Specific glycosaminoglycan chain length and sulfation patterns are required for cell uptake of tau vs. α-synuclein and β-amyloid aggregates

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Supporting information

Table S1

Gene	gRNA sequence
EXT1	GCCAGTGTTGAAGCTTCTCG
NDST1	CGTGCACGTGCAGCTGTACG
HS6ST2	GGTGCCGGACCCGTACCGCT

Table S1: gRNA sequences used for TIDE analysis

Figure S1:

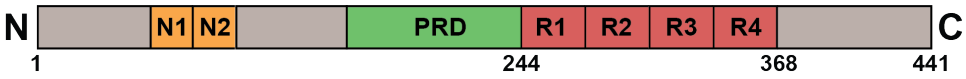

Figure S1: Tau polypeptide sequence and boundaries of RD domain used in FRET P301S biosensor cell line. The monoclonal FRET P301S biosensor cell line was made by overexpressing tau RD domain, comprising domains R1 to R4 (amino acids 244 to 368), tagged at the C-terminus to either CFP or YFP, in HEK293T cells (1,2). PRD = proline-rich domain. N1, N2 = N=terminal domains 1 and 2.

Figure S2A

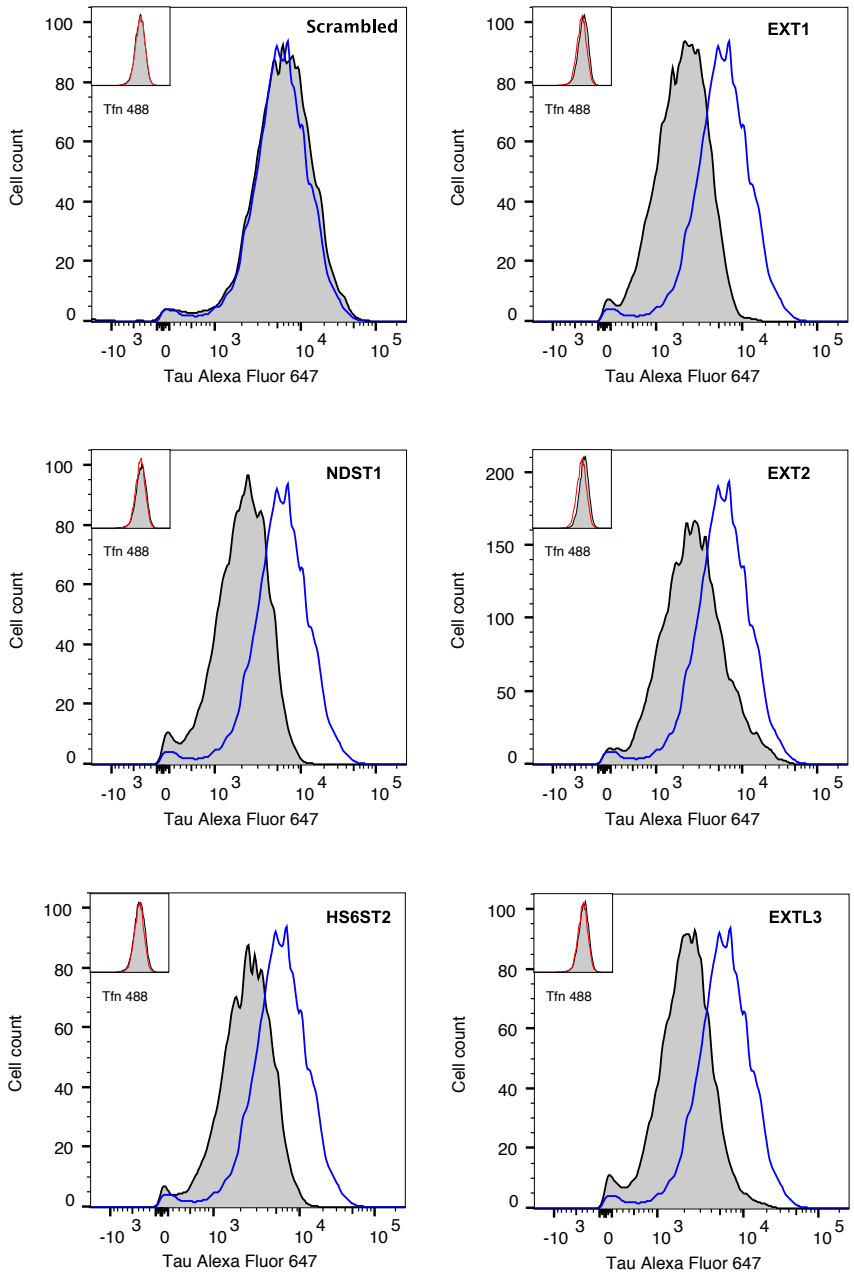
Scrambled
EXT1
NDST1
EXT2
HS6ST2
EXTL3
Tfn 488
Cell count
Tau Alexa Fluor 647

Figure S2B

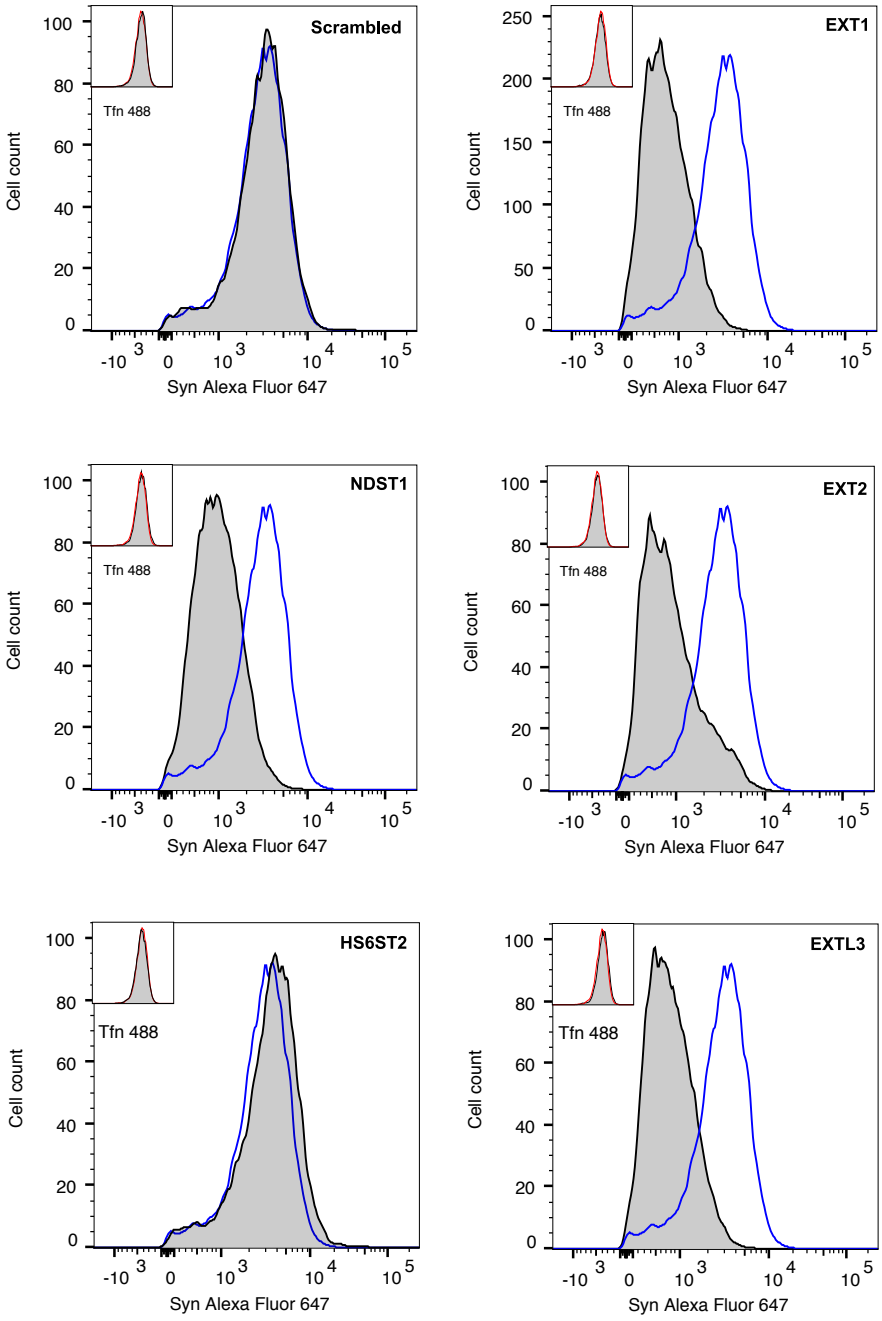

Figure S2: Selected histograms for genetic analyses. Histograms depict the fluorescence intensity of Alexa Fluor 647-labeled tau (A) and α-synuclein (B) fibril uptake in the setting of gene knockout (grey) vs. scrambled controls (blue line). Insets shows the fluorescence intensity profile for internalized transferrin labeled with Alexa Fluor 488 (Tfn 488) in the same cells (gene knockout cell lines in gray and scrambled control cell lines as a red line). *EXT1*, *EXT2*, *EXTL3*, and *NDST1* knockout appreciably reduced tau and α-synuclein uptake, while the knockout of *HS6ST2* only reduced tau uptake. None of the gene knockouts interfered with transferrin uptake.

Figure S3

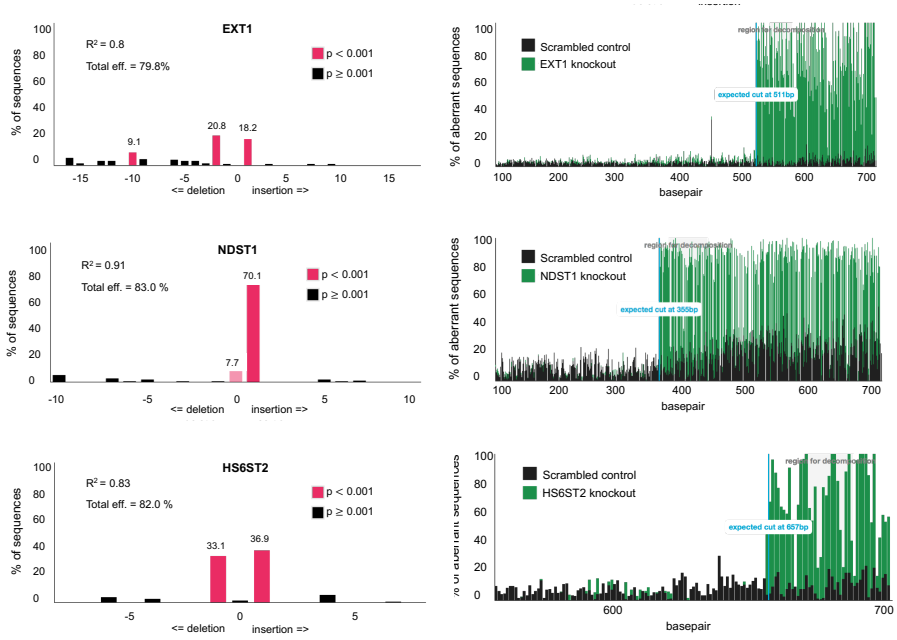

Figure S3: Analysis of gene editing by Tracking of Indels by Decomposition (TIDE). TIDE software was used for this analysis (https://tide.deskgen.com) (3). The plots represent the spectrum of indels and their frequencies for the genes of interest (*EXT1*, *NDST1* and *HS6ST2*) on the left side and the overlay of the control sequence (black, derived from scrambled control cell line) and the sequence derived from the knockout cell line (green) on the right side.

Figure S4

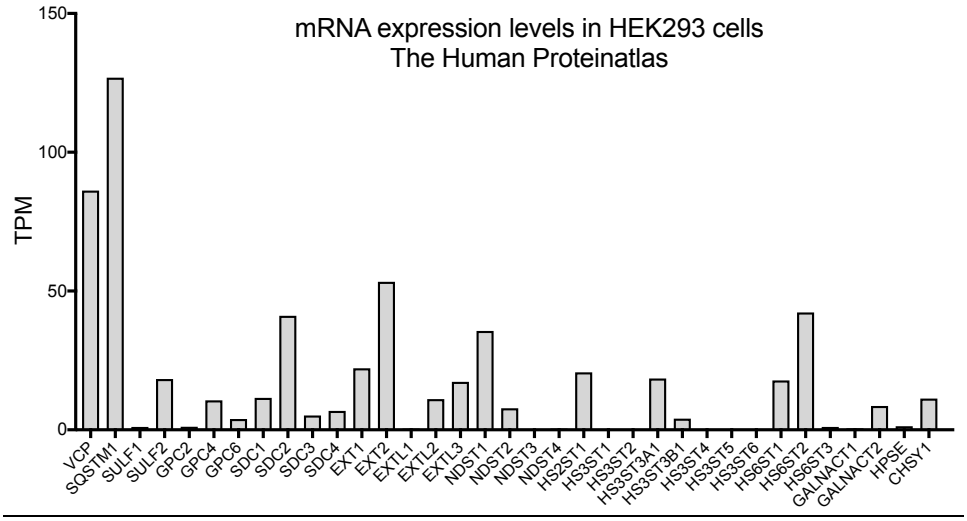

Figure S4: mRNA Expression levels in HEK293 cells of genes included in the candidate screen. The mRNA expression levels in HEK293 cells for all the genes included in the candidate screen are shown as "Transcripts per Million" (TPM). Of note, the genes of the HSPG pathway have overall lower mRNA levels compared to control genes (e.g. *VCP*, *SQSTM1*). All the genes that appeared as hits in our screen have a detectable mRNA expression level. Source: The Human Protein Atlas version 18 (www.proteinatlas.org) (4,5).

Figure S5

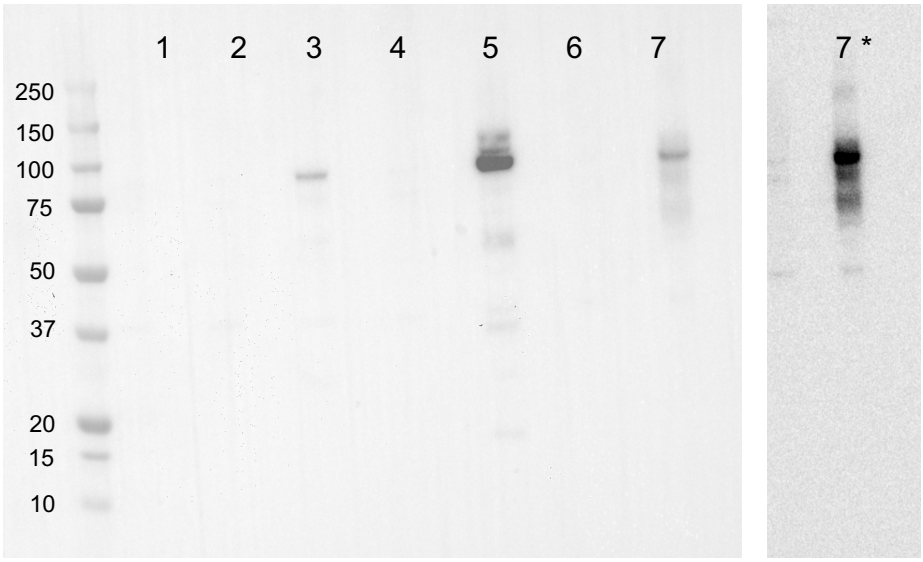

Figure S5: Western Blot of knockout and rescue cell lines. Western Blot with anti-HA antibody (1:4000) was used to confirm rescue of the genes of interest in HEK293T cells. Lane 1: scrambled control cell line, lane 2: *EXT1* knockout cell line, lane 3: *EXT1* rescue cell line, lane 4: *NDST1* knockout cell line, lane 5: *NDST1* rescue cell line, lane 6: *HS6ST2* knockout cell line, lane 7: *HS6ST2* rescue cell line, lane 7*: same blot/lane as in 7, but longer exposure to demonstrate lower bands. In the scrambled control cell line and the knockout cell lines, HA is not detectable (lane 1, 2, 4, 6). Expected protein sizes (including HA tag): EXT1 = 87.7 kDa, NDST1 = 102.4 kDa, HS6ST2 = 70.2 kDa. EXT1-HA and NDST1-HA bands are located as expected (lane 3 and 5 respectively). For HS6ST2-HA, several bands are visible between ~60 and 100 kDa when exposed longer, possibly due to protein aggregation or changes of protein properties due to the HA tag. In summary, HA tag was only detected in cells overexpressing our rescue constructs, which confirms successful expression of the cDNA. Total protein content of the cell lysate was quantified by Bradford assay and 10 µg was loaded for knockout cell lines and control cell lines. Protein standard (left side of the blot) is labeled in kDa.

References:

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