

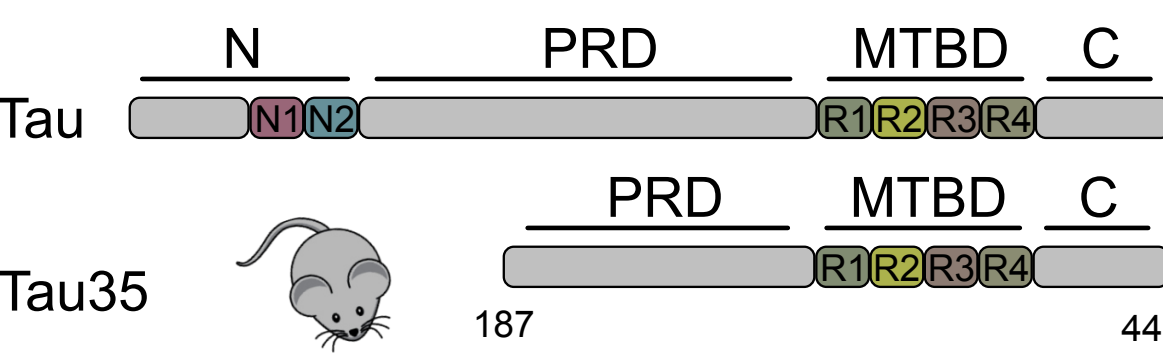
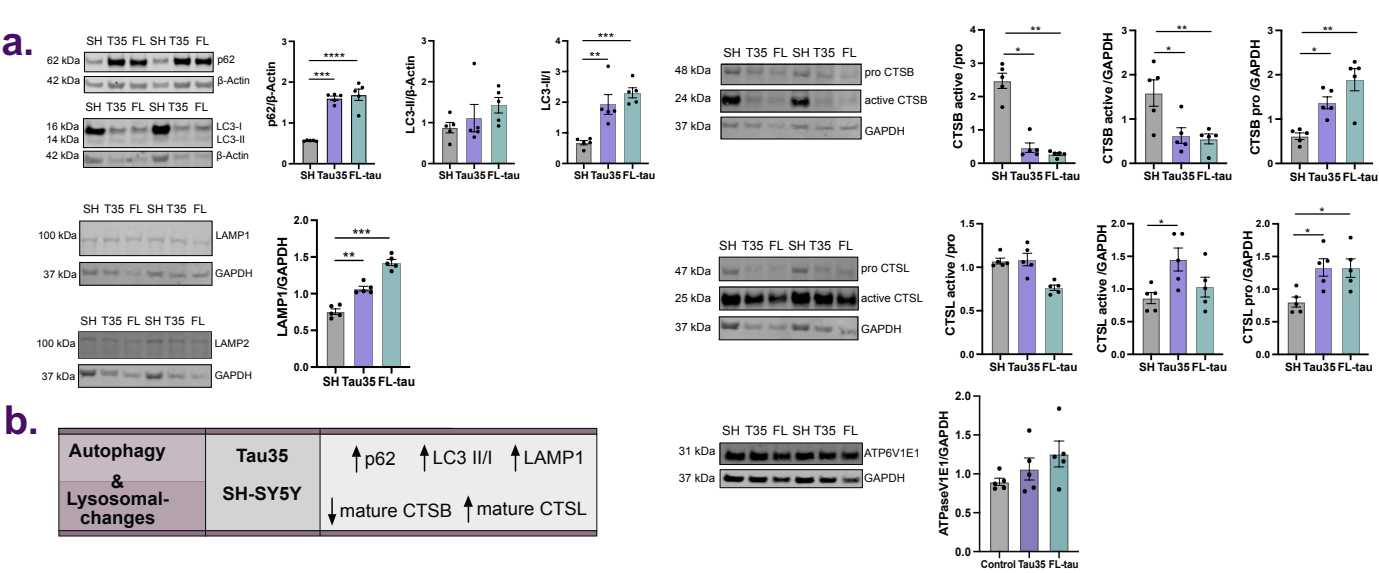
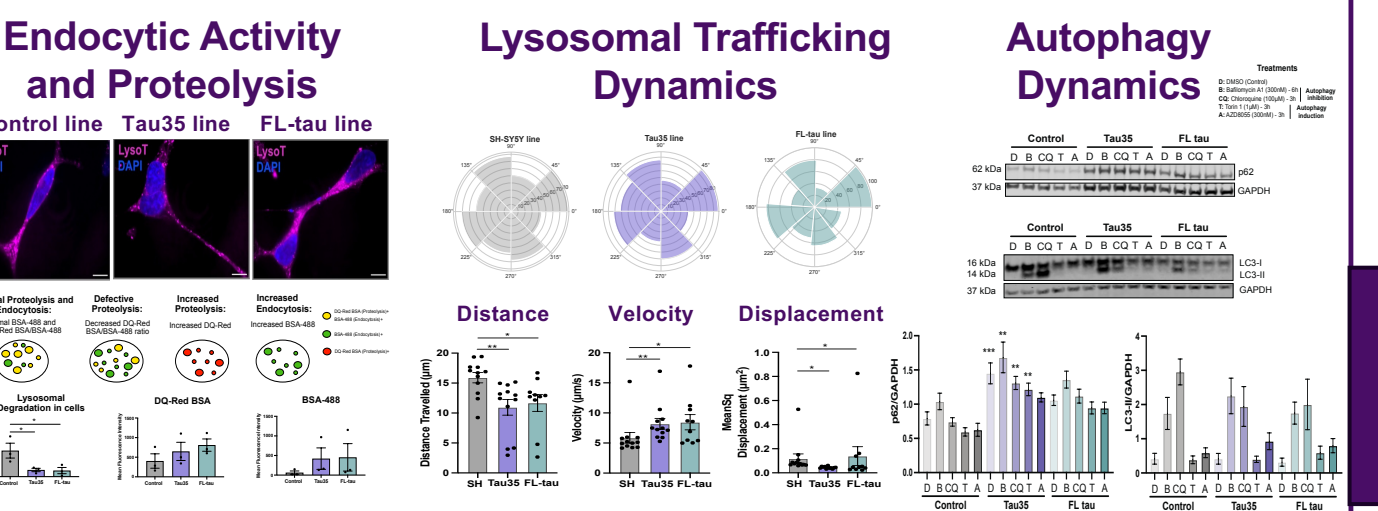
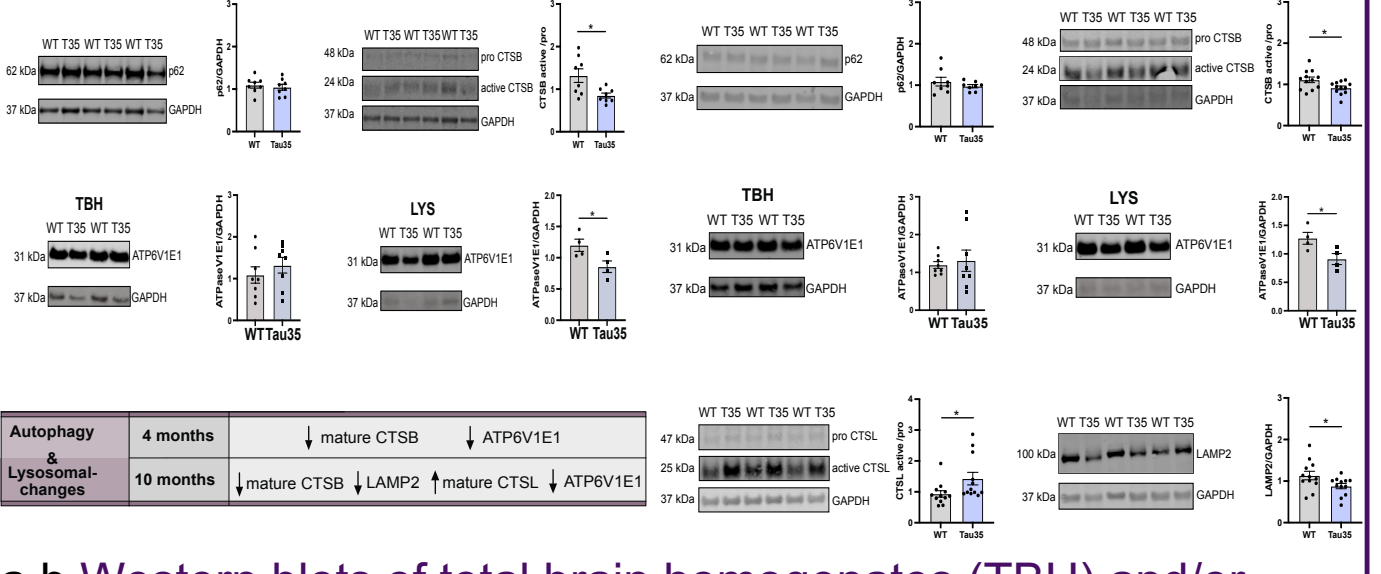
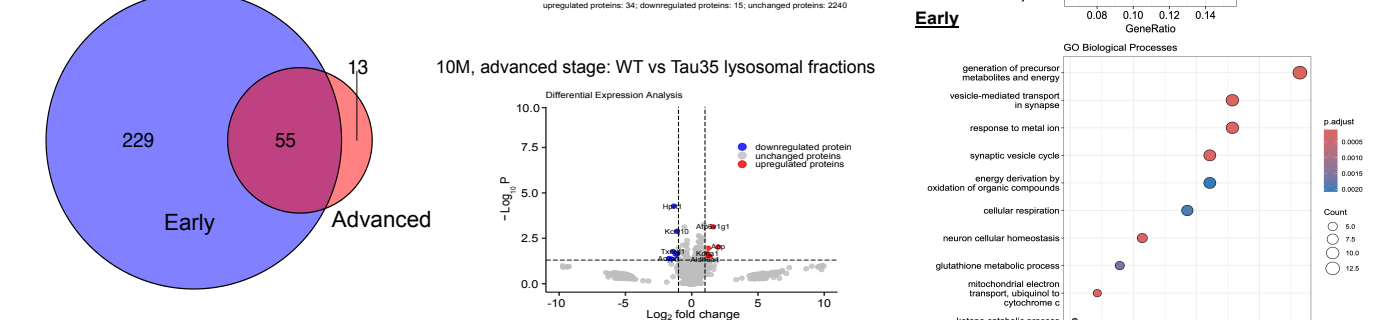
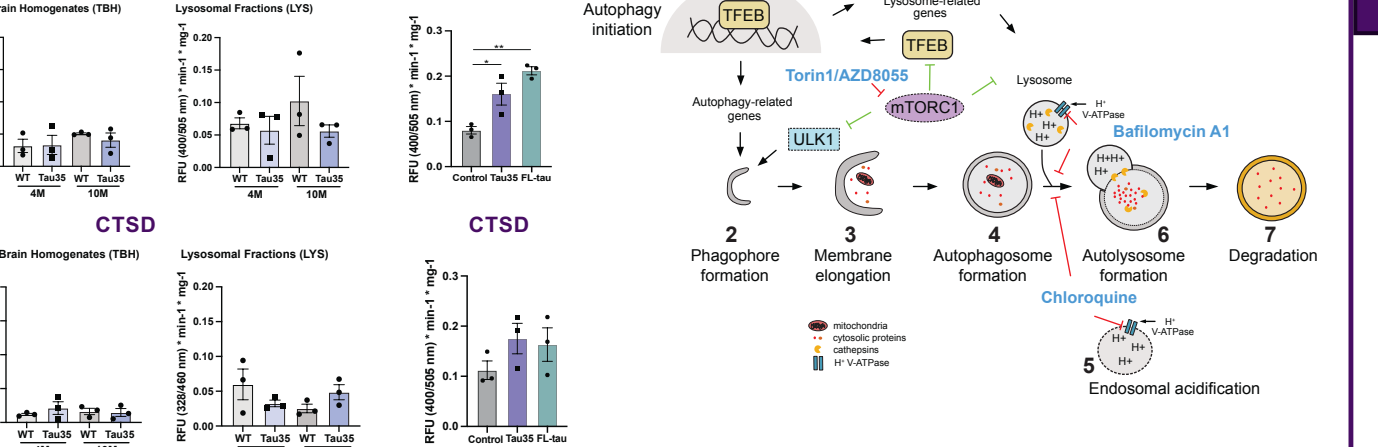
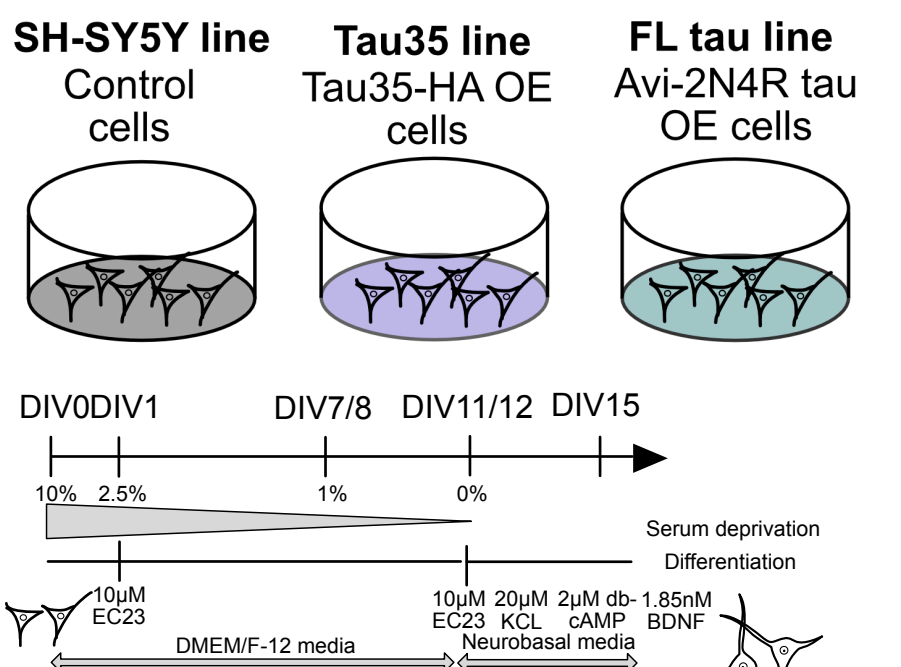
Truncated tau compromises cellular clearance: proteomic and functional insights into autophagy and lysosomal dysfunction

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Tau35 overexpression disrupts endo-lysosomal and autophagic pathways, driving early cellular dysfunction through impaired acidification, trafficking, and proteostatic resilience.

INTRODUCTION	METHODS	RESULTS	RESULTS	RESULTS
Tauopathies are marked by the accumulation of pathological tau species that interfere with intracellular clearance mechanisms, particularly the autophagy–lysosomal pathway (ALP), which is essential for neuronal protein homeostasis. Impairment of this system promotes cellular stress and neurodegeneration. Here, we examined the impact of the N-terminally truncated tau isoform Tau35 on autophagic and endo-lysosomal processes that regulate neuronal proteostasis.	Tau35 mouse  Lysosomal and autophagic alterations were evaluated by immunoblotting, quantitative proteomic analysis of lysosome-enriched brain fractions, cathepsin activity assays, endocytic uptake and proteolysis assays, and live-cell imaging with LysoTracker to quantify lysosomal distribution and motility.	II. Alterations in Tau35 and FL-tau SH-SY5Y lines Increased p62, LC3 II/I and LAMP1 expression and altered balance of active CTSB and CTSL in differentiated Tau35 cells  a, Western blots of total cell lysates from Tau35, FL-tau and control SH-SY5Y lines probed with antibodies to p62, LC3 II/I, ATP6V1E1, CTSB, CTSL, LAMP1, LAMP2, beta actin and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Quantification of the blots is shown in the graphs as mean ± SEM, n= 5 samples per group. Student's t-test, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. b, Table summarising changes in key lysosomal and autophagy markers (p62, LC3 II/I, CTSB, CTSL, LAMP1) in differentiated (14 DIV) SH-SY5Y cells.	IV. Autophagic and endo-lysosomal changes in Tau35 and FL-tau cells Excessive endocytic activity disrupts autophagic flux, lysosomal proteolytic function and trafficking dynamics  a, Super-resolution images of LysoTracker staining in control, Tau35, and FL-tau differentiated cells (14 DIV). LysoTracker Deep Red (magenta), NucBlue (blue); scale bar: 10 µm. Schematic depicts normal, defective, and altered endocytosis/proteolysis. Quantification of DQ-Red BSA/BSA-488 ratio and DQ-Red BSA and BSA-488 fluorescence intensities. Mean ± SEM; n=3 independent experiments; Student's t-test, P<0.05. b, Quantification of lysosome mobility from trajectory analysis showing distance travelled (µm), mean velocity (µm/s), and mean square displacement (µm²). Box plots; mean ± SEM; n=10–12 independent experiments; Kruskal–Wallis, P<0.05, *P<0.01. c, Basal and treatment-induced changes in p62 and LC3-II/I in control, Tau35, and FL-tau SH-SY5Y cells. Schematic summarises autophagy treatments; western blots probed for p62, LC3, and GAPDH. Quantification shown as mean ± SEM; n=5 independent experiments; two-way ANOVA, P<0.05, *P<0.01, **P<0.001.	a, Cathepsin B activity showed a non-significant reduction in Tau35 samples across early (4M) and advanced (10M) stages, most evident in 10M Tau35 LYS, while cathepsin D remained unchanged. b, In cell lysates, cathepsin B activity was significantly increased in Tau35 and FL-tau versus controls, whereas cathepsin D showed a non-significant upward trend. Data shown as mean ± SEM; n=3 independent experiments; Student's t-test, P<0.05, *P<0.01. c, Schematic overview of endo-lysosomal regulation and pharmacological modulation during autophagy.
METHODS	RESULTS	I. Alterations in Tau35 mouse brain Reduced LAMP2 and ATP6V1E1 expression and altered balance of active CTSB and CTSL in Tau35 mice  a,b Western blots of total brain homogenates (TBH) and/or lysosomal fractions (LYS) from Tau35 and control brains probed with antibodies to p62, ATP6V1E1, CTSB, CTSL, LAMP2 and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Quantification of the blots is shown in the graphs as mean ± SEM, n= 4-12 brains per group. Student's t-test, *P < 0.05. c, Table summarising changes in key lysosomal and autophagy markers (ATP6V1E1, CTSB, CTSL, LAMP2) at early (4M) and advanced (10M) disease stages in Tau35 mice.	III. Proteomic changes in Tau35 mouse brain Tau35 expression alters endo-lysosomal pathways involved in mitochondria and energy metabolism dynamics  a, Summary table of differentially expressed proteins (discovery analysis: p-cutoff: P>0.05, fc-cutoff: fc>0.5) in early and advanced cohorts. Venn diagram shows lysosomal protein alterations. A total of 55 proteins were significantly altered across all datasets. b, Volcano plots display log-fold changes (Log2FC) on the x-axis, and –log10 transformed p-values, adjusted for multiple testing using the Benjamini-Hochberg procedure on the y-axis. c, GO enrichment analysis of differentially expressed proteins highlighting key autophagy-lysosomal pathway (ALP) categories across early and advanced disease stages.	V. Cathepsin activity changes in Tau35 expressing systems Tau35 alters cathepsin activity and autophagy dynamics  a. Mouse Brain b. SH-SY5Y lines c. Endo-lysosomal drug effects
The effects of Tau35 on cellular degradation pathways were assessed using a Tau35 transgenic mouse model and SH-SY5Y cells stably expressing either Tau35 or full-length tau.	Tau35/FL-tau SH-SY5Y lines  SH-SY5Y line Control cells Tau35 line Tau35-HA OE cells FL tau line Avi-2N4R tau OE cells DIV0DIV1 DIV7/8 DIV11/12 DIV15 10µM EC23 10µM EC23 20µM KCL 2µM cAMP 10µM BDNF Neurobasal media DMEMF-12 media Serum deprivation			
				CONCLUSIONS - Proteomic analysis of lysosomal fractions identifies Tau35-specific protein dysregulation associated with disease pathology. - Parallel studies in Tau35 mice and Tau35/FL-tau SH-SY5Y cells reveal convergent cellular dysfunction, including increased endocytosis, impaired proteolysis, altered autophagic flux, and disrupted lysosomal motility. - These findings indicate that early endo-lysosomal dysfunction may drive pathogenic protein accumulation and cellular instability in tauopathies, supporting shared pathogenic mechanisms with lysosomal storage disorders.
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