Supporting Information

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SI Text

Role of Other Immunity Genes and Pathways in *irg-1* Induction by *P. aeruginosa*. In addition to the PMK-1 p38 MAPK pathway, we also tested the role of several other *Caenorhabditis elegans* immunity-related pathways in infection response gene 1 (*irg-1*) induction. The *dbl-1* TGF-beta pathway, which includes the TGF-beta ligand, *dbl-1*, and appears to be independent of the p38 MAPK pathway, has been implicated in *C. elegans* resistance to both bacterial and fungal pathogens (1, 2). The JNK pathway, including the terminal kinase *kgb-1*, has also been implicated in *C. elegans* immunity (3). In addition, the leucine-rich repeat GPCR *fshr-1* was recently shown to be important for resistance to bacterial pathogens and induction of an overlapping set of *Pseudomonas aeruginosa* response genes with the p38 MAPK pathway (4). Another pathway implicated in *C. elegans* immunity is the *bar-1*/β-catenin pathway. The β-catenin homolog *bar-1* is required for full induction of genes induced by infection with *Staphylococcus aureus* and for resistance to killing by this pathogen (5). We found that *P. aeruginosa* infection induced *irg-1* mRNA expression in *dbl-1*, *kgb-1*, *fshr-1*, and *bar-1* mutants at similar levels as in wild-type worms (Fig. S1 *B–E*). Therefore, these previously identified immune pathways do not appear to be required for *irg-1* induction.

We further investigated the roles of *hsf-1* and *elt-2*, two other transcription factors identified in our *irg-1::GFP* screen. qRT-PCR analysis of animals treated with *elt-2* RNAi showed that they were defective in the induction of all infection response genes that were tested (Fig. S4*B*). These included genes that are not predicted to have a GATA box (an *elt-2* binding site) in their promoter, such as *irg-1* and *irg-2*, suggesting that they are not directly regulated by ELT-2 protein. As described above, under the conditions of our RNAi treatment, *elt-2* RNAi causes an extensive reduction in intestinal cell volume. One possibility is that these shrunken intestinal cells are not able to make substantial quantities of RNA, thus explaining the general lack of induction of infection response genes that are primarily expressed in the intestine. Previous studies have indicated that *elt-2* is important for immunity in adult animals (6). Further analysis using postdevelopmental RNAi knock-down of *elt-2* in the adult animal is needed to resolve the role of *elt-2* in the early transcriptional response to infection. RNAi against *hsf-1* caused milder defects in infection-induced gene expression. However, like *elt-2*, *hsf-1* appeared to affect both PMK-1-independent and PMK-1-dependent induction. For example, *hsf-1* RNAi affected induction of *irg-1* (PMK-1-independent), as well as induction of *F49F1.6* (PMK-1-dependent) (Fig. S4*C*). Thus, *hsf-1* may generally affect several pathways, and not be specific to PMK-1-independent infection response gene induction, like *zip-2*.

SI Materials and Methods

Pathogen Treatment Experiments. For *S. aureus* experiments, overnight cultures of NCTC8325 were diluted 1:5 or 1:10, then seeded onto tryptic soy agar plates containing 5-μg/mL naladixic acid. Plates were incubated at 37 °C for 3 to 6 h, then animals were washed onto plates, incubated at 25 °C and viewed 8 to 20 h later. For *Salmonella enterica*, overnight cultures of SL1344 were seeded onto SK plates, incubated for 12 h at 37 °C, 2 h at 25 °C, then animals were washed onto plates and viewed 8 to 20 h later. For *Enterococcus faecalis*, overnight or 4-h cultures of OG1RF were seeded onto brain heart-infusion agar plates containing 100-μg/mL kanamycin, grown at 37 °C for 24 h, then animals were washed onto plates and viewed 8 to 20 h later. For control comparisons, the streptomycin-resistant *Escherichia coli* strain OP50-1 was used.

Construction of agIs17 (irg-1::GFP). The *irg-1* promoter-GFP fusion was made with overlap PCR using 3.8-kb upstream of *irg-1* and the first five amino acids fused in frame to GFP. This fusion product was injected into N2 animals, along with a *myo-2::mCherry* coinjection marker that expresses in the nuclei of pharyngeal muscle cells (gift of J. Kaplan). Three independent transgenic lines carrying the *irg-1::GFP* extrachromosomal array were isolated and all three lines induced GFP upon infection with *P. aeruginosa*. One of these lines was integrated using UV-irradiation to generate the integrated transgene *agIs17*. This strain was back-crossed to N2 four times to generate strain AU133, which was then used for experiments.

Cadmium Chloride Experiments. For $CdCl_2$ experiments, SK plates were poured with a final concentration of 100 μM $CdCl_2$ and seeded with concentrated OP50-1. Animals were transferred to $CdCl_2$ or control plates and then harvested 4 h later for qRT-PCR experiments.

1. Mallo GV, et al. (2002) Inducible antibacterial defense system in *C. elegans*. *Curr Biol* 12:1209–1214.
2. Zugasti O, Ewbank JJ (2009) Neuroimmune regulation of antimicrobial peptide expression by a noncanonical TGF-beta signaling pathway in *Caenorhabditis elegans* epidermis. *Nat Immunol* 10:249–256.
3. Kim DH, et al. (2004) Integration of *Caenorhabditis elegans* MAPK pathways mediating immunity and stress resistance by MEK-1 MAPK kinase and VHP-1 MAPK phosphatase. *Proc Natl Acad Sci USA* 101:10990–10994.
4. Powell JR, Kim DH, Ausubel FM (2009) The G protein-coupled receptor FSHR-1 is required for the *Caenorhabditis elegans* innate immune response. *Proc Natl Acad Sci USA* 106:2782–2787.
5. Irazoqui JE, Ng A, Xavier RJ, Ausubel FM (2008) Role for beta-catenin and HOX transcription factors in *Caenorhabditis elegans* and mammalian host epithelial-pathogen interactions. *Proc Natl Acad Sci USA* 105:17469–17474.
6. 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.

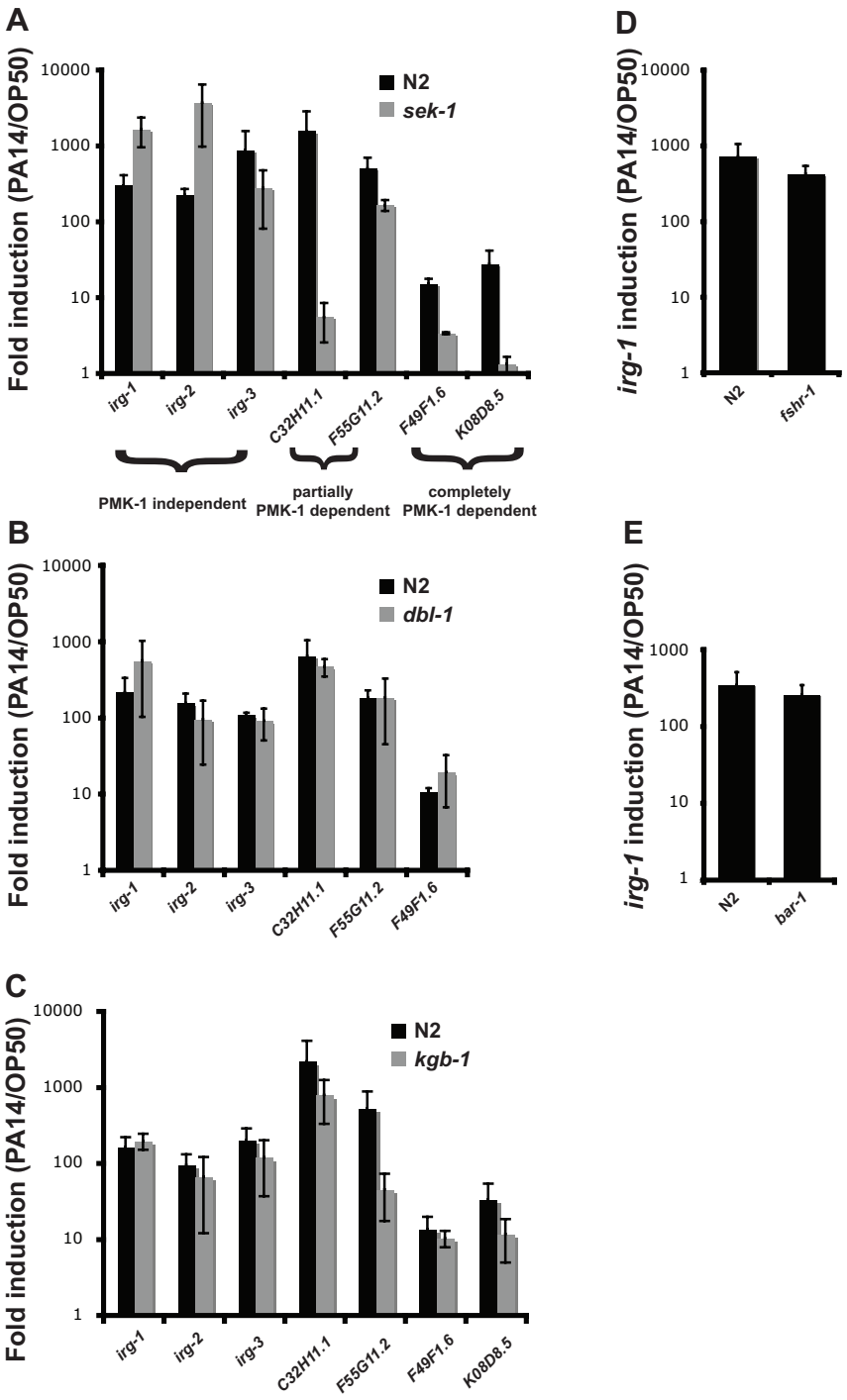

Fig. S1. Infection response genes are induced independently of previously identified defense pathways. qRT-PCR of infection response genes induced by *Pseudomonas aeruginosa* infection in wild-type (N2) animals and *sek-1* mutants (*A*), *dbl-1* mutants (*B*), *kgb-1* mutants (*C*), *fshr-1* mutants (*D*), and *bar-1* mutants (*E*). Results shown are the average of three *(A, B,* and *E),* or two *(C* and *D)* biological replicates. Error bars are SEM.

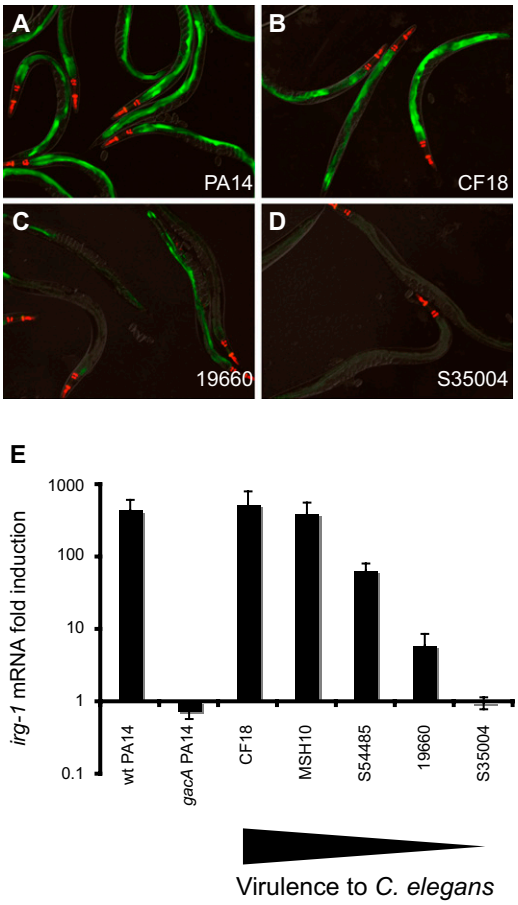

Fig. S2. *irg-1::GFP* induction on *P. aeruginosa* strains PA14 (*A*) (classified as strong induction), CF18 (*B*) (strong induction), 19660 (*C*) (medium induction), S35004 (*D*) (weak/no induction). (*E*) qRT-PCR of *irg-1* induction on *P. aeruginosa* strains with varying degrees of virulence. Results are the average of two independent biological replicates and error bars are SEM. See Table S1 for more data and for virulence information. *myo-2::mCherry* shows red pharyngeal expression as a marker for the presence of the transgene. All images were taken at the same time with the same camera exposure.

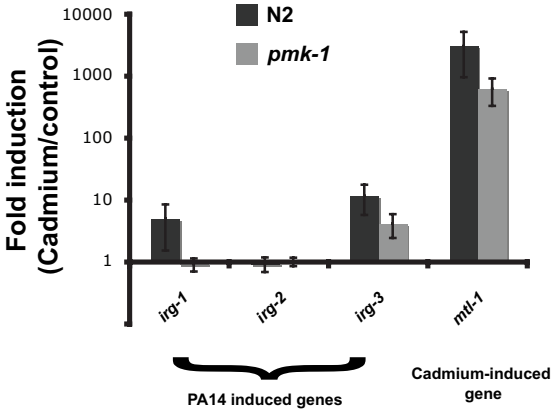

Fig. S3. qRT-PCR of cadmium-induced genes in N2 and *pmk-1(km25)* mutants.

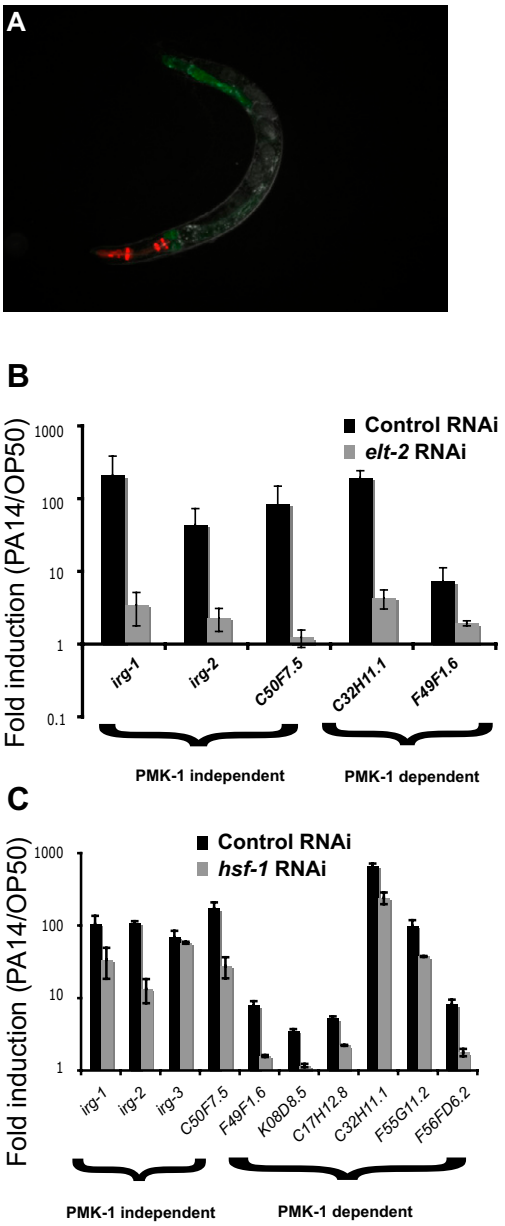

Fig. S4. Knock-down of transcription factors that affect *irg-1::GFP* induction. (*A*) *irg-1::GFP* expression in animals treated with *elt-2* RNAi (compare with control-treated animals shown in Fig. 1*F*—images were taken in same experiment with same camera exposure). qRT-PCR of infection response genes induced by *P. aeruginosa* infection in animals treated with control RNAi or *elt-2* RNAi (*B*) or *hsf-1* RNAi (*C*). qRT-PCR results shown are the average of three (*B*) or two (*C*) biological replicates. Error bars are SEM.

Fig. S5. Diagram of predicted *zip-2* coding region and *zip-2(tm4067)* deletion.

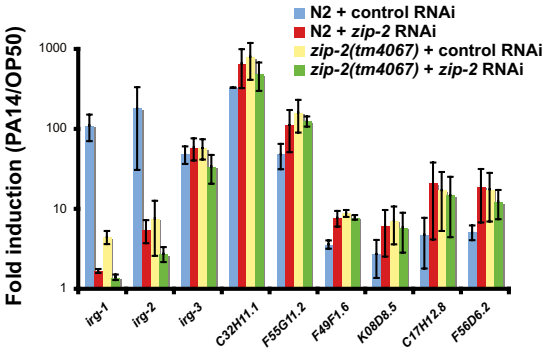

Fig. S6. qRT-PCR of *P. aeruginosa* infection response genes in N2 wild-type animals treated with control RNAi (L4440), N2 treated with *zip-2* RNAi, *zip-2 (tm4067)* treated with control RNAi (L4440), and *zip-2(tm4067)* treated with *zip-2* RNAi. Results are the average of three independent biological replicates, with all samples processed in parallel, and error bars are SEM.

Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOC)
Table S3 (DOC)
Table S4 (DOC)