Online Methods

Strains and genetics. Worms were raised on OP50 *E. coli*-seeded NGM plates at 20°C. Wild-type animals were N2 Bristol strain. KP4 *glr-1(n2461)* III was provided by the *Caenorhabditis* Genetics Center (CGC). AA699 *din-1(hd36)* II was a gift from the Antebi lab, *nuIS25* was a gift from the Kaplan lab, and *eri-1(mg366) IV; lin-15b(n765) X* was a gift from the Bargmann lab.

Cloning and constructs. Plasmids and transgenic strains were generated using standard techniques[44]. See Supplementary List of Constructs for more detail. *wyIs120 [Popt-3::glr-1::YFP::glr-1 3' UTR, Popt-3::snb-1::CFP::unc-54 3' UTR, Popt-3::mCherry::unc-54 3' UTR], wyEx2438 [Popt-3::mCherry::unc-54 3' UTR], wyEx3504 [fosmid WRM0615bA06 w/ grld-1 C-terminal GFP, Popt-3::mCherry::unc-54 3' UTR], wyEx3557 [fosmid WRM0615bA06 w/ grld-1 C-terminal GFP, Punc-4::NLS::mCherry::unc-54 3' UTR], wyEx3559 [fosmid WRM0615bA06 w/ grld-1 C-terminal GFP, Psra-6::NLS::mCherry::unc-54 3' UTR], wyEx3561 [fosmid WRM0615bA06 w/ grld-1 C-terminal GFP, Pglr-1::NLS::mCherry::unc-54 3' UTR] wyEx1669* and *wyEx2366 [Popt-3::grld-1::unc-10 3' UTR], wyEx2354* and *wyEx2355 [Pglr-1::grld-1::unc-10 3' UTR], wyEx3614 [Popt-3::mCherry::unc-54 3' UTR, Popt-3::grld-1::unc-10 3' UTR], wyEx2031 [Popt-3::grld -1(RRMs only)* $_{1–375}$*::unc-10 3' UTR], wyEx2048 [Popt-3::grld-1(spoc only)* $_{322–521}$*::unc-10 3' UTR], wyEx1704* and *wyEx1707 [Popt-3::glr-1::yfp::unc-10 3' UTR], wyEx2357* and *wyEx2359 [Popt-3::glr-1 cDNA::GFP::glr-1 3' UTR, Popt-3::mCherry::unc-54 3' UTR], wyEx1655 [Popt-3::GFP::grld-1::unc-54 3' UTR], wyEx2505 [Phsp16-2, hsp16-41::grld-1a::unc-54 3' UTR], wyEx1245 [Popt-3::cam-1::yfp::unc-10 3' UTR, Popt-3::SNB-1::cfp::unc-54 3' UTR]*, *wyEx4034*, *wyEx4035*, and *wyEx4036 [Popt-3::glr-*

1 genomic (minus introns 1–2)::gfp::glr-1 3' UTR, Popt-3::mCherry::unc-54 3' UTR], *wyEx4037*, *wyEx4038*, and *wyEx4039 [Popt-3::glr-1 genomic (minus introns 3–9)::gfp::glr-1 3' UTR, Popt-3::mCherry::unc-54 3' UTR]*, *wyEx4040*, *wyEx4041*, and *wyEx4042 [Popt-3::glr-1 genomic (minus introns 10–13)::gfp::glr-1 3' UTR, Popt-3::mCherry::unc-54 3' UTR]*, *wyEx4043*, *wyEx4044*, and *wyEx4045 [Popt-3::glr-1 genomic (minus introns 3–13)::gfp::glr-1 3' UTR, Popt-3::mCherry::unc-54 3' UTR]*,. Transgenic lines were injected with co-injection markers *Punc-122::dsRed* (P. Sengupta) at 30 ng/ul, pJM23, *lin-15* rescue construct at 50 ng/ul, or *Podr-1::RFP* at 40 ng/ul.

3' UTR swap and *glr-1* genomic-cDNA hybrid constructs. *wyEx1704*, *wyEx1707*, *wyEx2357*, *wyEx2359*, and *wyEx4034–wyEx4045*. These constructs were injected into worms at similar molar concentrations as *wyIs120*, the [*glr-1 genomic::glr-1 3' UTR*] construct. All constructs resulted in GLR-1 fluorescence distributed predominantly in cell body and dendrites, a similar pattern compared with *wyIs120*.

Nose-touch response assay. Experiments were completed as described in a previous study[45]. Only young adults were used. Statistical comparisons were made only from animals that were assayed blind on the same day.

Feeding RNAi. An enhanced RNAi strain, *eri-1(mg366)IV; lin-15b(n744)*, which allows detection of neuronal defects[46], was combined with the transgene *wyIs120* and used for the feeding RNAi experiments. RNAi bacterial clones, which target the entire open reading frames were obtained from the *C. elegans* ORF-RNAi Feeding library (Thermo Scientific). Bacteria

were grown in liquid LB with 100 μg/mL carbenicillin and 50 μg/mL tetracyclin at 37°C overnight, seeded onto NGM plates, and induced with 1 mM IPTG for 24 hours at room temperature. Five to eight L4 worms were introduced onto each plate, incubated at 20 °C, and potential phenotypes analyzed in the progeny.

Fluorescence Microscopy. Images were captured live as described[47] except images of GFP::GRLD-1 were on an AxioCam MRm.

Calculation of GLR-1, CAM-1, and mCherry intensity. All images used for quantification were taken on an AxioCam MRm. For the quantification of GLR-1::YFP/GFP in **Figures 1e**, **4a**, **5**, *glr-1 genomic::glr-1 3' UTR* of **6h**, **7b**, **Supplementary Figures 3**, and **6g** the AVE axon was first focused using the cytoplasmic mCherry, and then an image was taken of the YFP fluorescence. For other lines, the AVE axon was focused first in the channel in which the image was subsequently taken. Using ImageJ software, mean pixel intensities of the anterior AVE axon in the nerve ring was subtracted from background fluorescence.

Patch-clamp whole-cell recording. Experiments were conducted with standard whole-cell recordings using an EPC-10 patch-clamp amplifier (HEKA) and a protocol modified from previous studies[48–50]. Worms were glued on a sylgard-coated coverglass covered with bath solution. A small piece of cuticle in the worm head was cut open and pinned down to the coverglass to expose the neurons. The AVE neuron was identified by an mCherry fluorescence marker expressed as a transgene driven by the *opt-3* promoter. Recording pipettes were pulled from borosilicate glass and fire-polished to 10-13 MΩ. The pipette solution contained 115 mM

potassium gluconate, 25 mM KCl, 50 mM HEPES, 0.1 mM $CaCl_2$, 1 mM BAPTA, 5 MgATP, 0.5 mM NaGTP, 0.5 mM cAMP and 0.5 mM cGMP (310-315 mOsm, pH 7.35). The bath solution contained 150 mM NaCl, 5 mM KCl, 5 mM CaCl2, 1 mM MgCl2, 15 mM HEPES, 10 mM Glucose and 20 μM Glycine (320-325 mOsm, pH 7.35). 1 mM glutamate was applied by pressure ejection from a closely placed pipette. The membrane potential was held at -70 mV. Current data were sampled at 33 kHz. Series resistance and membrane capacitance were both compensated for during recording.

Statistical analysis. Statistical analysis was performed using two-tailed Student's *t*-tests or one-way ANOVA tests.