ZR | Zoological Research

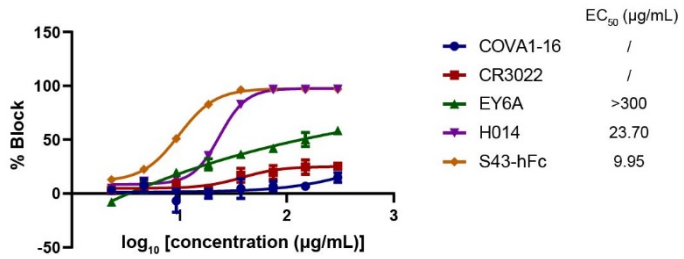
Supplementary Materials

Cross-species recognition of bat coronavirus RsYN04 and cross-reaction of SARS-CoV-2 antibodies against the virus

Runchu Zhao[1,2,#], Sheng Niu[3,#], Pu Han[2,#], Yue Gao[2,4], Dezhi Liu[1,2], Chunliang Luo[2,3], Honghui Liu[2], Bo Liu[2,3], Yanli Xu[5], Jianxun Qi[2], Zhihai Chen[5], Weifeng Shi[6], Lili Wu[2,*], George F. Gao[2,7], Qihui Wang[1,2,7,*]

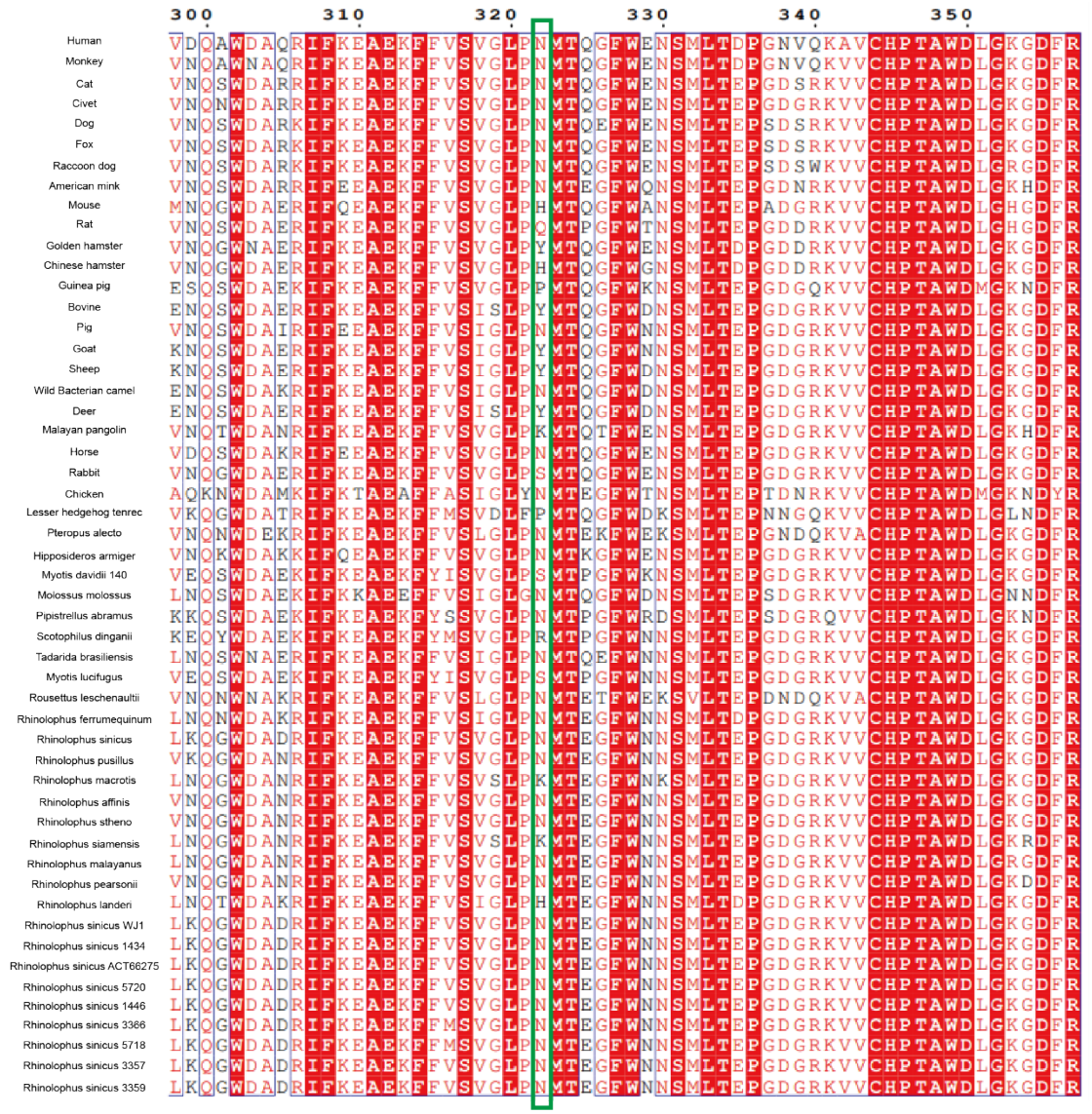
[1] *Institute of Physical Science and Information*, *Anhui University*, *Hefei*, *Anhui* 230039, *China*
[2] *CAS Key Laboratory of Pathogen Microbiology and Immunology*, *Institute of Microbiology*, *Chinese Academy of Sciences*, *Beijing* 100101, *China*
[3] *College of Veterinary Medicine*, *Shanxi Agricultural University*, *Jinzhong*, *Shanxi* 030801, *China*
[4] *School of Life Sciences*, *Hebei University*, *Baoding*, *Hebei* 071002, *China*
[5] *Center of Infectious Disease*, *Beijing Ditan Hospital*, *Capital Medical University*, *Beijing* 100015, *China*
[6] *Key Laboratory of Etiology and Epidemiology of Emerging Infectious Diseases in Universities of Shandong*, *Shandong First Medical University & Shandong Academy of Medical Sciences*, *Taian*, *Shandong* 271000, *China*
[7] *University of Chinese Academy of Sciences*, *Beijing* 100049, *China*

[#]Authors contributed equally to this work
[*]Corresponding authors, E-mail: wulili@im.ac.cn; wangqihui@im.ac.cn



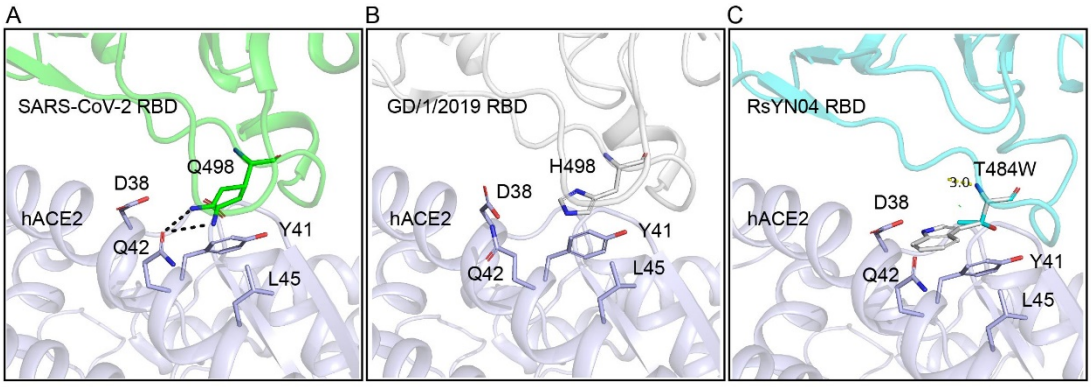
Supplementary Figure S1 Inhibition of *R. stheno* ACE2 and RsYN04 RBD binding by SARS-CoV-2 antibodies

Blocking curves and efficacies of antibodies. One representative result from two independent experiments with two replicates for each experiment (*n=2*) is shown.



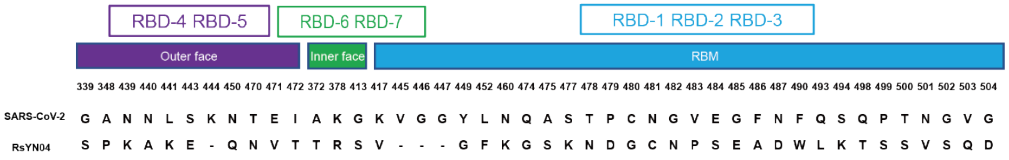
Supplementary Figure S2 Conservation analysis of N322 glycosylation among 52 ACE2 orthologs

Sequence alignment was conducted for ACE2 sequences from 52 animal species. Conserved residues are highlighted in red and 322 positions of ACE2 in each species are marked with green squares.



Supplementary Figure S3 Structural analysis of enhanced binding of RsYN04-T484W RBD to ACE2

A: Interaction of Q498 on SARS-CoV-2 RBD with hACE2 (PDB: 6LZG). **B:** Interaction of H498 on GD/1/2019 RBD with hACE2 (PDB:7DDO). **C:** T484W of RsYN04 RBD and potential residues on hACE2 are shown by superimposing the RsYN04 RBD-S43 complex onto the SARS-CoV-2 RBD-hACE2 complex. Modeled W484 is in gray.



Supplementary Figure S4 Amino acid substitution mapping of RBDs from SARS-CoV-2 prototype and RsYN04

Three major epitopes on SARS-CoV-2 RBD targeted by seven classes of monoclonal antibodies (RBD-1 to RBD-7), and residue substitution mapping of RBDs from SARS-CoV-2 and RsYN04.

Supplementary Table S1. Crystallographic data collection and refinement statistics.

	RsYN04 RBD-S43
Data collection	
Space group	$P4_12_12$
Wavelength (Å)	0.97918
Unit cell dimensions	
a, b, c (Å)	68.64, 68.64, 241.69
α, β, γ (°)	90.00, 90.00, 90.00
Resolution (Å)	66.03 - 2.75 (2.9 - 2.75)
Unique reflection	16001 (2255)
R_{merge}	0.123 (1.819)
I / σI	20.3 (2.5)
Completeness (%)	99.9 (100.0)
$CC_{1/2}$	0.999 (0.946)
Redundancy	25.0 (25.2)
Refinement	
Resolution (Å)	27.55-3.00
No. of reflections	21637
R_{work} / R_{free}	0.2334/0.2413
No. of atoms	
Protein	2542
Ligand/ion	0
Water	0
B-factors	
Protein	43.2
Ligand/ion	-
Water	-
R.m.s.deviations	
Bond length (Å)	0.003
Bond angles (°)	0.672
Ramachandran Statistics (%)	
Favored (%)	95.53
Allowed (%)	4.47
Disallowed (%)	0.00

Supplementary Table S2 Comparison of S43 binding to SARS-CoV-2 RBD and RsYN04 RBD.

RsYN04 RBD/ SARS-CoV-2 RBD	S43 (RsYN04 RBD)	S43 (SARS-CoV-2 RBD)
Y359/Y369 (15/10)	Y107(15)	Y107(10, **1**)
F364/F374 (1/1)	T108(1)	T108(1)
S365/S375 (5/9)	T108(1), G110(2), T112(2, **1**)	T108(1), G110(2), T112(6, **1**)
T366/T376 (8/9)	T108(1), T112(2), D113(5, **1**)	T112(4, **1**), D113(5, **1**)
F367/F377 (9/14)	Y106(1), Y107(4, **1**), T108(4)	Y106(1), Y107(9, **1**), T108(4, **1**)
R368/K378 (14/12)	V104(1), Y105(2), T112(1), D113(10, **1**)	V104(3), Y105(3), D113(6, **1**)
C369/C379 (10/10)	V104(2), Y105(8, **2**)	V104(3), Y105(7, **2**)
Y370/Y380 (5/6)	G103(1), V104(4)	G103(2), V104(4)
V372/V382 (4/5)	Y105(4)	Y105(5)
S373/S383 (1/0)	Y105(1)	
P374/P384 (4/7)	Y105(4)	Y105(2), Y106(2), Y107(3)
G394/G404 (4/6)	W111(4)	W111(5), T112(1)
V397/V407 (2/3)	T112(2)	T112(3)
R398/R408 (13/11)	E98(9, **1**), T112(3), G115(1)	E98(6, **1**), T112(3), G115(2)
P402/P412 (0/1)		S102(1)
S403/G413 (2/1)	Y101(2)	Y101(1)
Q404/Q414 (6/4)	E98(3, **1**), P99(3)	E98(3), P99(1, **1**)
D417/D427(0/1)		S102(1, **1**)
Q489/V503 (15/7)	E44(8), W111(7)	W111(7)
D490/G504 (6/2)	R45(1), W111(5)	W111(2)
Y494/Y508 (5/5)	W111(4), T112(1)	W111(4), T112(1)
Total	129, **8**	123, **12**

The numbers without underlining in parentheses of RsYN04 RBD and SARS-CoV-2 RBD residues represent the number of vdw contacts between the indicated RBD residues and S43. Underlined numbers indicate the number of potential hydrogen bonds between pairs of residues. The vdw contacts were analyzed at a cutoff of 4.0 Å and hydrogen bonds at a cutoff of 3.3 Å.