

1 **Table S1.** Crystallographic data, structure solution and refinement statistics.

	Derivative	Native 1	Native 2	GlcNAc-soaked
<i>Data</i>				
Synchrotron beamline	ALBA BL13-Xaloc	ALBA BL13-Xaloc	ESRF ID29	ESRF ID23-2
Data collection date	31 January 2014	31 January 2014	21 February 2014	31 October 2014
Detector	Dectris Pilatus 6M	Dectris Pilatus 6M	ADSC Quantum 315r	MAR Mosaic 225
Spacegroup	$C222_1$	$P2_1$	$P2_12_12_1$	$I2_13$
Cell parameters a, b, c (Å)	92.1, 164.3, 96.4	93.88, 100.60, 95.47	55.87, 91.11, 128.92	163.41, 163.41, 163.41
Cell angles $\alpha$ , $\beta$ , $\gamma$ (°)	90.0, 90.0, 90.0	90.00, 116.28, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Wavelength (Å)	1.0024	1.0023	0.9763	0.8729
Average mosaicity (°)	0.14	0.48	0.27	0.41
Resolution (Å)	96.4-2.76 (3.02-2.76)	29.7-1.80 (1.83-1.80)	34.1-1.70 (1.74-1.70)	28.9-2.00 (2.05-2.00)
Observed reflections	19199 (4473)	144435 (7096)	68912 (3722)	48930 (3642)
Completeness	0.997 (0.988)	0.982 (0.978)	0.943 (0.696)	1.000 (1.000)
Multiplicity	11.7 (8.0)	2.8 (2.9)	3.5 (1.9)	7.9 (7.8)
Rmerge	0.104 (0.589)	0.078 (0.334)	0.116 (0.233)	0.101 (0.404)
I/sigma(I)	19.6 (3.2)	7.1 (2.5)	6.3 (2.1)	13.2 (4.8)
CC1/2	0.998 (0.867)	0.989 (0.857)	0.983 (0.857)	0.998 (0.714)
Wilson B (Å <sup>2</sup> )	71.3	16.5	14.5	20.0
<i>Phasing</i>				
Number of heavy atoms	12 Hg	-	-	-
Correlation coefficient	0.462	-	-	-
Anomalous phasing power	0.753	-	-	-
Figure of merit	0.250	-	-	-
Solvent flattening (solvent fraction)	0.539	-	-	-
Hand score (original / inverted)	0.2471 / 0.1310	-	-	-
Correlation on $ E ^2$ / contrast	2.5866	-	-	-
<i>Refinement</i>				
Resolution	82.1-2.76 (2.83-2.76)	29.7-1.80 (1.90-1.80)	34.1-1.70 (1.82-1.70)	28.0-2.00 (2.05-2.00)
Reflections used	18197 (1269)	140696 (20353)	65280 (9409)	46439 (3444)
Reflections used for Rfree	985 (69)	3644 (499)	3559 (540)	2409 (165)
R-factor	0.203 (0.327)	0.168 (0.232)	0.172 (0.257)	0.165 (0.269)
Rfree	0.248 (0.377)	0.201 (0.253)	0.216 (0.266)	0.205 (0.294)
No. of atoms (total / protein / Hg / water / chloride / glycerol / sulfate / GlcNAc)	4578 / 4529 / 5 / 44 / 0 / 0 / 0 / 0	10928 / 9213 / 0 / 1664 / 8 / 42 / 0 / 0	5285 / 4607 / 0 / 672 / 0 / 6 / 0 / 0	5036 / 4580 / 0 / 350 / 0 / 6 / 55 / 45
Average B (overall / protein / Hg / water / chloride / glycerol / sulfate / GlcNAc)	62.4 / 62.6 / 70.0 / 41.6 / - / - / - / -	25.7 / 23.7 / - / 36.8 / 33.3 / 37.0 / - / -	21.1 / 19.8 / - / 30.1 / - / 48.1 / - / -	29.9 / 29.1 / - / 36.7 / - / 40.1 / 44.4 / 30.2
Ramachandran (favored / allowed)	0.929 / 0.990	0.968 / 1.000	0.976 / 1.000	0.971 / 0.998
R.m.s.d. bonds (Å) / angles (°)	0.009 / 1.5	0.013 / 1.6	0.012 / 1.5	0.010 / 1.4
PDB code	5N83	5N8D	5NBH	5NC1

2 Values in parentheses are for the highest resolution bin

3

4 **Table S2.** Abbreviations, names, print concentrations and primary structures of the glycans used in  
5 the microarray.

Abbreviation	Neoglycoconjugate/ glycoprotein	Print conc (mg/mL)	Structure
1 Fetuin	Fetuin	1	Bovine fetuin
2 Ov	Ovalbumin	1	Hen ovalbumin
3 4APHSA	4AP-HSA	1	4AP-HSA, linker alone attached to HSA
4 a-C	$\alpha$ -Crystallin from bovine lens	1	$\alpha$ -Crystallin from bovine lens, A and B subunits
5 M3BSA	Man $\alpha$ 1,3(Man $\alpha$ 1,6)Man-BSA	1	Man $\alpha$ -(1,3)-[Man $\alpha$ -(1,6)]-Man-BSA
6 GlcNAcBSA	GlcNAc-BSA	1	GlcNAc-Sp14-NH2(Lys)-BSA
7 3SLacHSA	3'-Sialyllactose-APD-HSA	1	Neu5Ac $\alpha$ -(2,3)-Gal- $\beta$ -(1,4)-Glc-APD-HSA
8 6SLacHSA	6'-Sialyllactose-APD-HSA	1	Neu5Ac $\alpha$ -(2,6)-Gal- $\beta$ -(1,4)-Glc-APD-HSA
9 H2BSA	H Type II-APE-BSA	1	Fuc $\alpha$ -(1,2)-Gal- $\beta$ -(1,4)-GlcNAc- $\beta$ -APE-BSA
10 GGGNHSA	Gal $\alpha$ 1,3Gal $\beta$ 1,4GlcNAc-HSA	1	Gal $\alpha$ -(1,3)-Gal- $\beta$ -(1,4)-GlcNAc-HSA
11 Ga3GBSA	Gal $\alpha$ 1,3Gal-BSA	1	Gal $\alpha$ -(1,3)-Gal-Sp3-BSA
12 4APBSA	4AP-BSA	1	4AP-BSA, linker alone attached to BSA
13 LNFP1BSA	Lacto-N-fucopentaose I-BSA	1	Fuc $\alpha$ -(1,2)-Gal- $\beta$ -(1,3)-GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-BSA
14 LebBSA	LNDI-BSA/ Lewis b-BSA	1	Fuc $\alpha$ -(1,2)-Gal- $\beta$ -(1,3)-[Fuc $\alpha$ -(1,4)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-APD-BSA
15 LexBSA	Lewis x-BSA	1	Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc-BSA
16 3LexHSA	Tri-Lex-APE-HSA	1	Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -O-APE-HSA
17 6SuLeaBSA	6-Sulfo Lewis a-BSA	1	(SO4)6Gal- $\beta$ -(1,3)-[Fuc $\alpha$ -(1,4)]GlcNAc-Sp3-BSA
18 LeyHSA	Lewis y-tetrasaccharide-APE-HSA	1	Fuc $\alpha$ -(1,2)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -O-APE-HSA
19 LNTHSA	Lacto-N-tetraose-APD-HSA	1	Gal- $\beta$ -(1,3)-GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-APD-HSA
20 GlobNTHSA	Globo-N-tetraose-APD-HSA	1	GalNAc- $\beta$ -(1,3)-Gal- $\alpha$ -(1,4)-Gal- $\beta$ -(1,4)-Glc-APD-HSA
21 GlobTHSA	Globotriose-APE-HSA	1	Gal $\alpha$ -(1,4)-Gal- $\beta$ -(1,4)-Glc- $\beta$ -APE-HSA
22 ASF	Asialofetuin	1	Bovine asialofetuin
23 PBS	PBS	-	Phosphate buffered saline
24 RB	RNase B	1	Ribonuclease B
25 Xferrin	Transferrin	1	Bovine transferrin
26 LacNAcBSA	LacNAc-BSA	1	Gal- $\beta$ -(1,4)-GlcNAc-Sp3-BSA
27 3SLNBSA	3'SialylLacNAc-BSA	1	Neu5Ac $\alpha$ -(2,3)-Gal- $\beta$ -(1,4)-GlcNAc-BSA
28 2FLBSA	2'Fucosyllactose-BSA	1	Fuc $\alpha$ -(1,2)-Gal- $\beta$ -(1,4)-Glc-Sp3-BSA
29 3SFLBSA	3'Sialyl-3-fucosyllactose-BSA	1	Neu5Ac $\alpha$ -(2,3)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]Glc-Sp3-BSA
30 BGABSA	Blood Group A-BSA	1	GalNAc $\alpha$ -(1,3)-[Fuc $\alpha$ -(1,2)]Gal- $\beta$ -(1,4)-GlcNAc-Sp6-BSA
31 BGBHSA	Blood Group B-BSA	1	Gal $\alpha$ -(1,3)-[Fuc $\alpha$ -(1,2)]Gal- $\beta$ -(1,4)-GlcNAc-Sp6-HSA
32 Gb4GBSA	Gal $\beta$ 1,4GalBSA	1	Gal- $\beta$ -(1,4)-Gal-Sp3-BSA
33 Ga2GBSA	Gal $\alpha$ 1,2GalBSA	1	Gal $\alpha$ -(1,2)-Gal-Sp3-BSA
34 LNFP1IIBSA	Lacto-N-fucopentaose II-BSA	1	Fuc $\alpha$ -(1,3)-Gal- $\beta$ -(1,3)-GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-BSA
35 LNFP1IIBSA	Lacto-N-fucopentaose III-BSA	1	Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-BSA
36 LNDHBSA	Lacto-N-difucotetraose I-BSA	1	Fuc $\alpha$ -(1,2)-Gal- $\beta$ -(1,3)-[Fuc $\alpha$ -(1,4)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-Sp3-BSA
37 DiLexBSA	Di-Lex-APE-BSA	1	Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -O-APE-BSA
38 DiLexHSA	Di-Lewis-APE-HSA	1	Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -O-APE-HSA
39 3SLexBSA3	3'Sialyl Lewis x-BSA	1	Neu5Ac $\alpha$ -(2,3)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc-Sp3-BSA
40 SLexBSA14	3'Sialyl Lewis x-BSA	1	Neu5Ac $\alpha$ -(2,3)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc-Sp14-BSA
41 6SuLexBSA	6-Sulfo Lewis x-BSA	1	(SO4)6Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc-Sp3-BSA
42 3SuLeaBSA	3-Sulfo Lewis a-BSA	1	(SO4)3Gal- $\beta$ 1-3-[Fuc $\alpha$ -(1,4)]GlcNAc-Sp3-BSA
43 3SuLexBSA	3-Sulfo Lewis x-BSA	1	(SO4)3Gal- $\beta$ 1-4-[Fuc $\alpha$ -(1,3)]GlcNAc-Sp3-BSA
44 DFPLNHSA	Difucosyl-para-lacto-N-hexaose-APD-HSA, (Lea/Lex)	1	Gal- $\beta$ -(1,3)-[Fuc $\alpha$ -(1,4)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-APD-HSA
45 LeaBSA	Lewis a-BSA	1	Gal- $\beta$ -(1,3)-[Fuc $\alpha$ -(1,4)]GlcNAc-Sp3-BSA
46 3FLexHSA	Tri-fucosyl-Ley-heptasaccharide-APE-HSA	1	Fuc $\alpha$ -(1,2)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -O-APE-HSA
47 LNnTHSA	Lacto-N-neotetraose-APD-HSA	1	Gal- $\beta$ -(1,4)-GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-APD-HSA
48 SLNFVHSA	Sialyl-LNF V-APD-HSA	1	Fuc $\alpha$ -(1,2)-Gal- $\beta$ -(1,3)-[NeuAc $\alpha$ -(2,6)]GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-APD-HSA
49 MMLNnHSA	Monofucosyl, monosialyllactose-N-neohexaose-APD-HSA	1	Neu5Ac $\alpha$ -(2,3)-Gal- $\beta$ -(1,4)-GlcNAc- $\beta$ -(1,3)-[Gal- $\beta$ -(1,4)-[Fuc $\alpha$ -(1,3)]GlcNAc- $\beta$ -(1,6)]Gal- $\beta$ -(1,4)-Glc-APD-HSA
50 SLNnTHSA	Sialyl-LNnT-penta-APD-HSA	1	Neu5Ac $\alpha$ -(2,3)-Gal- $\beta$ -(1,4)-GlcNAc- $\beta$ -(1,3)-Gal- $\beta$ -(1,4)-Glc-APD-HSA
51 GM1HSA	GM1-pentasaccharide-APD-HSA	1	Gal- $\beta$ -(1,3)-GalNAc- $\beta$ -(1,4)-[Neu5Ac $\alpha$ -(2,3)]Gal- $\beta$ -(1,4)-Glc-APD-HSA
52 aGM1HSA	Asialo-GM1-tetrasaccharide-APD-HSA	1	Gal- $\beta$ -(1,3)-GalNAc- $\beta$ -(1,4)-Gal- $\beta$ -(1,4)-Glc-APD-HSA
53 Inv	Invertase	1	Yeast invertase, grade VII
54 Fibrin	Fibrinogen	0,5	Fibrinogen from human plasma
55 A1AT	alpha-1-antitrypsin	1	alpha-1-antitrypsin
56 Cerulo	Ceruloplasmin	1	Ceruloplasmin, human, type III

Linker key  
Sp3 = 3 atom spacer  
Sp6 = 6 atom spacer  
Sp14 = 14 atom linker  
4AP = 4-aminophenyl  
APE = aminophenylethyl  
APD = acetylphenylenediamine

8 **Table S3.** List of gastrointestinal tract mucins and glycoproteins printed and printing conditions for  
9 the gastrointestinal tract mucin microarray.

Number	Source	Print concentration (mg/mL)	Print buffer
1	Equine stomach	0.25	PBS 0.01% Tween 20
2	Ovine abomasum antrum	0.1	PBS 0.01% Tween 20
3	HT29MTXE12	0.5	PBS 0.025% Tween 20
4	Ovine descending colon	0.15	PBS 0.01% Tween 20
5	Ovine ileum	0.15	PBS 0.01% Tween 20
6	Ovine spiral colon	0.5	PBS
7	Chicken proximal small intestine	0.25	PBS 0.025% Tween 20
8	Ovine jejunum	0.5	PBS 0.01% Tween 20
9	Ovine duodenum	0.15	PBS 0.01% Tween 20
10	Porcine gastric mucin	0.33	PBS 0.01% Tween 20
11	Chicken large intestine	0.2	PBS 0.01% Tween 20
12	Equine duodenum	0.3	PBS 0.01% Tween 20
13	Deer jejunum	0.25	PBS 0.025% Tween 20
14	Deer spiral ascending colon	0.75	PBS 0.025% Tween 20
15	Bovine abomasum	0.25	PBS 0.01% Tween 20
16	Bovine duodenum	0.5	PBS 0.01% Tween 20
17	Equine small intestine	0.25	PBS
18	Equine left ventral colon	0.25	PBS 0.01% Tween 20
19	Bovine spiral colon	0.25	PBS 0.01% Tween 20
20	Deer duodenum	0.5	PBS 0.025% Tween 20
21	Equine right ventral colon	0.15	PBS 0.01% Tween 20
22	Equine dorsal colon	0.25	PBS 0.01% Tween 20
23	Deer abomasum	0.25	PBS 0.01% Tween 20
24	Chicken cecum	0.5	PBS 0.025% Tween 20
25	LS174T	0.5	PBS 0.01% Tween 20
26	Porcine descending colon	0.5	PBS 0.025% Tween 20
27	Porcine jejunum	0.25	PBS
28	Porcine spiral colon	0.6	PBS 0.025% Tween 20
29	Porcine stomach	0.5	PBS 0.025% Tween 20
30	Porcine ceca	0.5	PBS 0.025% Tween 20
31	Mouse large intestine	0.4	PBS 0.025% Tween 20
32	Mouse cecum	0.3	PBS 0.025% Tween 20
33	Mouse stomach	0.5	PBS 0.025% Tween 20
34	Mouse small intestine	0.25	PBS 0.025% Tween 20
35	Rat ileum	0.5	PBS 0.025% Tween 20
36	Rat duodenum and jejunum	0.5	PBS 0.025% Tween 20
37	Rat cecum	0.5	PBS 0.025% Tween 20
38	Rat stomach	0.5	PBS 0.025% Tween 20
39	PBS		
40	Asialofetuin	1	PBS 0.05% Tween 20
41	RNase B	1	PBS 0.05% Tween 20
42	Fetuin	1	PBS 0.05% Tween 20
43	Transferrin	1	PBS 0.05% Tween 20
44	Ovomucoid	0.5	PBS 0.05% Tween 20
45	Human $\alpha$ 1-acid glycoprotein	1	PBS 0.05% Tween 20
46	PBS 0.025% Tween 20		

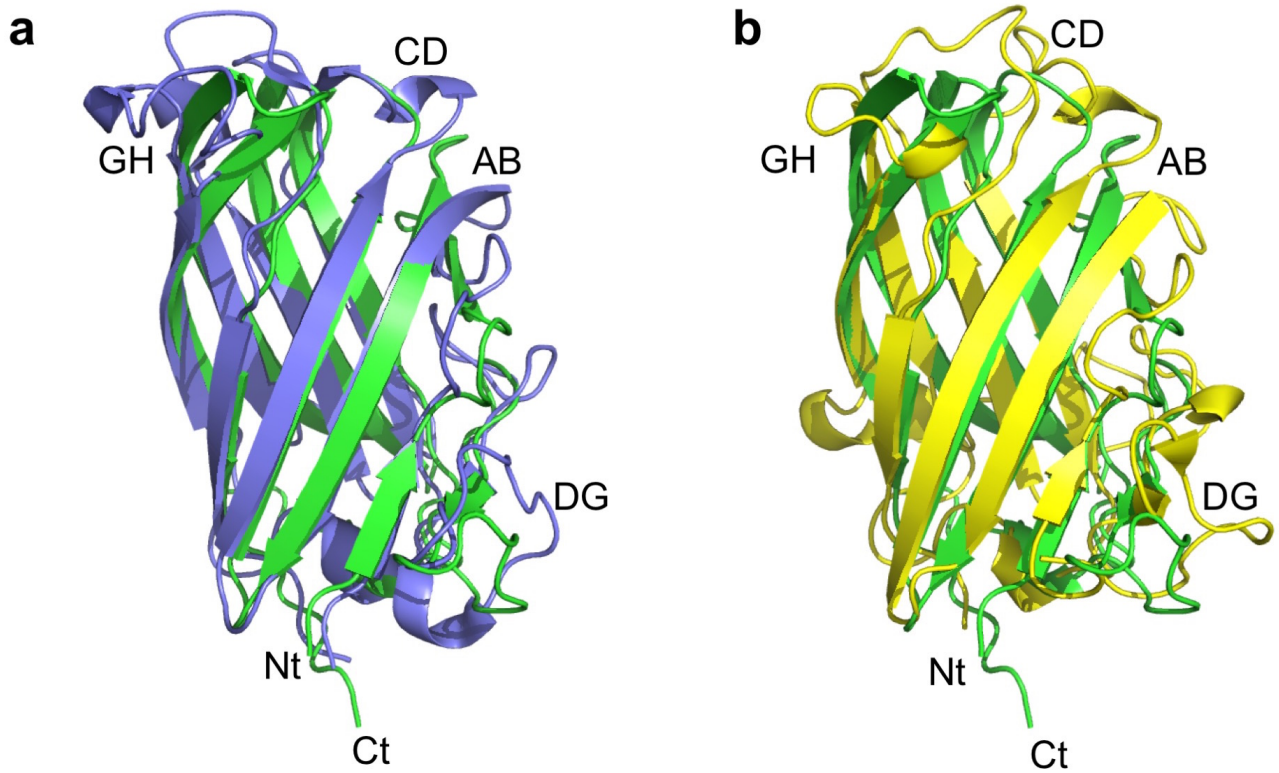
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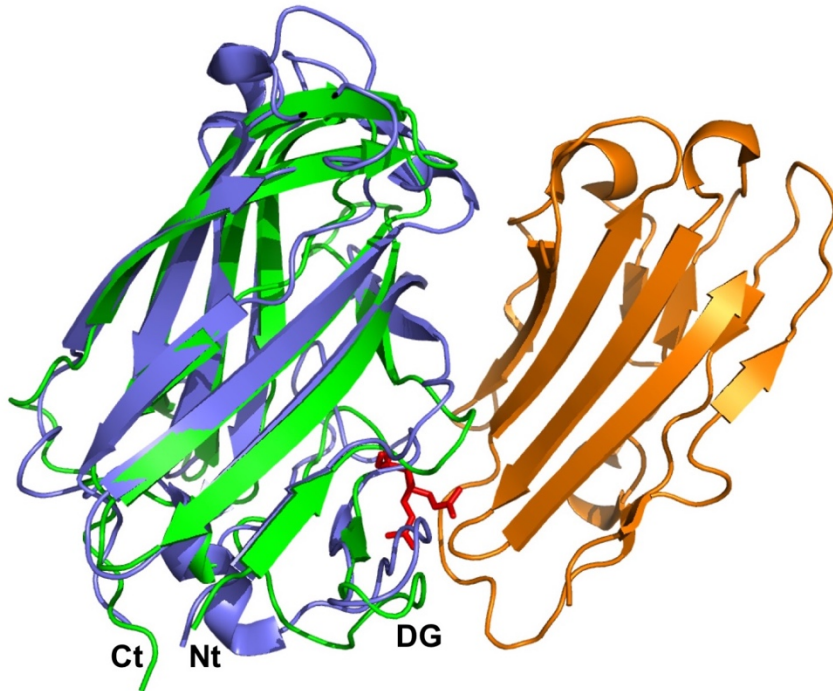
12 **Table S4.** Inhibition percentage of binding in the presence of 100 mM GlcNAc. Ten percent  
 13 inhibition or greater is highlighted by shaded cells.  
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<b>Binding probe</b>	<b>MAdV- 2fib(517-787)</b>	<b>MAdV- 2fib(586-787)</b>	<b>WGA</b>
Equine stomach mucin	0.0	23	86
Ovine abomasum antrum mucin	1.8	19	93
HT29MTXE12 mucin	39.4	44	63
Ovine descending colon mucin	41.9	61	83
Ovine ileum mucin	19.2	34	55
Ovine spiral colon mucin	43.2	64	45
Chicken proximal small intestine mucin	83.9	85	0
Ovine jejunum mucin	10.8	48	66
Ovine duodenum mucin	60.0	75	22
Porcine gastric mucin	37.7	79	0
Chicken large intestine mucin	43.9	65	0
Equine duodenum mucin	43.1	75	67
Deer jejunum mucin	0.0	0	0
Deer spiral ascending colon mucin	0.0	0	55
Bovine abomasum mucin	9.4	40	90
Bovine duodenum mucin	40.4	27	64
Equine small intestine mucin	62.4	80	88
Equine left ventral colon mucin	52.1	50	90
Bovine spiral colon mucin	0.0	0	0
Deer duodenum mucin	0.0	29	0
Equine right ventral colon mucin	0.0	3	91
Equine dorsal colon mucin	0.0	7	91
Deer abomasum mucin	100.0	100	0
Chicken cecum mucin	66.6	99	0
LS174T mucin	71.4	83	94
Porcine descending colon mucin	76.8	82	54
Porcine jejunum mucin	0.0	0	0
Porcine spiral colon mucin	69.7	75	90
Porcine stomach mucin	30.9	59	85
Porcine cecum mucin	37.2	42	0
Mouse large intestine mucin	81.7	84	70
Mouse cecum mucin	94.0	93	95
Mouse stomach mucin	43.4	76	11
Mouse small intestine mucin	85.5	94	78
Rat ileum mucin	72.7	83	18
Rat duodenum & jejunum mucin	93.3	95	0
Rat cecum mucin	81.0	69	0
Rat stomach mucin	90.9	85	82
Asialofetuin	0.0	43	0
RNase B	95.2	96	49
Fetuin	0.0	37	49
Transferrin	0.0	0	0
Ovomucoid	97.1	96	41
Human $\alpha$ 1-acid glycoprotein	0.0	16	0

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 17 **Fig. S1.** Comparison of the MAdV-2, CAdV-2 and HAdV-5 fibre head structures. A. MAdV-2 fibre  
 18 head monomer (in green) superposed onto the CAdV-2 fibre head monomer (in blue). B. MAdV-2  
 19 fibre head monomer (in green) superposed onto the HAdV-5 fibre head monomer (in yellow). The  
 20 N-termini, C-termini and AB-, CD-, DG- and GH-loops are labelled.  
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24 **b**

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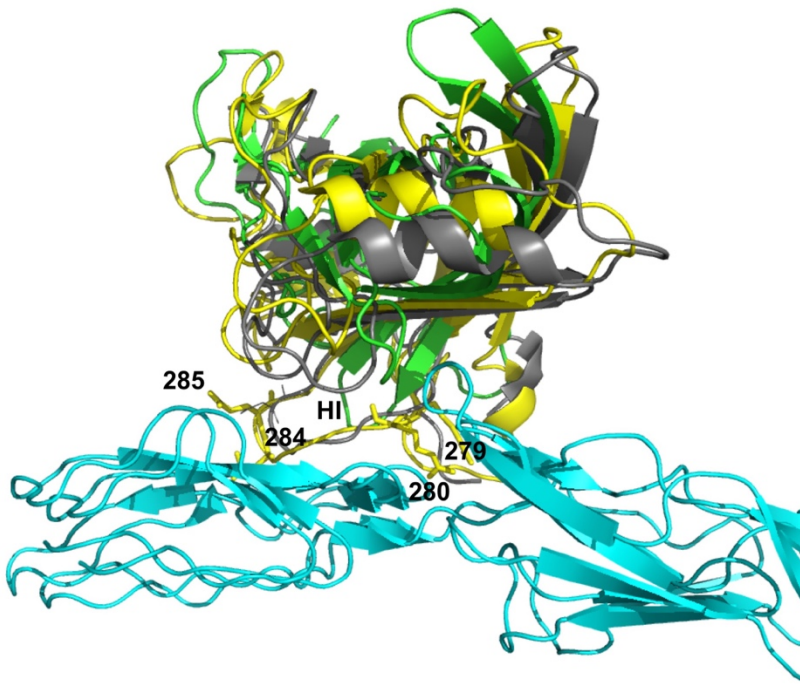
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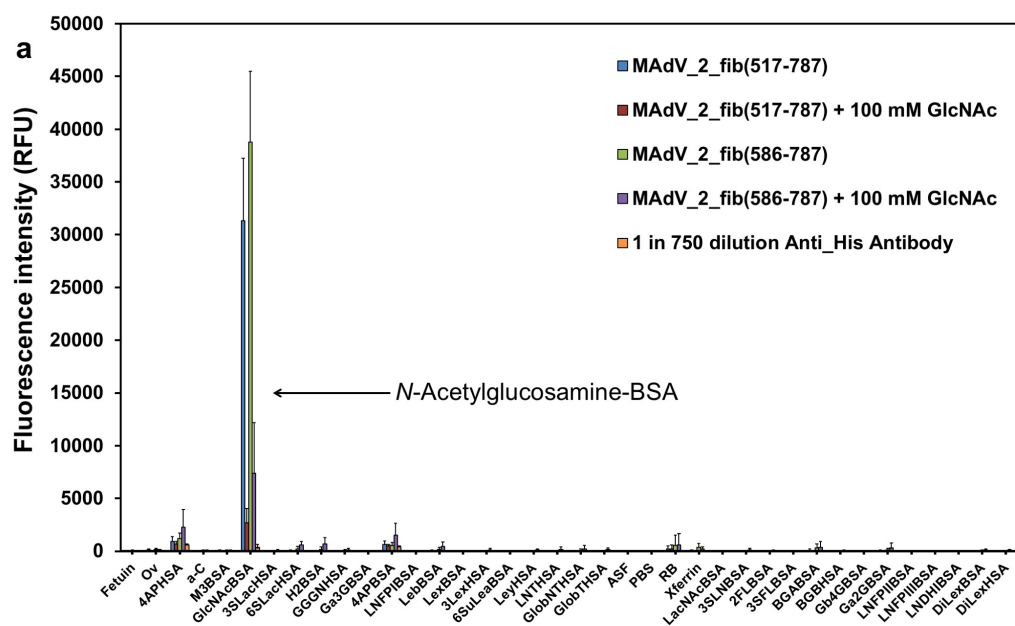
                ==AB==                =BC=                =CD==
MAdV-2  631  PVFVYQTPFNTPMRLRNNGTYNEY--ADAHIQVRFGTTVLFNIDVTGETNATGTQTWEL----- 687
CAdV-2  358  PPAAPITLWTGPGPSIINGFINDTFVIRCFICLTRDSNLVTVNASFVGGEGYRIVSPTQSQFSLIMEF 424
          * . * : . * ** * : .. * : * .. * . * .....* . . . . :
          :
          =====DG=====
MAdV-2  688  -----QFDGTLGSCLTGRMQVMGGTGEELDVTPTFILPTSDKSVYKQGFMPIV-C-----SENGEF 737
CAdV-2  425  DQFGQLMSTGNINSTTTWGEKPGWNNVQPRPSHTWKLCMPNREVYSTPAATISRCGLDSIAVDGAP 491
          *... * : *.. : : * : * .....* . * * : : *
          :
          =HI=                =IJ==
MAdV-2  738  KQ-----STYCSYALTYRLGNFYITLKSTTSGCKPIFQMSFMYESQIGIV 787
CAdV-2  492  SRSIDCMLIINKPKGVATYTLTFRFLNFN-RLSGGTLFKTDVLTFTYVGENQ---- 542
          .. : . : : * : * : * .. * . : : : : * . *

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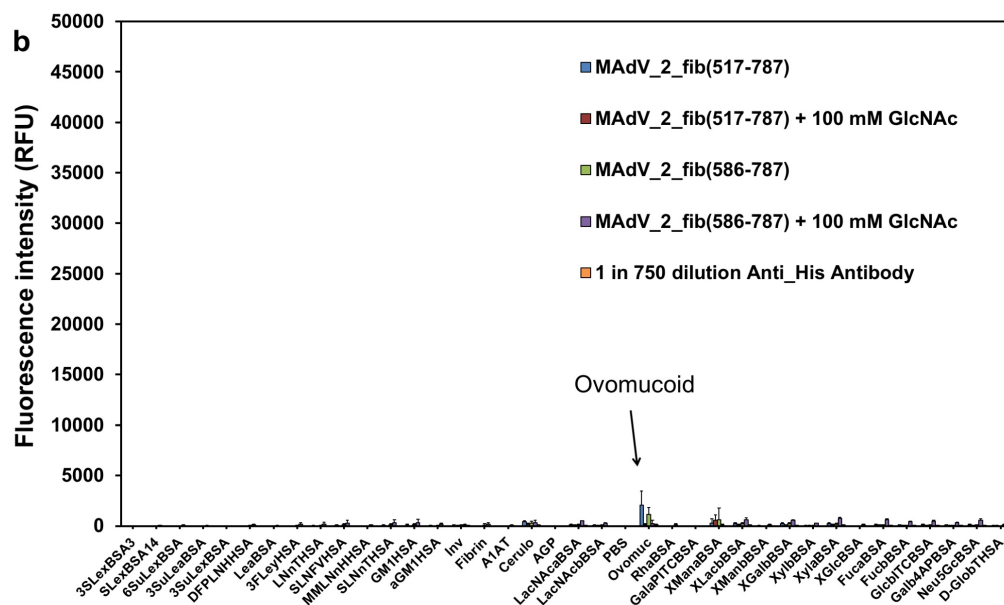
**Fig. S2.** MAdV-2 fibre head is unlikely to bind CAR. A. Monomer of the MAdV-2 fibre head (in green) superposed onto the CAdV-2 fibre head-CAR D1 complex (PDB entry 2J1K; CAdV-2 fibre head in blue, CAR D1 in orange). The DG-loop is labelled; Arg700 of the MAdV-2 fibre head structure is shown in red. N- and C-termini are also indicated. B. Alignment of the MAdV-2 fibre head sequence with the CAR-binding CAdV-2 fibre head sequence. Residues of the CAdV-2 fibre head which interact with CAR D1 domain are highlighted in yellow; Thr441 in green. Loops connecting different  $\beta$ -strands as observed in the MAdV-2 fibre head are labelled. Arg700 of the MAdV-2 fibre head is highlighted in red.



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51 **Fig. S3.** MAdV-2 fibre head is unlikely to bind CD46. Superposition of the MAdV-2 fibre head  
52 domain (in green) onto the HAdV-11 and HAdV21 fibre head domains bound to CD46 (HAdV11:  
53 PDB entry 3O8E, yellow; HAdV21: PDB entry 3I89; grey; CD46 in cyan). While the conformations  
54 of the HI-loops of HAdV-11 and HAdV-21 are similar to each other; the HI-loop of MAdV-2 is much  
55 shorter and apparently incompatible with CD46 binding. Arg279, Arg280, Asp284 and Glu285 of the  
56 HAdV-11 are shown and labelled.  
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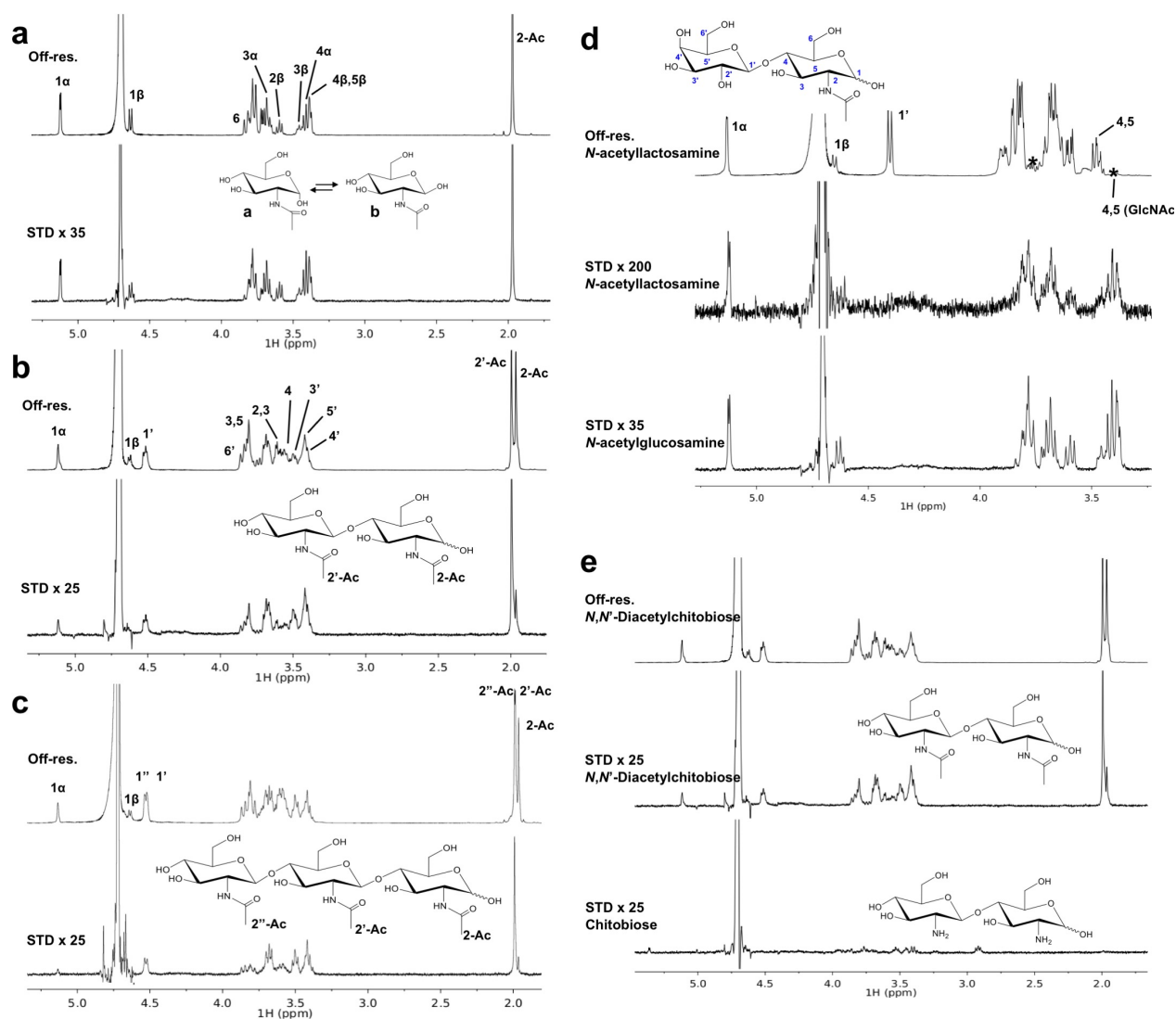
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60 **Fig. S4.** Binding profile of two isoforms of MAdV-2 fiber protein. Bar chart (divided in panels A and  
 61 B) representing the binding intensity of the long (MAdV-2-fib(517-787)) and short (MAdV-2-  
 62 fib(586-787)) forms of the MAdV-2 fiber to carbohydrates on a microarray surface. Binding was  
 63 detected using a fluorescently labelled anti-His antibody. The data represents the average of four  
 64 replicate experiments and the error bars depict one standard deviation of the mean calculated over  
 65 four microarray slides. The protein isoforms show comparable binding patterns with strong binding  
 66 to GlcNAc-BSA and lower binding to ovomucoid, both of which were inhibited by co-incubation  
 67 with 100 mM GlcNAc.





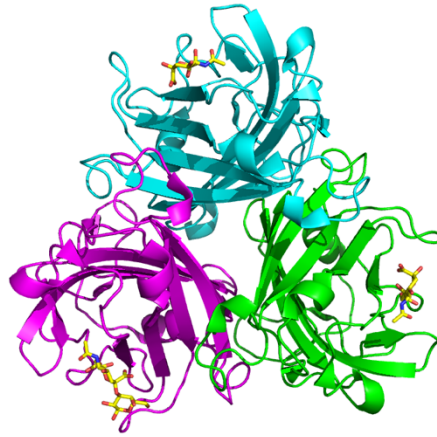
68  
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70 **Fig. S5.** A. *N*-acetyl-glucosamine binding to MAdV-2-fib(586-787) studied by STD-NMR. Top: Off-  
 71 resonance spectrum, with labels indicating the assignment for representative ligand signals. Bottom:  
 72 STD spectrum (up-scaled 35×). B. *N,N'*-diacetylchitobiose (GlcNAc-β-(1→4)-GlcNAc) binding of  
 73 MAdV-2-fib(586-787). Top: Off-resonance spectrum, with labels indicating the assignment for  
 74 representative ligand signals. Bottom: STD spectrum (up-scaled 25×). C. Binding of *N,N',N''*-  
 75 triacetylchitotriose (GlcNAc-β-(1→4)-GlcNAc-β-(1→4)-GlcNAc) to MAdV-2-fib(586-787). Top  
 76 spectrum: Off-resonance (reference) spectrum. Bottom spectrum: STD spectra up-scaled 25×. D.  
 77 Effect of deacetylation on the binding of *N,N'*-diacetylchitobiose to MAdV-2-fib(586-787). Top  
 78 spectrum: Off-resonance spectrum of *N,N'*-diacetylchitobiose. Middle spectrum: STD spectrum of  
 79 *N,N'*-diacetylchitobiose. Bottom spectrum: STD spectrum of chitobiose. E. STD-NMR experiment  
 80 performed on *N*-acetyllactosamine (Gal-β-(1→4)-GlcNAc), showing that the only species being  
 81 recognised in the sample are trace amounts of GlcNAc. Top spectrum: Off-resonance spectrum of *N*-  
 82 acetyllactosamine; middle spectrum: STD spectrum of the *N*-acetyllactosamine sample; bottom  
 83 spectrum: STD spectrum of GlcNAc.  
 84

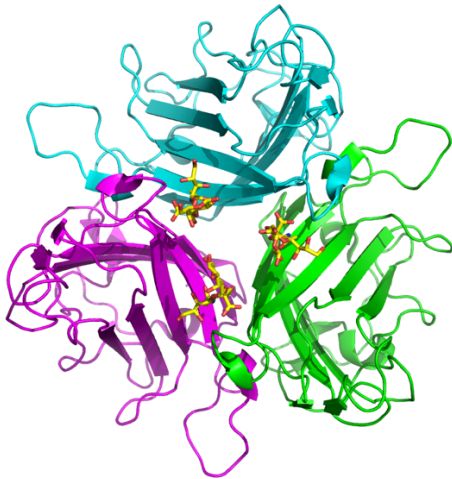
85 **a**



**b**



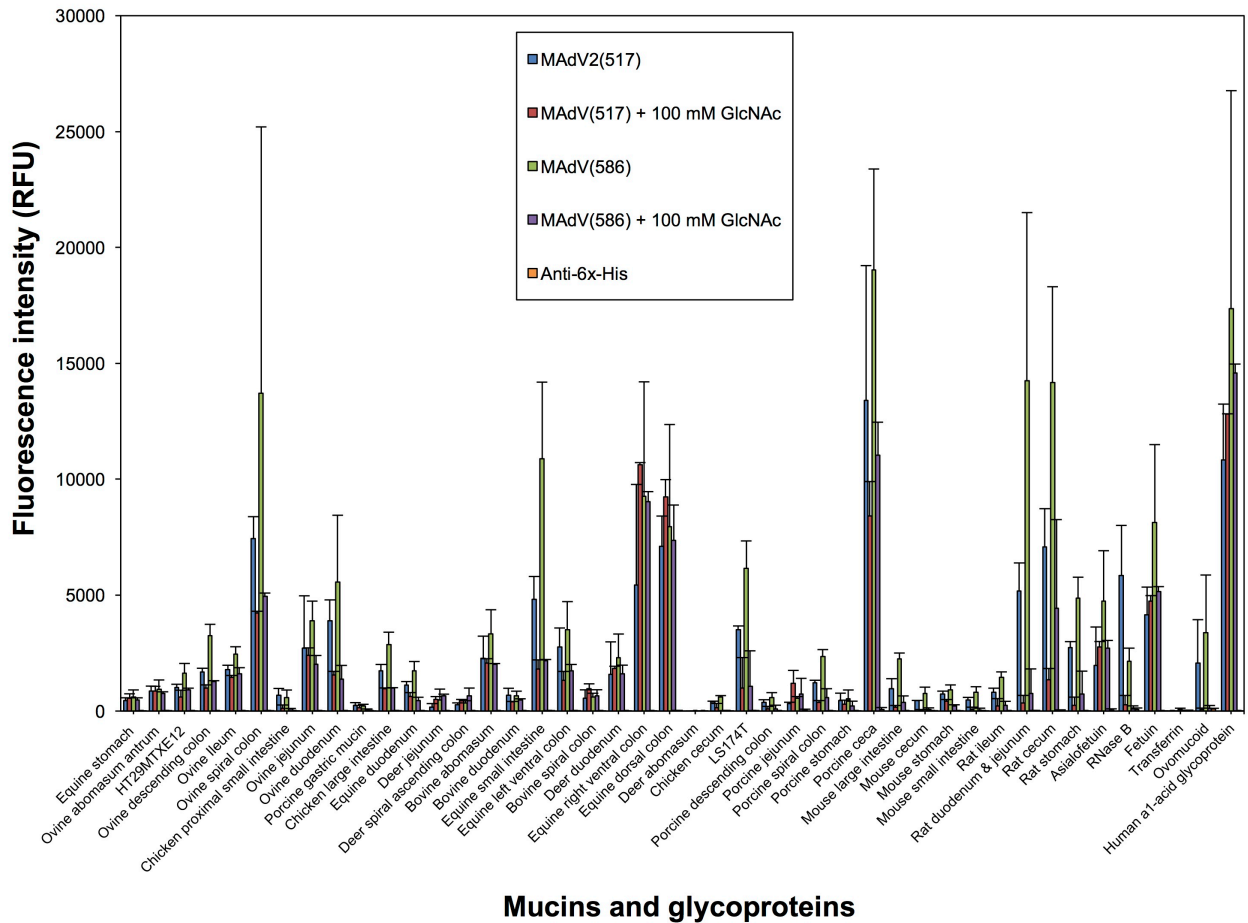
86  
87 **c**



**d**



88  
89 **Fig. S6.** Comparison of the ligand binding sites of MAdV-2 fibre head bound to GlcNAc (PDB entry  
90 5NC1; panel A), CAdV-2 fibre head bound to sialyllactose (PDB entry 2WBV; panel B), HAdV37  
91 fibre head bound to sialyllactose (PDB entry 1UXA; panel C) and HAdV-52 fibre head bound to  
92 trisialic acid (PDB entry 6G47; panel D). Top views with the trimers in comparable orientations are  
93 shown, i.e. from the viral-distal end of the fibre.  
94



95

96 **Fig. S7.** MAdV-2 fibre binding to gastrointestinal tract mucins. Bar chart representing the binding  
 97 intensity of the MAdV-2fib(586-787) and MAdV-2fib(517-787) fibre proteins to mucins and  
 98 glycoproteins on a mucin microarray in the presence and absence of 100 mM GlcNAc. Binding was  
 99 detected using a fluorescently labelled anti-His antibody. The data for the uninhibited proteins  
 100 represents the mean of three technical replicate microarray slides and the data for the inhibited  
 101 proteins represent the mean of two technical replicates. Error bars represent one standard deviation  
 102 of the mean.