

1 **Optogenetic control of gut bacterial metabolism to promote longevity**

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12 **Abstract**

13 Gut microbial metabolism is associated with host longevity. However, because it requires
14 direct manipulation of microbial metabolism *in situ*, establishing a causal link between these two
15 processes remains challenging. We demonstrate an optogenetic method to control gene
16 expression and metabolite production from bacteria residing in the host gut. We genetically
17 engineer an *Escherichia coli* strain that synthesizes and secretes colanic acid (CA) under the
18 quantitative control of light. Using this optogenetically-controlled strain to induce CA production
19 directly in the *Caenorhabditis elegans* gut, we reveal the local effect of CA in protecting
20 intestinal mitochondria from stress-induced hyper-fragmentation. We also exploit different
21 intensities of light to determine that the lifespan-extending effect of CA is positively correlated
22 with its levels produced from bacteria. Our results show that optogenetic control offers a rapid,
23 reversible and quantitative way to fine-tune gut bacterial metabolism and uncover its local and
24 systemic effects on host health and aging.

25

26 **Introduction**

27 Microbiome studies have identified correlations between bacteria and host aging (Kundu
28 et al., 2017; O'Toole and Jeffery, 2015). For example, 16S rRNA and metagenomic DNA
29 sequencing are used to associate the presence or abundance of specific bacteria to human
30 centenarians (Biagi et al., 2016; Claesson et al., 2012, 2010). However, given the complexity and
31 heterogeneity of the human gut environment, these approaches are unable to elucidate how a
32 specific microbial species contributes to longevity. The nematode *Caenorhabditis elegans* has a
33 short and easily-measured lifespan, features that have revolutionized our understanding of the
34 molecular genetics of aging and longevity (Kenyon, 2010). Studies using *C. elegans* also provide
35 mechanistic insight into the association between bacterial species and host longevity (Gusarov et
36 al., 2013; Kim, 2013). Importantly, recent studies have revealed that bacterial metabolism can
37 produce specific products to directly influence the aging process in the host *C. elegans* or
38 modulate the effects of environmental cues on *C. elegans* lifespan (Cabreiro et al., 2013; Pryor et
39 al., 2019; Virk et al., 2016). These findings highlight the significance of bacterial metabolism in
40 regulating host physiology during the aging process and have inspired interest in directly
41 manipulating bacterial metabolism *in situ* in the host gastrointestinal (GI) tract.

42 In several recent studies, researchers have administered antibiotic- or carbohydrate-based
43 small molecule inducers to modulate gene expression from gut bacteria (Kotula et al., 2014; Lim
44 et al., 2017; Mimee et al., 2015). While this approach has enabled the *in situ* analysis of a gut
45 bacterial-host interaction (Lim et al., 2017), chemical effectors may have unwanted side-effects
46 on host or microbial physiology and subject to slow and poorly-controlled transport and
47 degradation processes that ultimately limit their precision.

48 Optogenetics combines light and genetically-engineered photoreceptors to achieve
49 unrivaled control of biological processes (Olson and Tabor, 2014). Previously, we and others
50 have engineered bacterial photoreceptors that activate or repress gene expression in response to
51 specific wavelengths of light (Levskeya et al., 2005; Li et al., 2020; Ohlendorf et al., 2012; Ong
52 and Tabor, 2018; Ong et al., 2017; Ramakrishnan and Tabor, 2016; Ryu and Gomelsky, 2014;
53 Schmidl et al., 2014). These photoreceptors have been used to achieve precise quantitative
54 (Olson et al., 2017, 2014), temporal (Chait et al., 2017; Miliias-Argeitis et al., 2016; Olson et al.,
55 2017, 2014), and spatial (Chait et al., 2017; Levskeya et al., 2005; Ohlendorf et al., 2012; Tabor
56 et al., 2010) control of bacterial gene expression in culture conditions. They have also been used
57 to characterize and control transcriptional regulatory circuits (Chait et al., 2017; Olson et al.,
58 2014; Tabor et al., 2009) and bacterial metabolic pathways (Fernandez-Rodriguez et al., 2017;
59 Tandar et al., 2019) *in vitro*. Here, we hypothesized that optogenetic control of bacterial gene
60 expression might provide a new way to manipulate bacterial metabolism *in vivo* in the host GI
61 tract, with high temporal and spatial precision and no unwanted side-effects.

62 We address this possibility using the *E. coli-C. elegans* interaction model, a testable
63 system with known mechanistic links between bacterial metabolism and host longevity and with
64 complete optical transparency. In particular, CA is an exopolysaccharide synthesized and
65 secreted from *E. coli*, which can extend the lifespan of the host *C. elegans* through modulating
66 mitochondrial dynamics (Han et al., 2017). We thus have genetically engineered an *E. coli* strain
67 to put its biosynthesis of CA under a switchable control between green and red lights, and then
68 utilized green light to induce CA production from this strain in the gut of the host *C. elegans*. We
69 discovered that light-induced CA from bacteria residing in the host is sufficient to modulate
70 mitochondrial dynamics and lifespan, which gives even more potent effects than dietary

71 supplementation of CA. Furthermore, this optogenetic manipulation allowed us to investigate the
72 local effect of CA on intestinal cells in a time-controlled manner and its systemic effect on
73 organisms in a quantitative way, which are not possible with purified CA or CA-overproducing
74 genetic mutants. This work paves the road for future application of bacterial optogenetics in
75 understanding bacteria-host interaction with temporal, spatial and quantitative controls and
76 minimal chemical interference.

77

78 **Results**

79 To demonstrate optogenetic control over gut bacterial gene expression, we first
80 engineered *E. coli* strain LH01, wherein our previous light-switchable two-component system
81 CcaSR (Schmidl et al., 2014) controls expression of superfolder green fluorescent protein (*sfgfp*),
82 and *mcherry* is expressed constitutively to facilitate identification of the bacteria (**Fig. 1a**,
83 **Supplementary Fig. 1, Supplementary Tables 1-3**). In LH01, green light exposure switches
84 CcaS to an active state in the presence of chromophore phycocyanobilin (PCB), wherein it
85 phosphorylates the response regulator CcaR. Phosphorylated CcaR then activates transcription of
86 *sfgfp* from the P_{cpcG2-172} output promoter. Red light reverts active CcaS to the inactive form, de-
87 activating *sfgfp* expression (**Fig. 1a**).

88 We then reared two groups of *C. elegans* from the larval to the adult stage on plates of
89 LH01 under red or green light, respectively (**Fig. 1b**). Next, we washed away external bacteria,
90 applied the paralyzing agent levamisole to prevent expulsion of gut contents, and transferred the
91 worms to agar pads. Finally, we switched the light color from red to green, or green to red, and
92 used epi-fluorescence microscopy to image the resulting changes in fluorescence in the gut
93 lumen over time (**Fig. 1c**). In the red-to-green (step ON) experiment, we observed that sfGFP

94 fluorescence in the worm gut lumen starts low, begins to increase within 2 hours, and reaches a
95 saturated high level at 6 hours (**Fig. 1d**). In contrast, in the green-to-red (step OFF) experiment,
96 sfGFP fluorescence begins high, and decreases exponentially between hours 1-7 (**Fig. 1d**). This
97 light response is abolished when the PCB biosynthetic operon is removed (Δ PCB) (**Fig. 1d**),
98 demonstrating that sfGFP levels are specifically controlled by CcaSR.

99 Next, we used flow cytometry to achieve high-throughput single-cell resolution analysis
100 of this *in situ* bacterial light response. Specifically, we reared worms in red and green light as
101 before, but then washed, paralyzed, and placed them into microtubes prior to light switching
102 (**Fig. 1b**). At several time points over the course of 8 hours, we homogenized the animals,
103 harvested the gut contents, and sorted bacterial cells and measured fluorescence via cytometry.
104 This experiment revealed that bacteria in the host gut remain intact (**Supplementary Fig. 2**) and
105 respond to light in a unimodal fashion (**Fig. 1e-g**). Furthermore, the temporal dynamics of the
106 gene expression response and dependence on PCB recapitulate our microscopy results
107 (**Supplementary Fig. 3**). We also confirmed that residual bacteria on the exterior of worms do
108 not contribute to the flow cytometry measurements (**Supplementary Fig. 4**). Together, these
109 experiments demonstrate that we can use optogenetics to rapidly, reversibly induce gene
110 expression of *E. coli* residing in the *C. elegans* gut.

111 Next, we sought to utilize our optogenetic method to modulate the production of specific
112 metabolites in bacteria residing in the gut of live hosts. It is well-known that bacterial genes
113 involved in the same metabolic process are often clustered into operons and co-regulated at the
114 transcriptional level. We took advantage of this coordinated mode of regulation and chose the
115 *cps* operon and its transcription activator RcsA for testing optogenetic control of bacterial
116 metabolism. The *cps* operon in *E. coli* consists of 19 genes that encode enzymes required for the

117 biosynthesis and secretion of CA (Torres-Cabassa and Gottesman, 1987), and CA-overproducing
118 bacterial mutants Δlon and Δhns promote longevity in the host *C. elegans* (Han et al., 2017). To
119 place CA biosynthesis under optogenetic control, we engineered an *E. coli* strain (MVK29)
120 lacking genomic *rcaA* and expressing a heterologous copy of *rcaA* under the control of CcaSR
121 (**Fig. 2a, Supplementary Tables 1-3**). We first examined whether MVK29 could respond to
122 green light and induce CA production and secretion. To this end, we grew the strain in batch
123 culture under red or green light and quantified supernatant CA levels. In red light, MVK29
124 secretes CA to concentrations below the limit of detection of the assay, similar to the $\Delta rcaA$
125 mutant (**Fig. 2b**). Green light, on the other hand, induces MVK29 to secrete high levels of CA,
126 and removal of the PCB biosynthetic operon abolishes this response (**Fig. 2b**). Moreover,
127 mutation of the CcaS catalytic histidine to a non-functional alanine (H534A), or the CcaR
128 phosphorylation site from an aspartic acid to a non-functional asparagine (D51N) abolishes
129 detectable CA production (**Fig. 2b**). Importantly, the level of secreted CA increases sigmoidally
130 with green light intensity, similar to the response of CcaSR itself (**Fig. 2c**) (Schmidl et al., 2014).
131 We conclude that we have placed CA production under the control of the CcaSR system, and
132 that we can use light to tune the production of bacterial metabolites.

133 We then used this approach to study a gut bacterial metabolite-host interaction pathway
134 *in vivo*. We first reared worms expressing mitochondrially-localized GFP (mito-GFP)
135 (**Supplementary Table 3**) on MVK29 with red light. We then continued this red-light exposure
136 for one group, and switched a second to green, for an additional 6 hours, and immediately
137 imaged intestinal cell mitochondrial morphology using confocal microscopy (**Fig. 3b**). We found
138 that mitochondrial fragmentation increases in worms exposed to the bacteria with light-induced

139 CA secretion (**Fig. 3c**). This result recapitulates the phenotype observed in worms supplemented
140 with purified CA (Han et al., 2017).

141 Next, we induced CA production directly from bacteria residing within the host gut. To
142 this end, we paralyzed the worms carrying mito-GFP, split them into two groups, and treated one
143 with red and the second with green for 6 hours (**Fig. 3a**). We then analyzed mitochondrial
144 morphology in intestinal cells using confocal microscopy. We found that the 6-hour levamisole
145 treatment does not kill worms but leads to mitochondrial hyper-fragmentation (**Fig. 3d**), which
146 might be due to the inhibitory effect of levamisole on mitochondrial NADH-oxidizing enzymes
147 (Köhler and Bachmann, 1978). This stress-induced effect resembles mitochondrial decay related
148 to aging and age-related neurodegenerative diseases (Cho et al., 2009; Exner et al., 2007;
149 Sebastián et al., 2017). Interestingly, we found that green light exposure counteracts this hyper-
150 fragmentation in paralyzed worms bearing MVK29 in the gut (**Fig. 3d**). Importantly, we found
151 no such effects in worms bearing the Δ PCB, CcaS(H534A), CcaR(D51N) or Δ *rcaA* mutant
152 strains (**Fig. 3d**), suggesting that this protective effect is a result of light-induced CA
153 overproduction in the gut. These results not only show that optogenetics can be utilized to induce
154 CA secretion from gut-borne *E. coli in vivo*, but also reveal a local protective effect of CA on
155 intestinal cells.

156 Finally, we took the advantage of the quantitative control afforded by optogenetics to
157 investigate how the lifespan-extending effect of CA relates to CA levels. Beginning at the day-1
158 adult stage, we exposed worms bearing MVK29 to red, or two green light intensities resulting in
159 intermediate or high CA secretion, and measured their lifespans. We found that the lifespan
160 extension increases proportionally with green-light intensity (**Fig. 4a**), revealing the pro-
161 longevity effect of CA is dose dependent. We also noticed that the extent of lifespan extension

162 by light is much stronger than that caused by dietary supplementation of purified CA (Han et al.,
163 2017), suggesting the high efficacy of optogenetic induction to modulate host physiology. As a
164 control, we repeated the experiment with worms bearing the CA-overproducing Δlon mutant and
165 showed that the lifespan extension caused by Δlon is independent of light exposure (**Fig. 4b**).
166 The extent of lifespan extension is similar to the MVK29 intermediate green light condition, but
167 less than the MVK29 high green light condition (**Fig. 4a, b**), suggesting that MVK29 is capable
168 of producing higher levels of CA than Δlon . In addition, the lifespan of worms bearing the $\Delta rcsA$
169 mutant is also light-independent and is comparable to that of MVK29 worms under red light
170 (**Fig. 4a, c**). These results suggest that optogenetic control is sufficient to induce bacterial
171 production of pro-longevity compounds and improve host health, and can exert stronger
172 beneficial effects than administration of a bacterial mutant or supplementing purified
173 compounds. Importantly, unlike the traditional approach of introducing a bacterial mutant,
174 optogenetic control of bacterial metabolism can modulate a host-level phenotype in a
175 quantitative manner.

176

177 **Discussion**

178 Our method has broad applications for studying microbe-host interactions *in situ*. For
179 example, we have identified about two dozen additional *E. coli* genes that are unrelated to CA
180 biosynthesis and that enhance worm longevity when knocked out (Han et al., 2017), though the
181 mechanisms by which they act remain largely unclear. By using light to induce their expression
182 in the gut and measuring acute host responses such as changes in mitochondrial dynamics, the
183 role of these genes in gut microbe-host interactions could be further explored. In another
184 example, the quorum-sensing peptide CSF and nitric oxide, both of which are produced by

185 *Bacillus subtilis* during biofilm formation, have been found to extend worm lifespan through
186 downregulation of the insulin-like signaling pathway (Donato et al., 2017). We have recently
187 ported CcaSR into *B. subtilis* and demonstrated that it enables rapid and precise control of gene
188 expression dynamics (Castillo-Hair et al., 2019). The method we report here should enable *in*
189 *situ* studies of how gene expression and metabolite production from this important Gram-positive
190 model bacterium impact longevity as well.

191 Multiple photoreceptors could also be combined to study more complex microbe-host
192 interaction pathways. Specifically, we and others have co-expressed CcaSR with independently-
193 controllable blue/dark and red/far-red reversible light sensors in order to achieve simultaneous
194 and independent control of the expression of up to three genes in the same bacterial cell
195 (Fernandez-Rodriguez et al., 2017; Olson et al., 2017; Tabor et al., 2010). Such optogenetic
196 multiplexing could be performed *in situ* and used to study potential synergistic, antagonistic, or
197 other higher-order effects of multiple bacterial genes or pathways. A large number of eukaryotic
198 photoreceptors have also been developed, enabling optical control of many cell- and
199 neurobiological processes (Deisseroth, 2015; Gautier et al., 2014; Goglia and Toettcher, 2018;
200 Leopold et al., 2018). Bacterial and eukaryotic photoreceptors could be combined to enable
201 simultaneous optical manipulation of bacterial and host pathways in order to interrogate whether
202 or how they interact. Optogenetics could also be used to manipulate bacterial and/or host
203 pathways at specific locations within the gut to examine location- or tissue-dependent
204 phenomena.

205 Finally, our method could be extended to other bacteria or hosts. In particular, it should
206 be possible to port CcaSR or other bacterial photoreceptors into native *C. elegans* symbionts
207 (Zhang et al., 2017) or pathogens(Couillault and Ewbank, 2002). Because these strains stably

208 colonize the host, the use of these bacteria could eliminate the need for paralysis, and facilitate
209 longer-term experiments. It is likely that light can also be used to control gut bacterial gene
210 expression in other model hosts such as flies, zebrafish, or mammals. Red-shifted wavelengths
211 and corresponding optogenetic tools(Ong et al., 2017; Ryu and Gomelsky, 2014) may prove
212 superior for less optically transparent or larger animals. Overall, by enabling precision control of
213 bacterial gene expression and metabolism *in situ*, we believe that optogenetics will greatly
214 improve our understanding of a wide range of microbe-host interactions.

215

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225

226 **Author Contributions**

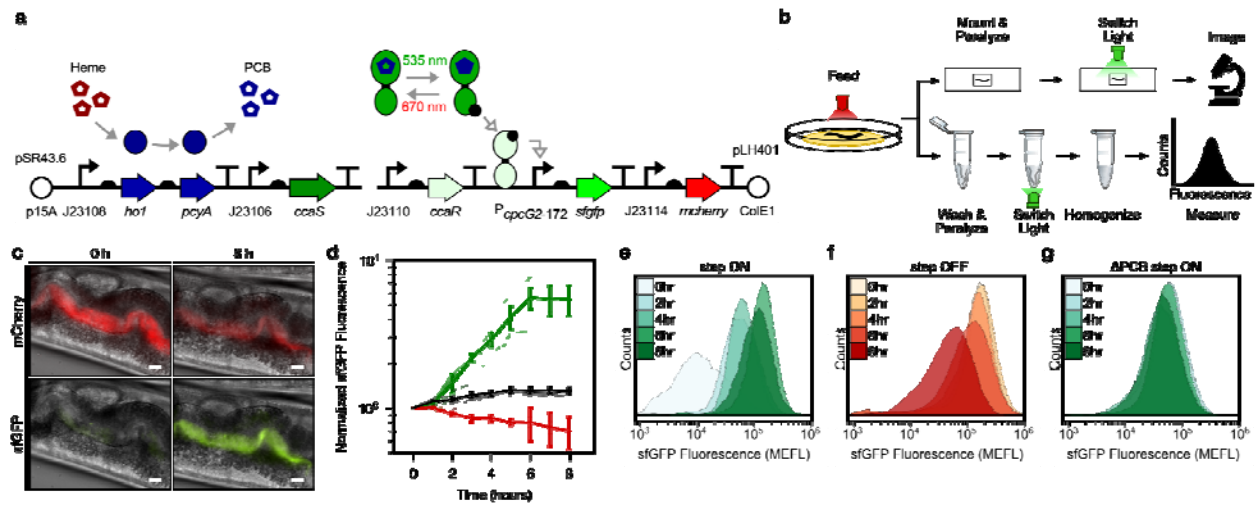
227 JJT and MW conceived of the study. LAH and MVK designed experiments. MVK and LAH
228 constructed plasmids and strains. LAH, MVK, BH, CJL, LG, and MW performed experiments.
229 CJL and LG scored single-blinded mitochondrial confocal micrographs. LAH, MVK, and MW
230 analyzed and interpreted results. LAH, MW, and JJT wrote the manuscript.

231

232 **Declaration of Interests**

233 The authors declare no competing interests.

234



235 **Figures and legends**

236

237 **Figure 1. Optogenetic control of *C. elegans* gut bacterial gene expression.** (a) Strain LH01.

238 (b) Microscopy and cytometry workflows. (c) Fluorescence microscopy images 0 and 8 h after

239 green light exposure in the step ON experiment. Scale bar: 10 μ m. (d) Response dynamics in the

240 step ON (green) and step OFF (red) microscopy experiments. Black: Δ PCB strain (step ON

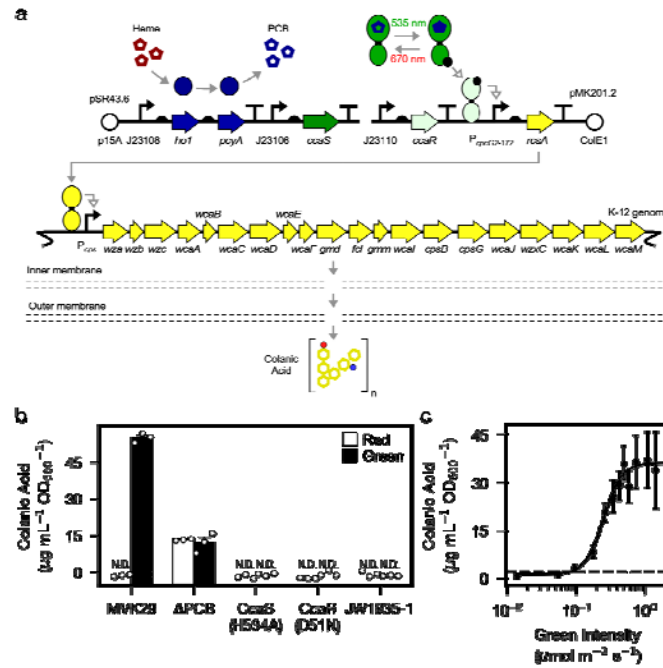
241 experiment). Individual- (light lines) and multi-worm average (dark lines) data are shown. $n = 7$,

242 4, 6 worms for green, red, black data sets (measured over 2, 3, 1 days, respectively). Error bars:

243 SEM. (e-g) Flow cytometry histograms for response dynamics experiments. MEFL: molecules of

244 equivalent fluorescein.

245



246

247 **Figure 2. Optogenetic control of colanic acid biosynthesis.** (a) Strain MVK29. (b) CA

248 secretion levels for MVK29 and control strains exposed to red and green light. JW1935-1 is the

249 *E. coli rcsA* background strain used in this study. N.D.: below assay limit of detection. (c)

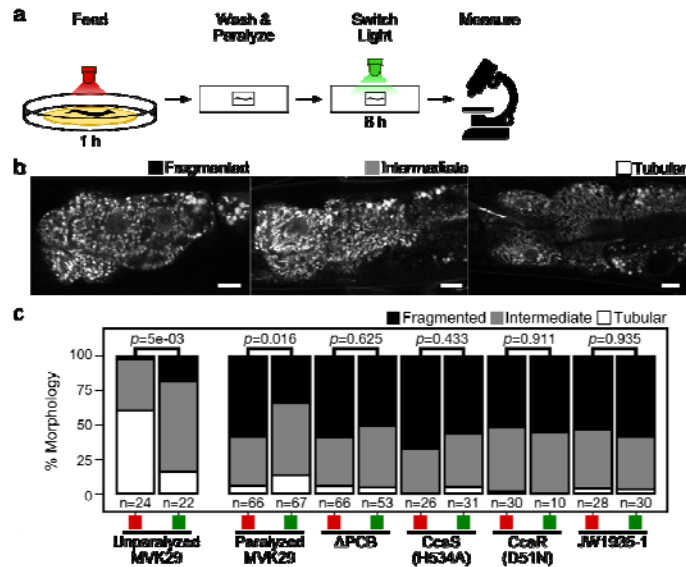
250 Green light intensity versus CA secretion level for MVK29. Data points represent 3 biological

251 replicates collected on a single day. Dashed line: limit of detection. Error bars indicate standard

252 deviation of the three biological replicates.

253

254



255

256 **Figure 3. Light-regulated CA secretion modulates *C. elegans* mitochondrial dynamics. (a)**

257 Schematic of experiment for activating CA biosynthesis *in situ*. (b) Representative images of the

258 mitochondrial network of anterior intestinal cells immediately distal to the pharynx are scored as

259 fragmented, intermediate, or tubular, as previously (Han et al., 2017). Scale bars: 10 μ m. (c)

260 Mitochondrial fragmentation profiles of un-paralyzed worms fed MVK29 while exposed to red

261 or green light for 6 h. (d) Fragmentation profiles for worms fed the indicated strain, then

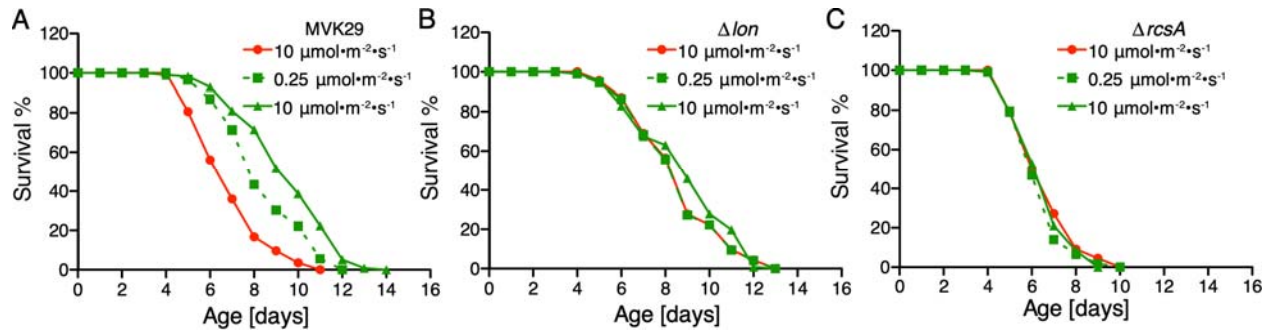
262 paralyzed for 6 h while exposed to red or green light. The number of worms included in each

263 condition is indicated below each bar. The Chi-Squared Test of Homogeneity was used to

264 calculate *p*-values between conditions.

265

266



267

268 **Figure 4. Optogenetically-regulated CA biosynthesis extends worm lifespan.** (a) When

269 exposed to green light, worms grown on MVK29 live longer than those exposed to red light, and

270 the magnitude of lifespan extension is proportional to green light intensity ($p < 0.0001$ green vs.

271 red, log-rank test). (b-c) The lifespans of worms grown on the Δlon (b) or the $\Delta rcsA$ (c) controls

272 are not affected by light condition ($p > 0.1$ green vs. red, log-rank test).

273

274

275 **Methods**

276 *E. coli* plasmids, strains, and media

277 Plasmids used in this study are described in **Supplementary Table 1**. Genbank accession
278 numbers are given in **Supplementary Table 2**. All plasmids constructed in this study were
279 assembled via Golden Gate cloning(Engler et al., 2009). Primers were ordered from IDT
280 (Coralville, IA). Assembled plasmids were transformed into *E. coli* NEB10 β (New England
281 Biolabs) for amplification and screening. All plasmid sequences were confirmed by Sanger
282 sequencing (Genewiz; S. Plainfield, NJ). To construct pLH401 and pLH405, pSR58.6(Schmidl
283 et al., 2014) was modified by inserting an *mCherry* expression cassette composed of a
284 constitutive promoter (J23114; <http://parts.igem.org/Promoters/Catalog/Anderson>), RBS
285 (BBa_B0034; http://parts.igem.org/Part:BBa_B0034), *mCherry*, and a synthetic transcriptional
286 terminator (L3S1P52 (Chen et al., 2013)). To construct pLH405, pLH401 was further modified
287 by exchanging the superfolder GFP gene (*sfgfp*) for *gfpmut3**. pMVK201.2 was built by
288 modifying pSR58.6 to control expression of *rcaA*.

289 All *E. coli* strains are described in **Supplementary Table 3**. $\Delta rcaA$ (JW1935-1) was
290 obtained from the Coli Genetic Stock Center. Δlon (JW0429-1) was obtained from the Keio *E.*
291 *coli* knockout library(Baba et al., 2006), a gift from the Herman lab. All *E. coli* strains were
292 maintained in LB media supplemented with appropriate antibiotics (chloramphenicol 34 μ g/mL,
293 spectinomycin 100 μ g/mL, kanamycin 100 μ g/mL) in a shaking incubator at 37 °C and 250 rpm
294 unless otherwise noted.

295

296 *C. elegans* strains and media

297 All *C. elegans* strains (**Supplementary Table 3**) were provided by the Caenorhabditis
298 Genetics Center (University of Minnesota), which is funded by the NIH office of Research
299 Infrastructure Programs (P40 OD010440). Worms were grown at 20°C on 1.7% NGM-agar
300 plates in 60 mm Petri dishes inoculated with a lawn of *E. coli* (CGSC str. BW28357), as
301 described in the CGC WormBook (wormbook.org), unless otherwise specified. The common
302 strain *E. coli* OP50 was not used for worm feeding, as it produces CA during normal growth (Han
303 et al., 2017). M9 buffer for *C. elegans* (abbreviated M9Ce to distinguish from *E. coli* M9 media)
304 was composed of 3 g KH₂PO₄, 6 g Na₂HPO₄, 5 g NaCl, 1 mL 1 M MgSO₄, H₂O to 1 L, and
305 sterilized by autoclaving (wormbook.org).

306

307 Optogenetic control of CA production

308 3 mL starter cultures of appropriate *E. coli* strains were inoculated from a -80°C freezer
309 and grown 12 h at 37°C. These starters were diluted to OD₆₀₀ = 1x10⁻² in M9 minimal media (1x
310 M9 salts, 0.4% w/v glucose, 0.2% w/v casamino acids, 2 mM MgSO₄, 100 μM CaCl₂)
311 supplemented with appropriate antibiotics. The M9/cell mixtures were then distributed into 3 mL
312 aliquots in 15 mL clear polystyrene culture tubes and grown at 37°C in a shaking incubator at
313 250 rpm while illuminated with the appropriate light wavelength and intensity, using the Light
314 Tube Array (LTA) device (Gerhardt et al., 2016). After 22 h, cultures were removed and iced to
315 halt growth and the OD₆₀₀ was measured. Culture samples were collected for CA quantification.

316

317 CA quantification

318 We adapted a previous CA quantification protocol(DISCHE, 1947; DISCHE and
319 SHETTLES, 1948) that takes advantage of the fact that it is the only exopolysaccharide
320 produced in our *E. coli* strains that incorporates fucose. In particular, we quantified the amount
321 of fucose in cell-derived exopolysaccharides (EPS), and used that value as a proxy CA levels.
322 First, EPS was liberated from cells by boiling 2 mL of culture for 15 min. in a 15 mL conical
323 tube. The sample was then centrifuged in 1.5 mL Eppendorf tubes for 15 min. at 21,000 x g.
324 Then, 0.7 mL of supernatant was dialyzed against water for at least 12 h using Pur-A-Lyzer Midi
325 3500 dialysis mini-tubes (Sigma-Aldrich, PURD35100-1KT) to remove monomeric fucose from
326 the sample.

327 Fucose monomers were then liberated from the EPS polymers by hydrolyzing 0.2 mL of
328 dialyzed media with 0.9 mL of H₂SO₄ solution (6:1 v/v acid:water). This mixture was boiled in a
329 15 mL conical for 20 min and then cooled to room temperature. The absorbance at 396 nm and
330 427 nm was measured. Next, 25 µL of 1 M L-cysteine HCl was added and mixed thoroughly by
331 pipetting. The absorbance at 396 nm and 427 nm was measured again. Simultaneously,
332 absorbance measurements of L-fucose standards pre- and post-L-cysteine addition were also
333 recorded. Absorbance change, given by *D* in the formula below, were used to compare the L-
334 fucose standard samples to the dialyzed culture samples and estimate the L-fucose concentration
335 in the dialyzed product.

$$D = \left((A_{post}^{396} - A_{pre}^{396}) - (A_{post}^{427} - A_{pre}^{427}) \right)$$

336

337 Preparation of NGM-agar plates for worm feeding

338 3 mL *E. coli* starter cultures were inoculated from -80 °C freezer stocks and grown for 12
339 h at 37 °C. These starters were then diluted to $OD_{600} = 1 \times 10^{-6}$ in M9 minimal media
340 supplemented with appropriate antibiotics. The M9/cell mixture was then distributed into 3mL
341 aliquots in 15 mL clear polystyrene culture tubes and grown at 37 °C in a shaking incubator at
342 250 rpm while illuminated with the appropriate light in the LTA. Once cultures reached $OD_{600} =$
343 0.1-0.4, tubes were iced for 10 min and subsequently concentrated to $OD_{600} \sim 20$ by
344 centrifugation (4 °C, 4000 rpm, 10 min) and resuspension in fresh M9 media. 400-600 μ L of
345 dense bacterial culture was then applied to sterile NGM-agar plates and allowed to dry in a dark
346 room, or a room with green overhead safety lights if cultures were preconditioned in green light.
347 Plates were wrapped in foil and refrigerated at 4 °C for no more than 5 days until needed.

348

349 Time-lapse microscopy

350 To obtain age-synchronized worm cultures, axenized *C. elegans* (strain *glo-1*) eggs were
351 isolated and allowed to arrest in L1 by starvation in M9 buffer (distinct from M9 media: 3 g
352 KH_2PO_4 , 6 g Na_2HPO_4 , 5 g NaCl, 1 mL 1M $MgSO_4$, and water to 1 L, sterilized by autoclaving
353 at 121 °C for 20 min) at room temperature for 12-18 h. 10-100 larvae were transferred to a
354 previously prepared NGM-agar plate containing a lawn of the appropriate bacterial strain. The
355 plate was then placed in a 20 °C incubator and illuminated with appropriate optogenetic light
356 provided by a single LED positioned 1cm above the Petri dish. Adult worms were transferred to
357 a fresh plate as necessary to maintain only a single generation.

358 Individual worms aged 1-3 days were removed from the dish and prepared for time-lapse
359 epifluorescence imaging. A 1.5% agar pad was prepared using M9 buffer as previously
360 described⁴⁵, and punched into ½” circles with a hollow punch. A 4 µL droplet of 2 mM
361 levamisole was deposited on a pad and 5 adult worms were transferred from the NGM plate to
362 the droplet. An additional 4 µL of levamisole solution was added and the worms were gently
363 washed to remove external bacteria. Worms were then transferred to a fresh pad with a 4 µL
364 droplet of levamisole solution, which was allowed to dry, thereby co-localizing and aligning the
365 worms on the pad. The pad was then inverted and placed into a 13 mm disposable microscopy
366 petri dish with a #1.5 coverslip on the bottom (Cell E&G; Houston, TX). Another coverslip was
367 placed on the top of the pad in the dish to curtail evaporation.

368 The dish was then placed on the stage of a Nikon Eclipse Ti-E inverted epifluorescence
369 microscope (Nikon Instruments, Inc; Melville, NY). Complete paralysis was induced by
370 incubating the dish at room temperature (~23 °C) for 30 min. Meanwhile, worms were exposed
371 to appropriate preconditioning light supplied by a circular array of 8 LEDs (4 x 660 nm, 4 x 525
372 nm) mounted to the microscope condenser ring, about 2 cm above the Petri dish. Light was then
373 switched from the preconditioning to the experimental wavelength, and worms were imaged
374 periodically using 10x, 40x, and 60x objectives. For each time point, the LEDs were turned off
375 and images acquired in the brightfield (DIC) and fluorescent channels. Afterwards, the LEDs
376 were turned on again to maintain optogenetic control.

377

378 Epifluorescence image analysis

379 All epifluorescence images were analyzed using the Nikon Elements software package
380 (Nikon Instruments, Inc; Melville, NY). The mCherry signal was used as a marker for the gut

381 lumen, and only cells in this region were included in the analysis. Image ROIs were created by
382 thresholding the sfGFP signal to identify the boundaries of cell populations. Out of focus regions
383 were eliminated from analysis. The average sfGFP pixel intensity inside the ROIs was calculated
384 and recorded for each time point.

385

386 Flow cytometry

387 1-3 day old *glo-1* worms were prepared for flow cytometry of the microbiome
388 constituents by washing, using a protocol adapted from previous work (Portal-Celhay et al.,
389 2012). Groups of 5 worms were washed 2x in a 5 μ L droplet of lytic solution: *C. elegans* M9
390 buffer containing 2 mM levamisole, 1% Triton X-100, and 100 mg/mL ampicillin. The worms
391 were then washed 2x in 5 μ L droplets of M9 buffer containing 2 mM levamisole only. Finally,
392 the worms were transferred to clear 0.5 mL Eppendorf tubes containing 50 μ L of M9 buffer + 2
393 mM levamisole, ensuring that 5 worms were deposited in the liquid contained in each tube. Each
394 tube was then exposed to light by placing it within one well of a 24-well plate (AWLS-303008,
395 ArcticWhite LLC) atop a Light Plate Apparatus (LPA) containing green and red LEDs⁴⁷ for 8 h
396 at room temperature. In separate control experiments, we demonstrated that any stray bacteria
397 that may escape the worms over this period, or which were inadvertently added to the 50 μ L of
398 M9 buffer, are incapable of responding to optogenetic light (**Supplementary Fig. 4**).

399 At the conclusion of the experiment, tubes were removed from the plate and immediately
400 chilled in an ice slurry for 10 min in the dark. Worms were homogenized using an anodized steel
401 probe sterilized between samples via 70% ethanol treatment and flame before being cooled.

402 Next, we used our previous antibiotic-based fluorescent protein maturation
403 protocol(Olson et al., 2014) to allow unfolded proteins to mature while preventing the production
404 of new protein. In particular, 250 μ L PBS containing 500 mg/mL Rifampicin was added to the
405 50 μ L homogenized worm samples and transferred to cytometry tubes. These tubes were
406 incubated in a 37°C water bath for precisely 1 h, then transferred back to an ice slurry.

407 These samples were measured on a BD FACScan flow cytometer. For gating, an
408 FSC/SSC polygon gate was first created using non-fluorescent bacteria grown *in vitro* at 37°C
409 (**Supplementary Fig. 2**). Events outside this region were excluded as non-bacterial material. To
410 isolate the engineered gut bacteria, only events with a high mCherry signal (FL3 > 1200 a.u.,
411 FL3 gain: 999) were included (**Supplementary Fig. 2**). Samples were measured until 20,000
412 events were recorded or the sample was exhausted.

413

414 Flow cytometry data analysis

415 All flow cytometry data (FCS format) were analyzed using FlowCal(Castillo-Hair et al.,
416 2016) and Python 2.7. We wrote a standard cytometry analysis workflow that truncated the
417 initial and final 10 events to prevent cross-sample contamination, removed events from saturated
418 detector bins at the ends of the detection range, and added 2D density gate on SSC/FSC retaining
419 the densest 75% of events (**Supplementary Fig. 2a**). GFPmut3* fluorescence units were
420 converted into standardized units of molecules of equivalent fluorescein (MEFL) using a
421 fluorescent bead standard (Rainbow calibration standard; cat. no. RCP-30-20A, Spherotech, Inc.)
422 as described previously(Castillo-Hair et al., 2016). Finally, to eliminate events associated with *C.*
423 *elegans* autofluorescence (**Supplementary Fig. 2b**), any events in the region $FL1 \leq 1200$ MEFL
424 were discarded.

425

426 Mitochondrial Fragmentation Assays

427 Synchronized L1 worms (strain *ges-1*) were applied to NGM agar plates containing
428 bacterial strain BW25113 and allowed to develop until adulthood. This parental bacterial strain is
429 used to allow all worms to develop at the same rate, avoiding any developmental/growth effects
430 the experimental strains may exert on the worms. All experimental bacterial strains were
431 preconditioned in red light with optogenetic light provided by a single LED positioned 1 cm
432 above the Petri dish.

433 After 3-5 days (between days 1-3 of adulthood), worms allocated for the experiment were
434 transferred to experimental strains for approximately 60-90 minutes to thoroughly inoculate the
435 GI tract. In the case of the unparalyzed worms, red or green light was then applied for an
436 additional 6 hours. For the paralyzed worm experiments, 1.5% low-melt agar pads were prepared
437 as described above and placed on individual slides. About 15 adult worms were transferred from
438 the experimental strain Petri dish to an agar pad containing 10 μ L of *C. elegans* M9 buffer + 2
439 mM levamisole (M9Ce+Lev), where worms were gently washed before being transferred to a
440 fresh pad also containing 10 μ L of M9Ce+Lev. The majority of M9Ce+Lev on the pad was
441 allowed to evaporate, which causes the worms to align longitudinally before a cover slip was
442 applied. Slides were then exposed to either red or green light by placing them under a single
443 LED positioned 1 cm above the Petri dish for 6 h. Afterward, the anterior intestinal cells were
444 imaged using confocal microscopy (Olympus Fluoview 3000) in the brightfield and GFP
445 channels.

446

447 Confocal Microscopy Image Analysis

448 All confocal images for an experiment were manually cropped to display only the
449 anterior intestinal cells of a single worm (in the GFP channel). These cropped images were then
450 anonymized, randomized and the mitochondrial networks of each were blindly classified by two
451 researchers independently as either tubular, fragmented, or intermediate. Tubular samples were
452 marked by a high degree of network connectivity throughout. Fragmented samples were
453 composed almost exclusively of isolated clusters of fluorescence with high circularity.
454 Intermediate samples contained regions of both types. Scores were then de-randomized and
455 aggregated. For each experimental strain, the red and green light conditions were compared for
456 statistical significance using the chi-squared test of homogeneity.

457

458 Lifespan experiments

459 3 mL starter cultures of Δlon , $\Delta rcsA$ or MVK29 were inoculated from -80°C freezer
460 stocks into LB supplemented with appropriate antibiotics and grown shaking for 12 h at 37 °C at
461 250 rpm. These cultures were diluted to $OD_{600} = 1 \times 10^{-6}$ in 27 mL M9 media supplemented with
462 appropriate antibiotics. 1.5 mL of each M9/cell mixture was added to each of 18 wells on three
463 24-well plates and grown in 3 LPA devices under the appropriate light conditions at 37 °C and
464 250 rpm. Once cultures reached $OD_{600} = 0.1-0.4$, all tubes were iced for 10 min and subsequently
465 concentrated 10 times by centrifugation (4°C, 4000 rpm, 10 min). Approximately 50 μ L of this
466 dense bacterial culture was then applied to sterile NGM-agar plates with no antibiotics and
467 allowed to dry in a dark room. The plates were then illuminated with the appropriate light
468 wavelength and intensity for 16 h at room temperature, and immediately used for lifespan assays.

469 During the longitudinal lifespan assay, exposure to white light is limited to the minimal
470 level. To reach this goal, the *sqt-3(e2117)* temperature sensitive mutant (**Supplementary Table**
471 **3**) was used to perform longitudinal analyses at 25 °C, which avoids time-consuming animal
472 transfer without interrupting normal reproduction. *sqt-3(e2117)* is a collagen mutant of *C.*
473 *elegans* that reproduces normally but is embryonically lethal at 26 °C, and has been used
474 previously in longitudinal studies (Han et al., 2017; Wang et al., 2014). Worms were age-
475 synchronized by bleach-based egg isolation followed by starvation in M9 buffer at the L1 stage
476 for 36 hours. Synchronized L1 worms were grown on BW25113 *E. coli* at 15 °C until the L4
477 stage, when worms were transferred to 24-well plates (~15 worms/well) with Δlon , $\Delta rcsA$ or
478 MVK29 (**Supplementary Table 3**). The plates were placed in LPA. The LPA LEDs were
479 programmed to illuminate wells with constant red (10 $\mu\text{mol}/\text{m}^2/\text{s}$), low-intensity green (0.25
480 $\mu\text{mol}/\text{m}^2/\text{s}$), or high-intensity green light (10 $\mu\text{mol}/\text{m}^2/\text{s}$). The apparatus was then transferred to a
481 26 °C incubator. The number of living worms remaining in each well was counted every day.
482 Death was indicated by total cessation of movement in response to gentle mechanical
483 stimulation. Statistical analyses were performed with SPSS (IBM Software) using Kaplan-Meier
484 survival analysis and the log-rank test (**Supplementary Table 4**).

485

486 **Supplemental Information Titles and Legends**

487 **Supplementary Figure 1. *In vitro* characterization of GFP reporter strains used in this**
488 **study.** All optogenetic strains carry our previously published CcaSR v2.0 system, which is
489 encoded on plasmids (a) pSR43.6 and (b) pSR58.6. (c) pLH401 (used for microscopy) and (d)
490 pLH405 (used for cytometry) genetic device schematics. We replaced *sfgfp* with *gfpmut3** in
491 pLH405 as we hypothesized that the latter may be less stable and thus result in faster response

492 dynamics. However, we observed no difference in dynamics. (e) Batch culture light responses of
493 CcaSR v2.0 and all GFP reporter strains used in this study. GFP fluorescence was measured by
494 flow cytometry. The dynamic range (ratio of GFP fluorescence in green versus red light) is
495 shown above each data set. We note that CcaSR v2.0 exhibits 77-fold dynamic range in the
496 reference strain BW29655 ($\Delta envZ$, $\Delta ompR$), which is similar to our previous measurement of
497 this strain at 120-fold (Schmidl et al., 2014). The calculated fold-change is sensitive to
498 fluctuations in the measured *E. coli* autofluorescence and red-light expression level, which likely
499 explains the slight discrepancy. CcaSR v2.0 dynamic range increases slightly to 84.2-fold in
500 $\Delta rcsA$ (JW1935-1), which is used throughout this work, due to higher sfGFP expression in green
501 light. The mCherry cassette in pLH401 decreases dynamic range to 38.3-fold due to higher leaky
502 sfGFP expression in red light. In worms, the fold-change in response to green light decreases
503 further to 5.52 ± 2.4 -fold (**Fig. 1d**). The use of *gfpmut3** in pLH405 further decreases dynamic
504 range to 13.5 ± 0.0 -fold for reasons that are not clear, while in worms the fold-change is $8.63 \pm$
505 3.6 -fold (**Fig. 1e**). Data represent the mean of three independent, autofluorescence-subtracted,
506 biological replicates acquired on 3 separate days. Error bars: standard deviation.

507

508 **Supplementary Figure 2. Flow cytometry gating.** (a) Strain LH05 (**Supplementary Table 3**),
509 which expresses only mCherry, was fed to worms, isolated, and analyzed by flow cytometry to
510 quantify *E. coli* autofluorescence through the FL1 (GFP) channel. To eliminate events
511 corresponding to cytometer noise and autofluorescence of homogenized worm samples, three
512 gates were then applied: (1) a density gate for the most homogeneous 75% of samples in forward
513 scatter (FSC) vs side-scatter (SSC) (the area within the bold black line in the plots in Column 1),
514 (2) a threshold gate on the FL1 (GFP) channel (marked by a red dashed line; **Methods**), and (3) a

515 threshold gate for events exhibiting high FL3 (mCherry, red dashed line; **Methods**). (b)
516 Applying these gates to samples from the step ON experiment (**Fig. 1e**) reveals robust isolation
517 of bacteria with a FSC/SSC profile consistent with our previous *in vitro* experiments^{24,47} and an
518 increase in expression of GFPmut3* in response to green light. Ungated data are shown for
519 comparison to gated data in all plots.

520

521 **Supplementary Figure 3. Gene expression dynamics from flow cytometry experiments.** (a)
522 LH03 and (b) LH04 (Δ PCB) step ON results. (c) LH03 and (d) LH04 step OFF results. Each
523 individual trajectory (faint lines) corresponds to a single biological replicate. A biological
524 replicate comprises the homogenized contents of five worms collected on a single day. Each
525 biological replicate on a given plot was run on a different day. Population medians taken across
526 all trajectories are shown in bold (**Methods**). Data are composed of six and eight trajectories in
527 the step ON and step OFF experiments, respectively. Error bars: SEM.

528

529 **Supplementary Figure 4. Bacteria on the exterior of worms do not contribute to the**
530 **measured light response in flow cytometry experiments.** Worms were suspended in clear
531 tubes containing M9Ce+Lev media and levamisole in the flow cytometry experiments. To
532 demonstrate that bacterial cells outside the worm gut (e.g. on the exterior of the worm) do not
533 respond to light in these conditions, samples of pre-conditioned bacteria from the NGM plates
534 were suspended in the M9Ce+Lev or *E. coli* M9 media supplemented with casamino acids and
535 glucose (M9Ec). These samples were then exposed to either green or red light for 8 h, then
536 measured via flow cytometry after the fluorophore maturation protocol (**Methods**). As expected,

537 the bacterial populations are only responsive to light when grown in M9Ec and when the PCB
538 operon is intact (strain JW1935-1/pLH405/pSR43.6, aka LH03). We conclude that the responses
539 shown in data in **Fig. 1e-f** and **Supplementary Fig. 3** are not due to bacteria outside the worm,
540 nor to bacteria that escape the worm over the course of the experiment, as such cells do not
541 respond to light in the experimental buffer (M9Ce). Data represent 7, 6, 3, and 5 replicates (left);
542 and 8, 3, 8, and 3 replicates (right) over 8 and 10 days, respectively; error bars: SEM. Individual
543 data points for each condition are overlaid as white markers.

544 **Supplementary Table 1. Plasmids used in this study**

545 **Supplementary Table 2. Genbank Accession Numbers**

546 **Supplementary Table 3. Bacterial and worm strains used in this study**

547 **Supplementary Table 4. Statistical analysis of worm lifespan experiments**

548

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