Heparan sulfate proteoglycans (HSPGs) serve as the mediator between monomeric tau and its subsequent intracellular ERK1/2 pathway activation

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Supplementary Materials

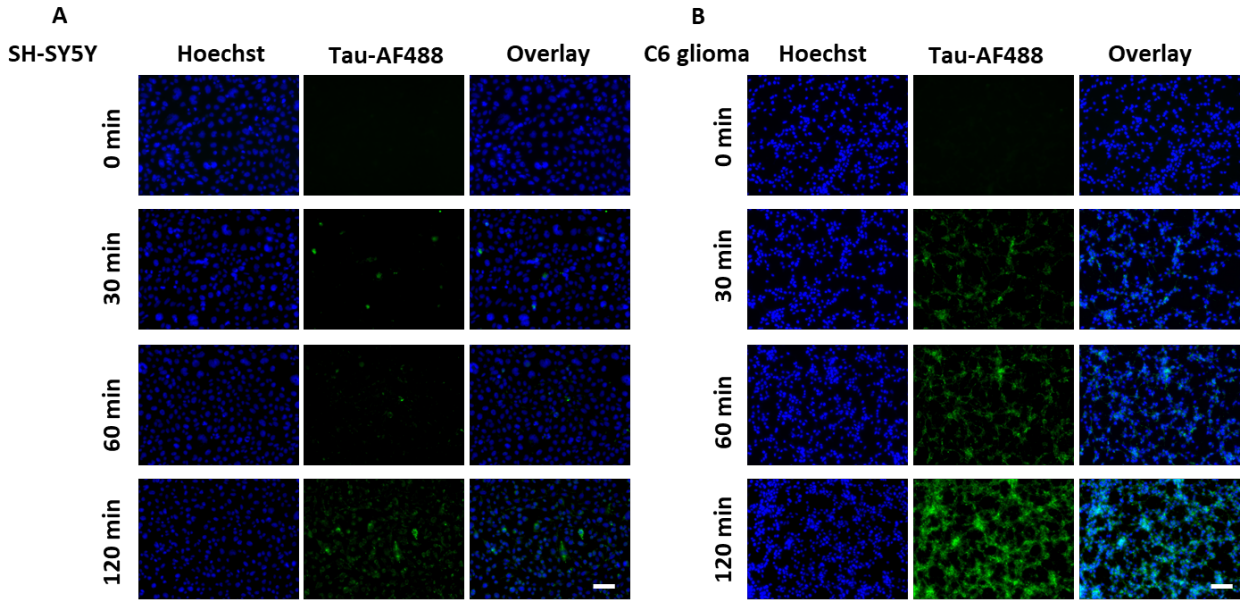

Fig.S1 C6 glioma cells rapidly take up tau. The amount of tau-AF488 internalized by both SH-SY5Y cells **(A)** and C6 glioma cells **(B)** increases over time from 0min to 120mins, while SH-SY5Y cells internalize much less tau protein than C6 glioma cells. Scale bar = 100μm.

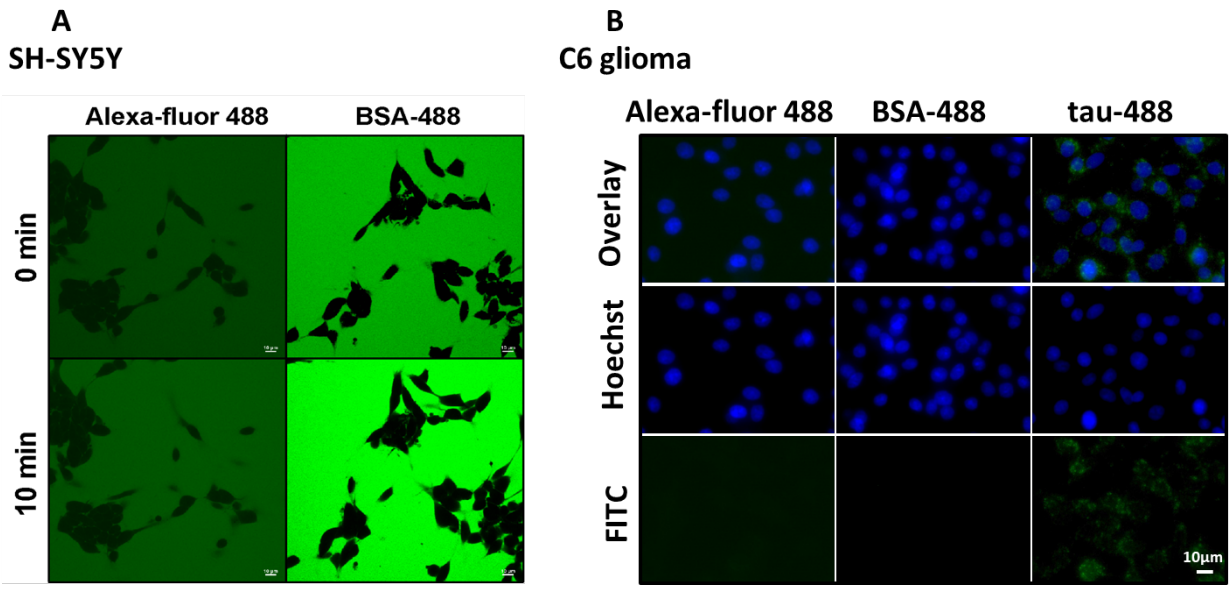

Fig.S2 No internalization of label or BSA occurs in both SH-SY5Y cells and C6 glioma cells over short imaging periods. (A) Both free Alexa fluor 488 and labeled BSA remain excluded from SH-SY5Y cells over a ten-minute imaging period. **(B)** Tau-488 primarily localized within cell body of C6 glioma cells after 15mins incubation, while no internalization of free label or BSA was observed.

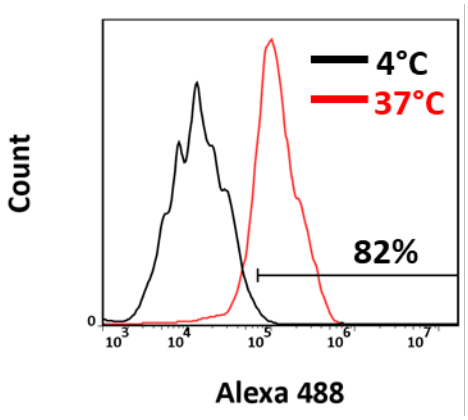

Fig. S3 The propensity of Alexa Fluor-488-labeled monomeric 0N4R tau uptake was quantified in C6 cells by flow cytometry at different temperatures, 4°C vs. 37°C. Representative flow cytometry histogram is shown. Black line: cells incubated at 4°C; red line: cells incubated at 37°C.

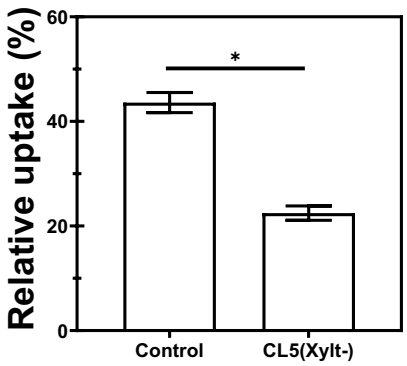

Fig. S4 HSPG deficiency decreases cellular uptake of tau. The relative uptake of tau (1μM) was quantified by flow cytometry after a thirty-minute incubation and compared to CL5. (*Indicates $p<0.05$ between different test conditions, n=3).

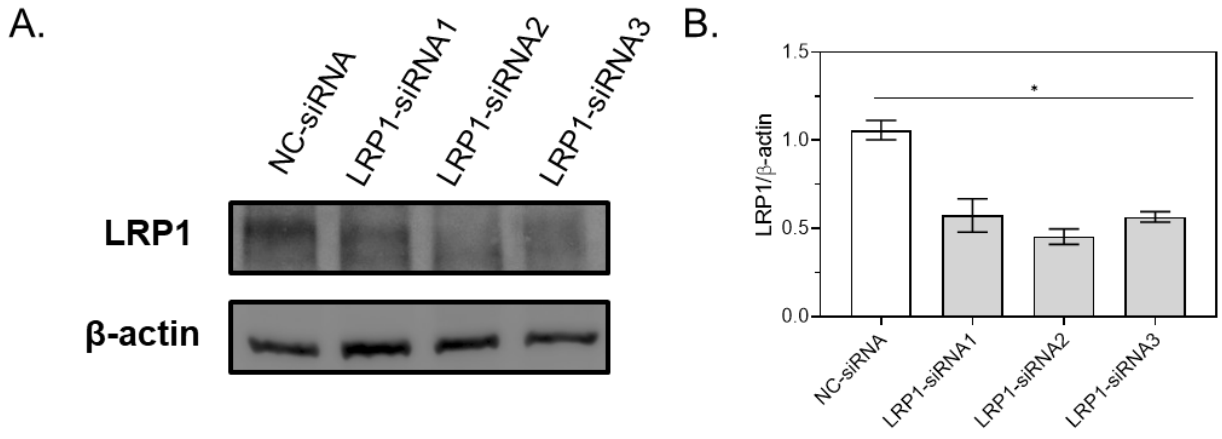

Fig. S5 Downregulation of LRP1 by transfecting C6 glioma cells with LRP1-specific siRNA. (A) Rat C6 glioma cells were transiently transfected with non-targeting siRNA (NC) or LRP1-specific siRNA1, siRNA2, and siRNA3 for 48 h. LRP1 downregulation was then evaluated by immunoblotting against LRP1 and β-actin. Quantitative data of LRP1/β-actin ratios from western blot experiments is shown in **(B)**. (* Indicates $p<0.05$ between different test conditions, n=3 biological replicates)

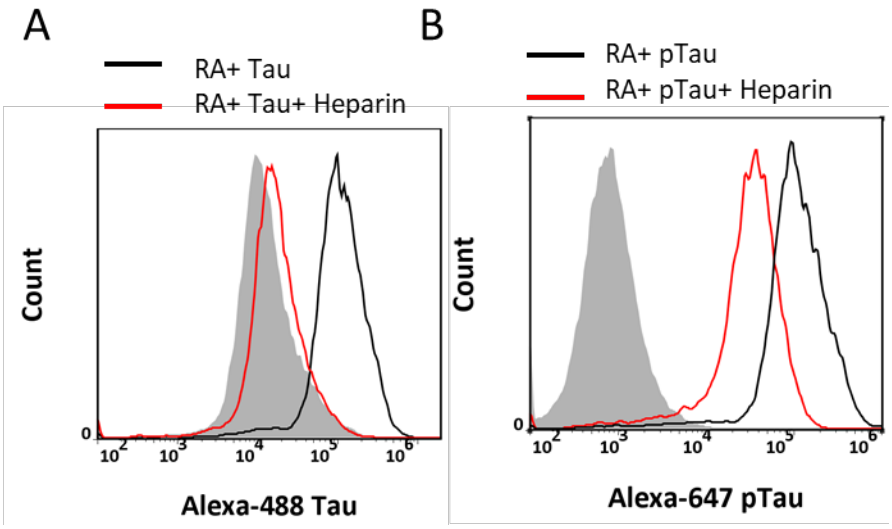

Fig. S6 Quantification of different tau isoforms uptake by primary astrocytes. The propensity of Alexa Fluor-488-labeled monomeric 0N4R tau uptake and Alexa Fluor-647-labeled ptau were quantified in primary astrocytes by flow cytometry. Grey filled line: negative control; Black line: cells without heparin; red line: cells with heparin incubation.

Supplemental Table S1. List of primers for sequencing, sgRNAs and RT-qPCR

Name	Forward Primer 5' to 3' (F)	Reverse Primer 5' to 3' (R)
Exon7 sgRNA	CACCGAATGTCTATGACGAACCAGA	AAACTCTGGTTCGTCATAGACATTC
Exon7 Sequencing	ACAGGTCATGGAGTCCCAGGGC	GCATCTTGCTCTGCCTGGGACC
Xylt1	GACACTGGAGACATGGATGATG	GTCAGTGCCAACCTCAGAAA
LRP1	GGCGTGGTGTTCTGGTATAA	GTAGGTAGGGTTTCCGATTTCC
Il-6	GAAGTTAGAGTCACAGAAGGAGTG	GTTTGCCGAGTAGACCTCATAG
TNF-α	ACCTTATCTACTCCCAGGTTCT	GGCTGACTTTCTCCTGGTATG
Il-1β	CTATGGCAACTGTCCCTGAA	GGCTTGGAAGCAATCCTTAATC
β-actin	ACAGGATGCAGAAGGAGATTAC	ACAGTGAGGCCAGGATAGA