

Supplementary Material

A bioluminescent and homogeneous SARS-CoV-2 spike RBD and hACE2 interaction assay for antiviral screening and monitoring patient neutralizing antibody levels

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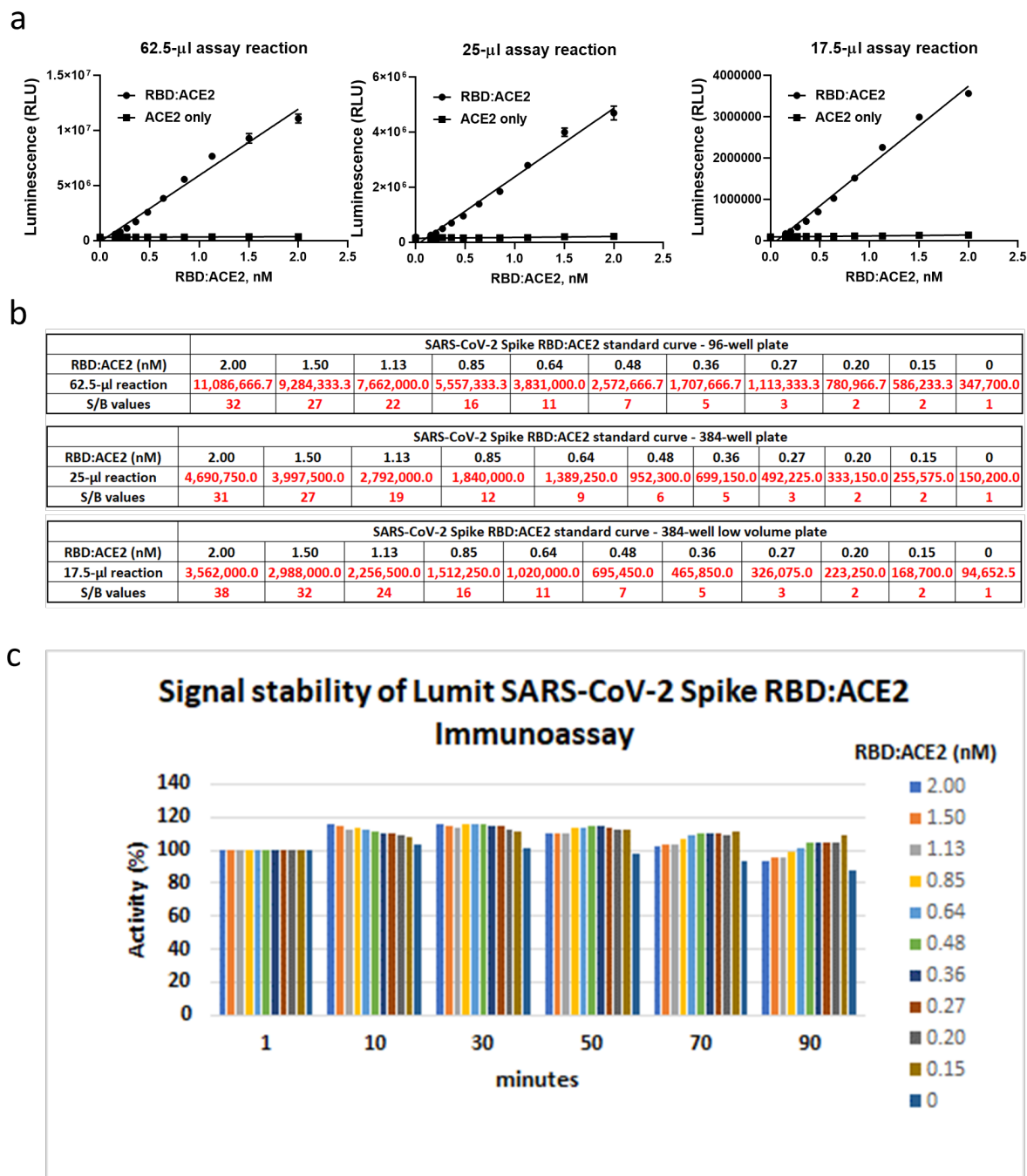
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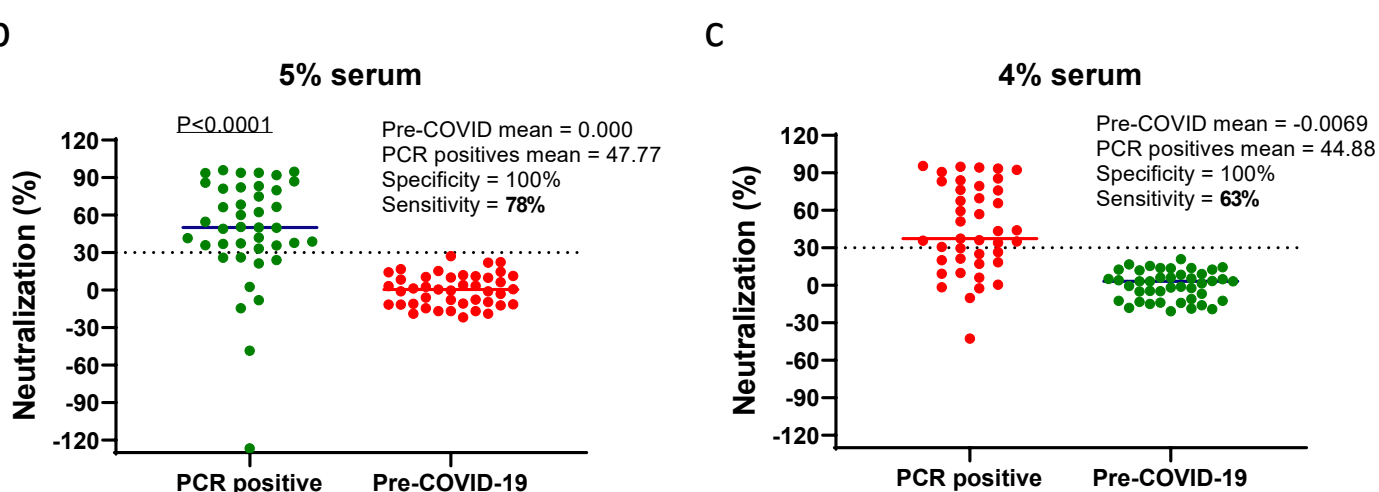
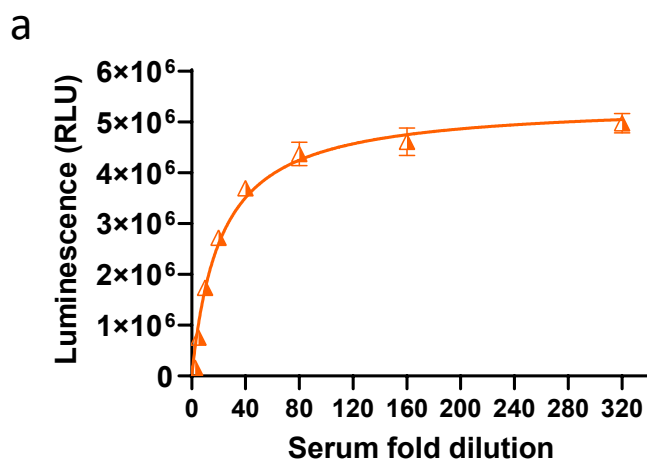
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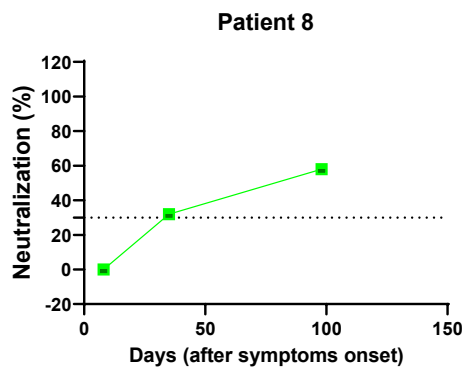
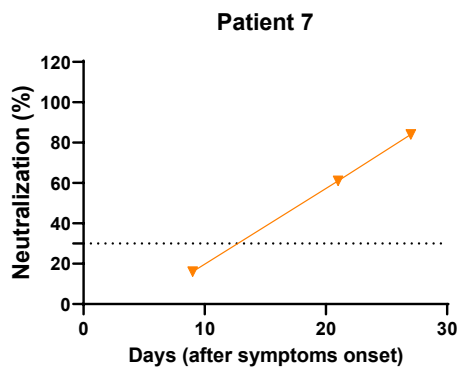
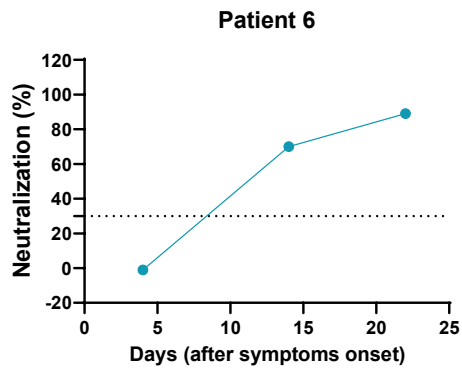
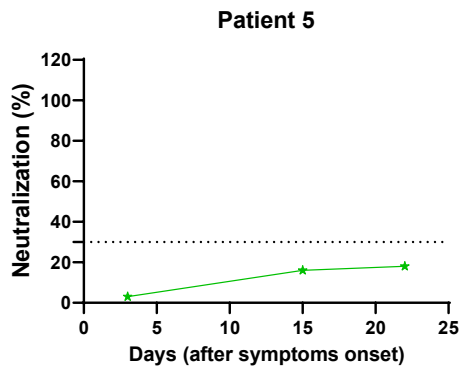
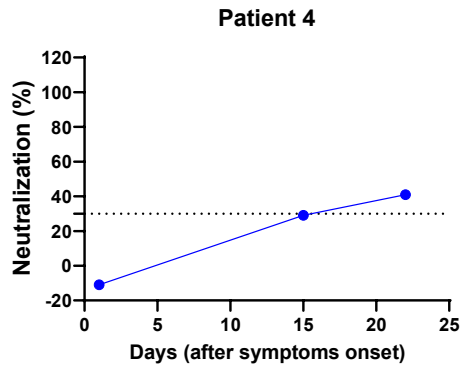
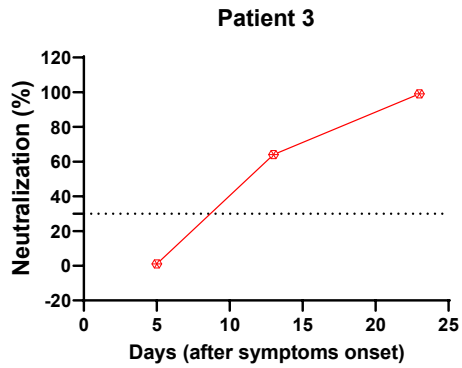
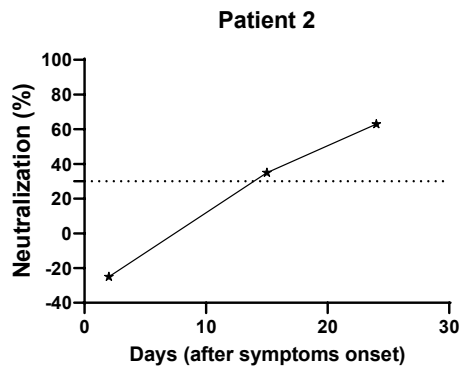
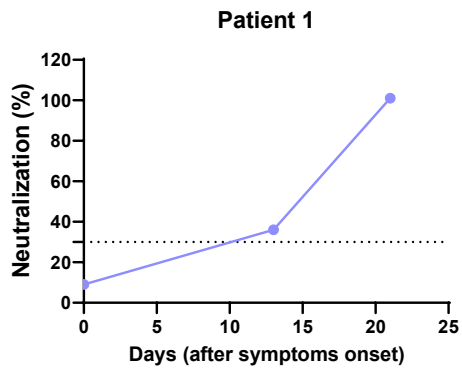
*Juliano Alves, Ph.D. email: juliano.alves@promega.com



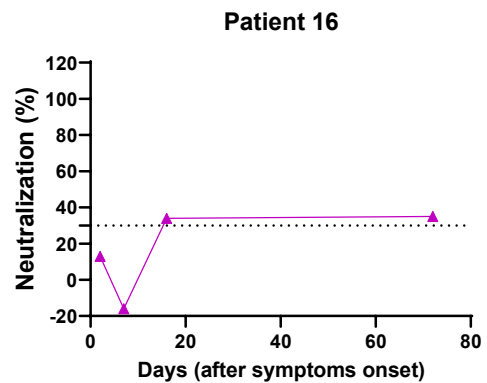
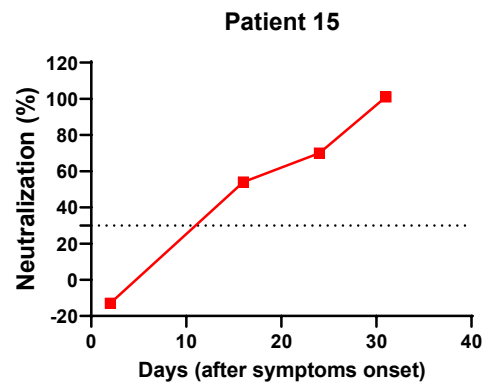
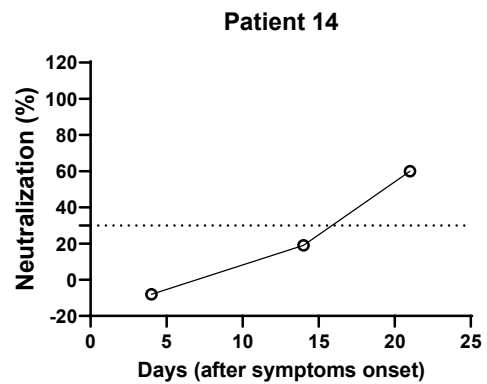
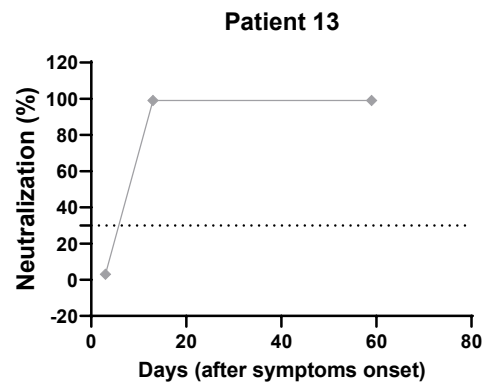
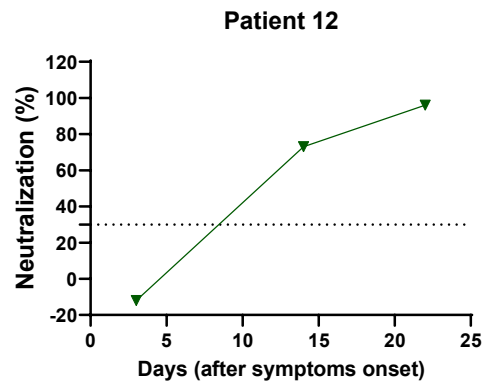
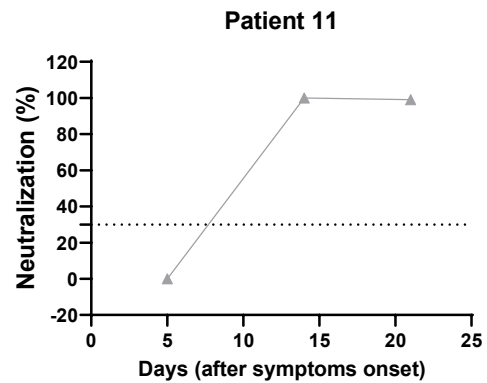
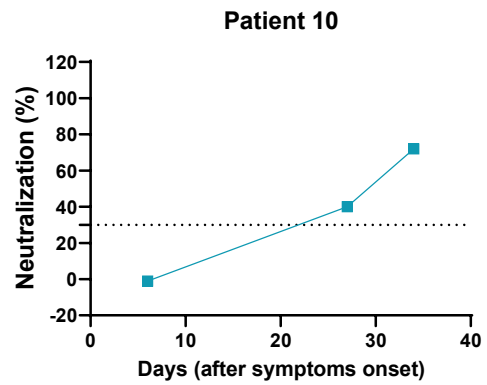
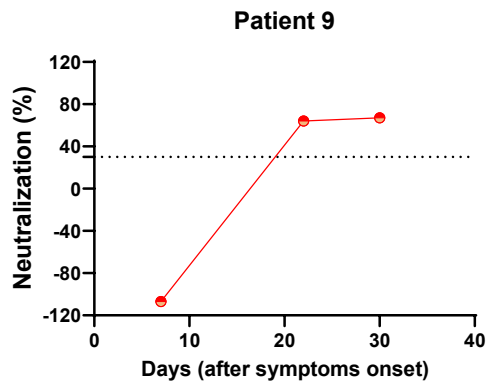
Supplementary Fig. 1: Lumit SARS-CoV-2 Spike RBD: ACE2 Immunoassay features. (a) SARS-CoV-2 Spike RBD: ACE2 linearity curves and determination of the limit of detection using different reaction volumes and plate formats (96-, 384- and 384-well low volume plates). SARS-CoV-2 Spike RBD: ACE2 titration was performed in 1X Buffer C. (b) Signal-to-background values for each SARS-CoV-2 Spike RBD: ACE2 titration are shown. (c) Signal stability as percent change from a 30-minute read after the addition of Lumit Detection Reagent. Results are presented as means $\pm$ S.E.M. (n=3).



Supplementary Fig. 2. Optimization of Lumit SARS-CoV-2 Spike RBD: ACE2 Immunoassay in patient-derived samples. (a) Effect of serum dilutions on light output generated by Lumit immunoassay. (b-c) Comparison of sensitivity and specificity of the Lumit assay in two different serum dilutions. COVID-19 positive (n=41) and negative pre-pandemic samples (n=43) were tested using Lumit SARS-CoV-2 Spike RBD: ACE2 Immunoassay at 5% and 4% serum. Serum samples were pre-incubated with Spike RBD-rabbit Fc for 30 minutes prior to the addition of the other assay components. A cutoff value of 30% was selected based on the distribution of COVID-19 positive and negative pre-pandemic samples and the assay improved specificity. The horizontal lines indicate the mean values, and the dotted lines represent the cutoff at 30% inhibition. The P values presented in a and b were calculated from unpaired two-tailed Student's t-tests. Results are representative of two independent experiments

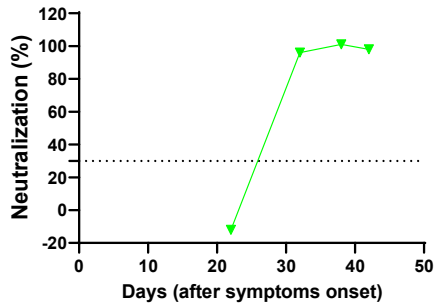


Supplementary Fig. 3. Time-course of patient-derived samples that were collected in separate dates after the initial symptom onset.

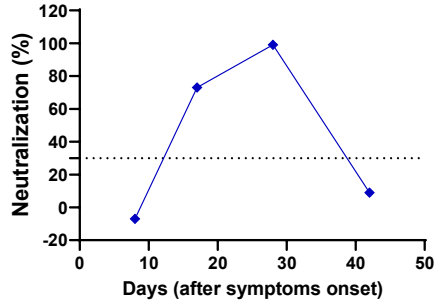


Supplementary Fig. 3 continued

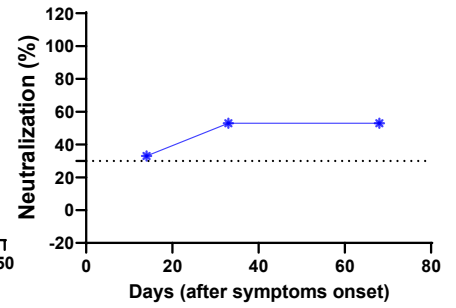
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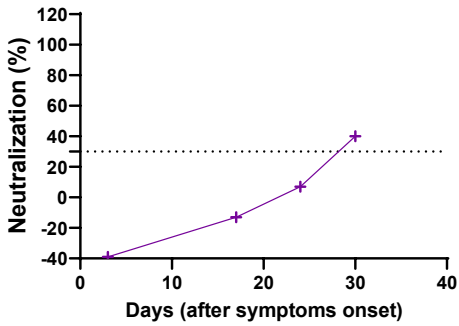
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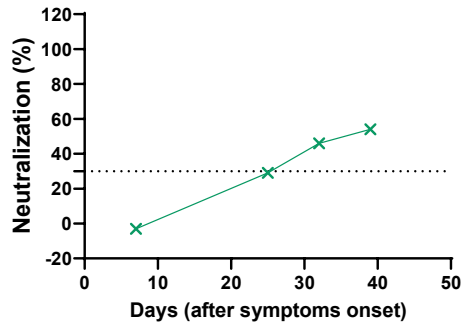
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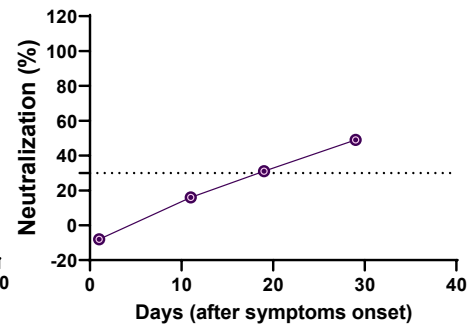
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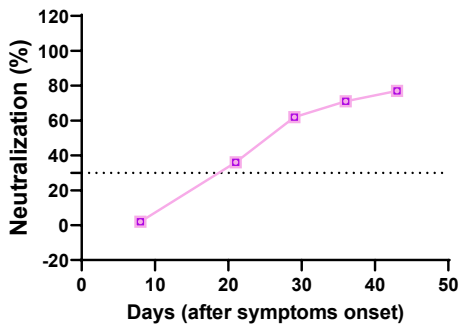
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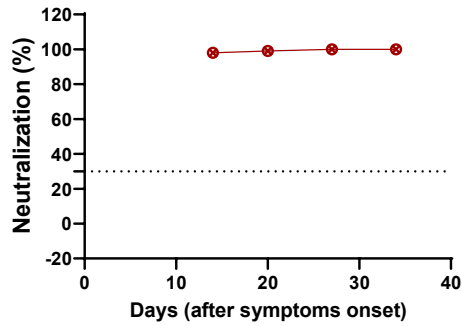
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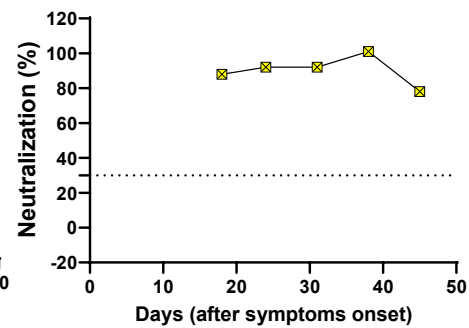
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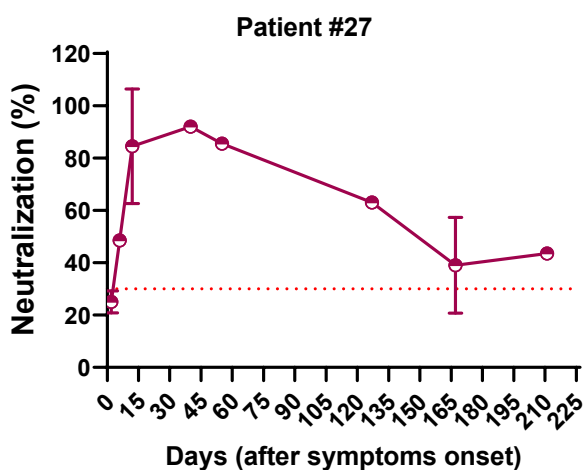
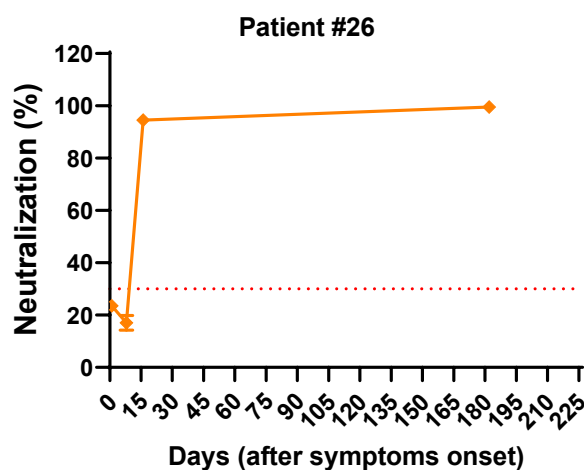
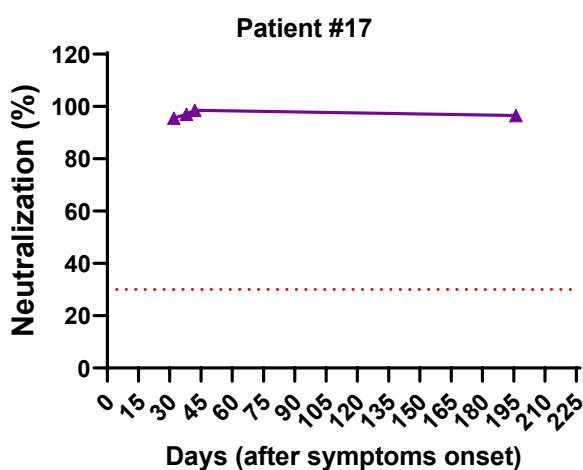
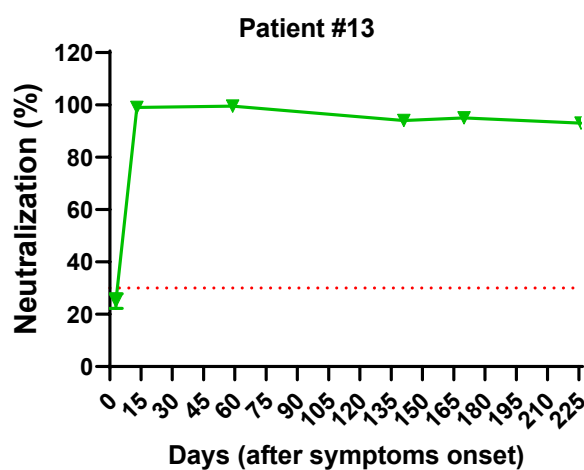
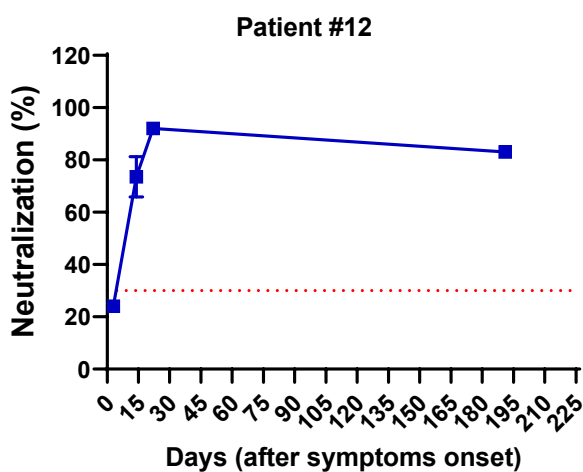
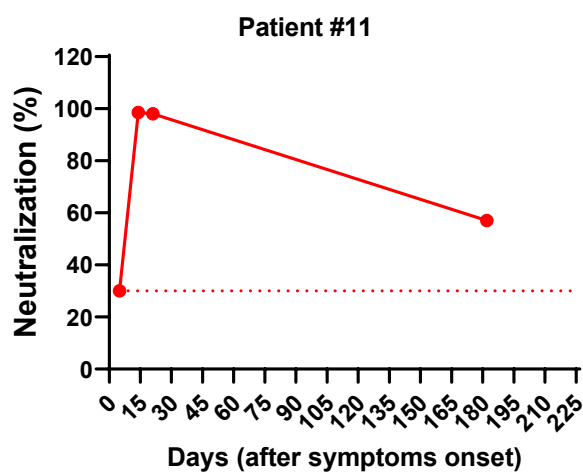
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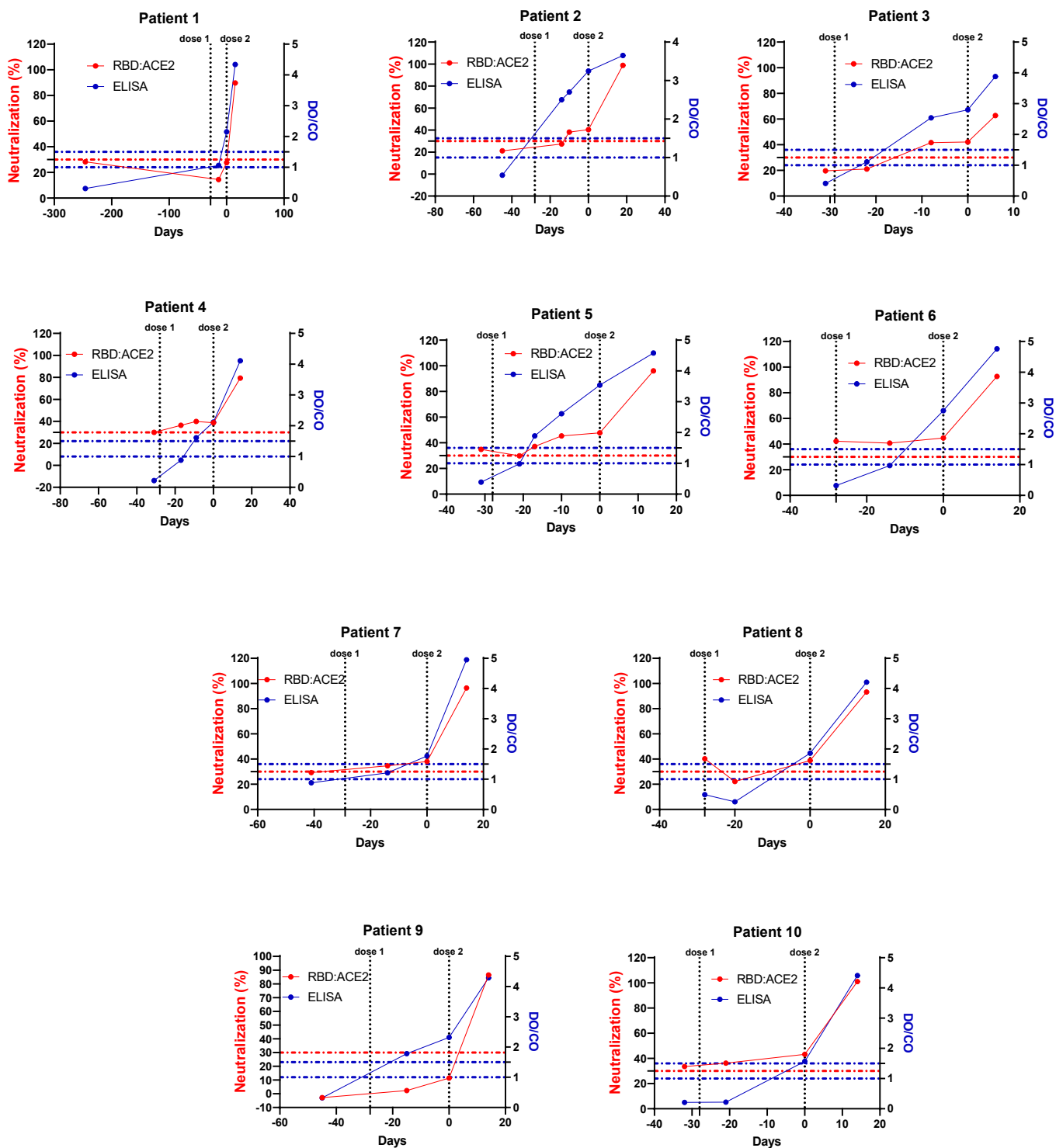
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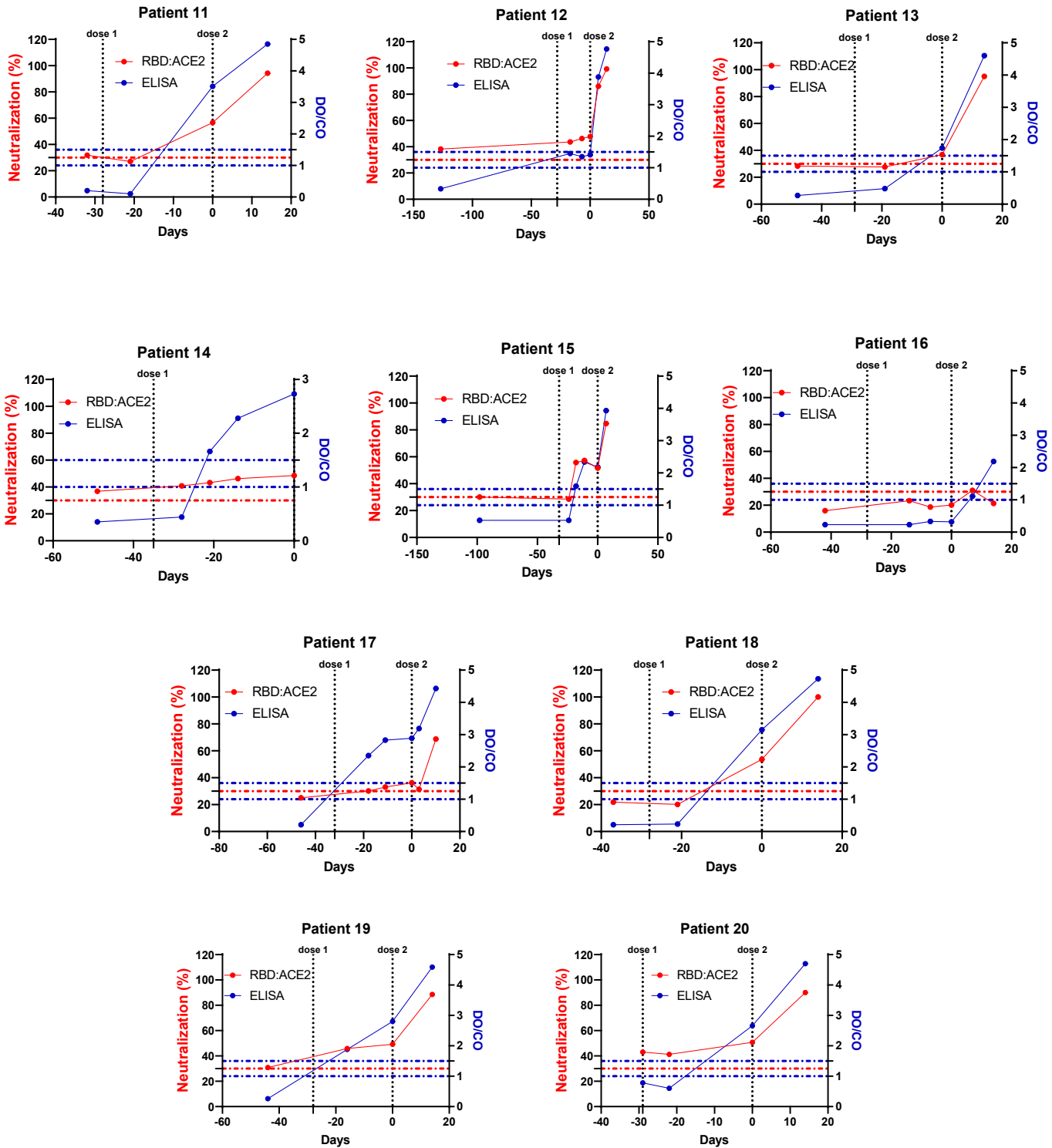
Supplementary Fig. 3 continued



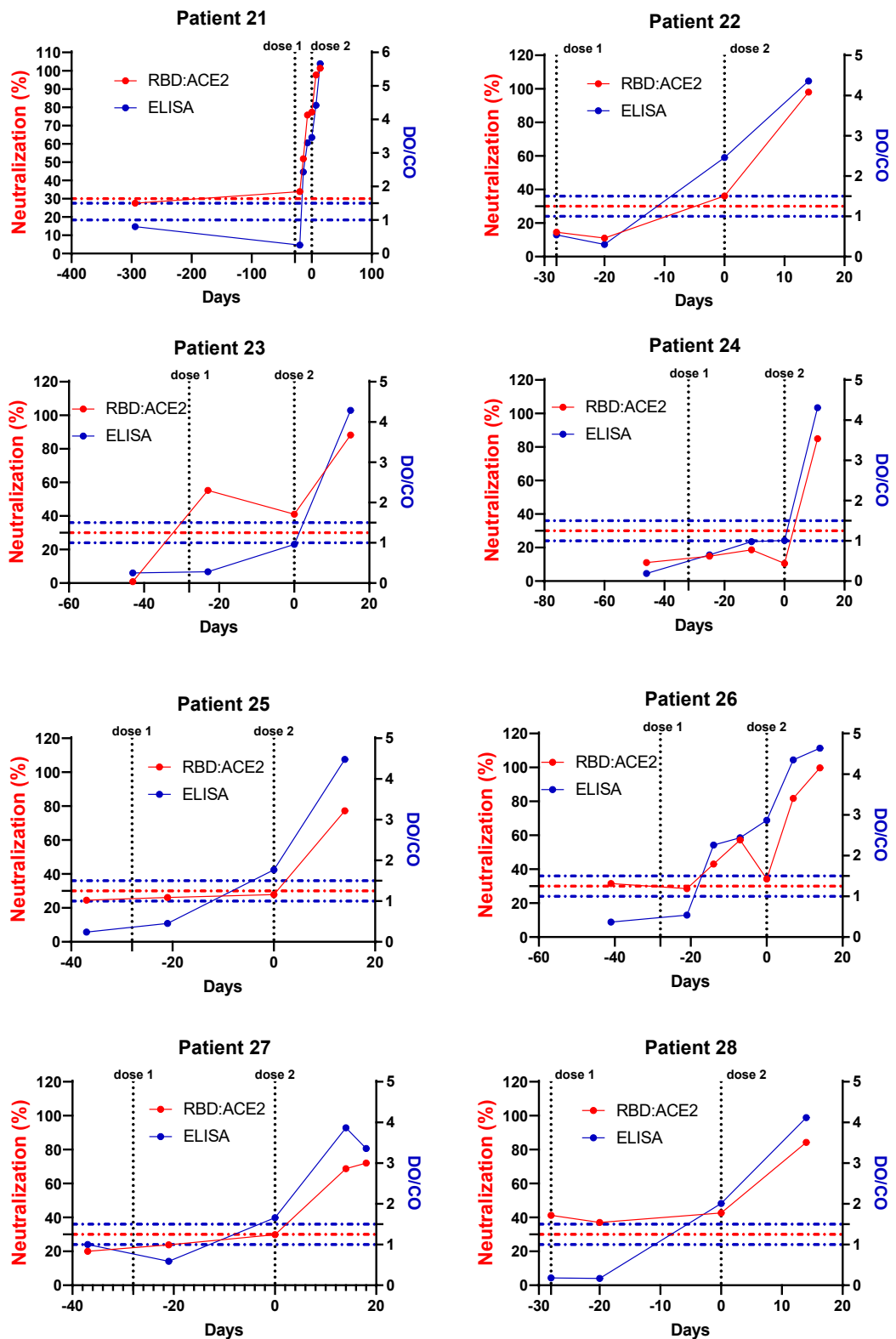
Supplementary Fig. 4. Longer time-course of patient-derived samples that were collected in separate dates after the initial symptom onset and beyond 6 months.



Supplementary Fig. 5. Time-course of patient-derived samples that were collected in separate dates before and after CoronaVac vaccination. Anti-Spike antibodies were detected using Lumit Spike RBD: ACE2 immunoassay and ELISA.



Supplementary Fig. 5 continued



Supplementary Fig. 5 continued

Supplementary Table 1: List of In silico-derived peptide inhibitors⁹ used in this study

Name	Peptide Sequence
AHB1	DEDLEELERLYRKAEVAKAKDASRRGDDERAKEQMERAMRLFDQVFELAQELQEKQTDGNRQKATHLDKAVKEAADELYQRVR
AHB2	ELEEQVMHVLDQVSELAHELLHKLTGEELERAAYFNWWATEMMLELIKSDDEREIREIEEEEARRILEHLEELARK
LCB2	SDDEDSVRYLLYMAELRYEQGNPEKAKKILEMAEFIAKRNNNEELERLVREVKKRL
LCB3	NDDELHMLMTDLVYEALHFAKDEEIKKRVFQLFELADKAYKNNDRQKLEKVVEELKELLERLLS
LCB4	QREKRLKQLEMALLEYAIERNDPYLMFDVAVEMLRLAEEENNDERIIERAKRILEEYE
LCB6	DREQRLVRFLVRLASKFNLSPEQILQLFEVLEELLERGVSEEEIRKQLEEVAKELG

Supplementary Table 2: Spike RBD mutants tested in the competition assay and their IC₅₀ values

Spike RBD	IC ₅₀ (nM)	Fold Change in affinity	Country of first report
K417N	94.84	5.7 (Decrease)	
G476S	38.88	2.4 (Decrease)	USA
Q414E	28.08	1.7 (Decrease)	
E484K	27.64	1.7 (Decrease)	
W436R	21.35	1.3 (Decrease)	Wuhan
L452R	20.37	1.2 (Decrease)	
F342L	20.35	1.2 (Decrease)	England
P384L	18.27	1	
K417N, E484K, N501Y	18.08	1	South Africa
T478I	17.64	1	
R408I	16.76	1	India
Wild Type	16.51	1	
A435S	15.75	1	Finland
K458R	15.54	1	
N354D	13.54	1.2 (Increase)	China
Q414R	12.05	1.4 (Increase)	
V483A	11.95	1.4 (Increase)	USA
V367F	11.54	1.4 (Increase)	Hong Kong/ France
V347F	11.51	1.4 (Increase)	
V341I	9.843	1.7 (Increase)	Wales
Y508H	6.865	2.4 (Increase)	
N439K	5.658	2.9 (Increase)	
S477N	4.62	3.6 (Increase)	Australia
Y453F	2.009	8.2 (Increase)	Denmark mink
N501Y	1.659	10.0 (Increase)	UK/South Africa/Brazil