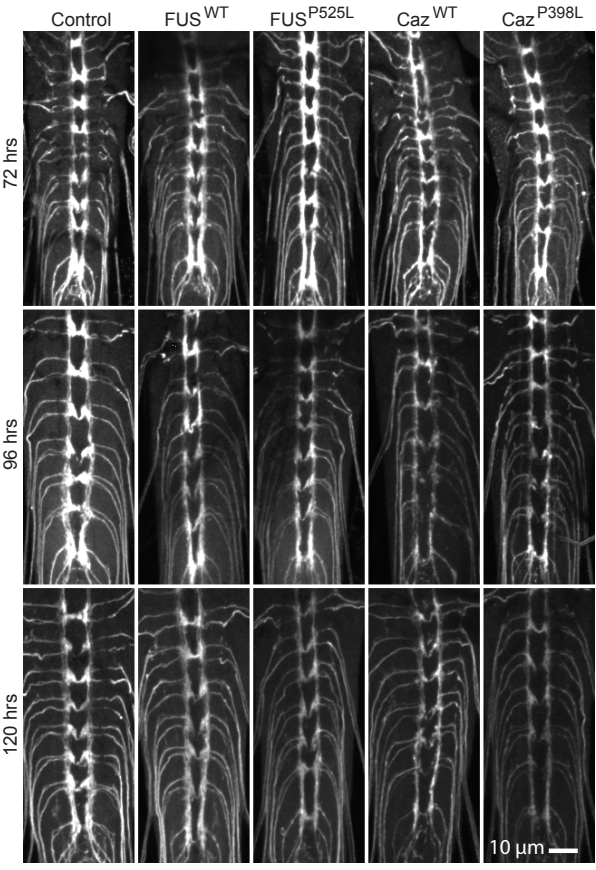
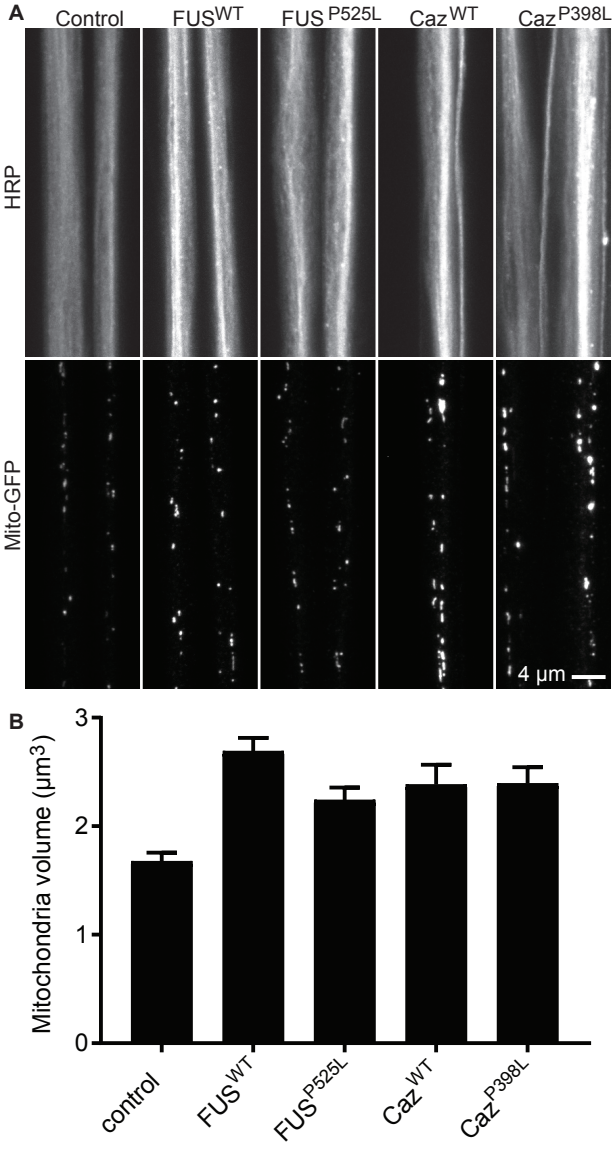
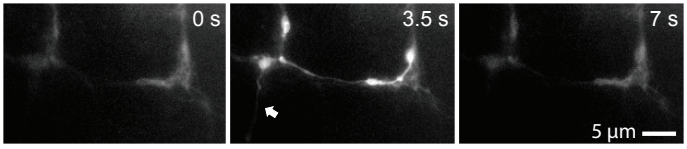
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental figure 1: FUS overexpression causes progressive neurodegeneration.

CD8-GFP was coexpressed with FUS^{WT}, FUS^{P525L}, Caz^{WT}, or Caz^{P398L} in da neurons using the Ppk-gal4 driver and progressive loss of neuronal processes was assayed at 72, 96, and 120 hours into development, corresponding to early, mid, and late third instar larval stage. At 72 hours, ventral ganglion neuronal projections were intact in all genotypes, but expression of FUS^{WT}, FUS^{P525L}, Caz^{WT} and Caz^{P398L} resulted in a progressive loss of CD8-GFP signal intensity, whereas animals expressing Caz^{WT} or Caz^{P398L} projections were progressively fragmented.

Supplemental figure 2: FUS overexpression alters the size and distribution of axonal mitochondria.

(A) Mito-GFP was coexpressed with FUS^{WT}, FUS^{P525L}, Caz^{WT}, or Caz^{P398L} in da neurons using the Ppk-gal4 driver and mitochondria within the segmental nerves were imaged in third instar larvae. (B) The size of mitochondria within the axons of da neurons was measured and a frequency distribution analysis was performed. In animals expressing FUS^{WT}, FUS^{P525L}, Caz^{WT}, or Caz^{P398L} a greater population of mitochondria with larger volumes were observed.

Supplemental figure 3: Ca^{2+} transients in da neuron synaptic projections are caused by action potentials

Spontaneous changes in GCAMP fluorescence were imaged in the ventral ganglia of third instar larvae coexpressing GCAMP5 with Caz^{P398L} under the control of the Ppk-GAL4 driver. Local increases in Ca^{2+} concentration within synaptic projections of da neurons are associated with simultaneous Ca^{2+} influx within the axons of these neurons, suggesting that voltage-gated Ca^{2+} channels mediate Ca^{2+} influx during action potentials.

Supplemental Methods

Analysis of progressive larval phenotypes. Mated flies of the indicated genotypes were passed on to fresh food and allowed to lay eggs for 8 hours. Larvae were dissected at 72, 96, and 120 hours after passage, stained and imaged.

Analysis of mitochondria size. Mitochondria size was measured using the surface segmentation tool in Imaris. N = 4 animals.