METHODS

***C. elegans* strains.** Wild-type strains were *C. elegans* variety Bristol, strain N2. Worms were grown at 23 °C on nematode growth medium (NGM) plates seeded with bacteria (*Escherichia coli* OP50) as a food source. All males contained either *him-8(e1489) IV* or *him-5(e1490) V* as indicated by strain. Male worms were picked at the fourth larval stage onto plates with ten other males (unless otherwise indicated), and allowed to moult into adults and age to the day indicated for each analysis or experiment.

Mutant alleles used in this study include: *him-8(e1489) IV*, *him-5(e1490) V*, *unc-31(e928) IV*, *nlg-1(ok259) X*, *nrx-1(ok1649) V*, *unc-119(ed3) III*, *nrx-1(wy778[unc-119(+)]) V*, *lim-6(nr2073) X*, *pkd-2(pt8) IV*, *unc-97(su110) X*, *unc-25(e156) III*, *unc-49(e407) III*, *nrx-1(ok1649)V*, and *nrx-1(gk246237)*.

All transgenic strains used in this study are listed in Supplementary Table 1 ordered by Figures and Extended Data Figures. All plasmids were injected at 25 ng μl^{-1} with coinjection marker *ttx-3::gfp* or *ttx-3::wCherry* also at 25 ng μl^{-1} to generate extrachromosomal arrays (unless otherwise noted).

Cloning and constructs. To generate *lim-6^{int4}::wCherry* (pMG198) and *lim-6^{int4}::gfp* (pMG141), a 291-bp fragment of the *lim-6* fourth intron was amplified with primers adding BamHI to forward (CCCCGGATCCTTAGCCAGTTGCATAAATAT) and MscI to reverse (GGGGTGGCCACTAAGCTTCTTGCTAAAATTC). This fragment was digested and ligated into a pPD95.75 vector with either GFP or codon-optimized mCherry (wCherry). Plasmids were injected at 5 ng μl^{-1} into a *pha-1(e2123)* mutant strain with pha-1(+) coinjection marker. Extrachromosomal arrays were integrated to yield *otIs541 and otIs525*. *lim-6^{int4}* was found to express brightly in DVB, dimly in AVL and RIS, and dimly in about 70% of worms in PVT.

To generate *lim-6^{int4}::gfp::rab-3* (pMH1), *lim-6^{int4}* was PCR-amplified from pMG193 using primers forward GATGGATACGCTAACAACTTGGAAATGA AATGGATCCTTAGCCAGTTGCATAAATATTAAAGTCAAATG and reverse GAAACATACCTTTGGGTCCTTTGGCCACTAAGCTTCTTGCTAAAATTCT CTTTGATTTG, and cloned into DACR10 (a gift from D. Colon-Ramos) to replace the *ttx-3* promoter using restriction free cloning. The resulting plasmid was injected at 45 ng μl^{-1} with coinjection marker *ttx-3::gfp* also at 45 ng μl^{-1}. An extrachromosomal array was integrated to yield *otIs659*.

To generate *lim-6^{int4}::ChR2::yfp* (pMH17), *lim-6^{int4}* was PCR-amplified from pMH1 using primers forward CTAGATCAAACAAGTTTGTACAAAAAAAGCTT GCATGCCTGGATCCTTAG and reverse CACTTTGTACAAGAAAGCTGGGTC CTAAGCTTCTTGCTAAAATTCTCTTTG, and cloned into pLR183 (*gar-3b::ChR2::yfp*, a gift from L. R. Garcia[16,30]) to replace the *gar-3b* promoter using restriction free cloning.

To generate *lim-6^{int4}::BirA::nrx-1^{LONG}* (pMH27), *lim-6^{int4}* was PCR-amplified from pMH1 using primers forward GAAATGAAATAAAGCTTGCATGAG CTTGCATGCCTGGATCCTTAG and reverse CTTTGGGTCCTTTGGCCAAT CCCGGCTAAGCTTCTTGCTAAAATTC, and cloned into pMO23[31] (*srg-13::BirA::nrx-1)* to replace the *srg-13* promoter using restriction free cloning.

To generate *lim-6^{int4}::BirA::nrx-1^{SHORT}* (pMH41), the first exon of the *nrx-1* short isoform was PCR-amplified from N2 genomic DNA using primers forward GAAGT GGAGGTGGAGGCTCCTCAGGTGTATTCCTTGAGCATTTGCGTGGTG and reverse GTTGGAAGGACTGGCGAGAAGAATCCAGTAGTCTCTCC GGACACATCATTC, and cloned into pMH27 to replace the first 23 exons of the long isoform of *nrx-1* using restriction free cloning.

To generate *lim-6^{int4}::BirA::nrx-1^{noPDZ}* (pMH44), the first exon of the *nrx-1* short isoform was PCR-amplified from N2 genomic DNA using primers forward CAACGGCCACAATGATGAGAAACGGAAACGGGAATGGGGTGGCATCT CGAGGAGCTCCCGAGATCTTCAGCGCTC and reverse CTACGAATGCTG AGCGCTGAAGATCTCGGGAGCTCCTCGAGATTATGCCACCCCATTCCC GTTTC, and cloned into pMH27 to delete the last 30 bp of *nrx-1* cDNA before the stop codon using restriction free cloning.

To generate *lim-6^{int4}::GFP::nrx-1* (pMH37), *eGFP* cDNA was PCR-amplified from pMH1 using primers forward CTATCGGAGCAGCATTCAATACTAGGCA TTTGGCTCAAAAAAGACTGTTACG and reverse CGACGATGAC GTAACAGTCTTTTTTGAGCCAAATGCCTAGTATTGAATG, and cloned into pMH27 to replace *birA* cDNA using restriction free cloning.

To generate *lim-6^{int4}::nlg-1::gfp1-10* (pMH18), *lim-6^{int4}* was PCR-amplified from pMH1 using primers forward CAAGCTTGCATGCGCGGCCGCACAGCTT GCATGCCTGGATCCTTAG and reverse GTCCTTTGGCCAATCCCGGGGATCT AAGCTTCTTGCTAAAATTCTCTTTG, and cloned into MVC6 (*gpa-6::nlg-1::gfp1-10*, a gift from M. VanHoven) to replace the *gpa-6* promoter using restriction free cloning.

To generate *gar-3b::nlg-1::gfp11* (pMH20), the *gar-3b* promoter was PCR-amplified from pLR183 using primers forward CAAGCTTGCATGCGCGGCCG CACCATAAGCATCATGAGCAACATCTCCACTTCTCGTGAGC and reverse GTCCTTTGGCCAATCCCGGGGATGATTAATAAATGTGCAGGAGGAGTA ATAATGGTGTATGT, and cloned into MVC12 (*flp-18p::nlg-1::gfp11*, a gift from M. VanHoven) to replace the *flp-18* promoter using restriction free cloning.

To generate *lim-6^{int4}::nlg-1::gfp11* (pMH8), *lim-6^{int4}* was PCR-amplified from pMH1 using primers forward CAAGCTTGCATGCGCGGCCGCACAGCT TGCATGCCTGGATCCTTAG and reverse GTCCTTTGGCCAATCCCGGGGAT CTAAGCTTCTTGCTAAAATTCTCTTTG, and cloned into MVC6 to replace the *gar-3b* promoter using restriction free cloning.

To generate *flp-13::nlg-1::gfp11* (pMH23), the *flp-13* promoter was PCR-amplified from N2 genomic DNA using primers forward CAAGCTTGCATGCGCGGC CGCACGCAGTGACGTCATCTTGTTCG and reverse GTCCTTTGGCCAATC CCGGGGATAAATTGTGCCTCCTGATGCTG, and cloned into pMH20 to replace the *gar-3b* promoter using restriction free cloning.

To generate *unc-103E::nlg-1::gfp11* (pMH21), the *unc-103E* promoter was PCR-amplified from N2 genomic DNA using primers forward CAAGCTTGCATGC GCGGCCGCACTCGCGGTGCCCAAAAGGTAGGTTATTGACGTATTCTCC and reverse GTCCTTTGGCCAATCCCGGGGATTACCACCACCACCACAAC CACCGATCGACGAC, and cloned into pMH20 to replace the *gar-3b* promoter using restriction free cloning.

To generate *unc-103F::nlg-1::gfp11* (pMH25), the *unc-103F* promoter was PCR-amplified from N2 genomic DNA using primers forward CAAGCTTGCA TGCGCGGCCGCACCACGCCTGCCTAAGGGATGCCTTAGCTC and reverse GTCCTTTGGCCAATCCCGGGGATGACATTGCCACGTGGTTGTGTGTGTG, and cloned into pMH20 to replace the *gar-3b* promoter using restriction free cloning.

To generate *lim-6^{int4}::HisCl1::gfp* (pMH3), the *lim-6^{int4}* promoter was PCR-amplified from N2 genomic DNA using primers forward GCATGCGCGGCCGCA CTGACTGGGCCGGCCGGATCCTTAGCCAGTTG and reverse CAATCCCGGG GATCCTCTAGAGGCGCGCCCTAAGCTTCTTGCTAAAATTC, and cloned into pNP471 to replace the *rig-3* promoter using restriction free cloning.

To generate *gar-3b::HisCl1::gfp* (pMH28), the *gar-3b* promoter was PCR-amplified from pMH20 genomic DNA using primers forward CTTGCAT GCGCGGCCGCACTGACTGGGCCGGCCCATAAGCATCATGAGCAACATC TC and reverse CAATCCCGGGGATCCTCTAGAGGCGCGCCAAAGCTGG GTCGATTAATAAATGTGCAG, and cloned into pMH3 to replace the *lim-6^{int4}* promoter using restriction free cloning.

Microscopy. Worms were anaesthetized using 100 mM sodium azide (NaN_3) and mounted on a pad of 5% agar on glass slides. Worms were analysed by Nomarski optics and fluorescence microscopy, using a Zeiss 880 confocal laser-scanning microscope. Multidimensional data were reconstructed as maximum intensity projections using Zeiss Zen software. Puncta were quantified by scanning the original full *Z*-stack for distinct dots in the area overlapping with the processes of the DVB neuron. Figures were prepared using Adobe Photoshop CS6 and Adobe Illustrator CS6.

Neurite tracing. Confocal *Z*-stacks were opened using FIJI, and loaded into the Simple Neurite Tracer plugin[32]. The primary neurite of DVB was traced from the centre of the cell soma to the point where the axon projects ventrally and then turns anteriorly, at the final branch point before it becomes a single process. Neurites were added by tracing off of this primary neurite, including all neurites emanating posterior of the last branch point. The simple neurite tracer plugin was used to analyse the skeletons for neurite length, which were summed to calculate total neurite length, and the number of neurite junctions (a proxy for the number of neurite branches).

Cell ablation. We performed laser ablations using a MicroPoint Laser System Basic Unit (N2 pulsed laser (dye pump), ANDOR Technology) attached to a Zeiss Axioplan 2IE widefield microscope (objective EC Plan-Neofluar 100 Å~/1.30 Oil M27). This laser delivers 120 μJoules of 337-nm energy with a 3-ns pulse length. Ablations were performed as previously described[33], with pulse repetition rates of ~15 Hz. Cell identification was performed with GFP or Cherry markers. Ablations were performed at the days of adulthood indicated, and worms were analysed ~20 h later. Mock-ablated worms were placed on same slide under the microscope but were not ablated, and were allowed to recover in a similar manner. Before relevant assays were performed (spicule protraction or aldicarb assays), worms were analysed for loss of cell fluorescence under a dissecting scope. When possible, after assays, worms were mounted on glass slides and analysed under a microscope to validate that cell ablation was successful.

Aldicarb spicule protraction assay. Aldicarb was added to warm liquid NGM agar medium to a final concentration of 5 mM and poured into plates. Worms were picked 12 or fewer at a time onto aldicarb plates and observed for spicule protractions longer than 5 s, when the time was recorded for each worm[13].

Mating assay. L4 male worms were picked and singled onto plates. Non-mated males were left individually on plates, whereas mated males had 10 *unc-31(e928)* hermaphrodites added to their plates. We exposed males to uncoordinated hermaphrodites (*unc-31*/CAPS), to ensure a successful mating experience. Following 48 h of being housed either individually or with 10 hermaphrodites, all