

Proteome Analysis

NanoLC-MS/MS—All analyses were performed on nanoLC-LTQ-Orbitrap mass spectrometers (Thermo, San Jose, CA) at a resolution of 60,000. For nanoLC, Eksigent nalo LC 1D plus systems were equipped with 3 mm ReproSil-Pur C18-AQ ((Dr. Maisch GmbH, Ammerbuch) trapping columns (packed in-house, i.d., 150 μ m; resin, 5 μ m) and 200 mm ReproSil-Pur C18-AQ (Dr. Maisch GmbH, Ammerbuch) analytical columns (packed in-house, i.d., 75 μ m; resin, 3 μ m). Solvents used were 0.5% formic acid water solution (buffer A) and 0.5 % formic acid acetonitrile (ACN) solution (buffer B). Trapping was performed at 2 μ l/min buffer A for 15 min, and elution was achieved with a gradient of 0–32% B in 80 min, 32–50% B in 6 min, 80% B for 6 min at a flow rate of 300 nL/min. Eluting peptide cations were converted to gas-phase ions by Nanospray Flex ion source with 1.8 kV . The mass spectrometer was operated in the data-dependent mode to automatically switch between MS and MS/MS. Survey full scan MS spectra were acquired from m/z 300 to m/z 1800, and the 10 most intense ions with charge state above 2 and above an intensity threshold 500 were fragmented in the linear ion trap using with normalized collision energy of 35%. For the Orbitrap, the AGC target value was set 1e6 and a maximum fill time for full MS was set 500 ms. Fragment ion spectra were acquired in the LTQ with AGC target value of 3e4 and a maximum fill-time of 150 ms. Dynamic exclusion for selected precursor ions was set at 120 s. lock mass option was enabled for the 462.14658 ion.

Table S1. Transcripts with differential expression (p value < 0.05) in HS-27a and in HS-5

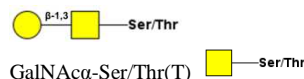


Number	symbol	gene accession	decription	p-value	fold-change
1	ACVRL1	NM_000020	activin A receptor type II-like 1	0.0232	1.6303
2	ALG5	NM_013338	dolichyl-phosphate beta-glucosyltransferase	0.0105	2.0100
3	ALG6	NM_013339	alpha-1,3-glucosyltransferase	0.0094	1.5451
4	ANGPT2	NM_001147	angiotensinogen 2	0.0000	2.1820
5	ARF1	NM_001024227	ADP-ribosylation factor 1	0.0007	0.5396
6	B3GAT3	NM_012200	beta-1,3-glucuronyltransferase 3	0.0131	1.6762
7	B4GALT1	NM_001497	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1	0.0005	2.7230
8	B4GALT3	NM_001199873	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 3	0.0316	0.7651
9	BDNF	NM_170735	brain-derived neurotrophic factor	0.0411	2.1718
10	BMP1	NM_001199	bone morphogenetic protein 1	0.0123	1.9679
11	BMP2	NM_001200	bone morphogenetic protein 2	0.0454	1.4188
12	BMP4	NM_001202	bone morphogenetic protein 4	0.0105	5.6399
13	BMPR2	NM_001204	bone morphogenetic protein receptor, type II (serine/threonine kinase)	0.0049	0.5928
14	CD164	NM_006016	CD164 molecule, sialomucin	0.0021	0.5161
15	CD83	NM_004233	CD83 molecule	0.0111	0.4551
16	CLU	NM_001831	clusterin	0.0037	1.8556
17	CSPG4	NM_001897	chondroitin sulfate proteoglycan 4	0.0002	2.8573
18	CTBS	NM_004388	chitinase, di-N-acetyl-	0.0048	1.6431
19	CTNS	NM_001031681	cystinosin, lysosomal cystine transporter	0.0146	0.6512
20	CX3CR1	NM_001171174	chemokine (C-X3-C motif) receptor 1	0.0481	0.4591
21	CXCR4	NM_001008540	chemokine (C-X-C motif) receptor 4	0.0488	0.4213
22	DDB1	NM_001923	damage-specific DNA binding protein 1, 127kDa	0.0031	0.7772
23	DDOST	NM_005216	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit (non-catalytic)	0.0250	1.4168
24	DDX5	NM_004396	DEAD (Asp-Glu-Ala-Asp) box helicase 5	0.0093	0.7302
25	DGCR2	NM_005137	DiGeorge syndrome critical region gene 2	0.0027	1.6079
26	DPAGT1	NM_001382	dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminophosphotransferase 1 (GlcNAc-1-P transferase)	0.0018	1.5650
27	EEF2	NM_001961	eukaryotic translation elongation factor 2	0.0048	0.5948
28	EIF4G2	NM_001418	eukaryotic translation initiation factor 4 gamma, 2	0.0224	1.4780
29	EXTL3	NM_001440	exostosin-like glycosyltransferase 3	0.0223	0.7791
30	FGF12	NM_021032	fibroblast growth factor 12	0.0444	0.5126
31	FGF2	NM_002006	fibroblast growth factor 2 (basic)	0.0000	0.2209
32	FGF5	NM_004464	fibroblast growth factor 5	0.0334	0.6119
33	FMOD	NM_002023	fibromodulin (FMOD)	0.0006	1.8819
34	FPGT	NM_003838	fucose-1-phosphate guanylyltransferase	0.0017	1.6267




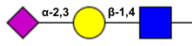
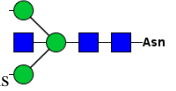


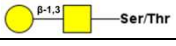
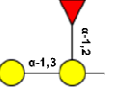

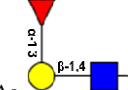
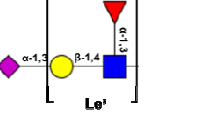


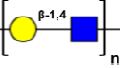


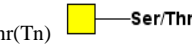
			fucosyltransferase 4 (alpha (1,3)		
35	FUT4	NM_002033	fucosyltransferase, myeloid-specific)	0.0305	0.7669
36	FZD1	NM_003505	frizzled class receptor 1	0.0073	4.4127
37	FZD4	NM_012193	frizzled class receptor 4	0.0001	11.4969
38	FZD6	NM_003506	frizzled class receptor 6	0.0093	1.4289
39	GAA	NM_000152	<u>glucosidase, alpha; acid</u>	0.0208	1.6588
			polypeptide N-acetylgalactosaminyltransferase		
40	GALNT1	NM_020474	1	0.0001	3.5644
			polypeptide N-acetylgalactosaminyltransferase		
41	GALNT3	NM_004482	3	0.0102	2.7903
42	GLA	NM_000169	galactosidase, alpha	0.0156	1.4760
43	GLB1	NM_000404	galactosidase, beta 1	0.0053	1.4922
44	GPC1	NM_002081	glypican 1	0.0113	1.6411
45	GPC3	NM_001164617	glypican 3	0.0000	0.2168
46	GPC4	NM_001448	glypican 4	0.0053	0.3048
47	GUSB	NM_000181	glucuronidase, beta	0.0005	2.6138
48	HDLBP	NM_005336	high density lipoprotein binding protein	0.0022	0.4632
49	HEXA	NM_000520	hexosaminidase A (alpha polypeptide)	0.0170	2.1401
			hepatocyte growth factor (hepapoietin A;		
50	HGF	NM_000601	scatter factor)	0.0050	1.6744
51	HK1	NM_000188	hexokinase 1 (HK1)	0.0053	0.4256
52	HPSE	NM_006665	heparanase (HPSE)	0.0090	3.3304
			heparan sulfate (glucosamine)		
53	HS3ST3B1	NM_006041	3-O-sulfotransferase 3B1	0.0001	0.1354
54	IFITM1	NM_003641	interferon induced transmembrane protein 1	0.0384	2.0131
55	IFITM2	NM_006435	interferon induced transmembrane protein 2	0.0112	2.4741
56	IGF1R	NM_000875	insulin-like growth factor 1 receptor	0.0088	0.5637
57	IGF2R	NM_000876	insulin-like growth factor 2 receptor	0.0286	0.6354
58	IGFBP3	NM_001013398	insulin-like growth factor binding protein 3	0.0001	19.5985
59	IL10RA	NM_001558	interleukin 10 receptor, alpha	0.0046	0.3984
60	IL15	NM_172175	interleukin 15	0.0475	1.5435
61	IL1A	NM_000575	interleukin 1, alpha	0.0022	0.0304
62	IL1B	NM_000576	interleukin 1, beta	0.0000	0.0206
63	IL2RG	NM_000206	interleukin 2 receptor, gamma	0.0468	0.4730
64	IL4R	NM_001257997	interleukin 4 receptor	0.0054	0.5427
65	IL6ST	NM_001190981	interleukin 6 signal transducer	0.0002	0.4061
66	IL7R	NM_002185	interleukin 7 receptor	0.0000	19.4497
67	ILF2	NM_001267809	interleukin enhancer binding factor 2	0.0048	0.5562
68	ILK	NM_001014794	integrin-linked kinase	0.0315	1.3726
69	INHBA	NM_002192	inhibin, beta A	0.0008	0.1031
70	JAG1	NM_000214	jagged 1	0.0006	2.3570
71	LAMP2	NM_001122606	lysosomal-associated membrane protein 2	0.0272	1.5585
72	LEPR	NM_002303	leptin receptor	0.0087	2.0899

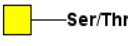

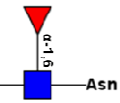
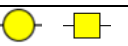

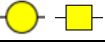
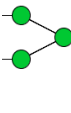



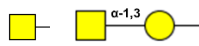

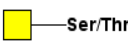
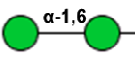

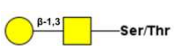


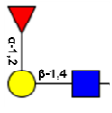


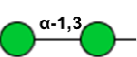
			lectin, galactoside-binding, soluble, 3 binding		
73	LGALS3BP	NM_005567	protein	0.0485	0.6530
74	LGALS7	NM_002307	lectin, galactoside-binding, soluble, 7	0.0064	0.7125
75	LIF	NM_002309	leukemia inhibitory factor	0.0022	0.1186
76	LTA4H	NM_001256643	eukotriene A4 hydrolase	0.0442	1.4304
77	LUM	NM_002345	lumican	0.0166	20.5943
78	MAN1A2	NM_006699	mannosidase, alpha, class 1A, member	0.0003	1.6048
79	MAN1B1	NM_016219	mannosidase, alpha, class 1B, member 1	0.0023	1.7176
80	MAN2A1	NM_002372	mannosidase, alpha, class 2A, member 1	0.0001	0.1816
81	MAN2A2	NM_006122	mannosidase, alpha, class 2A, member 2	0.0419	2.0416
82	MBL2	NM_000242	mannose-binding lectin (protein C) 2, soluble	0.0000	0.2249
83	MET	NM_001127500	proto-oncogene, receptor tyrosine kinase	0.0000	9.3319
			MFNG O-fucosylpeptide		
84	MFNG	NM_002405	3-beta-N-acetylglucosaminyltransferase	0.0282	0.5059
			mannosyl (alpha-1,6-)-glycoprotein		
85	MGAT2	NM_002408	beta-1,2-N-acetylglucosaminyltransferase	0.0001	2.0107
			mannosyl (beta-1,4-)-glycoprotein		
86	MGAT3	NM_002409	beta-1,4-N-acetylglucosaminyltransferase	0.0001	0.2618
			meningioma expressed antigen 5		
87	MGEA5	NM_001142434	(hyaluronidase)	0.0002	0.4656
88	MPI	NM_001289157	mannose phosphate isomerase	0.0070	1.6701
			N-deacetylase/N-sulfotransferase (heparan		
89	NDST2	NM_003635	glucosaminyl) 2	0.0000	0.4643
90	NEU1	NM_000434	sialidase 1	0.0006	2.2314
91	NGFR	NM_002507	nerve growth factor receptor	0.0417	0.6943
92	NONO	NM_001145409	non-POU domain containing, octamer-binding	0.0462	0.8189
93	OAZ1	NM_001301020	ornithine decarboxylase antizyme 1	0.0026	2.1715
			3'-phosphoadenosine 5'-phosphosulfate		
94	PAPSS2	NM_001015880	synthase 2	0.0037	0.6168
			platelet-derived growth factor receptor, alpha		
95	PDGFRA	NM_006206	polypeptide	0.0047	3.4092
			platelet-derived growth factor receptor, beta		
96	PDGFRB	NM_002609	polypeptide	0.0046	6.6757
97	PEG3	NM_006210	paternally expressed 3	0.0060	0.2436
			phosphatidylinositol glycan anchor		
98	PIGC	NM_002642	biosynthesis, class C	0.0006	0.5248
99	PODXL	NM_005397	podocalyxin-like	0.0177	0.4893
100	POMT1	NM_001136114	protein-O-mannosyltransferase 1	0.0068	1.7687
			proteoglycan 2, bone marrow (natural killer		
			cell activator, eosinophil granule major basic		
101	PRG2	NM_001243245	protein)	0.0015	1.9850
102	PRG4	NM_005807	proteoglycan 4	0.0004	0.4626
103	PSEN1	NM_000021	presenilin 1	0.0047	1.3395
104	PSMB2	NM_001199780	proteasome (prosome, macropain) subunit,	0.0015	1.5885

		beta type, 2		
		proteasome (prosome, macropain) subunit,		
105	PSMB3	NM_002795	beta type, 3	0.0280 1.4688
106	PTMA	NM_001099285	prothymosin, alpha	0.0041 0.1217
107	PTN	NM_002825	pleiotrophin	0.0273 1.8884
108	RPL21	NM_000982	ribosomal protein L21	0.0021 2.1900
109	RPL30	NM_000989	ribosomal protein L30	0.0102 1.6485
110	RPN1	NM_002950	ribophorin I	0.0015 1.6606
111	RPS11	NM_001015	ribosomal protein S11	0.0043 0.5843
112	RPS5	NM_001009	ribosomal protein S5	0.0257 1.5233
113	RPS6	NM_001010	ribosomal protein S6	0.0046 0.5787
114	S100A10	NM_002966	S100 calcium binding protein A10	0.0040 2.0152
115	SDC2	NM_002998	syndecan 2	0.0001 3.5068
116	SDC4	NM_002999	syndecan 4	0.0409 1.7705
117	SFRP1	NM_003012	secreted frizzled-related protein 1	0.0068 0.3294
118	SOD1	NM_000454	superoxide dismutase 1, soluble	0.0007 1.7724
119	SPAG7	NM_004890	sperm associated antigen 7	0.0306 0.7187
120	STS	NM_000351	steroid sulfatase (microsomal), isozyme S	0.0000 4.9218
		sulfotransferase family, cytosolic, 1A,		
121	SULT1A3	NM_177552	phenol-preferring, member 3	0.0248 1.2459
122	TCEA1	NM_201437	transcription elongation factor A (SII), 1	0.0229 1.4819
123	TGFB2	NM_001135599	transforming growth factor, beta 2	0.0247 2.9489
124	TGFBR2	NM_003242	transforming growth factor, beta receptor II	0.0028 0.5827
125	TGFBR3	NM_001195684	transforming growth factor, beta receptor III	0.0234 0.4331
126	UGDH	NM_001184701	UDP-glucose 6-dehydrogenase	0.0008 2.5279
127	UGP2	NM_001001521	UDP-glucose pyrophosphorylase 2	0.0394 1.4979
128	UST	NM_005715	uronyl-2-sulfotransferase	0.0003 0.3973
129	VEGFB	NM_003377	vascular endothelial growth factor B (VEGFB)	0.0296 1.8127
		wingless-type MMTV integration site family,		
130	WNT5A	NM_003392	member 5A	0.0000 0.0803

Table S2. The lectins and relative intense of HS27a/HS5

No.	Lectin	Binding structure	p-value	HS27a/HS5 ratio
1	Jacalin	Gal β 1-3GalNAc α -Ser/Thr(T)  GalNAc α -Ser/Thr(T)	0.002	3.243
2	ECA	Gal β -1,4GlcNAc 	0.204	0.794
3	HHL	Non-substituted α -1,6 Man 	0.073	1.472

4	WFA	GalNAc α / β 1-3/6Gal 	0.032	0.294
5	GSL-II	GlcNAc  Galactosylated N-glycans 	0.019	1.949
6	MAL-II	Sia α 2-3Gal β 1-4Glc(NAc) 	0.096	1.793
7	PHA-E	Bisecting GlcNAc and Biantennary N-glycans 	0.026	0.372
8	PTL-I	α GalNAc and Gal 	0.042	2.466
9	SJA	Terminal GalNAc and Gal 	0.060	0.538
10	PNA	Gal β 1-3GalNAc α -Ser/Thr(T) 	0.021	1.285
11	EEL	Gal α 1-3(Fuc α 1-2)Gal 	0.750	0.947
12	AAL	Terminal Fuc α -1,6GlcNAc  Fuc α -1,3Gal β -1,4GlcNAc 	0.033	0.490
13	LTL	sLe ^x , Le ^x  Fuc α -1,3GlcNAc (core)  ;	0.016	0.783
14	MPL	α GalNAc 	0.032	1.104
15	LEL	Poly-LacNAc  (GlcNAc) _n 	0.016	0.271
16	GSL-I	α GalNAc, α Gal  GalNAc α -Ser/Thr(Tn) 	0.507	1.088

17	DBA	GalNAc α -Ser/Thr(Tn)  GalNAc α 1-3Gal 	0.227	0.874
18	LCA	Fuc α -1,6GlcNAc (core) 	0.000	2.491
19	RCA120	Gal, GalNAc 	0.014	0.684
20	STL	(GlcNAc) _n 	0.005	0.117
21	BS-I	α -Gal, α -GalNAc 	0.043	1.074
22	ConA	Branched and terminal Man,  terminal GlcNAc 	0.000	4.758
23	PTL-II	Gal 	0.223	0.238
24	DSA	GlcNAc 	0.000	4.534
25	SBA	Terminal GalNAc (especially GalNAc α 1-3Gal) 	0.001	3.532
26	VVA	GalNAc  GalNAc α -Ser/Thr(Tn) 	0.049	1.539
27	NPA	Non-substituted α -1,6Man 	0.016	1.302
28	PSA	Fuc α -N-acetylchitobiose-Man 	0.002	1.836
29	ACA	Gal β 1-3GalNAc α -Ser/Thr 	0.677	0.924
30	WGA	Multivalent Sia  ; (GlcNAc) _n 	0.024	1.337
31	UEA-I	Fuc α 1-2Gal β 1-4Glc(NAc) 	0.007	1.801
32	PWM	GlcNAc 	0.005	0.554
33	MAL-I	Gal β -1,4GlcNAc 	0.118	1.302
34	GNA	Terminal α -1,3 Man 	0.811	1.097

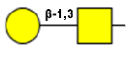
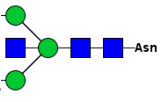
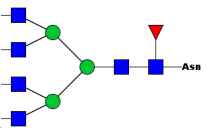

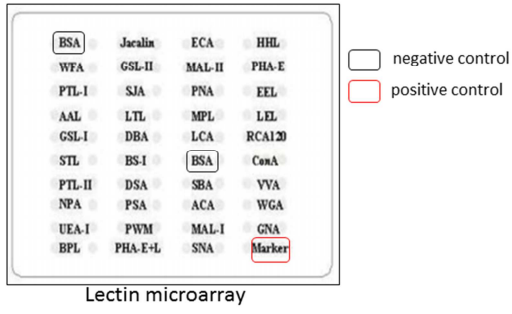
35	BPL	Gal β 1-3GalNAc 	0.964	1.016
36	PHA-E+L	<p>Bisecting GlcNAc and biantennary</p> <p>N-glycans </p> <p>Tetra-antennary complex-type</p> <p>N-glycan </p>	0.003	3.220
37	SNA	Sia2-6Gal β 1-4GlcNAc 	0.101	0.716

Figure S1

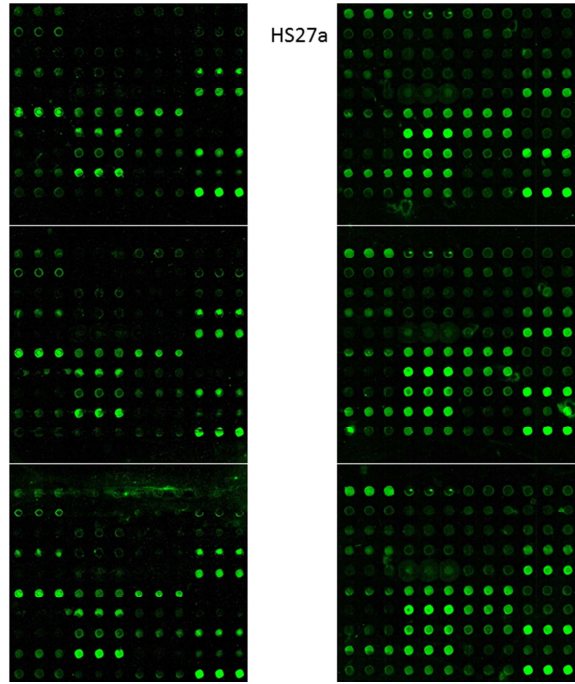
A



B

HS5

HS27a



S1: (A) the lectin array; (B) The scanning images of lectin array (three times).