Supporting Information

Role of Glycosyltransferase 25 domain 1 in Type I Collagen Glycosylation and Molecular Phenotypes

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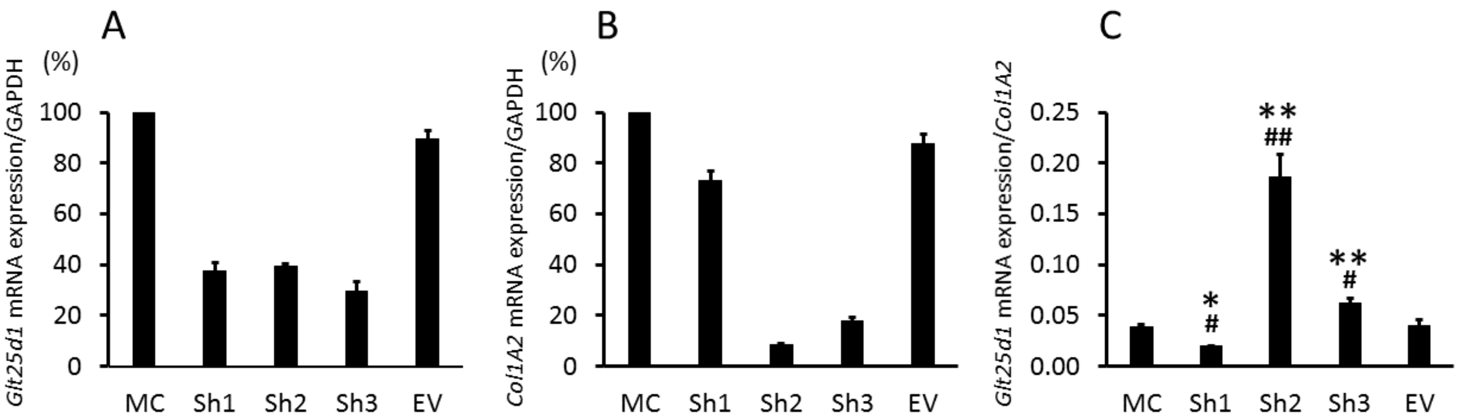

Figure S1. Gene expression of *Glt25d1* from transient transfection of short hairpin constructs targeting *Glt25d1*. Quantitative real-time PCR was performed to determine the suppression levels of *GLT25d1* and the expression of *Col1A2*. A, The *Glt25d1* mRNA expression was normalized to the expression of internal control (GAPDH). The non-transfected MC cells was 100%. B, The *Col1A2* mRNA expression was normalized to the expression of GAPDH. The non-transfected MC cells was 100%. C, The *Glt25d1* mRNA expression was normalized to the expression of *Col1A2*. *Error bars* indicate S.D. of three independent experiments. [*], significantly different from MC ($p<0.05$); [**], significantly different from MC ($p<0.01$); [#], significant different from EV ($p<0.05$); [##], significant different from EV ($p<0.01$); *Glt25d1*, glycosyltransferase 25 domain 1; Sh, short hairpin.

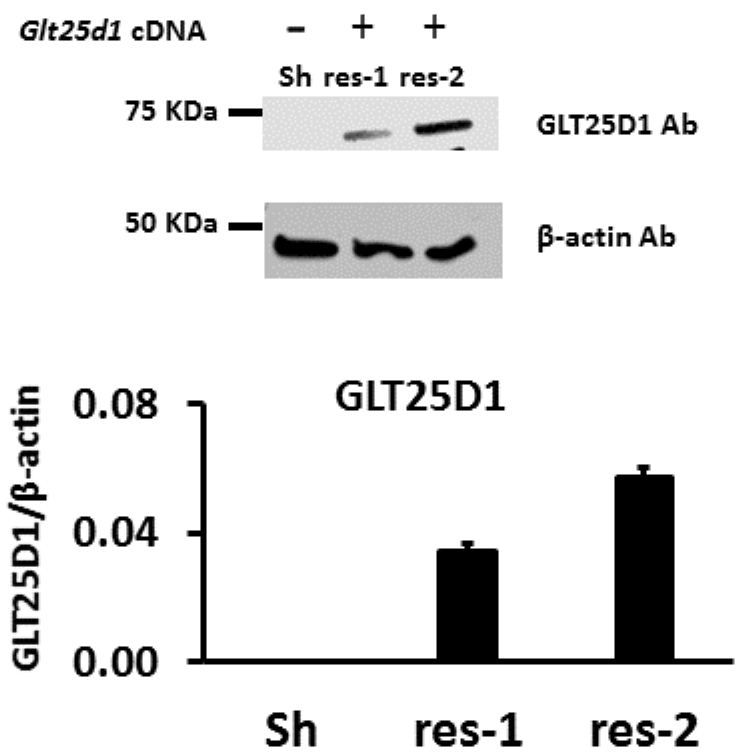

Figure S2. GLT25D1 levels produced by Sh clones with and without overexpression of *Glt25d1* cDNA. Western blot analyses were performed with cell lysates using anti-GLT25D1 and anti-β-actin antibodies. Ten micrograms of total protein from each sample was loaded. The proteins levels were assessed by their immunoreactivities relative to β-actin. GLT25D1, glycosyltransferase 25 domain 1; Ab, antibody; Sh, Sh (short hairpin) 1-1; res-1, rescue Sh 1-1; res-2, rescue Sh 1-2.

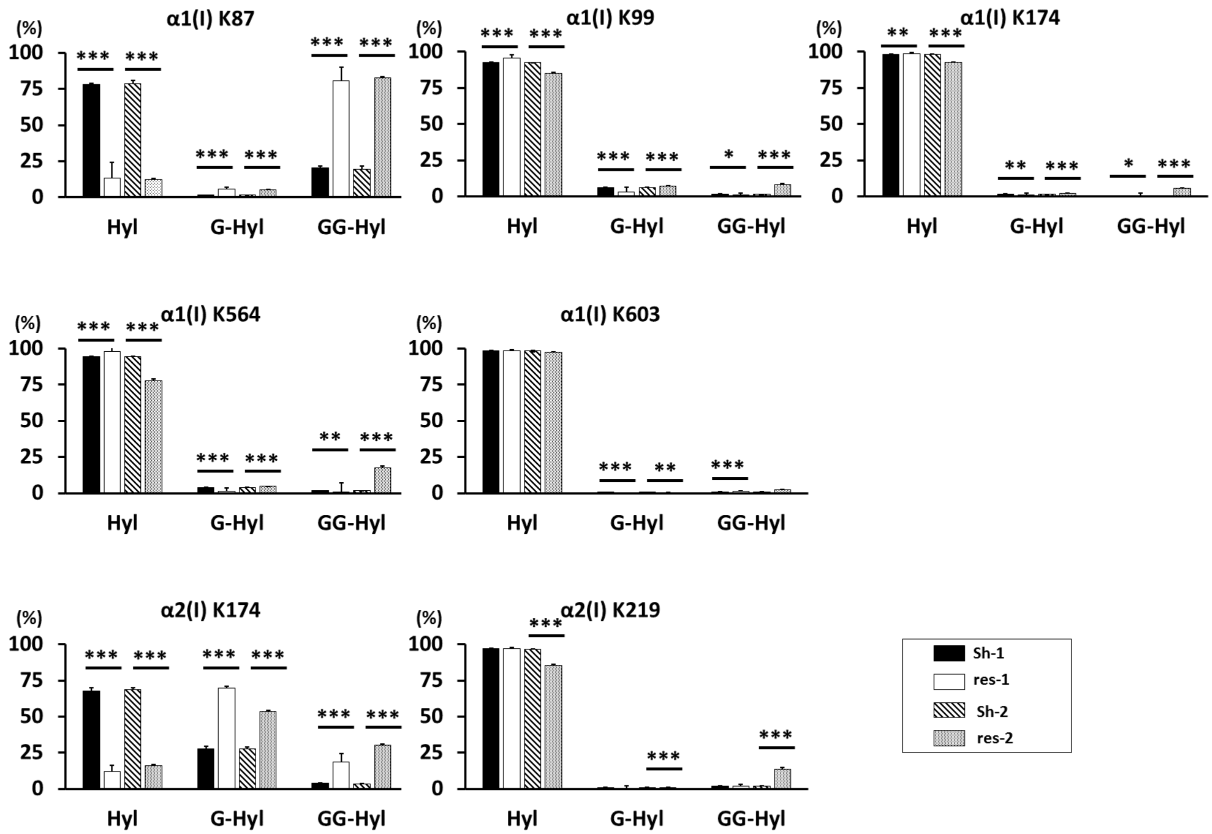

Figure S3. Mass spectrometric analysis of hydroxylysine glycosylation at specific molecular sites in type I collagen isolated from Sh clones with and without overexpression of *Glt25d1* cDNA. The percentage shows relative glycosylation levels of Hyl residue (Hyl + G-Hyl + GG-Hyl = 100%). Hyl, hydroxylysine; GG-, glucosyl galactosyl-; G-, galactosyl-; Sh, short hairpin. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ between Sh 1 (n=3) and rescue-1 clones (n=3) or between Sh 2 (n=3) and rescue-2 clones (n=3), respectively. Sh-1/-2 and rescue-Sh1/-2 clones, see Figure S2. Sh-1, Sh 1-1; Sh-2, Sh 1-2; res-1, rescue Sh 1-1; res-2, rescue Sh 1-2.

Table S1. Ratios of alpha chain and collagen type.

	α1(I)/α2(I) ratio	$[\alpha1(I)]_3$/ $\{[\alpha1(I)]_3 + [\alpha1(I)]_2\alpha2(I)\}$ (%)	Relative content (%)	
			Type I collagen	Type III collagen
MC	2.22 ± 0.07	6.7 ± 2.0	97.2 ± 1.0	2.8 ± 1.0
Sh1-1	2.25 ± 0.05	7.8 ± 1.5	97.8 ± 0.6	2.2 ± 0.6
Sh1-2	2.32 ± 0.07	9.5 ± 1.8	97.9 ± 0.3	2.1 ± 0.3
Sh1-3	2.26 ± 0.03	7.9 ± 1.0	95.7 ± 0.3	4.3 ± 0.3
EV	2.21 ± 0.05	6.6 ± 1.5	95.3 ± 0.2	4.7 ± 0.2

Type I collagen + Type III collagen = 100%. Values represent means (S.D. in parentheses) from three independent experiments. $p > 0.05$ between controls (MC and EV) and Sh. Sh, short hairpin; $[\alpha1(I)]_3$, type I collagen homotrimer; $[\alpha1(I)]_2\alpha2(I)$, type I collagen heterotrimer.

Table S2. Summary of site-specific modifications determined by mass spectrometry of non-cross-linked, hydroxylated and glycosylated residues in type I collagen from controls (MC and EV) and Sh clones.

Lys + Hyl + G-Hyl + GG-Hyl = 100%

The percentage in parentheses shows glycosylation of Hyl residues (Hyl + G-Hyl + GG-Hyl = 100%). Lys, lysine; Hyl, hydroxylysine; GG-, glucosylgalactosyl-; G-, galactosyl-; Sh, short hairpin.

		Site occupancy (%)				
		MC	Sh1-1	Sh1-2	Sh1-3	EV
α1(I) K87	Lys	0.8 ± 0.1	0.2 ± 0.0** ##	0.2 ± 0.0** ##	0.2 ± 0.0** ##	4.6 ± 0.4
	Hyl	12.1 ± 2.5	77.9 ± 0.9** ##	78.7 ± 1.6** ##	76.3 ± 0.1** ##	12.6 ± 2.9
	(%)	(12.2 ± 2.5)	(78.0 ± 0.9** ##)	(78.9 ± 1.6** ##)	(76.4 ± 0.1** ##)	(13.2 ± 3.0)
	G-Hyl	3.3 ± 0.2	1.6 ± 0.1** ##	1.5 ± 0.0** ##	1.7 ± 0.0** ##	3.5 ± 0.0
	(%)	(3.4 ± 0.2)	(1.6 ± 0.1** ##)	(1.5 ± 0.0** ##)	(1.7 ± 0.0** ##)	(3.7 ± 0.0)
	GG-Hyl	83.7 ± 2.5	20.3 ± 0.9** ##	19.5 ± 1.6** ##	21.9 ± 0.1** ##	79.3 ± 2.6
	(%)	(84.4 ± 2.6)	(20.4 ± 0.9** ##)	(19.6 ± 1.6** ##)	(21.9 ± 0.1** ##)	(83.1 ± 3.0)
α1(I) K99	Lys	64.8 ± 0.6	53.8 ± 0.6** ##	52.6 ± 1.1** ##	54.2 ± 1.0** ##	61.1 ± 0.6
	Hyl	24.9 ± 0.6	42.8 ± 0.6** ##	43.9 ± 1.0** ##	42.2 ± 1.1** ##	28.4 ± 0.8
	(%)	(70.7 ± 1.4)	(92.5 ± 0.3** ##)	(92.5 ± 0.1** ##)	(92.2 ± 0.3** ##)	(73.0 ± 2.5)
	G-Hyl	7.8 ± 0.3	2.8 ± 0.1** #	2.9 ± 0.1** #	2.9 ± 0.1** #	7.4 ± 0.7
	(%)	(22.2 ± 0.6)	(6.0 ± 0.2** ##)	(6.2 ± 0.1** ##)	(6.3 ± 0.2** ##)	(19.0 ± 1.6)
	GG-Hyl	2.5 ± 0.3	0.7 ± 0.0* #	0.6 ± 0.0* #	0.7 ± 0.0* #	3.1 ± 0.4
	(%)	(7.1 ± 2.8)	(1.4 ± 0.1* #)	(1.4 ± 0.1** #)	(1.5 ± 0.1* #)	(8.0 ± 1.0)
α1(I) K174	Lys	47.6 ± 0.6	38.6 ± 0.2** ##	38.7 ± 0.1** ##	38.7 ± 0.1** ##	43.7 ± 0.7
	Hyl	47.1 ± 0.6	60.3 ± 0.2** ##	60.2 ± 0.1** ##	60.1 ± 0.1** ##	51.3 ± 1.0
	(%)	(89.9 ± 0.8)	(98.3 ± 0.1** ##)	(98.2 ± 0.0** ##)	(98.1 ± 0.1** ##)	(91.1 ± 0.7)
	G-Hyl	3.5 ± 0.2	0.9 ± 0.1** ##	0.9 ± 0.0** ##	1.0 ± 0.0** ##	3.0 ± 0.2
	(%)	(6.7 ± 0.3)	(1.5 ± 0.1** ##)	(1.5 ± 0.0** ##)	(1.6 ± 0.1** ##)	(5.4 ± 0.5)
	GG-Hyl	1.8 ± 0.3	0.1 ± 0.0* ##	0.2 ± 0.0* ##	0.2 ± 0.0* ##	2.0 ± 0.2
	(%)	(3.4 ± 0.5)	(0.2 ± 0.0* ##)	(0.3 ± 0.0* ##)	(0.3 ± 0.0* ##)	(3.5 ± 0.4)
α1(I) K564	Lys	63.1 ± 1.8	61.5 ± 0.6	60.4 ± 0.6	61.4 ± 0.7	58.3 ± 2.4
	Hyl	29.2 ± 0.8	36.4 ± 0.6**	37.4 ± 0.5**	36.4 ± 0.5**	33.4 ± 1.5
	(%)	(79.1 ± 1.6)	(94.6 ± 0.3** ##)	(94.5 ± 0.1** ##)	(94.3 ± 0.4** ##)	(80.3 ± 1.5)
	G-Hyl	4.7 ± 0.4	1.4 ± 0.1** #	1.5 ± 0.1** #	1.5 ± 0.1** #	4.5 ± 0.6
	(%)	(12.7 ± 0.4)	(3.8 ± 0.2** ##)	(3.9 ± 0.1** ##)	(3.9 ± 0.2** ##)	(10.7 ± 0.8)

	GG-Hyl	3.0 ± 0.6	0.6 ± 0.0* #	0.7 ± 0.0* #	0.7 ± 0.1* #	3.8 ± 0.5
	(%)	(8.2 ± 1.2)	(1.6 ± 0.1* ##)	(1.7 ± 0.1* ##)	(1.8 ± 0.2* ##)	(9.0 ± 0.8)
α1(I) K603	Lys	22.5 ± 1.4	22.1 ± 1.1	22.7 ± 0.3	22.4 ± 1.9	18.7 ± 2.0
	Hyl	75.6 ± 1.4	76.8 ± 1.2	76.2 ± 0.3	76.3 ± 1.9	78.5 ± 2.1
	(%)	(97.5 ± 0.3)	(98.6 ± 0.0* ##)	(98.6 ± 0.1* ##)	(98.3 ± 0.0##)	(96.5 ± 0.2)
	G-Hyl	0.9 ± 0.1	0.4 ± 0.0* ##	0.4 ± 0.0* ##	0.6 ± 0.0* ##	1.4 ± 0.1
	(%)	(1.2 ± 0.1)	(0.6 ± 0.1* ##)	(0.6 ± 0.0* #)	(0.8 ± 0.1* #)	(1.8 ± 0.2)
	GG-Hyl	1.0 ± 0.1	0.6 ± 0.0* ##	0.7 ± 0.0* ##	0.7 ± 0.0* ##	1.4 ± 0.1
	(%)	(1.3 ± 0.1)	(0.8 ± 0.0* ##)	(0.9 ± 0.0* ##)	(0.9 ± 0.0* ##)	(1.7 ± 0.1)
α2(I) K174	Lys	14.1 ± 0.5	7.2 ± 0.2** ##	7.6 ± 0.2** ##	7.7 ± 0.1** ##	23.0 ± 1.3
	Hyl	9.3 ± 0.8	63.1 ± 0.8** ##	63.6 ± 1.1** ##	60.9 ± 0.5** ##	8.2 ± 1.4
	(%)	(10.8 ± 1.0)	(68.0 ± 0.7** ##)	(68.8 ± 1.0** ##)	(65.9 ± 0.6** ##)	(10.6 ± 1.6)
	G-Hyl	62.1 ± 1.3	26.0 ± 0.6** ##	25.6 ± 0.8** ##	27.8 ± 0.6** ##	55.0 ± 0.5
	(%)	(72.3 ± 1.8)	(28.0 ± 0.7** ##)	(27.7 ± 0.9** ##)	(30.2 ± 0.7** ##)	(71.5 ± 1.0)
	GG-Hyl	14.5 ± 1.7	3.7 ± 0.1* ##	3.2 ± 0.1* ##	3.6 ± 0.1* ##	13.8 ± 0.4
	(%)	(16.8 ± 1.8)	(4.0 ± 0.1** ##)	(3.5 ± 0.2** ##)	(3.9 ± 0.1** ##)	(17.9 ± 0.8)
α2 (I) K219	Lys	29.3 ± 0.9	19.2 ± 0.6** #	18.7 ± 0.7** #	18.8 ± 0.5** #	21.3 ± 0.7
	Hyl	59.0 ± 0.8	78.5 ± 0.9** ##	78.6 ± 0.6** ##	78.8 ± 0.3** ##	69.4 ± 1.4
	(%)	(83.4 ± 2.0)	(97.1 ± 0.5** #)	(96.7 ± 0.1* #)	(97.0 ± 0.4** #)	(88.2 ± 2.3)
	G-Hyl	3.4 ± 0.1	0.8 ± 0.2**	0.9 ± 0.1**	0.9 ± 0.1**	2.2 ± 0.5
	(%)	(4.8 ± 0.1)	(1.0 ± 0.2** #)	(1.1 ± 0.1**)	(1.1 ± 0.2**)	(2.8 ± 0.6)
	GG-Hyl	8.3 ± 1.5	1.5 ± 0.3* #	1.7 ± 0.0* #	1.6 ± 0.2* #	7.1 ± 1.4
	(%)	(11.8 ± 2.0)	(1.8 ± 0.3* #)	(2.1 ± 0.0* #)	(1.9 ± 0.3* #)	(9.0 ± 1.7)

*p<0.05 and **p<0.01 between MC and Sh; #p<0.05 and ##p<0.01 between EV and Sh, respectively.

Table S3. Collagen cross-links and glycosylation in controls (MC and EV) and Sh clones.

A.

Collagen cross-links. Values (moles/mole of collagen) represent means (S.D. in parentheses) from three independent experiments.

Cells/Clones	DHLNL[a]		HLNL		Pyr		Total aldehyde[b]	
MC	1.738	(0.20)	0.741	(0.09)	0.022	(0.005)	2.523	(0.22)
Sh1-1	1.077	(0.17)[d]	0.426	(0.05)[d]	0.046	(0.004)[d]	1.594	(0.13)[d]
Sh1-2	1.243	(0.05)[d]	0.499	(0.03)[d]	0.050	(0.001)[d]	1.842	(0.02)[d]
Sh1-3	1.140	(0.08)[d]	0.470	(0.02)[d]	0.043	(0.001)[d]	1.697	(0.10)[d]
EV	1.666	(0.03)	0.674	(0.08)	0.029	(0.002)	2.398	(0.06)

B.

Glycosylation and Maturation of DHLNL cross-link. Values represent means (S.D. in parentheses) from three independent experiments.

Cells/Clones	DHLNL[C]		GG-DHLNL		G-DHLNL		Free-DHLNL		Pyr/DHLNL	
	percentage		percentage		percentage		percentage		ratio	
MC	100	(0.0)	40.2	(1.8)	5.4	(2.3)	54.4	(0.9)	0.013	(0.001)
Sh1-1	100	(0.0)	20.8	(1.0)[d]	ND[e]		79.2	(1.0)[d]	0.069	(0.008)[d]
Sh1-2	100	(0.0)	23.0	(0.6)[d]	ND[e]		77.0	(0.6)[d]	0.045	(0.008)[d]
Sh1-3	100	(0.0)	18.4	(2.6)[d]	ND[e]		81.6	(2.6)[d]	0.048	(0.009)[d]
EV	100	(0.0)	40.9	(1.0)	5.5	(2.3)	53.6	(1.4)	0.018	(0.001)

[a]DHLNL (moles/mole of collagen) including GG- + G- + free-DHLNL

[b]Total aldehydes=total DHLNL+HLNL+2×Pyr

[c]DHLNL (100%)=GG- (%) +G- (%) + free-DHLNL (%)

[d]Significantly different from both controls (MC and EV)

[e]ND, not detected

DHLNL, dihydroxylysinonorleucine; HLNL, hydroxylysinonorleucine; Pyr, pyridinoline; GG-, glucosylgalactosyl-; G-, galactosyl-; Sh, short hairpin.