Supplemental Information

Identification of Human Single-Domain

Antibodies against SARS-CoV-2

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Supplemental Information

Figure S1. Analytical SEC-HPLC for eight human germline IGHV alleles with high yield and the SARS-CoV-2-specific single-domain neutralizing antibodies (n3088, n3130, n3086, n3113). Related to Figure 1.
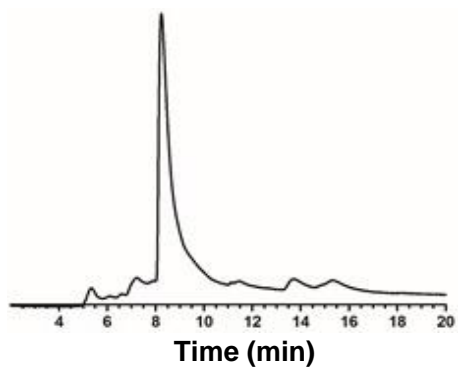
Figure S2. Competition of single-domain antibodies with ACE2, or the antibody CR3022 for SARS-CoV-2 RBD binding were measured by BLI. (**A**) Immobilized SARS-CoV-2 RBD was first saturated with the testing single-domain antibodies. The capacity of ACE2 binding to RBD was monitored by measuring further shifts after injecting the ACE2 in the presence of the testing single-domain antibody. The grams show binding patterns of ACE2 to SARS-CoV-2 RBD with (green curve) or without (purple curve) prior incubation with each testing single-domain antibody. (**B**) Immobilized SARS-CoV-2 RBD was first saturated with CR3022 and the capacity of single-domain antibody to bind RBD was monitored by measuring further shifts after incubating with the tested single-domain antibody in the presence of CR3022. Grams show binding patterns of single-domain antibody to SARS-CoV-2 RBD with (green curve) or without (purple curve) CR3022. Related to Figure 2.

Figure S3. Epitope mapping of the representative single-domain antibodies in competition groups A, D and E on SARS-CoV-2 RBD, as measured by ELISA. (**A**) Binding capacity of representative single-domain antibody n3021 in competition group A and antibody n3130 in competition group D to wild-type and variants of SARS-CoV-2 RBD, as measured by ELISA. The mutations are shown in red box, ACE2-binding sites are shown in cyan, and the epitopes of CR3022 are shown in yellow. The critical residues for the RBD binding of n3021 and n3130 are highlighted by red star and blue star, respectively. (**B**) Sequence alignment of three SARS-CoV-2 clinical isolates (nCoV-SH01, SZTH-004 and IDF0372) in which the mutations are highlighted in red box; binding capacity of neutralizing single-domain antibodies (group D antibody n3088 and group E antibody n3113) to RBD of three SARS-CoV-2 clinical isolates, as
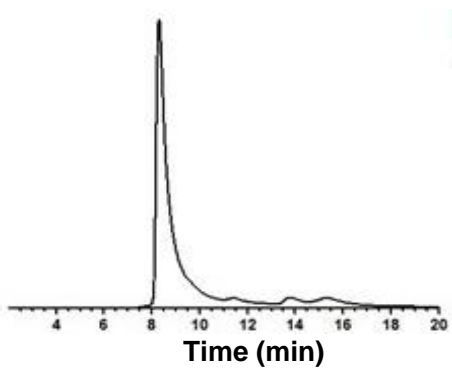
measured by ELISA, with an irrelevant protein (Tim-3) as control. Related to Figure 3.
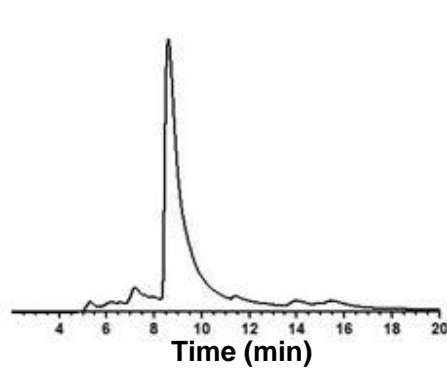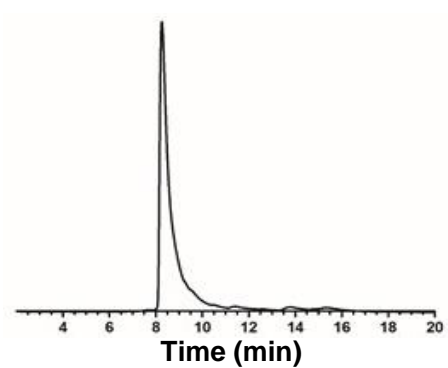
Figure S1
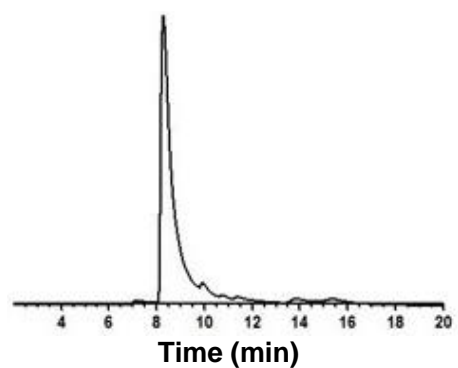
Figure S2

A

B

Figure S3

A

n3021

Absorbance at 405 nm

Log [Concentration], µg/mL

- W.T.
- Q414A/T415A
- T500A/N501A/G502A
- Y505A/Q506A/P507A

n3130

Absorbance at 405 nm

Log [Concentration], µg/mL
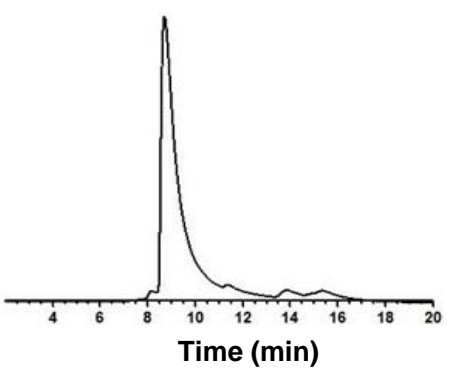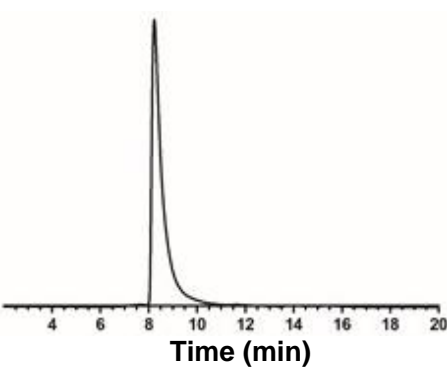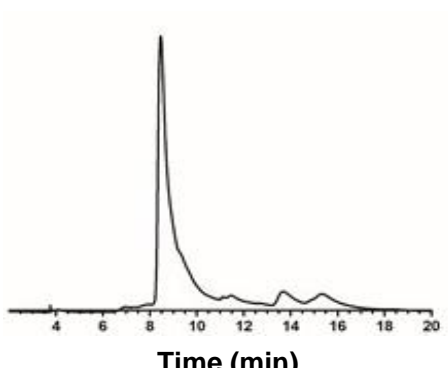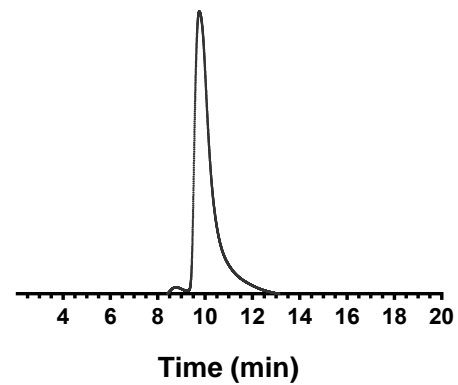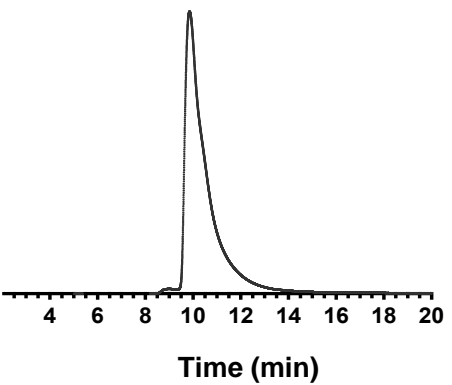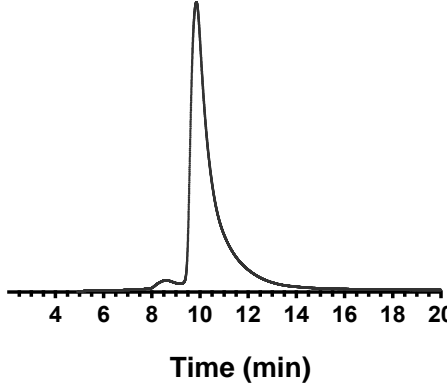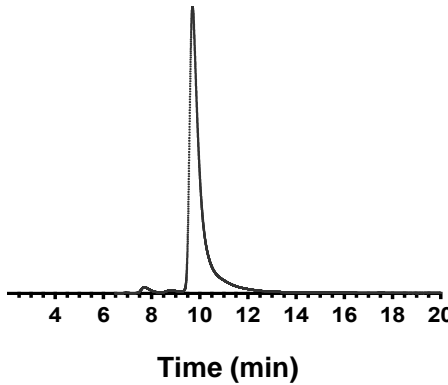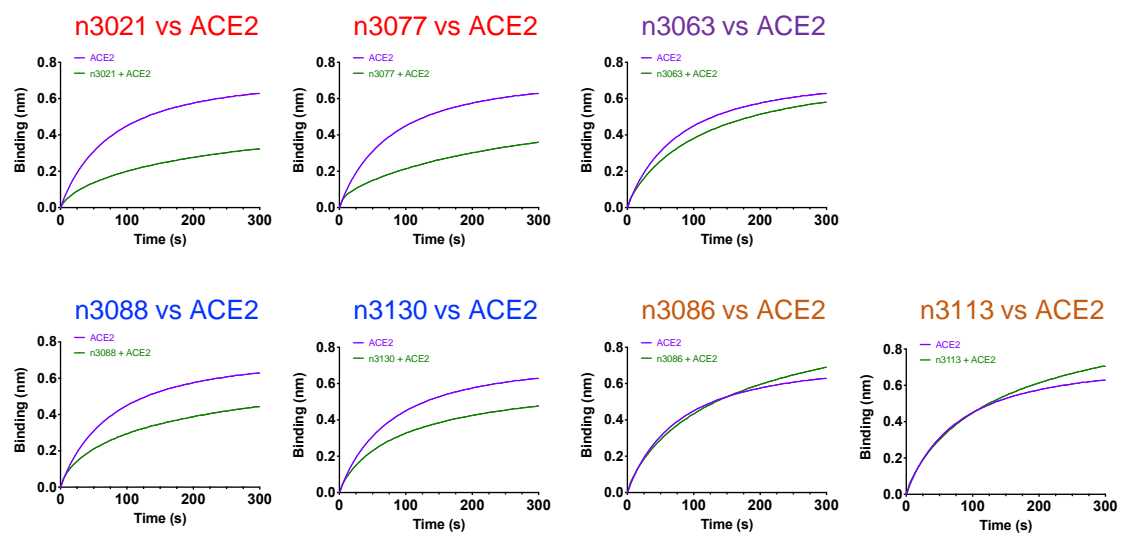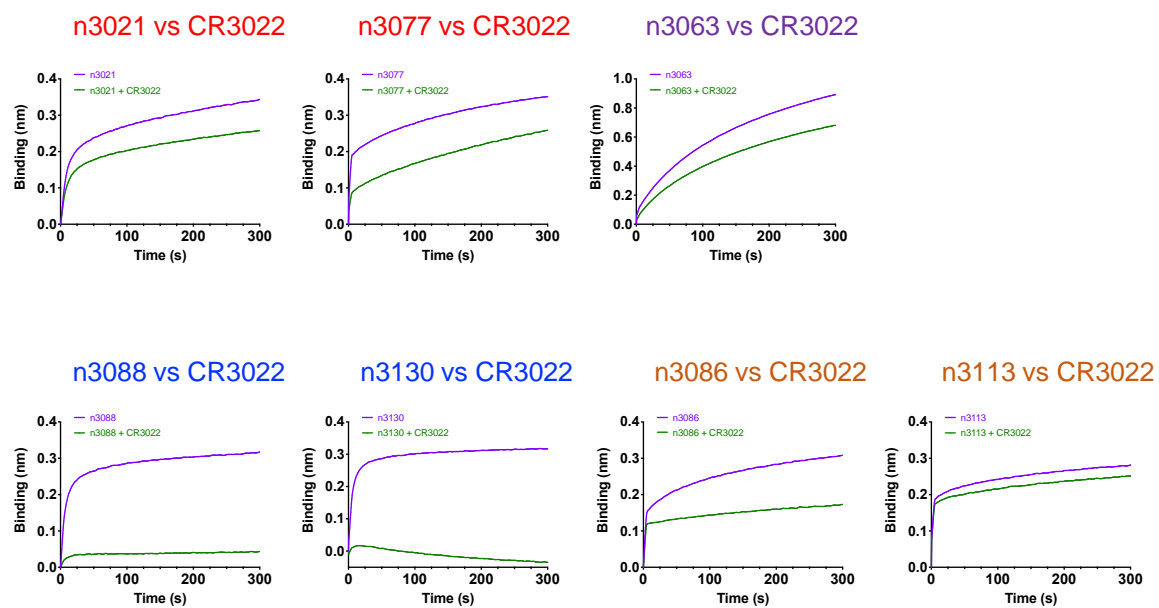
- W.T.
- F374A
- F377A
- S383A
- P384A
- T385A
- K386A
- L390A
- F392A
- D427A
- D428A
- F429A
- E516A
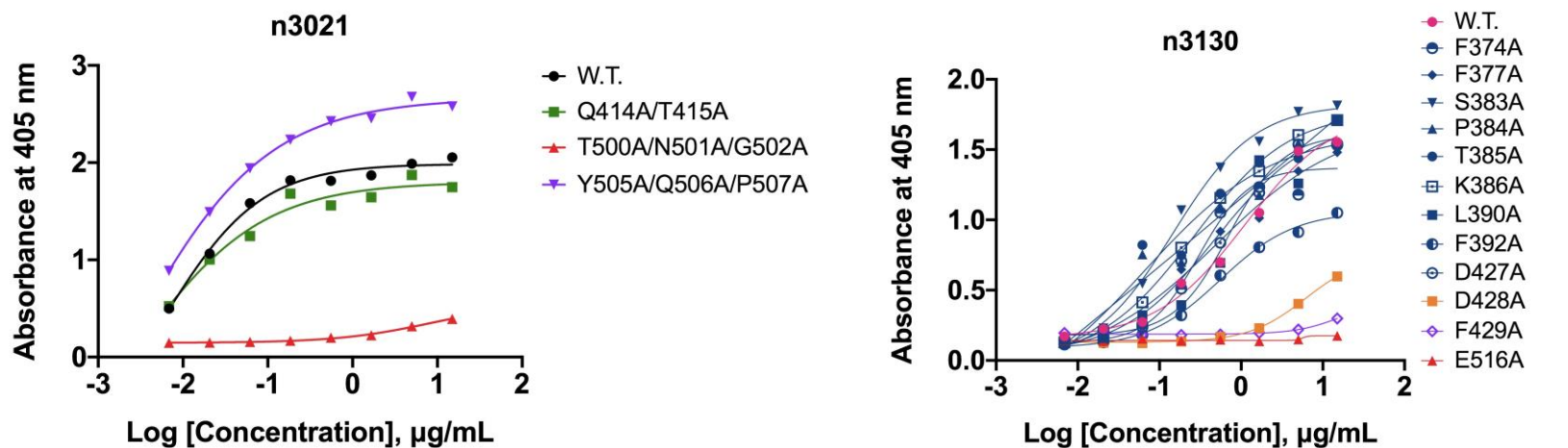
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K LNDL C F TNVYADSFVIRGDEVRQIAPG QT GKIADYNYKLP DDF TGCVIAWNS N NLDSKVGGN Y NY
L Y R L FRKSNLKPFERDISTEIYQ A GSTPCNGVEG F N C Y FPLQ S Y GFQP TNG VG Y QP YRVVVLS FE LL
H APATVCGPKKSTNLVKNKCVNFS

B

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              354           364  367
nCoV-SH01   YAW N RKRISNCVA D YS V LYNS
SZTH-004    YAW D RKRISNCVA Y YS V LYNS
IDF0372     YAW N RKRISNCVA D YS F LYNS
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n3088

Absorbance at 405 nm

Log [Concentration], µg/mL

- nCoV-SH01
- SZTH-004
- IDF0372
- N.C.

n3113

Absorbance at 405 nm

Log [Concentration], µg/mL

- nCoV-SH01
- SZTH-004
- IDF0372
- N.C.
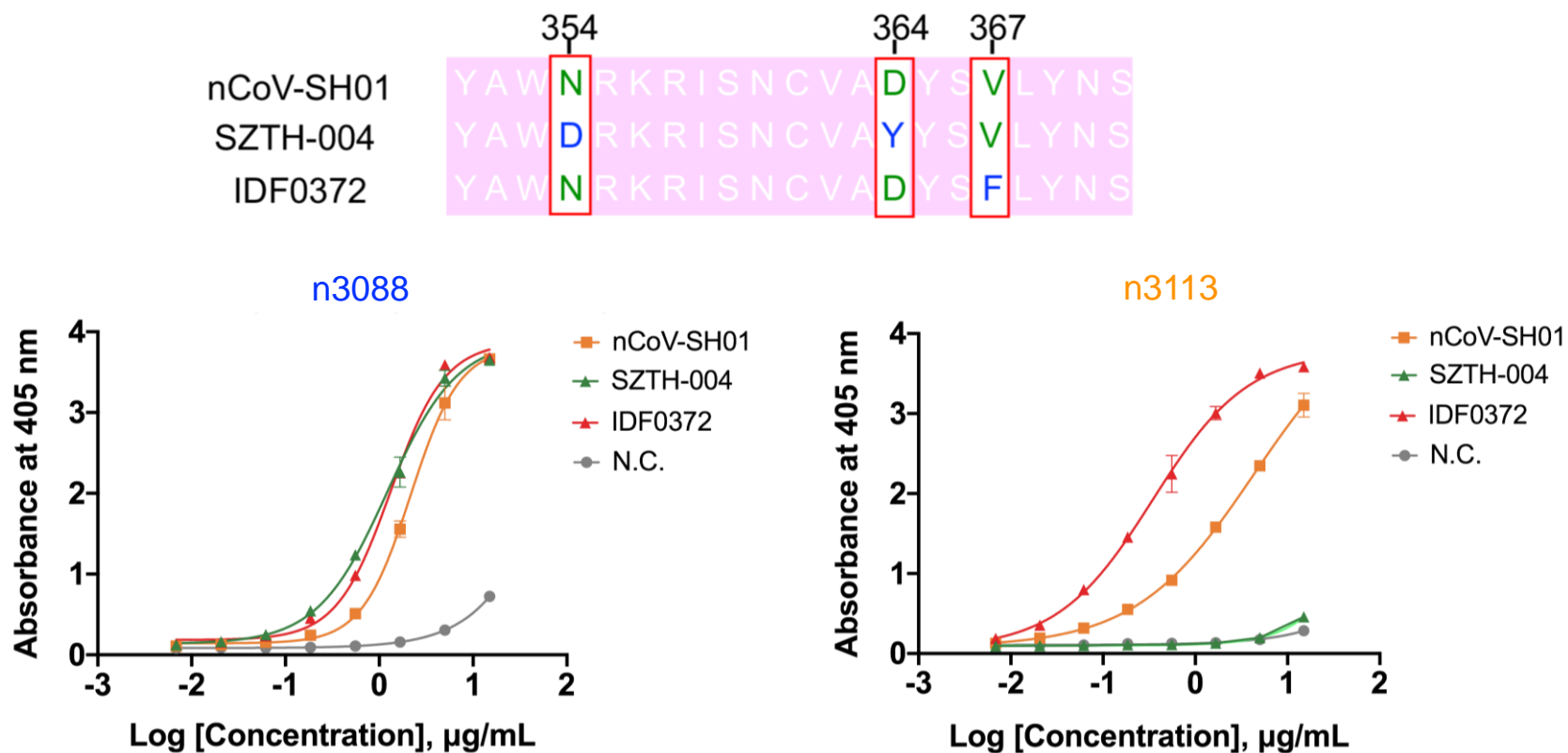
Table S1 Primers used for amplification of heavy-chain CDR1, CDR2, and CDR3 from naïve antibody libraries. Related to Figure 1.

Primer description	Name	Sequence	Product
H1 sense	H1F	CTGAGACTCTCCTGTGCAGCC TCT	CDR1
H1 antisense	H1R	TGGAGCCTGGCGGACCCAGCT CAT	
H2 sense	H2F1	ATGAGCTGGGTCCGCCAGGCTCCAGGACAASGSCTTGAGTGG	CDR2
	H2F2	ATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGCCCTGGAGTGG	
	H2F3	ATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGNCTRGAGTGG	
H2 antisense	H2R1	ATTGTCTCTGGAGATGGTGACCCTKYCCTGRAACTY	
	H2R2	ATTGTCTCTGGAGATGGTGAATCGGCCCTTCACNGA	
	H2R3	ATTGTCTCTGGAGATGGTGACTMGACTCTTGAGGGA	
	H2R4	ATTGTCTCTGGAGATGGTGACSTGGCCTTGGAAGGA	
	H2R5	ATTGTCTCTGGAGATGGTAAACCGTCCTGTGAAGCC	
H3 sense	H3F1	AGCCTGAGAGCCGAGGACACRGCYTTRTATTACTGT	CDR3
	H3F2	AGCCTGAGAGCCGAGGACACAGCCAYRTATTACTGT	
	H3F3	AGCCTGAGAGCCGAGGACACRGCYGTRTATTACTGT	
H3 antisense	H3R	GTGGCCGGCCTGGCCACTTGAGGAGACGGTGACC	

Table S2 List of binding kinetic parameters of human single-domain antibodies. Association-rate (k_{on}), dissociation-rate (k_{off}) and affinity (K_D) are shown. Representative single-domain antibodies in each of competition groups are shown in colors which correspond to the competition group designation in Figure 2A. Related to Figure 2.

	Antibody	K_D (M)	k_{on} (Ms^{-1})	k_{off} (s^{-1})	R^2 value
SARS-CoV-2 RBD	n3001	1.20×10^{-8}	9.10×10^4	1.09×10^{-3}	0.98
	n3002	1.46×10^{-8}	1.22×10^5	1.78×10^{-3}	0.97
	n3003	3.34×10^{-8}	8.65×10^4	2.89×10^{-3}	0.97
	n3004	1.48×10^{-8}	1.17×10^5	1.73×10^{-3}	0.97
	n3008	3.05×10^{-8}	6.30×10^4	1.92×10^{-3}	0.99
	n3009	1.69×10^{-8}	1.41×10^5	2.38×10^{-3}	0.96
	n3010	**7.16×10^{-8}**	**2.13×10^4**	**1.53×10^{-3}**	**0.99**
	n3011	9.94×10^{-9}	1.04×10^5	1.03×10^{-3}	0.97
	n3014	1.17×10^{-8}	1.02×10^5	1.20×10^{-3}	0.98
	n3020	4.71×10^{-8}	4.28×10^4	2.01×10^{-3}	0.99
	n3021	**6.32×10^{-10}**	**7.97×10^5**	**5.04×10^{-4}**	**0.99**
	n3025	1.18×10^{-8}	1.20×10^5	1.41×10^{-3}	0.97
	n3026	1.60×10^{-8}	1.59×10^5	2.53×10^{-3}	0.95
	n3047	2.20×10^{-8}	8.79×10^4	1.78×10^{-3}	0.97
	n3051	2.06×10^{-8}	1.58×10^5	3.24×10^{-3}	0.96
	n3055	2.09×10^{-8}	9.70×10^4	2.03×10^{-3}	0.98
	n3063	**2.93×10^{-8}**	**8.94×10^3**	**2.62×10^{-4}**	**0.98**
	n3065	2.22×10^{-8}	1.32×10^5	2.92×10^{-3}	0.97
	n3088	3.25×10^{-8}	7.34×10^4	2.38×10^{-3}	0.96
	n3130	1.26×10^{-8}	2.72×10^5	3.42×10^{-3}	0.97
	n3086	1.15×10^{-6}	1.57×10^5	0.18	0.99
	n3113	1.90×10^{-6}	6.81×10^4	0.13	0.99
	n3072	N.A.	N.A.	N.A.	N.A.
SARS-CoV-2 S1	**n3088**	**3.70×10^{-9}**	**7.63×10^4**	**2.82×10^{-4}**	**0.99**
	n3130	**5.54×10^{-8}**	**1.01×10^4**	**5.62×10^{-4}**	**0.99**
	n3086	**8.90×10^{-8}**	**7.42×10^3**	**6.60×10^{-4}**	**0.99**
	n3113	**5.70×10^{-8}**	**1.26×10^4**	**7.21×10^{-4}**	**0.99**
	n3072	5.06×10^{-8}	1.21×10^4	6.11×10^{-4}	0.99

Table S3 HCDR3 characteristics of the representative single-domain antibodies in each of competition groups. Related to Figure 2.

Human single-domain antibody	Competition group	HCDR3 length	HCDR3 sequence
n3021	A	9	VRDWLRFDY
n3063	B	11	AKDLLPGGADV
n3010	C	20	ARHQPPDYYDSSGKPYYFDY
n3088	D	21	ARVREYYDILTGYSDYYGMDV
n3130	D	13	ATRSPYGDYAFSY
n3086	E	10	ARDFNWGVDY
n3113	E	12	VSNWASGSTGDY