The ERK MAPK pathway modulates Gq-dependent locomotion in *Caenorhabditis elegans*

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Abstract

The heterotrimeric G protein Gq regulates neuronal activity through distinct downstream effector pathways. In addition to the canonical Gq effector phospholipase Cβ, the small GTPase Rho was recently identified as a conserved effector of Gq. To identify additional molecules important for Gq signaling in neurons, we performed a forward genetic screen in the nematode *Caenorhabditis elegans* for suppressors of the hyperactivity and exaggerated waveform of an activated Gq mutant. We isolated two mutations affecting the MAP kinase scaffold protein KSR-1 and found that KSR-1 modulates locomotion downstream of or in parallel to the Gq-Rho pathway. Through epistasis experiments, we found that the core ERK MAPK cascade is required for Gq-Rho regulation of locomotion, but that the canonical ERK activator LET-60/Ras may not be required. Through neuron-specific rescue experiments, we found that the ERK pathway functions in acetylcholine neurons to control Gq-dependent locomotion. Additionally, expression of activated LIN-45/Raf in acetylcholine neurons is sufficient to cause an exaggerated waveform phenotype and hypersensitivity to the acetylcholinesterase inhibitor aldicarb, similar to an activated Gq mutant. Taken together, our results suggest that the ERK MAPK pathway modulates the output of Gq-Rho signaling to control locomotion behavior in *C. elegans.*

Introduction

The heterotrimeric G protein Gq is a conserved regulator of neurotransmission in metazoans. Gq is highly expressed in neurons in mammals and in the nematode *C. elegans* (Wilkie *et al.* 1991; Lackner *et al.* 1999). In its canonical signaling pathway, Gq activates phospholipase Cβ (PLCβ) to cleave the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG) (Rhee 2001). An increased DAG concentration at the synapse helps trigger synaptic vesicle release (Miller *et al.* 1999; Lackner *et al.* 1999).

In addition to activating PLCβ, Gq directly binds and activates the Rho guanine nucleotide exchange factor (GEF) Trio, which in turn activates the small GTPase Rho (Lutz *et al.* 2005, 2007; Williams *et al.* 2007). In mature *C. elegans* neurons, the Rho ortholog RHO-1 regulates synaptic activity through multiple G protein-dependent mechanisms. First, RHO-1 acts downstream of the G_{12}-class G protein GPA-12 by binding to and inhibiting the diacylglycerol kinase DGK-1. Inhibition of DGK-1 allows DAG to accumulate at the synapse, thereby increasing synaptic vesicle release (McMullan *et al.* 2006; Hiley *et al.* 2006). Second, Gq-Rho signaling promotes neurotransmitter release by recruiting the sphingosine kinase SPHK-1 to presynaptic terminals (Chan *et al.* 2012). Third, Gq-Rho signaling positively regulates the NCA-1/NALCN cation channel to regulate locomotion (Topalidou *et al.* 2017a). Here we identify the extracellular signal-related kinase mitogen-activated protein kinase (ERK MAPK) pathway as a positive regulator of neuronal activity acting downstream of or in parallel to Gq and Rho in acetylcholine neurons.

ERK MAPK signaling acts extensively in animal development, cellular proliferation, and cancer signaling (Yoon and Seger 2006; Karnoub and Weinberg 2008; Sun *et al.* 2015). ERKs are highly expressed in mammalian neurons (Boulton *et al.* 1991; Ortiz *et al.* 1995) and act in both the nucleus and at the synapse to regulate synaptic activity and plasticity (Thomas and Huganir 2004; Sweatt 2004; Mao and Wang 2016b). In *C. elegans*, the ERK pathway is required for multiple developmental events including specification of the vulva (Sternberg 2005; Sundaram 2013), and also acts in several types of neurons to control behavior. ERK signaling is activated in response to odorants in the AWC sensory neuron to regulate chemotaxis to volatile odorants and in AIY interneurons to mediate odor adaptation (Hirotsu *et al.* 2000; Hirotsu and Iino 2005; Chen *et al.* 2011; Uozumi *et al.* 2012). ERK is also activated in the ASER sensory neuron to regulate chemotaxis to salt (Tomioka *et al.* 2006; Tomida *et al.* 2012). ERK signaling regulates foraging behavior by acting in the IL1, OLQ, and RMD neurons (Hamakawa *et al.* 2015). Finally, the ERK pathway has been shown to act in interneurons to regulate the nose touch response, a mechanosensory behavior (Hyde *et al.* 2011).

In the canonical ERK MAPK pathway, extracellular ligand binding activates transmembrane receptor tyrosine kinases (RTKs), and adaptor proteins recruit a GEF to activate the small GTPase Ras (LET-60 in *C. elegans*). Upon Ras activation, LIN-45/Raf translocates to the plasma membrane where it interacts with Ras and the scaffold protein KSR-1. KSR-1 facilitates the activation of LIN-45/Raf and the subsequent phosphorylation of the MAPK cascade consisting of LIN-45/Raf, MEK-2/MEK, and MPK-1/ERK (Sundaram 2013). In this study, we found that the ERK MAPK pathway

consisting of KSR-1, LIN-45/Raf, MEK-2/MEK and MPK-1/ERK modulates Gq-Rho signaling in acetylcholine neurons, but that surprisingly LET-60/Ras may not be required.

Materials and Methods

C. elegans strains

All strains were cultured using standard methods and were maintained at 20°C. Table S1 contains all the strains used in this study.

Isolation and mapping of the ksr-1(ox314) and ksr-1(yak10) mutations

The *ox314* and *yak10* mutants were isolated from an ENU mutagenesis suppressor screen of the activated Gq mutant *egl-30(tg26)* (Ailion *et al.* 2014). We mapped the *ox314* mutation by its activated Gq suppression phenotype using single nucleotide polymorphisms (SNPs) in the Hawaiian strain CB4856 as described (Davis *et al.* 2005).The *ox314* mutation was mapped to an approximately 709 kb region in the middle of the X chromosome between SNPs on cosmids F45E1 and F53A9 (SNPs F45E1[1] and pkP6158). This region included 159 predicted protein-coding genes. A complementation test of *ox314* and *yak10* in the *egl-30(tg26)* background showed these to be alleles of the same gene. Whole genome sequencing (see below) identified these as mutations in *ksr-1*, and we confirmed this by performing a complementation test with the deletion allele *ksr-1(ok786).*

Whole genome sequencing

Strains EG4198 *egl-30(tg26); ox314* and XZ1340 *egl-30(tg26); yak10* were sequenced to identify candidate mutations. DNA was purified according to the Hobert Lab protocol (http://hobertlab.org/whole-genome-sequencing/). Ion Torrent sequencing

was performed at the University of Utah DNA Sequencing Core Facility. Each data set contained roughly 18,400,000 reads of a mean read length of 160 bases, resulting in about 30X average coverage of the *C. elegans* genome. The sequencing data were processed on the Galaxy server at usegalaxy.org (Afgan *et al.* 2016). SNPs and indels were identified and annotated using the Unified Genotyper and SnpEff tools (DePristo *et al.* 2011; Cingolani *et al.* 2012). After filtering for mutations in open reading frames, we found each strain to have unique stop mutations in *ksr-1*, in the middle of the interval where we mapped *ox314*. *ox314* is a G to A transition that causes a stop codon at amino acid K463, and *yak10* is an A to T transversion that causes a stop codon at W254.

Locomotion assays

Track waveform and radial locomotion assays were performed on 10 cm nematode growth medium (NGM) plates seeded with 400 µl of OP50 *E. coli* culture and spread with sterile glass beads. Bacterial lawns were grown at 37°C for 16 hrs and the plates were stored at 4°C until needed. For track waveform measurements, five first day adult animals were placed on a plate and allowed to roam for 2-5 min. We then recorded each animal's tracks following forward locomotion. Track pictures were taken at 40X on a Nikon SMZ18 microscope with the DS-L3 camera control system. Pictures of worm tracks were processed using ImageJ. Period and 2X amplitude were measured freehand using the line tool. For each worm, we calculated the average period/amplitude ratio of five individual track bends (Figure 1C). For assays with the temperature sensitive allele *sos-1(cs41)*, all strains were grown at 20°C and shifted to

144 the non-permissive temperature of 25°C for 24 hours before being assayed. For radial
145 locomotion assays, ten to fifteen first day adult animals were picked to the center of a
146 plate and were then allowed to move freely for 40 minutes. The positions of worms were
147 marked and the distances of the worms from the starting point were measured. For all
148 waveform and radial locomotion assays, the experimenter was blind to the genotypes of
149 the strains assayed.
150
151 ***Microscopy***
152 Photographs of moving worms were taken at 60X on a Nikon SMZ18 microscope
153 with the DS-L3 camera control system. The worms were age-matched as first day
154 adults grown at 20°C.
155
156 ***Constructs and transgenes***

157 Plasmids were constructed using the three-slot multisite Gateway cloning system
158 (Invitrogen). Plasmids and primers used are found in Tables S2 and S3. The *ksr-1* and
159 *lin-45* cDNAs were amplified by PCR from worm cDNA library and cloned into [1-2]
160 Gateway entry vectors. Activating Raf mutations T626E/T629D were introduced into the
161 *lin-45* cDNA vector by two sequential site-directed mutagenesis reactions (Q5 kit, NEB)
162 with primers oBC094/095 and oBC096/097, respectively, and then confirmed by
163 sequencing. The *ksr-1* and activated *lin-45* cDNAs were cloned into expression
164 constructs under different neuronal promoters using the multisite Gateway system.
165 Proper expression of *ksr-1* and activated *lin-45* was confirmed by including an operon
166 GFP::H2B in the [2-3] slot of the expression constructs. The operon GFP template *tbb-2*

3'utr::gpd-2 operon::GFP::H2B:cye-1 3'utr (Frøkjær-Jensen *et al.* 2012) results in untagged proteins whose expression can be monitored by GFP expression.

Injections and chromosomal integrations

Worms carrying the activated *lin-45* transgenes *Punc-17::lin-45** and *Punc-17H::lin-45** as extrachromosomal arrays were generated by injecting pBC37 or pBC44 at 20 ng/µL or 10 ng/ µL respectively along with co-injection markers pCFJ104 (*Pmyo-3::mCherry*) at 5 ng/µL, pCFJ90 (*Pmyo-2::mCherry*) at 2.5 ng/µL, and the carrier DNA Litmus 38i to a final concentration of 100 ng/µL DNA (Mello *et al.* 1991). MosSCI lines were generated as described (Frøkjær-Jensen *et al.* 2012) using an injection mix containing 10-15 ng/µL targeting vector, 50 ng/µL pCFJ601 (*Peft-3::Mos1* transposase), negative selection markers pGH8 (*Prab-3::mCherry*) at 10 ng/µL, pCFJ104 (*Pmyo-3::mCherry*) at 5 ng/µL, pCFJ90 (*Pmyo-2::mCherry*) at 2.5 ng/µL, pMA122 (*Phsp16.41::peel-1*) at 10 ng/µL, and carrier DNA Litmus 38i to a final concentration of 100 ng/µL DNA.

Extrachromosomal arrays were integrated into the genome by exposure to 4000 rads of gamma irradiation. Irradiated young adult hermaphrodites were transferred to 10 cm OP50 plates (5 worms/plate) and grown to starvation. The plates were chunked and grown to starvation twice more to enrich for stably expressing lines. When nearly starved, 8 animals per plate were picked to individual plates. The progeny were then screened for 100% stable transmission, indicating integration into the genome. Integration was confirmed by mapping the transgene to a chromosome.

190 ***Aldicarb assays***

191 35 mm aldicarb assay plates were poured with NGM supplemented with 1 mM
192 aldicarb. The plates were seeded with 5 uL OP50 and dried at room temperature
193 overnight. Animals were picked onto the OP50 lawn to begin the assay (time 0) and
194 then kept at room temperature. Every 15 minutes, animals were scored for paralysis by
195 lightly touching the nose of the animal with an eyebrow hair. Animals were scored as
196 paralyzed if the worm displayed no locomotor response to three nose touches and had
197 no pharyngeal pumping. Animals that left the OP50 lawn were picked back onto the
198 food.

199

200 ***Statistical analysis***

201 P values were determined using GraphPad Prism 5. Normally distributed data
202 sets were analyzed with a one-way ANOVA and Bonferroni's *post hoc* test when group
203 size was unequal, or with Tukey's *post hoc* test when group size was equal. Data sets
204 with non-normal distribution (using the Shapiro-Wilk normality test) were analyzed with
205 a Kruskal-Wallis test and Dunn's *post hoc* test. Data sets with multiple independent
206 variables were analyzed by two-way ANOVA and Bonferroni's *post hoc* test.

207

208 ***Reagent and data availability***

209 Strains and plasmids are listed in Tables S1 and S2 and are available upon
210 request. Primers are listed in Table S3. The authors state that all data necessary for
211 confirming the conclusions presented in the article are represented fully within the
212 article and Supplemental Material.

213

Results

KSR-1 regulates locomotion downstream of Gq

In *C. elegans,* the heterotrimeric G protein Gq regulates synaptic vesicle release (Hu *et al.* 2015). Gq is a key regulator of neuromuscular activity, as loss-of-function mutants in *egl-30* are nearly paralyzed (Brundage *et al.* 1996) whereas the gain-of-function mutant *egl-30(tg26)* has hyperactive locomotion with an exaggerated loopy waveform (Doi and Iwasaki 2002; Bastiani *et al.* 2003) (Figure 1A, C, D). To identify pathways required for Gq signaling, we performed a forward genetic screen in *C. elegans* for suppressors of the activated Gq mutant *egl-30*(*tg26)*. Two suppressors identified in this screen, *ox314* and *yak10*, showed similar suppression of the loopy waveform and hyperactivity of *egl-30(tg26)* animals (Figure 1D). When crossed away from the *egl-30(tg26*) background, both mutants moved with wild-type waveform (Figure 1D), but at a slightly slower rate. We mapped the *ox314* allele near the center of the X chromosome (see Materials and Methods), and a complementation test showed that *ox314* and *yak10* are mutations in the same gene since they fail to complement in an *egl-30(tg26)* background.

We used whole genome sequencing to identify *ox314* and *yak10* as nonsense mutations in *ksr-1* (Figure 1B, see Materials and Methods). KSR-1 is a scaffold protein that facilitates the localization and interactions required for the Ras-mitogen activated protein kinase (MAPK) cascade consisting of Raf, MEK and ERK (Kornfeld *et al.* 1995b; Sundaram and Han 1995; Nguyen *et al.* 2002; Zhang *et al.* 2013). The deletion allele *ksr-1(ok786)* also suppressed the loopy waveform of the activated Gq mutant identically

to *ox314* and *yak10.* These results suggest that KSR-1 activity is required for regulation of locomotion rate and waveform by Gq.

The ERK MAPK cascade acts to promote Gq signaling

Because the loss of the MAPK scaffold *ksr-1* suppresses the activated Gq mutant *egl-30(tg26)*, we asked whether other components of the Ras-ERK pathway would also suppress. Since the core components of the Ras-ERK pathway are required for viability, we built double mutants of activated Gq with reduction-of-function mutations in genes at each step of the ERK cascade. Mutations in Raf (*lin-45(sy96)*), MEK (*mek-2(n1989)*, *mek-2(ku114)*), and ERK (*mpk-1(ga117)*, *mpk-1(oz140)*) all suppressed the loopy waveform of activated Gq animals similarly to *ksr-1(ok786)* (Figure 2A,B; Figure S1A,B). However, mutations in Ras (*let-60(n2021)*) and the upstream pathway activators EGF (*lin-3(e1417)*) and the EGFR (*let-23(sy12)*) did not suppress activated Gq (Figure 2C). Because *let-60* is required for viability, most *let-60* alleles including *n2021* are partial loss-of-function (Beitel *et al.* 1990). We also analyzed the dominant negative D119N allele *let-60(sy93)* that disrupts Ras binding to guanine nucleotides and thus prevents Ras activation (Han and Sternberg 1991). We found that *let-60(sy93)* also did not suppress the loopy waveform of activated Gq (Figure 2D), supporting the possibility that ERK activation occurs through a Ras-independent mechanism.

Because partial loss-of-function mutations in the ERK MAPK pathway genes downstream of Ras showed clear suppression of activated Gq, we were surprised to find that partial loss-of-function mutations in Ras did not suppress. If LET-60/Ras is

indeed not required, Gq might instead activate the ERK pathway via other Ras-subfamily proteins. To test this possibility, we made double mutants of activated Gq with putative null alleles of R-Ras/*ras-1,* M-Ras/*ras-2,* and Rap1/*rap-1* and found that they also did not suppress activated Gq (Figure S2A). To further investigate whether this pathway acts independently of Ras, we tested mutations in GEFs that activate Ras. The temperature-sensitive RasGEF mutant *sos-1(cs41)* did not suppress activated Gq when shifted to the non-permissive temperature (Figure S2B). Additionally, a null mutation in the neuronal RasGEF *rgef-1* also did not suppress activated Gq locomotion (Figure S2C), supporting the possibility that this pathway is Ras-independent. In genetic screens for vulval induction mutants, additional factors such as the PP2A subunit *sur-6* (Sieburth *et al.* 1999) and ion transporter *sur-7* (Yoder *et al.* 2004) were identified as positive regulators of Ras-ERK activity. However, the *sur-6(sv30)* and *sur-7(ku119)* mutations did not suppress activated Gq locomotion (data not shown). These data suggest either that only a low level of Ras activity is needed to properly activate ERK signaling downstream of Gq, or that ERK signaling acts independently of LET-60/Ras and other known *C. elegans* Ras family proteins to regulate locomotion downstream of Gq.

KSR-1 and the ERK MAPK cascade modulate Rho signaling

Three classes of suppressor mutations were isolated in our forward genetic screen of activated Gq, as characterized by their molecular role and unique suppression phenotypes (Topalidou *et al.* 2017a; b). We grouped together a class of suppressor mutations including *ox314, yak10*, and the RhoGEF Trio (*unc-73* in *C. elegans*) by their

282 characteristic strong suppression of the loopy waveform of activated Gq (Topalidou *et*
283 *al.* 2017a; b), suggesting that *ksr-1* might act in the same pathway as *unc-73*.

284 We have shown that Gq regulates locomotion via the small GTPase Rho (RHO-1
285 in *C. elegans*) (Topalidou *et al.* 2017a). Transgenic expression of an activated RHO-1
286 mutant (G14V) in acetylcholine neurons (here called "*rho-1(gf)*") causes worms to have
287 a loopy waveform and impaired locomotion (McMullan *et al.* 2006) (Figure 3A). To
288 examine whether *ksr-1* acts in the Gq-Rho pathway we tested whether mutations in *ksr-*
289 *1* suppress the phenotypes of *rho-1(gf)* worms. We found that the *ksr-1* alleles *ok786,*
290 *ox314,* and *yak10* all suppressed the loopy waveform of *rho-1(gf)* worms (Figure 3A).
291 Because *rho-1(gf)* worms have a slow locomotion rate and loopy waveform, these
292 mutants do not efficiently travel long distances. We used radial locomotion assays (see
293 Materials and Methods) to quantify the locomotion phenotype of *rho-1(gf)* worms. *rho-*
294 *1(gf) ksr-1* double mutants had an increased radial distance traveled compared to *rho-*
295 *1(gf)* alone (Figure 3B). These data suggest that KSR-1 acts downstream of or in
296 parallel to the Gq-Rho pathway to regulate locomotion.

297 Since *ksr-1* mutants suppress the exaggerated waveform of both activated Gq
298 and activated Rho animals, we expected that loss of other ERK pathway components
299 would also suppress activated Rho. We made double mutants of activated Rho (*rho-*
300 *1(gf)*) with reduction-of-function alleles of the Ras-ERK pathway and found that
301 mutations in Raf, MEK, and ERK suppressed the *rho-1(gf)* loopy waveform and
302 decreased locomotion phenotypes (Figure 3A,C). However, the *let-60(n2021)* Ras
303 mutation did not suppress the loopy waveform or radial locomotion defect of *rho-1(gf)*
304 worms (Figure 3A,C). These data suggest that the ERK pathway acts downstream of or

in parallel to the Gq-Rho pathway to regulate locomotion, possibly in a Ras-independent manner.

The ERK MAPK cascade acts in acetylcholine neurons to control locomotion

Members of the ERK pathway are expressed in neurons in *C. elegans* (Dent and Han 1998; Hunt-Newbury *et al.* 2007), and Gq and Rho act in acetylcholine neurons to promote synaptic release and regulate locomotion(Lackner *et al.* 1999; McMullan *et al.* 2006) To determine whether the ERK pathway also acts in neurons to modulate Gq signaling, we expressed the *ksr-1* cDNA under promoters driving expression in specific types of neurons. Single-copy transgenic expression of *ksr-1* under an acetylcholine neuron promoter (*Punc-17*) or under a head acetylcholine neuron promoter (*Punc-17H)* fully reversed the *ksr-1* suppression of the loopy waveform of activated Gq worms (Figure 4). *ksr-1* expression in ventral cord acetylcholine motor neurons (*Punc-17β*) or GABA neurons (*Punc-47)* did not significantly reverse the *ksr-1* suppression of activated Gq (Figure 4). This suggests that ERK signaling primarily functions in the acetylcholine interneurons or motor neurons of the head to modulate Gq-dependent locomotion.

We have shown that the ERK pathway is necessary for Gq-dependent effects on locomotion. To determine whether ERK signaling is sufficient to modulate locomotion, we expressed an activated form of *lin-45*/Raf specifically in acetylcholine neurons. Raf kinase activity is regulated via conserved phosphorylation events, and phosphomimetic mutations T626E/T629D in the kinase activation loop of *lin-45* are sufficient to confer constitutive Raf activity (Chong *et al.* 2001). We found that expression of activated Raf

in acetylcholine neurons (*Punc-17*) causes a loopy waveform similar to activated Gq and Rho mutants and similar limited dispersal in radial locomotion assays (Figure 5A, B).

To determine if Raf activation affects acetylcholine release, we assayed for sensitivity to the acetylcholinesterase inhibitor aldicarb. Mutants with reduced acetylcholine secretion are resistant to aldicarb, whereas mutants with increased acetylcholine secretion are hypersensitive to aldicarb (Mahoney *et al.* 2006). Activated Gq and Rho mutants have increased rates of paralysis when exposed to aldicarb (Lackner *et al.* 1999; McMullan *et al.* 2006) . We found that expression of activated Raf in acetylcholine neurons also led to aldicarb hypersensitivity, similar to activated Gq and Rho mutants (Figure 5C). However, we found that a *ksr-1* mutation does not suppress the aldicarb hypersensitivity of an activated Gq mutant, and the *ksr-1* mutant on its own has similar aldicarb sensitivity to wild type (Figure 5D). These results suggest that the ERK pathway is not necessary for synaptic transmission, but is sufficient to stimulate synaptic transmission when constitutively activated.

Discussion

In this study we identified KSR-1 and the ERK MAPK cascade as downstream modulators of Gq-Rho signaling. We found that ERK signaling acts in the acetylcholine interneurons or motor neurons of the head to modulate Gq-dependent locomotion. We also found that activation of the MAPKKK Raf/LIN-45 in acetylcholine neurons causes increased sensitivity to the acetylcholinesterase inhibitor aldicarb, probably reflecting increased release of acetylcholine at neuromuscular synapses. Our data support the model that Gq-Rho activation of the ERK pathway may be independent of its canonical regulator, the small GTPase Ras/LET-60 (Figure 6).

The ERK signaling cascade has been well-studied for its regulation of cellular proliferation and differentiation (Sun *et al.* 2015), but also plays important roles in mature neurons and has been associated with synaptic plasticity and memory (Impey *et al.* 1999). In addition to activating transcription, ERKs regulate synaptic plasticity both presynaptically and postsynaptically by phosphorylating relevant substrates. ERKs phosphorylate the presynaptic proteins synapsin I and Munc18-1, and postsynaptic proteins such as scaffolds, K_V4.2 potassium channels, and Group I metabotropic glutamate receptors (Jovanovic *et al.* 2000; Thomas and Huganir 2004; Sweatt 2004; Kushner *et al.* 2005; Boggio *et al.* 2007; Vara *et al.* 2009; Mao and Wang 2016a; Schmitz *et al.* 2016). Our findings suggest that the ERK pathway controls Gq-dependent locomotion in *C. elegans* and that activated ERK promotes synaptic transmission.

In contrast to many developmental and neuronal roles of Ras-dependent ERK signaling in *C. elegans,* our data suggest that Raf-MEK-ERK signaling may modulate Gq-Rho output independently of Ras/LET-60. One caveat to the conclusion that the

366 ERK pathway acts independently of Ras to control Gq-dependent locomotion is that the
367 alleles of *let-60*/Ras tested here are not null (Han and Sternberg 1990, 1991; Han *et al.*
368 1990; Beitel *et al.* 1990). However, other than *mpk-1(ga117)*, the alleles we used of *lin-*
369 *45*, *mek-2* and *mpk-1* are also not null (Han *et al.* 1993; Kornfeld *et al.* 1995a; Wu *et al.*
370 1995; Lackner and Kim 1998; Hsu *et al.* 2002)**,** yet were able to suppress an activated
371 Gq mutant. Furthermore, the *let*-60/Ras alleles used here have stronger phenotypes in
372 vulval development than the two weak *mek-2*/MEK alleles we used (*n1989* and *ku114*)
373 (Beitel *et al.* 1990; Kornfeld *et al.* 1995a; Wu *et al.* 1995) and the *let-60(n2021)*/Ras
374 mutant has comparable phenotypes to the *lin-45(sy96)*/Raf and *mpk-1(ga117*)/ERK
375 alleles for chemotaxis to odorants and the regulation of foraging behavior (Hirotsu *et al.*
376 2000; Hamakawa *et al.* 2015). Thus, either LET-60/Ras is not required to modulate Gq
377 signaling, or ERK signaling in the locomotor circuit requires a lower threshold of Ras
378 activity and the *let-60* mutants we used have sufficient levels of active Ras to modulate
379 Gq signaling, but not enough for vulval induction or other behaviors.
380 If Ras is not required, how instead might KSR-Raf-MEK-ERK signaling be
381 activated? Normally, active Ras recruits Raf to the plasma membrane but it has been
382 shown that artificial recruitment of Raf to the plasma membrane in the absence of Ras
383 is sufficient to activate Raf signaling (Stokoe *et al.* 1994; Marais *et al.* 1995). Thus,
384 another possible way to activate the ERK pathway would be for Raf to be recruited to
385 the membrane by proteins other than Ras. The most obvious candidates for recruiting
386 Raf are other members of the Ras family such as Rap1 or R-Ras that have a conserved
387 effector-binding domain (Reiner and Lundquist 2016); it has been reported that Rap1
388 can mediate Ras-independent activation of Raf downstream of a Gq-coupled receptor

(Guo *et al.* 2001). However, mutations in the worm orthologs of Rap1/RAP-1 and R-Ras/RAS-1 did not suppress the activated Gq mutant, indicating that these Ras-like proteins are not required, at least individually, to activate Raf in this pathway. It is possible that more than one of these Ras family members function redundantly to activate Raf, or that Raf is activated completely independently of Ras family proteins. There have been other reported cases of Ras-independent activation of the Raf-MEK-ERK pathway (Robbins *et al.* 1992; Honda *et al.* 1994; van Biesen *et al.* 1996; Ueda *et al.* 1996; Drosten *et al.* 2014), some of which involve G protein signaling and protein kinase C, though the precise mechanisms involved are unclear.

Gq, Rho, and ERK are also required for the *C. elegans* behavioral and immune response to infection by the bacterium *M. nematophilum* (Nicholas and Hodgkin 2004; McMullan *et al.* 2012). Interestingly, LET-60/Ras was reported to not be required for the immune response (Nicholas and Hodgkin 2004) or only partially required (McMullan *et al.* 2012). Additionally, LET-60/Ras was not required for the increased sensitivity to aldicarb caused by infection (McMullan *et al.* 2012), at least as assayed using the *let-60(n2021)* allele. Thus, the same neuronal Ras-independent ERK pathway we describe here appears to also modulate Gq signaling in the neuronal response to bacterial infection and possibly the innate immune response as well.

Our epistasis analysis suggests that ERK signaling acts genetically downstream of or in parallel to Gq-Rho signaling. How might Gq-Rho signaling lead to ERK activation? ERK activation could occur via a linear pathway downstream of Gq and Rho, or ERK could signal in parallel and converge downstream of Rho to affect neuronal activity (Figure 6). There is precedence for Gq activating ERK via a linear pathway. In

412 the pharyngeal muscle of *C. elegans*, ERK activity is increased by Gq-dependent
413 signaling through protein kinase C (You *et al.* 2006). In the AWC olfactory neuron, ERK
414 activity is increased downstream of Gq signaling via the RasGEF RGEF-1 (Chen *et al.*
415 2011; Uozumi *et al.* 2012). Protein kinase C and RGEF-1 are both activated by DAG,
416 probably produced by the canonical Gq-PLCβ pathway. By contrast, we found that ERK
417 signaling regulates locomotion by modulating the output of the Gq-Rho pathway that
418 acts in parallel to Gq-PLCβ, and does not depend on RGEF-1.
419 How might the ERK pathway modulate neuronal activity downstream of Gq-Rho
420 signaling? Rho regulates neuronal activity and synaptic release through several
421 mechanisms in *C. elegans* neurons, any of which could be targets of ERK signaling.
422 One possible ERK effector is the NCA/NALCN cation channel that acts genetically
423 downstream of Gq-Rho to regulate locomotion rate and waveform (Topalidou *et al.*
424 2017a; b). Though ERK has not been directly connected to NCA/NALCN, mammalian
425 ERK regulates neuronal excitability by directly phosphorylating voltage-gated sodium
426 and potassium channels (Schrader *et al.* 2006; Stamboulian *et al.* 2010) and by
427 regulating channel expression (Yang *et al.* 2015). Given the many possible ways that
428 ERK may regulate excitability or synaptic transmission, *C. elegans* genetics is well
429 suited to determine relevant effectors of Gq-Rho-ERK signaling.
430

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Figure Legends

Figure 1. *ksr-1* mutations suppress activated Gq.

(A) A *ksr-1* mutation suppresses the exaggerated body bends of activated Gq. The activated Gq mutant *egl-30(tg26)* has small size and deep body bends. *ksr-1(ok786)* suppresses the exaggerated body bends and small size of *egl-30(tg26)* worms.

(B) Gene structure of *ksr-1* locus. Locations of the *egl-30(tg26)* suppressor alleles *ox314* and *yak10* are indicated, as well as the position of the *ok786* deletion. The gene structure was drawn using Exon-Intron Graphic Maker (www.wormweb.org/exonintron)

(C) A *ksr-1* mutation suppresses the exaggerated body bend waveform of *egl-30(tg26)* mutants. Straightened images of tracks left in bacterial lawns show similar waveform for wild type and *ksr-1(ok786)* worms. *egl-30(tg26)* mutants have an exaggerated waveform, creating tracks with a large amplitude relative to the period. *ksr-1*(*ok786)* suppresses the exaggerated body bends of *egl-30(tg26)* mutants.

(D) A *ksr-1* mutation suppresses the *egl-30(tg26)* waveform. *ksr-1* nonsense alleles *ox314* and *yak10* and the deletion allele *ok786* strongly suppress the *egl-30(tg26)* exaggerated waveform. $N \geq 12$ *** $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test.

Figure 2. Mutations in the ERK MAPK pathway suppress activated Gq.

(A) Mutations in the MAPKKK *lin-45*/Raf, the MAPKK *mek-2*/MEK, and the MAPK *mpk-1*/ERK suppress the exaggerated waveform of *egl-30*(*tg26*) worms. Partial loss of Ras *let-60* activity does not suppress activated Gq waveform.

(B) Waveform quantification of ERK pathway mutants show levels of *egl-30*(*tg26*) suppression similar to *ksr-1* alleles. $N \geq 12$ *** $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test.

(C) Signaling pathways upstream of the ERK pathway do not suppress activated Gq. Mutations in the EGFR (*let-23*) or the EGF ligand (*lin-3*) do not affect the exaggerated waveform of *egl-30*(*tg26*) animals. The Ras partial loss-of-function mutation *let-60(n2021)* does not suppress activated Gq waveform. $N \geq 12$ *** $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test.

(D) The *let-60(sy93)* dominant negative mutation in Ras does not suppress activated Gq waveform. $N \geq 13$, n.s., not significant, one-way ANOVA with Bonferroni's *post hoc* test.

Figure 3. Mutations in *ksr-1* and the ERK pathway suppress activated Rho.

(A) Mutations in the ERK pathway visibly suppress the exaggerated body bends of animals expressing an activated Rho mutant (G14V) in acetylcholine neurons (*nzIs29[Punc-17::rho-1(gf)]*). Reduction of LET-60/Ras activity does not suppress the activated Rho waveform.

(B) The *ksr-1*(*ok786*), *ksr-1*(*ox314*), and *ksr-1*(*yak10*) mutations suppress the locomotion of activated Rho animals as shown by radial locomotion assay. $N \geq 38$ *** $P < 0.001$, ** $P < 0.01$, Kruskal-Wallis test with Dunn's *post hoc* test.

(C) Mutations in *lin-45* and *mek-2* suppresses the locomotion defect of activated Rho animals as shown by radial locomotion assays. The *let-60*(*n2021*) and *mpk-1*(*ga117*) mutations do not significantly suppress activated Rho locomotion. $N \geq 50$ *** $P < 0.001$, Kruskal-Wallis test with Dunn's *post hoc* test.

Figure 4. KSR-1 acts in acetylcholine neurons to modulate Gq signaling.
Single-copy expression of the *ksr-1* cDNA exclusively in acetylcholine neurons (*Punc-17*, *yakSi26* transgene) or head acetylcholine neurons (*Punc-17H, yakSi27* transgene) is sufficient to reverse the *ksr-1* suppression of the loopy waveform of activated Gq animals. *ksr-1* expression in ventral cord acetylcholine neurons (*Punc-17β*, *yakSi28* transgene) or GABA neurons (*Punc-47*, *yakSi29* transgene) does not reverse the *ksr-1* suppression of the activated Gq exaggerated waveform. $N \geq 12$ *** $P < 0.001$, ** $P < 0.01$, n.s., not significant, Kruskal-Wallis test with Dunn's *post hoc* test.

Figure 5. Increased ERK signaling in acetylcholine neurons is sufficient to regulate locomotion and increase acetylcholine release.
(A) Transgenic lines expressing an activated form of *lin-45* (T626E/T629D) in acetylcholine neurons (*yakIs34[Punc-17::lin-45(gf)]*) have exaggerated body bends and coiling behavior similar to the activated Gq mutant *egl-30*(*tg26*) and to animals expressing activated Rho in acetylcholine neurons (*nzIs29[Punc-17::rho-1(gf)]*). The wild type and *egl-30(tg26)* photos are the same as shown in Figure 1A, while the *rho-1(gf)* photos is the same as the one in Figure 3A.
(B) Expression of activated Rho (*nzIs29[Punc-17::rho-1(gf)]*) or Raf (*yakIs34[Punc-17::lin-45(gf)]*) in acetylcholine neurons impairs coordinated locomotion similarly to activated Gq (*egl-30*(*tg26*)). $N \geq 19$ * $P < 0.05$, *** $P < 0.001$, Kruskal-Wallis test with Dunn's *post hoc* test.

(C) Animals expressing activated Gq, Rho, or Raf are hypersensitive to the acetylcholinesterase inhibitor aldicarb. Activated Gq (*egl-30*(*tg26*)), activated Rho expressed in acetylcholine neurons (*nzIs29[Punc-17::rho-1(gf)]*), and activated Raf expressed in acetylcholine neurons *yakIs34[Punc-17::lin-45(gf)]*) become paralyzed significantly faster than wild type animals when exposed to 1 mM aldicarb. All strains are significantly different from wild type at t = 60, 75, 90, and 105 minutes. $N \geq 61$ *** $P < 0.001$, two-way ANOVA with Bonferroni's *post hoc* test.

(D) *ksr-1* is not necessary for the aldicarb hypersensitivity of activated Gq. Paralysis of *ksr-1*(*ok786*) animals on 1 mM aldicarb is not significantly different from wild type. The *ksr-1* deletion *ok786* does not suppress the aldicarb hypersensitivity of activated Gq (*egl-30*(*tg26*)). $N \geq 53$.

Figure 6. Model for Gq-Rho-ERK signaling. Gq directly activates Trio and Rho (solid arrow) (Lutz et al. 2007). The core ERK cascade acts either downstream of Rho or in parallel (dashed arrows) to modulate locomotion. ERK activation occurs independently of the extracellular growth factor LIN-3, its receptor LET-23, or Ras/LET-60. It is possible that Raf/LIN-45 is activated by a Ras family member other than the canonical worm Ras/LET-60.

Figure S1. Additional mutations in *mek-2* and *mpk-1* suppress activated Gq.

(A) The *mek-2(ku114)* mutation suppresses the exaggerated waveform of *egl-30*(*tg26*) worms. $N \geq 14$ *** $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test.

529 (B) The *mpk-1(oz140)* mutation suppresses the exaggerated waveform of *egl-30*(*tg26*)
530 worms. $N \geq 12$ *** $P < 0.001$, one-way ANOVA with Bonferroni's *post hoc* test.
531

532 **Figure S2.** Gq-dependent locomotion does not depend on the RasGEFs *sos-1* and
533 *rgef-1*, or the Ras family members *ras-1*, *ras-2*, and *rap-1*.
534 (A) Deletion mutations *ras-1(gk243)* and *ras-2(ok682),* or the nonsense mutation *rap-*
535 *1(pk2082)* do not suppress the waveform of the activated Gq mutant *egl-30(tg26)*. $N \geq$
536 18, n.s., not significant, one-way ANOVA with Bonferroni's *post hoc* test.
537 (B) Loss of the RasGEF *sos-1* does not suppress activated Gq waveform. Temperature
538 sensitive *egl-30(tg26); sos-1(cs41)* mutant animals were incubated for 24 hours at the
539 non-permissive temperature of 25° and assayed for their waveform. $N \geq 15$, n.s., not
540 significant, one-way ANOVA with Tukey's *post hoc* test.
541 (C) The RasGEF deletion mutation *rgef-1(ok675)* does not suppress the exaggerated
542 waveform of activated Gq. $N = 16$, n.s., not significant, one-way ANOVA with Tukey's
543 *post hoc* test.
544

545 **Figure S3.** Expression of activated LIN-45/Raf in head acetylcholine neurons is
546 sufficient to cause locomotion with exaggerated body bends.
547 The *yakIs34[Punc-17::lin-45(gf)]* integrated array expressing activated *lin-*
548 *45*(T626E/T629D) in all acetylcholine neurons and the *yakEx154[Punc-17H::lin-45(gf)]*
549 extrachromosomal array expressed in head acetylcholine neurons both cause
550 exaggerated body bends and coiled locomotion. $N \geq 12$ *** $P < 0.001$, * $P < 0.05$,
551 Kruskal-Wallis test with Dunn's *post hoc* test.

Table S1. List of strains

N2	Bristol wild strain
XZ1151	*egl-30(tg26) I*
EG6699	*ttTi5605 II; unc-119(ed3) III*
EG6207	*unc-119(ed3) III*
RB915	*ksr-1(ok786) X*
EG4782	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II*
MT4866	*let-60(n2021) IV*
PS436	*let-60(sy93) IV*
PS427	*lin-45(sy96) IV*
MT8666	*mek-2(n1989) I*
SD378	*mpk-1(ga117) / dpy-17(e164) unc-79(e1068) III*
CB1417	*lin-3(e1417) IV*
PS5131	*let-23(sy12) / mIn1[dpy-10(e128) mIs14] II*
UP604	*sos-1(cs41) V*
VC450	*ras-1(gk243) II*
RB852	*ras-2(ok682) III*
TZ181	*rap-1(pk2082) IV*
RB848	*rgef-1(ok675) V*
MH538	*mek-2(ku114) I; let-60(n1046) IV*
MT8186	*mpk-1(oz140)/unc-79(e1068) dpy-17(e164) III*

The following strains were produced during this study:

EG314	*ksr-1(ox314) X*
XZ10	*ksr-1(yak10) X*
EG4198	*egl-30(tg26) I ; ksr-1(ox314) X*
XZ1340	*egl-30(tg26) I ; ksr-1(yak10) X*
XZ1511	*egl-30(tg26) I; ksr-1(ok786) X*

XZ1465	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; ksr-1(ox314) X*
XZ1547	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; ksr-1(yak10) X*
XZ2042	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; ksr-1(ok786) X*
XZ1615	*egl-30(tg26) I; let-60(n2021) / nT1[qIs51(Pmyo-2::GFP; Ppes-10::GFP; F22B7.9::GFP)] IV*
XZ1626	*egl-30(tg26) I; lin-3(e1417) IV*
XZ1630	*egl-30(tg26) I; let-23(sy12) / mIn1[dpy-10(e128) mIs14] II*
XZ1677	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; let-60(n2021) / nT1[qIs51(Pmyo-2::GFP; Ppes-10::GFP; F22B7.9::GFP)] IV*
XZ1548	*egl-30(tg26) I; lin-45(sy96) IV*
XZ1556	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; lin-45(sy96) IV*
XZ1850	*egl-30(tg26) mek-2(n1989) / hT2[bli-4(e937) let(q782) qIs48] I*
XZ1851	*mek-2(n1989) / hT2[bli-4(e937) let(q782) qIs48] I; nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II*
XZ1700	*mpk-1(ga117) / qC1[dpy-19(e1259) glp-1(q339) qIs26(Plag-2::GFP, rol-6(su1006))] III*
XZ1668	*egl-30(tg26) I; mpk-1(ga117) / qC1[dpy-19(e1259) glp-1(q339) qIs26(Plag-2::GFP, rol-6(su1006))] III*
XZ2043	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; mpk-1(ga117) / qC1[dpy-19(e1259) glp-1(q339) qIs26(Plag-2::GFP, rol-6(su1006))] III*
XZ2046	*egl-30(tg26) I; sos-1(cs41) V*
XZ1855	*egl-30(tg26) I; ras-1(gk243) II*
XZ1856	*egl-30(tg26) I; ras-2(ok682) III*
XZ2045	*egl-30(tg26) I; rap-1(pk2082) IV*
XZ2101	*egl-30(tg26) I; rgef-1(ok675) V*
XZ1921	*egl-30(tg26) sur-6(sv30) / hT2[bli-4(e937) let(q782) qIs48] I*
XZ1857	*egl-30(tg26) I ; sur-7(ku119) X*
XZ1854	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; ras-2(ok682) III*
XZ1852	*nzIs29[Punc-17::rho-1(G14V), unc-122::gfp] II; rap-1(pk2082) IV*
XZ1880	*yakSi26[Punc-17::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II*

XZ1881	*yakSi27[Punc-17H::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II*
XZ1882	*yakSi28[Punc-17β::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II*
XZ1883	*yakSi29[Punc-47::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II*
XZ1884	*egl-30(tg26) I; yakSi26[Punc-17::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II; ksr-1(ok786) X*
XZ1885	*egl-30(tg26) I; yakSi27[Punc-17H::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II; ksr-1(ok786) X*
XZ1946	*egl-30(tg26) I; yakSi28[Punc-17β::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II; ksr-1(ok786) X*
XZ1947	*egl-30(tg26) I; yakSi29[Punc-47::ksr-1 cDNA::tbb-2 3'UTR::OPERON::GFP] II; ksr-1(ok786) X*
XZ2015	*unc-119(ed3) III; yakEx122[Punc-17::lin-45* cDNA::tbb-2 3'UTR::OPERON::GFP, Pmyo-3::mCherry]*
XZ2077	*egl-30(tg26) I; let-60(sy93) IV*
XZ2106	*egl-30(tg26) I; let-60(sy93) / nT1[qIs51(Pmyo-2::GFP; Ppes-10::GFP; F22B7.9::GFP)] IV*
XZ2050	*unc-119(ed3) III; yakIs34[Punc-17::lin-45* cDNA::tbb-2 3'UTR::OPERON::GFP, Pmyo-3::mCherry] X*
XZ2130	*egl-30(tg26) I; mpk-1(oz140) / oxTi619[Peft-3::TdTomato::H2B cb-unc-119(+)] III*
XZ2131	*egl-30(tg26) mek-2(ku114) I / hT2[bli-4(e937) let(q782) qIs48] I;III*
XZ2119	*ttTi5605 II; unc-119(ed3) III; yakEx154[Punc-17H::lin-45*::tbb-2 3'UTR::OPERON::GFP, Pmyo-2::mCherry, Pmyo-3::mCherry]*

Table S2. List of plasmids

Gateway destination vectors

pCFJ150	Gateway destination vector for insertion at chr II Mos site ttTi5605

Gateway entry clones

pEGB05	*Prab-3* [4-1]

pGH1	*Punc-17* [4-1]
pADA180	*Punc-17H* [4-1] (head acetylcholine neurons)
pMA23	*Punc-17β* [4-1] (body acetylcholine neurons)
pMH522	*Punc-47* [4-1]
pBC11	*ksr-1* cDNA [1-2]
pBC26	*lin-45* cDNA [1-2]
pBC35	*lin-45** (T626E/T629D) cDNA [1-2]
pCFJ326	*tbb-2* 3'UTR::OPERON::*GFP* [2-3]

Gateway expression constructs

pBC13	*Prab-3::ksr-1* cDNA::*tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC31	*Punc-17::ksr-1* cDNA*::tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC32	*Punc-17H*::*ksr-1* cDNA::*tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC33	*Punc-17β*::*ksr-1* cDNA::*tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC34	*Punc-47*::*ksr-1* cDNA::*tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC37	*Punc-17::lin-45** cDNA*::tbb-2* 3'UTR::OPERON::GFP_pCFJ150
pBC44	*Punc-17H::lin-45** cDNA*::tbb-2* 3'UTR::OPERON::GFP_pCFJ150

Table S3. List of primers

oBC011	Forward *ksr-1* cDNA for Gateway [1-2] ENTR vector
	GGGGACAAGTTTGTACAAAAAAGCAGGCTCAatgatgcaaacccaagttgc
oBC012	Reverse *ksr-1* cDNA for Gateway [1-2] ENTR vector
	GGGGACCACTTTGTACAAGAAAGCTGGGTGaaatgtcgactcgtaacttttcatc
oBC085	Forward *lin-45* cDNA for Gateway [1-2] ENTR vector
	GGGGACAAGTTTGTACAAAAAAGCAGGCTcaATGAGTCGGATTAATTTCAAAAAG
oBC086	Reverse *lin-45* cDNA for Gateway [1-2] ENTR vector
	GGGGACCACTTTGTACAAGAAAGCTGGGTgCTAAATGAGACCATAGACATTGTAGTATG

oBC094	Reverse *lin-45* T626E mutagenesis
	gttcactgtccatttcgttttgacctctgccaagccgaaatctccaatttt
oBC095	Forward *lin-45* T626E mutagenesis
	aaaattggagatttcggcttggcagaggtcaaaacgaaatggacagtgaac
oBC096	Reverse *lin-45* T629D mutagenesis
	cctccgttcactgtccatttatctttgacctctgccaagccga
oBC097	Forward *lin-45* T629D mutagenesis
	tcggcttggcagaggtcaaagataaatggacagtgaacggagg

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880

881

882

Figure 1

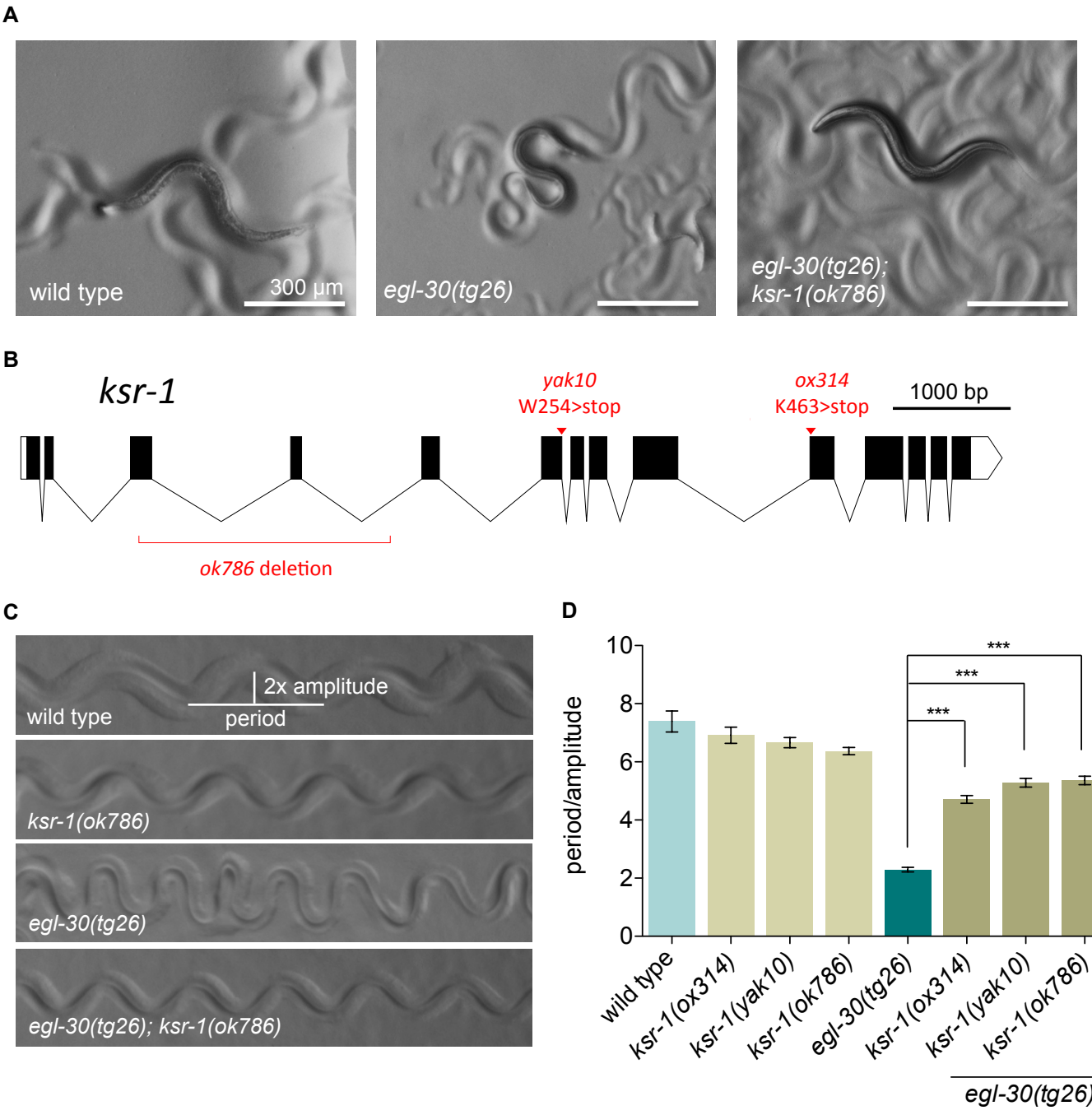

A
wild type
300 µm
egl-30(tg26)
egl-30(tg26);
ksr-1(ok786)
B
ksr-1
yak10
W254>stop
ox314
K463>stop
1000 bp
ok786 deletion
C
2x amplitude
wild type
period
ksr-1(ok786)
egl-30(tg26)
egl-30(tg26); ksr-1(ok786)
D
period/amplitude
10
8
6
4
2
0
***
***
***
wild type
ksr-1(ox314)
ksr-1(yak10)
ksr-1(ok786)
egl-30(tg26)
ksr-1(ox314)
ksr-1(yak10)
ksr-1(ok786)
egl-30(tg26)

Figure 2

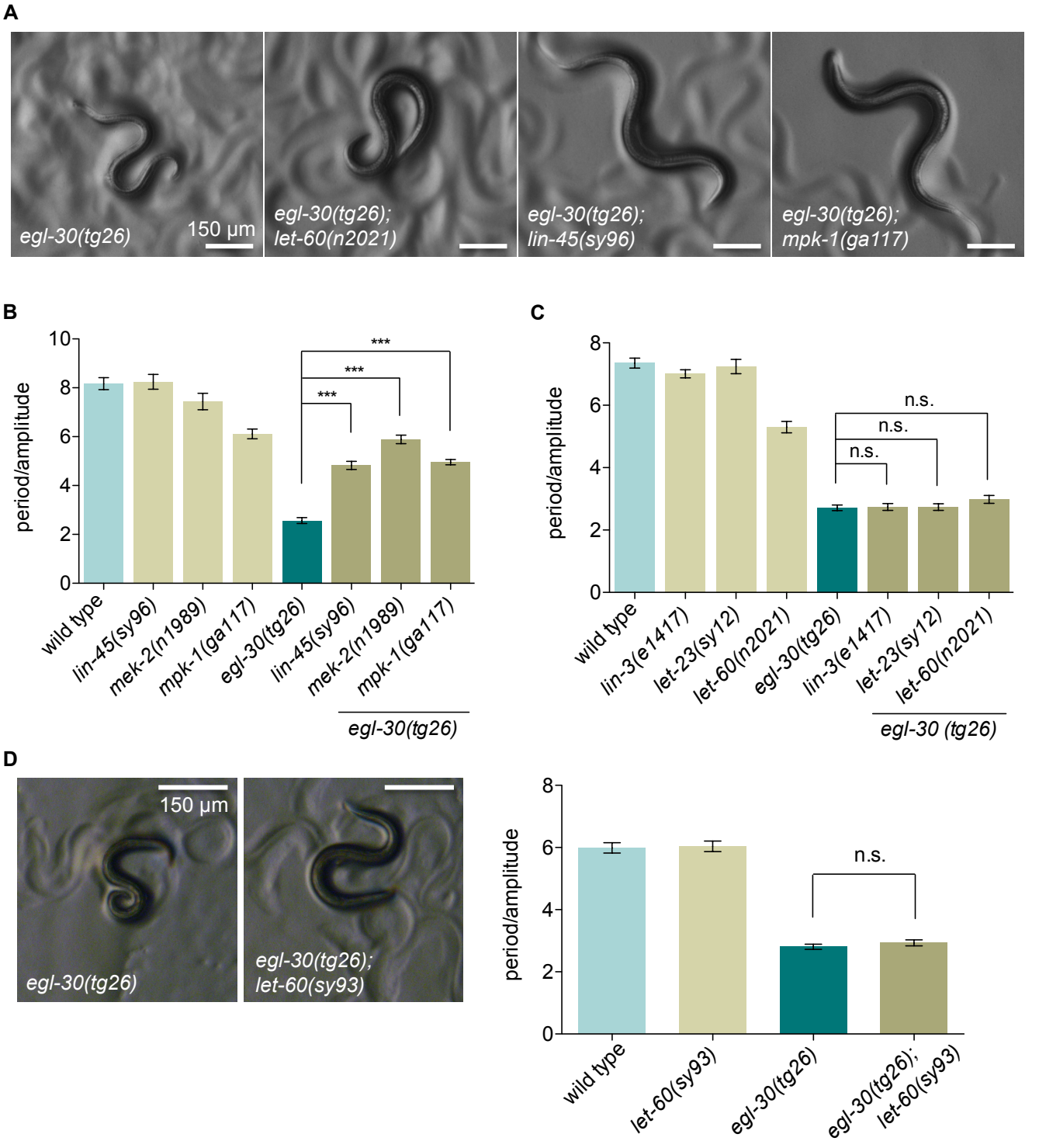
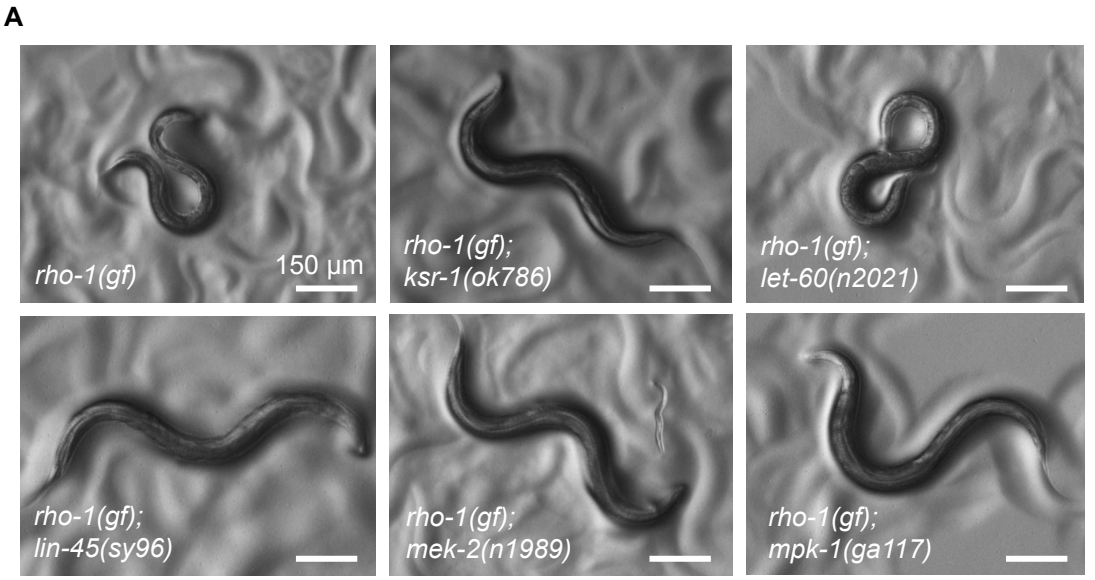
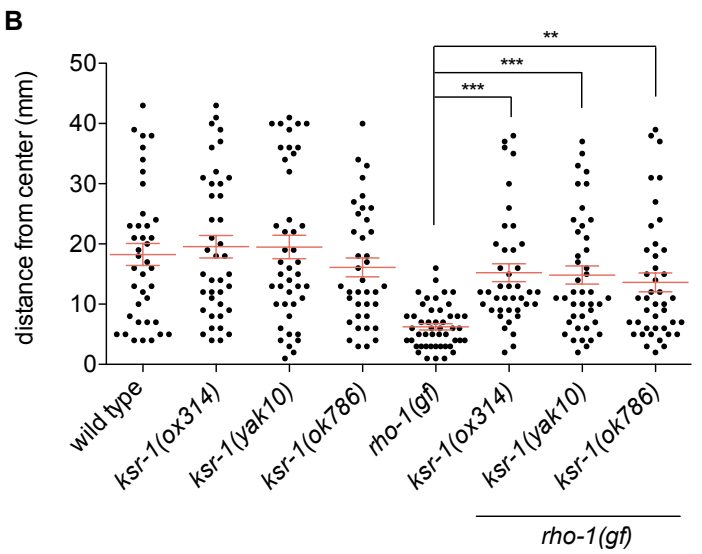

A
egl-30(tg26)
150 µm
egl-30(tg26); let-60(n2021)
egl-30(tg26); lin-45(sy96)
egl-30(tg26); mpk-1(ga117)
B
period/amplitude
wild type
lin-45(sy96)
mek-2(n1989)
mpk-1(ga117)
egl-30(tg26)
lin-45(sy96)
mek-2(n1989)
mpk-1(ga117)
***
***
***
egl-30(tg26)
C
period/amplitude
wild type
lin-3(e1417)
let-23(sy12)
let-60(n2021)
egl-30(tg26)
lin-3(e1417)
let-23(sy12)
let-60(n2021)
n.s.
n.s.
n.s.
egl-30 (tg26)
D
egl-30(tg26)
egl-30(tg26); let-60(sy93)
period/amplitude
wild type
let-60(sy93)
egl-30(tg26)
egl-30(tg26); let-60(sy93)
n.s.

Figure 3


A
rho-1(gf)
150 µm
rho-1(gf);
ksr-1(ok786)
rho-1(gf);
let-60(n2021)
rho-1(gf);
lin-45(sy96)
rho-1(gf);
mek-2(n1989)
rho-1(gf);
mpk-1(ga117)


B
distance from center (mm)
0
10
20
30
40
50
**
***
***
wild type
ksr-1(ox314)
ksr-1(yak10)
ksr-1(ok786)
rho-1(gf)
ksr-1(ox314)
ksr-1(yak10)
ksr-1(ok786)
rho-1(gf)

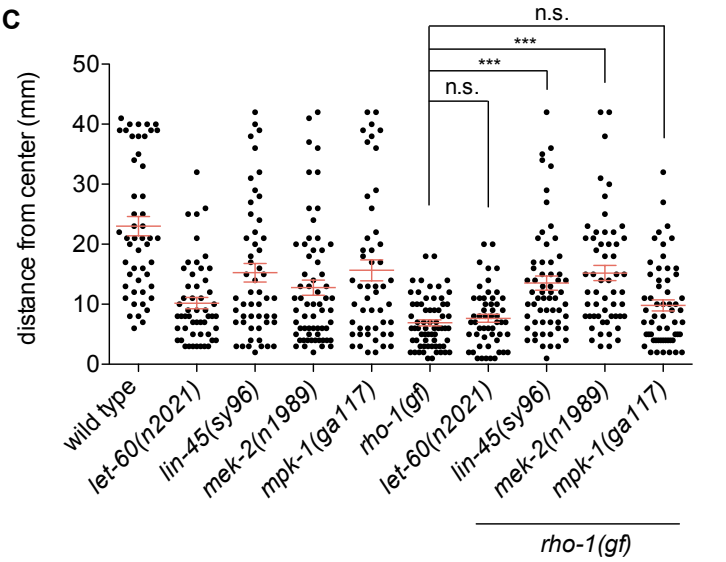

C
distance from center (mm)
0
10
20
30
40
50
n.s.
***
***
n.s.
wild type
let-60(n2021)
lin-45(sy96)
mek-2(n1989)
mpk-1(ga117)
rho-1(gf)
let-60(n2021)
lin-45(sy96)
mek-2(n1989)
mpk-1(ga117)
rho-1(gf)

Figure 4

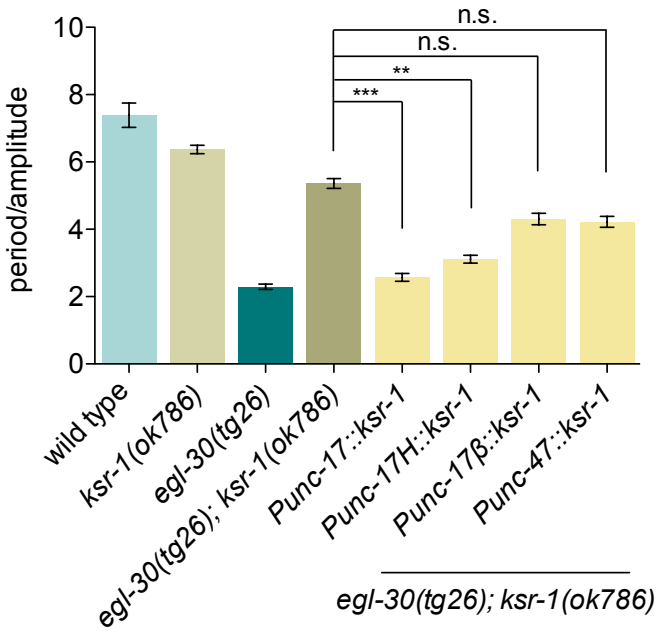

n.s.
n.s.
**
***
10
8
6
4
2
0
period/amplitude
wild type
ksr-1(ok786)
egl-30(tg26)
egl-30(tg26); ksr-1(ok786)
Punc-17::ksr-1
Punc-17H::ksr-1
Punc-17β::ksr-1
Punc-47::ksr-1
egl-30(tg26); ksr-1(ok786)

Figure 5

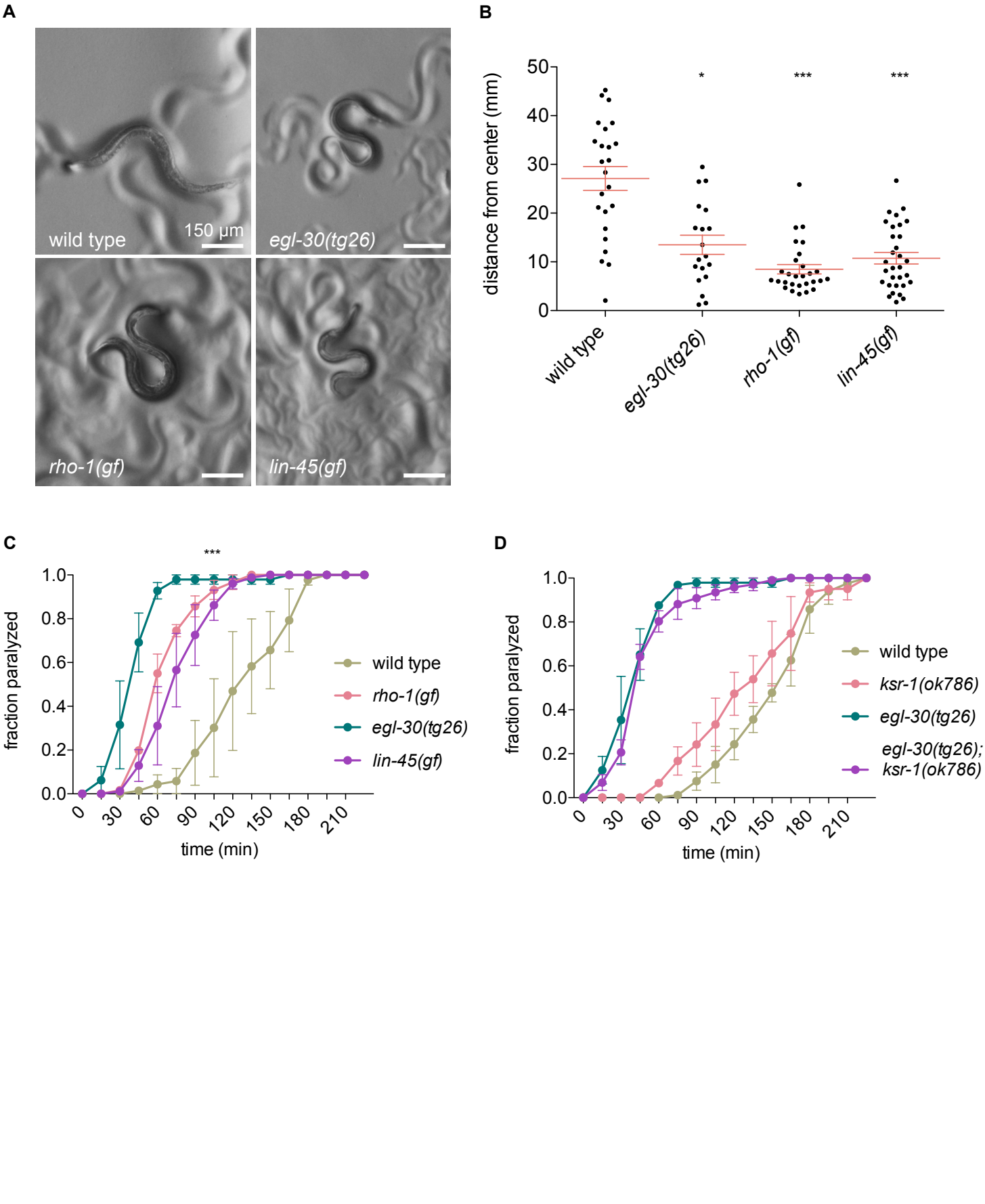

A
wild type
150 µm
egl-30(tg26)
rho-1(gf)
lin-45(gf)
B
distance from center (mm)
50
40
30
20
10
0
*
***
***
wild type
egl-30(tg26)
rho-1(gf)
lin-45(gf)
C
***
fraction paralyzed
1.0
0.8
0.6
0.4
0.2
0.0
0
30
60
90
120
150
180
210
time (min)
wild type
rho-1(gf)
egl-30(tg26)
lin-45(gf)
D
fraction paralyzed
1.0
0.8
0.6
0.4
0.2
0.0
0
30
60
90
120
150
180
210
time (min)
wild type
ksr-1(ok786)
egl-30(tg26)
egl-30(tg26);
ksr-1(ok786)

Figure 6

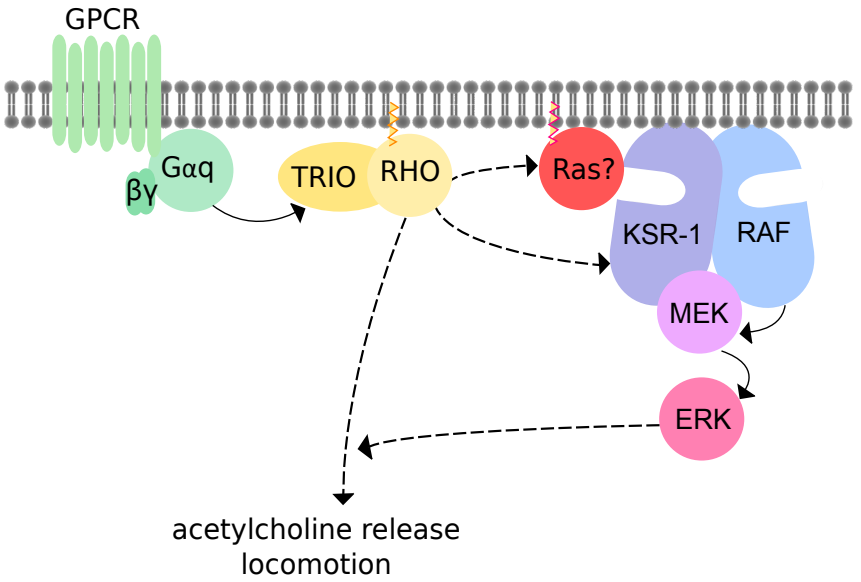

GPCR
Gαq
βγ
TRIO
RHO
Ras?
KSR-1
RAF
MEK
ERK
acetylcholine release
locomotion

Figure S1

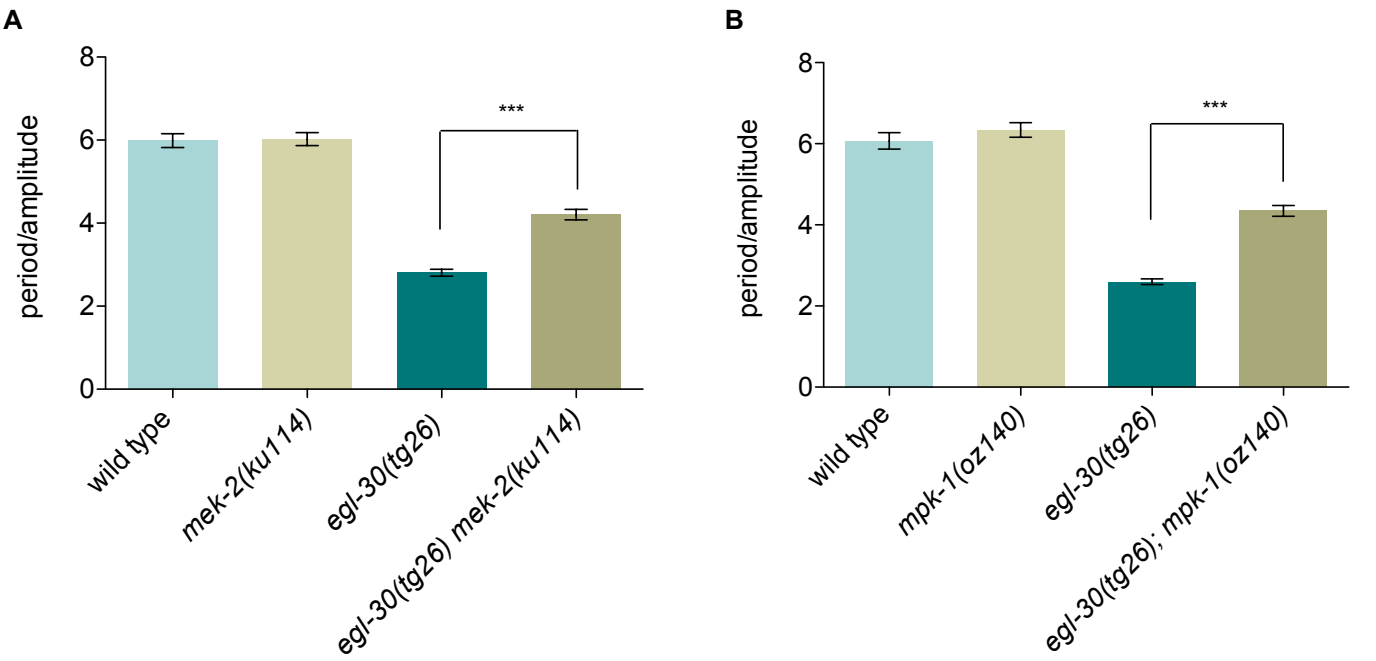

A
period/amplitude
8
6
4
2
0
***
wild type
mek-2(ku114)
egl-30(tg26)
egl-30(tg26) mek-2(ku114)
B
period/amplitude
8
6
4
2
0
***
wild type
mpk-1(oz140)
egl-30(tg26)
egl-30(tg26); mpk-1(oz140)

Figure S2

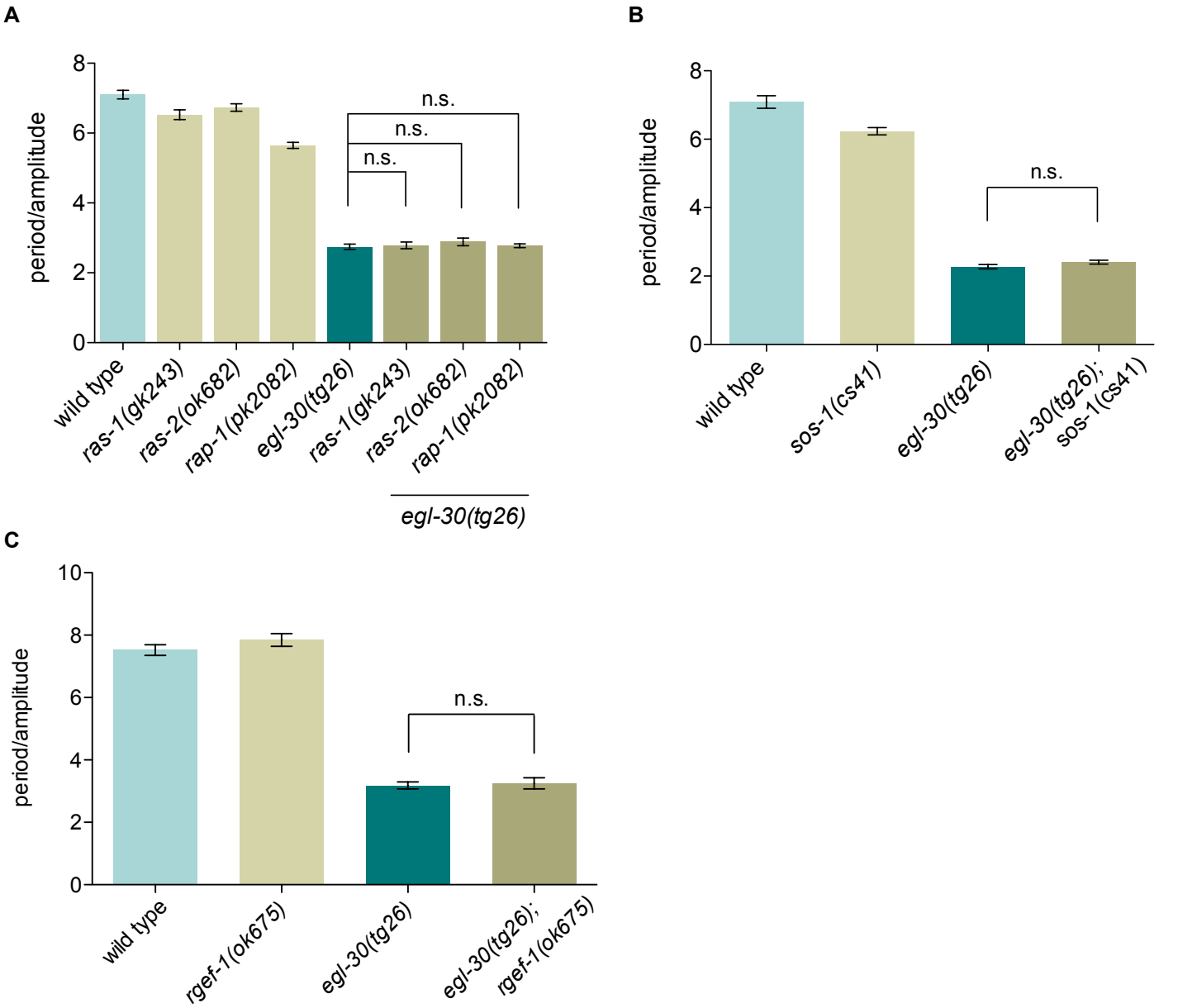
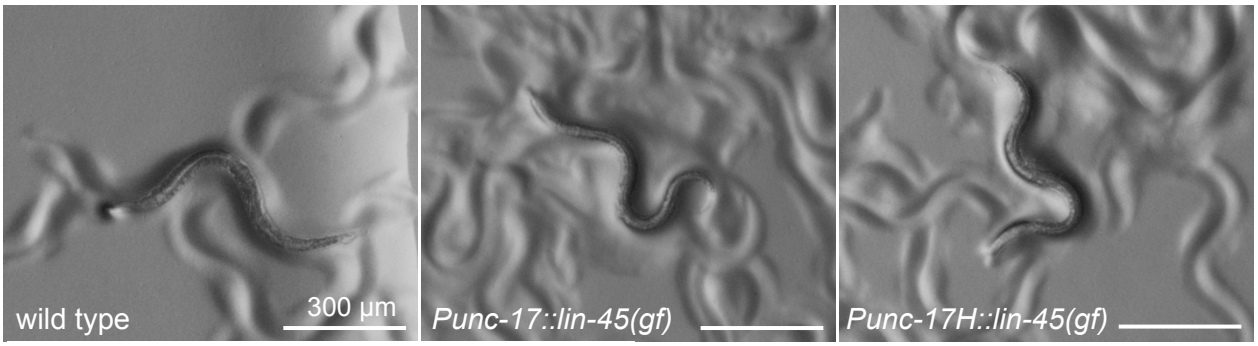
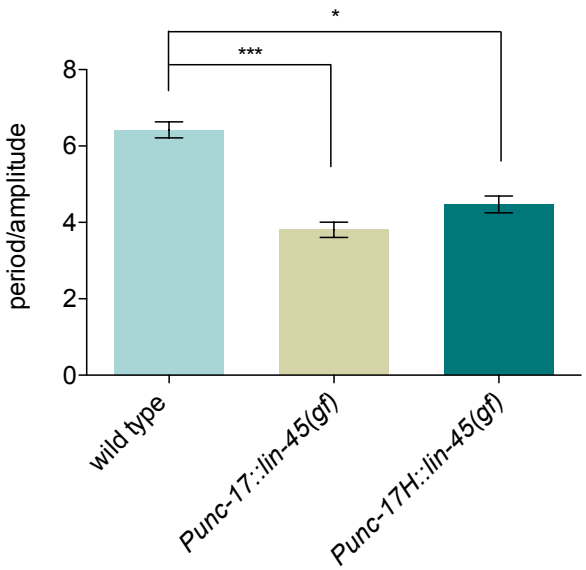

A
period/amplitude
8
6
4
2
0
n.s.
n.s.
n.s.
wild type
ras-1(gk243)
ras-2(ok682)
rap-1(pk2082)
egl-30(tg26)
ras-1(gk243)
ras-2(ok682)
rap-1(pk2082)
egl-30(tg26)
B
period/amplitude
8
6
4
2
0
n.s.
wild type
sos-1(cs41)
egl-30(tg26)
egl-30(tg26); sos-1(cs41)
C
period/amplitude
10
8
6
4
2
0
n.s.
wild type
rgef-1(ok675)
egl-30(tg26)
egl-30(tg26); rgef-1(ok675)

Figure S3


wild type
300 µm
Punc-17::lin-45(gf)
Punc-17H::lin-45(gf)


*
***
period/amplitude
8
6
4
2
0
wild type
Punc-17::lin-45(gf)
Punc-17H::lin-45(gf)