Supplementary Material

Role of white adipose lipolysis in the development of NASH induced by methionine- and choline-deficient diet

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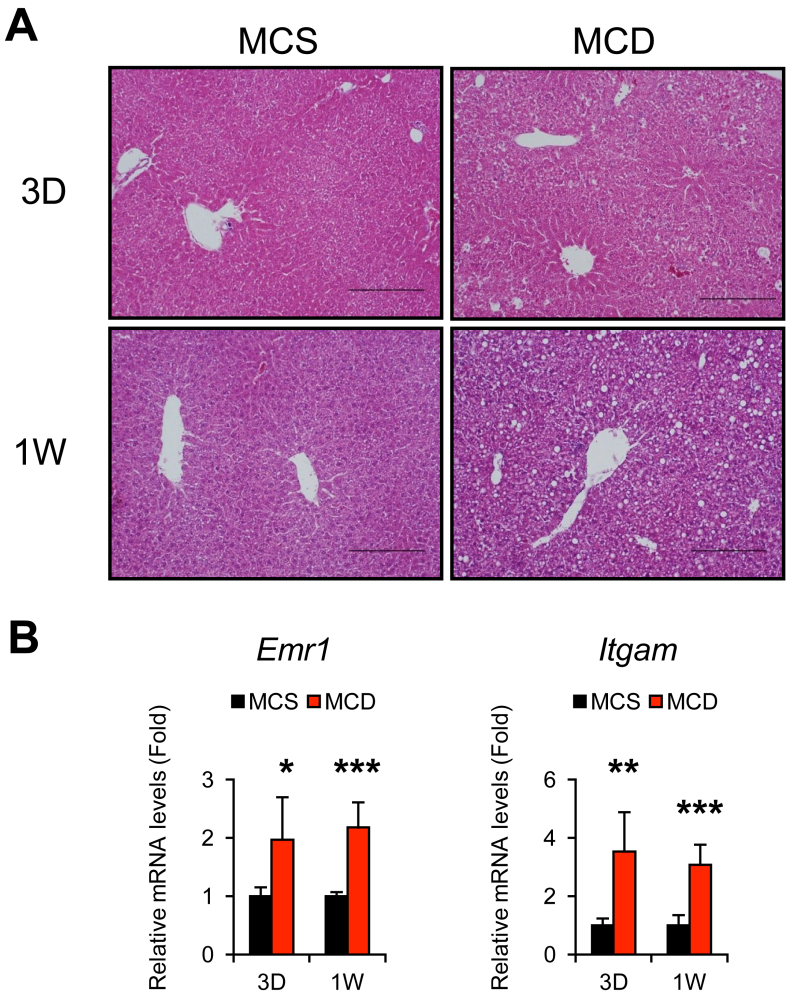
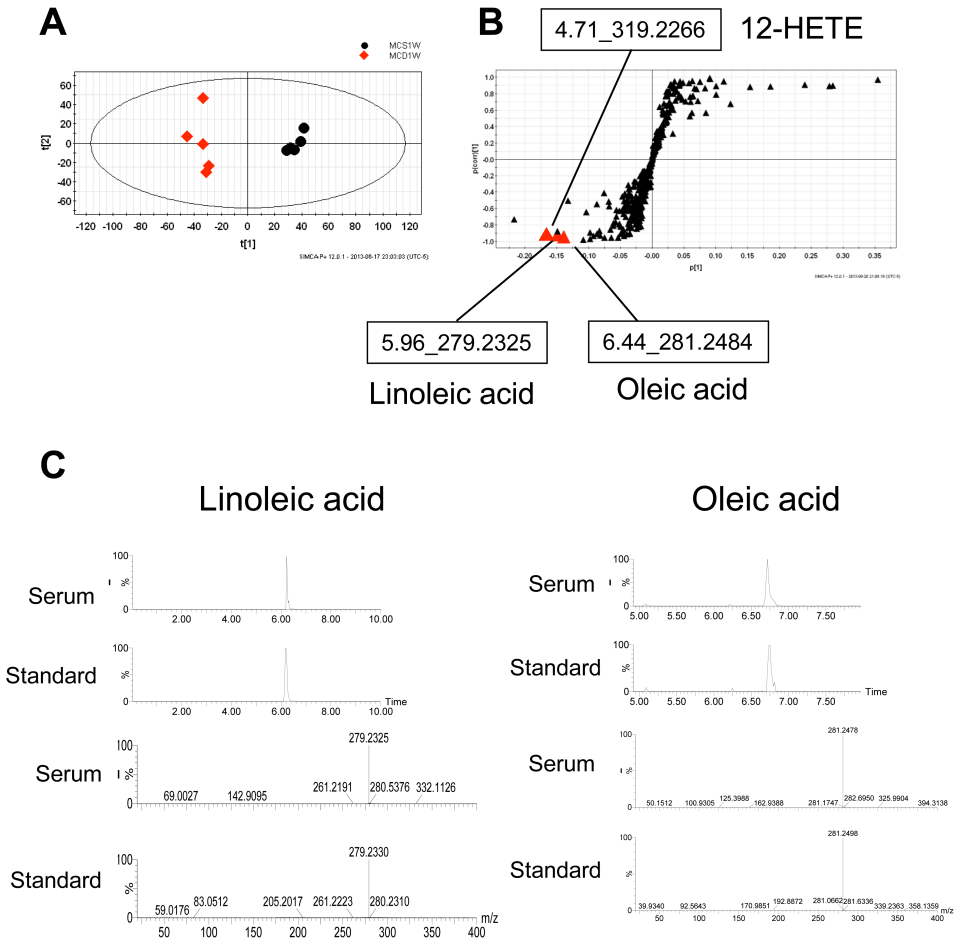
Numbers of Supplementary Figures and Tables: 6 figures and 2 tables.



Supplementary Fig. 1. Phenotypic changes in mice with 3-day or 1-week MCD treatment.

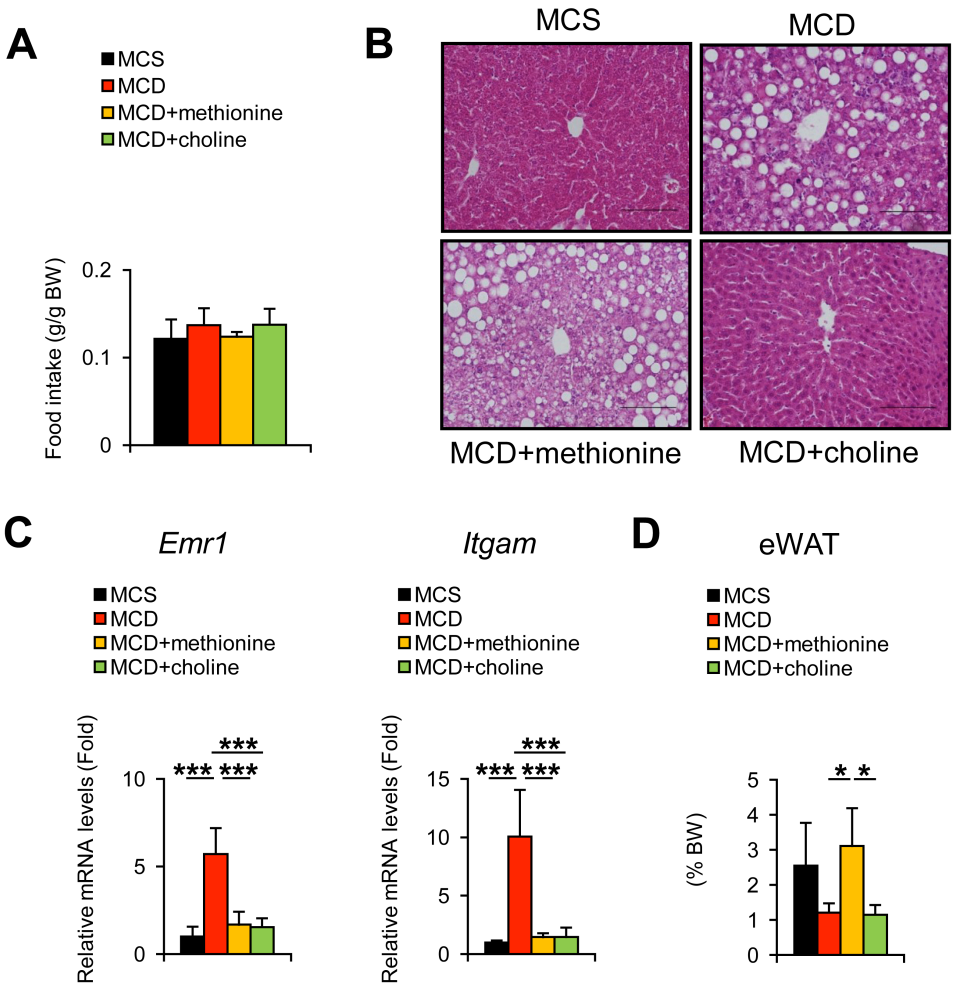
(A) Liver histology. Hematoxylin and eosin staining; Bar = 200 μm.
(B) The mRNA levels of *Emr1* and *Itgam*. The mRNA levels were normalized to those of 18S ribosomal mRNA and subsequently normalized to those of MCS-treated mice.

Statistical analysis was performed using the Student's *t*-test. n = 5 /group. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. MCS-treated mice in the same time point. The full terms of the gene names are listed in Supplementary Table 2.



Supplementary Fig. 2. Serum metabolomic analysis of mice with 1-week MCD treatment.

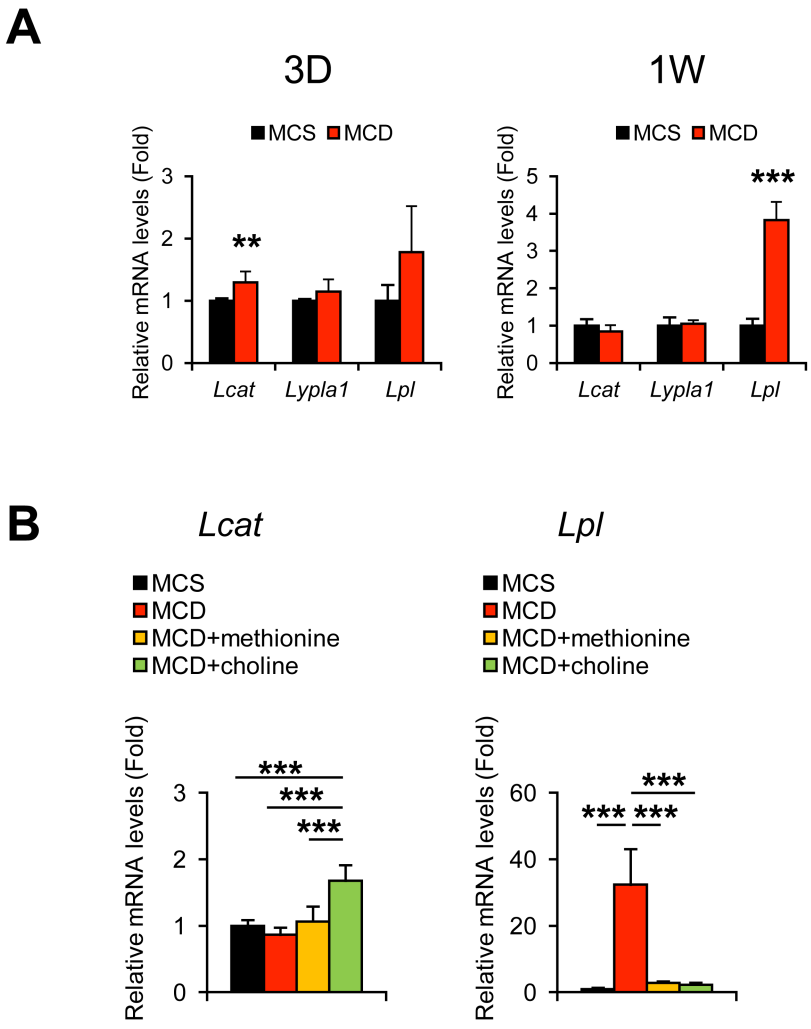
(A) PCA of serum metabolites between mice treated with 1-week MCD (red diamond, n = 5) and MCS (black circle, n = 5).
(B) S-plot of OPLA analysis using the same samples as (A). Retention time and molecular mass were indicated.
(C) MS/MS fragmentation patterns of oleic acid and linoleic acid.



Supplementary Fig.3. The effect of supplementation of methionine or choline to the phenotypic changes in mice with 2-week MCD treatment.

(A) Food intake.
(B) Liver histology. Hematoxylin and eosin staining; Bar = 100 μm.
(C) The mRNA levels of *Emr1* and *Itgam*. The mRNA levels were normalized to those of 18S ribosomal mRNA and subsequently normalized to those of MCS-treated mice.
(D) Epididymal WAT weight.

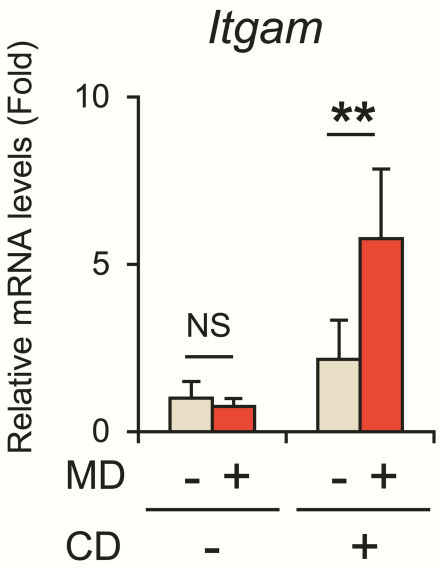
Statistical analysis was performed using the one-way ANOVA test with Bonferroni's correction. n = 5 /group; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.



Supplementary Fig. 4. The changes in mRNAs encoding *Lcat*, *Lypla1*, and *Lpl* in mice treated with MCD.

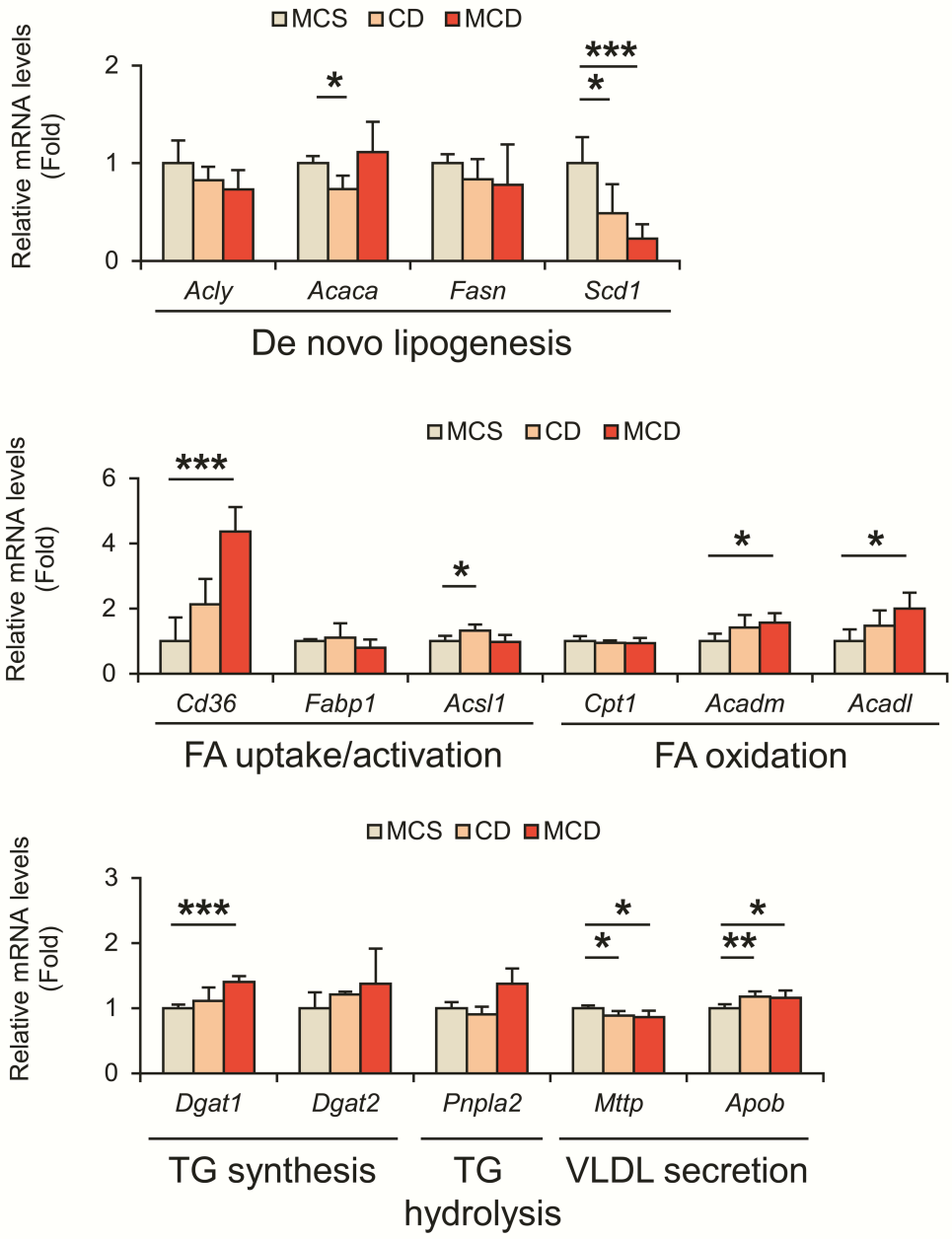
(A) The mRNA levels of mice treated with MCD for 3 days and 1 week. The same samples used in Supplementary Fig. 1 were conducted. Statistical analysis was performed using the Student's *t*-test. n = 5 /group. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. MCS-treated mice in the same time point.

(B) The effect of supplementation of methionine or choline on the levels of mRNAs encoding *Lcat* and *Lpl* in mice with 2-week MCD treatment. The same samples used in Fig. 4 and 5 were assayed. Statistical analysis was performed using the one-way ANOVA test with Bonferroni's correction. n = 5 /group. *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001.



Supplementary Fig. 5. The effect of dietary methionine deficiency on hepatic mRNA levels of *Itgam*.

The same samples used in Fig. 7 were conducted. The mRNA levels were normalized to those of 18S ribosomal mRNA and subsequently normalized to those of MCS-treated mice.
Statistical analysis was performed using the Student's *t*-test. $^{**}P<0.01$ vs. CD-treated mice (designated as CD+ MD-); NS, not significant.



Supplementary Fig. 6. The changes in mRNAs encoding genes associated with steatogenesis in mice treated with CD and MCD for 2 weeks.

The same samples in Fig. 7 were used for this qPCR analysis. The mRNA levels were normalized to those of 18S ribosomal mRNA and subsequently normalized to those of MCS-treated mice.
Statistical analysis was performed using the Student's *t*-test. n = 4-5 /group. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. MCS-treated mice. Full terms of the gene names were listed in Supplementary Table 2. FA, fatty acid; TG, triglyceride; VLDL, very-low-density lipoprotein.

Supplementary Table 1. Composition of the diet used in this study

	MCS	MCD	CD	MD
Cornstarch	100	100	100	100
Dextrin	100	100	100	100
Sucrose	392	409	407	394
Cellulose	50	50	50	50
Corn oil	50	50	50	50
Primex	100	100	100	100
Salt	35	35	35	35
Sodium bicarbonate	4.3	4.3	4.3	4.3
Vitamin mix	10	10	10	10
Choline Bitartrate	14.5	0	0	14.5
Ferric Citrate, U.S.P.	0.3	0.1	0.3	0.3
Amino acid	144	142	144	142
L-Alanine	5.1	5.1	5.1	5.1
L-Arginine	12.7	12.7	12.7	12.7
L-Aspartic Acid	15.8	15.8	15.8	15.8
L-Cystine	3.7	3.7	3.7	3.7
L-Glutamic Acid	28.9	28.9	28.9	28.9
Glycine	6.2	6.2	6.2	6.2
L-Histidine	3.4	3.4	3.4	3.4
L-Isoleucine	6.1	6.1	6.1	6.1
L-Leucine	10.5	10.5	10.5	10.5
L-Lysine-HCl	9.1	9.1	9.1	9.1
L-Methionine	1.7	0	1.7	0
L-Phenylalanine	7.3	7.3	7.3	7.3
L-Proline	7.6	7.6	7.6	7.6
L-Serine	7.2	7.2	7.2	7.2
L-Threonine	4.6	4.6	4.6	4.6
L-Tryptophan	1.8	1.8	1.8	1.8
L-Tyrosine	5.7	5.7	5.7	5.7
L-Valine	6.3	6.3	6.3	6.3

Values indicate gram/kilogram of diet.

Supplementary Table 2. Primer pairs used for qPCR

Gene	GenBank Accession Number		Primer Sequence (5'-3')
18S	NR_003278	F	ATTGGAGCTGGAATTACCGC
		R	CGGCTACCACATCCAAGGAA
Acadl	NM_007381	F	TCTTGCGATCAGCTCTTTCA
		R	GGTACATGTGGGAGTACCCG
Acadm	NM_007382	F	AGCTCTAGACGAAGCCACGA
		R	GCGAGCAGAAATGAAACTCC
Acaca	NM_133360	F	TGGTGCAGAGGTACCGAAGTG
		R	CGTAGTGGCCGTTCTGAAACT
Acly	NM_134037	F	GTGGCCCCAACTATCAAGAG
		R	AATGGCCGTCATGTGAGTTT
Acsl1	NM_007981	F	CTTCCAACCAACACCCTCAT
		R	ACCATCAGTGGTACCCGCTA
Apob	NM_009693	F	AAGCACCTCCGAAAGTACGTG
		R	CTCCAGCTCTACCTTACAGTTGA
Adrb1	NM_007419	F	GCTGATCTGGTCATGGGATT
		R	AAGTCCAGAGCTCGCAGAAG
Adrb2	NM_007420	F	ATTTTGGCAACTTCTGGTGC
		R	TAGCGATCCACTGCAATCAC
Adrb3	NM_013462	F	ACAGGAATGCCACTCCAATC
		R	GGGGAAGGTAGAAGGAGACG
Cd36	NM_007643	F	GCGACATGATTAATGGCACA
		R	CCTGCAAATGTCAGAGGAAA
Ces3	NM_053200	F	TGGTATTTGGTGTCCCATCA
		R	GCTTGGGCGATACTCAAACT
Cpt1a	NM_013495	F	GCCCATGTTGTACAGCTTCC
		R	ATGGCAGAGGCTCACCAAGC
Dgat1	NM_010046	F	GACGGCTACTGGGATCTGA
		R	TCACCACACACCAATTCAGG
Dgat2	NM_026384	F	CGCAGCGAAAACAAGAATAA
		R	GAAGATGTCTTGGAGGGCTG
Emr1	NM_010130	F	GGATGTACAGATGGGGGATG
		R	CATAAGCTGGGCAAGTGGTA
Fabp1	NM_017399	F	TGCAGAGCCAGGAGAACTTT
		R	GATTTCTGACACCCCCTTGA
Fabp4	NM_024406	F	CATGGCCAAGCCCAACAT
		R	CGCCCAGTTTGAAGGAAATC
Fasn	NM_007988	F	AAGTTGCCCGAGTCAGAGAACC
		R	ATCCATAGAGCCCAGCCTTCCATC
Fgf21	NM_020013	F	CCTGGGTGTCAAAGCCTCTA
		R	CTCCAGCAGCAGTTCTCTGA
Fsp27	NM_178373	F	TGGGAGGTCCAACACAATCCA
		R	GTGCTCACTGCCACATGCCT
Lipe	NM_010719	F	CCTCCAAGCAGGGCAAAGA
		R	GCGTAAATCCATGCTGTGTGA
Il1b	NM_008361	F	GGACCCATATGAGCTGAAAGCT
		R	TGTCGTTGCTTGGTTCTCCTT
Itgam	NM_008401	F	ATTCGGTGATCCCTTGGATT
		R	GTTTGTTGAAGGCATTTCCC
Lcat	NM_008490	F	GGTTTTATCTCTCTCGGGGC
		R	TATGTTGGACAGGATGGGGA

Lpl	NM_008509	F	TTTGGCTCCAGAGTTTGACC
		R	TGTGTCTTCAGGGGTCCTTAG
Lypla1	NM_008866	F	CCTTCACGGATTGGGAGATA
		R	GGGGCATGTGGACAGATGTA
Mttp	NM_008642	F	AGGTGCTGGGGGTCAGTT
		R	GGCAAAAGCCCTGGTCTCTT
Plin1	NM_175640	F	TGAAGCAGGGCCACTCTC
		R	GACACCACCTGCATGGCT
Pnpla2	NM_025802	F	CCACTCACATCTACGGAGCC
		R	TAATGTTGGCACCTGCTTCA
Scd1	NM_009127	F	ACGCCGACCCTCACAATTC
		R	CAGTTTTCCGCCCTTCTCTTT
Slc2a4	NM_009204	F	TTTTAAAACAAGATGCCGTCG
		R	CAGTGTTCCAGTCACTCGCT
Tnf	NM_013693	F	CCACCACGCTCTTCTGTCTAC
		R	AGGGTCTGGGCCATAGAACT

F, forward sequence; R, reverse sequence.

Acadl, acyl-CoA dehydrogenase, long-chain
Acadm, acyl-CoA dehydrogenase, medium-chain
Acaca, acetyl-CoA carboxylase alpha
Acly, ATP citrate lyase
Acsl1, acyl-CoA synthetase long-chain family member 1
Apob, apolipoprotein B
Adrb, adrenergic receptor, beta
Cd36, CD36 antigen (fatty acid translocase)
Ces3, carboxylesterase 3
Cpt1a, carnitine palmitoyltransferase 1a, liver
Dgat, diacylglycerol O-acyltransferase
Emr1, EGF-like module containing, mucin-like, hormone receptor-like sequence 1
Fabp, fatty acid-binding protein
Fasn, fatty acid synthase
Fgf21, fibroblast growth factor 21
Fsp27, fat-specific protein 27 (cell death-inducing DFFA-like effector c)
Il1b, interleukin 1 beta
Itgam, integrin alpha M
Lcat, lecithin cholesterol acyltransferase
Lipe, hormone-sensitive lipase
Lpl, lipoprotein lipase
Lypla1, lysophospholipase 1
Mttp, microsomal triglyceride transfer protein
Plin1, perilipin 1
Pnpla2, patatin-like phospholipase domain containing 2 (adipose triglyceride lipase)
Scd1, stearoyl-CoA desaturase 1
Slc2a4, solute carrier family 2, member 4 (glucose transporter type 4)
Tnf, tumor necrosis factor alpha