THE LANCET
Infectious Diseases

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Sheward DJ, Kim C, Fischbach J, et al. Evasion of neutralising antibodies by omicron sublineage BA.2.75. *Lancet Infect Dis* 2022; published online Sept 1. https://doi.org/10.1016/S1473-3099(22)00524-2.

Appendix

Evasion of neutralizing antibodies by Omicron sublineage BA.2.75

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Supplementary Figures and Tables

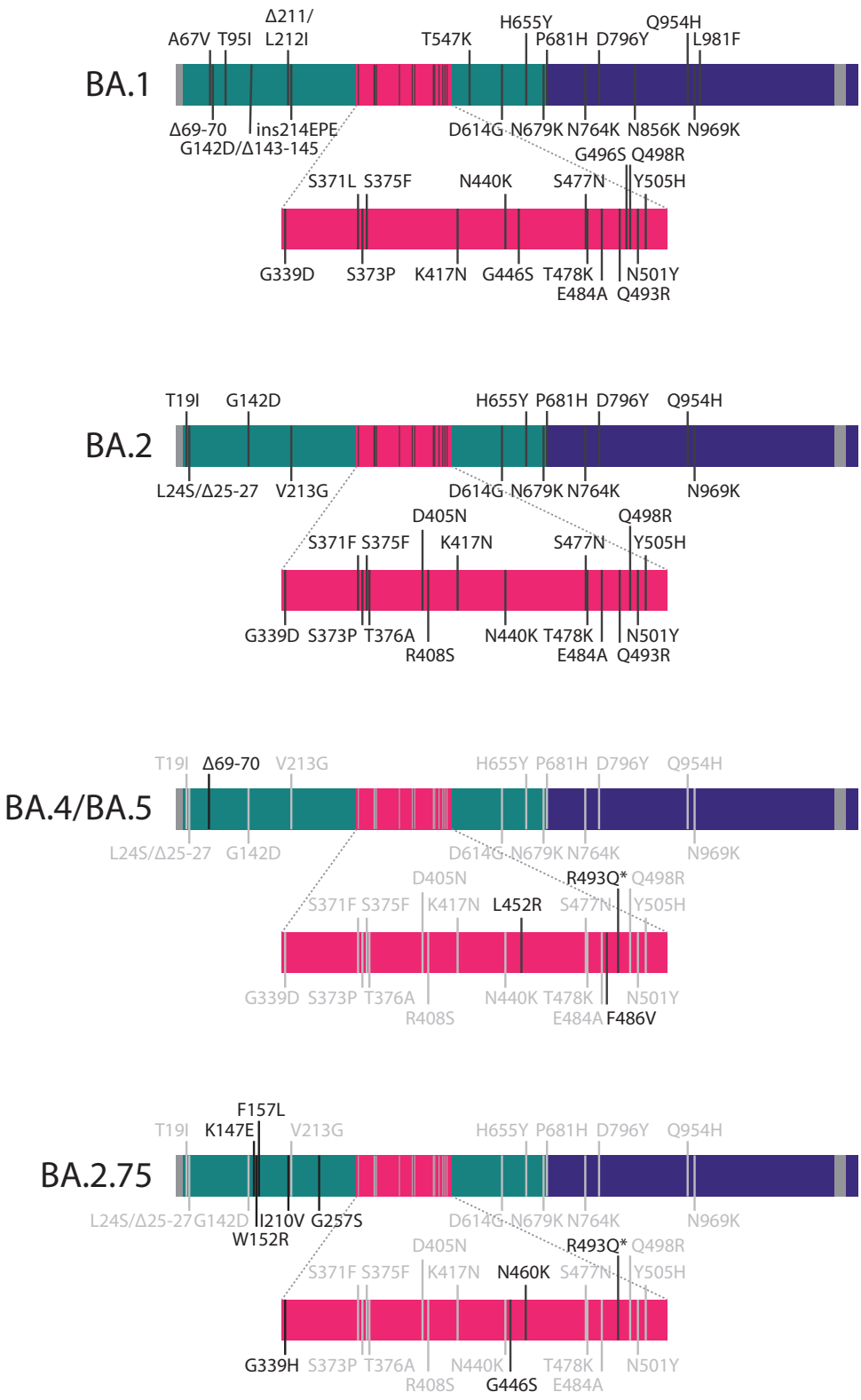

Figure S1. List of spike mutations in Omicron sub-variants BA.1, BA.2, BA.4/5, and BA.2.75.
*indicates a reversion to the ancestral amino acid.

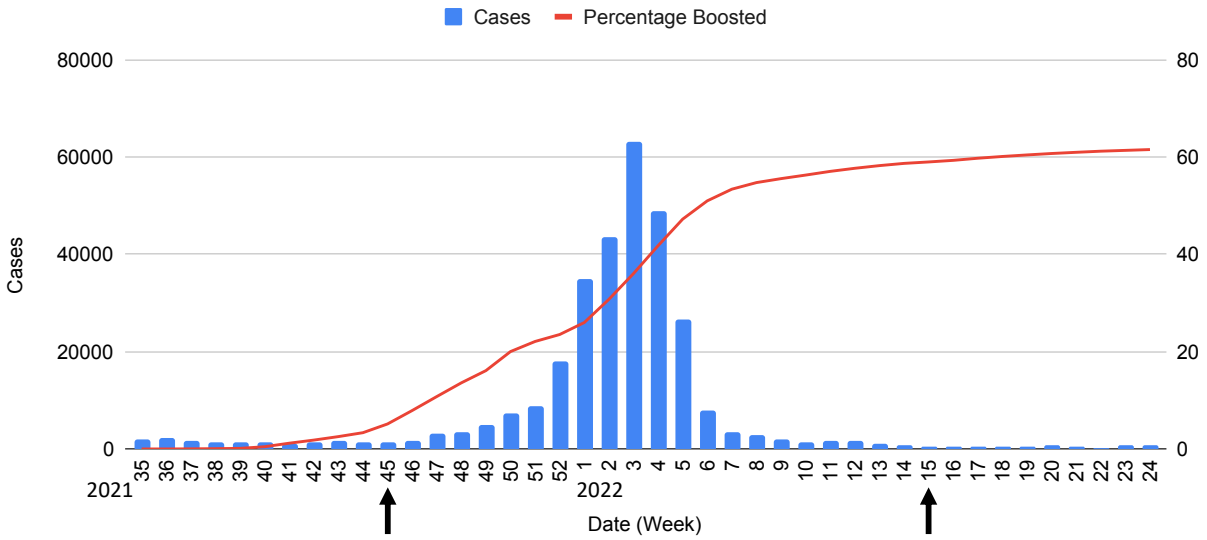

Figure S2. Timecourse of SARS-CoV-2 infections and vaccinations in Stockholm, Sweden. Depicted are numbers of confirmed SARS-CoV-2 cases in Stockholm per week[1] (blue), as well as cumulative percentage of individuals, 18 and older, who had received their 3rd dose "booster" vaccination[2] (red). The date range spans Sweden's first Omicron infection wave, which was initially dominated by BA.1, but followed by BA.2 cases[3]. Black arrows denote the weeks from which our serum samples were obtained. The first sampling point is prior to any confirmed Omicron infections, and the second is after the bulk of BA.1 and BA.2 infections, but prior to the arrival of BA.4 or BA.5.

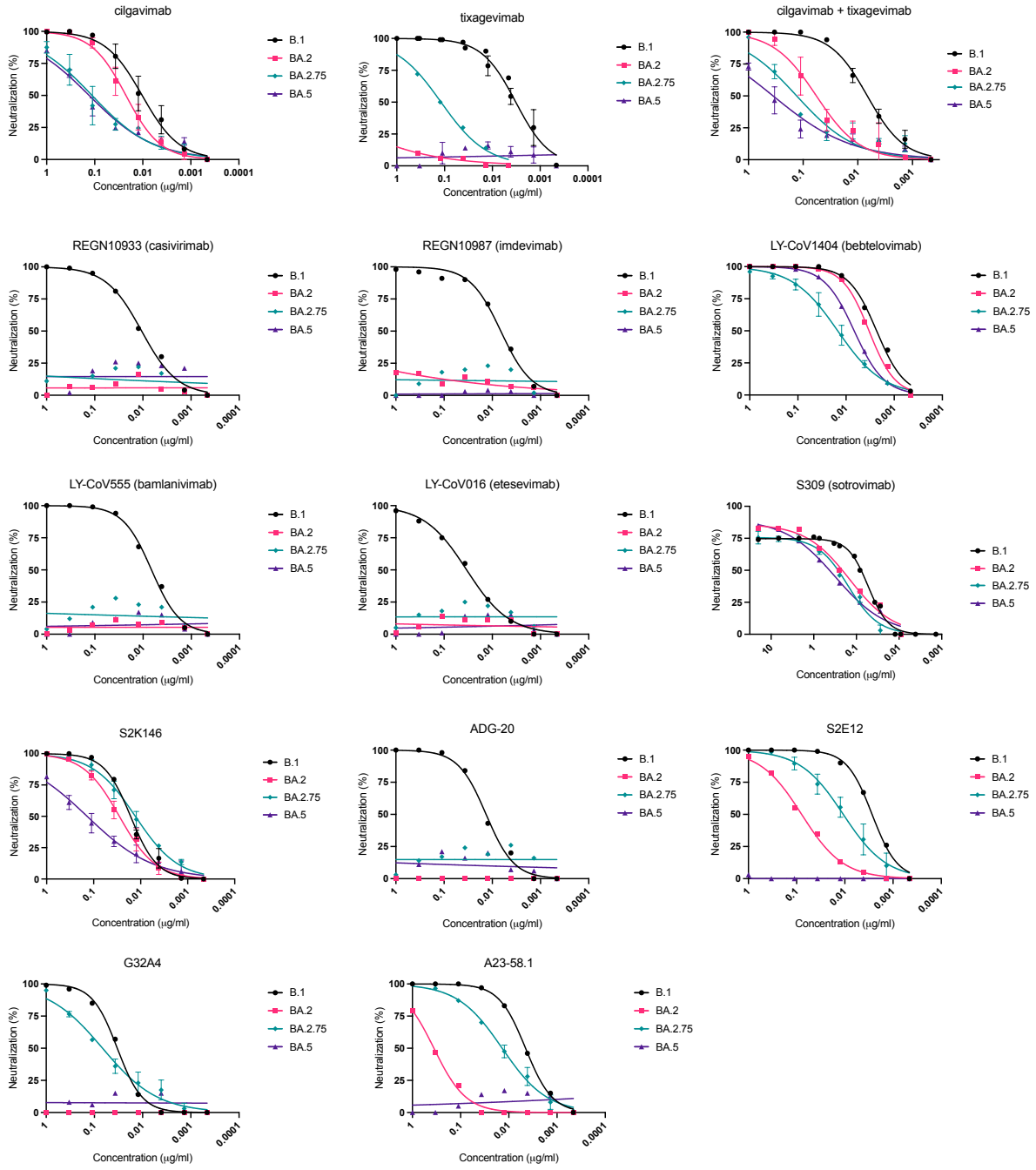

Figure S3. Neutralization curves for monoclonal antibodies against B.1 (D614G), and Omicron sublineages BA.2, BA.5 and BA.2.75.

Table S1. Relative sensitivity of BA.2.75 to therapeutic and pre-clinical monoclonal antibodies. IC_{50} titers (50% inhibitory concentration, in ng/μl) in a pseudovirus neutralization assay are tabulated for monoclonal antibodies against B.1 and Omicron sublineages BA.2, BA.5 and BA.2.75.

	B.1	BA.2	BA.5	BA.2.75
cilgavimab[4]	10	22	130	114
tixagevimab[4]	3	>1,000	>1,000	133
S309 (sotrovimab)[5]	79	214	426	269
REGN10933 (casivirimab)[6]	11	>1,000	>1,000	>1,000
REGN10987 (imdevimab)[6]	7	>1,000	>1,000	>1,000
LY-CoV1404 (bebtelovimab)[7]	2	3	7	15
LY-CoV555 (bamlanivimab)[8]	7	>1,000	>1,000	>1,000
LY-CoV016 (etesevimab)[9]	34	>1,000	>1,000	>1,000
S2E12[10]	3	79	>1,000	8
S2K146[11]	15	35	180	15
ADG20[12]	13	>1,000	>1,000	>1,000
A23-58.1[13]	4	358	>1,000	13
G32A4[14]	34	>1,000	>1,000	78

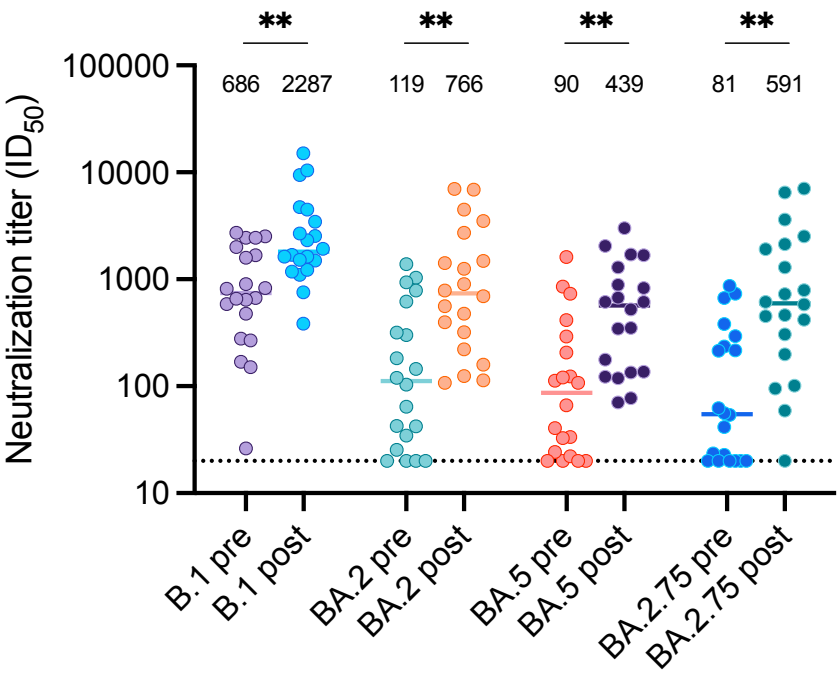

Fig S4. Comparison of 'pre-wave' and 'post-wave' neutralizing titers. Neutralizing ID50s (50% inhibitory dilution) are shown for serum from blood donated in Stockholm, Sweden during week 45, 2021 (8 Nov - 14 Nov), prior to a wave of infections dominated by BA.1 and BA.2 (**pre**), and week 15, 2022 (11 Apr - 17 Apr), after the infection wave (**post**). Depicted above are the geometric mean ID_{50} titres. Sera with ID_{50} less than the lowest dilution tested (20, dotted line) are plotted as 20. ** P<0.01; *** P<0.001 (Mann-Whitney U test, corrected for multiple comparisons using the Holm procedure).

Methods

Cell culture

HEK293T cells (ATCC CRL-3216) and HEK293T-ACE2 cells (stably expressing human ACE2) were cultured in Dulbecco's Modified Eagle Medium (high glucose, with sodium pyruvate) supplemented with 10% fetal bovine serum, 100 units/ml Penicillin, and 100 µg/ml Streptomycin. Cultures were maintained in a humidified 37°C incubator (5% CO_2).

Pseudovirus Neutralization Assay

Pseudovirus neutralization assay was performed as previously[15]. Briefly, spike-pseudotyped lentivirus particles were generated by co-transfection of HEK293T cells with a relevant spike plasmid, an HIV gag-pol packaging plasmid (Addgene #8455), and a lentiviral transfer plasmid encoding firefly luciferase (Addgene #170674) using polyethylenimine. The BA.2.75 spike plasmid was generated by introducing the following mutations into the BA.2 spike, by multi-site directed mutagenesis: K147E, W152R, F157L, I210V, G257S G339H, G446S, N460K, R493Q, which was subsequently confirmed by sequencing.

Neutralization was assessed in HEK293T-ACE2 cells. Pseudoviruses sufficient to produce ±100,000 RLU were incubated with serial 3-fold dilutions of serum for 60 minutes at 37°C in a black-walled 96-well plate. 10,000 HEK293T-ACE2 cells were then added to each well, and plates were incubated at 37°C for 48 hours. Luminescence was measured using Bright-Glo (Promega) on a GloMax Navigator Luminometer (Promega). Neutralization was calculated relative to the average of 8 control wells infected in the absence of serum.

Monoclonal antibodies

Cilgavimab and tixagevimab were evaluated as their clinical formulations. For the rest of the monoclonal antibodies evaluated, antibody sequences were extracted from deposited RCSB entries, synthesized as gene fragments, cloned into pTWIST transient expression vectors by Gibson assembly or restriction cloning, expressed and purified, all as previously described[16].

Serum samples

Serum samples from anonymized blood donors from Stockholm, Sweden, were obtained from week 45, 2021 (prior to the BA.1/BA.2 Omicron infection wave), and from week 15, 2022 (after the BA.1/BA.2 Omicron infection wave, but prior to the arrival of BA.4 or BA.5); see Fig S2. 25 serum samples from each time point were pre-screened for detectable neutralization activity against ancestral B.1 (D614G), and 20 samples with detectable activity against B.1 (D614G) for each time point were selected, randomly, for this study. Sera were heat inactivated at 56°C for 60 minutes prior to use in neutralization assays.

Ethical Statement

The blood donor samples were anonymized, and not subject to ethical approvals, as per advisory statement 2020–01807 from the Swedish Ethical Review Authority.

Statistical analysis

Individual ID_{50} and IC_{50} values for each sample against each variant were calculated in Prism v9 (GraphPad Software) by fitting a four-parameter logistic curve to neutralization by serial 3-fold dilutions of serum/antibody. Comparison of titers between variants was assessed using paired Wicoxon signed-rank tests. Comparison of titers pre- and post-wave was assessed

using unpaired Mann-Whitney tests. Correction for multiple comparisons was performed using the Holm procedure[17], implemented in MultipleTesting.jl, in the Julia language for Scientific Computing. P values are summarized as: ns P>0.05; * P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001.

Author contributions

Conceptualization, D.J.S., T.P.P., B.M.;
Formal analysis, D.J.S., B.M.;
Conducted the assays, D.J.S., C.K., J.F., T.P.P.;
Designed the methodology, D.J.S., C.K., R.E., T.P.P, B.M.;
Responsible for figures and tables, D.J.S., T.P.P., B.M.;
Resources, S.M., R.E., S.R, N.K.B., G.B.K.H., J.A., B.M.;
Oversaw the study, D.J.S., G.B.K.H., S.T.R., J.A., B.M;
Funding Acquisition, S.T.R., G.B.K.H., J.A., T.P.P., B.M.
Writing – original draft, D.J.S.;
Writing – review & editing, D.J.S., J.F., G.B.K.H., J.A., T.P.P., B.M.
D.J.S and B.M. were responsible for the decision to submit the manuscript for publication.

Acknowledgements

pCMV-dR8.2 dvpr was a gift from Bob Weinberg (Addgene plasmid # 8455; http://n2t.net/addgene:8455; RRID:Addgene_8455). pBOBI-FLuc was a gift from David Nemazee (Addgene plasmid # 170674; http://n2t.net/addgene:170674; RRID:Addgene_170674). We also wish to thank the researchers, globally, who contributed to the sequencing and identification of the emerging BA.2.75 variant.

Funding

This project was supported by funding from SciLifeLab's Pandemic Laboratory Preparedness program to B.M. (Reg no. VC-2022-0028) and to J.A. (Reg no. VC-2021-0033); from the Erling Persson Foundation to B.M and G.B.K.H; by the European Union's Horizon 2020 research and innovation programme under grant agreement no. 101003653 (CoroNAb) to G.B.K.H., S.T.R., and B.M; and by the G2P-UK National Virology consortium funded by MRC/UKRI (grant ref: MR/W005611/1) (T.P.P).

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