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1 Parallel signatures of mammalian domestication and human industrialization in the gut
2 microbiota
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15 **Abstract:** Domestication may have had convergent effects on the microbiota of domesticates and
16 humans through analogous ecological shifts. Comparing the gut microbiota of domestic and related
17 wild mammals plus humans and chimpanzees, we found consistent shifts in composition in
18 domestic animals and in humans from industrialized but not traditional societies. Reciprocal diet
19 switches in mice and canids demonstrated that diet played a dominant role in shaping the domestic
20 gut microbiota, with stronger responses in the member of the wild-domestic pair with higher
21 dietary and microbial diversity. Laboratory mice recovered wild-like microbial diversity and
22 responsiveness with experimental colonization. We conclude that domestication and
23 industrialization have similarly impacted the gut microbiota, emphasizing the utility of domestic
24 animal models and diets for understanding host-microbial interactions in rapidly changing
25 environments.

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26 Changes in industrialized human lifestyles have resulted in large shifts in the gut
27 microbiota relative to traditional populations or closely related primates, including reductions in
28 alpha-diversity and changes in composition (*1-4*) that have been implicated in the rise of various
29 metabolic and immunological diseases (*5-7*). Ecological differences between industrialized
30 humans and chimpanzees, and to a lesser extent between industrialized and non-industrialized
31 human populations, resemble those between domestic and wild animals, including shifts toward
32 non-seasonal calorically-dense diets, reduced physical activity, variations in movement and
33 density, changes in pathogen exposure and antibiotic use, and altered reproductive patterns (*8*).
34 Furthermore, the evolution of *Homo sapiens* has been argued to reflect self-domestication arising
35 due to selection for reduced social aggression (*9*). Despite these parallels, the global effects of
36 domestication on the gut microbiota and its relationship to the effects of human industrialization
37 remain unclear.

38 Notably, many of the altered ecological features experienced by domesticated animals and
39 industrialized humans have been independently observed to impact the gut microbiota, including
40 diet (*10, 11*) physical activity (*12, 13*) , the size and nature of social networks (*14, 15*), antibiotic
41 use (*16, 17*), and changes in birthing and lactation practices (*16, 18*). This overlap leads to the
42 predictions that (i) gut microbial communities will differ between domestic animals and their wild
43 counterparts, (ii) gut microbial communities of diverse domestic animals may exhibit convergent
44 characteristics in a microbial counterpart to the physiological domestication syndrome (*19*), and
45 (iii) gut microbial changes observed with domestication may parallel contrasts observed between
46 chimpanzees and industrialized humans. In addition, to the extent that domestication effects are
47 driven by ecology rather than host genotype, we should expect (iv) humans in traditional and
48 industrialized societies will differ, and (v) experimental control of environmental variables should
49 be able to overcome differences in the gut microbiota between closely related hosts.

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50 Here, we evaluate these predictions by reporting the effects of domestication on the
51 mammalian gut microbiota, comparing these effects to those of human industrialization, and
52 exploring the genetic and ecological forces driving these patterns. First, we characterized the fecal
53 microbiota of wild and domestic populations of nine pairs of artiodactyl, carnivore, lagomorph,
54 and rodent species (Fig. 1A) using 16S rRNA gene amplicon sequencing and qPCR. We found
55 consistent effects of domestication status on gut microbiota composition, despite observing no
56 single convergent profile. Domestication status contributed significantly to variation in microbial
57 communities ($P<0.001$, $R^2=0.16$, PERMANOVA), although the largest single factor was host pair
58 (e.g., pig/boar; $P<0.001$ $R^2=0.39$; Fig. 1B). Diet and digestive physiology were also determinants
59 ($P<0.001$, $R^2=0.11$ diet, $R^2=0.14$ physiology; Fig. S1), as seen in other surveys of mammals (*20*),
60 with effect sizes comparable to that of domestication status. Consistent with the idea that higher
61 ecological homogeneity may lead to more similar gut microbial communities in domesticates, we
62 found there was greater between-animal variability in wild gut communities than in domesticates
63 ($P=0.005$, $F=8.833$; permutation test for F).

64 To determine whether there was a consistent shift in microbial composition with
65 domestication, we calculated the difference between an individual's ordination coordinates and
66 the average of its host pair along the first and second NMDS axis. Domestic individuals were
67 typically further right (axis 1: $P<0.001$, Mann-Whitney U test; Fig. 1C) and further up (axis 2:
68 $P=0.007$; Fig. 1D) relative to the average of their host pair. Domestic species all displayed these
69 shifts, whether classified as laboratory, agricultural, or companion animals ($P<0.05$, Mann-
70 Whitney U tests; Fig. 1A, S2).

71 Microbial density quantified as copies of the 16S rRNA gene per gram of feces ($P=0.089$,
72 Mann-Whitney U test), OTU richness ($P=0.800$), and Shannon index ($P=0.200$; Fig. S3) did not
73 differ based on domestication status, indicating that the domestication signal overall is not

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74 primarily driven by species loss. By contrast, we observed changes in the abundances of certain
75 microbial taxa. Across host taxa, domestication was associated with higher abundances of the
76 phyla Bacteroidetes (P=0.023, Bonferroni-corrected Mann-Whitney U test; Fig. 1E, S3) and
77 Verrucomicrobia (P=0.001; Fig. S3). These phyla are known to be overrepresented in
78 industrialized compared with traditional human populations (*4*). Consistent with heightened
79 environmental exposure, wild animals generally had more diverse (P=0.001, Mann-Whitney U
80 test) and marginally more abundant (P=0.092; Fig. S3) communities of microbes recognized as
81 potential human pathogens. Among laboratory animals specifically, microbial richness (P=0.045,
82 Mann-Whitney U test), potential pathogen abundance (P<0.001), and pathogen richness (P<0.001)
83 were all substantially lower than among wild relatives, while total microbial load was higher
84 (P=0.006; Fig. S2). Agricultural animals had higher Shannon index values (P=0.001, Mann-
85 Whitney U test) and marginally higher pathogen abundances (P=0.067; Fig. S2) compared with
86 their wild counterparts. By contrast, companion animals did not differ significantly by
87 domestication status for microbial load, diversity, or pathogen metrics. The elevated pathogen
88 abundances found in wild populations overall may largely be ascribed to differences in laboratory
89 animals, which are maintained under conditions that minimize the likelihood of infection. Under
90 natural conditions, however, the domestic microbiota may exhibit reduced colonization resistance
91 or immune system functioning (*21, 22*), resulting in higher pathogen colonization, as observed in
92 agricultural animals.

93 Given the hypothesis that *Homo sapiens* has undergone a process of self-domestication (*9,*
94 *19*), we next tested whether the gut microbial communities of industrialized humans and
95 chimpanzees exhibit parallel shifts to those observed between domestic animals and their wild
96 counterparts when compared in the same ordination space. Indeed, this is what we found (P<0.001,
97 Mann-Whitney U tests; Fig. 1C, 1D). Microbial load (P=0.002, Mann-Whitney U test) and

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98 Shannon index (P=0.018; Fig. S3) also differed between industrialized humans and chimpanzees,
99 with industrialized humans harboring microbial communities with substantially lower alpha-
100 diversity. Consistent with the greater evolutionary and profound ecological distance between
101 humans and chimpanzees (*2*), the magnitude of the microbial difference between industrialized
102 humans and chimpanzees exceeded that observed for other animal pairs. To estimate the
103 divergence attributable to ecology versus host genotype, we proceeded to compare the gut
104 microbial communities of humans living in industrialized versus traditional societies. Reanalysis
105 of our cross-species comparison to include published data on human populations in rural Malawi
106 and Venezuela (*23*) (see *Methods*) found that the gut microbial communities of these traditional
107 populations differed substantially from those of two independent U.S. samples, clustering more
108 closely to those of chimpanzees (Fig. S4). These data indicate that the human gut microbiota does
109 not carry a global signal of domestication, as would be predicted under the human self-
110 domestication hypothesis. Rather, they suggest that gut microbial responses to domestication and
111 industrialization are more likely driven by common ecological factors, a conclusion further
112 supported by the observation that domestic animals were significantly more similar to those of
113 industrialized humans than their wild animal counterparts (P=0.002, Mann-Whitney U test).
114 Notably, the gut microbial communities of domestic animals and industrialized humans most
115 closely resembled one another for companion and laboratory animals (P<0.001, Kruskal-Wallis
116 test; Fig. S2), presumably reflecting their greater degree of overlap in ecological variables and
117 physical contact (*24*).

118 Importantly, the observation that gut microbial divergence is restricted to industrialized
119 populations implicates recent ecological changes as opposed to ecological changes with deeper
120 roots in human evolution. Many recent ecological changes involve accelerations of basic patterns
121 established during the evolution of *Homo*, including increased proportion of calories from fat and

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122 protein, increased dependence on animal source foods, and extensive food processing by thermal
123 and non-thermal means (*25*). Other ecological changes are likely specific to industrialization,
124 including reduced physical activity and antibiotic use. Further work will be required to illuminate
125 the combination of ecological factors driving similarities between the domesticated and
126 industrialized microbial profiles.

127 To begin to tease apart these ecological drivers, we performed a series of reciprocal diet
128 experiments that tested the extent to which gut microbial signatures of domestic-wild pairs could
129 be recapitulated and reversed solely by the administration of domestic versus wild diets. We first
130 conducted a fully factorial experiment in which wild-caught and laboratory mice (*Mus musculus*)
131 were maintained for 28 days on wild or domestic diets (Fig. 2A, Table S1). Overall, we found that
132 host genotype explained the largest amount of variation in composition (P<0.001, R^2=0.173,
133 PERMANOVA), but diet (P<0.001, R^2=0.042) and a genotype by diet interaction term (P<0.001,
134 R^2=0.020) were also significant (Fig. 2B, S5). Experimental groups varied in their microbial
135 responsiveness over the course of the experiment (axis 1: P=0.063, axis 2: P<0.001, Kruskal-
136 Wallis tests; Fig. 2C, S5). Generally, the microbiota of $Wild_G/Dom_D$ mice moved toward the
137 Dom_G/Dom_D mouse average community, the $Dom_G/Wild_D$ microbiota moved in the opposite
138 direction, and those of $Wild_G/Wild_D$ and Dom_G/Dom_D mice did not shift (Fig. 2B). Over the course
139 of the experiment, Shannon index values also changed significantly across treatment groups
140 (P=0.005, Kruskal-Wallis test), with $Dom_G/Wild_D$ mice becoming significantly more diverse
141 (P=0.002, one-sample Wilcoxon test) despite initial differences in alpha-diversity between wild
142 and domestic mice (P=0.009, Mann Whitney U test; Fig. S6).

143 Neither diet nor host genotype were associated with differences in microbial density over
144 the experiment (P=0.272, Kruskal-Wallis test; Fig. S6), but it is notable that the total amount of
145 feces produced, and thus likely the total number of bacteria, was lower in each host genotype when

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fed wild diet (P<0.001, Kruskal-Wallis test; Fig. S6). Despite similar trends in fecal production between the experimental groups, energy harvest responses differed markedly between experimental groups (P<0.001, Kruskal-Wallis test; Fig. S6). While wild mice were equally efficient consumers of both diets, laboratory mice captured 15% fewer calories when consuming the wild versus domestic diet. Nonetheless, weight gain in laboratory mice did not differ between diet groups, while $\text{Wild}_G/\text{Dom}_D$ mice tended to gain weight over the course of the experiment (P=0.250, one-sample Wilcoxon test; Fig. S6). Interestingly, the asymmetry in energy harvest between genotypes was also reflected in differential microbial responses to reciprocal diets. Whereas the microbial communities of $\text{Wild}_G/\text{Dom}_D$ mice eventually largely recapitulated those of untreated Dom_G mice, the microbial communities of $\text{Dom}_G/\text{Wild}_D$ mice remained distinct from untreated Wild_G mice throughout the experiment (P=0.042, Mann-Whitney U test; Fig. 2B). The inability to foster a wild-type microbiota may underpin the reduced digestive efficiency of the $\text{Dom}_G/\text{Wild}_D$ mice.

We hypothesized that these asymmetries were due to past extinction of relevant strains from laboratory microbial communities and no dispersal source of replacement strains (*26*). Therefore, we tested whether experimental dispersal from a wild microbial community in conjunction with feeding a wild diet could support a fully wild microbial community in laboratory mice (Fig. 3A). A single colonization treatment with a wild mouse cecal community (via gavage) led to significant shifts in the microbial community (Fig. 3B, S7), resulting in closer resemblance to the wild donor (P<0.001, Mann-Whitney U test; Fig. 3C). While laboratory mice fed a wild diet but given a control gavage (PBS) also moved toward the donor along NMDS axis 1 (P=0.002, one-sample Wilcoxon test; Fig. 3D), reflecting the influence of diet, the magnitude of the shift following the experimental colonization was substantially greater (P<0.001, Kruskal-Wallis test). There were no apparent differences in these shifts based on diet treatment among colonized mice

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170 (P=0.182, Mann-Whitney U test). Colonization with a wild community led to an increase in alpha-
171 diversity as measured by the Shannon index (P=0.042, Kruskal-Wallis test), and wild diet
172 treatment led to reductions in fecal production (P<0.001; Fig. S7). Although all mice exhibited an
173 increase in load over the course of the experiment (P<0.05, one-sample Wilcoxon tests),
174 colonization with a wild community did not lead to higher loads overall (P=0.742, Kruskal-Wallis
175 test; Fig. S7). This result suggests that differences observed with treatment reflected shifts in gut
176 microbial community structure rather than simple augmentation.

177 To test if these findings were generalizable to non-laboratory animals, we conducted an
178 analogous reciprocal diet experiment in a captive sympatric population of wolves and dogs (Fig.
179 4A). We tracked gut microbial dynamics in these canids for one week on their standard diet (raw
180 carcasses or commercial dog food, respectively) and one week on the reciprocal diet. As in the
181 mouse experiment, we found that host genotype explained the largest amount of variation in gut
182 microbiota composition (P<0.001, R^2=0.098, PERMANOVA), but diet (P<0.001, R^2=0.058) and
183 a genotype by diet interaction term (P<0.001, R^2=0.028) were also significant (Fig. 4B, S8). There
184 were significant differences between experimental groups in the magnitude of their shifts along
185 the first (P<0.001, Kruskal-Wallis test; Fig. 4C) and second (P=0.045; Fig. S8) NMDS axes over
186 the experimental periods. As in the mouse experiments, we observed animals on reciprocal diet
187 treatments moved significantly toward the diet control of the other species (P<0.05, one-sample
188 Wilcoxon tests; Fig. 4B), while the control animals did not shift predictably (P>0.100).

189 Again, we observed an asymmetry in the degree of microbial composition change between
190 domestic and wild animals. On experimental diets, dogs and wolves differed significantly in their
191 dissimilarity to diet controls (P<0.001, Kruskal-Wallis test, Fig. 4D), with the gut microbial
192 communities of dogs fed raw carcasses resembling those of wolves at baseline but the gut
193 microbial communities of wolves fed dog food remaining distinct from those of dogs at baseline

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194 (P=0.001, Mann-Whitney U test). The difference in the direction of asymmetry between the mouse
195 and canid experiments may be explained by the different trends in the diet ecology between
196 omnivores and carnivores during domestication. Carnivores, through the addition of extensive
197 carbohydrates to their diet (*27*), likely encounter more diverse diets in captivity than in the wild,
198 whereas herbivores and omnivores eat a smaller number of plant species or even just a single feed
199 mix. Supporting this, we found dogs initially had significantly higher OTU richness (P<0.001) and
200 Shannon index (P=0.003) than wolves (Fig. S9), but that reciprocal diets led to a switch in diversity
201 (richness: P=0.014, Shannon index: P=0.027, Mann-Whitney U tests), with wolves becoming more
202 diverse on dog food while dogs lost diversity on raw carcasses (Fig. 4E).

203 Our reciprocal diet experiments in mice and canids confirm that ecology plays a
204 predominant role in shaping the domestic gut microbiota. Moreover, that the effects of a single
205 ecological variable like diet were sufficiently profound to outweigh those of host genotype
206 suggests that suites of ecological variables changing together, such as during domestication or
207 industrialization, may have collectively exerted an even larger influence. However, microbiota
208 changes were certainly not the only pathway for domesticating animals to respond to changing
209 ecological factors. For example, in dogs, genetic changes have enhanced starch digestion (*27*). The
210 increased microbial diversity and shifts in microbial composition that we observed in dogs may
211 likewise contribute to carbohydrate digestion and may have been particularly important early in
212 domestication, before host evolution occurred, although that hypothesis remains to be tested.
213 Notably, the microbiota has been found to supplement evolutionary responses during dietary niche
214 expansion in wild animals that consume plants high in toxins (*28*). As such, the changes observed
215 in domestic animals are not necessarily maladaptive, as the industrialized human microbiome is
216 often characterized to be (*29*). Beyond host support of microbiota that can better digest a domestic
217 diet, humans may have selected for animals harboring a microbiota that helped them grow and

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218 reproduce well on such diets. Specialization for microbial performance domestic diets may have
219 come at the cost of broader digestive capacity, as seen in the domestic mouse microbiota, which
220 was better at harvesting energy from domestic diets than from wild diets (Fig. S6). Future studies
221 examining the trade-offs between microbially-mediated functions, like digestive capacity,
222 reproduction, and immunity, will help to illuminate the complex selection pressures shaping the
223 domestic holobiont.

224 Taken together, our data reveal strong parallels between the gut microbial signatures of
225 domestication and industrialization, most likely driven by convergent changes in ecology,
226 including diet. Because laboratory mice demonstrate some of the largest overall differences
227 relative to their wild counterparts, and in part emulate the variation observed between
228 industrialized humans and closely related primates, their translational potential as models for
229 studying the gut microbiota of industrialized populations may be greater than currently
230 appreciated. However, our data also suggest that laboratory animals may not be broadly
231 representative of natural host-microbe interactions or their evolutionary history (*30*). Nevertheless,
232 that laboratory mice were permissive of recolonization by wild strains indicates that the local
233 extinctions that occurred during domestication and/or generations in captivity can potentially be
234 mitigated. Previous work has relied on germfree mice colonized with a wild microbiota but fed
235 standard laboratory chow (*21*). A combination of these approaches— adding wild community
236 members and feeding wild diet—would be expected to best support a wild microbiota in laboratory
237 mice. A wild-microbiota laboratory-genotype model could be especially useful for studying
238 infection challenges, disentangling host gene versus microbiota contributions to disease
239 phenotypes, and testing for coevolution between host and microbes.

240 More generally, our data add to growing evidence that the gut microbiota is finely tuned to
241 variations in the environment, affording at once an opportunity for host-microbial mismatch and

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242 an opportunity for rapid microbiota-mediated host adaptation to novel environments (*31*). Further
243 work to characterize the ecological significance of gut microbial plasticity will help reveal the
244 fundamental nature of the host-microbial relationship, the conditions under which plasticity is
245 beneficial versus detrimental, and the ecological conditions promoting cooperative, commensal,
246 and competitive dynamics.

247 **Materials and Methods**

248 <u>Fecal sample collection</u>

249 Gut microbiota samples from a range of non-human species were collected by authors or
250 collaborators primarily from feces. Fecal samples from non-human mammals were collected from
251 the ground within seconds to hours of production. In the case of artiodactyl, carnivore, lagomorph,
252 and rodent feces, this approach precluded the need for institutional approval. Chimpanzee fecal
253 samples were collected under the approval of the UNM IACUC (Protocol 18-200739-MC) and
254 with permission of the Uganda Wildlife Authority and Uganda National Council for Science and
255 Technology. Human samples were self-collected by healthy study participants after providing
256 written informed consent under the approval of the Harvard University IRB (Protocol 17-1016).
257 All samples were flash-frozen or preserved in ethanol prior to permanent storage at -80°C.
258

259 *Domestic animals*

260 Domestic sheep (*Ovis aries*; N=11, 10 female), cattle (*Bos taurus*; N=10, sex unknown), and pig
261 (*Sus scrofa domesticus*; N=9, sex unknown) fecal samples were collected from a farm in Vershire,
262 Vermont. Domestic alpaca (*Vicugna pacos*; N=8, sex unknown) and domestic sheep (*Ovis aries*;
263 N=2, 2 female), fecal samples were collected from a farm in Groton, Massachusetts. Domestic
264 rabbit (*Oryctolagus cuniculus*; N=11, 4 female) fecal samples were collected from a shelter in
265 Billerica, Massachusetts. Mouse (*Mus musculus*, N=9, 0 female), rat (*Rattus norvegicus*; N=6, sex

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266 unknown), and guinea pig (*Cavia porcellus;* N=10, 0 female) fecal samples were collected from
267 animals in Harvard laboratory facilities. Dog (*Canis lupus familiaris*; N=7, 4 female) fecal samples
268 were collected from personal pets in Stacy, Minnesota.
269

270 *Wild animals*

271 Wild boar (*Sus scrofa*; N=16, 5 female) fecal samples were collected from adults and juveniles in
272 southeastern Alabama during fall 2017. Rat (*Rattus norvegicus*; N=10, 3 female) gut samples from
273 adults and juveniles were collected directly from the colon shortly following trapping in New York
274 City between February and May 2017 (*32*). Bison (*Bison bison*, N=20, sex unknown) fecal samples
275 were collected from a semi-free-ranging population in Elk Island National Park, Alberta, Canada
276 (*33*). Wild house mouse (*Mus musculus*, N=9, sex unknown) fecal samples were collected from
277 live-trapped animals in the Boston, Massachusetts area during winter 2018. Pursuant to
278 Massachusetts state law, permits were not necessary to trap animals indoors. Wild European rabbit
279 (*Oryctolagus cuniculus*; N=12, sex unknown) fecal samples were collected in Mértola, Portugal
280 during spring 2018. Bighorn sheep (*Ovis canadensis*; N=10, sex unknown) fecal samples were
281 collected during 2017 and 2018 in Wyoming. Vicuña (*Vicugna vicugna*; N=4, 2 female) fecal
282 samples were collected during spring 2018 from a captive population in Santiago, Chile that was
283 free-grazing but supplemented with hay. Wild guinea pig (*Cavia tschudii*, N=11, sex unknown)
284 fecal samples were collected at a facility in Lima, Peru during spring 2018. Wolf (*Canis lupus*;
285 N=9, sex unknown) fecal samples were collected during fall 2017 from captive packs at the
286 Wildlife Science Center in Stacy, Minnesota fed an exclusively raw diet. Wild chimpanzee (*Pan*
287 *troglodytes schweinfurthii*, N=7, 7 female) fecal samples were collected between September 2015
288 and January 2016 from adult members of the Kanyawara community in Kibale, Uganda.
289

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290 *Human*

291 Fecal samples were collected from healthy adult humans (N=7, 5 female) residing in the
292 Cambridge, Massachusetts area. All participants were provided with sterile study kits, and self-
293 collected fecal samples during the same 3-day period in December 2017. During this period,
294 participants freely consumed their habitual diets. Fecal samples were immediately stored at -20°C
295 and were transferred within 24 hours to permanent storage at -80°C.

296

297 *Human sample meta-analysis*

298 To compare the microbial differences between wild and domestic animals or US humans and
299 chimpanzees with other human populations, we also performed analyses including all of the
300 samples outlined above and a subset of published data from Yatsunenko and colleagues (*23*). We
301 subsampled 7 adult females from their Malawian, Venezuelan, and American populations,
302 downloading the data from MG-RAST. All sequences were trimmed to 100 bp before analysis (see
303 16S rRNA gene analysis below), and the published dataset was rarefied to 100,000 reads per
304 sample to ensure comparable sequencing depth with our data.

305

306 Animal experiments

307 *Wild mouse capture*

308 *Mus musculus* were introduced to North America from Western Europe and are now commonly
309 found in commensal settings (*34*). We set out Sherman live traps in the evenings in buildings and
310 barns during February 2018. Traps were baited with peanut butter and a chunk of fruit and outfitted
311 with sufficient bedding and food to sustain an adult mouse for at least 48 hr. They were checked
312 the following morning to minimize time spent in the traps. Rodents were immediately transferred
313 from their traps to a plastic bag, and unwanted rodent species were released immediately. Mice

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314 that were identified as *Mus musculus* (rather than *Peromyscus spp.*, also common in
315 Massachusetts) were transferred to temporary cages for transport to lab facilities. At time of
316 capture, we collected fecal samples and body swabs for zoonoses testing by Charles River. The
317 only agent of concern found was fur mites. Because animals were not treated for parasites or
318 pathogens in order to increase maintenance of the wild-state microbiota, they were housed under
319 non-SPF conditions at Harvard's Concord Field Station. Mice were allowed at least three days to
320 adjust to laboratory conditions without handling and provided with a wild mouse diet [a mix of
321 bird seed (Wagner's Eastern Regional Blend Deluxe Wild Bird Food) and freeze-dried mealworms;
322 Table S1] before the beginning of the experiment. All mice were housed singly from the time of
323 arrival at the Concord Field Station and had access to water and food ad libitum.
324
325 *Wild/laboratory mice reciprocal diet experiment*
326 A total of 10 wild mice were captured for this experiment. Of these, 2 were deemed too young for
327 inclusion in the study, 1 died before beginning the experiment, and 1 died during the course of the
328 experiment. As a result, we collected 6 wild mice ($Wild_G$) that were included in the full study. In
329 addition to the wild mice, male C57BL/6 mice 10-12 weeks of age with a conventional microbiota
330 were purchased from Charles River Laboratories for inclusion in the study (Dom_G). All mouse
331 experiments were conducted in accordance with the National Institutes of Health Guide for the
332 Care and Use of Laboratory Animals using protocols approved by the Harvard University
333 Institutional Animal Care & Use Committee (protocol number 17-11-315). All mice were housed
334 singly from the time of arrival at the Concord Field Station and had access to water and food ad
335 libitum. Mice were provided nesting material and plastic enrichment housing atop corncob
336 bedding. The mice were maintained in a room with natural light cycles kept at 20-22°C.
337

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338 Mice, both wild and laboratory, were randomly assigned to one of two dietary treatment groups
339 (N=10 laboratory mice or 3 wild mice per group). The first group (domestic diet: Dom_D) was
340 provided *ad libitum* mouse chow (Prolab Isopro RMH 3000) in hanging food hoppers, as is
341 standard in mouse studies. The second group (wild diet: $Wild_D$) was provided a mix of bird seed
342 (Wagner's Eastern Regional Blend Deluxe Wild Bird Food) and freeze-dried mealworms (Table
343 S1) in excess of predicted consumption. The food was placed in the corncob bedding to simulate
344 foraging.
345
346 Before initiating the dietary interventions, all individuals were weighed and multiple fecal samples
347 were collected. The mice were then returned to a new, clean cage with the treatment diet present.
348 Over the next week, fecal samples and weights were collected daily for each mouse. The amount
349 of food remaining was weighed and additional wild diet was added daily. One week after beginning
350 the experiment, mice were weighed and fecal samples collected then mice were moved to clean
351 cages. Weights and fecal samples were henceforth collected weekly (day 14, 21, 28) until the end
352 of the experiment, although additional food was added biweekly for individuals assigned to the
353 wild diet treatment. Additional chow was added to hoppers for individuals assigned to the
354 conventional diet treatment, and all water bottles were refilled as necessary. At the end of each
355 week, cage bedding was collected and sifted to quantify uneaten food ($Wild_D$) and total weekly
356 fecal production (all groups during week 3), as well as to provide fecal samples for bomb
357 calorimetry (6050 Calorimeter, Parr). All calorimetry results were adjusted for the average weekly
358 fecal production and average weekly food intake of each experimental group. At the end of the
359 experiment (day 28-30), mice were humanely sacrificed via CO_2 euthanasia.
360
361 *Wild/laboratory mice gavage experiment*

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362 Thirty 10 week old male C57BL/6 mice with a native microbiota were purchased from Charles
363 River Laboratories for inclusion in the study. Mice were cohoused in litter groups of 3-4 until
364 beginning the study. Cage groups were spread across the treatment groups, with individuals
365 randomly assigned to a diet and colonization treatment. There were three treatment groups: wild
366 colonized/wild diet ($Wild_C/Wild_D$); wild colonized/domestic diet ($Wild_C/Dom_D$); or PBS
367 gavage/wild diet ($PBS_C/Wild_D$). The latter served as a colonization control, emulating the
368 $Dom_G/Wild_D$ group from the reciprocal diet mouse experiment.

369

370 On the first day of study, fecal samples were collected from each mouse and the mice were weighed
371 before colonization. For mice receiving a wild microbiota, we experimentally colonized them with
372 cecal contents collected from one randomly selected $Wild_G/Wild_D$ individual in the wild/laboratory
373 experiment (see above). The cecal contents were prepared following (*21*). In short, frozen cecal
374 contents were resuspended in reduced PBS (1:1 g:ml) under anaerobic conditions then diluted
375 1:30. Each recipient mouse received a single dose of 100 to 150ul cecal solution via oral gavage.
376 PBS control mice received 100 to 150ul reduced PBS via oral gavage.

377

378 Following gavage, mice were transferred to single housing in new, clean cages with the treatment
379 diet present. Mice receiving domestic diet were provided ad libitum mouse chow (Prolab Isopro
380 RMH 3000) in hanging food hoppers. Wild mouse diet consisted of a mix of bird seed (Wagner's
381 Eastern Regional Blend Deluxe Wild Bird Food) and freeze-dried mealworms (Table S1), which
382 was provided in excess of predicted consumption and placed in the corncob bedding to simulate
383 foraging. All mice were provided with nesting material and plastic enrichment housing atop
384 corncob bedding.

385

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386 Additional fecal samples and weights were collected on days 1, 2, and 8 following gavage. After
387 weights and fecal samples were collected on day 8, mice were humanely sacrificed via CO_2
388 euthanasia. At the end of the experiment, cage material was collected and sifted to quantify uneaten
389 food ($Wild_D$) and total weekly fecal production (all groups).
390
391 *Wolf/dog reciprocal diet experiment*
392 Ten wolves (*Canis lupus*) and nine dogs (*Canis familiaris*) participated in the study. Wild-caught
393 or captive born wolves lived in packs ranging in size from 2-6 at the Wildlife Science Center
394 (WSC; Stacy, MN). They were exposed to natural light cycles and weather conditions, with access
395 to shelters and wolf-dug dens in their enclosures. Wolves had ad libitum access to water. Dogs
396 enrolled in this study were privately owned and were recruited to participate through their owners.
397 Dogs were kept in their typical environment throughout the experiment. All canid experimentation
398 was approved by the WSC IACUC (protocol number HAR-001). Wolves were enrolled in the
399 study from Dec. 5 – Dec. 20 2018; dogs from Dec. 24 2018 – Jan. 8 2019.
400
401 Every day of the study, animals were given inert glass beads via treats (~15g raw meatballs for
402 wolves). The beads can be passed naturally without harm to the animal and allowed for source
403 identification for fecal samples in cohoused animals. Fecal samples were collected daily in a sterile
404 manner then moved to -20°C storage before long-term storage at -80°C. For the first week of the
405 experiment all animals received a control diet that matched their genetic background (Table S1)—
406 raw chicken parts (4lbs/animal) for wolves ($Wild_G$/$Wild_D$) and commercial dog food (Nutrisource
407 Lamb Meal and Peas Grain Free) for dogs (Dom_G/Dom_D). Fecal samples were collected at least
408 once daily from wolf enclosures and the dogs' home environments without handling the animals.
409 On day 8, wolves were provided no new food, but were able to complete consumption of

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410 previously provided diet materials. Fecal samples collected on this day were considered baseline
411 samples for the next arm of the experiment. Beginning on day 8, a week of reciprocal diet feeding
412 was commenced. During this period, wolves were fed commercial dog food ($Wild_G/Dom_D$) and
413 dogs were fed raw chicken parts ($Dom_G/Wild_D$); glass beads continued to be administered via treats
414 thus wolves received small amounts (~15g) of raw meat daily. Daily fecal samples were again
415 collected. Following completion of the study, animals were returned to their standard diet.
416

417 16S rRNA gene analysis

418 *Extraction*

419 Following collection during observational or experimental animal work, fecal samples were
420 temporarily stored at -20°C then moved to -80°C for long term storage. Individual mouse pellets
421 or approximately 0.1g feces were used for DNA extraction using the E.Z.N.A. Soil DNA Kit
422 (Omega) following manufacturer's instructions.
423

424 *Sequencing*

425 We performed 16S rRNA gene amplicon sequencing on fecal samples to determine gut microbial
426 community structure. We used custom barcoded primers (*35*) targeting the 515F to 806Rb region
427 of the 16S rRNA gene following published protocols (*35-37*). Sequencing was conducted on an
428 Illumina HiSeq with single end 150bp reads in the Bauer Core Facility at Harvard University. Data
429 was processed using Qiime1.8 commands for closed reference OTU picking with 97% similarity.
430 Microbial taxonomy was assigned in reference to the GreenGenes database. We obtained
431 158611±109567 assigned reads per sample.
432

433 *qPCR*

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434 To estimate total bacterial load, quantitative PCR (qPCR) was performed on fecal DNA using the
435 same primers as used for sequencing. qPCR assays were run using PerfeCTa SYBR Green
436 SuperMix Reaction Mix (QuantaBio) on a BioRad CFX384 Touch (Applied Biosystems, Foster
437 City, CA) in the Bauer Core Facility at Harvard University. Cycle-threshold values were
438 standardized against a dilution curve of known concentration and then adjusted for the weight of
439 fecal matter extracted.
440
441 Statistical analyses
442 All statistical analyses were carried out in R (R core team, ver. 3.3). Alpha-diversity (Shannon
443 index, OTU richness) and beta-diversity (Bray-Curtis) metrics were calculated using the vegan
444 package (*38*). All statistical tests performed were non-parametric. Permutational MANOVA
445 (PERMANOVA) was carried out with the adonis function in vegan. Variability in a species'
446 microbial community composition was calculated with the permutest and betadisper functions in
447 vegan. For changes in phylum-level abundance, relative abundance data were multiplied by
448 bacterial load measurements; a Bonferroni correction for multiple hypothesis correction was then
449 applied to all test results. Phyla were included if they had an average abundance of at least 0.01%
450 across all samples.
451
452 Potential human pathogens were identified following published methods (*39, 40*). In short, we
453 obtained a list of potential human pathogens, compiled by Kembel and colleagues (*39*), then
454 manually compared that list to the taxa identified to genus or species level in analysis. A subset of
455 the data containing only these species was then analyzed for diversity with the same methods used
456 for the total dataset.
457

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458 To determine the consistency of gut microbial shifts with domestication or industrialization in
459 the observational study, we calculated the average of the species pair (e.g., pig/boar) for axis 1
460 and axis 2 of the NMDS then measured the shift along each axis for an individual sample and
461 tested for differences by domestication status. To estimate the direction and magnitude of
462 changes in beta-diversity during the experimental studies, we calculated the distance along axis 1
463 or 2 of the NMDS relative to a baseline sample for that individual. We estimated the direction
464 and magnitude of dissimilarity from the expected community composition (donor microbial
465 community in gavage experiment; baseline species average for Dom_G/Dom_D or $Wild_G$/$Wild_D$ in
466 diet experiments) as the length of the vector through the first two axes of ordination space.
467

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484 study, conducted analyses, and wrote the manuscript; K.S.C., C.E.D., M.B., P.C., and R.R.
485 performed experiments and edited the manuscript; M.E.T. provided samples and edited the
486 manuscript; R.N.C designed the study and wrote the manuscript. **Competing interests:** The
487 authors declare no competing interests.
488
489

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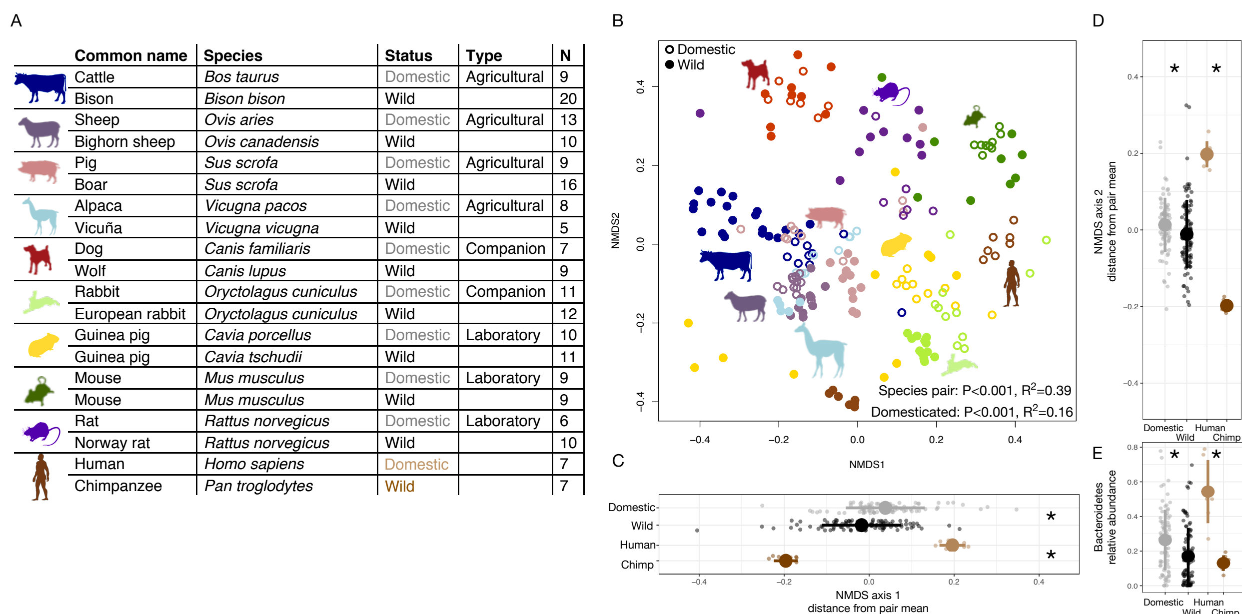

Common name	Species	Status	Type	N
Cattle	*Bos taurus*	Domestic	Agricultural	9
Bison	*Bison bison*	Wild		20
Sheep	*Ovis aries*	Domestic	Agricultural	13
Bighorn sheep	*Ovis canadensis*	Wild		10
Pig	*Sus scrofa*	Domestic	Agricultural	9
Boar	*Sus scrofa*	Wild		16
Alpaca	*Vicugna pacos*	Domestic	Agricultural	8
Vicuña	*Vicugna vicugna*	Wild		5
Dog	*Canis familiaris*	Domestic	Companion	7
Wolf	*Canis lupus*	Wild		9
Rabbit	*Oryctolagus cuniculus*	Domestic	Companion	11
European rabbit	*Oryctolagus cuniculus*	Wild		12
Guinea pig	*Cavia porcellus*	Domestic	Laboratory	10
Guinea pig	*Cavia tschudii*	Wild		11
Mouse	*Mus musculus*	Domestic	Laboratory	9
Mouse	*Mus musculus*	Wild		9
Rat	*Rattus norvegicus*	Domestic	Laboratory	6
Norway rat	*Rattus norvegicus*	Wild		10
Human	*Homo sapiens*	Domestic		7
Chimpanzee	*Pan troglodytes*	Wild		7

Fig. 1. The gut microbiota of wild and domestic mammals differ consistently and in a manner recapitulating differences between industrialized humans and chimpanzees. (**A**) Sampling scheme for cross-species study. (**B**) Nonmetric multidimensional scaling (NMDS) ordination of Bray-Curtis dissimilarities illustrates a significant signal of domestication (closed versus open circles) and clustering by species pair (color). (**C**) Individual shifts relative to species-pair mean along the first NMDS axis differ by domestication status. (**D**) Individual shifts relative to species-pair mean along the second NMDS axis differ by domestication status. (**E**) Relative abundance of the bacterial phylum Bacteroidetes differs by domestication status. Asterisks in (C-E) indicate P<0.05 Mann-Whitney U test by domestication status for animals or by species for human/chimpanzees. Large circles are means; bars show standard deviations.

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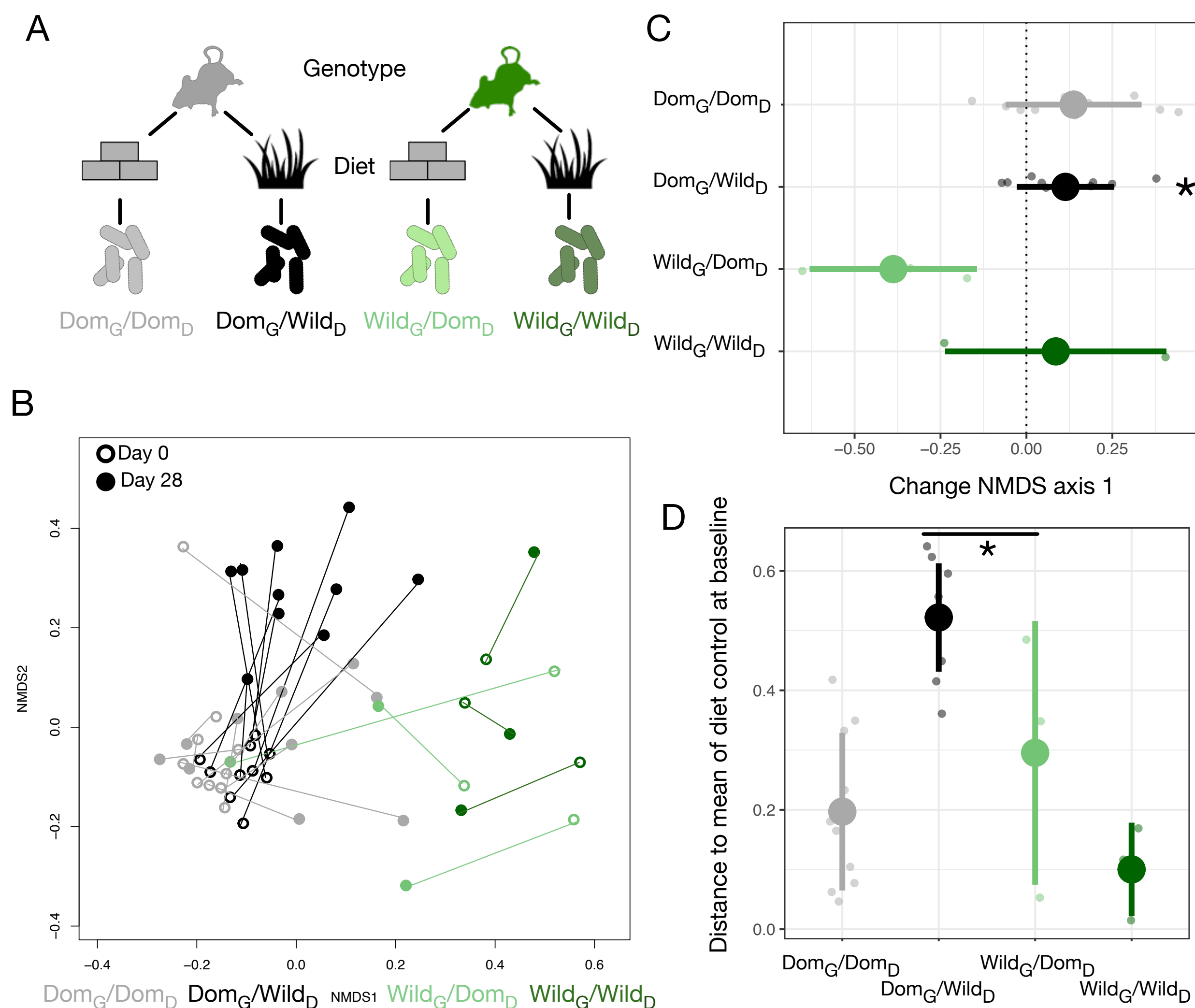

584
585 **Fig. 2**. Microbial differences between wild and domestic mice can be overcome by diet shifts. (**A**)
586 Design scheme for genotype/diet factorial mouse experiment. (**B**) Nonmetric multidimensional
587 scaling (NMDS) ordination of Bray-Curtis dissimilarities showing changes for mice from day 0
588 (open circle) to day 28 (closed circle) by experimental groups (color). (**C**) Animals on reciprocal
589 diets ($Dom_G/Wild_D$ and $Wild_G/Dom_D$) move in opposite directions along NMDS axis 1 from day
590 0 to day 28. Asterisk indicates P<0.05 one-sample Wilcoxon test. Dashed line indicates a shift of
591 0. (**D**) At the end of the experiment, distance to the mean of the diet control at baseline
592 (Dom_G/Dom_D and $Wild_G/Wild_D$) was lower for wild mice than lab mice. Asterisk indicates P<0.05
593 Mann-Whitney U test. Large circles are means; bars show standard deviations.

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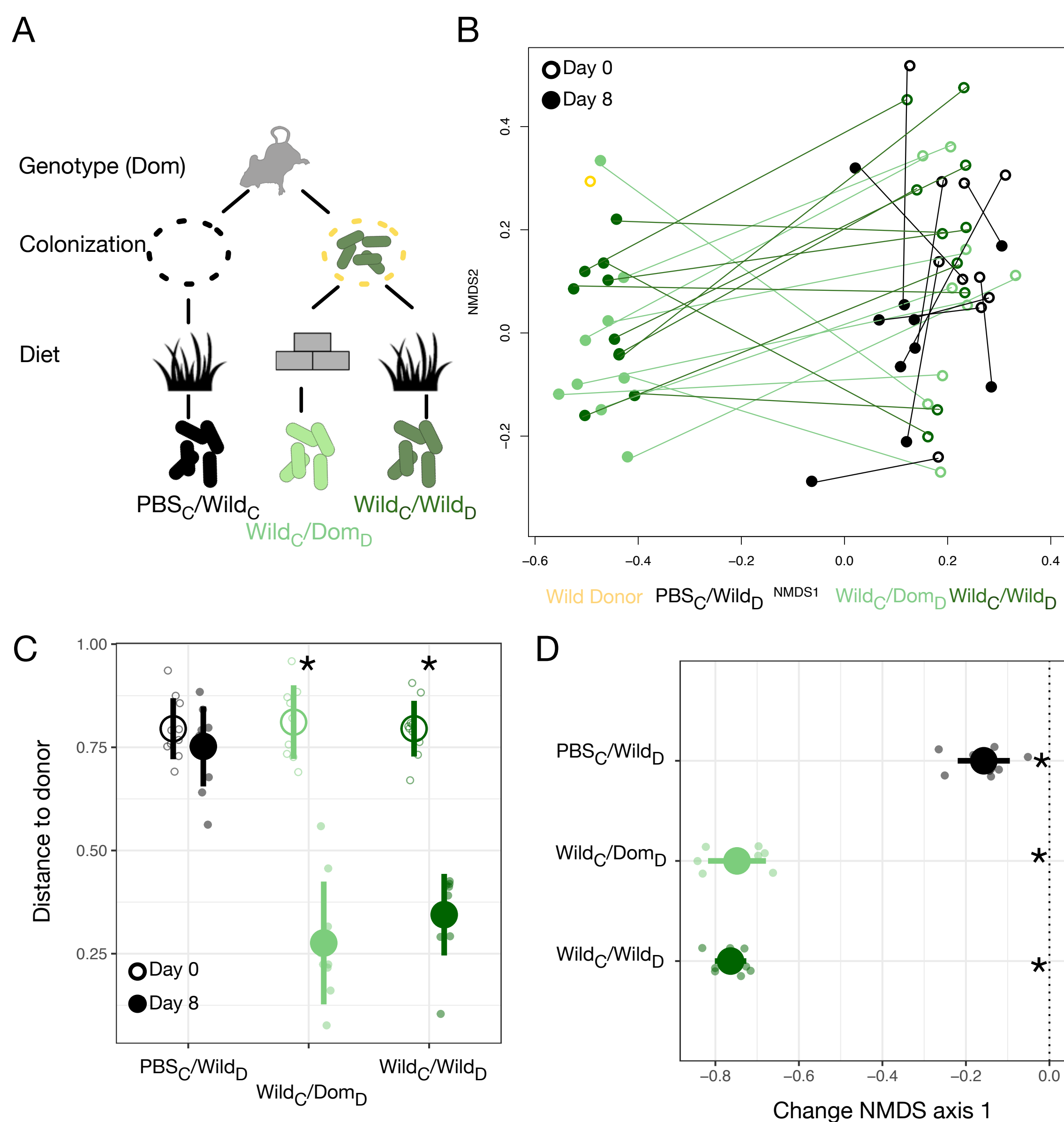

596
597 **Fig. 3.** Laboratory mice can be re-wilded through colonization with wild microbial community.
598 (**A**) Design scheme for colonization/diet mouse experiment. (**B**) Nonmetric multidimensional
599 scaling (NMDS) ordination of Bray-Curtis dissimilarities showing changes for mice from day 0
600 (open circles) to day 8 (closed circles) by experimental groups (color). (**C**-**D**) At the end of the
601 experiment (closed circle), distance to the wild community donor decreased most in animals
602 colonized with wild communities ($Wild_C/Dom_D$ and $Wild_C/Wild_D$; C), but all experimental groups
603 exhibited change along NMDS axis 1 (D) during the course of the experiment. Asterisks in (C)
604 indicate $P<0.05$ Mann-Whitney U test comparing day 0 to day 8 for each experimental group.
605 Asterisks in (D) indicate $P<0.05$ one-sample Wilcoxon test, and dashed line indicates a shift of 0.
606 Large circles are means; bars show standard deviations.

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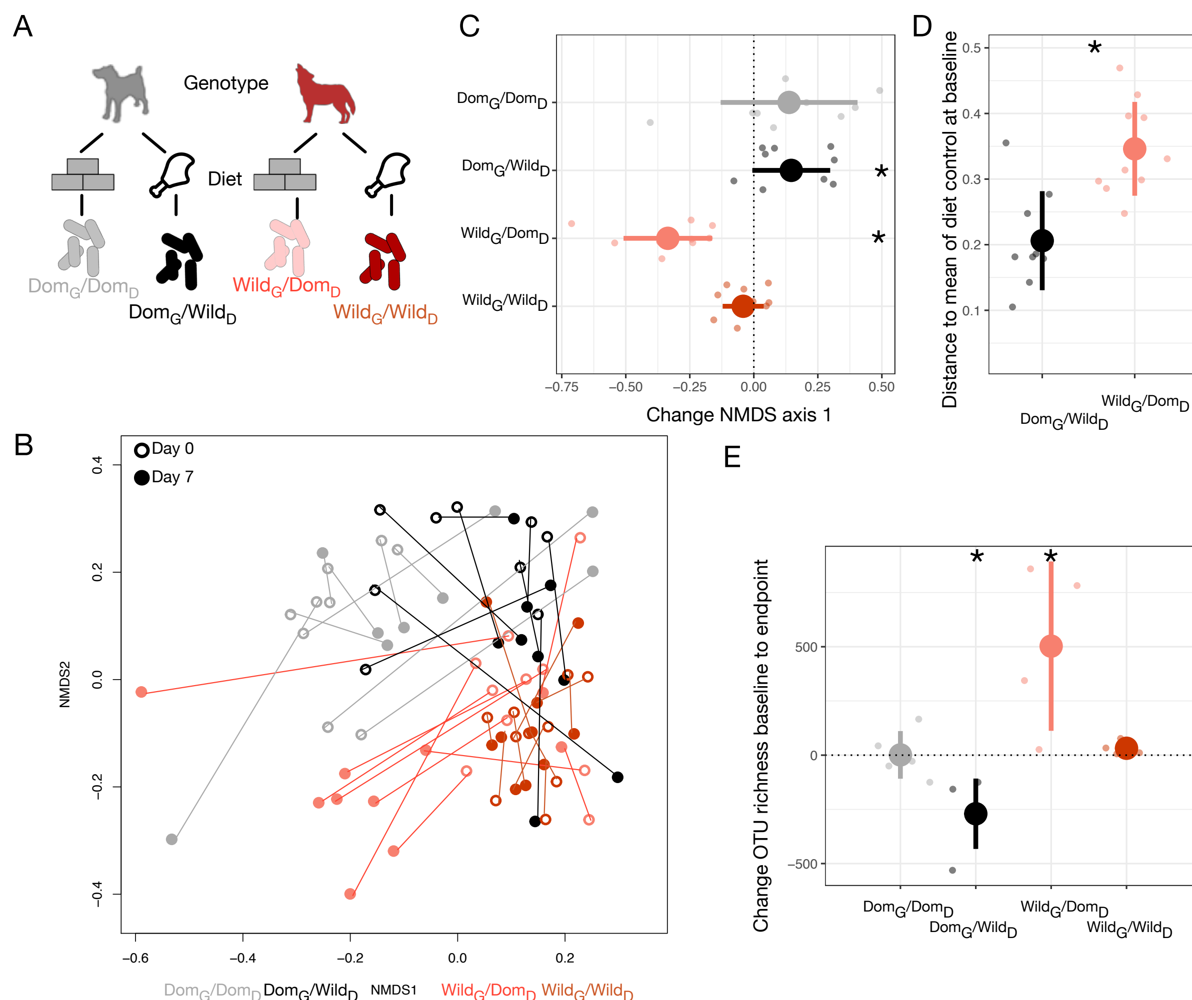

609
610 **Fig. 4.** Microbial differences between wild and domestic canids were overcome by diet shifts, as
611 in mice. (**A**) Design scheme for genotype/diet canid experiment. (**B**) Nonmetric multidimensional
612 scaling (NMDS) ordination of Bray-Curtis dissimilarities showing changes for canids from day 0
613 (open circle) to day 7 (closed circle) by experimental groups (color). (**C**) Canids on reciprocal diets
614 ($Dom_G/Wild_D$ and $Wild_G/Dom_D$) moved in opposite directions along NMDS axis 1 over time. (**D**)
615 At the end of the experiment, distance to the mean of diet controls at baseline (Dom_G/Dom_D and
616 $Wild_G/Wild_D$) was lower for dogs than wolves on reciprocal diets. Asterisk indicates P<0.05 Mann-
617 Whitney U test. (**E**) Change in OTU richness from day 0 to day 7 differed significantly from 0 in
618 opposite directions for animals on reciprocal diets ($Dom_G/Wild_D$ and $Wild_G/Dom_D$). Asterisks in
619 (C, E) indicate P<0.05 one-sample Wilcoxon test, and dashed line indicates a shift of 0. Large
620 circles are means; bars show standard deviations.
621

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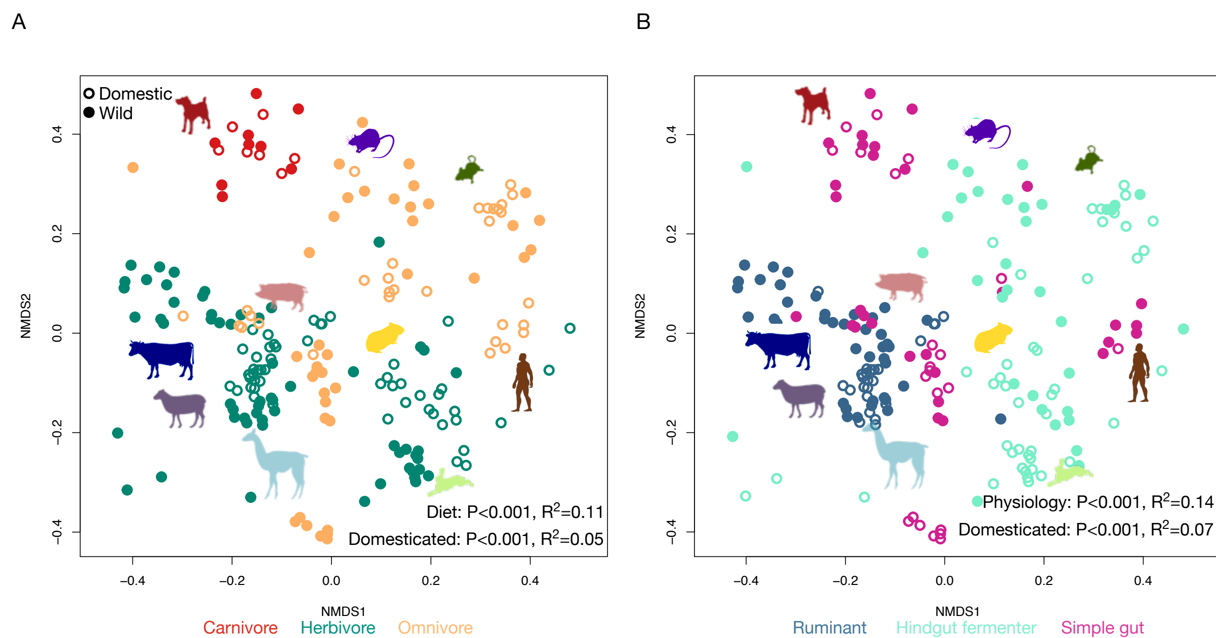

622
623 **Fig. S1.**
624 Diet type (**A**) and digestive physiology (**B**) were associated with variation in gut microbial
625 community composition amongst wild (closed circles) and domestic (open circles) mammals,
626 visualized here with nonmetric multidimensional scaling of Bray-Curtis dissimilarity.
627

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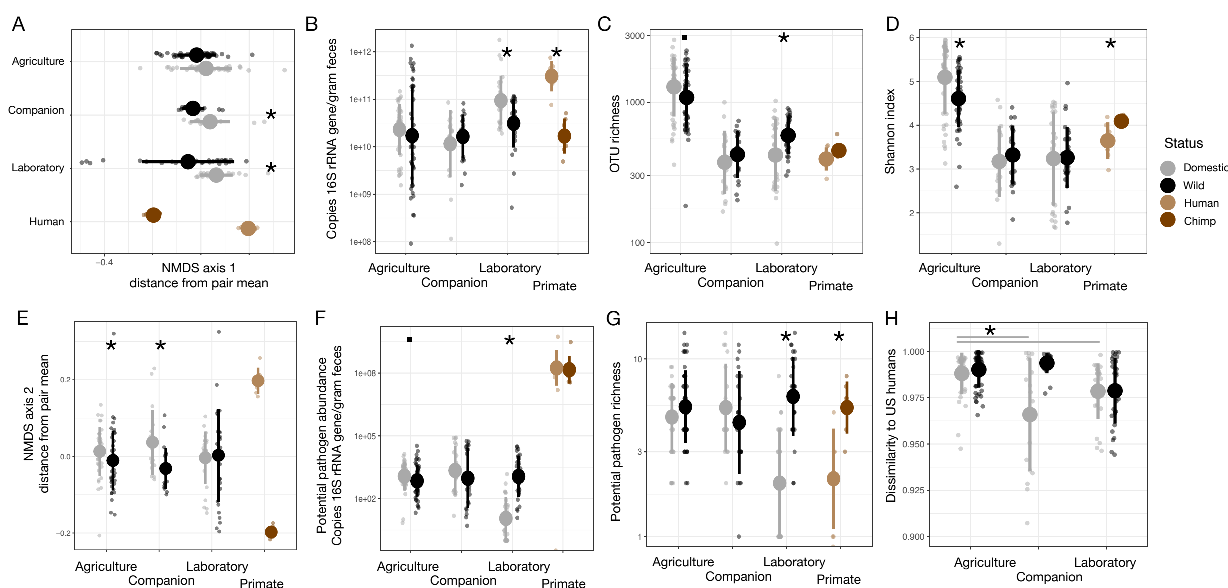

629

630 **Fig. S2.**

631 Ordination axis shifts (**A**, **E**), microbial load (**B**), OTU richness (**C**), Shannon index (**D**),
632 potential pathogen abundance (**F**), and potential pathogen richness (**G**) varied by domestication
633 status for at least one domestication type (agriculture, companion, or laboratory) in cross-species
634 dataset. Trends often mirrored those seen in comparing humans to chimpanzees. (**H**) Bray-Curtis
635 dissimilarity to industrialized humans varied by domestication status and domestication type.
636 Asterisks indicate $P<0.05$ and periods indicate $P<0.1$ Mann-Whitney U test.

637

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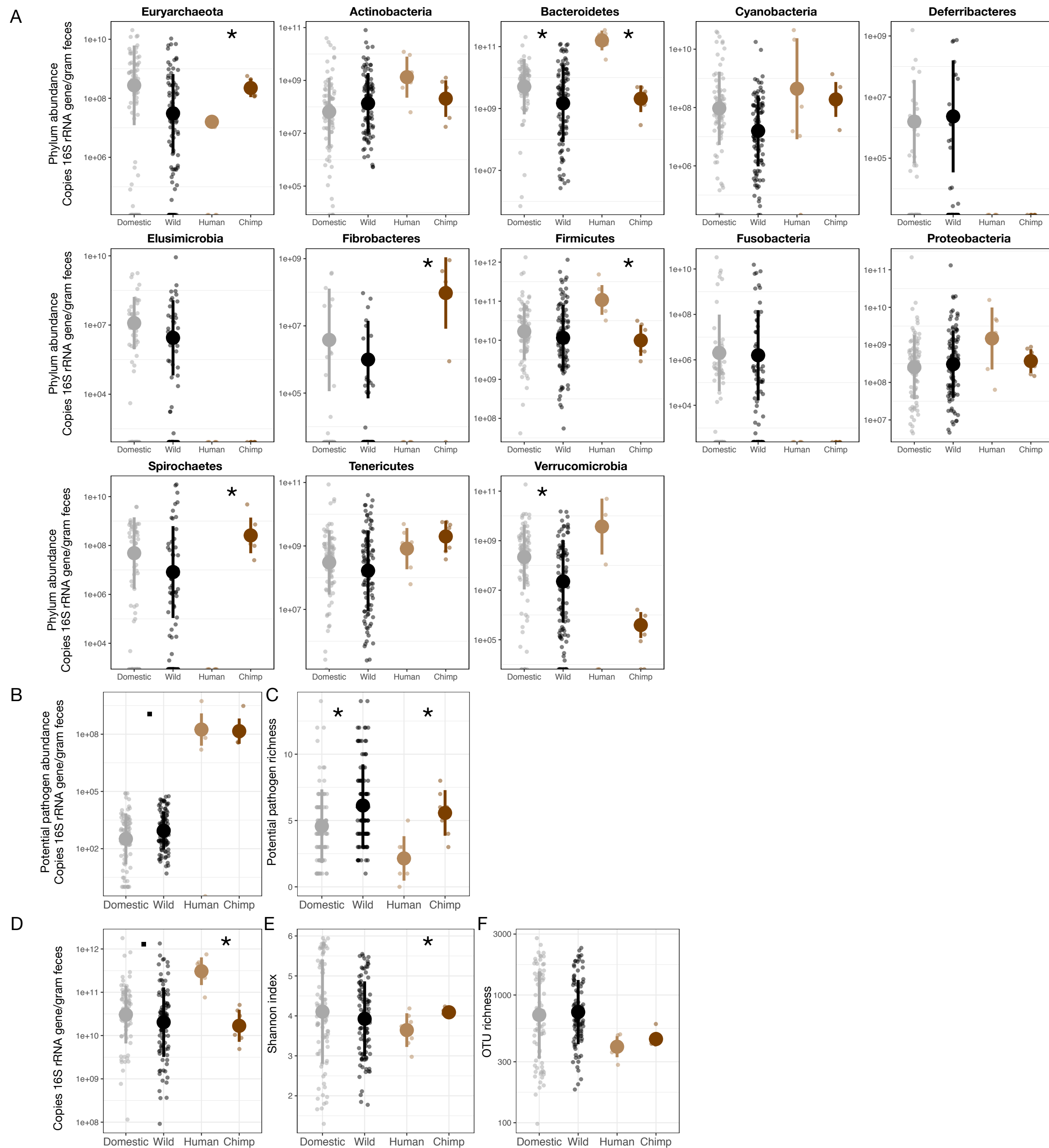
638



639

640 **Fig. S3.**

641 Some phylum abundances (**A**) and potential pathogen community characteristics (**B**, **C**) varied
642 with domestication in our cross-species dataset. Microbial density (quantified as 16S rRNA gene
643 copies per gram feces; **D**) and alpha-diversity metrics (Shannon index (**E**) and OTU richness (**F**))
644 did not vary consistently between wild and domestic animals. Asterisks indicate $P<0.05$ and
645 periods indicate $P<0.1$ Mann-Whitney U test. Analyses by phylum included Bonferroni multiple
646 hypothesis correction.
647

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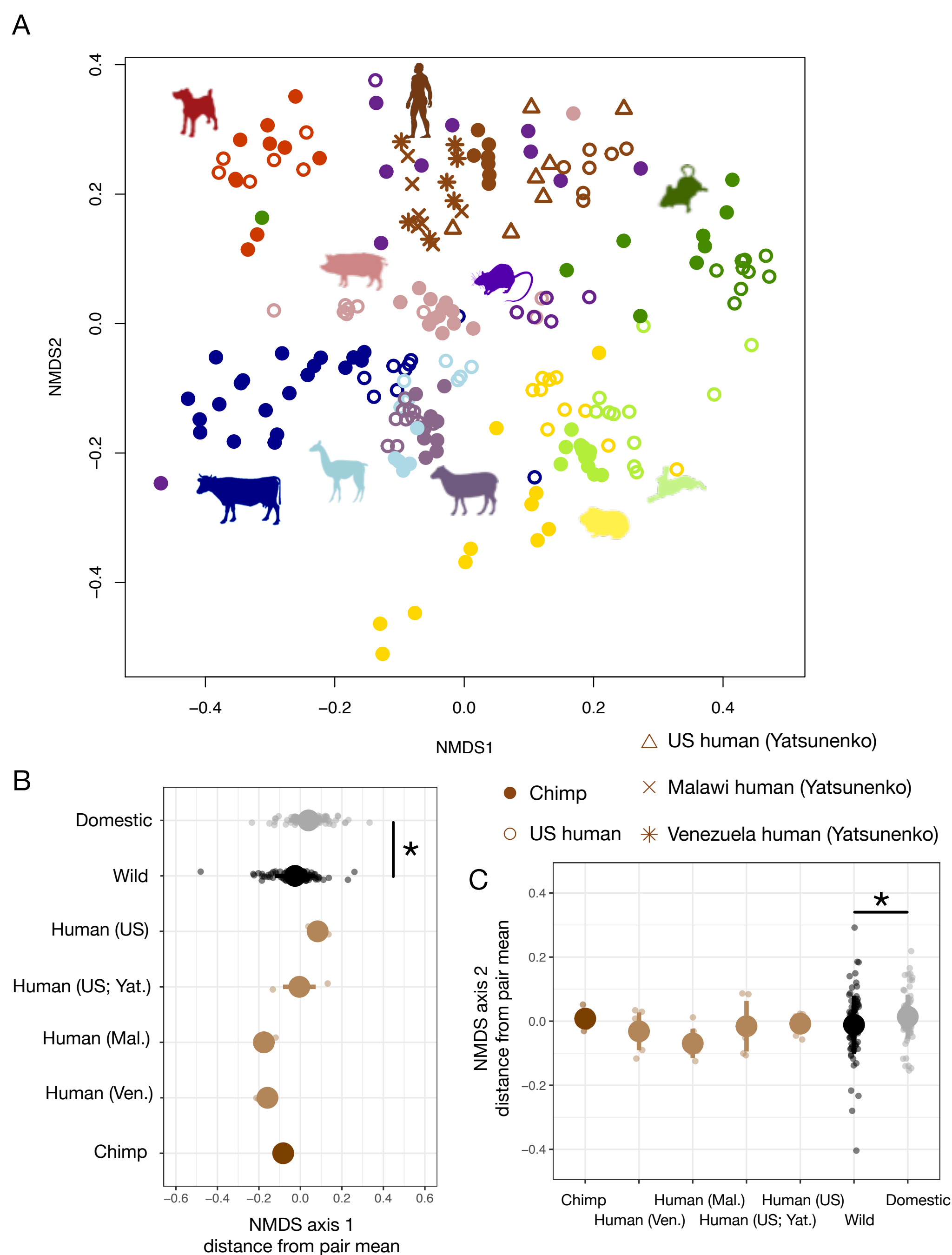

648

649 **Fig. S4.**

650 Inclusion of additional human gut microbiota samples shows that while humans and
651 chimpanzees cluster relative to other animals (**A**), traditional human populations do not
652 demonstrate the same shifts along nonmetric multidimensional scaling (NMDS) axis 1 (**B**) and 2
653 (**C**) as chimpanzees relative to industrialized humans or wild animals relative to domestic
654 animals. NMDS calculated with Bray-Curtis dissimilarity. Asterisks indicate P<0.05 Mann-
655 Whitney U test.
656

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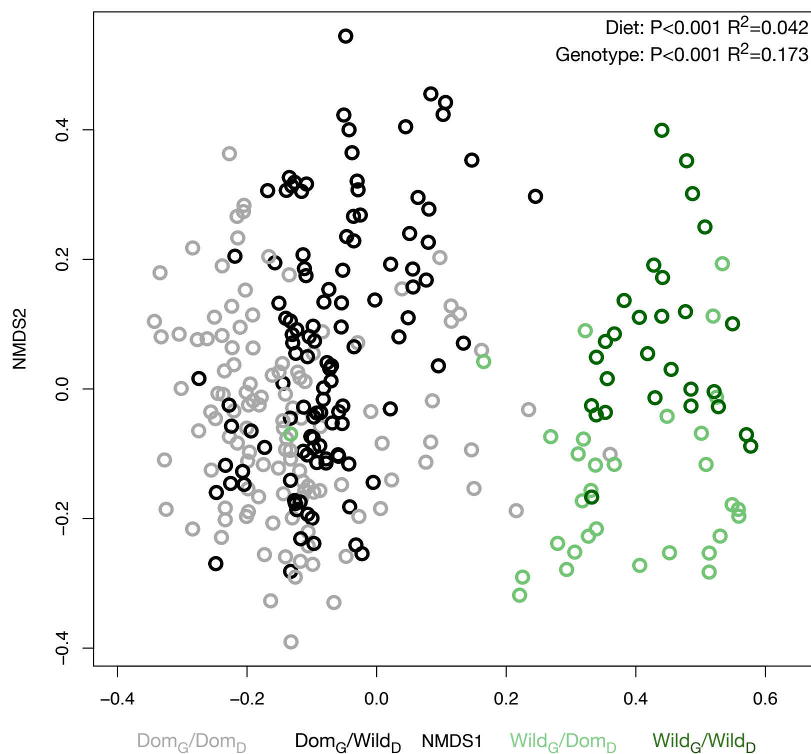
A



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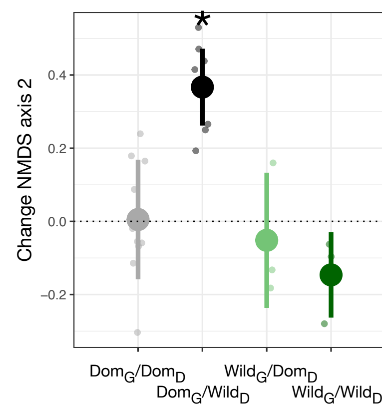

657
658 **Fig. S5.**

659 (**A**) Nonmetric multidimensional scaling (NMDS) of all time points illustrates significant effects
660 of genotype and diet on Bray-Curtis dissimilarity. (**B**) $Dom_G/Wild_D$ mice move significantly up
661 along the second NMDS axis between day 0 and 28 of the experiment. Asterisk indicates P<0.05
662 one-sample Wilcoxon test.
663

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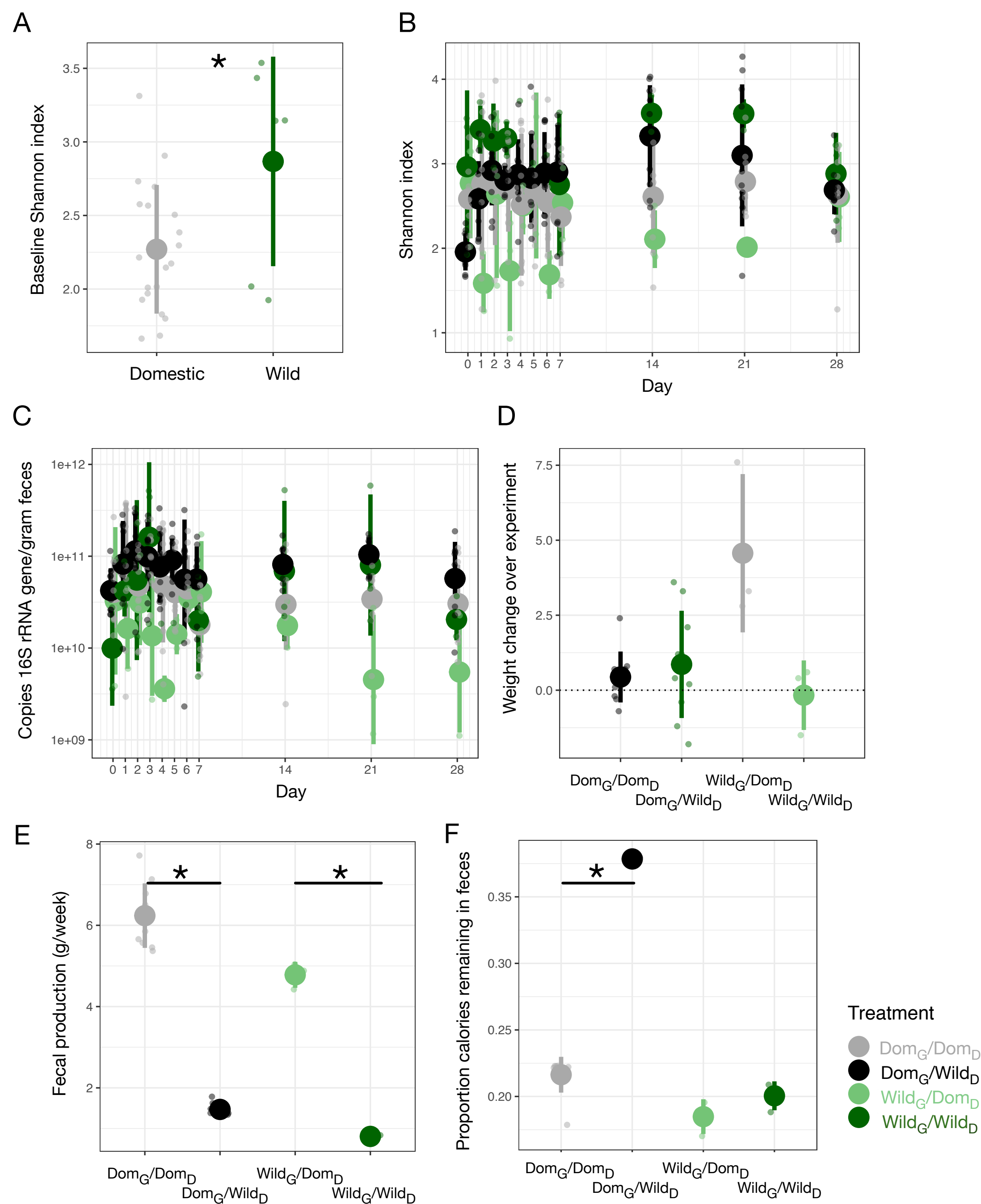

664

665 **Fig. S6.**

666 (**A**) Shannon index differed between genotypes on day 0. (**B**) Shannon index plotted by
667 experimental groups over time. (**C**) Microbial load (quantified as 16S rRNA gene copies per
668 gram feces) plotted by experimental groups over time. (**D**) Individual weight gain over the
669 course of the experiment was highest in $Wild_G/Dom_D$ mice. (**E**) Total fecal production over one
670 week differed between experimental groups. (**F**) Calories remaining in feces as a function of
671 total calories consumed varied by diet in Dom_G mice. Asterisks in (A, E, F) indicate $P<0.05$
672 Mann-Whitney U test.

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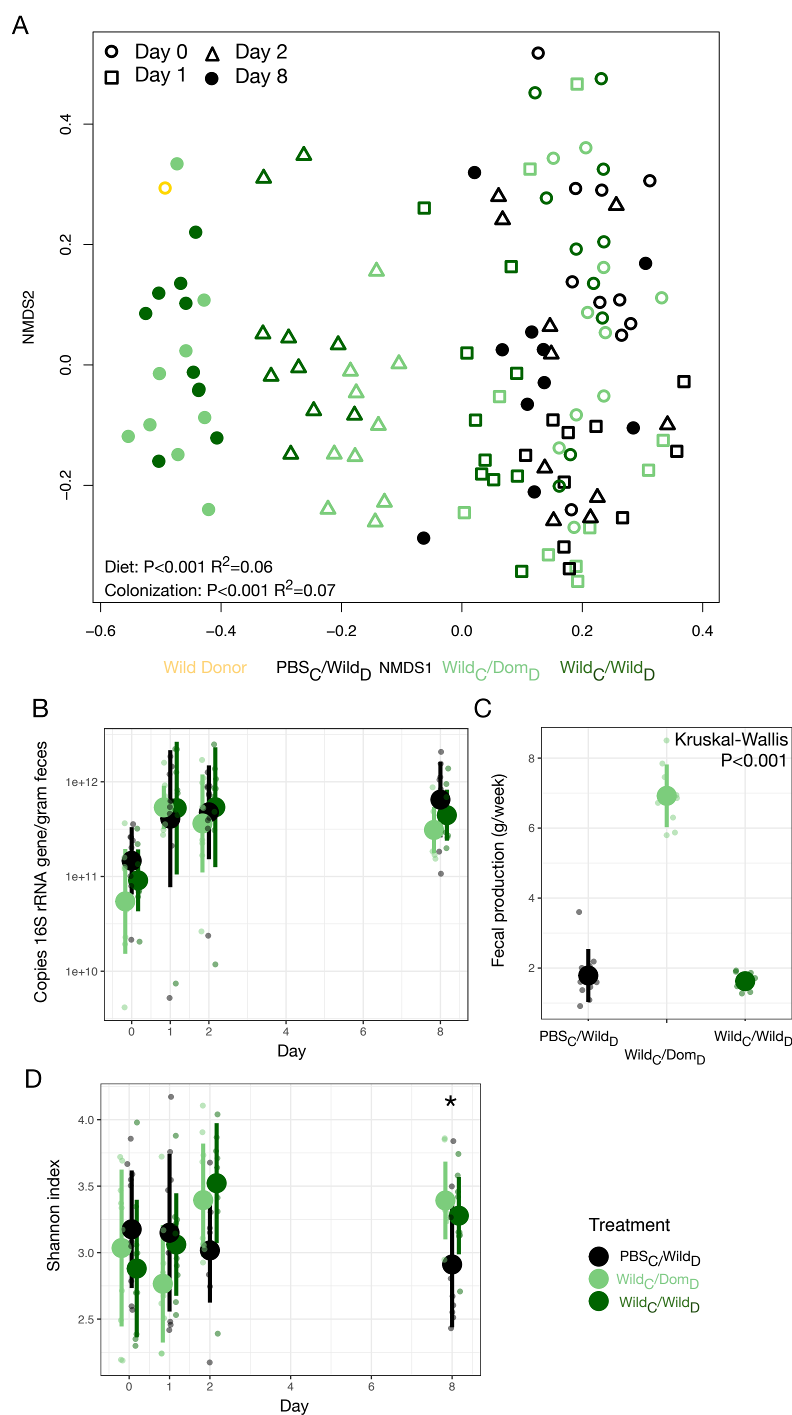

673

674 **Fig. S7.**

675 (**A**) Nonmetric multidimensional scaling (NMDS) of all time points illustrated significant effects of colonization and
676 diet treatment on Bray-Curtis dissimilarity. (**B**) Microbial load by experimental groups plotted over time. (**C**) Total
677 fecal production over one week differed between experimental groups. (**D**) Shannon index plotted by experimental
678 groups over time. Asterisks indicate P<0.05 Kruskal-Wallis test.

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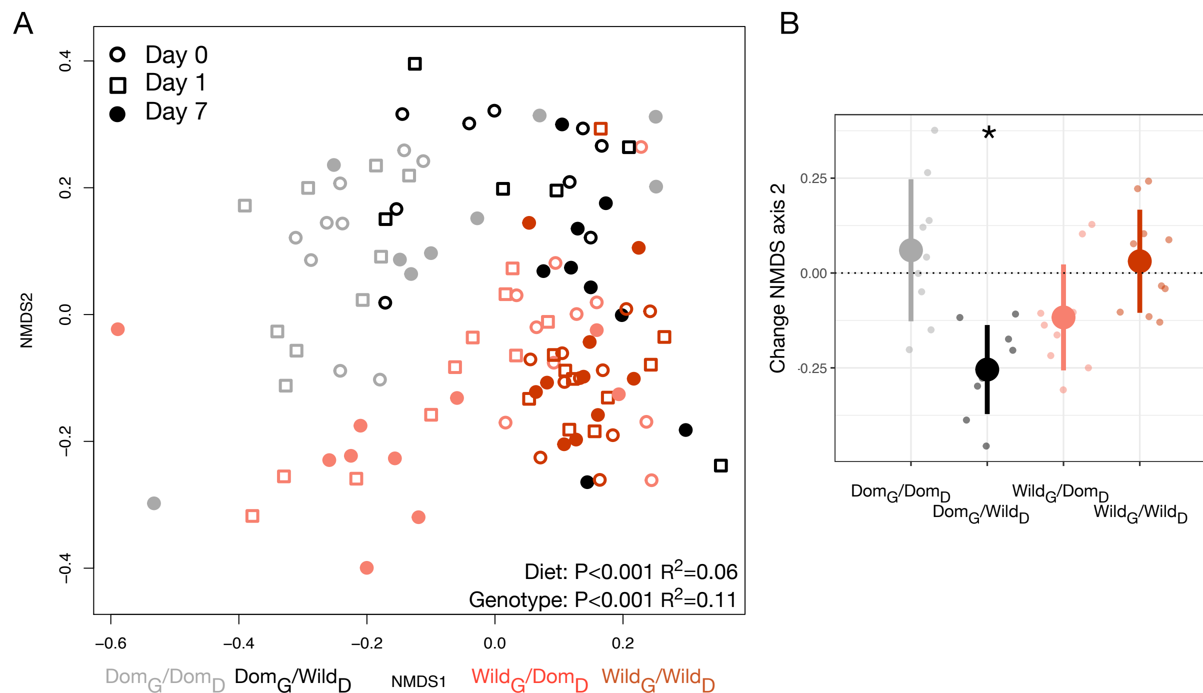

679

680 **Fig. S8.**

681 (**A**) Nonmetric multidimensional scaling (NMDS) of all time points illustrated significant effects
682 of genotype and diet on Bray-Curtis dissimilarity. (**B**) $Dom_G/Wild_D$ canids moved significantly
683 down along the second NMDS axis between day 0 and 7 of the experiment. Asterisk indicates
684 $P<0.05$ one-sample Wilcoxon test.

685

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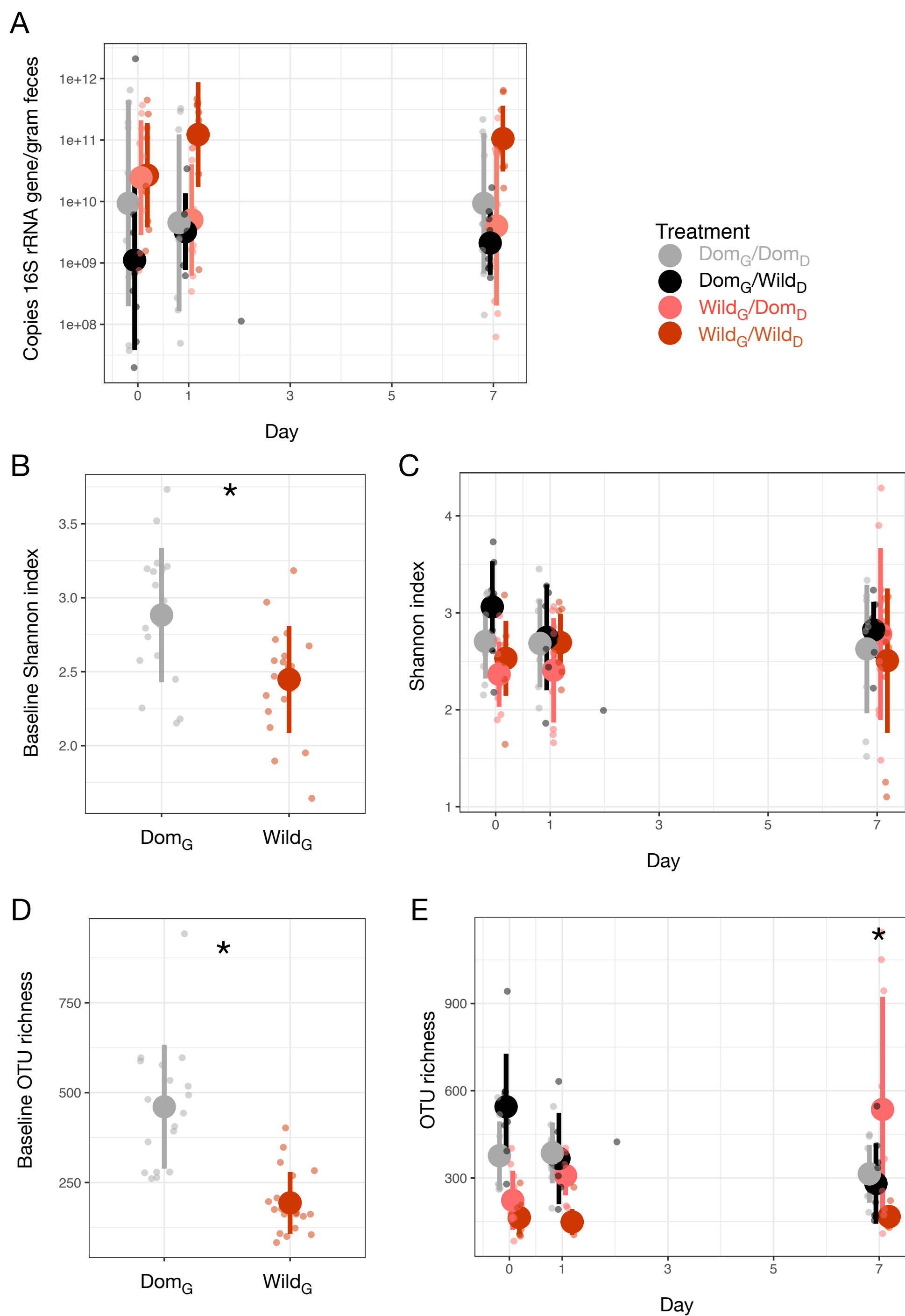

686

687 **Fig. S9.**

688 (**A**) Microbial load plotted by experimental groups over time. (**B**) Shannon index differed
689 between genotypes on day 0. (**C**) Shannon index plotted by experimental groups over time. (**D**)
690 OTU richness differed between genotypes on day 0. (**E**) OTU richness plotted by experimental
691 groups over time. Asterisks for (B, D) indicate P<0.05 Mann-Whitney U test. Asterisk for (E)
692 indicates P<0.05 Kruskal-Wallis test.

693

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694 **Table S1.**

695 Nutritional information for experimental diets.

696

	Mouse				Canid	
	Wild diet			Domestic diet	Wild diet	Domestic diet
	Mealworms (5% by weight)	Birdseed (95%)	Mix	Prolab Isopro RMH 3000	Raw chicken	Nutrisource Lamb Meal and Peas Grain Free Diet
Crude Protein (min)	50.0	9.0	11.1	22.0	14.1	28.7
Crude Fat (min	24.0	4.0	5.0	5.0	28.7	18.3
Crude Fiber (max)	9.5	15.0	14.7	5.0	0.0	3.7
Phosphorus (min)	0.5			0.8	0.8	1.2
Moisture (max)	10.0	13.0	12.9	10.0		9.5
Calories/gram			5143	4136	3190	4088

697