Supporting Information

Rumpf et al. 10.1073/pnas.1406898111

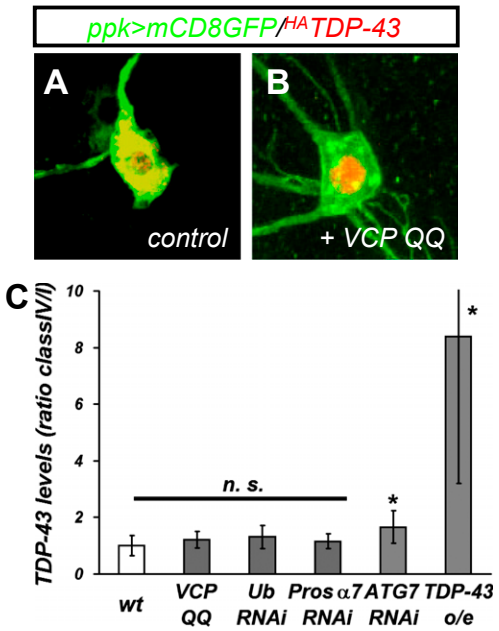

Fig. S1. Localization of transgenic tagged TAR–DNA-binding protein of 43 kilo-Dalton (TDP-43) and influence of ubiquitin–proteasome system (UPS) manipulations on TDP-43 levels. (*A*) Transgenic HA-tagged TDP-43 was expressed in class IV dendritic arborization (da) neurons and visualized with anti-HA (red), and class IV da neurons were visualized with anti-GFP staining (green). (*B*) HA–TDP-43 was coexpressed in class IV da neurons with Valosin-Containing Protein (VCP) QQ. (*C*) VCP and UPS manipulations do not affect TDP-43 levels. TDP-43 staining intensity was measured in class IV da neurons under the indicated UPS manipulations and normalized against TDP-43 staining intensity of adjacent class I da neurons ($n = 10$ each). Statistical comparison was done by Student t test.

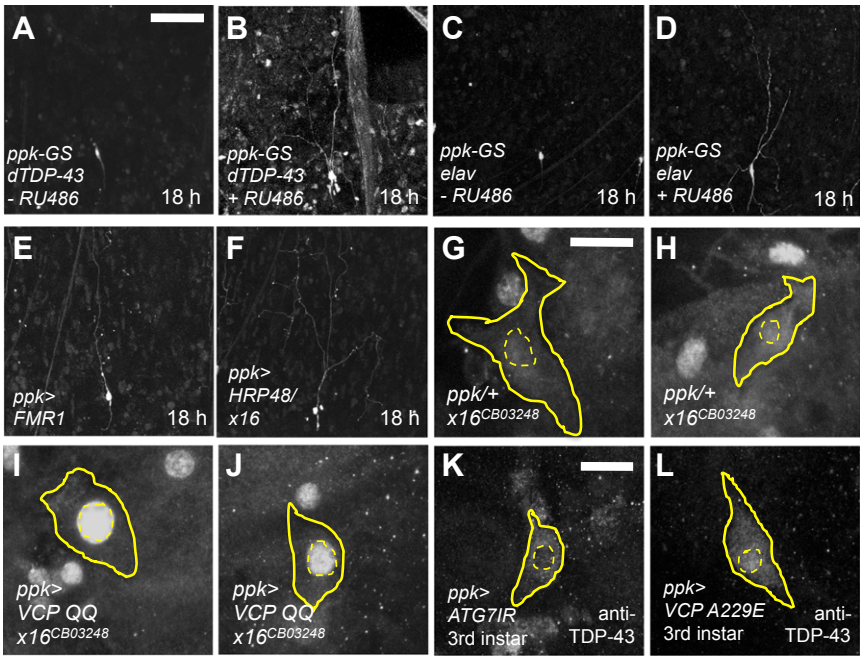

Fig. S2. Links between RNA-binding proteins, VCP, and class IV da neuron dendrite pruning. (*A–D*) Acuteness of TDP-43 and elav effects on pruning. TDP-43 or elav overexpression under *ppk-GeneSwitch* was induced by addition of 200 μM RU486 24 h before puparium formation, and pruning was assessed 18 h after puparium formation. (*A*) TDP-43, control without RU486 induction (0/16 neurons with dendrites still attached to soma). (*B*) TDP-43, with RU486 induction (25/35 with attached dendrites, $P < 0.005$). (*C*) elav, control without RU486 induction (0/62 with attached dendrites). (*D*) elav, with RU486 induction (44/76 with dendrites still attached to soma, $P < 0.005$). (*E*) FMR1 overexpression causes pruning defects in class IV da neurons (15/30 with attached dendrites, $P < 0.005$). (*F*) x16/HRP48 overexpression causes pruning defects (18/25 with attached dendrites, $P < 0.005$). (*G–J*) Levels of an ×16-GFP protein trap are increased upon VCP inhibition. (*G* and *H*) x16-GFP levels in control class IV da neurons. (*I* and *J*) x16-GFP levels in class IV da neurons upon VCP QQ coexpression under *ppk-GAL4*. (*K*) TDP-43 is largely cytoplasmic in class IV da neurons expressing ATG7 RNAi. (*L*) TDP-43 is largely nuclear in class IV da neurons expressing VCP A229E. (Scale bar in *A*, 50 μm; and in *G* and *K*, 10 μm.) Statistic analyses were with Fisher's exact test.

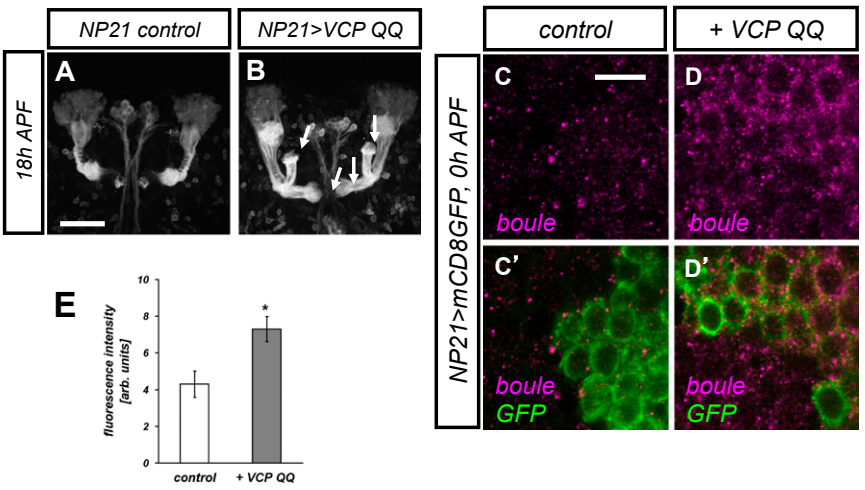

Fig. S3. VCP is required for mushroom body γ neuron axon pruning and affects boule levels. (*A*) Control γ neurons have pruned their axons at 18 h after puparium formation. (*B*) Neurons expressing VCP QQ have not pruned their axons at 18 h after puparium formation (18/18). (*C* and *C'*) Boule levels in control γ neurons labeled with NP21-GAL4 at the white pupal stage (0 h after puparium formation). (*D* and *D'*) Boule levels in γ neurons expressing VCP QQ. (*E*) Quantification of boule stainings in *C* and *D*. Boule staining intensity in γ neurons was normalized against staining intensity in cells not labeled by NP21-GAL4. (Scale bar in *A*, 100 μm; in *C*, 10 μm.)

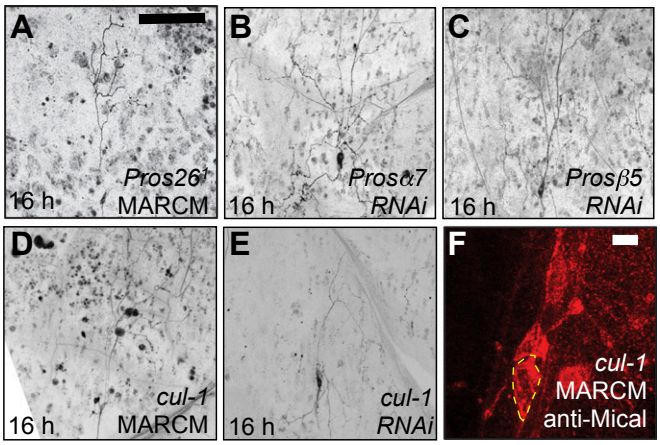

Fig. S4. 20S proteasome subunits and cullin-1 are required for pruning. (*A*) *Pros26*^{1} mutant class IV da neuron at 16 h after puparium formation (8/10 with attached dendrites, $P < 0.005$). (*B*) Expression of an RNAi construct directed against Prosα7 causes pruning defects (15/17 with attached dendrites, $P < 0.005$). (*C*) Expression of an RNAi construct directed against Prosβ5 causes pruning defects (10/11 neurons with attached dendrites, $P < 0.005$). (*D–F*) The Skp1/Cullin/F-box (SCF) subunit cul-1 is required for class IV da neuron dendrite pruning. (*D*) A *cul-1* mutant Mosaic Analysis with a Repressible Cell Marker class IV da neuron retains attached dendrites at 16 h after puparium formation. (*E*) *cul-1* RNAi expressed under *ppk-GAL4* leads to pruning defects at 16 h after puparium formation (24/36 with attached dendrites, $P < 0.005$). (*F*) Mical expression in class IV da neurons is not affected in a *cul-1* mutant class IV da neuron at 2 h after puparium formation. (Scale bar in *A*, 50 μm; in *F*, 10 μm.)

Table S1. RNA-binding proteins tested for overexpression phenotypes in class IV da neurons

RNA-binding protein	Expression construct	Phenotype, ppk-GAL4	Ref.
TDP-43	UAS	Pruning defect	1
elav	UAS	Pruning defect	2
FMR1	UAS	Pruning defect	3
x16/HRP48	GS15869	Pruning defect	
Cabeza (dFUS)	EP1564	—	
dAtx2	EP3145	—	4
boule	UAS	—	
Bruno-3	EP	—	
Staufen	UAS	—	5
nanos	UAS	—	5
Pumilio	UAS	—	5
tsunagi	EP	—	6
CG17176	EP	—	6

1. Lu Y, Ferris J, Gao FB (2009) Frontotemporal dementia and amyotrophic lateral sclerosis-associated disease protein TDP-43 promotes dendritic branching. *Mol Brain* 2:30–39.
2. Koushika SP, Lisbin MJ, White K (1996) ELAV, a *Drosophila* neuron-specific protein, mediates the generation of an alternatively spliced neural protein isoform. *Curr Biol* 6(12):1634–1641.
3. Zhang YQ, et al. (2001) *Drosophila* fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. *Cell* 107(5):591–603.
4. Satterfield TF, Jackson SM, Pallanck LJ (2002) A *Drosophila* homolog of the polyglutamine disease gene SCA2 is a dosage-sensitive regulator of actin filament formation. *Genetics* 162(4):1687–1702.
5. Ye B, et al. (2004) Nanos and Pumilio are essential for dendrite morphogenesis in *Drosophila* peripheral neurons. *Curr Biol* 14(4):314–321.
6. Couthouis J, et al. (2011) A yeast functional screen predicts new candidate ALS disease genes. *Proc Natl Acad Sci USA* 108(52):20881–20890.