

1 **Common proanthocyanidin-rich foods modulate gastrointestinal blooms of *Akkermansia***
2 ***muciniphila* in a diet-dependent manner**

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10

11 **Summary**

12 Developing methods to modulate growth of the mucin-degrading gut bacterium *Akkermansia muciniphila*
13 could benefit patients with different health needs, as *A. muciniphila* has been associated with both positive
14 metabolic health outcomes and detrimental neurodegenerative outcomes. Growth of *A. muciniphila* is
15 sensitive to plant-derived polyphenols, and particularly proanthocyanidins (PACs), when administered in
16 isolated form at supraphysiological doses. However, it remains unclear whether doses sufficient for these
17 effects are achievable via diet. Here, we explore the extent to which nutritionally relevant doses of common
18 polyphenol-rich foods – berries, wine, and coffee – influence *A. muciniphila* abundance in C57BL/6J mice
19 under varying dietary conditions. By administering polyphenol-rich whole foods, comparing polyphenol-
20 depleted and PAC-rich versus PAC-poor food supplements, and through gradient PAC-dosing experiments,
21 we show that PAC-rich foods uniquely induce *A. muciniphila* growth at doses that are feasibly achieved
22 through routine diet. Notably, the effects of PAC supplementation were detected against a high-fat diet but
23 not a low-fat control diet background, highlighting the importance of habitual diet strategies in either
24 amplifying or mitigating the prebiotic effects of PAC-rich food consumption. Ultimately, our work suggests
25 that both PACs and diet influence *A. muciniphila* abundance with downstream impacts for human health.

26 Introduction

27 Given compelling links between the gut microbiota and numerous aspects of human health, there is great
28 potential in developing therapies that target the gut microbial community. Although the gut microbiome
29 and its interactions with host physiology are highly sensitive to diet (Carmody et al., 2015; Chadaideh and
30 Carmody, 2021; David et al., 2014; Sonnenburg et al., 2016; Turnbaugh et al., 2010), the complex network
31 of microbial relationships in the gut often results in interventions having unpredictable or difficult-to-
32 replicate outcomes. Therefore, there is a pressing need to identify interventions that lead to predictable
33 increases or decreases in specific microbial taxa implicated as causal agents in specific health phenotypes.

34 One candidate intervention targeting the gut microbiota is the administration of diet-derived
35 polyphenols, which have been associated with lowered risks of chronic metabolic and inflammatory
36 diseases (Del Bo et al., 2019; Domínguez-Avila et al., 2020; Halliwell, 2007). Specifically, the subclass of
37 proanthocyanidins (PACs), which are responsible for the bitter taste of berries and found in wine and other
38 tannin-rich foods (Gu et al., 2004), have been strongly associated with attenuating obesity, insulin
39 resistance, and systemic inflammation in mice (Anhê et al., 2015; Dixon et al., 2004; Roopchand et al.,
40 2015; Zhang et al., 2018), as well as reducing cardiovascular disease and diabetes risk in humans (Blumberg
41 et al., 2013; Del Bo et al., 2019; Gu et al., 2004).

42 The mechanisms by which polyphenols confer beneficial metabolic effects are unclear, but their
43 role in cardiometabolic protection may at least partially derive from changes in the gut microbiota, and
44 specifically, increased abundance of the bacterial species *Akkermansia muciniphila* (Anhê et al., 2015;
45 Everard et al., 2013; Kim et al., 2020; Régnier et al., 2020; Roopchand et al., 2015). In studies investigating
46 polyphenol supplementation in mice, administration of isolated PACs at levels representing 1% of the diet
47 by weight was shown to increase the relative abundance of *A. muciniphila* from <2% to >40% within days
48 (Anhê et al., 2015; Roopchand et al., 2015). Interestingly, *A. muciniphila* blooms in mice preceded changes
49 in host gene expression relevant to low-grade systemic inflammation (Everard et al., 2013; Zhang et al.,
50 2018), and correlated with reduced adiposity and insulin insensitivity on a high-fat diet (Anhê et al., 2015;
51 Roopchand et al., 2015; Zhang et al., 2018). *A. muciniphila* enrichment was also recently shown to improve
52 metabolic health in a randomized double-blind placebo-controlled study in overweight/obese insulin-
53 resistant human subjects (Depommier et al., 2019). And while the specific mechanisms through which
54 polyphenol-induced enrichment of *A. muciniphila* improves metabolic health also remain unknown, *A.*
55 *muciniphila* secretes the protein P9, which was recently found to be sufficient to induce glucagon-like
56 peptide-1 (GLP-1) secretion and brown fat thermogenesis in mice fed a high-fat diet (Yoon et al., 2021).

57 Despite evidence that *A. muciniphila* enrichment improves metabolic health, increased abundance
58 of this microbe does not appear to be universally advantageous. For example, investigations into the role
59 of the gut-brain axis in neurological function have revealed correlations between *A. muciniphila* and

60 neurodegenerative disease (Fang et al., 2020). Recent studies investigating the gut microbiota of
61 Parkinson's patients demonstrated a link between increased relative abundance of the genus *Akkermansia*
62 and a progression of symptoms (Haikal et al., 2019; Heintz-Buschart et al., 2018; Romano et al., 2021;
63 Schepersjans et al., 2015; Vidal-Martinez et al., 2020), and *A. muciniphila* has been suggested as a biomarker
64 for diagnosing Parkinson's patients relative to healthy age-matched controls (Bedarf et al., 2017). One
65 explanation for the diverging roles of *A. muciniphila* may be that the genomic diversity of *A. muciniphila*
66 has yet to be fully characterized. Recent work using metagenome-assembled genomes (MAGs) has
67 identified distinct subspecies-level genomic variation in *A. muciniphila* that may be associated with
68 differences in functional capacity (Karcher et al., 2021). Furthermore, *A. muciniphila* has been shown to
69 induce altered host immune responses depending on broader gut microbial community composition in mice
70 (Ansaldi et al., 2019), and this context-dependence has been proposed as a reason for varying host health
71 outcomes associated with *A. muciniphila* enrichment (Haikal et al., 2019).

72 Given links between *A. muciniphila* and divergent metabolic and neurodegenerative outcomes,
73 there is great therapeutic potential in developing methods to modulate *A. muciniphila* abundance within the
74 gut. Previous studies in mice have reported strong *A. muciniphila* blooms in response to polyphenol
75 administration (Anhê et al., 2017; Régnier et al., 2020; Roopchand et al., 2015; Zhang et al., 2018), but
76 their experimental designs used purified compounds divorced from their whole food form. Isolated phenolic
77 compounds are volatile and degrade easily (Volf et al., 2014), making them difficult and costly to administer
78 commercially. Furthermore, although polyphenols are found in a wide range of foods commonly consumed
79 in industrialized populations (Manach et al., 2004), the doses previously tested in mice have been 20-50
80 times greater, after allometric scaling, than what would typically be consumed by humans in industrialized
81 settings (Del Bo et al., 2019). Therefore, it remains unclear whether ingesting polyphenols at levels
82 achievable via foods already consumed in an industrialized diet could similarly foster blooms of *A.*
83 *muciniphila*. In addition, to our knowledge, studies exploring how to selectively impede *A. muciniphila*
84 growth have yet to be performed. Understanding how environmental drivers like diet can shape gut
85 microbiota dynamics that influence *A. muciniphila* growth could advance new avenues to mitigate
86 metabolic and neurodegenerative disease via the gut microbiota.

87 In this study, we explore the extent to which consuming nutritionally relevant doses of common
88 polyphenol-rich foods influences the growth of *A. muciniphila* in mice under high-fat versus low-fat dietary
89 conditions. By feeding native and polyphenol-depleted versions of foods that vary in their underlying
90 composition of polyphenolic compounds, we show that the modulation of *A. muciniphila* is driven not by
91 total polyphenolic content, but rather by PAC content. By observing changes in the gut microbiome under
92 PAC-rich versus PAC-poor conditions, and through gradient PAC-dosing experiments, we show that PAC-
93 rich foods commonly consumed in industrialized settings uniquely induce *A. muciniphila* growth at doses

94 that can be achieved through routine diet. Notably, the effects of PAC supplementation were detected
95 against a high-fat but not a low-fat diet background, highlighting that the prebiotic effects of PACs may
96 depend on the broader diet strategy. Overall, our findings indicate that interactions between PAC-rich food
97 supplementation and overall diet composition may play a significant role in modulating *A. muciniphila*
98 growth.

99

100 **Results**

101 ***Dried blueberry consumption leads to rapid blooms of A. muciniphila***

102 To date, studies supporting an association between polyphenol consumption and blooms of *A. muciniphila*
103 have relied exclusively on purified polyphenol extracts (Anhê et al., 2015, 2016, 2017; Régnier et al., 2020;
104 Roopchand et al., 2015; Zhang et al., 2018). Therefore, our first goal was to establish whether polyphenols
105 administered in their whole food matrix could likewise induce *A. muciniphila* growth. To do this, we first
106 examined changes in gut microbial community composition among mice reared for 5 days on homogeneous
107 *ad libitum* diets of polyphenol-rich dried grapes (raisins), dried cranberries, or dried blueberries relative to
108 a standard chow control group (**Figure 1A**). Each of these berries is rich in polyphenolic compounds
109 although they differ in total polyphenolic abundance and composition (**Figure 1B-C**), and each berry has
110 been associated with metabolic benefits in humans (Curtis et al., 2019; Fulgoni et al., 2017; Pourmasoumi
111 et al., 2020; Stote et al., 2020; Wijayabahu et al., 2019).

112 The differing total polyphenol content (TPC) and proanthocyanidin (PAC) content of the raisins,
113 cranberries, and blueberries used in this study (**Figure 1B-C**) allowed us to evaluate any differential
114 phenotypic effects of polyphenol intake. Consumption of any of the berry treatments led to significantly
115 lower mRNA expression in the proximal colon of markers of gut inflammation (IL-6, IL-1 β and TNF α),
116 gut epithelial integrity (Muc2 and Ocln, but not Inectin), and, to some degree, antimicrobial peptides
117 (Reg3 γ and Pla2g2a) (**Figure S1F-M**). However, by the end of the 5-day intervention, mice consuming
118 blueberries (highest TPC and PAC content) exhibited a significant decrease in total body mass compared
119 to controls (**Figure 1D**), an effect not observed in mice consuming raisins (lowest TPC and PAC content)
120 or cranberries (intermediate TPC and low PAC content). Notably, accelerated loss of body mass was not
121 driven by reduced food consumption in the blueberry group, as food consumption was comparable across
122 groups ($p=0.356$, Kruskal-Wallis). Rather, change in body mass per unit food intake decreased significantly
123 among mice consuming blueberries ($p=0.002$, linear mixed effects model, **Figure S1A**), while the
124 relationship between change in body mass and total food intake among mice consuming either raisins or
125 cranberries did not significantly differ from controls (**Figure S1A**). Foreshadowing the possibility that
126 PACs exert unique effects, we also detected significant positive longitudinal relationships between

127 decreases in total body mass and total PAC intake ($p=4.92e-16$, linear mixed effects model) among mice in
128 all three berry treatment groups.

129 Gut microbial community signatures of mice were also distinct by treatment group at endpoint
130 ($p=0.001$, pseudo- $F=4.97$, PERMANOVA; **Figure 1E**). Blueberry consumption uniquely resulted in
131 significant changes in Bray-Curtis distances relative to baseline when compared to the control diet at every
132 day of the study, as well as when compared to either cranberries or raisins by Day 5 (**Figure 1F**). Changes
133 in whole community composition were not an artifact of changes in alpha-diversity (Shannon index), the
134 ratio of Firmicutes to Bacteroidetes relative abundance as an index of tradeoffs between the two dominant
135 bacterial phyla in the gut, or total bacterial abundance as assessed by universal 16S qPCR (**Figure S1C-E**).
136 However, increased PAC intake did significantly correspond with longitudinal increases in the relative
137 abundance of *A. muciniphila* ($p=6.94e-14$, linear mixed effects model). Correspondingly, blueberry
138 consumption correlated with striking blooms in both the relative and absolute abundance of *A. muciniphila*
139 compared to the control group (**Figure 1G-H**), including after controlling for cumulative food intake
140 ($p=0.0104$, linear mixed effects model; **Figure S1B**).

141 Because mice on the blueberry treatment exhibited strong correlations between *A. muciniphila*
142 relative abundance, PAC intake, and changes in body mass, we examined the degree to which *A.*
143 *muciniphila* enrichment was the result of PAC intake versus an artifact of weight loss. When controlling
144 for their associations with body mass, *A. muciniphila* relative abundance maintained a significant positive
145 relationship with cumulative PAC intake ($p=8.32e-04$, linear mixed effects model, **Figure 1I**). Furthermore,
146 whereas PAC intake maintained a positive effect on *A. muciniphila* relative abundance when controlling
147 for its relationship to body mass ($p=0.038$, linear mixed effects model, **Figure 1J**), body mass exhibited no
148 effect on *A. muciniphila* when controlling for its relationship to PAC intake ($p=0.698$, linear mixed effects
149 model, **Figure 1K**). These results suggest that PAC intake, not weight loss, is the primary driver of *A.*
150 *muciniphila* enrichment among mice consuming dried blueberries.

151

152 ***A. muciniphila* growth promoted by PAC-rich foods, not generally polyphenol-rich foods, against the**
153 ***backdrop of a high-fat diet***

154 Because blueberries were highest in TPC as well as PACs relative to the other berry groups (**Figure 1B-**
155 **C**), we next proceeded to confirm that such effects were unique to PAC-rich as opposed to generally
156 polyphenol-rich foods. To isolate gut microbial effects attributable to PACs versus total polyphenols and
157 to investigate whether the broader diet shapes the response of *A. muciniphila* to foods rich in these
158 phenolics, we gavaged mice with polyphenol-rich red wine or coffee, or polyphenol-depleted versions of
159 the same red wine or coffee, as daily prebiotic supplements on top of either a high-fat diet (HFD) or a
160 standard chow control diet (**Figure 2A**). We selected red wine and coffee for testing because they are

161 similarly rich in TPC but differ significantly in their polyphenol composition (Gu et al., 2004), with wine
162 being rich in PACs while coffee is not (**Figure 2B-C**). Supplementation was achieved by gavaging mice
163 daily with 100 μ L/25g of a solution containing 1.47g dry weight/mL dealcoholized red wine, 1.30g dry
164 weight/mL polyphenol-free dealcoholized wine (winePF), 0.606g dry weight/mL decaffeinated coffee, or
165 0.497g dry weight/mL polyphenol-free decaffeinated coffee (coffeePF), allometrically scaled doses
166 equivalent to 750mL (i.e., 1 bottle of wine or 25 oz of coffee) for a 65kg human (**Figure 2A**). We compared
167 each of these conditions to a vehicle control group, which was gavaged daily with 100 μ L/25g of nuclease-
168 free water. Under the hypothesis that PAC consumption drives *A. muciniphila* abundance, we would expect
169 increased *A. muciniphila* only among the wine treatment, as the other treatments are either experimentally
170 depleted in PAC (winePF) or high intrinsically low in PACs (coffee or coffeePF).

171 As seen in prior work (Carmody et al., 2015; Turnbaugh et al., 2006), transitioning from standard
172 chow to an HFD generally altered Bray-Curtis distances relative to baseline (**Figure S2B**), while mice
173 consuming the control diet exhibited minimal changes relative to baseline (**Figure S2O**). Importantly,
174 however, we observed distinct interaction effects between the diet base and prebiotic supplementation that
175 impacted gut microbial phenotypes. Among HFD-fed mice, gut microbial community composition differed
176 by prebiotic treatment by Day 12 of the study ($p=0.001$, pseudo- $F=2.87$, PERMANOVA; **Figure 2D**), but
177 such differentiation was not observed among mice fed the control diet ($p=0.373$, pseudo- $F=1.061$,
178 PERMANOVA; **Figure 2E**). The compositional changes with treatment among HFD-fed mice could not
179 be attributed to differences in alpha-diversity (Shannon index), Firmicutes to Bacteroidetes relative
180 abundance, or total bacterial abundance (**Figure S2C-E,P-R**). Instead, treatment differences seen on the
181 HFD were driven in large part by changes in *A. muciniphila* abundance ($p=1.38e-08$, $coef=5.568$,
182 MaAsLin2), with the HFD-wine supplementation inducing the greatest enrichment of *A. muciniphila* by
183 endpoint ($p=0.001$, $coef=9.063$, MaAsLin2).

184 Consistent with our hypothesis, the HFD-wine group experienced the only significant changes in
185 *A. muciniphila* abundance, with the HFD-wine group exhibiting increased *A. muciniphila* relative
186 abundance compared to the HFD-winePF group (**Figure 2H**), as well as increased absolute abundance
187 compared to the HFD-winePF, HFD-coffee, or HFD-vehicle groups (**Figure 2G**). As seen in the blueberry
188 case (**Figure 1I-K**), enrichment of *A. muciniphila* in the HFD-wine group was not attributable to underlying
189 changes in body mass, as change in body mass was similar among the HFD-wine, HFD-winePF, HFD-
190 coffee, and HFD-vehicle groups (**Figure 2F**). That wine supplementation did not induce similar effects on
191 *A. muciniphila* when administered under control diet feeding (**Figure 2G-H**), suggests that PAC-induced
192 blooms of *A. muciniphila* may be enhanced by a diet high in fat and low in fiber.

193 Overall, we observed no significant effects of either wine or coffee supplementation on host body
194 mass or adiposity relative to the vehicle control (**Figure 2F**, **Figure S2A,N**). Moreover, in contrast to

195 signatures of proximal colon expression seen in the dried berry study (**Figure S1F-M**), wine and coffee
196 treatments did not broadly alter expression of markers of inflammation or gut barrier integrity in the
197 proximal colon (**Figure S2F-M,S-Z**), although we did observe a consistent increase in *Ocln* expression
198 among both HFD-fed and control diet-fed mice on the wine treatment relative to winePF and coffee
199 supplementation (**Figure S2J,W**). The sources of these experimental differences are unknown, but may be
200 attributable to differences in effective dose between homogeneous (berry) and supplemented (coffee and
201 wine) diets, physicochemical differences between solid and liquid diets, or differences in macronutrient
202 composition across the test substrates. To discriminate among these variables, we next proceeded to test
203 the dose of PACs required to stimulate *A. muciniphila* blooms under conditions that controlled for
204 background diet.

205

206 ***PACs induce *A. muciniphila* blooms at a distinct, diet-relevant dose in mice consuming high-fat diets,
207 while mice consuming control diets exhibit a blunted response to PACs***

208 Our polyphenol composition experiment confirmed that supplementing mice with PAC-rich red wine, but
209 not PAC-depleted red wine or PAC-poor coffee, can increase *A. muciniphila* abundance regardless of
210 changes in body composition, and that the effects of PAC intake on the murine gut microbiome may be
211 enhanced against a HFD base. Although the dose of red wine we administered was selected to lie at the
212 high end of human nutritional relevance (the allometric equivalent of 1 bottle of wine for humans, or 1.47g
213 dry weight/mL per 25g mouse), we next sought to determine whether blooms in *A. muciniphila* could occur
214 at lower levels of PAC intake under a HFD and to validate our prior finding that the prebiotic effect of
215 PACs is relatively minimal on a control diet. To accomplish this, we performed a PAC dosing experiment
216 using the same purified grape-derived PACs used in previous studies (Roopchand et al., 2015; Zhang et al.,
217 2018). We selected the following range of PAC doses to administer to mice consuming either HFD or
218 control diet bases: (1) a high PAC concentration (9mg/25g, or 9PAC), which replicated a dose previously
219 used to elicit *A. muciniphila* growth in a 10-day feeding intervention (Zhang et al., 2018); (2) an
220 intermediate PAC concentration (6mg/25g, or 6PAC), which is comparable to the PAC level from wine
221 supplementation in our polyphenol composition study; (3) a low PAC concentration (3mg/25g, or 3PAC),
222 to test a reduced PAC level for potential modulation of *A. muciniphila*; and (4) a PAC-free vehicle control
223 (**Figure 3A**).

224 HFD-fed mice administered either the 6PAC or 9PAC dose exhibited a significant loss of body
225 mass by Day 10, while the 3PAC condition exhibited a marginal but nonsignificant decrease, compared to
226 the vehicle condition (**Figure 3D**). In contrast, mice did not exhibit any significant shifts in body mass
227 under control diet feeding (**Figure 3D**). Gut microbial community composition also differed by treatment
228 group at endpoint in HFD-fed mice ($p=0.001$, pseudo- $F=3.88$, PERMANOVA; **Figure 3B**), but not among

229 mice consuming the control diet ($p=0.629$, pseudo- $F=0.868$, PERMANOVA; **Figure 3C**). Interestingly,
230 the gut microbial communities of HFD-fed mice administered either 6PAC or 9PAC experienced a relative
231 increase in Bray-Curtis distances relative to baseline, a decrease in alpha-diversity (Shannon index), and
232 lower Firmicutes versus Bacteroidetes relative abundance compared to vehicle controls (**Figure S3A-C**).
233 Moreover, HFD intake induced a decrease in total bacterial abundance in mice administered either the
234 vehicle control or 3PAC supplements, but this bacterial loss was inhibited in the case of 6PAC and 9PAC
235 dosing (**Figure S3D**). These differences in alpha-diversity and beta-diversity, as well as longitudinal
236 maintenance of total absolute abundance, were in part attributable to blooms in *A. muciniphila*. On the
237 HFD, 6PAC and 9PAC dosing induced stark increases in *A. muciniphila* relative abundance (**Figure 3E**).
238 6PAC dosing also increased *A. muciniphila* absolute abundance, while 9PAC dosing induced a marginal
239 but nonsignificant increase, when compared to the vehicle control (**Figure 3F**). *A. muciniphila* was also
240 detected as an enriched biomarker of 6PAC ($p=0.001$, $\text{coef}=1.529$, MaAsLin2) and 9PAC ($p=0.003$,
241 $\text{coef}=1.501$, MaAsLin2) dosing, but the same was not true of 3PAC dosing ($p=0.529$, $\text{coef}=0.408$,
242 MaAsLin2) or any PAC dosing levels on the control diet by endpoint ($p \geq 0.639$, $\text{coef} \leq 5.89$, MaAsLin2).
243 PAC dosing did not significantly influence gene expression in the proximal colon for our biomarkers of gut
244 inflammation and epithelial integrity under either diet condition (**Figure S3E-L,Q-X**). Overall, we reliably
245 observed changes in gut microbial phenotypes, including expected *A. muciniphila* blooms, among HFD-
246 fed mice supplemented with at least 6mg/25g PAC, while no significant changes were observed at lower
247 PAC doses or under any control diet condition (**Figure 3E-F**; **Figure S3M-P**).
248

249 **Discussion**

250 Our data suggest that polyphenols sourced from whole foods can be used to modulate the abundance of *A.*
251 *muciniphila* in the murine gut microbiota, and show that these effects are amplified against the backdrop of
252 a high-fat, low-fiber diet. By rearing mice for 5 days on raisins, dried cranberries, or dried blueberries, we
253 determined that dried blueberries, which contained the highest TPC and PAC content, were the most
254 efficient in stimulating the growth of *A. muciniphila*. Additionally, through prebiotic supplementation with
255 dealcoholized wine (a polyphenol-rich, PAC-rich food) versus decaffeinated coffee (a polyphenol-rich,
256 PAC-poor food), along with polyphenol-free wine and coffee controls, we established that the effects of
257 polyphenols on *A. muciniphila* growth were most likely attributable to high PAC consumption as opposed
258 to high TPC or other compounds present in the food matrix.

259 We also determined that a daily mouse dose of 6mg/25g of body mass was sufficient for PAC-
260 induced changes in *A. muciniphila* abundance, which allometrically scales up to a daily dose of 1,265mg
261 PACs for a 65 kg human (Reagan-Shaw et al., 2008). For reference, the Tannat wine used in our polyphenol
262 composition study contains about 200mg of PACs per serving (5oz or 146mL), and one serving of fresh

263 blueberries (1/4 cup or about 40g) contains between 134-249mg of PACs (Gu et al., 2004). Based on these
264 concentrations, consuming approximately two servings of dealcoholized wine and three servings of
265 blueberries would deliver a PAC dose in the range necessary to promote *A. muciniphila* growth, assuming
266 that the necessary dose threshold is similar for humans and mice. Although parallel experiments in humans
267 will be necessary to confirm translatability, and additional work will be required to determine whether
268 common forms of processing, e.g. cooking (Carmody et al., 2019), affects the impact of PAC-rich foods on
269 *A. muciniphila*, our data suggest that *A. muciniphila* abundance might be regulated in humans by selectively
270 ingesting or limiting foods with high PAC content.

271 Finally, by performing experiments against both a HFD and control diet background, we found that
272 effects of dietary PACs on *A. muciniphila* abundance were amplified on the HFD while the control diet
273 repeatedly mitigated these effects. This finding replicates previous work highlighting the role of isolated
274 PACs in attenuating HFD-induced obesity via blooms in *A. muciniphila* (Anhê et al., 2015; Roopchand et
275 al., 2015) and suggests such effects are achievable via whole food consumption. However, Zhang et al.,
276 (2018), which served as the basis for our 9PAC treatment in the PAC titration study, reported increased
277 relative abundance of *A. muciniphila* on a low-fat diet, an effect not observed here whether PACs were diet-
278 derived or delivered in isolated form. It is possible that our inability to replicate these effects was due to the
279 low sample size in our control-9PAC condition, although we also did not detect any effects of control-
280 3PAC or control-6PAC supplementation despite larger sample sizes. It is also possible that differences in
281 baseline gut microbiota profiles across studies led to differential responsiveness of the gut microbiota to
282 PAC supplementation. In the study by Zhang and colleagues, the average baseline relative abundance of *A.*
283 *muciniphila* among mice consuming a low-fat diet and receiving the 9PAC equivalent dose was 0.79%
284 (Zhang et al., 2018) while mice in our 9PAC treatment group had an average baseline relative abundance
285 of only 0.33%, though the difference between studies did not reach significance ($p=0.794$, Mann-Whitney
286 U test). Finally, we cannot exclude the possibility that the effects of PAC on *A. muciniphila* abundance
287 depend on a concomitant change in diet that perturbs the gut microbiota and thereby allows for *A.*
288 *muciniphila* expansion. While this might in principle explain why we observed effects among mice
289 transitioned onto a HFD but not those remaining on a control diet, the findings of Zhang and colleagues
290 suggest that a dietary transition is not a prerequisite for *A. muciniphila* augmentation (Zhang et al., 2018).

291 That effects of PACs were amplified against the backdrop of a high-fat, low-fiber diet (comprised
292 of 45% of calories from fat, with a fat profile predominantly comprised of long-chain saturated fatty acids)
293 is intriguing because humans that habitually consume a similar diet profile tend to live in industrialized
294 societies (Chadaideh and Carmody, 2021) where both metabolic and neurodegenerative diseases are on the
295 rise (Corbett et al., 2018; Emard et al., 1995) and where *A. muciniphila* is also a prevalent gut commensal

296 (Smits et al., 2017). This suggests that PAC supplementation offers unique leverage in the same
297 environments where *A. muciniphila* modulation could achieve its highest potential health impacts.

298 While our study did not directly test the consequences of PAC-induced manipulation of *A.*
299 *muciniphila* for metabolic syndrome or neurodegenerative disease, we determined that interactions between
300 PAC-rich foods and overall diet can reliably influence the abundance of a key member of the gut microbiota
301 that has been repeatedly and, in many cases, causally linked to specific health outcomes. Previous work has
302 shown that blooms of *A. muciniphila* can be maintained with continued PAC treatment, and that early
303 blooms of *A. muciniphila* are predictive of long-term metabolic benefits under a HFD (Anhê et al., 2015;
304 Roopchand et al., 2015). Such data suggest that PAC-induced *A. muciniphila* blooms observed in our study
305 under HFD backgrounds could potentially persist with long-term interventions and confer a protective
306 metabolic effect in humans consuming typical industrialized diets. In contrast, traditional populations such
307 as the Hadza, which are known to consume fiber-rich diets in conjunction with polyphenol-rich foods
308 (Murray et al., 2001), exhibit low levels of *A. muciniphila* in their guts (Smits et al., 2017), which portends
309 that the low level of *A. muciniphila* among our control diet-fed mice may persist regardless of PAC intake.
310 Thus, fiber-rich diets could potentially be a beneficial long-term strategy for mitigating the growth of *A.*
311 *muciniphila* in Parkinson's patients, although this would need to be weighed against the parallel possibility
312 that short-chain fatty acids derived from the gut microbial fermentation of dietary fiber promote motor
313 deficits in Parkinson's disease (Sampson et al., 2016). Taken together, our work supports the promise of
314 PAC-rich whole foods and overall dietary recommendations for regulating *A. muciniphila* abundance
315 depending on individual health needs.

316

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323

324 **Author Contributions**

325 K.S.C. and R.N.C. designed the study; K.S.C., K.E.E., and B.E.M. optimized assays; K.S.C. and K.E.E.
326 ran the mouse experiments; K.S.C. and K.E.E. performed 16S rRNA gene sequencing; K.S.C. performed
327 universal 16S qPCR and RT-qPCR; K.S.C. analyzed the data; K.S.C. and R.N.C. wrote the manuscript; all
328 authors contributed to editing the final manuscript.

329

330 **Declaration of interest**

331 The authors declare no competing interests.

332

333 **Methods**

334 ***Animal experiments***

335 All mice were male C57BL/6J purchased from Jackson Laboratory (Bar Harbor, ME, USA) and, unless
336 otherwise noted, cagemates were distributed symmetrically across treatment groups. For the 5-day dried
337 berries experiment, 7-week-old mice were transported in groups of 3 mice per cage, with control chow
338 treatment mice sourced from an in-house colony of age-matched mice. For the 10-day PAC dosage by diet
339 experiment, 8-week-old mice were purchased in groups of 8 mice per cage. For the 12-day polyphenol
340 composition experiment, 7-week-old mice were purchased and transported in groups of 6 mice per cage.
341 Mice were singly housed at the Biological Research Infrastructure (BRI) facility at Harvard University
342 upon arrival and fed a standard low-fat chow (ProLab Isopro RMH 3000: 14.4% of calories from fat; 26.1%
343 from protein; 59.5% from carbohydrate) until their recruitment into the study. All mice were fed *ad libitum*,
344 provided free access to water, and maintained on a 12-hour light-dark cycle. Diets varied based on treatment
345 group and experiment, as described below. Protocols were approved by Harvard University Institutional
346 Care and Use Committee (IACUC 17-06-306).

347

348 ***Dried berries experiment***

349 For 5 days, C57BL/6J mice were provided *ad libitum* access to either dried blueberries, cranberries, raisins,
350 or a standard chow control diet (n=5-6 per treatment). Fecal samples and body mass measurements were
351 collected daily, during which berry treatments were replenished and food refusals were collected for
352 analysis of food intake. Mice were euthanized on Day 5 of treatment.

353

354 ***Polyphenol composition experiment***

355 To determine the impact of common whole food polyphenols on *A. muciniphila* abundance, we conducted
356 a 12-day intervention in which mice were supplemented with a daily oral gavage of either wine or coffee
357 as a source of polyphenols. C57BL/6J mice were administered one of the following experimental
358 treatments: alcohol-free wine (wine), alcohol-free and polyphenol-free wine (winePF), caffeine-free coffee
359 (coffee), caffeine-free and polyphenol-free coffee (coffeePF), or a vehicle control, with gavage solutions
360 prepared as described under “Wine and coffee gavage solution preparation” and “Polyvinylpolypyrrolidone
361 (PVPP) removal of polyphenols from food sources” below. Mice were either placed on a high-fat diet
362 (TD.170592: 45% of calories from fat; 15.3% from protein; 39.8% from carbohydrate) or maintained on
363 the standard low-fat chow control diet (ProLab Isopro RMH 3000: 14.4% of calories from fat; 26.1% from

364 protein; 59.5% from carbohydrate) during the 12-day treatment intervention (n=4-6 per condition). Each
365 mouse was measured for adiposity on a baseline and endpoint day. On Day 0, mice in HFD groups were
366 switched onto the HFD chow while the control diet groups continued to receive the standard low-fat chow.
367 For 12 days, beginning on Day 0, mice were gavaged daily with their assigned treatment. Fecal samples
368 were taken on the three days preceding the start of the dietary intervention and on Days 7, 10, and 12 of the
369 dietary intervention. Mice were euthanized on Day 12 of the intervention period.
370

371 *Proanthocyanidin (PAC) titration experiment*

372 To understand the effect of proanthocyanidins at more intuitive doses, such as recommended serving sizes
373 of berries or wine, and to evaluate the effect of base diet, mice were assigned to a range of isolated
374 commercial PAC doses. Mice were either placed on the high-fat diet (TD.170592) or maintained on the
375 standard low-fat chow control diet (ProLab Isopro RMH 3000) during the 10-day treatment intervention
376 (n=3-6 per condition). We administered one of the following 4 prebiotic supplements to cohorts of mice
377 consuming either the high-fat or control diet base groups: a maximum dose group (9PAC) receiving the
378 same dose as in Zhang et al. (2018) (9mg PACs/25g mouse), an intermediate dose group (6PAC) receiving
379 6mg PACs/25g mouse, a dose commensurate with the PACs administered via wine supplementation in our
380 polyphenol composition study, a low dose group (3PAC) receiving 3mg/25g mouse, and a vehicle control
381 group (0PAC) receiving a gavage of 0.5% ethanol in nuclease-free water. Mice received their prescribed
382 PAC dose via daily gavage for the duration of the 10-day intervention based on the protocol below.
383

384 *Wine and coffee gavage solution preparation*

385 For the safety of the mice, we used dealcoholized wine and decaffeinated coffee. For the polyphenol-free
386 treatments, polyphenols were removed via the polyvinylpolypyrrolidone (PVPP) removal method discussed
387 under “Polyvinylpolypyrrolidone (PVPP) removal of polyphenols from food sources” below.
388

Bodega El Porvenir de Cafayate 2018 Amauta Absoluto Tannat wine was used for this study. This
389 vintage was selected because of the high tannin content of its tannat grape varietal. Wine was dealcoholized
390 using a BÜCHI Rotavapor R-300 rotary evaporator (BÜCHI Labortechnik, Flawil, Switzerland). Alcohol
391 removal was confirmed by a 12.5% decrease in mass (the alcohol percentage of the wine). After removing
392 the alcohol, contents of a bottle of wine were homogenized and divided into two halves, with one half
393 destined for polyphenol depletion via the PVPP method discussed below. PVPP treated wine and untreated
394 dealcoholized wine were aliquoted into 50mL tubes and frozen at -80°C at a slight angle. After 24hrs, the
395 tubes were lyophilized for 72hrs to remove the water content from the frozen solutions. Samples were then
396 weighed and reconstituted with deionized water to the desired stock concentration. The concentration of
397 wine was determined based on allometric scaling of a human-equivalent dose of 1 bottle of dealcoholized

398 wine, an upper limit of biological relevance. Dry content in one bottle of wine was calculated based on the
399 density of the dry weight divided by the initial wet weight. The resulting human dose for this wine
400 concentration (0.55g dry wine/kg) was then converted to a mouse-equivalent dose based on the equation
401 for allometric dose translation between humans and mice (Reagan-Shaw et al., 2008): Animal Equivalent
402 Dose (mg/kg) = Animal dose (mg/kg) * (Animal Km ÷ Human Km), where Km is a scaling factor that
403 captures species differences in oxygen utilization, caloric expenditure, basal metabolism, blood volume,
404 circulating plasma proteins, and renal function. The Km values for humans and mice are 37 and 3,
405 respectively (Reagan-Shaw et al., 2008). Applying this allometric translation resulted in a mouse-equivalent
406 dose of 7.34g dry wine/kg mouse, which was reconstituted to a solution of 1.47g/mL. Polyphenol-free wine
407 was less dense (due to the removal of polyphenols), resulting in a stock concentration of 1.30g/mL dry
408 weight to deliver a 0.052g dry weight/kg human-equivalent dose, or 6.52g dry weight/kg mouse-equivalent
409 dose when scaled using the same allometric dose translation method.

410 Starbucks Decaffeinated Pike Place Roast was used in this study, and was brewed according to
411 package instructions. Briefly, 10g of ground coffee were added to the coffee filter for every 180mL of cold
412 water brewed. The coffee brew was divided into two halves, with one half destined for polyphenol depletion
413 via the PVPP method discussed below. Untreated coffee and PVPP coffee were aliquoted into 50mL tubes
414 and frozen at a slight angle at -80°C. After 24hrs, the tubes were placed into a lyophilizer for 72hrs. Tubes
415 were then weighed and reconstituted with water, reducing the final volume by 22% to maintain comparable
416 phenolic content to between the wine and coffee treatments. To accurately align the total polyphenol dose
417 with the wine condition, deionized water was added to the desired stock concentration of 0.606g dry
418 coffee/mL, which allows for delivery of a 2.97g/kg mouse dose within a 150µL gavage solution for a 30g
419 mouse. The polyphenol-free condition had a stock concentration of 0.497g dry/mL, which allowed for a
420 2.48g/kg mouse dose. To ensure that mice received an accurate dry-weight dose for their treatment group,
421 gavage solution volumes were calculated daily based on individual body mass. Mice in the vehicle control
422 group received a standard 100µL of solution.

423

424 ***Polyvinylpolypyrrolidone (PVPP) removal of polyphenols from food sources***

425 In order to control for compounds other than polyphenols in each treatment group, polyphenol-free stocks
426 of wine and coffee were prepared using the polyvinylpolypyrrolidone (PVPP) method of extracting
427 polyphenols (Ranatunge et al., 2017). 5mL syringes were placed into 15mL centrifuge tubes. Cotton wool
428 plugs were placed at the bottom of the 5mL syringes. 0.9g of PVPP powder was then added to the syringe
429 on top of the cotton plug, and powder was compressed using the syringe plunger. 3mL of treatment solution
430 was added to the top of each PVPP column and placed into a centrifuge at 2,000 revolutions per minute
431 (RPM) for 10min. After the centrifuge wash, extracted product in the centrifuge tubes were placed back

432 into the original solution container. 1mL of solution was then added to each PVPP column and centrifuged
433 again at 2,000RPM for 10min. The supernatant was collected into a 50mL tube. This process was repeated
434 5 more times before disposing of the PVPP columns. This polyphenol depletion protocol successfully
435 removed both TPC and PACs from the wine and coffee substrates (**Figure 2B-C**).
436

437 ***Measuring total polyphenol content (TPC)***

438 TPC was measured using the Folin-Ciocalteu assay (Ainsworth and Gillespie, 2007). Gallic acid was used
439 to create a dilution series and calibration curve of absorption versus polyphenol content. This curve can be
440 used to plot the absorbance data from the treatment groups to determine accurate measurements of
441 polyphenol content. Treatment group samples were diluted 5-fold by adding Milli Q water to the sample
442 solution. 100µL of sample was placed into 1.5mL tubes, and 100µL of 0.25 N Folin reagent (Sigma-
443 Aldrich) was added to each tube. Each tube was vortexed for 5sec and incubated at room temperature for
444 3min. 100µL of 1M sodium carbonate was then added to each tube. After tubes were incubated at room
445 temperature for 5min, 700µL of Milli Q water was added, and the tube was vortexed for 5sec. 200µL from
446 each sample tube was placed in a clear, flat-bottomed 96-well plate and the optical density values were read
447 at 726nm on an Epoch 2 BioTek plate reader (BioTek Instruments, Winooski, VT, USA). The standard
448 curve equation was used to determine total polyphenol content of each sample based on absorbance values.
449

450 ***Measuring proanthocyanidin (PAC) content***

451 PAC content of each sample was measured using the 4-dimethylaminocinnamaldehyde (DMAC) method
452 (Prior et al., 2010). Acidified ethanol was first prepared by adding 12.5mL of hydrochloric acid (Millipore
453 Sigma) to 12.5mL of Milli Q water and 75mL of HPLC grade ethanol (Thermo Fisher). The dilution
454 solution was prepared by adding 80mL of HPLC grade ethanol to 20mL of Milli Q water. DMAC reagent
455 was prepared by adding 0.05g of DMAC (Millipore Sigma) to 50mL of the acidified ethanol solution, which
456 was prepared fresh for each assay. The procyanidin A2 calibration standard was prepared by adding 5mg
457 of procyanidin A2 (Adipogen) to 50mL of HPLC grade ethanol, for a final concentration of 100µg/mL.
458 The procyanidin A2 control sample was prepared by adding 250µL of HPLC-grade ethanol to 1mL of
459 procyanidin A2 calibration standard, for a final quality control sample at 80µg/mL concentration. 32-fold
460 serial dilutions of triplicate stocks were created for the procyanidin A2 calibration standards, the
461 procyanidin A2 control samples, and for each treatment sample. A 32-fold dilution was also prepared for
462 the HPLC ethanol blanks. All samples were diluted using the previously prepared dilution solution. On a
463 clear-bottomed 96-well plate, 210µL of the DMAC reagent solution was added to 70µL of each
464 experimental solution. The 96-well plate was then placed on an Epoch 2 BioTek plate reader (BioTek
465 Instruments, Winooski, VT, USA) and read every minute for 30min at an optical density of 640nm at 25°C.

466 Maximum corrected absorbance readings were calculated by subtracting the average ethanol blank from
467 the maximum absorbance read of each sample. Proanthocyanidin concentrations were determined by
468 calculating the regression equation based on the procyanidin A2 calibration standard dilution series, with
469 triplicate reads averaged for each sample.

470

471 ***Preparing PAC dosing stocks***

472 Zhang et al. (2018) determined that a 10-day gavage with a dose of 360mg/kg isolated and commercially
473 available proanthocyanidins is sufficient to induce a bloom in *A. muciniphila* (Zhang et al., 2018), which is
474 the equivalent of 9mg of PACs administered daily to a 25g mouse. We decided to match this level and scale
475 down to produce two additional doses for our titration experiment. These additional doses corresponded to
476 2/3 of the reference dose, or 6mg per 25 g mouse (6PAC), which corresponds to the PAC dose delivered in
477 our wine supplementation experiment, and 1/3 of the reference dose, or 3mg/25g mouse (3PAC). Purified
478 grape seed oligomeric proanthocyanidins (Millipore Sigma) were dissolved in a 0.5% ethanol and Milli-Q
479 water solution. The final gavage stock solution concentrations were the following: 24mg PACs/mL (3PAC),
480 48mg PACs/mL (6PAC), 72mg PACs/mL (9PAC), and a 0.5% ethanol vehicle control condition (0PAC).
481 Gavage volumes were calculated based on daily body mass measures for each mouse across all PAC
482 treatment groups. Mice administered the vehicle control received a standard 100 μ L of solution.

483

484 ***16S rRNA gene sequencing and analysis***

485 DNA isolation was performed using the PowerSoil DNA Isolation Kit (Qiagen). For each sample, 3 μ L of
486 purified DNA was then amplified in triplicate via polymerase chain reaction (PCR), using 2 μ L of forward
487 and reverse barcoded bacterial primers amplifying the V4 region of the 16S rRNA gene (515F and 806R),
488 1 μ L of 25mM MgCl₂ (New England Biolabs), 10 μ L of 2.5X 5-Prime Host Mastermix (QuantaBio), and
489 11 μ L of nuclease-free water per reaction. Samples were amplified on a Bio-Rad T100 thermocycler (Bio-
490 Rad, Hercules, CA, USA) using the following cycle settings: 94°C for 3min, 35 cycles of 94°C for 45sec,
491 50°C for 30sec, and 72°C for 90sec, with a final extension at 72°C for 10min. Amplification of pooled PCR
492 products was confirmed using 1.5% gel electrophoresis. Any sample that failed to properly amplify was
493 cleaned using the DNeasy PowerClean Pro Cleanup Kit (Qiagen). PCR products were cleaned on a per-
494 sample basis using AMPure XP beads (Beckman Coulter), then quantified using the Picogreen dsDNA
495 reagent and read with a Spectramax Gemini XS (Molecular Devices Corporation, Sunnyvale, CA, USA) at
496 480EX/520EM. Amplicon concentrations were determined based on the standard curve, and barcoded
497 amplicons were pooled into a single tube at the calculated volume for 100ng of DNA per sample, with a
498 maximum volume of 30 μ L per amplicon. The collective pool was further cleaned with Qiaquick MinElute
499 Kit (Qiagen), then loaded onto a mini 1.5% agarose gel and run for 50min at 50V, and sequences of 381bp

500 length were re-extracted using the Qiaquick Gel Extraction kit (Qiagen). The final sample pool was diluted
501 to a 10nM concentration and sequenced on an Illumina HiSeq 2500 (Illumina, San Diego, CA, USA) at the
502 Harvard University Bauer Core Facility. Analysis of 16S rDNA sequencing was performed on the Harvard
503 Odyssey cluster using the QIIME 2 (Quantitative Insights Into Microbial Ecology) software package 2020.2
504 (Bolyen et al., 2019). Sequences were demultiplexed using the Demux plugin and denoised for quality
505 control using DADA2. Taxonomy was assigned using the Naive Bayes classifier trained on Greengenes
506 13_8 99% OTUs from the 515F/806R region. Bray-Curtis distances and PERMANOVA comparisons were
507 determined using the beta_group_significance plugin. Differential enrichment analyses were performed
508 using MaAsLin2 (Microbiome Multivariable Association with Linear Models) (Mallick et al., 2021).

509

510 ***16S universal qPCR***

511 To estimate gut bacterial density, we performed universal 16S quantitative PCR (qPCR) using the same
512 primer pairs employed for 16S rRNA gene sequencing (515F and 806R). 1 μ L of template DNA was
513 combined with 12.5 μ L PerfeCTa SYBR Green SuperMix Reaction Mix (QuantaBio), 6 μ L nuclease-free
514 H₂O, and 2.25 μ L of each primer. Amplification was performed on a Bio-Rad CFX384 Touch (Bio-Rad,
515 Hercules, CA, USA) in the Bauer Core Facility at Harvard University using the following cycle settings:
516 95°C for 10min, followed by 40 cycles of 95°C for 15sec, 60°C for 40sec and 72°C for 30sec. Reactions
517 were performed in triplicate with the mean value used in statistical analyses. Cycle-threshold values were
518 standardized against a dilution curve of *Escherichia coli* genomic DNA at the following concentrations
519 (ng/ μ L): 100, 50, 25, 10, 5, 1, 0.5, plus a no-template (negative) control. Imputed bacterial DNA
520 concentrations were normalized to whole genome equivalents based on a mean genome size of 4.50Mbp,
521 then multiplied by the total template DNA volume (50 μ L) and divided by the grams of feces utilized in the
522 isolation of template DNA (varied). To calculate absolute abundance of the species *Akkermansia*
523 *muciniphila* for each sample, total genome copies per gram of fecal matter was multiplied by the relative
524 abundance of *A. muciniphila* determined via 16S rRNA gene sequencing.

525

526 ***RT-qPCR of proximal colon samples***

527 Using a GentleMACS Octo Dissociator (Miltenyi Biotec, Bergisch Gladbach, Germany), 20-30mg of tissue
528 was dissociated in 700 μ L of TRK Lysis Buffer (Omega) treated with 2-mercaptoethanol. Tissue lysate was
529 then transferred to a Homogenizer Spin Column (Omega) and spun down for 5min at max speed. Total
530 RNA was extracted from the resulting supernatant using the E.Z.N.A Total RNA Kit I (Omega). DNases
531 were digested from each sample using the DNase I Digestion Set (Omega). Final RNA concentrations were
532 quantified using a Nanodrop One Microvolume UV-Vis spectrophotometer (Thermo Fisher Scientific,
533 Waltham, MA, USA), and RNA extracts were stored at -80°C.

534 mRNA from each sample was then reverse transcribed to cDNA. 2 μ g of mRNA were combined
535 with 1 μ L of random hexamers (Invitrogen) and 1 μ L of 10mM dNTPs (QuantaBio), then raised to 13 μ L
536 total volume using nuclease-free water. Samples were heated at 65°C for 5min then chilled on ice for 5min.
537 Next, first-strand synthesis was performed by adding 4 μ L of First-Strand Buffer (Thermo Fisher), 1 μ L of
538 DTT (Thermo Fisher), and 1.5 μ L of SuperScript II (Thermo Fisher). Samples were then incubated using
539 the following settings: 25°C for 7.5min, followed by 42°C for 75min, then 70°C for 15min. Second-strand
540 synthesis was then performed by adding 61.75 μ L of nuclease-free water, 10 μ L of NEBuffer2 (New England
541 Biolabs), an additional 3 μ L of 10mM dNTP (QuantaBio), 5 μ L of *E. coli* DNA polymerase (New England
542 Biolabs), and 0.25 μ L of RNaseH (New England Biolabs), then incubating samples at 16°C for 2hrs. Finally,
543 strands were ligated by adding 5 μ L of *E. coli* DNA ligase buffer (New England Biolabs), 1 μ L of *E. coli*
544 DNA ligase (New England Biolabs), and another 0.25 μ L of RNaseH (New England Biolabs), and
545 incubating samples at 16°C for 15min followed by heat-activation at 75°C for 20min. Samples were then
546 cleaned using the PCR Purification Kit (Qiagen) and quantified using a dsDNA HS Assay Kit (Qubit). RT-
547 PCR was then performed using 4 μ L of dsDNA (diluted to 4ng/ μ L), which was combined with 10 μ L of
548 KiCqStart SYBR Green PCR Ready Mix (Millipore Sigma), 1 μ L of forward primer, 1 μ L of reverse primer,
549 and 4 μ L nuclease-free water. The following primer pairs were used to measure gene expression:
550 IL-6 (forward AAGAAATGATGGATGCTACC, reverse GAGTTCTGTATCTCTCTGAAG),
551 IL-1 β (forward GGATGATGATGATAACCTGC, reverse CATGGAGAATATCACTTGTGG),
552 Tnf α (forward CTATGTCTCAGCCTCTTCTC, reverse CATTGGAACTTCTCATCC),
553 Muc2 (forward AAGAAATGCCCAATAAC, reverse CATCCATGTAGTGTTCCT),
554 Ocln (forward AAAGCAAGTTAAGGGATCTG, reverse TGGCATCTCTCTAAGGTTTC),
555 Insectin (forward CTCCTCCCTCTTCCCTT, reverse GCCTTCTGACTTCCACAT),
556 Reg3 γ (forward AACAGTGGCCAATATGTATG, reverse TCTCTCTCCACTTCAGAAATC),
557 Pla2g2a (forward GAACAAGAAACCATACCA, reverse ACAACCCTCAAATACAT),
558 Actb (forward GATGTATGAAGGCTTGGTC, reverse TGTGCACTTTATTGGTCTC).
559 Amplification was performed on a Bio-Rad CFX384 Touch (Bio-Rad, Hercules, CA, USA) in the Bauer
560 Core Facility at Harvard University using the following cycle settings: 95°C for 30min, followed by 40
561 cycles of 95°C for 15sec, 58°C for 30sec and 72°C for 15sec. Reactions were performed in duplicate with
562 the mean value used in statistical analyses. The relative amount of target mRNA was normalized to Actb
563 levels as an endogenous control gene, and data were analyzed according to the 2 $^{-\Delta CT}$ method.
564

565 **Statistical methods**

566 Statistical analyses and data visualization were performed using QIIME 2 (Bolyen et al., 2019) and/or R
567 version 4.1.1 (R Core Team, 2021). Error bars for all plots reflect means \pm standard error unless otherwise

568 specified. Student's t-tests, Mann-Whitney U tests, and MaAsLin2 analyses were corrected using the
569 Benjamini-Hochberg method for false discovery detection.

570

571 ***Data availability***

572 16S rRNA gene sequencing data have been deposited in the NCBI Sequence Read Archive (accession no.
573 PRJNA763652). Figure source data and additional study data are available upon request.

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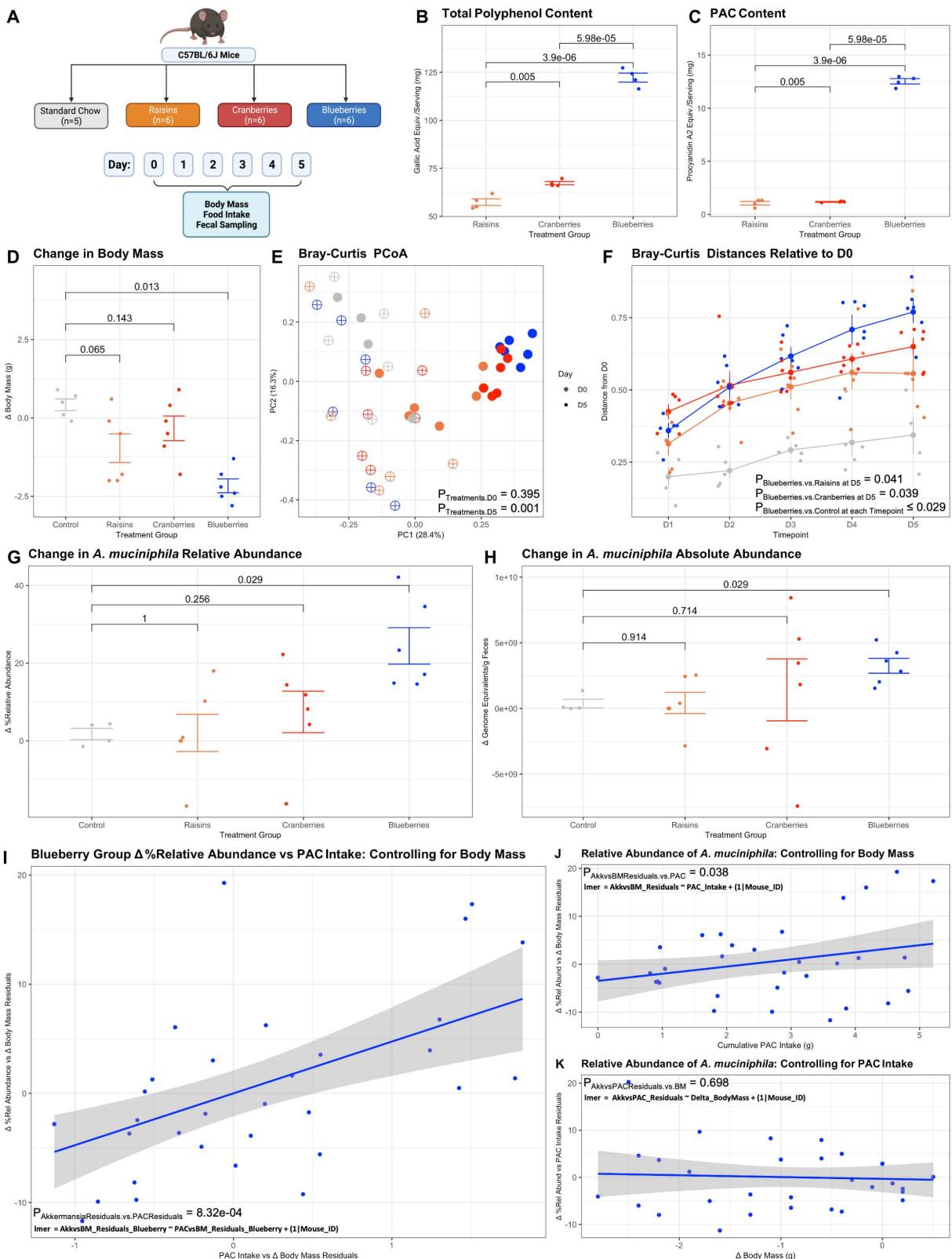


Figure 1: Dried blueberry consumption results in significant differences in body mass and gut microbial community structure in mice.

(A) We investigated the effects of dried berry consumption (blueberries, cranberries, and raisins) on host and microbial phenotypic effects in adult male C57BL/6J mice (n=6) compared to consumption of a standard chow control (n=5) over a 5-day dietary intervention. **(B)** Total polyphenol content (TPC) contained in a serving of each dried berry source (1/4 cup), measured using the Folin-Ciocalteau assay. **(C)** Proanthocyanidin (PAC) content per serving of dried berry source (1/4 cup), measured using the DMAC assay. **(D)** Change in body mass from Day 0 to Day 5. **(E)** Bray-Curtis PCoA plot for samples collected on Day 0 versus Day 5. Association of samples by treatment group determined at each timepoint using PERMANOVA. **(F)** Bray-Curtis distances of fecal samples at each timepoint for each mouse relative to its own baseline sample. **(G)** Change in relative abundance of *A. muciniphila* from Day 0 to Day 5. **(H)** Change in absolute abundance of *A. muciniphila* from Day 0 to Day 5. **(I)** Comparing the change in relative abundance of *A. muciniphila* in response to cumulative PAC intake and when controlling for their relationships to body mass. **(J)** The effect of cumulative PAC intake on *A. muciniphila* relative abundance when controlling for body mass. **(K)** The effect of changes in body mass on *A. muciniphila* relative abundance when controlling for PAC intake. **(B-C)** Statistics determined using student's t-test and Benjamini-Hochberg correction for false discovery rate. **(D, F-H)** Statistics determined using Mann-Whitney U test with Benjamini-Hochberg correction for false discovery rate. **(I-K)** Statistics determined using linear mixed effects model.

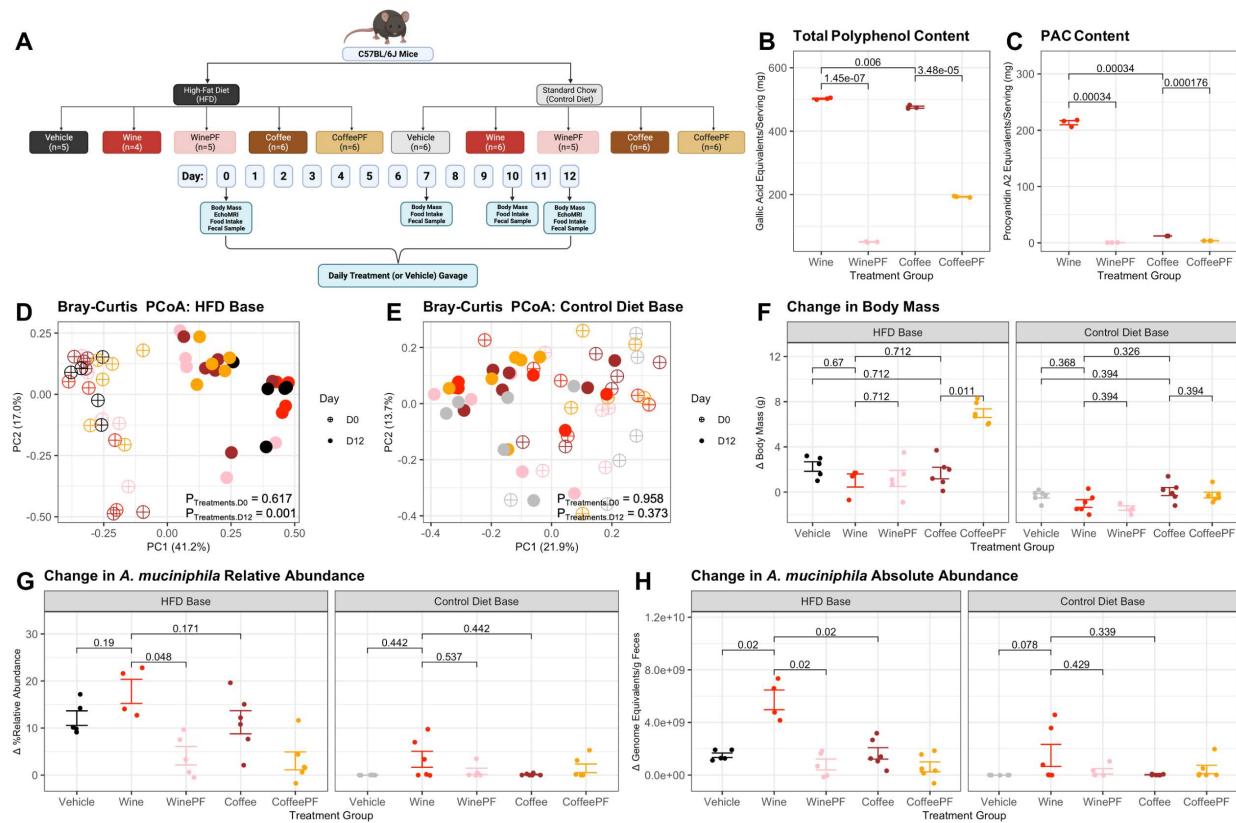


Figure 2: Wine versus coffee supplementation impacts microbial community structure under high-fat diet (HFD) but not control diet feeding in mice.

(A) Adult male C57BL/6J mice consuming either a HFD or control diet base were administered one of the following prebiotic treatments over a 12-day dietary intervention: alcohol-free wine (wine), alcohol-free and polyphenol-free wine (winePF), caffeine-free coffee (coffee), caffeine-free and polyphenol-free coffee (coffeePF), or a vehicle control (n=4-6 per condition). Treatments or vehicle controls were administered daily via oral gavage. **(B)** Total polyphenol content (TPC) per recommended serving of each treatment (5 oz of wine product or 6oz of coffee product) as measured by the Folin-Ciocalteau assay. **(C)** Proanthocyanidin (PAC) content per recommended serving of each treatment (5 oz of wine product or 6oz of coffee product) as measured by the DMAC assay. **(D-E)** Bray-Curtis PCoA plot for HFD treatments across Day 0 versus Day 12 of the study. **(E)** Bray-Curtis PCoA plot for control diet treatments across Day 0 versus Day 12 of the study. **(F)** Change in body mass from Day 0 to Day 12 across treatment groups administered each diet base. **(G)** Change in relative abundance of the *A. muciniphila* from Day 0 to Day 12 across treatment groups administered each diet base. **(H)** Change in absolute abundance of *A. muciniphila* from Day 0 to Day 12 across treatment groups administered each diet base. **(B-C)** Statistics determined using Student's t-test and Benjamini-Hochberg correction for false discovery rate. **(D-E)** Association of samples by treatment group determined at each timepoint using PERMANOVA. **(F-H)** Statistics determined using Mann-Whitney U test with Benjamini-Hochberg correction for false discovery rate.

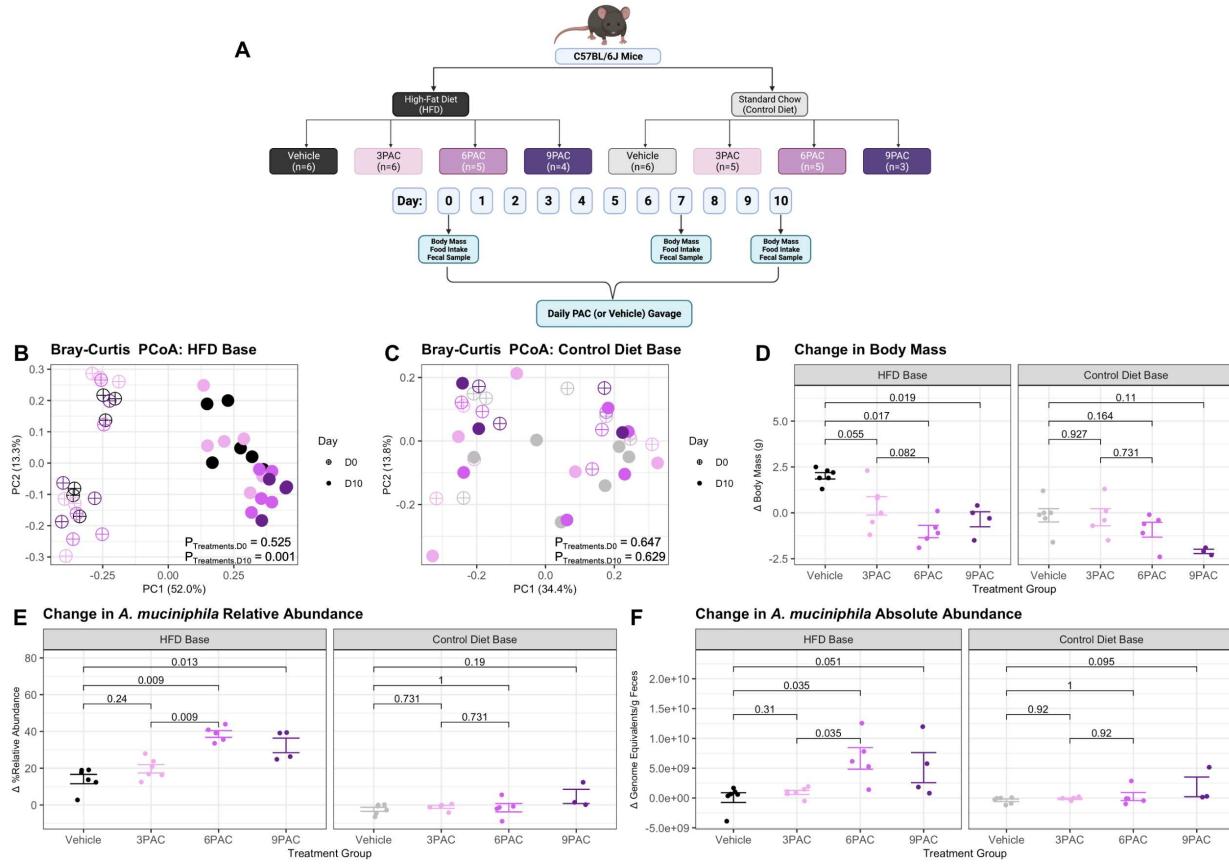


Figure 3: Proanthocyanidin (PAC) supplementation exhibits dose-dependent effects on body mass and microbial community structure under high-fat diet (HFD) but not control diet feeding in mice.

(A) Adult male C57BL/6J mice consuming either a HFD or control diet base were administered one of the following PAC doses per 25g of body mass over a 10-day intervention: 3mg (3PAC), 6mg (6PAC), 9mg (9PAC), or a PAC-free vehicle control (n=3-6 per condition). PAC doses or vehicle controls were administered daily via oral gavage. (B) Bray-Curtis PCoA plot for HFD conditions across Day 0 versus Day 10 of the study. (C) Bray-Curtis PCoA plot for control diet conditions across Day 0 versus Day 10 of the study. (D) Change in body mass from Day 0 to Day 10 across treatment groups administered each diet base. (E) Change in relative abundance of the *A. muciniphila* from Day 0 to Day 10 across treatment groups administered each diet base. (F) Change in absolute abundance of the *A. muciniphila* from Day 0 to Day 10 across treatment groups administered each diet base. (B-C) Association of samples by treatment group determined at each timepoint using PERMANOVA. (D-F) Statistics determined using Mann-Whitney U test with Benjamini-Hochberg correction for false discovery rate.

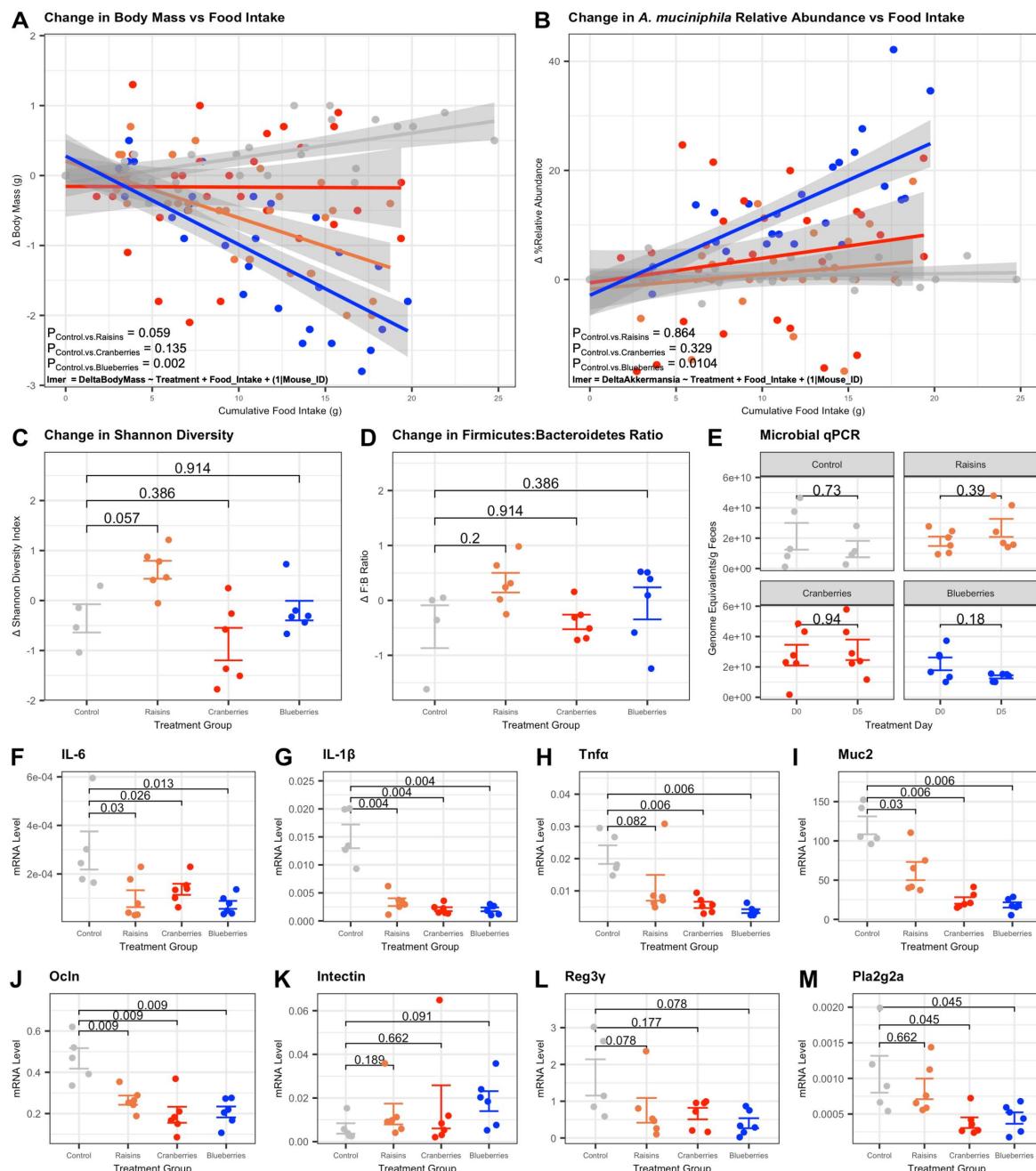
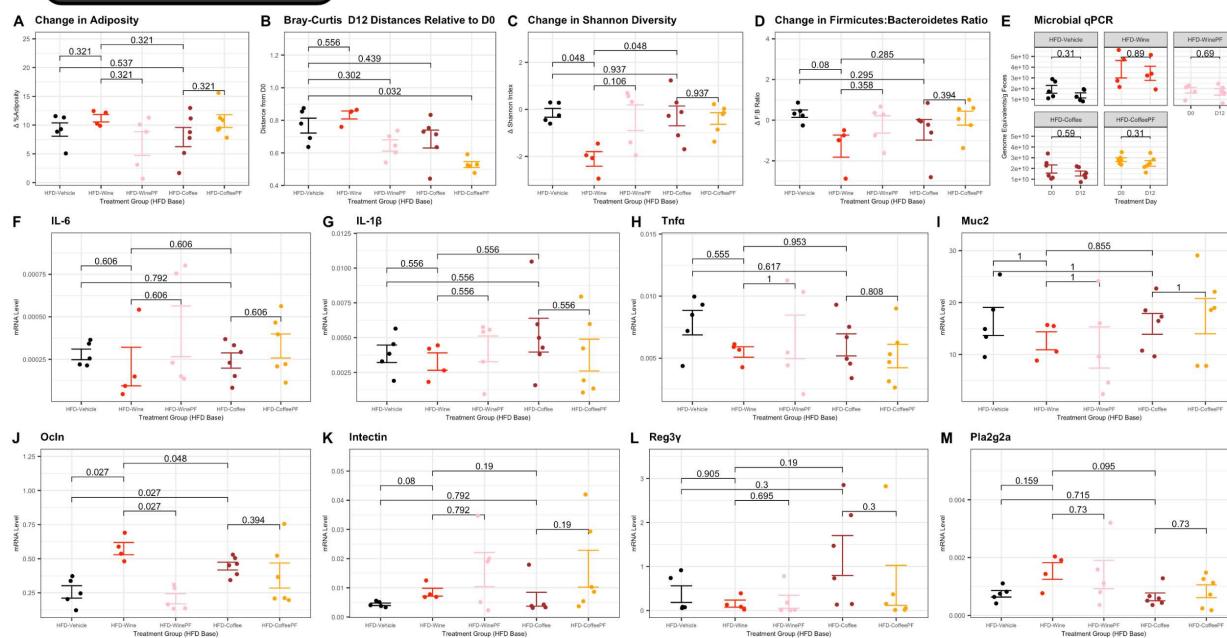


Figure S1: Longitudinal changes in host phenotype and gut microbial community structure across dried berry treatments.

(A) Change in body mass relative to cumulative food intake across treatment groups. **(B)** Change in the relative abundance of *A. muciniphila* relative to cumulative food intake across treatment groups. **(C)** Change in Shannon Diversity from Day 0 to Day 5. **(D)** Change in the ratio of relative abundance of Firmicutes to Bacteroidetes from Day 0 to Day 5. **(E)** Absolute bacterial abundance on Day 0 versus Day 5, determined using 16S qPCR. **(F-M)** Relative mRNA levels of indicated genes in proximal colon tissue collected from mice receiving each treatment group (tissue control group collected on Day 31 post-intervention, tissue from dried berry treatment groups collected on Day 5 post-intervention). Data represent qPCR of technical duplicates analyzed using the $2^{-\Delta CT}$ method, normalized to expression of the housekeeping gene *Actb*. **(A-B)** Statistics determined using linear mixed effects model. **(C-F)** Statistics determined using Mann-Whitney U test with Benjamini-Hochberg correction for false discovery rate.

HFD Base:



Control Diet Base:

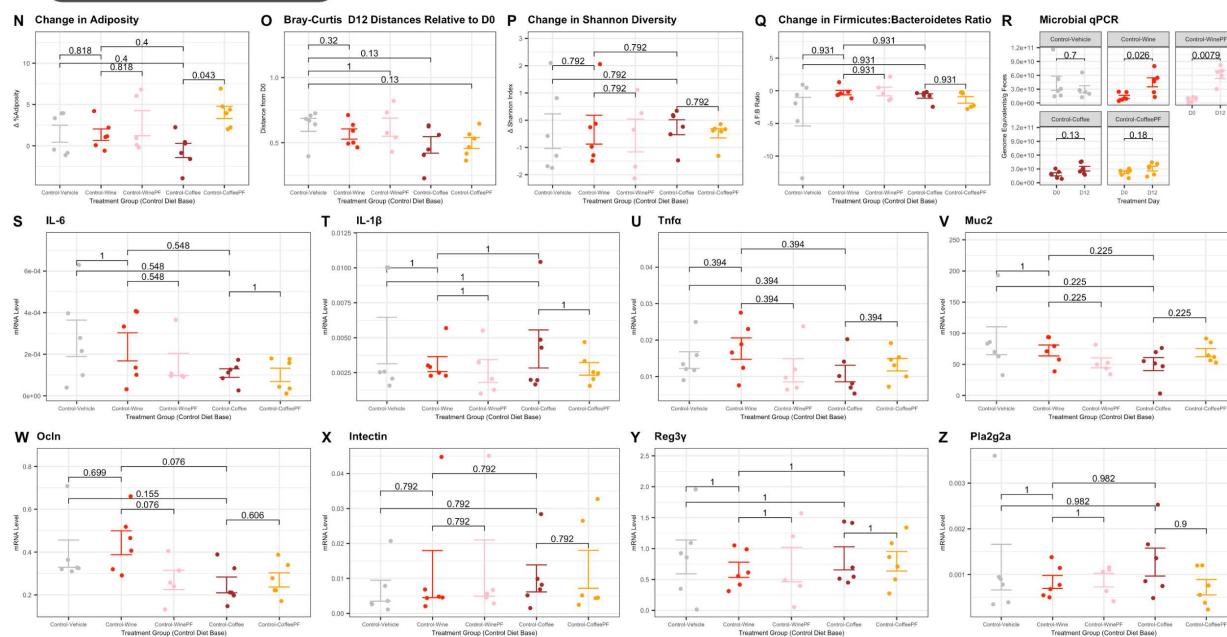
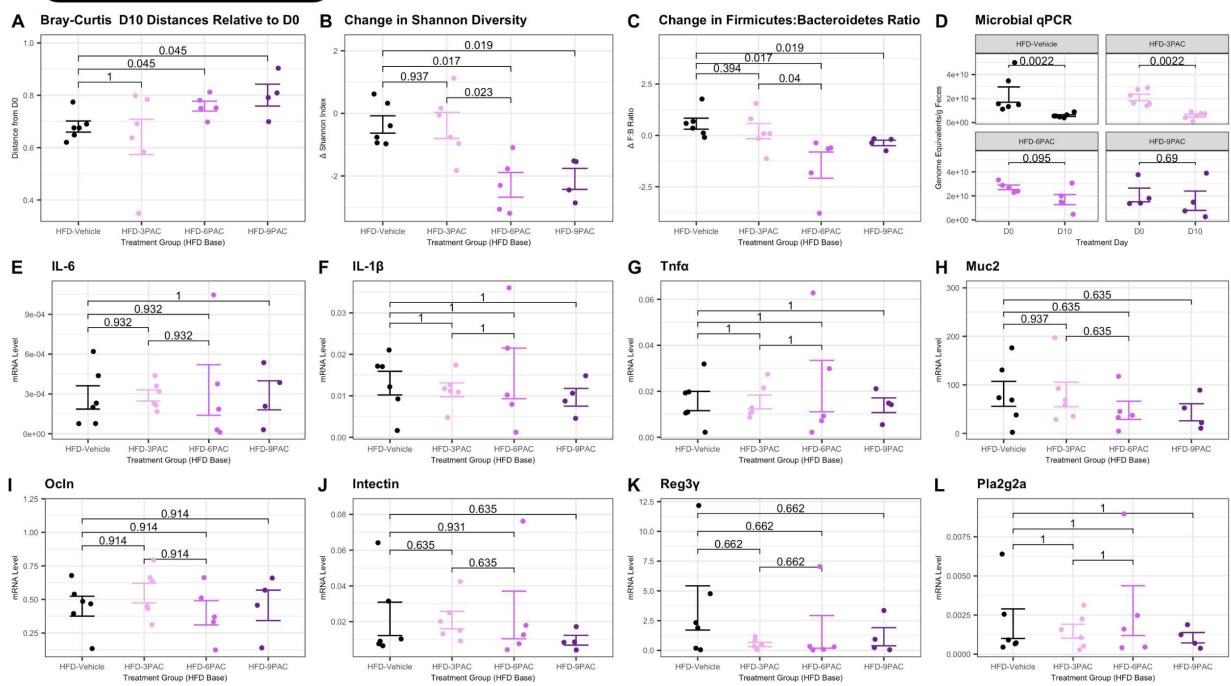


Figure S2: Effects of wine versus coffee supplementation under high-fat diet (HFD) or control diet feeding on host phenotype and microbial community structure.

(A) Change in percent adiposity from Day 0 to Day 12 across HFD conditions. **(B)** Bray-Curtis distances of fecal samples at Day 12 for mice across HFD conditions relative to their own baseline sample. **(C)** Change in Shannon Diversity from Day 0 to Day 12 across HFD conditions. **(D)** Change in the ratio of relative abundance of Firmicutes to Bacteroidetes from Day 0 to Day 10 across HFD conditions. **(E)** Absolute bacterial abundance on Day 0 vs Day 12 under HFD conditions, determined using 16S qPCR. **(F-M)** Relative mRNA levels of indicated genes in proximal colon tissue collected from mice across HFD conditions (tissue collected on Day 12). Data represent qPCR of technical duplicates analyzed using the $2^{-\Delta CT}$ method, normalized to the housekeeping gene *Actb*. **(N)** Change in percent adiposity from Day 0 to Day 12 across control diet conditions. **(O)** Bray-Curtis distances of fecal samples at Day 12 for mice across control diet conditions relative to their own baseline sample. **(P)** Change in Shannon Diversity from Day 0 to Day 12 across control diet conditions. **(Q)** Change in the ratio of relative abundance of Firmicutes to Bacteroidetes from Day 0 to Day 12 across control diet conditions. **(R)** Absolute bacterial abundance on Day 0 vs Day 12 under control diet conditions, determined using 16S qPCR. **(S-Z)** Relative mRNA levels of indicated genes in proximal colon tissue collected from mice across control diet conditions (tissue collected on Day 12). Data represent qPCR of technical duplicates analyzed using the $2^{-\Delta CT}$ method, normalized to expression of the housekeeping gene *Actb*. **(A-Z)** Statistics determined using Mann-Whitney U test with Benjamini-Hochberg correction for false discovery rate.

HFD Base:



Control Diet Base:

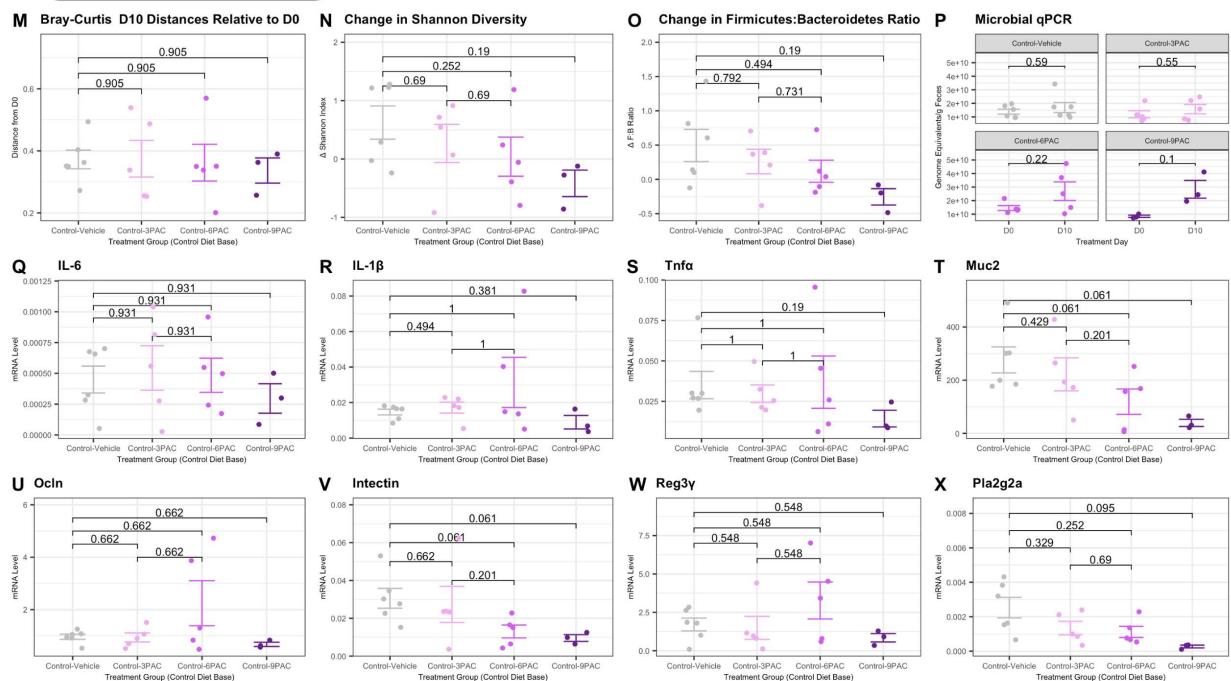


Figure S3: Effects of proanthocyanidin (PAC) supplementation under high-fat diet (HFD) or control diet feeding on host phenotype and gut microbial community structure.

(A) Bray-Curtis distances of fecal samples at Day 10 for mice across HFD conditions relative to their own baseline sample. **(B)** Change in Shannon Diversity from Day 0 to Day 12 across HFD conditions. **(C)** Change in the ratio of relative abundance of Firmicutes to Bacteroidetes across HFD conditions from Day 0 to Day 10. **(D)** Absolute bacterial abundance on Day 0 vs Day 10 under HFD conditions, determined using 16S qPCR. **(E-L)** Relative mRNA levels of indicated genes in proximal colon tissue collected from mice across HFD conditions (tissue collected on Day 10). Data represent qPCR of technical duplicates analyzed using the $2^{-\Delta CT}$ method, normalized to the housekeeping gene *Actb*. **(M)** Bray-Curtis distances of fecal samples at Day 10 for mice across control diet conditions relative to their own baseline sample. **(N)** Change in Shannon Diversity from Day 0 to Day 10 across control diet conditions. **(O)** Change in the ratio of relative abundance of Firmicutes to Bacteroidetes across control diet conditions from Day 0 to Day 10. **(P)** Absolute bacterial abundance on Day 0 vs Day 10 under control diet conditions, determined using 16S qPCR. **(Q-X)** Relative mRNA levels of indicated genes in proximal colon tissue collected from mice across control diet conditions (tissue collected on Day 10). Data represent qPCR of technical duplicates analyzed using the $2^{-\Delta CT}$ method, normalized to expression of the housekeeping gene *Actb*. **(A-X)** Statistics determined using Mann-Whitney U test with Benjamini-Hochberg correction for false discovery rate.