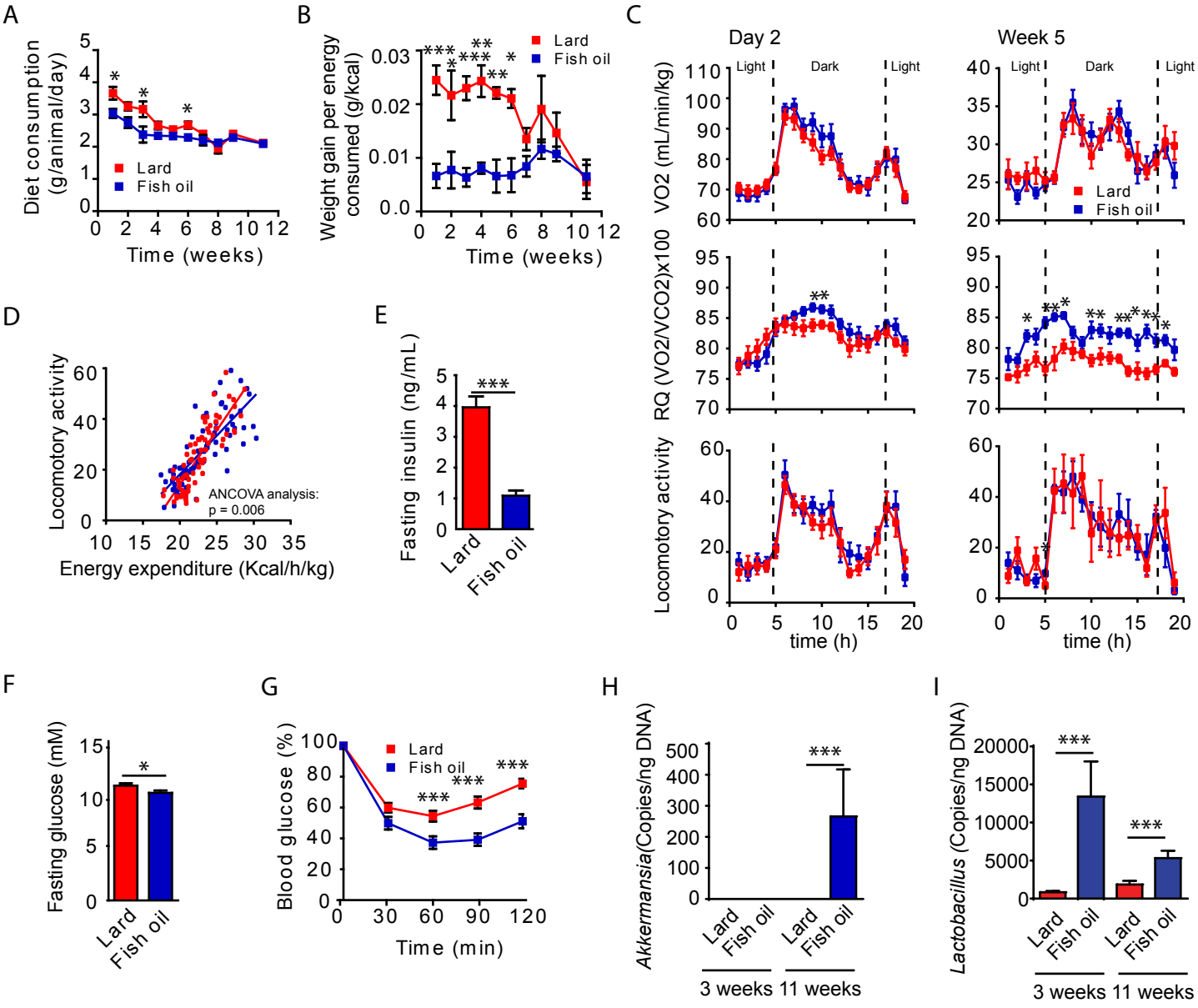
Cell Metabolism, Volume *22*

Supplemental Information

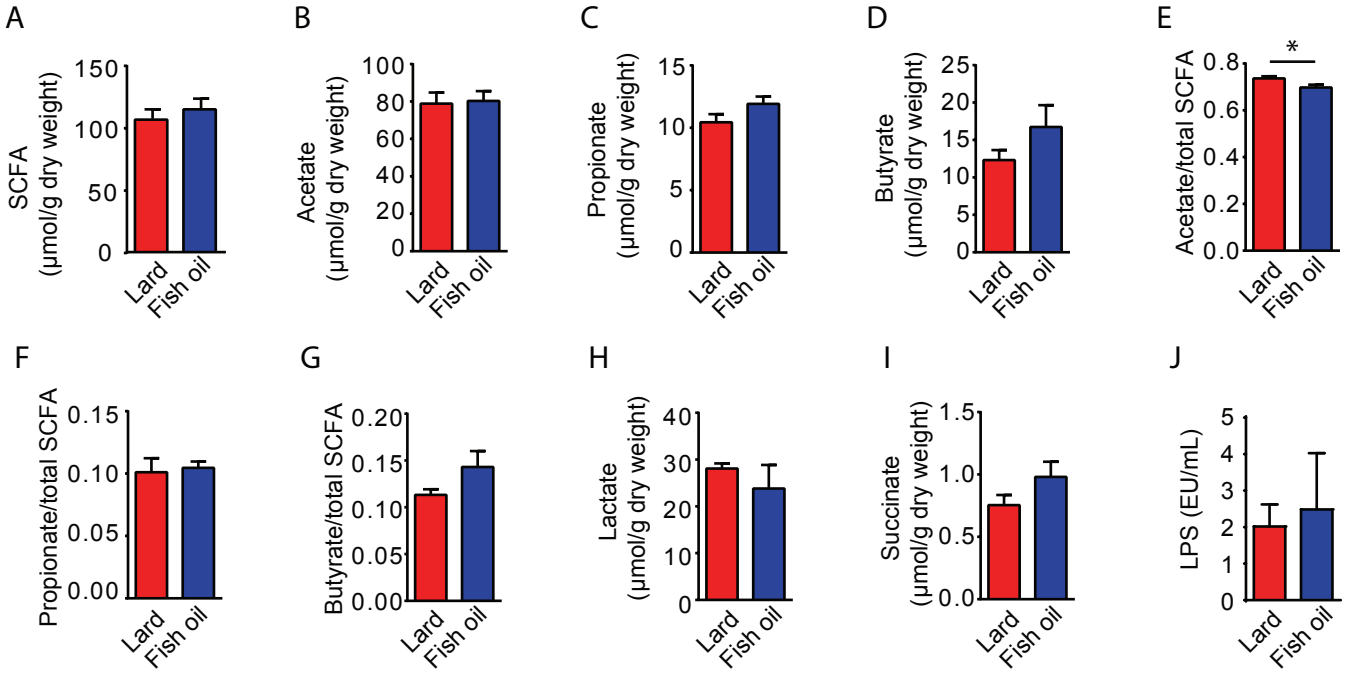
Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Signaling

Robert Caesar, Valentina Tremaroli, Petia Kovatcheva-Datchary, Patrice D. Cani, and Fredrik Bäckhed

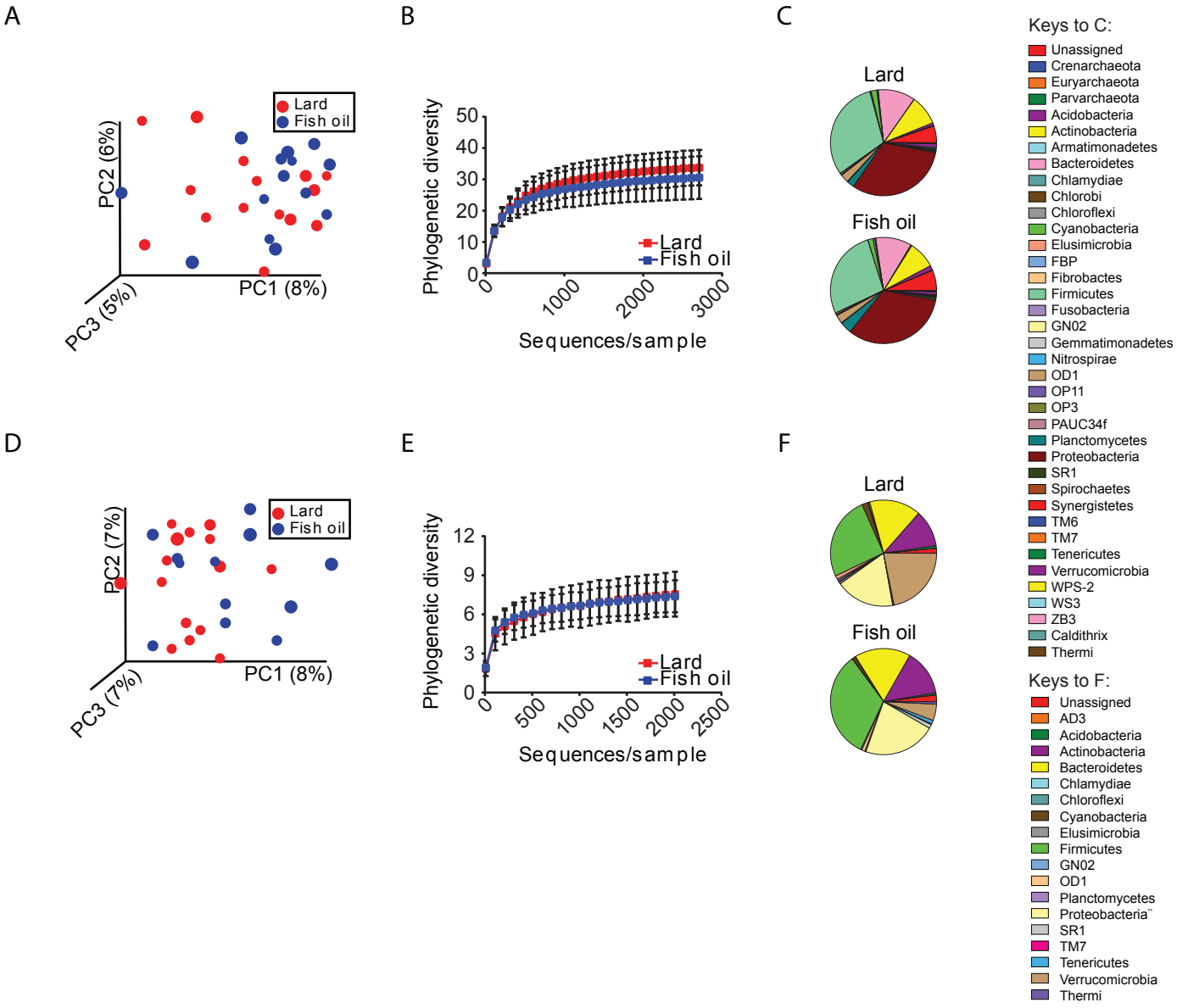


Supplementary Figure 1. Energy balance and glucose homeostasis in mice fed lard or fish oil. Related to Figure 1. (A) Food consumption during high-fat diet (n=3-4 cages/time-point). (B) Efficiency of feeding measured as gram body weight gained per kcal consumed (n=3-4 cages/time-point). (C) VO2 consumption, respiratory quotient (RQ) and locomotory activity measured over 18 h, including two light and one dark periods, in mice fed lard or fish oil for 2 days of for 5 weeks. Measurements performed on single-caged mice using SOMEDIC metabolic systems and G2 E-Mitter telemetry devices [n=8 (2 days); 5 (5 weeks)]. (D) Correlation analysis between energy expenditure and locomotory activity in mice fed lard or fish oil for 2 days. Correlation between locomotory activity and energy expenditure was based on average values collected over 18 h. Each dot represents data collected in 2 h intervals. 8 mice were used for each dietary group resulting in a total of 72 data points for each dietary group. Energy expenditure was calculated by the equation (3.815 + 1.232*RQ)×VO2. ANCOVA analysis was performed to determine difference in slope between linear regression lines. Fasting (E) insulin and (F) glucose levels in mice fed lard or fish oil for 11 weeks (n=9-10 mice per group). (G) Insulin tolerance in mice fed lard or fish oil (n=9-10 mice per group) for 10 weeks. Abundance of (H) *Akkermansia* and (I) *Lactobacillus* in cecum of mice fed lard or fish oil for 3 weeks (N=9 mice per group) and 11 weeks (N=15 mice per group). Quantification of bacteria was performed by qPCR.

Mean values ± SEM are plotted; *p<0.05, **p<0.01, ***p<0.001 versus fish oil.

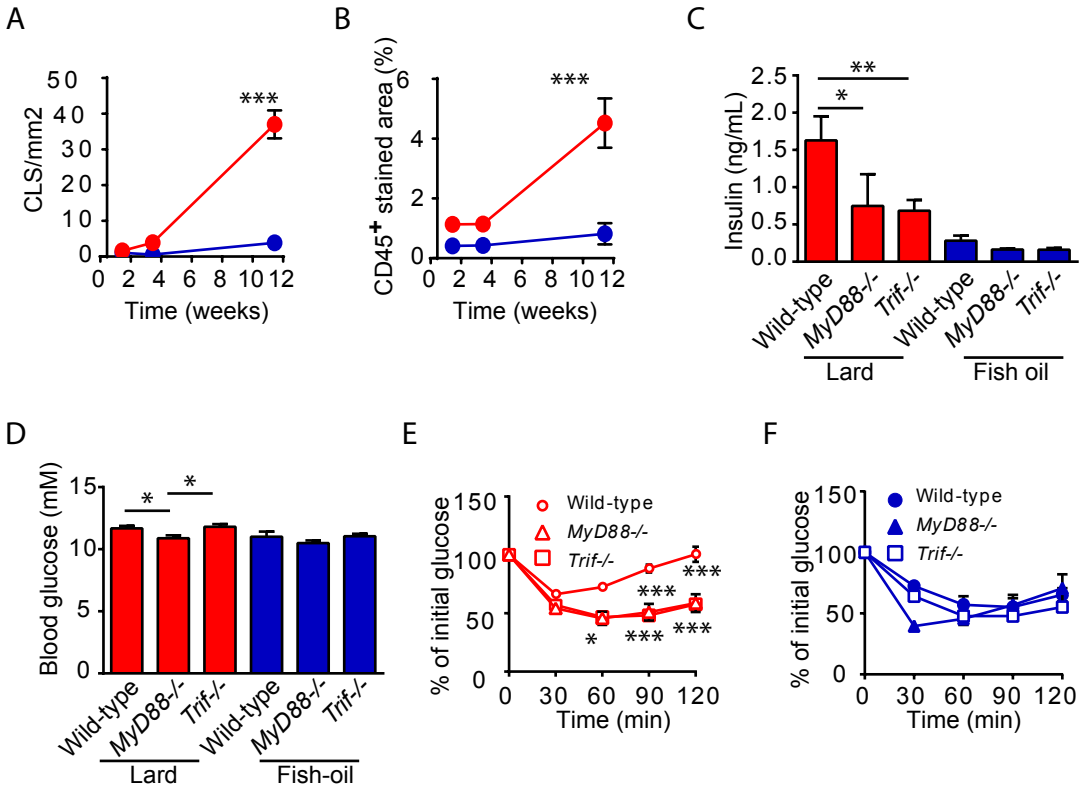


Supplementary Figure 2. Abundance of SCFA in mice fed lard or fish oil. Related to Figure 1.
Abundance of (A) total SCFA, (B) acetate, (C) propionate and (D) butyrate in cecum of mice fed lard diet or fish oil for 11 weeks (n=6 mice per group. Ratios between (E) acetate, (F) propionate and (G) butyrate and total abundance of SCFA (based on data presented in panels A-D). Abundance of (H) lactate and (I) succinate in cecum of mice fed lard or fish oil for 11 weeks. (J) Abundance of LPS in serum from mice fed lard or fish oil for 3 weeks (n=7-8 mice per group).
Mean values ± SEM are plotted; *$p<0.05$.

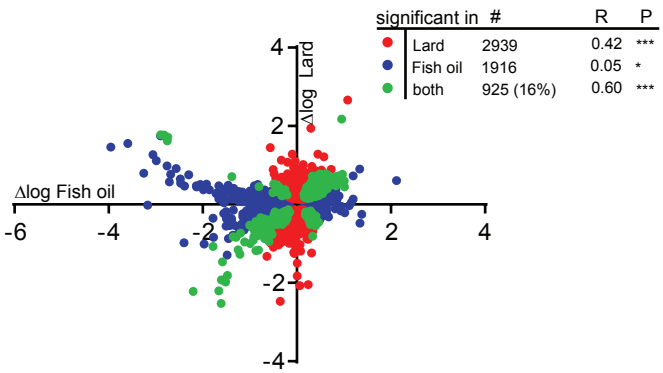


Supplementary Figure 3. Microbiota in blood and epididymal WAT of mice fed lard or fish oil for 11 weeks. Related to Figure 2.

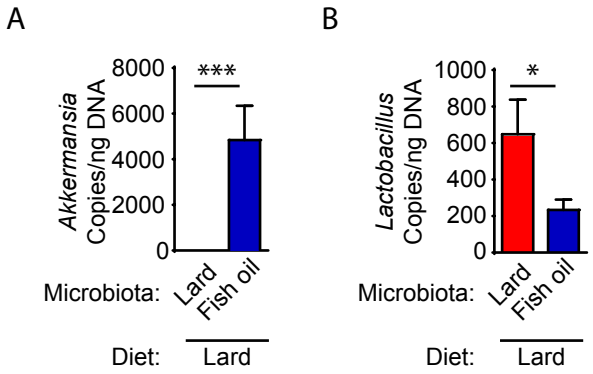
(A) Principal coordinate analysis of blood microbiota composition based on unweighted UniFrac in mice fed lard or fish oil [n=14 (lard), n=15 (fish oil)]; no amplification product with 515F and 806R primers could be obtained for one lard blood sample). (B) Rarefaction curves for phylogenetic diversity in blood microbiota from mice fed lard or fish oil (10-2710 sequences/sample). (C) Pie charts of blood microbial phyla composition in mice fed lard or fish oil for 11 weeks. (D) Principal coordinate analysis of WAT microbiota composition based on unweighted UniFrac in mice fed lard or fish oil [n=12 (lard), n=15 (fish oil)]; the depth of sequencing for three lard WAT samples was lower than 2000 sequences/samples so these samples were excluded from microbiota analyses). (E) Rarefaction curves for phylogenetic diversity in WAT microbiota from mice fed lard or fish oil (10-2010 sequences/sample). (F) Pie charts of WAT microbial phyla composition in mice fed lard or fish oil for 11 weeks.



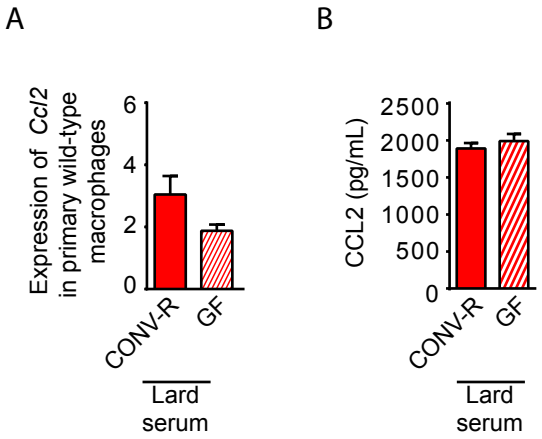
Supplementary Figure 4. WAT inflammation in wild-type mice after 1, 3 and 11 weeks of lard or fish oil diet and glucose homeostasis in wild-type, *Myd88-/-* and *Trif-/-* mice after 10 weeks of lard or fish oil diet. Related to Figure 3. (A) Quantification of crown-like structures in wild-type mice fed lard or fish-oil for 1, 3 or 11 weeks [n=10 (wild-type lard 1 week); 10 (wild-type lard 3 week); 7 (wild-type lard 11 week); 10 (wild-type fish oil 1 week); 10 (wild-type fish oil 3 weeks); 7 (wild-type fish oil 11 week)]. (B) Percentage of area occupied by $CD45^+$ cells in WAT from wild-type mice fed lard or fish-oil for 1, 3 or 11 weeks [n=10 (wild-type lard 1 week); 10 (wild-type lard 3 week); 7 (wild-type lard 11 week); 10 (wild-type fish oil 1 week); 10 (wild-type fish oil 3 weeks); 7 (wild-type fish oil 11 week)]. Fasting (C) insulin and (D) glucose levels in mice fed lard or fish oil for 11 weeks [n=6 (wild-type lard); 4 (*Myd88-/-* lard); 6 (*Trif-/-* lard); 3 (wild-type fish oil); 9 (*Myd88-/-* fish oil); 9 (*Trif-/-* fish oil)]. Insulin tolerance in mice fed (E) lard [n=3 (wild-type); 7 (*Myd88-/-*); 8 (*Trif-/-*)] or (F) fish oil [(n=3 (wild-type); 4 (*Myd88-/-*); 6 (*Trif-/-*)] for 10 weeks.
Mean values ±SEM are plotted; *p<0.05, **p<0.01, ***p<0.001.



Supplementary Figure 5. Gene regulation by the gut microbiota in mice fed lard or fish oil. Related to Figure 4. Regulation of WAT genes induced by the gut microbiota in mice fed lard (y-axis) or fish oil (x-axis). Genes significantly regulated ($p<0.05$, corrected for FDR) are displayed. The embedded table displays the number of regulated genes unique to either of the dietary groups or common to both and the R-value and p-value associated with a linear regression analysis of the dataset (n=6 mice).
Mean values ± SEM are plotted; *$p<0.05$, ***$p<0.001$.



Supplementary Figure 6. . Abundance of bacterial taxa in mice transplanted with cecal microbiota from donor mice fed lard or fish oil for 11 weeks. Related to Figure 5. (A-C) Abundance of *Akkermansia* and *Lactobacillus* in cecum of mice fed lard for 3 weeks and transplanted with cecal microbiota from donor mice fed lard or fish oil for 11 weeks (n=10 mice per group). Quantification of bacteria was performed by qPCR.
Mean values ±SEM are plotted; *p<0.05, ***p<0.001.



Supplementary Figure 7. Expression and secretion of CCL2 in primary macrophages. Related to Figure 6. (A) Expression of *Ccl2* and (B) CCL2 secretion in primary wild-type macrophages stimulated for 4 h with 2% plasma isolated from *vena cava* of CONV-R and GF mice fed lard (n=5-6 mice per group). Mean values ± SEM are plotted.

Supplementary Table 1. Diet composition. Related to Figure 1.

	D10011202 (lard diet)		D05122102 (Fish-oil diet)	
	g (%)	**kcal (%)**	**g (%)**	**kcal (%)**
Protein	24	20	24	20
Carbohydrate	41	35	41	35
Fat	23	45	23	45
Total		100		100
	kcal/g		**kcal/g**	
	4.72		4.73	
	g/unit	**kcal/unit**	**g/unit**	**kcal/unit**
Casein, 80 Mesh	200	800	200	800
L-Cysteine	3	12	3	12
Corn Starch	72.8	291	72.8	291
Maltodextrin 10	100	400	100	400
Sucrose	172.8	691	172.8	691
Cellulose	50		50	
Soybean oil	25	225	25	225
Lard	177.7	1598		
Menhaden Oil			177.7	1598
tert-Butylhydroquinone	0.0355			
Mineral Mix S10026	10		10	
DiCalcium Phosphate	13		13	
Calcium Carbonate	5.5		5.5	
Potassium Citrate	16.5		16.5	
Vitamin mix V10001	10	40	10	40
Choline Bitartrate	2		2	
Cholesterol	0.54			
Total	858.73	4057	858.15	4057
Cholesterol (g)	0.709		0.71	
Cholesterol (%)	0.083		0.083	
tBHQ (g)	0.035		0.035	

Supplementary Table 2. Relative abundance of phyla and of genera differentially abundant in gut microbiota from mice fed lard or fish oil for 11 weeks (p<0.05, FDR correction for multiple testing). Related to Figure 1.

		Relative abundance		Fold change
Phylum	**FDR**	**Lard**	**Fish oil**	**(Lard/Fish oil)**
Bacteroidetes	0.038	2.30E-01	1.46E-01	1.57
Unassigned;Other	0.31	4.18E-03	2.74E-03	1.53
Deferribacteres	0.32	2.72E-02	1.82E-02	1.49
Verrucomicrobia	0.026	4.59E-04	1.42E-03	0.32
TM7	0.026	1.15E-04	4.93E-04	0.23
Proteobacteria	0.038	5.11E-02	9.97E-02	0.51
Tenericutes	0.13	3.44E-04	6.07E-04	0.57
Actinobacteria	0.32	7.74E-03	8.82E-03	0.88
Cyanobacteria	0.32	1.67E-04	3.22E-04	0.52
Bacteria;Other	0.55	2.15E-03	2.42E-03	0.89
Firmicutes	0.78	6.77E-01	7.19E-01	0.94
Taxon				
p_Proteobacteria|c_Deltaproteobacteria|o_Desulfovibrionales|f_Desulfovibrionaceae|g_Bilophila	0.042	4.44E-03	1.14E-04	39.05
p_Bacteroidetes|c_Bacteroidia|o_Bacteroidales|f_|g_	0.012	9.12E-03	1.57E-03	5.79
p_Bacteroidetes|c_Bacteroidia|o_Bacteroidales|f_Bacteroidaceae|g_Bacteroides	0.038	1.31E-01	4.41E-02	2.98
p_Firmicutes|c_Clostridia|o_Clostridiales|f_Lachnospiraceae|Other	0.040	3.71E-02	2.10E-02	1.77
p_Firmicutes|c_Clostridia|o_Clostridiales|f_Ruminococcaceae|g_Ruminococcus	0.040	1.74E-02	1.02E-02	1.70
p_Bacteroidetes|c_Bacteroidia|o_Bacteroidales|f_S24-7|g_	0.041	5.26E-02	8.37E-02	0.63
p_Proteobacteria|c_Deltaproteobacteria|o_Desulfovibrionales|f_Desulfovibrionaceae|g_	0.041	4.57E-02	9.82E-02	0.47
p_Firmicutes|c_Bacilli|o_Lactobacillales|f_Streptococcaceae|g_Streptococcus	0.042	1.36E-04	3.98E-04	0.34
p_Verrucomicrobia|c_Verrucomicrobiae|o_Verrucomicrobiales|f_Verrucomicrobiaceae|g_Akkermansia	0.037	4.59E-04	1.42E-03	0.32
p_Firmicutes|c_Clostridia|o_Clostridiales|f_Lachnospiraceae|g_Coprococcus	0.012	6.63E-03	2.83E-02	0.23
p_TM7|c_TM7-3|o_CW040|f_F16|g_	0.038	1.15E-04	4.93E-04	0.23
p_Firmicutes|c_Bacilli|o_Lactobacillales|f_Lactobacillaceae|g_Lactobacillus	0.012	4.79E-03	2.39E-02	0.20
p_Firmicutes|c_Clostridia|o_Clostridiales|f_Clostridiaceae|g_Candidatus Arthromitus	0.040	0.00E+00	2.28E-04	0.00
p_Firmicutes|c_Clostridia|o_Clostridiales|f_Peptococcaceae|g_rc4-4	0.040	0.00E+00	1.19E-03	0.00

Supplementary Table 3. Functional analysis of gene regulation induced by diet (lard or fish oil for 11 weeks) in WAT. Related to Figure 4 and Supplementary Figure 5.

GO term	Description	Bonferroni
Categories enriched in WAT of mice fed lard		
278	mitotic cell cycle	$4.2x10^{-15}$
51301	cell division	$2.1x10^{-13}$
45321	leukocyte activation	$4.2x10^{-13}$
1775	cell activation	$1.7x10^{-12}$
48285	organelle fission	$5.8x10^{-12}$
35556	intracellular signal transduction	$2.6x10^{-08}$
1817	regulation of cytokine production	$1.4x10^{-07}$
30029	actin filament-based process	$1.8x10^{-06}$
6954	inflammatory response	$5.5x10^{-06}$
9611	response to wounding	$7.6x10^{-06}$
6260	DNA replication	$1.6x10^{-05}$
7059	chromosome segregation	$4.3x10^{-05}$
6793	phosphorus metabolic process	$4.6x10^{-05}$
6796	phosphate-containing compound metabolic process	$4.6x10^{-05}$
6897	endocytosis	$1.9x10^{-04}$
10324	membrane invagination	$1.9x10^{-04}$
6935	chemotaxis	$3.7x10^{-04}$
6259	DNA metabolic process	$3.9x10^{-04}$
6468	protein phosphorylation	$4.1x10^{-04}$
44093	positive regulation of molecular function	$1.3x10^{-03}$
32943	mononuclear cell proliferation	$2.7x10^{-03}$
8219	cell death	$1.2x10^{-02}$
16265	death	$2.1x10^{-02}$
7017	microtubule-based process	$2.2x10^{-02}$
42325	regulation of phosphorylation	$2.5x10^{-02}$
6665	sphingolipid metabolic process	$3.0x10^{-02}$
51174	regulation of phosphorus metabolic process	$4.6x10^{-02}$
Categories enriched in WAT of mice fed fish oil		
6091	generation of precursor metabolites and energy	$4.7x10^{-32}$
22900	electron transport chain	$3.4x10^{-24}$
6412	translation	$1.2x10^{-20}$
55114	oxidation-reduction process	$6.0x10^{-19}$
6732	coenzyme metabolic process	$4.6x10^{-10}$
51186	cofactor metabolic process	$3.1x10^{-08}$
19318	hexose metabolic process	$1.6x10^{-04}$
6631	fatty acid metabolic process	$9.2x10^{-04}$
7005	mitochondrion organization	$1.8x10^{-03}$
9081	branched-chain amino acid metabolic process	$4.1x10^{-02}$

Supplementary Table 4. Functional analysis of gene regulation induced by gut microbiota or interaction between gut microbiota and diet in WAT from mice fed lard or fish oil for 11 weeks. Related to Figure 4 and Supplementary Figure 5.

GO term	Description	Bonferroni
Categories enriched in genes increased by gut microbiota in WAT of mice fed lard		
6396	RNA processing	$1.8x10^{-12}$
6397	mRNA processing	$1.1x10^{-11}$
16071	mRNA metabolic process	$1.1x10^{-09}$
6091	generation of precursor metabolites and energy	$1.4x10^{-04}$
6457	protein folding	$1.6x10^{-04}$
22900	electron transport chain	$2.4x10^{-04}$
7005	mitochondrion organization	$1.5x10^{-03}$
44265	cellular macromolecule catabolic process	$8.3x10^{-03}$
22613	ribonucleoprotein complex biogenesis	$9.6x10^{-03}$
9057	macromolecule catabolic process	$1.2x10^{-02}$
2504	antigen processing and presentation of antigen MHC class II	$1.2x10^{-02}$
Categories enriched in genes reduced by gut microbiota in WAT of mice fed lard		
6091	generation of precursor metabolites and energy	$4.7x10^{-32}$
22900	electron transport chain	$3.4x10^{-24}$
6412	translation	$1.2x10^{-20}$
55114	oxidation-reduction process	$6.0x10^{-19}$
6732	coenzyme metabolic process	$4.6x10^{-10}$
51186	cofactor metabolic process	$3.1x10^{-08}$
19318	hexose metabolic process	$1.6x10^{-04}$
6631	fatty acid metabolic process	$9.2x10^{-04}$
7005	mitochondrion organization	$1.8x10^{-03}$
9081	branched-chain amino acid metabolic process	$4.1x10^{-02}$
Categories enriched in genes induced by gut microbiota in WAT of mice fed fish oil		
6412	translation	$1.9x10^{-42}$
6091	generation of precursor metabolites and energy	$4.4x10^{-38}$
22900	electron transport chain	$5.9x10^{-33}$
55114	oxidation-reduction process	$3.1x10^{-19}$
34660	ncRNA metabolic process	$3.3x10^{-12}$
7005	mitochondrion organization	$5.7x10^{-10}$
6399	tRNA metabolic process	$9.5x10^{-09}$
6396	RNA processing	$3.5x10^{-08}$
51186	cofactor metabolic process	$1.3x10^{-07}$
22613	ribonucleoprotein complex biogenesis	$5.2x10^{-07}$
34613	cellular protein localization	$1.5x10^{-05}$
6732	coenzyme metabolic process	$1.1x10^{-04}$
6413	translational initiation	$2.3x10^{-03}$
8610	lipid biosynthetic process	$3.2x10^{-03}$
Categories enriched in genes reduced by gut microbiota in WAT of mice fed fish oil		
9611	response to wounding	$1.1x10^{-11}$
51056	regulation of GTPase mediated signal transduction	$8.1x10^{-08}$
6954	inflammatory response	$8.7x10^{-06}$
50817	coagulation	$3.9x10^{-04}$
2252	immune effector process	$1.6x10^{-03}$
1775	cell activation	$7.5x10^{-03}$
6897	endocytosis	$1.1x10^{-02}$
Categories enriched in genes regulated by gut microbiota-diet interaction in Q1(Figure 3H)		
0009611	response to wounding	$1.4x10^{-11}$
0002526	acute inflammatory response	$6.9x10^{-08}$
0006955	immune response	$8.5x10^{-05}$
0050817	coagulation	$1.6x10^{-04}$
0009074	aromatic amino acid family catabolic process	$1.8x10^{-03}$
0055114	oxidation-reduction process	$1.9x10^{-03}$
0048584	positive regulation of response to stimulus	$6.2x10^{-03}$
0019439	aromatic compound catabolic process	$1.1x10^{-02}$
Categories enriched in genes regulated by gut microbiota-diet interaction in Q3 (Figure 3H)		
0015031	protein transport	$1.5x10^{-04}$
0051186	cofactor metabolic process	$1.5x10^{-04}$
0051188	cofactor biosynthetic process	$2.4x10^{-03}$
0006511	ubiquitin-dependent protein catabolic process	$1.0x10^{-02}$

Supplementary Experimental procedures

Telemetry, energy expenditure and food consumption measurements

Telemetry devices (G2 E-Mitter, Mini Mitter, Bend, OR) were implanted in mice two weeks before the experiment according to the surgery protocol provided by the manufacturer. Measurements were performed after 2 days or after 5 weeks of high-fat diet. Only one measurement was performed on each mouse. Oxygen consumption (VO2) and carbon dioxide production (VCO2) were recorded every 2 min for 23 h by indirect calorimetry with a SOMEDIC metabolic system (INCA®, Somedic Sales, Hörby, Sweden), which is built around a sealed chamber and ventilated with a constant flow of air. Animals had *ad libitum* access to food and water during the measurements and the temperature was set to 21°C. The indirect calorimeter was combined with the Mini Mitter telemetry system to measure locomotory activity, defined as the number of changes in direction per minute, and body temperature. The data for the first 3 h were discarded to allow for animal acclimatization to the testing conditions. The respiratory quotient (RQ) was calculated per hour as the VCO2/VO2 ratio. In all calculations, W0.75 was used to correct for body size, according to Kleiber's law (Kleiber, 1932). Energy expenditure was calculated by the equation (3.815 + 1.232*RQ)×VO2.

Insulin tolerance tests and measurements of plasma insulin levels

Insulin tolerance tests were performed after 10 weeks of high-fat diet by intraperitoneal injection of insulin (0.75 U/kg body weight in lard vs. fish-oil diet; 0.375 U/kg in wild-type vs. mutant mice), after a 4 h fast. Tail blood was collected at 0, 30, 60, 90 and 120 min after injection. Blood glucose levels were determined using a HemoCue glucose 201+ analyzer (HemoCue, Ängelholm, Sweden). Insulin was measured with a kit from Crystal Chem (Downers Grove, IL) according to the manufacturer’s protocol.

Extraction of Genomic DNA and Profiling of the 16S rRNA gene by Next Generation sequencing

Total genomic DNA was isolated from 60-100 mg of cecal content, 100 mg of WAT and 50 µl of frozen whole blood collected from the cava vein by using a repeated bead beating method as previously described (Salonen et al., 2010).

The cecal microbiota of mice fed lard or fish oil diets for 11 weeks was sequenced by 454 pyrosequencing of the 16 rRNA gene in the V1-V2 region, amplified with the 27F and barcoded 338R primers fused with sequencing adapters (Hamady et al., 2008). Samples were sequenced in a 454 GS FLX system with Titanium chemistry by GATC Biotech (Konstanz, Germany). Each sample was amplified in triplicate in reaction volumes of 25 µl containing 1.5 U of FastStart Taq DNA Polymerase (Roche), 200 nM of each primer and 20 ng of genomic DNA. PCR was carried out under the following conditions: initial denaturation for 3 min at 95°C, followed by 25 cycles of denaturation for 20 sec at 95°C, annealing for 20 sec at 52°C and elongation for 60 sec at 72°C, and a final elongation step for 8 min at 72°C.

The bacterial DNA present in WAT and blood samples of mice fed lard ad fish oil diets for 11 weeks, and the cecal microbiota of the transplanted mice were profiled by sequencing of the V4 region of the 16S rRNA gene on an Illumina MiSeq (llumina RTA v1.17.28; MCS v2.5) using 515F and 806R primers designed for dual indexing (Kozich et al., 2013) and the V2 kit (2x250 bp paired-end reads). Each sample was amplified in duplicate in reaction volumes of 25 µl containing 1x Five Prime Hot Master Mix (5 PRIME GmbH), 200 nM of each primer, 0.4 mg/ml BSA, 5% DMSO and 20 ng (cecal samples) or 100 ng (WAT and blood samples) of genomic DNA. PCR was carried out under the following conditions: initial denaturation for 3 min at 94°C, followed by 25 cycles (cecal samples) or 35 cycles (blood and WAT samples) of denaturation for 45 sec at 94°C, annealing for 60 sec at 52°C and elongation for 90 sec at 72°C, and a final elongation step for 10 min at 72°C.

Replicates were combined, purified with the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Germany) and quantified using the Quant-iT PicoGreen dsDNA kit (Invitrogen, Carlsbad, CA). Purified PCR products were diluted to 20 ng/µl and pooled in equal amounts. The pooled amplicons were purified again using Ampure magnetic purification beads (Agencourt, Danvers, MA) to remove short amplification products.

Raw pyrosequencing data was trimmed of the 454 adapter and barcodes, and filtered to remove sequences that were shorter than 200 nucleotides, longer than 1000 nucleotides, contained primer mismatches, ambiguous bases, uncorrectable barcodes, and homopolymer runs in excess of six bases.

Illumina paired-end reads were merged using PEAR (Zhang et al., 2014) and quality filtered with FASTX (Phred score $\geq$ 20 for 100% of the bases in a sequence). For WAT and blood samples sequences that were shorter than 200 nucleotides or longer than 400 were removed from the analysis.

Quality filtered 454 pyrosequencing and Illumina reads were analyzed with the software package QIIME (Caporaso et al., 2010) (version 1.8.0). Sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold using an open-reference OTU picking approach with UCLUST (Edgar, 2010) against the Greengenes reference database (DeSantis et al., 2006) (13_8 release). All sequences that failed to cluster when tested against the Greengenes database were used as input for picking OTUs *de novo*. 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 plots were visualized using Emperor (Vázquez-Baeza et al., 2013). Chimeric sequences were identified with ChimeraSlayer (Haas et al., 2011) and excluded from all downstream analyses. Similarly, sequences that could not be aligned with PyNAST, singletons and very low abundant sequences (relative abundance <0.005%) were also excluded.

To correct for differences in sequencing depth, the same amount of sequences was randomly sub-sampled for each sample and used for diversity analyses. The Wilcoxon rank-sum test was used to compare the abundance of OTUs at different taxonomical levels; significant differences were identified after correction for false discovery rate. Statistical significance of sample groupings was tested with a multivariate non-parametric analysis of variance (adonis, 999 permutations) (Anderson, 2001). LDA Effect Size (LEfSe) (Segata et al., 2011) was used to identify taxa that discriminated microbiota profiles according to the diet.

qPCR Analysis of 16S rRNA genes

Quantitative PCR (qPCR) was used to enumerate bacterial 16S rRNA gene copies in the genomic DNA extracted from cecal, WAT and blood samples. Samples were quantified in 25 µl reactions containing 1x SYBR Green Master Mix (Thermo Scientific, Waltham, MA), 200 nM of each primer and 5 ng of genomic DNA. Standard curves for quantification consisted in ten-fold serial dilutions in the range of 10^8 to 10^0 copies of target 16S rRNA genes from reference strains, amplified with primers 27F (5'- GTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGCTA CCTTGTTACGAC-3').

The total amount of bacterial DNA in WAT and blood samples was quantified with the universal primers UniF (5'-GTGSTGCAYGGYYGTCGTCA-3') and UniR (5'-ACGTCRTCCMCNCCTTCCTC-3') (Fuller et al., 2007) using the 16S rRNA gene of *Escherichia coli* W3310 as standard. In cecal samples, *Lactobacillus* with LactoF (5'-TGGAAACAGRTGCTAATACCG-3') and LactoR (5'-GTCCATTGTGGAAGATTCCC-3')

(Byun et al., 2004) and *Akkermansia* with AM1 (5'-CAGCACGTGAAGGTGGGGAC3') and AM2 (5'-CCTTGCGGTTGGCTTCAGAT-3'); the 16S rRNA genes of *Lactobacillus reuteri* SD2112 and *Akkermansia muciniphila* were used as standard, respectively. All measurements were performed in duplicates.

Measurement of TLR and NOD activation, SCFAs and LPS

Blood was collected from the *vena cava* of mice after an 4 h fast using a pyrogen-free syringe/needle, and plasma was immediately isolated and frozen in liquid nitrogen. TLR2, TLR4, TLR5, TLR9 and NOD2 agonists were assayed using HEK-Blue reporter cell lines expressing mTLR2, mTLR4, mTLR5, mTLR9 or mNOD2 (InvivoGen, San Diego, CA) using a modified version of the manufacturer's protocol. HEK-Blue cells were grown for two passages with medium supplemented with selective antibiotics provided by the manufacturer, and then passaged once in medium without any antibiotics. The assay was performed when cells were in passage 11-14 by plating 10^5 cells in 96-well plates containing 10% heat-inactivated (2 h at 56 °C) serum. Cells were then incubated with 2% (v/v) serum isolated from *vena cava* of fasted mice for 21 h at 37 °C under an atmosphere of 5% CO2/95% air. Twenty microlitres of the cell culture supernatants were added to 180 µl of the QUANTI-Blue substrate in a 96-well plate. The mixtures were then incubated at 37°C for 1-3 h and secreted embryonic alkaline phosphatase levels were determined using a spectrophotometer at 655 nm.

GC-MS was used for measurement of organic acids in caecal content samples from mice fed fish oil (N=6) and lard (N=6) based diets. 90 –140 mg of frozen caecal contents were transferred to glass tubes (16 × 125 mm) fitted with a screw cap, and a volume of 100 µl of internal standards stock solution (1M [1-^{13}C]acetate and [$^{2}H_6$]propionate, 0.5M [$^{13}C_4$]butyrate, 0.1M [1-$^{13}C_1$]isobutyrate and [1-^{13}C]isovalerate, [1,2-$^{13}C_2$]hexanoate, [^{13}C]lactate and [$^{13}C_4$]succinic acid each in 40 mM) was added to the tubes. Prior to extraction samples were freeze-dried at −50 °C for 3 h, yield 30–40 mg dry weight. Extraction, analysis and

quantification of the measured metabolites are described in detail by Ryan et al. (Ryan et al., 2014). LPS concentration in serum collected from *vena cava* was measured using Endosafe-MCS (Charles River, Lyon, France) based on the limulus amoebocyte lysate (LAL) kinetic chromogenic methodology as previously described (Caesar et al., 2012).

Immunohistochemistry of WAT

Immunohistochemistry on paraffin-embedded epididymal WAT sections (5 mm) was performed as previously described (Caesar et al., 2012). Cells were stained with MAC-2/galectin-3 antibody (CL8942AP, Cedarlane Laboratories, Burlington, ON) or CD45 antibody (ab25386, Abcam, Cambridge, UK) diluted 1:500. Adipocyte size, number of CLS and $CD45^+$ stained area were determined in histological sections of 15-50 mm^2 for each mouse. Adipocyte size and $CD45^+$ stained area were quantified using the software Biopix iQ 2.1.4 (Gothenburg, Sweden).

Microarray expression analysis

RNA was isolated from epididymal WAT using RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). 250 nanograms of total RNA from each sample were used to generate amplified and biotinylated sense-strand cDNA from the entire expressed genome according to the Ambion WT Expression Kit (P/N 4425209 Rev C 09/2009) and Affymetrix GeneChip® WT Terminal Labeling and Hybridization User Manual (P/N 702808 Rev 3, Affymetrix, Santa Clara, CA). The arrays (GeneChip® Mouse Gene 1.0 ST Array) were hybridized for 16 h in a 45°C incubator, rotated at 60 rpm, washed, stained and finally scanned using the GeneChip® Scanner 3000 7G according to the manufacturer's manual (PN 702731 Rev 3, Affymetrix).

The raw data were normalized using the robust multi-array average method (Li and Wong, 2001). Differential expression of microarray data was evaluated by Student's t-test followed by correction for false discovery rate. Analysis of interaction, using colonization status and

diet as independent variables, was evaluated by two-way analysis of interaction followed by correction for false discovery rate. Analysis of enrichment of regulated genes within functional categories [gene ontology categories (GO)] (Ashburner et al., 2000) was performed using the software David (Huang et al., 2008). The results of the enrichment calculation were filtered for GO categories that were significantly enriched ($p<0.05$) after Bonferroni correction. Redundancy within lists of GO terms was reduced by the Revigo software (Supek et al., 2011) with a similarity score set to 0.5. Principle component analysis was performed in MultiExperiment Viewer (MeV).

Quantitative RT-PCR

RNA was isolated from epididymal WAT using RNeasy kit with on-column DNase treatment (Qiagen). cDNA templates were synthesized from total RNA using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Primers used for *Tnfα* were CCAGACCCTCACACTCA (forward) and CACTTGGTGGTTTGCTACGAC (reverse) and primers used for *Ccl2* were AGGTCCCTGTCATGCTTCTGG (forward) and CTGCTGCTGGTGATCCTCTTG (reverse). PCR assays were performed in 25 µl reactions containing 1xSYBR Green Master Mix buffer (Thermo Scientific, Waltham, MA), and 900 nM gene-specific primers. Gene expression data were normalized to the ribosomal protein L32.

Primary cell harvest, cultivation and assays

Primary adipocytes were prepared by isolating preadipocytes from epididymal WAT of SPF mice fed standard chow diet. WAT was treated with collagenase type II (C6885, Sigma Aldrich, St Louis, MO) (10 mg/ml) in Hank's buffer supplemented with 2% BSA for 1 h at 37°C. The cells were run through a 70 µm cell strainer and the stromal vascular fraction was isolated by centrifugation. Erythrocytes were removed by ACK solution treatment.

Adipocytes were grown in ready-made mediums from ZenBio (OM-PM, OM-DM and OM-AM, ZenBio, Durham, NC) according to the manufacturer's protocol.

Primary macrophages were prepared by harvesting bone marrow from femur of SPF mice fed standard chow diet. Erythrocytes were removed by ACK solution treatment. Bone marrow cells were cultured in high-glucose Dulbecco modified Eagle medium supplemented with 10% FCS, 1% HEPES, 1% gentamycin, 0.01% β-mercaptoethanol, and 50 ng/mL M-CSF (416-ML-050, R&D Systems, McKinley Place, MN). Experiments were performed on differentiated macrophages after 10-12 days.

Primary cells were stimulated with 2% (v/v) of serum isolated from *vena cava* of fasted SPF or GF mice fed lard diet for 4 hours. The concentration of secreted CCL2 was determined by ELISA (MJE00, R&D Systems).

Supplementary References

Anderson, M.J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecol *26*, 32-46.

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., et al. (2000). Gene Ontology: tool for the unification of biology. Nat Genet *25*, 25-29.

Byun, R., Nadkarni, M.A., Chhour, K.L., Martin, F.E., Jacques, N.A., and Hunter, N. (2004). Quantitative analysis of diverse Lactobacillus species present in advanced dental caries. Journal of clinical microbiology *42*, 3128-3136.

Caesar, R., Reigstad, C.S., Bäckhed, H.K., Reinhardt, C., Ketonen, M., Östergren Lundén, G., Cani, P.D., and Bäckhed, F. (2012). Gut-derived lipopolysaccharide augments adipose macrophage accumulation but is not essential for impaired glucose or insulin tolerance in mice. Gut *61*, 1701-1707.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. Nat Methods *7*, 335-336.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., and Andersen, G.L. (2006). Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. Applied and Environmental Microbiology *72*, 5069-5072.

Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics *26*, 2460-2461.

Faith, D.P. (1992). Conservation evaluation and phylogenetic diversity. Biological Conservation *61*, 1-10.

Fuller, Z., Louis, P., Mihajlovski, A., Rungapamestry, V., Ratcliffe, B., and Duncan, A.J. (2007). Influence of cabbage processing methods and prebiotic manipulation of colonic microflora on glucosinolate breakdown in man. Br J Nutr *98*, 364-372.

Haas, B., Gevers, D., Earl, A., Feldgarden, M., Ward, D., Giannoukos, G., Ciulla, D., Tabbaa, D., Highlander, S., Sodergren, E., et al. (2011). Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res *21*, 494 - 504.

Hamady, M., Walker, J.J., Harris, J.K., Gold, N.J., and Knight, R. (2008). Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. Nat Meth *5*, 235-237.

Huang, D.W., Sherman, B.T., and Lempicki, R.A. (2008). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protocols *4*, 44-57.

Kleiber, M. (1932). Body size and metabolism. ENE *1*, E9.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol *79*, 5112-5120.

Li, C., and Wong, W.H. (2001). Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. Proceedings of the National Academy of Sciences *98*, 31-36.

Lozupone, C., and Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol *71*, 8228 - 8235.

Price, M.N., Dehal, P.S., and Arkin, A.P. (2010). FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. PLoS One *5*, e9490.

Ryan, K.K., Tremaroli, V., Clemmensen, C., Kovatcheva-Datchary, P., Myronovych, A., Karns, R., Wilson-Perez, H.E., Sandoval, D.A., Kohli, R., Backhed, F., et al. (2014). FXR is a molecular target for the effects of vertical sleeve gastrectomy. Nature *509*, 183-188.

Salonen, A., Nikkila, J., Jalanka-Tuovinen, J., Immonen, O., Rajilic-Stojanovic, M., Kekkonen, R.A., Palva, A., and de Vos, W.M. (2010). Comparative analysis of fecal DNA extraction methods with phylogenetic microarray: effective recovery of bacterial and archaeal DNA using mechanical cell lysis. J Microbiol Methods *81*, 127-134.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W., and Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. Genome Biology *12*, R60.
Supek, F., Bošnjak, M., Škunca, N., and Šmuc, T. (2011). REVIGO Summarizes and Visualizes Long Lists of Gene Ontology Terms. PLoS One *6*, e21800.
Wang, Q., Garrity, G., Tiedje, J., and Cole, J. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol *73*, 5261 - 5267.
Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A., and Knight, R. (2013). EMPeror: a tool for visualizing high-throughput microbial community data. GigaScience *2*, 16-16.
Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2014). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics *30*, 614-620.