Table S1

Disaccharide composition of CS chains from L, L-Wnt-3a, and L-Wnt-3a-C4ST1 transfectants

Values are expressed as pmol of disaccharide per mg of dried homogenates of these cells, and the means ± s.d. of three determinations

Composition	L	L-Wnt-3a	L-Wnt-3a-C4ST-1 clone 2	L-Wnt-3a-C4ST-1 clone 3	L-Wnt-3a-C4ST-1 clone 4	L-Wnt-3a-C4ST-1 clone 11
			pmol/mg (mol %)			
ΔDi-0S[a]	18.61±4.25（13.8）	8.17±0.52 (41.2)	13.50±1.88 (43.2)	13.26±0.52 (52.5)	14.02±0.94 (44.1)	13.24±2.10 (40.6)
ΔDi-6S	2.39±0.63 (1.8)	1.56±0.043 (7.9)	1.91±0.09 (6.2)	1.34±0.08 (5.3)	2.24±0.13 (7.0)	1.94±0.18 (6.0)
ΔDi-4S	99.46±22.72 (74.0)	9.24±0.66 (46.6)	13.62±1.19 (43.7)	9.62±0.19 (38.1)	13.64±0.31 (42.9)	14.27±2.29 (43.8)
ΔDi-diS_D	ND[b]	ND	ND	ND	ND	ND
ΔDi-diS_E	13.82±2.75 (10.4)	0.86±0.24 (4.3)	2.12±0.54 (6.9)	1.02±0.13 (4.1)	1.89±0.38 (6.0)	3.11±0.52 (9.6)
Total	134.27±30.03	19.83±0.41	31.15±2.87	25.22±0.41	31.79±1.07	32.57±5.00

[a]Abbreviations: ΔDi-0S, ΔHexUAα1-3GalNAc; ΔDi-6S, ΔHexUAα1-3GalNAc(6-*O*-sulfate); ΔDi-4S, ΔHexUAα1-3GalNAc(4-*O*-sulfate); ΔDi-diS_D, ΔHexUA(2-*O*-sulfate)α1-3GalNAc(6-*O*-sulfate); ΔDi-diS_E, ΔHexUAα1-3GalNAc(4-*O*-sulfate,6-*O*-sulfate)

[b]ND, not detected.

Supplementary Figure Legends

Fig. S1. (A) **Specific primer pairs for *human C4ST-1* and *GAPDH* used for measurement of the expression level of *C4ST-1* and *GAPDH* in HeLa cells cultured together with L or L-Wnt-3a cells hardly amplify *mouse C4ST-1* and *GAPDH*.** Total RNA was isolated from L, L-Wnt-3a, and HeLa cells, and then analyzed by real-time PCR using human primer pairs (top panel) or mouse primer pairs (bottom panel). (B) **HeLa cells can respond to Wnt-3a as well as L cells.** L and HeLa cells were treated with (+) or without (-) conditioned medium from L-Wnt-3a cells (L-Wnt-3a CM). After 2 h cells were lysed, and accumulated β-catenin in the cytosol was analyzed by immunoblotting.

Fig. S2. Strategy of luciferase reporter assay for measurement of secreted Wnt-3a. One day before transfection, C2C12 cells were seeded on a 24-well culture plate. 1 μg of pTCF7wt-luc as a reporter plasmid and 0.1 μg of pRL-TK (harboring thymidine kinase promoter just upstream of *Renilla* luciferase) as a reference plasmid were transfected into C2C12 cells using Lipofectamine 2000. After 12 h, cells were treated with the conditioned medium prepared from L, L-Wnt-3a, or L-Wnt-3a-C4ST-1 cells, and then cultured for 12 h. Firefly luciferase and *Renilla* luciferase activities were measured. “Relative activity” is defined as the ratio of firefly luciferase activity to *Renilla* luciferase activity.

Fig. S3. *C4ST-1* expression levels are positively correlated with E-disaccharide content and negatively correlated with Wnt-3a secretion. *C4ST-1* expression level versus E-disaccharide content (top panel), *C4ST-1* expression level versus secretion of Wnt-3a (middle panel), and E-disaccharide content versus secretion of Wnt-3a (bottom panel) were plotted.

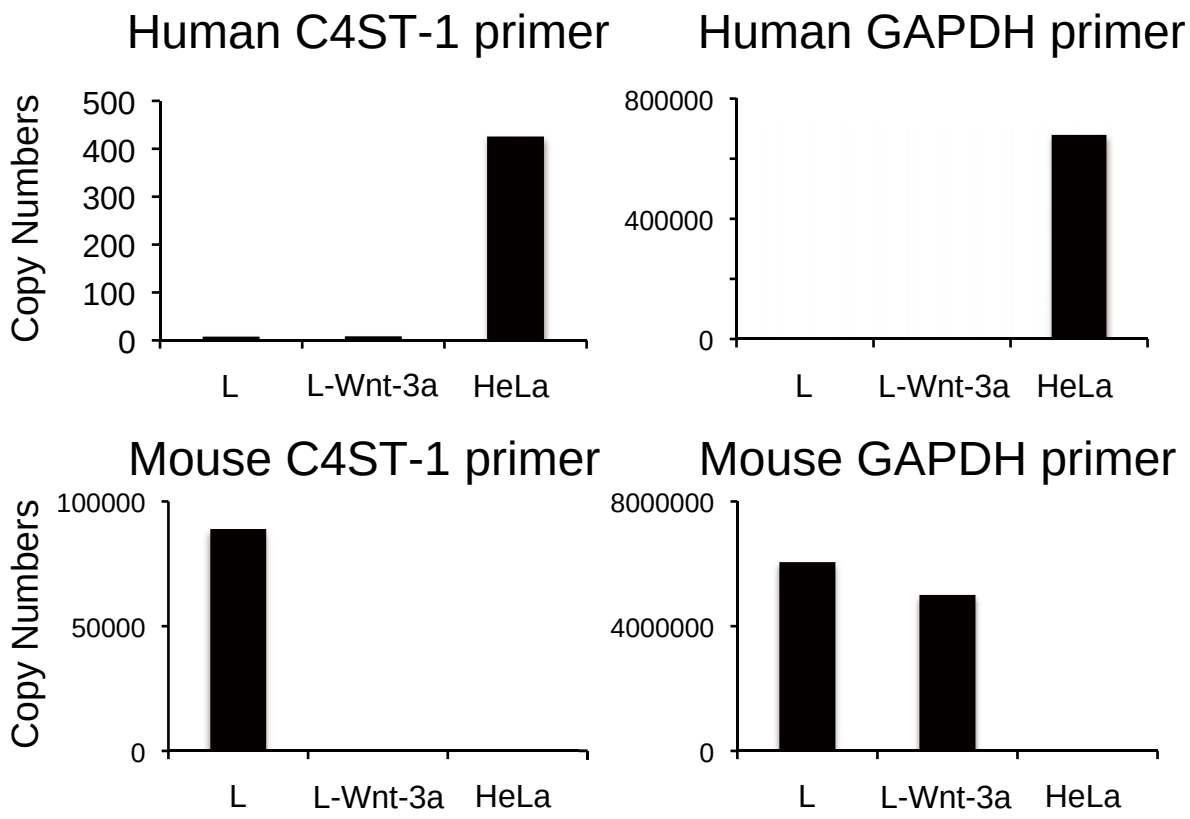
Fig. S4. Competitive inhibition assays by BIAcore using various CS isoforms and chemically synthesized tetrasaccharides as competitors. Recombinant mouse Wnt-3a (20 nM) was individually injected over the CS-E-immobilized sensor chip. Open and closed arrowheads indicate the start of the sample injection and the beginning of the dissociation phase initiated with running buffer, respectively. Values of the vertical axis, expressed in response units (RU), represent increases in mass concentration on the CS-E-sensor surface due to the binding of Wnt-3a analyte. Recombinant mouse Wnt-3a (20 nM) was pre-incubated with 20 μg/ml of CS-A, CS-C, CS-D, or CS-E for 0.5 h (A) or 20 μg/ml of tetrasaccharides (B), and

then injected over the CS-E-immobilized sensor chip. E-E tetra: GalNAc(4-*O*-sulfate,6-*O*-sulfate)-GlcUA-GalNAc(4-*O*-sulfate,6-*O*-sulfate)-GlcUA-*O*-*p*-methoxyphenyl, A-E tetra: GalNAc(4-*O*-sulfate)-GlcUA-GalNAc(4-*O*-sulfate,6-*O*-sulfate)-GlcUA-*O*-*p*-methoxyphenyl, C-C tetra: GalNAc(6-*O*-sulfate)-GlcUA-GalNAc(6-*O*-sulfate)-GlcUA-*O*-*p*-methoxyphenyl.

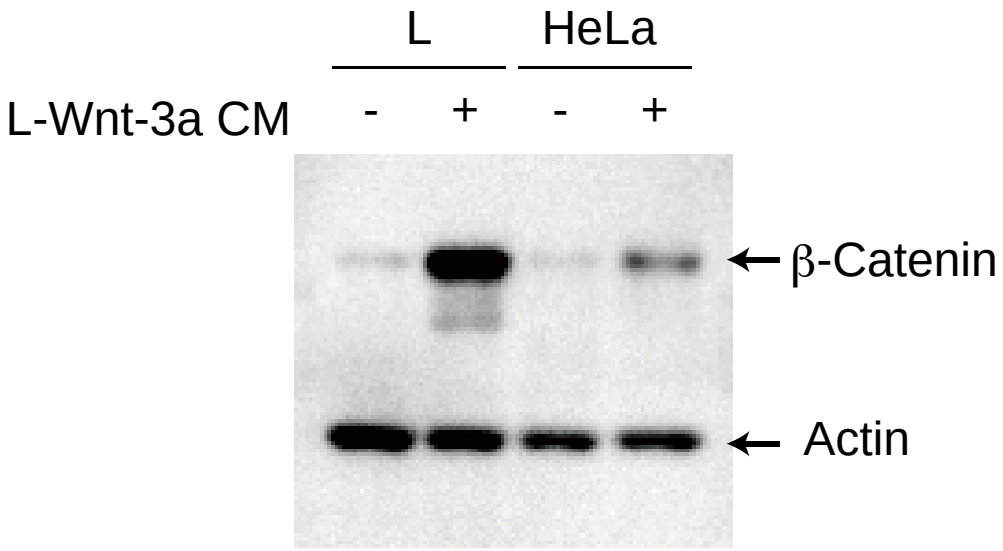
Fig. S5. Expression levels of β-catenin levels in the cytosol of L-Wnt-3a cells. Cytosolic β-catenin in L and L-Wnt-3a cells were extracted using 0.1% saponin buffer, and then analyzed by immunoblotting. The level of cytosolic β-catenin accumulation was measured as in Fig. 5*A*. The means from two independent experiments were plotted with the s.d. (error bars).

Supplementary Fig. S1

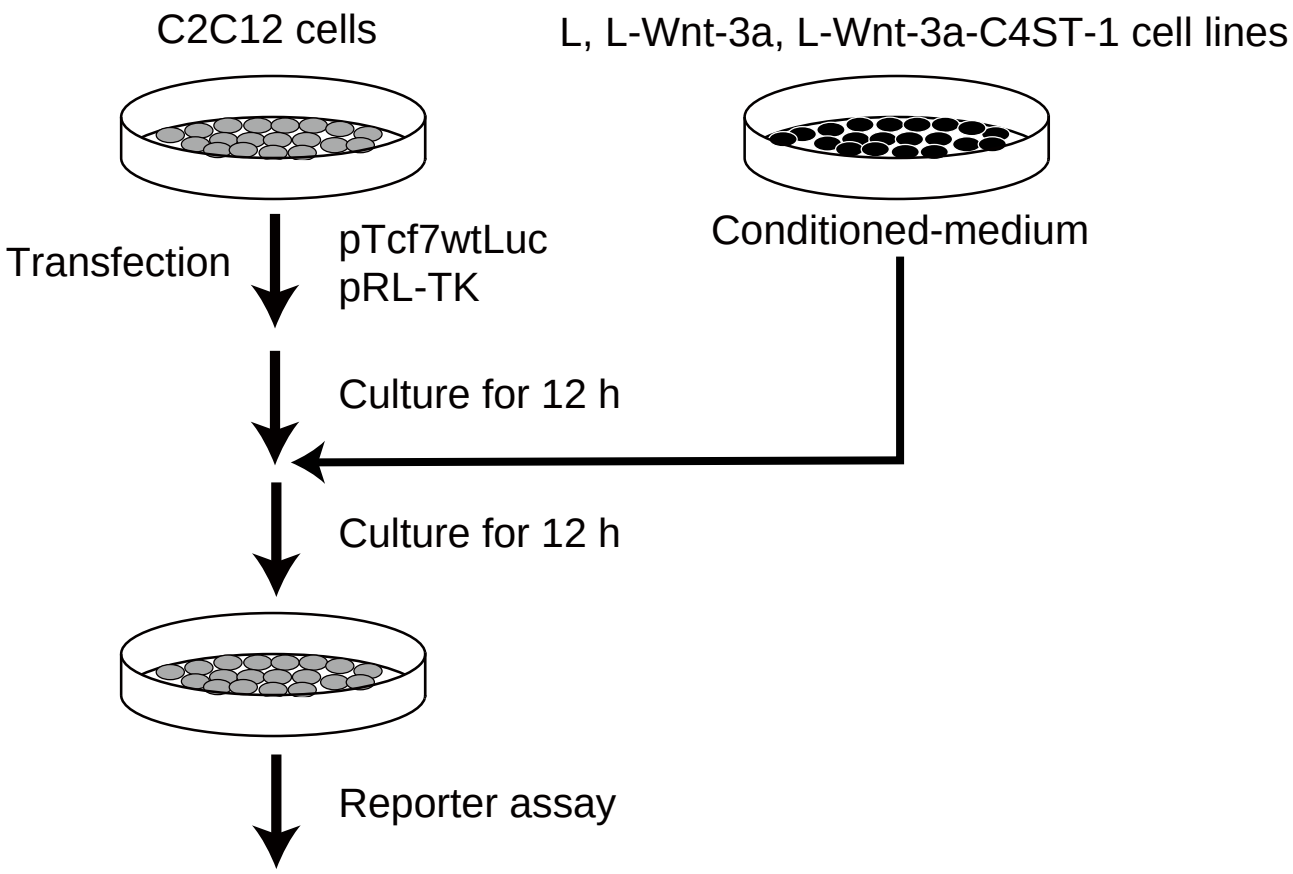
A



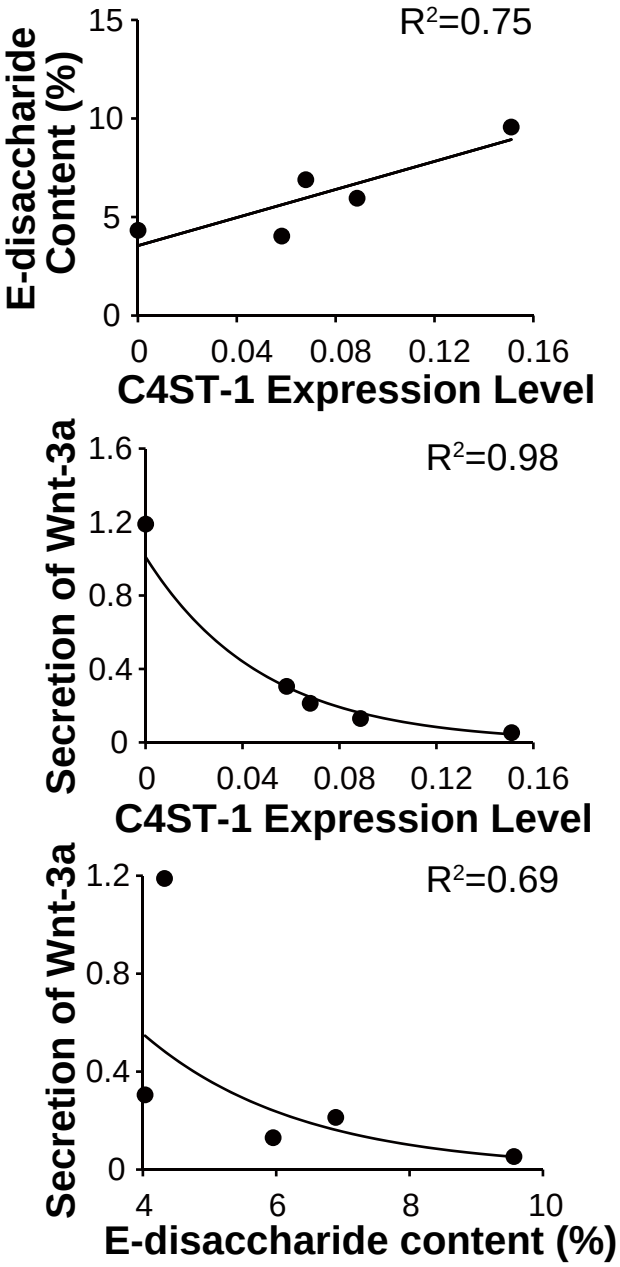
B



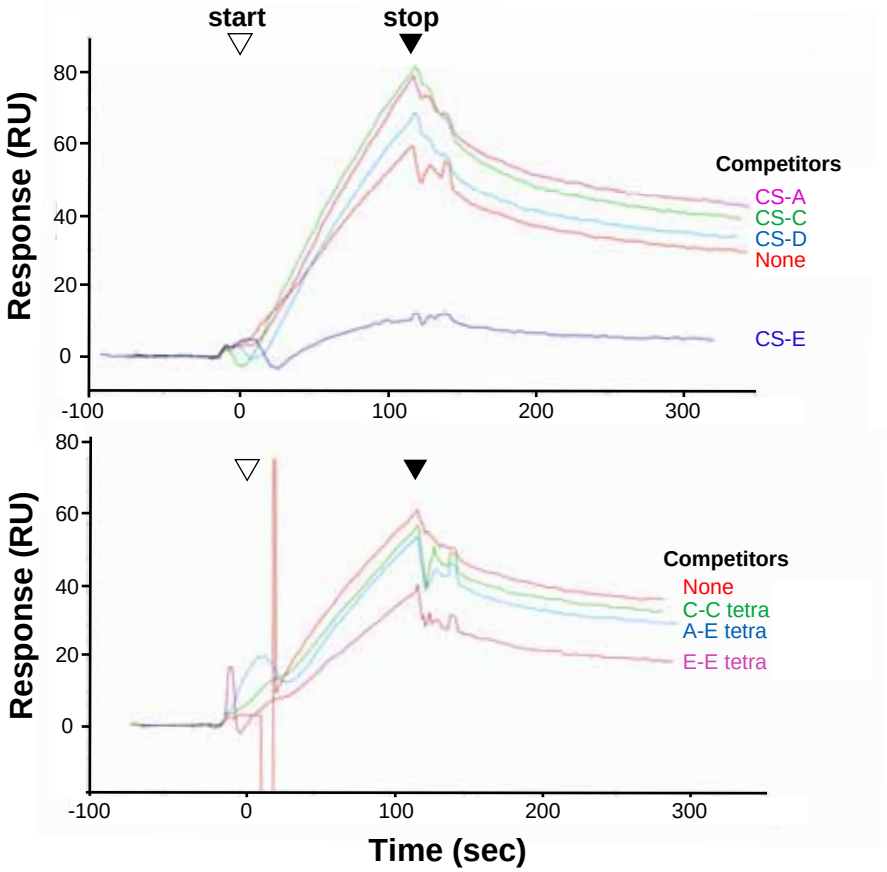
Supplementary Fig. S2



Supplementary Fig. S3



Supplementary Fig. S4



Supplementary Fig. S5