Supplementary Information for

Discovery of a Functional Sequon for Chondroitin Sulfate Glycosylation

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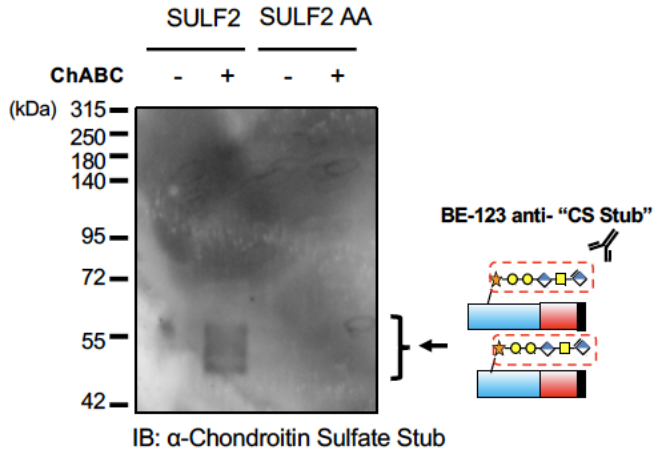

Fig. S1. Chondroitin sulfate (CS) modification in SULF2, as confirmed by an anti-CS stub antibody.

Western blotting was used to detect CS modification in purified SULF2 proteins using the BE-123 anti-CS stub antibody. The proteins were pretreated with chondroitinase ABC (ChABC), which is required for the BE-123 epitope.

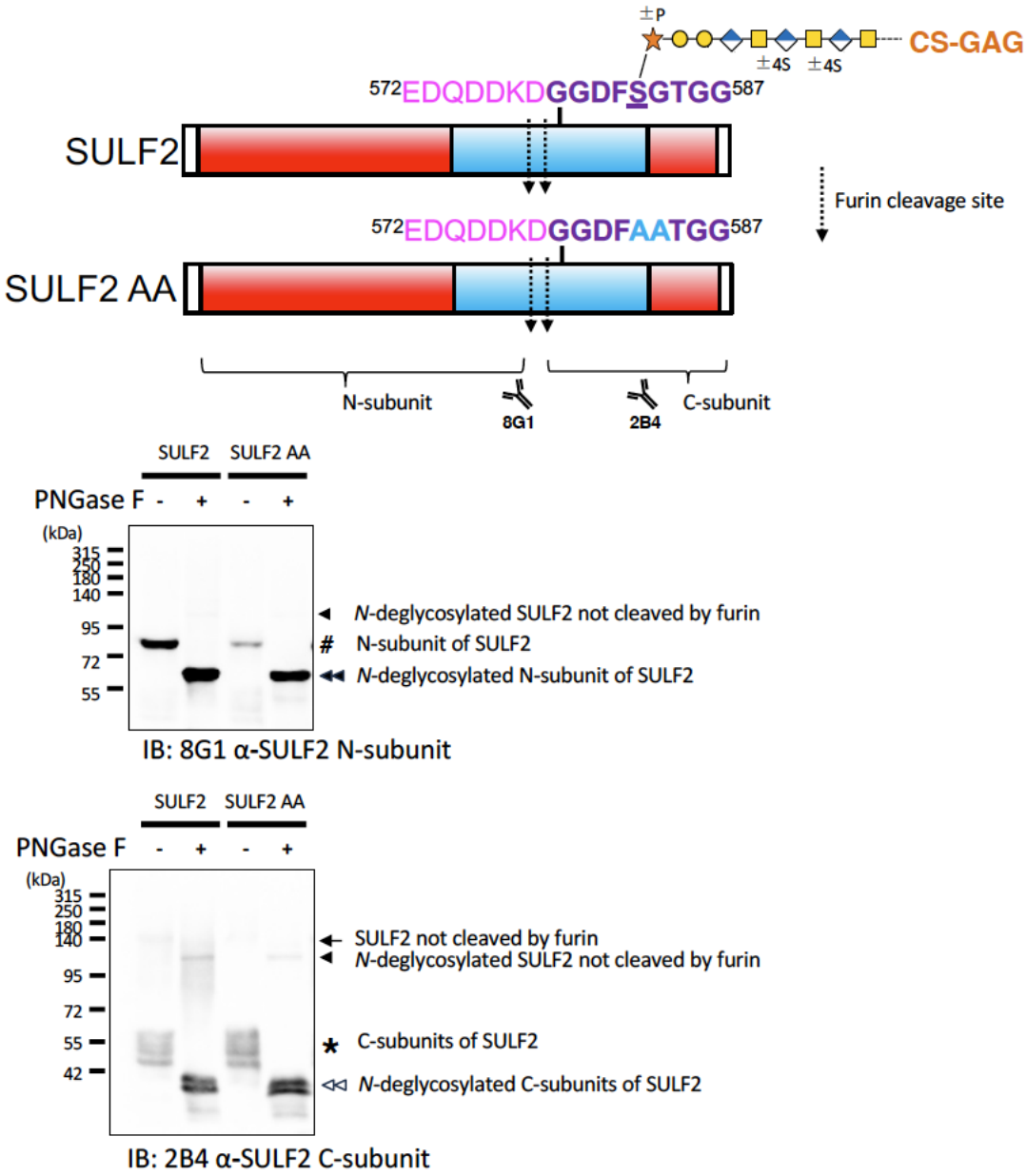

Fig. S2. N-Glycosylation of the N- and C-terminal subunits of SULF2.

Schematic representation of the SULF2 and its AA variant proteins is shown. The 8G1 and 2B4 anti-SULF2 antibodies recognize epitopes in the N- and C-subunits, respectively. Immunoblotting analysis of the expressed SULF2 and its AA variant in HEK293 cells using 8G1 and 2B4 antibodies after PNGase F treatment. The predicted components of the SULF2 protein are shown on the right. 8G1 preferentially recognizes the N-subunit of the furin-processed SULF2 protein and is less reactive with furin-uncleaved SULF2 proteins under reducing conditions.

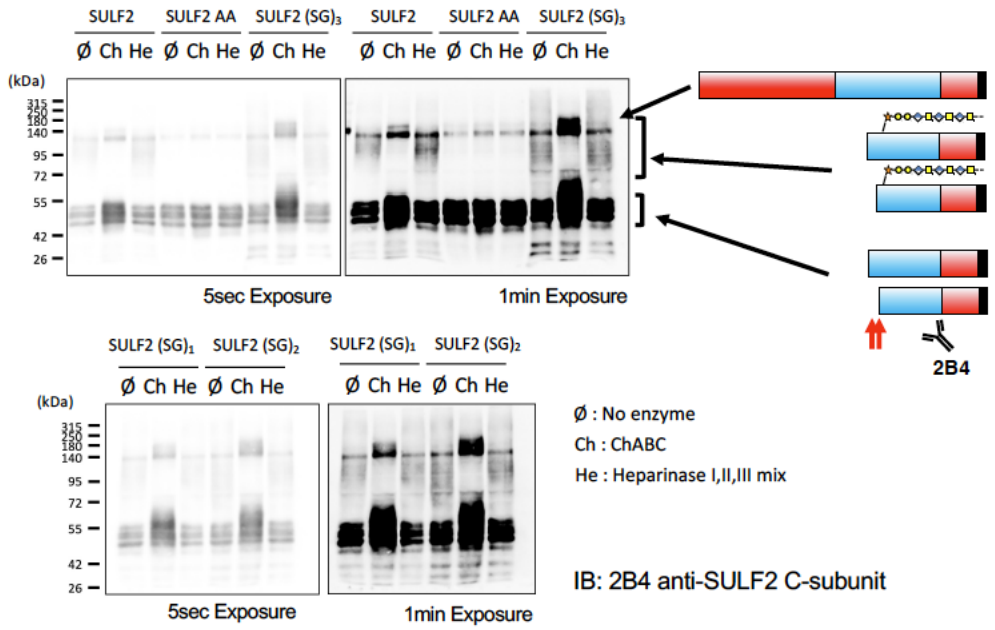

Fig. S3. The possibility of switching the synthesis of CS to HS by inserting repeated SG sequences is unlikely.

Immunoblotting analysis of the SULF2 variants with additional SG sequences using the 2B4 anti-SULF2 antibody was performed. The samples prepared from HEK293 cells were pretreated with ChABC or a mixture of heparinases I, II, and III. The smear band signals of CS-modified C-subunits were cleared by pretreating the samples with ChABC but not with a mixture of heparinases I, II, and III.

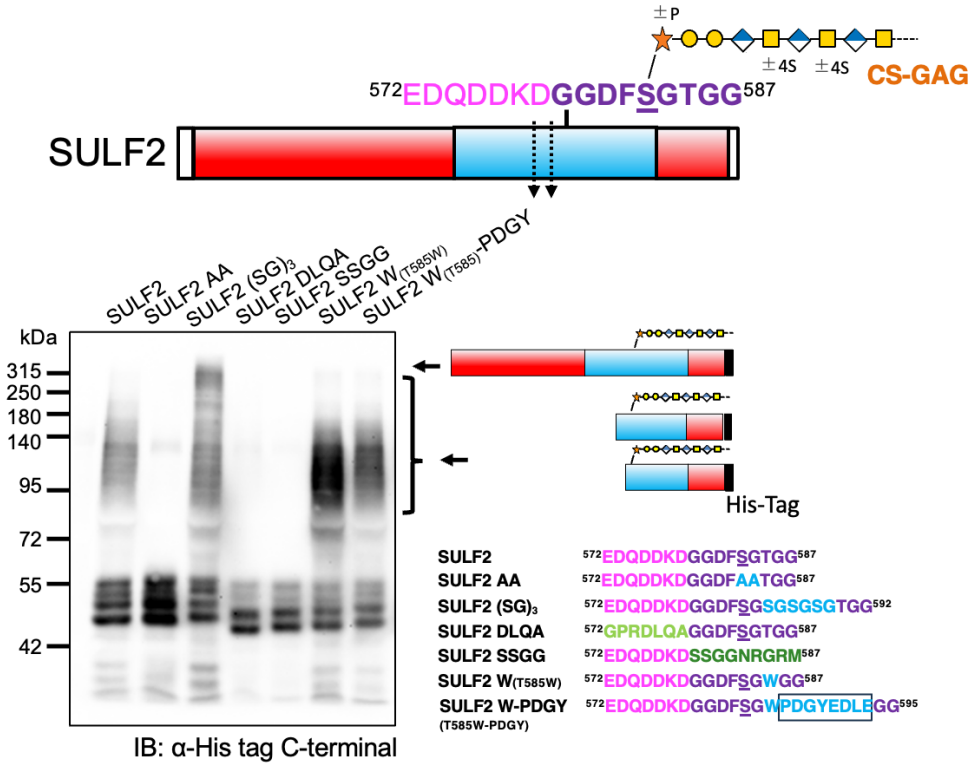

Fig. S4. The addition of the amino acid sequence PDGYEDLE with the Thr^{585} substitution to Trp produced a level of CS modification similar to the W ($T_{585}W$) variant.

Immunoblotting analysis was performed for the SULF2 W-PDGY ($T_{585}W$-PDGY) variant, which has the amino acids PDGYEDLE (*7*) inserted into the SULF2 W ($T_{585}W$) variant. In the $(SG)_3$ variant, the CS-modified C-subunit is also observed at ≥250 kDa. Schematic representations of the detected SULF2 components are shown on the right.

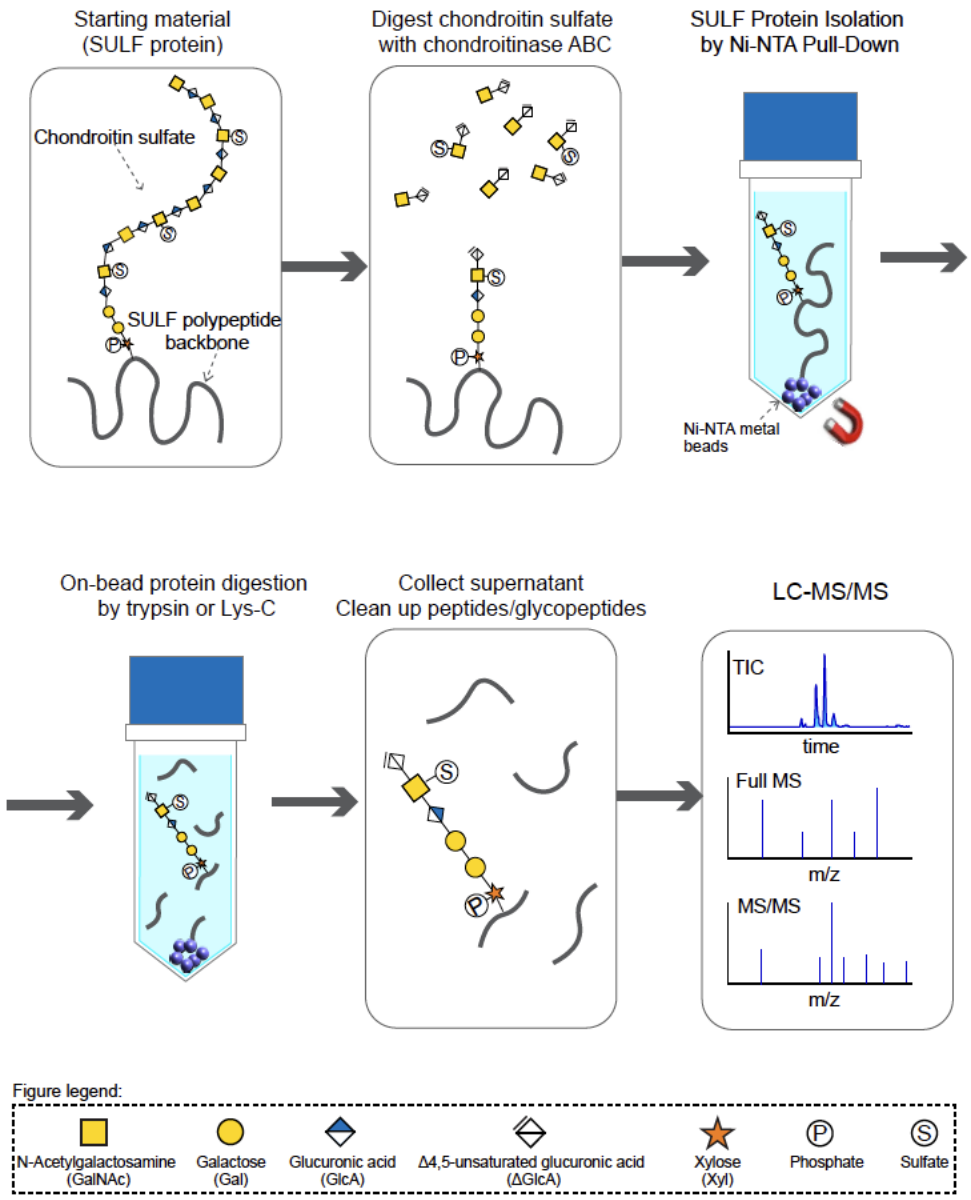

Fig. S5. Schematic workflow for MS analysis of CS-core glycopeptides of SULF proteins. The workflow illustrates the major steps for MS analysis of SULF proteins. Following chondroitinase ABC digestion, His-tagged proteins were captured using Ni-NTA magnetic beads and digested directly on-bead with trypsin or Lys-C to generate peptides and glycopeptides. On-bead digestion was selected to avoid contamination and sample loss associated with elution buffers, thereby improving sample cleanliness and MS detection. Nano LC-MS/MS analysis was performed (*18,19*).

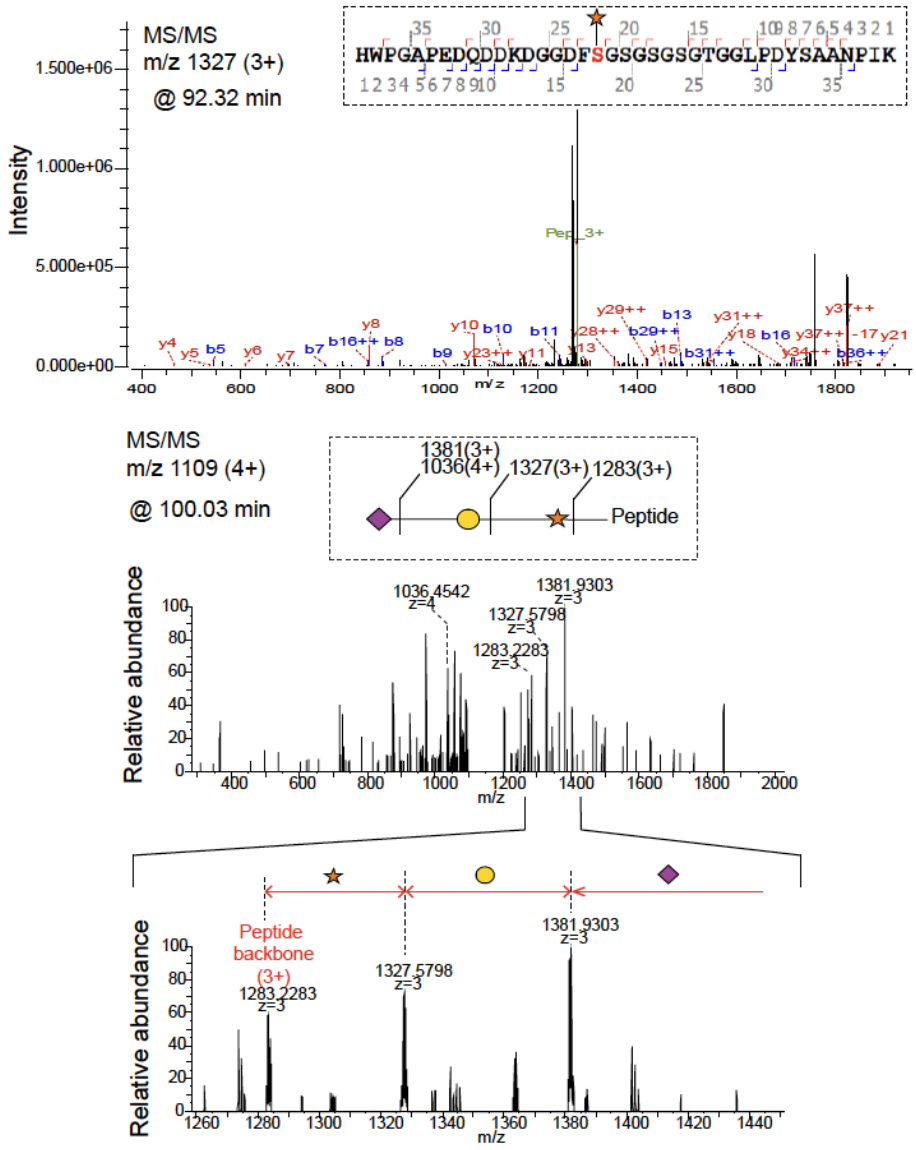

Fig. S6. Glycopeptides with truncated bridge structures detected in the SULF2 $(SG)_3$ variant.

MS/MS spectra of two representative glycopeptides with truncated bridge structures in the SULF2 $(SG)_3$ variant are shown. (A) Fragmentation-matched MS/MS spectrum of a glycopeptide carrying a single xylose with b- and y-type ions, confirming the peptide sequence and xylose glycosylation. (B) Collision-induced dissociation MS/MS spectrum of a glycopeptide ion at m/z 1109 (4+) corresponds to the peptide backbone from His^{566} to Lys^{604}, which carries $NeuAc_1Hexose_1Pentose_1$. The spectrum shows a series of neutral losses that are consistent with a NeuAc–hexose–pentose trisaccharide being attached to the peptide.

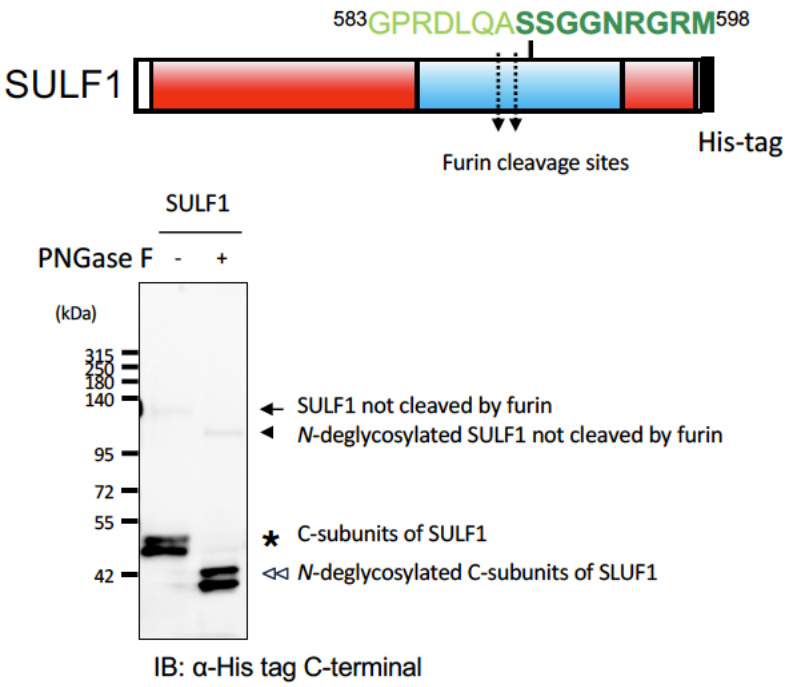

Fig. S7. N-Glycosylation of SULF1.

Schematic representation of the SULF1 protein is shown. Immunoblotting analysis of the expressed SULF1 in HEK293 cells using an anti-His tag antibody after PNGase F treatment. The predicted components of the SULF1 protein are shown on the right.

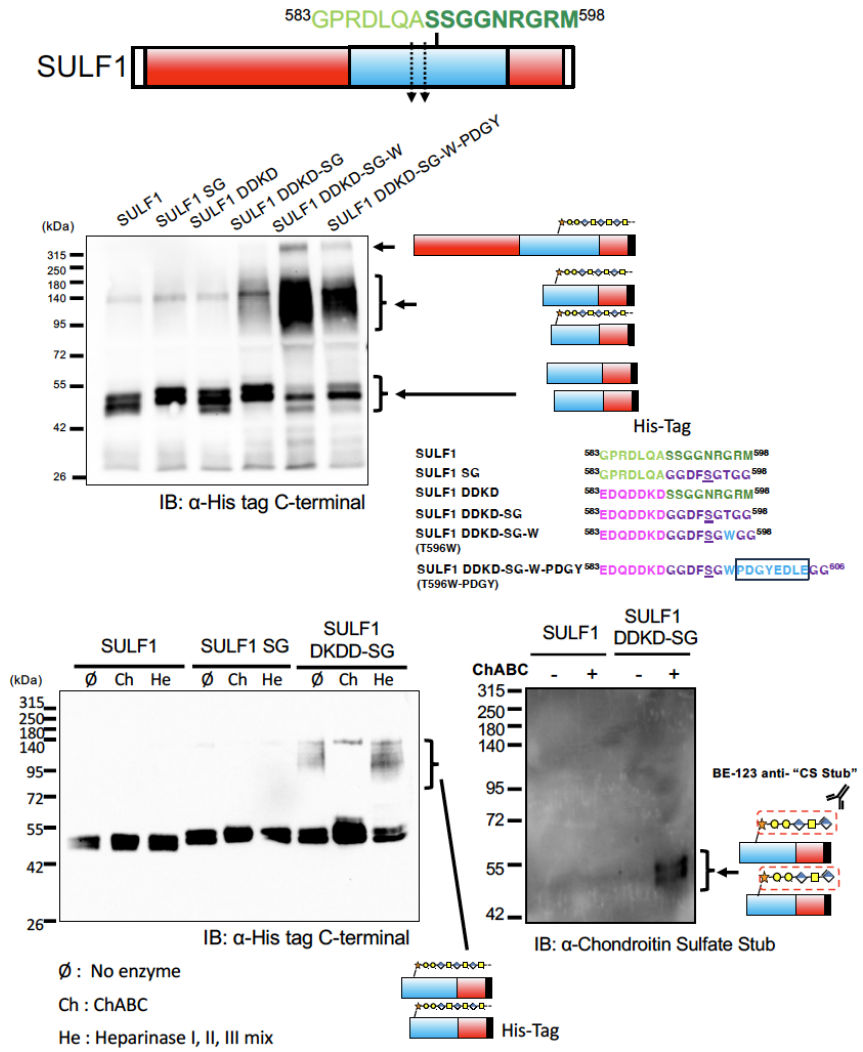

Fig. S8. The addition of the amino acid sequence PDGYEDLE with the Thr^{596} substitution to Trp produced a level of CS modification similar to the DDKD-SG-W ($T_{596}W$) variant. Immunoblotting analysis was performed for the SULF1 DDKD-SG-W-PDGY ($T_{596}W$-PDGY) variant, which has the amino acids PDGYEDLE (*7*) inserted into the SULF1 DDKD-SG-W ($T_{596}W$) variant. Schematic representations of the detected SULF1 components are shown on the right. Western blotting was used to detect CS modification in the purified SULF1 DDKD-SG variant protein. The smear band ranging from 95 to 180 kDa was cleared by pretreatment with chondroitinase ABC (ChABC), but not with a mixture of heparinases I, II, and III (lower left panel). BE-123 anti-CS stub antibody and ChABC pretreatment revealed C-subunit bands (lower right panel). Novel CS biosynthesis was confirmed in the SULF1 DDKD-SG variant.

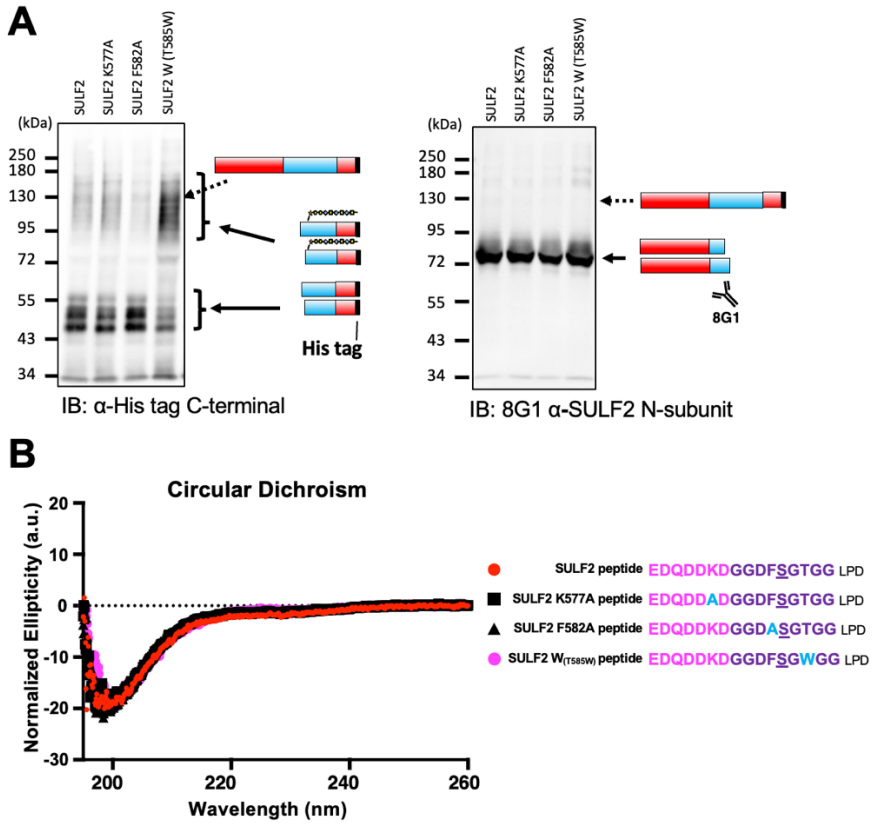

Fig. S9. Analysis of intrinsic secondary force interactions around Ser^{583} for CS modification in SULF2.

(**A**) The SULF2 variants harboring the mutations in Lys^{577}, a unique positively charged residue in the EDQDDKD cluster, or in Phe^{582}, an aromatic residue located near Ser^{583} were expressed in HEK293 cells. The concentrated conditioned medium from the transfected cells was subjected to Western blotting. The smear band signals of CS-modified C-subunits of the SULF2 $K_{577}A$ and SULF2 $F_{582}A$ variants are comparable to the SULF2. 8G1 preferentially recognizes the N-subunit of the furin-processed SULF2 protein and is less reactive with furin-uncleaved SULF2 proteins under reducing conditions. (**B**) Secondary structure of the peptides revealed by circular dichroism (CD) analysis. Normalized far-UV CD spectra of the synthesized SULF2 variant peptides are shown. These peptides form similar random coil structures.

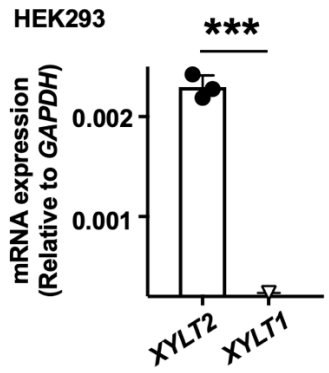

Fig S10. HEK293 cells predominantly express the xylosyltransferase 2 (XYLT2) gene, but not the XYLT1 gene.

The mRNA expression levels of XYLT2 and XYLT1 were quantified using qRT-PCR (n = 3 for each gene). Two of the three XYLT1 values; ≤ 0.0002. Data are means ± S.D. ***$P < 0.001$.

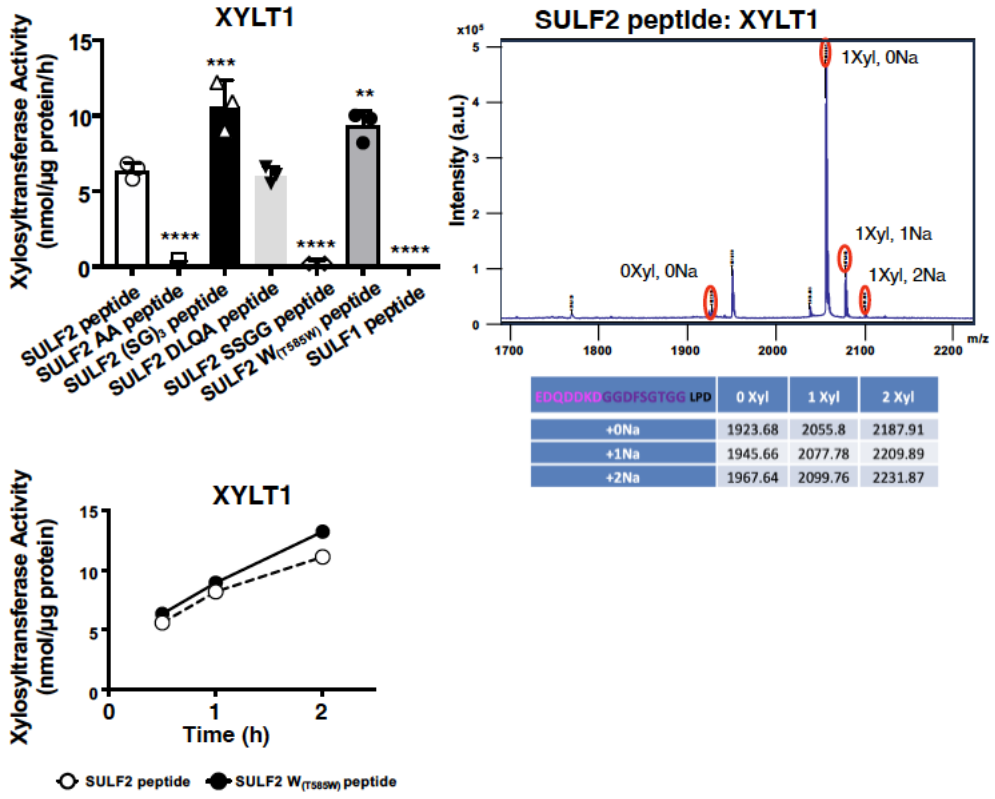

Fig. S11. Xylosyltransferase 1 activity toward the synthesized SULF peptides.

In vitro xylosyltransferase 1 (XYLT1) assay. XYLT1 activity with the SULF2 variant peptides were equal to or slightly higher than that with the SULF2 peptide (n = 3). Column with mean value less than 0.1 in the SULF1 peptide. Data are means ± SE. $^{**}P < 0.01$, $^{***}P < 0.001$. The activity of XYLT1 increases over time with both the SULF2 and the SULF2 W ($T_{585}W$) peptides. Monoxylosylation of the SULF2 peptide was verified using MALDI-TOF MS analysis.

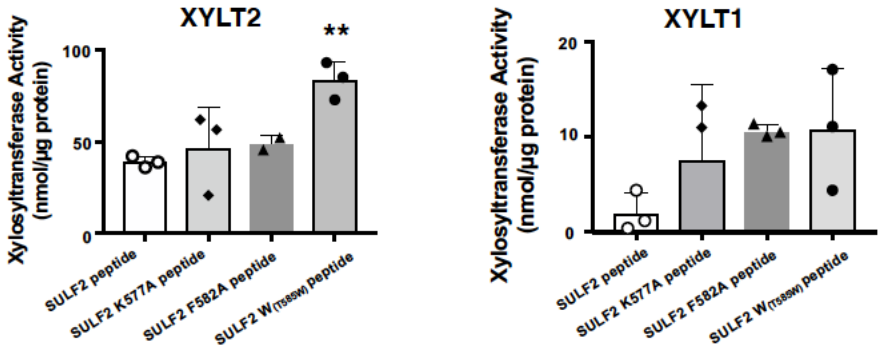

Fig. S12. In vitro assay of xylosyltransferase 2 (XYLT2) and XYLT1 for the peptides derived from the SULF2 K_{577}A and SULF2 F_{582}A variants.

Both the SULF2 K577A and SULF2 F582A peptides exhibit the same level of activity for XYLT2 and XYLT1 as the SULF2 peptide. The data were obtained from the 2hs incubation assay. Data are means ± SE. $^{**}P < 0.01$.

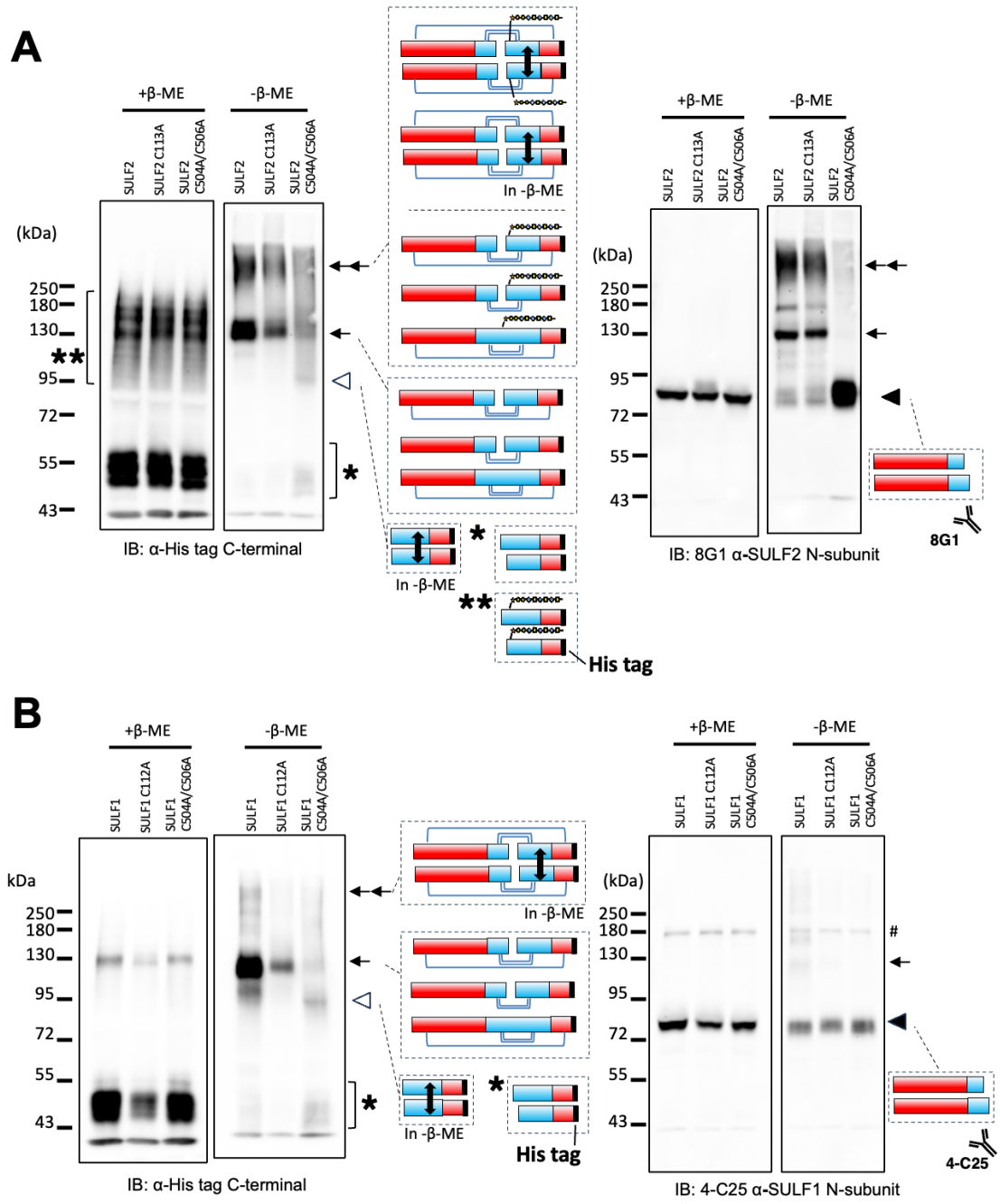

Fig. S13. The subunit organization of human SULFs.

The SULF pro-proteins are cleaved by a furin-type convertase into two subunit chains of 75 and 50 kDa, which form disulfide-linked heterodimers (*1,13*). The Cys residues that form these disulfide bonds are highly conserved in vertebrate and invertebrate SULFs and are found in the sulfatase domains of the N- and C-subunits (Cys^{113} and Cys^{822} in SULF2; Cys^{112} and Cys^{841} in SULF1) and in two C-X-C motifs in the hydrophilic domain (Cys^{504}, Cys^{506}, Cys^{660}, and Cys^{662} in SULF2; Cys^{504}, Cys^{506}, Cys^{675}, and Cys^{677} in SULF1) (see also **figure S17**). (**A**) SULF2

variants harboring Ala substitutions in Cys^{113} or Cys^{504}/Cys^{506} (SULF2 C113A and SULF2 C504A/C506A) and (**B**) SULF1 variants harboring Ala substitutions in Cys^{112} or Cys^{504}/Cys^{506} (SULF1 C112A and SULF1 C504A/C506A) were expressed in HEK293 cells. The concentrated CM were analyzed by Western blotting with or without β-mercaptoethanol (β-ME). The schematic representation of the predicted components of the SULF subunits is shown on the right. The bands with ≥ 250 kDa under non-reducing conditions partially contain potential SDS-resistant multimers of the SULFs. These minor species are formed via the coiled-coil motif (bidirectional arrow) in the hydrophilic domain as previously described (*1,13*). #; the bands also seen in an isotype-match control blot. Both 8G1 and 4-C25 preferentially recognize the N-subunit of furin-processed SULF proteins. These antibodies are less reactive against furin-uncleaved SULFs under reducing conditions.

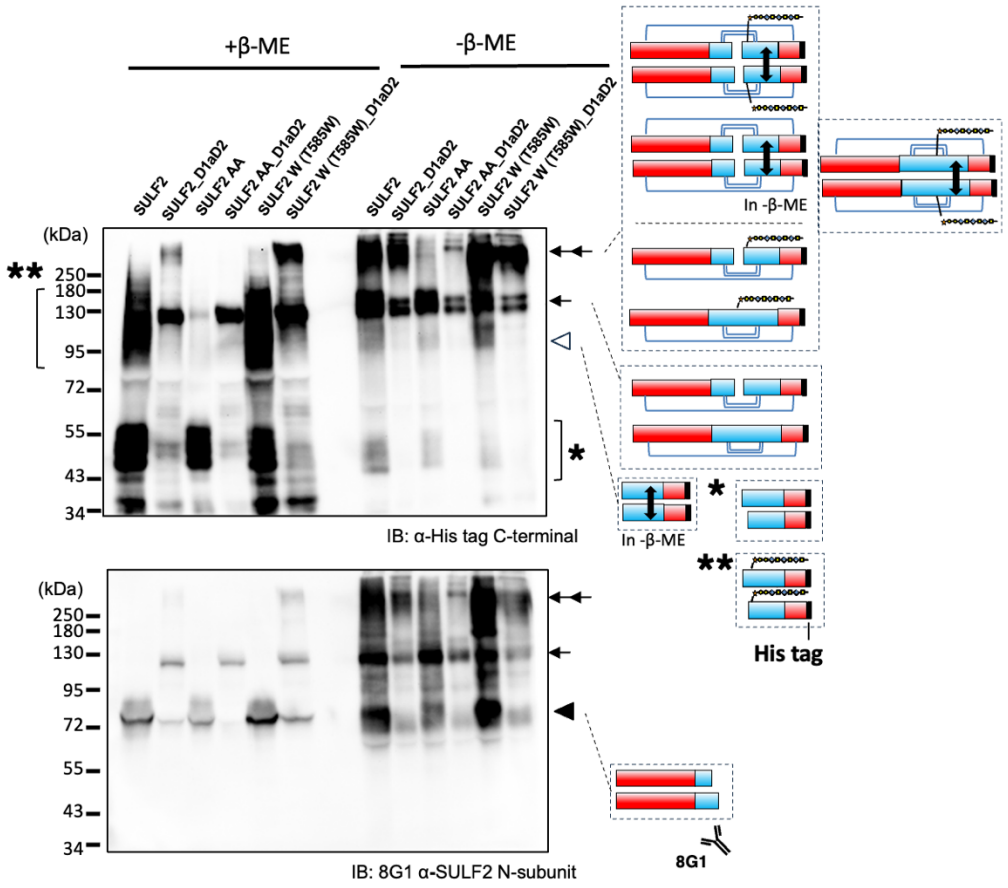

Fig. S14. Increase in the higher order formation of furin-uncleavable SULF2 by CS modification

The furin-type convertase cleaves at the Arg^{538} and Arg^{565} sites of the SULF2 protein. The furin recognition sites are ^{533}RSRSIR^{538} and ^{560}RNLTKR^{565}. The new variant of the protein SULF2 lacks these amino acids at both sites (SULF2 D1aD2). Immunoblotting analysis was carried out to detect the N- and C-subunits of the variants with or without β-mercaptoethanol (β-ME). The schematic representation of the predicted components of the SULF2 subunits is shown on the right. The bands with ≥ 250 kDa under non-reducing conditions partially contain SDS-resistant potential multimers of SULF2 that are formed via the coiled-coil motif in the hydrophilic domain (*13*).

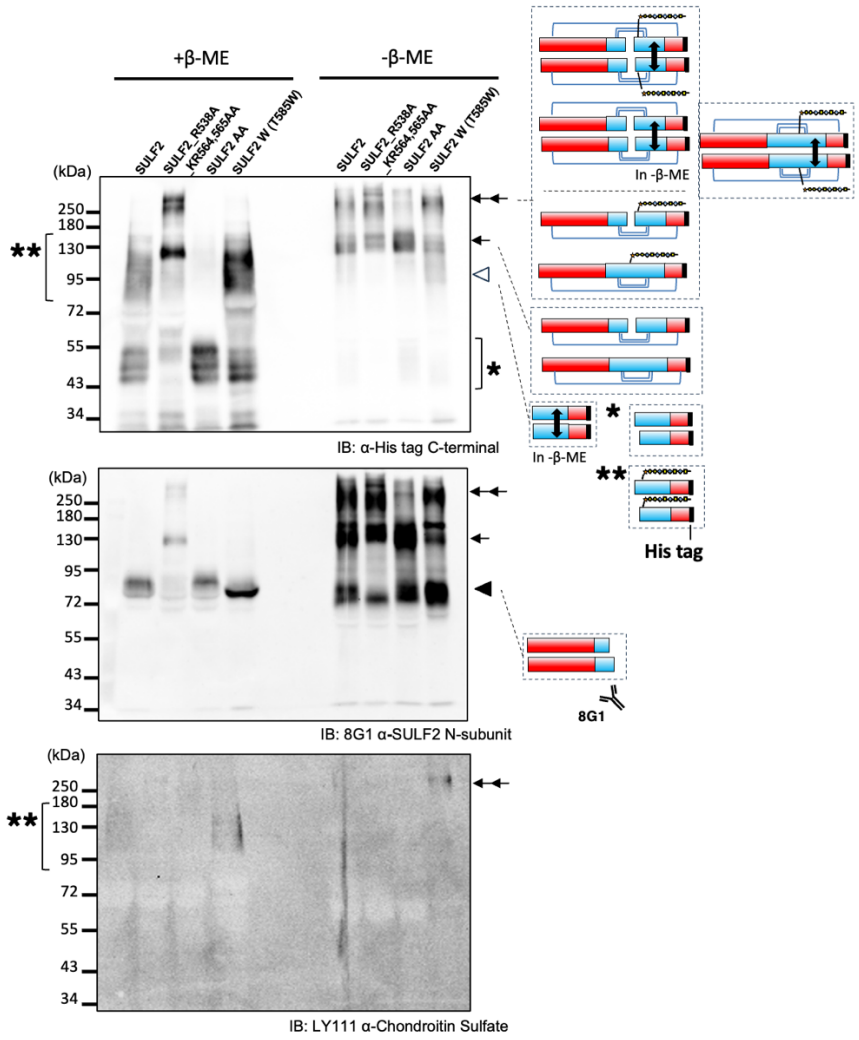

Fig. S15. CS modification in the higher order of SULF2

The furin-type convertase cleaves at the Arg^{538} and Arg^{565} sites of the SULF2 protein. The furin recognition sites are ^{533}RSRSIR^{538} and ^{560}RNLTKR^{565}. The new variant of the protein SULF2 (SULF2 R538A_KR564,565AA) contains the substitutions of these amino acids, Arg^{538} and Lys^{564}- Arg^{565} into Ala. Immunoblotting analysis was carried out to detect the N- and C-subunits of the variants (8G1 in the upper band and His tag in the lower band) with or without β-mercaptoethanol (β-ME). The samples were also immunoblotted with the LY111 anti-chondroitin sulfate (CS) antibody. The schematic representation of the predicted components of the SULF2 subunits is shown on the right. The bands with ≥ 250 kDa in non-reducing conditions partially contain SDS-resistant potential multimers of SULF2 that are formed via the coiled-coil motif in the hydrophilic domain (*13*).

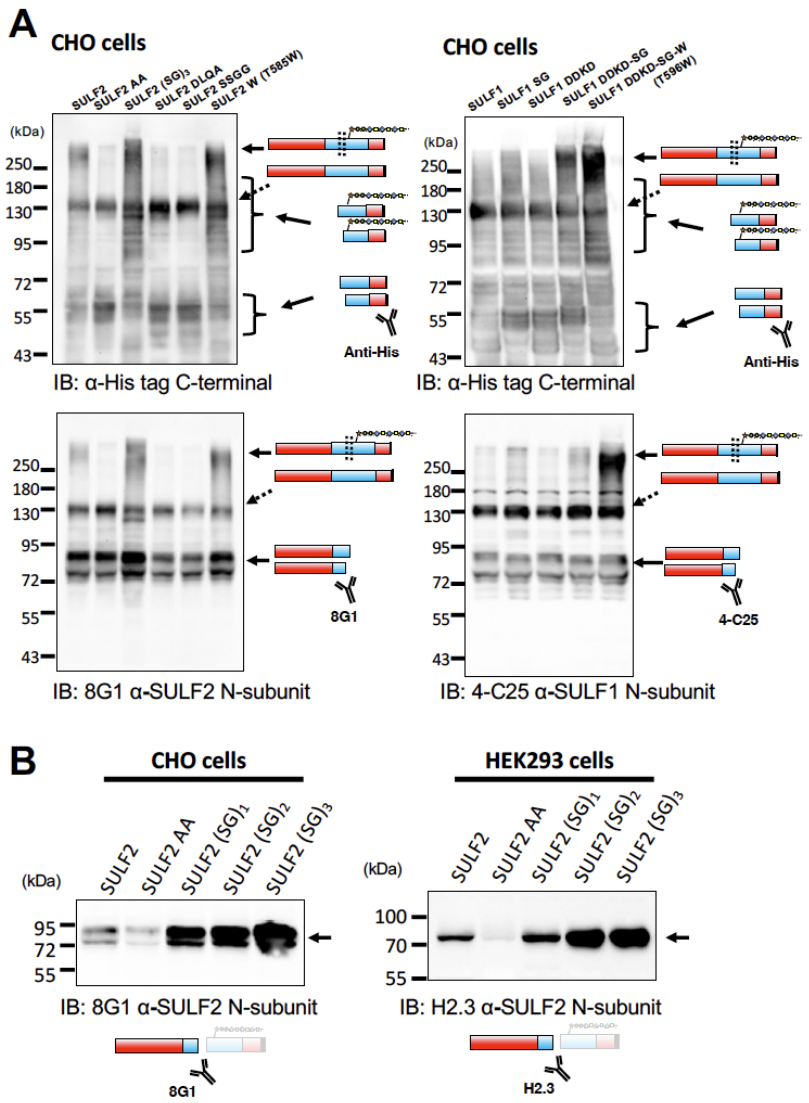

Fig. S16. The discovered CS sequons are functional for human SULFs in Chinese hamster ovary (CHO) cells.

(**A**) The C-terminal His-tagged human SULF2, SULF1, and their mutated variants were transiently expressed in CHO cells. The concentrated conditioned medium (CM) from the transfected CHO cells were subjected to Western blotting to detect the His tag or their N-subunits. The schematic representation of the predicted major components of the SULF subunits is shown on the right. (**B**) The level of CS-modified SULF2 proteins in the CM of the transfected CHO cells were analyzed to detect the N-subunits of the SULF2 and its variants. The insertion of Ser-Gly amino acids at Ser^{583}-Gly^{584} increases CS-modified SULF2 protein levels in the CM of transfected CHO cells, as observed in HEK293 cells.

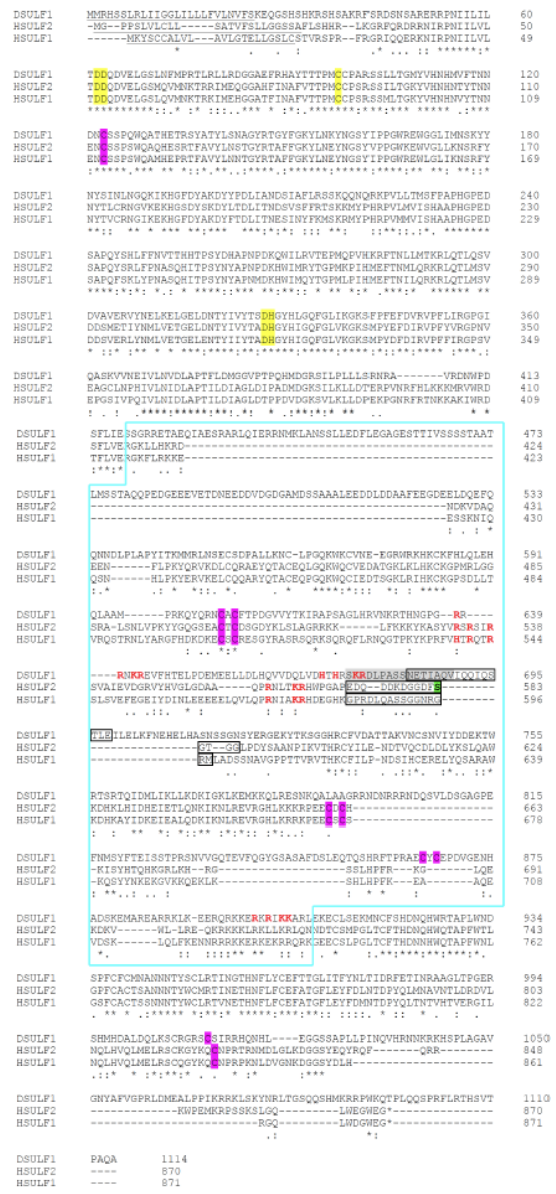

Fig. S17. Integration of the CS sequon EDQDDKDGGDFSGWGG into Drosophila Sulf1. The amino acid alignment of dSulf1 with human SULF2 and SULF1 is shown. The light blue line demarcates the hydrophilic domain. The signal peptide sequence is underlined. Yellow denotes the calcium ion-binding active sites, including the Cys residue that undergoes *N*-formylglycine modification (*1*). Purple indicates the Cys residues that form disulfide bonds between the N- and C-subunits. The double disulfide bonds between the two C-X-C residues in the hydrophilic domain are the primary bonds in the SULF family. See also **figure S13**. Consensus furin cleavage sites with basic amino acids in red are shown. See also **figure S14** and **figure S15**. Ser^{583} of SULF2 is highlighted in green.

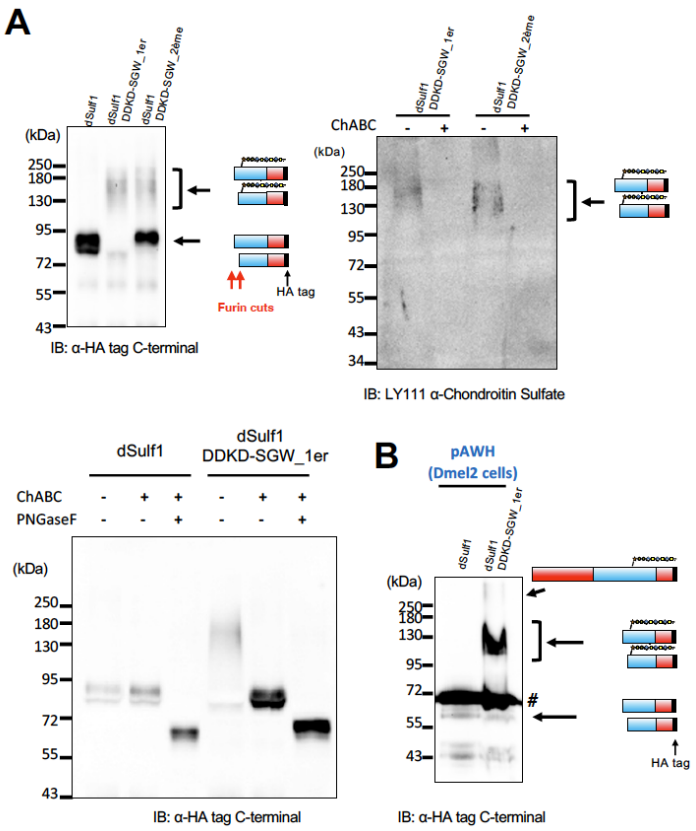

Fig. S18. The discovered CS sequon is sufficient alone to induce CS biosynthesis in Drosophila Sulf1.

(A) The concentrated conditioned medium (CM) from HEK293 cells that were transfected with dSulf1 or CS-sequon-integrated dSulf1 variants (dSulf1 DDKD-SGW-1er and dSulf1 DDKD-SGW-2ème) was subjected to Western blotting to detect the C-terminal HA tag, with or without chondroitinase ABC (ChABC) pretreatment. See **Figure 4C** and **figure S17** for detailed amino acid sequences. Schematic representation of the predicted components of the dSulf1 variants detected in the blot is shown on the right. The reduction in MW of the bands detected by PNGase F treatment indicates that dSulf1 and the dSulf1 DDKD-SGW-1er variant undergo *N*-glycan post-translational modification. (**B**) The concentrated CM prepared from Drosophila Dmel2 cells (*36*) that were transfected with dSulf1 or dSulf1 DDKD-SGW-1er, which was reconstructed in the pAWH vector (*36*), was subjected to Western blotting to detect the C-terminal HA tag. Induction of the CS modification appears to occur in dSulf1 with the discovered CS sequon. Given the MW of the detected band, it is likely that the CS chain length in the dSulf1 variant is shorter than that expressed in HEK293 cells. # denotes anti-HA reactive unknown materials present in the Dmel2 medium.

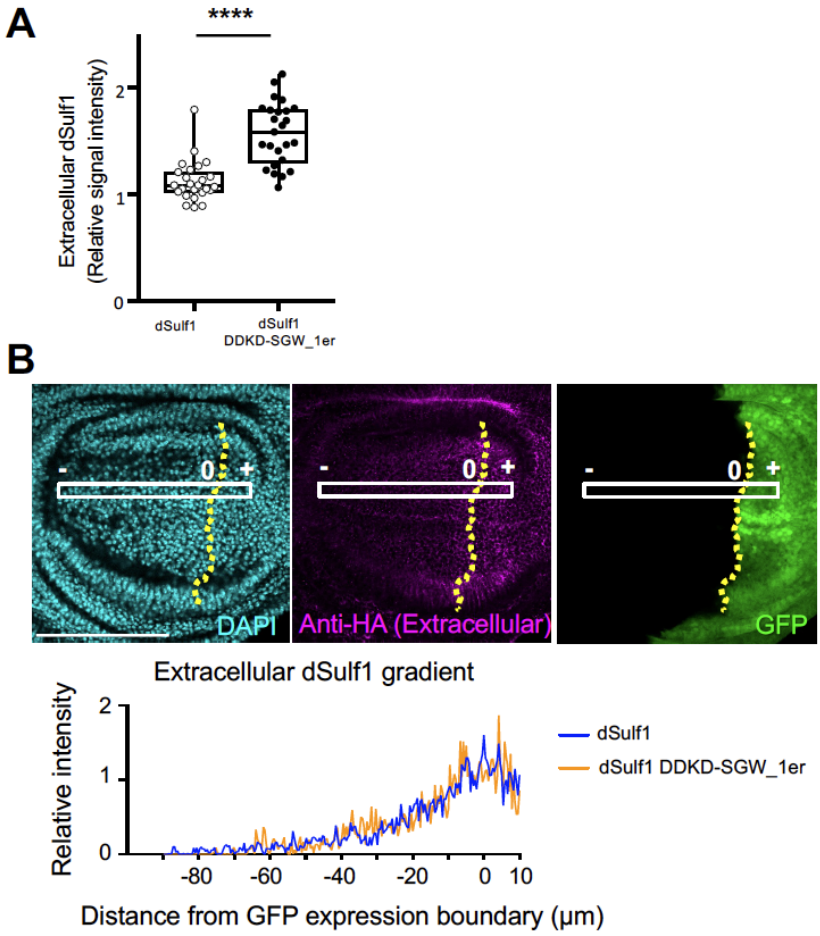

Fig. S19. Increased extracellular protein level of the Drosophila Sulf1 variant integrated with the discovered CS sequon in vivo.

Drosophila strains with either the UAS-dSulf1 transgene (dSulf1) or the UAS-CS sequon-integrated dSulf1 transgene (dSulf1 DDKD-SGW-1er) were generated. Expression of the transgenes was induced in the posterior compartment of the wing disc using a hedgehog (hh)-Gal4 driver. (**A**) Extracellular dSulfs within the anterior compartment of the wing pouch were quantified (n = 25 for each). See **Figure 4E** for the posterior compartment. The data are presented in the form of a box-and-whisker diagram. ****$P < 0.0001$. (**B**) The molecular gradient of the extracellular dSulf1 and the dSulf1 DDKD-SGW-1er variant in the wing pouch was measured. A comparable gradient pattern in hh>dSulf1 and hh>dSulf1 DDKD-SGW 1er is shown. DAPI was used to show cell nuclei. The C-terminal HA tag of the extracellular dSulf1 and dSulf1 DDKD-SGW-1er was revealed by extracellular immunostaining. The posterior compartment is marked by GFP expression (green). Scale bar: 100 μm.

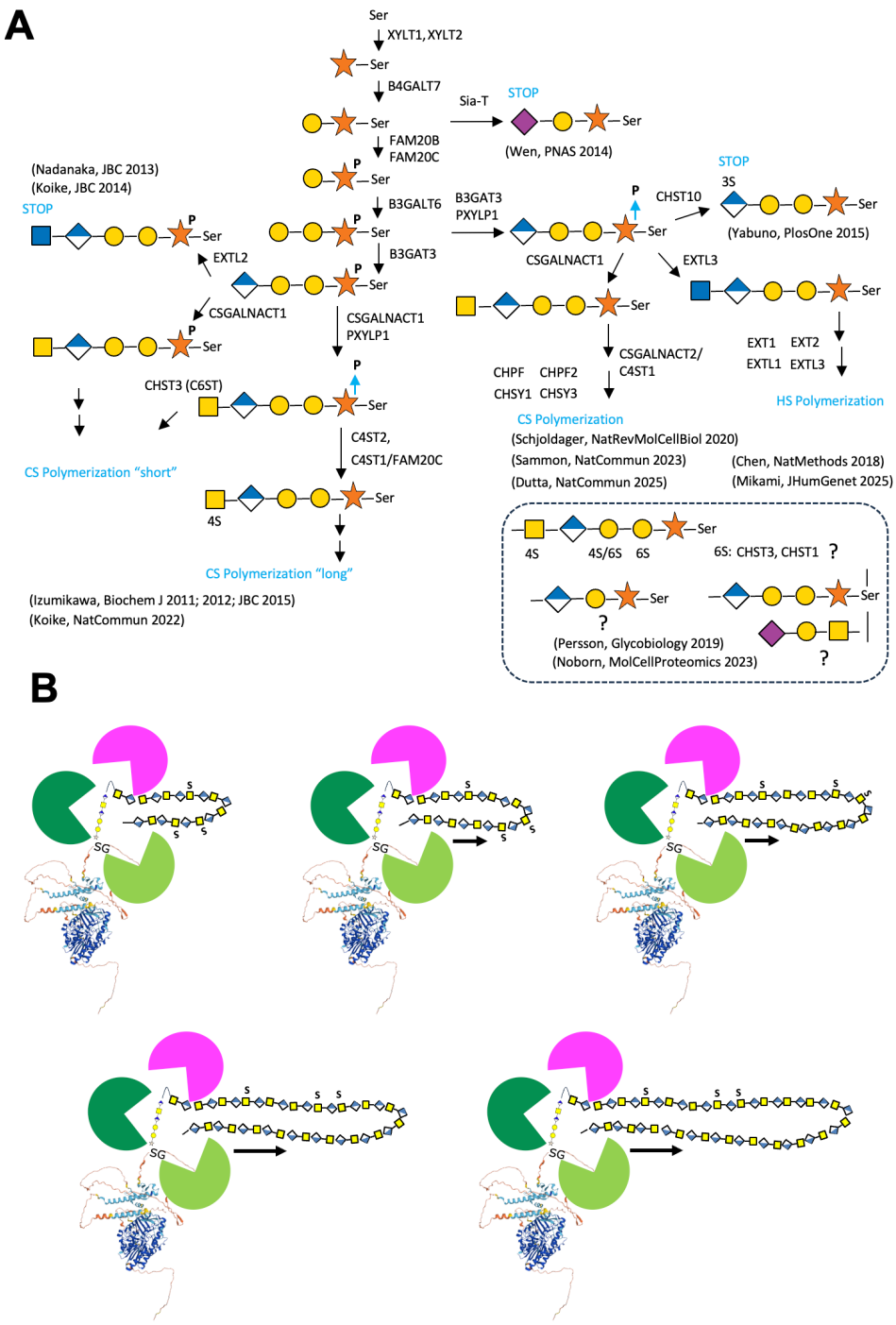

Fig. S20. A brief summary of the CS biosynthesis initiation pathways and a proposed model of the mechanism by which CS polymerization occurs near the core protein.

(**A**) A brief summary of CS biosynthesis initiation, starting with the mono-xylosylation of the core protein (*20-23, 29, 37-44, 49*). Symbols denote the following: xylose (orange star), galactose (yellow circle), glucuronic acid (divided blue diamond), *N*-acetylgalactosamine

(yellow square), *N*-acetylglucosamine (blue square), *N*-acetylneuraminic acid, predominant sialic acid (purple diamond), sulfate (S), and phosphate (P). (**B**) A proposed model based upon the results of this study implies that CS polymerization occurs in close proximity to the core protein. The non-reducing end of the CS chain is expected to constantly remain in the vicinity of the core protein during polymerization. Arrows indicate the direction of elongation. A complex of enzymes involved in CS chain polymerization is shown (pink, green, light green).

Table S1. Relative abundance of the CS peptides and glycopeptides detected in human SULF2 and its variants.

Analyte		CS-modification level	Protease used	Peptide sequence			Peak intensity area %									
				Start	End	Sequence	Without CS	With 1 Xyl	With 1 CS	With 1 CS + 1 P	With 1 CS + 1 S	With 1 CS + 1 P + 1 S	With 2 Xyl	With NeuAc-Gal-Xyl	With GalNAc-HexA-Gal-Gal-Xyl	With 1 CS, 1 Xyl
SULF2	WT	+	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFSGTGGLPDYSAANPIK(V)	70.82%	3.06%	12.83%	8.41%	4.88%	0.00%	0.00%	0.00%	0.00%	0.00%
	AA	-	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFAATGGLPDYSAANPIK(V)	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	SG3	++	Trypsin	566	604	(R)HWPGAPEDQDDKDGGDFSGSGSGSGTGGLPDYSAANPIK(V)	69.27%	22.76%	3.73%	2.54%	0.25%	0.00%	0.65%	0.54%	0.00%	0.27%
	DLQA	-	Trypsin	575	598	(R)DLQAGGDFSGTGGLPDYSAANPIK(V)	97.03%	1.10%	1.30%	0.30%	0.31%	0.00%	0.00%	0.00%	0.00%	0.00%
	SSGG	-	Lys-C	566	586	(R)HWPGAPEDQDDKDSSGGNRGR(M)	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	T585W	+++	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFSGWGGLPDYSAANPIK(V)	5.51%	7.60%	39.09%	35.29%	12.50%	0.00%	0.00%	0.00%	0.00%	0.00%

Table S2. Relative abundance of the CS peptides and glycopeptides detected in human SULF1 and its variants.

Analyte		CS-modification level	Protease used	Peptide sequence			Peak intensity area %									
				Start	End	Sequence	Without CS	With 1 Xyl	With 1 CS	With 1 CS + 1 P	With 1 CS + 1 S	With 1 CS + 1 P + 1 S	With 2 Xyl	With NeuAc-Gal-Xyl	With GalNAc-HexA-Gal-Gal-Xyl	With 1 CS, 1 Xyl
SULF1	WT	-	Trypsin	586	595	(R)DLQASSGGNR(G)	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	SG	-	Trypsin	586	613	(R)DLQAGGDFSGTGGLADSSNAVGPPTTVR(V)	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	DDKD	-	Trypsin	577	595	(R)HDEGHKEDQDDKDSSGGNR(G)	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	DDKD-SG	+	Trypsin	577	613	(R)HDEGHKEDQDDKDGGDFSGTGGLADSSNAVGPPTTVR(V)	69.20%	0.75%	6.38%	4.44%	10.55%	8.67%	0.00%	0.00%	0.00%	0.00%
	DDKD-SG-T596W	+++	Trypsin	577	613	(R)HDEGHKEDQDDKDGGDFSGWGGLADSSNAVGPPTTVR(V)	11.51%	2.06%	27.66%	12.87%	31.47%	14.43%	0.00%	0.00%	0.00%	0.00%

Table S3. The primers used to construct each plasmid.

Name		oligo	length mer	%GC	Tm °C	Ta °C	^{572}EDQDDKDGGDFSGTGG^{587}
HSulf2 AA	Forward	TGGGGACTTCgcagctACTGGAGGCCTTC	29	62	57	58	
	Reverse	CCATCCTTGTCATCTTGG	18	50	59		^{572}EDQDDKDGGDFAATGG^{587}
HSulf2 (SG)1	Forward	tctgggACTGGAGGCCTTCCCGAC	24	67	70	69	
	Reverse	GCCACTGAAGTCCCCACC	18	67	68		^{572}EDQDDKDGGDFSGSGTGG^{589}
HSulf2 (SG)2	Forward	agtggaACTGGAGGCCTTCCCGAC	24	63	70	66	
	Reverse	CCCAGAGCCACTGAAGTC	18	61	65		^{572}EDQDDKDGGDFSGSGSGTGG^{591}
HSulf2 (SG)3	Forward	tcgggaACTGGAGGCCTTCCCGAC	24	67	70	70	
	Reverse	TCCACTCCCAGAGCCACTG	19	63	69		^{572}EDQDDKDGGDFSGSGSGSGTGG^{592}
HSulf2 DLQA	Forward	tctccaggctGGTGGGGACTTCAGTGGCACTG	32	63	72	72	
	Reverse	tctcttggcccAGGGGCCCCTGGCCAGTG	29	72	77		^{572}GPRDLQAGGDFSGTGG^{587}
HSulf2 SSGG	Forward	CAGGGGCAGGATGCTTCCCGACTACTCAGCC	31	65	65	62	
	Reverse	TTGCCACCACTGGAATCCTTGTCATCTTGGTCC	33	52	61		^{572}EDQDDKDSSGGNRGRM^{587}
HSulf2 W (T585W)	Forward	CTTCAGTGGCtggGGAGGCCTTCC	24	67	62	63	
	Reverse	TCCCCACCATCCTTGTCA	18	56	65		^{572}EDQDDKDGGDFSGWGG^{587}
HSulf2 W (T585W)-PDGY	Forward	tgaagatctggagGCCAACCCCATTAAAGTGAC	33	48	64	65	
	Reverse	taaccatctggccaGCCACTGAAGTCCCCACC	32	59	68		^{572}EDQDDKDGGDFSGWPDGYEDLE^{593}
HSulf2 K577A	Forward	CCAAGATGACgcaGATGGTGGGGACTTCAGTGGCACTGG	39	59	72	72	
	Reverse	TCCTCAGGGGCCCCTGGC	18	78	76		^{572}EDQDDADGGDFSGTGG^{587}
HSulf2 F582A	Forward	TGGTGGGGACgcaAGTGGCACTG	23	65	62	61	
	Reverse	TCCTTGTCATCTTGGTCC	18	50	60		^{572}EDQDDKDGGDASGTGG^{587}
HSulf2 D1aD2 (for D1a)	Forward	TCAGTGGCCATCGAGGTG	18	61	67	64	
	Reverse	GACATAGCTGGCCTTGTAC	19	53	63		^{533}~~RSRSIR~~^{538} ... ^{560}~~RNLTKR~~^{565}
HSulf2 D1aD2 (for D2)	Forward	CACTGGCCAGGGGCCCCT	18	78	77	72	
	Reverse	GGGCTGGGCGGCATCACC	18	78	75		^{533}~~RSRSIR~~^{538} ... ^{560}~~RNLTKR~~^{565}
HSulf2 C113A	Forward	CAATGAGAACgctTCCTCGCCCTCC	25	60	68	59	
	Reverse	TTGGTGTAGGTGTTGTGG	18	50	62		^{113}ASSP
HSulf2 C504A/C506A	Forward	cgcaGACAGCGGGGACTACAAGCTCAGC	28	64	74	72	
	Reverse	gttgcGGCCTCGCTGCCCTGCCC	23	78	78		^{504}ATA^{506}

Name		oligo	length mer	%GC	Tm °C	Ta °C	^{583}GPRDLQASSGGNRGRM^{598}
HSulf1 SG	Forward	tggcactggaggcCTGGCAGATAGCAGCAAC	31	61	64	64	
	Reverse	ctgaagtccccaccAGCCTGGAGATCTCTTGG	32	59	63		^{583}GPRDLQAGGDFSGTGG^{598}
HSulf1 DDKD-SG	Forward	tgacaaggatGGTGGGGACTTCAGTGGC	28	57	68	67	
	Reverse	tcttggtcctcCTTGTGGCCTTCATCATGACG	32	53	66		^{583}EDQDDKDGGDFSGTGG^{598}
HSulf1 DDKD	Forward	tgacaaggatTCCAGTGGTGGCAACAGG	28	54	67	64	
	Reverse	tcttggtcctcCTTGTGGCCTTCATCATGAC	31	52	63		^{583}EDQDDKDSSGGNRGRM^{598}
HSulf1 DDKD-SG-W(T596W)	Forward	CTTCAGTGGCtggGGAGGCCTGG	23	70	62	63	
	Reverse	TCCCCACCATCCTTGTCA	18	56	65		^{583}EDQDDKDGGDFSGWGG^{598}
HSulf1 DDKD-SG-W(T596W)-PDGY	Forward	tgaagatctggagGCCGTGGGCCCACCTACC	31	65	75	72	
	Reverse	taaccatctggccaGCCACTGAAGTCCCCACCATCC	36	58	72		^{583}EDQDDKDGGDFSGWPDGYEDLE^{604}
Hsulf1 C112A	Forward	CAACGAGAACgcaTCTTCCCCCTC	24	58	55	56	
	Reverse	TTGGTGTAGACATTGTGATTG	21	38	60		^{112}ASSP
HSulf1 C504A/C506A	Forward	tgcaAGGGAGTCTGGTTACCGT	22	55	65	61	
	Reverse	cttgcCTCTTTGTCTTTGTCATGGAAG	27	44	60		^{504}ASA^{506}

Name		oligo	length mer	%GC	Tm °C	Ta °C	
dSulf1 DDKD-SGW-1er	Forward	ggggacttcagtggctggggaggcATCTTGGAGCTTAAATTCAACGAGC	49	55	65	66	
	Reverse	accatccttgtcatcttggtcctcGCTGCTTGCGGGCAAATC	42	55	68		^{683}NETIAQVIQQIQSTLE^{698} -> ^{683}EDQDDKDGGDFSGWGG^{698}
dSulf1 DDKD-SGW-2eme	Forward	tggctggggaggcATTCAGCAGATACAAAGC	31	55	56	58	
	Reverse	ctgaagtccccaccATCCTTGTCATCTTGGTC	32	53	58		^{674}SKRDLPASSNETIAQV^{689} -> ^{674}EDQDDKDGGDFSGWGG^{689}

Table S4. The retention times and m/z values and the integrated peak areas of the target peptides, CS-glycopeptides, and truncated CS-glycopeptides that were detected from SULF1, SULF2, and their variants.

The detected ions are highlighted in yellow.

Analyte		CS-modification level	Protease used	Peptide sequence			Without CS					with 1CS						with 1CS + 1S/P					with 1CS + 1S + 1P			
							Elution time region	Charge state				Elution time region	Charge state					Elution time region	Charge state				Elution time region	Charge state		
				Start	End	Sequence		2+	3+	4+	5+		2+	3+	4+	5+	6+		2+	3+	4+	5+		3+	4+	5+
SULF1	WT	-	Trypsin	586	595	(R)DLQASSGGNR(G)	18.4-20.2min	***502.7414***	335.4967	251.8744	201.7009	n.d.	999.3819	666.5904	500.1946	400.3571	333.7988	n.d.	1039.3603	693.2426	520.1838	416.3485	n.d.	719.8949	540.1730	432.3399
	SG	-	Trypsin	586	613	(R)DLQAGGDFSGTGGLADSSNAVGPPTTVR(V)	98.5-104.5min	***1324.1333***	***883.0913***	662.5703	530.2577	n.d.	1820.7738	1214.1850	910.8905	728.9139	607.5961	n.d.	1860.7522	1240.8372	930.8797	744.9053	n.d.	1267.4895	950.8689	760.8966
	DDKD	-	Trypsin	577	595	(R)HDEGHKEDQDDKDSSGGNR(G)	14.5-16.5min	1063.4419	***709.2971***	532.2246	425.9811	n.d.	1560.0824	1040.3907	780.5448	624.6373	520.6990	n.d.	1600.0608	1067.0430	800.5340	640.6287	n.d.	1093.6952	820.5232	656.6201
	DDKD-SG	+	Trypsin	577	613	(R)HDEGHKEDQDDKDGGDFSGTGGLADSSNAVGPPTTVR(V)	48-53min	1884.8338	***1256.8917***	***942.9206***	***754.5379***	53-59min	2381.4743	1587.9853	***1191.2408***	***953.1941***	***794.4963***	61-69min	2421.4527	1614.6376	***1211.2300***	***969.1855***	71-78min	***1641.2898***	***1231.2192***	***985.1768***
	DDKD-SG-T596W	+++	Trypsin	577	613	(R)HDEGHKEDQDDKDGGDFSGWGGLADSSNAVGPPTTVR(V)	75.5-79.5min	1927.3497	1285.2356	***964.1785***	***771.5442***	81-88.5min	2423.9902	1616.3292	***1212.4987***	***970.2004***	***808.6682***	90.5-99.0min	2463.9686	1642.9815	***1232.4879***	***986.1918***	100-106min	1669.6337	***1252.4771***	***1002.1832***
SULF2	WT	+	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFSGTGGLPDYSAANPIK(V)	97-103min	1707.7609	***1138.8430***	***854.3841***	683.7087	100-107min	2204.4014	***1469.9367***	***1102.7043***	882.3649	735.4720	111.5min	2244.3798	***1496.5889***	***1122.6935***	898.3563	n.d.	1523.2412	1142.6827	914.3476
	AA	-	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFAATGGLPDYSAANPIK(V)	100-105min	***1706.7713***	***1138.1833***	***853.8893***	683.3129	n.d.	2203.4118	1469.2769	1102.2095	881.9691	735.1421	n.d.	2243.3902	1495.9292	1122.1987	897.9604	n.d.	1522.5815	1142.1879	913.9518
	SG3	++	Trypsin	566	604	(R)HWPGAPEDQDDKDGGDFSGSGSGSGTGGLPDYSAANPIK(V)	93-98min	1923.8411	***1282.8965***	***962.4242***	770.1408	96-103min	2420.4816	1613.9902	***1210.7444***	968.7970	807.4987	110.5min	2460.4600	1640.6424	***1230.7336***	984.7884	n.d.	1667.2947	1250.7228	1000.7797
	DLQA	-	Trypsin	575	598	(R)DLQAGGDFSGTGGLPDYSAANPIK(V)	98.5-105min	***1176.0611***	***784.3765***	588.5342	471.0288	103.5-106min	***1672.7016***	***1115.4702***	836.8544	669.6850	558.2387	116.5min	***1712.6800***	***1142.1224***	856.8436	685.6764	n.d.	1168.7747	876.8328	701.6677
	SSGG	-	Lys-C	566	586	(R)HWPGAPEDQDDKDSSGGNRGR(M)	21.8-24.6min	1141.0025	***761.0041***	***571.0049***	457.0054	n.d.	1637.6430	1092.0977	819.3251	655.6615	546.5525	n.d.	1677.6214	1118.7500	839.3143	671.6529	n.d.	1145.4023	859.3035	687.6443
	T585W	+++	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFSGWGGLPDYSAANPIK(V)	110-115min	1750.2767	***1167.1869***	***875.6420***	700.7151	112.5-119.5min	2246.9172	***1498.2806***	***1123.9622***	899.3712	749.6439	119.5-124.5	2286.8956	***1524.9328***	***1143.9514***	915.3626	n.d.	1551.5851	1163.9406	931.3540

Analyte		CS-modification level	Protease used	Peptide sequence			with 1 Xyl					with 2 Xyl		with NeuAc-Gal-Xyl		with GalNAc-HexA-Gal-		with 1 CS, 1 Xyl	
							Elution time region	Charge state				Elution time region	Charge state	Elution time region	Charge state	Elution time region	Charge state	Elution time region	Charge state
				Start	End	Sequence		2+	3+	4+	5+		4+		4+		3+		4+
SULF1	DDKD-SG	+	Trypsin	577	613	(R)HDEGHKEDQDDKDGGDFSGTGGLADSSNAVGPPTTVR(V)	48-50min	1950.8550	1300.9058	***975.9311***	***780.9464***	n.d.	1008.9417	n.d.	1089.2182	n.d.	1535.3114	n.d.	1224.2514
	DDKD-SG-T596W	+++	Trypsin	577	613	(R)HDEGHKEDQDDKDGGDFSGWGGLADSSNAVGPPTTVR(V)	75.0-76.5min	1993.3708	1329.2497	***997.1891***	***797.9527***	n.d.	1030.1996	n.d.	1110.4761	n.d.	1563.6553	n.d.	1245.5093
SULF2	WT	+	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFSGTGGLPDYSAANPIK(V)	97-103min	1773.7820	***1182.8571***	***887.3947***	710.1172	n.d.	920.4052	n.d.	1000.6817	n.d.	1417.2628	n.d.	1135.7149
	SG3	++	Trypsin	566	604	(R)HWPGAPEDQDDKDGGDFSGSGSGSGTGGLPDYSAANPIK(V)	91-98.45min	1989.8623	***1326.9106***	***995.4348***	796.5493	90-95min	***1028.4454***	98.5-103min	***1108.7218***	n.d.	1561.3163	97.5-98.5min	***1243.7550***
	DLQA	-	Trypsin	575	598	(R)DLQAGGDFSGTGGLPDYSAANPIK(V)	97-98.5min	***1242.0823***	***828.3906***	621.5448	497.4373	n.d.	654.5554	n.d.	734.8318	n.d.	1062.7963	n.d.	869.8650
	T585W	+++	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFSGWGGLPDYSAANPIK(V)	109.5-113min	1816.2979	***1211.2010***	***908.6526***	727.1235	n.d.	941.6632	n.d.	1021.9396	n.d.	1445.6067	n.d.	1156.9728

Analyte		CS-modification level	Protease used	Peptide sequence			Peak intensity area								
				Start	End	Sequence	Without CS	with 1 Xyl	with 1 CS	with 1 CS + 1 S/P	with 1 CS + 1P + 1S	with 2 Xyl	with NeuAc-Gal-Xyl	with GalNAc-HexA-Gal-Gal-Xyl	with 1 CS, 1 Xyl
SULF1	WT	-	Trypsin	586	595	(R)DLQASSGGNR(G)	5.02E+06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	SG	-	Trypsin	586	613	(R)DLQAGGDFSGTGGLADSSNAVGPPTTVR(V)	1.25E+09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	DDKD	-	Trypsin	577	595	(R)HDEGHKEDQDDKDSSGGNR(G)	2.69E+05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	DDKD-SG	+	Trypsin	577	613	(R)HDEGHKEDQDDKDGGDFSGTGGLADSSNAVGPPTTVR(V)	6.76E+08	7.33E+06	6.23E+07	1.46E+08	8.47E+07	n.d.	n.d.	n.d.	n.d.
	DDKD-SG-T596W	+++	Trypsin	577	613	(R)HDEGHKEDQDDKDGGDFSGWGGLADSSNAVGPPTTVR(V)	6.52E+07	1.17E+07	1.57E+08	2.51E+08	8.18E+07	n.d.	n.d.	n.d.	n.d.
SULF2	WT	+	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFSGTGGLPDYSAANPIK(V)	7.91E+08	3.42E+07	1.43E+08	1.48E+08	n.d.	n.d.	n.d.	n.d.	n.d.
	AA	-	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFAATGGLPDYSAANPIK(V)	1.75E+09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	SG3	++	Trypsin	566	604	(R)HWPGAPEDQDDKDGGDFSGSGSGSGTGGLPDYSAANPIK(V)	9.17E+08	3.01E+08	4.93E+07	3.69E+07	n.d.	8.54E+06	7.18E+06	n.d.	3.51E+06
	DLQA	-	Trypsin	575	598	(R)DLQAGGDFSGTGGLPDYSAANPIK(V)	9.32E+08	1.06E+07	1.24E+07	5.46E+06	n.d.	n.d.	n.d.	n.d.	n.d.
	SSGG	-	LysC	566	586	(R)HWPGAPEDQDDKDSSGGNRGR(M)	9.21E+06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	T585W	+++	Trypsin	566	598	(R)HWPGAPEDQDDKDGGDFSGWGGLPDYSAANPIK(V)	2.83E+07	3.90E+07	2.01E+08	2.45E+08	n.d.	n.d.	n.d.	n.d.	n.d.

Table S5. Peak intensities of key fragment ions in the averaged MS/MS spectra of co-eluted CS-glycopeptides carrying a single sulfate or phosphate.

Analyte		Precursor ions		Fragment ions in averaged MS2 data					
				key fragment ion from GalNAc-S			Key fragment ion from Xyl-P		
		m/z	z	m/z	z	Peak intensity	m/z	z	Peak intensity
SULF1	DDKD-SG	1211.99	4	1301.5789	3	6.55E+04	1328.2325	3	2.76E+04
	DDKD-SG-T596W	1233.00	4	1329.9190	3	1.34E+05	1356.5757	3	5.47E+04
SULF2	WT	1123.20	4	1183.5265	3	4.24E+04	1209.8454	3	7.30E+04
	SG3	1231.25	4	1327.2435	3	1.30E+04	1354.2341	3	1.35E+05
	DLQA	1142.46	3	1242.5739	2	1.05E+05	1282.5608	2	1.14E+05
	T585W	1144.20	4	1211.5470	3	1.40E+04	1238.1910	3	3.95E+04

Table S6. Relative abundance of co-eluting CS glycopeptides with a single sulfate or phosphate modification.

		Relative % (MS2 based)	
	Analyte	With 1CS + 1S (on GalNAc)	With 1CS + 1P (on Xyl)
SULF1	DDKD-SG	70.38%	29.62%
	DDKD-SG-T596W	70.97%	29.03%
SULF2	WT	36.75%	63.25%
	SG3	8.81%	91.19%
	DLQA	47.93%	52.07%
	T585W	26.16%	73.84%

Data S1. The purity and molecular weight of the nine synthesized peptides identified using reversed-phase liquid chromatography and electrospray ionization mass spectrometry.

SULF2 peptide: EDQDDKDGG DFSGTGG LPD

Sample ID: J148DHC230–3
Sample Name: SULF2 pep – EDQDDKDGG DFSGTGG LPD

Pump A : 0.065% trifluoroacetic in 100% water(v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile(v/v)
Total Flow:1 ml/min
Wavelength:220 nm

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

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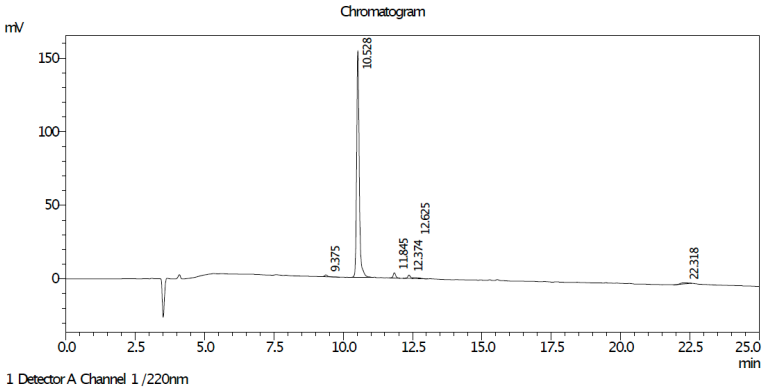
Column : Inertsil ODS–3 4.6 x 250 mm

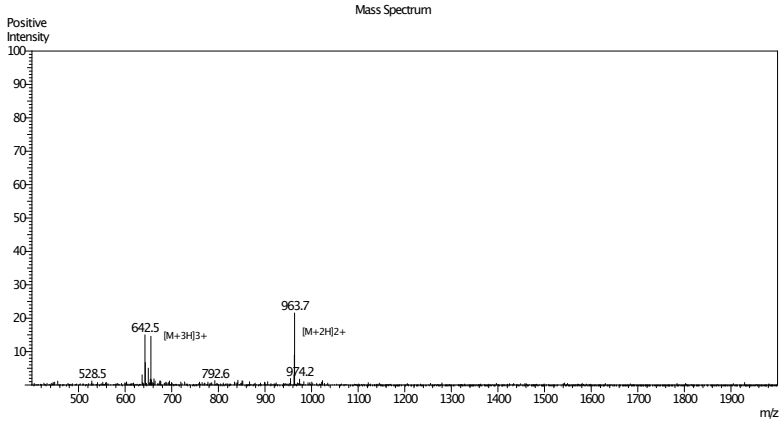


1 Detector A Channel 1 /220nm

Peak Table

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	9.375	9684	1218	0.894
2	10.528	1014587	146283	93.636
3	11.845	22135	3522	2.043
4	12.374	15591	2192	1.439
5	12.625	9734	565	0.898
6	22.318	11808	632	1.090
Total		1083538	154411	100.000



Sample Information

Injection Volume :	0.2
Sample Name :	SULF2 pep – EDQDDKDGG DFSGTGG LPD
Sample ID :	J148DHC230–3
Theoretical MW :	1924.85
Observed MW :	1925.4

Interface	:ESI
Nebulizing Gas Flow	:1.5L/min
CDL Temp	:250
Block Temp	:200

Equipment	: ZJ21010035
Interface Bias	: +4.5kV
Drying Gas Flow	:5 L/min
T.Flow	:0.2 ml/min
B.conc	:50%H2O/50%MeOH

SULF2 AA peptide: EDQDDKDGG DFAATGG LPD

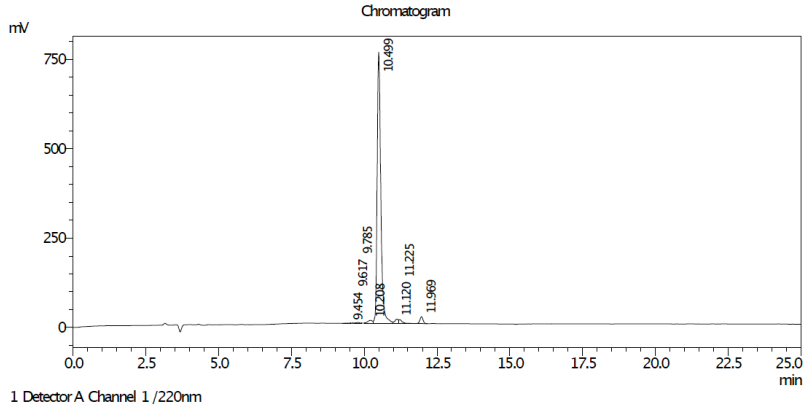
Sample ID: J148DHC230–17
Sample Name: SULF2 AA pep – EDQDDKDGG DFAATGG LPD

Pump A : 0.065% trifluoroacetic in 100% water(v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile(v/v)
Total Flow:1 ml/min
Wavelength:220 nm

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

<<Column Performance>>
<DetectorA>
Column : Inertsil ODS–3 4.6 x 250 mm

Chromatogram



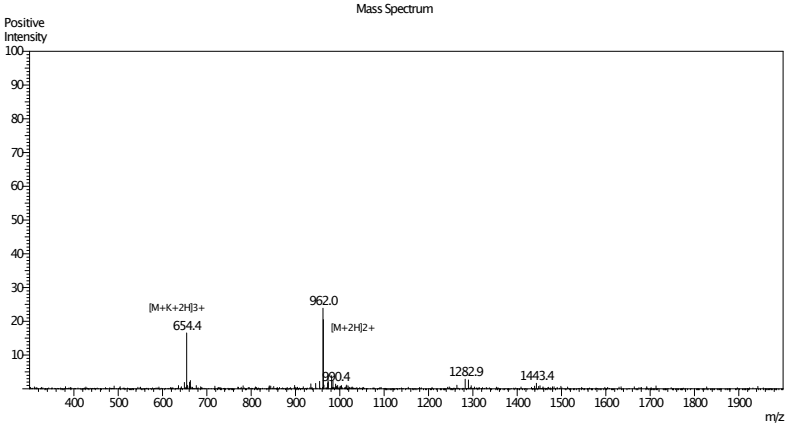
1 Detector A Channel 1 /220nm

Peak Table

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	9.454	12012	1060	0.170
2	9.617	8891	1161	0.126
3	9.785	20193	2378	0.286
4	10.208	94700	8168	1.341
5	10.499	6603085	758402	93.507
6	11.120	98482	11973	1.395
7	11.225	82397	10384	1.167
8	11.969	141850	19061	2.009
Total		7061610	812587	100.000

Mass Spectrum



Sample Information

Injection Volume :	0.2
Sample Name:	SULF2 AA pep – DQDDKDGG DFAATGG LPD
Sample ID :	J148DHC230–17
Theoretical MW :	1922.88
Observed MW :	1922.0

Interface	:ESI
Nebulizing Gas Flow	:1.5L/mir
CDL Temp	:250
Block Temp	:200

Equipment	: ZJ21010035
Interface Bias	: +4.5kV
Drying Gas Flow	:5 L/min
T.Flow	:0.2 ml/min
B.conc	:50%H2O/50%MeOH

SULF2 $(SG)_3$ peptide: EDQDDKDGG DFSGSGSGSGTGG LPD

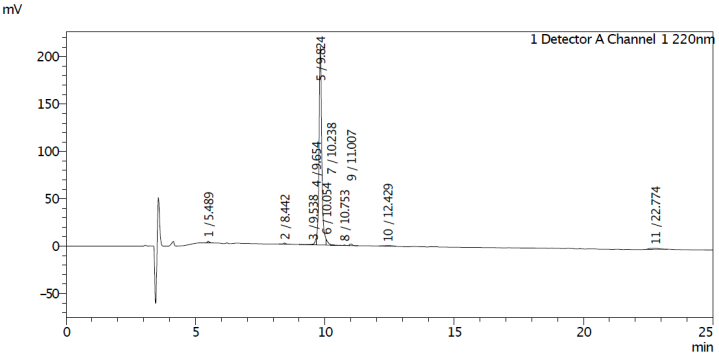
Sample ID: J148DHC230-19
Sample Name: SULF2 (SG)3 pep - EDQDDKDGG DFSGSGSGSGTGG LPD

PumpA : 0.065% trifluoroacetic in 100% water(v/v)
PumpB : 0.05% trifluoroacetic in 100% acetonitrile(v/v)
Total Flow:1 ml/min
Wavelength:220 nm
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

<<ColumnPerformance>>
<DetectorA>
Column :Inertsil ODS-3 4.6 x 250 mm
Equipment: ZJ19010140

<Chromatogram>

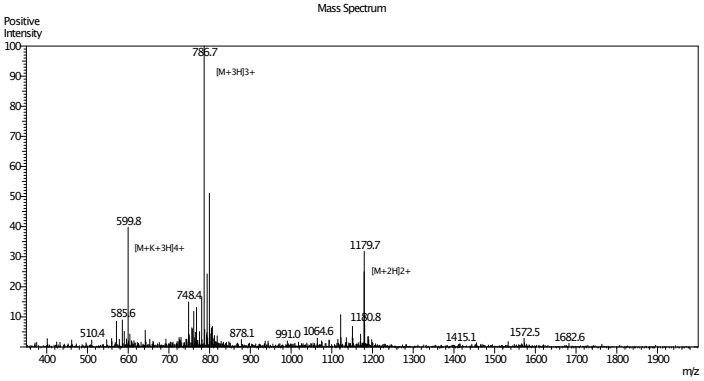


<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	5.489	9590	1661	0.574
2	8.442	5741	1031	0.344
3	9.538	7447	718	0.446
4	9.654	21273	6117	1.274
5	9.824	1538156	210031	92.101
6	10.054	30715	6177	1.839
7	10.238	3635	606	0.218
8	10.753	3285	419	0.197
9	11.007	11444	1756	0.685
10	12.429	12615	573	0.755
11	22.774	26172	938	1.567
Total		1670074	230026	100.000

Mass Spectrum



Sample Information

Injection Volume :	0.1
Sample Name :	SULF2 (SG)3 pep - EDQDDKDGG DFSGSGSGSGTGG LPD
Sample ID :	J148DHC230-19
Theoretical MW :	2357.24
Observed MW :	2357.1

Interface	:ESI
Nebulizing Gas Flow	:1.5L/min
CDL Temp	:250
Block Temp	:200

Equipment	: ZJ21010035
Interface Bias	: +4.5kV
Drying Gas Flow	:5 L/min
T.Flow	:0.2 ml/min
B.conc	:50%H2O/50%MeOH

SULF2 DLQA peptide: GPRDLQAGG DFSGTGG LPD

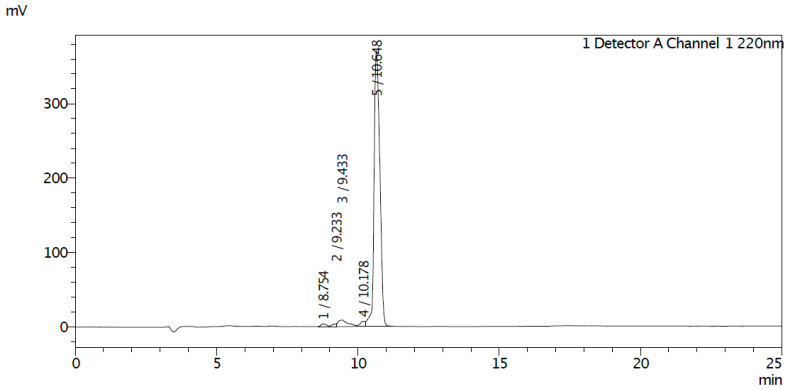
Sample ID: J 148DHC230-5
Sample Name: SULF2 DLQA pep - GPRDLQAGG DFSGTGG LPD

PumpA : 0.065% trifluoroacetic in 100% water(v/v)
PumpB : 0.05% trifluoroacetic in 100% acetonitrile(v/v)
Total Flow:1 ml/min
Wavelength:220 nm
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

<<Column Performance>>
<Detector A>
Column :Inertsil ODS-3 4.6 x 250 mm
Equipment: GR09010180

<Chromatogram>

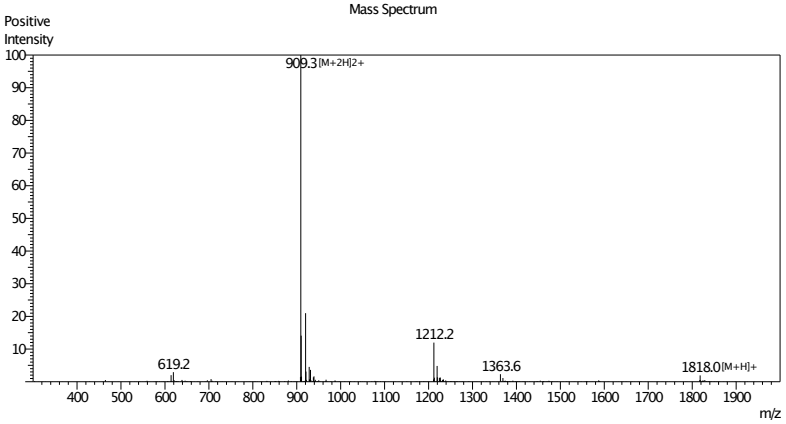


<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	8.754	48235	3540	0.851
2	9.233	36653	3623	0.647
3	9.433	213635	8672	3.769
4	10.178	84415	7076	1.489
5	10.648	5284960	370248	93.244
Total		5667898	393158	100.000

Mass Spectrum



Sample Information

Injection Volume : 0.2
Sample Name : SULF2 DLQA pep - GPRDLQAGG DFSGTGG LPD
Sample ID : J148DHC230-5
Theoretical MW : 1816.89
Observed MW : 1816.6

Interface :ESI
Nebulizing Gas Flow :1.5L/mir
CDL Temp :250
Block Temp :200

Equipment : GK11010007
Interface Bias : +4.5kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/mir
B.conc :50%H2O/50%M

SULF2 SSGG peptide: EDQDDKD SSGGNRGRM LPD

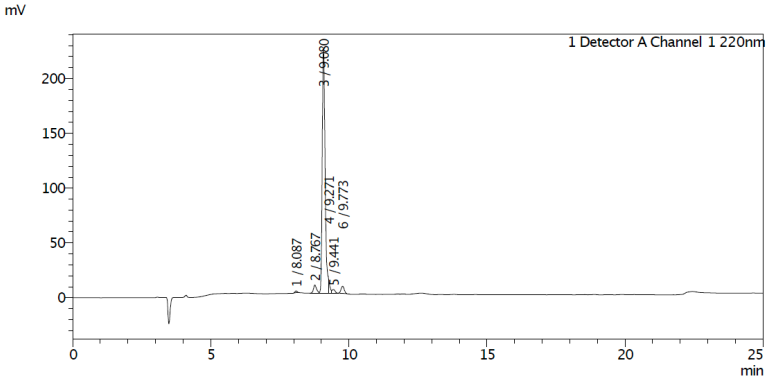
Sample ID: J 148DHC230-7
Sample Name: SULF2 SSGG pep - EDQDDKD SSGGNRGRM LPD

PumpA : 0.065% trifluoroacetic in 100% water(v/v)
PumpB : 0.05% trifluoroacetic in 100% acetonitrile(v/v)
Total Flow:1 ml/min
Wavelength:220 nm
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

<<Column Performance>>
<Detector A>
Column :Inertsil ODS-SP 4.6 x 250 mm
Equipment: SS-CM-0286

<Chromatogram>

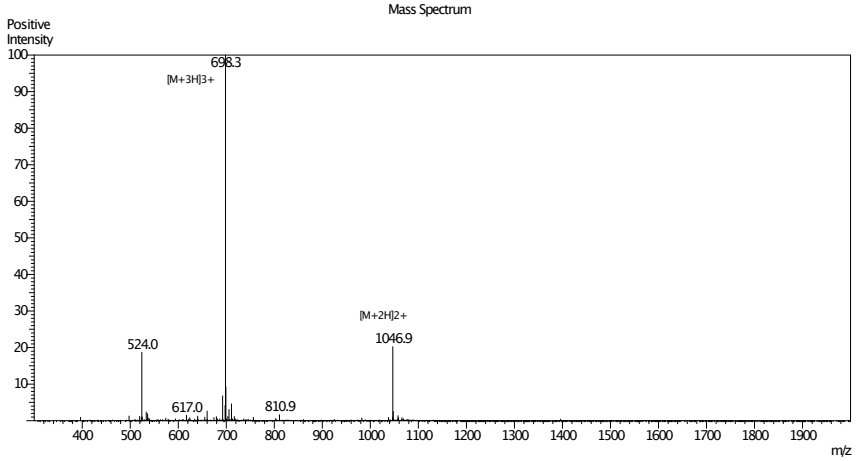


<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	8.087	7843	1531	0.437
2	8.767	58896	7477	3.278
3	9.080	1617544	222499	90.037
4	9.271	36608	12763	2.038
5	9.441	24697	3407	1.375
6	9.773	50942	6494	2.836
Total		1796529	254171	100.000

Mass Spectrum



Sample Information

Injection Volume: 0.2
Sample Name: SULF2 SSGG pep - EDQDDKD SSGGNRGRM LPD
Sample ID : J148DHC230-7
Theoretical MW : 2092.13
Observed MW : 2091.9

Interface :ESI
Nebulizing Gas Flow :1.5L/mir
CDL Temp :250
Block Temp :200

Equipment : ZJ21010035
Interface Bias : +4.5kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/min
B.conc :50%H2O/50%MeOH

SULF2 W (T585W) peptide: EDQDDKDGG DFSGWGG LPD

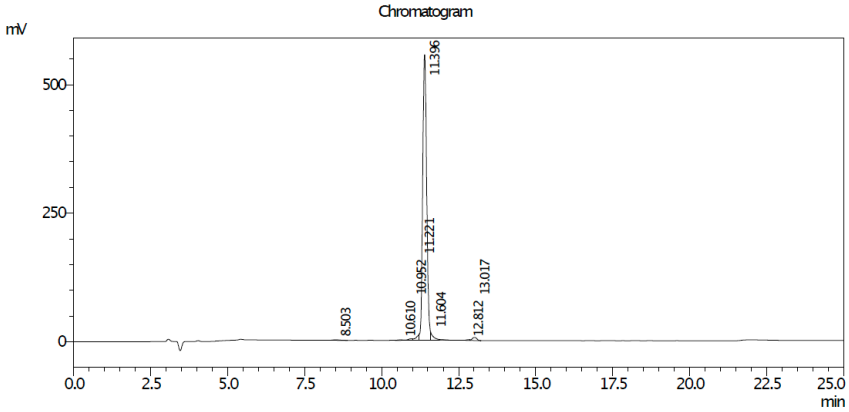
Sample ID: J148DHC230–13
Sample Name: SULF2 W (T585W) pep – EDQDDKDGG DFSGWGG LPD

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow: 1 ml/min
Wavelength: 220 nm

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

<<Column Performance>>
<Detector A>
Column : Inertsil ODS–3 4.6 x 250 mm

Chromatogram



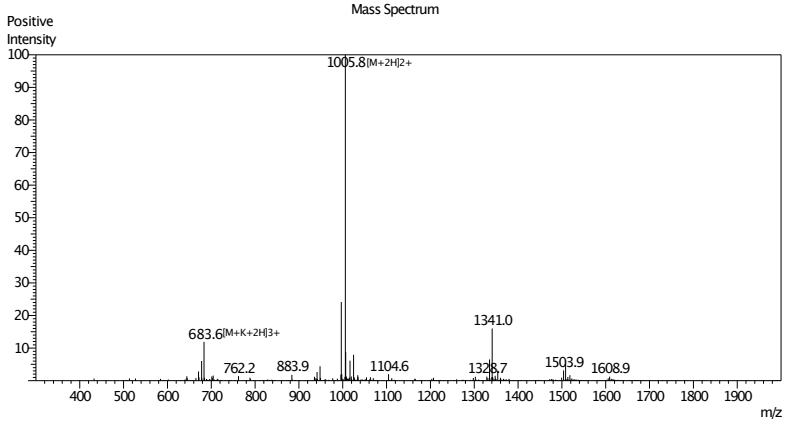
1 Detector A Channel 1 /220nm

Peak Table

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	8.503	14600	969	0.275
2	10.610	12115	1075	0.228
3	10.952	29912	2803	0.563
4	11.221	76943	11050	1.448
5	11.396	5007235	555060	94.249
6	11.604	99649	13975	1.876
7	12.812	11063	1390	0.208
8	13.017	61284	6063	1.154
Total		5312801	592386	100.000

Mass Spectrum



Sample Information

Injection Volume:	0.4	Interface	:ESI	Equipment	: GK11010007
Sample Name:	SULF2 W (T585W) pep – EDQDDKDGG DFSGWGG LPD	Nebulizing Gas Flow	:1.5L/min	Interface Bias	: +4.5kV
Sample ID :	J148DHC230–13	CDL Temp	:250	Drying Gas Flow	:5 L/min
Theoretical MW :	2009.96	Block Temp	:200	T.Flow	:0.2 ml/min
Observed MW :	2009.6			B.conc	:50%H2O/50%M

SULF2 K577A peptide: EDQDDADGG DFSGTGG LPD

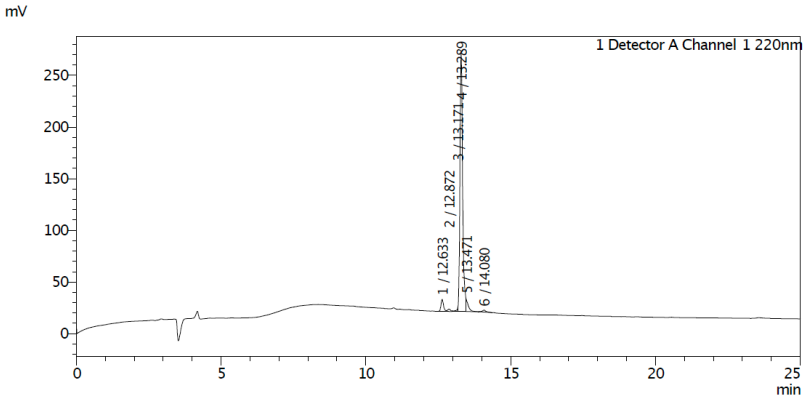
Sample ID: J 148DHC230-9
Sample Name: SULF2 K577A pep – EDQDDADGG DFSGTGG LPD

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

<<Column Performance>>
<Detector A>
Column :Inertsil ODS-3 4.6 x 250 mm
Equipment: ZJ17010508

<Chromatogram>

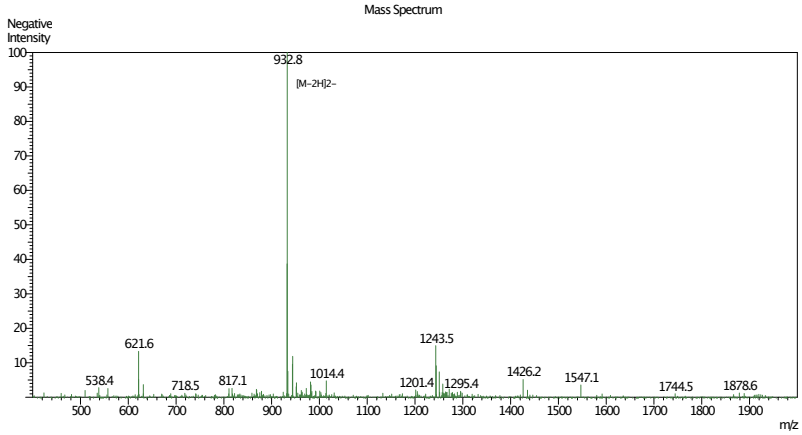


<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	12.633	70873	11577	3.959
2	12.872	15498	2252	0.866
3	13.171	12613	2657	0.704
4	13.289	1614732	250616	90.190
5	13.471	61001	11513	3.407
6	14.080	15644	1810	0.874
Total		1790361	280425	100.000

Mass Spectrum



Sample Information
Injection Volume : 0.2
Sample Name : SULF2 K577A pep – EDQDDADGG DFSGTGG LPD
Sample ID : J148DHC230-9
Theoretical MW : 1867.75
Observed MW : 1867.6

Interface :ESI
Nebulizing Gas Flow :1.5L/min
CDL Temp :250
Block Temp :200

Equipment : ZJ21010035
Interface Bias : -3.5kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/min
B.conc :50%H2O/50%MeOH

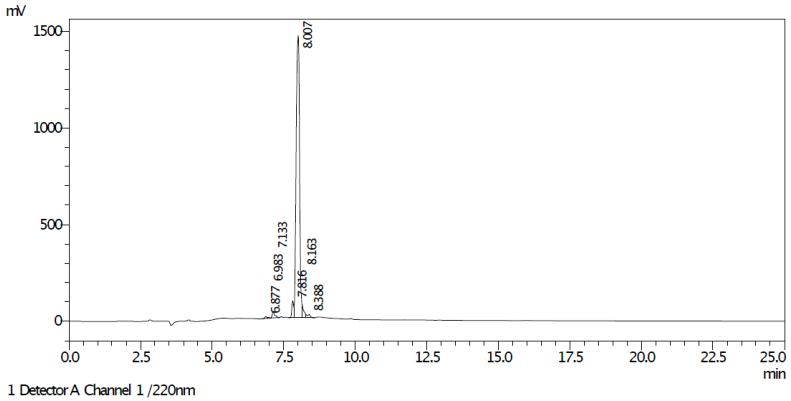
SULF2 F582A peptide: EDQDDKDGG DASGTGG LPD

Sample ID: J148DHC230-11
Sample Name: SULF2 F582A pep - EDQDDKDGG DASGTGG LPD

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow: 1 ml/min
Wavelength: 220 nm

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

<<Column Performance>>
<Detector A>
Column : Inertsil ODS-3 4.6 x 250 mm
Equipment: GK11010017



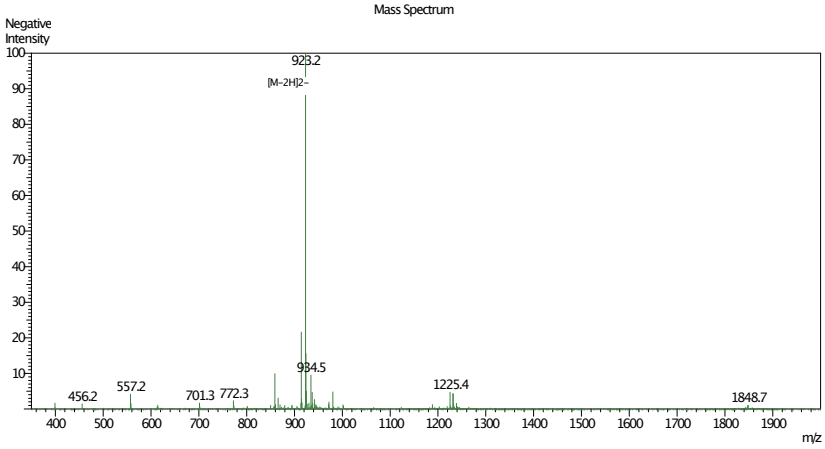
1 Detector A Channel 1 /220nm

Peak Table

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	6.877	48269	10357	0.369
2	6.983	18765	4793	0.144
3	7.133	190303	30981	1.456
4	7.816	378184	87121	2.894
5	8.007	12042124	1457553	92.158
6	8.163	250797	57015	1.919
7	8.388	138317	16514	1.059
Total		13066760	1664334	100.000

Mass Spectrum



Sample Information

Injection Volume : 0.1
Sample Name : SULF2 F582A pep - EDQDDKDGG DASGTGG LPD
Sample ID : J148DHC230-11
Theoretical MW : 1848.75
Observed MW : 1848.4

Interface :ESI
Nebulizing Gas Flow :1.5L/min
CDL Temp :250
Block Temp :200

Equipment : ZJ21010035
Interface Bias : -3.5kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/min
B.conc :50%H2O/50%MeOH

SULF1 peptide: GPRDLQA SSGGNRGRM LAD

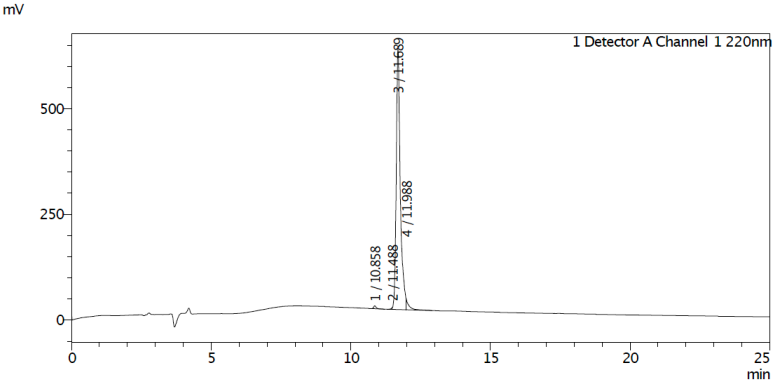
Sample ID: J 148DHC230-1
Sample Name: SULF1 pep – GPRDLQA SSGGNRGRM LAD

PumpA : 0.065% trifluoroacetic in 100% water(v/v)
PumpB : 0.05% trifluoroacetic in 100% acetonitrile(v/v)
Total Flow:1 ml/min
Wavelength:220 nm
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
25.00	Pumps	B.Conc	65
25.01	Pumps	B.Conc	95
27.00	Pumps	B.Conc	95
27.01	Pumps	B.Conc	5
35.00	Pumps	B.Conc	5
35.01	Controller	Stop	

<<Column Performance>>
<Detector A>
Column :Inertsil ODS-3 4.6 x 250 mm
Equipment: SS-CM-0310

<Chromatogram>

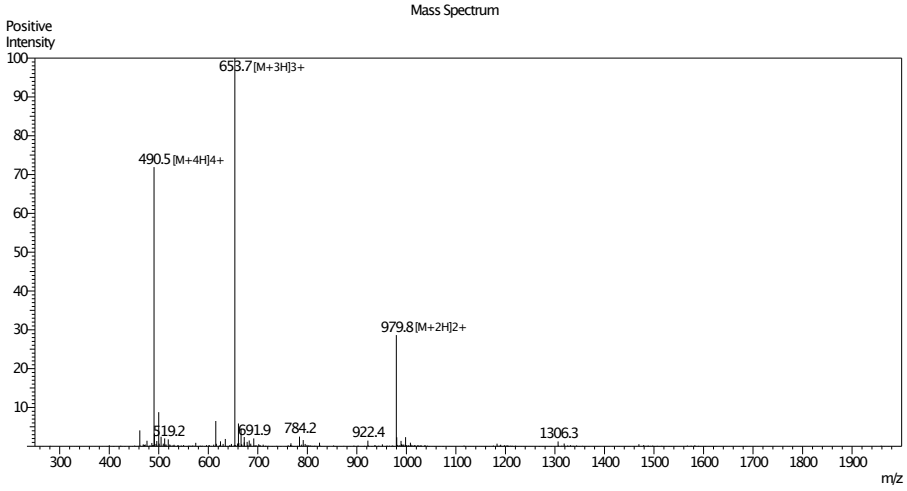


<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	10.858	45626	6872	0.702
2	11.488	13925	3688	0.214
3	11.689	6260876	616216	96.312
4	11.988	180180	25348	2.772
Total		6500608	652123	100.000

Mass Spectrum



Sample Information

Injection Volume: 0.4
Sample Name: SULF1 pep – GPRDLQA SSGGNRGRM LAD
Sample ID : J148DHC230-1
Theoretical MW : 1958.13
Observed MW : 1958.1

Interface :ESI
Nebulizing Gas Flow :1.5L/mir
CDL Temp :250
Block Temp :200

Equipment : GK11010007
Interface Bias : +4.5kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/min
B.conc :50%H2O/50%MeOH