004	0.050 ± 0.004	>10	0.014 ± 0.005	0.050 ± 0.010
Omi-26	0.002 ± 0.001	0.006 ± 0.002	0.005 ± 0.001	0.013 ± 0.001	0.018 ± 0.002	>10	0.010 ± 0.004	0.010 ± 0.000
Omi-27 (3-66)	0.008 ± 0.003	0.026 ± 0.006	0.034 ± 0.009	0.034 ± 0.005	0.026 ± 0.007	0.069 ± 0.023	6.672 ± 4.466	>10
Omi-28 (3-66)	0.022 ± 0.000	0.011 ± 0.004	0.009 ± 0.002	0.008 ± 0.000	0.019 ± 0.000	0.028 ± 0.009	0.133 ± 0.082	0.103 ± 0.048
Omi-29 (3-53)	0.014 ± 0.006	0.017 ± 0.003	0.016 ± 0.009	0.056 ± 0.014	0.064 ± 0.017	0.396 ± 0.007	>10	>10
Omi-30	0.012 ± 0.002	0.008 ± 0.003	0.008 ± 0.004	0.011 ± 0.002	0.015 ± 0.003	>10	0.008 ± 0.002	0.018 ± 0.001
Omi-31	0.376 ± 0.090	0.029 ± 0.002	0.031 ± 0.012	0.013 ± 0.002	0.013 ± 0.004	>10	0.014 ± 0.008	0.015 ± 0.001
Omi-32	0.010 ± 0.006	0.017 ± 0.000	>10	2.682 ± 0.553	1.018 ± 0.139	0.035 ± 0.016	0.354 ± 0.064	2.341 ± 0.282
Omi-33	0.027 ± 0.011	0.014 ± 0.005	0.042 ± 0.018	0.068 ± 0.022	0.133 ± 0.021	0.013 ± 0.004	0.053 ± 0.006	0.490 ± 0.156
Omi-34	0.007 ± 0.004	0.008 ± 0.001	0.062 ± 0.004	0.009 ± 0.003	0.014 ± 0.000	>10	0.005 ± 0.000	0.020 ± 0.001
Omi-35	0.018 ± 0.004	0.058 ± 0.006	0.381 ± 0.061	0.094 ± 0.004	0.044 ± 0.018	1.687 ± 0.441	0.020 ± 0.000	0.056 ± 0.012
Omi-36 (3-66)	0.022 ± 0.004	0.009 ± 0.003	0.009 ± 0.003	0.030 ± 0.014	0.178 ± 0.048	0.024 ± 0.006	>10	>10
Omi-38	0.015 ± 0.004	0.024 ± 0.015	>10	0.005 ± 0.000	0.008 ± 0.002	0.005 ± 0.001	0.011 ± 0.005	0.010 ± 0.001
Omi-39	0.014 ± 0.002	0.009 ± 0.004	>10	0.026 ± 0.011	0.014 ± 0.001	0.035 ± 0.003	0.027 ± 0.009	0.045 ± 0.017
Omi-41	>10	0.053 ± 0.028	0.037 ± 0.002	>10	0.032 ± 0.007	>10	>10	>10
Omi-42	0.013 ± 0.004	0.007 ± 0.004	0.006 ± 0.002	0.021 ± 0.011	0.025 ± 0.012	0.013 ± 0.001	0.003 ± 0.000	0.007 ± 0.002

(B) IC50 of commercial mAbs against PV BA.2.75 (related to Figure 5B)

	IC50 (µg/mL)						
	Victoria	BA.1	BA.1.1	BA.2	BA.3	BA.4/5	BA.2.75
REGN10987	0.002 ± 0.001	>10	>10	0.616 ± 0.347	>10	>10	>10
REGN10933	0.001 ± 0.002	>10	>10	>10	>10	>10	>10
AZD1061	0.002 ± 0.001	0.308 ± 0.058	>10	0.008 ± 0.003	0.019 ± 0.007	0.015 ± 0.004	0.021 ± 0.002
AZD8895	0.001 ± 0.000	0.246 ± 0.027	0.100 ± 0.053	1.333 ± 0.317	>10	>10	0.008 ± 0.000
AZD7442	0.001 ± 0.000	0.232 ± 0.113	0.806 ± 0.093	0.008 ± 0.001	0.065 ± 0.011	0.065 ± 0.007	0.017 ± 0.003
ADG10	0.007 ± 0.002	>10	>10	>10	>10	>10	>10
ADG20	0.003 ± 0.002	0.348 ± 0.169	0.253 ± 0.070	>10	>10	>10	>10
ADG30	0.014 ± 0.006	>10	>10	>10	>10	>10	>10
Ly-CoV555	0.002 ± 0.000	>10	>10	>10	>10	>10	>10
Ly-CoV16	0.014 ± 0.010	>10	>10	>10	>10	>10	>10
Ly-CoV1404	0.001 ± 0.000	0.002 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000
S309	0.079 ± 0.027	0.113 ± 0.006	0.142 ± 0.012	0.638 ± 0.154	0.311 ± 0.023	0.689 ± 0.041	0.202 ± 0.017

Table S2. IC50 of BA.1 mAbs against PV BA.2, BA.2.75 and BA.2 with each of the four BA.2.75 mutations (see also Figure S1, related to Figure 5)

mAbs	IC50 (µg/ml)					
	BA.2	BA.2+D339H	BA.2+R493Q	BA.2+G446S	BA.2.+ N460K	BA.2.75
Omi02	0.003 ± 0.000	0.007 ± 0.003	0.003 ± 0.000	0.007 ± 0.002	0.025 ± 0.003	0.009 ± 0.002
Omi03	0.008 ± 0.001	0.006 ± 0.000	0.002 ± 0.001	0.005 ± 0.001	0.401 ± 0.026	0.017 ± 0.000
Omi06	0.039 ± 0.008	0.012 ± 0.002	0.023 ± 0.010	0.087 ± 0.002	0.026 ± 0.002	0.063 ± 0.005
Omi08	0.114 ± 0.045	0.250 ± 0.009	0.194 ± 0.020	0.017 ± 0.001	0.552 ± 0.090	0.036 ± 0.002
Omi09	0.008 ± 0.002	0.005 ± 0.001	0.003 ± 0.000	0.006 ± 0.001	0.010 ± 0.002	0.003 ± 0.000
Omi12	0.003 ± 0.001	0.003 ± 0.001	0.001 ± 0.000	0.003 ± 0.001	0.011 ± 0.002	0.003 ± 0.001
Omi16	0.034 ± 0.012	0.014 ± 0.004	0.008 ± 0.003	0.018 ± 0.004	>10	>10
Omi17	0.060 ± 0.004	0.036 ± 0.015	0.013 ± 0.001	0.038 ± 0.002	>10	0.255 ± 0.169
Omi18	0.005 ± 0.000	0.003 ± 0.000	0.004 ± 0.000	0.003 ± 0.000	0.014 ± 0.002	0.035 ± 0.007
Omi20	0.015 ± 0.003	0.007 ± 0.000	0.005 ± 0.001	0.005 ± 0.001	0.315 ± 0.142	0.178 ± 0.075
Omi23	0.019 ± 0.005	0.006 ± 0.000	0.007 ± 0.000	0.010 ± 0.002	0.022 ± 0.005	0.011 ± 0.006
Omi24	0.007 ± 0.001	0.005 ± 0.001	0.004 ± 0.000	0.005 ± 0.000	0.014 ± 0.000	0.008 ± 0.004
Omi25	0.024 ± 0.004	0.016 ± 0.003	0.007 ± 0.002	0.022 ± 0.000	0.050 ± 0.010	0.014 ± 0.005
Omi26	0.013 ± 0.001	0.007 ± 0.002	0.008 ± 0.001	0.008 ± 0.002	0.010 ± 0.000	0.010 ± 0.004
Omi27	0.034 ± 0.006	0.007 ± 0.001	0.007 ± 0.001	0.011 ± 0.001	>10	6.672 ± 4.466
Omi28	0.008 ± 0.000	0.009 ± 0.001	0.010 ± 0.001	0.014 ± 0.000	0.103 ± 0.048	0.133 ± 0.082
Omi29	0.056 ± 0.014	0.018 ± 0.006	0.042 ± 0.012	0.024 ± 0.002	>10	>10
Omi30	0.013 ± 0.002	0.006 ± 0.001	0.002 ± 0.000	0.003 ± 0.000	0.018 ± 0.001	0.008 ± 0.002
Omi31	0.011 ± 0.002	0.005 ± 0.001	0.003 ± 0.000	0.005 ± 0.001	0.015 ± 0.001	0.014 ± 0.008
Omi32	2.614 ± 0.533	0.683 ± 0.179	0.312 ± 0.008	0.330 ± 0.010	2.341 ± 0.282	0.354 ± 0.064
Omi33	0.070 ± 0.024	0.177 ± 0.035	0.063 ± 0.008	0.043 ± 0.016	0.490 ± 0.156	0.053 ± 0.006
Omi34	0.009 ± 0.003	0.004 ± 0.000	0.002 ± 0.000	0.002 ± 0.000	0.020 ± 0.001	0.005 ± 0.000
Omi35	0.092 ± 0.004	0.012 ± 0.003	0.017 ± 0.011	0.014 ± 0.006	0.056 ± 0.012	0.020 ± 0.000
Omi36	0.030 ± 0.014	0.036 ± 0.002	0.013 ± 0.003	0.067 ± 0.015	>10	>10
Omi38	0.005 ± 0.000	0.011 ± 0.000	0.003 ± 0.001	0.010 ± 0.000	0.010 ± 0.001	0.011 ± 0.005
Omi39	0.026 ± 0.011	0.012 ± 0.002	0.021 ± 0.007	0.009 ± 0.002	0.045 ± 0.017	0.027 ± 0.009
Omi41	>10	>10	>10	>10	>10	>10
Omi42	0.021 ± 0.011	0.011 ± 0.002	0.006 ± 0.001	0.016 ± 0.002	0.007 ± 0.002	0.003 ± 0.000

	Delta-V1-Vaccine	Vaccine-V1_Delta	Delta	Gamma	Beta	Alpha	Early pandemic	BNT162b2 V3+28	AZV3+28	BA.4/5 infection	BA.2 infection	BA.1 infection
Participants												
Female	7	7	7	7	7	7	7	7	7	7	7	7
Male	4	4	3	4	5	6	9	10	21	5	4	7
Median Age (Y)	41 (Range 31-54)	40 (Range 28-70)	26 (Range 12-36)	32 (Range 23-49)	47 (Range 16-64)	57 (Range 29-76)	60 (Range 53-69)	45 (Range 30-59)	37 (Range 25-53)	42 (Range 20-94)	41 (Range 22-57)	22 (Range 21-56)

Table S3. Sample participant information.

Table S4. Primers used for site-directed PCR mutagenesis to generate the BA.2.75 construct using the BA.2 Spike construct as template (related to methods)

Primer ID	Sequence
D339H_pNeoF	5' -GGTTGCGTAGCTGAAACCGGTACCAATCTGTGCCCTTCCACGAGGTGTTCAATGCCACC-3'
G446S_F	5' -CAAAC TAGATTTCGAAAGTTAGCGGCAATTACAATTACCTG-3'
G446S_R	5' -CAGGTAATTGTAATTGCCGCTAACTTTCGAATCTAGTTTG-3'
N460K_F	5' -CAGACTGTTTCAGAAAGAGCAAACCTGAAGCCTTTCGAGAGAGAC-3'
N460K_R	5' -GTCTCTCTCGAAAGGCTTCAGTTTGCTCTTCTGAACAGTCTG-3'
R493Q_F (RBD)	5' -CAATTGCTACTTCCCTCTGCAGAGCTACGGCTTCAGACCTACC-3'
R493Q_R (RBD)	5' -GGTAGGTCTGAAGCCGTAGCTCTGCAGAGGGAAGTAGCAATTG-3'
RBD333_BAP_R	5' -GTCATTCAGCAAGCTCTTCTTGCCGCACACGGTAGC-3'
pNeoRBD333Omi_F	5' -GGTTGCGTAGCTGAAACCGGTCATCACCATCACCATCACACCAATCTGTGCCCTTTCGAC-3'
K147E_W152R_F157L_F	5' -CGTTTATTATCATGAGAACAACAAGAGCAGGATGGAGAGCGAGTTACGCGTATATTCGTCGGC-3'
K147E_W152R_F157L_R	5' -GCCGACGAATATACGCGTAACTCGCTCTCCATCCTGCTCTTGTGTTCTCATGATAATAAACG-3'
I210L_F	5' -CAGCAAGCACACACCCGTTAATCTGGGCAGAGACC-3'
I210L_R	5' -GGTCTCTGCCAGATTAACGGGTGTGTGCTTGCTG-3'
G275S_F	5' -GCGATTCGTCAAGCAGTTGGACCGCTGGAGC-3'
G275S_R	5' -GCTCCAGCGGTCCAACCTGCTTGACGAATCGC-3'
D339H_F	5' -CAATCTGTGCCCTTCCACGAGGTGTTCAATGC-3'
D339H_R	5' -GCATTGAACACCTCGTGGAAGGGCAGAGATTG-3'
G446S_N460K_F	5' -GAACTCTAACAACTAGATTTCGAAAGTTAGCGGCAATTACAATTACCTGTACAGACTGTTTCAGAAAGAGCAAGCTGAAGCCTTTCGAGAG-3'
G446S_N460K_R	5' -CTCTCGAAAGGCTTCAGCTTGCTCTTCTGAACAGTCTGTACAGGTAATTGTAATTGCCGCTAACTTTCGAATCTAGTTTGTAGAGTTC-3'
R493Q_F	5' -GCTTCAATTGCTACTTCCCTCTGCAGAGCTACGGCTTCAGACCTACC-3'
R493Q_R	5' -GGTAGGTCTGAAGCCGTAGCTCTGCAGAGGGAAGTAGCAATTGAAGC-3'

Table S5. X-ray data collection and structure refinement statistics (related to Figure 4)

^a Values in parentheses are for highest-resolution shell.

Structure	BA.2.75 RBD/ACE2
PDB ID	8ASY
Data collection	
Space group	P4 ₁ 2 ₁ 2
Cell dimensions	
a, b, c (Å)	105.3, 105.3, 220.8
a, b, g (°)	90, 90, 90
Resolution (Å)	76–2.85 (2.80–2.85) ^a
R _{merge}	0.443 (---)
R _{pim}	0.086 (1.401)
I/s(I)	7.6 (0.4)
CC _{1/2}	0.971 (0.279)
Completeness (%)	99.8 (96.9)
Redundancy	26.8 (25.7)
Refinement	
Resolution (Å)	76–2.85
No. reflections	2089/1439
R _{work} / R _{free}	0.217/0.265
No. atoms	
Protein	6464
Ligand/ion/water	167
B factors (Å ²)	
Protein	86
Ligand/ion/water	108
r.m.s. deviations	
Bond lengths (Å)	0.002
Bond angles (°)	0.4