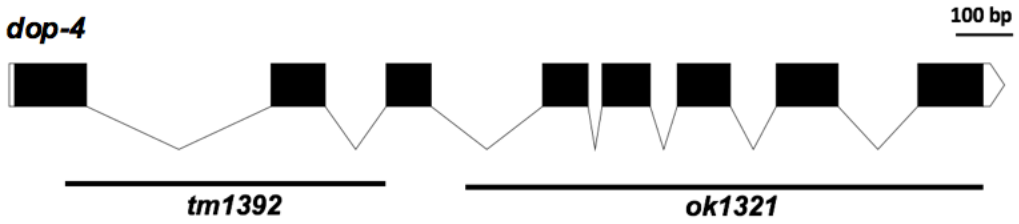

Figure S1, related to Fig. 2 – ***dop-4* gene and *dop-4* alleles.** The *tm1392* allele contains a 1086-bp deletion, and the *ok1321* allele contains a 1766-bp deletion.

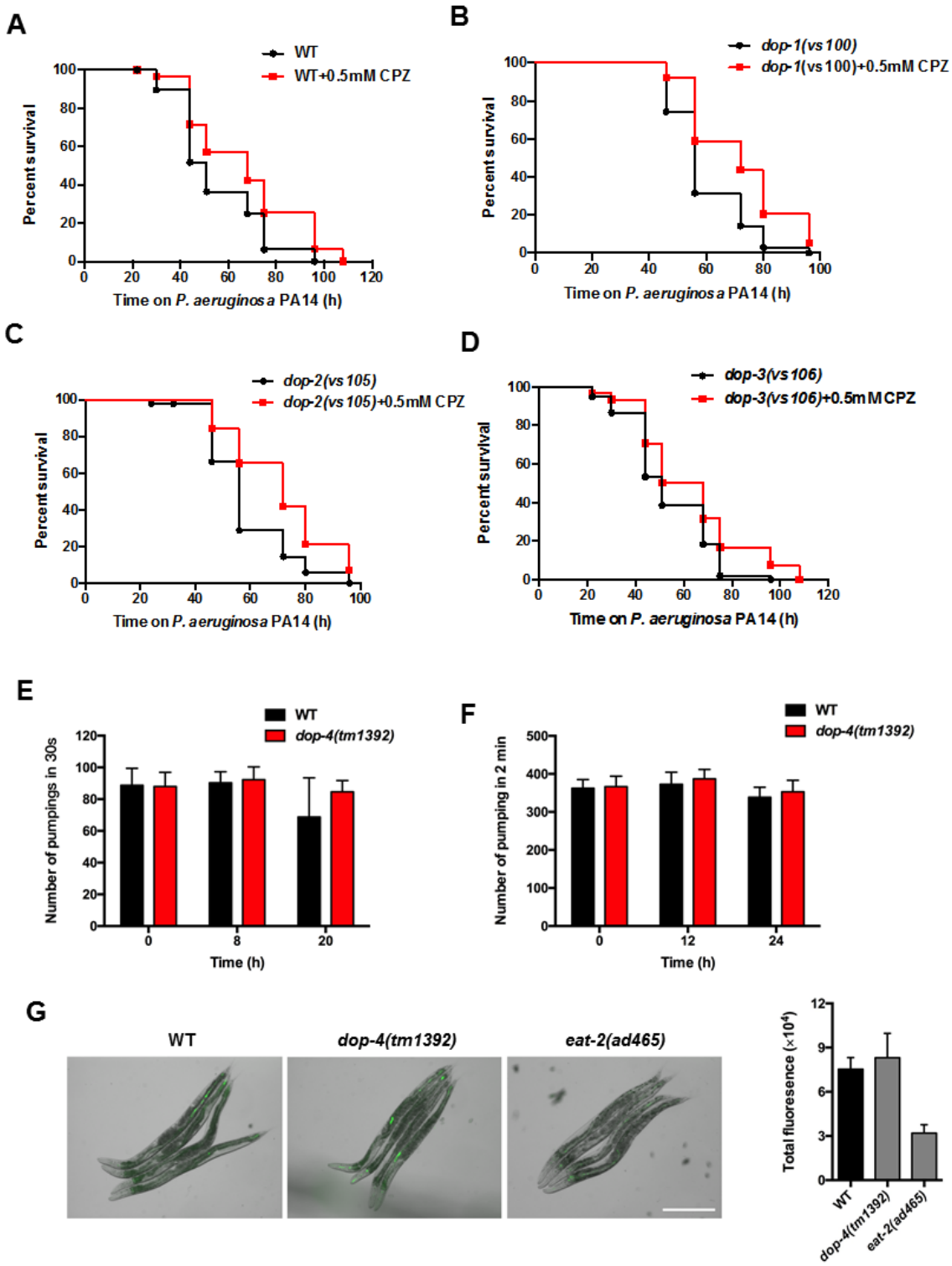

Figure S2, related to Fig. 2 – Chlorpromazine treatment of *dop-1(vs100)*, *dop-2(vs105)*, and *dop-3(vs106)* mutants and feeding behavior of *dop-4(tm1392)* animals.

(A) Wild-type, (B) *dop-1(vs100)*, (C) *dop-2(vs105)*, and (D) *dop-3(vs106)* animals were

exposed to *P. aeruginosa* PA14 and scored for survival in the presence or absence of the dopamine antagonist chlorpromazine (CPZ). WT vs. WT+CPZ: $p < 0.01$, *dop-1(vs100)* vs. *dop-1(vs100)*+CPZ: $p < 0.01$, *dop-2(vs105)* vs. *dop-2(vs105)*+CPZ: $p < 0.01$, *dop-3(vs106)* vs. *dop-3(vs106)*+CPZ: $p < 0.05$.

(E and F) The pumping rates of ten nematodes were determined before infection with *P. aeruginosa* or at 12 and 24 hours post-infection. (E) over 2 min; (F) over 30 s. Bars represent means ± SEM. (G) Wild-type, *eat-2(ad465)*, and *dop-4(tm1392)* animals were fed a 25:1mixture of *P. aeruginosa* PA14 and 0.2-μm fluorescent beads for 30 minutes and visualized using a Leica M165 FC fluorescence stereomicroscope. Scale bar = 0.4 mm. Fluorescence was quantified using software ImageJ, WT vs. *dop-4(tm1392)* $p > 0.1$, WT vs. *eat-2(ad465)* $p < 0.05$.

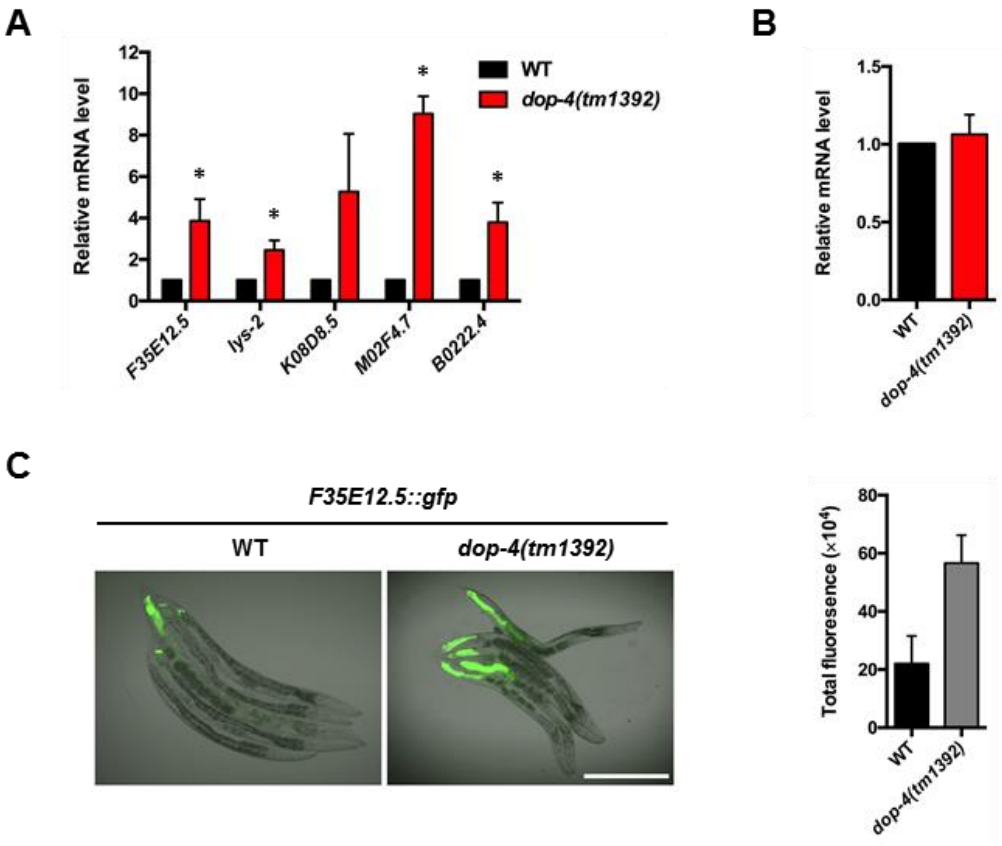

Figure S3, related to Fig. 3 – ***dop-4(tm1392)*** **animals show higher expression level of *pmk*-1–regulated genes upon *P. aeruginosa* infection.** (A) Quantitative RT-PCR analysis of *pmk-1*–regulated genes in *dop-4(tm1392)* compared with wild-type animals fed on *P. aeruginosa* PA14. (B) Expression level of *pmk-1* gene in *dop-4(tm1392)* compared with wild-type animals fed on *P. aeruginosa* PA14. $p > 0.1$ (C) Fluorescence image of wild-type and *dop-4(tm1392)* animals expressing *F35E12.5*::*gfp*. Young adult animals were fed on *P. aeruginosa* PA14 for 8 hours and visualized using a Leica M165 FC fluorescence stereomicroscope. Scale bar = 0.5 mm. Fluorescence was quantified using software ImageJ. All bars represent means ± SEM. $p < 0.05$.

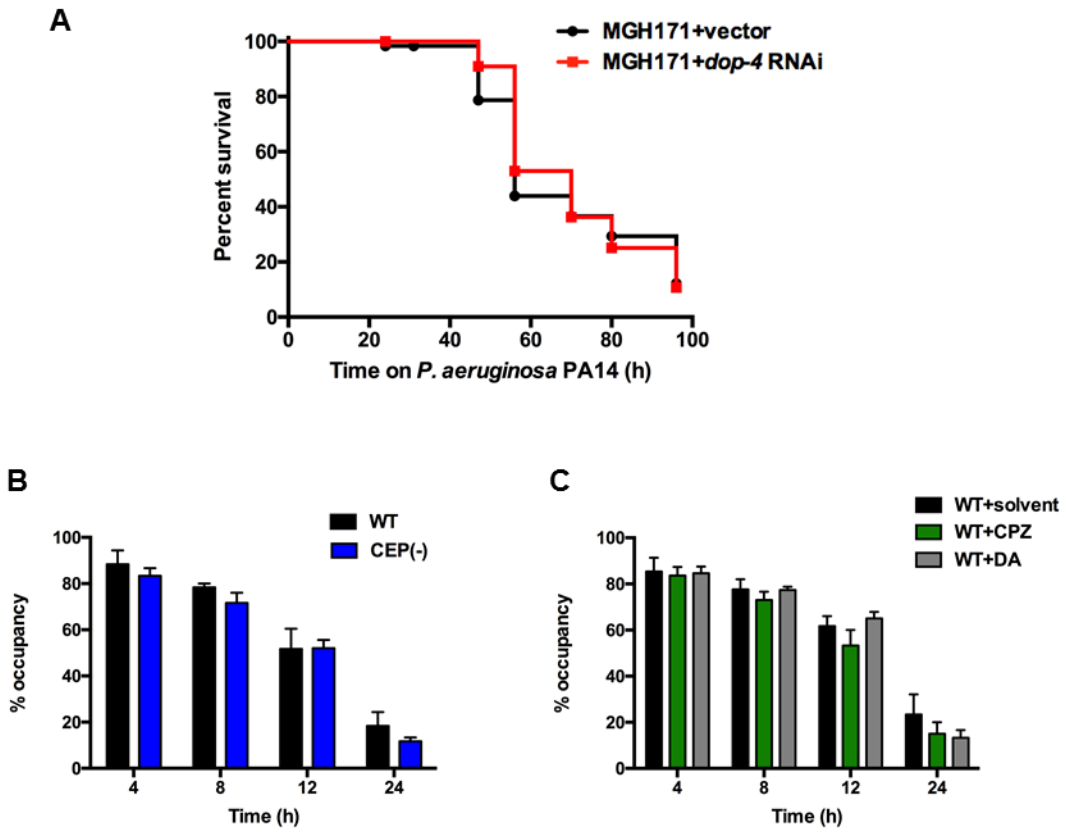

Figure S4, related to Fig. 4 – DOP-4 does not function in the intestine to regulate the immune response and neural ablation or drug treatment do not alter the avoidance behavior on *P. aeruginosa*. (A) The intestine-specific RNAi strain MGM171 fed vector control or *dop-4* RNAi bacteria was exposed to *P. aeruginosa* PA14 and scored for survival. $p > 0.1$. (B) Wild-type and CEP(-) animals were cultured on partial pathogen lawns, and the percentage of animals on the pathogen lawn was calculated for each time point. (C) Wild-type animals were cultured on partial pathogen lawns supplemented with S-Basal (control solvent), chlorpromazine (CPZ) and dopamine (DA), and the percentage of animals on the pathogen lawn was calculated for each time point. Identical concentrations of chlorpromazine (CPZ) and dopamine (DA) used in survival assay were applied. All bars represent means ± SEM.

Supplemental Experimental Procedures:

Nematode and Bacterial Strains

C. elegans strains were cultured and maintained using standard conditions as previously described [S1]. Bristol N2 was used as the wild-type control unless otherwise indicated. Strains LX645 *dop-1(vs100)*, LX702 *dop-2(vs105)*, LX703 *dop-3(vs106)*, FG58 *dop-4(tm1392)*, RB1254 *dop-4*(*ok1321*), AY101 *acIs101*[P*F35E12.5*::*gfp*, *rol-6*(*su1006*)] [S2] and TU3401 *sid-1*(*pk3321*);*uIs69*[P*myo-2*::*mCherry*,P*unc-119*::*sid-1*] were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN). Strain BY250 *vtIs7*[P*dat-1*::*gfp*] was obtained from Joel Meyer (Duke University, Durham, NC). MGH171 *sid-1(qt9)*;*Is*[P*vha-6*::*sid-1*::*SL2*::*gfp*] was provided by Gary Ruvkun (Massachusetts General Hospital, Harvard Medical School, Boston, MA). DA465 *eat-2(ad465)* was provided by Ryan Baugh (Duke University, Durham, NC). The following bacterial strains were used: *Escherichia coli* strain OP50-1 [Sm^R], *Pseudomonas aeruginosa* strain PA14 and *P. aeruginosa* strain PA14-GFP [Amp^R, Kan^R]. Bacterial cultures were grown overnight in 4 ml of Luria-Bertani (LB) broth at 37°C.

Plasmid Constructs and Generation of Transgenic Lines

Plasmids pXC1 (pPD95.77_ *ced-3*(p15)::NZ) and pXC2 (pPD95.77_ CZ::*ced-3*(p17)) were constructed by cloning reconstituted caspases into the *Bam*HI and *Eco*RI sites of the pPD95.77 vector (Fire Lab *C. elegans* Vector Kit; Addgene, Cambridge, MA) to replace the GFP coding sequence. The templates used were plasmids TU806 (pPD95.75_*Pmec-18*::*ced-3*(p15)::NZ) and TU807 (pPD95.75_*Pmec-18*::CZ::*ced-3*(p17)) (Addgene, Cambridge, MA) [S3]. Plasmid pXC3 was constructed by inserting 979bp of the *ace-1* promoter sequence upstream of *ced-3*(p15)::NZ and plasmid pXC4 was constructed by inserting 700bp of the *dat-1* promoter sequence upstream of CZ::*ced-3*(p17). To avoid creating fusion proteins, plasmid pRK1 (pPD95.77_*SL2*::*gfp*) was used, in which an intercistronic sequence between *gpd-2* and *gpd-3* was cloned and inserted into the pPD95.77 vector at a *Kpn*I site in front of the GFP coding sequence (Dr. Rie Kajino-Sakamoto, unpublished work). A 3.4kb genomic fragment containing the *dop-4* gene was cloned into the *Pst*I and *Bam*HI sites of the pRK1 vector, forming the *dop-4*::*SL2*::*gfp*

fusion. Plasmid pXC5 was constructed by inserting 691bp of the *gcy-15* promoter sequence upstream of the *dop-4* sequence. Strain AY114 *vtIs7*[P*dat-1*::*gfp*];*acEx*[*Pdat-1*::*ced-3*(p17); *Pace-1*::*ced-3*(p15); P*unc-122*::*gfp*] was created by injecting 25ng/µl of plasmids pXC3 and pXC4, respectively, together with 50ng/µl of the co-injection marker P*unc-122*::*gfp*. Strain AY115 *dop-4(tm1392)*;*acEx*[P*gcy-15*::*dop-4*::*SL2*::*gfp*] was created by injecting 50 ng/µl of plasmid pXC5. Strain AY116 *dop-4(tm1392)*; *acIs101*[P*F35E12*.5::*gfp, rol-6*(*su1006*)] was created using standard genetic crossing.

C. elegans Killing Assay

The bacterial lawns used for the *C. elegans* killing assays were prepared by placing 20µl of an overnight culture of the bacterial strains on modified NGM agar medium (0.35% instead of 0.25% peptone) in 3.5cm-diameter plates. The plates were incubated overnight at 37°C and cooled to room temperature for 1 hour before being seeded with animals. Full-lawn plates were prepared by spreading 30µl of overnight culture on the complete surface of modified NGM agar medium in 3.5-cm-diameter plates. Animals were synchronized by placing gravid adults on NGM plates containing *E. coli* OP50 for 2 hours at 25°C. The gravid adults were removed, leaving the eggs to hatch and develop at 20°C. Synchronized young adult *C. elegans* hermaphrodites were transferred to modified NGM plates containing bacterial lawns and incubated at 25°C. Animals were scored at the indicated times for survival and transferred to fresh pathogen lawns each day. Animals were considered dead when they failed to respond to touch and no pharyngeal pumping was observed. Each experiment was performed in triplicate. N=60 animals.

Drug Treatment

Bacterial cultures were grown as described above. Fifty µl of 0.5 mM chlorpromazine hydrochloride or 1 mM dopamine hydrochloride (Sigma-Aldrich, St. Louis, MO) was placed on the bacterial lawn in each plate and equilibrated for absorption for 1 hour at room temperature. The bacterial lawn was then used for the following experiments. S-Basal buffer was used as the solvent and in the control plates.

C. elegans Longevity Assay

Bacterial cultures were grown as described above. The overnight cultures were concentrated at a 1:10 ratio and heat-killed at 100°C for 1 hour. Next, the cultures were placed on LB plates to confirm the killing effect. A 50-µl drop of the killed bacteria was plated on a 3.5-cm plate of modified NGM agar containing 100 µg/ml ampicillin. The plates were incubated at 25°C. Animals were scored at the indicated times for survival, and live animals were transferred to fresh plates if needed. Animals dead due to matricide were removed before analysis.

Bacterial Lawn Avoidance Assay

Bacterial lawns were prepared as described above. Twenty young adult animals were placed in the center of each bacterial lawn. The plates were incubated at 25°C, and the number of animals within the lawn were counted at each time point.

Fluorescent bead Assay

The assay was done by feeding synchronized animals with 0.2-µm fluorescent polystyrene beads (Phosphorex, Inc., Hopkinton, MA). *P. aeruginosa* PA14 cultures were mixed with the beads at a 25:1 dilution and 50 µl of the mixture were placed onto the plate and incubated at 37 °C overnight. Young adult animals were transferred to the plate and incubated at 25 °C for 30 minutes. After that, live worms were mounted for microscopy in M9 buffer with 5 mM sodium azide. The area containing the fluorescent beads was analyzed using ImageJ software.

Pharyngeal Pumping Assay

Bacterial lawns were prepared as described above. Young adult animals were transferred to bacterial lawns, and the plates were incubated at 25°C. At the indicated time points, the number of contractions of the terminal bulb was counted over 30 seconds and 2 minutes. A contraction was defined as the backward movement of the grinder in the terminal bulb of the pharynx. The pumping rates for ten nematodes were recorded for each strain.

Visualization of Bacterial Accumulation in the Nematode Intestine

The *P. aeruginosa* PA14-GFP culture was grown as described above. The bacterial lawns

were prepared by placing 100 µl of the culture on modified NGM agar medium in 6-cm-diameter plates. Synchronized young adult *C. elegans* were transferred to the plates and incubated at 25°C for 30 hours. Animals were transferred to an NGM plate seeded with *E. coli* OP50 and then washed in M9 buffer to eliminate the fluorescent bacteria stuck to the cuticle. The animals were mounted onto agar pads with 30mM sodium azide and visualized using a Leica M165 FC fluorescence stereomicroscope.

Quantification of Intestinal Bacterial Loads

The quantification of colony forming units (cfu) was performed by feeding synchronized *C. elegans* with *P. aeruginosa* PA14-GFP as described above. Animals were transferred to an NGM plate seeded with *E. coli* OP50 for 20 minutes to eliminate the fluorescent bacteria stuck to the cuticle. Animals were then transferred to a new NGM plate seeded with *E. coli* OP50 for 30 minutes to further eliminate the remaining fluorescent bacteria. Ten nematodes per strain were transferred into 50 µl of phosphate-buffered saline (PBS) plus 0.1% Triton X-100 and ground with sterilized plastic sticks. Serial dilutions of the lysates (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) were spread onto LB/kanamycin plates and incubated overnight at 37°C to select for *P. aeruginosa* PA14-GFP colonies.

Western Blot Analysis

Whole-worm lysates were prepared in the presence of protease and phosphatase inhibitors (Thermo Scientific, Waltham, MA). Phosphorylated PMK-1 protein was detected using an anti-active p38 polyclonal antibody from rabbit (Promega, Fitchburg, WI) and β-actin was detected using a monoclonal anti-actin antibody from mouse (Sigma-Aldrich, St. Louis, MO). Blots were developed using SuperSignal chemiluminescence substrate (Thermo Scientific, Waltham, MA).

RNA Interference

RNA interference was used to generate loss-of-function RNAi phenotypes by feeding nematodes with *E. coli* strain HT115(DE3) expressing double-stranded RNA homologous to a target gene. *E. coli* HT115(DE3) was grown overnight in LB broth containing ampicillin (100 µg/ml) at 37°C and spread onto NGM plates containing 100µg/ml

ampicillin and 5mM isopropyl 1-thio-β-D-galactopyranoside. RNAi-expressing bacteria were allowed to grow overnight at 37°C. L2 to L3 larval stage animals were placed on RNAi or vector control plates for 2 days at 20°C. Gravid adults were then transferred to fresh RNAi-expressing bacterial lawns and allowed to lay eggs for 2 hours to synchronize a second-generation RNAi population. The gravid adults were removed, and the eggs were allowed to develop at 20°C to reach the young adult stage for experimental use. Because knocking down *skn-1* causes a maternal-lethal effect to the progeny, *skn-1* RNAi was initiated at the L1 stage, and it was performed by placing synchronized L1 animals on RNAi plates for one generation. In all experiments, *unc-22* RNAi was included as a positive control to account for the RNAi efficiency.

RNA Isolation and Quantitative Real-time PCR (qRT-PCR)

Synchronized L1 larval animals were placed on NGM plates seeded with *E. coli* OP50 and grown at 20°C until the animals reached the L4 larval stage. Animals were collected and washed with M9 buffer before being transferred to modified NGM plates containing *E. coli* OP50 or *P. aeruginosa* PA14 for 4 hours at 25°C. Animals were washed off the plates with M9 and frozen in TRIzol (Life Technologies, Carlsbad, CA). Total RNA was extracted using the RNeasy Plus Universal Kit (Qiagen, Netherlands). Residual genomic DNA was removed using TURBO DNase (Life Technologies, Carlsbad, CA). A total of 2 μl of total RNA was reverse transcribed with random primers using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). qRT-PCR was conducted using Power SYBR PCR Master Mix (Applied Biosystems, Foster City, CA) on an Applied Biosystems 7900HT real-time PCR machine in 96-well plate format. The relative fold-changes of the transcripts were calculated using the comparative C_T ($2^{-\Delta\Delta CT}$) method and normalized to pan-actin. All experiments were performed in triplicate. Primer sequences are available upon request.

Statistical Analysis

Survival curves were plotted using GraphPad Prism 6 (GraphPad Software, La Jolla, CA). Survival curves were considered different from the control when p-values were < 0.05. The Kaplan-Meier method was used to calculate the survival fractions, and

statistical significance between survival curves was determined using the log-rank test. The two-sample *t* test was used when needed, and p-values < 0.05 were considered significant. All experiments were done in triplicate. Each comparison was done with the corresponding control individually.

Supplemental References

S1. Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics 77, 71-94.

S2. Bolz, D.D., Tenor, J.L., and Aballay, A. (2010). A Conserved PMK-1/p38 MAPK is Required in Caenorhabditis elegans Tissue-specific Immune Response to Yersinia pestis Infection. Journal of Biological Chemistry 285, 10832-10840.

S3. Chelur, D.S., and Chalfie, M. (2007). Targeted cell killing by reconstituted caspases. Proceedings of the National Academy of Sciences 104, 2283-2288.