Supplemental Table S1. The normalised area under the curve ± standard deviation from EIC of each disaccharide (HA, CS, and HS) observed for standards and lysate samples.

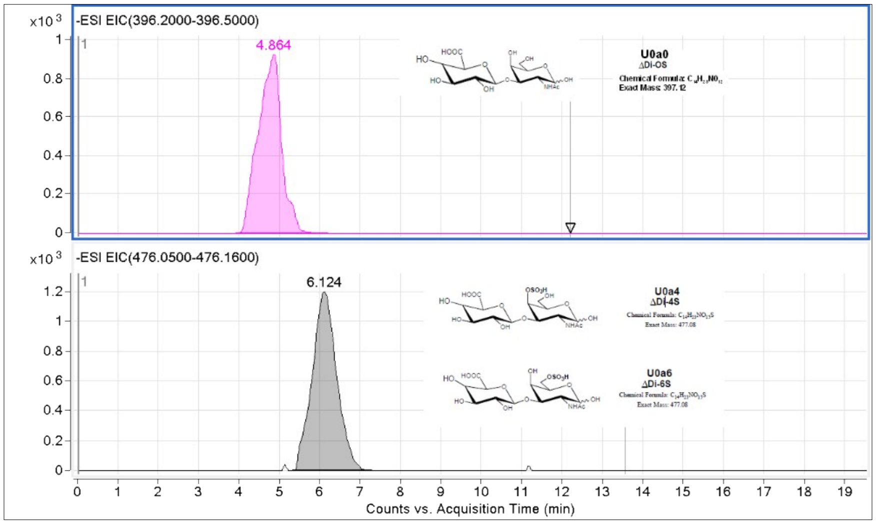
Samples	**Normalised area under the curve**
HA disaccharides	
	D0a0
Hyaluronic Acid -batch 01	5.28E+03 ± 5.14E+02
Hyaluronic Acid -batch 02	4.78E+03 ± 9.85E+01
Lysate- batch 01	1.92E+03 ± 6.42E+01
Lysate-batch 02	2.33E+03 ± 8.67E+02

CS disaccharides					
	D0a0	**D0a4/D0a6**	**D0a10/D2a4/D2a6**	**U0a0**	**U0a4/U0a6/U2a0**
Aggrecan-batch 01	5.99E+05 ± 2.29E+04	1.88E+07± 3.59E+06	4.69E+04± 1.77E+03	9.37E+02± 1.96E+02	5.89E+04± 5.85E+03
Aggrecan-batch 02	6.05E+05 ± 1.00E+04	1.67E+07± 1.41E+06	3.60E+04± 6.92E+02	1.18E+03± 3.74E+02	5.91E+04± 8.26E+03
Neurocan-batch01	1.04E+05 ± 4.67E+04	1.88E+06± 8.42E+05	1.04E+04± 5.72E+03	3.55E+03± 4.20E+02	1.05E+04± 4.35E+03
Syndecan-batch 01	1.23E+05± 2.56E+04	8.83E+05± 1.39E+05	3.06E+04± 9.74E+03	2.82E+04± 6.62E+03	3.08E+03± 7.36E+02
Syndecan-batch 02	1.27E+05± 1.38E+04	1.11E+06± 1.88E+04	2.55E+04± 6.52E+03	2.75E+04± 8.28E+02	3.89E+03± 1.03E+03
Lysate-batch 01	1.67E+04± 3.03E+03	7.11E+05± 4.43E+04	2.58E+04± 1.03E+03	Not observed	1.27E+03± 4.25E+02
Lysate-batch 02	1.96E+04± 1.13E+03	5.33E+05± 1.58E+04	2.40E+04± 2.89E+03	Not observed	8.72E+02± 4.31E+02

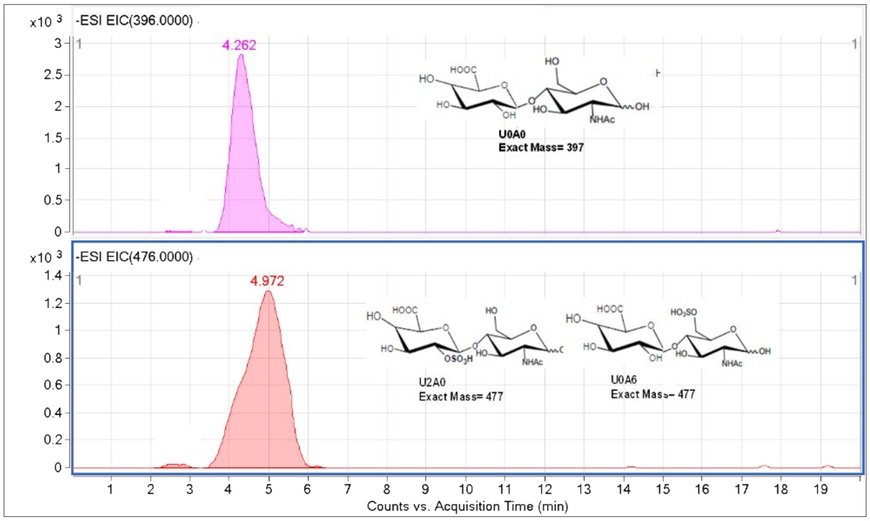
HS disaccharides								
	D0A0	**DOA6/D2A0**	**D2A6**	**D0S0**	**D0S6/D2S0**	**D2S6**	**U0A0**	**UOA6/U2A0**
HSBK-batch 01	4.42E+06 ± 2.84E+05	3.73E+06 ±4.08E+05	1.76E+06± 4.73E+04	2.65E+04 ± 1.98E+04	9.33E+05± 1.38E+05	1.11E+04± 3.71E+03	5.28E+04± 1.72E+04	2.97E+04± 1.91E+04
HSBK-batch 02	4.16E+06 ± 7.21E+01	2.46E+06± 5.26E+05	2.44E+06± 1.97E+05	4.08E+04 ± 3.17E+03	1.42E+06± 4.49E+03	1.48E+04± 7.56E+04	7.66E+04± 1.14E+03	3.11E+04± 2.00E+03
HSPIM-batch 01	2.25E+06 ± 1.19E+03	1.68E+06± 6.43E+05	1.800E+06± 7.86E+05	2.79E+05 ± 1.21E+05	3.60E+06± 3.76E+05	2.15E+06± 2.18E+05	1.12E+04± 4.19E+02	4.01E+04± 3.95E+03
HSPIM-batch 02	2.47E+06 ±	1.14E+07±	1.47E+06±	2.14E+05 ±	2.96E+06±	1.73E+06±	1.17E+04±	3.64E+04±

	4.38E+04	1.44E+05	2.75E+05	9.70E+04	3.88E+05	1.26E+05	7.49E+03	6.51E+03
Syndecan-batch 01	1.25E+06 ± 1.42E+05	1.61E+06± 9.64E+04	1.13E+06± 4.31E+04	2.89E+04 ± 7.39E+03	5.82E+05± 2.86E+04	6.65E+02± 5.43E+01	3.23E+04± 1.27E+04	1.50E+04± 3.12E+03
Lysate-batch 01	3.31E+05 ± 5.30E+04	2.57E+05± 4.51E+04	1.04E+05± 4.88E+03	7.62E+02 ± 9.91E+01	7.13E+04± 1.02E+04	Not observed	2.57E+03± 9.97 E+01	Not observed
Lysate-batch 02	4.56E+05 ± 2.29E+05	3.21E+05± 1.88E+05	1.11E+05 ± 1.11E+05	6.22E+02 ± 5.60E+01	5.69E+04± 6.60E+04	Not observed	2.71E+03± 7.89E+01	Not observed

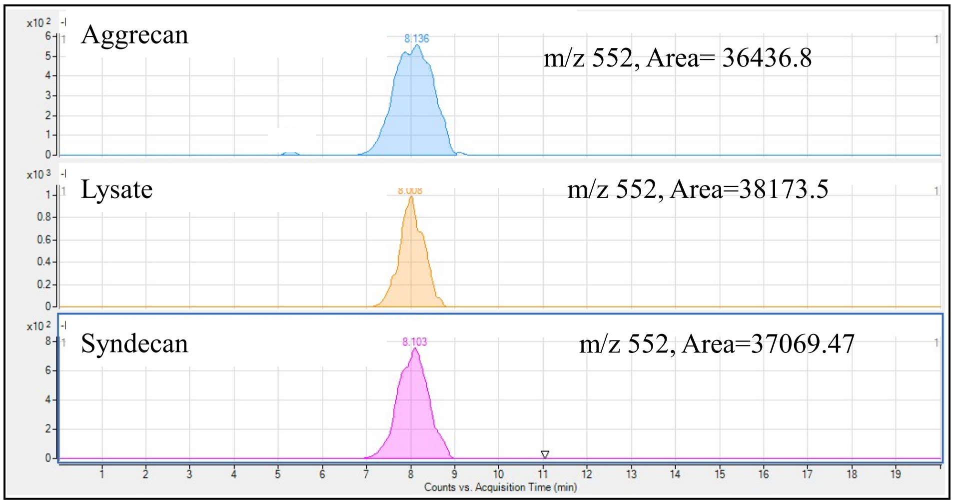
A,



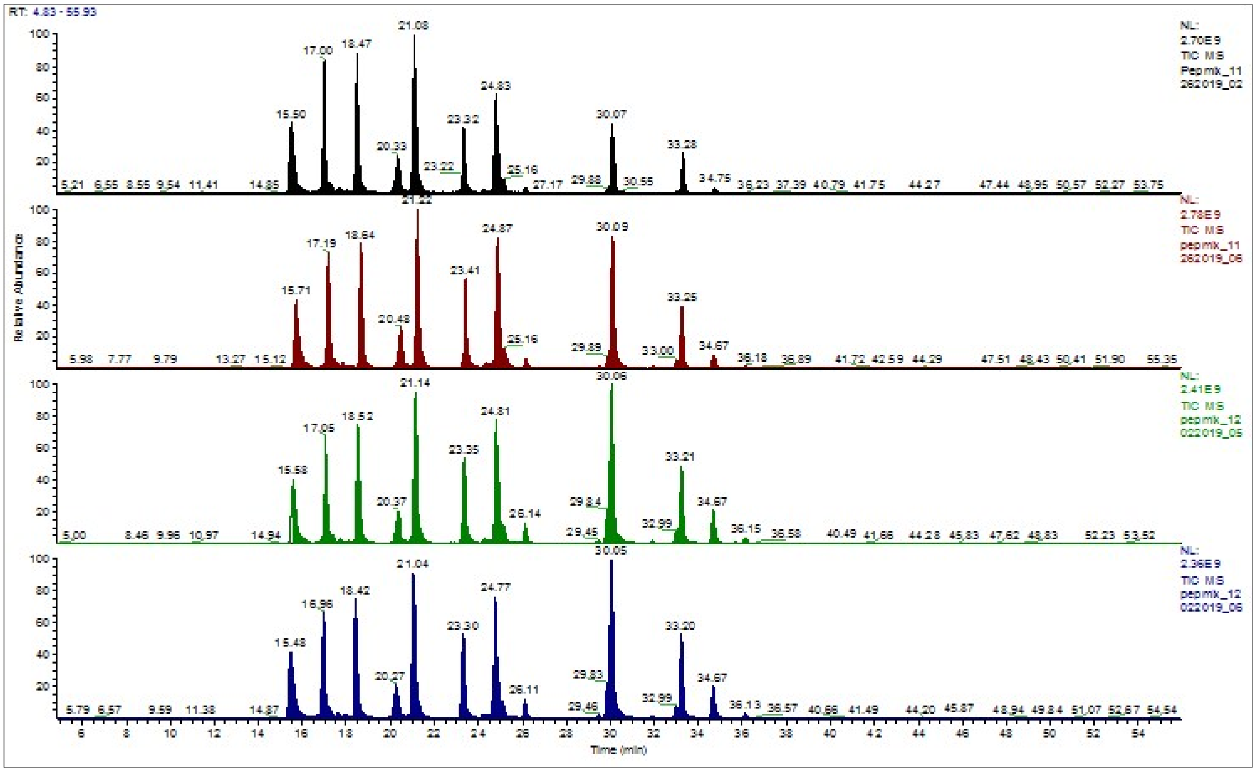
B,



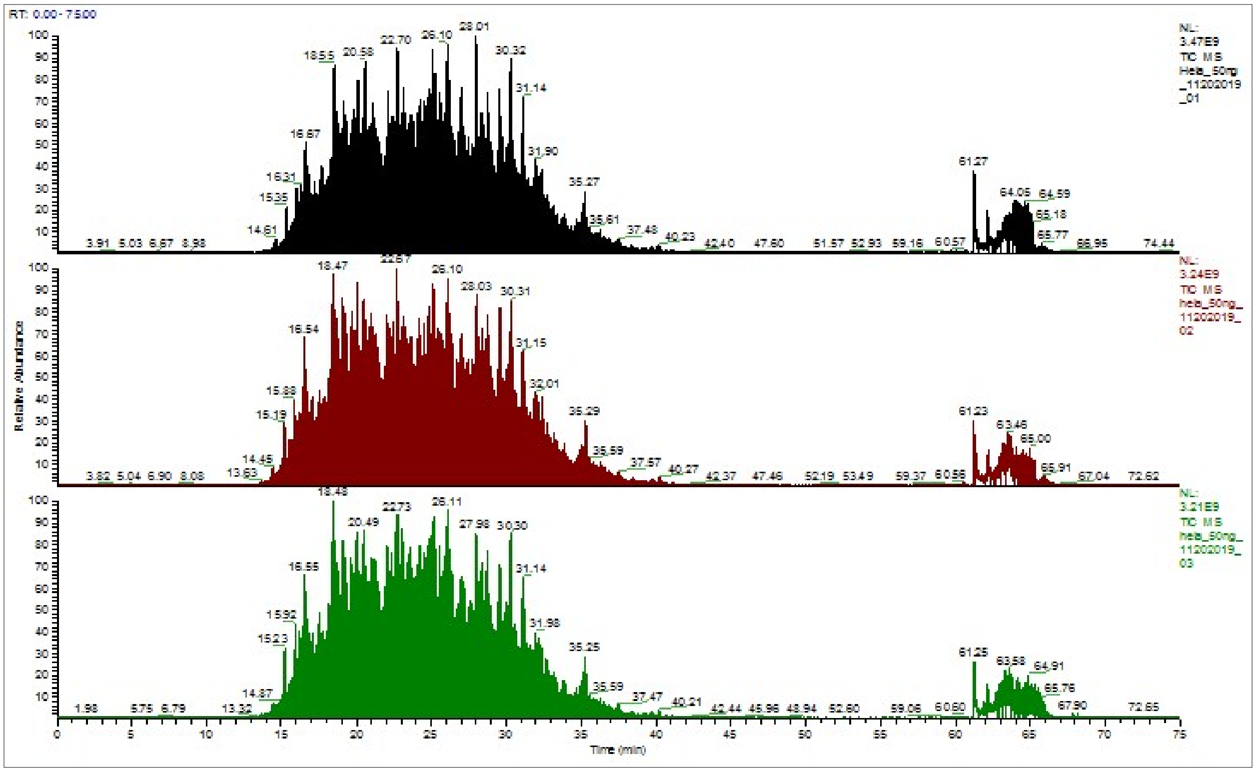
Supplemental Fig S1. A, Extracted ion chromatograms (EICs) for observed saturated CS disaccharides. **B,** Structures for observed saturated HS disaccharides.



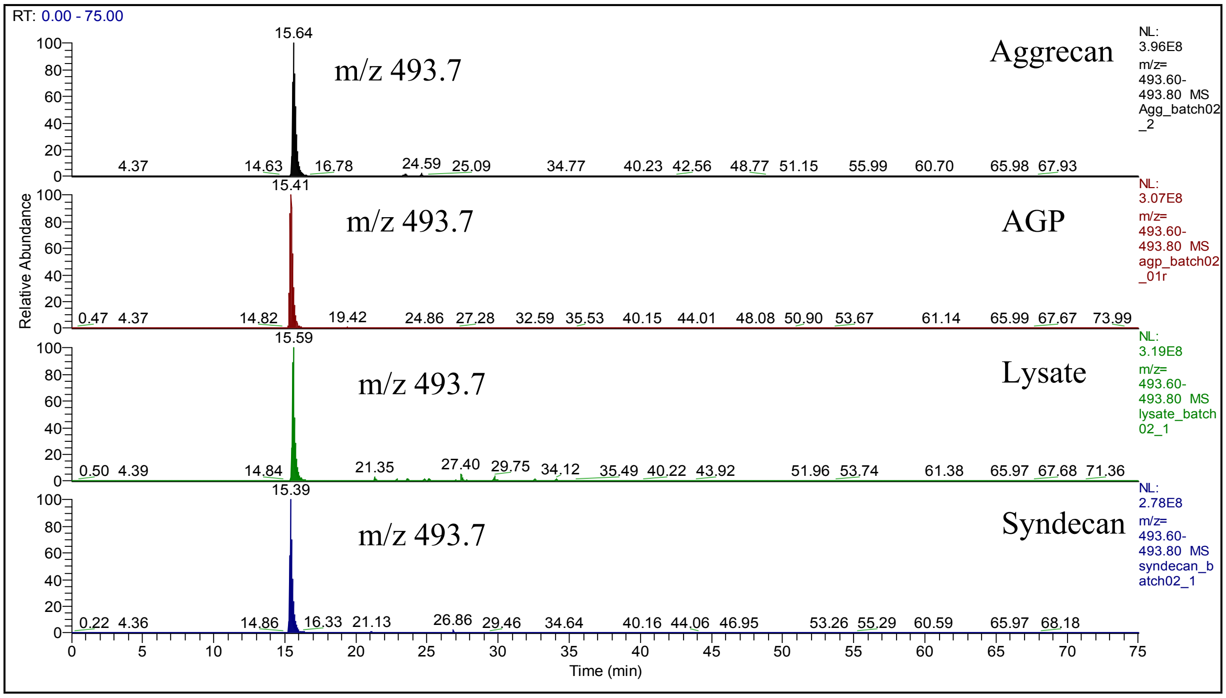
Supplemental Fig S2. The intensity and retention time for spiked internal standard m/z 552 (of ΔHexA2S-GlcNCoEt(6S) (Hd009, Iduron) is consistent among different samples ran GlycanPac AXH-1column mounted on an Agilent 1200 LC connected to an Agilent 6520 Q-TOF.



Supplemental Fig S3. A total ion chromatogram (TIC) for a retention time calibration peptide mixture (100 fmol) ran on Waters nano-Acquity UPLC connected to a Thermo QE-HF mass spectrometer to check instrument day-to-day performance.

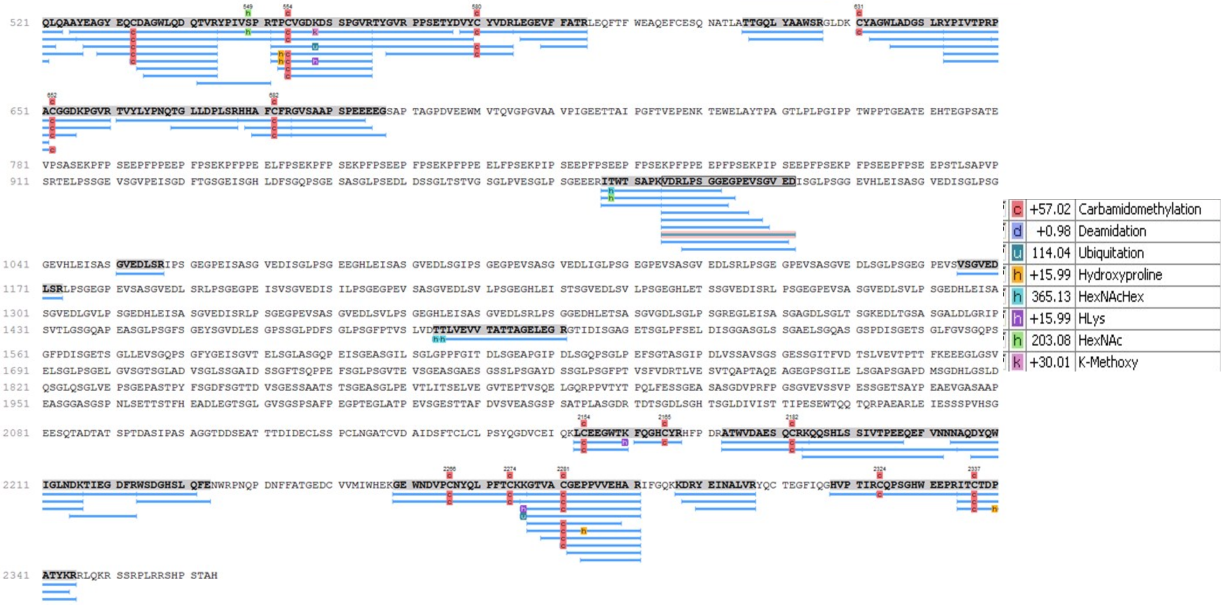


Supplemental Fig S4. A total ion chromatogram (TIC) for a commercially available digested Hela cell lysates (50 ng) ran on Waters nano-Acquity UPLC connected to a Thermo QE-HF mass spectrometer to check instrument performance before running actual samples.



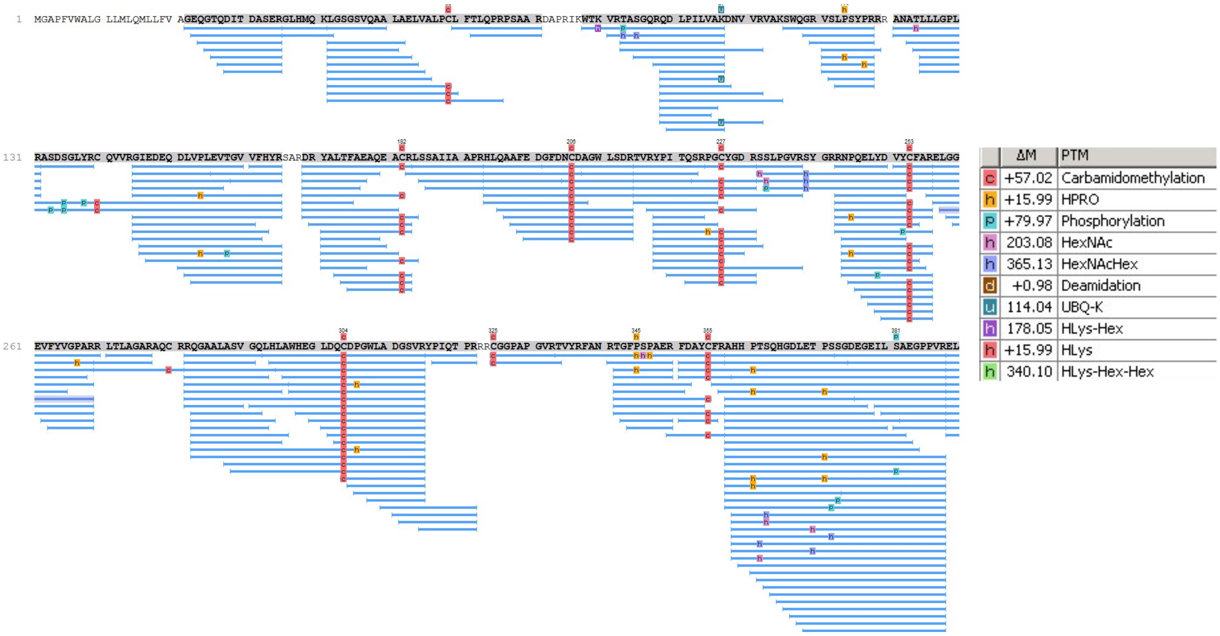
Supplemental Fig S5. An extracted ion chromatogram (EIC) for peptide m/z 493.7 from spiked internal control (pierce retention time calibration peptide mixture). The LC retention time and intensity over different samples were consistent.

A, Aggrecan



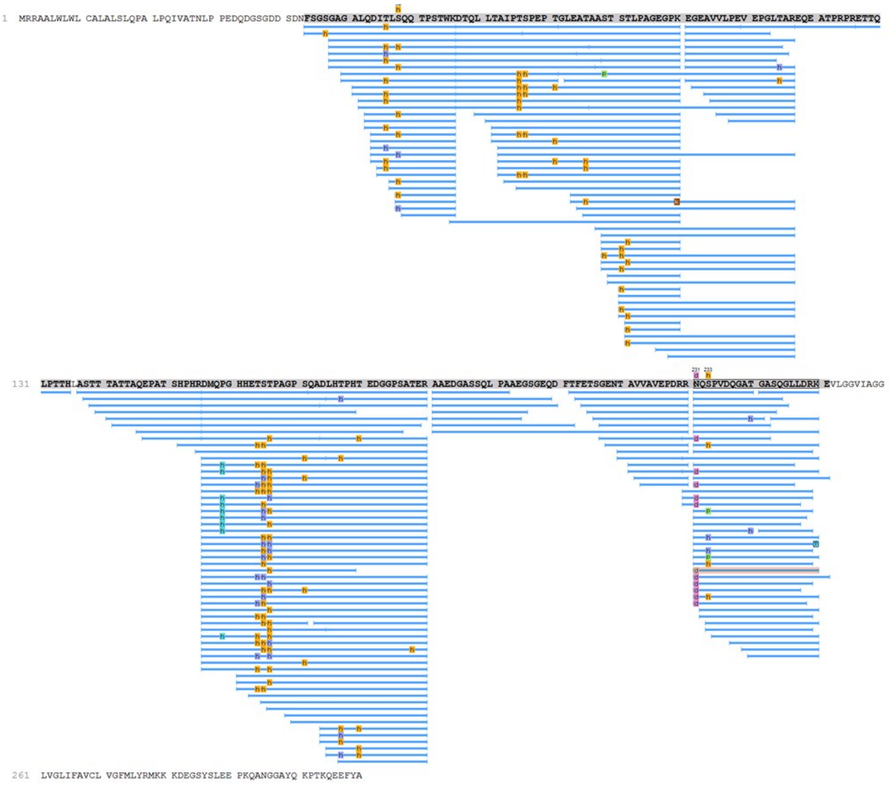
c	+57.02	Carbamidomethylation
d	+0.98	Deamidation
u	114.04	Ubiquitation
h	+15.99	Hydroxyproline
h	365.13	HexNAcHex
h	+15.99	HLys
h	203.08	HexNAc
k	+30.01	K-Methoxy

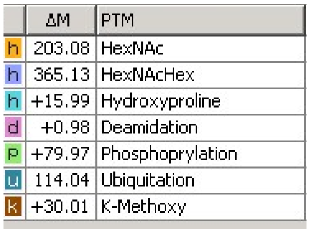
B, Neurocan



	ΔM	PTM
c	+57.02	Carbamidomethylation
h	+15.99	HPRO
P	+79.97	Phosphorylation
h	203.08	HexNAc
h	365.13	HexNAcHex
d	+0.98	Deamidation
u	114.04	UBQ-K
h	178.05	HLys-Hex
h	+15.99	HLys
h	340.10	HLys-Hex-Hex

C, Syndecan





	ΔM	PTM
h	203.08	HexNAc
h	365.13	HexNAcHex
h	+15.99	Hydroxyproline
d	+0.98	Deamidation
P	+79.97	Phosphoprylation
u	114.04	Ubiquitation
k	+30.01	K-Methoxy

Supplemental Fig S6. Coverage maps for **A,** Aggrecan, **B,** Neurocan, **C,** Syndecan illustrating PEAKS post-translational-modifications (PTMs) using PEAKS studio 8.5 software.