SUPPLEMENTARY INFORMATION

Dietary restriction

All mice were fed the same diet, so same quantities of diet represent identical amounts of energy content (2.9kcal/g). The food intake of leukemic/BaF mice was mimicked by restricting the amount of food given to healthy sham-injected (DR) mice in the same proportions as the quantity consumed by the BaF mice. For instance, on day 12, BaF mice consumed 74% of their basal energy intake (measured over the last 3 days before injection). Therefore, on day 12, DR mice received 74% of their basal energy intake (in two equal portions, at 7AM and 6PM) (Figure S2).

Morbidity score

The morbidity score was determined in a blinded fashion by an experienced investigator looking at the following features: mobility, coat and eye aspect, and posture. Score was attributed based on the classification table (Table 1) for assessing pain and distress in mice, published by Burkholder and colleagues (Burkholder et al, 2012).

Pain and distress assessment	Examples
1. No indication of pain and Distress	Normal; well groomed; alert; active; good condition; asleep or calm; normal appetite; BCS = 3,4, or 5
2. Mild or anticipated pain and distress	Not well groomed; awkward gait; slightly hunched; looks at wound or pulls away when area touched; mildly agitated; BCS = 2
3. Moderate pain and distress	Rough hair coat; dirty incision; squinted eyes; moves slowly; walks hunched and/or slowly; depressed or moderately agitated; slight dehydration; pruritic; restless; uncomfortable; not eating or drinking; BCS = 2–.
4. Severe pain and distress	Very rough hair coat; eyes sunken (severe dehydration); slow to move or nonresponsive when coaxed; hunched; large abdominal mass; dyspnea; self-mutilating; violent reaction to stimuli or when approached; BCS = 1

Table 1. Assessing Pain and Distress in Mice. BCS: Body Condition Scoring. Table published by Burkholder et al, 2012.

qPCR procedure for tissue mRNA

Samples. ileal, liver and muscle tissues were macro-dissected. Starting from the caecum, the three first cm of the ileum were removed and the next two cm were cleaned from adipose tissue, cut and

frozen in liquid nitrogen within two minutes. Liver was clamped in liquid nitrogen within one minute and muscle were withdrawn and frozen in liquid nitrogen within ten minutes.
Samples were stored at -80°C for 10 months (ileum), 10 days (liver), 10 months (tibialis), 12 days (gastrocnemius, synbiotic experiment) and 1 month (gastrocnemius, C26 model).

Nucleic acid extraction. Total RNA was isolated from tissues using the TriPure isolation reagent kit (Roche Diagnostics, Germany) according to manufacturer's instructions. Homogenization of the tissues in Tripure was done using a Tissue Lyzer (Qiagen, Germany). No DNAse treatment was performed. RNA concentration was determined and purity (A_{260}/A_{280}) was checked using a NanoDrop 2000 (Thermo Fisher Scientific, USA) or alternatively, a spectrophotometer (SpectraMax 190 Microplate Reader, Molecular Devices, USA). A_{260}/A_{280} values were between 1.6 and 2.1. For ileum, RNA integrity of a subset of 11 samples was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). All RIN values were above 7.2 (9.3 ± 0.2, mean & sem) and no difference between groups were observed. RIN was not checked for the liver and muscle of these experiments because comparable analyses performed on the same tissues in the similar conditions at the same period provided RIN values all above 9.

Reverse transcription. For each sample, 3µl of a RNA solution at 1µg/3µl was heated at 70°C for 10 min. Subsequently, 17µl of RT mix (final concentration: oligo$(dT)_{15}$ primer 0.5µg/20µl, dNTP 1mM, MgCl2 5mM, AMV reverse transcriptase 15U/20µl, RNAsin Ribonuclease 25U/20µl, RT buffer; Reverse Transcription System, Promega, USA) was added and the resulting solution was heated at 42°C for 15 min followed by a step at 95°C for 5 min. cDNA were stored at -20°C.

qPCR target information and qPCR oligonucleotides.
Gene symbol, primer sequences, and amplicon length that were targeted, are indicated in Table S1. Primers were previously published or designed using Primer3 (Untergasser et al, 2012) and checked for specificity using Primer-BLAST (Ye et al, 2012). For primers that we designed, if they are intron-skipping and which splicing variants are targeted are indicated in the Table S1. Primers were synthesized with RP-Cartridge-Gold purification (Eurogentec, Belgium).

qPCR protocole. Real-time polymerase chain reaction (PCR) was performed with a StepOnePlus Real-Time PCR System (Applied Biosystems, The Netherlands) using SYBR Green (MESA FAST qPCR MasterMix Plus for SYBR® Assay, Eurogentec, Belgium) for detection. All samples were run in duplicate in a single 96-well reaction plate. Final concentrations were as follow: cDNA 2µl/25µl, primers 300nM, and SyberGreen mix 1X (MeteorTaq DNA polymerase, dNTP, RT buffer, $MgCl_2$ 4mM,

SYBR® Green I, ROX passive reference and stabilizers, as provided by the manufacturer). Thermocycling conditions were as follow: initiation step at 95°C 10 min; cycling stage at 95°C 3 s, 60°C 26 s, 72°C 10 s, 40 cycles; melt curve stage at 95°C 15 s, 65°C 20 s, increment of 0.5°C every 15 s until reaching 95°C.

qPCR validation. The purity of the amplified product was verified by analyzing the melt curve performed at the end of amplification. Regarding the non-template control, a qPCR is considered valid if the Cq of the non-template control is at least 6 units higher than the Cq of the templates or, for targets with low expression levels, if the melt curve of the non-template control was different from the templates. Primers were validated by performing a calibration curve using serial dilution of cDNA in the tissue of interest. Primers were considered as acceptable when efficiency reaches values between 90% and 110%.

Data analysis. Threshold was manually adjusted to reach the linear range of the log-fluorescent curves and Cq were determined using the StepOnePlus PCR Software (Version 2.1, Applied Biosystems, The Netherlands). The ribosomal protein L19 (RPL19) gene was chosen as a reference gene based on de Jonge et al (de Jonge et al, 2007). Data were analyzed in Excel according to the $2^{-\Delta\Delta CT}$ method (Bookout et al, 2006). At least 80% of the duplicates show a variation lower than 0.5 Cq units. Outliers were determined either by visual inspection of the log-fluorescent curves or application of a statistical test (Grubs outlier test) on the normalized values.

Note. RNA specificity is assured through the use of oligo$(dT)_{15}$ primers for the reverse transcription and the use of intron-skipping qPCR primers (when the target allows it).

qPCR procedure for quantification of bacteria

Samples. Caecal contents were withdrawn, frozen in liquid nitrogen within five minutes, and stored at -80°C for 14 days (synbiotic treatment), 33 months (C26 model), 15 days (BaF model), 5 months (experiment A), 2 months (experiment B), and 5 days (dietary restriction).

Nucleic acid extraction. Genomic DNA was extracted from the caecal contents using a QIAamp DNA Stool Mini Kit (Qiagen, Germany) according to manufacturer's instructions, including a bead-beating step (glass bead 0.45µm, VWR, Belgium). Treatment with RNAse A was performed (10 units, Qiagen, Germany). DNA concentration was determined and purity (A_{260}/A_{280}) was checked using a NanoDrop 2000 (Thermo Fisher Scientific, USA) or alternatively, a spectrophotometer (SpectraMax 190

Microplate Reader, Molecular Devices, USA). A_{260}/A_{280} values were between 1.6 and 2.1. Dilutions of samples were done in TE buffer pH 8. DNA was stored at -20°C.

qPCR target information and qPCR oligonucleotides.

Bacterial name, primer sequences, and amplicon length are indicated in Table S1. Primers were previously published and checked for specificity using Primer-BLAST (Ye et al, 2012). Primers were synthesized with RP-Cartridge-Gold purification (Eurogentec, Belgium).

qPCR protocole. Real-time polymerase chain reaction (PCR) was performed with a StepOnePlus Real-Time PCR System (Applied Biosystems, The Netherlands) using SYBR Green (MESA FAST qPCR MasterMix Plus for SYBR® Assay, Eurogentec, Belgium) for detection. All samples (10ng/µl for all targets, except for total bacteria, 0.1ng/µl) were run in duplicate in a single 96-well reaction plate. Final concentrations were as follow: cDNA 2µl/25µl, primers 300nM, and SyberGreen mix 1X (MeteorTaq DNA polymerase, dNTP, RT buffer, $MgCl_2$ 4mM, SYBR® Green I, ROX passive reference and stabilizers, as provided by the manufacturer). Thermocycling conditions were as follow: initiation step at 95°C 10 min; cycling stage at 95°C 3 s, 60°C 26 s (unless a different annealing temperature is indicated in Table S1), 72°C 10 s, 40 cycles; melt curve stage at 95°C 15 s, 65°C 20 s, increment of 0.5°C every 15 s until reaching 95°C.

qPCR validation. The purity of the amplified product was verified by analyzing the melt curve performed at the end of amplification. Regarding the non-template control, a qPCR is considered valid if the Cq of the non-template control is at least 6 units higher than the Cq of the templates or, for targets with low expression levels, if the melt curve of the non-template control was different from the templates. Primers were validated by performing a calibration curve using serial dilution of bacterial DNA coming from pure culture (except for *Parabacteroides goldsteinii*/ASF 519, where DNA from caecal content was used). Primers were considered as acceptable when efficiency reaches values between 70% and 100%.

Standard curves. A standard curve (performed in duplicate) was included on each plate by diluting genomic DNA from pure culture (five-fold serial dilution). Cell counts were determined by plating and expressed as "colony-forming unit" (CFU) before DNA isolation. For total bacteria and *Lactobacillus* spp., *L. acidophilus* NCFM was used as standard. For *L. reuteri*, standard DNA was quantified based on *L. acidophilus* DNA (DSMZ, Braunshweig, Germany). For the Enterobacteriaceae family, *Escherichia coli* DH5-alpha was used as standard. For Parabacteroides goldsteinii/ASF59, serial dilution of DNA from caecal content was used to generate a relative curve (to take into account

primer efficiency in the calculations) and data were expressed in relative units. Same relative units applied for all the analyses in the present studies.

Data analysis. Threshold was manually adjusted to reach the linear range of the log-fluorescent curves and Cq were determined using the StepOnePlus PCR Software (Version 2.1, Applied Biosystems, The Netherlands). Data were analyzed in Excel. At least 80% of the duplicates show a variation lower than 0.5 Cq units. Outliers were determined either by visual inspection of the log-fluorescent curves or application of a statistical test (Grubs outlier test) on the normalized values.

Next-generation sequencing of DNA extracted from the caecal content

Amplicon sequencing of the caecal microbiome was done at the University of Minnesota Genomics Center, as previously described (Krumbeck et al, 2015). Briefly, the V5-V6 region of the 16S rRNA gene was PCR-enriched using the primer pair 784F (5'-RGGATTAGATACCC-3') and 1064R (5'-CGACRRCCATGCANCACCT-3') in a 25 µl PCR reaction containing 5 µl of template DNA, 5 µl of 2X HotStar PCR master mix, 500 nM of final concentration of primers and 0.025 U/µl of HostStar Taq+ polymerase (QIAGEN). PCR-enrichment reactions were conducted as follows, an initial denaturation step at 95°C for 5 min followed by 20-25 cycles of denaturation (50 s at 94°C), annealing (30 s at 40°C), and elongation (30 s at 72°C).

Next, the PCR-enriched samples were diluted 1:100 in water for input into library tailing PCR. The PCR reaction was analogous to the one conducted for enrichment except with a Taq polymerase concentration of 0.25 U/ µl, while the cycling conditions used were as follows, initial denaturation at 95°C for 5 min followed by 10-15 cycles of denaturation (50 s at 94°C), annealing (30 s at 40°C), and elongation (1 min at 72°C). The primers used for tailing are the following : F-indexing primer AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCG GCAGCGTC and R-indexing primer CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCG TGGGCTCGG, where [i5] and [i7] refer to the index sequence codes used by Illumina..

The resulting PCR products were quantitated by PicoGreen (Life Technologies). A subset of the amplicon libraries were spot-checked on a Bioanalyzer High-Sensitivity DNA Chip (Agilent Technologoies) for correct amplicon size. Next, samples were normalized to 2nM and pooled together. The total volume of the libraries was reduced by SpeedVac and amplicons were size-selected at 420 bp +/- 20% using the Caliper XT (Perkin Elmer). Next, library pools were cleaned-up by 1.8X AMPureXP beads (Beckman Coulter) and eluted in water. The final pool was quantitated by PicoGreen and normalized to 2 nM for input into Illumina MiSeq (v3 Kit) to produce 2x300 bp sequencing products. Clustering was done at 10 pM with a 5% spike of PhiX.

Initial quality filtering of the reads was performed with the Illumina Software, yielding an average of 148370 pass-filter reads per sample. Quality scores were visualized with the FastQC software (http://www.bioinformatics.babraham.ac.uk/publications.html), and reads were trimmed to 240 bp with the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Next, reads were merged with the merge-illumina-pairs application (with p-value = 0.03, enforced Q30 check, perfect matching to primers which are removed by the software, and otherwise default settings including no ambiguous nucleotides allowed) (Eren et al, 2013). For samples with >50000 merged reads, a subset of 50000 reads was randomly selected using Mothur 1.32.1 centos 5.5 for Linux (Schloss et al, 2009), to avoid large disparities in the number of sequences. Three samples with 36844, 45748 and 49310 quality-controlled merge reads, were not subsampled.

Subsequently, the UPARSE pipeline implemented in USEARCH v7.0.1001 (Edgar, 2013) was used to further process the sequences. Putative chimeras were identified against the Gold reference database and removed. Clustering was performed with 98% similarity cutoff to designate Operational Taxonomic Units (OTUs). Non-chimeric sequences were also subjected to taxonomic classification using the RDP MultiClassifier 1.1 from the Ribosomal Database Project (Cole et al, 2014), for phylum to genus characterization of the caecal microbiome.

The phylotypes were computed as percent proportions based on the total number of sequences in each sample. Alpha and beta diversity indexes were calculated using QIIME (Caporaso et al, 2010). PCoA plot of the beta-diversity indexes were obtained using EMPeror (Vazquez-Baeza et al, 2013).

Both parametric and non-parametric analyses were performed. For non-parametric analyses, we used LEfSe, to compute and calculate the LDA Effect size (Segata et al, 2011) (http://huttenhower.sph.harvard.edu/ galaxy/). For parametric analyses, we used t-test and one-way ANOVA. Specifically, significantly affected taxa and OTUs were identified by one-way ANOVA (3 groups) or t-test (2 groups). The p-value of the one-way ANOVA or the t-test was adjusted to control the false discovery rate (FDR) for multiple tests according to the Benjamini and Hochberg procedure (Benjamini and Hochberg, 1995) and robust (Huber) estimation was used to down weight outliers, as implemented in the Response screening platform, JMP 11. Tukey post test was computed for the significantly affected taxa and phylotypes using a script in R.

The sequences used for analysis can be found in the MG-RAST database under the project name "MicrobCan", with the following accession numbers: 4603996.3, 4603997.3, 4603998.3, 4603999.3, 4604000.3, 4604001.3, 4604002.3, 4604003.3, 4604004.3, 4604005.3, 4604006.3, 4604007.3, 4604008.3, 4604009.3, 4604010.3, 4604011.3, 4604012.3, 4604013.3, 4604014.3, 4604015.3, 4604016.3, 4604017.3, 4604018.3, 4604019.3, 4604020.3, 4604021.3, 4604022.3, 4604023.3, 4604024.3, 4604025.3, 4604026.3, 4604027.3, 4604028.3, 4604029.3, 4604030.3, 4604031.3, 4604032.3, 4604033.3, 4604034.3, 4604035.3, 4604036.3, 4604037.3, 4604038.3, 4604039.3,

4604040.3, 4604041.3, 4604042.3, 4604043.3, 4604044.3, 4604045.3, 4604046.3, 4604047.3, 4604048.3.

Metabolic profiling by 1H NMR spectroscopy

Sample preparation for NMR-based metabonomics

Every plasma sample (50 µL) was mixed with 10 µL of D_2O, vortexed and centrifuged 5 min at 10 000 g and the supernatant was transferred into 1.7 mm NMR capillary tubes.

NMR acquisition

All NMR spectra were acquired on a Bruker Avance DRX 700 MHz NMR Spectrometer (Bruker Biopsin, Rheinstetten, Germany) operating at 700.19 MHz and equipped with a CryoProbe™ from the same manufacturer. They were all acquired using a standard 1-dimensional (1D) pulse sequence [recycle delay (RD)-90°-t_1-90°-tm-90°-acquire free induction decay (FID)] with water suppression applied during RD of 2 s, a mixing time (tm) of 100 ms and a 90° pulse set at 7.70 µs. For each spectrum, a total of 512 scans were accumulated into 32K data points with a spectral width of 9803 Hz. FIDs were then processed in MestReNova software (Mestrelab Research S.L. version 10.0.1) using a zero-filling of 2 and were multiplied by an exponential function corresponding to 0.3 Hz line broadening. All spectra were manually phased. Baseline correction was performed automatically using a polynomial fit of order 1 and calibrated to the chemical shift of creatine (δ 3.04). Metabolites were assigned using our in house standard database, data from literature (Fan, 1996; Nicholson et al, 1995) and confirmed by 2D NMR experiments (^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC) performed on selected and pooled samples.

Statistical analysis

All spectral regions between 0.2 and 10 ppm were imported in Matlab version R2014b (Mathworks, U.K.) and spectral regions corresponding to solvent residuals of ethanol and water were removed (i.e. δ1.15 – 1.20, δ3.63 – 3.69 and δ4.70 – δ5.10). Statistical algorithms were provided by Korrigan Sciences (Korrigan Sciences Ltd., U.K.). Prior analysis, all data were mean-centred and scaled using unit variance. Data were first visualized by Principal Component Analysis followed by pair-wise Orthogonal Projection to Latent Structure Discriminant Analysis models (O-PLS-DA). The reliability of each model was evaluated for goodness of prediction (Q^2Y value) using 7-fold cross-validation. The O-PLS-DA correlation coefficients (r^2) were calculated for each variable and back-scaled into a spectral domain, so that the shape of NMR spectra and the sign of the coefficients were preserved (Cloarec et al, 2005). The weights of the variables were color-coded, according to the square of the O-PLS-DA correlation coefficients.

Predictions of bacterial populations from plasma metabolic profiles were performed using O-PLS regressions on selected OTUs and PCR transcripts (i.e. OTUs that were identified as significantly

altered by the condition). This was performed for every treatment group independently to avoid statistical bias due to the metabolic differences between groups. Each model uses a single component to predict the intensity of detection of each OTU or PCR transcript (Y predictors). Significance of models ($p < 0.05$) was tested using 1000 random permutations, and a P value was calculated by rank determination of the model actual Q^2Y value among the Q^2Y values calculated for the permutated models, as previously described (Eriksson et al, 2008).

Statistical analyses

Statistical analyses of data were performed using R (R Development Core Team, 2012), GraphPad Prism 5.0 (GraphPad Software, San Diego, CA) and JMP 11 (SAS Institute, Inc., Cary, NC). Outliers were removed based on the Grub's outlier test. Statistical significance of differences between groups was assessed by Student's t-test, when comparing two groups, or one-way ANOVA followed by post hoc Tukey's multiple comparison test, when comparing several groups. Evolution of the intake of food and energy was analysed by two-way ANOVA for repeated measures with Bonferroni post-tests. For gut microbiota analysis, the p-value of the one-way ANOVA was adjusted to control for the false discovery rate for multiple testing, according to the Benjamini and Hochberg procedure (Benjamini and Hochberg, 1995) and robust (Huber) estimation was used to down weight outliers, as implemented in JMP 11. Multiple correlation analyses were done in R, with adjustment of the p-value according to the Benjamini and Hochberg procedure. Data are presented as mean ± SEM or Whiskers Plot with minimum and maximum. $P < 0.05$ was considered as statistically significant.

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