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Supplemental information

SARS-CoV-2 variants C.1.2 and B.1.621 (Mu) partially evade neutralization by antibodies elicited upon infection or vaccination

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Supplementary Table 1: COVID-19 patient data (Related to Figure 2).

ID	Age group (y)	Gender	WHO classification upon ICU admission (mild, moderate, severe, critical)	Time between symptom onset and sample collection (d)	Time between ICU admission and sample collection (d)	SARS-CoV-2 PANGO lineage
15	65-74	M	critical	unknown	8	unknown
18	65-74	F	critical	unknown	unknown	unknown
20	55-64	M	critical	unknown	unknown	unknown
22	25-34	F	critical	unknown	unknown	unknown
23	65-74	F	severe	unknown	unknown	unknown
24	55-64	M	critical	unknown	unknown	unknown
27	45-54	M	critical	unknown	8	unknown
33	75-84	M	critical	unknown	8	unknown
51	65-74	M	critical	unknown	8	unknown
56	65-74	M	critical	unknown	1	unknown

Abbreviation: ICU = intensive care unit; d = days; y = years; M = Male; F = Female

Supplementary Table 2: Vaccinated patient data (Related to Figure 2).

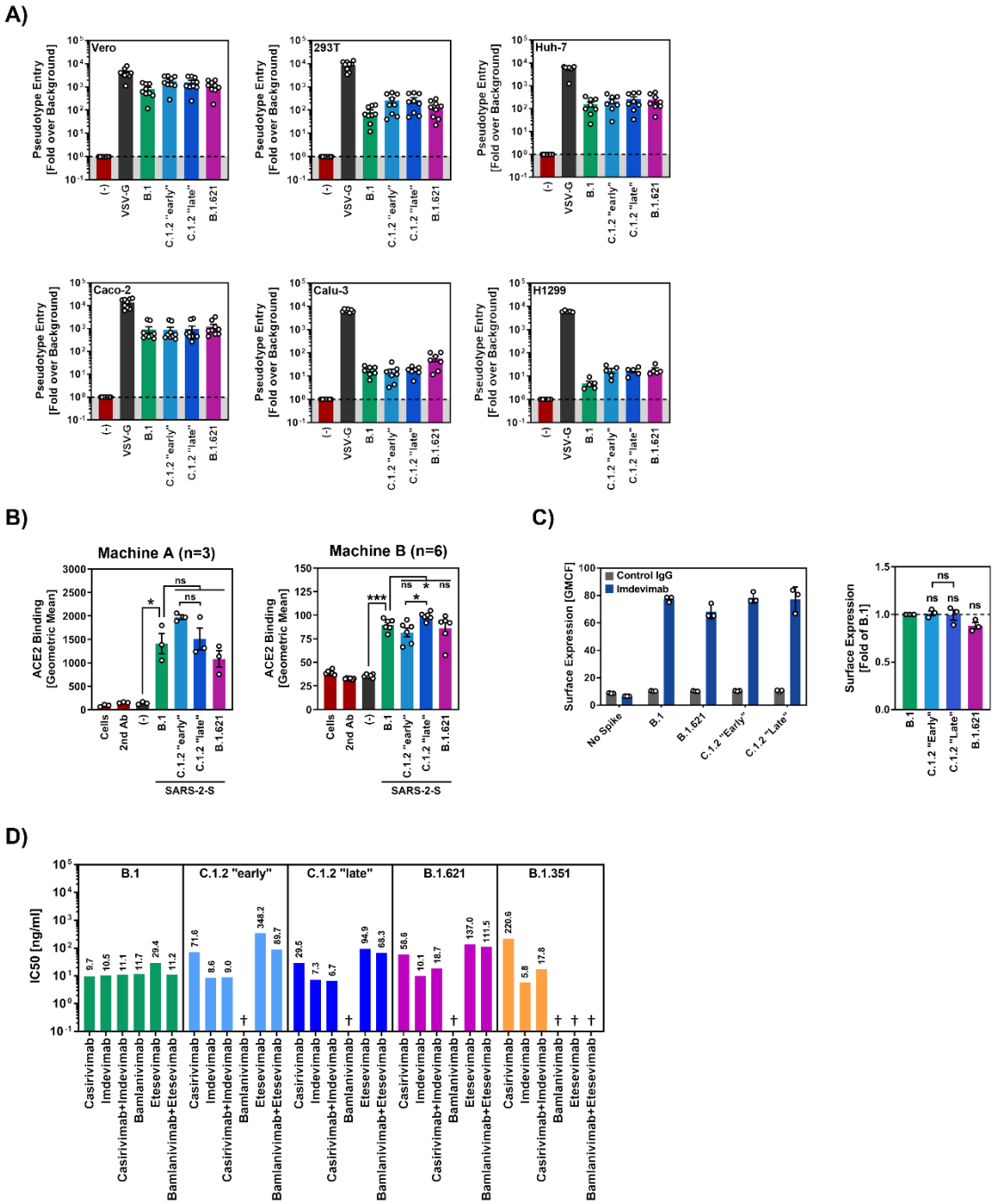
ID	Age group (y)	Gender	1st vaccination	2nd vaccination	Time between 1st & 2nd vaccination (d)	Time since 2nd vaccination (d)
L3	25-34	F	BNT162b2 (BNT)	BNT162b2 (BNT)	21	198
L4	35-44	F	BNT162b2 (BNT)	BNT162b2 (BNT)	21	197
L9	35-44	M	BNT162b2 (BNT)	BNT162b2 (BNT)	21	197
L11	18-24	M	BNT162b2 (BNT)	BNT162b2 (BNT)	23	190
L12	18-24	F	BNT162b2 (BNT)	BNT162b2 (BNT)	21	199
L13	18-24	F	BNT162b2 (BNT)	BNT162b2 (BNT)	21	204
L16	18-24	F	BNT162b2 (BNT)	BNT162b2 (BNT)	42	72
L22	18-24	M	BNT162b2 (BNT)	BNT162b2 (BNT)	21	199
L25	18-24	M	BNT162b2 (BNT)	BNT162b2 (BNT)	21	199
L26	18-24	M	BNT162b2 (BNT)	BNT162b2 (BNT)	21	186
6365	25-34	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	74	42
6205	55-64	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	71	27
6239	55-64	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	75	27
6297	45-54	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	81	29
6243	55-64	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	79	30
6262	55-64	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	71	30
6272	55-64	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	80	32
6321	25-34	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	77	33
6358	25-34	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	92	33
6236	55-64	F	ChAdOx1-SARS-COV-2 (AZ)	ChAdOx1-SARS-COV-2 (AZ)	76	34

Abbreviation: d = days; y = years; M = Male; F = Female

Supplementary Table 3: Primers used for cloning (Related to STAR Methods).

Primer name	Sequence
SARS-2-S (BamHI) F	AAGGCCGGATCCGCCACCATGTTCGTGTTTCTGGTGCTGC
SARS-2-SΔ18 (XbaI) R	AAGGCCTCTAGACTACTTGCAGCAGCTGCCACAG
SARS-2-S (P9L) F	GTTTCTGGTGCTGCTGCTTCTGGTGTCCAGCCAG
SARS-2-S (P9L) R	CTGGCTGGACACCAGAAGCAGCAGCACCAGAAAC
SARS-2-S (C136F) F	GCGAGTTCCAGTTCTTCAACGACCCCTTCCTG
SARS-2-S (C136F) R	CAGGAAGGGGTCGTTGAAGAACTGGAACTCGC
SARS-2-S (R190S) F	CAAGAACCTGAGCGAGTTCGTGTTC
SARS-2-S (R190S) R	ACACGAACTCGCTCAGGTTCTTGAAG
SARS-2-S (D215G) F	CAACCTCGTGCGGGGTCTGCCTCAGGGCTTC
SARS-2-S (D215G) R	GAAGCCCTGAGGCAGACCCCGCACGAGGTTG
SARS-2-S (Y449H) F	CAAAGTCGGCGGCAACCACAATTACCTGTACC
SARS-2-S (Y449H) R	GGTACAGGTAATTGTGGTTGCCGCCGACTTTG
SARS-2-S (E484K) F	TAACGGCGTGAAAGGCTTCAACTGCTACTTC
SARS-2-S (E484K) R	TGAAGCCTTTCACGCCGTTACAAGG
SARS-2-S (N501Y) F	TCAGCCCACATATGGCGTGGGCTATC
SARS-2-S (N501Y) R	CCCACGCCATATGTGGGCTGAAAGC
SARS-2-S (H655Y) F	CGGAGCCGAGTACGTGAACAATAGC
SARS-2-S (H655Y) R	TGTTCACGTACTCGGCTCCGATCAGAC
SARS-2-S (N679K) F	CCAGACACAGACAAAGAGCCCCAGACGGGCCAG
SARS-2-S (N679K) R	CTGGCCCGTCTGGGGCTCTTTGTCTGTGTCTGG
SARS-2-S (T716I) F	CTATCCCCATCAACTTCACCATCAGC
SARS-2-S (T716I) R	GTGAAGTTGATGGGGATAGCGATAGAGTTG
SARS-2-S (P25L) F	CAAGAACCCAGCTGCTTCCAGCCTACACCAAC
SARS-2-S (P25L) R	GTTGGTGTAGGCTGGAAGCAGCTGGGTTCTTG
SARS-2-S (Y144Δ/W152R) F	CCTTCCTGGGCGTCTATCACAAGAACAACAAGAGCCGGATGGAAAGCGAG TTCC
SARS-2-S (Y144Δ/W152R) R	GGAACTCGCTTTCCATCCGGCTCTTGTTGTTCTTGTGATAGACGCCCAGGAA GG
SARS-2-S (A243Δ/ L244Δ) F	GGTTTCAGACACTGCTGCACAGAAGCTACCTG
SARS-2-S (A243Δ/ L244Δ) R	CAGGTAGCTTCTGTGCAGCAGTGTCTGAAACC
SARS-2-S (T478K) F	CTATCAGGCCGGCAGCAAACCTTGTAACGGCGTG
SARS-2-S (T478K) R	CACGCCGTTACAAGGTTTGCTGCCGGCCTGATAG
SARS-2-S (T859N) F	GAAGTTTAACGGACTGAACGTGCTGCCACCACTG
SARS-2-S (T859N) R	CAGTGGTGGCAGCACGTTCAGTCCGTTAAACTTC
SARS-2-S (A879T) F	CACATCTGCCCTGCTGACCGGCACAATCACAAG
SARS-2-S (A879T) R	CTTGTGATTGTGCCGGTCAGCAGGGCAGATGTG
SARS-2-S (T95I) F	GTGTACTTTGCCAGCATCGAGAAGTCCAACATC
SARS-2-S (T95I) R	GATGTTGGACTTCTCGATGCTGGCAAAGTACAC
SARS-2-S (Y144S/Y145N) F	CCTTCCTGGGCGTCTCCAATCACAAGAACAACAAG
SARS-2-S (Y144S/Y145N) R	CTTGTTGTTCTTGTGATTGGAGACGCCCAGGAAGG
SARS-2-S (R346K) F	GTGTTCAATGCCACCAAATTCGCCTCTGTGTAC
SARS-2-S (R346K) R	GTACACAGAGGCGAATTTGGTGGCATTGAACAC
SARS-2-S (P681H) F	GACAAACAGCCACAGACGGGCCAGATCTG
SARS-2-S (P681H) R	GGCCCGTCTGTGGCTGTTTGTCTGTGTC
SARS-2-S (D950N) F	AAGCTGCAGAACGTGGTCAACCAGAATGCCCAGG
SARS-2-S (D950N) R	TGACCACGTTCTGCAGCTTTCCCAGGGCGCTTGC
SARS-2-S Seq-01	CAAGATCTACAGCAAGCACACC
SARS-2-S Seq-02	GTCGGCGGCAACTACAATTAC
SARS-2-S Seq-03	CTGTCTGATCGGAGCCGAGCAC
SARS-2-S Seq-04	TGAGATGATCGCCCAGTACAC
SARS-2-S Seq-05	GCCATCTGCCACGACGGCAAAG
pCG1 F	CCTGGGCAACGTGCTGGT
pCG1 R	GTCAGATGCTCAAGGGGCTTCA

Figure S1



Supplementary Figure 1 (Related to Figures 1 and 2):

(A) Pseudotype entry data presented in Figure 1E normalized against the assay background. The experiment was performed as described in the legend of Figure 1E with the difference that pseudotype entry was normalized against signals obtained from cells inoculated with particles bearing no viral glycoprotein (background, set as 1). Further, entry of particles bearing VSV-G is shown. Error bars indicate the standard error of the mean (SEM).

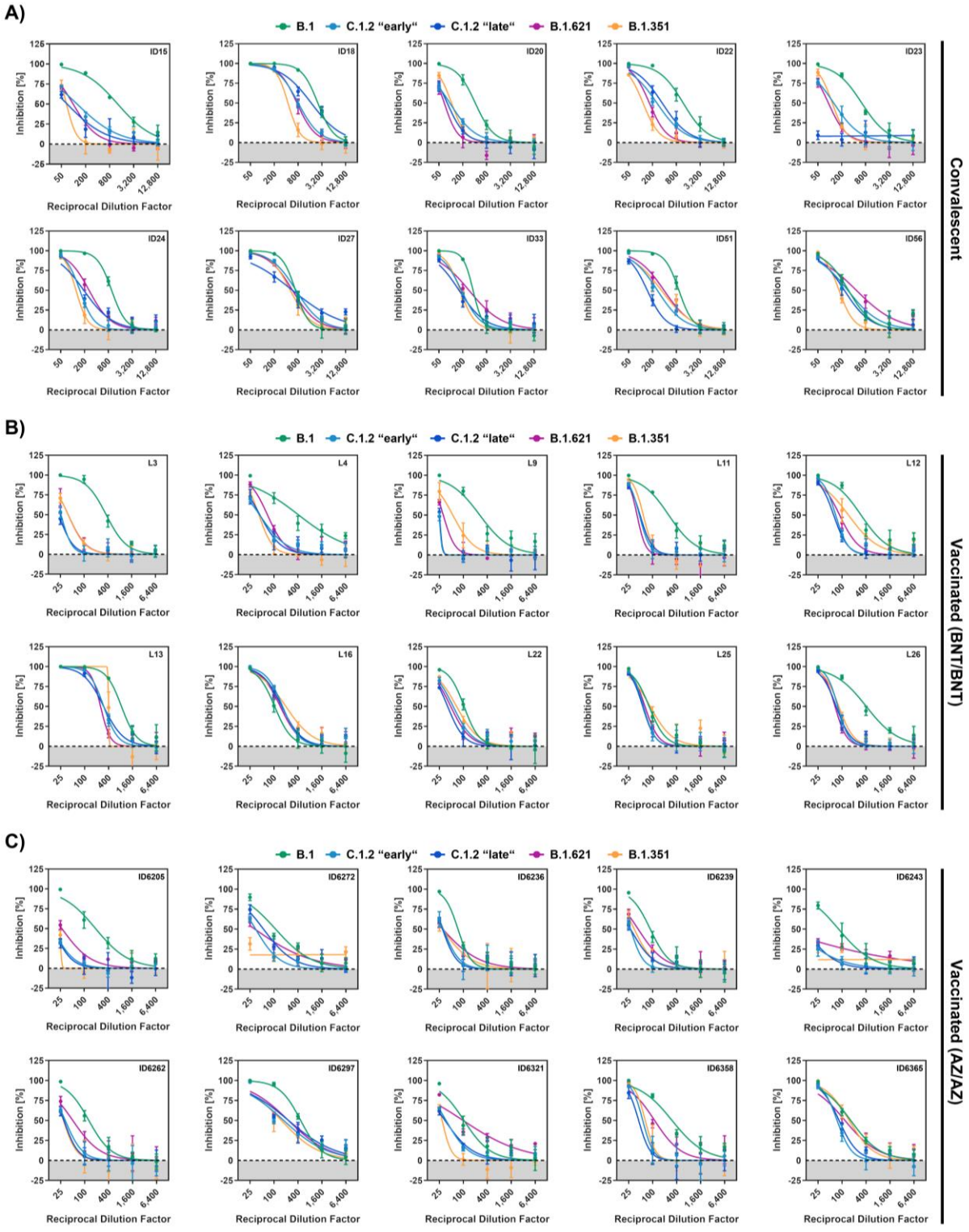
(B) Unprocessed data on ACE2 binding analyzed by flow cytometry (related to Figure 2C). 293T expressing the indicated S proteins following transfection were incubated with soluble ACE2 (ACE2 ectodomain fused to an Fc-tag derived from human IgG) and AlexaFluor-488-conjugated anti-human antibody and analyzed flow cytometry. The

following samples served as controls: (i) untransfected and unstained cells (Cells), (ii) empty vector-transfected cells incubated only with secondary antibody (2nd Ab), (iii) empty vector-transfected cells incubated with soluble ACE2 and secondary antibody (-). Efficiency of ACE2 binding was assessed by measuring the geometric mean channel fluorescence at 488 nm. Shown are the average (mean) data from three (left panel, machine A, ID7000 Spectral Analyser, Sony) and six (right panel, machine B, LSR II flow cytometer, BD Biosciences) biological replicates (each performed with single samples). Error bars indicate the standard deviation (SD). Statistical significance of differences in ACE2 binding was analyzed by two-tailed Students t-test with Welch's correction ($p > 0.05$, ns; $p < 0.05$, *; $p < 0.01$, **; $p < 0.001$, ***).

(C) Cell surface expression of the indicates S proteins in transfected 293T cells was analyzed by flow cytometry using the monoclonal anti-SARS-CoV-2 S antibody Imdevimab and AlexaFluor-488-conjugated anti-human secondary antibody (related to Figure 2C). Samples incubated with a control human isotype antibody (control IgG) instead of Imdevimab served as controls. Shown are the average (mean) data from three biological replicates that were each performed with single samples (left panel) or the respective normalized data (B.1 = 1, right panel). Error bars indicate the SD (left panel) or the SEM (right panel). Statistical significance of differences in S protein surface expression was analyzed by two-tailed Students t-test with Welch's correction ($p > 0.05$, not significant [ns]).

(D) mAb concentrations that reduce S protein-driven cell entry by 50% (inhibitory concentration 50, IC50) calculated based on the data presented in Fig. 2E.

Figure S2



Supplementary Figure 2: Individual neutralization data (Related to Figure 2).

Presented are the individual neutralization results for the data presented in Figure 2F (convalescent), 2G (AZ/AZ-vaccinated) and 2H (BNT/BNT-vaccinated). All data show the mean values of four technical replicates with error bars indicating the standard deviation. The curves were calculated based on a non-linear regression model with variable slope.