

### **Supplementary Materials**

#### Cross-species recognition of bat coronavirus RsYN04 and cross-

### reaction of SARS-CoV-2 antibodies against the virus

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

<sup>1</sup> Institute of Physical Science and Information, Anhui University, Hefei, Anhui 230039, China

<sup>2</sup> CAS Key Laboratory of Pathogen Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

<sup>3</sup> College of Veterinary Medicine, Shanxi Agricultural University, Jinzhong, Shanxi 030801, China <sup>4</sup> School of Life Sciences, Hebei University, Baoding, Hebei 071002, China

<sup>5</sup> Center of Infectious Disease, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China

<sup>6</sup> 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

<sup>7</sup> University of Chinese Academy of Sciences, Beijing 100049, China

<sup>#</sup>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.

	300	310	320	330	340	350
Human	VDOAWDAO	RIFKEAEKE	FVSVGLPN	MTOGFWENSMLT	PGNVOKAV	CHPTAWDLGKGDER
Monkey	VNÕAWNAÕ	RIFKEAEKF	FVSVGLPN	MTOGFWENSMLT	PGNVÕKVV	CHP TAWD LGKGDFR
Cat	VNOSWDAR	RIFKEAEKF	FVSVGLPN	MTOGFWENSMLT	PGDSRKVV	CHPTAWDLGKGDFR
Civet	VNONWDAR	RIFKEAEKE	FVSVGLPN	MTOGFWENSMLTI	EPGDGRKVV	CHPTAWDLGKGDFR
Dog	VNOSWDAR	KIFKEAEKF	FVSVGLPN	MTOEFWENSMLTI	E P S D S R K V V	CHPTAWDLGKGDFR
Fox	VNOSWDAR	KIFKEAEKF	FVSVGLPN	MTOGEWENSMLT	PSDSRKVV	CHP TAWD LGKGDF R
Raccoon dog	VNOSWDAR	KIFKEAEKF	FVSVGLPN	MTOGFWENSMLT	PSDSWKVV	CHPTAWDLGRGDFR
American mink	VNQSWDAR	RIFEEAEKE	FVSVGLPN	MTEGFWONSMLTI	EPGDNRKVV	CHPTAWDLGKHDFR
Mouse	MNQGWDAE	RIFQEAEKF	FVSVGLPH	MTOGFWANSMLTI	E P A D G R K V V	CHPTAWDLGHGDFR
Rat	VNQSWDAE	RIFKEAEKF	FVSVGLPQ	MTPGFWTNSMLTI	EPGDDRKVV	CHPTAWDLGHGDFR
Golden hamster	VNQGWNAE	RIFKEAEKF	FVSVGLPY	MTQGFWENSMLTI	DPGDDRKVV	CHPTAWDLGKGDFR
Chinese hamster	VNQGWDAE	RIFKEAEKF	FVSVGLPH	MTQGFWGNSMLTI	DPGDDRKVV	CHPTAWDLGKGDFR
Guinea pig	ESQSWDAE	K I F KE A E K F	FVSVGLPP	MTQGFWKNSMLTI	EPGDGQKVV	CHPTAWDMGKNDFR
Bovine	ENOSWDAE	RIFKEAEKE	FVSISLPY	MTOGFWDNSMLTI	EPGDGRKVV	CHPTAWDLGKGDFR
Pig	VNOSWDAI	RIFEEAEKF	FVSIGLPN	MTOGEWNNSMLT	EPGDGRKVV	CHPTAWDLGKGDFR
Goat	KNÕSWDAE	RIFKEAEKF	FVSIGLPY	MTOGFWNNSMLTI	EPGDGRKVV	CHP TAWD LGKGDFR
Sheep	KNOSWDAE	RIFKEAEKF	FVSIGLPY	MTOGFWDNSMLTI	PGDGRKVV	CHP TAWD LGKGDFR
Wild Bacterian camel	ENOSWDAK	RIFKEAEKE	FVSIGLPN	MTOGFWDNSMLTI	EPGDGRKVV	CHPTAWDLGKGDFR
Deer	ENOSWDAE	RIFKEAEKF	FVSISLPY	MTOGFWDNSMLTI	EPGDGRKVV	CHPTAWDLGKGDFR
Malayan pangolin	VNOTWDAN	RIFKEAEKF	FVSVGLPK	MTOTEWENSMLT	PGDGRKVV	CHP TAWD LGKHDFR
Horse	VDOSWDAK	RIFEEAEKF	FVSVGLPN	MTOGEWENSMLT	PGDGRKVV	CHPTAWDLGKGDFR
Rabbit	VNÕGWDAE	RIFKEAEKF	FVSVGLPS	MTÕGFWENSMLTI	EPGDGRKVV	CHPTAWDLGKGDFR
Chicken	AOKNWDAM	K I F K T A E A F	FASIGLYN	MTEGFWTNSMLT	E P T D N R K V V	CHPTAWDMGKNDYR
Lesser hedgehog tenrec	VKOGWDAT	RIFKEAEKF	FMSVDLFP	MTOGFWDKSMLTI	PNNGQKVV	CHPTAWDLGLNDFR
Pteropus alecto	VNONWDEK	RIFKEAEKE	FVSLGLPN	MTEKFWEKSMLTI	EPGNDÖKVA	CHPTAWDLGKGDFR
Hipposideros armiger	VNQKWDAK	KIFQEAEKF	FVSVGLPN	MTKGFWENSMLT	EPGDGRKVV	CHPTAWDLGKGDFR
Myotis davidii 140	VEOSWDAE	KIFKEAEKF	YISVGLPS	MTPGFWKNSMLTI	EPGDGRKVV	CHPTAWD <mark>LG</mark> KGDFR
Molossus molossus	LNQSWDAE	KIFKKAEEF	FVSIGLGN	MTQGFWDNSMLTI	E P S D G R K V V	CHPTAWDLGNNDFR
Pipistrellus abramus	KKOSWDAE	KIFKEAEKF	YSSVGLPN	MTPGFWRDSMLT	PSDGROVV	CHP TAWD LGKNDFR
Scotophilus dinganii	KEQYWDAE	KIFKEAEKF	YMSVGLPR	MTPGFWNNSMLTI	EPGDGRKVV	CHPTAWDLGKGDFR
Tadarida brasiliensis	LNQSWNAE	RIFKEAEKF	FVSIGLPN	MTQEFWNNSMLTI	EPGDGRKVV	CHPTAWDLGKGDFR
Myotis lucifugus	VEQSWDAE	KIFKEAEKF	YISVGLPS	MTPGFWNNSMLTI	EPGDGRKVV	CHPTAWDLGKGDFR
Rousettus leschenaultii	VNQNWNAK	RIFKEAEKF	FV <mark>S</mark> LG <b>L</b> PN	MTETFWEKSVLTI	E <mark>P</mark> D N D Q K V A	CHPTAWDLGKGDFR
Rhinolophus ferrumequinum	LNQNWDAK	RIFKEAEKF	FVSIGLPN	MTEGFWNNSMLTI	DPGDGRKVV	CHPTAWDL <mark>GKG</mark> DFR
Rhinolophus sinicus	LKQGWDAD	RIFKEAEKE	FVSVGLPN	MTEGFWNNSMLT	EPGDGRKVV	CHPTAWD <mark>L</mark> GKGDFR
Rhinolophus pusillus	VKQGMDAN	RIFKEAEKE	FVSVGLPN	MTEGFWNNSMLT	EPGDGRKVV	CHPTAWD <mark>L</mark> GKG <mark>D</mark> FR
Rhinolophus macrotis	LNQGWDAN	RIFKEAEKF	FVSVSLPK	MTEGFWNKSMLT	EPGDGRKVV	CHPTAWD <mark>L</mark> GKG <mark>D</mark> FR
Rhinolophus affinis	VNQGWDAN	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLTI	EPGDGRKVV	CHPTAWDL <mark>G</mark> KGDFR
Rhinolophus stheno	VNQGWDAN	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLTI	EPGDGRKVV	CHPTAWDL <mark>GKG</mark> DFR
Rhinolophus siamensis	LNQGWDAN	RIFKEAEKF	FVSVSLPK	MTEGFWNNSMLT	E <mark>P</mark> G D G R K V V	CHP TAWD L GKRDFR
Rhinolophus malayanus	LNQGWDAN	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLTI	E <mark>P</mark> G D G R K V V	CHP TAWD L GRGDFR
Rhinolophus pearsonii	VNQGWDAN	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLTI	E P G D G R K V V	CHPTAWDLGKDDFR
Rhinolophus landeri	LNQTWDAK	RIFKEAEKF	FVSIGLPH	MTEGFWNNSMLTI	DPGDGRKVV	CHPTAWDL <mark>GKG</mark> DFR
Rhinolophus sinicus WJ1	LKQGWDAD	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLT	EPGDGRKVV	CHPTAWDL <mark>G</mark> KG <mark>D</mark> FR
Rhinolophus sinicus 1434	LKQGWDAD	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLT	EPGDGRKVV	CHP TAWD L G K G D F R
Rhinolophus sinicus ACT66275	LKQGWDAD	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLTI	E <mark>P</mark> G D G R K V V	CHPTAWDL <mark>G</mark> KGDFR
Rhinolophus sinicus 5720	LKQGWDAD	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLT	E <mark>P</mark> G D G R K V V	CHPTAWDL <mark>G</mark> KGDFR
Rhinolophus sinicus 1446	LKQGWDAD	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLT	E P G D G R K V V	CHPTAWDLGKGDFR
Rhinolophus sinicus 3366	LKQGWDAD	RIFKEAEKF	FMSVGLPN	MTEGFWNNSMLTI	EPGDGRKVV	CHPTAWDLGKGDFR
Rhinolophus sinicus 5718	LKQGWDAD	RIFKEAEKF	FMSVGLPN	MTEGFWNNSMLT	E P G D G R K V V	CHPTAWDLGKGDFR
Rhinolophus sinicus 3357	LKQGWDAD	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLT	E P G D G R K V V	CHPTAWDLGKGDFR
Rhinolophus sinicus 3359	LKOGMDAD	RIFKEAEKF	FVSVGLPN	MTEGFWNNSMLT	EPGDGRKVV	CHPTAWDLGKGDFR

## 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.

	RsYN04 RBD-S43				
Data collection					
Space group	P4 <sub>1</sub> 2 <sub>1</sub> 2				
Wavelength (Å)	0.97918				
Unit cell dimensions					
a, b, c (Å)	68.64, 68.64, 241.69				
$\alpha, \beta, \gamma$ (°)	90.00, 90.00, 90.00				
Resolution (Å)	66.03 - 2.75 (2.9 - 2.75)				
Unique reflection	16001 (2255)				
R <sub>merge</sub>	0.123 (1.819)				
I / σI	20.3 (2.5)				
Completeness (%)	99.9 (100.0)				
CC <sub>1/2</sub>	0.999 (0.946)				
Redundancy	25.0 (25.2)				
Refinement					
Resolution (Å)	27.55-3.00				
No. of reflections	21637				
$R_{ m work}$ / $R_{ m free}$	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 S1. Crystallographic data collection and refinement statistics.

RsYN04 RBD/ SARS-CoV-2 RBD	S43 (RsYN04 RBD)	S43 (SARS-CoV-2 RBD)
Y359/Y369 (15/10)	Y107(15)	Y107(10, <u>1</u> )
F364/F374 (1/1)	T108(1)	T108(1)
8365/8375 (5/9)	T108(1), G110(2), T112(2, <u>1</u> )	T108(1), G110(2), T112(6, <u>1</u> )
T366/T376 (8/9)	T108(1), T112(2), D113(5, <u>1</u> )	T112(4, <u>1</u> ), D113(5, <u>1</u> )
F367/F377 (9/14)	Y106(1), Y107(4, <u>1</u> ), T108(4)	Y106(1), Y107(9, <u>1</u> ), T108(4, <u>1</u> )
R368/K378 (14/12)	V104(1), Y105(2), T112(1),	V104(3), Y105(3), D113(6, <u>1</u> )
	D113(10, 1)	
C369/C379 (10/10)	V104(2), Y105(8, <u>2</u> )	V104(3), Y105(7, <u>2</u> )
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, <u>1</u> ), T112(3), G115(1)	E98(6, <u>1</u> ), T112(3), G115(2)
P402/P412 (0/1)		S102(1)
S403/G413 (2/1)	Y101(2)	Y101(1)
Q404/Q414 (6/4)	E98(3, <u>1</u> ), P99(3)	E98(3), P99(1, <u>1</u> )
D417/D427(0/1)		S102(1, <u>1</u> )
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, <b>8</b>	123, <u>12</u>

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

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.