Supporting Information

Identification of novel binding sites for heparin in RPTPs: implications for proteoglycan signaling

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Schematic structure of RPTPσ recombinant proteins used in this study

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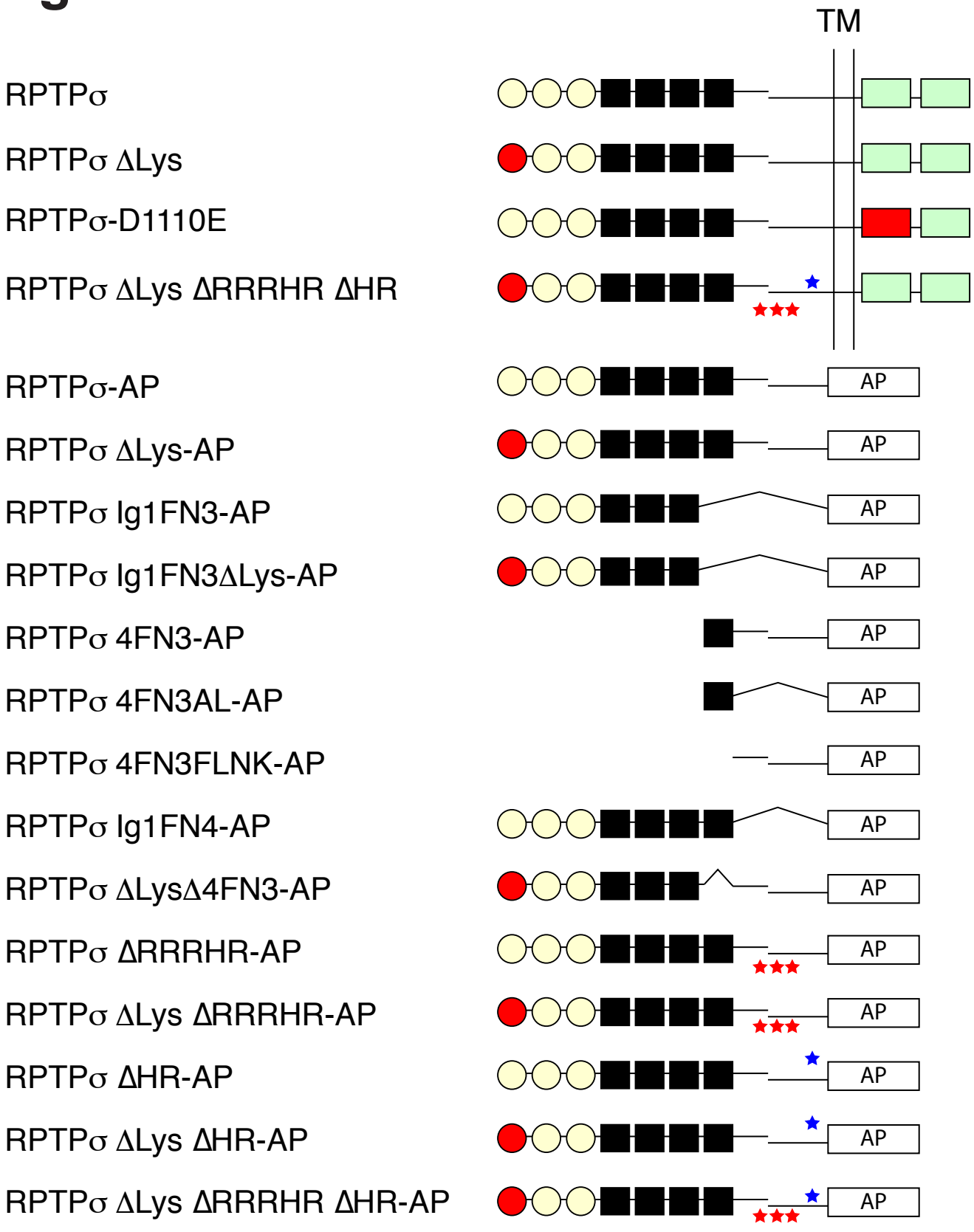
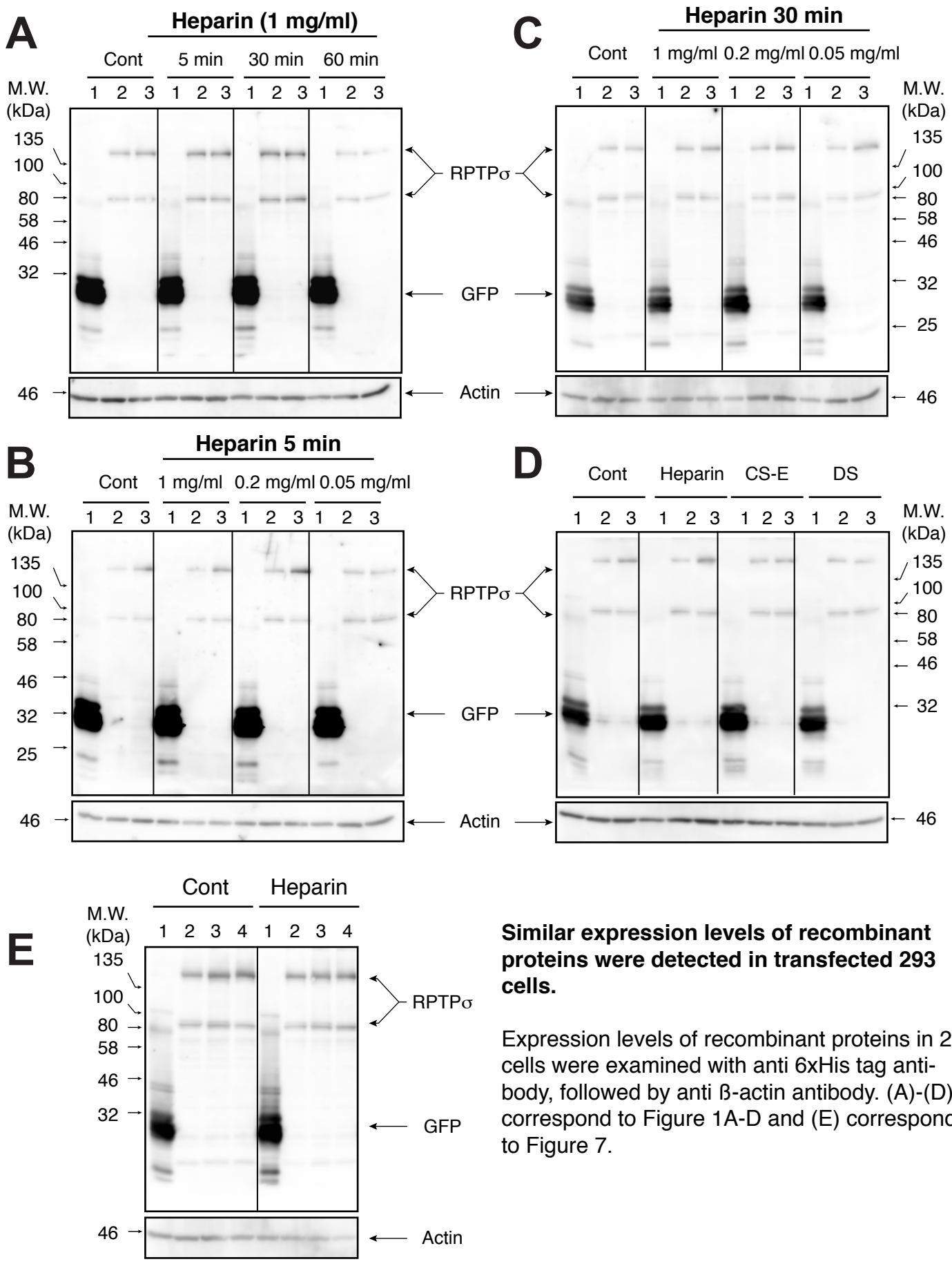
Figure S1



Schematic structure of RPTPσ recombinant proteins used in this study

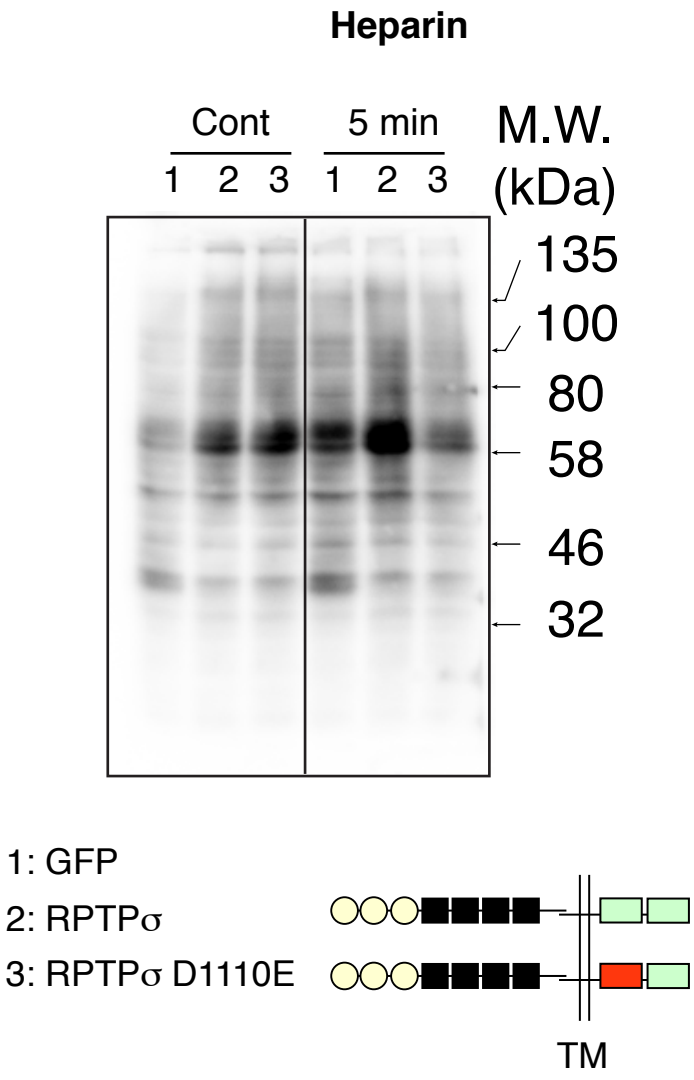
Figure S2



Similar expression levels of recombinant proteins were detected in transfected 293 cells.

Expression levels of recombinant proteins in 293 cells were examined with anti 6xHis tag antibody, followed by anti ß-actin antibody. (A)-(D) correspond to Figure 1A-D and (E) corresponds to Figure 7.

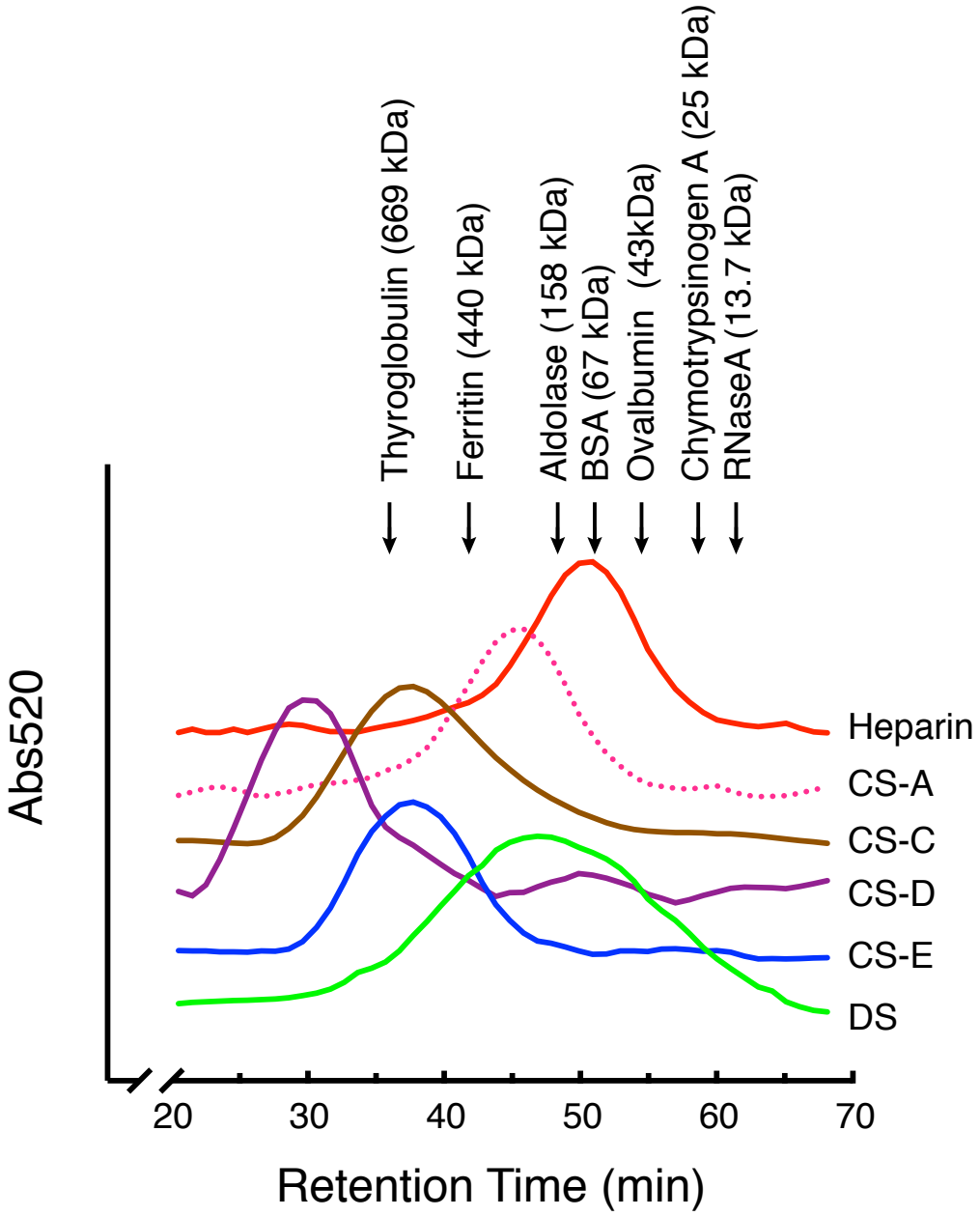
Figure S3



Intact phosphatase domain of RPTPσ is essential for the upregulated intracellular phosphorylation upon heparin exposure.

293 cells expressing (1) GFP, (2) RPTPσ, and (3) RPTPσ D1110E (catalytically inactive form) were treated with heparin (1 mg/ml) for 5 min and Tyr phosphorylation was examined by immunoblot.

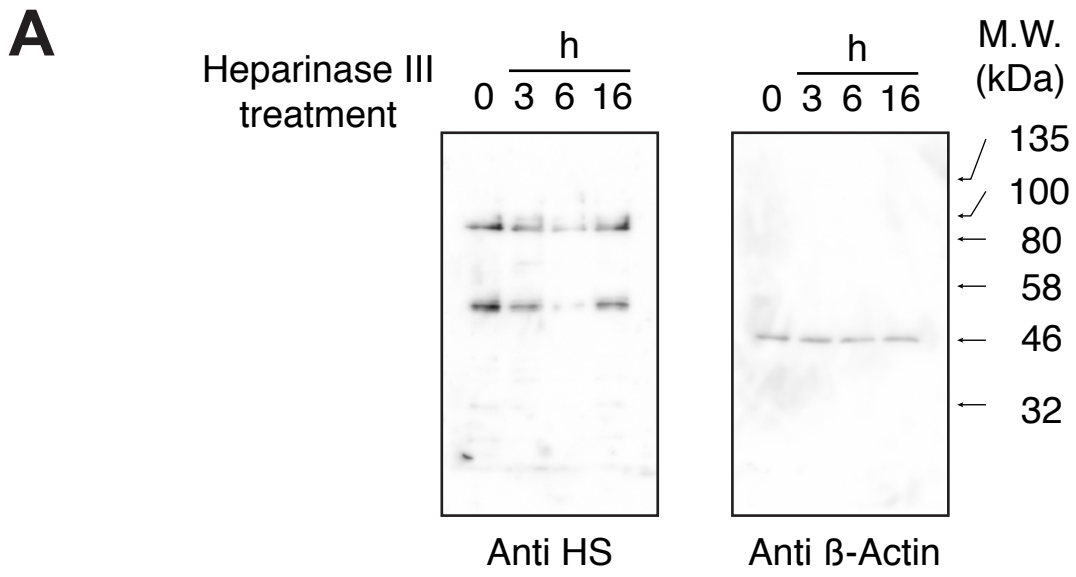
Figure S4

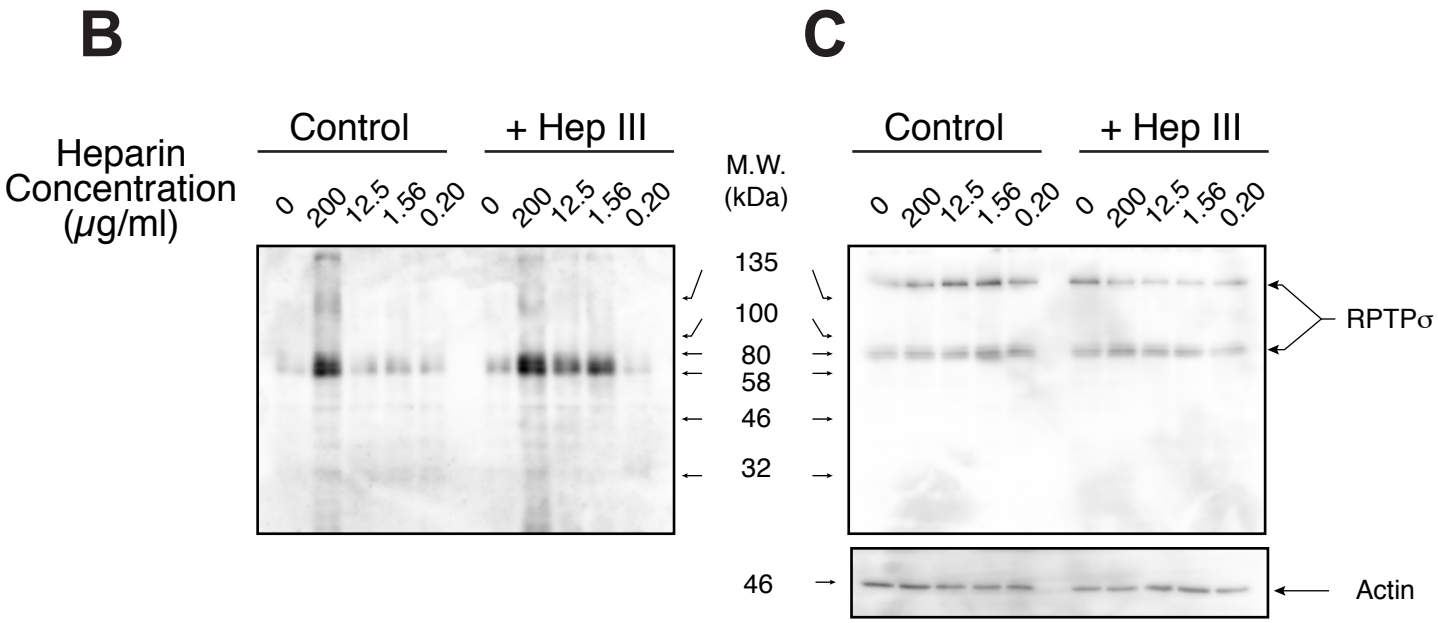


Size exclusion chromatography of GAGs used in this study

Each GAG was separated in 150 mM ammonium bicarbonate by size-exclusion chromatography (Superdex 200, 10 x 300 mm, GE Healthcare Life Sciences) attached to a Shimadzu UFLC system at a flow rate of 0.3 ml/min. Amount of uronic acid in each fraction was measured by the carbazole method.

Figure S5





Heparinase treatment of transfected cells increases the sensitivity to exogenous heparin

Transfected 293 cells with RPTPσ (full length) were treated with heparinase III (Sigma, H8891). (A) At indicated time, cell lysates were collected with 1% TritonX100 in PBS containing Protease Inhibitor Cocktail (Sigma #539137), followed by immunoblot with anti HS antibody (F58-10E4, AMSBIO, # 370255). After stripping, PVDF membrane was reprobed with anti ß-actin antibody. (B) After 8 h treatment with heparinase III, cells were treated with heparin for 5 min and harvested with 2 x SDS sample buffer. Tyrosine phosphorylation was detected by immunoblot with anti-phosphotyrosine antibody. (C) Similar expression level of RPTPσ was confirmed with anti-6xHis tag antibody, followed by reprobing with anti ß-actin antibody.

Table S1

Disaccharide composition analysis of chondroitin and dermatan sulfate

Mol %	ΔDi-0S[a]	ΔDi-4S	ΔDi-6S	ΔDi-2,6S	ΔDi-4,6S	ΔDi-2,4,6S
CS-A	6.3	54.2	39.5	0	0	0
CS-C	13.9	26.9	58.3	1.0	0	0
CS-D	2.2	12.8	42.3	42.7	0	0
CS-E	3.4	26.6	25.5	0	44.6	0
DS	7.7	74.6	16.0	0.9	0.9	0

Disaccharide composition analysis of heparin

Mol %	D0H0[b]	D0A0	D0H6	D2H0	D0S0	D0A6	D2A0	D2H6	D0S6	D2S0	D2A6	D2S6
Heparin	0	12.4	0	0	7.7	7.4	6.8	0	20.7	23.1	2.9	19.0

Unsaturated chondroitin disaccharides generated by digestion with chondroitinase ABC were analyzed by anion-exchange HPLC after labeling with the fluorophore 2AB as described in Experimental Procedures section. Analysis of heparin composition was performed by the UCSD Glycotechnology Core.

[a]ΔDi-0S=ΔHexUA-GalNAc; ΔDi-6S=ΔHexUA−GalNAc(6-*O*-sulfate); ΔDi-4S=ΔHexUA-GalNAc(4-*O*-sulfate); ΔDi-2,6S=ΔHexUA(2-*O*-sulfate)-GalNAc(6-*O*-sulfate); ΔDi-4,6S=ΔHexUA-GalNAc(4,6-*O*-disulfate); ΔDi-2,4,6S=ΔHexUA(2-*O*-sulfate)-GalNAc(4,6-*O*-sulfate).

[b]D0H0, ΔHexUA-GlcNH$_2$; D0A0, ΔHexUA-GlcNAc; D0H6, ΔHexUA-GlcNH$_2$- 6S; D2H0, ΔHexUA2S-GlcNH$_2$; D0S0, ΔHexUA-GlcNS; D0A6, ΔHexUA-GlcNAc6S; D2A0, ΔHexUA2S- GlcNAc; D2H6, ΔHexUA2S-GlcNH$_2$6S; D0S6, ΔHexUA-GlcNS6S; D2S0, ΔHexUA2S-GlcNS; D2A6, ΔHexUA2S-GlcNAc6S; D2S6, ΔHexUA2S- GlcNS6S.

Table S2

Potential heparin binding sites within the fourth FNIII flanking region selected by "heparin protection" assays

Peptides are sorted according to the position in RPTPσ. Modified amino acids are shown as the addition of numbers; [+226.07760] indicates a modification by biotin and [+15.99492] indicates Met oxidation.

Position	Sequence	Observed m/z	Calculated m/z	ppm error
667	F.VLTNR[+226.07760]GSSL.G	586.8114	586.8082	5.39
669	L.TNRGSSLGGLQQTVTAR[+226.07760]TAF.N	764.3949	764.3902	6.10
669	L.TNRGSSLGGLQQTVTAR[+226.07760].T	658.0101	658.0058	6.49
669	L.TNR[+226.07760]GSSLGGLQQTVTAR.T	658.0094	658.0058	5.41
669	L.TNR[+226.07760]GSSLGGLQQTVT.A	872.9407	872.936	5.47
669	L.TNR[+226.07760]GSSLGGLQQTV.T	822.4167	822.4121	5.55
671	N.R[+226.07760]GSSLGGLQQTVT.A	765.3954	765.3907	6.26
676	L.GGLQQTVTAR[+226.07760]TAF.N	788.4061	788.401	6.48
676	L.GGLQQTVTAR[+226.07760].T	628.8281	628.8244	5.89
684	T.AR[+226.07760]TAF.N	396.1991	396.1971	5.13
689	F.NM[+15.99492]LSGK[+226.07760]PSVAPKPDNDGF.I	706.0024	705.9994	4.22
689	F.NM[+15.99492]LSGK[+226.07760]PS.V	538.2523	538.2492	5.86
689	F.NMLSGK[+226.07760]PS.V	530.2546	530.2517	5.41
692	L.SGK[+226.07760]PSVAPKPDNDGFIVVY.L	739.378	739.3734	6.21
692	L.SGKPSVAPK[+226.07760]PDNDGF.I	871.42	871.4143	6.57
697	S.VAPK[+226.07760]PDNDGF.I	643.3	643.2977	3.56
724	Y.FIVM[+15.99492]VPL.R	834.4849	834.4793	6.68
731	L.RKSR[+226.07760]GGQFPVLL.G	528.636	528.6328	6.03
732	R.KSR[+226.07760]GGQFPVLL.G	714.3995	714.395	6.26
732	R.KSR[+226.07760]GGQFPVLL.G	714.3994	714.395	6.09
733	K.SR[+226.07760]GGQFPVLL.G	650.3516	650.3475	6.30
733	K.SR[+226.07760]GGQFPVLL.G	650.3512	650.3475	5.64
754	L.IQDISR[+226.07760]LQ.R	599.8191	599.8161	5.05
754	L.IQDISR[+226.07760]L.Q	535.7903	535.7868	6.55
783	F.SILPAVFHPGNQK[+226.07760]QY.G	962.9961	962.9905	5.76
788	A.VFHPGNQK[+226.07760]QY.G	722.3497	722.3455	5.75
790	F.HPGNQK[+226.07760]QYGGF.D	729.8372	729.8328	6.01
790	F.HPGNQK[+226.07760]QY.G	599.2809	599.2771	6.40
790	F.HPGNQK[+226.07760]Q.Y	517.7482	517.7454	5.26
801	F.DNRGLEPGHR[+226.07760]YVLF.V	633.6529	633.6492	5.82
801	F.DNRGLEPGHR[+226.07760]Y.V	513.9119	513.9089	5.93
815	F.VLAVLQK[+226.07760]NEPTF.A	792.9311	792.9263	6.00
817	L.AVLQK[+226.07760]NEPTF.A	686.8535	686.8501	4.97
820	L.QK[+226.07760]NEPTFAASPF.S	781.8738	781.869	6.17
820	L.QK[+226.07760]NEPTF.A	545.2583	545.2553	5.48

Table S3

DNA plasmids and oligonucleotides used in this study

DNA Plamid Name	Gene of Interest	Protein Region	Vector Backbone	Cloning site(s)	Mutations	Primers used	Accession Number
pTAG5-RPRPσ	RPTPσ	1-1502	pAPTAG5	NheI(5') and XhoI(3')	None	1,2	NM_001252456
pTAG5-RPRPσ ΔLys	RPTPσ	1-1502	pAPTAG5	NheI(5') and XhoI(3')	K68A, K69A, K71A, K72A	1,2,4,5	NM_001252456
pTAG5-RPRPσ D1110E	RPTPσ	1-1502	pAPTAG5	NheI(5') and XhoI(3')	D1110E	1,2,16,17	NM_001252456
pTAG5-RPRPσ ΔLys ΔRRRHR ΔHR	RPTPσ	1-1502	pAPTAG5	NheI(5') and XhoI(3')	K68A, K69A, K71A, K72A	1,2,4,5,10-13,18,19	NM_001252456
pAPTAG5-RPRPσ	RPTPσ	1-847	pAPTAG5	NheI(5') and HindIII (3')	None	1,3	NM_001252456
pAPTAG5-RPRPσ ΔLys	RPTPσ	1-847	pAPTAG5	NheI(5') and HindIII (3')	K68A, K69A, K71A, K72A	1,3-5	NM_001252456
pAPTAG5-RPRPσ Ig1FN3	RPTPσ	1-602	pAPTAG5	NheI(5') and HindIII (3')	None	1,6	NM_001252456
pAPTAG5-RPRPσ Ig1FN3 ΔLys	RPTPσ	1-602	pAPTAG5	NheI(5') and HindIII (3')	K68A, K69A, K71A, K72A	1,4-6	NM_001252456
pAPTAG5-RPRPσ 4FN3	RPTPσ	601-847	pAPTAG5	HindIII (5' and 3')	None	3,8	NM_001252456
pAPTAG5-RPTPσ Ig1FN4	RPTPσ	1-682	pAPTAG5	NheI(5') and HindIII (3')	None	1,7	NM_001252456
pAPTAG5-RPTPσ 4FN3 FLNK	RPTPσ	673-847	pAPTAG5	HindIII (5' and 3')	None	3,9	NM_001252456
pAPTAG5-RPTPσ 4FN3AL	RPTPσ	601-682	pAPTAG5	HindIII (5' and 3')	None	8,9	NM_001252456
pAPTAG5-RPTPσ Δ4FN3	RPTPσ	1-602, 673-847	pAPTAG5	NheI(5') and HindIII (3')	None	1,3,6,9	NM_001252456
pAPTAG5-RPTPσ Δ4FN3 ΔLys	RPTPσ	1-602, 673-847	pAPTAG5	NheI(5') and HindIII (3')	K68A, K69A, K71A, K72A	1,3-6,9	NM_001252456
pAPTAG5-RPTPσ ΔRRRHR	RPTPσ	1-847	pAPTAG5	NheI(5') and HindIII (3')	R762A, R763A, R766A, H767A, R769A	1,3,10-13	NM_001252456
pAPTAG5-RPTPσ ΔLys ΔRRRHR	RPTPσ	1-847	pAPTAG5	NheI(5') and HindIII (3')	K68A, K69A, K71A, K72A, R762A, R763A, R766A, H767A, R769A	1,3-5,10-13	NM_001252456
pAPTAG5-RPTPσ ΔHR	RPTPσ	1-847	pAPTAG5	NheI(5') and HindIII (3')	H809A, R810A	1,3.18,19	NM_001252456
pAPTAG5-RPTPσ ΔLys ΔHR	RPTPσ	1-847	pAPTAG5	NheI(5') and HindIII (3')	, K69A, K71A, K72A, H809, R	1,3-5,18,19	NM_001252456
pAPTAG5-RPTPσ ΔLys ΔRRRHR ΔHR	RPTPσ	1-847	pAPTAG5	NheI(5') and HindIII (3')	K68A, K69A, K71A, K72A, R762A, R763A, R766A, H767A, R769A, H809A, R810A	1,3-5,10-13,18,19	NM_001252456
pFUSE-RPTPσ	RPTPσ	1-847	pFUSE hIg1	EcoRI(5') and EcoRV(3')	None	14,15	NM_001252456
pGFPTAG5	EGFP	1-239	pAPTAG5	BspEI(5') and XhoI (3')	None	20,21	U55763
pGFPTAG5-RPTPσ	RPTPσ	1-847	pAPTAG5	NheI(5') and HindIII (3')	None	1,3,20,21	U55763, NM_001252456
pLNCX chick v-src	v-src		pLNCX				NM_205457

Table S3 (continued)

Oligonucleotides	
Name	Sequence
1	TGGCTAGCGCCGCCATGGCGCCCACCTGGAGTCCCA
2	GAGCGCTTCCTCGAGTCCTGTTGCATAATGATCAAAACTGC
3	GGACCCTCAGCCCATTGTGGAAGCTTAC
4	CCTAAGCCACGGGTGACCTGGAACgccgcGGGagcGgcAGTGAACTCACAGCGCTTCGAGACC
5	GGTCTCGAAGCGCTGTGAGTTCACTgcCgctCCCgcggcGTTCCAGGTCACCCGTGGCTTAGG
6	gtaagcttCCTGCAGCGTGCGCTGGCGCAC
7	ttaagcttCAGTGACCGTCTGCTGCAGG
8	atgaagcttacCTGCAGGCCATCTCCCCAAAG
9	cgaagcttaCAGCAGCCTGGGGGGCCTGC
10	GACATCTCCCGGCTGCAGgcGgctAGCCTGCGCCACTCCAG
11	CTGGAGTGGCGCAGGCTagcCgcCTGCAGCCGGGAGATGTC
12	CAGgcGgctAGCCTGgcCgcCTCtgcgCAGCTGGAGGTGCCTC
13	GAGGCACCTCCAGCTGcgcaGAGgcGgcCAGGCTagcCgcCTG
14	AAGAATTCAAGCTGGCTAGCGCCGCCATGG
15	TTGATATCCACAATGGGCTGAGGGTCC
16	TGGGGTACTCAGGTACtCCGTGtTCGGGCCATGCCGT
17	ACGGCATGGCCCGAaCACGGaGTACCTGAGTACCCCA
18	GCACAAAGAGGACATAtgcGgcGCCTGGCTCCAAGCC
19	GGCTTGGAGCCAGGCgcCgcaTATGTCCTCTTTGTGC
20	aatccggaATGGTGAGCAAGGGCGAGGAGCTG
21	aactcgagCTTGTACAGCTCGTCCATGC