Supplementary material and methods section

Animals

Nine weeks old male C57Bl/6J (Wild-type, WT) and Apoe$^{-/-}$ (KO) mice (Charles River Laboratories, L'Arbresle, France) were housed three mice per cage, in order to avoid the stress linked to mouse isolation that could modify kinetics of development of atherosclerotic plaques, and with a 12h light/dark cycle at 22°C, diet and water were *ad libitum*. Mice were fed an *n-3* PUFA-depleted (DEF) diet (D08041806, Research Diets, New Brunswick, NJ, USA) for 12 weeks. The composition of DEF diet is described in Table SM1. At week 10 of *n-3* PUFA depletion, mice were separated in two groups by genotypes and supplemented or not with inulin-type fructans (ITF) (Orafti®P95, Tienen, Belgium) at 250mg per mouse per day in the drinking water. ITF is oligofructose derived from inulin through partial enzymatic hydrolysis. The degree of polymerization ranges between 2 and 8. The data provided in the manuscript are issued from three separate experiments, performed in the same animal facility (n=3 per group, n=9 per group, and n=12 per group, for the first, second and third experiments, respectively).

At the end of the protocol, mice were anaesthetized in postprandial state (ketamine/xylazine i.p., 100 and 10 mg/kg of body weight, respectively) between 6.30 a.m. and 7 a.m. Venous blood was obtained by a puncture of the right ventricle, immediately frozen in heparinized calibrated tube (0.2mL) in liquid nitrogen for nitric oxide measurement. The remaining volume of venous blood was collected and centrifuged (3 min at 13,000g) before storage. Ileum, proximal colon, caecal tissue, caecal content and liver were removed, weighted and immediately frozen in liquid nitrogen. The samples were stored at -80°C for further analysis.

The experiments were approved by and performed in accordance with the guidelines of the local ethics committee. Housing conditions were specified by the Belgian Law of May 29, 2013, regarding the protection of laboratory animals (agreement n° LA1230314).

Blood biochemical analyses

Triglycerides (TG), total cholesterol (CHOL) and high density lipoprotein cholesterol (HDLc) concentrations were measured in non-thawed plasma using kits (Diasys Diagnostic and Systems, Holzheim, Germany) (Catry et al., 2016). Circulating levels of active glucagon-like peptide 1 (GLP-1) were measured by Elisa kit (Millipore, Billerica, USA) in portal plasma, treated with an inhibitor of dipeptidylpeptidase-IV.

Measurement of nitric oxide bioavailability by Electron Paramagnetic Resonance

The level of circulating heme-nitrosylated haemoglobin (Hb-NO) was assayed in whole blood of mice from the EPR signal of 5-coordinate-α-Hb-NO as previously described (Catry et al., 2016; Desjardins et al., 2008).

Measurement of vascular contraction and relaxation

Second and third order mesenteric arteries and carotid arteries were rapidly removed and carefully isolated from adipose and connective tissues in ice cold solution (Tyrode solution) gassed with carbogen. Artery segments of 2mm in length were mounted in a wire myograph (model 610M and 620M, Danish Myo Technology A/S, Aarhus, Denmark). Normalization of each artery was performed to calculate the effective transmural pressure. Arteries were set at a tension equivalent to that generated at 90 percent of the artery diameter at 100 mmHg. All procedures were performed in absence and presence of indomethacin (0.01mM). After 45 minutes of equilibration, arteries were challenged with a high KCl-solution to assess the viability and the maximal contraction. Endothelium-dependent relaxation was evaluated by cumulative addition of acetylcholine (from 10^{-8}M to 3.10^{-5}M) on pre-contracted arteries with a high KCl-solution, in the presence or the absence of nitric oxide synthase inhibitor Nω-Nitro-L-arginine methyl ester (L-NAME 100µM).

Microarray analysis of caecal tissue

Equal amounts of RNA from eight mice per group were pooled within each group. Microarrays were performed as previously described (Essaghir et al., 2009). Mouse gene ST microarray chips were used for hybridization (MoGene 2.0 ST, Affymetrix). The WT expression kit (Affymetrix) was used for complementary RNA preparation from the total RNA. Analysis procedure was previously described in (Duparc et al., 2016). Data normalization was performed on the Gene expression console® using full-gene RMA method. The detection above the background (DABG) p-values were computed using the Affymetrix Power Tools®. Genes with non-significant p-values in all the conditions were discarded from the analysis. Data are available under GEO accession number GSE87603.

Next-generation sequencing of DNA extracted from the caecal content

The gut microbiota analysis was performed on two of the three experiments, performed at different periods, in the same animal facility and housing conditions. Amplicon sequencing of the caecal microbiome was done at the University of Minnesota Genomics Center, as previously described (Bindels et al., 2016). 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 133532 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 220 bp (R1) and 200 bp (R2) with the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Next, reads were merged with the merge-illumina-pairs application v1.4.2 (with P = 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 >25000 merged reads, a subset of 25000 reads was randomly selected using Mothur v1.25.0 (Schloss et al., 2009), to avoid large disparities in the number of sequences. Five samples with 24329, 22535, 21540, 18821 and 21529 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) with a minimal cluster size of 2. 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 diversity indexes, beta diversity indexes, Adonis p-values and ANOSIM p-values were calculated using QIIME (Caporaso et al., 2010). PCoA plot of the beta-diversity indexes were obtained using EMPeror (Vazquez-Baeza et al., 2013).

Significantly affected taxa and OTUs were identified by one-way ANOVA followed by Tukey post-tests using a script in R. The p-value of the one-way ANOVA was adjusted (q-values) to control for the false discovery rate (FDR) for multiple tests according to the Benjamini and Hochberg procedure (Benjamini et al, 1995). We used LEfSe to compute and visualize the LDA Effect size (Segata et al, 2011). Prediction of the functional composition of the metagenome using 16S gene data and a database of reference genomes was performed using PICRUSt (Langille et al, 2013).

The sequences used for analysis can be found in the MG-RAST database under the project name “KDP” (ID 17493), with accession numbers from 4692382.3 to 4692450.3.

Liquid chromatography tandem mass spectrometry (LC-MS/MS)

The method allowed the quantification of 24 different BAs within the range of 1.5-2000 nmol/L [cholic acid (CA), glycocholic acid (GCA), taurocholic acid (TCA), chenodeoxycholic acid (CDCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), deoxycholic acid (DCA), glycodeoxycholic acid (GDCA),

taurochenodeoxycholic acid (TCDCA), lithocholic acid (LCA), glycolithocholic acid (GLCA), taurolithocholic acid (TLCA), ursodeoxycholic acid (UDCA), lycoursodeoxycholic acid (GUDCA), tauroursodeoxycholic acid (TUDCA), α-muricholic acid (αMCA), tauro-α-muricholic acid (TαMCA), β-muricholic acid (βMCA), tauro-β-muricholic acid (TβMCA), ω-muricholic acid (ωMCA), tauro-ω-muricholic acid (TωMCA), hyocholic acid (HCA), glycohyocholic acid (GHCA), taurohyocholic acid (THCA)), and the 5 deutered forms (cholic acid-2,2,4,4-D4 (CA-D4), glycochenodeoxycholic acid-2,2,4,4-D4 (GCDCA-D4), chenodeoxycholic acid-2,2,4,4-D4 (CDCA-D4), taurocholic acid-2,2,4,4-D4 (TCA-D4)].

LC–MS/MS analysis was performed on a UFLC-XR HPLC device (Shimadzu) coupled to a QTRAP® 5500 LC/MS/MS system hybrid triple quadrupole/linear ion trap mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with a Turbo VTM ion source. Instrument control, data acquisition and processing were performed using the associated Analyst 1.5.1 software. The separation was carried out on a Symmetry C18 Luna column (250 mm×2.1 mm, particle size 5 µm) (Phenomenex). The injection volume was 5 µL. Elution was performed at a flow rate of 500 µL/min with water containing 20 mM ammonium acetate, pH=8 as eluent A and acetonitrile as eluent B, employing a 5 min plateau with 28 % B and a linear gradient from 28 % B to 90 % B in 15 min, followed by a 2 min plateau with 90 % B. Then, column re-equilibration was performed for 4 min. The injection duty cycle was 26 min, taking into account the column equilibration time.

The mass spectrometer was initially tuned and calibrated using polypropylene glycol, reserpine and Agilent Tuning Mix (all AB Sciex, Foster City, CA, USA) according to the manufacturer's instructions. Q1 resolution was adjusted to 0.7 ± 0.1 a.m.u. FWHM, referred to as unit resolution. Q3 was also set to unit resolution in multiple reaction monitoring mode. MS analysis was carried out in negative ionization mode using an ion spray voltage of 4500 V. The nebulizer and the curtain gas flows were set at 25 psi using nitrogen. The Turbo VTM ion source was set at 550°C with the auxiliary gas flow (air) set at 50 psi. For all bile acids, the following MRM transitions were monitored with a dwell time of 50 ms: CA 407.2 → 407.2 (Collision Energy "CE" = 40eV); GCA 464.1 →74 (CE = 85eV); TCA 514.1 → 80 (CE = 130eV); CDCA 391.2 → 391.2 (CE = 40eV); GCDCA 448.1 → 74 (CE = 80eV); TCDCA 498.1 → 80 (CE = 125eV); DCA 391.2 → 391.2 (CE = 40eV); GDCA 448.1 → 74 (CE = 80eV); TDCA 498.1 → 80 (CE = 130eV); UDCA 391.1 → 391.1 (CE = 40eV); LCA 375.2 → 375.2 (CE = 40eV); TLCA 482.0 → 80 (CE = 130eV); TUDCA 498.1 → 80 (CE = 126eV); αMCA 407.2 → 407.2 (CE = 40eV); TαMCA 514.1 → 80 (CE = 130eV); βMCA 407.2 → 407.2 (CE = 40eV); TβMCA 514.1 → 80 (CE = 130eV); ωMCA 407.2 → 407.2 (CE = 40eV); TωMCA 514.1 → 80 (CE = 130eV); HCA 407.3 → 407.3 (CE = 40eV); GHCA 464.1 → 74 (CE = 80eV); THCA 514.1 → 80 (CE = 130eV); GUDCA 448.1 → 74 (CE = 80eV); GLCA 432.1

→ 74 (CE = 80eV); CA-D4 411.2 → 411.2 (CE = 40eV); GCDCA-D4 452.1 → 74 (CE = 80eV); GCA-D4 468.1 → 74 (CE = 85eV); CDCA-D4 395.1 → 395.1 (CE = 40eV); TCA-D4 518.2 → 79.8 (CE = 130eV). The inter- and intra- coefficients of variation for BA concentration assay are below 15 %, except for HCA and LCA.

Histological analyses

Second order mesenteric arteries and proximal colon, previously emptied of its content, were fixed in 10% formaldehyde for 24h and then immersed in ethanol 100% for 24 hours prior to processing for paraffin embedding. Paraffin sections of mesenteric arteries of 5 µm were cut at 3 different levels for each sample. Haematoxylin-eosin stained sections were digitalized at a 20x magnification using a SCN400 slide scanner (Leica, Wetzlar, Germany). On each section, intima and media thickness was determined after manual delineation using the freely available imaging software ImageJ (version 1.48r, National Institutes of Health, Bethesda, Maryland, USA). Results were expressed as µm. Paraffin sections of proximal colon of 10 µm were stained with primary antibodies against glucagon-like peptide 1 (GLP-1) as a marker of L cells and against Cytokeratin (CK) 8+18 as markers of the intestinal epithelium. Secondary antibodies conjugated with Alexa 594 and Alexa 488 were used for GLP-1 and CK8+18, respectively. Nuclear counterstaining was performed with Hoechst 33342. Antibodies are listed in Table SM2. Stained sections were digitalized at 20x magnification using a Mirax Midi Scanner (Carl Zeiss MicroImaging) equipped with a Zeiss Plan-Apochromat 20× NA 0.80 objective lens and a Hitachi HV-F22 acquisition camera, providing an object pixel size of 0.23µm. Image acquisition was controlled with the Mirax Scan software (Zeiss). Image files were compressed with 0.80× jpeg. A least 4 distinct sections separated by at least 100 µm were obtained. Quantification of the intestinal epithelial area was performed based thresholding of the Alexa 488 signal using ImageJ software. L cells were counted in a blind manner based on the Alexa 594 signal. L cell density was defined as the number of L cells / mm² of epithelial area

Quantitative real-time PCR

Total RNA was isolated from tissue by TriPure reagent (Roche, Basel, Switzerland). cDNA was prepared by reverse transcription of 1 µg total RNA using Reverse Transcription System kit (Promega, Leiden, The Nederlands). Real-time qPCRs were performed with a CFX96 Touch™ instrument and software v3.1. (Bio-Rad Laboratories, CA, USA) using Mesa Fast qPCR™ (Eurogentec, Seraing, Belgium) for detection. Ribosomal

protein L19 (RPL19) RNA was chosen as housekeeping gene. Sequences of the primers are summarized in Table SM3.

Western blot

Equal amount proteins from mesenteric arteries were separated by 10% SDS/PAGE and transferred to nitrocellulose membrane, blocked in tris-buffered saline tween-20 (TTBS) containing 5% non-fat dry milk or bovine serum albumin (BSA) for one hour at room temperature. The membranes were incubated overnight at 4°C with antibodies (listed in Table SM2) in TTBS containing 1% non-fat dry milk or BSA. Beta-actin was used as loading control. Gels were analysed and quantified by ImageQuant^{TM} TL instrument and software version 8.1 (GE Healthcare, Buckinghamshire, England).

Statistical analysis

The power calculation was based on previous results published in Catry *et al*. We have reported that n-3 PUFA-depleted WT mice present a residual KCl contraction of 45 % after 3.10^{-5} M of acetylcholine and n-3 PUFA-depleted Apoe-/- mice present a residual KCl contraction of 75 % after 3.10^{-5} M of acetylcholine. Our hypothesis is that ITF supplementation can restore the relaxation in response of acetylcholine by decreasing the residual contraction as the same percentage as that observed in WT mice. The sample size were evaluated using PASS 14 Power Analysis and Sample Size Software (2015) (NCSS, LLC. Kaysville, Utah, USA) for the four treatment groups by after considering a power of 0.80 and a significance level of 0.05, the mean residual contraction and the standard deviation.

Statistical significance between groups was assessed by one-way ANOVA followed by Tukey's multiple comparison tests. Grouped analyses were assessed by two-way ANOVA followed by Bonferroni's multiple comparison post-tests. Unpaired t test was used for statistical comparison between KO DEF and KO DEF PRE mice. Data with superscript symbol (* vs WT DEF, $^{\$}$ vs WT DEF PRE or $^{\#}$ vs KO DEF) were significantly different ($p<0.05$). Statistical analyses were performed using GraphPad Prism version 5.00 for windows.

References

Benjamini, Y. & Hochberg, Y. Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. J. R. Statist. Soc. B 57, 289-300 (1995).

Bindels, L.B., Neyrinck, A.M., Claus, S.P., Le Roy, C.I., Grangette, C., Pot, B., Martinez, I., Walter, J., Cani, P.D., and Delzenne, N.M. (2015). Synbiotic approach restores intestinal homeostasis and prolongs survival in leukaemic mice with cachexia. The ISME journal.

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., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature methods 7, 335-336.

Catry, E., Neyrinck, A.M., Lobysheva, I., Pachikian, B.D., Van Hul, M., Cani, P.D., Dessy, C., and Delzenne, N.M. (2016). Nutritional depletion in n-3 PUFA in apoE knock-out mice: a new model of endothelial dysfunction associated with fatty liver disease. Molecular nutrition & food research.

Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., and Tiedje, J.M. (2014). Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic acids research 42, D633-642.

Desjardins, F., Lobysheva, I., Pelat, M., Gallez, B., Feron, O., Dessy, C., and Balligand, J.L. (2008). Control of blood pressure variability in caveolin-1-deficient mice: role of nitric oxide identified in vivo through spectral analysis. Cardiovascular research 79, 527-536.

Duparc, T., Plovier, H., Marrachelli, V.G., Van Hul, M., Essaghir, A., Stahlman, M., Matamoros, S., Geurts, L., Pardo-Tendero, M.M., Druart, C., Delzenne, N.M., Demoulin, J.B., van der Merwe, S.W., van Pelt, J., Backhed, F., Monleon, D., Everard, A., and Cani, P.D. (2016). Hepatocyte MyD88 affects bile acids, gut microbiota and metabolome contributing to regulate glucose and lipid metabolism. Gut.

Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature methods 10, 996-998.

Eren, A.M., Vineis, J.H., Morrison, H.G., and Sogin, M.L. (2013). A filtering method to generate high quality short reads using illumina paired-end technology. PloS one 8, e66643.

Essaghir, A., Dif, N., Marbehant, C.Y., Coffer, P.J., and Demoulin, J.B. (2009). The transcription of FOXO genes is stimulated by FOXO3 and repressed by growth factors. The Journal of biological chemistry 284, 10334-10342

Langille, M.G., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., et al. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31, 814-821.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., and Weber, C.F. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and environmental microbiology 75, 7537-7541.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., and Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. Genome Biol 12, R60.

Vazquez-Baeza, Y., Pirrung, M., Gonzalez, A., and Knight, R. (2013). EMPeror: a tool for visualizing high-throughput microbial community data. GigaScience 2, 16.

Table SM1: Composition of n-3 PUFA depleted (DEF) diet.

Composition (% weight/weight)	DEF diet (D08041806)
Casein (%)	20
Total carbohydrates (%)	72.4
Corn starch (%)	44.2
Sucrose (%)	10
Maltodextrin (%)	13.2
Cellulose (%)	5
Sunflower oil (%)	5
C16:0 (%)	0.33
C18:0 (%)	0.26
C20:0 (%)	0.02
C16:1 (%)	0.02
C18:1 (%)	1.16
C18:2 n-6 (%)	3.19
C18:3 n-3 (%)	0.02
Mineral mix (%)	3.5
Vitamin mix (%)	1
kcal/g	3.9

Formulated by Research Diets. Parenthetical numbers indicate the manufacturer's diet number. (DEF, n-3 PUFA depleted diet)

Table SM2: List of antibodies for histological analyses and Western blot

Histological analyses	Firm	Reference	Used dilution
anti-GLP1	Abcam	Ab26278	1/1500
anti-CK(8+18)	Abcam	Ab53280	1/1500
Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor® 594 conjugate	Thermo Fisher	A-21203	1/500
Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate	Thermo Fisher	A-21206	1/500
Hoechst 33258	Life Technologies	H1399	1/10000

Western Blot	Firm	Reference	Used dilution
Phospho-eNOS (Ser1177)	Cell Signaling	9571S	1/500
Phospho-eNOS (Thr495)	BD Sciences	612707	1/500
eNOS/NOS	BD Sciences	610297	1/1000
Caveolin-1	BD Sciences	610407	1/5000
Beta-actin	Abcam	Ab6276	1/10000

Table SM3: Sequences of primers used for real-time quantitative PCR

Genes	Forward primer (5' to 3')	Reverse primer (5' to 3')
Anpep	ATGCAACTCTGGTGAACGAA	CAATGCTGATGATGGTGGAG
CD3g	GCAAGAATAGGAAGGCCAAG	GGTCCACAGAAGGCGATGT
Duox2	ACACAGCTCTGTGTCAAAGGT	TGATGAACGAGACTCGACAGG
DuoxA2	TCAGTCCATCCCGTGTTCAA	TTCAGACGCCAAGTGAAACG
Enpep	CGTCATCCGCTACATCTCCT	TTGAAGGGCTCAGCTATGGT
Gcg	TGGCAGCACGCCCTTC	GCGCTTCTGTCTGGGA
iNOS	AAACTGTGTGCCTGGAGGTT	TCTCTGCCTATCCGTCTCGT
LGR5	TGAGCGGGACCTTGAAGATT	AATAGGTGCTCACAGGGCTT
NeuroD1	AGGAATTCGCCCACGCAGAAG	CTCCTCTGCATTCATGGCTTCAAG
Ngn3	ACTCAGCAAACAGCGAAGAAG	CAGTGCCCAGATGTAGTTGTG
Nts	CATCCAAGATCAGCAAAGCA	TGCCAACAAGGTCGTCATC
OSTa	TACAAGAACACCCTTTGCCC	CGAGGAATCCAGAGACCAAA
PC1/3	TTGGCTGAAAGGGAAAGAGA	GATGTCCCATGCAAAATCAA
Reg3g	TTCCTGTCCTCCATGATCAAA	CATCCACCTCTGTTGGGTTC
RPL-19	GAAGGTCAAAGGGAATGTGTTCA	CCTTGTCTGCCTTCAGCTTGT
SAA1	CATTTGTTCACGAGGCTTTCC	GTTTTTCCAGTTAGCTTCCTTCATGT
SAA2	CCATTCTGAAACCCTTGTGG	GGGGTCTGGGCTTCCTATCT
SAA3	CGCAGCACGAGCAGGAT	CCAGGATCAAGATGCAAAGAATG
Slc10A2	TGGGTTTCTTCCTGGCTAGACT	TGTTCTGCATTCCAGTTTCCAA
Slc5A8	GCATATTCGGCATGGTTGGT	GGGCTCCAATTCCTACCCAT

Anpep, alanyl aminopeptidase; CD3g, cluster of differentiation 3 gamma; Duox2, dual oxidase 2; DuoxA2, dual oxidase A2; Enpep, glutamyl aminopeptidase; Gcg, proglucagon; iNOS, inducible nitric oxide synthase; LGR5, Leucine-Rich Repeat Containing G Protein-Coupled Receptor 5; NeuroD1, Neuronal Differentiation 1; Ngn3, neurogenin 3; Nts, neurotensin; OSTa, Organic solute transporter alpha; PC1/3, prohormone convertase 1/3; Reg3g, Regenerating islet-derived protein 3 gamma; RPL19, ribosomal protein L19; SAA, serum amyloid A; Slc10A2, solute carrier family 10 member 2 (or Apical Sodium Dependent Bile Acid Transporter); Slc5A8, solute carrier family 5 member 8.