SVIP is a molecular determinant of lysosomal dynamic stability, neurodegeneration and lifespan

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Missense mutations in Valosin-Containing Protein (VCP) are linked to diverse degenerative diseases including IBM, amyotrophic lateral sclerosis (ALS), muscular dystrophy and Parkinson’s disease. Here, we characterize a VCP-binding co-factor (SVIP) that specifically recruits VCP to lysosomes. SVIP is essential for lysosomal dynamic stability and autophagosome–lysosomal fusion. SVIP mutations cause muscle wasting and neuromuscular degeneration while muscle-specific SVIP over-expression increases lysosomal abundance and is sufficient to extend lifespan in a context, stress-dependent manner. We also establish multiple links between SVIP and VCP-dependent disease in our Drosophila model system. A biochemical screen identifies a disease-causing VCP mutation that prevents SVIP binding. Conversely, over-expression of an SVIP mutation that prevents VCP binding is deleterious. Finally, we identify a human SVIP mutation and confirm the pathogenicity of this mutation in our Drosophila model. We propose a model for VCP disease based on the differential, co-factor-dependent recruitment of VCP to intracellular organelles.
The Valosin-Containing Protein (VCP) gene encodes an AAA+ ATPase that has been linked to a broad spectrum of autosomal dominant degenerative diseases including IBMPFD (inclusion body myopathy with Paget’s disease and fronto-temporal dementia; Watts et al.), amyotrophic lateral sclerosis (ALS), spastic paraplegia, muscular dystrophy, and Parkinson’s disease. Patients with IBMPFD generally present to the clinic with muscle weakness in mid to late life. Approximately one-third of patients have degeneration of the central nervous system including the fronto-temporal lobes.

The VCP gene is cell lethal when deleted. As a likely consequence, disease-causing VCP mutations are primarily distributed in the N-terminal regulatory region, a substrate/co-factor binding domain. To date, despite a wealth of information, VCP has been proposed as cell biological consequences of disease pathogenesis. For example, there is considerable debate regarding whether disease-causing mutations are gain-of-function versus loss-of-function mutations. Furthermore, although defects in protein quality control mechanisms appear to be a root cause of VCP-related disease, consistent with the general characterization of VCP as a ubiquitin-dependent protein segregate, there remains debate regarding which protein clearance pathways are compromised given that VCP participates in ER-associated degradation (ERAD), autophagy, mitophagy, and ubiquitin-proteasome-associated degradation (UPS). Although pathogenic mutations in VCP do not seem to impair the UPS or ERAD protein degradation pathways, impaired autophagy, mitochondrial quality control, and lysosomal dysfunction have been proposed as cell biological consequences of disease-causing VCP mutations.

One possibility is that VCP is an essential enzymatic resource that is dynamically deployed to different organelle systems, as necessary, to resolve sites of local cellular stress (Fig. 1a). In this model, the critical determinants of cellular robustness are the co-factors that bind VCP and dictate the distribution of VCP throughout the cell. According to this model, disease-causing mutations in the co-factor binding domain could, conceivably, result in both gain and loss-of-function effects if a fixed amount of VCP enzyme becomes errantly redistributed within the cell in the absence of binding to a specific co-factor. These effects might be exacerbated under conditions of cellular stress and accelerate during disease progression. With this model in mind, we set out to investigate and characterize the function of VCP-binding proteins, focusing on currently unknown mechanisms that localize VCP to the lysosome.

We previously identified an essential activity for VCP at Drosophila muscle lysosomes. We demonstrated that muscle lysosomes form a dynamic, tubular lattice that extends throughout individual muscle cells (insect and mammalian muscle). When VCP is mutated, or when VCP is acutely depleted from the cytosol, tubular lysosomes fragment, and autophagosome–lysosome fusion is blocked, ultimately leading to impaired autophagy, impaired protein clearance, and mitochondrial defects. Fragmentation of muscle lysosomes is one of the earliest cellular defects known to occur following the disruption of VCP. Notably, patients with disease-causing mutations in VCP present to the clinic with primary muscle weakness, highlighting the potential relevance of VCP-dependent control of muscle lysosomes and autophagy. We sought to identify co-factor(s) that bind and recruit VCP to the newly identified, highly dynamic system of muscle tubular lysosomes.

In this study, we define the function of a VCP-interacting co-factor termed SVIP (Small VCP-Interacting Protein). SVIP was initially identified as a VCP-interacting protein and a negative regulator of ERAD. Prior work in immortalized human cells (HeLa) demonstrated that SVIP over-expression is sufficient to alter the subcellular distribution of VCP to “perinuclear granules” and the plasma membrane. SVIP knockdown correlated with altered LC3 lipidation in these cells. However, beyond this, little is known about SVIP function.

Here we define SVIP as an essential VCP co-factor that recruits VCP to lysosomes, an activity that is essential for the dynamic stability of the muscle tubular-lysosomal lattice. Loss of SVIP disrupted the muscle lysosomal network, impaired autophagosome–lysosome fusion, and caused myriad degenerative defects including muscle wasting, neuromuscular degeneration, dendrite degeneration in motoneurons, progressive motor dysfunction and decreased organismal lifespan. We link SVIP to VCP-dependent degenerative disease through phenotypic analysis of a known human disease-causing mutation in Drosophila VCP, and we present the identification of an SVIP mutation in a human patient diagnosed with fronto-temporal dementia (FTD). We subsequently confirmed the pathogenicity of this human SVIP mutation in our Drosophila model. Although we cannot conclude causality, this human mutation and our subsequent characterization in vivo in Drosophila argues that the function of SVIP has relevance to the human condition. Taken together, our data define a model for VCP disease based on the differential, co-factor-dependent recruitment of VCP to intracellular organelles. Further, our data underscore the relevance of lysosome dysfunction as an underlying cause for VCP-linked diseases of the peripheral and central nervous systems.

Results

Drosophila SVIP recruits VCP to tubular lysosomes. We identified a small ORF (CG32039) that shares high sequence homology to the human, mouse, and rat SVIP proteins. Critical residues are conserved in CG32039 including glycine 2 that is required for SVIP myristoylation and membrane tethering, as well as the region encompassing a putative VCP-interaction motif (VIM) (Fig. 1b). Thus, based on sequence homology, CG32039 (hereafter referred to as SVIP) is a strong candidate to be the Drosophila SVIP ortholog.

We first demonstrate that Drosophila SVIP directly binds to VCP. Purified recombinant GST-SVIP and MBP-VCP N-D1 (containing the N-terminal and first D1 ATPase domain) proteins directly bind and this interaction was lost when two arginine residues (R24, 25) in the VIM sequence of SVIP were mutated to alanine (Fig. 1c). Additionally, mutation of VCP aspartate 32, a residue previously demonstrated to disrupt VCP interaction with VIM motif also disrupted the SVIP–VCP interaction in vitro (Supplementary Fig. S1A, B). Thus, we define SVIP as a VCP-binding protein.

In order to define the subcellular distribution of SVIP, we took a two approaches. First, we generated an antibody against GST-SVIP to examine the endogenous expression patterns of Drosophila SVIP. SVIP is expressed in both 3rd instar larvae and adults, being expressed at relatively lower levels in the brain compared to the rest of the body (Supplementary Fig. S1C). Second, we used CRISPR to knock-in a superfold GFP (sFGP) tag at the C terminus of the endogenous SVIP gene (see “Methods” section). This approach allows us to assess SVIP localization in live tissue. We demonstrate that SVIP is expressed in the body wall muscles of 3rd instar larvae where it co-localizes with a lysosomal marker Spin-RFP (Fig. 1d, e).

We previously demonstrated that muscle lysosomes form a dynamic tubular lattice that extends throughout the muscle cytoplasm. This dynamic lattice can be observed by imaging an RFP tagged integral lysosomal membrane protein (Spin-RFP;
Supplementary Movie 1). This tubular-lysosomal system is present in a diversity of cells and organisms, inclusive of cultured mouse myoblasts (ref. 16 and unpublished observation). Importantly, tubular lysosomes are only visible in live tissue, collapsing into vesicular structures by a diverse array of fixation methods16.

Here we demonstrate that SVIP localizes at lysosome tubules that are distributed throughout the cytosol (see Fig. 1d, insets 1 and 2 as well as quantification in Fig. 1e).

To determine whether SVIP recruits VCP to muscle lysosomes, we tested whether over-expressing Drosophila SVIP directly recruits VCP to tubular lysosomes in muscles. a Distinct co-factors (shapes of different colors adjacent to arrows) spatially regulate VCP subcellular distribution and function. b Schematic of Drosophila CG32039 gene and protein organization (top) and sequence alignment with human, mouse, and rat SVIP orthologs (bottom). The region shaded in magenta corresponds to the predicted VCP-interaction motif (VIM), M indicates myristoylation site, asterisks indicate arginine residue mutated in part C, and serine highlighted in yellow indicates SVIP patient mutation identified (Fig. 9). c In vitro binding of recombinant MBP-VCP and GST-SVIP fusion proteins. d Co-localization of endogenously tagged SVIP-sfGFP and Spin-RFP (lysosomes) in Drosophila muscles. The dotted line indicates the nucleus. Boxes indicated insets, below at higher magnification. e Mander’s correlation coefficients for SVIP-sfGFP (GFP) and Spin-RFP (RFP) signal (n = 13 muscle cells examined over five independent animals). f Co-imaging of VCP-sfGFP and Spin-RFP in control and SVIP+ over-expression in muscles. Dotted lines as in d and f. Yellow arrow (middle row) highlights signal co-localization. g Nuclear to cytoplasmic ratios of VCP-sfGFP signal was quantified for the genotypes indicated (top graph). Spin-RFP signal was used to create a masked ROI (white lines, “mask”) and plots represent GFP/RFP signal intensities within the masked region (bottom graph, see “overlay” in g). Data, artificial imaging units. (n = 10 muscle cells examined over five independent animals). See also Supplementary Fig. S1. Scale bars 5 μm. Data presented as mean and SEM. Student’s t-test for individual comparisons (*p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001).
VCP with sgFPR at its endogenous locus. Using these tools, we demonstrate that endogenous VCP protein concentrates on the nuclear membranes in muscle and is weakly distributed throughout the rest of the muscle cell. Image analysis of extra-nuclear VCP reveals a significant concentration at tubular lysosomes (Fig. 1f, g; note that exposure time for VCP-sgFPR is extended in (g) compared to (f) to enable visualization of lysosome-associated VCP). Strikingly, when UAS-SVIP is over-expressed using the muscle-specific mhc-GAL4 driver, endogenously expressed VCP-sgFPR re-localizes to tubular lysosomes throughout the muscle cell (Fig. 1f, g). The SVIP-dependent re-localization of VCP causes depletion of VCP from the perinuclear compartment where VCP is enriched at a steady state (Fig. 1f, g). As a control, we over-expressed UAS-SVIPRR, an SVIP variant that cannot bind VCP (Fig. 1c). As expected, SVIPRR over-expression did not induce VCP re-localization to the tubular-lysosomal network (Fig. 1f, g). We quantified these effects by measuring two parameters. First, we quantified the juxta-nuclear to cytoplasmic (representative of lysosomal tubules located throughout the cytoplasm) ratio of endogenously tagged VCP-sgFPR (Fig. 1b). Second, we used the Spin-RFP channel to create a mask of the tubular-lysosomal network and quantified VCP-sgFPR intensity in this region of interest (Fig. 1g, h). Collectively, these data demonstrate that Drosophila SVIP binds VCP and is sufficient to re-localize endogenous VCP to the tubular-lysosomal system.

SVIP-dependent VCP recruitment is essential to stabilize lysosomal structure and function. To investigate the function of SVIP in vivo, we generated an SVIP mutant using the CRISPR-Cas9 system. We designed a gRNA to direct Cas9 to cut within the first exon of SVIP, which resulted in a frame-shift mutation and a pre-mature stop codon upstream of the VIM site (Fig. 2a). Using our SVIP antibody, we validated that the SVIP mutation is a protein null by western analysis (Fig. 2a; hereafter referred to as SVIPKO).

We previously demonstrated that VCP mutations cause fragmentation of the tubular lysosome system in Drosophila muscle. If SVIP is necessary to recruit VCP to lysosomes, then the loss of SVIP should phenocopy the VCP mutant. Prior live imaging studies demonstrate that lysosome tubules are highly dynamic with constant tubule extension, scission, and fusion throughout the muscle cytoplasm. We previously demonstrated that VCP is required for the dynamic lysosomal network in body wall muscles of 3rd instar larvae (Fig. 2b). Two measures were used to quantify effects on the lysosomal tubular network: (1) the number of junctions per tubule, which assesses the complexity of the tubule network, and (2) the number of tubules within a 100 \( \mu m^2 \) region of interest, as a measure of tubule density. Both measures are significantly decreased in the SVIPKO (Fig. 2b, pink). This effect requires the SVIP–VCP interaction because over-expression of wild-type UAS-SVIP in the SVIPKO fully rescues the tubular lysosome system (Fig. 2b, rescue, green), whereas over-expression of the UAS-SVIPRR mutant that abolishes SVIP–VCP binding does not rescue (Fig. 2b, SVIPRR, orange). Finally, we note that over-expression of UAS-SVIP not only restores the lysosomal network but increased tubule density beyond wild-type levels (\( p < 0.001 \)), suggesting that SVIP levels may be limiting for the dynamic stability of the lysosomal tubule network.

We also extend these observations to the adult Drosophila abdominal muscles, demonstrating that the SVIPKO disrupts tubular-lysosomal networks (Fig. 2c). We demonstrate a significant drop in tubule complexity (junctions per tubule), but there was only a trend toward fewer tubules. On closer inspection of the collapsed lysosomal network, we find that tubules can still emanate from vesicular lysosomes (Fig. 2c, white arrows). Thus, in the absence of SVIP-dependent recruitment of VCP, it appears that lysosomes retain the ability to generate de novo tubules at a basal rate, but tubules appear unable to fuse with other lysosomes and cannot sustain a stable lattice.

We previously demonstrated that VCP is required for the fusion of autophagosomes with lysosomes, a process that sustains the integrity of the tubular-lysosomal system. To test the involvement of SVIP in VCP-mediated autophagosome–lysosome fusion, we used a dual fluorescent autophagy sensor: mCherry-GFP-Atg8a. Atg8a resides in the membrane of mature autophagosomes. When autophagosomes fuse with lysosomes, GFP fluorescence is quenched by low lysosomal pH, but the mCherry signal is unaffected. Therefore, the efficiency of autophagosome–lysosome fusion can be measured by the amount of GFP fluorescence compared to the amount of mCherry signal. Here, we demonstrate that SVIPKO animals display significantly decreased GFP fluorescence compared to wild type (Fig. 2d; \( p < 0.001 \)). Thus, the ability of autophagosomes to fuse with lysosomes is defective. Consistent with such a defect, SVIPKO animals also accumulated detergent-insoluble protein aggregates (Supplementary Fig. S2). Collectively, these data argue that SVIP-mediated VCP recruitment to muscle lysosomes promotes both homotypic lysosome fusion (to sustain the tubular-lysosomal lattice) and heterotypic membrane fusion with autophagosomes (Fig. 2d).

### Synthetic reconstitution of VCP-lysosomal targeting rescues lysosomes in SVIPKO

To further address our emerging model, we asked whether synthetically driving VCP to lysosomes in the SVIPKO is sufficient to rescue the tubular lysosome lattice. To do so we designed a system based on FKBP-FRB (FK506 Binding Protein–FRB) dimerization to artificially drive VCP to lysosomes. A variant of FKBP (FKBP12.6) that binds to the rapamycin analog B/B dimerizer was fused to the N terminus of the lysosome-specific membrane protein Spinster. The complementing FRB tag was fused to the C terminus of Spinster and VCP, respectively (Fig. 2e). In SVIPKO animals, in the absence of the B/B dimerizer, lysosomes were vesicular and VCP appeared diffuse throughout the cytoplasm (Fig. 2f, top panels, control). However, after 4 h of B/B incubation, VCP re-localized to lysosomes, and the tubular-lysosomal lattice began regenerating (Fig. 2f, bottom panels, dimerizer). We find that the increase in tubule density in the presence of dimerizer is highly significant (\( p < 0.001 \)), and there is a strong trend toward increased tubule complexity (\( p = 0.09 \)). Thus, synthetic recruitment of VCP to lysosomes, performed in the absence of SVIP which normally localizes VCP to lysosomes, is sufficient to enhance the stability of a dynamic lysosomal network. The inability to fully generate a network, inclusive of significant increases in the number of junctions per tubule could be due to the short time (4 h) of dimerizer or reflect a slight impairment of reconstituted VCP activity due to the engineered dimerizer system.

SVIP functions in muscle, affecting organellar motility, and lifespan. We sought to assess the organellar effects of SVIPKO and define which tissues require SVIP function. In the 3rd instar larvae, we observed no apparent impairment of motility (Supplementary Fig. S3A) and no hallmarks of motor neuron or muscle degeneration (Supplementary Fig. S3B). However, we found that 28% of SVIPKO pupa failed to eclose to the adult stage,
compared to just 7% of wild-type animals (Supplementary Fig. S3C). These data argue that SVIP function is relevant to organismal development and may be relevant to health and lifespan. Thus, we turned to the analysis of adult animals where these parameters can be addressed.

In adult Drosophila, we find that SVIPKO animals have a reduced lifespan compared to controls (29% decrease in median lifespan) (Fig. 3a). We next asked where SVIP is required for a normal lifespan. Remarkably, diminished lifespan can be fully rescued by muscle-specific expression of UAS-SVIP (Fig. 3a). By contrast, neuronal expression of UAS-SVIP in the SVIPKO did not rescue viability (Supplementary Fig. S4A). Since SVIP is necessary and sufficient in skeletal muscle to control autophagosomal fusion and the dynamic stability of the lysosomal tubular network, we propose that these activities of SVIP, in muscle, are necessary for normal organismal lifespan.

Next, we asked whether the decrease in lifespan is preceded by impaired motor behavior. Motility was assessed using a commonly employed negative geotaxis assay, taking advantage of a powerful innate behavior in which animals climb the side of a vial immediately upon being knocked to the bottom. We observe a profound deficit in motor ability in SVIPKO animals that is first apparent at 14 days and progresses to a complete inability to climb the sides of a vial by 28 days (Fig. 3b). The SVIPKO at 28 days are fully viable and do not begin to show increased mortality for another 10 days (Fig. 3a). Thus, progressive motor dysfunction precedes early lethality, consistent with age-related, progressive degeneration in the motor system.

Next, we asked where SVIP is required in order to sustain normal motor behavior. To do so, we assessed climbing performance at 28 days. This time point was chosen as a moment when the SVIPKO is almost completely immobile, but when there remains no change in animal viability. Muscle-specific expression of UAS-SVIP in muscle cells (Fig. 3c) restores mobility to nearly wild-type values (~90% rescue of negative geotaxis, Fig. 3c), whereas pan-neuronal expression of UAS-SVIP in the SVIPKO provides only partial (~50%) rescue (Supplementary Fig. S4B). Thus, while there is some benefit to the expression of SVIP in the nervous system, it is apparent that wild-type animal viability and motility can be achieved when SVIP is expressed exclusively in muscle.
To further confirm the essential activity of muscle SVIP, and to link SVIP activity to the muscle-specific function of VCP, we compared the effects of muscle over-expression of wild-type UAS-SVIP versus mutant UAS-SVIP<sup>RR</sup> that is unable to bind VCP. Muscle-specific over-expression of UAS-SVIP<sup>WT</sup> was without effect, but muscle-specific over-expression of UAS-SVIP<sup>RR</sup> had a profound (~65%) decrease in animal mobility in our negative geotaxis assay (Fig. 3d). These data are consistent with SVIP-dependent recruitment of VCP to muscle lysosomes being essential.

Next, we returned to the effects of SVIP on organismal lifespan. Given the potent activity of SVIP in muscle, we asked whether muscle over-expression of SVIP, in a wild-type background, would further extend lifespan. Indeed, lifespan extension is statistically significant when SVIP is expressed in muscle, although the absolute magnitude of the effect is rather modest (Fig. 3e, purple). Finally, over-expression of the SVIP mutant that is unable to bind VCP (SVIP<sup>RR</sup>), significantly diminished lifespan, as predicted (Fig. 3e, orange).

What happens when animals are stressed by the administration of drugs that disrupt the lysosome or impair normal proteostasis? Would SVIP muscle over-expression have a beneficial effect? We raised wild type and SVIP<sup>KO</sup> animals on food containing the lysosomal inhibitor bafilomycin-A (BafA). Under this condition, wild-type mean lifespan is decreased from ~53 days (Fig. 3a) to ~30 days (Fig. 3f), highlighting the deleterious effects of this drug delivered at concentrations previously used for feeding experiments in Drosophila<sup>29</sup>. Importantly, we find that lifespan is even more severely affected in the SVIP<sup>KO</sup> compared to wild type (Fig. 3f, pink), with mean lifespan diminished to ~20 days. Thus, loss of SVIP sensitizes the organism to the effects of BafA, as might be expected given that BafA targets the lysosome.

Next, we tested whether muscle-specific SVIP over-expression might counter the deleterious effects of BafA. Indeed, muscle over-expression of SVIP in wild-type animals nearly doubles lifespan in the presence of BafA (Fig. 3g; mhc > SVIP<sup>WT</sup>). Thus, SVIP over-expression, which is sufficient to increase lysosomal density in muscle, has a beneficial effect on lifespan under conditions of lysosomal stress.

Finally, we asked whether SVIP over-expression is beneficial in the context of other pharmacological stressors. We reared animals on food containing Tunicamycin to stress the ER-resident unfolded protein response. A concentration of Tunicamycin on food containing Tunicamycin to stress the ER-resident unfolded protein response. A concentration of Tunicamycin (Fig. 3h, pink). Several possible explanations exist. Among these, SVIP<sup>KO</sup> animals could be Tunicamycin resistant because increased amounts of VCP can be deployed to the ER in the absence of SVIP-mediated localization of VCP to the lysosome. This is consistent with the initial identification of SVIP as a negative regulator of ER-associated protein degradation<sup>24</sup>. These data support our initial model in which the dynamic, co-factor-dependent deployment of VCP to different organellar systems can have powerful effects on organismal health and lifespan (Fig. 1a).
Age-related muscle defects in SVIP knockout animals. IBMPFD is an age-related disorder, with patients typically presenting with muscle weakness in their fifth to sixth decade. Thus, we sought to characterize the progression of muscle wasting and neuromuscular degeneration, over time, in adult Drosophila. We assessed muscle integrity at 1 week and 4 weeks of age, comparing wild type and SVIPKO. The Dlg antibody labels the highly organized junction of T-tubule membranes with the muscle plasma membrane. We quantified anti-Dlg signal intensity/area (see "Methods" section). The anti-Brp antibody labels pre-synaptic T-bars that reside at sites of neurotransmitter release within the neuromuscular junction. Loss of Brp is an early hallmark of neuromuscular degeneration in Drosophila.

In wild type, there is no change in Dlg over time (Fig. 4a, d), although minor changes in Dlg organization occur (Fig. 4a, inset). By contrast, major changes are observed in the SVIPKO. Dlg staining is slightly diminished compared to wild type at 1 week (Fig. 4b, d), but is nearly abolished by 4 weeks (Fig. 4b, d). In parallel, we observe that Brp puncta are dramatically reduced in number (Fig. 4b, bottom inset). We used Brp puncta as a proxy to calculate the length of the neuromuscular synapse and find a highly significant, age-dependent decrease in the SVIPKO (Fig. 4e).

Importantly, both the change in Dlg and Brp are fully rescued by muscle-specific resupply of SVIP in SVIPKO (Fig. 4c–e). We conclude that loss of SVIP causes progressive degeneration of muscle membrane systems and neuromuscular synapses.

In order to extend our anatomical analyses, we used phalloidin as a marker of normal muscle actin organization. These analyses were performed in the adult abdominal muscles. As in the jump muscle (Fig. 4a–e), Dlg labels the t-tubule network, although it
appears less striated in these muscles (Fig. 4f). In SVIPKO animals, Dlg becomes completely diffuse, consistent with disruption of the t-tubule network (Fig. 4f, h). In addition, the phalloidin-labeled, actin-rich bands are broader and less well-defined than the SVIPKO (Fig. 4f, g). These data are consistent with the analyses of flight muscle and became the substrate for a more detailed analysis by electron microscopy (see next section).

Ultrastructural visualization of muscle wasting in SVIP knockouts. Muscle wasting, quantified by immunohistochemical techniques, correlates with impaired organismal motility, arguing for progressive neuromuscular degeneration. To assess neuromuscular degeneration in greater detail, we examined neuromuscular ultrastructure in adult Drosophila. In Fig. 5a, b, we present longitudinal (Fig. 5a) and cross-sections (Fig. 5b) of a single adult wild type, poly-nucleate muscle cell. Nuclei (N) are present in the central region of the muscle cell (Fig. 5a), occupying a peripheral location in cross-section (Fig. 5b). Prominent, well-organized Z-bands are apparent (Z) with associated mitochondria. The neuromuscular synapses (Fig. 5a, red boxes, NMJ) are distributed along the muscle surface. Higher magnification of the NMJ (G) reveals concentrations of synaptic vesicles at electron densities with characteristic T-bar structures that are diagnostic of sites of neurotransmitter release termed active zones (AZ in Fig. 5g).

The SVIPKO mutants show signs of severe wasting, impaired mitochondria morphology, and hallmarks of neuromuscular synapse degeneration. The diameter of individual muscle cells is dramatically reduced (Fig. 5c, d, j). Note that two muscle cells are shown (differentially colored in Fig. 5c) in the same physical space that is normally occupied by a single wild-type muscle cell (Fig. 5a). In SVIPKO mutants the acto-myosin cytoskeleton is greatly diminished with disorganized Z-bands (Z). The muscle nuclei (N) are also variably positioned, consistent with impaired muscle cell health. Muscle mitochondria in SVIPKO mutants are altered with apparent vacuoles (Fig. 5c, red star). More commonly, however, mitochondria in the SVIPKO mutants show numerous, smaller voids within the intra-organellar membranes of unknown consequence (compare Fig. 5e, f; also see panel j and Supplementary Fig. S5B, C). The mitochondria phenotypes we observe are recapitulated by muscle-specific over-expression of UAS-SVIPR24,25A (Supplementary Fig. S5D) emphasizing that this phenotype is likely related to the loss of SVIP-dependent VCP recruitment to the lysosomal membrane system. We also note that defects in mitochondria are rescued by muscle-specific over-expression of UAS-SVIP in SVIPKO (Supplementary Fig. S5A). Since the primary effect of SVIP muscle over-expression is the potent re-localization of VCP to lysosomal membranes, we propose that the observed mitochondria defects are secondary to the...
integrity of the lysosomal system, a possibility that is consistent with a role for VCP in mitophagy. Finally, neuromuscular synapses show ultrastructural hallmarks of degeneration, consistent with immunohistochemical observations (Fig. 4). Neuromuscular junctions (NMJ) are positioned at the muscle surface in cross-section in both wild type and in SVIPKO mutants (Fig. 5a). In SVIPKO mutants NMJ are characterized by a darkened appearance and the proliferation of large vesicles (asterisks H, I; see also panel J for quantification) that are consistent with the appearance of enlarged endolysosomes and increased membrane internalization in other models of neuromuscular degeneration, both in Drosophila and mammalian systems.

Muscle SVIP expression rescues neuronal degeneration in SVIPKO mutants. In mammalian systems, ALS and related neuromuscular degenerative disorders are characterized by synaptic degeneration, muscle wasting as well as degeneration of lower and upper motoneurons. Thus, we tested whether degeneration is restricted to the NMJ, or whether it extends to motoneurons that participate in a simple motor circuit in the adult Drosophila central nervous system. We quantified neurodegeneration with single-cell resolution by using ShakB-Gal4 to drive expression of UAS-CD8-GFP in the tergolateral motor neuron (TTMn) (Fig. 6a). The TTMn neuron is born and matures morphologically during pupal development (Supplementary Fig. S6A). Therefore, we quantified neuronal morphological changes during pupal development as well as during adult lifespan, comparing wild type to SVIPKO animals. Pupal development was staged as a percentage of total developmental time. At 70% pupa development, the TTMn neurons are fully developed and there is no evidence of degeneration in SVIPKO animals (Supplementary Fig. S6B, C). However, in SVIPKO animals TTMn neurons rapidly begin to degenerate beginning at 1 day of adulthood and degeneration becomes progressively more severe over the first 30 days of adult life (Fig. 6b–e). By 30 days, 100% of SVIPKO animals showed severe signs of motoneuron degeneration, including complete loss of dendrites and somas (Fig. 6b–e). In contrast, wild-type animals exhibited little to no signs of dendrite degeneration by 30 days of adulthood (Fig. 6b–e).

As an independent assessment of neurodegeneration, we assessed the function of a simple, well-defined neural circuit termed the giant fiber (GF) circuit (Fig. 6a) that impinges on the TTMn motoneuron. Electrical stimulation of the GF neuron in the Drosophila head elicits activation of both the TTMn motoneuron (Fig. 6a, green) and the DLMn motoneuron (Fig. 6a, blue). It is possible to assess the integrity of this simple circuit by stimulating the GF and recording in the target musculature (Fig. 6a). Muscle responses are categorized as a failure if there is a complete absence of stimulus-time-locked muscle depolarization. Failures will occur if the neuromuscular synapse has completely uncoupled from the muscle, or if elements of the neural circuitry in the central nervous system have degenerated and broken the circuit. Complete synapse elimination at the NMJ is rarely observed (Fig. 4b, d, e), suggesting that complete failures likely reflect degeneration of elements within the central nervous system. Wild-type animals show a 100% success rate at 30 days in both target muscles (Fig. 6f, g). Young SVIPKO animals (2–5 days) have a 100% success rate, but by 30 days this has decreased to 0% success in the TTMn target muscle and to less than 40% success in the DLMn target muscle (Fig. 6f, g). These data are consistent with our anatomical analyses demonstrating severe motoneuron dendrite degeneration (Fig. 6b–e).

We next asked where SVIP is necessary to support the integrity of motoneurons in the CNS. Remarkably, we find that muscle-specific expression of UAS-SVIP completely rescues neurotransmission at the TTMn target muscle and doubles the success rate at the DLMn target muscle in SVIPKO animals (Fig. 6f, g). These results demonstrate that muscle-specific expression of UAS-SVIP is sufficient to rescue muscle health, NMJ integrity and it is also sufficient to improve motoneuron integrity. Thus, we suggest that SVIP-mediated recruitment of VCP to lysosomes in muscle can have profound cell non-autonomous consequences for the integrity of central neuronal circuitry and animal behavior. These data are consistent with a growing body of evidence that skeletal muscle proteostasis can have organism-wide effects on cellular health and organismal fitness (see “Discussion” section).

The ability of muscle SVIP expression to rescue motoneuron degeneration prompted us to determine the breadth of this effect. To do so, we quantified cell death in aged wild type and SVIPKO animals, as well as SVIPKO animals with muscle-specific SVIP rescue. Cell death was quantified by counting neuronal soma positively stained by ApopTag (see "Methods" section). In the region of the central nervous system where the majority of motoneurons reside that innervate the limbs, abdominal muscles, and flight muscles (termed ventral nerve cord), shown in Fig. 6 aged SVIPKO animals show a significant increase in cell death that is fully rescued by muscle-specific expression of SVIP (Fig. 6h, i). By contrast, although significantly elevated cell death was observed within the neural circuitry of the head and the visual system in the aged SVIPKO, this cell death was not rescued by muscle-specific expression of SVIP (Fig. 6j, k). These data agree with the muscle-specific, cell non-autonomous rescue of motoneurons at the single-cell level (Fig. 6b–g). These data also suggest that the restoration of muscle health by muscle-specific SVIP expression in SVIPKO is achieved by localized trophic support of motor circuitry rather than an organism-wide effect.

Identification of a VCP disease-associated mutation that abolishes VCP–SVIP binding. To this point, we have focused on the necessity of the SVIP gene. We next sought to connect SVIP activity to human disease-relevant mutations in the VCP gene. We hypothesize that pathogenic mutations in VCP could, in some instances, directly impair the VCP–SVIP interaction, precluding VCP-dependent VCP recruitment to lysosomes, causing multi-system failure. To test this hypothesis, we biochemically screened 10 conserved, disease-relevant, missense mutations in VCP (Supplementary Table 1) for loss of SVIP binding. Four mutations reside in the N-terminal protein–protein interaction domain, four reside in the D1 ATPase domain and two reside in the first linker region (Fig. 7a). Mutations in the N-terminal region had the largest effect of reducing SVIP–VCP affinity (Fig. 7b–e). The most dramatic effect was observed in the P134L mutant, which reduced SVIP–VCP affinity more than 30-fold (Fig. 7b, c).

Given the marked reduction in SVIP–VCP binding caused by the VCPP134L mutation, we sought to determine the in vivo consequences of this mutation, comparing them to the effects of the SVIPKO. To better mimic disease, we used CRISPR to knock-in the VCPP134L mutation at the endogenous VCP locus. An sfGFP tag was fused to the mutant transgene to enable localization studies (VCPP134L-sfGFP). As a control, we also generated a knock-in of wild-type VCP fused to sfGFP (VCPWT-sfGFP). While we were able to obtain homozygous VCPWT-sfGFP adult flies, we failed to obtain any homozygous VCPP134L-sfGFP adults, suggesting that the mutation is homozygous lethal. Given that IBMFFD is autosomal dominant, VCPP134L heterozygotes should genetically mimic the human disease and we further characterized the defects associated with the heterozygous mutant.
VCP protein self-oligomerizes into a hexamer. Since we are analyzing VCP\textsuperscript{P134L} heterozygous mutants, we wanted to examine whether the P134L mutant protein could assemble with wild-type VCP normally in vivo. To assess this, we performed co-immunoprecipitations. The epitope-tagged VCP\textsuperscript{P134L}-sfGFP allows easy resolution of the mutant protein compared to wild-type VCP by gel electrophoresis. VCP\textsuperscript{WT}-sfGFP heterozygous flies were used to control for the sfGFP tag interfering with VCP oligomerization. Both VCP\textsuperscript{WT}-sfGFP and VCP\textsuperscript{P134L}-sfGFP were able to co-immunoprecipitate wild-type VCP (Fig. 7f), indicating that the P134L mutation does not prevent VCP self-oligomerization. Since VCP hexamers should consist of both wild-type and mutant VCP proteins, this result supports our hypothesis that the P134L mutation does not interfere with VCP self-oligomerization.
wild-type and mutant VCP protein, we tested whether VCP mutant heterocomplexes displayed reduced binding to SVIP in vivo. Indeed, the VCP heterocomplex immunoprecipitated significantly less SVIP compared to a wild-type VCP complex (Fig. 7g; p < 0.05). Given this result, we examined lysosomes in the VCP\textsuperscript{P134L} mutant animals by imaging Spin-RFP. Similar to what we observed in SVIP\textsuperscript{KO} animals, the dynamic lysosomal network is significantly disrupted (Fig. 7h). We document significant defects in tubule length, tubule density, and tubule complexity (junctions per tubule) (Fig. 7h). Moreover, we note significantly less localization of VCP\textsuperscript{P134L} sfGFP at lysosomes (Supplementary Fig. S7A). Thus, a disease-causing mutation in VCP (P134L) impairs both SVIP binding and the lysosomal network in muscle.

Identification of a human SVIP mutation. In order to explore additional connections between SVIP and human disease, we screened a cohort of sporadic FTD patients (n = 341), with whole-genome sequencing data from the UCSF Memory and Aging Center (MAC), for putative pathogenic variants in SVIP. We found one potentially deleterious heterozygous nonsynonymous single nucleotide variant (SNV) in SVIP at genomic coordinates Chr11:22,844,669. This SNV results in a C to T substitution at nucleotide 230 in the coding sequence (NM_148893: c.230C>T) and was subsequently confirmed with Sanger sequencing. This variant causes a serine to leucine substitution, p.S77L, with the substituted amino acid being highly conserved from Drosophila to human (refer back to Fig. 1b). Importantly, we did not find the c.230C>T or any other non-synonymous SVIP variants in 106 clinically normal, healthy older controls or 318 early-onset Alzheimer’s disease patients from the UCSF MAC cohorts. Further, to the best of our knowledge, this variant has not been reported in public whole-genome and/or exome sequencing databases, including the Genome Aggregation Database (gnomAD) and Exome Aggregation Consortium (ExAC), suggesting that this variant is extremely rare in the general population. Finally, this patient was negative for known pathogenic mutations in the following genes: APP, C9ORF72, CHMP2B, FUS, GRN, MAPT, PSEN1, PSEN2, and TARDBP.

SVIP\textsuperscript{S82L} disrupts lysosome structure and function in Drosophila. To examine potential defects associated with the human S77L mutation, we first examined SVIP–VCP binding. We generated a GST fusion protein of Drosophila SVIP harboring the orthologous mutation (S82L, see Fig. 1b for reference) and found
that SVIP<sup>S82L</sup> can still bind VCP in vitro (Supplementary Fig. S8). Next, we generated a Drosophila SVIP<sup>S82L</sup> transgene. When SVIP<sup>S82L</sup> is over-expressed in an SVIP heterozygous knockout (SVIP<sup>KO/+</sup>) background we find that lysosomal tubules are no longer visible and lysosomal dynamics are severely perturbed (Fig. 9a; Supplementary Movies 1 and 2). Finally, an SVIP<sup>S82L</sup> knock-in, when tested in a lifespan assay, also showed resistance to the effects of tunicamycin feeding, similar to SVIP<sup>KO</sup> mutants (Fig. 9b). Thus, although SVIP<sup>S82L</sup> can still bind VCP in vitro, the mutant protein is sufficient to dominantly disrupt tubular lysosomes and affect animal physiology.

Finally, we imaged the mCh-GFP-Atg8a autophagy sensor to compare the proficiency of autophagosome–lysosome fusion in animals over-expressing SVIP<sup>WT</sup> or SVIP<sup>S82L</sup> in the SVIP heterozygous knockout background (SVIP<sup>KO/+</sup>) (Fig. 9c, d). When SVIP<sup>WT</sup> is over-expressed, we observed little to no GFP

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**Fig. 7 VCP disease mutation abrogates SVIP–VCP interaction.**

a Schematic of VCP with relative positions of disease-relevant mutations. b In vitro binding titration assay of GST-SVIP with MBP-VCP<sup>WT</sup> and MBP-VCP<sup>P134L</sup> recombinant proteins. c–e Binding curves of GST-SVIP with each MBP-VCP mutant as indicated. Affinities are listed in the tables for each mutant. f Co-immunoprecipitation of endogenously expressed VCP and VCP-sfGFP (transheterozygote). Diagram of hexameric VCP protein with proposed SVIP (green) binding at left. Quantification at right. (n = 3 independent experiments). g In vitro binding assay with recombinant GST-SVIP and VCP-sfGFP protein lysates extracted from whole flies. Quantitation of the amount of VCP-sfGFP protein bound to GST-SVIP. (n = 3 independent experiments). h Representative images of Spin-RFP in VCP<sup>WT</sup> and VCP<sup>P134L</sup> mutants. Quantification at right. (n = 8 muscle cells examined over four independent animals). Data presented as mean and SEM. Student’s t-test for individual comparisons (*p < 0.05; **p < 0.01; ***p < 0.001).
fluorescence where mCherry fluorescence was observed, indicating that autophagosomes are efficiently fusing with lysosomes. However, when mCh-GFP-Atg8a is co-expressed with SVIP(S82L), we observed a significant increase in GFP fluorescence, indicating autophagosome–lysosome fusion impairment (Fig. 9c, d). Collectively, these data suggest that the SVIP(S82L/77L) mutation is pathogenic in Drosophila muscle.

Discussion
IBMPFD is characterized by degeneration of tissues, including the neuromuscular junction and central neurons, causing similar symptoms associated with ALS, Parkinson’s, and FTD. Although IBMPFD is a comparatively rare disease, it has the potential to inform about generalized mechanisms that drive neurodegeneration more broadly. Here, we define SVIP as a cytoplasmic co-factor that is responsible for the translocation of VCP to a dynamic, tubular-lysosomal system in muscle. The importance of SVIP is underscored by our phenotypic analysis of SVIP knockouts and VCP transgenic knock-in mutants that disrupt the SVIP–VCP interaction. The SVIPKO shows a progressive, degenerative phenotype in muscle and motoneurons that is similar to that observed in disease-relevant VCP mutants as well as patients with IBMPFD and ALS. Degeneration is associated
with the disintegration of intracellular membrane systems and disrupted mitochondria. We show that these phenotypes extend to progressive deficits in organismal behavior and diminished lifespan.

Regulated deployment of VCP by SVIP and related co-factors. Our data speak to the possibility that VCP is deployed to different organelles based upon stress-related or cell-specific needs (Fig. 1a). This is consistent with the prior demonstration that VCP is recruited to mitochondria following pharmacological poisoning of oxidative phosphorylation with cyanide or phenyl-hydrazone (CCCP). Given the diverse activities of VCP, it was initially surprising that targeting the majority of VCP to the lysosomal system are less relevant compared to the increased abundance of VCP at the lysosomal compartment. In either case, SVIP-dependent recruitment of VCP to muscle lysosomes dramatically extends lifespan under conditions of lysosomal stress. Future efforts might be directed toward assessing whether SVIP over-expression can ameliorate the pathology and etiology of diseases associated with impaired lysosomal integrity. For example, lysosomal storage diseases are a group of ~50 rare metabolic disorders that primarily affect children, causing lethality within months or a few years of age.

It is important to emphasize that, while blocking SVIP-mediated VCP localization to lysosomes (SVIP knockout) is maladaptive if animals are fed bafilomycin-A, it is beneficial if animals are fed tunicamycin, stressing the ERAD pathway. We interpret this effect as being a consequence of more VCP being available to the ER system in the absence of lysosomal stressors. This finding is also consistent with our model regarding the dynamic deployment of a limited VCP resource to sites of cellular stress (Fig. 1a).

It is also worth noting that reduced proteostasis is a general feature of muscle aging and degeneration. Indeed, proteostasis mechanisms naturally decline with age and are thought to be a major contributing factor to the onset of neurodegenerative diseases. The predominant cellular degradation sites for clearing and recycling cellular waste products are lysosomes. Enhancing autophagy–lysosome-mediated proteostasis either pharmacologically or by caloric restriction has been shown to increase organismal healthspan in multiple species, including worms, flies, and mammals.
The sites of action for the beneficial effects of caloric restriction and enhanced autophagy–lysosome mediated proteostasis are under investigation. Intriguingly, there have been multiple examples of muscle proteostasis influencing the broader health of an organism. In particular, disrupting proteostasis in C. elegans muscles causes upregulation of the heat shock response system in distant tissues via the expression of specific chaperone proteins. In Drosophila, the muscle-specific expression of FOXO enhances organismal viability. These “tele-proteostasis” mechanisms may promote organismal robustness in a stressful environment by communicating the existence of proteotoxic stress to surrounding, healthy tissue, enabling a preparatory response. In essence, this is a type of feed-forward stress signaling mechanism that promotes organismal robustness.

Models of IBMPFD. Current Drosophila models of IBMPFD are based upon transgene over-expression, with unknown consequences on the normal regulation of VCP protein throughout the cell. In particular, it has been proposed, based on transgenic over-expression models, that IBMPFD is associated with adverse VCP gain of function activity. By extension, it was proposed that VCP inhibitors might be beneficial to disease progression and human health. However, the complexity of VCP signaling, acting at many sites within the cell (Fig. 1a) seems to argue for other therapeutic approaches.

To facilitate future therapeutic approaches, we have generated several mutants and transgenic animals that can be considered Drosophila models for exploration of IBMPFD. The VCP, specifically target the VCP-dependent recruitment of VCP to lysosomes. It is interesting that several pathogenic VCP mutations do not affect SVIP binding. Yet, there is clear phenotypic overlap comparing the loss of VCP to the pathogenic VCP mutations do not affect SVIP binding. The recruitment of VCP to lysosomes. It is interesting that several VCP, VCP variants, and human health. However, the complexity of VCP signaling, acting at many sites within the cell (Fig. 1a) seems to argue for other therapeutic approaches.

Endogenous gene replacements were generated using a scarless CRISPR strategy (http://flycrispr.molbio.wisc.edu/scarless). In brief, donor and gRNA plasmids were co-thermalized into Crispr9 embryos (either BDRC 56552 or 51324 stocks). The donor plasmid contained the transgene of interest, ~1 kb of homologous sequence upstream and downstream of the insertion sites, and a dSR cassette as a selection marker. Additionally, the PAM sites that were used to guide Cas9 cleavage of the endogenous genome were mutated in the donor plasmid to prevent cleavage of the transgenic after insertion. The DNA fragments and two gRNA sequences to direct Cas9 cleavage of sites upstream and downstream of the target genomic region to be replaced. The injection services of Bestgene were used to generate transgenic flies. Transgenic animals were verified by PCR and sequencing and the dsRed cassette was removed by crossing flies to 3xP3 transposase expressing flies. See Supplementary Table 2 for a list of all primers used in this study.

In vitro binding assays. MBP and GST fusion proteins were affinity purified in column buffer (20 mM Tris (pH 7.0), 150 mM NaCl, 2 mM EDTA, and 0.1% NP40) with Amylose Resin (NEB) or GST-bind resin (Millipore), respectively. MBP fusion proteins were eluted with maltose and GST fusion proteins were eluted with glutathione per manufacturers recommendations. For binding reactions, 1 μg of each protein was incubated with 50 μl GST-bind resins in NP40 buffer for 1 h. Beads were washed 3× with NP40 buffer before 50 μl of 5× Sample Buffer was added. Proteins were boiled for 10 min and resolved by SDS-PAGE on a 4–12% Bis-Tris gel (ThermoFisher). Protein gels were stained with Coomassie Blue (Bioreagents) or Bioimaging stained with Coomassie Blue (ThermoFisher). Protein bands were excised and digested with modified trypsin to generate peptides, which were analyzed by liquid chromatography coupled tandem mass spectrometry and identified with Pro蛋白质en (ThermoFisher) or Iontrap (AB SCIEX) instruments. Peptide mass fingerprints were compared to Drosophila databases in the protein database.

Microscopy methods. For live imaging, third instar larvae or adult flies were dissected in HL3 saline buffer with no calcium (70 mM NaCl, 5 mM KCl, 10 mM MgCl2, 10 mM NaHCO3, 115 mM sucrose, 4.2 mM trehalose, and 5 mM HEPES) and all live imaging was performed in saline. Imaging was performed on an inverted Axiovert 200 microscope (Zeiss) using a 100× Plan Achromat objective (1.4NA). Images were captured with a CoolSnap HQ2 CCD camera (Photometrics) and de-convolved using Slidebook 5.0 software (Intelligent imaging innovations, Denver, CO). Image quantification was performed with ImageJ software (NIH), Volume rendering was performed with Slidebook 5.0 software. Any adjustment of brightness or contrast was performed using Slidebook 5.0 software and always applied to the entire image.

For fixed imaging, adult brains were dissected in 0.01 M NaCl, 2.5 mM CaCl2, 4.4 mM MgCl2, 1.25 mM NaH2PO4, and 20.7 mM NaHCO3, pH 7.252 and fixed for 4 h for at least 1 h. Adult abdominal muscles, DLMs, and TTM were fixed in 4% PFA for 1 h. Muscles were stained with Phalloidin (ThermoFisher) overnight in a final concentration of 0.165 μM. Adult NMJs were visualized using mouse anti-BR1 (1:100; Developmental Studies Hybridoma Bank) and rabbit anti-DLG (1:1000). Conjugated secondary antibodies (Alexa Fluor 488 goat anti-mouse and Cy3 goat anti-rabbit) were used at 1:300 (Jackson immuno-research laboratories). The DLMs and TTM were dissected out of the thorax and identified by three parameters: size, shape, and location in the thorax. For co-localization analyses, Mander’s correlation coefficients were calculated using the Coloc2 plugin in FIJI.

ApopTag staining. The central nervous system was dissected out of 30-day-old adult Drosophila, across indicated genotypes. All fat bodies and trachea were removed from the nervous tissue because it is auto-fluorescent. Tissue was adhered to a glass coverslip with Vectabond (Vector labs) and 1:1 (2:1) for 10 min at 20 °C. ApopTag assay was performed as described in Millipore Sigma ApopTag Fluorescein kit protocol. Tissue was mounted in vectashield mounting media (Vector Labs) containing DAPI as a counterstain to image nuclei in the sample. ApopTag Fluorescein signal was imaged in the FITC channel. Samples were imaged on a Nikon Ti-E, 1.3 μM, 100x/0.75 Nikon objective. ApopTag Fluorescein positive nuclei were counted using Fiji 3D Object counter.

EM methods. Animals were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, for 2 h at room temp, rinsed in buffer, and post-fixed with 1% OsO4 in a buffer for 1 h at room temp. The post-fixed samples were then rinsed with water and incubated in 5% uranyl acetate in water, dehydrated in ethanol and propylene oxide, and embedded in Eponate 12 resin (Ted Pella, Inc., Redding, CA). 50 μm sections were cut with a Leica UCt ultramicrotome using a Diatome
diamond knife, picked up on slot grids with Pioloform films, stained with uranyl acetate and Sato’s lead, and examined with an FEI T12 TEM at 120 kV equipped with a Catan Ultrascan 4k × 4k camera.

**Lifespan and climbing assays.** Adult male flies were collected within 12 h of eclosion and maintained at 25°C on standard fly food. Flies were flipped to new food and scored for death every 2–3 days. Climbing assays were performed by flipping flies into a 100 ml graduated cylinder, tapping them to the bottom, and scoring the number of flies that could successfully climb to the 100 ml mark in 1 min. The statistical software, OASIS, was used to calculate the mean lifespans and perform log-rank tests to determine statistical significance.

**Electrophysiology methods.** Adult *Drosophila* was immobilized in dental wax, ventral side down and a glass microelectrode was directly placed into the identified muscle through the cuticle of the animal. Glass electrodes were prepared with a Master 8 stimulator through tungsten wire electrodes placed in each of the *Drosophila* eyes. An Axon Instruments Digidata 1322 A Data Acquisition System was used to digitize the data and recordings were collected with Clampex 10.7 software (Molecular Devices). Muscle depolarization at low (0.2 Hz) and high stimulus frequencies (100 Hz) were recorded to test the fidelity of the circuit in control and experimental animals.

**Sequencing data generation and processing.** Whole-genome sequencing of the patient’s genomic DNA isolated from whole blood was performed as described previously at HudsonAlpha Institute for Biotechnology (Huntsville, AL) on an Illumina HiSeq X with 150 bp paired-end reads to obtain 30× sequencing coverage. Paired-end reads were aligned to the GRCh37 build of the human reference genome with the Burrows-Wheeler Aligner (BWA-MEM v0.7.8) and processed using the Broad Institute’s Genome Analysis Toolkit (GATK) best-practices pipeline. The variant was confirmed with direct Sanger sequencing at UCSF.

**Statistical methods.** All Student’s *t*-test were performed with two-tailed analysis. All ANOVA are one-way with Tukey’s multiple comparisons. Lifespan data were compared using a log-rank test (see Lifespan, above). Analyses performed with Prism v9 (Graphpad).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the corresponding author Graeme Davis (Graeme.Davis@ucsf.edu). All unique/stable reagents generated in this study are available without restriction. This study did not generate data sets for public repositories or new data code. Source Data are provided with this paper.

Received: 18 February 2020; Accepted: 17 December 2020; Published online: 21 January 2021

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Acknowledgements
This study was funded by NINDS grant R35NS097212 (G.W.D.); NINDS grant K99/R00NS100988 (A.E.J.); NIH-NIA R01 AG049152 (J.S.Y.); NIH-NIA R01 AG062588 (J.S.Y.); Rainwater Charitable Foundation (J.S.Y.); NIA P01 AG019724 (B.L.M.); NIA P50 AG023501 (B.L.M.); and NIA P30 AG086242 (B.L.M.).

Author contributions
A.E.J. designed and conducted experiments including imaging, biochemistry, genetics, transgene and CRISPR transgenics, lifespan analyses, wrote, and edited the manuscript. B.O.O. contributed anatomical analysis of synapse and dendrite degeneration, cell death, and electrophysiology, and edited manuscript. R.D.F. contributed to electron microscopy and analysis. L.A.P. contributed to image analysis. E.G.G., A.J.M., J.S.Y., and B.L.M. contributed to clinical data. G.W.D. designed experiments, coordinated experiments, secured funding, wrote, and edited manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-20796-8.

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Peer review information Nature Communications thanks the anonymous reviewers for their contributions to the peer review of this work. Peer review reports are available

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