

# **Prediction of the binding interface between monoclonal antibody m102.4 and Nipah attachment glycoprotein using structure-guided alanine scanning and computational docking**

Phanthakarn Tit-oon<sup>1,#</sup>, Kannan Tharakaraman<sup>2,3,#</sup>, Charlermchai Artpradit<sup>1,#</sup>, Abhinav Godavarthi<sup>1,π</sup>, Preenart Sungkeeree<sup>1</sup>, Varun Sasisekharan<sup>1</sup>, Jarunee Kerdwong<sup>1</sup>, Nathaniel Loren Miller<sup>2,3,4</sup>, Bhuvna Mahajan<sup>1</sup>, Amnart Khongmanee<sup>1</sup>, Mathuros Ruchirawat<sup>1</sup>, Ram Sasisekharan<sup>2,3,\*</sup>, and Mayuree Fuangthong<sup>1,\*</sup>

<sup>1</sup>Translational Research Unit, Chulabhorn Research Institute, Bangkok, 10210, Thailand

<sup>2</sup>Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

<sup>3</sup>Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

<sup>4</sup>Harvard-MIT Division of Health Sciences & Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

<sup>π</sup>Present address: Yale University, New Haven, CT 06520, USA

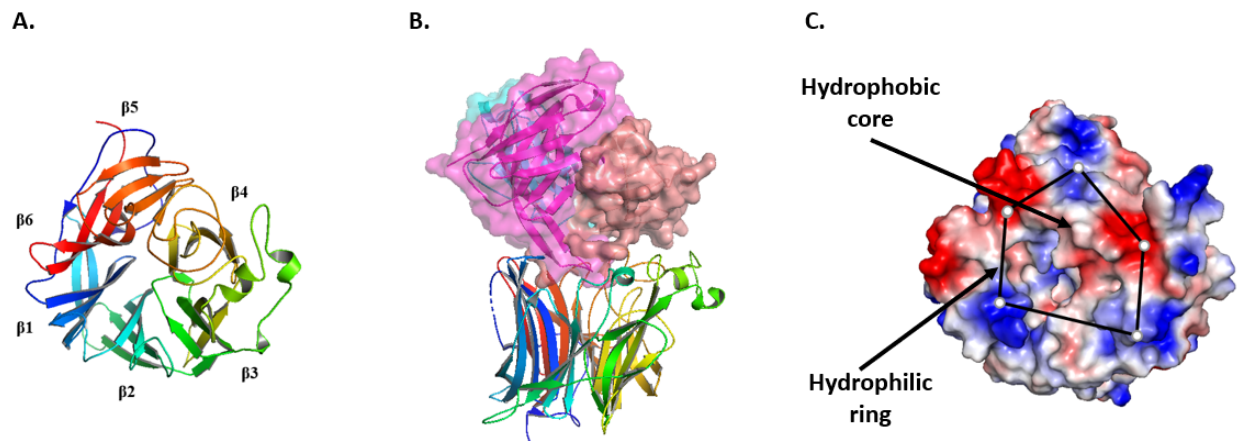
<sup>#</sup>These authors contributed equally to this work.

<sup>\*</sup>To whom the correspondence should be addressed.

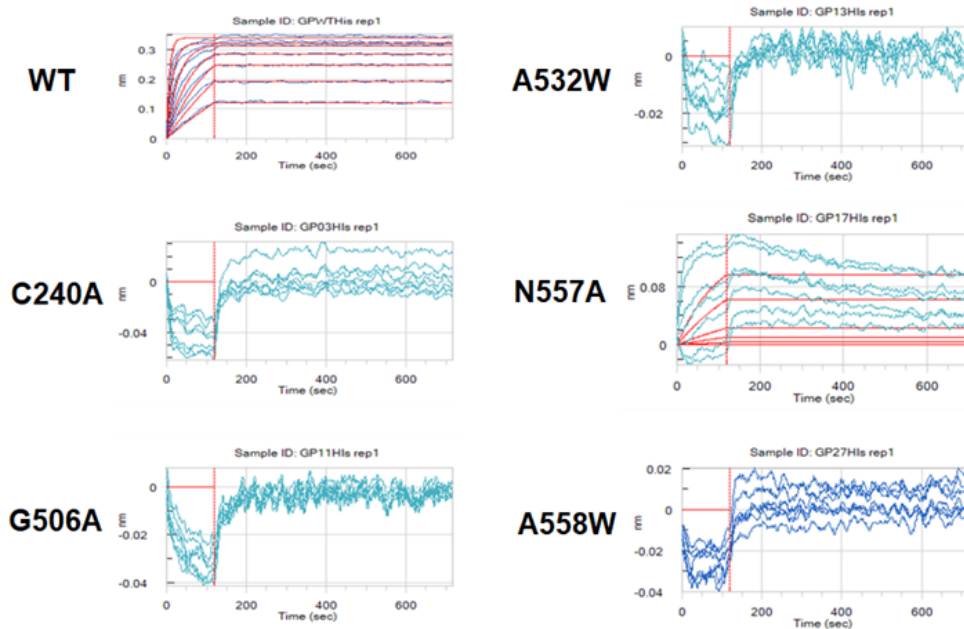
Email: [mayuree@cri.or.th](mailto:mayuree@cri.or.th); Tel: +66 2 553 8535; Fax: +66 2 553 8558;

Email: [rams@mit.edu](mailto:rams@mit.edu); Tel: +1617 258 9494

## SUPPLEMENTARY MATERIALS



**Supplementary Figure S1. Hendra GP structure and m102.3 epitope.** (A) the toroidal arrangement of deglycosylated GP formed by the six beta propeller blades around a central axis (labeled). The view of GP is along the line of central axis. GP is colored in rainbow color with the N terminus in blue and the C terminus in red; (B) GP bound to m102.3 (PDB: 6CMG) and its receptor Ephrin-B2 (PDB: 6PDL). View of the GP is perpendicular to the central axis. The Fv of m102.3 is colored in magenta and cyan, respectively, whereas the receptor is in light brown. (C) m102.3 epitope consisting of hydrophobic core in the center and hydrophilic pockets at the periphery highlighted on the electrostatic surface of GP.



**Supplementary Figure S2.** Sensorgram data showing fit curves (shown in red) generated from Octet QKe. Kinetic assays were performed by first capturing biotinylated wild-type Nipah antibody using streptavidin biosensor. The antibody-captured biosensors were then submerged in wells containing different concentrations of G-protein for 120 sec followed by dissociation period in PBST buffer. The wild-type G-protein generates signals that fit perfectly to the curves. No fit curves were generated from the mutant G-protein with C240A, G506A, A532W, N557A, or A558W mutations.

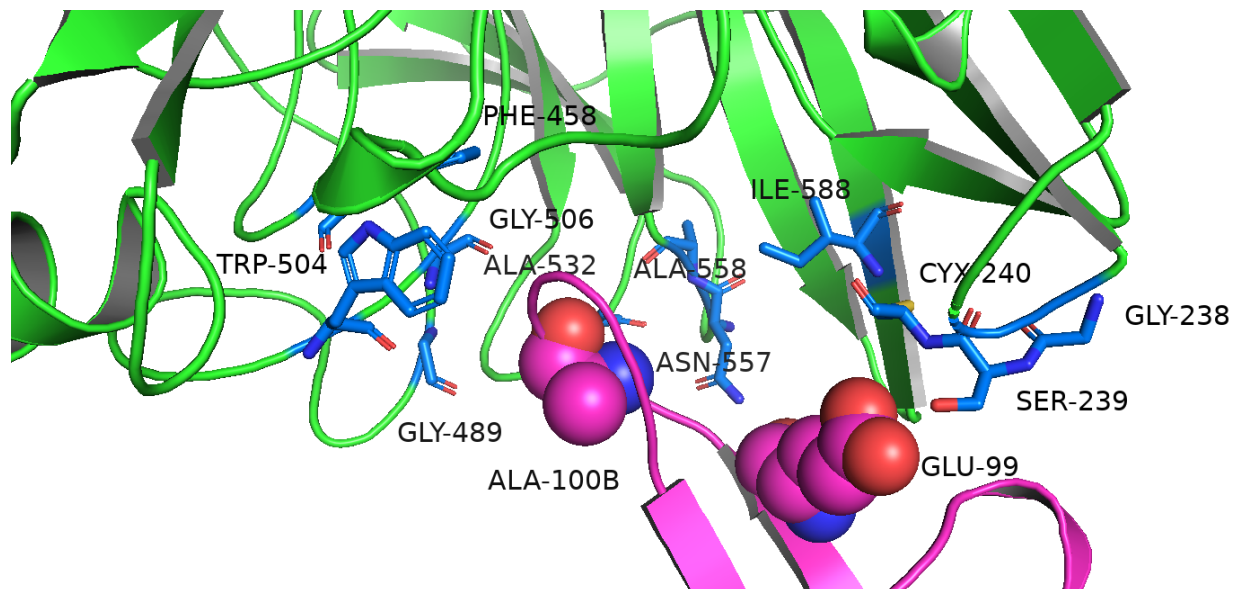
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                                *
m102.3  EIVMTQSPGTPSLSPGERATLSCRASQSIRSTYLAWYQQKPGQAPRLLIYGASSRATGIP
m102.4  EIVMTQSPGTLSLAPGERATLSCWASQSVRNNYLAWYQQKPGQAPRLVIYNGSTRATGIP

                                ***** *
m102.3  DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGRSP--SFGQGTKVEIK
m102.4  DRFSGSGSGTDFTLTISRLDPEDFAVYYCQQYGNSRRVTFGGGGTKVEIK

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**Supplementary Figure S3. Sequence alignment between LC of m102.3 and m102.4.** Amino acid differences are indicated in red. Amino acids that are different in the two chains and fall in the VH:VL interface are highlighted by an asterisk symbol. Chothia CDR loops are colored in yellow.



**Supplementary Figure S4.** Close-up view of m102.4-GP (NiV) interface from the docked model. The residues used as constraints for docking are highlighted by spheres (paratope) and sticks (epitope). The light chain is not seen in this orientation. The heavy chain of m102.4 and GP are colored in magenta and green, respectively.

EGVSNLVGLPNNICLQKTSNQILKPKLISYTLPVVGQSGTCITDPLLAMDEGYFAYSHLE  
 RIG↓S↓C↓SRGVSKQRIIGVGEVLDRGDEVPSLFMTNVWTPPNPNTVYHCSAVYNNEFYVLC  
 AVSTVGDPIILNSTYWSGSLMMTRLAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSPG  
 IKQGD TLYFPAVGFLVRTEFKYND SNCPITKCQY SKPENCR L SMGIRPN SHYILRSGLLK  
 YNLS DGENPKVVFIEISDQRLSIGSPSKIYDSL GQPVFYQASF SWDTMIKFGDVLT VNPL  
 VVNRNNTVISRPGQSQCPRFNTCPEICWEGVYNDAFLIDRINWISAGVFLDSNQTAENP  
 VFTVFKDNEILYRAQLAS↓ED↓NAQ↓KTITNCFLLNKIWCISLVEI↓Y↓DT↓G↓DNVIRPKLFAV  
 KIPEQCTA

**Supplementary Figure S5.** m102.3 and m102.4 epitope residues mapped on NiV GP sequence. Epitope residues that are common to both antibodies are colored in purple, whereas amino acids unique to m102.3 and m102.4 are in red and blue, respectively. Residues involved in H-bonds or salt bridge contact with both antibodies are indicated by downward pointing black arrows; Residues involved in H-bonds or salt bridge contact with m102.3 or m102.4 but not both are indicated by yellow (m102.3) and green (m102.4) arrows, respectively.

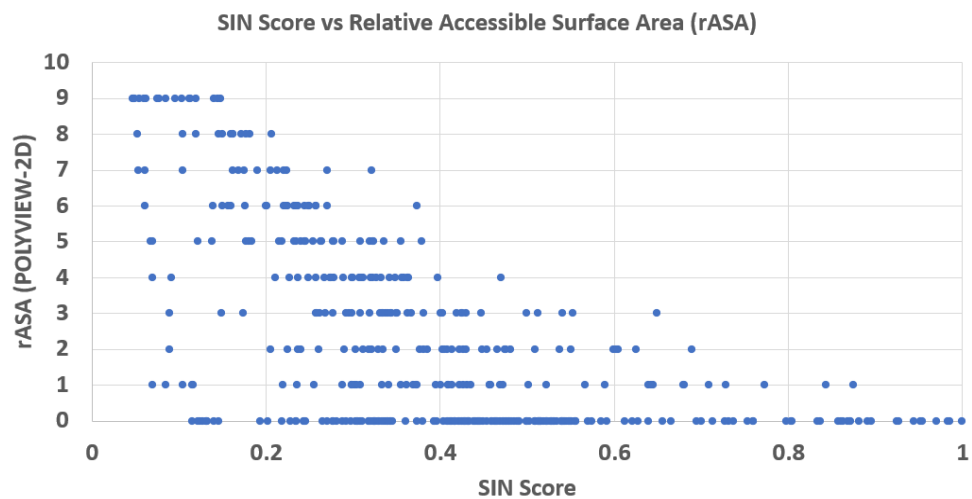
## A.

NiV-GP	EGVSNLVGLPNNICLQKTSNQILKPKLISYTLPPVVGQSGTCTDPELLAMDEGYFAYSHLE
HeV-GP	QGVSDLVGLPNQICLQKTTSTILKPRLISYTLPINTR <b>EGVCIT</b> DPELLAVDNGFFAYSHLE
NiV-GP	RI <b>GS</b> CSR <b>G</b> VSKQRIIGVGEVLDRGDEVPSLFMTNVWTPPNPNTVYHCSAVYNNEFYVLC
HeV-GP	KI <b>GSCTR</b> GIAKQRIIGVGEVLDRGDKVPSMFMTNVWTPPNPSTIHHCSSTYHEDFYVLC
NiV-GP	AVSTV <b>GD</b> PILNSTYWSGSLMMTRLAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSPG
HeV-GP	AVSHV <b>GD</b> PILNSTSWTESLSLIRLAVRPKSDSGDYNQKYIAITKVERGKYDKVMPYGPSPG
NiV-GP	IKQGD <sup>T</sup> LYFPAVGFLVRTEFKYNDSNCPITKCQYKSPENCRLSMGIRPNSHYILRSGLLK
HeV-GP	IKQGD <sup>T</sup> LYFPAVGFLPRTEFQYNDSNCPIIHCKYKAENCRLSMGVNSKSHYILRSGLLK
NiV-GP	YNLSLDGENPKVVFIEISDQRLSIGSPSKIYDSLQGPVFYQASFSWDTMIKFGDVLTVNPL
HeV-GP	YNLSLGGDIIILQFIEIADNRLTIGSPSKIYNSLGQPVFYQASYSWDTMIKLGVDVTDVPL
NiV-GP	VVNWRNNTVISR <b>PGQ</b> SQCPRFNTCPEIC <b>WEGVY</b> ND AFLIDRINWISAGVFLDSN <b>QTA</b> ENP
HeV-GP	RVQWRNNSVISR <b>PGQ</b> SQCPRFNVCPEVC <b>WEGT</b> YND AFLIDRLNWSAGVYLNSN <b>QTA</b> ENP
NiV-GP	VFTVFKDNEILYRAQLASEDT <b>NAQ</b> KTITNCFLLNKIWCISLV <b>EIYDTGDNVI</b> RPKLFV
HeV-GP	VFAVFKDNEILYQVPLAE <b>DDNAQ</b> KTITDCFLENIWCISLV <b>EIYDTGDSVI</b> RPKLFV
NiV-GP	KIPEQCTA
HeV-GP	KIPAQCSE

## B.

m102.4-VH	EVQVIQSGADVKKPGSSVKVSCKSSGGTFS <b>KY</b> AINWVRQAPGQGLEWMGGI <b>IPILGI</b> ANY
m102.3-VH	EVQLVQSGAEVKKRGSVKVSCKSSGGTFS <b>NY</b> AINWVRQAPGQGLEWMGGI <b>IPILGI</b> ANY
m102.4-VH	AQKFQGRVTITTTDESTSTAYMELSSLRSEDTAVYYCARGW <b>GREQLAPHPSQYYYY</b> YGM
m102.3-VH	AQKFQGRVTITTTDESTSTAYMELSSLRSEDTAVYYCARGW <b>GREQLAPHPSQYYYY</b> YGM
m102.4-VH	VWGQGT <sup>T</sup> TVTVSS
m102.3-VH	VWGQGT <sup>T</sup> TVTVSS
m102.4-VL	EIVMTQSPGTL <sup>S</sup> SLAPGERATLSCWASQSV <b>RNN</b> YLAWYQQKPGQAPRLVIYNGSTRATGIP
m102.3-VL	EIVMTQSPGTP <sup>S</sup> LSLPGERATLSCRASQSI <b>RST</b> YLAWYQQKPGQAPRLLIYGASSRATGIP
m102.4-VL	DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGNRRVTFGGG <b>TKVEIK</b>
m102.3-VL	DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY <b>G--R</b> SFSGG <b>TKVEIK</b>

**Supplementary Figure S6.** Residues involved in antibody–antigen interactions: m102.3-HeV (PDB:6CMG) and m102.4-NiV (pose\_8193). **A.** Antibody contacts on the amino acid sequence alignment of NiV and HeV GP. Contacts made by VH and VL are colored in red and blue, respectively. **B.** Antigen contacts on the amino acid sequence alignment of VH and VL of m102.4 and m102.3, respectively. VH and VL-based contacts are colored in red and blue, respectively, consistent with the coloring employed in **A.**



**Supplementary Figure S7.** Scatter plot of SIN scores and solvent accessibility (expressed as relative accessible surface area calculated from POLYVIEW-2D, <http://polyview.cchmc.org/>) computed for the X-ray crystal structure of Nipah GP (PDB: 3D11).



**Supplementary Table S1.** Sequence of DNA primers for G-protein mutation using Gibson assembly.

Purpose	Primer ID - Sequence (5'-3')
G238A mutation	gP007-CGAAAGAATCGCTTCGTGCTCGC
	gP008-GCGAGCACGAAGCGATTCTTTTCG
S239A mutation	gP009-AAGAATCGGCGCATGCTCGCGGG
	gP010-CCC GCGAGCATGCGCCGATTCTT
C240A mutation	gP011-AATCGGCTCGGCTTCGCGGGGGG
	gP012-CCCCCGCGAAGCCGAGCCGATT
S241A mutation	gP013-CGGCTCGTGCGCACGGGGGGTGT
	gP014-ACACCCCCCGTGCACGAGCCG
R242A mutation	gP015-CTCGTGCTCGGCAGGGGTGTCAAAG
	gP016-CTTTGACACCCTGCCGAGCACGAG
L305A mutation	gP017-AGATCCTATTGCAAACCTCCACCTACTGGTCCGGTTC
	gP018-GAACCGGACCAGTAGGTGGAGTTTGAATAGGATCT
F458A mutation	gP019-CCAAGCGTCCGCTTCTGGGACAC
	gP020-GTGTCCCAGGAAGCGGACGCTTGG
P488A mutation	gP053-GGCACTGGCTCTGTCCGGCCCCGGAG
	gP054-CTCCCGGGCCGGACAGAGCCAGTGCC
G489A mutation	gP055-GGCACTGGCTCTGTGCGGGCCGGAG
	gP056-CTCCCGGGCCGCACAGAGCCAGTGCC
Q490A mutation	gP021-CCGGCCCCGAGCCAGCCAGTGCC
	gP022-GGCACTGGCTGGCTCCGGGCCGG
W504A mutation	gP023-GGAAATCTGCGCCGAGGGGGTGTACAATG
	gP024-CATTGTACACCCCCTCGGCGCAGATTTCC
E505A mutation	gP025-AATCTGCTGGGCAGGGGTGTACAATG
	gP026-CATTGTACACCCCCTGCCAGCAGATT
G506A mutation	gP027-CTGCTGGGAGGCTGTGTACAATG
	gP028-CATTGTACACAGCCTCCCAGCAG
V507A mutation	gP057-GGCGTCATTGTATGCCCCCTCCCAG
	gP058-CTGGGAGGGGGCATAACAATGACGCC
T531A mutation	gP029-TAGCAACCAGGCCGCGGAGAACC
	gP030-GGTTCTCCGCGGCCTGGTTGCTA
A532W mutation	gP031-CAACCAGACCTGGGAGAACCCAG

	gP032-CTGGGTTCTCCAGGTCTGGTTG
E533A mutation	gP033-CCAGACCGCGGCTAACCCAGTGT gP034-ACACTGGGTTAGCCGCGGTCTGG
E554A mutation	gP035-ACTGGCGTCGGCTGACACCAACG gP036-CGTTGGTGTGACCCGACGCCAGT
D555A mutation	gP037-GGCGTCGGAGGCCACCAACGCTC gP038-GAGCGTTGGTGGCCTCCGACGCC
N557A mutation	gP039-GGAGGACACCGCCGCTCAAAAGACCATTACCAACTGTTTTTC gP040-GAAAACAGTTGGTAATGGTCTTTTGAGCGGCGGTGTCCCTCC
A558W mutation	gP059-CAGTTGGTAATGGTCTTTTGCCAGTTGGTGTG  gP060-GACACCAACTGGCAAAAGACCATTACCAACTG
Q559A mutation	gP041-CACCAACGCTGCCAAGACCATTACCAACTG gP042-CAGTTGGTAATGGTCTTGGCAGCGTTGGTG
E579A mutation	gP061-GGTGTCGTAGATTGCCACGAGGGAG  gP062-CTCCCTCGTGGCAATCTACGACACC
Y581A mutation	gP043-CGTGGAATCGCCGACACCGGTG gP044-CACCGGTGTCGGCGATTCCACG
T583A mutation	gP045-AATCTACGACGCAGGTGATAACG gP046-CGTTATCACCTGCGTCGTAGATT
D585A mutation	gP047-CGACACCGGTGCTAACGTCATTC gP048-GAATGACGTTAGCACCGGTGTCG
N586A mutation	gP049-CACCGGTGATGCAGTCATTCGCC gP050-GGCGAATGACTGCATCACCGGTG
I588A mutation	gP051-TGATAACGTCGCCCCGCCCTAAACTG gP052-CAGTTTAGGGCGGGCGACGTTATCA
Forward primer for Gibson cloning	fw-CGGCCGCCACTGTGCTGGATTCTAGAGGATCGAACCCCTTCAC
Reverse primer for Gibson cloning	gPRv-TGTGGTGGAAATTCTGCAGATGAATTCATCATTTCCCCGGGGAC
Forward primer for His-tag cloning	gPEcoRI-GGTGGAATTCTCTAGAGGATCGAACCCCTTCAC
Reverse primer for His-tag cloning	gPHistag-TCATCAATGATGATGATGATGATGTGTGCACTGCTCGGGGATC

**Supplementary Table S2.** Sequence of DNA primers for Nipah antibody mutation using Gibson assembly.

<b>Purpose</b>	<b>Primer ID - Sequence (5'-3')</b>
K31A mutation	Nmab007-AACCTTCTCCGCCTACGCGATTAAGTGGGTCCGC
	Nmab008-GCGGACCCAGTTAATCGCGTAGGCGGAGAAGGTT
L55A mutation	Nmab009-CATCCAATTGCAGGGATCGCCAACACTACGC
	Nmab010-CTGTCCCCAGACATCCATCCCGTAGTAATAGGCGTAGTATTGG
G56A mutation	Nmab011-CCCAATTCTGGCAATCGCCAACACTACG
	Nmab012-CGTAGTTGGCGATTGCCAGAATTGGG
I57A mutation	Nmab013-AATTCTGGGGGCCGCCAACACTACGC
	Nmab014-GCGTAGTTGGCGGCCCCCAGAATT
R102A mutation	Nmab015-GGGTTGGGGAGCTGAACAGTTGGCG
	Nmab016-CGCCAACTGTTTCAGCTCCCCAACCC
E103A mutation	Nmab017-TTGGGGAAGGGCCCAGTTGGCGC
	Nmab018-GCGCCAACCTGGGCCCTTCCCCAA
Q104A mutation	Nmab019-GGGAAGGGAAGCCTTGGCGCCCCACCCG
	Nmab020-CGGGTGGGGCGCCAAGGCTTCCCTTCC
L105A mutation	Nmab021-AAGGGAACAGGCTGCGCCCCACCCGTC
	Nmab022-GACGGGTGGGGCGCAGCCTGTTCCTT
A106W mutation	Nmab023-GGAACAGTTGTGGCCCCACCCGTCCCAATAC
	Nmab024-GTATTGGGACGGGTGGGGCCACAACACTGTTCC
P107A mutation	Nmab025-ACAGTTGGCGGCCACCCGTCCC
	Nmab026-GGGACGGGTGGGCCGCCAACACTGT
H108A mutation	Nmab027-GTTGGCGCCCGCTCCGTCCCAATAC
	Nmab028-TATTGGGACGGGGCGGGCGCCAAC
P109A mutation	Nmab029-GGCGCCCCACGTTCCCAATACT
	Nmab030-AGTATTGGGAAGCGTGGGGCGCC
S110A mutation	Nmab031-GCCCCACCCGGCACAATACTACT
	Nmab032-AGTAGTATTGTGCCGGGTGGGGC
Q111A mutation	Nmab033-CCACCCGTCCGCATACTACTACTATTACTAC
	Nmab034-GTAGTAATAGTAGTAGTATGCGGACGGGTGG
Y112A mutation	Nmab035-CCCGTCCCAAGCTTACTACTATTACTACGGGATGGATG
	Nmab036-CATCCATCCCGTAGTAATAGTAGTAAGCTTGGGACGGG

<b>Purpose</b>	<b>Primer ID - Sequence (5'-3')</b>
Y113A mutation	Nmab037-GTCCCAATACGCCTACTATTACTACGGGATGGATGTCTGGG Nmab046-CCCAGACATCCATCCCCTCGTAATAGTAGGCGTATTGGGAC
Y114A mutation	Nmab039-CCAATACTACGCCTACTACTACGGGATGGATGTCTGGGGACAG Nmab040-CTGTCCCCAGACATCCATCCCCTAGTAATAGGCGTAGTATTGG
L55A mutation	Nmab048-CATCATCCCAGCACTGGGGATCGCC Nmab047-GGCGATCCCCAGTGCTGGGATGATG
Forward primer for Gibson assembly	fw-CGGCCGCCACTGTGCTGGATTCTAGAGGATCGAACCCCTCAC
Reverse primer for Gibson assembly	VHrv- TGTGGTGGAAATTCTGCAGATTCATCATTTACCCGGCGACAACGACAGTG

**Supplementary Table S3.** Theoretical prediction of changes in Gibbs free energy of binding ( $\Delta\Delta G$ ) computed using mCSM-AB<sup>1</sup>. The positions inferred by Xu *et al.*<sup>2</sup> are highlighted in yellow. As seen below, 7/13 alanine mutations reduce antigen binding, contradicting the experimentally generated data.

CHAIN	WILD_RES	RES_POS	MUT_RES	RSA	PRED_ $\Delta\Delta G$	Predicted Affinity Change
B	R	30	A	58.0	-0.146	Reduce
B	T	32	A	66.6	-0.658	Reduce
B	R	94	A	58.7	-1.572	Reduce
C	N	31	A	64.5	0.152	Increase
C	I	54	A	48.9	-0.336	Reduce
C	G	56	A	88.0	0.191	Increase
C	I	57	A	11.0	-0.291	Reduce
C	R	102	A	47.0	-1.662	Reduce
C	E	103	A	8.2	-1.503	Reduce
C	Q	104	A	9.9	-0.575	Reduce
C	L	105	A	0.6	0.643	Increase
C	A	106	W	0.0	-1.257	Reduce
C	P	107	A	0.1	-0.297	Reduce
C	H	108	A	9.7	0.825	Increase
C	P	109	A	3.3	0.26	Increase
C	S	110	A	32.4	0.079	Increase
C	Q	111	A	58.3	0.035	Increase
C	Y	112	A	11.4	-0.304	Reduce
C	Y	113	A	9.4	-0.951	Reduce
C	Y	114	A	8.8	-1.242	Reduce
C	Y	115	A	16.5	-1.518	Reduce

\*\*RSA: Relative Solvent Accessibility

**Supplementary Table S4.** Amino acid sequences of VH and VL regions of m102.3 and m102.4 disclosed in US 2009/0214428 A1 (2009) and Xu *et al.*<sup>2</sup>. Amino acid differences between the two published sources are highlighted in bold and colored in red.

Mab	Source	VH	VL
m102.3	US 2009/0214428 A1 (2009)	EVQ <b>VI</b> QSGADVKK <b>P</b> GSSVKVSKSSGGTFS <b>K</b> YAINWVRQAPGGLEWMGGIIPILGIANYAQ KFQGRVTITTTDESTSTAYMELSSLRSEDVAV YYCARGWGREQQLAPHPSEQYYYYYGMVDVWGQ GTTVTVSS	EIVMTQSPGTPSLSPGERATLSCRASQ SIRSTYLAWYQQKPGQAPRLLIYGASS RATGIPDRFSGSGSGTDFTLTISRLEP EDFAVYYCQQYGRSPSFGQGTKVEIK
	Xu <i>et al.</i> <sup>2</sup>	EVQ <b>LV</b> QSGA <b>EV</b> KK <b>R</b> GSSVKVSKSSGGTFS <b>N</b> YAINWVRQAPGGLEWMGGIIPILGIANYAQ KFQGRVTITTTDESTSTAYMELSSLRSEDVAV YYCARGWGREQQLAPHPSEQYYYYYGMVDVWGQ GTTVTVSS	EIVMTQSPGTPSLSPGERATLSCRASQ SIRSTYLAWYQQKPGQAPRLLIYGASS RATGIPDRFSGSGSGTDFTLTISRLEP EDFAVYYCQQYGRSPSFGQGTKVEIK
m102.4	US 2009/0214428 A1 (2009)	EVQ <b>VI</b> QSGADVKK <b>P</b> GSSVKVSKSSGGTFS <b>K</b> YAINWVRQAPGGLEWMGGIIPILGIANYAQ KFQGRVTITTTDESTSTAYMELSSLRSEDVAV YYCARGWGREQQLAPHPSEQYYYYYGMVDVWGQ GTTVTVSS	EIVMTQSPGTLAPGERATLSCWASQ SVRNNYLAWYQQKPGQAPRLVIYNGST RATGIPDRFSGSGSGTDFTLTISRLEP EDFAVYYCQQYGNRRVTFGGGTKVEI K
	Xu <i>et al.</i> <sup>2</sup>	EVQ <b>LV</b> QSGA <b>EV</b> KK <b>R</b> GSSVKVSKSSGGTFS <b>N</b> YAINWVRQAPGGLEWMGGIIPILGIANYAQ KFQGRVTITTTDESTSTAYMELSSLRSEDVAV YYCARGWGREQQLAPHPSEQYYYYYGMVDVWGQ GTTVTVSS	EIVMTQSPGTLAPGERATLSCWASQ SVRNNYLAWYQQKPGQAPRLVIYNGST RATGIPDRFSGSGSGTDFTLTISRLEP EDFAVYYCQQYGNRRVTFGGGTKVEI K

## References

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2. Xu, K. *et al.* Crystal structure of the Hendra virus attachment G glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).