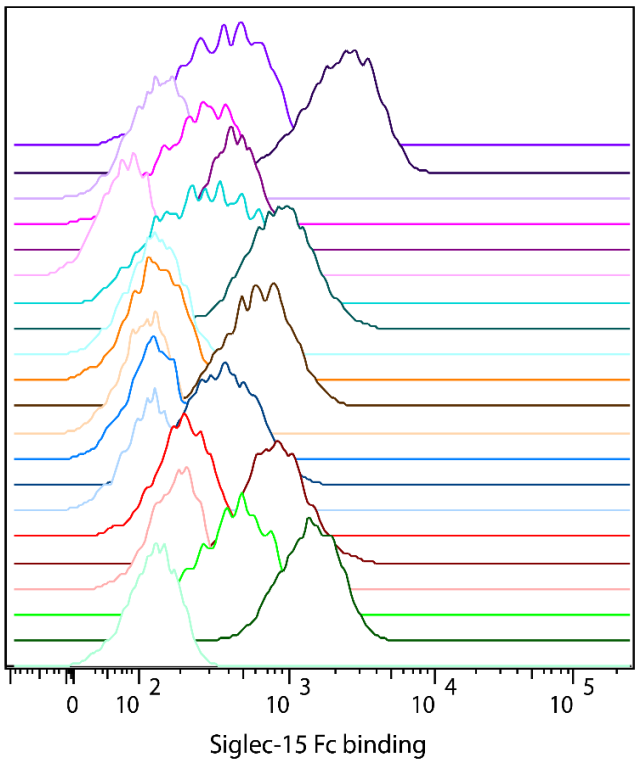
Supplementary data

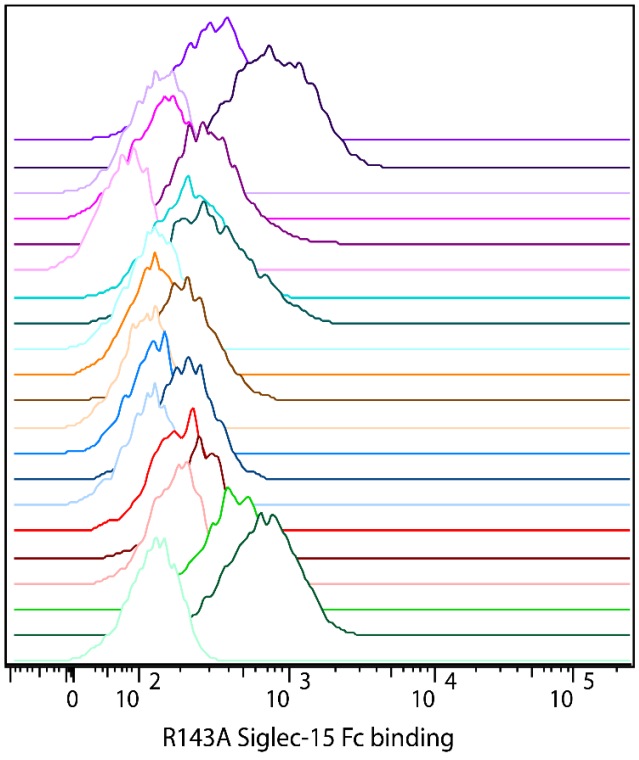
Figure S1

A.



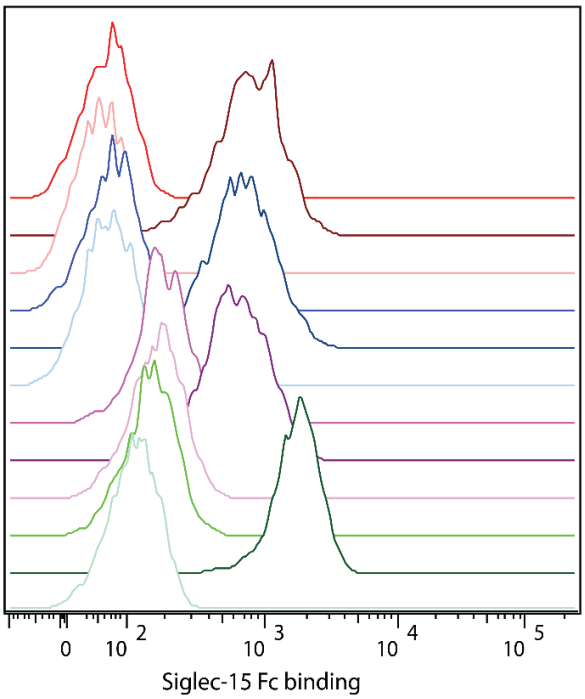
Sample	MFI
SKMES1 Sialidase Siglec-15 Fc	360
SKMES1 Siglec-15 Fc	2202
SKMES1 Anti-hulgG Fc	135
H292 Sialidase Siglec-15 Fc	248
H292 Siglec-15 Fc	402
H292 Anti-hulgG Fc	70.1
H2405 Sialidase Siglec-15 Fc	286
H2405 Siglec-15 Fc	812
H2405 Anti-hulgG Fc	121
H460 Sialidase Siglec-15 Fc	127
H460 Siglec-15 Fc	603
H460 Anti-hulgG Fc	105
HCC827 Sialidase Siglec-15 Fc	121
HCC827 Siglec-15 Fc	340
HCC827 Anti-hulgG Fc	114
HCC44 Sialidase Siglec-15 Fc	197
HCC44 Siglec-15 Fc	761
HCC44 Anti-hulgG Fc	175
A549 Sialidase Siglec-15 Fc	427
A549 Siglec-15 Fc	1323
A549 Anti-hulgG Fc	122

B.



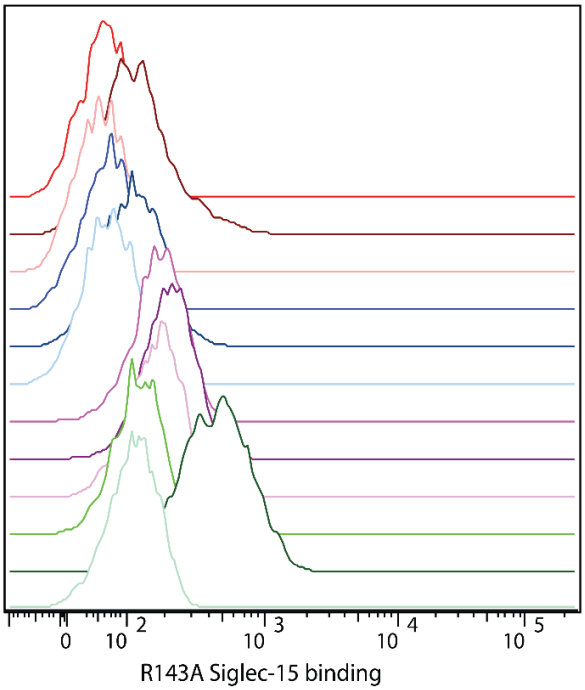
Sample	MFI
SKMES1 Sialidase R143A Siglec-15	293
SKMES1 R143A Siglec-15 Fc	689
SKMES1 Anti-hulgG Fc	135
H292 Sialidase R143A Siglec-15	144
H292 R143A Siglec-15 Fc	272
H292 Anti-hulgG Fc	70.1
H2405 Sialidase R143A Siglec-15	213
H2405 R143A Siglec-15 Fc	270
H2405 Anti-hulgG Fc	121
H460 Sialidase R143A Siglec-15	121
H460 R143A Siglec-15 Fc	197
H460 Anti-hulgG Fc	105
HCC827 Sialidase R143A Siglec-15 Fc	122
HCC827 R143A Siglec-15 Fc	198
HCC827 Anti-hulgG Fc	114
HCC44 Sialidase R143A Siglec-15	184
HCC44 R143A Siglec-15 Fc	242
HCC44 Anti-hulgG Fc	175
A549 Sialidase R143A Siglec-15 Fc	402
A549 R143A Siglec-15 Fc	627
A549 Anti-hulgG Fc	122

C.



Sample	MFI
T47D Sialidase Siglec-15 Fc	62.2
T47D Siglec-15 Fc	758
T47D Anti-hulgG Fc	48.8
MDA MB 231 Sialidase Siglec-15 Fc	69.3
MDA MB 231 Siglec-15 Fc	650
MDA MB 231 Anti-hulgG Fc	63.6
CAL120 Sialidase Siglec-15 Fc	182
CAL120 Siglec-15 Fc	613
CAL120 Anti-hulgG Fc	173
BT549 Sialidase Siglec-15 Fc	155
BT549 Siglec-15 Fc	1753
BT549 Anti-hulgG Fc	114

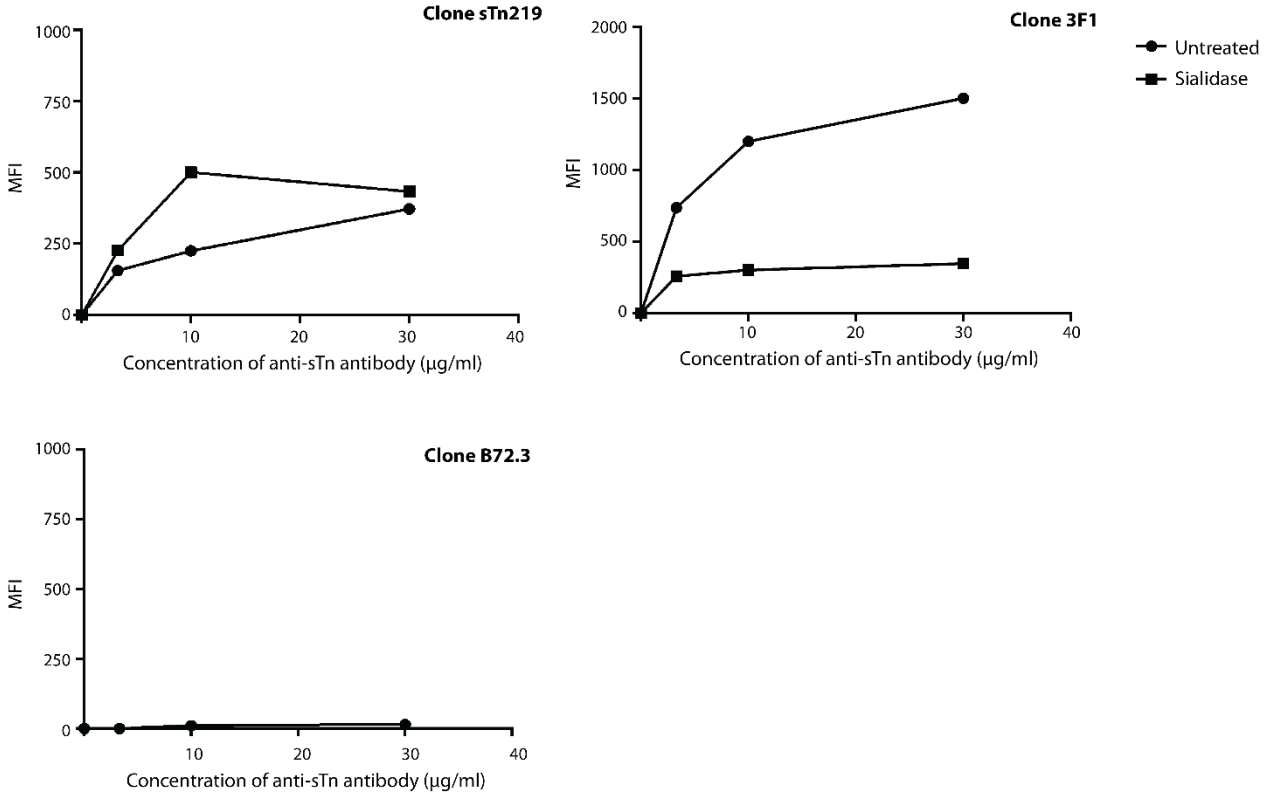
D.



Sample	MFI
T47D Sialidase R143A Siglec-15 Fc	56.1
T47D R143A Siglec-15 Fc	114
T47D Anti-hulgG Fc	48.8
MDA MB 231 Sialidase R143A Siglec-15 Fc	62.2
MDA MB 231 R143A Siglec-15 Fc	111
MDA MB 231 Anti-hulgG Fc	63.6
CAL120 Sialidase R143A Siglec-15 Fc	179
CAL120 R143A Siglec-15 Fc	213
CAL120 Anti-hulgG Fc	173
BT549 Sialidase R143A Siglec-15 Fc	123
BT549 R143A Siglec-15 Fc	440
BT549 Anti-hulgG Fc	114

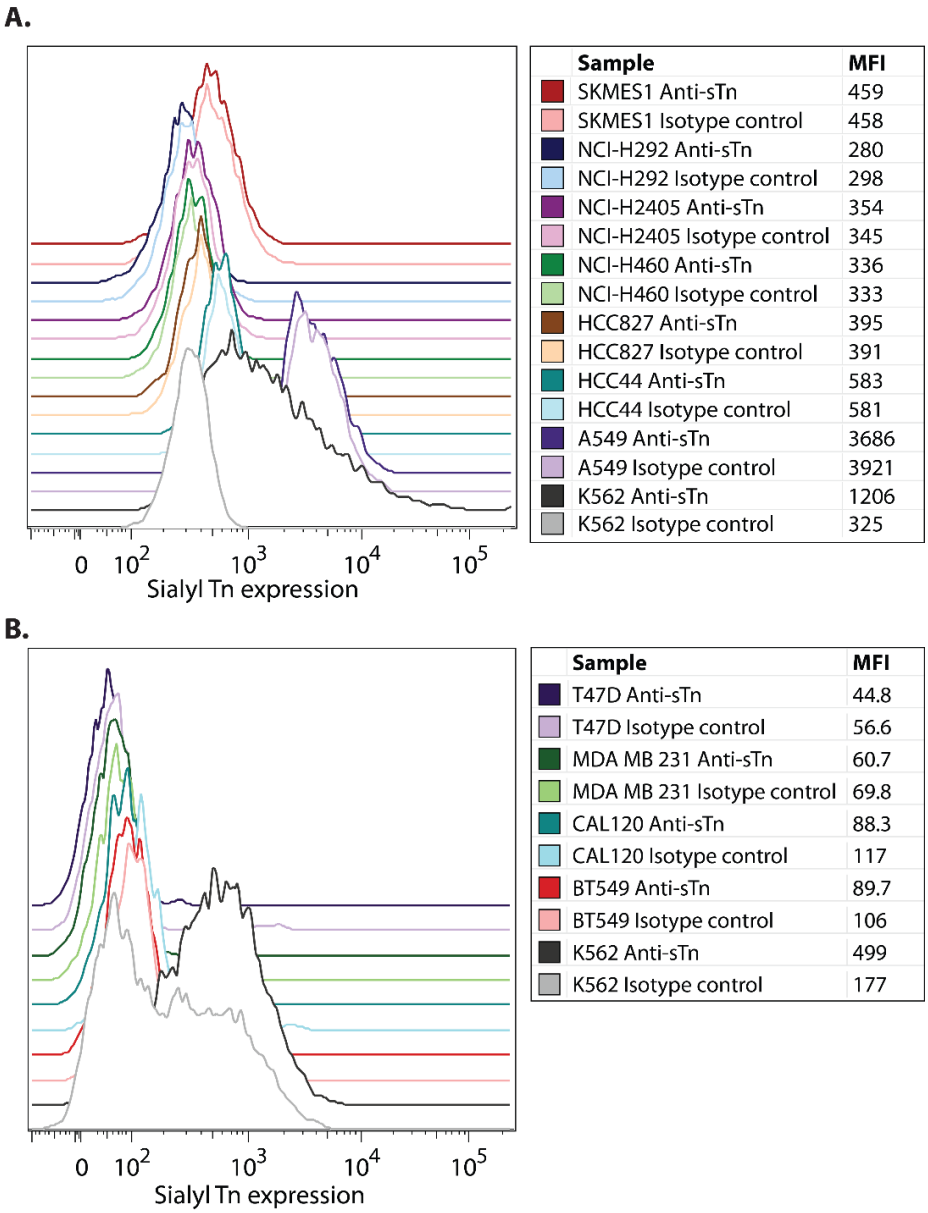
Supplementary Figure S1. Binding of Lung cancer cell lines were analysed for binding to recombinant Siglec-15-Fc wildtype **(A)** or R143A mutant **(B)** (each at 1 µg/ml) precomplexed with anti-human IgG-Fc FITC (1 µg/ml). Breast cancer cell lines were analysed for binding to Siglec-15-Fc wildtype **(C)** or R143A mutant **(D)** (each at 1 µg/ml) precomplexed with anti-human IgG Fc-FITC (1 µg/ml). Cells were pretreated with sialidase to determine whether Siglec-15 binding is sialic acid-dependent. Histograms are representative of three independent experiments.

Figure S2



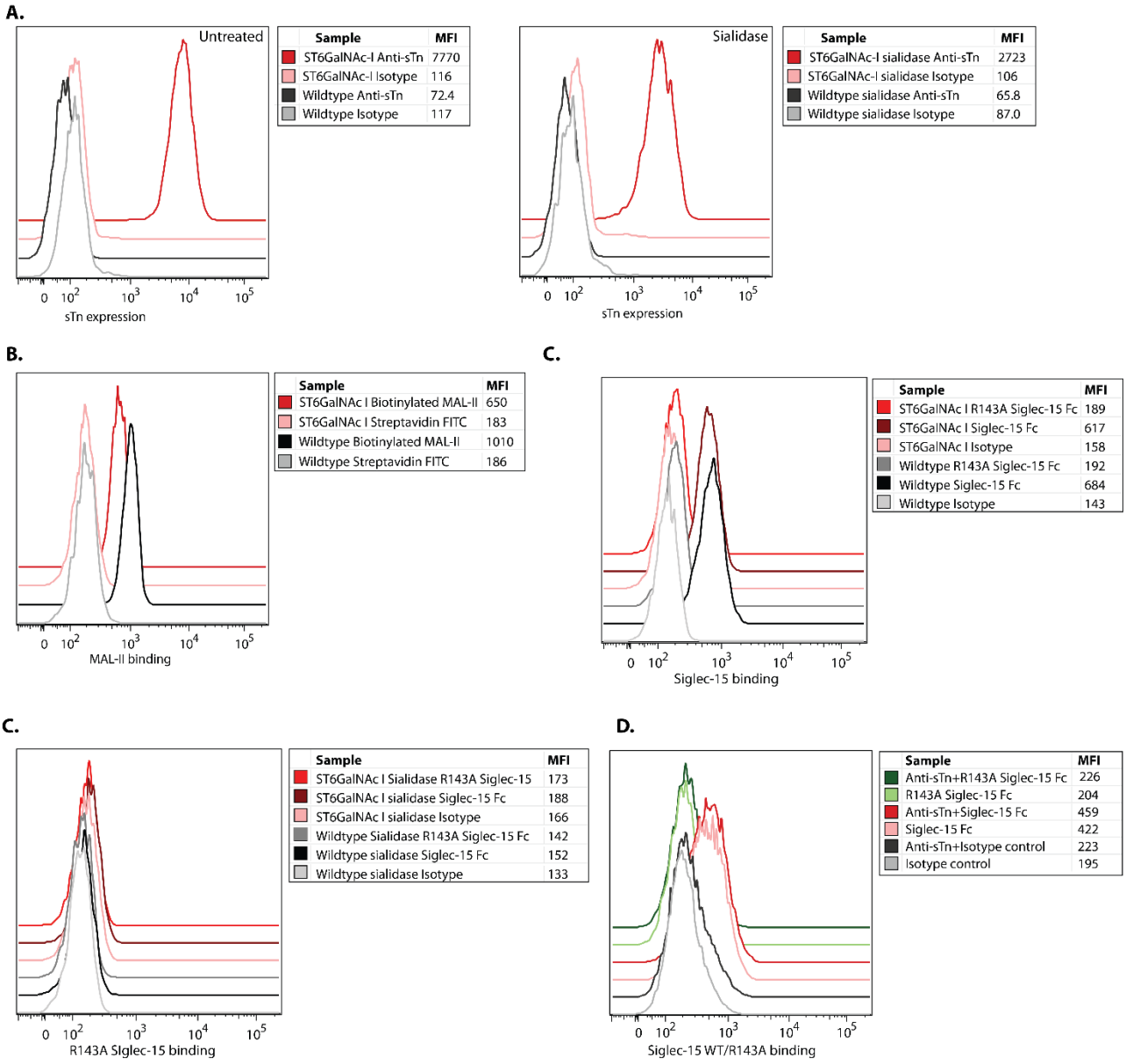
Supplementary Figure S2. Three different clones of anti-sTn antibodies were incubated with K562 cells at increasing concentrations (0, 3.3, 10 and 30 µg/ml) and tested for binding using PE-conjugated anti-mouse IgG secondary antibody. Sialidase-treated K562 cells were used as the negative control. MFI values were plotted against concentration of the anti-sTn antibody. Representative of two independent experiments.

Figure S3



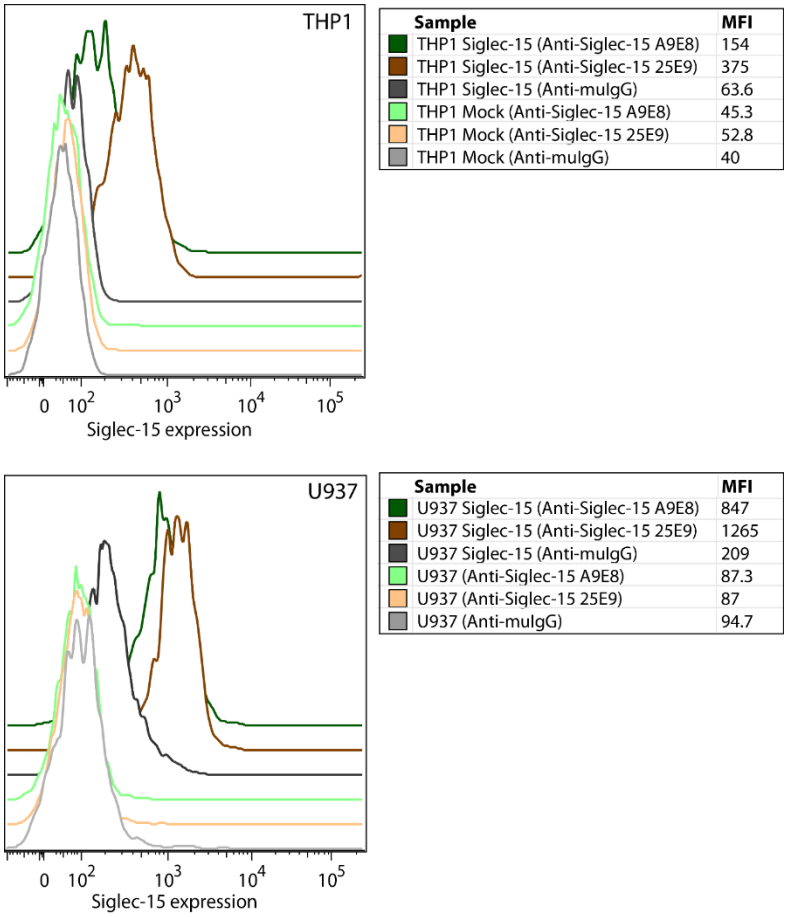
Supplementary Figure S3. STn expression on lung **(A)** and breast **(B)** carcinoma cell lines was analysed by incubating the cells with anti-sTn antibody clone 3F1 followed by APC-conjugated anti-mouse IgG secondary antibody. K562 cells were used as a positive control and isotype-matched mouse IgG was used as a negative control. Histograms are representative of three independent experiments.

Figure S4



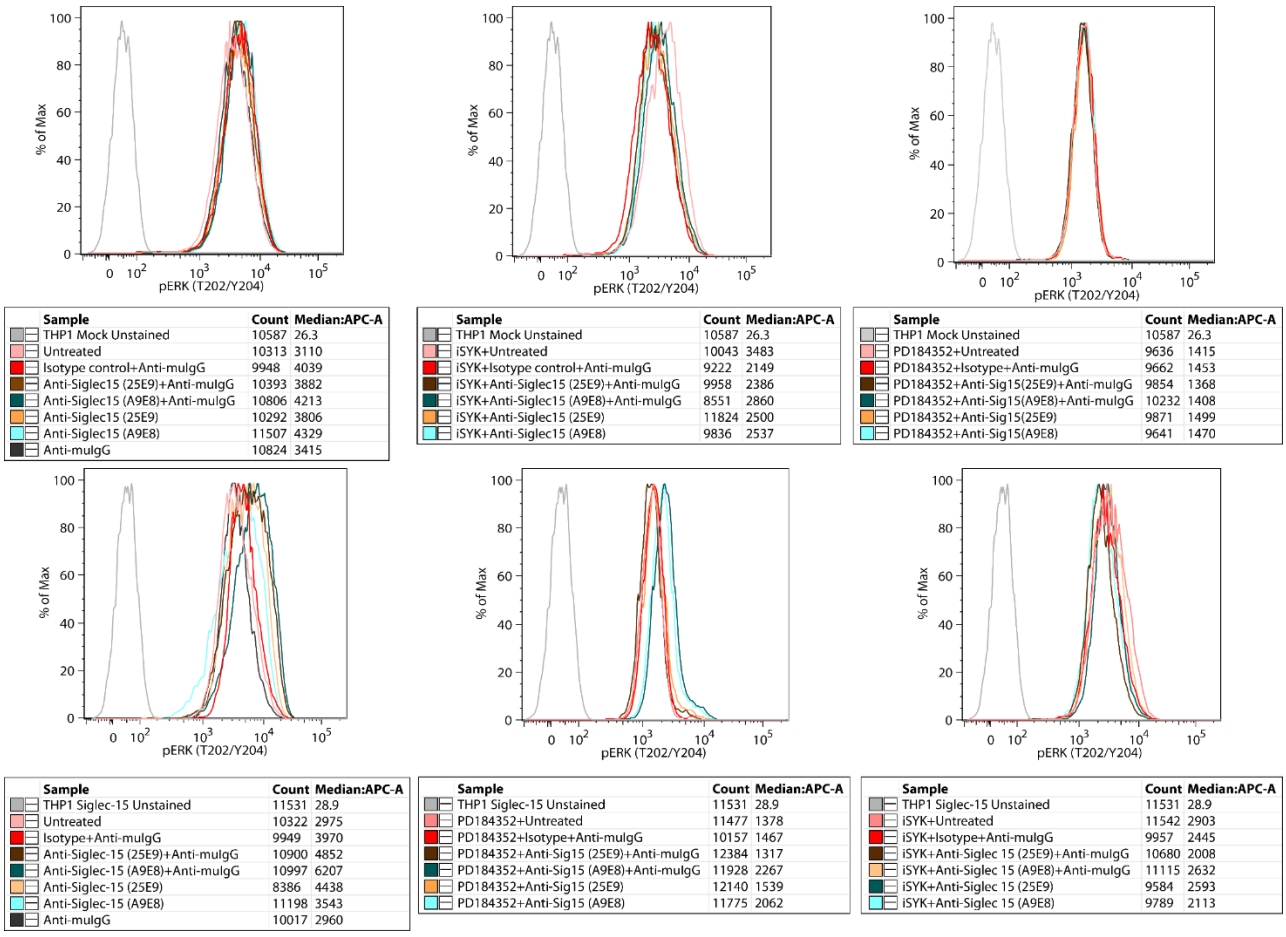
Supplementary Figure S4. (A) H460 wildtype and ST6GalNAc-I transferase-expressing cells, pretreated without or with sialidase, were tested for sTn expression using anti-sTn antibody (clone 3F1) followed by PE-conjugated anti-mouse IgG secondary antibody. **(B)** H460 cells were tested for expression of α2-3-linked sialic acids using biotinylated MAL-II (2 µg/ml) followed by streptavidin-FITC. **(C)** H460 cells with or without pre-treatment with sialidase were incubated with Siglec-15-Fc wildtype or R143A mutant precomplexed with anti-human IgG Fc-FITC. **(D)** sTn expressed on the surface of K562 cells was blocked with anti-sTn antibody (30 µg/ml) followed by Siglec-15-Fc wildtype or R143A mutant precomplexed with anti-human IgG Fc-FITC. Human isotype-matched IgG precomplexed with anti-IgG Fc-FITC was used as negative control. Histograms are representative of three independent experiments.

Figure S5



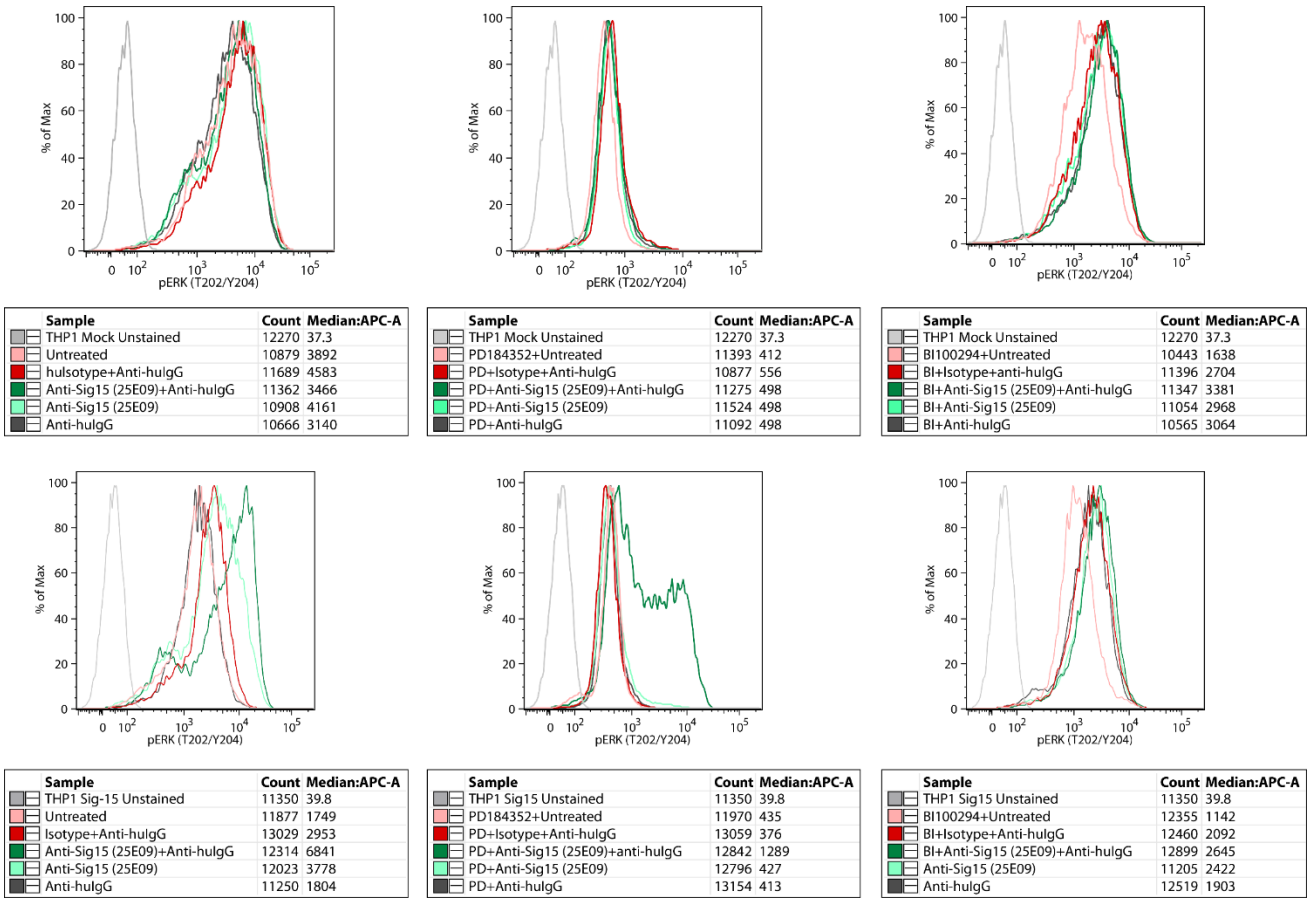
Supplementary Figure S5. Human monocytic cell lines THP-1 and U937 stably expressing human Siglec-15 were generated using the retroviral system. Expression of Siglec-15 on these cell lines was analysed by using two mouse monoclonal antibodies (clones 25E9 and A9E8) against Siglec-15 followed by anti-mouse IgG conjugated to PE.

Figure S6



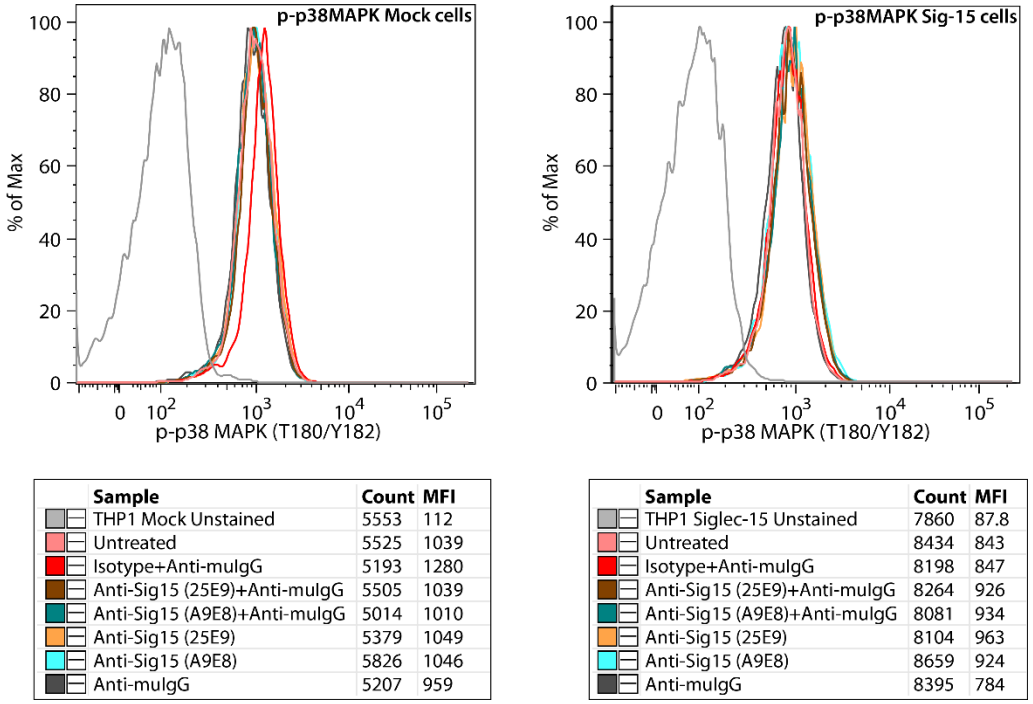
Supplementary Figure S6. THP-1 mock-transfected and Siglec-15-expressing cells, with or without pre-treatment with PD184352 (2 µM) or iSYK/BI1002494 (10 µM), were incubated with mouse anti-human Siglec-15 antibodies (6 µg/ml) (clones 25E9 and A9E8) followed by cross-linking with anti-mouse IgG F(ab')2 secondary antibody (3 µg/ml). Cells were fixed, permeabilised and stained using rabbit anti-ERK pT202/Y204 phosphospecific antibody followed by anti-rabbit IgG $F(ab')_2$-AF647 secondary antibody. Isotype-matched mouse antibody was used as the negative control. Histograms are representative of three independent experiments.

Figure S7



Supplementary Figure S7. THP-1 mock-transfected and Siglec-15-expressing cells, with or without pre-treatment with PD184352 (2 µM) or iSYK/BI1002494 (10 µM), were incubated with anti-Siglec-15 human IgG1 KO antibody (6 µg/ml) (clone 25E09) followed by cross linking with anti-human IgG F(ab')2 secondary antibody (3 µg/ml). Cells were fixed, permeabilised and stained using rabbit anti-ERK pT202/Y204 phosphospecific antibody followed by anti-rabbit IgG $F(ab')_2$-AF647 secondary antibody. Human isotype-matched antibody was used as the negative control. Histograms are representative of three independent experiments.

Figure S8



Sample	Count	MFI
THP1 Mock Unstained	5553	112
Untreated	5525	1039
Isotype+Anti-mulgG	5193	1280
Anti-Sig15 (25E9)+Anti-mulgG	5505	1039
Anti-Sig15 (A9E8)+Anti-mulgG	5014	1010
Anti-Sig15 (25E9)	5379	1049
Anti-Sig15 (A9E8)	5826	1046
Anti-mulgG	5207	959

Sample	Count	MFI
THP1 Siglec-15 Unstained	7860	87.8
Untreated	8434	843
Isotype+Anti-mulgG	8198	847
Anti-Sig15 (25E9)+Anti-mulgG	8264	926
Anti-Sig15 (A9E8)+Anti-mulgG	8081	934
Anti-Sig15 (25E9)	8104	963
Anti-Sig15 (A9E8)	8659	924
Anti-mulgG	8395	784

Supplementary Figure S8. THP1 mock-transfected and Siglec-15-expressing cells were incubated with mouse anti-human Siglec-15 antibodies (6 µg/ml) (clones 25E9 and A9E8) followed by cross linking with anti-mouse IgG F(ab')2 secondary antibody (3 µg/ml). Cells were fixed, permeabilised and stained using rabbit anti-p38 MAPK pT180/Y182 phosphospecific antibody followed by anti-rabbit IgG F(ab')$_2$-AF647 secondary antibody. Isotype-matched mouse IgG was used as the negative control. Histograms are representative of two independent experiments.

Supplementary Table SI. Glycan microarray document based on MIRAGE Glycan Microarray Guidelines (doi:10.3762/mirage.3)

<table>
<tr><th>Classification</th><th>Guidelines</th></tr>
<tr><td colspan="2">1. Sample: Glycan Binding Sample</td></tr>
<tr><td>Description of Sample</td><td>Sample names:
Human Siglec-15 wildtype (hSiglec-15 WT) and Arg134Ala mutant (hSiglec-15 ArgR143Ala) are recombinant and were expressed as Fc-fusion proteins as described in materials methods section in the main text.
Anti-sialyl Tn monoclonal antibody (anti-STn 3F1-IgG) was from SBH Sciences (USA).</td></tr>
<tr><td>Sample modifications</td><td>Not relevant.</td></tr>
<tr><td>Assay protocol</td><td>Assay procedures were according established protocols (Liu et al., Methods Mol Biol. 2012)(Liu et al. 2012). In brief, the arrayed slides were blocked with 2% w/v BSA in 100 mM Phosphate Buffer Saline pH 7.4 (2%BSA/PBS). The Siglec-15 chimeras were analysed as multimers at 2 µg/ml, prepared by pre-complexing with the biotinylated goat anti-human IgG (VECTOR, BA300) at 1:1 ratio (by weight). The Siglec-15-antibody complex was prepared by pre-incubating the protein and antibody, diluted in the final required volume of blocking buffer, for 1h at 4ºC. The anti-sialyl Tn antibody 3F1 was analysed at 50µg/ml prepared in binding buffer (1% BSA/PBS), followed by incubation (1h) with 10 µg/ml biotinylated anti-mouse IgG (SIGMA, B-7264) .</td></tr>
<tr><td colspan="2">2. Glycan Library</td></tr>
<tr><td>Glycan description for defined glycans</td><td>Sixty amino-terminating glycans (aminoalkyl-, phenyl- or glycine-terminating, or natural serine or threonine terminating amino acids) were used to prepare neoglycolipids (NGLs). These are listed and referenced below, together with 22 reducing oligosaccharides or glycosylceramides used as reference glycans.
<table>
<tr><th>Probe Designation</th><th>Pos.[a]</th><th>Sequence[b]</th><th>Glycan sources</th></tr>
<tr><td colspan="4">Amino-terminating glycans</td></tr>
<tr><td>Lac-C2-N</td><td>1</td><td>Galβ-4Glcβ-O-C2-NH2</td><td>W Chai and colleagues (submitted)</td></tr>
<tr><td>2'FL-Gly</td><td>2</td><td>Fucα-2Galβ-4Glcβ-NH-Gly</td><td>Human milk (Likhosherstov et al. 2012)</td></tr>
<tr><td>B-tri-C3-N</td><td>3</td><td>Galα-3Galβ-O-C3-NH2
|
Fucα-2</td><td>Elicityl</td></tr>
<tr><td>3'SA-Lac-C2-N</td><td>4</td><td>NeuAcα-3Galβ-4Glcβ-O-C2-NH2</td><td>Elicityl</td></tr>
<tr><td>3'SA-Lac-Gly</td><td>5</td><td>NeuAcα-3Galβ-4Glcβ-NH-Gly</td><td>Human milk (Likhosherstov et al. 2016)</td></tr>
<tr><td>6'SA-Lac-Gly</td><td>6</td><td>NeuAcα-6Galβ-4Glcβ-NH-Gly</td><td>Human milk (Likhosherstov et al. 2016)</td></tr>
<tr><td>LNFP I-Gly</td><td>7</td><td>Fucα-2Galβ-3GlcNAcβ-3Galβ-4Glcβ-NH-Gly</td><td>Human milk (Likhosherstov et al. 2012)</td></tr>
<tr><td>LSTb-Gly</td><td>8</td><td>Galβ-3GlcNAcβ-3Galβ-4Glcβ-NH-Gly
|
NeuAcα-6</td><td>Human milk (Likhosherstov et al. 2016)</td></tr>
<tr><td>DSLNT-Gly</td><td>9</td><td>NeuAcα-3Galß-3GlcNAcß-3Galß-4Glcß-NH-Gly
|
NeuAcα-6</td><td>Human milk (Likhosherstov et al. 2016)</td></tr>
<tr><td>DSMFLNH-Gly</td><td>10</td><td>Galß-4GlcNAcß-6
|
Fucα-3 Galß-4Glcβ-NH-Gly
|
NeuAcα-3Galß-3GlcNAcß-3
|
NeuAcα-6</td><td>Human milk (Likhosherstov et al. 2016)</td></tr>
<tr><td>ChitoBiose-Gly</td><td>11</td><td>GlcNAcβ-4GlcNAcβ-NH-Gly</td><td>Chitin (Nishimura et al. 1989)</td></tr>
<tr><td>Fuc(3)GlcNAc-Gly</td><td>12</td><td>GlcNAcβ-NH-Gly
|
Fucα-3</td><td>Chemical synthesis (Likhosherstov et al. 2015)</td></tr>
</table>
</td></tr>
</table>

		Fuc(6)GlcNAc-Gly	13	Fucα-6GlcNAcβ-NH-Gly	Chemical synthesis (Likhosherstov et al. 2015)
		DFuc(3,6)GlcNAc-Gly	14	Fucα-6GlcNAcβ-NH-Gly | Fucα-3	Chemical synthesis (Likhosherstov et al. 2015)
		Man5-Gly	15	Manα-6 | Manα-3Manα-6 | Manβ-4GlcNAcβ-4GlcNAcβ-NH-Gly | Manα-3	Chicken egg white (Piskarev et al. 1990)
		Man6-Gly	16	Manα-6 | Manα-3Manα-6 | Manβ-4GlcNAcβ-4GlcNAcβ-NH-Gly | Manα-2Manα-3	Chicken egg white (Piskarev et al. 1990)
		NA2-Gly	17	Galβ-4GlcNAcβ-2Manα-6 | Manβ-4GlcNAcβ-4GlcNAcβ-NH-Gly | Galβ-4GlcNAcβ-2Manα-3	Chicken egg white (Piskarev et al. 1990)
		NA2F-Gly	18	Galβ-4GlcNAcβ-2Manα-6 Fucα-6 | | Manβ-4GlcNAcβ-4GlcNAcβ-NH-Gly | Galβ-4GlcNAcβ-2Manα-3	Chicken egg white (Piskarev et al. 1990)
		SM1a-C3-N	19	Galβ-3GalNAcβ-4Galβ-4Glcβ-O-C3-NH2 | SU-3	W Chai and colleagues (submitted)
		SM1a(2S)-C3-N	20	Galβ-3GalNAcβ-4Galβ-4Glcβ-O-C3-NH2 | SU-2	W Chai and colleagues (submitted)
		GalNAcα-ON	21	GalNAcα-O-NH2	Elicityl
		GalNAcα-Ser	22	GalNAcα-O-Ser	Sussex Research
		GalNAcα-Thr	23	GalNAcα-O-Thr	Dextra Lab
		GalNAcβ-Ser	24	GalNAcβ-O-Ser	W Chai and colleagues (submitted)
		GalNAcβ-Thr	25	GalNAcβ-O-Thr	W Chai and colleagues (submitted)
		GlcNAcβ-Ser	26	GlcNAcβ-O-Ser	W Chai and colleagues (submitted)
		GlcNAcβ-Thr	27	GlcNAcβ-O-Thr	W Chai and colleagues (submitted)
		STn-Ser	28	NeuAcα-6GalNAcα-O-Ser	W Chai and colleagues (submitted)
		STn-Thr	29	NeuAcα-6GalNAcα-O-Thr	W Chai and colleagues (submitted)
		Core 1-Ser	30	Galβ-3GalNAcα-O-Ser	Dextra Lab
		Core 1-Thr	31	Galβ-3GalNAcα-O-Thr	Sussex Research
		SA1(2-3)-Core 1-Ser	32	NeuAcα-3Galβ-3GalNAcα-O-Ser	W Chai and colleagues (submitted)
		SA1(2-3)-Core 1-Thr	33	NeuAcα-3Galβ-3GalNAcα-O-Thr	W Chai and colleagues (submitted)
		SA1(2-6)-Core 1-Ser	34	Galβ-3GalNAcα-O-Ser | NeuAcα-6	Human urine (Parkkinen and Finne 1983)
		SA1(2-6)-Core 1-Thr	35	Galβ-3GalNAcα-O-Thr | NeuAcα-6	Human urine (Parkkinen and Finne 1983)
		SA2-Core 1-Ser	36	NeuAcα-3Galβ-3GalNAcα-O-Ser | NeuAcα-6	Human urine (Parkkinen and Finne 1983)
		SA2-Core 1-Thr	37	NeuAcα-3Galβ-3GalNAcα-O-Thr | NeuAcα-6	Human urine (Parkkinen and Finne 1983)
		Core 2-C3-N	38	Galβ-3GalNAcα-O-C3-NH2 | GlcNAcβ-6	Nicolai Bovin
		Core 2-Thr	39	Galβ-3GalNAcα-O-Thr | GlcNAcβ-6	Sussex Research
		Core 3-Ser	40	GlcNAcβ-3GalNAcα-O-Ser	Sussex Research
		Core 3-Thr	41	GlcNAcβ-3GalNAcα-O-Thr	Sussex Research
		Gal-Core 3-Ser	42	Galβ-4GlcNAcβ-3GalNAcα-O-Ser	W Chai and colleagues (submitted)

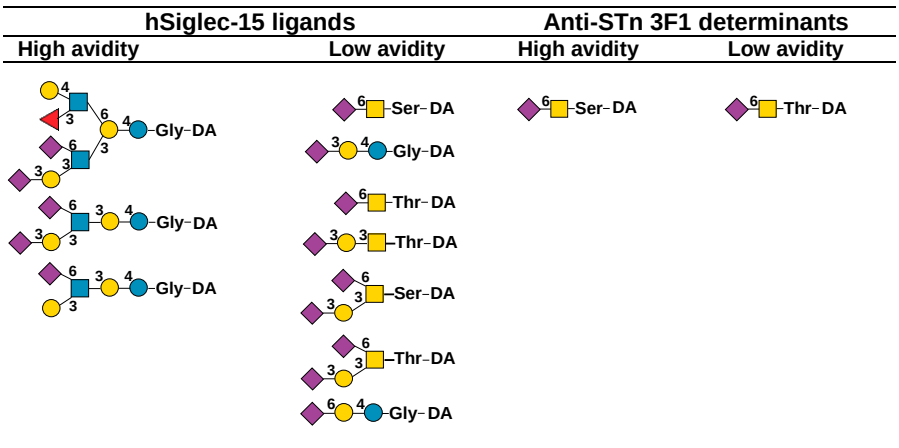
		Gal(3S)-Core 3-Ser	43	Gal(3S)β-4GlcNAcβ-3GalNAcα-O-Ser	W Chai and colleagues (submitted)
		GalNAc-G(3S)-Core 3-Ser	44	GalNAcβ-4Gal(3S)β-4GlcNAcβ-3GalNAcα-O-Ser	W Chai and colleagues (submitted)
		GSC 967-Ser	45	Galβ-3GalNAcβ-4Gal(3S)β-4GlcNAcβ-3GalNAcα-O-Ser	W Chai and colleagues (submitted)
		Core 4-C3-N	46	GlcNAcβ-3GalNAcα-O-C3-NH2 | GlcNAcβ-6	Nicolai Bovin
		Core 4-Thr	47	GlcNAcβ-3GalNAcα-O-Thr | GlcNAcβ-6	Sussex Research
		Man-PentaPep	48	YAT(Man)AV	Chemical synthesis (Bartels et al. 2016)
		Man-HexaPep	49	CYAT(Man)AV-NMe	Chemical synthesis (Bartels et al. 2016)
		Man-UndecaPep	50	Sp-TriEG-SQSLEET(Man)ISPR	Chemical synthesis (Bartels et al. 2016)
		Hep-4-NAc-PhN	51	GlcNAcα-4GlcAβ-4GlcNAcα-4GlcAβ-O-Ph-NH2	Chemo-enzymatic synthesis (Jian Liu)
		Hep-4-NS-PhN	52	GlcNSα-4GlcAβ-4GlcNSα-4GlcAβ-O-Ph-NH2	Chemo-enzymatic synthesis (Jian Liu)
		Glc12-C5-N	53	Glcβ-3Glcβ-3Glcβ-3Glcβ-[3Glcβ-3Glcβ]$_3$-3Glcβ-3Glcβ-O-C5-NH2	Chemical synthesis (Weishaupt et al. 2013, 2017)
		Glc15-C2-N	54	Glcβ-3Glcβ-3Glcβ-3Glcβ-[3Glcβ-3Glcβ]$_3$-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-O-C2-NH2	Chemical synthesis Novartis
		Glc13(B10)-C5-N	55	Glcβ-3Glcβ-3Glcβ-3Glcβ-[3Glcβ-3Glcβ]$_3$-3Glcβ-3Glcβ-O-C5-NH2 | Glcβ-6	Chemical synthesis (Weishaupt et al. 2013, 2017)
		Gal-C2-N	56	Galβ-O-C2-NH2	W Chai and colleagues (submitted)
		Gal-Ph-N	57	Galβ-O-Ph-NH2	W Chai and colleagues (submitted)
		Glc-C2-N	58	Glcβ-O-C2-NH2	W Chai and colleagues (submitted)
		Man-C2-N	59	Manβ-O-C2-NH2	W Chai and colleagues (submitted)
		Xyl-C2-N	60	Xylβ-O-C2-NH2	W Chai and colleagues (submitted)
		Reducing glycans and glycosylceramides			
		Lac	61	Galβ-4Glc	(Palma et al. 2011; Vendele et al. 2020)
		B-Tetra-T2	62	Galα-3Galβ-4GlcNAc | Fucα-2	W Chai and colleagues (submitted)
		NeuAcα-(3')Lac	63	NeuAcα-3Galβ-4Glc	(Palma et al. 2011; Vendele et al. 2020)
		LNFP-I	64	Fucα-2Galβ-3GlcNAcβ-3Galβ-4Glc	(Palma et al. 2011; Vendele et al. 2020)
		LNFP-III	65	Galβ-4GlcNAcβ-3Galβ-4Glc | Fucα-3	(Palma et al. 2011; Vendele et al. 2020)
		LSTb	66	Galβ-3GlcNAcβ-3Galβ-4Glc | NeuAcα-6	(Palma et al. 2011; Vendele et al. 2020)
		H2 (with H2+Fuc)	67	Fucα-2Galβ-4GlcNAcβ-3Galβ-4GlcNAcβ-3Galβ-4Glc(β-Cer)*	(Gao et al. 2014)
		TFiLNO	68	Galβ-3GlcNAcβ-3Galβ-4GlcNAcβ-6 | | | Fucα-4 Fucα-3 Galβ-4Glc | Galβ-3GlcNAcβ-3 | Fucα-4	(Palma et al. 2011; Vendele et al. 2020)
		SM1a	69	Galβ-3GalNAcβ-4Galβ-4Glc(β-Cer) | SU-3	(Palma et al. 2011; Vendele et al. 2020)
		Asialo-GM1-Tetra	70	Galβ-3GalNAcβ-4Galβ-4Glc	(Palma et al. 2011; Vendele et al. 2020)
		SB1a	71	SU-3Galβ-3GalNAcβ-4Galβ-4Glc(β-Cer) | SU-3	(Palma et al. 2011; Vendele et al. 2020)
		GalNAc-Ser	72	GalNAcα-Ser	(Palma et al. 2011; Vendele et al. 2020)
		GalNAc-Thr	73	GalNAcα-Thr	(Palma et al. 2011; Vendele et al. 2020)
		BSM-Di-A1	74	NeuGcα-6GalNAc	(Chai et al. 1992)
		BSM-Di-A2	75	NeuAcα-6GalNAc	(Chai et al. 1992)
		Galβ-3GalNAc	76	Galβ-3GalNAc	(Palma et al. 2011; Vendele et al. 2020)
		Galβ-6GalNAc	77	Galβ-6GalNAc	(Palma et al. 2011; Vendele et al. 2020)

DST	78	NeuAcα-3Galβ-3GalNAc | NeuAcα-6	(Palma et al. 2011; Vendele et al. 2020)
Curd-13	79	Glcβ-3Glcβ-3Glcβ-3Glcβ-[3Glcβ-3Glcβ]$_3$-3Glcβ-3Glcβ-3Glc*	(Palma et al. 2015)
Cello-3	80	Glcβ-4Glcβ-4Glc	(Palma et al. 2015)
GN2	81	GlcNAcβ-4GlcNAc	(Palma et al. 2011; Vendele et al. 2020)
GN6	82	GlcNAcβ-4GlcNAcβ-4GlcNAcβ-4GlcNAcβ-4GlcNAcβ-4GlcNAc*	(Vendele et al. 2020)

[a] Pos, position in the microarray with glycans sorted according to backbone type.
[b] Abbreviations of reducing terminal structures: Gly, glycine; Ph, phenyl; Ser, serine; Thr, threonine; C2, -CH_2-CH_2-; C3, -CH_2-CH_2-CH_2-; C5, -CH_2-$(CH_2)_3$-CH_2-.
*Asterisks indicate that major components are depicted.

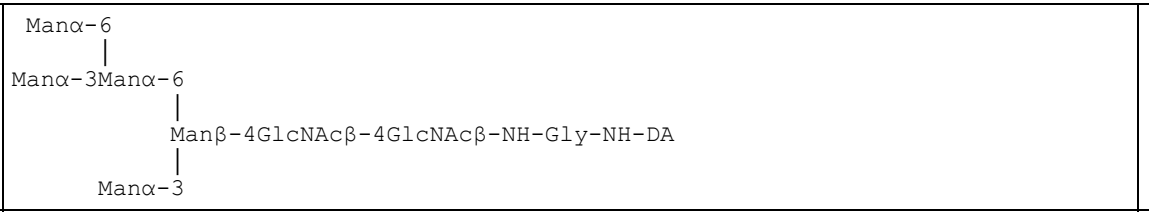
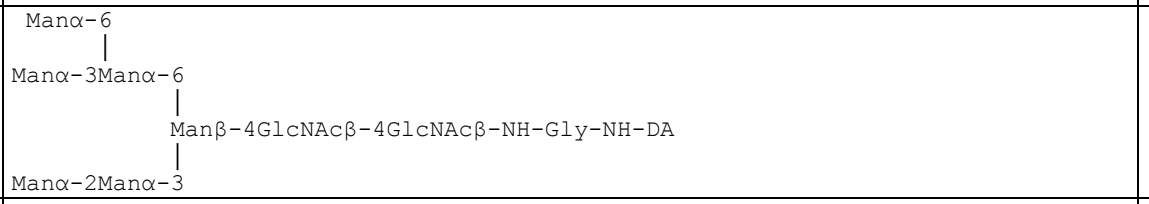
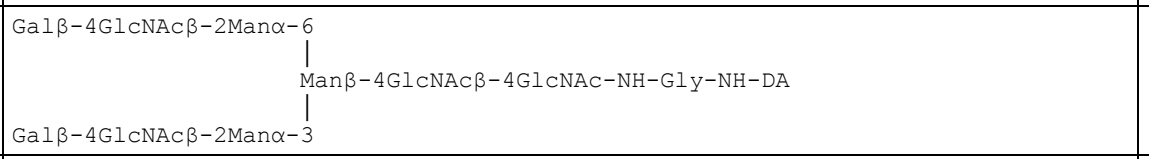
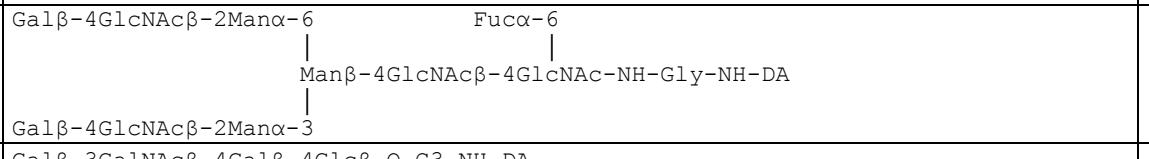
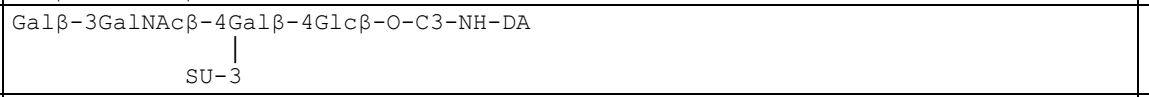
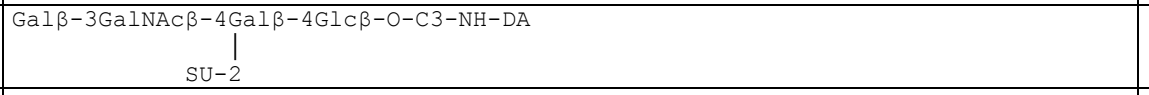
Glycan description for undefined glycans	Not applicable.
Glycan modifications	The amino-terminating glycans were conjugated to a new aldehyde-functionalized phospholipid reagent *N*-(4-formylbenzamide)-1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine, abbreviated to DA (W Chai and colleagues, submitted). The NGLs were designated DA-NGLs. DH, NGLs, prepared from reducing oligosaccharides by reductive amination with the amino lipid, 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine [(DHPE) (Chai et al., Methods Enzymol. 2003)](Chai et al. 2003); AO, NGLs prepared from reducing oligosaccharides by oxime ligation with an aminooxy functionalized DHPE [(AOPE) (Liu et al., Chem. Biol. 2007)](Liu et al. 2007).
3. Printing Surface; e.g., Microarray Slide	
Description of surface	Nitrocellulose-coated glass microarray slides.
Manufacturer	16-pad UniSart® 3D Microarray Slide from Sartorius (Goettingen, Germany).
Custom preparation of surface	Not relevant.
Non-covalent Immobilization	The lipid-linked oligosaccharide probes were formulated as liposomes by adding carrier lipids, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) and cholesterol for arraying and non-covalent immobilization on nitrocellulose-coated glass slides (Liu et al., Methods Mol. Biol. 2012)(Liu et al. 2012).
4. Arrayer (Printer)	
Description of Arrayer	Nano-Plotter 2.1 (GeSiM, Radeberg, Germany).
Dispensing mechanism	Non-contact liquid delivery with four dispensing tips.
Glycan deposition	Approximately 0.33 nl was printed per spot. Each glycan probe was printed at 2 levels (2 and 5 fmol per spot), in duplicate.
Printing conditions	The printing solutions were aqueous-based. Printing was performed at ambient temperature and relative humidity of 58%. The NGL printing solutions contained 100 pmol/µl of DHPC and cholesterol (both from SIGMA) as lipid carriers in addition to the lipid-linked glycan probes. The concentrations of the NGL probes were 5 and 15 pmol/µl for the 2 and 5 fmol per spot levels, respectively. The printing solutions also contained Cy3 NHS ester (GE Healthcare) at 20 ng/ml (26 fmol/µl) as a marker to monitor the printing process.
5. Glycan Microarray with "Map"	

Array layout	Each array slide contained 16-pad subarrays. Each pad was set up for printing 64 probes maximum, each at 2 levels in duplicate (four spots for one probe in a row); up to 256 spots (16x16) in total for each subarray. The probes were printed on multiple subarrays for parallel binding analyses.
Glycan identification and quality control	Quality control was performed with sequence-specific proteins: biotinylated plant lectins - Wheat Germ Agglutinin (WGA), *Vicia Villosa* Lectin (VVL), Peanut agglutinin (PNA), *Helix pomatia* lectin (HPA), *Ricinus Communis* agglutinin I (RCA_{120}), Concanavalin A (ConA) and *Aleuria aurantia* lectin (AAL) (Vector Laboratories); Fc-tagged-MGL (CLEC10A) (Abcam); his-tagged carbohydrate-binding modules of bacterial glycoside hydrolases (*Cm*CBM6-2, *Tm*CBM4-2); CTD110.6 – anti-O-GlcNAc (Santa Cruz) and anti-blood group H monoclonal antibodies (17-206, BRIC231) (Abcam) (W Chai and colleagues, submitted).
6. Detector and Data Processing	
Scanning hardware	GenePix 4300A (Molecular Devices)
Scanner settings	Scanning resolution: 10 µm / pixel; Laser channel: red (scan wavelength 635nm); PMT Voltages: 350 Scan power: Adjusted for each sample to achieve maximum signal without saturation of any single spot.
Image analysis software	GenePix® Pro 7 (Molecular Devices)
Data processing	The gpr file was entered into an in-house microarray database using software (designed by Mark Stoll, http://www.beilstein-institut.de/en/publications/proceedings/glyco-2009) for data processing. No particular normalization method or statistical analysis was used.
7. Glycan Microarray Data Presentation	
Data presentation	In Figure 4, the binding results are presented as 2D bar graphs with bars representing averaged mean signal of duplicate spots at 5 fmol/spot and error bars representing standard deviation. The fluorescence intensity numerical scores are depicted in **Supplementary Table S2**.
8. Interpretation and Conclusion from Microarray Data	
Data interpretation	No software or algorithms were used to interpret processed data.
Conclusions	Human Siglec-15 is shown to bind to sialylated glycans apart from sTn. Mutation of the conserved Arg143 that is critical for interaction with sialic acid to Ala markedly reduced but not abolished the binding. The binding of hSiglec-15 contrasts with the restricted binding observed with the 3F1-IgG antibody to the sTn antigen.



Supplementary Table SII. List of the lipid-linked glycan probes included in microarrays and the fluorescence binding intensities elicited with human Siglec-15 proteins and 3F1 antibody.

			Fluorescence intensities[3] (5fmol/spot)		
Probe name[1]	**Pos.**	**Probe Sequence[2]**	**hSiglec-15 WT**	**hSiglec-15 Arg143Ala**	**Anti-STn 3F1-IgG**
DA-NGLs					
Lac-C2-N-	1	Galβ-4Glcβ-O-C2-NX-DA	-	-	-
2'FL-Gly-	2	Fucα-2Galβ-4Glc-NH-Gly-NH-DA	-	-	-
B-tri-C3-N-	3	Galα-3Galβ-O-C3-NX-DA Fucα-2	-	-	-
3'SA-Lac-C2-N-	4	NeuAcα-3Galβ-4Glcβ-O-C3-NX-DA	-	-	-
3'SA-Lac-Gly-	5	NeuAcα-3Galβ-4Glcβ-NH-Gly-NH-DA	1 451	-	-
6'SA-Lac-Gly-	6	NeuAcα-6Galβ-4Glcβ-NH-Gly-NH-DA	451	-	-
LNFP I-Gly-	7	Fucα-2Galβ-3GlcNAcβ-3Galβ-4Glc-NH-Gly-NH-DA	-	-	-
LSTb-Gly-	8	Galβ-3GlcNAcβ-3Galβ-4Glcβ-NH-Gly-NH-DA NeuAcα-6	4 766	-	-
DSLNT-Gly-	9	NeuAcα-3Galß-3GlcNAcß-3Galß-4Glcß-NH-Gly-NH-DA NeuAcα-6	4 597	-	-
DSMFLNH-Gly-	10	Galß-4GlcNAcß-6 Fucα-3 Galß-4Glcβ-NH-Gly-NH-DA NeuAcα-3Galß-3GlcNAcß-3 NeuAcα-6	6 347	759	-
ChitoBiose-Gly-	11	GlcNAcβ-4GlcNAcβ-NH-Gly-NH-DA	-	-	-
Fuc(3)GlcNAc-Gly-	12	GlcNAcβ-NH-Gly-NH-DA Fucα-3	-	-	-
Fuc(6)GlcNAc-Gly-	13	Fucα-6GlcNAcβ-NH-Gly-NH-DA	-	-	-
DFuc(3,6)GlcNAc-Gly-	14	Fucα-6GlcNAcβ-NH-Gly-NH-DA Fucα-3	-	-	-

Man5-Gly-	15		-	-	-
Man6-Gly-	16		-	-	-
NA2-Gly-	17		-	-	-
NA2F-Gly-	18		-	-	-
SM1a-C3-N-	19		-	-	-
SM1a(2S)-C3-N-	20		-	-	-
GalNAcα-ON-	21	GalNAcα-O-N=DA	-	-	-
GalNAcα-Ser-	22	GalNAcα-O-Ser-NH-DA	-	-	-
GalNAcα-Thr-	23	GalNAcα-O-Thr-NH-DA	-	-	-
GalNAcβ-Ser-	24	GalNAcβ-O-Ser-NH-DA	-	-	-
GalNAcβ-Thr-	25	GalNAcβ-O-Thr-NH-DA	-	-	-
GlcNAcβ-Ser-	26	GlcNAcβ-O-Ser-NH-DA	-	-	-
GlcNAcβ-Thr-	27	GlcNAcβ-O-Thr-NH-DA	-	-	-
STn-Ser-	28	NeuAcα-6GalNAcα-O-Ser-NH-DA	1 687	410	8 226
STn-Thr-	29	NeuAcα-6GalNAcα-O-Thr-NH-DA	543	-	789
Core 1-Ser-	30	Galβ-3GalNAcα-O-Ser-NH-DA	-	-	-

Core 1-Thr-	31	Galβ-3GalNAcα-O-Thr-NH-DA	-	-	-
SA1(2-3)-Core 1-Ser-	32	NeuAcα-3Galβ-3GalNAcα-O-Ser-NH-DA	-	-	-
SA1(2-3)-Core 1-Thr-	33	NeuAcα-3Galβ-3GalNAcα-O-Thr-NH-DA	852	-	-
SA1(2-6)-Core 1-Ser-	34	Galβ-3GalNAcα-O-Ser-NH-DA | NeuAcα-6	-	-	-
SA1(2-6)-Core 1-Thr-	35	Galβ-3GalNAcα-O-Thr-NH-DA | NeuAcα-6	224	-	-
SA2-Core 1-Ser-	36	NeuAcα-3Galβ-3GalNAcα-O-Ser-NH-DA | NeuAcα-6	748	-	-
SA2-Core 1-Thr-	37	NeuAcα-3Galβ-3GalNAcα-O-Thr-NH-DA | NeuAcα-6	436	-	-
Core 2-C3-N-	38	Galβ-3GalNAcα-O-C3-NH-DA | GlcNAcβ-6	-	-	-
Core 2-Thr-	39	Galβ-3GalNAcα-O-Thr-NH-DA | GlcNAcβ-6	40	-	-
Core 3-Ser-	40	GlcNAcβ-3GalNAcα-O-Ser-NH-DA	-	-	-
Core 3-Thr-	41	GlcNAcβ-3GalNAcα-O-Thr-NH-DA	1 450	549	-
Gal-Core 3-Ser-	42	Galβ-4GlcNAcβ-3GalNAcα-O-Ser-NH-DA	40	-	-
Gal(3S)-Core 3-Ser-	43	Gal(3S)β-4GlcNAcβ-3GalNAcα-O-Ser-NH-DA	151	16	-
GalNAc-Gal(3S)-Core 3-Ser-	44	GalNAcβ-4Gal(3S)β-4GlcNAcβ-3GalNAcα-O-Ser-NH-DA	-	-	-
GSC 967-Ser-	45	Galβ-3GalNAcβ-4Gal(3S)β-4GlcNAcβ-3GalNAcα-O-Ser-NH-DA	-	-	-
Core 4-C3-N-	46	GlcNAcβ-3GalNAcα-O-C3-NH-DA | GlcNAcβ-6	-	-	-
Core 4-Thr-	47	GlcNAcβ-3GalNAcα-O-Thr-NH-DA | GlcNAcβ-6	-	-	-
Man-PentaPep-	48	YAT(Man)AV-NH-DA[4]	-	-	-
Man-HexaPep-	49	CYAT(Man)AV-NMe-DA[4]	477	-	-
Man-UndecaPep-	50	Sp-TriEG-SQSLEET(Man)ISPR-NH-DA[3]	-	-	-

Hep-4-NAc-PhN-	51	GlcNAcα-4GlcAβ-4GlcNAcα-4GlcAβ1-O-Ph-NH-DA	1 106	-	-
Hep-4-NS-PhN-	52	GlcNSα-4GlcAβ-4GlcNSα-4GlcAβ1-O-Ph-NH-DA	904	-	-
Glc12-C5-N-	53	Glcβ-3Glcβ-3Glcβ-3Glcβ-[3Glcβ-3Glcβ]$_3$-3Glcβ-3Glcβ-O-C5-NH-DA	-	-	-
Glc15-C2-N-	54	Glcβ-3Glcβ-3Glcβ-3Glcβ-[3Glcβ-3Glcβ]$_3$-3Glcβ-3Glcβ-3Glcβ-3Glcβ-3Glcβ-O-C2-NH-DA	-	-	-
Glc13(B10)-	55	Glcβ-3Glcβ-3Glcβ-3Glcβ-[3Glcβ-3Glcβ]$_3$-3Glcβ-3Glcβ-O-C5-NH-DA Glcβ-6	-	-	-
Gal-C2-N-	56	Galβ-O-C2-NX-DA	-	-	-
Gal-Ph-N-	57	Galβ-O-Ph-NH-DA	-	-	-
Glc-C2-N-	58	Glcβ-O-C2-NX-DA	-	-	-
Man-C2-N-	59	Manβ-O-C2-NH-DA	-	-	-
Xyl-C2-N-	60	Xylβ-O-C2-NX-DA	-	-	-
Cer, AO- and DH-NGLs					
Lac-	61	Galβ-4Glc-AO	-	-	-
B-Tetra-T2-	62	Galα-3Galβ-4GlcNAc-AO Fucα-2	-	-	-
NeuAcα-(3')Lac-	63	NeuAcα-3Galβ-4Glc-AO	4 711	-	-
LNFP-I-	64	Fucα-2Galβ-3GlcNAcβ-3Galβ-4Glc-AO	-	-	-
LNFP-III-	65	Galβ-4GlcNAcβ-3Galβ-4Glc-DH Fucα-3	-	-	-
LSTb-	66	Galβ-3GlcNAcβ-3Galβ-4Glc-DH NeuAcα-6	5 610	-	-
H2 (with H2+Fuc)-	67	Fucα-2Galβ-4GlcNAcβ-3Galβ-4GlcNAcβ-3Galβ-4Glcβ-Cer*	-	-	-
TFiLNO-	68	Galβ-3GlcNAcβ-3Galβ-4GlcNAcβ-6 Fucα-4 Fucα-3 Galβ-4Glc-DH Galβ-3GlcNAcβ-3 Fucα-4	-	-	-
Asialo-GM1-Tetra-	69	Galβ-3GalNAcβ-4Galβ-4Glc-DH	-	-	-
SM1a-	70	Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer SU-3	-	-	-

SB1a-	71	SU-3Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer | SU-3	-	-	-
GalNAc-Ser-	72	GalNAcα-Ser-DH	-	-	-
GalNAc-Thr-	73	GalNAcα-Thr-DH	-	-	-
BSM-Di-A1-	74	NeuGcα-6GalNAc-AO	633	-	-
BSM-Di-A2-	75	NeuAcα-6GalNAc-AO	3 112	-	-
Galβ-3GalNAc-	76	Galβ-3GalNAc-AO	-	-	-
Galβ-6GalNAc-	77	Galβ-6GalNAc-AO	-	-	-
DST-	78	NeuAcα-3Galβ-3GalNAc-AO | NeuAcα-6	3 889	-	-
Curd-13-	79	Glcβ-3Glcβ-3Glcβ-3Glcβ-[3Glcβ-3Glcβ]$_3$-3Glcβ-3Glcβ-3Glc-AO*	-	-	-
Cello-3-	80	Glcβ-4Glcβ-4Glc-AO	-	-	-
GN2-	81	GlcNAcβ-4GlcNAc-AO	-	-	-
GN6-	82	GlcNAcβ-4GlcNAcβ-4GlcNAcβ-4GlcNAcβ-4GlcNAcβ-4GlcNAc-AO*	-	-	-

[1]The oligosaccharide probes are all lipid-linked, neoglycolipids (NGLs) or glycosylceramides and are sorted by glycan backbone type referred to a position as in the binding charts in **Figure 4**.

[2]Abbreviations are as follows: DA, NGLs prepared using a new aldehyde-terminating lipid reagent to conjugate with amino-terminating glycans; NX, NH or NMe; C2, -CH_2-CH_2-; C3, -CH_2-CH_2-CH_2-; C5, -CH_2-CH_2-CH_2-CH_2-CH_2-; Gly, glycine; Ser, serine; Thr, threonine; Ph, phenyl; Gly, glycine. For the control probes: DH, NGLs prepared from reducing oligosaccharides by reductive amination with the amino lipid, 1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine (DHPE)(Chai et al. 2003); AO, NGLs prepared from reducing oligosaccharides by oxime ligation with an aminooxy (AO) functionalized DHPE(Liu et al. 2007); Cer, natural glycolipids with various ceramide moieties.

[3]Means of fluorescence intensities of duplicate spots printed at the high level of probe arrayed (5fmol/spot); –, less than 1.

[4]*O*-mannosyl glycopeptide.

*Asterisks indicate that major components are depicted.

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