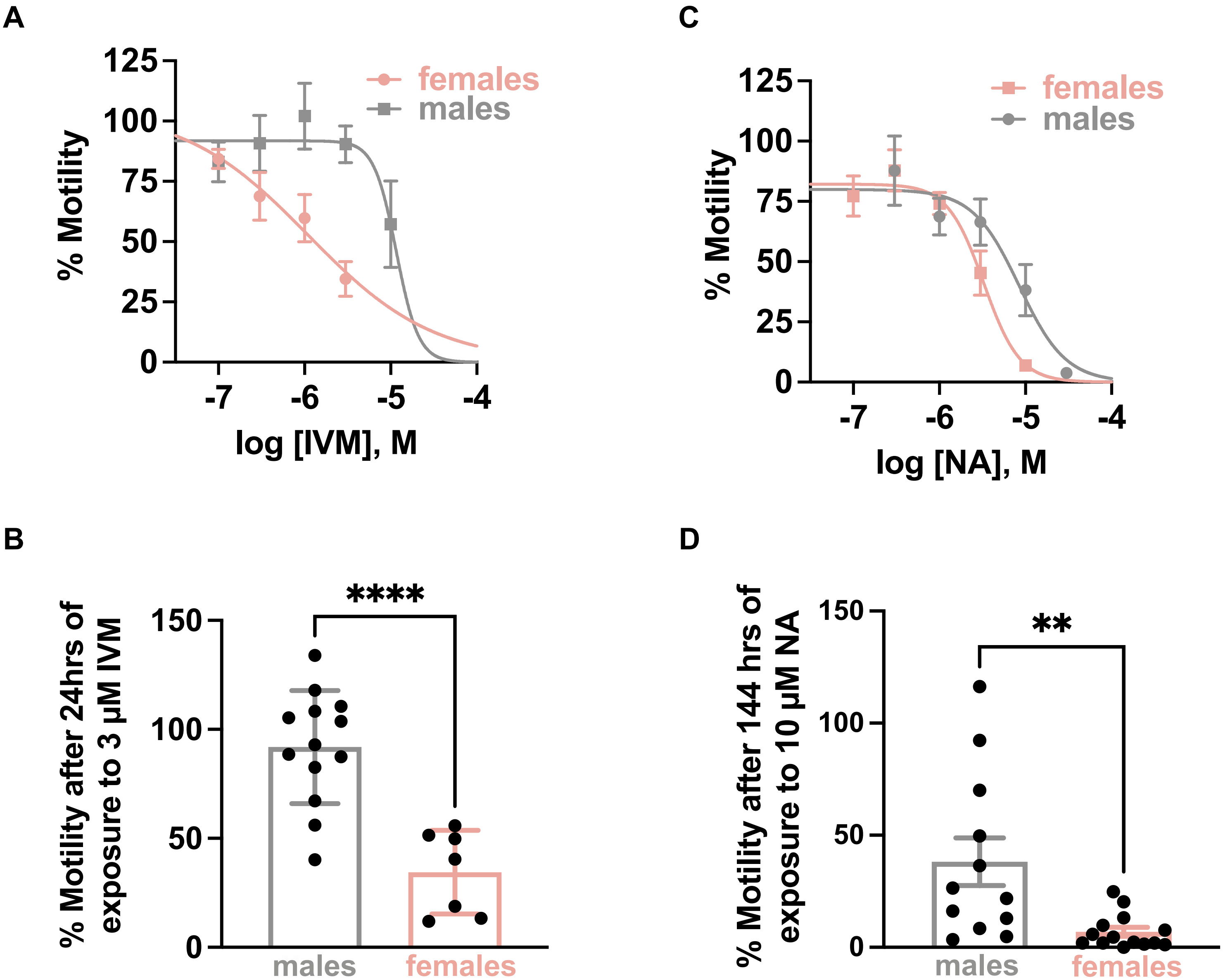

Fig. S1 Effect of IVM and NA on adult worm motility. (*A*) Concentration-response relationship of IVM in adult male and female worms after 24 hrs of exposure. The estimated IC_{50}, pIC_{50} and n_H values in male worms were 11.6 µM, 4.9 ± 0.2 and -3.4 ± 12.4. In females, the estimated IC_{50}, pIC_{50} and n_H values were 1.1 µM, 5.9 ± 0.4 and -0.6 ± 0.3. (*B*) Sex-dependent effect of 3 µM IVM in adult worms after 24 hrs of exposure. The mean response (expressed as mean ± SEM, %.) for males and females was 92.0 ± 7.2 and 34.0 ± 7.3, respectively. (*C*) Concentration-response relationship of NA in adult male and female worms after 144 hrs of exposure. In male worms the estimated IC_{50} was 8.5 µM, pIC_{50} was 5.1 ± 0.1 and n_H was -2.1 ± 0.8. In females, the estimated IC_{50}, pIC_{50} and n_H values were 3.3 µM, 5.5 ± 0.1 and -1.6 ± 0.7. (*D*) Sex-dependent effect of 10 µM NA in adult worms after 144 hrs of exposure. The mean response (expressed as mean ± SEM, %) for males and females was 38.0 ± 11.0 and 6.9 ± 2.0, respectively. ($^{****}P \leq 0.0001$, $^{**}P \leq 0.005$; significantly different as indicated, Two-tailed unpaired t-test).

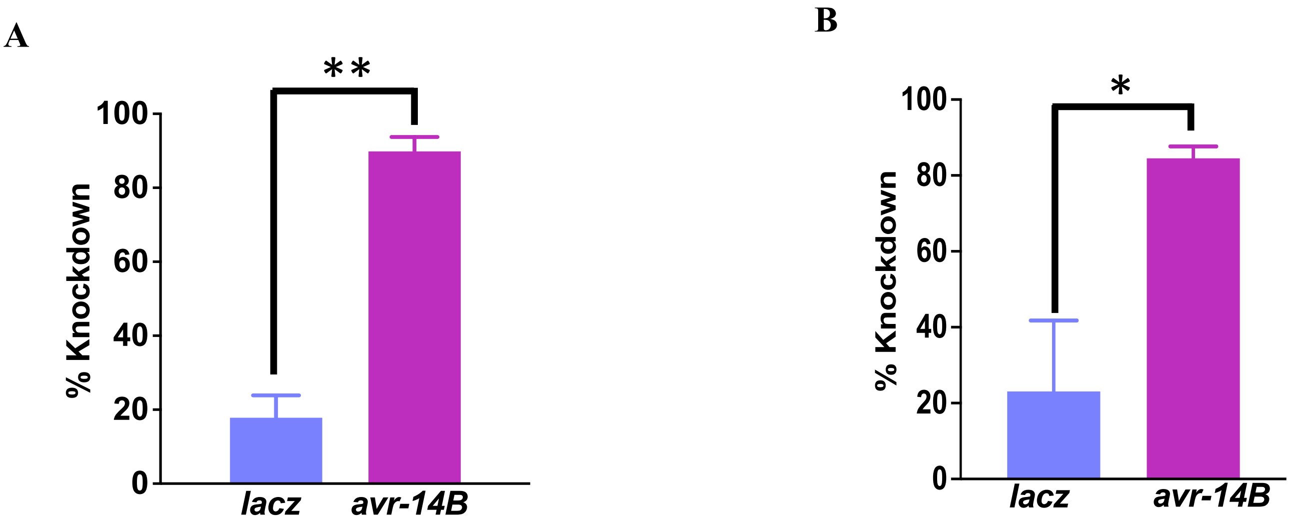

Fig. S2. (*A*) Knock down of *avr-14B* and *lacz* transcript in female worms after 120 hrs of incubation was assayed using qPCR. Knock down of the *avr-14B* transcript (expressed as mean ± SEM, %) was 90 ± 3.9 and 18 ± 6.1 in worms treated with *avr-14B* and control *LacZ* dsRNA, respectively. (*B*) Knock down of *avr-14B* transcript in male worms after 24 hrs of incubation was assayed using qPCR. Knock down of the *avr-14B* transcript (expressed as mean ± SEM, %) was 84 ± 3.1 and 23 ± 18.7 in worms treated with *avr-14B* and control *LacZ* dsRNA, respectively. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$; significantly different as indicated; Two-tailed paired t-test.

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Bma-AVR-14B     ---MNGCMICWIFTILILVMAKKKLKEQEIIQRTLKDYDWRVRPRGSNLSWPDTGGPVLV     57
Cel-AVR-14B     MWHYRLTTTILLIISIIHSIRAKRKLKEQEIIQRILKDYDWRVRPRGMNATWPDTGGPVLV     60
                        *  *::*:  : **:*********** ************ *  :**********

                      G                  D
Bma-AVR-14B     SVNIYLRSISKIDDVNMEYSAQFTFREEWNDARLAYERLAD-ENTQVPPFVVLAASEQAD     116
Cel-AVR-14B     TVNIYLRSISKIDDVNMEYSAQFTFREEWTDQRLAYERYEESGDTEVPPFVVLATSENAD     120
                :****************************.* ******   :   :*:********:**:**

                          A                                E         Cys-loop
Bma-AVR-14B     LTQQIWMPDTFFQNEKEARRHLIDKPNVLIRIHPDGQILYSVRLSLVLSCPMSLEYYPLD     176
Cel-AVR-14B     QSQQIWMPDTFFQNEKEARRHLIDKPNVLIRIHKNGQILYSVRLSLVLSCPMSLEFYPLD     180
                 :****************************** :*******************:****

                         B                    F                    C
Bma-AVR-14B     RQTCLIDLASYAYTTDDIKYEWKLKNPIQQKEGLRQSLPSFELQDVLTDYCTSKTNTGEY     236
Cel-AVR-14B     RQNCLIDLASYAYTTQDIKYEWKEKKPIQQKDGLRQSLPSFELQDVVTDYCTSLTNTGEY     240
                **.************:******* *:*****:**************:****** ******

                                    TM1                         TM2
Bma-AVR-14B     SCARVMLLLRREYSYYLIQLYIPCIMLVVVSWVSFWLDKDAVPARVSLGVTTLLTMTTQA     296
Cel-AVR-14B     SCARVVLRLRREYSYYLIQLYIPCIMLVVVSWVSFWLDKDAVPARVSLGVTTLLTMTTQA     300
                *****:* ****************************************************

                                          TM3
Bma-AVR-14B     SGINAKLPPVSYIKAVDIWIGVCLAFIFGALLEYALVNYYGRQEFLKKEKKKKTEFKGCL     356
Cel-AVR-14B     SGINSKLPPVSYIKAVDVWIGVCLAFIFGALLEYAVVNYYGRKEFLRKEKKKKTRIDDCV     360
                ****:************:*****************:******:***:*******.:..*:

                                                                    TM4
Bma-AVR-14B     CPSDHPFNQDLRQSLRLDMNTYRRKRWTK--FWLNRYLCGNTEVSKRVDLISRFAFPTFF     414
Cel-AVR-14B     CPSDRPP-------LRLDLSAYRSVKRLPIIKRISEILSTNIDISRRVDLMSRLTFPLTF     413
                ****:*        ****:.:**  :       :.. *. * ::*:****:**::**  *

Bma-AVR-14B     ACFLVLYYVNYVN---- 427
Cel-AVR-14B     FSFLIFYYVAYVKQSRD 430
                 .**::*** **:
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Fig. S3. Amino acid sequence alignment of *B. malayi* AVR-14B with *C. elegans* AVR-14B**.** Color code: Green = ligand binding loops A – G and residues that make contacts with bound glutamate are highlighted in maroon within different loops; Orange = Cys-loop characteristic of the Cys-loop ligand-gated ion channel superfamily; Regions highlighted in olive green = transmembrane regions 1 - 4 as predicted using TMHMM Server v. 2.0; Underlined region = PAR motif characteristic of ligand-gated anion channels; S260 in TM2 in the *C. elegans* GluCl alpha (GLC-1) subunit that is critical for ivermectin binding as described by Hibbs and Gouaux, 2011, is substituted by Alanine (A) highlighted in red in the TM2 region of both *Bma*-AVR-14B (A296) and *Cel*-AVR-14B (A300); the corresponding E114, V235 and L256 amino acid residues that confer ivermectin sensitivity in *Cooperia oncophora* AVR-14 are color coded in blue in *Bma*-AVR-14B (Q102, L223 and L244) and in *Cel*-AVR-14B (E106, V227 and R248) (Dent et al., 2000; Hibbs and Gouaux, 2011; Njue et al., 2004; Wolstenholme, 2012). Residues involved in van der Waals interactions with ivermectin *C. elegans* GluCl alpha (GLC-1) are highlighted in grey in the transmembrane regions and between TM2-TM3 loop (Hibbs and Gouaux, 2011).

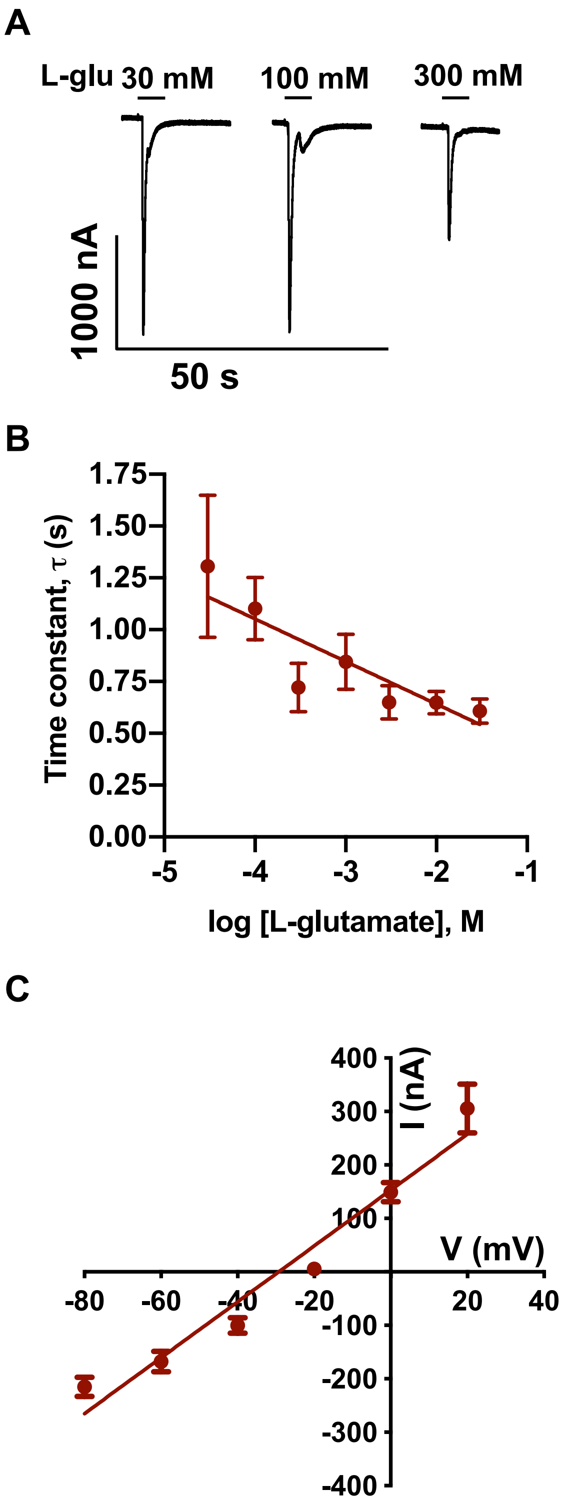

Fig. S4. (*A*) Representative inward current traces for 30 mM, 100 Mm and 300 mM L-glutamate mediated response. (*B)* Desensitization fit for L-glutamate gated currents. Desensitization time constants (τ) obtained over the entire dose-response relationship are plotted as a function of the agonist concentration. (*C*) Current-voltage (I-V) relationship plot for L-glutamate-gated currents. Oocytes were held at membrane potentials ranging from –80 to +20 mV and challenged with 1 mM L-glutamate at each holding potential. The reversal potential (E_{rev}) was equal to –28.9 ± 2.9 mV, n = 5.

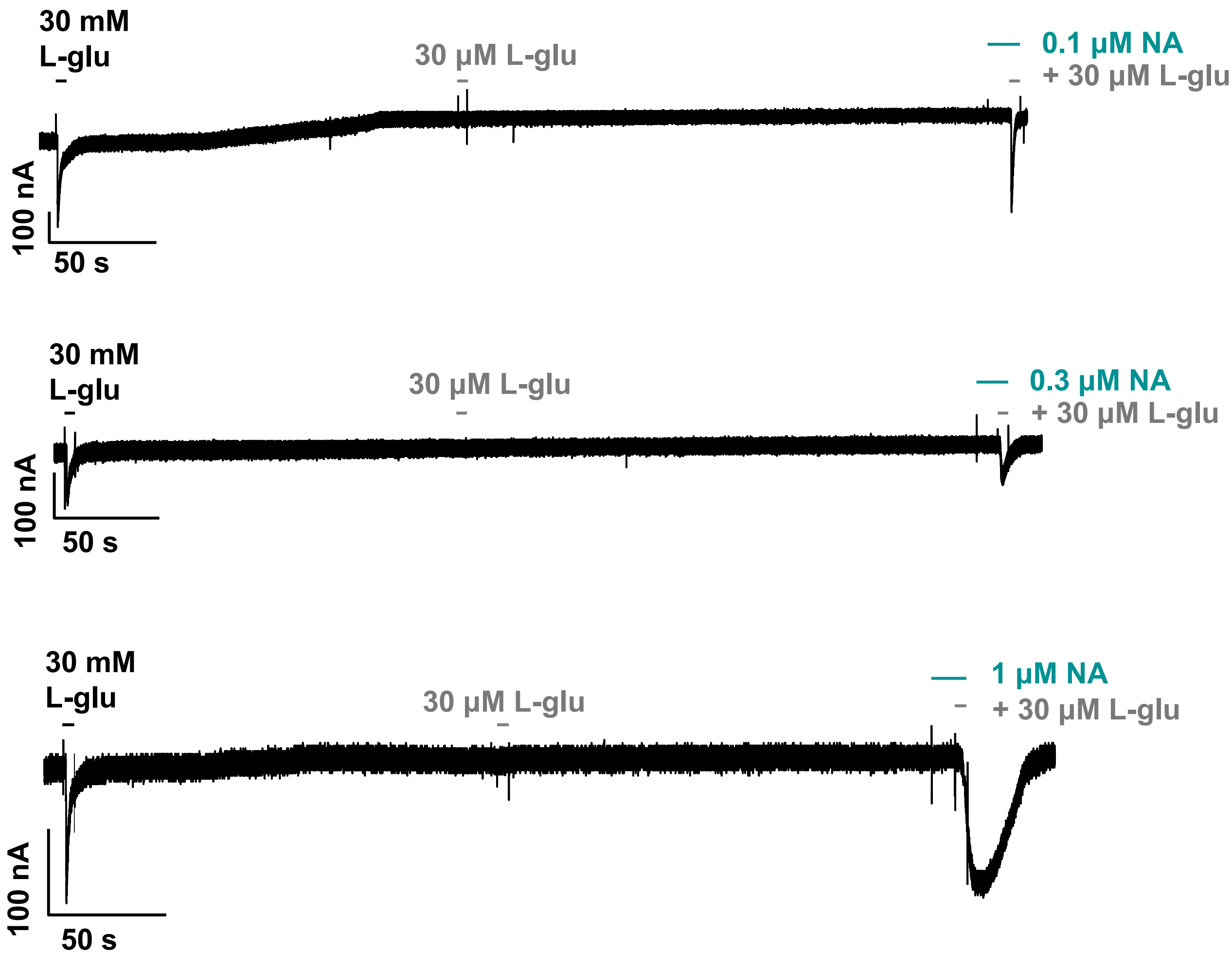

Fig. S5. Representative inward current response from oocyte used for studying the positive modulatory effect of NA on L-glutamate (L-glu; 30 µM) gated current response (n ≥ 5).

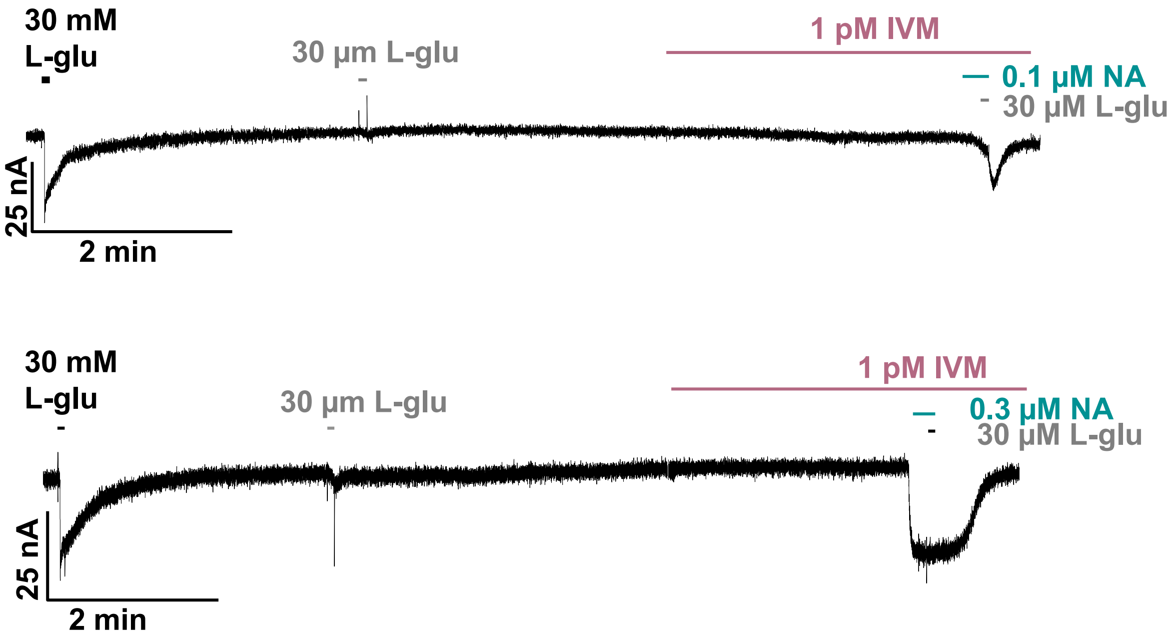

Fig. S6. Representative inward current response from oocyte used for studying the modulatory effect 1 pM IVM on NA (0.1µM and 0.3µM) and L-glutamate (L-glu; 30 µM) gated current response (n ≥ 5).

Table 1. A summary of AVR-14 GluCls from various nematodes

Organism	GluCl gene	Subunit	Tissue distribution	Pharmacological properties		
				Glutamate	IVM	PTX
C. elegans	*avr-14*	AVR-14A/ GluClα3A	neurons in ring ganglion, ventral cord, and mechanosensory neurons[1]	No response[1]	No response[1]	Not determined
		AVR-14B/ GluClα3B	neurons in ring ganglion, ventral cord, and mechanosensory neurons[1]	Sensitive to 10 mM glutamate [1]	Sensitive to 10μM IVM[1]	Not determined
H. contortus	*avr-14/ gbr-2/ GluClα3*	AVR-14A/ GluClα3A	amphidial neurons, motor neuron commissures, lateral and ventral nerve cords, and nerve ring[2]	No response[3]	No response[3]	Not determined
		AVR-14B/ GluClα3B	pharyngeal neurons, amphidial neurons, motor neuron commissures, lateral and ventral nerve cords, and nerve ring[2]	EC_{50} = 27.6 ± 2.7 μM[3]	estimated EC_{50} =~0.1 ± 1.0 nM[3]	Inhibits glutamate and IVM induced currents[3]
D. immits	*avr-14*	AVR-14A/ GluClα3A	Not determined	No response[4]	Not determined	Not determined
		AVR-14B/ GluClα3B	Not determined	Sensitive to glutamate (1–100 mM)[4]	Sensitive to 1μM IVM[4]	Not determined
C. oncophora	IVM sensitive (IVS) and IVM resistant (IVR) *GluClα3*	IVS AVR-14/ GluClα3	Not determined	EC_{50} = 29.7 ± 4 μM[5]	EC_{50} = 0.5 ± 0.12 μM[5]	Not determined
		IVR AVR-14/ GluClα3	Not determined	EC_{50} =171.6 ± 20.7 μM[5]	EC_{50} = 1.3 ± 0.11 μM[5]	Not determined
A. suum	*gbr-2*	GBR-2/ AVR-14	Nerve cords[6]	Not determined	Not determined	Not determined

[1] J. A. Dent, M. M. Smith, D. K. Vassilatis, L. Avery, The genetics of ivermectin resistance in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences* **97**, 2674 (2000).
[2] V. Portillo, S. Jagannathan, A. J. Wolstenholme, Distribution of glutamate-gated chloride channel subunits in the parasitic nematode Haemonchus contortus. *Journal of Comparative Neurology* **462**, 213-222 (2003).

[3] S. McCavera, A. T. Rogers, D. M. Yates, D. J. Woods, A. J. Wolstenholme, An ivermectin-sensitive glutamate-gated chloride channel from the parasitic nematode Haemonchus contortus. *Molecular pharmacology* **75**, 1347-1355 (2009).
[4] D. M. Yates, A. J. Wolstenholme, An ivermectin-sensitive glutamate-gated chloride channel subunit from Dirofilaria immitis. *International Journal for Parasitology* **34**, 1075-1081 (2004).
[5] N. I. Njue, R. K. Prichard, Genetic variability of glutamate-gated chloride channel genes in ivermectin-susceptible and -resistant strains of Cooperia oncophora. *Genetic variability of glutamate-gated chloride channel genes in ivermectin-susceptible and -resistant strains of Cooperia oncophora* **129**, 741-751 (2004).
[6] J S. Jagannathan *et al.*, Ligand-gated chloride channel subunits encoded by the Haemonchus contortus and Ascaris suum orthologues of the Caenorhabditis elegans gbr-2 (avr-14) gene. *Molecular and Biochemical Parasitology* **103**, 129-140 (1999).

Table S2. List of primers used in the study along with the description and nucleotide sequences.

Primer name	Description	Sequence 5'-3'
avr-14f	*Bma avr-14* dsRNA 5'	GATTGGTGTATGCTTGGCA
avr-14r	*Bma avr-14* dsRNA 3'	ACGATTGCCTTAGGTCCTG
gapdhf	*Bma gapdh* dsRNA 5'	GACGCTTCAAGGGAAGTGTTTCTG
gapdhr	*Bma gapdh* dsRNA 3'	GTTTTGGCCAGCACCACGAC
LacZf	*LacZ* dsRNA 5'	CGTAATCATGGTCATAGCTGTTTC
LacZr	*LacZ* dsRNA 3'	CTTTTGCTGGCCTTTTGCTC