

Lysyl hydroxylase 2 mediated collagen post-translational modifications and functional outcomes

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Supplementary Table 1. Collagen type analysis by mass spectrometry of cell/matrix layer from controls (MC and EV), and KO clones.

Percentages of type I collagen calculated by type I collagen / (type I + type III collagen).

	MC	EV	KO-1	KO-2	KO-3
Type I collagen (%)	98.3	98.6	97.9* ##	96.0** ##	97.4** ###
(S.D.)	(0.04)	(0.13)	(0.10)	(0.28)	(0.08)

Values represent mean the percentages of type I collagen $\pm$ S.D. (n=3) of triplicate analysis of the hydrolysates.

* $p < 0.05$ and ** $p < 0.01$ between MC and KO; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ between EV and KO, respectively. MC, MC3T3-E1; EV, empty vector; KO, knock-out.

Supplementary Table S2. Summary of site-specific modification analysis by mass spectrometry of prolyl 3-hydroxylation in type I collagen from controls (MC and EV) and KO clones.

Prolyl 3-hydroxylation (%) represents the relative levels of prolyl 3-hydroxylation at $\alpha 1$ Pro-986 ($0-1 \times 3$ -Hyp), $\alpha 1$ Pro-707, 716, 719 ($0-3 \times 3$ -Hyp) and $\alpha 2$ Pro-707, 716, 719 ($0-3 \times 3$ -Hyp) (total of $0-3 \times 3$ -Hyp = 100%). Hyp, hydroxyproline; MC; MC3T3-E1; EV, empty vector; KO, knock-out.

		Site occupancy (%)				
		MC	EV	KO-1	KO-2	KO-3
$\alpha 1(I)$ [975-990]	0×3 -Hyp	8.7 ± 0.2	6.8 ± 0.1	$6.1 \pm 0.0^{** \#}$	$5.9 \pm 0.1^{*** \#\#}$	$2.4 \pm 0.1^{*** \#\#}$
	1×3 -Hyp	91.3 ± 0.2	93.2 ± 0.1	$93.9 \pm 0.0^{** \#}$	$94.1 \pm 0.1^{*** \#}$	$97.6 \pm 0.1^{*** \#\#}$
$\alpha 1(I)$ [705-725]	0×3 -Hyp	83.3 ± 1.2	84.4 ± 0.5	$79.6 \pm 0.4^* \#\#\#$	$79.7 \pm 0.3^* \#\#\#$	$71.0 \pm 0.1^{** \#\#}$
	1×3 -Hyp	16.1 ± 0.8	14.9 ± 0.3	$19.0 \pm 0.3^* \#\#\#$	$18.8 \pm 0.3^* \#\#\#$	$26.9 \pm 0.1^{** \#\#}$
	2×3 -Hyp	0.6 ± 0.4	0.7 ± 0.1	$1.5 \pm 0.1^{\#}$	$1.6 \pm 0.0^{\#}$	$1.9 \pm 0.0^* \#$
	3×3 -Hyp	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$0.1 \pm 0.0^* \#$
$\alpha 2(I)$ [705-725]	0×3 -Hyp	66.9 ± 0.4	67.4 ± 0.3	$63.4 \pm 0.2^{** \#\#\#}$	$63.3 \pm 0.6^{** \#}$	$48.4 \pm 0.6^{*** \#\#\#}$
	1×3 -Hyp	31.7 ± 0.1	31.4 ± 0.4	$33.8 \pm 0.1^{*** \#}$	$33.9 \pm 0.5^* \#$	$47.5 \pm 0.5^{*** \#\#\#}$
	2×3 -Hyp	1.4 ± 0.5	1.3 ± 0.1	$2.8 \pm 0.1^* \#\#\#$	$2.8 \pm 0.1^* \#\#\#$	$4.2 \pm 0.2^{*** \#\#\#}$
	3×3 -Hyp	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

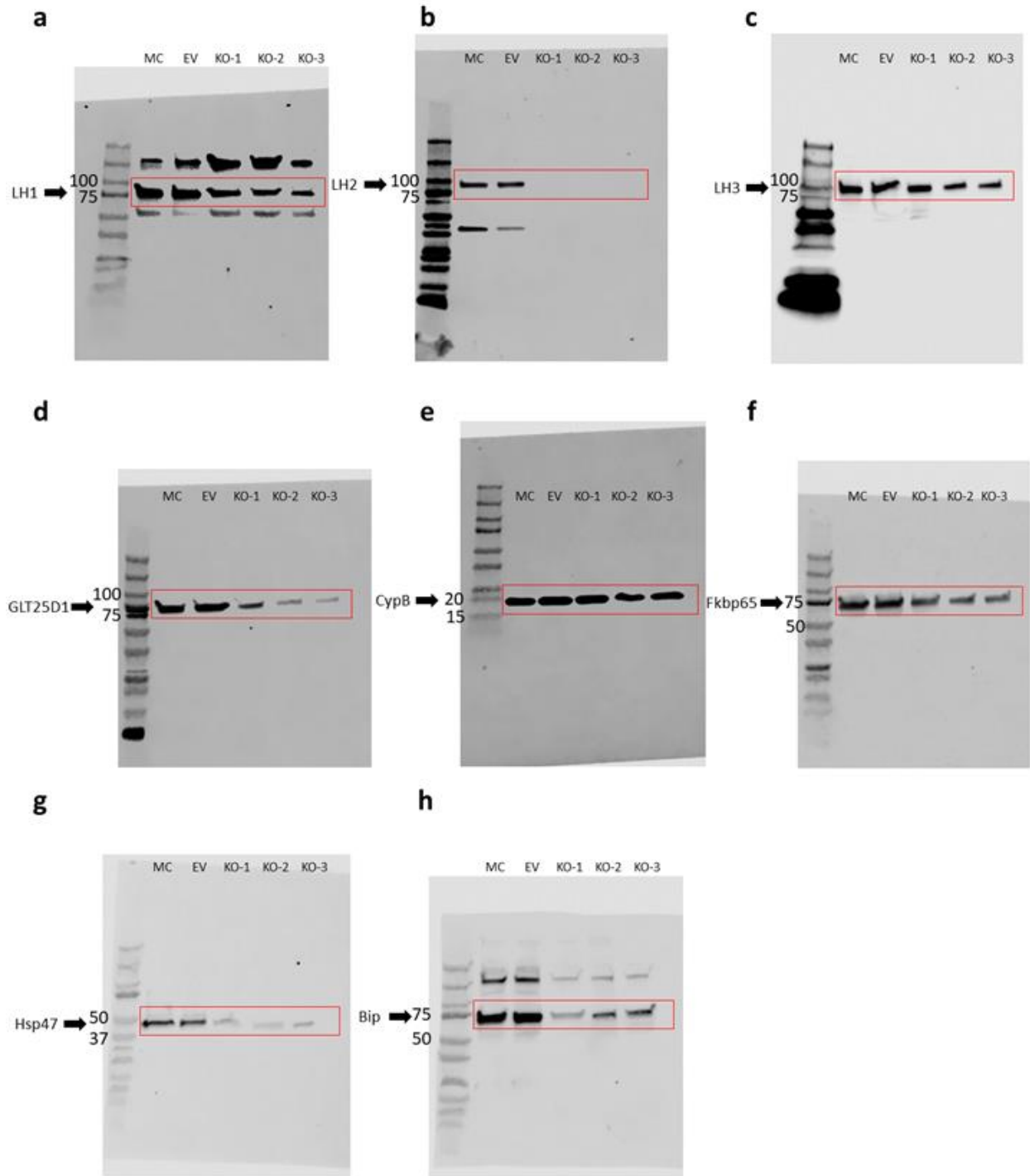
Values represent mean $\pm$ S.D. (n=3) of triplicate for each group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ between MC and KO; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ between EV and KO, respectively.

Supplementary Table S3. Ratio of galactosyl (G)- to glucosylgalactosyl (GG)-hydroxylysine in type I collagen isolated from MC and KO clone.

Values represent ratio of Hyl glycosylation calculated as G-Hyl/GG-Hyl. Hyl, hydroxylysine; G-, galactosyl-; GG-, glucosylgalactosyl-; MC, MC3T3-E1; EV, empty vector; KO, knock-out.

	ratio (G-Hyl/GG-Hyl)						
	MC	EV	KO-1		KO-2		KO-3
$\alpha 1(I)$ K87	0.09 $\pm$ 0.00	0.09 $\pm$ 0.00	0.15 $\pm$ 0.01	***, ###	0.14 $\pm$ 0.01	***, ###	0.09 $\pm$ 0.01
$\alpha 1(I)$ K99	1.85 $\pm$ 0.10	1.90 $\pm$ 0.07	2.73 $\pm$ 0.10	***, ###	2.61 $\pm$ 0.14	**, ##	1.77 $\pm$ 0.05
$\alpha 1(I)$ K174	1.32 $\pm$ 0.10	1.32 $\pm$ 0.12	2.71 $\pm$ 0.18	***, ###	2.52 $\pm$ 0.28	**, ##	1.93 $\pm$ 0.06
$\alpha 1(I)$ K564	0.89 $\pm$ 0.06	0.96 $\pm$ 0.10	1.71 $\pm$ 0.17	**, ##	1.79 $\pm$ 0.27	**, ##	1.39 $\pm$ 0.07
$\alpha 2(I)$ K174	1.89 $\pm$ 0.17	1.92 $\pm$ 0.04	3.25 $\pm$ 0.13	***, ###	3.02 $\pm$ 0.12	***, ###	1.76 $\pm$ 0.06
$\alpha 2(I)$ K219	0.18 $\pm$ 0.07	0.23 $\pm$ 0.01	0.39 $\pm$ 0.12		0.31 $\pm$ 0.08		0.20 $\pm$ 0.02

Values represent mean $\pm$ S.D. (n=3) of triplicate for each group. **p<0.01 and ***p<0.001 between MC and KO; ##p<0.01 and ###p<0.001 between EV and KO (n=3), respectively.



Supplementary Figure S1. Uncropped immunoblot images. (a) LH1, (b) LH2, (c) LH3, (d) GLT25D1, (e) CypB, (f) Fkbp65, (g) Hsp47, (h) Bip. LH, lysyl hydroxylase; GLT25D1, glycosyltransferase 25 domain containing 1; CypB, cyclophilin B; Fkbp65, FK506-binding protein 65; Hsp47, heat shock protein 47; Bip, immunoglobulin heavy-chain-binding protein; MC, MC3T3-E1; EV, empty vector; KO, knock-out.