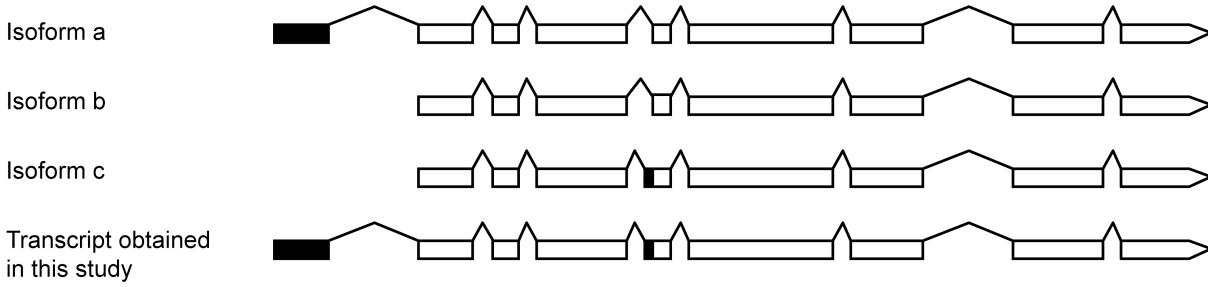


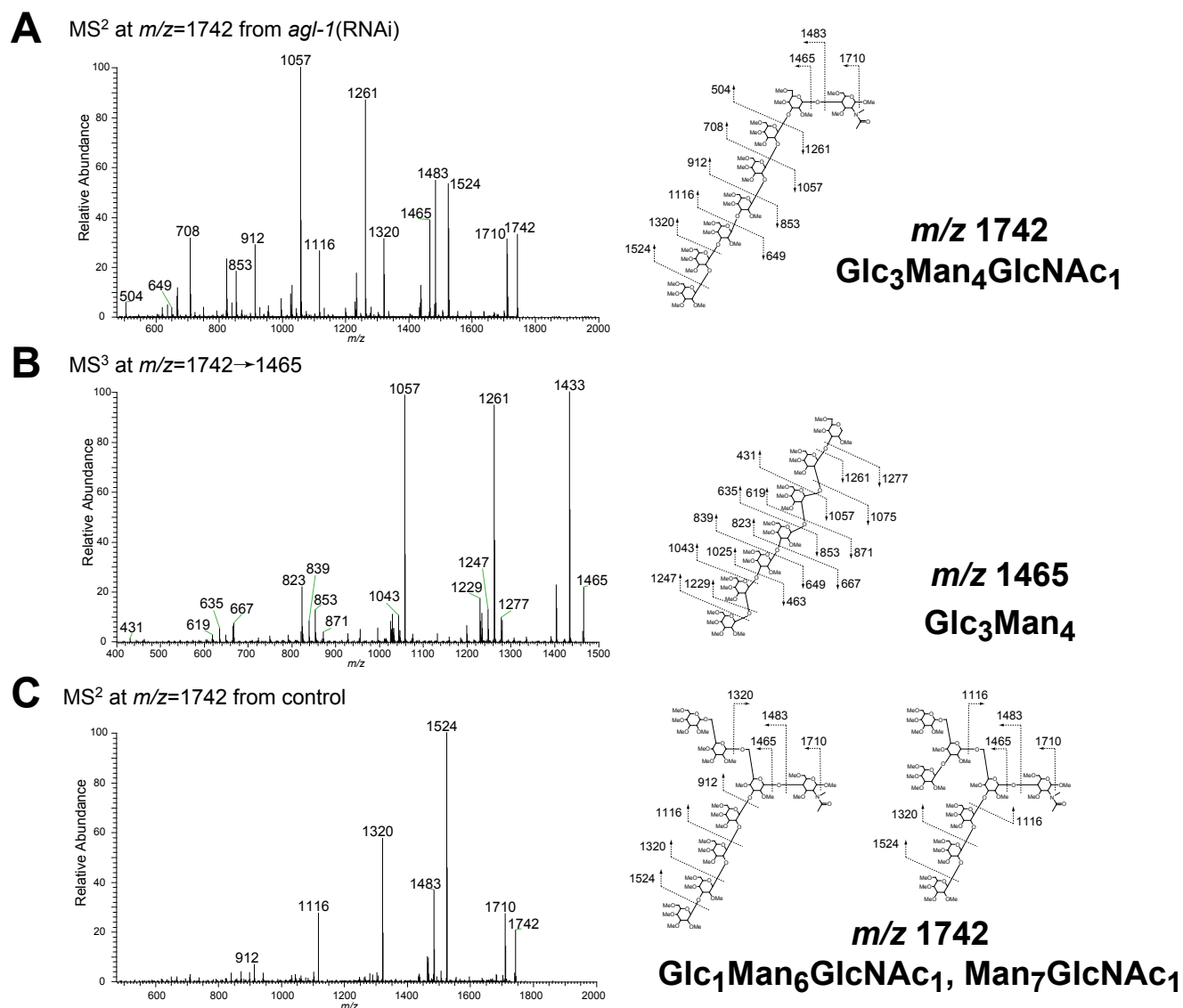
A



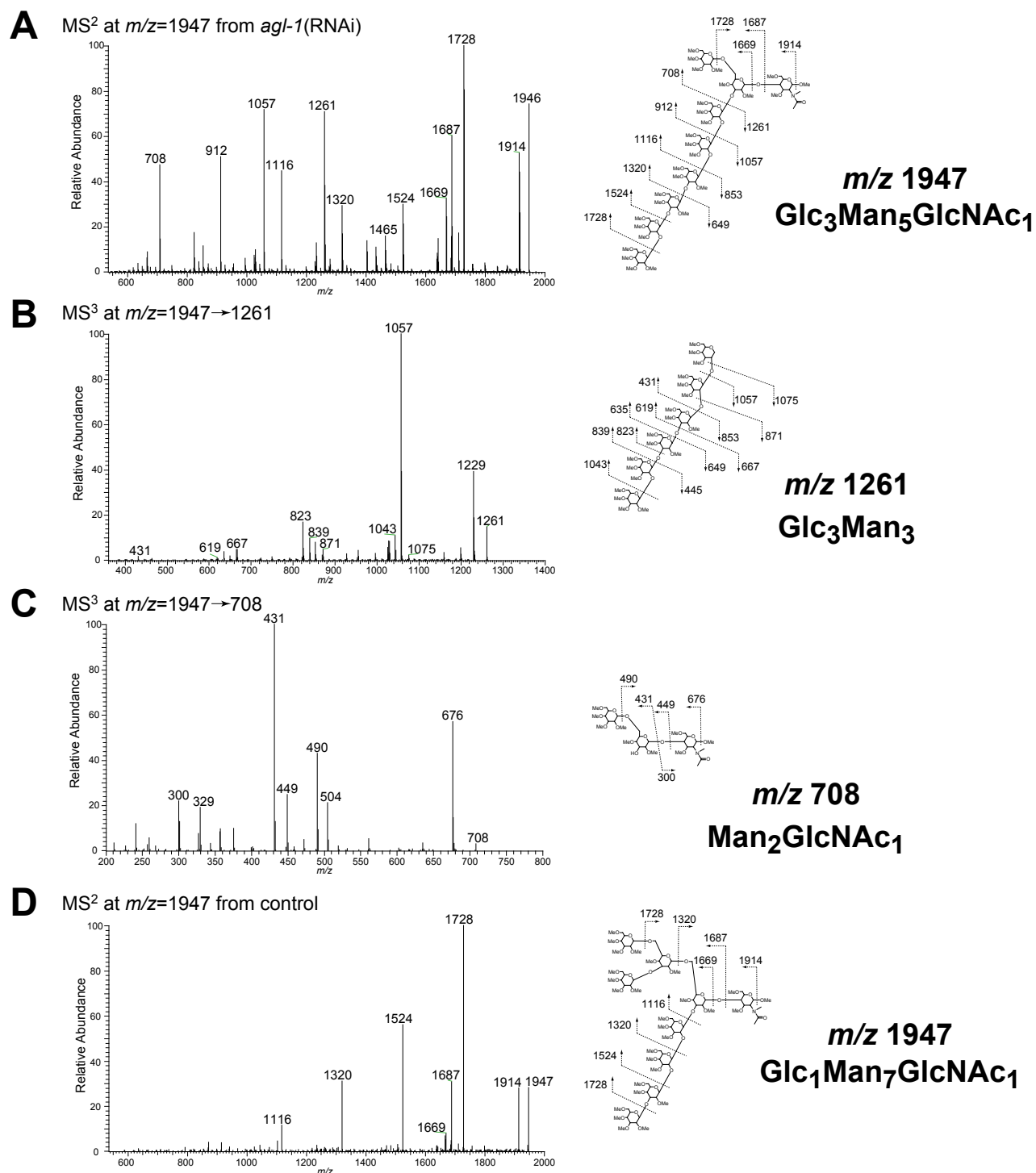
B

mouse	1	MAR---GE	RRRAAAAEAGARFLERAR	---A	AGRRDRGRAGGARGSASGAALAVVVLALAFGLS	GRVVLAWLR	---V	RRALTLHPAP	---S	ALPDS	---S	SPAVAP	----	EL	P	W	G	T	R	F	H	V	F	G	M	K	R	S	P	
CHO	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
human	1	MAR---	GE	RRRAVPAEGV	RTAERA	ARGGPR	RRDRGRGGPR	STAGG	VALVVL	SLALGMS	GRVVLAWYR	---A	ARRAVTLHSA	P	---	P	V	L	D	A	S	---	S	P	A	V	A	P		
D.melanogaster	1	MARNTGSS	SGSTPA	ASSSSSS	AAAAA	AAAAA	AAAAA	NAKQ	KQ	RS	FR	SL	SSNL	L	L	D	K	W	K	T	M	I	C	V	C	L	A	I	A	S
A.thaliana	1	MTG---	ASRR	SARGRIK	SSLS	PGS	DEGS	AYPS	IR	R	G	K	EL	V	S	I	G	A	F	K	T	L	L	L	L	L	L	L	L	
C.elegans agl-1	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
S.cerevisiae	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	

Supplementary figure S1. (A) The gene structure of *agl-1*. Three isoforms (a-c), composed of 8 or 9 exons, have been previously reported in WormBase. The black bars indicate the differences in exons between them. The transcript obtained in this study is a hybrid form of isoform a and isoform c. **(B)** Alignment of ER α -glucosidase I from *C. elegans* and its homologues. The deduced amino acid sequence of *agl-1* from *C. elegans* is compared with human GCS1 (GenBank accession number AAI17253), Chinese hamster (CHO, BAC80205), mouse (AAD00906), *Drosophila melanogaster* (AAF48028), *Arabidopsis thaliana* (NP_176916), and *Saccharomyces cerevisiae* (AAC49157). Positions of a mutation identified in CHO Lec23 cell (equivalent to S440F in human) and two mutations from a CDG IIb patient (R486T and F562L) are indicated by asterisks.



Supplementary figure S2. Fragmentation analysis by NSI-MSⁿ of a FOS at m/z 1742. (A) MS² fragmentation pattern of ion at m/z 1742 from *agl-1*(RNAi) animals, which is deduced to be a linear structure of Hex₇HexNAc₁ (Glc₃Man₄GlcNAc₁) based on the detection of a diagnostic fragment ion of Hex₆ at m/z 1261 (B-ion) and its counter ion at m/z 504 (Y-ion). (B) When an ion at m/z 1465 in (B) was subjected to MS³, a fragment ion at m/z 1261 was seen again. This provides further evidence that the main component of Hex₇HexNAc₁ from *agl-1*(RNAi) is a linear structure, Glc₃Man₄GlcNAc₁. (C) MS² fragment pattern of ion at m/z 1742 from control animals, which is deduced to be a branched structure of Hex₇HexNAc₁ (Glc₁Man₆GlcNAc₁/Man₇GlcNAc₁); note lack of 1261.



Supplementary figure S3. Fragmentation analysis by NSI-MSⁿ of a FOS at m/z 1947. (A) MS² fragmentation pattern of ion at m/z 1947 from *agl-1*(RNAi) animals, which is deduced to be a triglycosylated structure of Hex₈HexNAc₁ (Glc₃Man₅GlcNAc₁) based on the diagnostic B-ion of a fragment of Hex₆ at m/z 1261 and its counter Y-ion at m/z 708. MS³ fragment of an ion at m/z 1261 and at m/z 708 in (A) are shown in (B) and (C), respectively. (D) MS² fragmentation pattern of ion at m/z 1947 from control animals. Glc₁Man₇GlcNAc₁ is a possible structure fitting the composition Hex₈HexNAc₁ deduced from this fragmentation pattern.

Supplemental table I

Permethylated N-glycans from C. elegans wild-type and agl-1 knock-down detected by NSI-MS-FT(Orbitrap) in the positive ion mode

Assignment	Predicted m/z ^a			Observed m/z ^b			% of Total Profile ^c	
	[M+Na] ⁺	[M+2Na] ²⁺	[M+3Na] ³⁺	[M+Na] ⁺	[M+2Na] ²⁺	[M+3Na] ³⁺	Control	<i>agl-1</i> (RNAi)
Hex3HexNAc2	1171.58	597.29	405.85	1171.59	nd ^d	nd	15.5±2.9	4.7±0.2
Hex4HexNAc2	1375.68	699.34	473.89	1375.69	699.33	nd	2.9±0.4	0.9±0.1
Hex5HexNAc2	1579.78	801.39	541.92	1579.79	801.39	nd	9.5±0.7	4.4±0.2
Hex6HexNAc2	1783.88	903.44	609.95	1783.89	903.44	nd	4.6±0.2	1.7±0.1
Hex7HexNAc2	1987.98	1005.49	677.99	1988.99	1005.49	nd	5.2±0.4	1.8±0.1
Hex8HexNAc2	2192.08	1107.54	746.02	ol ^e	1108.04	nd	8.2±0.9	2.1±0.1
Hex9HexNAc2	2396.18	1209.59	814.05	ol	1210.09	814.39	17.5±1.5	7.5±0.1
Hex10HexNAc2	2600.28	1311.64	882.09	ol	1312.14	882.42	3.3±0.6	16.7±0.1
Hex11HexNAc2	2804.38	1413.69	950.12	ol	1414.19	950.46	0.1±0.0	24.4±0.1
Hex12HexNAc2	3008.48	1515.74	1018.15	ol	1516.24	1018.49	0.0±0.0	9.9±0.4
Hex8HexNAc2dHex1	2366.17	1194.58	804.05	ol	1195.58	nd	0.3±0.1	0.1±0.0
Hex9HexNAc2dHex1	2570.27	1296.63	872.08	ol	1297.13	nd	0.0±0.0	0.2±0.0
Hex10HexNAc2dHex1	2774.37	1398.68	940.12	ol	1399.18	940.45	0.3±0.2	2.1±0.1
Hex11HexNAc2dHex1	2978.47	1500.73	1008.15	ol	1501.23	1008.49	0.0±0.0	8.1±0.1
Hex12HexNAc2dHex1	3182.57	1602.78	1076.18	ol	1603.78	1076.52	0.1±0.0	0.1±0.0
Hex2HexNAc2dHex1	1141.57	582.28	395.85	1141.58	nd	nd	0.9±0.2	0.5±0.1
Hex3HexNAc2dHex1	1345.67	684.33	463.88	1345.68	684.33	nd	8.3±1.0	4.4±0.2
Hex4HexNAc2dHex1	1549.77	786.38	531.92	1549.78	786.38	nd	1.6±0.2	0.1±0.0
Hex5HexNAc2dHex1	1753.87	888.43	599.95	1753.88	888.43	nd	0.9±0.1	0.1±0.0
Hex3HexNAc2dHex2	1519.76	771.38	521.91	1519.76	771.4	nd	2.2±0.3	1.6±0.3
Hex4HexNAc2dHex2	1723.86	873.43	589.95	1723.87	873.43	nd	2.7±0.2	0.9±0.0
Hex5HexNAc2dHex2	1927.96	975.48	657.98	1927.97	975.48	nd	2.9±0.4	0.7±0.1
Hex6HexNAc2dHex2	2132.06	1077.53	726.01	ol	1077.53	nd	0.6±0.2	0.2±0.0
Hex4HexNAc2dHex3	1897.95	960.47	647.98	1897.95	960.47	nd	1.0±0.5	0.3±0.1
Hex5HexNAc2dHex3	2102.05	1062.52	716.01	ol	1062.52	nd	1.7±0.5	0.8±0.0
Hex6HexNAc2dHex3	2306.15	1164.57	784.04	ol	1164.57	nd	1.3±0.4	0.7±0.0
Hex7HexNAc2dHex3	2510.25	1266.62	852.08	ol	1266.62	nd	0.7±0.3	0.4±0.0
Hex4HexNAc2dHex4	2072.04	1047.52	706.01	ol	1048.02	nd	0.4±0.2	0.3±0.0
Hex5HexNAc2dHex4	2276.14	1149.56	774.04	ol	1150.07	nd	0.7±0.4	0.6±0.0
Hex6HexNAc2dHex4	2480.24	1251.61	842.07	ol	1252.12	nd	0.9±0.4	0.9±0.1
Hex7HexNAc2dHex4	2684.34	1353.66	910.11	ol	1354.17	nd	0.8±0.2	0.8±0.0
Hex3HexNAc3	1416.71	719.85	487.56	1416.71	719.85	nd	2.4±0.4	1.0±0.1
Hex4HexNAc3	1620.81	821.90	555.60	1620.81	nd	nd	0.1±0.0	0.0±0.0
Hex3HexNAc3dHex1	1590.80	806.89	545.59	1590.81	nd	nd	0.5±0.1	0.1±0.0
Hex3HexNAc4	1661.84	842.41	569.27	1661.84	842.41	nd	1.2±0.1	0.3±0.1
Hex3HexNAc4dHex1	1835.93	929.46	627.30	1835.92	929.46	nd	0.7±0.0	0.4±0.1

a, Monoisotopic mass

b, Most intense ion within detected isotopic peaks.

c, Percentages of total identified glycan ions. Values are shown as mean ± standard error for three independent samples.

d, Not detected.

e, Over limit ($m/z > 2000$)

Supplemental table II

Permethylated free oligosaccharides (FOS) from C. elegans wild-type and agl-1 knock-down detected by NSI-MS-FT (Orbitrap) in the positive ion mode

Assignment	Predicted m/z ^a			Observed m/z ^b			% of Total Profile ^c	
	[M+Na] ⁺	[M+2Na] ²⁺	[M+3Na] ³⁺	[M+Na] ⁺	[M+2Na] ²⁺	[M+3Na] ³⁺	Control	<i>agl-1</i> (RNAi)
FOS-GN1 species								
Hex2HexNAc1	722.36	372.67	256.11	722.36	nd ^d	nd	30.5±3.5	10.5±2.8
Hex3HexNAc1	926.46	474.72	324.15	926.46	474.72	nd	22.3±1.1	9.3±1.4
Hex4HexNAc1	1130.56	576.77	392.18	1130.56	576.77	nd	9.2±1.4	6.6±1.6
Hex5HexNAc1	1334.66	678.82	460.21	1334.66	678.82	nd	13.1±0.8	9.9±1.0
Hex6HexNAc1	1538.76	780.87	528.25	1538.76	780.87	nd	7.3±0.6	8.9±2.5
Hex7HexNAc1	1742.86	882.92	596.28	1742.86	882.92	nd	3.6±0.9	20.7±2.1
Hex8HexNAc1	1946.96	984.97	664.31	1946.96	984.97	nd	1.9±0.6	18.8±3.4
Hex9HexNAc1	2151.06	1087.02	732.35	ol ^e	1087.52	732.68	0.7±0.5	3.3±0.2
Hex10HexNAc1	2355.16	1189.07	800.38	ol	1189.58	800.71	0.1±0.1	2.8±1.2
Hex11HexNAc1	2559.26	1291.12	868.41	ol	1291.62	868.75	0.1±0.1	0.9±0.5
Hex12HexNAc1	2763.36	1393.17	936.45	ol	nd	936.92	0.0±0.0	0.2±0.2
FOS-GN2 species								
Hex2HexNAc2	967.48	495.24	337.82	967.48	495.24	nd	2.8±0.2	1.4±0.1
Hex3HexNAc2	1171.58	597.29	405.85	1171.58	597.29	nd	3.8±0.5	1.8±0.2
Hex4HexNAc2	1375.68	699.34	473.89	1375.68	699.34	nd	1.8±0.3	1.0±0.1
Hex5HexNAc2	1579.78	801.39	541.92	1579.78	801.39	nd	0.8±0.5	0.6±0.3
Hex6HexNAc2	1783.88	903.44	609.95	1783.89	903.44	nd	1.3±0.8	0.6±0.2
Hex7HexNAc2	1987.98	1005.49	677.99	1987.98	1005.99	677.99	0.4±0.3	1.6±0.6
Hex8HexNAc2	2192.08	1107.54	746.02	ol	1107.54	746.02	0.3±0.4	1.1±0.4
Hex9HexNAc2	2396.18	1209.59	814.05	ol	1209.57	nd	0.1±0.1	0.2±0.1

a, Monoisotopic mass

b, Most intense ion within detected isotopic peaks.

c, Percentages of total identified glycan ions. Values are shown as mean ± standard error for three independent samples.

d, Not detected.

e, Over limit ($m/z > 2000$)