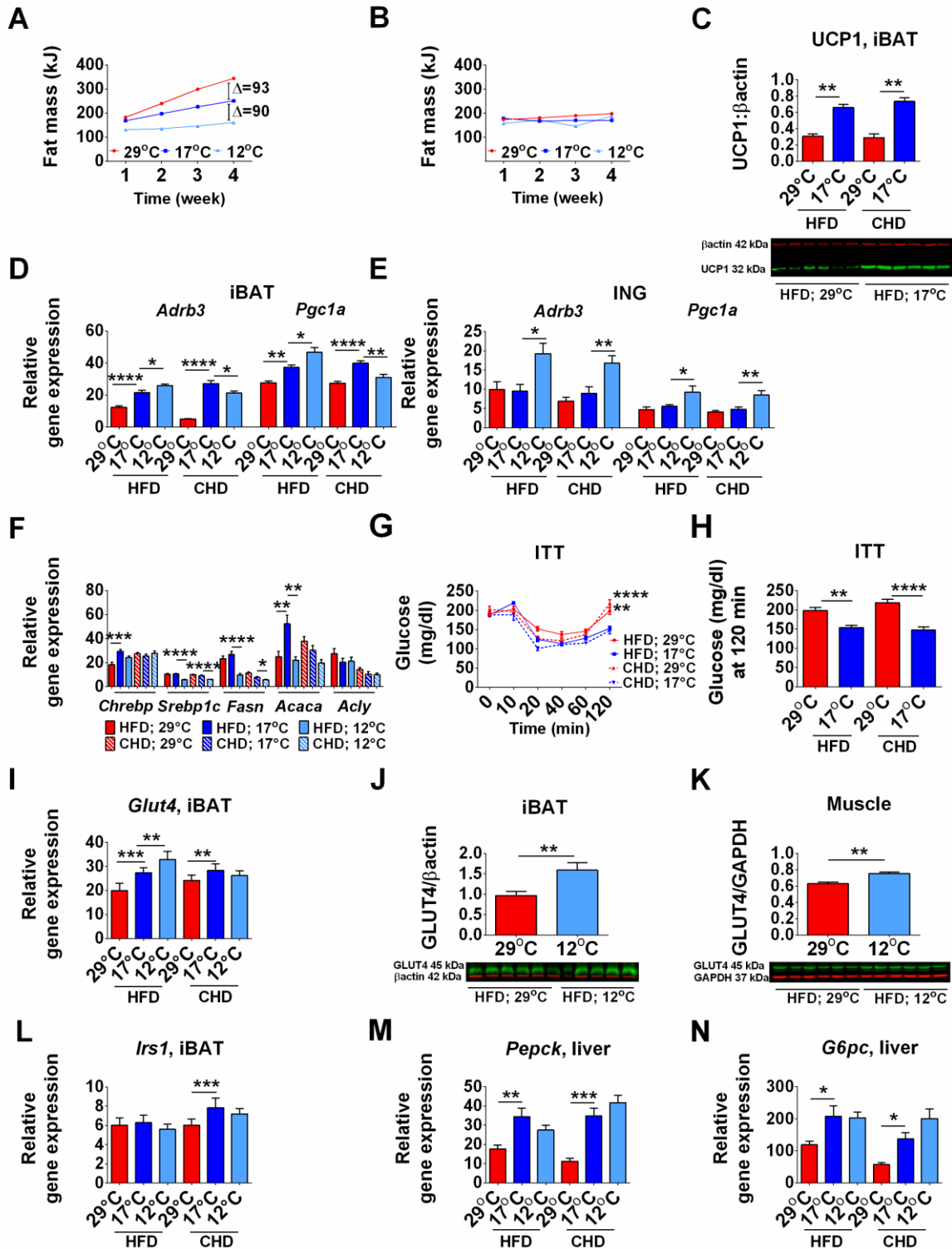**Supplemental Information**

Altered Microbiota Contributes

to Reduced Diet-Induced Obesity upon Cold Exposure

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## Supplemental Information

Figure S1, Related to Figure 1. Effect of Reduced Ambient Temperature on Energy Balance Phenotype and Glucose Metabolism in Mice Fed a HFD or CHD for Four Weeks. Cumulative increase in fat mass in mice fed HFD (A) or CHD (B). UCP1 protein levels in iBAT (C). Induction of *Adrb3* and *Pgc1a* in iBAT (D) and ING (E). Hepatic expression of genes involved in lipogenesis (F). Insulin tolerance test (G) and glucose level presented at 120 min after insulin injection (H). *Glut4* expression in iBAT (I); GLUT4 protein expression in iBAT (J) and skeletal muscle (K). *Irs1* (L) expression in iBAT. *Pepck* (M) and *G6pc* (N) expression in liver.
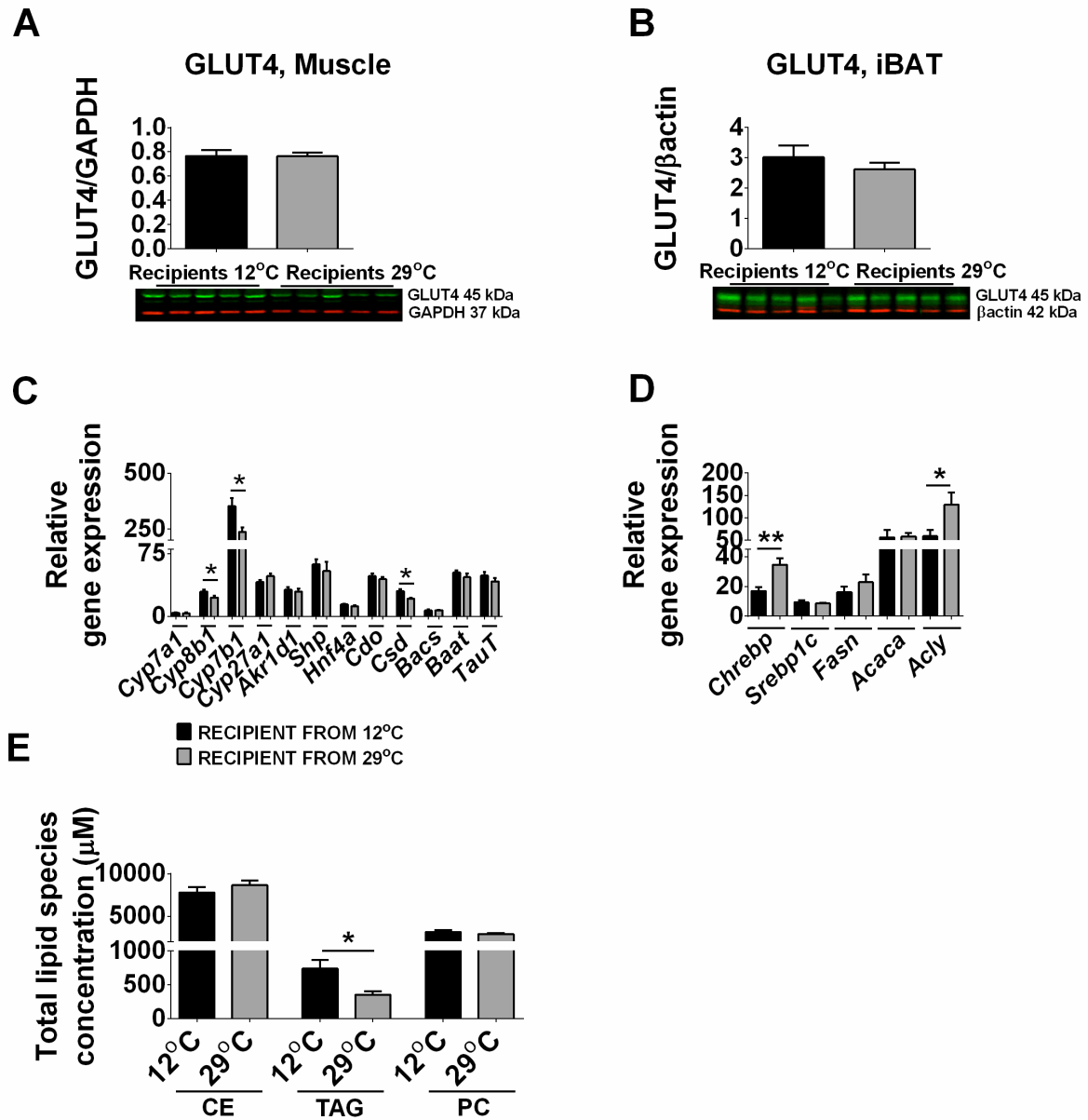
Quantification of the results from immunoblots is expressed relative to β-actin. Data are presented as mean ± SEM. Differences between groups were analyzed by one-way ANOVA followed by Tukey`s post hoc multiple comparison test and Mann-Whitney test (immunoblot). N=8-10 (ITT and qRT-PCR) or 5-6 (immunoblot). $* p < 0.05$; $** p < 0.01$; $*** p < 0.001$; $**** p < 0.0001$.
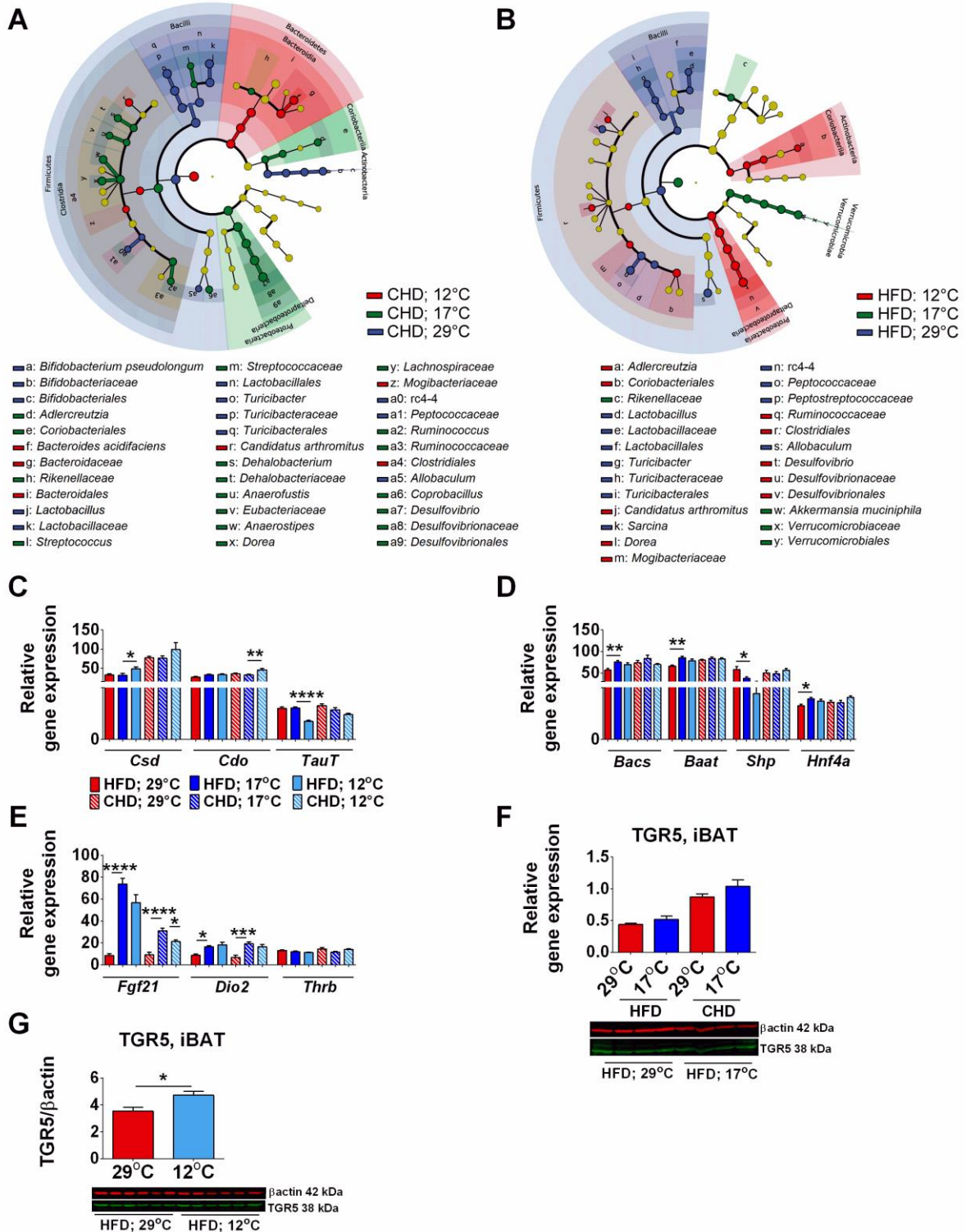
Figure S2, Related to Figure 2. Reduced Ambient Temperature Influences Gut Microbiota and Hepatic BAs Metabolism. Cladogram representing OTUs of statistical and biological difference between caecum microbiota of mice fed a CHD (A) and HFD (B) in response to ambient temperature. Each circle diameter is proportional to OTU's abundance. Hepatic expression of genes involved in taurine biosynthesis and transport (C) and BAs conjugation (D). The induction of *Fgf21*, *Dio2* and *Thrb* (thyroid hormone receptor beta) mRNA levels in iBAT (E). Protein levels of TGR5 (F, G) measured in iBAT. The data are given as mean ± SEM. Differences between groups were analyzed by one-way ANOVA followed by Tukey`s post hoc multiple comparison test or Mann-Whitney test (immunoblot). N=8-10 (qRT-PCR and cladogram) or 4-6 (Western blot). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.
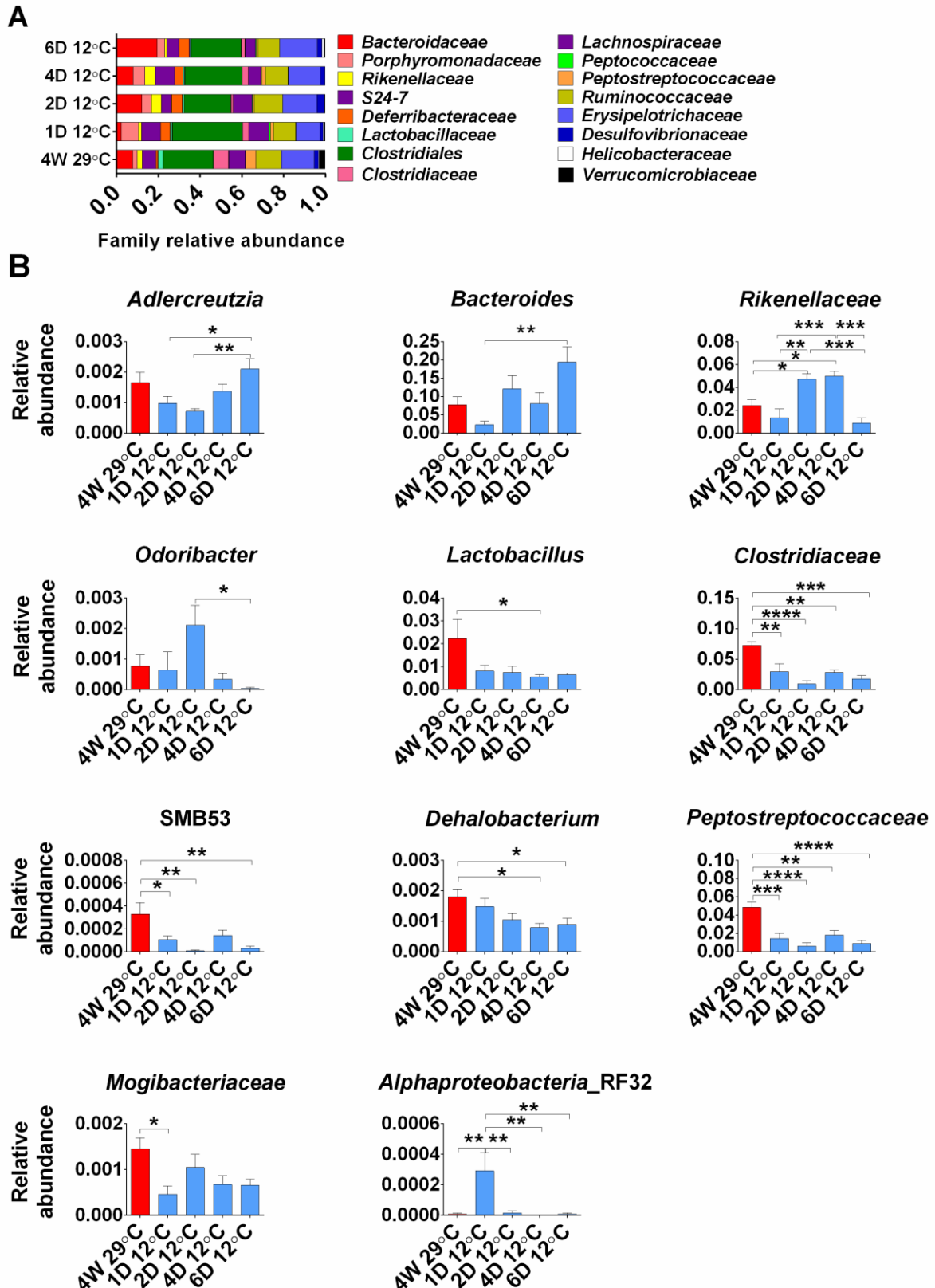
Figure S3, Related to Figure 3. Cold-dependent Changes in Gut Microbiota Composition. Relative abundance at family level (A) and major changes in the caecal microbiota (B) of mice with DIO exposed to cold for 6 days. The data are given as mean ± SEM. Differences between groups were analyzed by one-way ANOVA followed by Tukey`s post hoc multiple comparison test. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001 versus 4W 29°C. N=6.

A GLUT4, Muscle

B GLUT4, iBAT

C Relative gene expression

D Relative gene expression

E Total lipid species concentration (μM)

■ RECIPIENT FROM 12°C
□ RECIPIENT FROM 29°C

Figure S4, Related to Figure 4. Effect of Gut Microbiota on Glucose, BAs and Lipid Metabolism in Recipient Mice Colonized With Microbiota from Donor Mice Kept at 12°C and 29°C. Immunoblot of GLUT4 expressed in muscle (A) and iBAT (B). Changes in expression of hepatic genes involved in BAs synthesis (C) and hepatic lipogenesis (D). Total lipid species measured in plasma in recipient mice (E). The data are given as mean ± SEM. Differences between groups were analyzed Mann-Whitney test. N=4-5. CE: cholesteryl ester; TAG: triacylglycerol; PC: phosphatidylcholine. * $p < 0.05$; ** $p < 0.01$.

Supplemental Experimental Procedures

Quantitative real-time PCR.
Total RNA was isolated from homogenized adipose tissues and liver using TRI reagent per manufacturer's directions (Molecular Research Centre, USA). RNA was stored at -80°C in RNase-free water with SUPERase In (Ambion, USA) an inhibitor of RNases. Quality of isolated RNA was confirmed by its integrity on agarose gel electrophoresis with UV light and the quantity by spectrophotometry (NanoDrop1000, ThermoScientific, USA). TaqMan probes were used for genes quantification using the TaqMan one-step PCR master mix reagents kit (Applied Biosystem, USA) on ABI Prism 7900 HT Sequence Detection System (Applied Biosystem, USA). All samples with standards were run in duplicate and normalized to cyclophilin. Probe and primer sequences were designed using Primer-Express™, version 2.0.0 (Applied Biosystems, USA) or by Applied Biosystem.

Primer and Probe Sequences Used for Gene Expression Analysis.

Gene	Forward and reverse primers (F, R) Sequence 5` - 3`	Probe Sequence 5` (6FAM) - 3` (TAMRA)
Acaca	F:ATGTCCGCACTGACTGTAACCA R:TGCTCCGCACAGATTCTTCA	TCCTCAACTTTGTGCCCACGGTCA
Acly	F:CACCCCGCTGCTCGACT R:TCAGGATAAGATTTGGCTTCTTGG	TGCCCTGGAAGTGGAGAAGATTACCACC
Adrb3	F:TCCCAGCCAGCCCTGTT R:CGCACCTTCATAGCCATCAA	CAGGCAGAGTCCACCGCTCAACAG
Cyclophilin	F:GGTGGAGAGCACCAAGACAGA R:GCCGGAGTCGACAATGATG	ATCCTTCAGTGGCTTGTCCCGGCT
Dio2	F:GCTGCGCTGTGTCTGGAA R:TGGAATTGGGATCATCTTCAC	AGCTTCCTCCTAGATGCCTACAAACAGG TTAAACTG
Fasn	F: CCTGGACTCGCTCATGGGT R: ATTTCCTGAAGTTTCCGCAGC	CGTCAGATCCTGGAACGAGAACACGA
Glut4	F:GATTCCATCCCACAAGGCAC R:TCATGCCACCCACAGAGAAG	CTACGCTCTGGGCTCTCTCCGTGG
G6pc	F:GGAGTCTTGTCAGGCATTGCT R:GCGCGAAACCAAACAAGAAG	CAGCCTCCGGAAGTATTGTCTCATCACC
Pepck	F:GAGGAGGAGTACGGGCAGTTG R:TCGATCCTGGCCACATCTC	TATGACAACTGTTGGCTGGCTCTCACTG AC
Pgc1a	F:CATTTGATGCACTGACAGATGGA R:CCGTCAGGCATGGAGGAA	CCGTGACCACTGACAACGAGGCC
Ucp1	F:CACCTTCCCGCTGGACACT R:CCCTAGGACACCTTTATACCTAATGG	AGCCTGGCCTTCACCTTGGATCTGA

Primers and TaqMan Probes Used for Quantification of Target Genes.

Gene	Assay ID
Akr1d1	Mm01165275_m1
Baat	Mm00476075_m1
Bacs	Mm00447768_m1
Cdo	Mm00473573_m1
Csd	Mm00520087_m1
Cpt1a	Mm01231183_m1
Cyp7a1	Mm00484150_m1
Cyp7b1	Mm00484157_m1
Cyp8b1	Mm00501637_s1
Cyp27a1	Mm00470430_m1
Fgf21	Mm00840165_g1
Hnf4a	Mm01247712_m1
Irs1	Mm01278327_m1
Chrebp	Mm02342723_m1
Shp	Mm00442278_m1
Srebp1c	Mm00550338_m1
TauT	Mm00436909_m1
Thrb	Mm00437044_m1

Western blot analysis.

Western blot analysis was performed according to Xue et al. (Xue et al., 2005). Polyclonal antibodies used were anti-UCP1 (goat anti-UCP1, Santa Cruz Biotechnology, USA) and anti-TGR5 (goat anti-TGR5, Santa Cruz Biotechnology, USA), DIO2 (rabbit anti-DIO2, Abcam, USA), AMPK (rabbit anti-AMPK, Cell Signaling, USA), phospho-AMPK (rabbit anti-pAMPK, Cell Signaling, USA), GLUT4 (rabbit anti-GLUT4, Abcam, USA), PGC1α (rabbit anti-PGC1α, Abcam, USA), ACC (rabbit anti-ACC, Cell Signaling, USA), phospho-ACC (rabbit anti-pACC, Cell Signaling, USA). β-actin (mouse anti-β-actin, Abcam, USA) or GAPDH (mouse anti-GAPDH, Abcam, USA) were used as internal loading controls. Bands were visualized and quantified using the Odyssey imaging system (Li-Cor, USA) with fluorescent-labeled antibodies IRDye800 and IRDye700 (Rockland, USA).

Caecal transplantation experiment.

Adult GF B6 male mice (4-5 in group) were maintained in plastic gnotobiotic research isolators and fed HFD (40.6 % kcal from fat; TD.09683 Harlan Teklad, double irradiated) for a week. Thereafter, the GF mice were colonized with mouse caecum microbiota from CONV-R mice fed HFD (58 % kcal from fat, AIN-76A 9G03 Research Diets) at 12°C or 29°C for 6 weeks in a blinded fashion. Colonization was performed by diluting the caecum of a donor mouse (app. 50 mg) in 2 ml reduced PBS. After the material was re-suspended, a 1 ml syringe was used to recover a 200 μl aliquot of the suspension, which was subsequently introduced by intragastric gavage of each GF recipient. Mice with transferred caecal microbiota were fed a HFD (40.6 % kcal from fat; TD.09683 Harlan Teklad, double irradiated), housed at 23°C for 6 weeks. Body composition was defined using EchoMRI (EchoMRI, Houston, TX) 1 day and 6 weeks after colonization. Intraperitoneal glucose tolerance test (IPGTT) was performed at the end of the experiment. Blood for BA analysis was collected from the vena porta under deep isoflurane-induced anesthesia. Liver, gall-bladder, iBAT, ileum, caecum, and colon were harvested. All tissues were immediately snap-frozen in liquid nitrogen and stored in -80°C until further processed. Caecum transplantation experiments were performed using protocols approved by the University of Gothenburg Animal Studies Committee.

Glucose and insulin tolerance test (GTT, ITT).

Mice were fasted for 4h before intraperitoneal injection of 20 % glucose solution (2 mg/ g body weight). Blood glucose levels from tail vein were measured at baseline, 20, 40, 60 min. by using an Accu-Chek glucometer (Roche Diagnostics, France). The area under the curve (AUC) was calculated to determine IPGTT between the groups. For ITT, the blood glucose levels were measured at baseline, 10, 20, 40, 60, 120 min. after injection with bovine insulin (0.75 U/ kg body weight).

16S RNA amplification.

The V4 region of the bacterial 16S rRNA gene was amplified using primers and the sequencing approach and protocol of Kozich et al (Kozich et al., 2013). PCR was carried out as described by De Vadder et al (De Vadder et al., 2014). The forward and reverse reads from the pair-end sequencing were joined by benefiting from the long overlap between both reads using in-house codes. Identical bases in the overlap sequence increase the assurance accuracy of the sequencing and therefore we assigned the highest possible quality score for those matching bases. The FASTX-Toolkit has been used to filter out the low quality reads. The reads that have quality Phread scores of more than 20 in at least 98 % of their sequences passed the filter. Subsequently, the sequencing data were analyzed using the software package Quantitative Insights Into Microbial Ecology (QIIME), version 1.8.0. Sequences were clustered into operational taxonomic units (OTUs) at a 97 % identity threshold using a closed-reference OTU picking approach with UCLUST (Edgar, 2010) against the Greengenes reference database (DeSantis et al., 2006) (13_8 release). Representative sequences for the OTUs were Greengenes reference sequences or cluster seeds and were taxonomically assigned using the Greengenes taxonomy and the Ribosomal Database Project Classifier (Wang et al., 2007). Representative OTUs were aligned using PyNAST (Caporaso et al., 2010) and used to build a phylogenetic tree with FastTree (Price et al., 2010), which was used to estimate α- and β-diversity of samples using phylogenetic diversity (Faith, 1992) and unweighted UniFrac (Lozupone and Knight, 2005). Three-dimensional principal coordinates analysis (PCoA) plots were visualized using Emperor (Vazquez-Baeza et al., 2013). Chimeric sequences were identified with ChimeraSlayer (Haas et al., 2011) and excluded from all downstream analyses. Singletons were also excluded. LDA Effect Size algorithm (Segata et al., 2011) was used to identify taxa that discriminated caecal microbiota profiles according to the colonization origin.

Analysis of bile acids.

The extraction and analysis of BAs were based on a previously published method using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) (Tremaroli et al., 2015).

Lipid analysis

Tissue lipid extraction was performed using the Folch procedure (Folch et al., 1957) while plasma lipids were extracted with the automated BUME method (Lofgren et al., 2012). Internal standards for triglycerides and cholesteryl esters (glyceryl-d_5 trihexadecanoate and cholesteryl-d_6 octadecanoate) were attained from CDN Isotopes through QMX Laboratories Ltd. (Essex, UK) and 1,2-diheptadecanoyl-*sn*-glycero-3-phosphocholine was purchased from Avanti Lipids (Alabaster, AL, USA). Lipids were detected and quantified using direct infusion mass spectrometry according to previous publication (Stahlman et al., 2013).

Total levels of CE included CE 14:0, CE 16:0, CE 16:1, CE 18:1, CE 18:2, CE 18:3, CE 20:0, CE 20:1, CE 20:2, CE 20:3, CE 20:4, CE 20:5, CE 22:5, CE 22:6.Total levels of TAG included TAG 48:0 TAG 48:1, TAG 48:2, TAG 50:0, TAG 50:1, TAG 50:2, TAG 50:3, TAG 52:0, TAG 52:1, TAG 52:2, TAG 52:3, TAG 52:4, TAG 54:1, TAG 54:2, TAG 54:3, TAG 54:4, TAG 54:5, TAG 56:3, TAG 56:4, TAG 56:5, TAG 56:6, TAG 56:7. Total levels of PC included PC 30:0, PC 32:2, PC 32:1, PC 32:0, PC 34:7, PC 34:4, PC 34:3, PC 34:2, PC 34:1, PC 36:5, PC 36:4, PC 36:3, PC 36:2, PC 36:1, PC 38:6, PC 38:5, PC 38:4, PC 38:3, PC 40:8, PC 40:7, PC 40:6, PC 40:5.

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