Supplemental Data
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Antifungal Innate Immunity in *C. elegans*: PKCδ Links G Protein Signaling and a Conserved p38 MAPK Cascade

Katja Ziegler, C. Léopold Kurz, Sophie Cypowyj, Carole Couillault, Matthieu Pophillat, Nathalie Pujol, and Jonathan J. Ewbank

Supplemental Experimental Procedures

Number of Animals Quantified with the COPAS Biosort for Each Figure

Figure	Number of worms used in each sample, from left to right
3A	145, 125, 62, 150, 158, 68, 192, 87, 53, 93, 100, 38, 225, 116, 52, 45, 53, and 29
3B	208, 194, 369, 141, 114, 86, 189, 136, 83, 162, 186, 124, 115, 150, 141, 47, 56, and 69.
4A	725, 795, 821, 1241, 1135, 1209, 1140, 948, 864, and 652
4C	102, 125, 100, 225, 377, and 88
4D	142, 280, 135, 183, 228, and 147
5A	82, 302, 75, 70, 127, 55, 62, 152, 63, 63, 207, and 46
5B	147, 169, 64, 141, 152, 55, 178, 286, and 78
5C	67, 120, 94, and 241
6A	57, 52, 192, 126, 145, and 105
6B	107, 98, 124, 159, 118, and 120
6C	79, 160, 80, 112, 119, 92, 53, 92, 59, 98, 45, and 108
6D	242, 263, 317, 262, 238, and 229

Primers and Enzymes for SNP Mapping *fr1* on Chromosome IV

sition (chromosome: cM)	SNP name	Primers	Restriction enzyme and products (nt)
IV: -26.99	Y38C1A[2]	5'CGTCTAGTAAGTCATCTGTCGG 5'GGAGGACTAACGGTTCTGAGG	XbaI N2: 537 CB4856: 395 + 142
IV: -25.9	pKP4049	5'GATTCCGTCACTGGTTACTGAG 5'TTTCAATACGGCGTCCTG	MboI N2: 493 CB4856: 271 + 222
IV: -23.6	K11H12[1]	5'CGCACTGAAAATGGCTGAAATCTG 5'TCGGTTTCCGTTCATTCCTCCC	MfeI N2: 500 + 500 CB4856: 1000
IV: -23.11	Y55F3BR[6]	5'GGTGGCCTAGAAACTTTGAAAATC 5'GATATTCCATTTTCGCAATATTTTTC	HhaI N2: 120 + 80 CB4856: 200

Protein Extraction and Labeling

Worms were collected from plates 5 h after infection with *D. coniospora* and washed several times in M9 buffer to eliminate a maximum of bacteria and spores. Protein extracts were prepared using the Amersham 2D Clean Up kit and proteins were re-suspended in DiGE compatible buffer containing 7M Urea, 2 M Thiourea, Tris 25mM, CHAPS 4% at a pH of 8.5**.** DiGE labeling reactions were performed following the manufacturer's instructions (GE Healthcare). The combined samples were brought to a final volume of 380 µl with the destreack rehydration buffer containing 2% carrier ampholytes (IPG GE Healthcare) and immediately used for isoelectric focusing IEF.

Two-Dimensional Gel Electrophoresis

The IEF was performed using 150 µg of the mixed labelled protein loaded on 11 cm gels with an immobilized linear pH gradient of 3-10 (Immobiline DryStrips, GE Healthcare) in IPGphor strip holders (GE Healthcare) with an IPGphorIII machine (GE Healthcare) as follows: 30 V f gradient for 1.5h, 300 V gradient for 1.5h, 1000 V gradient for 1.5 hr, 8000 V gradient for 7 hr and 8000 V step for 4h, all at 20°C. Prior to SDS PAGE, IPG strips were soaked in equilibration buffer (6M urea, Tris pH8.8 50mM, SDS 2%, 65mM DTT, *glycerol 38.5%*) for 20 minutes. The second dimension was performed using a Criterion Dodeca Cell separation unit (Biorad) and pre-cast 10% SDS PAGE gels (Biorad) at 20°C. Electrophoresis was conducted according to the manufacturer's protocol. After SDS-PAGE, cyanine dye-labelled protein gels were scanned using the Ettan DiGE Imager scanner (GE Healthcare) at a 100 µm resolution. After fixing, gels were stained using the Imperial Protein Stain from Pierce.

Determination of protein abundance and statistics based on 2D DIGE were carried out with the DeCyder software package (version 6.5; GE Healthcare) with spot detection 10000 and area filter 30000. For the differential analysis 2 groups comparison was performed using the T-Test between

infected and non-infected samples. Differential spots were selected for a ratio> 30% and a p-value < 1%.

In-Gel Digestion and MALDI-TOF MS

Protein spots excised from Imperial protein stain gels were subjected to in-gel digestion with trypsin (Sequencing grade modified porcine trypsine; Promega, Madison, WI, USA) according to a modified protocol from Shevchenko et al. (Shevchenko et al., 1996).

Tryptic peptides were then extracted from the gel by successive treatment with 5% formic acid and 60% acetonitrile/5% formic acid. Extracts were pooled and dried in a Speedvac evaporator. Peptides resuspended in an α–cyano-4-hydroxycinnamic acid matrix solution (prepared by diluting 6 times a saturated solution in 50 % acetonitrile/0.3 % TFA), were then spotted on the metal target. Mass analyses were performed on a MALDI-TOF Bruker Ultraflex spectrometer (Bruker Daltonics, Wissembourg, France). Mass spectra were internally calibrated using autolytic peptides from trypsin.

Database Searching and Data Interpretation

The peptide mass lists were used to identify proteins using Mascot software available online. Criteria used for protein identification are given by Mascot as a Probability Based Mowse Score. Ions score is -10*Log(P), where P is the probability that the observed match is a random event. Protein scores greater than X (X is a number between 60 and 74, from one search to the other) are significant ($p<0.05$).

***tpa-1*-Specific RNAi Construct**

Primers with PstI restriction sites were used for PCR on *tpa-1A* cDNA. The product is 500nt long and corresponds to a sequence shared by *tpa-1A* and *tpa-1B* isoforms. PCR product was cloned into

pGEMTeasy vector (Promega) then the vector was digested with PstI and the insert cloned into pPD129.36 for transformation into HT115 *E. coli* competent cells.

Primers were:

5'AAACTGCAGGATGGTCACATAAAGCTTGCCG

5'AAACTGCAGCGCCGCCTTCTCGTTCGTGA

Supplemental Reference

Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996). Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Analytical Chemistry *68*, 850-858.

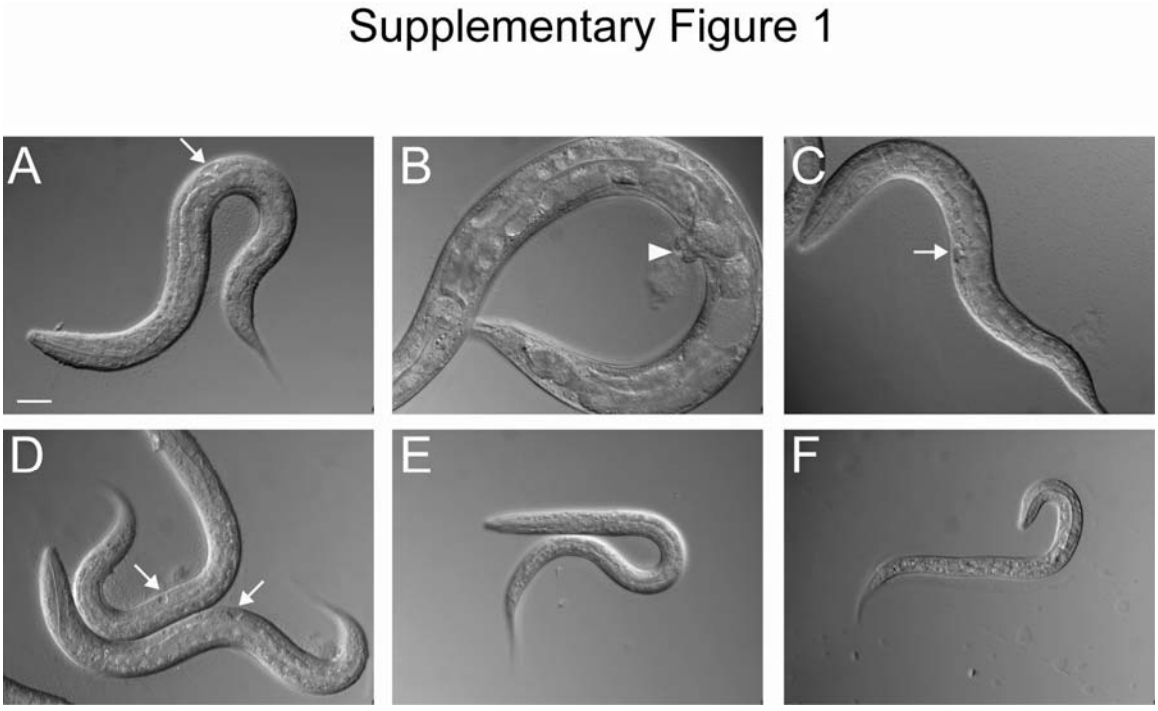

Figure S1. *tpa-1* Mutant Worms Are Resistant to PMA

(A–F) Nomarski images of WT animals (A), *tpa-1(fr1)* (B), *tir-1(tm3036)* (C), *pmk-1(km25)* (D), and *nipi-3(fr4)* mutants (E) exposed to PMA from L1 stage and allowed to develop for seven days at 25°C (see Experimental Procedures for details). The only strain able to reproduce is *tpa-1(fr1)* but the adult present a protruding vulva (arrowhead in B) and the F1 are blocked at the L1 stage as shown in (F). The wild type *tir-1* and *pmk-1* worms are the size of L2 larvae but are arrested at the L4 stage (arrow shows the vulval primordium), while *nipi-3(fr4)* mutants arrest at a more precocious stage. Scale bar represents 20 μm.

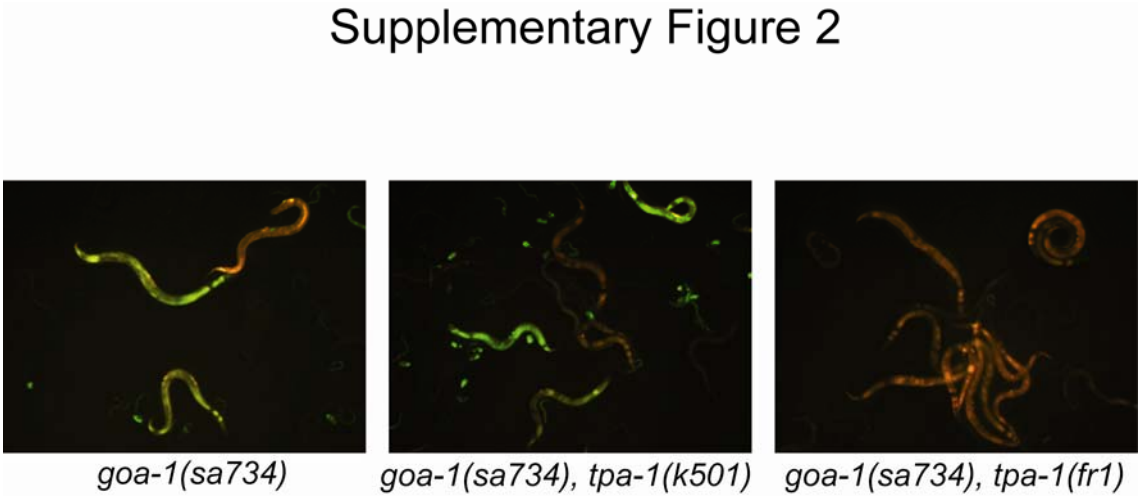

Figure S2. The Increased p*nlp-29::gfp* Expression Associated with *goa-1* Mutation Is Abrogated by the *tpa-1(fr1)* Allele but Not by *tpa-1(k501)*

Fluorescence pictures of representative *goa-1(sa734)*;*frIs7*, *tpa-1(k501);goa-1(sa734)*;*frIs7* and *tpa-1(fr1);goa-1(sa734)*;*frIs7* adult mutants in control conditions visualized with a filter allowing red and green fluorescence to be seen simultaneously.

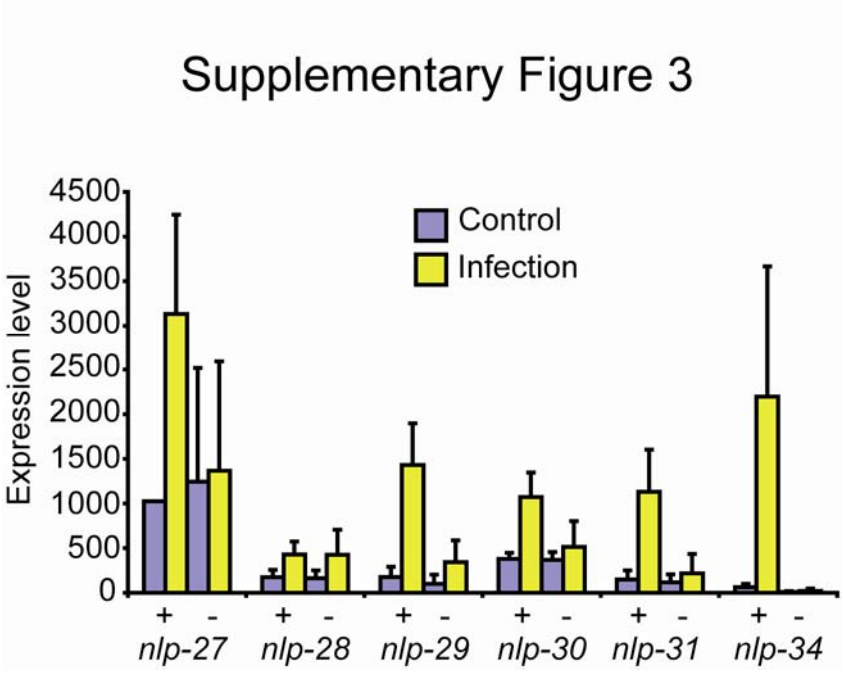

Figure S3. *tpa-1(fr1)* Mutation Impairs Induction of the Whole *nlp-29* Locus upon Infection

Relative expression of the *nlp* genes of the *nlp-29* locus in control and infected WT (+) and *tpa-1(fr1)* mutant (-) animals. Values represent means of three independent experiments; the bars are standard errors of the mean. With the exception of *nlp-28*, which in this series of experiments only showed a marginal induction in wild-type worms, the difference in the level of expression of the genes of *nlp-29* cluster was in all cases significantly higher ($p < 0.05$; one-paired Student's t test) after infection in the wild-type background than in the *tpa-1(fr1)* mutant.

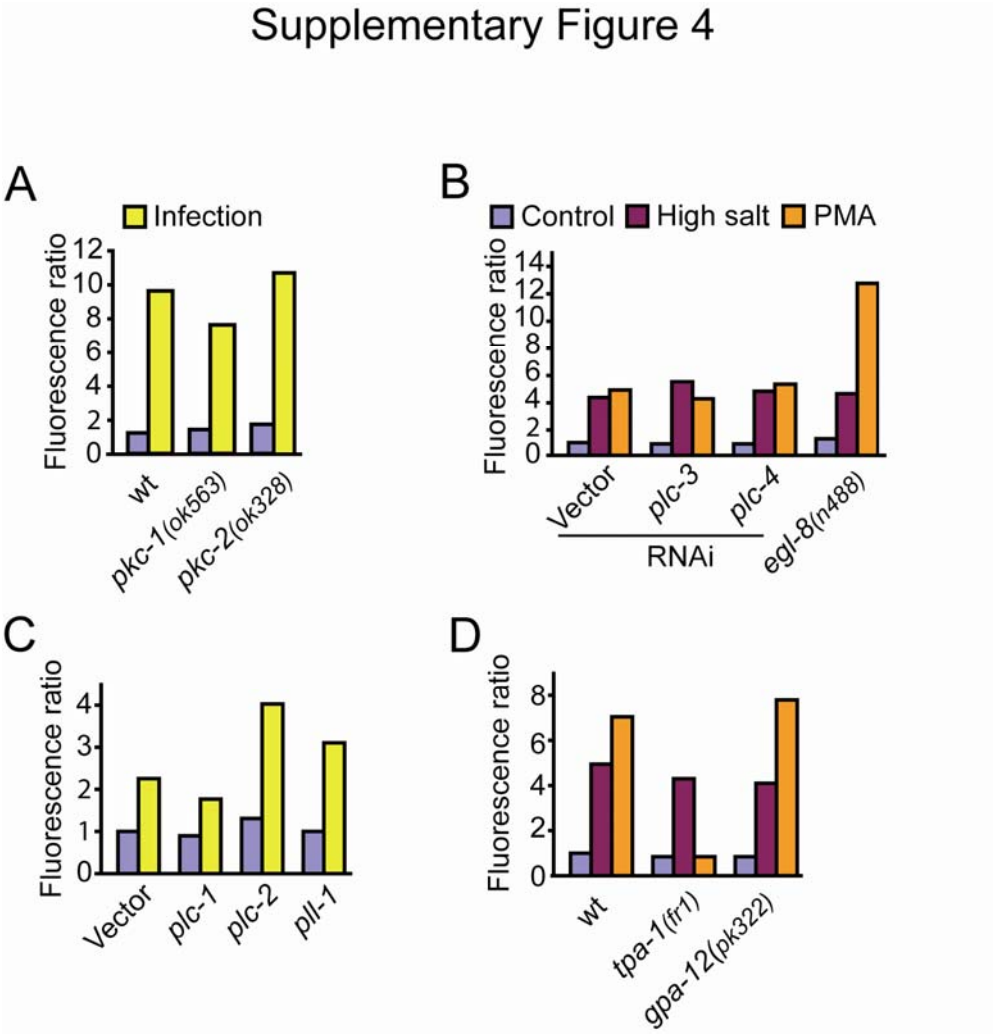

Figure S4. *gpa-12*, *plc-3,* and *egl-8* Are Not Required for *nlp-29* Induction upon Osmotic Stress or Exposure to PMA

(A–D) Normalized fluorescence ratio of worms carrying the *frIs7* reporter transgene. The p*nlp-29::*GFP transgene expression was quantified in different RNAi-treated animals or mutants following osmotic stress and exposure to PMA (B and D) or infection (A and C). Vector corresponds to RNAi with bacteria containing a plasmid without any insert. The number of worms used in each sample from left to right in (A): 155, 239, 187, 220, 218 and 205; in (B): 135, 175, 158, 89, 151, 287, 61, 195, 278, 23, 43 and 28; in (C): 661, 577, 750, 810, 753, 685, 1384 and 903; and in (D): 92, 111, 150, 160, 184, 211, 203, 149 and 142.

Supplementary Figure 5

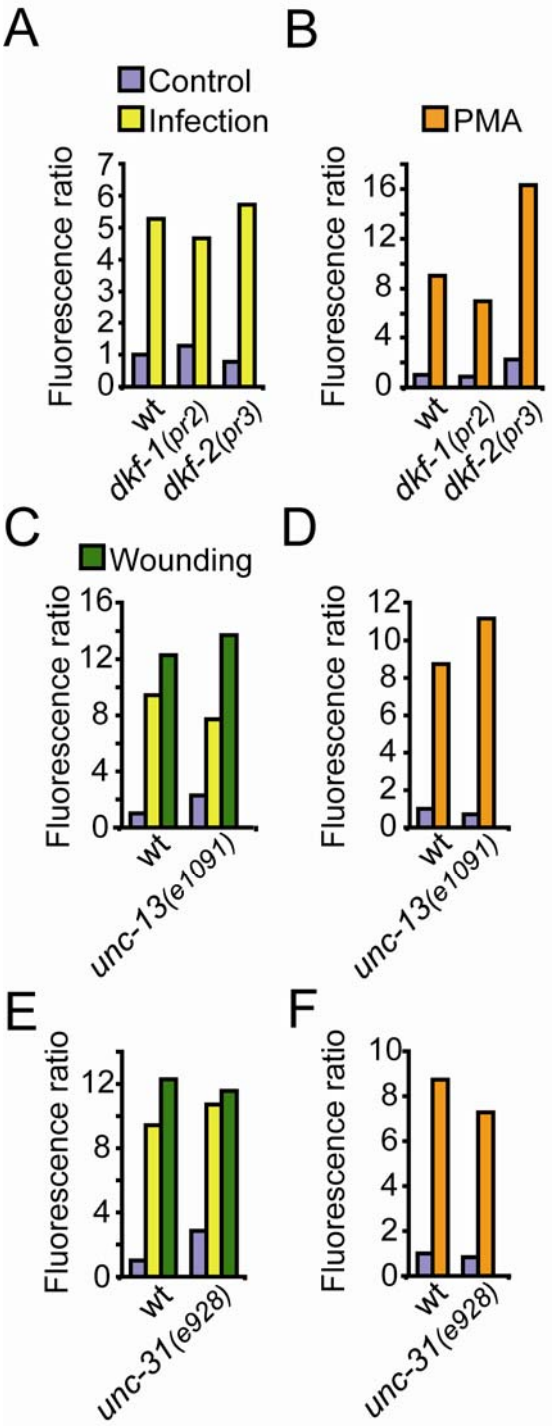

Figure S5. PKDs and Neurosecretion Do Not Regulate p*nlp-29::gfp* Expression

Normalized fluorescence ratio of *frIs7*worms in wild-type (WT), *dkf-1(pr2)* and *dkf-2(pr3)* (A and B), *unc-13(e1091)* (C and D), and *unc-31(e928)* (E and F) mutant backgrounds following infection (A, C, and E), wounding (C and E) and PMA treatment (B, D, and F). The number of worms used in each sample from left to right in (A): 285, 347, 439, 429, 115 and 221; in (B): 872, 454, 741, 541, 153 and 164; in (C): 103, 166, 69, 434, 361 and 23; in (D): 205, 218, 121 and 70; in (E): 103, 166, 69, 615, 456 and 44; and in (F): 51, 106, 118 and 78.

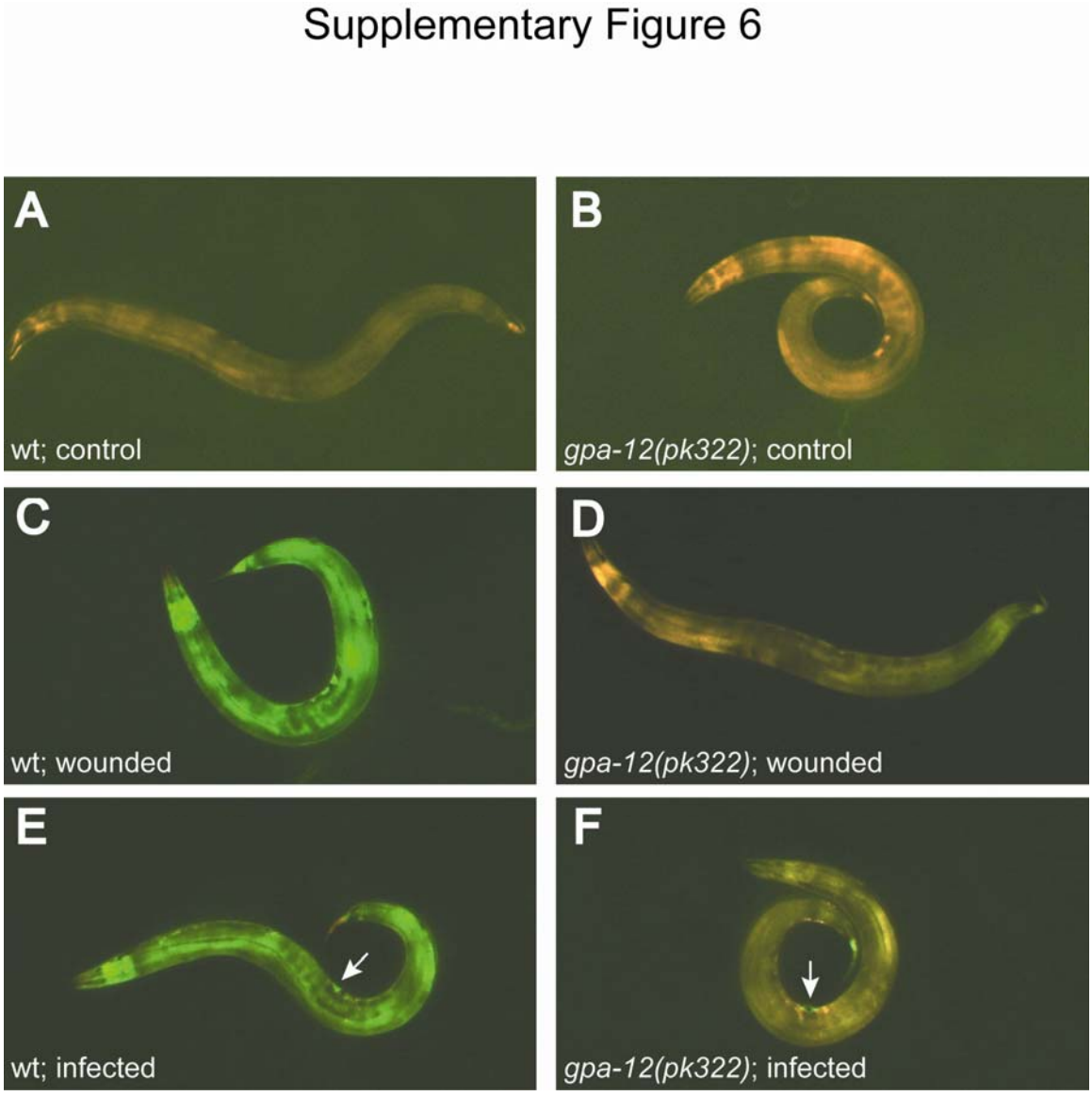

Figure S6. *gpa-12* Regulates *nlp-29* Expression in the hyp7 Epidermal Syncitium

Fluorescence pictures of representative individual WT;*frIs7* adults (A, C, and E) and *gpa-12(pk322)*;*frIs7* mutants (B, D, and F) in control conditions (A and B), after injury (C and D), or upon fungal infection (E and F), visualized with a filter allowing red and green fluorescence to be seen simultaneously. The white arrow points to perivulval cells expressing the p*nlp-29::gfp* transgene specifically upon fungal infection. Animals under control conditions do not express enough p*nlp-29::*GFP to be seen clearly.

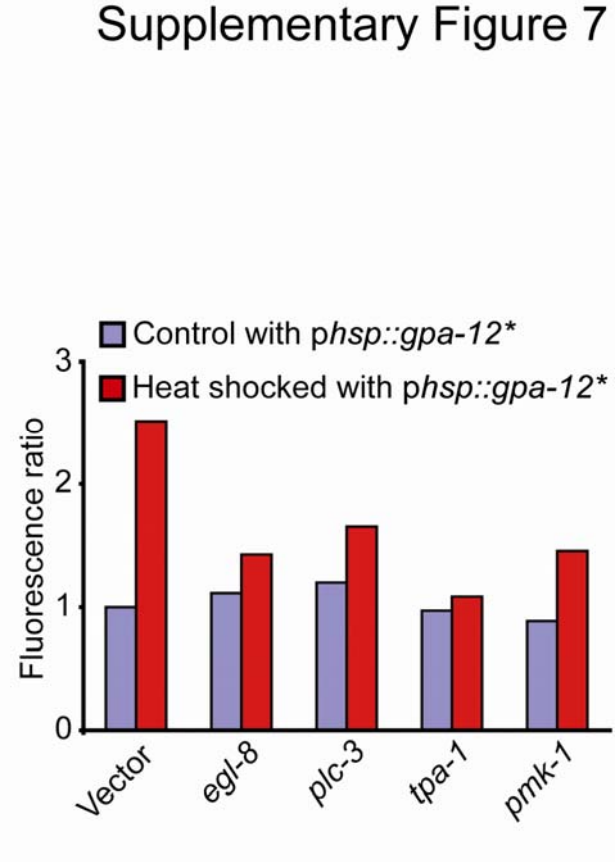

Figure S7. *gpa-12* Act Upstream of the PLCs and the p38 Pathway

Normalized fluorescence ratios of RNAi-treated *frIs7* worms carrying an additional transgene expressing a constitutively active form of GPA-12 under the control of a heat shock promoter (p*hsp::gpa-12**) were compared to worms without the transgene (-) before and after heat shock. Vector corresponds to RNAi with bacteria containing the plasmid without any insert. The number of worms used in each sample from left to right is 92, 55, 68, 46, 81, 49, 86, 53, 85 and 60.

Supplementary Figure 8

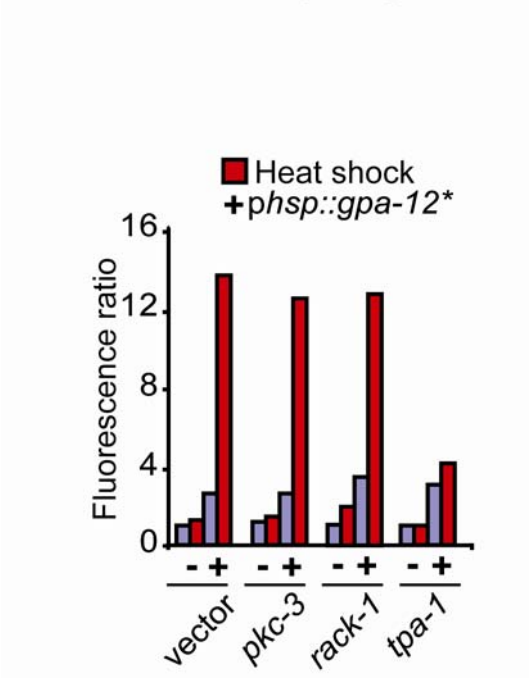

Figure S8. *pkc-3* or *rack-1* RNAi Do Not Impair p*nlp-29::gfp* Induction Associated with *gpa-12* Gain of Function

Normalized fluorescence ratios of RNAi-treated *frIs7* worms carrying an additional transgene expressing a constitutively active form of GPA-12 under the control of a heat-shock promoter (p*hsp::gpa-12**) before and after heat-shock. Vector corresponds to RNAi with bacteria containing the plasmid without any insert. The number of worms used in each sample from left to right is 280, 443, 484, 205, 480, 328, 303, 157, 527, 474, 542, 137, 273, 306, 541 and 164.

Supplementary Figure 9

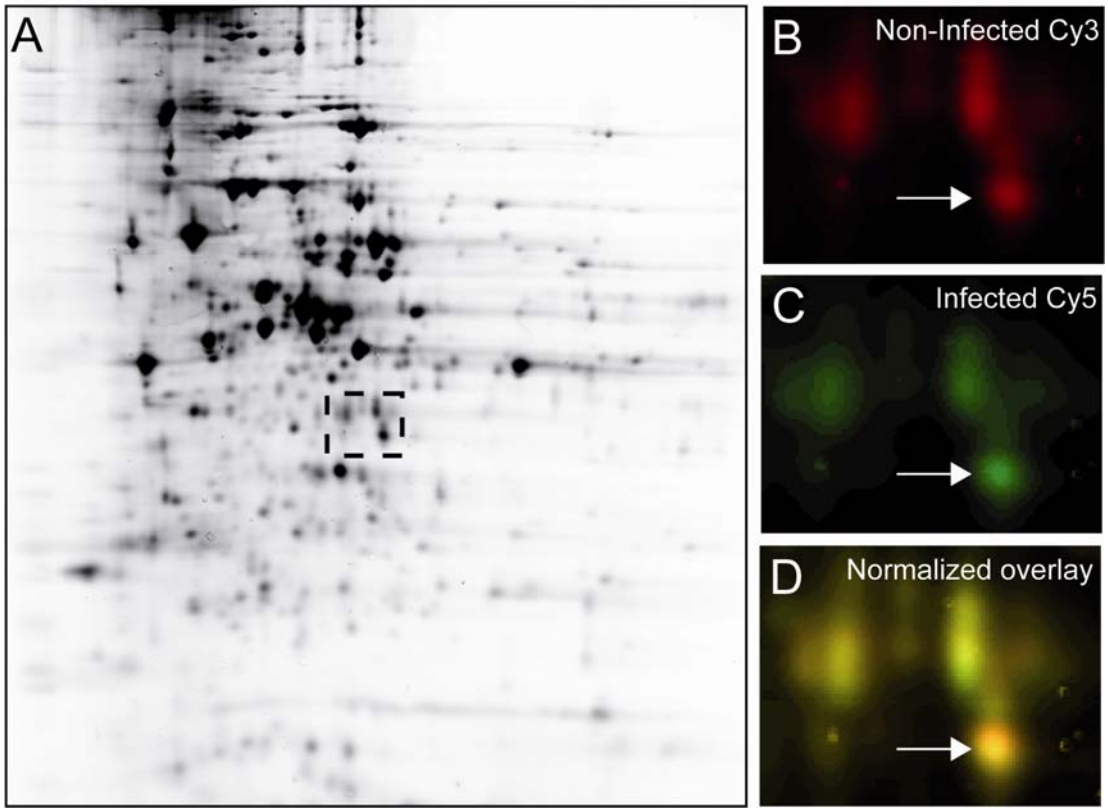

Figure S9. Proteomic Analysis Reveals that RACK-1 Is Changed between Infected and Noninfected Nematodes

(A) Example of a raw 2D gel corresponding to fraction 4 of infected nematodes. The box corresponds to the area shown in (B), (C), and (D).

(B) Nonnormalized image of the 2D gel dyed with Cy3 and corresponding to the fraction 4 of noninfected animals.

(C) Nonnormalized image of the 2D gel dyed with Cy5 and corresponding to the fraction 4 of infected animals.

(D) Normalized overlay of the images shown in (B) and (C). The orange color indicates a prominent change between infected and noninfected worms. The white arrow points to the spot corresponding to RACK-1.

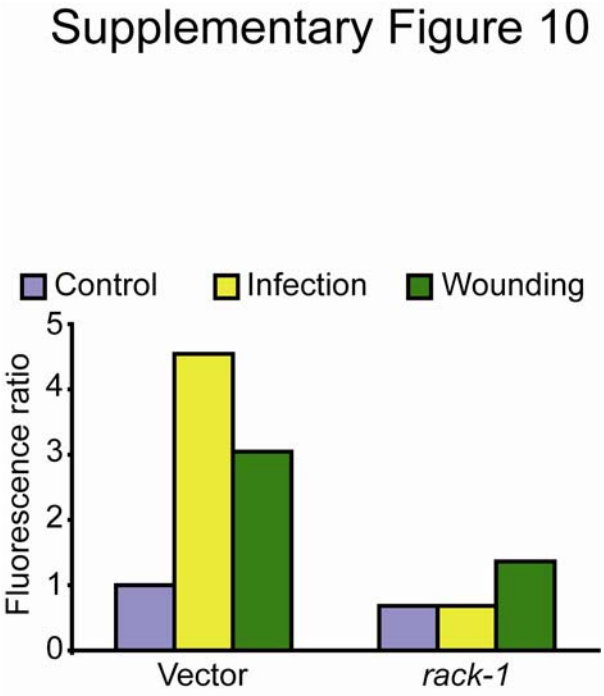

Figure S10. *rack-1* Controls *nlp-29* Induction upon Infection and Injury

Normalized fluorescence ratio of *frIs7* worms. The *nlp-29* transgene expression was quantified in *rack-1* RNAi-treated animals following fungal infection or infection. Vector corresponds to RNAi with bacteria containing the plasmid without any insert. The number of worms used in each sample from left to right is 256, 213, 9, 286, 312, and 16.

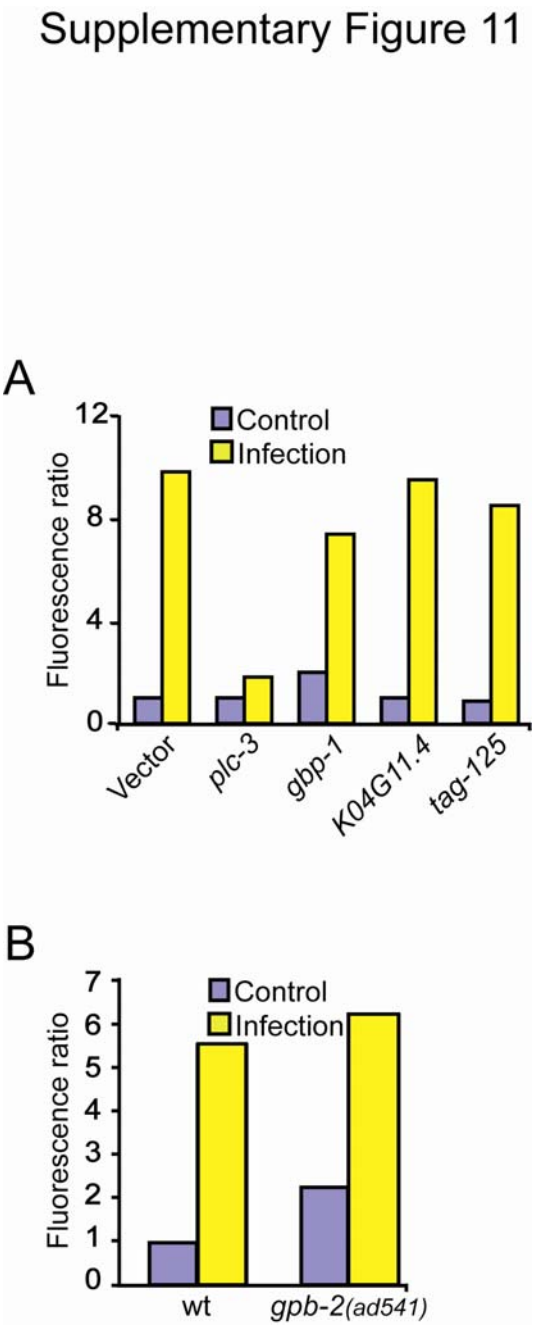

Figure S11. *gbp-1* RNAi and *gbp-2* Mutation Do Not Alter *nlp-29* Induction upon Infection

(A and B) Normalized fluorescence ratio of *frIs7* worms. The *nlp-29* transgene expression was quantified in *gbp-1* RNAi-treated animals (A) and *gbp-2(ad541)* mutants (B) following fungal infection. Vector corresponds to RNAi with bacteria containing the plasmid without any insert. The number of worms used in each sample from left to right in (A) is: 174, 300, 192, 198, 201, 207, 339, 154, 254 and 254 and in (B) is: 78, 104, 47 and 51.

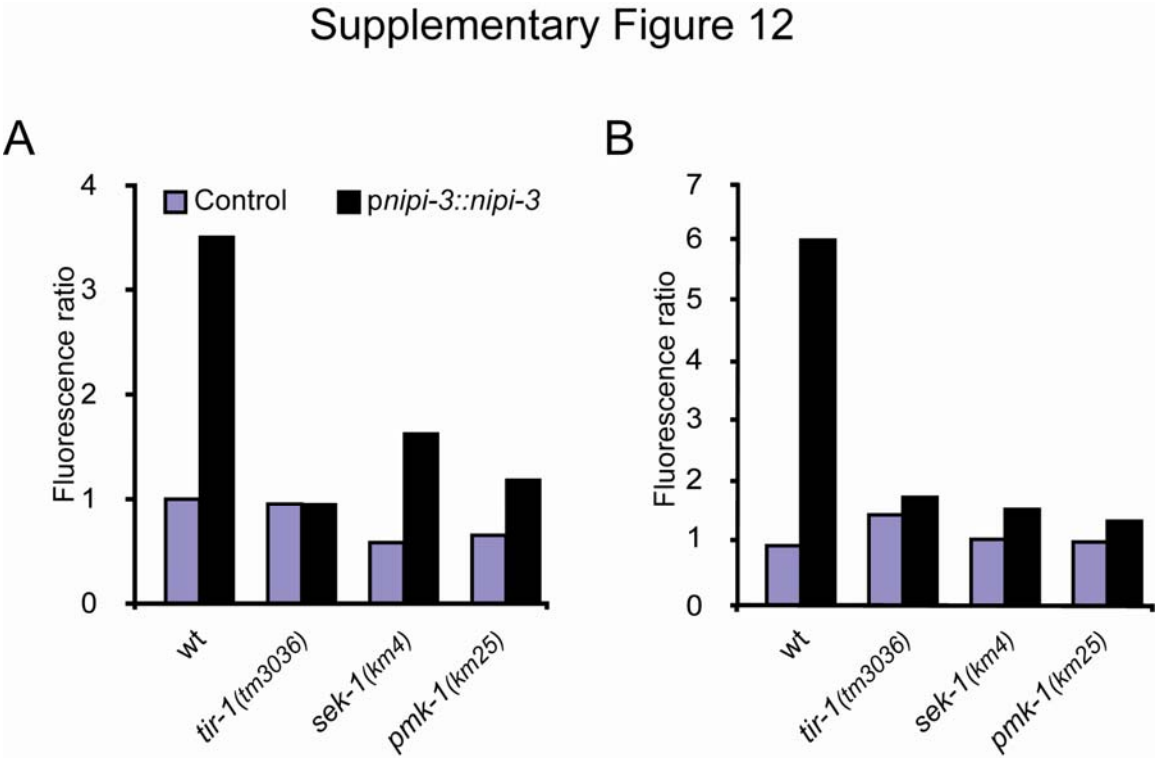

Figure S12. *nipi-3* Is Genetically Upstream of *tir-1* and *pmk-1* for *nlp-29* Regulation

(A and B) Normalized fluorescence ratio of *frIs7* worms. *nlp-29* expression was quantified in WT, *tir-1(tm3036), sek-1(km4)* and *pmk-1(km25)* mutants with (black bars) or without (blue bars) a transgene overexpressing *nipi-3* under the control of its own promoter. The p*nipi-3::nipi-3* transgene provokes a robust *nlp-29* upregulation under non-infectious conditions. Results from two independent experiments are shown. The number of worms used in each sample from left to right in (A) is: 205, 137, 151, 119, 226, 55, 69 and 138; in (B): 159, 101, 123, 88, 228, 94, 154 and 94.

Table S1. Complementation Tests with F1 from Different Crosses

F1 from (males x hermaphrodites)	PMA	Fungal infection
WT x *tpa-1(k530)*	+	NT
tpa-1(fr1)* x *tpa-1(fr1)	-	-
WT x *tpa-1(fr1)*	+	+
tpa-1(fr1)* x *tpa-1(k501)	-	+

Animals were tested for p*nlp-29::gfp* induction upon PMA exposure or fungal infection. + means induction, - means no induction, and NT means Not Tested.