Microbial byproducts determine reproductive fitness of free-living and parasitic nematodes

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Abstract

Trichuris nematodes reproduce within the microbiota-rich mammalian intestine, yet microbial byproducts that facilitate the parasite lifecycle are unknown. Here, we report a novel pipeline to identify microbial factors with conserved roles in the reproduction of nematodes. A screen for *E. coli* mutants that impair *C. elegans* fertility identified genes in fatty acid biosynthesis and ethanolamine utilization pathways, including *fabH* and *eutN*. *Trichuris muris* eggs displayed defective hatching in the presence of *E. coli* deficient in *fabH* or *eutN* due to reduction in arginine or elevated levels of aldehydes, respectively. Remarkably, *T. muris* reared in gnotobiotic mice colonized with these *E. coli* mutants failed to lay viable eggs. These findings indicate that microbial byproducts mediate evolutionarily conserved transkingdom interactions that impact reproductive fitness of distantly-related nematodes.

One-Sentence Summary: Byproducts from the microbiota contribute to the life cycles of distantly-related free-living and parasitic worms.

Main text

The reproductive success of the parasitic nematode *Trichuris trichiura* is evidenced by the over 400 million individuals colonized by this soil-transmitted helminth (1). Its enormous reproductive capacity contributes to its infectious spread and hampers parasite eradication efforts. A distinct feature of *Trichuris* species is that reproductive development, from egg hatching to egg laying, is completed exclusively within the host digestive tract (2, 3). A new infection begins when embryonated eggs ingested from the environment hatch in the host cecum, a region within the gastrointestinal tract populated by a dense community of bacteria. Although bacteria mediate egg hatching for *Trichuris muris* (4-6), the *Trichuris* species that infects mice, a role for bacteria at later stages of *Trichuris* development remains unclear. Our group and others have shown that *Trichuris* infections in mice and humans affect the bacterial composition of the gut microbiota (6-13). Additionally, the *T. muris* microbiota harbors taxa similar to those observed in the murine host microbiota, with a notable enrichment of Proteobacteria, including Gram-negative commensals like *E. coli* (6).

For the free-living nematode *C. elegans*, reducing the quantity or quality of the bacterial diet, or interfering with nutrient uptake, can impair germline development with consequences for fertility (14-19). Because of the high degree of conservation of body plan and neuromuscular system organization within the phylum Nematoda, *C. elegans* has been used as a model for the discovery of anthelmintics for more than four decades (20, 21). Therefore, we hypothesized that bacteria-derived essential requirements for reproductive development in the well-characterized, model organism *C. elegans* are conserved in *Trichuris*.

To identify bacteria-derived factors that impact nematode reproduction, we assessed fertility of young adult *C. elegans* raised on a library of viable *E. coli* mutants. We used *C. elegans* bearing a temperature-sensitive mutation in the gene encoding the GLP-1 Notch receptor, *glp-1(e2141)*, to facilitate quantification of fertility. At the semi-permissive temperature of 20°C, the number of germline progenitor cells is roughly half of the wild type (22), thereby sensitizing the worms to fertility defects when signaling pathways conveying nutritional sufficiency are altered (14, 18). The *C. elegans* strain also carried fluorescent markers for the pharynx and for embryos (23) to facilitate counting and staging the worms, and to determine whether individual worms contained embryos (gravid) (Fig. 1, A-B). Wells were excluded from subsequent analyses if *E. coli* failed to grow, or if *C. elegans* exhibited developmental arrest or severely delayed growth. Using a series of selection criteria that considered both plate-by-plate statistical comparisons and penetrance, our primary screen of ~3000 of the 3985 *E. coli* mutants in the Keio library identified 315 bacterial mutants that reduced the percentage of gravid worms at the established time point (Fig. 1, C-D).

After these 315 *E. coli* mutants were re-screened in biological triplicate, ten mutants significantly reduced the penetrance of embryo-bearing *C. elegans* compared to our wild-type (WT) *E. coli* strain (GC1547) in at least two out of three biological replicates (Fig. 1C and S1, A-B). Seven of the ten *E. coli* genes fell into three functional groups related to ethanolamine utilization, fatty acid biosynthesis or lipopolysaccharide synthesis (Table S1).

We focused on the *E. coli* mutants $\Delta fabF$, $\Delta fabH$, $\Delta eutD$, and $\Delta eutN$, because they were associated with the greatest reduction in embryo-bearing *C. elegans* (Fig.

1E). Complementation with a plasmid carrying the wild-type *fabH* or *eutN* sequences confirmed their functional relevance in the *C. elegans* fertility delay phenotype (Fig. S1C). For *fabF*, we rederived the *E. coli* deletion mutant by using P1 phage to transduce the original $\Delta fabF::kan$ allele from the Keio library strain into the Keio progenitor strain BW25113 (Fig. S1, D-E). Similar to the original mutant, the transductant caused a significant reduction in the percentage of embryo-bearing *C. elegans*.

To assess whether these *E. coli* mutants delayed somatic and/or germline development of *C. elegans*, we performed a time-course analysis of vulval development (24) and assayed for enhancement of the Pro phenotype caused by a weak *glp-1(gf)* mutation (25). The latter assay is a proxy for delayed germline development relative to somatic development (26). Among the four mutants, only worms fed $\Delta eutN$ exhibited delayed somatic development, with an additional germline delay relative to somatic development (Fig. 1, F-G). Worms fed $\Delta fabF$ displayed delayed germline development without the somatic delay, while $\Delta fabH$ and $\Delta eutD$ developed comparably with worms fed WT *E. coli*. When we prolonged the assay by scoring the worms on Day 2—24 hours after the initial screening time-point (Day 1)—the percentage of gravid worms significantly increased, indicating that feeding on these *E. coli* mutants delayed *C. elegans* fertility rather than induced permanent sterility (Fig. 1H). We also found no significant differences when comparing the number of germline progenitor zone (PZ) nuclei in *C. elegans* raised on each mutant or WT *E. coli* at the L4-to-adult molt (Fig. S1, F-G), indicating that the delay in fertility is not likely due to slower accumulation of the PZ during larval stages (14, 27, 28).

DAF-2 insulin/IGF-like and DAF-7 TGF β signaling pathways link nutrition and fertility (16-18, 22). However, constitutive activation of these pathways (by loss of DAF-16 FOXO or DAF-5, respectively) did not substantially mitigate the delay in fertility caused by the *E. coli* mutants. Loss of *daf-16* actually exacerbated the penetrance of non-gravid worms, even with WT *E. coli* (Fig. S1H), whereas constitutive activation of the DAF-7 TGF β pathway via loss of *daf-5* had no effect on fertility of *C. elegans* raised on Δ *eutD* and a modest effect with Δ *eutN*, Δ *fabF* and Δ *fabH* (Fig. S1I). Loss of *daf-5* also elevated penetrance of gravid *C. elegans* raised on WT *E. coli* to a similar or greater extent. We conclude that neither pathway likely mediates the effects of these *E. coli* mutants on the timing of *C. elegans* fertility.

Having identified *E. coli* mutants that impede fertility of a free-living nematode, we examined whether these bacteria impact a distantly-related parasitic nematode. *T. muris* egg hatching was shown to be the reproductive stage sensitive to the presence of bacteria (4, 6). Thus, to begin investigating possible effects of the mutants identified in our *C. elegans* screen on the *Trichuris* lifecycle, we adapted a previously described method to quantify *E. coli*-mediated hatching kinetics of embryonated *T. muris* eggs *in vitro* (4) (Fig. 2A). All *E. coli* mutants were confirmed to have similar growth kinetics to WT *E. coli* (Fig. S2). Two of the mutants tested, Δ *eutN* and Δ *fabH*, elicited significantly reduced hatching compared to rates mediated by WT *E. coli* (Fig. 2B).

Complementation of Δ *eutN* or Δ *fabH* with their wild-type genes rescued hatching (Fig. S3).

To determine whether reduced egg hatching is due to the production of a toxic factor, we performed the hatching assay utilizing WT *E. coli* mixed with each mutant in a

9:1 or 1:1 ratio (Fig. 2C). As little as 10% $\Delta eutN$ in the mixture significantly inhibited the ability of WT *E. coli* to mediate hatching, whereas the presence of $\Delta fabH$ had no effect. Thus, the inhibitory effect of $\Delta eutN$ is dominant over WT *E. coli*, implicating the production of a toxic factor by this mutant, while the reduced hatching rate of embryonated eggs incubated with $\Delta fabH$ is likely due to deficiency of a pro-hatching factor.

Hybrid metabolomics revealed an unexpectedly low relative level of arginine and ornithine in $\Delta fabH$ cell lysates compared to WT *E. coli* (Fig. 2D). Supplementation of $\Delta fabH$ cultures with 500 μ M arginine partly suppressed the *C. elegans* fertility delay without elevating the baseline fertility seen in the control (Fig. 2E and S4, A-B). We observed a similar result with ornithine supplementation (Fig. 2F and S4C). Supplementation of $\Delta fabH$ cultures with arginine or ornithine also successfully rescued *T. muris* egg hatching to levels similar to WT *E. coli* (Fig. 2, G-H and S4, D-E). Consistent with a role for the arginine biosynthesis pathway, we found that *Trichuris* egg hatching was diminished in the presence of $\Delta argE$ or $\Delta argH$ *E. coli*, mutants defective in ornithine and arginine synthesis, respectively (Fig. 2, I-J).

Although optimal *T. muris* egg hatching *in vitro* requires physical contact with *E. coli* (4, 5), a reduced amount of hatching can still occur in a transwell system that separates bacteria and eggs into different chambers (5), suggesting that both contact-dependent and independent factors contribute to the process. Similar to previous reports, eggs hatched at approximately 50% their maximum rate when incubated separately from WT *E. coli* in a transwell system (Fig. 2, K-L). Hatching was further reduced when $\Delta fabH$ was used as the bacterium. Supplementation of ornithine restored

hatching in the presence of $\Delta fabH$ to levels similar to those elicited by WT *E. coli* in the transwell system. Altogether, these findings indicate that nematode pathways regulated by ornithine and arginine contribute to contact-independent *E. coli*-mediated hatching of *T. muris* embryonated eggs.

To understand why *T. muris* eggs incubated with $\Delta eutN$ *E. coli* displayed the greatest reduction in hatching, we first determined whether disrupting other parts of the ethanolamine utilization pathway (Fig. 3A) led to similar outcomes. *E. coli* mutants lacking enzymes in the pathway— $\Delta eutB$, $\Delta eutC$, $\Delta eutD$, $\Delta eutE$, and $\Delta eutG$ —facilitated egg hatching at rates similar to WT *E. coli* (Fig. 3B). In contrast to these *eut* operon genes, *eutN* encodes a subunit of a proteinaceous shell that enables the efficient colocalization of the *eut* enzymes with their cofactors and substrates within a microcompartment and prevents diffusion of pathway intermediates (29). It is possible that disruption of the microcompartment architecture allows the release of a soluble toxic intermediate. Therefore, we tested whether transferring the supernatant from $\Delta eutN$ culture was sufficient to inhibit the ability of WT *E. coli* to mediate hatching (Fig. 3C). Although transferring culture supernatant from $\Delta eutN$ grown in isolation had no effect, supernatant isolated from $\Delta eutN$ that had been incubated with eggs reduced hatching mediated by WT *E. coli* (Fig. 3D). This suggests that $\Delta eutN$ produces a soluble toxic factor in the presence of *T. muris* embryonated eggs.

Acetaldehyde is a known intermediate of the ethanolamine utilization pathway that can readily form irreversible adducts with proteins, changing their conformation and function (30, 31). Additionally, deletion of *eutN* in *Salmonella* resulted in the greatest increase in acetaldehyde volatility among all *eut* operon mutants during the first hours of

growth (32). Using a fluorogenic hydrazone transfer (DarkZone) system to label aldehydes (33), we detected elevated levels of aldehydes in the culture supernatant of $\Delta eutN$ that had been incubated with *T. muris* eggs, but not in the culture supernatant of the complemented $\Delta eutN$ strain or when the mutant was grown in the absence of eggs (Fig. 3E and S5A). Aldehyde levels were also elevated in $\Delta eutD$, the other *E. coli* mutant of this pathway identified by the *C. elegans* screen, although the increase following egg incubation was not as striking as for $\Delta eutN$, potentially explaining why deletion of *eutD* did not inhibit hatching (Fig. 3E). Next, we deleted the rate limiting enzyme of the ethanolamine utilization pathway, *eutC*, in the $\Delta eutN$ background ($\Delta eutN\Delta eutC$) to prevent the formation of toxic intermediates (32, 34). We found that aldehyde levels of $\Delta eutN\Delta eutC$ were similar to that of WT *E. coli* and that *T. muris* egg hatching was completely restored when the assay was performed with this double mutant (Fig. 3E-F). Additionally, we found that supplementation with acetaldehyde was sufficient to inhibit hatching elicited by WT *E. coli* in a concentration-dependent manner and confirmed that bacteria viability was unaffected (Fig. S5, B-C).

In vitro T. muris egg hatching serves as a sensitive assay to investigate nematode-bacteria interactions, but the role of intestinal bacteria in subsequent steps of *Trichuris* development remains obscure because they are difficult to recreate outside the mammalian host. Given that the *C. elegans* screen was focused on development of the reproductive tract, we established a bacterial monocolonization mouse model to investigate the role of bacterial *fabH*, *eutN*, and *eutD* in *T. muris* development and reproduction (Fig. 4A). After we verified that each *E. coli* mutant colonized germ-free mice to levels similar to WT *E. coli* (Fig. S6A), monocolonized mice were gavaged twice

with 100 eggs and sacrificed two weeks after the second gavage. We were able to recover adult worms from the cecum of mice monocolonized with WT *E. coli* but not germ-free mice (Fig. S6B), consistent with previous observations (6), indicating that our assay allows investigation of mechanisms by which *E. coli* contributes to *T. muris* development in an intact host. Our experimental setup in which mice receive high doses of embryonated eggs was designed to overcome the potential reduced hatching efficiency that may occur in mice lacking a complex microbiota. Therefore, as expected, we did not detect any differences in worm burden when comparing mice colonized with WT *E. coli* versus $\Delta fabH$, $\Delta eutN$, and $\Delta eutD$ strains (Fig. S6B). However, many of the *T. muris* recovered from mice colonized with these mutants displayed a striking deformity characterized by shortened length (Fig. 4, B-C; indicated by arrowheads). Although worms from WT *E. coli*-colonized mice were exclusively long in length, mice colonized with $\Delta fabH$, $\Delta eutN$, and $\Delta eutD$ *E. coli* produced a mixture of both long and short length worms, defined as < 1cm. The defect was most severe in mice monocolonized with $\Delta eutN$ from which most worms recovered were short.

The average number of freshly laid eggs per worm was not significantly different between the conditions (Figure S6C). However, in stark contrast to worms from WT *E. coli*-colonized mice, which laid eggs of the typical ovoid structure, eggs laid by worms harvested from mice colonized with $\Delta fabH$, $\Delta eutD$, and $\Delta eutN$ *E. coli* displayed an aberrant rounded morphology, and in some cases lacked opercula and a distinct shell, and the inner contents were disorganized (Fig. 4, D-E). These structurally aberrant eggs took longer to reach embryonated morphology, and even after a prolonged incubation period, significantly fewer eggs appeared embryonated in the $\Delta eutN$ and $\Delta eutD$ groups

(Fig. 4F). Eggs laid by worms from all three mutant-colonized groups of mice had significantly lower hatching rates compared to eggs laid by worms from WT *E. coli*-colonized mice when tested for bacteria-mediated hatching *in vitro* (Fig. 4G). Finally, monocolonization with $\Delta eutN\Delta eutC$ abrogated the length and egg fitness defects of *T. muris* observed in $\Delta eutN$ -colonized mice (Fig. S7). Thus, bacterial products are important for the reproductive development of *T. muris* *in vivo*.

Despite the wide taxonomic divergence between *C. elegans* and *T. muris*, our screen for bacterial factors impacting fertility in *C. elegans* identified two pathways that impact *T. muris* biology. Our finding that bacterial deficiencies decrease *T. muris* worm size and egg fitness is in line with recent evidence showing that another parasitic nematode *Heligmosomoides polygyrus*, lays fewer eggs and exhibits similar length defects when reared in a germ-free host (35). Our results also provide evidence for a previously undescribed role for byproducts of microbial metabolism on nematode development, a role that appears evolutionarily conserved. Despite lacking many genes specific to parasitism, our findings support the utility of *C. elegans* as a model for bacteria-nematode interactions because of its high sensitivity to its bacterial milieu. Focusing on microbiota-nematode interactions has also revealed vulnerabilities of *Trichuris* that have implications for transmission dynamics and may be useful for controlling life-long infections in areas where helminths are endemic because of ineffective deworming treatment and high rates of reinfection. We propose that *C. elegans* can be used as a novel system for investigating the effects of specific microbial genes and pathways in supporting the parasitic nematode life cycle and aid in further understanding the transkingdom interactions that sustain human disease.

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Author contributions

M.V., R.D., E.J.A.H, and K.C. conceived the study and designed the experiments. M.V. and R.D. performed, analyzed, and interpreted all the experiments for *T. muris* and *C. elegans*, respectively. D.J.L. performed the bacterial cloning. H.S.P and E.T.K provided DarkZone and technical advice. K.C., E.J.A.H, and J.G.B oversaw analysis and interpretation of all experiments described. M.V., R.D., E.J.A.H., and K.C. wrote the manuscript with inputs from all authors.

Competing interests

K.C. has received research support from Pfizer, Takeda, Pacific Biosciences, Genentech, and Abbvie. K.C. has consulted for or received honoraria from Puretech Health, Genentech, and Abbvie. K.C. holds U.S. patent 10,722,600 and provisional patent 62/935,035 and 63/157,225, and E.J.A.H. holds US patent 6,087,153.

Data and materials availability

All data needed to evaluate the conclusions in this study are available in the main text and the supplementary materials or available from the corresponding authors upon request.

Supplementary Materials

Materials and Methods

Figs. S1 to S7

Tables S1-S5

Fig. 1 Identification of *E. coli* mutants that delay *C. elegans* fertility. (A) Concept of high throughput screening of *C. elegans* raised on *E. coli* mutants from the Keio library.

(B) Example of reduced penetrance of *C. elegans* bearing embryos at a single time-point; embryos marked with GFP and pharynx with mCherry. **(C)** Detailed flowchart for schematic in (A). **(D)** Raw primary screen results recorded as percentage of worms without embryos in each well. **(E)** Percentage of *C. elegans* bearing embryos in replicates of four *E. coli* mutants of interest from the screen. In each case, $p \leq 0.001$ for each mutant versus its control from the same plates; total n number of worms scored are (left to right): 409, 923, 242, 387, 189, 522, 163, 501. **(F)** Somatic development is delayed in *C. elegans* raised on $\Delta eutN$. Percent of worms at indicated stage at 56 hours after L1 seeding to wells containing the control or mutant bacteria. $n \geq 38$ worms were scored for each *E. coli* mutant. $\Delta eutN$ is the only mutant that is statistically significantly different from the control GC1547 ($p \leq 0.001$). n number of worms scored (left to right): 52, 59, 61, 40, 54. **(G)** $\Delta eutN$ and $\Delta fabF$ delay germline development relative to somatic development. Percent of gonad arms displaying characteristic Pro phenotype. $p \leq 0.001$ for $\Delta eutN$ and $\Delta fabF$. Pooled from two independent trials; n number of worms scored are (left to right): 87, 103, 79, 41, 61. **(H)** Fertility is delayed. Percent of worms with embryos on Day 1 and Day 2. $p \geq 0.05$ (not significant) for any mutant versus the control on Day 2. n number of worms scored (left to right): 547, 297, 242, 242, 60, 46, 124, 121, 253, 261. (D, G) Circles represent mean of each of independent biological replicate and bar is SEM; pairwise Fisher's exact test versus controls (D-F) or Day 1 versus Day 2 (G). ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Fig. 2 *E. coli* mutants with altered metabolic byproducts delay *C. elegans* fertility and inhibit *T. muris* egg hatching. (A) Experimental approach for *in vitro* hatching

assay. **(B)** Percent *in vitro* hatched *T. muris* eggs after incubation with overnight cultures of four *E. coli* mutants identified from the *C. elegans* screen or WT *E. coli*. Hatched eggs were checked using light microscopy at timepoints indicated (n = 4); h = hours. **(C)** Hatching rates for mixtures of WT *E. coli* and either $\Delta fabH$ or $\Delta eutN$ overnight cultures in the ratios indicated. Total concentration of each mixture was maintained at 1×10^8 CFU/mL (n = 3). **(D)** Hierarchical clustering of metabolites that were significantly (p < 0.05, Student's t-test) downregulated (blue) or upregulated (red) in $\Delta fabH$ relative to WT. **(E, F)** Percentage of *C. elegans* Day 1 adults with embryos raised on $\Delta fabH$ *E. coli* with arginine or ornithine supplementation. Fisher's exact test for $\Delta fabH$ alone or with supplementation with 500 μ M arginine or 100 μ M ornithine compared to WT; Total n number of worms scored (left to right): **(E)** 208, 237, 300 and **(F)** 207, 216, 149. **(G, H)** Percent *in vitro* hatched *T. muris* eggs elicited by $\Delta fabH$ and cultures supplemented with (G) 500 μ M arginine or (H) 70 μ M ornithine where indicated (n=3). **(I)** Arginine biosynthesis pathway in *E. coli*. **(J)** Hatching rates for $\Delta argE$ and $\Delta argH$ (n = 4). **(K)** Experimental approach for hatching assay in a transwell system. **(L)** Hatching quantified for eggs contained within a transwell chamber separated from bacterial culture in the outer chamber by a membrane with 0.4 μ m pores. Samples supplemented with ornithine as in H are indicated (n = 3). (B) One-way analysis of variance (ANOVA) of area under curve compared to WT. (C, G-H, J, L) One-way analysis of variance (ANOVA) with Dunnett's post-test compared to WT. For all panels, measurements were taken from distinct samples. Graphs show means and SEM. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001. ns = not significant.

Fig. 3 Toxic intermediates of the *E. coli* ethanolamine utilization pathway inhibit *T. muris* egg hatching. (A) Ethanolamine utilization pathway in *E. coli*. Modified from (36). (B) Hatching rates for *eut* mutants in the Keio library and WT controls (n = 3-6). (C) Experimental approach to assess the toxicity of soluble factors produced by *E. coli* Δ *eutN*. Filtered supernatant from Δ *eutN* *E. coli* or controls grown with or without *T. muris* eggs for four hours were transferred to a dish containing WT *E. coli* and *T. muris* eggs.

487 The ability of WT *E. coli* to mediate egg hatching in the presence of supernatant was
 488 examined four hours later. **(D)** Quantification of hatching rates in mixtures containing
 489 WT *E. coli* cells and the indicated filtered supernatants (n = 3). **(E)** Aldehyde
 490 concentrations in supernatants of indicated *E. coli* strains incubated with or without *T.*
 491 *muris* eggs; $\Delta eutN$ comp. = $\Delta eutN$ strain where *eutN* has been complemented on a
 492 plasmid. **(F)** Hatching rates for $\Delta eutN\Delta eutC$ double mutant (n = 4). (B, D-F)
 493 Measurements were taken from distinct samples. The average per independent
 494 experiment for three technical replicates shown. Graphs show means and SEM. One-
 495 way analysis of variance (ANOVA) with Dunnett's post-test compared to WT. * p < 0.05,
 496 *** p < 0.001, **** p < 0.0001. ns = not significant.

Fig. 4 *T. muris* infection of mice colonized with *E. coli* mutants leads to defects in worm morphology and fertility. (A) Experimental approach for *T. muris* infection of mice monocolonized with WT or mutant *E. coli*. (B) Proportion of short and long worms harvested per mouse colonized by indicated *E. coli* strains; N.D. = not detected (n = 6-9

502 mice per group). **(C)** Representative images of worms harvested from mice
 503 monocolonized with WT or $\Delta eutN$ *E. coli*. A representative short worm (indicated by
 504 arrowhead) next to a long worm is shown in the inset for $\Delta eutN$ *E. coli*. **(D)**
 505 Representative images of eggs laid by worms from (B). Worms were incubated in RPMI
 506 medium overnight to trigger egg laying. **(E)** Proportion of total eggs laid per worm with
 507 normal morphology. Circles represent individual worms (WT, n = 46; $\Delta fabH$, n = 68;
 508 $\Delta eutD$, n = 58; $\Delta eutN$, n = 50). **(F)** Percentage of embryonated eggs following 8-week
 509 incubation. **(G)** Percentage of eggs in (F) that hatched after incubation with WT *E. coli*
 510 for 4 hours at 37°C. (B, E, F-G) Measurements were taken from distinct samples. Data
 511 shown are pooled from two independent experiments. Graphs show (B) means and
 512 SEM or (E, F-G) medians. One-way analysis of variance (ANOVA) with Dunnett's post-
 513 test compared to WT. ** p < 0.01, **** p < 0.0001. ns = not significant.

Supplementary Materials

Materials and Methods

Keio Library growth and culture

The frozen Keio collection was stamped out onto 150mm LB kanamycin (30µg/ml) plates and grown overnight at 37°C. For *C. elegans* experiments, individual colonies were inoculated into 700 µl of LB-Kan (30µg/ml) in 96 deep well plates using a compatible pin replicator. The plates were sealed with pure link air porous tape (Invitrogen cat no. 12262010) and incubated for 16-18 hours at 37°C, shaking at 250 rpm. Cultures were diluted 10X in S-media in 96 well format and OD at 600 nm was recorded for future reference to ensure that candidate positive mutants grew to an OD similar to the GC1547 wild-type control wells in the same plate. (This was done since poor bacterial growth can reduce the percentage of gravid *C. elegans*; all 315 candidates from the primary screen passed this control). Bacterial cultures were pelleted by centrifugation at 2000 rpm for 30 minutes. Supernatant was discarded and the pellet was resuspended in 100µl of S-media. 40µl of the culture was transferred into two separate plates (two technical replicates; black-walled, clear bottom 96-well microplates; Corning Cat. No. 3904) using a multi-channel pipette. The GC1547 control wild-type strain was a kanamycin resistant transformant of the Keio library starting strain BW25113, carrying the pET28a plasmid. For *T. muris in vitro* hatching assays, *E. coli* was grown overnight for 12-14 hours in Luria-Bertani broth with shaking at 225 rpm at 37 °C.

Primary screen

C. elegans GC1474 (Table S1; derived by outcrossing GC1373 (23) of the genotype *glp-1(e2141); end-1::gfp; myo-2p::mCherry*) was maintained on OP50 *E. coli* on NGM plates at 20°C and synchronized in the L1 larval stage using hypochlorite treatment followed by overnight hatching in egg buffer (37). 40-50 L1 larvae were seeded into 96-well plates in technical duplicate with 40µl of *E. coli* mutant cultures and incubated at

20°C with mild shaking in a humidified chamber for 62-65 hours. At this timepoint, control worms were fully gravid adults; control GC1547 bacteria were included on each plate in wells void of library bacteria. Prior to imaging, 40µl of 2mM levamisole was added to each well to immobilize the worms. The plates were then sealed with aluminum foil (Corning Cat. No. 6570) and imaged to detect both green (embryos) and red (pharynx) fluorescent markers using arrayscan (Thermo Scientific VTI). Images were captured at 2.5X magnification 2x2 binning in a 16 mm² field and digitally archived. Images captured on the red and green channels were merged in ImageJ using macros written to automate the process. Each adult animal was scored visually as “with embryos” (green embryos visible in the uterus) or “without embryos” (no green embryos visible in the uterus), and the number of worms without embryos and the total number of worms (excluding any that were arrested or any that crossed the edge of the well). Wells in which many animals had not reached adulthood were marked and were not considered further in our analysis. Ten such bacterial mutants grew well by OD but did not support worm development.

Additional culling of potential hits was performed. A variety of technical factors eliminated fewer than 10% of the total: 54 library wells were devoid of bacteria as per the plate map; 58 had a gene name in the library but the bacteria did not grow (either on solid or liquid culture); 41 were missed due to human error while scoring (noted as largely fertile but not counted individually); 5 had low n; 4 were not included due to reasons classified as ‘other’ (e.g., poor image) and 10 wells had poor worm growth in both replicates.

Worm counts (with/without embryos) were exported into ActivityBase (IDBS, <https://www.idbs.com>; a Scientific informatics software platform) and the robust Z score (a statistical measure that takes into account the median plate “without embryos” percent) was calculated for every well to minimize problems associated with plate to plate variation. Each well was analyzed with respect to percent worms without embryos, consistency (across technical replicates) and robust Z score. A cut-off of robust Z ≥ 1.5 was selected as “Z positive” ($\leq 10\%$ of the wells per plate scored above this threshold).

We also calculated the penetrance of worms without embryos for pooled technical replicates.

Criteria for selection in the primary screen weighed the robust Z and penetrance, with special consideration for cases where only one replicate was available. We included those wells for which only one technical replicate was available provided that well made the RobZ cut off (≥ 1.5) and/or percent non-gravid worms as further described below. Reasons for single-replicate included low n (62 wells), human error (49 wells), worm arrest (31 wells), poor image quality (22 wells) and 1 well marked as 'other'. Considering Z score, penetrance and replicates, the candidates were further classified as follows: High, A: both replicates Z positive and average penetrance of non-gravid worms $> 20\%$, or B: one replicate available and it is Z positive and $> 20\%$, or C: both replicates available but only one is Z positive and non-gravid $\geq 30\%$. Medium, A: both replicates Z positive and pooled non-gravid percent 13-20%, or B: only one replicate available which is Z positive and 13-20% penetrance of worms without embryos, or C: both replicates available but only one is Z positive with pooled non-gravid penetrance between 20-30%. Low: both replicates available, but only one is Z positive and non-gravid percent between 12.5-20%. This analysis yielded 315 primary screen candidates.

We then plotted the percent non-gravid in descending order for every well per plate and noted the inflection point (see (23)). We placed a subset of the mutants that passed the criteria above onto these plots and observed a strong correlation: wells with larger Z scores all appeared above the inflection point.

Secondary screen: reproducibility

The 315 candidates from the primary screen were re-selected from the original Keio library and gridded onto secondary screen plates. Two plates included only high priority candidates or a combination of randomly selected high, medium and low priority candidates to help assess whether there was a correlation between penetrance and reproducibility. No such correlation emerged, but these plates were considered as additional replicates in the analysis going forward (hence 6 plates) though 315 mutants.

Control bacteria were included in all secondary screen plates. Three biological replicates were performed in technical triplicate and scored and analyzed as described above. Technical replicate non-gravid/gravid counts were pooled and analyzed in pairwise comparison with the control on the same plates using Fisher's exact test. Candidates with p-values ≤ 0.05 in at least 2 out of 3 biological replicates were chosen for further analysis. This analysis yielded 10 candidates.

PCR validation of selected Keio mutants (completed for all ten hits)

We amplified and sequenced PCR fragments ~150 bp upstream and downstream of the predicted gene from each mutant and the control. Nested primers were used to sequence the PCR fragment and confirm the presence of the FRT and kanamycin cassette in the mutant DNA (Tables S3, S5).

P1 transduction

Mutant alleles comprising a kanamycin-resistance marker within a gene deletion were introduced into BW25113 by P1 transduction (38) from the Keio collection (39). The kanamycin-resistance cassette was subsequently excised to generate a markerless in-frame gene deletion by transformation with the temperature-sensitive plasmid pCP20 (40), which encodes FLP recombinase, and growth at 30°C. Finally, pCP20 was eliminated from the cells by growth at 42°C.

Complementation

For *fabH* and *eutN*, the bacterial gene coding regions plus 20 bp upstream were inserted into pBR322 downstream of a plasmid promoter by Gibson Assembly. The wild-type genomic DNA was amplified and assembled with a PCR fragment obtained from the pBR322 vector (specific primers are listed in Table S3; the fragment excluded part of the Amp^R gene between *EcoRI* and *PstI* restriction site). PCR products were purified using gel extraction kit (QIAEX II cat no. 20021) and 100 ng of vector was used

in 1:1 molar reaction with the genomic fragment. The assembly reaction was then transformed into high efficiency DH5alpha cells (NEB C2987I). Plasmid preparations (Qiagen mini prep kit Cat no.27106) were sequenced using pBR322 specific primers (Table S4). Plasmids were confirmed by DNA sequence and transformed into the respective mutant (named as strains GC1558 and GC1559 for *eutN* rescue and *fabH* rescue, respectively) and tested for reduced penetrance of the *C. elegans* non-gravid phenotype upon feeding.

Developmental timing and germline analysis

Somatic development assay

C. elegans were maintained on OP50, synchronized by hypochlorite treatment and allowed to hatch at 20°C with shaking. 30-40 L1 larvae were seeded in 96 deep well plates with each well carrying a specific mutant clone. Worms were incubated at 20°C with shaking for 48 hours and monitored at 400X on a Zeiss Z1 Axio Imager for vulval morphology (24) at specific time points.

Pro phenotype assay

glp-1(ar202) animals were scored for the Pro phenotype upon feeding with mutant bacteria using the same strategy as for the primary screen. Worms were maintained at 15°C on OP50 synchronized and grown in 96 deep well format as above. Day 1 (~72 hours post seeding) worms were fixed and DAPI stained and each gonad arm was scored for the Pro phenotype (25) at 400X.

Fertility at Day 2

GC1474 worms were maintained at 20°C on OP50, synchronized as L1 larvae and grown in 96 deep well format as above. Worms were anesthetized and imaged as in the screen on Day 1 and Day 2 to determine changes in penetrance of fertile worms.

Progenitor zone nuclei counts at L4-Adult molt

Worms grown on GC1547 control, *eutN*, *eutD*, and *fabH* mutant bacteria were monitored for vulval development and isolated at the L4-adult molt. Worms were then stained with DAPI (22) and 0.5µm Z-stack images were collected and analyzed using ImageJ to determine the number of germ cells in the PZ. In approximately 30% of the worms, the transition zone was not clear. These were censored from the analysis.

Assay for dependence on DAF-7/TGFB and DAF-2 IIS pathways

C. elegans strains GC1474, GC1545 and GC1250 (Table S2) were hypochlorite-synchronized and hatched overnight at 20°C. L1 larvae were seeded into 96 deep well plates containing specific bacterial mutants and scored for the presence of embryos in adults on Day 1 on Zeiss compound microscope at 100X.

Parasite maintenance

Stock eggs of *T. muris* E strain (7) were maintained in the NOD.Cg-*Prkdc*^{scid}/J (Jax) mouse strain in a specific pathogen free (SPF) facility and propagated as previously described (41). Each egg batch was confirmed to hatch at ≥ 80% *in vitro* using method below and WT *E. coli* before use in subsequent experiments.

***In vitro* hatching of *T. muris* eggs**

T. muris eggs were hatched *in vitro* by mixing 25µL of embryonated eggs at a concentration of 1 egg/1µL suspended in sterile water with 10µL of *E. coli* overnight culture and 15µL sterile LB in individual wells of a 48 well plate. Plates were incubated at 37°C and checked every hour for a total of four hours on the Zeiss Primovert microscope to enumerate hatched eggs. Rates describe hatching after four hours of incubation unless otherwise indicated. Experiments utilizing transwell inserts (Millicell) were performed as previously described (5). For experiments where cell-free supernatant was used, supernatant and cells were isolated by centrifugation and filtration through a 0.22µm syringe filter or after wash with autoclaved ddH₂O,

respectively. Incubation with embryonated eggs was performed by mixing *T. muris* eggs at a concentration of 15 eggs/2 mL culture and keeping at 37°C for four hours.

Metabolomics

Sample preparation

Individual colonies (obtained after streaking frozen bacteria onto LB-kanamycin plates and incubating at 37°C) were inoculated in 30 ml of LB-kanamycin (30µg/ml) liquid in a 50ml falcon tube. Cultures were grown for 16-18hr at 37°C with 250 rpm shaking and then bacteria were pelleted by centrifugation at 1500 rpm for 20-30 minutes at 4°C. The supernatant was transferred to a fresh tube, filtered through 0.2µm filter (Corning (431229) and frozen at -80°C for future analysis. Pellets were resuspended in 7.5 ml of S-media (for ~250ml of S-media the recipe was 240ml of S-basal, 2.5ml of 1M potassium citrate- final concentration of 10mM, 2.5ml of 1M trace metals for final concentration of 10mM, 750µl of 1M MgSO₄ for final concentration of 3mM, 750µl of 1M CaCl₂ for final concentration of 3mM, 72µl of 100mg/ml of kanamycin for final concentration of 30µg/ml). This solution was filter sterilized using a 500ml Nalgene filter (291-3320 Fisher). Post filter sterilization, 250µl of 5mg/ml cholesterol – final concentration of 5µg/ml and 2.5ml of 250µg/ml fungizone for final concentration of 2.5µg/ml (BP264550 Fisher).

The number of CFU/ml was estimated for each culture by plating dilutions (10µl of culture was mixed with 900µl of S-media to get 10² or 1:100 dilution, which was then serially diluted to 1:10⁵ or 1:10⁶ on LB agar plates and counting colonies after growth at 37°C. The remaining culture was incubated at 20°C with mild shaking overnight to mimic *C. elegans* growth conditions. Based on CFU/ml measurements, 10¹⁰ cells were fast filtered (EZFIT Vacuum Manifold, Millipore EZFITLOW03 with Microfil V, Millipore MVHAWG124) and metabolite extracts were isolated as previously described (42). Three independent biological samples were extracted on three independent days and subsequently analyzed by the NYU Langone Metabolomics Core Resource Library.

LC-MS/MS with the hybrid metabolomics method

Samples were subjected to an LCMS analysis to detect and quantify known peaks. A metabolite extraction was carried out on each sample based on a previously described method (43). The LC column was a MilliporeTM ZIC-pHILIC (2.1 x150mm, 5µm) coupled to a Dionex Ultimate 3000TM system and the column oven temperature was set to 25°C for the gradient elution. A flow rate of 100µL/min was used with the following buffers: A) 10mM ammonium carbonate in water, pH 9.0, and B) neat acetonitrile. The gradient profile was as follows; 80-20%B (0-30 min), 20-80%B (30-31 min), 80-80%B (31-42 min). Injection volume was set to 2µL for all analyses (42 min total run time per injection).

MS analyses were carried out by coupling the LC system to a Thermo Q Exactive HFTM mass spectrometer operating in heated electrospray ionization mode (HESI). Method duration was 30 min with a polarity switching data-dependent Top 5 method for both positive and negative modes. Spray voltage for both positive and negative modes was 3.5kV and capillary temperature was set to 320°C with a sheath gas rate of 35, aux gas of 10, and max spray current of 100µA. The full MS scan for both polarities utilized 120,000 resolution with an AGC target of 3e6 and a maximum IT of 100ms, and the scan range was from 67-1000 *m/z*. Tandem MS spectra for both positive and negative mode used a resolution of 15,000, AGC target of 1e5, maximum IT of 50ms, isolation window of 0.4*m/z*, isolation offset of 0.1*m/z*, fixed first mass of 50*m/z*, and 3-way multiplexed normalized collision energies (nCE) of 10, 35, 80. The minimum AGC target was 1e4 with an intensity threshold of 2e5. All data were acquired in profile mode.

Hybrid Metabolomics Data Processing

Relative quantification of metabolites

The resulting ThermoTM RAW files were converted to mzXML format using ReAdW.exe version 4.3.1 to enable peak detection and quantification. The centroided data were searched using an in-house python script Mighty_skeleton version 0.0.2 and peak

heights were extracted from the mzXML files based on a previously established library of metabolite retention times and accurate masses adapted from the Whitehead Institute (44), and verified with authentic standards and/or high resolution MS/MS spectral manually curated against the NIST14MS/MS (45) and METLIN (2017)(46) tandem mass spectral libraries. Metabolite peaks were extracted based on the theoretical m/z of the expected ion type e.g., $[M+H]^+$, with a ± 5 part-per-million (ppm) tolerance, and a ± 7.5 second peak apex retention time tolerance within an initial retention time search window of ± 0.5 min across the study samples. The resulting data matrix of metabolite intensities for all samples and blank controls was processed with an in-house statistical pipeline Metabolyze version 1.0 and final peak detection was calculated based on a signal to noise ratio (S/N) of 3X compared to blank controls, with a floor of 10,000 (arbitrary units). For samples where the peak intensity was lower than the blank threshold, metabolites were annotated as not detected, and the threshold value was imputed for any statistical comparisons to enable an estimate of the fold change as applicable. The resulting blank corrected data matrix was then used for all group-wise comparisons, and t-tests were performed with the Python SciPy (1.1.0) (47) library to test for differences and generate statistics for downstream analyses. Any metabolite with p-value < 0.05 was considered significantly regulated (up or down). Heatmaps were generated with hierarchical clustering performed on the imputed matrix values utilizing the R library pheatmap (1.0.12) (48). Volcano plots were generated utilizing the R library, Manhatanly (0.2.0). In order to adjust for significant covariate effects (as applicable) in the experimental design the R package, DESeq2 (1.24.0) (49) was used to test for significant differences. Data processing for this correction required the blank corrected matrix to be imputed with zeroes for non-detected values instead of the blank threshold to avoid false positives. This corrected matrix was then analyzed utilizing DESeq2 to calculate the adjusted p-value in the covariate model.

Arginine and ornithine supplementation assay

fabH mutant *E. coli* or wild-type control was grown with arginine (400 μ M or 500 μ M) or ornithine (40-100mM) in LB-kanamycin overnight at 37°C with 250 rpm shaking. For C.

C. elegans experiments, worms were imaged on Day 1 as described for the primary screen. For *T. muris* experiments, hatching assay was performed as described above.

DarkZone labeling of aldehydes

Cell-free supernatants isolated from *E. coli* overnight cultures were incubated with 20μM DarkZone dye pre-dissolved in DMSO, 5mM 2,4 dimethoxyaniline catalyst (TCI America) pre-dissolved in DMSO, and buffer (100mM Tris pH 6.8, 150 mM NaCl) in a 96 well optical flat-bottom plate (Thermo-Fisher) in duplicate at 37°C for 1 hour. Adhesive plate seals were used to prevent evaporation of aldehydes. Fluorescence was measured using an EnVision 2 103 Multi-label Reader (PerkinElmer). Duplicate measurements were averaged.

Mice

Gnotobiotics

Previously described germ-free C57BL/6J mice (50) were maintained in flexible film isolators, and absence of faecal bacteria and fungi was confirmed by aerobic culture in brain heart infusion, sabaraud and nutrient broth (Sigma), and qPCR for bacterial 16S and eukaryotic 18S ribosomal RNA genes through sampling of stool from individual cages in each isolator on a monthly basis. Mice were transferred into individually ventilated Tecniplast ISOcages for infections to maintain sterility under positive air pressure. All animal studies were performed according to approved protocols and ethical guidelines established by the NYU Grossman School of Medicine Institutional Animal Care and Use Committee (IACUC) and Institutional Review Board.

Murine in vivo infections

Female mice were monocolonized at 6-8 weeks of age by oral gavage with 1×10^8 colony forming units per mL (CFU/mL) of indicated *E. coli* strains. Overnight *E. coli* cultures were diluted 1:100 in Luria-Bertani broth followed by 2-3 hours of growth until 1×10^8 CFU/mL was reached. Bacterial density was confirmed by dilution plating.

Cultures were pelleted by centrifugation at 2437g for 10 minutes and washed once with sterile 1x PBS. Pellets were then resuspended in sterile 1x PBS and mice were inoculated by oral gavage with 1×10^8 CFU in a volume of 100 μ L. Inoculum was verified using dilution plating of aliquots.

7 and 28 days later, mice were infected by oral gavage with ~100 embryonated *T. muris* eggs. Individual worms were collected from cecal contents of all infected mice and washed in RPMI 1640 (Invitrogen) supplemented with penicillin (100U/ml) and streptomycin (100 μ g/ml; Sigma). To evaluate egg laying, each worm was placed into individual wells of a 48 well plate with 200 μ L supplemented RPMI. Plates were then incubated at 37°C overnight in a closed tupperware (Sistema) lined with damp paper towels. The following day, eggs laid were enumerated using a Zeiss Primovert light microscope at 100X. Samples containing ~1000 eggs per condition were mounted on glass slides with glycerol mounting medium (Sigma-Aldrich) and analyzed using a Zeiss Axio Observer at 400X with oil emersion for color images or EVOS FL Auto (Life Technologies) at 200X for black and white images. Images were processed using ImageJ.

Statistical analysis

Statistical tests and parameters used, including the definition of central value and the exact number (n) of mice or worms per group, are annotated in the corresponding figure legend. All analyses were performed with Graphpad Prism version 8.4.3 for Mac (GraphPad). The numbers of animals or biological replicates used herein were estimated on the basis of a power analysis with the following assumptions: the standard deviation will be roughly 20% of the mean; *p* values will be less than 0.05 when the null hypothesis is false; and the effect size (Cohen's *d*) is between 1.0 and 2.0. We have also carefully chosen the indicated sample size on the basis of empirical evidence of what is necessary to interpret the data and statistical significance.

Fig. S1 Primary screen selection criteria, validation of *E. coli* mutants, and effect of mutant *E. coli* on *C. elegans*. (A) Representative bubble plot from secondary screen replicates. One of the six 96-well plates used in the secondary screen is shown here rendered using Vortex software data visualization tool. The size of the bubble

841 represents percent of worms without embryos that is calculated from three technical
842 replicates, while the color indicates the three biological trials. Boxed wells H12 and C3
843 indicate the negative control and a positive hit in two out of three replicates based on
844 statistical tests (see Methods). **(B)** Scatter plot showing the Robust Z (Rob Z) score for
845 all primary screen wells scored. Scatter plot was generated using Vortex dotmatics
846 software. Yellow dots indicate wells containing the negative control bacteria (GC1547).
847 Dashed line indicates cut-off for Rob Z criterion. **(C)** $\Delta eutN$ and $\Delta fabH$ complementation
848 suppresses *C. elegans* fertility defect. $p \leq 0.0001$ by Fisher's exact test compared to
849 WT; n total number of worms scored in each case is (left to right): 925, 220, 197, 744,
850 1078. **(D, E)** $\Delta fabF$ phage transductants mimic *C. elegans* fertility defect of $\Delta fabF$
851 original clone. Circles represent mean of four independent biological replicates and bar
852 is SEM. (D) $p \leq 0.007$ for original *fabF* clone (n = 330, 214 worms scored in WT and
853 $\Delta fabF$, respectively), and (E) $p \leq 5 \times 10^{-5}$ (n = 231, 66), Fisher's exact test, compared to
854 control GC1547 bacteria in each case. **(F)** The number of progenitor zone nuclei of *glp-*
855 *1(e2141)* at the L4 to Adult molt is not altered in worms raised on $\Delta eutN$, $\Delta fabH$ or
856 $\Delta eutD$ versus wild-type *E. coli*. Scatter plots showing number of progenitor zone germ
857 cell counts; each dot represents one gonad arm. We noted that in ~30% of *eutN*
858 mutants (relative to 10% in control), the border of the PZ was difficult to detect; these
859 were censored from PZ counts. The elevated penetrance of this phenotype in the
860 $\Delta eutN$ -fed worms may represent a meiotic entry defect, accounting for the delay in
861 germline development relative to somatic development. **(G)** No significant difference is
862 observed between the number of progenitors in worms grown on BW25113 versus
863 OP50 on standard NGM solid media ($p \geq 0.05$, Student's t-test). We note that the
864 number of progenitors is markedly lower in the *glp-1(e2141)* mutant background after
865 growth on liquid versus growth on solid media; the Keio starting strain was used in (G)
866 since OP50 is sensitive to kanamycin. **(H, I)** Fertility delay is not suppressed by loss of
867 *daf-16* or *daf-5*. Percent of worms with embryos on Day 1 in (H) *daf-16(+)*; *daf-2(e1370)*
868 *glp-1(e2141)* or *daf-16(m26)*; *daf-2(e1370) glp-1(e2141)* and (I) *daf-5(+)*; *glp-1(e2141)*
869 or *daf-5(e1386)*; *glp-1(e2141)*. Although a significant difference was detected by
870 Fisher's exact test between *daf-5(+)* and *daf-5(-)* for $\Delta eutN$ ($p < 0.0001$) and $\Delta fabH$ ($p <$
871 0.0001), this was not considered biologically meaningful since the *daf-5(e1386)* worms

872 raised on GC1547 bore significantly more embryos in comparison to the *daf-5(+)* worms
873 raised on the GC1547 control ($p \leq 0.0027$). Circles represent mean of independent
874 biological replicates and bar is SEM. n total number of worms scored are (left to right):
875 **(I)** 269, 203, 207, 245, 179, 255, 229, 214, 200, 233 and **(J)** 59, 65, 71, 62, 179, 73, 64,
876 54, 56, 57.

Fig. S2 *E. coli* mutants used for *T. muris* egg hatching assay display similar *in vitro* growth kinetics as wild-type bacteria.

Bacterial density of monoclonal cultures of each of the strains indicated measured by dilution plating; CFU = colony forming units, h = hours.

Fig. S3 Complementation of $\Delta fabH$ and $\Delta eutN$ *E. coli* rescue *T. muris* egg hatching.

Hatching elicited by WT *E. coli* (WT), mutants **(A)** $\Delta eutN$ and **(B)** $\Delta fabH$, and strains where deletions were complemented with a plasmid-borne gene (n = 3-4). Measurements were taken from distinct samples. Data represent the average of at least three independent experiments. Graphs show means and SEM. One-way analysis of variance (ANOVA) with Dunnett's post-test compared with WT. * p < 0.05, **** p < 0.0001. ns = not significant.

Fig. S4 Ornithine or arginine supplementation of $\Delta fabH$ *E. coli* rescues defects in *C. elegans* and *T. muris*, related to Fig. 2. (A-C) Percentage of *C. elegans* Day 1 adults with embryos; WT = WT *E. coli*. (A) Arginine supplementation does not alter

895 baseline penetrance of *C. elegans* with embryos in *glp-1(e2141)*. Total n worms scored
 896 (left to right): 173, 118, 108. **(B-C)** Reproduction of Fig. 2, E-F with additional
 897 concentrations of (B) arginine and (C) ornithine. Total n worms scored (left to right) for
 898 (B) are 208, 237, 200, 300 and (C) 207, 216, 39, 20,149; p values calculated by Fisher's
 899 exact test. **(D-E)** Reproduction of Fig. 2, G-H with additional concentrations of (D)
 900 ornithine and (E) arginine. Percent *in vitro* hatched *T. muris* eggs elicited by *E. coli*
 901 cultures indicated (n=3). One-way analysis of variance (ANOVA) with Dunnett's post-
 902 test compared with WT. For all panels, measurements were taken from distinct
 903 samples. The average per independent experiment for two to three technical replicates
 904 shown. Graphs show means and SEM. * p < 0.05, ** p < 0.01, **** p < 0.0001. ns = not
 905 significant.

Fig. S5 Acetaldehyde is sufficient to reduce egg hatching, related to Fig. 3.

(A) DarkZone standard curve for acetaldehyde. Fluorescence intensity of samples incubated with known concentrations of acetaldehyde and 20 μM DarkZone dye ($n = 2$). AU, arbitrary units. **(B)** Hatching rates for *T. muris* eggs incubated with WT *E. coli* cultures supplemented with acetaldehyde at concentrations indicated ($n = 3$). **(C)** Bacterial density of conditions indicated from B measured by dilution plating ($n = 3$). Measurements were taken from distinct samples. Graph shows means and SEM. B-C, One-way analysis of variance (ANOVA) with Dunnett's post-test compared to 0. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

Fig. S6 *T. muris* infection of germ-free and monocolonized mice, related to Fig 4.

(A) *E. coli* burden 7 dpi in stool of mice gavaged with 1×10^8 CFU of strains indicated (n = 4-5 mice per group). **(B)** Total worms harvested per mouse for germ-free (GF) and mice monocolonized with *E. coli* strains indicated; N.D. = not detected (n = 6-9 mice per group). **(C)** Quantification of eggs laid per worm. Harvested worms were incubated in individual wells of a 48-well plate with RPMI medium overnight and eggs laid were quantified using light microscopy. Measurements were taken from distinct samples. Graph shows means (A-B) or medians (C).

Fig. S7 Deleting *eutC* reverses *T. muris* fitness defects induced by $\Delta eutN$ *E. coli* in mice. (A) Representative photos of worms harvested from WT and $\Delta eutN\Delta eutC$ monocolonized C57BL/6 mice. (B) Total worms harvested per mouse for monocolonized groups indicated. Circles represent individual mice (WT, n = 9;

930 $\Delta eutN\Delta eutC$, n = 11). **(C)** Proportion of long worms (> 1cm) harvested per mouse. **(D)**
 931 Quantification of eggs laid per worm. Harvested worms were incubated with RPMI
 932 media overnight and eggs laid were quantified using light microscopy. Circles represent
 933 individual worms (WT, n = 43; $\Delta eutN\Delta eutC$, n = 57). **(E)** Proportion of total eggs laid per
 934 worm in (D) with normal morphology. **(F)** Representative images of eggs laid by worms
 935 harvested from monocolonized mice groups indicated. **(G)** Percentage of embryonated
 936 eggs following 8-week incubation. **(H)** Percentage of eggs in (G) that hatched after
 937 incubation with WT *E. coli* for 4 hours at 37°C. (B-E, G-H) Two-tailed, unpaired t-test. ns
 938 = not significant. Data shown are pooled from two independent experiments.
 939 Measurements were taken from distinct samples. Graph shows means and SEM.

Table S1 Top candidates and bacterial gene function (EcoCyc). *Fab* and *eut* genes
were chosen for further characterization.

GENE	PROTEIN FUNCTION	FUNCTIONAL GROUP
<i>fabF</i>	β -ketoacyl-[acyl carrier protein] synthase II	Fatty acid biosynthesis
<i>fabH</i>	β -ketoacyl-[acyl carrier protein] synthase III	Fatty acid biosynthesis
<i>eutD</i>	phosphate acetyltransferase	Ethanolamine utilization
<i>eutN</i>	putative ethanolamine catabolic microcompartment shell protein	Ethanolamine utilization
<i>pta</i>	phosphate acetyltransferase	Ethanolamine utilization
<i>rfaF</i>	ADP-heptose—LPS heptosyltransferase 2	Lipopolysaccharide biosynthesis
<i>rfaH</i>	transcription antiterminator RfaH	Lipopolysaccharide biosynthesis
<i>cmoA</i>	carboxy-S-adenosyl-L-methionine synthase	Uridine modification in RNA
<i>yhgN</i>	putative inner membrane protein	Antibiotic resistance
<i>holD</i>	DNA polymerase III subunit ψ	DNA replication

944 **Table S2 Strains used in this study.**

STRAIN	SPECIES	GENOTYPE	REFERENCE
BW25113	<i>E. coli</i>	<i>F</i> ⁻ , Δ (<i>araD-araB</i>)567, Δ <i>lacZ</i> 4787(:: <i>rrnB</i> -3), λ ⁻ , <i>rph</i> -1, Δ (<i>rhaD-rhaB</i>)568, <i>hsdR</i> 514	(39)
GC833	<i>C. elegans</i>	<i>glp-1(ar202)</i> III	(25)
GC1250	<i>C. elegans</i>	<i>daf-16(m26)</i> I; <i>daf-2(e1370)</i> <i>glp-1(e2141)</i> III	(22)
GC1474	<i>C. elegans</i>	<i>glp-1(e2141)</i> III; <i>hjsi20</i> [<i>Pmyo-2P::mCherry::unc-54</i> 3'UTR] IV; <i>zuls70</i> [pJN152: <i>end-1P::gfp::caax</i> ; <i>unc-119(+)</i>] V	This study
GC1545	<i>C. elegans</i>	<i>daf-5 (e1386)</i> II; <i>glp-1(e2141)</i> III	This study
GC1547	<i>E. coli</i>	BW25113 transformed with pET28	This study
GC1549	<i>E. coli</i>	BW25113 Δ <i>fabF::kan</i>	This study
GC1558	<i>E. coli</i>	BW25113 Δ <i>eutN::kan</i> , pGC735	This study
GC1559	<i>E. coli</i>	BW25113 Δ <i>fabH::kan</i> , pGC738	This study
GC1643	<i>E. coli</i>	BW25113 Δ <i>eutC::kan</i>	This study
GC1644	<i>E. coli</i>	BW25113 Δ <i>eutC</i>	This study
GC1645	<i>E. coli</i>	BW25113 Δ <i>eutN::kan</i>	This study
GC1646	<i>E. coli</i>	BW25113 Δ <i>eutN</i>	This study
GC1647	<i>E. coli</i>	BW25113 Δ <i>eutC</i> Δ <i>eutN::kan</i>	This study
GC1648	<i>E. coli</i>	BW25113 Δ <i>eutC::kan</i> Δ <i>eutN</i>	This study
JW1077	<i>E. coli</i>	BW25113 Δ <i>fabH::kan</i>	(39)
JW2442	<i>E. coli</i>	BW25113 Δ <i>eutD::kan</i>	
JW2440	<i>E. coli</i>	BW25113 Δ <i>eutN::kan</i>	
JW2294	<i>E. coli</i>	BW25113 Δ <i>pta::kan</i>	
JW3595	<i>E. coli</i>	BW25113 Δ <i>rfaF::kan</i>	
JW3818	<i>E. coli</i>	BW25113 Δ <i>rfaH::kan</i>	
JW4334	<i>E. coli</i>	BW25113 Δ <i>holD::kan</i>	
JW1859	<i>E. coli</i>	BW25113 Δ <i>cmoA::kan</i>	
JW3397	<i>E. coli</i>	BW25113 Δ <i>yhgN::kan</i>	

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946

Table S3 Primers used in this study.

Primer	Details
GCo2700 – 5'.... CCGCGCTGGTTCGTTTCtagTGCAGCAATGGCAAC AACGTTG	Forward primer- vector fragment to amplify pBR322 for Gibson assembly with PCR product bearing wild-type <i>fabH</i> sequence
GCo2701 – 5'.... ACGTTGTTGCCATTGCTGCAActaGAAACGAACCAG CGCG	Reverse primer- vector fragment to amplify pBR322 for Gibson assembly with PCR product bearing wild-type <i>fabH</i> sequence
GCo2702 – 5'.... AGCTGTCAAACATGAGAATTATAACCGAAAAGTGA CTGAGCGTACa	Forward primer- <i>fabH</i> coding sequence for Gibson assembly with pBR322
GCo2703 – 5'.... CTCAGTCACTTTTCGGTTATAATTCTCATGTTTGA CAGCTTATCATCGATAAG	Reverse primer- <i>fabH</i> coding sequence for Gibson assembly with pBR322
GCo2708 – 5'.... agctgtcaaacatgagaattAATACGCCACGGAGGCG	Forward primer- <i>eutN</i> coding sequence for Gibson assembly with pBR322
GCo2709 – 5'.... acgttgttgccattgtgcattaTTTGTGAAAATTACCTGAC CGCC	Reverse primer- <i>eutN</i> coding sequence for Gibson assembly with pBR322
GCo2710 – 5'.... ACCCGCCTCCGTGGCGTATTaattctcatgttgacagcttat catcgataag	Forward primer- vector fragment to amplify pBR322 for Gibson assembly with PCR product bearing wild-type <i>eutN</i> sequence
GCo2711 – 5'.... AGGTAATTTTCCACAAAtaatgcagcaatggcaacaacgt	Reverse primer- vector fragment to amplify pBR322 for Gibson assembly with PCR product bearing wild-type <i>eutN</i> sequence
GCo2744 – 5'.... GCCGGGAAGCTAGAGTAAGTAGT	pBR322 forward (used to confirm gene sequence)

951 **Table S4 Plasmid names and descriptions.**

Plasmid name	Description
pGC735	<i>eutN</i> cloned into pBR322 by Gibson assembly
pGC737	pET28
pGC738	<i>fabH</i> cloned into pBR322 by Gibson assembly
pBR322	pBR322

952

953 **Table S5 Primer sequences to verify kan cassette insertion within target genes.**

GCo296 AGCTTTGAAGTATTACGCCCGGAC	Forward PCR primer <i>eutD</i>
GCo2797 CCTTTCGAGCACGGTCTACG	Forward sequencing primer <i>eutD</i>
GCo2798 AACACCAACCAGCTTGACGC	Reverse PCR primer <i>eutD</i>
GCo2799 AGGCCCCGGGTTTCGATC	Reverse sequencing primer <i>eutD</i>
GCo2800 CTTCCCGATCGGCCTGAAAGG	Forward PCR primer <i>eutN</i>
GCo2801 CTGTAATCACCTGTGTGACGTCG	Forward sequencing primer <i>eutN</i>
GCo2802 ACTTTGGCTGCCGCAACGGC	Reverse PCR primer <i>eutN</i>
GCo2803 CGCGAAAACGCCCATCTCATG	Reverse sequencing primer <i>eutN</i>
GCo2804 AATTGCCGCTCGCCTGGA	Forward PCR primer <i>fabH</i>

GCo2805 GGTTTTGAGCTGCTGGACGG	Forward sequencing primer <i>fabH</i>
GCo2806 GCCGCAGAAGCTTCAGCAAAC	Reverse PCR primer <i>fabH</i>
GCo2807 GGATAGCTCGCCGCCATATCA	Reverse sequencing primer <i>fabH</i>
GCo2808 GTCCTGCGGTGGTTATCCCAA	Forward PCR primer <i>pta</i>
GCo2809 AAGAACTGGTTATCGCGCAAG	Forward sequencing primer <i>pta</i>
GCo2810 AAGTGGGATGGCGCAATTCAT	Reverse PCR primer <i>pta</i>
GCo2811 GCAGCGCAGTTAAGCAAGATAA	Reverse sequencing primer <i>pta</i>
GCo2812 AATCACCACCGTTCAGGCTGC	Forward PCR primer <i>fabF</i>
GCo2813 GCCACCAGGCGTAAGTGAAC	Forward sequencing primer <i>fabF</i>
GCo2814 CCGTTAATTAAGAACATACCGGCTCC	Reverse PCR primer <i>fabF</i>
GCo2815 AGTGTGGCAGCATGTTCACCTACG	Reverse sequencing primer <i>fabF</i>
GCo2832 AACTGAAAGGCCGCTATCAGG	Forward PCR primer <i>rfaF</i>

GCo2833 GGTTACGACAAACCGTTCAAAACC	Forward sequencing primer <i>rfaF</i>
GCo2834 GCCAGGAAGGAATCTGTGCGAA	Reverse PCR primer <i>rfaF</i>
GCo2835 CCACCCAGTCAAACCTTAATCCCTG	Reverse sequencing primer <i>rfaF</i>
GCo2836 GGCGTTCATCTTTGCGATGCTG	Forward PCR primer <i>rfaH</i>
GCo2837 CTGACGGTATAACGCAAACCGG	Forward sequencing primer <i>rfaH</i>
GCo2838 TCTCACGCCAAAGCCATCATCC	Reverse PCR primer <i>rfaH</i>
GCo2839 CCATATTTTGCAACGTATTGCGCAC	Reverse sequencing primer <i>rfaH</i>
GCo2840 CTCTTGGCACATCTTTCACCATACA	Forward PCR primer <i>yhgN</i>
GCo2841 CGAAGCCATCAGTAATGCGACTT	Forward sequencing primer <i>yhgN</i>
GCo2842 CCGAAACGCTGAAAACCTGGAC	Reverse PCR primer <i>yhgN</i>
GCo2843 GGATGATTGCGTTCCAGCTGTT	Reverse sequencing primer <i>yhgN</i>
GCo2844 TGTGCGCTGTTGCTGATGGT	Forward PCR primer <i>cmoA</i>

GCo2845 TTTGGTATATGCCCTGGGAGTTGG	Forward sequencing primer <i>cmoA</i>
GCo2846 GGTAGAACTCCACCGCATTTGA	Reverse PCR primer <i>cmoA</i>
GCo2847 TTAAACAACCCGTGCTGCTGCT	Reverse sequencing primer <i>cmoA</i>
GCo2848 TGTGACGCAGCAAGACTTCACT	Forward PCR primer <i>holD</i>
GCo2849 ATGCAGACATGGAATGCTCCTCAA	Forward sequencing primer <i>holD</i>
GCo2850 ACCACTTGCGTAATCGCAAACG	Reverse PCR primer <i>holD</i>
GCo2851 TTGCCGTTTTGCGTAACTGAAAGT	Reverse sequencing primer <i>holD</i>