

UPR^{ER}–immunity axis acts as physiological food evaluation system that promotes aversion behavior in sensing low-quality food

Reviewed Preprint

v2 • August 5, 2024

Revised by authors

Reviewed Preprint

v1 • January 23, 2024

Pengfei Liu, Xinyi Liu, Bin Qi ✉

Southwest United Graduate School, Yunnan Key Laboratory of Cell Metabolism and Diseases, State Key Laboratory of Conservation and Utilization of Bio-resources in Yunnan, Center for Life Sciences, School of Life Sciences, Yunnan University, Kunming, China

 https://en.wikipedia.org/wiki/Open_access
 Copyright information

Abstract

To survive in challenging environments, animals must develop a system to assess food quality and adjust their feeding behavior accordingly. However, the mechanisms that regulate this chronic physiological food evaluation system, which monitors specific nutrients from ingested food and influences food-response behavior, are still not fully understood. Here, we established a low-quality food evaluation assay system and found that heat-killed *E. coli* (HK-*E. coli*), a low sugar food, triggers cellular UPR^{ER} and immune response. This encourages animals to avoid low-quality food. The physiological system for evaluating low-quality food depends on the UPR^{ER} (IRE-1/XBP-1) – Innate immunity (PMK-1/p38 MAPK) axis, particularly its neuronal function, which subsequently regulates feeding behaviors. Moreover, animals can adapt to a low-quality food environment through sugar supplementation, which inhibits the UPR^{ER} – PMK-1 regulated stress response by increasing vitamin C biosynthesis. This study reveals the role of the cellular stress response pathway as physiological food evaluation system for assessing nutritional deficiencies in food, thereby enhancing survival in natural environments.

eLife assessment

This **valuable** work uses unbiased approaches to discover critical molecules in *C. elegans* and its bacterial food for nutrition sensing and food choice, providing a framework for other studies. The data **convincingly** support their model that *C. elegans* uses UPR^{ER} and immune response pathways to evaluate sugar contents in the bacteria to change their behaviors.

<https://doi.org/10.7554/eLife.94181.2.sa3>

Introduction

Food is essential for the survival, growth and fitness of all animals. To adapt to fluctuating environments with a wide range of food sources, animals have developed a food evaluation system. This system enables them to identify nutrient-rich food and avoids low-quality or toxic food, thereby maximizing their survival prospects ^{1,2,3}. Various sensory neuron evaluation systems in animals has evolved to evaluate food quality through vision ^{4,5}, olfactory ^{3,6,7,8,9,10,11,12} and gustatory senses ^{13,14,15}. Besides these sensory systems that facilitate quick feeding decisions, animals may also initiate cellular stress response programs to detect nutrition/toxin and trigger food response behaviors ^{16,17}. This could be one of physiological food quality evaluation systems that monitor the nutritional status of consumed food. However, the signaling events in cellular stress responses involved in evaluating of specific nutrients and the mechanisms that connect these signaling activities to food behaviors are largely unexplored. More specifically, while cellular stress response through UPR^{ER} ¹⁸ and PMK-1/p38 MAPK ¹⁹ dependent immunity in response to pathogens have been extensively studied, the functions of these cellular stress response in sensing and evaluating specific nutrients from food remain unclear.

Vitamin C is an essential micronutrient that cannot be synthesized by humans due to the loss of a key enzyme in the biosynthetic pathway ²⁰. Animals obtain vitamin C from their diet and possibly also from gut microbes ²¹. Vitamin C is an important physiological antioxidant and a cofactor for a family of biosynthetic and gene regulatory monooxygenase and dioxygenase enzymes. It is also required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters ^{22,23}. Vitamin C has been associated with various human diseases including scurvy, immune defect and cardiovascular disease ²⁰. Therefore, in animals, having robust food evaluation systems to detect vitamin C levels could significantly impact their survival in the wild. However, the potential involvement of the cellular stress response pathway in this food evaluation system for sensing and assessing vitamin C remains largely unexplored.

In this study, using the low-quality food evaluation assay system we established ²⁴, we elucidated the mechanism by which the cellular stress response pathway operates as a physiological food evaluation system. This pathway assesses the deficiency of D-glucose in food and the subsequent vitamin C content in animals through the unfolded protein response (UPR^{ER}) – innate immunity (PMK-1/p38 MAPK) axis. This mechanism promotes animals to leave low-quality food and is critical for their survival in nature environments.

Results

Low-quality food induces stress response in animals

Our previous studies have shown that Heat-killed *E. coli* (HK-*E. coli*), which lacks certain molecules, is considered a low-quality food that is unable to support animal growth ^{24,25}. Moreover, through metabolic-seq analysis, we identified significant changes in a large numbers of derivatives (Figure 1 – figure supplement 1A, Table S1), including lipids and their derivatives (Figure 1-figure supplement 1B, Table S1), amino acid and their metabolites (Figure 1-figure supplement 1C, Table S1), as well as coenzymes and vitamins (Figure 1-figure supplement 1D, Table S1). Interestingly, we observed a significant decrease in carboxylic acids and their derivatives (Figure 1-figure supplement 1E, Table S1) in *E. coli* after being heat-killed (Figure 1-figure supplement 1F, Table S1). This suggests that HK-*E. coli* is nutritionally deficient for *C. elegans* when compared to normal *E. coli* food.

Next, we conducted two behavior assays to facilitate the analysis of the food evaluation process in animals by seeding L1 animals in assay plates (**Figure 1A** and **1B**). In the avoidance assay, wild-type animals avoided the HK-*E. coli* OP50 (HK-OP50) food (**Figure 1A**). Interestingly, in the food choice assay, animals initially showed no preference between the two types of food (1-2h), but eventually exhibited a preference for high-quality food (Live *E. coli*) up until the 17h mark (**Figure 1B**, Figure 1-figure supplement 1G). This suggests that worms depart from the HK-*E. coli* after recognizing it as low-quality food source through ingestion.

In order to investigate the pathways in animals that respond to HK-*E. coli*, we performed transcriptomics analysis on worms that were cultured with both HK-*E. coli* and Live *E. coli*. Gene-expression profiling revealed that stress response genes, including those related to biotic stimulus, immune response and response to stress, are up-regulated in animals fed with HK-*E. coli* OP50 (HK-OP50) (**Figure 1C**, Table S2). Among these up-regulated genes, we identified 11 out of 62 of UPR^{ER} target genes (**Figure 1D**, Figure 1-figure supplement 1H and Table S2). Additionally, about 50%-80% of up-regulated genes overlap with genes responding pathogenic bacteria ²⁶, ²⁷ (**Figure 1E**, Table S2). Consistent with the results of the RNA sequencing (RNA-seq) analysis, the UPR^{ER} reporter (*Phsp-4::GFP*)²⁸ and immunity reporter (*Pirg-5::GFP*)²⁹ were strongly induced in intestine (**Figure 1F-G**) and neurons (Figure 1-figure supplement 2A) by feeding unfavorable food (HK-*E. coli* OP50), suggesting that UPR^{ER} and immune pathways may respond to low-quality food (HK-*E. coli* OP50). As intestinal fluorescence (*Phsp-4::GFP* or *Pirg-5::GFP*) is easy observation and scoring, the further analyses were done in the intestine.

Moreover, UPR^{Mt} reporter (*Phsp-6::GFP*)³⁰ was weakly induced under HK-*E. coli* feeding condition (**Figure 1**-figure supplement 2B), and starved worm did not induce UPR^{ER} and immunity (Figure 1-figure supplement 2C-D).

Together, these findings suggest that low-quality food (HK-*E. coli* OP50) triggers a stress response pathway in animals, including UPR^{ER} and innate immune pathway. This implies that animals may assess the quality of food through UPR^{ER} and innate immune pathway.

Animals evaluate food quality through UPR^{ER}-immune-dependent physiological food quality evaluation system

To determine whether the UPR^{ER} and innate immune pathways play a role in evaluating low-quality food, we first examined whether the activation of the UPR^{ER} by HK-*E. coli* was dependent on the known signaling components of the UPR^{ER} branches, including IRE/XBP-1, PERK/ATF-4 and ATF-6 ³¹, ³². We observed no difference in *Phsp-4::GFP* induction with *atf-4* (Figure 2-figure supplement 1A) or *atf-6* (Figure 2-figure supplement 1B) RNAi-mediated knockdown in animals fed with HK-*E. coli*. However, knockdown of *ire-1/xbp-1* or mutation of *xbp-1* reduced GFP fluorescence (**Figure 2A**, 2B). Among the 11 differentially expressed UPR^{ER} target genes in animals fed with HK-*E. coli* from RNA-seq (**Figure 1D**), 64% of the genes are IRE-mediated genes (Figure 1 – figure supplement 1H, Table S2). The mRNA level of IRE-1-mediated splicing of *xbp-1*²⁸ is also induced in animals fed with HK-*E. coli* OP50 (Figure 2-figure supplement 1C). However, UPR^{ER} is not affect in animals feeding live-*E. coli* by RNAi of *ire-1*, *xpb-1*, *atf-4* and *atf-6* (Figure 2-figure supplement 1D). These data suggest that activation of the UPR^{ER} by low-quality food (HK-*E. coli*) depends on the IRE-1/XBP-1.

To further analyze whether XBP-1-dependent UPR^{ER} activation is critical for animals to leave low-quality food, we tested food avoidance behavior using *xbp-1* mutant. The results show that *xbp-1* mutants had a significantly decreased likelihood of leaving of HK-*E. coli*, which was rescued by expressing *xbp-1* in neuron rather than intestine (**Figure 2C**). This indicates that XBP-1-dependent UPR^{ER} activation in neuron is critical for animals to specific evaluate low-quality food (HK-*E. coli*).

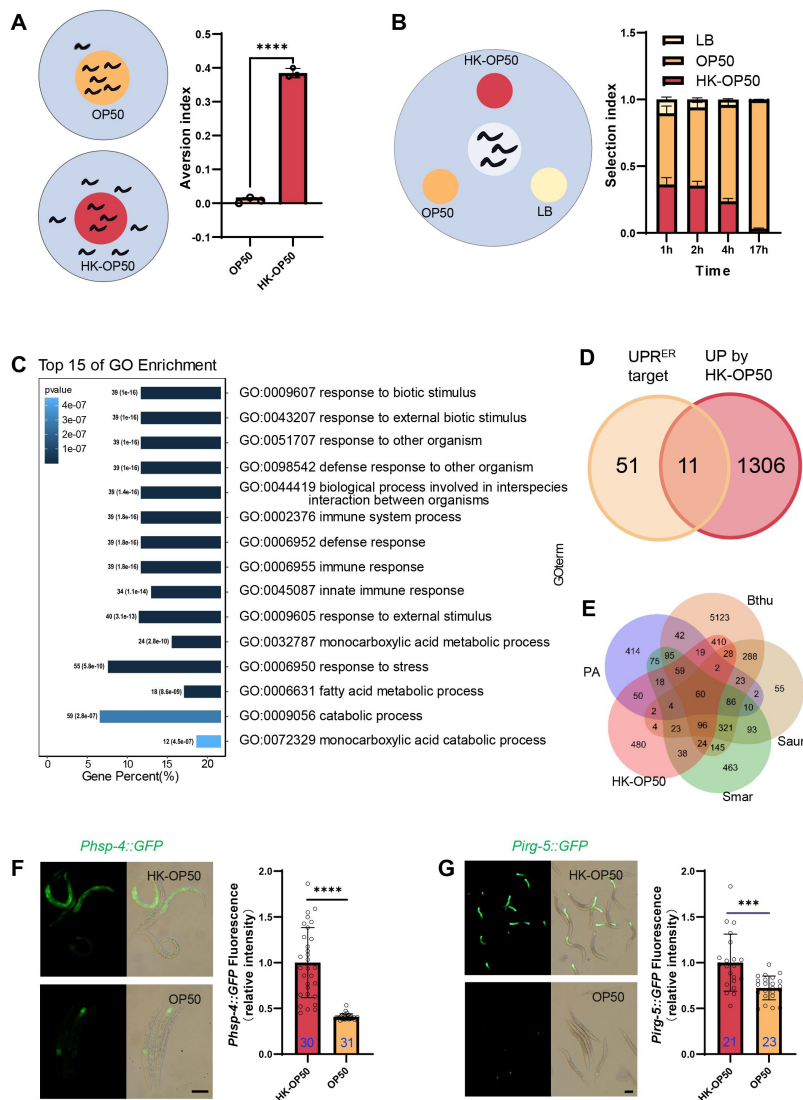


Figure 1.

The stress response is induced in animals fed low-quality food, HK-*E. coli*.

(A) Schematic drawing and quantitative data of the food aversion assay. Circles indicate the food spot for live (yellow) and HK-OP50 (red) bacteria, respectively. The animals were scored 16-17 hours after L1 worms were placed on the food spot. Data are represented as mean \pm SD from three independent experiments, 79-129 animals/assay.

(B) Schematic method and quantitative data of the food selection assay. Live (yellow), heat-killed (red) *E. coli* and LB as the buffer for *E. coli* were placed on indicated position. Synchronized L1 worms were placed in the center spot. The selection index was calculated at the indicated time. Data are represented as mean \pm SD from eight independent experiments, 123-792 animals/assay.

(C) GO enrichment analysis of up-regulated genes in animals fed with HK-*E. coli* vs live *E. coli*.

(D) Venn diagram showing numbers of UPR^{ER} target genes and up-regulated genes in animals fed HK-*E. coli*, and their overlap.

(E) Venn diagram showing numbers of induction genes by four pathogenic bacteria and HK-*E. coli* induced genes, and their overlap. The gene expression data was extracted from published data of animals' infection with *Pseudomonas aeruginosa* (PA) ²⁶, *Bacillus thuringiensis* (Bthu) ²⁷, *Staphylococcus aureus* (Saur) ²⁷, and *Serratia marcescens* (Smar) ²⁷.

(F-G) GFP fluorescence images and bar graph showing that *Phsp-4::GFP* (F) and *Pirg-5::GFP* (G) were induced in animals fed with HK-*E. coli*. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

For all panels, Scale bar shows on indicated figures, 50 μ m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

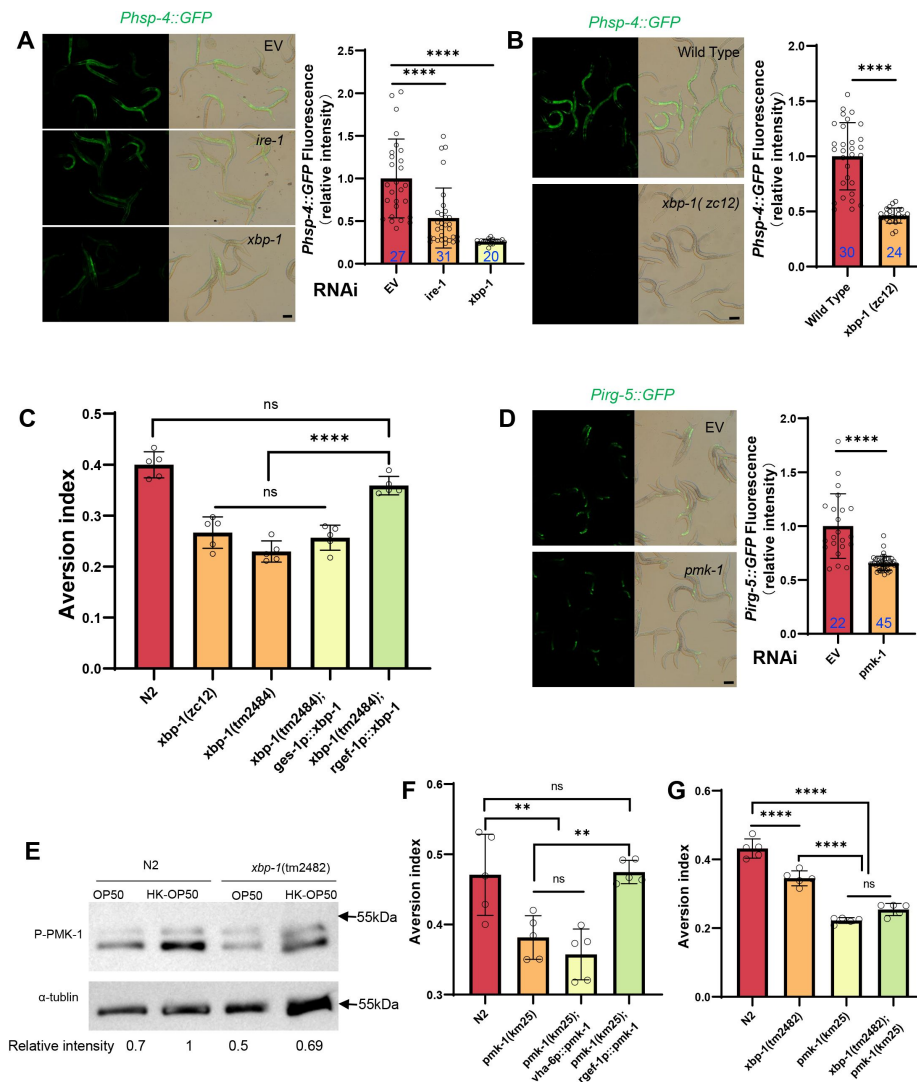


Figure 2.

Animals evaluate food quality through UPR^{ER} (*ire-1/xbp-1*) - Innate immunity (*pmk-1* MAPK) axis.

(A-B) GFP fluorescence images and bar graph showing that HK-*E. coli* induced *Phsp-4::GFP* was decreased in animals with *ire-1* or *xbp-1* RNAi treatment (A) or *xbp-1* mutation (B). Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean ± SD.

(C) Food aversion assay showing that *xbp-1* mutation eliminated the discrimination against HK-*E. coli*. However, this effect is rescued by expressing *xbp-1* in neurons rather than intestine. Data are represented as mean ± SD from five independent experiments, 156-763 animals/assay.

(D) GFP fluorescence images and bar graph showing that HK-*E. coli* induced *Pirg-5::GFP* was decreased in animals with *pmk-1* RNAi treatment. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean ± SD.

(E) Western blot images showing the level of p-PMK-1 in L1 animals (Wild-type N2 and *xbp-1* mutant) fed with OP50 or HK-OP50 for 4 h. The level of p-PMK-1 is induced in animals fed HK-OP50.

(F) Food aversion assay showing that *pmk-1* mutation eliminated the discrimination against HK-*E. coli*. However, this effect is rescued by expressing *pmk-1* in neurons rather than intestine. Data are represented as mean ± SD from five independent experiments, 168-492 animals/assay.

(G) Food aversion assay in wild-type, *xbp-1*, *pmk-1* and double mutant. Data are represented as mean ± SD from five independent experiments, 259-490 animals/assay.

For all panels, Scale bar shows on indicated figures, 50 μm. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, ns: no significant difference. Precise P values are provided in Raw Data.

We then investigated which innate immune pathway is involved in evaluating low-quality food. First, we analyzed HK-*E. coli* induced genes from RNA-seq. Among these up-regulated genes, 82 out of 409 of PMK-1-dependent genes³³ were identified (Figure 2-figure supplement 1E, Table S2). Second, we confirmed the induction of several well-known PMK-1 target genes in RNA-seq data³⁴ (Figure 2-figure supplement 1F) and reporter analysis (Figure 2-figure supplement 1G-H). Moreover, the induction of *Pirg-5::GFP* was abolished in *pmk-1* knockdown animals fed with HK-*E. coli* (Figure 2D). Third, we found that the phosphorylated PMK-1 (p-PMK-1) level was prominently increased in wild-type N2 animals fed HK-*E. coli* compared to feeding *E. coli* OP50 (Figure 2E). Finally, *pmk-1* mutants had a decreased likelihood of leaving of HK-*E. coli*, which was rescued by expressing *pmk-1* in neurons rather than intestine (Figure 2F). Moreover, *Pirg-5::GFP* is not affected in animals feeding live-*E. coli* by RNAi of *pmk-1* (Figure 2-figure supplement 1I). These data suggest that PMK-1 regulated immune pathway evaluates low-quality food, especially the neuronal PMK-1 has a critical function for food quality response.

XBP-1 and PMK-1 are in the same pathway for evaluating food quality

Next, we explored the connection between UPR^{ER} (IRE-1/XBP-1) and innate immunity (PMK-1 p38 MAPK) in food quality evaluation. We found that *Pirg-5::GFP* induction (Figure 2-figure supplement 1I, 2A) and PMK-1 activation (Figure 2E) were decreased in animals with *xbp-1* mutation or knockdown when fed with HK-*E. coli*, suggesting that XBP-1 could regulate PMK-1 under this condition. Additionally, *Phsp-4::GFP* induction under HK-*E. coli* was not affected in animals with *pmk-1* RNAi (Figure 2-figure supplement 1D, 2B), indicating that XBP-1-dependent UPR^{ER} activation is not regulated by PMK-1. Finally, we constructed a double mutant of *xbp-1* and *pmk-1* and found that the food avoidance phenotype of the double mutant was similar to the *pmk-1* mutant (Figure 2G), indicating that PMK-1 is downstream of XBP-1 in responding to low-quality food.

We then asked whether UPR^{ER} (IRE-1/XBP-1) – Innate immunity (PMK-1/p38 MAPK) axis is specific to evaluate low-quality food (HK-*E. coli*). We performed behavior assay in N2, *pmk-1* and *xbp-1* mutant animals by feeding normal *E. coli* food, inedible food (*Saprophytic staphylococci*)³⁵ and pathogenic food (*Pseudomonas aeruginosa*-PA14)¹⁸. We found that N2, *pmk-1*, and *xbp-1* mutant worms did not exhibit avoidance behavior when presented with normal food (OP50). However, both N2 and *xbp-1* mutant worms were able to escape from inedible food (N2 was predominantly found on the border areas of the bacterial lawn and *xbp-1* mutant worms on border and in), *Saprophytic staphylococci*, whereas *pmk-1* mutant worms did not exhibit this avoidance behavior. Notably, N2 and *xbp-1* mutant worms exhibited even more pronounced avoidance behavior when exposed to *Pseudomonas aeruginosa*, whereas *pmk-1* mutant worms were more susceptible to infection by this pathogen (Figure 2-figure supplement 2C). These findings suggest that the UPR-Immunity pathway plays a crucial role in helping animals avoid low-quality food (HK-*E. coli*) by triggering an avoidance response. In contrast, the Innate immunity pathway, which is mediated by PMK-1/p38 MAPK, appears to play a key role in evaluating unfavorable food sources, such as HK-*E. coli*, *Saprophytic staphylococci*, and *Pseudomonas aeruginosa*, and helping animals avoid these environments.

Sugar deficiency in HK-*E. coli* food induces stress response and avoidance behavior in animals

We then investigated which nutrients/metabolites are sensed by animals through the XBP-1-PMK-1 axis for food quality evaluation. First, we hypothesized that the nutrition status is improved in *E. coli* mutant (HK-treatment), which could inhibit UPR^{ER} and immune response in animals. We established a system for screening the *E. coli* mutant Keio library (Figure 3 – figure supplement 1A), and identified 20 *E. coli* mutants that did not induce *Phsp-4::GFP* through the UPR^{ER} reporter (*Phsp-4::GFP*) after three rounds of screening (Table S3). From these 20 *E. coli* mutants, we

identified 9 *E. coli* mutants that did not induce *Pirg-5::GFP* through the immunity reporter (*Pirg-5::GFP*) screening (Figure 3-figure supplement 1B-C, Table S3). Animals fed HK-*yfbR*, which catalyzes carbohydrate derivative metabolic process³⁶, had a decreased ability to leave food (Figure 3A, Table S3), indicating that HK-*yfbR* may be a higher quality food for animals compared to HK-K12.

Secondly, we performed a metabolomics analysis of different quality food (HK-K12, HK-*yfbR* and Live-K12). We found that the level of 13 metabolites were similar between HK-*yfbR* and Live-K12, but significantly changed in HK-K12 (Figure 3B, Figure 3-figure supplement 2A, and Table S1). We also found that genes involved in glycolysis/gluconeogenesis were up-regulated in animals fed with HK-*E. coli* (Figure 3-figure supplement 2B), suggesting that glycolysis/gluconeogenesis metabolism is disordered in animals fed with HK-*E. coli*, which may result from changes in sugar/carbohydrate intake. The carbohydrates (D-trehalose, lactose, and D-(+)-sucrose) were also decreased in HK-*E. coli* (Figure 3B), suggesting that carbohydrate deficiency may induce stress response and avoidance behavior in animals feeding HK-*E. coli*.

Thirdly, to determine which carbohydrate inhibits stress response in animals, we supplemented each metabolite to HK-*E. coli* and found that only Lactose, and D-(+)-sucrose inhibited HK-*E. coli* induced UPR^{ER} (Figure 3C and 3D, Figure 3-figure supplement 2C). Moreover, we found from our metabolomic data that the sugar level, including lactose, and D-(+)-sucrose, and D-(+)-glucose, was also decreased in HK-*E. coli* (Figure 3B, Table S1). Since lactose and D-(+)-sucrose are hydrolyzed to produce glucose^{37, 38}, we wondered whether glucose also inhibits the stress response in animals. We found that D-(+)-glucose supplementation also inhibited HK-*E. coli* induced UPR^{ER} (Figure 3E), immune response (Figure 3F, 3G and Figure 3-figure supplement 2D) and avoidance (Figure 3H). Moreover, sugar supplementation did not affect UPR^{ER} and immunity in normal food (OP50) or starved condition (NGM) (Figure 3 – figure supplement 2E-F). While sugar effectively inhibits the HK-*E. coli*-induced UPR^{ER} and immune response, it does not fully suppress it to the extent observed with live-*E. coli* (Figure 3C-F). This implies that additional nutrients present in live-*E. coli* might also contribute to the inhibition of UPR^{ER} and immune response.

Previous studies have shown that heat-killed *E. coli* (HK-*E. coli*) is a low-quality food source that cannot support the growth of *C. elegans* larvae^{24, 25}, whereas supplementation with vitamin B2 (VB2) can restore animal growth²⁴. Here, we found that sugar deficiency in HK-*E. coli* induces the UPR^{ER}-immune response and avoidance behavior in *C. elegans*. Given this, we investigated whether sugar supplementation could promote animal growth when fed HK-*E. coli*. To our surprise, supplementing HK-*E. coli* with carbohydrates (D-Glc, D-GlcA) did not support animal development (Figure 3-figure supplement 2G), suggesting that carbohydrates are not essential for supporting animal growth on this food source. However, we did find that carbohydrates are critical for inhibiting the UPR^{ER}-immune response induced by sugar deficiency in HK-*E. coli*.

Together, these findings suggest that HK-*E. coli* induces a stress response and avoidance behavior in animals, which can be inhibited by D-(+)-glucose supplementation. This implies that animals may evaluate the sugar deficiency from HK-*E. coli* through the activation of UPR^{ER} and immune responses.

Animals could overcome a low-quality food environment by sugar supplementation through vitamin C biosynthesis

We discovered that D-(+)-glucose supplementation inhibited HK-*E. coli* induced UPR^{ER} (Figure 3E), immune response (Figure 3F, 3G and Figure 3-figure supplement 2D) and avoidance (Figure 3H). Simultaneously, vitamin C (VC), which is synthesized by glucuronate pathway using D-glucose^{39, 40} (Figure 4A), was found to contribute to neuroprotective^{41, 42}, immune

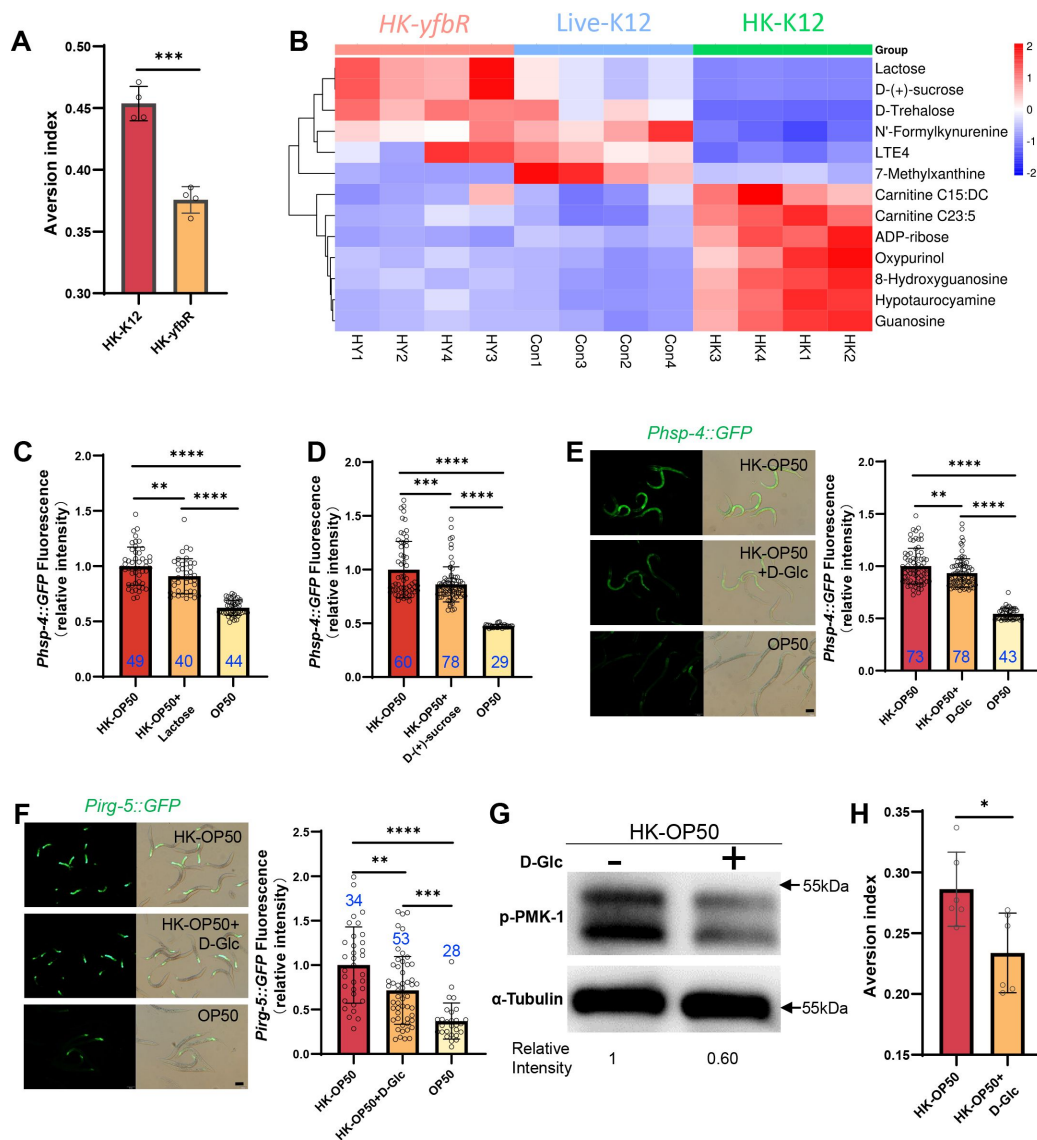


Figure 3.

HK-*E. coli* is low sugar food, which induce stress response and avoidance behavior in animals.

(A) Food aversion assay showing that wild-type animals eliminated the discrimination against HK-*E. coli* when *yfbR* is mutated in *E. coli*. Data are represented as mean \pm SD four independent experiments, 251-490 animals/assay.

(B) Heat map showing the thirteen differential metabolites from HK-K12, HK-*yfbR*, and K12 in 4 independent experiments. Color indicates the relative level of each metabolite.

(C-D) The bar graph showing that HK-*E. coli* induced *Phsp-4::GFP* was decreased in animals with lactose (C) or D-(+)-sucrose (D) supplementation. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(E-F) GFP fluorescence images and bar graph showing that HK-*E. coli* induced *Phsp-4::GFP* (E) and *Pirg-5::GFP* (F) were decreased in animals with D-(+)-glucose (D-Glc) supplementation. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(G) Western blot images showing the level of p-PMK-1 in L1 animals fed HK-*E. coli* with or without D-(+)-glucose (D-Glc) supplementation for 4 h. The level of p-PMK-1 is decreased in animals fed HK-OP50+D-Glc.

(H) Food aversion assay showing that wild-type animals eliminated the discrimination against HK-*E. coli* with D-Glc supplementation. Data are represented as mean \pm SD six independent experiments, 190-492 animals/assay.

For all panels, Scale bar shows on indicated figures, 50 μ m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

defense⁴³,⁴⁴, and inhibits inflammatory and ER stress⁴⁵,⁴⁶. This led us to question whether the vitamin C biosynthesis pathway is involved in evaluating low-quality food by using D-glucose.

Firstly, we observed an increase in the vitamin C level in *C. elegans* when fed with HK-*yfbR* (Figure 4B), a high carbohydrate food compared to HK-*E. coli* (Figure 3B and Figure 1-figure supplement 1E). However, the VC level in bacteria is the same (Figure 4-figure supplement 1A). The VC level also increased when D-glucose (Figure 4-figure supplement 1B) or D-glucuronate (D-GlcA) was added to HK-*E. coli* (Figure 4C), which was abolished by knocking down VC biosynthesis genes (Figure 4C and Figure 4-figure supplement 1B). This suggests that addition of sugar supplementation promotes VC synthesis in animals fed with HK-*E. coli*.

Secondly, we hypothesized that animals could overcome a low-quality food (HK-*E. coli*) environment by inhibiting the stress response through increasing vitamin C biosynthesis. We found that VC or D-glucuronate (D-GlcA) supplementation inhibits HK-*E. coli* induced UPR^{ER} (Figure 4D), immune response including *irg-5/sysm-1* reporter expression (Figure 4E and Figure 4-figure supplement 1C) and p-PMK-1 (Figure 4F and 4G), as well as food avoidance (Figure 4H).

Finally, we asked whether inhibition of stress response and avoidance by sugar supplementation depends on the vitamin C biosynthesis pathway. We found that suppression of HK-*E. coli* induced UPR^{ER} (Figure 4I), immune response (Figure 4J and Figure 4-figure supplement 1D) and food avoidance (Figure 4K) by D-GlcA/sugar supplementation was abolished in animals with RNAi of VC biosynthesis genes. Food selection behavior assays showed that D-GlcA/sugar supplementation increased the preference for heat-killed bacteria, which was also suppressed by knocking down VC biosynthesis genes (Figure 4-figure supplement 1E). However, VC supplementation still suppressed the UPR^{ER} (Figure 4I), immune response (Figure 4J and Figure 4-figure supplement 1D) and food avoidance (Figure 4K), and increased the food preference (Figure 4-figure supplement 1E) in animals with or without RNAi of VC biosynthesis genes. This suggests that VC, as the final metabolite synthesized from D-glucose, is critical for evaluating low-quality food response in animals.

Together, these data indicate that the vitamin C biosynthesis pathway is critical for evaluating whether food is of higher quality and can be eaten by animals. It also suggests that animals could improve their VC levels to adapt to bad food environment.

Animals evaluate sugar and vitamin C through neuronal XBP-1 and PMK-1

As D-GlcA/sugar and VC supplementation suppressed HK-*E. coli* induced UPR^{ER}, immune response and food avoidance behavior, we investigated whether animals evaluate sugar and VC through XBP and PMK-1 dependent pathways. We performed a food selection behavior assay by adding D-Glc, D-GlcA or VC to the NGM, *E. coli* and HK-*E. coli* (Figure 5A). The food selection behavior assays revealed that supplementation with D-Glc, D-GlcA, or VC inhibits the animals' choice of sugar or VC on *E. coli*-OP50 feeding conditions (Figure 5-figure supplement 1A). This suggests that supplementation with D-Glc, D-GlcA, or VC may alter the metabolites of live bacteria, leading to avoidance by the animals. There was no preference observed on NGM plate (no food condition) supplementation with D-Glc and VC (Figure 5-figure supplement 1B), indicating that the intake of sugar or VC alone does not influence animal preference. However, alone D-GlcA could influence worm physiology which induces preference change (Figure 5-figure supplement 1B). Interestingly, D-Glc and D-GlcA (Figure 5B and 5C) or VC (Figure 5D) supplementation increased the preference for heat-killed bacteria, which was suppressed in *xbp-1* or *pmk-1* mutant animals. However, this preference was also rescued in *xbp-1* or *pmk-1* mutant animals by expressing XBP-1

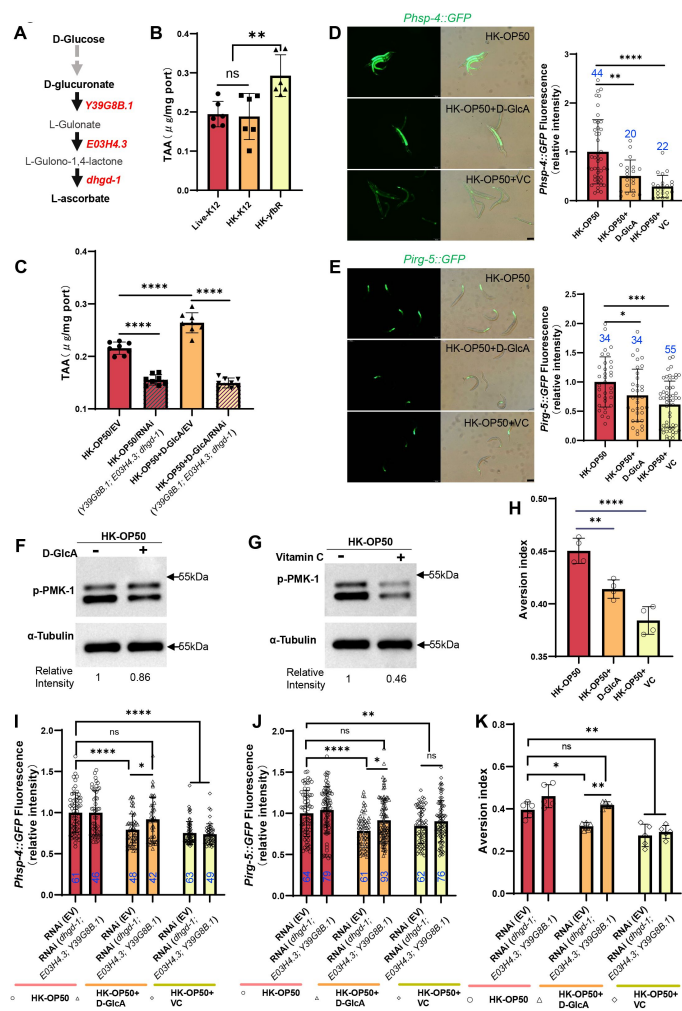


Figure 4.

Vitamin C biosynthesis pathway is critically involved in evaluating sugar in the food

(A) Cartoon illustration of a simplified, Vitamin C biosynthesis pathway in *C. elegans*. The relevant coding genes of enzymes were labeled with red.

(B) The level of total L-ascorbic acid (TAA), also known as vitamin C, in animals fed with Live-K12, HK-K12, or HK-*yfbr*. Data are represented as mean \pm SD from six independent experiments.

(C) The level of total L-ascorbic acid (TAA) in animals (control or knockdown of Vitamin C biosynthesis genes) fed with HK-*E. coli* with or without D-glucuronate (D-GlcA) supplementation. Data are represented as mean \pm SD from eight independent experiments.

(D-E) GFP fluorescence images and bar graph showing that HK-*E. coli* induced *Phsp-4::GFP* (D) and *Pirg-5::GFP* (E) were decreased in animals with D-GlcA or Vitamin C supplementation. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(F-G) Western blot images showing the level of p-PMK-1 in L1 animals fed with HK-*E. coli* with D-GlcA or Vitamin C supplementation for 4 h. The level of p-PMK-1 is decreased in animals with D-GlcA (F) or Vitamin C (G) supplementation.

(H) Food aversion assay showing that wild-type animals eliminated the discrimination against HK-*E. coli* with D-GlcA or Vitamin C supplementation. Data are represented as mean \pm SD from four independent experiments, 153-292 animals/assay.

(I-K) The bar graph showing that suppression of HK-*E. coli* induced *Phsp-4::GFP* (I), *Pirg-5::GFP* (J) and food avoidance (K) by D-GlcA supplementation was abolished in animals with RNAi of VC biosynthesis genes, which was not affected by Vitamin C supplementation. Blue numbers are the number of worms scored from at least three independent experiments and Data are represented as mean \pm SD(I-J). Data are represented as mean \pm SD from five independent experiments, 252-537 animals/assay (K).

For all panels, Scale bar shows on indicated figures, 50 μ m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

or PMK-1 in neurons rather than intestine (**Figure 5B-D**), indicating that neuronal XBP-1 and PMK-1 are critical for physiological food evaluation system for monitoring the level of sugar and VC under low-quality food condition.

Discussion

To better survive, animals must evolve a system to recognize and evaluate the quality of their food. This includes the sensory neuron evaluation system for immediate response and feeding decision², as well as physiological food evaluation system for chronic response to ingested food. In our previous study, we discovered that the TORC1-ELT-2 pathway, acting as master regulators in intestine, evaluates vitamin B2 deficiency in low-quality food (HK-*E. coli*) and regulates gut digestive activity to impact animal's food behavior²⁴. To further identified the mechanism by which animals evaluate low-quality food (HK-*E. coli*), we performed metabolomics and transcriptomics analyses to identify specific nutrition deficiencies in low-quality food and the cellular response pathways that are involved in food evaluation pathway. This study identified a physiological food evaluation mechanism by which animals recognize food quality through UPR^{ER} (IRE-1/XBP-1) – Innate immunity (PMK-1/p38 MAPK) regulated cellular stress response program in neurons that dictates food avoidance and selection behaviors (**Figure 6**).

One of the cellular stress response pathways, the Unfolded Protein Response (UPR^{ER}), is activated by various stresses, including infection and nutrition deficiency, which disrupt the homeostasis of the endoplasmic reticulum (ER)³¹. The activation of UPR^{ER}, specifically in the nervous system, has been shown to promote changes in feeding and foraging behavior⁴⁷. The p38 PMK-1 pathway is also crucial for regulating the expression of secreted innate immune effectors and is essential for survival during infection⁴⁸. Therefore, these two pathways play a critical role in ensuring animals' survival in changing environments. However, it is still unclear whether UPR^{ER} and innate immunity evaluate food quality under physiological conditions. Our study provides evidence that low-quality food (HK-*E. coli*) activates both UPR^{ER} and p-PMK-1, leading to animals leaving the low-quality food. Previously finding have shown that inhibition of ER function promotes *C. elegans* avoid to the toxic food, which employs the MLK-1/MEK-1/KGB-1 pathway⁴⁹. Notably, HK-*E. coli* induced avoidance behavior is independent of the KGB-1 pathway (Figure 5-figure supplement 1C). Additionally, our study reveals that neuronal UPR^{ER} and PMK-1 are essential for evaluating low-quality food, suggesting that the nervous system plays a critical role in assessing food quality.

Previous studies have shown that XBP1 deficiency in intestinal epithelial cells leads to IRE1a hyperactivation and increased JNK phosphorylation in the epithelial compartment in vivo⁵⁰. The IRE1-XBP1 axis has been identified as a critical protective branch of the Unfolded Protein Response (UPR) induced secondary to an innate immune response in the presence of *P. aeruginosa*^{18, 51}. The p38 MAPK has also been shown to directly act on the phosphorylation of IRE-1 to promote the stress response^{52, 53}. Interestingly, IRE-1 has been found to confer cold resistance independently of XBP-1 by activating JNK-1 MAPK⁴⁹. In contrast, our study reveals a new mechanism where the UPR^{ER} (IRE-1/XBP-1) positively regulates Innate Immunity (PMK-1/p38 MAPK) under HK-*E. coli* food conditions, establishing a novel physiological food evaluation system that activates the cellular stress response program.

A previous study has shown that activating innate immunity (PMK-1 MAPK) leads to a reduction in translation⁵⁴. Our own previous research has also demonstrated that PMK-1 activation causes a shutdown of food digestion in animals³⁵, likely to reduce protein translation and cellular metabolism. To investigate this further, we measured the translation level of animals fed with HK-*E. coli* and found that total translation ability is significantly reduced in these animals (Figure 5-

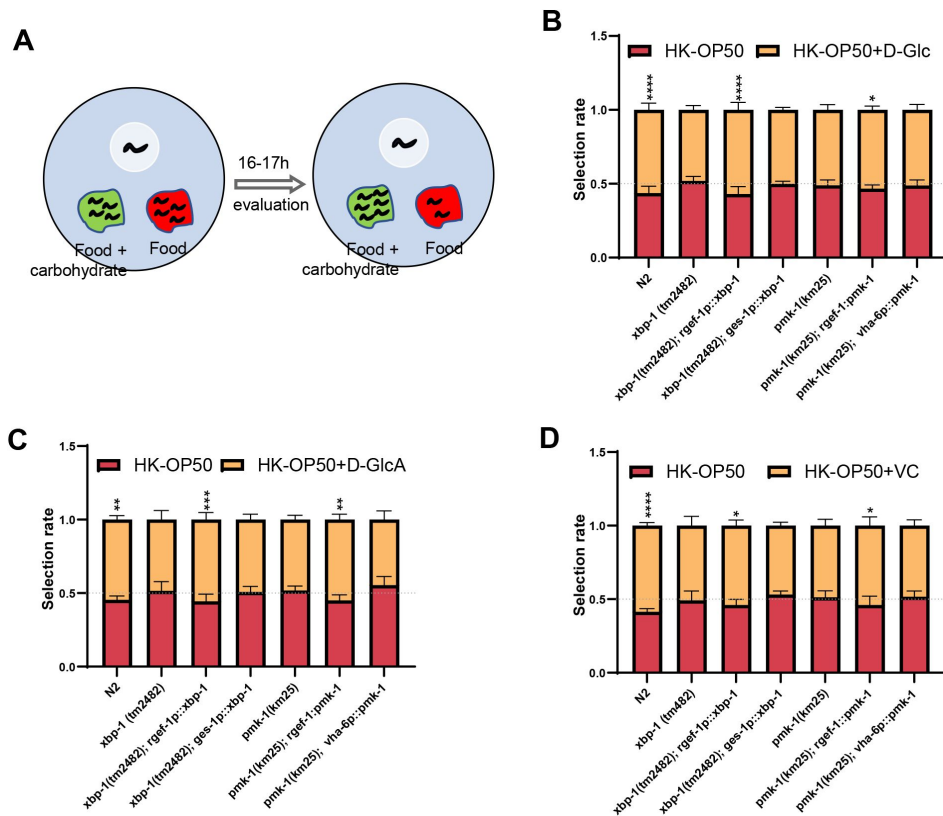


Figure 5.

Animals evaluate sugar and vitamin C through neuronal XBP-1 and PMK-1

(A) Schematic method of the food selection assay. Food (red) and food with carbohydrate (D-Glc, D-GlcA, or VC) supplementation (green) was placed on indicated position. Synchronized L1 worms were then placed in plate. After 16-17h, the selection index was calculated.

(B-D) Food selection assay showing that *xbp-1* or *pmk-1* mutation eliminated the preference of HK-*E. coli* with D-Glc (B), D-GlcA (C) or Vitamin C (D) supplementation, which was rescued in *xbp-1* or *pmk-1* mutant animals by expressing XBP-1 or PMK-1 in neurons rather than intestine.

Data are represented as mean \pm SD from five independent experiments, 68-647 animals/assay (B).

Data are represented as mean \pm SD from six independent experiments, 83-701 animals/assay (C).

Data are represented as mean \pm SD from six independent experiments, 67-1035 animals/assay (D).

For all panels, Scale bar shows on indicated figures, 50 μ m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

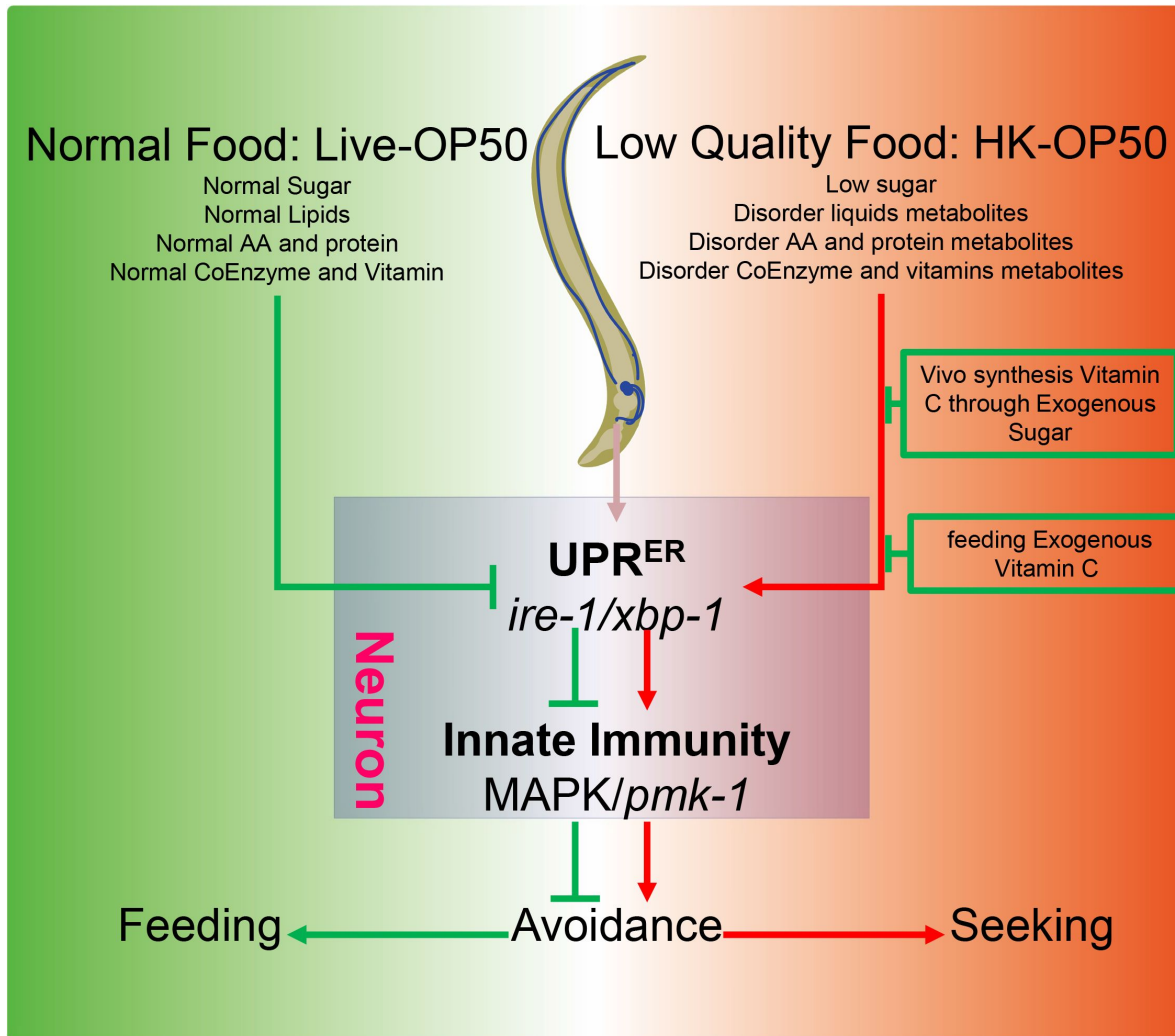


Figure 6.

Schematic model of physiological food evaluation system in evaluating/sensing sugar and vitamin C through UPR^{ER} (IRE-1/XBP-1) - Innate immunity (PMK-1/p38 MAPK) axis.

Vitamin C level is low in animals fed low sugar food, HK-*E. coli*. Sugar and Vitamin C deficiency activate cellular UPR^{ER} and immune response, which promote animals to leave low-quality food and seek better food for survival.

This cellular stress regulated physiological food evaluation system depends UPR^{ER} (IRE-1/XBP-1) - Innate immunity (PMK-1/p38 MAPK) axis in neuron.

figure supplement 1D). This finding suggests that activating innate immunity (PMK-1 MAPK) may serve as a mechanism to slow down translation progress, thereby alleviating the pressure on the unfolded protein response (UPR) and preventing excessive UPR^{ER} activation.

Vitamin C (VC) is an important physiological antioxidant and a cofactor for a family of biosynthetic and gene regulatory monooxygenase and dioxygenase enzymes. It is also required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters ²², ²³. Meanwhile, VC helps animals to protect neuron ⁴¹, ⁴², defend excessive immune ⁴³, ⁴⁴, and inhibit inflammatory and ER stress ⁴⁵, ⁴⁶ in order to better survive. The synthesis of vitamin C (VC) occurs through the glucuronate pathway, utilizing D-glucose as a precursor ³⁹, ⁴⁰ (Figure 4A). This led us to investigate whether the vitamin C biosynthesis pathway is involved in evaluating low-quality food by using D-glucose. In this study, we found that animals feeding live *E. coli*, which should produce more VC, exhibit higher glucose levels. However, our results show that animals maintain similar VC levels when fed ideal food (live *E. coli*) compared to low-quality food (HK-*E. coli*) (Figure 4B), suggesting that animals do not stimulate VC biosynthesis under favorable food conditions. In contrast, when animals are fed low-quality food (HK-OP50), we found that supplementing D-GlcA in HK-*E. coli* or *E. coli-yfbR* mutation can improve VC levels and inhibit UPR^{ER}-immunity (Figure 4C). These data indicate that glucose boosts the animal's ability to adapt to unfavorable food environments by increasing VC levels, but not in favorable food conditions.

Unlike the sensory neuron evaluation system, which permits rapid feeding decisions through smell and taste, the cellular stress response as physiological food evaluation system describe here requires a slow and multi-step signal transduction process after the ingestion of food. The disruption of cellular homeostasis by ingested of low-quality or toxic food can activate stress response mechanisms that both increase the cellular ability to withstand and adapt to this disruption of homeostasis and promote behavioral strategies to avoid these conditions and lessen their impact on the organism. These cellular stress response mechanisms include heat shock response, unfolded protein response, oxidative stress response ⁵⁵. Therefore, this slow physiological food evaluation system is an evolutionary adaptation mechanism for detecting nutrition deficiencies in food that was not detected by quick sensory nervous system.

One limitation of our study is the lack of explanation for why HK-*E. coli* activates UPR^{ER} and immunity. We hypothesized that when heat-killed, HK-*E. coli* may lack or contain altered levels of certain metabolites that either activate or inhibit UPR^{ER} and immunity, respectively. Additionally, we speculated that *E. coli* mutants killed by heat may lack metabolites that activate UPR^{ER} and immunity, or conversely, have increased levels of metabolites that inhibit these pathways. Fortunately, our investigation led to the discovery of the *E. coli* mutant *yfbR*, which inhibits UPR^{ER} and immunity by increasing carbohydrates that aid in resisting these stress pathways. Moving forward, we intend to further explore the intricate relationship between HK-*E. coli* and UPR^{ER}-immunity. This will be a key focus of our future research efforts.

Collectively, this study uncovers the unexpected function of UPR^{ER} (IRE-1/XBP-1) – Innate immunity (PMK-1/p38 MAPK) as a physiological food evaluation system for evaluating and sensing food quality in animals. It also highlights the utility of the HK-*E. coli* (low-quality food) – *C. elegans* interaction as a means to dissect the mechanism of food evaluation system in assessing food. Most importantly, it reveals that animals are capable of altering their nutrient (Vitamin C) levels through in vivo synthesis or food intake to adapt to a poor food environment when better food choices are not available.

Author Contributions

P. L. designed, performed experiments, analyzed data. X. L. constructed all transgenic animals. B.Q. designed research, supervised this study, and wrote the paper with inputs from P. L.

Acknowledgements

We thank the *Caenorhabditis* Genetics Center (CGC) (funded by NIH P40OD010440) for strains; Dr. Zhao Shan for suggestions. This work was supported by the Ministry of Science and Technology of the People's Republic of China (2019YFA0802100, 2019YFA0803100), the National Natural Science Foundation of China (32170794), Yunnan Applied Basic Research Projects (202302AP370005, 202201AT070196, K264202230211, 202001AV070011), the Yunnan University Startup Program.

Declaration of interests

The authors declare no competing interests.

Supplemental Figure legends

Figure 1 – figure supplement 1.

Food selection assay of animals fed HK-*E. coli* or *E. coli*. Relative to Figure 1 [↗](#).

(A-E) Metabolomics analysis of different quality food (HK-K12, HK-*yfbR* and Live-K12). Cluster analysis of all metabolites (A), lipids and their derivatives (B), amino acids and their derivatives (C), coenzymes and vitamins (D), and carbohydrates and their derivatives (E) from Live-K12, HK-K12, and HK-*yfbR*. Color indicates the relative level of each metabolite. HK-K12: heat-killed *E. coli* wild-type K12; HK-*yfbR*: heat-killed *E. coli* mutant *yfbR*; K12: live *E. coli* wild-type K12. z-score for standardizing data, complete for bi-clustering algorithm, and Euclidean for distance method.

(F) Principal component analysis to test the repeatability of the metabolic experiment. HK-K12: heat-killed *E. coli* wild-type K12; HK-*yfbR*: heat-killed *E. coli* mutant *yfbR*; K12: live *E. coli* wild-type K12. LB: LB buffer for culturing *E. coli*.

(G) The number of worms in each position calculated at the indicated time, indicating that animals initially select both foods (1-2h), but eventually favor high-quality food (Live *E. coli*) until 17h.

(H) GO enrichment analysis of UPR^{ER} dependent IRE-1 branch.

For all panels, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

Figure 1 – figure supplement 2.

Stress response of animals fed HK-*E. coli* or *E. coli*. Relative to Figure 1 [↗](#).

(A) UPR^{ER} reporter (*Phsp-4::GFP*) and immunity reporter (*Pirg-5::GFP*) were induced in the neuron and intestine of animals fed with HK-*E. coli*. Neurons are highlighted with a red arrow.

(B) UPR^{mit} reporter (*Phsp-6::GFP*) was weakly induced in animals fed with HK-*E. coli*.

(C) UPR^{ER} reporter (*Phsp-4::GFP*) expression in animals under normal food (OP50), low quality food (HK-OP50), and starved (M9: hatching L1 worm in M9, NGM) condition; L1 animals were cultured in OP50, HK-OP50 or starved NGM for 20h.

(D) Immunity reporter (*Pirg-5::GFP*) expression in animals under normal food (OP50), low quality food (HK-OP50), and starved (M9: hatching L1 worm in M9, NGM). L1 animals were cultured in OP50, HK-OP50 or starved NGM for 20h. For all panels, Scale bar shows on indicated figures, 50 μ m.

Figure 2 – figure supplement 1.

UPR^{ER} and innate immunity pathway in animals are critical for evaluating HK-*E. coli*. Relative to Figure 2 [↗](#).

(A-B) GFP fluorescence images and bar graph showing that HK-*E. coli* induced *Phsp-4::GFP* was not affected in animals with *atf-4* (A) or *atf-6* (B) RNAi treatment. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(C) qPCR showing that IRE-1-mediated splicing of *xbp-1* mRNA is induced in animals fed with HK-*E. coli*.

(D) UPR^{ER} reporter (*Phsp-4::GFP*) expression in animals with candidate RNAi feeding OP50 or HK-OP50.

(E) Venn diagram showing the numbers of PMK-1 dependent genes [↗](#) and up-regulated genes in animals fed HK-*E. coli*, and their overlap.

(F) The expression of PMK-1 dependent genes which was extracted from RNA-seq data from animals fed with HK-*E. coli*. The data from average of three independent experiments.

(G-H) GFP fluorescence images and bar graph showing that *Psypm-1::GFP* (G) and *Pirg-1::GFP* (H) were induced in animals fed HK-*E. coli*. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(I) Immunity reporter (*Pirg-5::GFP*) expression in animals with candidate RNAi feeding OP50 or HK-OP50.

For all panels, Scale bar shows on indicated figures, 50 μ m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

Figure 2 – figure supplement 2.

UPR^{ER} positively regulates innate immunity pathway in animals. Relative to Figure 2 [↗](#).

(A) GFP fluorescence images and bar graph showing that HK-*E. coli* induced *Pirg-5::GFP* was decreased in animals with *ire-1* or *xbp-1* RNAi treatment. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(B) GFP fluorescence images and bar graph showing that *Phsp-4::GFP* was not affected in animals with *pmk-1* RNAi treatment. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(C) Images and bar graph showing that the avoidance behavior of N2, *xbp-1* mutant, and *pmk-1* mutant in response to different food source (*Saprophytic staphylococci*-SS, *Pseudomonas aeruginosa*-PA14, or OP50).

“Broder” indicates regions with thicker SS boundaries; “In” denotes areas inside SS; “Out” refers to areas without SS. The scale is as follows: 2 for animals capable of escaping from PA14 and feeding on PA14 located with edges; 1 for animals capable of escaping from PA14 but not feeding on PA14 located with edges; 0 for animals unable to escape from PA14 and being killed by PA14. Data are represented as mean \pm SD from five independent experiments, 359-670 animals/assay. For all panels, Scale bar shows on indicated figures, 50 μ m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

Figure 3 – figure supplement 1.

E. coli Keio mutant screening. Relative to Figure 3 [↗](#).

(A) Flow chart of strategy for *E. coli* Keio mutant screening. We identified 20 *E. coli* mutants that did not induce *hsp-4::GFP* through the UPR^{ER} reporter (*Pirg-5::GFP*) after three rounds of screening (Table S3). From these 20 *E. coli* mutants, we identified 9 *E. coli* mutants that did not induce *Pirg-5::GFP* through the immunity reporter (*Pirg-5::GFP*) screening (Table S3).

(B-C) The bar graph showing that HK-*E. coli* induced *Phsp-4::GFP* (B) and *Pirg-5::GFP* (C) was decreased in animals fed mutant *E. coli* (Heat-killed). Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

For all panels, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

Figure 3 – figure supplement 2.

Low sugar food, HK-*E. coli*, induce stress response and avoidance behavior in animals. Relative to Figure 3 [↗](#).

(A) Venn diagram showing the number of differentially metabolites in HK-*E. coli*-K12, HK-*E. coli*-*yfbR* and *E. coli*.

(B) KEGG enrichment analysis of differentially expressed genes in animals fed HK-*E. coli* vs live *E. coli*. We noticed that most of glycolysis/gluconeogenesis genes are up-regulated in animals fed HK-*E. coli*.

(C) The bar graph showing that HK-*E. coli* induced *Phsp-4::GFP* was not affected in animals with D-(+)-trehalose supplementation. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(D) GFP fluorescence images and bar graph showing that HK-*E. coli* induced *Psym-1::GFP* was decreased in animals with D-(+)-glucose (D-Glc) supplementation. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(E) UPR^{ER} reporter (*Phsp-4::GFP*) expression animals with D-Glc supplementation under OP50, HK-OP50, or NGM condition.

(F) immunity reporter (*Pirg-5::GFP*) expression animals with D-Glc supplementation under OP50, HK-OP50, or NGM condition.

(G) Development of animals after 48h fed with OP50, HK-OP50, HK-OP50+D-Glc, and HK-OP50+D-GlcA. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

For all panels, Scale bar shows on indicated figures, 50 μ m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

Figure 4 – figure supplement 1.

Vitamin C biosynthesis pathway is critical for evaluating low sugar. Relative to Figure 4 [↗](#).

(A) The level of total L-ascorbic acid (TAA) in Live-K12, HK-K12, or HK-*yfbR*. Data are represented as mean \pm SD from six independent experiments.

(B) The level of total L-ascorbic acid (TAA) in animals fed HK-*E. coli* with or without D-Glc supplementation. Data are represented as mean \pm SD from eight independent experiments.

(C) GFP fluorescence images and bar graph showing that HK-*E. coli* induced *Psym-1::GFP* was decreased in animals with D-GlcA or vitamin C supplementation. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(D) The bar graph showing that suppression of HK-*E. coli* induced *Psym-1::GFP* by D-GlcA supplementation was abolished in animals with RNAi of VC biosynthesis genes, which was not affected by vitamin C supplementation. Blue numbers are the number of worms scored from at least three independent experiments. Data are represented as mean \pm SD.

(E) Food selection assay showing that the preference of HK-*E. coli* with D-GlcA supplementation was abolished in animals by RNAi of vitamin C biosynthesis genes. Data are represented as mean \pm SD from six independent experiments, 427-775 animals/assay.

For all panels, Scale bar shows on indicated figures, 50 μ m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

Figure 5 – figure supplement 1.

Food behavior of animals. Relative to Figure 5 [↗](#).

(A) Food selection assay for OP50 & OP50 + D-Glc, D-GlcA, or VC, respectively. Data are represented as mean \pm SD from five independent experiments, 241-1182 animals/assay.

(B) Food selection assay for buffer (H₂O) & buffer (H₂O) + D-Glc, D-GlcA, or VC, respectively. Data are represented as mean \pm SD from five independent experiments, 8-153 animals/assay.

(C) Food avoidance assay for N2 & kgb-1 mutant animals fed with HK-*E. coli*. Data are represented as mean \pm SD from six independent experiments, 348-660 animals/assay.

(D) Translation ability of animals fed with OP50 or HK-OP50 are presented by Western blot of puromycin-labeled peptides.

For all panels, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns: no significant difference. Precise P values are provided in Raw Data.

Supplemental Tables

Table S1. Metabolites analysis.

Table S2. RNA-seq analysis.

Table S3. Screening data for *E. coli* mutant keio library.

Table S4. Metabolomics-seq data of HK-K12, HK-*yfbR* and K12.

Table S5. RNA-seq data of animals fed with HK-*E. coli* OP50 and *E. coli* OP50.

Raw-data. Raw data for experiments.

Star Methods

Resource Availability

Lead contact

Further information and requests for reagents may be directed to the lead contact Bin Qi (qb@yun.edu.cn).

Materials availability

All reagents and strains generated by this study are available through request to the lead contact with a completed Material Transfer Agreement.

Data and code availability

Metabolomics-seq data are accessible in Table S4

RNA-seq data are accessible in Table S5.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Experimental model and subject details

C. elegans strains and maintenance

Nematode stocks were maintained on nematode growth medium (NGM) plates seeded with bacteria (*E. coli* OP50) at 20°C.

- 1) The following strains/alleles were obtained from the Caenorhabditis Genetics Center (CGC) or as indicated:
 N2 Bristol (wild type control strain);
 AU78: *agIs219 [T24B8.5p::GFP::unc-54 3' UTR + Ptx-3::GFP::unc-54 3' UTR]*;
 SJ4005: *zCIs4 [Phsp-4::GFP]*;
 AY101: *acIs101 [F35E12.5p::GFP + rol-6(su1006)]*;
 SJ17: *xbp-1 (zc12)*;
 KU25: *pmk-1(km25)*;
 AY102: *pmk-1(km25) IV; acEx102 [Pvha-6::pmk-1::GFP + rol-6(su1006)]*;
 YNU108: *Ex[Prgef-1::pmk-1::GFP;Podr-1::RFP]³⁵*; *xbp-1(tm2482)*⁵⁶;
 KU21: *kgb-1(km21)*;
 AU133: *agIs17 [Pmyo-2::mCherry + Pirg-1::GFP] IV*;
 SJ4100: *zCIs13 [Phsp-6::GFP + lin-15(+)]*.
 2) The following strains were constructed by this study:
 YNU242: *xbp-1(tm2482); pmk-1(km25)* double mutant was constructed by crossing: *xbp-1(tm2482)* with KU25[*pmk-1(km25)*].
 YNU240: *ylfEx149 [xbp-1(tm2482); Prgef-1::xbp-1::GFP; Podr-1::RFP]* transgene strain was constructed by injecting plasmid *Prgef-1::xbp-1::GFP* with *Podr-1::RFP* in *xbp-1(tm2482)* background
 YNU241: *ylfEx150 [xbp-1(tm2482); Pges-1::xbp-1::GFP; Podr-1::RFP]* transgene strain was constructed by injecting plasmid *Pges-1::xbp-1::GFP* with *Podr-1::RFP* in *xbp-1(tm2482)* background

Bacterial strains

E. coli-OP50, *Saprophytic staphylococci*, *Pseudomonas aeruginosa*-PA14, *E. coli*-K12 (BW25113), and *E. coli*-K12 mutant were cultured at 37°C in LB medium. A standard overnight cultured bacteria was then spread onto each Nematode growth media (NGM) plate.

Culture Medium

MGN Medium: Sigma agar: 20g/L; Bacto Peptone: 2.5g/L; NaCl: 3g/L; MgSO₄: 0.12g/L; CaCl₂: 0.111g/L; PPB: (KH₂PO₄ 0.8M; K₂HPO₄·3H₂O 0.2M) 25ml/L; Cholesterol: 0.005g/L.

LB broth: TPYPTONE: 10g/L; Yeast Extract: 5g/L; NaCl 5g/L.

Method Details

Generation of transgenes

- 1) To construct the *C. elegans* plasmid for expression of *xbp-1* in neuron, 3057bp promoter of *rgef-1* and genomic DNA of *xbp-1* was inserted into the PPD95.77 vector. DNA plasmid mixture containing *Prgef-1::xbp-1::GFP* (25ng/ul) and *Podr-1::RFP* (25ng/ul) was injected into the gonads of adult *xbp-1(tm2482)*. *C. elegans* plasmid for expression of *xbp-1* in neuron, 3057bp promoter of *rgef-1* and genomic DNA of *xbp-1* was inserted into the PPD95.77 vector. DNA plasmid mixture containing *Prgef-1::xbp-1::GFP* (25ng/ul) and *Podr-1::RFP* (25ng/ul) was injected into the gonads of adult *xbp-1(tm2482)*.
- 2) To construct the *C. elegans* plasmid for expression of *xbp-1* in intestine, 2549 bp promoter of *ges-1* and genomic DNA of *xbp-1* was inserted into the PPD95.77 vector. DNA plasmid mixture containing *Pges-1::xbp-1::GFP* (25ng/ul) and *Podr-1::RFP* (25ng/ul) was injected into the gonads of adult *xbp-1(tm2482)*. *C. elegans* plasmid for expression of *xbp-1*

2) To construct the *C. elegans* plasmid for expression of *xbp-1* in intestine, 2549 bp promoter of *ges-1* and genomic DNA of *xbp-1* was inserted into the PPD95.77 vector. DNA plasmid mixture containing *Pges-1::xbp-1::GFP* (25ng/ul) and *Podr-1::RFP* (25ng/ul) was injected into the gonads of adult *xbp-1(tm2482)*. *C. elegans* plasmid for expression of *xbp-1* in intestine, 2549 bp promoter of *ges-1* and genomic DNA of *xbp-1* was inserted into the PPD95.77 vector. DNA plasmid mixture containing *Pges-1::xbp-1::GFP* (25ng/ul) and *Podr-1::RFP* (25ng/ul) was injected into the gonads of adult *xbp-1(tm2482)*.

Preparation and feeding of worm food

We followed an established protocol ²⁴, ²⁵ to prepare heat-killed (HK) *E. coli*. Briefly, a standard OD₆₀₀=0.5-0.6 of *E. coli* OP50 and *E. coli* K12 grown in LB broth was concentrated to 1/20 vol and was then heat-killed at 80°C for 180 min. About 150ul of the heat-killed bacteria was spread onto each 35mm NGM plate.

Preparation of HK-*E. coli* + carbohydrate or vitamin C food

- 1) 100ul of water, 100ul of L-ascorbic acid (dissolved in water at a concentration of 100mg/ml, Sangon Biotech, 100143-0100) or 100 ul of D-glucuronic acid (dissolved in water at a concentration of 100mg/ml, Adamas, 1102520) was mixed with 500 ul of HK-*E. coli*, then 150ul of the mixture was spread onto 35mm NGM plates. *E. coli*, then 150ul of the mixture was spread onto 35mm NGM plates.
- 2) 12.5ul of water or 12.5ul of D-(+)-glucose (dissolved in water at a concentration of 100mg/ml, Sangon Biotech, A501991-0500) was mixed with 500 ul of HK-*E. coli*, then 150ul of the mixture was spread onto 35mm NGM plates. *E. coli*, then 150ul of the mixture was spread onto 35mm NGM plates.

Behavioral assay

C. elegans selection assays

For *C. elegans* to have enough space to evaluate food, we add 18ul of the sample onto a 35mm NGM plate. This creates a round lawn with a radius of 5mm, which occupies about 8% of the total plate area.



$$\frac{a}{b} = \frac{\pi r_1^2}{\pi r_2^2} = \frac{5mm^2}{17.5mm^2} = 8\%$$

a: the area of bacterial lawn
b: the space of worm life (area of culture dish)

- 1) 18ul of heat-killed OP50, live OP50, and LB broth (as the buffer for bacteria) was added into 35mm NGM plate in an equilateral triangle pattern. Then, synchronized L1 worms were seeded in the center of NGM plate for 16-17h at 20°C (as indicated in Figure1B).
- 2) 18ul of heat-killed OP50 and heat-killed OP50 with D-GlcA or vitamin C was added into 35mm NGM plate in an equilateral triangle pattern, then synchronized L1 worms were seeded on equilateral triangle of NGM plate for 16-17h at 20°C (as indicated in Figure 5A). **Figure 5A** [↗](#).

Here is Selection rate formula:

$$\text{selection rate} = \frac{\frac{\text{worm amount}}{\text{one lawn area}}}{\frac{\text{worm amount}}{\text{lawn1 area}} + \frac{\text{worm amount}}{\text{lawn2 area}} + \frac{\text{worm amount}}{\text{lawn3 area}} + \dots}$$

***C. elegans* aversion assays**

18ul food was spread out the center of NGM plate, then synchronized L1 by bleach solution (NaOH: 1M, NaClO:4-6%) worms were seeded on center of food for 16-17h at 20 °C.

$$\text{Aversion index} = \frac{\text{worm amount of out of lawn}}{\text{worm amount of (out + in) lawn}}$$

Three to ten replicates for each condition were performed for each assay, and the experiments were duplicated on different days.

Analysis of the fluorescence intensity in worms

The synchronized L1 worms carrying either UPR^{ER} reporter (*Phsp-4::GFP*) or innate immunity reporter (*Pirg-5::GFP*; *Psym-1p::GFP*; *Pirg-1p::GFP*) were seeded on NGM with indicated food and incubated for 24h at 20°C. For fluorescence imaging, worms were anesthetized with 25 mM levamisole and photographed using either an Olympus BX53 microscope or Olympus MVX10 dissecting microscope equipped with a DP80 camera.

The fluorescence intensity in entire intestinal region was quantified using ImageJ software and normalized to the body area.

***E. coli* Keio collection screen**

The whole Keio *E. coli* single mutant collection (Baba et al., 2006) was screened. Mutant bacteria strains, as well as the wild-type control strain BW25113, were cultured in LB medium with 50 µg/ml kanamycin at 37°C until an OD₆₀₀ of 0.5-0.6 was reached. The bacteria were then heat-killed following our established protocol [24](#), and 150 µl of the heat-killed mutant *E. coli* was spread onto 35 mm NGM plates. Synchronized L1 worms carrying UPR^{ER} reporter (*Phsp-4::GFP*) were seeded and cultured for 24h at 20°C. The fluorescence was then examined by using an Olympus MVX10 dissecting microscope, progressive screening three times. Next, a 4th screen was performed using immune reporter (*Pirg-5::GFP*) animals fed with HK-*E. coli* mutants that reduced the *Phsp-4::GFP* fluorescence. Finally, an aversion behavior assay was performed using HK-*E. coli* mutants that both reduced *Phsp-4::GFP* and *Pirg-5::GFP*. HK-*E. coli* mutants that reduced UPR^{ER}, immune and avoidance behavior were identified through this screening.

RNAi treatment

RNAi plasmid is delivered in a *E. coli* strain, HT115, from either the MRC RNAi library [57](#) or the ORF-RNAi Library [58](#). RNAi plates were prepared by adding IPTG to NGM agar to a final concentration of 1 mM. Overnight *E. coli* cultures (LB broth containing 100 µg/ml ampicillin and 100 µM IPTG) of specific RNAi strains and the control HT115 strain were seeded onto RNAi feeding plates and cultured at room temperature until dry. Synchronized L1 worms were treated RNAi by feeding (Ahringer, Reverse genetics, WormBook 2006) for the first generation and allowed to grow to maturity. The worms were then bleached and hatched in M9 buffer for 18hr. The synchronized L1 worms were then seeded on the indicated feeding plate.

Western blot

To measure the level of p-PMK-1, worms (feeding different food for 4h) were analyzed by standard western blot methods and probed with anti-p38 (dilution = 1:5,000; Cell Signaling, 9212S), anti-p38 (dilution = 1:5,000; Cell Signaling, 4511S) and anti-α-tubulin (dilution = 1:10,000; Sigma T5168) as a loading control.

To measure the level of protein translation, worms (feeding different food for 24h) were analyzed by standard western blot methods and probed with anti-Puromycin (dilution = 1:10,000; Sigma-Aldrich, MABE343) and anti-α-tubulin (dilution = 1:10,000; Sigma T5168) as a loading control.

Total content of ascorbic acid (TAA) assay

The total content of ascorbic acid was measured using the kits (Beijing Biotech-Pack-analytical Scientific Co., Ltd., Beijing, China, BKWB132 <http://biotech-pack-analytical.foodmate.net/>) according to the manufacturer's protocol. Briefly, L1 worms were seeded on the different feeding assay plate and cultured for 4 hours. The worms were then lysed in ice-cold conditions using lysis buffer. Equal amounts of protein were used for the normalization. Here is formula for getting TAA concentration

$$\text{TAA}(\mu\text{g}/\text{mg prot}) = ((\Delta A - a) \div b) \div (\text{Cpr} \times V1) \times D$$

V1 – the volume of supernatant of for experiment

Cpr – the concentration of supernatant protein

D – Dilution ratio of supernatant

a – the intercept of standard curve

b – the slope of standard curve

the standard curve $y = 0.0611x + 0.0003$ for [Figure 4C](#); $y = 0.0258x + 0.0066$ for [Figure 4B](#), [Figure 4-figure supplement 1A](#) and [Figure 4-figure supplement 1B](#).

Preparation of samples for RNA sequencing

RNA-seq was done with three biological replicates that were independently generated, collected, and processed. Adult wild type (N2) worms were bleached and then the eggs were incubated in M9 for 18 hours to obtain synchronized L1 worms. L1 worms were cultured in the NGM plate with *HK-E. coli* or *E. coli* for 4hrs at 20°C. L1 worms were then collected for sequencing.

RNA sequencing and data processing

For the RNA sequencing assay, cDNA libraries were constructed, and single-end libraries were sequenced using the Illumina platform (Novogene, Beijing, China). HISAT2⁵⁹ was used to map the clean reads to the reference gene sequence (Species: *Caenorhabditis elegans*; Source: NCBI; Reference Genome Version: GCF_000002985.6_WBcel235), and then “featureCounts” tool in subread software⁶⁰ was used to calculate the gene expression level of each sample. Read counts were inputted into DESeq2⁶¹ to calculate differential gene expression and statistical significance. Differentially expressed genes (DEGs) were screened using following criteria: $|\log_2(\text{FoldChange})| > 1$ & $\text{padj} \leq 0.05$.

Preparation of samples for metabolome sequencing

Metabolome-seq of bacterial was done with four biological replicates that were independently generated, collected, and processed. Total of three group *E. coli* sample including: *E. coli* K12 (Con), *HK-E. coli* K12 (HK), and *HK-E. coli yfbR* mutant (HY). All bacteria were overnight cultured to the same OD ($\text{OD}_{600}=1$). *E. coli* K12 and *E. coli yfbR* mutant are heat-killed (80, 180min), *E. coli* K12, *HK-E. coli* K12 and *HK-E. coli yfbR* mutant were then spread out NGM plate for 72hs at room temperature. Finally, sample was collected into 1.5ml tube by using sterile cell scraping.

Metabolome sequencing and data processing

Metabolome were sequenced using the Ultra Performance Liquid Chromatography (UPLC) (ExionLC AD, <https://sciex.com.cn/>) and Quadrupole-Time of Flight (TripleTOF 6600, AB SCIEX) for Non-targeted; Ultra Performance Liquid Chromatography (UPLC) (ExionLC AD, <https://sciex.com.cn/>) and Tandem mass spectrometry (MS/MS) (QTRAP®, <https://sciex.com/>) for Broad targeting (Metware, Wuhan, China). Multiple reaction monitoring (MRM) was used to calculate the expression level of each metabolite. Differential metabolites were screened through Fold change ≥ 2 or Fold change ≤ 0.5 and $\text{VIP} \geq 1$ (Variable Importance in Projection of OPLS-DA model).

Microscopy

Analysis of fluorescence was performed with an Olympus BX53 microscope, CLSM (Zeiss LSM900), or Olympus MVX10 dissecting with a DP80 camera.

Quantification and statistical analysis

Quantification

ImageJ software was used for quantifying fluorescence intensity of UPR^{ER} and Innate immunity reporter. ImageJ software was used for counting the number of worms about selection and aversion behavior.

Statistical analysis

All statistical analyses were performed in Graphpad prism 8.0. Two-tailed unpaired t test was used for statistical analysis of two groups of samples, one-way or two-way ANOVA was used for statistical analysis of more than two groups of samples. Data are presented as Mean \pm SD, and $p < 0.05$ was considered a significant difference, “*” represents $p < 0.05$, “**” represents $p < 0.01$, “***” is represents < 0.001 , “*****” represents $p < 0.0001$, “ns” represents no significant difference. For all figures, “n” represents the number of worms scored from at least three independent experiments.

References

1. Filosa A, Barker AJ, Dal Maschio M, Baier H (2016) **Feeding State Modulates Behavioral Choice and Processing of Prey Stimuli in the Zebrafish Tectum** *Neuron* **90**:596–608
2. Florsheim EB, Sullivan ZA, Khoury-Hanold W, Medzhitov R (2021) **Food allergy as a biological food quality control system** *Cell* **184**:1440–1454
3. McLachlan IG, et al. (2022) **Diverse states and stimuli tune olfactory receptor expression levels to modulate food-seeking behavior** *Elife* **11**
4. Avery JA, Liu AG, Ingeholm JE, Gotts SJ, Martin A (2021) **Viewing images of foods evokes taste quality-specific activity in gustatory insular cortex** *Proc Natl Acad Sci U S A* **118**
5. Melin AD, et al. (2019) **Fruit scent and observer colour vision shape food-selection strategies in wild capuchin monkeys** *Nat Commun* **10**
6. Bargmann CI (2006) **Chemosensation in C. elegans** *WormBook* :1–29
7. Sengupta P, Chou JH (1996) **Bargmann CI. odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl** *Cell* **84**:899–909
8. Troemel ER, Kimmel BE, Bargmann CI (1997) **Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in C. elegans** *Cell* **91**:161–169
9. Fiala A (2007) **Olfaction and olfactory learning in Drosophila: recent progress** *Curr Opin Neurobiol* **17**:720–726
10. Chalasani SH, et al. (2007) **Dissecting a circuit for olfactory behaviour in Caenorhabditis elegans** *Nature* **450**:63–70
11. Zhang B, Jun H, Wu J, Liu J, Xu XZS (2021) **Olfactory perception of food abundance regulates dietary restriction-mediated longevity via a brain-to-gut signal** *Nat Aging* **1**:255–268
12. Ha HI, et al. (2010) **Functional organization of a neural network for aversive olfactory learning in Caenorhabditis elegans** *Neuron* **68**:1173–1186
13. Scott K (2018) **Gustatory Processing in Drosophila melanogaster** *Annu Rev Entomol* **63**:15–30
14. Hukema RK, Rademakers S, Dekkers MP, Burghoorn J, Jansen G (2006) **Antagonistic sensory cues generate gustatory plasticity in Caenorhabditis elegans** *Embo J* **25**:312–322
15. Ni L, et al. (2013) **A gustatory receptor paralogue controls rapid warmth avoidance in Drosophila** *Nature* **500**:580–584
16. Jones D, Candido EPM (1999) **Feeding is inhibited by sublethal concentrations of toxicants and by heat stress in the nematode Caenorhabditis elegans: Relationship to the cellular stress response** *J Exp Zool* **284**:147–157

17. Xie Z, et al. (2022) **The gut-to-brain axis for toxin-induced defensive responses** *Cell* **185**:4298–4316
18. Richardson CE, Kooistra T, Kim DH (2010) **An essential role for XBP-1 in host protection against immune activation in *C. elegans*** *Nature* **463**:1092–1095
19. Kim DH, et al. (2002) **A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity** *Science* **297**:623–626
20. Carr AC, Maggini S (2017) **Vitamin C and Immune Function** *Nutrients* **9**
21. Steinert RE, Lee YK, Sybesma W (2020) **Vitamins for the Gut Microbiome** *Trends Mol Med* **26**:137–140
22. Li Y, Schellhorn HE (2007) **New developments and novel therapeutic perspectives for vitamin C** *J Nutr* **137**:2171–2184
23. Carr AC, Frei B (1999) **Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans** *Am J Clin Nutr* **69**:1086–1107
24. Qi B, Kniazeva M, Han M (2017) **A vitamin-B2-sensing mechanism that regulates gut protease activity to impact animal's food behavior and growth** *eLife* **6**
25. Qi B, Han M (2018) **Microbial Siderophore Enterobactin Promotes Mitochondrial Iron Uptake and Development of the Host via Interaction with ATP Synthase** *Cell* **175**:571–582
26. Nakad R, et al. (2016) **Contrasting invertebrate immune defense behaviors caused by a single gene, the *Caenorhabditis elegans* neuropeptide receptor gene *npr-1*** *BMC Genomics* **17**
27. Sinha A, Rae R, Iatsenko I, Sommer RJ (2012) **System wide analysis of the evolution of innate immunity in the nematode model species *Caenorhabditis elegans* and *Pristionchus pacificus*** *PLoS One* **7**
28. Calfon M, et al. (2002) **IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA** *Nature* **415**:92–96
29. Bolz DD, Tenor JL, Aballay A (2010) **A Conserved PMK-1/p38 MAPK Is Required in *Caenorhabditis elegans* Tissue-specific Immune Response to *Yersinia pestis* Infection*** *The Journal of Biological Chemistry* **285**:10832–10840
30. Yoneda T, Benedetti C, Urano F, Clark SG, Harding HP, Ron D (2004) **Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones** *Journal of Cell Science* **117**:4055–4066
31. Hetz C, Zhang K, Kaufman RJ (2020) **Mechanisms, regulation and functions of the unfolded protein response** *Nat Rev Mol Cell Biol* **21**:421–438
32. Ron D, Walter P (2007) **Signal integration in the endoplasmic reticulum unfolded protein response** *Nat Rev Mol Cell Biol* **8**:519–529
33. Fletcher M, Tillman EJ, Butty VL, Levine SS, Kim DH (2019) **Global transcriptional regulation of innate immunity by ATF-7 in *C. elegans*** *Plos Genet* **15**

34. Foster KJ, McEwan DL, Pukkila-Worley R (2020) **Measurements of Innate Immune Function in *C. elegans*** *Methods Mol Biol* **2144**:145–160
35. Geng S, et al. (2022) **Gut commensal *E. coli* outer membrane proteins activate the host food digestive system through neural-immune communication** *Cell Host Microbe* **30**:1401–1416
36. Weiss B (2007) **The Deoxycytidine Pathway for Thymidylate Synthesis in *Escherichia coli*** *Journal of Bacteriology* **189**:7922–7926
37. Franceus J, Desmet T (2020) **Sucrose Phosphorylase and Related Enzymes in Glycoside Hydrolase Family 13: Discovery, Application and Engineering** *International Journal of Molecular Sciences* **21**
38. Xing S, et al. (2019) **Lactose induced redox-dependent senescence and activated Nrf2 pathway** *Int J Clin Exp Pathol* **12**:2034–2045
39. Patananan AN, Budenholzer LM, Pedraza ME, Torres ER, Adler LN, Clarke SG (2015) **The invertebrate *Caenorhabditis elegans* biosynthesizes ascorbate** *Arch Biochem Biophys* **569**:32–44
40. Yabuta Y, et al. (2020) **L-Ascorbate Biosynthesis Involves Carbon Skeleton Rearrangement in the Nematode *Caenorhabditis elegans*** *Metabolites* **10**
41. Moritz B, Schmitz AE, Rodrigues ALS, Dafre AL, Cunha MP (2020) **The role of vitamin C in stress-related disorders** *The Journal of Nutritional Biochemistry* **85**
42. Rice ME (2000) **Ascorbate regulation and its neuroprotective role in the brain** *Trends in Neurosciences* **23**:209–216
43. Webb AL, Villamor E (2007) **Update: Effects of Antioxidant and Non-Antioxidant Vitamin Supplementation on Immune Function** *Nutrition Reviews* **65**:181–217
44. Maggini S, Wintergerst ES, Beveridge S, Hornig DH (2007) **Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses** *British Journal of Nutrition* **98**:S29–S35
45. Luo X, et al. (2022) **Vitamin C protects against hypoxia, inflammation, and ER stress in primary human preadipocytes and adipocytes** *Molecular and Cellular Endocrinology* **556**
46. Su M, Liang X, Xu X, Wu X, Yang B (2019) **Hepatoprotective benefits of vitamin C against perfluorooctane sulfonate-induced liver damage in mice through suppressing inflammatory reaction and ER stress** *Environmental Toxicology and Pharmacology* **65**:60–65
47. Ozbey NP, et al. (2020) **Tyramine Acts Downstream of Neuronal XBP-1s to Coordinate Inter-tissue UPR(ER) Activation and Behavior in *C. elegans*** *Dev Cell* **55**:754–770
48. Troemel ER, Chu SW, Reinke V, Lee SS, Ausubel FM, Kim DH (2006) **p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*** *Plos Genet* **2**

49. Melo JA, Ruvkun G (2012) **Inactivation of conserved *C. elegans* genes engages pathogen- and xenobiotic-associated defenses** *Cell* **149**:452–466
50. Kaser A, et al. (2008) **XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease** *Cell* **134**:743–756
51. Kaser A, Blumberg RS (2010) **Survive an innate immune response through XBP1** *Cell Research* **20**:506–507
52. Guan L, Zhan Z, Yang Y, Miao Y, Huang X, Ding M (2020) **Alleviating chronic ER stress by p38-Ire1-Xbp1 pathway and insulin-associated autophagy in *C. elegans* neurons** *PLOS Genetics* **16**
53. Qu M, Liu Y, Xu K, Wang D (2019) **Activation of p38 MAPK Signaling-Mediated Endoplasmic Reticulum Unfolded Protein Response by Nanopolystyrene Particles** *Advanced Biosystems* **3**
54. Weaver BP, Weaver YM, Omi S, Yuan W, Ewbank JJ, Han M (2020) **Non-Canonical Caspase Activity Antagonizes p38 MAPK Stress-Priming Function to Support Development** *Dev Cell* **53**:358–369
55. Ozbey NP, Thompson MA, Taylor RC (2021) **The regulation of animal behavior by cellular stress responses** *Exp Cell Res* **405**
56. Richardson CE, Kinkel S, Kim DH (2011) **Physiological IRE-1-XBP-1 and PEK-1 signaling in *Caenorhabditis elegans* larval development and immunity** *PLoS Genet* **7**
57. Kamath RS, et al. (2003) **Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi** *Nature* **421**:231–237
58. Rual JF, et al. (2004) **Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library** *Genome Res* **14**:2162–2168
59. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B (2008) **Mapping and quantifying mammalian transcriptomes by RNA-Seq** *Nat Methods* **5**:621–628
60. Liao Y, Smyth GK, Shi W (2014) **featureCounts: an efficient general purpose program for assigning sequence reads to genomic features** *Bioinformatics* **30**:923–930
61. Love MI, Huber W, Anders S (2014) **Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2** *Genome Biol* **15**

Editors

Reviewing Editor

Martin Denzel

Altos Labs, Cambridge, United Kingdom

Senior Editor

Wendy Garrett

Harvard T.H. Chan School of Public Health, Boston, United States of America

Reviewer #2 (Public Review):**Summary:**

In this work, the authors aim to better understand how *C. elegans* detects and responds to heat-killed (HK) *E. coli*, a low-quality food. They find that HK food activates two canonical stress pathways, ER-UPR and innate immunity, in the nervous system to promote food aversion. Through the creative use of *E. coli* genetics and metabolomics, the authors provide evidence that the altered carbohydrate content of HK food is the trigger for the activation of these stress responses and that supplementation of HK food with sugars (or their biosynthetic product, vitamin C), reduces stress pathway induction and food avoidance. This work makes a valuable addition to the literature on metabolite detection as a mechanism for evaluation of nutritional value; it also provides some new insight into physiologically relevant roles of well-known stress pathways in modulating behavior.

Strengths:

- The work addresses an important question by focusing on understanding how the nervous system evaluates food quality and couples this to behavioral change.
- The work takes full advantage of the tools available in this powerful system and builds on extensive previous studies on feeding behavior and stress responses in *C. elegans*.
- Creative use of *E. coli* genetics and metabolite profiling enabled identification of carbohydrate metabolism as a candidate source of food-quality signals.
- For the most part, the studies are rigorous and logically designed, providing good support for the authors' model.

Weaknesses:

- The authors' claim that they can detect induction of *hsp-4* and *irg-5* expression in neurons (Fig 1-S2A) requires further support. The two tail cells shown are quite a bit larger than would be typically expected for neurons. The rescue they observe by neuronal expression is largely convincing, so it's quite possible that these pathways do indeed function in neurons, but that their level of induction in the nervous system is below reporter detection limits (or is 'swamped out' by much higher levels of expression in the intestine).
- The authors conclude that "the induction of *Pirg-5::GFP* was abolished in *pmk-1* knockdown animals fed with HK-*E. coli*" (Fig 2D). Because a negative control for induction (e.g., animals fed with control *E. coli*) is not shown, this conclusion must be regarded as tentative.
- The effect sizes in the food-preference assay shown in Figure 5 are extremely small and do not provide strong support for the strong conclusions about the role of stress response pathways in food preference behavior.

<https://doi.org/10.7554/eLife.94181.2.sa2>

Reviewer #3 (Public Review):**Summary:**

Animals can evaluate food quality in many ways. In contrast to the rapid sensory evaluation with smell and taste, the mechanism of slow nutrient sensation and its impact on food choice is unexplored. The authors utilize *C. elegans* larvae and their bacterial food as an elegant model to tackle this question and reveal the detailed molecular mechanism to avoid nutrient-poor foods.

Strength:

The strength of this study is that they identified the molecular identities of the critical players in bacterial food and *C. elegans* using unbiased approaches, namely metabolome analysis, *E. coli* mutant screening, and RNA sequencing. Furthermore, they strengthened their findings

by thorough experiments combining multiple methods such as genetics, fluorescent reporter analysis, and Western blot.

Weakness:

The major caveat of this study is the reporter genes; specifically, transcriptional reporters used to monitor the UPRER and immune responses in the intestine of *C. elegans*. However, their tissue-specific rescue experiments suggest that the genes in the UPRER and immune response function in the neurons. Thus, we should carefully interpret the results of the reporter genes. Another point to be aware of is that although they show that lack of carbohydrates elicits the response to "low-quality" food, carbohydrate supplementation with heat-killed *E. coli* was insufficient to support animal growth.

Overall, this work provides convincing data to support their model. In the *C. elegans* field, the behaviors of larvae are not well studied compared to adults. This work will pose an interesting question about the difference between larvae and adults in nutrition sensing in *C. elegans* and provide a framework and candidate molecules to be studied in other organisms.

<https://doi.org/10.7554/eLife.94181.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public Review):

Summary:

This manuscript by Liu et al explores the role of the UPR and immune regulators in the evaluation of nutritional quality in C. elegans. They identify neuronal UPR activation and the MAPK PMK-1 as key responders to low food quality. In particular, the data suggest that these pathways are activated by low levels of vitamin C synthesis that result from the low sugar levels present in heat-killed E. coli.

Strengths:

The results are intriguing and expand our understanding both of physiological food evaluation systems, and of the known roles of stress response pathways in organismal physiology. The authors use a range of techniques, encompassing imaging, metabolomic analysis, gene expression analysis, and behavioural assays, to support their claims.

Thank you for your thorough review and acknowledgment of the strengths of our study.

Weaknesses:

There is limited mechanistic analysis in the study. In particular, how does low vitamin C trigger UPR activation? This is an intriguing finding that, if followed up, could potentially reveal a novel mechanism of UPR activation. In addition, how is the activation of the PMK-1 pathway driven by/coordinated with UPR activation? The data in some figures is not as convincing as it could be: the magnitude of the effect size is small in the supplementation experiments, and the statistical tests used are not always appropriate to enable multiple comparisons.

(1) There is limited mechanistic analysis in the study. In particular, how does low vitamin C trigger UPR activation? This is an intriguing finding that, if followed up, could

| potentially reveal a novel mechanism of UPR activation.

Thank you for highlighting the need for further mechanistic analysis in our study. We appreciate the opportunity to clarify the process by which low vitamin C triggers UPR activation.

Our investigation revealed that the vitamin C content in heat-killed *E. coli* (HK-*E. coli*) is comparable to that of live *E. coli* or HK-*yfbR* mutant *E. coli* (Figure 4-figure supplement 1A), indicating that the induction of unfolded protein response (UPR) in *C. elegans* by HK-*E. coli* is not solely attributed to low vitamin C levels but rather involves other unidentified factors.

Through metabolomic analysis, we observed significant decreases in sugar levels, including lactose, D-(+)-sucrose, and D-(+)-glucose, in HK-*E. coli* (Figure 3B, Table S1). Notably, supplementing D-(+)-glucose effectively inhibited UPRER, immune response, and avoidance behavior induced by HK-*E. coli* (Figure 3E-H). These findings suggest that the deficiency in sugars in HK-*E. coli* triggers a stress response and avoidance behavior in animals, which can be alleviated by D-(+)-glucose supplementation.

Furthermore, when comparing heat-killed *E. coli* mutant *yfbR* (HK-*yfbR*) to HK-*E. coli*, we observed significantly higher sugar levels, including lactose and D-(+)-sucrose, in HK-*yfbR* (Figure 3B). This was accompanied by reduced UPRER in animals feeding on HK-*yfbR* (Figure 3-figure supplement 1B), indicating that higher sugar levels may inhibit the induction of UPRER by low-quality food.

Considering that the synthesis of vitamin C (VC) occurs through the glucuronate pathway, utilizing D-glucose as a precursor 1, 2 (Figure 4A), we investigated whether the vitamin C biosynthesis pathway is involved in evaluating low-quality food using D-glucose. Contrary to our initial hypothesis, animals fed live *E. coli* did not exhibit higher glucose levels compared to those fed low-quality food (HK-*E. coli*). Our results indicate that animals maintain similar VC levels when fed ideal food (live *E. coli*) compared to low-quality food (HK-*E. coli*) (Figure 4B), suggesting that animals do not stimulate VC biosynthesis under favorable food conditions. However, supplementation of D-GlcA or *E. coli-yfbR* mutation in HK-*E. coli* significantly improved VC levels when animals were fed low-quality food (HK-OP50) (Figure 4B, 4C). Moreover, VC or D-glucuronate (D-GlcA) supplementation inhibited HK-*E. coli*-induced UPRER (Figure 4D), indicating that glucose boosts the animal's ability to adapt to unfavorable food environments by increasing VC levels, thereby inhibiting UPRER, but not under favorable food conditions.

These findings shed light on the complex interplay between vitamin C, sugar levels, and UPR activation, providing valuable insights into the mechanisms underlying food evaluation and stress response pathways in organisms.

Overall, we are grateful for the reviewer's constructive feedback, which motivates us to continue our efforts to understanding how the UPR response contributes to the complexities of food evaluation and behavioral responses in organisms.

| (2) In addition, how is the activation of the PMK-1 pathway driven by/coordinated with UPR activation?

Thank you for your insightful inquiry. In our discussion section, we have addressed this question by integrating new data and discussion to provide insights into the coordination between PMK-1 pathway activation and UPR activation.

Previous studies have demonstrated that activating innate immunity, specifically the PMK-1 MAPK pathway, results in a reduction in translation³, as well as a shutdown of food digestion in animals⁴, likely aimed at reducing protein translation and cellular metabolism. To further

investigate this relationship, we measured the translation level of animals fed with heat-killed *E. coli* (HK-*E. coli*) and found a significant reduction in total translation ability in these animals (Figure 5-figure supplement 1D). This observation suggests that activating innate immunity through the PMK-1 MAPK pathway may serve as a mechanism to slow down translation progress, thereby alleviating the pressure on the unfolded protein response (UPR) and preventing excessive UPRER activation.

By integrating these findings, we propose a model wherein activation of the PMK-1 pathway coordinates with UPR activation to regulate translation and cellular metabolism in response to low-quality food. This coordinated response likely serves to maintain cellular homeostasis and prevent detrimental effects associated with excessive UPRER activation.

These insights contribute to our understanding of the intricate interplay between innate immunity, cellular stress responses, and metabolic regulation in organisms facing nutritional challenges.

(3) The data in some figures is not as convincing as it could be: the magnitude of the effect size is small in the supplementation experiments, and the statistical tests used are not always appropriate to enable multiple comparisons.

We appreciate the reviewers' concerns regarding the data presentation and statistical analyses in some of our figures. In response to this feedback, we have made revisions to improve the robustness and clarity of our statistical methods.

All statistical analyses were conducted using GraphPad Prism 8.0 software. Specifically, a two-tailed unpaired t-test was employed for the statistical analysis of two groups of samples, while one-way or two-way ANOVA was utilized for the statistical analysis of more than two groups of samples. These adjustments ensure appropriate statistical comparisons and enhance the reliability of our findings.

Reviewer #2 (Public Review):

Summary:

*In this work, the authors aim to better understand how *C. elegans* detects and responds to heat-killed (HK) *E. coli*, a low-quality food. They find that HK food activates two canonical stress pathways, ER-UPR, and innate immunity, in the nervous system to promote food aversion. Through the creative use of *E. coli* genetics and metabolomics, the authors provide evidence that the altered carbohydrate content of HK food is the trigger for the activation of these stress responses and that supplementation of HK food with sugars (or their biosynthetic product, vitamin C), reduces stress pathway induction and food avoidance. This work makes a valuable addition to the literature on metabolite detection as a mechanism for the evaluation of nutritional value; it also provides some new insight into the physiologically relevant roles of well-known stress pathways in modulating behavior.*

Strengths:

*-The work addresses an important question by focusing on understanding how the nervous system evaluates food quality and couples this with behavioral change. -The work takes full advantage of the tools available in this powerful system and builds on extensive previous studies on feeding behavior and stress responses in *C. elegans*.*

*-Creative use of *E. coli* genetics and metabolite profiling enabled the identification of carbohydrate metabolism as a candidate source of food-quality signals.*

-For the most part, the studies are rigorous and logically designed, providing good support for the authors' model.

We deeply appreciate the reviewer's insightful assessment of our study's strengths.

Weaknesses:

-It is not clear how the mechanism identified here is connected to previously described, related processes. In particular, it is not clear whether this mechanism has a role in the detection of other low-quality foods. Further, the specificity of the ability of sugar/vitamin C to suppress stress pathway induction is unclear (i.e., does sugar/vitamin C have any effect on the activation of these pathways through other means?). Additionally, the relationship of this pathway to the vitamin B2-sensing mechanism previously described by the senior author is unclear. These issues do not weaken confidence in the authors' conclusions, but they do reduce the potential significance of the work.

(1) In particular, it is not clear whether this mechanism has a role in the detection of other low-quality foods.

Thank you for your valuable feedback. In response to your inquiry, we investigated whether the UPRER (IRE-1/XBP-1) - Innate immunity (PMK-1/p38 MAPK) axis is specific to evaluating low-quality food (HK-*E. coli*) or if it plays a broader role in food detection.

We conducted behavioral assays using N2, *pmk-1*, and *xbp-1* mutant animals fed with normal *E. coli* food, inedible food (*Saprophytic staphylococci*)⁴, and pathogenic food (*Pseudomonas aeruginosa*-PA14)⁵. We found that N2, *pmk-1*, and *xbp-1* mutant worms did not exhibit avoidance behavior when presented with normal food (OP50). However, both N2 and *xbp-1* mutant worms were able to escape from inedible food (N2 was predominantly found on the border areas of the bacterial lawn and *xbp-1* mutant worms on border and in), *Saprophytic staphylococci*, whereas *pmk-1* mutant worms did not exhibit this avoidance behavior. Notably, N2 and *xbp-1* mutant worms exhibited even more pronounced avoidance behavior when exposed to *Pseudomonas aeruginosa*, whereas *pmk-1* mutant worms were more susceptible to infection by this pathogen (Figure 2-figure supplement 2C). These findings suggest that the UPR-Immunity pathway plays a crucial role in helping animals avoid low-quality food (HK-*E. coli*) by triggering an avoidance response. In contrast, the Innate immunity pathway, mediated by PMK-1/p38 MAPK, appears to play a key role in evaluating unfavorable food sources, such as HK-*E. coli*, *Saprophytic staphylococci*, and *Pseudomonas aeruginosa*, and helping animals avoid these environments.

(2) Further, the specificity of the ability of sugar/vitamin C to suppress stress pathway induction is unclear (i.e., does sugar/vitamin C have any effect on the activation of these pathways through other means?).

Thank you for your inquiry regarding the specificity of the ability of sugar/vitamin C to suppress stress pathway induction. We aimed to address this question by investigating whether high levels of VC inhibit other stress-induced UPRER pathways.

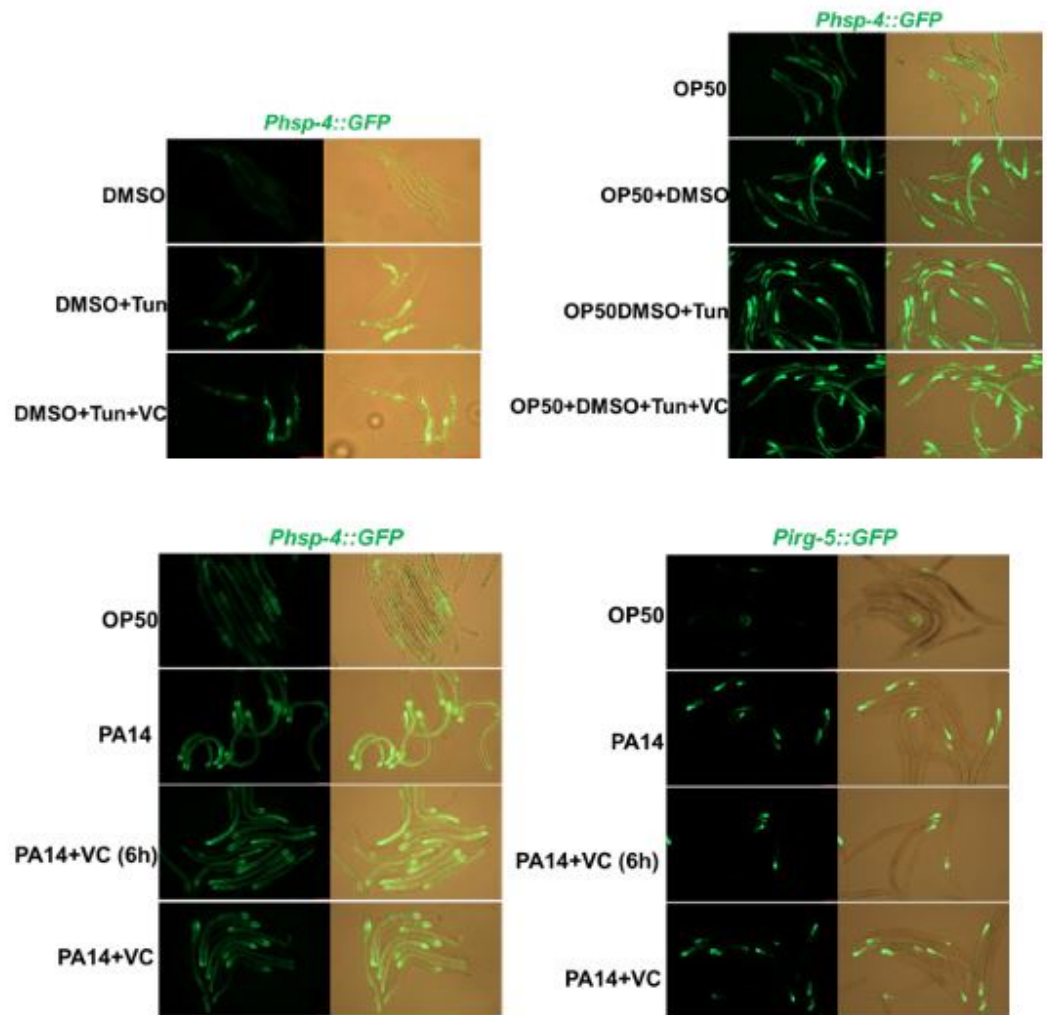
Previous studies have shown that both Tunicamycin⁶ and pathogenic bacteria, such as *Pseudomonas aeruginosa*-PA14⁵, induce UPRER in *C. elegans*. In response to your query, we conducted experiments to examine whether VC supplementation inhibits UPRER induced by these stressors. Our findings indicate that VC supplementation does not inhibit UPRER induced by either Tunicamycin or PA14 (Author response image 1).

These results suggest that while sugar/vitamin C may suppress stress pathway induction in the context of low-quality food, its effects may not extend to other stressors that induce

UPRER through different mechanisms. This insight helps clarify the specificity of sugar/vitamin C's role in modulating stress pathway activation, contributing to a better understanding of the broader regulatory networks involved in stress response in *C. elegans*.

Author response image 1.

VC supplementation does not inhibit Tunicamycin or PA14-induced UPRER.



(3) Additionally, the relationship of this pathway to the vitamin B2-sensing mechanism previously described by the senior author is unclear.

In response to your comment, we would like to clarify the relationship of our pathway to the previously described vitamin B2-sensing mechanism we found. Previous studies have demonstrated that heat-killed *E. coli* (HK-*E. coli*) serves as a low-quality food source incapable of supporting the growth of *C. elegans* larvae, whereas supplementation with vitamin B2 (VB2) can restore animal growth⁷

This study investigates the role of sugar deficiency in HK-*E. coli*, which induces the UPRER-immune response and avoidance behavior in *C. elegans*. Surprisingly, our findings indicate that supplementing HK-*E. coli* with carbohydrates such as D-Glc and D-GlcA does not promote animal development (Figure 3-figure supplement 2G), suggesting that carbohydrates are not

essential for supporting animal growth on this food source. However, we did observe that carbohydrates play a critical role in inhibiting the UPRER-immune response induced by sugar deficiency in HK-*E. coli*.

-The authors claim that the induction of the innate immune pathway reporter irg-5::GFP is "abolished" in pmk-1(RNAi) animals, but Figure S2K seems to show a clear GFP signal when these animals are fed HK-OP50. Similarly, the claim that feeding WT animals HK-OP50 enriches phospho-PMK-1 levels (Fig 2E) is unconvincing - only one western blot is shown, with no quantification, and there is a smear in the critical first lane.

(1) The authors claim that the induction of the innate immune pathway reporter irg-5::GFP is "abolished" in pmk-1(RNAi) animals, but Figure S2K seems to show a clear GFP signal when these animals are fed HK-OP50.

We sincerely appreciate the reviewer's attention. To address this concern, we have replaced the images with higher resolution, larger ones in Figure 2-figure supplement 1-I. These updated images provide a clearer representation of the data, ensuring that all details are readily visible and enabling a more accurate interpretation of the results.

(2) Similarly, the claim that feeding WT animals HK-OP50 enriches phospho-PMK-1 levels (Fig 2E) is unconvincing - only one western blot is shown, with no quantification, and there is a smear in the critical first lane.

Thank you, following reviewer's suggestion, we also repeated some of the western. We now replace the Figure 2E and quantified relative intensity of pPMK-1/tublin. We also provide the uncropped western blots images as source data ("raw-data WB" file).

*-The rationales for some of the paper's hypotheses could be improved. For example, the rationale for screening the E. coli mutant library is that some mutants, when heat-killed, may be missing a metabolite that induces the ER-UPR. A more straightforward hypothesis might be that some mutant E. coli strains aberrantly induce the ER-UPR when *not* heat-killed, because they are missing a metabolite that prevents stress pathway induction. This is not in itself a major concern, but it would be useful for the authors to provide a rationale for their hypothesis.*

Thank you for the insightful suggestion. We acknowledge the importance of providing a clear rationale for our hypotheses in the paper. In response to this feedback, we have enhanced the discussion section to better elucidate the rationale behind our hypotheses.

One limitation of our study is the lack of explanation for why HK-*E. coli* activates UPRER and immunity. We hypothesized that when heat-killed, HK-*E. coli* may lack or contain altered levels of certain metabolites that either activate or inhibit UPRER and immunity, respectively. Additionally, we speculated that *E. coli* mutants killed by heat may lack metabolites that activate UPRER and immunity, or conversely, have increased levels of metabolites that inhibit these pathways.

Fortunately, our investigation led to the discovery of the *E. coli* mutant *yfbR*, which inhibits UPRER and immunity by increasing carbohydrates that aid in resisting these stress pathways. Moving forward, we intend to further explore the intricate relationship between HK-*E. coli* and UPRER-immunity. This will be a key focus of our future research efforts.

-The authors do not provide any explanation for some unexpected results from the E. coli screen. Earlier in the paper, the authors found that innate immune signaling is downstream of ER-UPR activation. However, of the 20 E. coli mutants that, when heat-killed, "did not induce... the UPR-ER reporter," 9 of them still activate the innate immune

response. This seems at odds with the authors' simple model since it suggests that low-quality food can induce innate immune signaling independently of the ER-UPR. Further, only one of the 9 has an effect on behavior, even though failure to activate the innate immune pathway might be expected to lead to a behavioral defect in all of these.

Thank you for your understanding, and we apologize for any confusion caused by our earlier statement. To provide clarification, our study revealed that out of the 20 *E. coli* mutants examined, none activated the UPRER. Among these mutants, 9 did not induce immunity, and interestingly, one out of these 9 mutants demonstrated the ability to inhibit avoidance behavior.

This diversity in phenotypic outcomes can be attributed to the varied metabolites present in different *E. coli* mutants. To thoroughly evaluate the effects of these mutants, we conducted a comprehensive three-step screening process, utilizing UPRER marker, immunity marker, and avoidance behavior assays.

Through this rigorous approach, we identified the *E. coli* mutant, *yfbR*, which exhibited the desired inhibitory effects on UPRER, immunity, and avoidance behavior.

Subsequently, we conducted a metabolomics analysis of various food qualities (HK-K12, HK-*yfbR*, and Live-K12). Our findings revealed higher sugar levels in

HK-*yfbR* and Live-K12 compared to HK-K12 (Figure 3B, Figure 3-figure supplement 2A, and Table S1), indicating that sugar deficiency might trigger the UPRER, immunity responses, and subsequent avoidance behavior.

-In a number of places, the writing style can make the authors' arguments difficult to follow.

Thanks for the reviewer's efforts. We changed all of these errors and polish the language of this paper.

*-Some of the effect sizes observed by the authors are exceedingly small (e.g, the suppression of *hsp-4::gfp* induction by sugar supplementation in Figs 3C-E), raising some concern about the biological significance of the effect.*

Thank you for your feedback. In response to your concern, we have included additional clarification in the manuscript.

We have added the following statement: "While sugar effectively inhibits the HK-*E. coli*-induced UPRER and immune response, it does not fully suppress it to the extent observed with live-*E. coli* (Figure 3C-F). This implies that additional nutrients present in live-*E. coli* might also contribute to the inhibition of UPRER and immune response."

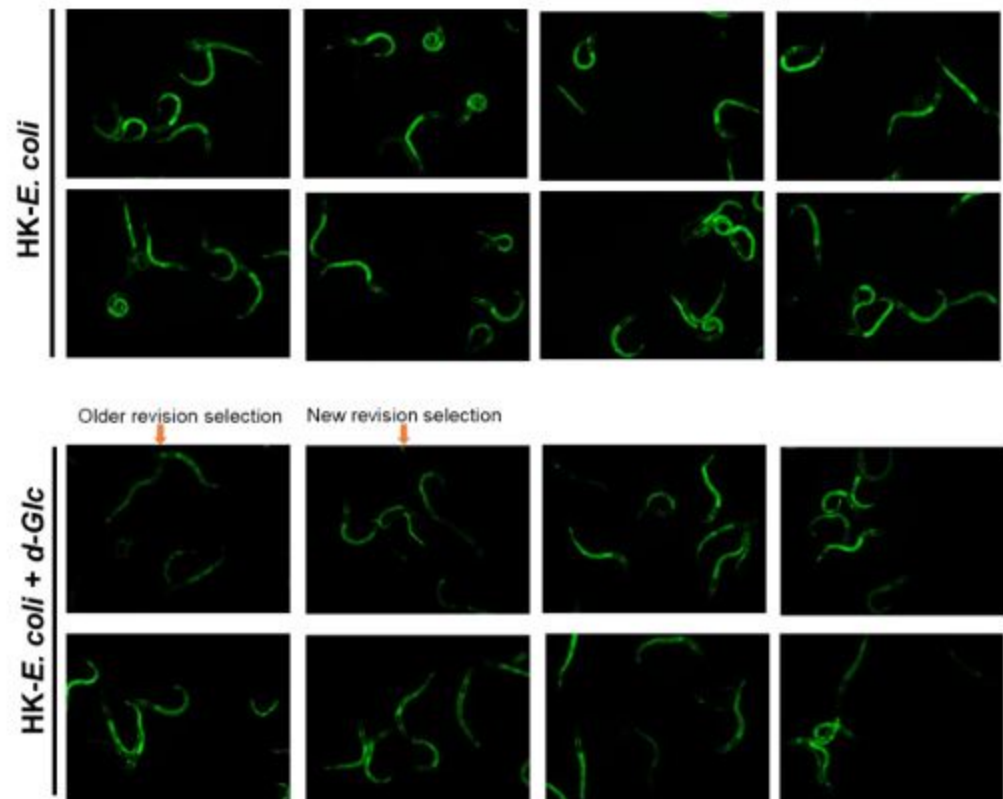
This addition helps to address the observation that some effect sizes appear small, providing context and suggesting potential factors that may influence the outcomes.

-In some cases, there is a discrepancy between the fluorescence images and their quantitation (e.g., Figure 3E, where the effect of glucose on GFP fluorescence seems much stronger in the image than in the graph).

Thank you for your valuable suggestion. In response, we have revised our image selection process to ensure impartiality. We now randomly select images to ensure they accurately represent the quantified data without bias. More details regarding this update can be found in Author response image 2.

Author response image 2.

More original picture corresponding to Figure 3E



Reviewer #3 (Public Review):

Summary:

*Animals can evaluate food quality in many ways. In contrast to the rapid sensory evaluation with smell and taste, the mechanism of slow nutrient sensation and its impact on food choice is unexplored. The authors utilize *C. elegans* larvae and their bacterial food as an elegant model to tackle this question and reveal the detailed molecular mechanism to avoid nutrient-poor foods.*

Strengths:

*The strength of this study is that they identified the molecular identities of the critical players in bacterial food and *C. elegans* using unbiased approaches, namely metabolome analysis, *E. coli* mutant screening, and RNA sequencing. Furthermore, they strengthen their findings by thorough experiments combining multiple methods such as genetics, fluorescent reporter analysis, and Western blot.*

Thank you for highlighting the strengths of our study.

Weaknesses:

*The major caveat of this study is the reporter genes. The transcriptional reporters were used to monitor the UPRER and immune responses in the intestine of *C. elegans*.*

However, their tissue-specific rescue experiments suggest that the genes in the UPRER and immune response function in the neurons. Thus, we should carefully interpret the results of the reporter genes.

Thank you for your insightful comment. We appreciate the opportunity to address your concerns regarding the interpretation of our reporter gene data.

Upon reevaluation, we observed strong induction of the UPRER reporter

(*Phsp-4::GFP*)⁸ and immunity reporter (*Pirg-5::GFP*)⁹ both in the intestine (Figure 1F-G) and in neurons (Figure 1-figure supplement 2A) in response to feeding unfavorable food (HK-*E. coli*). This suggests that both the UPRER and immune pathways may indeed respond to low-quality food (HK-*E. coli*) in multiple tissues of *C. elegans*. While we acknowledge that our tissue-specific rescue experiments suggest a role for these pathways in neurons, the intestinal fluorescence of *Phsp-4::GFP* or *Pirg-5::GFP* is easily observable and scorable. Therefore, we chose to focus our further analyses on the intestine for practical reasons.

Overall, this work provides convincing data to support their model. In the C. elegans field, the behaviors of larvae are not well studied compared to adults. This work will pose an interesting question about the difference between larvae and adults in nutrition sensing in C. elegans and provide a framework and candidate molecules to be studied in other organisms.

Recommendations for the authors:

Reviewer #1 (Recommendations For The Authors):

Major suggestions:

(1) My major overall comment is that the paper would be substantially strengthened by more mechanistic analysis. In particular, how does low vitamin C trigger UPR activation? This is an intriguing finding and it would be important to see it more fully explored.

Our study revealed that the vitamin C content in HK-*E. coli* is comparable to that of live *E. coli* or HK-*yfbR* (Figure 4-figure supplement 1A), suggesting that the induction of unfolded protein response (UPR) in *C. elegans* by HK-*E. coli* is not attributed to low vitamin C levels, but rather to unknown factors.

Metabolomic analysis showed that the sugar levels, including lactose, D-(+)-sucrose, and D-(+)-glucose, were significantly decreased in HK-*E. coli* (Figure 3B, Table S1).

Furthermore, we found that supplementing D-(+)-glucose effectively inhibited UPRER (Figure 3E), immune response (Figure 3F, 3G, and Figure 3-figure supplement 2D), and avoidance behavior (Figure 3H) induced by HK-*E. coli*. Our findings suggest that the deficiency in sugars in HK-*E. coli* triggers a stress response and avoidance behavior in animals, which can be alleviated by D-(+)-glucose supplementation.

Notably, when *E. coli* was heat-killed, we observed that the sugar levels, including lactose and D-(+)-sucrose, were significantly higher in the heat-killed *E. coli* mutant *yfbR* (HK-*yfbR*) compared to HK-*E. coli* (Figure 3B). Moreover, we found that UPRER was reduced in animals feeding HK-*yfbR* (Figure 3-figure supplement 1B), indicating that higher sugar levels may inhibit the induction of UPRER by low-quality food.

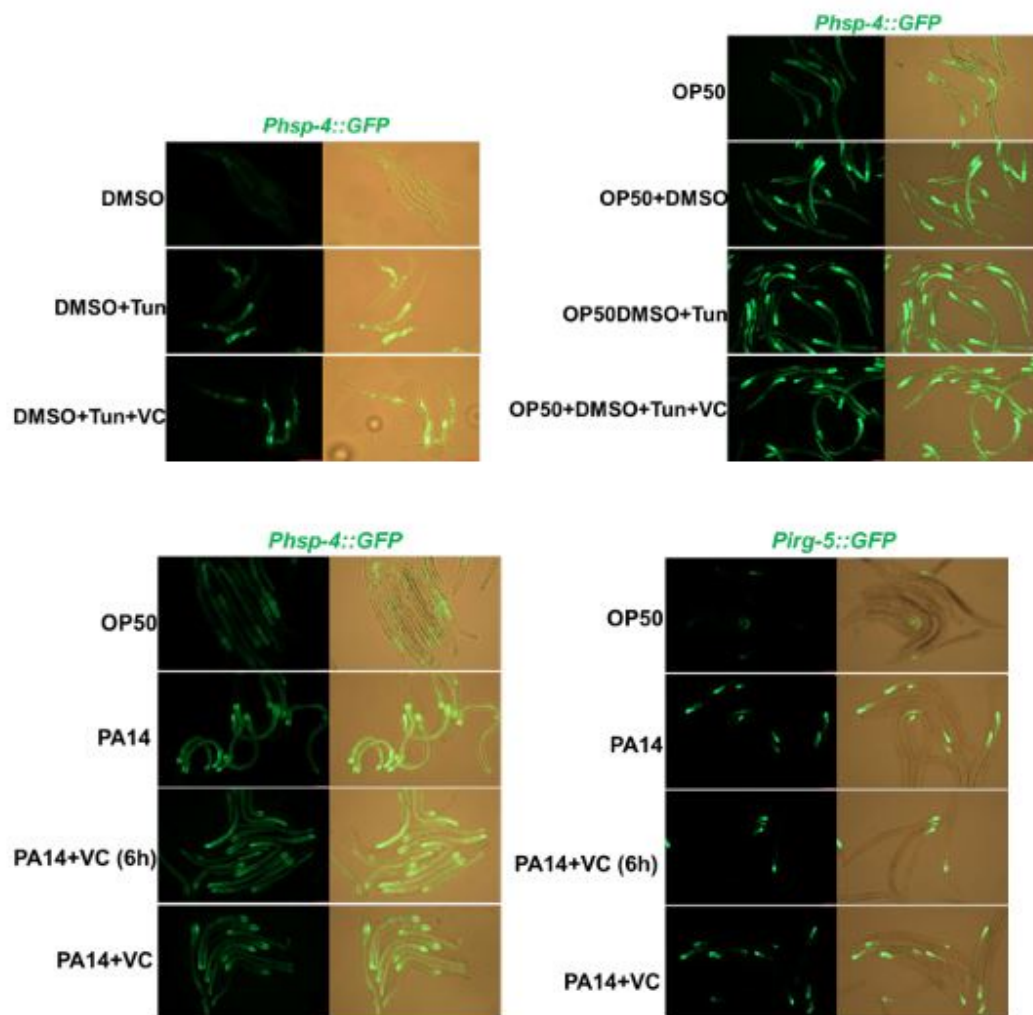
The synthesis of vitamin C (VC) occurs through the glucuronate pathway, utilizing D-glucose as a precursor 1, 2 (Figure 4A). This led us to investigate whether the vitamin C biosynthesis pathway is involved in evaluating low-quality food by using D-glucose. In this study, we found

that animals feeding live *E. coli*, which should produce more VC, exhibit higher glucose levels. However, our results show that animals maintain similar VC levels when fed ideal food (live *E. coli*) compared to low-quality food (HK-*E. coli*) (Figure 4B), suggesting that animals do not stimulate VC biosynthesis under favorable food conditions. In contrast, when animals are fed low-quality food (HK-OP50), we found that supplementing D-GlcA (Figure 4C) or *E. coli-yfbr* mutation (Figure 4B) in HK-*E. coli* can improve VC levels. Moreover, we found that VC or D-glucuronate (D-GlcA) supplementation inhibited HK-*E. coli* induced UPRER (Figure 4D). These data indicate that glucose boosts the animal's ability to adapt to unfavorable food environments by increasing VC levels, thereby inhibiting UPRER, but not in favorable food conditions.

In addition, we asked whether high level of VC inhibits other stress induced UPRER. Previous study shown that Tunicamycin6 and pathogenic bacteria-*Pseudomonas aeruginosa*-PA145 induce UPRER in *C. elegans*. We found that VC supplementation does not inhibit Tunicamycin or PA14-induced UPRER (Author response image 3).

Author response image 3.

VC supplementation does not inhibit Tunicamycin or PA14-induced UPRER.



In addition, how is the activation of the PMK-1 pathway driven by/coordinated with UPR activation?

If the authors do not want to pursue these directions experimentally in this study, the discussion would be strengthened by considering these questions and identifying candidate regulatory mechanisms for further exploration.

In this study, we found that heat-killed *E. coli* (HK-*E. coli*), a low-sugar food, triggers cellular unfolded protein response (UPRER) and immune response. We also demonstrated that 1) the activation of UPRER by low-quality food depends on the IRE-1/XBP-1, 2) activation of immune response (PMK-1) is downstream of XBP-1 in responding to low-quality food.

how is the activation of the PMK-1 pathway driven by/coordinated with UPR activation?

In our discussion part, we added new data and discussion to answer reviewer's question.

A previous study has shown that activating innate immunity (PMK-1 MAPK) leads to a reduction in translation³. Our own previous research has also demonstrated that PMK-1 activation causes a shutdown of food digestion in animals⁴, likely to reduce protein translation and cellular metabolism. To investigate this further, we measured the translation level of animals fed with HK-*E. coli* and found that total translation ability is significantly reduced in these animals (Figure 5-figure supplement 1D). This finding suggests that activating innate immunity (PMK-1 MAPK) may serve as a mechanism to slow down translation progress, thereby alleviating the pressure on the unfolded protein response (UPR) and preventing excessive UPRER activation.

(2) Figure 2C: The data shows that xbp-1 mutants are significantly more likely to leave heat-killed E. coli. However, no other conditions are examined. Is this avoidance defect specific to heat-killed E. coli, or is it a more general effect of xbp-1 mutants - that is, are other conditions that evoke avoidance also affected by mutation of xbp-1? Is feeding behavior on regular E. coli altered in this background? The finding would be more relevant if the authors could clarify or provide more context for their claims here.

We then asked whether UPRER (IRE-1/XBP-1) - Innate immunity (PMK-1/p38 MAPK) axis is specific to evaluate low-quality food (HK-*E. coli*). We examined the avoidance behavior phenotype of wild-type and mutant L1 animals by placing them on various food conditions, including normal *E. coli* food, inedible food (*Saprophytic staphylococci*) and pathogenic food (*Pseudomonas aeruginosa*-PA14), for a 24-hour period. We found that N2, *pmk-1*, and *xbp-1* mutant worms did not exhibit avoidance behavior when presented with normal food (OP50). However, both N2 and *xbp-1* mutant worms were able to escape from inedible food, *Saprophytic staphylococci*, whereas *pmk-1* mutant worms did not show this avoidance. Notably, *xbp-1* mutant worms exhibited even more pronounced avoidance behavior when exposed to *Pseudomonas aeruginosa*, whereas *pmk-1* mutant worms were more susceptible to infection by this pathogen (Figure 2-figure supplement 2C). These findings suggest that the UPR-Immunity pathway plays a crucial role in helping animals avoid low-quality food by triggering an avoidance response. In contrast, the Innate immunity pathway, which is mediated by PMK-1/p38 MAPK, appears to play a key role in evaluating unfavorable food sources, such as HK-*E. coli*, *Saprophytic staphylococci*, and *Pseudomonas aeruginosa*, and helping animals avoid these environments.

(3) Figure 3C-F: The magnitude of the changes between conditions shown in these panels is small. To what extent does this supplementation represent a full rescue? The findings would be strengthened if figures/images for the control condition (non-HK E. coli) were

shown for comparison to allow the reader to assess the extent to which UPR/PMK-1 activation is rescued.

In response to a reviewer's suggestion, we included live-*E. coli* as a control in our study. Notably, our data revealed that the addition of lactose, D-(+)-sucrose, and D-(+)-glucose partially inhibited the HK-*E. coli*-induced unfolded protein response (UPRER) and immune response, suggesting that other nutrients present in live-*E. coli* may also play a role in inhibiting UPRER.

We added this in manuscript: "While sugar effectively inhibits the HK-*E. coli*-induced UPRER and immune response, it does not fully suppress it to the extent observed with live-*E. coli* (Figure 3C-F). This implies that additional nutrients present in live-*E. coli* might also contribute to the inhibition of UPRER and immune response."

(4) Figure 5B-D: The magnitude of changes shown between conditions here again appear to be very small, even those labelled as statistically significant. It is important to ensure that the correct statistical tests have been used to assess the significance of these differences (see below).

All statistical analyses were performed in Graphpad prism 8.0. Two-tailed unpaired t test was used for statistical analysis of two groups of samples, one-way or two-way ANOVA was used for statistical analysis of more than two groups of samples.

(5) Methods: In the "Statistical analysis" section, the authors state that "All statistical analyses were performed using Student's t-test". However, this is not the appropriate test to use in experiments where multiple comparisons are made, which is true in several instances across the paper. In these cases, a more appropriate statistical test should be used.

All statistical analyses were performed in Graphpad prism 8.0. Two-tailed unpaired t test was used for statistical analysis of two groups of samples, one-way or two-way ANOVA was used for statistical analysis of more than two groups of samples.

Minor suggestions:

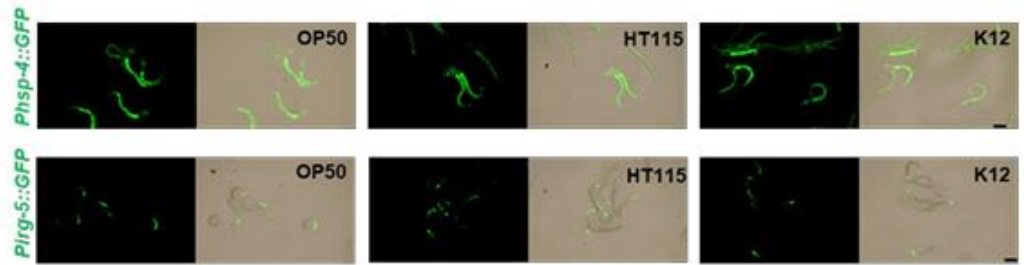
(1) Figure S2: RNAi is usually delivered in a different E. coli strain, HT115. Is this the case with the RNAi knockdowns in Figure S2, and given that diet can influence UPR activation, is it possible that this different diet could change the phenotypes observed?

This should be clarified by the authors.

In this study, all RNAi experiments involved bleaching adult animals under RNAi strain culture conditions to obtain L1 animals. Subsequently, L1 animals were transferred to HK-*E. coli* OP50 for phenotype analysis. In response to a reviewer's suggestion, we observed that L1 animals obtained from mothers fed *E. coli* strains OP50, HT115, or K12 exhibited similar UPR induction under HK-*E. coli* OP50 feeding conditions (Author response image 4). These findings suggest that variations in diet did not alter the UPR phenotypes.

Author response image 4.

L1 animals obtained from mothers fed *E. coli* strains OP50, HT115, or K12 exhibited similar UPR induction under HK-*E. coli* OP50 feeding conditions



Reviewer #2 (Recommendations For The Authors):

Line 182: "irg-5::GFP" should be "hsp-4::gfp".

Thanks for the reviewer's efforts. We have changed this error.

Reviewer #3 (Recommendations For The Authors):

Major comments:

(1) The reporter genes of UPRER and immune response were analyzed in the intestine throughout the study. On the other hand, their rescue experiments suggest that these pathways function in the neurons. They should provide the fluorescence data in the neurons at least for Figures 1F and 1G to confirm that the intestinal response matches the neuronal response and mention that further analyses were done in the intestine for easy scoring.

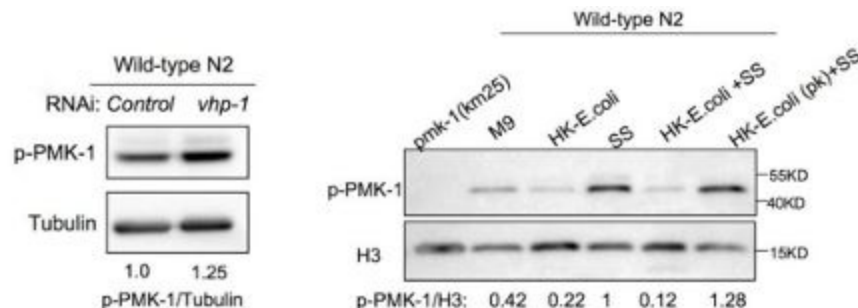
Consistent with the results of the RNA sequencing (RNA-seq) analysis, the UPRER reporter (*Phsp-4::GFP*)⁸ and immunity reporter (*Pirg-5::GFP*)⁹ were strongly induced in intestinal (Figure 1F-G) and neurons (Figure 1-figure supplement 2A) by feeding unfavorable food (HK-*E. coli*), suggesting that UPRER and immune pathways may respond to low-quality food (HK-*E. coli*). As intestinal fluorescence (*Phsp-4::GFP* or *Pirg-5::GFP*) is easy observation and scoring, the further analyses were done in the intestine.

(2) I have concerns about the interpretation of the p-PMK-1 data. Although the authors described that "p-PMK-1 is prominently increased" in the text (Line 150), it is unclear on the data (Figure 2E). Similarly, the authors' statement "p-PMK-1 is decreased in animals with D-GlcA (F).." was not fully supported by the data in Figure 4F. The experiment should be repeated and quantified. Moreover, pPMK-1 showed single bands in Figure 2E, but double bands in Figure 3G, 4F, and 4G. The authors should explain why that is the case and which band we should look at for Figures 3G, 4F, and 4G.

As reviewer's suggestion, we also repeated some of the western. We found that after longer expose, there are two bands for pPMK-1 (Figure 2E, new data; and "raw-data WB" file). The VHP-1 phosphatase is known to inhibit PMK-13. In our previous study, we found that worms treated with *vhp-1*(RNAi), which hyperactivates p-PMK-1 (lower band) 4. In contrast, the two bands are disappeared in *pmk-1* mutant (Author response image 5). Thus, the lower band indicates the pPMK-1. We now replace the Figure 2E and quantified relative intensity of pPMK-1/tubulin. We also provide the uncropped western blots images as source data ("raw-data WB" file).

Author response image 5.

In our previous study, we found that worms treated with vhp-1(RNAi), which hyperactivates p-PMK-1 (lower band) 4. In contrast, the two bands are disappeared in pmk-1 mutant. These pictures are extracted from our previous study4.



(3) Heat-killed *E. coli* (HK-*E. coli*) is low-quality because the lack of sugar cannot support the growth of *C. elegans* larvae (Qi and Han, Cell, 2018). Thus, animals do not show the UPRER-immune response and avoidance when HK-*E. coli* is supplemented with sugars such as glucose (Line 225-227). If these sugars are the key, *C. elegans* larvae should be able to grow better with HK-*E. coli* supplemented with glucose. Authors should address this possibility.

Previous studies have shown that heat-killed *E. coli* (HK-*E. coli*) is a low-quality food source that cannot support the growth of *C. elegans* larvae⁷. Here, we found that sugar deficiency in HK-*E. coli* induces the UPRER-immune response and avoidance behavior in *C. elegans*. Given this, we investigated whether sugar supplementation could promote animal growth when fed HK-*E. coli*. To our surprise, supplementing HK-*E. coli* with carbohydrates (D-Glc, D-GlcA) did not support animal development (Figure 3-figure supplement 2G), suggesting that carbohydrates are not essential for supporting animal growth on this food source. However, we did find that carbohydrates are critical for inhibiting the UPRER-immune response induced by sugar deficiency in HK-*E. coli*.

(4) Line 884: Instead of the Student's t-test, the ANOVA should be used for multiple comparisons.

All statistical analyses were performed in Graphpad prism 8.0. Two-tailed unpaired t test was used for statistical analysis of two groups of samples, one-way or two-way ANOVA was used for statistical analysis of more than two groups of samples.

(5) Although the results are interesting and convincing, the manuscript needs some careful editing and proofreading. As far as I could catch, there are more than 100 errors and typos, as I summarized in minor comments. I recommend the authors proofread thoroughly to make this work easier to read.

Thanks for the reviewer's efforts. We changed all of these errors and polish the language of this paper.

Minor comments:

(1) Line 30: nature -> natural

(2) Line 86: elegnas -> elegans

- (3) Line 93: the17h -> the 17h
- (4) Line 97: response -> respond
- (5) Line106: responded -> respond
- (6) Line 107-109: Add references for the three reporters
- (7) Line 114: immune -> immune pathway
- (8) Line 118: immune depended -> immune-dependent
- (9) Line 128, 594, 596: deferentially -> differentially
- (10) Line 131: Explain what IRE-1-mediated splicing of xbp-1 with references
- (11) Line 170: XPB-1 -> XBP-1
- (12) Line 179: URP -> UPR
- (13) Line 181: hsp-4::GFP -> Phsp-4::GFP
- (14) Line 183: Italicize *E. coli*; mutant -> mutants
- (15) Line 184: irg-5::GFP -> Pirg-5::GFP (2 places)
- (16) Line 197, 203, 206, 207: Lactose -> lactose
- (17) Line 206, 209, 217, 225, 228, 232, 237, 262, 442, 445, 604, 739: Glucose -> glucose
- (18) Line 218: Sugars deficiency -> sugar deficiency
- (19) Line 229: found contribute to -> found to contribute to
- (20) Line 235, 537, 539, 587, 599, 642, 855: Italicize *E. coli*
- (21) Line 236: same -> the same
- (22) Line 239: I recommend adding "in *C. elegans*". This study uses both *E. coli* and *C. elegans* genetics. Sometimes, it is confusing which organism was mentioned. It should be applied where it is necessary.
- (23) Line 240: additional -> addition
- (24) Line 339, 642: Italicize *kgb-1*
- (25) Line 390: Italicize *Pseudomonas aeruginosa*, *Bacillus thuringiensis*, *Staphylococcus aureus*, and *Serratia marcescens*
- (26) Line 394: wiht -> with
- (27) Line 400, 550: Change ER to superscript; Italicize *ire-1*, *xbp-1*, and *pmk-1*
- (28) Line 415: xpb-1 -> xbp-1
- (29) Line 460, 525, 531, 532, 617, 655: Italicize *yfbR*
- (30) Line 457, 468, 472, 475, 482, 497, 513, 624, 629, 633, 733, 758: Vitamin -> vitamin
- (31) Line 459: Make it clear what is the relationship between vitamin C and TAA

- (32) Line 527: Do not italicize mutant
- (33) Line 538: Phsp-6:GFP -> Phsp-6::GFP (to match other descriptions)
- (34) Line 540: Phsp-4:GFP -> Phsp-4::GFP (to match other descriptions)
- (35) Line 540: Italicize hsp-4
- (36) Line 543: Pirg-5:GFP -> Pirg-5::GFP (to match other descriptions) and italicize irg-5
- (37) Line 550, 881: Innate -> innate
- (38) Line 557, 560, 564, 838: Do not italicize HK
- (39) Line 561: Remove the extra space before "three"
- (40) Line 575, 577: Reporter -> reporter
- (41) Line 575, 607: Italicize Phsp-4::GFP
- (42) Line 577: immunity -> Immunity; Italicize Pirg-5::GFP
- (43) Line 585, 653: keio -> Keio
- (44) Line 586: hsp-4::GFP -> Phsp-4::GFP
- (45) Line 586, 589 (2 places): irg-5::GFP -> Pirg-5::GFP
- (46) Line 597: Remove "all"
- (47) Line 600: Trehalose -> trehalose
- (48) Line 609: Italicize Pirg-5::GFP
- (49) Line 615: critically -> critical
- (50) Line 636: Remove "+"
- (51) Line 656 (2 places), 682: Do not italicize OP50
- (52) Line 664: Lead -> lead
- (53) Line 681: Describe the composition of NGM or show the reference. Since this paper examines nutrition, the composition of the medium is crucial.
- (54) Line 686-706: Italicize all allele names. Be consistent with how to write the promoter to avoid confusion (e.g., ttx-3p -> Pttx-3). Be consistent with how to describe the transgene (e.g., Phsp-4::GFP(zcIs4) -> zcIs4[Phsp-4::GFP])
- (55) Line 710: Describe the composition of LB or show the reference. Since this paper examines nutrition, the composition of the medium is crucial.
- (56) Line 709, 856 (2 places), 858: Do not italicize K12 to make it consistent
- (57) Line 719: Podr-1p:RFP -> Podr-1::RFP
- (58) Line 722, 724: Italicize ges-1 and xbp-1
- (59) Line 723: Pges-1::xbp-1::GFP -> Pges-1::xbp-1::GFP
- (60) Line 735: Glucuronic -> glucuronic

- (61) Line 748: I believe it is 5 mm instead of 0.5 mm
- (62) Line 750: The equation should be $(5 \text{ mm})^2 / (17.5 \text{ mm})^2$
- (63) Line 759: Remove the period after "pattern".
- (64) Line 766: Describe how they were synchronized
- (65) Line 774: Italicize *Psym-1p::GFP*
- (66) Line 785: Insert a space before "until"
- (67) Line 787: the mutant -> mutant
- (68) Line 789, 792, 793, 795 (2 places): GFP -> GFP
- (69) Line 791: next -> Next; an -> a
- (70) Line 799: Remove a space before "MRC".
- (71) Line 804: I do not understand what "until adulthood" means in this context;
Remove a space before "by". (I recommend searching double space and correcting it.)
- (72) Line 853: Metabolome -> metabolome
- (73) Line 893-1082: Species and gene names should be italicized in Reference
- (74) Figures 1F, 1G, S2F, S2G: The panels' order should match the bar graphs' order. The apparent difference in the representative data does not match the marginal difference in the bar graph in Fig. 1G. The authors should double-check the results.
- (75) Figure 1F, 2A, 2B, 3C, 3D, 3E, 4D, 4I, S1J, S2A, S2B, S2I, S3B, S3F, S3H: hsp-4::GFP -> Phsp-4::GFP
- (76) Figure 1G, 2D, 3F, 4E, 4J, S1K, S2H, S3C, S3I: irg-5::GFP -> Pirg-5::GFP
- (77) Figure 6: Liquids -> Lipids; Italicize *ire-1*, *xbp-1*, *pmk-1*
- (78) Figure S1I: hsp-6::GFP -> Phsp-6::GFP
- (79) In the legend for Figure S1 after Figure S1, (A), (B)... were duplicated. It is OK in the corresponding main text (Line 530)
- (80) Figure S2F, S3G, S4C, S4D: sysm-1::GFP -> Psym-1::GFP
- (81) Figure S2G: irg-1::GFP -> Pirg-1::GFP
- (82) Figure S3H and S3I: Describe which ones are Glu + conditions

References:

- (1) Patananan AN, Budenholzer LM, Pedraza ME, Torres ER, Adler LN, Clarke SG. The invertebrate *Caenorhabditis elegans* biosynthesizes ascorbate. *Arch Biochem Biophys* 569, 32-44 (2015).
- (2) Yabuta Y_, et al. L-Ascorbate Biosynthesis Involves Carbon Skeleton Rearrangement in the Nematode *Caenorhabditis elegans*. *Metabolites* 10, (2020).
- (3) Weaver BP, Weaver YM, Omi S, Yuan W, Ewbank JJ, Han M. Non-Canonical Caspase Activity Antagonizes p38 MAPK Stress-Priming Function to Support Development. *Dev Cell* 53,

358-369 e356 (2020).

(4) Geng S_, et al._ Gut commensal *E. coli* outer membrane proteins activate the host food digestive system through neural-immune communication. *Cell Host Microbe* 30, 1401-1416 e1408 (2022).

(5) Richardson CE, Kooistra T, Kim DH. An essential role for XBP-1 in host protection against immune activation in *C. elegans*. *Nature* 463, 1092-1095 (2010).

(6) Harding HP_, et al._ An Integrated Stress Response Regulates Amino Acid Metabolism and Resistance to Oxidative Stress. *Molecular Cell* 11, 619-633 (2003).

(7) Qi B, Kniazeva M, Han M. A vitamin-B2-sensing mechanism that regulates gut protease activity to impact animal's food behavior and growth. *eLife* 6, e26243 (2017).

(8) Calfon M_, et al._ IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415, 92-96 (2002).

(9) Bolz DD, Tenor JL, Aballay A. A Conserved PMK-1/p38 MAPK Is Required in *Caenorhabditis elegans* Tissue-specific Immune Response to *Yersinia pestis* Infection*. *The Journal of Biological Chemistry* 285, 10832 - 10840 (2010).

<https://doi.org/10.7554/eLife.94181.2.sa0>