Reddy et al, Supplemental Information

Supplemental Figures and Figure Legends

Figure S1

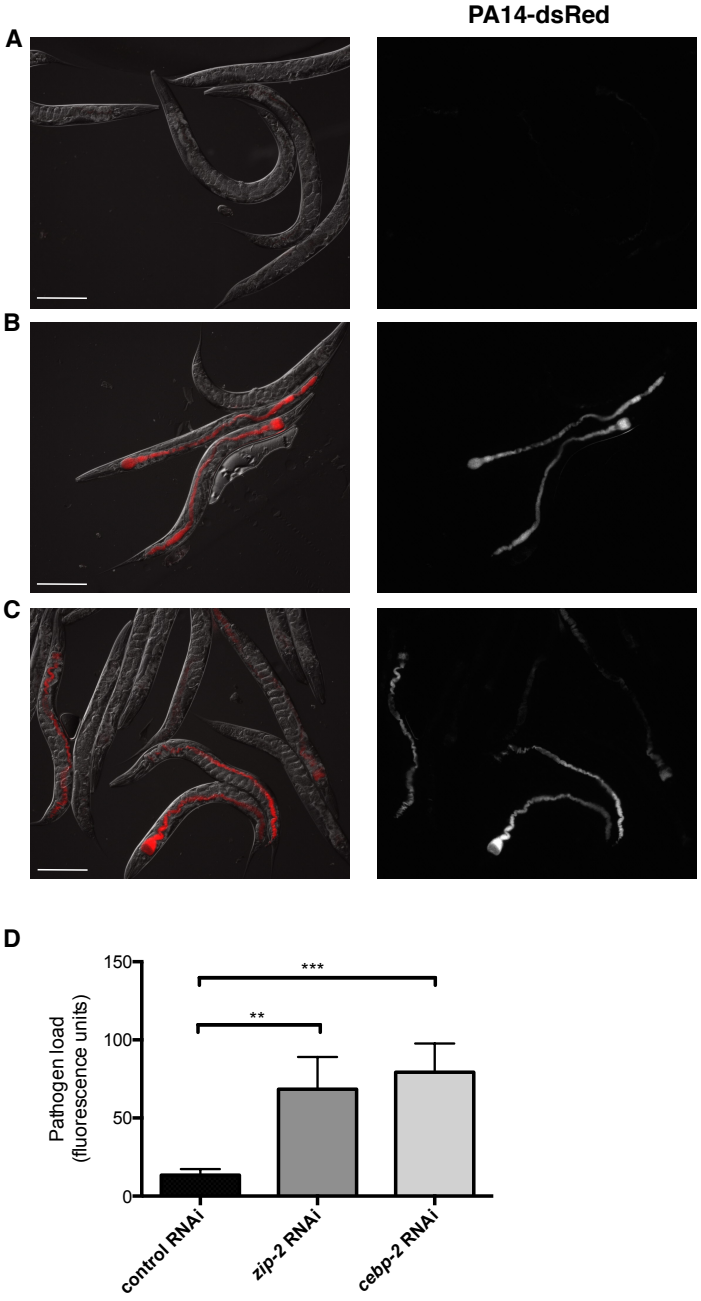

Figure S1. *cebp-2* and *zip-2* control *P. aeruginosa* pathogen burden. Related to Figure 2.

(A-C) Images of (A) control RNAi, (B) *zip-2* RNAi, and (C) *cebp-2* RNAi treated animals after 16 hours of infection with dsRed-expressing PA14. In each panel, the left image shows an overlay of Nomarski with red fluorescence and the right image shows red fluorescence alone. Scale bar, 200 μm. (D) Quantification of dsRed fluorescence levels in the intestine of control RNAi (L4440), *zip-2* RNAi, and *cebp-2* RNAi treated animals after 16 hours of exposure to dsRed-expressing PA14. Results shown are a representative assay of two independent replicates, with at least 30 animals measured for each sample. *** $p < 0.001$, ** $p < 0.01$ with a two-tailed t test.

Figure S2

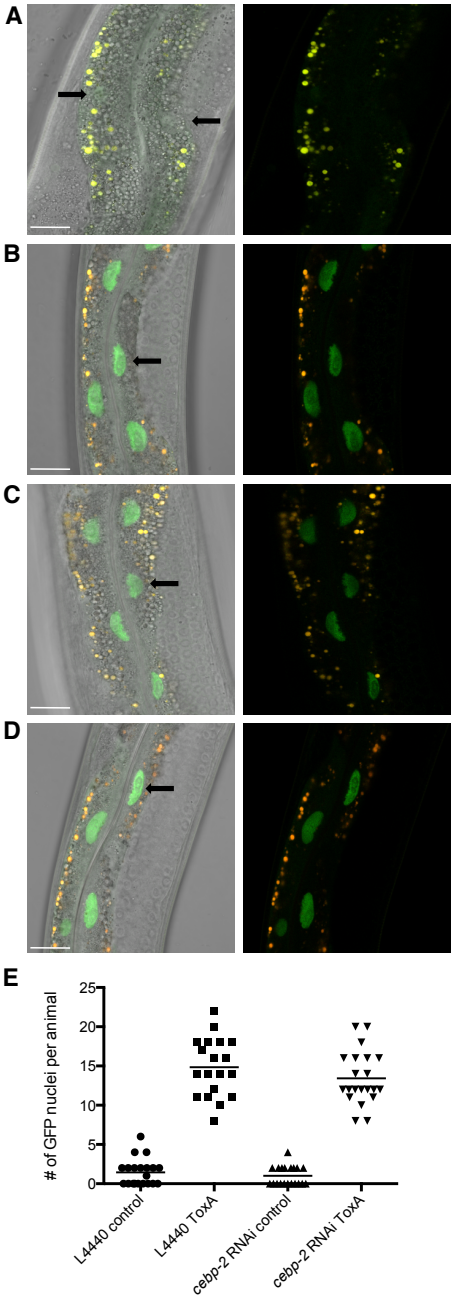
A
B
C
D
E
# of GFP nuclei per animal
25
20
15
10
5
0
L4440 control
L4440 ToxA
cebp-2 RNAi control
cebp-2 RNAi ToxA

Figure S2. *ZIP-2::GFP* transgene is induced by exposure to the translation inhibitor ToxA, independently of *cebp-2*. Related to Figure 3.

(A) *ZIP-2::GFP* transgenic animals do not have GFP expression after feeding on *E. coli* carrying the empty expression vector. (B) *ZIP-2::GFP* transgenic animals have nuclear GFP expression in the intestine after feeding on *E. coli* expressing ToxA. (C-D) *ZIP-2::GFP* transgenic animals treated with either (C) RNAi control L4440 or (D) *cebp-2(RNAi)* have GFP expression in intestinal nuclei after feeding on *E. coli* expressing ToxA. (A-D) In each panel, the right image shows GFP fluorescence in green and autofluorescence in yellow. The left panel shows an overlay of Nomarski with the right image with black arrows indicating intestinal nuclei. Scale bar, 20 μm. (E) Number of intestinal nuclei with GFP expression per animal after 24 hours of feeding on *E. coli* expressing either the empty expression vector or ToxA. Each dot represents an animal, and the horizontal bar indicates the mean. ***, $p < 0.001$ with a two-tailed t test; n.s., difference is not significant ($p = 0.22$ with a two-tailed t test).

Figure S3

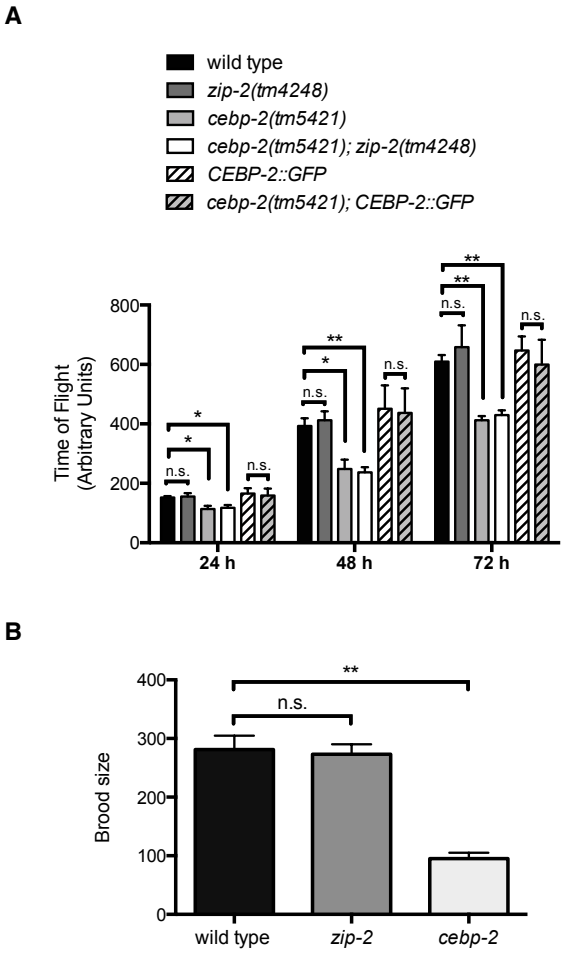
A
wild type
zip-2(tm4248)
cebp-2(tm5421)
cebp-2(tm5421); zip-2(tm4248)
CEBP-2::GFP
cebp-2(tm5421); CEBP-2::GFP
Time of Flight (Arbitrary Units)
800
600
400
200
0
n.s.
*
**
24 h
48 h
72 h
B
Brood size
400
300
200
100
0
wild type
zip-2
cebp-2

Figure S3. *cebp-2* mutants have smaller body size and smaller brood size. Related to Figure 1.

A. Average time of flight (length) of animals measured at 24, 48, and 72 hours after the L1 larval stage. Results shown are the average of three independent replicates. Error bars are SD. (B) Average progeny produced per animal in wild-type animals, *zip-2(tm4248)* mutants, and *cebp-2(tm5421)* mutants. Results shown are the average of two independent replicates. Error bars are SD. For (A) and (B), ** $p < 0.01$, * $p < 0.05$ with a two-tailed t test. n.s., not significant.

Figure S4

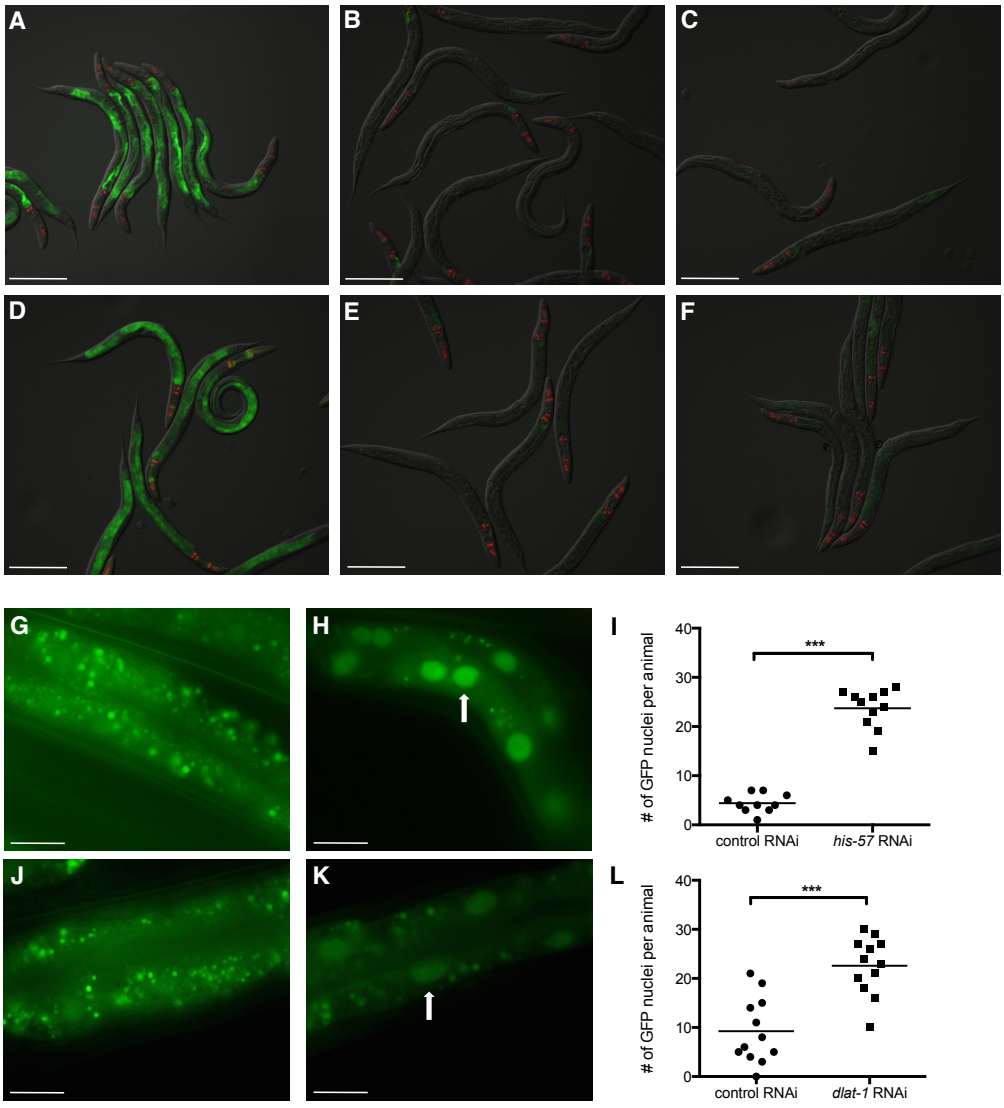
A
B
C
D
E
F
G
H
I
J
K
L
# of GFP nuclei per animal
40
30
20
10
0
***
control RNAi
his-57 RNAi
dlat-1 RNAi

Figure S4. *cebp-2* is required for gene induction upon perturbation of other core processes. Related to Figure 4.

(A-C) *his-57* RNAi induces *irg-1p::GFP* expression in wild-type animals (A), but not in *zip-2(tm4248)* mutants (B), or *cebp-2(tm5421)* mutants (C). (D-F) *dlat-1* RNAi induces *irg-1p::GFP* expression in wild-type animals (D), but not in *zip-2(tm4248)* mutants (E), or *cebp-2(tm5421)* mutants (F). (A-F) Images are overlays of green, red, and Nomarski channels and were taken with the same camera exposure for all. Green is *irg-1p::GFP*, red is *myo-2::mCherry* expression in the pharynx as a marker for presence of the transgene. Scale bar, 200 μm. (G), (J) *ZIP-2::GFP* transgenic animals do not have GFP expression after treatment with control RNAi (L4440). *ZIP-2::GFP* transgenic animals have nuclear GFP expression in the intestine after treatment with (H) *his-57* RNAi or (K) *dlat-1* RNAi. In (G-H), (J-K), GFP fluorescence and intestinal autofluorescence are both in green. White arrows indicate intestinal nuclei expressing GFP. Scale bar, 20 μm. (I), (L) Number of intestinal nuclei with GFP expression per animal after treatment with control RNAi (L4440), *his-57* RNAi, or *dlat-1* RNAi. Each dot represents an animal, and the horizontal bar indicates the mean. ***, $p < 0.001$ with a two-tailed t test.

Supplemental Experimental Procedures

Strains

C. elegans were maintained on NGM plates seeded with OP50, as described. We used N2 wild-type animals. The deletion allele *cebp-2(tm5421)* was a gift of the National BioResource Project, and was back-crossed three times to N2 before experiments to generate ERT214 *cebp-2(tm5421)*. The *cebp-2(tm5421)* deletion was confirmed by sequencing a PCR product and found to remove most of the *cebp-2* coding region, thus likely representing a null mutation. The

ERT214 strain was crossed to AU133 *agIs17[irg-1p::GFP]* to generate strain ERT215 *cebp-2(tm5421);agIs17*. The *CEBP-2::GFP* transgene was generated by using PCR to amplify a fragment from genomic DNA encompassing 1.1 kb of upstream sequence and the entire coding sequence, which was then inserted into the pPD95.77 vector. This construct was injected into N2 worms with the *myo-3::mCherry* co-injection marker to generate the strain CMH2 *mskEx[cebp-2p::CEBP-2::GFP]*. This strain was crossed into the *cebp-2(tm5421)* mutant to generate strain ERT409. Other *C. elegans* strains used in this study were AU133 *agIs17[irg-1p::GFP]*, ERT61 *zip-2(tm4248)*, ERT105 *agIs17; zip-2(tm4248)*, and ERT216 *cebp-2(tm5421);zip-2(tm4248)*. The *ZIP-2::GFP* construct pET233 was generated by using PCR to amplify a fragment from genomic DNA encompassing 1.3 kb of upstream sequence and the entire coding sequence, which was then inserted into the pPD95.75 vector. This construct was injected into N2 animals with the *myo-2p::mCherry* co-injection marker and the resulting extrachromosomal array was integrated using trimethylpsoralen/UV irradiation. The strain carrying the integrated transgene was backcrossed three times to N2 to generate the strain ERT201 *jyIs29[zip-2p::ZIP-2::GFP]*. The *cebp-2* RNAi clone was made with PCR and consists of 254 base pairs of internal *cebp-2* coding sequence. This sequence was amplified from N2 genomic DNA and cloned into the L4440 RNAi vector, and then transformed into HT115 bacteria for feeding RNAi experiments.

Fluorescence imaging of C. elegans

Images of *ZIP-2::GFP* in Figure 3 were captured with a Zeiss LSM700 confocal microscope, as previously described (Dunbar et al., 2012). All other *C. elegans* fluorescent images were captured with a Zeiss AxioImager.

Pathogen load experiments

Animals were infected with a PA14-dsRed strain (Djonovic et al., 2013), using the standard *P. aeruginosa* infection protocol, except adding 50 µg/ml ampicillin to SK plates to select for dsRed plasmid-containing PA14. Animals at the L4 stage were washed onto PA14-dsRed plates, incubated at 25°C for 16 hours, then analyzed with either a COPAS Biosort machine (Union Biometrica) (Pulak, 2006) or by imaging on a Zeiss AxioImager microscope. Animals assessed with the COPAS Biosort were washed off PA14-dsRed plates and assayed for the amount of red fluorescence inside each animal. Animals visualized on the microscope were mounted onto agarose slides and viewed with epifluorescence. Images were captured using the same exposure time for all samples. Average fluorescence was quantified for each sample using the measure tool in Axiovision software.

Size measurement

Synchronized L1 stage animals were washed onto NGM plates seeded with OP50, and incubated at 20°C. The COPAS Biosort machine was used to measure the time of flight (length) of worms at the indicated timepoints. 200 to 300 animals were measured for each strain at every timepoint.

Brood size measurement

Single L4 stage animals were placed onto NGM plates seeded with OP50 and incubated at 20°C. Each worm was transferred to a fresh plate every 24 hours until the completion of egg production, and the number of eggs and hatched worms was counted on each plate. At least 10 worms were used for each strain per experiment.