Nementin is a Nematode-Selective Small Molecule Agonist of Neurotransmitter Release

Sean Harrington[1,2], Jessica J. Knox[2,3], Andrew R. Burns[2,3], Ken-Loon Choo[4], Aaron Au[2,5], Megan Kitner[6], Cecile Haeberli[7,8], Jacob Pyche[1,2], Cassandra D'Amata[2], Yong-Hyun Kim[4], Jonathan R. Volpatti[9,10], Maximillano Guiliani[2,5], Jamie Snider[2], Victoria Wong[2], Bruna M. Palmeira[11], Elizabeth M. Redman[11], Aditya S. Vaidya[12,13], John S. Gilleard[11], Igor Stagljar[2,3,14,15,16], Sean R. Cutler[12,13], Daniel Kulke[17, †,‡], James J. Dowling[9,10], Christopher M. Yip[2,5], Jennifer Keiser[7,8], Inga Zasada[6], Mark Lautens[4], and Peter J. Roy[1,2,3,20*]

Affiliations:

[1]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, M5S 1A8, Canada.

[2]The Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, M5S 3E1, Canada.

[3]Department of Molecular Genetics, University of Toronto; Toronto, ON, M5S 1A8, Canada.

[4]The Department of Chemistry, University of Toronto, 80 St. George Street, M5S 3H6, Toronto, Canada.

[5]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada.

[6]USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330, USA.

[7]Department of Medical Parasitology and Infection Biology; Swiss-Tropical and Public Health Institute, (Swiss TPH), Socinstr. 57, CH–4051 Basel, Switzerland.

[8]Faculty of Science; University of Basel, Petersplatz 1, 4001 Basel.

[9]Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 0A4.

[10]Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 0A4.

[11]Department of Comparative Biology and Experimental Medicine, Host-Parasite Interactions (HPI) Program, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada.

[12]Institute for Integrative Genome Biology, University of California, Riverside, Riverside, CA 92521, USA.

[13]Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA 92521, USA.

[14]Department of Biochemistry, University of Toronto, Toronto, ON, M5S 1A8, Canada.

[15]Mediterranean Institute for Life Sciences, Meštrovićevo Šetalište 45, HR-21000, Split, Croatia.

[16]School of Medicine, University of Split, Šoltanska ul. 2, 21000, Split, Croatia.

[17]Research Parasiticides, Bayer Animal Health GmbH, Monheim, Germany.

[†]Current affiliation, Department of Biomedical Sciences; Iowa State University, Ames, Iowa 50011, USA.

[‡]Current affiliation, Global Innovation, Boehringer Ingelheim Vetmedica GmbH; Binger Str. 173, 55218 Ingelheim am Rhein, Germany.

*Corresponding author.

The Donnelly Centre, Rm 1202, 160 College St., Toronto, ON, M5S 3E1, CANADA

4f16-978-6116, peter.roy@utoronto.ca

Preprint Servers: An earlier version of this manuscript is available on BioRxiv. doi: https://doi.org/10.1101/2022.03.11.483951. It is made available under a CC-BY 4.0 International license.

Classification: Biological Sciences, Genetics

Keywords: chemical genetics, nematicide, anthelmintic, *C. elegans*, parasitic nematodes, *Strongyloides ratti*, *Meloidogyne hapla*, small molecule screens, early-stage drug discovery, dense core vesicles, convulsions, dgk-1, egl-30, goa-1, unc-13, unc-17, unc-31, unc-43, unc-64

One-Sentence Summary: A *C. elegans*-based screening pipeline identifies a selective nematicide that also potentiates acetylcholinesterase inhibitors.

Abstract: Nematode parasites of humans, livestock and crops pose a significant burden on human health and welfare. Alarmingly, parasitic nematodes of animals have rapidly evolved resistance to anthelmintic drugs, and traditional nematicides that protect crops are facing increasing restrictions because of poor phylogenetic selectivity. Here, we present a pipeline that exploits multiple motor outputs of the model nematode *C. elegans* for nematicide discovery. This pipeline yielded multiple compounds that selectively kill and/or immobilize diverse nematode parasites. We focus on one compound that induces violent convulsions and paralysis that we call Nementin. We find that Nementin agonizes neuronal dense core vesicle release, which in turn agonizes cholinergic signaling. Consequently, Nementin synergistically enhances the potency of widely-used non-selective acetylcholinesterase inhibitors (AChEIs), but in a nematode-selective manner. Nementin therefore has the potential to reduce the environmental impact of toxic AChEI pesticides used to control nematode infections and infestations.

80 **Significance Statement:**

81 Parasitic nematodes pose a considerable burden to human health and food security.
82 Small molecules that have traditionally been used to control these parasites have either
83 been banned because of toxicity concerns or are being rendered ineffective because of
84 the evolution of resistance. Significant gaps in our nematicidal toolkit are therefore
85 becoming an alarming problem. Here, we describe our discovery of Nementin, a small
86 molecule that disrupts the nematode nervous system but is ineffective against non-
87 targeted organisms. We find that Nementin also enhances the activity of non-selective
88 pesticides but does so in a nematode-selective manner. Hence, Nementin is an
89 innovative solution to combat parasitic nematodes in a safe and phylum-selective
90 manner.

Introduction

Parasitic nematodes are a scourge to humanity. Not only do helminths currently infect more than 1.5 billion people (WHO Soil Transmitted Helminth Fact Sheet, 2022) but nematode parasitism also destroys tens of billions of dollars (USD) worth of livestock annually[1]. Alarmingly, the rate of destruction is growing due to the evolution of anthelmintic resistance[2]. Plant-parasitic nematodes (PPNs) are even more destructive as they ruin over 125 billions of dollars (USD) worth of food crops annually[3]. Traditional nematicides that protect crops have been justifiably banned or severely restricted due to a lack of phylum selectivity[4], but these restrictions severely impact food security[5]. Compounding these issues, global food demand is expected to increase by 70% by the year 2050[6]. Hence, the development of new and selective nematicides is essential to our collective welfare.

Here, we present a novel motor-centric screening pipeline to identify novel candidate nematicides. Disrupting a parasite's motor control has repeatedly proven effective in mitigating nematode infection[7,8]. We therefore screened our collection of worm-active (wactive) compounds[9] using two successive behavioural assays of the free-living nematode *Caenorhabditis elegans*. We identified four molecules that fail to elicit phenotypes in off-target systems at concentrations that kill multiple nematode species. We focused on one of these, called Nementin, that induces *C. elegans* hyperactivity within seconds of exposure, followed by whole-body convulsions minutes later. Nementin and its analogs selectively immobilize and/or kill nematode parasites of plants and mammals. Chemical-genetic analyses reveal that Nementin agonizes neuronal dense core vesicle release, which in turn, agonizes synaptic vesicle release. This

114 insight led to the finding that Nementin enhances the paralytic effects of
115 organophosphate and carbamate acetylcholinesterase inhibitors not only in *C. elegans*,
116 but in nematode parasites of mammals and plants. We conclude that Nementin is a
117 novel nematode-selective scaffold that may also improve the selectivity of broad-acting
118 pesticides.
119

Results

26 Compounds Disrupt Multiple *C. elegans* Motor Activities

To identify small molecules that disrupt nematode neuromuscular activity, we designed a 96-well plate imaging system that measures the egg-laying rate of adult *C. elegans* hermaphrodites (Fig. 1a-c). The regulation of *C. elegans* egg-laying relies on well-characterized cholinergic, serotonergic, GABAergic and peptidergic circuits[10], and is therefore a paragon to assess neuromuscular perturbation by small molecules. To identify egg-laying stimulators, we screened small molecules in the background of M9 worm buffer[9]. Animals incubated in M9-vehicle control wells lay 1.1 eggs per hour on average (Fig. 1d; Supplementary Data 1). To identify egg-laying inhibitors, we screened small molecules in the background of exogenous serotonin plus nicotine that stimulates animals to lay an average of 7.4 eggs per hour (Fig. 1d; Supplementary Data 1).

A screen of the 486 molecules from our worm-active library[9] revealed 29 stimulators and 29 inhibitors of egg-laying (Fig. 1e; Supplementary Data 2). We cross-referenced the list of acute egg-laying modulators to the list of 247 wactive molecules that are lethal in six-day viability assays[9]. We found a 1.8-fold enrichment of lethality among the stimulators ($p<0.001$) (Fig. 1f), suggesting that excessive neuromuscular modulation may lead to death. A 1.4-fold enrichment of lethality among the inhibitors was also found ($p<0.05$), which is not unexpected given that egg-laying inhibition is certain to be a consequence of molecules that kill rapidly. A Tanimoto structural similarity cut-off of 0.55 reveals that the 58 egg-laying modulators can parsed into 37 distinct core scaffolds, consisting of 11 clusters and 26 singletons[11] (Fig. 1g). 10 of the 11 clusters

142 are composed of molecules with the identical egg-laying phenotype, which validates the
143 phenotypic assignments.

144 We reasoned that molecules that disrupt multiple motor circuits are more likely to
145 disrupt the parasitic nematode lifecycle. We therefore surveyed the 58 egg-laying
146 modulators for those that disrupt the normal sinusoidal locomotion of wild type *C.*
147 *elegans*. 26 of the 58 egg-laying modulators (45%) induce at least one locomotory
148 phenotype such as convulsions, coiling, and paralysis (Supplementary Table 1; Fig. 1g;
149 Supplementary Movies 1-6; Supplementary Data 3). The molecules that induce slow
150 movement and paralysis are dominated (88%) by molecules that inhibit egg-laying
151 (Supplementary Table 1). This is consistent with the idea that the egg-laying inhibitor
152 class may be enriched with acutely lethal molecules. By contrast, the molecules that
153 induce convulsions, shaking, and coiling are dominated (80%) by those that enhance
154 egg-laying (Supplementary Table 1).

155

156 **Nementin-1 is Effective Against Multiple Parasitic Nematodes**

157 To prioritize the 26 neuromodulatory molecules, we eliminated those from consideration
158 that: i) are cytotoxic to human HEK293 cells; ii) induce developmental defects in
159 zebrafish; iii) have lackluster lethality against the five nematodes previously assayed; iv)
160 induce subtle locomotory phenotypes (reversal-defective), or v) were closely related to
161 the commercial nematicide fluopyram[9] (Supplementary Table 1; Supplementary Data 4).
162 This left worm-active (wact) molecules 10, 13, 15, 55, 120, 128, and 444 for further
163 consideration (Fig. 2a; Supplementary Table 1).

164 We further assessed the activity of our seven hits against seven nematode species
165 from three phylogenetic clades (Fig. 2b). Four of these seven species are parasites of
166 mammals. We also analyzed (or reanalyzed) the molecules' activity against the non-
167 target models *D. rerio* fish, HEK293 cells, and *Arabidopsis thaliana* plants (Fig. 2b;
168 Supplementary Fig. 1). We prioritized wact-55 because it arguably demonstrates the
169 best combination of broad nematode activity and selectivity. SciFinder-based literature
170 searches failed to reveal prior annotation of nematicidal activity for wact-55's alkyl
171 phenylpiperidine core scaffold[12].

172 A detailed temporal analysis showed that within 90 seconds of exposure of 60 μM
173 wact-55, *C. elegans* exhibits a hyperactive nose movement phenotype (Fig. 2c). The
174 hyperactive phenotype dissipates at the expense of spastic convulsions over the course
175 of 2 hours (Fig. 2c). By 24 hours, 100% of the adult animals incubated in wact-55 are
176 dead (non-moving and/or disintegrating; three trials, n = 18 animals per trial). For
177 reasons outlined below, we renamed the wact-55 molecule 'Nementin-1' (nematode
178 enhancer of neurotransmission-1).

179 To determine whether Nementin-1 might have a canonical mechanism-of-action
180 (MOA), we tested whether any of the *C. elegans* mutant strains that resist the effects of
181 seven popular anthelmintics also resist the lethal effects of Nementin-1. All drug-
182 resistant strains remain sensitive to Nementin-1 (Supplementary Fig. 2). Furthermore,
183 we previously showed that *C. elegans* cannot be easily mutated to resist Nementin-1's
184 lethality; 290,000 randomly mutated genomes failed to yield wact-55-resistant mutants[9].
185 These results suggest that: i) Nementin-1 does not share an MOA with canonical
186 anthelmintics, ii) Nementin-1's MOA may not be limited to a single protein target, and,

187 iii) genetic resistance to Nementin-1 may be difficult to achieve in the field. All of these
188 properties make Nementin-1 an attractive hit for further investigation.

189

190 **Nementin Convulsions are Dependent on Dense Core Vesicle Release**

191 We reasoned that mutations in the pathway targeted by Nementin-1 might phenocopy
192 the motor defects induced by the molecule and provide mechanistic insight. A survey of
193 the literature yielded eight mutants that share at least some of Nementin-1's
194 phenotypes, including *acr-2, unc-2, unc-43, unc-58, unc-93, sup-9, sup-10*, and *twk-*
195 *18*[13-21] (Supplementary Table 2). We tested the hypothesis that Nementin-1 disrupts the
196 pathway disrupted by each of these mutants. We did this by asking whether known
197 suppressors of the phenocopying mutants can suppress Nementin-1's effects (see
198 Supplementary Table 2). Of the potential suppressing mutants tested, only the *unc-43*
199 gain-of-function (GF) mutant resisted Nementin-1's convulsions (Fig. 3a). We also found
200 the converse to be true; *unc-43* reduction-of-function (RF) alleles enhance Nementin-1
201 convulsions (Fig. 3a). Remarkably, the interaction between the *unc-43* GF and
202 Nementin-1 is mutual in that Nementin-1 can rescue the paralysis of *unc-43* GF (Fig.
203 3b). This observation argues against the idea that the *unc-43* GF mutant alters
204 Nementin-1 absorption or metabolism. These results suggest that Nementin-1 may be
205 disrupting UNC-43 pathway activity.

206 UNC-43 is the *C. elegans* ortholog of CaMKII (calcium/calmodulin-dependent protein
207 kinase II) and is a key negative regulator of dense core vesicle (DCV) release in *C.*
208 *elegans* neurons[14,22] (Fig. 3c). In animals with reduced UNC-43 function, neuronal DCV

209 content is released in excess and is likely responsible for the mutant's convulsive and
210 constitutive egg-laying phenotypes[13,22]. In *unc-43* GF animals, DCVs accumulate, and
211 their limited release is likely responsible for the mutant's severely lethargic locomotion[22].

212 To examine DCV behaviour in response to Nementin-1, we exploited the
213 neuropeptide reporter (INS-22::GFP) that is packaged into DCVs in cholinergic motor
214 neurons[22]. Like *unc-43* RF mutants[22], worms treated with either Nementin-1 or a second
215 analog called Nementin-12 have reduced axonal INS-22::GFP signal ($p<0.05$) (Fig. 3d-
216 f,h; Supplementary Fig. 3c,d). Coincidentally, INS-22::GFP accumulates in the
217 pseudocoelomic fluid-scavenging cells, called coelomocytes, of Nementin-treated
218 animals ( ).

219 We reasoned that if Nementin disrupts motor behavior by agonizing DCV release,
220 then disruption of UNC-31 (calcium-dependent activator protein for secretion (CAPS)),
221 which is required for DCV release[22,23], or UNC-64 syntaxin, which is required for all
222 vesical fusions[24,25], should suppress the Nementin-1-driven locomotory defects. Indeed,
223 we find that both the weaker temperature-sensitive *unc-31(e169)*, the *unc-31(e928)* null,
224 and the canonical *unc-64(e246)* RF mutants suppress the convulsions induced by
225 Nementin-1 (Fig. 4a). Together, these data indicate that Nementin-induced DCV release
226 is responsible for convulsions.

227 Collectively, these results beg the question of whether UNC-43 is likely to be the
228 physiologically relevant target of Nementin-1. Several observations argue against this.
229 First, we note that the terminal phenotype of Nementin-1 is death, yet presumptive null
230 alleles of *unc-43* (*ce685* and *n1186*) are viable[14,22]. Second, if Nementin-1 inhibits UNC-

231 43, then Nementin-1 should not be able to enhance the convulsion phenotype of *unc-43*
232 null mutants. We find that Nementin-1 does indeed enhance presumptive *unc-43* null
233 mutants (*ce685* and *n1186*) (Fig. 3a). Third, if UNC-43 was Nementin-1's sole
234 physiological target, then *unc-43* GF mutations would have been identified in our
235 genetic suppressor screens[9], but no resistant mutants arose. Finally, the *unc-43* GF
236 mutant (and *unc-31* and *unc-64* RF mutants) remain sensitive to the lethal effects of
237 Nementin-1 (Supplementary Fig. 4). Together, these data argue against the idea that
238 UNC-43 is Nementin-1's sole physiologically relevant target. Instead, the similarities
239 between Nementin-1-induced phenotypes and the *unc-43* RF mutants suggest that the
240 compound targets a component(s) that acts with UNC-43 (Fig. 3c, purple glow).

241

242 **Nementin-1 Agonizes Cholinergic Signaling**

243 We next investigated whether cholinergic signaling is also required for Nementin-1
244 activity. We tested whether mutants of the UNC-17 vesicular acetylcholine transporter
245 (VAChT), which loads synaptic vesicles (SVs) with acetylcholine, or mutants of UNC-13,
246 which is specifically required for SV release[26,27], could suppress Nementin-1-induced
247 convulsions. Reduction-of-function mutants of UNC-17 and UNC-13 remained sensitive
248 to Nementin-1 (Fig. 4a). Incidentally, we found that Nementin-1 could partially rescue
249 the lethargic locomotion of *unc-17* RF mutants (Fig. 4b). These data show that despite
250 cholinergic signalling being dispensable for Nementin-1-induced convulsions, Nementin-
251 1 may nevertheless agonize cholinergic signalling.

252 We further investigated the idea that Nementin-1 agonizes cholinergic signalling. To
253 do so, we used the acetylcholinesterase (AChE) inhibitor aldicarb, which is a well-
254 characterized tool used to investigate perturbations of cholinergic signaling in *C.*
255 *elegans*[28]. AChE catabolizes acetylcholine at the neuromuscular junction (NMJ) and its
256 inhibition increases acetylcholine levels that in turn paralyzes the animal[29] (see Fig. 3c).
257 We found that Nementin-1 sensitizes animals to the paralytic effects of aldicarb (Fig.
258 4c), suggesting that Nementin-1 agonizes acetylcholine release at the NMJ. The
259 relationship between Nementin-1 and aldicarb is synergistic in nature, yielding a zero-
260 interaction potency (ZIP) δ-score of 31.1 with a δ-score of 64.8 over the most
261 synergistic set of concentrations (ZIP scores >10 are considered synergistic[30,31] (Fig.
262 4d,e)). Nementin-1 also enhances the paralysis induced by dichlorvos and trichlorfon,
263 which are two other AChE inhibitors that are also commercial anthelmintics, by 12.7 and
264 18.7-fold, respectively (Fig. 4c). By contrast, Nementin-1 fails to enhance the effects of
265 an AChE-inhibitor on the fruit fly *D. melanogaster* (Fig. 4c). These data provide
266 additional support for the idea that Nementin-1 agonizes cholinergic signalling and does
267 so in a nematode-selective manner.
268

269 **Cholinergic Agonism is a Secondary Effect of DCV Release**

270 Neuropeptides released from DCVs are known to interact with G-protein coupled-
271 receptors (GPCRs) that signal through G-proteins[32]. In *C. elegans* neurons, the $G\alpha_q$
272 protein EGL-30 stimulates DAG production via the EGL-8 and PLC-3 phospholipases[33]
273 (see Fig. 3c). By contrast, the $G\alpha_o$ protein GOA-1 reduces DAG abundance by
274 triggering its phosphorylation by diacylglycerol kinase DGK-1[34]. Neuronal DAG primes

275 both DCV a. nd SV release via interaction with PKC-1(PKC-ε/η)[35] and UNC-1 [36],
276 respectively. The bioamines and neuropeptides that are released from the DCVs can
277 have both paracrine and autocrine activity[37,38]. This circuitry raised the possibility that
278 Nementin-1-induced DCV release may initiate a positive feedback loop that amplifies
279 DCV release and agonizes SV release as a secondary consequence (see green and
280 yellow pathways in Fig. 3c).

281 We tested this hypothesis in three ways. First, we examined the impact of mutant
282 components of the 'green' circuit depicted in Fig. 3c. We found that *egl-30* RF, which
283 reduces DAG production, suppresses Nementin-1 convulsions (Fig. 4a). By contrast,
284 *goa-1* RF and *dgk-1* loss-of-function (LF) mutants, which lack regulation of membrane
285 DAG, enhance Nementin-1 convulsions. We also found that disruption of PKC-1
286 reduces Nementin-1 convulsions (Fig. 4a). Second, we asked whether the cell
287 permeable DAG mimetic phorbol myristate acetate (PMA) enhances Nementin-1
288 convulsions and found that it did (Fig. 4a). Consistent with the lack of convulsions in
289 *goa-1* RF mutants (not treated with Nementin; Figure 4a), PMA treatment (without
290 Nementin) does not induce convulsions in wild type animals (Figure 4a). This indicates
291 that PKC-1-agonism alone cannot severely disrupt motor activity. Finally, we asked
292 whether Nementin-1's agonism of cholinergic signaling is dependent on the machinery
293 needed for DCV release. Indeed, we found that the *unc-31(e169)* RF mutation and the
294 *unc-43(n498)* GF mutation suppress Nementin-1's agonism of cholinergic signaling (Fig.
295 4c). Together, these observations are consistent with a model whereby Nementin-1
296 agonizes DCV release whose contents may stimulate autocrine/paracrine G-protein
297 signaling, which in turn agonizes both more DCV release and cholinergic signaling.

298 **Nementin Disrupts Parasitic Nematodes of Plants and Animals**

299 We carried out a small structure-activity relationship (SAR) analysis of Nementin
300 analogs assayed against free-living nematodes, parasitic nematodes, and non-target
301 models (Supplementary Table 3; Supplementary Data 4). We purchased 18
302 commercially available analogs of Nementin-1 and found that Nementin-12 was the
303 most potent inducer of acute motor phenotypes (Supplementary Table 3). We expanded
304 the SAR by synthesizing 22 analogs of Nementin-12 (see Materials and Methods).
305 Nementins 1, 12-5, 13 and 14 demonstrated broad anthelmintic activity without inducing
306 obvious phenotype against non-target organisms. In addition, Nementin analogs were
307 identified with improved activity in each parasitic nematode model tested. These data
308 suggest that the Nementin scaffold may be further refined to improve selectivity and
309 potency.

310 Given that Nementin-1 synergistically enhances the activity of AChE inhibitors in *C.*
311 *elegans*, we tested whether it might act similarly against parasitic nematodes. We tested
312 Nementin-1's interaction with the organophosphate dichlorvos against *Strongyloides*
313 *ratti*, a nematode parasite of mammals. Dichlorvos is approved for anthelmintic use in
314 mammals[39,40]. Indeed, we find that Nementin-1 synergistically enhances dichlorvos'
315 activity against *S. ratti* (Fig. 4f,g). Nementin-1 was also found to synergistically paralyze
316 the plant-parasitic nematode *Meloidogyne hapla* with the carbamate AChE inhibitor
317 oxamyl, which is approved for field use[39,40] (Fig. 4h,i). The Nementins may therefore
318 have added benefit against parasitic nematodes when used in combination with
319 pesticides that are AChE inhibitors.

320 **Discussion**

321 Here, we have developed a *C. elegans*-based pipeline to identify novel anthelmintic
322 small molecules with phylum-selectivity. The pipeline was designed to yield broad-
323 acting anthelmintics that acutely affect nematode behaviour. The strategy yielded four
324 small molecules that exhibit phylum-selectivity within the limited range of organisms
325 tested. We focused on one of these molecules, called Nementin-1, which induces *C.*
326 *elegans* hyperactivity within seconds of exposure, followed by spastic convulsions
327 minutes later. Several Nementin analogs affect parasitic nematodes from distinct clades
328 at concentrations that fail to elicit obvious phenotypes from non-target organisms.
329 Despite significant effort, we were not able to generate *C. elegans* mutants that resist
330 the lethal effects of Nementin, suggesting that resistance in the wild may be difficult to
331 achieve.

332 Our data support a model whereby Nementin exposure initiates DCV release, which
333 in turn agonizes SV release that promotes cholinergic signaling. Nementin-induced
334 convulsions depend on DCV release but not cholinergic signaling, indicating that
335 agonized DCV release is key to Nementin's ability to disrupt motor behavior. How
336 Nementin agonizes DCV release remains unclear. Despite the phenotypic similarities
337 between Nementin exposure and *unc-43* RF mutants, our data suggests that Nementin
338 is unlikely to inhibit UNC-43 in any canonical fashion. However, because of CaMKII's
339 complexity and its many isoforms[14,41], we cannot rule out the possibility that Nementin
340 interacts with CaMKII in a complex and perhaps tissue-specific manner.

341

342 Convulsions in *C. elegans* and other animals are thought to manifest from an
343 imbalance of excitatory and inhibitory neurotransmitter signals, termed E/I imbalance [42].
344 Gain-of-function mutations in motor neuron cation channels like the ACR-2
345 acetylcholine receptor or the UNC-2 voltage-gated calcium channel simultaneously
346 increase excitatory cholinergic signals and decrease inhibitory GABAergic signals that
347 are relayed to muscles. This in turn generates an excitation-dominated E/I imbalance
348 that culminate in convulsions [18,19,38]. Several parallels exist between Nementin-treated
349 wild-type worms and either the *unc-2* and/or *acr-2* gain-of-function mutants including
350 hyperactive locomotion, convulsions, a phenotypic dependence on UNC-31 CAPS, and
351 aldicarb hypersensitivity [18,19,38]. Nementin-induced convulsions are independent of
352 ACR-2, UNC-2, and robust cholinergic signaling. We therefore deduce that the likely E/I
353 imbalance induced by Nementin must originate from signals that are released from
354 neuronal dense core vesicles.

355 Nementin has several features that make it an attractive anthelmintic for further
356 development. First, *in vitro* tests demonstrate that it exhibits potentially broad-spectrum
357 activity while maintaining phylogenetic selectivity. Such a feature is key to the
358 development of an environmentally safe anthelmintic. Second, Nementin analogs
359 exhibit an *in vitro* potency that is comparable to several commercial anthelmintics. Third,
360 the Nementin synthesis route is relatively simple and uses inexpensive starting
361 materials ('Chemistry' in Supplementary Materials)[43]. Finally, Nementin has the potential
362 to reduce the amount of non-selective pesticides that are notoriously released into the
363 environment.

364 Despite the toxicity concerns over indiscriminate activity, many AChE inhibitors such
365 as trichlorfon, dichlorvos, coumaphos, aldicarb, fosthiazate, chlorpyrifos and oxamyl
366 remain approved for nematicidal field use as of 2021 because of limited control
367 options[39] (see the FDA Approved Animal Drug Products at https://tinyurl.com/by83ks98,
368 the US Environmental Protection Agency at https://tinyurl.com/3n3kf34n; and the US
369 Geological Survey pesticide study at https://tinyurl.com/bp5h5em4). Because of
370 Nementin's selectivity and synergistic effects, using it in combination with AChE
371 inhibitors has the potential to maintain nematode-selectivity whilst simultaneously
372 reducing the amount of non-selective pesticide applied to the environment.
373
374 **Materials and Methods**
375
376 *Materials and Methods are provided in the Supplementary Information for this initial
377 submission.

References

1 Rashid, M. *et al.* A systematic review on modelling approaches for economic losses studies caused by parasites and their associated diseases in cattle. *Parasitology* **146**, 129-141, doi:10.1017/S0031182018001282 (2019).

2 Geerts, S. & Gryseels, B. Drug resistance in human helminths: current situation and lessons from livestock. *Clin Microbiol Rev* **13**, 207-222, doi:10.1128/CMR.13.2.207 (2000).

3 Chitwood, D. J. Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture-Agricultural Research Service. *Pest Manag Sci* **59**, 748-753, doi:10.1002/ps.684 (2003).

4 Affairs, U. N. D. o. E. a. S. Consolidated list of products whose consumption and / or sale have been banned, withdrawn, severely restricted or not approved by Governments. (New York, 2009).

5 Godfray, H. C. & Garnett, T. Food security and sustainable intensification. *Philos Trans R Soc Lond B Biol Sci* **369**, 20120273, doi:10.1098/rstb.2012.0273 (2014).

6 Cole, M. B., Augustin, M. A., Robertson, M. J. & Manners, J. M. The science of food security. *NPJ Sci Food* **2**, 14, doi:10.1038/s41538-018-0021-9 (2018).

7 Geary, T. G., Klein, R. D., Vanover, L., Bowman, J. W. & Thompson, D. P. The nervous systems of helminths as targets for drugs. *J Parasitol* **78**, 215-230 (1992).

8 Holden-Dye, L. & Walker, R. J. Anthelmintic drugs and nematicides: studies in Caenorhabditis elegans. *WormBook*, 1-29, doi:10.1895/wormbook.1.143.2 (2014).

401 9 Burns, A. R. *et al.* Caenorhabditis elegans is a useful model for anthelmintic
402 discovery. *Nat Commun* **6**, 7485, doi:10.1038/ncomms8485 (2015).
403 10 Schafer, W. F. Genetics of egg-laying in worms. *Annu Rev Genet* **40**, 487-509,
404 doi:10.1146/annurev.genet.40.110405.090527 (2006).
405 11 Bajusz, D., Racz, A. & Heberger, K. Why is Tanimoto index an appropriate
406 choice for fingerprint-based similarity calculations? *J Cheminform* **7**, 20,
407 doi:10.1186/s13321-015-0069-3 (2015).
408 12 Somerville, A. N. SciFinder Scholar. *Journal of Chemical Education* **75**, 959-960,
409 doi:doi.org/10.1021/ed075p959 (1998).
410 13 Brenner, S. The genetics of Caenorhabditis elegans. *Genetics* **77**, 71-94 (1974).
411 14 Reiner, D. J., Newton, E. M., Tian, H. & Thomas, J. H. Diverse behavioural
412 defects caused by mutations in Caenorhabditis elegans unc-43 CaM kinase II.
413 *Nature* **402**, 199-203, doi:10.1038/46072 (1999).
414 15 Wei, A., Jegla, T. & Salkoff, L. Eight potassium channel families revealed by the
415 C. elegans genome project. *Neuropharmacology* **35**, 805-829, doi:10.1016/0028-
416 3908(96)00126-8 (1996).
417 16 de la Cruz, I. P., Levin, J. Z., Cummins, C., Anderson, P. & Horvitz, H. R. sup-9,
418 sup-10, and unc-93 may encode components of a two-pore K+ channel that
419 coordinates muscle contraction in Caenorhabditis elegans. *J Neurosci* **23**, 9133-
420 9145 (2003).
421 17 Greenwald, I. S. & Horvitz, H. R. unc-93(e1500): A behavioral mutant of
422 Caenorhabditis elegans that defines a gene with a wild-type null phenotype.
423 *Genetics* **96**, 147-164 (1980).

424 18 Huang, Y. C. *et al.* Gain-of-function mutations in the UNC-2/CaV2alpha channel
425 lead to excitation-dominant synaptic transmission in Caenorhabditis elegans.
426 *Elife* **8**, doi:10.7554/eLife.45905 (2019).
427 19 Jospin, M. *et al.* A neuronal acetylcholine receptor regulates the balance of
428 muscle excitation and inhibition in Caenorhabditis elegans. *PLoS Biol* **7**,
429 e1000265, doi:10.1371/journal.pbio.1000265 (2009).
430 20 Salkoff, L. *et al.* Potassium channels in C. elegans. *WormBook*, 1-15,
431 doi:10.1895/wormbook.1.42.1 (2005).
432 21 Kasap, M. & Dwyer, D. S. Clozapine, nimodipine and endosulfan differentially
433 suppress behavioral defects caused by gain-of-function mutations in a two-pore
434 domain K(+) channel (UNC-58). *Neurosci Res*, doi:10.1016/j.neures.2020.07.006
435 (2020).
436 22 Hoover, C. M. *et al.* A novel CaM kinase II pathway controls the location of
437 neuropeptide release from Caenorhabditis elegans motor neurons. *Genetics* **196**,
438 745-765, doi:10.1534/genetics.113.158568 (2014).
439 23 Speese, S. *et al.* UNC-31 (CAPS) is required for dense-core vesicle but not
440 synaptic vesicle exocytosis in Caenorhabditis elegans. *J Neurosci* **27**, 6150-
441 6162, doi:10.1523/JNEUROSCI.1466-07.2007 (2007).
442 24 Hammarlund, M., Watanabe, S., Schuske, K. & Jorgensen, E. M. CAPS and
443 syntaxin dock dense core vesicles to the plasma membrane in neurons. *J Cell*
444 *Biol* **180**, 483-491, doi:10.1083/jcb.200708018 (2008).

445 25 Laurent, P. *et al.* Genetic dissection of neuropeptide cell biology at high and low
446 activity in a defined sensory neuron. *Proc Natl Acad Sci U S A* **115**, E6890-
447 E6899, doi:10.1073/pnas.1714610115 (2018).
448 26 Lackner, M. R., Nurrish, S. J. & Kaplan, J. M. Facilitation of synaptic transmission
449 by EGL-30 Gqalpha and EGL-8 PLCbeta: DAG binding to UNC-13 is required to
450 stimulate acetylcholine release. *Neuron* **24**, 335-346, doi:10.1016/s0896-
451 6273(00)80848-x (1999).
452 27 Alfonso, A., Grundahl, K., Duerr, J. S., Han, H. P. & Rand, J. B. The
453 Caenorhabditis elegans unc-17 gene: a putative vesicular acetylcholine
454 transporter. *Science* **261**, 617-619, doi:10.1126/science.8342028 (1993).
455 28 Nguyen, M., Alfonso, A., Johnson, C. D. & Rand, J. B. Caenorhabditis elegans
456 mutants resistant to inhibitors of acetylcholinesterase. *Genetics* **140**, 527-535
457 (1995).
458 29 Cambon, C., Declume, C. & Derache, R. Effect of the insecticidal carbamate
459 derivatives (carbofuran, pirimicarb, aldicarb) on the activity of
460 acetylcholinesterase in tissues from pregnant rats and fetuses. *Toxicol Appl*
461 *Pharmacol* **49**, 203-208, doi:10.1016/0041-008x(79)90242-4 (1979).
462 30 Ianevski, A., Giri, A. K. & Aittokallio, T. SynergyFinder 2.0: visual analytics of
463 multi-drug combination synergies. *Nucleic Acids Res* **48**, W488-W493,
464 doi:10.1093/nar/gkaa216 (2020).
465 31 Yadav, G. & Ganguly, S. Structure activity relationship (SAR) study of
466 benzimidazole scaffold for different biological activities: A mini-review. *Eur J Med*
467 *Chem* **97**, 419-443, doi:10.1016/j.ejmech.2014.11.053 (2015).

468 32 Koelle, M. R. Neurotransmitter signaling through heterotrimeric G proteins:
469 insights from studies in C. elegans. *WormBook* **2018**, 1-52,
470 doi:10.1895/wormbook.1.75.2 (2018).
471 33 Robatzek, M. & Thomas, J. H. Calcium/calmodulin-dependent protein kinase II
472 regulates Caenorhabditis elegans locomotion in concert with a G(o)/G(q)
473 signaling network. *Genetics* **156**, 1069-1082 (2000).
474 34 Miller, K. G., Emerson, M. D. & Rand, J. B. Goalpha and diacylglycerol kinase
475 negatively regulate the Gqalpha pathway in C. elegans. *Neuron* **24**, 323-333,
476 doi:10.1016/s0896-6273(00)80847-8 (1999).
477 35 Sieburth, D., Madison, J. M. & Kaplan, J. M. PKC-1 regulates secretion of
478 neuropeptides. *Nat Neurosci* **10**, 49-57, doi:10.1038/nn1810 (2007).
479 36 Richmond, J. E., Davis, W. S. & Jorgensen, E. M. UNC-13 is required for
480 synaptic vesicle fusion in C. elegans. *Nat Neurosci* **2**, 959-964,
481 doi:10.1038/14755 (1999).
482 37 Steuer Costa, W., Yu, S. C., Liewald, J. F. & Gottschalk, A. Fast cAMP
483 Modulation of Neurotransmission via Neuropeptide Signals and Vesicle Loading.
484 *Curr Biol* **27**, 495-507, doi:10.1016/j.cub.2016.12.055 (2017).
485 38 Stawicki, T. M., Takayanagi-Kiya, S., Zhou, K. & Jin, Y. Neuropeptides function in
486 a homeostatic manner to modulate excitation-inhibition imbalance in C. elegans.
487 *PLoS Genet* **9**, e1003472, doi:10.1371/journal.pgen.1003472 (2013).
488 39 Nixon, S. A. *et al.* Where are all the anthelmintics? Challenges and opportunities
489 on the path to new anthelmintics. *Int J Parasitol Drugs Drug Resist* **14**, 8-16,
490 doi:10.1016/j.ijpddr.2020.07.001 (2020).

491 40 Abdel-Sattar, M., Haikal, A. M. & Hammad, S. E. Meloidogyne incognita
492 population control and nutritional status and productivity of Thompson seedless
493 grapevines managed with different treatments. *PLoS One* **15**, e0239993,
494 doi:10.1371/journal.pone.0239993 (2020).
495 41 Myers, J. B. *et al.* The CaMKII holoenzyme structure in activation-competent
496 conformations. *Nat Commun* **8**, 15742, doi:10.1038/ncomms15742 (2017).
497 42 Shao, L. R., Habela, C. W. & Stafstrom, C. E. Pediatric Epilepsy Mechanisms:
498 Expanding the Paradigm of Excitation/Inhibition Imbalance. *Children (Basel)* **6**,
499 doi:10.3390/children6020023 (2019).
500 43 Ertl, P. & Schuffenhauer, A. Estimation of synthetic accessibility score of drug-
501 like molecules based on molecular complexity and fragment contributions. *J*
502 *Cheminform* **1**, 8, doi:10.1186/1758-2946-1-8 (2009).
503

504 **Acknowledgements:**

505 We are grateful for the strains given to us by the *Caenorhabditis* Genetics Center
506 (University of Minnesota), Marie-Anne Félix (IBENS, Paris), Ken Miller (Oklahoma
507 Medical Research Foundation), and Mei Zhen and Wesley Hung (Lunenfeld-
508 Tanenbaum Research Institute, Toronto). Many thanks to Dr. Doug Colwell and Dawn
509 Gray at the Lethbridge and Agri-Food Canada Research Centre for supplying faeces
510 from infected calves for *Cooperia oncophora* egg collection.

511 **Ethics Statement**

512 DK: Experiments on *D. immitis* and *N. brasiliensis* were performed in the laboratories of
513 Bayer Animal Health GmbH (Monheim, Germany) in accordance with the local Animal
514 Care and Use Committee and governmental authorities. JK: The generation of *N.*
515 *americanis*, *T. muris*, S. ratti, and *H. polygyrus* and in vitro studies were carried out at
516 the Swiss Tropical Institute (Basel, Switzerland), in accordance with both cantonal
517 (license no. 2070) and Swiss national regulations on animal experimentation. JD: All
518 zebrafish experiments were performed in compliance with any relevant ethical
519 regulations, specifically following an institutionally reviewed and approved animal use
520 protocol as well as the policies and guidelines of the Canadian Council on Animal Care
521 and Animals for Research Act of Ontario.

522 **Funding:**

523 Research in the I.Z. lab is partially supported by the United States Department of
524 Agriculture, Agricultural Research Service. P.J.R. is supported by CIHR project grants
525 (313296 and 173448). P.J.R. is a Canada Research Chair (Tier 1) in Chemical
526 Genetics.

527 **Author Contributions**

528 Conceptualization: PJR, SH

529 Methodology: SH, JJK, K-L C, AA, MK, CH, JP, JRV, MG, JS, VW, BMP, EMR, ASV,

530 Investigation: SH, JJK, AB, MK, CH, JP, C'DA, Y-H K, JRV, MG, JS, VW, BMP, EMR,
531 ADS

532 Visualization: PJR, SH

533 Funding acquisition: PJR

534 Project administration: PJR

535 Supervision: PJR, JG, IS, SRC, JJD, CMY, JK, IZ, ML

536 Writing – original draft: PJR, SH, JJK, K-LC, AA, SRC, DK, JK

537 Writing – review & editing: PJR, SH, AB, JJK, SC, DK, JK, IZ, ML

538 **Competing interests:**

539 Mention of trade names or commercial products in this publication is solely for the
540 purpose of providing specific information and does not imply recommendation or
541 endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity
542 provider and employer. S.H., J.K., A.R.B., K-L.C., J.P., M.L. and P.J.R. have patents
543 pending related to Nementin.

544 **Data and materials availability:**

545 Original data for all analyses presented are included in Supplementary Data 1-S4.
546 Original images for analyses can be made available per request through the
547 corresponding author P.J.R.

548 **Supplementary Materials**

549 Materials and Methods

550 Supplementary Figures 1-4

551 Supplementary Table 1-3

552 Supplementary Movies 1 to 6 (description)

553 Supplementary Data 1 to S4 (description)

554

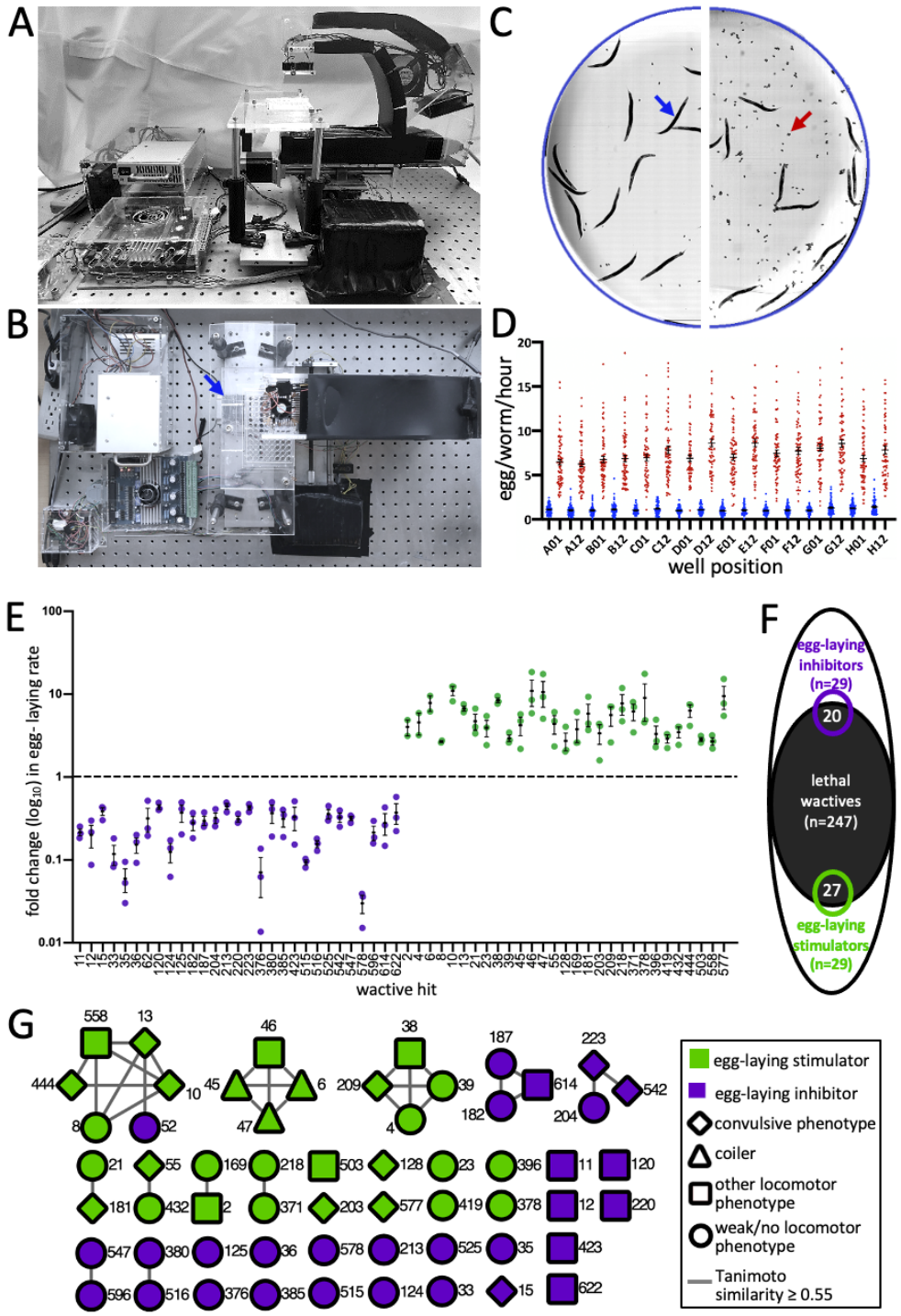
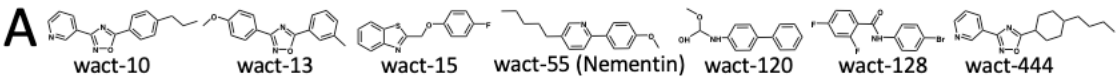


Fig. 1 The Identification of 58 Small Molecules that Modulate the *C. elegans* Nervous System.

558 (**a** and **b**) Two perspectives of the '2020 Imager' showing one 96-well plate in the
559 imager holder (arrow). (**c**) Representative half-well images of vehicle controls in the
560 basal (M9-only) background (left) and the 'stimulatory cocktail' of 12.3 mM serotonin
561 creatine sulfate and 7.7 mM nicotine (right) after 1 hour. A blue arrow points to an adult
562 hermaphrodite and a red arrow points to a pair of embryos. (**d**) The distribution of
563 counts of eggs/worm/hour for the basal background (blue) and the stimulatory
564 background (red) from the indicated well-coordinates over 22 experimental replicates
565 (96-well plates). The mean and SEM is shown. (**e**) A screen of the worm active
566 (wactive) library yields 29 inhibitors (purple) and 29 stimulators (green) of the *C.*
567 *elegans* egg-laying rate. Mean Egl rate and SEM is shown. (**f**) Venn diagram showing
568 the overlap of the wactive molecules that are lethal (at 60 μM) with those that modulate
569 egg-laying among the 486 molecules assayed in both assays. (**g**) A structure similarity
570 network of the 58 egg-laying modulators. Each node represents a numbered wactive
571 molecule. Connecting lines indicate shared structural similarity. Additional information is
572 provided in Supplementary Table 1.
573



B

	free-living nematodes						parasites				non-target models			
wactive	*C. elegans* lethality	*C. elegans* motor phene	*P. pacificus* lethality	*P. pacificus* motor phene	*R. diutinus* lethality	*R. diutinus* motor phene	*H. contortus* egg hatch	*C. oncophora* egg hatch	*D. immitis* motility	*N. strongylus* secretion	HEK293 viability	*D. rerio* development	*D. rerio* motor	*A. thaliana* greening
wact-10	5.0	14.5	6.1	>60	<1.9	9.8	2.4	3.3	5.4	5.4	30.9	9.8	17	>45
wact-13	4.5	19.0	8.0	>60	2.6	39.6			10.3	6.9	>60	>45	>45	>45
wact-15	5.4	18.8	12.8	16.8	32.8	15.6			11.2	10.1	>60	>45	>45	>45
wact-55	8.7	26.0	9.8	9.0	8.7	2.8	1.5	4.0	5.4	5.4	>60	>45	>45	>45
wact-120	6.6	32.0	6.8	13.6	6.6	3.8	1.8	1.7	48.7	27.0	>60	4.6	10.7	>45
wact-128	1.9	12.8	3.9	53.8	1.9	31.3	11.2	13.2	50.0	13.4	>60	>45	>45	5
wact-444	13.6	23.2	8.4	27.8	3.4	7.8	3.8	7.6	9.1	27.0	>60	>45	>45	>45

0 compound EC50 (μM) 60 active in non-target model

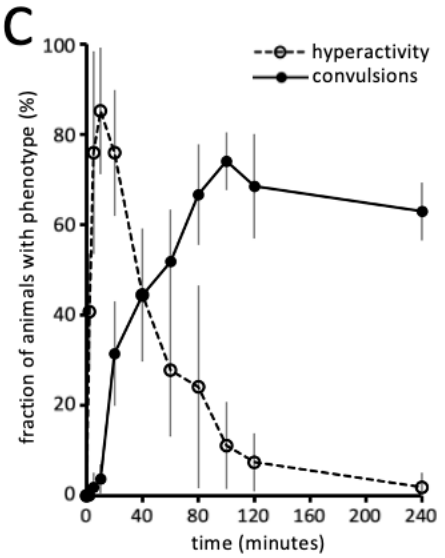
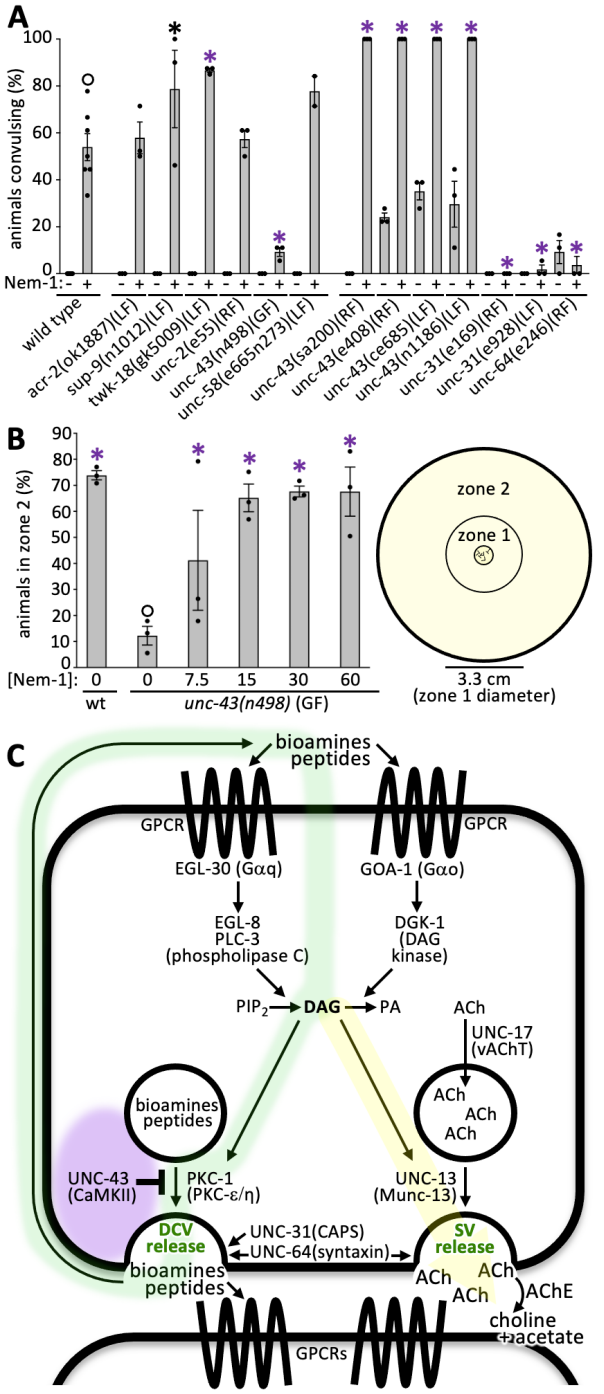


Fig. 2 The motor-centric pipeline yields convulsing-inducing Nementin-1. (**a**) Structures of the 7 prioritized neuromodulators. (**b**) Bioactivity of the seven prioritized molecules. Data are EC50s except for the *A. thaliana* greening assay, which is the lowest concentration that yields a discernible difference from control. (**c**) A time-course analysis of 60 μM Nementin-1-induced locomotory phenotypes. Data are the mean of biological triplicate measurement of 18 animals per each time point. The standard deviation is shown.


A
animals convulsing (%)
Nem-1:
wild type
acr-2(ok1887)(LF)
sup-9(n1012)(LF)
twk-18(gk5009)(LF)
unc-2(e55)(RF)
unc-43(n498)(GF)
unc-58(e665n273)(LF)
unc-43(sa200)(RF)
unc-43(e408)(RF)
unc-43(ce685)(LF)
unc-43(n1186)(LF)
unc-31(e169)(RF)
unc-31(e928)(LF)
unc-64(e246)(RF)
B
animals in zone 2 (%)
[Nem-1]: 0 0 7.5 15 30 60
wt
unc-43(n498) (GF)
zone 2
zone 1
3.3 cm
(zone 1 diameter)
C
bioamines
peptides
GPCR
GPCR
EGL-30 (Gαq)
GOA-1 (Gαo)
EGL-8
PLC-3
(phospholipase C)
DGK-1
(DAG
kinase)
PIP2
DAG
PA
ACh
UNC-17
(vAChT)
bioamines
peptides
ACh
ACh
ACh
UNC-43
(CaMKII)
PKC-1
(PKC-ε/η)
UNC-13
(Munc-13)
DCV
release
UNC-31(CAPS)
UNC-64(syntaxin)
SV
release
bioamines
peptides
ACh
ACh
ACh
AChE
choline
+acetate
GPCRs
D
E
control
dc
co
cb
vc
20 μM
F
Nem-1
dc
co
cb
vc
20 μM
G
coelomocytes
GFP/RFP fluorescence ratio
control
Nem-1
Nem-12
H
dorsal axons
mean GFP fluorescence (AU)
control
Nem-1
Nem-12

583

584

585 **Fig. 3 Nementin agonizes dense core vesicle release.** (**a**) Convulsions induced by
586 Nementin-1 (Nem-1) (60 μM) in the indicated genetic background after 80 minutes. The
587 mean of three biological replicates with 18 animals per trial is shown. (**b**) The mean
588 percentage of animals of the indicated genotype (three trials with ~150 animals per trial)
589 that locomote to zone 2 over 180 minutes after being placed in the centre of a plate (see
590 schematic on right). For A and B, black and purple asterisks represent $p<0.05$ and
591 $p<0.001$, Chi-square test of independence with Bonferroni correction relative to control
592 (open circle); the standard error of the mean (SEM) is shown. (**c**) A schematic of
593 neuronal signaling pathways relevant to Nementin activity. Purple glow indicates area of
594 possible Nementin action; the green glow indicates how DCV neurotransmitter cargo
595 may activate $G\alpha_q$ and $G\alpha_o$ signaling pathways; the yellow glow indicates how the
596 secondary messenger diacylglycerol (DAG) can also promote synaptic vesicle release.
597 (**d**) Schematic of adult (strain KG4247) expressing INS-22::GFP (green dots; packaged
598 into DCVs) and secreted mCherry (fuchsia) in cholinergic motor neurons (cell bodies
599 are oval). mCherry is constitutively secreted and taken up by the coelomocytes (three
600 pairs of pink objects in the animal). Box indicates the area shown in E and F. Anterior is
601 the left and dorsal is up. (**e** and **f**) Images of the midbody region of control and 60 µM
602 Nementin-1-treated KG4247 animals after 4 hours. dc, dorsal cord; vc, ventral cord; co,
603 coelomocytes; cb, cell bodies. (**g**) Quantification of the midbody coelomocytes (ratio of
604 measured GFP/RFP) and dorsal cord fluorescence (relative to background tissues) of
605 the animal. AU, arbitrary units., 1-way ANOVA with Dunnett correction for multiple

606 comparisons; ** $p<0.01$; *** $p<0.001$; SEM is shown. Other regions of the animal are

607 quantified in Supplementary Fig. 3.

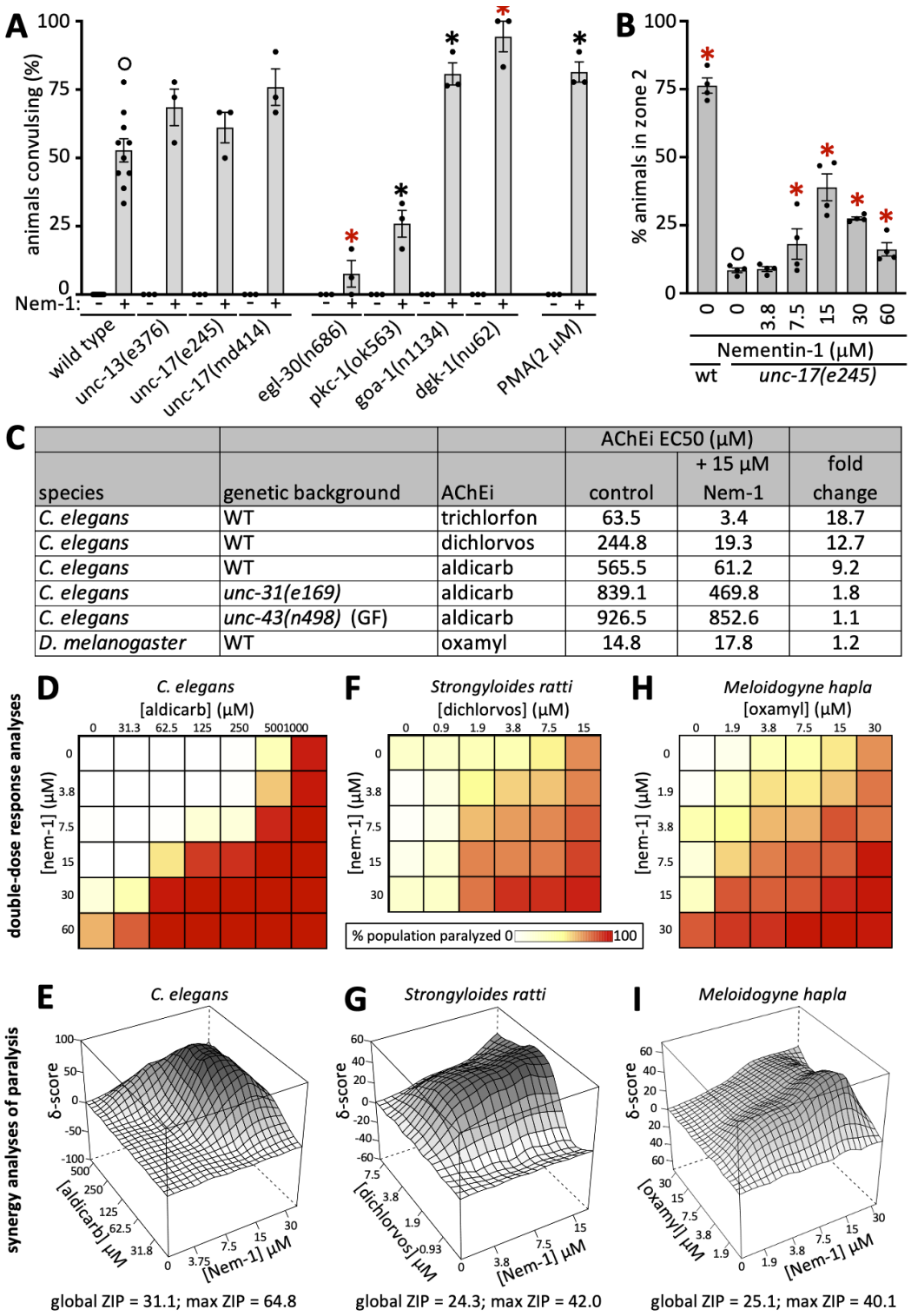


Fig. 4 Nementin enhances AChE inhibitor activity via agonism of DCV release. (**a**) Convulsions induced after 80 minutes of exposure to Nementin-1 (Nem-1) (60 µM) or vehicle control in the background of the indicated mutant gene, or in the background of

612 24 hours of preincubation with the DAG memetic PMA. (**b**) The ability of wild type (WT)
613 or *unc-17* mutant animals to productively locomote to zone 2 over 180 minutes after
614 being placed in the centre of a plate (see schematic in Fig. 3b) in the indicated
615 Nementin concentration. For both A and B, black asterisk $p<0.01$, red asterisk $p<0.001$,
616 Bonferroni corrected Chi-square test of independence relative to control (open circle);
617 SEM is shown. (**c**) Acetylcholinesterase inhibitor (AChEi) EC50s for different species
618 and genetic backgrounds. Oxamyl is the only AChEi tested that exhibited activity
619 against flies. Data are the mean of 3 biological replicates scoring 18 animals per
620 condition. (**d-f**) Double dose-response analyses of Nementin-1 (y-axis) vs the indicated
621 AChEi (x-axis) in the indicated nematode species. The scale for the percentage of the
622 population paralyzed applies to d-f. (**g-i**) The corresponding zero interaction potency
623 (ZIP) synergy score heatmaps for the double dose-response analyses presented in d-f.
624 Max ZIP-score is defined as the maximal ZIP-score obtained from a combination of
625 three concentrations of each compound in the double dose response. Shading
626 highlights the topology of the plot and differs between g-i. N=3 trials for *elegans* with
627 n=18 within each trial, N=6 trials for *ratti* with $n \geq 30$ within each trial, $N \geq 3$ trials for *hapla*
628 with $n \geq 15$ within each trial.