small methods

Supporting Information

for *Small Methods,* DOI: 10.1002/smtd.202200387

A Class of Shark-Derived Single-Domain Antibodies can Broadly Neutralize SARS-Related Coronaviruses and the Structural Basis of Neutralization and Omicron Escape

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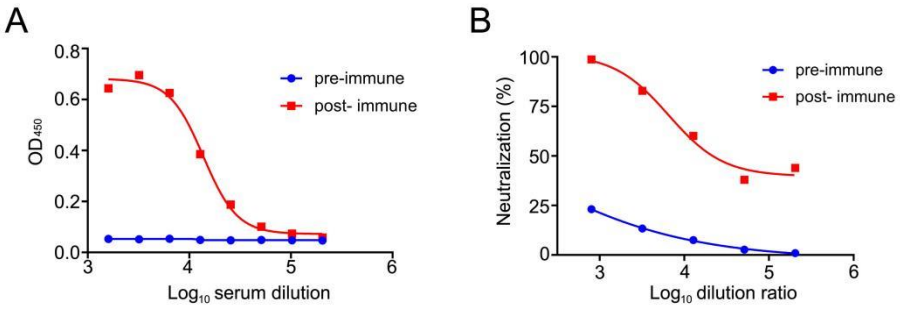

Figure S1 Development of RBD-specific shark anti-sera for potent SARS-CoV-2 neutralization.

(A) Detection of strong and specific serologic activities after immunization of SARS-CoV-2 S1. (B) The neutralization potency of the immunized shark's serum were calculated based on pseudotyped SARS-CoV-2 neutralization (luciferase). Blue and red lines denote immunized and pre-immunized shark's serum.

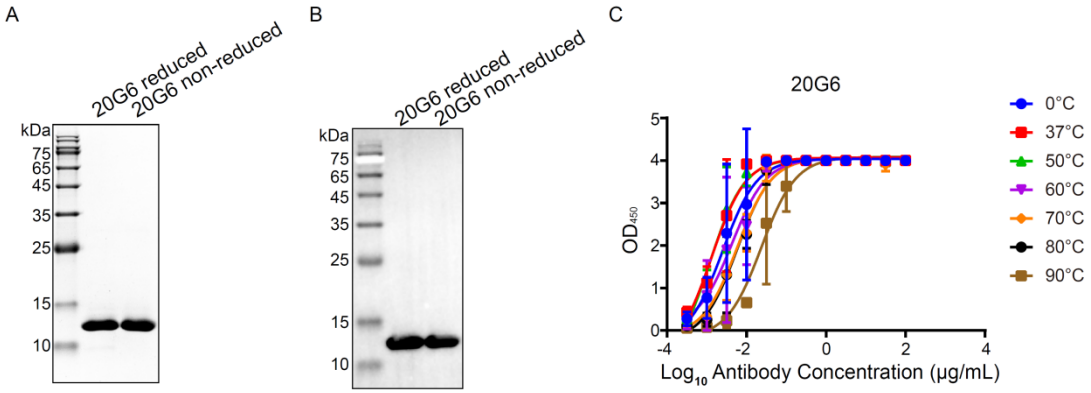

Figure S2 Biophysical features of 20G6.

(A-B) SDS-PAGE (A) and Western blot (B) to verify the purification of 20G6. (C) Thermostability of 20G6. 20G6 was heated at different temperatures for 1 hour, and its binding activity to RBD was detected by ELISA.

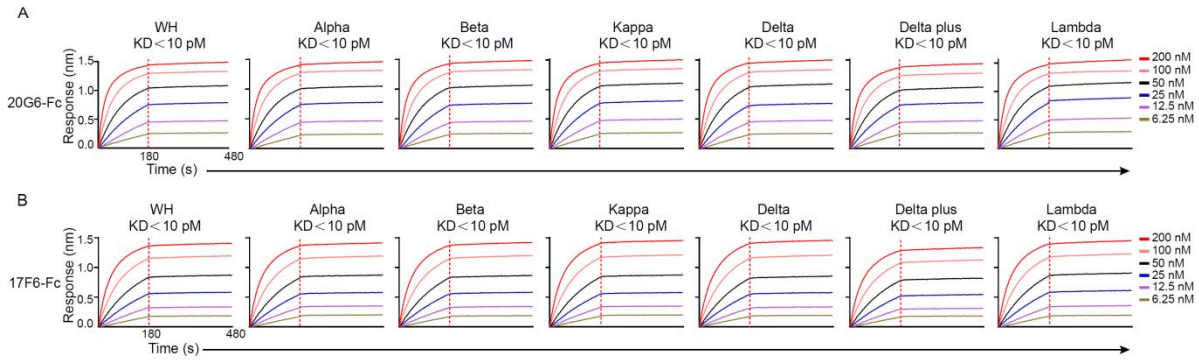

Figure S3　Binding capacity of 20G6-Fc and 17F6-Fc to RBDs of SARS-CoV-2 variants.

(A) The BLI binding kinetics of different concentrations of 20G6-Fc to immobilized RBD variants. (B) The BLI binding kinetics of different concentrations of 17F6-Fc to immobilized RBD variants.

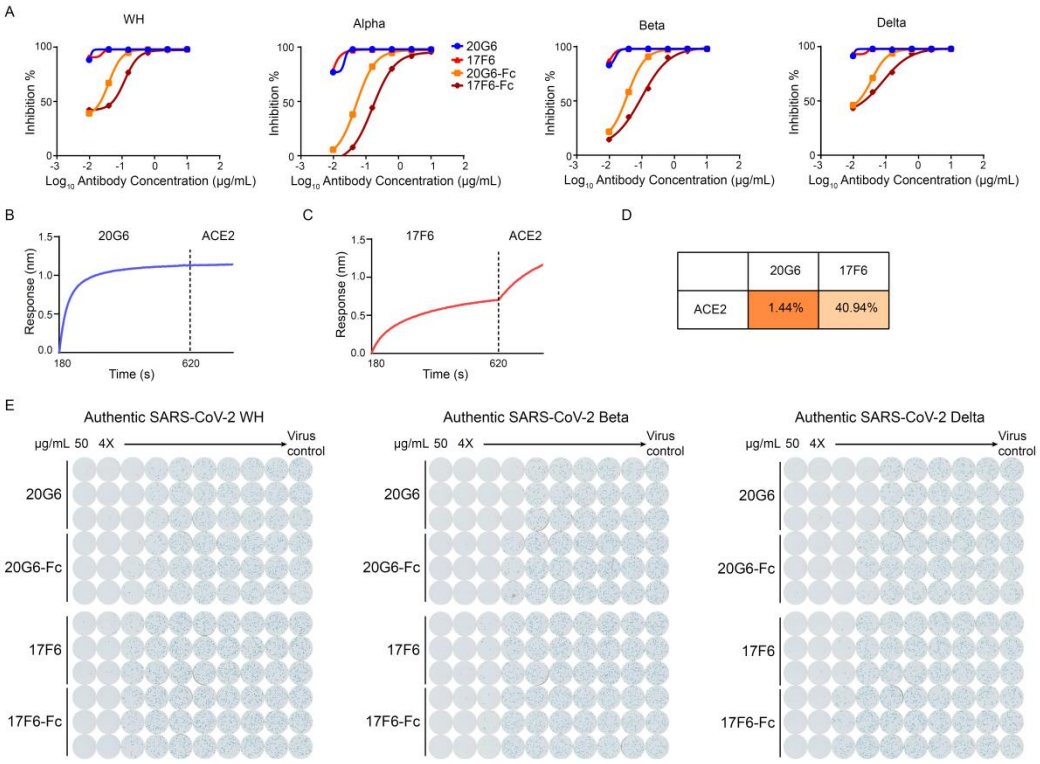

Figure S4 The neutralization potency measurements of two vnarbodies.

(A) Competition analysis of 20G6 or 17F6 with ACE2 for binding to SARS-CoV-2 RBD variants (WH, Alpha, Beta, Delta) by sVNT. (B) Competition of 20G6 with ACE2 for binding to SARS-CoV-2 RBD. (C) Competition of 17F6 with ACE2 for binding to SARS-CoV-2 RBD. The vertical dashed line indicates the start of the association of ACE2 to immobilized RBD. (D) Competition tolerance was shown for 20G6 and 17F6 with ACE2. (E) Plaques formed in Vero E6 cells inoculated with 200 FFU SARS-CoV-2 WH, Beta and Delta variants and 4-fold diluted vnarbodies.

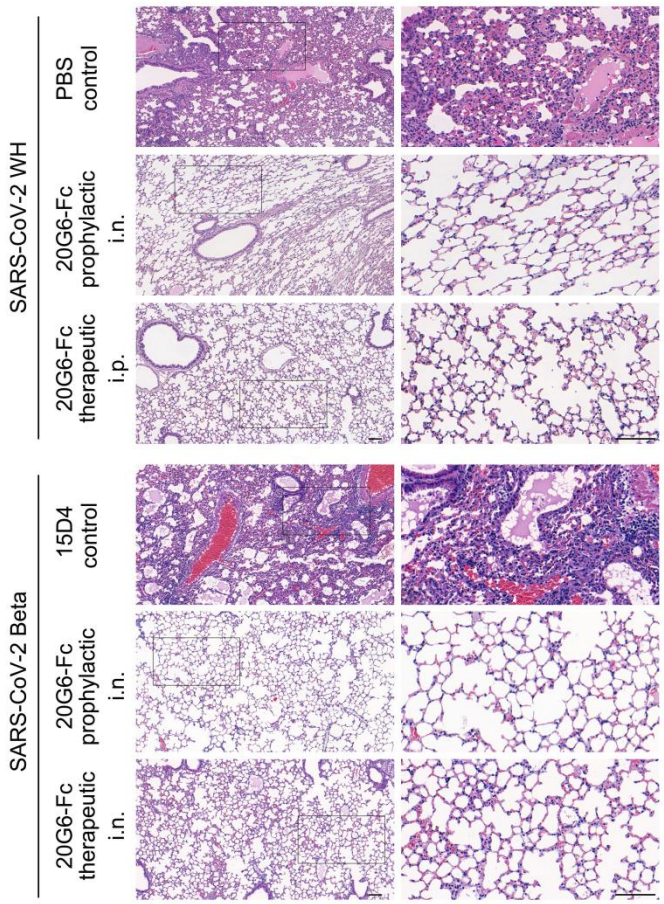

Figure S5 Lung pathology of SARS-CoV-2 infected mice treated with 20G6-Fc prophylactically and therapeutically.

Sections of paraffin-embedded lungs from SARS-CoV-2 WH and SARS-CoV-2 Beta infected mice at 3 d.p.i. were stained with hematoxylin and eosin. Histopathological analysis of lung sections from the phosphate-buffered saline (PBS) control or isotype control-treated mice showed immune cell infiltration, alveolar edema filled with liquid and vascular thrombosis. 20G6-Fc treated mice showed minimal lung pathology. Scale bar, 100 μm.

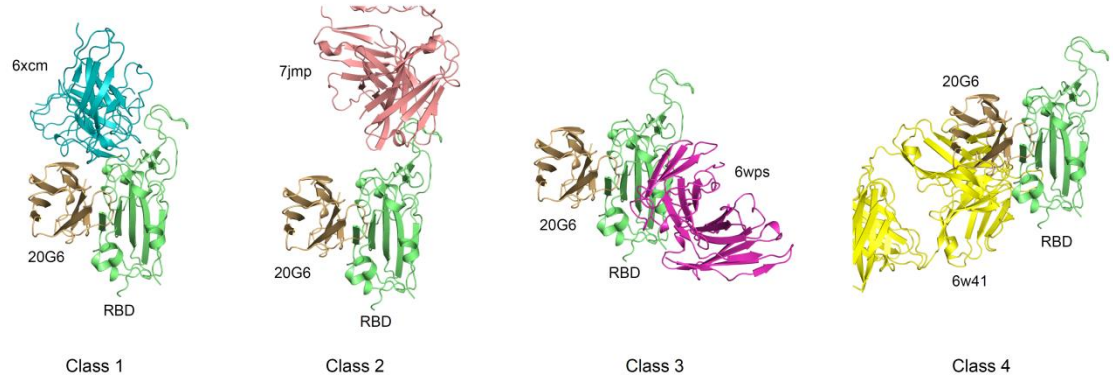

Figure S6 Comparison of 20G6 with other four RBD binding epitopes.

RBD is colored in green, 20G6 is colored in gold, PDB ID: 6xcm is colored in cyan, PDB ID: 7jmp is colored in pink, PDB ID: 6wps is colored in magenta, and PDB ID: 6w41 is colored in yellow.

Figure S7 Sequence alignment of vnarbodies 20G6 and 17F6.

The CDRs and hypervariable regions are colored in red, the FRs are colored in blue, the amino acids that bind to RBD are marked with black circles.

Neutralization IC 50 (μg/ml)					
		20G6	**20G6 Fc**	**17F6**	**17F6 Fc**
Pseudovirus	**WH**	0.043 (2.86 nM)	0.114 (1.46 nM)	0.61 (40.66 nM)	0.678 (8.69 nM)
	Beta	0.101 (6.73 nM)	0.172 (2.20 nM)	0.885 (59.00 nM)	0.459 (5.88 nM)
	Kappa	0.095 (6.33 nM)	0.225 (2.88 nM)	0.345 (23.00 nM)	0.230 (2.94 nM)
	Delta	0.055 (3.66 nM)	0.144 (1.84 nM)	0.422 (28.13 nM)	0.746 (9.56 nM)
	Delta plus	0.074 (4.93 nM)	0.261 (3.34 nM)	0.170 (11.33 nM)	0.233 (2.98 nM)
	Lambda	0.135 (9.00 nM)	0.177 (2.26 nM)	0.337 (22.46 nM)	0.573 (7.34 nM)
	Omicron	>10	>10	>10	>10
	SARS-CoV-1	>10	>10	>10	>10
	Pangolin GD1	0.001 (0.06 nM)	0.012(0.15 nM)	0.531 (35.4 nM)	0.058(0.72 nM)
	Bat RaTG13	0.004(0.26 nM)	0.001 (0.01 nM)	0.162 (10.8 nM)	0.019 (0.23 nM)
	MERS	>10	>10	>10	>10
Authentic virus	**WH**	0.61 (40.67 nM)	0.92 (11.79 nM)	2.72 (181.33 nM)	2.68 (34.36 nM)
	Beta	0.37 (24.67 nM)	0.73 (9.36 nM)	0.92 (61.33 nM)	1.55 (19.87 nM)
	Delta	0.30 (20.00 nM)	0.80 (10.26 nM)	1.02 (68.00 nM)	2.64 (33.85 nM)

Table S1 Summary of the neutralization potency of vnarbodies 20G6 and 17F6.

The IC50 was calculated from Figure 1D-E, Figure 4D.

	17F6-RBD	20G6-RBD$_{N501Y}$
Data collection		
Wavelength (Å)	0.97915	0.97915
Space group	$P2_1$	$P22_12_1$
Unit cell (Å, º)	a=82.02, b=73.75, c=270.72 β=90	a=53.51, b=90.53, c=162.46
Molecules per asymmetric unit	16	4
Resolution range (Å)[a]	135.36-2.85 (2.91-2.85)	19.82-1.90 (1.94-1.90)
Mosaicity (º)	-	0.19
Unique reflections	76077 (4462)	63067 (4175)
Completeness (%)	99.8 (99.2)	99.9 (99.7)
<I/σ(I)>	7.2 (1.8)	16.5 (2.0)
R_{pim}[b] (%)	6.4 (30.6)	2.1 (35.1)
Average redundancy	5.1	12.5
Refinement statistics		
Resolution range (Å)	90.40-2.85	19.82-1.90
R-factor[c]/R-free[d] (%)	25.4/30.0	19.7/23.9
RMSD[e] bond lengths (Å)	0.012	0.013
RMSD bond angles (º)	1.794	1.906
Mean B factors (Å^2)		
Protein	53.82	85.36
Sugar	83.10	119.6
Water	47.87	59.08
Ramachandran plot		
Most favored (%)	96.3	97.6
Outlier (%)	0.1	0.0
PDB entry	7FBJ	7FBK

[a]The values in parentheses refer to statistics in the highest resolution bin.

[b]*R*pim is the precision-indication merging R factor.

[c]R-factor =$\sum_h$|Fo(h)－Fc(h)|/$\sum_h$Fo(h), where Fo and Fc are the observed and calculated structure-factor amplitudes, respectively.

[d]R-free was calculated with 5% of the data excluded from the refinement.

[e]Root-mean square-deviation from ideal values.

Table S2 Data collection and refinement statistics