Altered (neo-) lacto series glycolipid biosynthesis impairs α2-6 sialylation on *N*-glycoproteins in ovarian cancer cells

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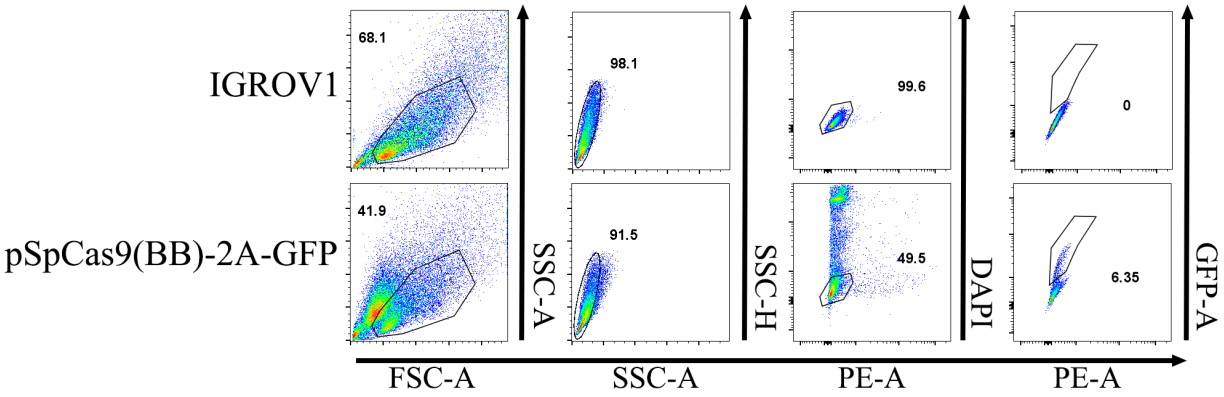
Supplementary information

Supplemental Fig. S1

Single- cell selection after transient transfection of CRISPR- *Cas9* encoding plasmids. Representative gating of fluorescence activated single cell sorting into 96-well plates after transient delivery of pSpCas9(BB)-2A-GFP (PX458) encoding sgRNA.



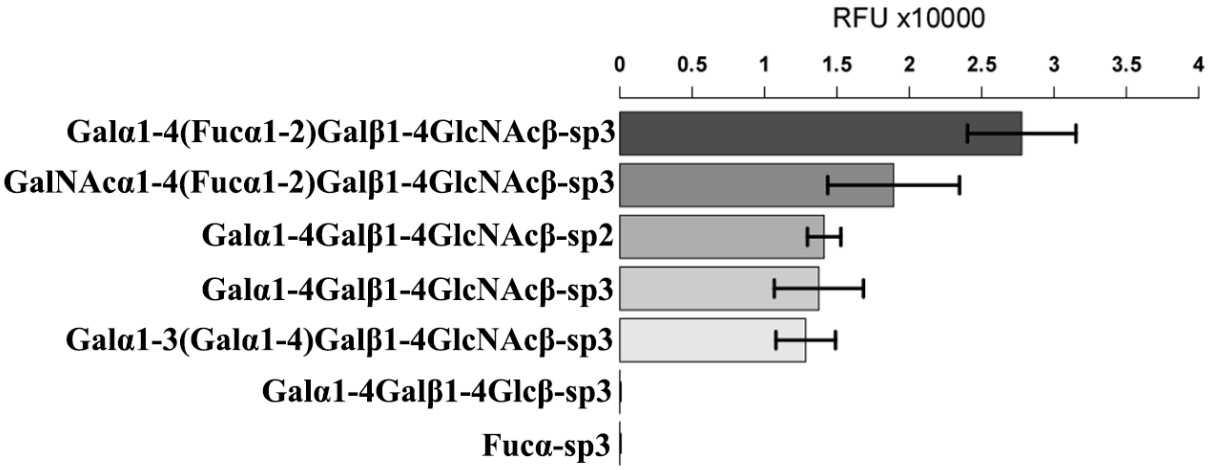
Supplemental Fig. S2

Determination and verification of potential off targets. *In silico* analysis revealed 50 and 17 potential off-targets for sgRNA_1 and sgRNA_2, respectively. The sgRNA_1 had only one predicted off-target in gene NM_001242377 chr5:+112339780 whereas sgRNA_2 was not predicted for an off-target site in a gene. Genomic sequences of predicted off-targets with highest score for each sgRNA were further amplified and sequenced, in which no mutations were observed. Off target DNA sequencing results for B3GNT5 wildtype (IGROV1) and knockout (clone KO_1 and KO_2) cells performed in triplicates. Genomic DNA sequence according to February 2009 human reference sequence (http://genome.ucsc.edu/index.html, GRCh37). Standard SP6 DNA Sequencing primer was used.

Off target	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′		GENOME
01_SP6_	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′	OK	clone KO_1
02_SP6_	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′	OK	clone KO_1
03_SP6_	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′	OK	clone KO_1
04_SP6_	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′	OK	clone KO_2
05_SP6_	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′	OK	clone KO_2
06_SP6_	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′	OK	clone KO_2
07_SP6_	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′	OK	IGROV1
08_SP6_	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′	OK	IGROV1
09_SP6_	5′- ata cac aat tac agt ctt ttc aga aat tta gat -3′	OK	IGROV1

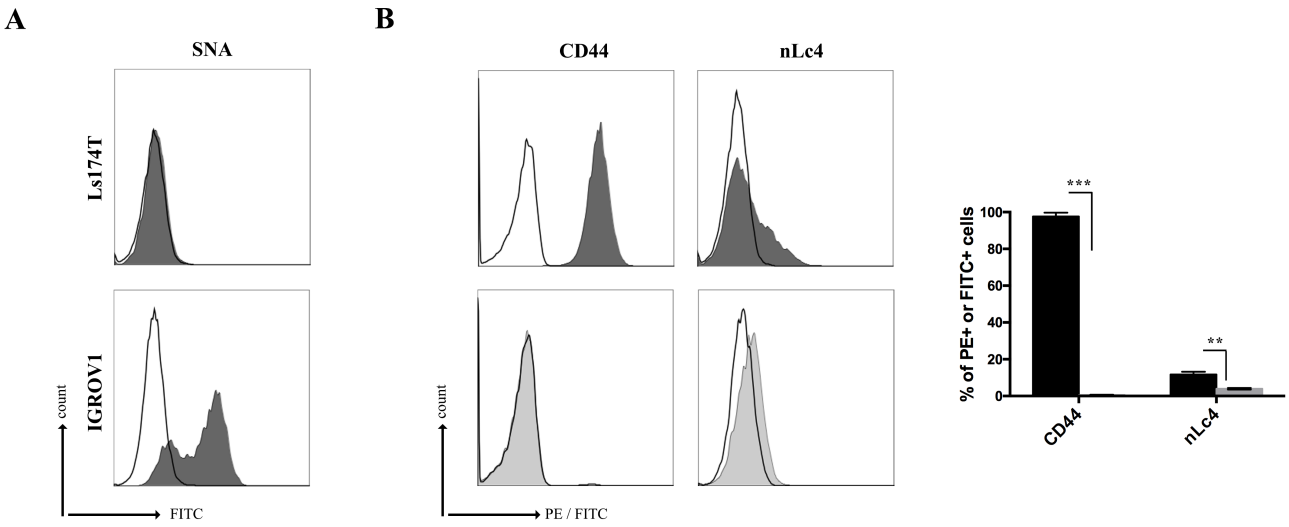
Supplemental Fig. S3

Determination of anti- P_1 IgM binding epitopes using printed glycan array. Barchart demonstrating the binding specificity for human anti-P_1 IgM P3NIL100 antibodies. We performed a broad screening of glycan binding specificity of the monoclonal antibody P3NIL100 across 381 glycans by utilizing glycan array technology. The results clearly demonstrated that antibody bound specifically to glycans with Galα1-4Galβ1-4GlcNAcβ as well as glycans terminating with Galα1-3(Galα1-4)Galβ1-4 and GalNAcα1-4Galβ1-4, with some of these structures bearing α1-2-linked fucose. As expected, there was no binding of the antibody to Gb3 glycan (Galα1-4Galβ1-4Glcβ-sp).



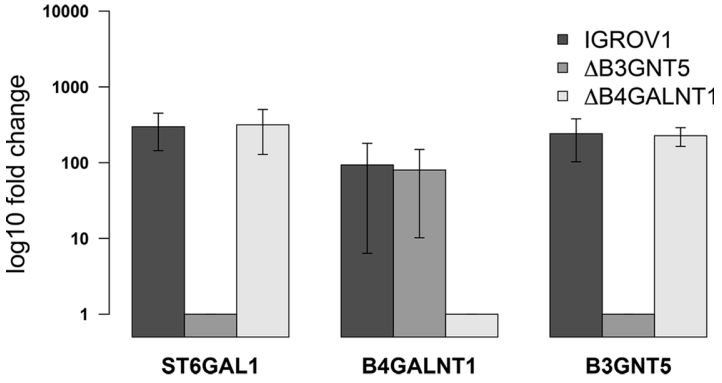
Supplemental Fig. S4

The epitope for anti- nLc4 IgM is present on glycoproteins in colon cancer cell line Ls174T. A) Histogram showing the absence of α2-6 sialylation in Ls174T cells. **B)** Proteinase K treatment on Ls174T cells following staining for CD44 and nLc4. Representative histogram for unstained (white), Proteinase K - untreated (dark gray) and -treated cells (light gray). Data are represented as mean ± s.d, *** $p < 0.001$, ** $p < 0.01$.



Supplemental Fig. S5

Quantitative gene expression of *ST6GAL1*, *B4GALNT1*, and *B3GNT5* in IGROV1, Δ*B3GNT5-, and* Δ*B4GALNT1-* cells. Barchart represents three independent experiments. Data are represented as mean ± s.d. RT-qPCR was performed in triplicates on three independent experiments.



Supplemental Table S1

Oligonucleotides used in the study.

single guided RNAs				
	oligonulceotide 5 '- 3'		Gene	Method
sgRNA 1	CAC CGA TAC ACG ATT ATA GCC GTT T	AAA CAA ACG GCT ATA ATC GTG TAT C	B3GNT5	Gene-editing
sgRNA 2	CAC CGA TCG ACG TTC CGG AAT TAG A	AAA CTC TAA TTC CGG AAC GTC GAT C	B3GNT5	Gene-editing
sgRNA 1	CAC CGT GGA TGC CGC GGT TTC GAC G	AAA CCG TCG AAA CCG CGG CAT CCA C	B4GALNT1	Gene-editing
sgRNA 2	CAA CGC CTT CAA ATA GTC CTC GGG A	AAA CTC CCG AGG ACT ATT TGA AGG C	B4GALNT1	Gene-editing
genotyping PCR primer				
	oligonulceotide 5 '- 3' (Forward)	oligonulceotide 5 '- 3' (Reverse)	Gene	Method
PCR_1	GTA TCT GCT TTC ATC CTG ACC AT	TGC CCA ACT GAA CTG CAT AAG	B3GNT5	Genotyping
PCR_2	GTA TCT GCT TTC ATC CTG ACC AT	TCG AGA CCA TAG AAC TTC GTG T	B3GNT5	Genotyping
PCR_3	GGC CTC GCT ACC AAT ACT T	TGC CCA ACT GAA CTG CAT AAG	B3GNT5	Genotyping
PCR_4	GAT GAG GAT AAA GCA GTG CAG AT	GCC TCA GCC CTG CTT CAA ATC	chr5:113003768+113004235	off target
PCR_1	AGA GCG TTA GAC AGG TCA GT	TGG AGG AAG GAG AGG ACA GA	B4GALNT1	Genotyping
PCR_2	CTG TGC GCT CTG GTC CTT	TGG AGG AAG GAG AGG ACA GA	B4GALNT1	Genotyping
PCR_3	CCA CTA CTT GCT CCT TGA TCC	TGG AGG AAG GAG AGG ACA GA	B4GALNT1	Genotyping
PCR_4	CCA CTA CTT GCT CCT TGA TCC	CTG TGC GCT CTG GTC CTT	B4GALNT1	Genotyping
PCR_5	GCA CAT ACT GAA GCG TTC ACA	ATC CCA GCT CCT CAG CTA CA	chr22:30296425-30296628	off target
PCR_6	TTG GCC AGA GGT TCA TGC TA	ACC GTC ACC CTA CTC AAG TG	chr12:52518025-52518321	off target

Supplemental Table S2

Details of target and reference genes, primers and amplicons investigated in this study by RT-qPCR. QPCR parameters are provided including efficiency (E in %; calculated based on the standard curve according to the equation $E=10^{(-1/slope)}-1) \times 100$ and expressed as percentage) and correlation coefficient (R^2).

Symbol	Gene name	Accession number	Chr. location	Forward Primer 5'-3'	Reverse Primer 5'-3	Amplicon size in bp	E in %	R^2
B3GNT5	UDP-GlcNAc β -1,3-*N*-acetylglucosaminyl-transferase 5	NM_032047.4	3q28	GGCTTGAACTTCGT GAGTTTCGC	TCGAGACCATAGAA CTTCGTGT	305	92.2	0.994
ST6GALNAC1	GalNAc α -2,6-Sialyltransferase I	NM_018414.3	17q25.1	CGAAATAGGAGGCC TTCAGACGAC	TTTCTGGAGCCACA GCGACTTG	76	74.1	0.999
ST6GALNAC2	GalNAc α -2,6-Sialyltransferase II	NM_006456.2	1q31.1	ATCGAATTCTTGGA CAGGAAAGGG	GATTGAACAGGCCA CGGAAGTG	92	83.9	0.899
ST6GALNAC3	GalNAc α -2,6-Sialyltransferase III	NM_001160011.1	1q31.1	AGCGCATGAGTTAC TGTGATGG	TGCATGGTCACTCT GTACTGTCC	69	103.9	0.999
ST6GALNAC4	GalNAc α -2,6-Sialyltransferase IV	NM_175039.3	9q34.11	ACCAGATCTTCCAG GACGAGAC	TCTTCTCCCTGCAGT AGCTGTC	150	86.7	0.998
ST6GALNAC5	GalNAc α -2,6-Sialyltransferase V	NM_030965.1	1q31.1	TGACAATTGCACTG GAGCTCTG	GTGATTGGGATCCC TGCAGAAG	80	131.6	0.955
ST6GALNAC6	GalNAc α -2,6-Sialyltransferase VI	NM_013443.3	9q34.11	CATGCGGCAATTTG ACGACCTC	CACGAATGAGACTT CTCCCTGTCC	66	106.1	0.999
ST6GAL1	β -galactosamide α-2,6-sialyltransferase 1	NM_173217.2	3q27-q28b	CCATCCTCTGGGAT GCTTGGTATC	ACGTCAGTCTTGCG CTTGGATG	95	98.1	0.997
ST6GAL2	β -galactosamide α-2,6-sialyltransferase 2	NM_001142351.1	2q12.3	TTCCTTGGGCGAGG AAATAGATTC	TCATAACCACGTGT AGGAGCAGAG	72	99.2	0.991
B4GALNT1	β -1,4-*N*-Acetyl-Galactosaminyl Transferase 1	NM_001276468	12q13.3	TGTGCGCTCTGGTCC TTCTG	TTGATCCTGACCGG GATGTGT	181	74.1	0.999
HSPCB	Heat shock protein 90kDa alpha (cytosolic)	NM_007355	6p12	TCTGGGTATCGGAA AGCAAGCC	GTGCACTTCCTCAG GCATCTTG	80	87.2	0.999
YWHAZ	Tyrosine 3-monooxygenase/ tryptophan 5-monooxygenase activation protein	NM_003406	8q23.1	ACTTTTGGTACATTG TGGCTTCAA	CCGCCAGGACAAAC CAGTAT	94	94.1	0.999
SDHA	Succinate dehydrogenase complex, subunit A	NM_004168	5p15	TGGGAACAAGAGGG CATCTG	CCACCACTGCATCA AATTCATG	86	100.9	0.999