SUPPLEMENTARY DATA

Supplementary Table 1. **The number of genes with DNA methylation change**

	Number of genes with DNA hypermethylation		Number of genes with DNA hypomethylation	
	Total number	**and decreased mRNA expression**	**Total number**	**and increased mRNA expression**
From e18.5 to D2	1	0	1	0
From D2 to D16	1721	433	568	249
From D16 to D28	15	0	129	26

Supplementary Table 2. GO terms enriched in the genes with DNA methylation change in the liver of D16 relative to D2

GO terms (Biological process)	***P*-values**
GO terms enriched in the genes with DNA hypermethylation and decreased mRNA expression	
Regulation of lymphocyte activation	3.1E-7
Cell activation	8.5E-6
Regulation of T cell activation	7.8E-6
Regulation of lymphocyte proliferation	1.9E-4
Regulation of mononuclear cell proliferation	1.9E-4
Immune effector process	0.0032
Cytoskeleton organization	0.0054
Regulation of protein amino acid phosphorylation	0.0076
Leukocyte mediated immunity	0.035
Regulation of B cell activation	0.036
GO terms enriched in the genes with DNA hypomethylation and increased mRNA expression	
Nitrogen compound biosynthetic process	3.2E-5
Cellular amino acid biosynthetic process	6.0E-5
Organic acid catabolic process	0.0040
Cofactor metabolic process	0.011
Steroid metabolic process	0.016
Cellular amino acid catabolic process	0.016
Aspartate family amino acid metabolic process	0.025
Lipid transport	0.030
Acylglycerol metabolic process	0.030
Coagulation	0.033

SUPPLEMENTARY DATA

Supplementary Table 3. Transcriptional factor binding motifs enriched in the promoter regions of genes with DNA methylation change

Binding matrices	***P*-values**
Motifs enriched in the genes with DNA hypomethylation and increased mRNA expression in the liver of D16 relative to D2	
V$PPARG	8.20E-06
V$DR1	0.0015
V$PPAR_DR1	0.0015
V$IPF1	0.0018
V$PPARA	0.0049
V$COUP_DR1	0.0055
V$ETS	0.0055
V$HNF4	0.0061
Motifs enriched in the genes with DNA hypermethylation in the liver of PPARα-KO mice relative to WT mice on D2	
V$PPAR_DR1	5.95E-07
V$DR1	1.66E-06
V$CP2	0.00035
V$PAX3	0.000843

Supplementary Table 4. **Pathway analysis of genes with DNA methylation change**

KEGG ID	Pathway names	*P*-values
Pathways highlighted in the genes with DNA hypomethylation and increased mRNA expression in the liver of D16 relative to D2		
mmu03320	PPAR signaling pathway	2.88E-04
mmu00260	Glycine, serine and threonine metabolism	0.010
mmu00561	Glycerolipid metabolism	0.011
mmu00071	Fatty acid metabolism	0.012
mmu00330	Arginine and proline metabolism	0.012
mmu00410	beta-Alanine metabolism	0.015
Pathways highlighted in the genes with DNA hypermethylation in the liver of PPARα -KO mice relative to WT mice on D2		
mmu00071	Fatty acid metabolism	0.014
Pathways highlighted in the genes with DNA demethylation in the offspring liver on D2, obtained from the ligand-administered dams		
mmu00071	Fatty acid metabolism	1.25E-05
mmu03320	PPAR signaling pathway	0.0031
Pathways highlighted in the genes with DNA hypomethylation and increased mRNA expression in the adult human liver relative to fetal liver		
hsa03320	PPAR signaling pathway	0.018
hsa00071	Fatty acid metabolism	0.067

SUPPLEMENTARY DATA

PCR primers for the genes in Supplementary Fig. 2*A*

Ppara
forward: AGGAAGCCGTTCTGTGACAT
reverse : AATCCCCTCCTGCAACTTCT

Decr1
forward: GCTTTGTAATGCCAAGTTCTTCAG
reverse : TCAGCTGCAAGTGACTTATTCATG

Decr2
forward: GCTTCCGGATCGCAGAGA
reverse : TCCTGCCGACGATGACAGT

Peci
forward: CGGAAGCATGCTCCTCTTACA
reverse : GCATCTCAGCTGCCTTTGC

Echs1
forward: TGGTCAGCCGCGTGTTC
reverse : CAGGGCAAAGGCTGCATT

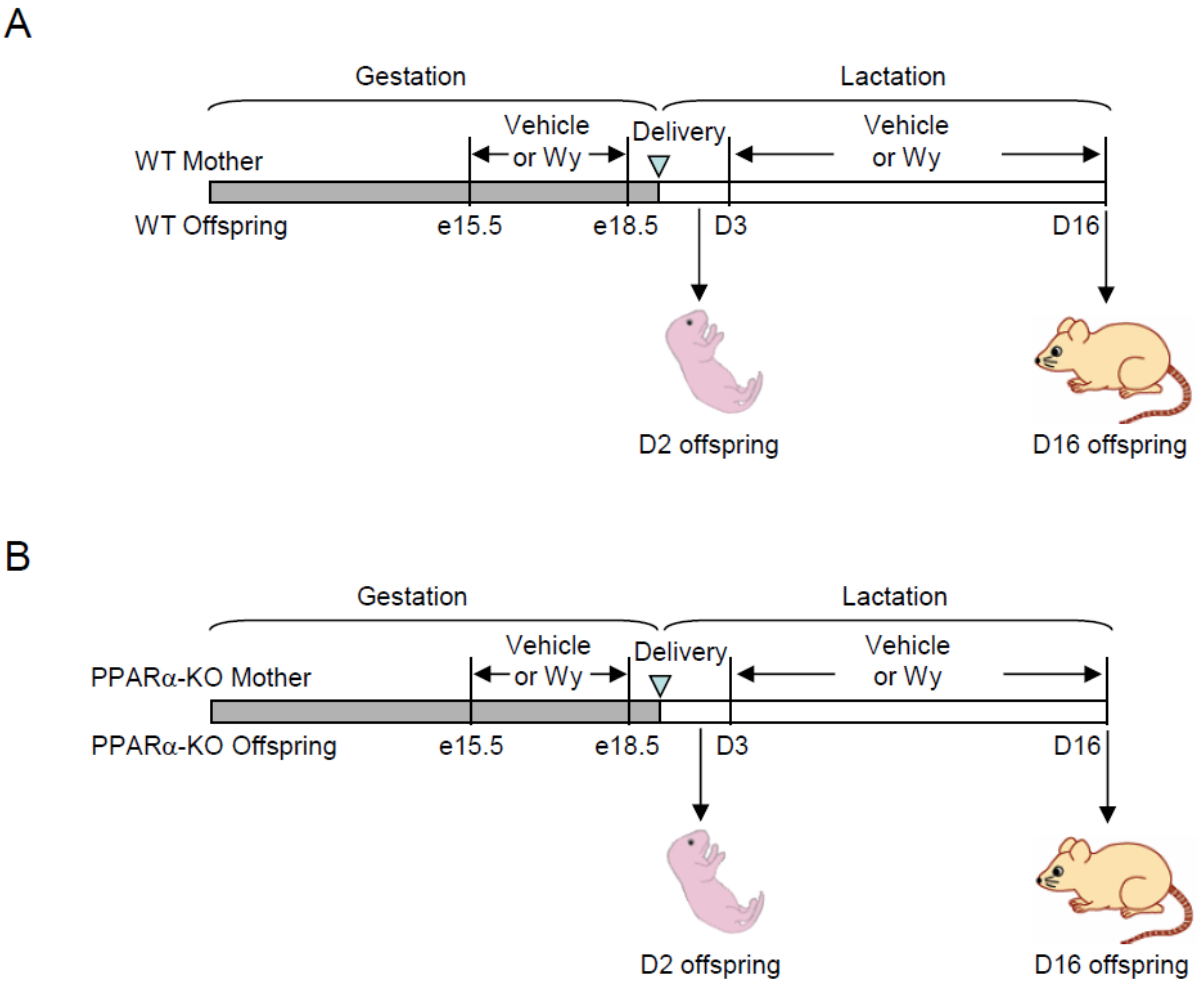
Acaa1a
forward: CAAGTCAGGCCGTCTACTGTGT
reverse : CCAGCGGGTTCACCTTCTC

Acaa1b
forward: GAAAGCAGGGCTGACTGTGAAT
reverse : GTAGACGGCCTGACTTGCAAA

DNA Methylation Profiling
The Microarray-based Integrated Analysis of Methylation by Isoschizomers (MIAMI) analysis was performed as follows. One microgram of genomic DNAs from the two samples were digested with the methylation-sensitive restriction enzyme *Hpa*II followed by adaptor ligation and PCR-amplification. Amplified DNA from one sample was labeled with Cy3 and the other with Cy5, then they were co-hybridized to the gene promoter arrays containing 41,332 probes (for mouse) (22) or 38,172 probes (for human) (23). The fluorescence signals obtained were normalized with the global normalization method and signal differences between samples (*i.e.* Cy3 signal intensity/Cy5 signal intensity), which indicate DNA methylation differences between samples, were determined. Genomic DNAs were also digested with methylation-insensitive isoschizomer *Msp*I followed by the same procedure to correct for false-positives caused by single nucleotide polymorphisms or incomplete digestion. To describe the difference in DNA methylation between samples, scatter plot graphs were depicted with the log-transformed values of *Hpa*II signal differences (horizontal axis) and *Msp*I signal differences (vertical axis) (Figs. 1*A*, 2*A*, 3*F*, and 4*A*). For each probe, *Hpa*II/*Msp*I signal difference was determined as the value of methylation difference. The criteria of methylation difference judged as DNA methylation change are shown in **Figure legends**. In the analysis of human fetal and adult liver, we performed three independent MIAMI experiments (male 18 weeks after fertilization vs. male 59 years old, male 22 weeks after fertilization vs. male 41 years old, and female 18 weeks after fertilization vs. male 20 years old.), and the genes that showed decreased DNA methylation among these experiments were subjected to further analysis. The complete experimental protocol is available at http://epigenome.dept.showa.gunma-u.ac.jp/~hatada/miami/image/MIAMI%20Protocol%20V4.pdf.

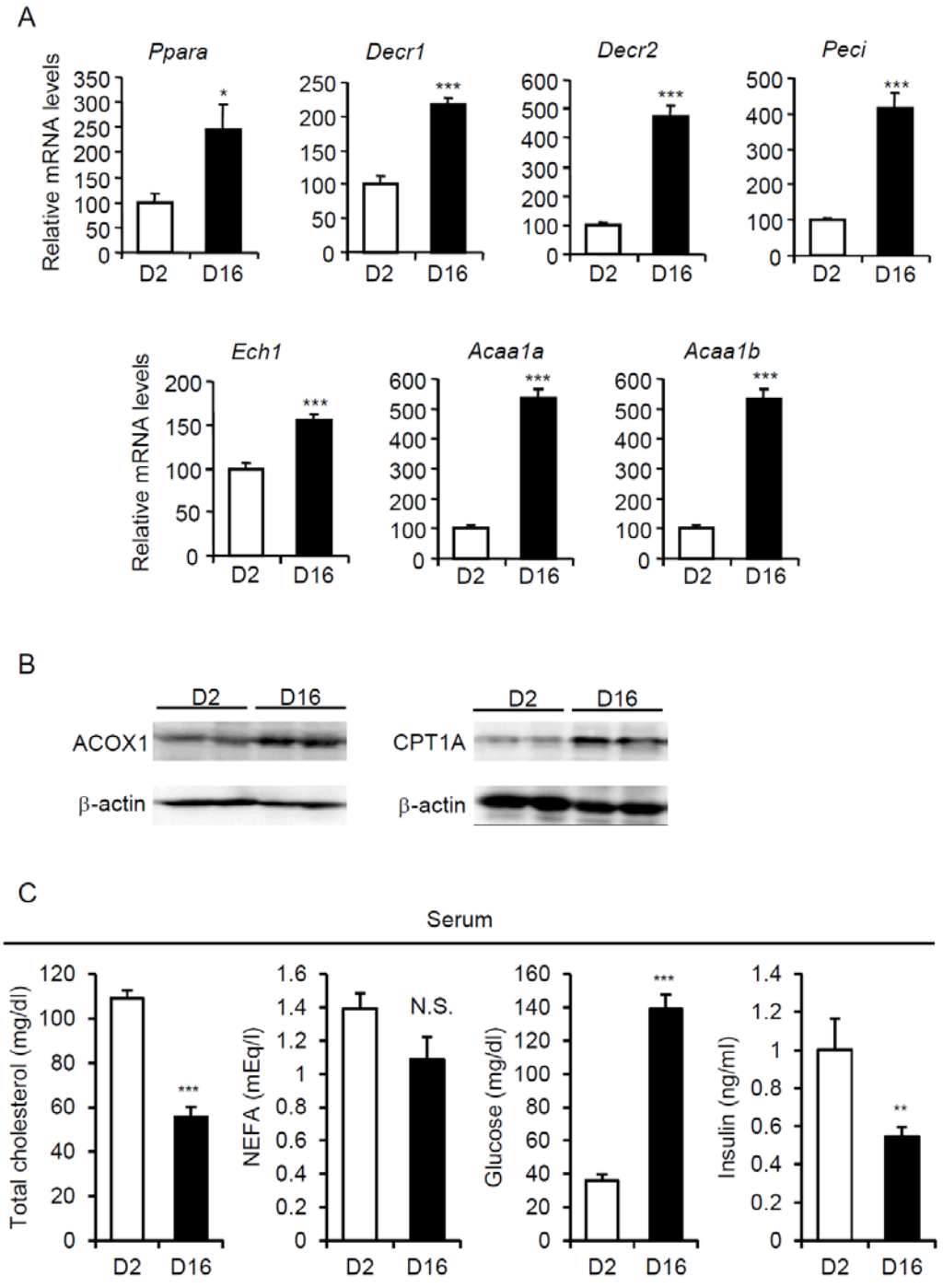
SUPPLEMENTARY DATA

Supplementary Figure 1. Experimental protocols of maternal Wy-administration. ***A*****:** Maternal administration of Wy to WT pregnant female mice. The offspring livers were sampled on D2 and D16. *B*: Maternal administration of Wy to PPARα-KO pregnant female mice. The offspring livers were sampled on D2 and D16.



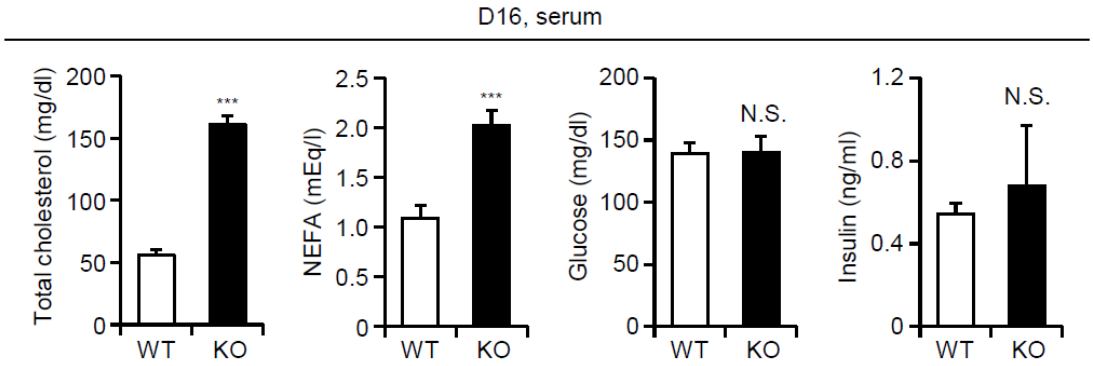
SUPPLEMENTARY DATA

Supplementary Figure 2. Relative mRNA levels of β-oxidation genes, protein levels of ACOX1 and CPT1A and serum metabolic parameters in the liver of D2 and D16 mice. ***A*:** Relative mRNA levels of β-oxidation genes. Values for D2 are set at 100. Primer information is described in Supplementary Information. *B*: Representative Western blots of ACOX1 and CPT1A. β-actin was used as a loading control. *C*: Serum metabolic parameters. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 vs. D2, N.S., not significant, *n* = 5. Data are expressed as the mean ± SEM.

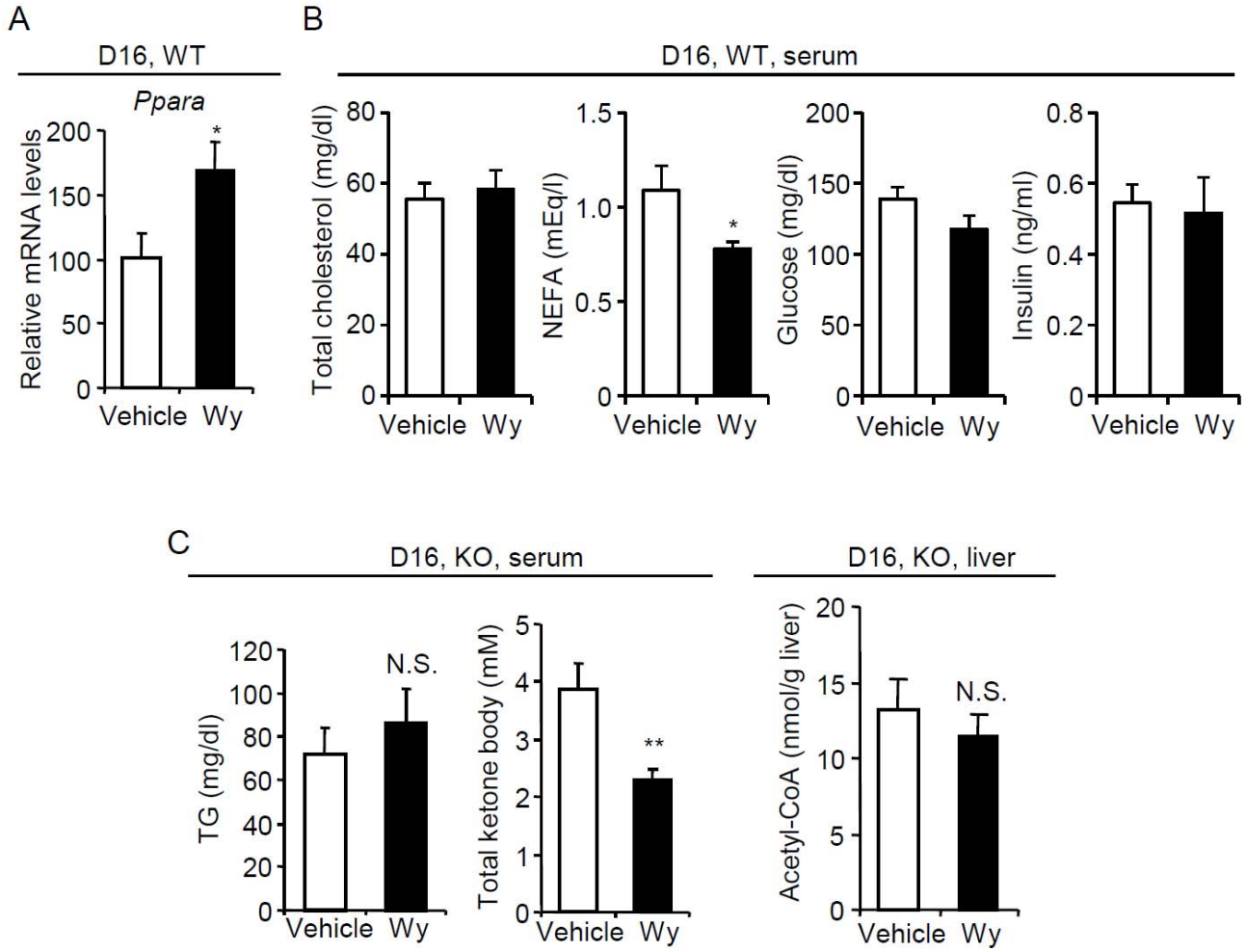


SUPPLEMENTARY DATA

Supplementary Figure 3. Serum metabolic parameters in the liver of D16 KO mice. ***$P < 0.001$ vs. D2, N.S., not significant, $n = 5$. Data are expressed as the mean ± SEM.



Supplementary Figure 4. **Gene expression of PPARα and serum metabolic parameters in the offspring derived from Wy-administered WT and PPARα-KO dams.** Relative hepatic mRNA levels of PPARα (***A***) and serum metabolic parameters (***B***) in the D16 offspring from the Wy-administered WT dams. $n = 5$. ***C*:** serum TG and total ketone body concentrations and hepatic acetyl-CoA levels in the D16 offspring derived from the Wy-administered KO dams. **$P < 0.01$, *$P < 0.05$, N.S., not significant vs. vehicle, $n = 8$. Data are expressed as the mean ± SEM.



SUPPLEMENTARY DATA

Supplementary Figure 5. Schematic illustartion showing that the ligand-activated PPARα-dependent DNA demethylation regulates the fatty acid β-oxidation genes in the postnatal liver. Before birth, when glucose is provided via the cord blood, expression of the fatty acid β-oxidation genes may be suppressed in a DNA methylation-dependent manner, which is partly because PPARα ligands are unavailable. After birth, activation of hepatic PPARα by milk lipid ligands may result in the activation of the fatty acid β-oxidation pathway via a DNA demethylation mechanism.