Supplement 1

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              Cytoplasmic Tail    Transmembrane Domain
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hC2GnT1    MLRTLLRRRLFSYPTK YYFMVLVLSLITFSVLRIH

cC2GnT1    MLRTLLRRRLFSYPTK YYFMVLVLSLITFSVLRIH

dC2GnT1    MLRTLLRRRLFSYPTK YYCLLLVFSVVTFSVLRIH

bC2GnT1    MLRKLWRRKLFSFPTK YYFLFLAFSVVTFTVLRIH

mC2GnT1    MLRNLFRRRLFSCPTK YYFMLLVLSLITFSVLRIH

rC2GnT1    MLRNLFRRRLFSYPTK YYFMVLVLSLITFSVVRIH
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Supplement 1. Alignment of the deduced amino acid sequences of C2GnT1 from human (*Homo sapiens*) (h), Chimpanzee (*Pan troglodytes*) (c), rat (*Rattus norvegicus*) (r), mouse (*Mus musculus*) (m), bovine (*Bos Taurus*) (b), and dog (*Canis lupus*) (d). Multiple sequence alignment was performed by Vector NTI 11 software. The accession numbers for these proteins are: hC2GnT1, (NP_001481); cC2GnT1, (XP_001145936); dC2GnT1, (XP_541274); bC2GnT1, (NP_803476); mC2GnT1, (NP_775618); rC2GnT1, (NP_071612). The amino acids predicted to be crucial for binding to GOLPH3 are underlined.

Supplement 2

Table 1. C2GnT1 cytoplasmic tail interactors identified by yeast two-hybrid screen [a]

Class	Identity	GenBank Accession	Occurrence [b]	Function
Protein transport	CLTC	NM_004859	1	Component of the clathrin coat, involved in the intracellular trafficking of receptors and endocytosis.
	GOLPH3	NM_022130	2	Involved in mTOR signaling and maintainance of Golgi form.
	SEC24D	NM_014822	1	Component of the COPII coat.
	VPS36	NM_016075	1	Formation and sorting of endosomal cargo proteins.
Cytoskeleton binding	PARVA	NM_018222	1	A member of the parvin family of actin-binding proteins.
	STMN2	NM_007029	1	Regulate microtubules instability.
Protein folding	CCT7	NM_006429	1	A molecular chaperone that is a member of the chaperonin containing TCP1 complex.
	HSPA14	NM_016299	1	Heat shock 70 kDa protein 14, a molecular chaperone.
Other	CCCDC80	NM_199512	2	Coiled-coil domain containing protein.
	LAP3	NM_015907	8	Aminopeptidase that is involved in the regulation and turnover of several proteins.
	OSBPL1A	NM_018030	1	Oxysterol binding protein, contains pleckstrin homology domain.
	RSU1	NM_012425	1	Plays a role in the Ras signal transduction pathway.

[a] Human universal cDNA library was screened using a yeast-two-hybrid system. The bait was a Gal4-binding domain fused to a MLRTLLRRRLFSYPTKY peptide, which contains the C2GnT1 cytoplasmic tail. [b] Number of independent clones identified in the screen that encoded the same protein.

Supplement 3.

GOLPH3 depletion in KG1a cells does not alter the Golgi localization of C1GalT1 or ST3GALT1 and has no apparent effect on N- and O-Glycans as assessed by various lectins.   A) Confocal immunofluorescence images of KG1a cells showing the colocalization of C1GalT1 (top) and ST3Gal1 (bottom) with the Golgi marker, Giantin after transfection with non-targeting (mock) or GOLPH3 siRNAs. B) FACS analysis of KG1a cells after labelling with fluorescein-labeled DSA, PNA, Jackalin or VVA lectin. Cells transfected with non-targeting siRNA (mock), dotted line; or GOLPH3 siRNA, solid line. DSA binds complex-type N-glycans, PNA binds nonsialylated core 1 O-glycan (T-antigen), Jackalin binds core 1 O-glycans, and VVA binds Tn antigen.