

Supplemental information

**The effect of spike mutations
on SARS-CoV-2 neutralization**

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Supplemental Information

Figure S1. Substitution in SARS-CoV-2 based on SARS-CoV sequence, related to Figure 1.

Figure S2. Binding and neutralization titers across two infection cohorts, related to Figure 3.

Figure S1. Substitution in SARS-CoV-2 based on SARS-CoV sequence, related to Figure 1.

(A) Alignment of RBD from pseudotype SARS-CoV-2 Spike plasmid with SARS-CoV consensus amino acids. Sequences were aligned using the Clustal Omega (1.2.4) tool. The amino acid positions removed in the Spike plasmid are highlighted in grey and the residues of SARS-CoV that replaced them are indicated in red text. Amino acid positions for the first residue changed in each individual mutant is positioned above the first mutated residue in any given mutant. A ¶ by the residue number indicates the pseudotyped virus carrying this substitution did not yield sufficiently infectious titre for analysis of antibody sensitivity. The RBM region in both sequences is underlined. Conservation symbols are listed below the aligned residues and are as follows: an * (asterisk) indicates positions that have a single, fully conserved residue; a : (colon) indicates conservation between groups of strongly similar properties; a . (period) indicates conservation between groups of weakly similar properties; a (gap) indicates no conservation.

(B) SARS CoV-2 RBD (PDB code 6VXX) showing WT (top) and mutated (bottom) amino acid side chains at residues of interest. Using UCSF Chimera, amino acid side chains were altered at select positions using Dunbrack 2010 backbone-dependent rotamer library (Shapovalov & Dunbrack, 2011). Note that the image does not show structure alterations that may occur as a result of mutated residues.

(C) Each SARS-CoV-2 pseudotype mutant is listed on the left and the mAb clusters are shown on the right. Mutations that result in a complete loss of function are indicated by a line joining the mutant to the mAb. Mutations that result in a partial loss of function are indicated by a dashed line joining the mutant to the mAb. Two mutations that had minimal/no effect on function are not linked to any mAb.

Shapovalov, M. V., & Dunbrack, R. L., Jr. (2011). A smoothed backbone-dependent rotamer library for proteins derived from adaptive kernel density estimates and regressions. *Structure*, 19(6), 844-858. doi:10.1016/j.str.2011.03.019

pcDNA-SARS-CoV2 Spike SETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRK
SARS-CoV-consensus AELKCSVKSEFIDKGIYQTSNFRVVPVSGDVVRFPNITNLCPFGEVFNATKFPVSYAWERK
:* **:*** :***** *: :*****:*****:* *****:*

pcDNA-SARS-CoV2 Spike RISNCVADYSVLYNASFSFTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTG
SARS-CoV-consensus KISNCVADYSVLYNS~~TF~~STFKCYGVSA~~TK~~LNDLCFSNVYADSFVVKGDDVRQIAPGQTG
:*****: ***** *****:*****:***:*****

pcDNA-SARS-CoV2 Spike KIADYNYKLPDDFTGCVIAWNSN~~N~~LD~~SK~~VGGN~~Y~~NYLYRLFRKSNLKPFFERDISTEIIYQAG
SARS-CoV-consensus VIADYNYKLPDDFMGCVLAWNT~~R~~IN~~DA~~TSTGN~~Y~~NYKYR~~Y~~LRHGKLRPFFERDIS~~NVPFSPD~~
***** *:***:*.:. ***** ** :*:.*.*****. :. .

pcDNA-SARS-CoV2 Spike STPCNGVEGFNCYFPLQSYGFGQPTNGVGYPYRVVLSFELLHAPATVCGPKKSTNLVKN
SARS-CoV-consensus GK~~PCTP~~-PALNCYWPLNDYGFYTTTGIGYQPYRVVLSFELLNAPATVCGPKLSTDLIKN
... :***:*.:.***** *:***:*****:***** ***** ** :***:*

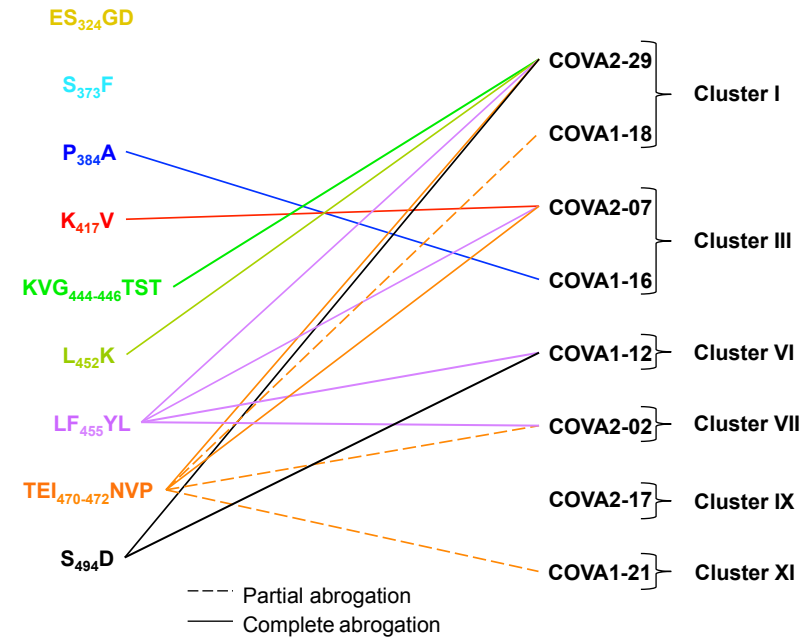


Figure S2. Binding and neutralization titers across two infection cohorts, related to Figure 3.

Binding and neutralization titers were generated as described in the Methods. Concentrations of S1-specific serum IgG (pg) at ID₅₀ dilutions were calculated using the IgG titers quantified via the semi-quantitative ELISA and the known ID₅₀ value. Only sera that gave a measurable titer in both semi-quantitative ELISA and pseudotype neutralization assay were included. Column statistics below were generated in Prism Graphpad and the data were analyzed by a non-parametric Mann-Whitney U test.

		Binding titer (S1 IgG µg/ml)		Neutralization titer (ID ₅₀)		Specific IgG at ID ₅₀	
		Mild	Severe	Mild	Severe	Mild	Severe
n =		105	94	99	93	92	91
Minimum		0.3	2	50	50	12.86	68.06
25% Percentile		1.6	17	201.3	513.8	253.9	971.3
Median		3.9	46.5	342.3	1466	558.6	1757
75% Percentile		7.2	122.8	666.4	2556	1155	3073
Maximum		68	529	4152	20601	2907	45053
Mean		5.562	89.56	603.8	2347	736.5	3181
Std. Deviation		7.429	108.2	718.5	3008	604.7	5540
Std. Error of Mean		0.725	11.16	72.21	311.9	63.04	580.8
Lower 95% CI of mean		4.124	67.4	460.5	1727	611.3	2027
Upper 95% CI of mean		6.999	111.7	747.1	2966	861.7	4335
		Mann Whitney test		Mann Whitney test		Mann Whitney test	
P value		< 0.0001		< 0.0001		< 0.0001	
Exact or approximate P value?		Exact		Exact		Exact	
P value summary		****		****		****	
Significant (P < 0.05)		Yes		Yes		Yes	
One- or two-tailed P value?		Two-tailed		Two-tailed		Two-tailed	
Sum of ranks in column A,B		6217, 13684		6862, 11667		5777, 11059	
Mann-Whitney U		651.5		1912		1499	