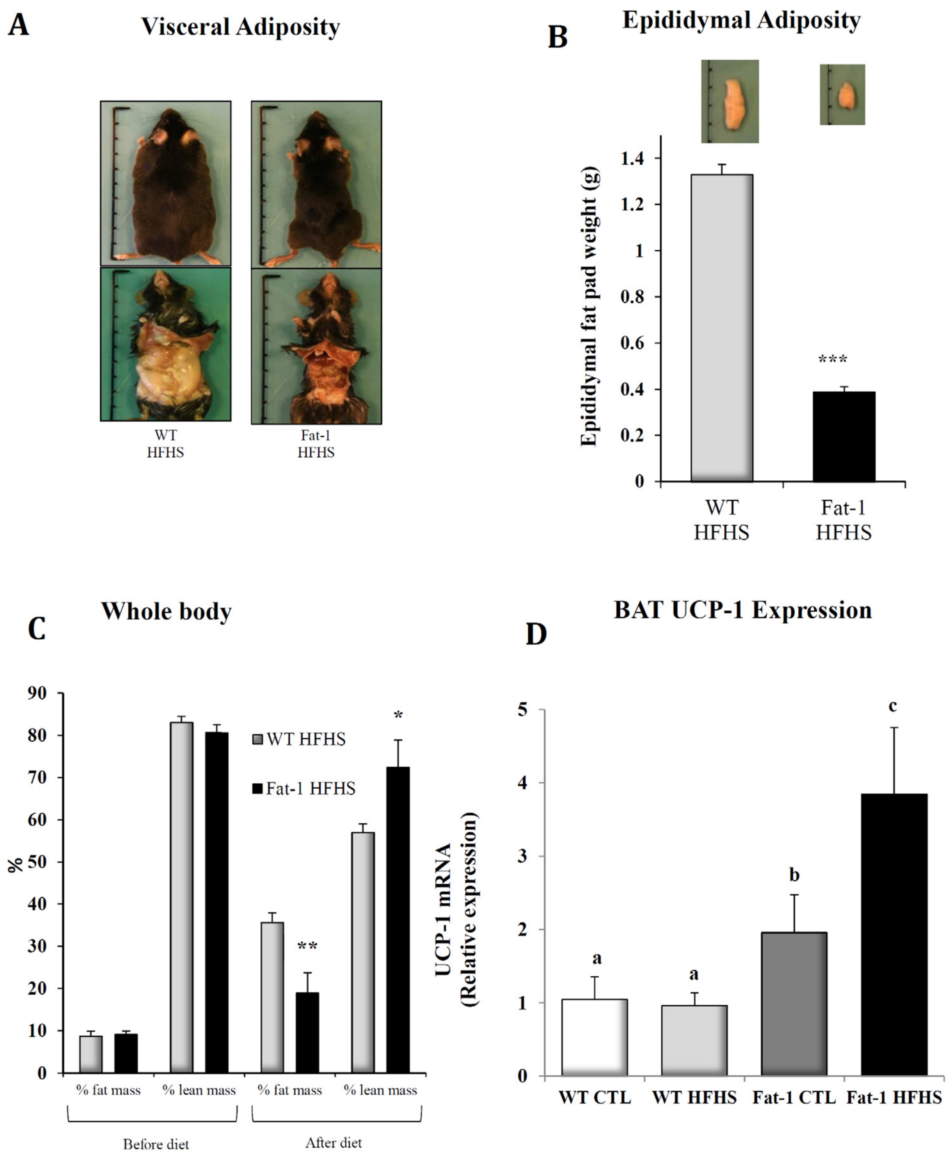
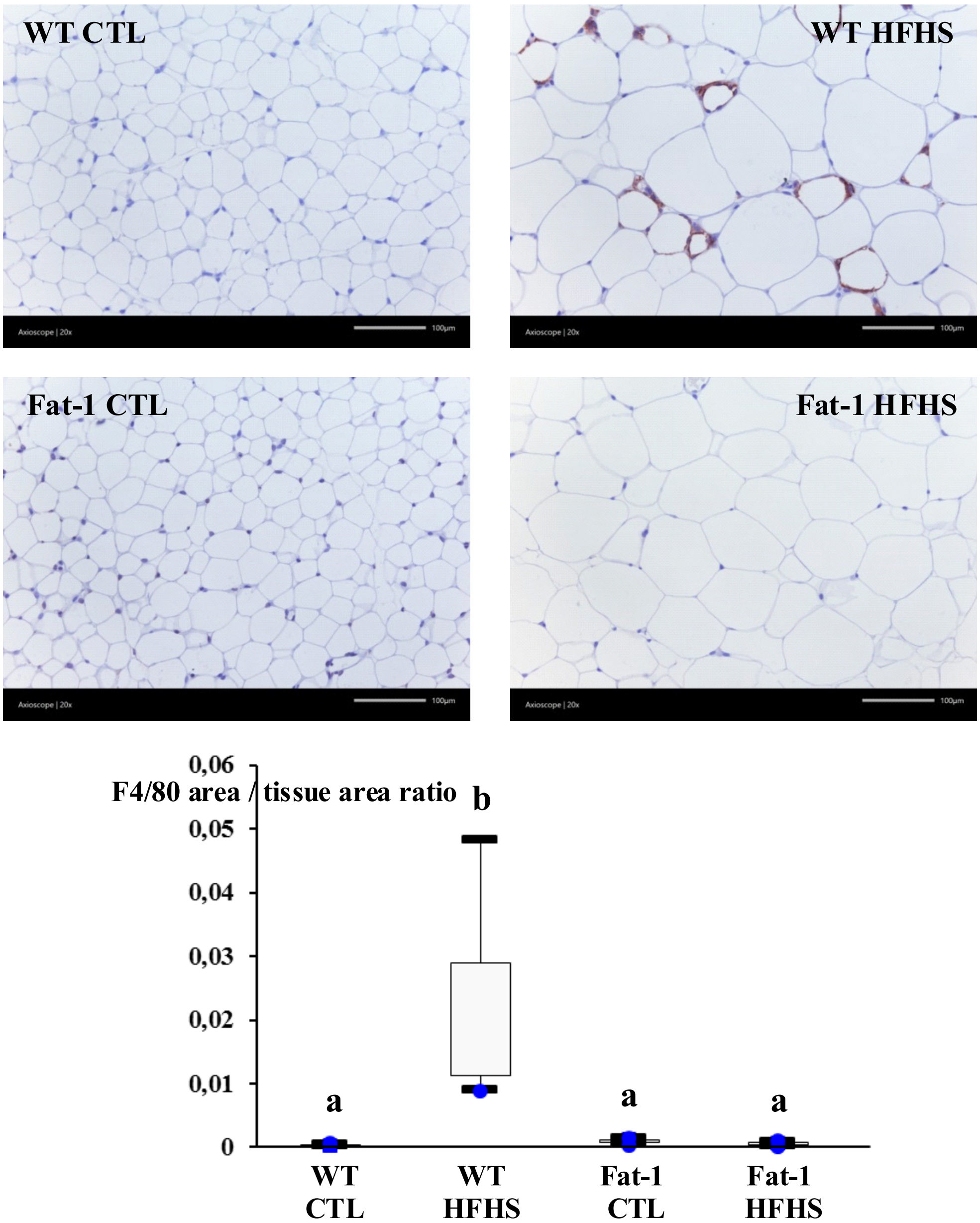
SUPPLEMENTARY DATA

Supplementary Figure S1. **Fat-1 mice exhibit reduced adiposity when fed an HFHS diet.** WT and fat-1 mice were fed either a control or an HFHS diet for 18 weeks. **A:** Representative photographs for a side-by-side comparison of male WT and transgenic mice fed the HFHS diet for 18 weeks. **B:** Epididymal fat mass in WT and fat-1 mice fed a HFHS diet (n=8 per group). **C:** Body composition analysis (fat mass and lean mass) of WT and fat-1 mice before and after 18 weeks on the HFHS diet using dual-energy X-ray absorptiometry. n= 10 to 12 for control and HFHS groups. **P<0.05; **P<0.01* (Mann-Whitney test test). Similar results were obtained in five independent experiments. D: UCP1 mRNA expression in interscapular brown adipose tissue. Groups with different superscript letters are significantly different (P<0.05, according to Kruskal-Wallis test and the Dunn's multiple comparisons test).



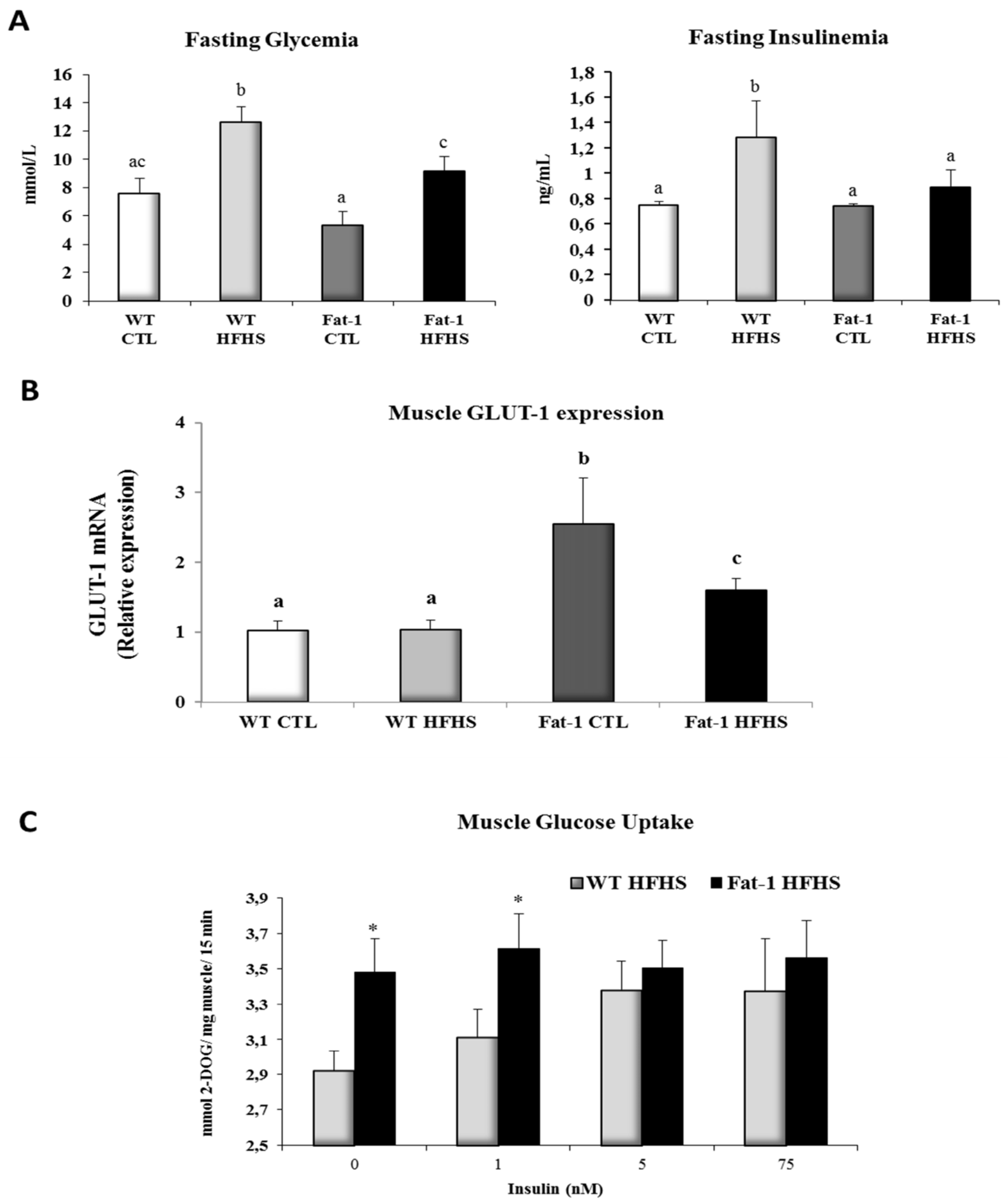
SUPPLEMENTARY DATA

Supplementary Figure S2. F4/80 immunohistochemistry labeling in adipose tissue from wild type and fat-1 mice fed a control or high fat high sucrose diet. Box and whisker diagram showing labelling index (ratio between F4/80 area and tissue area) for the different groups. Groups with different superscript letters are significantly different (P<0.05, according to Kruskal-Wallis test and the Dunn's multiple comparisons test).



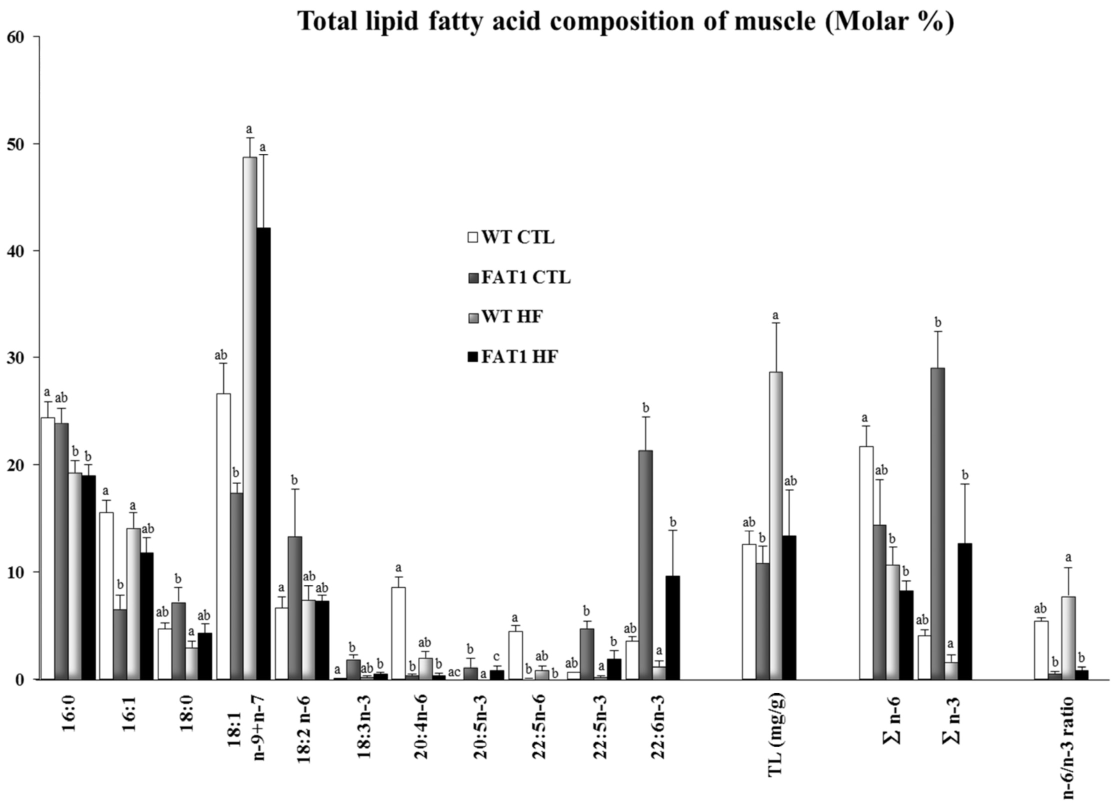
SUPPLEMENTARY DATA

Supplementary Figure S3. Fat-1 mice display resistance to HFHS-induced hyperglycemia and insulin resistance. WT and fat-1 mice were fed either a control or an HFHS diet for 18 weeks. **A:** The mice were fasted for 12h for glycemia and insulinemia determination. **B:** GLUT1 mRNA expression in skeletal muscle **C:** Dose-response histograms of insulin-stimulated glucose transport in isolated gastrocnemius muscles from WT and fat-1 mice fed an HFHS diet after overnight food deprivation. n=4 (A and B); n=8 (C); n=6 (D). Data are expressed as the mean ± SEM. Groups with different superscript letters are significantly different (P<0.05, according to Kruskal-Wallis test and the Dunn's multiple comparisons test). **P<0.05; **P<0.01* (Mann-Whitney test). Similar results were obtained in five independent experiments.



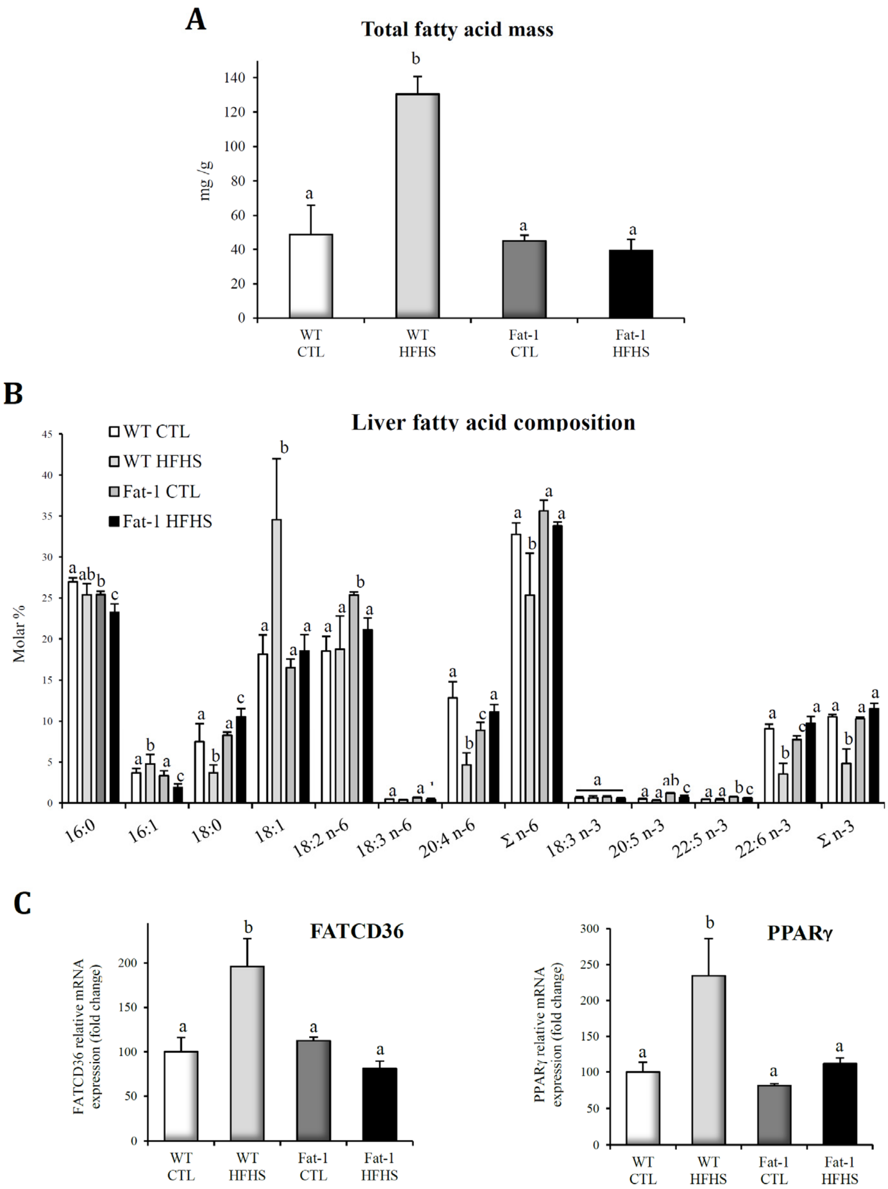
SUPPLEMENTARY DATA

Supplementary Figure S4. **Muscle lipidomic analysis.** Wild-type and fat-1 mice were fed either a control or an HFHS diet for 18 weeks. Muscle major fatty acid composition, total omega 6, total omega 3 and omega6/omega3 ratios are indicated for WT and fat-1 mice fed with control or HFHS diets (n=6). The results are expressed as the mean ± SEM. Data with different superscript letters are significantly different at *P*<0.05, according to the Kruskal-Wallis test and the Dunn's multiple comparisons test.



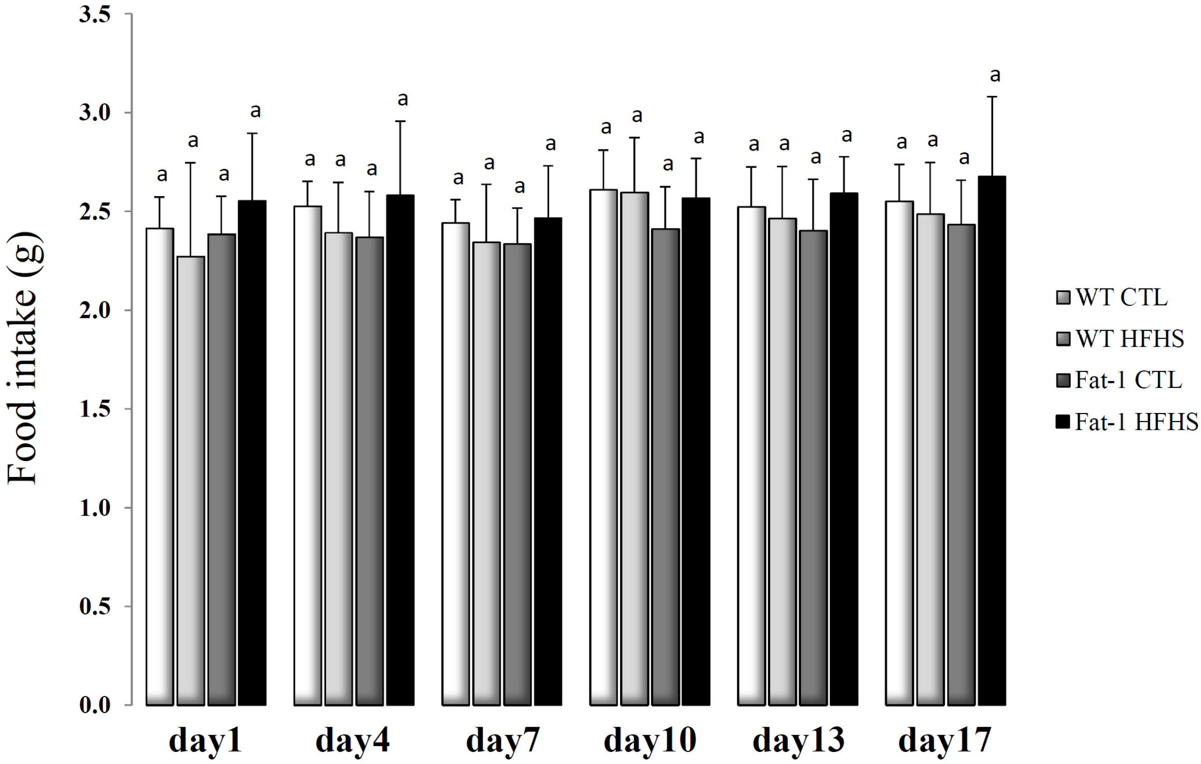
SUPPLEMENTARY DATA

Supplementary Figure S5. **Omega 3 fatty acid enrichment protects fat-1 mice against liver HFHS-induced lipogenesis.** Wild-type and fat-1 mice were fed either a control or an HFHS diet for 18 weeks. **A:** Liver total lipid levels in mice fed either control or HFHS diet. **B:** Liver major fatty acid composition, total omega 6 and total omega 3 are indicated for WT and fat-1 mice fed with control or HFHS diets (n=8). **C:** Hepatic FATCD36 and PPARγ mRNA levels were measured by qRT-PCR. All mRNA levels were normalized relative to β-actin (n=10). The results are expressed as the mean ± SEM. Groups with different superscript letters are significantly different ($P<0.05$, according to Kruskal-Wallis test and the Dunn's multiple comparisons test). FATCD36: Fatty acid transporter cluster of differentiation 36; PPARγ: peroxisome proliferator-activated receptor gamma. Similar results were obtained in five independent experiments.



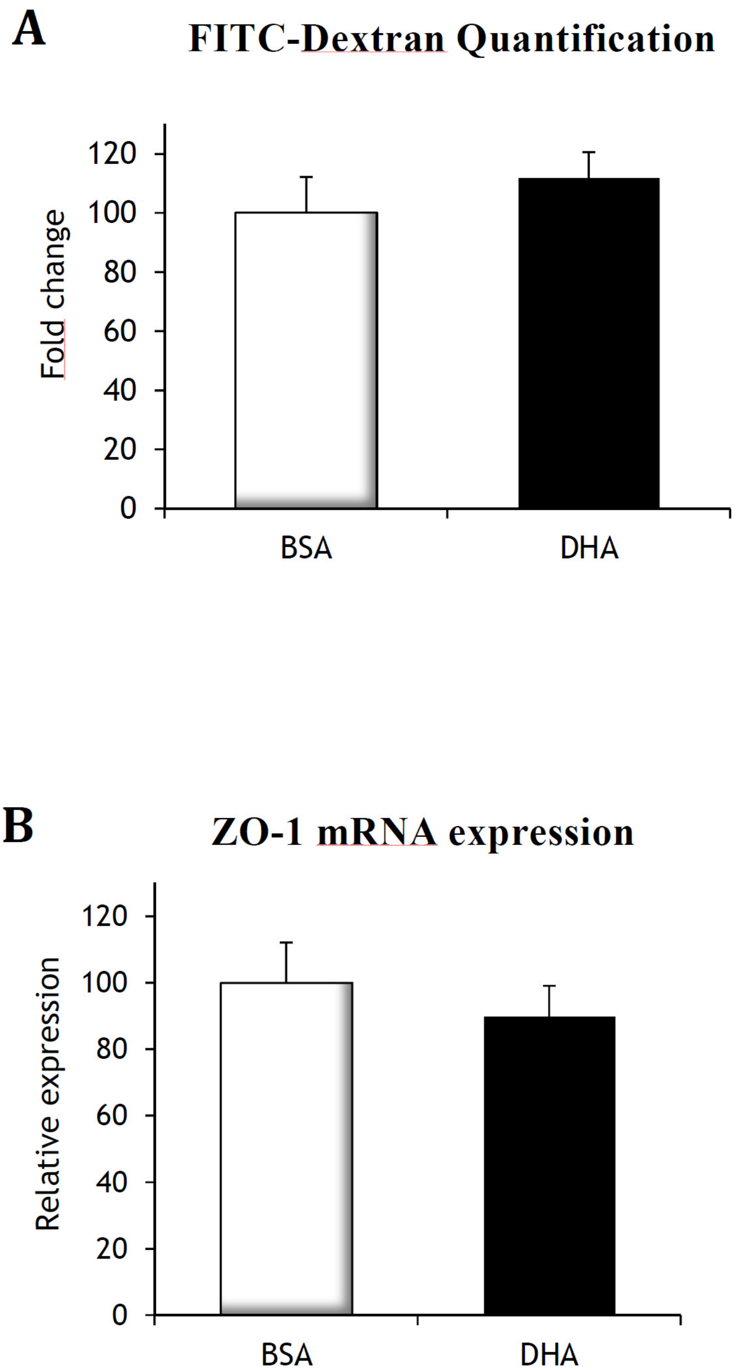
SUPPLEMENTARY DATA

Supplementary Figure S6. **Food intake in fat-1 and WT mice fed the control or HFHS diet.** 24h food intake (recorded every 3 days over 3 weeks) is expressed in wild-type and fat-1 transgenic mice fed a control diet (CTL) and high fat/high sucrose (HFHS) diet. n= 7 for each group. The results are expressed as the mean ± SEM. Groups with different superscript letters are significantly different (P<0.05, according to Kruskal-Wallis test and the Dunn's multiple comparisons test).



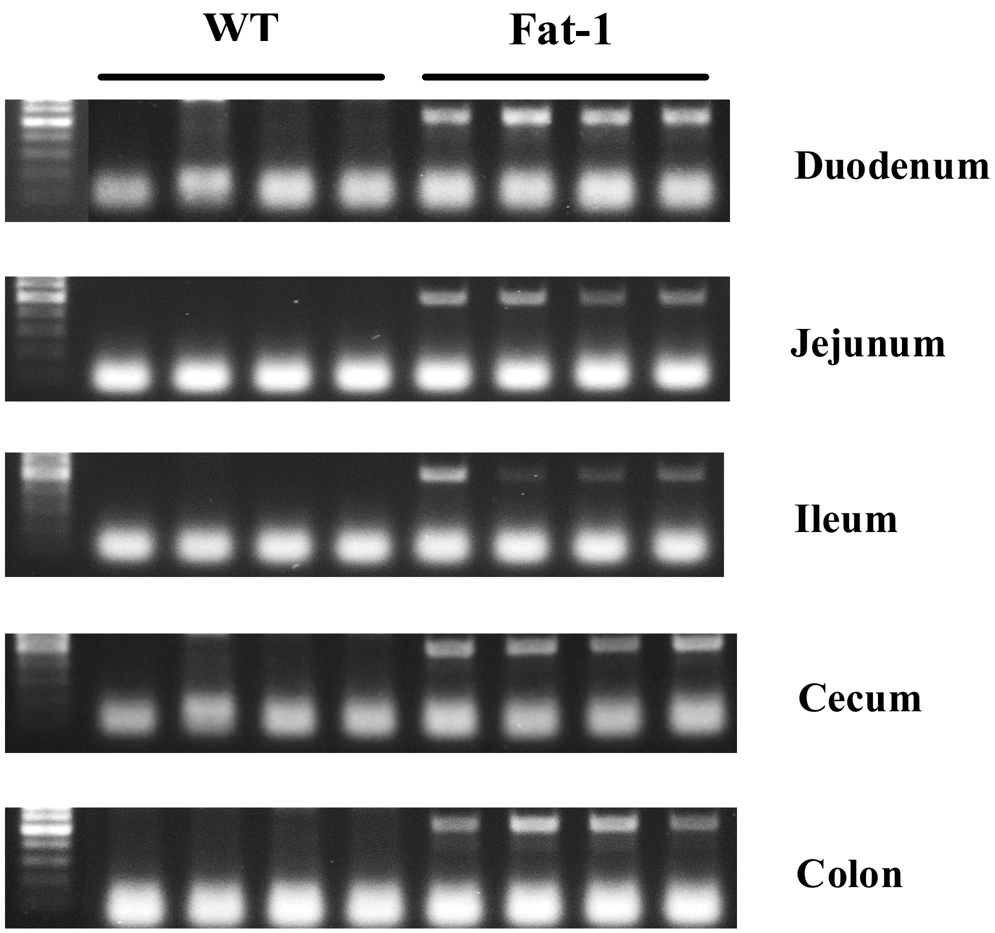
SUPPLEMENTARY DATA

Supplementary Figure S7. **DHA supplementation has no direct effect on permeability of cultured Caco2-TC7 monolayers.** **A**: Effect of DHA on FITC-Dextran permeability. **B**: ZO-1 mRNA expression. Cells were treated with 100µM of DHA for 24 h and BSA treatment served as control. Results are presented as mean ± SEM. FITC-Dextran: Fluorescein Isothiocyanate-dextran; ZO-1: Zonula Occludens 1; DHA: docosahexaenoic acid; BSA: bovine serum albumin.



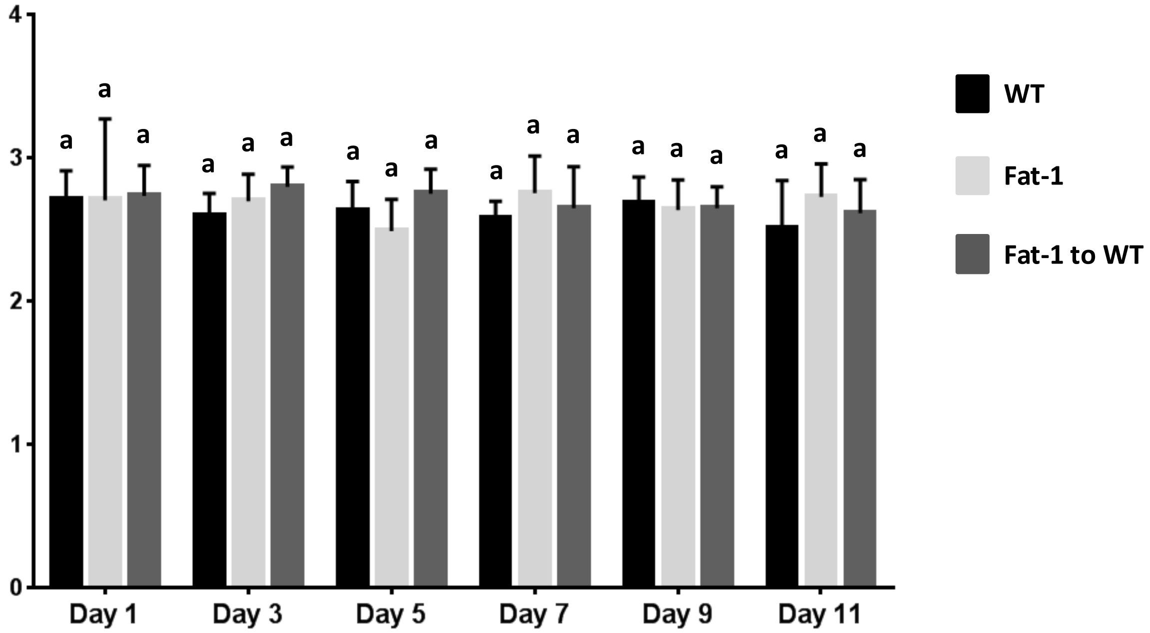
SUPPLEMENTARY DATA

Supplementary Figure S8. **Fat-1 transgene expression along the intestinal tract.** The expression of the fat-1 transgene was assessed in the distinct segments of the intestinal tract (duodenum, jejunum, ileum, caecum and colon) in wild-type and transgenic animals.



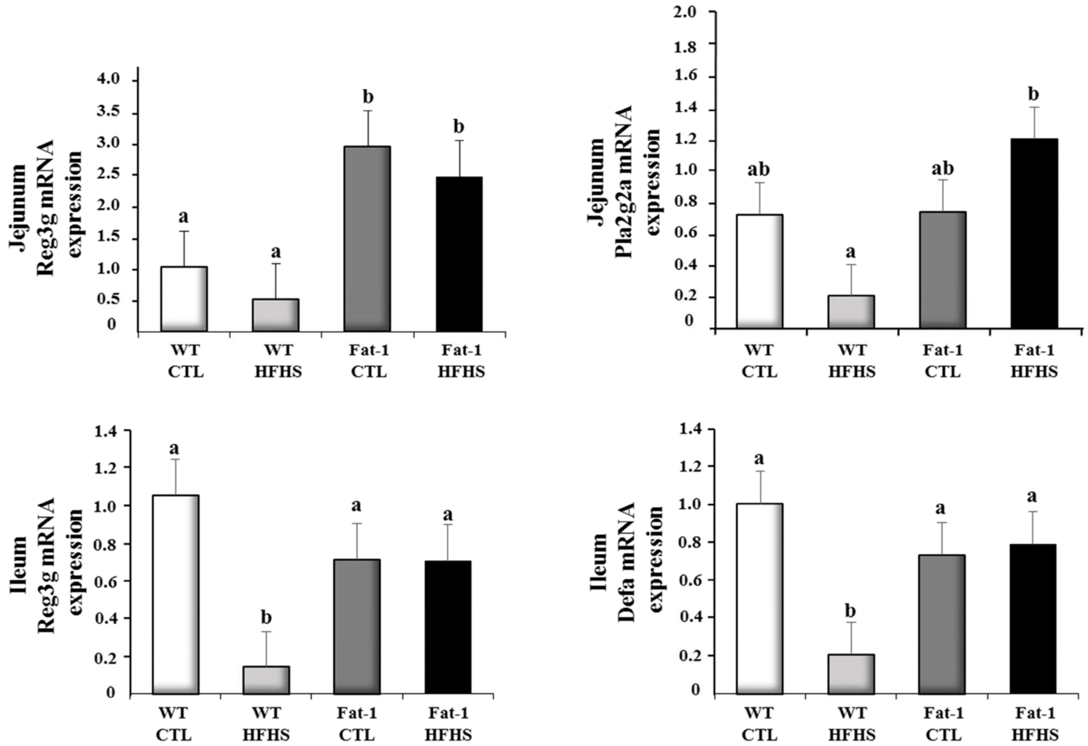
SUPPLEMENTARY DATA

Supplementary Figure S9. **Food intake in WT, Fat-1 and Fat-1-to-WT mice fed the HFHS diet.** The levels of 24h food intake (recorded every 2 days for a 2 weeks period) is expressed in the wild-type, Fat-1 and Fat-1 to WT mice fed a high fat/high sucrose (HFHS) diet. N= 6 for each group. The results are expressed as the mean ± SEM. Groups with different superscript letters are significantly different ($P<0.05$, according to Kruskal-Wallis test and the Dunn's multiple comparisons test).



SUPPLEMENTARY DATA

Supplementary Figure S10. **Antimicrobial peptides expression in the jejunum and ileum of control and high fat high sucrose-fed WT and fat-1 mice.** Expression of Regenerating islet-derived 3-gamma (RegIIIγ, encoded by Reg3g); Phospholipase A2 group II (encoded by Pla2g2) and α-defensins (encoded by Defa), measured in the jejunum and ileum of control diet-fed mice (CTL) and high fat high sucrose-fed mice (HFHS). N= 6 for each group. The results are expressed as the mean ± SEM. Groups with different superscript letters are significantly different ($P<0.05$, according to Kruskal-Wallis test and the Dunn's multiple comparisons test).



SUPPLEMENTARY DATA

Supplementary Table 1. The sequences of primers used in Realtime PCR assays.

GENES	Forward Primer	Reverse Primer
FAT/CD36	5'-AATTAGTAGAACCGGGCCAC-3'	5'-CCAACTCCCAGGTACAATCA-3'
PPARγ	5'-ATCTTAACTGCCGGATCCAC-3'	5'-AGGCACTTCTGAAACCGACA-3'
ZO-1	5'-GACCTTGAGCAGCCGTCATA-3'	5'-CCGTAGGCGATGGTCATAGTT-3'
UCP-1	5'-GCTTAATGACTGGAGGTGTGG-3'	5'-CTCTGGGCTTGCATTCTGAC-3'
GLUT-1	5'-GTGACGATCTGAGCTACGGG-3'	5'-TCACCTTCTTGCTGCTGGG-3'
Defa	5'-GGTGATCATCAGACCCCAGCATCAGT-3'	5'-AAGAGACTAAAACTGAGGAGCAGC-3'
Reg3g	5'-TTCCTGTCCTCCATGATCAAA-3'	5'-CATCCACCTCTGTTGGGTTC-3'
Pla2g2a	5'-AGGATTCCCCCAAGGATGCCAC-3'	5'-CAGCCGTTTCTGACAGGAGTTCTGG-3'
β Actin	5'-ATGCTCCCCGGGCTGTAT-3'	5'-CATAGGAGTCCTTCTGACCCATTC-3'

Supplementary Table 2. Analyses of the composition of fatty acids in Diets.

	Molar %	
Fatty acid	**D12450B (CTL)**	**D12451 (HFHS)**
14:0	3.8	2.8
16:0	30.1	28.8
18:0	17.5	18.6
20:0	0.3	0.3
SFA	**51.7**	**50.5**
16:1	3.2	2.8
18:1	37.6	36.1
20:1	0.5	0.4
MUFA	**41.3**	**39.3**
18:2N-6	6.4	8.8
18:3N-3	0.4	0.7
20:2N-6	0.2	0.7
PUFA	**7.0**	**10.2**
n-6/n-3	**16.5**	**13.6**

Abbreviations: SFA, short-chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.