Supporting information

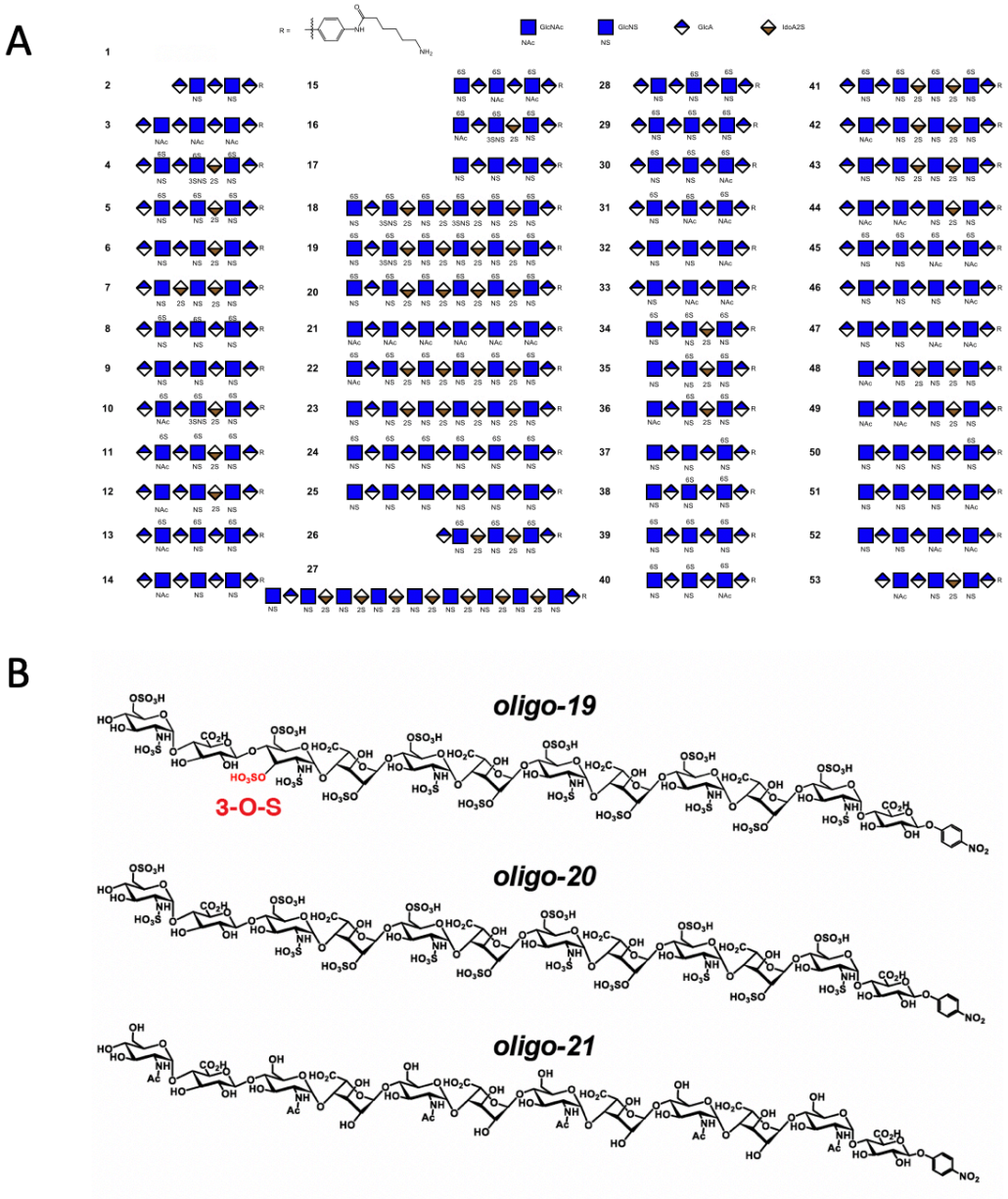

Fig. S1. HS oligosaccharides immobilized on microarray chip (A) and chemical structure of oligo-19, 20 and 21 (B).

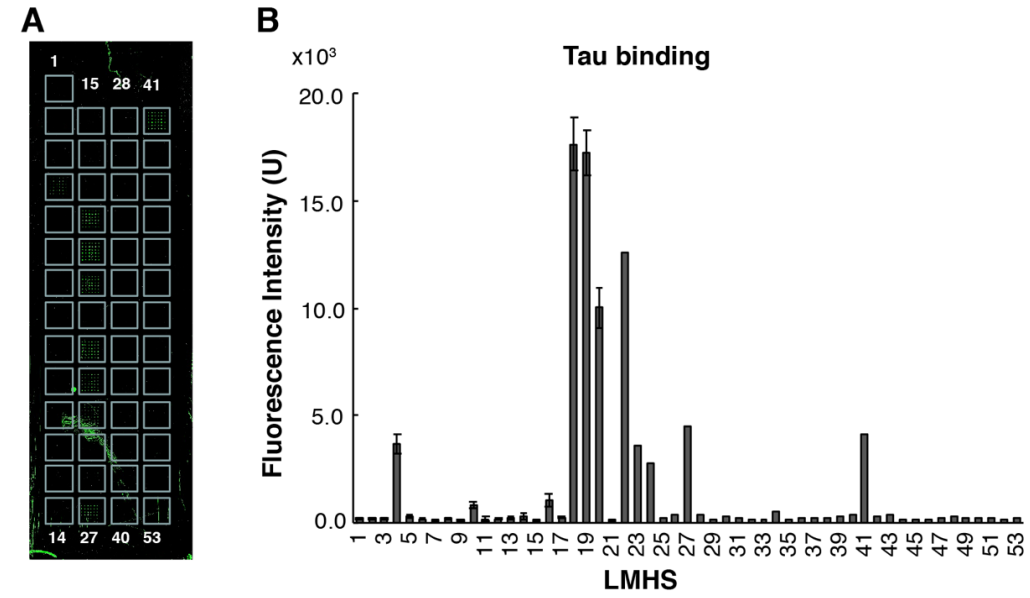

Fig. S2. Fluorescence image (A) and intensity (B) of each spot on LMHS array.

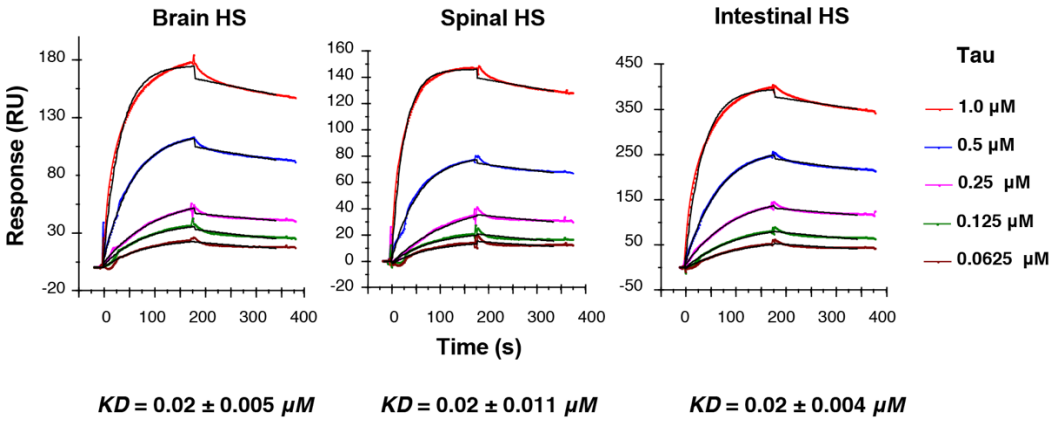

Fig. S3. Porcine Brain, spinal and intestinal HS exhibited similar binding pattern to full-length tau, with the binding affinity (K_D) of 0.02 μM. The association and dissociation curve of different tau concentrations were fitted (black line) by a 1:1 Langmuir kinetics model in Bio-evaluation.

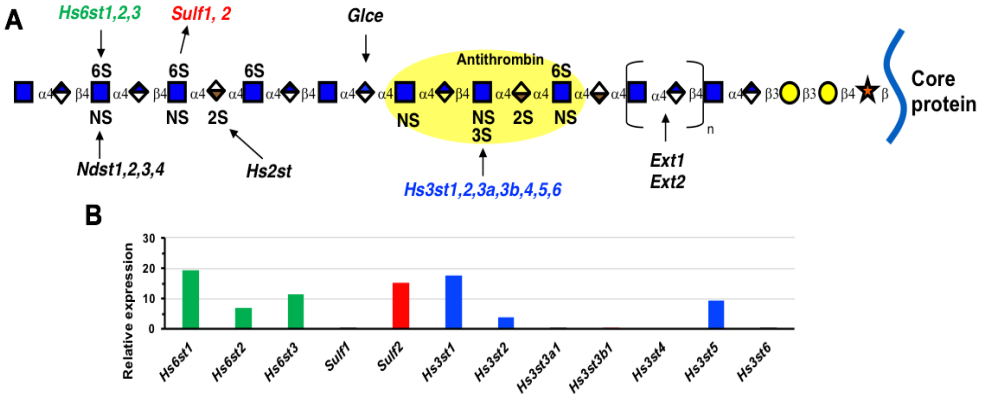

Fig. S4. Gene target selection in HS synthesis pathway for generating neuro-specific HS deficient mice with specific sulfation pattern. (A). HS structure and biosynthetic/remodeling genes. In mammals, the 3S level is determined by *Hs3st*. In MLECs *Hs3st1* is the most abundantly expressed *Hs3st*. **(B).** The expression profiles of *Hs6st*, *Sulfs* and *Hs3sts* in primary mouse cerebral cortex neurons determined by RNA-seq.

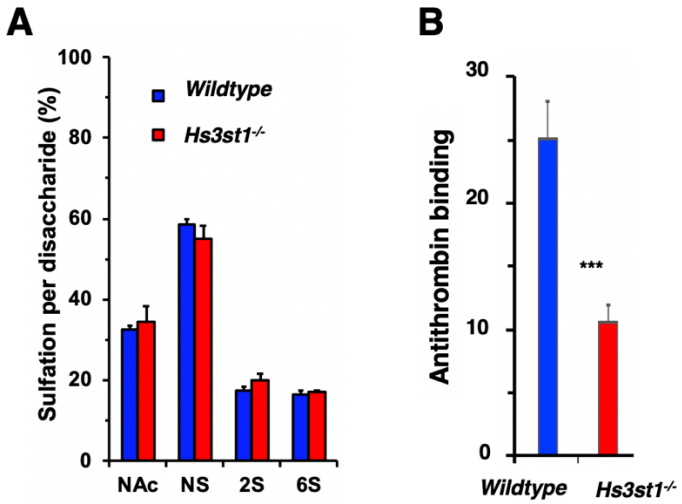

Fig. S5. The *Hs3st1*$^{-/-}$ MLEC line expressed normal levels of NS, 6-*O*-S and 2-*O*-S (A), but reduced cell surface binding to antithrombin III (B).

Experimental Section

Materials. The overexpression and purification of full-length tau protein were performed as previously described [1,2]. Chemoenzymatic synthesis of low molecular weight heparan sulfate (LMHS) was completed according to methods published previously [3] [4]. Heparan sulfate extracted from porcine intestine is a commercial product obtained as a side stream in the manufacture of porcine intestinal heparin [5]. Heparan sulfates from porcine brain and spine were purified and characterized as previously described [6].

The wildtype and *Hs3st1*$^{-/-}$ MLEC lines were developed in our lab recently using Crispr-Cas9 or conditional Cre-LoxP gene editing technologies [7]. The *Hs3st1* deletion selectively reduces 3-*O*-S and correspondingly, can be applied to specifically determine the requirement of 3-*O*-S in interaction with a protein ligand, respectively, in a cellular setting.

The binding preference of tau to HS using microarray assay. Full-length tau protein was labeled with fluorescence dye Alexa Fluor 488 5-SDP Ester (Life Technologies) according to the supplier's instructions. The degree of labeling (DOL) was 1-2 moles/mole of protein. A series of structurally defined HS oligosaccharides are immobilized on a microarray chip using a robotic printer as previously described [8]. The fluorescently labeled tau protein is incubated with the slide for 1 h at room temperature and then washed. The wash process was repeated twice before analyzing the slide using the excitation wavelength of 488 nm on a GenePix 4300 scanner (Molecular Dynamics). Resolution was set at 10 μm. The array images were analyzed by GenePix Pro 7.2.29.002 software. Spots were automatically found and spot deviations were manually fit to correct. Mean median fluorescence intensities of arrays were obtained by Array Quality Control of software.

Characterization of tau-HS interaction by SPR assays.

Preparation of the HS biochip. Biotinylated HS was prepared by reacting sulfo-N-hydroxysuccinimide long-chain biotin (Thermo Scientific, Waltham, MA) with free amino groups of unsubstituted glucosamine residues in the polysaccharide chain according to a published procedure [9]. The biotinylated HS was immobilized to a SA chip based on the manufacturer's

protocol. In brief, a 20 μL solution of the HS-biotin conjugate (0.1 mg/mL) in HBS-EP running buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, and 0.005% surfactant P20 (pH 7.4)) was injected over flow cell 2 (FC2) of the SA chip at a flow rate of 10 μL/min. Successful immobilization of HS was confirmed by the observation of an ~250 resonance unit increase in the sensor chip. The control flow cell (FC1) was prepared by a 1 min injection with saturated biotin.

Binding affinity of HS-tau interaction. Lyophilized full-length tau protein was resuspended in HBS-EP buffer. Different concentrations of the protein (0.1 μM, 0.25 μM, 0.5 μM, 1.0 μM, and 2.0 μM) were injected at a flow rate of 30 μL/min for 3 min. At the end of the sample injection, HBS-EP buffer was flowed over the sensor surface to facilitate dissociation. After a 3 min dissociation time, the sensor surface was regenerated by injection with 30 mL of 2 M NaCl. The response was monitored as a function of time (sensorgram) at 25 °C.

Competition assay of 12-mer. Competition SPR experiments were performed to study how the presence of 3-*O*-S impacts the inhibition of 12-mer on tau-HS interaction. Tau protein was premixed with three different 12-mer, separately, and flowed over the HS chip at a flow rate of 30 μL/min. After each injection, dissociation and regeneration were performed as described above. For each set of competition experiments on SPR, a control experiment (with only tau protein and no 12-mer) was performed to confirm that the surface was completely regenerated and that the results obtained between runs were comparable. A series of concentrations of 12-mer was tested and IC_{50} was obtained by fitting the data using the '[Agonist] vs. normalized response' equation in GraphPad Prism 8 software, $Y=100*X^H/(IC_{50}^H + X^H)$, where Y is the normalized binding of tau to HS biochip, X is the concentration of 12-mer, and H is the Hill slope describing the steepness of the curve.

Cell surface tau binding assay. ELISA was performed to determine cell surface tau binding. In brief, 3×10^4 MLECs, including wildtype and *Hs3st1*$^{-/-}$cells, were seeded at 200 μl/well in DMEM containing 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin in a 96 well plate. After culturing overnight, the cells were washed with DPBS (3 times x 5 min) and then fixed with 4% PFA (15 min, RT), washed with DPBS (3 times x 5 min) and blocked with DPBS containing 1% BSA (90 min, RT), the cells were incubated with 100 μl DPBS containing BSA (50 ng), biotinylated Tau (50 ng/ml), or biotinylated Tau (50 ng) mixed with heparin (50 ng), oligo-19 (25

ng), oligo-20 (25 ng) or oligo-21 (25 ng) for 90 min at RT. Following, the cells were washed with DPBS (3 times x 5 min), incubated with Streptavidin-HRP (1:2000 dilution in DPBS containing 1% BSA, 30 min, RT), and then cell surface bound tau (represented by HRP activity) was measured using an Ultra TMB-ELISA kit (34028, Thermo Scientific) according to the manufacturer's protocol.

Tau internalization assay. MLECs (5×10^5), including wild type and *Hs3st1*$^{-/-}$ cells, were seeded at 600 μL /well DMEM containing 10%FBS, 100 U/ml penicillin and 100 μg/ml streptomycin in a 12 well plate. After culturing overnight, the cells were washed with DPBS twice and then incubated with 500 μL/well DMEM containing BSA (2 μg/ml), Tau-Alexa (2 μg/ml) or Tau-Alexa (2 μg/ml) mixed with heparin (10 μg/ml), oligo-19 (2.5 μg/ml), oligo-20 (2.5 μg/ml) or oligo-21 (2.5 μg/ml) at 37 °C for 3 h. Followingly, the cells were processed for image or flowcytometry analyses. For image analysis, the cells were covered with mounting medium DAPI and examined for internalized tau under confocal microscope for flowcytometry analysis, the cells were trypsinized and resuspended in DPBS containing 2 mM EDTA, 1% BSA and PI which stain dead cells, and then measured for internalized tau-Alexa with flow cytometer.

3-*O*-S Binding site mapping by NMR. NMR experiments with oligo-4 (HS 7-mer with 3-*O*-S) and oligo-5 (HS 7-mer without 3-*O*-S) were performed on full-length tau to map the binding site of 3-*O*-S on tau. ^{1}H-^{15}N HSQC spectra were recorded on a 150 μM full-length tau sample (in 25 mM NaH_2PO_4, 25 mM NaCl, 0.3 mM DTT, 2.5 mM EDTA, 10% D_2O, pH 6.5) on an 800 MHz spectrometer at 20 °C before and after the adding of a 1:0.6 ratio of oligo-4 and oligo-5, separately. Normalized chemical shift perturbation (CSP) of tau for amide ^{1}H and ^{15}N chemical shifts upon HS 7-mer addition were calculated using the equation $CSP = \sqrt{100 \times \Delta H^2 + \Delta N^2}$, where ΔH and ΔN are the differences between the chemical shifts of the free and bound forms of tau, respectively. As the only difference between oligo-4 and oligo-5 is an additional 3-*O*-S, a CSP difference (ΔCSP) calculated by CSP (due to oligo-4) minus CSP (due to oligo-5) was plotted against the residue number to map the binding site of 3-*O*-S in tau.

References

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