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Lamin Dysfunction Mediates Neurodegeneration in Tauopathies

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Summary

The filamentous meshwork formed by the lamin nucleoskeleton provides a scaffold for the anchoring of highly condensed heterochromatic DNA to the nuclear envelope, thereby establishing the three-dimensional architecture of the genome [1]. Insight into the importance of lamins to cellular viability can be gleaned from the laminopathies, severe disorders caused by mutations in genes encoding lamins. A cellular consequence of lamin dysfunction in laminopathies is relaxation of heterochromatic DNA [1]. Similarly, we have recently reported the widespread relaxation of heterochromatin in tauopathies [1]: age-related progressive neurodegenerative disorders, including Alzheimer's disease, which are pathologically characterized by aggregates of phosphorylated tau protein in the brain [2, 3]. Here we demonstrate that acquired lamin misregulation though aberrant cytoskeletal-nucleoskeletal coupling promotes relaxation of heterochromatin and neuronal death in an *in vivo* model of neurodegenerative tauopathy. Genetic manipulation of lamin function significantly modifies neurodegeneration *in vivo*, demonstrating that lamin pathology plays a causal role in tau-mediated neurotoxicity. We show that lamin dysfunction is conserved in human tauopathy, as super-resolution microscopy reveals a significantly disrupted nuclear lamina in postmortem tissue from human Alzheimer's disease brain. Our study provides strong evidence that tauopathies are neurodegenerative laminopathies, and identifies a new pathway mediating neuronal death in currently untreatable human neurodegenerative disorders, including Alzheimer's disease.

Results

The model organism *Drosophila* provides a genetically tractable platform that can be used to identify and validate molecular mechanisms. In addition, panneuronal expression of

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Author Contributions

B.F. and M.B.F. conceptualized the study, participated in interpreting data and wrote the manuscript. B.F. and F.H.B. performed experiments. M.B.F. supervised the research.

The authors declare no conflicts of interest.

transgenic human tau in *Drosophila* (Figure S1A) recapitulates aspects of human tauopathies such as heterochromatin relaxation [4], DNA damage [5], activation of the cell cycle in postmitotic neurons [6], synapse loss [7], and progressive neurodegeneration [8]. Since pathogenic tau induces heterochromatin relaxation [4], and the lamin nucleoskeleton regulates chromatin dynamics [1], we investigated a potential role for lamin dysfunction in tauopathy. We first determined if lamin levels are altered in the brains of adult *Drosophila* panneuronally expressing a disease-associated mutant form of human tau [9], tau^{R406W}. Like vertebrate B-type lamin, *Drosophila* "Lamin" is expressed in most cells and developmental stages, and has a CaaX box that targets it to the nuclear membrane [10]. In 10-day-old tau^{R406W} transgenic *Drosophila* heads, Lamin protein levels were significantly decreased (Figure 1A), whereas protein levels of *Drosophila* A-type lamin, termed "Lamin C," were not affected (Figure S1B). Since Lamin C was unchanged in tau^{R406W} transgenic *Drosophila*, we focused subsequent studies on the *Drosophila* B-type lamin.

Abnormal nuclear morphology, including invagination of the nuclear envelope, is common in cells from laminopathy patients [11]. Similarly, we observed invaginations of the nuclear envelope in tau^{R406W} transgenic *Drosophila*. Costaining with elav, a neuron-specific protein, indicated that nuclei harboring invaginations were neuronal (Figure 1B–C). In addition to decreased Lamin protein levels in tau transgenic *Drosophila* brains, morphological changes of the lamin nucleoskeleton suggest that tau causes dysfunction of neuronal Lamin. We next determined if Lamin invaginations coincide with pathological tau. Tau phosphorylation is a well-characterized pathogenic event in Alzheimer's disease and related tauopathies [12]. 86% of nuclei containing Lamin invaginations in tau^{R406W} transgenic *Drosophila* were positive for tau phosphorylated at serine 214, a disease-associated tau phosphopepitope (Figure 1D). Taken together, these data suggest that pathological tau reduces Lamin protein levels and causes lamin dysfunction by altering the three-dimensional morphology of the lamin nucleoskeleton.

To determine whether Lamin reduction and disorganization are specific to tau^{R406W} versus a general feature of tau pathology, we utilized *Drosophila* transgenic for human wild-type tau (tau^{WT}) [8] or a pseudohyperphosphorylated form of human tau (tau^{E14}) [13]. Panneuronal expression of tau^{WT} or tau^{E14} significantly reduced Lamin levels in adult *Drosophila* brains (Figure S1C–D), and caused Lamin to invaginate (Figure S1E–F). The extent of Lamin reduction and invagination caused by expression of tau^{WT} or tau^{E14} correlated with their toxicities, which cause substantially less and more, respectively, neuronal toxicity than tau^{R406W} [8, 13]. These data suggest that Lamin pathology is a general feature of tau-induced toxicity and is downstream of aberrant tau phosphorylation. Since expression of tau^{R406W} provides a level of toxicity that is well suited for genetic manipulation and biochemical analysis [8], 10-day-old tau^{R406W} transgenic flies were used in our subsequent experiments, and are referred to as tau hereafter for simplicity.

We next determined if Lamin dysfunction plays a causal role in promoting cell death. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) detects DNA fragmentation, and has been used to quantify neuronal death in tau transgenic *Drosophila* [4, 6, 14]. Further depletion of Lamin levels in neurons of tau transgenic *Drosophila* by RNAi-mediated reduction of *Lamin* significantly increased neuronal death compared to tau

expressed alone (Figure 1E). As an additional control, we measured TUNEL levels in control and tau transgenic *Drosophila* with RNAi targeting *luciferase* (Figure S1G). We next determined if Lamin depletion activates the cell cycle in postmitotic neurons of tau transgenic flies. Ectopic expression of cell cycle markers in postmitotic neurons and their coincidence with tau pathology is a well-described feature of tauopathies [15, 16], and abnormal activation of the cell cycle in neurons can drive apoptosis in tau transgenic *Drosophila* [6]. Based on staining with proliferating cell nuclear antigen (PCNA), we found a significant increase in ectopic cell cycle activation in tau transgenic *Drosophila* brains when *Lamin* was panneuronally depleted via RNAi (Figure 1F). RNAi-mediated *Lamin* reduction enhanced locomotor defects [4] and shortened median lifespan [8] of tau transgenic *Drosophila* (Figure S1H–I), suggesting that Lamin depletion affects the overall health of tau transgenic *Drosophila*. We validated *Lamin* mRNA knockdown (Figure S1J), and Lamin protein knockdown in control and tau transgenic *Drosophila* heads (Figure S1K–S1L). RNAi targeted to *Lamin* did not change total levels of transgenic tau protein (Figure S1M). Together, these genetic manipulations are consistent with a causal role for tau-induced lamin dysfunction in driving cell cycle activation and subsequent apoptotic cell death of postmitotic neurons.

To determine if Lamin reduction and disorganization are specific to tauopathy versus a general consequence of neurodegeneration, we utilized a *Drosophila* model of spinocerebellar ataxia type 3 (SCA3; Machado-Joseph disease) [17]. We did not detect reduced Lamin levels or a disrupted Lamin nucleoskeleton in brains of adult SCA3 model *Drosophila* (Figure S1N–O). As previously reported, we observed loss of Kenyon cells, the projection neurons of the mushroom bodies, in brains of SCA3 model *Drosophila* [18]; however, genetic reduction of *Lamin* did not exacerbate Kenyon cell loss (Figure S1P). These data suggest that Lamin reduction and disorganization are not a result of general neurodegeneration.

While knockout of B-type lamins in the mouse forebrain reduces neuronal number [19], the effects of lamin dysfunction in the adult brain are not well characterized. We thus determined whether Lamin pathology can drive heterochromatin relaxation, DNA damage, cell cycle activation, and apoptosis in neurons. Since modest reduction of *Lamin* levels mediated by transgenic RNAi was not toxic in the absence of transgenic human tau expression, while null or strong *Lamin* mutations are reported to be lethal or semilethal [20, 21], we used a homozygous partial loss of function allele, Lam^{A25}, to genetically induce Lamin dysfunction in *Drosophila*. Lam^{A25} removes sequences encoding the CaaX domain responsible for localizing Lamin to the inner nuclear membrane [22] (Figure S2A). Like pathological tau [4], Lamin dysfunction significantly reduced total levels of dimethylated lysine 9 of histone 3 (H3K9me2) and Heterochromatin Protein 1α (HP1α), markers of heterochromatic DNA, in adult *Drosophila* brains (Figure 2A). Like many other cell types, *Drosophila* neurons possess cytologically distinct regions of DAPI-dense heterochromatic DNA termed “chromocenters.” We observed loss of chromocenter staining in neurons of Lam^{A25} mutant *Drosophila* (Figure 2B–C, Figure S2B–C), suggesting that, like pathological tau [4], Lamin dysfunction disrupts genomic architecture in neurons. We have previously reported that tau-induced relaxation of heterochromatin causes increased expression of

genes in the brain that are normally silenced by heterochromatin [4]. Three of the six genes that we previously reported as most significantly affected by heterochromatin relaxation in tau transgenic fly heads [4] were also expressed at higher levels in Lam^{A25} mutant fly heads (Figure S2D). Since low baseline gene expression causes variability in RT-PCR, it is possible that modest increases in gene expression were undetected. DNA damage is a cellular hallmark of tauopathies in both *Drosophila* and humans [5, 16], and is a known consequence of heterochromatin relaxation [23]. Significantly more neurons of flies expressing transgenic tau or harboring the Lam^{A25} mutation had DNA damage compared to control (Figure 2D). In tau transgenic flies, all nuclei harboring DNA damage also had a lamin invagination (Figure S2E), providing further evidence of the strong connection between lamin pathology and DNA damage in adult neurons.

Having established that Lamin dysfunction is sufficient to cause both heterochromatin relaxation and DNA damage in neurons, we next asked if Lamin pathology induces neuronal cell cycle activation and apoptosis. Both PCNA (Figure 2E, average of 23.5 in six Lam^{A25} mutant brains, SEM=4.91, control=0) and phosphorylated histone 3 (Figure S2F) staining of adult fly brains indicated that Lamin misregulation causes ectopic activation of the cell cycle in postmitotic neurons. Similarly, TUNEL staining revealed neuronal apoptosis in Lam^{A25}-mutant brains (Figure 2F, average of 87.5 in six Lam^{A25} mutant brains, SEM=10.46, control=0), demonstrating that blocking incorporation of Lamin into the nuclear envelope triggers cell death in postmitotic neurons. Lam^{A25} mutant animals also had significantly reduced locomotor activity (Figure 2G) and lifespan (Figure 2H), both of which are features of tauopathy [4, 8, 24].

As additional evidence that lamin pathology lies in the pathway of tau-induced neurotoxicity, heterozygous Lam^{A25} mutation synergizes with transgenic tau in the brain to induce significantly more neurodegeneration (Figure S2G) and neuronal cell cycle activation (Figure S2H) than either does alone. Similarly, in the background of heterozygous Lam^{A25} mutation, transgenic tau causes further heterochromatin relaxation (Figure S2I). Together, these experiments provide strong evidence that Lamin dysfunction is upstream of heterochromatin relaxation, DNA damage, neuronal cell cycle activation, and apoptosis in the pathway of tau-induced neurotoxicity.

We next investigated the mechanism by which pathological tau reduces Lamin protein levels and alters its nuclear distribution. In addition to its role as a microtubule-binding protein, tau also binds actin and induces overstabilization and bundling of filamentous actin (F-actin) in tauopathy [25, 26]. Genetically reversing actin overstabilization significantly suppresses tau neurotoxicity [14, 26], demonstrating that overstabilization of F-actin is a causal event in tau-induced neurodegeneration. Since the actin cytoskeleton has intimate connections to the lamin nucleoskeleton through the nuclear envelope-spanning linker of nucleoskeleton and cytoskeleton (LINC) complex [27], we determined if manipulating levels of F-actin affects the nuclear lamina. In tau transgenic *Drosophila* brains, reversing F-actin stabilization by overexpressing Gelsolin [14], an actin-severing protein, significantly increased Lamin protein levels (Figure 3A–B), strongly supporting the hypothesis that tau-induced actin stabilization drives neurotoxicity by disrupting the nuclear lamina. Gelsolin overexpression significantly decreased the number of neurons harboring disruptions in the nuclear lamina in

tau transgenic *Drosophila* (Figure 3C). In addition, Gelsolin overexpression significantly improved locomotion and prolonged the median lifespan of tau transgenic *Drosophila* (Figure S3A–B), consistent with our previous finding that Gelsolin overexpression reduces tau-induced apoptosis in *Drosophila* brains [14]. To further test if stabilization of F-actin disrupts Lamin levels and localization, we promoted formation of Factin in neurons by overexpressing WASp (Figure S3C) or the RD domain of spire (Figure S3D) [28, 29] in the absence of transgenic tau. Like transgenic expression of human tau, ectopic stabilization of F-actin in neurons significantly decreased Lamin protein levels in the brain (Figures 3D–E). In addition, genetically promoting actin polymerization induced invagination of the nuclear lamina (Figure 3F). We observed concentrations of F-actin [30, 31] in close proximity to Lamin invaginations in tau transgenic flies (Figures 3G–H), supporting the hypothesis that tau-induced stabilization of actin filaments causes local disruptions of the nuclear lamina. In addition to regulating actin dynamics in the cytoplasm, nuclear Gelsolin, WASp, or spire [32–34] could potentially affect Lamin levels and/or morphology.

Since the LINC complex acts as a physical bridge between the actin cytoskeleton and the lamin nucleoskeleton, we determined if the LINC complex facilitates F-actin-induced disruption of the lamin nucleoskeleton in tauopathy. In *Drosophila*, Lamin binds directly to LINC complex component koi, the *Drosophila* homolog of human SUN1 [35]. Koi traverses the inner nuclear envelope and binds Msp300, the *Drosophila* homolog of human nesprin, in the perinuclear space. Together, koi and Msp300 span the nuclear envelope, and Msp300 binds F-actin in the cytoplasm [36] (Figure 3I). Overstabilizing F-actin via transgenic tau or overexpression of WASp or spireRD altered the distribution of neuronal koi. While koi lined the nuclear envelope in neurons of control brains, we observed a significant increase in koi-positive blebs and focal concentrations of koi along the nuclear envelope in tau transgenic and WASp or spireRD-overexpressing neurons (Figure 3J). Reducing the interaction between F-actin and the LINC complex by RNAi-mediated reduction of *Msp300* (Figure S3E) or a transposable element insertion into *Msp300*, *Msp300^{MB00410}* [37], significantly increased Lamin levels in tau transgenic *Drosophila* brains (Figure 3K) and reduced neuronal apoptosis and aberrant cell cycle activation (Figure 3L–M). Neither of these suppressors of tau toxicity altered levels of total transgenic tau protein (Figure S3F). Collectively, these experiments provide strong support for a model in which tau-induced overstabilization of F-actin disrupts LINC complex organization and reduces lamin levels and localization, which promotes heterochromatin relaxation, subsequent aberrant cell cycle activation and neurodegeneration in tauopathy.

We next investigated lamin in human Alzheimer's disease, the most common tauopathy. As predicted, we detected a disruption of the nuclear lamina in neurons from Alzheimer's disease brains, where a significant number of neurons harbored invaginations of the nuclear lamina into the deep nuclear interior (Figure 4A, Movie S1). Quantitative analysis of lamin B revealed a significant reduction in lamin B protein levels in neurons from brains of patients with Alzheimer's disease versus controls (Figure 4B). Lamina-associated protein 2β LAP2β) also lined the nuclear invaginations, suggesting that invaginations are not restricted to the lamin protein itself (Figure 4C). In addition, membrane invaginations were lined with nuclear pores (Figure 4D), suggesting that inner and outer membranes of the nuclear

envelope invaginate, and that invaginations are likely filled with cytoplasm. To visualize the interaction between heterochromatin and lamin, we costained neuronal nuclei with lamin and H3K9me2 or HP1α, both of which are altered in Alzheimer's disease brains [4]. Unlike neuronal nuclei from controls, in which we observed formation of typical perinucleolar chromocenters, nuclei from Alzheimer's disease brains did not exhibit chromocenter staining in neurons harboring lamin invaginations (Figures 4E and 4F). Finally, we observed that F-actin (Figure 4G) and disease-associated phosphotau (Movie S2) lined the nuclear envelope invaginations in neurons from Alzheimer's disease brains, supporting our hypothesis that tau-induced stabilization of F-actin disrupts the lamin nucleoskeleton.

Discussion

Our data suggest that tauopathies are neurodegenerative laminopathies, and establish that B-type lamins are required for maintaining genomic architecture, genomic integrity, function and survival of adult neurons. We find that lamin abnormalities are present in both *Drosophila* and human tauopathy, emphasizing the clinical relevance of these data. While irregularities in nuclear shape in Alzheimer's disease have been previously reported [38], our genetic data from tau transgenic *Drosophila* suggest that lamin pathology and nuclear envelope invagination are early events that precede heterochromatin relaxation, neuronal cell cycle activation, and apoptosis. Mechanistically, we show that tau-induced stabilization of F-actin [26] causes LINC complex dysfunction and reduction and disorganization of lamin in neurons, allowing untethering of heterochromatin from the nuclear periphery, subsequent heterochromatin relaxation, DNA damage, cell cycle activation and apoptosis. Tau-induced overstabilization of F-actin is also known to disrupt mitochondrial dynamics, causing increased oxidative stress [14]. Since oxidative stress is known to cause DNA damage in the central nervous system [39], it is possible that lamin pathology and oxidative stress both contribute to some of the observed phenotypes. Our studies identify cellular mechanisms that are shared between tauopathies and laminopathies, and lay the groundwork for exploration of lamin and the LINC complex as novel therapeutic targets for the treatment of tauopathies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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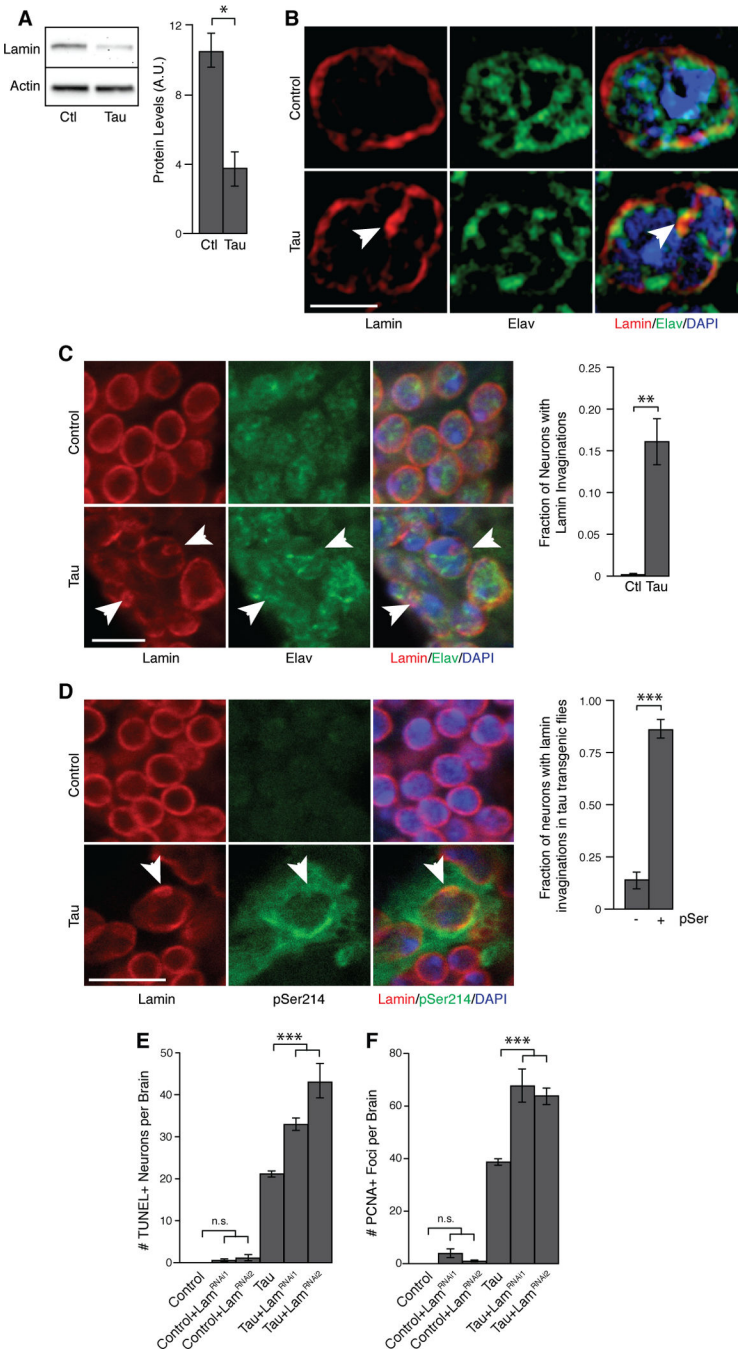

Figure 1. Lamin pathology in tau transgenic *Drosophila*

(A) Western blot for Lamin in homogenates from control and tau transgenic *Drosophila* heads, n=3.

(B) Super-resolution microscopy of Lamin and elav immunostaining in control and tau transgenic *Drosophila* brains. Arrowhead indicates Lamin invagination.

(C) Immunostaining of Lamin and elav in control and tau transgenic *Drosophila* brains. Arrowheads indicate neurons with Lamin invaginations, n=3.

(D) Immunostaining of Lamin and tau phosphorylated at serine 214 in control and tau transgenic *Drosophila* brains. Arrowhead indicates Lamin invagination in a pSer214-positive neuron, n=3.
(E) Neuronal degeneration assayed by TUNEL staining in brains of control and tau transgenic *Drosophila* harboring RNAi transgenes targeted to *Lamin*, n=6.
(F) Cell cycle activation assayed by PCNA staining in brains of control and tau transgenic *Drosophila* harboring RNAi transgenes targeted to *Lamin*, n=6.
All flies are 10 days old. Controls are *elav-GAL4/+*. Scale bars are 1 μm in (B) and 5 μm in (C) and (D). Data are presented as mean ± SEM, unpaired t-test or ANOVA, **p<0.01, ***p<0.001. See also Figure S1.

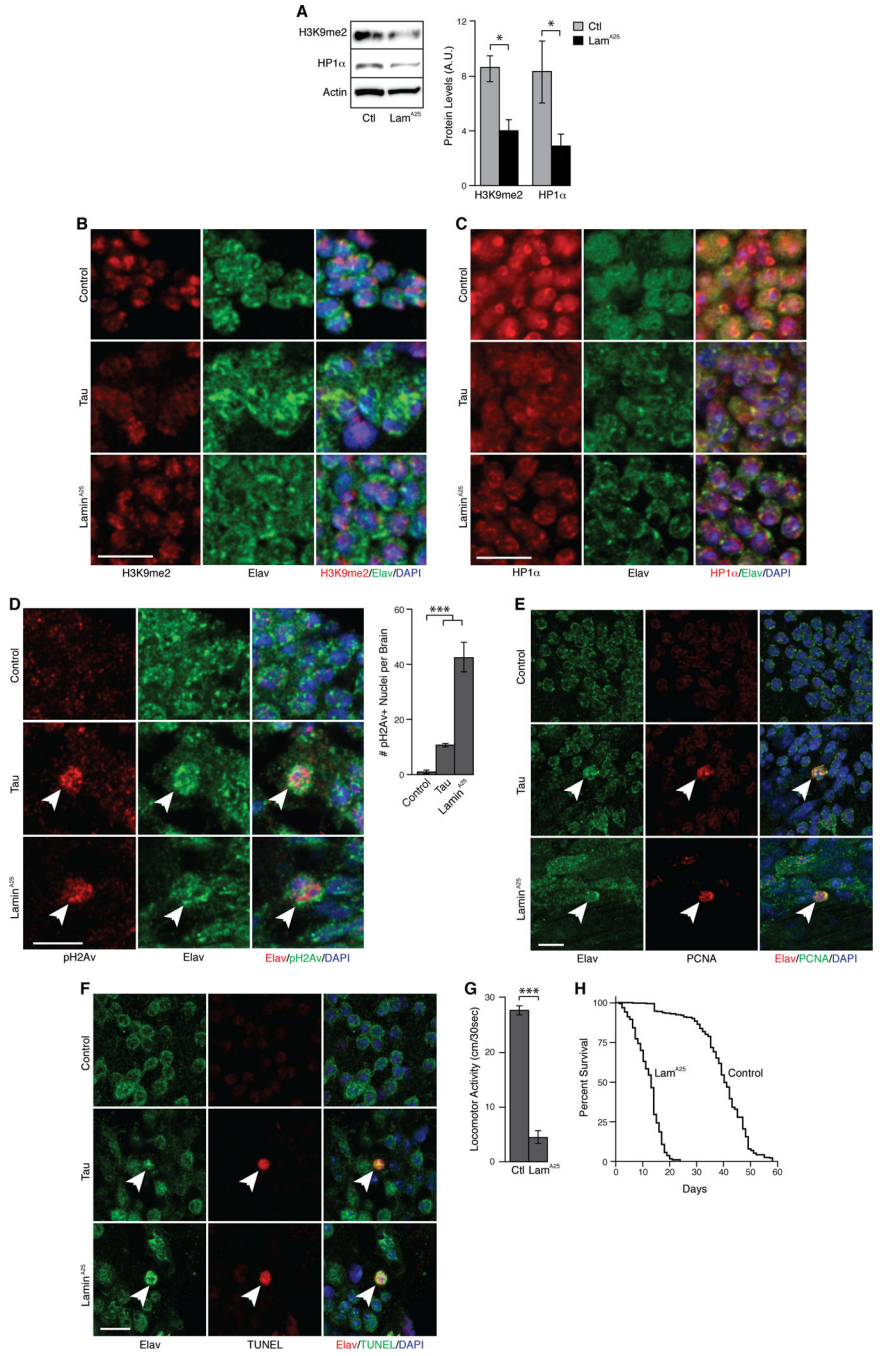

Figure 2. Lamin dysfunction causes neurodegeneration, heterochromatin relaxation, and DNA damage in the adult *Drosophila* brain

(A) Western blot for markers of heterochromatin in homogenates from control and *Lam*^{A25} mutant *Drosophila* heads, n=3.

(B) Neuronal heterochromatin assayed by immunostaining of H3K9me2 and elav in control, tau transgenic, and *Lam*^{A25} mutant fly brains.

(C) Neuronal heterochromatin assayed by immunostaining of HP1α and elav in control, tau transgenic, and *Lam*^{A25} mutant fly brains.

(D) Neuronal DNA damage assayed by immunostaining of pH2Av and elav in control, tau transgenic, and Lam^{A25} mutant fly brains. Arrows indicate DNA damage in elav-positive neurons, n=6.
(E) Neuronal cell cycle activation assayed by immunostaining of control, tau transgenic, and Lam^{A25} mutant fly brains with elav and PCNA. Arrows indicate cell cycle activation in Elav-positive neurons. n=6.
(F) Neuronal degeneration assayed by immunostaining of control, tau transgenic, and Lam^{A25} mutant fly brains with elav and TUNEL. Arrows indicates TUNEL staining in elav-positive neurons. n=6.
(G) Locomotor activity of control and Lam^{A25} mutant *Drosophila.* n=18.
(H) Lifespan of control and Lam^{A25} mutant *Drosophila*. n=300, $p<0.0001$, log-rank test. Flies are 10 days old except in (H). Control is w^{1118} in (A, G, and H) and *elav-GAL4/+* in (B–F). Lam^{A25} mutants are homozygous. Scale bars are 5 μm. Data are presented as mean ± SEM, t-test or ANOVA unless otherwise stated, *$p<0.05$, ***$p<0.001$. See also Figure S2.

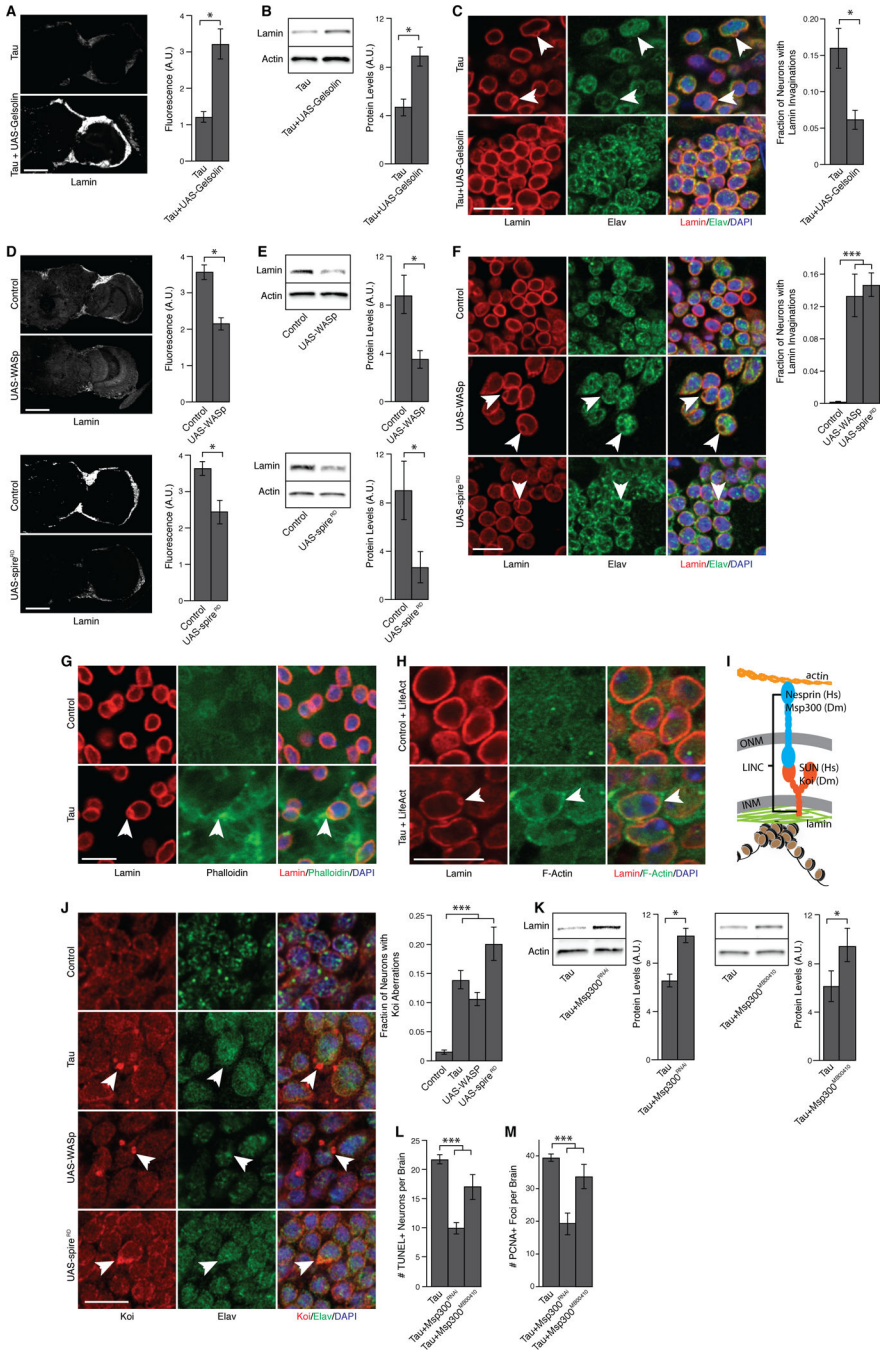

Figure 3. Tau disrupts lamin via stabilization of F-actin and LINC complex dysfunction in *Drosophila*

(A) Lamin immunofluorescence in cortical neurons of tau transgenic flies with and without overexpression of Gelsolin, n=3.

(B) Lamin protein levels in brains of tau transgenic flies with and without overexpression of Gelsolin, n=3.

(C) Immunofluorescence of Lamin in elav-positive neurons of tau transgenic flies with and without overexpression of Gelsolin, n=3, arrows indicate Lamin invaginations, n=3.

(D) Lamin immunofluorescence in cortical neurons of *Drosophila* overexpressing WASp or spireRD, n=3.
(E) Lamin protein levels in brains of *Drosophila* overexpressing WASp or spireRD, n=3.
(F) Immunofluorescence of Lamin in elav-positive neurons of *Drosophila* overexpressiong WASp or spireRD, n=3, arrows indicate Lamin invaginations.
(G) Immunostaining of Lamin and phalloidin in control and tau transgenic fly brains, arrow indicates Lamin disruption adjacent to F-actin.
(H) Immunostaining of Lamin and GFP in control and tau model *Drosophila* transgenic for a GFP-based reporter of F-actin, arrow indicates Lamin disruption adjacent to F-actin.
(I) Schematic diagram of the LINC complex.
(J) Immunostaining of koi in control, tau transgenic, and WASp- and spireRD-overexpressing fly brains, n=3, arrows indicate altered koi distribution in elav-positive neurons, n=3.
(K) Lamin protein levels in brains of tau transgenic *Drosophila* versus tau transgenic *Drosophila* with *Msp300*^{MB00410} or RNAi targeted to *Msp300*, n=3.
(L) Neuronal apoptosis assayed by TUNEL staining in tau transgenic *Drosophila* versus tau transgenic *Drosophila* with *Msp300*^{MB00410} or RNAi targeted to *Msp300*, n=6.
(M) Cell cycle activation assayed by PCNA staining in tau transgenic *Drosophila* versus tau transgenic *Drosophila* with *Msp300*^{MB00410} or RNAi targeted to *Msp300*, n=6.
Control is *elav-GAL4/+*. All flies are 10 days old. Scale bars are 30 μm in (A) and (D), and 5 μm in (C), (F–H), and (J). Data are presented as mean ± SEM, unpaired t-test or ANOVA, *p<0.05, **p<0.01, ***p<0.001. See also Figure S3.

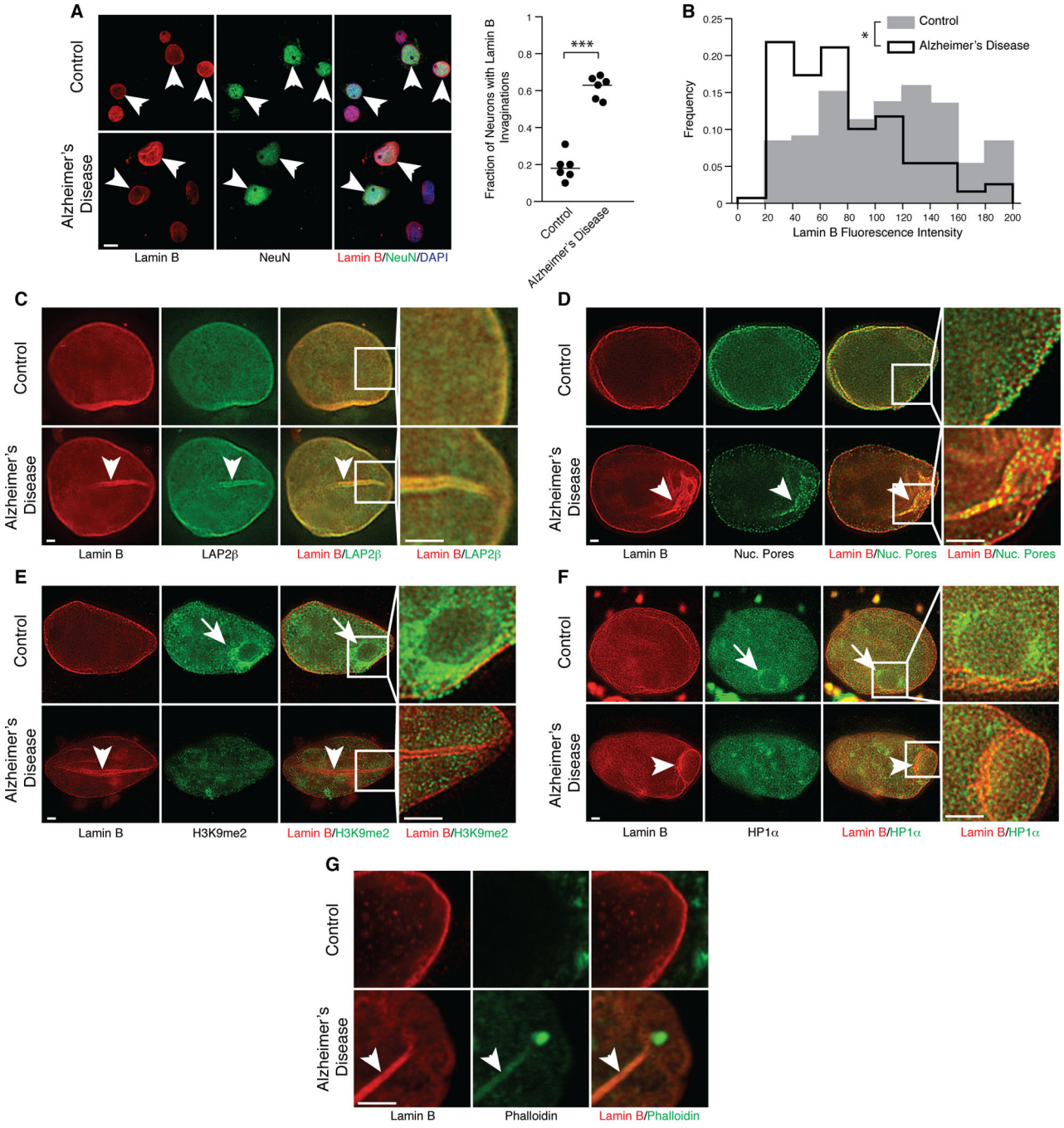

Figure 4. Lamin pathology in human Alzheimer's disease

(A) Lamin B and NeuN immunostaining in nuclei from postmortem control and Alzheimer's disease frontal cortex. Arrows indicate NeuN-positive neurons. n=6, ***p<0.001, t-test.

(B) Lamin B levels in NeuN-positive neurons from control and Alzheimer's disease frontal cortex. n=6, *p=0.02, Mixed Effect Model.

(C) Super-resolution microscopy of Lamin B and LAP2β in neurons from control and Alzheimer's disease frontal cortex.

(D) Super-resolution microscopy of Lamin B and nuclear pores in neurons from control and Alzheimer's disease frontal cortex.
(E) Super-resolution microscopy of Lamin B and H3K9me2 in neurons from control and Alzheimer's disease frontal cortex.
(F) Super-resolution microscopy of Lamin B and HP1α in neurons from control and Alzheimer's disease frontal cortex.
(G) Super-resolution microscopy of Lamin B and F-actin visualized via phalloidin staining in neurons from control and Alzheimer's disease frontal cortex.
Arrowheads indicate lamin invaginations and arrows indicate perinucleolar staining of heterochromatin in (E–F). Scale bars are 5 μm in (A) and 1 μm in (C–G). See also Movies S1 and S2.