

**Transgenerational lipid-reducing activity of
benzyloisoquinoline alkaloids in *Caenorhabditis elegans***

Yit-Lai Chow^{1*} and Fumihiko Sato^{1,2*}

Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University,
Kitashirakawa, Sakyo, Kyoto 606-8502, Japan

*Corresponding authors:

¹Address: Division of Integrated Life Science, Graduate School of Biostudies, Kyoto
University, Kitashirakawa, Sakyo, Kyoto 606-8502, Japan

²Present address: Graduate School of Science, Osaka Prefecture University, 1-1
Gakuen-cho, Naka-ku, Sakai, Osaka 599-8531, Japan

Tel: +81-(0)75-753-9222

Fax: +81-(0)75-753-9229

Email: yitlaichow@yahoo.com

Email: fsato@lif.kyoto-u.ac.jp

Running title: Transgenerational lipid reduction

21 Keywords: transgenerational inheritance, Nile red fluorescence, lipid, berberine,
22 sanguinarine, *Caenorhabditis elegans*, epigenetics
23
24 Number of figures: 7
25 Supplementary figures: 2
26

Abstract

Epigenetic mechanisms allow for transgenerational memory of an ancestor's environment and can affect the gene expression, physiology and phenotype of that ancestor's descendants, independent of DNA sequence alteration. Among many model organisms, *Caenorhabditis elegans* has been instrumental in studies of transgenerational inheritance, most of which have focused on the effects of external stressors of the parent worm on the lifespan and stress resistance of future generations. In this work, we used Nile red staining of accumulated lipids in *C. elegans* to investigate the transgenerational effect of two benzylisoquinoline alkaloids, namely, berberine and sanguinarine. Our results showed that a reduction in Nile red fluorescence can be propagated to subsequent worm generations. Using mutant worms, we found that the transgenerational effect requires the ASH-2 component of the histone H3K4me3 complex and the HRDE-1 worm Argonaute protein. *Ash-2* is also required for transgenerational inheritance of the xenobiotic response in the worm. Our study offers new insights into transmissible drug effects across multiple generations and suggests the importance of such analyses in the drug development process.

1 INTRODUCTION

Sublethal stress pretreatments in diverse combinations of stressors and recipient organisms have been shown to increase germination in plants, animal cell proliferation in culture, protein turnover, subsequent stress resistance, and lifespan extension. An increased lifespan has been reported to result from diverse types of stressors, such as heat, cold, oxidative stress, radiation, electrical stimulation, exercise, and fasting (Ristow et al., 2011; Lagisz et al., 2013; Matsuyama et al., 2014; Chuang et al., 2016; Rechavi et al., 2014). However, the biological mechanisms of these stress treatment effects are not fully understood. Advances in molecular genetics research have revealed that environmental stimuli and exogenous stressors on an organism can induce responses that are passed on to subsequent generations. This phenomenon is called epigenetic inheritance, and epigenetic changes are defined as heritable changes in gene expression that are not due to alterations in the DNA sequence (Holliday, 1987).

Many studies on epigenetic inheritance have been carried out using the model organism *Caenorhabditis elegans* due to its numerous physiological advantages, such as a rapid reproductive cycle, small size and short lifespan, as well as its genetic homology with mammalian models. Some of the earliest work in *C. elegans* showed that sublethal heat treatment of the worms affected their heat resistance and lifespan (Lithgow et al., 1994; 1995). In recent years, studies have revealed that those responses are transmitted to subsequent generations through epigenetic modifications such as changes in chromatin

structure, including DNA methylation, histone modification and variant incorporation, and noncoding RNAs (Egger et al., 2004; Greer et al., 2011, Hourri-Ze'evi et al., 2016; Klosin et al., 2017). As these studies have reported, transgenerational inheritance often involves external stimuli or stressors, which result in the increased resistance and survival of the exposed organisms.

In this work, we investigated whether the effects of two benzyloquinoline alkaloids, namely, berberine and sanguinarine, on lipid reduction in *C. elegans* (Chow & Sato, 2013) are propagated to its descendants. Our results indicated that the effects of alkaloids are transgenerational.

2 RESULTS

2.1 Treatment with berberine and sanguinarine reduced Nile red fluorescence of accumulated lipids in *C. elegans*, and the effect was transmitted to subsequent generations

Previously, we reported that berberine and sanguinarine reduced lipid accumulation in *C. elegans* using Oil red O staining. Such activity was consistently observed with 400 to 500 μ M berberine and 10 to 25 μ M sanguinarine in the worms. A similar reduction was also observed with Nile red staining in an evaluation of lipid accumulation in *C. elegans* (Chow and Sato, 2013). Although several reports have claimed that the measurement of lipid

droplets in *C. elegans* using Nile red staining is invalid (Brooks et al., 2009; O'Rourke et al., 2009; Zhang et al., 2010), our results from assessments using both Oil red O and Nile red staining methods are concordant. Thus, we used Nile red vital dye in this study as it allows for rapid and live in vivo observation of lipid accumulation in worms. Nile red is widely used for the analysis of lipid droplets in *C. elegans* and has led to the identification of numerous evolutionarily conserved fat regulatory genes and small molecules that affect fat metabolism (Van Gilst et al., 2005; Srinivasan et al., 2008; Chen et al., 2009; Cohen et al., 2009; Mullaney et al., 2010; Lemieux et al., 2011; Pathare et al., 2012). However, to clarify our interpretation of lipid accumulation in *C. elegans* in this study, we used the difference in Nile red fluorescence intensity between treated and control worm groups as the indicator of in vivo lipid-level changes.

As Figure 1a shows, treatment of L4 larval stage wild-type N2 worms with 500 μ M berberine and 10 μ M sanguinarine for 48 hours significantly reduced Nile red fluorescence. The treated parent worms (P0) were then transferred to culture plates containing fresh nematode growth medium (NGM) without alkaloids and allowed to lay eggs. Adults of the next generation, F1, were then stained with Nile red to observe their fluorescence, and a portion of the worms were transferred to new NGM plates to lay eggs that hatched into F2 worms. This procedure was repeated until the F3 worms were evaluated. Nile red staining showed that reduced fluorescence in berberine- and sanguinarine-treated P0 worms was also exhibited by their F1, F2, and F3 offspring (Figure 1b, c, d). These results imply that the

memory of an ancestor's environment (e.g., alkaloid treatment of the P0 generation) is transmissible and can affect the gene expression and physiology of future generations.

2.2 The transgenerational effect of lipid reduction by berberine and sanguinarine treatment of parent worms was abolished in the F1 to F3 progeny of *ash-2* (*tm1726*) and *hrde-1* (*tm1200*) mutants

Epigenetic modifications are reported to be involved in the transgenerational inheritance of parental phenotypes (Lim and Brunet, 2013; Heard and Martienssen, 2014). Thus, we hypothesized that xenobiotics, i.e., alkaloid treatment of the *C. elegans* parent generation, would induce epigenetic alterations, which could be maintained and transmitted to future generations that are unexposed to alkaloids. To test this hypothesis, we used mutant strains, namely, *ash-2* and *hrde-1*. ASH-2 is one of the histone H3 lysine 4 trimethylase (H3K4me3) regulatory components of chromatin modification (Greer et al., 2011), and HRDE-1 is one of several *C. elegans* WAGO Argonaute proteins that bind RNAi-associated 22G-RNAs in the germline. HRDE-1 localizes to the nucleus to affect transcriptional gene silencing and transgenerational RNAi (Buckley et al., 2012; Luteijn et al., 2012; Shirayama et al., 2012).

Both *ash-2* and *hrde-1* deletion mutants are homozygous knockouts generated by the National BioResource Project of Japan (The *C. elegans* Deletion Mutant Consortium, 2012).

When we treated the wild-type N2, *ash-2* and *hrde-1* worms with berberine and sanguinarine, all the P0 generations showed reduced Nile red fluorescence (Figures 2a, 3a). This result

indicates that *ash-2* and *hrde-1* did not abrogate the lipid-reduction effect of alkaloid treatment in the parent worms. However, we observed that the deletion mutants of ASH-2 and HRDE-1 failed to reduce the Nile red fluorescence in the F1 to F3 descendants (Figure 2b-d, 3b-d). These results suggest that the inheritance of lipid-reduction effects requires specific histone modification factors, such as the H3K4 trimethylation complex or possibly additional epigenetic mediators, and may involve the nuclear Argonaute protein bound to PIWI-interacting RNAs for transgenerational silencing (Ashe et al., 2012).

A previous study using *C. elegans* reported that exposure to heavy metal stressors in the parent generation induced epigenetic alterations that could be maintained and transmitted to the descendants (Kishimoto et al., 2017). The environmental stressor increased the lifespan of the exposed parent worms and subsequently increased the resistance of the descendants to oxidative stress. However, knockdown of the H3K4me3 complex components (*wdr-5.1*, *ash-2* and *set-2*) in the P0 generations was found to have no effect on the increased stress resistance of the parents, whereas it failed to increase the resistance of the F1 progeny. These results suggest that the parent worms acquire stress-induced effects independently of these histone modifiers.

In our experiments, the worms were exposed to alkaloids, which mimicked a xenobiotic stressor. The lipid-reduction effect of berberine and sanguinarine was transmissible to the F1 to F3 descendants of wild-type N2 worms. However, this effect was abolished in *ash-2* and *hrde-1* offspring. Assuming that alkaloids are exogenous stressors to the worms, we

hypothesized that the lipid reduction activities of berberine and sanguinarine were the result of xenobiotic effects. Thus, we next investigated the xenobiotic response of the worms and the correlation between this response and the lipid metabolism of the worms in the P0 to F3 generations.

2.3 Xenobiotic response of *C. elegans* to berberine and sanguinarine treatment

Plant alkaloids have been suggested to function in the defense against herbivores and pathogens. Therefore, these compounds are likely to induce a xenobiotic response in *C. elegans* to metabolize and consequently eliminate the chemical toxins from their bodies. The isoquinoline alkaloids berberine and sanguinarine were reported to exhibit cytotoxicity and inhibit mitochondrial respiratory complex I (Adhami et al., 2003; Turner et al., 2008). As a result of such inhibition, berberine and sanguinarine were found to activate the cellular energy sensor AMP-activated protein kinase (AMPK) due to an increase in cellular AMP levels and reduced ATP levels. AMPK activation leads to inhibition of acetyl-coenzyme A carboxylase (ACC) in the cells, which in turn inhibits triglyceride synthesis. Therefore, we expected that berberine and sanguinarine would induce a xenobiotic response in worms. *C. elegans* has many genes encoding enzymes for their xenobiotic responses, including four main classes of detoxification enzymes: cytochrome P450 (CYP), short-chain dehydrogenases (SDR), UDP-glucuronosyl or glycosyl transferases (UGT), and glutathione-S-transferases (GST) (Lindblom and Dodd, 2006). Previously, we investigated the genome-wide response of the worms to berberine and sanguinarine treatment by microarray analysis.

The results were subsequently verified by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and indicated that defense response and detoxification genes, including a CUB-like domain-containing protein gene (*F08G5.6*) affecting innate immune response, a cytochrome P450 family gene (*cyp-35C1*) affecting xenobiotic response, a glutathione *S*-transferase gene (*gst-5*), and UDP-glucuronosyltransferase gene (*ugt-21* and *ugt-25*) affecting detoxification, were the most significantly upregulated (Chow et al., 2014).

In this study, we evaluated the xenobiotic response of *C. elegans* to berberine and sanguinarine treatment by quantifying the expression of these genes in P0 parents and their untreated F1, F2, and F3 offspring by qRT-PCR. The results showed that the detoxification response genes *cyp-35C1*, *gst-5*, *ugt-21*, *ugt-25* and *F08G5.6* were significantly upregulated in treated wild-type N2 parent worms (Figure 4a). The induction of these genes in alkaloid-treated worms was still observed in the F1 worms, although the levels were diminished, especially in the offspring of sanguinarine-treated worms. The detoxification response genes were also found to be significantly upregulated in the F2 worms, and some genes remained significantly induced in the F3 offspring of sanguinarine-treated P0 worms (Figure 4b-d).

However, stress-response genes, such as a superoxide dismutase gene (*sod-5*) and a heat shock protein gene (*hsp-16.2*), that are induced in response to osmotic, heat shock or other environmental stresses were repressed in P0 worms treated with an alkaloid. However, the expression of these genes was upregulated in F2 worms, and the *hsp-16.2* gene remained upregulated in the F3 offspring of sanguinarine-treated P0 worms. This result suggests that

xenobiotic stimulation could increase stress resistance in the descendants of affected ancestor worms. This phenomenon supports the hypothesis that transgenerational epigenetic inheritance acts to protect future progeny from the effects of external stress (Kaati et al., 2007; Baugh, 2013; Schott et al., 2014).

2.4 The transgenerational effect of the xenobiotic response was abolished in the F1 to F3 progeny of *ash-2* but not *hrde-1* worms

When we treated the *ash-2* (*tm1726*) and *hrde-1* (*tm1200*) worms with berberine and sanguinarine in the parent generation, the detoxification response genes were upregulated, as was also observed for wild-type N2 worms (Figures 5 & 6). However, this response was reduced or abolished in *ash-2* F1 to F3 progeny, especially in F3 progeny of sanguinarine-treated P0 (Figure 5d).

In contrast, the induction of detoxification response genes in *hrde-1* was reduced but still detected in the F1 to F3 descendants of alkaloid-treated P0 worms. This trend was similar to that of wild-type worms. The stress-response gene *hsp-16.2* was upregulated in the P0 and F3 generations of the *hrde-1* worms (Figure 6) and remained upregulated in the F1 to F3 offspring of the sanguinarine-treated ancestor.

These results suggest that alkaloid treatment possibly acts as a stressor, inducing transgenerational effects and subsequently increasing stress resistance in worms. As was

reported in other studies using *C. elegans*, the epigenetic memory of such stress exposure requires ASH-2, which is a component of the histone H3K4me3 regulatory complex (Greer et al., 2011; Kishimoto et al., 2017). Our results also show that *ash-2* is involved in the transgenerational inheritance of detoxification response in the worms, but *hrde-1* is not.

2.5 The upregulated xenobiotic response might reduce fertility in the F3 generation of wild-type and *hrde-1* mutant worms

The cytotoxicity of berberine and sanguinarine has been previously reported (Chow et al., 2016; 2017). Furthermore, these alkaloids induce detoxification-responsive genes, indicating their toxicity to worms. The xenobiotic response was still observed in the F3 offspring that were unexposed to alkaloids. Therefore, we wanted to determine whether this xenobiotic effect would alter reproduction in future generations of alkaloid-treated P0 worms. However, after 24 hours, the mean number of offspring that hatched from the F3 generation of berberine and sanguinarine-treated worms was lower than that of the untreated N2 control, although the difference was statistically nonsignificant. This trend was also observed in the *hrde-1* and *ash-2* mutants (Figure 7).

Although the mechanisms controlling the transmission and duration of epigenetic changes across generations are still unknown, the number of generations where epigenetic inheritance was found varied from as few as one to more than forty. A switch between a transitory, reversible effect and a stable, inherited effect was observed when only one

generation of worms was exposed to volatile odorants compared to exposure of four generations continuously (Remy, 2010). Our results showed that berberine and sanguinarine induced a relatively strong detoxification response in the parent generation that ingested the alkaloids, followed by a transient shift close to the basal level in the immediate F1 progeny, but the memory of the xenobiotic response was then inherited in the generations thereafter.

Exposures of *C. elegans* to various stressors, e.g., oxidative and osmotic stress, electrical pulses, UV light, radiation, and caloric restriction, have been reported to increase the lifespan of the worms. It has also been suggested that the number of progeny decreases as energy is conserved for lifespan extension. The induction of xenobiotic response genes other than those in the CYP-35 family that have a direct impact on worms' reproduction remains to be elucidated.

3 DISCUSSION

In this study, we investigated the transgenerational effect of two benzylisoquinoline alkaloids, namely, berberine and sanguinarine, in *C. elegans*. Our results suggested that these alkaloids affected the worms' lipid metabolism and xenobiotic responses transgenerationally (Figures 1 & 4). Through epigenetic modifications, an ancestor's experience can affect its gene expression, physiology, and lifespan, which are transmissible to its descendants. Through quantification of Nile red fluorescence in *C. elegans*, we found that the lipid-reduction effects of alkaloids were inherited by the offspring of wild-type

worms but diminished in *ash-2* and *hrde-1* worms (Figures 1-3). This result indicates that the ASH-2 component of the histone H3K4me3 complex and the HRDE-1 worm Argonaute protein are involved in transgenerational inheritance of the lipid-reduction phenotype.

We hypothesized that the lipid reduction activities of alkaloid treatment are the result of the xenobiotic response of the worms and the changes in gene expression in the P0 and F1 to F3 generations. Our results showed that the upregulation of detoxification response genes in P0 worms was inherited by F3 wild-type offspring, whereas such effects were decreased in *hrde-1* worms and diminished in *ash-2* worms (Figures 4-6). This finding suggests that *ash-2* is required for transgenerational inheritance of the xenobiotic response, whereas *hrde-1* is not. We also observed that alkaloid treatment of the P0 worms resulted in reduced fertility in their F3 offspring (Figure 7). Of the various detoxification genes, *cyp-35C1* was noticeably highly induced in alkaloid-treated P0 worms, and its enhanced expression was maintained at a diminished level in the F1 to F3 offspring of these worms. Incidentally, a reduction in the expression of *cyp-35C1* seemed to restore the fertility of F3 *ash-2* and *hrde-1* worms with sanguinarine-treated ancestors but not that of berberine-treated worms. This result supports the finding of a previous report that the *cyp-35A/C* gene reduces the reproduction of *C. elegans* exposed to xenobiotics (Menzel et al., 2005). This result also suggests that *cyp-35C1* is a useful indicator of the worm's xenobiotic response for transgenerational inheritance experiments.

Since berberine and sanguinarine induced transgenerational lipid-reducing activity in *C. elegans*, we also analyzed the expression of several genes involved in the worm's lipid metabolism (Supplementary Figures S1 and S2). AAK-1 and AAK-2 are the *C. elegans* homologs of the catalytic α -subunits of AMPK that regulate triglyceride synthesis. Our qRT-PCR results show enhanced *aak-1* expression in alkaloid-treated P0 wild-type, *ash-2* and *hrde-1* worms but a gradual reduction in *aak-1* expression in the F3 generations (Supplementary Figure S1). Although the role of AAK-1 in the transmissible lipid-reduction effect is unclear, a similar change in *aak-1* expression found in all three worm strains suggests that it is not directly involved in the transgenerational effect on lipid accumulation. Additionally, the qRT-PCR results showed that *nhr-49*, the key regulator of fat consumption, and its downstream target genes were downregulated in F3 wild-type, *ash-2* and *hrde-1* worms (Supplementary Figure S2). Since the *nhr-49* mutant exhibits a high-fat phenotype due to repression of fatty-acid β -oxidation genes, the reduction in lipid accumulation in F3 wild-type worms treated with alkaloids also suggests that the effect is independent of fatty-acid β -oxidation. These results indicate that further investigation is necessary to identify the mechanisms involved.

Our results show that *hrde-1* is not required for transgenerational inheritance of the xenobiotic response. However, HRDE-1 is involved in the transgenerational inheritance of the lipid-reduction effect of alkaloid treatment in P0 worms (Figure 3). HRDE-1 is essential for transgenerational shuttling of heritable small RNAs. In a transcriptome-sequencing experiment using starvation as a stressor in *C. elegans*, Rechavi et al. (2014) reported that

96.8% of the heritable small RNAs were not inherited in *hrde-1* mutants. Future small RNA transcriptome analysis could help identify the heritable small RNAs and their target genes that are responsible for the transgenerational lipid-reducing effect in alkaloid-treated worms.

Investigation of transgenerational inheritance of drug effects is pertinent to the fields of pharmacology and toxicology since these drug effects impact the offspring in future generations. Our study reveals that the biological activity of a xenobiotic in *C. elegans* is transmissible to descendants. Although the exact mechanisms are largely unknown, we found that the epigenetic memory of the lipid-reduction effect of berberine and sanguinarine requires *ash-2* and *hrde-1*. qRT-PCR results of detoxification response and lipid metabolism gene expression show that sanguinarine treatment has a stronger effect in F3 offspring than berberine treatment, suggesting that the impact of transgenerational inheritance is specific to each stimulus and is not a generalized epigenetic memory of the ancestor's exposure to xenobiotics. In this study, we evaluated the transgenerational inheritance of alkaloids' lipid-reducing effect in *C. elegans*. Taki et al. (2013) reported that nicotine exposure induced heritable behavioral changes in the F1 and F2 offspring of *C. elegans*. These results offer new insights into transgenerational drug effects on the descendants of treated subjects and suggest the importance of such analysis beyond pharmacokinetics in drug development.

4 EXPERIMENTAL PROCEDURES

4.1 Chemicals and reagents

Berberine sulfate was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and sanguinarine chloride and Nile red were purchased from Sigma-Aldrich (St. Louis, U.S.A.). All other reagents were purchased from Wako Pure Chemicals (Osaka, Japan) unless otherwise stated. Berberine and sanguinarine were dissolved in distilled water. Nile red stock solution was prepared with acetone at 250 mg/ml and diluted in phosphate buffer solution to 0.5 µg/ml.

4.2 Nematode strains

The following worm strains were used: the wild-type N2 (Bristol) and two knockout mutants classified as homozygous viable by the National BioResource Project of Japan (The *C. elegans* Deletion Mutant Consortium, 2012), namely, *hrde-1* (*tm1200*; a mutant with a deletion of 374 bp at 32391-32764, chromosome III) and *ash-2* (*tm1726*; a mutant with a deletion of 469 bp at 12372-12840, chromosome II). All worms were maintained on NGM at 20°C according to standard culture methods (Stiernagle, 2006).

4.3 Nematode treatment

L4 larval stage worms were treated with berberine, sanguinarine or distilled water (as a control) and 50 ng/ml Nile red in 24-well plates for 48 hours at 20°C under 180 rpm (for aeration) in liquid S-medium with *Escherichia coli* OP50 as a food source.

Approximately 10-12 worms were randomly chosen from two different plates for Nile red fluorescence measurement, and the rest of the treated parent worms (P0) were rinsed

in M9 buffer and transferred to fresh NGM plates with OP50 without alkaloids and allowed to lay eggs. Adult worms of the next generation, F1, were then stained with Nile red to observe their lipid accumulation, and a portion of these worms was then transferred to new NGM plates to lay eggs that hatched into F2 worms. The procedure was repeated until F3 worms were evaluated.

4.4 Nile red fluorescence quantification

P0-treated worms after 48 hours and adult worms of the F1, F2, and F3 generations were sampled randomly from two plates for microscopic observation. Nile red fluorescence images were acquired at the same exposure time using the Keyence Biorevo BZ-9000 imaging system (Keyence Co., Osaka, Japan) after the same staining treatment. Fluorescence images of ten to twelve nematodes were used to quantify lipid droplets using ImageJ software (<http://rsbweb.nih.gov/ij/>). The most anterior four pairs of intestinal cells were selected, and the fluorescence intensity per unit area for each sample was quantified (Lemieux, 2011). The results were verified by reproducibility in two independent experiments.

4.5 Egg laying assay

Five adult worms were randomly chosen from the F3 generation and transferred to new NGM plates with OP50 and allowed to lay eggs for 24 hours at 20°C, after which the adult worms were removed from the plates. Eggs were incubated at 20°C for another 24 hours, and the number of eggs that hatched into larvae was counted.

4.6 Quantitative RT-PCR (qRT-PCR)

Worms were rinsed three times with M9 buffer. Total RNA was extracted from the worm pellet (> 1000 worms) with Sepasol-RNA I Super G (Nacalai Tesque, Kyoto, Japan), purified with an RNeasy Mini Kit (Qiagen, Tokyo, Japan), and reverse-transcribed into cDNA using the PrimeScript RT-PCR Kit (Takara Bio, Kusatsu, Japan) with oligo(dT) primer. cDNA (at a final concentration of 500 pg/μl) was subjected to qRT-PCR analysis using a CFX96 Real-Time PCR system (Bio-Rad Laboratories, Inc., Tokyo, Japan) with IQ SYBR Green Super Mix (Bio-Rad). The conditions for PCR were as follows: 95°C for 15 min, followed by 40 cycles of 95°C for 10 s, 60°C for 20 s and 72°C for 20 s. Melting curve analysis was performed after each run at 72°C to 95°C to check the specificity of amplification. Data were analyzed using Bio-Rad CFX Manager (Bio-Rad). The number of transcripts in a sample was determined by comparing the number of cycles (c) required for the reaction to reach a common threshold (t). The mRNA abundance values are plotted as the average of triplicate cDNA templates applied to run qRT-PCR, and the results were normalized by the amplification of *cdc-42* as an internal control.

The sequences of forward and reverse primers used in the quantitative RT-PCR analysis were as follows:

sod-5 forward, 5'-TTGGCTTACCCAGAAAGCCGAAGGT-3'; reverse, 5'-GACGTACATCCATCGGTTGAGTCTC-3'

395 *hsp-16.2* forward, 5'-CTCCAGTCTGCAGAATCTCTCCAT-3'; reverse, 5'-
 396 GTGAGACGTTGAGATTGATGGCA-3'
 397 *cyp-35C1* forward, 5'-AATTGGAGGACATCCTGTCTG-3'; reverse, 5'-
 398 AAATACAGCTCGGCTCTTGC-3';
 399 *gst-5* forward, 5'-TCAAGCTCAACGGAAAAACC-3'; reverse, 5'-
 400 CCGAAGCCTTCAAGAAGTTG -3';
 401 *ugt-21* forward, 5'-AGGGAGAAATGCACAAATGC-3'; reverse, 5'-
 402 CTTGCTGCAAATTCCACGTA-3';
 403 *ugt-25* forward, 5'-AAATCCGAGCCAAATGTCAC-3'; reverse, 5'-
 404 TGCAAGCATATTCGCATTTC-3';
 405 *F08G5.6* forward, 5'-GTCCCACTGTCACAAGCTCA-3'; reverse, 5'-
 406 GTTTCGACCGAGAAATCGAG-3';
 407 *aak-1* forward, 5'-TGGTTCCGTATCGATCTTCC-3'; reverse, 5'-
 408 TCCCAAAAATCTTCCATTGC-3'
 409 *aak-2* forward, 5'-CCGGAATTCGTGGAAGTAGA-3'; reverse, 5'-
 410 AACGAGCCAGTGTTCCAATC-3'
 411 *nhr-49* forward, 5'-TTAAATCCAGCCGGATCAGT-3'; reverse, 5'-
 412 CTGCTCACTGTTCAAAAATGGAA-3'
 413 *acs-2* forward, 5'-TGACGTGCTCAAGTCTCCAC-3'; reverse, 5'-
 414 CTTACCATCTTCTCGCACA-3';
 415 *cpt-5* forward, 5'-TGCGATGGAGCTGAGTTAGA-3'; reverse, 5'-
 416 GTGACAGTCGCAATCTCCAA-3';

417 *ech-1* forward, 5'-GAGGCTAAGGCATTTGGTGA-3'; reverse, 5'-
418 CGATTTCATTGACCGGAAGT-3';
419 *gei-7* forward, 5'-GGAAATCCTTTTCGCTCACCGCCCAA-3'; reverse, 5'-
420 ATATCAGCCTGAACTTGGTTGCGCT-3'
421 *cdc-42* forward, 5'-AGCTTCATTTCGAGAATGTCC-3'; reverse, 5'-
422 CTCGAGCATTCTGGATCAT-3'.
423

424 **4.7 Statistical analysis**

425 Data are expressed as the mean $\pm$ standard deviation (SD) unless otherwise noted and were
426 analyzed for significance using Student's *t*-test or ANOVA for multiple-comparison tests. A
427 probability value of $p < 0.05$ indicates statistical significance.
428

429 **ACKNOWLEDGMENTS**

430 The *C. elegans* mutant strains *hrde-1* (*tm1200*) and *ash-2* (*tm1726*) were provided by
431 the Mitani Laboratory through the National BioResource Project of the Ministry of
432 Education, Culture, Sports, Science and Technology of Japan (MEXT). This research
433 was supported by the MEXT [Grant-in-Aid for Scientific Research (S); 26221201 to FS].
434

435 **Author Contributions**

436 Y-LC designed and performed the experiments; Y-LC and FS analyzed the data; and Y-
437 LC and FS wrote the manuscript. Both authors discussed and approved the final version
438 of the manuscript.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

Adhami, V.M., Aziz, M.H., Mukhtar, H., & Ahmad, N. (2003). Activation of prodeath Bcl-2 family proteins and mitochondrial apoptosis pathway by sanguinarine in immortalized human HaCaT keratinocytes. *Clinical Cancer Research*. 9, 3176-3182.

Ashe, A., Sapetschnig, A., Weick, E.M., Mitchell, J., Bagijn, M.P., Cording, A.C...Miska, E.A. (2012). piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. *Cell*. 150, 88-99. <https://doi.org/10.1016/j.cell.2012.06.018>

Baugh, L.R. (2013). To grow or not to grow: nutritional control of development during *Caenorhabditis elegans* L1 arrest. *Genetics*. 194, 539-555. <https://doi.org/10.1534/genetics.113.150847>

Brooks, K.K., Liang, B., & Watts, J.L. (2009). The influence of bacterial diet on fat storage in *C. elegans*. *PLoS One*. 10, e7545. <https://doi.org/10.1371/journal.pone.0007545>.

Buckley, B.A., Burkhart, K.B., Gu, S.G., Spracklin, G., Kershner, A., Fritz, H....Kennedy S.
 (2012). A nuclear Argonaute promotes multigenerational epigenetic inheritance and
 germline immortality. *Nature*. 489, 447-451. [https: doi: 10.1038/nature11352](https://doi.org/10.1038/nature11352)

Chen, S., Whetstine, J.R., Ghosh, S., Hanover, J.A., Gali, R.R., Grosu, P., & Shi, Y. (2009).
 The conserved NAD(H)-dependent corepressor CTBP-1 regulates *Caenorhabditis elegans*
 lifespan. *Proc Natl Acad Sci USA*. 106, 1496–1501. [https: doi: 10.1073/pnas.0802674106](https://doi.org/10.1073/pnas.0802674106)

Chow, Y.L., & Sato, F. (2013). Screening of isoquinoline alkaloids for potent lipid
 metabolism modulation with *Caenorhabditis elegans*. *Bioscience Biotechnology*
Biochemistry. 77, 2405-2412. [https: DOI: 10.1271/bbb.130523](https://doi.org/10.1271/bbb.130523)

Chow, Y.L, Kawasaki, Y., & Sato, F. (2014). Knockdown of the NHR-8 nuclear receptor
 enhanced sensitivity to the lipid-reducing activity of alkaloids in *Caenorhabditis elegans*.
Bioscience Biotechnology Biochemistry. 78, 2008-2013. [https: doi:
 10.1080/09168451.2014.940278](https://doi.org/10.1080/09168451.2014.940278)

Chow, Y.L., Sogame, M., & Sato, F. (2016). 13-Methylberberine, a berberine analogue with
 stronger anti-adipogenic effects on mouse 3T3-L1 cells. *Scientific Reports*. 6, 38129. [https:
 doi: 10.1038/srep38129](https://doi.org/10.1038/srep38129)

482 Chow, Y.L., Iwata, Y., & Sato, F. (2017). Dihydrosanguinarine enhances glucose uptake in
 483 mouse 3T3-L1 Cells. *ACS Omega*. 2, 6916-6925. [https: doi: 10.1021/acsomega.7b01134](https://doi.org/10.1021/acsomega.7b01134)
 484

485 Chuang, H.S., Kuo, W.J, Lee, C.L., Chu, I.H., & Chen, C.S. (2016). Exercise in an
 486 electrotactic flow chamber ameliorates age-related degeneration in *Caenorhabditis elegans*.
 487 *Scientific Reports*. 6, 28064. [https: doi: 10.1038/srep28064](https://doi.org/10.1038/srep28064)
 488

489 Cohen, M., Reale, V., Olofsson, B., Knights, A., Evans, P., & de Bono, M. (2009).
 490 Coordinated regulation of foraging and metabolism in *C. elegans* by RFamide neuropeptide
 491 signaling. *Cell Metabolism*. 4, 375-385. [https: doi: 10.1016/j.cmet.2009.02.003](https://doi.org/10.1016/j.cmet.2009.02.003)
 492

493 Egger, G., Liang, G., Aparicio, A., & Jones, P.A. (2004). Epigenetics in human disease and
 494 prospects for epigenetic therapy. *Nature*. 429, 457-463. [https: DOI:10.1038/nature02625](https://doi.org/10.1038/nature02625)
 495

496 Greer, E.L. Maures, T.J., Ucar, D., Hauswirth, A.G., Mancini, E., Lim, J.P.....Brunet, A.
 497 (2011). Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*.
 498 *Nature*. 479, 365-371. [https: doi: 10.1038/nature10572](https://doi.org/10.1038/nature10572).
 499

500 Heard, E., & Martienssen, R.A. (2014). Transgenerational epigenetic inheritance: myths and
 501 mechanisms. *Cell*. 157, 95-109. [https: doi: 10.1016/j.cell.2014.02.045](https://doi.org/10.1016/j.cell.2014.02.045)
 502

503 Holliday, R. (1987). The inheritance of epigenetic defects. *Science*. 238, 163-170.

504

505 Houri-Ze'evi, L., Korem, Y., Sheftel, H., Faigenbloom, L., Toker, I.A.,
506 Dagan, Y.,Rechavi, O. (2016). A Tunable Mechanism Determines the Duration of the
507 Transgenerational Small RNA Inheritance in *C. elegans*. *Cell*. 165, 88-99. [https: doi:](https://doi.org/10.1016/j.cell.2016.02.057)
508 10.1016/j.cell.2016.02.057

509

510 Kaati, G., Bygren, L.O., Pembrey, M., & Sjöström, M. (2007). Transgenerational response
511 to nutrition, early life circumstances and longevity. *European Journal Human Genetics*. 15,
512 784-790. [https: DOI: 10.1038/sj.ejhg.5201832](https://doi.org/10.1038/sj.ejhg.5201832)

513

514 Kishimoto, S., Uno, M., Okabe, E., Nono, M., & Nishida, E. (2017). Environmental stresses
515 induce transgenerationally inheritable survival advantages via germline-to-soma
516 communication in *Caenorhabditis elegans*. *Nature Communication*. 8,14031. [https: doi:](https://doi.org/10.1038/ncomms14031)
517 10.1038/ncomms14031

518

519 Klosin, A., Casas, E., Hidalgo-Carcedo, C., Vavouri, T., & Lehner, B. (2017).
520 Transgenerational transmission of environmental information in *C. elegans*. *Science*. 356,
521 320-323. [https: DOI: 10.1126/science.aah6412](https://doi.org/10.1126/science.aah6412)

522

523 Lagisz, M., Hector, K.L., & Nakagawa, S. (2013) Life extension after heat shock exposure:
524 assessing meta-analytic evidence for hormesis. *Ageing Research Reviews*. 12, 653-660. DOI:
525 10.1016/J.ARR.2013.03.005

526

527 Lemieux, G.A., Liu, J., Mayer, N., Bainton, R.J., Ashrafi, K., & Werb, Z. (2011). A whole-
528 organism screen identifies new regulators of fat storage. *Nature Chemical Biology*. 7, 206-
529 213. <https://doi.org/10.1038/nchembio.534>

530

531 Lim, J.P., & Brunet, A. (2013). Bridging the transgenerational gap with epigenetic memory.
532 *Trends Genetics*. 29, 176-186. <https://doi.org/10.1016/j.tig.2012.12.008>

533

534 Lindblom, T.H., & Dodd, A.K. (2006). Xenobiotic detoxification in the nematode
535 *Caenorhabditis elegans*. *Journal Experimental Zoology part A: Comparative Experimental*
536 *Biology*. 305, 720-730. <https://doi.org/10.1002/jez.a.324>

537

538 Lithgow, G.J., White, T.M., Hinerfeld, D.A., & Johnson, T.E. (1994) Thermotolerance of a
539 long-lived mutant of *Caenorhabditis elegans*. *Journals Gerontology*. 49, B270-276.

540

541 Lithgow, G.J., White, T.M., Melov, S., & Johnson, T.E. (1995) Thermotolerance and
542 extended life-span conferred by single-gene mutations and induced by thermal stress.
543 *Proceedings National Academy Science U.S.A.* 92, 7540-7544.

544

545 Luteijn, M.J., van Bergeijk, P., Kaaij, L.J., Almeida, M.V., Roovers, E.F., Berezikov, E., &
546 Ketting, R.F. (2012). Extremely stable Piwi-induced gene silencing in *Caenorhabditis*
547 *elegans*. *EMBO Journal*. 31, 3422-3430. [doi: 10.1038/emboj.2012.213](https://doi.org/10.1038/emboj.2012.213)

548

549 Matsuyama, S. Moriuchi, M., Suico, M.A., Yano, S., Morino-Koga, S., Shuto, T....Kai, H.
550 (2014). Mild electrical stimulation increases stress resistance and suppresses fat
551 accumulation via activation of LKB1-AMPK signaling pathway in *C. elegans*. *PLoS One*. 9,
552 e114690. <https://doi.org/10.1371/journal.pone.0114690>

553

554 Menzel, R., Rodel, M., Kulas, J., & Steinberg, C.E. (2005). CYP35: xenobiotically induced
555 gene expression in the nematode *Caenorhabditis elegans*. *Archives Biochemistry Biophysics*.
556 438, 93-102. <https://doi.org/10.1016/j.abb.2005.03.020>

557

558 Mullaney, B.C., Blind, R.D., Lemieux, G.A., Perez, C.L., Elle, I.C., Faergeman,
559 N.J....Ashrafi, K. (2010). Regulation of *C. elegans* fat uptake and storage by Acyl-CoA
560 Synthase-3 is dependent on NR5A family nuclear hormone receptor nhr-25. *Cell*
561 *Metabolism*. 12, 398–410. <https://doi.org/10.1016/j.cmet.2010.08.013>.

562

563 O'Rourke, E.J., Soukas, A.A., Carr, C.E., & Ruvkun, G. (2009). *C. elegans* major fats are
564 stored in vesicles distinct from lysosome-related organelles. *Cell Metabolism*. 5, 430–435.
565 <https://doi.org/10.1016/j.cmet.2009.10.002>

566

567 Pathare, P.P., Lin, A., Bornfeldt, K.E., Taubert, S., & Van Gilst, M.R. (2012). Coordinate
568 regulation of lipid metabolism by novel nuclear receptor partnerships. *PLoS Genetics*. 8:
569 e1002645. <https://doi.org/10.1371/journal.pgen.1002645>.

570

571 Rechavi, O., Houri-Ze'evi, L., Anava, S., Goh, W.S.S., Kerk, S.Y., Hannon, G.J....Hobert, O.
572 (2014). Starvation-induced transgenerational inheritance of small RNAs in *C. elegans*. *Cell*.
573 158, 277-287. doi: 10.1016/j.cell.2014.06.020

574

575 Remy, J.J. (2010). Stable inheritance of an acquired behavior in *Caenorhabditis elegans*.
576 *Current Biology*. 20, R877-878. https: doi: 10.1016/j.cub.2010.08.013

577

578 Ristow, M., & Schmeisser, S. (2011) Extending life span by increasing oxidative stress.
579 *Free Radical Biology and Medicine*. 51, 327-336. https: DOI:
580 10.1016/j.freeradbiomed.2011.05.010

581

582 Schott, D., Yanai, I., & Hunter, C.P. (2014). Natural RNA interference directs a heritable
583 response to the environment. *Scientific Reports*. 4, 7387. https: doi: 10.1038/srep07387

584

585 Shirayama, M., Seth, M., Lee, H.C., Gu, W., Ishidate, T., Conte, D. Jr., & Mello, C.C.
586 (2012). piRNAs initiate an epigenetic memory of nonself RNA in the *C. elegans* germline.
587 *Cell*. 150, 65-77. https: doi: 10.1016/j.cell.2012.06.015

588

589 Srinivasan, S., Sadegh, L., Elle, I.C., Christensen, A.G., Faergeman, N.J., & Ashrafi, K.
590 (2008). Serotonin regulates *C. elegans* fat and feeding through independent molecular
591 mechanisms. *Cell Metabolism*. 6, 533-544. https: doi: 10.1016/j.cmet.2008.04.012.

592

593 Stiernagle, T. (2006). *WormBook*, ed. The *C. elegans* Research Community, WormBook,
594 <https://doi.org/10.1895/wormbook.1.101.1>

595

596 Taki, F.A., Pan, X., & Zhang, B. (2013). Nicotine exposure caused significant
597 transgenerational heritable behavioral changes in *Caenorhabditis elegans*. *EXCLI Journal*.
598 *12*, 793-806.

599

600 The *C. elegans* Deletion Mutant Consortium (2012) Large scale screening for targeted
601 knockouts in the *Caenorhabditis elegans* genome. *G3: Genes, Genomes, Genetics*, *2*, 1415-
602 1425, doi.org/10.1534/g3.112.003830

603

604 Turner, N., Li, J.Y., Gosby, A., To, S.W., Cheng, Z., Miyoshi, H., et al. (2008). Berberine
605 and its more biologically available derivative, dihydroberberine, inhibit mitochondrial
606 respiratory complex I: a mechanism for the action of berberine to activate AMP-activated
607 protein kinase and improve insulin action. *Diabetes*. *57*, 1414-1418. [https://doi:](https://doi.org/10.2337/db07-1552)
608 [10.2337/db07-1552](https://doi.org/10.2337/db07-1552)

609

610 Van Gilst, M.R, Hadjivassiliou, H., Jolly, A. & Yamamoto, K.R. (2005). Nuclear hormone
611 receptor NHR-49 controls fat consumption and fatty acid composition in *C. elegans*. *PLoS*
612 *Biology*. *3*:e53. [https://doi: 10.1371/journal.pbio.0030053](https://doi.org/10.1371/journal.pbio.0030053).

613

614 Zhang, S.O., Trimble, R., Guo, F., & Mak, H.Y. (2010). Lipid droplets as ubiquitous fat
615 storage organelles in *C. elegans*. *BMC Cell Biology*. 11, 96. [https: doi: 10.1186/1471-2121-](https://doi.org/10.1186/1471-2121-11-96)
616 11-96.
617

Figure Legends

Figure 1. Nile red staining in wild-type N2 *C. elegans* in the P0, F1, F2 and F3 generations. Worms were treated without alkaloids (control) or with alkaloids (B500: 500 μ M berberine, S10: 10 μ M sanguinarine) at the L4 larval stage in the P0 generation for 48 hours; then, offspring were obtained on fresh NGM medium without alkaloids. Relative Nile red fluorescence intensity (Relative Intensity) was determined based on the value of the control sample (P0, 0.022; F1, 0.018; F2, 0.016; and F3, 0.015) measured using ImageJ software. Panels (a) to (d) refer to parent worms P0, and F1, F2, F3 untreated offspring, respectively. The scale bar indicates 100 μ m. Error bar = standard deviation (SD). * indicates statistical significance; * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$ vs control; two-tailed Student's *t*-test. The fluorescence data are averages from 10 to 12 worms, and the experiments were validated by duplication.

Figure 2. Nile red staining in *ash-2* mutant worms in the P0, F1, F2 and F3 generations. Worms were treated without alkaloids (control) or with alkaloids (B500: 500 μ M berberine, S10: 10 μ M sanguinarine) at the L4 larval stage in the P0 generation for 48 hours; then, offspring were obtained on fresh NGM medium without alkaloids. Relative Nile red fluorescence intensity was determined based on the value of the control sample (P0, 0.018; F1, 0.021; F2, 0.014; and F3, 0.016) measured using ImageJ software. Panels (a) to (d) refer to parent worms P0, and F1, F2, F3 untreated offspring, respectively. The scale bar indicates 100 μ m. Error bar = standard deviation (SD). * indicates statistical significance; * $p < 0.05$,

Figure 2. Nile red staining in *hrde-1* mutant worms in the P0, F1, F2 and F3 generations. Worms were treated without alkaloids (control) or with alkaloids (B500: 500 μ M berberine, S10: 10 μ M sanguinarine) at the L4 larval stage in the P0 generation for 48 hours; then, offspring were obtained on fresh NGM medium without alkaloids. Relative Nile red fluorescence intensity was determined based on the value of the control sample (P0, 0.019; F1, 0.020; F2, 0.012; and F3, 0.015) measured using ImageJ software. Panels (a) to (d) refer to parent worms P0, and F1, F2, F3 untreated offspring, respectively. The scale bar indicates 100 μ m. Error bar = standard deviation (SD). * indicates statistical significance; * p < 0.05, ** p < 0.005, and *** p < 0.001 vs control; two-tailed Student's t -test. The fluorescence data are averages from 10 to 12 worms, and the experiments were validated by duplication.

Figure 3. Nile red staining in *hrde-1* mutant worms in the P0, F1, F2 and F3 generations. Worms were treated without alkaloids (control) or with alkaloids (B500: 500 μ M berberine, S10: 10 μ M sanguinarine) at the L4 larval stage in the P0 generation for 48 hours; then, offspring were obtained on fresh NGM medium without alkaloids. Relative Nile red fluorescence intensity was determined based on the value of the control sample (P0, 0.019; F1, 0.020; F2, 0.012; and F3, 0.015) measured using ImageJ software. Panels (a) to (d) refer to parent worms P0, and F1, F2, F3 untreated offspring, respectively. The scale bar indicates 100 μ m. Error bar = standard deviation (SD). * indicates statistical significance; * p < 0.05, ** p < 0.005, and *** p < 0.001 vs control; two-tailed Student's t -test. The fluorescence data are averages from 10 to 12 worms, and the experiments were validated by duplication.

Figure 4. Quantitative RT-PCR of xenobiotic response genes in wild-type N2 worms in the P0 (a), F1 (b), F2 (c), and F3 (d) generations. C (black bar), control; B (white bar), 500 μ M berberine treatment; S (gray bar), 10 μ M sanguinarine treatment. n = 3; Error bar = SD. * indicates statistical significance; * p < 0.05, ** p < 0.005, and *** p < 0.001 vs control; two-tailed Student's t -test.

Figure 5. Quantitative RT-PCR of xenobiotic response genes in *ash-2* worms in the P0 (a), F1 (b), F2 (c), and F3 (d) generations. C (black bar), control; B (white bar), 500 μ M

berberine treatment; S (gray bar), 10 μ M sanguinarine treatment. n = 3; error bar = SD. * indicates statistical significance; * p < 0.05, ** p < 0.005, and *** p < 0.001 vs control; two-tailed Student's t -test.

Figure 6. Quantitative RT-PCR of xenobiotic response genes in *hrde-1* worms in the P0 (a), F1 (b), F2 (c), and F3 (d) generations. C (black bar), control; B (white bar), 500 μ M berberine treatment; S (gray bar), 10 μ M sanguinarine treatment. n = 3; error bar = SD. * indicates statistical significance; * p < 0.05, ** p < 0.005, and *** p < 0.001 vs control; two-tailed Student's t -test.

Figure 7. Box plot showing the number of offspring hatched from F3 worms after 24 hours of egg laying. N2 wild-type, *ash-2* and *hrde-1* mutants were treated without alkaloids (control) or with alkaloids (B500: 500 μ M berberine, S10: 10 μ M sanguinarine) in the P0 generation. The offspring of five worms were measured. Different letters indicate statistical significance at p < 0.05 (ANOVA followed by Dunnett's multiple comparisons test).

Supplementary Figure S1. Quantitative RT-PCR of AAK genes in P0 (a) and F3 (b) generation worms. Control (C; black bar), 500 μ M berberine (B; white bar), 10 μ M sanguinarine (S; gray bar) treatments. n = 3; Error bar = SD. * indicates statistical significance; * p < 0.05, ** p < 0.005, and *** p < 0.001 vs control; two-tailed Student's t -test.

684

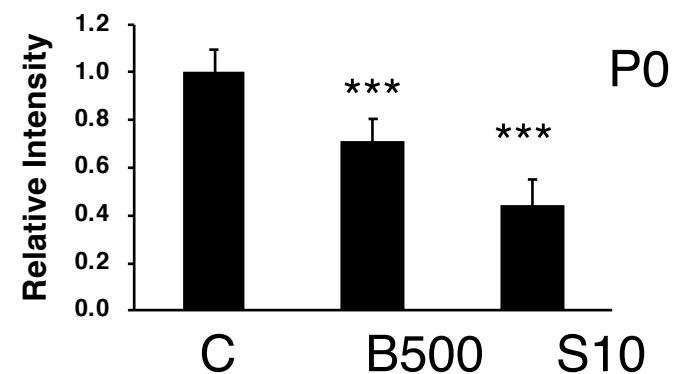
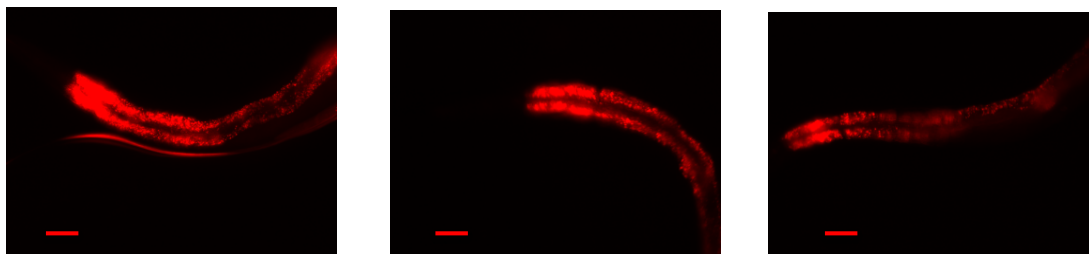
685 **Supplementary Figure S2.** Quantitative RT-PCR of lipid metabolism-related genes in P0

686 (a) and F3 (b) generation worms. Control (C; black bar), 500 μ M berberine (B; white bar),

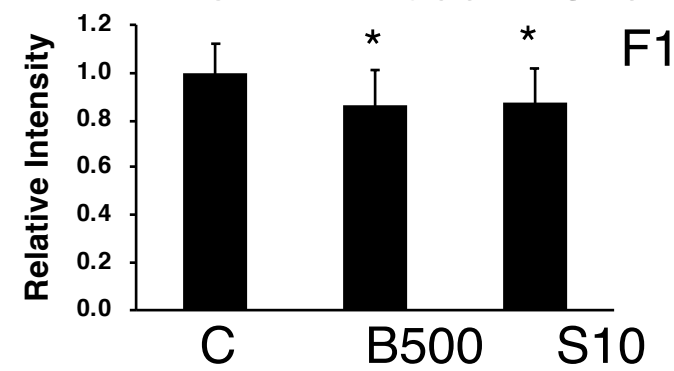
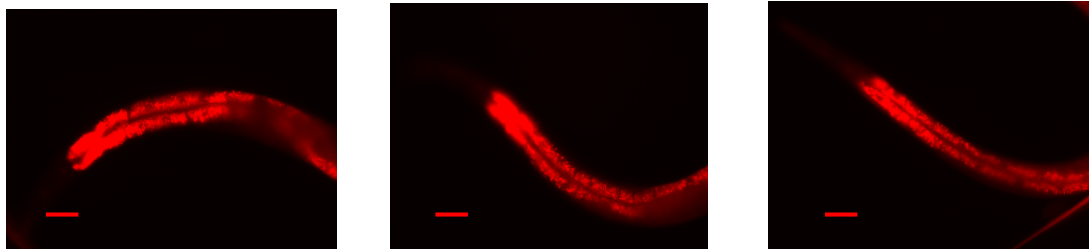
687 10 μ M sanguinarine (S; gray bar) treatments. n = 3; Error bar = SD. * indicates statistical

688 significance; * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$ vs control; two-tailed Student's t -test.

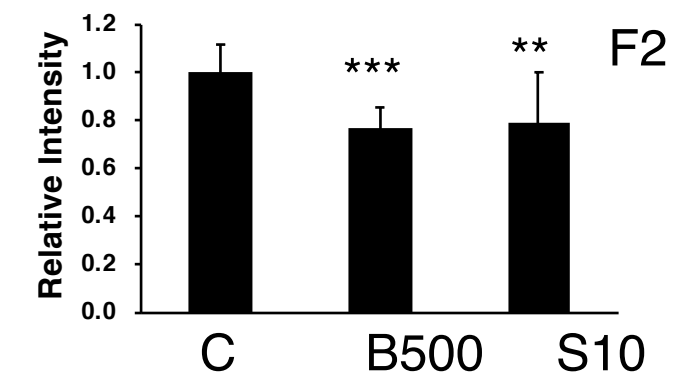
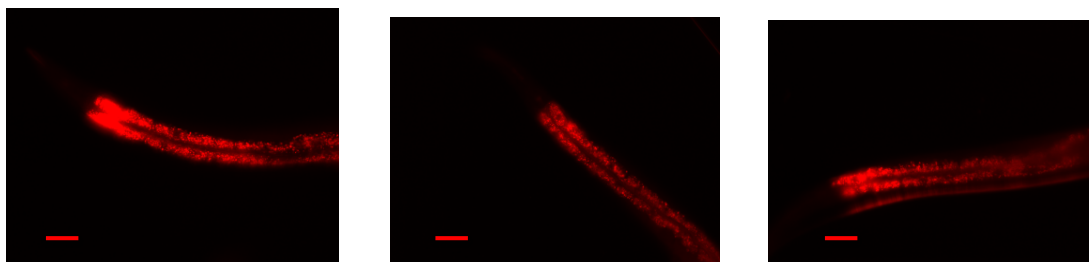
a) N2 P0 treated



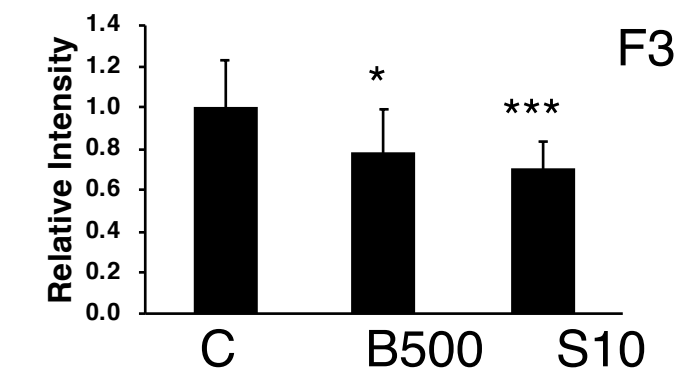
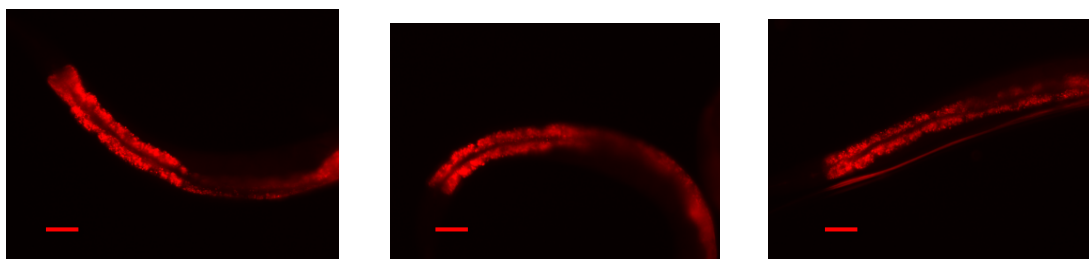
b) N2 F1



c) N2 F2



d) N2 F3

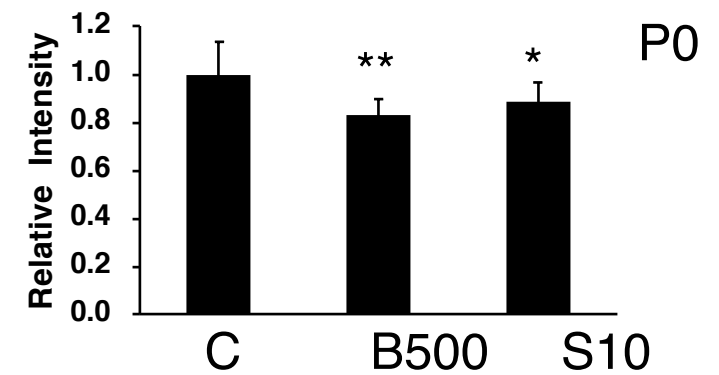
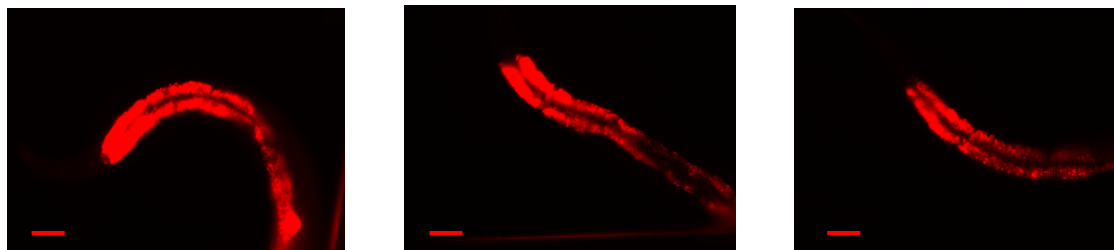


Control

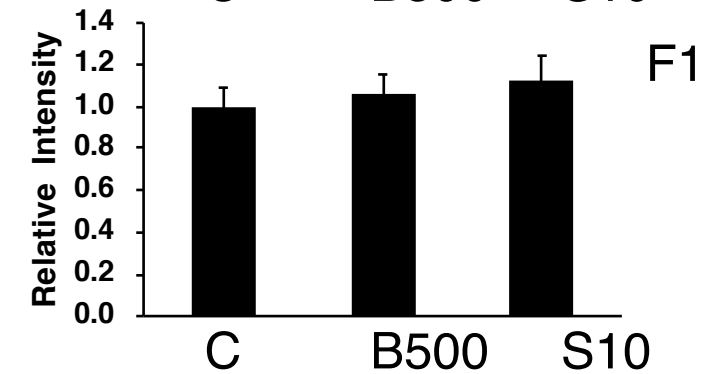
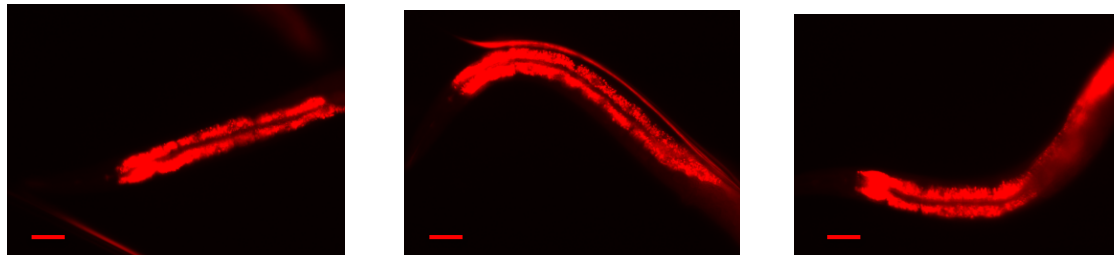
Berberine 500 μ M

Sanguinarine 10 μ M

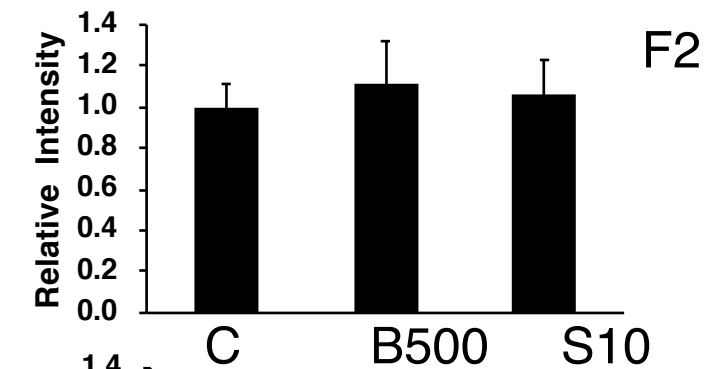
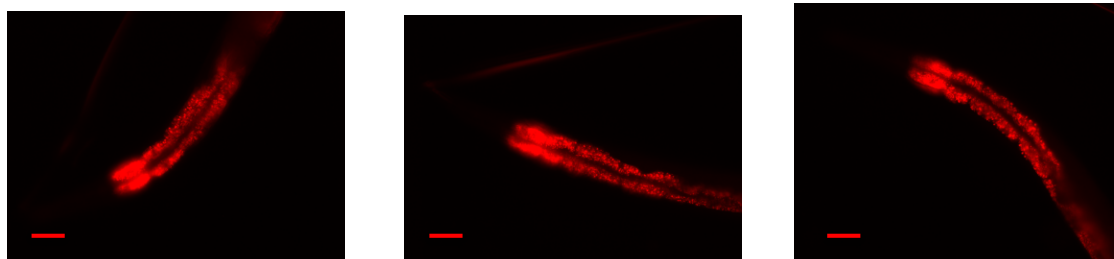
a) *ash-2* P0 treated



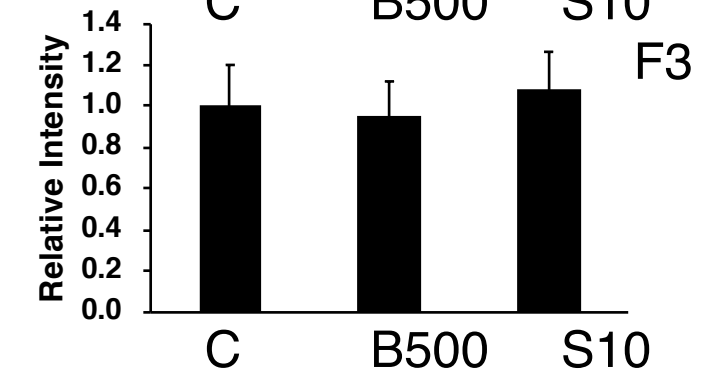
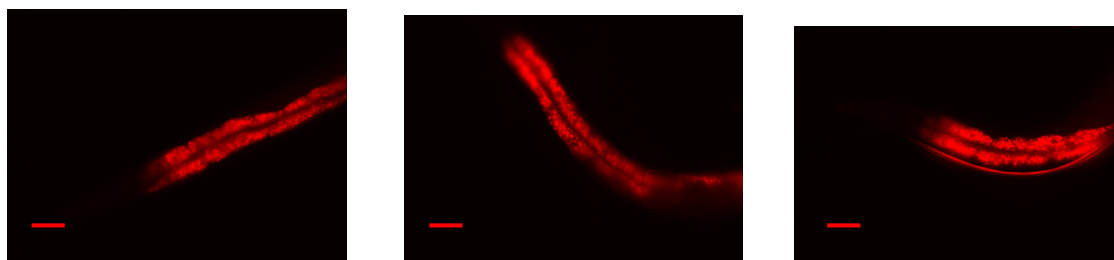
b) *ash-2* F1



c) *ash-2* F2



d) *ash-2* F3

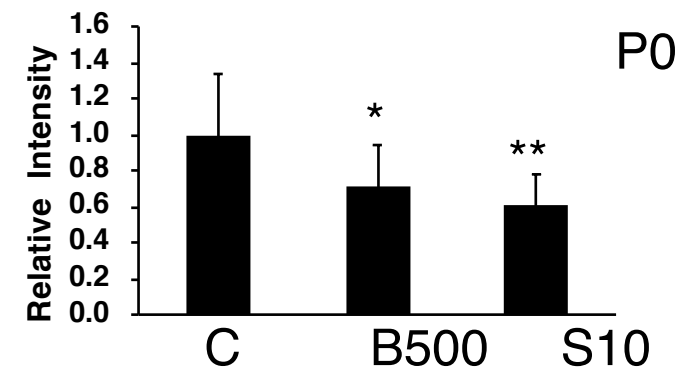
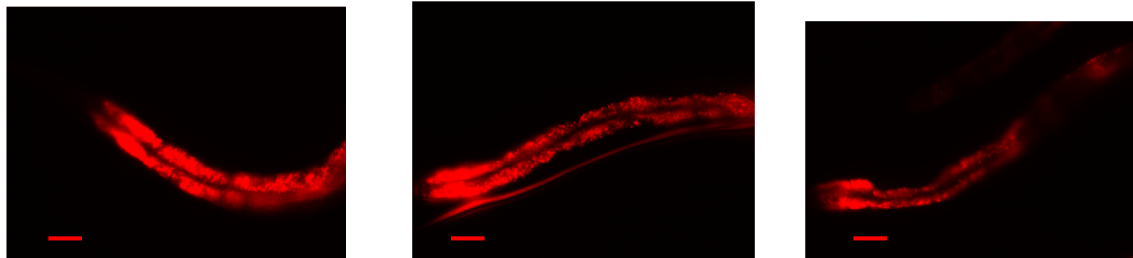


Control

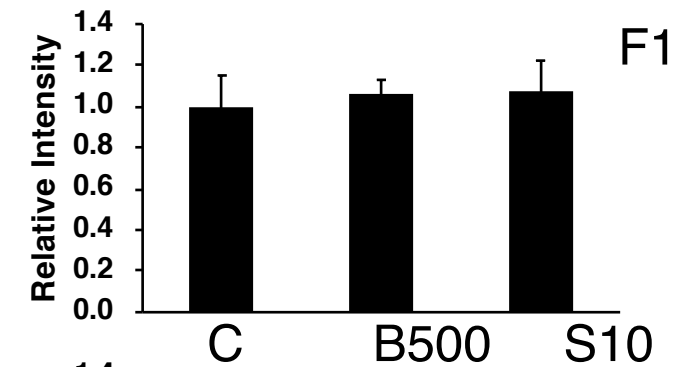
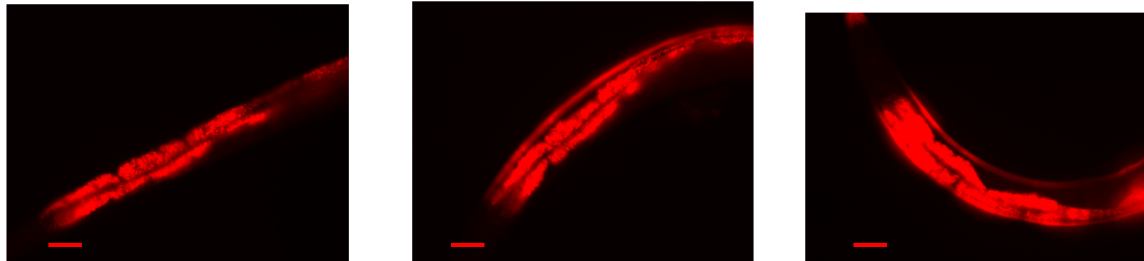
Berberine 500 μ M

Sanguinarine 10 μ M

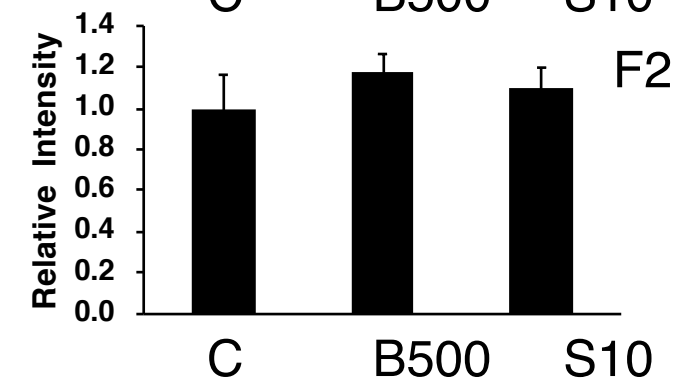
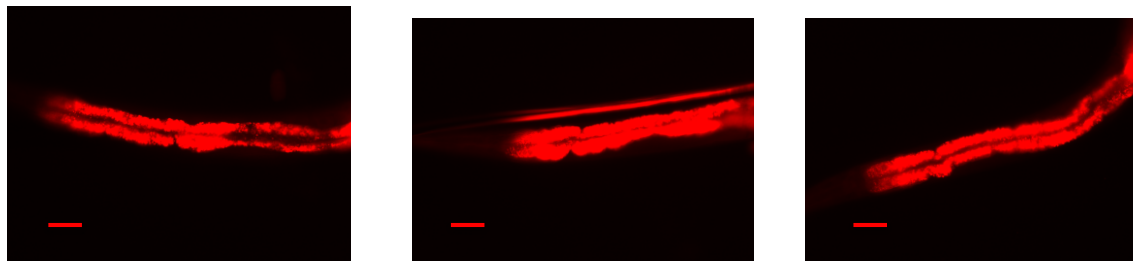
a) *hrde-1* P0 treated



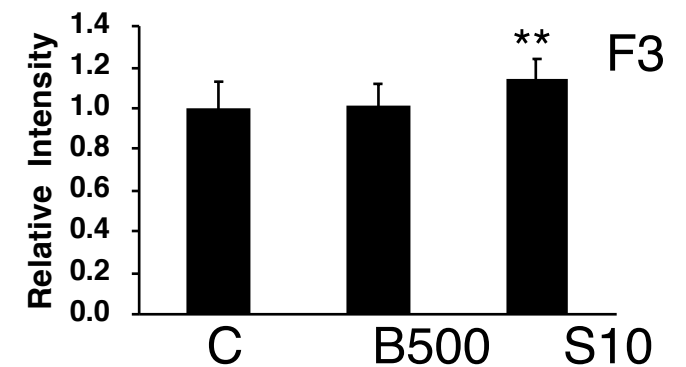
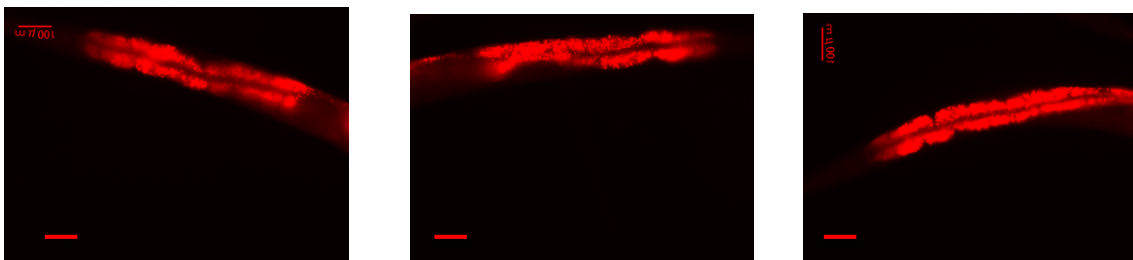
b) *hrde-1* F1



c) *hrde-1* F2



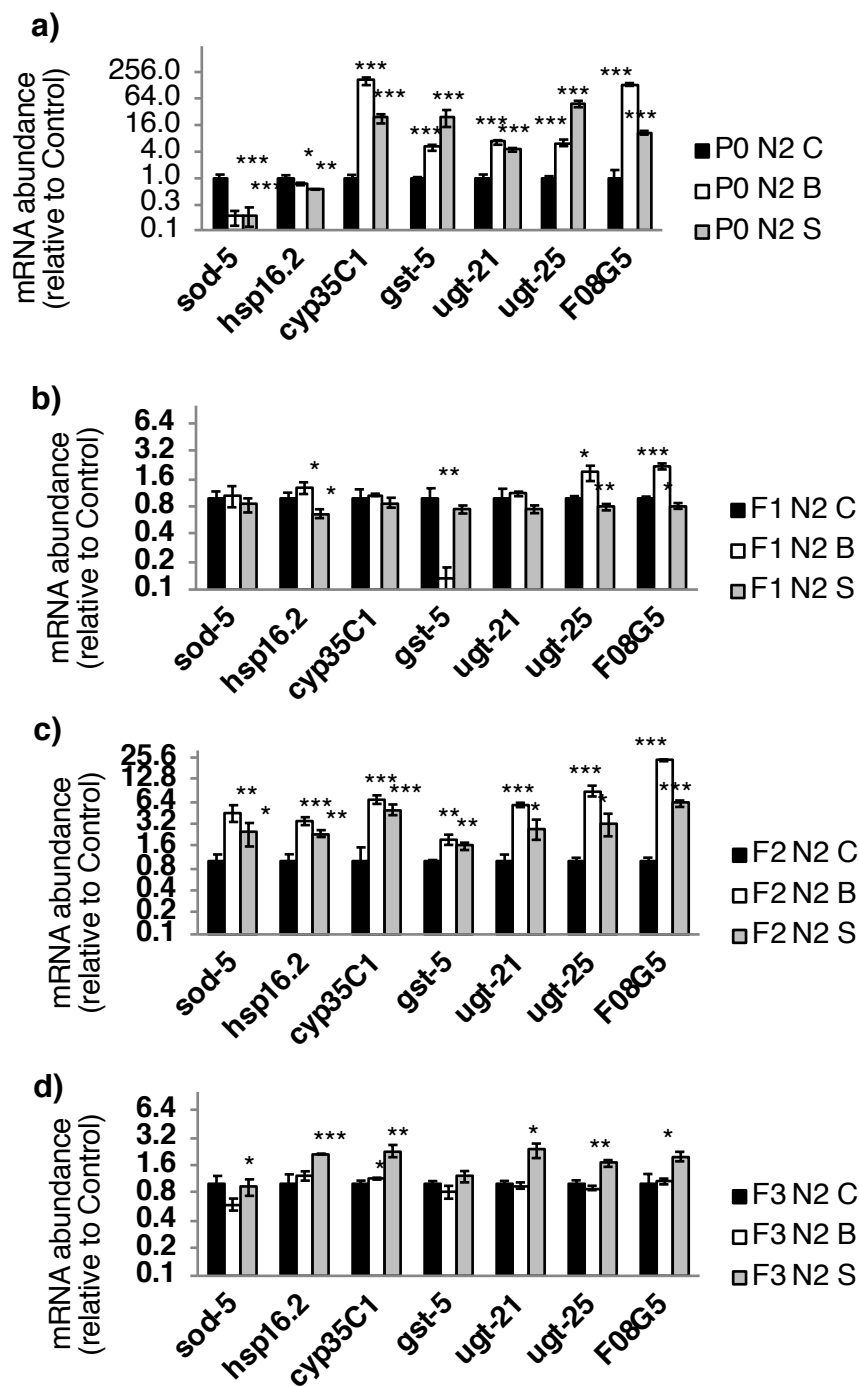
d) *hrde-1* F3

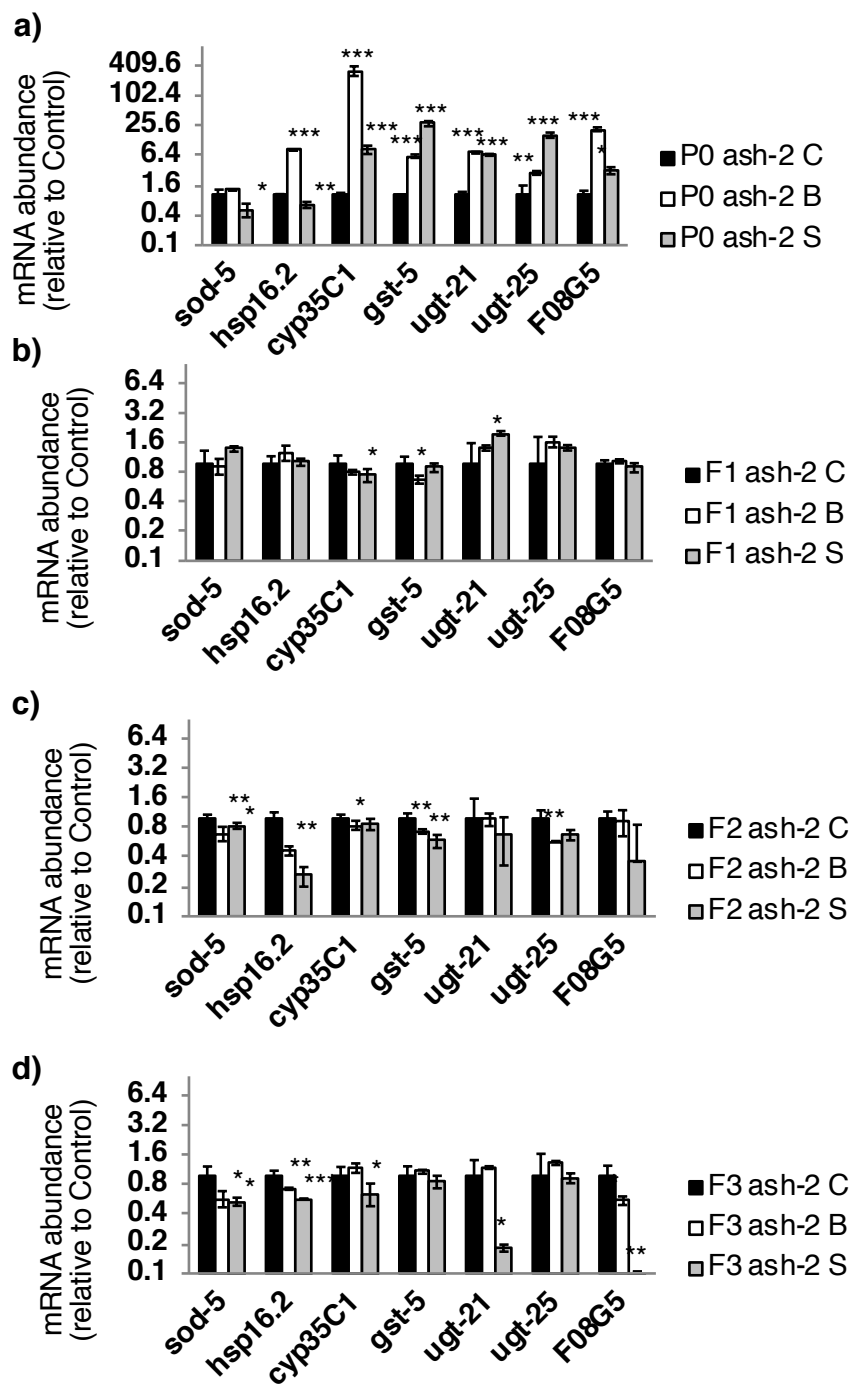


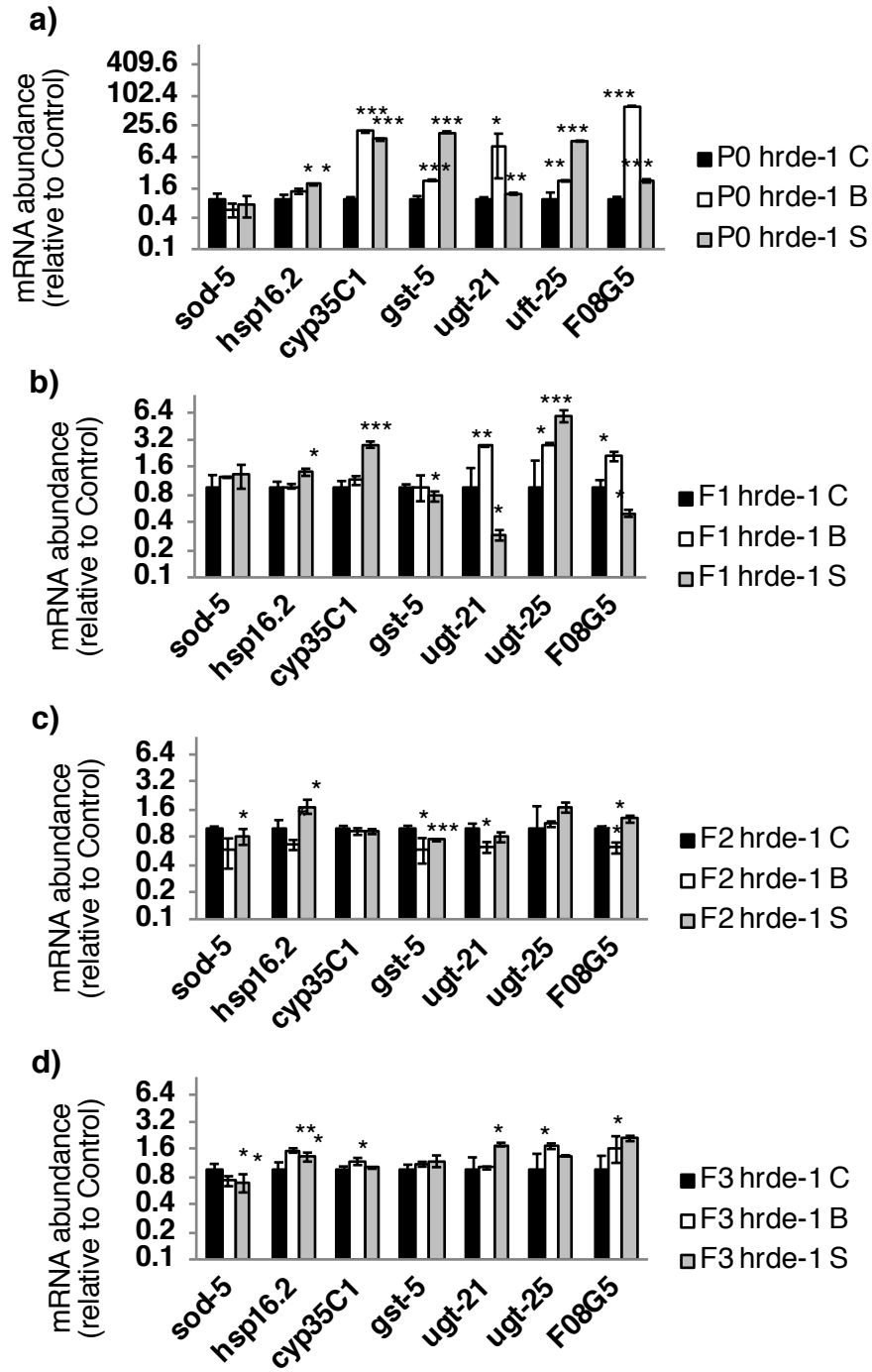
Control

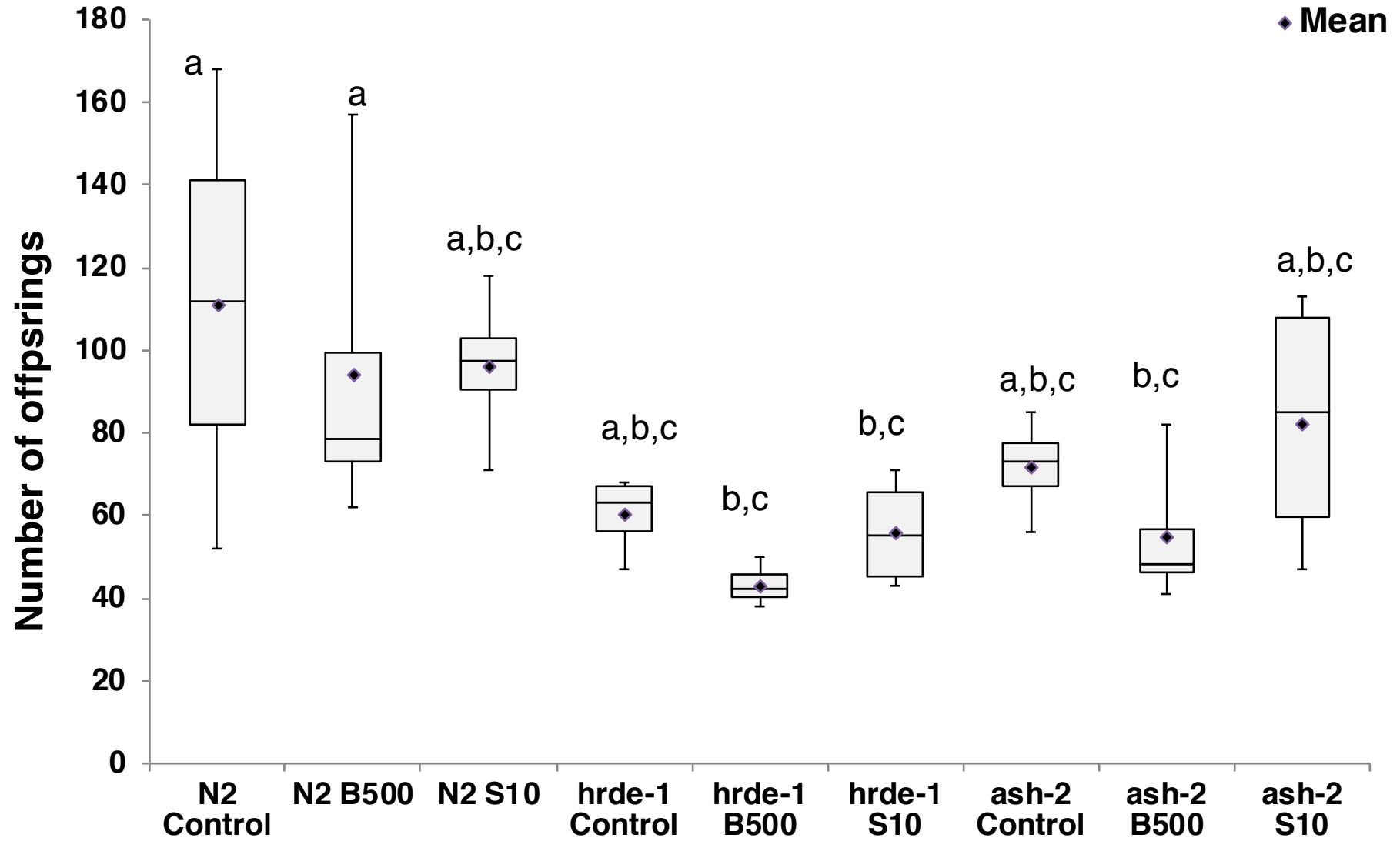
Berberine 500 μ M

Sanguinarine 10 μ M









Transgenerational lipid-reducing activity of benzyloquinoline alkaloids in *Caenorhabditis elegans*

Yit-Lai Chow and Fumihiko Sato

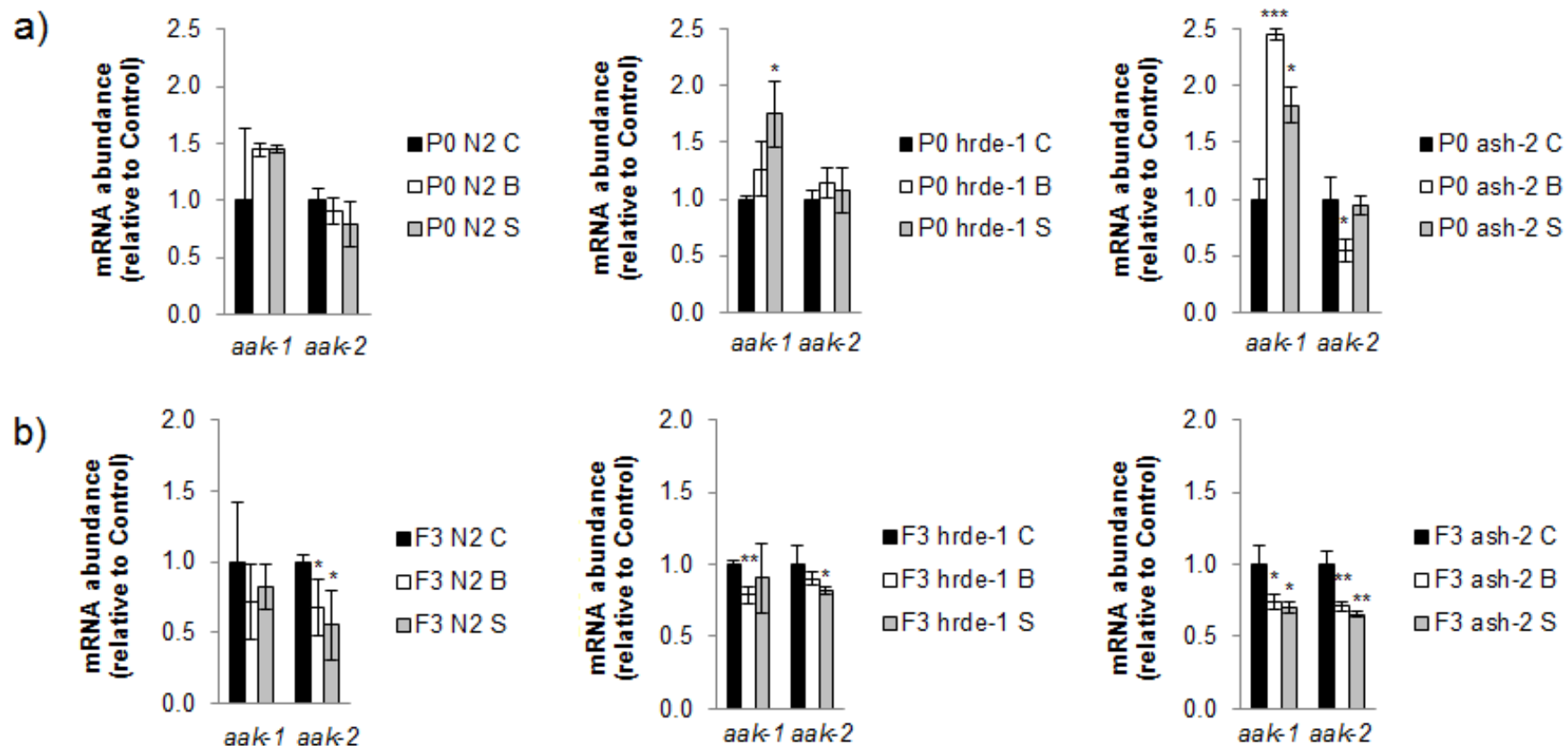
Division of Integrated Life Science, Graduate School of
Biostudies, Kyoto University, Kitashirakawa, Sakyo, Kyoto
606-8502, Japan

Supplementary Figure S1.

Quantitative RT-PCR of AAK genes in P0 (a) and F3 (b)
generation worms.

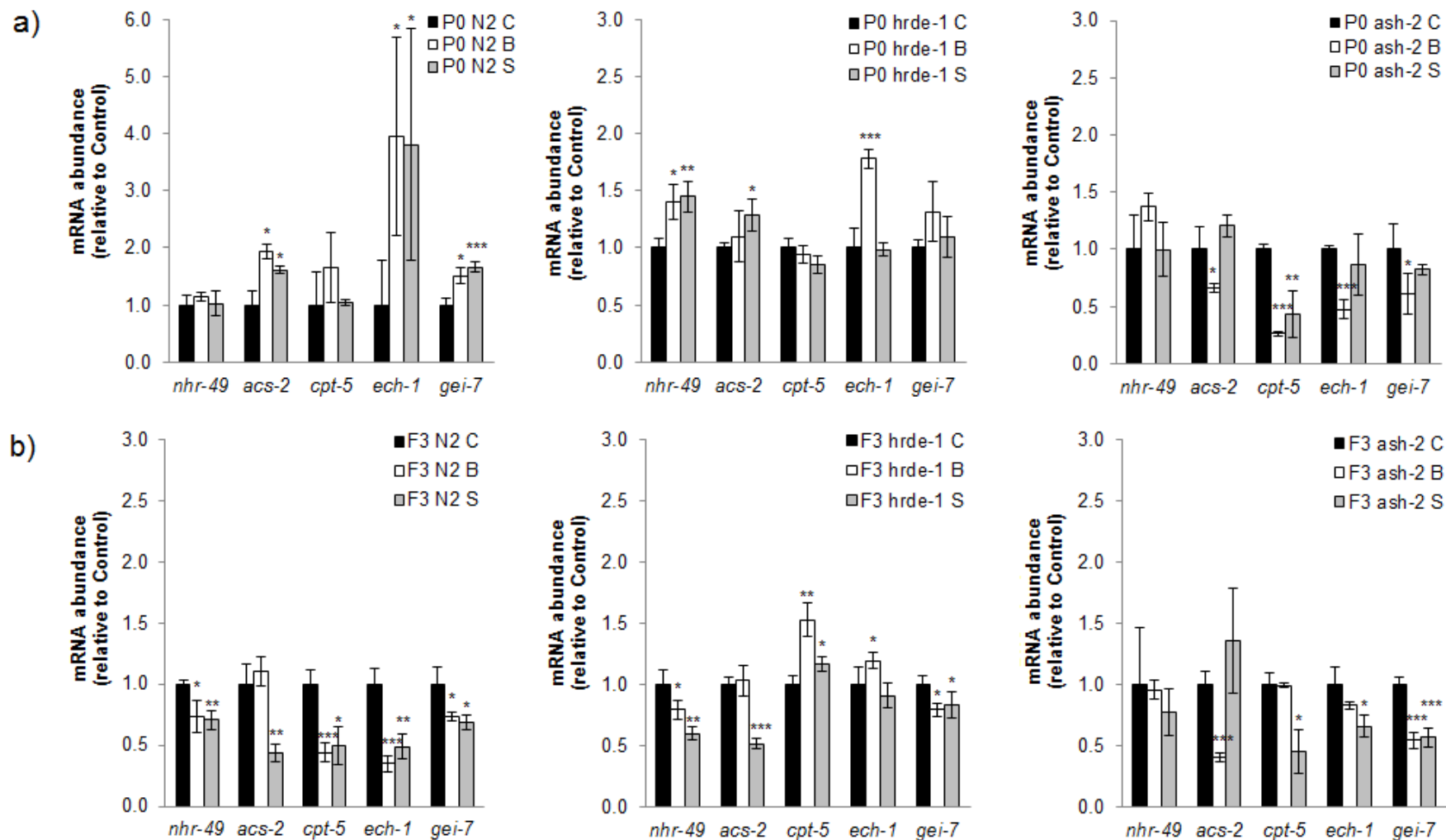
Supplementary Figure S2.

Quantitative RT-PCR of lipid metabolism-related genes in
P0 (a) and F3 (b) generation worms.



Supplementary Figure S1.

Quantitative RT-PCR of AAK genes in P0 (a) and F3 (b) generation worms. Control (C; black bar), 500 mM berberine (B; white bar) , 10 mM sanguinarine (S; gray bar) treatments. n = 3; Error bar = SD. * indicates statistical significance; * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$ vs control; two-tailed Student's t -test.



Supplementary Figure S2.

Quantitative RT-PCR of lipid metabolism-related genes in P0 (a) and F3 (b) generation worms. Control (C; black bar), 500 mM berberine (B; white bar) , 10 mM sanguinarine (S; gray bar) treatments. n = 3; Error bar = SD. * indicates statistical significance; * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$ vs control; two-tailed Student's t -test.