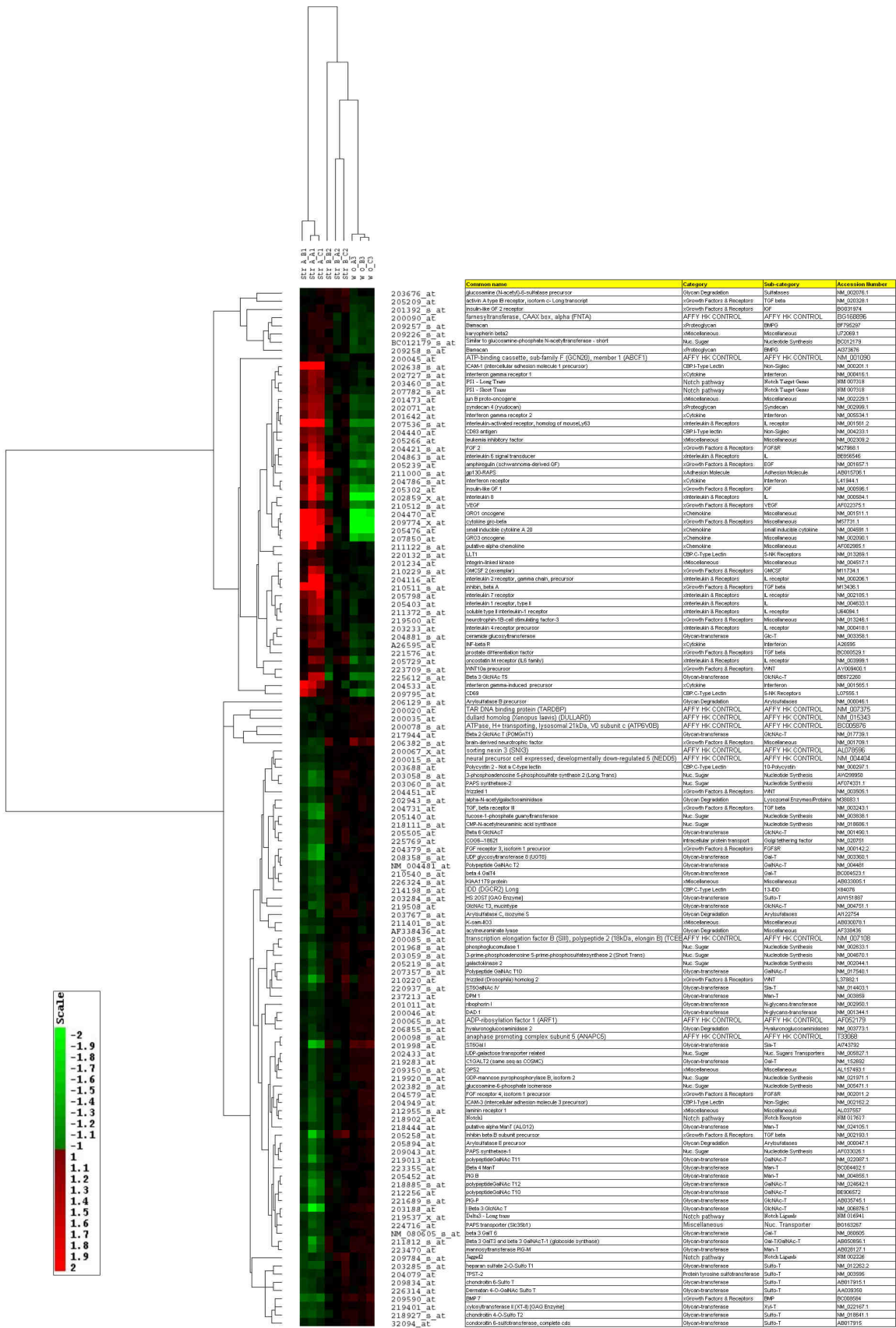


Supplementary Material 1



Legend of Supplementary Figure 1. Heat-map generated using class comparison methods and clustered using analysis of variance test (ANOVA), as described in the Methods section.

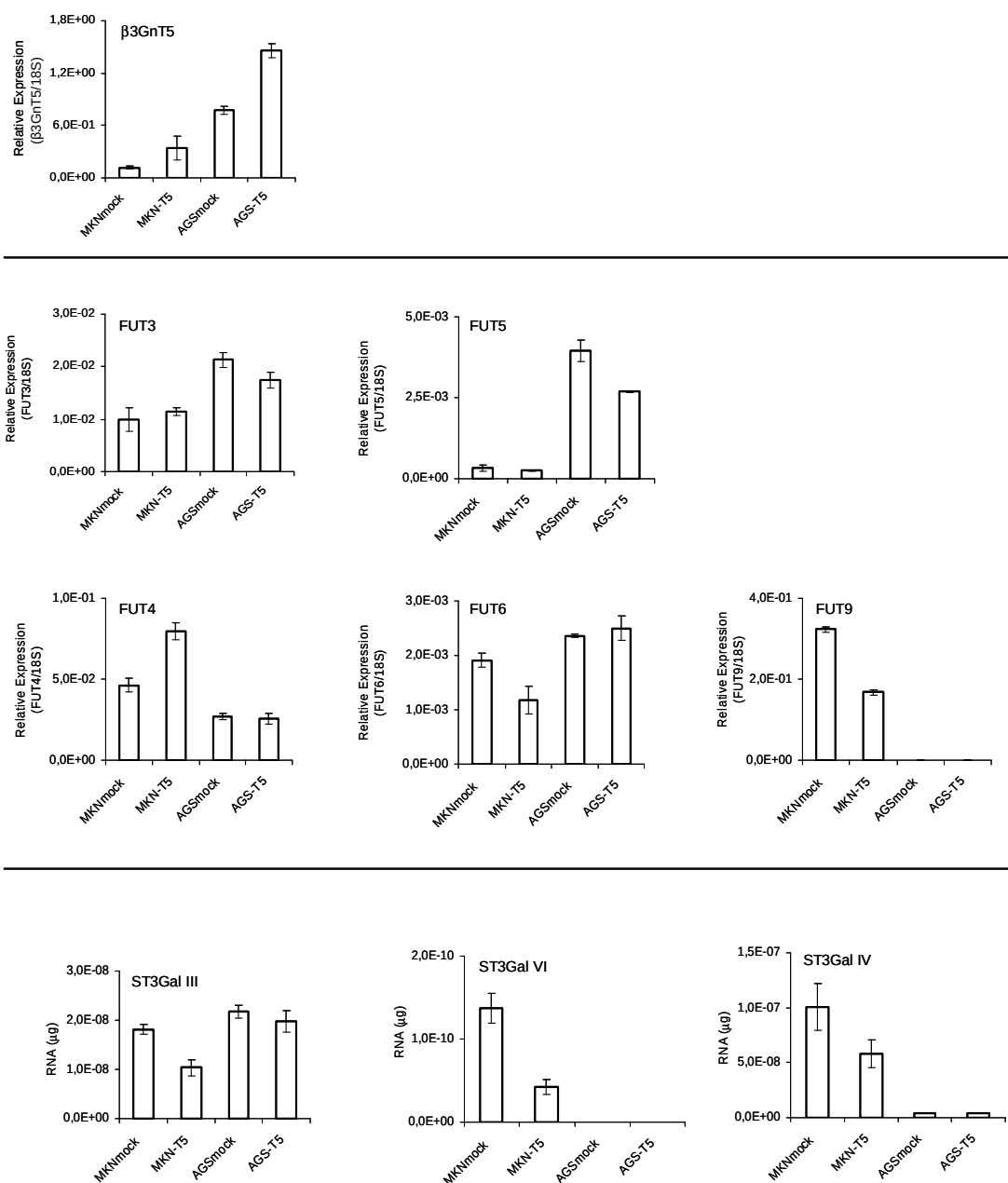
Supplementary Material 2

Methods

Quantitative real-time PCR (qRT-PCR). cDNA was generated from 5 µg of total RNA using the RNeasy Plus MiniKit (Quiagen) in a 20 µl final reaction volume. Real-time PCR reactions were performed in triplicate using one dilution (1/50; 4 µl /well) of each cDNA, primers described in Suppl. Table 4, POWER SYBR Green PCR Master Mix (Applied Biosystems) in a volume of 20 µl in 96-well optical plates. PCR reactions were run on an ABI Prism 7000 (Applied Biosystems). 18S expression was also measured in triplicate for each sample and used for normalization of target gene abundance. Specificity of amplification was confirmed by melt curve analysis. Standard curve was determined for each gene and results are presented as a ratio between the quantity of glycosyltransferase product and 18S. Absolute quantification of RNA was determined for each sialyltransferase (ST) gene using pcDNA3.1/ST constructs.

Results

Expression of terminal glycosyltransferases. Sialyl-Le^x antigen synthesis depends on several terminal glycosyltransferases, namely sialyl- and fucosyltransferases. The mRNA levels of some of these genes were quantified in MKN-T5 and AGS-T5 and compared to their mock-transfected counterparts. MKN-T5 and AGS-T5 showed no significant alterations in expression of α 1,3/4 fucosyltransferases with exception of FUT4 and FUT9 (Supplemental Figure 1). FUT4 mRNA level increased 2.3-fold while FUT9 decreased 1.9-fold in MKN-T5. On the other hand mRNA levels of ST3Gal III and ST3Gal IV decreased 1.7-fold and ST3Gal VI decreased 3.2-fold in MKN-T5 while no alterations were observed in AGS-T5 (Supplementary Figure 2).



Legend of Supplementary Figure 2. qRT-PCR expression of glycosyltransferases in MKN45 and AGS cell lines after stable transfection with β 3GnT5, MKN-T5 and AGS-T5, respectively. MKN45*mock* and AGS*mock* cells were used as controls of the transfection.

Supplementary Table 1. Differentially expressed genes related to glycan biosynthesis, modification and degradation.

Gene common name	Accession Number	Fold differences ^S	
		High-Hp vs. Control	Low-Hp vs. Control
Glycan-transferase			
<i>GalNAc-Transferases</i>			
Polypeptide GalNAc T4	NM_003774	1,321	
Polypeptide GalNAc T10	NM_017540.1	0,694	
Polypeptide GalNAc T10	BE906572	0,538	
PolypeptideGalNAc T11	NM_022087.1	0,551	
PolypeptideGalNAc T12	NM_024642.1	0,537	
<i>Sialyl- Transferases</i>			
ST3Gal IV	NM_006278.1	0,764	
ST6GalNAc IV	NM_014403.1	0,675	
ST6GalNAc I	Y11339.2	0,468	
ST6Gal I	AI743792	0,338	0,674
<i>GlcNAc- Transferases</i>			
Beta 3 GlcNAc T5	BE672260	3,887	
PIG A isoform 1	NM_002641.1	2,479	1,304
Beta 2 GlcNAc T (POMGnT1)	NM_017739.1	0,871	
Beta 3 GnT3 transmembrane Ptn. 3	NM_014256.1	0,706	
Dolichol phosphate GlcNAc-1-P T	Z82022	0,676	
PIG-P	AB035745.1	0,661	
Beta 6 GlcNAcT	NM_001490.1	0,606	
I Beta 3 GlcNAc T	NM_006876.1	0,287	0,808
<i>Glc- Transferases</i>			
Ceramide glucosyltransferase	NM_003358.1	1,891	
<i>Gal-GalNAc Transferases</i>			
Beta 3 GalT3 / beta 3 GalNAcT-1 (globoside synthase)	AB050856.1	0,511	
<i>Gal- Transferases</i>			
chondroitin polymerizing factor/FLJ22678 (CHPF)	NM_024536	0,806	
beta 4 GalT4	BC004523.1	0,742	
C1GALT2 (COSMC)	NM_152692	0,737	
beta 3 GalT 6	NM_080605	0,695	
UDP glycosyltransferase 8 (UGT8)	NM_003360.1	0,479	
MGC39558	NM_152490		1,198
beta 3 GalT6	H93450	0,695	
<i>Fuc- Transferases</i>			
FUT8	NM_004480.1	1,598	
FUT4 (ELAM-1 ligand)	AF305083.1		0,766
<i>Man- Transferases</i>			
Beta 4 ManT	BC004402.1	0,738	
Not56 (D. melanogaster)-like protein	NM_005787.1	0,738	
Man T3	NM_018973.1	0,716	
PIG B	NM_004855.1	0,599	
mannosyltransferase PIG-M	AB028127.1	0,542	
<i>N-glycans-transferase</i>			
ribophorin I	NM_002950.1	0,817	0,882
DAD 1	NM_001344.1	0,763	0,880
<i>Miscellaneous</i>			
EXT1 HS copolymerase [GAG Enzyme]	NM_000127.1	1,364	
C5 Glucuronyl Epimerase [GAG Enzyme]	XM_035390	0,763	
<i>Sulfo- Transferases</i>			
HNK-1 Sulfo T	NM_004854.1	0,804	
HS 2OST [GAG Enzyme]	AW151887	0,778	1,174
condroitin 6-sulfotransferase	AB017915	0,766	
NDST1 [GAG Enzyme]	AL526632	0,761	
chondroitin 6-Sulfo T	AB017915.1	0,736	
Dermatan 4-O-GalNAc Sulfo T	AA039350	0,732	
Heparan sulfate 2-O-Sulfo T1	NM_012262.2	0,644	
chondroitin 4-O-Sulfo T2	NM_018641.1	0,61	
Glycan Degradation			
solute carrier 17-5	NM_012434.1	1,309	
lysosomal acid alpha-mannosidase	U68567.1	1,133	
Neu1	BC000722	0,896	
alpha-L-iduronidase precursor	NM_000203.1	0,808	
glucosidase, beta; acid	K02920.1	0,794	
cystinosis, nephropathic	NM_004937.1	0,783	
fucosidase, alpha-L- 1, tissue	NM_000147.1	0,758	
hyaluronoglucosaminidase 2	NM_003773.1	0,746	0,878
alpha-N-acetylgalactosaminidase	M38083.1	0,735	
acylneuraminase lyase	AF338436	0,731	
Arylsulfatase C, isozyme S	AI122754	0,677	
Arylsulfatase E precursor	NM_000047.1	0,647	
glucosamine (N-acetyl)-6-sulfatase precursor	NM_002076.1		1,304
Proteoglycan			
syndecan 4 (ryudocan)	NM_002999.1	3,25	1,445
Bamacan	BF795297	1,507	1,475
Bamacan	AI373676	1,465	1,354
CD44 (Epican)	NM_000610.1	0,831	
Glypican 4 (Gpc 4)	AF064826.1	0,649	

§ light filling indicates >2-fold increase and dark filling indicates >2-fold decrease

Supplementary Table 2. Differentially expressed genes related to inflammation and host immune response.

Gene common name	Accession Number	Fold differences [§]	
		High-Hp vs. Control	Low-Hp vs. Control
Chemokines			
small inducible cytokine A 20	NM_004591.1	66.2	6.9
GRO1 oncogene	NM_001511.1	15.3	4.4
GRO3 oncogene	NM_002090.1	12.5	3.1
putative alpha chemokine	AF002985.1	2.7	
CCR11	AF193507	2.4	
Cytokines			
interferon gamma-induced precursor	NM_001565.1	3,755	
interferon receptor	L41944.1	3,428	1,592
interferon gamma receptor 2	NM_005534.1	2,659	
interferon gamma receptor 1	NM_000416.1	2,275	
INF-beta R	A26595	2,021	1,353
interferon receptor 2	NM_000874.1	1,666	
Interleukins and Receptors			
interleukin-activated receptor, homol. mouseLy63 (TNF receptor member 9)	NM_001561.2	30,994	1,919
interleukin 8	NM_000584.1	15,868	4,641
interleukin 6 signal transducer	BE856546	4,349	1,553
interleukin 2 receptor, gamma chain, precursor	NM_000206.1	3,166	
interleukin 7 receptor	NM_002185.1	2,774	
oncostatin M receptor (IL6 family)	NM_003999.1	2,384	1,816
soluble type II interleukin-1 receptor	U64094.1	2,314	
interleukin 1 receptor, type II	NM_004633.1	2,183	
interleukin 4 receptor precursor	NM_000418.1	1,604	
I-Type Lectin			
ICAM-1	NM_000201.1	18,479	
CD83 antigen	NM_004233.1	2,686	1,389
ICAM-3 (intercellular adhesion molecule 3 precursor)	NM_002162.2	0,62	
C-Type Lectin			
CD69	L07555.1	3,756	1,933
LLT1	NM_013269.1	1,253	
IDD (DGCR2) Long	X84076	0,72	
Polycystin 2 - Not a C-type lectin	NM_000297.1	0,7	
Collectin K1	AK002131		1,303
S-Type Lectin			
Galectin 8	AI659005		1,639
Prostate carcinoma antigen (Galectin 8)	L78132.1		1,52
Galectin 8	AF074000.1		1,436

§ light filling indicates >2-fold increase and dark filling indicates >2-fold decrease

Supplementary Table 3. Differentially expressed genes related to signalling pathways, cell-cell signalling, nucleotide synthesis, and others.

Gene common name	Accession Number	Fold differences [§]	
		High-Hp vs. Control	Low-Hp vs. Control
Growth Factors and Receptors			
<i>TGF beta</i>			
inhibin, beta A	M13436.1	23,608	
activin A type I receptor precursor	NM_001105.2	2,75	1,386
prostate differentiation factor	BC000529.1	1,312	
activin A typeIB recept., isof. c-L-transc.	NM_020328.1	1,139	1,331
TGF-beta	M60316.1	0,582	
TGF, beta receptor III	NM_003243.1	0,513	
inhibin beta B subunit precursor	NM_002193.1		
<i>BMP</i>			
BMP 2 Short transcript	AA583044	4,46	
BMP 2 Long transcript	NM_001200.1	3,765	
BMP 2b	M22490	0,508	0,813
BMP 4	D30751.1	0,495	0,783
BMP 7	BC008584	0,445	
<i>FGF</i>			
FGF 2	M27968.1	3,442	1,623
FGF receptor	M60485.1	0,867	
FGF receptor 2, isoform 2 precursor	NM_022969.1	0,71	
FGF receptor 4, isoform 1 precursor	NM_002011.2	0,678	
FGF receptor 3, isoform 1 precursor	NM_000142.2	0,478	
<i>WNT</i>			
WNT10a precursor	AY009400.1	1,381	
frizzled (Drosophila) homolog 2	L37882.1	0,515	
frizzled 6	NM_003506.1		1,305
frizzled 1	NM_003505.1		
<i>PDGF</i>			
PDGF beta polypeptide	NM_002608.1	1,981	
<i>IGF</i>			
insulin-like GF 1	NM_000596.1	9,989	2,4
insulin-like GF 2 receptor	BG031974	1,307	1,325
<i>VEGF</i>			
VEGF	AF022375.1	3,026	2,06
VEGF B	NM_003377.1	0,73	
<i>EGF</i>			
amphiregulin (schwannoma-deriv. GF)	NM_001657.1	7,835	2,137
<i>Other Growth Factors and Receptors</i>			
cytokine gro-beta	M57731.1	23,046	6,129
GMCSF 2 (exemplar)	M11734.1	2,398	
patched (Drosophila) homolog	BG054916		1,599
neurotrophin-1B-cell stimulating fac.-3	NM_013246.1	1,53	
Adhesion Molecule			
gp130-RAPS	AB015706.1	3,984	1,689
Protein tyrosine sulfotransferase			
TPST-2	NM_003595	0,719	
Notch pathway			
PS1 - Short Trans	NM_007318	2,041	
PS1 - Long Trans	NM_007318	1,962	
APH1	NM_016022	0,824	
PS2 - Long Trans	NM_000447	0,746	
Jagged2	NM_002226	0,734	
Notch1	NM_017617	0,636	
Delta3 - Long trans	NM_016941	0,662	
Intracellular protein transport			
COG6--18621	NM_020751	0,668	
COG1- Short	NM_018714	0,645	
COG5 - Possible Long trans	NM_006348		1,215
Nucleotide Synthesis			
glutamine-fructose-6-phosphate transaminase 1	NM_002056.1	1,556	
glucosamine-phosphate N-acetyltransferase – short	BC012179	1,43	
glutamine-fructose-6-phosphate transaminase 2	NM_005110.1	1,38	
uridine diphosphoglucose pyrophosphorylase	BC000173	0,866	
phosphomannomutase 1	NM_002676.1	0,781	
glucosamine-6-phosphate isomerase	NM_005471.1	0,767	
GMDS (GDP-mannose 4,6-dehydratase)	NM_001500.1	0,766	
CMP-N-acetylneuraminic acid synthase	NM_018686.1	0,755	
galactokinase 2	NM_002044.1	0,753	
UDP-galactose transporter related	NM_005827.1	0,709	0,857
3-prime-phosphoadenosine 5-prime-phosphosulfatesynthase 2 (Short)	NM_004670.1	0,707	
GDP-fucose	NM_018389.1	0,683	
phosphoglucomutase 1	NM_002633.1	0,681	
GDP-mannose pyrophosphorylase B, isoform 2	NM_021971.1	0,666	0,803
PAPS synthetase-1	AF033026.1	0,648	
CMP-sialic acid transporter	NM_006416.1	0,614	
3-phosphoadenosine 5-phosphosulfate synthase 2 (L)	AW299958	0,58	
PAPS synthetase-2	AF074331.1	0,579	

fucose-1-phosphate guanylttransferase	NM_003838.1	0,551	
<i>Other genes</i>			
leukemia inhibitory factor	NM_002309.2	3,088	1,499
jun B proto-oncogene	NM_002229.1	2,841	1,499
jun D proto-oncogene	AI762296	1,743	
karyopherin beta2	U72069.1	1,491	1,285
integrin-linked kinase	NM_004517.1	1,347	
ribosomal protein L38	BC000603.1	1,157	
proteasome 26S subunit,ATPase, 1	NM_002802.1	0,871	
K-sam-IIQ3	AB030078.1	0,835	
GPS2	AL157493.1	0,68	0,844
laminin receptor 1	AL037557	0,642	
+ transporter (Slc35b1)	BG163267	0,631	
KIAA1179 protein	AB033005.1		

§ light filling indicates >2-fold increase and dark filling indicates >2-fold decrease

Supplementary Table 4. qRT-PCR conditions and primer sequences for analysis of gene expression.

Gene	Primers	Amplicon	PCR
<i>α1,3/4 fucosyltransferases</i>			
FUT3	for 5'- caaaatgccaagggtggaca -3' rev 5'- ttggcctcaatcaatcctcct -3'	89 bp	15s 95°C 60s 60°C
FUT4	for 5'- aagccgttgaggcgggtt -3' rev 5'- acagttgtgtatgagattggaagct -3'	88 bp	15s 95°C 60s 60°C
FUT5	for 5'- gccctggacggatacttcaa -3' rev 5'- gcagagccacgggtgtgtta -3'	70 bp	15s 95°C 60s 60°C
FUT6	for 5'- caaagccacatcgattgaa -3' rev 5'- atccccgttcagaacca -3'	95 bp	15s 95°C 60s 60°C
FUT9	for 5'- tcccatgcagtctgatccat -3' rev 5'- gaagggtggcctagcttgct -3'	78 bp	15s 95°C 60s 60°C
<i>α2,3 sialyltransferases</i>			
ST3Gal III	for 5'- ggtggcagtcgcaggattt -3' rev 5'- catgcgaacggctctcatagtagtg -3'	76 bp	15s 95°C 60s 60°C
ST3Gal IV	for 5'- cctggtagctttcaaggcaatg -3' rev 5'- cctttcgacccgcttct -3'	74 bp	15s 95°C 60s 60°C
ST3Gal VI	for 5'- gtcgaaaatattccgctgatgtt -3' rev 5'- cggctgattttagaagattgctt -3'	90 bp	15s 95°C 60s 60°C