EXTENDED DATA FIGURE LEGENDS

Extended Data Fig. 1. Study design. The (**A**) plant-based and (**B**) animal-based diets were fed to subjects for five consecutive days. All dates are defined relative to the start of these diet arms (day 0). Study volunteers were observed for four days before each diet (the baseline period; days -4 to -1) and for six days after each diet arm (the washout period; days 5 to 10), in order to measure subjects' eating habits and assess their recovery from each diet arm. Subjects were instructed to eat normally during both the baseline and washout periods. Stool samples were collected daily on both diet arms and 16S rRNA and fungal ITS sequencing was performed on all available samples. Subjects also kept daily diet logs. Several analyses (RNA-Seq, short-chain fatty acids, and bile acids) were performed primarily using only two samples per person per diet (*i.e.* a baseline and diet arm comparison). Comparative sampling did not always occur using exactly the same study days, due to limited sample availability for some subjects. Because we expected the animal-based diet to promote ketogenesis, we only measured urinary ketones on the animal-based diet. To test the hypothesis that microbes from fermented foods on the animal-based diet survived transit through the gastrointestinal tract, we cultured bacteria and fungi before and after the animal-based diet.

Extended Data Fig. 2. A vegetarian's microbiota. One of the our study's subjects is a lifelong vegetarian (Subject 6). (**A**) Relative abundances of *Prevotella and Bacteroides* are shown across the plant-based diet for Subject 6 (orange circles), as well as for all other subjects (green circles). Consecutive daily samples from Subject 6 are linked by

dashed lines. For reference, median baseline abundances are depicted using larger circles. (**B**) Relative abundances are also shown for samples taken on the animal-based diet. Labeled points correspond to diet days where the Subject 6's gut microbiota exhibited an increase in *Bacteroides* relative abundance. (**C**) A principal coordinates-based characterization of overall community structure for Subject 6, as well as all other subjects. QIIME[30] was used to compute microbial β-diversity with the Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac statistics. Sample similarities were projected onto two dimensions using principal coordinates analysis. (**C: Upper row**) When colored by subject, samples from Subject 6 (green triangles) partition apart from the other subjects' samples. (**C: Lower row**) Of all of Subject 6's diet samples, the ones most similar to the other subjects' are the samples taken while consuming the animal-based diet.

Extended Data Fig. 3. Subject physiology across diet arms. (**A**) Gastrointestinal motility, as measured by the initial appearance of a non-absorbable dye added to the first and last lunch of each diet. The median time until dye appearance is indicated with red arrows. Subject motility was significantly lower ($p<0.05$, Mann-Whitney U test) on the animal-based diet (median transit time of 1.5 days) than on the plant-based one (1.0 days). (**B**) Range (shaded boxes) and median (solid line) of subjects' weights over time. Subjects' weight did not change significantly on the plant-based diet relative to baseline periods, but did decrease significantly on the animal-based diet (asterisks denote $q<0.05$, Bonferroni-corrected Mann-Whitney U test). Subjects lost a median of 1.6% and 2.5% of body weight by days 3 and 4, respectively, of the animal-based diet arm. (**C**)

Measurements of subjects' urinary ketone levels. Individual subjects are shown with black dots, and median values are connected with the black solid line. Urinary ketone readings were taken from day 0 of the animal-based diet onwards. Ketone levels were compared to the readings on day 0, and asterisks denote days with significant ketone increases ($q<0.05$, Bonferroni-corrected Mann-Whitney U test; significance tests were not carried out for days where less than 4 subjects reported their readings.). All subjects on the animal-based diet showed elevated levels of ketones in their urine by day 2 of the diet (≥15 mg/dL as compared to 0 mg/dL during initial readings), indicating they experienced ketonuria during the diet arm. This metabolic state is characterized by the restricted availability of glucose and the compensatory extraction of energy from fat tissue[31].

Extended Data Fig. 4. Baseline *Prevotella* abundance is associated with long-term fiber intake. *Prevotella* fractions were computed by summing the fractional 16S rRNA abundance of all OTUs whose genus name was *Prevotella*. Daily intake of dietary fiber over the previous year was estimated by Diet History Questionnaire[32] (variable name "TOTAL_DIETARY_FIBER_G_NDSR"). There is a significant positive correlation between subjects' baseline *Prevotella* abundance and their long-term dietary fiber intake (Spearman's $\rho=0.78$, $p=0.008$).

Extended Data Fig. 5. Significant correlations between SCFAs and cluster abundances across subjects. SCFAs are drawn in rectangles and colored maroon or green if they are produced from amino acid or carbohydrate fermentation, respectively.

Clusters whose members include known bile-tolerant or amino-acid fermenting bacteria[15,16] are colored maroon, whereas clusters including known saccharolytic bacteria[3] are colored green. Uncolored clusters and SCFAs are not associated with saccharolytic or putrefactive pathways. Significant positive and negative correlations are shown with black arrows and gray arrows, respectively ($q<0.05$; Spearman correlation).

Extended Data Fig. 6. Inter-individual microbial community variation according to diet and sequencing technique. To measure the degree to which diet influences inter-individual differences in gut microbial gene expression, we clustered RNA-seq profiles from (**A**) baseline and (**B**) diet periods. Dots indicate pairs of samples that cluster by subject. The potential for diet to partition samples was measured by splitting trees at the arrowed branches and testing the significance of the resulting 2×2 contingency table (diet vs. partition; Fisher's exact test). To avoid skewed significance values caused by non-independent samples, we only clustered a single sample per subject, per diet period. In the case of multiple baseline samples, the sample closest to the diet intervention was used. In the case of multiple diet samples, the last sample during the diet intervention was kept. A single sample was randomly chosen if there were multiple samples from the same person on the same day. No association between diet and partitioning was found for partitions I-VI ($p>0.05$). However, a significant association was observed for partition VII ($p=0.003$). (**C**) To determine if diet affects inter-individual differences in gut microbial community structure, we hierarchically clustered 16S rRNA data from the last day of each diet arm. Samples grouped weakly by diet: subtrees partitioned at the arrowed node showed a minor enrichment for plant-based diet

samples in one subtree and animal-based diet samples in the other (p=0.07; Fisher Exact Test). Still, samples from five subjects grouped by individual, not diet (indicated by black nodes), indicating that diet does not reproducibly overcome inter-individual differences in gut microbial community structure.

Extended Data Fig. 7. Food-associated microbes and their enteric abundance over time. (**A**) Major bacterial and fungal taxa found in plant-based diet menu items were determined using 16S rRNA and ITS sequencing, respectively, at the species (s), genus (g), and order level (o). The majority of 16S rRNA gene sequences are Streptophyta, representing chloroplasts from the ingested plant matter. (**B**) One of the fungi from (**A**), *Candida* sp., showed a significance increase in fecal abundance on the plant-based diet ($p<0.05$, Wilcoxon Signed-Rank test). (**C**) Levels of bacteria and fungi associated with the animal-based diet are plotted over the plant- and animal-based diet arms. Taxa are identified on the genus (g) and species (s) level. The abundance of foodborne bacteria was near our detection limit by 16S rRNA gene sequencing; to minimize resulting measurement errors, we have plotted the fraction of samples in which bacteria are present or absent. *Lactococcus lactis*, *Pediococcus acidilactici*, and *Staphylococcus*-associated reads all show significantly increased abundance on the animal-based diet ($p<0.05$, Wilcoxon Signed-Rank Test). Fungal concentrations were measured using ITS sequencing and are plotted in terms of log-fractional abundance. Significant increases in *Penicillium*-related fungi were observed, along with significant decreases in the concentration of *Debaryomyces* and a *Candida sp.* ($p<0.05$, Wilcoxon signed-rank test). One possible explanation for the surprising decrease in the

concentration of food-associated fungi is that the more than 10-fold increase in *Penicillium* levels lowered the relative abundance of all other fungi, even those that increased in terms of absolute abundance.

Extended Data Fig. 8. Eukaryotic and viral taxa detected via RNA-Seq. (A) Identified plant and other viruses. The most common virus is a DNA virus (lambda phage) and may be an artifact of the sequencing process. (**B**) Identified fungi, protists, and other eukaryotes. Taxa that were re-annotated using manually curated BLAST searches are indicated with asterisks and their original taxonomic assignments are shown in parentheses (see *Supplementary Methods* for more details).

Extended Data Fig. 9. Fecal bile acid concentrations on baseline, plant-, and animal-based diets. Median bile acid concentrations are shown for all individuals on the (**A**) plant-based and (**B**) animal-based diets (error bars denote median absolute deviations). Detailed experimental protocols are in the “Bulk bile acid quantification” section of the *Supplementary Methods*. Bile acid levels did not significant change on the plant-based diet, relative to baseline levels ($p>0.1$, Mann-Whitney U test). However, bile acid levels trended upwards on the animal-based diet, rising from 1.48 μmol / 100 mg dry stool during the baseline to 2.37 μmol / 100 mg dry stool ($p<0.10$, Mann-Whitney U test).

Extended Data Fig. 10. The dissimilatory sulfate reduction pathway. (**A**) Microbes reduce sulfate to hydrogen sulfide by first converting sulfate to adenosine 5'-

phosphosulfate (APS) via the enzyme ATP sulfurylase (sat). Next, APS is reduced to sulfite by the enzyme APS reductase (apr). Finally, the end product hydrogen sulfide is reached by reducing sulfite through the enzyme sulfite reductase (dsrA). This last step of the pathway can be performed by *Bilophila* and is thought to contribute to intestinal inflammation[6]. (**B**) No significant changes in apr gene abundance were observed on any diet ($p>0.05$, Mann-Whitney U test; n=10 samples/diet arm). Values are mean±sem. However, dsrA abundance increased on the animal-based diet (**Fig. 5d**).

EXTENDED DATA LEGEND REFERENCES

31 Walker, H. K., Hall, W. D., Hurst, J. W., Comstock, J. P. & Garber, A. J. *Ketonuria*. 3rd edn, (Butterworths, 1990).

32 Diet History Questionnaire, Version 2.0 (National Institutes of Health, Applied Research Program, National Cancer Institute, 2010).