A standardized gnotobiotic mouse model harboring a minimal 15-member mouse gut microbiota recapitulates SOPF phenotype

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Abstract

Mus musculus is the classic mammalian model for biomedical research. Despite global efforts in standardizing breeding and experimental procedures, the undefined nature and inter-individual diversity of laboratory mouse microbiota remains a limitation. In an attempt to standardize preclinical studies, we have developed a simplified mouse microbiota composed of 15 strains from 7 of the 20 most prevalent bacterial families representative of the fecal microbiota found in specific opportunistic- and pathogen-free (SOPF) C57BL/6J animals and derived a standardized gnotobiotic mouse model called GM15. GM15 recapitulates extensively the functionalities found in C57BL/6J SOPF microbiota metagenome and GM15 animals are phenotypically similar to SOPF. They even perform better in a model of post-weaning malnutrition. The GM15 model ensures an increased reproducibility and robustness of preclinical studies by limiting the confounding effect of microbiota composition fluctuation and offers new possibilities for research focusing on how the microbiota shapes host physiology in health and diseases.

41 **Background**

42 The intestinal microbiota is a complex and dynamic ecosystem largely composed of bacteria
43 whose activity profoundly influences our health and diseases [1]. Advances in sequencing and
44 analytical technologies coupled with improved computing tools have revolutionized the field of
45 host-microbiota interaction [2]. These developments have provided an increased depth and
46 accuracy in the study of intestinal microbial assemblages and activity for correlative studies
47 with human health or disease traits. Despite these sophisticated descriptions of host-
48 microbiome interaction phenomena, the underlying causal mechanisms remain largely elusive
49 [3].

50 The use of model organisms plays a decisive role in the challenge to move from
51 correlation to causal links in the host-microbiome field as they have long enabled researchers
52 to identify the shared biological functions among living organisms, and facilitated the discovery
53 of conserved molecular mechanisms governing the fundamental principles of biology [4].
54 Thanks to its genetic and physiological similarities to humans, in addition to its rapid and prolific
55 breeding, the mouse has been a classic mammalian model of choice for the past decades for
56 biomedical research and the host-microbiome field is no exception [5]. While the use of defined
57 genetic backgrounds, as well as the absence of specific pathogens [6] is now common practice
58 in mouse studies, an important confounding factor is the variability in the composition of the
59 intestinal microbiota among experimental animals and animal facilities [7,8]. This parameter is
60 under the influence of multiple elements such as genetics, diets, biological rhythms and
61 breeding conditions [9]. As a consequence, to restrain microbial diversity, efforts have been
62 made to tailor protocols for microbiota-related mice studies and to standardize mouse
63 microbiota composition [9, 10, 11, 12, 13].

64 The mouse gut microbiota richness is usually estimated at more than 300 bacterial
65 genera [5] and common inhabitants of the mouse intestine belong to seven bacterial phyla with
66 *Firmicutes*, *Bacteroidetes* and *Proteobacteria* being the most abundant ones [12, 14, 15]. The
67 first attempt in standardizing the mouse microbial environment (initially to study
68 immunocompromised mouse models) arose 15 years ago with the wide implementation of the

69 Specific Pathogen-Free (SPF) hygienic status of mouse husbandries [6] by re-deriving mouse
70 strains by two-cell-stage embryo transfer to SPF recipients and subsequent post-natal
71 inoculation with a cocktail of bacteria devoid of pathogens to homogenize microbial
72 colonization among a given animal facility. SPF, and then SOPF inbred lines (lacking Specific
73 Opportunist Pathogens, such as *Staphylococcus aureus* or *Pseudomonas aeruginosa*) now
74 represents the common standard health status for experimental mouse breeding. However,
75 despite the global efforts in standardizing the SPF procedures, the undefined nature and
76 important inter-individual diversity of the SPF microbiota remains a limitation in host-
77 microbiome studies, since the scientific community still lacks a common SPF standard cocktail
78 and rather use a facility-specific cocktail of bacteria [9]. Indeed, the microbiota fluctuates a lot
79 with diet and environment, so it is strictly impossible to have the exact same microbiota of SPF
80 mice in two different facilities.

81 Microbial cultivation and gnotobiology offer attractive strategies to standardize the
82 microbiota of mouse models. Germ-Free (GF) animals (i.e. animals devoid of any living micro-
83 organisms) are the originators of gnotobiotic animals (i.e. animals with a controlled microbiota)
84 obtained by colonization with pure culture or cocktails of bacterial strains [16]. Recent efforts
85 have been put into isolating, cultivating and archiving isolated culture of the dominant members
86 of the mouse microbiota [17]. Gnotobiotic animals can be kept in isolators for several
87 generations and offer the possibility of a strict control of their microbial status. Gnotobiotic
88 models offering different degree of microbial complexity have been developed in the past
89 ranging from mono-colonization (monoxenic animals) to high diversity microbiota models such
90 as conventionalized ex-GF animals using a donor microbiota [9, 12, 18]. Two models have
91 emerged for breeding and long-term experimental purpose: the Altered Schaedler Flora (ASF)
92 model and the recent oligo-Mouse-Microbiota$_{12}$ (oligoMM$_{12}$) model [19, 20]. These models offer
93 an enlarged microbial potential as compared to monoxenic mice, while keeping the model
94 simple and experimentally tractable as compared to conventionalized animals (SPF or SOPF).

95 The ASF was developed in the late 70s by adding bacterial strains which better
96 represented the microbiota of conventional mice to the initial Schaedler flora, a minimal

97 microbial consortium that protected ex-GF mice from opportunistic pathogen colonization
98 during breeding [21]. The ASF is composed of 8 defined bacteria, which are stable over mouse
99 generations. Mice colonized with ASF display a certain degree of immune normalization when
100 compared with SPF mice [19]. Limitations of the ASF model are that the strains are not publicly
101 available of the strains and that they are not all representative of the dominant members of the
102 mouse microbiota [9, 12]. In addition, ASF mice differ substantially from SPF mice with respect
103 to microbiota-associated traits beyond immune parameters and resistance to opportunistic
104 pathogen colonization, probably owing to the limited phylogenic diversity and metabolic
105 capabilities of the ASF consortium [9, 12, 22]. Recently, the oligoMM$_{12}$ model was developed
106 [20]. It is a minimal microbiota gnotobiotic model composed of 12 defined cultivable mouse
107 commensal bacteria from the miBC collection representing members of the major bacterial
108 phyla of the mouse gut [17, 23, 24]. The community is transmissible and stable over
109 consecutive mouse generations and unlike ASF offers colonization resistance against
110 *Salmonella enterica* serovar Typhimurium albeit not to the degree of a conventional complex
111 microbiota [20]. However, how the oligoMM$_{12}$ model performs relative to the SPF for
112 microbiota-associated traits beyond *S.* Typhimurium colonization resistance remains elusive.
113 In an attempt to standardize preclinical studies in the host microbiome field, we have
114 developed a simplified mouse microbiota that is representative of SOPF microbiota at the
115 functional level and derived a standardized gnotobiotic mouse model called GM15, which
116 phenotypically mimics SOPF mice under standard diet conditions. We show that, under
117 conditions of chronic physiological stress such as post-weaning malnutrition on a low-protein
118 diet, a dietary condition triggering stunting, GM15 microbiota seems superior to an SOPF
119 microbiota to buffer the deleterious effect of a depleted diet on mouse juvenile growth.
120

121 ## Results and Discussion

122 ### *In silico* identification of the main bacterial families of the C57BL/6J SOPF fecal
123 microbiota

124 To define a minimal microbiota containing representative and prevalent bacteria from the gut
125 of C57BL/6J SOPF mice, we analyzed the composition of fecal pellets from 4 C57BL/6J SOPF
126 mice (two females and two males) by whole-genome sequencing. An average of 13.4 million
127 of paired-end reads was obtained per sample with a length of 300 bp. The metagenomics data
128 sets generated were classified using the Centrifuge software [25] and compared to the RefSeq
129 complete genome database [26], and 20 dominant families consistently present in all mice
130 were identified (Fig. 1a). The profiling of metagenomic sequencing data pointed out a
131 comparable distribution of bacterial families among the four tested C57BL/6J SOPF mice.
132 Moreover, bacterial species identification was possible for genome sequences with good
133 phylogenetic resolution and already referenced in taxonomy databases. Interestingly, among
134 the identified species, *Bacteroides acidifaciens, Clostridium cocleatum, Lactobacillus*
135 *johnsonii, Lactobacillus murinus* and *Lactobacillus reuteri* were previously identified as mouse-
136 enriched and dominant intestinal bacteria [17] and *Lactobacillus murinus*, *Parabacteroides*
137 *goldsteinii, Clostridium* ASF 356 and *Clostridium* ASF 502 were already part of the ASF model
138 [19].
139

140 **Isolation and taxonomic characterization of GM15 bacterial strains**

141 We established four different strategies in order to isolate and culture a maximal number of
142 representative strains of the 20 dominant bacteria families identified in our metagenomic
143 sequencing analysis (Fig. 1b). First, we isolated the most prevalent strains from fecal pellets
144 of C57BL/6J SOPF mice using non-selective agar media. Then, we used antibiotic selection
145 to isolate resistant strains. We also used rumen enrichment to isolate strains from caecal
146 content. Finally, we used fecal pellets of ASF mice to isolate additional strains. We obtained a
147 collection of approximately 400 cultivable bacterial isolates. All isolates were pre-screened by
148 MALDI-TOF MS for dereplication prior to the first taxonomic identification by 16S rRNA gene
149 Sanger sequencing. We selected eleven strains covering seven of the most representative and
150 prevalent families of the intestinal microbiota of C57Bl/6J SOPF mice and obtained four
151 additional strains from the DSMZ miBC collection to establish the GM15 consortium that covers

152 most of the dominant bacteria families found in C57Bl/6J SOPF animals (Fig.1a,b). In
153 summary, GM15 is composed of two strains of Bacteroidaceae, one strain of Tannerellaceae,
154 six strains of Lachnospiraceae, three strains of Lactobacillaceae, one strain of
155 Erysipelotrichaceae, one strain of Ruminococcaceae, and one strain of Enterobacteriaceae
156 (Additional file 1: Figure S1). The genomes of the fifteen strains were sequenced and the draft
157 genomes sequences aligned against the NCBI database [27], which allowed the identification
158 of 14 strains at the species level and 1 strain at the family level.
159

160 ***In silico* functional metagenomics analysis of the GM15 strains**

161 To gain insights into the functionalities encoded in the individual genomes of the GM15
162 members, the coding sequences of the 15 strains were converted into their respective protein
163 sequences, which were annotated for clustering into KO (KEGG Orthology) groups. By
164 merging the 15 assembled individual genomes, we found that the GM15 metagenome
165 possesses 3890 non-redundant KO groups. Besides, all GM15 strains possessed 3 to 64
166 unique functions, although *E. coli* Mt1B1 exhibited a vast repertoire of unique KO groups (10_3),
167 indicating that *E. coli* Mt1B1 is responsible for the GM15's functional metagenomic profile at
168 33%, while the 14 other strains contribute all together at 8% and the 59% remaining are
169 associated to non-unique KO groups (Fig. 1c). It is noteworthy that KEGG module analysis is
170 biased towards gene sets, pathways and functional groups of well-characterized bacteria such
171 as *E. coli*, which is by far the most studied bacterial species to date.

172 Next, we highlighted the *in silico* functionalities of the 15 selected strains associated
173 with known enzymatic activities in the gut [28] (Fig. 1d). Again *E. coli* Mt1B1 is a major
174 contributor, but each functionality is also covered by other strains at equivalent or lower levels.
175 As expected, the enzymatic activities in the gut are correlated with the phylogenetic
176 membership of the strains. For example, Lactobacilli, which are a major part of the lactic acid
177 bacteria group, the principal contributor to lactate dehydrogenase [29], clustered together.
178 Additionally, Lachnospiraceae clustered with Ruminococcaeae and Erysipelotrichaceae,
179 which include mainly bacteria with sporulation capabilities [30, 31, 32]. Finally, Bacteroides

180 clustered with Parabacteroides, whose species are predominant in the colonic mucus barrier
181 and promote mucinase activity [33], and are significant producers of succinate, a major
182 metabolic by-product [34, 35]. Thus, different strains of a bacteria family and of other closely
183 related bacteria families are capable of the same enzymatic activities in the gut. This can be
184 essential for the generation of simplified non-specific gnotobiotic models.

185 Then, we determined the functional coverage of the GM15 metagenome (i.e. the sum
186 of the genomes of the 15 strains) relative to the KEGG modules of C57BL/6J SOPF mouse
187 microbiota found in our initial metagenomic analysis (Fig.1a). In addition, the KEGG modules
188 from the $OligoMM_{12}$ and ASF microbiota were included for comparative analysis, as these
189 consortia were previously used to generate gnotobiotic mice with a stable and defined mouse-
190 derived microbiota [23, 36]. The presence and completeness of KEGG modules was
191 determined for each metagenome and used for hierarchical clustering (Fig. 1e). One cluster
192 contained highly conserved modules in all mouse models (Fig. 1e, cluster 4; Additional file 3:
193 Table S2), including 140 pathways. We also identified clusters of modules that were not
194 represented among the ASF and $OligoMM_{12}$ consortia, but specifically common to GM15 and
195 SOPF (Fig. 1e, clusters 9 and 6; Additional file 3: Table S2), comprising a total of 155 pathways
196 indicating that qualitatively the GM15 metagenome covers functionalities found in SOPF
197 microbiota that were lacking in ASF and $OligoMM_{12}$ models. Quantitatively, the defined
198 consortia of GM15, $OligoMM_{12}$ and ASF covered respectively 72, 54 and 48% of the KEGG
199 modules of the C57BL/6J SOPF microbiome suggesting a superior functional potential of the
200 GM15 community as compared to $OligoMM_{12}$ and ASF models. Thus, taken collectively our *in*
201 *silico* analysis suggests that the GM15 community carries a significant potential for enzymatic
202 activities in the gut and recapitulates widely the functionalities found in C57BL/6J SOPF murine
203 metagenome.

204

205 **Monitoring and stability assessment of GM15 gut microbiota**

206 To explore *in vivo* the functional potential of the GM15 community, we next generated GM15
207 isobiotic animals. First, we investigated whether the GM15 consortium can stably colonize the

208 mouse intestine over several generations. To this end, we developed a strain-specific
209 quantitative polymerase chain reaction (qPCR) microfluidic assay, which allows simultaneous
210 absolute quantification of the 15 strains, along with the global bacterial load in a given biological
211 sample (Additional file 2: Table S1). Fecal samples from GF and SOPF mice were used as
212 negative and positive controls. Only *C. clostridioforme* YL32, which was obtained from the
213 DSMZ miBC collection, was not detected in our SOPF mice. Co-monitoring of specific and total
214 bacteria aimed to detect any bacterial load imbalance caused by a contamination in gnotobiotic
215 isolators.

216 Eight-week-old GF mice (five breeding pairs; GM15 founders, F0; Additional file 4:
217 Figure S2) were inoculated by oral gavage with the frozen mixtures of the 15 strains and bred
218 in sterilized positive pressure isolators up to the F4 filial generation. All strains except
219 *Lachnospiraceae* bacterium COE1, *Lachnospiraceae* sp. MD329, and Lachnospiraceae sp.
220 MD308 were above the detection limit of our qPCR microfluidic assay in the fecal samples of
221 most individual mice (GM15 founders and progenies from the 4 consecutive generations;
222 Additional file 5: Figure S3a; Fig. 2a). *Anaerotruncus colihominis* JM4-15, *Clostridium* ASF
223 356, *Clostridium* ASF 502, and *Clostridium cocleatum* I50 were occasionally below detection
224 limit. The detection of strains was reproducible between fecal and caecal samples from
225 individual mice of the second filial generation (Additional file 5: Figure S3b). These results
226 indicate a stable colonization and effective vertical transmission of at least 12 strains out of the
227 15 inoculated. Based on the results of our qPCR assay, we consider that the 3 remaining
228 strains: *Lachnospiraceae* bacterium COE1, *Lachnospiraceae* sp. MD329, and
229 *Lachnospiraceae* sp. MD308, either did not efficiently colonize the animals, live in the caecum
230 or the colon below the detection limit of our qPCR assay, or predominantly live in other
231 gastrointestinal niches than those sampled.

232 Next, we evaluated the effect of aging on the GM15 community by following individual
233 mice of the first filial generation between 2 and 12 months of age (Additional file 4: Figure S2).
234 Overall, no changes in the qualitative and quantitative composition of the GM15 consortium
235 were detected (Fig. 2b). Then, we asked if the composition of the GM15 community was

236 modulated by substituting the breeding diet by an alternative maintenance diet. This alternative
237 diet is quasi isocaloric but its nutritional composition differs by 1.3 and 1.6-fold fewer proteins
238 and lipids respectively, and 1.2-fold more carbohydrates than the breeding diet. An alternative
239 diet was administrated to 8-week-old GM15 mice for 4 weeks. We collected fecal samples
240 before diet change, after 2 and 4 weeks, and again after 4 weeks back to the breeding diet
241 (Additional file 4: Figure S2). We did not detect any significant changes in the GM15
242 composition under these conditions (Fig. 2c). Additionally, successful fecal microbiota
243 transplantation (FMT) from GM15 founders to GF mice was confirmed by strain-specific qPCR
244 of the ex-GF mice' feces collected at week 1, 2 and 3 post-transplantation (Additional file 4:
245 Figure S2a; Fig. 2d).

246 When all the data are analyzed together (Fig. 2e), we notice a limited fluctuation
247 (maximum 1Log10-fold change) of each member of the GM15 gut microbiota's load among
248 the conditions tested (Fig. 2e). Therefore, we conclude that the GM15 community is stable
249 upon adult colonization, among filial generations, during aging, upon mild dietary fluctuations
250 and can be transmitted efficiently by FMT.

251

252 **The GM15 microbial community recapitulates SOPF macroscopic phenotype**

253 GF and published gnotobiotic mice display anatomical alterations compared to SOPF mice,
254 such as enlarged caecum, along with physiological and metabolic differences [20, 37]. To
255 phenotypically assess the gnotobiotic GM15 model, we designed a comparative study to
256 evaluate the steady state macroscopic, immune, metabolic and endocrine phenotypes of GF,
257 SOPF and GM15 mice. In addition, all phenotyping was achieved across two generations, F1
258 and F2, to strengthen data analysis.

259 Initially, we evaluated the reproduction performance of the GM15 model by recording
260 the period from mating to offspring delivery (Fig. 3a), the number of pups per litter (Fig. 3b),
261 and the perinatal mortality (Fig. 3c). GM15 mice behaved like SOPF mice with the exception
262 of one less progeny per mean litter. Then, we quantified the food intake relative to body weight
263 after weaning at 4 weeks of age and observed no significant difference between the three

264 groups despite a marked increased variation among GF animals which is not detected in GM15
265 and SOPF animals (Fig. 3d). Next, we studied post-natal growth parameters. Male and female
266 GM15 animals gained weight (Fig. 3e) and size (Fig. 3f) like SOPF mice, although the growth
267 curves from the two sexes differ. We then studied internal organ size. As expected, the
268 characteristic caecum enlargement seen in GF animals was reduced in GM15 mice (Fig. 3g).
269 The weights of GM15 and SOPF brain, liver and spleen were equivalent and larger than those
270 of GF mice (Fig. 3g). Bone size was also identical in GM15 and SOPF mice and larger than
271 that of GF mice (Fig. 3h). Taken together, these results confirm that the gut microbiota is
272 associated with somatic tissue growth [38, 39], and that the GM15 simplified microbiota is
273 sufficient to largely recapitulate the breeding and growth performance of SOPF mice by
274 compensating the physiological limitations of GF mice.
275

276 **The GM15 simplified microbiota partially restores SOPF immune phenotype**

277 It is now well established that host-specific bacteria consortia influence intestinal and systemic
278 immune maturation [40, 41]. We thus profiled the basal immune parameters of GM15 animals
279 and compared them to SOPF and GF animals at 7-8 weeks of age. We observed that the
280 GM15 community induces the production of IgA in the gut and in serum at levels equivalent to
281 those detected in SOPF mice (Fig. 4a). Notably, the highest levels of circulating IgA were not
282 correlated with the highest levels of fecal IgA, and intragroup variability was not related to gut
283 microbiota composition, which was homogeneous between individuals. Additionally, IgG2b
284 serum levels were equivalent in GM15 and SOPF mice compared to GF mice, IgG3 levels
285 were increased in GM15 mice compared to GF and SOPF mice, and IgM levels were slightly
286 increased in GM15 mice compared to SOPF (Fig. 4a), which was consistent with published
287 comparative analyses carried out with SPF mice even though IgG3 and IgM levels were lower
288 in our SOPF mice [42, 43]. On the other part, no difference of circulating IgG1 was observed
289 between GF, GM15 and SOPF, and all IgG2a levels were below levels of detection (data not
290 shown). Surprisingly, GM15 mice presented elevated levels of IgE in serum compared to GF
291 and SOPF mice (Fig. 4a). High IgE levels may result from parasitic infections, or

292 immunodeficiencies and long-lived IgE producing plasma cells generated by food antigens in
293 mice with low-diversity microbiota during early life [42, 44, 45]. Independent tests following
294 FELASA guidelines (CR EU RADS, France) rejected the infection hypothesis of the GM15
295 mice, which were negative for ectoparasites and endoparasites, respiratory and intestinal
296 specific pathogenic bacteria and infectious agents, as well as viruses. It is also known that
297 Clostridia-abundant juvenile mice have lower IgE levels [46]. Of note, our GF mice do not show
298 increased IgE levels as SOPF, and GM15 mice harbor a similar load of Clostridia as SOPF
299 despite elevated levels of IgE. Therefore, we speculate that specific members of the GM15
300 consortium are inducers of the observed high IgE titer.

301 Next, we assayed the circulating levels of key intestinal cytokines IL-17a and IL-22,
302 whose production is induced by commensal bacterial via TLR2 activation in the gut mucosa
303 [47], and linked to local inflammation [48, 49] or associated with anti-microbial responses and
304 control of bacterial colonization [50, 51]. GM15 microbiota induced release of both cytokines
305 compared to GF mice (Fig. 4b) and in a similar manner to SOPF mice for IL-22. To note, serum
306 IL-17a concentrations in our SOPF mice detected by Luminex were slightly lower than those
307 published for SOPF animals in previous studies using Luminex detection, but do not explain
308 the difference observed between GM15 and SOPF mice levels [52]. We also measured IL-1b,
309 IL-10, IL-12p70, and IL-17f, which are cytokines involved in autoimmune and inflammatory
310 bowel diseases, but we did not detect any difference between the three groups of mice.

311 In parallel, we assessed viable leukocytes by measuring CD45+ cell counts and
312 analyzed T cells, B cells, NK cells and monocytes or dendritic cells (DC) populations in whole
313 blood and several lymphoid organs (spleen, thymus, Peyer's patches (PP) and mesenteric
314 lymph nodes (MLN)). It has been previously shown that isolated lymphoid structures such as
315 PP and associated cellularity were strongly increased with microbiota diversity whereas the
316 total cell numbers in MLN were comparable between GF and SPF mice [53]. As described in
317 literature, the number of PP collected where higher in SOPF mice compared to GF and partially
318 restored in GM15 mice (data not shown), and no or minor difference were observed between
319 the 3 groups for CD45+ cells count that showed slight increased levels in SOPF compared to

320 GF mice with apparent intermediary levels in GM15 mice in spleen, thymus and PP, and no
321 difference in MLN (Fig. 4c).]. In 2018, Kennedy *et al.* published an overview of the literature
322 describing the main immune cell populations modifications observed in GF mice in different
323 organs [54]. In our study, very few immune cell populations seemed to be impacted by flora
324 composition. No difference between the 3 groups was observed for MLN DC, circulating and
325 splenic CD4+ and CD8+ T cells and splenic B cells as described in literature (data not shown).
326 Minor differences were observed for PP CD4+ T cells and splenic DC between GM15 mice
327 and SOPF but not with GF mice whereas both populations were described to be decreased in
328 GF mice (Additional file 6: Fig. S4b/e). Only PP NK cells showed similar and increased levels
329 in GM15 and GF mice compared to SOPF mice (Additional file 6: Fig. S4e). Interestingly, the
330 great majority of populations impacted showed similar levels in GM15 mice and SOPF
331 compared to GF mice (Additional file 6: Fig. S4a-e). Circulating monocytes, MLN CD8+ T cells
332 and splenic NK cells were increased in GM15 and SOPF compared to GF as described in
333 literature for circulating monocytes and MLN CD8+ T cells and though splenic NK cells were
334 not described to be modified in GF mice. Thymic CD4+ T cells, circulating and MLN B cells
335 were decreased in GM15 and SOPF mice compared to GF though circulating B cells were
336 described to be decreased in GF mice.

337 Taken collectively, our results indicate that the GM15 community is sufficient to dampen
338 the characteristic immunodeficiencies of GF animals and restore an immunophenotype close
339 to that detected in SOPF animals with a peculiar signature of the GM15 model with high IL17a
340 and IgE levels in sera. To note, no correlation was observed between those two markers in
341 our study although positive or negative correlations have been both described in literature [55,
342 56, 57, 58]. Furthermore, as for IgE, IL-17a levels modulations have been described to be
343 associated to particular flora compositions [42, 59, 60, 61, 62, 63, 64, 65, 66, 67]. Such an
344 observation paves the way to further investigation to decipher which and how members of the
345 GM15 community trigger these immunophenotypes.

346

347 **Low-complexity GM15 microbiota shares more metabolic traits with SOPF than GF mice**

348 The gut microbiota influences multiple host metabolic pathways by providing metabolites to its
349 host and also shapes inter-organ communication within the body by influencing the production
350 and activity of endocrine signals [68, 69]. One-dimensional proton nuclear magnetic resonance
351 spectrometry ($_1$H NMR) was previously applied to investigate how the gut microbiota impacts
352 host metabolism using mouse models [70, 71], or human cohorts [72]. Using this technology,
353 we analyzed the metabolic profile of plasma samples from GF, GM15 and SOPF mice and
354 were able to quantify a total of 51 polar metabolites and 5 non-polar metabolites whose levels
355 were not significantly affected by sex (Additional file 7: Table S3; Additional file 8: Table S4).
356 A principal component analysis based on quantified polar metabolites showed sequential
357 alignment on first principal component (8.8% of total variance) according to microbiota
358 complexity of GF, GM15 and SOPF samples, independently of F1 and F2 generations (Fig.
359 5a). The metabolic composition of GF plasma resulted only from the host metabolic activity
360 and represented a basal phenotype. At the other end, the SOPF mice harboring a very diverse
361 gut microbiota, exhibited a larger panel of metabolic activities based on host-bacterial and
362 bacterial-bacterial interactions. In-between, the GM15 low-complexity community contributed
363 in a lower extent to the metabolic phenotype. We performed the equivalent statistical analysis
364 using binned NMR spectra instead of quantified metabolites and as expected we obtained the
365 same results (Additional file 9: Figure S5). Next, we performed a discriminant analysis across
366 all samples to highlight the specific metabolic signatures of each group. Eight metabolites
367 emphasized significant variation, although it is noteworthy that the calculated distance placed
368 GM15 closer to SOPF than to GF (Fig. 5b). As previously reported, GF mice had higher plasma
369 levels of glycine [73] and reduced plasma acetate concentration [74]. On the contrary, SOPF
370 mice harboring a diverse microbiota had higher plasma levels of acetate, acetoacetate and
371 dimethyl sulfone [75]. As for GM15 mice, less methanol and more citrate, ethanol and pi-
372 methylhistidine were detected. Interestingly, methanol may occur as a result of fermentation
373 by gut bacteria and can stimulate citric acid fermentation [76, 77]. Thus, the simplified gut
374 microbiota of GM15 mice may produce less methanol and/or microbially-produced methanol
375 may be used to form citrate. On the other part, ethanol is produced by some intestinal bacteria,

376 such as *E. coli* and lactic acid bacteria, which are well represented in the GM15 community
377 [78]. These specific metabolic signatures may be used in the future as a panel of biomarkers
378 to identify the GM15 model. Besides, the analysis of the plasma non-polar metabolites
379 indicated that GM15 colonization was sufficient to reduce free cholesterol and
380 phosphatidylcholine as observed in SOPF mice (Fig. 5c). The three additional non-polar
381 metabolites detected were equivalent in all mice (Additional file 8: Table S4).

382 Finally, we investigated the circulating levels of key metabolic hormones and growth
383 factors. To allow blood sample mutualization for different analyses, mice were not fasted.
384 Ghrelin was below limit of detection for all groups, and glucagon, insulin and leptin did not
385 show any difference between GM15 and GF mice, whereas levels detected in SOPF mice
386 were similar to those previously published under basal conditions [79, 80, 81] (data not shown).
387 However, as compared to levels detected in GF animals the GM15 gut microbiota was able to
388 restore the levels of circulating insulin growth factor 1 (IGF-1) as seen in SOPF animals. IGF-
389 1 is an essential growth factor promoting systemic and tissue growth [82, 83] and this
390 observation correlates well with the improved macroscopic growth of GM15 mice compared to
391 GF animals (Fig. 5d, Fig. 3e-h). Corticosterone levels, which are high in GF and low in SOPF
392 animals were also normalized by GM15 bacterial colonization [84] (Fig. 5e), indicating that
393 GM15 community seems as efficient as a complex SOPF microbiota at utilizing host
394 metabolites and promoting steroidogenesis and growth factor production.

395 Collectively, our results reveal that GM15 animals stand out from GF mice and
396 recapitulate many of the SOPF metabolic features even though some differences exist.

397

398 **GM15 mice are less sensitive to diet-induced stunting than SOPF mice**

399 Previous work by our lab and others [85, 86, 38] has shown that the gut microbiota influences
400 pathogenesis associated to chronic undernutrition, particularly diet-induced stunting.
401 Consequently, we sought to study our newly-established gnotobiotic mouse model under a
402 severe nutritional stress, induced by a macronutrient-depleted diet (containing 4% protein and

403 2% lipids) expected to trigger stunting. We thus fed male mice a breeding diet (BD) or a
404 depleted diet (DD) from post-natal day 21 (i.e. the day of weaning) until post-natal day 56.
405 As shown in Fig. 6a-b and similarly to our previous observation (Fig. 3e-f) GM15 and
406 SOPF mice grew well on the BD as they show similar body weight and size gains. Next, we
407 confirmed that the DD triggered an almost full stunting of both the juvenile GM15 and SOPF
408 mice, characterized by the flattering of their weight and size curves (Fig. 6a-b). However, the
409 GM15 mice performed slightly better in terms of growth as both their body weight and size
410 were significantly less impacted than SOPF animals by the DD. While the caecum of GM15
411 was enlarged (Fig. 6c, Additional file 10: Fig. S6a), this variation could not account for the total
412 weight difference between GM15 and SOPF animals. We thus compared the sizes and weights
413 of nine other organs (Fig. 6d-f, Additional file 10: Fig. S6b-g) in order to account for these
414 variations and using two-way ANOVA, we confirmed that diet was the main driver of the growth
415 phenotype. However, we found that the increase in body size observed in GM15 mice on the
416 DD could be correlated to a significant increase in the size of the tibia (Fig. 6d). Although we
417 did not find any other significant difference in other single organ size or weight between GM15
418 and SOPF animals on DD, we observed a clear tendency of an increase in GM15 compared
419 to SOPF animals for most parameters tested (Fig. 6e-f, Additional file 10: Fig. S6b-g). We
420 tested this tendency by integrating all the phenotypical parameters in a Principal Component
421 Analysis (PCA). We first established which parameters were correlated to the phenotype
422 (Additional file 10: Fig. S6h), thus excluding brown adipose tissue from the analysis as a non-
423 correlating parameter. The PCA revealed that, under BD, the GM15 phenotype was part of the
424 spectrum of the SOPF phenotype (Fig. 6g). However, under DD, there is a clear shift between
425 the GM15 and SOPF phenotypical space (Fig 6g, dotted lines). Our results thus indicate that
426 under nutritional stress, GM15 microbiota buffers diet-induced stunting slightly more effectively
427 than an SOPF microbiota. As IGF1 is an important driver of the diet and microbiota mediated
428 growth promotion [38], we assessed IGF1 levels in animals and while the levels massively
429 drop in the DD conditions, we did not detect any difference between GM15 and SOPF mice at
430 day 56 on the DD (Fig. 6h). However, we cannot exclude that slight variation in size between

431 GM15 and SOPF animals on DD results from a differential secretion of IGF1 before day 56,
432 when the growth rate of the animal is maximal (Fig. 6a-b).

433 Taken together, our data show that animals bearing the simplified GM15 microbial
434 community perform similarly than animal bearing a complex SOPF microbiota under non
435 stressful conditions, however the GM15 microbiota seem partially protective against the
436 deleterious effects of chronic undernutrition, as compared to SOPF mice.

437

438 **Conclusions and perspectives**

439 Here we describe GM15, a simplified and controlled murine gut microbiota and its related
440 GM15 C57BL/6J gnotobiotic mouse model. The GM15 community is composed of pure
441 cultures of 15 strains from 7 of the 20 most prevalent bacteria family present in the fecal
442 microbiota of SOPF C57BL/6J mice. GM15 carries significant potential for enzymatic activities
443 in the gut and recapitulates extensively the functionalities found in C57BL/6J SOPF gut
444 microbiome. *In vivo* GM15 is stable upon adult colonization for up to 12 months, during natural
445 transmission among 4 filial generations, upon mild dietary fluctuations and can be transmitted
446 efficiently by FMT. GM15 compensates the steady state breeding, growth, immune, endocrinal
447 and metabolic limitations of GF mice and recapitulates many of the SOPF phenotypical
448 features. Taken together our results establish that GM15 is a novel controlled preclinical model
449 phenotypically similar to SOPF with the potential to ensure an increased reproducibility and
450 robustness of preclinical studies by limiting the confounding effect of microbiota composition
451 fluctuation. Importantly, the reduced microbial complexity of the GM15 community, the
452 tractability of its members and the control offered to the experimenter on its composition allow
453 easy quantification of gut microbiota dynamics. Indeed, upon manipulation of the GM15
454 community composition, the correlation of such dynamics with fluctuating host traits allows the
455 establishment of causal relationships between specific microbiota members and host traits.
456 GM15 model offers exciting perspectives for improvement. Indeed, under stressful nutritional
457 environment, the simplified GM15 microbiota performs slightly better than a complex SOPF
458 community in terms of physiological host response. We have previously identified a

459 *Lactobacillus* strain that is capable of buffering the deleterious effects of such challenge in
460 monocolonized mice [38]. Interestingly, out of its 15 strains, GM15 microbiota contains 3
461 *Lactobacillus* strains. Further genetic manipulations coupled to gnotobiotic studies focusing on
462 modifying the Lactobacilli components of GM15 will pave the way to understanding how this
463 minimal bacterial community buffers the host response to chronic undernutrition.

464 As all experimental models GM15 has its limitations. First, phenotypically, despite being
465 macroscopically similar, GM15 and SOPF animals differ in specific immune and metabolic
466 signatures. This is not particularly surprising given the marked reduced microbial diversity that
467 the GM15 model carries compare to SOPF microbiota (15 strains vs hundreds of species in
468 SOPF animals). These specific immune and metabolic signatures of the GM15 models pave
469 the way to further studies aiming at defining if the presence or absence of specific community
470 members triggers these phenotypes. Another limitation worth mentioning here is that the GM15
471 model is a standardized model, and such standardization, essential for establishing robust
472 causal relationships between a microbiota configuration and a host trait, may decrease the
473 translational potential of the observation.

474 In addition, microbiota-mediated resistance to *Salmonella* infection may be tested in
475 GM15 animals and compared to ASF and $OligoMM_{12}$ animals as recently done by Stecher and
476 colleagues [20]. Second, the GM15 model, by its low complexity, offers the possibility to use it
477 as a template for establishing further complex or complete consortia, e.g. by complementing it
478 with representative strains of the missing prevalent bacteria family found in SOPF microbiota
479 such as Deferribacteraceae, Oscillospiraceae, Clostridiaceae and Eubacteriaceae. Despite
480 these limitations and room for improvement, our study establishes that the GM15 model offers
481 new possibilities for preclinical research focusing on host-microbes and microbe-microbe
482 interactions, and how the microbiota shapes the environmental impact on health and diseases
483 or drug efficacy.

484

485

486

487 ## Methods

488 ### Bacterial strains isolation and identification

489 Fresh caecal contents and fecal pellets of C57BL/6J SOPF mice (Charles River Lab., France)
490 were resuspended (1/10 wt/vol) in reduced broth media for direct dilution plating on agar plates
491 and growth at 37°C under anaerobic atmosphere (90% N_2, 5% H_2, 5% CO_2). *Lactobacillus*
492 *johnsonii* NCC 533 was isolated on MRS agar. *Lactobacillus murinus* 313 and *Parabacteroides*
493 *goldsteinii* WAL 12034 were isolated on Colombia CNA agar with 5% sheep blood. *Bacteroides*
494 *acidifaciens* A40 and *Lachnospiraceae* sp. MD308 were isolated on GAM agar. *Bacteroides*
495 *caecimuris* I48 and *Lactobacillus reuteri* I49 were isolated on GAM agar supplemented
496 respectively with 32 µg/mL vancomycin and 32 µg/mL erythromycin. *Lachnospiraceae*
497 bacterium COE1 and *Lachnospiraceae* sp. MD329 were isolated on M2GSC agar (modified
498 Hobson, containing (per 100 mL) 1 g of casitone, 0.25 g of yeast extract, 0.4 g of NaHCO3,
499 0.2 g of glucose, 0.2 g of cellobiose, 0.2 g of soluble starch, 30 mL of clarified rumen fluid, 0.1
500 g of cysteine, 0.045 g of K2HPO4, 0.045 g of KH2PO4, 0.09 g of (NH4)2SO4, 0.09 g of NaCl,
501 0.009 g of MgSO4.7H2O, 0.009 g of CaCl2, 0.1 mg of resazurin, and 1.5 g agar).
502 *Lachnospiraceae* bacterium COE1, *Lachnospiraceae* sp. MD329, and *Lachnospiraceae* sp.
503 MD308 were isolated from caecal contents, the rest from fecal pellets. Fecal pellets of ASF
504 mice (Taconic, USA) were cryopreserved at -80°C, and then resuspended in reduced broth
505 media for direct FMT in GF mice. Fresh caecal content and fecal pellets were resuspended in
506 reduced broth media for direct dilution plating on agar plates and growth at 37°C under
507 anaerobic atmosphere (90% N_2, 5% H_2, 5% CO_2). *Clostridium* ASF356 and *Clostridium*
508 ASF502 were isolated on M2GSC agar, respectively from caecal content and fecal pellets. For
509 identification of isolates, colonies were pre-screened for dereplication by MALDI-TOF MS
510 (Vitek MS, Biomérieux) according to the manufacturer's instructions and database enrichment
511 using RUO mode. Then, gDNA was extracted from pure cultures and analyzed by 16S rRNA
512 gene sequencing (GATC Biotech). Following Edgar's recommendation [87], a full-length 16S
513 rRNA sequence similarity ≥99% using either NCBI blast [27], Ribosomal Database Project [88],
514 or EzTaxon [89] programs allowed the identification of 13 isolates at the species level, and

515 isolates MD329 and MD308 could only be assigned to the *Lachnospiraceae* family. A more
516 precise annotation could be given for the 2 isolates *Clostridium* ASF356 and *Clostridium*
517 ASF502 obtained from the defined ASF microbial consortium. *Anaerotruncus colihominis* JM4-
518 15 (DSM-28734), *Clostridium clostridioforme* YL32 (DSM-26114), *Clostridium cocleatum* I50
519 (DSM-1551), *Escherichia coli* Mt1B1 (DSM-28618) were obtained from DSMZ.
520

521 **Culture conditions**

522 Freshly grown cultures of individual bacterial strains were supplemented with 20% glycerol
523 prior cryopreservation at -80°C. Each culture was systematically validated for culture purity
524 and identity by MALDI-TOF. Culture media and material were introduced in the anaerobic
525 chamber at least 2 days before use. Anaerobic bacterial strains were grown in GAM, except
526 *Clostridium* ASF502, *Lachnospiraceae* sp. MD308, *Lachnospiraceae bacterium* COE1 and
527 *Lachnospiraceae* sp. MD329 in M2GSC, and *A. colihominis* JM4-15 in Bifidobacterium
528 medium. For mouse colonization and absolute quantification of bacteria, a single colony of
529 each of the 15 bacterial strains was grown and amplified at 37°C. Each bacterial pellet was
530 resuspended in medium, 1 mL was cryopreserved with 10% glycerol, 1 mL was centrifuged
531 and bacterial pellet was stored at -20°C for gDNA extraction, and the rest was used for
532 numeration by dilution plating on agar plates. A frozen mixture of the GM15 bacterial
533 community containing the 15 individual strains at equivalent concentration (6.67E+06 CFU)
534 was prepared to enable easy inoculation.
535

536 **WGS**

537 DNA samples from the 15 bacterial cultures were prepared for whole-genome sequencing,
538 using the Nextera XT DNA library preparation kit (Illumina, Cat.Nos. FC-XXX, California, USA)
539 according to manufacturer's recommendations. The resulting libraries were checked for their
540 quality using the High-sensitivity DNA chip using the Agilent 2100 Bioanalyzer (Waldbroon,
541 Germany) and quantified using the QuantiFluor One dsDNA kit (Promega). Paired-end

542 (2x300bp) sequencing was performed on a MiSeq sequencer using the “MiSeq v3 kit (600
543 cycles)” (Illumina, California, USA).
544
545 ***De novo* genome assembly**
546 After a quality control with FastQC [90], the paired-end reads were assembled *de novo* using
547 the “A5-miseq” assembly pipeline [91], comprising the following steps: adapter trimming,
548 quality trimming and filtering, error correction, contigging and scaffolding. The 15 *de novo*
549 assemblies resulted in draft genomes composed of few scaffolds (from 30 to 268) with high
550 N50 values (from 13 099 to 943 892). Genomes were then ordered using Mauve [92] and
551 annoted with PGAP of the NCBI database. Default parameters were used for all software tools.
552
553 **Taxonomic annotation**
554 WGS generated data have been trimmed and quality controlled by AfterQC software [93]. A k-
555 mer counting strategy with the Centrifuge software [25] have been privileged to infer taxonomy,
556 and reads were confronted to the RefSeq complete genome database [26], with bacteria,
557 archaea and viruses domains and the mouse representative genome (taxid 10090), in order
558 to estimate the amount of host DNA contamination and remove it from sequenced data.
559
560 **Genomic functional analysis**
561 Genes were predicted and turned into protein sequences using Prodigal [94]. Marker genes
562 were searched using the HMM3 package [95]. Predicted proteins sequences of genomes were
563 blasted against the KEGG microbial database [96] which contains 13 millions of proteins
564 sequences and trimmed with following parameters: best-hit with an expected value threshold
565 $< 10^{-5}$. The matrix obtained was consolidated into KEGG orthologs count (KO, which
566 represents a set of genes the have sequence similarities and do the same function), into KEGG
567 modules (which represent short enzymatic pathways, involving few proteins and doing a
568 targeted function), and into KEGG pathways (i.e. large metabolic pathways). KO were
569 analyzed for their presence absence among genomes. The modules were analyzed for their

570 completion (4 levels: Full, lack 1 enzymes, lack 2 enzymes, absent), and only modules with a
571 score with 3 or 4 were presented and integrated for their KO count. The KEGG pathways were
572 analyzed for their number of related KO count affiliated to them. A list of functions of interest
573 have been designed and their presence among genomes have been analyzed in details
574 (Additional file 3: Table S2). Because pathways and functions of interest did not have the same
575 number of KO of interest and a different distribution among the genomes, functional data were
576 normalized among each function in order to obtain values that can be comparable. For each
577 function/pathway, the number of different KO was normalized by the total number of KO
578 retrieved. Data were then log-transformed +1. Clusterization of both functions and communities
579 was performed using Euclidian distance and ward's method, and a kmeans clustering was
580 performed in order to define the community clusters.
581
582 **Identification of specific regions for primers design**
583 NUCmer, a part of the MUMmer package [97], was used to perform pairwise alignment of the
584 15 genomes. Based on these alignments, PanCake [98] was used with default parameters to
585 identify specific regions of each genome. Specific regions with a length of 200 bp were
586 extracted, meeting the following criteria: GC content between 48 and 52%, distance to a border
587 of a scaffold higher than 300bp, unique in the draft genome. The specificity of each 200bp
588 region was double-checked with BLAST [27] on the 15-genome database and on the NCBI *nr*
589 database. The design of primers on the specific regions was performed by Fluidigm. The
590 primer specificity was checked with BLAST.
591
592 **Animal experiment**
593 All mice were bred according standardized procedures in the gnotobiology unit of BIOASTER
594 at the ANSES animal facility (Lyon, France), housed in sterilized positive pressure isolators
595 (Noroit) under a 12h light/dark cycle at 22±2°C and 50±30% of humidity, and fed ad libitum
596 with irradiated R03-40 diet (3395 kcal/kg, 25.2% kcal proteins, 61.3% kcal carbohydrates,
597 13.5% kcal lipids; Safe) and autoclaved water. Irradiated corn-cob granules (Safe) were used

598 as bedding. Sterile enrichment was provided in all cages and was constituted by cotton rolls
599 as nesting material, poplar bricks and a polycarbonate red mouse igloo (Safe). Nesting material
600 and poplar bricks were renewed every two weeks. All breeders were mated by trio (2 females
601 and 1 male) between 8 weeks and 6 months of age, and all mice were weaned at 4 weeks
602 after birth. C57BL/6J GF mice were obtained by aseptic hysterectomy of a C57BL/6J SOPF
603 female (Charles River Lab.), and neonates were fostered on C3H GF mothers (CDTA). Axenic
604 status was assessed weekly by gram staining and cultures of fecal suspension on solid and
605 liquid media. GM15 founders were 8-week-old C57BL/6J GF mice colonized by oral
606 inoculations of 215 µL of the GM15 bacterial community, twice at 48h interval. GM15
607 microbiota composition was analyzed by qPCR microfluidic assay from feces collected at 6-
608 week-old. Alternative diet R04-40 (3339 kcal/kg, 19.3% kcal proteins, 72.4% kcal
609 carbohydrates, 8.4% kcal lipids; Safe) was given at 8-week-old GM15 mice for 4 weeks. FMT
610 was done by inoculating orally a suspension of fresh fecal pellets to 7-week-old C57BL/6J GF
611 mice, twice at 48h interval. For undernutrition experiments, GM15 and SOPF mice were bred
612 and randomly assigned to be given either the above R03-40 diet or a custom-made low-protein
613 diet (3500 kcal/kg, 4.7% kcal proteins, 90.1% kcal carbohydrates, 5.3% kcal lipids, Envigo) for
614 5 weeks after weaning. Mice were killed by cervical dislocation and biocollections were
615 performed aseptically. All animal procedures were approved by ANSES/ENVA/UPEC ethic
616 committee (APAFIS#4529-2016022616404045v3; APAFIS#785-2015042819315178v2;
617 APAFIS#18918-2019020118003843v3) and were conducted in accordance with the National
618 and European legislation on protection of animals used for scientific purposes.
619

620 **gDNA extraction from caecal contents and fecal pellets**

621 Caecal and fecal gDNA were extracted using the DNeasy® PowerLyzer® PowerSoil® Kit
622 (Qiagen) following the manufacturer's instructions with modifications. Samples (approximately
623 0.1g) were heat-treated at 65°C for 10 min, and 95°C for 10 min, before a double bead beating
624 at 30 Hz for 5 min. 50µL of DNA were obtained with two consecutive elutions.
625

626 **Quantitative PCR microfluidic assay**

627 In order to quantify specific and global bacteria load per g of caecal or fecal samples (wet
628 weight), qPCR microfluidic assay was performed using respectively specific primers for GM15
629 and "universal" primers amplifying the genes encoding 16S rRNA from most bacteria groups
630 [99]. Amplicons generated using these primers range between 60 and 99 base pairs. qPCR
631 microfluidic assays were conducted in 48.48 Dynamic Array™ IFCs for EvaGreen Fast Gene
632 Expression on a Biomark HD (Fluidigm) according to the manufacturer's instructions, with
633 cDNA diluted 100-fold, preamplified with pooled primers, and diluted again 100-fold. Each IFC
634 included triplicate reactions per DNA sample, standards and negative control. Standards were
635 generated by serial dilution of a gDNA extract from pure bacterial cultures of known
636 concentration. The efficiency of each qPCR reaction was calculated based on the slope of
637 standard curves and within the range of 78-107%. An equivolume mixture of standards was
638 used to normalize data between runs. Due to the different individual detection limits of the
639 qPCR assay for each primer, the detection limit for GM15 ranged between 2.73 x 10^2 and 6.57
640 x 10^5 CFU/g (Additional file 2: Table S1).

641

642 **Sample preparation for immunophenotyping**

643 For flow cytometry analyses whole blood was collected on EDTA tube. Spleen, thymus, MLN
644 and PP were collected in RPMI (Gibco). Single-cell suspensions were achieved using a 100
645 µm cell-stainer (Becton Dickinson) and a 5 mL syringe plunger in RPMI supplemented with 2%
646 heat-inactivated fetal bovine serum (Sigma) and 100 µg/mL DNASE1 (Roche). Cells were then
647 spun at 400xg for 5 min at room temperature. Medium was discarded and cells were washed
648 using 5 mL of supplemented medium. For whole blood, spleen and thymus samples, red blood
649 cells were lysed by resuspension in 1 mL PharmLyse 1X (Becton Dickinson) for 10 min. Cells
650 were then spun at 400xg for 5 min at room temperature. Lysing solution was discarded and
651 cells were washed using 2 mL of PBS (Gibco). Cells were pelleted a second time and
652 resuspended in PBS supplemented with 2% heat-inactivated fetal bovine serum (Sigma).
653 Numeration and viability were determined using Propidium Iodide marker exclusion and

654 MACSQUANT Flow cytometer (Miltenyi). Cells were then resuspended to a working
655 concentration of 10_6 cells / tube for organs and 100 µL / tube for whole blood, and analyzed by
656 flow cytometry.
657 Whole blood was collected on dry Eppendorf tube for sera analysis. Sera were obtained by
658 centrifugation 2000xg for 15 min at 4°C and stored at -20°C before Luminex and ELISA
659 analyses. Feces were collected in Eppendorf low-binding tubes and stored at -80°C before
660 Luminex analysis. Feces supernatant was obtained by disrupting 100 mg feces in 1mL PBS-
661 Protease Inhibitor 1X (Sigma) using Lysing Matrix E Tube (MP Biomedicals) and Fast Prep
662 homogenizer (MP Biomedicals). Samples were spun at 8000xg for 15 min at 4°C and
663 supernatants were collected for IgA Luminex analysis.
664
665 **Flow Cytometry**
666 10_6 cells or totality of cells for some of PP samples were stained for surface markers.
667 Leukocytes were stained using anti-CD45 Viogreen (Miltenyi, clone30F11) and were checked
668 for viability using Zombie Green Fixable Viability (Biolegend). T cells were stained using anti-
669 CD3 BV421 (BD, clone 145-2C11), anti-CD4 PE (Miltenyi, clone REA604) and anti-CD8a PE
670 Vio615 (Miltenyi, clone REA601). B cells were stained using anti-CD45 RB220 PE Vio770
671 (Miltenyi, clone RA3-6B2). NK cells were stained using anti-CD335 APC (NKp46) (Miltenyi,
672 clone 29A1.4.9). Monocytes were stained using anti-CD11b APC Vio770 (Miltenyi, clone
673 REA592) in spleen, thymus and whole blood samples. Dendritic cells were stained using anti-
674 CD11c APC Vio770 (Miltenyi, clone REA754) in spleen, PP and MLN samples. Cells were
675 analyzed using a MACSQuant Ten Flow cytometer (Miltenyi) and raw data were analyzed
676 using FlowJo software (Tree Star, Becton Dickinson). For CD45+ cell count comparison in
677 PP, results were normalized to 10_6 cells using number of cells stained for each mouse when
678 less than 10_6 viable cells were isolated from PP. Data normalization was not possible for whole
679 blood sample, thus CD45+ cell count comparison has not been performed for this
680 compartment. For frequency results, data are represented as a percentage of CD45+ cells for
681 all organs.

682

683 **Metabolic Panel, Ig and Cytokines Luminex**

684 Serum concentrations of Metabolic Panel (Ghrelin, Glucagon, Insulin and Leptin) were
685 determined using the Mouse Metabolic Magnetic Bead Panel Milliplex MAP kit (Millipore).
686 Samples were not diluted, and assay was performed according to the manufacturer's
687 instructions. Serum concentrations of Ig Panel (IgA, IgG1, IgG2a, IgG2b; IgG3, IgM, IgE) and
688 IgA in feces supernatant were determined using the Mouse Immunoglobulin Isotyping
689 Magnetic Bead Panel Milliplex MAP kit (Millipore) and Mouse IgE Single Plex Magnetic Bead
690 Milliplex MAP kit (Millipore). Samples were diluted 1:12500 (IgA, IgG1, IgG2a, IgG2b; IgG3,
691 IgM), 1:100 (IgE), and 1:100 (IgA in feces supernatant). Assays were performed according to
692 the manufacturer's instructions. Serum concentrations of Cytokines Panel (IL-1b, IL-10,
693 IL12p70, IL-17a, IL-17f, IL-22) were determined using the Mouse Th17 Magnetic Bead Panel
694 Milliplex MAP kit (Millipore). Samples were no diluted and assay was performed according to
695 the manufacturer' instructions. Samples were assayed in monoplicate.

696

697 **Corticosterone and IGF-1 ELISAs**

698 Serum concentrations of Corticosterone were determined using the Corticosterone ELISA kit
699 (Abnova). Samples were diluted 1:50, and assay was performed according to the
700 manufacturer' instructions. Serum concentrations of IGF-1 were determined using the
701 Mouse/Rat IGF-1 Quantikine ELISA kit (R&D Systems). Samples were diluted 1:500, and
702 assay was performed according to the manufacturer' instructions. Samples were assayed in
703 monoplicate.

704

705 **Sample preparation for metabophenotyping**

706 It is known that the quantification of some plasma metabolites, such as tryptophan and
707 tyrosine, is biased since they bind to albumin, a highly abundant protein in plasma [100]. In
708 addition, protein precipitation methods with organic solvents can induce loss of volatile
709 metabolites and overlay of residual broad resonances of lipids with some polar metabolites

710 [101]. Thus, the polar and non-polar metabolites extraction from the same plasma sample were
711 prepared with some in-house modifications to previously described sequential approaches
712 [102, 103]. Briefly, we deproteinized the plasma samples by acidified ultrafiltration in order to
713 increase desorption yields of aromatic amino acids and then, quantify the polar metabolites.
714 We also extracted the filter residue of the same sample to analyze the non-polar metabolites.
715

716 **Polar metabolites preparation**

717 Frozen mice plasma samples are thawed in thermoshaker Eppendorf (10 min, 10°C and 1000
718 rpm). The entire amount of plasma about 180 µL is filtered using 0.2 µm centrifugal tube from
719 VWR (5 min, 10°C, 10000xg). Next, the lipoproteins removal was performed by mixing 150 µL
720 of filtered plasma with 50 µL milli-Q water and 10 µL deuterated formic acid (2.1% in milli-Q
721 water) on a clean 10 kDa cut-off ultracentrifugation tube (VWR) using thermoshaker Eppendorf
722 (10 min, 10°C, 750 rpm) and then centrifuged (30 min, 10°C, 10000xg, soft ramp). Vortexed
723 10 kDa-filtred plasma samples (135 µL) were transferred in 0.5 mL 96-wellplate Agilent and
724 mixed with phosphate buffer solution (45 µL, 0.6 M, pH=7.4) containing internal standard DSS-
725 d4 (1.54 mM), pH-reference standard DFTMP (4 mM) and D_2O (40%) on thermoshaker
726 Eppendorf (1.5 min, 10°C, 650 rpm). Finally, the resulting sample solutions (155 µL) were
727 transferred in 3 mm SampleJet NMR tubes. The DSS-d4 concentration was calibrated by $_1$H
728 NMR using sodium succinate dibasic hexahydrate standard solution. This protocol was
729 systematically applied to prepare blank and quality control samples. The concentration of the
730 formic acid was optimized to allow desorption of the metabolites like tryptophan from plasma
731 albumin. Deuterated formic acid instead of protonated form was used to decrease the
732 exogenous NMR signal and allow the quantification of the endogenous formiate. All
733 ultracentrifugation filters are previously recovered from tubes and washed separately from
734 residual glycerol in a 250 mL Duran bottle, plunged in milli-Q water and then sonicated in a
735 bath for 10 min. This washing procedure is repeated five times. After the last step of the
736 washing the ultracentrifugation filters could be stored in water for at least 3 months. Just before
737 use traces of milli-Q water from ultracentrifugation filters are dispersed outside with nitrogen

738 stream. We observed that this procedure of washing removes better the residual glycerol than
739 supplier's protocol and gives higher yields of ultrafiltred plasma.
740
741 **Non-polar metabolites extraction after ultrafiltration**
742 The lipoproteins on the 10 kDa filters was further diluted with 150 µL phosphate buffer solution
743 (1 M, pH=7.4) and mixed on thermoshaker Eppendorf (10 min, 10°C, 750 rpm). The
744 lipoproteins samples were then transferred in clean Eppendorf tubes and extracted with 400
745 µL methanol-dichlormethan (1:2 v/v). Samples were centrifuged (5 min, 10°C, 10000xg) for
746 better phase separation. The dichlormethan layer was transferred in clean Eppendorf tube and
747 the aqueous phase was extracted again with dichlormethan. The pulled organic phase was
748 evaporated under nitrogen stream. The dry lipidic residue was dissolved with 200 µL
749 deuterated chloroform containing 0.03% TMS internal standard and 155 µL of resulting solution
750 was transferred in 3 mm SampleJet NMR tube. The TMS concentration was calibrated by NMR
751 using 1,3,5-tritertbutylbenzen standard solution and it was found to be 0.435 mM.
752
753 **Metabolites analysis and quantification**
754 The 1D ^{1}H NMR spectra were acquired at 298 K with 600 MHz Ascend (Avance III HD)
755 spectrometer from Bruker equipped with a 5 mm QCI cryoprobe. All samples were stored at
756 6°C in the SampleJet autosampler. Polar metabolites were analyzed using *noesygppr1d* pulse
757 sequence. For each spectrum, 128 scans were collected into 32k data points within 14 ppm
758 spectroscopic width and a recycling delay of 4 s. The mixing time was calibrated to 50 ms and
759 the acquisition time was 3.9 s. The non-polar metabolites NMR spectra were acquired using
760 *zg30* pulse sequence. The spectra were recording using 256 scans, into 32k data point and a
761 spectroscopic width of 20 ppm. The relaxation delay was 4 s. The FIDs were zero-filled to 64k
762 points and Fourier transformed using 0.3 Hz exponential line-broadening function. All spectra
763 were aligned to DSS-d4 and TMS respectively internal standard. The concentrations of the
764 polar and non-polar metabolites were quantified using Chenomx NMR suite 8.1. The Chenomx
765 software was applied also for spectra binning of 10^{-3} ppm width for each bin. The triacyl glycerol

766 (TAG), phosphatidylcholine (PC), lysophosphatidylcholine (LysoPC), sphingomyelin (SM), free
767 cholesterol (FC) and cholesterol ester (EC) quantification an in-house lipid database within
768 Chenomx was built with authentic lipid standards. The lipids database spectra were recorded
769 using the same parameters as described above for non-polar metabolites.
770

771 **Metabolomics data analysis**

772 Discriminant analyses were performed using the partial least square (PLS-DA) algorithm to
773 extract metabolomics signatures [104, 105]. A variable selection algorithm based on Elastic-
774 Net was used to improve model performance by selecting most significant metabolic
775 signatures that explain the groups (GF, GM15, SOPF). The statistical performances of the
776 regression models were assessed using the balanced error rate with and without cross-
777 validation (E2 and CV-E2) and permutation tests. Permutation tests consisted in building the
778 null distribution of the balanced error rate E2 by randomly permuting observations. Regression
779 models were thus challenged by testing if the cross-validation error rate CV-E2 is significantly
780 different from the null distribution with a p-value < 0.05. Metabolites involved in the cross-
781 validated signatures were ranked by order of importance in the PLS-DA model using their VIP
782 (variable importance in projection) scores. The biplot allows projecting onto the two first
783 components, the samples and the metabolites that significantly discriminate each sample
784 groups. Metabolites that are positively correlated (or positively contribute) to a sample group
785 will point to the direction of this group. They will point to the reverse direction for a negative
786 correlation.
787

788 **Statistics**

789 Reproductive performance and body growth were analyzed respectively by 1-way and 2-way
790 ANOVA. Phenotyping data impacted by age, filial generation or sex, were analyzed by the F-
791 test for multiple linear regressions (R v3.4.2), otherwise by 1-way ANOVA and Tukey's multiple
792 comparison parametric test or Kruskal-Wallis and Dunn's multiple comparison non-parametric
793 test after D'Agostino et Pearson test for data set normality assessment (GraphPad Prism v7

794 and v8). PCA for phenotyping analysis was performed using R v3.4.2 and the ade4 package
795 [106].
796

797 ## Additional files

798 **Additional file 1: Figure S1.** Morphological features of the GM15 strains. (TIFF 2312 kb)
799 **Additional file 2: Table S1.** GM15 strains-specific primers. (XLSX 11 kb)
800 **Additional file 3: Table S2.** KEGG clusters. (XLSX 142 kb)
801 **Additional file 4: Figure S2.** *In vivo* experimental design. (TIFF 591 kb)
802 **Additional file 5: Figure S3.** Assessment of gut microbiota stability of GM15 founders, and of
803 reproducibility between fecal and caecal samples of individual mice. (TIFF 799 kb)
804 **Additional file 6: Figure S4.** Immune cell populations profiling. (TIFF 471 kb)
805 **Additional file 7: Table S3.** Polar metabolites. (XLSX 18 kb)
806 **Additional file 8: Table S4.** Non-polar metabolites. (XLSX 16 kb)
807 **Additional file 9: Figure S5.** PCA based on binned NMR spectra. (TIFF 1374 kb)
808 **Additional file 10: Figure S6.** Measurements of several organs after nutritional challenge.
809 (TIFF 1011 kb)
810

811 **Abbreviations**

812 ASF: Altered Schaedler flora; BD: Breeding diet; bp: Base pair; CFU: colony forming units;
813 CNA: Colistin and naladixic acid; DD: Depleted diet; FMT: Fecal microbiota transplantation;
814 GAM: Gifu anaerobic medium; gDNA: Genomic deoxyribonucleic acid; GF: Germ-free; IFC:
815 Integrated fluidic circuit; KO: KEGG orthology; MLN: mesenteric lymph nodes; MRS: Man
816 Rogosa Sharpe medium; OTU: Operational taxonomic unit; PCA: Principal coordinate
817 analysis; PP: Peyer's patches; NMR: Nuclear magnetic resonance; qPCR: Quantitative
818 polymerase chain reaction; SEM: Standard error of the mean; SOPF: Specific opportunistic
819 pathogen-free; WGS: Whole genome sequencing
820

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Availability of data and materials

The 15 assembled genomes and the corresponding sequencing reads have been deposited in the DDBJ/ENA/GenBank data banks and the Sequence Read Archive respectively, under the BioProject number PRJNA551571 (GenBank accession no.: VIRE00000000, VIRD00000000, VIRC00000000, VIRB00000000, VIRA00000000, VIQZ00000000, VIQY00000000, VIQX00000000, VIQW00000000, VIQV00000000, VIQU00000000, VIQT00000000, VIQS00000000, VIQR00000000, VIQQ00000000 ; SRA accession no.: SRR9696643 to SRR9696657).

Author’s contributions

MD, FL and AT conceived the project, analyzed, interpreted and integrated all the data. MD and FL wrote the manuscript. MD prepared figures. LB and JY achieved *in silico* design. MD performed bacteria isolation and identification, gDNA extraction, developed the qPCR microfluidic assay and analyzed macroscopic phenotyping data. AS carried out WGS, CE identified specific regions for primers design, and AM performed taxonomic annotation and functional bioinformatic analyses. PB, HD and MD performed animal experiments. CC, AD, JC and SP performed the immunophenotyping, analyzed and interpreted data. AB and DAO performed the metabophenotyping, analyzed and interpreted data. MS helped design the diet

849 induced stunting protocol. FDV performed, analyzed and contributed to the text and figures
850 related to the stunting model experiments. ALB provided technical support during the stunting
851 model experiments. All authors read and approved the final manuscript.
852
853 **Ethics approval**
854 Mouse experiments were performed as approved under the MESR and ANSES/ENVA/UPEC
855 Ethical Committee, protocols 2015042819315178 and 2016022616404045.
856
857 **Consent for publication**
858 Not applicable.
859
860 **Competing interests**
861 The authors declare that they have no competing interests.
862
863 **References**

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1162 formers and other misplaced clostridia. Environ Microbiol. 2013;15:2631-41.
1163
1164
1165 **Legends**
1166 **Fig. 1. Selection, isolation and functional analysis of GM15 strains. a** Composition of
1167 representative and prevalent bacterial families identified from fecal samples of 4 C57BL/6J
1168 mice of our colony. **b** 11 bacterial strains were isolated from fecal or caecal samples of either
1169 C57BL/6J or ASF mice using various culture methods. 4 additional strains were obtained from
1170 the DSMZ collection. **c** Unique KO (KEGG Orthology) groups are those present in only one
1171 GM15 member. The unique contribution of each GM15 member is relatively evenly distributed
1172 (<2%), except *E. coli* whose unique functions contribute for 33%, although non-unique KO
1173 groups are primarly responsible for the GM15's metagenomic profile with 59% of contribution.
1174 **d** Heat map of hierarchical clustering of enzymatic activities in the gut for the 15 strains of the
1175 GM15 model. In quorum sensing (QS) function, only effector proteins were screened (because
1176 receptors suffer of similarities with nonspecific QS receptors). **e** Heat map of hierarchical

1177 clustering of KEGG modules distribution in the metagenomes of GM15, SOPF, ASF and Oligo-
1178 MM_{12} models. Clusters of KEGG modules are highlighted with their respective size (number of
1179 KEGG modules). The list of KEGG modules and clusters is shown in Additional file 3: Table
1180 S2.
1181
1182 **Fig. 2. Stability assessments of the GM15 mice gut microbiota over filial generations, in**
1183 **aging, under diet change and through FMT**. SOPF groups show the distribution of each
1184 GM15 strains in the complex gut microbiota of 8-week-old SOPF mice. The absolute
1185 quantification of each strain was determined by specific qPCR microfluidic assay. To gain
1186 clarity, each GM15 strain is associated to a number between 1 and 15. * Strains 4, 6, 7 and 14
1187 were at the detection limit of the qPCR microfluidic assay, and thus were not detected in all
1188 samples. ** Strains 8, 9 and 10 were below detection limit of the qPCR microfluidic assay.
1189 Strain 5, obtained from the DSMZ collection, was not detected in our SOPF colony. **a** Radar
1190 plot showing the GM15 strains distribution in feces of GF C57Bl/6J mice colonized with the
1191 GM15 community (F0, n=10) and bred for consecutive generations (F1 to F4, n=19 to 8). **b**
1192 Radar plot showing the overall stability of the GM15 community composition in feces collected
1193 from 9 mice between 2 and 12 months of age (2 mice died at 12-month-old). **c** Radar plot
1194 showing that an alternative diet, such as a maintenance diet, can be used for 4 weeks, and
1195 then reversed to the breeding diet for 4 more weeks, without modifying the composition of the
1196 gut microbiota of GM15 mice (n=18) compared to mice fed all along with the breeding diet
1197 (n=9). **d** Radar plot showing the feasibility of GM15 fecal microbiota transplantation to GF mice
1198 (n=9). **e** Box plots showing the low variability of the GM15 strains concentrations considering
1199 all fecal samples of mice from generation F1 to F4 (n=47), from generation F1 at 12-month-old
1200 (n=7), from generation F1 under diet change (n=18), and fecal samples collected 3 weeks post
1201 fecal microbiota transplantation in GF mice. Whiskers represent min and max data.
1202
1203 **Fig. 3. Macroscopic phenotyping on mice from two consecutive filial generations (F1-**
1204 **F2). a-c** 1-way ANOVA analyses of the reproductive performance, where dots, lines and error

1205 bars represent respectively individual litters (34 GF, 31 GM15 and 19 SOPF), means and SEM.
1206 **a** GM15, like SOPF, delivered pups around 24 days after mating, against 33 days for GF mice.
1207 **b** GM15 mean litter of 5 pups was in between GF (4 pups) and SOPF (6 pups). **c** Perinatal
1208 mortality was similar for GM15 and SOPF (12 and 11%), and much lower than for GF mice
1209 (45%). **d** Box plots showing no difference between GF, GM15 and SOPF mice in terms of feed
1210 intake, although much variability was observed with GF mice. Whiskers represent min and max
1211 data. **e-f** 2-way ANOVA analyses of body growth of mice bred with their mothers until week 4,
1212 where lines and error bars represent respectively means and SEM. Body weight and size
1213 curves of GM15 mice (9 females (F), 12 males (M)) were similar or equivalent to those of SOPF
1214 mice (10 F, 10 M), whereas GF mice showed a significant delayed growth (7 F, 10 M). **g-h**
1215 Organs weight or size impacted by age, filial generation or sex, were analyzed by the F-test
1216 for multiple linear regressions, otherwise by 1-way ANOVA. Each animal is represented by a
1217 dot at the age of the sacrifice. **g** The characteristic caecum enlargement of GF mice was
1218 partially, but significantly, reduced in GM15 mice. The weights of the brain, liver and spleen
1219 were equivalent in GM15 and SOPF mice, and significantly bigger than those of GF mice. **h**
1220 Femur and tibia lengths were also equivalent in GM15 and SOPF mice, and significantly bigger
1221 than those of GF mice. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.
1222

1223 **Fig. 4. Immune phenotyping through serum and fecal immunoglobulin subtyping,**
1224 **circulating cytokines analysis and immune cell populations analysis in different organs**
1225 **a-c** Dot plots where dots, lines and error bars represent respectively individual mice (15-17
1226 GF, 20-21 GM15 and 20 SOPF), means and SEM. **a** Fecal IgA, serum IgA, IgG2b, IgG3, IgM
1227 and IgE Luminex analysis. F-test for multiple linear regression analyses (fecal IgA) and Dunn's
1228 multiple comparison showing restoration of fecal IgA, serum IgA and serum IgG2b levels in
1229 GM15-colonized mice at the same level than SOPF, slightly increased IgM levels in GM15
1230 mice compared to SOPF mice and elevated levels of IgG3 and IgE levels in GM15 mice
1231 compared to SOPF and GF mice. **b** Circulating IL-17a and IL-22 levels Luminex analysis.
1232 Dunn's multiple comparison showing elevated IL-17a levels in GM15 mice compared to SOPF

1233 and GF mice and restoration of IL-22 levels in GM15 mice at the same level than SOPF. **c**
1234 CD45+ count comparison in spleen, thymus, MLN and PP by flow cytometry. Dunn's multiple
1235 comparison showing increased CD45+ cell count in spleen, thymus, and PP of SOPF mice
1236 compared to GF mice, levels of GM15 mice are in between, and no difference between groups
1237 for MLN. Tukey's multiple comparison showing increased PP in SOPF mice, then in GM15
1238 mice, compared to GF mice. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.
1239
1240 **Fig. 5. Metabolic phenotyping of GM15 animals**. **a** PCA score plot representing the
1241 distribution of the sample in the two first principal components. b Biplot representing the
1242 projection the samples in the PLS-DA score plot given by the two first components and the
1243 projection of the contribution of significant metabolites. **c** F-test for multiple linear regressions
1244 showing equivalent decreased levels of Free Cholesterol and Phosphatidylcholine in GM15
1245 and SOPF mice compared to GF mice. **d-e** Circulating hormones Luminex analysis. Dot plots
1246 where dots, lines and error bars represent respectively individual mice (16 GF, 21 GM15 and
1247 19-20 SOPF), means and SEM. **d** Dunn's multiple comparison showing restoration of serum
1248 IGF-1 titers in GM15-colonized mice at the same level than SOPF. **e** F-test for multiple linear
1249 regressions showing equivalent and reduced serum corticosterone concentrations in GM15
1250 and SOPF mice, compared to GF mice.
1251
1252 **Fig. 6. GM15 mice and SOPF respond differently under post-weaning chronic**
1253 **undernutrition**. Body weight (**a**) and body size (**b**) were measured every week from weaning
1254 to post-natal day 56. **c.** Representative picture of caecum, subcutaneous fat and right femur
1255 of mice at day 56. Tibia length (**d**), liver (**e**) and epididymal white adipose tissue (**f**) weight were
1256 measured. **g.** PCA of tissue weight and size showed clustering of GM15 and SOPF under
1257 control conditions but a distinct pattern under nutritional stress. Percentages on each axis
1258 indicate the variance associated to each coordinate. **h**. Serum IGF1 at day 56. BD: breeding
1259 diet; DD: depleted diet. P-values after two-way ANOVA were adjusted for Sidak's post-hoc test
1260 for multiple comparisons. *P<0.05, **P<0.01, ***P<0.001.

1261

1262 **Additional file 1: Figure S1. Morphological features of the GM15 strains**. Each bacterial
1263 strain was grown individually from a single colony isolated on agar medium and amplified in
1264 liquid culture to exponential growth phase. Bacteria were Gram-stained and imaged by light
1265 microscopy (80-fold magnification, NanoZoomer S60, Hamamatsu). Members of
1266 Bacteroidaceae, Tannerellaceae and Enterobacteriaceae stained Gram-negative, while
1267 Lactobacillaceae, Erysipelotrichaceae and Ruminococcaceae stained Gram-positive.
1268 Lachnospiraceae stained Gram-positive, except *Lachnospiraceae* sp. MD329 and
1269 *Lachnospiraceae* bacterium COE1, which stained Gram-negative likely due to their cell wall
1270 structure as already reported for other Clostridiales [107].

1271

1272 **Additional file 2: Table S1. GM15 strains-specific primers**. Sequences of primers designed
1273 in this study and detection limits by qPCR microfluidic assay.

1274

1275 **Additional file 3: Table S2. KEGG clusters**.

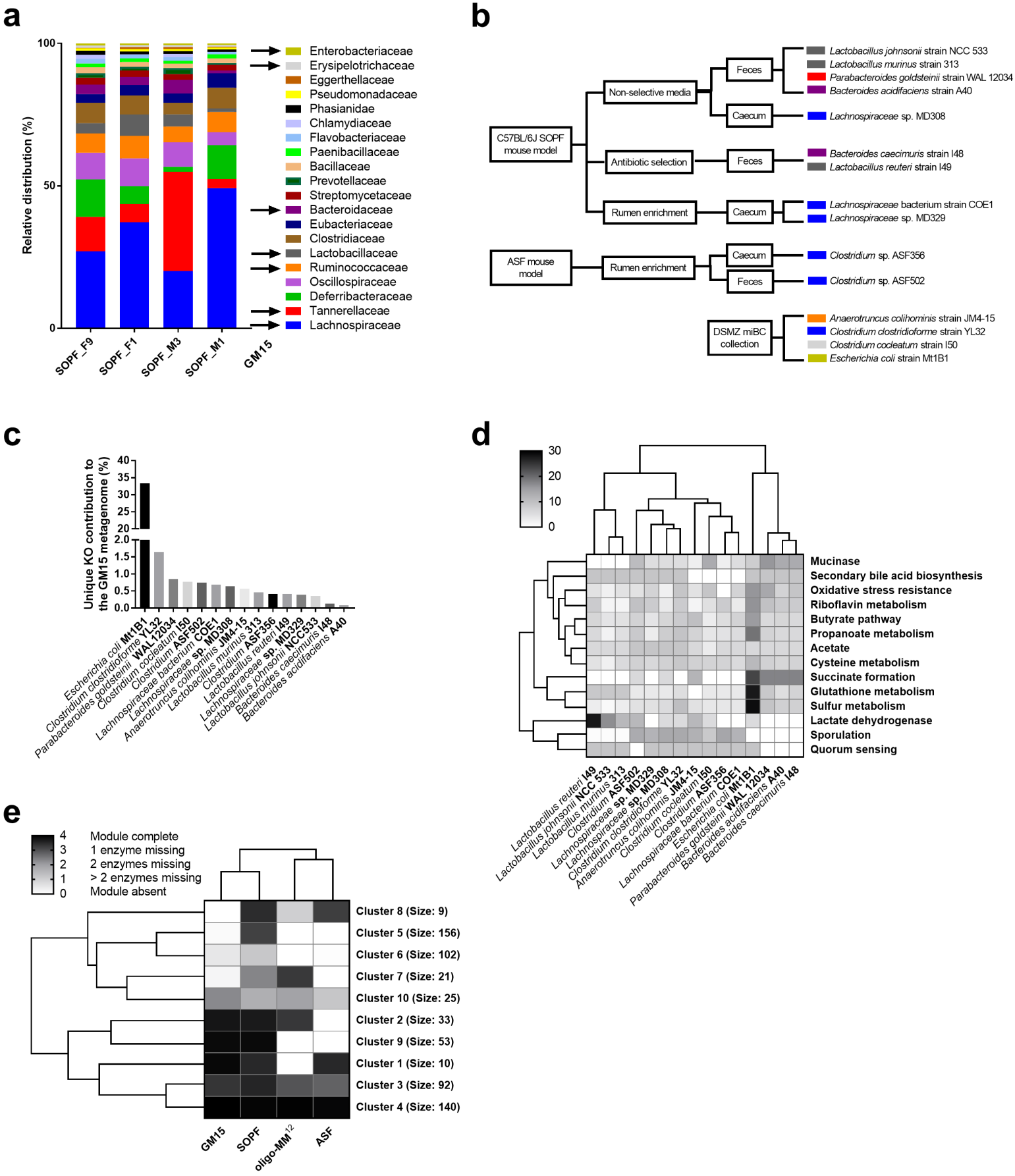
1276

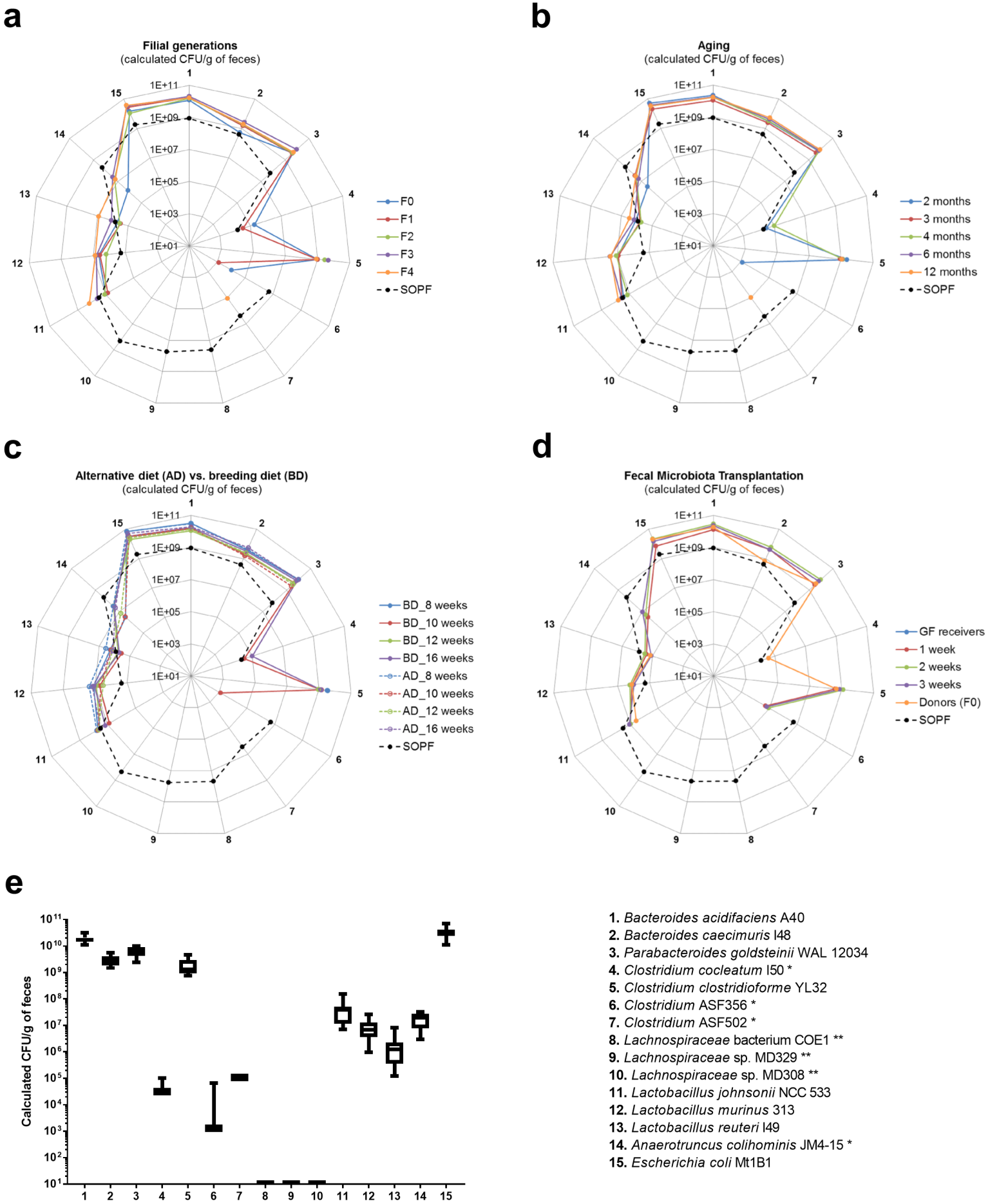
1277 **Additional file 4: Figure S2. *In vivo* experimental design**. First, 5 couples of 8 week-old
1278 C57BL/6J GF mice were colonized by oral inoculations of the GM15 bacterial community, twice
1279 at 48h interval (F0). GM15 microbiota stability was assessed by qPCR microfluidic assay from
1280 feces collected after 1, 2 and 3 weeks. Then, in order to evaluate the reproducible transfer of
1281 the GM15 microbiota by fecal microbiota transplantation, 7 week-old C57BL/6J GF mice were
1282 colonized by oral inoculations of a suspension of fresh fecal pellets from GM15 mice (F0),
1283 twice at 48h interval. Again, qPCR microfluidic assay from feces collected after 1, 2 and 3
1284 weeks was carried out. Next, the GM15 mouse line was amplified to monitor the GM15
1285 microbiota through four filial generations at 6 weeks of age (F1-F4), and allow the phenotyping
1286 study from two consecutive generations (F1.2 and F2.1). Reproduction performance and
1287 perinatal mortality were recorded, 4 week-old mice were randomly selected at weaning,
1288 monitored weekly for body weight and size, and feed intake, until sacrifice at 8-9 weeks of age.

1289 GF and SOPF mice were also studied as control groups. Besides, a comparative analysis was
1290 done on the fecal microbiota of 8 week-old GM15 mice (F1.1) either fed with the breeding diet
1291 or an alternative isocaloric diet given for 4 weeks. Finally, the fecal microbiota of 6 month-old
1292 and 12 month-old control GM15 mice fed with the breeding diet was analyzed.
1293
1294 **Additional file 5: Figure S3. Assessment of gut microbiota stability in GM15 founders**
1295 **(F0, n=10), and of reproducibility between fecal and caecal samples of individual GM15**
1296 **mice (F2, n=11).** SOPF group shows the distribution of each GM15 strains in the complex gut
1297 microbiota of 8-week-old SOPF mice. The absolute quantification of each strain was
1298 determined by specific qPCR microfluidic assay. To gain clarity, each GM15 strain is
1299 associated to a number between 1 and 15. * Strains 4, 6, 7 and 14 were at the detection limit
1300 of the qPCR microfluidic assay, and thus were not detected in all samples. ** Strains 8, 9 and
1301 10 were below detection limit of the qPCR microfluidic assay. Strain 5, obtained from the DSMZ
1302 collection, was not detected in our SOPF colony. **a** Radar plot showing the GM15 strains
1303 distribution in feces of GF C57Bl/6J mice before and 1, 2 and 3 weeks after the oral colonization
1304 with the GM15 community. **b** Radar plot showing the reproducible detection of the GM15
1305 strains in feces and caecum collected from the same GM15 mice.
1306
1307 **Additional file 6: Figure S4. Immune markers. Immune cell populations profiling analysis**
1308 **by flow cytometry in different organs a-e** Dot plots where dots, lines and error bars
1309 represent respectively individual mice (13-17 GF, 21 GM15 and 19-20 SOPF), means and
1310 SEM. **a** Tukey's multiple comparison showing restoration of whole blood monocytes levels in
1311 GM15 mice at the same levels than in SOPF mice and difference of B cells levels between the
1312 3 groups. **b** Tukey's multiple comparison showing splenic dendritic levels slightly decreased
1313 in GM15 mice compared to SOPF, and Dunn's multiple comparison showing restoration of NK
1314 cells levels in GM15 mice at the same levels than in SOPF mice. **c** Tukey's multiple comparison
1315 showing restoration of MLN CD8+ T cells and B cells levels in GM15 mice at the same levels
1316 than in SOPF mice. **d** Tukey's multiple comparison thymic CD4+ T cells levels restored in

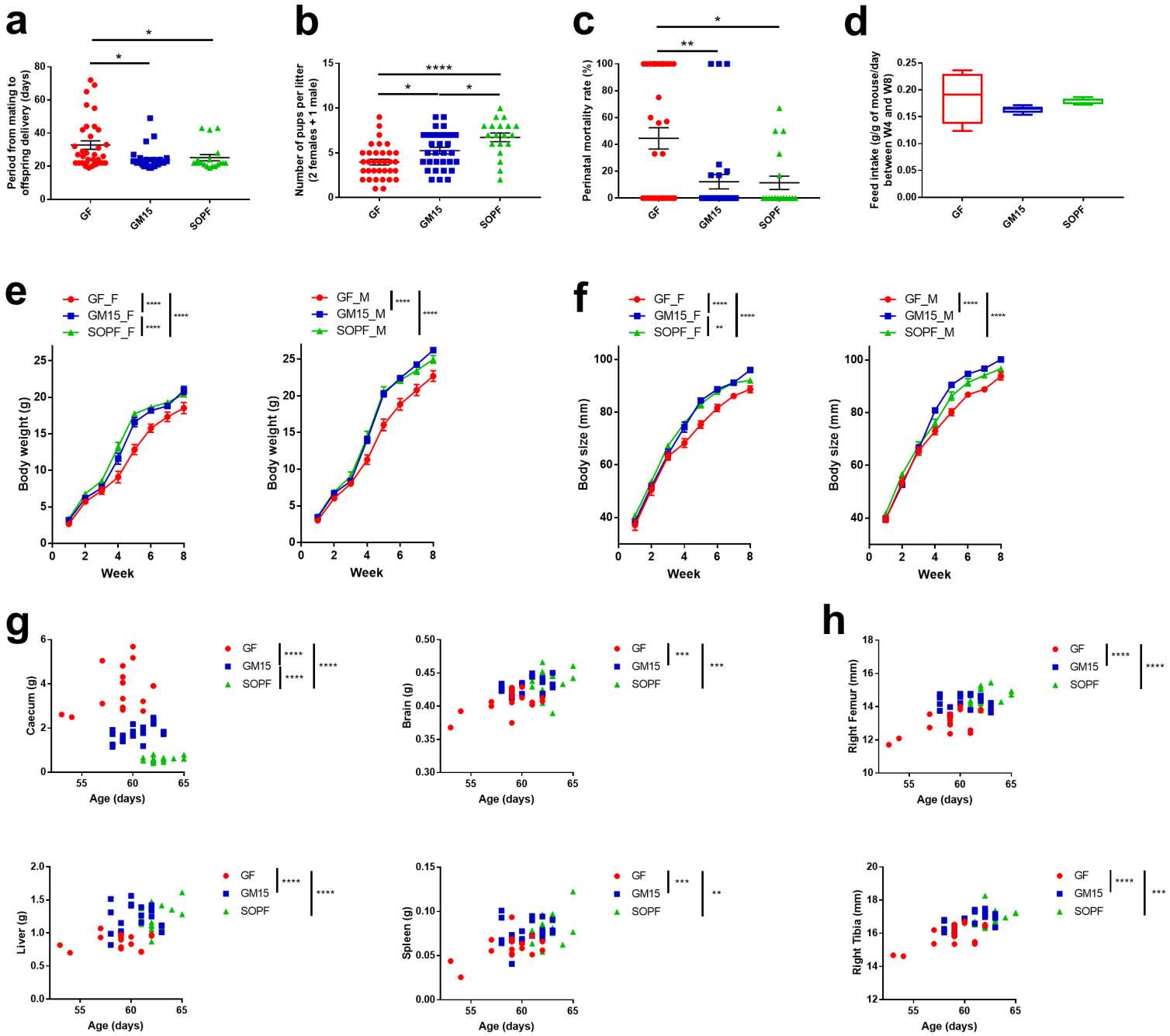
1317 GM15 mice at the same levels than in SOPF. **e** Tukey's multiple comparison showing slightly
1318 decreased PP CD4+ T cells in GM15 mice compared to SOPF and Dunn's multiple comparison
1319 showing increased PP dendritic cells levels in GF mice compared to SOPF mice and increased
1320 NK cells levels in GF and GM15 mice compared to SOPF.
1321
1322 **Additional file 7: Table S3. Polar metabolites concentration in plasma samples of GF,**
1323 **GM15 and SOPF mice**. The metabolites quantification was performed with the help of
1324 Chenomx NMR suite 8.3. The concentrations are reported as mean and standard deviation.
1325
1326 **Additional file 8: Table S4. Non-polar metabolites concentration in plasma samples of**
1327 **GF, GM15 and SOPF mice.** The metabolites quantification was performed with the help of
1328 Chenomx NMR suite 8.3. The non-polar database profiles were created with the help of
1329 Compound builder module using $_1$H NMR spectra of authentic lipid standards. The
1330 concentrations are reported as mean and standard deviation.
1331
1332 **Additional file 9: Figure S5. PCA screen plot based on $_1$H NMR plasma fingerprints.**
1333 Processed $_1$H NMR plasma spectra of polar metabolites were binned with AMIX v3.9.14
1334 software from Bruker Biospin, using 0.04ppm width from 0.5 to 10ppm spectral window. The
1335 residual water region from 4.68 to 4.88pp was excluded from analysis. All spectra were
1336 normalized to the total spectral area and the data table was exported into SIMCA v13.0.3
1337 software for statistical analysis. The PCA analysis was performed using UV-scaling of data
1338 and the model was autofit using cross validation rules to determine the number of significant
1339 components. The clouds of sample points GF, GM15 and SOPF are distributed according to
1340 microbiota complexity from left to right on PC1 (18%) and top to down on PC2 (12%).
1341
1342 **Additional file 10: Figure S6. Measurements of several organs after nutritional**
1343 **challenge**. Measurements at day 56 of weights of caecum (**a**), visceral (**b**) and subcutaneous
1344 (**c**) white adipose tissue, brown adipose tissue (**d**), kidneys (**e**) and soleus muscle (**f**) and length

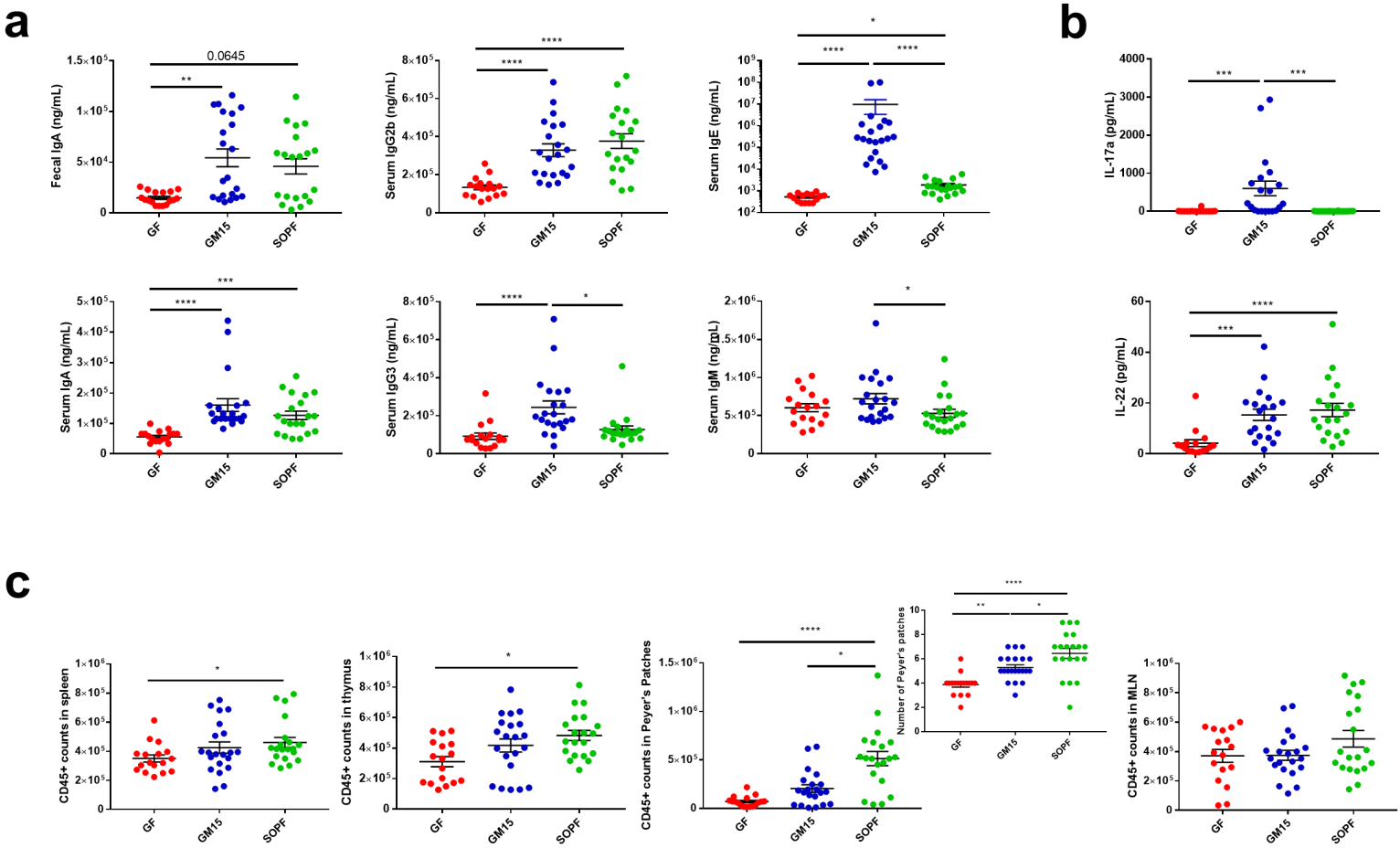
of right femur (**g**). P-values after two-way ANOVA were adjusted for Sidak's post-hoc test for multiple comparisons. **h.** Correlation matrix showing Pearson r values of all the measured parameters. For PCA, brown adipose tissue was excluded, given its lack of correlation with other parameters.

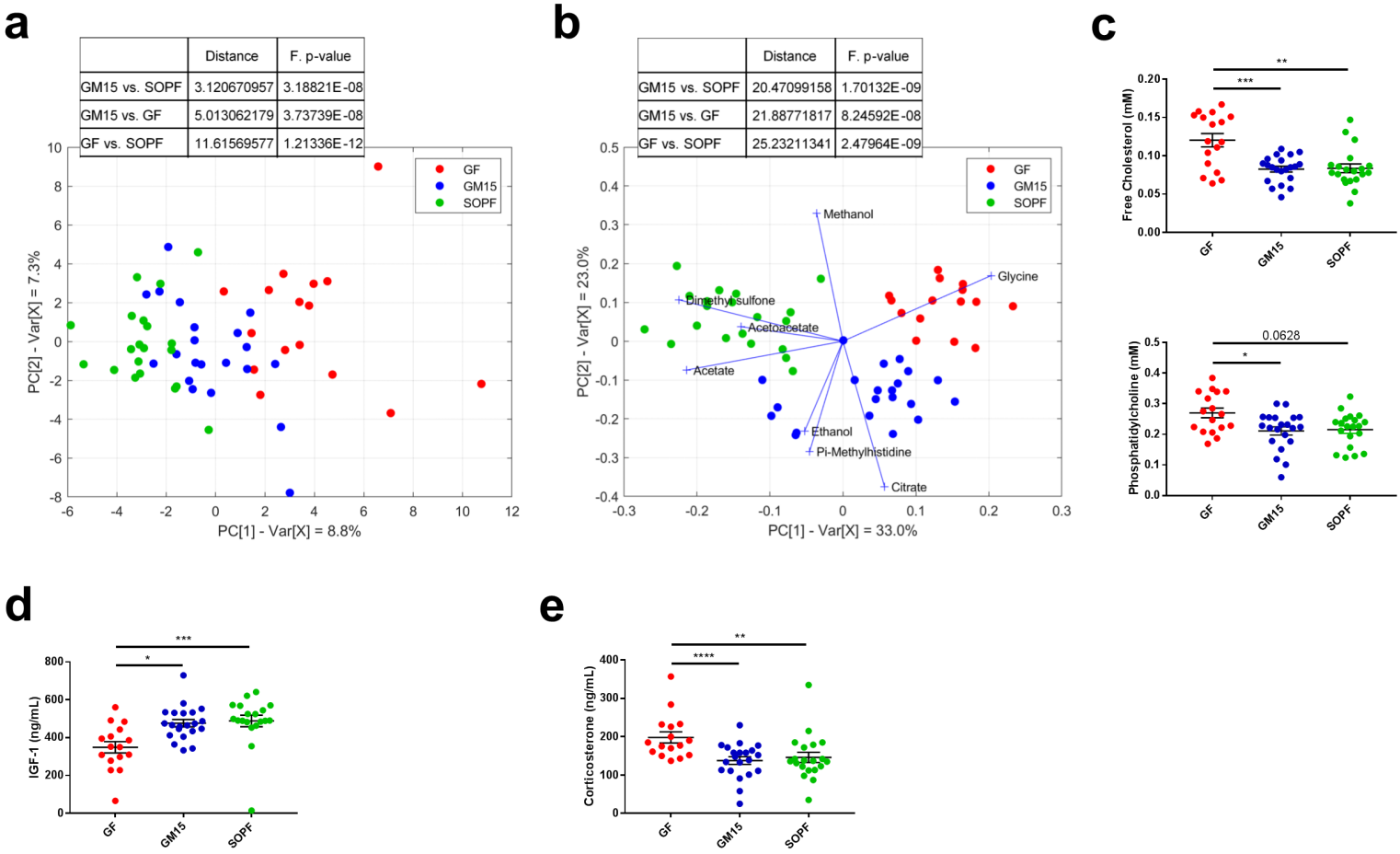

a
Relative distribution (%)
100
50
0
SOPF_F9
SOPF_F1
SOPF_M3
SOPF_M1
GM15
Enterobacteriaceae
Erysipelotrichaceae
Eggerthellaceae
Pseudomonadaceae
Phasianidae
Chlamydiaceae
Flavobacteriaceae
Paenibacillaceae
Bacillaceae
Prevotellaceae
Streptomycetaceae
Bacteroidaceae
Eubacteriaceae
Clostridiaceae
Lactobacillaceae
Ruminococcaceae
Oscillospiraceae
Deferribacteraceae
Tannerellaceae
Lachnospiraceae
b
C57BL/6J SOPF mouse model
Non-selective media
Feces
Lactobacillus johnsonii strain NCC 533
Lactobacillus murinus strain 313
Parabacteroides goldsteinii strain WAL 12034
Bacteroides acidifaciens strain A40
Caecum
Lachnospiraceae sp. MD308
Antibiotic selection
Feces
Bacteroides caecimuris strain I48
Lactobacillus reuteri strain I49
Rumen enrichment
Caecum
Lachnospiraceae bacterium strain COE1
Lachnospiraceae sp. MD329
ASF mouse model
Rumen enrichment
Caecum
Clostridium sp. ASF356
Feces
Clostridium sp. ASF502
DSMZ miBC collection
Anaerotruncus colihominis strain JM4-15
Clostridium clostridioforme strain YL32
Clostridium cocleatum strain I50
Escherichia coli strain Mt1B1
c
Unique KO contribution to the GM15 metagenome (%)
40
35
30
25
20
2.0
1.5
1.0
0.5
0.0
Escherichia coli Mt1B1
Clostridium clostridioforme YL32
Parabacteroides goldsteinii WAL 12034
Clostridium cocleatum I50
Clostridium ASF502
Lachnospiraceae bacterium COE1
Lachnospiraceae sp. MD308
Anaerotruncus colihominis JM4-15
Lactobacillus murinus 313
Clostridium ASF356
Lactobacillus reuteri I49
Lachnospiraceae sp. MD329
Lactobacillus johnsonii NCC533
Bacteroides caecimuris I48
Bacteroides acidifaciens A40
d
30
20
10
0
Mucinase
Secondary bile acid biosynthesis
Oxidative stress resistance
Riboflavin metabolism
Butyrate pathway
Propanoate metabolism
Acetate
Cysteine metabolism
Succinate formation
Glutathione metabolism
Sulfur metabolism
Lactate dehydrogenase
Sporulation
Quorum sensing
Lactobacillus reuteri I49
Lactobacillus johnsonii NCC 533
Lactobacillus murinus 313
Clostridium ASF502
Lachnospiraceae sp. MD329
Lachnospiraceae sp. MD308
Clostridium clostridioforme YL32
Anaerotruncus colihominis JM4-15
Clostridium cocleatum I50
Clostridium ASF356
Lachnospiraceae bacterium COE1
Escherichia coli Mt1B1
Parabacteroides goldsteinii WAL 12034
Bacteroides acidifaciens A40
Bacteroides caecimuris I48
e
4
3
2
1
0
Module complete
1 enzyme missing
2 enzymes missing
> 2 enzymes missing
Module absent
Cluster 8 (Size: 9)
Cluster 5 (Size: 156)
Cluster 6 (Size: 102)
Cluster 7 (Size: 21)
Cluster 10 (Size: 25)
Cluster 2 (Size: 33)
Cluster 9 (Size: 53)
Cluster 1 (Size: 10)
Cluster 3 (Size: 92)
Cluster 4 (Size: 140)
GM15
SOPF
oligo-MM12
ASF

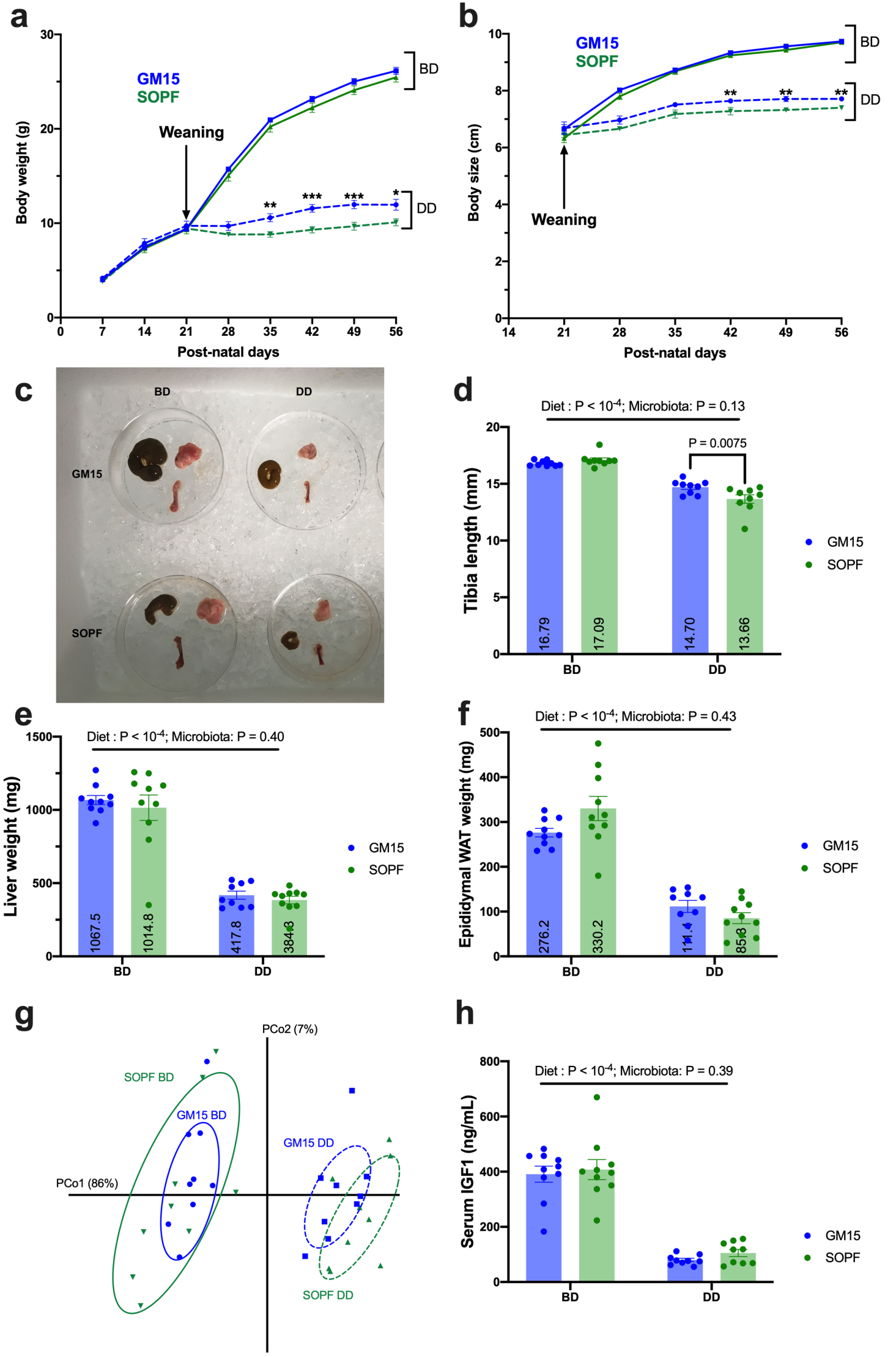


1. *Bacteroides acidifaciens* A40
2. *Bacteroides caecimuris* I48
3. *Parabacteroides goldsteinii* WAL 12034
4. *Clostridium cocleatum* I50 *
5. *Clostridium clostridioforme* YL32
6. *Clostridium* ASF356 *
7. *Clostridium* ASF502 *
8. *Lachnospiraceae* bacterium COE1 **
9. *Lachnospiraceae* sp. MD329 **
10. *Lachnospiraceae* sp. MD308 **
11. *Lactobacillus johnsonii* NCC 533
12. *Lactobacillus murinus* 313
13. *Lactobacillus reuteri* I49
14. *Anaerotruncus colihominis* JM4-15 *
15. *Escherichia coli* Mt1B1


a
Period from mating to offspring delivery (days)
GF
GM15
SOPF
b
Number of pups per litter (2 females + 1 male)
c
Perinatal mortality rate (%)
d
Feed intake (g/g of mouse/day between W4 and W8)
e
GF_F
GM15_F
SOPF_F
GF_M
GM15_M
SOPF_M
Body weight (g)
Week
f
Body size (mm)
g
Caecum (g)
Brain (g)
Liver (g)
Spleen (g)
Age (days)
h
Right Femur (mm)
Right Tibia (mm)


a
b
c
Fecal IgA (ng/mL)
Serum IgG2b (ng/mL)
Serum IgE (ng/mL)
Serum IgA (ng/mL)
Serum IgG3 (ng/mL)
Serum IgM (ng/mL)
IL-17a (pg/mL)
IL-22 (pg/mL)
CD45+ counts in spleen
CD45+ counts in thymus
CD45+ counts in Peyer's Patches
Number of Peyer's patches
CD45+ counts in MLN
GF
GM15
SOPF
0.0645


a
Distance
F. p-value
GM15 vs. SOPF 3.120670957 3.18821E-08
GM15 vs. GF 5.013062179 3.73739E-08
GF vs. SOPF 11.61569577 1.21336E-12
GF
GM15
SOPF
PC[2] - Var[X] = 7.3%
PC[1] - Var[X] = 8.8%
b
Distance
F. p-value
GM15 vs. SOPF 20.47099158 1.70132E-09
GM15 vs. GF 21.88771817 8.24592E-08
GF vs. SOPF 25.23211341 2.47964E-09
Methanol
Glycine
Dimethylsulfone
Acetoacetate
Acetate
Ethanol
Pi-Methylhistidine
Citrate
PC[2] - Var[X] = 23.0%
PC[1] - Var[X] = 33.0%
c
Free Cholesterol (mM)
Phosphatidylcholine (mM)
0.0628
d
IGF-1 (ng/mL)
e
Corticosterone (ng/mL)


a
GM15
SOPF
Weaning
BD
DD
Body weight (g)
Post-natal days
b
GM15
SOPF
Weaning
BD
DD
Body size (cm)
Post-natal days
c
BD
DD
GM15
SOPF
d
Diet : P < 10-4; Microbiota: P = 0.13
P = 0.0075
Tibia length (mm)
16.79
17.09
14.70
13.66
BD
DD
GM15
SOPF
e
Diet : P < 10-4; Microbiota: P = 0.40
Liver weight (mg)
1067.5
1014.8
417.8
384.3
BD
DD
GM15
SOPF
f
Diet : P < 10-4; Microbiota: P = 0.43
Epididymal WAT weight (mg)
276.2
330.2
111.4
85.3
BD
DD
GM15
SOPF
g
PCo2 (7%)
PCo1 (86%)
SOPF BD
GM15 BD
GM15 DD
SOPF DD
h
Diet : P < 10-4; Microbiota: P = 0.39
Serum IGF1 (ng/mL)
BD
DD
GM15
SOPF