Supporting Information

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SI Materials and Methods

Nematode and Bacteria Strains. All *C. elegans* strains were cultured on *Escherichia coli* OP50-1 and maintained under standard conditions at 20 or 25 °C (3). The *egl-8(n488)*, *egl-30(n686)*, *daf-2 (e1370)*, *daf-16(mu86)*, *sek-1(km4)*, and *IsDAF-16::GFP* (TJ356) strains were obtained from the *C. elegans* Genetic Center. The *egl-30(n686)* mutant harbors a splice site acceptor mutation that results in reduction of gene function (4). *egl-8(n488)* is a null allele with a 1.8-kb deletion that removes exons 10 and 11 of *egl-8* and disrupts the reading frame before the catalytic Y domain of the PLCβ (5). *egl-8(md1971)* is a loss of function allele with an early stop codon that probably prevents formation of part of the catalytic Y domain and the C-terminal region of the EGL-8 protein (5). The VP303 (*rde-1(ne219); [nhx-2:rde-1, rol-6]*) and NR222 (*rde-1 (ne219); [lin-26:rde-1, rol-6]*) strains were a kind gift from Kevin Strange, Vanderbilt University. Double mutants were constructed and verified by using standard genetic and molecular methods. *P. aeruginosa* strain PA14 was used for nematode infection (6). *E. coli* HT115 clones expressing double-stranded RNA for RNAi were obtained from Geneservice and Open Biosystems.

***C. elegans* Survival Assay.** *C. elegans* survival assays were performed as described (7). To avoid the confounding effects of varying brood sizes, egg laying rates, and progeny hatching within the infected worms on worm mortality, we used worms rendered sterile by RNAi of *pos-1*, loss of which results in inviable embryos (7, 8). Statistical analysis was performed by using Kaplan–Meier nonparametric survival analysis using the software Statview (version 5.0.1; SAS Institute). Worms that died because of desiccation on the walls of the Petri dish or because of bursting vulva were censored from further analysis.

Feeding RNAi. Feeding RNAi was used to knockdown the genes of interest by using RNAi clones obtained from Geneservice and Open Biosystems. Identity of each clone was verified by PCR and/or sequencing. Standard NGM plates containing 1 mM IPTG were used to induce double-stranded RNA (dsRNA) synthesis (9). Neuronal tissues are known to be less sensitive to gene inactivation by RNAi (10). We overcame this challenge by feeding animals with dsRNA corresponding to the gene of interest for two successive generations (Table S1). The VP303 *rde-1(ne219); [nhx-2:rde-1, rol-6]* strain, which restricts RNAi-mediate gene knockdown only to intestinal cells (11, 12), was used to inactivate the gene of interest only in the intestine. The NR222 *rde-1(ne219); [lin-26:rde-1, rol-6]* worms, in which RNAi-mediated gene knockdown is restricted to the epidermis (13), was used as a control. For adult *skn-1* RNAi, worms were grown to young adult stage on *pos-1* RNAi clone at 25 °C, after which they were transferred to *skn-1* RNAi clones for 48 h to inactivate *skn-1* only in the adult stage.

PMA Treatment. Phorbol 12-myristate 13-acetate (PMA) and its inactive analog, 4α-phorbol 12-myristate 13-acetate (4α-PMA) (Sigma-Aldrich) were dissolved in DMSO to obtain a stock solution of 1 mg/mL. PMA plates were prepared by spreading the PMA stock solution to the required effective concentration on survival assay plates along with OP50-1. 4α-PMA at the same concentration was used as a control in all experiments involving PMA. Plates were incubated overnight in the dark to achieve even diffusion of PMA in the agar as well as sufficient growth of OP50-1 as food for worms. After a 6- to 8-h exposure to PMA or 4α-PMA, 1-d-old adult worms were used for oxidative stress resistance assay or immunoblot analysis. Aldicarb-induced paralysis was used to ascertain the efficacy of PMA-induced hypersecretion (14).

Immunoblot Analysis. Worm samples were washed with M9 buffer and snap frozen in liquid nitrogen and stored at −80 °C until further analysis. Samples were homogenized in disruption buffer, and protein content was measured with the protein estimation Pierce BCA kit (product no. 23250, Pierce). Equal amount of protein was loaded on to 12% reducing SDS-acrylamide gels and electrophoresis, and transfer was performed by using standard techniques. The phospho p38 antibody (Promega), p38 antibody (Cell Signaling Technology), and the anti-actin antibody (Sigma-Aldrich) were used for immunoblot analysis.

Oxidative Stress Sensitivity Assay. Standard NGM plates were coated with a final concentration of 3 mM sodium arsenite (Sigma-Aldrich) and allowed to dry overnight (15). Plates were seeded with appropriate amount of 50× concentrated culture of *E. coli* OP50-1. Worms were transferred to these plates and assayed at 25 °C. For paraquat (methyl viologen; Sigma-Aldrich) sensitivity, worms were treated with 100 mM paraquat in M9 buffer containing 5% of 25× concentrated OP50-1 in 96-well plates at 25 °C. For each experiment ≈120 animals were scored for survival. Plates were scored every 12 h, and worms that did not move on their own or respond when prodded by a platinum wire were considered dead.

Aldicarb Resistance Assay. Aldicarb (2-methyl-2-[methylthio]-propionaloxime; Chem Services) stocks were dissolved in acetone. Standard NGM worm plates were treated with Aldicarb to a final concentration of 0.7 mM and allowed to dry at room temperature (16). For each experiment, animals were picked to pretreated plates and scored for resistance to the drug. Approximately 120 worms were used per strain per treatment for each experiment and monitored every 4–6 h for paralysis. Worms were considered paralyzed if they did not move on their own or respond when prodded with a platinum wire.

qRT-PCR Analysis of Gene Expression. RNA extraction and qRT-PCR analysis of antimicrobial gene targets was performed on age-matched 1-d-old hermaphrodites as described (17). After specific experimental conditions, RNA was extracted from ≈1,000 to 2,000 worms by using TRIzol. qRT-PCR was performed by using 100 ng of RNA using the iScript One-Step RT-PCR kit with SYBR Green (Bio-Rad Laboratories). Bio-Rad iCycler was used for amplification and quantitation of the products. Specificity of amplification was confirmed by melt curve analysis after amplification. Normalized threshold cycle values were used to calculate fold increases or decreases of RNA levels in samples from test condition/animal as compared with controls. Actin RNA levels (obtained with the pan-actin primers that amplify *act-1, 3*, and *4*), *ama-1*, and F44B9.5 were used for normalization. ANOVA analysis using Dunnett's test was performed by using normalized threshold cycle values, a $P < 0.05$ was considered significant. Primer sequences are available upon request.

DAF-16 Nuclear Delocalization Assay. Assay was performed as described (18). Briefly, the *cdc25.1* gene was inactivated by RNAi in TJ356, or the *egl-8(n488), egl-30(n686)*, or *ins-7(tm1907)* mutant, each harboring the DAF-16::GFP transgene that was crossed-in from TJ356 animals. This treatment produced animals without germ-line and constitutive localization of DAF-16::GFP to the intestinal nuclei. One-day-old adult animals were then exposed to

PA14 or OP50-1 for 17–21 h. The number of intestinal nuclei that accumulate DAF-16::GFP were enumerated at 200× total magnification by using a Leica DMRXA2 microscope using I3 filter cube. A total of 20 animals were assayed per strain per condition.

1. Kawli T, Tan M-W (2008) Neuroendocrine signals modulate the innate immunity of Caenorhabditis elegans through insulin signaling. *Nat Immunol* 9:1415–1424.
2. Tullet JMA, et al. (2008) Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. *Cell* 132:1025–1038.
3. Brenner S (1974) The genetics of Caenorhabditis elegans. *Genetics* 77:71–94.
4. Brundage L, et al. (1996) Mutations in a C. elegans Gqalpha gene disrupt movement, egg laying, and viability. *Neuron* 16:999–1009.
5. Miller KG, Emerson MD, Rand JB (1999) Goalpha and diacylglycerol kinase negatively regulate the Gqalpha pathway in C. elegans. *Neuron* 24:323–333.
6. Tan MW, Mahajan-Miklos S, Ausubel FM (1999) Killing of Caenorhabditis elegans by Pseudomonas aeruginosa used to model mammalian bacterial pathogenesis. *Proc Natl Acad Sci USA* 96:715–720.
7. Shapira M, Tan MW (2008) Genetic analysis of Caenorhabditis elegans innate immunity. *Methods Mol Biol* 415:429–442.
8. Tabara H, Hill RJ, Mello CC, Priess JR, Kohara Y (1999) pos-1 encodes a cytoplasmic zinc-finger protein essential for germline specification in C. elegans. *Development* 126:1–11.
9. Kamath RS, et al. (2001) Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in Caenorhabditis elegans. *Genome Biol.* 2:research0002.1–0002.10.
10. Tavernarakis N, Wang SL, Dorovkov M, Ryazanov A, Driscoll M (2000) Heritable and inducible genetic interference by double-stranded RNA encoded by transgenes. *Nat Genet* 24:180–183.
11. Espelt MV, Estevez AY, Yin X, Strange K (2005) Oscillatory Ca2+ signaling in the isolated Caenorhabditis elegans intestine: Role of the inositol-1,4,5-trisphosphate receptor and phospholipases C beta and gamma. *J Gen Physiol* 126:379–392.
12. Lorin-Nebel C, Xing J, Yan X, Strange K (2007) CRAC channel activity in C. elegans is mediated by Orai1 and STIM1 homologues and is essential for ovulation and fertility. *J Physiol* 580:67–85.
13. Qadota H, et al. (2007) Establishment of a tissue-specific RNAi system in C. elegans. *Gene* 400:166–173.
14. Miller KG, et al. (1996) A genetic selection for Caenorhabditis elegans synaptic transmission mutants. *Proc Natl Acad Sci USA* 93:12593–12598.
15. Inoue H, et al. (2005) The C. elegans p38 MAPK pathway regulates nuclear localization of the transcription factor SKN-1 in oxidative stress response. *Genes Dev* 19:2278–2283.
16. Yook KJ, Proulx SR, Jorgensen EM (2001) Rules of nonallelic noncomplementation at the synapse in Caenorhabditis elegans. *Genetics* 158:209–220.
17. Shapira M, et al. (2006) A conserved role for a GATA transcription factor in regulating epithelial innate immune responses. *Proc Natl Acad Sci USA* 103:14086–14091.
18. Evans EA, Chen WC, Tan MW (2008) The DAF-2 insulin-like signaling pathway independently regulates aging and immunity in C. elegans. *Aging Cell* 7:879–893.

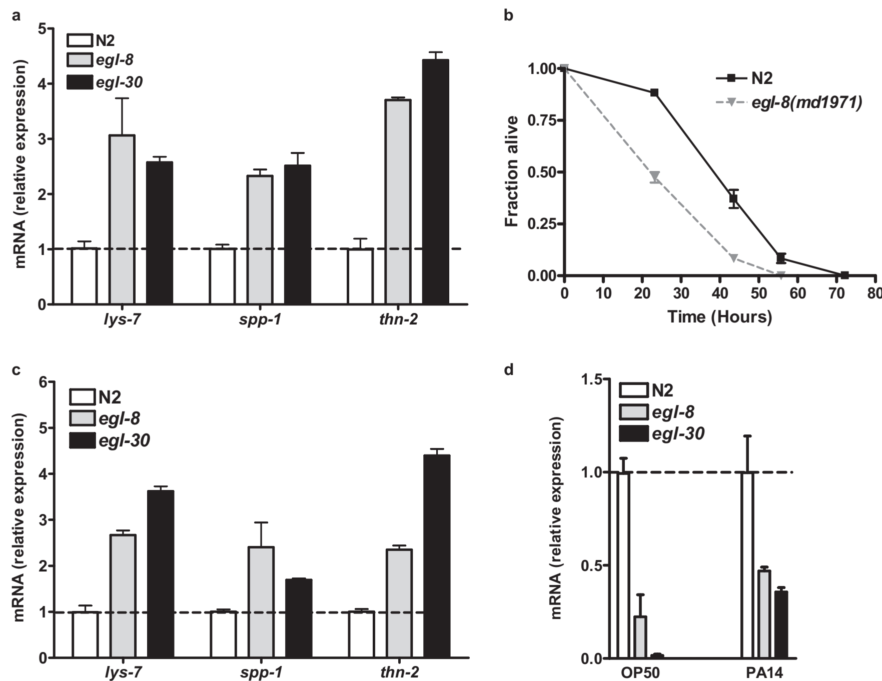

Fig. S1. Elevated expression of IIS-regulated immune genes in *egl-8(md1971)* and *egl-30(n686)* mutants. (*A*) mRNA levels of immune genes in *egl-8(md1971)* and *egl-30(n686)* relative to wild-type (N2) under conditions of normal growth on *E. coli* as measured by qRT-PCR. (*B*) *egl-8(md1971)* mutants are sensitive to pathogen. Fraction of *egl-8(md1971)* and wild-type (N2) alive is plotted as a function of time of exposure to *P. aeruginosa*. Shown is a representative of two experiments for a cohort of 100–120 age-matched adults for each strain. Log rank $P < 0.001$ relative to N2. (*C* and *D*) *egl-8* mutants maintain reduced IIS upon infection. mRNA levels of *lys-7*, *spp-1*, and *thn-2* (*C*) and *ins-7* (*D*) in *egl-8(md1971)* and *egl-30(n686)* relative to wild-type (N2) after 12 h exposure to *P. aeruginosa* as measured by qRT-PCR. In *A*, *C*, and *D*, mRNA level of each gene was compared with N2, which was set at 1. Shown is the mean ± SD for a representative of experiment. mRNA expression data were analyzed by using ANOVA (Dunnett's test); all datasets were significantly different from wild-type ($P < 0.05$).

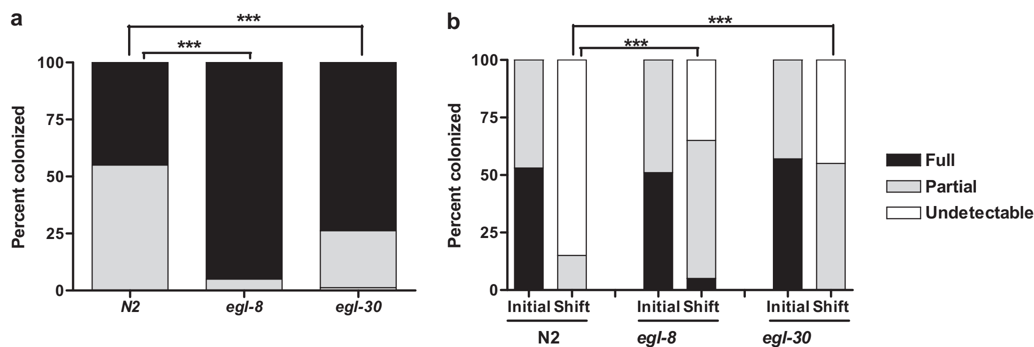

Fig. S2. *egl-30(n686)* and *egl-8(n488)* worms were more extensively colonized (*A*) and cleared pathogen less effectively than wild-type (*B*). (*A*) Worms were exposed to PA14-GFP for 24 h, after which the extent of colonization was measured. (*B*) Worms were exposed to PA14-GFP for varying amount of times to achieve equal extent of colonization in the population (Initial). These worms were then shifted to OP50-1 lawns, and the extent of PA14-GFP colonization was assessed after 24 h (shift). Graphs shown in *A* and *B* are representatives of two independent experiments with 80 worms per sample. ***$P < 0.001$ by χ^2 analysis. Worms were categorized into three visual categories as described (1).

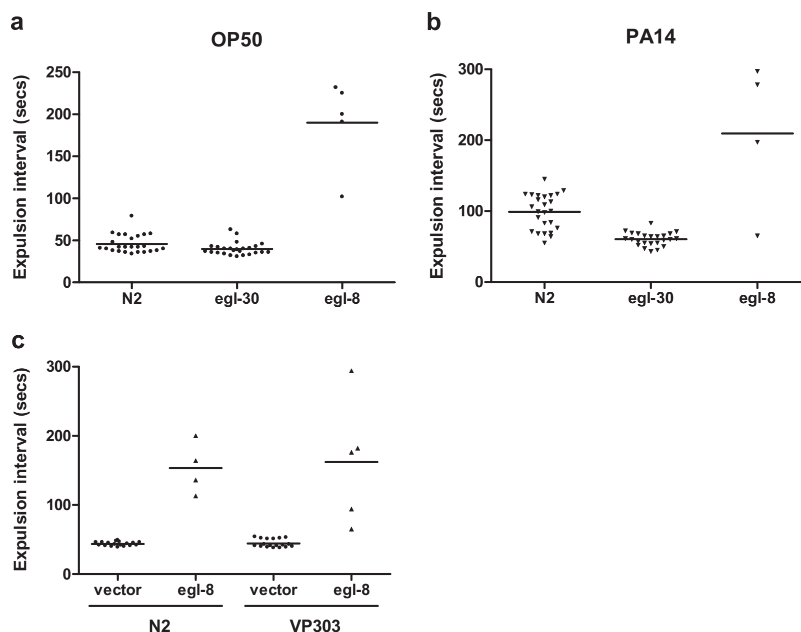

Fig. S3. (*A* and *B*) Pathogen expulsion does not contribute to pathogen clearance. Five expulsion intervals for five worms each are shown in seconds (*y* axis) for age-matched wild-type (N2), *egl-30(n686)*, and *egl-8(n488)* adult worms on OP50 (*A*) and 24 h after PA14 (*B*) infection. *egl-8(n488)* animals were observed for 5 min on OP50 (*A*) and 10 min on PA14 (*B*) and any events during this time period were recorded. (*C*) Loss of *egl-8* in the intestine is sufficient to cause a defecation defect. Effect of *egl-8* RNAi or vector RNAi in the entire animal (N2) or only in the intestine (VP303) on expulsion intervals. RNAi was performed for two generations. For vector RNAi, six expulsion intervals were measured for three worms each. Because of the extended expulsion intervals, *egl-8* RNAi animals were observed for 5 min and any events during this time period were recorded.

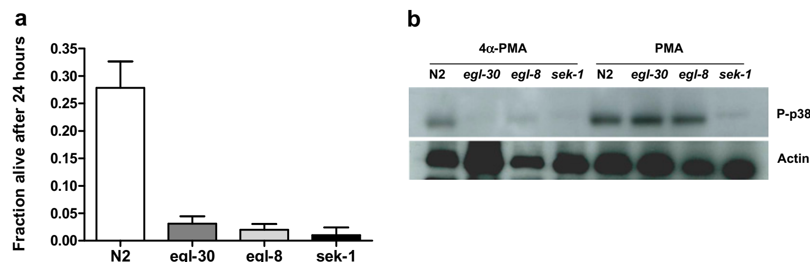

Fig. S4. (*A*) Gqα-PLCβ signaling mutants are sensitive to paraquat-induced oxidative stress. Shown is survival of wild-type (N2), *egl-30(n686)*, *egl-8(n488)*, and *sek-1(km4)* adult worms after exposure to 100 mM paraquat in liquid at 25 °C. (*B*) PMA, a DAG mimetic, rescues the PMK-1 activation defect in Gqα-PLCβ signaling mutants. Activation of PMK-1 by the DAG mimetic, PMA in wild-type (N2), *egl-30(n686)*, and *egl-8(n488)* animals. Wild-type (N2), *egl-30(n686)*, *egl-8 (n488)*, and *sek-1(km4)* animals were treated with 100 ng/mL PMA or 4α-PMA for 6 h, and the extent of PMK-1 phosphorylation was determined by Western blot. PMK-1 phosphorylation was detected by using p38-phosphospecific antibody (P-p38). Anti-actin antibody was used as a loading control for protein levels.

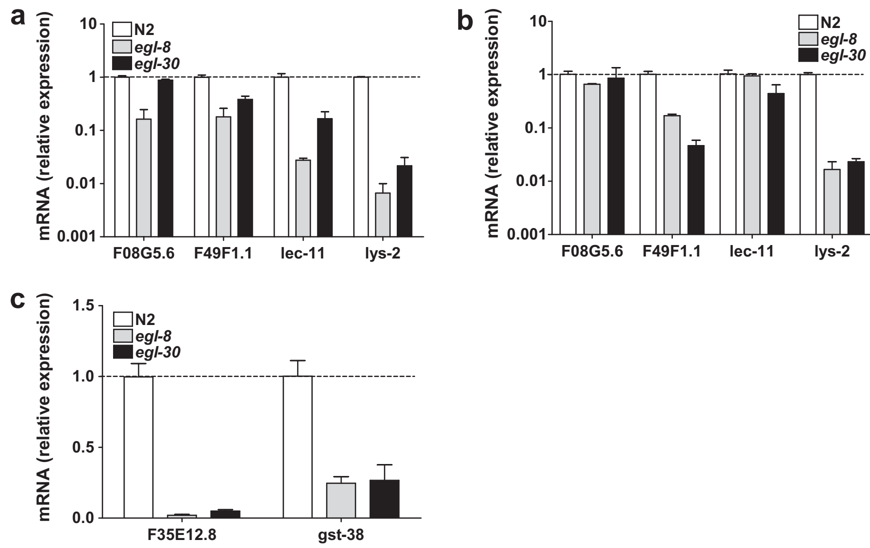

Fig. S5. *egl-8(md1971)* and *egl-30(n686)* mutants have reduced levels of p38 MAPK-regulated immune genes. (*A–C*) Decreased expression of p38 MAPK-regulated immune and oxidative stress response genes in *egl-8(md1971)* and *egl-30(n686)* mutants. mRNA levels of p38 MAPK-regulated immune genes in *egl-8(md1971)* and *egl-30(n686)* relative to wild-type (N2) adults during normal growth on *E. coli* (*A*) and after exposure to *P. aeruginosa* (*B*). (*C*) mRNA levels of p38 MAPK-regulated oxidative stress protective genes in *egl-8(md1971)* and *egl-30(n686)* relative to wild-type (N2) adults during normal growth on *E. coli*. For each graph, mRNA level of immune or stress genes is shown relative to the respective N2, which was set at 1. Shown are relative mean ± SD. Data were analyzed by using ANOVA (Dunnett's test); all datasets were significantly different from wild-type ($P < 0.05$).

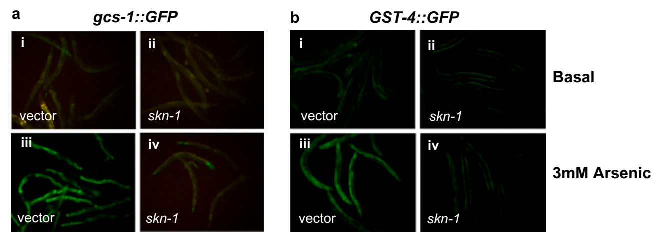

Fig. S6. *skn-1* functions in adult *C. elegans* to influence oxidative stress resistance. Effect of *skn-1* on the expression of *gcs-1*::GFP (*A*) and GST-4::GFP (*B*). Basal expression on *gcs-1* and *gst-4* under conditions of normal growth (*i* and *ii*) and upon exposure to 3 mM arsenic for 3 h (*iii* and *iv*) in control (*i* and *iii*) and *skn-1* (*ii* and *iv*) RNAi-treated animals. In *A*, *iii* taken at 10 times reduced exposure to avoid overexposure (6 ms as compared with 60 ms exposure for other images).

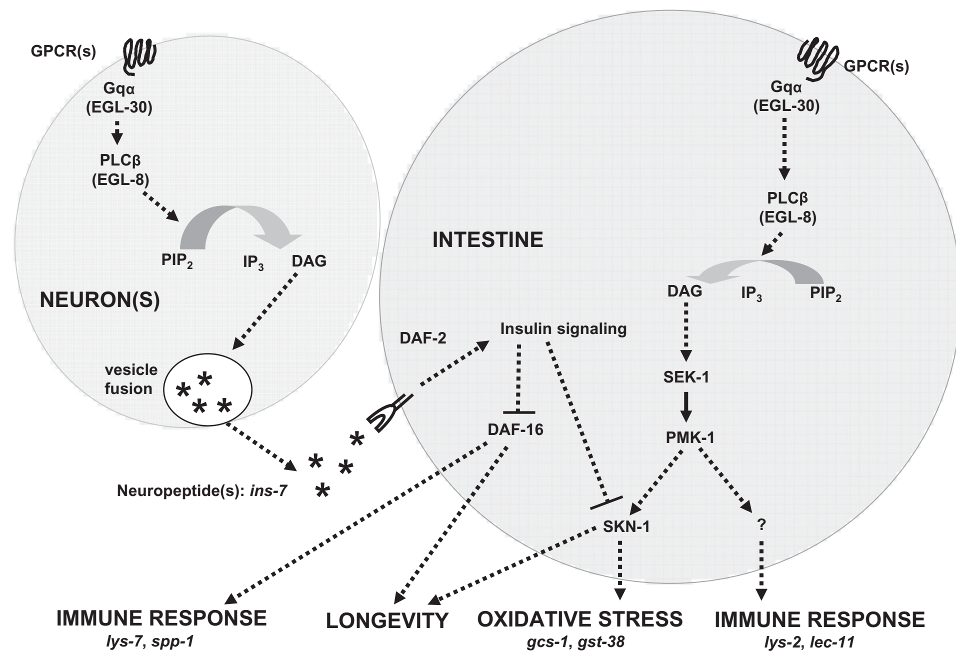

Fig. S7. Regulation of immunity, oxidative stress response, and longevity by Gqα-PLCβ signaling. Gqα-PLCβ signaling in the neurons affects IIS in the intestine through regulated release of insulin agonist, such as *ins-7*, and affects longevity and immune gene expression. Within the intestine, Gqα-PLCβ signaling is mediated by DAG to affect p38 MAPK activity and primarily affects oxidative stress response and immunity. SKN-1 acts downstream of p38 MAPK to influence oxidative stress response, and probably downstream of IIS, but independent of DAF-16 to influence longevity (2). In adult animals, SKN-1 is however dispensable for immune functions. Increased pathogen sensitivity of Gqα-PLCβ signaling-defective mutants to an intestinal pathogen is an aggregate of the protective effects of diminished IIS and detrimental effects of diminished p38 MAPK signaling, with the latter being more prominent.

Table S1. Comparison of the effect of loss of function mutations and RNAi-mediated knockdown of *egl-8* and *egl-30* on the sensitivity to aldicarb mediated paralysis

Gene inactivated	Genetic lesion	RNAi-mediated knockdown
None	8.1 ± 0.5 (87/89)	7.9 ± 0.7 (88/89)
egl-8	12.6 ± 1.3* (89/90) (1.5X)	10.3 ± 1.0* (91/91) (1.3X)
egl-30	13.1 ± 1.2* (90/90) (1.6X)	11.4 ± 1.0* (87/88) (1.4X)

*Log rank $P < 0.001$ relative to vector.

Aldicarb sensitivity of N2, *egl-8(n488)*, and *egl-30(n686)* as compared with N2 animals treated with vector, *egl-8*, or *egl-30* RNAi for two generations. Number of animals used is shown in parentheses, as (No. scored/Total). "No. scored" represents the number of animals analyzed, and "Total" represents the number of animals at the start of experiment. The number in parentheses (X) represents the relative strength of the phenotype as compared with the control population of N2 worms grown under identical conditions. Animals that were stuck to the wall of the plates or died because of bursting of the vulva were censored. TDmean is the mean time to paralysis as calculated by using Kaplan–Meier nonparametric analysis.

Table S2. Epidermal Gqα and PLCβ do not contribute to pathogen sensitivity, oxidative stress sensitivity, and life span

RNAi	Whole-animal gene knockdown (N2)	Epidermis-restricted gene knockdown (NR222)
Sensitivity to paralysis by 0.7 mM aldicarb		
Vector	7.9 ± 0.7 (88/89)	8.3 ± 0.6 (89/89)
egl-8	10.3 ± 1.0* (91/91)	7.8 ± 1.2 (90/91)
egl-30	11.4 ± 1.0* (87/88)	8.2 ± 0.7 (88/88)
sek-1	7.2 ± 0.7 (90/90)	8.1 ± 0.8 (93/93)
Life span at 25 °C		
Vector	182.2 ± 2.7 (103/116)	167.3 ± 6.6 (104/119)
egl-8	199.5 ± 2.7* (115/126)	165.9 ± 5.8 (112/120)
egl-30	201.6 ± 3.1* (116/123)	164.7 ± 4.0 (117/121)
sek-1	180.3 ± 4.1 (105/124)	166.1 ± 3.2 (119/122)
Sensitivity to *P. aeruginosa*-mediated killing		
Vector	59. 8 ± 1.9 (107/114)	45.3 ± 1.1 (108/120)
egl-8	46.7 ± 1.7* (85/86)	43.2 ± 3.0 (118/122)
egl-30	42.5 ± 1.4* (82/87)	44.7 ± 1.4 (119/121)
sek-1	31.5 ± 1.0* (66/69)	45.9 ± 1.7 (112/117)
Oxidative stress sensitivity (3 mM arsenite)		
Vector	54.4 ± 3.7 (82/84)	38.9 ± 2.2 (87/89)
egl-8	35.4 ± 1.8* (79/84)	39.5 ± 1.1 (89/90)
egl-30	36.8 ± 2.0* (84/88)	38.7 ± 2.1 (90/90)
sek-1	15.5 ± 1.1* (67/85)	37.6 ± 2.0 (86/88)

*Log rank $P < 0.001$ relative to vector.

Number of animals used is shown in parentheses, as (No. scored/Total). "No. scored" represents the number of animals analyzed, and "Total" represents the number of animals at the start of experiment. Animals that were stuck to the wall of the plates or died because of bursting of the vulva were censored. TDmean is the mean time to death (or mean time to paralysis for the aldicarb analysis) as calculated by using Kaplan–Meier non-parametric analysis.