

**Table S1.** Statistically corrected inter-fragment interaction energy (SCIFIE), inter-fragment interaction energy (IFIE) and its energy components electrostatic (ES), exchange repulsion (EX), charge transfer and higher order mixed term (CT+mix), and dispersion (DI). These were calculated as the interaction energy between each sugar residue of the tetrasaccharide and heparanase.

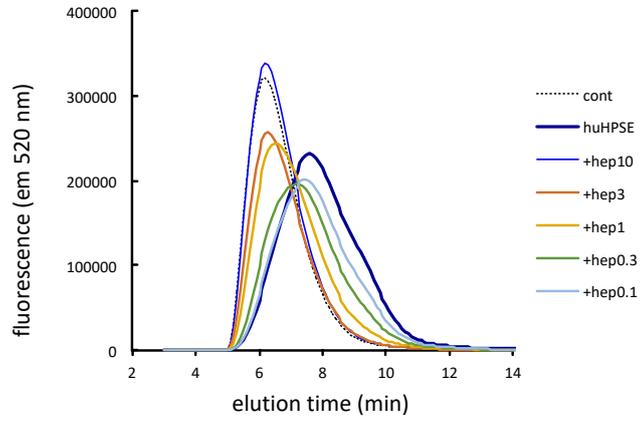
	<b>SCIFIE</b>	<b>IFIE</b>	<b>ES</b>	<b>EX</b>	<b>CT+mix</b>	<b>DI</b>
<b>hs-HA4</b>	<b>-2395.4</b>	-3123.0	-3122.1	212.8	-78.5	-135.2
(+1)	-633.5	-842.1	-830.7	80.7	-35.5	-56.6
(-1)	-562.6	-779.5	-774.8	38.1	-12.7	-30.1
(-2)	-635.3	-804.1	-817.9	58.6	-16.3	-28.4
(-3)	-521.4	-697.3	-698.8	35.5	-14.0	-20.1
<b>Dp4</b>	<b>-2500.5</b>	-3276.9	-3306.6	289.5	-99.3	-160.5
(+1)	-700.6	-920.0	-919.0	77.5	-30.7	-47.8
(-1)	-630.1	-809.6	-844.7	119.2	-29.0	-55.0
(-2)	-644.7	-809.3	-806.7	62.9	-26.4	-39.1
(-3)	-525.1	-738.1	-736.3	29.9	-13.2	-18.6

**Table S2.** Statistically corrected inter-fragment interaction energy (SCIFIE) between each sugar residue of the tetrasaccharide and two heparin binding domains (HBDs).

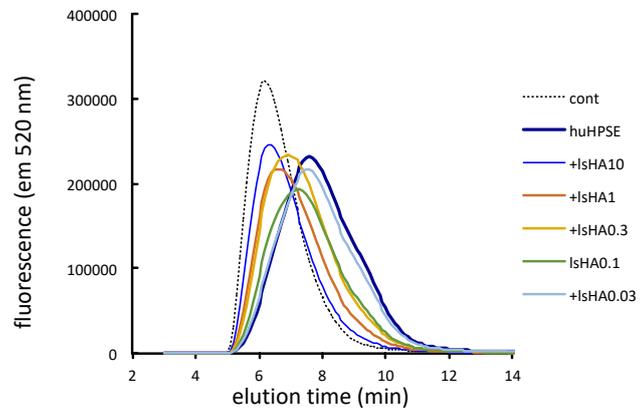
<b>HBD-1 (Lys159-Asp171)</b>					
	Total	(+1)	(-1)	(-2)	(-3)
<b>hs-HA4</b>	-659.8	-65.7	-128.5	-339.1	-126.5
<b>Dp4</b>	-589.7	-101.0	-93.9	-214.9	-180.0
<b>difference</b>	-70.0	35.2	-34.6	-124.2	53.5
<b>HBD-2 (Gln270-Lys280)</b>					
	Total	(+1)	(-1)	(-2)	(-3)
<b>hs-HA4</b>	-553.4	-297.3	-112.9	-69.4	-73.8
<b>Dp4</b>	-526.0	-270.8	-116.8	-78.3	-60.0
<b>difference</b>	-27.4	-26.5	3.9	8.9	-13.8

**Figure S1.** Gel filtration of fluoresceine-labeled heparan sulfate degraded by human heparanase (huHPse). Inhibitors were added at different concentrations which are shown as  $\mu\text{g/ml}$ .

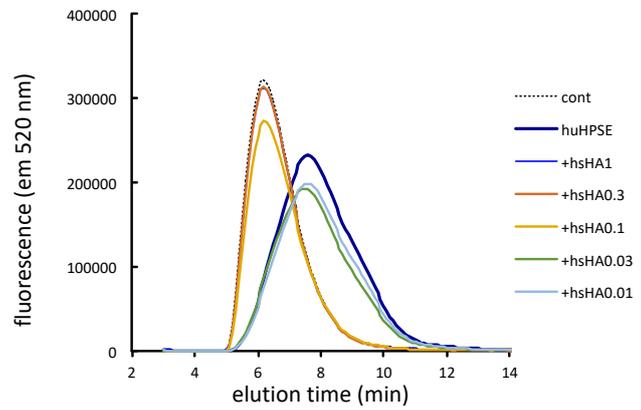
**A: heparin (hep)**



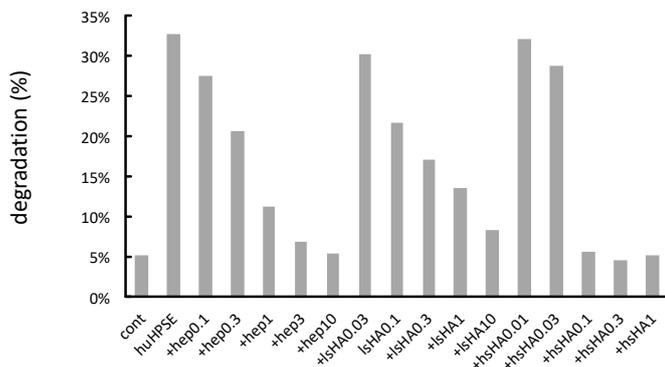
**B: low-sulfated hyaluronan (ls-HA)**



**C: high-sulfated hyaluronan (hs-HA)**

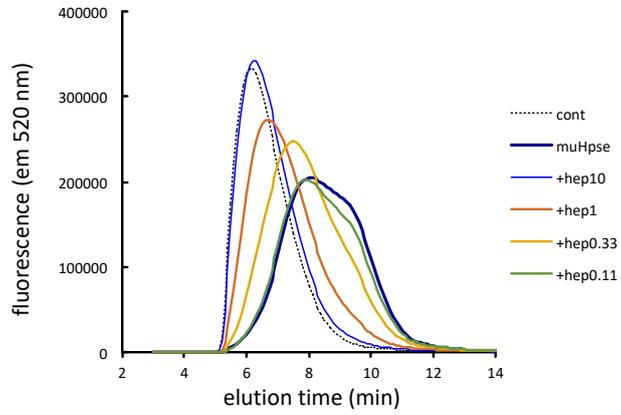


**D: degradation ratio of panel A-C**

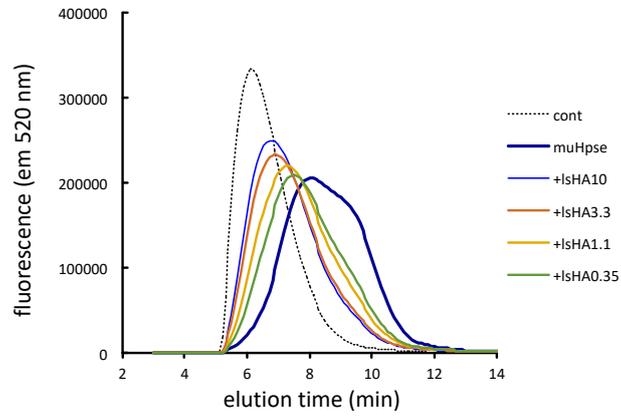


**Figure S2.** Gel filtration of fluorescently-labeled HS degraded by muHpse. Inhibitors were added at different concentrations which are shown as  $\mu\text{g/ml}$ .

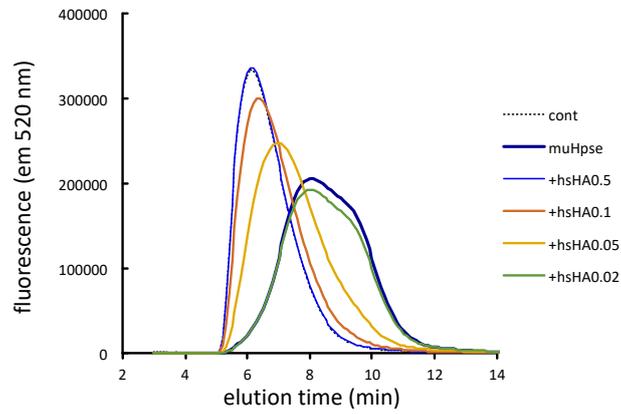
**A: heparin**



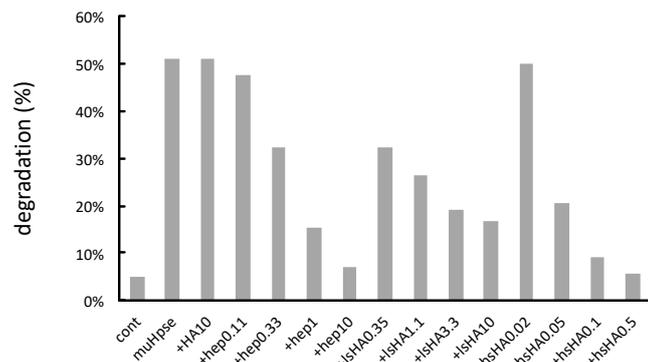
**B: ls-HA**



**C: hs-HA**

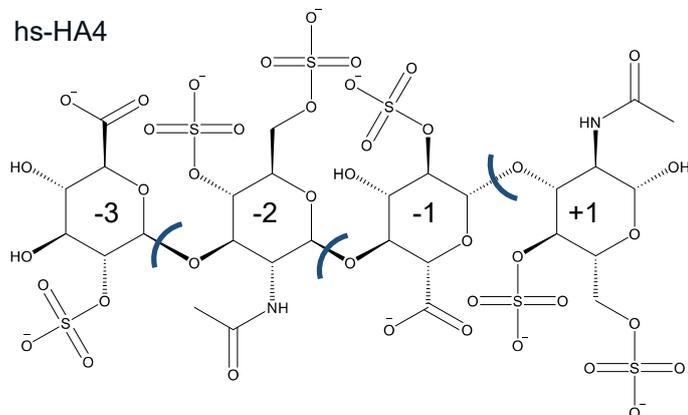


**D: degradation ratio of panel A-C**

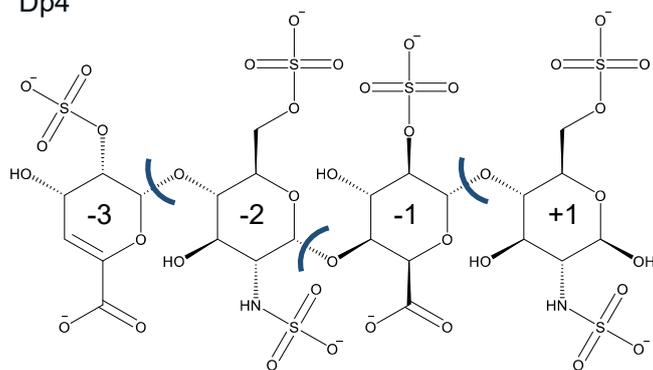


**Figure S3.** Structures of hs-HA4 and Dp4 tetrasaccharides. The delimiter lines indicate the fragmentation positions.

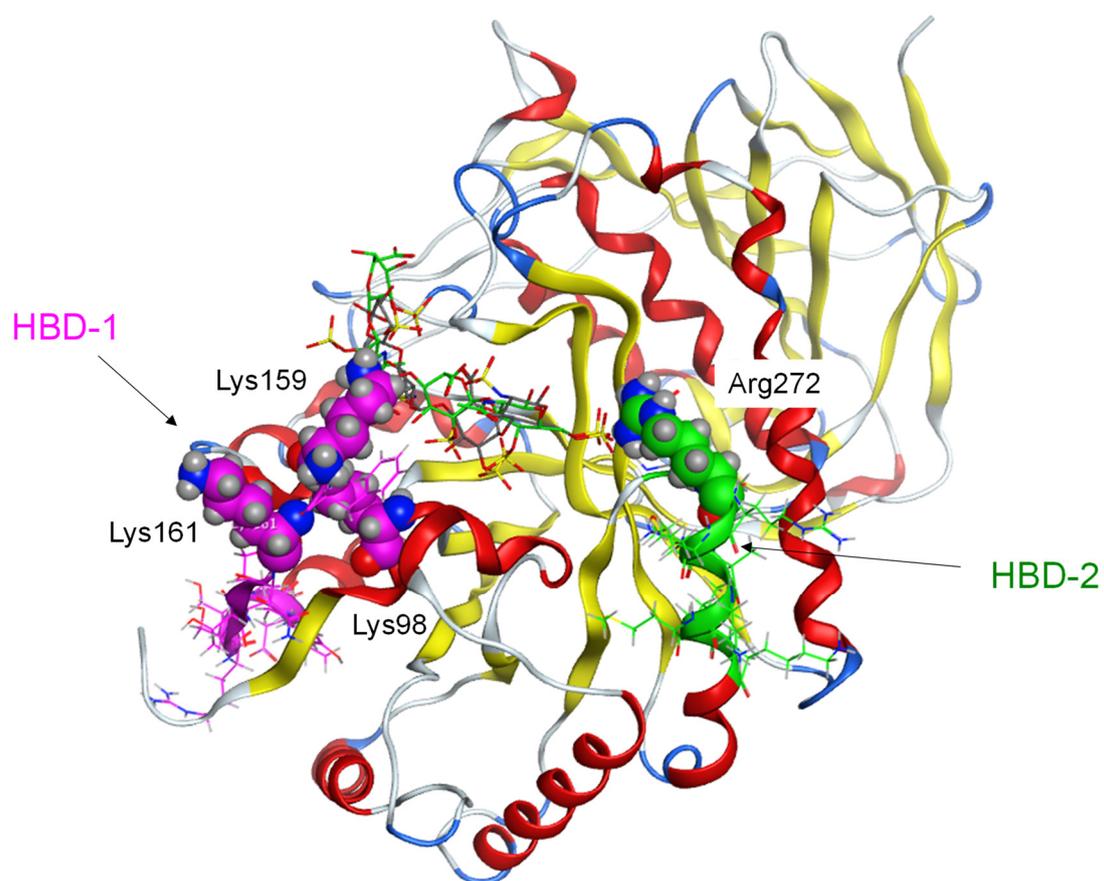
**A** hs-HA4



**B** Dp4

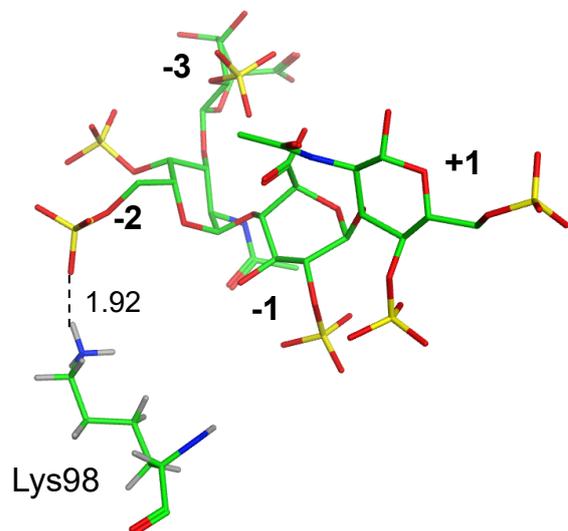


**Figure S4.** Location of tetrasaccharides and heparin binding domains (HBDs). Atoms in the tetrasaccharides are indicated by sticks, and HBD-1 and HBD-2 are indicated by pink and green ribbons, respectively.

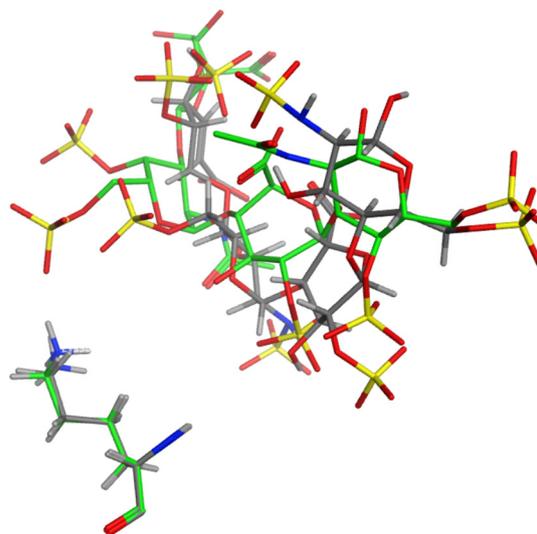


**Figure S5.** Interaction of the 6-*O*-sulfate group of the (-2) sugar residue of the tetrasaccharides with Lys98 of huHpse.

**A: hs-HA4**



**C: Superimposed image**



**B: Dp4**

