Supplementary Information

Altered sulfation status of FAM20C-dependent chondroitin sulfate is associated with osteosclerotic bone dysplasia.

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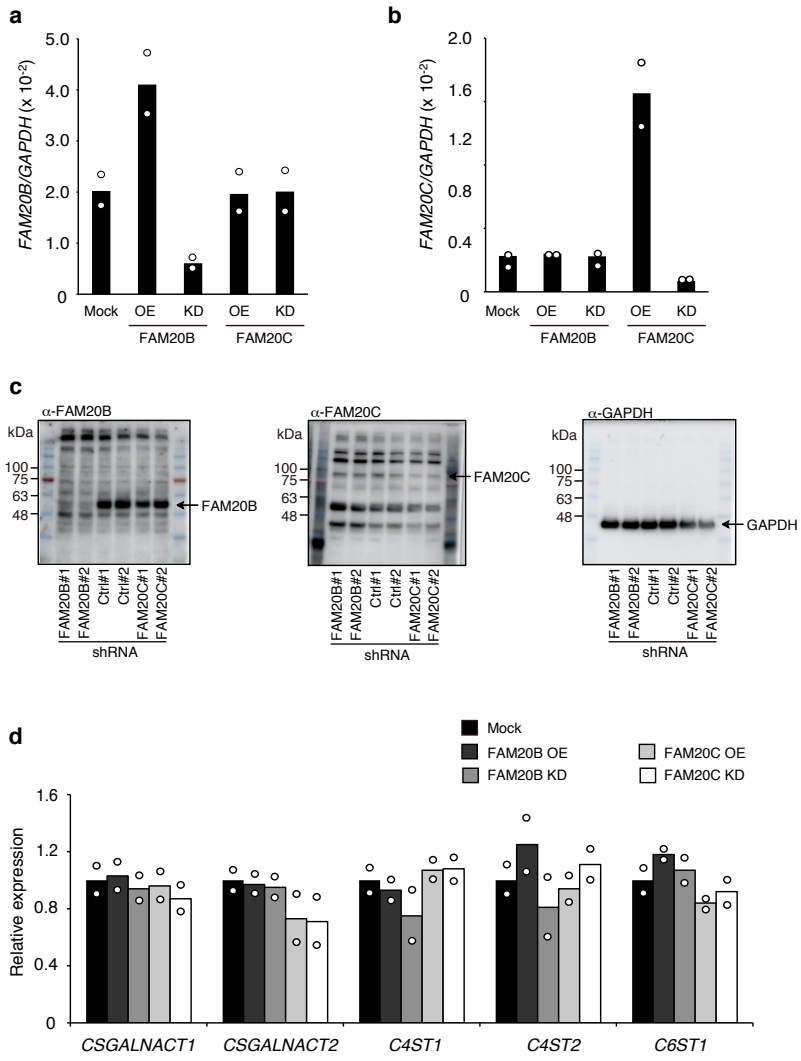
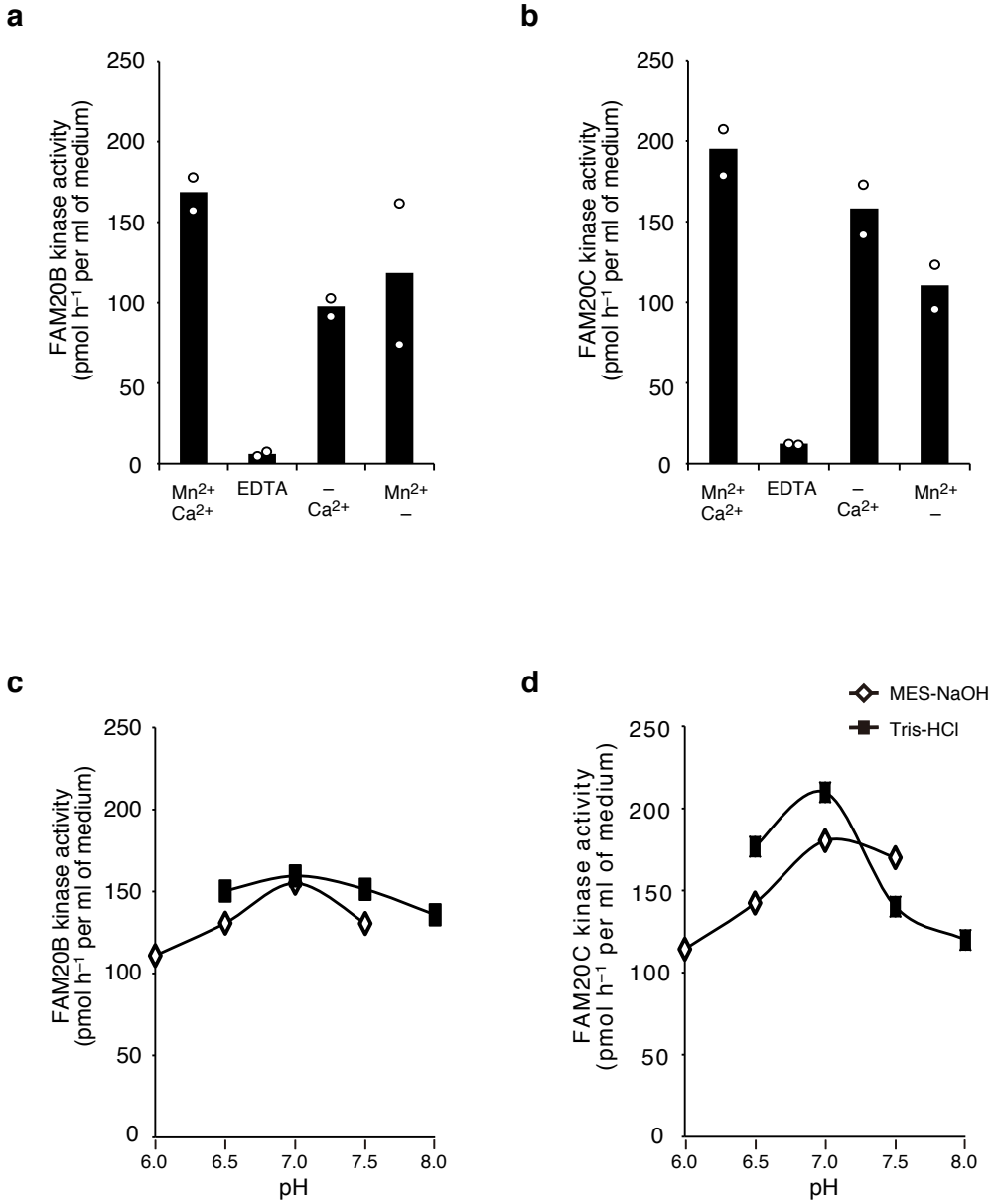
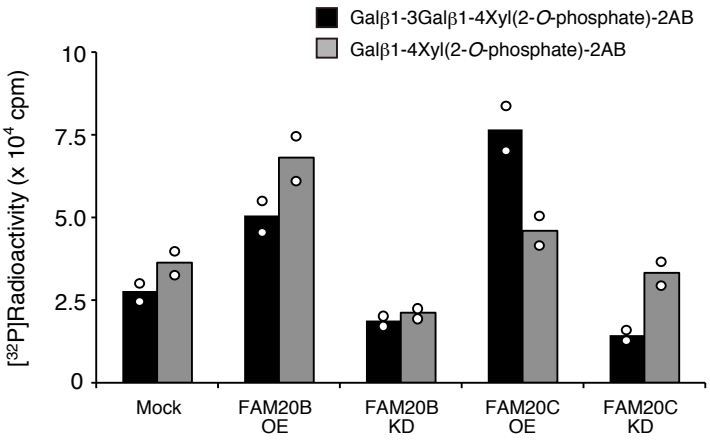


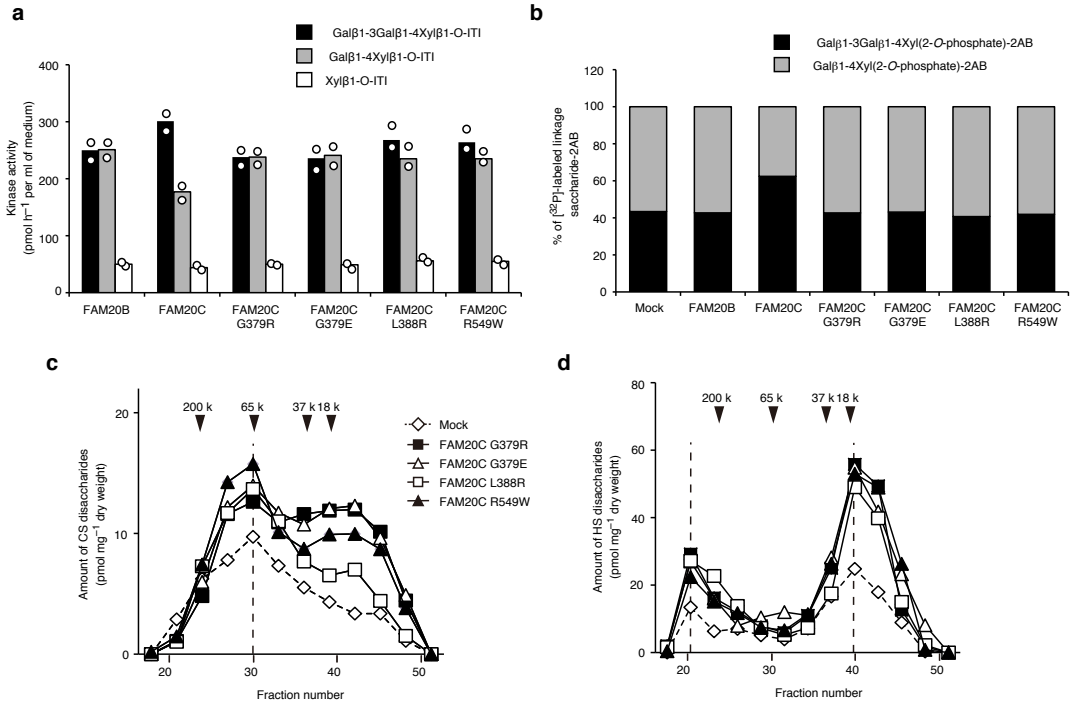
Supplementary Figure 1 Establishment of FAM20B-related or FAM20C-related stable HeLa cell lines. (**a**,**b**) Relative expression level of the transcripts for FAM20B (**a**) or FAM20C (**b**) (normalized to that of *GAPDH*) in mock-transfected (Mock), FAM20B overexpressing (OE), FAM20B knockdown (KD), FAM20C OE, and FAM20C KD HeLa cell lines (n =2 independent experiments). (**c**) Cell lysates from FAM20B KD (FAM20B#1 and #2), non-targeting control (Ctrl#1 and #2), and FAM20C KD (FAM20C#1 and #2) HeLa cell lines were analyzed by immunoblotting to evaluate knockdown efficiency for FAM20B and FAM20C. GAPDH was used as a loading control. Data are obtained from three independent experiments and representative images are shown. (**d**) Expression profiles of genes involved in CS biosynthesis in stable HeLa cell lines. The expression level of the respective transcripts was normalized to that of *GAPDH* (n = 2 independent experiments). Source data are provided as a Source Data file.



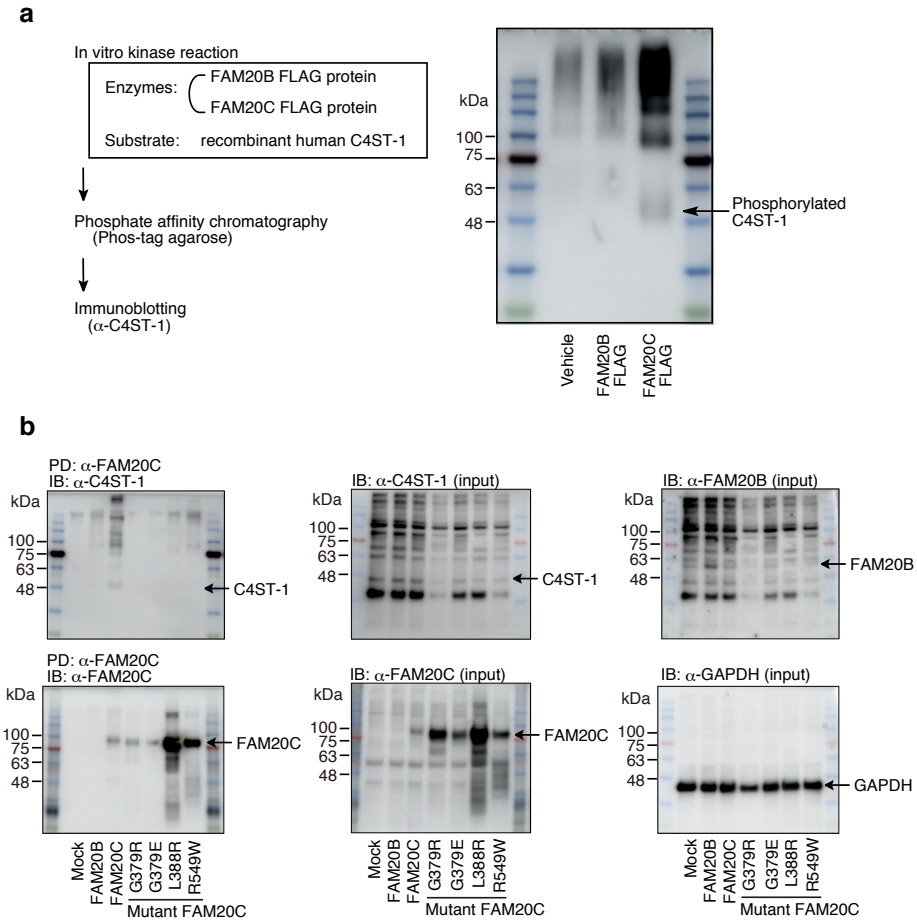
Supplementary Figure 2 Enzymatic characteristics of soluble forms of FAM20B and FAM20C. The metal ion dependence (**a**,**b**) and the pH dependence (**c**,**d**) for Xyl kinase activities of FAM20B (**a**,**c**) and FAM20C (**b**,**d**) toward GlcAβ1-3Galβ1-3Galβ1-4Xylβ1-*O*-SerGlyTrpProAspGly were determined under standard assay conditions in the presence of divalent cations or EDTA in different buffers. Data in **a** and **b** are the mean values from two independent experiments. Data in **c** and **d** correspond to raw data from single experiment. Source data are provided as a Source Data file.



Supplementary Figure 3 Detection of the GAG linkage saccharide intermediates from FAM20B-related or FAM20C-related stable HeLa cell lines. Data are the mean values from two independent experiments. Source data are provided as a Source Data file.

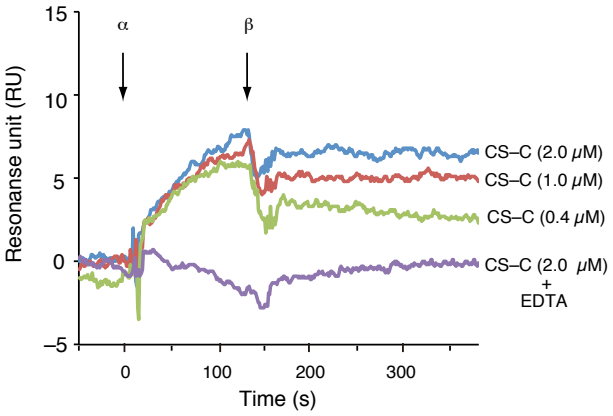


Supplementary Figure 4 XYLK activity-dependent GAG biosynthesis is not affected by Raine syndrome mutations in FAM20C. (**a**) Kinase activities of soluble forms of FAM20 proteins toward ITI preparations. Data are the mean values from two independent experiments. (**b**) The relative proportions of the linkage saccharide intermediates from stable HeLa cell lines overexpressing wild-type FAM20B, wild-type FAM20C or mutant FAM20C proteins. Data are the mean values from two independent experiments. (**c**, **d**) Gel filtration elution profiles of (**c**) CS and (**d**) HS chains from stable HeLa cell lines overexpressing each of the mutant FAM20C proteins. Numbered arrowheads (200 k, 65 k, 37 k, and 18 k) indicate the elution position of 200-, 65-, 37-, and 18-kDa saccharides derived from size-defined commercial dextran, respectively. The profile data for mock cells in **c** and **d** are identical to those in Figs. 1**d** and **e**, respectively. Results represent the average of two series of independent experiments. Source data are provided as a Source Data file.

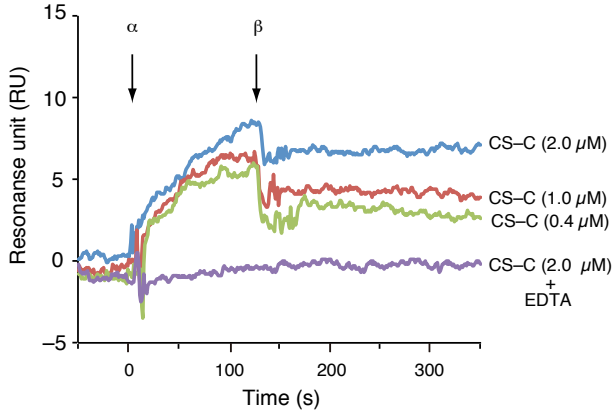


Supplementary Figure 5 FAM20C interacts with C4ST-1. (**a**) Recombinant C4ST-1 can be phosphorylated by a soluble form of FLAG-tagged FAM20C, but not that of FAM20B, *in vitro*. An *in vitro* kinase assay was performed by incubating recombinant C4ST-1 and the purified FLAG-tagged FAM20 proteins. The phosphorylated proteins in the reaction mixtures were isolated by phosphate affinity chromatography using phos-tag agarose. The resultant samples were analyzed by immunoblotting using an anti-C4ST-1 antibody (L18). Data are obtained from three independent experiments and a representative image is shown. (**b**) Wild-type FAM20C, but not its Raine syndrome mutants, can physically interact with endogenous C4ST-1 in Saos-2 cells. The anti-FAM20C immunoprecipitates from formaldehyde-treated cell lysates of stable Saos cell lines overexpressing FAM20 proteins were analyzed by immunoblotting using anti-C4ST-1 antibody (L18) and anti-FAM20C antibody. Data are obtained from three independent experiments and representative images are shown. Source data are provided as a Source Data file.

a



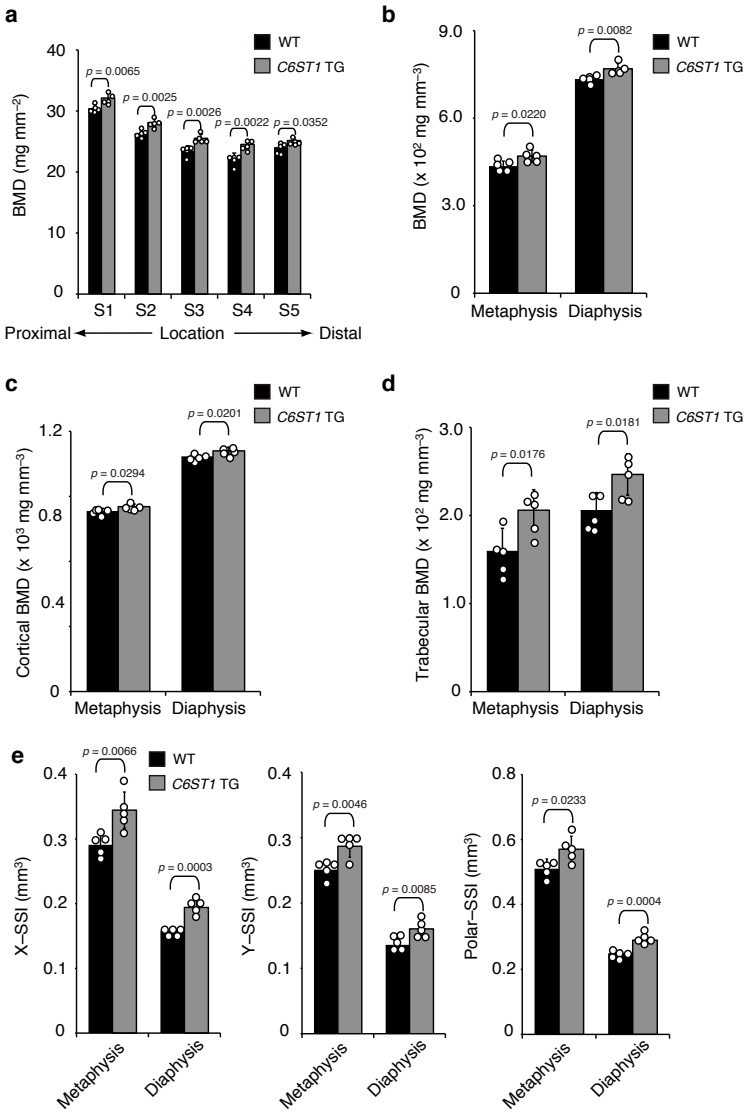
b



c

GAG vs protein	*ka*	*kd*	K_d
	M^{-1} S^{-1}	S^{-1}	nM
CS–C vs N-cadherin	4.0 x 10^3	6.8 x 10^{-4}	1.7 x 10^2
CS–C vs Cadherin-11	3.6 x 10^3	6.9 x 10^{-4}	1.9 x 10^2

Supplementary Figure 6 CS-C binds to N-cadherin and cadherin-11. (**a**,**b**) Overlaid sensorgrams for the binding of CS-C to immobilized N-cadherin (**a**) or cadherin-11 (**b**) in a BIAcore system. Various concentrations of CS-C were individually injected over the N-cadherin- or cadherin-11-immobilized sensor chip in the presence of 3 mM $CaCl_2$ or 3 mM EDTA. The beginning of the association and dissociation phases is marked by arrows labeled α and β, respectively. RU, resonance units. (**c**) Kinetic parameters for the interaction of CS-C with immobilized N-cadherin or cadherin-11. Values of the apparent association (*ka*) and dissociation (*kd*) rate constants, and equilibrium dissociation constants (*Kd*), are expressed as mean ± s.d. Source data are provided as a Source Data file.



Supplementary Figure 7 Assessment of the BMD of tibias from 16-week-old wild-type (WT) or *C6ST1* transgenic (TG) male mice. (**a**) BMD of each of 5 equal longitudinal divisions of the tibias were determined by peripheral quantitative computed tomography, pQCT (n = 5 bones from total, each from different litters, unpaired Student's *t* test, two-sided). (**b-d**) Dual energy X-ray absorptiometry (DXA)-derived measurements of BMD at the tibial metaphysis and diaphysis (n = 5 bones from total, each from different litters, unpaired Student's *t* test, two-sided). (**e**) X-axis, Y-axis, and polar stress-strain indexes (SSIs) of tibias from 16-week-old WT or *C6ST1* TG male mice were calculated as surrogate measurements of bone mechanical strength (n = 5 bones from total, each from different litters, unpaired Student's *t* test, two-sided). Data in **a-e** are represented as mean ± s.d. Source data are provided as a Source Data file.

Supplementary Table 1 Disaccharide composition of GAG chains from stable clones of HeLa cells.

	pmol mg^{-1} (mol%)[a]						
CS disaccharides	FAM20C KD	Mock	FAM20C OE	FAM20C G379R	FAM20C G379E	FAM20C L388R	FAM20C R549W
ΔHexA[b]-GalNAc	1.1 ± 1.0 (3)	1.7 ± 0.3 (3)	1.7 ± 0.5 (2)	2.1 ± 0.6 (3)	3.5 ± 0.4 (4)	1.1 ± 0.1 (2)	3.1 ± 2.4 (3)
ΔHexA-GalNAc(6S)	7.0 ± 0.8 (20)	8.7 ± 1.5 (17)	9.5 ± 1.1 (10)	15.3 ± 1.0 (18)	16.6 ± 0.8 (18)	14.3 ± 1.2 (20)	17.8 ± 1.7 (20)
ΔHexA-GalNAc(4S)	24.9 ± 0.6 (71)	37.9 ± 4.0 (74)	73.0 ± 11.0 (79)	61.1 ± 4.0 (73)	66.2 ± 3.7 (72)	52.4 ± 2.3 (73)	65.2 ± 5.7 (72)
ΔHexA(2S)-GalNAc(6S)	0.7 ± 0.3 (2)	1.2 ± 0.3 (2)	0.7 ± 0.6 (1)	1.8 ± 0.2 (2)	2.0 ± 0.2 (2)	1.5 ± 0.5 (2)	1.6 ± 0.3 (2)
ΔHexA-GalNAc(4S,6S)	1.1 ± 0.4 (3)	1.9 ± 0.7 (4)	7.5 ± 1.6 (8)	3.5 ± 0.4 (4)	3.6 ± 0.4 (4)	2.6 ± 0.5 (4)	3.1 ± 0.3 (3)
Total CS disaccharides	34.9 ± 1.8 (100)	51.4 ± 3.7 (100)	92.4 ± 9.7 (100)	83.7 ± 3.7 (100)	91.9 ± 2.3 (100)	71.8 ± 4.2 (100)	91.0 ± 5.4 (100)
HS disaccharides	FAM20C KD	Mock	FAM20C OE	FAM20C G379R	FAM20C G379E	FAM20C L388R	FAM20C R549W
ΔHexA-GlcNAc	33.6 ± 2.2 (48)	49.4 ± 5.7 (44)	101.1 ± 2.9 (50)	111.3 ± 4.1 (49)	116.6 ± 9.0 (48)	92.6 ± 2.2 (47)	112.1 ± 5.0 (52)
ΔHexA-GlcNAc(6S)	0.7 ± 0.4 (1)	1.8 ± 0.5 (2)	2.3 ± 0.7 (1)	2.3 ± 0.7 (1)	2.4 ± 0.4 (1)	2.0 ± 0.2 (1)	2.3 ± 0.6 (1)
ΔHexA-GlcN(NS)	20.3 ± 6.1 (29)	36.2 ± 6.0 (32)	72.2 ± 1.7 (36)	79.5 ± 12.1 (35)	87.5 ± 9.4 (36)	69.0 ± 2.8 (35)	81.8 ± 4.6 (38)
ΔHexA-GlcN(NS,6S)	0.8 ± 0.4 (1)	2.5 ± 0.8 (2)	4.5 ± 0.9 (2)	4.5 ± 1.2 (2)	4.9 ± 0.3 (2)	3.9 ± 0.2 (2)	4.7 ± 1.1 (2)
ΔHexA(2S)-GlcN(NS)	7.7 ± 3.6 (11)	10.9 ± 1.3 (10)	13.8 ± 7.7 (7)	18.2 ± 1.8 (8)	19.4 ± 2.3 (8)	15.8 ± 0.2 (8)	18.7 ± 1.5 (8)
ΔHexA(2S)-GlcN(6S,NS)	6.9 ± 3.7 (10)	12.2 ± 1.8 (11)	9.7 ± 1.1 (5)	11.4 ± 2.3 (5)	12.2 ± 0.7 (5)	13.8 ± 1.9 (7)	14.0 ± 1.7 (6)
Total HS disaccharides	69.9 ± 9.9 (100)	113.0 ± 13.3 (100)	203.6 ± 13.2 (100)	227.2 ± 18.3 (100)	243.0 ± 19.8 (100)	197.0 ± 5.3 (100)	233.6 ± 14.1 (100)
Relative expression[c]	0.3	1.0	5.4	9.4	10.9	4.5	9.5

[a]The values are expressed as pmol of disaccharide per mg of dried homogenate and represent the mean ± s.d. (n = 3).

[b]ΔHexA, GalNAc, GlcNAc, and GlcN represent unsaturated hexuronic acid, *N*-acetylgalactosamine, *N*-acetylglucosamine, and glucosamine, respectively; 6S, 4S, 2S, and NS represent 6-*O*-sulfate, 4-*O*-sulfate, 2-*O*-sulfate, and 2-*N*-sulfate, respectively.

[c]Quantitative real-time RT-PCR was used to measure relative amounts of the *FAM20C* transcript. *FAM20C* mRNA levels were normalized to that of *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase).

Source data are provided as a Source Data file.

Supplementary Table 2 Comparison of kinase activities of fusion proteins secreted into the culture medium by the transfected COS-1 cells.

Acceptor substrate	FAM20B	FAM20C	FAM20B(D309G)	FAM20B(D478G)
	Kinase activity (pmol h^{-1} per ml of medium)[a]			
α-TM[b]	171.2 ± 17.6	188.6 ± 18.1	–[c]	–
GlcAβ1-3Galβ1-3Galβ1-4Xylβ1-*O*-SerGlyTrpProAspGly	155.6 ± 2.9	178.2 ± 46.1	ND[d]	ND
Galβ1-3Galβ1-4Xylβ1-*O*-Ser	50.2 ± 3.5	57.7 ± 14.0	–	–
Galβ1-3Galβ1-4Xyl(2-*O*-phosphate)β1-*O*-Ser	ND	ND	–	–

[a]The values are the mean ± s.d. For FAM20B and FAM20C, n = 3; For FAM20B(D309G) and FAM20C(D478G), n = 2.

[b]α-TM contains a tetrasaccharide linkage, GlcAβ1-3Galβ1-3Galβ1-4Xyl[58].

[c]–, not tested.

[d]ND, not detected (< 0.1 pmol h^{-1} per ml of medium).

Source data are provided as a Source Data file.

Supplementary Table 3 Comparison of kinase activities of FAM20B and FAM20C toward ITI preparations.

Acceptor substrate	FAM20B	FAM20C
	Kinase activity (pmol h^{-1} per ml of medium) [a]	
Galβ1-3Galβ1-4Xylβ1-*O*-ITI	249.8 ± 33.0	298.6 ± 18.3
Galβ1-4Xylβ1-*O*-ITI	251.2 ± 32.4	176.7 ± 21.4
Xylβ1-*O*-ITI	49.9 ± 11.6	44.1 ± 8.9

[a]The values are the mean ± s.d. (n = 3).

Source data are provided as a Source Data file.

Supplementary Table 4 Disaccharide composition of CS chains from stable clones of murine fibroblastic L and its mutant cell line, sog9 cells.

	pmol mg^{-1} (mol%)[a]				
CS disaccharides	L-mock	L-FAM20B-1	L-FAM20B-2	L-FAM20C-1	L-FAM20B-2
ΔHexA-GalNAc	36.9 ± 0.7 (10)	45.2 ± 2.8 (9)	48.6 ± 6.7 (10)	35.0 ± 2.8 (8)	33.5 ± 0.7 (6)
ΔHexA-GalNAc(6S)	11.2 ± 0.2 (3)	18.1 ± 1.3 (4)	22.8 ± 3.7 (5)	10.9 ± 2.7 (2)	11.9 ± 2.7 (2)
ΔHexA-GalNAc(4S)	275.5 ± 1.7 (76)	365.3 ± 13.4 (76)	374.3 ± 46.7 (74)	348.8 ± 16.8 (78)	442.5 ± 14.5 (80)
ΔHexA(2S)-GalNAc(6S)	ND[b]	ND	ND	ND	ND
ΔHexA-GalNAc(4S,6S)	41.1 ± 1.0 (11)	54.9 ± 4.1 (11)	57.6 ± 7.9 (11)	51.1 ± 3.0 (11)	65.2 ± 1.3 (12)
Total CS disaccharides	364.8 ± 1.1 (100)	483.5 ± 21.4 (100)	503.3 ± 64.9 (100)	445.7 ± 19.0 (100)	553.1 ± 14.7 (100)
Relative expression of *FAM20B*[c]	1.0	2.0	3.5	1.0	1.1
Relative expression of *FAM20C*[c]	1.0	1.2	1.0	2.1	3.6
CS disaccharides	sog9-mock	sog9-FAM20B-1	sog9-FAM20B-2	sog9-FAM20C-1	sog9-FAM20B-2
ΔHexA-GalNAc	44.2 ± 2.0 (50)	67.4 ± 2.7 (60)	73.8 ± 7.1 (60)	76.3 ± 2.5 (62)	81.4 ± 7.1 (63)
ΔHexA-GalNAc(6S)	15.2 ± 0.3 (17)	21.0 ± 1.7 (19)	23.9 ± 1.4 (19)	22.2 ± 1.9 (18)	24.1 ± 0.6 (19)
ΔHexA-GalNAc(4S)	27.9 ± 1.0 (32)	23.9 ± 0.4 (21)	24.4 ± 2.8 (20)	23.2 ± 1.0 (19)	23.0 ± 2.9 (18)
ΔHexA(2S)-GalNAc(6S)	ND	ND	ND	ND	ND
ΔHexA-GalNAc(4S,6S)	1.0 ± 0.2 (1)	0.6 ± 0.2 (1)	1.0 ± 0.3 (1)	1.1 ± 0.1 (1)	0.9 ± 0.4 (1)
Total CS disaccharides	88.2 ± 3.5 (100)	113.0 ± 3.3 (100)	123.1 ± 11.5 (100)	122.9 ± 1.6 (100)	129.5 ± 6.9 (100)
Relative expression of *FAM20B*	1.0	2.3	3.1	1.2	1.1
Relative expression of *FAM20C*	1.0	1.0	1.1	2.6	3.6

[a]The values are expressed as pmol of disaccharide per mg of dried homogenate and represent the mean ± s.d. (n = 3).

[b]ND, not detected.

[c]Quantitative real-time RT-PCR was used to measure relative amounts of the *FAM20B/Fam20b* or *FAM20C/Fam20c* transcripts. The respective mRNA levels were normalized to that of *Gapdh*.

Source data are provided as a Source Data file.

Supplementary Table 5 Disaccharide composition of CS chains from stable clones of MC3T3-E1 cells.

	pmol mg^{-1} (mol%)[a]			
CS disaccharides	Parental	Mock	C6ST-1 OE #1	C6ST-1 OE #2
ΔHexA-GalNAc	22.0 ± 3.0 (5)	24.4 ± 1.4 (6)	22.8 ± 1.0 (5)	20.3 ± 2.2 (5)
ΔHexA-GalNAc(6S)	7.3 ± 0.6 (2)	7.2 ± 0.6 (2)	11.0 ± 0.9 (3)	16.4 ± 3.8 (4)
ΔHexA-GalNAc(4S)	366.4 ± 24.0 (90)	377.1 ± 22.5 (90)	376.3 ± 2.8 (89)	383.1 ± 18.8 (89)
ΔHexA(2S)-GalNAc(6S)	ND[b]	ND	ND	ND
ΔHexA-GalNAc(4S,6S)	11.8 ± 1.2 (3)	12.6 ± 1.3 (3)	12.2 ± 1.4 (3)	12.1 ± 1.2 (3)
Total CS disaccharides	407.6 ± 28.6 (100)	421.3 ± 24.5 (100)	422.4 ± 2.9 (100)	431.9 ± 20.4 (100)
Relative expression[c]	1.0	1.0	1.8	2.7

[a]The values are expressed as pmol of disaccharide per mg of dried homogenate and represent the mean ± s.d. (n = 3).

[b]ND, not detected.

[c]Quantitative real-time RT-PCR was used to measure relative amounts of the *C6st1* transcript. *C6st1* mRNA levels were normalized to that of *Gapdh*.

Source data are provided as a Source Data file.

Supplementary Table 6 Disaccharide composition of CS chains from BMSCs derived from wild-type or *C6ST1* transgenic mice.

CS disaccharides	pmol mg^{-1} (mol%)[a]	
	Wild-type	*C6ST1* transgenic
ΔHexA-GalNAc	7.3 ± 2.0 (1)	6.3 ± 2.7 (1)
ΔHexA-GalNAc(6S)	14.3 ± 3.2 (2)	49.5 ± 5.7 (6)
ΔHexA-GalNAc(4S)	783.6 ± 31.9 (94)	716.4 ± 58.3 (89)
ΔHexA(2S)-GalNAc(6S)	2.5 ± 0.8 (0)	8.0 ± 2.3 (1)
ΔHexA-GalNAc(4S,6S)	29.5 ± 1.4 (4)	22.2 ± 4.8 (3)
Total CS disaccharides	837.2 ± 38.8 (100)	802.4 ± 73.3 (100)

[a]The values are expressed as pmol of disaccharide per mg of dried homogenate and represent the mean ± s.d. (n = 3).

Source data are provided as a Source Data file.

Supplementary Table 7 Antibodies used in this study

Antibody (clone)	Source of species	Cat. No.	Dilution etc.	Applications	Suppliers
Anti-FLAG (M2)	mouse	#F1804	1:1,000	Western blotting	Sigma-Aldrich
Anti-His-tag	rabbit	#2365	1:1,000	Western blotting	CST
Anti-FAM20C	rabbit	#25395-1-AP	1:1,000	Western blotting	Proteintech
			1 μg/sample	Immunoprecipitation	
Anti-FAM20B (1018512)	mouse	#MAB8427	1:250	Western blotting	R&D systems
Anti-C4ST-1 (L18)	mouse	#sc-100868	1:200	Western blotting	Santa Cruz
Anti-GAPDH (5A12)	mouse	#014-25524	1:1,000	Western blotting	Fujifilm Wako
Anti-N-cadherin (GC4)	mouse	#C3865	5 ng ml^{-1}	Neutralization	Sigma-Aldrich
Anti-cadherin-11 (16G5)	mouse	#ab151446	5 ng ml^{-1}	Neutralization	Abcam
Anti-ERK1/2	rabbit	#9102	1:1,000	Western blotting	CST
Anti-phospho-ERK1/2	rabbit	#9101	1:1,000	Western blotting	CST
Anti-Smad1	rabbit	#9743	1:1,000	Western blotting	CST
Anti-phospho-Smad1/5/8	rabbit	#9511	1:1,000	Western blotting	CST
Anti-Smad3	rabbit	#9523	1:1,000	Western blotting	CST
Anti-phospho-Smad3	rabbit	#9520	1:1,000	Western blotting	CST
Anti-mouse IgG HRP-linked	sheep	#NA931	1:5,000	Western blotting	Cytiva
Anti-rabbit IgG HRP-linked	donkey	#NA934	1:10,000	Western blotting	Cytiva
Anti-goat IgG HRP-linked	mouse	#sc-2354	1:5,000	Western blotting	Santa Cruz

Supplementary Table 8 Primers used for construction of expression plasmids

Construct		Forward/sense (5'–3')	Reverse/antisense (5'–3')
pCMV-FAM20Cs		CAAGATCT[a]GGCAGAACGCGCTCCAGG	CAAGATCTCCCCTCTGTCTCTGTCCC
pCMV-FAM20C (G379R)	IM[b]	CACGCCCTGTGCAGGAAGCCAGACC	GGTCTGGCTTCCTGCACAGGGCGTG
pCMV-FAM20C (G379E)	IM	CACGCCCTGTGCGAGAAGCCAGACC	GGTCTGGCTTCTCGCACAGGGCGTG
pCMV-FAM20C (L388R)	IM	TCGAGGGCTCGCGGGCGGCCTTCCT	AGGAAGGCCGCCCGCGAGCCCTCGA
pCMV-FAM20C (R549W)	IM	GCCCTGGACCGGTGGCTCCGCGTCGT	ACGACGCGGAGCCACCGGTCCAGGGC
pEF-BOS/IP-FAM20B		CGGGATCCTCAGCTGCCAACCGGGAGGAC	CGGGATCCACTTCCAATCCATCTCATACC
pEF-BOS/IP-FAM20B (D309G)	IM	GCTCATCCTTCTTGGTAATGCCAAAAGCTT	AAGCTTTTGGCATTACCAAGAAGGATGAGC
pEF-BOS/IP-FAM20Cs		CAAGATCTGAGCCCGGCTGTTCGTG	CAAGATCTCCCCTCTGTCTCTGTCCC
pEF-BOS/IP-FAM20C (D478G)	IM	TTCATCATCCACTTAGGCAATGGAAGAGGG	CCCTCTTCCATTGCCTAAGTGGATGATGAA
p3XFLAG-CMV-8-FAM20B		GCTCTAGATCAGCTGCCAACCGGGAGGAC	GCTCTAGAACTTCCAATCCATCTCATACC
p3XFLAG-CMV-8-FAM20Cs		GCTCTAGAGAGCCCGGCTGTTCGTG	GCTCTAGACCCCTCTGTCTCTGTCCC
pcDNA3Ins-His-C4ST-1		GCTCTAGACTGCAGGAACTCTACAAC	CGGGATCCTAAAAAGCATGATTCTCTC
pEF-BOS/IP-XylT-1		GAAGATCTGACAGCAACAACGAGAACTCTG	GAAGATCTCTTTCCCGTTGAGATCCTGCTG
pEF-BOS/IP-GalT-I		CGGGATCCTCTGGGGACGTGGCCCGG	CGGGATCCTCAGCTGAATGTGCACCA
pEF-BOS/IP-GalT-II		CCGGATCCGAGCCCGGGGACCCCAGG	CGGGATCCTCAGGGGATGCCCTCCCTTCT

[a]The underlines indicate restriction enzyme sites.

[b]IM, internal mutagenic primers.

Supplementary Table 9 Primers used for quantitative real-time RT-PCR.

Human gene	Forward (5'–3')	Reverse (5'–3')
GAPDH	ATGGGTGTGAACCATGAGAAGTA	GGCAGTGATGGCATGGAC
FAM20B[a]	GCCTTAAAATCTGCCATGGC	GGCATCCTGTCTTCCACCAG
FAM20C[a]	GAGAGGCACAATGCGGAGAT	GGATCTCCTTGGTCATGTTG
CSGALNACT1	AGAAGAAATAAATGAAGTCAAAGGAATAC	GAAGTAGATGTCCACATCACAG
CSGALNACT2	CCTAGAATCTGTCACCAGT	GTTAAGGAATTCGGCTGAGAAATA
C4ST1	AAACGCCAGCGGAAGAA	GGGATGGCAGAGTGAGTAGA
C4CT2	GAGGGAAAGTTCTTTGTTTAAGTG	CGGCCTTAACAGCCATAAT
C6ST1	TCTGCCATTGGCTTGAAC	CATGCAGACATGAAATAGCAAAC
Mouse gene	Forward (5'–3')	Reverse (5'–3')
Gapdh	CATCTGAGGGCCCACTG	GAGGCCATGTAGGCCATGA
C6st1	CTGGCATTTGTGGTCATAGTTT	AAGAGAGATGCATTCTCCGATAAG
Akp2	CCTGACTGACCCTTCGC	GTCAAGGTGTCTTTCTGGGA

[a]These primer sets are applicable for quantitative real-time RT-PCR of the respective mouse counterparts.