Cell line	Culture additive	%Neu5Gc/total Sia
THP-I	none	n.s.
	5mM Neu5Gc	62.54
	100µM GalNGc	n.s.
	10mM GalNGc	0.70
	100µM perGalNGc	0.65
EMeg32$^{+/-}$	none	n.s.
	5mM Neu5Gc	65.25
	100µM GalNGc	n.s.
	10mM GalNGc	2.47
	100µM perGalNGc	0.87
EMeg32$^{-/-}$	none	n.s.
	5mM Neu5Gc	81.05
	100µM GalNGc	n.s.
	10mM GalNGc	12.43
	100µM perGalNGc	2.24
BJA-B K20	none	n.s.
	5mM Neu5Gc	86.72
	100µM GalNGc	n.s.
	10mM GalNGc	n.s.
	100µM perGalNGc	n.s.
BJA-B K88	none	n.s.
	5mM Neu5Gc	74.03
	100µM GalNGc	n.s.
	10mM GalNGc	5.17
	100µM perGalNGc	0.78

Figure S1: **DMB-HPLC analysis of cells fed *N*-glycolylgalactosamine.** Various cell lines were cultured under Neu5Gc-free conditions using 5% human serum. Thereafter, different *N*-glycolylated compounds (Neu5Gc, GalNGc, or perGalNGc) were added to the culturing media of the cells. After 3 days incubation, cells were harvested, washed well, and lysed. The cell lysates were treated with acid to release all bound sialic acids and total sialic acid content (Neu5Ac and Neu5Gc) was analyzed by DMB-HPLC. The percentage of Neu5Gc was calculated of the total cellular sialic acid amount (Neu5Ac+Neu5Gc). Samples that did not contain a peak at the elution time for Neu5Gc were labeled n.s. (not significant) in this table.

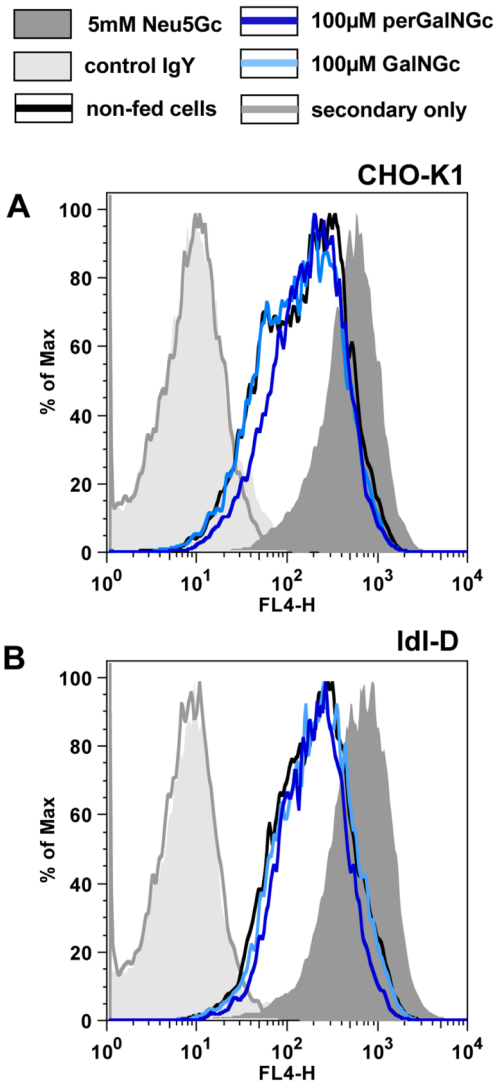
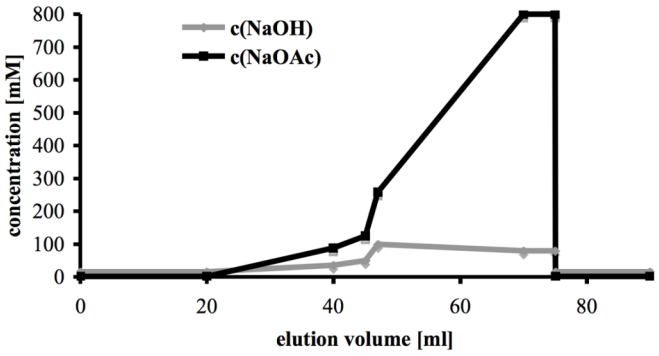


Figure S2: **K1 and ldl-D CHO cells comprise significant endogenous expression of Neu5Gc.** CHO-K1 cells (**A**) and ldl-D cells (**B**) were cultivated in Neu5Gc-free medium containing 5% human serum. Thereafter, the media were supplemented with either 5mM Neu5Gc (positive control; shaded dark grey), 100µM GalNGc (light blue line), 10mM GalNGc (magenta line), or 100µM peracetylated GalNGc (perGalNGc, dark blue line). In parallel, cells were kept in 5% human serum without feeding (negative control; black line). After 3-day feedings, cells were harvested and analyzed by flow cytometry using αNeu5Gc IgY for detection of cell-surface glycosidically-bound Neu5Gc. As an additional negative control, cells fed 5mM Neu5Gc were also stained with control chicken IgY antibody (control IgY; shaded light grey) or incubated only with the secondary antibody (secondary only, dark grey line). Non-fed cells (black lines) reveal the presence of significant levels of cell-surface Neu5Gc although grown in Neu5Gc-free medium. This is likely explained by the presence of a functional *Cmah* gene, which causes endogenous expression of Neu5Gc in such hamster cells.

A



B

elution volume [ml]	c(NaOH) [mM]	c(NaOAc) [mM]
0	15.1	1
20	15.1	1
40	35	87.4
45	50	125
47	100	257.5
70	80	800
75	80	800
75.1	15.1	1
90	15.1	1

FIGURE S3: **HPAEC-PAD HPLC analyses. A**, Profile of the gradient used in the present study to separate UDP-aminosugars by HPLC using a PA-1 column under alkaline conditions. The gradient is composed of several individual linear gradients and the concentrations of sodium hydroxide (NaOH, grey line) and sodium acetate (NaOAc, black line) are depicted for the whole gradient. The flow rate was set to 1ml/min. **B**, summary mentioning exact concentrations of NaOH and NaOAc at the start- and end-points of all linear gradients involved in this method.

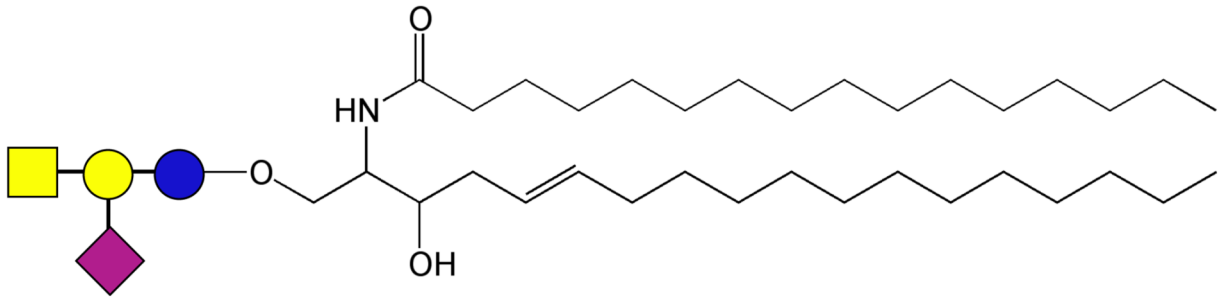


Figure S4: **Possible structure of GM2 ganglioside.** Analysis of isolated gangliosides from GalNAc-fed M-21 cells by mass spectrometry revealed the presence of an *m/z* 1354.98 ion (Fig. 8A). Further MS/MS fragmentation of this ion indicates a GM2 ganglioside composed of the depicted tetrasaccharide, leaving an *m/z* 536.63 fragment ion behind for the ceramide moiety of the ganglioside (Fig. 8C). The above drawn ceramide moiety represent one possible structure (among others) to highlight that the *m/z* 536.63 fragment ion would indeed fit the molecular mass of a common ceramide backbone.

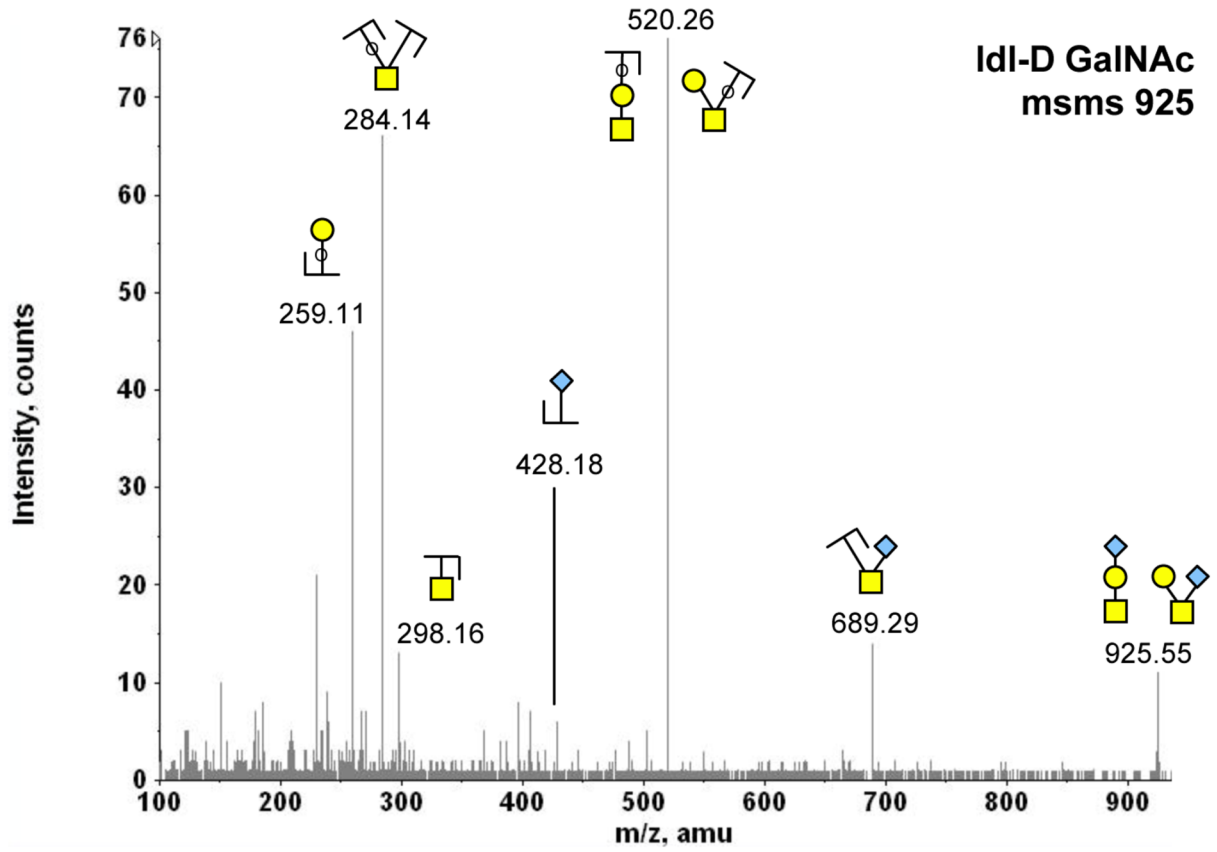


FIGURE S5: **Nature of sialic acid impacts branching of O-glycans.** CHO ldl-D cells were kept under Neu5Gc-free conditions using 5% human serum. The media was supplemented with 10mM GalNGc or 10mM GalNAc for the last 3 days before cells reach confluency. Thereafter, cells were harvested with 20mM EDTA in PBS, pelleted, and washed well. O-glycans were released from cell lysates by alkaline borohydrate treatment and purified O-glycans were permethylated and analyzed by MALDI. The spectrum of GalNAc-fed ldl-D cells contains an *m/z* 925.55 ion (Fig. 10A). Further MS/MS fragmentation confirms the presence of a Neu5Gc residue as depicted here and reveals strong fragment ions pointing to a mainly branched O-glycan structure. In contrast, MS/MS fragmentation analysis of the corresponding Neu5Ac-containing O-glycan structure (*m/z* 895.53, Fig. 10A) indicates a linear O-glycan structure.