Supplementary Information

Disruption of phospholipid and bile acid homeostasis in mice with nonalcoholic steatohepatitis

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Supplementary table 1. Primer pairs used for qPCR

Gene	GeneBank Accession Number		Primer Sequence (5'-3')
18S	NR_003278	F	ATTGGAGCTGGAATTACCGC
		R	CGGCTACCACATCCAAGGAA
Abcb11	NM_021022	F	CCAGAACATGACAAACGGAA
		R	AAGGACAGCCACACCAACTC
Abcc1	NM_008576	F	GATGGCTCCGATCCACTCT
		R	AGGTAGAAACAAGGCACCCA
Abcc2	NM_013806	F	TCCAGGACCAAGAGATTTGC
		R	TCTGTGAGTGCAAGAGACAGGT
Abcc4	NM_009804	F	AGCTTCAACGGTACTGGGATA
		R	TCGTCGGGGTCATACTTCTC
Abcc5	NM_013790	F	GCCCTGGGTACAGAAGTGAC
		R	TCTTGGCATTCCAACGATCT
Alox12	NM_007440	F	GATCACTGAAGTGGGGCTGT
		R	CACACATGGTGAGGAAATGG
Alox12b	NM_009659	F	CTTCCCAGCTTACCAGTGGA
		R	GGATAGGGAGTGTGTCGTCTG
Alox15	NM_009660	F	CGGTCTACTTGTCTCCCTGC
		R	ATCCGCTTCAAACAGAGTGC
Alox15b	NM_009661	F	CGCCAGAAGGAGCTTGAGT
		R	TCACAGTCTCGTGGTCAAGG
Cybb	NM_007807	F	CTTTCTCAGGGGTTCCAGTG
		R	TGCAGTGCTATCATCCAAGC
Cyp7a1	NM_007824	F	GGGAATGCCATTTACTTGGA
		R	GTCCGGATATTCAAGGATGC
Cyp8b1	NM_010012	F	TCCTCAGGGTGGTACAGGAG
		R	GATAGGGGAAGAGAGCCACC
Cyp27a1	NM_024264	F	CTATGTGCTGCACTTGCCC
		R	GGGCACTAGCCAGATTCACA
Egr1	NM_007913	F	GAGCGAACAACCCTATGAGC
		R	TGGGATAACTCGTCTCCACC
Enpp2	NM_015744	F	TCGAGGGCGAGAGAAGTTTA
		R	AAAAGAATGTCCCGGCTCTC
Il6	NM_031168	F	TGATGCACTTGCAGAAAACA
		R	ACCAGAGGAAATTTTCAATAGGC
Lcat	NM_008490	F	GGTTTTATCTCTCTCGGGGC
		R	TATGTTGGACAGGATGGGGA
Lpcat1	NM_145376	F	CACGAGCTGCGACTGAGC
		R	ATGAAAGCAGCGAACAGGAG
Lpcat2	NM_173014	F	ACCTGTTTCCGATGTCCTGA
		R	CCAGGCCGATCACATACTCT
Lpcat3	NM_145130	F	AGCCTTAACAAGTTGGCGAC
		R	ATGCCGGTAAAACAGAGCC
Lpcat4	NM_207206	F	GAGTTACACCTCTCCGGCCT
		R	GGCCAGAGGAGAAAGAGGAC
Lypla1	NM_008866	F	CCTTCACGGATTGGGAGATA
		R	GGGGCATGTGGACAGATGTA
Ostb	NM_178933	F	AGAGAAAGCTGCAGCCAATG
		R	CCAGGACCAGGATGGAATAA
Slc10a1	NM_011387	F	AGGGGGACATGAACCTCAG
		R	TCCGTCGTAGATTCCTTTGC
Slco1a1	NM_013797	F	ACTCCCATAATGCCCTTGG
		R	TAATCGGGCCAACAATCTTC
Slco1b2	NM_020495	F	ACCAAACTCAGCATCCAAGC
		R	TAGCTGAATGAGAGGGCTGC
Tgfb1	NM_011577	F	GGAGAGCCCTGGATACCAAC
		R	CAACCCAGGTCCTTCCTAAA
Tnfa	NM_013693	F	CCACCACGCTCTTCTGTCTAC
		R	AGGGTCTGGGCCATAGAACT

F, forward sequence; R, reverse sequence.

Supplementary table 2. Top ten of serum ions that were significantly altered in mice treated with MCD diet for 2 weeks compared with in mice treated with MCS diet

Increased ions

Rank	RT (min)	Found (m/z)	Identity	Mass error (ppm)	Elemental Composition
1	4.71	319.2271	12-Hydroxyeicosatetraenoic acid	0.63	C20H32O3
2	2.55	514.2843	Tauro-β-muricholate	0.97	C26H45NO7S
3	2.92	514.2829	Taurocholate	1.75	C26H45NO7S
4	5.97	279.2318	Linoleic acid	2.15	C18H32O2
5	6.44	281.2474	Oleic acid	2.49	C18H34O2
6	0.31	92.9297	Not determined		
7	0.26	112.9862	Not determined		
8	5.81	327.2319	Docosahexaenoic acid	1.53	C22H32O2
9	6.44	381.1738	Not determined		
10	2.09	187.0977	Azelaic acid	3.74	C9H16O4

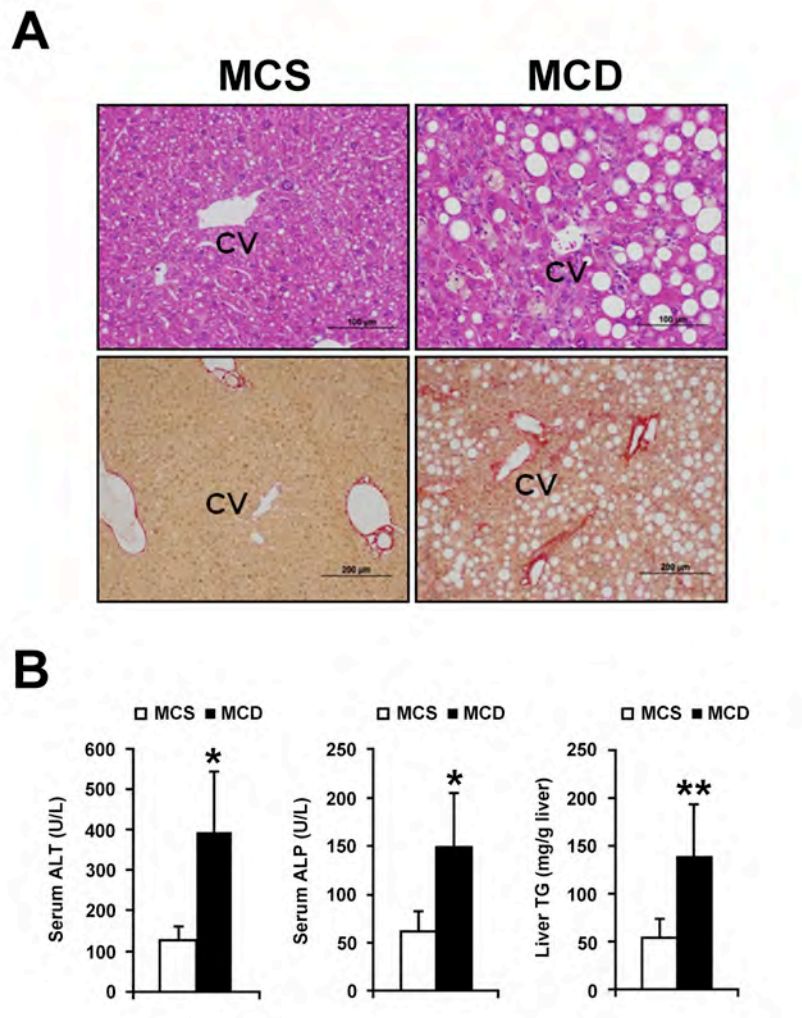
Decreased ions

Rank	RT (min)	Found (m/z)	Identity	Mass error (ppm)	Elemental Composition
1	5.40	568.3615	Stearoyl-LPC (18:0-LPC)	0.18	C27H56NO9P
2	4.78	540.3303	Palmitoyl-LPC (16:0-LPC)	0.37	C25H52NO9P
3	4.94	566.3463	Oleoyl-LPC (18:1-LPC)	0.88	C27H54NO9P
4	4.53	612.3291	Docosahexanoyl-LPC (22:6-LPC)	1.63	C31H52NO9P
5	4.57	564.3303	Linoleoyl-LPC (18:2-LPC)	0.35	C27H52NO9P
6	0.30	215.0321	Not determined		
7	4.57	588.3306	Arachidonoyl-LPC (20:4-LPC)	0.85	C29H52NO9P
8	4.54	500.2777	Not determined		
9	7.38	745.5478	Not determined		
10	4.40	538.3137	Palmitoleoyl-LPC (16:1-LPC)	1.49	C25H50NO9P

The ion ranking, based on OPLS analysis, shows the highest confidence and greatest contribution to separation between MCD and MCS diet treatment. RT, retention time; LPC, lysophosphatidylcholine.

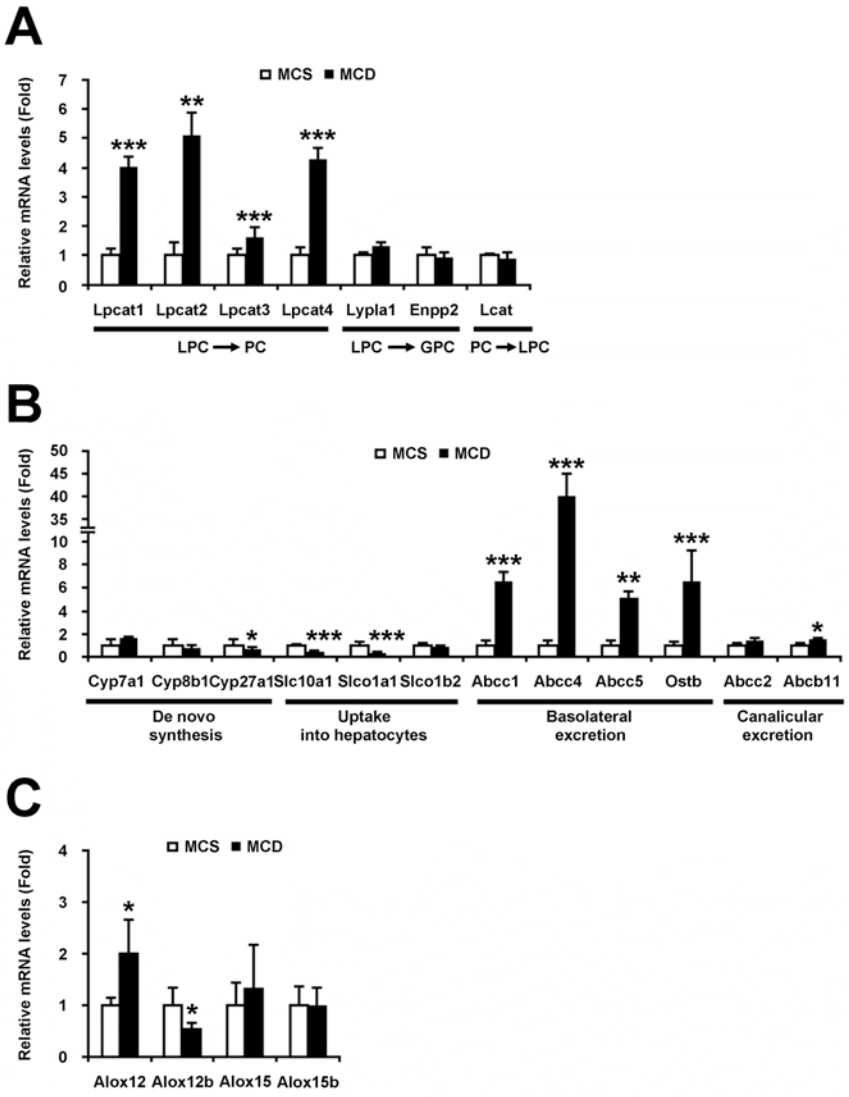
Supplementary figure 1. Histological and biochemical findings in mice with 8-week MCD diet treatment

(**A**) Liver histology. Liver sections were stained by the hematoxylin and eosin (upper rows) and Sirius red methods (lower rows), respectively. Bars represent 100 μm (upper row) and 200 μm (lower row), respectively. CV, central vein.
(**B**) Serum levels of ALT and ALP and hepatic TG contents. Statistical analysis was performed using the two-tailed Student's *t*-test (n = 4 in each group). *, $P<0.05$; **, $P<0.01$.



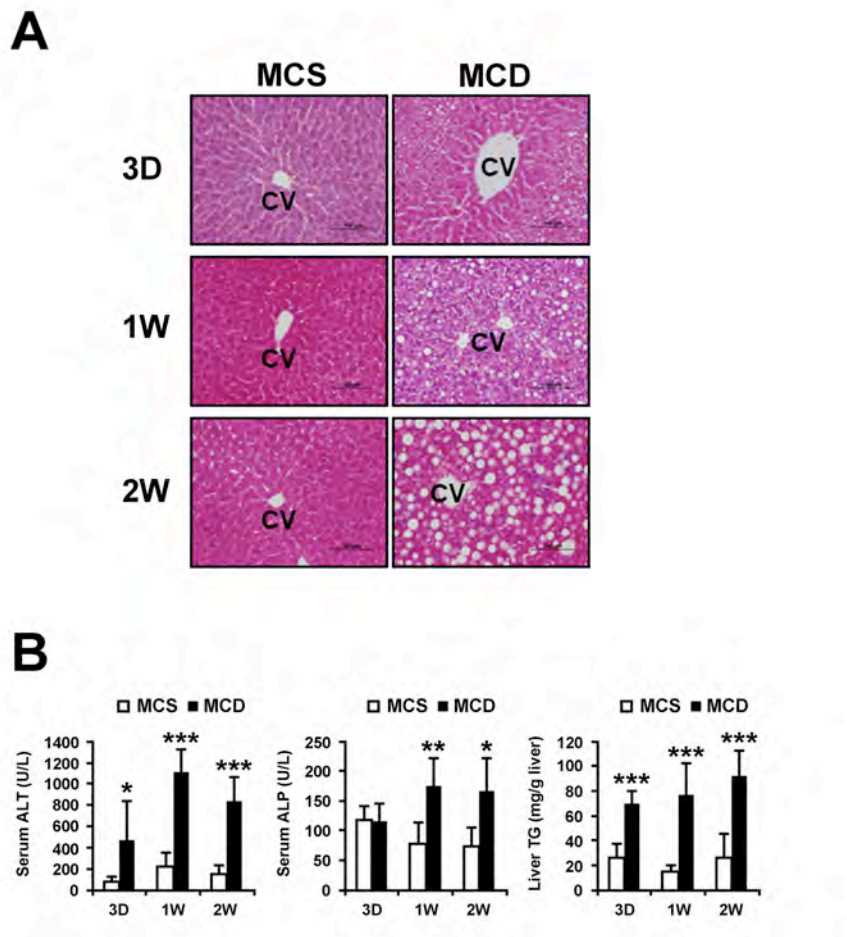
Supplementary figure 2. Hepatic mRNA levels of genes associated with metabolism of LPC (A), bile acids (B) and 12-HETE (C) in mice with 2-week MCD diet treatment

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 two-tailed Student's *t*-test (n = 4 in each group). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; PC, phosphatidylcholine; GPC, glycerophosphocholine.



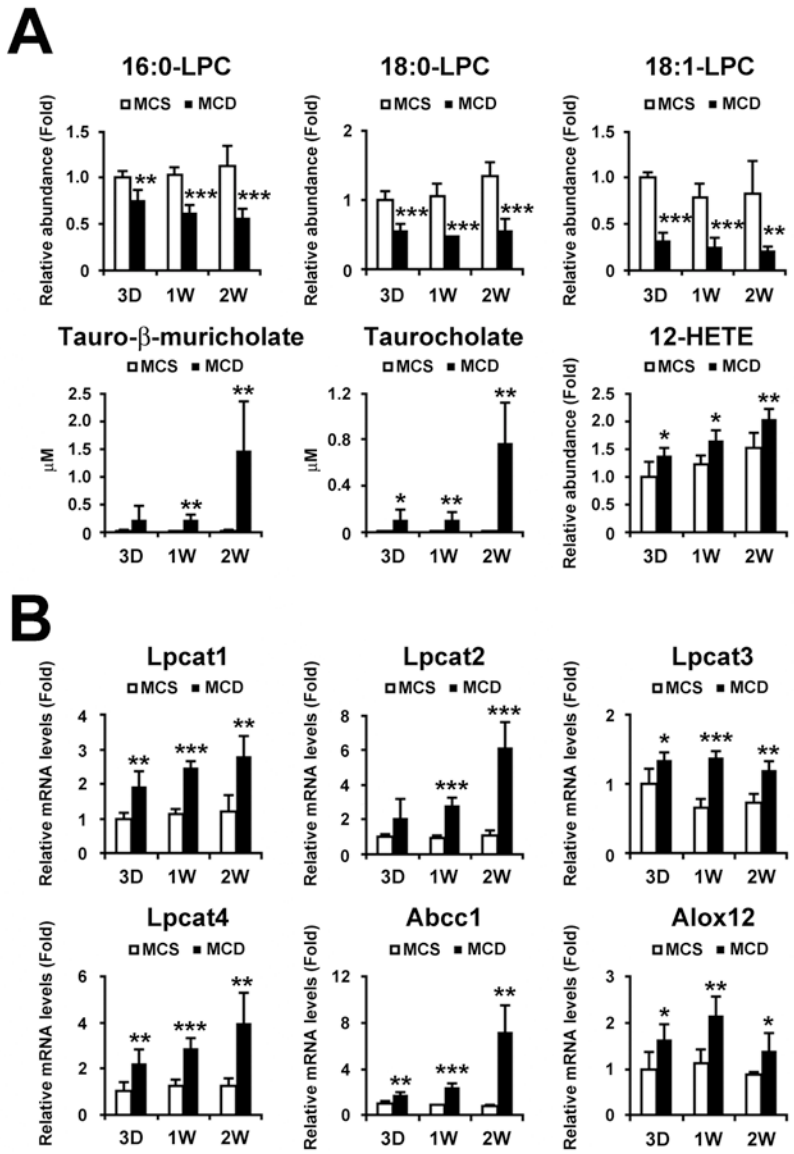
Supplementary figure 3. Histological and biochemical findings in mice with 3-day, 1-week, and 2-week MCD diet treatment

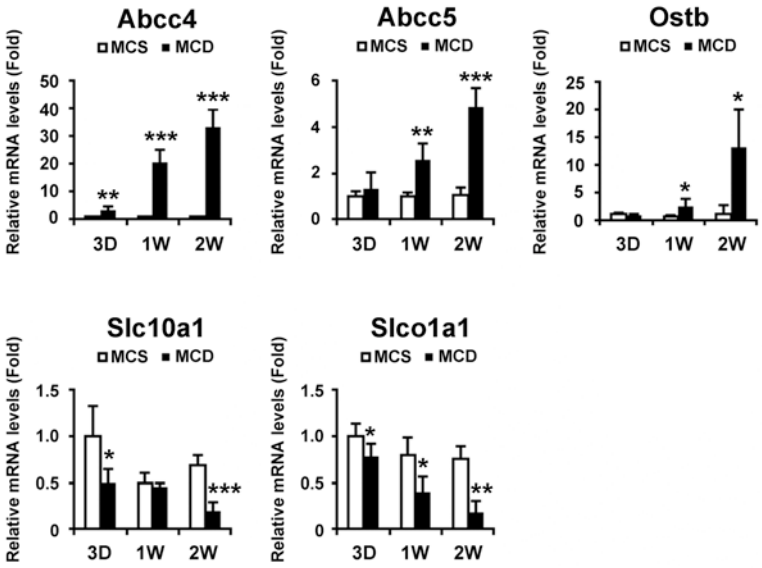
(**A**) Liver histology. Liver sections were stained by the hematoxylin and eosin method. Bars represent 100 μm. CV, central vein.
(**B**) Serum levels of ALT and ALP and hepatic TG contents. Statistical analysis was performed using the two-tailed Student's *t*-test in the same treatment period (n = 5 in each group). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.



Supplementary figure 4. Time course of serum metabolite concentrations (A) and mRNA levels of the related genes (B)

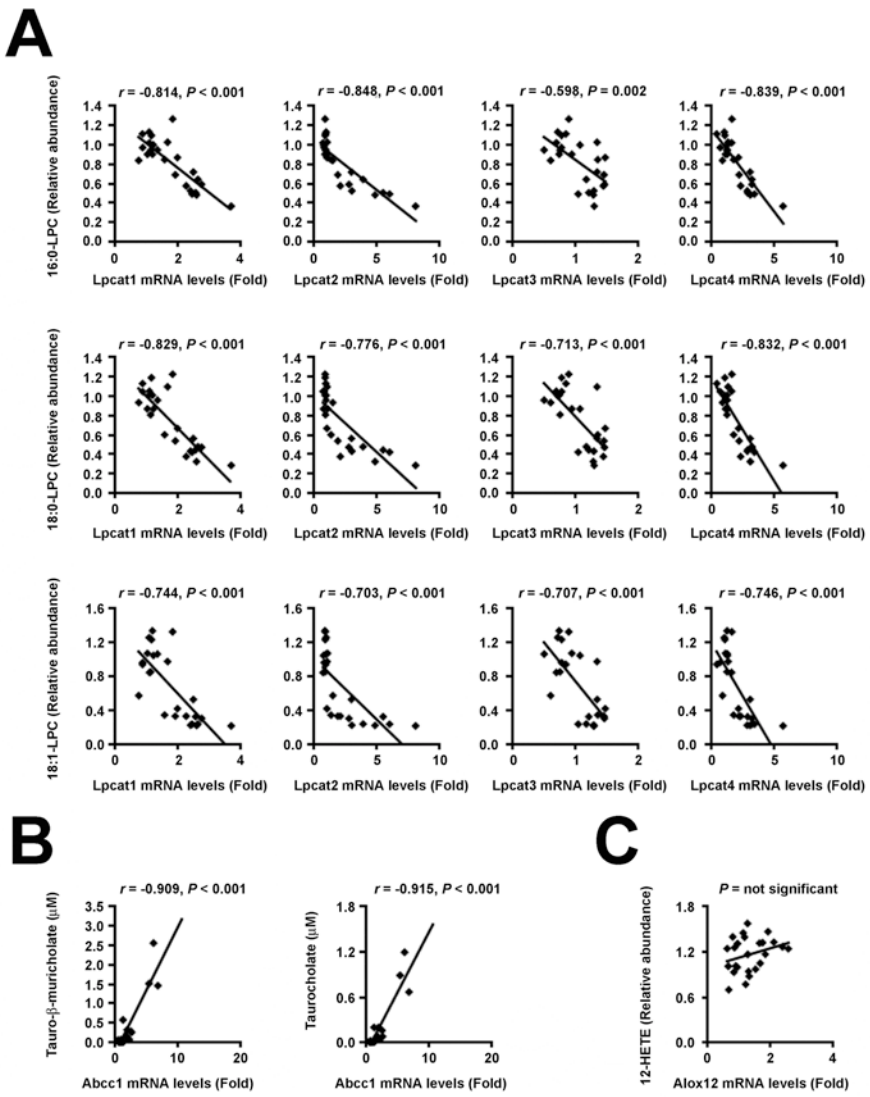
In panel (**A**), the levels of LPC and 12-HETE were normalized to those of mice with 3-day MCS diet treatment and were expressed as relative abundance. In panel (**B**), the mRNA levels were normalized to those of 18S ribosomal mRNA and subsequently normalized to those of mice with 3-day MCS diet treatment. Statistical analysis was performed using the two-tailed Student's *t*-test in the same treatment period (n = 5 in each group). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.




Abcc4
MCS MCD
Relative mRNA levels (Fold)
50
40
30
20
10
0
**
***
***
3D 1W 2W
Abcc5
MCS MCD
Relative mRNA levels (Fold)
6
4
2
0
**
***
3D 1W 2W
Ostb
MCS MCD
Relative mRNA levels (Fold)
25
20
15
10
5
0
*
*
3D 1W 2W
Slc10a1
MCS MCD
Relative mRNA levels (Fold)
1.5
1.0
0.5
0
*
***
3D 1W 2W
Slco1a1
MCS MCD
Relative mRNA levels (Fold)
1.5
1.0
0.5
0
*
*
**
3D 1W 2W

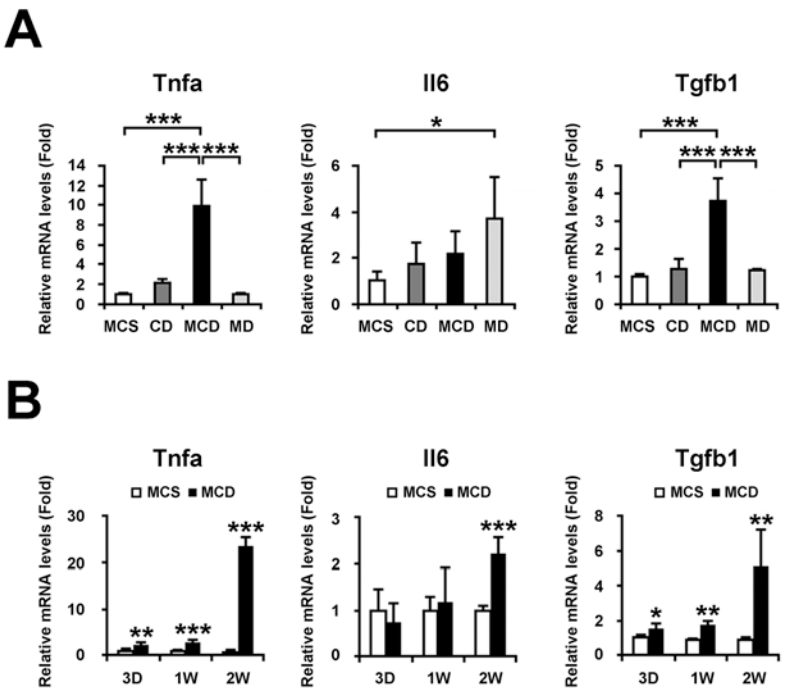
Supplementary figure 5. Correlation between serum LPC, bile acids, and 12-HETE and the mRNA levels of the related genes.

Data obtained from mice with MCD or MCS diet treatment for 3 days, 1 week, and 2 weeks were adopted (n = 30). Correlation coefficients were calculated by means of the Pearson's method.



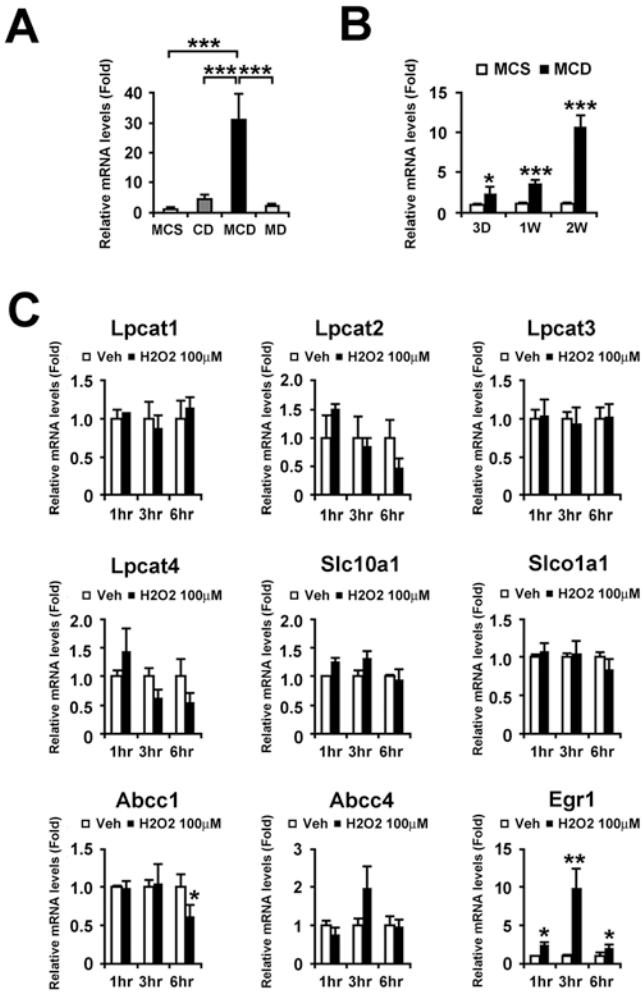
Supplementary figure 6. Hepatic expression of pro-inflammatory cytokines in NASH

(**A**) The effect of methionine and choline supplementation to MCD diet treatment on the mRNA levels of pro-inflammatory cytokines. 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 one-way ANOVA with Tukey's test (n = 5 in each group). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; MD, methionine-deficient treatment; CD, choline-deficient treatment.
(**B**) Time course of the mRNA levels of pro-inflammatory cytokines. The mRNA levels were normalized to those of 18S ribosomal mRNA and subsequently normalized to those of mice with 3-day MCS diet treatment. Statistical analysis was performed using the two-tailed Student's *t*-test in the same treatment period (n = 5 in each group). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.



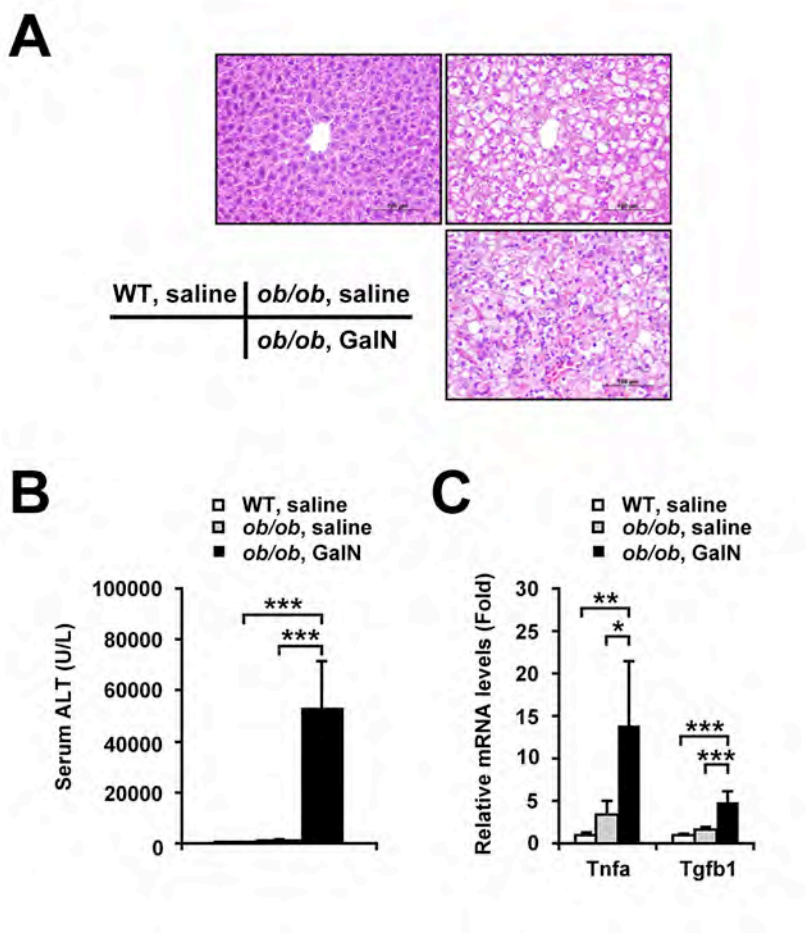
Supplementary figure 7. Hepatic expression of NADPH oxidase 2 (Cybb) in NASH and the changes in the expression of Lpcats and bile acid transporters by H2O2 in primary hepatocytes

(**A**) The effect of methionine and choline supplementation to MCD diet treatment on the Cybb mRNA levels. Statistical analysis was performed using the one-way ANOVA with Tukey's test (n = 5 in each group). ***, $P<0.001$; MD, methionine-deficient treatment; CD, choline-deficient treatment.
(**B**) Time course of the mRNA levels of Cybb. The mRNA levels were normalized to those of 18S ribosomal mRNA and subsequently normalized to those of mice with 3-day MCS diet treatment. Statistical analysis was performed using the two-tailed Student's *t*-test in the same treatment period (n = 5 in each group). *, $P<0.05$; ***, $P<0.001$.
(**C**) Primary hepatocytes were treated with H2O2 (100 μM) for 1, 3, and 6 hours, respectively, and harvested to extract total RNA. The mRNA levels were normalized to those of 18S ribosomal mRNA and subsequently normalized to those of vehicle-treated hepatocytes in the same time point. Early growth response 1 (Egr1) mRNA levels were used as a positive control. Statistical analysis was performed using the two-tailed Student's *t*-test in the same period (n = 3 in each group). *, $P<0.05$; **, $P<0.01$.



Supplementary figure 8. Phenotypical changes in *ob/ob* mice with D-galactosamine (GalN) injection

(**A**) Liver histology. Liver sections were stained by the hematoxylin and eosin method. Bars represent 100 μm. WT, C57BL/6J wild-type mice.
(**B** and **C**) Serum ALT levels and hepatic mRNA levels of pro-inflammatory cytokines. Statistical analysis was performed using the one-way ANOVA with Tukey's test (n = 5 in each group). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.



Supplementary figure 9. Changes in serum metabolite concentrations (A) and mRNA levels of the related genes (B) in *ob/ob* mice with GalN injection

In panel (**A**), serum levels of LPC were normalized to those of C57BL/6J wild-type (WT) mice with vehicle injection and were expressed as relative abundance. TMCβ and TC indicate tauro-β-muricholate and taurocholate, respectively. In panel (**B**), the mRNA levels were normalized to those of 18S ribosomal mRNA and subsequently normalized to those of WT mice with vehicle injection. Statistical analysis was performed using the one-way ANOVA with Tukey's test (n = 5 in each group). *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.