Supplementary Material

Additional File 1 contains 3 Figures and 2 Tables

An Antibody Cocktail with Broadened Mutational Resistance and Effective Protection Against SARS-CoV-2

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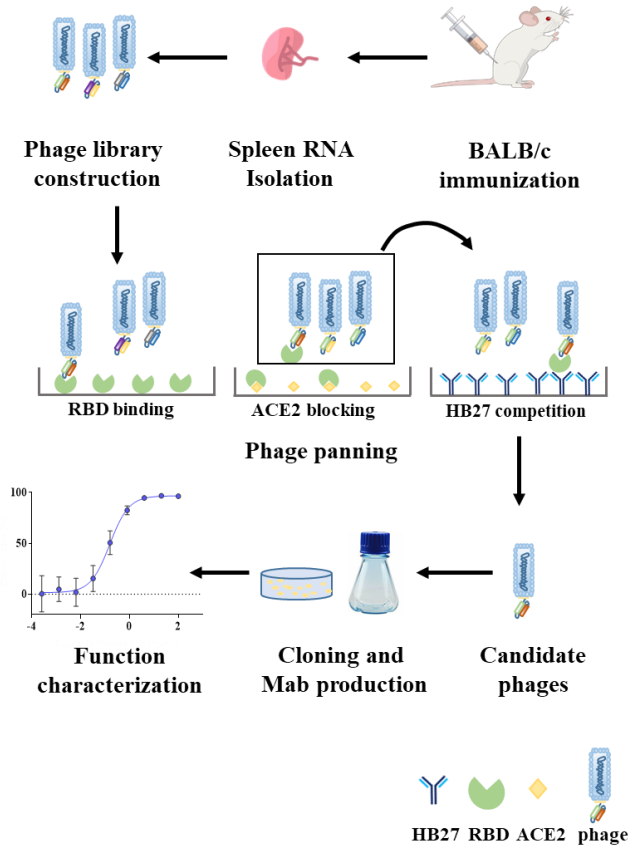
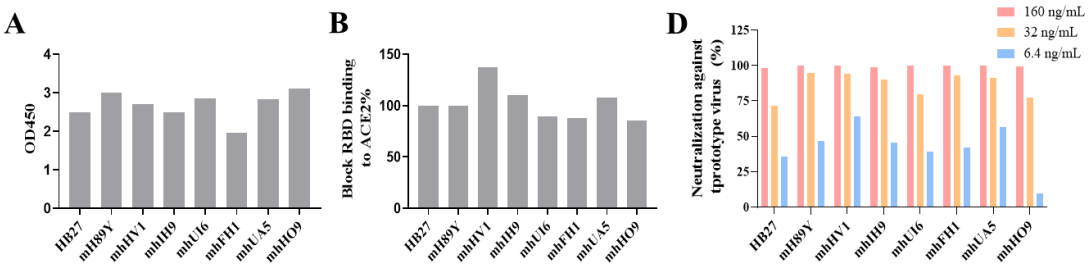


Figure S1 Diagram of H89Y screening. BALB/c mice were immunized with recombinant SARS-CoV-2 RBD protein. Antibody variable regions (VH and VL) were amplified by RT-PCR, and single-chain variable fragments (scFvs) were generated by linking VH and VL fragments and cloned into a phage vector pComb3x for library construction. Phages displaying scFv were harvested for several rounds of biopanning to screen scFvs positive for SARS-CoV-2 RBD binding, ACE2-RBD interaction blocking and non-competitive binding of HB27 to RBD. The selected phage clones were used as a template for amplification to obtain the light and heavy chain variable region sequence and construct the expression vectors. Mouse-human chimeric antibodies were produced in HEK293 cell.



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mAb	HB27	mh89Y	mhHV1	mhIH9	mhUI6	mhFH1	mhUA5	mhHO9
HB27	96%	17%	9%	6%	9%	-65%	-13%	-43%
mh89Y	14%	87%	/	/	/	/	/	/
mhHV1	4%	/	83%	/	/	/	/	/
mhIH9	1%	/	/	89%	/	/	/	/
mhUI6	-9%	/	/	/	38%	/	/	/
mhFH1	-17%	/	/	/	/	77%	/	/
mhUA5	-16%	/	/	/	/	/	59%	/
mhHO9	-15%	/	/	/	/	/	/	78%

Figure S2 Identification of leading antibody paired with HB27. A. Binding of the candidate antibodies to recombinant prototype RBD by ELISA. **B.** Blocking of prototype RBD binding to ACE2 by HB27 and candidate antibodies by ELISA. **C.** Competing with HB27 for RBD binding by ELISA. Each antibody was tested in two orientations: as a solid phase on the plate, and as a liquid phase in solution. Grey shading blanks indicate the self-competing of the antibody. **D.** PsV neutralization against the SARS-CoV-2 prototype strains in Vero E6 cells.

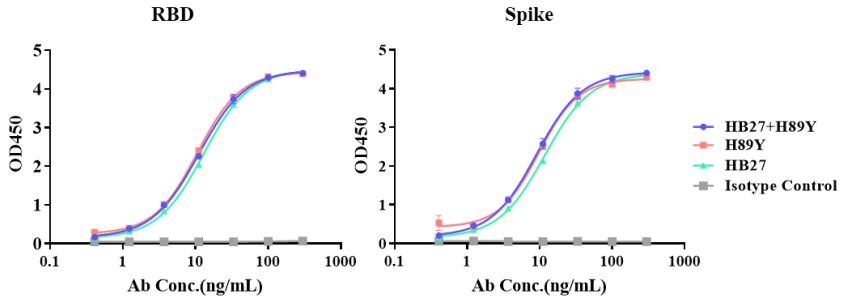

Figure S3. HB27 and H89Y cocktail specific binding to RBD and spike protein. Binding of individual HB27, H89Y and the HB27+H89Y antibody cocktail to recombinant prototype RBD or spike protein measured by ELISA. Isotype control was a non-spike targeting antibody with the Fc LALA mutation.

Table S1 HB27 and H89Y non-competitive binding to prototype SARS-CoV-2

Antigen	Antibody 1 50 μg/mL	Response-1	Antibody 2 100 μg/mL	Response-2	Subtraction
Spike-his-biotin	H89Y	0.54	HB27	0.59	0.55
	H89Y	0.57	H89Y	0.04	
	HB27	0.58	H89Y	0.54	0.51
	HB27	0.58	HB27	0.03	
RBD-his-biotin	H89Y	3.76	HB27	1.99	1.53
	H89Y	3.81	H89Y	0.46	
	HB27	3.45	H89Y	2.23	1.85
	HB27	3.81	HB27	0.38	

Table S2. NGS analysis from escape mutant screening

NGS results of P1							
Location	**REF**	**ALT**	**Mutation Type**	**HB27**	**H89Y**	**HB27+H89Y**	**Isotype Control**
P272P	CCT	CCG	Synonymous	0/1, 29.9%	0/0	0/0	0/0
S477F	TCT	TTT	Non-Synonymous	0/0	0/1, 44.7%	0/0	0/0
F486S	TTC	TCC	Non-Synonymous	0/0	0/1, 29.9%	0/0	0/0
P499S	CCT	TCT	Non-Synonymous	0/1, 56.5%	0/0	0/0	0/0
R682Q	CGG	CAG	Non-Synonymous	0/1, 60.2%	1/1, 73.2%	1/1, 89.8%	0/1, 80.2%
H1058H	CAC	CAT	Synonymous	0/1, 62.4%	0/1, 75.95%	1/1, 90.5%	0/1, 81.9%
NGS results of P2							
Location	**REF**	**ALT**	**Mutation Type**	**HB27**	**H89Y**	**HB27+H89Y**	**Isotype Control**
V3M	GTG	ATG	Non-Synonymous	0/0	0/0	0/1, 17.6%	0/0
P272P	CCT	CCG	Synonymous	0/1, 43.8%	0/0	0/0	0/0
S477F	TCT	TTT	Non-Synonymous	0/0	0/1, 74.0%	0/0	0/0
F486S	TTC	TCC	Non-Synonymous	0/0	0/1, 18.8%	0/0	0/0
R682Q	CGG	CAG	Non-Synonymous	0/1, 95.2%	0/1, 95.4%	1/1, 95.6%	0/1, 99.0%
H1058H	CAC	CAT	Synonymous	0/1, 97.6%	0/1, 94%	1/1, 100%	0/1, 96.1%

NGS analysis of result of P1 and P2. 0/0= homozygous and is consistent with reference base (REF); 0/1= heterozygous of two alleles, one is alternate base (ALT) and the other is REF; 1/1= homozygous with both ALTs. The percentage represents the proportion of the number of ALT base reads to total base reads. The alphabets highlighted in red represent the bases altered after selection. Non-synonymous mutation such as R682Q was near the furin cleavage site and spontaneously occurred during SARS-CoV-2 passage(Johnson et al., 2020; Lau et al., 2020).

References

Johnson, M. C., Lyddon, T. D., Suarez, R., Salcedo, B., LePique, M., Graham, M. and Ricana, C. (2020). Optimized Pseudotyping Conditions for the SARS-COV-2 Spike Glycoprotein. J Virol 94, e01062-01020.

Lau, S. Y., Wang, P., Mok, B. W., Zhang, A. J., Chu, H. and Lee, A. C. (2020). Attenuated SARS-CoV-2 variants with deletions at the S1/S2 junction. Emerg Microbes Infect 9, 837-842.