Expanded View Figures

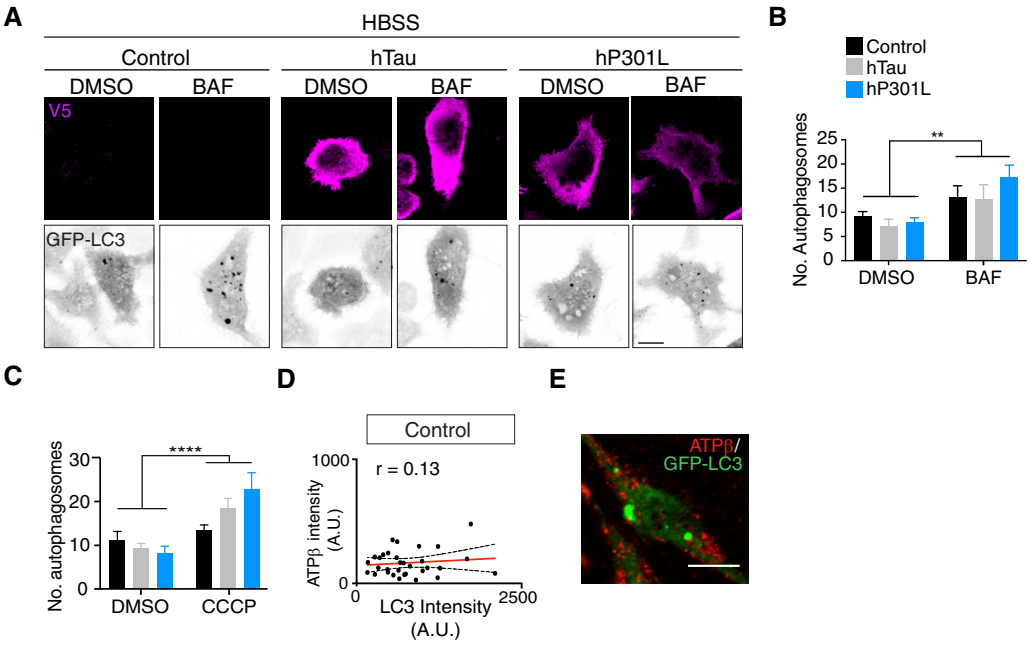

Figure EV1. Transient transfection of Tau does not impair autophagic flux.

A Representative images of N2a cells expressing GFP-LC3 and hTau, hP301L or control vector, that were starved in HBSS and treated with bafilomycin A (BAF), illustrating an increase in LC3-positive autophagosomes with BAF treatment.

B Quantification of LC3-positive autophagosomes after 24 h of tau expression in starved cells that were treated with DMSO or BAF. Data were analysed by two-way ANOVA, illustrating a main effect of BAF treatment, $F(1, 135) = 10.3$, $P = 0.0017$, but no main effect of tau expression, $F(2, 135) = 0.5425$, $P = 0.5826$ and no significant interaction effect $F(2, 135) = 0.6667$, $P = 0.5151$, $n = 19$–35 cells/group.

C Quantification of LC3-positive autophagosomes after 24 h of tau expression and 4 h of CCCP treatment, revealing no inhibition of autophagosome formation by tau. Data were analysed by two-way ANOVA, illustrating a main effect of CCCP treatment, $F(1, 158) = 17.81$, $P < 0.0001$, but no main effect of tau expression, $F(2, 158) = 0.7444$, $P = 0.4767$ and no significant interaction effect $F(2, 158) = 2.939$, $P = 0.0558$, $n = 19$–34 cells/group.

D, E LC3 fluorescence intensity does not correlate with the mitochondrial signal, indicating a lack of colocalisation ($r = 0.125$, $P = 0.4867$), $n = 33$ cells.

Data information: Data are given as mean and SEM, $^{**}P < 0.01$, $^{****}P < 0.0001$. Scale bar = 10 μm.
Source data are available online for this figure.

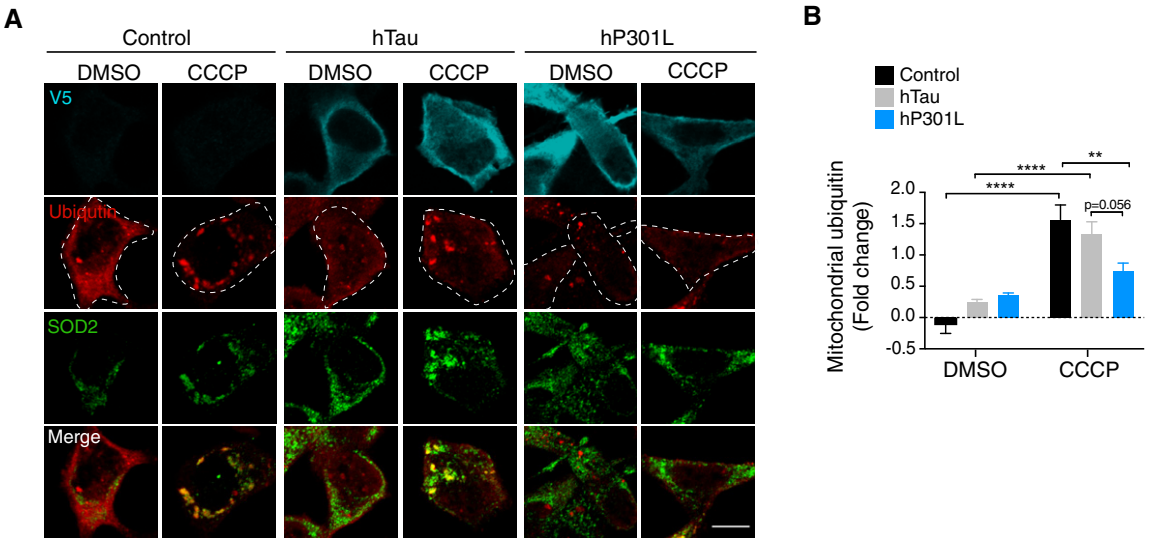

Figure EV2. Ubiquitination of mitochondrial targets is reduced by hP301L.

A Representative images of N2a cells transfected with hTau, hP301L, or empty vector control and myc-Parkin for 24 h and treated with CCCP for 4 h. Immunostaining was performed for mitochondria (SOD2) and ubiquitin. Scale bar = 10 μm.

B Mitochondrial ubiquitin was quantified by assessing its fluorescence intensity in mitochondrial ROIs and calculating the fold change over whole-cell ubiquitin intensity. Data were analysed by two-way ANOVA, revealing a main effect of CCCP treatment $F(1, 124) = 58.7$, $P < 0.0001$, no main effect of tau species $F(2, 124) = 1.21$, $P = 0.3004$, and a significant interaction effect $F(2, 124) = 7.16$, $P = 0.0011$. Data are given as mean and SEM, $^{**}P < 0.01$, $^{****}P < 0.0001$ for simple effects, $n = 15–29$ cells/group.

Source data are available online for this figure.

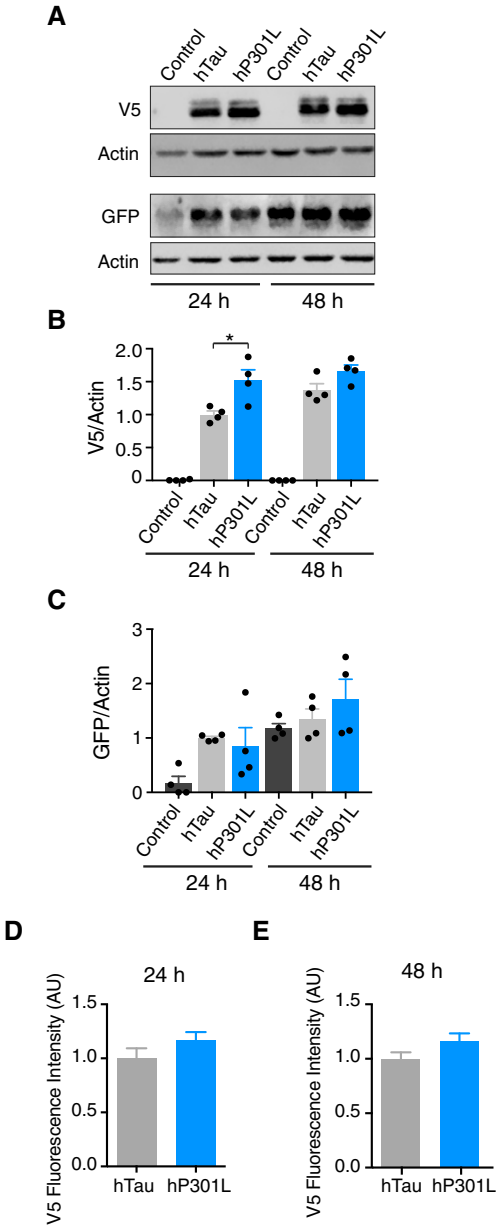

Figure EV3. Tau and Parkin expression levels.

A Immunoblots illustrating population expression level of Tau-V5 and GFP-Parkin at 24 and 48 h post-transfection.

B Quantification of human tau levels from immunoblots. Tau levels were compared using two-way ANOVA, with the control group excluded from the statistical analysis, revealing a main effect of expression time, $F(1, 12) = 5.865$, $P = 0.0322$, a main effect of tau species, $F(1, 12) = 14.66$, $P = 0.0024$ and no interaction effect $F(1, 12) = 1.187$, $P = 0.2972$.

C Quantification of GFP-Parkin levels from immunoblots. Parkin levels were compared using two-way ANOVA, revealing a main effect of expression time, $F(1, 18) = 16.29$, $P = 0.0008$, a main effect of tau species, $F(2, 18) = 4.249$, $P = 0.0308$ and no interaction effect $F(2, 18) = 1.176$, $P = 0.3313$.

D, E Tau expression level in cells analysed for Parkin translocation, as represented by V5 fluorescence intensity following immunostaining, at 24 h (unpaired t-test $t = 1.412$, $P = 0.1692$) and at 48 h ($t = 1.673$, $P = 0.0979$).

Data information: Data are given as mean and SEM, with $^*P < 0.05$ for simple effects.
Source data are available online for this figure.

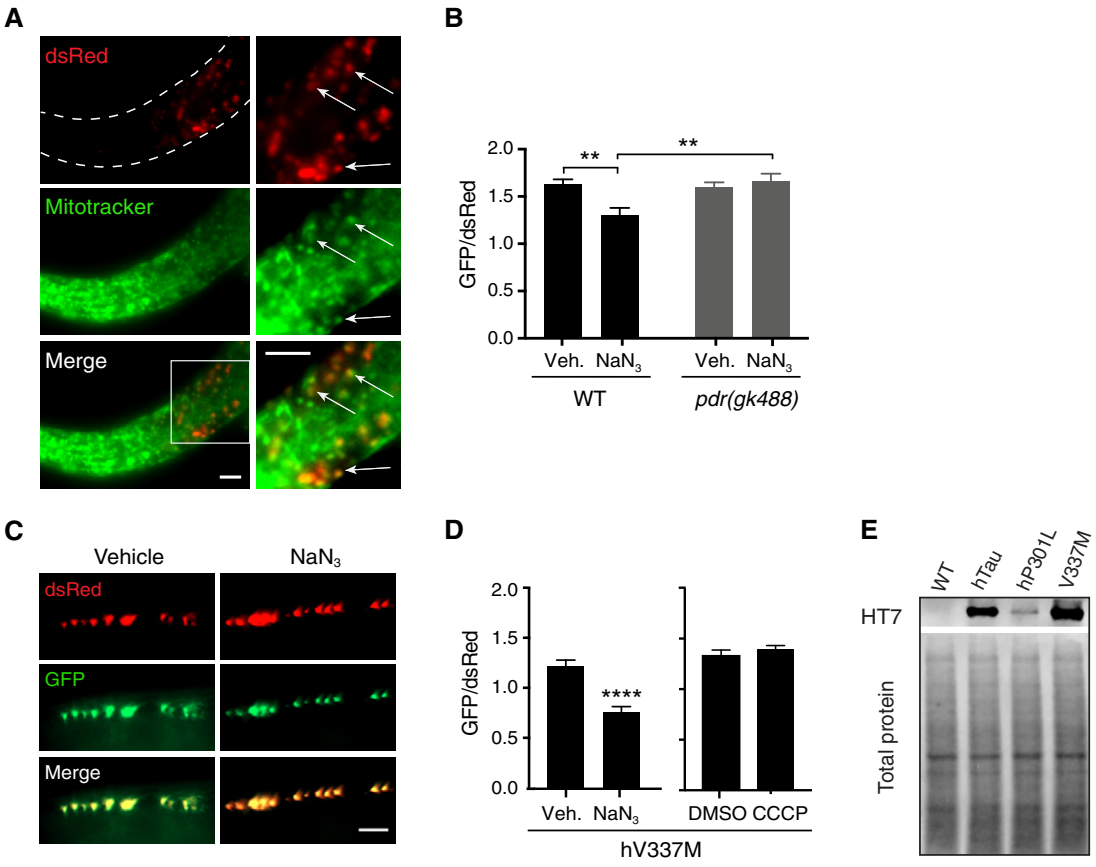

Figure EV4. Characterisation of mito-Rosella *Caenorhabditis elegans* and the effect of hV337M-Tau on mitophagy in *C. elegans* neurons.

A Mitotracker Deep Red staining illustrating colocalisation between Mitotracker (pseudo-coloured green for better visualisation) and mito-Rosella (dsRed). Scale bars = 5 μm.

B Analysis of mitophagy in wild-type worms and pdr-1(gk488) mutant animals in response to NaN_3. Data were analysed using two-way ANOVA, revealing a main effect of genotype $F(1, 106) = 6.415$, $P = 0.0128$; a main effect of NaN_3 treatment $F(1, 106)$, $P = 0.047$; and a significant interaction effect $F(1, 106)$, $P = 0.0041$; $n = 26–29$ animals/group.

C, D Analysis of mitophagy in worms expressing hV337M-Tau in response to NaN_3 (Mann–Whitney U-test, $U = 25$, $P < 0.0001$, $n = 16, 19$ for vehicle and NaN_3, respectively), and CCCP treatment (unpaired t-test, $t = 0.8113$, $P = 0.4211$, $n = 25, 27$ for DMSO and CCCP, respectively).

E Tau expression in worm lysates detected with the human tau-specific antibody HT7 (extended immunoblot from Fig 6I).

Data information: Data are given as mean and SEM. $^{**}P < 0.01$, $^{****}P < 0.0001$. Scale bar = 10 μm.
Source data are available online for this figure.