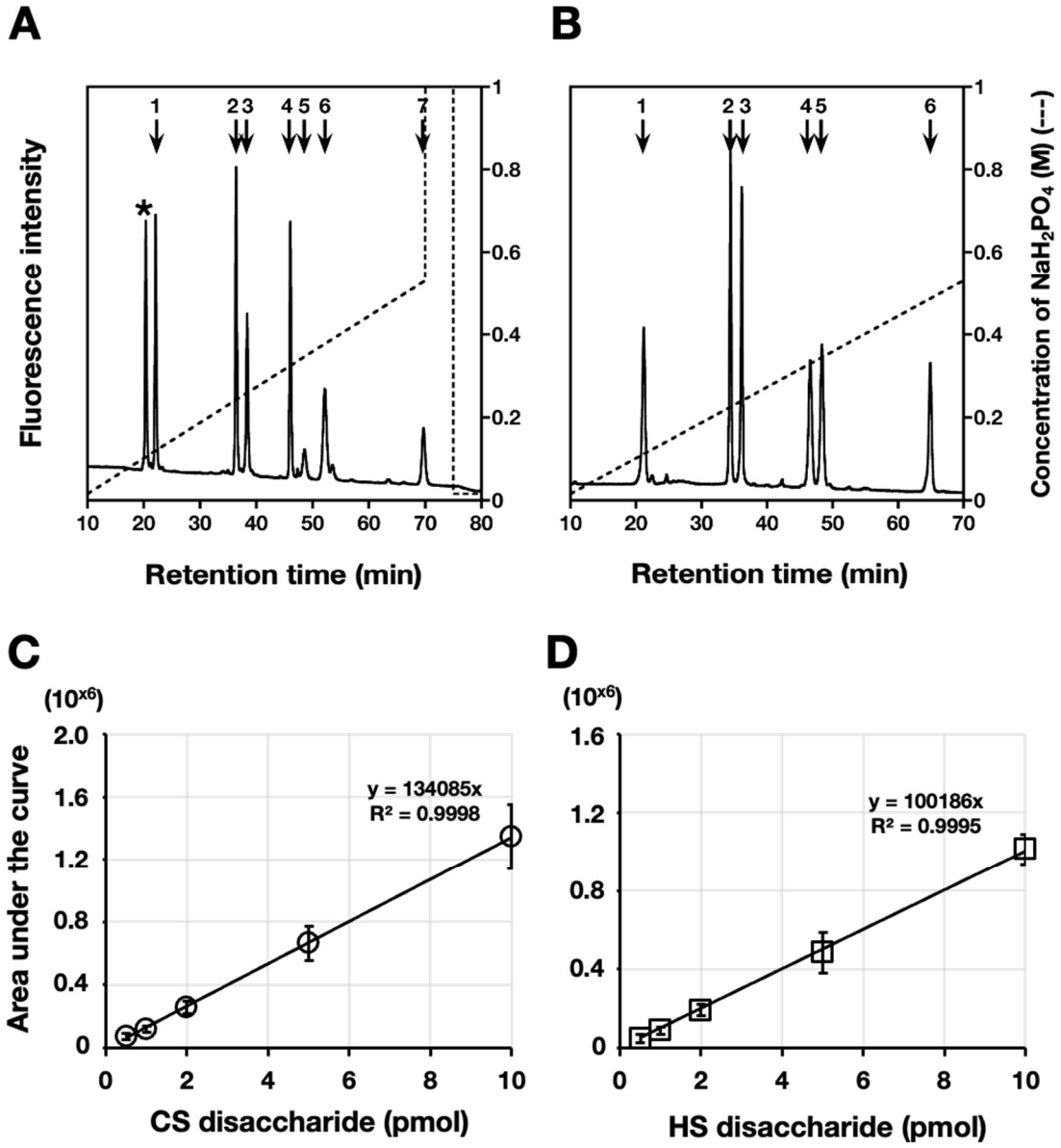
Supplementary Figures

Supplementary Figure S1



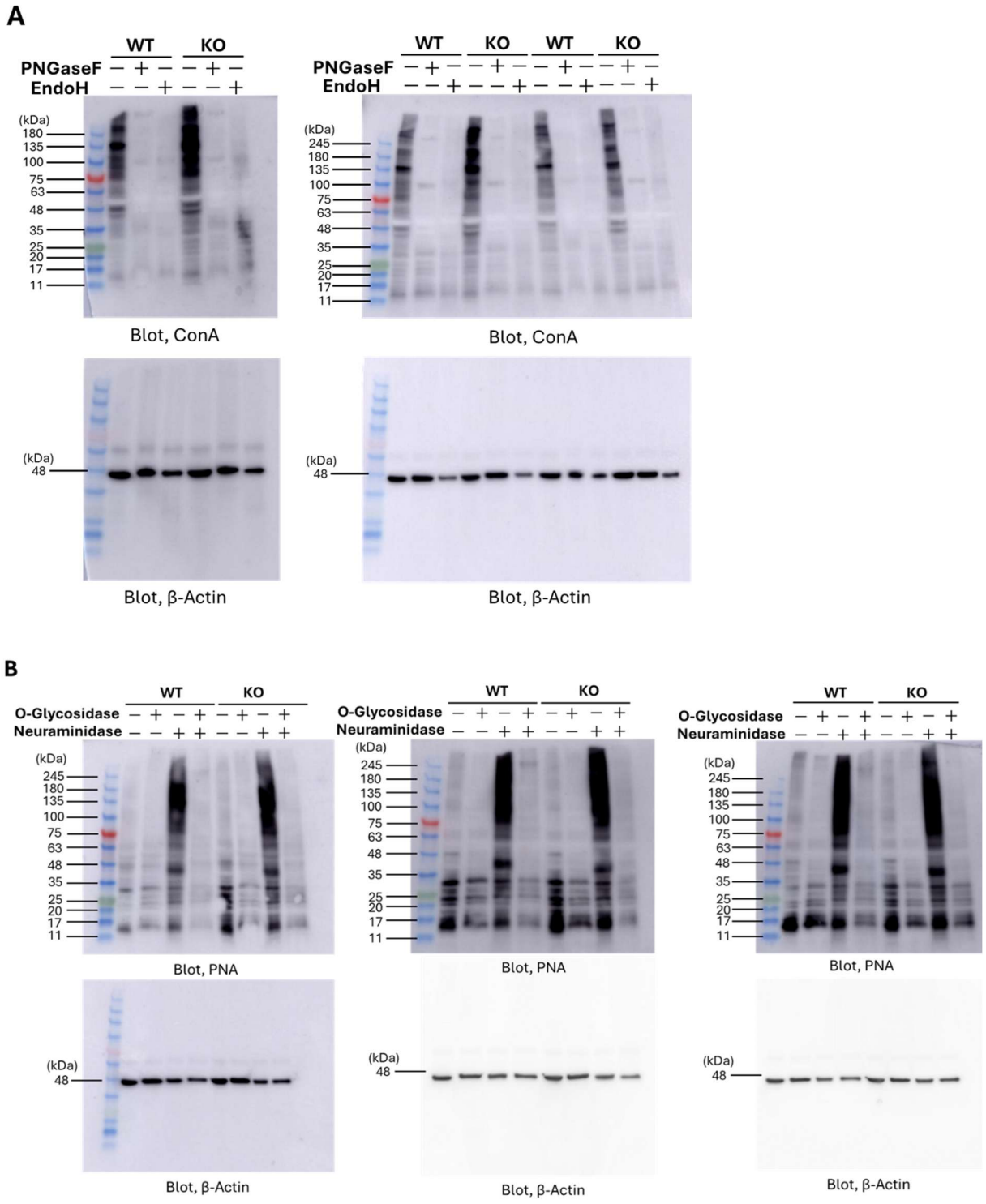
Supplementary Figure S1. HPLC profiles of the authentic CS and HS standard disaccharides, and their standard curves.

(A, B) Standard disaccharides (0.5~10 pmol each) were separated by anion-exchange HPLC on an amine-bound silica PA-G column using a linear gradient of NaH_2PO_4 as indicated by the dashed line. The elution positions of 2-aminobenzamide (2AB)-labeled CS disaccharides (2 pmol each) are indicated by numbered arrows (A). (1) ΔHexUA-GalNAc; (2) ΔHexUA-GalNAc(6S); (3) ΔHexUA-GalNAc(4S); (4) ΔHexUA(2S)- GalNAc(6S); (5) ΔHexUA(2S)-GalNAc(4S); (6) ΔHexUA-

GalNAc(4S,6S); (7) ΔHexUA(2S)-GalNAc(4S,6S). The asterisk indicates the position of 2AB-labeled ΔHexUA-GlcNAc derived from hyaluronan, which is also depolymerized by chondroitinases. The elution positions of authentic 2AB-labeled HS disaccharides (3 pmol each) are indicated by numbered arrows (B): 1, ΔHexUA-GlcNAc; 2, ΔHexUA-GlcNAc(6S); 3, ΔHexUA-GlcN(NS); 4, ΔHexUA-GlcN(NS,6S); 5, ΔHexUA(2S)-GlcN(NS); 6, ΔHexUA(2S)-GlcN(NS,6S). Based on the peak area of these standard disaccharides, the amounts of GAG disaccharides from mouse brain tissues were determined. *Abbreviations*: ΔHexUA, 4,5-unsaturated hexuronic acid; GalNAc, *N*-acetyl-D-galactosamine; GlcN, D-glucosamine; GlcNAc, *N*-acetyl-D-glucosamine; 2S, NS, 4S, and 6S, 2-*O*-, 2-*N*-, 4-*O*-, and 6-*O*-sulfate, respectively.

(C, D) Standard curves of CS (C) and HS (D) disaccharides. The average of peak area from each disaccharide standard was plotted. The error bars indicate standard deviation.

Supplementary Figure S2



Supplementary Figure S2. Representative lectin blotting images used for quantitative analysis in Figure 3.

Original lectin blot image for Figure 3 and additional lectin blot images from two independent

experiments (n = 3), each performed using brain tissues from different individual mice, are shown. These data constitute the three independent biological replicates used for quantitative analysis. Brain tissue lysates from wild-type (WT) and *Slc35a3*-KO (KO) mice were analyzed by lectin blotting using (A) concanavalin A (ConA) for *N*-glycans and (B) peanut agglutinin (PNA) for *O*-glycans following the indicated enzymatic treatments.

Corresponding β-actin blots are shown below each lectin blot as loading controls. These data demonstrate the reproducibility of the lectin blot signal patterns across independent experiments.