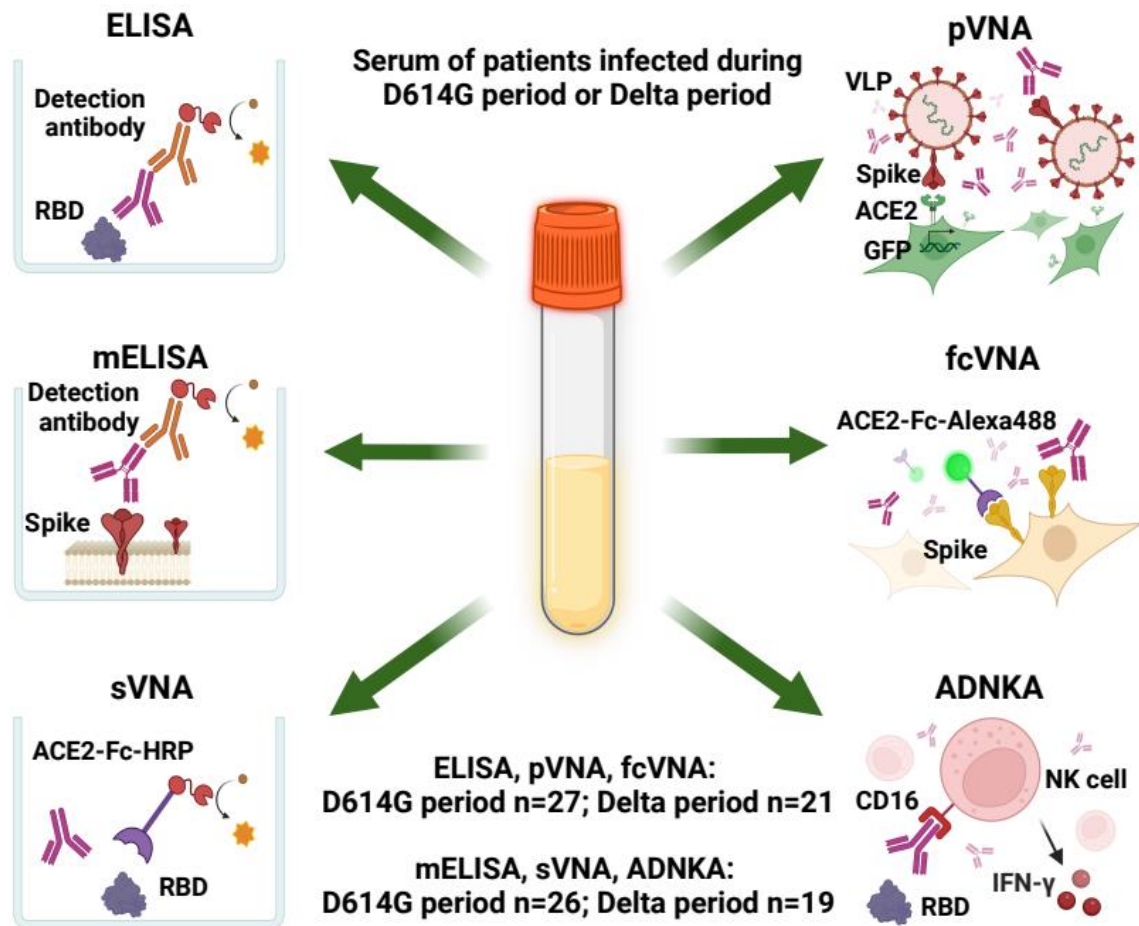


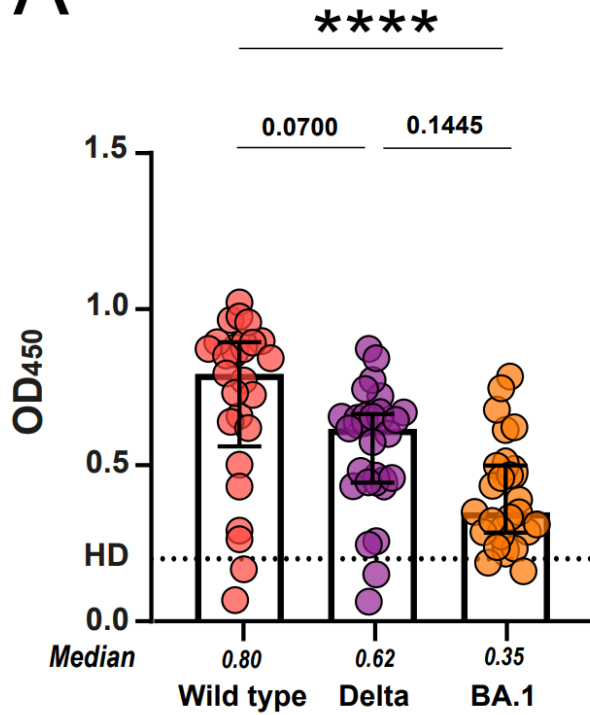
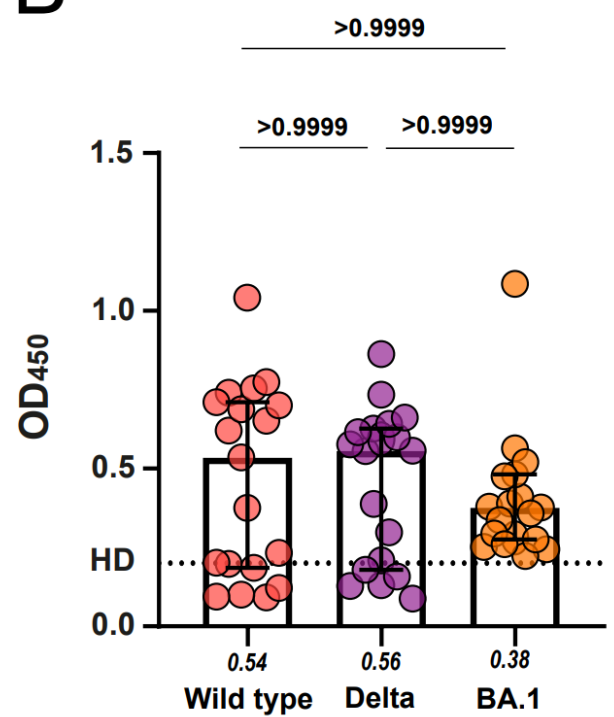
Supplementary Table S1. Patients' characteristics.

Characteristics	D614G period	Delta period	Total	<i>p</i> -value
Females, n (%)	13 (48.1%)	13 (61.9%)	26 (54.2%)	-
Age (years), median (IQR)	68 (IQR 61-72)	73 (IQR 63-82)	68 (IQR 62-76)	0.2127
Days from symptoms' onset to sampling, median (IQR)	21 (IQR 17-35)	21 (IQR 18-32)	21 (IQR 18-33)	0.5757
COVID-19 severity, n (moderate/severe)	9/18	8/13	17/31	-
Vaccine status, Not vaccinated	100% (27/27)	100% (21/21)	100% (48/48)	-
Period of infection	May–June 2020	October–November 2021		-

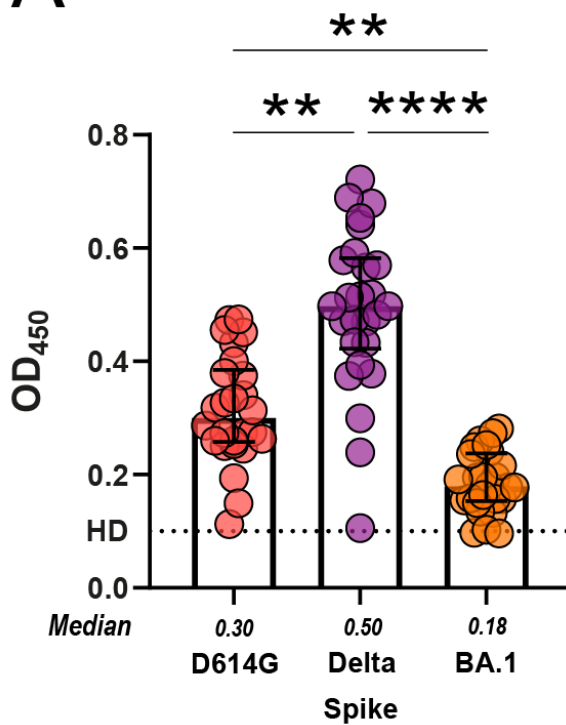
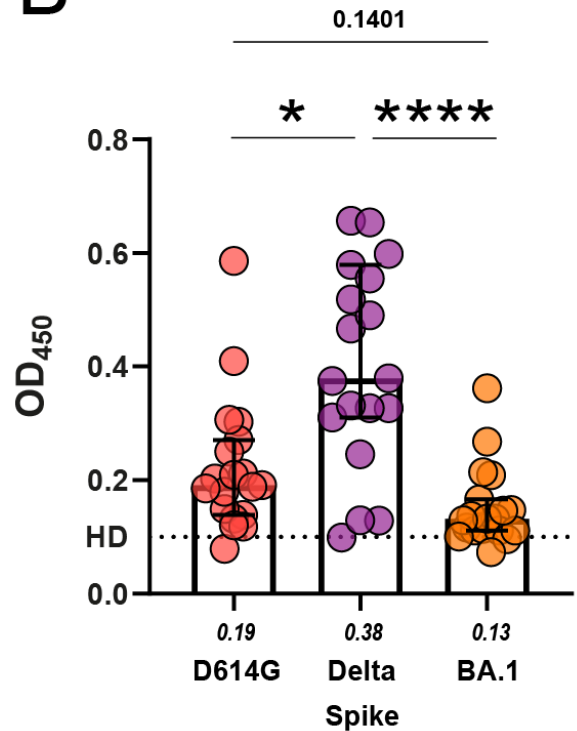


Supplementary Figure S1. Study design.

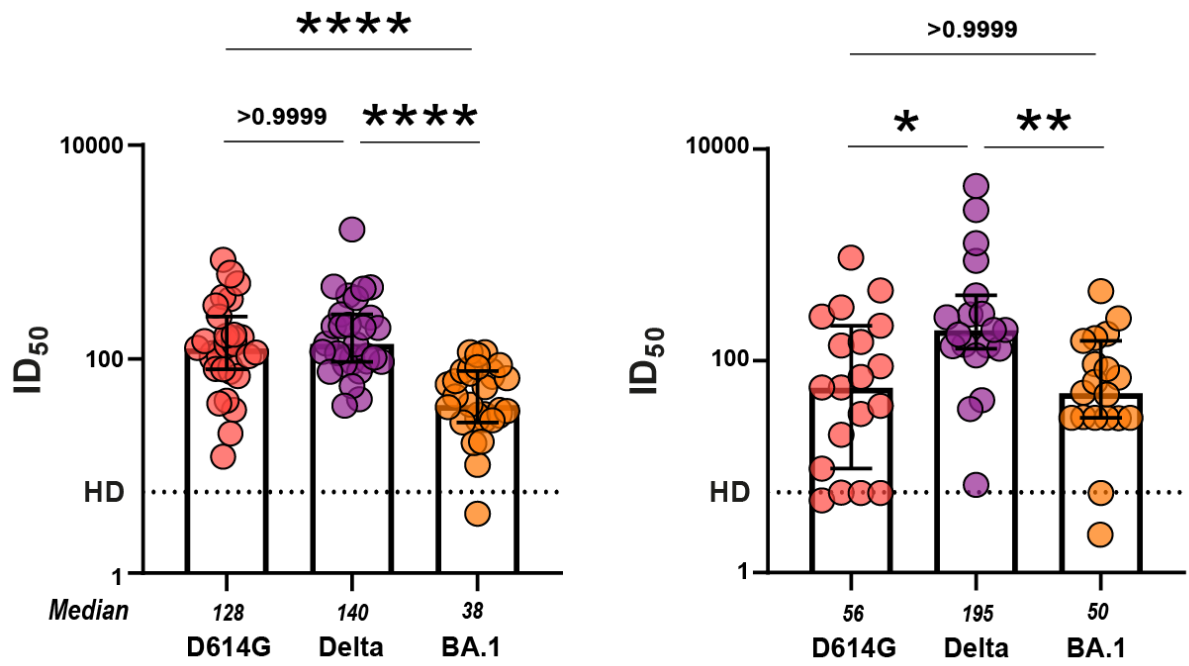
The study included two groups of patients hospitalized for acute COVID-19. Serum samples were collected from patients who had been infected during D614G (n = 27) or Delta (n = 21) periods. Sera were tested for SARS-CoV-2 binding and neutralizing antibodies with six assays based on standard ELISA, cell-membrane-based ELISA (mELISA), surrogate or pseudotyped virus-neutralization assay (sVNA or pVNA), flow-cytometry-based surrogate virus-neutralization assay (fcVNA) and antibody-dependent NK cell activation (ADNKA) with RBD and Spike protein from wild type, Delta and Omicron BA.1 variants as antigen.

A**B****Supplementary Figure S2.**

Reactivity of D614G (A) and Delta (B) period serum samples with WT, Delta, and BA.1 RBD in ELISA.

A**B****Supplementary Figure S3.**

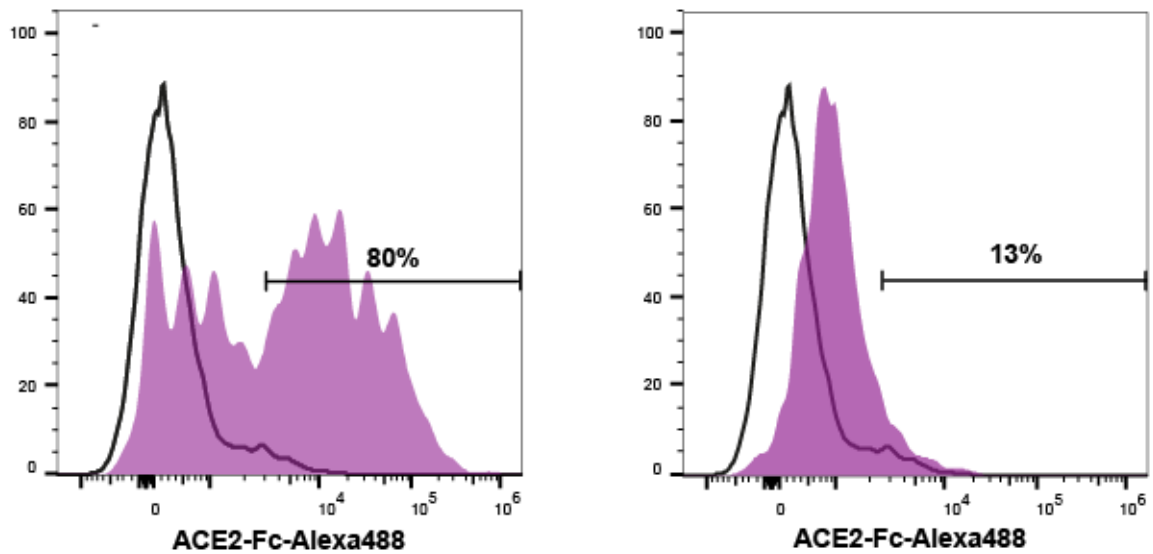
Reactivity of D614G (A) and Delta (B) period serum samples with D614G, Delta, and BA.1 S protein in membrane based ELISA (mELISA).



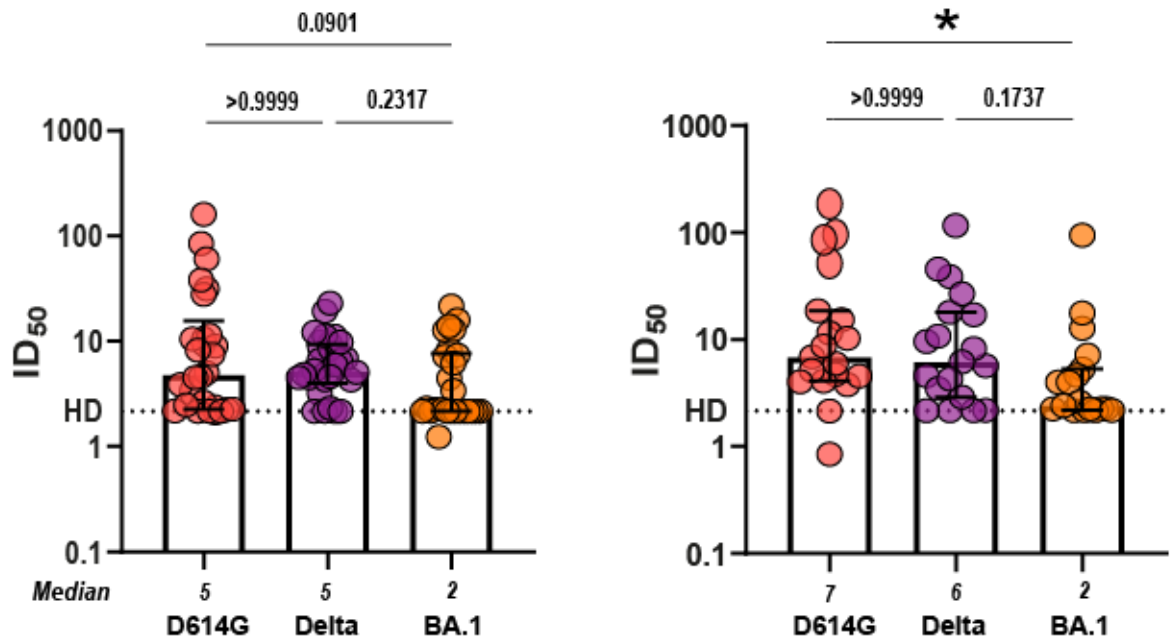
Supplementary Figure S4.

Neutralization antibody titers (ID₅₀ values) against VLP pseudotyped with D614G, Delta, and BA.1 Spike variants in pseudotyped virus neutralization assay (pVNA) of D614G (left) and Delta (right) period serum samples.

A

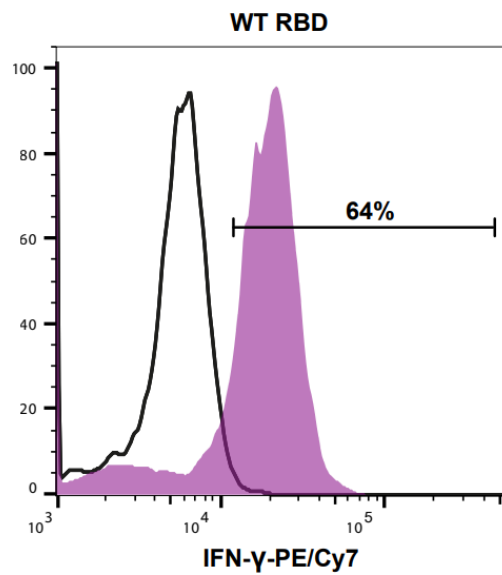
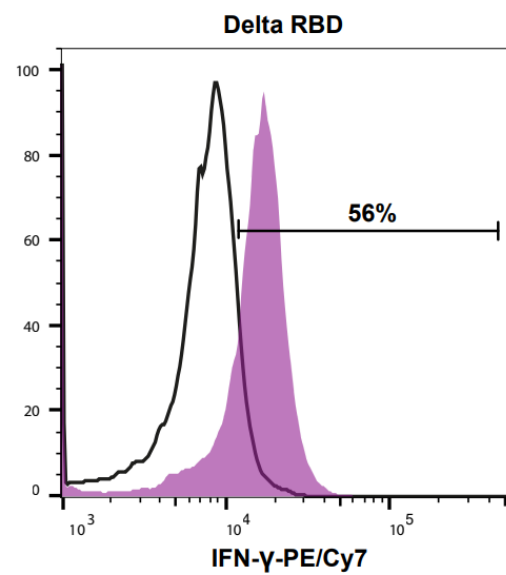


B



Supplementary Figure S5. Flow cytometry based surrogate virus neutralization assay (fcVNA).

A - representative flow plots for HEK293 cells transiently transfected with D614G Spike and stained with ACE2-Fc-Alexa Fluor 488 without addition of patient serum (left panel) or in the presence of patient serum (right panel). Untransfected cells stained with ACE2-Fc-Alexa Fluor 488 were used as a control (open plots). B - neutralization antibody titers (ID₅₀ values) against D614G, Delta, and BA.1 Spike variants in fcVNA of D614G (left) and Delta (right) period serum samples.

A**B**

Supplementary Figure S6. Representative flow plots of antibody-dependent NK cell activation assay (ADNKA).

NK cells were incubated in wells coated with WT (A) or Delta (B) RBD, then permeabilized and stained with PE/Cy5-labeled antibody for IFN- γ . Numbers indicate the percentage of positive events. NK cells from uncoated wells stained with for IFN- γ -PE/Cy7 were used as a control (open plots).