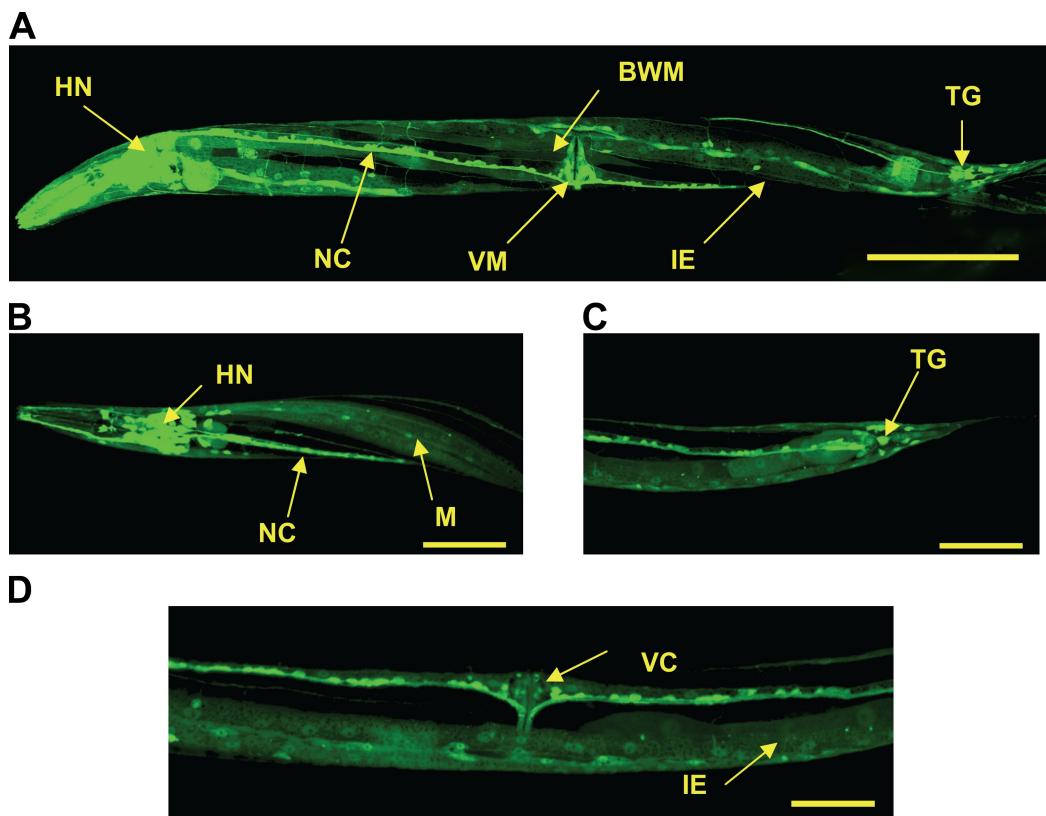
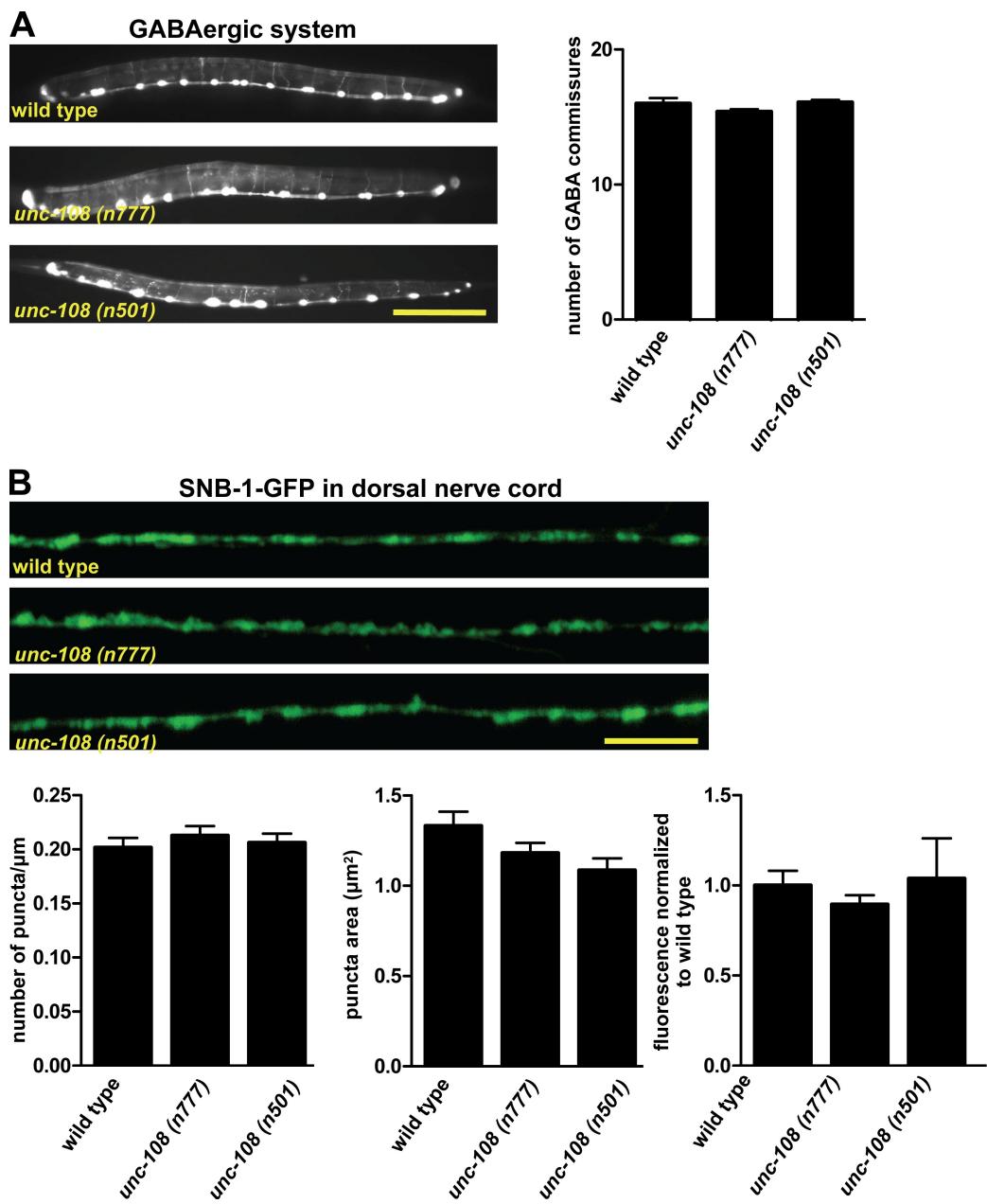


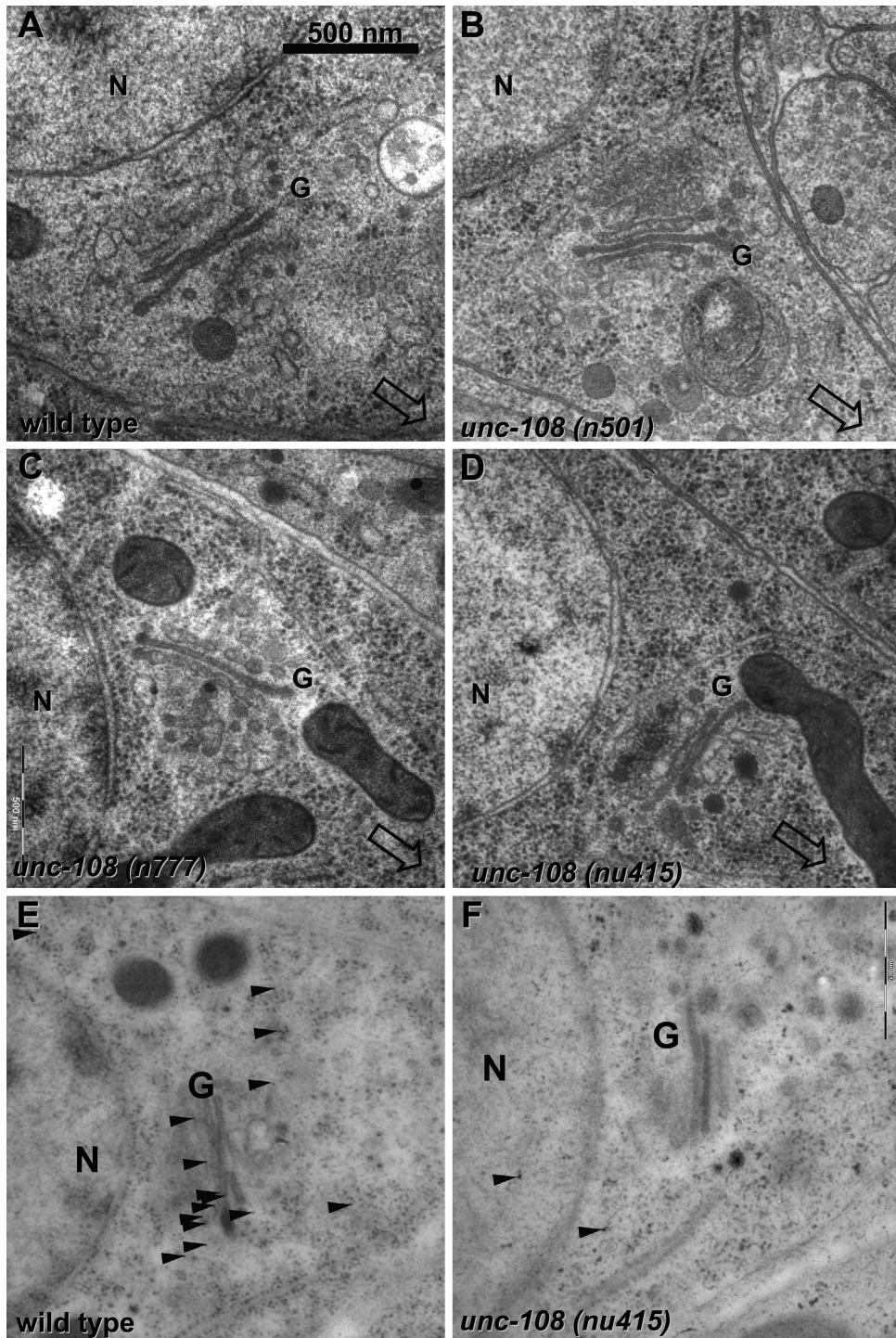
Sumakovic et al., <http://www.jcb.org/cgi/content/full/jcb.200902096/DC1>



**Figure S1.** *rab-2* shows ubiquitous expression with high expression levels in the nervous system. (A–C) GFP was expressed under the *rab-2* promoter. *rab-2* is expressed in the muscle (M) but shows strong expression levels in the nervous system. BWM, body wall muscle; HN, head neurons; NC, nerve cord; TG, tail ganglia; VM, vulva muscle. (D) Expression of *rab-2* in vulval cells (VC) and intestinal epithelium (IE). Bars: (A) 30  $\mu$ m; (B–D) 20  $\mu$ m.

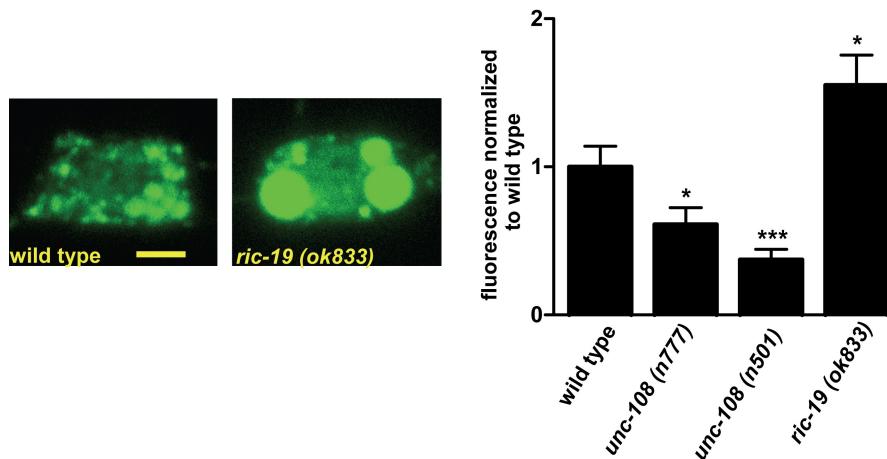


**Figure S2.** *unc-108* mutants have no defect in synaptic morphology nor GABAergic system development. (A) GABAergic motor neurons were visualized using a strain expressing GFP under *unc-47*, a GABA transporter promoter. The development of the GABAergic system appears to be normal in *unc-108* mutants, as judged by the number of commissures that GABAergic neurons send from the ventral to the dorsal side. Error bars = SEM. (B) Animals expressing SNB-1-GFP in cholinergic motor neurons were crossed into the *unc-108* background. The synapse morphology appears to be similar between wild type and *unc-108* mutants, as judged by SNB-1-GFP puncta number, size, and mean fluorescence. Error bars = SEM ( $n = 10$ ). Bars: (A) 20  $\mu\text{m}$ ; (B) 10  $\mu\text{m}$ .

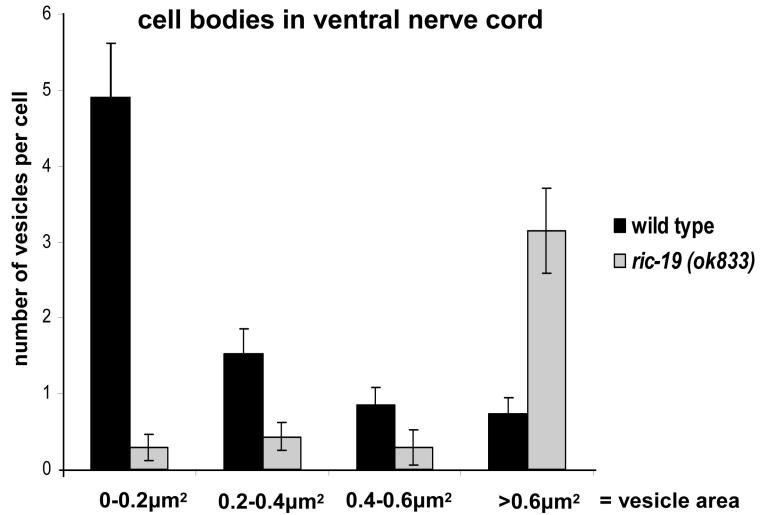


**Figure S3. Golgi morphology in neuronal cell bodies is not altered in *unc-108* mutants.** (A–D) Motor neuron cell bodies are shown for wild-type (A) and *unc-108*(n501) (B), *unc-108*(n777) (C), and *unc-108*(nu415) (D) alleles. The morphology of the Golgi complexes in dominant or recessive *unc-108* mutants appears normal when compared with wild type using HPF EM. Arrows indicate the direction of axonal processes. (E and F) Immunolabeling of endogenous RAB-2 in the wild-type (E) and null *unc-108*(nu415) strain (F). Arrowheads point to gold labels. N, nucleus; G, Golgi stack.

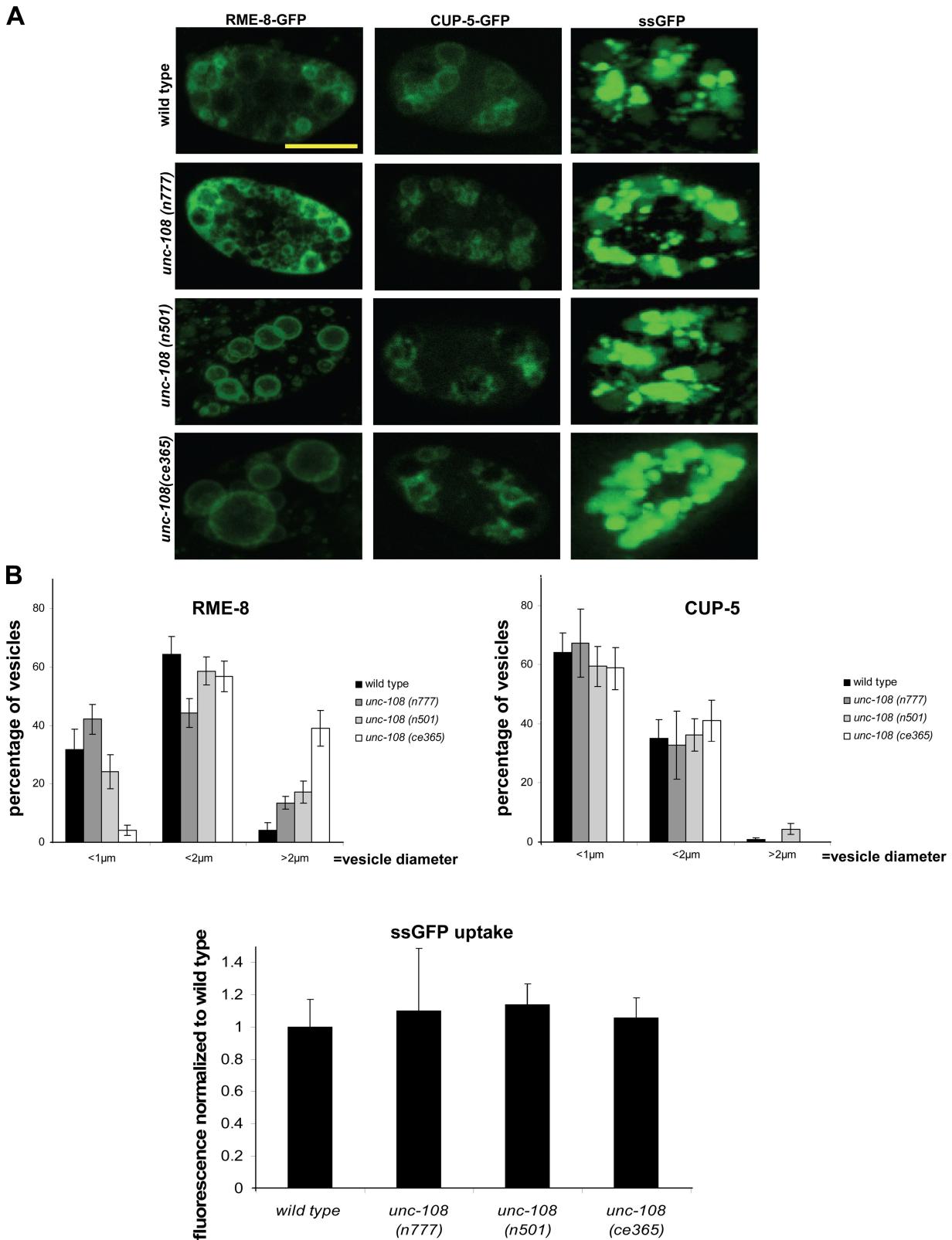
### A NLP-21-YFP in cell bodies in ventral nerve cord



### B cell bodies in ventral nerve cord



**Figure S4. The amount of neuropeptide is decreased in the neuronal cell bodies in the VNC.** (A) NLP-21-YFP is expressed in cholinergic motor neurons from integrated array *nul183*, and representative images of neuronal cell bodies in the VNC of wild-type animals and *ric-19* mutants are shown. The amount of neuropeptide present in the neuronal cell bodies of *unc-108* mutants is decreased compared with wild type, whereas it is increased in the *ric-19* deletion strain. Error bars = SEM (\*, P < 0.05; \*\*\*, P < 0.005; Student's t test; n = 10). (B) NLP-21-YFP neuropeptide-positive vesicles have different size distribution in *ric-19* mutants compared with wild type. Error bars = SEM (n = 10). Bar, 2 μm.



**Figure S5. *unc-108* mutants have no defect in fluid phase uptake in coelomocytes.** (A) Localization of RME-8 (early and late endosomal marker) and CUP-5 (endosomal/lysosomal marker) in wild-type and *unc-108* coelomocytes and uptake of GFP secreted from body wall muscle (ssGFP). (B) Size of RME-8- and CUP-5-positive vesicles in wild type and *unc-108* mutants was sorted as a percentage of the total number of vesicles into three categories based on their diameter, <1 μm, 1–2 μm, and >2 μm. *ce365* mutants have more of the large RME-8-positive vesicles (top), whereas the uptake of ssGFP for all *unc-108* strains is similar to wild type (bottom). Error bars = SEM ( $n = 10$ ). Bar, 5 μm.

Table S1. HPLC–MALDI-TOF MS analysis of wild type and *unc-108* mutants

Gene	Peptide sequence	Wild type	<i>unc-108(n777)</i>	<i>unc-108(n501)</i>
<i>nlp-1</i>	VNLDPNSFRMSFa	xx	xx	xx
<i>nlp-6</i>	YKPRSFAMGFa	x/ND	x/ND	ND
<i>nlp-6</i>	AAMRSFNMGFa	x/ND	x/ND	ND
<i>nlp-7</i>	MILPSLADLHRYTMYD	xx	x/ND	ND
<i>nlp-8</i>	YPYLIFPASPSSGDSRRLV	x/ND	xx	xx
<i>nlp-8</i>	SFDRMGGTEFGLM	x/ND	ND	ND
<i>nlp-9</i>	TPIAEAQGAPEDVDDRRELE	xx	x/ND	ND
<i>nlp-11</i>	SAPMASDYGNQFQMYNRLIDAa	xx	x/ND	ND
<i>nlp-11</i>	HISPSYDVEIDAGNMRNLLDla	xx	x/ND	x/ND
<i>nlp-11</i>	SPAISPAYQFENAFGLSEALERa	xx	xx	xx
<i>nlp-12</i>	DYRPLQFa	xx	xx	xx
<i>nlp-12</i>	DGYRPLQFa	xx	xx	xx
<i>nlp-13</i>	SPVDYDRPIMAFa	xx	xx	ND
<i>nlp-13</i>	SAPSDFSRDIMSFa	xx	x/ND	ND
<i>nlp-13</i>	AEDYERQIMAFa	x/ND	X/ND	ND
<i>nlp-14</i>	ALNSLDGNGCFGDF	x/ND	ND	ND
<i>nlp-14</i>	ALNSLDGQQGFGFE	x/ND	ND	ND
<i>nlp-16</i>	NAEDHHEHQ	xx	xx	xx
<i>nlp-18</i>	SPYRTFAFA	xx	xx	xx
<i>nlp-20</i>	SGPQAHEGAGMRFa	x/ND	ND	ND
<i>nlp-21</i>	GGARAFLTEM	x/ND	x/ND	ND
<i>nlp-21</i>	GGARAFYDE	x/ND	x/ND	ND
<i>nlp-24</i>	FTGPYGGYFa	x/ND	ND	ND
<i>nlp-35</i>	AVVSGYDNIYQVLAPRF	xx	xx	xx
<i>nlp-37</i>	NNAEVVNHIKKNFGALDRLGDVa	xx	xx	x/ND
<i>nlp-38</i>	SPAQWQRANGLWa	xx	xx	xx
<i>nlp-38</i>	ASDDRVLGWNKAHGLWa	xx	xx	ND
<i>nlp-39</i>	EVPNFQADNVPEAGGRV	xx	xx	ND
<i>nlp-40</i>	APSAPAGLEEKLR	xx	xx	x/ND
<i>nlp-41</i>	APGLFELPSRSVRLI	xx	xx	xx
<i>fip-1</i>	SDPNFLRFa	xx	xx	xx
<i>fip-1</i>	SADPNFLRFa	xx	xx	xx
<i>fip-1</i>	AGSDPNFLRFa	xx	xx	xx
<i>fip-1</i>	SQPNFLRFa	xx	xx	xx
<i>fip-2</i>	SPREPIRFa	xx	xx	x/ND
<i>fip-3</i>	NPENDTPFGTMRFa	xx	xx	ND
<i>fip-3</i>	NPLGTMRFa	x/ND	ND	ND
<i>fip-3</i>	SADDsapFGTMRFa	x/ND	x/ND	ND
<i>fip-3</i>	SAEPFGTMRFa	x/ND	x/ND	ND
<i>fip-3</i>	ASEDALFGTMRFa	x/ND	x/ND	ND
<i>fip-4</i>	ASPSFIRFa	xx	xx	ND
<i>fip-5</i>	GAKFIRFa	xx	xx	x/ND
<i>fip-5</i>	AGAKFIRFa	xx	xx	xx
<i>fip-5</i>	APKPKFIRFa	xx	xx	xx
<i>fip-6</i>	KSAYMRFa	xx	x/ND	ND
<i>fip-7</i>	TPMQRSSMVRFa	xx	xx	xx
<i>fip-7</i>	SPMQRSSMVRFa	xx	x/ND	ND
<i>fip-8</i>	KNEFIRFa	x/ND	x/ND	ND
<i>fip-9</i>	KPSFVRFa	xx	xx	xx
<i>fip-11</i>	NGAPQPFPVRFa	xx	xx	xx
<i>fip-11</i>	AMRNALVRFa	xx	x/ND	xx
<i>fip-11</i>	ASGGMRNALVRFa	xx	xx	xx
<i>fip-12</i>	RNKFEFIRFa	x/ND	ND	ND
<i>fip-13</i>	AADGAPLIRFa	xx	xx	xx
<i>fip-13</i>	ASSAPLIRFa	xx	xx	xx
<i>fip-13</i>	APEASPFIRFa	xx	xx	xx

Table S1. HPLC–MALDI-TOF MS analysis of wild type and *unc-108* mutants (Continued)

Gene	Peptide sequence	Wild type	<i>unc-108(n777)</i>	<i>unc-108(n501)</i>
<i>flp-13</i>	ASPSAPLIRFa	xx	xx	xx
<i>flp-13</i>	SPSAVPLIRFa	xx	xx	xx
<i>flp-14</i>	KHEYLRFa	xx	xx	xx
<i>flp-15</i>	GGPQGPLRFa	xx	xx	xx
<i>flp-15</i>	RGPSPGPLRFa	xx	xx	xx
<i>flp-16</i>	GQTFVRFa	xx	xx	xx
<i>flp-16</i>	AQTFVRFa	xx	xx	xx
<i>flp-17</i>	KSAFVRFa	xx	x/ND	xx
<i>flp-18</i>	DFDGAMPGVLRFa	xx	xx	xx
<i>flp-18</i>	EIPGVLRFa	xx	xx	xx
<i>flp-18</i>	EMPGVLRFa	xx	xx	ND
<i>flp-18</i>	SEVPGVLRFa	xx	xx	xx
<i>flp-18</i>	DVPGVLRFa	xx	x/ND	xx
<i>flp-18</i>	SVPGVLRFa	xx	xx	xx
<i>flp-19</i>	WANQVRFa	xx	xx	xx
<i>flp-19</i>	ASWASSVRFa	xx	xx	ND
<i>flp-22</i>	SPSAKWMRFA	xx	x/ND	x/ND
<i>flp-24</i>	VPSAGDMMVRFa	xx	xx	ND
<i>flp-25</i>	AAPIKKASYDYIRFa	xx	xx	x/ND
<i>flp-26</i>	EFNADDITLRFa	xx	xx	ND
<i>flp-26</i>	GGAGEPLAFSPDMILSLRFa	xx	xx	xx
<i>flp-28</i>	APNRVLMRFa	xx	xx	ND
<i>flp-33</i>	APLEGFEDMSGFLRTIDGIQKPRFa	xx	xx	ND

HPLC, high performance liquid chromatography; ND, not detected. Two trials were performed for each strain. The detected neuropeptide in each trial was designated with an x.

Table S2. Strains used in this study

Strain name	Genotype
MT1093	<i>unc-108(n501)</i>
MT1656	<i>unc-108(n777)</i>
KG1279	<i>unc-108(ce363)</i>
KG1281	<i>unc-108(ce365)</i>
KG1900	<i>unc-108(nu415)</i>
NM791	<i>rab-3(fs49)</i>
VC461	<i>egl-3(gk238)</i>
RB946	<i>ric-19(ok833)</i>
RB1131	<i>lev-10(ok1154)</i>
	<i>nuls183 [punc-129::nlp-21-mvenus]</i>
GQ025	<i>unc-108(n777); nuls183 [punc-129::nlp-21-mvenus]</i>
GQ026	<i>unc-108(n501); nuls183 [punc-129::nlp-21-mvenus]</i>
GQ142	<i>unc-108(nu415); nuls183 [punc-129::nlp-21-mvenus]</i>
GQ143	<i>egl-3(gk238); nuls183 [punc-129::nlp-21-mvenus]</i>
GQ144	<i>egl-3(gk238); unc-108(nu415); nuls183 [punc-129::nlp-21-mvenus]</i>
GQ145	<i>egl-3(gk238); unc-108(n501); nuls183 [punc-129::nlp-21-mvenus]</i>
NL4256	<i>rrf-3(pk1426)</i>
GQ146	<i>unc-108(n501); rrf-3(pk1426); nuls183 [punc-129::nlp-21-mvenus]</i>
GQ147	<i>rrf-3(pk1426); nuls183 [punc-129::nlp-21-mvenus]</i>
GQ148	<i>ric-19(ok833); rrf-3(pk1426); nuls183 [punc-129::nlp-21-mvenus]</i>
EG1285	<i>oxls12 [punc-47::gfp]</i>
GQ027	<i>unc-108(n777); oxls12 [punc-47::gfp]</i>
GQ028	<i>unc-108(n501); oxls12 [punc-47::gfp]</i>
	<i>nuls152 [punc-129::gfp-snb-1]</i>
GQ029	<i>unc-108(n777); nuls152 [punc-129::gfp-snb-1]</i>
GQ030	<i>unc-108(n501); nuls152 [punc-129::gfp-snb-1]</i>
	<i>arls37 [pmyo-3::ssgfp]</i>
GQ112	<i>unc-108(n501); arls37 [pmyo-3::ssgfp]</i>
GQ113	<i>unc-108(n777); arls37 [pmyo-3::ssgfp]</i>
GQ114	<i>unc-108(ce365); arls37 [pmyo-3::ssgfp]</i>
TV199	<i>wyls22 [unc-86::gfp-rab-3]</i>
GQ023	<i>wyls22 [unc-86::gfp-rab-3]; gz100 [prab-3::mCherry-rab-2]</i>
DH1336	<i>bls34 [prme-8::rme-8-gfp]</i>
GQ115	<i>unc-108(n501); bls34 [prme-8::rme-8-gfp]</i>
GQ116	<i>unc-108(n777); bls34 [prme-8::rme-8-gfp]</i>
GQ117	<i>unc-108(ce365); bls34 [prme-8::rme-8-gfp]</i>
NP745	<i>cdls40 [unc-122::gfp-cup-5]</i>
GQ118	<i>unc-108(n501); cdls40 [unc-122::gfp-cup-5]</i>
GQ119	<i>unc-108(n777); cdls40 [unc-122::gfp-cup-5]</i>
GQ120	<i>unc-108(ce365); cdls40 [unc-122::gfp-cup-5]</i>
CB5600	<i>ccls4251; him-8(e1489)</i>
NL2003	<i>ric-19(pk690)</i>
GQ121	<i>gz102 [prab-3::gfp-cb-5; prab-3::mCherry-rab-2]</i>
GQ122	<i>gz101 [prab-3::mannosidase II-gfp; prab-3::mCherry-rab-2]</i>
GQ123	<i>gz103 [prab-3::gfp-2xfyve domain; prab-3::mCherry-rab-2]</i>
GQ124	<i>gz104 [prab-3::rfp-ecop; prab-3::myfp-rab-2]</i>
GQ125	<i>gz105 [prab-3::mCherry-rab-3; prab-3::myfp-rab-2]</i>
GQ126	<i>gz112 [prab-3::mCherry-rab-2(S20N)DN; prab-3::ric-19-yfp]</i>
GQ127	<i>gz113 [prab-3::mCherry-rab-2(WT); prab-3::ric-19-yfp]</i>
GQ128	<i>gz114 [prab-3::mCherry-rab-2(Q65I)DA; prab-3::ric-19-yfp]</i>
GQ129	<i>nuls183 [punc-129::nlp-21-mvenus]; gz100 [prab-3::mCherry-rab-2]</i>
GQ130	<i>nuls183 [punc-129::nlp-21-mvenus]; gz106 [prab-3::mCherry-syn-6]</i>
GQ131	<i>nuls183 [punc-129::nlp-21-mvenus]; gz107 [prab-3::mCherry-rab-5]</i>
GQ138	<i>nuls183 [punc-129::nlp-21-mvenus]; gz108 [prab-3::mCherry-rab-7]</i>
GQ139	<i>nuls183 [punc-129::nlp-21-mvenus]; gz109 [prab-3::imp-1-mCherry]</i>
GQ149	<i>unc-108(n501); nuls183 [punc-129::nlp-21-mvenus]; gz106 [prab-3::mCherry-syn-6]</i>
GQ150	<i>unc-108(n501); nuls183 [punc-129::nlp-21-mvenus]; gz107 [prab-3::mCherry-rab-5]</i>

Table S2. Strains used in this study (Continued)

Strain name	Genotype
GQ151	<i>unc-108(n501); nuls183 [punc-129::nlp-21-mvenus]; gz108 [prab-3::mCherry-rab-7]</i>
GQ152	<i>unc-108(n501); nuls183 [punc-129::nlp-21-mvenus]; gz109 [prab-3::lmp-1-mCherry]</i>
GQ153	<i>nuls183 [punc-129::nlp-21-mvenus]; gz110 [prab-3::mCherry-rab-5(Q78L)DA]</i>
GQ154	<i>unc-108(n501); nuls183 [punc-129::nlp-21-mvenus]; gz110 [prab-3::mCherry-rab-5(Q78L)DA]</i>
GQ137	<i>ric-19(ok833); nuls183 [punc-129::nlp-21-mvenus]; gz111 [prab-3::ric-19]</i>
GQ136	<i>nuls183 [punc-129::nlp-21-mvenus]; gz111 [prab-3::ric-19]</i>
GQ135	<i>gz116 [prab-3::ric-19-yfp]</i>
GQ133	<i>unc-108(n501); gz116 [prab-3::ric-19-yfp]</i>
GQ134	<i>unc-108(n777); gz116 [prab-3::ric-19-yfp]</i>
GQ132	<i>gz115 [prab-2::gfp]</i>
DA509	<i>unc-31(e928)</i>

WT, wild type. Genotypes without a strain name were provided by other laboratories, and strain names were not available (see Materials and methods).

Table S3. Transgenic arrays used in this study

Array name	Plasmid	Markers
gz102	10 ng/μl <i>prab-3::GFP-Cb-5; 5 ng/μl prab-3::mCherry-rab-2</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz101	10 ng/μl <i>prab-3::mannosidase II-gfp; 5 ng/μl prab-3::mCherry-rab-2</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz103	10 ng/μl <i>prab-3::gfp-2xfyve domain; 5 ng/μl prab-3::mCherry-rab-2</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz104	10 ng/μl <i>prab-3::rfp-ecop; 5 ng/μl prab-3::myfp-rab-2</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz105	5 ng/μl <i>prab-3::mCherry-rab-3; 5 ng/μl prab-3::myfp-rab-2</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz100	5 ng/μl <i>prab-3::mCherry-rab-2</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz106	10 ng/μl <i>prab-3::mCherry-syn-6</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz107	5 ng/μl <i>prab-3::mCherry-rab-5</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz108	5 ng/μl <i>prab-3::mCherry-rab-7</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz109	20 ng/μl <i>prab-3::lmp-1-mCherry</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz110	5 ng/μl <i>prab-3::mCherry-rab-5(Q78L)DA</i>	20 ng/μl <i>ptx-3::rfp</i>
gz111	20 ng/μl <i>prab-3::ric-19</i>	20 ng/μl <i>ptx-3::rfp</i>
gz112	5 ng/μl <i>prab-3::mCherry-rab-2(S20N)DN; 20 ng/μl prab-3::ric-19-yfp</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz113	5 ng/μl <i>prab-3::mCherry-rab-2(WT); 20 ng/μl prab-3::ric-19-yfp</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz114	5 ng/μl <i>prab-3::mCherry-rab-2(Q65L)DA; 20 ng/μl prab-3::ric-19-yfp</i>	40 ng/μl <i>pRF4(rol-6(su1006)); 20 ng/μl ptx-3::gfp</i>
gz115	40 ng/μl <i>prab-2::gfp</i>	40 ng/μl <i>pRF4(rol-6(su1006))</i>
gz116	20 ng/μl <i>prab-3::ric-19-yfp</i>	40 ng/μl <i>pRF4(rol-6(su1006))</i>

WT, wild type.