Figure S1

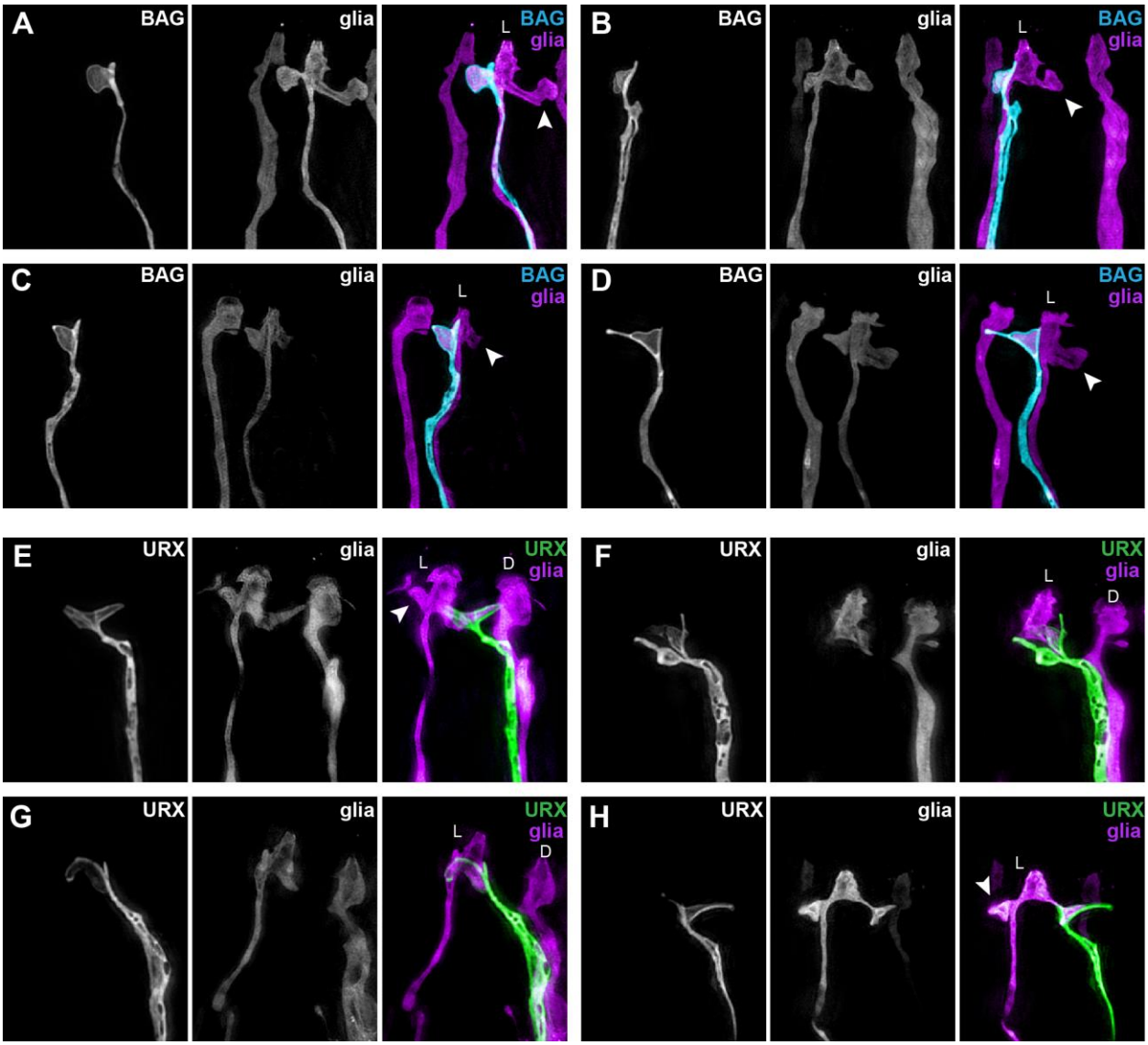


Fig. S1. BAG and URX dendrites form specialized contacts with a single glial cell. Related to Fig. 1.

Examples showing variability in morphology of the dendrite-glia contact across individuals. Single-wavelength and merged superresolution images of BAG (A-D, blue, *flp-17*pro) or URX (E-H, green, *flp-8*pro) and ILso glia (purple, *grl-18*pro) acquired with structured illumination microscopy. In addition to the glial protrusion that is contacted by the labeled neuron, a second

glial protrusion (arrowhead) likely represents the site of contact with the unlabeled neuron (A-D, URX unlabeled; E-H, BAG unlabeled). In two examples (F, G) the second glial protrusion is hidden from view due to the orientation of the animal. The glial process in F and the dorsal glial cell in H are not visible due to these cells extending outside the optical stack. L, lateral. D, dorsal.

Figure S2

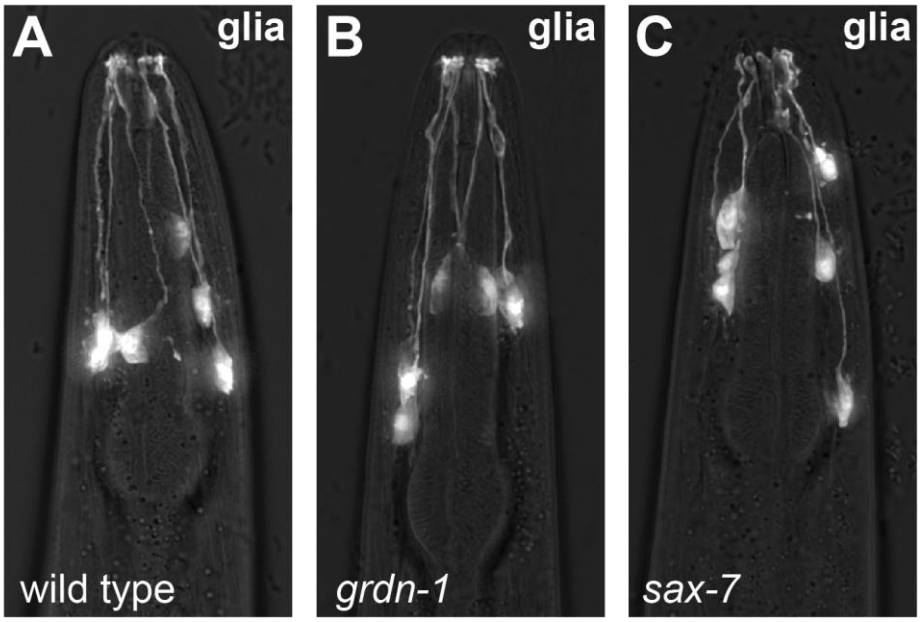


Fig. S2. Glial morphology in *grdn-1* and *sax-7* mutants. Related to Fig. 3. (A) Wild-type, (B) *grdn-1*, and (C) *sax-7* animals expressing the ILso marker *grl-18*pro. Glial processes extend normally to the nose. Some cell body positioning defects are apparent in *sax-7* animals.

Figure S3

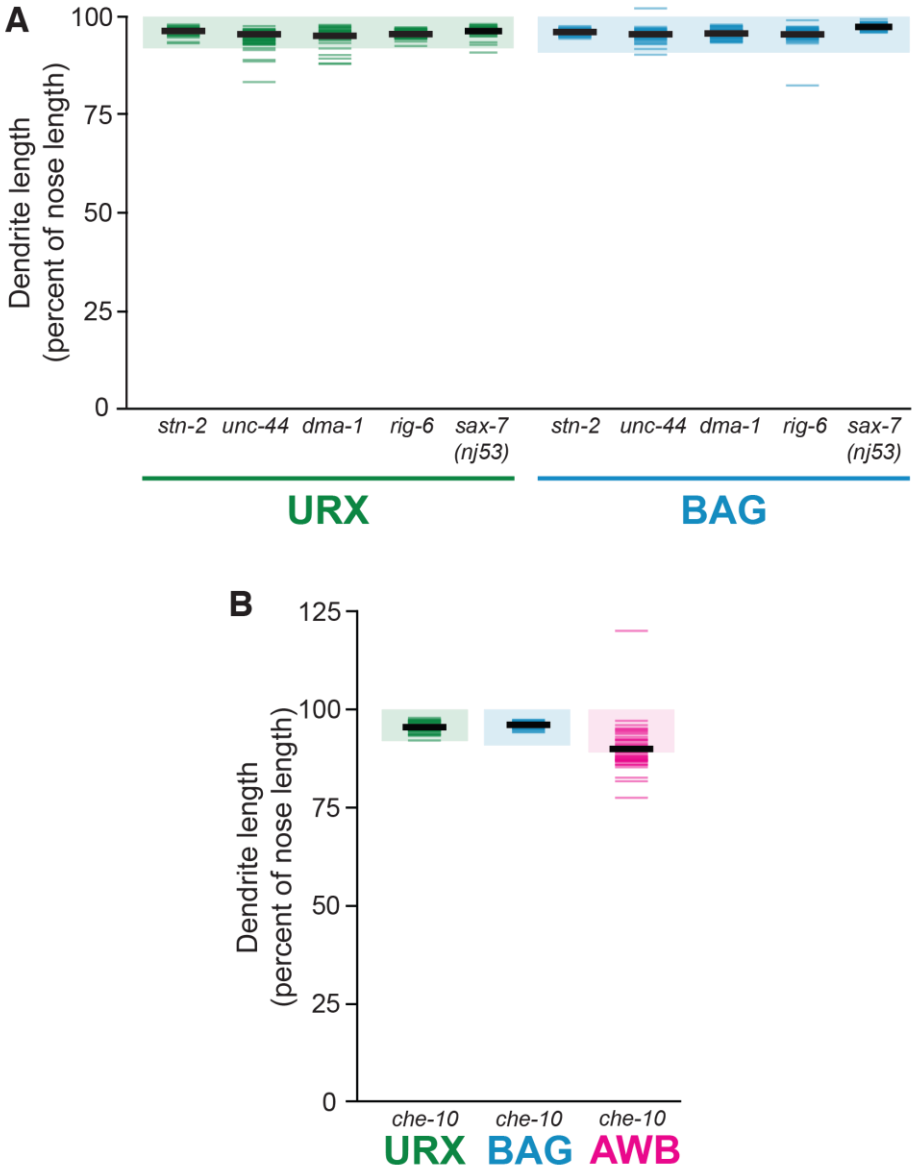


Fig. S3. Factors that act with SAX-7 and GRDN-1 in neuronal development are not required for URX or BAG dendrite extension. Related to Fig. 3.

Quantification of URX (*flp-8*pro, green), BAG (*flp-17*pro, blue), and AWB (amphid neuron, *str-1pro*, pink) dendrite lengths, expressed as a percentage of the distance from cell body to nose. Colored bars represent individual dendrites (n≥48 per genotype); black bars represent population

averages; shaded regions represent wild-type mean ± 5 s.d. for each neuron type, which we define as "full length". (A) Quantification of *stn-2(tm1869), unc-44(e362), dma-1(wy686), rig-6(ok1589),* and *sax-7(nj53)* dendrite lengths. SAX-7 physically interacts with UNC-44, STN-2, DMA-1, and RIG-6 to affect neuronal development, but these factors do not strongly affect URX or BAG dendrite lengths. *sax-7(nj53)* specifically affects the SAX-7L isoform, which has different adhesive properties than the SAX-7S isoform used for rescue experiments (Bénard et al., 2012). In *unc-44* mutants, URX and BAG dendrites often exhibited abnormal branching, and one BAG dendrite exhibited overgrowth. *rig-6* mutants also showed other, low-penetrance morphological defects in URX and BAG including ectopic dendrite branching and cell body positioning defects. (B) Quantification of *che-10(e1809)* dendrite lengths. GRDN-1 acts through CHE-10 in cilia development (Nechipurenko et al., 2016) and loss of CHE-10 causes dendrite extension defects in some amphid neurons, but not in URX or BAG. One AWB dendrite exhibited overgrowth.

Figure S4

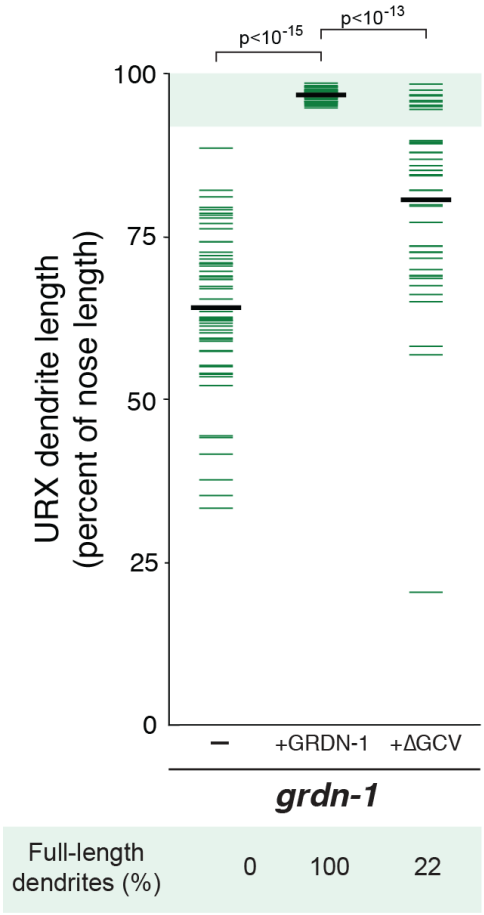


Fig. S4. The GRDN-1a isoform promotes dendrite extension, and requires its PDZ-binding motif for full activity. Related to Fig. 3.

Quantification of full-length URX dendrites in wild-type or *grdn-1(ns303)* animals bearing no transgene (–) (same data as Fig. 3G) or a transgene composed of a *grdn-1* promoter sequence driving expression of full-length GRDN-1a cDNA (+GRDN-1, same data as Fig. 7A) or GRDN-1a cDNA lacking its carboxy-terminal PDZ-binding motif (+ΔGCV). Colored bars represent individual dendrites (n≥44 per genotype); black bars represent population averages. Shaded region represents wild-type mean ± 5 s.d. and the percentage of dendrites in this range ("full length dendrites") is indicated below the plot. p-values, Wilcoxon Rank Sum test.

Figure S5

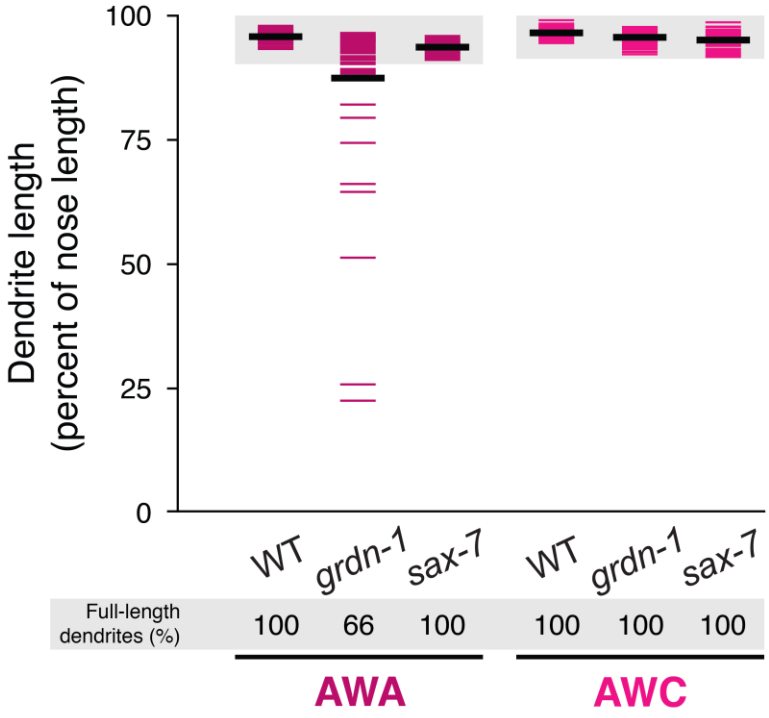


Fig. S5. Dendrite extension defects in amphid neurons. Related to Fig. 4.

Dendrite extension defects are observed in *grdn-1* mutants for the amphid neurons AWB (Fig. 4) and AWA, but not AWC, and these defects are not present in *sax-7* mutants. Quantification of AWA (*gpa-4*Δpro) and AWC (*odr-1*pro) dendrite lengths in wild-type, *grdn-1* and *sax-7* animals, expressed as a percentage of the distance from cell body to nose. Colored bars represent individual dendrites (n≥50 per genotype); black bars represent population averages. Shaded region represents wild-type mean ± 5 s.d. and the percentage of dendrites in this range ("full length dendrites") is indicated below the plot.

Figure S6

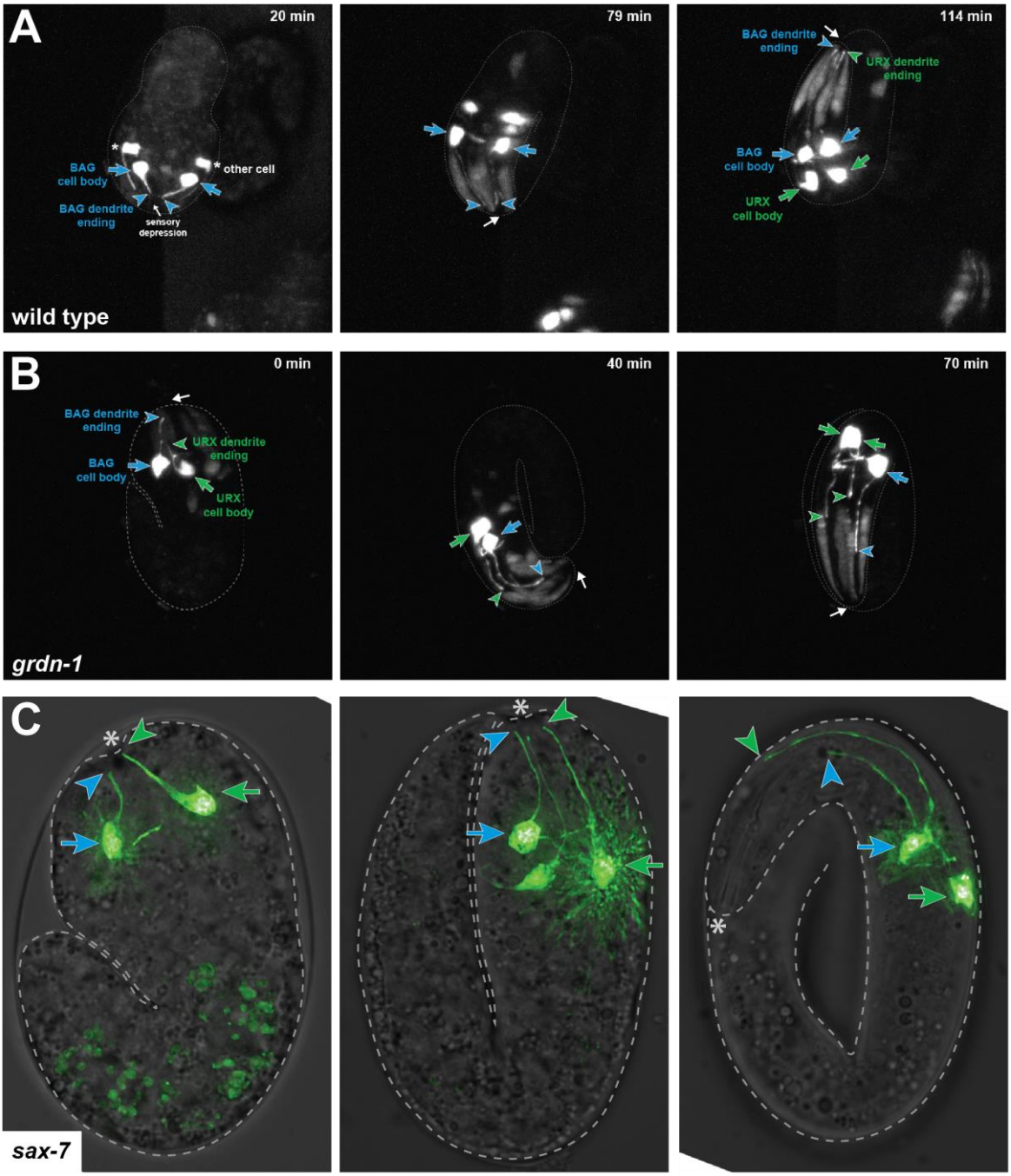


Fig. S6. URX and BAG dendrite length defects arise during embryo elongation. Related to Fig. 5 and Movies S1 and S2.

(A) In a wild-type embryo, BAG and URX extend short dendrites that contact the presumptive nose and appear to extend by stretch during embryo elongation. (B) In a *grdn-1* embryo, dendrites detach from the nose and fail to extend further. Time-lapse movies (Movies S1 and S2) of embryos expressing *egl-13*pro:GFP were acquired using dual-inverted selective plane illumination microscopy (diSPIM), and selected frames are shown. Time stamp is relative to start of movie, not developmental stage. At the beginning of the movies, the embryo in (A) is at a developmental stage ~40 min earlier than in (B). White arrows indicate the nose. Colored arrows and arrowheads indicate cell bodies and dendrite endings, respectively (BAG, blue; URX, green). Approximate outline of each embryo is drawn. Changes in orientation of the animal are due to rapid twitching and writhing within the eggshell. The marker is also strongly expressed in an additional neuron (asterisk) and more weakly in elongated cells in the anterior. (C) URX and BAG dendrite extension in *sax-7* embryos at 1.5- fold, 2-fold, and pretzel stages. URX and BAG cell bodies and dendrite endings are marked with arrows and arrowheads, respectively (URX, green; BAG, blue). Asterisk, embryo nose. Dotted line, outline of embryo.

Figure S7

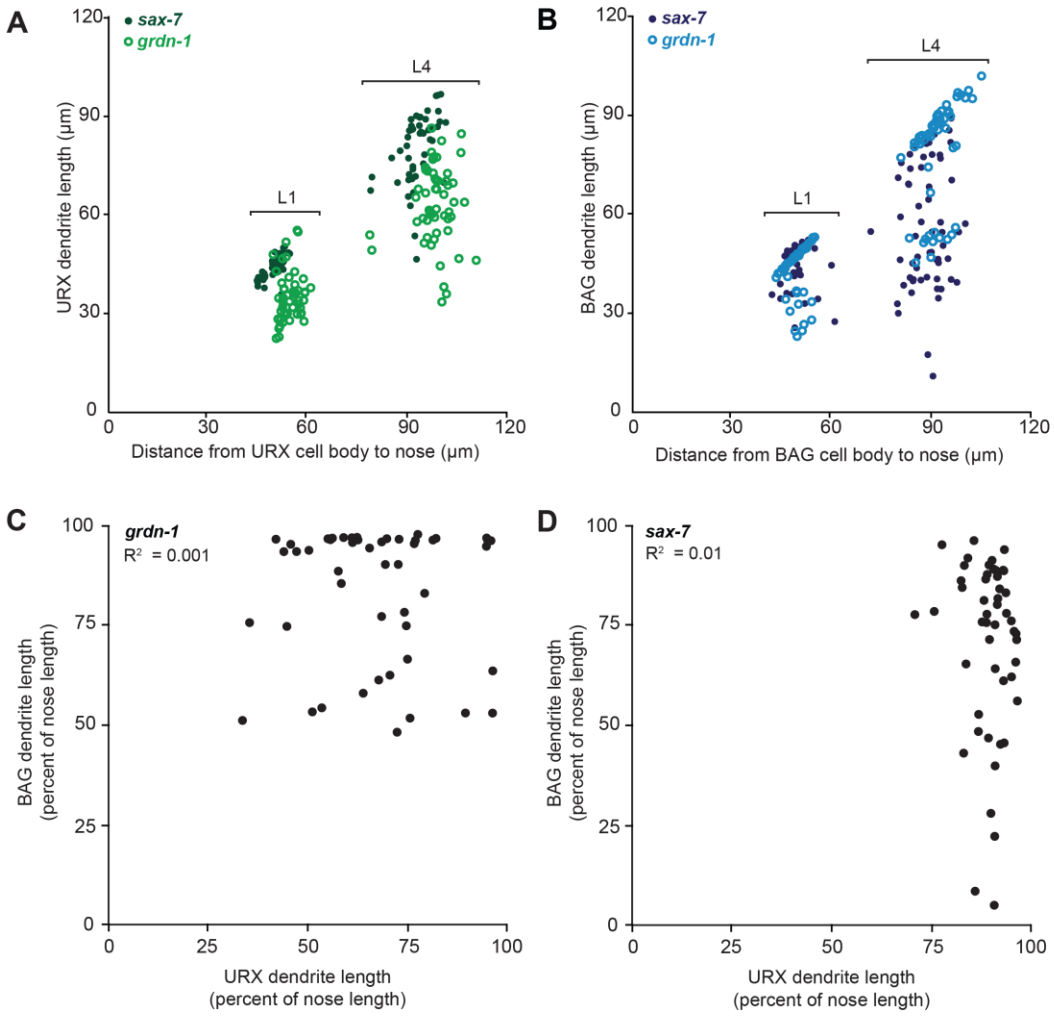


Fig. S7. Shortened URX and BAG dendrites keep pace with larval growth and are not correlated in length. Related to Fig. 5.

(A, B) URX and BAG dendrite lengths increase with larval growth. (A) URX and (B) BAG dendrite lengths in *sax-7* (closed circles) and *grdn-1* (open circles) animals were scored at the first (L1) and fourth (L4) larval stages and plotted as a function of the distance from the cell body to the nose. Populations of L1 and L4 animals were selected by overall morphology on a dissecting stereomicroscope. As animals grow from L1 to L4 (~48 h), the length of the head

("Distance from cell body to nose") approximately doubles from ~40-60 µm to ~80-120 µm. During this period, the lengths of the shortened URX and BAG dendrites increase similarly. n≥49 dendrites per genotype per neuron at each larval stage. (C, D) URX and BAG dendrite lengths are not correlated. Ipsilateral URX and BAG neurons were visualized simultaneously using *flp-8*pro:GFP and *flp-17*pro:mApple, respectively. Dendrite lengths were measured as a percentage of the distance from each cell body to nose. The dendrite lengths of ipsilateral URX and BAG neurons in (C) *grdn-1* and (D) *sax-7* animals are shown. URX and BAG dendrite lengths were not correlated (R^2 = 0.001, *grdn-1*; R^2 = 0.01, *sax-7*). n = 50 for each genotype.

Figure S8

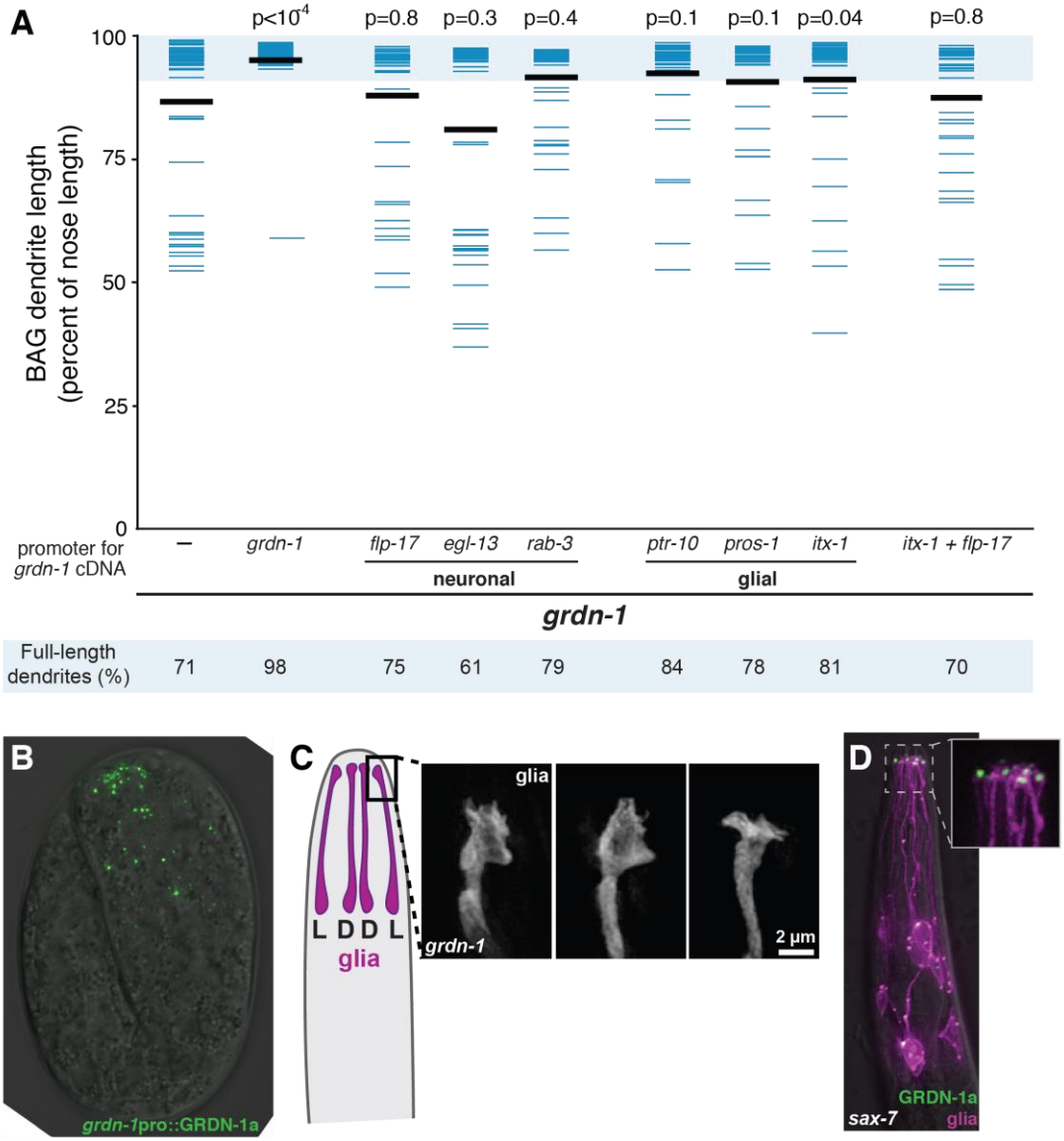
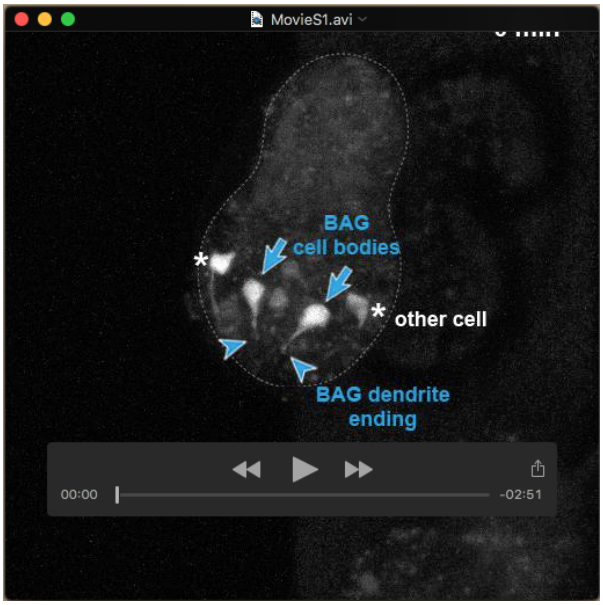


Fig. S8. GRDN-1 activity and localization in glia. Related to Figure 7.

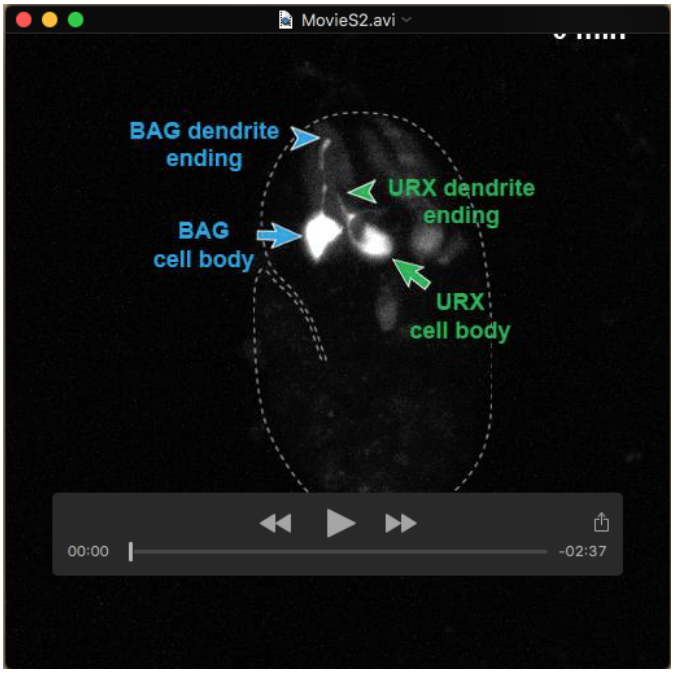
(A) Transgenes containing GRDN-1a cDNA under control of the indicated promoters, or no transgene (–), were introduced into *grdn-1* animals and BAG dendrite lengths were measured as a percentage of the distance from the cell body to the nose. Expression of GRDN-1a under its endogenous promoter rescued dendrite extension defects ($p<0.0001$). Mild rescue using other

promoters was not statistically significant ($p>0.05$ for all promoters shown except $p=0.04$ for *itx-1*pro). Colored bars represent individual dendrites ($n\geq40$ per genotype); black bars represent population averages. Shaded region represents wild-type mean ± 5 s.d. and the percentage of dendrites in this range ("full length dendrites") is indicated below the plot. p-values (Wilcoxon Rank Sum test) compared to *grdn-1* with no transgene (–) are at top. (B) Wild-type embryo expressing sfGFP-GRDN-1 under control of the *grdn-1* promoter. GRDN-1 is broadly expressed and localizes in puncta throughout the embryonic head. (C) Three examples of the lateral ILso glial ending in *grdn-1* animals in which both URX and BAG dendrites are short, suggesting that the elaborate protrusions characteristic of the wild-type structure are reduced or absent. Superresolution images of ILso glia (*grl-18*pro) acquired with structured illumination microscopy. Individuals with short BAG dendrites were selected for imaging; URX is always short in *grdn-1*. (D) Localization of glial-expressed GRDN-1a appeared unchanged in *sax-7* mutants. YFP-GRDN-1a (*itx-1*pro, yellow) and myristyl-mCherry (*itx-1*pro, red) were expressed together in glia. GRDN-1 localized to puncta at glial endings.



Movie 1. URX and BAG dendrites extend by stretch during embryo elongation. Related to Fig. 5.

Time-lapse movie of a wild-type embryo expressing *egl-13*pro:GFP, collected with dual-inverted selective plane illumination microscopy (diSPIM). BAG and URX extend short dendrites that contact the presumptive nose early in development and are then stretched to their full length during embryo elongation. The first eight frames were taken at 5 min intervals (40 min total, before the onset of embryo twitching), while the remaining frames were taken at 5 sec intervals to allow tracking of cells during rapid embryo movement. The movie is shown at a frame rate of 6 frames/sec. Selected frames have been annotated. Time stamp indicates minutes relative to start of movie. Approximate outline of embryo is drawn. Arrows and arrowheads indicate cell bodies and dendrite endings, respectively (BAG, blue; URX, green). The marker is also strongly expressed in an additional neuron (asterisk) and more weakly in elongated cells in the anterior.



Movie 2. URX and BAG dendrite defects in *grdn-1* mutants arise during embryo elongation. Related to Fig. 5.

Time-lapse movie of a *grdn-1* mutant embryo expressing *egl-13*pro:GFP, collected with dual-inverted selective plane illumination microscopy (diSPIM). BAG and URX extend short dendrites that contact the presumptive nose early in development, but then detach from the nose and fail to extend. Frames were taken at 5-sec intervals, and the movie is shown at a frame rate of 6 frames/sec. Selected frames have been annotated. Time stamp indicates minutes relative to start of movie. Approximate outline of embryo is drawn. Arrows and arrowheads indicate cell bodies and dendrite endings, respectively (BAG, blue; URX, green). The marker is also strongly expressed in an additional neuron (asterisk) and more weakly in elongated cells in the anterior.

Table S1. Strains. Related to all figures.

Strains generated for this study

ID	Genotype	Figures
CHB2450	*hmnEx1378*[*flp-17*pro:mApple + *flp-17*pro:GCY-9-mApple + *grl-18*pro:GFP + *grl-18*pro:myrGFP + pRF4]	1, S1, S2
CHB2792	*hmnEx1575*[*flp-8*pro:mApple + *flp-8*pro:myrmApple + *grl-18*pro:GFP + *grl-18*pro:myrGFP + pRF4]	1, S1
CHB3098	*hmnEx1747*[*ops-1*pro:mCherry + *T02B11*pro:GFP + pRF4]	2
CHB95	*oyIs44*[*odr-1*pro:dsRed]; *dyf-7(ns119)*	2
CHB13	*dex-1(ns42); oyIs44*[*odr-1*pro:dsRed]	2
CHB3232	*dex-1(ns42); oyIs44*[*odr-1*pro:dsRed]; *ynIs78*[*flp-8*pro:GFP]	2
CHB1458	*oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]; *dyf-7(ns119)*	2
CHB3231	*dex-1(ns42); oyIs44*[*odr-1*pro:dsRed]; o*yIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	2
CHB2509	*grdn-1(ns303); hmnEx1378*[*flp-17*pro:mApple + *flp-17*pro:GCY-9-mApple + *grl-18*pro:GFP + *grl-18*pro:myrGFP + pRF4]	S2, S8
CHB2510	*sax-7(hmn12); hmnEx1378*[*flp-17*pro:mApple + *flp-17*pro:GCY-9-mApple + *grl-18*pro:GFP + *grl-18*pro:myrGFP + pRF4]	S2
CHB1408	*sax-7(hmn12)*; *ynIs78*[*flp-8*pro:GFP]	3, 4, 6, S7
CHB1416	*sax-7(hmn12)*; *oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	3, 4, 6, S7
CHB1407	*sax-7(hmn3)*; *ynIs78*[*flp-8*pro:GFP]	3
CHB1415	*sax-7(hmn3)*; *oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	3
CHB3203	*sax-7(hmn147)*; *ynIs78*[*flp-8*pro:GFP]	3
CHB1038	*sax-7(hmn147)*; *oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	3
CHB6	*grdn-1(ns302) oyIs44*[*odr-1*pro:dsRed]; *ynIs78*[*flp-8*pro:GFP]	3
CHB1409	*grdn-1(ns302)*; *oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	3
CHB1402	*grdn-1(ns303)*; *ynIs78*[*flp-8*pro:GFP]	3, 4, 7, S4, S7
CHB1410	*grdn-1(ns303)*; *oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	3, 4, S7,

		S8
CHB1403	*grdn-1(hmn1)*; *ynIs78*[*flp-8*pro:GFP]	3
CHB1411	*grdn-1(hmn1)*; *oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	3
CHB1404	*grdn-1(hmn4)*; *ynIs78*[*flp-8*pro:GFP]	3
CHB1412	*grdn-1(hmn4)*; *oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	3
CHB1405	*grdn-1(hmn7)*; *ynIs78*[*flp-8*pro:GFP]	3
CHB1413	*grdn-1(hmn7)*; *oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	3
CHB1406	*grdn-1(hmn8)*; *ynIs78*[*flp-8*pro:GFP]	3
CHB1414	*grdn-1(hmn8)*; *oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	3
CHB2409	*stn-2(tm1869); hmnEx1364*[*flp-17*pro:mApple + *flp-8*pro:GFP + pRF4]	S3
CHB3500	*unc-44(e362); ynIs78*[*flp-8*pro:GFP]	S3
CHB2369	*unc-44(e362); oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	S3
CHB3230	*dma-1(wy686); hmnEx1367*[*flp-17*pro:mApple + *flp-8*pro:GFP]	S3
CHB3837	*rig-6(ok1589); ynIs78*[*flp-8*pro:GFP]	S3
CHB2421	*rig-6(ok1589); oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	S3
CHB3836	*sax-7(nj53); ynIs78*[*flp-8*pro:GFP]	S3
CHB2868	*sax-7(nj53); oyIs82*[*flp-17*pro:GFP + *unc-122*pro:dsRed]	S3
CHB2086	*che-10(e1809); ynIs78*[*flp-8*pro:GFP]	S3
CHB2085	*che-10(e1809); oyIs82*[*flp-17*pro:GFP]	S3
CHB3386	*kyIs104* [*str-1*pro:GFP]; *sax-7(hmn12)*	4
CHB1506	*hmnEx782*[*dat-1*pro:GFP + *flp-8*pro:mCherry + pRF4]	4
CHB2227	*grdn-1(ns303)*; *hmnEx782*[*dat-1*pro:GFP + *flp-8*pro:mCherry + pRF4]	4
CHB1515	*sax-7(hmn12)*; *hmnEx782*[*dat-1*pro:GFP + *flp-8*pro:mCherry + pRF4]	4
CHB1307	*hmnEx644*[*itx-1*pro(short)*myo-2*pro:GFP + *klp-6*pro:mCherry + pRF4]	4
CHB1508	*grdn-1(ns303)*; *hmnEx644*[*itx-1*pro(short):GFP + *klp-6*pro:mCherry + pRF4]	4

CHB1514	*sax-7(hmn12)*; *hmnEx644*[*itx-1*pro(short)*myo-2*pro:GFP + *klp-6*pro:mCherry + pRF4]	4
CHB1304	*hmnEx641*[*ocr-4*pro:mCherry + pRF4]	4
CHB1507	*grdn-1(ns303)*; *ynIs78*; *hmnEx641*[*ocr-4*pro:mCherry + pRF4]	4
CHB1513	*sax-7(hmn12)*; *oyIs82*; *hmnEx641*[*ocr-4*pro:mCherry + pRF4]	4
CHB1517	*hmnEx788*[*tol-1*pro:GFP + pRF4]	4
CHB1519	*grdn-1(ns303)*; *hmnEx788*[*tol-1*pro:GFP + pRF4]	4
CHB2234	*sax-7(hmn12)*; *hmnEx788*[*tol-1*pro:GFP + pRF4]	4
CHB1302	*grdn-1(ns303)*; *ynIs78*; *hmnEx639*[*grdn-1*pro:GRDN-1aΔGCV + pRF4]	S4
CHB1460	*hmnEx741*[*egl-13*pro:GFP + *itx-1*pro(short)*myo-2*pro:myr-mCherry+ pRF4]	5, S6
CHB1490	*grdn-1(ns303)*; *hmnEx741*[*egl-13*pro:GFP + *itx-1*pro(short)*myo-2*pro:myr-mCherry + pRF4]	5, S6
CHB2065	*sax-7(hmn12)*; *ynIs78*; *hmnEx1191*[*grdn-1*pro:SAX-7s + pRF4]	6
CHB3596	*oyIs61*[*gpa-4*Δpro:GFP]; *sax-7(hmn12)*	S5
CHB26	*grdn-1(ns303) oyIs44*[*odr-1*pro:dsRed]; *ynIs78*[*flp-8*pro:GFP]	S5
CHB70	*sax-7(hmn12)*; *oyIs44*[*odr-1*pro:dsRed]	S5
CHB2066	*sax-7(hmn12)*; *ynIs78*; *hmnEx1193*[*flp-8*pro:SAX-7s + pRF4]	6
CHB2063	*sax-7(hmn12)*; *ynIs78*; *hmnEx1189*[*itx-1*pro:SAX-7s + pRF4]	6
CHB2067	*sax-7(hmn12)*; *ynIs78*; *hmnEx1194*[*flp-8*pro:SAX-7s + *itx-1*pro:SAX-7s + pRF4]	6
CHB2064	*sax-7(hmn12)*; *oyIs82*; *hmnEx1191*[*grdn-1*pro:SAX-7s + pRF4]	6
CHB1807	*sax-7(hmn12)*; *oyIs82*; *hmnEx991*[*flp-17*pro:SAX-7s + pRF4]	6
CHB2062	*sax-7(hmn12)*; *oyIs82*; *hmnEx1189*[*itx-1*pro:SAX-7s + pRF4]	6
CHB1808	*sax-7(hmn12)*; *oyIs82*; *hmnEx992*[*flp-17*pro:SAX-7s + *itx-1*pro:SAX-7s + pRF4]	6
CHB1818	*hmnEx997*[*flp-17*pro:CFP + *itx-1(3kb)*pro:SAX-7s-YFP + *itx-1(1kb)-myo-2min*pro:myrmCherry]	6

CHB1601	*sax-7(hmn12); hmnEx741*[*egl-13*pro:GFP + *itx-1*pro(short)*myo-2*pro:myr-mCherry+ pRF4]	S6
CHB2228	*grdn-1(ns303)*; *ynIs78*; *hmnEx1952*[*grdn-1*pro:GRDN-1a + *flp-8*pro::CFP + pRF4]	7
CHB287	*grdn-1(ns303)*; *ynIs78*; *hmnEx56*[*grdn-1*pro:GRDN-1a + pRF4]	7, S4
CHB476	*grdn-1(ns303)*; *ynIs78*; *hmnEx159*[*flp-8*pro:GRDN-1a + pRF4]	7
CHB1398	*grdn-1(ns303)*; *ynIs78*; *hmnEx701*[*egl-13*pro:GRDN-1a + pRF4]	7
CHB1390	*grdn-1(ns303)*; *ynIs78*; *hmnEx693*[*rab-3*pro:GRDN-1a + pRF4]	7
CHB2229	*grdn-1(ns303)*; *ynIs78*; *hmnEx697*[*ptr-10*pro:GRDN-1a + pRF4]	7
CHB2230	*grdn-1(ns303)*; *ynIs78*; *hmnEx698*[*itx-1*pro:GRDN-1a + pRF4]	7
CHB2231	*grdn-1(ns303)*; *ynIs78*; *hmnEx696*[*pros-1*pro:GRDN-1a + pRF4]	7
CHB3872	*grdn-1(ns303)*; ynIs78; *hmnEx1280*[*itx-1*pro:GRDN-1a + *flp-8*pro:GRDN-1a + pRF4]	
CHB2073	*hmnEx1200*[*grdn-1*pro:HIS-24-mCherry + *itx-1*pro(short)*myo-2*pro:myr-GFP + pRF4]	7
CHB1853	*hmnEx1034*[*itx-1*pro:sfGFP-GRDN-1a + *egl-13*pro:mCherry + pRF4]	7
CHB1746	*hmnEx956*[*flp-8*pro:CFP + *itx-1*pro:YFP-GRDN-1a + *itx-1*pro(short)*myo-2*pro:myr-mCherry]	7
CHB1749	*hmnEx959*[*flp-17*pro:CFP + *itx-1*pro:YFP-GRDN-1a + *itx-1*pro(short)*myo-2*pro:myr-mCherry]	7
CHB2068	*hmnEx1195*[*itx-1*pro(short)*myo-2*pro:CD4-spGFP(11) + *flp-8*pro:CD4-spGFP(1-10) + *flp-8*pro:mCherry + pRF4]	7
CHB1815	*hmnEx999*[*flp-8*pro:CD4-spGFP(1-10) + *itx-1*pro(short)*myo-2*pro:CD4-spGFP(11) + *itx-1*pro:mApple-GRDN-1a + pRF4]	7
CHB3852	*sax-7(hmn12); hmnEx1369*[*flp-17*pro:mApple + *flp-8*pro:GFP + *unc-122*pro:RFP]	S7
CHB3851	*grdn-1(ns303); hmnEx1369*[*flp-17*pro:mApple + *flp-8*pro:GFP + *unc-122*pro:RFP]	S7
CHB1469	*grdn-1(ns303)*; *oyIs82*; *hmnEx750*[*flp-17*pro:GRDN-1a + pRF4]	S8
CHB2232	*grdn-1(ns303)*; *oyIs82*; *hmnEx693*[*rab-3*pro:GRDN-1a + pRF4]	S8

CHB2233	*grdn-1(ns303)*; *oyIs82*; *hmnEx701*[*egl-13*pro:GRDN-1a + pRF4]	S8
CHB1394	*grdn-1(ns303)*; *oyIs82*; *hmnEx697*[*ptr-10*pro:GRDN-1a + pRF4]	S8
CHB1395	*grdn-1(ns303)*; *oyIs82*; *hmnEx698*[*itx-1*pro:GRDN-1a + pRF4]	S8
CHB1393	*grdn-1(ns303)*; *oyIs82*; *hmnEx696*[*pros-1*pro:GRDN-1a + pRF4]	S8
CHB3878	*grdn-1(ns303)*; *oyIs82*; *hmnEx2186*[*itx-1*pro:GRDN-1a + *flp-17*pro:GRDN-1a + pRF4]	S8
CHB1511	*hmnEx785*[*grdn-1*pro:sfGFP-GRDN-1a + *itx-1*pro(short)*myo-2*pro:myr-mCherry + pRF4]	S8
CHB2235	*sax-7(hmn12); hmnEx959*[*flp-17*pro:CFP + *itx-1*pro:YFP-GRDN-1a + *itx-1*pro(short)*myo-1*pro:myr-mCherry]	S8

Strains generated in previous studies. Related to Figures 2-7 and Figures S3, S4, S5, S8.

ID	Genotype	Figure	Source/Reference
CHB4	*oyIs44*[*odr-1*pro:dsRed]	2, S5	Lanjuin et al., 2003 (as PY2417)
CHB1738	*oyIs44*[*odr-1*pro:dsRed]; *dyf-7(ns119); hmnEx952*[*flp-8*pro:CFP + *tol-1*pro:YFP]	2	Low and Williams et al., 2019
PY6342	*che-10(e1809); kyIs104*[*str-1*pro:GFP]	S3	Nechipurenko et al., 2016
PY1089	*kyIs104*[*str-1*pro:GFP]	4	Troemel et al., 1997
PY8658	*grdn-1(ns303)*; *kyIs104*[*str-1*pro:GFP]	4	Nechipurenko et al., 2016
PY6101	*oyIs61*[*gpa-4*Δpro:GFP]	S5	Olivier-Mason et al., 2013
PY8836	*oyIs61*[*gpa-4*Δpro:GFP]; *grdn-1(ns303)*	S5	Nechipurenko et al., 2016
CHB1929	*ynIs78*[*flp-8*pro:GFP]	2, 3, 4, 6, 7, S4	Kim and Li, 2004 (as NY2078)
CHB851	*oyIs82*[*flp-17*pro:GFP, *unc-122*pro:dsRed]	2, 3, 4, 6, S8	Astrid Cornils and Piali Sengupta (as PY8503)

Table S2. Plasmids generated for this study.

ID	Name
pEL3	*flp-17*pro:GRDN-1a
pEL5	*flp-17*pro:CFP
pEL7	*flp-17*pro:mCherry
pEL15	*itx-1*pro(short)*myo-2*pro:CD4-spGFP(11)
pEL16	*flp-17*pro:mApple
pEL25	*egl-13*pro:mCherry
pEL137	*grl-18*pro:GFP
pEL153	*flp-17*pro:GCY-9-mApple
pEL168	*grl-18*pro:myrGFP
pEL208	*flp-8*pro:mApple
pEL209	*flp-8*pro:myrmApple
pIM5	*grdn-1*pro:GRDN-1a
pIM8	*flp-8*pro:GRDN-1a
pIM20	*grdn-1*pro:GRDN-1aΔGCV
pIM37	*itx-1*pro(short)*myo-2*pro:GFP
pIM38	*itx-1*pro(short)*myo-2*pro:myr-mCherry
pIM39	*itx-1*pro(short)*myo-2*pro:myr-GFP
pIM44	*rab-3*pro:GRDN-1a
pIM45	*dpy-7*pro:GRDN-1a
pIM47	*itx-1*pro:sfGFP-GRDN-1a
pIM48	*egl-13*pro:GFP
pIM49	*egl-13*pro:GRDN-1a
pIM50	*grdn-1*pro:sfGFP-GRDN-1a
pIM53	*pros-1*pro:GRDN-1a
pIM54	*ptr-10*pro:GRDN-1a

pIM55	*itx-1*pro:GRDN-1a
pIM57	*itx-1*pro:YFP-GRDN-1a
pIM61	*itx-1*pro:SAX-7s-YFP
pIM62	*itx-1*pro:sfGFP-GRDN-1aΔGCV
pIM67	*itx-1*pro:SAX-7s
pIM68	*flp-17*pro:SAX-7s
pIM69	*flp-8*pro:SAX-7s
pIM93	*itx-1*pro:mApple-GRDN-1a
pIM94	*flp-8*pro:CD4-spGFP(1-10)
pIM95	*grdn-1*pro:SAX-7s
pIM96	*grdn-1*pro:HIS-24-mCherry
pMH333	*flp-8*pro:GFP
pMH407	*dat-1*pro:GFP
pMH415	*tol-1*pro:GFP
pMH421	*flp-8*pro:CFP
pMH445	*klp-6*pro:mCherry
pMH458	*ocr-4*pro:mCherry
pMH462	*flp-8*pro:mCherry

Table S3. Primers

Primers of general interest

Name	Sequence
grdn-1pro_fwd	AGTATCAGCCTGCAGGCTTCGTAAATCTACAAAACATTTTCAACGTGC
grdn-1pro_rev	GAGATTCGGCGCGCCCCTTGATATTTTCGCTTGTTTTTTTTTTCAGAAGG
egl-13pro_fwd	GATACCTGCAGGTGGGAGTTTGGTGCTTCC
egl-13pro_rev	GATAGGCGCGCCGTCTACGGCTGATGCTGG
grl-18pro_fwd	GTACTCCTGCAGGTGTGTTGTAGATTTGAGCTCCC
grl-18pro_rev	GATCGGCGCGCCTTGCGATTGAATAGAATGTATCAG
pros-1pro_fwd	TAGCTCCTGCAGGGGTGATATCGAAAGTAACCAACG
pros-1pro_rev	TAGCTGGCGCGCCTGAGATTGATGACGTCACTAGC
itx-1pro_fwd	TGATCCCTGCAGGCGGTAAAATTGCAAAATAAATTG
itx-1pro_rev	TGATCGGCGCGCCGGTACGGCTGAAATAGAGAGC
itx-1pro(short)_rev	TGTTGGCGCGCCTCTCTGCGTGTCTTTGCC

Table S4. Mutant alleles. Related to Figure 3.

Alleles isolated in this study. Substitutions are bracketed with the mutant sequence underlined.

Uppercase corresponds to predicted exons; lowercase to predicted introns.

Allele	**Sequence**
grdn-1(ns302)	atttttca[g>a]CGACTGCG
grdn-1(ns303)	GAAAACTG[G>A]AGCCATCC
grdn-1(hmn1)	tctagGAT[C>T]AACTCAGC
grdn-1(hmn4)	TACTGACC[C>T]AGAACAAG
grdn-1(hmn7)	AAATCAGC[C>T]AGTTTTAC
grdn-1(hmn8)	ATTTACCC[C>T]AAAACCCT
sax-7(hmn3)	TGAAGGAG[g>a]taggacat
sax-7(hmn12)	ACTTGCAT[G>A]GAATGGTG
sax-7(hmn147)	TCACAATG[G>A]AGAACTCA
sax-7(hmn159)	ACTTGCAT[G>A]GAATGGTG