SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

Strains

The following mutant alleles were used in this study: *oig-4(kr39)*, *oig-4(kr193)*, *lev-10(kr26::Mos1)*, *lev-9(ox177::Mos1)*, *unc-29(x29)*, *unc-38(x20)*, *acr-16(ok789)*, *unc-49(e407), lin-15(n765ts)*, *oig-4(tm3753/+)*, *oig-4(kr39)*;*unc-29(x29)*, *oig-4(kr39)*;*lev-10(kr26::Mos1)*, *oig-4(kr39)*;*lev-9(ox177::Mos1)*, *unc-63(kr98::YFP)*, *oig-4(kr39);unc-63(kr98::YFP)*, *oig-4(kr193);unc-63(kr98::YFP), lev-9(kr184::T7), oig-4(kr39);lev-9(kr184::T7)*, *oig-4(kr193);lev-9(kr184::T7)*, *unc-49(e407);oig-4(kr39)*, *unc-49(e407);oig-4(kr193), unc-49(e407);acr-16(ok789)*, *unc-49(e407);acr-16(ok789);oig-4(kr39)*, *unc-49(e407);acr-16(ok789);oig-4(kr193),*

Strains for mapping: CB4856, *rol-6(e187) oig-4(kr39), sqt-2(sc3) oig-4(kr39), bli-1(e935) oig-4(kr39)* and the Hawaian isolate.

Strains for haplo-insufficency study: *rol-6(e187) oig-4(kr39) and the deficiency mnDf99 – the genotype oig-4(mnDf99/kr39) corresponds to the strain oig-4(mnDf99/kr39) unc-4(+/e120) rol-6(0/e187).*

The following transgenic lines were generated for this study:

Arrays in *oig-4(kr39): krEx381 [Pmyo-2::gfp)],* krEx519, 535-537 *[Pmyo-2::gfp;Poig-4::oig-4(DNA genomic fragment)], krEx510-512, 517-518 [Pmyo-2::gfp; Pmyo-3::oig-4(cDNA)], krEx736-739 [Pmyo-2::gfp; Pmyo-3::oig-4(R83A,D85A)]*

Arrays in *oig-4(kr193): krEx574-579 [Pmyo-2::gfp;Poig-4::oig-4(DNA genomic fragment)]*

Arrays in *oig-4(kr39); lin-15(n765ts): krEx524-527,540-541 [lin-15(+);Poig-4::oig-4(DNA genomic fragment)- SL2- gfp)], krEx551-554 [lin-15(+); Poig-4::gfp-oig-4(DNA)], krEx555-557 [lin-15(+); Pmyo-3::gfp-oig-4(cDNA)], krEx593,672-675 [lin-15(+);Pmyo-3::gfp-oig-4(G84R)(translational fusion)], krIs53-54 [lin-15(+); Poig-4::gfp-oig-4(translational fusion)].* The last inserted array (Is) was also inserted in strains *oig-4(kr39); unc-29(x29), oig-4(kr39); lev-9(ox177::Mos1), oig-4(kr39); lev-10(kr26::Mos1).*

Arrays in *oig-4(kr39);unc-29(x29): krEx743-745 [rol-6(su1006); Pmyo-3::gfp-oig-4(cDNA)].* These last arrays were also obtained in strain *oig-4(kr39);unc-29(+).*

Plasmid construction and PCR amplification

pGR27 *[Poig-4::oig-4(DNA genomic fragment)]:* A genomic fragment of 2400bp corresponding to *C. elegans R07G3.9* was PCR amplified from N2 genomic DNA using Taq polymerase (Invitrogen) (primers 5'- catttcccatcgttataatcttcat -3' (oGR91) and 5'- cccaattaaaagaagaaaaatttgg -3' (oGR92). This PCR fragment was subcloned in the backbone vector by TA cloning (Invitrogen).

pGR32 *[Poig-4::oig-4(DNA genomic fragment)- SL2- gfp)]:* The *oig-4* genomic fragment of 2400bp was PCR amplified from vector pGR27 using Phusion polymerase (Finnzymes) (primers 5'- gcatgccatttcccatcgttataatcttcat-3' (oGR95) and 5'- ggtaccttgatttgtgaaaaatctttattc-3' (oGR96). This PCR fragment was subcloned at the restriction sites *SphI* and *KpnI* of vector pMG023 (Gendrel et al., 2009).

pGR38 *[Pmyo-3::oig-4(cDNA)]:* The *oig-4* cDNA was amplified from pGR28 using Taq polymerase (Invitrogen) (primers 5'- aggtaccatgagcttccgattatggg-3' (oGR106) and 5'- ggaattctcaatacgtatattctgct-3' (oGR107) and subcloned in vector pPD115.62 at the restriction sites *KpnI* and *EcoRI*.

pGR43 *[Poig-4:: gfp-oig-4(DNA)]:* The *gfp* sequence was amplified from pPD114.95 (GFP(S65T) using Taq polymerase (Invitrogen) (primers 5'- atcgattccagaaattttaaagcagaatatacg-3' (oGR108) and 5'- ctctagaatccatgccatgtgtaatccca-3' (oGR109) containing restriction sites *ClaI* and *XbaI* (vector pGR39). The *gfp* was inserted in the vector pGR28, after the 69th base pair of *oig-4* cDNA, at the restriction sites *ClaI* and *XbaI:* vector pGR40. Consequently, the GFP is inserted after the signal peptide of the protein. The *gfp-oig-4* DNA fragment from vector pGR40 was subcloned in the backbone pGR27 using the restriction sites *ClaI* and *NheI.*

pGR46 *[Pmyo-3::gfp-oig-4(cDNA)]:* The *gfp-oig-4*(cDNA) fragment from vector pGR40 was subcloned in backbone pGR38 using the restriction sites *ClaI* and *NheI.*

pGR49 *[Pmyo-3::gfp-oig-4(G84R)]:* The Glycine(G) to Arginine(R) mutation in the amino acid position 84 of OIG-4 was constructed by site directed mutagenesis in the vector pGR46 (primers 5'-tgcaaagctgctggagatcctagaccgaca-3' (oGR114) and 5'-ggatctccagcagctttgcaaatgataag-3' (oGR115).

Other vectors used (as co-injection markers): pPD118.33 *(Pmyo-2::gfp)*, EKL15 *[lin-15(+)]*, pPD115.62(*Pmyo-3::gfp*), pRF4 *[rol-6(su1006)].*

C. elegans germline transformation

Transformation was performed by microinjection of a DNA mixture of plasmids into the gonad of young adults (Mello et al., 1991).

For gene rescue experiments: *oig-4(kr39)* worms were injected with a DNA mixture containing either pGR27 (10 ng/µL), pPD118.33 (10 ng/µL) and 1 kb^+ ladder (Invitrogen) (80 ng/µL) or pGR27 (1 ng/µL), pPD115.62(5 ng/µL), pGH8 (10 ng/µL) and 1 kb^+ ladder (Invitrogen) (84 ng/µL).

oig-4(kr193) worms were injected with pGR27 (10 ng/µL), pPD118.33 (10 ng/µL) and 1 kb^+ ladder (Invitrogen) (10 ng/µL).

For tissue-specific rescue experiments: *oig-4(kr39)* worms were injected with pGR38 (10 ng/µL), pPD118.33 (10 ng/µL) and 1 kb^+ ladder (Invitrogen) (80 ng/µL).

For experiments of expression of transcriptional reporter: *oig-4(kr39);lin-15(n765ts)* worms were injected with pGR32 (20 ng/µL), EKL15 (20 ng/µL) and 1 kb^+ ladder (Invitrogen) (60 ng/µL).

For expression and integration of *gfp-oig-4* translational fusion: *oig-4(kr39);lin-15(n765ts)* worms were injected with pGR43 (20 ng/µL), EKL15 (20 ng/µL) and 1 kb^+ ladder (Invitrogen) (60ng/µL) or (10 ng/µL), EKL15 (20 ng/µL) and 1 kb^+ ladder (Invitrogen) (70ng/µL). *oig-4(kr39);unc-29(x29)* worms were injected with pGR46 (50 ng/µL), pRF4 (50 ng/µL).

For tissue specific expression of *gfp-oig-4* translational fusion: *oig-4(kr39);lin-15(n765ts)* worms were injected with pGR46 (20 ng/µL), EKL15 (80 ng/µL).

For expression of mutated *oig-4* sequences: *oig-4(kr39);lin-15(n765ts)* worms were injected with pGR49 (5 ng/µL), EKL15 (80 ng/µL) and 1 kb^+ ladder (Invitrogen) (15 ng/µL) or pGR49 (0,5 ng/µL), EKL15 (85 ng/µL) and 1 kb^+ ladder (Invitrogen) (15 ng/µL) or with pGR53 (1 ng/µL) and EKL15 (99 ng/µL) or pGR53 (20 ng/µL) and EKL15 (80 ng/µL).

For gfp transgenic *oig-4(kr39)* mutants: *oig-4(kr39)* worms were injected with pPD118.33 (20 ng/µL) and 1 kb^+ ladder (Invitrogen) (80 ng/µL).

Levamisole assay

Assays for levamisole sensitivity after acute and overnight exposure was performed as previously described (Gendrel et al., 2009).

(–)-Tetramisole hydrochloride (Sigma) was dissolved in water and added to 55°C-equilibrated NG agar at a concentration of 0.2mM, 0.6mM, or 1mM just before plates were poured. Levamisole containing plates were seeded with OP50 *E. coli.*

Levamisole acute response and overnight exposure assay: Young adult worms were put on plates containing 0.2mM, 0.6mM, or 1mM levamisole. For acute dose responses, animals were scored blindly for paralysis after 2 hour exposure on levamisole. The plates were tapped ten times on the bench then moving worms were scored For overnight dose responses, animals were let overnight at 20°C and surviving animals were then scored. For rescue experiments plates with 0.6mM levamisole were used.

Microscopy

Animals were anesthetized (solution of 3.8mM tricaine, 0.42mM tetramisole, and 20mM sodium azide in M9 buffer), mounted on pads (2% agarose in H_2O) and examined using either a Leica 5000B microscope (Nussloch, Germany) or an SP2 confocal microscope.

Live imaging and scoring of the UNC-63-YFP L-AChR subunit was performed in Axioscope compound microscope (Zeiss).

20 animals were mounted, per slide and examined immediately after anesthetizing. They were scored blindly for the presence (group a, similar to wild type) or absence (group b) of fluorescent clusters in the nerve cords.

Immunofluorescence labeling was examined either under the Leica 5000B microscope or the spinning disk CSU10 (Yokogawa). Image reconstruction and merges were obtained with Image J (NIH).

Immunocytochemical staining

Worms were prepared with the freeze-crack method described previously (Duerr et al., 1999; Gendrel et al., 2009). The methanol/ acetone fixation was used for all staining conditions except when using anti-T7 antibodies (PAF fixation).

The antibodies were used at the following dilutions: anti-UNC-17 at a 1:3000 dilution, mouse monoclonal anti-T7 (Novagen) at a 1:500 dilution and rabbit anti-T7 (Genetex) at a 1:1000 dilution, anti-UNC-49 and anti-UNC-38 antibodies at a 1:800 dilution, anti-GFP mouse monoclonal (Roche, ref. 1814460) or rabbit polyclonal (molecular probes, ref. A11122) both at a 1:500 dilution, anti-LEV-10 at 1:200 dilution, anti-ACR-16 at 1/50 dilution.

When used in single labeling experiments antibodies were incubated o/n at 4°C. In double-labeling experiments, incubation conditions were the following: anti-UNC-17 antibodies were incubated 1h at room temperature, then after 1h of washing anti-LEV-10 antibodies were

incubated overnight at 4°C, anti-UNC-38 or anti-GFP rabbit polyclonal antibodies were incubated overnight at 4°C, then after 1h of washing anti-UNC-17 antibodies were incubated 1h at room temperature, anti-UNC-38 antibodies were incubated overnight at 4°C, then after 1h of washing anti-GFP mouse monoclonal antibodies were incubated 1h at room temperature, anti-ACR-16 and anti-UNC-38 antibodies. anti-ACR-16 and anti-UNC-49 antibodies were incubated together overnight at 4°C, anti-T7 mouse monoclonal antibodies were incubated overnight at 4°C together with anti-UNC-38 or anti-LEV-10 antibodies.

As for the secondary antibodies, the Cy3-labelled goat anti-rabbit IgG (H+L) (Jackson ImmunoResearch Laboratories), the Cy3-labelled goat anti-guinea pig IgG (H+L) (Jackson ImmunoResearch Laboratories) and the Cy3-labelled goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories) were used at a 1:1000 dilution, the Alexa488-labelled goat anti-mouse (Molecular Probes) and the Alexa488-labelled goat anti-rabbit IgG (H+L) (Molecular Probes) were used at a 1:500 dilution.

SUPPLEMENTARY REFERENCES

Duerr, J.S., Frisby, D.L., Gaskin, J., Duke, A., Asermely, K., Huddleston, D., Eiden, L.E. and Rand, J.B. (1999) The cat-1 gene of Caenorhabditis elegans encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. *J Neurosci*, **19**, 72-84.

Gendrel, M., Rapti, G., Richmond, J.E. and Bessereau, J.-L. (2009) A secreted complement-control-related protein ensures acetylcholine receptor clustering. *Nature*, **461**, 992-996.

Mello, C.C., Kramer, J.M., Stinchcomb, D. and Ambros, V. (1991) Efficient gene transfer in C.elegans: extrachromosomal maintenance and integration of transforming sequences. *EMBO J*, **10**, 3959-3970.

SUPPLEMENTARY FIGURES

Figure S1

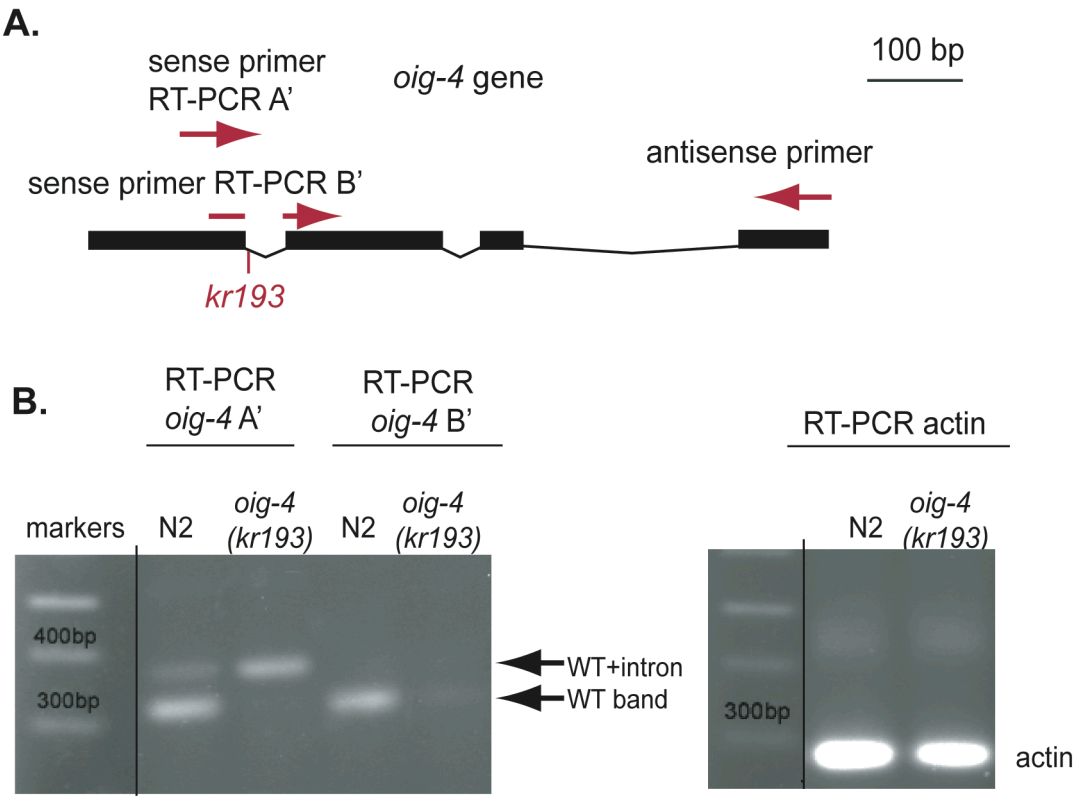


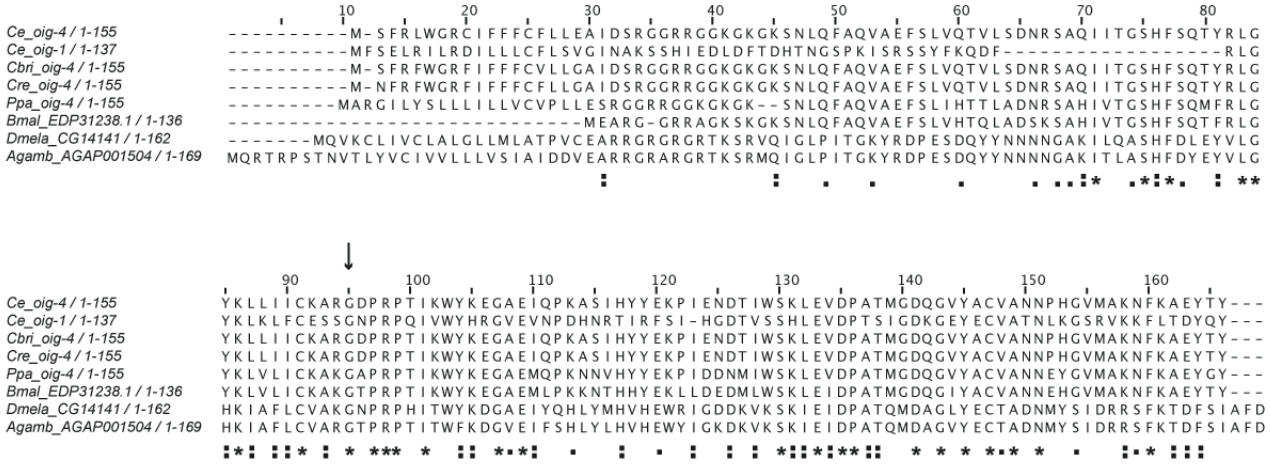
Figure S1. The *oig-4(kr193)* is a splicing mutant with 10% normally spliced *oig-4* transcripts thus is predicted to be a hypomorphic allele.

A. RT-PCR Strategy: PCR was performed on reverse transcription products using sense primers located either in the first exon of *oig-4* to detect spliced and non-spliced transcripts (RT-PCR A') or overlapping the junction between the 1st and the 2nd exon to detect only spliced *oig-4* transcripts that do not contain the 1st intron (RT-PCR B').

B. In wild-type (N2) animals RT-PCR A' detects mainly the spliced transcript (WT band, 321bp) and weakly the non-spliced transcript containing the 1st intron (370bp). In the *oig-4(kr193)* mutants the non-spliced transcript is only detected by this PCR. The RT-PCR B' detects the WT band in mutant animals at very low levels compared to the N2 animals (less than 10 %). RT-PCR of the actin mRNA was performed as a control. (n= 2 independent experiments for the 2 RT-PCR strategies)

Figure S2

A.



B.

Protein	*C. elegans* OIG-1	*C. briggsae* OIG-4	*C. remanei* OIG-4	*P. pacificus* OIG-4	*B. malayi EDP31238.1*	*D. melanogaster* CG14141	*A. gambiae* AGAP001504
Identity with *C. elegans* OIG-4	34.4%	97.4%	97.4%	71.2%	67.1%	32%	33.5%

Figure S2. Alignment of OIG-4 homologs in ecdysozoans

A. Alignment of proteins encoded by *oig-4* in *C. elegans (Ce_oig-4)* with the *C. elegans* paralog *oig-1 (Ce_oig-1)* and homologs in *C. briggsae (Cbri_oig-4), P. pacificus (Ppa_oig-4)*, *B. malayi (Bmai_ EDP31238.1), D. melanogaster (Dmela_CG14141)* and *A. gambiae (Agamb_AGAP001504)*. The numbering of amino acids in the figure is based on the longer sequence of *A. gambiae*. In the *C. elegans* OIG-4 protein the Ig domain spans amino acids 71 to 143 (positions 82-154 in the figure). All the homologs are almost identical to *C. elegans* OIG-4 in the region of the Ig domain. The glycine residue mutated in the *oig-4(kr39)* mutant (indicated with an arrow) is conserved in all OIG-4 homologs in contrast to its flanking residues. The alignment was performed with ClustalW2 and presented with Jalview software. ("*" for residues identical in all sequences, ":" for residues with conserved substitutions, "." for residues with semi-conserved substitutions.)

B. Identity of the *C. elegans* protein OIG-4 with the homologs presented above. Identities were retrieved from alignments using the EMBOSS needle Pairwise Alignment Algorithm (http://www.ebi.ac.uk/Tools/emboss/align/). Signal peptides were not excluded for identity calculations.