Glucose uptake in pigment glia suppresses tau-induced inflammation and photoreceptor degeneration in *Drosophila*

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Highlights

- Tau expression in the fly retina induces glial activation
- Pigment glial cells mediate inflammatory phenotypes in the degenerating retina
- Enhanced glucose uptake in the pigment glia suppresses inflammation and photoreceptor neurodegeneration caused by tau expression

Summary

Brain inflammation contributes to the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD). Glucose hypometabolism and glial activation are pathological features seen in AD brains; however, the connection between the two is not fully understood. Using a *Drosophila* model of AD, we identified that glucose metabolism in glia plays a critical role in neuroinflammation under disease conditions. Expression of human tau in the retinal cells, including photoreceptor neurons and pigment glia, causes photoreceptor degeneration accompanied by inclusion formation and swelling of lamina glial cells. We found that inclusions are formed by glial phagocytosis, and swelling of the laminal cortex correlates with the expression of antimicrobial peptides (AMPs). Co-expression of human glucose transporter 3 (*GLUT3*) with tau in the retina does not affect tau levels but suppresses these inflammatory responses and photoreceptor degeneration. We also found that expression of *GLUT3*, specifically in the pigment glia, is sufficient to suppress inflammatory phenotypes and mitigate photoreceptor degeneration in the tau-expressing retina. Our results suggest that glial glucose metabolism contributes to inflammatory responses and neurodegeneration in tauopathy.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia among older populations (Knopman et al., 2021). AD is characterized by the extracellular deposition of β-amyloid and intracellular accumulation of abnormally phosphorylated forms of the microtubule-associated protein tau (Samudra et al., 2023). Neuroinflammation is another core pathology in AD and other neurodegenerative disorders (Sobue et al., 2023). Inflammatory responses in the central nervous system are mediated by mainly glial cells, including microglia and astrocytes, to protect against infections and injuries; however, under neurodegenerative disease conditions, glial cells lose their neuroprotective functions and acquire reactive phenotypes (Ransohoff, 2016; Streit, 2002). Although they can remove protein aggregates by phagocytosis, activated glial cells can also harm neurons via excess phagocytosis and the release of neurotoxic cytokines (Chen and Holtzman, 2022). However, what triggers glial activation under disease conditions is not fully understood.

A decline in brain glucose metabolism has been pathologically linked to AD (Butterfield and Halliwell, 2019). Most of the ATP required to support brain function is supplied by glucose metabolism (Bélanger et al., 2011), and glucose utilization is reduced in the brains of AD patients (Dukart et al., 2013; Huang et al., 2020; Oka et al., 2021). Dysregulated glucose metabolism in AD may be due to cerebral hypoperfusion (Park et al., 2019) or downregulation of glucose transporters (GLUTs) that mediate glucose uptake across the blood-brain barrier and delivery to glial cells (Bélanger et al., 2011; Koepsell, 2020; Kyrtata et al., 2021; Liu et al., 2008). Glial cells also rely on glucose, and cellular metabolism can regulate their activation (Wang et al., 2019; Xiang et al., 2021). The mitochondrial oxidative phosphorylation is reduced in the activated microglial cells (Nair et al., 2019), and mitochondrial dysfunction in astrocytes has been reported in amyotrophic lateral sclerosis and neuroinflammation models (Fiebig et al., 2019; Martínez-Palma et al., 2019). While these reports suggest that targeting glial metabolism may be a strategy to decrease neuroinflammation and suppress neurodegeneration, mechanistic investigation in an *in vivo* model is required to explore this possibility.

Here, we investigated the roles of glucose metabolism in glial cells on tau-induced neurodegeneration in a *Drosophila* model. We identified glia-associated phenotypes in the fly retina expressing human tau, such as inclusion-like structures by phagocytosis and glial swelling

by inflammation. *Drosophila* retina contains neurons and several glial cells, and we found that pigment glia plays a critical role in tau-induced photoreceptor degeneration. Enhancement of glucose uptake in pigment glial cells ameliorates tau-induced glial swelling as well as photoreceptor degeneration. Our results suggest that glucose hypometabolism in glial cells contributes to neurodegeneration in tauopathy.

Results

Tau expression in the fly retina causes photoreceptor degeneration, swelling of the laminal cortex, and inclusion formation

Expression of human tau in *Drosophila* using the pan-retinal *GMR-GAL4* driver causes a rough eye phenotype in the compound eye surface accompanied by formation of vacuoles caused by apoptosis in the retina and histological signs of axonal degeneration in the lamina (Fig 1A) (Iijima-Ando et al., 2012; Jackson et al., 2002; Wittmann et al., 2001). Transmission electron microscopy (TEM) revealed degenerating photoreceptor neurons with disoriented rhabdomere in tau-expressing flies (Fig. 1B and C, an ommatidium colored in yellow). We also noticed morphological changes in glial cells in the retina. We observed swelling of the laminal cortex, which consists of layers of surface glia (perineurial glia; PNG and subperineurial glia; SPG) and cortex glia between lamina and retina (shaded in blue in Fig. 1A) (Chotard and Salecker, 2007; Kremer et al., 2017). Tau expression caused the formation of dense inclusion-like structures in the retina and lamina (Fig. 1A). TEM analysis of these structures revealed two distinct ultrastructures: invaginated corneal lens structures (arrowheads in Fig. 1A and D) and debris-containing inclusions wrapped by glial cells (arrows in Fig. 1A and E). These results suggest that glial cells are also affected by tau expression.

Tau expression induces glial phagocytosis and AMP expression

The appearance of inclusion-like structures in the lamina (Fig. 1E) was similar to the glial bodies (Dutta et al., 2016), which are membranous structures representing glial hyper-wrapping. We were motivated to test if these inclusions are formed via glial phagocytosis. The glial phagocytic

activities in the adult fly brain are mediated by two glial transmembrane phagocytic receptors, Draper (Drpr) and Nimrod C4 (NimC4, also known as SIMU) (Kurant et al., 2008; Scheib et al., 2012). We found that blocking glial phagocytosis by RNAi-mediated knockdown of *drpr* and *NimC4* reduced the number of these inclusions in the tau-expressing retina (Fig. 2A and Supplement Fig. 1). Swelling of the laminal cortex was also suppressed (Fig. 2A). These results indicate that the inclusions are formed by glial engulfment activity.

Proinflammatory responses in *Drosophila* involve the expression of antimicrobial peptides (AMPs) downstream of Toll and Immune deficiency (Imd) pathways (Harnish et al., 2021; Hedengren et al., 1999; Sakakibara et al., 2023; Shukla et al., 2019). We found that *GMR-GAL4* driven tau-expressing flies exhibited significantly elevated expression levels of AMPs, including *Drosomycin (Drs), Cecropin A1 (CecA1), Metchnikowin (Mtk), Diptericin B (DptB), and Attacin C (AttC)* (Fig. 2B). These results suggest that tau expression in the fly retina induces glial phagocytosis and inflammatory responses, which are hallmarks of glial activation.

Enhancement of glucose uptake suppresses tau-induced photoreceptor degeneration and swelling of the laminal cortex

We analyzed the effects of enhancement of glucose uptake in tau-induced phenotypes in the retina by expressing human GLUT3 (encoded by the *SLC2A3* gene), which has been shown to enhance glucose uptake in cells effectively (Besson et al., 2015; Manzo et al., 2019; Oka et al., 2021). Co-expression of GLUT3 increased the eye size in tau flies (Fig. 3A, compare *Tau and Tau+GLUT3*, $p<0.001$). The eyes co-expressing tau and CD8::RFP were similar to those in flies expressing tau alone, indicating that the increase in the eye size caused by co-expression of *GLUT3* is not due to non-specific effects of an exogenous protein or dilution of GAL4 activity (Fig. 3A, compare *Tau* and *Tau+CD8::RFP*, $p>0.05$). The expression of GLUT3 alone did not affect eye size (Fig. 3A, compared to Control and *GLUT3*, $p>0.05$). Rhodopsin 1 (Rh1) protein level is significantly lower in tau flies, likely due to loss of photoreceptor neurons (Fig 3B, compare control and *tau*). Co-expression of GLUT3 restored Rh1 protein levels (Fig. 3B, compare *Tau* and *Tau+GLUT3*, $p<0.001$). The vacuoles in the retina of flies co-expressing tau and GLUT3 were also significantly less than those expressing tau alone (Fig. 3C-D, $p<0.01$). At ultrastructural levels, while tau-expressing retina contains degenerating photoreceptor neurons

with disoriented rhabdomere in tau-expressing flies (Fig. 1D, an ommatidium colored in yellow), ommatidium from flies co-expressing tau and GLUT3 retained rhabdomeres with proper orientation (Fig. 3F, *Tau+GLUT3*). Co-expression of GLUT3 did not affect the number of inclusion-like structures (Fig. 3E), while suppressed laminal cortex swelling (Fig. 3F). Overexpression of *Drosophila* Glut1, a fly homolog of human GLUT3, also suppressed tau-induced retinal degeneration and the lamina cortex swelling(Fig. 3G-I). These results indicate that glucose uptake enhancement suppresses tau-induced neurodegeneration and glial phenotypes.

Enhancement of glucose uptake does not affect tau phosphorylation and accumulation

To understand the mechanisms underlying the neuroprotective effects of enhanced glucose uptake, we first analyzed the effect of GLUT3 expression on tau phosphorylation and accumulation by western blot with monoclonal antibodies. Co-expression of GLUT3 did not affect the levels of total tau (Total tau) or its phosphorylation at disease-associated sites, including Ser262 (pSer262), Ser396/404 (PHF1), and Ser202 (CP13) (Fig. 4). These results suggest that enhanced glucose uptake mitigates retinal degeneration downstream of tau phosphorylation and accumulation.

Enhancement of glucose uptake in pigment glial cells suppresses tau-induced neurodegeneration

GMR (glass multiple reporter) element used in *GMR-GAL4* drives expression in all the cells behind the morphogenetic furrow, including pigment glial cells (Freeman, 1996). Pigment glial cells surround photoreceptor neurons and support their functions by providing nutrition and up taking toxic materials (Liu et al., 2017). To dissect the effects of *GLUT3* expression in glial cells in tau-induced retinal degeneration, we used *GMR-tau (P301L)*, which expresses human tau carrying pathological mutation (P301L) directly under the control of the GMR regulatory sequence (Jackson et al., 2002), in combination with *GAL4/UAS* system to express *GLUT3* using *54C-GAL4*, a pigment glia-specific driver (Nagaraj and Banerjee, 2007). We found that glial phenotypes, such as swelling of the laminal cortex (Fig. 5A) and AMP expression (Fig. 5B) were suppressed by expression of *GLUT3* in pigment glia. Intriguingly, glial expression of *GLUT3* in

the tau-expressing retina also suppressed photoreceptor degeneration (Fig. 5C-E). Eye size was significantly increased and Rh1 protein level was restored upon *GLUT3* expression in the pigment glia (Fig. 5D). Vacuole formation in the lamina was also mitigated (Fig. 5E). In contrast, expression of *GLUT3* in photoreceptor neurons by *Rh1-GAL4* did not mitigate photoreceptor degeneration (Fig 5F-H), indicating that the protective effects of *GLUT3* co-expression against tau toxicity observed with *GMR-GAL4* (Fig 3) is mediated by pigment glia. Taken together, these results suggest that enhancement of glucose uptake in pigment glia mitigates inflammatory responses and tau-induced photoreceptor degeneration.

Discussion

Enhancing glucose uptake by neuronal overexpression of GLUTs has been reported to ameliorate neurodegeneration in several neurodegenerative conditions such as huntingtin, β-amyloid, and TDP-43 toxicity in *Drosophila* (Besson et al., 2015; Manzo et al., 2019; Niccoli et al., 2016; Cabirol-Pol et al., 2018) as well as in age-related neuronal dysfunction (Oka et al., 2021). However, beneficial effect of enhancing glucose uptake in glial cells has not been examined. In this study, we characterized glial phenotypes in the fly retina expressing human tau and examined the effects of enhanced glucose uptake in glia on them. We found that tau expression causes phenotypes mediated by glia, such as inclusion formation, swelling in the laminal cortex, and AMP expression (Figure 1-3). We also found that the expression of glucose transporters in the pigment glia suppresses many of these phenotypes and mitigates photoreceptor degeneration downstream of tau (Figures 4 and 5). These results suggest that glucose metabolism in glial cells contributes to neuroinflammation.

The role of glucose metabolism in glia to metabolically support neurons has been well established (Morrison et al., 2013; Pellerin and Magistretti, 1994): Glial cells consume glucose to supply neurons with metabolites, such as lactate (Chatterjee and Perrimon, 2021). In the *Drosophila* retina, pigment glial cells also support neurons by receiving oxidized lipids from neurons to combat neuronal oxidative stress (Liu et al., 2015). In this study, we revealed an underappreciated role of glucose in the glia to regulate its inflammatory responses.

The mechanism by which enhanced glucose uptake in glial cells suppresses neurodegeneration caused by tau expression seems to be coupled with its ability to suppress neuroinflammatory responses. However, additional studies are required to elucidate the precise mechanism. Changes in glial activity under disease conditions are often associated with alterations in the metabolic signatures including glucose metabolism pathways (Edison, 2020; Jha and Morrison, 2018; Wang, 2019). Multiple signaling pathways that sense metabolic status are conserved across species and also known to regulate inflammatory responses (Saito et al., 2019). For example, Liver kinase B1 (LKB1) is a key regulator of metabolism (Compton et al., 2023), and one of the downstream kinases of LKB1, AMP-activated protein kinase (AMPK), has been reported to promote anti-inflammatory responses (Compton et al., 2023) via negatively regulating the nuclear factor κB (NF-κB) signaling in microglia (Ramamurthy and Ronnett, 2006). Another critical energy regulator, Sirtuin 1 (SURT1), can suppress NF-κB signaling (Zhang et al., 2020). Many regulatory mechanisms of NF-κB signaling are conserved among species (Buchon et al., 2014), and these pathways can also regulate inflammatory responses in *Drosophila* (Hetru and Hoffmann, 2009). Since multiple AMPs that function downstream of NF-κB signaling are activated upon tau-expression and can be further suppressed upon GLUT3 co-expression, further studies using metabolic and transcriptomic approaches will likely facilitate the mechanistic understanding.

Our study also revealed that pigment glial cells can function as immune cells in the fly retina which are activated in response to tau-induced lesions. Pigment glia ensheathes individual ommatidium, the minimum unit of the compound eye, to provide optical insulation that prevents extraneous light rays from inappropriately activating the photoreceptors (Tomlinson, 2012). It also accumulates lipid droplets in response to elevated oxidative stress in photoreceptor neurons (Liu et al., 2015) and lipid accumulation has been linked to microglial activation in mammalian microglia (Jung and Mook-Jung, 2020; Marschallinger et al., 2020). Comprehensive single-cell RNA seq revealed that AMP expression overlaps with a pigment glia marker "*santa-maria*" (Li et al., 2022; Yeung et al., 2022). Further characterization of pigment glial cells to identify the equivalent mammalian glial cell types will make the *Drosophila* retina an *in vivo* platform to study neuroinflammation and neuron-glia interaction.

Materials and Methods

Fly stocks and husbandry

The following fly stocks were obtained from the Bloomington *Drosophila* Stock Center (BDRC): *UAS-CD8::RFP* (27391), *UAS-Luciferase* (35788), *UAS-Luciferase RNAi* (31603), *GMR-tau* P301L (51377), *UAS-CD8::GFP* (5137), *GMR-GAL4* (9146), *Rh1-GAL4* (8691), and *54C-GAL4* (27328). *UAS-Glut1*^{d05758}, which is a P{XP} insertion containing UAS elements upstream of *Glut1*, was obtained from Harvard Medical School (http://flybase.org/reports/FBti0055936.htm, Exelixis *Drosophila* Collection) (Thibault et al., 2004). *UAS-drpr RNAi* (HMJ30231) and *UAS-NimC4 RNAi* (HMJ23355) were obtained from the Japanese National Institute of Genetics (NIG-FLY). *w*^{1118} and *UAS-GLUT3* were a gift from Marie Thérèse Besson (Besson et al., 2015). *UAS-tau* (wildtype 0N4R) was a gift from Mel. B. Feany (Wittmann et al., 2001). Genotypes of the flies used in the experiments are described in Table S1 and S2. Flies were reared in a standard medium containing 10% glucose, 0.7% agar, 9% cornmeal, 4% Brewer's yeast, 0.3% propionic acid, and 0.1% n-butyl p-hydroxybenzoate [w/v]. Flies were maintained at 25°C under light-dark cycles of 12:12 hours.

Measurement of eye size

Images of fly eyes were captured with an Olympus digital microscope DSX110 or the Leica MZ16 stereomicroscope, and the area of the eye surface was measured using Fiji (NIH). Eyes from more than 4 flies were analyzed for each genotype.

Western blotting

Western blotting was carried out as described previously (Saito et al., 2021). The membranes were blotted with the antibodies described below, incubated with appropriate secondary antibodies, and detected using Immobilon Western Chemiluminescent HRP Substrate (Merck Millipore). One of the membranes was probed with anti-actin (sigma) and used as the loading control for other blots. Anti-tau pS202 (CP13) and anti-phospho-Ser396/404-tau antibody (PHF1) were a gift from Peter Davis (Albert Einstein College of Medicine, USA). Anti-tau antibody (T46, Thermo, #13–6400), Anti-pospho-Ser262-tau antibody (pSer262, abcam, #ab92627) anti-Rhodopsin 1 antibody (4c5, Developmental Studies Hybridoma Bank), and anti-

actin antibody (Sigma, #A2066) were purchased. The chemiluminescent signal was detected by Fusion FX (Vilber) and intensity was quantified using Fiji (NIH). Western blots were repeated a minimum of 3 times with different animals and representative blots are shown. Flies used for western blotting were 5-7 days old after eclosion.

Histological analysis

Fly heads at 3-5 days after eclosion were fixed in Bouin's fixative for 48 hours at room temperature (R.T.) incubated for 24 hours in leaching buffer (50 mM Tris/150 mM NaCl) and embedded in paraffin. Serial sections (6 μm thickness) through the entire heads were stained with hematoxylin and eosin and examined by bright-field microscopy. Images of the sections that include the lamina were captured with the microscope BZ-X700 (Keyence) or the microscope Mica (Leica). The retinal degeneration was analyzed using Fiji (NIH). Heads from more than 3 flies (more than 5 hemispheres) were analyzed for each genotype.

Electron microscopy

Decapitated heads were cut in half vertically, then incubated in primary fixative solution (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer) at R.T. for 2 hours. After washing heads with 3% sucrose in 0.1 M sodium cacodylate buffer, fly heads were post-fixed for 1 hour in secondary fixation (1% osmium tetroxide in 0.1 M sodium cacodylate buffer) on ice. After washing with H2O, heads were dehydrated with ethanol and infiltrated with propylene oxide and Epon mixture (TAAB and Nissin EM) for 3 hours. After infiltration, specimens were embedded with Epon mixture at 70°C for 2~3 days. Some longitudinal sections cut out by glass knives were stained with toluidine blue to check the position. Thin sections (70 nm) of retinal sections were collected on copper grids. The sections were stained with 2% uranyl acetate in 70% ethanol and Reynolds' lead citrate solution. Electron micrographs were obtained with a VELETA CCD Camera (Olympus Soft Imaging Solutions GMBH) mounted on a JEM-1010 electron microscope (Jeol Ltd.).

RNA extraction and quantitative real-time PCR analysis

Heads from more than 25 flies were mechanically isolated, and total RNA was extracted using ISOGEN (NipponGene) followed by reverse-transcription using PrimeScript RT reagent kit

(Takara). The resulting cDNA was used as a template for PCR with THUNDERBIRD SYBR qPCR mix (TOYOBO) on a Thermal Cycler Dice real-time system TP800 (Takara). Expression of genes of interest was standardized relative to *rp49* or *actin*. Relative expression values were determined by the ΔΔCT method (Livak and Schmittgen, 2001). Experiments were repeated three times, and a representative result was shown. Primers were designed using DRSC FlyPrimerBank (Harvard Medical School). Primer sequences are shown below.

Attacin C for 5’-CTGCACTGGACTACTCCCACATCA-3′

Attacin C rev 5’-CGATCCTGCGACTGCCAAAGATTG-3′

Cecropin A1 for 5′-CATTGGACAATCGGAAGCTGGGTG-3′

Cecropin A1 rev 5’-TAATCATCGTGGTCAACCTCGGGC-3′

Diptericin B for 5′-AGGATTCGATCTGAGCCTCAACGG-3′

Diptericin B rev 5’-TGAAGGTATACACTCCACCGGCTC-3′

Drosomycin for 5′-AGTACTTGTTCGCCCTCTTCGCTG-3′

Drosomycin rev 5’-CCTTGTATCTTCCGGACAGGCAGT-3′

Metchnikowin for 5′-CATCAATCAATTCCCGCCACCGAG-3′

Metchnikowin rev 5’-AAATGGGTCCCTGGTGACGATGAG-3′

drpr for 5’-GCAGATGCCTGAATAACTCCTC-3′

drpr rev 5’-TCCTTGCATTCCATGCCGTAG-3′

NimC4 for 5’-GAACGAGACGATACGAGCCAC-3′

NimC4 rev 5’-GGTGACTTGTTCCTCCTCTGA-3′

rp49 for 5′-GCTAAGCTGTCGCACAAATG-3′

rp49 rev 5′- GTTCGATCCGTAACCGATGT-3′

actin 5C for 5′-TGCACCGCAAGTGCTTCTAA-3′

actin 5C rev 5′-TGCTGCACTCCAAACTTCCA-3′

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Author contributions

M.O. and K.A. convinced and designed this project. M.O. and S.N. conducted experiments and analyzed the results. E.S. supervised the electron microscope experiments. S.Y. provided critical comments and edited the manuscript. M.O. and K.A. wrote and revised the manuscript.

Ethics declarations

The study was approved by the Research Ethics Committee of Tokyo Metropolitan University.

Competing interests

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed in this study are available from the corresponding author upon request.

Figure legends

Figure 1. Human tau expression in the fly retina causes photoreceptor degeneration, swelling of the laminal cortex, and inclusion formation.
(A) Schematic representation of the retina (Left) and hematoxylin and eosin (H&E)-stained sections (Right). The surface glia and cortex glia in the laminal cortex are indicated. Tau expression showed degeneration indicated by vacuoles in the retina (dashed line), swelling laminal cortex (blue shade), and inclusions (arrows and arrowheads). human tau expression was driven by GMR-GAL4, and luciferase was expressed as a control (Control). Flies were 5-day-old. Scale bar, 100 µm. (B-C) Representative transmission electron microscopy (TEM) images of eyes in 5-day-old flies expressing luciferase (*Control*) or human tau (*Tau*). Tau expression showed photoreceptor degeneration with disoriented rhabdomere (yellow shade). (D-E) Toluidine blue staining and TEM images of inclusion in the retina. Tau expression showed two types of inclusions, invaginated corneal lens (D) and debris-containing inclusions wrapped by glial cells (E).

Figure 2. Tau expression induces glial phagocytosis and AMP expression.
(A) Inclusions in the tau-expressing retina are formed by glial phagocytosis. RNAi-mediated knockdown of *drpr* or *NimC4* reduced the number of inclusions and swelling of the laminal cortex in the retina of tau-expressing flies. Representative images of H&E-stained tissue sections and quantification. Scale bar, 100 µm. Mean±SE, n=7-8, **; $p<0.01$, *; $p<0.05$, One-way ANOVA followed by Tuckey's HSD multiple comparisons test. Flies were 7 days after eclosion. (B) Tau induces antimicrobial peptide (AMP) expression. Heads carrying *GMR-GAL4* driven luciferase (Control) or human tau (Tau) were subjected to qRT-PCR to analyze the levels of AMPs. Mean±SE, n=3, ***; $p<0.001$; Student's t-test. Flies were 7 days after eclosion.

Figure 3. Enhancement of glucose uptake suppresses tau-induced photoreceptor degeneration and swelling of the laminal cortex.
(A) The reduction in the eye size caused by tau expression (compare Control and Tau) was suppressed by GLUT3 expression (compare *Tau* and *Tau+GLUT3)*. Expression of a control protein CD8::RFP did not affect tau-induced eye phenotype (*compare Tau* and *Tau+CD8::RFP*), and expression of GLUT3 alone did not *affect eye size (compare Control and GLUT3*). Scale bar, 250 µm. Mean±SE, n=5-8, ***; $p<0.001$, One-way ANOVA followed by Tuckey's HSD multiple comparisons test. (B-F) Human GLUT3 expression suppresses tau-induced photoreceptor degeneration and glial phenotype. (B) GLUT3 expression suppressed tau-induced reduction in Rhodopsin 1 protein levels (Rh1). Fly heads expressing tau with a control protein CD8::RFP (*Tau*) or tau and GLUT3 (*Tau + GLUT3*) were subjected to western blotting with an anti-Rh1 antibody. Actin was used as a loading control. Representative blot and quantification are shown. Mean±SE, n=3, n.s., ***; $p<0.001$, Student's t-test. (C-F) GLUT3 co-expression suppressed tau-induced neurodegeneration and glial phenotypes. (C) representative images of sections. (D) Mean±SE, n=7-8, **; $p<0.01$, Student's t-test. (D) GLUT3 co-expression suppressed tau-induced retinal degeneration. Quantitation of areas of the retina vacuole (Mean±SE, n=7-9, **; $p<0.01$, ***; $p<0.001$, Student's t-test). (E) GLUT3 co-expression did not affect inclusions but suppressed swelling of the laminal cortex (Mean±SE, n=7-8, *; $p<0.05$, n.s.; $p>0.05$, Student's t-test) (F) Representative TEM images of photoreceptors. GLUT3 co-expression mitigated rhabdomere disorientation in tau-expressing eyes (yellow shade). (G-I) *Drosophila* Glut1

expression also suppressed tau-induced retinal degeneration and swelling of the laminal cortex. Representative images of retinal sections (G) and quantification of areas of the retina and vacuole (H), the number of inclusions, and size of the laminal cortex (I). Mean±SE, n=6-8, *; p<0.05, **; p<0.01, ***; p<0.001, One-way ANOVA followed by Tuckey's HSD multiple comparisons test (G and I). Flies were 7 days after eclosion. Scale bar, 100 µm.

Figure 4. Enhancement of glucose uptake does not affect tau phosphorylation and accumulation. Fly heads expressing human tau with *CD8::GFP* as a control (Tau) or those co-expressing tau and *GLUT3* (*Tau + GLUT3*) were subjected to western blotting with anti-tau (total tau) or anti-phospho-tau monoclonal antibodies (pS262, PHF1 and CP13). Actin was used as a loading control. Mean±SE, n=4-6, n.s., Student's t-test. Flies were 3 days after eclosion.

Figure 5. Enhancement of glucose uptake in the pigment glia suppresses tau-induced neurodegeneration
(A-D) Pigment glia-specific GLUT3 expression using *54C-GAL4* rescued neurodegeneration and glial phenotypes in GMR-tau flies. GMR-tau, which expresses human tau directly under the control of the GMR regulatory sequence, was combined with GLUT3 expression driven by *54C-GAL4*, a pigment glia-specific driver. CD8::GFP was used as a control for GLUT3. (A) Pigment glia-specific GLUT3 expression suppressed photoreceptor degeneration (vacuole formation in the lamina) and glial swelling (increased glial cortex size) (Mean±SE, n=8, *; p<0.05, **; p<0.01, Student's t-test, Scale bar, 100 µm). Flies were 7 days after eclosion. (B) GLUT3 expression in the pigment glia suppressed AMP expressions in GMR-tau flies. AMP expression levels were measured by qRT-PCR (B, Mean±SE, n=3, **; p<0.01, ***; p<0.001, One-way ANOVA followed by Tuckey's HSD multiple comparisons test). (C-D) GLUT3 expression in the pigment cells suppressed the loss of photoreceptor neurons in GMR-tau flies. (C) Sizes of external eyes (Mean±SE, n=4, *; p<0.05, Student's t-test, Scale bar, 250 µm) and (D) Rh1 protein levels analyzed by Western blotting (Mean±SE, n=3, p=0.0517, Student's t-test). (E-G) Photoreceptor neuron-specific GLUT3 expression using *Rh1-GAL4* did not suppress photoreceptor degeneration in GMR-tau flies. GMR-tau, which expresses human tau directly under the control of the GMR regulatory sequence, was combined with GLUT3 expression driven by *Rh1-GAL4*, photoreceptor neurons-specific driver. CD8::GFP was used as a control for GLUT3. (E) Sizes of external eyes (Mean±SE, n=4-5, n.s.; p>0.05, Student's t-test, Scale bar, 250 µm), (F) Rh1 protein levels analyzed by Western blotting (Mean±SE, n=3, p=0.2457, Student's t-test) (G) Photoreceptor neuron-specific GLUT3 expression did not suppress photoreceptor degeneration (vacuole formation in the lamina) and glial swelling (increased glial cortex size) (Mean±SE, n=3, n.s.; p>0.05, One-way ANOVA followed by Tuckey's HSD multiple comparisons test, Scale bar, 100 µm).

Figure S1. RNAi-mediated knockdown for drpr and NimC4. Fly heads expressing *UAS-drpr* RNAi or UAS-*NimC4* RNAi driven by GMR-GAL4 were subjected to qRT-PCR. Mean±SE, n=3, ***; p<0.001, Student's t-test.

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Figure 1

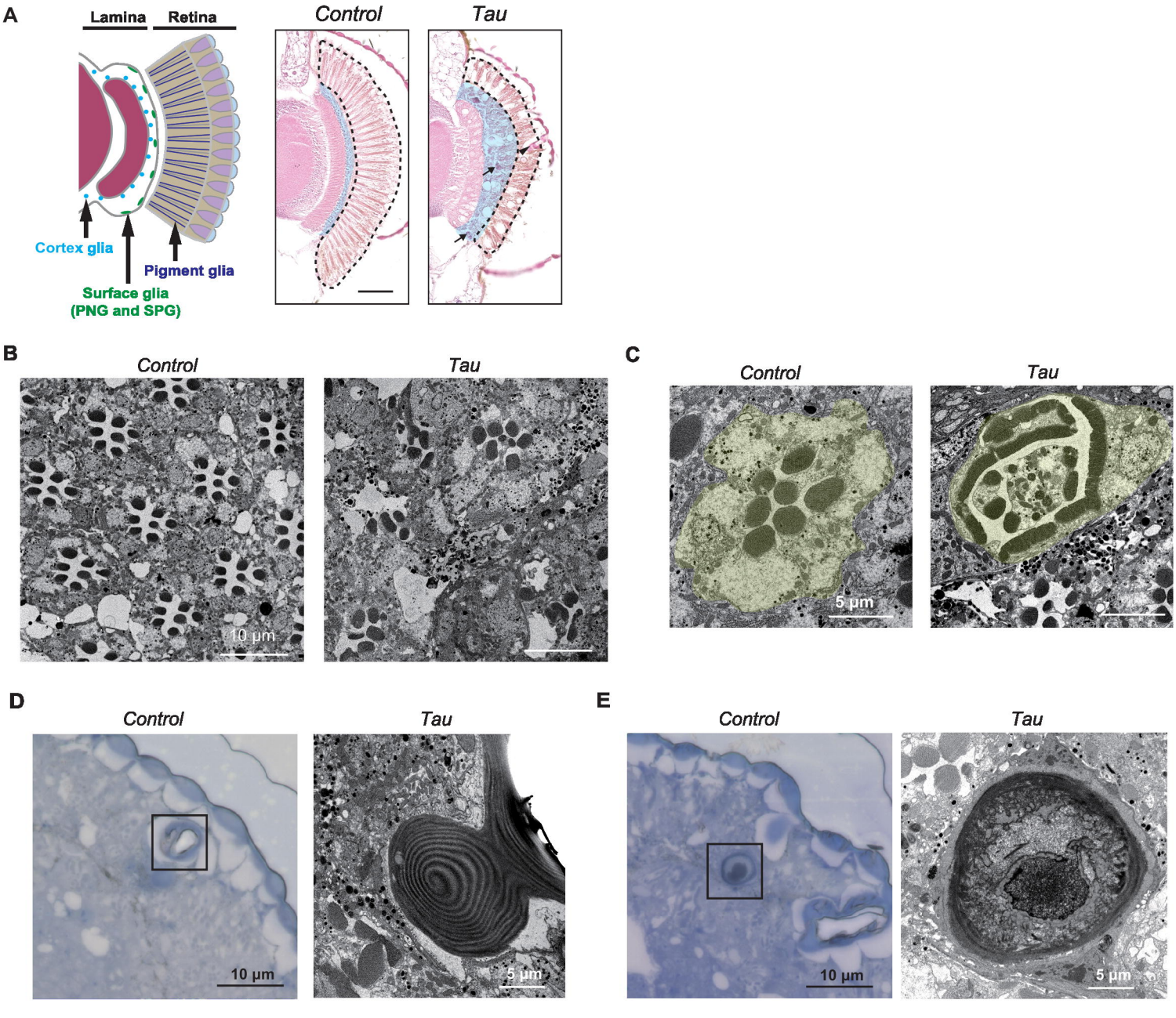
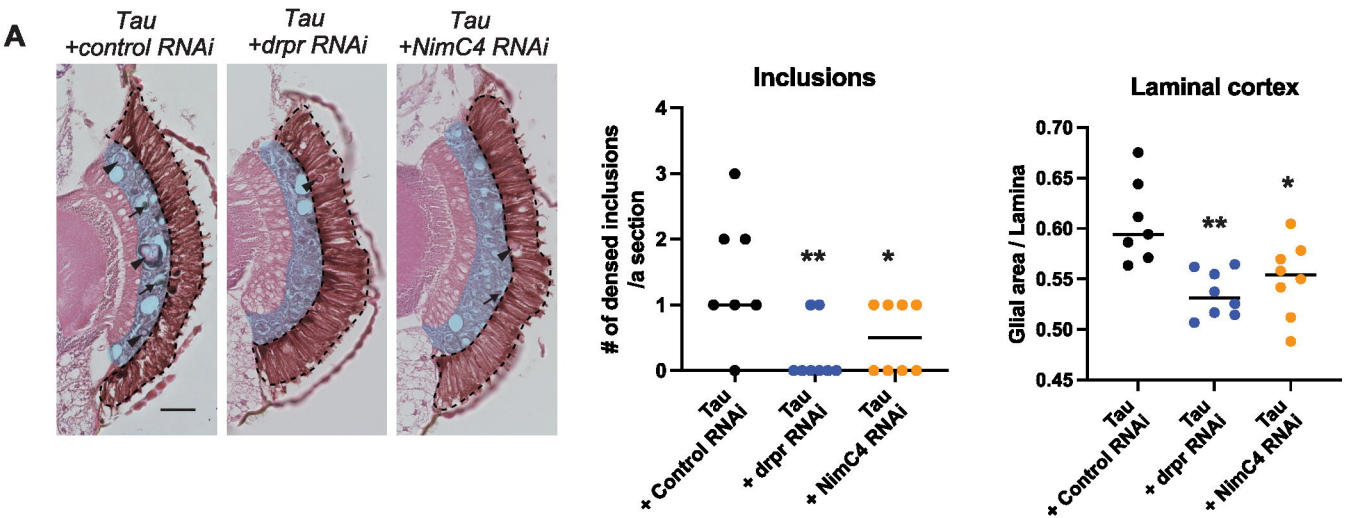

A
Lamina
Retina
Cortex glia
Pigment glia
Surface glia
(PNG and SPG)
Control
Tau
B
Control
Tau
10 µm
C
Control
Tau
5 µm
D
Control
Tau
10 µm
5 µm
E
Control
Tau
10 µm
5 µm

Figure 2


A
Tau +control RNAi
Tau +drpr RNAi
Tau +NimC4 RNAi
Inclusions
# of densed inclusions /a section
Tau + Control RNAi
Tau + drpr RNAi
Tau + NimC4 RNAi
Laminal cortex
Glial area / Lamina

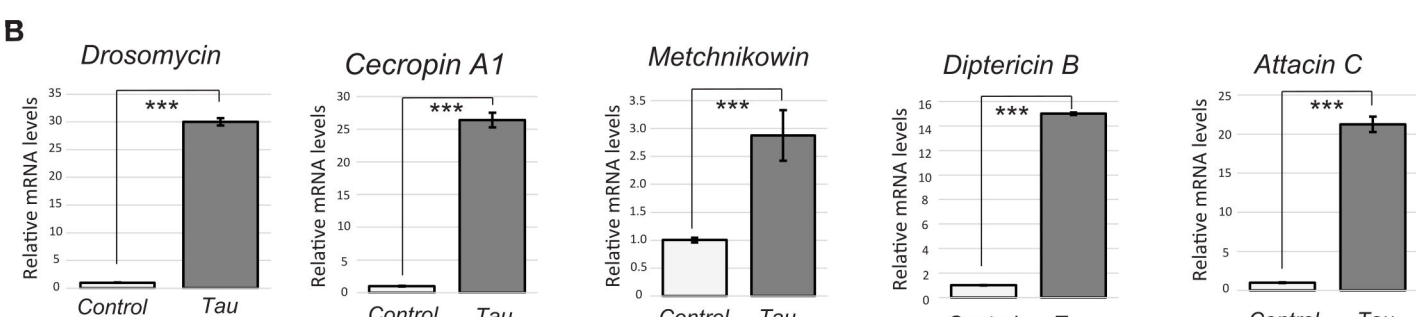

B
Drosomycin
Cecropin A1
Metchnikowin
Diptericin B
Attacin C
Relative mRNA levels
Control
Tau

Figure 3

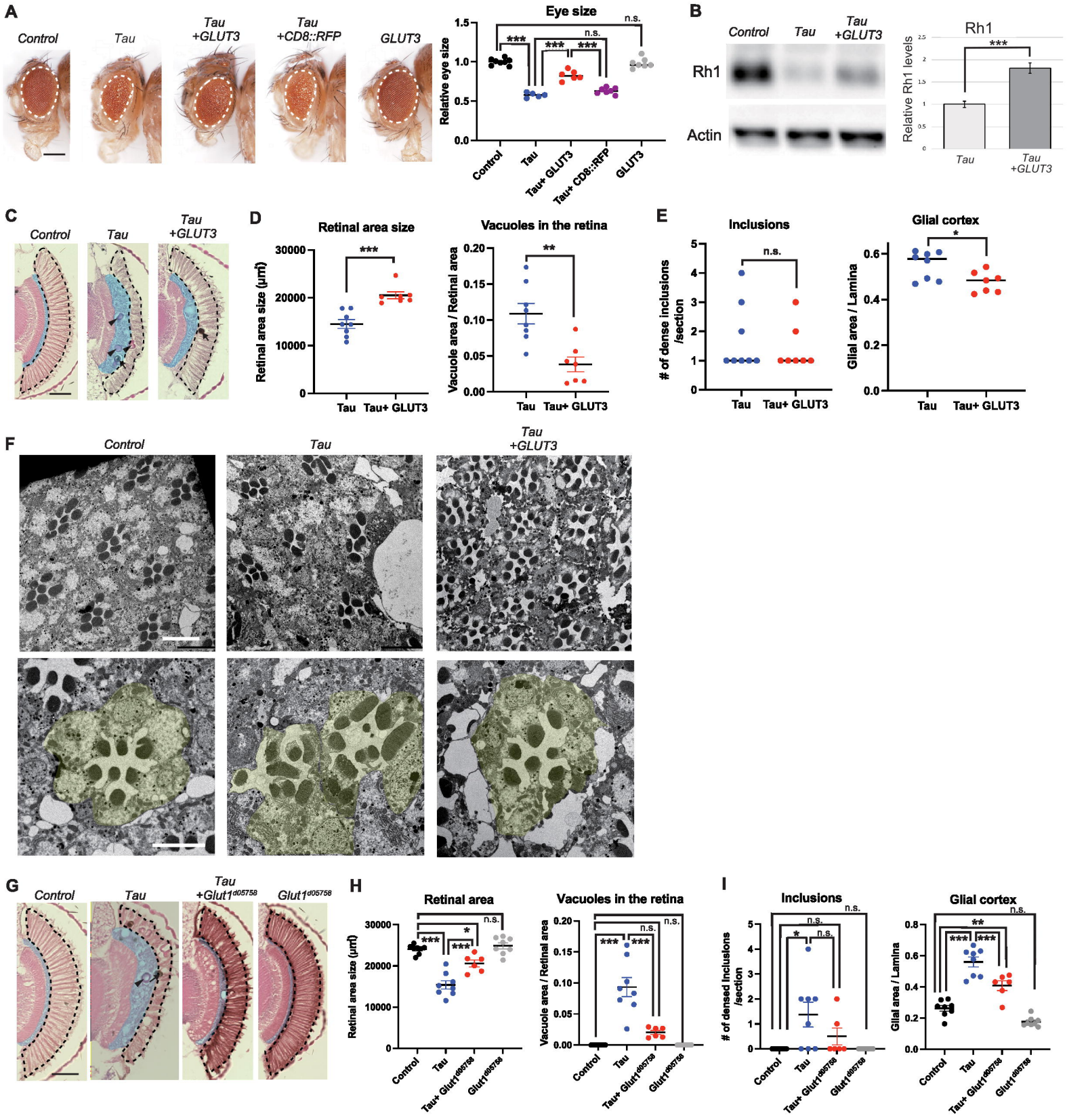

A
Control
Tau
Tau +GLUT3
Tau +CD8::RFP
GLUT3
Eye size
Relative eye size
Control
Tau
Tau+ GLUT3
Tau+ CD8::RFP
GLUT3
B
Control
Tau
Tau +GLUT3
Rh1
Actin
Rh1
Relative Rh1 levels
C
Control
Tau
Tau +GLUT3
D
Retinal area size
Retinal area size (µm²)
Vacuoles in the retina
Vacuole area / Retinal area
Tau
Tau+ GLUT3
E
Inclusions
# of dense inclusions /section
Glial cortex
Glial area / Lamina
F
Control
Tau
Tau +GLUT3
G
Control
Tau
Tau +Glut1d05758
Glut1d05758
H
Retinal area
Retinal area size (µm²)
Vacuoles in the retina
Vacuole area / Retinal area
Control
Tau
Tau+ Glut1d05758
Glut1d05758
I
Inclusions
# of densed Inclusions /section
Glial cortex
Glial area / Lamina

Figure 4

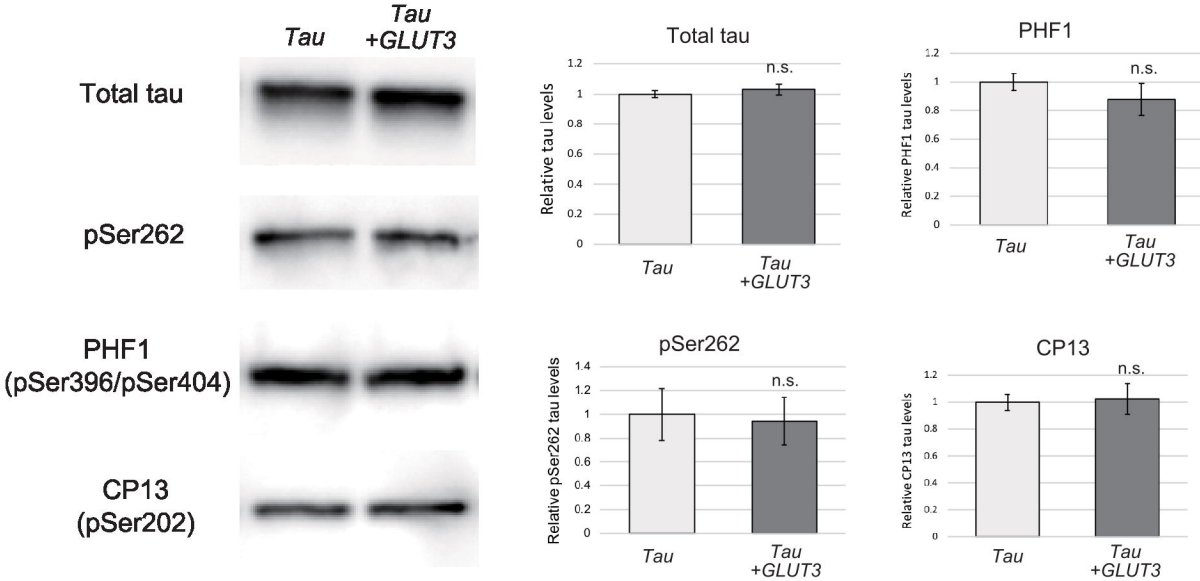
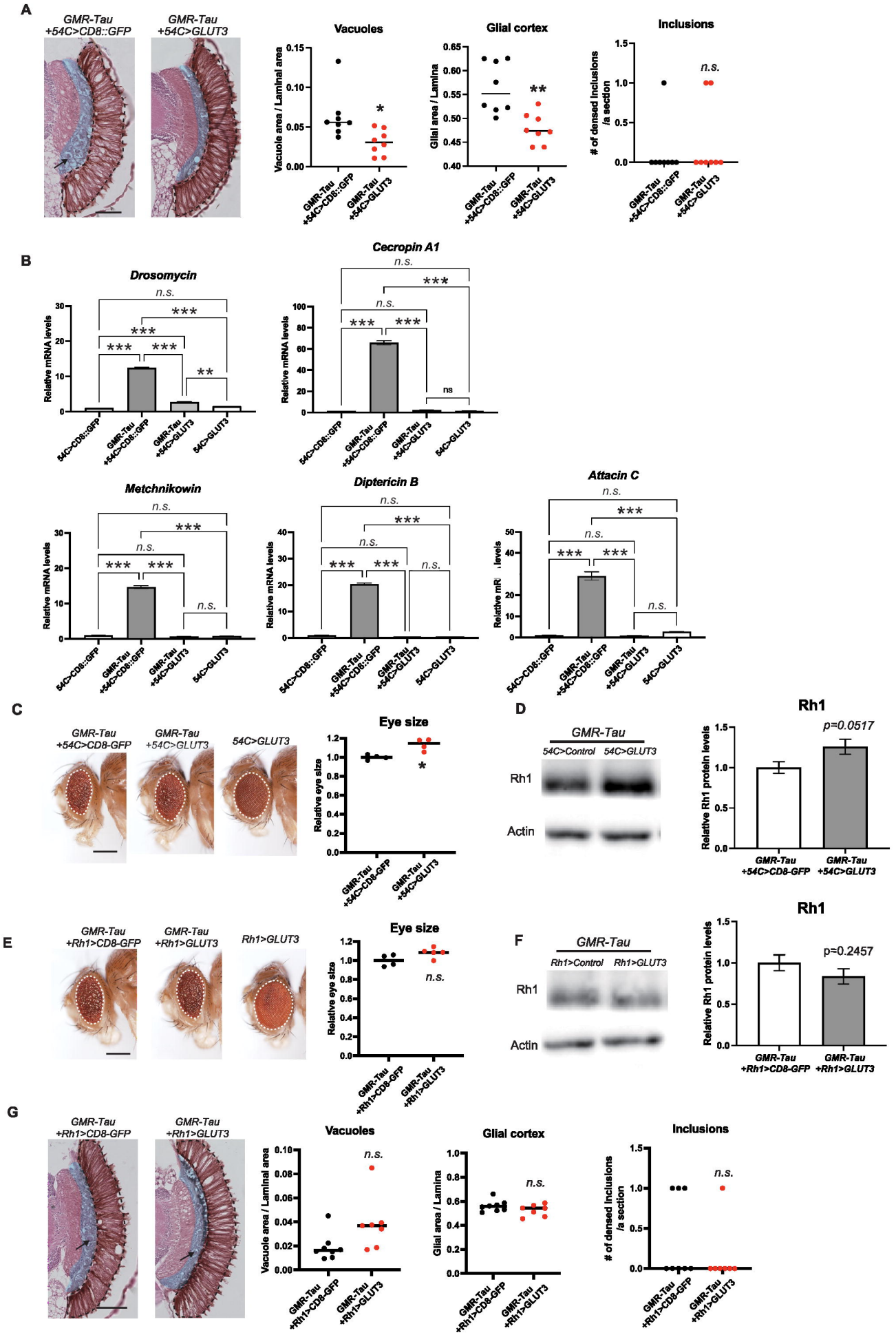

Tau
Tau +GLUT3
Total tau
pSer262
PHF1 (pSer396/pSer404)
CP13 (pSer202)
Total tau
Relative tau levels
n.s.
PHF1
Relative PHF1 tau levels
n.s.
pSer262
Relative pSer262 tau levels
n.s.
CP13
Relative CP13 tau levels
n.s.
Tau
Tau +GLUT3

Figure 5


A
GMR-Tau +54C>CD8::GFP
GMR-Tau +54C>GLUT3
Vacuoles
Vacuole area / Laminal area
Glial cortex
Glial area / Lamina
Inclusions
# of densed Inclusions /a section
n.s.
B
Drosomycin
Cecropin A1
Metchnikowin
Diptericin B
Attacin C
Relative mRNA levels
54C>CD8::GFP
GMR-Tau +54C>CD8::GFP
GMR-Tau +54C>GLUT3
54C>GLUT3
C
GMR-Tau +54C>CD8-GFP
GMR-Tau +54C>GLUT3
54C>GLUT3
Eye size
Relative eye size
D
GMR-Tau
54C>Control
54C>GLUT3
Rh1
Actin
Rh1
p=0.0517
Relative Rh1 protein levels
E
GMR-Tau +Rh1>CD8-GFP
GMR-Tau +Rh1>GLUT3
Rh1>GLUT3
Eye size
Relative eye size
F
GMR-Tau
Rh1>Control
Rh1>GLUT3
Rh1
Actin
Rh1
p=0.2457
Relative Rh1 protein levels
G
GMR-Tau +Rh1>CD8-GFP
GMR-Tau +Rh1>GLUT3
Vacuoles
Glial cortex
Inclusions

Supplemental Figure 1

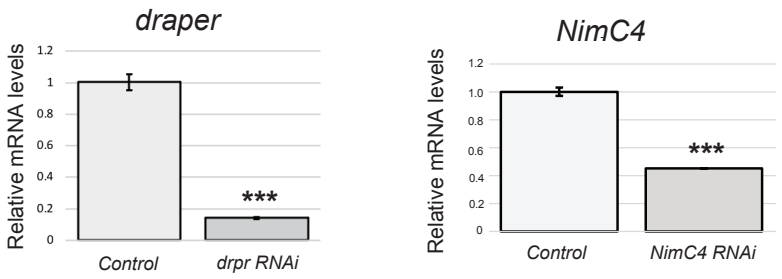


Figure S1. RNAi-mediated knockdown for drpr and NimC4. Fly heads expressing UAS-drpr RNAi or UAS-NimC4 RNAi driven by GMR-GAL4 were subjected to qRT-PCR. Mean±SE, n=3, ***; $p<0.001$, Student's t-test.

Table S1. The genotypes of flies used in each experiment related to Figure 1-5

Figure	Name	*Genotype*
Figure 1	Control	*w^{1118}; GMR-GAL4/+*
	Tau	*w^{1118}; GMR-GAL4/+; UAS-tau/+*
Figure 2A	Tau+luc RNAi	*w^{1118}; GMR-GAL4/+; UAS-tau/UAS-Luciferase RNAi*
	Tau+drpr RNAi	*w^{1118}; GMR-GAL4/UAS-drpr RNAi; UAS-tau/+*
	Tau+NimC4 RNAi	*w^{1118}; GMR-GAL4/UAS-NimC4 RNAi; UAS-tau/+*
Figure 2B	Control	*w^{1118}; GMR-GAL4/+*
	Tau	*w^{1118}; GMR-GAL4/+; UAS-tau/+*
Figure 3A	Control	*w^{1118}; GMR-GAL4/+*
	Tau	*w^{1118}; GMR-GAL4/+; UAS-tau/+*
	Tau+GLUT3	*w^{1118}; GMR-GAL4/UAS-GLUT3; UAS-tau/+*
	Tau+CD8::RFP	*w^{1118}; GMR-GAL4/UAS-mCD8::ChRFP; UAS-tau/+*
	GLUT3	*w^{1118}; GMR-GAL4/UAS-GLUT3*
Figure 3B-F	Control	*w^{1118}; GMR-GAL4/+*
	Tau	*w^{1118}; GMR-GAL4/+; UAS-tau/+*
	Tau+GLUT3	*w^{1118}; GMR-GAL4/UAS-GLUT3; UAS-tau/+*
Figure 3H-J	Control	*w^{1118}; GMR-GAL4/UAS-mCD8::ChRFP*
	Tau	*w^{1118}; GMR-GAL4/ UAS-mCD8::ChRFP; UAS-tau/+*
	Tau+Glut1^{d05758}	*w^{1118}; GMR-GAL4/+; UAS-tau/ UAS- Glut1^{d05758}*
	Glut1^{d05758}	*w^{1118}; GMR-GAL4/+; UAS- Glut1^{d05758}/+*
Figure 4	Tau	*w^{1118}; GMR-GAL4/+; UAS-tau/+*
	Tau+GLUT3	*w^{1118}; GMR-GAL4/UAS-GLUT3; UAS-tau/+*
Figure 5A-D	54C>CD8::GFP	*w^{1118}; 54C-GAL4/ UAS-mCD8::GFP*
	GMR-Tau +54C>CD8::GFP+GMR-Tau	*GMR-Tau/+; 54C-GAL4/UAS-mCD8::GFP*
	GMR-Tau +54C>GLUT3+GMR-Tau	*GMR-Tau/+; 54C-GAL4/UAS-GLUT3*
	54C>GLUT3	*w^{1118}; 54C-GAL4/UAS-GLUT3*
Figure 5E-G	Rh1>CD8::GFP	*w^{1118}; UAS-mCD8::GFP /+; Rh1-GAL4/+*

	GMR-Tau +Rh1>CD8::GFP+GMR-Tau	*GMR-Tau/+; UAS-mCD8::GFP/+; Rh1-GAL4/+*
	GMR-Tau +Rh1>GLUT3+GMR-Tau	*GMR-Tau/+; UAS-GLUT3/+; Rh1-GAL4/+*
	Rh1>GLUT3	*w^{1118}; UAS-GLUT3/+; Rh1-GAL4/+*

Table S2. The genotypes of flies used in each experiment related to Supplemental Figure 1

Figure	Name	*Genotype*
Supplemental Fig 1	Control	*w^{1118}; GMR-GAL4/+; UAS-Luciferase RNAi/+*
	drpr RNAi	*w^{1118}; GMR-GAL4/UAS-drpr RNAi*
	NimC4 RNAi	*w^{1118}; GMR-GAL4/UAS-NimC4 RNAi*