Supplementary Information

Environmental spread of microbes drives the development of metabolic phenotypes in mice transplanted with microbial communities from humans

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Content:

Supplementary experimental procedures

Physiological measurements of human donors and faecal collection

Sixteen obese and 16 normal weight children and adolescents, aged between 6.8 and 17.8 years, were recruited as human donors of faeces. Their Body Mass Index (BMI) z-score was calculated by the lambda-mu-sigma (LMS) method, which converts BMI into a normal distribution by sex and age using the median coefficient of variation and a measure of the skewness (Cole and Green, 1992), based on the Box-Cox power plot on Danish BMI charts (Nysom *et al.*, 2001). The total body/fat/bone mass was measured by whole body dual-energy X-ray absorptiometry using a GE Lunar iDXA system (GE Healthcare, Madison, WI, USA) and the enCORE software v16. The total body fat percentage was calculated as: fat mass/(total body mass - total bone mass). Blood samples were drawn intravenously from an antecubital vein between 7 a.m. and 9 a.m. after fasting overnight. Samples for obtainment of plasma were collected in fluoride containing tubes, and samples for obtainment of serum were collected in Venosafe tubes containing serum separating gel (Terumo Europe, Leuven, Belgium). All samples were centrifuged at 2 500 g, 4 °C for 10 min to obtain plasma/serum. Plasma glucose, serum triglycerides, serum total cholesterol, and serum high density lipoprotein (HDL) cholesterol were measured on a Dimension Vista 1500 analyser (Siemens, Munich, Germany). Low density lipoprotein (LDL) cholesterol was calculated as: total cholesterol - (triglycerides x 0.45) + HDL cholesterol. Plasma insulin was measured on a Cobas 6000 analyser (Roche, Basel, Switzerland).

For each human donor, physiological measurements and faecal collection were performed within two days. Faecal samples (10-20 g from each donor) were transferred into 15 mL cryo-tubes and stored at 4 °C. One day after collection, samples for use in transplantation studies were transferred to an anaerobic chamber, homogenized 1:1 in pre-reduced water with 50% glycerol, divided into aliquots and stored at -80 °C in 2 mL cryo-tubes. Another part of the samples were homogenized 1:1 in water and stored at -80 °C until DNA extraction.

Animal experiments

The conditions in the facility were: temperature 21-23 °C, humidity 55% ± 5% with a strict 12 h light cycle. At the endpoint, mice were anaesthetized with a Hypnorm/Dormicum mixture (0.315 mg/mL Fentanyl, 10 mg/mL Fluanisone and 5 mg/mL Midazolam in a 1:1:2 water solution) injected subcutaneously (0.006 mL/g body weight).

16S rRNA gene sequencing

The 16S rRNA gene sequencing was performed essentially as previously described (Tulstrup *et al.*, 2015). DNA was extracted from faeces and luminal contents using the PowerLyzer PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). DNA concentrations were measured using the NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The V3 region of the 16S rRNA gene was amplified using an universal forward primer with a unique 10-12 bp barcode (IonXpress barcode, Ion Torrent) for each bacterial community (PBU 5'-A-adapter-TCAG-barcode-GAT-CCTACGGGAGGCAGCAG-3') and a universal reverse primer (PBR 5'-trP1-adapter-ATTACCGCGGCTGCTGG-3'). The PCR reactions were conducted with 5 ng template DNA, 10 mmol/L dNTP, 1 µmol/L forward/reverse primer, 4 µL HF-buffer and 0.2 µL Phusion High-Fidelity DNA polymerase (Thermo Scientific, Vilnius, Lithuania) in a total reaction volume of 20 uL. PCR conditions were 30 s at 98 °C, 24 cycles of 15 s at 98 °C, 30 s at 72 °C, followed by 5 min at 72 °C. For a few samples that did not yield sufficient PCR products, the PCR cycles were increased to 30. PCR products were separated on a 1.5% agarose gel at 3.5 V/cm for 90 minutes, and bands of the expected size (approximately 260 bp) were excised from the gel. DNA was purified from the excised gel using the MinElute Gel Extraction Kit (Qiagen, Hilden, Germany). DNA concentrations were determined using the Qubit 2.0 fluorometer (Invitrogen, CA, California, USA) with the dsDNA HS assay (Invitrogen, Eugene, OR, USA), and an equal amount of PCR product from each community was pooled to construct a library. Sequencing was performed using the Ion PGM Template OT2 200 Kit and the Ion PGM Hi-Q Sequencing Kit with the 318-chip (Ion Torrent).

Sequencing data analysis

Sequences were de-multiplexed, trimmed to eliminate primers, and filtered with a length range of 125-180 nt using the CLC Genomic Workbench v7.0.3 (Qiagen, Arhus, Denmark). Each sequence was classified to the lowest possible taxonomic rank (assignment confidence $\geqslant$ 50%) using the Ribosomal Database Project (RDP) Classifier v2.10.1 (Wang *et al.*, 2007), and collapsed at different taxonomic ranks, resulting in a phylotype data table. The phylotype data table had sequencing depths (range, median) as follows: 56 883-88 394, 68 467 for human faecal samples; 29 727-76 262, 47 197 for mouse intestinal samples; 35 203-120 852, 66 985 for mouse faecal samples.

Operational taxonomic units (OTUs) were generated using UPARSE v8.0.1623 (Edgar, 2013). All sequences were subjected to quality filtering with a cut-off of maxee 2.0 (discards reads with > 2.0 total expected errors for all bases in

the read). Unique sequences except singletons were clustered at 97% sequence homology. Chimeras were firstly filtered by the UPARSE-OTU algorithm and then by the UCHIME algorithm (Edgar *et al.*, 2011) using the RDP classifier training database v9 and the default threshold. Taxonomies of OTU representative sequences were also assigned by the RDP classifier, resulting in an OTU table. Since the RDP database does not include sufficient information about Christensenellaceae, we additionally aligned the OTU representative sequences to the Greengene database (gg_13_8 97% OTU representative sequences) with the RDP classifier, and used the taxonomy information only for the determination of Christensenellaceae. The OTU table had depths (range, median) as follows: 46 714-70 845, 56 445 for human faecal samples; 11 727-59 643, 22 385 for mouse intestinal samples; 519-62 028, 29 129 for mouse faecal samples.

Microbial α diversity (Shannon index and OTU richness) and β diversity were analysed based on the OTU table rarefied to 10 000 reads per sample using Qiime 1.8.0 (Caporaso, Kuczynski, *et al.*, 2010). To build a phylogenetic tree, OTU representative sequences were pooled with a 16S rRNA gene sequence assigned as *Methanosarcina* within the Archaea, and aligned to the Greengenes core set (Greengenes 13_5 PyNAST aligned 85% OTU representative sequences) (McDonald *et al.*, 2012) using PyNAST (Caporaso, Bittinger, *et al.*, 2010). A phylogenetic tree was created using FastTree (Price *et al.*, 2009). Using Dendroscope V3.3.2, the tree was re-rooted with the Archaea outgroup, and the outgroup was pruned from the tree, thereby generating a phylogenetic tree for downstream analyses. Principle coordinate analyses (PCoA) were performed on UniFrac distances (Lozupone and Knight, 2005) between microbial communities. An ADONIS test was performed to assess the differential clustering of microbial communities using the *vegan* R package v2.3-0 (Oksanen *et al.*, 2015).

Principle component analysis (PCA) was performed on the relative abundances of genera in human faecal samples and mice faecal samples (dpc 1, 2, 4, 7, 28 and 49) using the LatentiX 2.12 software (Latent5, http://www.latentix.com). The phylotype table was rarefied at 35 203 reads per sample. Genera that were present in more than 50% of the samples with average relative abundances higher than 0.01% in either human faecal samples or in mice faecal samples from at least one time point were included in the PCA. Data were log-transformed and auto-scaled, and a detection limit value of 0.0005% (0.5 out of 100 000 reads) was assigned to zero measurements.

To build co-occurrence networks of bacterial genera in given gut compartments, the phylotype table was rarefied to 29 727 reads per sample, and genera that were present in more than 50% of the samples with average relative abundances higher than 0.01% were kept for the correlation analysis using SparCC (Friedman and Alm, 2012). Each correlation was computed as the median of 50 iterations, and 1 000 bootstraps were applied to assign p values. Correlation coefficients with two-tailed p values smaller than 0.002 were imported into Cytoscape v3.2.1 (Shannon *et al.*, 2003) to build the co-occurrence network, where each node represents a genus and the edges between the nods represent the correlation coefficients between genera.

Overlap between OTUs/genera present in human microbiotas and the corresponding mouse colonic microbiotas was calculated from the OTU table rarefied at 11 172 reads per sample and the phylotype table rarefied at 29 727 reads per sample. For each pair (human donor and corresponding transplanted mouse), OTUs/genera with relative abundances higher than 0.01% were kept.

To discover features, i.e. OTUs and bacterial groups at genus/family/phylum levels that were differentially abundant between the obese and lean groups or between the four isolator groups, features that were present in less than 50% of samples in all groups and features with average relative abundances lower than 0.01% in all groups were filtered out from each data set. For comparison between the obese and lean groups, the matrices of relative abundances were permutated 10 000 times, with p values representing the fraction of times that permutated differences assessed by Welch's t test were greater than or equal to real differences. For comparison among groups, Kruskal-Wallis test followed by Dunn's multiple comparisons test was performed. P values from multiple testing were adjusted (q value) using the Benjamini-Hochberg false discovery rate (FDR) with a significance level of 0.05 (Benjamini and Hochberg, 1995).

To identify genera that distinguished the microbiota of donor 13 from that of the other 15 control donors, genera that met the following two criteria were used: Firstly, the genus should be present in donor 13 or in more than 50% of the other 15 control donors, and secondly, the relative abundance of the genus in donor 13 or the average relative abundance of the genus in the other 15 control donors should be higher than 0.01%. A Gaussian distribution was built from the log-transformed relative abundances of each genus in the 15 control donors using a maximum-likelihood estimation, and p values represented the probability that genera of donor 13 were contained in the distributions. P

values were then adjusted by Benjamini-Hochberg FDR correction. The same strategy was applied to identify differences between the microbiota in different gut compartments (ileum, caecum and colon) of the mouse transplanted with donor 13's microbiota and the rest of mice in the Control 2 group.

Assessment of faecal gross energy

Faeces collected at 42 and 49 dpc were pooled at equal amounts to obtain a sufficient sample weight. Faeces were dried at 50 °C for 48 h, and stored in air tight containers at 4 °C for maximally one week prior to calorimetric assessments. Amounts of 0.1-0.4 g dried faeces were mixed with 0.7-0.9 g benzoic acid tablets (IKA, Staufen, Germany), and the gross energy was determined using the isoperibol mode of a bomb calorimeter (C6000, IKA). Measurements followed the international standard ISO 9831:1998 (E) without correction for heat from formation of acids.

Short chain fatty acid (SCFA) analysis

SCFAs were analysed as previously described (Nejrup *et al.*, 2015) with minor modifications. All standards were purchased from Sigma-Aldrich (St. Louis, MO, US).

Frozen caecum contents were thawed on ice. Around 200 mg of each sample was mixed with 1.5 mL Milli-Q (Merck Millipore, Bedford, MA, USA) water and 0.1 mL internal standard (100 mmol/L 2-ethylbutyric acid in 12% formic acid), and homogenized with a bead-beater for 2 min. Acidity was adjusted to pH = 2-3 using 3 mol/L HCl. The samples were then centrifuged at 10 000 g for 10 min, and supernatants were filtered through 0.45 µm Phenex-NY syringe filters (Phenomenex, Værløse, Denmark). External calibration was performed using standard solution mixtures of acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, iso-valeric acid, caproic acid and 2-ethylbutyric acid in the concentrations ranging from 10 µmol/L to 8 mmol/L.

Aliquots (3 µL) of each sample were injected into a HP 6890 GC system (Agilent Technologies, Santa Clara, CA, USA) with a CP-FFA WCOT fused silica capillary column (25 m x 0.53 mm i.d. coated with 1 µm film thickness, Chrompack, EA Middelburg, The Netherlands). The carrier gas was helium at a flow rate of 20 mL/min. The initial oven temperature of 60 °C was maintained for 0.25 min, raised to 180 °C at 8 °C/min and held for 3 min, then increased to 215 °C at 20 °C/min, and finally kept at 215 °C for 5 min. The temperature of the front inlet detector and the injector was 250 °C. The flow rates of hydrogen, air and helium as makeup gas were 40, 450, and 45 mL/min, respectively. The

run time for each analysis was 22 min. Data handling was performed with the OpenLAB Chromatography Data System ChemStation Edition software (Rev.A.10.02). The concentration of SCFA was calculated against the individual external standards, and adjusted according to the loss of internal standard.

Serum bile acid profiling

LC-MS grade acetonitrile, methanol, acetic acid were obtained from Sigma-Aldrich. All aqueous solutions were prepared using ultrapure water obtained from a Millipore Milli-Q Gradient A10 system (Millipore). Oasis HLB 1cc Cartridges (30 mg/mL) were purchased from Waters (Milford, MA, USA). Authentic compounds of 9 unconjugated, 9 taurine conjugated, and 2 glycine conjugated bile acids were ordered from either Sigma-Aldrich or Steraloids (Newport, RI, USA). Of these, four bile acids, which were absent in mouse serum, were used as internal standards, namely dehydrocholic acid (DHCA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), and taurolithocholic acid (TLCA). The other 16 bile acids, which were to be quantified, included tauro-ω-muricholic acid (TωMCA), tauro-α-muricholic acid (TαMCA), tauro-β-muricholic acid (TβMCA), tauroursodeoxycholic acid (TUDCA), taurohyodeoxycholic acid (THDCA), taurocholic acid (TCA), ω-muricholic acid (ωMCA), α-muricholic acid (αMCA), taurochenodeoxycholic acid (TCDCA), β-muricholic acid (βMCA), taurodeoxycholic acid (TDCA), cholic acid (CA), ursodeoxycholic acid (UDCA), hyodeoxycholic acid (HDCA), chenodeoxycholic acid (CDCA), and deoxycholic acid (DCA). Details on the authentic compounds are provided in Table S1.

Stock solutions (1 mg/mL) of 20 bile acids were individually prepared from their authentic compounds. Conjugated bile acids were prepared in 50% methanol and unconjugated bile acids were prepared in methanol. An internal standard solution containing four bile acids, namely dehydrocholic acid, glycocholic acid, glycochenodeoxycholic acid, and taurolitho-cholic acid (1 µg/mL each), was prepared in 50% methanol. The other 16 bile acids were mixed (BA mix) and further diluted with 50% methanol to give final concentrations of 0.01 µg/mL, 0.1 µg/mL, 1 µg/mL, and 5 µg/mL. For preparation of the calibration curves, 100 µL of the internal standard solution was placed into Eppendorf tubes and dried with nitrogen. Subsequently, 100 µL of the BA mix (for each BA mix concentration) was added to a tube containing internal standard.

Aliqouts of the internal standard solution (50 µL) were placed into individual Eppendorf tubes and dried with nitrogen. To each tube, a 40 µL of serum and 100 µL of acetonitrile were added. The tubes were vortexed and left in the -20 °C

freezer for 1 hour to precipitate the proteins. Subsequently, the tubes were centrifuged at 16 000 g, 4 °C for 10 min in a micro-centrifuge and each supernatant was loaded to a HLB Cartridge (each Cartridge was preconditioned with 1 mL of 70% acetonitrile prior to use) to deplete phospholipids. After sample loading, the flow-through fraction was collected in a new Eppendorf tube. Subsequently, each cartridge was washed with 300 µL of 70% acetonitrile and the flow-fractions were pooled, centrifuged at 16 000g, 4 °C for 1 min and dried with nitrogen. Dried samples were stored at -20 °C until use.

For each sample, the residues were reconstituted in 50 µL of 50% methanol. 5 µL of each sample was pooled resulting in a quality control (QC) sample. Serum samples were analysed in random order with a QC sample injected for every ten samples throughout the analysis. Also, the four BA mix solutions were analysed once for every 20 serum samples to obtain a standard curve for every 20 samples. For each sample, a volume of 2 µL was injected into a UPLC-QTOF-MS system consisting of a Dionex Ultimate 3000 RS liquid chromatograph (Thermo Scientific, Sunnyvale, CA, USA) coupled to a Bruker maXis time of flight mass spectrometer equipped with an electrospray interphase (Bruker Daltonics, Bremen, Germany) operating in negative mode. The analytes were separated on a Poroshell 120 SB-C18 column with a dimension of 2.1 x 100 mm and 2.7 µm particle size (Agilent Technologies). The column was held at 45 °C and the sampler at 10 °C. The UPLC mobile phases consisted of 0.01% acetic acid in water (solution A) and acetonitrile (solution B). While maintaining a constant flow rate of 0.4 ml/min, the analytes were eluted using the following gradient: Solvent programming started with a linear gradient from 10 to 60% B in 18 min, then a linear gradient up to 100% B at 20 min. The final gradient composition, 100% B, was held constant until 22 min, followed by a return of the solvent composition to initial conditions (10% B) at 22.1 min and reequilibrated until 25 min. Mass spectrometry data were collected in full scan mode at 2 Hz with a scan range of 50-1000 mass/charge (*m/z*). The following electrospray interphase settings were used: Nebulizer pressure 2 bar, drying gas 10 L/min, 200 °C, capillary voltage 4500 V. To improve the measurement accuracy, external and internal calibrations were done using sodium formate clusters (Sigma-Aldrich) and in addition a lock-mass calibration was applied (hexakis(1H,1H,2H-perfluoroethoxy)phosphazene, Apollo Scientific, Manchester, UK).

Bile acids were detected by extraction of ion chromatograms using the exact mass of the bile acids ± 0.002 Da and quantified by use of standard curves and internal standards with similar retention times as listed in Table S1. Data were processed using QuantAnalysis v2.2 (Bruker Daltonics) and three calibration curves were obtained for each bile

acid, which were used to calculate the concentrations of the serum bile acids (Table S2). Except for TαMCA, TUDCA and HDCA, the bile acids were successfully quantified with a coefficient of variance below 30% as assessed by the quality control (QC) sample.

Statistical Analyses

Unless specified, student's t test (if normally distributed) or Mann-Whitney test (if not normally distributed) were performed to compare the obese and control groups, and for comparisons among groups, one-way ANOVA followed by Tukey's multiple comparisons test (if normally distributed) or Kruskal-Wallis test followed by Dunn's multiple comparisons test (if not normally distributed) were applied using GraphPad Prism 6.02. Maximally one outlier from each isolator group detected by Grubbs' test (http://www.graphpad.com/quickcalcs/Grubbs1.cfm, alpha = 0.05) was excluded before these tests if the cause of the outlier could not be explained. Spearman's rank correlation was performed to find associations, and p values from multiple correlations between mice phenotypes, SCFAs/bile acids and microbial features were adjusted by FDR. P or q values smaller than 0.05 were considered significant unless otherwise specified.

Supplementary results

Assessment of Christensenellaceae abundance

Since the family Christensenellaceae has been shown to be associated with a low BMI in humans and reduced weight gain in germ-free mice inoculated with human faecal samples (Goodrich *et al.*, 2014), we checked the relative abundance of this particular family based on the Greengene database, while the rest of the analyses were based on the RDP database, which doesn't include sufficient data about Christensenellaceae. No difference was found between obese and control donors (Figure S6a), although donors in Obese 2 showed a tendency ($p = 0.07$, Dunn's multiple comparisons test, Figure S6b) towards higher levels of Christensenellaceae as compared with Obese 1 (Figure S6b). This family was only detected in the gut of 50% of the mice, and its abundance was highest in the caecum of Control 1 (Figure S6f), which had the lowest weight gain (Figure 2d, 2e). However, the abundance of Christensenellaceae did not correlate with either human BMI z-score orfat percentage or mice weight gain (Figure S6c, S6d, S6h).

Supplementary References

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Supplementary Tables

Table S1. List of authentic compounds

No.	Commom name	Abbreviation	Source	Catalogue No.	Molecular Formula	[M-H]-	Rt / min	Internal standard[a]
1	Tauro-ω-muricholic acid	TωMCA	Steraloids	C1889-000	C26H45NO7S	514.2833	7.3	DHCA
2	Tauro-α-muricholic acid	TαMCA	Steraloids	C1893-000	C26H45NO7S	514.2833	7.4	DHCA
3	Tauro-β-muricholic acid	TβMCA	Steraloids	C1899-000	C26H45NO7S	514.2833	7.5	DHCA
4	Tauroursodeoxycholic acid	TUDCA	Steraloids	C1052-000	C26H45NO6S	498.2884	8.8	DHCA
5	Taurohyodeoxycholic acid	THDCA	Steraloids	C0890-000	C26H45NO6S	498.2884	8.9	DHCA
6	Taurocholic acid	TCA	Sigma	T4009	C26H45NO7S	514.2833	9.0	DHCA
7	**Dehydrocholic acid (internal std)**	**DHCA**	**Sigma**	**30830**	**C24H34O5**	**401.2323**	**10.0**	**-**
8	**Glycocholic acid (internal std)**	**GCA**	**Sigma**	**G2878**	**C26H43NO6**	**464.3007**	**10.3**	**-**
9	ω-muricholic acid	ωMCA	Steraloids	C1888-000	C24H40O5	407.2792	10.4	GCA
10	α-muricholic acid	αMCA	Steraloids	C1890-000	C24H40O5	407.2792	10.6	GCA
11	Taurochenodeoxycholic acid	TCDCA	Sigma	T6260	C26H45NO6S	498.2884	10.6	GCA
12	β-muricholic acid	βMCA	Steraloids	C1895-000	C24H40O5	407.2792	11.0	GCA
13	Taurodeoxycholic acid	TDCA	Sigma	T0875	C26H45NO6S	498.2884	11.0	GCA
14	Cholic acid	CA	Sigma	C1129	C24H40O5	407.2792	12.4	GCDCA
15	**Glycochenodeoxycholic acid (internal std)**	**GCDCA**	**Sigma**	**G0759**	**C26H43NO5**	**448.3058**	**12.6**	**-**
16	Ursodeoxycholic acid	UDCA	Sigma	U5127	C24H40O4	391.2843	12.7	GCDCA
17	Hyodeoxycholic acid	HDCA	Sigma	H3878	C24H40O4	391.2843	13.0	GCDCA
18	**Taurolithocholic acid (internal std)**	**TLCA**	**Sigma**	**T7515**	**C26H45NO5S**	**482.2935**	**13.1**	**-**
19	Chenodeoxycholic acid	CDCA	Sigma	C9377	C24H40O4	391.2843	15.2	TLCA
20	Deoxycholic acid	DCA	Sigma	D2510	C24H40O4	391.2843	15.5	TLCA

Bile acids marked in black were used as internal standards.
[a]The listed internal standard was used to quantify the given bile acid.

Table S2. Calibration curves

No.	Bile acid	Abbreviation	Calibration curves[a]	R^2	QC CV%[b]
1	Tauro-ω-muricholic acid	TωMCA	y = 0.299994 x + 0.001230	0.999996	21%
			y = 0.287431 x - 0.002111	1	
			y = 0.241061 x + 0.000000	1	
2	Tauro-α-muricholic acid	TαMCA	y = 1.041492 x + 0.001411	0.999999	-
			y = 1.050563 x - 0.006930	0.999902	
			y = 0.904483 x + 0.005305	0.999999	
3	Tauro-β-muricholic acid	TβMCA	$y = 0.024995 x^2 + 0.832533 x + 0.001838$	0.999999	6%
			$y = 0.012118 x^2 + 0.803867 x - 0.000726$	1	
			$y = -0.000655 x^2 + 0.879739 x - 0.002542$	1	
4	Tauroursodeoxycholic acid	TUDCA	-	-	-
			-	-	
			-	-	
5	Taurohyodeoxycholic acid	THDCA	y = 1.604915 x - 0.003178	0.999931	29%
			y = 2.861145 x - 0.005989	0.999966	
			y = 2.712683 x + 0.008061	1	
6	Taurocholic acid	TCA	$y = 0.030730 x^2 + 1.078610 x + 0.006360$	1	4%
			$y = -0.010441 x^2 + 1.155236 x - 0.000742$	1	
			$y = 0.006305 x^2 + 1.099722 x + 0.007466$	1	
9	ω-muricholic acid	ωMCA	y = 0.387601 x + 0.000103	0.999996	12%
			y = 0.404355 x - 0.001328	1	
			y = 0.392709 x + 0.001453	0.999996	
10	α-muricholic acid	αMCA	y = 0.327873 x + 0.001579	0.999998	8%
			y = 0.367380 x + 0.000275	1	
			y = 0.370607 x + 0.002211	0.999982	
11	Taurochenodeoxycholic acid	TCDCA	y = 1.583165 x - 0.009830	0.999901	4%
			y = 1.510813 x - 0.005831	0.999945	
			y = 1.478769 x - 0.000279	0.999978	
12	β-muricholic acid	βMCA	y = 0.573200 x + 0.001089	0.999999	4%
			y = 0.582219 x + 0.001372	0.999999	
			y = 0.572018 x + 0.003713	0.999989	
13	Taurodeoxycholic acid	TDCA	y = 1.713798 x - 0.003950	0.999976	4%
			y = 1.670810 x - 0.001890	0.999945	
			y = 1.753457 x - 0.002823	0.999948	

14	Cholic acid	CA	y = 1.153204 x + 0.011784	0.999999	6%
			y = 1.239431 x + 0.003744	0.99994	
			y = 1.161192 x + 0.008412	0.999999	
16	Ursodeoxycholic acid	UDCA	y = 0.732963 x + 0.004028	0.999999	7%
			y = 0.779593 x - 0.002583	0.999973	
			y = 0.761977 x + 0.001268	0.999988	
17	Hyodeoxycholic acid	HDCA	y = 0.738140 x + 0.002053	1	139%
			y = 0.782574 x - 0.000537	0.999999	
			y = 0.758829 x + 0.001317	0.999984	
19	Chenodeoxycholic acid	CDCA	y = 0.395053 x + 0.002407	0.999978	10%
			y = 0.448499 x + 0.001449	0.99999	
			y = 0.423538 x + 0.003555	0.999982	
20	Deoxycholic acid	DCA	y = 0.810474 x - 0.001199	0.999953	24%
			y = 0.859060 x - 0.000385	0.999993	
			y = 0.843631 x - 0.001641	0.999968	

Bile acids marked in red were not included in the statistical analysis, as these bile acids were not detected in serum or not reproducible due to very low abundance.

[a]Three calibration curves were for each bile acid obtained during the run. For bile acids measured in low abundance (<1 µg/mL) in serum, a linear calibration curve was calculated (based on BA mix 0.01 µg/mL, 0.1 µg/mL and 1 µg/mL). For bile acids measured in high abundance (> 1 µg/mL) in serum, a quadratic calibration curve was calculated (based on BA mix 0.01 µg/mL, 0.1 µg/mL, 1 µg/mL, and 5 µg/mL) due to ion suppression at high concentrations.

[b]Coefficient of variance (CV) for each bile acid was determined based on pooled quality control (QC) samples.

Supplementary Figures

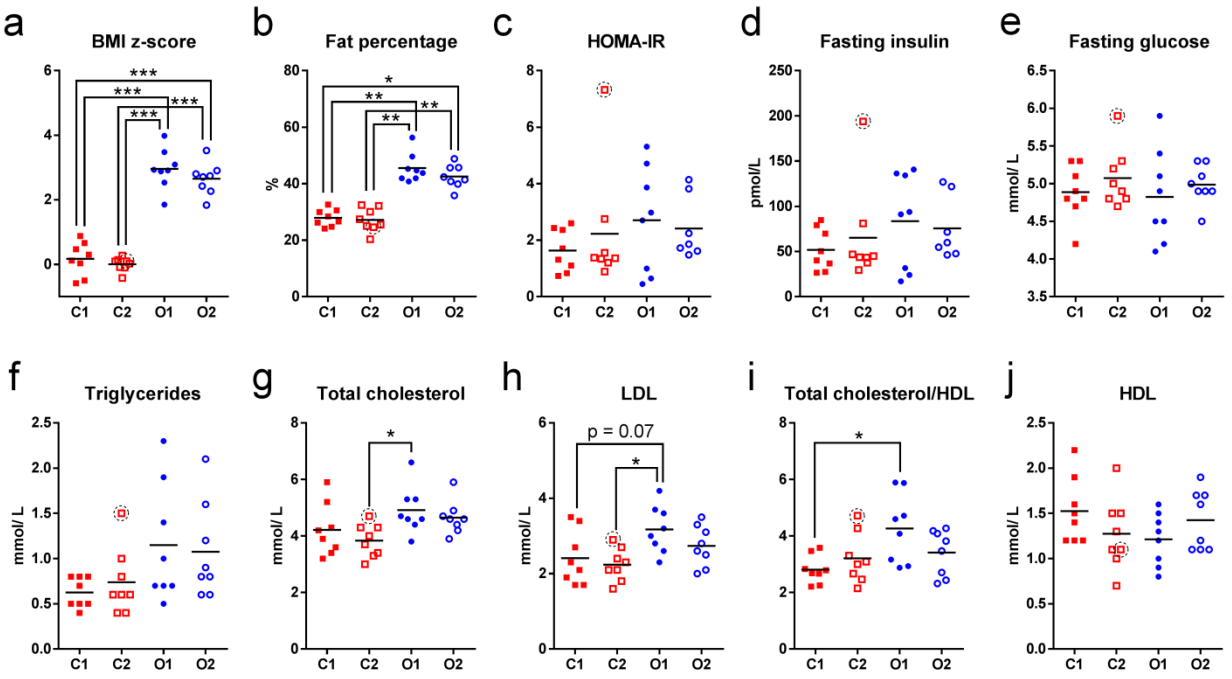

Figure S1. Phenotypes of human donors compared according to isolator group of the corresponding colonised mice, (a) BMI z-score, (b) fat percentage, (c) HOMA-IR, (d) fasting insulin in plasma, (e) fasting glucose in plasma, (f) fasting triglycerides, (g) total cholesterol, (h) LDL cholesterol, (i) ratio of total cholesterol to HDL cholesterol, and (j) HDL cholesterol in serum (n = 8 per group). The mean of each group is shown by a horizontal line, and asterisks represent significant differences between groups (*p < 0.05, **p < 0.01, ***p < 0.001, Tukey's multiple comparisons test or Dunn's multiple comparisons test). C1, Control 1; C2, Control 2; O1, Obese 1; O2, Obese 2.

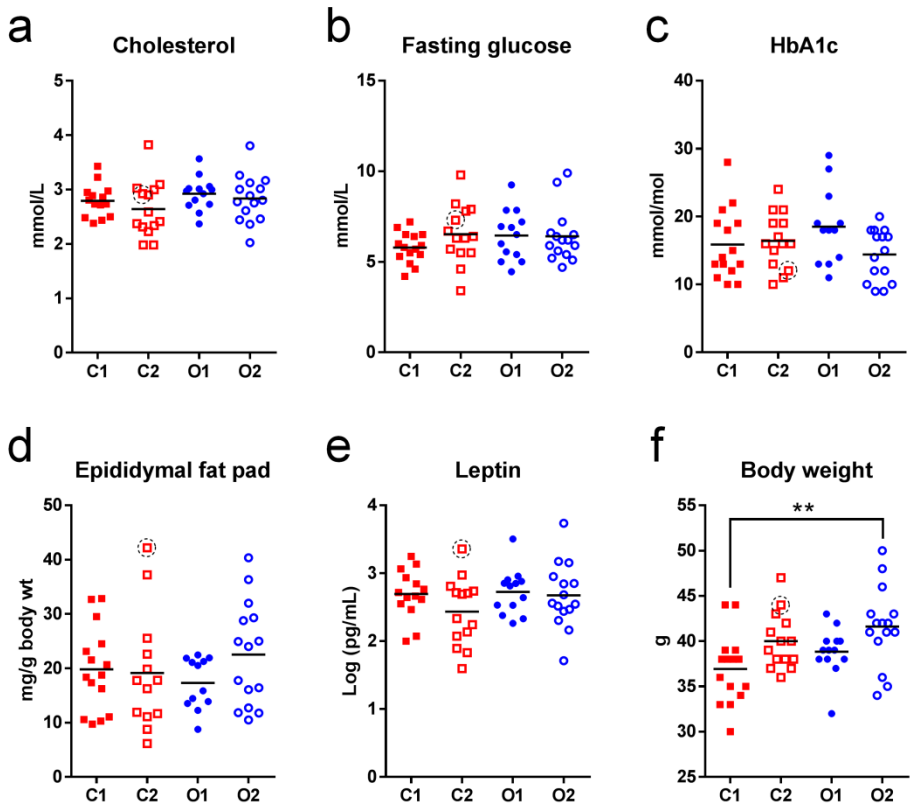

Figure S2. Metabolic features of transplanted mice including (a) serum cholesterol, (b) fasting glucose, (c) HbA1c, (d) weight of epididymal fat pad normalized to total body weight, (e) serum fasting leptin, and (f) body weight (n = 13-15 per group). The mean of each group is shown by a horizontal line, and asterisks represent significant differences between groups (**p < 0.01, Tukey's multiple comparisons test or Dunn's multiple comparisons test). C1, Control 1; C2, Control 2; O1, Obese 1; O2, Obese2.

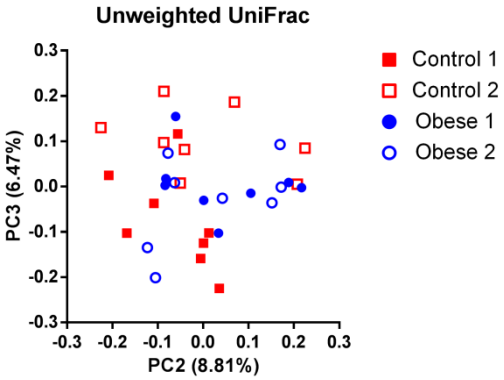

Figure S3. PCoA based on unweighted UniFrac distances between mouse faecal microbiotas at dpc 7 (n = 8 per group). The OTU table was rarefied to 2190 reads per sample.

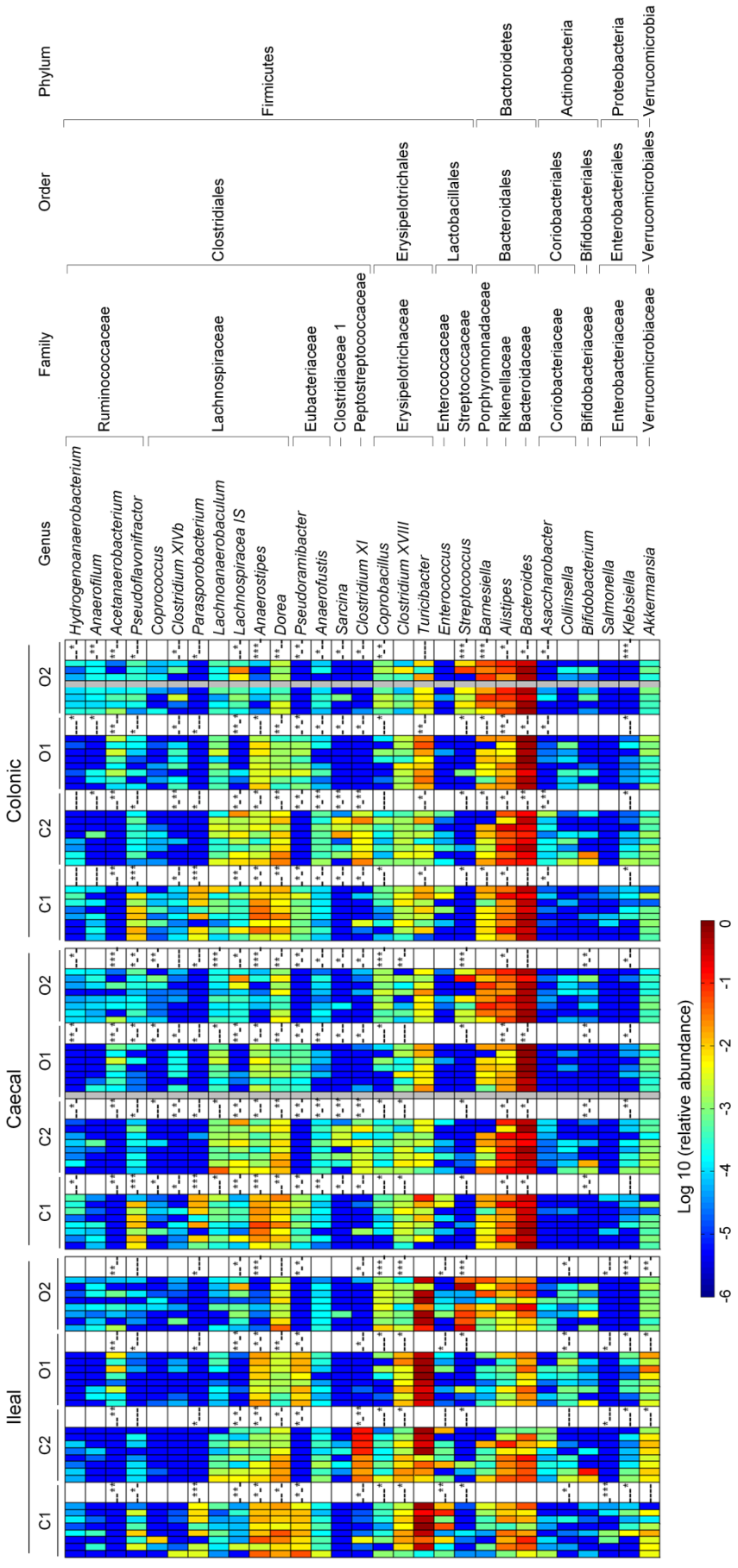

Figure S4. Heatmap showing the relative abundances of mice ileal, caecal and colonic genera differing between groups (n = 8 per group). Kruskal-Wallis test followed by FDR correction was performed to detect differing genera. For genera with $q < 0.05$, each group has a significance indication including four characters representing the four groups Control 1, Control 2, Obese 1 and Obese 2. A dash (-) in a given position indicates that the phylum abundance is not different from the rest of the groups, while an asterisk (*) indicates significant difference from the group at that position ($p < 0.05$, Dunn's multiple comparisons test).

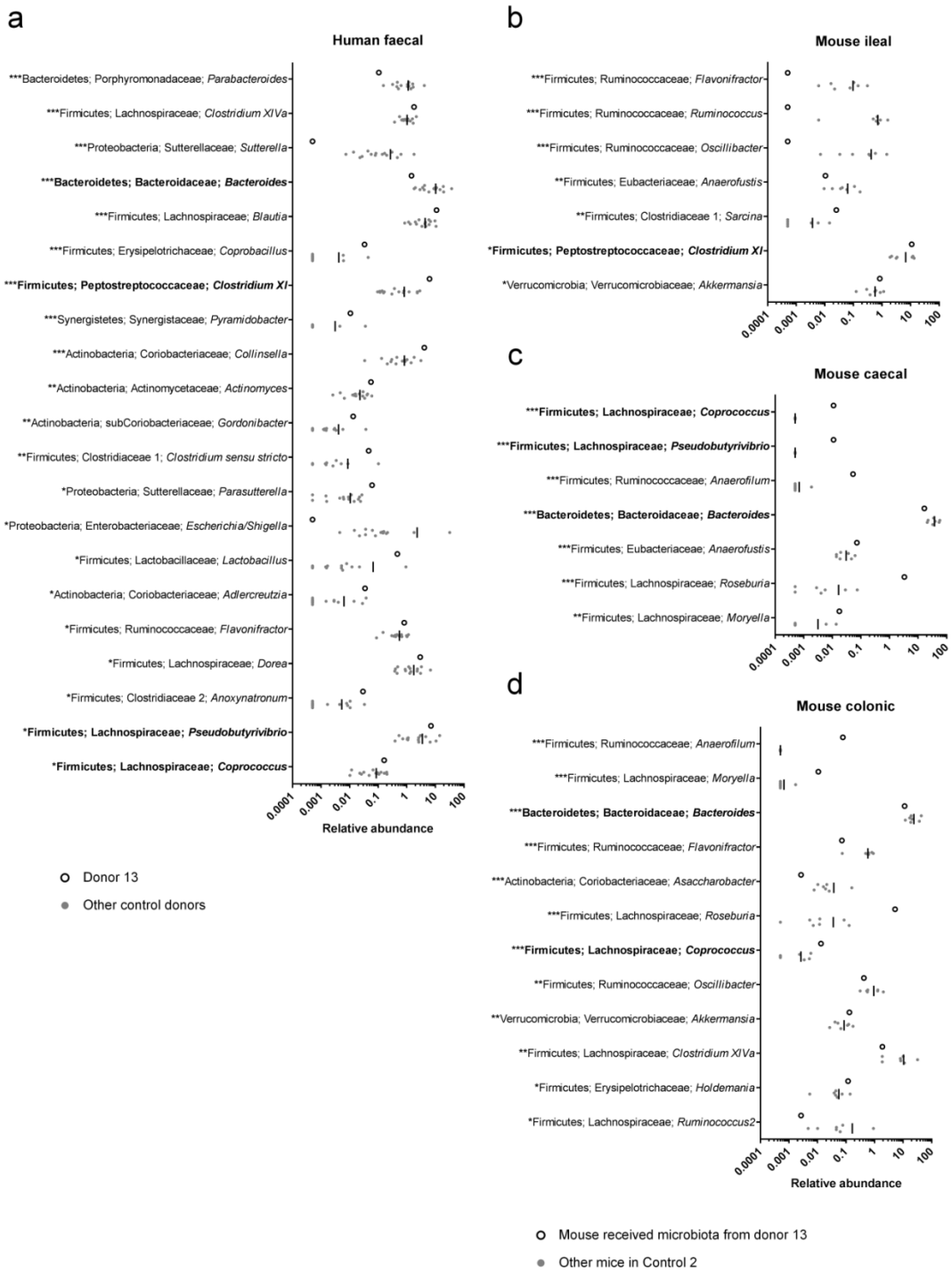

Figure S5. Genera differently abundant in (a) faecal microbiota of donor 13 and other control donors, as well as genera differently abundant in (b) ileal, (c) caecal and (d) colonic samples of mice transplanted with microbiota from donor 13 and other mice in Control 2 (n = 16 control humans or 8 mice). A Gaussian distribution was built from the log transformed relative abundances of each genus in control donors excluding donor 13 using a maximum-likelihood estimation, and p values represented the probability that the genus of donor 13 was contained in the distribution. The same strategy was applied to transplanted mice in Control 2. Differentiating genera shared by human and mouse were indicated by bold characters. Asterisks represent FDR adjusted p values (*q < 0.05, **q < 0.01, ***q < 0.001).

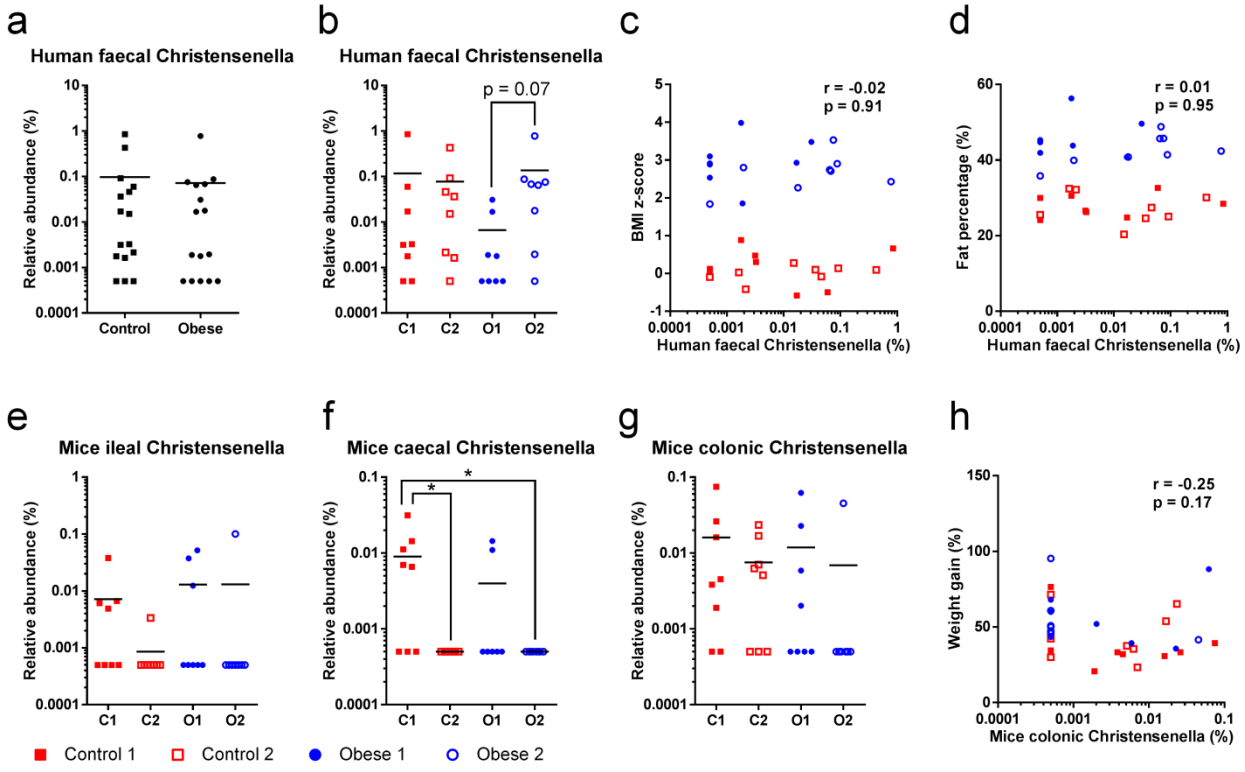

Figure S6. Christensenellaceae in human faecal microbiota and mouse gut microbiota presented as relative abundances of Christensenellaceae in human samples shown (a) by control/obese groups or (b) by isolator groups. Spearman correlations between the relative abundance of Christensenellaceae in human faecal microbiota and (c) BMI z-score or (d) fat percentage, respectively. Relative abundances of Christensenellaceae in (e) ileal, (f) caecal and (g) colonic samples from mice. Spearman correlation (h) between the relative abundance of Christensenellaceae in mice colonic microbiota and weight gain, n = 8 humans or 7-8 transplanted mice per group. In a-b and e-g, the mean of each group is shown by a horizontal line, and asterisks represent significant differences between groups (*p < 0.05, Dunn's multiple comparisons test). In c, d and h, correlation coefficients, r, and significance levels, p, from Spearman correlations are listed. C1, Control 1; C2, Control 2; O1, Obese 1; O2, Obese2.

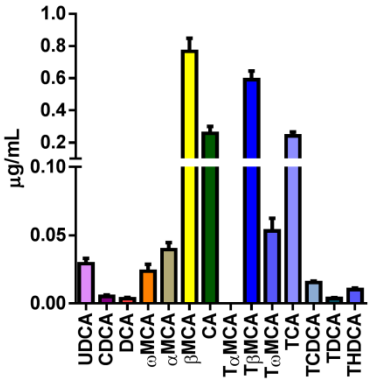

Figure S7. Serum bile acids. Data are shown as mean values ± SEM. n = 52 mice.

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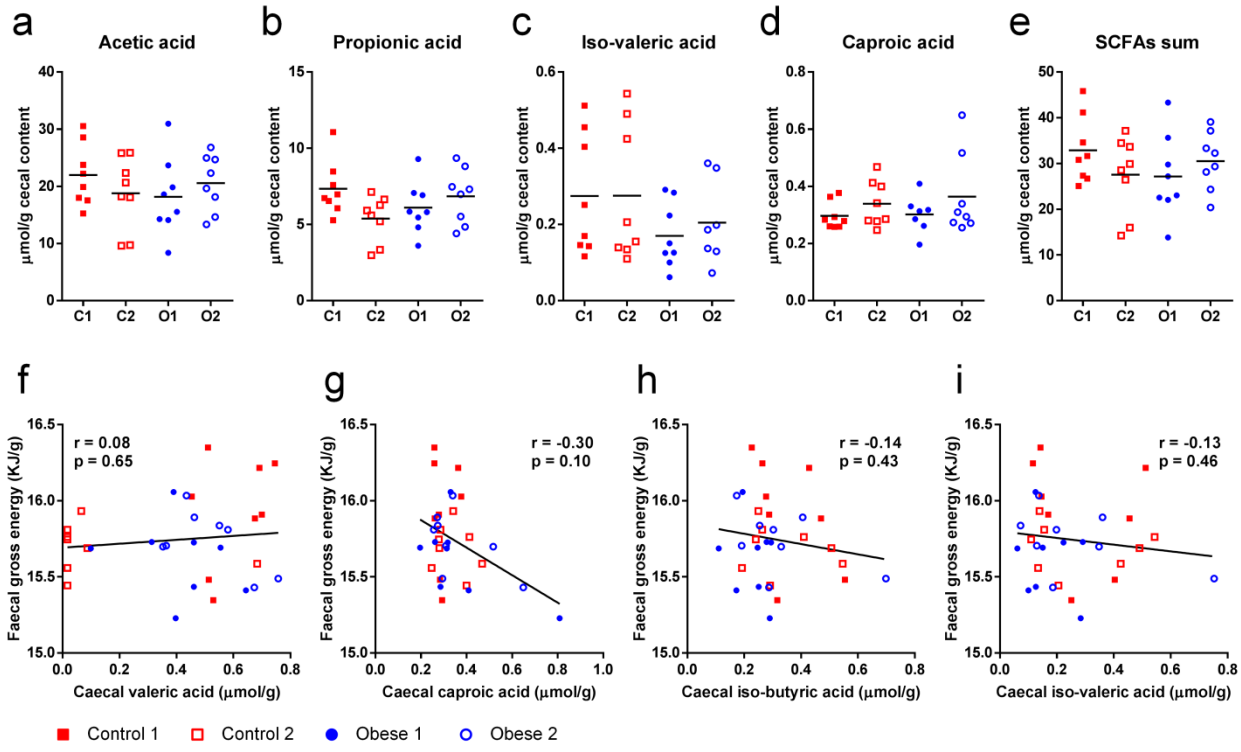

Figure S8. SCFAs in caecal content including (a) acetic acid, (b) propionic acid, (c) iso-valeric acid, (d) caprioic acid and (e) the sum of seven SCFAs. Means of each group are shown by horizontal lines. Spearman correlations between faecal gross energy and caecal (f) valeric acid, (g) caprioic acid, (h) iso-butyric acid, (i) iso-valeric acid (n = 8 per group). Correlation coefficients, r, and significance levels, p, from Spearman correlations are listed and linear regression lines are shown.

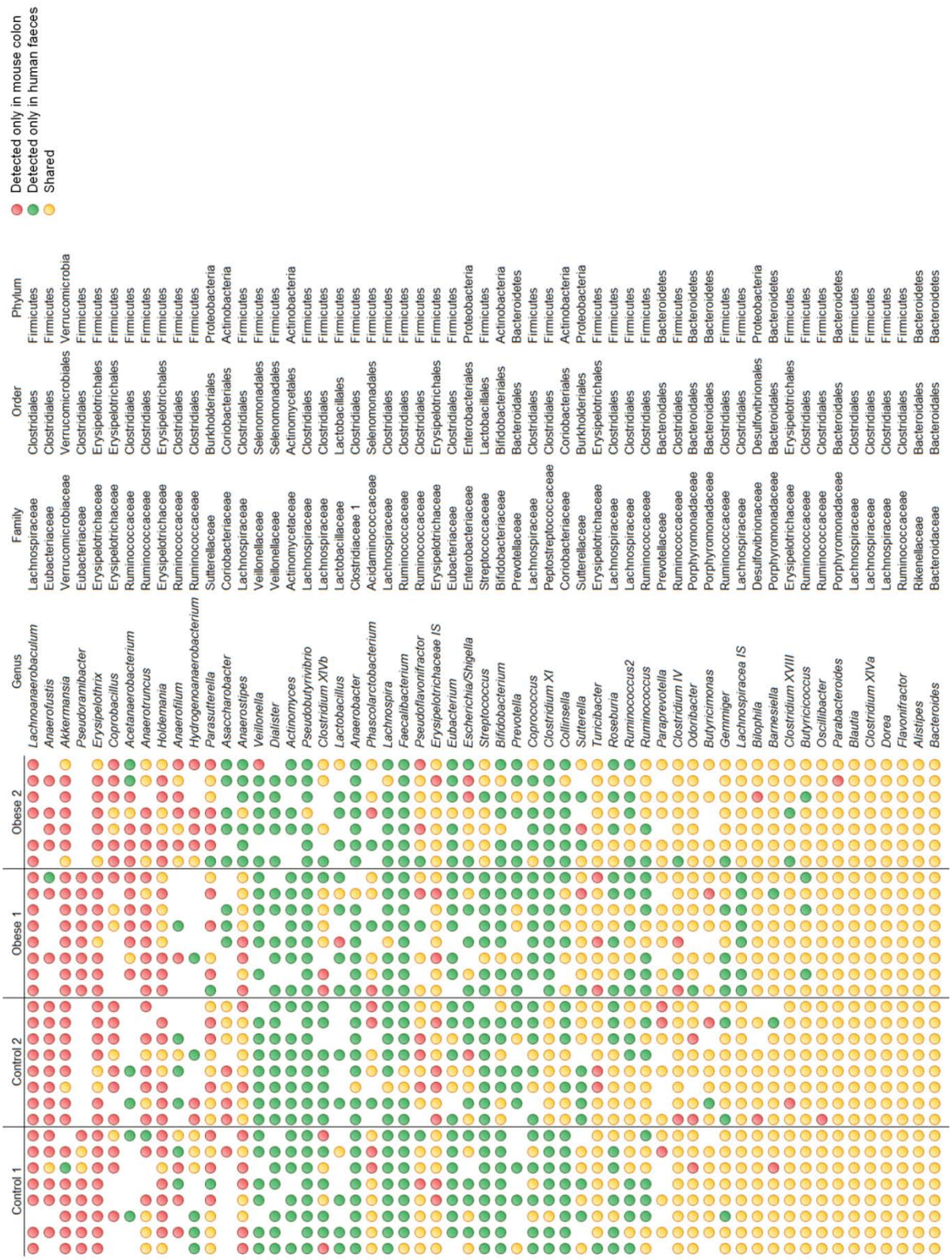

Figure S9. A map visualizing overlaps between detected genera (relative abundance > 0.01%) in human faecal microbiota and mice colonic microbiota for each human-mouse pair. Genera that were not detected in human nor in mice are shown as blanks, and genera with more than 16 blanks are not shown.