Supplementary Information

Preparation of HS samples HS-L1 and HS-L2, HS-B1and HS-B2

Fresh porcine liver (1800 g) and bovine brain (3 brains) tissue were chopped into small pieces and washed with cold PBS at 4˚C to remove blood and vessels thoroughly. The tissues were freeze-dried. The dried liver (440g) and brain (220 g) were ground into a fine powder. The powder was defatted with chloroform/methanol (2:1, 1:1, and 1:2) at room temperature with stirring (overnight extraction), subsequently and 250 g defatted liver and 50 g defatted brain were obtained after drying. Defatted liver (25 g) and defatted brain (25 g) were extracted in two sequential washes with 4 M guanidine-hydrochloride buffer containing a protease inhibitor cocktail and 2% CHAPS, 50 mM sodium acetate, at pH 6.0 for 24 h at 4˚C with stirring. Two fractions for each tissue were obtained, labeled as L1 and L2, B1and B2, respectively. For higher extraction efficiency, it is important to have a large supernatant volume and a small pellet. The extracted factions were ultrafiltrated using 10 kDa cutoff membrane against 8 M urea containing 2% CHAPS to concentrate and buffer exchange. The extracted, buffer exchanged fractions were next purified by strong anion exchage (SAX). A Vivapure MAXI Q H spin column was prepared by equilibrating with 3 ml of 8 M urea containing 2% CHAPS (pH 8.3). Clarified, filtered samples were loaded onto and run through the Vivapure MAXI QH spin columns under centrifugal force (500 × *g*). The columns were first washed with 3 ml of 8 M urea containing 2% CHAPS at pH 8.3. The columns were then washed five-times with 5 ml of 200 mM NaCl. Proteoglycan (PG) samples were released from the spin column by washing 3-times with 1 ml of 16% NaCl. Methanol (12 ml) was added to the PG solution in sodium chloride to afford an 80 vol%

solution and the mixture was equilibrated at 4° C for 18 h. The resulting precipitate was recovered by centrifugation (2500 × *g*) for 15 min. The recovered precipitate was dissolved in 0.5 ml of water. The PGs from above method is the mixture of HSPG and CSPG.

To obtain HSPG, chondroitinase ABC was used to remove the CSPG. After chondroitinase ABC digestion, the samples were subjected to purification using Vivapure MAXI Q H spin column with same protocol used above. The samples were labeled as HS-L1 and HS-L2, HS-B1and HS-B2. The HS disaccharides were obtained by exhaustive treatment with an equi-unit mixture of heparin lyase I, II and III.

To determine these samples using LC/MS, 250, 150, 210, 100 ng of disaccharide mixtures (determined by carbazole assay) obtained by heparin lyase digestion of 360, 300, 350, 220 ng of HS-L1, HS-L2, HS-B1 and HS-B2 (determined by carbazole assay), respectively, were combined with isotopically labeled disaccharide standards (15 ng of each disaccharide as determined by carbazole). The intensities of the peaks corresponding to the molecular ion of unlabeled disaccharides were compared to those of the corresponding isotopically labeled disaccharides and the amount of each peak was calculated.

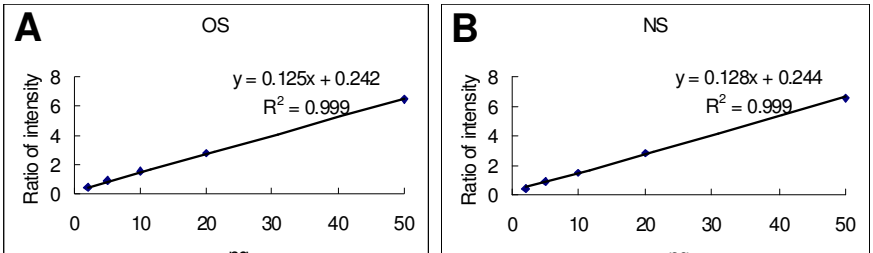

Figure 1S. The calibrate curves and linear equations of disaccharides based on the corresponding isotopically labeled internal standards. The ratio of MS intensity between each disaccharide and corresponding amount consistent internal standard is the function of their amounts.