Supplementary Material

Supplementary Methods

Quantitative PCR Experiments

For real-time PCR experiments, late L4 and early adult *C. elegans* were harvested and quantitative RT-PCR was performed as detailed previously (SIMON *et al.* 2008).

RIG-3 polyclonal Antibody generation and Immunoprecipitation

To generate the RIG-3 polyclonal antibodies, RIG-3 was expressed in pGEX4T-1 as a GST tagged fusion protein. Protein induction was done at 37°C for 3-4 hrs. The size of induced protein was approximately 93 KDa (67, RIG-3; 26, GST tag). This clone was used for custom polyclonal antibody generation from Merck.

Primers and Constructs:

pBAB#0013: P*myo-3*::CAM-1(FL)::GFP

PRS051 Forward (*Xba*I site) CTAGTCTAGAatgtctccccgaccagaagac

PRS080 Reverse (*BamH*I site) CGCGGATCCatcagaatcaccatcctctgaata

pBAB#0014: P*myo-3*::CAM-1(ΔIg):: GFP

PRS051 (*Xba*I site) CTAGTCTAGAatgtctccccgaccagaagac

PRS052 (*Sac*II) TCCCCGCGGctcgtctcccgaacttttggt

PRS053 (*Sac*II) TCCCCGCGGgtctcaaatccagcagcctc

PRS054 (*Xho*I) CCGCTCGAGactcgttggtcatc

Deletion of the Ig domain was obtained by overlap extension PCR. PRS051 and PRS 052 were used to amplify the N-terminal region of the Ig domains. PRS053 and PRS054 were used to amplify C-terminal region of Ig domain. Finally the fragment with Ig domain deletion was amplified by overlap extension PCR by using oligos, PRS051 and PRS054 and cloned into the *Xba*I and *Xho*I sites in CAM-1 containing construct.

pBAB#0038: P*myo-3*::CAM-1(FL)

To obtain this construct, pBAB#0013 was digested with *Kpn*I and *Sac*I. This removed the GFP fragment and the sticky ends were made blunt by adding T4 DNA polymerase to perform 3'

overhang removal. Following this ligation was performed and the expected construct was obtained by transformation.

pBAB#0039: P*myo-3*::CAM-1(ΔIg)

This construct was obtained from BAB#0014 by following the same protocol as given above for pBAB#0038.

pBAB#0015: RIG-3::GST

PRS074 (*BamH*I) CGCGGATCCggacgactacttgccaag

PRS075 (*Not*I) ATGCGGCCGCcgtggcaagggcaagagg

pBAB#0021: P*unc-17*::LIN-44

PRS029 (*Nhe*I) CTAGCTAGCatgcgagcagctccttttg

PRS030 (*Kpn*I) GGGGTACCgcttttcggcggtgtcccat

pBAB#0023: P*unc-129*::LIN-44::mCherry

PRS029 (*Nhe*I) CTAGCTAGCatgcgagcagctccttttg

PRS030 (*Kpn*I) GGGGTACCgcttttcggcggtgtcccat

pBAB#0037*: Pmyo-3*::HMP-2

PRS335 (*Nhe*I) CTAGCTAGAatgcttcttcactctaccaactc

PRS336 (*Kpn*I) GGGGTACCcacaattggtacgataccgatttg

pBAB#0040: P*myo-3*::CAM-1(FL)::VC155

pBAB#0041: P*myo-3*::CAM-1(ΔIg)::VC155

pBAB#0042: P*unc-17*::RIG-3::VN173

pBAB#0043: P*unc-17*::RIG-3(GPI anchor swapped with TM domain of NLG-1)

PRS389 (*Nhe*I) CTAGCTAGCatgggacgactacttgcc

PRS390 (*Kpn*I) GGGGTACCCttagacctgtatctcttccaatgtc

pBAB#0044: P*myo-3*::CAM-1(ΔKr)::VC155

pBAB#0045: P*myo-3*::CAM-1(ΔCRD)::VC155

pBAB#0046: P*myo-3*::CAM-1(Ig domain replaced with non-coding domain)::VC155

pBAB#0047: P*myo-3*::CAM-1(Kr & CRD replaced with Ig domains)::VC155

pBAB#379 : LIN-44::GFP

pBAB#0048: P*unc-129*:: CAM-1(FL)::VC155

pBAB#0049: P*unc-129*:: CAM-1(ΔIg)::VC155

The inserts for the plasmids pBAB#0040-47 were custom generated and cloned into *XbaI* and *KpnI* sites under the *myo-3* promoter (pBAB#0040-41 and 44-47) or the *unc-17* promoter (pBAB#0042, 43) in the pPD49.26 vector.

Custom synthesized constructs for Bi-Fluorescence Complementation (BiFC) Experiments

RIG-3::VN173

CTAGCTAGCatgggacgactacttgccaagatgctcttccctcttgcgatgtgtcttttcgtttccgcagtttccgcatcagatagtcc
atcttccaatgaggcaaatcctatcgtcatttcatcagaaGTGAGCAAGGGCGAGGAGCTGTTCACCGGGG
TGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGT
GTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATC
TGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTA
CGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCA
AGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGA
CGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAAC
CGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACA
AGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAA
GAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGggcggcggcggctccggc
ggcggcggctccggcggcggcggctccggcggcggcgcCATGGATTACGACACAAACACAATCACAG
TCAGAGAAGGAAAGAAGTTAATGGTTAGTTGTGTATTTGAAAGTGACGAACAAAT
CCACAAAAGTGATCTTCTTTGGAAACAAGCTAATGGAAACAATATCGACGGAGAA
TCCAATCCAAGTCTTTTCTCTGTGATTTTGAACGAAAAGGGGAGCAAACATCGCAA
AACATCGCTACATTTCTCATCGGTTCACACGAGAGACACCGGACTCTACACGTGCA
CAGGAAGAACTGCTGGGGGAGAAAATTTCGAGAAGACCATCAAATTGGTCGTACT
CCCTGCCATTGAATGGAATGATAAAGATACTGTGAAGGGAGCACTTCTTGGAGAG
CCCATCACAATTGACTGTGGAGTTAAGGGGCCATCCGGTAAAGAACCAATGATTC
AGATGACAAATGGAAATGGTGAGCCACTCGATGAAGAAATCTGGACAATTGCTGG
AAATGAAGCCACCATTGATAGCTTGAAAAAAGAGCATGCCGAGTTAACAGTGTCT
TGTATTACTATTGAGATGCACCAGGAAACAAGCAAGGAAGAATTCCCAGTTGTTG
ACAGAAAAGATGTTAACATCGAAGTTTACACCCTTCCCGAGTTTGAGACGGAAGA
ATCTGTGCAGTACACAGTTATTGATAACCACGTCCGTGATGCAATTATCTACTGTA
ATGTGACACATTCCTTCCCACCAGTTCGTCACTACACTTTTTATCACGGAGACGAG
GAGATCAAGATGAGCGATAAATTCAACATTTTTGTGAATGTCGGAGTTTCTCAAG
GAGCACATCTCAAAATTCACAATGTCAATGAGAACGATTTGGGCACTTACAAATG
CGAAGCTAACAATATCAAAGCAAAATCCTACCATACAATTCATCTTAGAGAAGCC
AATGCCCCAGCTGAGCCAAAAGTAACTCTTATCGAGGACAAGAGGCACTCCATCA
TCTGGAAAGTAGAATCTATCGATCGAGATCCAGATCTTCCAATGACCGCCGTTGA
AATTCGCCACCTCCGAGCCGGAACCGCCGAAGCTTCTGGAGTGTCCGATGAGGAT
ATTTCTGATGCCTACTGGAAGAGTCACTCAATTTTCATGCAGAGAAATATCAAGGA
TGATGGAATTTACGAAATTAATGGGCTAAGACATGGACATGAATATGTCTGGAGA
TTCCGACAGATTAATGAAGCTGGATTTGGAGATAGTGTGGTATTGCGTGCAAAAA
CGTTAGATGATATGATGGATTCGGCATCTGACAGCAAATTTCCTCTTGCCCTTGCC
ACGTTATTTTTTGTCTGTCTTTTTATCTAAGGGTACCC

CAM-1(FL)::VC155

atgtctccccgaccagaagacgacgatctcgtgatagaaccagccgacgatgagggtcttcactacggaaatgcatcaatggagggta
catcaactggtcaacgaccgtacatccgtctcacttctcaacttcgaaatgccaccCAGAAGAACGGCATCAAGGCC
AACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACT
ACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTA
CCTGAGCTACCAGTCCAAACTGAGCAAAGACCCCAACGAGAAGCGCGATCACAT

GGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTG
TACAAGggcggcggcggctccggcggcggcggctccggcggcggcggctccggcggcggcgcAAAAGTTCGGG
AGACGAGGTTCGGTTTAAGTGCGAAGCACTTGGAACCCCACCACTCAAATTTATA
TGGCTCAAAAACAATGGTCCAGTGGAAAAGACGAAACGAGTGAAAATTCGTGAT
AAGGAGAATTCATCTCGACTAGTCATTACACAATTGGACGTATTAGACAGTGGAT
ACTATCAATGCATTGTCTCAAATCCAGCAGCCTCGGTTAACACGACAAGTGTGCT
CAGGGTGAACAACGTGCCGGATGCGGTGAAATTGTCACAGAAGAAAGGATCACA
TCACTCGACAAAACATATTGCATTCGACGAGTACGAGGACTACGAGATGATGGAT
CGTGGACGGTTACCAGACGAAGAAGACGCAGACCTCTACCGTGTCCCTGACTCAG
CAGCTGGCTCCAACTACGCCCCTGTCGCTGTATCAGAACGATGGCTAGATGGTAT
CAAATACCGTGTCGGCGACTGTGTCCAGTATCGTGGTGAAGCGTGTCGACAGTAC
TTGTCCAACAAGTTTGTAATGATGACCAACGAGTCTCGAGAGGAAATGTATGACA
TTGACCGTAATCTTCGAGCCGCCATGTTGTTCATCAACGGAGCACCGACGATCTCT
CAAAAGTGTCGGCAACTATCACAGGCGGTCGCTTGTCATCATATGTATAAAGTTT
GCGAATCGGATTCTAACAATCAAATTGTTTCGATTTGCAAGCACGATTGTGACGTT
ATTCAGAATGACGAATGCCCATCAGAATTGGCACTTGCCGCGCAACACGAATTGG
TTGGGGATACACCAAAGGCGTTATTCCCGTTGTGTTCTCGGTTATCATCTACATCA
AATTGTATTCCGGTTATGAGCACGGCGCTTCAGAGTAGCCCCGTTGCCGAAGTAA
ATCGTGGTCATCTTACCCATTGGTGTTATGTGAACAGTGGTACACAATACGAAGG
AACCGTTGCCCAAACATCATCTGGAAAGCAATGTGCTCCATGGATCGACTCGACA
TCTCGTGACTTTAATGTTCATCGTTTCCCAGAACTTATGAATTCTAAAAACTATTG
CCGAAACCCAGGTGGCAAGAAGAGCCGTCCATGGTGCTATTCAAAGCCAATGGG
CAAGAGGAATACTGTGATGTTCCACAATGTCCAAGTGATATGTATCCTCATTTGA
ATGATAAGAAAGTGGAGGGAAGCACAAAAGGAGGCGTCTCAGAGTCTGTAACAG
CTCTTTGGGATTCTCTTGATCCTACAATGCAAGTAGCTCTTGTTGGTGGTGGAGTA
TTCTTTTCCCTTTTACTTCTTCTTTTGTTCTGCTGTGCGTGTTGCTGTCGAGCAAAG
AAGAAGTCTCAAAAGACACGTCATCAGAATGCACATTGCTCCAGTGCTCCTTCTG
TGATCAATAGTGCCGCCAATTCTGCATATTATCGGAAATTAAATGGAACAAGTAC
ACCTATAATGGGACGGGTACCTTGGCTCCAACTACGCCCCTGTCGCTGTATCAGA
ACGATGGCTAGATGGTATCAAATACCGTGTCGGCGACTGTGTCCAGTATCGTGGT
GAAGCGTGTCGACAGTACTGCCAAAAGGGTTCCCCG

The sequences shown above represent the constructs utilized for the venus based BiFC interaction studies. The sequence shows signal sequence (lower case blue), part of the YFP variant venus (green), linker sequence (lower case red), Ig domain (lower case purple) and the gene sequence (black). These inserts were custom generated from Thermofisher GENEART. Six additional constructs have been generated and used as controls in these studies. In case of RIG-3(TMD), a construct was generated where the C-terminal 23 amino acids, encoding for the region allowing for the GPI anchor attachment in RIG-3 has been replaced with the transmembrane and cytosolic regions of the NLG-1 protein, the sequence of which is:

GCTCTCGGTGGCGTTATTTTCATCGGTTGTGGATTCCTCATTATGAACGTTTGCCTA
TTAATTGCTGTTCGTAGAGAATGGGGAAAGAAGCGGCGGAACGAGAAGAAGTTTC
AACTGCAGTATCAGACTTATAACTCCAACCATGGCGGCGGCGCGGAACAATACAA
CAGCTTAAACTCGCCGGAACCCTTACTATCCGCATCGCACAAGAACTCAACTTCGA

TGCGACCCGCGGGAATATCACCAACGTGTCCACGTCACGGACGTGCCGCGCTTGC
GCTTCAAAATAGCCGAGGTAACAGTTTGACTGCTGCTCAAGCACCGACATTGGAA
GAGATACAGGTCTAA

In case of CAM-1, various domain encoding regions were deleted or swapped. The domain encoding regions are marked in the sequence above and are detailed here:

Ig domain (nucleotides 463-638, amino acids 155-213 indicated in purple), CRD domain (nucleotides 906-1305, 303-435, amino acids indicated in orange); Kringle domain (nucleotides 1353-1593, amino acids 452-531 indicated in brown).

In CAM-1(ΔIg) ::VC155, the Ig domain region was deleted. For the rest of the constructs we have swapped various domains as discussed below:

1. CAM-1(ΔKr, ΔCRD)+2 Ig domains::VC155- In this construct both CRD and Kringle domains have been replaced with Ig encoding amino acids from CAM-1 itself.

2. CAM-1(ΔCRD)::VC155- In this construct the CRD encoding amino acids have been removed

3. CAM-1 (ΔKr)::VC155- Here we have deleted the Kringle-dpmain encoding region

4. CAM-1 (ΔIg)+non-functional domain::VC155- Here the Ig domain of CAM-1 was replaced with a putative non-domain encoding DNA and the sequence of which is:

TCGGTTAACACGACAAGTGTGCTCAGGGTGAACAACGTGCCGGATGCGGTGAAAT
TGTCACAGAAGAAAGGATCACATCACTCGACAAAACATATTGCATTCGACGAGTA
CGAGGACTACGAGATGATGGATCGTGGACGGTTACCAGACGAAGAAGACGCAGA
CCTCTACCGT

Table S1. Description of mutant strains used in the study

Genotype	Strain name and allele description	References
rig-3(ok2156)X	**RB1712**. *rig-3(ok2156)* X. The *ok2156* mutation deletes 1.5kb of the *rig-3* gene, spanning exons 2–5 (including most of the Ig domains); consequently, *ok2156* is likely to give rise to a null mutation.	(SCHWARZ *et al.* 2009; BABU *et al.* 2011)
cam-1(ak37)II	**VM3896.** The *cam-1(ak37)* deletion Mutation is a predicted null allele that causes uncoordinated locomotion. The *cam-1(ak37)* mutation removes 2.5 kb of the coding sequence, leaving intact only the coding sequence for the Ig and CRD domains.	(FRANCIS *et al.* 2005)

lin-44(n1792)I	**MT5383.** At 20°C about 50% of the hermaphrodites are *egl.* This mutant has a point mutation that causes a W100 to Amber change and is likely a null allele.	(HERMAN *et al.* 1995)
hmp-2(qm39)I	**MQ468** This mutataion has some embryonic lethality and a high degree of larval lethality is seen. In later stages the anterior half of the body is short but well developed while the posterior is half is thinner.	(HEKIMI *et al.* 1995)

Table S2- List of Integrated lines:

S.no.	Plasmid	Integrated Line number	Source and reference
1	P*myo-3*::ACR-16::GFP	*nuIs299*	Josh Kaplan Lab (BABU *et al.* 2011)
2	CAM-1::GFP	*cwIs6*	Wayne Forrester Lab (KIM AND FORRESTER 2003)
3	P*unc-17*::RFP	*nuIs321*	Josh Kaplan Lab (BABU *et al.* 2011)
4	P*unc-25*::GFP	*juIs76*	CGC (WU *et al.* 2007)
5	P*unc-129*:: SYD-2::GFP	*nuIs160*	Josh Kaplan Lab (SIEBURTH *et al.* 2005)
6	P*acr-2*::mCherry::RAB-3	*ufIs63*	Mike Francis lab (PETRASH *et al.* 2013)

Table S3- List of Transgenes and Arrays:

S.no.	Plasmid	Plasmid number	Array number
1	P*myo-3*::CAM-1(FL)::YFP	BAB#0013	*indEx11*
2	P*myo-3*::CAM-1(ΔIg)::YFP	BAB#0014	*indEx17*
3	P*myo-3*::CAM-1(FL)	BAB#0038	*indEx25*
4	P*myo-3*::CAM-1(ΔIg)	BAB#0039	*indEx26*
5	P*rig-3*::mCherry::RIG-3 from (BABU *et al.* 2011)	KP#6298	*indEx21*
6	P*myo-3*::CAM-1(FL)::VC155	BAB#0040	*indEx41*
7	P*myo-3*::CAM-1(ΔIg)::VC155	BAB#0041	*indEx42*
8	P*unc-17*::RIG-3::VN173	BAB#0042	*indEx43*
9	P*unc-17*::RIG-3::VN173 and P*myo-3*::CAM-1(FL)::VC155	BAB#0042 and BAB#0040	*indEx44*
10	P*unc-17*::RIG-3::VN173 and P*myo-3*::CAM-1(ΔIg)::VC155	BAB#0042 and BAB#0041	*indEx45*
11	P*unc-17*::LIN-44	BAB#0021	*indEx28, indEx29, indEx30*

12	P*unc-129*::LIN-44::mCherry	BAB#0023	*indEx32*
13	P*myo-3*::HMP-2	BAB#0037	*indEx46, indEx47*
14	P*myo-3*::CAM-1(FL)::VC155 (injected into *cam-1*)	BAB#0040	*indEx48, indEx49*
15	P*myo-3*::CAM-1(ΔIg)::VC155 (injected into *cam-1*)	BAB#0041	*indEx50, indEx51*
16	P*unc-17*::RIG-3::VN173 in *rig-3* (injected into *rig-3*)	BAB#0042	*indEx52, indEx53*
17	P*unc-17*::RIG-3(TMD)::VN173 and P*myo-3*::CAM-1::VC155	BAB#0043 and BAB#0040	*indEx54, indEx55*
18	P*unc-17*::RIG-3::VN173 and P*myo-3*::CAM-1(ΔKr)::VC155	BAB#0042 and BAB#0044	*indEx56, indEx57*
19	P*unc-17*::RIG-3::VN173 and P*myo-3*::CAM-1(ΔCRD)::VC155	BAB#0042 and BAB#0045	*indEx58, indEx59*
20	P*unc-17*::RIG-3::VN173 and P*myo-3*::CAM-1(ΔIg domain+non-coding domain)::VC155	BAB#0042 and BAB#0046	*indEx60, indEx61*
21	P*unc-17*::RIG-3::VN173 and P*myo-3*::CAM-1(ΔKr, ΔCRD) +2XIg domains::VC155	BAB#0042 and BAB#0047	*indEx62, indEx63*
22	P*unc-129*::LIN-44::mCherry in *mig-14*	BAB#0023	*indEx34, indEx35*
23	P*myo-3*::CAM-1(ΔIg) in N2	BAB#0013	*indEx71*
24	*cwIs6*; P*myo-3*::CAM-1(ΔIg)	BAB#0014	*indEx72*
25	P*unc-129*::RIG-3::VN173 and P*myo-3*::CAM-1::VC155	BAB#48 and BAB#0040	*indEx73*
26	P*unc-129*::RIG-3::VN173 and P*myo-3*::CAM-1(ΔIg)::VC155	BAB#48 and BAB#0041	*indEx74*
27	P*unc-129*::RIG-3(TMD)::VN173 and P*myo-3*::CAM-1::VC155	BAB#49 and BAB#0040	*indEx75*
28	P*unc-129*::RIG-3(TMD)::VN173 and P*myo-3*::CAM-1(ΔIg)::::VC155	BAB#49 and BAB#0041	*indEx76*

Table S4- List of strains:

S. no.	Genotype	Strain number	Source and reference
1	*rig-3(ok2156)*	RB1712	CGC
2	*cam-1(ak37)*	VM3896	CGC
3	*lin-44(n1792)*	MT5383	CGC
12	*hmp-2(qm39)*	MQ468	CGC
20	*lin-44(n1792); rig-3(ok2156)*	BAB035	
21	*lin-44(n1792); cam-1(ak37)*	BAB036	
22	*cam-1(ak37); rig-3(ok2156)*	BAB037	
26	*hmp-2(qm39); rig-3(ok2156)*	BAB085	
27	*hmp-2(qm39); cam-1(ak37)*	BAB086	
28	*lin-44(n1792); cam-1(ak37); rig-3(ok2156)*	BAB087	
31	*rig-3(ok2156); nuIs299*	BAB060	
32	*cam-1(ak37); nuIs299*	BAB062	
33	*lin-44(n1792); nuIs299*	BAB069	

34	*lin-44(n1792); rig-3(ok2156); nuIs299*	BAB070	
35	*lin-44(n1792); cam-1(ak37); nuIs299*	BAB067	
32	*rig-3(ok2156); zwIs132*	BAB802	
33	*cam-1(ak37); zwIs132*	BAB805	
34	*lin-44(n1792); zwIs132*	BAB803	
35	*lin-44(n1792); rig-3(ok2156); zwIs132*	BAB804	
36	*lin-44(n1792); cam-1(ak37); zwIs132*	BAB801	
37	*lin-44; zwIs132; indEx29*	BAB806	
38	*hmp-2(qm39); nuIs299*	BAB073	
39	*lin-44(n1792); nuIs321*	BAB064	
40	*rig-3(ok2156); indEx21*	BAB079	
41	*cam-1(ak37); indEx11*	BAB066	
42	*cam-1(ak37); indEx17*	BAB067	
43	*cam-1(ak37); nuIs299; indEx25*	BAB057	
44	*cam-1(ak37); nuIs299; indEx26*	BAB058	
45	*cwIs6; indEx21*	BAB094	
46	*indEx41*	BAB826	
47	*indEx42*	BAB827	
48	*indEx43*	BAB828	
49	*indEx44*	BAB829	
50	*indEx45*	BAB830	
51	*lin-44(n1792); indEx28*	BAB078	
52	*lin-44(n1792); indEx29*	BAB082	
53	*lin-44(n1792); indEx30*	BAB083	
54	*indEx32*	BAB077	
55	*rig-3(ok2156); indEx32*	BAB079	
57	*hmp-2(qm39); indEx46*	BAB091	
58	*hmp-2(qm39); indEx47*	BAB097	
59	*rig-3(ok2156); indEx52*	BAB831	
60	*rig-3(ok2156); indEx53*	BAB832	
61	*cam-1(ak37); indEx48*	BAB833	
62	*cam-1(ak37); indEx49*	BAB834	
63	*cam-1(ak37); indEx50*	BAB835	
64	*cam-1(ak37); indEx51*	BAB836	
65	*hmp-2(qm39);rig-3(ok2156);nuIs299*	BAB819	
66	*indEx54*	BAB838	
67	*indEx56*	BAB839	
68	*indEx58*	BAB840	
69	*indEx60*	BAB841	
70	*indEx62*	BAB842	
71	*indEx64*	BAB843	
72	*indEx68*	BAB844	
73	*indEx69*	BAB845	
74	*indEx70*	BAB846	
75	*indEx71*	BAB847	
76	*indEx72*	BAB848	

77	*indEx73*	BAB849	
78	*indEx74*	BAB850	
79	*indEx75*	BAB851	
80	*indEx76*	BAB852	
81	*nuIs160; lin-44*	BAB853	
82	*lin-44;* P*unc-25*::SYD-2::GFP	BAB854	

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Supplementary Figures

Fig. S1

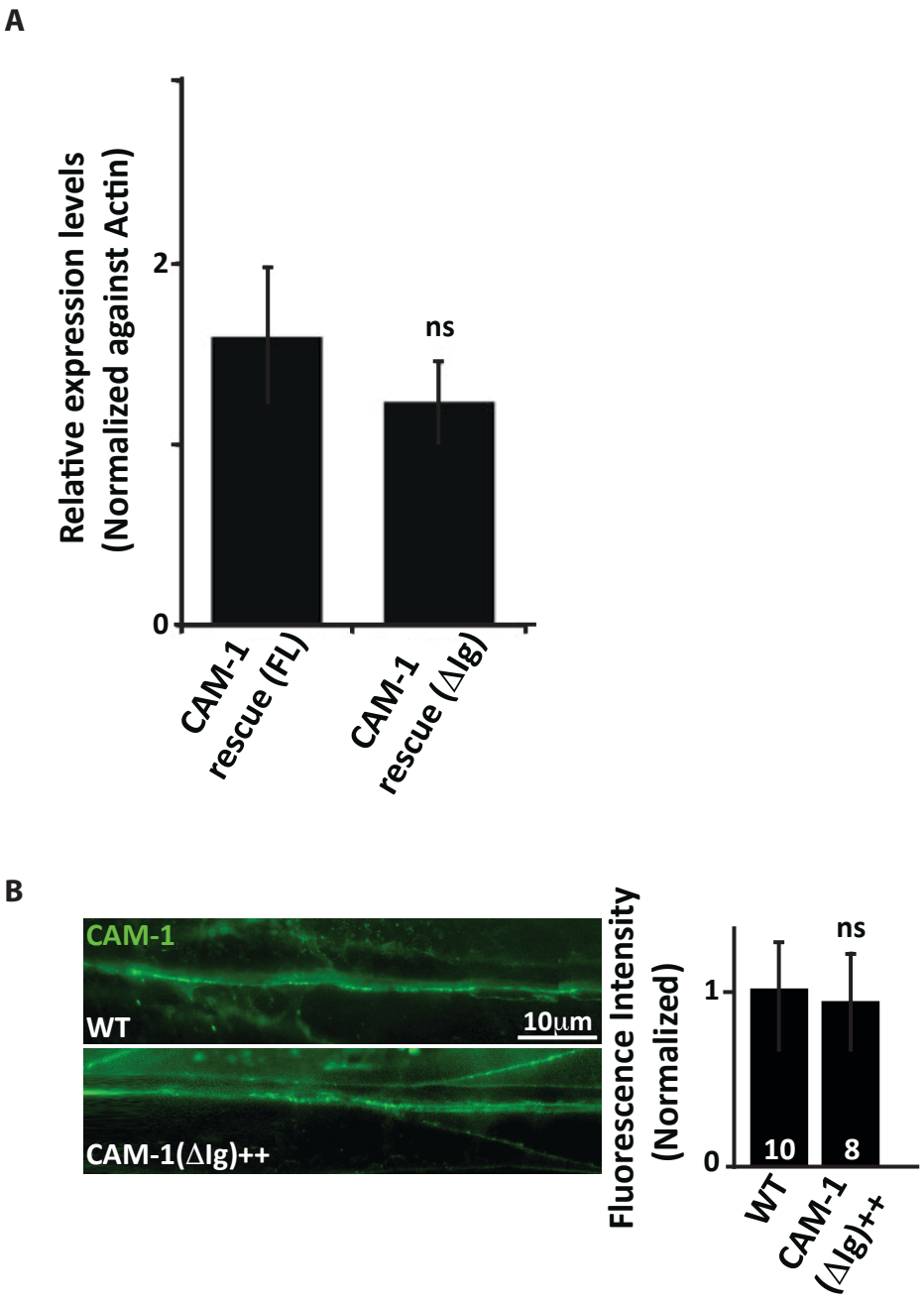


Figure S1. Expression analysis of Pmyo-3::CAM-1(FL) and Pmyo-3::CAM-1(ΔIg) rescue lines (Supplement to Fig. 1):
S1A. The graph shows quantitative PCR values of the P*myo-3*::CAM-1(FL) and P*myo-3*::CAM-1(ΔIg) array lines. The quantitative Reverse Transcription PCR values are plotted as normalized values against actin levels. It is evident from the graph that the expression pattern is similar for both the rescue constructs that were used in this experiment.
S1B. CAM-1::GFP fluorescent images using the *cwIs6* strain. This assay compared ventral cord fluorescence in *cwIs6* animals and cwIs6 *C. elegans* expressing the P*myo-3*::CAM-1(ΔIg) construct. To the right is a bar graph quantitating CAM-1::GFP fluorescence along the ventral cord in the two lines. Error bars indicate S.E.M.
In all figures "ns" indicates not significant and the numbers at the base of bar graphs indicate the number of animals imaged for that experiment.

Fig. S2

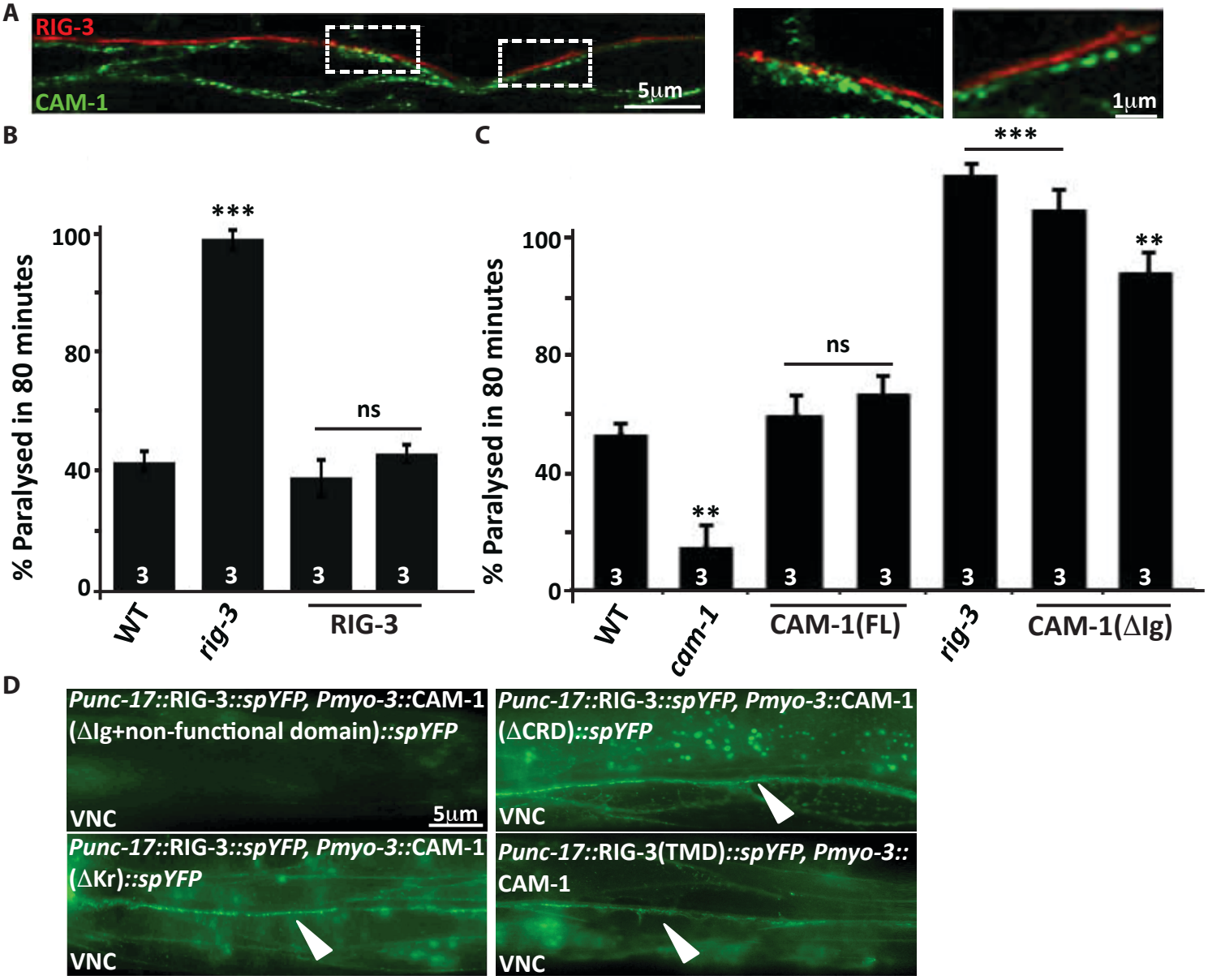


Figure S2. Interaction between RIG-3 and CAM-1 (Supplement to Fig. 2):

S2A. The expression pattern of RIG-3 and CAM-1 both tagged with fluorescence markers and expressed under their endogenous promoters indicates that they are expressed in regions opposing each other at the NMJ. The images shown in this panel are along the dorsal nerve cord of the animal. Shown in the lower panel are the zoomed-in regions of the boxed area in the top panel.

S2B and C. Aldicarb analysis of fusion proteins used in BiFC experiments: Aldicarb assays were performed with 1mM aldicarb and the number of *C. elegans* paralyzed at 80 mins were plotted as indicated in the bar graphs. RIG-3 rescue was done in cholinergic neurons under the *unc-17* promoter, while the CAM-1 rescue was done in body-wall muscle using the *myo-3* promoter. Two independent rescue array lines have been used in these experiments. In all aldicarb graphs the numbers at the base of the bar graphs indicate the number of times the experiment was performed with 15-25 *C. elegans* used in each trial, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$ and "***" indicates $p < 0.001$ in all figures. Unless otherwise indicated all p-values are with respect to WT animals. Error bars indicate S.E.M. in both panels.

S2D. BiFC between CAM-1 and RIG-3: The first panel indicates a CAM-1::*spYFP* construct where the Ig domain of CAM-1 was replaced by a non-functional domain and co-injected with RIG-3::*spYFP*. In the second and third panels Kringle and CRD domains were deleted from the CAM-1::*spYFP* construct and injected along with RIG-3::*spYFP*. The last panel shows RIG-3(TMD), where the GPI anchor region of RIG-3 is replaced with a transmembrane domain which was co-injected with CAM-1::*spYFP*. In all the experiments CAM-1 constructs were expressed in body-wall muscles while RIG-3 contructs were expressed in cholinergic neurons. In all cases except the absence of a functional Ig domain, CAM-1 and RIG-3 showed interaction as seen from the reconstituted YFP (arrowheads). All panels show representative images along the venral nerve cord (VNC) of the animals. For each strain 10-12 animals were analyzed.

Fig. S3

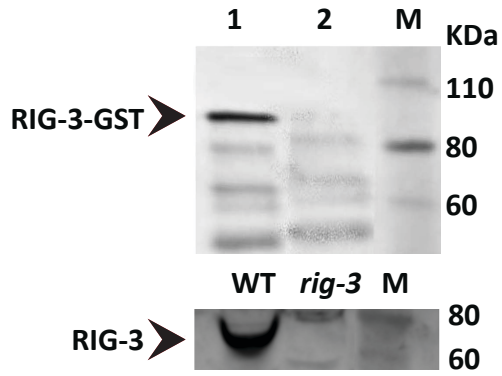


Figure S3. RIG-3 antibody probe (Supplement to Figure 3):
The upper panel shows an immunoblot of the RIG-3::GST fusion protein induced at 37^{o}C for 3 hrs. Lane 1 shows the induced protein, lane 2 shows the un-induced control and lane M indicates the prestained marker. The lower panel shows the blot for protein extracted from WT and *rig-3* mutant *C. elegans* that has been probed with the RIG-3 antibody.

Fig. S4

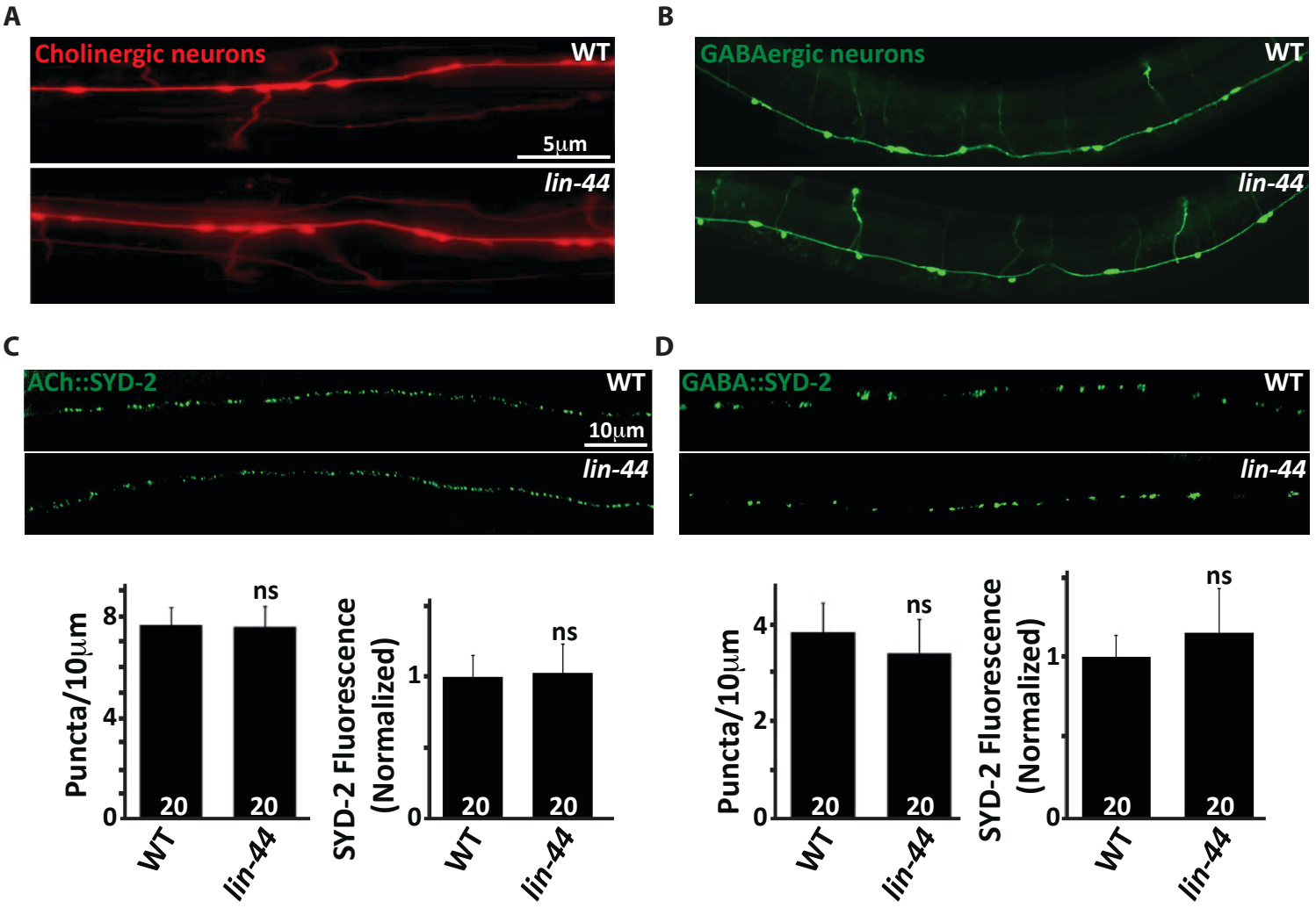


Figure S4. Developmental analysis of *Wnt/lin-44* mutants (Supplement to Fig. 4):
S4A and B. This panel shows representative images of P*unc-17*::RFP expression and P*unc-25*::GFP in WT and *Wnt/lin-44* mutant animals. There were no obvious defects seen in the *Wnt/lin-44* mutants in neuronal number, position or axon guidance using the P*unc-17*::RFP and P*unc-25*::GFP markers. 10-12 animals were analyzed for each genotype.
S4 C and D. Representative images and the summary data for an active zone protein SYD-2 (α-Liprin) in the dorsal nerve cord of WT and *Wnt/lin-44* mutants. SYD-2 protein is GFP-tagged and expressed in DA motor neurons (using the *unc-129* promoter) and GABAergic motor neurons (using the *unc-25* promoter).
SYD-2 fluorescence intensity and the punctal density were analysed and plotted as represented in the graphs shown below the images. The number of animals analyzed is indicated for each genotype at the base of each bar graph. Error bars indicate SEM. No significant differences were observed.

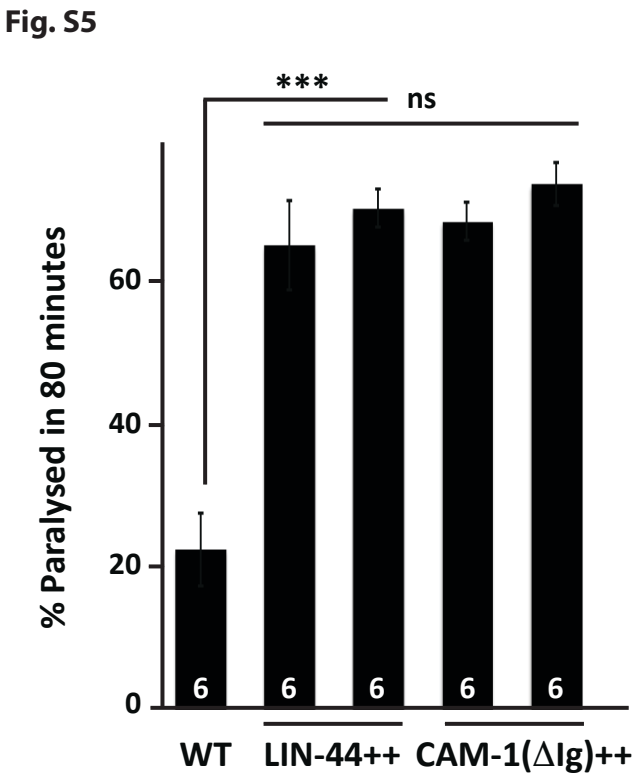
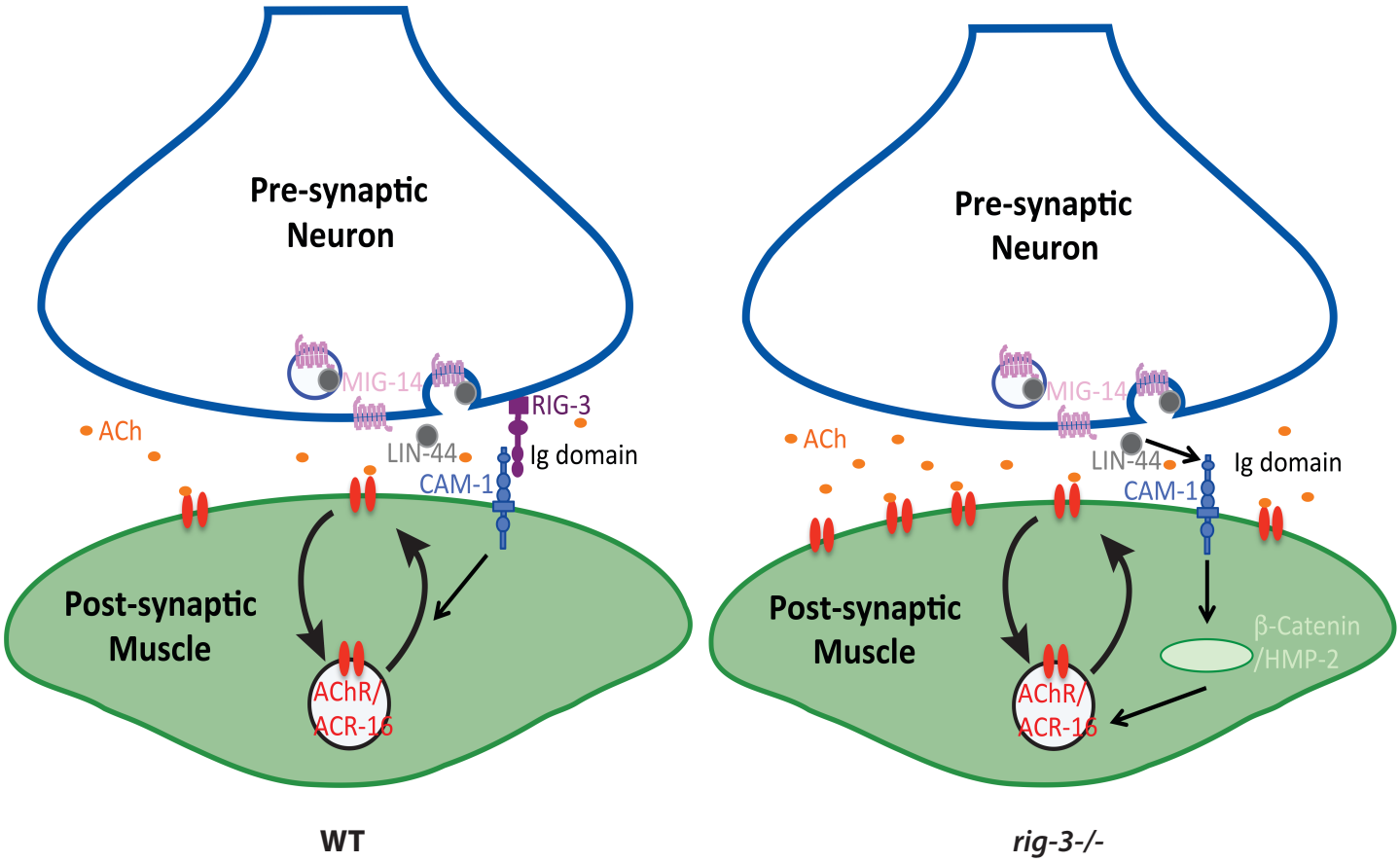


Figure S5. Aldicarb Assays with LIN-44 and CAM-1(ΔIg) overexpression strains (Supplement to Fig. 5): Aldicarb assay of Wnt/LIN-44 expressed in cholinergic neurons (under the *unc-17* promoter) and CAM-1(ΔIg) expressed in body-wall muscles (under the *myo-3* promoter). The bars represent *C. elegans* paralysed at 80 mins. Two independent array lines were used for each construct. Error bars indicate S.E.M.

Fig. S6



S6. Possible model for RIG-3 function at the NMJ:
RIG-3 functions pre-synaptically in cholinergic neurons (BABU *et al.* 2011), while CAM-1 functions post-synaptically in body-wall muscle (FRANCIS *et al.* 2005) to maintain normal AChR/ACR-16 at the *C. elegans* NMJ. Previous work has shown that the Wnt signaling pathway through CWN-2 functions to maintain normal AChR/ACR-16 levels at the *C. elegans* NMJ in wild type conditions (JENSEN *et al.* 2012). We show here that in the presence of increased activity brought about by aldicarb treatment, RIG-3 (expressed in cholinergic neurons) and CAM-1 (expressed in muscles) interact trans-synaptically at the NMJ and prevent the Wnt/LIN-44 from functioning through CAM-1, thereby causing an increase in AChR/ACR-16 levels at the NMJ (shown in the image on the left-hand side). In the absence of *rig-3* and again in the presence of increased activity brought about by aldicarb exposure, there is more Wnt/LIN-44 signaling through CAM-1 and an increase in AChR/ACR-16 at the NMJ (shown in the second image).