

1           **Role and impact of the gut microbiota in a *Drosophila* model for parkinsonism**

2

3                           Virzhiniya Feltzin<sup>1,2</sup>, Kenneth H. Wan<sup>3</sup>

4                           Susan E. Celniker<sup>3</sup> and Nancy M. Bonini<sup>1,\*</sup>

5

6

7

8

9           <sup>1</sup> Department of Biology, University of Pennsylvania, Philadelphia, PA 19104-6018

10           <sup>2</sup> Cell and Molecular Biology Graduate Group, Perelman School of Medicine, University of  
11           Pennsylvania, Philadelphia, PA 19104-6018

12           <sup>3</sup> Department of Bioengineering and Biomedical Sciences, Biological Systems and Engineering  
13           Division, Lawrence Berkeley National Laboratory, Berkeley, CA

14

15

16           \*Lead Contact & Corresponding author: [nbonini@sas.upenn.edu](mailto:nbonini@sas.upenn.edu)

17 **ABSTRACT:**

18 *Drosophila* is poised to be a powerful model organism for studies of the gut-brain axis due to the  
19 relative simplicity of its microbiota, similarity to mammals, and efficient methods to rear germ-  
20 free flies. We examined the gut-brain axis in *Drosophila* models of autosomal recessive  
21 parkinsonism and discovered a relationship between the gut microbiota and *parkin* loss of function.  
22 The number of live bacteria was increased approximately five-fold in the gut of aged *parkin* null  
23 animals. Conditional RNAi showed that *parkin* is required in gut enterocytes and not in neurons  
24 or muscle to maintain microbial load homeostasis. To examine the significance of gut microbiota,  
25 we reared germ-free *parkin* flies and discovered that removal of microbes in the gut improves the  
26 animals' resistance to paraquat. Sequencing of 16S rDNA revealed microbial species with altered  
27 relative abundance in *parkin* null flies compared to controls. These data reveal a role for *parkin*  
28 activity in maintaining microbial composition and abundance in the gut, suggesting a relationship  
29 between *parkin* function and the gut microbiota, and deepening our understanding of *parkin* and  
30 the impacts upon loss of *parkin* function.

31

32

33

34

35 **Key words:**

36 Gut microflora, dysbiosis, oxidative stress, axenic animals, *Drosophila* models of  
37 neurodegenerative disease

38

## 39 Introduction

40 Current studies have uncovered a fascinating link between the gut microbiota and the brain  
41 (Mayer et al., 2014; Sharon et al., 2016). For instance, alterations in the gut microbiota have been  
42 shown to affect host neurotransmitter levels, and anxiety- and depression-like symptoms (Bravo  
43 et al., 2011; Wong et al., 2016). In addition, studies suggest that changes in the gut microbiota  
44 are correlated with the development and severity of diseases such as autism and Parkinson's  
45 disease (Hsiao et al., 2013; Sampson et al., 2016; Scheperjans et al., 2015). As promising as  
46 these initial studies are, in-depth research into the link between microbes in the gut and disease  
47 of the brain is challenging given the complexity of the mammalian microbiota and the intricacies  
48 presented by mammalian models.

49 The genetics powerhouse of *Drosophila* has the potential to facilitate breakthrough studies of the  
50 gut microbiota and their relation to disease. The microbiome of the fly gut is simpler than that of  
51 mammals, with up to 20 species comprising more than 90 percent of all bacteria in the gut (Fink  
52 et al., 2013; Wong et al., 2013), allowing for powerful reductionist studies. Of the well-known  
53 residents of the fly gut, the genera *Lactobacillus* and *Enterococcus* are also commonly present in  
54 the human gut microbiome (Arumugam et al., 2011; Eckburg et al., 2005; Qin et al., 2010). One  
55 can rear germ-free flies efficiently and at lower cost compared to mammals, enabling  
56 experimental screens and studies that examine the impact of the gut microbiota on various  
57 disease models. The *Drosophila* microbiota are passed from parent to larvae through  
58 contamination of the embryonic shell (chorion), which the larvae consume after hatching. The  
59 larval microbiota develop as the growing larvae eat, until reaching a plateau at the third instar  
60 stage, and it is then eliminated during the pupal stage. Newly eclosed adult flies have a very low  
61 number of live bacteria in the gut, and the gut microbiota grow in number and evolve in  
62 composition as the animals age (Broderick and Lemaitre, 2012).

63 We sought to harness the potential of the fly with a screen to investigate the gut/brain axis in fly  
64 models of human disease. *Drosophila* disease models have contributed to crucial discoveries of  
65 disease mechanisms and etiology due to the wide array of available molecular genetic tools and  
66 the many conserved genes and pathways (Bier, 2005; Marsh and Thompson, 2006). We initiated  
67 our studies by measuring the gut microbial abundance in loss-of-function mutants for genes  
68 associated with recessive parkinsonism: *parkin* (*park*), *PTEN-induced putative kinase 1* (*pink1*),  
69 and *DJ-1*. It is thought that the main contribution of Pink1 and Parkin to development of PD is  
70 through a pathway in which both proteins work towards maintaining mitochondrial fidelity  
71 (Greene et al., 2003; Park et al., 2006). In healthy mitochondria, Pink1 is rapidly degraded, but  
72 mitochondrial damage and depolarization causes Pink1 to accumulate on the outer mitochondrial  
73 membrane (OMM) (Jin et al., 2010; Meissner et al., 2011; Narendra et al., 2010). Pink1  
74 phosphorylates Parkin resulting in recruitment of Parkin to the mitochondria and activation  
75 (Kane et al., 2014; Kazlauskaitė et al., 2014; Kondapalli et al., 2012; Koyano et al., 2014; Shiba-  
76 Fukushima et al., 2012; Shiba-Fukushima et al., 2014), eventually leading to engulfment of the  
77 damaged mitochondrion (Sarraf et al., 2013). Parkin has also been shown to regulate  
78 mitochondrial fission and fusion, protect against intracellular bacterial pathogens, and together  
79 with Pink1 play a role in intestinal stem cell proliferation (Deng et al., 2008; Manzanillo et al.,  
80 2013; Park et al., 2006; Poole et al., 2008). DJ-1 senses oxidative stress through oxidation of its  
81 cysteine residues and protects the cell from the harmful effects of reactive oxygen species  
82 (Canet-Avilés et al., 2004; Hayashi et al., 2009; Martinat et al., 2004; Taira et al., 2004).

83 In examining the gut microbiota in these genes associated with parkinsonism, here we report a  
84 link between the gut microbiome and *parkin* mutant flies. We find the abundance of gut  
85 microbiota is increased in aged mutant *parkin* animals, and that the absence of gut microbiota  
86 ameliorates paraquat sensitivity in *parkin* animals. These findings suggest a bidirectional  
87 relationship between the gut microbiota and *parkin* gene function that affects the severity and  
88 progression of the gene mutation effects.

## 89 RESULTS

90 **Microbial abundance is increased with age in *parkin* null animals.** To explore the idea of  
91 interactions between *Drosophila* models of neurodegenerative disease and disturbances in the  
92 gut microbiota, we measured microbial abundance in the autosomal recessive parkinsonism  
93 models *parkin*<sup>1</sup>, *pink1*<sup>B9</sup>, and a double knockout for the two *DJI* homologs in *Drosophila*, *DJ-1a*  
94 and *DJ-1β* (DJ-1 DKO). An abnormally high or low number of live bacteria in the gut indicates  
95 disruption of microbial homeostasis. Microbial abundance was quantified by dissecting the gut,  
96 homogenizing it through bead-beating, and spreading the homogenate in serial 10-fold dilutions  
97 on MRS-agar plates, a medium commonly used to rear the gut-associated microbes of  
98 *Drosophila* (Guo et al., 2014). The number of colonies that grew on the plates was counted and  
99 used to calculate the Colony Forming Units (CFU), representative of the number of live bacteria  
100 in the gut. We used males of ages 3d (young flies with a sparse microbiome) and 20d (older flies  
101 with a well-established abundant microbiome).

102 Consistent with previous findings (Guo et al., 2014)(Broderick et al., 2014), young flies had few  
103 living bacteria in the gut ( $\sim 10^3$ ), and this number rose steeply in older flies ( $\sim 10^5$ ) (Fig. 1a).  
104 There was no difference in microbial load between control flies and any of the parkinsonism  
105 gene models at 3d. At 20d, however, we observed a significant increase in the number of live  
106 microbes per gut of *parkin* null flies compared to control animals ( $\sim 10^6$ ) (Fig. 1a). Surprisingly,  
107 *pink1* and *DJ-1* mutant animals did not show a significant microbial load increase, even though  
108 Parkin and Pink1 are thought to regulate mitochondrial homeostasis and shape dynamics through  
109 the same pathway (Pickrell and Youle, 2015). This indicated a disturbance in the gut microbiota  
110 of *parkin* mutants, and that Parkin may play this role independently of Pink1.

111 We performed a series of control experiments to assess whether the increase in microbial load in  
112 *parkin* nulls was simply related to a change in eating or elimination from the gut. The rate of  
113 feeding was measured using proboscis print assays. Young and old wild-type and *parkin* male  
114 flies were placed individually on a microscope slide covered with sucrose-gelatin for 20  
115 min (Edgecomb et al., 1994). As the fly ingests gelatin, the proboscis leaves a print on the surface  
116 of the slide, which was observed and scored using Differential Interference Contrast (DIC)  
117 microscopy (Fig. 1c). The number of proboscis prints left on the slide at the end of the assay  
118 reflects the rate of feeding. We determined that *parkin* flies eat significantly less than wild-type  
119 controls at 3d and 20d (Fig. 1d), suggesting the increase in microbial load cannot be due to  
120 increased feeding. To measure the volume of food in the gut, the flies were fed standard food  
121 supplemented with FD&C Blue Dye #1, then guts were dissected, homogenized, and the  
122 absorbance of the sample at 630nm was measured. The assay revealed no significant difference  
123 in gut volume between old and young *parkin* mutants and wild-type controls (Fig. 1b).  
124 Therefore, neither a higher rate of feeding, nor a larger volume of food in the gut explains the  
125 increased microbial load in the gut of *parkin* mutants.

126 Since the mutant animals eat at the same rate as wild-type animals, we examined the possibility  
127 that the rate of elimination could be slower, causing more bacteria to accumulate in the gut, by  
128 conducting defecation assays with young and old *parkin* mutants, as well as with wild-type  
129 controls. To measure the rate of defecation, cohorts of 40 animals per age and genotype were  
130 placed on fly food containing FD&C Blue Dye #1. After 24h allowing the blue food to reach  
131 steady state in the gut, animals were transferred to fresh blue food vials, and the number of blue  
132 fecal spots deposited on the walls of the vial was counted after 24h. Food vials were laid on their  
133 side, so that the climbing defects of *parkin* mutants would not affect the results of the  
134 experiment. We observed that young *parkin* mutants had significantly lower rates of defecation  
135 compared to wild-type controls (Supplementary Fig. S1). Older flies showed no difference in  
136 defecation rate, and, together with cell-type specific *parkin* RNAi experiments (see below), these  
137 results suggested elimination from the gut is unlikely to be the sole contributor to the elevated  
138 microbial load in *parkin* mutants.

139 ***Parkin* is required in gut enterocytes to maintain microbial load homeostasis.** To determine  
140 which specific cell types required *parkin* activity to maintain gut microbial homeostasis, we  
141 characterized a *parkin* RNAi line and confirmed that ubiquitous *parkin* knockdown using this  
142 line led to a decrease in *parkin* RNA expression, muscle degeneration reflective of *parkin* loss of  
143 function, as well as the increase in gut microbial load (Fig. 2a-g). We then examined the role of  
144 tissues implicated in *parkin* function (the nervous system, muscle), as well as specific cell types  
145 within the gut for a role in the gut microbial phenotype. Knockdown of *parkin* in gut enterocytes  
146 (*NPI-GAL4* driver) resulted in the increased microbial load (Fig. 2h), whereas we observed no  
147 change in microbial load upon *parkin* depletion in gut stem cells (*esg-GAL4* driver), neurons  
148 (*elav-GAL4* driver), or muscle (*24B-GAL4* driver) (Fig. 2i-k). These results suggest that *parkin*  
149 gene function is required in gut enterocytes to maintain microbial load within the wild-type  
150 range.

151 **The gut microbiota impact *parkin* sensitivity to paraquat.** The fly gut microbiota are  
152 beneficial for the host, promoting larval development under conditions of nutrient scarcity (Shin  
153 et al., 2011; Téfrit and Leulier, 2017). We considered whether the increased microbial abundance  
154 in *parkin* flies may contribute to the *parkin* mutant phenotype. To assess this, we created germ-  
155 free animals by dechoriation of embryos followed by rearing on food supplemented with  
156 antibiotics (Guo et al., 2014; Ren et al., 2007). Flies mutant for *parkin* have a known increased  
157 sensitivity to oxidative toxins such as paraquat (Pesah et al., 2004). We assessed whether this  
158 phenotype was altered in germ-free animals, by subjecting germ-free and conventionally raised  
159 male flies to a paraquat sensitivity assay. Interestingly, we found that germ-free *parkin* flies  
160 survived longer on paraquat compared to conventional *parkin* animals (Fig. 3a). This finding  
161 suggests that the gut microbiota increase sensitivity of the *parkin* mutant to paraquat stress.

162 We confirmed that improved paraquat resistance of germ-free flies was not due to the animals  
163 eating less and thus ingesting less of the toxin, as proboscis print assays showed no difference in  
164 the rate of feeding between germ-free and conventional *parkin* males (Fig. 3B). The proboscis  
165 print assay showed no significant difference in feeding between *parkin* and wild-type males  
166 unlike the previous assay that showed *parkin* flies eat less (see Fig. 1d). Proboscis print assays on  
167 wild-type and *parkin* males reared on standard food and treated with starvation caused no  
168 difference in feeding rate analogous to the assay with males from germ-free lines (Fig. 3c),  
169 leading us to conclude that *parkin* and wild-type flies eat equally in response to starvation.

170 We further investigated whether *parkin* knockdown in the gut selectively affects paraquat  
171 sensitivity, or alternatively, if paraquat sensitivity is a non-gut phenotype that is affected by the  
172 presence of the gut microbiota. To examine this, we used conditional *parkin* RNAi followed by  
173 paraquat sensitivity assays. Ubiquitous RNAi of *parkin* phenocopied the increased toxin  
174 sensitivity of the *parkin* mutant (Fig. 3d). Intriguingly, *parkin* RNAi knockdown selectively in  
175 gut enterocytes did not cause a significant change in paraquat sensitivity (Fig 3E). Taken  
176 together with a recent study suggesting that increased paraquat sensitivity in *parkin* mutants may  
177 be due to *parkin* loss of function in muscle and brain (de Oliveira Souza et al., 2017), these  
178 results indicate that paraquat sensitivity is not a gut-specific effect but that altering the gut  
179 microbiota can influence non-gut animal characteristics, namely sensitivity to toxins.

180 **The gut microbiota are altered in composition in aged *parkin* mutants.** Given the impact of  
181 *parkin* gene function on gut microbial abundance, we determined whether there were alterations  
182 in the composition of microbes in the *parkin* gut. To define the microbial types, we sequenced  
183 16S rDNA V1-V2 variable region amplicons using DNA extracted from dissected guts of 7d and  
184 20d wild-type and *parkin* males. For the young timepoint, we chose 7d rather than 3d due to the  
185 very low microbial abundance in 3d guts. Sequences were clustered into Operational Taxonomic  
186 Units (OTUs) by aligning against “seed” sequences from the Greengenes database (Caporaso et  
187 al., 2010), or if clustering with Greengenes failed, by aligning against each other (open-reference  
188 OTU picking). The taxonomic identity of each OTU was assigned using the RDP  
189 classifier (Wang et al., 2007). We found no significant difference in  $\alpha$ -diversity between *parkin*  
190 and wild-type microbiomes using several diversity metrics (Supplementary Table S1). Weighted  
191 UniFrac showed no difference at 7d in microbial composition between *parkin* null and control  
192 males (Fig 4A). At 20d, however, the composition of the gut microbiota of *parkin* nulls and  
193 wild-type flies diverged from each other and from the microbiome of 7d males (Fig. 4a). These  
194 data indicate that aged *parkin* mutants not only have a higher gut bacterial load, but also an  
195 altered gut genera composition compared to normal animals.

196 We defined the variation underlying the divergent microbiome of aged *parkin* animals by  
197 analyzing the most abundant gut genera, defined as comprising at least 5% of the total reads in  
198 any one sample. These data showed that 20d *parkin* mutants have a decreased relative abundance  
199 of *Paenibacillus* and *Clostridium* reads (Fig. 4c). To interrogate differences at the species level,  
200 representative sequences from each OTU were fetched and batch-aligned to the BLAST 16S  
201 rRNA sequence database using nucleotide BLAST. The top hit with more than 99% identity to a  
202 sequence from an identified species in the database, defined the species identity and was  
203 assigned to the OTU (see Supplementary Figures S2-S4 for representative alignments). Species-  
204 level analysis revealed a switch of the dominant *Acetobacter* species from *A. orleanensis* to *A.*  
205 *pasteurianus* in 20d *parkin* males (Fig. 4e).

## 206 DISCUSSION

207 In this study we examined the relationship between microbes in the gut and *parkin* gene function.  
208 We discovered a five-fold increase of microbial load in the guts of aged *parkin* flies compared to  
209 wild-type controls. *In vivo* RNAi of *parkin* in gut enterocytes revealed that *parkin* gene function  
210 in the gut specifically impacts microbial load. Paraquat sensitivity assays with germ-free flies  
211 showed a beneficial effect on paraquat sensitivity in germ-free *parkin* animals compared to  
212 conventionally reared controls. Using 16S rDNA sequencing, we assessed the effect of the

213 *parkin* mutation on gut microbial composition and observed an altered bacterial genera and  
214 species abundance in aged *parkin* flies.

215 Unexpectedly, the increase compared to controls of live microbes in the guts of 20d *parkin* flies  
216 was not also observed in *pink1* flies, even though Pink1 and Parkin share many age-associated  
217 adult-onset phenotypes, and regulate mitophagy and mitochondrial fission/fusion as parts of the  
218 same pathway (Pickrell and Youle, 2015). In mammals, Parkin has been shown to ubiquitinate  
219 and activate NEMO, a member of the NF- $\kappa$ B pathway, in a manner that is independent of Pink1  
220 function (Müller-Rischart et al., 2013). Parkin also mediates ubiquitination of intracellular  
221 pathogens; whether Pink1 is required for this activity is not known (Manzanillo et al., 2013).  
222 Taken together, these observations suggest that Parkin has roles that are independent of Pink1  
223 gene function; regulation of microbial homeostasis may be one such function.

224 Our data suggest that *parkin* gene function impacts gut microbial load and abundance. There are  
225 a number of ways in which an increase in microbial load may be linked to a change in microbial  
226 composition. The increase may lead to a spike in inflammation and oxidative stress, rendering  
227 the gut inhospitable for some taxa that otherwise would be present. It is also possible that *parkin*  
228 loss of function causes a decrease in relative abundance of some microbes that would normally  
229 limit proliferation of other taxa, leading to overgrowth of the remaining taxa.

230 It is unlikely that the effects of *parkin* loss of function on the gut microbiota are secondary  
231 effects of the known function of *parkin* to disrupt mitochondrial homeostasis, since *pink1*  
232 mutants have similar effects on the mitochondria but not microbial load. Although we cannot  
233 fully rule out an effect on microbiota due to a change in defecation rate, we speculate that Parkin  
234 may regulate gut microbial homeostasis via interactions with *Drosophila* innate immunity  
235 pathways. Two immunity pathways are known to regulate microbes in the fly gut: the Dual  
236 oxidase (Duox) and Imd pathways (Broderick and Lemaitre, 2012). Duox, a member of the  
237 NADPH oxidase family, produces reactive oxygen species that restrict bacterial viability (Kim  
238 and Lee, 2014). The enzyme activity is known to be upregulated by bacterial-derived uracil (Lee  
239 et al., 2015). To our knowledge, no link between Parkin activity and Duox is known at present.  
240 Alternatively, the Imd pathway is the *Drosophila* analog of the mammalian NF- $\kappa$ B  
241 pathway (Myllymäki et al., 2014). In the fly, the pathway promotes transcription and ultimately  
242 secretion of antimicrobial peptides (AMPs) in response to DAP-type peptidoglycan, a component  
243 of bacterial cell walls (Myllymäki et al., 2014). Interestingly, Parkin in mammals ubiquitinates a  
244 member of the NF- $\kappa$ B pathway, NEMO (Müller-Rischart et al., 2013), which is essential for NF-  
245  $\kappa$ B pathway activation. This activity is independent of Pink1 (Müller-Rischart et al., 2013). The  
246 *Drosophila* NEMO homolog, IKK- $\gamma$ , also plays a role in activation of the Imd pathway (Ertürk-  
247 Hasdemir et al., 2009; Rutschmann et al., 2000). In mice, conditional ablation of NEMO leads to  
248 impaired AMP secretion, intestinal epithelial cell apoptosis, and translocation of bacteria into the  
249 intestinal mucosa (Nenci et al., 2007).

250 A surprising result is that the presence of a gut microbiome is detrimental to *parkin* mutants  
251 exposed to paraquat. Given the improved toxin resistance of germ-free *parkin* flies, metabolism  
252 of paraquat by microbes found in the *parkin* gut may increase paraquat toxicity. Many bacteria  
253 have been shown to be able to use paraquat as an electron carrier in the redox cycle, generating  
254 reactive oxygen species (ROS) (Haley, 1979). ROS generated by gut bacteria through redox  
255 cycling would not only be toxic in themselves, but also increase gut permeability, allowing even  
256 more toxic paraquat to be taken up by the fly. Paraquat can also be used as a coenzyme by

257 bacteria in the reduction of sulfate, thiosulfate, hydroxylamine, nitrate, among other compounds  
258 (Haley, 1979). It is possible that paraquat could mediate increased secretion of a gut bacterial  
259 metabolite which in turn is toxic to the host.

260 Our results suggest Parkin plays a before-undocumented role in regulation of gut microbial  
261 homeostasis, and conversely, that the gut microbiota impact parkinsonism as modeled in the fly.  
262 This study deepens our understanding of the *parkin* mutant phenotype and sets a foundation for  
263 further studies on the importance of the gut microbiota to parkinsonism in mammals.

264

265

266

### 267 **Acknowledgements**

268 We thank members of the Bonini laboratory for critical reading and helpful comments. We thank  
269 Karl Kumbier (UCB Statistics Department) and Jian-Hua Mao for reviewing our statistical  
270 analyses. This work was supported by funding from NIH grants R21-NS088370, R35-NS097275,  
271 and a Glenn Award for Research in Biological Mechanisms of Aging (to N.M.B.). Additional  
272 support was provided by Lawrence Berkeley National Laboratory (LBNL) Directed Research and  
273 Development (LDRD) program funding under the Microbes to Biomes (M2B) initiative (K.H.W.  
274 and S.E.C.). LBNL is a multi-program national laboratory operated by the University of California  
275 for the DOE under Contract DE AC02-05CH11231.

### 276 **Author Contributions**

277 V.F. and N.M.B. conceived and designed experiments. V.F. performed experiments and  
278 analyzed data. K.H.W. prepared and sequenced libraries. S.E.C. provided input,  
279 experimental advice and equipment. V.F. and N.M.B. wrote the manuscript with input from  
280 S.E.C.

### 281 **Competing interests**

282 The authors declare no competing interests.

283

284



## 285 METHODS

286 **Fly lines:** Flies were grown in standard cornmeal-molasses-agar medium at 25°C. *parkin*<sup>1</sup> (*w*\*;  
287 *P[EP]park1/TM3, Sb1 Ser1*, FlyBase ID: FBst0034747) and *parkin* RNAi (*y1 sc\* v1*;  
288 *P[TRiP.HMS01800]attP2/TM3, Sb1*, FlyBase ID: FBst0038333) flies were obtained from the  
289 Bloomington Stock Center. NP1-GAL4 flies were obtained from Sara Cherry (University of  
290 Pennsylvania) and *pink* (*pink1<sup>B9</sup>/FM6*) flies were obtained from Jongkyeong Chung (Seoul  
291 National University). The *parkin*<sup>1</sup> and *pink1<sup>B9</sup>* alleles were backcrossed into a homogenous wild-  
292 type background (*w<sup>1118</sup>*, FlyBaseID: FBst0005905) for five generations. DJ1 DKO (*w<sup>1118</sup>;DJ-*  
293 *1α<sup>A72</sup>; DJ-1β<sup>A93</sup>/TM6,Tb*) flies are described (Meulener et al., 2005).

294 **Gut dissection and CFU counting:** Animals used in different replicates were collected from  
295 different bottles and aged in different vials. All animals were collected as virgins and aged on  
296 standard cornmeal-molasses-agar medium at a density of 20 flies per vial. Fly density has been  
297 shown to impact the relative abundances of *Acetobacter* and *Lactobacillus* (Wong et al., 2015).  
298 Animals were transferred to fresh food vials every other day. *parkin* and wild-type animals were  
299 aged in the same vials. For all experiments, controls and experimentals were aged on the same  
300 batch of food and transferred on the same day. For the tissue specific expression experiments, the  
301 driver line was compared to the RNAi line using the same food and under the same conditions,  
302 as different driver lines represent different background.

303 For gut dissection, flies were anesthetized, washed 1X in 1mL 10% bleach, 1X in 1mL 100%  
304 ethanol, and finally rinsed 3X in 1 mL sterile PBS (Sigma-Aldrich). 200 μL of the final rinse  
305 were spread on an MRS-agar plate (BD Diagnostic Systems) as a control for the efficacy of the  
306 wash. Each gut was dissected in a drop of PBS on a sterile microscope slide and placed in 200  
307 μL PBS. The gut was homogenized by bead-beating with 1mm tissue-disruption beads (Research  
308 Products International) for 30s at maximum speed. 10-, 100-, and 1000-fold dilutions of the  
309 homogenate were spread on MRS-agar plates. The fly gut is aerobic, allowing culture of bacteria  
310 with most standard media and conditions, including the microbes defined here (Guo et al., 2014;  
311 He et al., 2007). All plates were incubated at 30°C for 48h. Bacterial colonies were counted and  
312 multiplied by the dilution factor to calculate the number of Colony Forming Units (CFU) per gut.

313 **Proboscis prints, blue dye, and defecation assays:** Proboscis print assays were modified from  
314 Edgecomb et al. (1994). Clean microscope slides (Fisher) were briefly dipped in 10% sucrose  
315 1% gelatin and left to dry at room temperature in a covered area for 3-4h. Flies were anesthetized  
316 and placed in individual wells of a 96-well plate. Groups of ten flies were arranged in two  
317 columns of five wells. Each group was covered by a strip of wax paper and a gelatinated  
318 microscope slide. Flies were incubated for 30 min at room temperature to recover from the  
319 anesthesia, during which time the outline of each well was traced on the slide using a thin  
320 permanent marker. At the end of the incubation period, the strip of wax paper was swiftly  
321 removed allowing contact between the fly and the sweet gelatin coat. Plates were inverted,  
322 allowing the flies to walk on top of the slide for 20 min at room temperature. The number of  
323 prints left on each slide was counted using Differential Interference Contrast (DIC) microscopy.

324 For the blue dye assays, flies were fed for 48h on standard food supplemented with 2.5% w/v  
325 FD&C Blue Dye #1 (SPS Alfachem). Five guts per age and genotype were dissected, homogenized  
326 and the absorbance of the sample at 630nm was measured with a spectrophotometer.

327 For the defecation assays, cohorts of 40 animals per age and genotype were tested using ten flies  
328 per vial on fly food containing 2.5% w/v FD&C Blue Dye. Animals were left on the dye for 24h.  
329 Flies were transferred to fresh blue food vials and the number of blue fecal spots deposited on the  
330 walls of the vials was counted after 24h.

331 **Germ-free flies:** The germ-free fly protocol was adapted from previously described  
332 techniques.(Guo et al., 2014; Koyle et al., 2016; Ma et al., 2015) Standard cornmeal-molasses-  
333 agar fly food was autoclaved and upon cooling supplemented with yeast extract (Fisher) to 100  
334 g/L. An antibiotic cocktail of kanamycin (1mM; Fisher), ampicillin (650  $\mu$ M; MediaTech), and  
335 doxycycline (650  $\mu$ M; Sigma-Aldrich) was added to the food as previously described(Ren et al.,  
336 2007). Food was dispensed in empty fly bottles at 50 mL per bottle in a laminar flow cabinet and  
337 left to solidify. A 12h collection of fly embryos was rinsed in 100% ethanol to cleanse and  
338 sterilize any leftover agar from collection plates, dechorionated in 10% bleach for 2 min, and  
339 immediately rinsed 3X in sterile PBS. Embryos were placed on the prepared fly food and  
340 overlaid with sterile glycerol. Germ-free fly lines were maintained on sterile food for up to 3-4  
341 generations using a laminar flow cabinet. Flies were monitored for bacterial contamination by  
342 homogenizing larvae and testing for bacterial growth on MRS-agar plates.

343 **Paraquat sensitivity assays:** Flies were transferred to empty vials at 20 flies per vial (Genesee  
344 Scientific), starved for 6h, then transferred to vials containing 2.5% agar (LabScientific), 10%  
345 sucrose (Sigma-Aldrich), 25mM Paraquat (MP Biomedicals). Vials were incubated at 25°C and  
346 the number of dead flies in each vial was counted every 8h until all flies were dead or until 168  
347 hr (7d) had passed.

348 **16S rDNA sequencing:** Animals from different replicates were collected from different bottles  
349 and aged in different vials to ensure replicates were biologically independent. Flies were aged at  
350 a density of 20 flies per vial and transferred to fresh food vials every other day. Wild-type and  
351 *parkin* flies were aged on the same batch of food and transferred at the same time. All twenty  
352 flies from a vial were used for each biological replicate. Twenty guts per sample were dissected  
353 as described above and subjected to DNA extraction using the PSP Spin Stool DNA Purification  
354 Kit (Stratec Biomedical). PCR of the V1-V2 variable regions was performed using the 27F –  
355 338R primer pair (27F: 5'-AGAGTTTGATCMTGGCTCAG-3'; 338R: 5'-  
356 TGCTGCCTCCCGTAGGAGT-3') with the following program: 94°C for 4 min, 94°C for 30s,  
357 58°C for 30s, 72°C for 40s, 30 total amplification cycles, 72°C for 10 min, then hold 4°C. Three  
358 PCR reactions were pooled and the PCR product was purified using the Agencourt AMPure XP  
359 PCR purification kit (Beckman Coulter) and sequenced using MiSeq (Illumina).

360 Sequencing analysis was carried out using the QIIME suite(Caporaso et al., 2010). Paired reads  
361 were joined and quality filtered using a Phred score cutoff of 20. OTUs were picked using an  
362 open-reference OTU picking algorithm with the Uclust alignment method and 99% identity.  
363 OTUs with less than 10 reads were removed from the analysis. The most abundant sequence was  
364 selected as a representative sequence for each OTU and used to assign a taxonomic classification  
365 for each OTU using the RDP classifier version 2.12(Wang et al., 2007). The resulting OTUs and  
366 their taxonomy were compiled in a QIIME OTU table.

367 **Climbing assays, thoracic indentations, and abnormal wing posture scoring:** Flies were  
368 raised and aged on standard cornmeal molasses agar food vials at a density 20 flies per vial.  
369 Number of flies with abnormal wing posture was scored on anaesthetized animals in the vial. For  
370 climbing assays, flies were flip-transferred in empty vials (Genesee Scientific) with a line

371 marking a distance 8 cm above the bottom of the vial, near the top. Vials were tapped and the  
372 number of animals that crossed the mark 10s after tapping was recorded. The presence or  
373 absence of thoracic indents was scored on anesthetized animals on a fly pad. Experiments were  
374 repeated in 3 independent biological replicates with 55-60 flies per replicate.

375 **Real-time quantitative PCR:** Total RNA from crushed whole males was purified using the  
376 Trizol reagent (Ambion) following the reagent manual. The RNA was DNase treated using  
377 TURBO DNase (Ambion) according to the kit instructions. After DNase treatment, the RNA was  
378 Trizol purified again. Reverse transcription was carried out using the High-Capacity cDNA  
379 Reverse Transcription Kit (Applied Biosystems) according to the kit manual. Real time PCR was  
380 carried out using the Fast SYBR Green Mastermix (Applied Biosystems) following the kit  
381 instructions. Primers used for RT PCR had the following sequences: *parkin* F: 5'-  
382 CGGATGTGAGTGATACCGTGT-3'; *parkin* R: 5'-ATAAACTGACGCTCGCCCAA-3'.

383 **Statistics:** Statistical analyses pertaining to the processing of 16S rDNA sequencing results were  
384 carried out using QIIME's built-in functions (Caporaso et al., 2010). All other statistical tests  
385 were performed using GraphPad Prism (GraphPad Software, La Jolla, CA). For treatment and  
386 mutant analyses, we used the Analysis of Variance (ANOVA) test to determine differences  
387 between three or more means. If significance was detected, Tukey's post-test was used to  
388 identify those values that were significantly different.

389

390

391

392

393

394

395

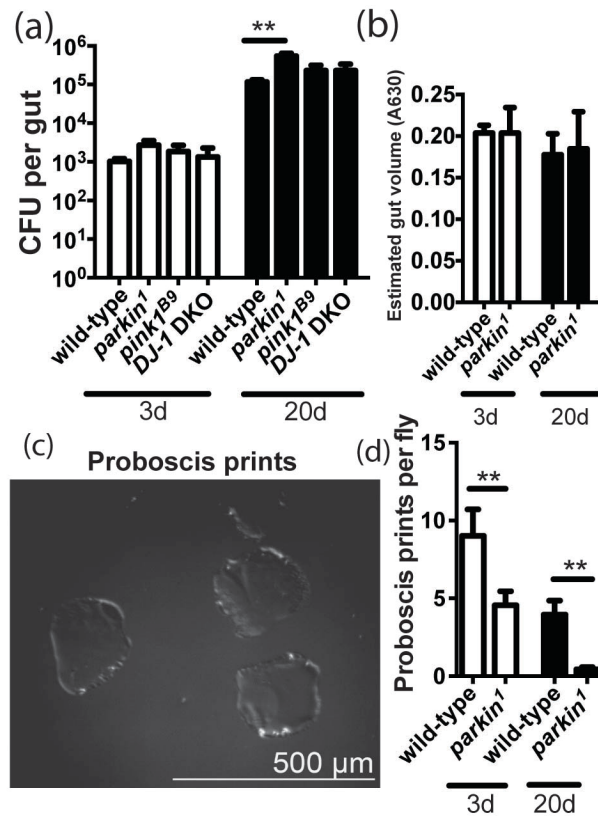
396 **REFERENCES**

- 397 Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes,  
398 G.R., Tap, J., Bruls, T., Batto, J.M., *et al.* (2011). Enterotypes of the human gut microbiome.  
399 *Nature* 473, 174-180.
- 400 Bier, E. (2005). *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nat Rev*  
401 *Genet* 6, 9-23.
- 402 Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G.,  
403 Bienenstock, J., and Cryan, J.F. (2011). Ingestion of *Lactobacillus* strain regulates emotional  
404 behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl*  
405 *Acad Sci U S A* 108, 16050-16055.
- 406 Broderick, N.A., Buchon, N., and Lemaitre, B. (2014). Microbiota-induced changes in *Drosophila*  
407 *melanogaster* host gene expression and gut morphology. *MBio* 5, e01117-01114.
- 408 Broderick, N.A., and Lemaitre, B. (2012). Gut-associated microbes of *Drosophila melanogaster*.  
409 *Gut Microbes* 3, 307-321.
- 410 Canet-Avilés, R.M., Wilson, M.A., Miller, D.W., Ahmad, R., McLendon, C., Bandyopadhyay, S.,  
411 Baptista, M.J., Ringe, D., Petsko, G.A., and Cookson, M.R. (2004). The Parkinson's disease  
412 protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization.  
413 *Proc Natl Acad Sci U S A* 101, 9103-9108.
- 414 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K.,  
415 Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., *et al.* (2010). QIIME allows analysis of  
416 high-throughput community sequencing data. *Nat Methods* 7, 335-336.
- 417 de Oliveira Souza, A., Couto-Lima, C.A., Rosa Machado, M.C., Espreafico, E.M., Pinheiro  
418 Ramos, R.G., and Alberici, L.C. (2017). Protective action of Omega-3 on paraquat  
419 intoxication in *Drosophila melanogaster*. *J Toxicol Environ Health A* 80, 1050-1063.
- 420 Deng, H., Dodson, M.W., Huang, H., and Guo, M. (2008). The Parkinson's disease genes pink1  
421 and parkin promote mitochondrial fission and/or inhibit fusion in *Drosophila*. *Proc Natl Acad*  
422 *Sci U S A* 105, 14503-14508.
- 423 Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R.,  
424 Nelson, K.E., and Relman, D.A. (2005). Diversity of the human intestinal microbial flora.  
425 *Science* 308, 1635-1638.
- 426 Edgecomb, R.S., Harth, C.E., and Schneiderman, A.M. (1994). Regulation of feeding behavior  
427 in adult *Drosophila melanogaster* varies with feeding regime and nutritional state. *J Exp Biol*  
428 197, 215-235.
- 429 Ertürk-Hasdemir, D., Broemer, M., Leulier, F., Lane, W.S., Paquette, N., Hwang, D., Kim, C.H.,  
430 Stöven, S., Meier, P., and Silverman, N. (2009). Two roles for the *Drosophila* IKK complex in  
431 the activation of Relish and the induction of antimicrobial peptide genes. *Proc Natl Acad Sci*  
432 *U S A* 106, 9779-9784.
- 433 Fink, C., Staubach, F., Kuenzel, S., Baines, J.F., and Roeder, T. (2013). Noninvasive analysis  
434 of microbiome dynamics in the fruit fly *Drosophila melanogaster*. *Appl Environ Microbiol* 79,  
435 6984-6988.
- 436 Greene, J.C., Whitworth, A.J., Kuo, I., Andrews, L.A., Feany, M.B., and Pallanck, L.J. (2003).  
437 Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila parkin* mutants.  
438 *Proc Natl Acad Sci U S A* 100, 4078-4083.

- 439 Guo, L., Karpac, J., Tran, S.L., and Jasper, H. (2014). PGRP-SC2 promotes gut immune  
440 homeostasis to limit commensal dysbiosis and extend lifespan. *Cell* 156, 109-122.
- 441 Haley, T.J. (1979). Review of the toxicology of paraquat (1,1'-dimethyl-4,4'-bipyridinium  
442 chloride). *Clin Toxicol* 14, 1-46.
- 443 Hayashi, T., Ishimori, C., Takahashi-Niki, K., Taira, T., Kim, Y.C., Maita, H., Maita, C., Ariga, H.,  
444 and Iguchi-Ariga, S.M. (2009). DJ-1 binds to mitochondrial complex I and maintains its  
445 activity. *Biochem Biophys Res Commun* 390, 667-672.
- 446 He, Z., Kisla, D., Zhang, L., Yuan, C., Green-Church, K.B., and Yousef, A.E. (2007). Isolation  
447 and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and  
448 polymyxin. *Appl Environ Microbiol* 73, 168-178.
- 449 Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow,  
450 J., Reisman, S.E., Petrosino, J.F., *et al.* (2013). Microbiota modulate behavioral and  
451 physiological abnormalities associated with neurodevelopmental disorders. *Cell* 155, 1451-  
452 1463.
- 453 Jin, S.M., Lazarou, M., Wang, C., Kane, L.A., Narendra, D.P., and Youle, R.J. (2010).  
454 Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by  
455 PARL. *J Cell Biol* 191, 933-942.
- 456 Kane, L.A., Lazarou, M., Fogel, A.I., Li, Y., Yamano, K., Sarraf, S.A., Banerjee, S., and Youle,  
457 R.J. (2014). PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J*  
458 *Cell Biol* 205, 143-153.
- 459 Kazlauskaitė, A., Kondapalli, C., Gourlay, R., Campbell, D.G., Ritorto, M.S., Hofmann, K.,  
460 Alessi, D.R., Knebel, A., Trost, M., and Muqit, M.M. (2014). Parkin is activated by PINK1-  
461 dependent phosphorylation of ubiquitin at Ser65. *Biochem J* 460, 127-139.
- 462 Kim, S.H., and Lee, W.J. (2014). Role of DUOX in gut inflammation: lessons from *Drosophila*  
463 model of gut-microbiota interactions. *Front Cell Infect Microbiol* 3, 116.
- 464 Kondapalli, C., Kazlauskaitė, A., Zhang, N., Woodroof, H.I., Campbell, D.G., Gourlay, R.,  
465 Burchell, L., Walden, H., Macartney, T.J., Deak, M., *et al.* (2012). PINK1 is activated by  
466 mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by  
467 phosphorylating Serine 65. *Open Biol* 2, 120080.
- 468 Koyano, F., Okatsu, K., Kosako, H., Tamura, Y., Go, E., Kimura, M., Kimura, Y., Tsuchiya, H.,  
469 Yoshihara, H., Hirokawa, T., *et al.* (2014). Ubiquitin is phosphorylated by PINK1 to activate  
470 parkin. *Nature* 510, 162-166.
- 471 Koyle, M.L., Veloz, M., Judd, A.M., Wong, A.C., Newell, P.D., Douglas, A.E., and Chaston, J.M.  
472 (2016). Rearing the Fruit Fly *Drosophila melanogaster* Under Axenic and Gnotobiotic  
473 Conditions. *J Vis Exp*.
- 474 Lee, K.A., Kim, B., You, H., and Lee, W.J. (2015). Uracil-induced signaling pathways for DUOX-  
475 dependent gut immunity. *Fly (Austin)* 9, 115-120.
- 476 Ma, D., Storelli, G., Mitchell, M., and Leulier, F. (2015). Studying host-microbiota mutualism in  
477 *Drosophila*: Harnessing the power of gnotobiotic flies. *Biomed J* 38, 285-293.
- 478 Manzanillo, P.S., Ayres, J.S., Watson, R.O., Collins, A.C., Souza, G., Rae, C.S., Schneider,  
479 D.S., Nakamura, K., Shiloh, M.U., and Cox, J.S. (2013). The ubiquitin ligase parkin mediates  
480 resistance to intracellular pathogens. *Nature* 501, 512-516.

- 481 Marsh, J.L., and Thompson, L.M. (2006). *Drosophila* in the study of neurodegenerative disease.  
482 Neuron 52, 169-178.
- 483 Martinat, C., Shendelman, S., Jonason, A., Leete, T., Beal, M.F., Yang, L., Floss, T., and  
484 Abeliovich, A. (2004). Sensitivity to oxidative stress in DJ-1-deficient dopamine neurons: an  
485 ES- derived cell model of primary Parkinsonism. PLoS Biol 2, e327.
- 486 Mayer, E.A., Savidge, T., and Shulman, R.J. (2014). Brain-gut microbiome interactions and  
487 functional bowel disorders. Gastroenterology 146, 1500-1512.
- 488 Meissner, C., Lorenz, H., Weihofen, A., Selkoe, D.J., and Lemberg, M.K. (2011). The  
489 mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1  
490 trafficking. J Neurochem 117, 856-867.
- 491 Meulener, M., Whitworth, A.J., Armstrong-Gold, C.E., Rizzu, P., Heutink, P., Wes, P.D.,  
492 Pallanck, L.J., and Bonini, N.M. (2005). *Drosophila* DJ-1 mutants are selectively sensitive to  
493 environmental toxins associated with Parkinson's disease. Curr Biol 15, 1572-1577.
- 494 Müller-Rischart, A.K., Pilsl, A., Beaudette, P., Patra, M., Hadian, K., Funke, M., Peis, R.,  
495 Deinlein, A., Schweimer, C., Kuhn, P.H., *et al.* (2013). The E3 ligase parkin maintains  
496 mitochondrial integrity by increasing linear ubiquitination of NEMO. Mol Cell 49, 908-921.
- 497 Myllymäki, H., Valanne, S., and Rämet, M. (2014). The *Drosophila* imd signaling pathway. J  
498 Immunol 192, 3455-3462.
- 499 Narendra, D.P., Jin, S.M., Tanaka, A., Suen, D.F., Gautier, C.A., Shen, J., Cookson, M.R., and  
500 Youle, R.J. (2010). PINK1 is selectively stabilized on impaired mitochondria to activate  
501 Parkin. PLoS Biol 8, e1000298.
- 502 Nenci, A., Becker, C., Wullaert, A., Gareus, R., van Loo, G., Danese, S., Huth, M., Nikolaev, A.,  
503 Neufert, C., Madison, B., *et al.* (2007). Epithelial NEMO links innate immunity to chronic  
504 intestinal inflammation. Nature 446, 557-561.
- 505 Park, J., Lee, S.B., Lee, S., Kim, Y., Song, S., Kim, S., Bae, E., Kim, J., Shong, M., Kim, J.M., *et*  
506 *al.* (2006). Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by  
507 parkin. Nature 441, 1157-1161.
- 508 Pesah, Y., Pham, T., Burgess, H., Middlebrooks, B., Verstreken, P., Zhou, Y., Harding, M.,  
509 Bellen, H., and Mardon, G. (2004). *Drosophila* parkin mutants have decreased mass and  
510 cell size and increased sensitivity to oxygen radical stress. Development 131, 2183-2194.
- 511 Pickrell, A.M., and Youle, R.J. (2015). The roles of PINK1, parkin, and mitochondrial fidelity in  
512 Parkinson's disease. Neuron 85, 257-273.
- 513 Poole, A.C., Thomas, R.E., Andrews, L.A., McBride, H.M., Whitworth, A.J., and Pallanck, L.J.  
514 (2008). The PINK1/Parkin pathway regulates mitochondrial morphology. Proc Natl Acad Sci  
515 U S A 105, 1638-1643.
- 516 Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N.,  
517 Levenez, F., Yamada, T., *et al.* (2010). A human gut microbial gene catalogue established  
518 by metagenomic sequencing. Nature 464, 59-65.
- 519 Ren, C., Webster, P., Finkel, S.E., and Tower, J. (2007). Increased internal and external  
520 bacterial load during *Drosophila* aging without life-span trade-off. Cell Metab 6, 144-152.

- 521 Rutschmann, S., Jung, A.C., Zhou, R., Silverman, N., Hoffmann, J.A., and Ferrandon, D.  
522 (2000). Role of *Drosophila* IKK gamma in a toll-independent antibacterial immune response.  
523 *Nat Immunol* 1, 342-347.
- 524 Sampson, T.R., Debelius, J.W., Thron, T., Janssen, S., Shastri, G.G., Ilhan, Z.E., Challis, C.,  
525 Schretter, C.E., Rocha, S., Gradinaru, V., *et al.* (2016). Gut Microbiota Regulate Motor  
526 Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* 167, 1469-1480  
527 e1412.
- 528 Sarraf, S.A., Raman, M., Guarani-Pereira, V., Sowa, M.E., Huttlin, E.L., Gygi, S.P., and Harper,  
529 J.W. (2013). Landscape of the PARKIN-dependent ubiquitylome in response to  
530 mitochondrial depolarization. *Nature* 496, 372-376.
- 531 Scheperjans, F., Aho, V., Pereira, P.A., Koskinen, K., Paulin, L., Pekkonen, E., Haapaniemi, E.,  
532 Kaakkola, S., Eerola-Rautio, J., Pohja, M., *et al.* (2015). Gut microbiota are related to  
533 Parkinson's disease and clinical phenotype. *Mov Disord* 30, 350-358.
- 534 Sharon, G., Sampson, T.R., Geschwind, D.H., and Mazmanian, S.K. (2016). The Central  
535 Nervous System and the Gut Microbiome. *Cell* 167, 915-932.
- 536 Shiba-Fukushima, K., Imai, Y., Yoshida, S., Ishihama, Y., Kanao, T., Sato, S., and Hattori, N.  
537 (2012). PINK1-mediated phosphorylation of the Parkin ubiquitin-like domain primes  
538 mitochondrial translocation of Parkin and regulates mitophagy. *Sci Rep* 2, 1002.
- 539 Shiba-Fukushima, K., Inoshita, T., Hattori, N., and Imai, Y. (2014). PINK1-mediated  
540 phosphorylation of Parkin boosts Parkin activity in *Drosophila*. *PLoS Genet* 10, e1004391.
- 541 Shin, S.C., Kim, S.H., You, H., Kim, B., Kim, A.C., Lee, K.A., Yoon, J.H., Ryu, J.H., and Lee,  
542 W.J. (2011). *Drosophila* microbiome modulates host developmental and metabolic  
543 homeostasis via insulin signaling. *Science* 334, 670-674.
- 544 Taira, T., Saito, Y., Niki, T., Iguchi-Ariga, S.M., Takahashi, K., and Ariga, H. (2004). DJ-1 has a  
545 role in antioxidative stress to prevent cell death. *EMBO Rep* 5, 213-218.
- 546 Téfit, M.A., and Leulier, F. (2017). *Lactobacillus plantarum* favors the early emergence of fit and  
547 fertile adult *Drosophila* upon chronic undernutrition. *J Exp Biol* 220, 900-907.
- 548 Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007). Naive Bayesian classifier for rapid  
549 assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* 73,  
550 5261-5267.
- 551 Wong, A.C., Chaston, J.M., and Douglas, A.E. (2013). The inconstant gut microbiota of  
552 *Drosophila* species revealed by 16S rRNA gene analysis. *ISME J* 7, 1922-1932.
- 553 Wong, A.C., Luo, Y., Jing, X., Franzenburg, S., Bost, A., and Douglas, A.E. (2015). The Host as  
554 the Driver of the Microbiota in the Gut and External Environment of *Drosophila*  
555 *melanogaster*. *Appl Environ Microbiol* 81, 6232-6240.
- 556 Wong, M.L., Inserra, A., Lewis, M.D., Mastronardi, C.A., Leong, L., Choo, J., Kentish, S., Xie,  
557 P., Morrison, M., Wesselingh, S.L., *et al.* (2016). Inflammasome signaling affects anxiety-  
558 and depressive-like behavior and gut microbiome composition. *Mol Psychiatry* 21, 797-805.
- 559
- 560



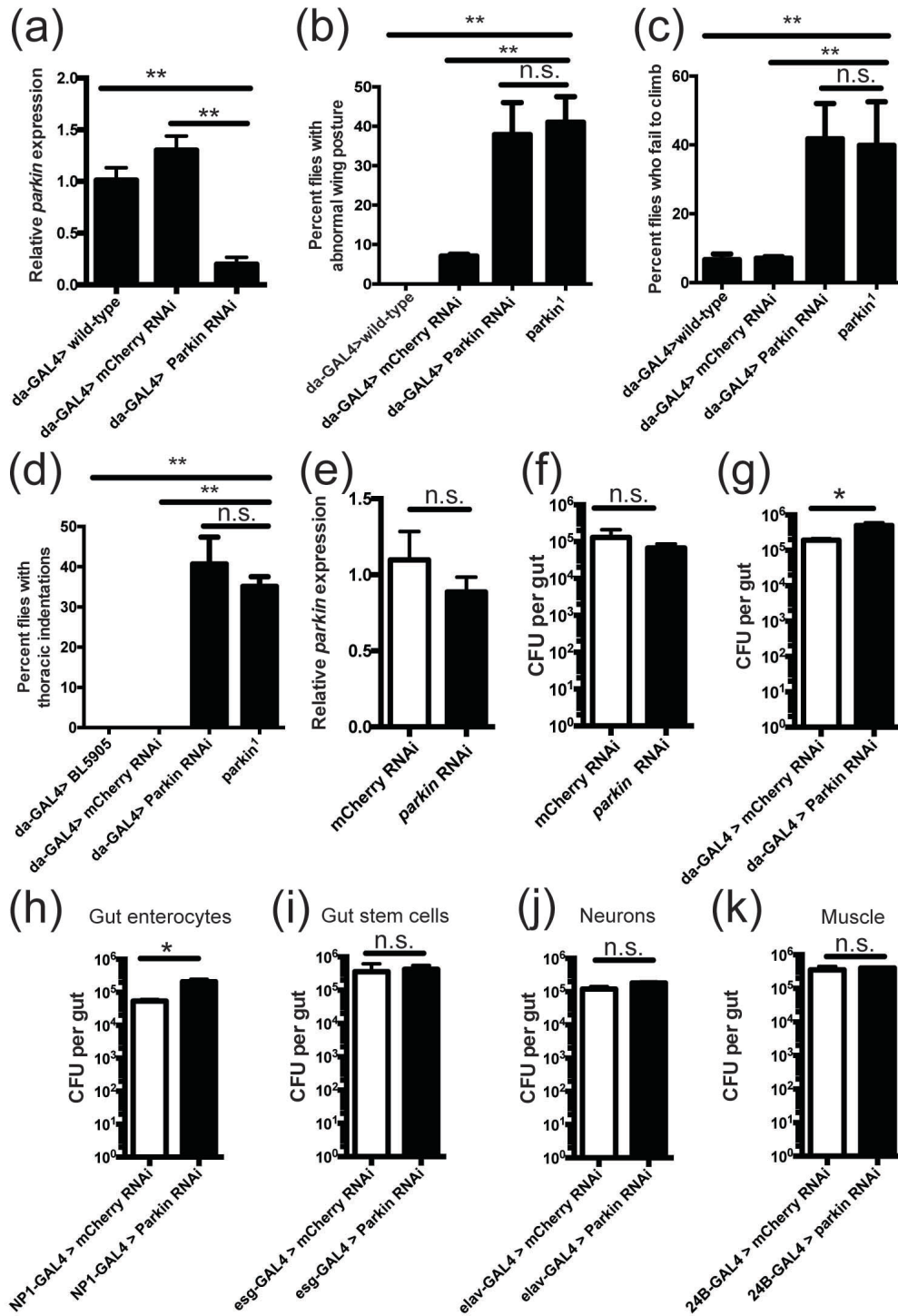
561

562 **Figure 1. *parkin* mutants exhibit an elevated microbial load with age.** (A) Microbial load of  
563 wild-type (*w<sup>1118</sup>*) males and male mutants for parkinsonism-associated genes at ages 3d and 20d.  
564 Dissected and homogenized individual guts were serially diluted and a fraction of the diluted  
565 homogenate was spread on MRS-agar plates. Colonies grown were counted and used to calculate  
566 the colony forming units (CFU) per gut. The experiment was repeated in four independent  
567 biological replicates of six individual guts each per age and genotype. DJ1 DKO stands for DJ1  
568 double knockout: *DJ-1 $\alpha$ <sup>472</sup>*; *DJ-1 $\beta$ <sup>493</sup>*. \*\**p*<0.01, ANOVA for significance, followed by Tukey's  
569 post-test. Comparisons not marked with a double asterisk (\*\*) are not statistically significant. (B)  
570 Blue-dye feeding assay to measure volume of food in the gut of wild-type (*w<sup>1118</sup>*) and *parkin<sup>1</sup>*  
571 mutant males at 3d and 20d. Flies were placed on food containing 2.5% w/v FD&C blue dye #1  
572 for 48 hr. Five guts per genotype/age group were dissected in PBS, homogenized, and the  
573 absorbance at 630 nm was measured. The experiment was repeated in three independent  
574 biological replicates. n.s. not significant, ANOVA followed by Tukey's post-test. (C) Example  
575 image of prints left by the fly proboscis on a 1% gelatin-, 5% sucrose- coated slide. (D)  
576 Proboscis print assay to measure the rate of feeding of wild-type (*w<sup>1118</sup>*) and *parkin<sup>1</sup>* mutant  
577 males at 3d and 20d. Animals were enclosed in individual chambers on top of a 1% gelatin-, 5%  
578 sucrose- coated slide and incubated for 20 min without disturbance. The number of proboscis  
579 prints left on the surface of the slide was counted. The experiment was repeated in ten  
580 independent biological replicates of ten individual flies each per age and genotype. \*\**p*<0.01,  
581 ANOVA followed by Tukey's post-test.

582

583





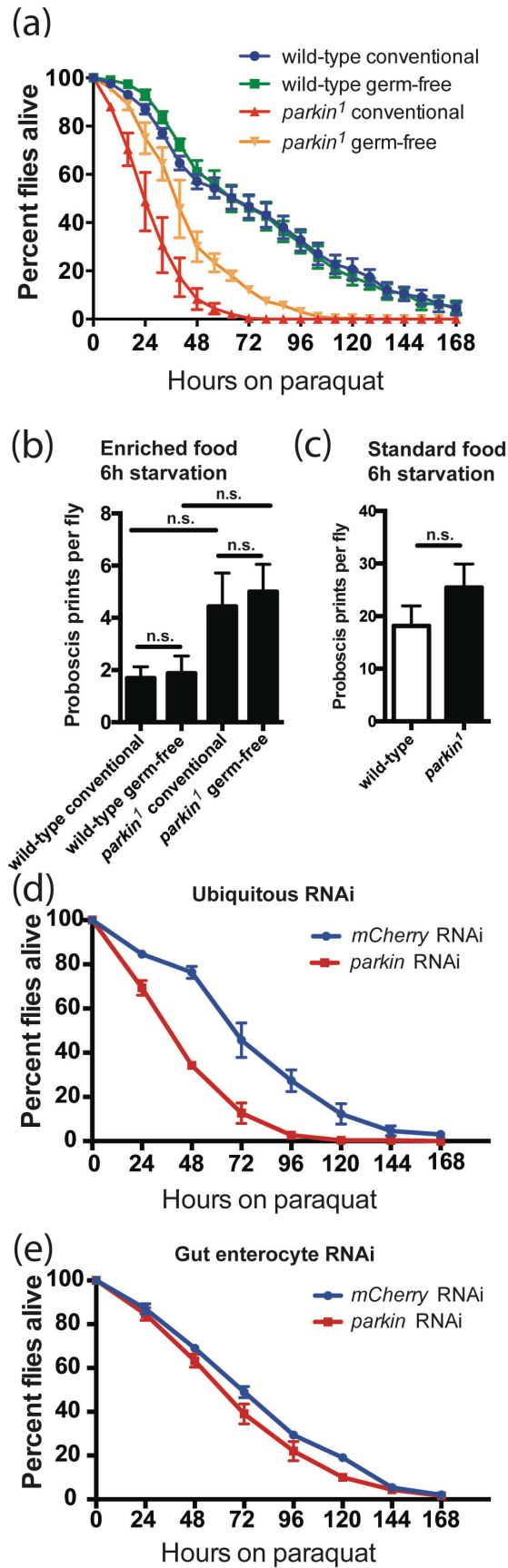
584

585

586 **Figure 2. Parkin is required in gut enterocytes to maintain microbial homeostasis. (A-D)**  
587 Validation of effective knockdown of *parkin* by in vivo expression of *siRNA* hairpin. (A) Real-  
588 time PCR for *parkin* in total RNA from whole 7d males expressing no hairpin, a hairpin against  
589 mCherry, or a hairpin against *parkin*. \*\* $p < 0.01$ , ANOVA followed by Tukey's post test. (B)  
590 Fraction of flies exhibiting abnormal wing posture among 7d males expressing no hairpin, a  
591 hairpin against mCherry, or the hairpin against *parkin*, compared to *parkin*<sup>1</sup> flies. Flies were aged  
592 on standard food and the number of animals with held-up wings was counted. The experiment  
593 was conducted in biological triplicate using 55-60 flies per replicate. \*\* $p < 0.01$ , n.s. – not  
594 significant, ANOVA followed by Tukey's post test. (C) Climbing assay of 7d males expressing  
595 no hairpin, a hairpin against mCherry, or the hairpin against *parkin*, as well as *parkin*<sup>1</sup> null flies.  
596 Flies were aged on standard food and placed in empty vials. The number of animals that climbed  
597 to the top of the vial 10s after tapping was recorded. Experiment was repeated in 3 independent  
598 biological replicates with 55-60 flies per replicate. \*\* $p < 0.01$ , n.s. – not significant, ANOVA  
599 followed by Tukey's post test. (D) Fraction of flies exhibiting thoracic indentations among 7d  
600 males expressing no hairpin, a hairpin against mCherry, or the hairpin against *parkin*, compared  
601 to *parkin*<sup>1</sup> flies. Flies were aged on standard food and the number of animals with a collapsed  
602 thorax was counted. Experiment was repeated in 3 independent biological replicates with 55-60  
603 flies per replicate. \*\* $p < 0.01$ , n.s. – not significant, ANOVA followed by Tukey's post test. (E)  
604 Real-time PCR for *parkin* in total RNA from whole 7d control or *parkin* RNAi males, in which  
605 the UAS-hairpin line was crossed to a wild-type line with no driver. n.s. – not significant,  
606 Student's t-test. (F) Gut microbial load of 20d control or *parkin* RNAi males, in which the UAS-  
607 hairpin line was crossed to a wild-type line with no driver. n.s. – not significant, Student's t-test.  
608 (G) Gut dissection followed by live colony counting in flies expressing mCherry or *parkin* RNAi  
609 ubiquitously. The gut dissection procedure was as in Fig 1A. The experiment was repeated in  
610 four independent biological replicates of six individual guts each per age and genotype. \* $p < 0.05$ ,  
611 Student's t-test. (H-K) Microbial load in guts of 20d control or *parkin* RNAi males, in which  
612 knockdown was carried out selectively in (H) gut enterocytes, (I) gut stem cells, (J) neurons, or  
613 (K) muscle cells with indicated GAL4 drivers. Guts were dissected as in Fig 1A. The experiment  
614 was repeated in four independent biological replicates of six individual guts each per age and  
615 genotype. \* $p < 0.05$ , n.s. – not significant, Student's t-test.

616

617

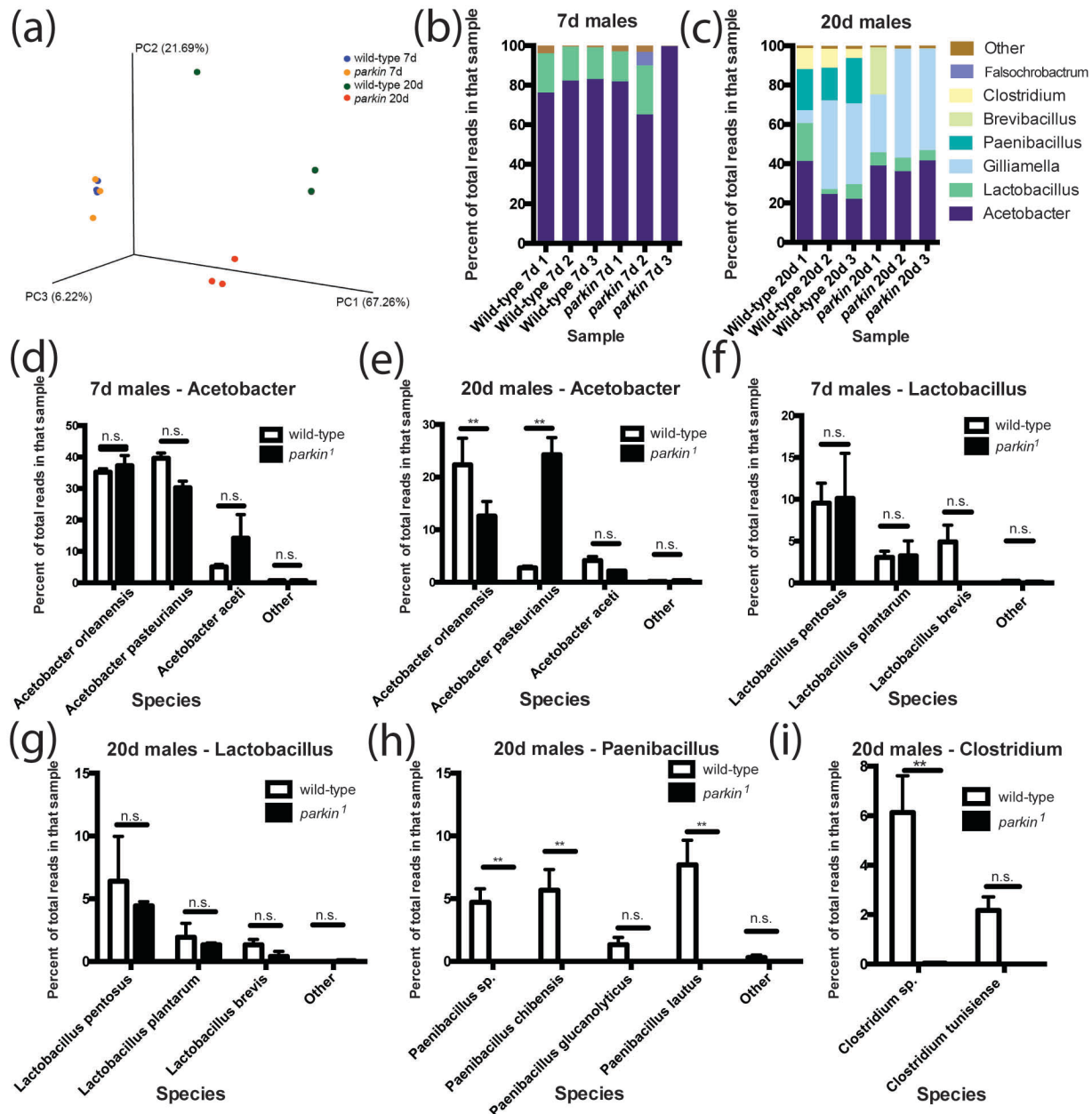


619 **Figure 3. Absence of the gut microbiota affects paraquat sensitivity of *parkin* mutants. (A)**  
620 Survival curve on 20 mM paraquat of 0-3d conventional or germ free wild-type and *parkin*<sup>1</sup>  
621 mutant males. 100 animals per treatment and genotype were starved for 6h then placed on 10%  
622 sucrose-, 2.5% agar- food containing 20 mM paraquat. Survival was measured every 8h for 168h  
623 (over 7d). The experiment was repeated in three independent biological replicates. *parkin*  
624 conventional and germ-free animals had significantly different survival curves to one another  
625 and to their respective wild-type controls (p<0.0001, Log-Rank test).

626 . **(B)** Proboscis print assay to measure the rate of feeding of conventionally reared and germ-free  
627 wild-type and *parkin*<sup>1</sup> mutant males at ages 0-3d. Assay was carried out as in Fig 1 but with flies  
628 grown on food supplemented with 100 g/L yeast and starved for 6h prior to the assay. n.s. - not  
629 significant, ANOVA followed by Tukey post-test. **(C)** Proboscis print assay to measure the rate  
630 of feeding of control and *parkin*<sup>1</sup> mutant males at ages 0-3d grown on standard fly food and  
631 starved for 6h prior to the assay. n.s. - not significant, Student's t-test. **(D-E)** Paraquat sensitivity  
632 assays with 0-3d control or *parkin* RNAi males, in which knockdown was carried out  
633 ubiquitously (D, da-GAL4 driver) or selectively in gut enterocytes (D, NP1-GAL4 driver).  
634 Paraquat sensitivity assays were carried out as in Fig 3A. The experiment was repeated in three  
635 independent biological replicates of 100 individual animals each per experimental group. (D)  
636 \*\*\*p<0.0001, log-rank test. (E) Not significant, log-rank test.

637

638



639

640 **Figure 4. *parkin* loss of function affects gut microbial composition.** 16S rDNA amplicon  
 641 sequencing of guts from 7d and 20d wild-type and *parkin* males. (A) Principle coordinate  
 642 analysis shows similar microbial composition at age 7d, but at age 20d compositions of the gut  
 643 microbiota of wild-type and *parkin*<sup>1</sup> mutant diverge. (B-C) Most common genera (defined as  
 644 more than 5% of total reads in at least one sample) in (B) 7d male guts and (C) 20d male guts.  
 645 (D-I) Relative abundance (measured as percentage of total reads in that sample) of *Acetobacter*  
 646 species detected in (D) 7d males and (E) 20d males, *Lactobacillus* species detected in (F) 7d  
 647 males and (G) 20d males, *Paenibacillus* species detected in (H) 20d males, and *Clostridium*  
 648 species detected in (I) 20d males. \*\*p < 0.01, n.s. – not significant, Student's t-test with Holm-  
 649 Sidak correction for multiple testing.

650

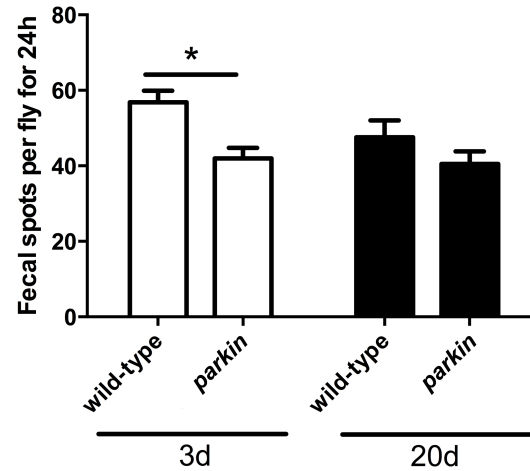
651 **Supplementary Table 1**

	Simpson index	Shannon index	Chao1	PD whole tree
Wild-type 7d	0.71±0.02	2.36±0.12	327.78±36.57	10.81±1.06
<i>parkin</i> 7d	0.72±0.03	2.31±0.29	299.20±71.22	10.81±1.06
p-value	0.69	0.91	0.74	0.82
Wild-type 20d	0.80±0.03	3.18±0.13	379.29±29.14	11.74±0.61
<i>parkin</i> 20d	0.69±0.06	2.34±0.33	327.72±8.44	10.69±0.54
p-value	0.24	0.11	0.21	0.27

Multiple metrics were used to best capture and compare the biodiversity that exists between the different gut bacterial communities. Diversity indices of wild-type and *parkin* 7d and 20d gut microbiomes using measures available from QIIME (Caporaso et al., 2010). The Simpson index is used to measure species richness (number of different species) and the Shannon index is used to measure species evenness (relative abundance of species). The Chao1 metric is used to analyze data sets with low-abundance classes. PD whole tree assesses phylogenetic diversity. Values are means ± SEM, n=3. *p*-values were calculated using Student's *t*-test comparing wild-type and *parkin* values at the specified age. The *p*-values are all >.05 demonstrating no significant differences between *parkin* and wild-type flies.

652

653



654

655

656

657 **Supplementary Figure 1: Decreased defecation rate in young *parkin* male guts.** Cohorts of 40 males  
658 were incubated on fly food containing FD&C Blue Dye #1 for 24h. Flies were transferred to fresh blue  
659 food vials, and after another 24h incubation period, the number of blue fecal spots on the walls of the  
660 vials were counted. The experiment was repeated in four independent biological replicates. \* $p < 0.05$ ,  
661 ANOVA with Tukey's post test.

662

663

664

665

666

```
VF12_6          GAGTTTGGATGATGGCTCAGAGCGAACGCTGGCGGCATGCTTAACACATGCAAGTCGCAC
NR_028614.1-Acetobacter_orleanensis -GAGTTTGGATCCTGGCTCAGAGCGAACGCTGGCGGCATGCTTAACACATGCAAGTCGCAC
VF12_41        GAGTTTGGATGATGGCTCAGAGCGAACGCTGGCGGCATGCTTAACACATGCAAGTCGCAC
NR_117457.1-Acetobacter_pasteurianus GAGTTTGGATGATGGCTCAGAGCGAACGCTGGCGGCATGCTTAACACATGCAAGTCGCAC
VF12_20943    GAGTTTGGATGATGGCTCAGAGCGAACGCTGGCGGCATGCTTAACACATGCAAGTCGCAC
NR_026121.1-Acetobacter_aceti      GAGTTTGGATGATGGCTCAGAGCGAACGCTGGCGGCATGCTTAACACATGCAAGTCGCAC
*****

VF12_6          GAAGGTTTCGGCCTTAGTGGCGGACGGGTGAGTAACGCGTAGGATCTATCCAAGGGTGG
NR_028614.1-Acetobacter_orleanensis GAAGGTTTCGGCCTTAGTGGCGGACGGGTGAGTAACGCGTAGGATCTATCCAAGGGTGG
VF12_41        GAAGGTTTCGGCCTTAGTGGCGGACGGGTGAGTAACGCGTAGGATCTATCCAAGGGTGG
NR_117457.1-Acetobacter_pasteurianus GAAGGTTTCGGCCTTAGTGGCGGACGGGTGAGTAACGCGTAGGATCTATCCAAGGGTGG
VF12_20943    GAAGGTTTCGGCCTTAGTGGCGGACGGGTGAGTAACGCGTAGGATCTATCCAAGGGTGG
NR_026121.1-Acetobacter_aceti      GAAGGTTTCGGCCTTAGTGGCGGACGGGTGAGTAACGCGTAGGATCTATCCAAGGGTGG
*****

VF12_6          GGGATAACTCCGGAAACTGGAGCTAATACCGCATGATACCTGAGGGTCAAAGGCCAAG
NR_028614.1-Acetobacter_orleanensis GGGATAACTCCGGAAACTGGAGCTAATACCGCATGATACCTGAGGGTCAAAGGCCAAG
VF12_41        GGGATAACTCCGGAAACTGGAGCTAATACCGCATGATACCTGAGGGTCAAAGGCCAAG
NR_117457.1-Acetobacter_pasteurianus GGGATAACTCCGGAAACTGGAGCTAATACCGCATGATACCTGAGGGTCAAAGGCCAAG
VF12_20943    GGGATAACTCCGGAAACTGGAGCTAATACCGCATGATACCTGAGGGTCAAAGGCCAAG
NR_026121.1-Acetobacter_aceti      GGGATAACTCCGGAAACTGGAGCTAATACCGCATGATACCTGAGGGTCAAAGGCCAAG
*****

VF12_6          TCGCCTGTGGAGGAGCTGCGTTTGATTAGCTGTGGTGGGTAAAGGCCTACCAAGGC
NR_028614.1-Acetobacter_orleanensis TCGCCTGTGGAGGAGCTGCGTTTGATTAGCTGTGGTGGGTAAAGGCCTACCAAGGC
VF12_41        TCGCCTGTGGAGGAGCTGCGTTTGATTAGCTGTGGTGGGTAAAGGCCTACCAAGGC
NR_117457.1-Acetobacter_pasteurianus TCGCCTGTGGAGGAGCTGCGTTTGATTAGCTGTGGTGGGTAAAGGCCTACCAAGGC
VF12_20943    TCGCCTGTGGAGGAGCTGCGTTTGATTAGCTGTGGTGGGTAAAGGCCTACCAAGGC
NR_026121.1-Acetobacter_aceti      TCGCCTGTGGAGGAGCTGCGTTTGATTAGCTGTGGTGGGTAAAGGCCTACCAAGGC
*****

VF12_6          GATGATCAATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAG
NR_028614.1-Acetobacter_orleanensis GATGATCAATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAG
VF12_41        GATGATCAATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAG
NR_117457.1-Acetobacter_pasteurianus GATGATCAATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAG
VF12_20943    GATGATCAATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAG
NR_026121.1-Acetobacter_aceti      GATGATCAATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCAG
*****

VF12_6          ACTCCTACGGGAGGCAGCA
NR_028614.1-Acetobacter_orleanensis ACTCCTACGGGAGGCAGCA
VF12_41        ACTCCTACGGGAGGCAGCA
NR_117457.1-Acetobacter_pasteurianus ACTCCTACGGGAGGCAGCA
VF12_20943    ACTCCTACGGGAGGCAGCA
NR_026121.1-Acetobacter_aceti      ACTCCTACGGGAGGCAGCA
*****
```

667

668

669

670 **Supplementary Figure 2: Alignment between sequenced *Acetobacter* 16S rDNA amplicons and best**  
671 **matches from BLAST search.** Reads were fetched from the set of representative sequences for each  
672 OTU and BLAST searched against the NCBI 16S rDNA sequence database. Three pairs of reads and their  
673 BLAST top hit were aligned using Clustal Omega. Mismatching nucleotides that can be used to  
674 differentiate between species are highlighted in red. VF12\_6 was identified as *Acetobacter orleanensis*.  
675 VF12\_41 was identified as *Acetobacter pasteurianus*. VF12\_20943 was identified as *Acetobacter aceti*.

676

677

678

679

680

681





697

```
VF32_4730694 AGAGTTTGATCATGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGC
NR_115623.1-Paenibacillus_chibensis -----CGTGCCTAATACATGCAAGTCGAGC
VF32_5232385 AGAGTTTGATCATGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGC
NR_040883.1-Paenibacillus_glucanolyticus -----GATCATGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGC
VF32_4749292 AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGC
NR_115599.1-Paenibacillus_lautus AGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGC
*****
```

```
VF32_4730694 GGACTTGAAGAGGTGCTTGCACTCTGATACCTAGCGGCGGACGGGTGAGTAACACGTAG
NR_115623.1-Paenibacillus_chibensis GGACTTGAAGAGGTGCTTGCACTCTGATACCTAGCGGCGGACGGGTGAGTAACACGTAG
VF32_5232385 GGACTTGAAGAGGTGCTTGCACTCTGATACCTAGCGGCGGACGGGTGAGTAACACGTAG
NR_040883.1-Paenibacillus_glucanolyticus GGACTTGAAGAGGTGCTTGCACTCTGATACCTAGCGGCGGACGGGTGAGTAACACGTAG
VF32_4749292 GGACTTGAAGAGGTGCTTGCACTCTGATACCTAGCGGCGGACGGGTGAGTAACACGTAG
NR_115599.1-Paenibacillus_lautus GGACTTGAAGAGGTGCTTGCACTCTGATACCTAGCGGCGGACGGGTGAGTAACACGTAG
*** ** * ***** ** * *****
```

```
VF32_4730694 GAAACCTGCCCTGAAGACTGGGATAACTACCGGAAACGGTAGCTAATACCGGATAATTTA
NR_115623.1-Paenibacillus_chibensis GAAACCTGCCCTGAAGACTGGGATAACTACCGGAAACGGTAGCTAATACCGGATAATTTA
VF32_5232385 GAAACCTGCCCTGAAGACTGGGATAACTACCGGAAACGGTAGCTAATACCGGATAATTTA
NR_040883.1-Paenibacillus_glucanolyticus GAAACCTGCCCTGAAGACTGGGATAACTACCGGAAACGGTAGCTAATACCGGATAATTTA
VF32_4749292 GAAACCTGCCCTGAAGACTGGGATAACTACCGGAAACGGTAGCTAATACCGGATAATTTA
NR_115599.1-Paenibacillus_lautus GAAACCTGCCCTGAAGACTGGGATAACTACCGGAAACGGTAGCTAATACCGGATAATTTA
* *****
```

```
VF32_4730694 TTTCCTTCCTGGAGATAATGAAAGACGGAGCAATCTGTCACCTACAGATGGCCTGC
NR_115623.1-Paenibacillus_chibensis TTTCCTTCCTGGAGATAATGAAAGACGGAGCAATCTGTCACCTACAGATGGCCTGC
VF32_5232385 TTACATGCACTAATGATAATGAAAGACGGAGCAATCTGTCACCTACAGATGGCCTGC
NR_040883.1-Paenibacillus_glucanolyticus TTACATGCACTAATGATAATGAAAGACGGAGCAATCTGTCACCTACAGATGGCCTGC
VF32_4749292 TTTCCTTCCTGGAGATAATGAAAGACGGAGCAATCTGTCACCTACAGATGGCCTGC
NR_115599.1-Paenibacillus_lautus TTTCCTTCCTGGAGATAATGAAAGACGGAGCAATCTGTCACCTACAGATGGCCTGC
** * * * ***** ** * *****
```

```
VF32_4730694 GGCGCATTAGCTAGTTGGTGGGTAAAGGCACCAAGGCGACGATGCGTAGCCGACCTG
NR_115623.1-Paenibacillus_chibensis GGCGCATTAGCTAGTTGGTGGGTAAAGGCACCAAGGCGACGATGCGTAGCCGACCTG
VF32_5232385 GGCGCATTAGCTAGTTGGTGGGTAAAGGCACCAAGGCGACGATGCGTAGCCGACCTG
NR_040883.1-Paenibacillus_glucanolyticus GGCGCATTAGCTAGTTGGTGGGTAAAGGCACCAAGGCGACGATGCGTAGCCGACCTG
VF32_4749292 GGCGCATTAGCTAGTTGGTGGGTAAAGGCACCAAGGCGACGATGCGTAGCCGACCTG
NR_115599.1-Paenibacillus_lautus GGCGCATTAGCTAGTTGGTGGGTAAAGGCACCAAGGCGACGATGCGTAGCCGACCTG
*****
```

```
VF32_4730694 AGAGGGTGAACGGCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAG--
NR_115623.1-Paenibacillus_chibensis AGAGGGTGAACGGCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCA
VF32_5232385 AGAGGGTGAACGGCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCA
NR_040883.1-Paenibacillus_glucanolyticus AGAGGGTGAACGGCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCA
VF32_4749292 AGAGGGTGAACGGCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAG--
NR_115599.1-Paenibacillus_lautus AGAGGGTGAACGGCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCA
*****
```

698

699

700

701 **Supplementary Figure 4: Alignment between sequenced *Paenibacillus* 16S rDNA amplicons and**  
702 **best matches from BLAST search.** Reads were fetched from the set of representative sequences for each  
703 OTU and BLAST searched against the NCBI 16S rDNA sequence database. Three pairs of reads and their  
704 BLAST top hit were aligned using Clustal Omega. Mismatching nucleotides that can be used to  
705 differentiate between species are highlighted in red. VF32\_4730694 was identified as *Paenibacillus*  
706 *chibensis*. VF32\_5232385 was identified as *Paenibacillus glucanolyticus*. VF32\_4749292 was identified  
707 as *Paenibacillus lautus*.

708

709

710