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Supplementary appendix

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Supplement to: Zhao X, Zheng A, Li D, et al. Neutralisation of ZF2001-elicited antisera to SARS-CoV-2 variants. *Lancet Microbe* 2021; published online August 20. https://doi.org/10.1016/S2666-5247(21)00217-2.

Appendix

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Materials and Methods

Serum samples

The 28 serum samples were collected from vaccine recipients after 3 doses of 25 μg ZF2001 vaccine on 0, 1, 2 Months (16 vaccinees) or 0, 1, (4-6) Months (12 vaccinees)[1,2]. The participants including 13 males and 15 females. Detailed information was provide in Table S1. All candidates signed the written informed consent.

Pseudotyped virus neutralization assay

The construction of VSV-ΔG-GFP based SARS-CoV-2 pseudotyped virus was mentioned in previous work with slight modifications[3-6]. The codon-optimized SARS-CoV-2 wild type (Wuhan-1 reference strain) and variants spike protein (with mutations shown in Fig.S1) with an 18 amino acid truncation at the C-terminal were constructed into the pCAGGS vector. 30 μg of the construct was transfected into HEK 293T cells. VSV-ΔG-G-GFP pseudovirus were added 24 h after the transfection and removed after 1 h incubation. Medium were changed into fresh complete DMEM medium with anti-VSV-G antibody (I1HybridomaATCC® CRL2700™). Supernatants were collected after another 30 h incubation, passed through a 0.45 μm filter (Millipore, Cat#SLHP033RB), aliquoted, and stored at -80 °C.

For the neutralization assay, the heat-inactivated (56 °C for 30min) serum samples from vaccinees were 2-fold serial diluted started from 1:20. 50 μL of the serially diluted sera were incubated with 50 μL of each pseudovirus at 1000 transducing units (TU) at 37 °C for 1 h, and added onto pre-plated Vero cells (ATCC CCL81) in 96 well plate. The TU numbers were calculated after a 15 h incubation on a CQ1 confocal image

cytometer (Yokogawa) [3].

Data and statistics analyses

The TU numbers were calculated by CQ1 software and statistical analyses were performed by GraphPad Prism 8.0 (GraphPad Software Inc.) for all experiments. $pVNT_{50}$ titers were determined by non-linear regression (Fig. S2). $pVNT_{50}$ below the lower limit of detection (<20) were recorded as 10 in the geometric mean calculation. Neutralization values between different strains were compared using a two-tailed Wilcoxon matched-pairs signed-rank test.

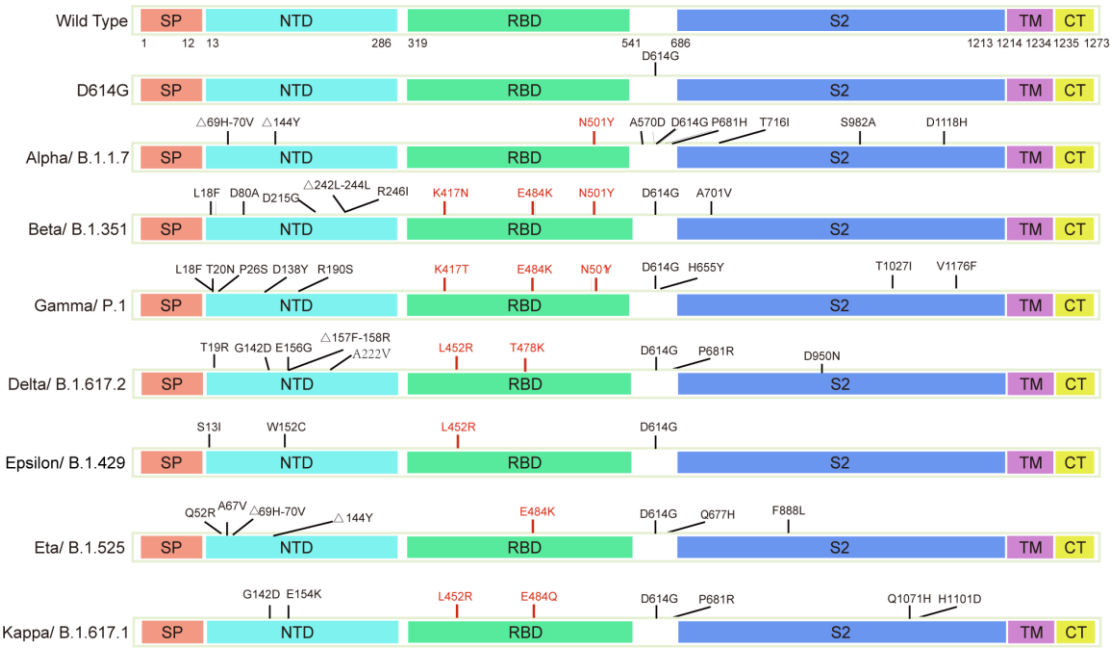
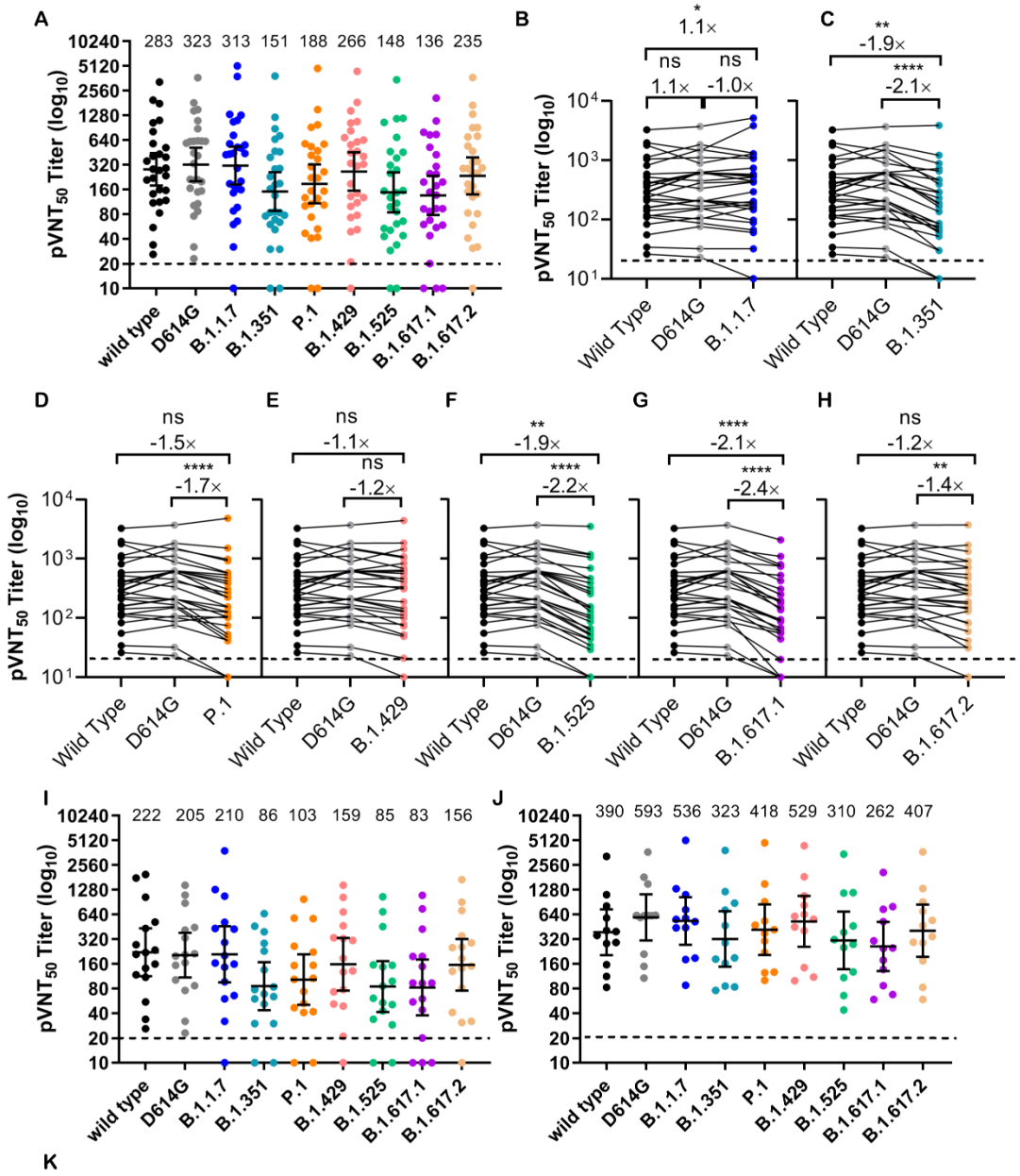


Figure S1: Schematic representation of SARS-CoV-2 Spike proteins including wild type (WT) and eight variants. The mutation sites of the variants were indicated, with the mutations on RBD highlighted in red. (SP: signal peptide, NTD: N-terminal domain, RBD: receptor-binding domain, TM: transmembrane domain, CT: C-terminal cytoplasmic domain).



pVNT50 (to WT)	Three dose at 0, 1, 2 Months							
	D614G	B.1.1.7	B.1.351	P.1	B.1.429	B.1.525	B.1.617.1	B.1.617.2
Fold change	-1.1	-1.1	-2.6	-2.2	-1.4	-2.6	-2.7	-1.4
P value	0.1973	0.8504	0.0003	0.0003	0.0131	<0.0001	<0.0001	0.0020

Three dose at 0, 1, (4-6) Months							
D614G	B.1.1.7	B.1.351	P.1	B.1.429	B.1.525	B.1.617.1	B.1.617.2
1.5	1.4	-1.2	1.1	1.4	-1.3	-1.5	1.0
0.0005	0.0005	0.9097	0.3394	0.0024	0.7910	0.0005	0.1099

Figure S2: Pseudotyped virus neutralization of SARS-CoV-2 variants in ZF2001-elicited serum samples.

Panel A shows 50% pseudovirus neutralization titer ($pVNT_{50}$) against VSV-expressing wild type spike or eight spike variants of SARS-CoV-2 in sera from 28 volunteers receiving three doses of ZF2001 vaccination. The variants including the parental variant D614G, four VOCs (B.1.1.7, B.1.351, P.1 and B.1.617.2 linages), and three variants of interest (B.1.429, B.1.525 and B.1.617.1 linages) listed by WHO. The geometric mean titer (GMT) was marked on top of each column and lined with 95% confidential interval (CI) shown as horizontal bars. The dashed line indicates the lower limit of detection. The GMTs lower than 20 were considered negative and calculated as 10 in the statistical analysis. **Panels B-H** shows the $pVNT_{50}$ titer against each of the seven variants compared with wild type and D614G. Fold change and significance compared with both wild type and D614G were shown in each panel. (*, $p<0.05$; **, $p<0.01$; **, $p<0.001$; ****, $p<0.0001$). **Panel I** shows the $pVNT_{50}$ against variants in volunteers (n=16) with three doses at 0, 1, 2 Months. **Panel J** shows the $pVNT_{50}$ against variants in volunteers (n=12) with three doses at 0, 1, 4-6 Months. **Panel K** summarization the fold-change and p-value of the $pVNT_{50}$ for the variants from the wild type. Background color in pink indicates significant GMT increase and in light blue indicates significant GMT decrease, compared with the GMT to the wild type. White means no significant change. Neutralization values between different variants were analyzed with two-tailed Wilcoxon matched-pairs signed-rank test. $pVNT_{50}$ of each sample is tested by two repeats.

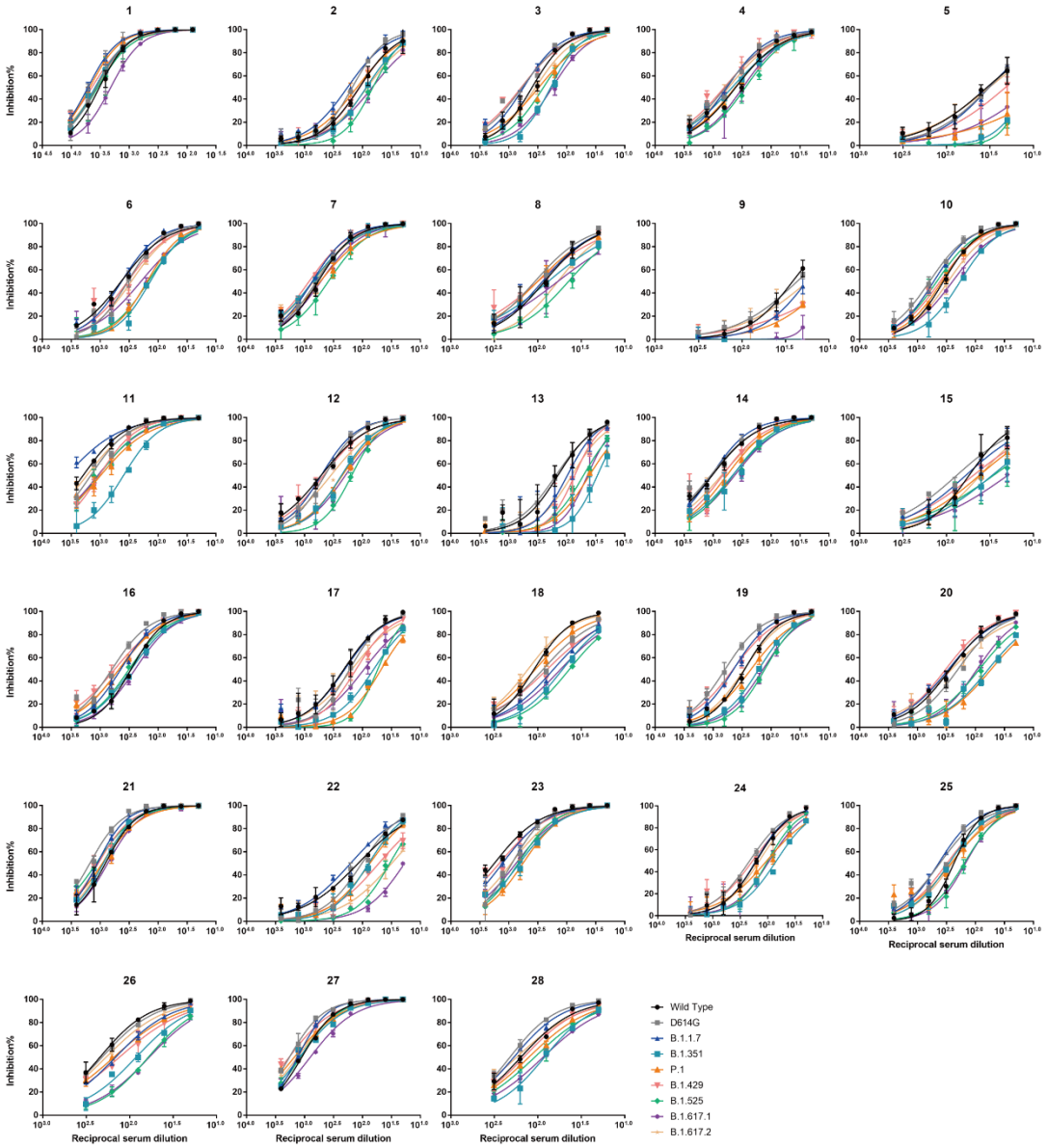


Figure S3: Neutralization assays of ZF2001 vaccinee sera against pseudovirus-based SARS-CoV-2 variants. The neutralization assay of sera from 28 vaccinated volunteers were detected against WT, D614G, B.1.1.7, B.1.351, P.1, B.1.429, B.1.525, B.1.617.1 and B.1.617.2 lineages. Each sample was tested with repeats.

Table S1. Characteristics of vaccine recipients.

Characteristics	Vaccine recipients
No. of participants	28
Age (median, range)	30.0 (24-43)
Sex	
Male (%)	13 (46.4)
Female (%)	15 (53.6)
Time interval between the first and second does (Median day, range)	
0, 1, 2 Months group (16 persons)	28.0 (24-29)
0, 1, (4-6) Months group (12 persons)	27.5 (27-35)
Time interval between the second and third does (Median day, range)	
0, 1, 2 Months group (16 persons)	35.0 (35-39)
0, 1, (4-6) Months group (12 persons)	113.0 (82-137)
Time interval between the third dose and blood sampling (Median day, range)	
0, 1, 2 Months group (16 persons)	14.0 (14-14)
0, 1, (4-6) Months group (12 persons)	64.5 (30-104)

Supplemental references:

1. Yang S, Li Y, Dai L, et al. Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD-based protein subunit vaccine (ZF2001) against COVID-19 in adults: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials. *The Lancet Infectious Diseases* 2021; **S1473-3099**(21).
2. Dai L, Gao GF. Viral targets for vaccines against COVID-19. *Nat Rev Immunol* 2021; **21**(2): 73-82.
3. Liu K, Pan X, Li L, et al. Binding and molecular basis of the bat coronavirus RaTG13 virus to ACE-2 in humans and other species. *Cell* 2021; **184**(13): 3438-51.
4. Li Q, Wu J, Nie J, et al. The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. *Cell* 2020; **182**(5): 1284-94 e9.
5. Zhang Z, Zhang Y, Liu K, et al. The molecular basis for SARS-CoV-2 binding to dog ACE2. *Nature communications* 2021; **12**(1): 4195.
6. Nie J, Li Q, Wu J, et al. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. *Emerging microbes & infections* 2020; **9**(1): 680-6.

Other related references:

7. Hoffmann M, Arora P, Gross R, et al. SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. *Cell* 2021; **184**(9): 2384-93 e12.
8. Zhang M, Xiao J, Deng A, et al. Transmission dynamics of an outbreak of the COVID-19 Delta variant B.1.617.2 — Guangdong Province, China, May–June 2021. *China CDC Weekly* 2021; **3**(27): 584-6.
9. Campbell F, Archer B, Laurenson-Schafer H, et al. Increased transmissibility and global spread of SARS-CoV-2 variants of concern as at June 2021. *Euro surveillance* 2021; **26**(24).
10. Wall EC, Wu M, Harvey R, et al. Neutralising antibody activity against SARS-CoV-2 VOCs B.1.617.2 and B.1.351 by BNT162b2 vaccination. *The Lancet* 2021; **397**(10292): 2331-3.
11. Liu C, Ginn HM, Dejnirattisai W, et al. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. *Cell* 2021; **184**: 1-17.
12. Edara VV, Pinsky BA, Suthar MS, et al. Infection and vaccine-induced neutralizing-antibody responses to the SARS-CoV-2 B.1.617 variants. *The New England journal of medicine* 2021. DOI: 10.1056/NEJMc2107799
13. Mlcochova P, Kemp S, Dhar MS, et al. SARS-CoV-2 B.1.617.2 Delta variant emergence and vaccine breakthrough. Research Square; 2021. DOI:10.21203/rs.3.rs-637724/v1
14. Planas D, Veyer D, Baidaliuk A, et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. *Nature* 2021. DOI: 10.1038/s41586-021-03777-9
15. Bernal JL, Andrews N, Gower C, et al. Effectiveness of COVID-19 vaccines against the B.1.617.2 variant. *medRxiv : the preprint server for health sciences* 2021: 2021.05.22.21257658.
16. Sheikh A, McMenamin J, Taylor B, Robertson C. SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness. *The Lancet* 2021; **397**(10293): 2461-2.