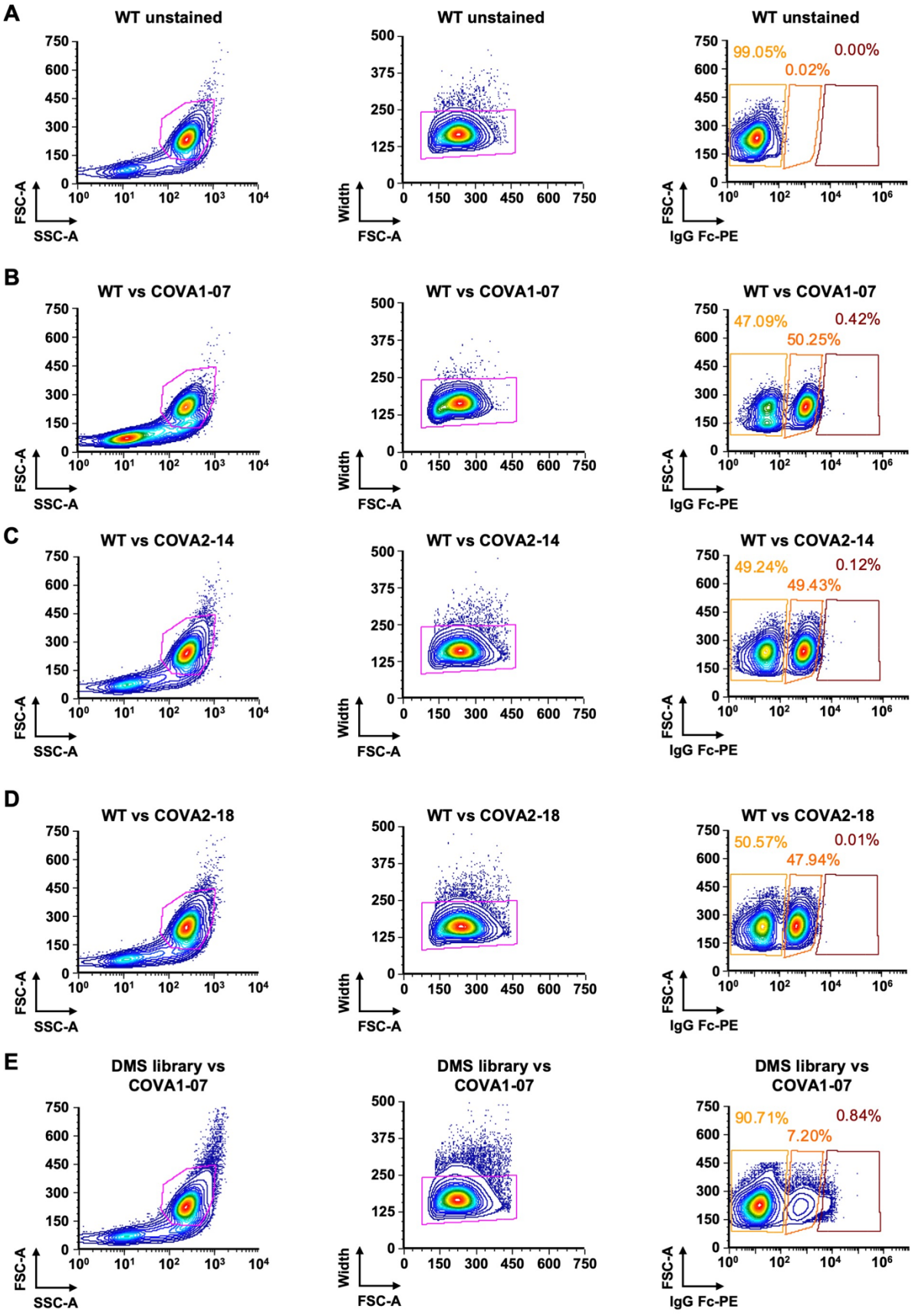

A
WT unstained
WT unstained
WT unstained
99.05%
0.02%
0.00%
B
WT vs COVA1-07
WT vs COVA1-07
WT vs COVA1-07
47.09%
50.25%
0.42%
C
WT vs COVA2-14
WT vs COVA2-14
WT vs COVA2-14
49.24%
49.43%
0.12%
D
WT vs COVA2-18
WT vs COVA2-18
WT vs COVA2-18
50.57%
47.94%
0.01%
E
DMS library vs COVA1-07
DMS library vs COVA1-07
DMS library vs COVA1-07
90.71%
7.20%
0.84%
FSC-A
SSC-A
Width
IgG Fc-PE

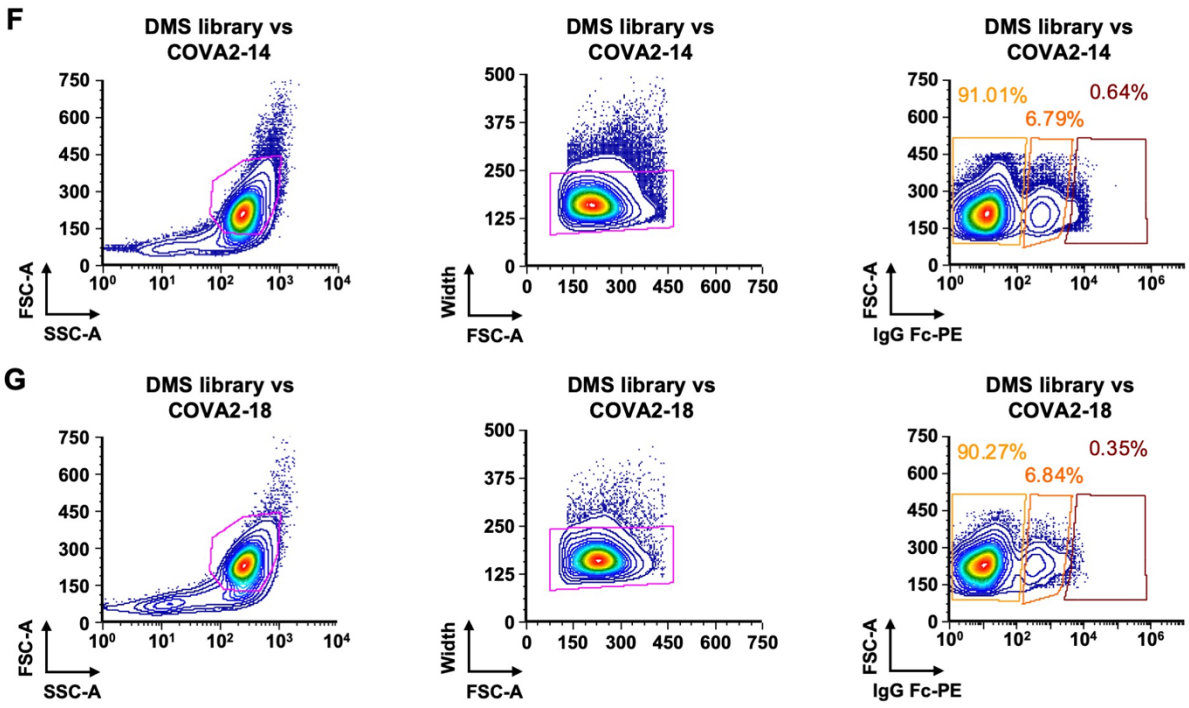


Figure S1. Gating strategy for FACS of DMS library. (A) Flow cytometry plots of the parental HEK293T landing pad cells without expression of SARS-CoV-2 spike. Primary antibody used was COVA1-07. **(B-D)** Flow cytometry plots of HEK293T landing pad cells stably expressing WT SARS-CoV-2 spike. Primary antibodies used were **(B)** COVA1-07, **(C)** COVA2-14, and **(D)** COVA2-18. **(E-G)** Flow cytometry plots of HEK293T landing pad cells stably expressing the DMS library. Primary antibodies used were **(E)** COVA1-07, **(F)** COVA2-14, and **(G)** COVA2-18.

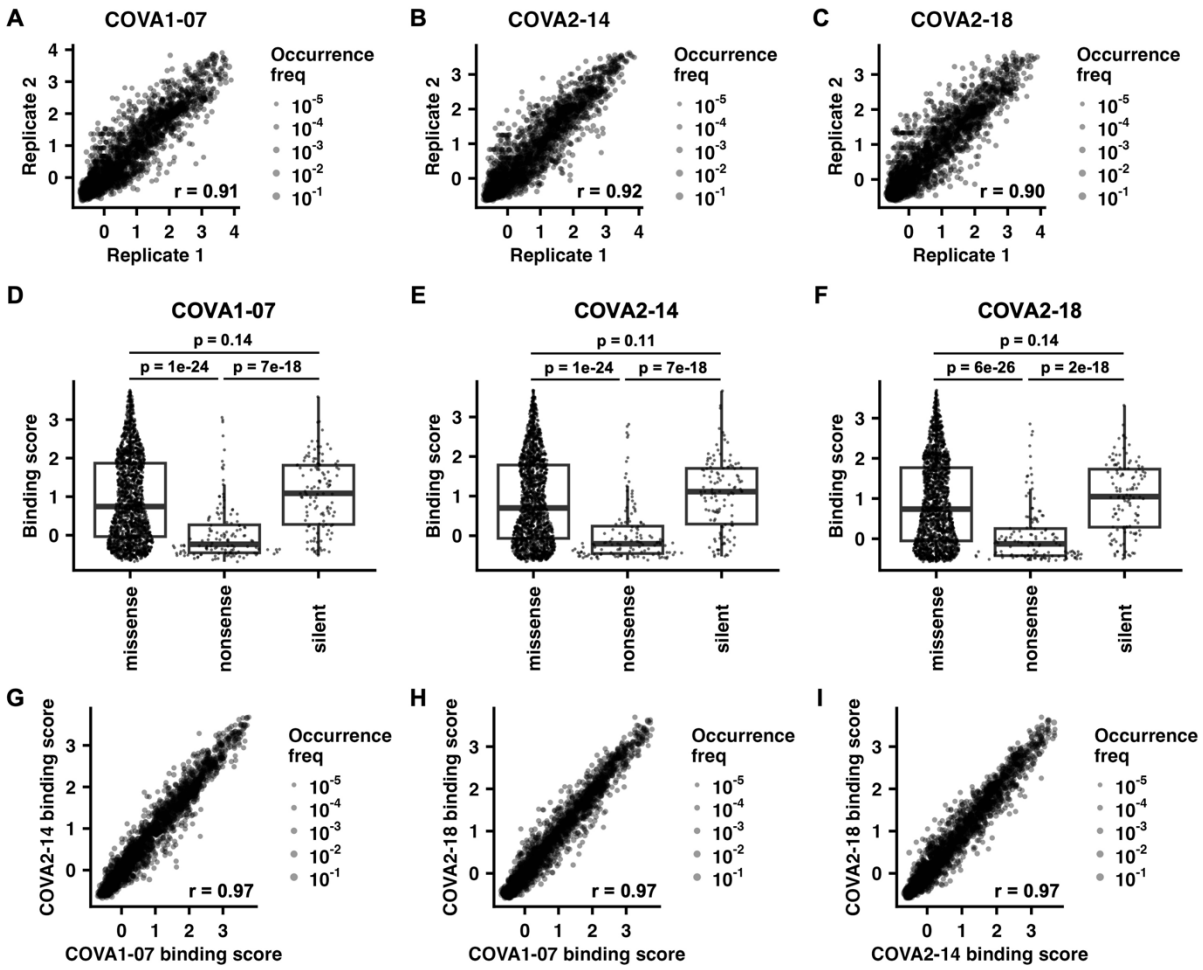
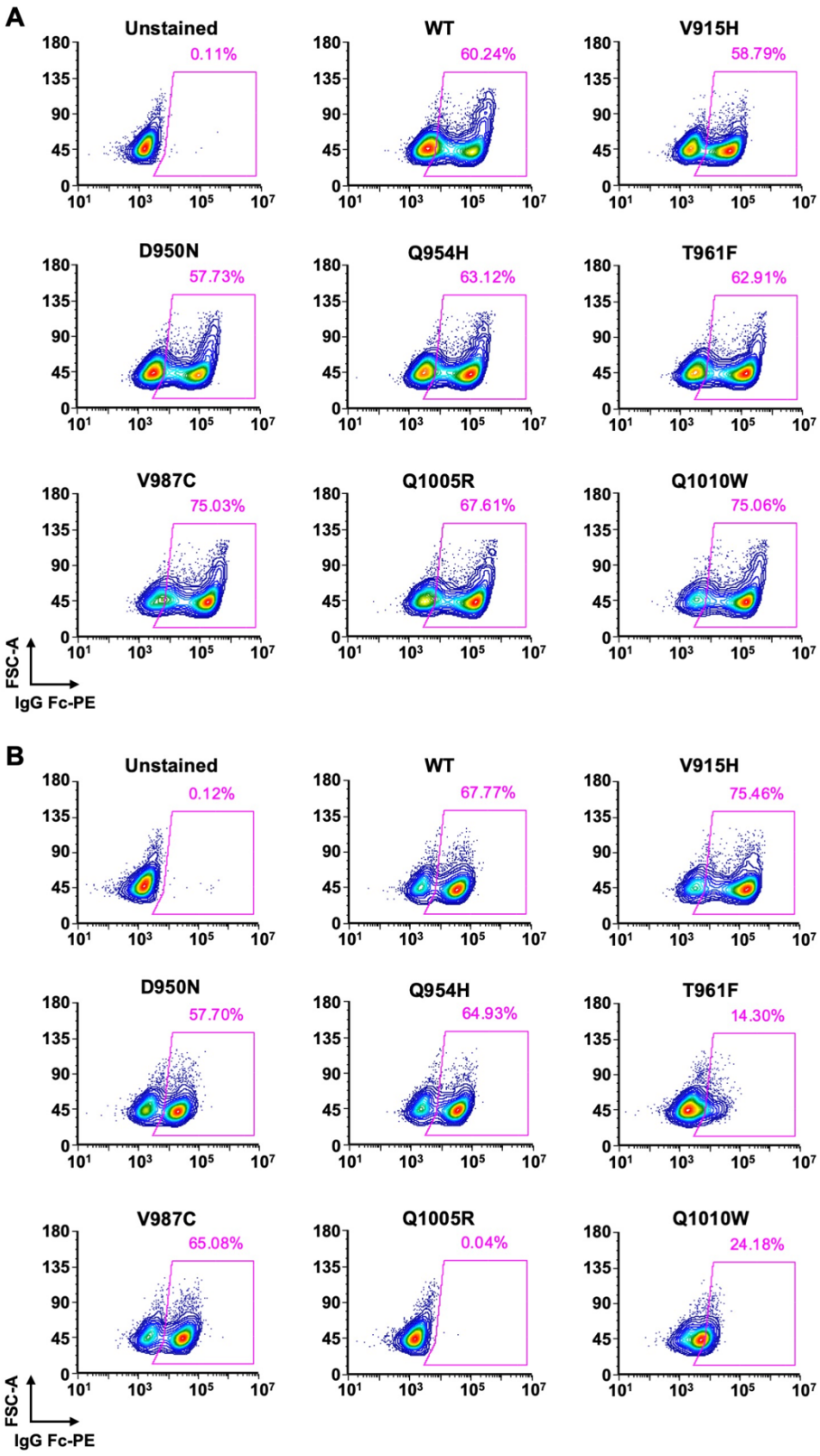


Figure S2. Reproducibility and quality of the deep mutational scanning data. (A-C) Correlation of binding scores to **(A)** COVA1-07, **(B)** COVA2-14, and **(C)** COVA2-18 between two biological replicates. Pearson's correlation coefficient, *r*, for each plot is indicated. **(D-F)** Box plots showing distribution of average binding scores to **(D)** COVA1-07, **(E)** COVA2-14, and **(F)** COVA2-18 according to different mutation classes (missense, nonsense, and silent). Two-tailed Student's *t* tests were performed, and *p*-values are shown. **(G-I)** Correlation plots between the binding scores to **(G)** COVA1-07 and COVA2-14, **(H)** COVA1-07 and COVA2-18, and **(I)** COVA2-14 and COVA2-18. Occurrence frequency of each mutant is the average of six sequencing read frequencies (**see Methods**).


A
Unstained
0.11%
WT
60.24%
V915H
58.79%
D950N
57.73%
Q954H
63.12%
T961F
62.91%
V987C
75.03%
Q1005R
67.61%
Q1010W
75.06%
FSC-A
IgG Fc-PE
B
Unstained
0.12%
WT
67.77%
V915H
75.46%
D950N
57.70%
Q954H
64.93%
T961F
14.30%
V987C
65.08%
Q1005R
0.04%
Q1010W
24.18%
FSC-A
IgG Fc-PE

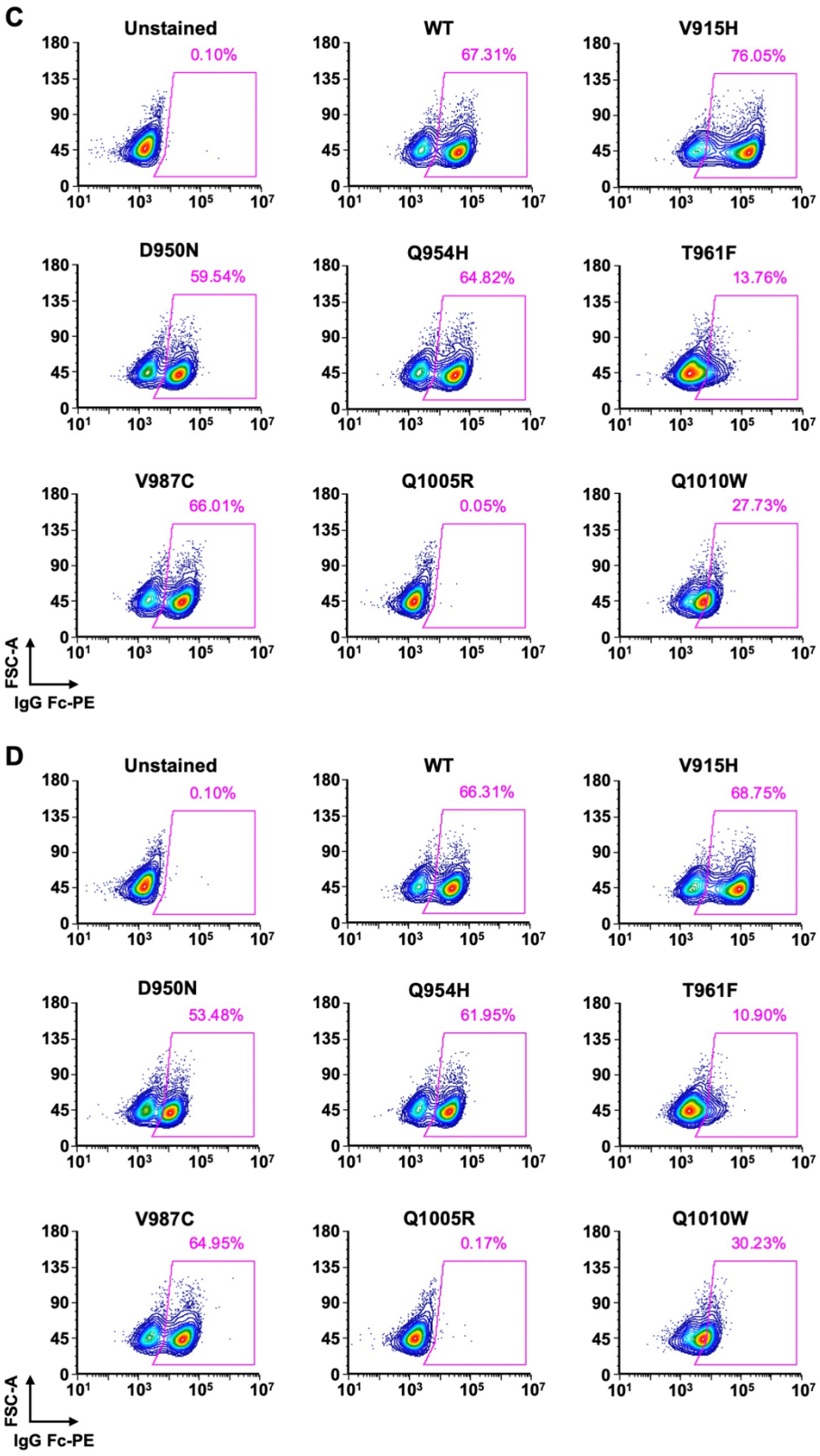

C
Unstained
0.10%
WT
67.31%
V915H
76.05%
D950N
59.54%
Q954H
64.82%
T961F
13.76%
V987C
66.01%
Q1005R
0.05%
Q1010W
27.73%
FSC-A
IgG Fc-PE
D
Unstained
0.10%
WT
66.31%
V915H
68.75%
D950N
53.48%
Q954H
61.95%
T961F
10.90%
V987C
64.95%
Q1005R
0.17%
Q1010W
30.23%
FSC-A
IgG Fc-PE

Figure S3. Flow cytometry plots for validation of expression and binding. (A) Cytometry plots for validation and quantification of surface expression of WT or mutant S using CC12.3, which is an RBD antibody [S1]. **(B-D)** Cytometry plots for validation and quantification of binding of WT or mutant S to **(B)** COVA1-07, **(C)** COVA2-14, or **(D)** COVA2-18.

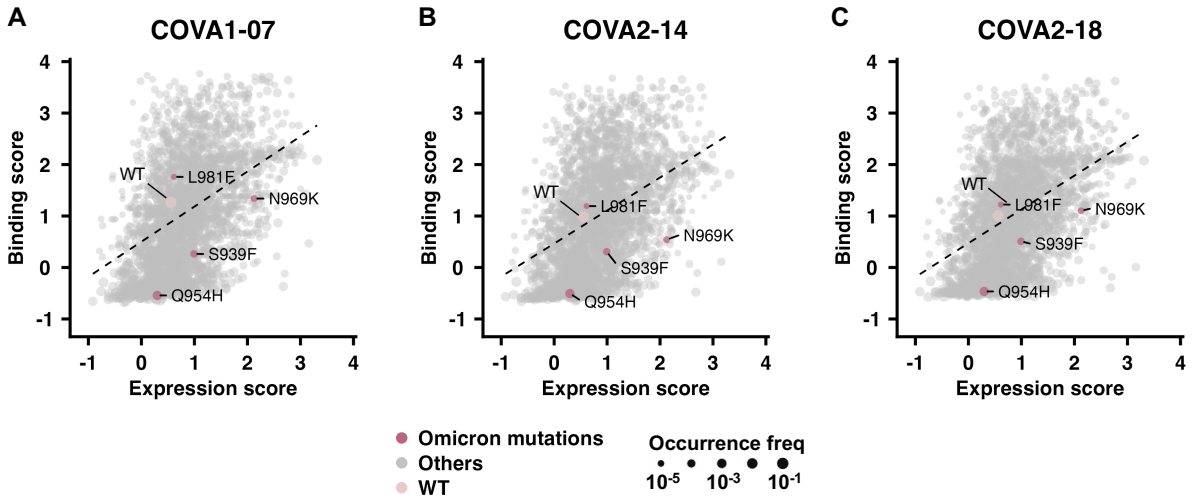


Figure S4. Binding and expression scores of mutations found in recent Omicron lineages. Plots of binding scores to **(A)** COVA1-07, **(B)** COVA2-14, and **(C)** COVA2-18 against expression scores. One datapoint represents one amino acid mutation in our mutant library. Mutations found in recent Omicron lineages are highlighted. Expression scores were obtained from our previous study[25]. Pearson's correlation coefficient is shown. Occurrence frequency of each mutant is the average of six sequencing read frequencies (**see Methods**). Each data point is sized according to their occurrence frequency. Biological replicates of expression and binding sorting experiments were performed starting from the sorting step on the same library (**see Methods**).

Table S1. Number of cells sorted into each bin in fluorescence-activated cell sorting.

COVA1-07			
	Bin 0	**Bin 1**	**Bin 2**
Replicate 1	3.69×10^6	5.65×10^5	1.11×10^5
Replicate 2	4.14×10^6	6.72×10^5	8.03×10^4
COVA2-14			
	Bin 0	**Bin 1**	**Bin 2**
Replicate 1	3.51×10^6	9.43×10^5	1.52×10^5
Replicate 2	3.98×10^6	5.64×10^5	9.10×10^4
COVA2-18			
	Bin 0	**Bin 1**	**Bin 2**
Replicate 1	3.22×10^6	5.90×10^5	1.38×10^5
Replicate 2	4.11×10^6	9.76×10^5	1.19×10^5

SUPPLEMENTAL REFERENCES

S1. Yuan, M., Liu, H., Wu, N.C., Lee, C.D., Zhu, X., Zhao, F., Huang, D., Yu, W., Hua, Y., Tien, H., et al. (2020). Structural basis of a shared antibody response to SARS-CoV-2. Science *369*, 1119-1123. 10.1126/science.abd2321.