Supporting Information

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SI Methods

Transgenic Strains. Expression clones were made in the pSM vector, a derivative of pPD49.26 (A gift from A. Fire, Stanford, CA 94305) with extra cloning sites (S. McCarroll and C. I. Bargmann, personal communication). The following plasmids and transgenic strains were generated using standard techniques: *wyIs93 (Pglr-3::glr-1::GFP, Pglr-3::mCherry::rab-3)*, *wyEx1410 (Pglr-3::acr-16::GFP, Pglr-3::mCherry::rab-3)*, *wyEx1153 (Pglr-3::cam-1::YFP, Pglr-3::mCherry::rab-3)*, *wyEx2097 (Pglr-3::snb-1::YFP, Pglr-3::mCherry)*, *wyEx2132 (Pglr-3::sng-1::YFP, Pglr-3::mCherry)*, *wyEx2692 (Pitr-1 pB::cam-1::YFP, Pitr-1 pB::mCherry)*, *wyIs121 (Popt-3::glr-1::YFP, Popt-3::snb-1::CFP, Popt-3::mCherry)*, *wyEx2269 (Pglr-3::unc-101)*, *wyEx2270 (Phsp16–2, hsp16–41::unc-101)*, *wyEx1758 (Pglr-3::glr-1$_{\Delta 876-962}$::YFP, Pglr-3::mCherry)*, *wyEx1713 (Pglr-3::cam-1$_{\Delta 467-902}$::YFP, Pglr-3::snb-1::CFP)*, *wyEx1883 [Pglr-3::pat-3(TM)::YFP, Pglr-3::mCherry]*, *wyEx1886 [Pglr-3:: pat-3(TM):: glr-1$_{876-962}$::YFP, Pglr-3::mCherry]*, *wyEx1946 [Pglr-3::pat-3(TM):: cam-1$_{467-902}$::YFP, Pglr-3::mCherry]*, *wyEx2032 [Pglr-3::pat-3(TM)::acr-16$_{314-472}$::YFP, Pglr-3::mCherry]*, *wyEx1900 [Pglr-3::pat-3(TM)::glr-1$_{876-905}$::YFP, Pglr-3::mCherry]*, *wyEx1989 [Pglr-3::pat-3(TM)::glr-1$_{906-962}$::YFP, Pglr-3::mCherry]*, *wyEx2165 (Pglr-3:: glr-1$_{\Delta 876-905}$::YFP, Pglr-3::mCherry)*, *wyEx1921 (Pglr-3:: glr-1$_{\Delta 906-962}$::YFP, Pglr-3::mCherry)*, *wyEx1837 (Pglr-3::glr-1YR880AA::YFP, Pglr-3::mCherry)*, *wyEx1897 (Pglr-3::unc-101::YFP, Pglr-3::MANS::mCherry)*, *wyEx2403 (Pitr-1::unc-101::YFP, Pitr-1::mCherry::rab-3)*, *wyEx2302 [Pglr-3::dyn-1(K46A)]*, *wyEx2391 [Pglr-3::dyn-1(K46A)::YFP, Pglr-3::mCherry]*.

All RIA constructs were injected at 20 ng μl^{-1}, except for *wyEx2269*, *wyEx2270*, and *wyEx2302*, which were injected at 50 ng μl^{-1}, and *wyEx2391*, which was injected at 30 ng μl^{-1}. For RIA constructs, we used the coinjection markers *Punc-122::dsRED* or *GFP* injected at 30 ng μl^{-1}. For DA9 and AVE constructs, we used coinjection markers *Podr-1::dsRED* (30 ng μl^{-1}) and *lin-15* (50 ng μl^{-1}), respectively. Multiple transgenic lines for each transgene were examined for fluorescence expression, rescue, and localization patterns.

Heat Shock Experiments. We subcloned *unc-101* cDNA into the plasmid pPD49.83, which contains inducible heat-shock promoters *hsp16–2* and *hsp16–4* (A gift from A. Fire, Stanford, CA 94305). The transgenic animals carrying the *Phs::unc-101* construct were heat shocked for 2 h at 33 °C at the L1/L2 stage. The degree of rescue was assessed 24 h later.

Fluorescence Microscopy. The majority of images of fluorescently tagged fusion proteins were captured in live *C. elegans* using a Plan-Apochromat 63X/1.4 objective on a Zeiss LSM510 confocal microscope. Images of CAM-1::YFP in DA9 neurons were captured using Zeiss Observer Z1 with a QuantEM camera. Worms were immobilized using 10 mM levamisole (Sigma).

Calculation of the Polarity Index and Statistics. For each genotype or construct, confocal images of 15–20 animals in L3, L4, or young adult stage were obtained. Z-stack slices corresponding to one of the RIA neurons were projected using Zeiss LSM Image Browser, and the subsequent image was analyzed using ImageJ software. Briefly, the areas corresponding to the dendritic or axonal region of RIA were traced using Freehand Selection function, and the mean pixel intensity was obtained. The mean pixel intensity was also obtained for the background fluorescence. RIA dendrite was anatomically defined as extending from the cell body to the most ventral tip of the neurite. Axon was defined as the part of the process that extends from the point of the nerve ring reentry (where it first meets the contralateral RIA process) to the most dorsal part of the neurite. The polarity index for each animal was obtained by using formula PI = (D−B)/[(D−B) + (A − B)], where D represents the dendritic mean pixel intensity, A is the axonal intensity, and B is the background intensity. The resulting PI is 0 for a perfectly axonally localized protein, 1 for a completely dendritic protein, and 0.5 for an unpolarized protein. Mean pixel intensity was used for polarity index calculations because left and right RIA axonal segments overlap, but their intensity contributions should be similar and thus correctly represented by the average pixel intensity metric. Student's *t* test was used to evaluate statistically significant differences between different constructs or genetic backgrounds.

Molecular Biology. The following promoters were PCR amplified and subcloned into the pSM vector:

Pglr-3 primers 5′-GGCCGGCCTCGGAAATGCGGAAGTTCTTTCCG and 3′-GGCGCGCCATGTTAATAGCAAATATTGAAGATTC, using FseI, AscI.

Popt-3 primers 5′-GGCCGGCCTAACAGAATTAGTAAGAAGGTGGG and 3′-GGCGCGCCCAGACACGGGAGAGGCGG using FseI, AscI.

Pitr pB primers and cloning strategy are detailed in ref. 1.

The *Pglr-3 pSM* vector was converted to Gateway destination vectors by inserting the Gateway reading frame A into NheI, KpnI sites.

wyIs93. *Pglr-3::glr-1::GFP: Pglr-3* promoter was cloned into *Popt-3::glr-1::GFP* (adapted from KP196, see below) using SphI, XmaI sites. *Pglr-3::mCherry::rab-3*: a NheI-ApaI fragment containing *rab-3::unc-10 3′UTR* from *ttx-3::mCherry::rab-3* (A gift from D. Colon-Ramos, New Haven, CT 06510) was subcloned into *Pglr-3::mCherry::pSM* (with mCherry placed between XmaI and NheI sites). The array was integrated into chromosome X using trimethylpsoralen/UV mutagenesis.

wyEx1410. *Pglr-3::acr-16::GFP* was generated using BfuIA, XbaI enzymes to replace *Pmyo-3* promoter in *pDM906* (A gift from V. Maricq, Salt Lake City, UT 84112); we added FseI and AscI restriction sites between *Pglr-3* promoter sequence and BfuIA, XbaI sites to increase compatibility with Shen Lab pSM vectors. The following primers were used to amplify *Pglr-3* sequence: 5′-AACTTGGGCTGCAGGTGGCCGGCCTCG-GAAATGCGGAAGTTCTTTCCG 3′-AATCTAGAATAG-GCGCGCCATGTTAATAGCAAATATTGAAG.

wyEx1153. The *cam-1* entry clone was obtained from the ORFeome Project (http://worfdb.dfci.harvard.edu/) and cloned into the destination vector *Pglr-3::gateway::YFP* using the Gateway strategy with LR Clonase (Invitrogen) to make *Pglr-3::cam-1::YFP*.

wyEx2097. *Pglr-3::snb-1::YFP* was generated by cloning *snb-1::YFP::unc-54 3′UTR* fragment from *Pmig-13::snb-1::YFP* (1) into *Pglr-3::pSM* vector using NheI, ApaI sites.

wyEx2132. The *sng-1* entry clone was obtained from the ORFeome Project (http://worfdb.dfci.harvard.edu/) and cloned into the destination vector *Pglr-3::gateway::YFP* using the Gateway

strategy with LR Clonase (Invitrogen) to make *Pglr-3*::*sng-1*::*YFP*.

wyEx2692. Cloning of *Pitr-1 pB*::*cam-1*::*YFP* is described in ref. 1.

wyIs121. *Popt-3* promoter was cloned into KP196 (A gift from J. Kaplan, Boston, MA 02114) using NotI, SalI restriction sites to generate *Popt-3*::*glr-1*::*GFP*. We added SphI and XmaI restriction sites between *Popt-3* promoter sequence and NotI, SalI sites to increase compatibility with Shen Lab pSM vectors. The following primers were used to amplify *Popt-3* sequence: 5′-GAAAGGGCGGCCGCATGCtaacagaattagtaagaaggtggg, 3′-GAAAGGGTCGACCCGGGcagacacgggagaggcgg. Furthermore, GFP was replaced by YFP to generate *Popt-3*::*glr-1*::*YFP*.

wyEx2269. The *unc-101* entry clone was obtained from the ORFeome Project (http://worfdb.dfci.harvard.edu/) and cloned into the destination vector *Pglr-3*::*gateway* using the Gateway strategy with LR Clonase (Invitrogen) to make *Pglr-3*::*unc-101*.

wyEx2270. A NheI, ApaI fragment containing *gateway*::*unc-54 3′ UTR* from *Pglr-3*::*gateway* vector was cloned into pPD49.83 (A gift from A. Fire, Stanford, CA 94305), which contains *hsp16–2* and *hsp16–41* heat-shock elements. Subsequently, the *unc-101* entry clone obtained from the ORFeome Project was cloned into the destination vector *Phs*::*gateway* using the Gateway strategy with LR Clonase (Invitrogen) to make *Phs*::*unc-101*.

wyEx1758. To create GLR-$1_{\Delta 876–962}$, we PCR amplified genomic *glr-1* lacking its C terminus from *Pglr-3*::*glr-1*::*GFP* using primers 5′-aaTGGCCAatgttttcttcgttttc-ttttttgaacatgttcgg and 3′-GGTACCccAGATCCAGATCCAGATCCAGATCCAGA-TCCgagtgcagccaacattgaaatgacc. The resulting fragment was cloned into *Pglr-3*::*pSM*::*YFP* using MscI, KpnI sites. For additional flexibility, GSGSGSGSGS linker was added between the last transmembrane domain of GLR-1 and the fluorophore, as well as 2 bp to maintain the frame.

wyEx1713. To create CAM-$1_{\Delta 467–902}$, we PCR amplified *cam-1* lacking its C terminus from *Pglr-3*::*cam-1*::*YFP* using primers 5′-aaGCTAGCatggagggtacatcaactggtcaacgand3′-ttGGTACCccA-GATCCAGATCCAGATCCAGATCCAGATCCtgctcgacagc-aacacgcacagcag. The resulting fragment was cloned into *Pglr-3*::*pSM*::*YFP* using NheI, KpnI sites. For additional flexibility, GSGSGSGSGS linker was added between the CAM-1 transmembrane domain and the fluorophore, as well as 2 bp to maintain the frame.

wyEx1883. *Pglr-3*::*pat-3(TM)*::*YFP* was created by PCR amplifying a 350-bp fragment containing *pat-3* signal sequence and transmembrane domain (A gift from C. Rongo, Piscataway, NJ 08854) using primers 5′-gaaaggGCTAGCatgccaccttcaacatcattgc and 3′-cctttcGGTACCGGTccAGATCCAG-ATCCttcggatctat-catgaagtac. The resulting fragment was cloned into *Pglr-3*::*pSM*::*YFP* using NheI, KpnI sites. For additional flexibility, a short GSGS linker was added at the end of PAT-3 transmembrane domain, as well as 2 bp to maintain the frame and a combined AgeI/KpnI site for more cloning flexibility.

wyEx1886. GLR-1 C-terminus sequence (876–962 aa), including introns and YFP, was PCR amplified from *Pglr-3*::*glr-1*::*YFP* using primers 5′-gaaaggGGTACCGgggga-attcttgtatcgaag and 3′-cctttcGGTACCcaagacaagaatttacatt. The resulting PCR product was cloned into *Pglr-3*::*pat-3(TM)* using KpnI sites, resulting in *Pglr-3*:: *pat-3(TM)*:: *glr-*$1_{876–962}$::*YFP*. One base pair was added at the beginning of *glr-1* C-terminus sequence to maintain the frame with PAT-3(TM).

wyEx1946. CAM-1 C-terminus sequence (497–902 aa) was PCR amplified from *Pglr-3*::*cam-1*::*YFP* using primers 5′-gaaaggAC-CGGTGaagaagaagtctcaaaagac and 3′-CCTTTCaccggtCCatca-gaatcaccatcctctg. The resulting PCR product was cloned into *Pglr-3*::*pat-3(TM)*::*YFP* using AgeI sites, resulting in *Pglr-3*::*pat-3(TM)*::*cam-*$1_{467–902}$::*YFP*. One base pair was added at the beginning of *cam-1* C-terminus sequence to maintain the frame with *pat-3*, and 2 bp were added at the end to maintain the frame with YFP.

wyEx2032. ACR-16 M3–M4 loop sequence (314–472 aa) was PCR amplified from an *acr-16* entry clone obtained from the ORFeome Project (http://worfdb.dfci.harvard.edu/) using primers 5′-gaaaggGGTACCGctcaacttacattaccgtac and 3′-cctttcGG-TACCCCgcg-gtccacaaccatggccgc. The resulting PCR product was cloned into *Pglr-3*::*pat-3(TM)*::*YFP* using KpnI sites, resulting in *Pglr-3*::*pat-3(TM)*::*acr-*$16_{314–472}$::*YFP*. One base pair was added at the beginning of *acr-16* loop sequence to maintain the frame with *pat-3*, and 2 bp were added at the end to maintain the frame with YFP.

wyEx1900. GLR-1 juxtamembrane C-terminus sequence (876–905 aa) was PCR amplified from *Pglr-3*::*glr-1*::*YFP* using 5′ primer designed for *wyEx1886* and 3′ primer cctttcGGTAC-CccAGATCCAGATCCAGATCCtgcacttttcaaatttttctg. The resulting PCR product was cloned into *Pglr-3*::*pat-3(TM)*::*YFP* using KpnI sites, resulting in *Pglr-3*::*pat-3(TM)*::*glr-*$1_{876–905}$::*YFP*. For additional flexibility, GSGSGS linker was added following GLR-1 juxtamembrane sequence, as well as 2 bp to maintain the frame with YFP.

wyEx1989. GLR-1 distal C-terminus sequence (906–962 aa), including introns and YFP, was PCR amplified from *Pglr-3*::*glr-1*::*YFP* using 5′ primer gaaaggGGTACCGttgt-catctcaattaagattatc and 3′ primer designed for *wyEx1886*. The resulting PCR product was cloned into *Pglr-3*::*pat-3(TM)* using KpnI sites, resulting in *Pglr-3*::*pat-3(TM)*::*glr-*$1_{906–962}$::*YFP*. One base pair was added at the beginning of *glr-1* distal C-terminus sequence to maintain the frame with PAT-3(TM).

wyEx2165. GLR-1 lacking juxtamembrane 30 aa was created by cloning a KpnI-KpnI *glr-*$1_{906–962}$::*YFP* fragment from *Pglr-3*::*pat-3(TM)*::*glr-*$1_{906–962}$::*YFP* into *Pglr-3*::*glr-*$1_{\Delta 876–962}$ (GLR-1 lacking its C terminus), resulting in *Pglr-3*:: *glr-*$1_{\Delta 876–905}$::*YFP*.

wyEx1921. GLR-1 lacking distal 56 C-terminus amino acids was PCR amplified from *Pglr-3*::*glr-1*::*YFP* using 5′ primer designed for *wyEx1758* and 3′ primer designed for *wyEx1900*. The resulting PCR product was cloned into *Pglr-3*::*pSM*::*YFP* using MscI, KpnI sites, resulting in *Pglr-3*:: *glr-*$1_{\Delta 906–962}$::*YFP*.

wyEx1837. *Pglr-3*::*glr-1YR880AA*::*YFP* was generated using site-specific mutagenesis (Stratagene QuikChange). P*glr-3*::*glr-1*::*YFP* was used as a template. The primers were as follows: 5′-GGCTGCACTCGGGGAATTCTTG**GCCGC**-CAGTAGGA-TTGAAGCGAGG and 3′-CCTCGCT-TCAATCCTACTGGCGGCCAAGAATTCC-CCGAGTG-CAGCC.

wyEx1897. The *unc-101* entry clone was obtained from the ORFeome Project (http://worfdb.dfci.harvard.edu/) and cloned into the destination vector *Pglr-3*::*gateway*::*YFP* using the Gateway strategy with LR Clonase (Invitrogen) to make *Pglr-3*::*unc-101*::*YFP*.

wyEx2403. The *unc-101* entry clone was obtained from the ORFeome Project and cloned into the destination vector *Pitr-1*

pB::*gateway*::*YFP* using the Gateway strategy with LR Clonase (Invitrogen) to make *Pitr-1*::*unc-101*::*YFP*. *Pitr-1 pB*::*Cherry*::*rab-3* was made by PCR amplifying *Pitr-1 pB* promoter and subcloning it into *Pglr-3*::*mCherry*::*rab-3* using FseI, AscI sites.

wyEx2302. The *dyn-1* (isoform a) entry clone was obtained from the ORFeome Project (http://worfdb.dfci.harvard.edu/). The K46A mutation was introduced by finding 2 unique cloning sites flanking the desired K46A mutation site (BstBI on the 5′ end, XhoI on the 3′ end). Primers were made that PCR amplified the segment of *dyn-1* between BstBI and XhoI sites, while also introducing the K46A mutation on the 5′ end: 5′-gaaaggTTCGAActtccacagatcgccgtcgtcggaggacagtccgctggaGCCtcgtcggtgctgg and 3′-gaaaggCTCGAGgatctcgcgagcatcggttccc. This modified fragment was cloned into the *dyn-1* entry clone using BstBI and XhoI. Finally, the modified *dyn-1(K46A)* entry clone was cloned into the destination vector *Pglr-3*::*gateway* using the Gateway strategy with LR Clonase (Invitrogen) to make *Pglr-3*::*dyn-1(K46A)*.

wyEx2391. The modified *dyn-1(K46A)* entry clone was cloned into the destination vector *Pglr-3*::*gateway*::*YFP* using the Gateway strategy with LR Clonase (Invitrogen) to make *Pglr-3*::*dyn-1(K46A)*::*YFP*.

1. Klassen MP, Shen K (2007) Wnt signaling positions neuromuscular connectivity by inhibiting synapse formation in C. elegans. *Cell* 130:704–716.

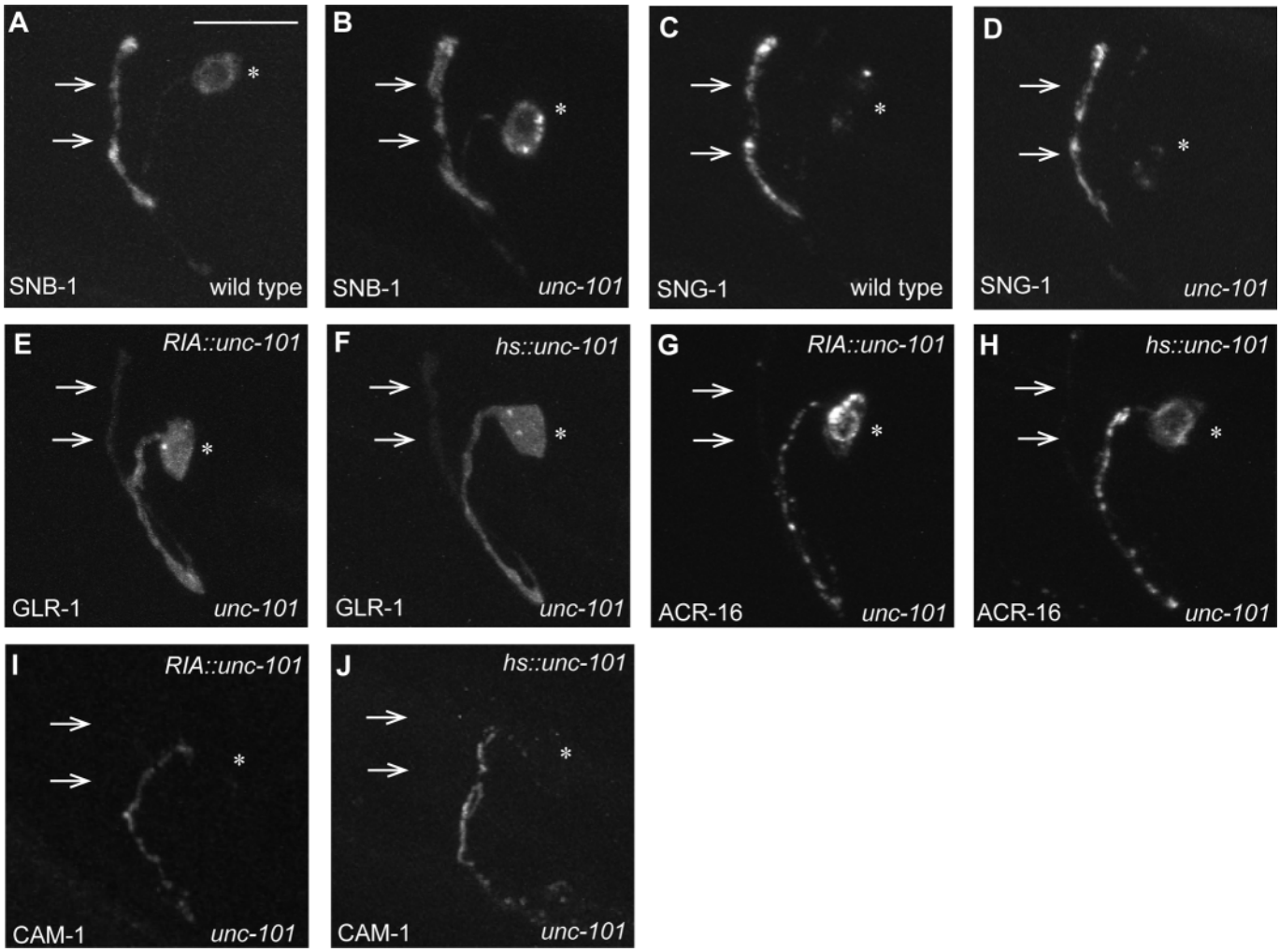

Fig. S1. Images of presynaptic protein localization in wild-type and μ1/*unc-101* mutants, and images of cell-autonomous and heat-shock rescue of GLR-1, ACR-16, and CAM-1 in *unc-101* mutants. (*A* and *B*) Integral membrane synaptic vesicle protein synaptobrevin SNB-1::YFP localizes presynaptically in wild-type animals (*A*) and *unc-101(m1)* mutants (*B*). (*C* and *D*) Integral membrane synaptic vesicle protein synaptogyrin SNG-1::YFP localizes presynaptically in wild-type animals (*C*) and *unc-101(m1)* mutants (*D*). (*E*, *G*, and *I*) Localization of GLR-1::GFP (*E*), ACR-16::GFP (*G*), and CAM-1::YFP (*I*) in *unc-101(m1)* animals expressing *unc-101* cDNA under the control of RIA-specific *glr-3* promoter. (*F*, *H*, and *J*) Localization of GLR-1::GFP (*F*), ACR-16::GFP (*H*), and CAM-1::YFP (*J*) in heat-shocked *unc-101(m1)* animals expressing *unc-101* cDNA under the control of the heat-shock promoter. Asterisk denotes RIA cell body; arrows point to the distal (presynaptic) region of the RIA neurite. Anterior is to the left; ventral is down. (Scale bar: 10 μm.)

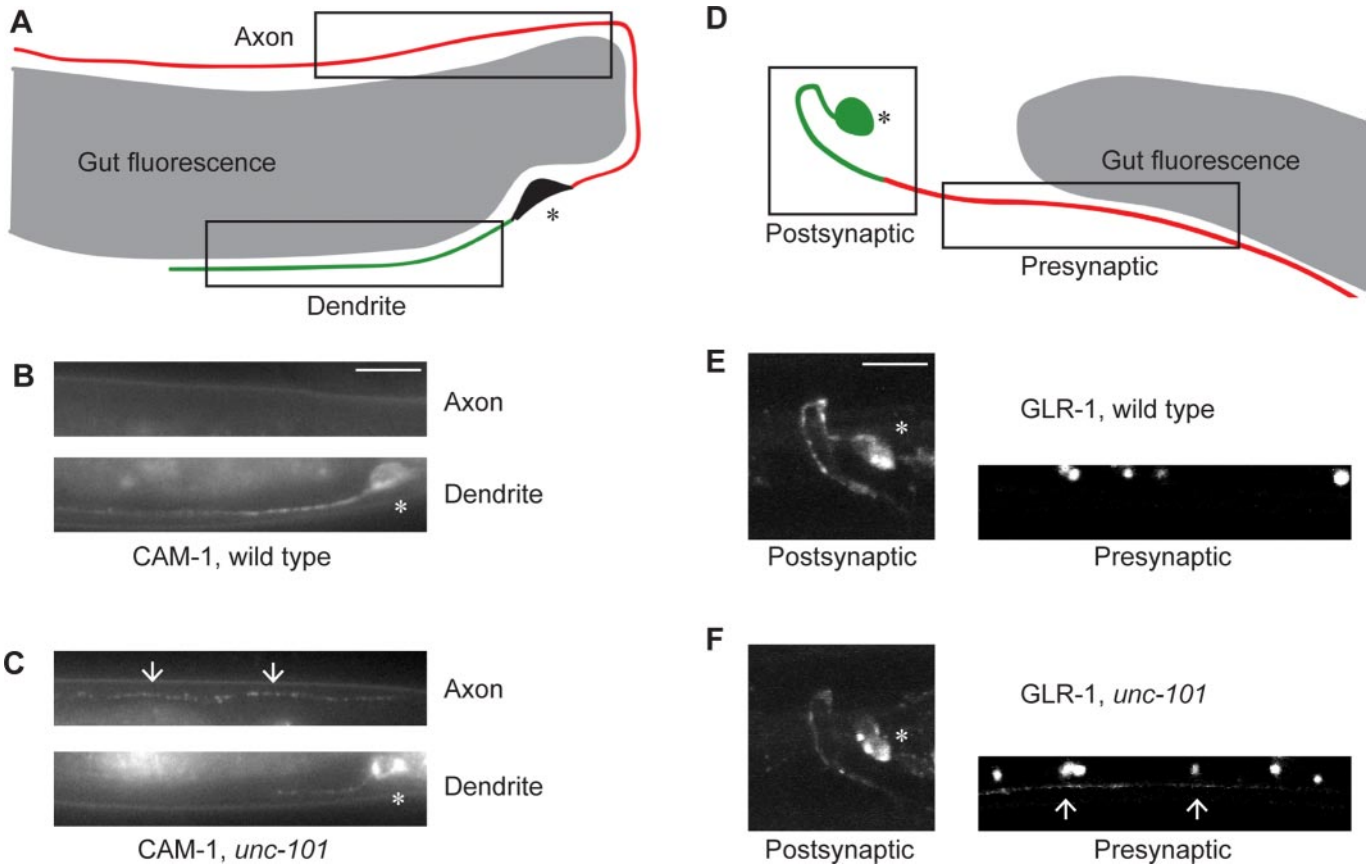

Fig. S2. μ1/*unc-101* is required for dendritic localization of postsynaptic receptors in polarized DA9 and AVE neurons. (*A*) Schematic diagram of DA9 neuron as viewed from the left side of the worm. DA9 cell body (asterisk) is located in the tail of the worm and extends 2 neurites: a dendrite anteriorly (green) and an axon commissurally and then anteriorly along the dorsal nerve cord (red). (*B*) Representative wild-type adult animal expressing CAM-1::YFP under the control of DA9-selective *Pitr-1 pB* promoter. (*Top*) Dorsal portion of the DA9 axon. (*Bottom*) DA9 cell body and dendrite. CAM-1::YFP localizes to the dendrite and proximal axon of the DA9 neuron. (*C*) In *unc-101(m1)* mutants, CAM-1::YFP is enriched in the cell body and faint in the DA9 dendrite (92.8%, $n = 83$; *Bottom*). In addition, CAM-1::YFP puncta can be observed in the axon of DA9 in 40.9% of *unc-101(m1)* animals ($n = 83$; *Top*, arrows). Finally, 100% ($n = 83$) of *unc-101* animals display a shortening of the DA9 dendrite to approximately half of the wild-type length. (*D*) Schematic diagram of the AVE neuron as viewed from the left side of the worm. AVE cell body (asterisk) is located in the lateral ganglion in the head of the worm and extends a single neurite that is exclusively postsynaptic proximally (green) and presynaptic distally (red). (*E*) Representative wild-type L3 animal expressing GLR-1::YFP under the control of AVE-selective *opt-3* promoter. GLR-1::YFP localizes exclusively to the postsynaptic region in the great majority (96.2%, $n = 52$) of wild-type animals and is absent from the presynaptic region. (*F*) In *unc-101(m1)* mutants, 100% of animals have diminished GLR-1::YFP levels in the postsynaptic AVE region, and 94.0% have faint GLR-1::YFP fluorescence visible in the distal, presynaptic region (arrows; $n = 50$). In addition, 82.0% ($n = 50$) of *unc-101(m1)* animals displayed AVE neurite overgrowth, with the process extending further than half of the worm body length. Anterior is to the left; ventral is down. (Scale bar: 10 μm.)

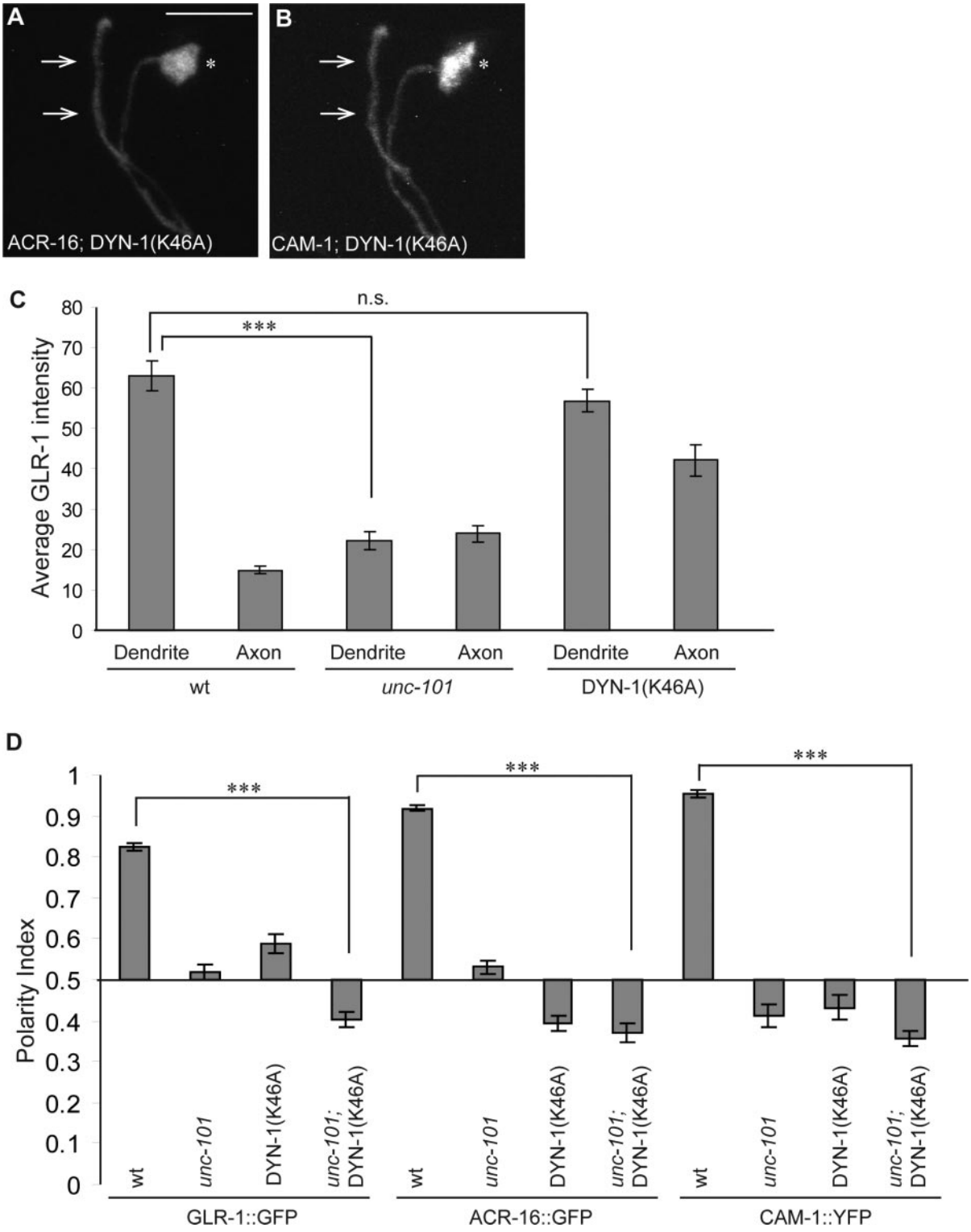

Fig. S3. Further characterization of *unc-101(m1)*, DYN-1(K46A), and *unc-101(m1)*; DYN-1(K46A) animals. (*A*) Animals expressing DYN-1(K46A) show mislocalization of ACR-16::GFP to the presynaptic region of RIA (arrows). (*B*) Animals expressing DYN-1(K46A) show mislocalization of CAM-1::YFP to the presynaptic region of RIA (arrows). Anterior is to the left; ventral is down. (*C*) Average pixel intensity of GLR-1::GFP in postsynaptic (''dendrite'') and presynaptic (''axon'') regions of RIA in wild-type, *unc-101(m1)*, and DYN-1(K46A) animals. These animals were imaged using identical confocal settings. Average pixel intensities for RIA dendrite, axon, and background were obtained as described in *Methods*. Average background intensity was subtracted from the dendrite and axon intensities, and these values plotted in the diagram. (*D*) Polarity index quantification for GLR-1::GFP, ACR-16::GFP, and CAM-1::YFP in *unc-101(m1)* animals expressing DYN-1(K46A) transgene (''double mutants''). (Scale bar: 10 μm.) ***$P < 0.001$; error bars, SEM.