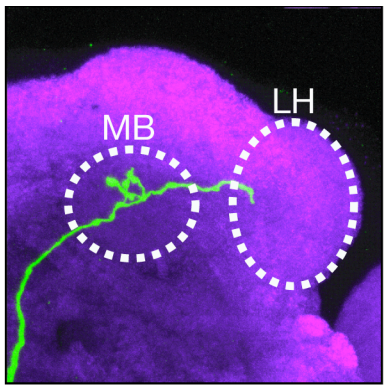
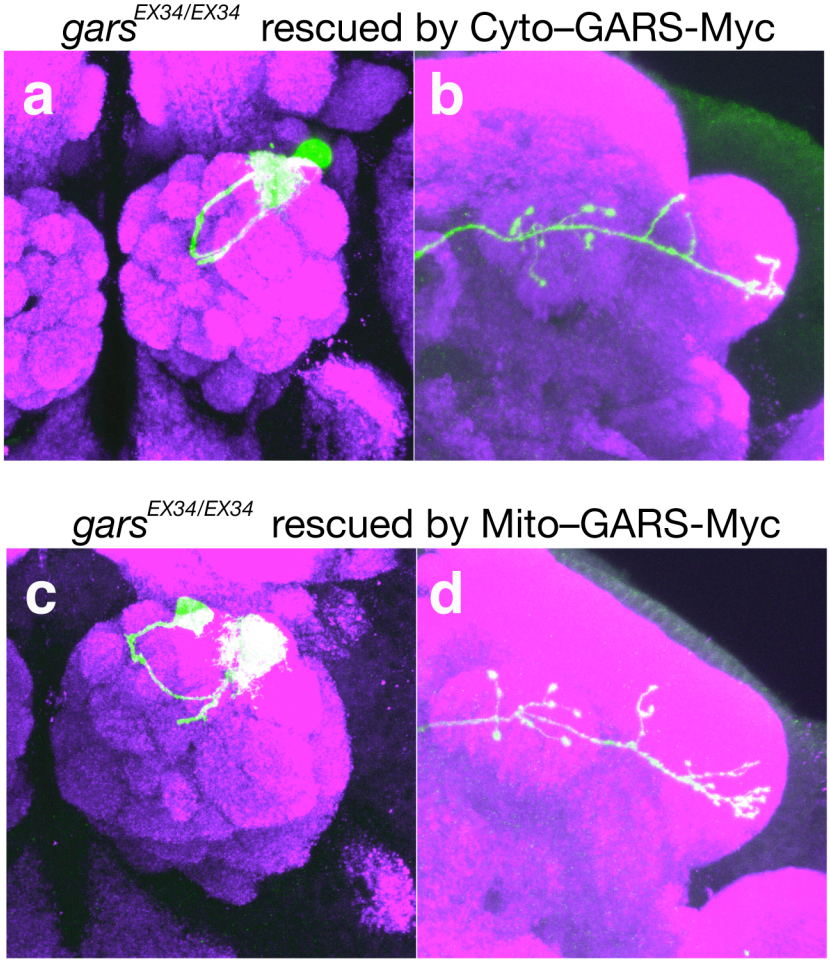
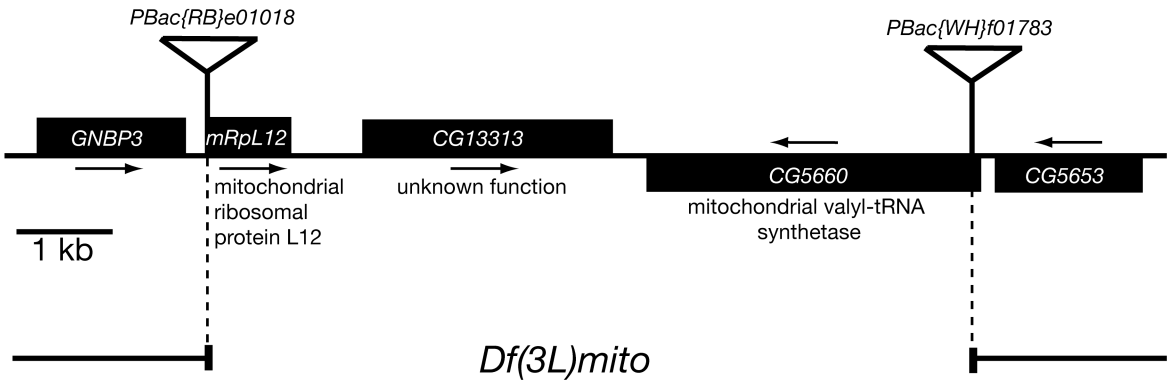


Supplementary Figure 1 Lack of axon terminal arborization in *team*$^{-/-}$ PNs. Axon of *gars*$^{team/team}$ PN clone occasionally reaches the lateral horn (n = 3/10), but their terminal arborization fails to elaborate. PN axon (green) is stained with anti-mouse CD8 antibody and presynaptic regions (magenta) are stained with nc82 antibody. Dotted circles indicate the calyx region of mushroom body (MB) and lateral horn (LH).



Supplementary Figure 2 MARCM rescue experiments of *gars*$^{EX34/EX34}$ PN clone with either cytoplasmic or mitochondrial GARS. Post-mitotic expressions (*Gal4-GH146*) of either Cyto–GARS-Myc (**a** and **b**) or Mito–GARS-Myc (**c** and **d**) were able to rescue both dendritic and axonal phenotypes of *gars*$^{EX34/EX34}$ PN clone. No differential rescuing capacity was detected. This is likely caused by leaky expression of Mito–GARS-Myc in the cytoplasm as was reported previously in yeast[1]. Staining with nc82 and anti-mouse CD8 antibodies are shown in magenta and green, respectively.

1. Chang, K.J. & Wang, C.C. Translation initiation from a naturally occurring non-AUG codon in Saccharomyces cerevisiae. *J Biol Chem* **279**, 13778-85 (2004).



Supplementary Figure 3 Generation of *Df(3L)mito*. The genomic region between *PBac{RB}e01018* and *PBac{WH}f01783* was deleted according to Parks *et al*[1]. The genomic deletion was confirmed by genomic PCR and lethal complementation test. Three genes including two genes related to mitochondrial protein translation should be completely disrupted in *Df(3L)mito* deletion stock.

1. Parks, A.L. et al. Systematic generation of high-resolution deletion coverage of the Drosophila melanogaster genome. *Nat Genet* **36**, 288-92 (2004).

Supplementary Methods

MARCM-based forward genetic screen

We used ethylmethane sulfonate (EMS) to mutagenize flies carrying FRT^{2A} and FRT^{82B} transgenes, which are sites for the FLP-mediated recombination in third chromosome left and right arms, respectively. Males carrying isogenized third chromosomes homozygous for FRT^{2A}, FRT^{82B}, *P[y+]* were treated with 25 mM EMS. After establishing individual mutant stocks and confirming the lethality of mutations located on *FRT*-containing third chromosomes, we crossed these mutants to "MARCM-ready fly stock". (For third chromosome left arm screen: *y w, hs-flp122, UAS-mCD8-GFP; Gal4-GH146, UAS-mCD8-GFP / CyO; tubP-Gal80,* FRT^{2A} */ TM3 Sb*. For third chromosome right arm screen: *y w, hs-flp122, UAS-mCD8-GFP; Gal4-GH146, UAS-mCD8-GFP / CyO;* FRT^{82B}*, tubP-Gal80 / TM3 Sb*.) We heat-shocked progenies of these crosses at 4–20 hr after larval hatching for 1 hr at 37°C. Then, we dissected out adult fly brains of the appropriate genotype and analyzed the PN dendritic and axonal projection patterns visualized by expression of mCD8-GFP in whole mount live brains under a compound fluorescence microscope.

Genetic mapping of *team* (*gars*^{team}) mutant

Genetic mapping for the causal gene of *team* was first performed with SNP-based recombination mappings[1]. This mapping showed that the responsible mutation for the PN phenotypes in $team^{-/-}$ MARCM clone is at a cytological location between 70D5 and 72C1. Next, we performed lethal complementation test with available deficiency lines within this candidate region, assuming *team* is homozygous lethal. *Df(3L)Brd12, Df(3L)Brd15, Df(3L)XG6, Df(3L)XG14, Df(3L)XG15, Df(3L)XG16* failed to complement lethality of *team* whereas *Df(3L)XG4, Df(3L)XG7* complemented, suggesting that *team* chromosome maps within genomic region from 71A3 to 71B8 (~280 kb). Next, we utilized the mapping method measuring the recombination rate from genetically mapped P-element insertion site[2]. Genetic distances from the *team* lethal mutation to P-element insertions; *GS3025, GS13002, GS9061, EP3609* and *KG02744* were 0.000, 0.050, 0.162, 0.407 and 0.262 cM, respectively, suggesting that the lethal mutation is around the genomic region from 71B3 to 71B7 (~120 kb). Then, we collected

genomic DNA from embryos homozygous for *team* selected by single embryo PCR, and sequenced open reading frames, splicing acceptor and donor sites of all genes in this region. A missense mutation in *CG6778, glycyl-tRNA synthetase* (*gars*) was confirmed three times by sequencing independent PCR products amplified from genomic DNA homozygous or heterozygous for *team* and parental alleles.

Fly stocks

We used the following additional mutants to assess the function of protein translation in neuronal morphogenesis: *P{EPgy2}Aats-gly*^{EY09021}, *P{PZ}Aats-gln*^{05461}, *tko*^{3}(ref.[3]), *wars*^{4}(ref.[4]), *P{tko*$^{25t\text{-}H85L}$*}*^{32}. To generate *gars* null mutant, *gars*^{EX34}, imprecise excision of *P{EPgy2}Aats-gly*^{EY09021} was performed using the *delta2-3* transposase. Loss of the *mini-white* transgene marker was used as an initial screen, and the deleted genomic region was confirmed by genomic PCR. *Df(3L)mito* was generated according to the method described by Parks *et al*[5]. Genotypes used in this study are: (1) *gars* loss-of-function in PN (**Fig. 1c, d, g, h**, **2d, e**, **3e**–**h**): *y w, hs-FLP122, UAS-mCD8-GFP/UAS-syt-HA* or *+; Gal4-GH146, UAS-mCD8-GFP/+; gars*$^{team\ or\ EX34}$*, FRT*^{2A} */tubP-GAL80, FRT*^{2A}. (2) *gars* loss-of-function in MB neuron (**Fig. 7f** and **g**): *y w, hs-FLP, UAS-mCD8-GFP/Y* or *+; UAS-mCD8-GFP/+; gars*^{EX34}*, FRT*^{2A} */tubP-GAL80, FRT*^{2A}*; Gal4-OK107/+*. (3) *gars* rescue (**Fig. 2f, g** and **8c**–**h**): *y w, hs-FLP122, UAS-mCD8-GFP; Gal4-GH146, UAS-mCD8-GFP/UAS-gars transgenes (various types); gars*$^{team\ or\ EX34}$*, FRT*^{2A} */tubP-GAL80, FRT*^{2A}. (4) *qars* or *wars* loss-of-function in PN (**Fig. 5**): *y w, hs-FLP122, UAS-mCD8-GFP; Gal4-GH146, UAS-mCD8-GFP/+; FRT*^{82B}*, qars or wars/ FRT*^{82B}*, tubP-GAL80.* (5) *wars*^{4} loss-of-function in MB neuron (**Fig. 7h** and **i**): *y w, hs-FLP, UAS-mCD8-GFP/Y or +; UAS-mCD8-GFP/+; FRT*^{82B}*, wars*^{4}*/ FRT*^{82B}*, tubP-GAL80; Gal4-OK107/+*. (6) *tko*^{3} loss-of-function in PN (**Fig. 6c**–**f**): *tko*^{3}*, FRT*^{19A}*/y w, hs-FLP122, tubP-GAL80, FRT*^{19A}*; Gal4-GH146, UAS-mCD8-GFP/Gal4-GH146, UAS-mCD8-GFP*. (7) *tko*^{3} loss-of-function in MB neuron (**Fig. 7j** and **k**): *tko*^{3}*, FRT*^{19A}*/y w, hs-FLP, tubP-GAL80, FRT*^{19A}*; UAS-mCD8-GFP/+; ;Gal4-OK107/+*. (8) *Df(3L)mito* MARCM analysis (images not shown, quantified in **Fig. 6g** and **h**): *y w, hs-FLP122, UAS-mCD8-GFP; Gal4-GH146, UAS-mCD8-GFP/+; Df(3L)mito, FRT*^{2A}*/tubP-GAL80, FRT*^{2A}. (6) *Drosophila* GARS-Myc localization in PN (**Fig. 4a**, **c**, **e**): *y w; Gal4-Mz19, UAS-mCD8-GFP / UAS-(full, cyto or*

mito)–gars-myc. (**Fig. 4g** and **h**): *y w; Gal4-Mz19 / UAS-full–gars-myc; UAS-mitoGFP / +*.

Immunostaining

Fixation, immunostaining, and imaging were carried out as described[6]. Additional antibodies used in this study were anti-HA (12CA5), 1:1000; rabbit anti-GFP, 1:500 (Molecular Probe); rat anti-*D*N-Cadherin, 1:30; mouse anti-Myc (9E10), 1:250 (Developmental Studies Hybridoma Bank at the University of Iowa). Triple immunofluorescent stainings with anti-mouse CD8 (rat), anti-HA (mouse), and nc82 (mouse) antibodies were carried out with Zenon Alexa Fluo 647 mouse IgG1 labeling kit (Molecular Probe). mCD8-GFP was used primarily to visualize mutant neurons as it is an extremely stable protein (e.g. ORN axons and terminals separated from cell bodies for 50 days still retain mCD8-GFP staining, see MacDonald *et al.* [7]).

Transfection and MitoTracker staining

Cos-7 cells were cultured and transfected by using Lipofectamine (BRL). For *Drosophila* GARS-myc localization, we co-trasnfected *UAS-gars-myc* and *pSN3* vector that expresses GAL4 under control of CA promoter in Cos-7 cells. For human GARS-Myc localization, we transfected *pcDNA3-human-gars-myc* into Cos-7 cells. For MitoTracker staining and immunostaining, transfected Cos-7 cells were incubated with DMEM culture medium containing 200 nM of MitoTracker red CMXRos (Invitrogen) for 30 min before fixation. After fixation with 4% paraformaldehyde/PBS, cells were stained with mouse anti-Myc (9E10) at 1:250 dilution (Developmental Studies Hybridoma Bank at the University of Iowa).

1. Berger, J. et al. Genetic mapping with SNP markers in Drosophila. *Nat Genet* **29**, 475-81 (2001).
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