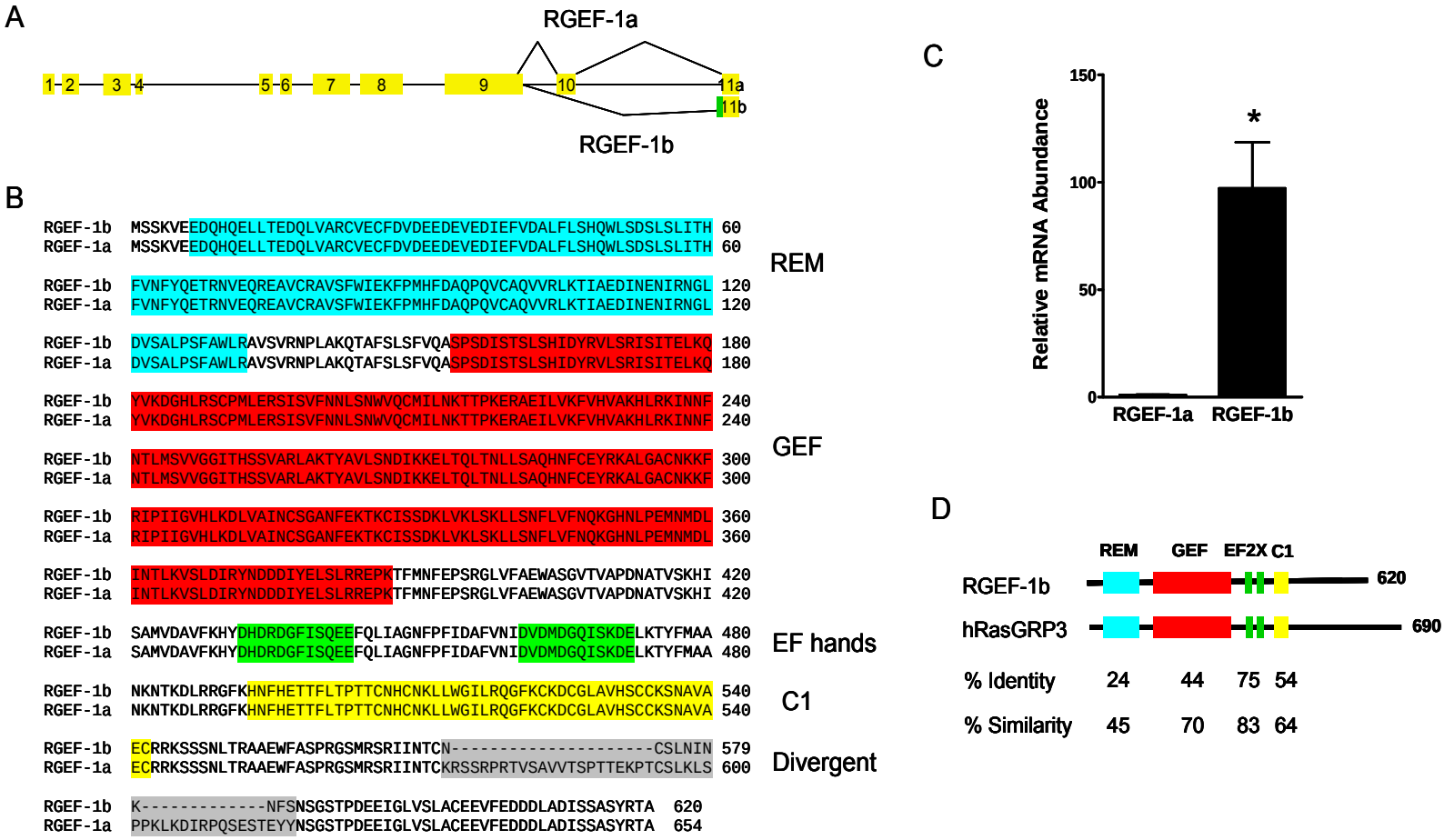

Figure S1. The *rgef-1* gene encodes prototypical RasGRPs (Related to Fig. 1). ***A***, the intron-exon organization of *rgef-1* gene was determined by aligning RGEF-1b and RGEF-1a cDNA sequences with *C. elegans* genome sequences (WormBase). Exons (yellow rectangles) 1-9 are shared by RGEF-1a and RGEF-1b mRNAs. Only RGEF-1a mRNA contains exon 10. The last exon of RGEF-1B (11b) is 30 nucleotides (marked in green) longer than the terminal exon of RGEF-1a. ***B*** shows derived amino acid sequences of RGEF-1b and RGEF-1a, and locations of conserved domains and a divergent region. ***C*****,** amounts of RGEF-1b and RGEF-1a mRNAs were quantified by qR-PCR and normalized to actin (ACT-1) mRNA. Data were plotted by taking the amount of RGEF-1a mRNA as 1.0 (n = 10, *P<0.0001). Error bars represent SEM. ***D*** depicts locations of conserved domains along polypeptide chains of RGEF-1b and human RasGRP3. (% similarity = % identity + % conserved amino acids.) ***E*****,** The complete sequence of RGEF-1b cDNA is shown. The 1860 bp ORF begins at nucleotide 110 and ends with a translation stop codon at nts 1970-1972. SL-1 and poly A addition site sequences are shown in blue. The sequence was determined as described under Supplemental Experimental Procedures.

E

RGEF-1b cDNA

```
   1  GGTTTAATTACCCAAGTTTGAGACCATTACCAACGTTCTTTCTAGATCTTGAGACAATCT  SL-1
  61  TCCTTCTGCTCAATCGTTCGTCGTGAAGACGGCATCGACGACGACGACGATGAGCAGTAA  Start
 121  AGTTGAAGAAGATCAACATCAAGAACTTCTCACAGAAGATCAACTTGTGGCAAGATGTGT
 181  TGAATGCTTTGATGTCGACGAGGAGGATGAGGTTGAAGATATTGAATTTGTTGACGCCTT
 241  GTTTTTAAGTCATCAATGGTTGAGTGATAGTTTATCACTTATTACACATTTCGTGAACTT
 301  CTATCAGGAAACTCGAAACGTCGAACAAAGAGAAGCAGTATGTCGAGCGGTATCATTTTG
 361  GATCGAAAAGTTTCCAATGCATTTCGATGCGCAACCTCAAGTCTGTGCACAAGTTGTTCG
 421  ATTGAAAACGATAGCAGAAGATATAAATGAAAATATTCGAAATGGTCTAGATGTCAGTGC
 481  ATTACCGTCATTTGCGTGGCTTCGAGCAGTTTCAGTTAGAAATCCATTGGCTAAACAGAC
 541  TGCCTTCTCTCTTTCATTTGTCCAAGCATCCCCTAGCGATATTTCGACGTCACTTTCTCA
 601  TATCGACTATCGCGTTTTGTCCAGAATTTCGATAACTGAGCTCAAGCAATATGTGAAAGA
 661  TGGACATCTAAGATCATGTCCAATGTTGGAAAGAAGTATTTCAGTATTTAATAATCTTTC
 721  CAATTGGGTACAGTGCATGATCCTTAACAAGACAACACCAAAAGAACGAGCAGAGATCCT
 781  TGTGAAGTTTGTTCATGTAGCAAAGCATCTACGAAAGATCAATAATTTTAACACTTTGAT
 841  GTCCGTTGTTGGTGGAATAACTCATTCATCAGTGGCTCGACTTGCCAAAACATATGCAGT
 901  TCTGTCAAACGATATCAAAAAAGAACTGACTCAGTTGACAAATCTTTTATCTGCTCAACA
 961  CAATTTCTGCGAGTATCGAAAAGCATTAGGAGCGTGTAACAAAAAGTTCCGGATACCTAT
1021  AATCGGTGTACATCTCAAAGACCTAGTCGCAATCAATTGTTCGGGTGCAAATTTTGAGAA
1081  AACAAAATGCATCAGTAGTGATAAATTGGTGAAACTTTCCAAACTGCTTTCAAATTTTCT
1141  GGTTTTCAATCAAAAAGGACATAATCTTCCCGAAATGAATATGGATTTGATAAACACATT
1201  GAAAGTGTCTTTGGACATTCGGTACAACGATGATGATATTTACGAGCTGTCGTTGAGACG
1261  GGAACCAAAAACTTTTATGAATTTCGAACCATCTCGTGGACTCGTATTCGCTGAGTGGGC
1321  CTCTGGAGTTACAGTTGCACCTGATAATGCAACAGTTTCAAAACATATCAGTGCAATGGT
1381  GGACGCAGTATTCAAACACTATGATCATGATAGAGATGGATTCATTTCTCAAGAAGAATT
1441  TCAGTTGATTGCTGGAAATTTCCCTTTTATTGACGCATTTGTGAACATAGATGTTGATAT
1501  GGATGGACAGATATCAAAAGATGAATTGAAGACCTATTTTATGGCTGCAAACAAGAATAC
1561  AAAAGATTTACGAAGAGGATTCAAGCACAATTTCCATGAAACTACATTCTTAACTCCAAC
1621  GACTTGCAATCATTGCAACAAATTGTTGTGGGGGATTTTGAGACAAGGCTTCAAGTGCAA
1681  AGATTGTGGATTAGCAGTTCATAGTTGCTGTAAATCAAATGCTGTAGCCGAATGCAGAAG
1741  AAAAAGTAGCAGCAATTTAACAAGGGCTGCCGAGTGGTTCGCTTCACCACGTGGCAGTAT
1801  GAGAAGTAGGATAATAAATACATGCAATTGTTCATTAAACATCAATAAGAATTTCAGTAA
1861  TTCCGGAAGTACACCAGATGAAGAAATTGGTCTTGTAAGCCTTGCTTGTGAAGAAGTCTT
1921  CGAAGATGATGATCTAGCAGATATTTCATCTGCGTCATACCGTACCGCCTGATTTGTCTC  Stop
1981  CATCTATCTCTTTCTCTGCACTCTCTTTATTTTTAGCAAGAATATATTACACAATACGCC
2041  TTCTCAGGATTTAGTGTTCTCGTAATATGAGAGTACAATATTAAGTTTATTCTTCTCATC
2101  TATTTTTCTTCAAATTAATGCATACGAGTATTTTTTCCTGCAAAATGCGATTCATTTTTT
2161  AACCATATCACTCAATTTCTCAAATTTATTTGTTTTTTCTTTCAGTACATTCCCTCACAA
2221  TTCGAGCTTGCTTTTTCCTATCCCACTTGTTTGATCTCTACGCTAAATATTTCCACGTGT
2281  AAAGTATTTTTCACCAGTCTTCCACACACTTTCCTCTTTTTTCGAATGAATTTTTGCTGA  Poly A
2341  ACAAAAAAAAAAAAAAAAAAA                                          addition
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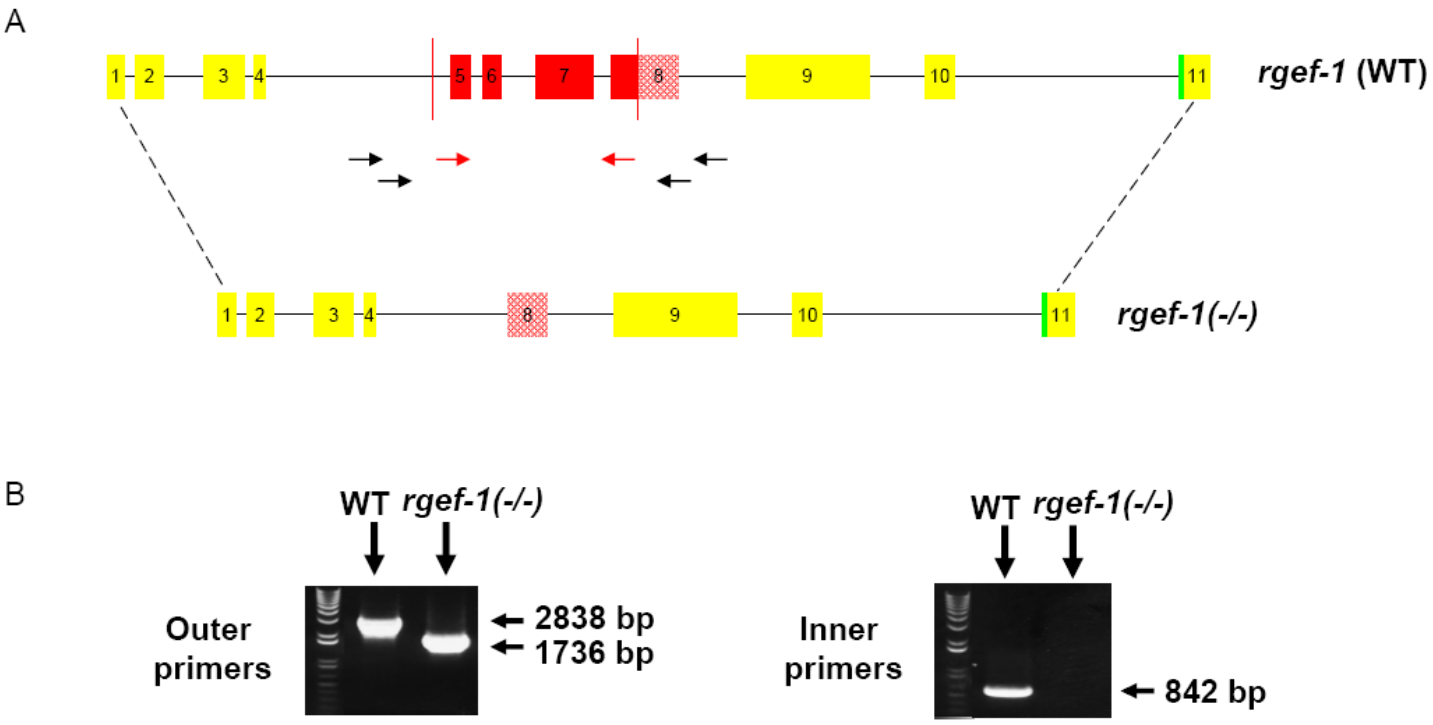

Figure S2. Disruption of the *rgef-1* gene (related to Fig. 3A). *A*, the *rgef-1* gene structure is depicted schematically. Nucleotides 1493-2594 of the *rgef-1* gene are deleted in strain RB848 (*rgef-1(-/-)*). The excised exons and boundaries of the deletion are marked in *red* on the *rgef-1* (WT) gene. Arrows show the positions of primers used to follow the deleted gene during back-crossing. ***B,*** PCR analysis was performed on genomic DNA templates isolated from individual animals obtained during back-crossing (*rgef-1(-/-)* x WT). The *left panel* shows amplified DNA generated by using nested primers that hybridize with segments of the *rgef-1* gene that precede or follow the deletion (***A*****,** *black arrows*). The *right panel* shows DNA synthesized when primers corresponding to the deleted sequence were used (***A,*** *red arrows*). DNA products were size-fractionated in a 1% agarose gel and stained with ethidium bromide. The first lane of each gel received DNA size markers.

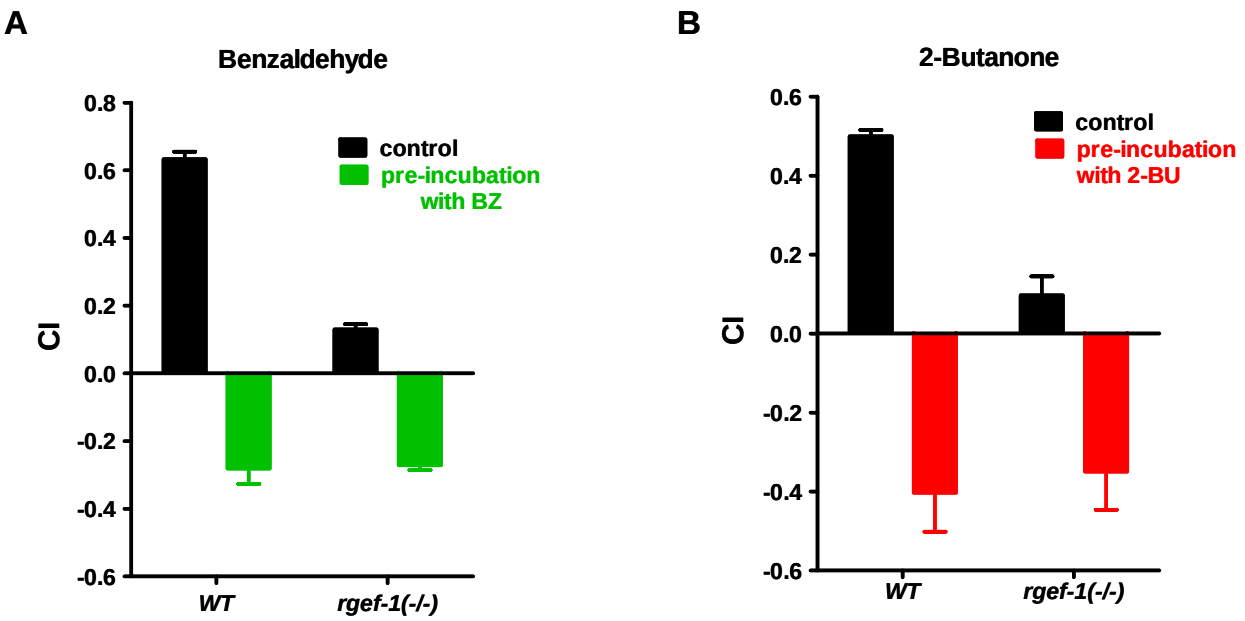

Figure S3. Odorant detection and avoidance are not impaired by RGEF-1b deficiency (related to Figs. 3E, 3F). - WT *C. elegans* and *rgef-1* null animals were pre-incubated for 60 min in buffer alone (control) or buffer containing a 1:20,000 dilution of BZ (A) or 2-BU (B). After washing, animals were assayed for chemotaxis to BZ (A) and 2-BU (B) as described under Experimental Procedures. Assays were performed in triplicate; error bars are SEM. Similar results were obtained in three experiments.

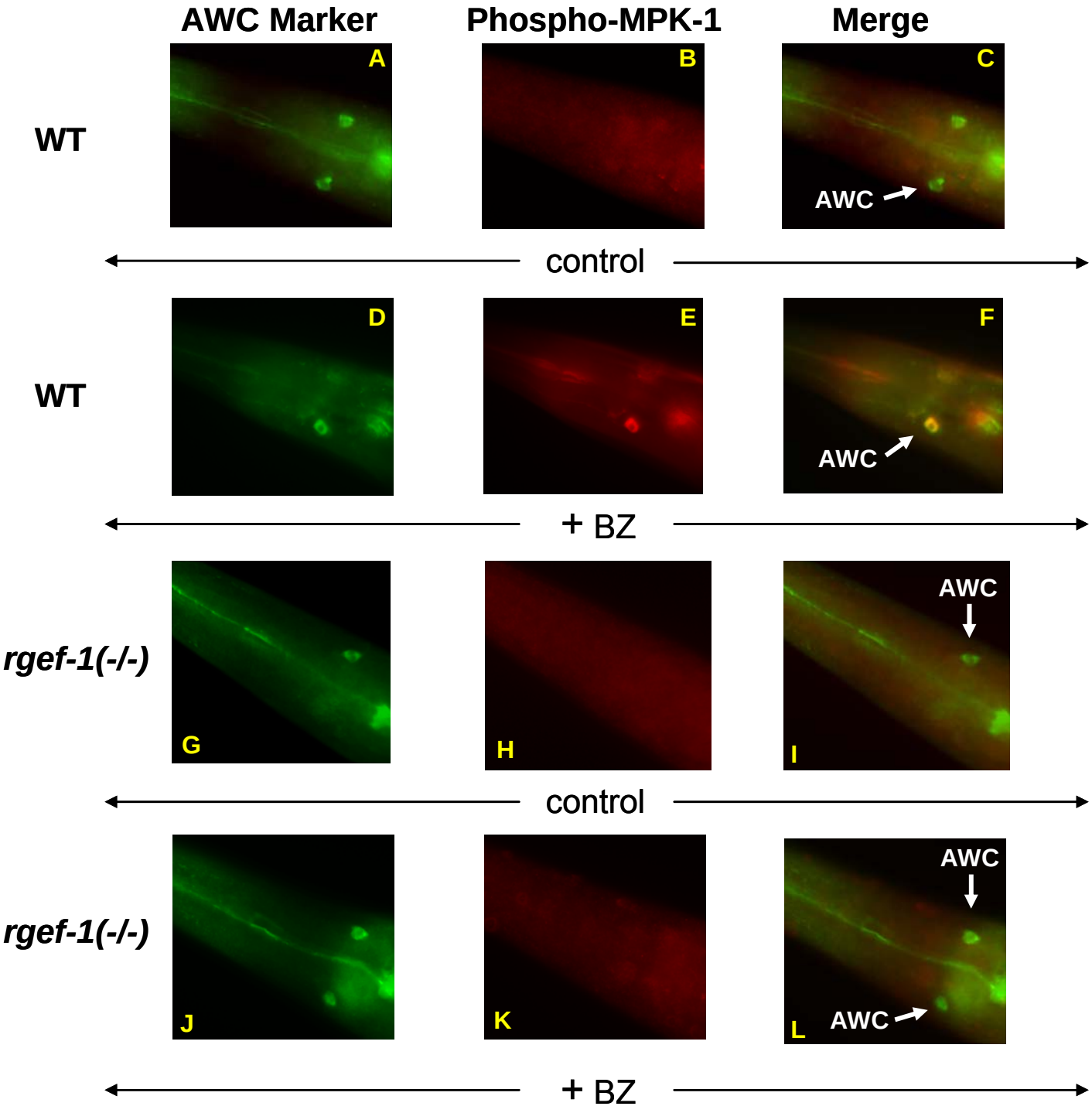

Figure S4. BZ elicits phosphorylation/activation of MPK-1 in AWC neurons (A-F); RGEF-1b depletion disrupts odor-induced MPK-1 activation (G-L) - The *odr-1* promoter directed expression of an RFP marker protein in AWC neurons of WT or *rgef-1(-/-)* animals. Transgenic animals were incubated with buffer (control) or BZ for 3 min prior to fixation, permeabilization and incubation with primary antibodies directed against the di-phosphorylated A-loop of MPK-1 and RFP. Locations of endogenous phospho-MPK-1-IgG and RFP-IgG complexes were visualized by fluorescence microscopy (see Experimental Procedures) after incubation with secondary antibodies conjugated to DyLight 549 (red) and DyLight 488 (green) fluorophores, respectively. Representative micrographs are shown.

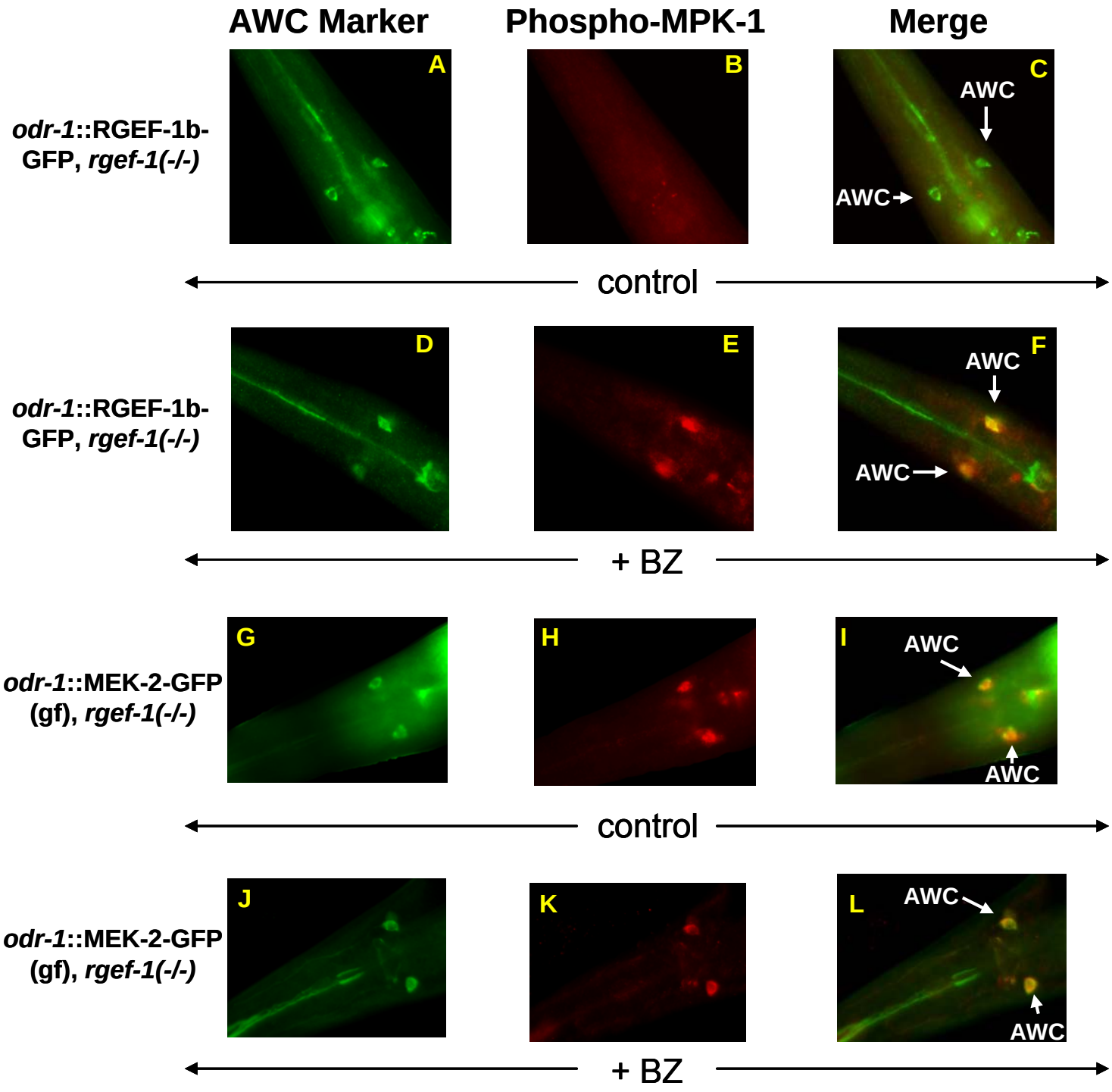

Figure S5. RGEF-1b is essential for odorant-induced MPK-1 phosphorylation in AWC neurons (A-F); MEK-2 activates MPK-1 in AWC neurons (G-L). The *odr-1* promoter directed expression of RFP protein (marker) and either RGEF-1b-GFP (**A-F**) or MEK-2E223 D227-GFP (MEK-2-GFP(gf)) (**G-L**) in AWC neurons of *rgef-1* null animals. Transgenic animals were incubated with buffer (control) or BZ for 3 min prior to fixation, permeabilization and incubation with primary antibodies directed against the di-phosphorylated A-loop of MPK-1 and RFP. Locations of phospho-MPK-1-IgG and RFP-IgG complexes were visualized by fluorescence microscopy (see Experimental Procedures) after incubation with secondary antibodies conjugated to DyLight 549 (red) and DyLight 488 (green) fluorophores, respectively. Representative micrographs are shown.

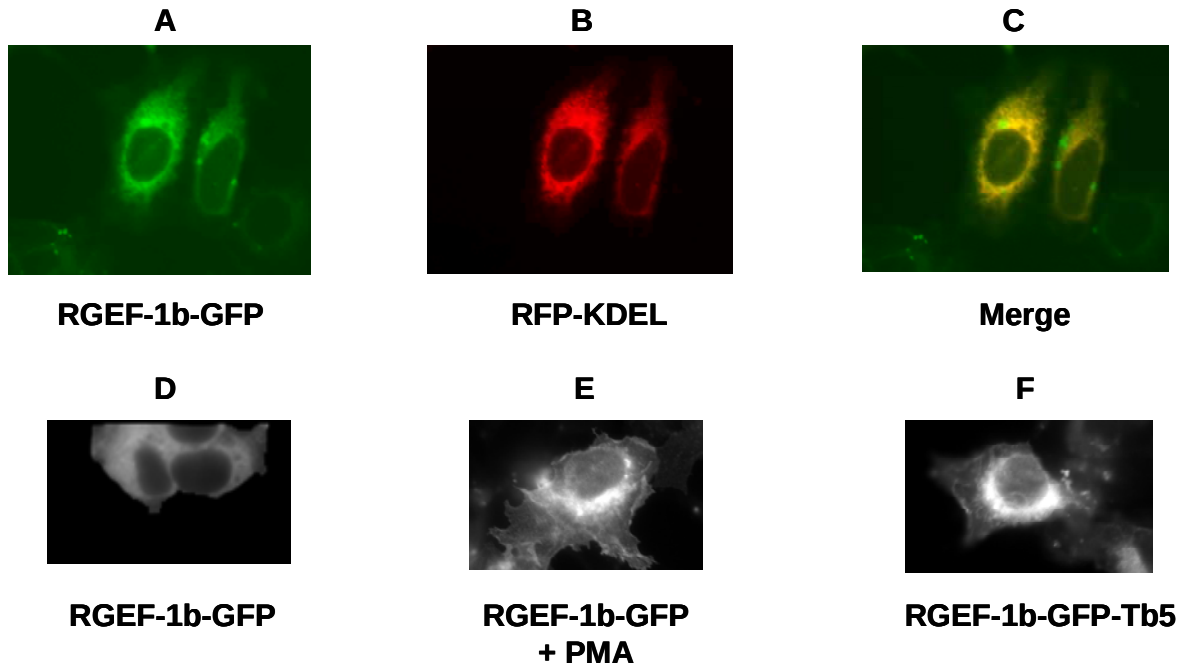

Figure S6. RGEF-1b is recruited to the ER by PMA and a targeting domain derived from cytochrome b5 (related to Fig. 7A) – PMA promoted translocation of RGEF-1b from cytoplasm to perinuclear membranes (see Fig. 7A). ***A-C*****,** the intracellular site of RGEF-1b recruitment was identified by co-transfecting HEK 293 cells with transgenes encoding RGEF-1b-GFP and RFP tagged with a C terminal tetrapeptide Lys-Asp-Glu-Leu (KDEL). RFP-KDEL is selectively retained in the ER. Cells were incubated with 50 nM PMA for 10 min and fluorescence signals derived from RGEF-1b-GFP **(*A*)** and the ER marker, RFP-KDEL **(*B*)** were recorded by microscopy as described under Supplemental Experimental Procedures. ***C*****,** merging panels ***A*** and ***B*** shows that RGEF-1b is targeted to the ER**.** ***D-F*****,** cells were transfected with transgenes encoding RGEF-1b-GFP or RGEF-1b-GFP-Tb5, as indicated. Incubation with PMA and fluorescence microscopy were performed as described for ***A*** and ***B*** above. ***D*****,** RGEF-1b is dispersed in the cytoplasm of unstimulated cells. ***E*****,** PMA elicits translocation of RGEF-1b to ER**.** ***F*****,** the C terminal ER targeting domain of cytochrome b5 (designated Tb5, see Supplemental Experimental Procedures for details) anchors RGEF-1b-GFP-Tb5 at the cytoplasmic surface of the ER in unstimulated cells.

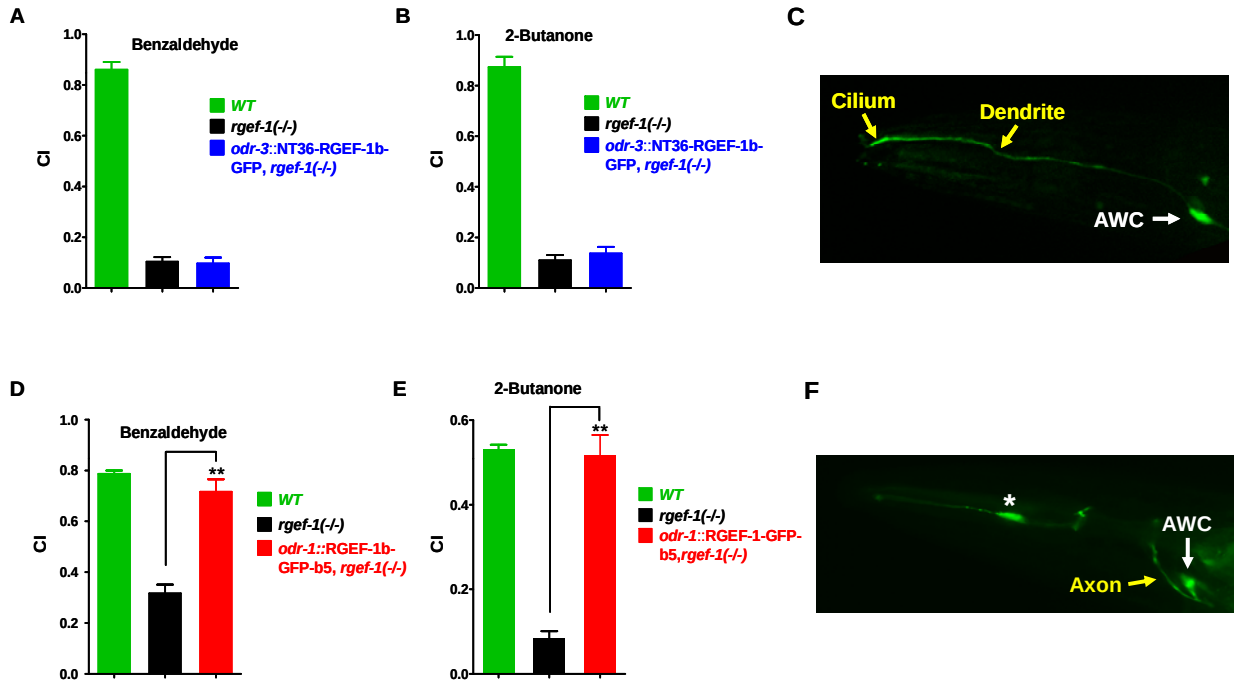

Figure S7. Intracellular localization profoundly affects RGEF-1b function (related to Figs. 7E, 7F) - Transgenes encoding NT36-RGEF-1b-GFP and RGEF-1b-GFP-cytochrome b5 were expressed and differentially localized in AWC neurons of *rgef-1* null animals (see Experimental Procedures). RGEF-1b-GFP was targeted to the dendrite, cilium and the cell body (white arrow) by fusion with amino acids 1-36 of the Gαi/o homolog, ODR-3 (***C***). Alternatively, RGEF-1b-GFP was routed to the cell body (white arrow) and axon by fusion with *C. elegans* cytochrome b5 (***F***).The *odr-1*::RGEF-1b-GFP-b5 transgene was also expressed in the I1 neuron (asterisk). WT, *rgef-1* null and transgenic animals were assayed for chemotaxis to BZ (***A,D***) and 2-BU (***B,E***). Assays were performed in triplicate; error bars are SEM. ***P*< 0.001 compared with *rgef-1(-/-)* animals, Student's *t* test. Similar results were obtained in three experiments.

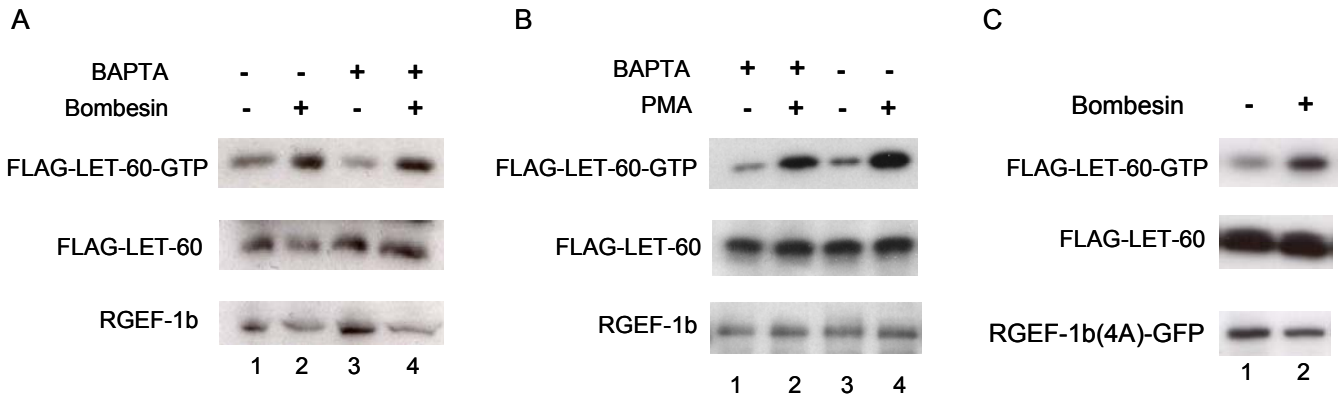

Figure S8. Ca^{2+} binding by RGEF-1b EF hands is not required for LET-60 activation (related to Fig. 8A). ***A*****,** HEK293 cells were transfected with RGEF-1b, FLAG-LET-60 and bombesin receptor transgenes. Cells were treated with 20 μM BAPTA-AM (30 min) and/or 200 nM bombesin (10 min), as indicated. Subsequently, cells were lysed with RAL buffer. Samples were processed as described in Figure 1A and Experimental Procedures. X-ray films from Western blots are shown. ***B*****,** HEK293 cells were transfected with expression plasmids encoding RGEF-1b and FLAG-LET-60. Cells were treated with 20 μM BAPTA-AM (30 min) and/or 50 nM PMA (15 min), as indicated. Subsequently, cells were lysed with RAL buffer. Samples were processed as indicated in ***A*** above. ***C*****,** HEK293 cells were transfected with transgenes encoding FLAG-LET-60, bombesin receptor and RGEF-1b(4A)-GFP. The RGEF-1b(4A) GTP exchanger contains 4 EF hand mutations that markedly decrease Ca^{2+} binding activity. Cells were incubated with vehicle or 200 nM bombesin for 10 min. Levels of LET-60, LET-60-GTP and RGEF-1b(4A)-GFP were monitored as described in Figure 1 A and "Experimental Procedures". Experiments were replicated 3 times; similar results were obtained in each instance. Typical data are shown.

Supplemental Table 1

Measurements of Western Immunoblot Signal Intensities

Figure	Lane	Integrated Signal Intensity (arbitrary units)	GTP-loading (fold-induction)
1A	3	9615	
1A	4	55313	5.8
1B	3	21674	
1B	4	88864	4.1
1C	1	9257	
1C	2	23946	2.6
1C	3	24746	
1C	4	70528	2.9
3C	1	3837	
3C	2	12846	3.3
3C	3	4180	
3C	4	12792	3.1
4A	1	3447	
4A	2	26175	7.6
4B	1	20738	
4B	2	61089	
4B	3	15859	
4B	4	41892	
4B	5	10030	
4B	6	23963	
4B	7	9368	
4B	8	10084	
5B	1	30016	
5B	2	31711	1.1
5B	3	12351	
5B	4	12632	1.0

7C	1	7137	
7C	2	29947	4.2
7C	3	598	
7C	4	5451	9.1
7C	5	8017	
7C	6	22685	2.8
7D	1, upper	3868	
7D	2, upper	7353	
7D	3, upper	25445	
7D	4, upper	29278	
7D	5, upper	32068	
7D	1, lower	26438	
7D	2, lower	26253	
7D	3, lower	25479	
7D	4, lower	29351	
7D	5, lower	30058	
7F	1	2027	
7F	2	18999	9.4
7F	3	509	
7F	4	4135	8.1
8B	3	1744	
8B	4	6776	3.9

Chemiluminescence signals derived from bands in the indicated lanes were quantified and normalized, using ImageQuant software (Experimental Procedures). Except for Fig. 8B*, which shows data for phosphorylation of Ser^{135}, the values represent amounts of FLAG-LET-60-GTP (or FLAG-RAP-1-GTP). Where appropriate, ratios (fold-increase in GTP loading) are provided. Lanes which had no signals are not listed.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Characterization of RGEF-1a and RGEF-1b cDNAs - Cosmid F25B3 (GenBank accession number Z70752), which contains the *rgef-1* gene (4893 bp) and flanking DNA, was obtained from Sanger Institute, Hinxton, UK. Conceptual splicing of 11 predicted *rgef-1* exons (Genefinder software) yields a putative mRNA with an open reading frame (ORF) of 1962 nucleotides (nt) (Sequence F25B3.3, WormBase, http://www.wormbase.org). To characterize authentic mRNAs encoded by the *rgef-1* gene, we performed RT-PCR analysis. Total RNA was extracted from an asynchronous population of WT *C. elegans* using TRIZOL Reagent (Invitrogen). RNA was purified on an RNeasy column (Qiagen). Superscript III reverse transcriptase (Invitrogen) catalyzed first strand cDNA synthesis, using purified RNA as a template and oligo dT as primer. The majority of *C. elegans* mRNAs contain a shared 5` splice leader sequence (SL-1). Thus, an SL-1 oligonucleotide and oligonucleotides derived from predicted 5` and 3` UTRs of *rgef-1* transcripts were prepared (Gene Link). cDNAs that contain complete RGEF-1 ORFs were synthesized via PCR, using KOD DNA polymerase (Novagen). Either an SL-1 oligonucleotide primer (nucleotides 1-22, Fig. S1E) or a 5`UTR primer (nucleotides 69-88, Fig. S1E) was used in conjunction with nested 3` UTR primers (inverse complements of nucleotides 2203-2224 and 2291-2309, Fig. S1E) to direct cDNA synthesis. Sequencing of amplified, cloned cDNAs revealed that two transcripts are generated by alternative splicing (see Fig. S1A and associated text under Results). Sequencing of a partial cDNA (yk317g10, provided by Y. Kohara, National Institute of Genetics, Mishima, Japan) verified results obtained for the RGEF-1b 3` UTR and established the site of polyadenylation. RGEF-1a cDNA has the ORF predicted by WormBase and encodes a GEF composed of 654 amino acids. RGEF-1b cDNA (Fig. S1E) encodes a highly homologous, but distinct GEF comprising 620 amino acids. Amino acid sequences and locations of structural, regulatory and catalytic domains of RGEF-1b and RGEF-1a proteins are shown in Fig. S1B.

Preparation of transgenes, recombinant expression vectors and transgenic animals – cDNA encoding full length RGEF-1b protein was synthesized by PCR, using primers that included BamHI (5`) and NotI (3`) restriction sites. After digestion with BamHI and NotI restriction enzymes, RGEF-1b cDNA was cloned into the mammalian expression plasmid pCDNA 3.1 (Invitrogen). This placed the transgene under control of a strong, constitutively active CMV promoter and enabled expression of RGEF-1b in cultured cells.

For expression in *C. elegans*, the RGEF-1b ORF was synthesized via PCR, using primers that added PstI (5`) and BamHI (3`) restriction sites. The cDNA was cleaved with PstI and BamHI and cloned into the *C. elegans* expression plasmid pPD 95.77 (Fire et al., 1990). Primer sequences used for cloning cDNAs and gene promoters into plasmid pPD 95.77 are provided below in two tables. We eliminated the translation stop codon to enable in-frame fusion with vector DNA encoding GFP. Promoter-enhancer DNA (2670 bp) that precedes the initiator ATG codon of the *rgef-1* structural gene was synthesized by PCR, using KOD DNA polymerase in concert with a genomic DNA template (cosmid F25B3) and primers that added SphI (5`) and PstI (3`) restriction sites. After digestion with SphI and PstI, *rgef-1* promoter-enhancer DNA was cloned upstream from RGEF-1b cDNA in the recombinant pPD 95.77 plasmid (which was also cut with Sph1 and PstI). Fusion of *rgef-1* promoter DNA with the 5`end of RGEF-1b cDNA generated an *rgef-1*::RGEF-1b-GFP transgene.

cDNAs encoding full length LET-60, RAP-1 and MEK-2 proteins were synthesized and cloned in frame with GFP in the pPD 95.77 expression vector as described above. PCR primers were derived from verified cDNA and gene sequences provided in Wormbase (sequences ZK792.6, C27B7.8, Y54E10BL.6). LET-60 and RAP-1 cDNAs were also cloned in a modified pCDNA 3.1 plasmid for expression in cultured cells. A module in the plasmid DNA appends a FLAG epitope

tag at the N-termini of LET-60 and RAP-1. A transgene encoding the human bombesin receptor was expressed in transfected HEK293 cells as previously reported (Feng et al., 2006).

In vivo expression of WT and mutant RGEF-1b-GFP, FLAG-LET-60, FLAG-RAP-1 and MEK-2-GFP transgenes was driven by *rgef-1, odr-1, odr-3, gpa-3* or *hsp-16.2* promoters as indicated in Results and Figs. 3-8, S3-S5 and S7. Construction of recombinant pPD 95.77 that contains *rgef-1* promoter-enhancer DNA is described above. The same strategy was used to synthesize and clone previously characterized *odr-1* (2403 bp)*, odr-3* (2754 bp)*, gpa-3* (4599 bp) and *hsp-16.2* (392 bp) promoters into the pPD 95.77 vector (Jones et al., 1986; L'Etoile and Bargmann, 2000; Lans et al., 2004). Cosmids named R01E6, C34D1, E02C12 and Y46H3A (Sanger Institute, Hinxton, UK), respectively, were used as templates. All cloned promoters and transgenes were verified by DNA sequencing. Sequences of PCR primers used for cloning cDNAs (transgenes) and promoter-enhancer DNAs are presented in separate Tables below. *C. elegans* was microinjected with recombinant plasmid DNA and transgenic animals were selected by monitoring GFP fluorescence as previously described (Fire et al., 1991; Freedman et al., 1993).

Targeting RGEF-1b to the endoplasmic reticulum – RGEF-1b-GFP was anchored to the cytoplasmic surface of the endoplasmic reticulum by attaching a targeting domain at the C-terminus of GFP. The targeting domain (designated Tb5) contains a synthetic cytoplasmic linker peptide (24 amino acids), a membrane spanning region (17 amino acids) and a C-terminal segment (22 amino acids) that inserts into the lumen of the endoplasmic reticulum (Bulbarelli et al., 2002). The transmembrane and C-terminal regions, which are derived from the resident endoplasmic reticulum protein cytochrome b5, mediate polarized insertion into the lumen of the endoplasmic reticulum. This ensures that proteins fused to the N-terminal linker peptide are retained at the cytoplasmic surface of the membrane. A recombinant pCDNA 3.1 plasmid (GFP-

ER-17) that contains unique NheI and HindIII restriction sites was provided by Dr. Erik Snapp (Department of Anatomy and Structural Biology, Albert Einstein College of Medicine). The HindIII site precedes a cDNA insert that encodes a GFP-linker-b5 transmembrane domain-b5 C-terminal segment chimeric protein. RGEF-1b cDNA was amplified using primers that appended NheI (5`) and HindIII (3`) restriction sites, as described above. After cleaving with restriction enzymes, RGEF-1b cDNA was ligated (in-frame) to the 5` end of DNA encoding GFP in the GFP-ER-17 plasmid. The resulting transgene directs synthesis of a modified RGEF-1b-GFP-Tb5 protein which is persistently associated with the cytoplasmic surface of the endoplasmic reticulum in transfected cells.

A pCDNA3.1 expression plasmid that contains cDNA encoding RFP-KDEL was generated and provided by Dr. Erik Snapp (Altan-Bonnet et al., 2006). RFP-KDEL is an established organelle marker that is selectively routed to and retained in the ER.

Mutagenesis - Amino acid substitutions were introduced by a PCR-based, site-directed mutagenesis protocol (QuickChange^{TM} kit, Stratagene) as previously described (Chen et al., 1997; Glantz et al., 1993; Zhang et al., 2001). Mutants were verified by DNA sequencing.

DNA and protein data analysis - Analysis of sequence data, sequence comparisons, and data base searches were performed using Blast programs (NCBI Server, National Institutes of Health, Bethesda, MD), the SMART website, ClustalW, and pattern/motif search programs provided by the Swiss Institute of Bioinformatics (Geneva, Switzerland). Information about *C. elegans* genomic DNA, cDNAs, ESTs and proteins was obtained from the WormBase website.

Protein determination - Protein concentrations were determined by using a modified Lowry assay, the BioRad *DC* Protein Assay. Bovine albumin was employed as a standard.

Quantitative real-time PCR analysis – qR-PCR was performed and data were analyzed as previously described (Ren et al., 2009).

Characterization of a disrupted *rgef-1* gene by single worm PCR analysis - An *rgef-1* deletion strain (RB848) was obtained from the *C. elegans* gene knockout consortium. After egg laying was completed, individual adult worms were incubated with 5µl of lysis buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.2, 2.5 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween 20, 0.01% gelatin, and 5% proteinase K) at 65 °C for 1 h to extract genomic DNA. Large fragments of the *rgef-1* gene were amplified by nested PCR, using genomic DNA (1:50 dilution) as the template. The strategy used to characterize the disrupted *rgef-1* gene is presented in "Results" and Fig. S2. To identify animals homozygous for the *rgef-1* null mutation after various crosses, PCR was first executed with an external left primer (5`-CGAGCGGTATCATTTTGGAT-3`) and an external right primer (5`-TGTCGGCTTCTCTGTTGTTG-3`). A second round of PCR used internal left (5`-CATACTGCCACGTGGTGAAG-3`) and internal right (5`-GGAATTGCGAGCTATGGTGT-3`) primers. Primers (5`-GACTATCGCGTTTTGTCCAG-3` and 5`-GCACCCGAACAATTGATTGC-3`) corresponding to the deleted region of the gene were used to distinguish WT and heterozygous *C. elegans* from homozygous *rgef-1(-/-)* null animals. Sizes of amplified DNA segments were estimated by electrophoresis in a 1% agarose gel containing a ladder of DNA standards. Exact deletion boundaries were determined by sequencing amplified segments of genomic DNA.

Synthesis of peptide and phosphopeptide antigens and production of antisera - Peptides corresponding to the C terminus of RGEF-1b, (EEVFEDDDLADISSASYRTA, amino acids 601-620), the phospho-peptide FAWLRAVpSVRNPLAKQ (amino acids 128-143) and the non-phosphorylated peptide FAWLRAVSVRNPLAKQ (amino acids 128-143) were synthesized by Proteintech Group Inc. (Chicago, IL) and conjugated to Keyhole Limpet Hemocyanin (KLH), a large carrier protein. Cys was added to the N termini of peptides to enable covalent coupling

with maleimide-activated hemocyanin carrier protein (for antigen injection), or SulfoLink agarose beads (Pierce Biotechnology) to generate affinity chromatography resins. Antigen was injected into rabbits (0.4-mg protein initial injection; 0.2 mg for each of four booster injections) at Proteintech Group Inc. at 3-week intervals. Antiserum was collected at 3-week intervals.

Affinity purification of anti-RGEF-1b peptide IgGs and anti-phospho-RGEF-1b peptide IgGs – A microlink^{TM} Protein Coupling Kit (Pierce) was used to couple peptide antigens to SulfoLink agarose beads (Pierce Biotechnology). IgGs were bound and eluted as described in previous studies (Land et al., 1994; Wu et al., 1998). In the case of anti-phospho-RGEF-1b peptide IgGs, antiserum was cycled over a column of non-phosphorylated peptide three times. The flow through was applied to a phosphorylated peptide-agarose column for affinity purification of desired IgGs. Purified IgGs were dialyzed against phosphate-buffered saline containing 50% glycerol and stored at -20 °C.

Immunofluorescence analysis of transfected cells - HEK293 cells transfected with WT and mutant RGEF-1b-GFP transgenes were grown on 20-mm glass coverslips that were coated with poly-L-Lysine. Cells were fixed (4% paraformaldehyde) for 20 min as described previously (Li et al., 1996). Next, cells were washed with PBS three times. After air drying, 15μl of 50% glycerol in PBS, containing 1mg/ml p-phenylenediamine (antibleaching agent), was placed on the specimens, and coverslips were mounted on slides. Fluorescence signals derived from recombinant GFP-tagged or RFP-tagged proteins were recorded using a Zeiss Axio Imager Z1 microscope and Zeiss AxioVision software.

***C. elegans* strains and culture -** *C. elegans* strains were cultivated at 20°C on agar plates seeded with *E. coli* OP50. Wild-type (WT) *C. elegans* (N2 Bristol) was obtained from Caenorhabditis Genetics Center (CGC, University of Minnesota). The *rgef-1*(rb848) null mutant (*rgef-1(-/-)*) was obtained from the *C. elegans* Knockout Consortium (Vancouver, BC). Mutants

were backcrossed (6X) into WT background. Various transgenic strains were generated for studies reported here: *rgef-1*::RGEF-1b-GFP, *rgef-1*::RGEF-1b^{R290A}-GFP, *rgef-1*::RGEF-1b^{P503G}-GFP, *rgef-1*::RGEF-1b^{4A}-GFP, *rgef-1*::RGEF-1b^{S135A}-GFP, *odr-1*::RGEF-1b-GFP, *odr-3*::RGEF-1b-GFP, *gpa-3*::RGEF-1b-GFP, *odr-1*::LET-60^{V12}, *rgef-1*::RAP-1^{V12}, *rgef-1*::MEK-2^{E223D227}-GFP(gf) and *odr-1*::MEK-2^{E223D227}-GFP(gf) transgenes were expressed in the *rgef-1*(-/-) null background. *rgef-1*:: GFP, *rgef-1*::RGEF-1b^{R290A}-GFP, *odr-1*::LET-60^{N17}, *odr-1*::RAP-1^{N17}, *rgef-1*::MEK-2^{A223A227}-GFP(dn) and *odr-1*:: MEK-2^{A223A227}-GFP(dn) transgenes were expressed in WT worms. All transgenic *C. elegans* were generated by methods described above.

Animals carrying a temperature sensitive *sos-1* allele (*sos-1(up604*)) were obtained from the CGC. SOS-1 catalyzes loading of GTP onto LET-60 during embryonic and larval development. The *sos-1(up604)* allele encodes a full length SOS-1 protein that contains a Glu^{980} to Lys mutation (Rocheleau et al., 2002). At 20ºC, SOS-1^{E980K} functions normally and supports proper development and a WT phenotype. Incubation of animals at 25ºC causes SOS-1^{E980K} inactivation, which is accompanied by embryonic and larval lethality. For chemotaxis experiments, synchronized young adult animals were incubated at the non-permissive temperature (25ºC) for 24 h prior to assay in order to eliminate GEF activity. Loss of SOS-1^{E980K} function was confirmed by the expected observation of extensive embryonic and larval lethality in offspring of animals maintained at 25ºC for 18-24 h.

The *C. elegans age-1*(*mg305*) strain was provided by Dr. Gary Ruvkun (Harvard Medical School). The *age-1(mg305)* allele encodes a temperature sensitive AGE-1 protein (Wang and Ruvkun, 2004). The animals express an AGE-1 (PI3K) variant that has normal activity when the nematodes are maintained at 18ºC. However, the kinase is inactivated at 25ºC. Synchronized, young adult *age-1*(*mg305*) animals (grown at 18º) were shifted to 25ºC for 24 hours to eliminate AGE-1 activity. Animals that have a hypomorphic mutation in AGE-1 (*age-1(hx546)*) were obtained from the CGC.

Analysis of transgenic animals by fluorescence microscopy – The *rgef-1* promoter (2670 bp) was ligated to either an RGEF-1b-GFP or GFP transgene in the *C. elegans* expression vector pPD 95.77, as described above. Transgenic animals were generated and selected by monitoring GFP fluorescence as previously described (Fire et al., 1991; Freedman et al., 1993). Slides were prepared as previously described (Feng et al., 2007). Fluorescence signals emanating from GFP or RGEF-1b-GFP in individual cells of living or fixed worms were recorded by using a Zeiss Axio Imager Z1 microscope.

Immunohistochemical detection of phospho-MPK-1 and RFP in intact animals - Immunostaining was performed to visualize and quantify endogenous phospho-MPK-1 in AWC neurons. All animals also expressed an *odr-1*::RFP transgene (provided by Dr. Noelle L'etoile, University of California, Davis CA), which enabled identification of AWC via anti-RFP IgGs. RFP expression was robust in AWC neurons, minimal and intermittent in AWB neurons and undetectable in other cells. Methods used for fixation and permeabilization of *C. elegans* are modifications of previously reported procedures (Hirotsu et al., 2000; Nonet et al., 1993). WT, mutant and transgenic *C. elegans* were grown on NGM plates under standard conditions. Animals were collected, washed and incubated in CTX buffer (5 mM KH_2PO_4/K_2HPO_4, pH 6.6, 1 mM $CaCl_2$, 1 mM $MgSO_4$), for 3h at 21°C before exposure to odorant. (Operations were performed at 21°C, unless noted otherwise.) Nematodes were then pelleted by gravity and resuspended in 100 µl of either BZ (diluted 1:10,000 in CTX buffer), PMA (300 nM in CTX buffer), BZ plus PMA, or CTX buffer alone (control). After a 3 min incubation period, fixation and permeabilization of the animals was initiated by adding 1 ml of modified Bouin's fixative (Bouin's solution/methanol/β-mercaptoethanol (βME), 40:40:1). Samples were slowly rocked on a reciprocating shaker for 30 min and then frozen in liquid N_2. Next, the worm suspension was quickly thawed at 21°C and incubated for an additional 30 min. Specimens were then pelleted by centrifugation at 300 x g for 10 sec and washed three times with 1.4 ml BTB (20 mM sodium borate, pH 9.2, containing 0.5% Triton X-100 and 2% βME). Samples were then incubated for

1h in 1 ml BTB. Subsequently. specimens were washed once with BT buffer (BTB lacking βME), and twice with ABA buffer (phosphate buffered saline, pH 7.4, containing 1% BSA, 0.5% Triton X-100, 10 mM NaN_3 and 1mM EDTA). Animals were then incubated in ABA buffer for 30 min prior to addition of primary IgGs.

Fixed animals were incubated with primary antibodies in 200 µl of ABA buffer at 21^{o}C for 16h; incubations with secondary antibodies were performed in 200 µl of ABB buffer (ABA containing less BSA (0.2%)) at 21^{o}C for 2h. One primary antibody was a mouse monoclonal IgG (M8159, Sigma-Aldrich) directed against a diphosphorylated A-loop peptide that is identical in *C. elegans* MPK-1 and mammalian ERKs. It was used at a dilution of 1:500. Rabbit polyclonal IgGs that bind multiple epitopes in RFP (DS red) were also employed as primary antibody. This reagent was purchased from BioVision (Mountain View, CA) and used at a dilution of 1:100. The secondary antibodies, DyLight-549 conjugated goat anti-mouse IgG antibodies (1:200) and DyLight-488 conjugated goat anti-rabbit IgG antibodies (1:200) were obtained from Jackson Immunoresearch.

Slides were prepared as previously described (Feng et al., 2007). Fluorescence signals derived from phospho-MPK-1-antibody complexes in AWC neurons were recorded, analyzed and quantified with a Zeiss Axio Imager Z1 microscopy system and Zeiss AxioVision and ImageJ software as previously described (Fu et al., 2009). DyLight-549 fluorescence intensity was normalized to the mean fluorescence of FluoSphere beads (Molecular Probes).

Intra-neuronal targeting of RGEF-1b- RGEF-1b GFP was differentially targeted within AWC neurons by attaching *C. elegans* cytochrome b5 (138 amino acids) to the C terminus of GFP. A short, hydrophobic, C-terminal domain selectively anchors cytochrome b5 by inserting into the ER membrane (Rolls et al., 2002; Saheki and Bargmann, 2009). The longer N-terminal and central portions of cytochrome b5 are exposed to the cytoplasm. DNA encoding *C. elegans*

cytochrome b5 (named C31E10.7 in WormBase) was amplified by PCR, using a cDNA library as a template for KOD DNA polymerase. Nhe-I (5`) and Eco-RI (3`) restriction sites were appended to the cDNA via the primers. An *odr-1*::RGEF-1b-GFP transgene was assembled in the pPD 95.77 expression plasmid as described above. Mutagenesis was used to eliminate the translation termination codon of GFP and add a 3` Nhe-I restriction site. Cytochrome b5 cDNA was cloned, in frame with the RGEF-1b-GFP ORF, into recombinant pPD 95.77 by using Nhe-I and Eco-RI restriction sites. The chimeric transgene (designated *odr-1*::RGEF-1b-GFP-b5) encodes an RGEF-1b-GFP-cytochrome b5 fusion protein that localized in the AWC axon and cell body.

The N-terminal 36 amino acids of ODR-3 contain myristoylation and palmitoylation sites that target reporter proteins to plasma membrane in dendrites, cilia and cell bodies of AWC neurons (Roayaie et al., 1998). Consequently, a DNA fragment, which includes 2683 bp of *odr-3* promoter-enhancer DNA and 108 bp (codons 1-36) of contiguous exon 1, was amplified by PCR using *C. elegans* genomic DNA as a template. Sph-I (5`) and Pst-I (3`) restriction sites were added in the primers. This enabled cloning of the DNA fragment into the pPD 95.77 expression vector as described above. RGEF-1b cDNA was cloned downstream from *odr-3* codon 36 and upstream from GFP by using Pst-I and Bam-H1 restriction sites, as described above. The resulting chimeric transgene encodes a fusion protein (designated NT36-RGEF-1b-GFP), in which the N-terminal targeting domain of ODR-3 routes the RGEF-1b-GFP GEF to the AWC dendrite, cilium and cell body. Intra-neuronal locations of the RGEF-1b-GFP fusion proteins were determined by immunofluorescence microscopy as described above.

Primers used for cloning gene promoters in pPD 95.77

promoter	5` primer	3`primer
rgef-1 (2670bp)	5`-GACTGCATGCCGAGTCAA CTGAAATCCG-3`	5`-GACTGACTCTGCAGCGTC GTCGTCGTCGATGC-3`
odr-1 (2403bp)	5`-GACTGCATGCCCACTGAG TCCACATCACATTC-3`	5`-GACTCTGCAGTTTGAGCA GAAGGCCAATTATCG-3`
odr-3 (2754bp)	5`- GACTCTGCAGATCTAAAA AAACAATGATCTATGAG-3`	5`-GACTGCATGCGCAACTCG CTCCTTTCATTG-3`
gpa-3 (4599bp)	5`-GACTGCATGCCCAGCTGG AATAGTGTCA-3`	5`- GACTCTGCAGAATTGTTC TTTGGCTAATAAT-3`
hsp-16-2 (392bp)	5`-GACTGCATGCGATCAAGA GCATTTGAATCAG-3`	5`-GACTCCCGGGGATTATAG TTTGAAGATTTC-3`

Primers used for cloning cDNAs in pPD 95.77

cDNA	5` primer	3`primer
RGEF-1 cDNA outer	5`-GGTTTAATTA CCCAAGTT TGAG-3` (SL1)	5`-GTGTGTGGAAGACTGGTG A-3`
RGEF-1 cDNA innner	5`-GACTCTGCAGCTCAATCG TTCGTCGTGAAG -3`	5`-GACTGGATCCCGAATTGT GAGGGAATGTACTG-3`
MEK-1 cDNA outer	5`-CGCTAAATTTTCCCATTT TCCCC-3`	5`-GAGCATCCTAGACAGTGA GAGAC-3`
MEK-1 cDNA inner	5'-GACTCCCGGGAGTGGATTC AACTTTGGAG-3`	5'- GACTCTGCAGCAGATAAT GTCGAGCGGA -3`
LET-60 outer	5`-GACTCTGCAGGTAATGA CGGAGTACAAGCTTG- 3`	5`-GAGAACAAGTTCAATCGA TGC- 3`
LET-60 inner	5`-GACTCTGCAGGTAATGA CGGAGTACAAGCTTG- 3`	5`-GACTGGATCCTCACATTA TTTGACACTTCTTC- 3`
RAP-1 outer	5`-CTCTCTCCACGTTCACTC ATC- 3`	5`-GTAGATAAAGATAATCAC ATGATGAC- 3`
RAP-1 inner	5`-GACTCTGCAGAGAATGCG GGAGTATAAGATTG- 3`	5`-GATCGGATCCGTCACATG ATGACACACGAGCAGCAC- 3`

SUPPLEMENTAL REFERENCES

Altan-Bonnet, N., Sougrat, R., Liu, W., Snapp, E.L., Ward, T., and Lippincott-Schwartz, J. (2006). Golgi inheritance in mammalian cells is mediated through endoplasmic reticulum export activities. Mol. Biol. Cell *17*, 990-1005.

Chen, Q., Lin, R.Y., and Rubin, C.S. (1997). Organelle-specific targeting of protein kinase AII (PKAII). Molecular and in situ characterization of murine A kinase anchor proteins that recruit regulatory subunits of PKAII to the cytoplasmic surface of mitochondria. J. Biol. Chem. *272*, 15247-15257.

Feng, H., Ren, M., Wu, S.L., Hall, D.H., and Rubin, C.S. (2006). Characterization of a novel protein kinase D: Caenorhabditis elegans DKF-1 is activated by translocation-phosphorylation and regulates movement and growth in vivo. J. Biol. Chem. *281*, 17801-17814.

Fire, A., Albertson, D., Harrison, S.W., and Moerman, D.G. (1991). Production of antisense RNA leads to effective and specific inhibition of gene expression in C. elegans muscle. Development *113*, 503-514.

Fire, A., Harrison, S.W., and Dixon, D. (1990). A modular set of lacZ fusion vectors for studying gene expression in Caenorhabditis elegans. Gene *93*, 189-198.

Freedman, J.H., Slice, L.W., Dixon, D., Fire, A., and Rubin, C.S. (1993). The novel metallothionein genes of Caenorhabditis elegans. Structural organization and inducible, cell-specific expression. J Biol Chem *268*, 2554-2564.

Fu, Y., Ren, M., Feng, H., Chen, L., Altun, Z.F., and Rubin, C.S. (2009). Neuronal and intestinal protein kinase D isoforms mediate Na+ (salt taste)-induced learning. Sci Signal *2*, ra42.

Glantz, S.B., Li, Y., and Rubin, C.S. (1993). Characterization of distinct tethering and intracellular targeting domains in AKAP75, a protein that links cAMP-dependent protein kinase II beta to the cytoskeleton. J. Biol. Chem. *268*, 12796-12804.

Jones, D., Russnak, R.H., Kay, R.J., and Candido, E.P. (1986). Structure, expression, and evolution of a heat shock gene locus in Caenorhabditis elegans that is flanked by repetitive elements. J. Biol. Chem. *261*, 12006-12015.

Land, M., Islas-Trejo, A., Freedman, J.H., and Rubin, C.S. (1994). Structure and expression of a novel, neuronal protein kinase C (PKC1B) from Caenorhabditis elegans. PKC1B is expressed selectively in neurons that receive, transmit, and process environmental signals. J. Biol. Chem. *269*, 9234-9244.

Li, Y., Ndubuka, C., and Rubin, C.S. (1996). A kinase anchor protein 75 targets regulatory (RII) subunits of cAMP-dependent protein kinase II to the cortical actin cytoskeleton in non-neuronal cells. J Biol Chem *271*, 16862-16869.

Nonet, M.L., Grundahl, K., Meyer, B.J., and Rand, J.B. (1993). Synaptic function is impaired but not eliminated in C. elegans mutants lacking synaptotagmin. Cell *73*, 1291-1305.

Ren, M., Feng, H., Fu, Y., Land, M., and Rubin, C.S. (2009). Protein kinase D is an essential regulator of C. elegans innate immunity. Immunity *30*, 521-532.

Roayaie, K., Crump, J.G., Sagasti, A., and Bargmann, C.I. (1998). The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in C. elegans olfactory neurons. Neuron *20*, 55-67.

Rolls, M.M., Hall, D.H., Victor, M., Stelzer, E.H., and Rapoport, T.A. (2002). Targeting of rough endoplasmic reticulum membrane proteins and ribosomes in invertebrate neurons. Mol. Biol. Cell *13*, 1778-1791.

Saheki, Y., and Bargmann, C.I. (2009). Presynaptic CaV2 calcium channel traffic requires CALF-1 and the alpha(2)delta subunit UNC-36. Nat. Neurosci. *12*, 1257-1265.

Wu, S.L., Staudinger, J., Olson, E.N., and Rubin, C.S. (1998). Structure, expression, and properties of an atypical protein kinase C (PKC3) from Caenorhabditis elegans. PKC3 is required for the normal progression of embryogenesis and viability of the organism. J. Biol. Chem. *273*, 1130-1143.

Zhang, L., Wu, S.L., and Rubin, C.S. (2001). A novel adapter protein employs a phosphotyrosine binding domain and exceptionally basic N-terminal domains to capture and localize an atypical protein kinase C: characterization of Caenorhabditis elegans C kinase adapter 1, a protein that avidly binds protein kinase C3. J. Biol. Chem. *276*, 10463-10475.