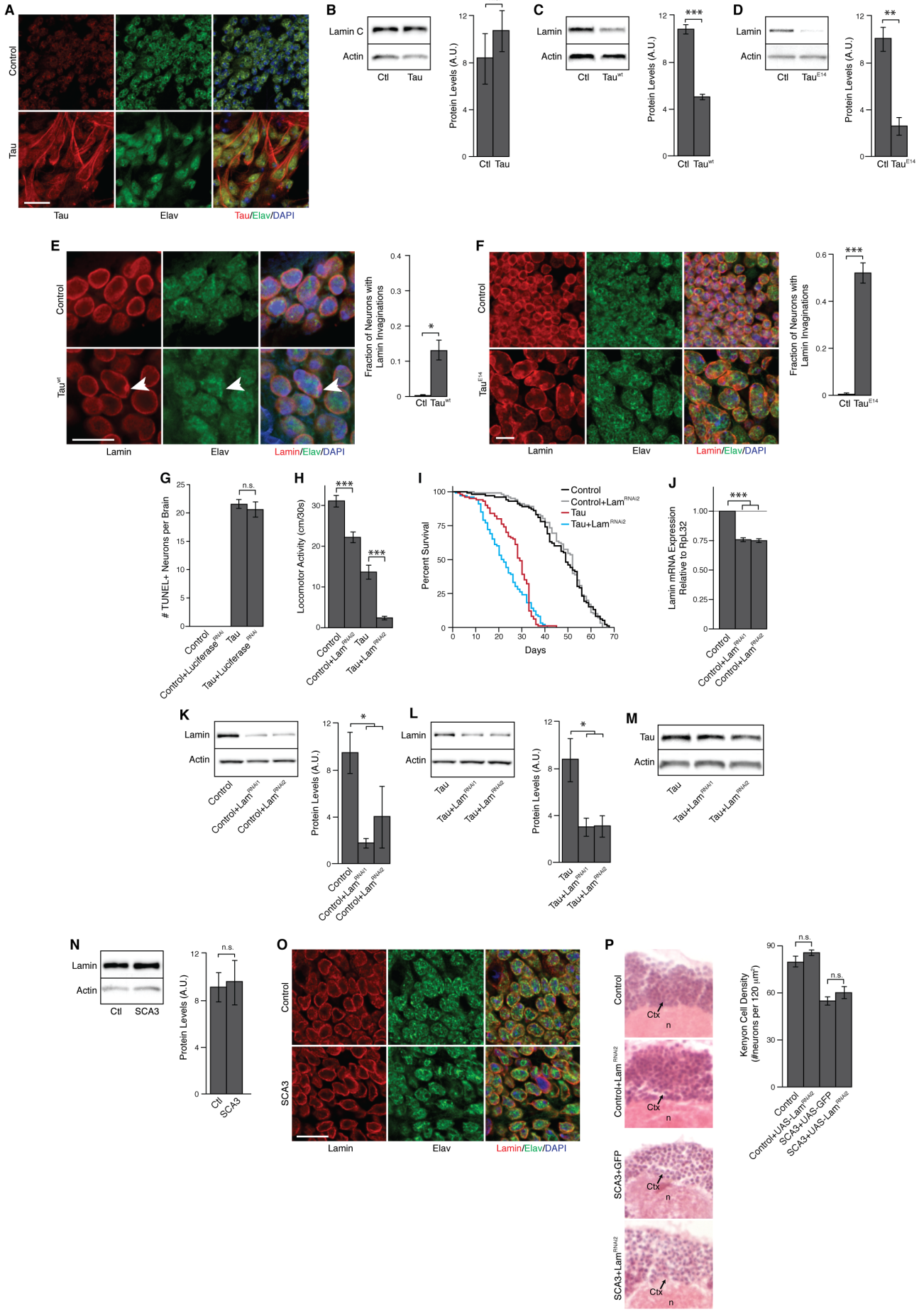
A
Control
Tau
Tau
Elav
Tau/Elav/DAPI
B
Lamin C
Actin
Ctl
Tau
n.s.
Protein Levels (A.U.)
C
Lamin
Actin
Ctl
Tau^wt
***
Protein Levels (A.U.)
D
Lamin
Actin
Ctl
Tau^E14
**
Protein Levels (A.U.)
E
Control
Tau^wt
Lamin
Elav
Lamin/Elav/DAPI
Fraction of Neurons with Lamin Invaginations
*
Ctl
Tau^wt
F
Control
Tau^E14
Lamin
Elav
Lamin/Elav/DAPI
Fraction of Neurons with Lamin Invaginations
***
Ctl
Tau^E14
G
TUNEL+ Neurons per Brain
n.s.
Control
Control+Luciferase^RNAi
Tau
Tau+Luciferase ^RNAi
H
Locomotor Activity (cm/30s)
***
***
Control
Control+Lam^RNAi2
Tau
Tau+Lam^RNAi2
I
Percent Survival
Days
Control
Control+Lam^RNAi2
Tau
Tau+Lam^RNAi2
J
Lamin mRNA Expression Relative to Rpl32
***
Control
Control+Lam^RNAi1
Control+Lam^RNAi2
K
Lamin
Actin
Control
Control+Lam^RNAi1
Control+Lam^RNAi2
*
Protein Levels (A.U.)
L
Lamin
Actin
Tau
Tau+Lam^RNAi1
Tau+Lam^RNAi2
*
Protein Levels (A.U.)
M
Tau
Actin
Tau
Tau+Lam^RNAi1
Tau+Lam^RNAi2
N
Lamin
Actin
Ctl
SCA3
n.s.
Protein Levels (A.U.)
O
Control
SCA3
Lamin
Elav
Lamin/Elav/DAPI
P
Control
Control+Lam^RNAi2
SCA3+GFP
SCA3+Lam^RNAi2
Ctx
n
n.s.
n.s.
Kenyon Cell Density (#neurons per 120 µm²)
Control
Control+UAS-Lam^RNAi2
SCA3+UAS-GFP
SCA3+UAS-Lam^RNAi2

Figure S1, Related to Figure 1

(A) Immunostaining of control and tau transgenic fly brains with antibodies recognizing elav and human tau.

(B) Lamin C protein levels in head homogenates from control and tau transgenic *Drosophila*, n=3.

(C) Lamin protein levels in head homogenates from control and tau^{WT} transgenic *Drosophila*, n=3.

(D) Lamin protein levels in head homogenates from control and tau^{E14} transgenic *Drosophila*, n=3.

(E) Immunostaining of Lamin and elav in control and tau^{WT} transgenic *Drosophila* brains. Arrow indicates neuron with Lamin invagination, n=3.

(F) Immunostaining of Lamin and elav in control and tau^{E14} transgenic *Drosophila* brains. Lamin invaginations are widespread, n=3.

(G) Neuronal apoptosis assayed by TUNEL staining in control and tau transgenic *Drosophila* with RNAi targeted to *Luciferase*, n=6.

(H) Locomotor activity of control and tau transgenic *Drosophila* harboring an RNAi transgene targeted to *Lamin*, n=18.

(I) Lifespan of control and tau transgenic *Drosophila* harboring an RNAi transgene targeted to *Lamin.* n=100, $p<0.0001$, log-rank test.

(J) *Lamin* mRNA levels in *Drosophila* harboring RNAi transgenes targeted to *Lamin*, n=3, flies are one day old.

(K) Lamin protein levels in head homogenates from *Drosophila* harboring RNAi transgenes targeted to *Lamin*, n=3.

(L) Lamin protein levels in head homogenates from tau transgenic *Drosophila* and tau transgenic *Drosophila* harboring RNAi transgenes targeted to *Lamin*, n=3.

(M) Lamin protein levels in head homogenates from *Drosophila* with and without RNAi-mediated reduction of *Lamin*.

(N) Lamin protein levels in head homogenates from control and SCA3 model *Drosophila*, n=3.

(O) Immunostaining of Lamin and elav in control and SCA3 model *Drosophila* brains.

(P) Hematoxylin and eosin staining of mushroom bodies of control and SCA3 model *Drosophila* with RNAi targeted to *Lamin*, n=6. Ctx=cortex, n=neuropil.

Flies are 10 days in A-H and K-M, and 7 days old in N-P. Scale bars are 5 mm. Controls are *elav-GAL4/+*. Data are presented as mean ± SEM, t-test or ANOVA, n.s.=not significant, $*p<0.05$, $**p<0.01$, $***p<0.001$.

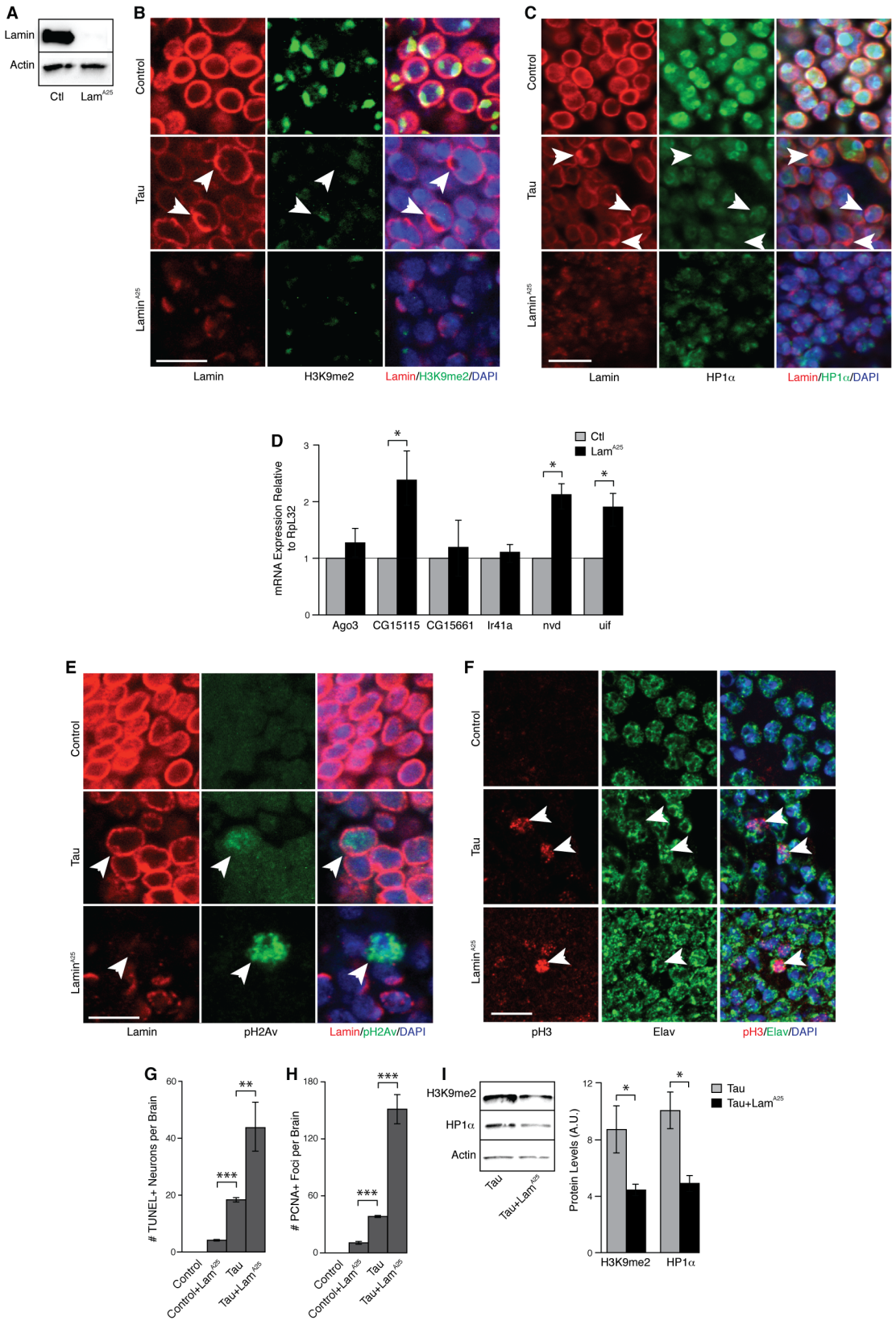

Figure S2, Related to Figure 2
(A) Western blot for C-terminal Lamin in homogenates from control *Drosophila* and *Drosophila* harboring the *Lam*^{A25} C-terminal Lamin truncation.

(B) Costaining of H3K9me2 and Lamin in control, tau transgenic, and Lam^{A25} mutant fly brains. Arrows indicate nuclei with lamin invagination.
(C) Costaining of HP1a and Lamin in control, tau transgenic, and Lam^{A25} mutant fly brains. Arrows indicate nuclei with lamin invagination.
(D) RT-PCR of the indicated mRNAs in homogenates from control and Lam^{A25} mutant fly heads. n=3.
(E) Costaining of Lamin and pH2Av in control, tau transgenic, and Lam^{A25} mutant *Drosophila* brains, n=3. Arrows indicate lamin invagination in a pH2Av-positive neuron.
(F) Neuronal cell cycle activation assayed by immunostaining of phosphorylated histone 3 (pH3) and elav in control, tau transgenic, and Lam^{A25} mutant fly brains. Arrows indicate cell cycle activation in elav-positive neurons.
(G) Neuronal degeneration assayed by TUNEL staining in brains of control and tau transgenic *Drosophila* heterozygous for the Lam^{A25} Lamin truncation, n=6.
(H) Cell cycle activation assayed by PCNA staining in brains of control and tau transgenic *Drosophila* heterozygous for the Lam^{A25} Lamin truncation, n=6.
(I) Western blot for markers of heterochromatin in head homogenates from tau transgenic *Drosophila* with and without the Lam^{A25} Lamin truncation, n=3.
All flies are 10 days old. Control is *elav-GAL4/+*. Lam^{A25} flies are homozygous in A-F and heterozygous in G-I. Scale bars are 5 mm. Data are presented as mean ± SEM, t-test or ANOVA, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

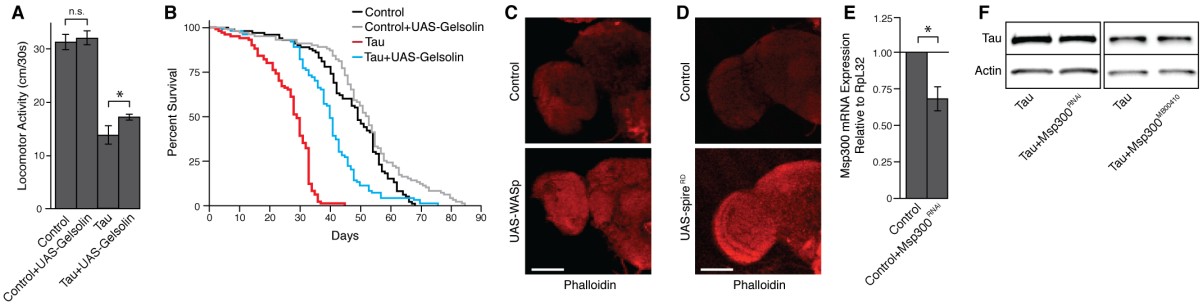

Figure S3, Related to Figure 3

(A) Locomotor activity of control and tau transgenic *Drosophila* with UAS-mediated overexpression of *Gelsolin.* n=18.

(B) Lifespan of control and tau transgenic *Drosophila* with UAS-mediated overexpression of *Gelsolin*. n=100, $p<0.0001$, log-rank test.

(C) Filamentous actin visualized by phalloidin staining of brains of control flies and flies panneuronally overexpressing WASp.

(D) Filamentous actin visualized by phalloidin staining of brains of control flies and flies panneuronally overexpressing the RD domain of spire.

(E) *Msp300* mRNA levels in *Drosophila* heads with RNAi-mediated reduction of *Msp300*, n=3. Data are presented as mean ± s.e.m, $*p<0.05$.

(F) Total levels of transgenic tau protein in whole head homogenates from *Drosophila* with and without RNAi-mediated reduction of *Msp300* or a transposable element insertion into *Msp300*.

Flies are 10 days old except in (B). Control is *elav-GAL4*. Scale bars are 30 mm. Data are presented as mean ± SEM, t-test or ANOVA unless otherwise stated, n.s.=not significant, $*p<0.05$, $***p<0.001$.

Antibody	Western	Immunostaining	Source
Actin (Dm)	1:10,000		DSHB JLA20, J.J. Lin
Elav (Dm)		1:5	DSHB 9F8A9, G.M Rubin
Elav (Dm)		1:5	DSHB 7E8A10, G.M. Rubin
GFP		1:200	Life Technologies #11122
H3K9me2		1:200	Abcam #1220
HP1α (Dm)		1:100	DSHB C1A9, L.L. Wallrath
HP1α (Hs)		1:100	Cell Signaling #2616
Koi (Dm)		1:50	Michael Welte
Lamin aa 336-410 (Dm)		1:100	DSHB, ADL40, Paul A. Fisher
Lamin aa 548-620 (Dm)	1:5,000	1:100	DSHB, ADL67.10, Paul A. Fisher
Lamin B1/2 (Hs)		1:100	Abcam Ab16048
Lamin B1/2 (Hs)		1:100	Santa Cruz Biotechnology sc-6216
Lamin C (Dm)	1:5,000		DSHB, LC28.26, Paul A. Fisher
LAP2β (Hs)		1:100	BD Biosciences #611000
NeuN (Hs)		1:50	Chemicon #MAB377
Nuclear pores (Hs)		1:200	Abcam #24609
pH2Av (Dm)		1:500 (DAB), 1:100 (IF)	Rockland #600-401-914
pH3		1:2,000	Cell Signaling #9701
PCNA (Dm)		1:500 (DAB), 1:100 (IF)	Dako #M0879
Tau	1:5,000,000	1:500	Dako #A0024
Tau AT8		1:100	Thermo Scientific #MN1020
Tau pSer214		1:100	Biosource #44-742G

Supplemental Table 1

Table S1. Antibody information. Related to Experimental Procedures.
"Dm" indicates antibodies that were used for staining *Drosophila melanogaster* proteins, "Hm" indicates antibodies that were used for staining human proteins.

Supplemental Experimental Procedures

Genetics and Animal Models

Drosophila melanogaster crosses and aging were performed at 25°C. Transgenic flies expressing human tau^{R406W} have been described previously [S1-S5]. Neuronal expression of RNAi and transgenes in *Drosophila* was achieved using the GAL4/UAS system with the panneuronal *elav* promoter driving GAL4 expression. The following *Drosophila* lines were obtained from the Bloomington *Drosophila* Stock Center: *Lam^{RNAi1}* (TRiP line number GL00577), *Lam^{A25}, UAS-Gelsolin, UAS-WASp, UAS-spireRD*, *Msp300^{RNAi}* (TRiP line number HMS00632)*, Msp300^{MB00410}, UAS-Lifeact-GFP*. *Lam^{RNAi2}* was obtained from the Vienna *Drosophila* RNAi center (line number 107419). *UAS-SCA3* transgenic flies were provided by Nancy Bonini.

Western blotting

Frozen *Drosophila* heads or brains were homogenized in 15ml Laemmli sample buffer (Sigma), boiled for 10 minutes, and analyzed by 15% SDS-PAGE (Lonza). After transferring to nitrocellulose membranes, antigen retrieval was performed by microwaving membranes in one liter of PBS for nine minutes. Equal loading was assessed by Ponceau S staining. After blocking membranes in PBS plus 0.05% Tween and 0.5% milk, membranes were incubated with primary antibodies overnight at 4°C. After washing, membranes were incubated with HRP-conjugated secondary antibodies for 2-3 hours at room temperature. Blots were developed with an enhanced chemiluminescent substrate (Thermo Scientific).

Immunocytochemistry and Histology

Antibodies are summarized in Table S1. To visualize Lamin/tau pSer214, Lamin/H3K9me2, Lamin/HP1a, Lamin/pH2Av, and Lamin/GFP in *Drosophila*, formalin-fixed, paraffin-embedded sections from *Drosophila* heads were used for immunostaining experiments. Sodium citrate-based antigen retrieval was performed prior to staining. For elav costaining, *Drosophila* brains were dissected in PBS and fixed in methanol prior to staining. For, Lamin/phalloidin costaining, *Drosophila* brains were dissected in PBS and fixed in 4% PFA prior to staining. Apoptosis was assessed by TUNEL staining (Calbiochem), which recognizes nuclear DNA fragmentation. TUNEL- and PCNA-positive nuclei were counted throughout the entire brain. Secondary detection was performed using fluorescently labeled secondary antibodies except in the case of TUNEL and PCNA, in which secondary detection was performed with DAB. For human immunostaining, touch preparations were performed from frozen frontal cortex and tissues were fixed with 4% PFA for 10 minutes. Fresh brains were used for phalloidin staining in *Drosophila* and postmortem human brain (Cytoskeleton, Acti-Stain 555 Phalloidin, 1:100 for 20 minutes). For confocal microscopy, brains were imaged on a Leica SP8 upright confocal microscope. For super-resolution microscopy, brains were imaged either on a Leica SP8 upright confocal microscope with STED depletion lasers or a Zeiss ELYRA. Images were analyzed with ImageJ. All images shown are a single slice. To quantify Lamin invaginations in *Drosophila,* 100 elav-positive cortical nuclei were scored for the presence of lamin invaginations in three controls and three tau transgenic brains.

Locomotor behavioral assay and lifespan analysis

The locomotor assay was performed as described previously [S6]. To assess lifespan, flies were collected at 1 day after eclosion and were transferred to fresh food every two days, at which time they were also scored for survival.

Reverse Transcription Quantitative PCR

Quantitative RT-PCR was performed as described previously [S6]. Primer sequences for *Lamin* were CGTCTGCTCGATGACACAG (forward) and CGACTCGTACATGCGGACATT (reverse). Primer sequences for *Msp300* were ATTGGCCAAGTGACGACTGT (forward) and TTTTCGTGCCAGGAGCGTAT (reverse). *RpL32* was used as an internal control gene.

Human brain analyses

Control brains included two males and five females, Braak stages I/II, with a median age of 71 and an age range of 65-80 years. Alzheimer's disease brains were from two males and five females, Braak stages V/VI, with a median age of 78 and an age range of 69-81 years. Postmortem intervals in cases and controls were similar, and all less than 24 hours. To quantify lamin B invaginations in human control and

Alzheimer's disease brains, 50 NeuN-positive nuclei were counted in six cases and six controls and scored for presence of lamin invaginations. Nuclei were scored as positive if invaginations extended into the nuclear interior and occupied at least 10% of the total nuclear diameter. To quantify lamin B levels in nuclei from human brain, ImageJ was used to measure lamin B fluorescence intensity in neuronal nuclei from ten microscopic fields for each of six control brains and six Alzheimer's disease brains. Intensities were assigned to bins comprised of 20 fluorescence units, and the number of nuclei per bin was calculated as a fraction of the total number of nuclei counted, which was 100-150.

Statistical Analyses

All n's reported are biological replicates. For experiments in human tissue, sample sizes were based on power analysis calculations, which demonstrated that a 30% difference between 6 cases and controls achieves a power of 80%. Statistical analysis was performed using a one-way ANOVA when making multiple comparisons, and an unpaired student's t-test when comparing two samples. For statistical analysis of lamin B levels in human brain, we employed a mixed effects model, including a random effect to adjust for correlation of measurements within brains, and tested whether the mean intensities were different for Alzheimer's disease versus controls.

Supplemental References

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S3. Khurana, V., Lu, Y., Steinhilb, M.L., Oldham, S., Shulman, J.M., and Feany, M.B. (2006). TOR-mediated cell-cycle activation causes neurodegeneration in a Drosophila tauopathy model. Current biology : CB *16*, 230-241.

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