

Supplementary Materials for:

A conserved behavioral role for a nematode interneuron neuropeptide receptor

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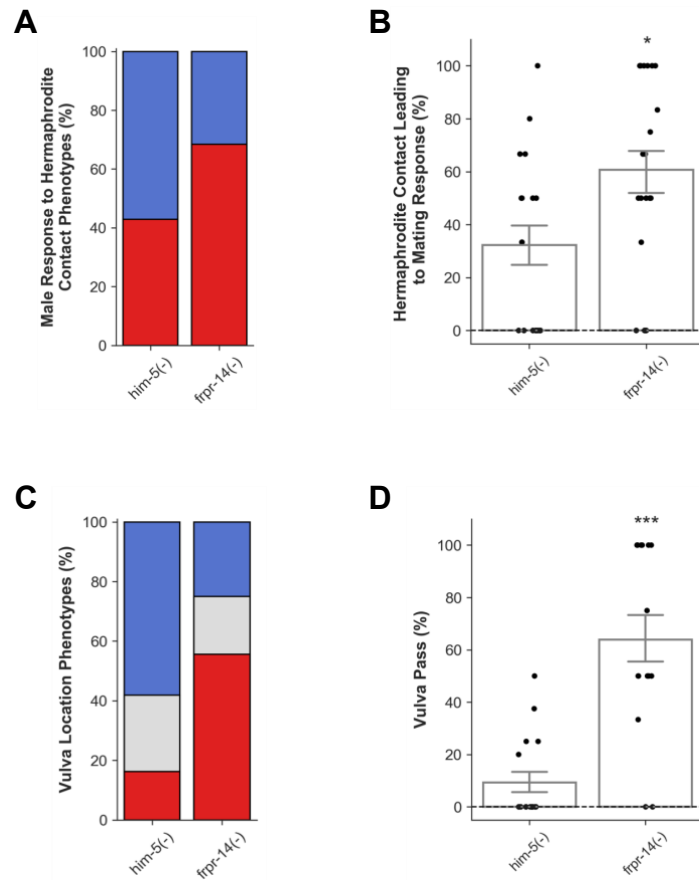


Figure S1: *frpr-14* mutant males are defective in vulva location and initiating mating response upon hermaphrodite contact.

(A) Proportions of pooled male response to hermaphrodite contact phenotypes. Red – contact not leading to mating response, blue – contact leading to mating response. *him-5(-)* (n=35 events), *frpr-14(-)* (n=57 events).

(B) Percentage of hermaphrodite contact events leading to male mating response. *him-5(-)* (n=18 animals), *frpr-14(-)* (n=21 animals). Wilcoxon rank sum test. * p<0.05. p=1.916e-02.

(C) Proportions of pooled vulva location phenotypes. Red – vulva pass, gray – hesitation upon vulva encounter, blue – successful vulva location. *him-5(-)* (n=43 encounters), *frpr-14(-)* (n=36 encounters).

(D) Percentage of vulva encounters resulting in vulva passing. *him-5(-)* (n=17 animals), *frpr-14(-)* (n=15 animals). Wilcoxon rank sum test. *** p<0.001. p=6.538e-05.

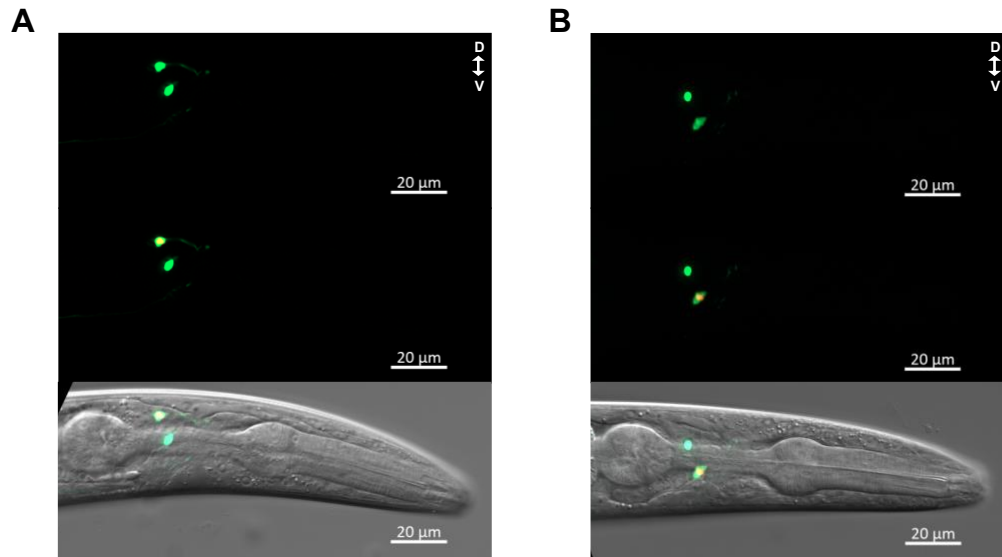


Figure S2: *frpr-14* translational GFP reporter colocalizes with AVH and AIB-specific markers.

Representative images of *frpr-14* translational GFP reporter co-expressed with (A) AVH::*mCherry-H2B* and (B) AIB::*mCherry-H2B* in L4 stage animals. Top – GFP only. Middle – GFP and mCherry merge. Bottom – DIC, GFP, and mCherry merge.

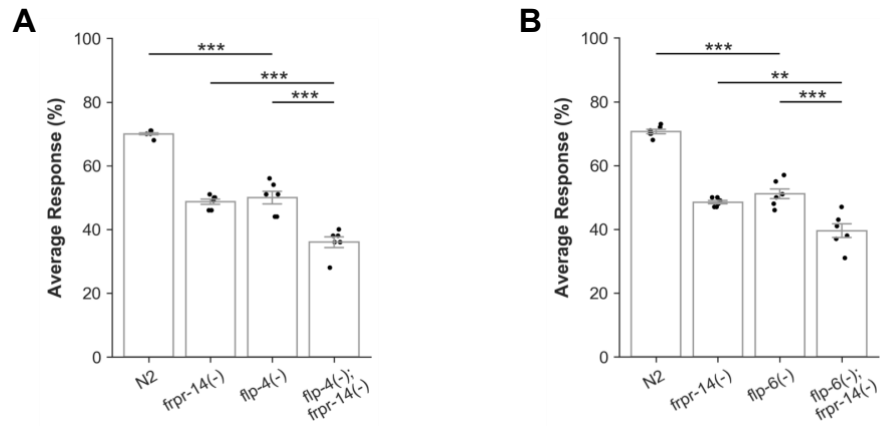


Figure S3: *flp-4* and *flp-6* receptor double mutant epistasis analysis for touch-evoked escape response.

(A) *flp-4(-); frpr-14(-)*. Error bars represent standard error of the mean. N=6 batches. One-way ANOVA followed by Tukey's HSD posthoc. *** $p < 0.001$, n.s.=no significance. N2 – *flp-4(-)* ($p = 2.035e-08$), *flp-4(-)* – *frpr-14(-)* ($p = 9.100e-01$), *flp-4(-)* – *flp-4(-); frpr-14(-)* ($p = 5.331e-6$).

(B) *flp-6(-); frpr-14(-)*. Error bars represent standard error of the mean. N=6 batches. One-way ANOVA followed by Tukey's HSD posthoc. *** $p < 0.001$, n.s.=no significance. N2 – *flp-6(-)* ($p = 5.920e-08$), *flp-6(-)* – *frpr-14(-)* ($p = 5.896e-01$), *flp-6(-)* – *flp-6(-); frpr-14(-)* ($p = 1.030e-4$).

CE-FRPR-14	MEPSTIELLDFEPPPIFCNSCLGSTDPEYLTYNLAVSGVLLAIVGMIGLIGNMLLVKTYL	60
CBR-FRPR-14	MEASTSQMLNFEPPPIFCNSCLGSTDPEYLTYNLAVSGVLLAIVGMIGLIGNILVVKTYL	60
	** * * : * : ***** : *****	
CE-FRPR-14	HPEQAIHSTSIYLAALAFSDFFLVLTAMFLFVLEAWRHHDYPTLAYLYVIGAPIVFPVAA	120
CBR-FRPR-14	HPELAIHSTSIYLAALGFSDFFLVLTAMFLFVLEAWRHHDYPTLAYLYVIGAPIVFPVAA	120
	** * ***** , *****	
CE-FRPR-14	VFQTSVVYFCVAAAVDCFIMVVLPEVSKQLYCTPRRAKITCVVLMICFIYNIPHFFELE	180
CBR-FRPR-14	VFQTSVVYFCVAAAVDCFIVVVLPEVSKVLYCTPRRAKMTCLILVICCAYNVPHPFELE	180
	***** : ***** ***** : * : * : * * * * : *****	
CE-FRPR-14	KVDCLDEDGRDSMQICPTDIRLDPAYYAIYYTYMYTFFLAIGPLTLLILLNICVVFTVVT	240
CBR-FRPR-14	KVDCLDDNGLDSMQICPTDIRLDAAYYAIYYTYMYTFFLAIGPLSLLILLNVCVVFTVVT	240
	***** : * ***** ***** : ***** : *****	
CE-FRPR-14	KGSSNENGEDDTISLILVVFFFIFCNFTALMVNFMEIILNDPMSMLVYFVDLSNLLVVVNG	300
CBR-FRPR-14	KGSNEENGEDDTISLILVVFFFIFCNFTALMVNFMEIIFDDPTMLVYFVDLSNLLVVVNG	300
	** * . ***** : * : * : *****	
CE-FRPR-14	TANFFCYLIFGTSFRATLKKVVLGSPAKRSVAVLWINDEEHKNQOSHALI	349
CBR-FRPR-14	TANFFCYFIFGTSFRNTLKKTILGAPAKRSVAVLWINDQENKNQQTSLI-	348
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Figure S4: Multiple sequence alignment of *C. elegans* CE-FRPR-14 and *C. briggsae* CBR-FRPR-14.

CE-FRPR-14 and CBR-FRPR-14 share 88.51% identity. Alignment performed in Clustal Omega.

<i>flp</i>	Strain	Allele
1	PS8997	<i>sy1599</i>
2	PS7370	<i>ok3351</i>
3	PS7379	<i>ok3265</i>
4	PS9050	<i>sy1606</i>
5	VC3280	<i>gk3123</i>
6	VC2324	<i>ok3056</i>
7	RB1990	<i>ok2625</i>
8	PT501	<i>pk360</i>
9	PS5793	<i>yn36</i>
10	PS7474	<i>ok2624</i>
11	PS7727	<i>tm2706</i>
12	RB1863	<i>ok2409</i>
13	PS6813	<i>tm2427</i>
14	VC1957	<i>gk1055</i>
15	VC2504	<i>gk1186</i>
16	RB2275	<i>ok3085</i>
17	PS7473	<i>n4894</i>
18	AX1410	<i>db99</i>
19	RB1902	<i>ok2460</i>
20	RB2188	<i>ok2964</i>
21	RB982	<i>ok889</i>
22	PS9052	<i>sy1608</i>
24	VC1971	<i>gk3109</i>
25	VC1982	<i>gk1016</i>
32	PS7219	<i>sy853</i>
34	PS7220	<i>sy810</i>

Table S1. List of *flp* mutant strains screened for spontaneous motility phenotype.