LETTER

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Diet-induced extinctions in the gut microbiota compound over generations

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The gut is home to trillions of microorganisms that have fundamental roles in many aspects of human biology, including immune function and metabolism[1,2]. The reduced diversity of the gut microbiota in Western populations compared to that in populations living traditional lifestyles presents the question of which factors have driven microbiota change during modernization. Microbiota-accessible carbohydrates (MACs) found in dietary fibre have a crucial involvement in shaping this microbial ecosystem, and are notably reduced in the Western diet (high in fat and simple carbohydrates, low in fibre) compared with a more traditional diet[3]. Here we show that changes in the microbiota of mice consuming a low-MAC diet and harbouring a human microbiota are largely reversible within a single generation. However, over several generations, a low-MAC diet results in a progressive loss of diversity, which is not recoverable after the reintroduction of dietary MACs. To restore the microbiota to its original state requires the administration of missing taxa in combination with dietary MAC consumption. Our data illustrate that taxa driven to low abundance when dietary MACs are scarce are inefficiently transferred to the next generation, and are at increased risk of becoming extinct within an isolated population. As more diseases are linked to the Western microbiota and the microbiota is targeted therapeutically, microbiota reprogramming may need to involve strategies that incorporate dietary MACs as well as taxa not currently present in the Western gut.

The gut microbiota of hunter–gatherers and populations consuming a rural agrarian diet is distinct, and contains greater diversity than the microbiota of Westerners[4–9] (Extended Data Fig. 1). One possible explanation for the greater microbiota diversity seen in hunter–gatherers and agrarians is the large quantity of dietary fibre they consume relative to Westerners[4,6,10,11]. MACs, which are abundant in dietary fibre, serve as the primary source of carbon and energy for the distal gut microbiota[3,12]. Therefore, we sought to determine whether a diet low in MACs could drive loss of taxa within the gut microbiota.

Humanized mice (4 weeks old, $n = 10$) were fed a diet rich in fibre derived from a variety of plants (high-MAC) for 6 weeks, and randomly divided into two groups (Extended Data Fig. 2). One group was switched to a low-MAC diet for 7 weeks, after which they were returned to the high-MAC diet for 6 weeks (Fig. 1a and Extended Data Table 1). The control group was maintained on the high-MAC diet throughout the experiment. At the start of the experiment, the microbiota composition from both groups of mice was indistinguishable ($P = 0.2$, Student's *t*-test; UniFrac distance; no significant difference in operational taxonomic unit (OTU) frequency observed between groups, Mann–Whitney *U* test). The diet-switching mice, while consuming the low-MAC diet, had an altered composition relative to controls ($P = 10^{-25}$, Student's *t*-test; UniFrac distance). Weeks after returning to the high-MAC diet, the microbiota of the diet-switching mice remained distinct from controls ($P = 3 \times 10^{-8}$, Student's *t*-test; UniFrac distance at 15 weeks) (Fig. 1b). To determine whether taxa had been lost over the course of the diet perturbation, we focused on a subset of OTUs that met stringent measures of prevalence and abundance and could be confidently monitored over the course of the experiment ('high-confidence' OTUs, see Methods). We identified 208 high-confidence OTUs in the diet-switching group and 213 high-confidence OTUs in the control group (Extended Data Table 2). When mice were switched from the high-MAC diet to the low-MAC diet, we observed that 60% of taxa (124 out of 208) decreased in abundance at least fourfold compared with only 11% of the control group (25 out of 213) (Supplementary Table 1). When these mice were returned to a high-MAC diet, 33% (71 out of 208) were fourfold less abundant. The control group did not change significantly (10% were fourfold less abundant; 22 out of 213) (Fig. 1c and Supplementary Table 2). These data reveal two divergent qualities of the microbiota. First, 59 of the 208 high-confidence OTUs that exhibit diet-induced decline in abundance recovered (were no longer at least twofold less abundant) with the reintroduction of MACs illustrating microbiota resilience over short time scales (Supplementary Table 1). Second, however, the low-MAC-diet perturbation induced 'scars' on the microbiota.

We proposed that diet-induced microbiota diversity loss would be magnified over generations. Mice from the previous experiment consuming the low-MAC-diet or the high-MAC diet were used to generate a litter of pups. Pups were weaned onto the respective diets of their parents. This breeding strategy was repeated for four generations. For each generation, low-MAC-diet parents were switched to the high-MAC-diet after their pups were weaned, to see whether taxa that became undetectable while MACs were scarce would bloom in the presence of MACs (Fig. 2a and Extended Data Fig. 3). At 5 weeks old, mice propagated in the low-MAC diet condition (born to low-MAC-diet parents and consuming a low-MAC diet) had a lower-diversity microbiota than high-MAC-fed controls ($P = 3 \times 10^{-6}$, $P = 8 \times 10^{-5}$ and $P = 8 \times 10^{-6}$, Student's *t*-test, Shannon index; generations two, three and four, respectively) (Fig. 2b, top). Even after mice were switched to the high-MAC diet for several weeks, their microbiota diversity did not recover to control levels ($P = 2 \times 10^{-6}$, $P = 1 \times 10^{-8}$ and $P = 1 \times 10^{-4}$, Student's *t*-test; generations two, three and four, respectively, at week 15) (Fig. 2b, bottom). With each generation, the microbiota composition of the diet-switching group showed increasing departure from that of controls (Fig. 2c). Weaning the diet-switching lineage directly onto the high-MAC diet did not correct the diversity loss relative to controls ($P = 3 \times 10^{-6}$ Student's *t*-test, Shannon index), and there was no difference in composition between this group and the generation-four diet-switching group ($P = 0.9$, Student's *t*-test; UniFrac distance; week 13, generation four, and week 5, generation five) (Extended Data Fig. 4).

Plotting the relative abundance of the high-confidence OTUs over time revealed a pattern of taxa loss over generations in the

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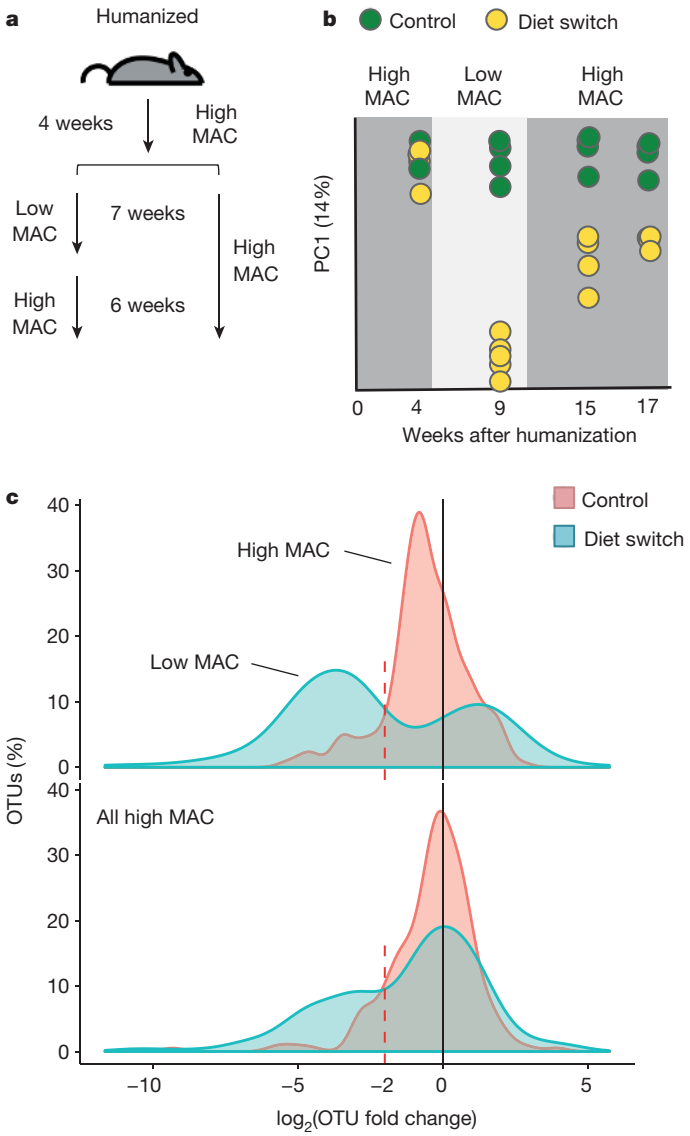

Figure 1 | Taxa reduction observed in low-MAC diet is largely reversible in a single generation. a, Schematic of mouse experiment. Humanized mice ($n = 10$) were maintained on a high-MAC diet for 4 weeks, after which one-half of the mice were switched to a low-MAC diet for 7 weeks. These mice were then switched back to the high-MAC diet for 6 weeks. **b**, Principle coordinate (PC) analysis of the UniFrac distance for 16S ribosomal RNA amplicon profiles from faecal samples collected from the diet-switching mice (yellow, $n = 5$) and control high-MAC-diet mice (green, $n = 5$). **c**, Distribution of OTU fold changes for diet-switching (blue, $n = 5$) or control (red, $n = 5$) groups comparing baseline (4 weeks post-humanization) versus week 9 (5 weeks post-low-MAC diet for 'diet switch' group; top panel) and baseline versus week 15 (4 weeks after return to high-MAC diet for 'diet switch' group, bottom panel).

diet-switching group (Fig. 2d). Specifically, in the diet-switching group, generations one, two and three exhibited a progressive loss in high-confidence OTUs while consuming the low-MAC diet (72%; 150 out of 208 lost by generation four, week 15) (Supplementary Table 3). In each generation, switching to the high-MAC diet allowed for the recovery of a small number of taxa (grey versus yellow highlighted rows within each generation in Fig. 2d), but most did not return (141 out of 208 were undetectable by generation four, week 15) (Supplementary Table 3). Most of the lost taxa (112 out of the 141) were from the Bacteroidales order with an additional loss of 26 taxa from the Clostridiales, making the Clostridiales the most numerous high confidence taxon present in the fourth generation (Extended Data Fig. 5a, b).

To determine whether the carbohydrate degrading capacity of the microbiota had been altered over the four generations, we compared imputed glycoside hydrolases between the first and fourth generations of both the diet-switching and control groups after validating this method[13,14] (Supplementary Table 4). Although representation of glycoside hydrolase families is not a perfect correlate of specificity for polysaccharide degradation (for example, owing to combinatorial activity or polyspecificity within a family), loss of representation within glycoside hydrolase families provides one measure of changes in glycan-degrading capacity. Twenty-two glycoside hydrolase families showed a loss in abundance in the diet-switching group between the initial time point in generation one versus generation four, 4 weeks after the switch to the high-MAC diet ($P < 0.05$ plus at least a twofold change, Bonferroni-corrected Student's t-test) (Extended Data Fig. 5c and Supplementary Table 5). No differences in glycoside hydrolase families were observed in the control group. An overall loss in glycoside hydrolase diversity occurred between generation-four diet-switching mice on the high-MAC diet relative to generation-one mice ($P = 0.0002$, Shannon index of glycoside hydrolase subfamilies; Supplementary Table 6 and Extended Data Table 3). No difference in glycoside hydrolase subfamilies was observed in the control group. These data demonstrate that, in addition to the loss of high-confidence OTUs, the diet-switching group sustained a widespread and marked loss in glycoside hydrolase repertoire over the four generations. Future experiments will be needed to reveal the functional consequences of these observations in terms of fibre-degrading capacity.

We next wanted to determine whether low-abundance OTUs that bloom when MACs are reintroduced are more likely to be lost owing to inefficient inter-generational transmission. Low-abundance taxa (average abundance <25 reads) in a given generation were less efficiently transferred to the next generation (average abundance >10 reads to be considered present, 4 weeks after high-MAC diet) compared to high-abundance taxa (average abundance >25 reads) ($P = 0.002$, $P = 4 \times 10^{-15}$ and $P = 0.01$, hypergeometric distribution inheritance between generations one and two, two and three, and three and four, respectively) (Supplementary Table 7). Notably, overall diversity and composition did not change between the third and fourth generations (Fig. 2b, c); however, we wondered whether additional loss could be obscured owing to lack of resolution of OTUs. Therefore, we identified 280 high-confidence sub-OTUs in the control group and 261 high-confidence sub-OTUs in the diet-switching group using a cluster-free filtering approach[15] (Supplementary Table 8). A similar decline in the number of sub-OTUs with each generation was observed (114 out of 261 sub-OTUs were undetectable by generation four, week 15) (Extended Data Fig. 6 and Supplementary Table 8). Most of the lost taxa (77 out of the 114) were from the Bacteroidales order with an additional loss of 32 taxa from the Clostridiales. Between generation three and four, we detected loss of 22 sub-OTUs, compared to four using the lower-resolution high-confidence OTUs (Supplementary Table 8).

Because high dietary MACs were insufficient to restore microbiota composition or diversity to control levels, we tested whether reintroduction of lost bacteria was required. Fourth-generation, diet-switching mice were gavaged with faecal samples (faecal microbiota transplant (FMT) group) from fourth-generation high-MAC-diet controls. Because the low-MAC diet does not support full microbiota diversity (Fig. 2d), the fourth-generation FMT recipients were fed a high-MAC diet for 2 weeks (Fig. 3a). Within 10 days, microbiota composition and diversity of the FMT group was indistinguishable from fourth-generation high-MAC-diet controls ($P = 0.4$, Student's t-test; UniFrac distance; $P = 0.4$, Student's t-test, Shannon index) (Fig. 3b, c), and 110 taxa were restored (average abundance ≥1 sequencing read; no taxa restored in no FMT controls) (Fig. 3d and Supplementary Table 9). Restored taxa were predominantly from the Bacteroidales (99 taxa), which experienced the greatest loss in high-confidence OTUs (Extended Data Fig. 7a). Similar results were observed using the high-confidence sub-OTUs (Extended Data Fig. 7b and Supplementary Table 10).

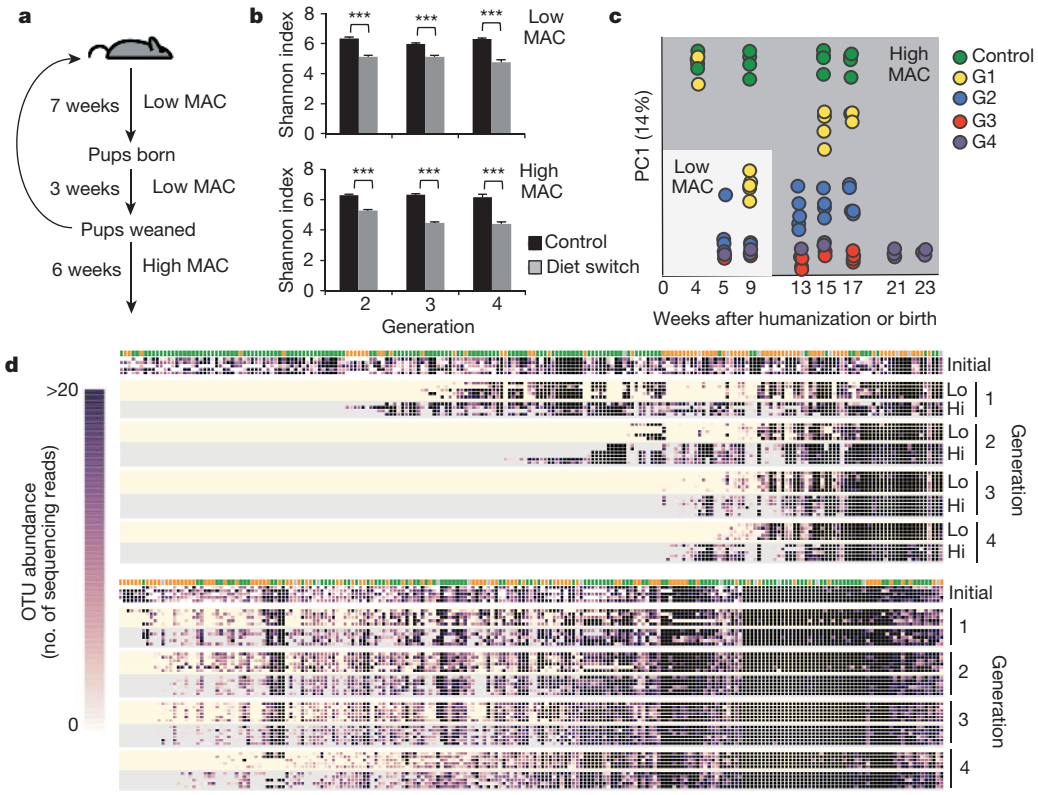

Figure 2 | Inefficient inter-generational transfer of taxa driven to low abundance by low dietary MACs. a, Schematic of multigeneration mouse experiment. Second- ($n = 6$), third- ($n = 6$) and fourth- ($n = 6$) generation mice were weaned onto a low-MAC diet. After mice generated a litter of pups that were weaned, low-MAC-diet mice were switched to the high-MAC diet for 6 weeks. A parallel group of control mice were maintained on the high-MAC diet throughout (generation 2, $n = 6$; generation 3, $n = 6$; generation 4, $n = 5$). **b**, Microbiota diversity as measured by Shannon index observed in the microbiota of mice at 5 weeks old (top panel, $n = 6$ for each group) or 4 weeks after shift to high-MAC diet (bottom panel, $n = 6$ for each group) from three generations of diet-switching mice (grey) or control high-MAC-diet mice (black). Error bars are s.e.m, and P values are from two-tailed Student's t-test. **c**, Principal coordinate analysis of UniFrac distance for 16S rRNA amplicon profiles from faecal samples collected from first-generation mice from the control group consuming a high-MAC diet (green, $n = 5$) or the diet-switching group from generation one (G1; yellow, $n = 5$), two (G2; blue, $n = 6$), three (G3; red, $n = 6$) and four (G4; purple, $n = 6$). **d**, Heat map of abundance of high-confidence OTUs (number of sequencing reads, columns) from the diet-switching group (top) and controls (bottom); taxonomic assignment is indicated at the top of each column (Bacteroidetes, green; Firmicutes, orange; other, grey). Each row represents an individual mouse microbiota from 4 weeks post-humanization (initial), while consuming the low-MAC diet (week 9, lo, shaded yellow), and 4 weeks after switching to the high-MAC diet (week 15, hi, shaded grey). Corresponding time points from controls are similarly shaded. $n = 5$, 6, 6 and 6 for the diet-switching group and $n = 5$, 6, 6 and 5 for the control group for generations one to four, respectively.

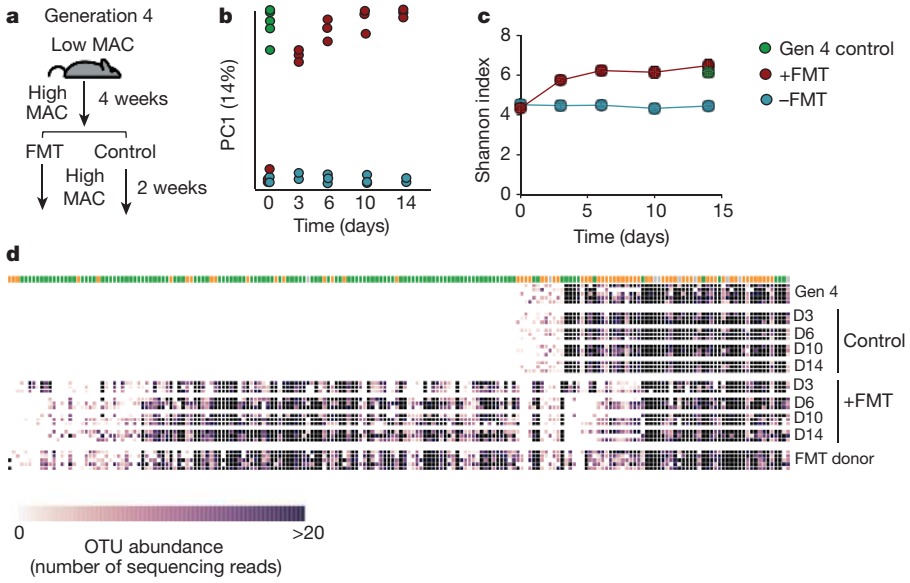

Figure 3 | Reintroduction of lost taxa and a high-MAC diet restores microbiota diversity and composition. a, Schematic of faecal transplant mouse experiment. **b**, Principal coordinate analysis of UniFrac distance for 16S rRNA amplicon profiles from faecal samples collected from fourth-generation control mice on a high-MAC diet (green, $n = 6$), fourth-generation diet-switching mice that received a faecal transplant (red, $n = 3$), or did not (blue, $n = 3$). **c**, Microbiota diversity as measured by Shannon index observed in the microbiota of mice that received a faecal transplant (red, $n = 3$) or did not (blue, $n = 3$). A green circle denotes the number of OTUs observed in fourth-generation control mice consuming a high-MAC diet ($n = 6$). Error bars are s.e.m. **d**, Heat map of abundance of high-confidence OTUs (number of sequencing reads) from fourth-generation diet-switching mice ($n = 3$) 3–14 days after FMT, and no-FMT controls ($n = 3$); taxonomic assignment is indicated at the top of each column (Bacteroidetes, green; Firmicutes, orange; other, grey). FMT donor (fourth-generation control mice, $n = 5$) and fourth-generation diet-switching mice ($n = 5$) 4 weeks after consuming high-MAC diet are also shown.

These data demonstrate a diet-induced ratcheting effect in which certain taxa decrease in abundance upon reduced MACs and are not effectively transferred to the next generation. Notably, most of the lost taxa are Bacteroidales, an order that is proficient in consumption of dietary fibre[16]. Introduction of dietary MACs are insufficient to regain 'lost' taxa in the absence of their deliberate re-introduction.

Over our history, humans have experienced major dietary changes from gathered to farmed foods during the agricultural revolution, and more recently to the mass consumption of processed foods in the industrialized world. Each dietary shift was probably accompanied by a concomitant adjustment in the microbiota. Here we have used a model in which mice have been colonized with a human microbiota from a Westerner to determine the effect of fibre deprivation over four generations on the gut microbiota. This model does not allow us to address microbiota changes that may have occurred as humans shifted from a hunter–gatherer lifestyle to one from a modern industrialized country. Our data support a model in which consuming a modern diet low in fibre contributes to the loss of taxa over generations, and may be responsible for the lower-diversity microbiota observed in the industrialized world compared to present-day hunter–gatherers and rural agrarians. The data we present also hint that further deterioration of the Western microbiota is possible.

The gut microbiota regulates numerous facets of human biology suggesting that our human genome has been shaped by interactions with these microorganisms over our co-evolutionary history. However, the microbiota can change on a timescale that is much faster than the host allowing for the possibility that the microbiota, if pressed by severe selective forces, could undergo change so rapidly that it cannot be accommodated by our human biology. While the roles of different types of microbiota diversity in host health remain to be defined, it is possible that rewilding the modern microbiota with extinct species may be necessary to restore evolutionarily important functionality to our gut.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions E.D.S. and J.L.S. conceived and designed the project. E.D.S., J.L.S. and S.K.H. designed and supervised the experiments. E.D.S. and S.K.H. performed the experiments. E.D.S., S.A.S. and M.T. analysed the experimental data. N.S.W. designed and supervised data analysis. E.D.S. and S.A.S. wrote the manuscript. All authors discussed the results and commented on the manuscript.

Author Information The 16S sequence data have been deposited in the Sequence Read Archive (SRA) under the accession PRJNA303185. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.L.S. (jsonnenburg@stanford.edu).

METHODS

No statistical methods were used to predetermine sample size.

Meta-analysis of human populations. 16S rRNA data sets from Hadza ($n = 27$), Malawian and Venezuelan ($n = 213$) and American ($n = 315$)[4,5] were trimmed to match FLX chemistry as previously described (MG-RAST Projects 111, 528, 7058)[12]. OTUs were picked on the Greengenes 13.8 database with a 97% similarity threshold using UCLUST[17]. Alpha- and beta-diversity measures were calculated using unweighted UniFrac[18] on rarified OTU tables. Principal coordinates were computed using QIIME 1.8 (ref. 19). Faecal 16S rRNA data were not included from other studies of traditional populations because (1) they did not use the V4 region of the 16S rRNA for amplification and were thus not comparable (Papua New Guinea[9]); (2) they have not made the data publicly available (Yanomami[7]); or (3) the control Western data was not comparable to other studies (Matses[8]).

Mice. All mouse experiments were performed in accordance with A-PLAC, the Stanford IACUC on mixed gender germ-free Swiss Webster mice that were humanized by oral gavage of human faecal sample obtained from a healthy anonymous donor (American male living in the San Francisco Bay Area, California, age 36, omnivorous diet) as previously described[20]. Humanized mice closely reconstituted the diversity and phylogenetic make-up of the donor (Pearson's $r = 0.96$; Extended Data Fig. 2). The investigators were not blinded to allocation during experiments and outcome assessment. Mice were randomly assigned to two groups and were fed either a high-MAC diet (LabDiet 5010) or a low-MAC diet (Harlan TD.86489). The high-MAC diet is a plant polysaccharide-rich diet in which the MACs come from a diverse source of plants including corn, soybean, wheat, oats, alfalfa and beet. The reported neutral detergent fibre content of the high-MAC diet is 15% by weight. The low-MAC diet is defined as a diet in which carbohydrates are from sucrose (31% by weight), corn starch (31% by weight) and cellulose (5% by weight). The accessibility of cellulose to gut microbiota is known to be extremely low and we have been unable to isolate bacteria from the microbiota that use this substrate[20]. Mice from the faecal transplant experiment were from the fourth generation of mice from the diet-switching group. Faecal transplants were carried out by gavage using freshly collected faecal samples from fourth-generation control mice consuming a high-MAC diet using a procedure identical to the original humanization[21]. Faecal samples were collected throughout all mouse experiments and stored at −80 °C.

16S rRNA amplicon sequencing and analysis. 16S rRNA amplicons were generated for the v4 region from faecal pellets collected and the 16,878,145 Illumina generated sequencing reads were analysed using Qiime 1.8 as described previously[19]. Sequencing data underwent quality filtering as described previously and data were rarefied to the lowest number of reads observed in a single sample (28,596 reads)[22]. OTUs were identified by open-reference picking using the UCLUST algorithm and taxonomy was assigned using the Greengenes 13.8 database. Plots of UniFrac distances are from unweighted analyses and UniFrac distance values are reported as within group versus between groups. Microbiota diversity was measured by Shannon index, which takes into account both overall richness and evenness. High-confidence OTUs were identified using the following criteria: present in at least three mice at the start of the experiment (4 weeks after humanization) and had a collective abundance of greater than 25 reads. High-confidence sub-OTUs were selected by first filtering out duplicate sequences whose distribution across samples is highly correlated (thresholded at 0.95 dynamical similarity). These duplicates correspond to the same bacterial populations, either as uncommonly frequent errors or as multiple 16S copies within the same bacterium. Second, for each experimental group all sequences whose initial raw abundance was at least 5 reads in at least 3 out of 5 mice in that isolator were selected as high-confidence sub-OTUs. Sub-OTU level analysis was performed as described previously[15].

Glycoside hydrolase profiles. This method was validated using samples for which 16S rRNA profiles and metagenomic data were available (imputed glycoside hydrolase profiles explained 84% of the annotated metagenomic data using a simple linear fit without any model corrections; $P = 10^{-28}$)[5]. Glycoside hydrolase imputations were performed by annotating 2,746 reference genomes with glycoside hydrolase families using validated hidden Markov models many of which were derived from conserved domain database models that are capable of identifying several domains within a putative enzyme with increased sensitivity[23,24]. The glycoside hydrolases from taxonomically assigned communities were then calculated by applying a weighted average of the lowest taxonomic level with representative genomes. To account for the fact that many glycoside hydrolase families have wide ranging functions while each glycoside hydrolase subfamily may possess distinct function, we further clustered the annotated glycoside hydrolases into subfamilies as previously described[23,25,26]. Fold-changes of glycoside hydrolase family copy numbers were determined by comparing generation-four mice to the mean generation-one glycoside hydrolase profiles with significance testing performed across treatment and control groups.

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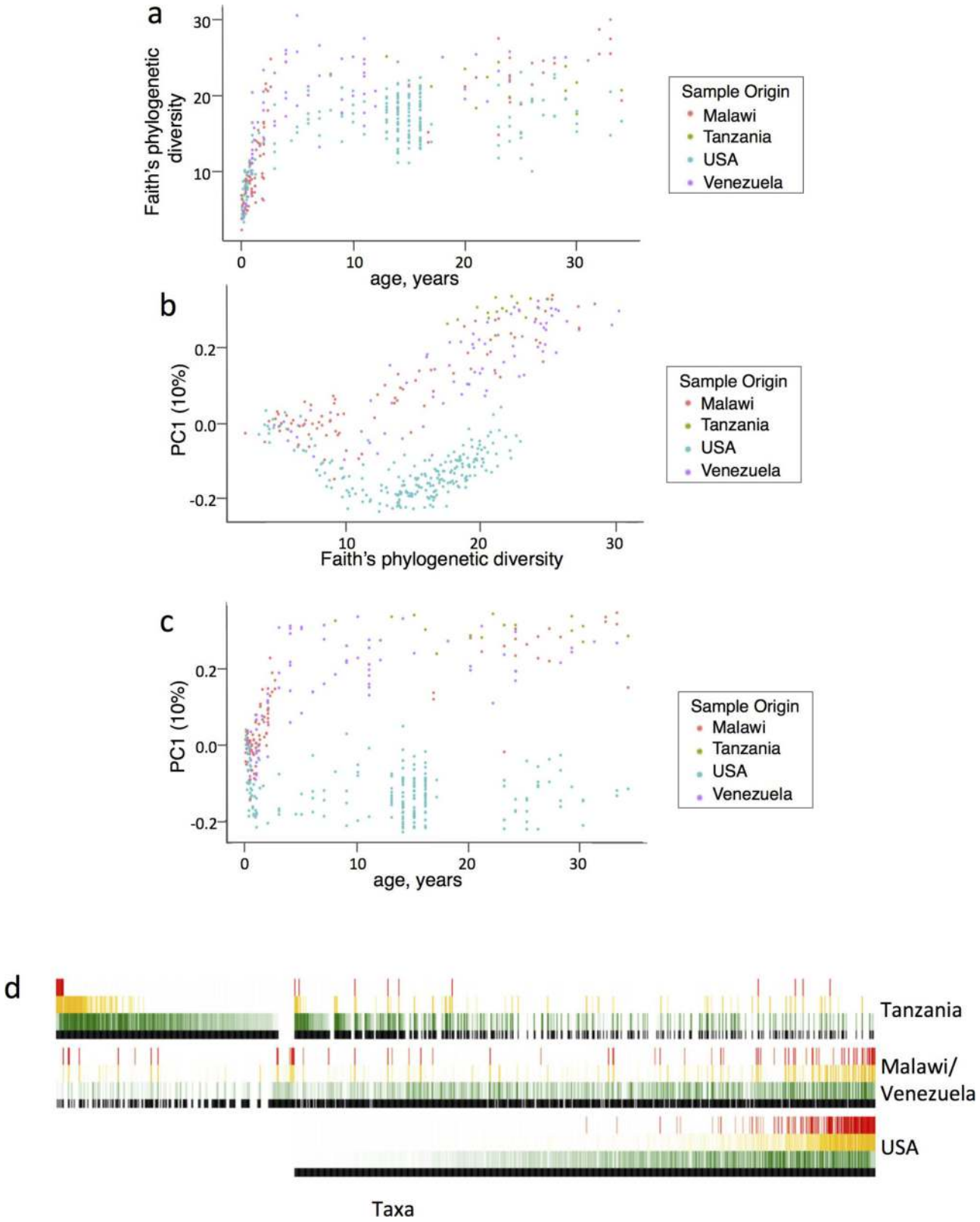
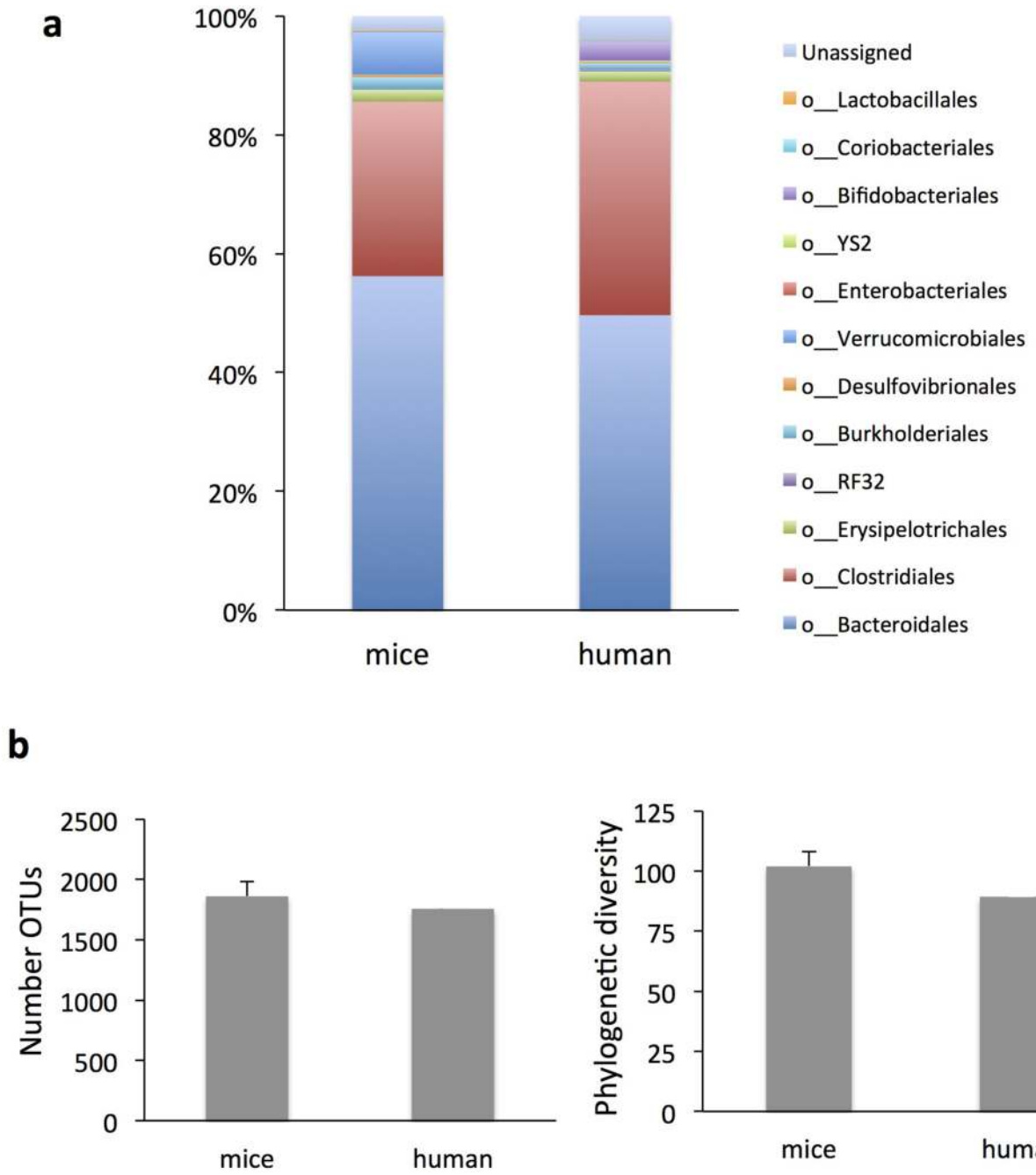
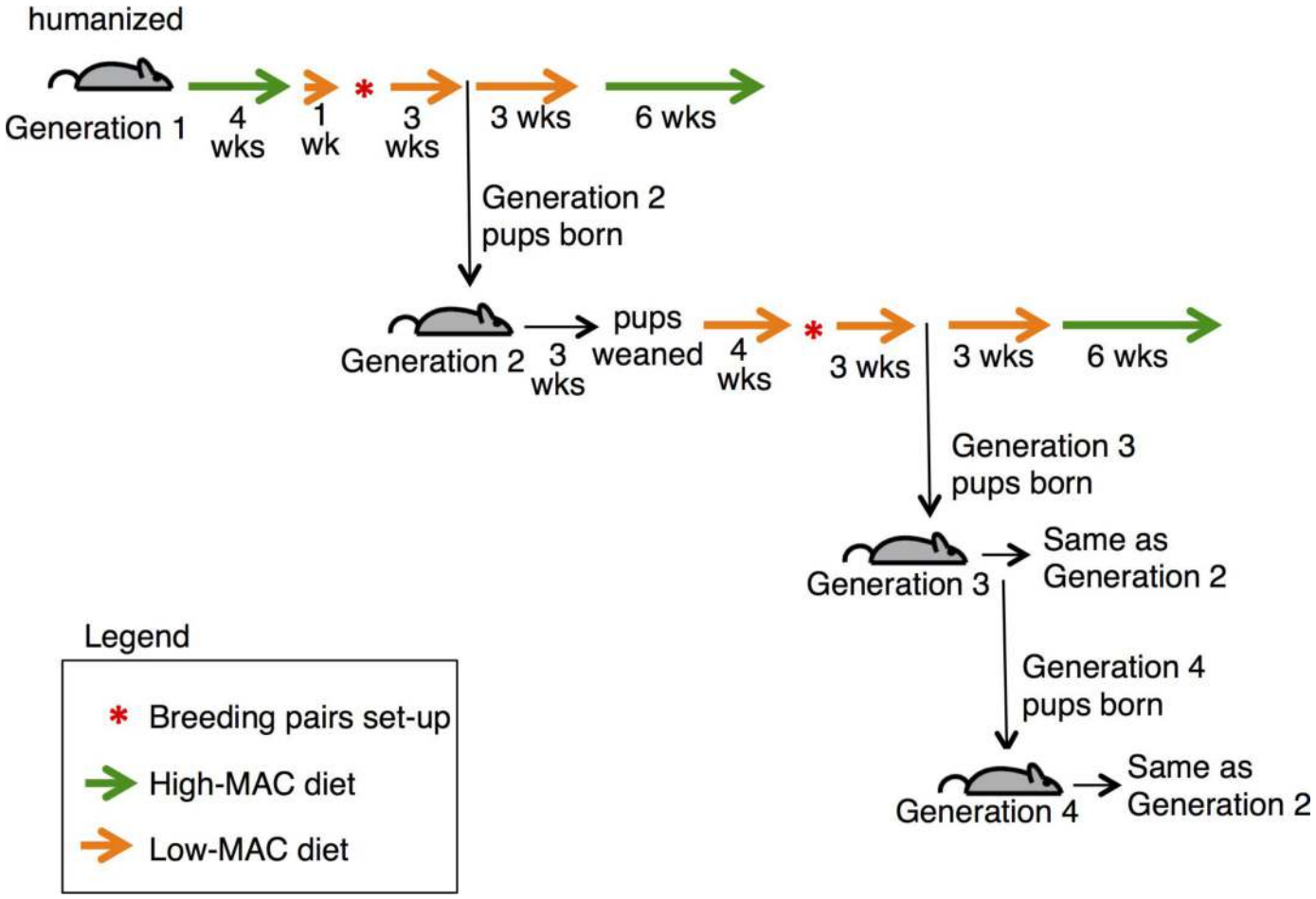


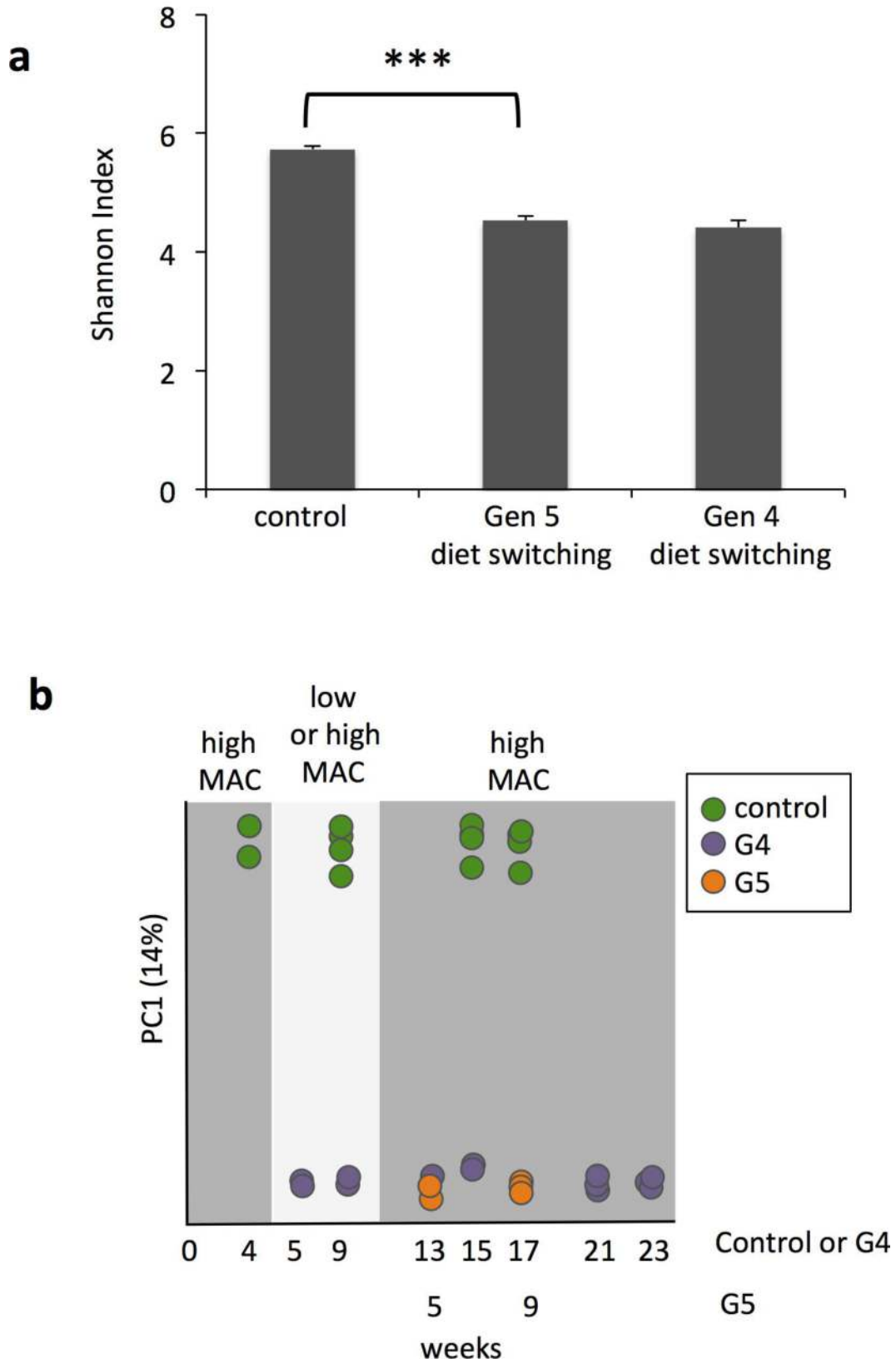
Extended Data Figure 1 | Collating data from studies of the microbiota of hunter–gatherers in Tanzania, agrarians from Malawi and Venezuela, and Westerners from the United States reveals that Western populations have depleted alpha-diversity from birth through childbearing years and are missing bacterial taxa present in the traditional groups. **a**, Scatterplot of faecal microbiota of individuals plotted by phylogenetic diversity against age of the Hadza hunter–gatherers from Tanzania ($n = 16$, green), agrarians from Malawi ($n = 81$, red) and Venezuela ($n = 78$, purple) and Americans ($n = 213$, blue) **b**, Individuals plotted by unweighted UniFrac PC1 versus phylogenetic diversity. **c**, Individuals plotted by unweighted UniFrac PC1 versus age. **d**, Line plot of unique OTUs from faecal microbiota across populations (Americans, $n = 315$; Malawi and Venezuela, $n = 213$; Tanzania, $n = 27$). OTUs (x axis; black, present; white, absent) are considered present if represented by $\geq 0.001\%$ of reads within each population. OTUs were sorted along the x axis by their relative abundance in the US and Tanzanian populations and further subdivided by their distributions within a population into tracks (red $>0.05\%$, yellow $\leq 0.05\%$, and green $\leq 0.01\%$, relative abundance). The opacity of the line is the proportion of that population that meets the criteria for that respective track.



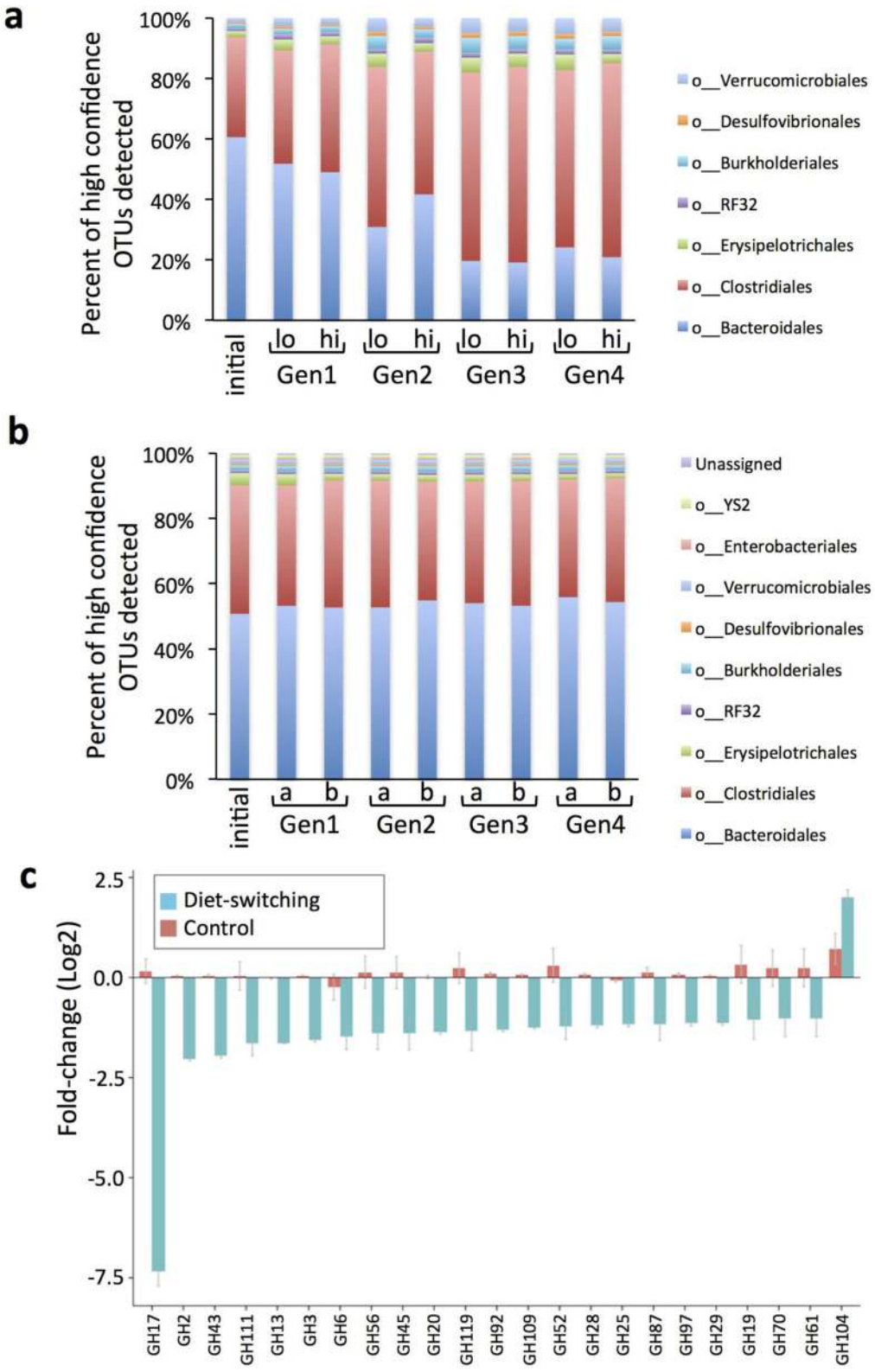
Extended Data Figure 2 | Comparison of human donor and humanized mice. a, Taxa summary plot of the relative abundance of taxa from humanized mice faeces (mice) ($n = 10$) and human donor faeces (human) ($n = 1$). **b**, Alpha-diversity of the faecal microbiota from humanized mice (mice) and human donor (human) expressed as number of OTUs (top) and phylogenetic diversity (bottom). Error bars are s.e.m.



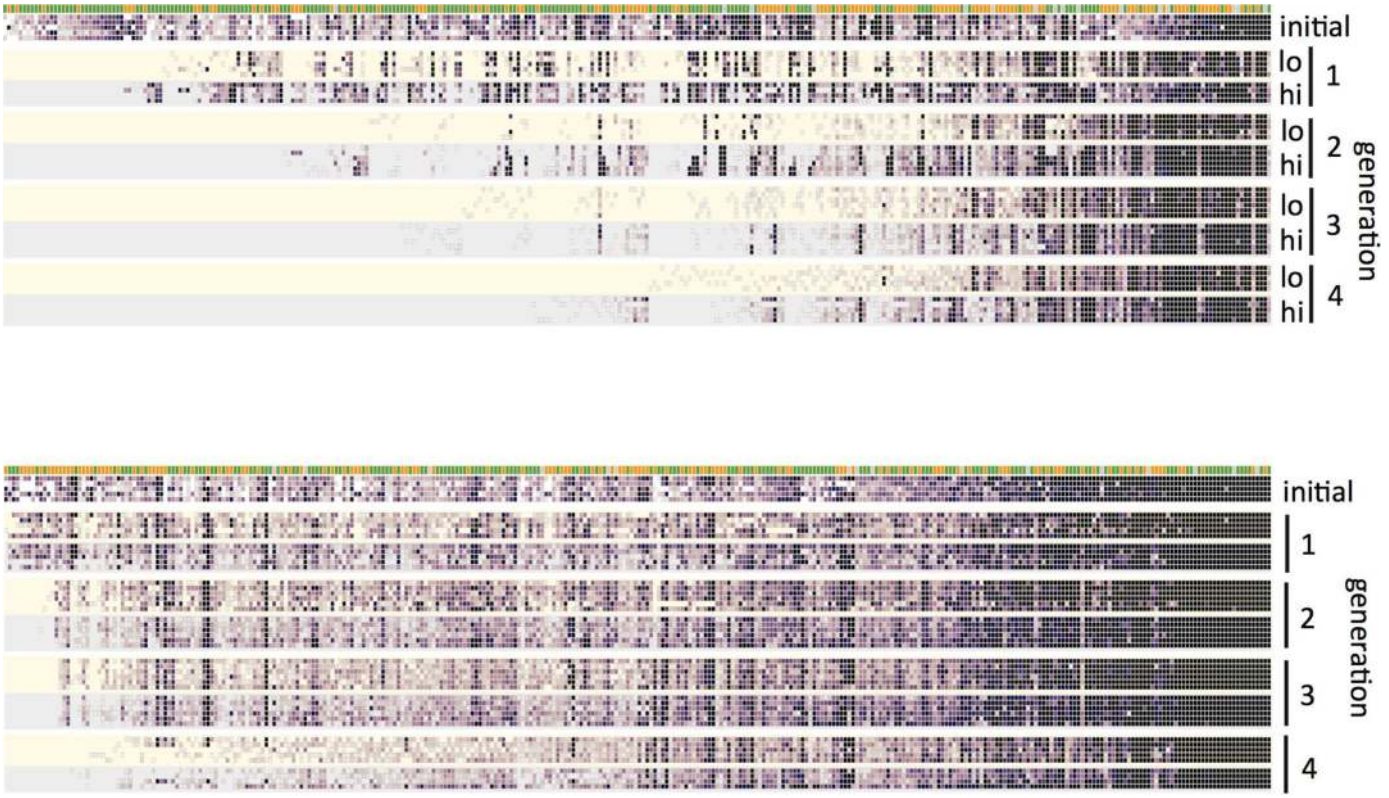
Extended Data Figure 3 | Detailed schematic of multigeneration experiment. Generation one: humanized mice were fed a high-MAC diet for 4 weeks then switched to a low-MAC diet. One week after diet switch, the mice were bred to generate a litter of pups. After three additional weeks on the low-MAC diet, generation-two pups were born and remained in the cage with their mother, suckling for 3 weeks (generation one still consuming the low-MAC diet). After pups were weaned, generation-one mice were returned to the high-MAC diet for 6 weeks. Generation two: pups were weaned from their mother at 3 weeks old onto a low-MAC diet, which they consumed for 10 weeks. Breeding pairs for generation-two mice were set-up at 7 weeks old. After three additional weeks on the low-MAC diet, generation-three pups were born and remained in the cage with their mother, suckling for 3 weeks (generation-two mice still consuming the low-MAC diet). After pups were weaned, generation-two mice were returned to the high-MAC diet for 6 weeks. Generations three and four followed the same protocol as generation two described above.



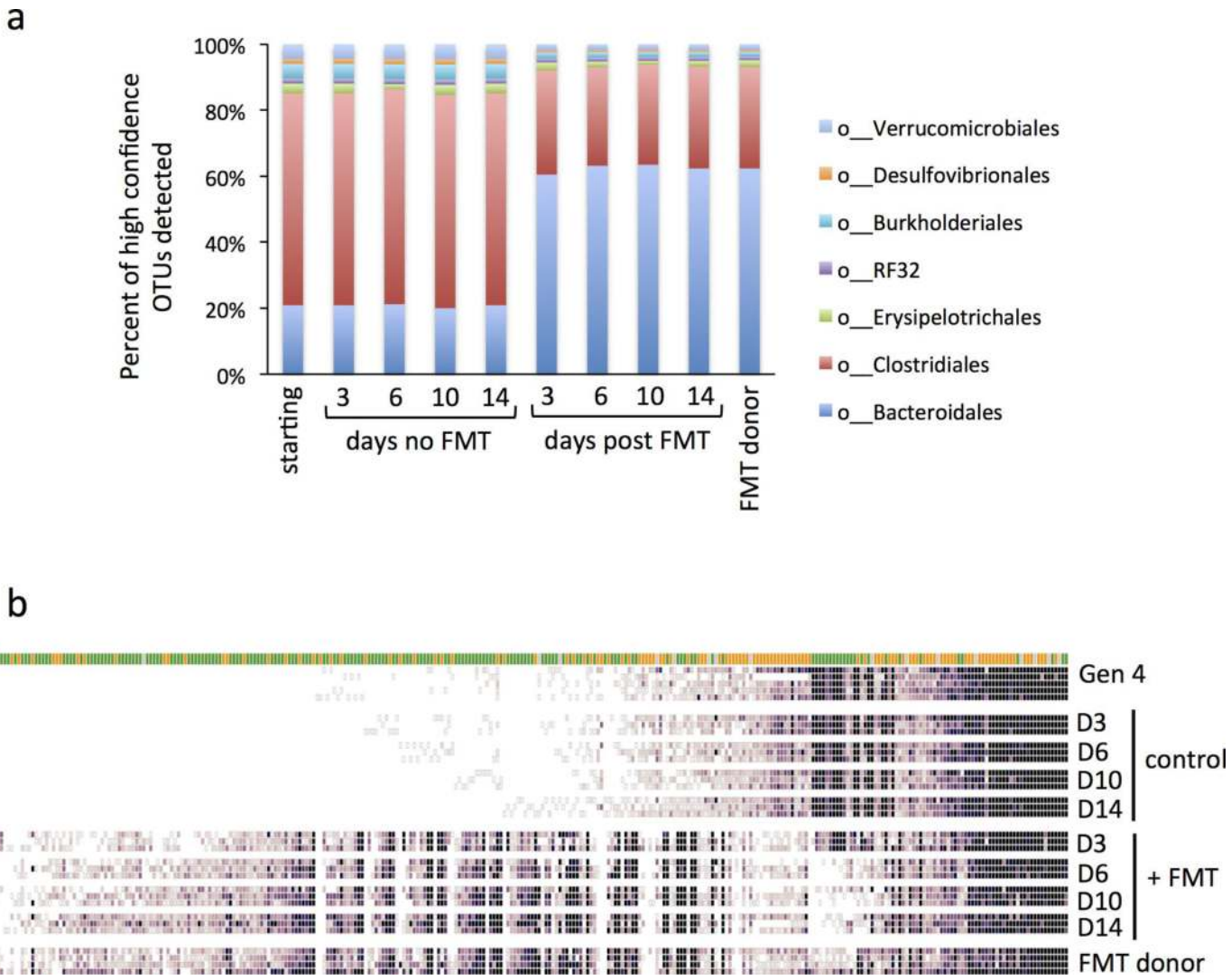
Extended Data Figure 4 | Microbiota diversity is not regained after direct weaning the diet-switching group onto the high-MAC diet. **a**, Alpha-diversity as measured by Shannon index of faecal microbiota from generation-five mice from the high-MAC-diet control (control) ($n = 6$), generation-five diet-switching group that was weaned directly onto the high-MAC diet (Gen 5 diet switching) ($n = 6$), and generation-four mice from the diet-switching group after weaning and maintenance on the low-MAC diet for 13 weeks and returned to the high-MAC diet for 4 weeks (Gen 4 diet switching) ($n = 5$). Error bars are s.e.m. and P values are from a two-tailed Student's t-test **b**, Principal coordinate analysis of unweighted UniFrac distance for 16S rRNA amplicon profiles from faecal samples collected from first-generation control mice on a high-MAC diet (green), fourth-generation diet-switching mice (purple), and fifth-generation mice from the diet-switching lineage weaned directly onto the high-MAC diet (orange). Control is plotted as weeks post-humanization and generations four and five are plotted as age.



Extended Data Figure 5 | Fraction of high-confidence OTUs from the Clostridiales order increases and from the Bacteroidales order decreases over several generations in the low-MAC-consuming mice. **a**, Percentage of high-confidence OTUs, grouped by order, detected in mice faeces over four generations in the diet-switching lineage on the low-MAC diet (lo) and high-MAC diet (hi) ($n = 5$ for Gen 1; $n = 6$ for Gen 2–4). **b**, Percentage of high-confidence OTUs, grouped by order, detected in mice faeces over four generations in the control high-MAC diet lineage at the equivalent time points to the high-MAC diet (**a**) and low-MAC diet (**b**) of the diet-switching group ($n = 5$ for Gen 1; $n = 6$ for Gen 2–4). **c**, Imputed gycloside hydrolase (GH) family members that show significant differences (at least twofold change and $P < 0.05$, Bonferroni-corrected *t*-test) between generation-four diet-switching mice after 4 weeks on the high-MAC diet (teal) ($n = 5$) and the starting generation-one mice (salmon) ($n = 10$). Error bars depict s.e.m. No glycoside hydrolase families showed significant changes in the control group.



Extended Data Figure 6 | Inefficient inter-generational transfer of taxa driven to low abundance by low dietary MACs. Heat map of abundance of high-confidence sub-OTUs (number of sequencing reads, columns) from faeces of the diet-switching (top) and control (bottom) group. Each row represents an individual mouse faecal microbiota from 4 weeks post-humanization (initial), while consuming the low-MAC diet (week 9, lo, shaded yellow), and 4 weeks after switching to the high-MAC diet (week 15, hi, shaded grey). Corresponding time points from controls are also shaded. Top row shows the taxonomic assignment for the OTUs plotted: Bacteroidetes are green, Firmicutes are orange, and others are grey.



Extended Data Figure 7 | Reintroduction of lost taxa and a high-MAC diet restores microbiota diversity and composition with Clostridiales order decreasing and Bacteroidales order increasing in low-MAC-consuming mice that receive a faecal transplant. a, Plot of percentage representation of high-confidence OTUs from generation-four mice faeces in the diet-switching group at day 0 before the FMT (starting) ($n = 6$) and then 3–14 days no-FMT control ($n = 3$) or post-FMT ($n = 3$). FMT donor is plotted on the right. **b**, Heat map of abundance of high-confidence sub-OTUs (number of sequencing reads, columns) from the faeces of the diet-switching group at day 0 (Gen 4), days 3–14 that did not receive an FMT (control) ($n = 3$ for each day), days 3–14 that received an FMT (+FMT), and the FMT donor. Each row represents an individual mouse faecal microbiota. Top row shows the taxonomic assignment for the OTUs plotted: Bacteroidetes are green, Firmicutes are orange, and others are grey.

Extended Data Table 1 | Nutritional information of mouse diets

Diet Name	Supplier/Product Name	Protein (% by weight)	Carbohydrates (% by weight)	Fat (% by weight)	Fibre (% by weight)	Neutral Detergent Fibre (% by weight)
High-MAC	LabDiet 5010	25	50	5	5	15
Low-MAC	Harlan TD.86489*	18	62	5	5	5**

* Remaining 5% composed of vitamins and minerals

** Exclusively from added cellulose

Extended Data Table 2 | High-confidence OTUs at experiment start

Taxonomy	Control Isolator (# OTUs)	Experimental Isolator (# OTUs)
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__[Odoribacteraceae]: g__Butyricimonas: s__	0	1
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Bacteroidaceae: g__Bacteroides: s__	49	48
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Bacteroidaceae: g__Bacteroides: s__fragilis	2	2
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Bacteroidaceae: g__Bacteroides: s__ovatus	2	2
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Bacteroidaceae: g__Bacteroides: s__uniformis	5	6
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Porphyromonadaceae: g__Parabacteroides: s__	3	5
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Porphyromonadaceae: g__Parabacteroides: s__distasonis	2	1
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Porphyromonadaceae: g__Parabacteroides: s__gordonii	1	2
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__Rikenellaceae: g__: s__	1	1
p__Bacteroidetes: c__Bacteroidia: o__Bacteroidales: f__S24-7: g__: s__	43	58
p__Cyanobacteria: c__4C0d-2: o__YS2: f__: g__: s__	2	0
p__Firmicutes: c__Clostridia: o__Clostridiales: f__: g__: s__	3	3
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Lachnospiraceae: g__: s__	22	17
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Lachnospiraceae: g__[Ruminococcus]: s__	3	3
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Lachnospiraceae: g__[Ruminococcus]: s__gnavus	6	0
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Lachnospiraceae: g__Blautia: s__	20	17
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Lachnospiraceae: g__Blautia: s__producta	5	3
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Lachnospiraceae: g__Coprococcus: s__	6	4
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Lachnospiraceae: g__Dorea: s__	4	3
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Ruminococcaceae: g__: s__	6	9
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Ruminococcaceae: g__Faecalibacterium: s__prausnitzii	0	4
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Ruminococcaceae: g__Oscillospira: s__	4	3
p__Firmicutes: c__Clostridia: o__Clostridiales: f__Ruminococcaceae: g__Ruminococcus: s__	5	3
p__Firmicutes: c__Erysipelotrichi: o__Erysipelotrichales: f__Erysipelotrichaceae: g__: s__	4	1
p__Firmicutes: c__Erysipelotrichi: o__Erysipelotrichales: f__Erysipelotrichaceae: g__[Eubacterium]: s__dolichum	2	2
p__Firmicutes: c__Erysipelotrichi: o__Erysipelotrichales: f__Erysipelotrichaceae: g__Coprobacillus: s__	1	1
p__Firmicutes: c__Erysipelotrichi: o__Erysipelotrichales: f__Erysipelotrichaceae: g__Holdemania: s__	1	0
p__Proteobacteria: c__Alphaproteobacteria: o__RF32: f__: g__: s__	2	2
p__Proteobacteria: c__Betaproteobacteria: o__Burkholderiales: f__Alcaligenaceae: g__Sutterella: s__	3	3
p__Proteobacteria: c__Deltaproteobacteria: o__Desulfovibrionales: f__Desulfovibrionaceae: g__Bilophila: s__	1	1
p__Proteobacteria: c__Gammaproteobacteria: o__Enterobacteriales: f__Enterobacteriaceae: g__: s__	1	0
p__Verrucomicrobia: c__Verrucomicrobiae: o__Verrucomicrobiales: f__Verrucomicrobiaceae: g__Akkermansia: s__muciniphila	3	3
Unassigned	1	0

Extended Data Table 3 | Shannon index of glycoside hydrolase subfamilies

Comparisons	Gen1 Mouse1	Gen1 Mouse2	Gen1 Mouse3	Gen1 Mouse4	Gen1 Mouse5	Gen4 Mouse1	Gen4 Mouse2	Gen4 Mouse3	Gen4 Mouse4	Gen4 Mouse5	p value	significance sign
Diet-switching	9.39	9.42	9.34	9.35	9.40	9.23	9.21	9.12	9.26	9.17	2.2E-04	**
Control	9.41	9.39	9.45	9.48	9.45	9.42	9.34	9.42	9.41	9.41	0.13	