Supplementary methods

Quantification of aerobic and anaerobic bacteria in the ileum contents

Upon harvesting, the ileum contents was manipulated in a specific anaerobic chamber. It was serially diluted ten-fold in Wilkins–Chalgren broth (WC, Oxoid, Basingstoke, UK). The bacteria present in the dilutions were cultivated on non-selective medium agar plates containing defibrinated sheep blood (BioMérieux) [1]. The plates were incubated in aerobic or anaerobic conditions for seven days at 37°C. The cultures were then analyzed according to the criteria in Bergey's manual of Systematic Bacteriology [2].

Chip-MS technology

Peptide mixtures were analyzed on a nanoHPLC-Chip-MS/MS system consisting of a nanoHPLC-microfluidic chip cube (Agilent) interfaced to an Amazon ETD mass spectrometer (Bruker Daltonics). For peptide profiling and sequencing, the Amazon ETD mass spectrometer was operated in full scan MS. Scan MS were acquired on the 300–1500 m/z range in the enhanced resolution mode.

A set of target peptides was obtained after data analysis and an inclusion list was built to fragment preferentially relevant peptides. MS/MS spectra were acquired by using the scheduled precursor list but the mass spectrometer was allowed to acquire spectra in empty time intervals. The most intense ions per scan were selected for CID fragmentation and dynamic exclusion was employed within 12 s to prevent repetitive selection of the same peptide. The fragmentation amplitude was set to 0.6 V.

Proteomics

Each ileum contents sample was incubated with 0.5µg of trypsin/tube overnight at 37°C. Peptides were extracted, dissolved in 10% acetonitrile 0.05% TFA and injected into a nano-LC chip-cube Agilent system (chip C18, 150mm) coupled to a mass spectrometer ESI-IonTrap-MS (Bruker Amazon ETD). After elution with a gradient of acetonitrile in water (0.1% formic acid) the mass spectrometer analyzed the eluent in simple mode with enhanced resolution to generate .d files.

Protein analysis

For protein concentration determination the ileum contents were homogenized in 0.5% CHAPS to lyse bacterial membranes. The protein concentration was quantified by using the Bradford method (OD=595nm).

Chip-MS data analysis

The data files (.d files in folder) generated with the Chip-MS technology were loaded to Progenesis LC-MS version 4.0 (Nonlinear Dynamics, UK). Automatic alignment was performed using an algorithm that corrects retention time shifts. After checking manually all runs, peak picking was performed using automatic parameters for sensitivity and retention time window. To lower the noise, the sensitivity was set to the lowest level. Normalization to all features was also processed. To select features of interest, statistical filters were set and only features that matched all filters were kept. The filters used were: p-value < 5%, max fold change > 1.5, power > 80%, max coefficient of variation (CV) within condition < 20% and q-value<1%. A .csv file was then exported from Progenesis and loaded to R 2.13.2. Bioinformatics analyses were performed on LC-MS Progenesis and R softwares to identify the peptides. Individuals factor maps (from Principal Component Analysis) were used to identify peptides differentially present between individuals. Biological variability between and within groups was analyzed to validate that statistical analysis of differences between groups was strictly related to biological features.

Peptide identification was performed by MS/MS only on the differentially identified peptides. The data files were analysed by bioinformatics using MASCOT software to perform Blast searches of the SwissProt, IPI-Mouse and NR databases.

Hepatic triglyceride measurement

Liver (50mg) was ground in PBS (Sigma Aldrich, St. Louis, MO) and triglycerides were extracted from 50 µL of lysate by adding 450 µl of absolute ethanol. The concentrations of free glycerol and total glycerol were determined by using the Free Glycerol Reagent and Triglyceride Reagent, respectively (Sigma Aldrich, St. Louis, MO). The free glycerol concentration was subtracted from the total glycerol concentration to calculate the triglyceride concentration.

Endotoxin Quantification

A kinetic chromogenic Limulus amoebocyte lysate assay kit (Charles River) was used to quantify endotoxin in murine serum samples. Optimization of the commercial assay and sample storage conditions were evaluated and validated prior to the study. Samples were diluted 1:200 in endotoxin/pyrogen-free water (PFW) and heat treated at 60°C for 45 min, allowed to cool. Control Standard Endotoxin (CSE) was made up to 1000 EU/ml (1 EU = 100 pg). The stock CSE was used to make up a 6 point standard curve of 0.25, 0.062, 0.016, 0.0039, 0.00097, 0.00024 EU/ml. The negative control was 100 µl of PFW added to 2 wells of a pyrogen-free microtiter plate; 100 µl of each standard were added in duplicate, 100 µl of

each sample were added into wells in quadruplicate; 10 µl of 0.5 EU/ml were added to 1 of the 4 sample wells to give 0.05 EU/ml positive controls; and 100 µl of LAL reagent at standardised concentration were added to every well containing sample or standard. The microtiter plate was placed in a temperature-controlled ELISA plate reader at 37°C and 405 nm readings recorded every 60 s. The reading stopped automatically when the negative controls (PFW) reacted to generate an absorbance increase of 0.1 over background readings. The reaction time values from each well were compared to the reaction times of the standard curve and, after correcting for the dilution factor, produced mean EU/ml values (the mean of the triplicate wells for each sample).

References

1 Lakhssassi N, Elhajoui N, Lodter JP, Pineill JL, Sixou M. Antimicrobial susceptibility variation of 50 anaerobic periopathogens in aggressive periodontitis: an interindividual variability study. Oral Microbiol Immunol 2005;**20**:244-52.

2 Naser SM, Hagen KE, Vancanneyt M, Cleenwerck I, Swings J, Tompkins TA. Lactobacillus suntoryeus Cachat and Priest 2005 is a later synonym of Lactobacillus helveticus (Orla-Jensen 1919) Bergey et al. 1925 (Approved Lists 1980). Int J Syst Evol Microbiol 2006;**56**:355-60.

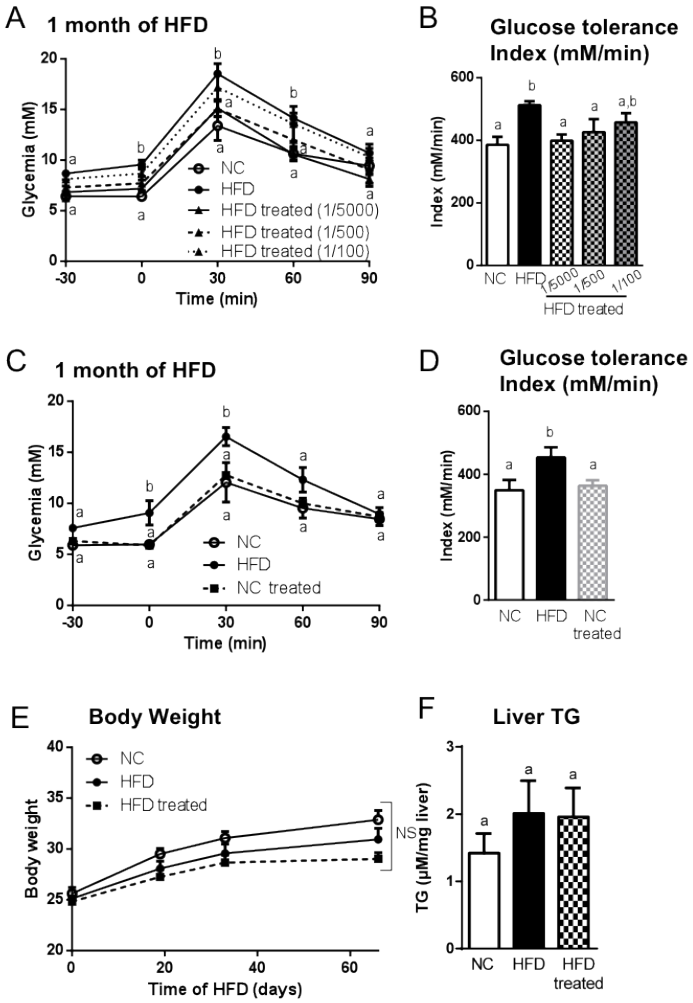
Primers for Fluidigm and qPCR

Primer	Forward Sequence	Reverse Sequence
CCL2	AGCAGCAGGTGTCCCAAA	TTCTTGGGGTCAGCACAGAC
CSF1	CCACATGATTGGGAATGGACAC	GCAATCTGGCATGAAGTCTCC
GATA3	CCTACCGGGTTCGGATGTAA	CCGCAGTTCACACACTCC
IFNgamma	GGCACAGTCATTGAAAGCCTA	GCCAGTTCCTCCAGATATCCA
IL1beta	TGGCAACTGTTCCTGAACTCA	GGGTCCGTCAACTTCAAAGAAC
IL4	ACGGAGATGGATGTGCCAAA	GCACCTTGGAAGCCCTACA
IL10	AAAGGACCAGCTGGACAACA	TAAGGCTTGGCAACCCAAGTA
leptin	AGACCATTGTCACCAGGATCA	ATGAAGTCCAAGCCAGTGAC
PAI1	CATTGAATGGCTGCGCTTCC	CGCTCTCGTGGATGGACAC
T bet	CAAGTTCAACCAGCACCAGAC	CCACGGTGAAGGACAGGAA
CD11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTCA
F4/80	TGACAACCAGACGGCTTGTG	GCAGGCGAGGAAAAGATAGTGT
TCRβ	CACAATCCTCGCAACCACTTC	GTGAGCCCTCTGGCCACTT
iNOS	CAGCTGGGCTGTACAAACCTT	CATTGGAAGTGAAGCGTTTCG
TNFα	TCCCAGGTTCTCTTCAAGGGA	GGTGAGGAGCACGTAGTCGG
IL6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
IL17F	CCCATGGGATTACAACATCACTC	CACTGGGCCTCAGCGATC
IL17A	CTCCAGAAGGCCCTCAGACTAC	GGGTCTTCATTGCGGTGG
Foxp3	CCCATCCCCAGGAGTCTTG	ACCATGACTAGGGGCACTGTA
RPL19	GAAGGTCAAAGGGAATGTGTTCA	CCTTGTCTGCCTTCAGCTTGT
Occludin	ATGTCCGGCCGATGCTCTC	TTTGGCTGCTCTTGGGTCTGTAT
ZO-1	ACCCGAAACTGATGCTGTGGATAG	AAATGGCCGGGCAGAACTTGTGTA
Claudin 2	CCCAGGCCATGATGGTGA	TCATGCCCACCACAGAGATAAT
Claudin 7	CCTGGTGTTGGGCTTCTTAGC	CCCACAGCGTGTGCACTTC
JAM-A	CTGATCTTTGACCCCGTGAC	ACCAGACGCCAAAAATCAAG
Mucin-1	CCCTATGAGGAGGTTTCGGC	GTGGGGTGACTTGCTCCTAC

Mucin-2	GGGTAGGGTCACCTCCATCT	TCAACCCTTCCCACCATCCT
Mucin-13	TCAACCCTTCCCACCATCCT	CCACTCATTAACTCTGACCCCTT
Angiogenin 4	TTGGCTTGGCATCATAGT	CCAGCTTTGGAATCACTG
Pla2g2a	CTATGCCTTCTATGGATGCCAC	CAGCCGTTTCTGACAGGAGT
Reg3β	TGGGAATGGAGTAACAATG	GGCAACTTCACCTCACAT
Reg3γ	CCATCTTCACGTAGCAGC	CAAGATGTCCTGAGGGC
Lyz1	GAGACCGAAGCACCGACTATG	CGGTTTTGACATTGTGTTCGC
Defa2	TGCTCTTCATGAAAAATCGTCGAG	GCCTCAGAGCTGATGGTTGT
Defa3	GACCAGGCTGTGTCTGTCTC	ACCAGATCTCTCAACGATTCCT
Defa6	GTCTCCACTTGCAGCCTTTTC	GGCATGAGCCTCCATAGCTC
Defb1	TCCTGGTGATGATATTTTTCTTTTCT	TGTTCTTCGTCCCAAGACTTGTGA

Supplementary Figures

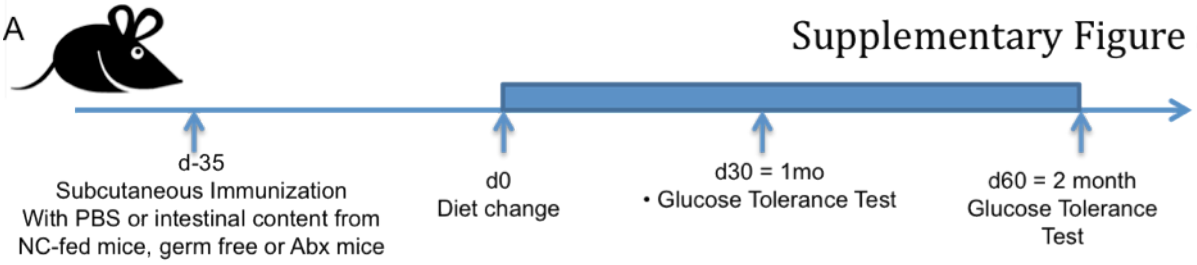
Supplementary Figure S1

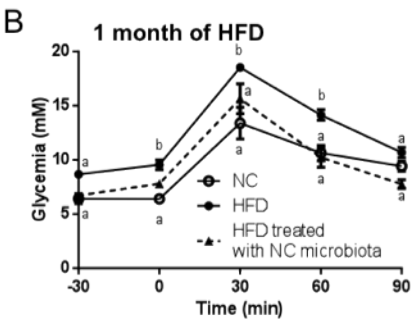


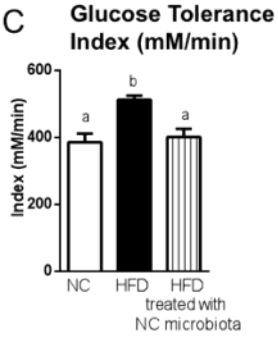
Supplementary Figure S1: Immunization with ileum contents protects against HFD-induced insulin resistance and dysglycemia.

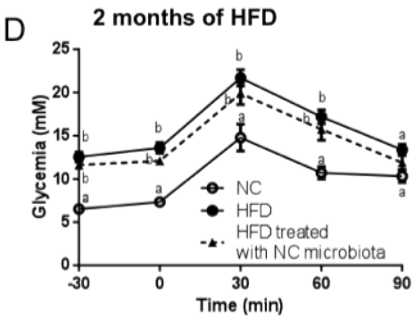
(A,B) Mice were injected s.c. with PBS (•,o) or with various doses of ileum contents from mice fed a HFD (treated). Thirty-five days later, the mice were fed either a HFD or NC. After one month of HFD, an intraperitoneal GTT (A) was performed and the glucose tolerance index (B) was calculated. (C,D) Mice were injected s.c. with PBS (•,o) or with ileum contents from mice fed a HFD (treated). Thirty-five days later, the mice injected with PBS were fed either a HFD or NC and mice injected with ileum content were fed a NC (NC treated). After one month of HFD, an intraperitoneal GTT (C) was performed and the glucose tolerance index (D) was calculated. (E-F) Body weight (E), and liver tryglicerides (F) were measured in mice injected with PBS ((NC; (o), HFD (•)), or injected with ileum microbiota from mice fed a HFD diet with dilution of 1/5000 (HFD treated (■)). (A-F) Data are means ± SEM; n=5 mice per group. Data with similar superscript letters (a,b) indicate data non-statistically different between each other at a probability threshold $p>0.05$.

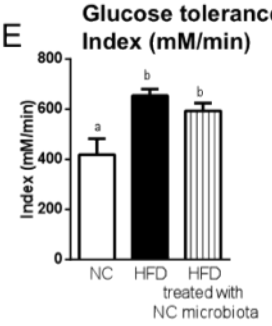
Supplementary Figure S2

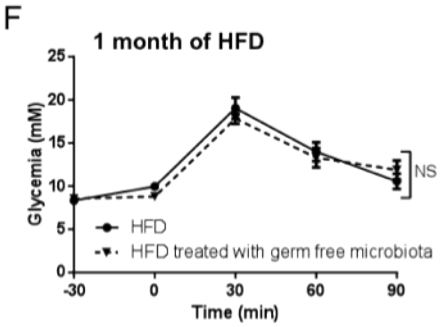


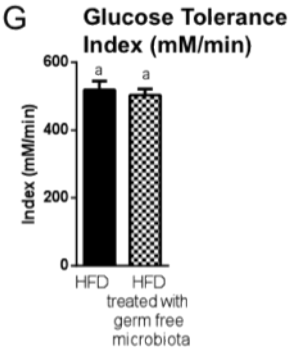


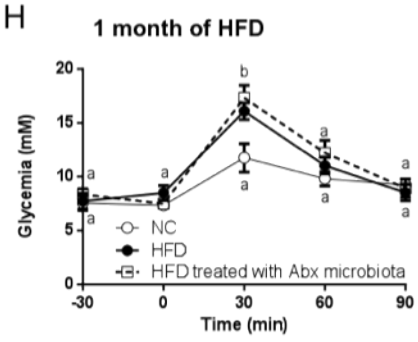


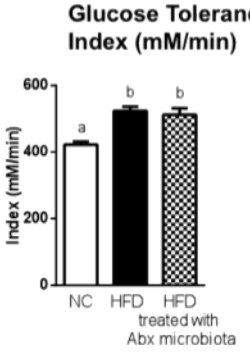






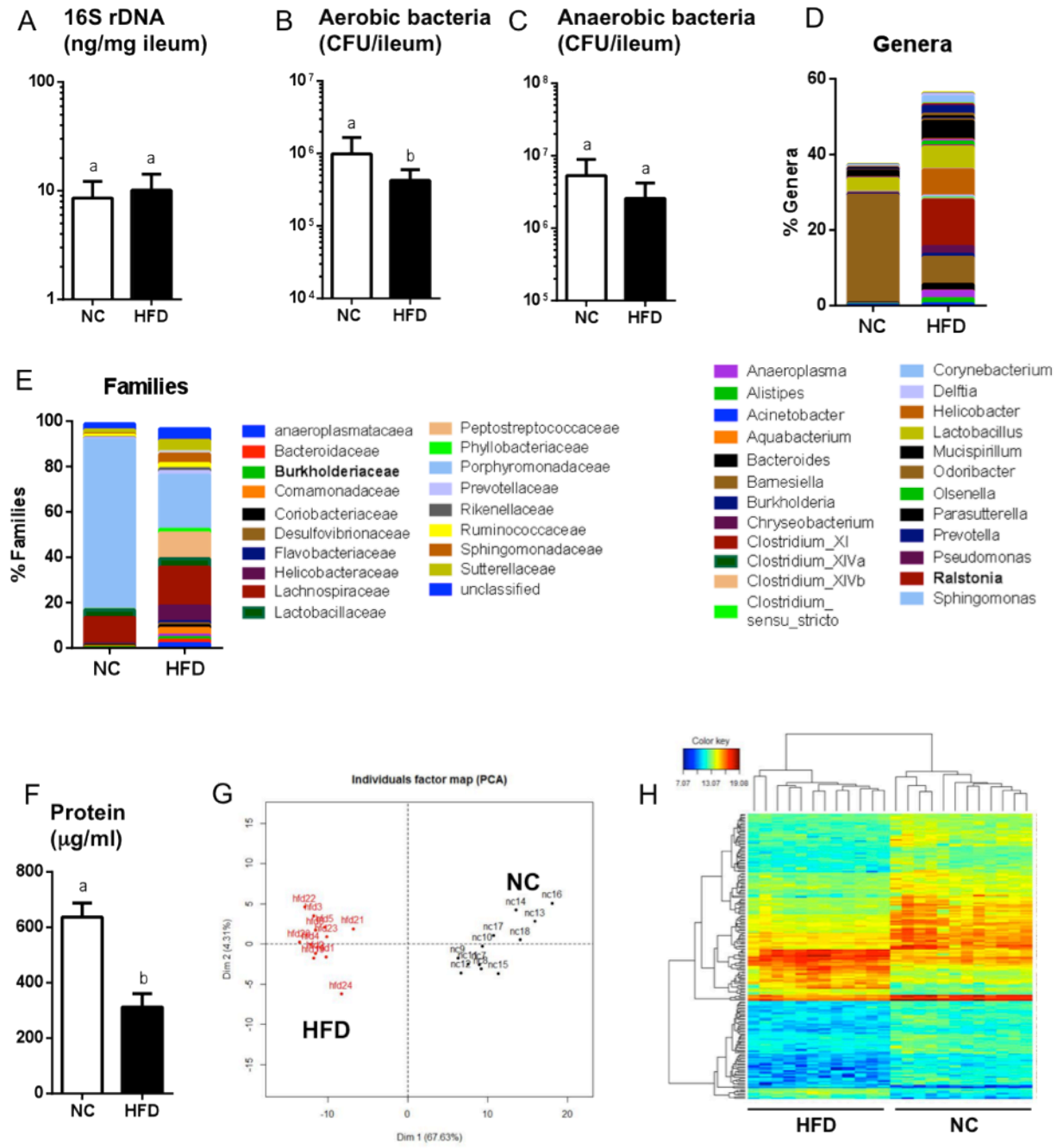






Supplementary Figure S2: Bacteria in the ileum contents used for immunization are required for protection against HFD-induced glucose intolerance.

(A,E) Mice were injected s. c. with PBS (NC (ο), HFD (•)), or with ileum contents from mice fed a NC diet (treated with NC microbiota (▲)) as illustrated in (A). After one month (B,C) or two months (D,E) of HFD, an intraperitoneal GTT (B,D) was performed and the glucose tolerance index (C,E) was calculated. (n = 8 mice per group). (F,G) Mice were injected s. c. with PBS (•) or with germ-free ileum contents (treated with germ free microbiota (▼)) as illustrated in (A). Thirty-five days later, the mice were fed a HFD and one month later an intraperitoneal GTT (F) was performed and the glucose tolerance index (G) was calculated. (n = 10 mice per group). (H,I) Mice were injected s. c. with either PBS (•,ο) or with ileum contents from antibiotic-treated mice fed the HFD (treated with Abx microbiota (□)) as illustrated in (A). Thirty-five days later, the mice were fed a HFD. One month later, an intraperitoneal GTT (H) was performed and the glucose tolerance index (I) was calculated. (n = 6 mice per group). All data are means ± SEM. Data with similar superscript letters (a,b) indicate data non-statistically different between each other at a probability threshold $p>0.05$.



Supplementary Figure S3: The microbiome in the ileum of mice fed NC differs from that of mice fed the HFD.

Analysis of the ileum contents of eight-week-old mice fed a normal diet (NC) or a HFD (HFD). (A) 16S rDNA concentration (ng/mg, n=6 mice/group). (B) Numbers of aerobic and (C) anaerobic bacteria (n = 4 mice/group). (D,E) Frequency of bacterial genera and families

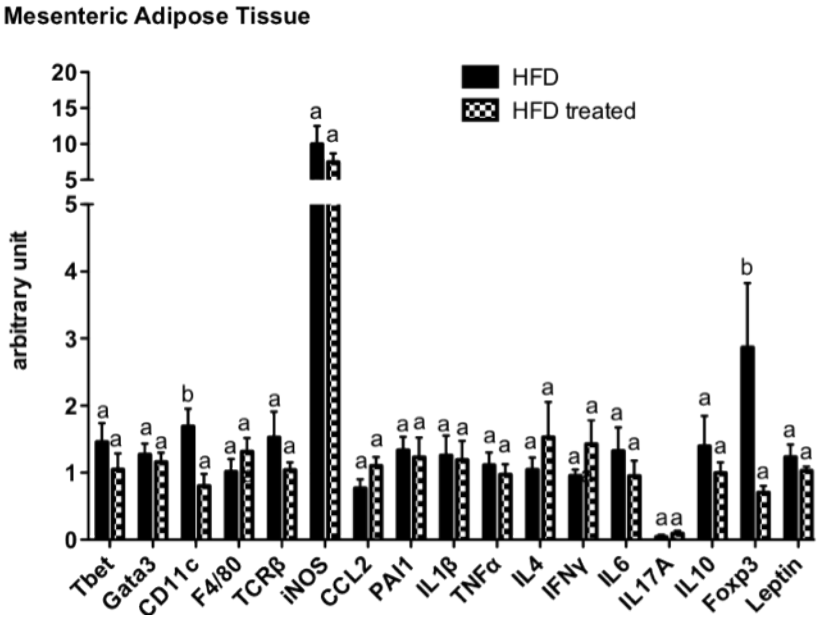
obtained from ileum contents 16s rRNA-DNA gene sequencing analysis (n=5 mice/group). (F) Protein concentration (n=6 mice/group). (G) Principal component analysis of the peptides that differ more than a two-fold between NC-fed and HFD-fed mice (q>0.05). Of the 264,114 peptides analyzed, 35,798 changed by more than two-fold in HFD-fed mice when compared to controls and 197 had a "q" value <0.05. The individual factor map (from PCA) clearly showed that mouse intestinal contents could be perfectly separated based on their differential peptides and explained 67% of the variance. (H) Heatmap of the peptides in which the columns represent the extracts from different HFD-fed and NC-fed mice and the rows indicate different peptides. Pearson's tree analyses of extracts and peptides are shown. The vast majority of the 197 peptides could not be identified in the databases. A heatmap generated from the data on the 197 peptides shows that some peptides were either under- or over-represented in the extracts from NC or HFD mice. All data are means ± SEM. Data with similar superscript letters (a,b) indicate data non-statistically different between each other at a probability threshold p>0.05.

Supplementary Table S1: Intestinal (ileum) bacterial proteins differentially present between NC and HFD-fed mice

MS/MS spectra were acquired for peptide sequencing and subsequent protein identification. Comparisons with protein databases identified six known bacteria

UVRC_FINM2	Sp:B0S0L4, UVRC_FINM2UvrABC system protein C OS=Finegoldia magna (ATCC 29328) GN=uvrC PE=3 SV=1
AROC_LEPBJ	Sp:Q04W40 AROC_LEPBJ Chorismate synthase OS=Leptospira borgpetersenii serovar Hardjo-bovis (strain JB197) GN=aroC PE=3 SV=1
GUAC_LACCB	Sp: B3WCK9, GUAC_LACCB GMP reductase OS=Lactobacillus casei (strain BL23) GN=GuaC PE=3 SV=1
HSLU_NITWN	Sp: Q3SWE4, HSLU_NITWN ATP-dependent protease ATPase subunit HslU OS=Nitrobacter winogradskyi (strain Nb-255, ATCC25391) GN=hslU PE=3 SV=1
CHMP3_DICDI; CAPSD_FDVS	Sp: Q54P63, CHMP3_DICDI Charged multivesicular body protein 3 OS=Dictyostelium discoideum GN=chmp3 PE=3 SV=1
SYR_LACAC	Sp: Q5FIQ9, SYR_LACAC Arginine-tRNA ligase OS= Lactobacillus acidophilus NCFM GN=argS PE=3 SV=1
HAT1_MOUSE	Sp: Q8BY71, HAT1_MOUSE Histone acetyltransferase type B catalytic subunit OS=Mus musculus GN=Hat1 PE=2 SV=1

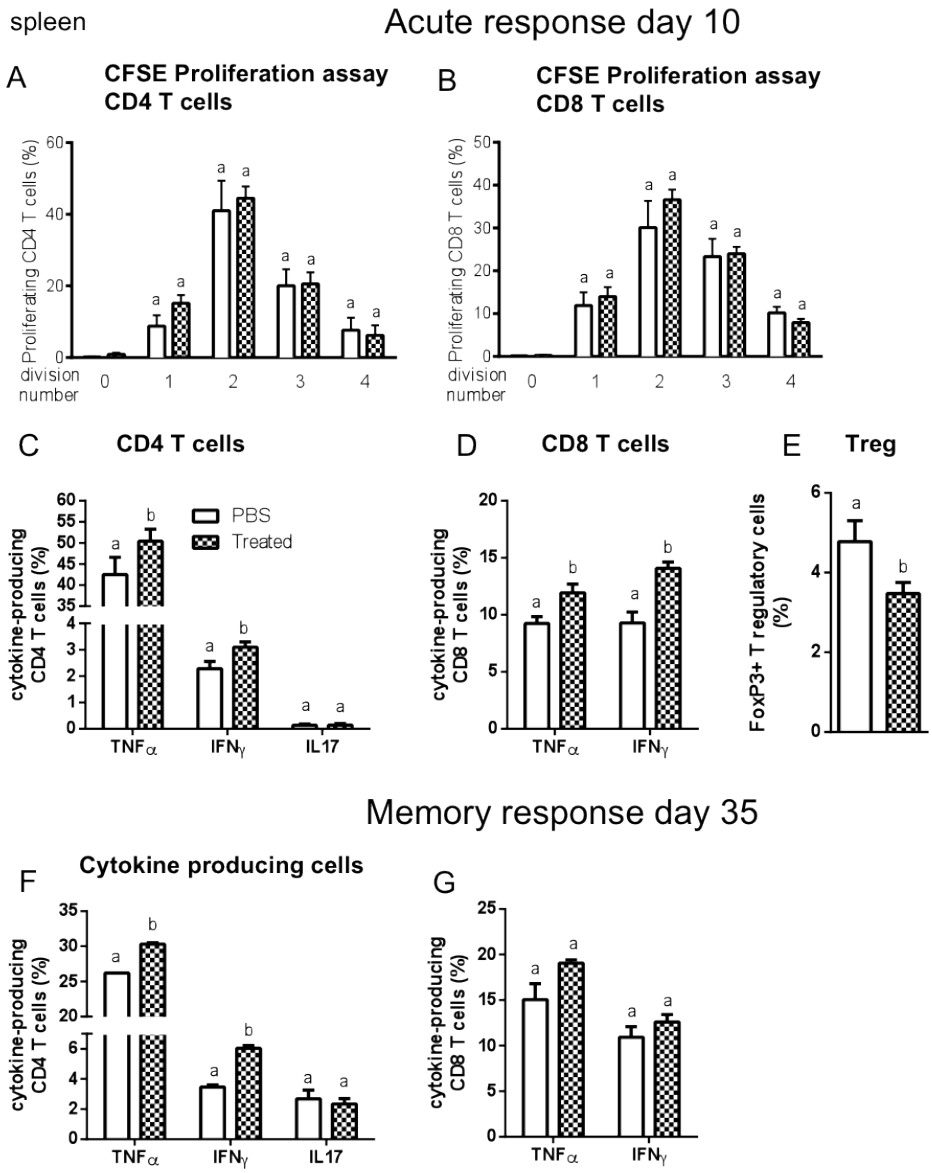
Supplementary Figure S4



Supplementary Figure S4: Immunization with ileum contents decreases adipose tissue inflammation.

C57Bl/6 mice were injected s.c with 200μl of PBS or with ileum contents from mice fed a HFD. Thirty-five days later, the mice were fed a HFD. After one month of HFD, proinflammatory and anti-inflammatory cytokine mRNAs were quantified from mesenteric adipose tissue from the mice injected with PBS (HFD) or from the mice injected with ileum contents (HFD treated). Relative RNA quantity was determined by the 2-ΔCt method using RPL19 as reference transcript. All data are means ± SEM; n=5-6 mice per group. Data with similar superscript letters (a,b) indicate data non-statistically different between each other at a probability threshold $p>0.05$.

Supplementary Figure S5

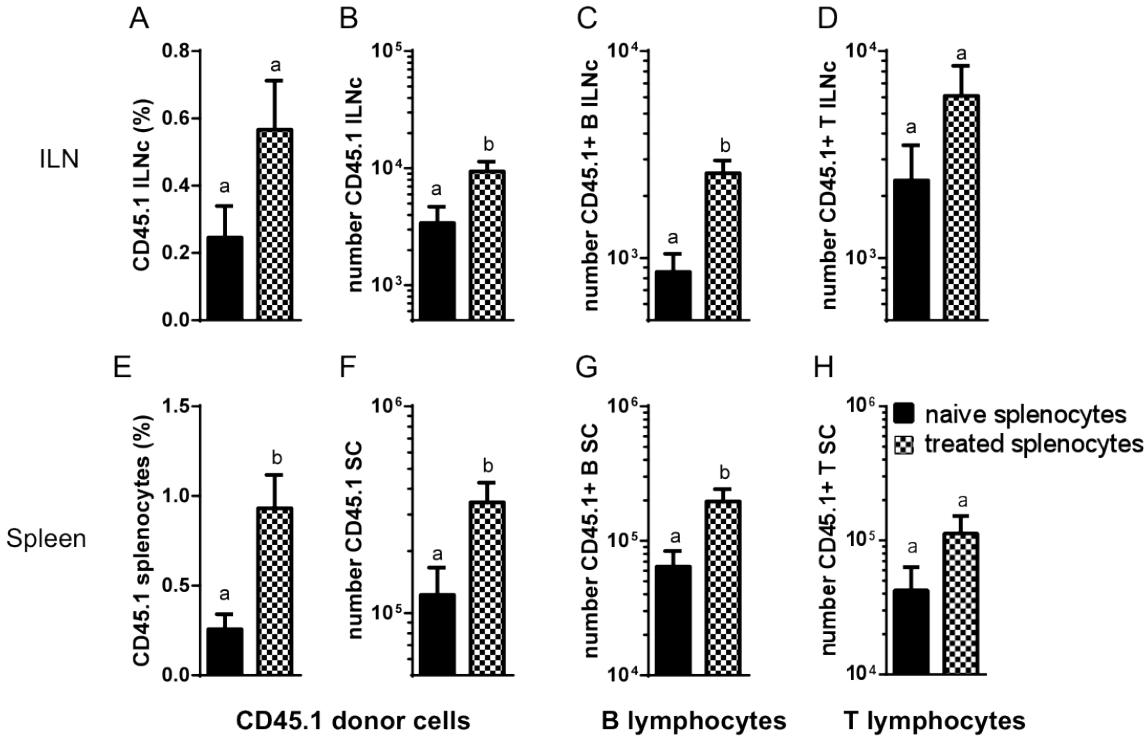


Supplementary Figure S5: Immunization with ileum contents increases the proportion of responsive T cells in the spleen.

Mice were injected s.c. with PBS or HFD microbiota (treated). Ten days later, the splenocytes were isolated and (A-B) labeled with CFSE to assess T cell proliferation during stimulation with plate-bound anti-CD3/CD28 for 3 days. The proportion of CD4 T cells (A) and CD8 T cells (B) at each round of division was determined. (C-E) The functionality of T cells in the isolated splenocyte populations was measured by ex vivo stimulation. The proportion of CD4 T cells (C) and CD8 T cells (D) in the splenocyte populations that produce IFNγ, TNFα, or IL17 in response to stimulation. (E) The proportion of Foxp3+ T regulatory cells in the splenocyte populations. (F-G) Splenocytes were isolated 35 days after immunization, and the proportions of cytokine (IFNγ, TNFα, IL17)-producing CD4 (F) and

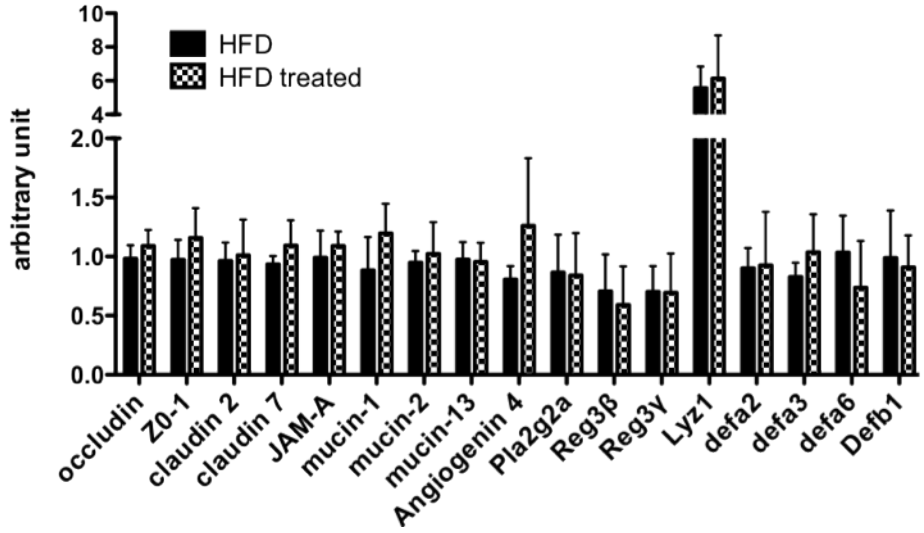
CD8 (G) T cells was quantified as in parts c and d (n= 6 mice per group). Data with similar superscript letters (a,b) indicate data non-statistically different between each other at a probability threshold p>0.05.

Supplementary Figure S6



Supplementary Figure S6: CD45.1 donor cells outcome following adoptive transfer.
CD45.1 mice were injected s.c. with either PBS (naïve) or with the ileum contents from mice fed the HFD (treated). Their splenocytes were isolated and were transferred into naïve CD45.2 recipients (3 x 10^7 splenocytes per animal). The number of CD45.1 donor cells in the lymph nodes (ILN; A-D) and spleens (E-H) of the recipients. The graphs show the proportion of total cells (A,E) or the absolute number (B,F) of CD45.1 (donor) B lymphocytes (C,G) or T lymphocytes (D,H) in each tissue. Data are means ± SEM. Data with similar superscript letters (a,b) indicate data non-statistically different between each other at a probability threshold p>0.05.

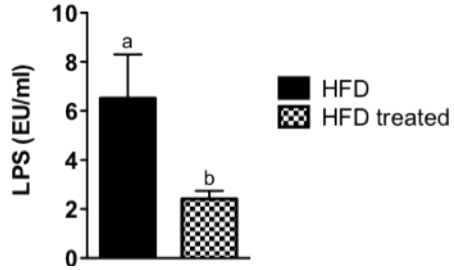
Supplementary Figure S7



Supplementary Figure S7: Ileum mRNA levels for epithelial junctions and antimicrobial peptides were not affected by the immunization procedure.

Mice were injected s.c. with PBS (HFD) or with ileum contents from HFD (HFD treated). Thirty-five days later, the mice were fed a HFD. After 10 days of HFD, epithelial junctions and antimicrobial peptides mRNAs were quantified from ileum mucosa from the mice injected with PBS (HFD) or from the mice injected with ileum contents (HFD treated). Data are means ± SEM, n=6 mice per group. Data with similar superscript letters (a,b) indicate data non-statistically different between each other at a probability threshold $p>0.05$.

Supplementary Figure S8



Supplementary Figure S8: Endotoxin concentration is decreased after immunization.
Mice were injected s.c. with PBS (HFD) or with ileum contents from HFD (HFD treated). Thirty-five days later, the mice were fed a HFD. After 30 days of HFD, plasmatic LPS concentration were quantified. Data are means ± SEM. Data with similar superscript letters (a,b) indicate data non-statistically different between each other at a probability threshold $p>0.05$.