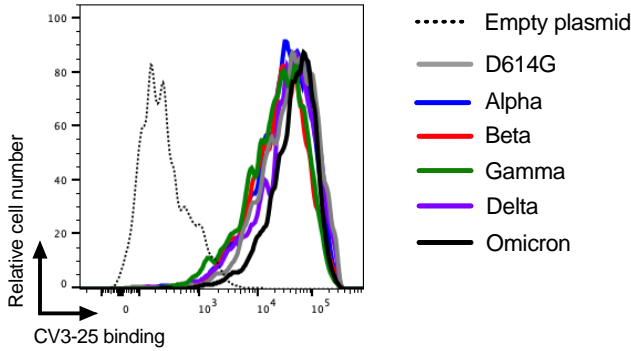
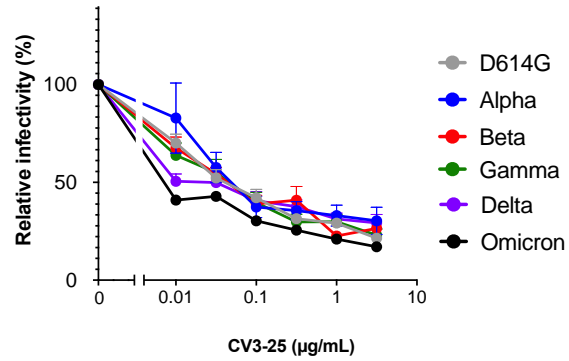


Supplemental information

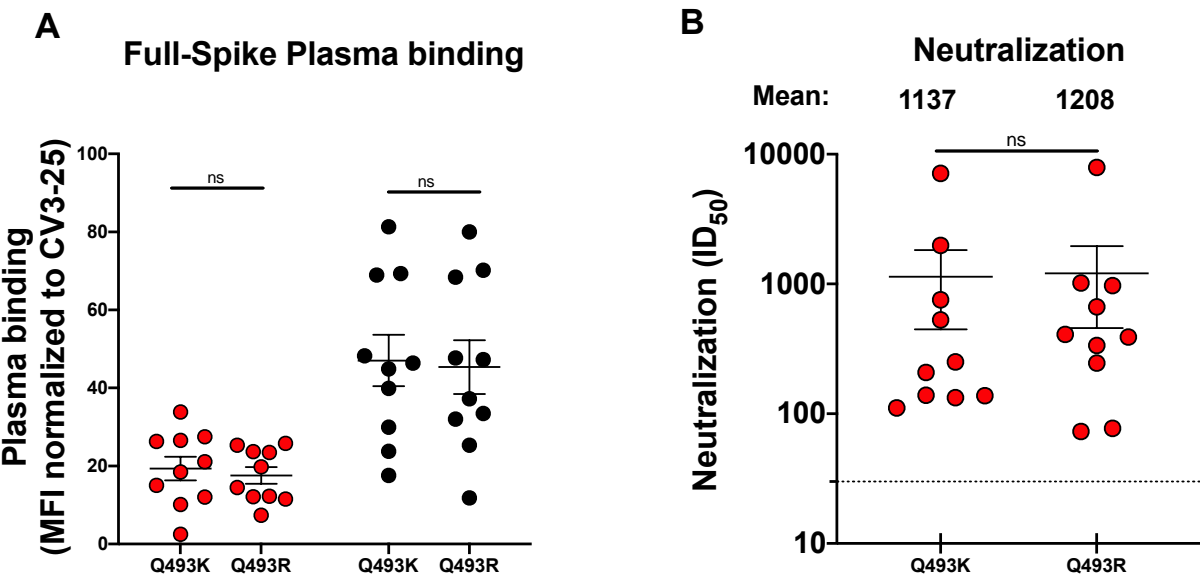
**SARS-CoV-2 Omicron Spike recognition by plasma
from individuals receiving BNT162b2 mRNA
vaccination with a 16-week interval between doses**

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A**Full-Spike binding****B****Neutralization**

Supplemental Figure 1. Recognition and neutralization of different VOCs Spikes by the anti-S2 neutralizing CV3-25 antibody. Related to Figure 1.

(A) 293T cells were transfected with the full-length Spikes from different VOCs (D614G, Alpha, Beta, Gamma, Delta and Omicron), stained with the CV3-25 Ab and analyzed by flow cytometry. Shown are histograms showing a representative staining on Spike-expressing GFP+ cells. (B) CV3-25 neutralizing activity against pseudoviral particles bearing the different VOC Spikes. Data shown are the mean \pm SEM from at least two independent experiments.



Supplemental Figure 2. Recognition and neutralization of Omicron Spikes with Q493K or Q493R changes. Related to Figures 1 and 2.

(A) 293T cells were transfected with the full-length Spikes of Omicron possessing either Q493K or Q493R mutation, stained with the CV3-25 Ab or with plasma collected 3 weeks (V3) after the second dose with a 16-week interval from naïve or previously-infected donors, represented by red and black points respectively and analyzed by flow cytometry. (B) Neutralizing activity was measured by incubating pseudoviruses bearing SARS-CoV-2 Spikes of the two different Omicron mutants as mentioned above, with serial dilutions of plasma collected 3 weeks (V3) after the second dose with a 16-week interval from naïve donors for 1 h at 37°C before infecting 293T-ACE2 cells. Neutralization half maximal inhibitory serum dilution (ID_{50}) values were determined using a normalized non-linear regression using GraphPad Prism software. Error bars indicate means \pm SEM. For each group $n=10$ donors have been used. Each symbol/points indicates one donor. Statistical significance was tested using a Wilcoxon test (ns, non-significant).