Novel diets as nutraceuticals to alter lifespan and healthspan trajectories in *C. elegans*

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ABSTRACT

Diet is one of the more variable aspects in life due to the variety of options that organisms are exposed to in their natural habitats. In the laboratory, *C. elegans* are raised on bacterial monocultures, traditionally the *E.coli B* strain OP50, and spontaneously occurring microbial contaminants are removed to limit experimental variability because diet - including the presence of contaminants, can exert a potent influence over animal physiology. In order to diversify the menu available to culture *C. elegans* in the lab, we have isolated and cultured three such microbes: *Methylobacterium, Xanthomonas*, and *Sphingomonas.* The nutritional composition of these diets is unique, and when fed to *C. elegans,* can differentially alter multiple life history traits including development, reproduction, and metabolism. In light of the influence each diet has on specific physiological attributes, we comprehensively assessed the impact of these diets on animal health and devised a blueprint for utilizing different diet combinations over the lifespan, as a nutraceutical for optimal longevity. The expansion of the bacterial diet options to use in the laboratory will provide a critical tool to better understand the complexities of gene-diet interactions for health.

INTRODUCTION

Since the discovery that aging can be improved by dietary, genetic, and pharmacological interventions, there has been an increase in studies aiming to elucidate how each of these interventions can promote long life and healthy aging. Many dietary interventions including calorie restriction, intermittent fasting, and dietary restriction [1] have been shown to not only increase maximal lifespan, but average lifespan of the cohort and healthspan as well. New diets and fads have become popularized within society [2, 3], however, these dietary fads focus on removing one aspect of diet (carbohydrates, sugars, fats, proteins, etc.) instead of focusing on the overarching complexity of food.

Food is imperative for all organisms in order to provide nourishment to fuel growth and essential cellular functions. Diet is one of the more variable aspects in life due to the vast options that organisms are regularly exposed to in their natural habitats. Survival within these environments require organisms to select high quality food sources from a range of nutritiously diverse alternatives. For centuries, society has accepted that diet is important for health and longevity; “You are what you eat.” However, our understanding of why diet holds such profound influence over our health and how we can use this knowledge to improve overall health and longevity requires further study.

Many diet studies have employed the use of the model organism *Caenorhabditis elegans* [4-6] due in part to shared metabolic pathways with mammals including AMPK, TOR, and insulin/IGF-1 (IIS) signaling [6]. These bacterivore nematodes can be used for the identification of dietary effects due to their invariant and short developmental and reproductive periods, which are followed by an averaged 3-week lifespan[7].

The impact of diet on physiology is vast and certainly integrates into multiple life history traits including development [8], reproduction[9], healthspan [10], and longevity [4, 11-13]. Additional studies have identified diet-induced phenotypic effects that are accompanied by measurable differences in metabolic profiles [11, 14, 15], fat content [5, 6, 8, 16], and feeding behavior [8, 13, 17, 18]. Under normal laboratory conditions, *C. elegans* are cultured using the standardized bacterial species *Escherichia coli* OP50. This diet was not chosen because of its association with nematodes in the wild, but because of availability in the laboratory setting [16]. Maintenance of *C. elegans* in the laboratory includes the routine removal of spontaneously occurring bacterial contaminants in order to limit experimental variations. Surprisingly, very few options to study the impact of different diets on *C. elegans* healthspan and lifespan are defined in the laboratory setting [16, 19].

C. elegans interact with a variety of bacterial species in their natural environment, including Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria [20] These bacterial species have shaped many aspects of *C. elegans* biology that have become undetectable in the artificial laboratory environment [16, 19-22]. Here we expand the menu of bacterial diets for the study of gene-diet interactions which allows for further insight into the contribution diets have on *C. elegans* physiology [4, 23]. Our studies provide a blueprint for how to use diet as a nutraceutical for manipulation of lifespan trajectories.

RESULTS

Identification and characterization of three distinct bacterial diets

In their natural environment, *C. elegans* need to adapt to a variety of microbes they might encounter as a source of food. The necessity to cope with varied food sources have likely shaped multiple aspects of their biology, which are masked in the artificial laboratory environment where they are fed the unnatural diet of *E. coli* [7]. In order to elucidate the underlying connection between diet and physiology, it is important to evaluate how different diets influence behavior and physiology. Occasionally, contaminating microbes "pop up" spontaneously on culture media and are often quickly removed to maintain a standardized monoculture diet. We routinely noticed that *C. elegans* are found feeding on contaminants, when present, rather than the *E. coli* food source supplied. With this in mind, we looked at these "contaminants" as an opportunity to examine the impact that different food sources have on physiology.

We isolated and cultured three bacterial species as novel *C. elegans* diets that we call "Red", "Orange", and "Yellow" due to their pigments when grown on plates (**Figure 1A**). Using 16S ribosomal sequence alignment, we identified the genus of each new diet: Red is *Methylobacterium*; Orange is *Xanthomonas*, and Yellow is *Sphingomonas.* Intriguingly, these three novel bacterial diet genera can be found in *C. elegans* natural environments [16]. We next identified optimal growth parameters to ensure monoculture growth for each diet in LB broth and plates (figure supplement 1A). Although most species were able to grow at 37°C on LB solid media and in LB liquid media, Red/*Methylobacterium* required a growth temperature of 30°C on LB solid media, while Yellow/*Sphingomonas* required a growth temperature of 26°C in LB liquid media. The growth rate of each bacterial species was similar, except Red/*Methylobacterium*, which was markedly reduced (**Figure 1B**).

To assess the nutritional value of each diet, we first performed bomb calorimetry to define the total caloric composition of each bacteria. Surprisingly, the total caloric composition was not significantly different in any of the diets in comparison to the *E. coli* OP50/B diet. In order to better assess the differential nutrient composition of each bacteria, we next measured the concentrations of glucose, glycerol, glycogen, triglyceride, and water to define a nutritional profile for each diet (**Figure 1C** and figure supplement 1B-G). The Orange/*Xanthomonas* diet was the most significantly different diet among the three novel diets because glucose, glycerol, glycogen, and water content were all higher than the levels found in OP50/B. The Red/*Methylobacterium* diet displayed a similar trend with an increase in glycerol, glucose, and water content, but not triglycerides. Finally, the Yellow/*Sphingomonas* diet only carried more glycerol and water content, compared to OP50/B. Interestingly, HB101/B&K-12 was the only diet with an increase in triglyceride content relative to OP50/B. We also noted that glucose and glycogen content were highest in Orange/*Xanthomonas*, glycerol and triglyceride content were highest in HB101/B&K-12, and water content was highest in Yellow/*Sphingomonas.* Taken together, Red/*Methylobacterium*, Orange/*Xanthomonas*, and Yellow/*Sphingomonas* represent three new potential *C. elegans* diets, with similar calorie content, but differential nutritional composition to OP50/B that could be used to investigate dietary effects on animal physiology.

Novel diets direct unique transcriptional signatures

We next confirmed that worms could be cultured on each diet as the sole source of nutrition to enable the systematic characterization of the impact of these diets on *C. elegans* health. After maintaining animals on each diet for more than 20 generations, we then compared animals reared on these new laboratory diets alongside populations grown on the three most common *E. coli* diets (OP50/B, HT115/K-12 and HB101/B&K-12).

Because diet can impact multiple cellular processes, we first assessed the steady state transcriptional response to each diet by RNA-seq (**Figure 2A** and supplementary file 1). Each diet evoked a unique transcriptional signature (**Figure 2B-F** and supplementary file 1) when fed to the animal over multiple generations, which revealed the impacts of diet on multiple physiological responses. We compared the relative expression for each gene to age-matched animals fed the OP50/B diet and discovered that there were 740

genes altered in animals fed HT115/K-12, 3329 genes altered in animals fed HB101/B&K-12, 456 genes altered in animals fed Red/*Methylobacterium* diet, 1146 genes altered in animals fed Orange/*Xanthomonas*, and 5619 genes altered in animals fed Yellow/*Sphingomonas.* Of these, 100 genes were uniquely altered in animals fed the HT115/K-12 diet, 324 on the HB101/B&K-12 hybrid, 41 on the Red/*Methylobacterium* diet, 24 on the Orange/*Xanthomonas* diet, and strikingly 2421 on the Yellow/*Sphingomonas* diet (**Figure 2G** and supplementary file 1). In addition to finding genes that were shared on two, three, and four diet types, we also identified 140 genes that were altered on all five diets, as compared to OP50/B fed animals.

An analysis of the Gene Ontology (GO)-terms of the unique genes that were differentially expressed in animals fed each bacteria type revealed specific molecular signatures for each diet (**Figure 2H),** as well as phenotypic profiles and cell/tissue enrichment (supplementary file 1) for each diet. Notably, we observed expression changes for genes that could alter multiple physiological processes including development, metabolism, reproduction, and aging. In consideration of this enrichment, we explored the impact of the different diets on each of these critical physiological attributes.

Diet-induced acceleration of developmental timing

Our ability to culture animals on each diet revealed that they were sufficient to support life, but as diet can influence multiple life history traits, we further examined the physiological responses to the diets relative to the standard OP50/B diet. Diet can impact developmental timing [24] and as such, we asked whether any diet altered the time to reach each larval stage using the *mlt-10*::GFP-PEST reporter strain, which marks the transition of each animal as it progresses through its four developmental molts [25]; noting that on the OP50/B diet larval development occurs with invariant timing (**Figure 3A**). We determined that all diets facilitated successful development to reproductive adulthood but remarkably, each diet resulted in faster development relative to OP50/B-reared animals (**Figure 3B-F and** figure supplement 2). We noted three important variables in this precocious development: first, diets could accelerate development of specific larval stages – HT115/K-12 (**Figure 3B**) and Red/*Methylobacterium* (**Figure 3C**) diets resulted in early transition from larval stage 2 (L2) to larval stage 3 (L3), Orange/*Xanthomonas* (**Figure 3D**) diet resulted in early transition from L3 to larval stage 4 (L4), and HB101/B&K-12 (**Figure 3E**) and Yellow/*Sphingomonas* (**Figure 3F**) diets resulted in early transition from L4 to adulthood. Second, with the exception of the precocious transition between two larval stages listed above, the time spent in each larval stage (peak to peak time) was one to three hours shorter compared to OP50/B raised animals (figure supplement 2). Third, the time to molt (trough to trough time) was mostly unchanged (**Figure 3A).** In addition, we measured animal size at each developmental stage, which showed some small but significant differences in body size on different diets, similar to observations from other studies [26-29]. Worms raised on HT115/K-12 contained a bigger area from L4-day 1 adult stage, Orange/*Xanthomonas* worms had a smaller area until day 2 of adulthood, and the other diets resulted in a fluctuation of larger and smaller areas throughout developmental stages, relative to OP50/B (figure supplement 2). These data reveal that animals fed the new Red/*Methylobacterium*, Orange/Xanthomonas, and Yellow/*Sphingomonas* diets or the commonly used *E.coli* diets HT115/K-12 or HB101/B&K-12, reach reproductive maturity faster, as compared to the standard OP50/B diet, which could influence animal fitness.

Due to both the difference in total time to reproduction and time to progress through each developmental stage, we examined the expression of *C. elegans* genes important for development (**Figure 3G)**. Based on GO-term classifications, worms fed the Yellow/*Sphingomonas* diet had the greatest number of differentially expressed developmental genes while worms fed the Red/*Methylobacterium* diet had the fewest. Intriguingly, of the 140 genes with shared expression changes between the five diets, 12 of these genes are involved in the molting cycle (*sqt-2,sqt-3,ptr-4,ptr-18,mlt-7,mlt-10,qua-1,bli-1,dpy-3,rol-6,acn-1* and *noah-1*) [25] [30].

Bacterial diets differentially affect lipid homeostasis

Diet directly impacts overall metabolism, which is under tight genetic control [23, 31]. The unique metabolic profiles of each bacteria drove us to explore how these different microbial diets could affect organismal metabolism. As changes in intracellular lipid stores are easily measured by microscopy, we stained age-matched populations of fixed animals with two lipophilic dyes: Nile Red (NR) to measure total lipid content

(**Figures 4A-G**) and Oil red O (ORO) to identify lipid distribution (**Figure 4H-N and** figure supplement 3). Relative to OP50/B-fed animals (**Figure A**), overall lipid levels were increased in animals fed the HT115/K-12 (**Figure B,G**) and Orange/*Xanthomonas* diets (**Figure E,G**), but were markedly decreased in HB101/B&K-12 (**Figure C,G**), Red/*Methylobacterium* (**Figure D,G**), and Yellow/*Sphingomonas* (**Figure F,G**) diets. Under most diets, lipid distribution across tissues were similar (**Figure 4H-L,N and** figure supplement 3), except for animals fed the Yellow/*Sphingomonas* diet, which resulted in the depletion of intestinal lipids in late reproductive adults, a phenotype known as age-dependent somatic depletion of fat (Asdf) at day 3 of adulthood (**Figure 4M,N**) [32, 33].

The fact that animals fed each of the diets developed faster than OP50/B-reared animals indicates animals were not nutritionally deprived, which can result in developmental delay [20, 34-38]. Nevertheless, different microbes have been shown to alter rates of pharyngeal pumping, which regulates ingestion of bacteria [39]. Pharyngeal pumping rates were examined in both the L4 stage and day 1 adult animals, but none of the diets significantly altered pharyngeal pumping (**Figure 4O** and figure supplement 3).

Because each diet represented a specific metabolic profile and *C. elegans* reared on these diets differed in the amounts of stored intracellular lipids, we examined the expression of genes that regulate lipid metabolism and homeostasis (**Figure 4P**). Of the total number of genes in each diet that were differentially expressed, about 1/3 of the genes in each diet were related to metabolism. Feeding animals the Yellow/*Sphingomonas* diet evoked the largest number of metabolism-related gene changes, which correlated with our phenotypic observation that animals fed this diet store the lowest levels of fat at the L4 stage (**Figure 4G**) and it was the only diet to drive the Asdf phenotype later in life (**Figure 4M**). Among the genes differentially expressed on these new diets were *fat-5* and *fat-7*, which may suggest changes in lipid biosynthesis pathways, as well as multiple lipases (Lipl-class), suggesting changes in lipid utilization (**Figure 4G**).

Red/Methylobacterium and Yellow/Sphingomonas reduce reproductive output

We next investigated how each diet influenced fertility by measuring the total number of viable progeny laid by individual animals over a ten-day reproductive span. The total number of progeny was reduced ~25% when animals were fed the Red/*Methylobacterium* diet, modestly reduced (~10%) in animals fed the Yellow/*Sphingomonas* diet, and unchanged on other microbial diets (**Figure 5A**). Although animals reached peak reproductive output at approximately the same time, animals fed the Red/*Methylobacterium* and HB101/B&K-12 diet ceased reproduction sooner than all other groups (**Figure 5B and** figure supplement 4). Reproductive output of *C. elegans* hermaphrodites is determined by the number of spermatids generated at the L4 larval stage [40, 41]. To determine if the decreased progeny production and early loss of reproductive output were due to diminished sperm availability in Red/*Methylobacterium*-reared worms, we mated hermaphrodites with males, which increases total reproductive output (**Figure 5C**). Males raised on either the OP50/B or Red/*Methylobacterium* diets could increase total reproductive output similarly when mated to OP50/B-reared or Red/*Methylobacterium*-reared hermaphrodites, which indicated that sperm are functional when in excess. Surprisingly, when mated, hermaphrodites raised on Red/*Methylobacterium* have significantly more progeny as compared to hermaphrodites raised on OP50/B. We also counted the number of unfertilized oocytes laid by unmated hermaphrodites, which revealed HT115/K-12 and HB101/B&K-12, Red/*Methylobacterium*, and Orange/*Xanthomonas* diets resulted in markedly reduced expulsion of unfertilized gametes (**Figure 5D**) while hermaphrodites on Yellow/*Sphingomonas* were similar to animals fed the OP50/B diet. An analysis of the reproduction-related genes that were differentially changed in animals fed each of the diets revealed several diet-specific changes (**Figure 5E**).

Novel diets differentially alter organismal life expectancy and health

The quantity of diet ingested has been shown to influence lifespan across organisms [42, 43]. Similarly, diet quality and composition can also influence lifespan, but the underlying mechanisms of healthspan improvement, and its relationship to diet, remain underdeveloped. As such, additional models to explore how diet can impact life- and healthspan are of great interest. To this end, we examined impact of each new diet on organismal lifespan. Similar to previous studies [13] animals raised on HB101/B&K-12 had a modest increase

in mean lifespan, with the most significant impact on the last quartile of life (**Figure 6A** and supplementary file 2). In contrast, worms raised on HT115/K-12, Red/*Methylobacterium*, and Yellow/*Sphingomonas* displayed significantly increased lifespan (**Figure 6B-D** and supplementary file 2) while animals raised on Orange/*Xanthomonas* were short-lived (**Figure 6E** and supplementary file 2). Taken together, our results show that Red/*Methylobacterium*, Yellow/*Sphingomonas,* and Orange/*Xanthomonas* represent three diets of sufficient nutritional quality to accelerate development, but differentially alter life expectancy in a diet-dependent manner.

We examined the transcriptional profiles of genes previously annotated to be involved in lifespan and discovered several interesting trends that may explain the lifespan-altering effects of each diet (**Figure 6F**). Feeding the Yellow/*Sphingomonas* results in an increase in lifespan and, correspondingly, genes in the insulin-like signaling pathway (*daf-2*/Insulin receptor and *age-1*/PI3K) were downregulated, while DAF-16/FoxO target genes (Dao and Dod) were upregulated. Similarly, although less significantly, animals fed the Red/*Methylobacterium* diet have increased expression of several genes regulated by DAF-16. Moreover, the HT115/K-12, Red/*Methylobacterium* and Yellow/*Sphingomonas* all extend lifespan and share 11 genes with altered expression, all of which are up regulated on these diets. In previous studies, these 11 genes (*abu-1, abu-11, abu-14, gst-10, kat-1, lys-1, pqn-2, pqn-54, lpr-5, glf-1, bus-8*) lead to a shortened lifespan phenotype when the expression is reduced by RNAi [30]. Finally, feeding worms the Orange/*Xanthomonas* diet significantly decreased lifespan and although we did not identify any known longevity genes to display transcriptional changes, we found that *kgb-1, pha-4,* and *vhp-1* were all up regulated on this diet, while previous studies have observed extended lifespan when these genes are targeted by RNAi [30]. Taken together, while longevity is a complex phenotype, feeding any of these diets can evoke transcriptional changes in genes associated with life expectancy.

Since lifespan can be uncoupled from healthspan [10, 44] we examined the impact of each diet on animal muscle function via thrashing as a surrogate for health (**Figure 7A-E** and figure supplement 4). We measured thrashing rate at five specific timepoints that were selected for their significance in relation to other phenotypes: L4, based on the RNAseq data and fat staining analysis; day 1 adult, based on the pharyngeal pumping assay; day 3 adult, based on the presentation of the Asdf phenotype; day 8 adult, due to 50% of the Orange/*Xanthomonas* population being dead; and day 11 adult when 50% of the OP50/B population had perished. Surprisingly, animals raised HT115/K-12, HB101/B&K-12, Red/*Methylobacterium* and Yellow/*Sphingomonas* had faster muscle movement while Orange/*Xanthomonas* were indistinguishable from OP50/B-fed animals at L4 stage (**Figure 7A** and supplementary file 3) and at day 1 of adulthood (**Figure 7B** and supplementary file 3). Although most day 3 animals had similar muscle function, animals fed HT115/K-12 and Orange/*Xanthomonas* had modestly slower movements (**Figure 7C** and supplementary file 3). By day 8 of adulthood, Red/*Methylobacterium*-fed animals displayed significantly slower movements (**Figure 7D** and supplementary file 3). Day 11 adults raised on HT115/K-12, HB101/B&K-12, Yellow/*Sphingomonas,* and Orange/*Xanthomonas* moved half as fast as they did at the end of development, similar to OP50/B-raised control animals, but Red/*Methylobacterium*-fed animals we significantly slower (~75% reduction) (**Figure 7E** and supplementary file 3). Taken together, despite some diets enhancing early life muscle function, age-related decline remained similar, and was even enhanced in animals fed Red/*Methylobacterium* (**Figure 7F**). It is notable that when examining the GO-term analysis of the 30 shared genes that are deregulated on all five of the diets, as compared to OP50/B, all 30 genes have been annotated as causing movement defects in *C. elegans* when the expression of that gene is altered [45-47].

Diet as a nutraceutical to alter lifespan trajectories

Previous studies have demonstrated that effects of calorie restriction (CR) can be realized even when initiated later in life [48, 49]. Moreover, when fed ad libitum after a period of CR, mortality is shifted as if CR never occurred. With this model in mind, we asked if switching diet type, rather than abundance, could be wielded to alter lifespan outcomes. To address these questions, we switched growth conditions at major life stages (development, reproduction, post-reproduction) and measured lifespan (**Figure 8A**). We used both the Orange/*Xanthomonas* diet, which decreases lifespan, and Red/*Methylobacterium* diet, which increases

lifespan, as the basis of our model. Remarkably, after assessing eight combinations of diet switching, we discovered that the diet fed during development (diet 1) and reproductive period (diet 2) contributed little to overall lifespan and the last diet exposed (diet 3) exerted the most impact. In brief, when animals were exposed to the Red/*Methylobacterium* diet after experiencing the Orange/*Xanthomonas* diet, the normally shortened lifespan resulting from ingestion of Orange/*Xanthomonas* is suppressed (**Figures 8B-D** and supplementary file 4). Conversely, exposure to the Orange/*Xanthomonas* diet suppresses the normally extended lifespan linked to whole-life feeding of the Red/*Methylobacterium* diet (**Figures 8E-G** and supplementary file 4). Intriguingly, when compared to animals raised on the OP50/B diet, ingestion of the Red/*Methylobacterium* post-reproductively, regardless of ingestion of the Orange/*Xanthomonas* diet at any other life stage, results in a relatively normal lifespan; not shortened (figure supplement 6). Moreover, if animals that eat the Red/*Methylobacterium* diet post-reproductively have an extended lifespan, which is further enhanced if the diet is introduced after development. Taken together, our studies reveal that expanding the available diets to feed *C. elegans* is a powerful tool to study the impact of diet on lifespan and healthspan. Future studies to integrate genetic analyses to define new gene-diet pairs [6, 50] and gene-environment interactions in general, will be of significant interest.

DISCUSSION

C. elegans is a well-established model to study diet and aging. Here we augment that model by introducing a comprehensive phenotypic analysis of *C. elegans* fed three novel laboratory diets: Red/*Methylobacterium*, Orange/*Xanthomonas*, and Yellow/*Sphingomonas*. Interestingly, these microbes have been found in the normal *C. elegans* environments [16] and when compared to the three most common *E. coli* diets OP50/B, HT115/K-12, HB101/B&K-12, our study is a critical tool to aid in our understanding of how these two distinct environments influence physiological and transcriptomic responses over the lifespan [11, 16]. Previous works have identified how *C. elegans* react to the three standard *E. coli* diets provided in the lab [5, 8, 50-52] and yet, to our knowledge, a “head-to-head" comparison of the age-related and healthspan-relevant outcomes that result from feeding these diets, is lacking. Because of this, we decided to investigate the effects of diet on both physiological attributes and transcriptional signatures of *C. elegans* raised on different bacterial species for over twenty generations, to avoid acute stress responses to the diet.

Recent studies have shown that diet can alter transcriptional responses in *C. elegans* [53, 54]. Our work supports this observation with three new menu options for *C. elegans* culture. Although each of these diets evokes a unique transcriptional signature, several specific classes of genes are shared among multiple, or even all of the, diets. Our RNAseq analysis was limited to gene expression changes observed as animals enter adulthood, prior to reproduction. Given the extent of changes in reproductive capacity, health (movement and fat), and aging, future work to examine how gene expression is altered on each diet over the lifespan will be of great interest. Moreover, a fine-tuned analysis of transcription at each development stage will be informative based on our observation that different diets can accelerate the transition of animals across specific developmental stages. Nevertheless, it is clear that the standard laboratory *E. coli* diets and our three new diets induce a transcriptional response of phenotypically relevant genes.

Bacteria serve as a live food source for *C. elegans* both in their natural environment and laboratory setting. Many aspects of the bacterial communities themselves have been shown to influence multiple attributes in *C. elegans,* including food preference, feeding rates, brood size, and lifespan [55, 56]. Previous studies have shown animals pumping at similar rates in the presence of bacteria they can and cannot eat [12, 57]. The rates of pharyngeal pumping were not significantly different on any of the diets in L4 stage and day 1 adult animals (**Figure 4O** and figure supplement 4A). Although actual bacterial ingestion can be measured, it is technically challenging, and the faster developmental timing observed (**Figure 3**) and large-sized broods (**Figure 5A**), demonstrate that each diet is providing sufficient nutrition to the worms feeding on them. Nevertheless, based on the similarities of animals eating Red/*Methylobacterium* and animals undergoing dietary restriction, it remains possible that this bacterium allows ad libitum ingestion with the physiological benefits of reduced eating.

Clearly, changing diets can potently impact multiple phenotypic attributes and several of these aging-relevant phenotypes are interrelated. For example, reproduction, fat, and stress resistance are intrinsically tied to overall life expectancy[9]. In support of this model, the Red/*Methylobacterium* and Yellow/*Sphingomonas* diets, which reduce overall reproduction, indeed increase lifespan. However, our study reveals that this relationship is more complex as these two diets have different effects on lipid storage and the Yellow/*Sphingomonas* diet evokes an Asdf response, which is a phenotype observed in animals exposed to pathogens [32, 33]. It may be that *C. elegans* perceive the ingested Yellow/*Sphingomonas* diet as a pathogen, however the increased lifespan that follows on this diet, suggests this diet is health-promoting. Lastly, genetic and environmental mechanisms that delay developmental timing have been tied to increased longevity in adulthood [36, 58, 59]. Each of the diets we tested results in faster development into a reproductive adult, but only HB101/B&K-12, HT115/K-12, Red/*Methylobacterium*, and Yellow/*Sphingomonas* increase lifespan, while Orange/*Xanthomonas* decreases lifespan. Taken together our study reveals that each diet can exert a specific life history changing response, but also questions previously-established models of aging.

Inspired by previously described dietary interventions that are capable of extending lifespan in multiple species [43, 60, 61], we wanted to investigate whether, instead of keeping animals on one diet their entire life, altering dietary exposure at critical life stages could affect overall lifespan. We asked whether these diets could alter lifespan without any previous generational exposure. Strikingly, acute exposure to these diets was capable of altering lifespan (**Figure 8**)**,** but intriguingly the magnitude of the response differed from animals that have been on these diets for 20+ generations (**Figure 6**)**.** Furthermore, our study revealed that the diet fed during the post-reproductive period exerted the strongest influence over the lifespan of the cohort. This result, however, is potentially confounded by the amount of time (longitudinally) that is spent on each diet. Regardless, our study reveals that any potential early-life (development and reproductive span) exposure to diets that normally shorten lifespan can be mitigated by eating a lifespan-promoting food option. This result is reminiscent of previous studies showing the mortality rate of dietary restricted (DR)-treated animals is accelerated when switched to an ad libitum diet while mortality rate is reduced when ad libitum-fed animals are switched to a DR diet [62]. In our case, calories are perhaps not different on the Red/*Methylobacterium* or the Orange/*Xanthomonas* diets, but rather the overall nutritional composition of the diet is different. Given that in our model animals are chronically exposed to as much food as they can eat throughout their life, we posit that if a similar human diet were discovered that this treatment protocol would be more accessible as food restriction is difficult, socially and psychologically [63-65].

Taken together, our study expands the menu of bacterial diets available to researchers in the laboratory, identifies diets with the ability to drive unique physiological outcomes, and provides a diet quality approach to better understand the complexities of gene-diet interactions for health over the lifespan.

FIGURES

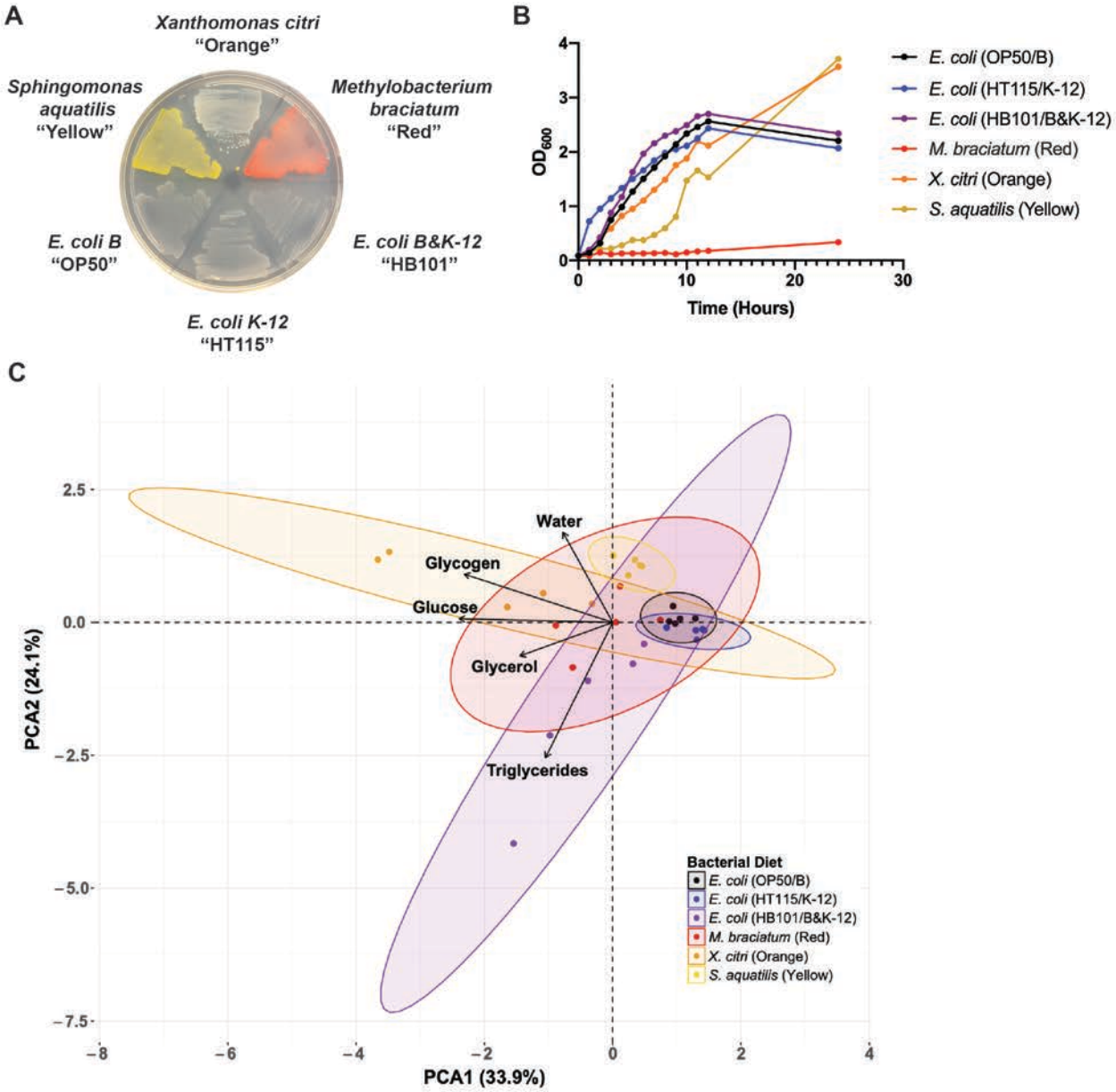


Figure 1. Characterization of bacterial diets. (A) Bacteria streak plate with the six bacterial diets fed to *C. elegans*. (B) Growth curves of the bacteria with optical density measurements every hour for 12 hours and another measurement taken at 24 hours. (C) PCA of metabolite concentrations in the different bacterial diets.

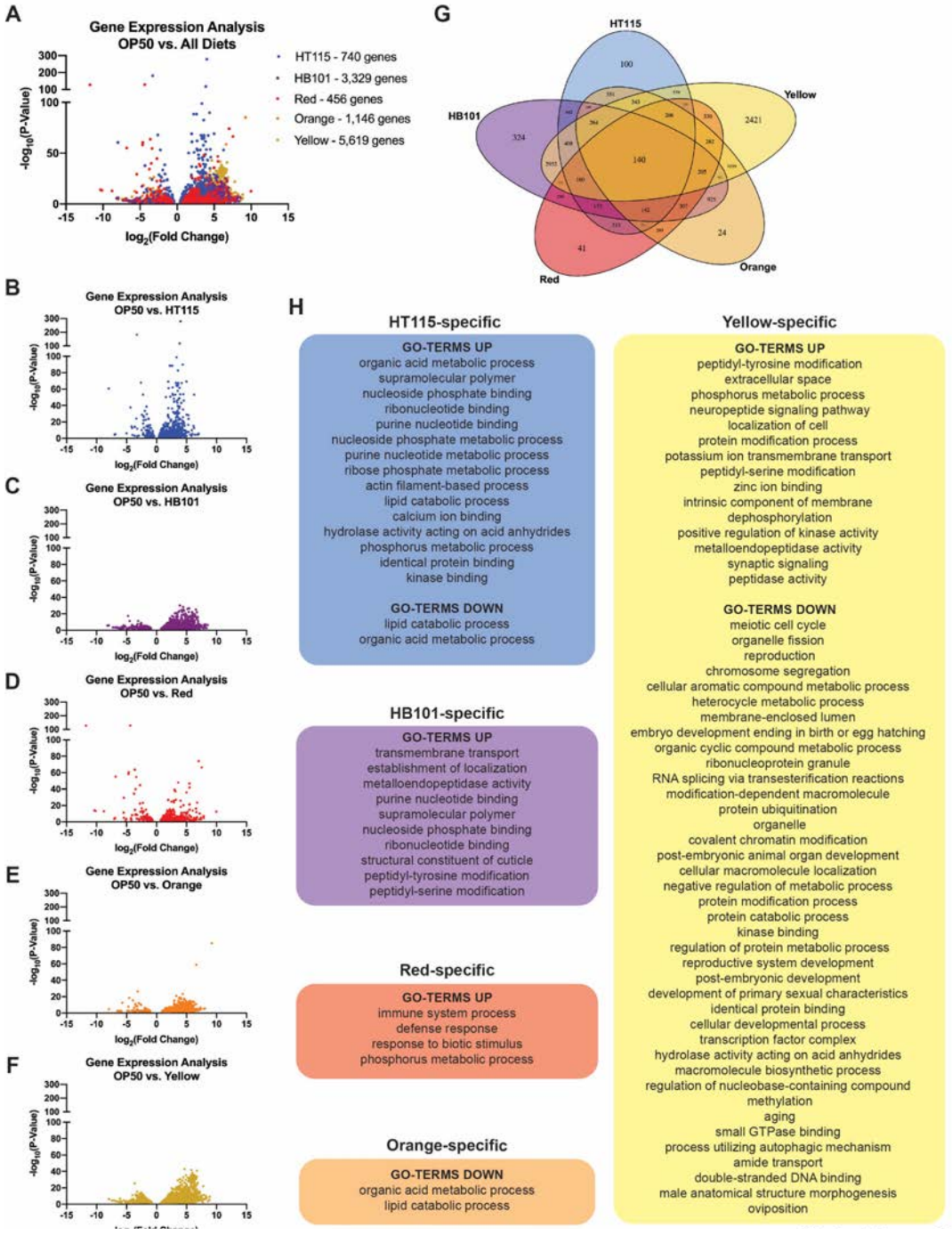


Figure 2. Gene expression analysis of L4 *C. elegans* on each of the diets after twenty generations. Gene Ontology (GO) term enrichment analysis of RNAseq data in larval stage 4 animals. (A) Volcano plot of all differentially-expressed genes in each diet relative to OP50/B-reared worms. All genes considered to be significant have a p-value <0.01. (B-F) Volcano plots of each individual diet with all significant genes that are differentially expressed. (G) Venn diagram of all significant genes. Shows the number of genes shared between two, three, four and five of the diets, along with the number of genes unique to each diet. GO-terms for uniquely-expressed genes are noted below and split between genes that were up-regulated and down-regulated. Further information on genes unique to each diet and specific GO terms can be found in supplementary file 1.

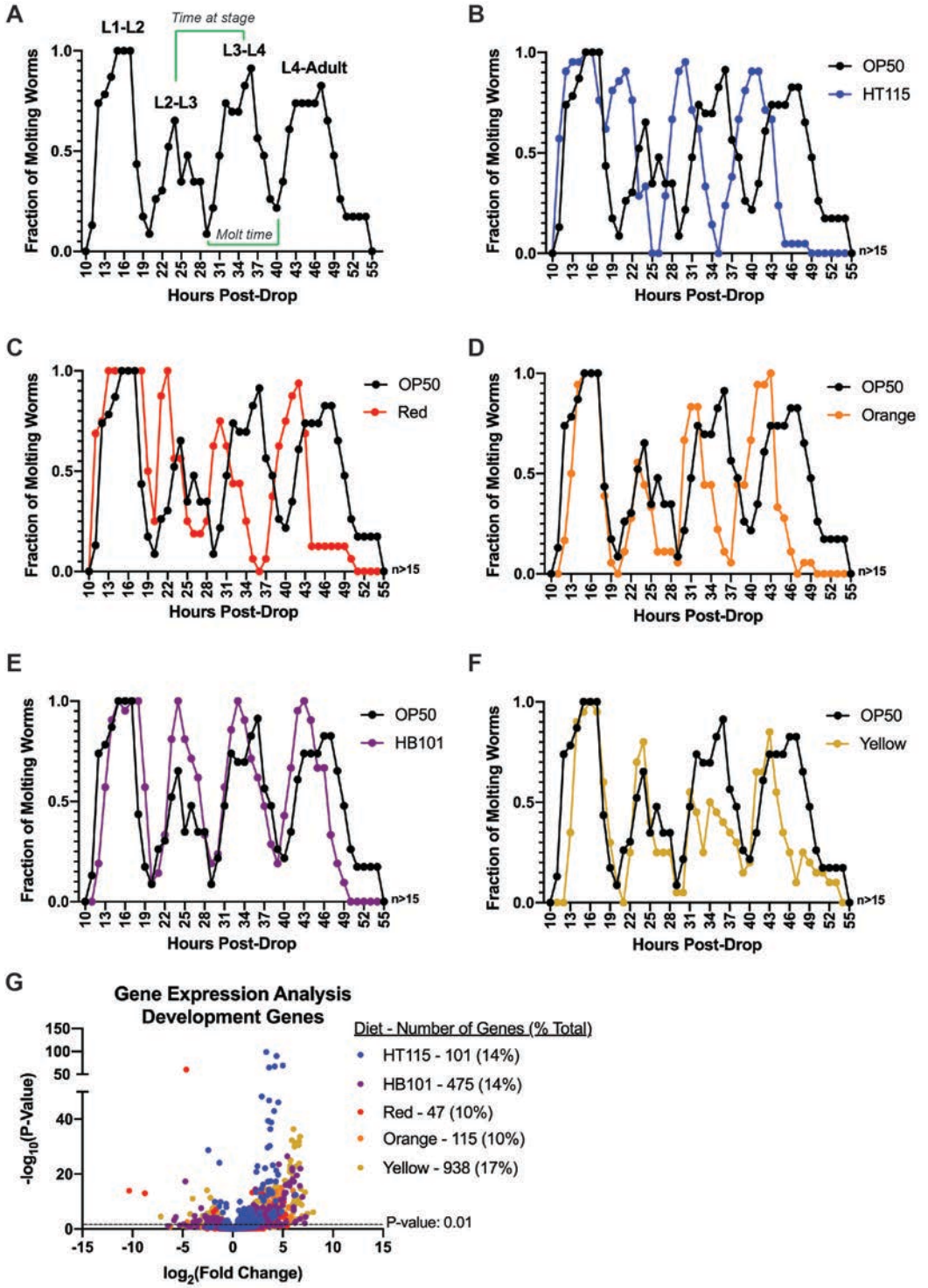


Figure 3. Developmental timing of *C. elegans* is dependent upon bacterial diet. (A) The molting reporter strain *mgIs49[mlt-10p::gfp-pest ttx-3p::gfp] IV* allows for the visualization of molting between larval stages of development into adulthood. The time between peaks represents the period at each larval stage, while time between troughs represent time spent molting. Analysis was carried out after this strain was raised on each diet for twenty generations. (B-F) Developmental timing of worms raised on each diet relative to OP50/B. All diets showed accelerated development into adulthood when compared to worms raised on OP50/B. (G) Volcano plot showing how many of the differentially expressed genes are related to development. The number of genes in each diet are labeled in the legend. All genes have a p-value <0.01.

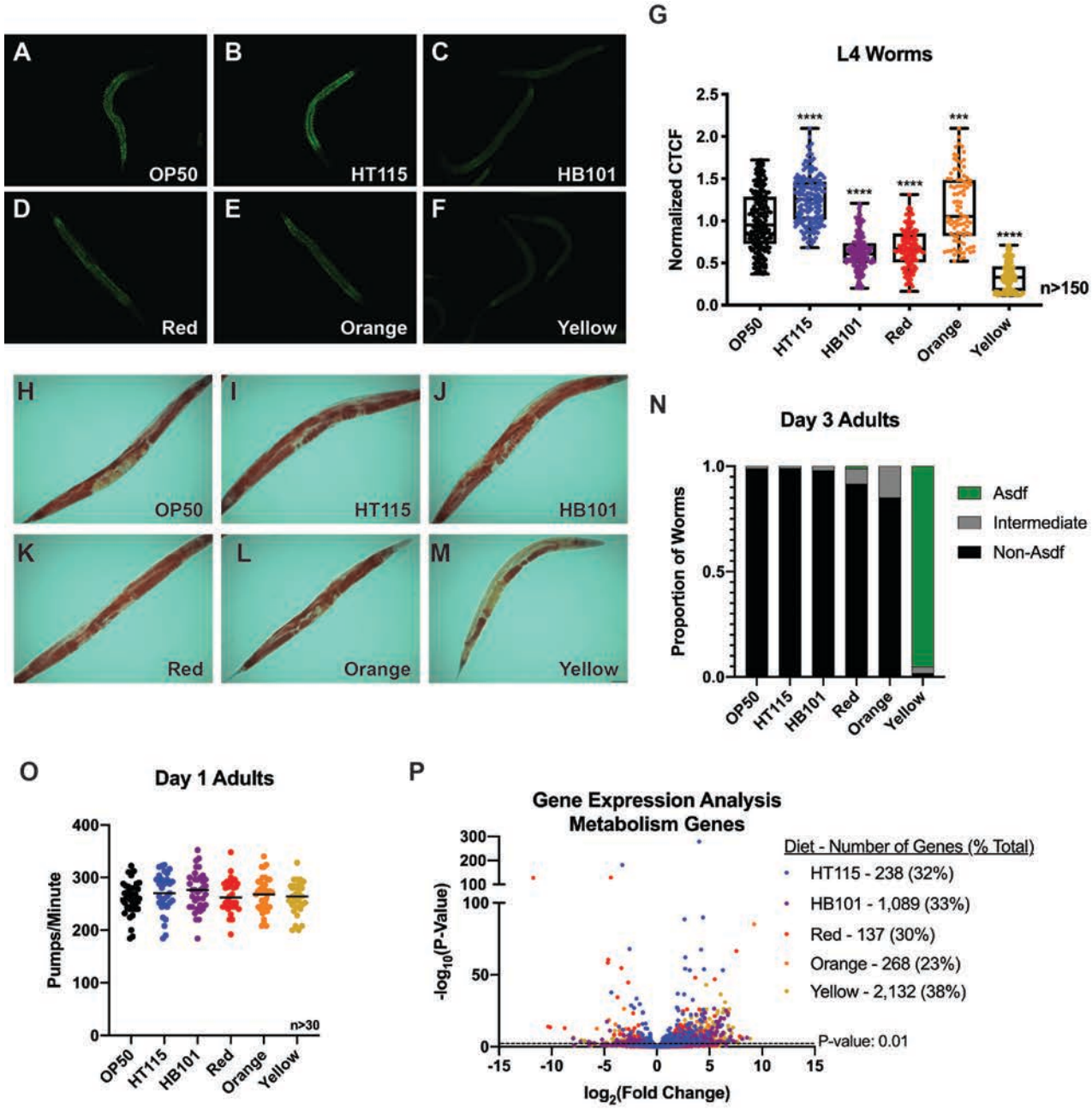
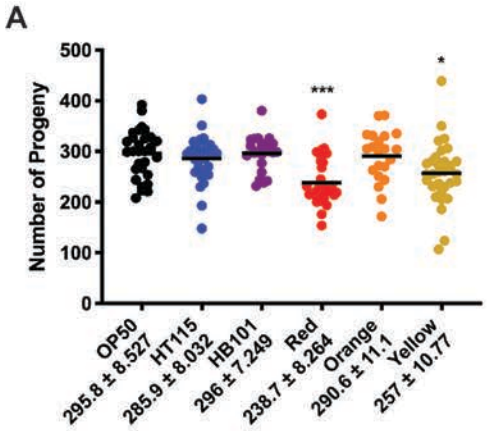
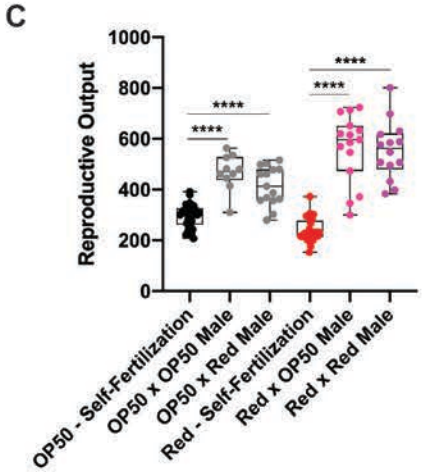


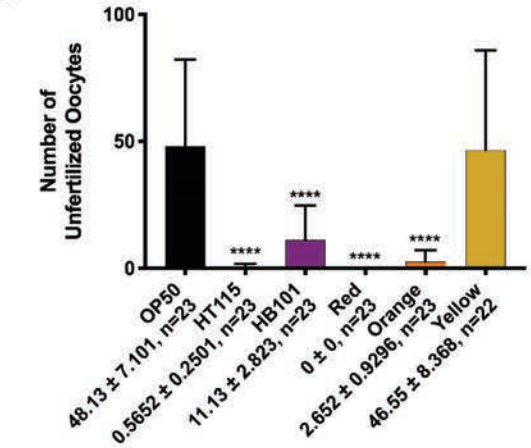
Figure 4. Fat content and distribution vary depending on the diet *C. elegans* are exposed to. (A-F) Nile Red staining of L4 worms. (G) Quantification of Nile Red staining with comparisons made to the OP50/B control. GFP fluorescence is measured and then normalized to area before being normalized to OP50/B. HT115/K-12 (B) and Orange/*Xanthomonas* (E) have significantly higher fat content while HB101/B&K-12 (C), Red/*Methylobacterium* (D) and Yellow/*Sphingomonas* (F) have lower fat contents. Statistical comparisons by Tukey's multiple comparison test. ***, p<0.001; ****, p<0.0001. (h-m) Oil Red O lipid staining in day 3 adult *C. elegans*. (H-L) OP50/B, HT115/K-12, HB101/B&K-12, Red/*Methylobacterium* and Orange/*Xanthomonas* show similar lipid distribution of fat throughout the animal (representative image). (M) Yellow/*Sphingomonas*-reared worms display age-dependent somatic depletion of fat (Asdf) with loss of fat in the intestine while fat is retained in the germline (representative image). (N) Quantification of lipid distribution across tissues. (O) Pumping in day 1 adult worms is not significantly different between any of the diets and OP50/B. (P) Volcano plot of differentially expressed metabolism genes in all diets compared to OP50/B. All genes have a p-value <0.01. All studies performed in biological triplicate.



B

	OP50 Avg. Progeny (p-value)	HT115 Avg. Progeny (p-value)	HB101 Avg. Progeny (p-value)	Red Avg. Progeny (p-value)	Orange Avg. Progeny (p-value)	Yellow Avg. Progeny (p-value)
D1	55.1	57.9 n.s.	99.0 ****	80.1 **	74.3 n.s.	55.1 n.s.
D2	141.4	180.9 ****	182.3 ****	154.5 n.s	166.9 *	154.2 n.s.
D3	90.9	47.9 ****	14.1 ****	17.0 ****	48.4 ***	54.2 ***
D4	15.5	3.1 ****	0.2 ****	0.2 ****	0.9 ****	3.7 ****
D5	2.7	0.1 ****	0.3 ****	0 ****	0.1 ****	0.3 ****





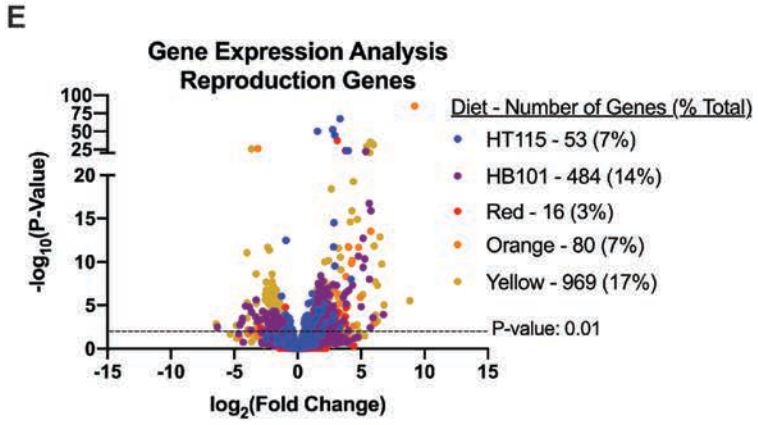


Figure 5. *C. elegans* raised on Red/*Methylobacterium* and Yellow/*Sphingomonas* have decreased reproductive output. (A) Hermaphrodites have reduced brood size when fed the Red/*Methylobacterium* or Yellow/*Sphingomonas* diet. (B) Reproductive timing is also altered, revealing differences between all diets and OP50/B in total output each day of their reproductive span. HB101/B&K-12 and Red/*Methylobacterium* halt output of viable progeny two days before OP50, HT115, Orange and Yellow. (C) OP50/B and Red/*Methylobacterium* hermaphrodites mated with OP50/B or Red/*Methylobacterium* males yield similar number of progeny. (D) Yellow/*Sphingomonas* has similar number of unfertilized oocytes compared to OP50/B. HT115/K-12, HB101/B&K-12, Red/*Methylobacterium* and Orange/*Xanthomonas* have significantly fewer unfertilized oocytes. Statistical comparisons by Tukey's multiple comparison test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$. (e) Volcano plot of differentially expressed reproduction genes in all diets compared to OP50/B. The number of significant genes have a p-value of <0.01.

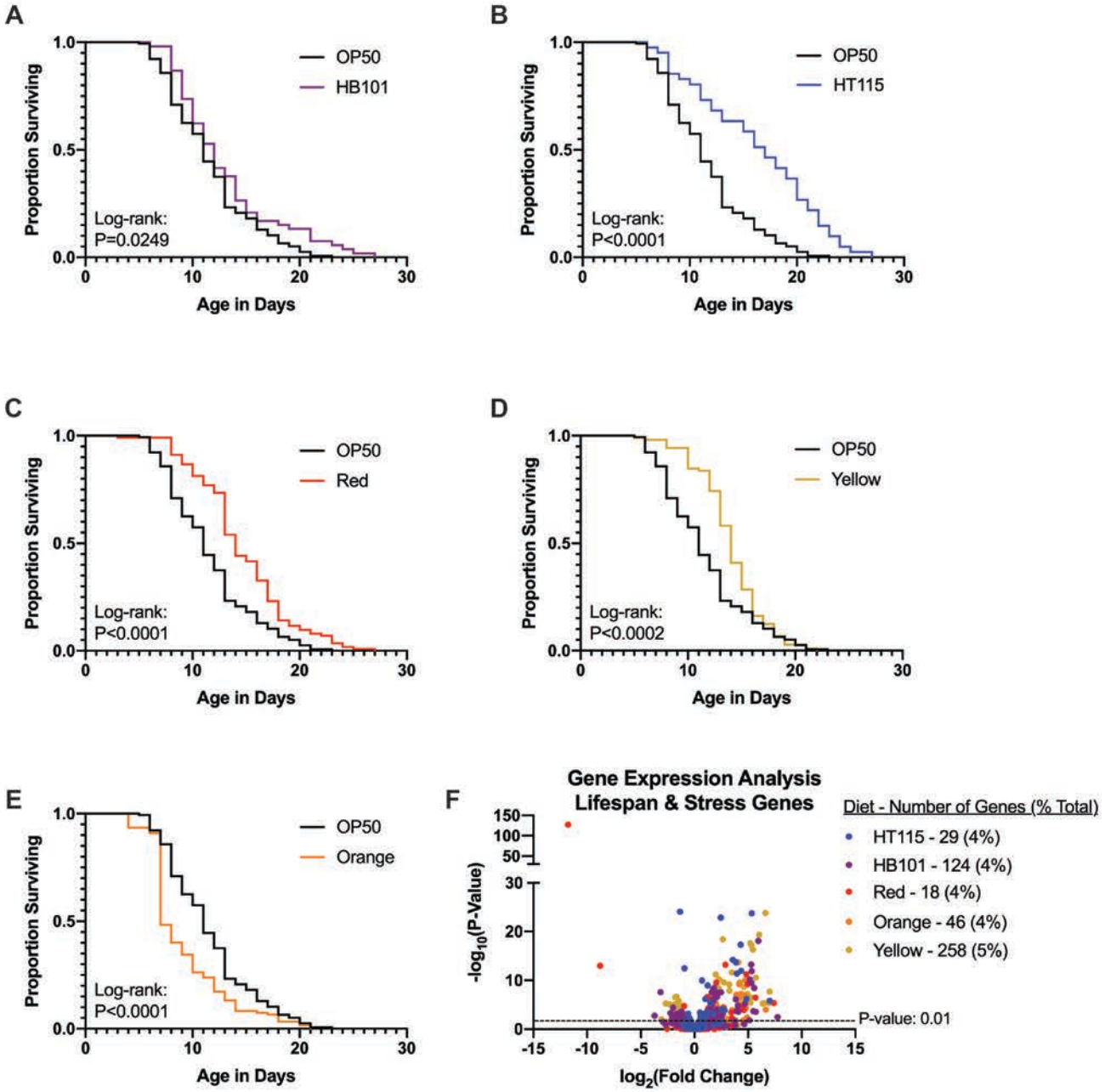


Figure 6. Lifespan of *C. elegans* on each diet. (A-E) Lifespan comparisons of OP50/B versus each bacterial diet. HB101/B&K-12 (A) had a very small significant difference in lifespan compared to OP50/B. HT115/K-12 (B), Red/*Methylobacterium* (C) and Yellow/*Sphingomonas* (D) worms had increased lifespans and Orange/*Xanthomonas* (E) worms had a shorter lifespan. Lifespan comparisons between OP50/B-reared worms and the other diets made with Log-rank test. (F) Volcano plot of differentially expressed genes related to lifespan in all diets compared to OP50/B. The number of significant genes have a p-value of <0.01.

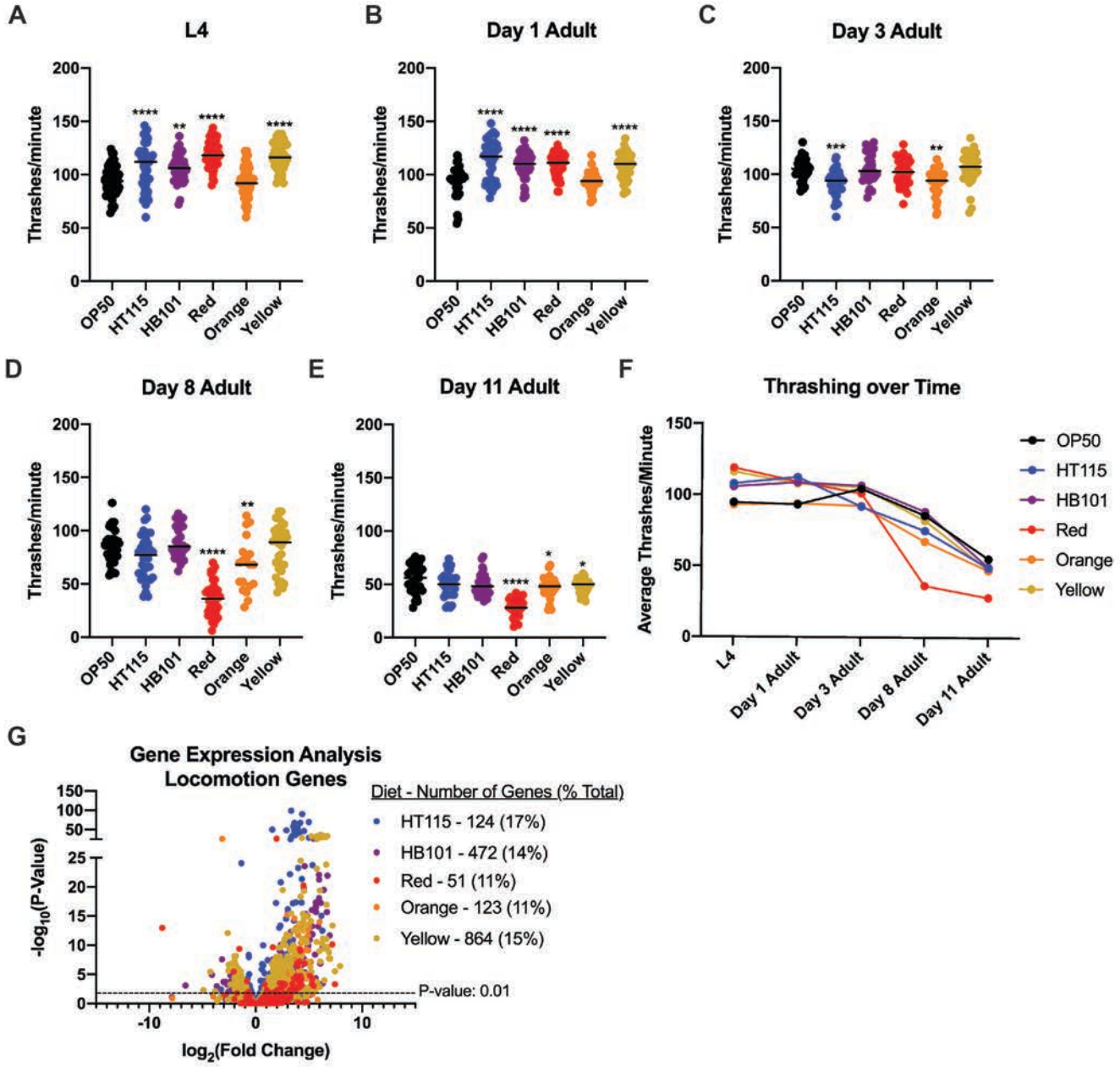


Figure 7. *C. elegans* have a diet-dependent decline in thrashing with age. (A-E) The rate of thrashing in worms raised on each diet at different developmental stages. All comparisons were made to OP50/B-reared worms. (A) All diets except for Orange/*Xanthomonas* showed a significantly higher thrashing rate at the L4 stage. (B) All diets except for Orange/*Xanthomonas* showed a significantly higher thrashing rate at the day 1 adult stage. (C) HT115/K-12 and Orange/*Xanthomonas* thrashed significantly less than OP50/B while the other diets were similar in thrashing rate at the day 3 adult stage. (D) Red/*Methylobacterium* and Orange/*Xanthomonas* had significantly lower thrashing rates compared to OP50/B and all other diets at the day 8 adult stage. (E) Red/*Methylobacterium*, Orange/*Xanthomonas* and Yellow/*Sphingomonas* had lower thrashing rates at day 11 adult when compared to OP50/B. Statistical comparisons by Tukey's multiple comparison test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$. (F) Average rate of thrashing over time on each bacterial diet. All studies performed in biological triplicate; refer to supplementary file 3 for n for each comparison. (G) Volcano plot of differentially expressed locomotion genes in all diets compared to OP50/B. The number of significant genes have a p-value of <0.01.

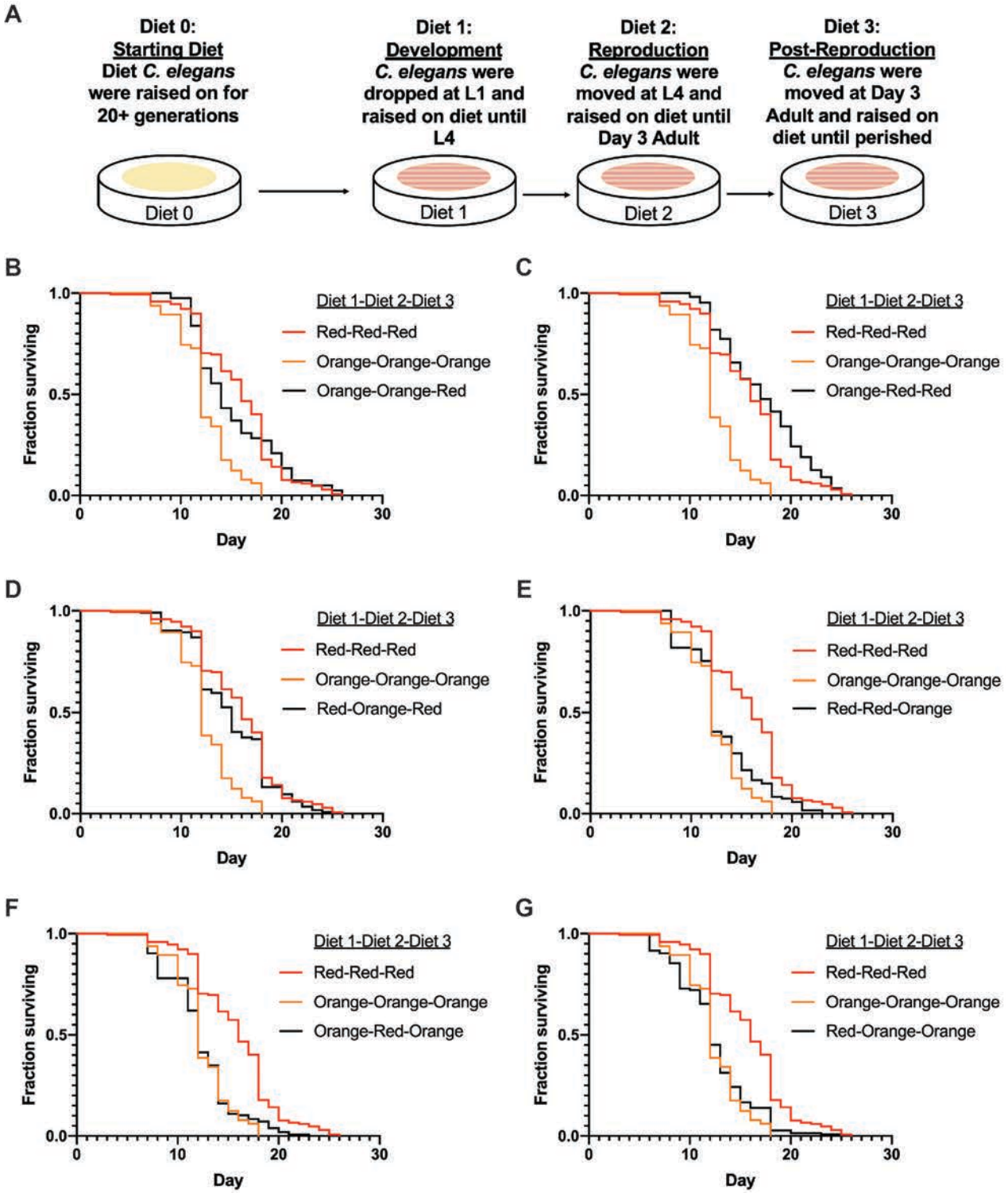


Figure 8. The introduction of the Red/*Methylobacterium* diet at the post-reproductive stage in *C. elegans* extends lifespan. (A) Schematic of the diet as a nutraceutical experiment. Diet 0 was OP50/B for all worms. After synchronization and allowing L1s to hatch overnight, L1s were dropped on diet 1 and moved at L4 to diet 2 before being moved to diet 3 at day 3 of adulthood. Lifespans were then measured. (B-G) Lifespan curves of diet as a nutraceutical experiments. Lifespan comparisons between the diet combinations and Red/*Methylobacterium*-only, and Orange/*Xanthomonas*-only were made with Log-rank test; refer to supplementary file 4. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

SUPPLEMENTAL FIGURE LEGENDS

A

Name	Bacteria	Doubling Time	Antibiotic Sensitivity	Growth Conditions for Experiments in Liquid Culture	Growth Conditions on Plate
OP50	*Escherichia coli*	2 Hours	Ampicillin	LB + Streptomycin at 37C	LB + Streptomycin at 37C
HT115	*Escherichia coli*	1 Hour	Streptomycin	LB + Ampicillin at 37C	LB + Ampicillin at 37C
HB101	*Escherichia coli*	3 Hours	Ampicillin	LB + Streptomycin at 37C	LB + Streptomycin at 37C
Red	*Methylobacterium*	23 Hours	Streptomycin	LB + Ampicillin at 37C	LB + Ampicillin at 30C
Orange	*Xanthomonas*	5 Hours	Streptomycin	LB + Ampicillin at 37C	LB + Kanamycin at 37C
Yellow	*Sphingomonas*	7 Hours	Streptomycin	LB + Ampicillin at 26C	LB + Ampicillin at 37C

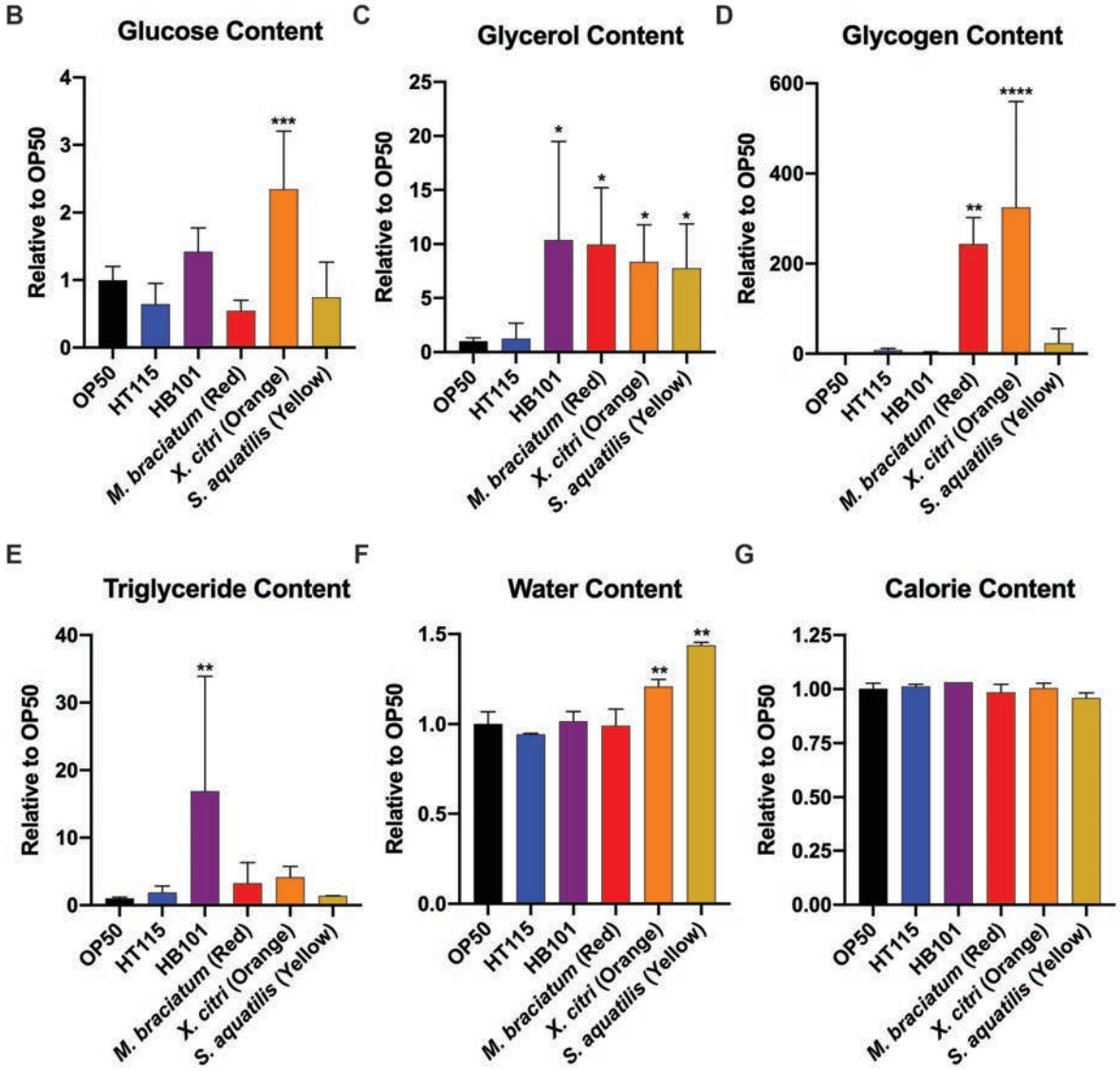


Figure S1. Bacterial growth conditions and metabolite concentrations. (A) Table with optimal growth conditions for each bacterial diet. (B-G) Metabolite concentrations in each diet relative to OP50/B. The different metabolites measured were glucose (B), glycerol (C), glycogen (D), triglycerides (E) and water content (F). Statistical comparisons by Tukey's multiple comparison test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$. All studies were performed in biological triplicate. (G) Calorie content of all diets were similar.

A

Diet	Time to 50% of the Population Molting (Hours)				Total Time to Adulthood	Hours in each molt (Average number of hours worms were GFP positive)				% Faster than OP50-reared worms
	L1 Molt	L2 Molt	L3 Molt	L4 Molt		L1 Molt	L2 Molt	L3 Molt	L4 Molt	
OP50	11	22	30	41	50	6	4	6	7	-
HT115	11	18	27	38	44	9	3	5	6	20%
HB101	12	22	30	40	47	6	5	6	6	14.5%
Red	11	20	28	38	44	9	4	4	6	20%
Orange	13	23	29	39	46	5	3	4	5	16%
Yellow	13	22	30	40	46	5	3	4	4	16%

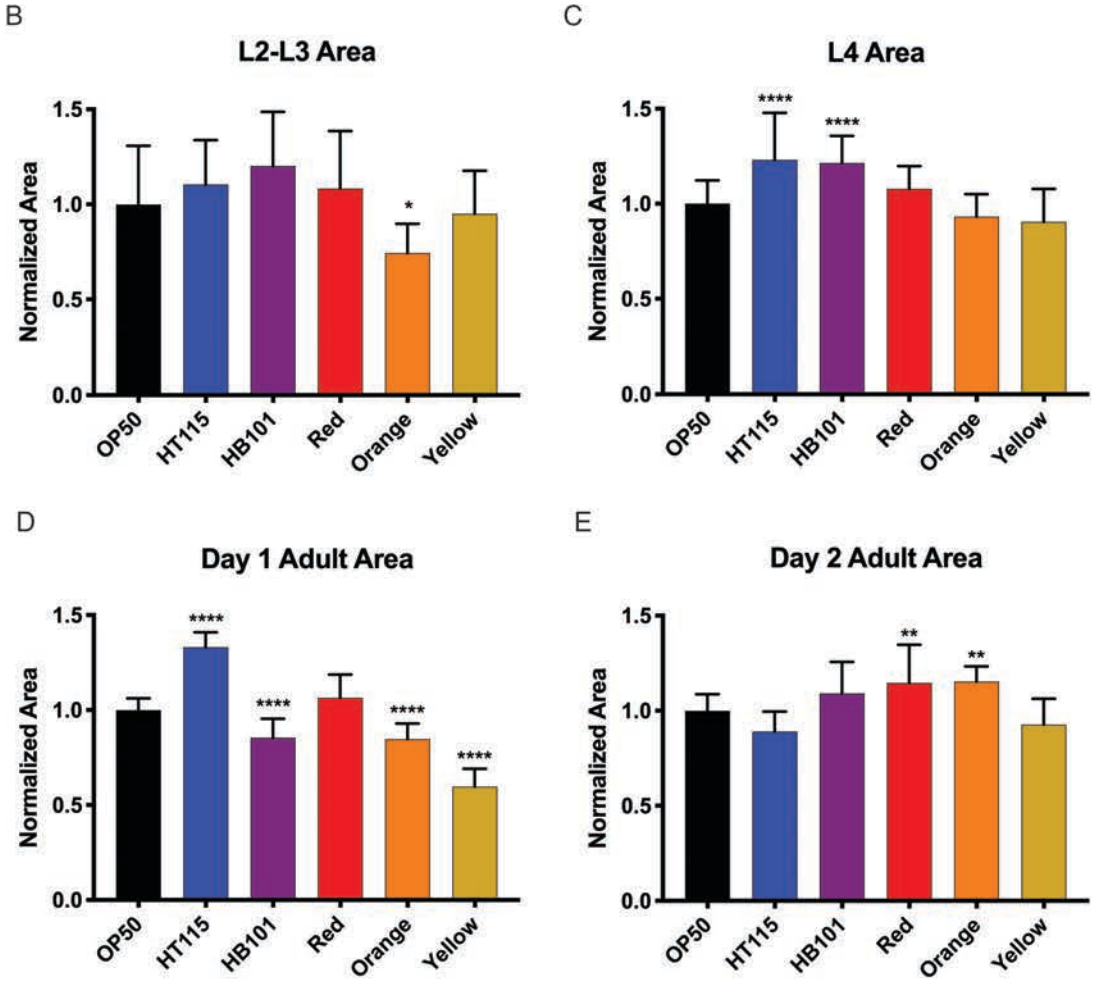


Figure S2. Time in each developmental stage and worm area is altered based on bacterial diet raised on. (A) Table showing differences in time to each molt along with hours spend in each stage of development. (B-E) Area measurements of worms on each diet relative to OP50/B. (B) Orange/*Xanthomonas*-reared worms showed a slightly smaller area than OP50/B and other diets. (C) *C. elegans* on HT115/K-12 and HB101/B&K-12 had significantly larger areas at the L4 stage. (D) Worms raised on HT115/K-12 have a larger area than worms raised on OP50/B at day 1 of adulthood. HB101/B&K-12, Orange/*Xanthomonas* and Yellow/*Sphingomonas* worms have smaller area at day 1 of adulthood. (E) *C. elegans* raised on Red/*Methylobacterium* and Orange/*Xanthomonas* have larger areas at day 2 of adulthood compared to OP50/B and the other diets. Statistical comparisons by Tukey's multiple comparison test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$. All studies were performed in biological triplicate.

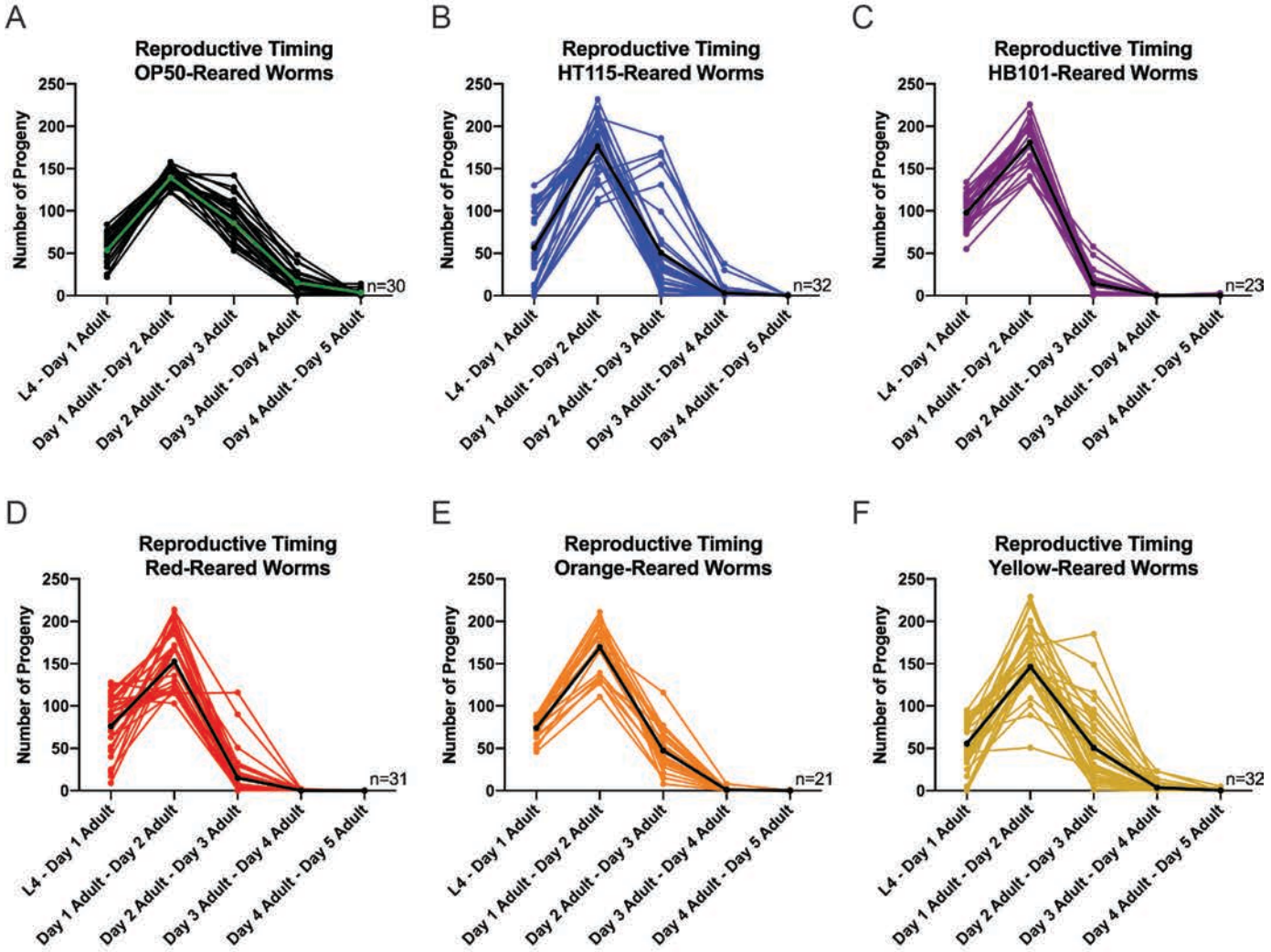


Figure S3. Reproductive timing is altered in *C. elegans* raised on the different bacterial diets. Reproductive output of individual worms (each line represents a different worm) at each day of its reproductive span. The thicker green line (A) and black line (B-F) on the graph represents the average output per day of reproduction. Worms were moved every 24 hours and progeny were counted. Each diet had a reproductive peak from day 1 to day 2 of adulthood and then started producing fewer progeny per day until day 5 of adulthood. HB101/B&K-12 (C) and Red/*Methylobacterium* (D) worms have a significant decline in reproductive output by day 3 of adulthood.

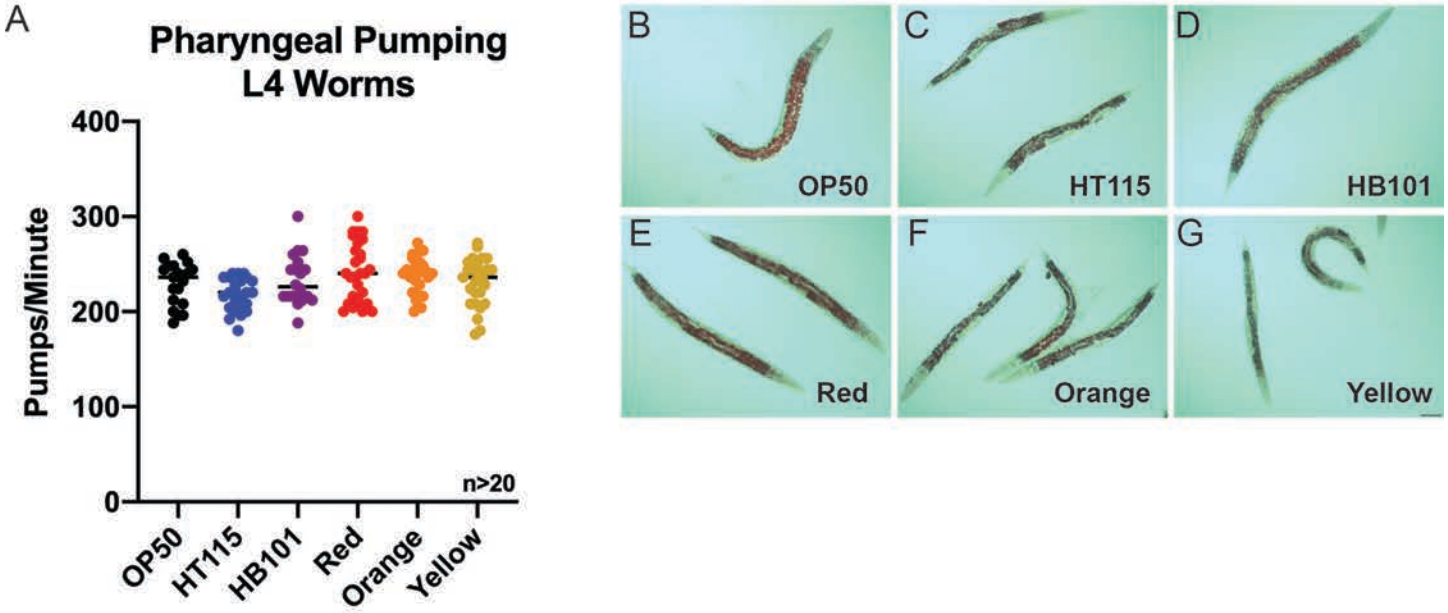


Figure S4. L4 Pumping and Oil Red O staining. (A) L4 pumping of worms raised on each bacterial diet. There was no significant difference between worms raised on the different diets. Statistical comparisons by Tukey's multiple comparison test. Study was performed in biological triplicate. (B-G) Oil Red O staining for lipid distribution in L4 *C. elegans*.

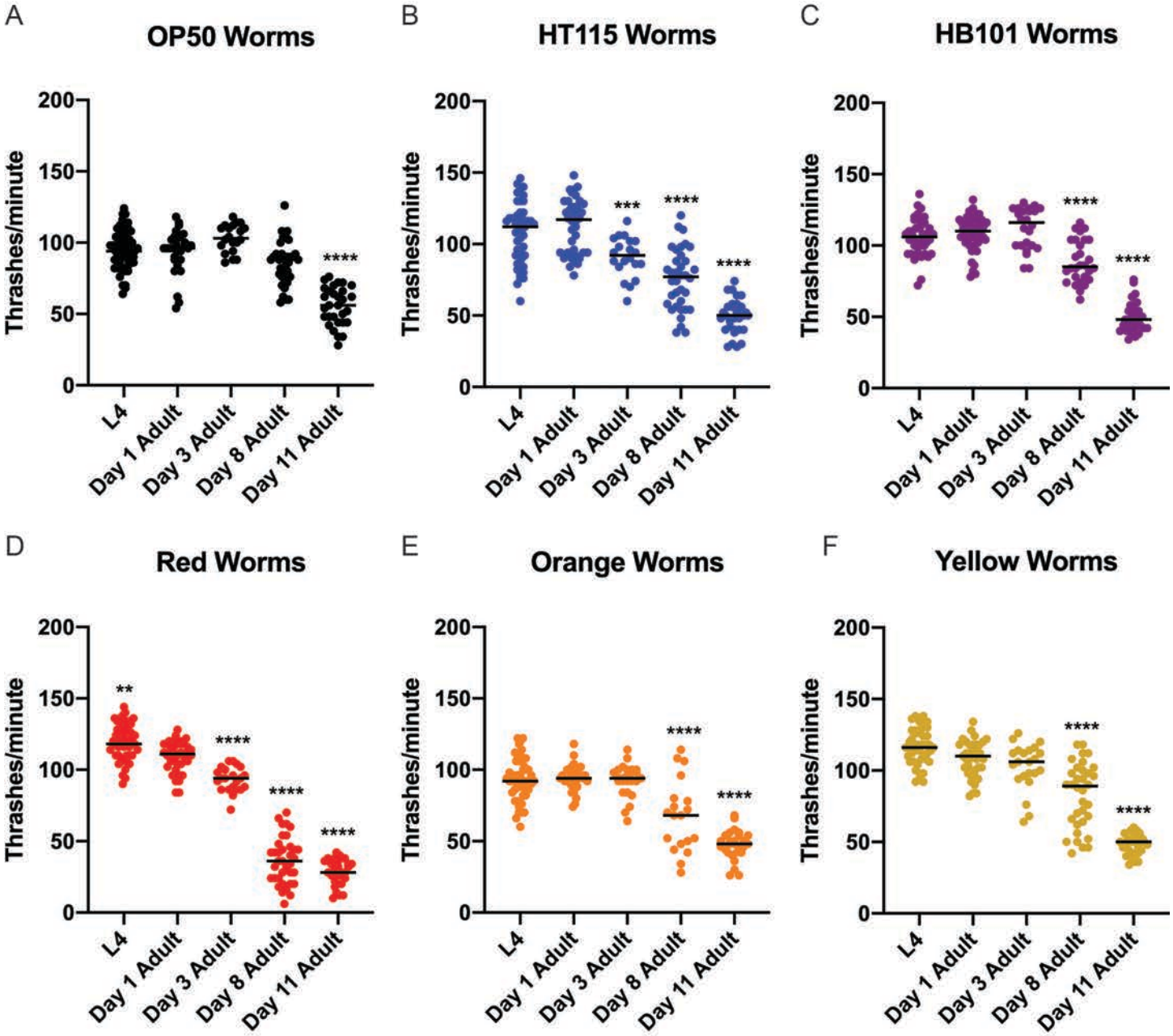


Figure S5. Thrashing declines with age in a diet-dependent manner. *C. elegans* at each stage of development on each diet. Comparisons were made to day 1 of adulthood. Statistical comparisons by Tukey's multiple comparison test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$. All studies were performed in biological triplicate. (A) OP50/B thrashing starts to show a decline at day 11 of adulthood. (B) HT115/K-12 thrashing declines starting at day 3 of adulthood and continues to do so up until day 11 of adulthood. (C, E-F) HB101/B&K-12, Orange/*Xanthomonas* and Yellow/*Sphingomonas* decline in thrashing rate at day 8 of adulthood and continues into day 11 of adulthood. (D) Red/*Methylobacterium* worms decline in thrashing from day 1 to day 11 of adulthood. Red/*Methylobacterium*-reared worms have the most significant rate of decline in thrashing compared to the other diets.

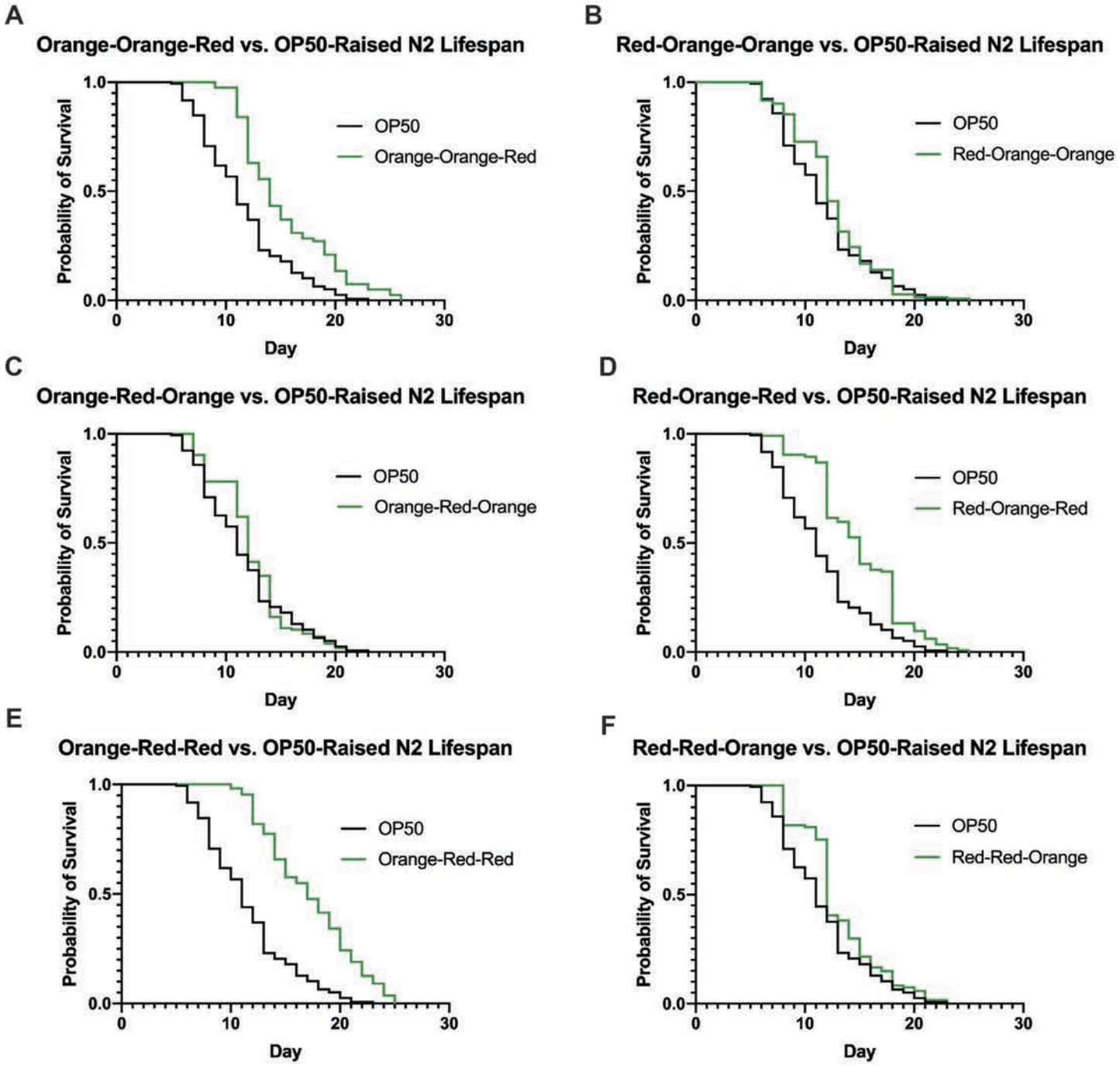


Figure S6. Nutraceutical diet combinations compared to *C. elegans* raised on OP50. Lifespan comparisons of OP50/B versus. each nutraceutical diet combination. Lifespan comparisons made with Log-rank test (supplementary file 2).

SOURCE DATA FILES

Table S1. RNAseq analysis in L4 *C. elegans*.

Table S2. Lifespan quartile comparisons.

Table S3. Thrashing replicates at each life stage.

Table S4. Diet as a nutraceutical Log-rank analysis compared to OP50/B, Red/*Methylobacterium* and Orange/*Xanthomonas.*

ACKNOWLEDGEMENTS
H. Dalton for early work on this project including isolation of new bacterial contaminants, C-A Yen for experimental assistance, J. Gonzalez for technical assistance, A. Hammerquist for critical reading of the manuscript, A. Frand for the strain GR1395 (*mgIs49*[*mlt-10p*::*gfp-pest*, *ttx-3*::*gfp*]IV]), and members of the Curran lab for thoughtful suggestions.

AUTHOR CONTRIBUTIONS
S.P.C. designed the study; N.L.S. performed the experiments; N.L.S. and S.P.C. analyzed data. S.P.C. wrote the draft manuscript, and N.L.S. and S.P.C. revised the final manuscript. This work was funded by the NIH R01GM109028 and R01AG058610 to S.P.C. and T32AG052374 to N.L.S.

DECLARATIONS OF INTERESTS
The authors declare no competing interests.

MATERIALS & METHODS

C. elegans strains and maintenance

C. elegans were raised on 6-cm nematode growth media plates supplemented with streptomycin and seeded with each bacteria diet. For experiments, nematode growth media plates without streptomycin were seeded with each bacteria at the optical density of 0.8 A_{600}.. All worm strains were grown at 20°C and unstarved for at least three generations before being used. Strains used in this study were N2 Bristol (wild type) and GR1395 (*mgIs49*[*mlt-10p*::*gfp-pest*, *ttx-3*::*gfp*]IV]). Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

E. coli strains used: OP50, HT115(DE3), HB101. Red/*Methylobacterium*, Yellow/*Sphingomonas,* and Orange/*Xanthomonas* bacteria were isolated from stock plates in the laboratory and selected for with antibiotics before inoculating.

Bacterial Growth Curves

Three test tubes with 5mL of LB without antibiotics were inoculated with each bacteria before being place in 37°C (except for Yellow/*Sphingomonas*, which was placed at 26°C). Optical density measurements were taken in duplicate every hour for 12 hours. The final measurement was taken 24 hours later. Optical density curves were made by averaging the readings together after performing experiment in biological triplicate.

Metabolite Kits

Bacteria was grown overnight in liquid culture with corresponding antibiotics. The next day, cultures were spun down and bacteria was washed in water three times before being spun down and frozen at -80°C until use. Red/*Methylobacterium* samples were seeded onto LB plates and then allowed to grow overnight before scraping off and collecting. Bacteria were homogenized based on metabolite kit instructions and corresponding metabolites were measured via Bio Vison kit instructions. Both glycogen and glucose measurements were obtained from the Glycogen Colorimetric/Fluorometric Assay Kit (K646) and the triglyceride and glycerol measurements were obtained from the Triglyceride Quantification Colorimetric/Fluorometric Kit (K622).

Bomb calorimetry

Bacteria was grown overnight in liquid culture with corresponding antibiotics. The next day, bacteria were seeded onto LB plates and allowed to grow overnight. Bacteria was then collected with autoclaved water from the seeded plates and spun down to remove excess water. Samples were frozen at -80°C until being sent in triplicate to the Department of Nutrition Sciences at the University of Alabama at Birmingham for sample drying and bomb calorimetry. Water content measurements were also obtained after measuring wet weight and dry weight of the samples.

RNA-sequencing

Wild type worms grown on each diet for at least 30 generations were egg prepped and eggs were allowed to hatch overnight for a synchronous L1 population. The next day, L1s were dropped on NGM plates seeded with 0.8 OD of each bacterial diet. 48 hours post drop, L4 animals were washed 3 times with M9 buffer and frozen in TRI reagent at -80°C until use. Animals were homogenized and RNA extraction was performed via the Zymo Direct-zol RNA Miniprep kit (Cat. #R2052). Qubit™ RNA BR Assay Kit was used to determine RNA concentration. The RNA samples were sequenced and read counts were reported by Novogene. Read counts were then used for differential expression (DE) analysis using R packages DESeq2 and edgeR. Significant genes found with each package were then compared and only genes appearing in both methods were used for comparisons.

Microscopy

All images in this study were acquired using ZEN software and Zeiss Axio Imager. Worm area comparisons were imaged at 10x magnification (L2-L3 and L4 stage worms) and 5x magnification (day 1 adult and day 2

adult) with the DIC filter. Worm areas were measured in ImageJ using the polygon tool. For GFP reporter strains, worms were mounted in M9 with 10mM levamisole and imaged with DIC and GFP filters.

Developmental timing by *mlt-10::gfp*
GR1395 worms grown on each diet for at least 20 generations were egg prepped and eggs were allowed to hatch overnight for a synchronous L1 population. One 24-well plate per diet, each containing a single L1 worm, were visualized by fluorescence microscopy every hour for 55 hours. Each hour worms were scored as green (molting) or non-green (not molting). Wells without worms, wells with two worms and worms that crawled to the side of the plate were censored.

Lifespan analysis
Worm strains on each diet were egg prepped to generate a synchronous L1 population and dropped on the corresponding diet. Worms were kept at 20°C and moved every other day during progeny output (day 2, day 4, day 6, day 8). Every diet was moved at the same time. Worms were scored daily for survival by gentle prodding with a platinum wire. Animals that burst or crawled to the side of the plate were censored and discarded from this assay.

Reproduction assay
Wild type worms grown on each diet for at least 30 generations were egg prepped and eggs were allowed to hatch overnight for a synchronous L1 population. The next day, L1s were dropped on NGM plates seeded with each bacterial diet (OD 0.8). L4s hermaphrodites were singled 48 hours later onto individual plates and moved every 12 hours until egg laying ceased. Progeny were counted 48 hours after the singled hermaphrodite was moved to another plate. Progeny were removed from the plate during counting in order to ensure accuracy. Unfertilized oocytes were counted 24 hours after the singled hermaphrodite was moved to another plate.

Mated reproduction
Male wild type populations were raised for at least 10 generations before mating to wild type hermaphrodite populations grown for 30+ generations on each diet. Wild type males raised on Red and OP50 were egg prepped at the same time as wild type hermaphrodite populations raised on Red and OP50 and allowed to hatch overnight in M9 to create synchronous L1 populations. The next day, L1s were dropped onto each corresponding diet and allowed to grow for 48 hours into L4s. L4 hermaphrodites were singled onto a plate with 30μL of OP50 or Red bacteria seeded in the center of the NGM plate. A single male was placed with each hermaphrodite and allowed to mate for 24 hours before the hermaphrodite was moved to a new plate and allowed to egg lay. Hermaphrodites were moved every day to a new plate until egg laying ceases. Progeny were counted 48 hours after moving the hermaphrodite and plates were checked for ~50% males to ensure the hermaphrodite was mated. Progeny were removed from the plate during counting in order to ensure accuracy.

Nile Red Staining
Worms were grown on each bacterial diet for 48 or 72 hours, to the L4 stage or day 1 adult stage respectively and washed with PBST. Worms were then rocked for 3 minutes in 40% isopropyl alcohol before being pelleted and treated with Nile Red in 40% isopropyl alcohol for 2 hours. Worms were pelleted after 2 hours and washed in PBST for 30 minutes before being imaged at 10x magnification (L4) or 5x magnification (day 1 adult) with DIC and GFP filters on the Zeiss Axio Imager. Fluorescence is measured via corrected total cell fluorescence (CTCF) via ImageJ and Microsoft Excel. CTCF = Worm Integrated Density-(Area of selected cell X Mean fluorescence of background readings) and normalized to the control of OP50-reared worms.

Oil Red O Staining
Worms were grown on each bacterial diet for 48 or 120 hours, to the L4 stage or day 3 adult stage respectively and washed with PBST. Worms were then rocked for 3 minutes in 40% isopropyl alcohol before being pelleted and treated with Oil Red O in diH2O for 2 hours. Worms were pelleted after 2 hours and washed in PBST for 30 minutes before being imaged at 10x magnification (L4) or 5x magnification (day 3 adult) with the DIC filter on the Zeiss Axio Imager Erc color camera.

Pharyngeal pumping assays
Wild type worms grown on each diet for at least 30 generations were egg prepped and eggs were allowed to hatch overnight for a synchronous L1 population. The next day, L1s were dropped on NGM plates seeded with a bacterial diet (OP50/B, HT115/K-12, HB101/B&K-12, Red*/Methylobacterium,* Orange/*Xanthomonas* or Yellow*/Sphingomonas).* Before imaging, worms were singled onto plates seeded with a bacterial diet 1-2 hours before videoing pumping. For L4 pumping analysis, 10-12 worms were imaged 48 hours later via Movie Recorder at 8ms exposure using the ZEN 2 software at 10X magnification (Zeiss Axio Imager). For day 1 adult pumping analysis, 10-12 worms were imaged 72 hours after dropping L1s.

M9 thrashing assays
Wild type worms grown on each diet for at least 30 generations were egg prepped and eggs were allowed to hatch overnight for a synchronous L1 population. The next day, L1s were dropped on NGM plates seeded with each bacterial diet (OD 0.8). Worms were grown on each bacterial diet until the L4, day 1 adult, day 3 adult, day 8 adult or day 11 adult stage. Worms were then moved to an unseeded NGM plate to remove bacteria from the cuticle of the worms. After an hour, worms were washed with M9 and dropped in 5μL M9 drops onto a fresh NGM plate. After one minute, 10-12 worms were imaged via Movie Recorder at 50ms exposure using the ZEN 2 software (Zeiss Axio Imager).

Statistical analysis
Data are presented as mean ± SEM. Comparisons and significance were analyzed in Graphpad Prism 8. Comparisons between more than two groups were done using ANOVA. For multiple comparisons, Tukey's multiple comparison test was used and p-values are *$p<0.05$ **$p<0.01$ *** $p<0.001$ ****<0.0001. Lifespan comparisons were done with Log-rank test.

REFERENCES

1. Fontana, L., L. Partridge, and V.D. Longo, *Extending healthy life span--from yeast to humans.* Science, 2010. **328**(5976): p. 321-6.
2. Fitzpatrick, M., *Food fads.* Lancet, 2004. **363**(9405): p. 338.
3. Agatston, A.S., *The end of the diet debates? All fats and carbs are not created equal.* Cleve Clin J Med, 2005. **72**(10): p. 946-50.
4. Macneil, L.T. and A.J. Walhout, *Food, pathogen, signal: The multifaceted nature of a bacterial diet.* Worm, 2013. **2**(4): p. e26454.
5. Brooks, K.K., B. Liang, and J.L. Watts, *The influence of bacterial diet on fat storage in C. elegans.* PloS one, 2009. **4**(10): p. e7545.
6. Yen, C.A. and S.P. Curran, *Gene-diet interactions and aging in C. elegans.* Exp Gerontol, 2016. **86**: p. 106-112.
7. Brenner, S., *The genetics of Caenorhabditis elegans.* Genetics, 1974. **77**(1): p. 71-94.
8. Soukas, A.A., et al., *Rictor/TORC2 regulates fat metabolism, feeding, growth, and life span in Caenorhabditis elegans.* Genes & development, 2009. **23**(4): p. 496-511.
9. Hansen, M., T. Flatt, and H. Aguilaniu, *Reproduction, fat metabolism, and life span: what is the connection?* Cell metabolism, 2013. **17**(1): p. 10-9.
10. Bansal, A., et al., *Uncoupling lifespan and healthspan in Caenorhabditis elegans longevity mutants.* Proc Natl Acad Sci U S A, 2015. **112**(3): p. E277-86.
11. Coolon, J.D., et al., *Caenorhabditis elegans genomic response to soil bacteria predicts environment-specific genetic effects on life history traits.* PLoS Genet, 2009. **5**(6): p. e1000503.
12. Shtonda, B.B. and L. Avery, *Dietary choice behavior in Caenorhabditis elegans.* J Exp Biol, 2006. **209**(Pt 1): p. 89-102.
13. Maier, W., et al., *A neuromedin U receptor acts with the sensory system to modulate food type-dependent effects on C. elegans lifespan.* PLoS Biol, 2010. **8**(5): p. e1000376.
14. Watson, E., et al., *Integration of metabolic and gene regulatory networks modulates the C. elegans dietary response.* Cell, 2013. **153**(1): p. 253-66.
15. Watson, E., et al., *Interspecies Systems Biology Uncovers Metabolites Affecting C. elegans Gene Expression and Life History Traits.* Cell, 2014. **156**(6): p. 1336-1337.
16. Samuel, B.S., et al., *Caenorhabditis elegans responses to bacteria from its natural habitats.* Proc Natl Acad Sci U S A, 2016. **113**(27): p. E3941-9.
17. Xiao, R., et al., *RNAi Interrogation of Dietary Modulation of Development, Metabolism, Behavior, and Aging in C. elegans.* Cell Rep, 2015. **11**(7): p. 1123-33.
18. You, Y.J., et al., *Insulin, cGMP, and TGF-beta signals regulate food intake and quiescence in C. elegans: a model for satiety.* Cell Metab, 2008. **7**(3): p. 249-57.
19. Schulenburg, H. and M.A. Felix, *The Natural Biotic Environment of Caenorhabditis elegans.* Genetics, 2017. **206**(1): p. 55-86.
20. Frezal, L. and M.A. Felix, *C. elegans outside the Petri dish.* Elife, 2015. **4**.
21. Felix, M.A. and F. Duveau, *Population dynamics and habitat sharing of natural populations of Caenorhabditis elegans and C. briggsae.* BMC Biol, 2012. **10**: p. 59.
22. Petersen, C., P. Dirksen, and H. Schulenburg, *Why we need more ecology for genetic models such as C. elegans.* Trends Genet, 2015. **31**(3): p. 120-7.
23. Watson, E. and A.J. Walhout, *Caenorhabditis elegans metabolic gene regulatory networks govern the cellular economy.* Trends Endocrinol Metab, 2014. **25**(10): p. 502-8.
24. MacNeil, L.T., et al., *Diet-induced developmental acceleration independent of TOR and insulin in C. elegans.* Cell, 2013. **153**(1): p. 240-52.

25. Frand, A.R., S. Russel, and G. Ruvkun, *Functional genomic analysis of C. elegans molting.* PLoS Biol, 2005. **3**(10): p. e312.
26. Tuck, S., *The control of cell growth and body size in Caenorhabditis elegans.* Exp Cell Res, 2014. **321**(1): p. 71-6.
27. So, S., K. Miyahara, and Y. Ohshima, *Control of body size in C. elegans dependent on food and insulin/IGF-1 signal.* Genes Cells, 2011. **16**(6): p. 639-51.
28. Nagashima, T., S. Ishiura, and S. Suo, *Regulation of body size in Caenorhabditis elegans: effects of environmental factors and the nervous system.* Int J Dev Biol, 2017. **61**(6-7): p. 367-374.
29. Nagashima, T., et al., *Dopamine regulates body size in Caenorhabditis elegans.* Dev Biol, 2016. **412**(1): p. 128-138.
30. Wormbase, http://www.wormbase.org, 2006. **WS164**.
31. Zecic, A., I. Dhondt, and B.P. Braeckman, *The nutritional requirements of Caenorhabditis elegans.* Genes Nutr, 2019. **14**: p. 15.
32. Lynn, D.A., et al., *Omega-3 and -6 fatty acids allocate somatic and germline lipids to ensure fitness during nutrient and oxidative stress in Caenorhabditis elegans.* Proc Natl Acad Sci U S A, 2015. **112**(50): p. 15378-83.
33. Nhan, J.D., et al., *Redirection of SKN-1 abates the negative metabolic outcomes of a perceived pathogen infection.* Proc Natl Acad Sci U S A, 2019. **116**(44): p. 22322-22330.
34. Curran, S. and G. Ruvkun, *Lifespan regulation by evolutionarily conserved genes essential for viability.* PLoS Genet, 2007. **3**(4): p. e56.
35. Tacutu, R., et al., *Prediction of C. elegans Longevity Genes by Human and Worm Longevity Networks.* PloS one, 2012. **7**(10): p. e48282.
36. Dalton, H.M. and S.P. Curran, *Hypodermal responses to protein synthesis inhibition induce systemic developmental arrest and AMPK-dependent survival in Caenorhabditis elegans.* PLoS Genet, 2018. **14**(7): p. e1007520.
37. Cassada, R. and R. Russell, *The dauerlarva, a post-embryonic developmental variant of the nematode Caenorhabditis elegans.* Dev Biol, 1975. **46**(2): p. 326-42.
38. Baugh, L. and P. Sternberg, *DAF-16/FOXO regulates transcription of cki-1/Cip/Kip and repression of lin-4 during C. elegans L1 arrest.* Curr Biol, 2006. **16**(8): p. 780-5.
39. Avery, L., *The genetics of feeding in Caenorhabditis elegans.* Genetics, 1993. **133**(4): p. 897-917.
40. Hirsh, D., D. Oppenheim, and M. Klass, *Development of the reproductive system of Caenorhabditis elegans.* Dev Biol, 1976. **49**(1): p. 200-19.
41. Ward, S. and J.S. Carrel, *Fertilization and sperm competition in the nematode Caenorhabditis elegans.* Developmental biology, 1979. **73**(2): p. 304-21.
42. Kapahi, P., M. Kaeberlein, and M. Hansen, *Dietary restriction and lifespan: Lessons from invertebrate models.* Ageing Res Rev, 2017. **39**: p. 3-14.
43. Matai, L., et al., *Dietary restriction improves proteostasis and increases life span through endoplasmic reticulum hormesis.* Proc Natl Acad Sci U S A, 2019. **116**(35): p. 17383-17392.
44. Keith, S.A., et al., *The C. elegans healthspan and stress-resistance assay toolkit.* Methods, 2014. **68**(3): p. 476-86.
45. Brooks, D.R., et al., *An essential role in molting and morphogenesis of Caenorhabditis elegans for ACN-1, a novel member of the angiotensin-converting enzyme family that lacks a metallopeptidase active site.* J Biol Chem, 2003. **278**(52): p. 52340-6.
46. Meli, V.S., et al., *MLT-10 defines a family of DUF644 and proline-rich repeat proteins involved in the molting cycle of Caenorhabditis elegans.* Mol Biol Cell, 2010. **21**(10): p. 1648-61.
47. Zugasti, O., J. Rajan, and P.E. Kuwabara, *The function and expansion of the Patched- and Hedgehog-related homologs in C. elegans.* Genome Res, 2005. **15**(10): p. 1402-10.

48. Mair, W., et al., *Demography of dietary restriction and death in Drosophila.* Science, 2003. **301**(5640): p. 1731-3.
49. Greer, E.L. and A. Brunet, *Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in C. elegans.* Aging cell, 2009. **8**(2): p. 113-27.
50. Pang, S. and S.P. Curran, *Adaptive Capacity to Bacterial Diet Modulates Aging in C. elegans.* Cell metabolism, 2014. **19**(2): p. 221-31.
51. Gomez-Orte, E., et al., *Effect of the diet type and temperature on the C. elegans transcriptome.* Oncotarget, 2018. **9**(11): p. 9556-9571.
52. Yen, C.A., et al., *Loss of flavin adenine dinucleotide (FAD) impairs sperm function and male reproductive advantage in C. elegans.* Elife, 2020. **9**.
53. Volkers, R.J., et al., *Gene-environment and protein-degradation signatures characterize genomic and phenotypic diversity in wild Caenorhabditis elegans populations.* BMC Biol, 2013. **11**: p. 93.
54. Schumacker, S.T., et al., *RNA sequencing dataset characterizing transcriptomic responses to dietary changes in Caenorhabditis elegans.* Data Brief, 2019. **25**: p. 104006.
55. Kim, D.H., *Bacteria and the aging and longevity of Caenorhabditis elegans.* Annu Rev Genet, 2013. **47**: p. 233-46.
56. Zhang, J., et al., *A Delicate Balance between Bacterial Iron and Reactive Oxygen Species Supports Optimal C. elegans Development.* Cell Host Microbe, 2019. **26**(3): p. 400-411 e3.
57. Avery, L. and B.B. Shtonda, *Food transport in the C. elegans pharynx.* J Exp Biol, 2003. **206**(Pt 14): p. 2441-57.
58. Moore, R.S., R. Kaletsky, and C.T. Murphy, *Piwi/PRG-1 Argonaute and TGF-beta Mediate Transgenerational Learned Pathogenic Avoidance.* Cell, 2019. **177**(7): p. 1827-1841 e12.
59. Denzel, M.S., L.R. Lapierre, and H.I.D. Mack, *Emerging topics in C. elegans aging research: Transcriptional regulation, stress response and epigenetics.* Mech Ageing Dev, 2019. **177**: p. 4-21.
60. Rollins, J.A., et al., *Dietary restriction induces posttranscriptional regulation of longevity genes.* Life Sci Alliance, 2019. **2**(4).
61. Kumar, S., et al., *Lifespan Extension in C. elegans Caused by Bacterial Colonization of the Intestine and Subsequent Activation of an Innate Immune Response.* Dev Cell, 2019. **49**(1): p. 100-117 e6.
62. Catterson, J.H., et al., *Short-Term, Intermittent Fasting Induces Long-Lasting Gut Health and TOR-Independent Lifespan Extension.* Curr Biol, 2018. **28**(11): p. 1714-1724 e4.
63. Polivy, J., *Psychological consequences of food restriction.* J Am Diet Assoc, 1996. **96**(6): p. 589-92; quiz 593-4.
64. Redman, L.M., et al., *Effect of caloric restriction in non-obese humans on physiological, psychological and behavioral outcomes.* Physiol Behav, 2008. **94**(5): p. 643-8.
65. Norwood, R., et al., *The psychological characteristics of people consuming vegetarian, vegan, paleo, gluten free and weight loss dietary patterns.* Obes Sci Pract, 2019. **5**(2): p. 148-158.