Supplemental Information

Host Translational Inhibition by *Pseudomonas aeruginosa* Exotoxin A Triggers an Immune Response in *Caenorhabditis elegans*

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Inventory of Supplemental Information

- Figure S1 relates to Figure 1.
- Figure S2 relates to Figure 2 as it confirms that the ToxA immune response mimics a *P. aeruginosa* immune response.
- Figure S3 relates to Figure 3 and tests the hypothesis that ToxA is responsible for the increased susceptibility of immunocompromised worms to *P. aeruginosa*. Whereas Figure 3 shows that ToxA is sufficient to kill nematodes, these results demonstrate that ToxA is not necessary for nematode lethality during *P. aeruginosa* infections.
- Figure S4 relates to Figure 5 and contains hygromycin-related data that supports the conclusions made in Figure 5 which, while important for understanding the specificity of hygromycin, do not fit into the main text.
- Tables S1 and S2 contain the relevant microarray data and are too large to include in the main text.
Figure S1

- **E. coli** with vector control
- **E. coli** with ToxA

**P. aeruginosa**

- F35E12.5::GFP

**S. aureus**

- clec-60::GFP

**Fold change (vector)**

- PA14
- ToxA
Figure S2
Figure S3
Figure S4

A

B

C

D

E

Transcription regulation (222)
Innate immunity (43)
Protein kinase cascade (124)
Ras GTPase-related (55)
F-box domain (49)
Other (233)

Transcription regulation (33)
Innate immunity (19)
Other (13)
**Figure S1: ToxA does not broadly activate C. elegans immune genes.** (A and B) C. elegans strains containing the *P. aeruginosa* reporter F35E12.5::GFP (A) or the *S. aureus* reporter clec-60::GFP (B) were exposed for 24 hours to *P. aeruginosa* PA14, *S. aureus* NCTC8325, or *E. coli* expressing ToxA or an empty expression vector. Images for each strain were taken at the same time using the same camera settings. Red pharyngeal expression is due to the co-injection marker *myo-2::mCherry* which confirms the transgene is present. Scale bar represents 100 μm. Insets are the corresponding bright field image. (B) qRT-PCR analysis of genes that respond to *P. aeruginosa* but not ToxA. Results shown are an average of 3 biological replicates. Error bars represent SEM.

**Figure S2: The ToxA response does not mimic other intestinal stressors.** Venn diagrams summarizing the overlap between genes upregulated by ToxA, *P. aeruginosa* PA14, and the heavy metal cadmium (left) or the pore-forming toxin Cry5B (right) (Huffman et al., 2004). All microarrays were conducted with the Affymetrix platform using animals infected at the L4/young adult stage and collected after 24 hours (ToxA), 4 hours (PA14), or 3 hours (cadmium, Cry5B).

**Figure S3: ToxA is dispensable for P. aeruginosa PA14-mediated C. elegans lethality.** Lifespan comparison between N2 and *pmk-1(km25)* animals exposed to either wild-type *P. aeruginosa* PA14 or a toxA mutants starting at the L4 stage.

**Figure S4: Hygromycin upregulates ToxA-induced genes and a subset of P. aeruginosa-induced genes.** (A) Lifespan comparison of N2 animals exposed hygromycin or G418 starting at the L4 stage. (B) Venn diagram summarizing the overlap between genes upregulated by hygromycin and ToxA. (C) Functional classes enriched in each data set as determined by DAVID analysis. Number of genes in each category is indicated in parenthesis. Classes with few genes [≤40 (hygromycin) or ≤5 (ToxA)] are categorized as “other”. (D-E) Venn diagrams
summarizing the overlap between genes upregulated by hygromycin and the pathogens *P. aeruginosa* PA14 (Troemel et al., 2006), *C. albicans* (Pukkila-Worley et al., 2011), and *S. aureus* (Irazoqui et al., 2010a) (D); or hygromycin, PA14, and cadmium or Cry5B (Huffman et al., 2004) (E). All microarrays were conducted with the Affymetrix platform using animals infected at the L4/young adult stage and collected after 24 hours (ToxA), 8 hours (*S. aureus*), 4 hours (PA14, *C. albicans*), or 3 hours (cadmium, Cry5B).

**Table S1: C. elegans genes differentially expressed following ToxA exposure.** This table lists all the Affymetrix probe sets differentially expressed in N2 animals fed *E. coli* expressing ToxA or an empty vector for 24 hours (≥2-fold change and a modified Wilcoxon rank test >1.45).

**Table S2: C. elegans genes differentially expressed following hygromycin treatment.** This table lists all the Affymetrix probe sets differentially expressed in N2 animals fed *E. coli* with an empty vector and exposed to hygromycin or no inhibitor for 24 hours (≥2-fold change and a modified Wilcoxon rank test >1.45).