

1 **Title**

2 *Escherichia coli* Metabolite Profiling Leads to the Development of an RNA Interference
3 Strain for *Caenorhabditis elegans*

4
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1 **Abstract**

2 The relationship of genotypes to phenotypes can be modified by environmental
3 inputs. Such crucial environmental inputs include metabolic cues derived from microbes
4 living together with animals. Thus the analysis of genetic effects on animals' physiology
5 can be confounded by variations in the metabolic profile of microbes. *Caenorhabditis*
6 *elegans* exposed to distinct bacterial strains and species exhibit phenotypes different at
7 cellular, developmental and behavioral levels. Here we reported metabolomic profiles of
8 three *Escherichia coli* strains, B strain OP50, K-12 strain MG1655, and B-K-12 hybrid
9 strain HB101, and also different mitochondrial and fat storage phenotypes of *C. elegans*
10 exposed to MG1655 and HB101 versus OP50. We found that these metabolic
11 phenotypes of *C. elegans* are not correlated with overall metabolic patterning of
12 bacterial strains, but their specific metabolites. In particular, the fat storage phenotype is
13 traced to the betaine level in different bacterial strains. HT115 is another K-12 *E. coli*
14 strain that is commonly utilized to elicit an RNA interference response, and we showed
15 that *C. elegans* exposed to OP50 and HT115 exhibit differences in mitochondrial
16 morphology and fat storage levels. We thus generated an RNA interference competent
17 OP50 (iOP50) strain that can robustly and consistently knockdown endogenous *C.*
18 *elegans* genes in different tissues. Together, these studies suggest the importance of
19 specific bacterial metabolites in regulating the host's physiology, and provide a tool to
20 prevent confounding effects when analyzing genotype-phenotype interactions under
21 different bacterial backgrounds.

22 **Introduction**

1 Every ecosystem presents its own particular pressures on the plants, animals,
2 and microbes living within. The lives of these macro and microorganisms are often
3 tightly intertwined, and have undergone countless generations of evolution together.
4 The metabolic interaction between microbes and hosts is a crucial aspect of their
5 interplay and is a key factor in shaping metabolic adaptation in the host. In particular,
6 the animal *Caenorhabditis elegans* is known as a powerful model organism for genetic
7 analysis of complex biological processes such as development, behavior, metabolism,
8 and aging. Although long considered as “soil dwelling” nematodes (Brenner, 1974),
9 advances in the field reveal that these animals may be more aptly described as dwelling
10 in “rotting vegetation”(Félix and Braendle, 2010). In this environment, *C. elegans* are
11 exposed to a multitude of bacterial species and strains, many of which can be prey,
12 pathogen, or commensals. These bacteria have different metabolic profiles, contributing
13 to their nutritional inputs, virulence factors, or communication cues, all of which can
14 stimulate different responses in *C. elegans*. The ability of *C. elegans* to detect those
15 metabolic cues derived from bacteria and adjust their physiology accordingly is required
16 for both themselves and their progeny to survive and reproduce in the environment
17 presented to them (Han et al., 2017; Lin and Wang, 2017; Sowa et al., 2015).

18 Historically, a strain of *Escherichia coli*, known as OP50 has been chosen for
19 growing *C. elegans* in laboratory conditions (Brenner, 1974). OP50 is a uracil-requiring
20 B-type *E. coli* and carries a number of positive traits for *C. elegans* husbandry: it forms a
21 thin bacterial lawn due to its uracil-requirements, it is relatively sticky which facilitates
22 worm transfer, and it is mostly considered non-pathogenic (Couillault and Ewbank,
23 2002). Alternatively, an *E. coli* B-K-12 hybrid strain HB101 and a ‘wild type’ *E. coli* K-12

1 strain MG1655 are both healthier than OP50 and can grow into a thicker lawn to support
2 a larger number of *C. elegans* (Lin and Wang, 2017; MacNeil and Walhout, 2013) .
3 Furthermore, another *E. coli* K-12 strain, HT115, has been utilized for *C. elegans* gene
4 knockdown using RNA interference (RNAi). HT115 has been rendered capable of
5 housing RNAi inducing double stranded RNA through two major mutations, the loss of
6 the *rnc* allele encoding RNase III, and the introduction of an IPTG inducible T7
7 polymerase. The phenotypic differences between *C. elegans* grown on OP50 and
8 alternative often K-12 derived strains have been a recent focus of the scientific
9 community. *C. elegans* have been shown to exhibit bacteria dependent molecular and
10 physiological changes, including alterations to development, lifespan, reproductive
11 lifespan, and metabolism (Coolon et al., 2009; MacNeil, et al., 2013; Pang and Curran,
12 2014; Soukas et al., 2009; Sowa et al., 2015). These phenotypic differences in *C.*
13 *elegans* are likely associated with metabolic cues derived from different bacterial
14 strains.

15 In our studies, we have performed high-throughput metabolomic profiling to
16 systemically analyze metabolic difference among three different *E. coli* strains, OP50,
17 HB101 and MG1655. We found that specific bacterial metabolites, rather than gross
18 metabolic patterns, actively influence phenotypic characteristics of *C. elegans* grown on
19 these bacterial strains. Given that *C. elegans* exhibit drastic differences in their
20 mitochondrial morphology, fat storage, and reproductive span when grown on OP50
21 and HT115, we have generated an OP50 strain that can effectively induce RNAi
22 knockdown and confirmed its efficacy for different tissues and for a variety of
23 endogenous genes. Together the metabolite profiles of different bacterial strains and

1 the OP50 RNAi strain provide useful resources for understanding microbe-host
2 interaction in regulating different physiological activities and for large-scale genetic
3 analysis using RNAi under different bacterial backgrounds.

4

5 **Results and Discussion**

6 **Revealing metabolomic diversity among different *E. coli* strains**

7 A microbial community carries diverse species of bacteria, and within each
8 species, there are different strains. Interestingly, *C. elegans* exposed to different strains
9 of *E. coli* exhibit different physiological phenotypes (Brooks et al., 2009; Han et al.,
10 2017; MacNeil, et al., 2013; Sowa et al., 2015), for example, the reproductive lifespan
11 and intestinal fat levels are increased when *C. elegans* are grown on the OP50 *E. coli*
12 strain compared to those animals grown on the HB101 or MG1655 *E. coli* strains
13 (Brooks et al., 2009; Lin and Wang, 2017; Sowa et al., 2015). Using stimulated Raman
14 scattering (SRS) microscopy (Ramachandran et al., 2015; Yong Yu et al., 2014), we
15 quantitatively examined fat content levels in *C. elegans* exposed to those different
16 bacterial strains. In line with previous studies (Brooks et al., 2009), we found that *C.*
17 *elegans* on OP50 show more fat storage than those on HB101 or MG1655 in the
18 intestine, the major fat storage tissue (Figure 1A). In parallel, we examined
19 mitochondrial fission-fusion dynamics, which have previously been linked to host's lipid
20 metabolism in response to bacterial inputs (Lin and Wang, 2017). Using a *C. elegans*
21 transgenic reporter strain expressing mitochondrial localized GFP (mito-GFP) under the
22 control of an intestine specific promoter, we imaged mitochondrial morphology and

1 scored the images as one of three distinct categories: filamented, intermediate and
2 fragmented (Figure 1B). Similar to the difference in fat storage, worms grown on OP50
3 show distinct mitochondrial morphology when compared to those grown on HB101 or
4 MG1655, as evidenced by increased mitochondrial fragmentation in the intestine
5 (Figure 1B).

6 To understand whether the difference in these physiological features of *C.*
7 *elegans* are associated with the difference in the metabolic features of *E. coli* strains,
8 we systemically analyzed the metabolomic profiles of OP50, HB101, and MG1655 *E.*
9 *coli* (Supplemental table 1). To our surprise, PCA analysis of all detected metabolites
10 shows that the metabolic profiles of HB101 and OP50 cluster together more readily than
11 OP50 or HB101 do with MG1655 (Figure 1C & 1D). Thus the phenotypic difference of
12 *C. elegans* on different *E. coli* strains is not simply a response to gross metabolic input
13 alterations in those *E. coli* strains, but might be associated with specific metabolites
14 derived from those *E. coli* strains.

15 We then searched for metabolites that show differences in both HB101 and
16 MG1655 when compared to OP50, which might contribute to the observed *C. elegans*
17 phenotypic differences. Our analysis identified a total of 42 carbohydrate-related
18 metabolites in our bacterial samples (Supplemental table 1), 12 of these metabolites
19 show an increase in both HB101 and MG1655 when compared to OP50 and one shows
20 a decrease (Figure 1E). An inverse relationship is observed in regards to lipid-related
21 metabolites when comparing HB101 and MG1655 to OP50, seven metabolites are
22 differentially observed and six out of the seven exhibit decreased levels in HB101 and
23 MG1655 (Figure 1E, Supplemental table 1). These results suggest that HB101 and

1 MG1655 may have a higher activity of glycolysis and less fermentation than OP50.
2 Among the groups of amino acids and nucleotides, there are also other specific
3 metabolites that show similar changes in HB101 and MG1655, although there is no
4 clear trend as a group.

5

6 **Bacterial betaine regulates lipid metabolism in the host**

7 Our previous studies have linked the bacterial one-carbon methyl cycle with lipid
8 metabolism in the host (Lin and Wang, 2017). Among the four metabolites directly
9 involved in the methyl cycle (Figure 2A), methionine is decreased by 50% in HB101
10 compared to OP50, but not in MG1655, neither dimethylglycine nor homocysteine is
11 significantly changed in HB101 and MG1655, but betaine is increased by 2-fold in
12 HB101 and by 3-fold in MG1655 compared to OP50 (Figure 2B).

13 Next, we examined whether changes in bacterial betaine levels are associated
14 with fat storage differences in the host *C. elegans*. Interestingly, the supplementation of
15 exogenous betaine is sufficient to reduce the high fat storage of *C. elegans* on OP50 to
16 a level comparable to those grown on HB101 or MG1655 (Figure 2C), while betaine
17 supplementation does not further decrease fat storage in worms on HB101 or MG1655.
18 Given the significant induction of carbohydrate-related metabolites in HB101 and
19 MG1655 compared to OP50 (Figure 1E), we also examined whether increased sugar
20 levels contribute to the low fat content levels in *C. elegans* grown on HB101 and
21 MG1655. We supplemented glucose to *C. elegans* grown on different *E. coli* strains,
22 and found that the glucose supplementation is not sufficient to suppress the fat storage

1 difference (Figure 2C). Together, these results suggest betaine as a key metabolite in
2 regulating lipid metabolism in the host *C. elegans*.

3 We also examined whether betaine regulates mitochondrial fission-fusion
4 dynamics, a trait that has been linked to host's lipid metabolism in response to bacterial
5 inputs (Lin and Wang, 2017). We supplemented betaine to worms grown on different
6 bacterial strains and examined the effect of this supplementation on mitochondrial
7 dynamics. We found that unlike the fat phenotype, betaine supplementation is not
8 sufficient to suppress the mitochondrial fragmentation phenotype observed in worms
9 grown on OP50 (Figure 2D). In addition, the supplementation of glucose to worms
10 grown on MG1655, HB101 or OP50 also fails to significantly alter mitochondrial
11 dynamics trends when compared the trends observed in non-supplemented animals
12 (Figure 1B). Therefore, betaine can regulate lipid metabolism in the host via a
13 mitochondrial dynamics independent mechanism.

14 Together the high-throughput metabolite profiles demonstrate that *E. coli* B and
15 K-12 strains exhibit drastic difference in their metabolism, however these global
16 metabolomics patterns of bacteria are not directly associated with their distinct impacts
17 on the physiology of the host. Instead, specific bacterial metabolites contribute to those
18 differences. In the example put forward, bacterial betaine specifically regulates fat
19 content levels in the host *C. elegans*, but has no effect on mitochondrial dynamics.
20 Thus, the association between bacterial metabolism and *C. elegans* physiology is
21 complex and multifaceted, and specific metabolite signals derived from different
22 bacterial strains can be key confounders interfering with the genetic analyses of *C.*
23 *elegans* phenotypes. In particular, the *E. coli* strain HT115, which is a K-12 strain, has

1 been utilized for over a decade to perform RNAi based screens, verify mutant
2 phenotypes, and generate hypomorphic conditions for otherwise lethal loss of function
3 mutations (Kamath et al., 2001).

4

5 **Development of an RNA interference strain using OP50**

6 Almost two decades ago, two large RNAi libraries were generated by Ahringer
7 and Vidal laboratories (Kamath et al., 2003; Rual et al., 2004). Both of these libraries
8 house their RNAi vectors in the HT115 (d3) strain of *E. coli*, which contains a deletion
9 RNase III (*rnc*) allele and an IPTG-inducible T7 RNA polymerase, changes which render
10 the individual bacterium capable of producing and maintaining double stranded RNA
11 from the L4440 double-T7 vector (derived from pPD129.36 (Timmons and Fire, 1998)).
12 Similar to MG1655 in being a K-12 derived strain, HT115 causes reduced fat storage in
13 *C. elegans* when compared to OP50 (Figure 3A), and the reduction level is similar to
14 that caused by either HB101 or MG1655 (Figure 1A). We also examined intestinal
15 mitochondrial morphology and found that worms on HT115 show a more filamented
16 mitochondrial network than that seen in worms on OP50 (Figure 3B). In addition, other
17 physiological characteristics are also different between worms grown on OP50 and on
18 HT115, including reproductive lifespan (Sowa et al., 2015), developmental timing
19 (MacNeil et al., 2013), and lifespan (Pang et al., 2014). Therefore, when comparing
20 phenotypes of *C. elegans* caused by HT115 induced RNAi knockdown, to those
21 exhibited in genetic mutants grown on OP50, the bacterial strain backgrounds introduce
22 an additional confounder that might mislead the interpretation.

1 To override this problem, we have developed an RNAi competent OP50 bacterial
2 strain using phage transduction of two loci required for double stranded RNA production
3 and retention (Figure 3C). The RNAIII RNase (*rnc*) allele from HT115 (*rnc:14::ΔTn10*)
4 was transduced into the CGC supplied “wild type” OP50 bacteria. This allele provides
5 tetracycline resistance, a trait used for selection of bacterial colonies. Allele introduction
6 was verified using PCR of the appropriate loci (Supplemental figure 1). Following
7 introduction of tetracycline resistance, the *laczyA::T7pol camFRT* allele was introduced
8 by phage transduction to facilitate production of double stranded RNA from the L4440
9 double T7 vector found in both the Ahringer and Vidal RNAi libraries. This allele is
10 selected for using Chloramphenicol, and its presence was further confirmed using PCR
11 (Supplemental figure1). Following these two phage transduction events, continued
12 rounds of selection on Tetracycline and Chloramphenicol were performed to purify the
13 background of the RNA interference competent OP50 strain (*rnc:14::ΔTn10*;
14 *laczyA::T7pol camFRT*) herein termed as RNAi competent OP50 or iOP50.

15 iOP50 *E. coli* carrying the L4440 plasmid show a distinct growth pattern when
16 compared to HT115. We grew iOP50 *E. coli* at 37°C overnight in LB with Carbenicillin
17 (50ug/ml) and measured OD hourly (Figure 3D). We found that a 10 to 14 hour growth
18 period is required for HT115 to enter a stationary phase, while iOP50 requires
19 approximately 18 hours to reach a stationary phase, a growth rate similar to that of non-
20 transformed OP50. Thus, an increased incubation time is necessary for iOP50 strain to
21 provide sufficient, robust and repeatable RNAi knockdown.

22

1 **Efficacy of OP50 RNAi strains in gene inactivation**

2 To confirm the knockdown efficacy of iOP50 in different tissues, we first
3 transformed iOP50 with the GFP RNAi plasmid, and supplied those GFP RNAi strains
4 to transgenic *C. elegans* strains expressing GFP in the intestine, muscle, and
5 hypodermis. We found iOP50 induced knockdown of GFP in a qualitatively comparable
6 level to that observed when inducing knockdown using HT115 (Figure 4A). Thus,
7 iOP50-mediated RNAi is sufficient to knockdown genes in different tissues. For these
8 experiments, various growth times have been assessed for iOP50, overnight liquid
9 culture periods ranging from 18 to 22 hours followed by overnight growth on standard
10 NGM plates with 1mM IPTG gives the most robust knockdown (data not shown).

11 Next, we examined the knockdown efficacy of iOP50 for numerous endogenous
12 genes, where RNAi can induce diverse phenotypes. When comparing the phenotypes
13 of *C. elegans* caused by RNAi knockdown using either iOP50 or HT115, we observed
14 two major categories of results, those where knockdown efficacy is comparable
15 between iOP50 and HT115 (Figure 4B), and those where knockdown using iOP50 gives
16 alternative phenotypes (Figure 4C). The first class includes *K04C2.2*, *prp-8*, *nud-1*, *dpy-*
17 *13*, *act-5* and *par-1*. Among them, RNAi knockdown of *prp-8* using HT115 causes
18 developmental arrest by the second larval stage (L2), similarly in iOP50, the majority of
19 animals arrest by L2, although there are few escapers that arrest at the third larval
20 stage (L3); and RNAi knockdown of *dpy-13* leads to decreased body size, but worms
21 are slightly larger when grown on iOP50 than on HT115. The second class includes *let-*
22 *711*, *gpb-1*, *npp-9*, *cars-1* and *qars-1*. RNAi knockdown of *let-711* using HT115 causes
23 over 90% of the worms to arrest at L3, however when using iOP50, over 90% of worms

1 manage to reach adulthood, but become sterile. For RNAi knockdown of *gpb-1*, the
2 HT115 background generates over 60% dead adults due to explosion through the vulva,
3 but the iOP50 background generates over 60% sterile adults with increased body
4 length. RNAi knockdown of *npp-9* using HT115 gives sterile sick adults, but its
5 knockdown using iOP50 gives grossly healthy adults whose progeny is embryonic
6 lethal. For *cars-1* or *qars-1*, its RNAi inactivation causes developmental arrest when
7 using HT115, but completely penetrant adulthood sickliness and partial adulthood
8 sterility when using iOP50. These phenotypic differences between HT115 and iOP50
9 RNAi might be related to the strength of gene knockdown, which may be weaker with
10 iOP50, and might also be related to the specific role of certain *C. elegans* genes in
11 response to different bacterial strains. Together, these studies demonstrate the efficacy
12 of iOP50 in executing RNAi knockdown in different tissues and for various genes, and
13 also highlight the importance of examining the effect of *C. elegans* genes under different
14 bacterial strain backgrounds.

15

16 **Conclusion**

17 In summary, *C. elegans* exhibit drastically different phenotypes when exposed to
18 different bacterial strains, which are unrelated to genetic alterations in *C. elegans*.
19 These differences could introduce confounders that complicate analysis of genetic
20 regulation, but also provide researchers the opportunity to investigate environment-
21 microbe-host interactions. Our metabolomics studies systematically reveal metabolite
22 difference among different *E. coli* strains, and found that *C. elegans* phenotypic
23 changes are directly associated with specific bacterial metabolites. In particular, we

1 have linked betaine, a metabolite derived from the methyl cycle, with lipid metabolism in
2 worms, which however does not contribute to mitochondrial dynamics or reproductive
3 span (Supplemental figure 2). This one-to-one relationship between a microbial
4 metabolite and a host's phenotype highlights the importance of investigating the
5 mechanistic impact of microbial metabolism on host's physiology beyond profiling
6 microbial phylogenetic composition. Moreover, to expand the analysis toolkit of genetic
7 regulation and microbe-host interaction, we have rendered the common *E. coli* strain
8 OP50 competent for the induction of the RNAi response in *C. elegans*. This bacterial
9 strain contains the same two alleles that render HT115 capable of inducing the RNAi
10 response. These changes allow iOP50 to induce RNAi in multiple tissues to a level
11 similar to that found in HT115 fed animals. A similar OP50 RNAi strain has also been
12 generated and used for lifespan screens (Xiao et al., 2015). iOP50 has recently been
13 used in our lab as the basis for a small scale RNAi screen (96 wells) and has proven
14 amenable to en-mass transformation. Phenotypes observed and described in the iOP50
15 screen hold true for the reciprocal HT115 screen (data not shown). The strain is
16 available by request, and has been deposited with the CGC for academic use. If your
17 mutant *C. elegans* strain fails to demonstrate the same phenotype as your RNAi fed
18 animals or vice-versa, we implore you to pause and question whether the bacterial
19 strain is responsible for the discrepancy and apply iOP50 into your phenotypic
20 validation.

21

22 **Figure Legends**

1 **Figure 1: Metabolomic profiles of three *E. coli* strains and their distinct effects on *C.***
2 ***elegans* fat storage and mitochondrial dynamics.**

3 **A)** Fat content levels of day-2-old *C. elegans* adults are quantified using SRS microscopy
4 imaging. When compared to those grown on OP50, worms grown on HB101 and MG1655 show
5 decreased fat storage in the intestine. Example SRS images are presented from three
6 independent trials and intestinal areas for quantification are outlined. **B)** Mitochondrial
7 morphology is examined in day-3-old *C. elegans* adults carrying a mitochondria-localized-GFP
8 reporter in the intestine, and classified into three categories, filamented, fragmented or
9 intermediate for quantification. When compared to those grown on OP50, worms grown on
10 HB101 and MG1655 show decreased mitochondrial fragmentation in the intestine. Phenotypes
11 were noted in a double-blind fashion from three independent trials. **C)** PCA analysis of *E. coli*
12 metabolomics profiles shows clustering between OP50 and HB101 that is separated from
13 MG1655. **D)** Clustering of different *E. coli* samples based upon metabolite levels shows that
14 HB101 and OP50 have a similar pattern distinct to MG1655. **E)** Heat maps show relative fold
15 changes in a log scale of significantly altered metabolites in MG1655 and HB101 when
16 compared to OP50. Five independent metabolomics profiles for each strain are presented.
17 Statistical significance indicated by asterisk, * $P = <0.05$, ** $P = <0.005$. Error bars represent
18 SEM. Statistical analysis performed using Welch's two-sample two-sided t-test (E), students t-
19 test (A), or Chi-Squared test (B).

20

21 **Figure 2: Bacterial metabolite Betaine regulates fat storage in the host.**

22 **A)** Simplified one-carbon methyl cycle in *E. coli*, highlighting the methyl donation interactions
23 between four key metabolites, methionine, homocysteine, dimethylglycine, and betaine. Arrows
24 indicate alterations in MG1655 (green) and HB101 (purple) when compared to OP50. **B)**

1 Quantitative comparison of one-carbon methyl cycle metabolite levels show that betaine levels
2 are increased over 3-fold and 2-fold in MG1655 and HB101, relative to OP50, respectively, and
3 the methionine level in HB101 is decreased compared to that in OP50. **C)** Supplementation of
4 betaine but not glucose suppresses the fat storage increase in *C. elegans* grown on OP50.
5 Graphical representation of relative whole intestine fat content levels as measured by SRS
6 microscopy from three independent trials. **D)** Neither betaine nor glucose supplementation
7 affects morphological difference of mitochondria in *C. elegans* grown on HB101, MG1655 and
8 OP50. Graphical representation of mitochondrial dynamics in the intestinal cells from double-
9 blind analyses of three independent trials.

10 Statistical significance indicated by asterisk, * $P = <0.05$, ** $P = <0.005$. Error bars represent
11 SEM. Statistical analysis performed using students T-test (B & C), or Chi-Squared test (D).

12

13 **Figure 3: Development of an RNA interference competent OP50 strain.**

14 **A)** Quantification based on SRS microscopic imaging shows that worms grown on HT115 have
15 decreased fat storage compared to those on OP50. Example SRS images are presented with
16 intestinal areas outlined for quantification. Graphical quantification is from three independent
17 trials. **B)** Mitochondrial fragmentation, visualized by the intestinal mito-GFP reporter, is reduced
18 in worms grown on HT115 compared to those on OP50. Representative data from two
19 independent trials. **C)** Schematic for the generation of an RNA interference capable OP50
20 bacterial cell line. **D)** The growth rate of OP50 is lower than that of HT115. Bacterial growth
21 curves for vector carrying OP50 or HT115 show OD_{600} measured every two hours for 20 hours,
22 representative of three independent trials.

23 Statistical significance indicated by asterisk, * $P = <0.05$, ** $P = <0.005$. Error bars represent
24 SEM. Statistical analysis performed using students T-test (A), or Chi-Squared test (B).

1 **Figure 4: OP50 induced RNA interference is efficient to induce gene knockdown in**
2 **different tissues.**

3 **A)** Example images of GFP knockdown using RNAi in either HT115 or iOP50 background show
4 efficacy in different tissues including the intestine, hypodermis, or muscle. **B)** Gene knockdown
5 using RNAi in either HT115 or iOP50 background gives almost indistinguishable phenotypes. **C)**
6 A number of RNAi lines cause variable phenotype when using HT115 versus iOP50, with less
7 severity using iOP50 than using HT115.

8 **Supplemental Table 1** – Metabolite levels for five samples of each strain of OP50, HB101, and
9 MG1655. Levels are normalized to total pellet weight and Bradford protein.

10 **Supplemental Figure 1** – 2% agarose gel depicting three colonies of iOP50 with the *rnc*
11 deletion allele amplified, and the same three colonies of iOP50 with the T7 RNA polymerase
12 allele identified.

13 **Supplemental Figure 2** – Reproductive lifespan of animals grown on OP50 or HB101 and
14 supplemented with Betaine or vehicle controls.

15

16 **Materials & Methods**

17 ***C. elegans* strains used**

18 The *C. elegans* strains used in this study were either provided by the Caenorhabditis
19 Genetics Center (CGC), which is funded by NIH Office of Research Infrastructure
20 Programs (P40 OD010440), or made in house by gonadal microinjection of DNA
21 mixtures at the young adult stage. Integration of extrachromosomal arrays were induced
22 by gamma irradiation exposures (4,500 rad for 5.9min) at the L4 stage, and the
23 integrated progeny were backcrossed to N2 at least five times. Worm strains: N2

1 (Bristol), *raxIs46*[*Pges-1::sl2::gfp*], *raxIs49*[*Pcol-12::mitogfp*], *raxIs51*[*Pges-1::mitoGFP*],
2 *raxIs52*[*Pmyo-3::mitogfp*].

3

4 **Bacterial strains used**

5 The following bacterial strains were used in this study. *E. coli* wild type (MG1655) was a
6 gift from J.J. Wang, and CH1681 was a gift from C. Herman. The *E. coli* strains OP50,
7 and HB101 were obtained from the CGC. The *E. coli* strain HT115(d3) was obtained
8 from the Ahringer (Kamath et al., 2003) RNAi library.

9

10 **Generation of RNA interference (RNAi) competent OP50 bacteria**

11 Standard OP50 (CGC) was rendered RNAi competent through two modifications
12 facilitated by phage transduction. The OP50 RNAIII RNase (*rnc*) allele was replaced
13 with the deletion allele (*rnc14::ΔTn10*) found in HT115(D3). Subsequently a genomically
14 encoded IPTG-inducible T7 RNA polymerase (*lacγA::T7pol camFRT*) was introduced
15 into OP50(*rnc14::ΔTn10*) from CH1681. In brief, OP50 overnight cultures were
16 transduced with HT115(*rnc14::ΔTn10*) lysate, insertion events were selected for using
17 tetracycline (Tet) resistance. Individual Tet resistant colonies were grown in LB + Tet
18 (50ug/ml) and banked, PCR was then used to confirm the insertion of the *rnc14::ΔTn10*
19 allele by PCR. OP50(*rnc14::ΔTn10*) overnight culture was then transduced with CH1681
20 (*lacγA::T7pol camFRT*) P1 lysate. Insertion events were selected for using
21 Chloramphenicol (CAM, 17ug/ml). Three rounds of population expansion and
22 bottlenecking using Tet + CAM (50ug/ml, and 17ug/ml) selection was used to purify the
23 RNAi inducible OP50 (iOP50) background. Individual colonies were banked, and PCR

1 was used to confirm the presence of *rnc14::ΔTn10* and *lacZγA::T7pol camFRT*
2 (Supplemental figure 1). iOP50 was then transformed with L4440 plasmids targeting
3 GFP, and RNAi efficacy was determined in *C. elegans* strain *raxIs46[Pges-1::sl2::gfp]*.

5 **Producing chemically competent iOP50 bacteria**

6 All steps are performed using sterile technique. Bacterial iOP50 stocks from -80°C were
7 streaked onto Tetracycline (Tet at 50ug/ml) and Chloramphenicol (CAM at 17ug/ml)
8 containing LB and grown overnight at 37°C. Two tubes of 2ml LB with Tet and CAM
9 (50ug/ml and 17ug/ml) were inoculated with single colonies of iOP50 and grown
10 overnight in a 37°C shaker. The next day, 1ml of overnight culture is used to inoculate
11 100ml of LB without antibiotics. The culture is shaken at 37°C for 1.5 to 3 hours, until
12 the OD600 is between 0.2 and 0.5. During the shaking, sterile microcentrifuge tubes
13 (>50), 50ml centrifuge tubes, 100mM CaCl₂, and 100mM CaCl₂/15% Glycerol are
14 cooled to 4°C. The bacteria are then transferred to two 50ml cold centrifuge tubes. Chill
15 the cells on ice for 10 minutes, all future steps are conducted in the 4°C cold room, in a
16 pre-chilled 4°C centrifuge or with the bacteria kept on ice. Harvest the cells by
17 centrifugation at 4000rpm for 3 minutes, then remove and discard the supernatant. Cells
18 are then gently re-suspended in 5ml of cold 100mM CaCl₂. Incubate the cells on ice for
19 20 minutes and then harvest the cells by centrifugation at 4000rpm for 3 minutes.
20 Return the cells to ice and discard the supernatant. Gently re-suspend the cells in 2ml
21 of cold 100mM CaCl₂/15% Glycerol and then dispense into microcentrifuge tubes in
22 50μl aliquots. Store the cells at -80°C. Thaw the cells on ice and transform with custom

1 plasmids, or those obtained from the Ahringer or Vidal library (Kamath et al., 2003; Rual
2 et al., 2004).

3

4 **Transformation of iOP50**

5 Competent cells were transformed using standard methodologies described in the New
6 England Biotechnology protocol (C2987H/C2987I).

7

8 **Metabolomics analysis**

9 *E. coli* metabolite profiling was performed at Metabolon, Inc (Durham, NC) with 5
10 biological replicates of each test bacterial strain (MG1655, OP50, HB101). *E. coli*
11 colonies were inoculated in LB, grown overnight, and then plated on Nematode Growth
12 Medium (NGM) Agar plates to form lawns for three days at 20°C. Lawns were then
13 scraped off of the surface of the agar and collected in distilled deionized water, samples
14 were then washed 3 times. Each sample contained approximately 0.1mg of pooled
15 bacterial pellet which was then flash frozen using liquid nitrogen. Metabolomics profiling
16 was performed in the method described previously (Evans et al., 2009). Statistical
17 analysis was performed using Welch's two-sample t-test to identify compounds that
18 differed significantly between bacterial strains.

19

20 **Measurement of lipid content levels in *C. elegans* intestine by SRS microscopy**

21 *C. elegans* () were raised from L1 on the specified food source/supplement (Betaine at
22 200mM or Glucose at 0.4% final volume compared to agarose). Day-2-old adults (~10
23 for each of three biological replicates) were anesthetized in 1% sodium azide in M9

1 buffer (3g KH₂PO₄, 6g Na₂HPO₄, 5g NaCl, 1ml 1M MgSO₄, H₂O to 1 liter, sterilized by
2 autoclaving), and mounted on 2% agarose pads sandwiched between glass
3 microscopic slides and coverslips. Images were taken using the SRS system as
4 described in previous studies (Ramachandran et al., 2015; Wang et al., 2011). In brief,
5 when using Stokes beam at 1064nm and pump beam at 817nm, the energy difference
6 between the Stokes and pump photons resonates with the vibrational frequency of CH₂
7 bonds (2845cm⁻¹). Due to the fact that the CH₂ chemical bonds are highly enriched in
8 lipid carbon chains, we quantitatively image total fat content levels by detecting the
9 emitted Raman signals of CH₂ bonds.

10

11 **Mitochondrial morphology observation and dynamics measurement**

12 The intestinal mitochondrial morphology was examined using day-3-old adult transgenic
13 *C. elegans* strain *raxIs51[Pges-1::mitoGFP]* raised from L1 on the specified food
14 source, with the specified supplement (Betaine at 200mM or Glucose at 0.4% final
15 volume compared to agarose). Images are presented as single-layer con-focal images
16 in anterior regions for consistency. For sample preparation, worms were anesthetized in
17 1% sodium azide (NaAz) in M9 buffer, and mounted on 2% agarose pads sandwiched
18 between glass microscopic slides and coverslips. Confocal images were taken using an
19 IX81 microscope (Olympus) connected to an AxioCam ICc3 camera (Zeiss). For
20 morphological categorization, if the lengths of majority of mitochondrial filaments in a
21 cell were longer than ~4μm, it would be considered “filamented”; if the lengths of
22 majority of mitochondrial filaments in a cell were shorter than ~2μm, it would be
23 considered “fragmented”; the rest belonged to the category “intermediate”. Categorical

1 rankings were determined by three blinded scorers, rankings were only determined for
2 cells on which at least 2 out of 3 of the scorers agreed. An individual cell was utilized as
3 the unit, at least three trials were performed and the accumulated counts from each
4 genotype and environmental condition were summed up across biological replicates,
5 percentages were calculated and reported as bar representations. The chi-squared test
6 for trend was conducted for statistical analysis.

7 8 **Inducing RNAi using HT115 or iPO50**

9 HT115 or iOP50 bacterial cultures were grown in LB with Carbenicillin (2.64 μ M) for 14h,
10 and 18h respectively. 150 μ l of bacterial culture was then plated on RNAi plates
11 (standard nematode growth medium (NGM) agarose plates with IPTG (1mM) &
12 Carbenicillin (2.64 μ M)) and allowed to dry. Production of dsRNA was induced overnight
13 (18h) at room temperature. *C. elegans* were then added to the plates. All noted
14 phenotypes or images were taken in P0 animals grown 62-72h at 20°C.

15 16 **Florescent imaging for determining RNAi efficacy**

17 Well-fed N2 animals were raised for two generations on standard NGM plates seeded
18 with OP50. Animals were synchronized using a variation of the “egg prep” methodology
19 described in (Porta-de-la-Riva et al., 2012). ~100 animals were grown from the L1 stage
20 on RNAi plates with bacteria containing L4440 based plasmids targeting GFP. At 72h
21 post plating, ~10 day 1 adult animals were picked into a pool of 8 μ l 2% sodium azide in
22 M9 buffer located in the center of a freshly made empty NGM plate. Animals were
23 paralyzed, and then arranged by gentle prodding and by increasing the surface area of

1 the NaAz pool, while allowing it to evaporate. Plates were then immediately imaged
2 using a Hamamatsu digital camera (C11440), utilizing the x-cite xylis light source, and a
3 Nikon SM218 microscope.

4

5 **Determining RNAi induced phenotypic alterations**

6 Well-fed N2 animals were raised for two generations on standard NGM plates seeded
7 with OP50. Animals were synchronized using a variation of the “egg prep” methodology
8 described in (Porta-de-la-Riva et al., 2012). 6cm plates of bacteria containing L4440
9 based plasmids targeting the loci of interest from the Ahringer or Vidal library were
10 prepared. ~100 animals were grown from the L1 stage on each of the 6cm RNAi plates.
11 Five independent trials were conducted and results were collated. Animals were grown
12 for ~72h at 20°C, phenotypes were then noted for all animals on the plates and
13 penetrance was determined.

14

15 **Methodology for performing Reproductive Lifespans**

16 Reproductive lifespans (RLS) were performed at 20°C on animals that were
17 developmentally synchronized using a variation of the “egg prep” methodology
18 described in (Porta-de-la-Riva et al., 2012). Well-fed animals are raised for two
19 generations on standard Nematode Growth Medium (NGM) plates seeded with OP50.
20 The third generation of animals are plated on antibiotic free NGM plates seeded with the
21 test bacteria, and if necessary, an inducible agent (IPTG) or supplement (Betaine at
22 20mM during development, and 100mM after L4, based upon plate volume). Upon
23 development to the L4 stage, worms are singled to individual plates containing their

1 specified food source, the animals are then transferred to fresh plates every day at the
2 same time until reproduction cessation, determined when no progeny are produced for
3 three continuous days. Two days after the individual worms are transferred, plates are
4 checked and then double checked for progeny. Animals which bag or die are noted as
5 censors. Reproductive span curves were calculated using Kaplan-Meier survival
6 analysis and compared using the log-rank test. At least two independent trials were
7 conducted.

8

9 **Author Contributions**

10 I.A.A.N, J.N.S., C.J.L. and M.C.W. conceived the study, I.A.A.N., J.N.S., and C.J.L.
11 conducted experiments and analysis. Y.Y. and L.H. conducted clustering analysis.
12 J.N.S, P.S. and C.H. created the iOP50 line. I.A.A.N. and M.C.W. wrote the manuscript.

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1

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Figure 1

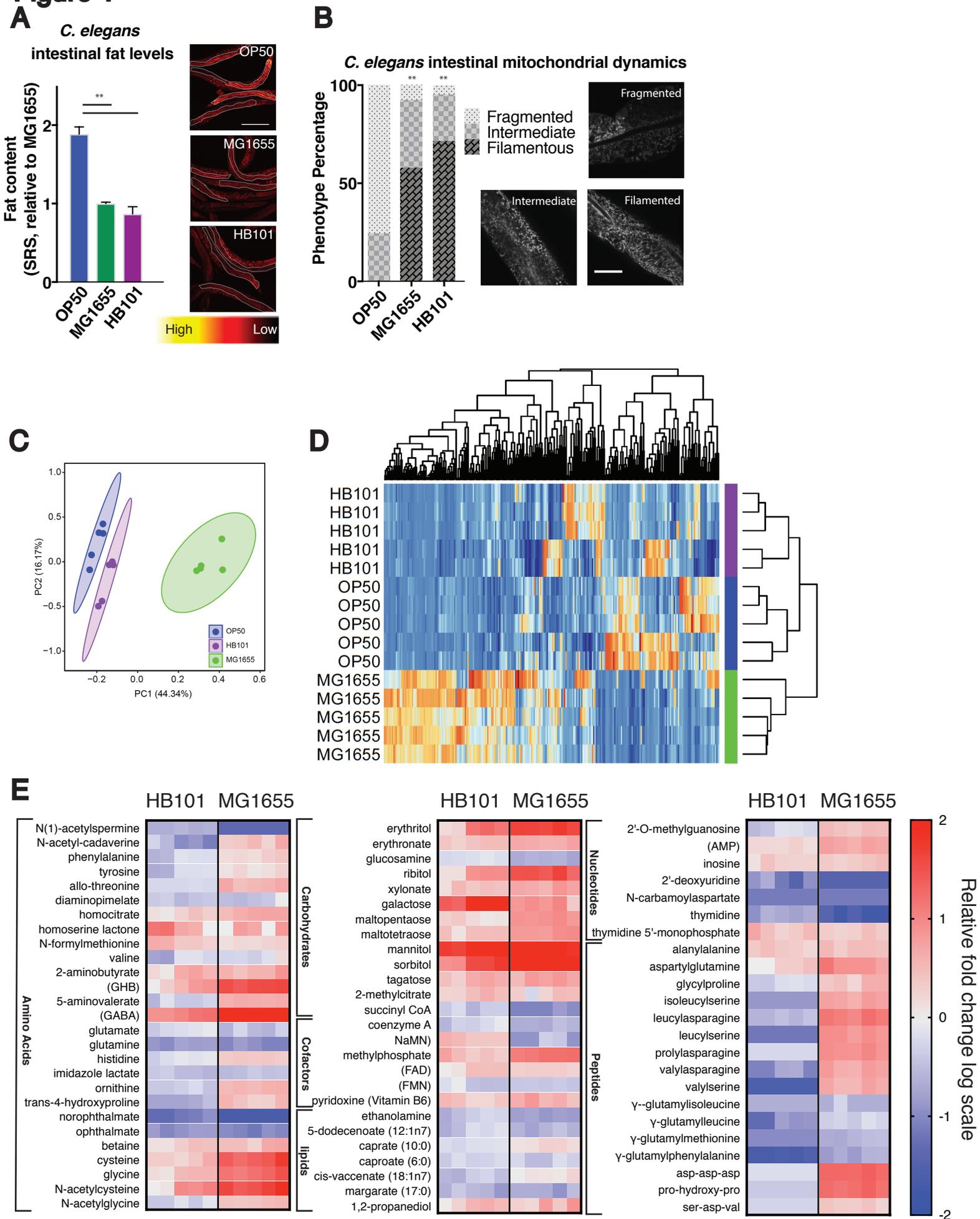
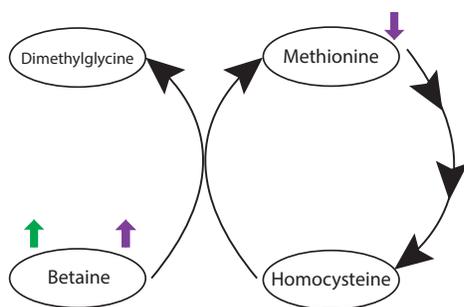
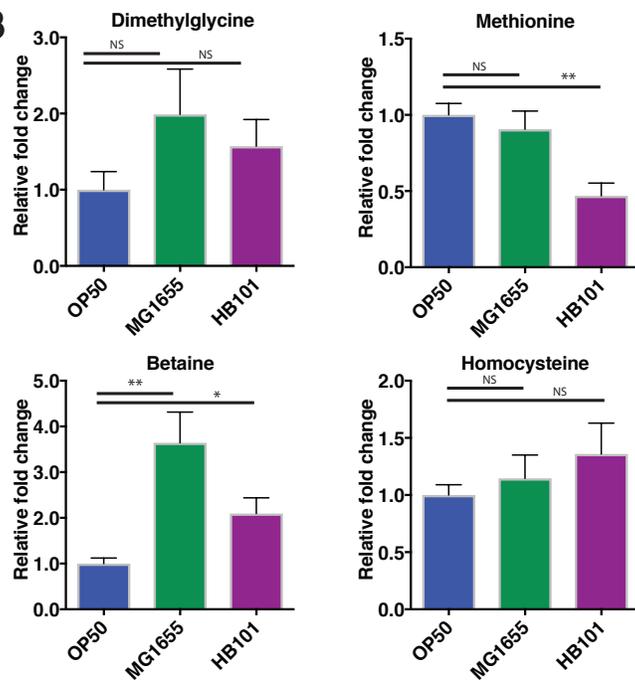


Figure 2

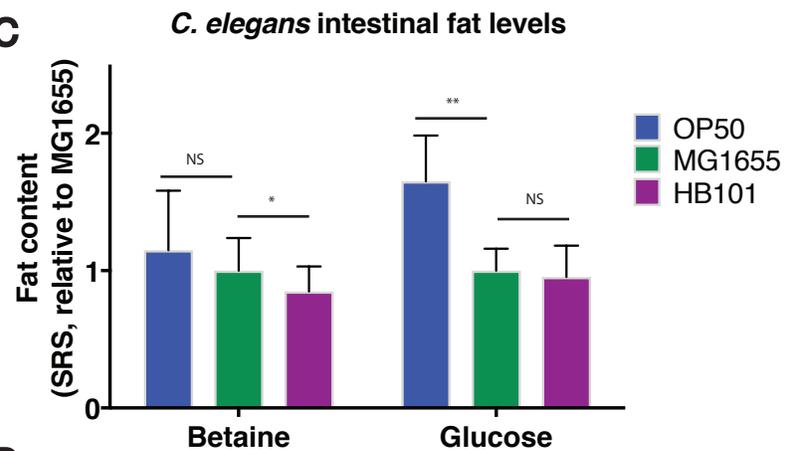
A



B



C



D

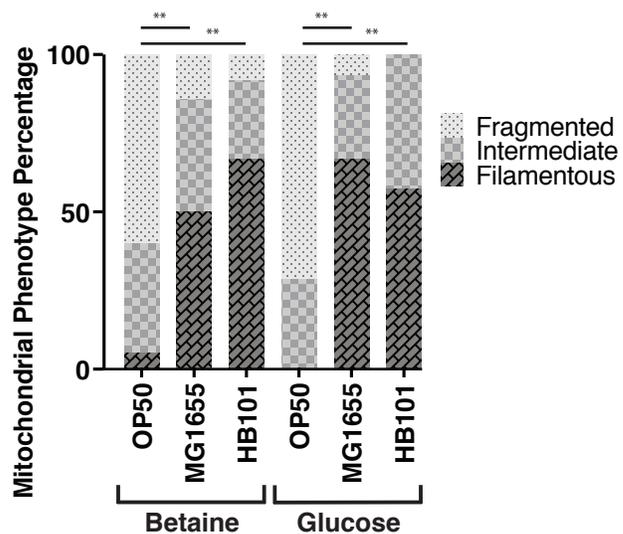
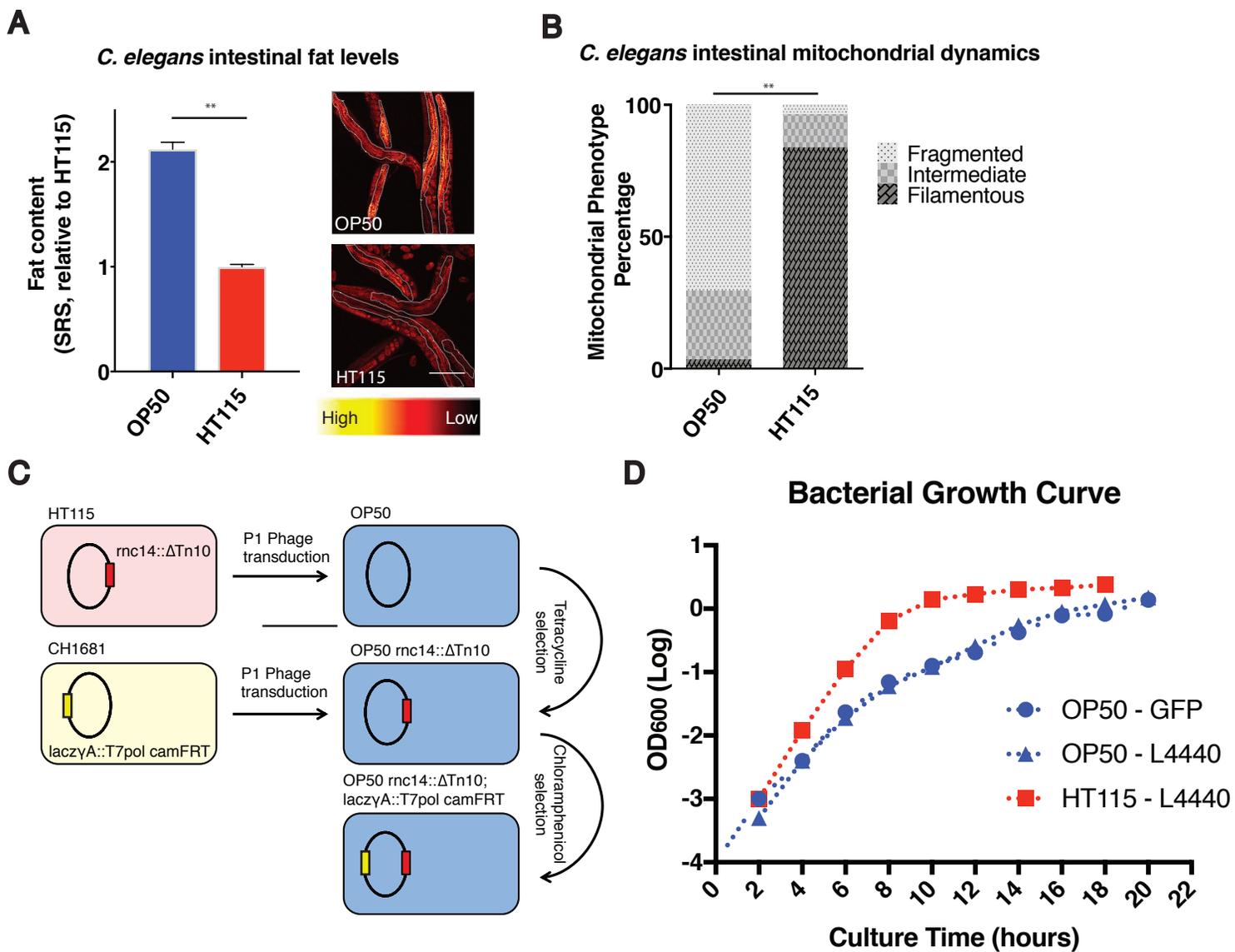
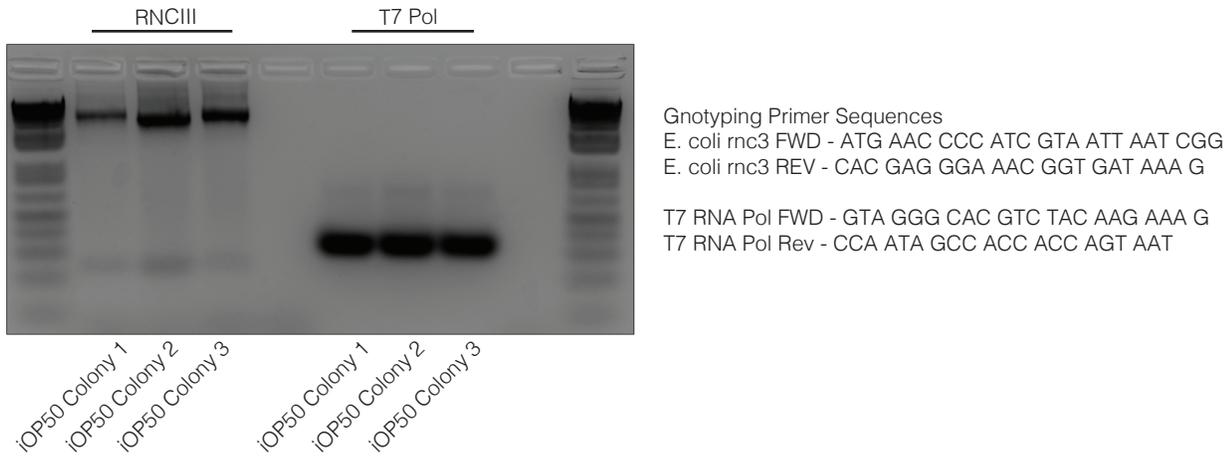


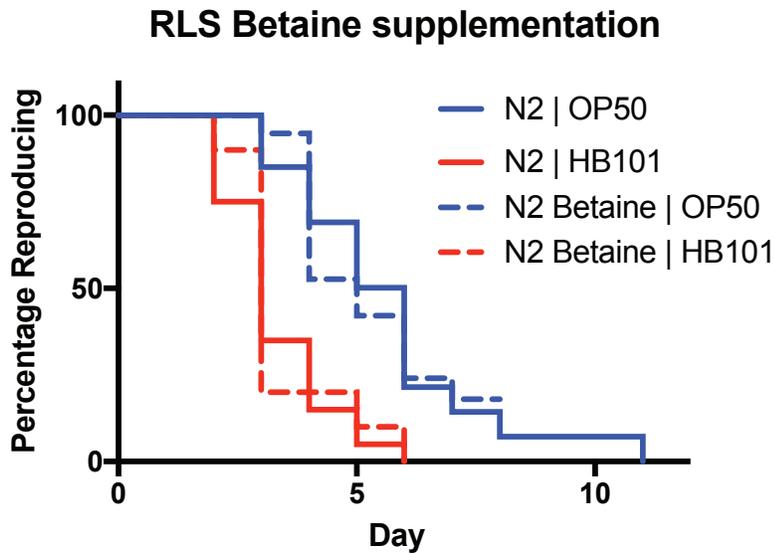
Figure 3

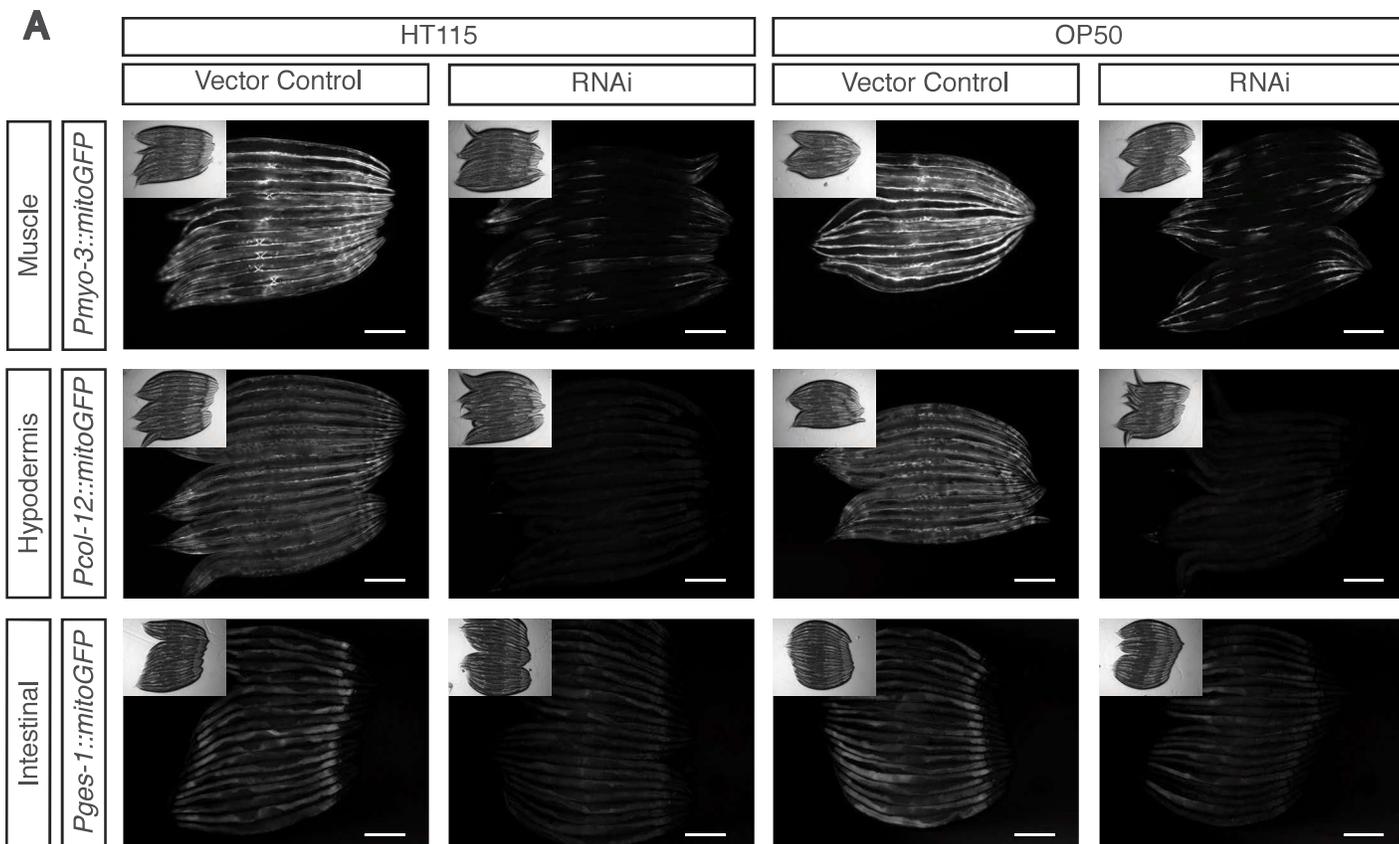


Supplemental Figure 1



Supplemental Figure 2





B

Gene Name	HT115	OP50	Phenotypes
<i>K04C2.2</i>	100%	100%	Sterile, ~2% explode through vulva
<i>prp-8</i>	100%	100%	Developmental arrest at L2/L3, more L3 animals on OP50 than HT115
<i>nud-1</i>	88%	88%	Sterile adult
	12%	12%	Viable, bagging, reduced progeny
<i>dpy-13</i>	100%	99%	Dumpy adults, slightly larger OP50 animals than HT115 animals
<i>act-5</i>	100%	100%	Developmental arrest at L2
<i>par-1</i>	100%	100%	Brood size ~20% that of controls

C

Gene Name	HT115	OP50	Phenotypes
<i>let-711</i>	> 90%		Developmental arrest by L3
		< 10%	Exploded through vulva
	< 10%	> 90%	Sterile adult
<i>gpb-1</i>	> 60%	< 20%	Exploded through vulva
	< 20%	> 60%	Long, sterile adult
	< 20%	< 20%	Viable, protruding vulva
<i>npp-9</i>	All	< 10%	Sterile, sick and thin, protruding vulva.
		> 90%	Grossly healthy adult animals, >98% of eggs laid fail to hatch.
<i>cars-1</i>	> 90%	< 10%	Variable developmental delay and arrest (L2-L4)
	< 10%	> 90%	Thin and sickly adults, reduced progeny number ~10% of controls
<i>qars-1</i>	> 90%	< 10%	Variable developmental delay and arrest (L2-L4)
	< 10%	> 90%	Thin and sickly adults, reduced progeny number ~10% of controls