Hepatology

Supporting materials and methods

Mice and diets

IRF9 KO mice were backcrossed with C57BL/6 mice for 6 or more generations. The genotyping forward primer 5'-TCAATGTTCCGATGTGGCAGTTCAAAGGATC-3' was used in combination with the reverse primer 5'-TATCGAATACTGCCAGCAGGAACCC-3' to detect endogenous IRF9 or 5'-TCCTGCTTTACGCTATCGCCGCTCCCGATT-3' to detect mutant IRF9 mRNA. We initiated our animal experiments using eight-week-old male mice. The mice were housed at controlled temperature $(23 \pm 2^{\circ}C)$ with a 12-hour light and a 12-hour dark cycle and given free access to water and a standard rodent diet prior to our study. Mouse body weight and fasting blood glucose levels were measured every four weeks, and food intake was monitored weekly.

Tissue processing and histological analyses

After 26 weeks of diet treatment, the mice were anesthetized with 3% pentobarbital sodium, and macroscopic pictures of their physical appearance were taken. Next, the mice were sacrificed, and their livers were rapidly harvested and weighed. Each liver was cut into two parts: one part was immediately frozen in liquid nitrogen for molecular biological analysis, and the other part was either fixed in 10% formalin or frozen with Tissue-Tek ® OCT[™] Compound (Japan) in dry ice and then embedded. Liver tissues were cut into sections and stained with H&E (5 µm per section) to assess hepatic steatosis. For Oil red O staining, frozen liver sections (4 µm) were stained with Oil red O (Sigma) for 30 minutes. The sections were counterstained with Mayer hematoxylin after destaining in 60% isopropanol. Liver sections were also stained with anti-IRF9 antibody (Santa Cruz, sc10793), anti-HNF4 antibody (Abcam, ab41898) and immunofluorescent secondary antibody (Invitrogen,

A11011). All digital images were obtained with a light microscope (Olympus DX51, Japan). IRF9-positive cells were counted with Image Pro Plus 6.0.

Metabolic studies and serum cytokine analyses

For GTTs, an i.p. injection of 1 g/kg glucose (Sigma-Aldrich Co. St. Louis, MO, USA) was administered. For ITTs, an i.p. injection of 0.75 U/kg insulin (Novolin R, Novo Nordisk Co., Bagsvaerd, Denmark) was administered. Blood glucose levels were measured with a glucometer (One Touch Ultra Easy, Life Scan) before glucose or insulin injection (after a 6-hour fast) and 15, 30, 60, and 120 minutes after injection. Serum fasting insulin was measured by ELISA (Millipore). The homeostasis model assessment of the IR index was calculated as HOMA-IR= [FBG (mmol/l) × FIns(mIU/l)]/22.5. Hepatic triglyceride, total cholesterol, and non-esterified fatty acid (NEFA) levels were determined using commercial kits (Wako). Triglycerides, total cholesterol, HDL-C, LDL-C, non-esterified fatty acid (NEFA), and β -hydroxybutyrate serum levels were determined using commercial kits (Wako and Abcam). Serum cytokines IL-1 β , IL-6, IL-4, TNF- α , MCP-1, IL-10, leptin, resistin, and adiponectin were measured by ELISA (R&D, MBL, RayBio, Invitrogen, Peprotech). Additional details are included in the Supporting table 1.

Assessment of liver function

The hepatic enzyme ALT, AST and ALP serum activities were measured with a spectrophotometer (Chemix 180i, Sysmex Shanghai Ltd.), according to the manufacturer's instructions. Additional details are included in the Supporting table 1.

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RNA isolation and quantitative real-time PCR

Total RNA from liver tissue and primary hepatocytes was isolated using TRIzol reagent (7950567275, Roche). Two micrograms of total RNA was used for cDNA synthesis with Transcriptor First Stand cDNA Synthesis Kit (04896866001, Roche). Quantitative real-time PCR reactions were performed in 20 μ l volumes (Light Cycler 480 SYBR Green I Master, 04887352001, Roche) using the LightCycler® 480 Real-time PCR system (Roche) according to the manufacturer's instructions. Gene expression levels were calculated after normalization to the standard housekeeping gene β -actin and expressed as relative mRNA levels compared with the internal control. See the Supporting table 2 for the primer pairs used.

Western blot

Liver tissue was lysed with RIPA lysis buffer containing 20 µl PMSF, 100 µl Complete protease inhibitors, 100 µl PhosSTOP, 50 µl NaF, and 10 µl Na₃VO₄. Total protein was quantified with the BCA kit (Thermo). Fifty micrograms of each protein sample was used for SDS/PAGE electrophoresis, followed by transfer to a PVDF membrane (Millipore). Protein expression levels were quantified and normalized to GAPDH as a loading control. All the antibodies used in this study are listed in the Supporting table 3.

Recombinant adenoviral vectors and in vivo adenovirus-mediated gene transfer

Hepatic IRF9 overexpression in two insulin-resistant mouse models (male C57BL/6 mice fed an HFD for 20 weeks and male *ob/ob* mice) was accomplished via jugular vein injection of GFP or Flag-tagged IRF9 (5×10^9 pfu) adenoviruses. PPAR α was overexpressed in the livers of 20-week,

HFD-fed C57BL/6 mice and IRF9 KO mice by jugular vein injection of GFP or Flag-tagged PPAR α adenoviruses (5×10⁹ pfu). Four weeks post-adenoviral injection, all the mice in each group were sacrificed, and the tissues were rapidly removed; one section of the liver was frozen in liquid nitrogen and stored at -80 °C for further analysis. Other sections were fixed for future histology analysis.

Primary hepatocyte isolation and adenoviral infection

Mice were anesthetized with 3% Pentobarbital Sodium. Livers were perfused in situ via the superior vena cava with perfusion buffer (0.9% saline), followed by digestion buffer (D Hanks' solution supplemented with 0.05% trypsin 25200, [GIBCO]). The cell suspension was sterile filtered and centrifuged at 50 g for 5 min to obtain hepatocytes. The hepatocytes were resuspended in DMEM media (15% FBS, 5 µg/ml insulin, 100 U/ml penicillin, 100 U/ml streptomycin) until the supernatant was clear. Hepatocyte viability was determined by trypan blue exclusion using the Countess (Invitrogen). Hepatocytes were seeded on collagen-coated dishes (Sigma) and cultivated for 24 h at 5% CO₂, 37 °C. The cultivation medium was replaced with fresh FBS-free media, and the cells were additionally incubated for 24 h before adenovirus administration. The cells were washed in PBS followed by incubation with the adenoviruses expressing mutant IRF9, Flag-tagged full-length IRF9 or PPARα for 24 h.

Plasmid Constructs

EGFP-myc-IRF9 recombinant was constructed by cloning encoding region of IRF9 gene of human into *Eco*RI and *Xho*I sites of the pEGFP-myc-C1. HA-IRF9 was PCR amplified with IRF9-5' and

IRF9-3' which were showed in Supporting Table 4. To obtain the IRF9 fragment consisting of residues 1 to 120, 210 to 393, and 120 to 220, HA-IRF9 were PCR amplified with IRF9-5' and IRF9-N1-3', IRF9-C1-5' and IRF9-3', respectively. The products were digested with *Eco*RI and *XhoI* and ligated into pEGFP-myc-C1 to create an in-frame fusion with EGFP-myc. To obtain IRF9 deletion mutant lacking 120-218, EGFP-myc-IRF9 was amplified with IRF9-dP-5' and IRF9-dP-3'. The resulting PCR products were phosphorylated with T4 PNK and then ligated to create EGFP-myc-IRF9 Δ 120-218. The Flag-PPAR α construct was generated by amplifying the encoding region of PPAR α gene with primers PPAR α -5' and PPAR α -3' from Myc-PPAR α and subcloning into pCMV-tag2B. To obtain the PPAR α fragment consisting of residues 1 to 101, 101-173, 173-278, and 278 to 468, Flag-PPAR α were PCR amplified with PPAR α -5' and PPAR α -A/B-3', PPAR α -C-5' and PPAR α -C-3', PPAR α -D-5' and PPAR α -D-3', PPAR α -E-5' and PPAR α -3', respectively. The products were digested with *Bam*HI and *SaI*I and ligated into pCMV-tag2B to create an in-frame fusion with Flag. All plasmids were verified by sequencing. The primers for making constructs are shown in the Supporting table 4.

Immunoprecipitation

For immunoprecipitation, cultured HepG2 cells were cotransfected with HA-IRF9 and FLAG-PPAR α for 48 h and lysed in NETN buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, and 0.5% NP-40) supplemented with protease inhibitor cocktail (Roche). Cell homogenates were incubated for 20 minutes at 4°C with constant agitation and then centrifuged (13,000 g for 10 minutes at 4°C). For each immunoprecipitation, 500 µl of the sample was incubated with 10 µl Protein A/G-agarose beads (11719394001, 11719386001, Roche) and 1 µg

antibody on a rocking platform (overnight at 4°C). according to the manufacturer's recommendations. Finally, immunoprecipitates were washed 5-6 times with cold NETN buffer before adding 1×loading buffer. Cell lysates and immunoprecipitates were immunoblotted using the indicated primary antibodies, the corresponding secondary antibodies and the SuperSignal chemiluminescence kit (Millipore).

GST pull-down assay

The GST-IRF9 constructed from pGEX-4T-1 was expressed in prokayotic (Rosetta (DE3) E. coli). For the pull-down assay, 10 ml E. coli (after IPTG induction) was harvested, and the purified GST fusion protein was immobilized on the glutathione-Sepharose 4B beads (GE healthcare Bio-Sciences AB). The GST-IRF9 beads were incubated with EGFP-Myc-PPARα-transfected 293T cell lysates in immunoprecipitation buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, and 0.5% NP-40 supplemented with protease inhibitor cocktail) for 4 hours at 4 °C. GST tag was used as the negative control under the same conditions. The samples were analyzed by western blot using anti-Myc antibodies after washing with immunoprecipitation lysis buffer (no cocktail) four times.

Confocal microscopy of primary mouse hepatocytes

Primary mouse hepatocytes were seeded in a 12-well plate containing cover slips. After pCherry-IRF9 and pEGFP-PPARα cotransfection for 48 h, the cells were fixed in 4% fresh paraformaldehyde for 15 minutes, permeabilized with 0.2% Triton X-100 in PBS for 5 minutes, then incubated in Image-ITTM FX signal enhancer (I36933, Invitrogen) for 30 minutes. The cells were washed with TBS-T three times and stained with DAPI (1 g/ml, 15 minutes). Finally, the slides were

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58 59 60 mounted with mounting solution (D2522, Sigma). Hepatocytes treated with secondary antibodies alone were used as a control. Images were obtained with a confocal laser-scanning microscope (Fluoview 1000; Olympus).

Supporting figure legends

Supporting Figure 1. IRF9 deficiency does not affect food intake but enhances hepatic gluconeogenesis.

(A) Energy of food intake by wild-type and IRF9 KO mice fed with the normal chow or HFD was calculated every week. n=28-39 per group. (B) Relative mRNA levels of glucogenesis genes PEPCK, G6Pase in liver extracts. n=6-12 per group. For all the statistical significance is indicated and compared with the WT HFD group, **p < 0.01.

Supporting Figure 2. IRF9 deficiency aggravates the hepatic steatosis upon HFD feeding.

(A) Liver function was examined by measuring the levels of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in the serum by reagent kits, n=7 per group. (B) Immunoblot analysis indicated the inhibition of AMPK signaling in liver extracts from IRF9 KO mice. Protein expression levels were quantified and normalized to loading control GAPDH. All values are expressed as the mean \pm SEM. The statistical significance is indicated and compared with the WT HFD group,**p<0.01, ***p < 0.001; compared with the WT NC group, ^{##}p < 0.01.

Supporting Figure 3. Hepatic IRF9 overexpression improves metabolism in diet-induced obese mice.

(A) Immunoblot showed the IRF9 expression in livers, <u>epididymal fat pads and gastrocnemius</u> <u>muscles</u> four weeks after jugular vein injection of adenovirus within IRF9 cDNA or GFP sequence control. (B) Representative immunofluorescent images of liver section slides, which were stained with antibodies against hepatic nuclear factor 4 (HNF4, green) and IRF9 (red). DAPI (blue) was used

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to show the nuclei. Scale bar indicates 50 μ m. (C) The levels of TG, cholesterol and NEFA were extracted in liver tissue from HFD group between WT with KO mice. n= 5 each group. (D) Liver function was examined by measuring the levels of ALT, AST and ALP in the serum by reagent kits, n=6 per group. (E) The mRNA levels of proinflammatory and anti-inflammatory markers in liver were measured by real-time PCR. n=12 for each group. All values are expressed as mean ± SEM. The statistical significance is indicated and compared with the GFP adenovirus injected group, *p < 0.05, **p < 0.01.

Supporting Figure 4. Hepatic IRF9 overexpression improves metabolism in *ob/ob* mice.

(A) Immunoblot showed the IRF9 expression in livers, epididymal fat pads and gastrocnemius muscles four weeks after jugular vein injection of adenovirus within IRF9 cDNA or GFP sequence control. (B) Representative immunofluorescent images of liver section slides, which were stained with antibodies against hepatic nuclear factor 4 (HNF4, green) and IRF9 (red). DAPI (blue) was used to show the nuclei. Scale bar indicates 50 μ m. (C) The levels of TG cholesterol and NEFA were extracted in liver tissue from HFD group between WT with KO mice, n= 5 each group. (D) Liver function was examined by measuring the levels of ALT, AST and ALP in the serum by reagent kits, n=7 per group. (E) The mRNA levels of proinflammatory and anti-inflammatory markers in liver were measured by real-time PCR. n=12 for each group. All values are expressed as mean ± SEM. The statistical significance is indicated and compared with the GFP adenovirus injected group, *p < 0.05, **p < 0.01.

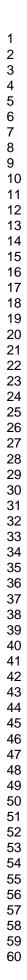
Supporting Figure 5. IRF9 interacts with PPARa to activate PPARa target genes.

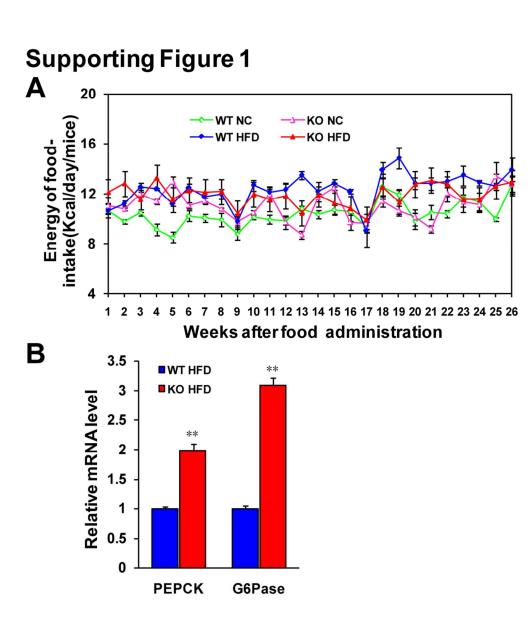
(A) The mRNA levels of PPAR α target genes in the primary mouse hepatocytes transfected with GFP or IRF9 plasmid are examined by real-time PCR. (B) The mRNA levels of PPAR α target genes in the primary mouse hepatocytes transfected with GFP or mutant IRF9 plasmid of which the PPAR α -interaction domain was deleted. (C) The mRNA levels of PPAR α target genes in the liver of WT mice fed with HFD injected with adenovirus containing GFP or IRF9. (D) The mRNA levels of PPAR α target genes in the liver of *ob/ob* mice injected with adenovirus containing GFP or IRF9. In (A) to (D), values are presented as the mean ± SEM, and statistical significance is indicated and compared with the Ad-GFP group, *p < 0.05, **p < 0.01.

Supporting Figure 6. Hepatic PPARα overexpression rescues deregulated metabolism in IRF9 KO mice.

(A) The mRNA levels of PPAR α target genes in primary mouse hepatocytes infected with adenovirus expressing IRF9 or vector controls. (B) Immunoblot showed the PPAR α expression in livers four weeks after jugular vein injection of adenovirus within PPAR α cDNA or GFP sequence control. (C) The mRNA levels of PPAR α and its target genes were determined by real-time PCR. (D) The levels of TG, cholesterol and NEFA were extracted in liver tissue from HFD group between WT with KO mice. n= 5 for each group. (E) Liver function was examined by measuring the levels of ALT, AST and ALP in the serum by reagent kits, n=7-9 per group. (F and G) The mRNA levels of proinflammatory and anti-inflammatory markers in livers of WT and IRF9 KO mice were measured by real-time PCR. n=9-12 for each group. All values are expressed as mean ± SEM. The statistical significance is indicated and compared with the GFP adenovirus injected WT group, *p < 0.05; compared with PPAR α adenovirus injected WT group, *p<0.05; compared with the GFP adenovirus

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Relative Protein Expression

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Ρ-ΑΜΡΚα

Τ-ΑΜΡΚα

Ρ-ΑΜΡΚβ1

Τ-ΑΜΡΚβ1

P-ACC2

T-ACC2

GAPDH

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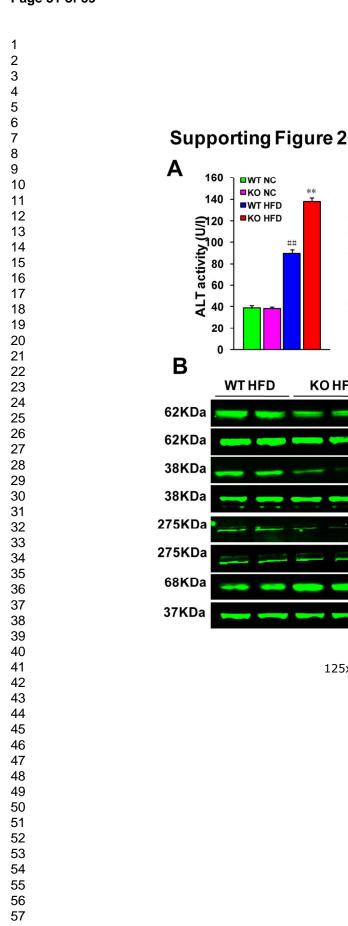
WT NC

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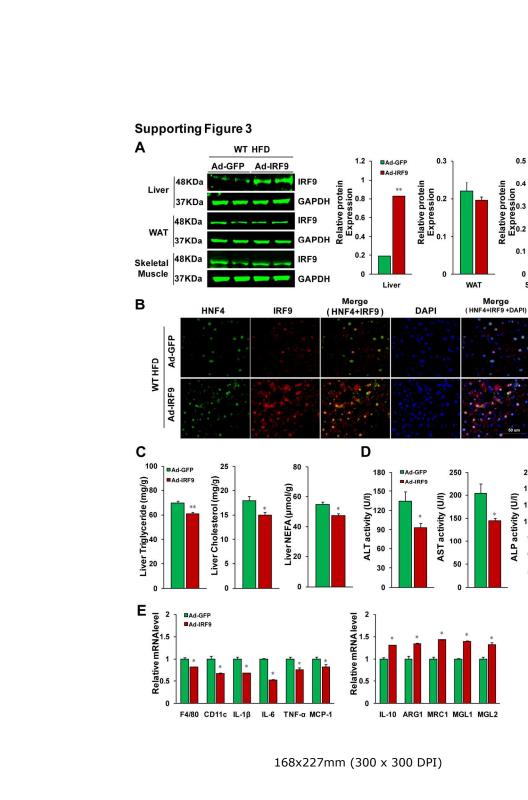
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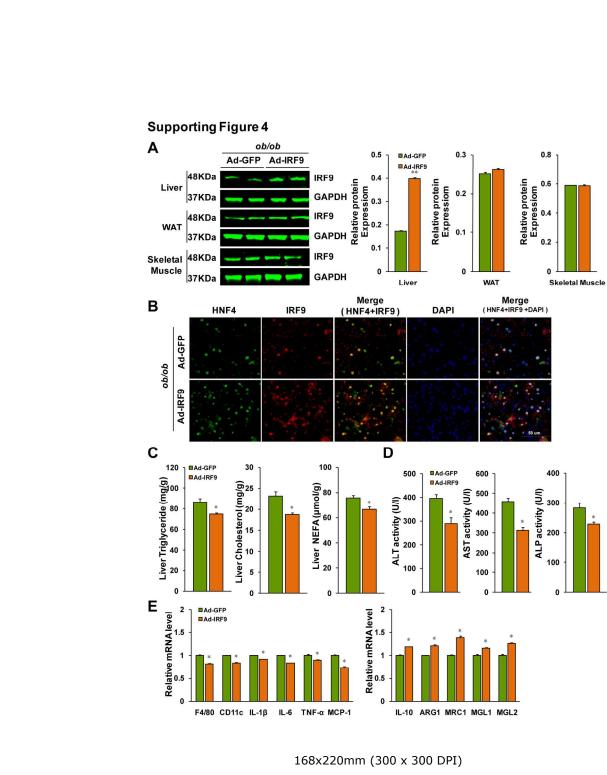
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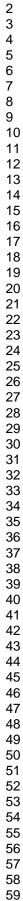
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ALP activity (U/I)

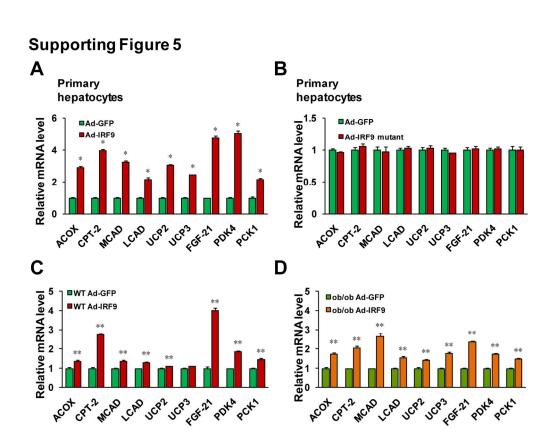
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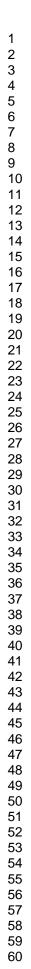


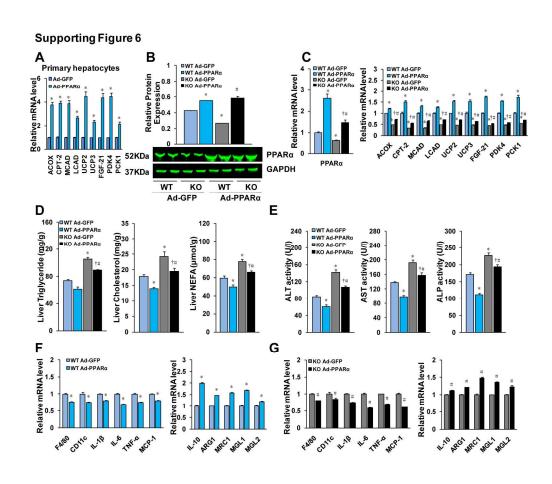






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Supporting Tables

Supporting table 1. Serum biochemical and cytokine, hormone analysis and liver function

anal	lysis	kits.
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Item	Manufacturer	Cat No.
Mouse CCL2/JE/MCP-1 Quantikine ELISA Kit	R&D	MJE00
Mouse Adiponectin ELISA Assay Kit	MBL international corporation	#JM-K4902-100
RayBio® Mouse IL-1βELISA Kit	RayBio	ELM-IL1beta-001
RayBio® Mouse Leptin ELISA Kit	RayBio	ELM-LEPTIN-00
RayBio® Mouse Resistin ELISA Kit	RayBio	ELM-Resistin-00
Mouse IL-10	Invitrogen	KMC0101
Mouse IL-4	Invitrogen	KMC0041
Mouse IL-6	Invitrogen	KMC0061
Mouse TNF-α	Peprotech	ADI-900-047
rat / mouse insulin ELISA kit	Millipore	EZRMI-13K
beta Hydroxybutyrate (beta HB) Assay Kit	abcam	ab83390
Cholesterol E, Total	Wako	439-17501
L-Type LDL-C Reagent 1	Wako	993-00404
L-Type LDL-C Reagent 2	Wako	999-00504
HDL-C/LDL-C Calibrator	Wako	990-28011
HDL-Cholesterol E	Wako	431-52501
L-Type TG M Enzyme Color A	Wako	461-08992
L-Type TG M Enzyme Color B	Wako	461-09092
Multi-Calibrator Lipid	Wako	464-01601
HR Series NEFA-HR(2) Color Reagent A	Wako	999-34691
HR Series NEFA-HR(2) Solvent A	Wako	995-34791
HR Series NEFA-HR(2) Color Reagent B	Wako	991-34891
HR Series NEFA-HR(2) Solvent B	Wako	993-35191
Wako NEFA Linearity Set	Wako	997-76491

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	NEFA Standard Solution	Wako	276-76491
	AST-R1	SYSMEX (Shanghai)	290505
	AST-R2	SYSMEX (Shanghai)	290506
	ALT-R1	SYSMEX (Shanghai)	290503
	ALT-R2	SYSMEX (Shanghai)	290504
	ALP-R1	SYSMEX (Shanghai)	290501
_	ALP-R2	SYSMEX (Shanghai)	290502

Supporting table 2. Primers for Real-time PCR detection.

Gene		Sequence5'3'
β-actin	forward	AGATCATTGCTCCTCCTGAGCGCA
	reverse	AAACGCAGCTCAGTAACAGTCCGC
IRF9	forward	ACAACTGAGGCCACCATTAGAGA
	reverse	CACCACTCGGCCACCATAG
PEPCK	forward	TGCCCCAGGCAGTGAGGAAGTT
	reverse	GTCAGTGAGAGCCAGCCAACAGT
G6Pase	forward	TCTGTCCCGGATCTACCTTG
	reverse	GCTGGCAAAGGGTGTAGTGT
HMGCR	forward	ATCATGTGCTGCTTCGGCTGCAT
	reverse	AAATTGGACGACCCTCACGGCT
LDLR	forward	ATGAGTGGCCACAGAACTGCC
	reverse	ATGCAGGAGCCATCTGCACACT
LXR-α	forward	TTGCCAAACAGCTCCCTGGCTT
	reverse	TTGATGAACTCCACCTGCAGCCCT
ABCA1	forward	AGGCACTCAAGCCACTGCTTGT
	reverse	TGCCTCTGCTGTCTAACAGCGT
ABCG1	forward	TGAACCCGTTTCTTTGGCACCG
	reverse	AGTCCCGCATGATGCTGAGGAA
ABCG5	forward	ACACCGGCATGCTCAATGCTGT
	reverse	AAATGACCGTGGCGATGACGCT
ABCG8	forward	AGGAAGTGCGTTGCGCATGT
	reverse	TCTTCCACCCGTTTGTCACGCT
CYP7A1	forward	TCAAAGAGCGCTGTCTGGGTCA
	reverse	TTTCCCGGGCTTTATGTGCGGT
SREBP-1c	forward	CACTTCTGGAGACATCGCAAAC
	reverse	ATGGTAGACAACAGCCGCATC
ΑССα	forward	GGCCAGTGCTATGCTGAGAT

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	reverse	AGGGTCAAGTGCTGCTCCA
FAS	forward	CTGCGGAAACTTCAGGAAATG
	reverse	GGTTCGGAATGCTATCCAGG
SCD1	forward	TCTTCCTTATCATTGCCAACACCA
	reverse	GCGTTGAGCACCAGAGTGTATCG
CD36	forward	TGGGTTTTGCACATCAAAGA
	reverse	GATGGACCTGCAAATGTCAGA
FABP1	forward	TGGTCCGCAATGAGTTCACCCT
	reverse	CCAGCTTGACGACTGCCTTGACTT
FATP1	forward	TGCACAGCAGGTACTACCGCAT
	reverse	TGCGCAGTACCACCGTCAAC
DGAT1	forward	TCCAGTGGGTTCCGTGTTTGCT
	reverse	ATAGCTCACAGCTTGCTGGGCA
DGAT2	forward	AGATCGCAGTGGGTGCGAAACT
	reverse	TTCCTGGTGGTCAGCAGGTTGT
GPAT	forward	TTCTCTTCACCGCCAGCAAGTCCT
	reverse	TCCTGCTCGTGTGGGTGATTGTGA
ATGL	forward	TGGATGGCGGCATTTCAGACA
	reverse	TGACGCGAAGCTCGTGGATGTT
HSL	forward	AAACGCAACGAGACAGGCCTCA
	reverse	ATGCCATGTTGGCCAGAGACGA
PPAR-α	forward	TATTCGGCTGAAGCTGGTGTAC
	reverse	CTGGCATTTGTTCCGGTTCT
ACOX	forward	CGGAAGATACATAAAGGAGACC
	reverse	AAGTAGGACACCATACCACCC
CPT-1a	forward	AGGACCCTGAGGCATCTATT
	reverse	ATGACCTCCTGGCATTCTCC
CPT-2	forward	CATCGTACCCACCATGCACT
	reverse	CTCCTTCCCAATGCCGTTCT
MCAD	forward	TGGCGTATGGGTGTACAGGG
	reverse	CCAAATACTTCTTTTTTTGTTGATCA
LCAD	forward	GGAGTAAGAACGAACGCCAA
	reverse	GCCACGACGATCACGAGAT
UCP2	forward	GCTGGTGGTGGTCGGAGATA
	reverse	ACTGGCCCAAGGCAGAGTT
UCP3	forward	TGCTGAGATGGTGACCTACGA
	reverse	CCAAAGGCAGAGACAAAGTGA
FGF-21	forward	AAGACACTGAAGCCCACCTG
	reverse	CTGCAGGCCTCAGGATCAAA

PDK4 forward TTCACACCTTCACCACATGC reverse AAAGGGCGGTTTTCTTGATG PCK1 forward CAGTCATCATCACCCAAGAGCA reverse GGGCGAGTCTGTCAGTTCAATAC F4/80 forward TCCTGCTGTGTCGTGCTGTTCA reverse ATCCCGCAATGATGGCACAAGC	1			
5reverseAAAGGGCGGTTTTCTIGAIG6PCK1forwardCAGTCATCATCACCCAAGAGCA7reverseGGGCGAGTCTGTCACTCACTCATAATAC9F4/80forwardTCCTGCTGTGTGGTGTGTCAATAC10reverseATCCCGCAATGATGGCACAAGC12CD11cforwardTTCCTGGCTGTTGGCTGTGCAGTT13reverseTGGACACTCCTGCTGTGCAGATA14IL-1βforwardCCGTGGACCTTCCAGGATGA15IL-1βforwardCCGTGGACCTTCCAGGAGAA16forwardAGTTGCCTTCTTGGGACTGA17IL-6forwardAGTTGCTTCTCAAAAATTCGAGTGACAAA18IL-6forwardCATCTTCTCAAAATTCGAGTGACAAA19reverseTGGGAGTAGACAAGGTACAACACC19reverseTGGGAGTAGACAAGGTACAAACC20TNF-αforwardTAAAAACCTGGATCGGAAACCAAA21reverseGCATTAGCTTCAGAATTACGGGT22reverseGCATTAGCTTAGGAAAATCG23MCP1forwardTCCACGGCTTAGCGAAAATCG24iNOSforwardTGCAGCACTGAGGAAACCTGAT25reverseATGGATGCTGCTGAGGGCTCTGTT26iNOSforwardACCCAGGCACCACACACAGAGGAGCACCA24forwardACCAGGACCACCACACTGACTGTT35MRC1forwardACACAGAAGGAGGACGACCACCA36reverseACCCAGGCCGTTTCCAGGCTT37MGL1forwardAACCCAGGAGCACCACCA38MGL1forwardAGCCCACTGCAGCGGATAACT32reverseGCTGGCTTTGCCCAGGCTGTGT38MGL	2 3	PDK4	forward	TTCACACCTTCACCACATGC
APCK1forwardCAGTCATCATCACCCAAGAGCA reverseGGCGAGTCTGTCAGTTCAATACreverseGGCGAGTCTGTCAGTTCAATACPAF4/80forwardTCCTGCTGTGCGTGCTGTTCA reverseAreverseATCCCGCAATGATGGCACAAGCCD11cforwardTTCCTGGCTGTTGGCTGTGGCAGTTIIreverseTGGACACTCCTGCTGTGCAGTTIIreverseGGGAACGTCACACACCAGCAIIreverseGGGAACGTCACACACCAGCAIIreverseGGGAACGTCACACACCAGCAIIreverseTCCACGATTTCCCAGAGAACIIreverseTCCACGATTTCCCAGAGAACIIreverseTCCACGATTTCCCAGAGAACAAAIIreverseTGGGAGTAGACAAGGTACAAACCCIITNF-αforwardIIreverseTGGGAGTAGACAAGGTACAAACCCIIreverseGCAACCTTATCGGAAACAAAIIreverseGCAAGCCTTATCGGAAACAAAAIIreverseGCAAGCCTTATCGGAAATGAIIreverseTTTCACAGGGGAGAAATCGIIiNOSforwardIIreverseATGGATGCTGCTGAGGAAAGCTGGTIIreverseACCCAGCACCACACACAGAGGACTGCTIIARG1forwardIIforwardAACCCAAGAGAGGAAGCACGCIIforwardAACCCAAGAGAGGACTGCGTIIforwardAACCCAAGAGAGGACTGCGTIIforwardAACCCAAGAGAGGACTGCGTIIforwardAACCCAAGAGAGGACTGCGTIIforwardAACCCAAGAGAGGACTGCGTIIforwardAACCCAAGAGAGCACGGGATAACTII	4 5		reverse	AAAGGGCGGTTTTCTTGATG
3FeverseGGGCGAGTCTGTGTCAGTTCAATAC9F4/80forwardTCCTGCTGTGTGGGTGTGTGTCA10reverseATCCCGCAATGATGGCACAAGC12CD11cforwardTTCCTGGCTGTTGGCTGTGGGT13reverseTGGACACTCCTGCTGTGCAGTT14IL-1βforwardCCGTGGACCTTCCAGGATGA15IL-1βforwardCGGTGGACCTTCCAGGATGA16reverseGGGAACGTCACACACACAGCA17IL-6forwardAGTTGCCTTCTTGGGACTGA18IL-6forwardCATCTTCTCAAAATTCGAGTGACAA19reverseTCGAGGATAGACAAGGTACAACCC20TNF-αforwardCATCTTCTCAAAATTCGAGTGACAA22reverseTGGGAGTAGACAAGGTACAACCC23MCP1forwardTAAAAACCTGGATCGGAAACCAAA24reverseGCAAGCCTTATCGGAAATGA25reverseTTTTCACAGGGGAGAAATCG26iNOSforwardTGCGCCTTTGCTCATGACATCGA27IL-10forwardTGCAGCACTGAGGAAAGCTGGT28reverseATGGATGCTGCTGAGGGACTGCTGTT32ARG1forwardTGCAGCACTGAGGAAAGCTGGT34reverseACCCAGCACCACACAGAGGAGGACTGCGT35MRC1forwardAACCCAAGAGAGGAGGACTGCGT36MGL1forwardAACCCAAGAGAGGAGGACCACCA39meverseATCCAATCACGGAGAGACCACCA34MGL2forwardAGCCACTGCAGCCGGATAACT34meverseATCCAATCACGAGAGCGACCACCA35MGL1forward36reverseACCCA	6	PCK1	forward	CAGTCATCATCACCCAAGAGCA
AF4/80forwardTCCTGCTGTGTCGTGCTGTTCA reverse10reverseATCCCGCAATGATGGCACAAGC12CD11cforwardTTCCTGGCTGTGGCTGTGGCAGTT13reverseTGGACACTCCTGCTGTGCAGTT14IL-1βforwardCCGTGGACCTTCCAGGATGA15IL-1βforwardCCGTGGACCTCCAGGACGA16reverseGGGAACGTCACACACACACAGCA17IL-6forwardAGTGCCTTCTTGGGACTGA18IL-6forwardCATCTTCCAAAATTCGAGTGACAA19reverseTCCACGATTTCCCAGAGAAC20TNF-αforwardCATCTTCTCAAAATTCGAGTGACAAA21TNF-αforwardTAAAAACCTGGATCGGAACCAAA22reverseTGGGAGTAGACAAGGTACAACCC23MCP1forwardCCAAGCCTTATCGGAAATGA24reverseTTTTCACAGGGGAGAAATCG25IL-10forwardTGCGCCTTTGCTCATGACATCGA26INOSforwardTGCAGCACTGAGGACAACGCGTGTT27IL-10forwardTGCAGCACTGAGGAAAAGCTGGT28reverseATGGATGCTGCTGAGGGACTGCTT29INOSforwardTGCAGCACTGAGGAAAGCTGGT36MRC1forwardACAACAGACAGGAGGACTGCGT37MGL1forwardAACCAAGAGCCTGGTAAAGCAGC38MGL1forwardAACCCAAGAGCCGGACAACCA39reverseATCCAATCACGGAGACGACCACCA34mGL2forwardAGGCCACTGCAGCCGGATAACT34reverseGCGGCTTTGCCCAGCCGGATAACT	7		reverse	GGGCGAGTCTGTCAGTTCAATAC
reverse ATCCCGCAATGATGGCACAAGC CD11c forward TTCCTGGCTGTTGGCTTGTGGT reverse TGGACACTCCGCGTGTGCAGTT IL-1β forward CCGTGGACCTTCCAGGATGA reverse GGGAACGTCACACACCAGCA IL-6 forward AGTTGCCTTCTTGGGACTGA reverse GGGAACGTCACACACCAGCA IL-6 forward AGTTGCCTTCTTGGGACTGA reverse TCCACGATTTCCCAGAGAAC O reverse TCCACGATTTCCCAGAGAAC CATCTTCTCAAAATTCGAGTGACAA reverse TGGGAGTAGACAAGGTACAACCC MCP1 forward TAAAAACCTGGAACGAAAAGGTACAACCC MCP1 forward TAAAAACCTGGAAACGAAACGAGAACCAAA reverse GCATTAGCTTATCGGAAATGA reverse TTTCACAGGGAGAAATCG forward TGCGCCTTGCTCATGACATCGAA RG1 forward TGCAGCACTGAGGAAATCG RG1 forward TGCAGCACTGAGAAAGCTGGT RG1 forward TGCAGCACTGAGAAAGCTGACTTGTT RG1 forward TGCAGCACTGAGAAAGCTGGT RG1 forward TGCAGCACTGAGAAAGCTGGT RG1 forward TGCAGCACTGAGAGACACGCACTT	8 9	F4/80	forward	
11CD11cforwardTTCCTGGCTGTTGGCTTGTGGT13reverseTGGACACTCCTGCTGTGCAGTT14IL-1βforwardCCGTGGACCTTCCAGGATGA15IL-1βforwardCCGTGGACCTCCAGCACCAGCA16reverseGGGAACGTCACACACCAGCA17IL-6forwardAGTTGCCTTCTTGGGACTGA18IL-6forwardAGTTGCCTTCTCGGACAGA19reverseTCCACGATTTCCCAGAGAAC20TNF-αforwardCATCTTCTCAAAATTCGAGTGACAA21TNF-αforwardTAAAAACCTGGATCGGAACCAAA22reverseGCATTAGCTTCAGATTTACGGGT23MCP1forwardTGCACCTTATCGGAAATGA24reverseGCATTAGCTTCAGATTACGGAGA25reverseTTTCACAGGGGAGAAATCG26IL-10forwardTGCGCCTTAGTCATGACATCGA27IL-10forwardTGCAGCACTGAGGAAAACCGA28iNOSforwardTGCAGCACTGAGGAAAAGCTGGT29iNOSforwardTGCAGCACTGAGGAAAGCTGGT31reverseATGGATGCTGCTGAGGGACAACCATT32ARG1forwardACAACAGACAGGAGGACTGCGT33reverseACCCAGCACCACACACAGAGAGCAGCACCACA34MGL1forwardAACCCAAGAGCCTGCAAAAGCAGC35MGL1forwardAGCCACTGCAGCCGGATAACT36MGL1forwardAGCCACTGCAGCCGGATAACT37reverseATCCAATCACGGAGCCGGCGGATAACT38MGL2forwardAGCCACTGCAGCCGGCTCTGTT39reverseATCCAATCACGGAGCCGCGGATAA	10	1 1/00		
13 reverse TGGACACTCCTGCTGTGCAGTT 14 IL-1β forward CCGTGGACCTTCCAGGATGA 15 IL-1β forward CCGTGGACCTTCCAGGATGA 16 reverse GGGAACGTCACACACCAGCA 17 IL-6 forward AGTTGCCTTCTTGGGACTGA 18 IL-6 forward AGTTGCCTTCTGGGACTGA 19 reverse TCCACGATTTCCCAGAGAAC 20 TNF-α forward CATCTTCTCAAAATTCGAGTGACAA 21 TNF-α forward CATCTTCTCAAAATTCGAGTGACAAA 22 reverse TGGGAGTAGACAAGGTACAAAGCCC 23 MCP1 forward TAAAAACCTGGATCGGAAACAAA 24 reverse GCAAGCCTTATCGGAAATGA 25 reverse GCAAGCCTTATCGGAAAATGA 26 IL-10 forward TGCACCATGAGGAGAAATCG 27 IL-10 forward TGGAACCATGAGAGAAATCG 28 reverse ATGAACAGGAGAGAAACCGA 29 iNOS forward TGCAACCACAGGAGAAAGCTGGT 31 reverse ATGGATGCTGCTGAGGGAAAGCTGGT 32 ARG1 forward	11	CD11c		
1InverseIn		CDITE		
6 reverse GGGAACGTCACACACACAGCA 78 IL-6 forward AGTTGCCTTCTTGGGACTGA 78 reverse TCCACGATTTCCCAGAGAAC 79 reverse TCCACGATTTCCCAGAGAAC 70 TNF-α forward CATCTTCTCAAAATTCGAGTGACAAA 72 reverse TGGGAGTAGACAAGGTACAACCC 73 MCP1 forward TAAAAACCTGGATCGGAACCAAA 74 reverse GCATTAGCTTCAGATTACGGGT 75 IL-10 forward CCAAGCCTTATCGGAAATGA 76 IL-10 forward TGCGCCTTTGCTCATGACATCGA 76 IL-10 forward TGCGCCTTTGCTCATGACATCGA 76 INOS forward TGCGCCTTTGCTCATGACATCGA 77 IL-10 forward TGCGCCTTTGCTCATGACATCGA 78 reverse ATGGATGCTGCTGAGGGACAATCG 79 iNOS forward TGCAGCACTGAGGAAAGCTGGT 78 reverse ATGGATGCTGCTGAGGAAAGCTGGT 79 ARG1 forward ACCCAGCACCACACAGGAGGACTGCGT 74 reverse ACCCATGCCGTTTCCAGCCTT 75 MRC1 <t< td=""><td>4</td><td>TT 10</td><td></td><td></td></t<>	4	TT 10		
InvesseGOOMACOTORICATECATORATIONAL18IL-6forwardAGTTGCCTTCTTGGGACTGA18reverseTCCACGATTTCCCAGAGAAC19reverseTGCACGATTTCCCAGAGTACAAACCC10TNF-αforwardCATCTTCTCAAAATTCGAGTGACAAA12reverseTGGGAGTAGACAAGGTACAACCC11MCP1forwardTAAAAACCTGGATCGGAACCAAA12reverseGCATTAGCTTCAGATTTACGGGT12IL-10forwardCCAAGCCTTATCGGAAATGA13reverseTTTTCACAGGGGAGAAATCG14reverseTTTTCACAGGGGAGAAATCG15MRC1forwardTGCAGCACTGAGGAAAGCTGGT16forwardTGCAGCACTGAGGAGACTGCGT17reverseACCCAGGACCACAGGAGGACTGCGT18MGL1forwardAACCCAAGAGCTGGAAAGCAGC19MGL2forwardAGGCCACTGCAGCCGGATAACT10MGL2forwardAGGCCACTGCAGCCGGATAACT19MGL2forwardAGGCCACTGCAGCCGGATAACT10MGL2forwardAGGCCACTGCAGCCGGATAACT10MGL2forwardAGCCATGCCGTTTGCCCAGCTTGTT		IL-IB	forward	
8IL-6forwardAGTTGCCTTCTTGGGACTGA9reverseTCCACGATTTCCCAGAGAAC20TNF-αforwardCATCTTCTCAAAATTCGAGTGACAA22reverseTGGGAGTAGACAAGGTACAACCC23MCP1forwardTAAAAACCTGGATCGGAACCAAA24reverseGCATTAGCTTCAGATTACGGGT25IL-10forwardCCAAGCCTTATCGGAAATGA26IL-10forwardCCAAGCCTTGCTCATGACATCG27IL-10forwardTGCGCCTTTGCTCATGACATCGA28reverseTTTTCACAGGGGAGAAATCG29iNOSforwardTGCGCCTTTGCTCATGACATCGA29iNOSforwardTGCAGCACTGAGGAAAGCTGGT20reverseATGGATGCTGCTGAGGGACAGCACGAGA21reverseACCCAGCACCACACAGAGGAGGACTGCGT22MRC1forwardACAACAGACAGGAGGACTGCGT23MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC24reverseATCCAATCACGGAGACGACCACCA25MGL1forwardAGCCAATGACGGAGACGACCACCA26reverseATCCAATCACGGAGACGACCACCA27reverseACCCATGCAGCCGGATAACT28MGL2forwardAGCCACTGCAGCCGGATAACT29reverseACCCATGCAGCCGGATAACT20reverseACCCAAGCAGCGGACGACCACCA21reverseACCCATGCAGCCGGATAACT22reverseGCTGGCTTTGCCCAGCTCTGTT			reverse	GGGAACGTCACACACCAGCA
20 21TNF-αforwardCATCTTCTCAAAATTCGAGTGACAA reverse22reverseTGGGAGTAGACAAGGTACAACCC23MCP1forwardTAAAAACCTGGATCGGAACCAAA reverse24reverseGCATTAGCTTCAGATTTACGGGT25IL-10forwardCCAAGCCTTATCGGAAATGA reverse26IL-10forwardCCAAGGGGAGAAATCG27iNOSforwardTGCGCCTTTGCTCATGACATCGA reverse28reverseATGGATGCTGCTGAGGGCTCTGTT29iNOSforwardTGCAGCACTGAGGACAACGAGA reverse30reverseATGGATGCTGCTGAGGACATCGA reverse31reverseACCCAGCACCACACAGAGGAGACTGCGT reverse32ARG1forwardACAACAGACAGGAGGACTGCGT reverse33mRC1forwardACAACAGACAGGAGGACTGCGT reverse34MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC reverse35MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC reverse36MGL1forwardAGGCCACTGCAGCCGGATAACT reverse37mGL2forwardAGGCCACTGCAGCCGGATAACT38MGL2forwardAGGCCACTGCAGCCGCGATAACT		IL-6	forward	AGTTGCCTTCTTGGGACTGA
INF-αforwardCATCITICICAAAATTCGAGTGACAAreverseTGGGAGTAGACAAGGTACAACCCMCP1forwardTAAAAACCTGGATCGGAACCAAAreverseGCATTAGCTTCAGATTTACGGGTIL-10forwardCCAAGCCTTATCGGAAATGAreverseTTTTCACAGGGGAGAAATCGINOSforwardTGCGCCTTTGCTCATGACATCGAreverseATGGATGCTGCTGAGGGCTCTGTTARG1forwardTGCAGCACTGAGGAAAGCTGGTreverseACCCAGCACCACACTGACTCTTMRC1forwardACAACAGACAGGAGACAGCGCGTreverseMGL1forwardAACCCAAGAGCCTGGTAAAGCAGCreverseATCCAATCACGGAGACGACCACCAreverseATCCAATCACGGAGACGACCACCAreverseACCCATGCCGTTTCCAGCCTTMGL2MGL2forwardAGCCACTGCAGCCGGATAACTreverseACCCATGCCGCTTTGCCCAGCCTGTT			reverse	TCCACGATTTCCCAGAGAAC
reverseTGGGAGTAGACAAGGTACAACCCMCP1forwardTAAAAACCTGGATCGGAACCAAAreverseGCATTAGCTTCAGATTTACGGGTIL-10forwardCCAAGCCTTATCGGAAATGAreverseTTTTCACAGGGGAGAAATCGiNOSforwardTGCGCCTTTGCTCATGACATCGAreverseATGGATGCTGCTGAGGGCTCTGTTARG1forwardTGCAGCACTGAGGAAAAGCTGGTreverseACCCAGCACCACACAGGAGAACTGCTMRC1forwardACCCATGCCGTTTCCAGCCTTMGL1forwardAACCCAAGAGCCTGGTAAAGCAGCMGL2forwardAGCCACTGCAGCCGGATAACTreverseATCCAATCACGGAGACCACCACACCACACCACACCACAC		TNF-α	forward	CATCTTCTCAAAATTCGAGTGACAA
MCP1forwardFAAAAACC FGGATCGGAACCGAAC126reverseGCATTAGCTTCAGATTTACGGGT126IL-10forwardCCAAGCCTTATCGGAAATGA127reverseTTTTCACAGGGGAGAAATCG128reverseTTTTCACAGGGGAGAAATCG129iNOSforwardTGCGCCTTTGCTCATGACATCGA120reverseATGGATGCTGCTGAGGGCTCTGTT121ARG1forwardTGCAGCACTGAGGAAAGCTGGT122ARG1forwardTGCAGCACCACACACTGACTCTT133reverseACCCAGCACCACACAGGAGGACTGCGT14reverseACCCATGCCGTTTCCAGCCTT15MRC1forwardACAACAGACAGGAGGACTGCGT16reverseAACCCATGCCGTTTCCAGCCTT17MGL1forwardAGGCCACTGCAGCCGGATAACT12reverseGCTGGCTTTGCCCAGCTGTTAACT			reverse	TGGGAGTAGACAAGGTACAACCC
reverse GCATTAGCTTCAGATTTACGGGT IL-10 forward CCAAGCCTTATCGGAAATGA reverse TTTTCACAGGGGAGAAATCG iNOS forward TGCGCCTTTGCTCATGACATCGA reverse ATGGATGCTGCTGAGGGCTCTGTT ARG1 forward TGCAGCACTGAGGAAAGCTGGT reverse ACCCAGCACCACACTGACTCTT MRC1 forward ACAACAGACAGGAGGACTGCGT reverse AACCCATGCCGTTTCCAGCCTT MGL1 forward ACCCAAGAGCCGGATAACT reverse ATCCAATCACGGAGACGACCACCA MGL2 forward AGGCCACTGCAGCCGGATAACT reverse GCTGGCTTTGCCCAGCTCTGTT		MCP1	forward	TAAAAACCTGGATCGGAACCAAA
IL-10forwardCCAAGCCTTATCGGAAATGA reverse77iNOSforwardTGCGCCTTATCGGAGAAATCG89iNOSforwardTGCGCCTTTGCTCATGACATCGA reverse60reverseATGGATGCTGCTGAGGGCTCTGTT72ARG1forwardTGCAGCACTGAGGAAAGCTGGT reverse73reverseACCCAGCACCACACTGACTCTT74forwardACAACAGACAGGAGGACTGCGT reverse75MRC1forwardACAACAGACAGGAGGACTGCGT reverse76MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC reverse76MGL2forwardAGGCCACTGCAGCCGGATAACT reverse72reverseGCTGGCTTTGCCCAGCTCTGTT			reverse	
reverse TTTTCACAGGGGAGAAATCG iNOS forward TGCGCCTTTGCTCATGACATCGA reverse ATGGATGCTGCTGAGGGCTCTGTT ARG1 forward TGCAGCACTGAGGAAAGCTGGT reverse ACCCAGCACCACACTGACTCTT MRC1 forward ACAACAGACAGGAGGACTGCGT reverse AACCCATGCCGTTTCCAGCCTT MGL1 forward AACCCAAGAGCCGGAGAAGCAGC reverse ATCCAATCACGGAGACCACCA MGL2 forward AGGCCACTGCAGCCGGATAACT reverse GCTGGCTTTGCCCAGCTCTGTT		П 10		
iNOS forward TGCGCCTTTGCTCATGACATCGA reverse ATGGATGCTGCTGAGGGCTCTGTT ARG1 forward TGCAGCACTGAGGAAAGCTGGT reverse ACCCAGCACCACACTGACTCTT MRC1 forward ACAACAGACAGGAGGACTGCGT reverse AACCCATGCCGTTTCCAGCCTT MGL1 forward AACCCAAGAGCCTGGTAAAGCAGC reverse ATCCAATCACGGAGACGACCACCA MGL2 forward AGGCCACTGCAGCCGGATAACT reverse GCTGGCTTTGCCCAGCTCTGTT	27	1L-10		
30INOSIonwardIOCOCCTITICCICATOACATCOA31reverseATGGATGCTGCTGAGGGCTCTGTT32ARG1forwardTGCAGCACTGAGGAAAGCTGGT33reverseACCCAGCACCACACAGGACTGCGT34reverseACCCAGGACAGGAGGACTGCGT35MRC1forwardACAACAGACAGGAGGACTGCGT36reverseAACCCATGCCGTTTCCAGCCTT37MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC38MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC39reverseATCCAATCACGGAGACGACCACCA40MGL2forwardAGGCCACTGCAGCCGGATAACT42reverseGCTGGCTTTGCCCAGCTCTGTT				
reverseATGGATGCTGCTGAGGGCTCTGTT2ARG1forwardTGCAGCACTGAGGAAAGCTGGT3reverseACCCAGCACCACACTGACTCTT4reverseACCCAGCACCACAGGAGGACTGCGT55MRC1forwardACAACAGACAGGAGGACTGCGT66reverseAACCCATGCCGTTTCCAGCCTT77MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC88MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC99reverseATCCAATCACGGAGACGACCACCA1MGL2forwardAGGCCACTGCAGCCGGATAACT2reverseGCTGGCTTTGCCCAGCTCTGTT		iNOS	forward	TGCGCCTTTGCTCATGACATCGA
reverse ACCCAGCACCACACTGACTCTT MRC1 forward ACAACAGACAGGAGGACTGCGT reverse AACCCATGCCGTTTCCAGCCTT MGL1 forward AACCCAAGAGCCTGGTAAAGCAGC reverse ATCCAATCACGGAGACGACCACCA MGL2 forward AGGCCACTGCAGCCGGATAACT reverse GCTGGCTTTGCCCAGCTCTGTT			reverse	ATGGATGCTGCTGAGGGCTCTGTT
AreverseACCCAGCACCACACTGACTCTT35MRC1forwardACAACAGACAGGAGGACTGCGT36reverseAACCCATGCCGTTTCCAGCCTT37MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC38MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC39reverseATCCAATCACGGAGACGACCACCA40MGL2forwardAGGCCACTGCAGCCGGATAACT42reverseGCTGGCTTTGCCCAGCTCTGTT		ARG1	forward	TGCAGCACTGAGGAAAGCTGGT
MRC1 forward ACAACAGACAGGAGGACTGCGT reverse AACCCATGCCGTTTCCAGCCTT MGL1 forward AACCCAAGAGCCTGGTAAAGCAGC reverse ATCCAATCACGGAGACGACCACCA MGL2 forward AGGCCACTGCAGCCGGATAACT reverse GCTGGCTTTGCCCAGCTCTGTT			reverse	ACCCAGCACCACACTGACTCTT
36reverseAACCCATGCCGTTTCCAGCCTT37MGL1forwardAACCCAAGAGCCTGGTAAAGCAGC39reverseATCCAATCACGGAGACGACCACCA40MGL2forwardAGGCCACTGCAGCCGGATAACT42reverseGCTGGCTTTGCCCAGCTCTGTT		MRC1	forward	ACAACAGACAGGAGGACTGCGT
MGL1 forward AACCCAAGAGCCTGGTAAAGCAGC reverse ATCCAATCACGGAGACGACCACCA MGL2 forward AGGCCACTGCAGCCGGATAACT reverse GCTGGCTTTGCCCAGCTCTGTT	86		reverse	
39 reverse ATCCAATCACGGAGACGACCACCA 40 MGL2 forward AGGCCACTGCAGCCGGATAACT 42 reverse GCTGGCTTTGCCCAGCTCTGTT		MGI 1		
MGL2 forward AGGCCACTGCAGCCGGATAACT reverse GCTGGCTTTGCCCAGCTCTGTT		INIGE1		
reverse GCTGGCTTTGCCCAGCTCTGTT				
		MGL2	forward	
			reverse	GCTGGCTTTGCCCAGCTCTGTT

Supporting table 3. Antibodies for immunoblot analyses.

	Protein interaction			Sources of	Molecular Weight	
Antibody	sites	Cat No.	Manufacturer	species	(KDa)	Dilution
GAPDH		MB001	Bioworld	mouse	37	1/10000
IRF9		sc10793	santa	rabbit	48	1/200
PPARα		ab8934	abcam	rabbit	52	1/500
Ρ-ΑΜΡΚα	thr172	2535	cst	rabbit	62	1/1000

T-AMPKβ4150cstrabbit381/1000P-ACC2ser219/ser221sc-30446-Rsantarabbit2801/200T-ACC23676cstrabbit2801/1000SREBP-1cab3259abcammouse120,601/1000P-IRS-1Tyr60809-432milliporerabbit1701/1000T-IRS-12382cstrabbit1801/1000P-AKTser4734060cstrabbit601/1000	219/ser221	4150 sc-30446-R 3676	cst santa cst	rabbit rabbit rabbit	38 280 280	1/1000
P-ACC2 ser219/ser221 sc-30446-R santa rabbit 280 1/200 T-ACC2 3676 cst rabbit 280 1/1000 SREBP-1c ab3259 abcam mouse 120,60 1/1000 P-IRS-1 Tyr608 09-432 millipore rabbit 170 1/1000 T-IRS-1 2382 cst rabbit 180 1/1000 P-AKT ser473 4060 cst rabbit 60 1/1000	219/ser221	sc-30446-R 3676	santa cst	rabbit rabbit	280 280	1/200 1/1000
T-ACC2 3676 cst rabbit 280 1/1000 SREBP-1c ab3259 abcam mouse 120,60 1/1000 P-IRS-1 Tyr608 09-432 millipore rabbit 170 1/1000 T-IRS-1 2382 cst rabbit 180 1/1000 P-AKT ser473 4060 cst rabbit 60 1/1000		3676	cst	rabbit	280	1/1000
SREBP-1c ab3259 abcam mouse 120,60 1/1000 P-IRS-1 Tyr608 09-432 millipore rabbit 170 1/1000 T-IRS-1 2382 cst rabbit 180 1/1000 P-AKT ser473 4060 cst rabbit 60 1/1000						
P-IRS-1 Tyr608 09-432 millipore rabbit 170 1/1000 T-IRS-1 2382 cst rabbit 180 1/1000 P-AKT ser473 4060 cst rabbit 60 1/1000		ab3259	abcam	moura	100 (0	
T-IRS-1 2382 cst rabbit 180 1/1000 P-AKT ser473 4060 cst rabbit 60 1/1000			uovum	mouse	120,60	1/1000
P-AKT ser473 4060 cst rabbit 60 1/1000	r608	09-432	millipore	rabbit	170	1/1000
		2382	cst	rabbit	180	1/1000
T-AKT 4691 cst rabbit 60 1/1000	:473	4060	cst	rabbit	60	1/1000
		4691	cst	rabbit	60	1/1000
		4691	cst	rabbit	60	1/100
	[4	473	2382 473 4060 4691	2382 cst 473 4060 cst	473 4060 cst rabbit 4691 cst rabbit	2382 cst rabbit 180 473 4060 cst rabbit 60 4691 cst rabbit 60

Supporting table 4. The primers for making constructs.

Primer name	Primer sequence (5'-3')
IRF9-5'	CCG <u>GAATTC</u> [#] ATGGCATCAGGCAGGGCACG
	CCG <u>CTCGAG</u> CTACACCAGGGACAGAATGGC
IRF9-3'	TG
	CCG <u>CTCGAG</u> CTAGACGATTCCTGGTGGCAGC
IRF9-N1-3'	A
IRF9-C1-5'	CCG <u>GAATTC</u> TTTCTGCTTCCTCCAGAGCC
IRF9-N2-5'	CCG <u>GAATTC</u> GTCTCTGGCCAGCCAGGGAC
	CCG <u>CTCGAG</u> CTACAGTGAGTAGTCTGGCTCT
IRF9-N2-3'	G
IRF9-dP-5'	TCACTGCTGCTCACCTTCATC
IRF9-dP-3'	GATTCCTGGTGGCAGCAACTG
PPARa-5'	CGC <u>GGATCC</u> ATGGTGGACACGGAAAGCCC
PPARa-3'	ACGC <u>GTCGAC</u> TCAGTACATGTCCCTGTAGAT
	ACGC <u>GTCGAC</u> TCATTCGATGTTCAATGCTCC
PPARa-A/B-3'	AC
PPARa-C-5'	CGC <u>GGATCC</u> GAATGTAGAATCTGCGGGGA
	ACGC <u>GTCGAC</u> TCAAAAACGAATCGCGTTGT
PPARa-C-3'	GTG
PPARα-D-5'	CGC <u>GGATCC</u> TTTGGACGAATGCCAAGATC
	ACGC <u>GTCGAC</u> TCAGCACTGGCAGCAGTGAA
PPARα-D-3'	AGA
PPARα-E-5'	CGC <u>GGATCC</u> TGCACGTCAGTGGAGACCGT

[#]Sites for restriction enzyme are underlined.