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Tau-induced deficits in nonsense-mediated mRNA decay contribute to neurodegeneration

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Abstract

While brains of patients with Alzheimer's disease and related tauopathies have evidence of altered RNA processing, we lack mechanistic understanding of how altered RNA processing arises in these disorders and if such changes are causally linked to neurodegeneration. Using *Drosophila melanogaster* models of tauopathy, we find that overall activity of nonsense-mediated mRNA decay (NMD), a key RNA quality control mechanism, is reduced. Genetic manipulation of NMD machinery significantly modifies tau-induced neurotoxicity, suggesting that deficits in NMD are causally linked to neurodegeneration. Mechanistically, we find that deficits in NMD are a consequence of aberrant RNA export and RNA accumulation within nuclear envelope invaginations in tauopathy. We identify a pharmacological activator of NMD that suppresses neurodegeneration in tau transgenic *Drosophila*, indicating that tau-induced deficits in RNA quality control are druggable. Our studies suggest that NMD activators should be explored for their potential therapeutic value to patients with tauopathies.

Keywords

Tauopathy; Alzheimer's disease; nonsense-mediated RNA decay; neurodegeneration; *Drosophila* ; nucleus

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CONTRIBUTIONS

GZ and BF developed the conceptual framework of the study. Experiments were performed by GZ, JDM, MG, SL and PR. GZ analyzed data and prepared the manuscript. BF contributed to data analysis and manuscript preparation.

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1 | NARRATIVE

1.1 | Contextual Background

There are over 20 neurodegenerative disorders known collectively as "tauopathies," which are pathologically defined by characteristic inclusions of the microtubule-associated protein tau (MAPT) in brains of affected individuals. Mutations in the *MAPT* gene cause autosomal dominant forms of frontotemporal dementia, collectively known as frontotemporal lobar dementia (FTLD)-tau with *MAPT* mutation[1–4]. While most tauopathies, including Alzheimer's disease, feature deposition of wild-type tau protein, this genetic link between *MAPT* mutation and neurodegeneration clearly demonstrate that tau dysfunction is sufficient to drive neurodegeneration. Despite lack of *MAPT* mutation in sporadic tauopathies, animal models featuring transgenic expression of human tau harboring disease-associated mutations have been quite valuable for identifying disease mechanisms that are conserved in Alzheimer's disease. In *Drosophila*, for example, transgenic expression of human wild-type tau or *MAPT* mutations drive neurodegeneration through conserved mechanisms including but not limited to actin-overstabilization[5,6], heterochromatin decondensation[7], disruption in nuclear architecture[8], and deficits in autophagy[6]. Importantly, these features of *Drosophila* models of tauopathy are also present in brains of patients with Alzheimer's disease[7–11]. Importantly, animal models of tauopathy allow us to determine whether such observations in human brain are causally linked to neurodegeneration. Identifying novel cellular mediators of tau-induced neurodegeneration is essential for the development of disease-modifying, mechanism-based therapies that will improve outcomes for patients who, to date, are treated symptomatically with inconsistent outcomes.

The cellular nucleus is a membrane-bound organelle that encapsulates the genome and houses the transcriptional machinery that activates or silences gene expression. When a gene is activated, transcriptional machinery home to the gene and generate a pre-messenger RNA transcript. Within the nucleus, pre-messenger RNAs are processed by A) Splicing, a process whereby introns are removed and exons are retained within the transcript, B) Polyadenylation, in which a string of adenosines are added to the 3' end of the transcript, and C) 5' capping of the transcript with a guanine. After processing, mature messenger RNA (mRNA) transcripts are exported from the cell nucleus through nuclear pores into the cytoplasm where they are translated by ribosomes into protein.

While the above described "DNA makes RNA makes protein" is the central dogma of biology, not all protein-coding RNA transcripts are translated into functional proteins. Errors in transcription and RNA processing are common in healthy cells[12,13]. Cells have multiple strategies that dictate which RNAs are translated into protein, including quality control mechanisms that surveil the transcriptome and degrade or stop the translation of error-containing, "faulty" RNAs. While expression of a single faulty RNA is most often inconsequential, failure to clear faulty RNAs generated from error-prone transcription of thousands of genes can have catastrophic consequences for organismal fitness. For example, RNA transcripts harboring nonsense errors that generate a premature termination codon (PTC) contribute to about one third of inherited genetic disorders[14]. NMD is an evolutionarily conserved, translation-dependent RNA surveillance mechanism

that recognizes and degrades PTC- and other error-containing transcripts as well as many naturally-occurring, error-free transcripts. The ability of NMD to also target 'normal' mRNA transcripts is important for a wide range of physiological pathways including differentiation and proliferation[15,16], development[17–20], viral defense[21,22], stress response[23–25], and neuronal activity (e.g. synaptic plasticity and axonal guidance)[26–28]. NMD machinery consists of several core factors that were first discovered in yeast as the up-frameshift-1 (*Upf1*), *Upf2*, and *Upf3* genes[29]. Four additional conserved NMD factors were subsequently discovered in *C. elegans*: suppressor of morphological defects on genitalia 1 (*Smg1*), *Smg5*, *Smg6*, and *Smg7*[30]. Knockdown of NMD core factors Upf1[19], Upf2[31], Smg1[32,33] or Smg6[34] are lethal in various model systems, indicating that proper function of NMD factors is essential for survival.

Studies in *C. elegans* report that neurons, unlike other cell types, retain NMD activity throughout aging[35]. Mutations in genes encoding NMD core factors Upf3B[36–38], Upf3A[39], Upf2[40] and Smg6[39] are associated with a wide clinical spectrum of intellectual disability in humans[39], suggesting that proper clearance of RNAs by NMD is critical for brain health. Why might neurons require more persistent NMD throughout aging than other cell types? Unlike skin cells that can be replaced if lost or damaged, mature neurons are postmitotic, meaning they do not divide and thereby cannot 'dilute' toxic RNA during mitosis. In addition, neurons are morphologically complex and have a higher level of alternative splicing than other cell types in the body, leading to more diversity within the transcriptome and proteome[41]. Given that approximately one third of alternative transcripts harbor PTCs[42], a significant portion of alternatively spliced transcripts are cleared via NMD.

Clearance of RNAs via NMD occurs at the cytoplasmic face of nuclear pores during or soon after mRNA export[43–45], suggesting that there is a spatially-defined checkpoint beyond which RNAs could evade NMD. The speed at which an RNA is exported from the nucleus is largely dependent on its proximity to nuclear pores that are embedded within the nuclear envelope[46]. Unlike the generally spherical shape of nuclei in healthy cells, nuclei of aged cells[47], laminopathies[48–50], various cancers[51–53], and neurons from patients with Alzheimer's disease[54] have morphological changes including lobulations and invaginations of the nuclear membrane into the nuclear interior. Analyses of post-mortem human Alzheimer's disease brain tissue reveal that nuclear envelope invaginations are lined with nuclear pore complexes[54], indicating that both the inner and outer nuclear membranes invaginate and that nuclear envelope invaginations contain a core of cytoplasm. Nuclear envelope invaginations are also present in *Drosophila*[54] and mouse[55] models of tauopathy as well as in induced pluripotent stem cell-derived neurons from patients carrying *MAPT* mutations[56], suggesting that nuclear envelope invaginations observed in human Alzheimer's disease brain are a consequence of pathological forms of tau. Genetic approaches to repair nuclear architecture significantly suppress neurodegeneration in brains of tau transgenic *Drosophila*, indicating that tau-induced disruption of nuclear morphology is a causal factor driving neuronal death[54,57]. Polyadenylated (polyA) RNA accumulates within nuclear envelope invaginations in neurons of tau transgenic *Drosophila*, and genetic or pharmacologic reduction of RNA export reduces the burden of RNA within nuclear envelope invaginations and suppresses tau-induced neurodegeneration[58]. In the current study, we utilize tau transgenic *Drosophila* to test the overall hypothesis that accumulation

of RNA within nuclear envelope invaginations limits the extent to which faulty RNAs are cleared from the cell.

1.2 | Study design and main results

Using *Drosophila* models of tauopathy, we find that transgenic expression of disease-associated mutant human tau (tau^{R406W}) and wild-type human tau (tau^{WT})[59] cause an overall deficit in NMD, and that NMD-sensitive transcripts that evade clearance can be translated into protein. We find that NMD targets accumulate within tau-induced nuclear envelope invaginations in brains of tau transgenic *Drosophila*. Experimentally activating NMD decreases accumulation of RNAs within nuclear envelope invaginations and suppresses neurotoxicity in tau transgenic *Drosophila*, suggesting that tau-induced deficits in NMD are causally associated with neurodegeneration. Mechanistically, we find that genetically decreasing RNA export reduces levels of transcripts that are normally cleared by NMD, raising the possibility that tau-induced increases in RNA export overwhelm the NMD machinery.

Recent work reports that tranilast (N-[3',4'-dimethoxycinnamoyl]-anthranilic acid), an analog of a tryptophan metabolite approved for the treatment of bronchial asthma in Japan and South Korea[60], is an effective activator of NMD in *Drosophila*[61]. We find that a ten-day treatment of tau transgenic *Drosophila* with tranilast activates NMD and suppresses tau-induced neurodegeneration and locomotor dysfunction, indicating that tau-induced deficits in NMD are druggable. Overall, our data provide new insights into how tau drives neuronal death and identify RNA quality control as a target for further investigation in the context of Alzheimer's disease and related tauopathies.

1.3 | Study conclusions, translational relevance, and therapeutic opportunities

Cellular homeostasis relies on genes being expressed at the right time, in the right place, at the right amount. Clearance of error-containing or faulty transcripts by NMD is integral to ensuring faithful expression of the genome at the protein level. Alterations in RNA processing and transport can lead to an abundance of error-containing transcripts that are likely to be degraded by NMD[62,63]. Elevation of such error-containing transcripts are observed in tauopathy[64] and as a consequence of physiological aging in *C. elegans*[35], suggesting that faulty RNA is either produced in higher quantities and/or that faulty RNA is not properly cleared from the cell in the context of tauopathy and aging. In tau transgenic mice and post-mortem human Alzheimer's disease brain tissue, pathogenic forms of tau are reported to disrupt proper nucleocytoplasmic transport of RNA and protein[55,57]. Studies in a *Drosophila* model of tauopathy indicate that tau-induced increase in RNA export is a causal factor driving neurodegeneration and that RNAs accumulate within tau-induced invaginations of the nuclear envelope[58]. In the current study, we identify deficits in RNA quality control as a novel mechanism connecting aberrant RNA export to nuclear envelope invagination and neurodegeneration in tau transgenic *Drosophila*.

Overall, we find that mutant and wild-type forms of human tau limit NMD in the *Drosophila* brain, that genetic manipulation of NMD modifies tau-induced neuronal death, and that tau-induced deficits in NMD are amenable to pharmacological intervention. Our finding

that overexpression of the NMD core factor Upf1 is neuroprotective in tauopathy is similar to reports in yeast[65], rodent[66,67], fly and human[61,68] models of ALS-FTD, suggesting an overlapping pathophysiology of tauopathies and ALS, a motor neuron disease involving RNA dysregulation. Unlike our study, however, recent work in human cells harboring *C9orf72* expansion[68], ALS-causing mutations in *FUS*[69], and *C9orf72* motor neurons cultured from ALS patients report an increase in NMD activity in the disease context. Our studies point toward an alternative mechanism in tauopathy in which RNAs escape clearance via NMD as a consequence of increased RNA export and accumulation within nuclear envelope invaginations.

We[58] and others[68,69] detect RNA and NMD machinery within nuclear envelope invaginations. While these data suggest that RNA surveillance can occur within nuclear invaginations, we find that most RNA-enriched invaginations lack Upf1 in brains of tau transgenic *Drosophila*. As Upf1 is required for NMD in *Drosophila*[18,70], these data suggest that a large majority of RNAs within invaginations are not subject to RNA surveillance via NMD in the context of tauopathy. We further find that genetic reduction of RNA export significantly reduces transcript levels of NMD targets in tau transgenic *Drosophila*. We speculate that existing NMD machinery is saturated in tauopathy due to increased RNA export, and that genetically elevating Upf1 suppresses tau neurotoxicity by increasing the pool of Upf1 that is available to clear RNA. Based on our studies to date, we favor a simple model in which RNA is exported through nuclear pores directly into tau-induced nuclear envelope invaginations, where it is shielded from cellular RNA surveillance mechanisms and is translated into faulty protein. To directly test this model, live cell imaging will be necessary to clarify the export path of RNAs, to quantify the longevity of NMD-sensitive RNAs that are exported into nuclear invaginations in tauopathy, and to monitor the location and degree to which faulty RNA transcripts are translated into protein.

Many cellular phenotypes of *Drosophila* tauopathy models are conserved in human Alzheimer's disease, including but not limited to oxidative stress[71], DNA damage[72], actin overstabilization[5], nuclear pleomorphisms[54], epigenetic changes[7], transposable element activation[73,74], aberrant activation of the cell cycle in postmitotic neurons[75], and progressive neurodegeneration. There are clear limitations, however, of using *Drosophila* to model human neurological disorders. *Drosophila* neurons are smaller and have less processes extending from the cell body than their vertebrate counterparts[76,77], lack axon-specific neurofilaments[78], and represent the majority of cells in the central nervous system[79]. Glia are ten times more abundant than neurons in the human brain, suggesting that an increased glia:neuron ratio represents greater brain complexity. Vertebrate-specific neuronal features may explain why large aggregates of misfolded tau protein form neurofibrillary tangles in brains of Alzheimer's disease patients but not in *Drosophila* models of tauopathy[80]. Abnormally phosphorylated tau and disease-associated conformations of tau do, however, accumulate with age in brains of tau transgenic *Drosophila* similar to early stages of human Alzheimer's disease[80,81].

Based on our reductionist approach that leverages simple *Drosophila* models of tauopathy in which transgenic human tau is expressed in all neurons of the adult fly brain, we speculate that cells harboring pathogenic forms of tau in Alzheimer's disease and related tauopathies

would exhibit a deficit in NMD. Ideally, one would sort pathogenic tau-containing neurons from patient brain samples and perform long-read RNA sequencing to quantify the burden of NMD targets in pathogenic tau-containing neurons versus those that lack pathogenic forms of tau. Similarly, one could analyze NMD targets in specific circuits of the human brain that are particularly affected in tauopathies. These experiments will, however, be costly and will require large amounts of brain tissue. In the absence of such studies, two recent analyses provide some clue that NMD may be functioning at a deficit in human Alzheimer's disease. Both studies report increased intron retention in transcriptomes from patients with Alzheimer's disease[82,83]. *Drosophila* models of tauopathy also exhibit intron retention[82], suggesting that human Alzheimer's disease-associated intron retention is a consequence of pathogenic forms of tau. Intron retention is known to trigger NMD, as introns often introduce a PTC into a mature mRNA[84,85]. While no analyses of human Alzheimer's disease have specifically focused on NMD, an increase in transcripts with intron retention in brains of patients with human Alzheimer's disease brains is consistent with deficits in NMD.

Aged *Drosophila* have significantly altered splicing patterns, including increased intron retention of genes, some of which are associated with Alzheimer's disease[82]. Since intron retention may trigger NMD, these data suggest a potential link between aberrant splicing, NMD, and age-related diseases like Alzheimer's disease. Analysis of gene expression profiles in cells lacking core NMD factors indicate that NMD targets about 10% of the transcriptome in *Drosophila*[18] and humans[86], suggesting that while the select targets may differ, the biological impact of deficits in NMD is shared between the two species. The steady-state level of RNA depends on the ratio between synthesis and degradation, making the use of steady-state RNA levels to infer NMD activity intrinsically limited. These limitations can be overcome by methods more amenable to cell culture that compare a PTC-containing, presumably NMD-targeted transcript relative to the errorless, NMD-insensitive transcript[87,88]. Our studies in *Drosophila* models of tauopathy should thus be validated in other model systems and critically and cautiously applied to human Alzheimer's disease and related tauopathies.

Overall, our data identifies tau-induced deficits in NMD as a pharmacologically targetable driver of neurodegeneration that occurs early in the disease process. We find that tranilast, a well-tolerated and low toxicity drug that penetrates the blood brain barrier[60], activates NMD and suppresses tau-induced neurotoxicity in *Drosophila*. The mechanism of tranilast-induced NMD activation is unclear and additional research on its action in the brain is required to assess the potential of tranilast as a tau therapy. Based on our findings that deficits in NMD activity occur prior to neurodegeneration in tau transgenic *Drosophila*, it is possible that NMD activators have disease-modifying potential during the early asymptomatic stages of Alzheimer's disease and related tauopathies. Our experiments are thus designed to identify and target early events, and to focus on mechanisms in which genetic manipulation modifies tau-induced neuronal death. While compounds that activate NMD would also be attractive for some cancer types[89–92], there are no publicly available NMD-activating compounds. A previous screen for NMD inhibitors happened to also identify 14 candidate compounds that enhance NMD activity in human cells[87], among which four were further studied in an ALS-FTD model. Only tranilast was validated as an NMD activator *in vivo*[61]. To our knowledge, there are no other studies testing NMD activators in the context of neurodegeneration.

A major challenge to the development of NMD activators is knowing which NMD machinery to target. The mechanisms by which Upf1 is phosphorylated[93,94], PTCs are recognized[95,96], and mRNA is degraded[97,98] vary across species. In fact, the efficiency of NMD is tissue-specific[35,99] and highly variable from cell-to-cell[100] and between individuals[38,101]. The search for small molecules that increase NMD activity will require more rigorous testing of the current model in which aberrant translation termination is thought to be the primary activation signal for NMD. Genetic modifier screening and small molecule screening using cell-based reporters of NMD activity may identify additional targets and approaches for further therapeutic development. The data described herein suggest that small molecules that specifically activate NMD in the brain, or gene therapy designed to modulate expression of NMD core factors in neurons are plausible NMD activation strategies for tauopathies. While our findings require further testing in vertebrate models, our study suggest that patients with Alzheimer's disease and related tauopathies could potentially benefit from therapy based on NMD activation.

2 | CONSOLIDATED RESULTS AND STUDY DESIGN

We detect deficits in NMD in brains of adult tau transgenic *Drosophila* using two complementary approaches. We find that panneuronal expression of human tau^{R406W} and tau^{WT} in the *Drosophila* brain disrupts the ability of NMD to clear a NMD-sensitive fluorescent reporter[102] and RNA transcripts of endogenous NMD targets *Gadd45*[61,103,104] and *Arc1*[61]. As phenotypes of tau^{WT} transgenic *Drosophila* are delayed compared to tau^{R406W} transgenic *Drosophila*, and tau^{R406W} transgenic *Drosophila* feature a moderate degree of neurodegeneration at 10 days of age that is convenient for genetic analyses[59], we used this *MAPT*-mutation based model for subsequent mechanistic inquiry into the cause and consequence of blunted NMD in tauopathy. We find that NMD activity is at a deficit in tau^{R406W} transgenic *Drosophila* compared to age-matched controls prior to overt neurodegeneration.

To determine if NMD deficits causally mediate tau-induced neurodegeneration in *Drosophila*, we next asked if genetic manipulation of NMD core factors alters the degree of neurotoxicity in brains of tau^{R406W} transgenic *Drosophila*. We quantified tau^{R406W}-induced neurodegeneration in response to genetic manipulation of NMD core factors Upf1, Upf2, Upf3 and Smg5 using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), which detects DNA fragmentation associated with apoptosis[7,75,80]. Panneuronal RNAi-mediated depletion of Upf1, Upf3 and Smg5 and expression of a dominant-negative form of Upf2 significantly enhance tau^{R406W}-induced neurodegeneration in *Drosophila*, while panneuronal overexpression of wild-type Upf1 and Upf2 significantly suppress tau^{R406W}-induced neurodegeneration.

Having previously reported that polyA RNA accumulates within nuclear envelope invaginations in tauopathy[58], we next asked if faulty RNAs also accumulate within invaginations. We detect protein encoded by a reporter transcript that is normally cleared by NMD within nuclear invaginations of tau^{R406W} transgenic *Drosophila*. This finding suggests that NMD-sensitive targets are present within nuclear envelope invaginations in brains of tau^{R406W} transgenic *Drosophila*, and that such NMD-sensitive transcripts

are effectively translated into protein within invaginations. We hypothesized that RNA within nuclear invaginations may not have access to NMD machinery. We visualized polyA RNA, Upf1, and the lamin nucleoskeleton using fluorescence *in situ* hybridization combined with immunofluorescence (FISH-IF). A quantitative evaluation revealed that Upf1 is largely excluded from RNA-enriched nuclear envelope invaginations in brains of tau^{R406W} transgenic *Drosophila*, suggesting that Upf1 is physically restricted from RNA within these invaginations, that faulty RNA is in excess to endogenous Upf1, or that Upf1 is recruited to some but not all RNAs within invaginations.

To determine if overexpression of Upf1 prevents neurodegeneration by enhancing the ability of NMD to clear RNA transcripts, in particular RNA within nuclear invaginations, we quantified the fraction of nuclear envelope invaginations that harbor RNA in brains of tau^{R406W} transgenic *Drosophila* with and without panneuronal overexpression of Upf1. We find that genetic activation of NMD significantly decreases the incidence of RNA-containing invaginations, as predicted. These results suggest that RNAs within nuclear invaginations are subject to degradation by NMD, and that activation of NMD may be an effective strategy to decrease neuronal loss in tauopathy.

Given that many of the clinical trial failures for Alzheimer's disease and related tauopathies stem from an inadequate understanding of the mechanisms driving neurodegeneration, we took additional steps to examine a potential mechanism underlying tau-induced deficits in NMD. Having previously reported that reducing RNA export clears RNA from nuclear envelope invaginations and suppresses tau-induced neurotoxicity[58], we hypothesized that tau-mediated increase in RNA export overwhelms the ability of NMD machinery to clear target RNA transcripts. We find that panneuronal RNAi-mediated reduction of nuclear export factors sbr and Nxt1, *Drosophila* homologues of human nuclear transport factor 2 like export factor 1 (*NXT1*) and nuclear RNA export factor 1 (*NXF1*), significantly reduces protein levels of the NMD-sensitive fluorescent reporter in *Drosophila* and reduces transcript levels of *Gadd45* and *Arc1* back to control levels in tau^{R406W} transgenic *Drosophila*. These findings suggest that an increase in nuclear RNA export limits NMD in tauopathy.

Equally important for future drug development efforts is to determine if tau-induced deficits in NMD are pharmaceutically targetable. Tranilast is an anti-inflammatory drug and NMD activator in *Drosophila*[61]. We find that tau^{R406W} transgenic *Drosophila* fed tranilast have greater NMD activity and significantly reduced neurodegeneration and locomotor deficits. Taken together, our findings reveal a new, non-canonical function of tau and highlight tau-induced deficits in NMD as a pharmacologically targetable, mechanistic driver of neurodegeneration with therapeutic potential for Alzheimer's disease and related tauopathies.

3 | DETAILED METHODS AND RESULTS

3.1.1 | *Drosophila* genetics

Drosophila melanogaster crosses and aging were performed at 25°C with a 12 hour light/dark cycle on standard fly food (Bloomington formulation) or fly food containing drug (as described below). Aging flies were transferred to fresh food every other day. In all

experiments, expression of transgenes and RNAi-mediated knockdown in *Drosophila* was achieved using the panneuronal *elav* promoter and the GAL4-*UAS* system. *Drosophila* lines were obtained from Bloomington *Drosophila* Stock Center. *Drosophila* RNAi Screening Center (DRSC) - Transgenic RNAi Project (TRiP)[105] and Vienna *Drosophila* Resource Center (VDRC, www.vdrc.at)[106] supplied transgenic RNAi fly lines (Supplemental Table 1). UAS-tau^{R406W} and UAS-tau^{WT} flies were provided by Dr. Mel Feany[54] and *Drosophila* expressing the NMD fluorescent reporter were provided by Dr. Mark Metzstein[102]. All experiments used an equal number of male and female flies unless otherwise noted.

3.1.2 | Fluorescence *in situ* hybridization with immunofluorescence

For fluorescence *in situ* hybridization combined with immunofluorescence, *Drosophila* brains were dissected in 1X PBS and fixed in 4% PFA for 30 minutes. After washing once in PBS plus 0.3% Triton, samples were incubated with 1 ng/μL 5'3'-DIG labelled probe in pre-heated hybridization buffer for at least three hours at 37°C. Samples were washed in 2X SSC and blocked in PBS plus 2% milk and 0.3% Triton for at least 30 mins. Primary antibodies (Supplemental Table 2) were added to samples for overnight incubation at room temperature. After washing, secondary antibodies donkey anti-mouse Alexa Fluor 555, anti-rabbit Alexa Fluor 488, or anti-goat Alexa Fluor 647 (1:200, Thermo Fisher Scientific) were incubated at room temperature for two hours before mounting samples with Fluoromount G with DAPI (Southern Biotech). The number of nuclear invaginations containing polyA RNA were scored as previously reported[54,58]. RNA was observed as a focal increase contained completely within a nuclear envelope invagination as imaged with lamin staining. All comparisons were made between samples processed at the same time.

For Upf1 and lamin immunofluorescence, we used *Drosophila* heads fixed in formalin, embedded in paraffin and serially sectioned at 4 μm through the entire brain. Slides were subjected to antigen retrieval in sodium citrate for 20 minutes. After washing, brains were blocked with 2% milk in 0.3% Triton in PBS and incubated with a primary antibody overnight in a humidifier chamber at room temperature. Slides were washed again prior to incubation with Alexa Fluor-conjugated secondary antibodies for two hours at room temperature. *Drosophila* brains were visualized on a Zeiss confocal microscope using Airyscanning technology to image nuclei in a single slice with high resolution.

To quantify TUNEL-positive neurons in *Drosophila* brains, we used a commercially available kit for TUNEL staining (Calbiochem, TdT FragEL) on formalin-fixed, paraffin embedded *Drosophila* heads sectioned at 4 μm. Following the kit protocol, DAB was used for secondary detection of biotin-labelled deoxynucleotides at exposed ends of DNA fragments. Brightfield microscopy was used to quantify TUNEL-positive neurons throughout the entire fly brain.

3.1.3 | Immunoblotting

One male and one female *Drosophila* head were homogenized in 2X Laemmelli buffer (Invitrogen) and boiled for 10 minutes. Samples were analyzed by 10% SDS-PAGE and transferred to nitrocellulose membranes in transfer buffer with methanol. Ponceau S was added to membranes to assess equal loading before blocking membranes for 10–30 mins

with 2% milk in PBS plus 0.05% Tween. Membranes were incubated overnight at 4°C with primary antibody. After washing with 1X PBS plus 0.05% Tween, membranes were incubated with HRP-conjugated secondary antibodies for two hours at room temperature and developed with an enhanced chemiluminescent substrate. Blots were visualized with a FluorChem HD2 imager (ProteinSimple) and the relative density of each band was quantified in ImageJ.

3.1.4 | Drug treatments

Tranilast (Sigma-Aldrich, T0318) was dissolved in DMSO and 1 μL was added to 1 mL fly food to a final concentration of 10μM. *Drosophila* were transferred to vials containing Tranilast- or DMSO-treated food at day one of adulthood and flipped into fresh drug or vehicle treated food every other day. At day ten of adulthood, flies were frozen or fixed for subsequent analyses.

3.1.5 | Locomotor Assay

Drosophila fed tranilast or vehicle (DMSO) for ten or twenty days were individually transferred to drug-treated food at least 24 hours prior to measuring walking activity, as described previously[7].

3.1.6 | NMD reporter assays

Drosophila harboring *UAS-nlsDsRed2::SV40 3′UTR* on the X chromosome (NMD reporter) were balanced with FM7. Virgins of genotype *UAS-nlsDsRed2::SV40 3′UTR/FM7* were mated to *elav-Gal4*/+ or *elav-Gal4*;+/+;Tau^{R406W}/+ males. Female progeny of genotype *elav-Gal4/UAS-nlsDsRed2::SV40 3′UTR*;+/+;Tau^{R406W}/+, *elav-Gal4/UAS-nlsDsRed2::SV40 3′UTR*;+/+;Tau^{WT}/+ and *elav-Gal4/UAS-nlsDsRed2::SV40 3′UTR*;+/+;+/+ were aged to ten or twenty days post-eclosion and frozen at −80°C prior to digital PCR (dPCR) or ELISA assays.

Since experiments utilizing the NMD reporter require two genetic elements (NMD reporter and *elav-Gal4*) on the X chromosome, only female flies were used for analysis. For each biological sample, five frozen *Drosophila* heads were homogenized in 15 μL RIPA buffer with protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific, 78440), diluted in 185 μL Assay Diluent, and 200 μL of each sample added to the anti-RFP antibody-coated plate in duplicate for quantification of RFP according to the RFP ELISA protocol (Cell BioLabs, AKR-122). Absorbance for each well was measured 5 mins after stopping the enzyme reaction on a GloMax Discover Plate Reader (Promega) using 450 nm as the primary wavelength.

3.1.7 | Digital PCR

Absolute quantification of *Gadd45, Arc1*, and NMD factors (*Upf1, Upf2, Upf3*, and *Smg5*) was performed using the Applied Biosystems QuantStudio 3D Digital PCR system. To quantify RNA expression, six *Drosophila* heads were homogenized in RIPA buffer plus protease and phosphatase inhibitor cocktail and RNA extracted using TRIzol (Invitrogen, 15596026) according to the manufacturer's protocol. RNA concentrations were measured using Nanodrop 8000 spectrophotometer (Thermo Scientific) and equal amounts of RNA

added to a reverse transcription reaction (cDNA Reverse Transcription Kit, Applied Biosystems). cDNA was loaded into a PCR chip, sealed, and amplified (up to 24 chips at one time) before analysis to determine the absolute concentration in copies/μL. The primers and probe for each *Drosophila* mRNA target were predesigned TaqMan gene expression (Supplemental Table 3).

3.1.8 | Statistical analyses

For all experiments, we performed either G*Power analysis[107] or used previously reported sample sizes[7,54,58,73] to determine sample size for each experiment. All reported n's represent biological replicates. Statistical analyses were performed using Prism 7 (GraphPad Software Inc., La Jolla, CA). Unless otherwise specified, results were considered significant if $P<0.05$ by ANOVA and Tukey's test for multiple comparisons, and student's *t*-test for single comparisons. To limit bias, samples were randomized and investigators were blinded to genotype in all immunofluorescence, immunohistochemistry, and locomotor assays when possible.

3.2 | RESULTS

3.2.1 | Deficits in NMD occur prior to neurodegeneration in tau^{R406W} transgenic *Drosophila*—

To evaluate overall activity of NMD in tauopathy, we quantified NMD at days one and ten of adulthood in brains of control and tau^{R406W} transgenic *Drosophila* using two complementary approaches. We first utilized a fluorescent reporter previously developed to monitor NMD activity in *Drosophila*[102]. The NMD reporter utilizes the SV40 3' small T antigen intron and a polyA signal in the 3' UTR to trigger NMD-mediated degradation of a red fluorescent protein (RFP) transcript. High RFP levels indicate that the transcript has evaded clearance by NMD and has been translated into protein. We first validated that the NMD reporter is activated as expected when NMD is dysfunctional in the adult *Drosophila* brain. We find that panneuronal knockdown of an NMD core factor, Smg5, effectively activates the NMD-sensitive reporter based on RFP ELISA, thus validating the utility of the reporter in the adult *Drosophila* brain (Fig. 1A). Similarly, we find robust activation of the NMD reporter in brains of tau^{R406W} transgenic *Drosophila* compared to control, indicating that tau^{R406W} transgenic flies fail to clear the "faulty" RNA encoded by the reporter and that the faulty RNA is effectively translated into protein. We find that tau^{R406W}-induced deficits in NMD occur early in the disease process, as the NMD reporter is activated at day one of adulthood, which is prior to neurodegeneration in this model, and at day 10 of adulthood, which is approximately 33% of the maximum lifespan of tau^{R406W} transgenic *Drosophila*[80] (Fig. 1B).

As a second approach to quantify NMD activity, we measured RNA levels of *Gadd45* and *Arc1*, which are well-established normal, non-error containing transcripts that are known to be cleared by NMD[61,103,104]. As expected, we find that panneuronal knockdown of Smg5 increases transcript levels of *Gadd45 and Arc1* in the adult *Drosophila* brain, thus validating that these RNAs are indeed NMD targets (Fig. 1C). Consistent with our findings using the NMD-sensitive reporter, we find that *Gadd45* (Fig. 1D) and *Arc1* (Fig. 1E) transcript levels are elevated at both day one and day ten of adulthood in brains of tau^{R406W} transgenic

Drosophila. Taken together, these data suggest that tau^{R406W} transgenic *Drosophila* fail to clear faulty and normal transcripts that are typically degraded via NMD.

While *Drosophila* featuring panneuronal expression of wild-type tau, which models sporadic tauopathies, feature delayed phenotypes and a milder degree of neurotoxicity compared to tau^{R406W} *Drosophila*, previous studies in *Drosophila* report that transgenic expression of human wild-type tau or *MAPT* mutations drive neurodegeneration through conserved mechanisms[5–8]. To determine if deficits in NMD occur in a *Drosophila* model of sporadic tauopathy, we analyzed NMD in flies overexpressing wild-type human tau[108]. We analyzed NMD at day twenty of adulthood due to the milder phenotypes of this model. We find that panneuronal transgenic expression of tau^{WT} significantly increases fluorescence of the NMD-sensitive reporter at twenty days of adulthood (Fig. 1F), indicating defective NMD. In addition, we find elevated levels of *Gadd45* and *Arc1* transcripts in head of twenty-day-old tau^{WT} flies (Fig. 1G, H), further suggesting blunted NMD in this model. These findings suggest that the NMD deficits we observe in tau^{R406W} transgenic *Drosophila* are also relevant to sporadic forms of tauopathy.

3.2.2 | Genetic manipulation of NMD modifies tau^{R406W}-induced neurodegeneration—We next determined if tau-induced deficits in NMD are causally associated with neurodegeneration. For this analysis and subsequent experiments, we utilized the tau^{R406W} transgenic flies, as this model features a moderate level of degeneration at day ten of adulthood that is convenient for genetic analyses and precedes an exponential decline in survivorship[80,109]. We first quantified overall transcript levels of NMD core factors *Upf1*, *Upf2*, *Upf3* and *Smg5* using dPCR, and find that these transcripts are unchanged in brains of control versus tau^{R406W} transgenic *Drosophila* (Fig. 2A). We genetically manipulated NMD core factors Upf1, Upf2, Upf3 and Smg5 in tau^{R406W} transgenic *Drosophila* and quantified tau^{R406W}-induced neurotoxicity using TUNEL. We find that panneuronal expression of a dominant-negative form of Upf2 (Upf2^{E801R}, henceforth referred to as "Upf2^{DN}")[17] and panneuronal RNAi-mediated reduction of Upf1, Upf3 or Smg5 significantly enhance tau^{R406W}-induced neurodegeneration (Fig. 2B). Conversely, we find that panneuronal overexpression of wild-type Upf1 or Upf2 significantly suppress tau^{R406W}-induced neurodegeneration (Fig. 2C). Overall protein levels of transgenic tau^{R406W} are unchanged as a consequence of genetic manipulation of NMD core factors (Fig. 2D, E), excluding the possibility that modification of tau^{R406W} neurotoxicity is a result of differential transgenic tau^{R406W} expression. These findings indicate that tau-induced deficits in NMD causally mediate neurodegeneration.

3.2.3 | Tau-induced nuclear envelope invaginations lack NMD machinery and accumulate protein encoded by faulty RNA in the *Drosophila* brain.—We became interested in a potential link between failed NMD and tau-induced nuclear envelope invaginations based on previous studies from our lab and others reporting that pathogenic forms of tau cause nuclear envelope invaginations[56,109] and that RNA accumulates within and adjacent to tau-induced nuclear envelope invaginations[58]. We hypothesized that RNA may accumulate within such invaginations because it is failed to be cleared by NMD. We first determined if NMD machinery is present alongside the RNA

that accumulates within tau^{R406W}-induced nuclear invaginations. We visualized RNA, Upf1, and their relationship with nuclear envelope invaginations by combining poly(dT) FISH with immunofluorescence-based detection of the lamin nucleoskeleton, which lines nuclear invaginations[44], and Upf1 (Fig. 3A). In line with previous studies, we find that Upf1 and polyA RNA are enriched at the nuclear envelope in cells of the *Drosophila* brain and are also present within the cytoplasm and nucleus (Supplemental Fig. 1). While we detect both Upf1 and RNA within about 38% of invaginations in brains of tau^{R406W} transgenic *Drosophila*, we find that the majority (~62%) of RNA-enriched invaginations lack Upf1 (Fig. 3B). As Upf1 is required for NMD in *Drosophila*[18,70], these data indicate that most RNA-containing nuclear envelope invaginations are not actively clearing faulty RNA via NMD.

We next asked if NMD targets accumulate within nuclear envelope invaginations of tau^{R406W} transgenic *Drosophila.* Using the NMD-sensitive reporter as measure of general NMD, we stained brains of tau^{R406W} transgenic *Drosophila* with antibodies detecting dsRed, which detects protein expressed by the NMD reporter when its transcript fails to be cleared by NMD, and lamin. We observe dsRed puncta within ~40% of nuclear invaginations of tau^{R406W} *Drosophila* (Fig. 3C, D), suggesting that NMD targets are not only present within nuclear invaginations, but that they are actively translated into protein.

3.2.4 | Experimental activation of NMD decreases RNA accumulation in nuclear invaginations in brains of tau^{R406W} transgenic *Drosophila*—Having established that genetically increasing NMD suppresses tau^{R406W}-induced neurotoxicity, that tau^{R406W}-induced nuclear envelope invaginations generally lack Upf1, and that protein encoded by faulty RNA is present within tau^{R406W}-induced nuclear envelope invaginations, we next asked if RNA can be cleared from nuclear envelope invaginations by experimentally increasing NMD via Upf1 overexpression. We first quantified the degree of Upf1 overexpression and NMD activity in tau transgenic *Drosophila* with panneuronal overexpression of Upf1. We detect a four-fold increase in *Upf1* RNA levels in heads of Upf1^{OE} tau^{R406W} transgenic *Drosophila* (Fig. 4A) alongside a two-fold increase in Upf1 protein levels (Fig. 4B). To determine the extent to which Upf1 overexpression generally functions to clear NMD targets, we determined the ability of Upf1 overexpression to reduce activation of the NMD-sensitive reporter. This experiment was performed in the absence of the human tau^{R406W} transgene due to the number of transgenes that would be required for this analysis in tau^{R406W} transgenic *Drosophila*. We find that panneuronal overexpression of Upf1 significantly reduces activation of the NMD sensitive reporter (Fig. 4C), suggesting that Upf1 overexpression is sufficient to activate NMD in the adult *Drosophila* brain. We further find that panneuronal Upf1 overexpression is sufficient to significantly reduce *Gadd45* (Fig. 4D), but not *Arc1* (Fig. 4E) transcript levels in brains of tau^{R406W} transgenic *Drosophila*, suggesting that Upf1 overexpression effectively clears some, but not all, endogenous targets of NMD. Having found that panneuronal Upf1 overexpression effectively increases Upf1 protein levels and activates NMD, we next asked if Upf1 overexpression affects the burden of RNAs that accumulate within tau^{R406W}-induced nuclear envelope invaginations. While we frequently detect enrichment of RNA within nuclear invaginations based on poly(dT)/lamin FISH-IF in brains of tau^{R406W} transgenic *Drosophila*, we find significantly less polyA RNA enrichment within nuclear envelope

invaginations in brains of tau^{R406W} transgenic *Drosophila* with Upf1 overexpression (Fig. 4F, G), providing additional evidence that RNAs that accumulate in nuclear envelope invaginations are targets of NMD.

3.2.5 | Genetic reduction of RNA export suppresses NMD deficits in tau^{R406W} transgenic *Drosophila*—We have previously reported that experimentally reducing RNA export factors sbr and Nxt1 significantly diminishes deposition of RNA within tau-induced nuclear envelope invaginations and suppresses tau neurotoxicity in *Drosophila*[58], suggesting that RNAs are actively transported into invaginations. We hypothesized that the tau-induced increase in RNA export could perhaps overwhelm NMD machinery and thus reduce the ability of NMD to effectively degrade its targets. To test this hypothesis, we analyzed *Gadd45* and *Arc1* transcript levels in tau^{R406W} transgenic *Drosophila* in response to panneuronal RNAi-mediated knockdown of sbr or Nxt1. Indeed, we find that reduction of sbr or Nxt1 significantly reduces RNA levels of *Gadd45* in tau^{R406W} transgenic *Drosophila* (Fig. 5A), and that Nxt1^{RNAi} effectively reduces RNA levels of *Arc1* in tau^{R406W} transgenic *Drosophila* (Fig. 5B), suggesting that genetic manipulation of RNA export mediates NMD in tau^{R406W} transgenic *Drosophila*. While experimentally reducing RNA export is not sufficient to significantly reduce *Gadd45* or *Arc1* RNA levels in flies lacking transgenic tau, we find that that experimentally reducing RNA export is sufficient to reduce protein encoded by the NMD reporter in healthy controls (Fig. 5C). While this finding raises the possibility that experimentally decreasing RNA export may allow NMD to more effectively clear some, but not all, faulty RNAs in healthy fly brains, it is also possible that experimentally reducing RNA export may simply result in less reporter RNA that is available for translation. Overall, these data suggest that reduction and/or slowing of RNA export in tau^{R406W} transgenic *Drosophila* decreases levels of RNAs that are usually cleared by NMD, and mechanistically links tau-induced increase in nuclear RNA export to a functional reduction in NMD.

3.2.6 | Pharmacological activation of NMD is neuroprotective in tau^{R406W} transgenic *Drosophila*—We next asked if tau-induced deficits in NMD are pharmacologically targetable. While an increasing number of preclinical drugs that inhibit NMD are in development for the treatment of cancer and human genetic disorders caused by a single-base pair mutation[110–113], NMD-activating drugs are less common. We first determined if tranilast, a previously identified NMD activator in *Drosophila*[61], effectively activates NMD in tauopathy. We treated control and tau^{R406W} transgenic *Drosophila* with vehicle (dimethyl sulfoxide, DMSO) or 10 μM tranilast in food from day one to ten of adulthood. We find that tranilast significantly activates general NMD in brains of tau^{R406W} transgenic *Drosophila* based on decreased expression of the NMD-sensitive reporter (Fig. 6A). In addition, tranilast treatment significantly decreases transcript levels of *Gadd45* and *Arc1* in tau^{R406W} transgenic *Drosophila* but does not alter their levels in controls (Fig. 6B, C). These results confirm the NMD-activating effects of tranilast and suggest that tranilast-mediated activation of NMD is efficacious only in an NMD-deficient background. Having established that tranilast activates NMD in tau^{R406W} transgenic *Drosophila*, we next asked if pharmacological activation of NMD protects against tau-induced toxicity. We find that ten days of tranilast treatment significantly ameliorates tau-induced neurodegeneration based on TUNEL staining (Fig. 6D). As an overall measure of organismal fitness, we analyzed

the effects of tranilast on locomotor activity. We find that ten days of tranilast treatment significantly suppresses tau-induced locomotor deficits (Fig. 6E), but that this effect is lost after 20 days of treatment, an age at which tau^{R406W} transgenic *Drosophila* are significantly impaired (Fig. 6F). Total levels of transgenic tau^{R406W} protein are not changed by tranilast treatment (Fig. 6G, H). Together, these results suggest that NMD is a pharmacologically targetable pathway with therapeutic potential for tauopathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HUMAN SUBJECTS

Human subjects were not involved in this study and consent was not necessary.

DISCLOSURES

Frost Disclosures

Support received for the submitted work

Federal (paid to institution)

Period: 02/15/19 – 11/30/23

Project #: 1 R01 AG057896

Funding Agency: NIA/NINDS

Title: Mechanisms of tau- and aging-induced neurological dysfunction: Focus on the nucleus

Status: Active

Role: Principal Investigator

Support received outside the submitted work

Grants and contracts over the past 36 months, all paid to institution:

Federal

Period: 02/15/19 – 11/30/23

Project #: 1 R01 AG057896

Funding Agency: NIA/NINDS

Title: Mechanisms of tau- and aging-induced neurological dysfunction: Focus on the nucleus

Status: Active

Role: Principal Investigator

Period: 09/01/19 – 05/31/24

Project #: 1 R01 AG062475-01A1

Funding Agency: NIA/NINDS

Title: Regulation of neuronal clearance pathways via nuclear calcium signaling in Alzheimer's disease

Status: Active

Role: Co-Investigator, PI: Radek Dobrowolski

Period: 07/01/19 – 06/30/24

Project #: 1 RF1 NS112391-01

Funding Agency: NIA/NINDS

Title: Investigating the role of transposable element dysregulation as a driver of neurotoxicity in tauopathy

Status: Active

Role: Principal Investigator

Period: 03/01/19 – 11/30/23

Project #: 1 R01 AG058778-01

Funding Agency: NIH/NINDS

Title: Regulation of ER-stress Activated UPR kinase PERK in Neurodegenerative Diseases

Status: Active

Role: Co-Investigator, PI: James Lechleiter

Total Direct Costs to Frost: $325,050

Grant Detail: The major goal of this grant is to understand how cytosolic and luminal regulation of PERK activity contribute to neurodegenerative disorders

Period: 08/01/14 – 11/30/19

Project #: 1 K99/R00 NS 088429

Funding Agency: NIH/NINDS

Title: Mechanisms and Consequences of Heterochromatin Loss in Tauopathies

Status: Complete

Role: Principal Investigator

Institutional

Period: 09/01/19 – 08/31/21

Project #: N/A

Funding Agency: UTHSCSA Pepper Center

Title: Evaluating the extent of transposable element activation in brain and fluid from patients with Alzheimer's disease

Status: Active

Role: Principal Investigator

Period: 01/01/19 – 12/31/19

Project #: N/A

Funding Agency: Center for Biomedical Neurosciences Pilot Grant

Title: Testing 3TC as a novel therapeutic for transposable element activation and consequent neurodegenerationin a mouse model of tauopathy

Status: Complete

Role: Principal Investigator

Private

Period: 08/01/19 – 07/31/21

Project #: N/A

Funding Agency: Rainwater Foundation/Tau Consortium

Title: Development of a *Drosophila* model of prion-like tau propagation for future genetic modifier and drug screening Status: Active

Role: Principal Investigator

Period: 06/01/19 – 05/31/21

Project #: N/A

Funding Agency: William and Ella Owens Medical Research Foundation

Title: Dysregulation of Transposable Element Sequences as a Novel Disease Mechanism in Alzheimer's Diseaseand Related Tauopathies

Status: Active

Role: Principal Investigator

Period: 08/01/18 – 08/31/20

Project #: N/A

Funding Agency: Rainwater Foundation/Tau Consortium

Title: Investigating transposable element activation as a causal mediator of neuronal death in tauopathy

Status: Active

Role: Principal Investigator

Sponsored Research Agreements

Period: 01/01/21 – 12/31/21

Project #: N/A

Funding Agency: MD Anderson Neurodegeneration Consortium

Title: Nuclear architecture and transposable element activation as a therapeutic target in human Alzheimer's disease and associated tauopathies

Status: Active

Role: Principal Investigator

Period: 01/01/20 – 12/31/20

Project #: N/A

Funding Agency: MD Anderson Neurodegeneration Consortium

Title: Transposable element activation as a therapeutic target in human Alzheimer's disease and associated tauopathies

Status: Complete

Role: Principal Investigator

Period: 06/01/20 – 05/31/21

Project #: N/A

Entity: Transposon Therapeutics

Title: Testing reverse transcriptase inhibitors in *Drosophila* models of tauopathy

Status: Complete

Role: Principal Investigator

Period: 05/01/19 – 04/31/20

Project #: N/A

Funding Agency: MD Anderson Neurodegeneration Consortium

Title: Reverse transcriptase inhibitors for Alzheimer's disease

Status: Complete

Role: Principal Investigator

Consulting over the past 36 months, paid to institution:

2019: $50,000.00

2021: $50,000.00

Source: MD Anderson Neurodegeneration Consortium

Paid honoraria over the past 36 months, paid to individual:

2020: University of Texas Austin, Department of Neuroscience Seminar Series, *invited speaker*

2019: University of Texas Southwestern, Symposium on Neurodegenerative Diseases, *invited speaker*

Paid travel over the past 36 months, paid to individual:

2020: Tau2020 and Tau Consortium Investigator's Meeting, Washington, DC, Paid travel from Rainwater Foundation, *invited speaker*.

2019: Gerontological Society of America, Austin, TX. Paid travel from GSA, *invited speaker*.

2019: Alzheimer's Association International Conference, Los Angeles, CA. Paid travel from Alzheimer's Association, *invited speaker*.

2019: Tau Consortium Meeting, Dallas, TX. Paid travel from Rainwater Foundation.

2018: American Federation for Aging Research New Investigator in Alzheimer's Disease Meeting, Santa Barbara, CA. Paid travel from AFAR, *invited speaker*.

2018: PSP & CBD International Research Symposium, London, United Kingdom. Paid travel from CurePSP, *keynote speaker*.

2018: Keystone Meeting, Advances in Neurodegenerative Disease Research, Keystone, CO (Invited Speaker), Paid travel from Keystone, *invited speaker*.

Leadership roles on boards over the past 36 months (unpaid)

2021: Scientific Advisory Board for CurePSP

Zuniga Disclosures

Support received for the submitted work

Federal (paid to institution)

Period: 07/01/18 – 06/30/23

Project #: T32 GM113896

Funding Agency: NIGMS

Title: South Texas Medical Scientist Training Program (STX-MSTP)

Status: Active

Role: Trainee

Principal Investigator: José Cavazos, M.D., Ph.D.

Period: 05/24/18– 04/30/23

Project #: TL1 TR002647

Funding Agency: NCATS

Title: NRSA Training Core (TL1)

Status: Complete

Role: Trainee

Principal Investigator: Linda McManus, Ph.D. and Christopher Frei, Ph.D.

Period: 07/01/13 – 06/30/18

Project #: T32 NS082145

Funding Agency: NINDS

Title: Integrated Graduate Training Program in Neuroscience, UTHS

Status: Complete

Role: Trainee

Principal Investigator: David Morilak, Ph.D.

Support received outside the submitted work

Grants and contracts over the past 36 months, all paid to institution:

Federal

Period: 07/01/21 – 06/30/22

Project #: T32 AG021890

Funding Agency: NIA

Title: Training Grant on Biology of Aging

Status: Active

Role: Trainee

Principal Investigator: Nicolas Musi, M.D.

Paid travel over the past 36 months, paid to individual:

2020: Tau2020 and Tau Consortium Investigator's Meeting, Washington, DC, Paid travel from Rainwater Foundation, *invited fellow.*

2019: Alzheimer's Drug Discovery Foundation Young Investigators Scholarship, 13th Annual Drug Discovery for Neurodegeneration Conference, An Educational Course on Translating Research into Drugs. Long Beach, CA, Paid travel from ADDF, *invited fellow.*

Levy Disclosures

Paid travel over the past 36 months, paid to individual:

2019: Tau Consortium Investigators' Meeting, San Diego, CA, Paid travel from Rainwater Foundation, *invited fellow.*

2019: Keystone Symposium on Neurodegenerative Disorders, Keystone, CO, Paid travel from UT Health San Antonio Graduate School of Biomedical Sciences Summer Travel Award

Ramirez Disclosures

Support received outside the submitted work

Grants and contracts over the past 36 months, all paid to institution:

Federal

Period: 08/01/19 – 08/31/21

Project #: R25 GM095480-10

Funding Agency: NIGMS

Title: Recruiting and Retaining Underrepresented Students

Status: Active

Role: Trainee

Principal Investigator: Nicquet Blake, PhD

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RESEARCH IN CONTEXT

1. Systematic review: Pathological forms of tau drive neuronal death in Alzheimer's disease and related tauopathies. Accumulating evidence indicate that tau pathology impairs RNA processing in the brain and may contribute to downstream neuronal loss. We previously reported that increased RNA export contributes to tau-induced neurodegeneration through an unknown mechanism.
2. Interpretation: We have discovered that tau-induced increase in RNA export leads to a deficit in NMD, a key RNA surveillance mechanism that clears faulty transcripts. We leverage genetic and pharmacological approaches in *Drosophila* to demonstrate that deficits in NMD causally mediate tau-induced neurotoxicity.
3. Future direction: Our study provides rationale for determining if NMD is at a deficit in human tauopathies. Given that activation of NMD is neuroprotective in *Drosophila*, future studies include 1) screening for and identifying compounds that activate NMD, and 2) investigating the therapeutic effect of highly selective NMD activators in a vertebrate model of tauopathy.

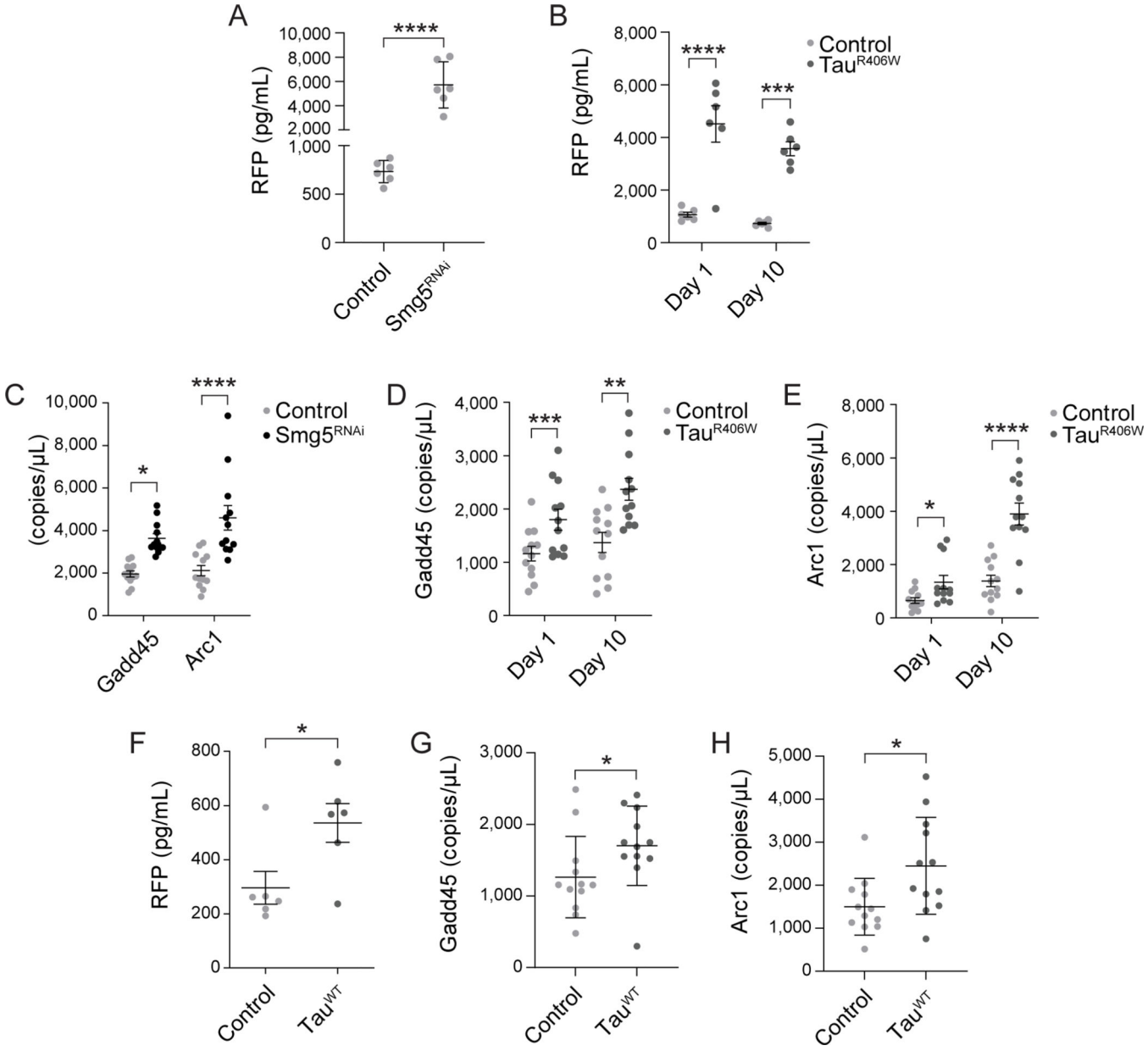

Figure 1 |. Reduced clearance of aberrant transcripts by NMD in tau transgenic *Drosophila*.

A) Panneuronal RNAi-mediated reduction of Smg5 limits NMD in *Drosophila* based on activation of an NMD sensitive reporter. n=6 biological replicates per genotype, per age**.** **B)** Panneuronal expression of transgenic human tau^{R406W} limits NMD in *Drosophila* at day one and day ten of adulthood based on activation of an NMD sensitive reporter. n=6 biological replicates per genotype, per age. Transcript levels of *Gadd45* and *Arc1* are significantly elevated in *Drosophila* with panneuronal RNAi-mediated reduction of Smg5 **(C)** and in tau^{R406W} transgenic *Drosophila* at day one and day ten of adulthood **(D, E)**. **F)** Panneuronal expression of transgenic human tau^{WT} limits NMD in *Drosophila* at day twenty of adulthood based on activation of an NMD sensitive reporter. n=6 biological replicates per genotype. Panneuronal expression of tau^{WT} elevates *Gadd45* **(G)** and *Arc1* **(H)** transcript

levels compared to controls. n=12 biological replicates per genotype. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, unpaired t-test and multiple unpaired t-test. Error bars=SEM. Full genotypes are included in Supplemental Table 1.

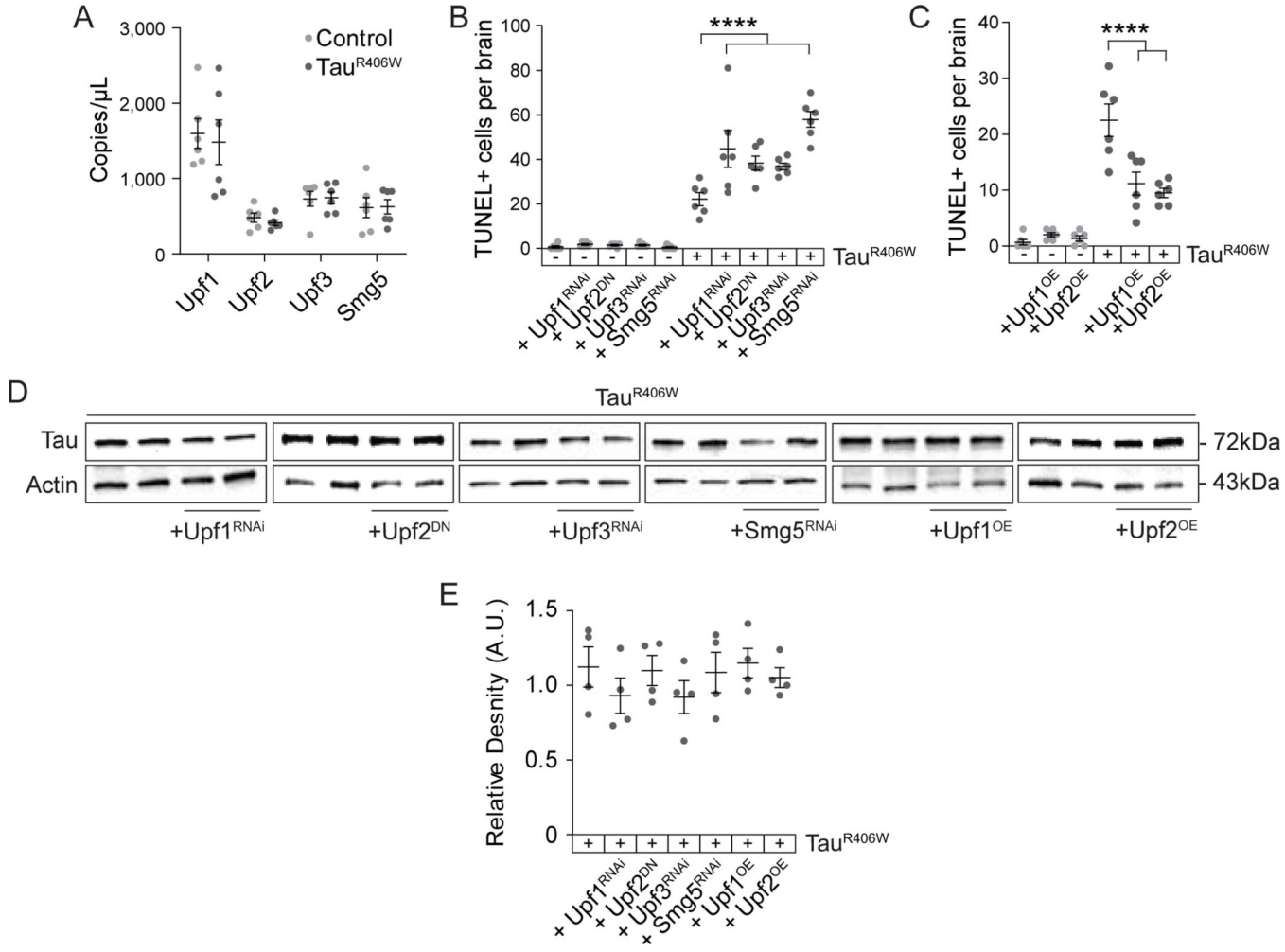

Figure 2 |. Genetic manipulation of NMD modifies tau^{R406W}-induced neurodegeneration in *Drosophila*.

A) Transcripts levels of *Upf1, Upf2, Upf3*, and *Smg5* are unchanged in control versus tau^{R406W} *Drosophila* head lysates based on dPCR. **B)** RNAi-mediated reduction of Upf1, Upf3, and Smg5, and overexpression of Upf2 harboring a dominant-negative mutation significantly enhance tau^{R406W}-induced neuronal death based on TUNEL staining in *Drosophila* brains. **C)** Panneuronal overexpression of wild-type Upf1 or Upf2 significantly suppress tau^{R406W}-induced neuronal death based on TUNEL staining in *Drosophila* brains. **D)** Genetic manipulation of NMD core factors does not change total protein levels of transgenic tau^{R406W} based on western blotting. **E)** Quantification of (D), total levels of tau protein normalized to actin. n=4–6 biological replicates per genotype. All assays were performed at day ten of adulthood. ****p<0.0001, one-way ANOVA. Error bars=SEM. Full genotypes are included in Supplemental Table 1.

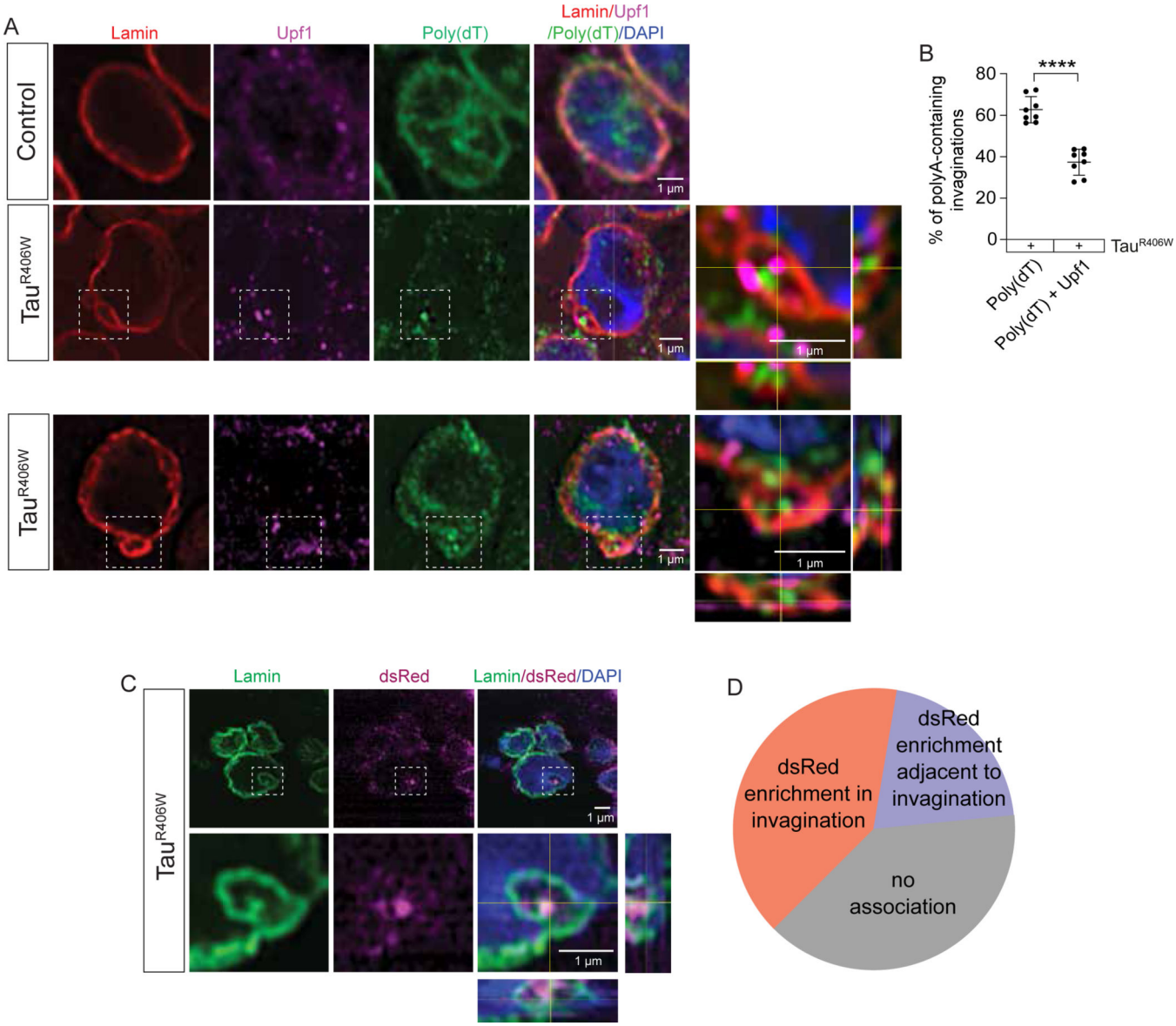

Figure 3 |. Tau^{R406W}-induced nuclear envelope invaginations lack NMD machinery and accumulate protein encoded by faulty RNA in the *Drosophila* brain.

A) FISH/IF based detection of Upf1, Lamin and polyA RNA in brains of tau^{R406W} transgenic *Drosophila* shows polyA RNA-enriched nuclear envelope invaginations containing Upf1 and lacking Upf1. Orthogonal views of the zoom region depicted show polyA RNA and/or Upf1 staining within lamin invaginations. **B)** Quantification of (A), percentage of polyA RNA-positive nuclear envelope invaginations containing versus lacking Upf1. **C)** Co-staining brains of tau^{R406W} transgenic *Drosophila* harboring the NMD-sensitive reporter with antibodies detecting lamin and dsRed indicate that reporter expression is present within nuclear envelope invaginations. **D)** Quantification of (C). All assays were performed at day ten of adulthood. n=6–8 biological replicates per genotype. p****<0.0001, unpaired t-test. Error bars=SEM. Full genotypes are included in Supplemental Table 1.

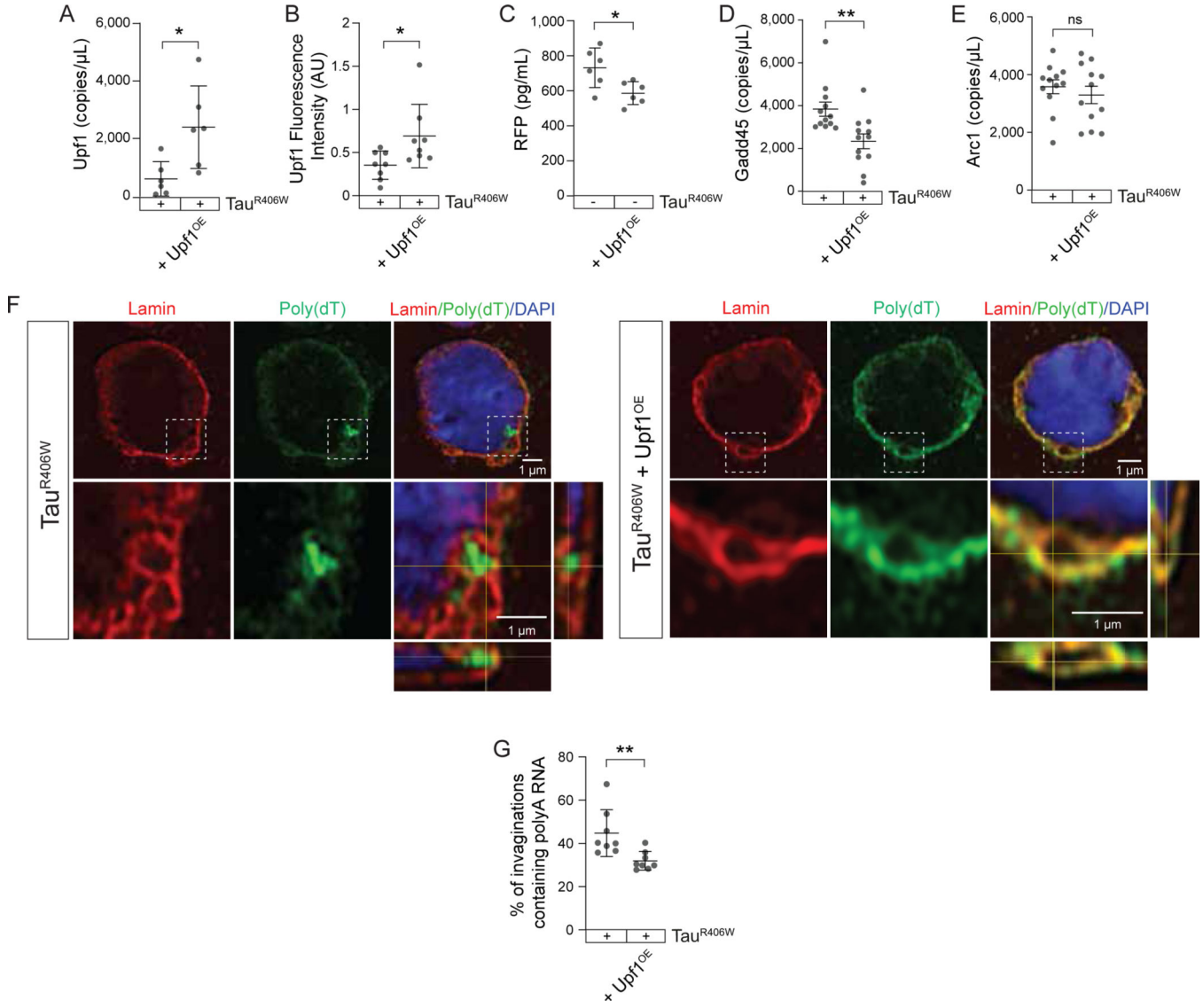

Figure 4 |. Experimentally elevating NMD decreases RNA accumulation in nuclear envelope invaginations in brains of tau^{R406W} transgenic *Drosophila*.

A) Upf1 RNA levels in tau^{R406W} *Drosophila* with and without Upf1 overexpression. **B)** Immunofluorescence-based quantification of Upf1 protein levels in tau^{R406W} *Drosophila* with and without Upf1 overexpression. **C)** ELISA-based NMD-sensitive reporter activity in control *Drosophila* with and without Upf1 overexpression. *Gadd45* **(D)** and Arc1 **(E)** transcript levels based on dPCR in tau^{R406W} *Drosophila* with and without Upf1 overexpression. **F)** FISH/IF-based detection of polyA RNA and lamin in tau^{R406W} *Drosophila* with and without Upf1 overexpression. Representative images include an orthogonal view of zoom region depicted showing polyA RNA-positive and -negative invaginations. **G)** Quantification of (F), percentage of nuclear envelope invaginations containing polyA RNA in flies of the indicated genotype. All assays were performed at day ten of adulthood. n=6–12 biological replicates per genotype. p*<0.05, one-way ANOVA. Error bars=SEM. Full genotypes are included in Supplemental Table 1.

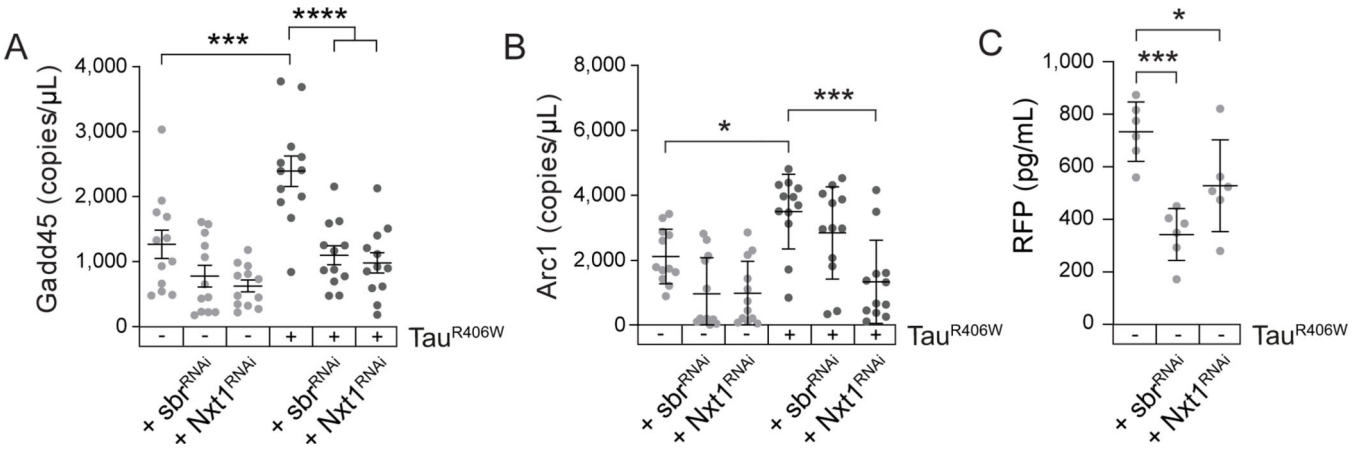

Figure 5 |. Genetic reduction of RNA export significantly increases NMD in brains of tau^{R406W} transgenic *Drosophila*.

RNAi-mediated reduction of sbr or Nxt1 stabilizes expression of NMD-sensitive *Gadd45* **(A)** and *Arc1* **(B)** in tau^{R406W} transgenic *Drosophila*. n=12 biological replicates per genotype. **C)** Panneuronal RNAi-mediated reduction of sbr or Nxt1 increase NMD in control flies based on the NMD-sensitive reporter. n=6 biological replicates per genotype. All assays were performed at day ten of adulthood. *p<0.05, ***p<0.001, ****p<0.0001, one-way ANOVA. Error bars=SEM. Full genotypes are included in Supplemental Table 1.

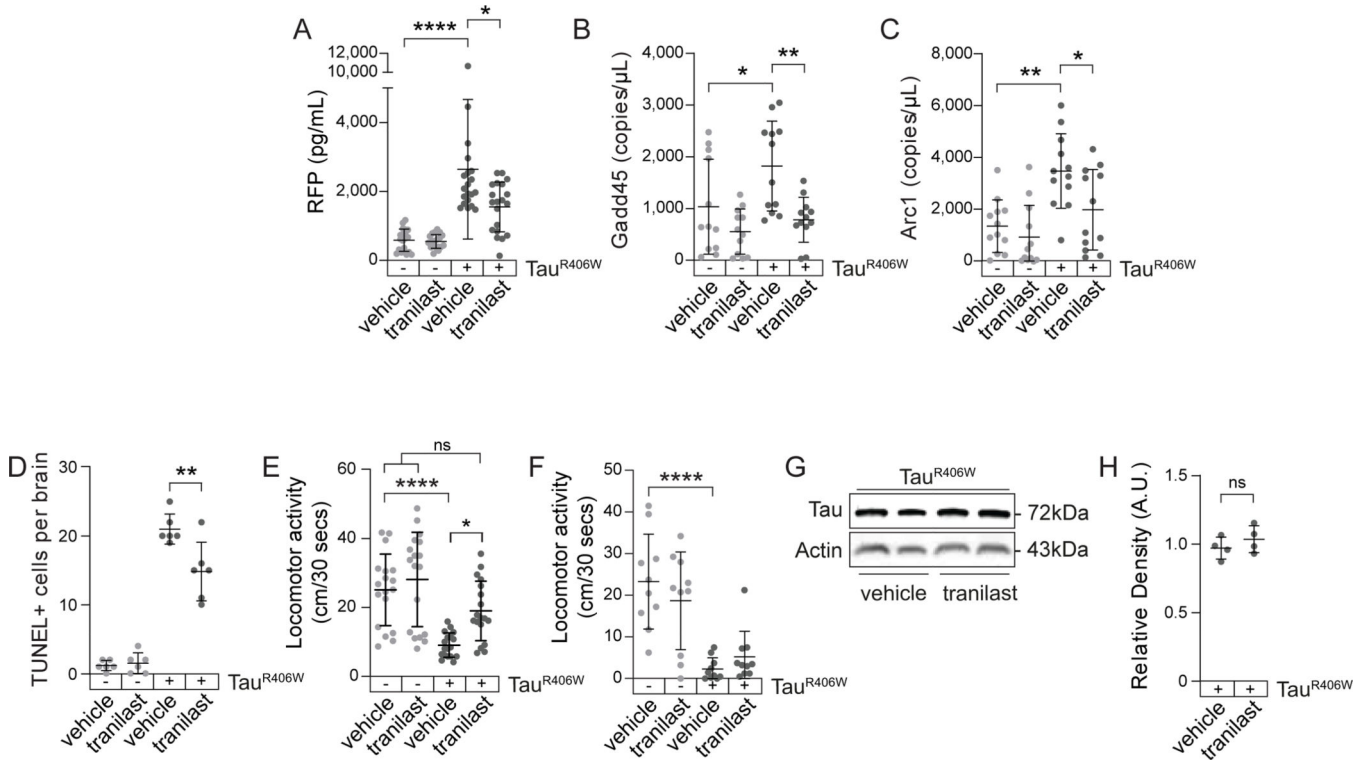

Figure 6 |. 10 µM tranilast activates NMD and suppresses tau^{R406W}-induced neurodegeneration in *Drosophila*.

A) Tranilast alleviates NMD deficits in tau^{R406W} transgenic *Drosophila* based on reduced expression of the NMD reporter. n=12 biological replicates per genotype, per treatment. RNA levels of the NMD-sensitive transcript *Gadd45* **(B)** and *Arc1* **(C)** are reduced by tranilast treatment of tau^{R406W} transgenic *Drosophila*. n=12 biological replicates per genotype, per treatment. **D)** Pharmacological activation of NMD by tranilast significantly suppresses neuronal death in tau^{R406W} transgenic *Drosophila* based on TUNEL staining. n=6 biological replicates per genotype, per treatment. **E)** Ten days of tranilast treatment significantly suppresses tau^{R406W}-induced locomotor deficits. n=18 biological replicates per genotype, per treatment. **F)** Twenty days of tranilast treatment does not suppress tau^{R406W} -induced locomotor deficits. n=10 biological replicates per genotype, per treatment. **G)** Transgenic tau protein levels in DMSO- and tranilast-treated tau^{R406W} flies based on western blotting. **H)** Quantification of (G). n=4 biological replicates per treatment. *p<0.05, **p<0.01, unpaired t-test and one-way ANOVA. Error bars=SEM. Full genotypes are included in Supplemental Table 1.