## **Supplementary Figure 1**

## LC-MS/MS Identification of the O-Glycosylation and Hydroxylation of Amino Acid Residues of Collagen α-1 (II) chain from Bovine Cartilage

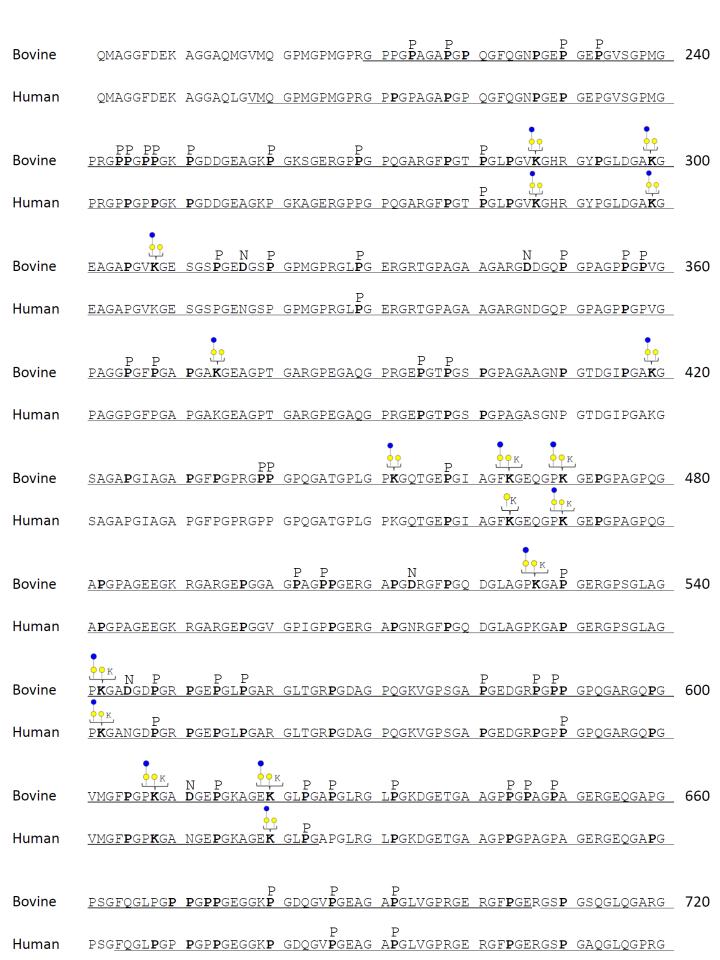
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**Keywords:** type II collagen, CO2A1, hydroxylation, glycosylation, glycopeptides, LC-MS/MS, quantitation



Bovine	P L <b>P</b> GT <b>P</b> GTDGP	KGAAGPAG <b>PP</b>	P GAQG <b>PP</b> GLQG	M <b>P</b> GERGAAGI	AGP <b>K</b> GDRGDV	P GEKGPEGA <b>P</b> G	780
Human	LPGTPGTDGP	KGASGPAGPP	GAQGPPG <u>LQG</u>	M <b>P</b> GERGAAGI	AGPKGDRGDV	GE <b>K</b> GPEGA <b>P</b> G	
Bovine	KDGGRGLTGP	PP N IGP <b>P</b> G <b>P</b> AGA <b>D</b>	GE <b>K</b> GEVGP <b>P</b> G	PAGTAGARGA	<b>P</b> GERGETGP <b>P</b>	GPAGFAGP <b>P</b> G	840
Human	KDGGRGLTGP	IGPPGPAGAN	K ge <b>k</b> gevgp <b>p</b> g	PAGSAGARGA	<b>P</b> GERGETGP <b>P</b>	gpag <u>fagp<b>p</b>g</u>	
Bovine	P ADGOPGAKGE	ogeago <b>k</b> gda	GA <b>P</b> GPQGPSG	P A <b>P</b> GPQGPTGV	TGP <b>K</b> GARGAQ	GP <b>P</b> GATGF <b>P</b> G	900
Human	<u>ADGQ</u> PGA <b>K</b> GE	QGEAGQ <b>K</b> GDA	GA <b>P</b> GPQGPSG	APGPQGPTGV	TGPKGARGAQ	GP <b>P</b> GATGF <b>P</b> G	
Bovine		N N DGDPGPPGPP					960
Human	AAGRVGP <b>P</b> GS	NGN <b>P</b> GP <b>P</b> GP <b>P</b>	<u>GPSGKDG</u> PKG	ARGDSGP <b>P</b> GR	AGE <b>P</b> GLQGPA	K P gp <b>p</b> ge <b>k</b> ge <b>p</b> g	
Bovine	DDGPSGPDGP	<b>P</b> GPQGLAGQR	GIVGL <b>P</b> GQRG	ERGF <b>P</b> GL <b>P</b> GP	P sge <b>p</b> gkoga <b>p</b>	gasgdrg <b>pp</b> g	1020
Human	DDGPSGAEGP	PGPQGLAGQR	GIVGL <b>P</b> GQRG	ERGF <b>P</b> GL <b>P</b> GP	P P SGE <b>P</b> GKQGA <b>P</b>	GASGDRGP <b>P</b> G	
Bovine	P PVGP <b>P</b> GLTGP	AGE <b>P</b> GREGS <b>P</b>	gadgp <b>p</b> grdg	AAGV <b>K</b> GDRGE	P P Tgavga <b>p</b> ga <b>p</b>	P P g <b>pp</b> gs <b>p</b> gpag	1080
Human	PVGPPGLTGP	AGE <b>P</b> GREGS <b>P</b>	GADGP <b>P</b> GRDG	AAGVKGDRGE	TGAVGAPGAP	GPPGSPGPAG	
Bovine	PIGKQGDRGE	P AGAQGPMG <b>P</b> A	P P G <b>P</b> AGARGM <b>P</b> G	PQGPRGD <b>K</b> GE	<u>TGEAGER</u> GLK	GHRGFTGLQG	1140
Human	PTGKQGDRGE	AGAQGPMGPS	GPAGARGIQG	PQGPRGD <b>K</b> GE	<u>AG</u> EPGERGLK	GHRGFTGLQG	
Bovine	P PP L <b>P</b> G <b>PP</b> GPSGD	QGASGPAGPS	P gprgp <b>p</b> gpvg	PSGKDGANGI	<b>P</b> GPIG <b>PP</b> GPR	<u>GRSGE</u> TGPAG	1200
Human	L <b>P</b> GP <b>P</b> GPSGD	QGASGPAGPS	GPRGPPGPVG	PSGKDGANGI	PGPIGPPGPR	GRSGETGPAG	
Bovine	PPGNPGPPGP	PGPPGPGIDM	SAFAGLGQRE	KGPDPLQYMR	ADEAAGNLRQ	НD	1260
Human	PPGNPGPPGP	PGPPGPGIDM	SAFAGLGPRE	KGPDPLQYMR	ADQAAGGLRQ	HD	

- Identified amino acids are underlined. Hydroxylated proline or lysine residues are bold while unmodified forms are not. If modified and unmodified were detected, additional letters P or K would be added in the upper line of the sequence. Glc-Gal or Gal modified lysines are represented.
- Reference: Van den Steen, P. E.; Proost, P.; Brand, D. D.; Kang, A. H.; Van Damme, J.; Opdenakker, G., Generation of glycosylated remnant epitopes from human collagen type II by gelatinase B. *Biochemistry*. 2004, 43, (33), 10809-16