GRLD-1 regulates cell-wide abundance of glutamate receptor through post-transcriptional regulation

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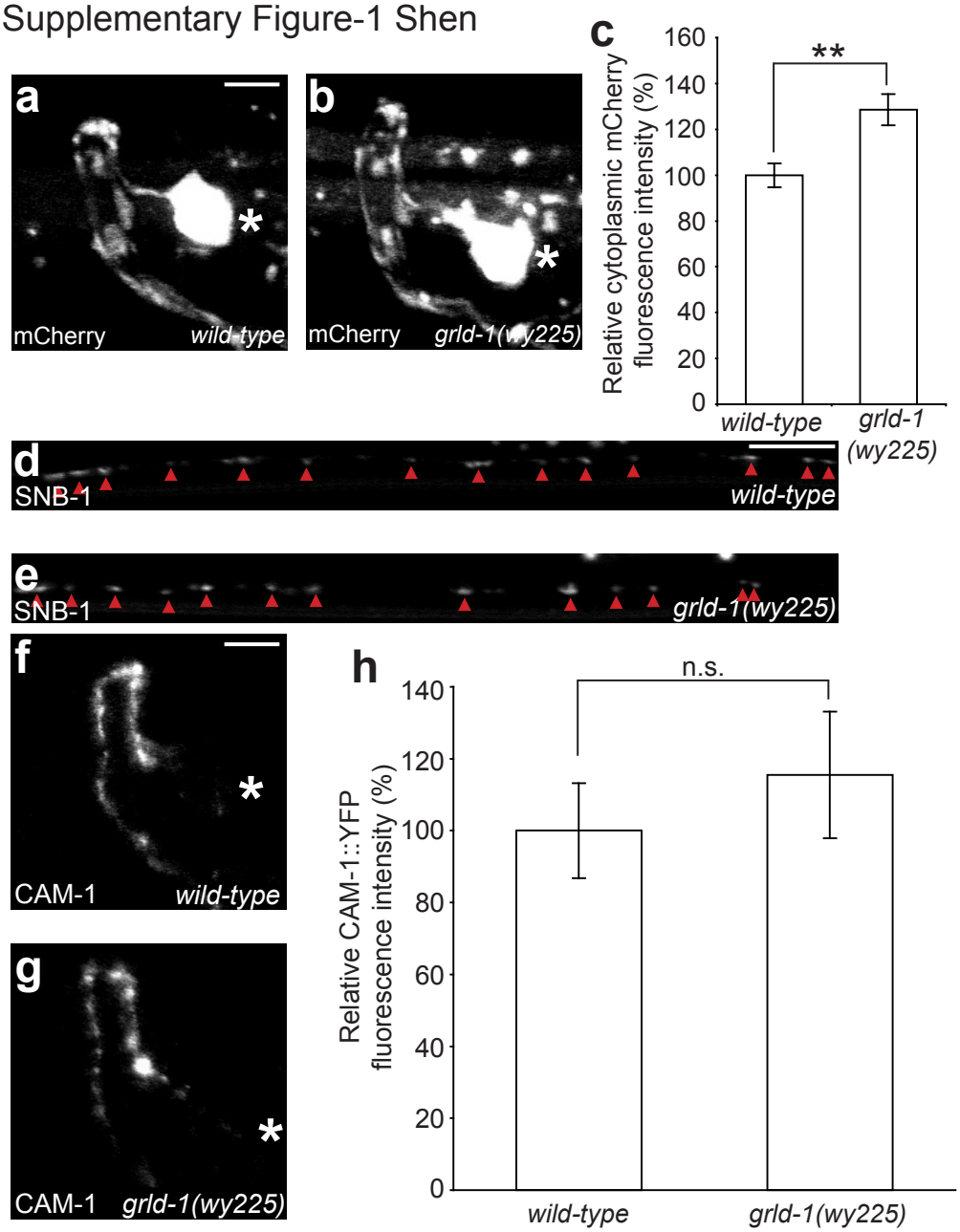
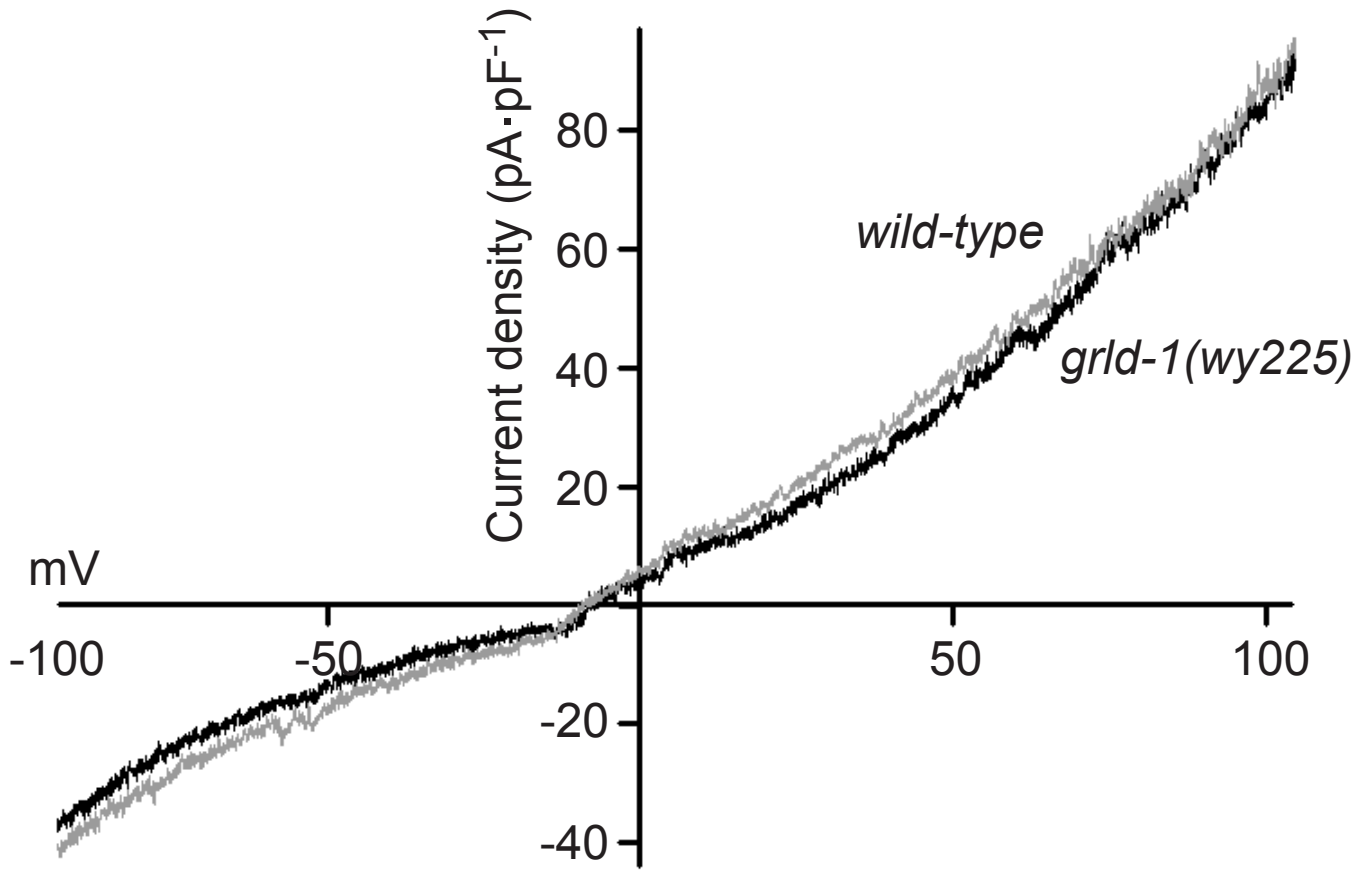
Supplementary Figure-1 Shen



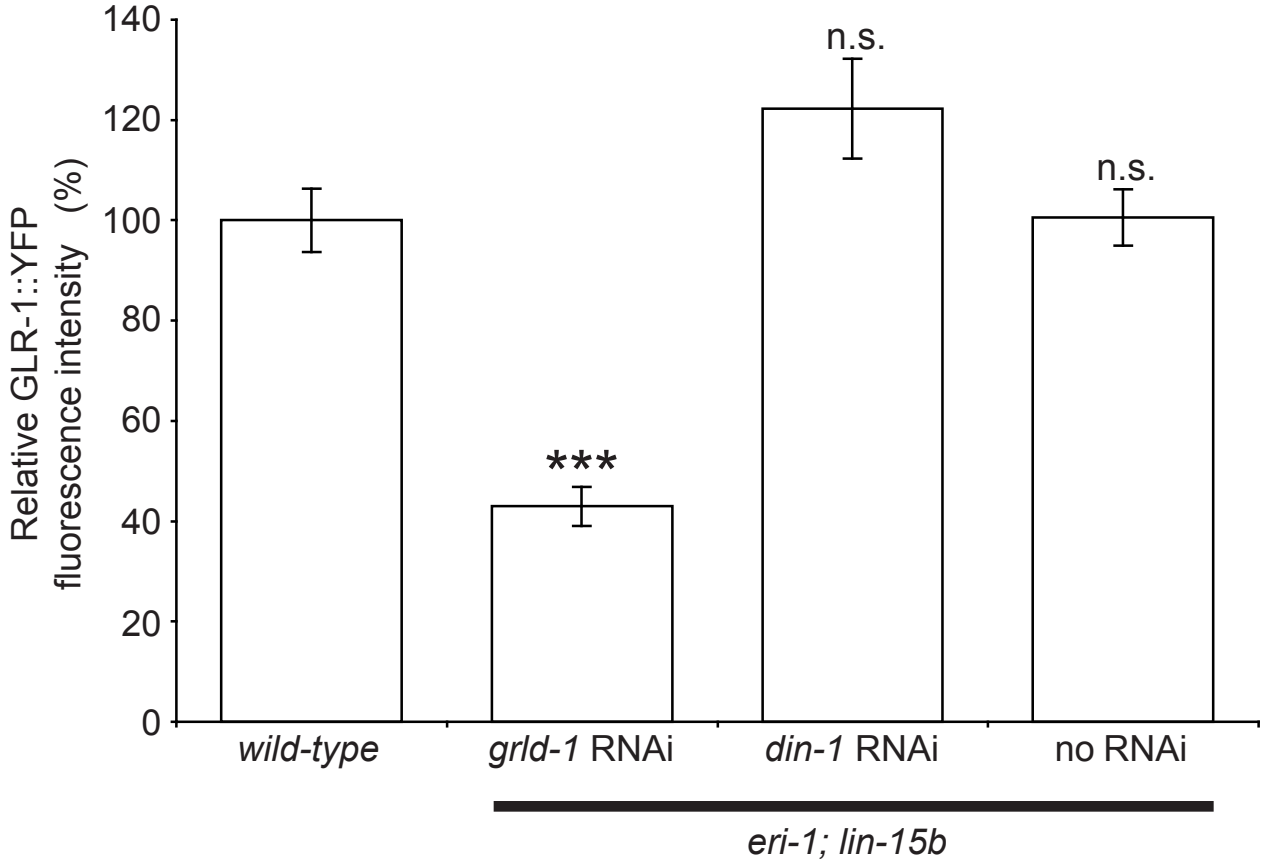
Supplementary Figure 1 Cytoplasmic mCherry, SNB-1::CFP, and CAM-1::YFP are not mislocalized or reduced in *grld-1(wy225)*. (**a**, **b**) Representative L2-stage wild-type (**a**) and *grld-1(wy225)* (**b**) animals expressing cytoplasmic mCherry. Asterisk denotes the AVE cell body. Scale bar, 2 μm. (**c**) Comparison of cytoplasmic mCherry fluorescence intensity (normalized to wild-type) between wild-type and *grld-1* mutants. n = 35. Error bars, s.e.m. **P < 0.01, *t*-test. (**d**, **e**) Representative L2 stage wild-type (**d**) and *grld-1(wy225)* (**e**) animals expressing SNB-1::YFP. Same region as dashed box in **Figure 1a**. Triangles denote SNB-1::CFP puncta. Scale bar, 2 μm. (**f**, **g**) Representative L2 wild-type (**f**) and *grld-1(wy225)* (**g**) animals expressing CAM-1::YFP. Scale bar, 2 μm. Asterisk denotes the AVE cell body. (**h**) Comparison of CAM-1::YFP fluorescence intensity (normalized to wild-type) between wild-type and *grld-1* mutants. n = 20. Error bars, s.e.m. n.s. = not significant, compared to wild-type animals, *t*-test.

Supplementary Figure-2 Shen



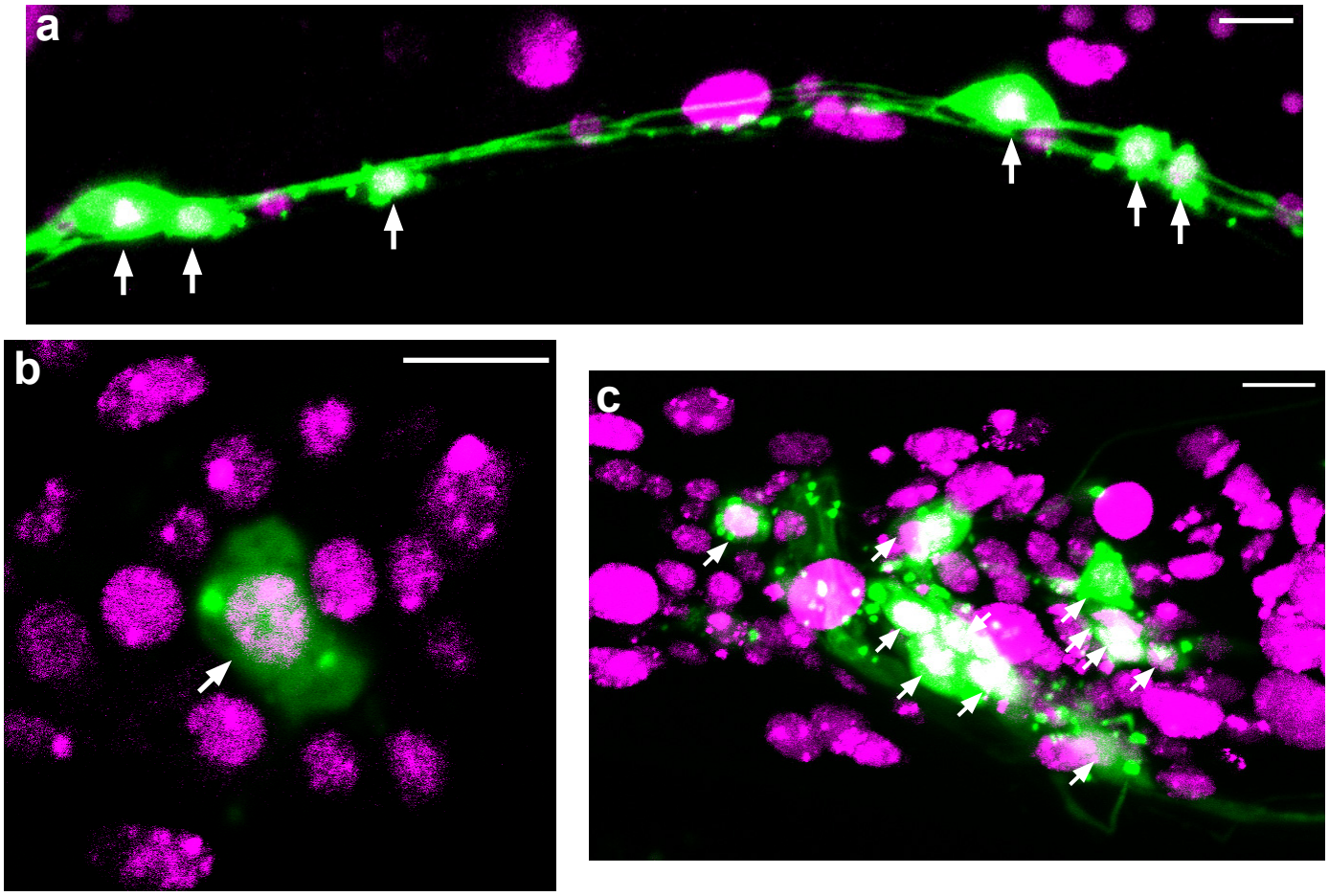
Supplementary Figure 2 Voltage-dependent currents are similar in wild-type and *grld-1(wy225)* mutants. Gray, wild-type AVEs; Black, *grld-1(wy225)* mutant AVEs.

Supplementary Figure-3 Shen



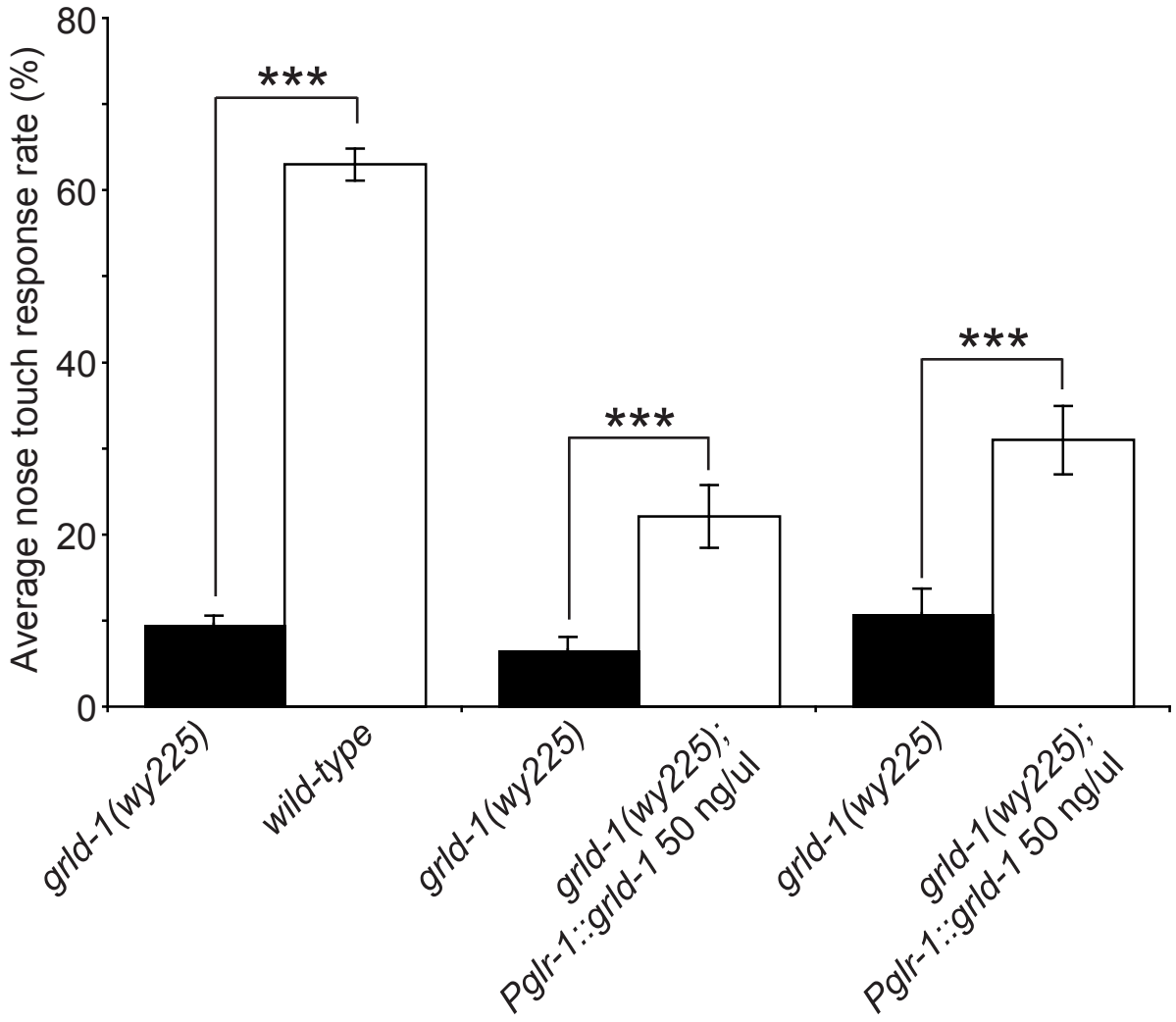
Supplementary Figure 3 *grld-1* RNAi exhibits decreased levels of GLR-1 in AVE. Comparison of GLR-1::YFP fluorescence intensity (normalized to wild-type) between wild-type, *eri-1(mg366)*; *lin-15b(n765)* RNAi of *grld-1*, *eri-1(mg366)*; *lin-15b(n765)* RNAi of *din-1*, and *eri-1(mg366)*; *lin-15b(n765)* no RNAi worms. n = 23. Error bars, s.e.m. ***P < 0.001, n.s. = not significant, *t*-test.

Supplementary Figure-4 Shen



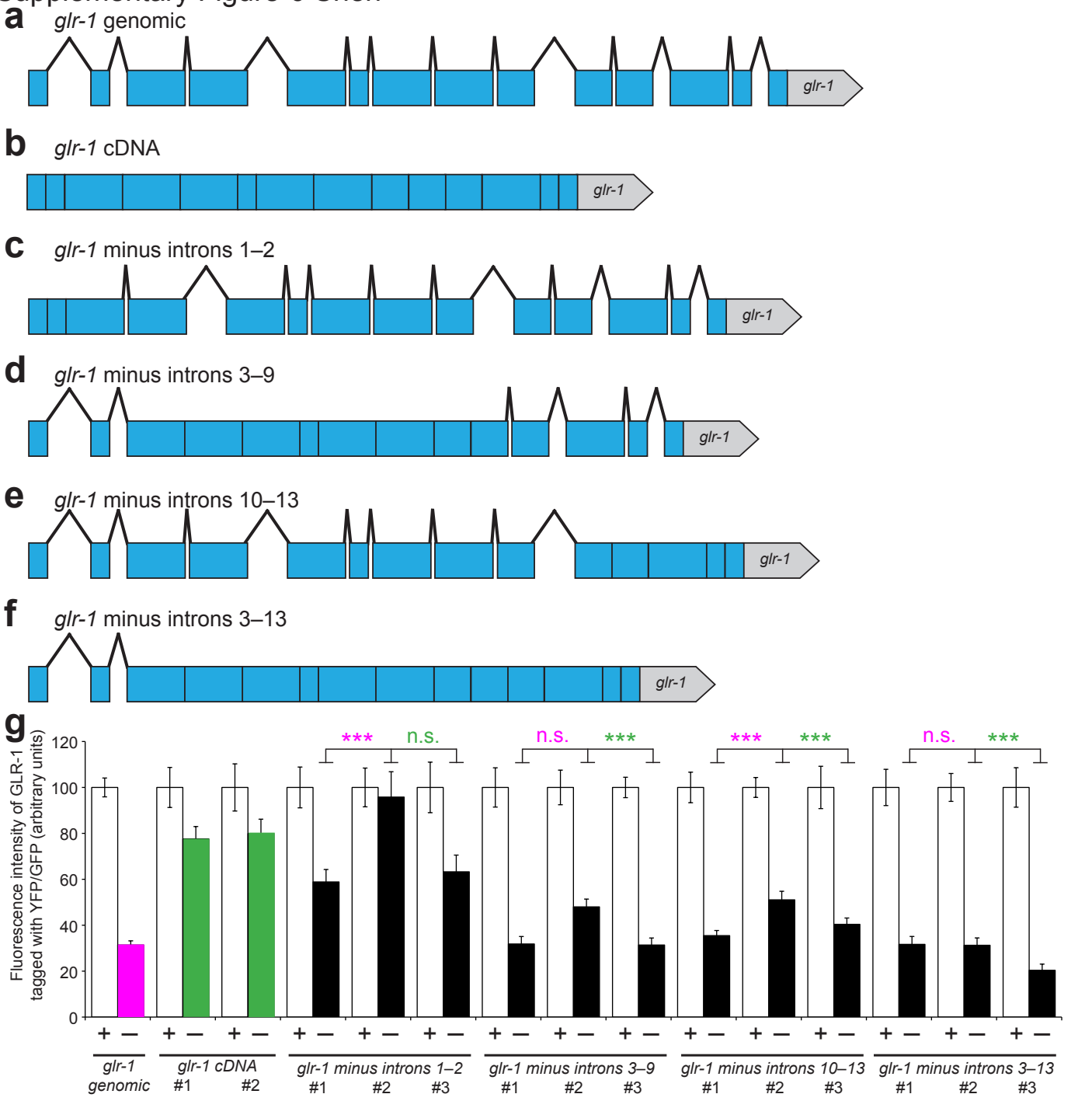
Supplementary Figure 4 *grld-1* is expressed in many other neurons. (**a**) *grld-1* is expressed in A-type motor neurons. NLS::mCherry (pseudo-colored green) is expressed in A-type motor neurons by the *unc-4* promoter and GFP-tagged GRLD-1 (pseudo-colored magenta) is expressed with fosmid recombineering. Arrows indicate cell bodies from left to right: DA3, VA4, VC2, VA5, DA4, and VC3. The image is a confocal stack of a young-adult worm. Scale bar, 5 µm. (**b**) *grld-1* is expressed in ASH. NLS::mCherry (pseudo-colored green) is expressed in ASH by the *sra-6* promoter and GFP-tagged GRLD-1 (pseudo-colored magenta) is expressed with fosmid recombineering. The arrow indicates the ASH cell body and anterior is left. The image is a single confocal plane (~1 µm) of an L2-staged worm. Scale bar, 5 µm. (**c**) *grld-1* is expressed in many *glr-1* expressing neurons. NLS::mCherry (pseudo-colored green) is expressed by the *glr-1* promoter and GFP-tagged GRLD-1 (pseudo-colored magenta) is expressed with fosmid recombineering. Arrows indicate cell bodies of *glr-1*-expressing neurons with expression of GFP-tagged GRLD-1. The image is a confocal stack of an L4-staged worm. Scale bar, 5 µm.

Supplementary Figure-5 Shen



Supplementary Figure 5 Comparison of nose-touch behavioural response between wild-type, *grld-1(wy225)*, and *grld-1(wy225)* expressing *grld-1* under the *glr-1* promoter animals. The compared genotypes were assayed on the same set of days. Wild-type compared to *grld-1(wy225)* mutants: n = 177, other comparisons: n = 24. Error bars, s.e.m. ***P < 0.001, *t*-test.

Supplementary Figure-6 Shen



Supplementary Figure 6 *grld-1* regulates *glr-1* through introns 1–2. (**a**–**f**) Schematic cartoon of the *glr-1* constructs. Lines, introns; blue boxes, exons; pentagons, 3' UTR. Note this *glr-1* depiction consists all of the introns and exons. *glr-1 genomic::glr-1 3' UTR*: all endogenous exons, introns, and *3' UTR*. All constructs have YFP or GFP inserted in the last exon (**a**). *glr-1 cDNA::glr-1 3' UTR*: all endogenous exons, no introns, and with *glr-1 3' UTR* (**b**). *glr-1 genomic (minus introns 1–2)::glr-1 3' UTR*: all endogenous exons, introns 3–13, and with *glr-1 3' UTR* (**c**). *glr-1 genomic (minus introns 3–9)::glr-1 3' UTR*: all endogenous exons, introns 1–2 and 10–13, and with *glr-1 3' UTR* (**d**). *glr-1 genomic (minus introns 10–13)::glr-1 3' UTR*: all endogenous exons, introns 1–9, and with *glr-1 3' UTR* (**e**). *glr-1 genomic (minus introns 3–13)::glr-1 3' UTR*: all endogenous exons, introns 1–2, and with *glr-1 3' UTR* (**f**). (**g**) Effectiveness of the *glr-1* constructs in rescuing the GLR-1 fluorescent phenotypes. The *glrd-1(wy225)* intensities were normalized to their respective expression constructs. #1, #2, and #3 indicate different extra-chromosomal arrays for each construct. n ≥ 20. + is wildtype and – is *grld-1(wy225)*. For each *glr-1* genomic-cDNA hybrid construct, the three arrays in *grld-1(wy225)* mutants are normalized and averaged together, then compared to *grld-1(wy225)* mutants expressing the *glr-1* genomic construct (in purple) and the averaged *glr-1* cDNA constructs (in green). Error bars, s.e.m. ***P < 0.001, n.s. = not significant, *t*-test.

Supplementary List of Constructs

Constructs and Transgenic Strains

Expression clones were made in the pSM vector, a derivative of pPD49.26 from A. Fire (Stanford University) with extra cloning sites from C. I. Bargmann (Rockefeller University).

Popt-3 was amplified using the following primers: 5' GGCCGGCCTAACAGAATTAGTAAGAAGGTGGG, 3' GGCGCGCCCAGACACGGGAGAGGCGG and subcloned into the pSM vector using FseI and AscI. *Popt-3* was subsequently moved to other vectors with SphI (upstream of FseI in pSM) and XmaI (downstream of AscI in pSM) unless otherwise noted.

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

All expression vectors were made with cDNA unless noted.

wyIs120: *Popt-3::glr-1 genomic::YFP::glr-1 3' UTR* (GW58-2) at 25 ng/ul: *Popt-3* promoter was cloned into KP#196 (J. Kaplan) using NotI, SalI restriction sites to generate *Popt-3::glr-1::GFP::glr-1 3' UTR* (GW31-1). 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::glr-1 3' UTR*. *Popt-3::snb-1::CFP::unc-54 3' UTR* (JK6-1) at 5 ng/ul: *snb-1* was cloned into *Pttx-3::CFP::unc-54 3' UTR*[1] using NheI and KpnI. *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) at 1 ng/ul: The GFP from pPD95.79 from A. Fire (Stanford University) was cut with KpnI and EcoRI (blunted with mung bean nuclease) and replaced with mCherry from (GW38-3) cut with KpnI and SacII (blunted with mung bean

nuclease). The mCherry from GW38-3 was generated via PCR from J. We (University of California, San Diego) with the following primers: 5' gaaaggGGTACCgATGggatccATGGTCTCAAAGG, 3' GAAAGGccgcggttaCTTATACAATTCATCCATGCCACCTG. The array was integrated into chromosome I using trimethylpsoralen/UV mutagenesis.

wyEx2438: *Popt-3::mCherry::unc-54 3' UTR* (GW90-2) at 0.5 ng/ul: cloned as above in *wyIs120*.

wyEx3504: *fosmid WRM0615bA06 w/ grld-1 C-terminal GFP* (GW238-3) at 1 ng/ul: *GFP* (and a linker) was amplified using the following primers: 5' attgcccattcttgctcggagctcttgccgtccgggctcctggaactccaGGTAGTGGAAGCGGCTCTatgagtaaaggag aagaacttttcactgg, 3' gagaataaattgtataaatcgagttatttacaagctcttggatggagatcctatttgtatagttcatccatgccatgtg from pBALU-1[2], the PCR product was recombineered[2], into fosmid WRM0615bA06 C-terminal of *grld-1* replacing the stop codon. *Popt-3::mCherry::unc-54 3' UTR* (GW90-2) at 2 ng/ul: cloned as above in *wyIs120*.

wyEx3557: fosmid WRM0615bA06 w/ grld-1 C-terminal GFP (GW238-3) at 1 ng/ul: cloned as above in *wyEx3504*. *Punc-4::NLS::mCherry::unc-54 3' UTR* (MS74-2) at 20 ng/ul: *NLS::mCherry* was amplified using the following primers: 5' (containing NLS sequence) ggggtaccatgCCAAAGAAGAAGCGTAAGGTAGTCTCAAAGGGTGAAGAAGAT, 3' gggaattcTTAggatccactagtCTTATACAATTCATCCATGCC and subcloned into pSM using KpnI and EcoRI. *Punc-4* was amplified using the following primers: 5' ACATGCATGCctgcagcctctgaaaatatatcaatgc, 3' TTTTTTGGCGCGCCtttcacttttggaagaagaagatcc and subcloned using SphI and AscI.

wyEx3559: fosmid WRM0615bA06 w/ grld-1 C-terminal GFP (GW238-3) at 1 ng/ul: cloned as above in *wyEx3504*. *Psra-6::NLS::mCherry::unc-54 3' UTR* (GW244-1) at 20 ng/ul:

NLS::mCherry::unc-54 3' UTR from MS74-2 was subcloned into a pSM derivative with *Psra-6*[3] using NheI and ApaI.

wyEx3561: fosmid WRM0615bA06 w/ grld-1 C-terminal GFP (GW238-3) at 1 ng/ul: cloned as above in *wyEx3504*. *Pglr-1::NLS::mCherry::unc-54 3' UTR* (GW245-1) at 10 ng/ul: *Pglr-1* was amplified using the following primers: 5' GAAAGGGCGGCCGCCCCGGGCTGCAGCATTTTTTAAAAG, 3' GAAAGGTCTAGATGTGAATGTGTCAGATTGGGTGCC and subcloned into a pSM derivative using NotI and XbaI and then subcloned into MS74-2 using SphI and XbaI.

wyEx1669: *Popt-3::GRLD-1::unc-10 3' UTR* (GW130-1) at 50 ng/ul: *unc-10 3' UTR* was amplified from *GFP::rab-3a::unc-10 3' UTR* (M. Nonet) using the following primers: 5' CGGAATTCCGGCCGCGGATAACAAATTTCATATG, 3' CGCGGGCCCACTAGTTGGCGTTAATATTTAAATG and subcloned into pSM using EcoRI and ApaI. The *grld-1* entry clone was obtained from the ORFeome project (http://worfdb.dfci.harvard.edu/) and cloned into the destination vector *Popt-3::gateway::unc-10 3' UTR* (GW68) using the Gateway strategy with LR clonase (Invitrogen) to make *Popt-3::grld-1::unc-10 3' UTR*. Injection were made into *wyIs120; grld-1(wy225)*.

wyEx2366: *Popt-3::GRLD-1::unc-10 3' UTR* (GW130-1) as *wyEx1669* at 20 ng/ul. Co-injection marker: *Podr-1::RFP* at 40 ng/ul injected into *grld-1(wy225)*.

wyEx2354: *Pglr-1::grld-1::unc-10 3' UTR* (GW169-2) at 50 ng/ul: *Pglr-1* was amplified using the following primers: 5' GAAAGGGCGGCCGCCCCGGGCTGCAGCATTTTTTAAAAG 3' GAAAGGTCTAGATGTGAATGTGTCAGATTGGGTGCC and subcloned into GW130 using NotI and XbaI.

wyEx2355 is a different isolate from the same injection as *wyEX2354*.

wyEx3614: *Popt-3::grld-1::unc-10 3' UTR* (GW130-2) as *wyEx1669* at 50 ng/ul. *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) as *wyIs120* at 2 ng/ul. Co-injection marker: *Punc-122::RFP* at 40 ng/ul. Injection was made into *wyIs120; wy225* and *wyIs120* was subsequently outcrossed for described experiments.

wyEx2661: *Pnmr-1::GRLD-1::unc-10 3' UTR* (GW191-1) at 50 ng/ul: *Pnmr-1* was amplified using the following primers: 5' gaaagggcatgcggactgatttgcaaccttgaccattcatg, 3' gaaaggggcgcgccatctgtaacaaaactaaagtttgtcgtgttcc and subcloned into pSM using SphI and AscI, then subcloned into GW58 using SphI and XmaI and then subcloned into GW130 using AscI and NotI.

wyEx2031: *Popt-3::GRLD-1* $_{1\text{-}375}$ *RRMs only::unc-10* (GW147-1) at 30 ng/ul: *RRMs* were amplified from the *grld-1* entry clone (see *wyEX1669)* using the following primers: 5' atggcagaagaacgcggtagacctc, 3' ttaagtagccgcataggcctcctccagc and was cloned into the pCR8/GW/TOPO/ TA vector (Invitrogen, GW144). Subsequently, the *RRMs* were cloned into the destination vector *Popt-3::gateway::unc-10 3' UTR* (GW126) using the Gateway strategy with LR clonase (Invitrogen). Injections were made into *wyIS120; grld-1(wy225)*.

wyEx2048: *Popt-3::GRLD-1* $_{322\text{-}521}$ *SPOC only::unc-10 3' UTR (*GW148-1) at 30 ng/ul: *SPOC* was amplified from the *grld-1* entry clone (see *wyEX1669)* using the following primers: 5' atgtacgccaaagatttgacggcgcaaccc, 3' ttatggagttccaggagcccggacg and was subcloned into the pCR8/GW/TOPO/ TA vector (Invitrogen, GW145). Subsequently, the *SPOC* was cloned into the destination vector *Popt-3::gateway::unc-10 3' UTR* (GW126) using the Gateway strategy with LR clonase (Invitrogen). Injections were made into *wyIs120; grld-1(wy225)*.

wyEx1704: *Popt-3::glr-1 genomic::YFP::unc-10 3' UTR* (GW137-14) at 25 ng/ul : *glr-1 genomic::YFP* was amplified from GW58 (see *wyIS120*) using the following primers: 5' gaaaggccgcggtcagacagctgtgttgtagagagtgtttg, 3' gaaaggggcgcgcctcgacgtcgccggcacccaatctga and

was subcloned into *Popt-3::gateway::YFP::unc-10 3' UTR* (GW17-3) with AscI and SacII. Injections were made into *grld-1(wy225)*.

wyEx1707 is a different isolate from the same injection as *wyEx1704*.

wyEx2357: *Popt-3::glr-1 cDNA::GFP::glr-1 3' UTR* (GW174-1) at 20 ng/ul: this construct was made created using the KP#196 (J. Kaplan) backbone. The *glr-1* cDNA was obtained from the ORFeome project (http://worfdb.dfci.harvard.edu/). Nucleotide errors were corrected by swapping out fragments of *glr-1* from KP#196 and a cDNA library generated using standard protocols. First the middle region of the *glr-1 cDNA::GFP* was subcloned into KP#196 to generated GW167 using NheI and XhoI. The 3' region of the *glr-1 cDNA::GFP* was then inserted into GW167 with the XhoI fragment from KP#196 (J. Kaplan) using XhoI to make GW168. Finally the 5' region of *glr-1* cDNA was generated with the following primers: GWp200: 5' gtcgacgtcgccggcacccaatctgacacattcacaatgttttcttcgttttctt, GWp201: 3' gctagcagttcactaattggaggtctcac and was subcloned into GW168-4 with NheI and SalI. The GFP was inserted into the *glr-1* cDNA using HindIII from KP#196. *Popt-3* was cloned as in *wyIs120*. *Popt-3::mCherry::unc-54 3' UTR* (GW90-2) at 1 ng/ul was generated as in *wyIs120*.

wyEx2359 is a different isolate from the same injection as *wyEx2357*.

wyEx1655: *Popt-3::GFP::GRLD-1::unc-54 3' UTR* (GW128-1) at 1 ng/ul: the *grld-1* entry clone was obtained from the ORFeome project (http://worfdb.dfci.harvard.edu/) and cloned into the destination vector *Popt-3::GFP::gateway::unc-54 3' UTR* (GW75-8) using the Gateway strategy with LR clonase (Invitrogen).

wyEx2505: *Phsp16-2, hsp16-41::grld-1a::unc-54 3' UTR* (GW188-2) at 50 ng/ul: We subcloned *grld-1a* cDNA into the plasmid pPD49.83, which contains inducible heat-shock promoters *hsp16-2* and *hsp16-41*. Injections were made into *wyIS120; grld-1(wy225)*.

wyEx1245: *Popt-3::cam-1::YFP::unc-10 3' UTR* (GW101-1) at 10 ng/ul: The *cam-1* entry clone was obtained from the ORFeome project (http://worfdb.dfci.harvard.edu/) and cloned into the destination vector *Popt-3::gateway::YFP::unc-10 3' UTR* (GW17-2B) using the Gateway strategy with LR clonase (Invitrogen). *Popt-3::snb-1::CFP::unc-54 3' UTR* (JK6-1) at 5 ng/ul: described above in *wyIS120*. Injections were made into *glo-1(zu391)* from the CGC and was subsequently outcrossed into N2.

wyEx4035: *Popt-3::glr-1 genomic (minus introns 1-2)::GFP::glr-1 3' UTR* (GW261-1) at 22ng/ul: *glr-1 genomic (minus introns 1-2)* was created by cloning BamHI, NheI from GW174 (see *wyEx2357*) into BamHI, NheI of GW31 (see *wyIS120*). *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) at 1 ng/ul was generated as in *wyIs120*. Injections were made into *grld-1(wy225)*.

wyEx4036: *Popt-3::glr-1 genomic (minus introns 1-2)::GFP::glr-1 3' UTR* (GW261-1) at 22ng/ul: *glr-1 genomic (minus introns 1-2)* was created by cloning BamHI, NheI from GW174 (see *wyEx2357*) into BamHI, NheI of GW31 (see *wyIS120*). *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) at 1 ng/ul was generated as in *wyIs120*.

wyEx4034: *Popt-3::glr-1 genomic (minus introns 1-2)::GFP::glr-1 3' UTR* (GW261-1) at 22ng/ul: *glr-1 genomic (minus introns 1-2)* was created by cloning BamHI, NheI from GW174 (see *wyEx2357*) into BamHI, NheI of GW31 (see *wyIS120*). *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) at 1 ng/ul was generated as in *wyIs120*. This was a different injection than *wyEx4036*.

wyEx4037: *Popt-3::glr-1 genomic (minus introns 3-9)::GFP::glr-1 3' UTR* (GW262-1) at 21ng/ul: *glr-1 genomic (minus introns 3-9)* was created by cloning NheI, BstAPI from GW174 (see *wyEx2357*) into NheI, BstAPI of GW31 (see *wyIS120*). *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) at 1 ng/ul was generated as in *wyIs120*. Injections were made into *grld-1(wy225)*.

wyEx4038: *Popt-3::glr-1 genomic (minus introns 3-9)::GFP::glr-1 3' UTR* (GW262-1) at 21ng/ul: *glr-1 genomic (minus introns 3-9)* was created by cloning NheI, BstAPI from GW174

(see *wyEx2357*) into NheI, BstAPI of GW31 (see *wyIS120*). *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) at 1 ng/ul was generated as in *wyIs120*.

wyEx4039 is a different isolate from the same injection as *wyEx4038*.

wyEx4040: *Popt-3::glr-1 genomic (minus introns 10-13)::GFP::glr-1 3' UTR* (GW262-1) at 21ng/ul: *glr-1 genomic (minus introns 10-13)* was created by cloning BstAPI, KpnI from GW174 (see *wyEx2357*) into BstAPI, KpnI of GW31 (see *wyIS120*). *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) at 1 ng/ul was generated as in *wyIs120*. Injections were made into *grld-1(wy225)*.

wyEx4041 is a different isolate from the same injection as *wyEx4040*.

wyEx4042: *Popt-3::glr-1 genomic (minus introns 10-13)::GFP::glr-1 3' UTR* (GW262-1) at 21ng/ul: *glr-1 genomic (minus introns 10-13)* was created by cloning BstAPI, KpnI from GW174 (see *wyEx2357*) into BstAPI, KpnI of GW31 (see *wyIS120*). *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) at 1 ng/ul was generated as in *wyIs120*.

wyEx4043: *Popt-3::glr-1 genomic (minus introns 3-13)::GFP::glr-1 3' UTR* (GW262-1) at 21ng/ul: *glr-1 genomic (minus introns 3-13)* was created by cloning BamHI, NheI from GW31 (see *wyIS120*) into BamHI, NheI of GW174 (see *wyEx2357*). *Popt-3::mCherry::unc-54 3' UTR* (GW90-1) at 1 ng/ul was generated as in *wyIs120*.

wyEx4044 and *wyEx4045* is a different isolate from the same injection as *wyEx4043*.

References

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2. Tursun, B., Cochella, L., Carrera, I. & Hobert, O. A toolkit and robust pipeline for the generation of fosmid-based reporter genes in C. elegans. *PLoS One* **4**, e4625 (2009).
3. Shen, K. & Bargmann, C.I. The immunoglobulin superfamily protein SYG-1 determines the location of specific synapses in C. elegans. *Cell* **112**, 619-630 (2003).