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Supplementary information

Converting non-neutralizing SARS-CoV-2 antibodies into broad-spectrum inhibitors

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Converting non-neutralizing SARS-CoV-2 antibodies into broad-spectrum inhibitors

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Supplementary note:

Signal Peptide

Hexa-His tag

TEV Site

ACE2

CV10-ACE2 Fusion scFv

MGWSCIILFLVATATGVHSQVQLQESGPGLVKPSETLSLTCNVSGGSISSYYWSWIRQP
PGKGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGFD
YWGQGTLVTVSSASGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQSVS

SIYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQ
YAGSPWTFGQGTKVEIKGGSGS*HHHHHH*ASTGGGSGGPSGQAGAAASE**ENLYFQG**SLF
VSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQN
MNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTI
LNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLR
PLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIK
PLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNID
VTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTA
WDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNGANEGFHEAV
GEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLLKQALTIVGTLPFTYMLEKWRWMVF
KGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQ
FQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTLALENVVGAKNMN
VRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD

CV27-ACE2 Fusion scFv

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVR
QAPGKGLEWVALISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC
ARSFGGSYYYGMDVWGQGTTVTASGGGGSGGGGSGGGGSQSALTQPASVSGSPGQSIT
ISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSGNTASLTIS
GLQAEDEADYYCSSYTSSSTPYVFGTGTKVGGSGS*HHHHHH*ASTGGGSGGPSGQAGAA
ASE**ENLYFQG**SLFVSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAEDLFYQSSLASWN
YNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSV

LSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAWE
SWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQL
IEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSL
TVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGN
VQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRN
GANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLLKQALTIVGTLPFTYM
LEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPASLFHVSNDYS
FIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTLAL
ENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD

COVA2-14-ACE2 Fusion scFv

MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIIWVRQ
APGQGLEWMGGIIPIFGTANYAQKFQGRVTITTDESTSTAYMELSSLRSEDTAVYYCAR
VRYYDSSGYYEDYWGQGTLVTVSSASGGGGSGGGGSGGGGSEIVLTQSPATLSLSPGER
ATLSCRASQSVSSYLAWYQQEPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLE
PEDFAVYYCQQRSNWPPMYTFGQGTKVEIKGGSGS*HHHHHH*ASTGGGSGGPSGQAGA
AASE**ENLYFQG**SLFVSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAEDLFYQSSLASW
NYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSS
VLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAW
ESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQ
LIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYS
LTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPG

NVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLLR
NGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLLKQALTIVGTLPFTY
MLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPASLFHVSNDY
SFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPWTLA
LENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD

COV2-2449-ACE2 Fusion scFv

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCATSGFTFSSFALHWVRQ
APGKGLEWVTVISDDGNNKYYVDSVKGRFTISRDNSKNTLFLQMNSLRVEDTAIYYCA
RASYNSNWSIGEYFRDWGQGTLVTVSSASGGGGSGGGGSGGGGSDIVMTQSPDSLAVS
LGERATINCKSSQSLLYTSNNKNYLAWYQQKPGQPPKLLIYWASTRESGVPDRFSGSGS
GTDFTLTISSLQAEDVAVYYCQQYYSPPWTFGQGTKVEIKGGSGS*HHHHHH*ASTGGGS
GGPSGQAGAAASE**ENLYFQG**SLFVSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAED
LFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQ
LQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSL
DYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNG
VDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDM
WGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGF
WENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDM
AYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLLKQA
LTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDP

ASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLR

LGKSEPWTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD

COV2-2143-ACE2 Fusion scFv

MGWSCIILFLVATATGVHSEVQLVESGGGLVQPGGSLRLSCAASGFTVSSNYMSWVR

QAPGKGLEWVSVIYSAGSTYYADSVKGRFSISRDKSKNTLYLQMNSLRAEDTAVYYCA

KEGGSGSLRYYYYGMDVWGQGTTVTVSSASGGGGSGGGGSGGGGSQSVVTQPPSASG

TPGQRVTISCSGSSSNIGYNIVNWYQQLPGTAPKLLIYSNNQRPSGVPDRFSGSKSGTSAS

LSISGLQSEDEADYYCAAWDDSLNGYVFGTGTKVTVLGGSGS*HHHHHH*ASTGGGSGGP

SGQAGAAASE**ENLYFQG**SLFVSNHAYGGSGGEARVSTIEEQAKTFLDKFNHEAEDLFY

QSSLASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQ

ALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDY

NERLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVD

GYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWG

RFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWE

NSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAY

AAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLLKQALTI

VGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPAS

LFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLG

KSEPWTLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD

Signal Peptide

Hexa-His tag

TEV Site

ACE2

COV2-2449-LC-ACE2 Fusion

MGWSCIILFLVATATGVHSDIVMTQSPDSLAVSLGERATINCKSSQSLLYTSNNKNYLA
WYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYS
PPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN
ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GECGGSGS*HHHHHH*ASTGGGSGGPSGQAGAAASE**ENLYFQG**SLFVSNHAYGGSGGEA
RVSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNMNNAGDKWSAFLK
EQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTMSTIYSTGKVC
NPDNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYVVLKNEM
ARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYVRAK
LMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPFGQKPNIDVTDAMVDQAWDA
QRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCT
KVTMDDFLTAHHEMGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLK
SIGLLSPDFQEDNETEINFLLKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKW
WEMKREIVGVVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHE

GPLHKCDISNSTEAGQKLFNMLRLGKSEPWTLALENVVGAKNMNVRPLLNYFEPLFTW

LKDQNKNSFVGWSTDWSPYAD

Signal Peptide

Knob/Hole Mutations

CrossMAb Swaps

COV2-2449-HC-Knob

MGWSCIILFLVATATGVHSQVQLVESGGGVVQPGRSLRLSCATSGFTFSSFALHWVRQ

APGKGLEWVTVISDDGNNKYYVDSVKGRFTISRDNSKNTLFLQMNSLRVEDTAIYYCA

RASYNSNWSIGEYFRDWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY

FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT

KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE

DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN

KALPAPIEKTISKAKGQPREPQVYTLPP***C***RDELTKNQVSL***W***CLVKGFYPSDIAVEWESNG

QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL

SPGK

CV10-LC-CrossMAb

MGWSCIILFLVATATGVHSEIVLTQSPGTLSLSPGERATLSCRASQSVSSIYLAWYQQK

PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYAGSPWTFGQ

GTKVEIKSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH

TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

CV10-HC-CrossMAb-Hole

MGWSCIILFLVATATGVHSQVQLQESGPGLVKPSETLSLTCNVSGGSISSYYWSWIRQP

PGKGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGFD

YWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA

LQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG

ECDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY

VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI

SKAKGQPREPQV***C***TLPPSRDELTKNQVSL***S***C***A***VKGFYPSDIAVEWESNGQPENNYKTTP

PVLDSDGSFFL***V***SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Tables:

Supplementary Table 1 – live virus neutralization

Inhibitor	Wuhan-1 50% CPE nM	BA.1 50% CPE nM	BA.2 50% CPE nM
scFv-based ReconnAbs			
CV10	8.1	29	56
CV27	3.3	306	211
2449	10	101	57
IgG-based ReconnAb			
CrossMab	21	171	106
CrossMAb + TEV	375	346	>500.00

Scale	
<200nM	>200nM

Supplementary Table 1 – IC_{50} values from a limiting dilution live SARS-CoV-2 neutralization assay shows ReconnAbs neutralize live viruses as expected. Cytopathic effect (CPE) was scored as binary, either infection or no infection and IC_{50} was calculated using the Spearman-Karber method. Data presented is the average of 3 assays, if one assay was above the limit of detection, the average was computed assuming the limit of detection was the value.