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Supplemental information

Tribbles pseudokinase NIPI-3 regulates intestinal immunity in *Caenorhabditis elegans* by controlling SKN-1/Nrf activity

Chenggang Wu, Ozgur Karakuzu, and Danielle A. Garsin

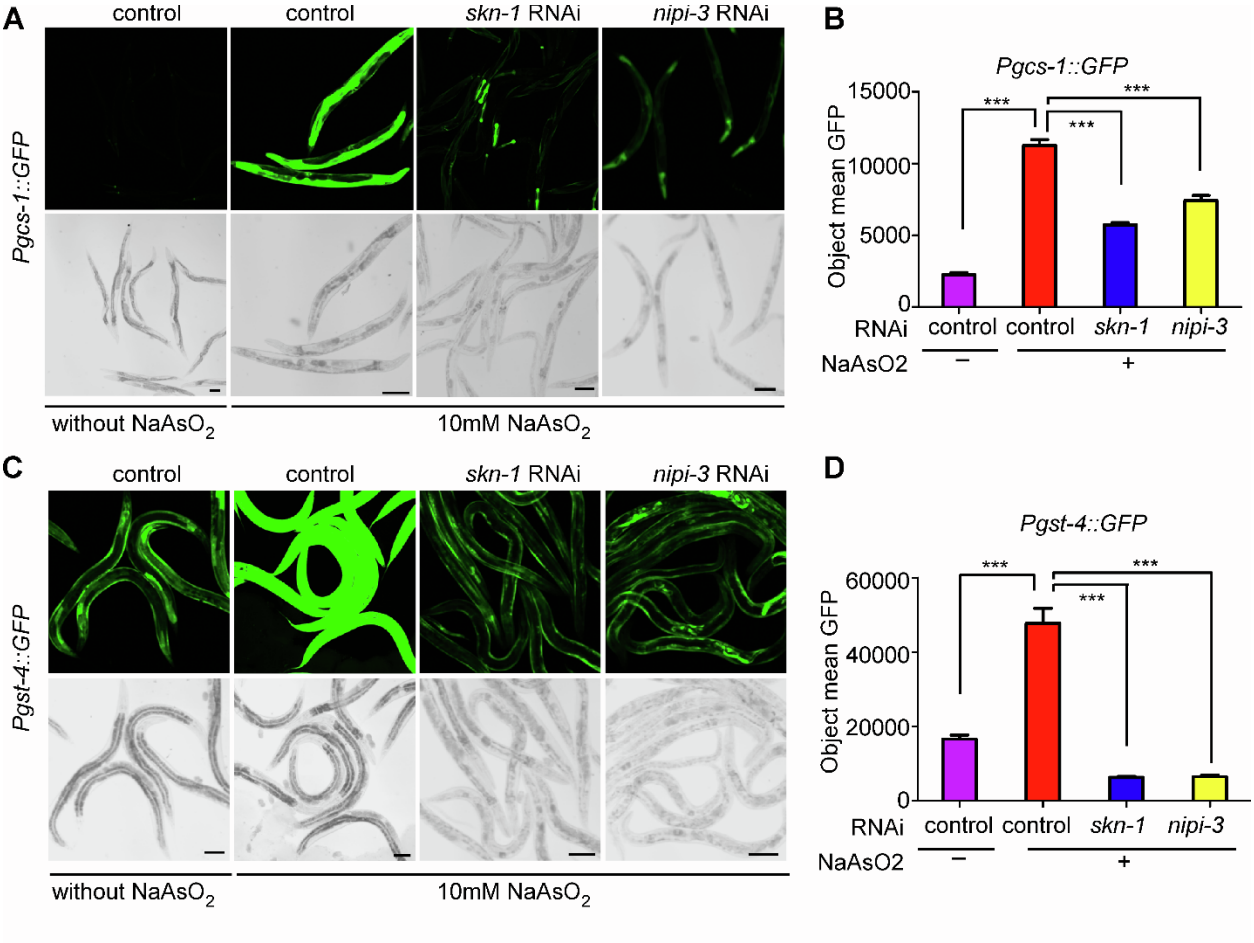


Figure S1: Loss of NIPI-3 reduces the expression of SKN-1 regulated genes in response to oxidant. Related to Figure 1. Fluorescence microscopy was used to analyze *Pgcs-1::gfp* (**A**) and *Pgst-4::gfp* (**C**) expression in wild type animals exposed to vector control, *skn-1* or *nipi-3* RNAi prior to a five hour exposure to 10 mM sodium arsenate in NGM plates **(A)** or M9 buffer **(B)**. *Pgcs-1::gfp* and *Pgst-4::gfp* expression was less induced in the intestines of animals treated with *skn-1* and *nipi-3* RNAi as compared to vector control, and was comparable to the level of the control animals not exposed to oxidant. The scale bars are 100 μm. The amount of GFP fluorescence produced by animals containing the *Pgcs-1::gfp* reporter (**B**) and the *Pgst-4::gfp* reporter (**D**) was quantified. The average gene expression of biological replicates is shown, and the error bars represent the SEM. *p < 0.05, **p < 0.01, ***p < 0.01.

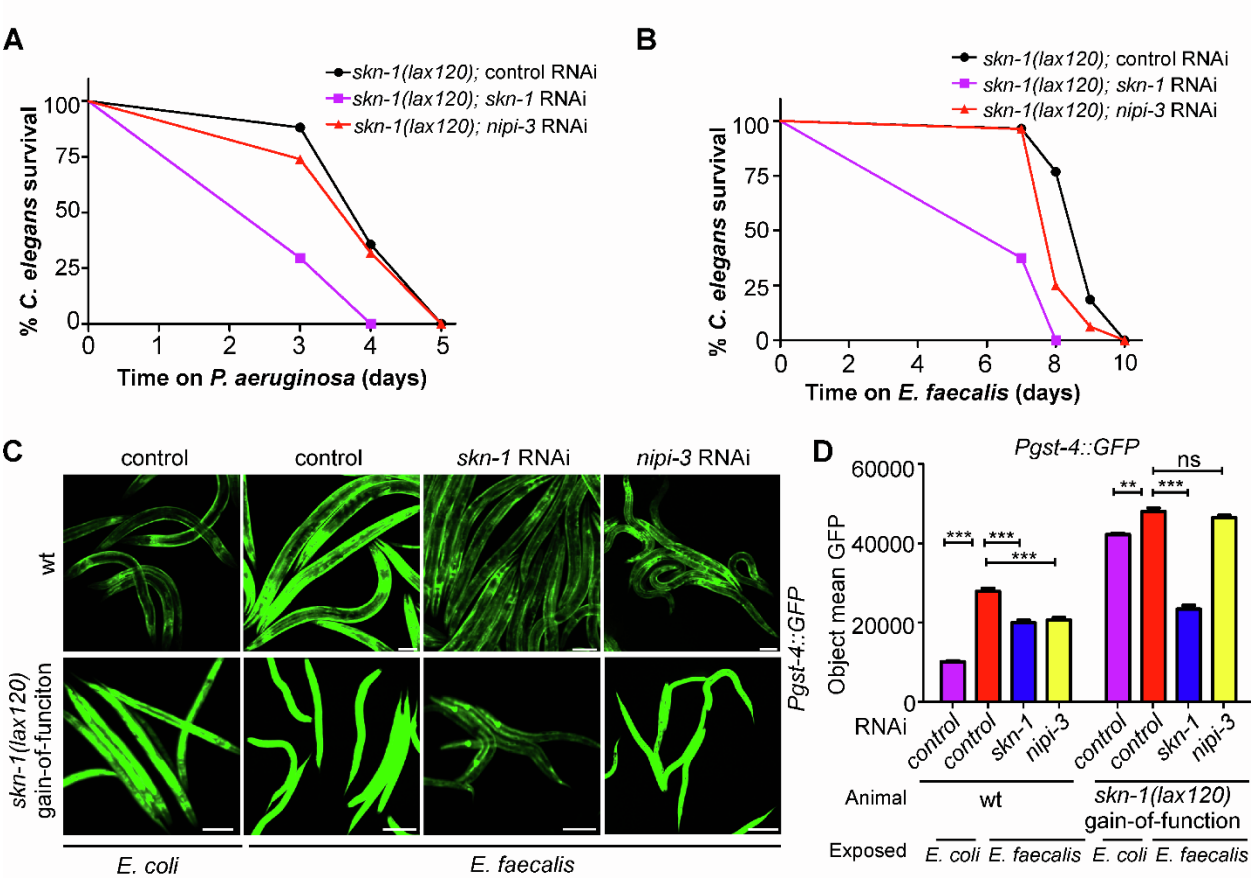


Figure S2: A *skn-1* gain-of-function mutant is unaffected by loss of NIPI-3. Related to Figure 2. *skn-1* gain-of-function animals (*skn-1(lax120))* exposed to vector control, *skn-1*, or *nipi-3* RNAi prior to exposure to *P. aeruginosa* **(A)** or *E. faecalis* **(B)**. $p < 0.0001$ for *skn-1* RNAi compared to *skn-1* or *nipi-3* RNAi. p = ns and $p < 0.05$ for control vs. *nipi-3* RNAi on *P. aeruginosa* and *E. faecalis* respectively. Median survival and p values are listed in Table S1 along with replicates of all experiments. **(C)** Confocal microscopy of vector control, *skn-1*, or *nipi-3* RNAi treated *skn-1(lax120)* animals carrying *Pgst-4::gfp* after exposure to *E. faecalis* or *E. coli* for 16 hours. The images are representative of three independent experiments (n > 150 animals). Scale bars indicate 100 μm. **(D)** Quantification of mean GFP fluorescence for the animals in **(C)**. Three biological replicates with at least 50 worms/replicate were analyzed. Error bars represent the SEM. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, ns not significant.

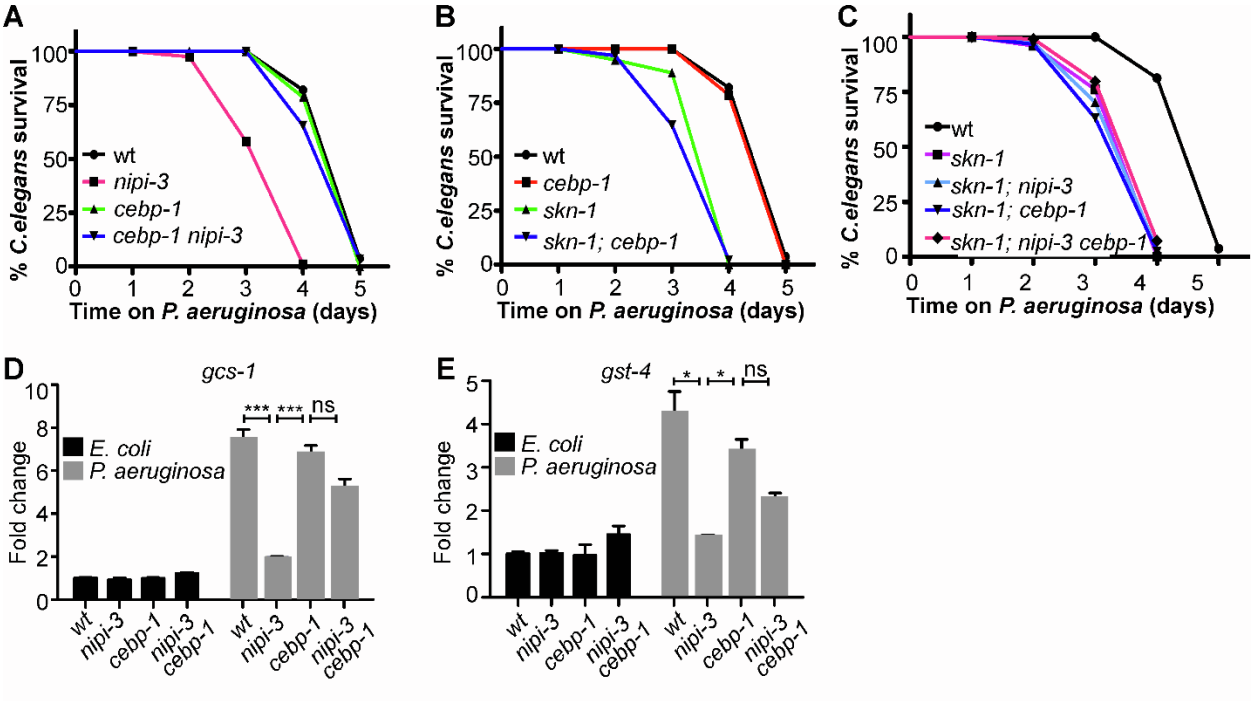
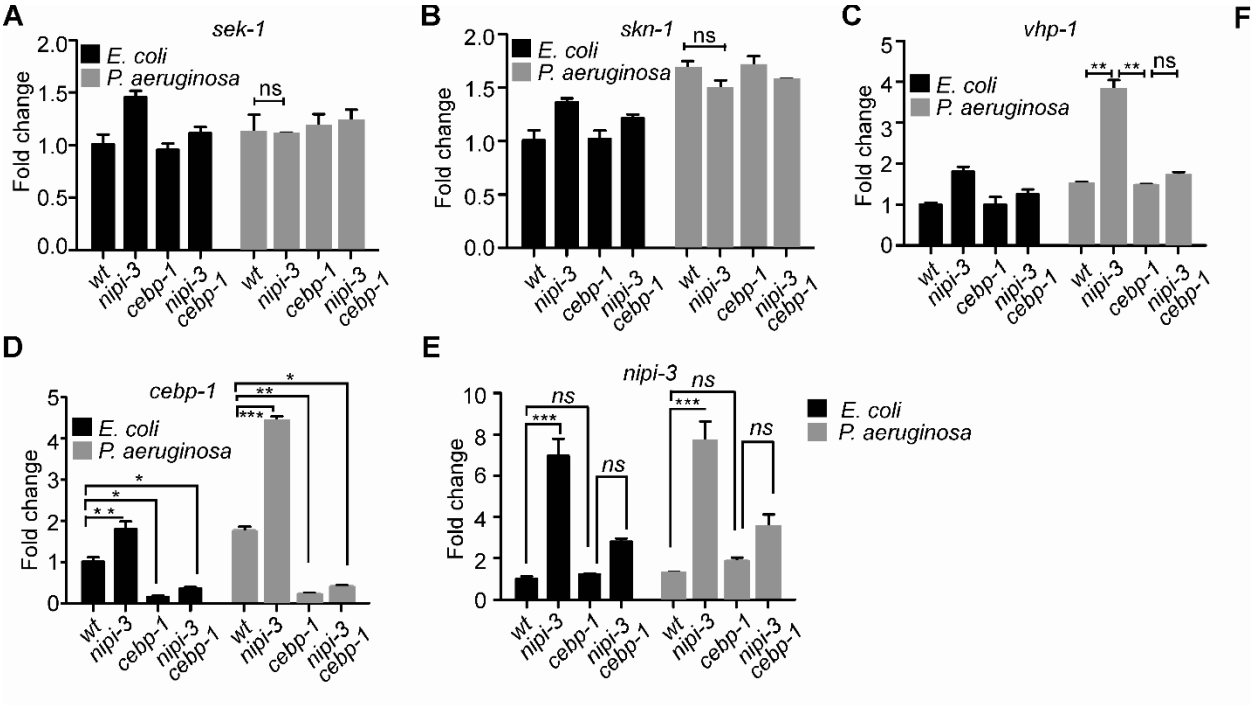


Figure S3: NIPI-3's effect on SKN-1 activation requires CEBP-1. Related to Figure 3. (**A**) Survival curves of wild type, *nipi-3(fr4)*, *cebp-1(tm2807)* and *nipi-3(fr4) cebp-1(tm2807)* on *P. aeruginosa* PA14. $p < 0.0001$ for *nipi-3* compared to all other strains. p = ns for wild type, *cebp-1* and *cebp-1 nipi-3* compared to each other. **(B)** Survival curves of wild type, *cebp-1(tm2807)*, *skn-1(zu135)* and *skn-1(zu135); cebp-1(tm2807)* on *P. aeruginosa* PA14. $p < 0.0001$ for wild type compared to *skn-1* and *skn-1; cebp-1* and for *cebp-1* compared to *skn-1* and *skn-1; cebp-1.* p = ns for wild type compared to *cebp-1* and for *skn-1* compared to *skn-1; cebp-1.* **(C)** Survival curves of wild type, *skn-1, skn-1; nipi-3*, *skn-1; cebp-1, skn-1; nipi-3 cebp-1* on *P. aeruginosa* PA14. $p < 0.0001$ for wild type compared to all strains. p = ns for comparisons between all strains except wild type. For the survival experiments, animals were exposed to *P. aeruginosa* after treatment with *cdc-25.1* RNAi. Killing assays were performed in triplicate and repeated independently three times. Sample sizes, median survival, and p values of all trials are shown in Tables S1. (**D & E**) The defect in *gcs-1* (**D**) and *gst-4* (**E**) expression levels in the *nipi-3* background are suppressed by loss of *cebp-1* as revealed by qRT-PCR. Wild type (N2), *nipi-3(fr4), cebp-1(tm2807)* and *nipi-3(fr4) cebp-1(tm2807)* animals were collected following exposure to *P. aeruginosa* or *E. coli* for 8 hours. Gene expression values are relative to the *act-1* housekeeping gene, with the N2 animals exposed to *E. coli* set to 1. The data shown are the mean ± SEM of the three independent experiments, each of which was performed in triplicate. *$p < 0.05$, ***$p < 0.001$, ns not significant.



Figures S4: CEBP-1 affects *vhp-1*, *cebp-1* and *nipi-3* transcript levels. **Related to Figure 4.** qRT-PCR analysis of *sek-1* **(A)**, *skn-1* **(B)**, *vhp-1* **(C)**, *cebp-1* **(D)**, *nipi-3* **(E)** expression levels in animals of the indicated genotypes (wild type (N2), *nipi-3(fr4), cebp-1(tm2807), nipi-3(fr4) cebp-1(tm2807)*) exposed to *P. aeruginosa* or *E. coli* for 8 hours. The data shown are the mean ± SEM of three independent experiments, each of which was performed triplicate. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns not significant.

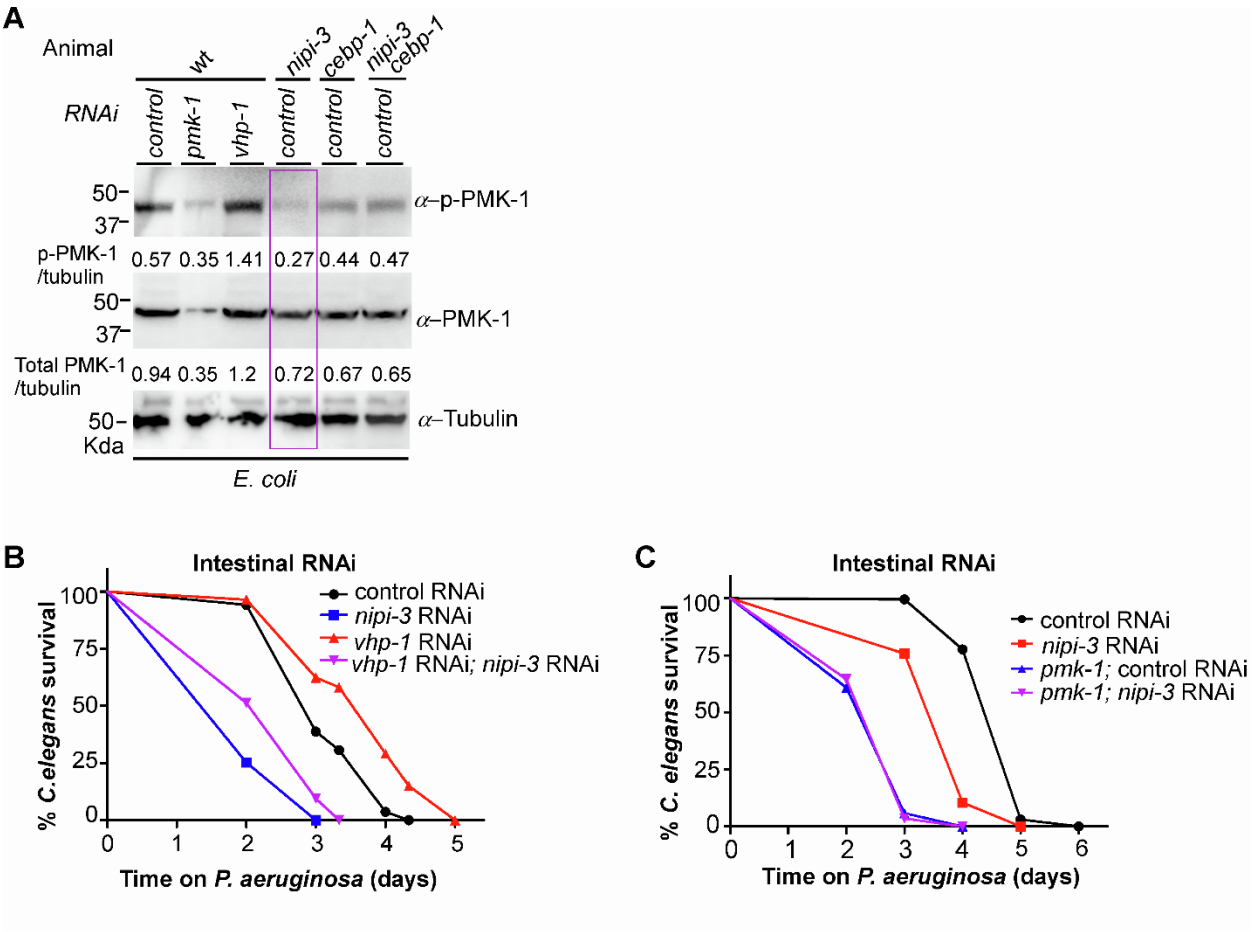


Figure S5: **NIPI-3 regulates the PMK-1 p38 MAPK pathway via the phosphatase VHP-1. Related to Figure 5. (A)** Loss of *nipi-3* results in lower levels of PMK-1 phosphorylation. Immunoblot analysis using an α-phospho-p38 antibody, an α-p38 antibody and an α-tubulin antibody (loading control) from lysates of the indicated animals exposed to *E. coli* for 16 hours. Band intensities relative to α–tubulin are indicated below each blot with the control set to 1. The lane containing the *nipi-3* lysate is boxed. **(B)** Epistasis analysis of intestine-only RNAi animals, either wild type or also containing the *pmk-1(ku25)* allele, exposed to *nipi-3* and *vhp-1* RNAi. $p < 0.0001$ for all strain comparisons. **(C)** Epistasis analysis of intestine-only RNAi animals exposed to *nipi-3* RNAi in both wild type and *pmk-1(km25)* mutant backgrounds. $p < 0.0001$ for all strain comparisons except for *pmk-1;* control RNAi to *pmk-1; nipi-3* RNAi, which is not significant. The VP303 background was used for all strains to enable intestinal specific RNAi, and *C. elegans* survival on *P. aeruginosa* was tracked over time. Data are representative of three trials. Sample sizes, median survival, and p values of all trials are shown in Table S1.

TABLE S2. Oligonucleotides related to STAR methods and Key Resources Table.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
confirm *skn-1(zu135)* genotype FWD primer zu135-seq-F: TTGTGGGGATTGAAGTGGGT	Sigma-Aldrich	N/A
confirm *skn-1(zu135)* genotype REV primer zu135-seq-R: CTCAAATAGGGCAAGTGGGT	Sigma-Aldrich	N/A
confirm *nipi-3(fr4)* genotype FWD primer nipi-3 seq-F: CATCTCATCCATGACCGCTG	Sigma-Aldrich	N/A
confirm *nipi-3(fr4)* genotype REV primer nipi-3-seq-R: TTGAACTGCAAACCTCCG	Sigma-Aldrich	N/A
confirm *pmk-1(km25)* genotype FWD primer pmk-1-F: ACTTTCTCGTTAATCCTATAAGTTG	Sigma-Aldrich	N/A
confirm *pmk-1(km25)* genotype REV primer pmk-1-R: TTTGATAGATCAGCTCCCATCAACATTG	Sigma-Aldrich	N/A
confirm *skn-1(lax120)* genotype FWD primer lax120-F: TGAAAAATAAAAAAATGTTGAATG	Sigma-Aldrich	N/A
confirm *skn-1(lax120)* genotype REV primer lax120-R: ATTGATAGTTGGAATGAGAAGGTATAGG	Sigma-Aldrich	N/A
confirm *cebp-1(tm2807)* genotype FWD primer cebp-1-F: GTTTGTACTTTCTTGCTCTGGTCTGTGG	Sigma-Aldrich	N/A
Confirm *cebp-1(tm2807)* genotype REV primer cebp-1-R: ATATCTATTTTTTATCCATTTTCATAGTGTTGG	Sigma-Aldrich	N/A
confirm intestinal special RNAi animals *rde-I(ne219)*-F: TGTTCATTTACACTATTCACAAGCATTGG	Sigma-Aldrich	N/A
confirm intestinal special RNAi animals *rde-I(ne219)*-R: TTCAGGTCGAGATTGACAGAACG	Sigma-Aldrich	N/A
confirm *vhp-1(sa366)* genotype FWD primer vhp-1-F: ATGAAAATGGGAAGCGATGATGCATACAG	Sigma-Aldrich	N/A
confirm *vhp-1(sa366)* genotype REV primer vhp-1-R: ATTCTTGATGGAAGTCCAAGAGCTTTTGG	Sigma-Aldrich	N/A

qRT-PCR for *act-1* FWD primer RT-actin F: CCATCATGAAGTGCGACATTG	Sigma-Aldrich	N/A
qRT-PCR for *act-1* REV primer RT-actin R: CATGGTTGATGGGGCAAGAG	Sigma-Aldrich	N/A
qRT-PCR for *cebp-1* FWD primer RT-cebp-1 F: GCAAGACAAGACTCTCTTAC	Sigma-Aldrich	N/A
qRT-PCR for *cebp-1* REV primer RT-cebp-1 R: CCAAGGTCCAGCTCAGTTTC	Sigma-Aldrich	N/A
qRT-PCR for *nipi-3* FWD primer RT-nipi-3-F: GATGAAATCCGTTTAAGCGAAC	Sigma-Aldrich	N/A
qRT-PCR for *nipi-3* REV primer RT-nipi-3-R: GGCGTAGGAGTGAATTGTAG	Sigma-Aldrich	N/A
qRT-PCR for *gcs-1* FWD primer RT-gcs-1F: AATGCCTTACGGAGGTCTC	Sigma-Aldrich	N/A
qRT-PCR for *gcs-1* REV primer RT-gcs-1R: AAGAGATGGGAACGATATCG	Sigma-Aldrich	N/A
qRT-PCR for *gst-4* FWD primer RT-gst-4 F: AAAGCTGAAGCCAACGACTC	Sigma-Aldrich	N/A
qRT-PCR for *gst-4* REV primer RT-gst-4 R: TCTGCAGTTTTTCCAGCG	Sigma-Aldrich	N/A
qRT-PCR for *vhp-1* FWD primer RT-vhp-1-F: CCTCTCACAACCATGTCTATCC	Sigma-Aldrich	N/A
qRT-PCR for *vhp-1* REV primer RT-vhp-1-R: CATGGTCTCATCCAAGCTATCA	Sigma-Aldrich	N/A
qRT-PCR for *skn-1* FWD primer RT-skn1-1F: CTGGCATCCTCTACCACCAC	Sigma-Aldrich	N/A
qRT-PCR for *skn-1* REV primer RT-skn1-1R: TTGGTGATGATGGCCGTGTT	Sigma-Aldrich	N/A
qRT-PCR for *sek-1* FWD primer RT-sek-1F: CACTGTTTGGCGACGATGAG	Sigma-Aldrich	N/A
qRT-PCR for *sek-1* REV primer RT-sek-1R: ATTCCGTCCACGTTGCTGAT	Sigma-Aldrich	N/A