Figure EV1. Tau is detected in the secretome of DnaJCS-expressing cells only.

A, B Extracted ion chromatographs of DnaJCS-overexpressing cells compared to control within the same time and mass window. Tau peptide is only detected in the 15N recombinant standard; no endogenous tau is shown. Overlay of DnaJCS (blue) and control (red) peak intensities indicates that endogenous peptide is only detected in DnaJCS-expressing cells.
Figure EV2. DnaJC5 does not decrease mRNA levels, proteasomal turnover, or cell toxicity.

A mRNA levels of human tau, α-synuclein, and TDP-43 mRNA expression in HEK293T cells overexpressing FLAG-DnaJC5 at 24 and 48 h post-transfection. Data are mean ± SEM and are representative of three independent repeats.

B Proteasome inhibition does not block FLAG-DnaJC5-mediated tau release. Cells were treated for 6 h with the proteasome inhibitor epoxomicin. Extracellular protein levels were analyzed by dot blot (quantification below, mean ± SEM, n = 3), and intracellular proteins levels were analyzed by Western blot; DnaJC5 was detected by FLAG antibody.

C DnaJC5 overexpression does not cause cell toxicity. Cytotoxicity was analyzed using LDH, MTS, and Alamar Blue assays performed 48 h post-transfection on HEK293T cells overexpressing FLAG-DnaJC5 and tau. Data are mean ± SEM, n = 8.

D Wild-type Ataxin-3 (25Q) is not released by DnaJC5. Media dot blot from HEK293T cells overexpressing tau or ATX325Q-myc with DnaJC5 and DnaJC8 as a control.

Source data are available online for this figure.
Figure EV3. Tau levels are reduced in DnaJC5-overexpressing synapses.

A Confocal micrographs of tau (red pseudocolor) and SNAP25 (green pseudocolor) colocalization (merged image) in primary neurons overexpressing GFP-AAV9 or DnaJC5-AAV9. Only neurons overexpressing virus were imaged. Boxed areas are 5× digital zoom on a 60× lens shown below. Scale bars are 20 μm and 5 μm (insets).

B Quantification of tau–SNAP25 colocalization (mean Pearson’s coefficient ± SEM, n = 16, *P < 0.05 by one-way analysis of variance with Tukey’s multiple comparisons post hoc analysis).

C Quantification of tau intensity ± SEM, n = 16, *P < 0.05 by one-way analysis of variance with Tukey’s multiple comparisons post hoc analysis in SNAP25 colocalization experiment.

D Confocal micrographs of tau (red pseudocolor) and synaptophysin (green pseudocolor) colocalization (merged image) in primary neurons overexpressing GFP-AAV9 or DnaJC5-AAV9. Only neurons overexpressing virus were imaged. Boxed areas are 5× digital zoom on a 60× lens shown below. Scale bars are 20 μm and 5 μm (insets).

E Quantification of tau–synaptophysin colocalization (mean Pearson’s coefficient ± SEM, n = 14, **P < 0.01 by one-way analysis of variance with Tukey’s multiple comparisons post hoc analysis).

F Quantification of tau intensity ± SEM, n = 16, *P < 0.05 by one-way analysis of variance with Tukey’s multiple comparisons post hoc analysis in synaptophysin colocalization experiment.
Figure EV4. Subcellular localization of TDP-43, α-synuclein, and tau.
M17 neuroblastoma cells were transfected with TDP-43, α-synuclein, and tau (green, all panels) co-expressed with empty vector or DnaJC5 and counterstained with DAPI (blue, all panels). Images were taken with a 60× objective. At least 10 fields containing 5 or more positive cells were imaged. Scale bar represents 20 μm.
Figure EV5. Ratio of extracellular to intracellular tau levels. Alternative quantification of Fig 6D showing extracellular tau levels relative to intracellular tau levels. The ratio is shown as average ± SEM, n = 3, *P < 0.05 one-way analysis of variance with Tukey’s multiple comparison post hoc analysis.