Supplemental Figures

Figure S1

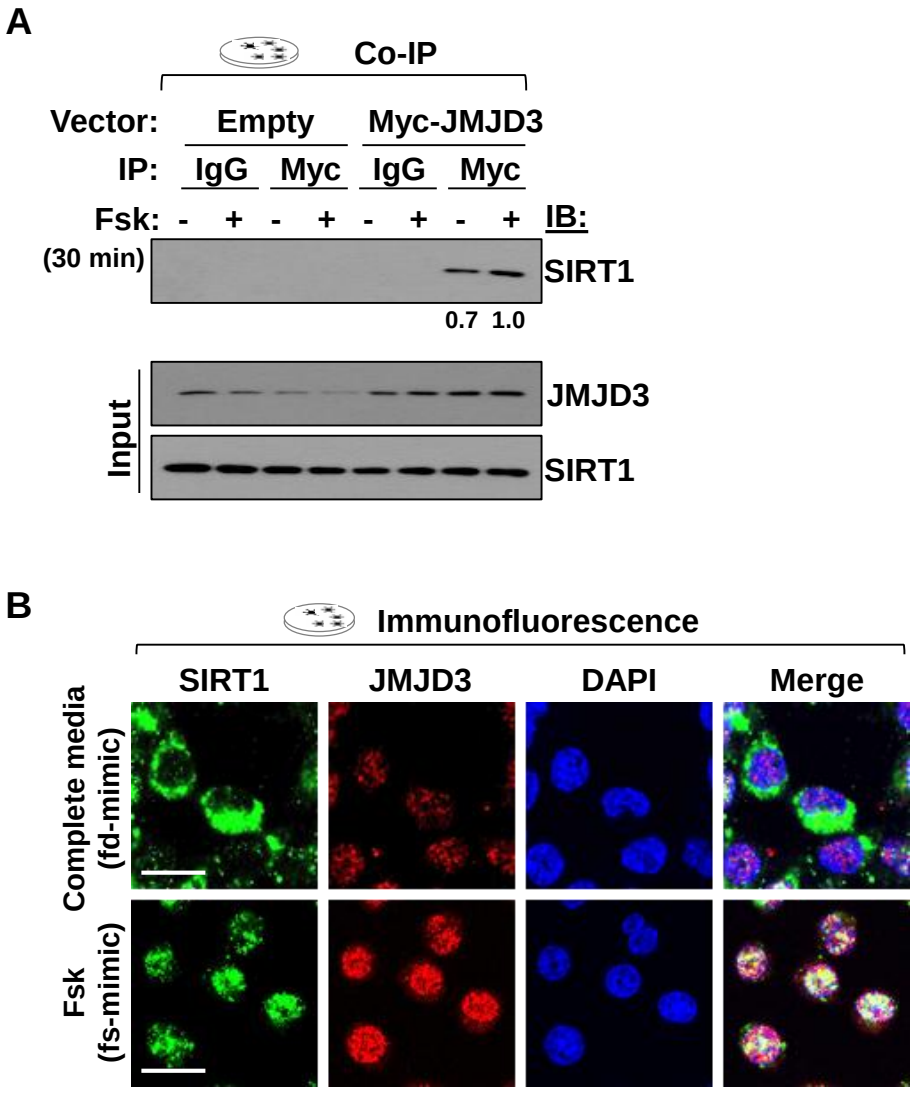


Figure S1. JMJD3 is a potential SIRT1-interacting protein in Fsk-treated hepatic cells. (A) CoIP of SIRT1 and exogenously expressed Myc-JMJD3. Hepa1c1c7 cells were transfected with Myc-JMJD3 or empty vector for 24 h, and treated with vehicle or Fsk (10 μM) for 30 min. SIRT1 in IgG (control) or anti-Myc (Myc-JMJD3) immunoprecipitates was detected by IB. JMJD3 and SIRT1 in the input were detected by IB using SIRT1 or JMJD3 (not Myc) antibody. **(B) Fsk treatment results in increased nuclear localization of both JMJD3 and SIRT1 in hepatic cells.** Hepa1c1c7 cells were treated with vehicle or Fsk for 6 h and JMJD3 and SIRT1 was detected by immunofluorescence. The white bar indicates 20 μm.

Figure S2

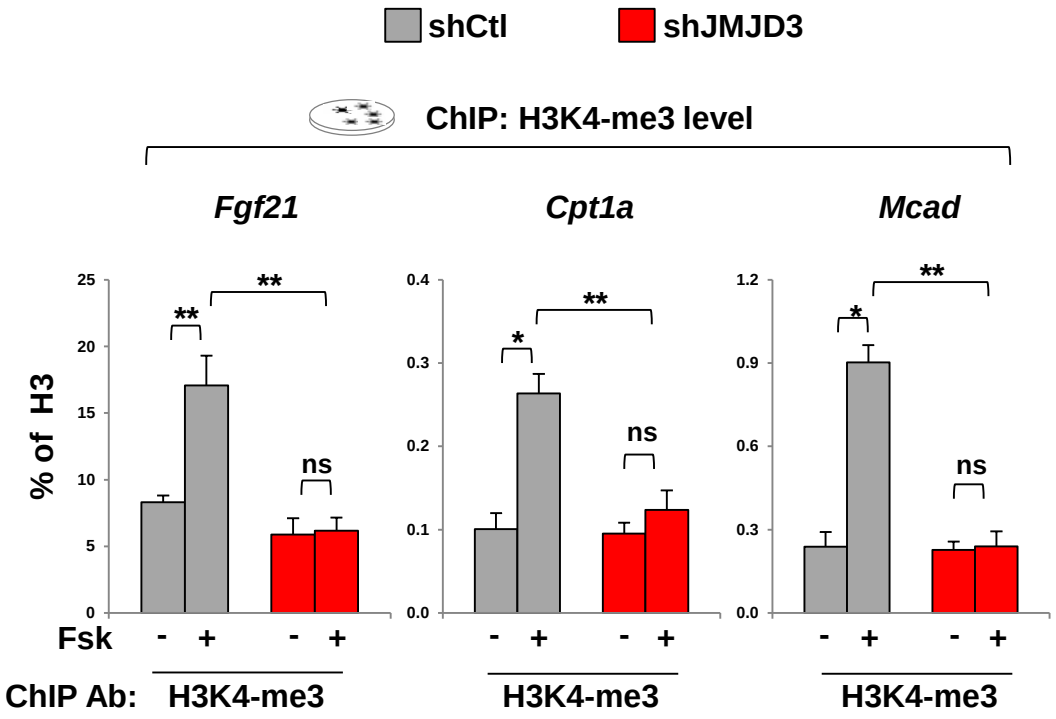


Figure S2. Effects of JMJD3 downregulation on histone H3K4-me3 levels at β-oxidation genes. Hepatocytes were infected with lenti-shRNA for JMJD3 (shJMJD3) or control RNA (shCtl), and 60 h later, cells were treated with 10 μM Fsk for 3 h. Levels of histone H3 and H3K4-me3 were determined by ChIP assay at the indicated genes. The ratio of H3K4-me3 to total H3 is plotted. Statistical significance was determined by two-way ANOVA with the FDR test (SEM, n=3, *P<0.05, **P<0.01, ns, statistically not significant).

Figure S3

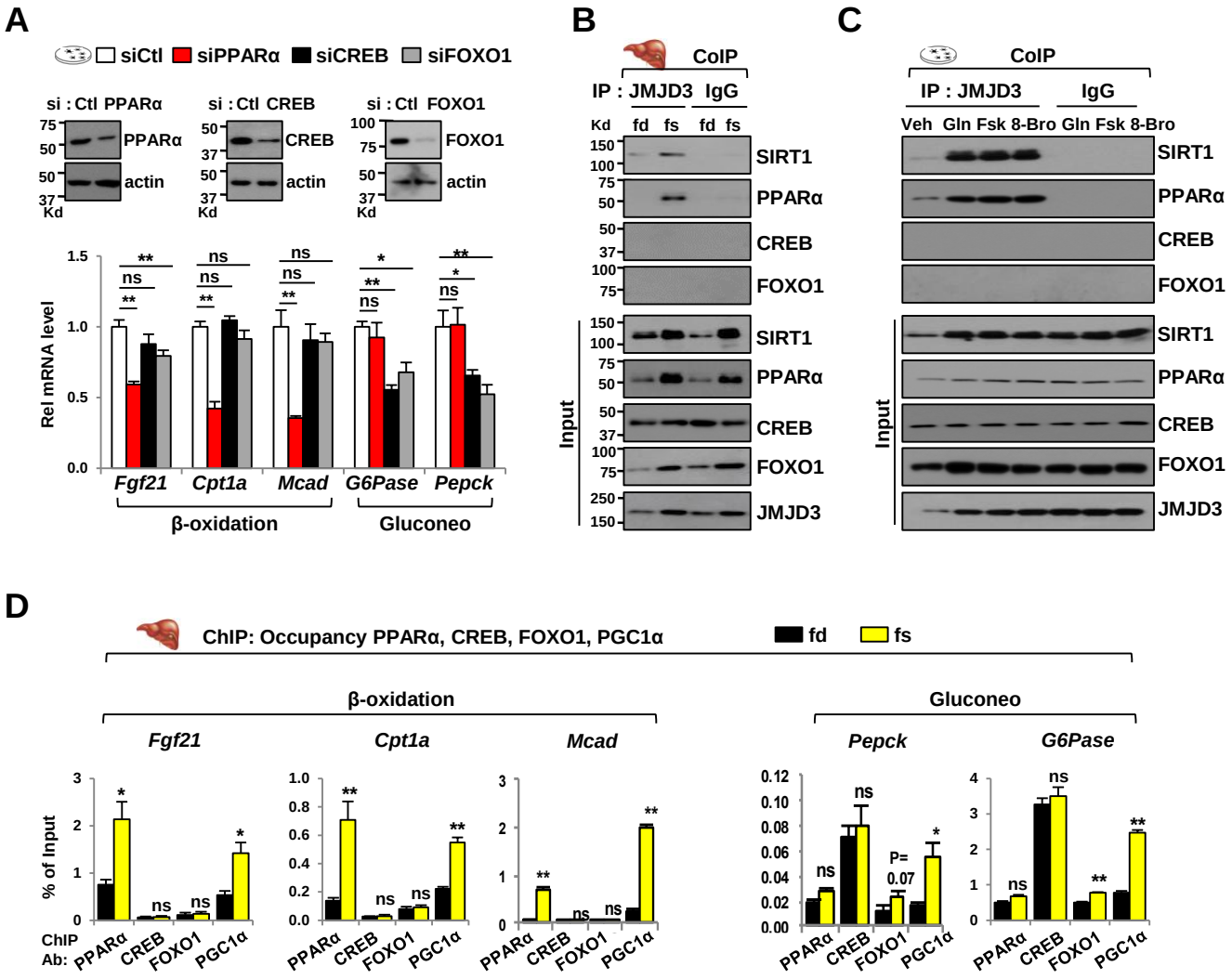
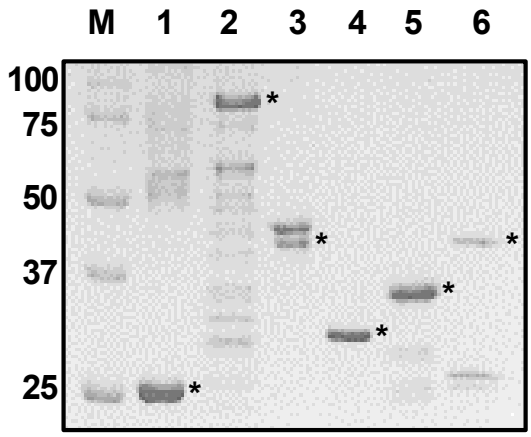


Figure S3. SIRT1 and PPARα are important for JMJD3 recruitment to β-oxidation genes. (A) Effects of downregulation of PPARα, CREB, and FOXO1 on mRNA levels of β-oxidation and gluconeogenic genes: Hepatocytes were transfected with siRNA for the indicated genes for 48 h and were treated with 10 μM Fsk for 6 h. Protein levels were determined by IB (top) and mRNA levels were determined by q-RTPCR (bottom) (n=8). **(B) CoIP: Effects of fasting on JMJD3 interaction with SIRT1, PPARα, CREB, and FOXO1:** Mice were fasted for 16 h (representative blot of 3 independent experiments) or **(C) CoIP:** Hepatocytes were treated with 10 nM glucagon (Gln), 10 μM forskolin (Fsk), or 0.1 mM 8-bromo-cAMP (8-Bro) for 3 h. CoIP assays were done using whole cell extracts. **(D) ChIP: Effects of fasting on occupancy of PPARα, CREB, FOXO1, and PGC-1α at *Fgf21, Cpt1a, Mcad, Pepck and G6Pase* genes:** Mice (n=3) were fasted (fs) for 16 h or fed (fd) for 6 h after fasting and ChIP assays using whole liver extracts were done with antibodies for the indicated proteins. **(A, D)** Statistical significance was determined by **(A)** one-way ANOVA with the FDR test or **(D)** the Mann-Whitney test (SEM, *P<0.05, **P<0.01, ns, statistically not significant).

Figure S4



1_ GST only
2_ GST-PPARα (1-468)_FL
3_ GST-PPARα (1-99)
4_ GST-PPARα (100-173)
5_ GST-PPARα (174-280)
6_ GST-PPARα (281-468)

Figure S4. GST-PPARα fusion proteins: SDS-PAGE analysis of GST-PPARα fusion proteins that were used in GST pull down experiments (Fig. 3B).

Figure S5

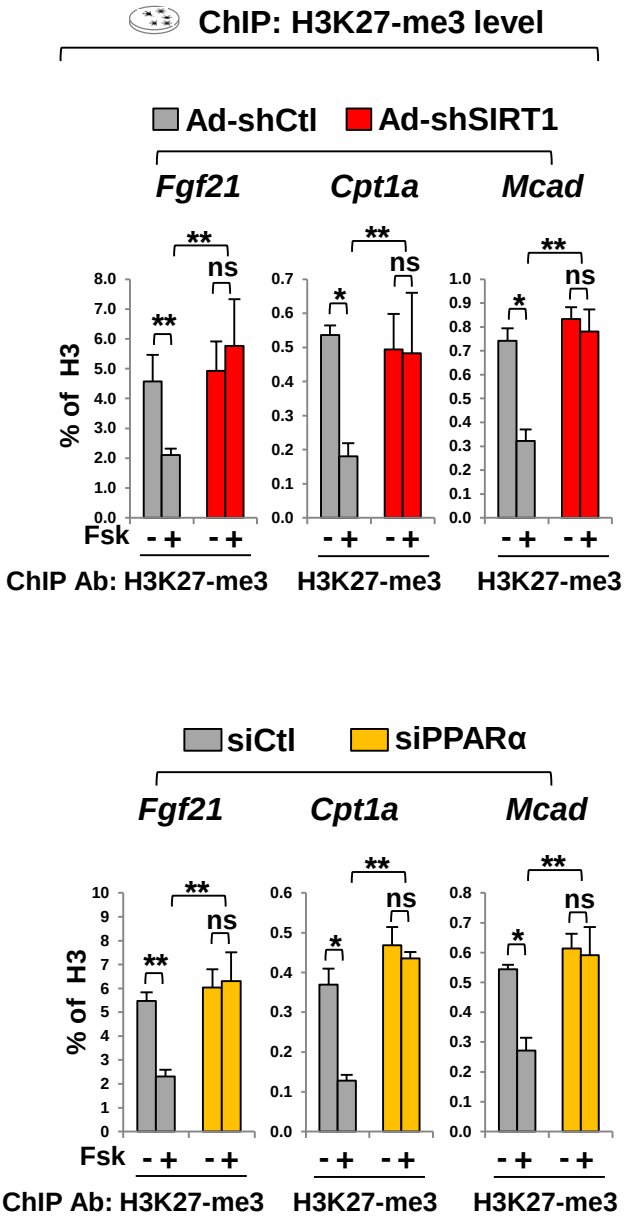


Figure S5. Fsk-mediated decreases in histone H3K27-me3 levels at β-oxidation genes are blunted by downregulation of either SIRT1 or PPARα. Hepatocytes were infected with Ad-shSIRT1 or Ad-shCtl for 48 h (left), or transfected with PPARα siRNA (siPPARα) or control RNA (siCtl) for 48 h (right), and treated with 10 μM Fsk for 3 h. The ratios of H3K27-me3 levels to total histone H3 levels determined by ChIP assay are shown. Statistical significance was determined by two-way ANOVA with the FDR test (SEM, n=3, *P<0.05, **P<0.01, ns, statistically not significant).

Figure S6

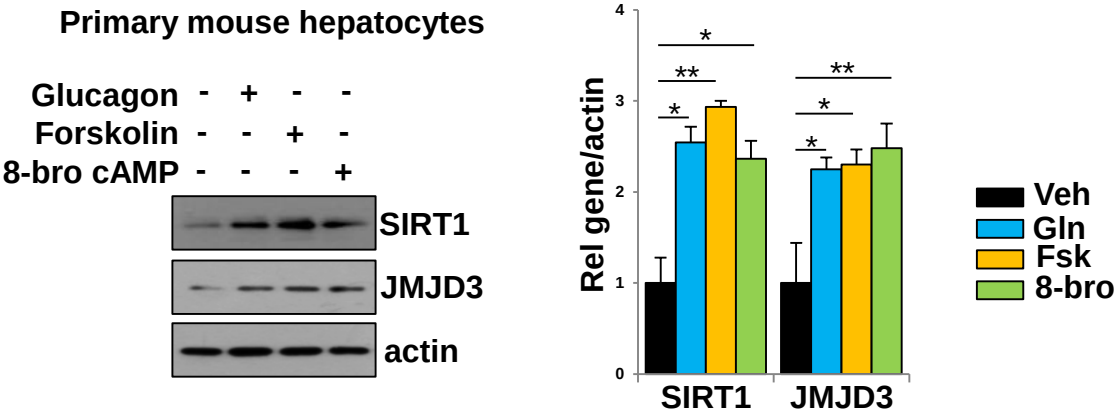


Figure S6. Treatment with glucagon, Fsk, or 8-bromo cAMP leads to increased protein levels of SIRT1 and JMJD3. (A) Hepatocytes were treated with 10 nM glucagon (Gln), 10 μM forskolin (Fsk), or 0.1 mM 8-bromo-cAMP (8-Bro) for 6 h and protein levels of the indicated proteins were determined by IB (left) and band intensities (n=3) were quantified (right). Statistical significance was determined by one-way ANOVA with the FDR test (SEM, *P<0.05, **P<0.01, ns, statistically not significant).

Figure S7

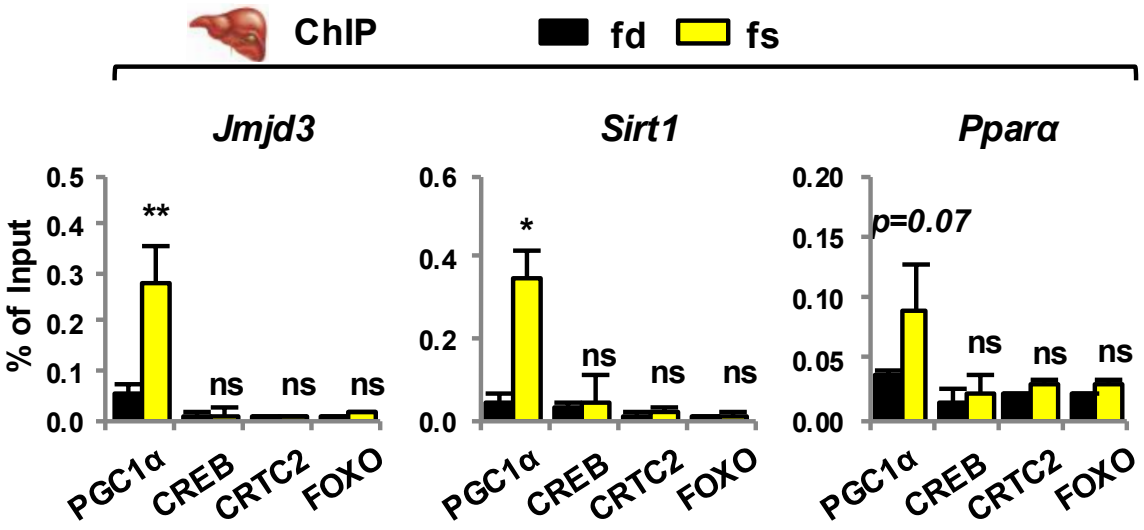


Figure S7. Effects of fasting on occupancy of PGC-1α, CREB, CRTC2, FOXO1 at *Jmjd3, Sirt1, and Pparα.* Mice (n=3) were fasted (fs) for 16 h or fed (fd) for 6 h after fasting. Occupancy of the indicated proteins at hepatic *Jmjd3, Sirt1, and Pparα* genes was determined by ChIP. Statistical significance was determined by the Mann-Whitney test (SEM, n=3 mice/group, *P<0.05, **P<0.01, ns, statistically not significant).

Figure S8

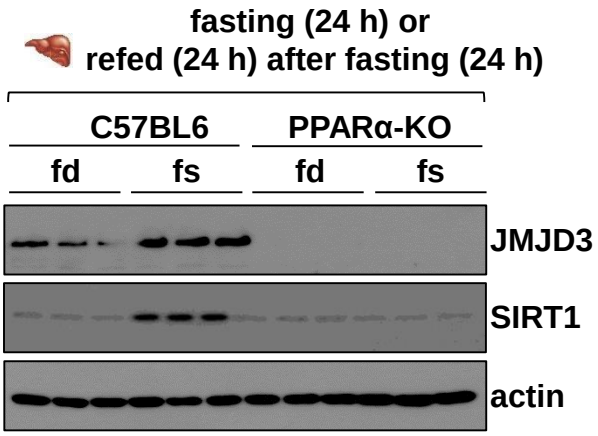


Figure S8. Fasting-induced increases in protein levels of JMJD3 and SIRT1 is blunted in PPARα-KO mice. C57BL6 mice and PPARα-KO mice were fasted for 24 h or refed for 24 h after fasting and protein levels of JMJD3 and SIRT1 in liver extracts were determined by IB.

Figure S9

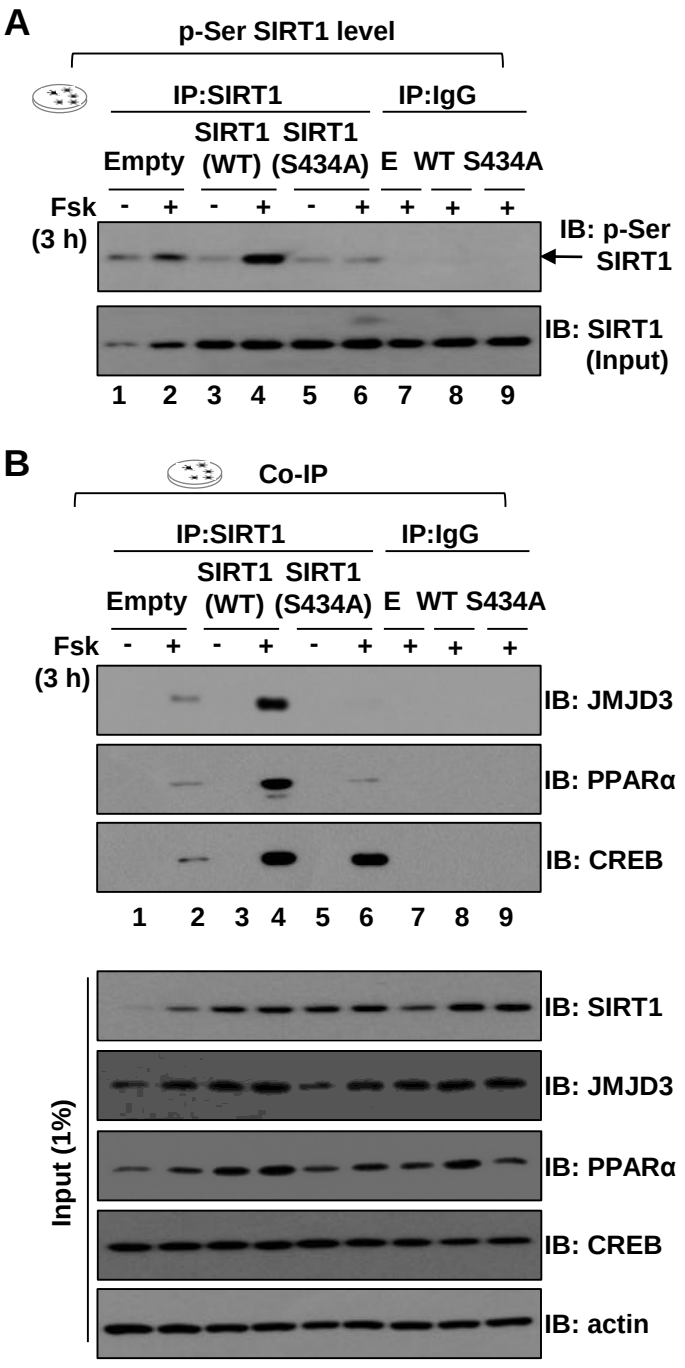


Figure S9. PKA-induced S434-SIRT1 phosphorylation is important for its functional interaction with JMJD3 and PPARα. Hepatocytes were transfected with expression vectors for SIRT1-WT or S434A-SIRT1 or control empty vector for 24 h, and treated with 10 μM Fsk for 3h **(A, B)** or 6 h **(C)**. **(A)** The p-Ser SIRT1 levels determined by IP/IB. **(B)** JMJD3, PPARα, and CREB in anti-SIRT1 immunoprecipitates from whole cells detected by IB (top) Input levels of the indicated proteins determined by IB. **(A, B)** Consistent results were observed in 2 independent assays. **(C)** The mRNA levels of *Pepck*. Statistical significance was determined by two-way ANOVA with the FDR test (SEM, n=6, **P<0.01).

Figure S10

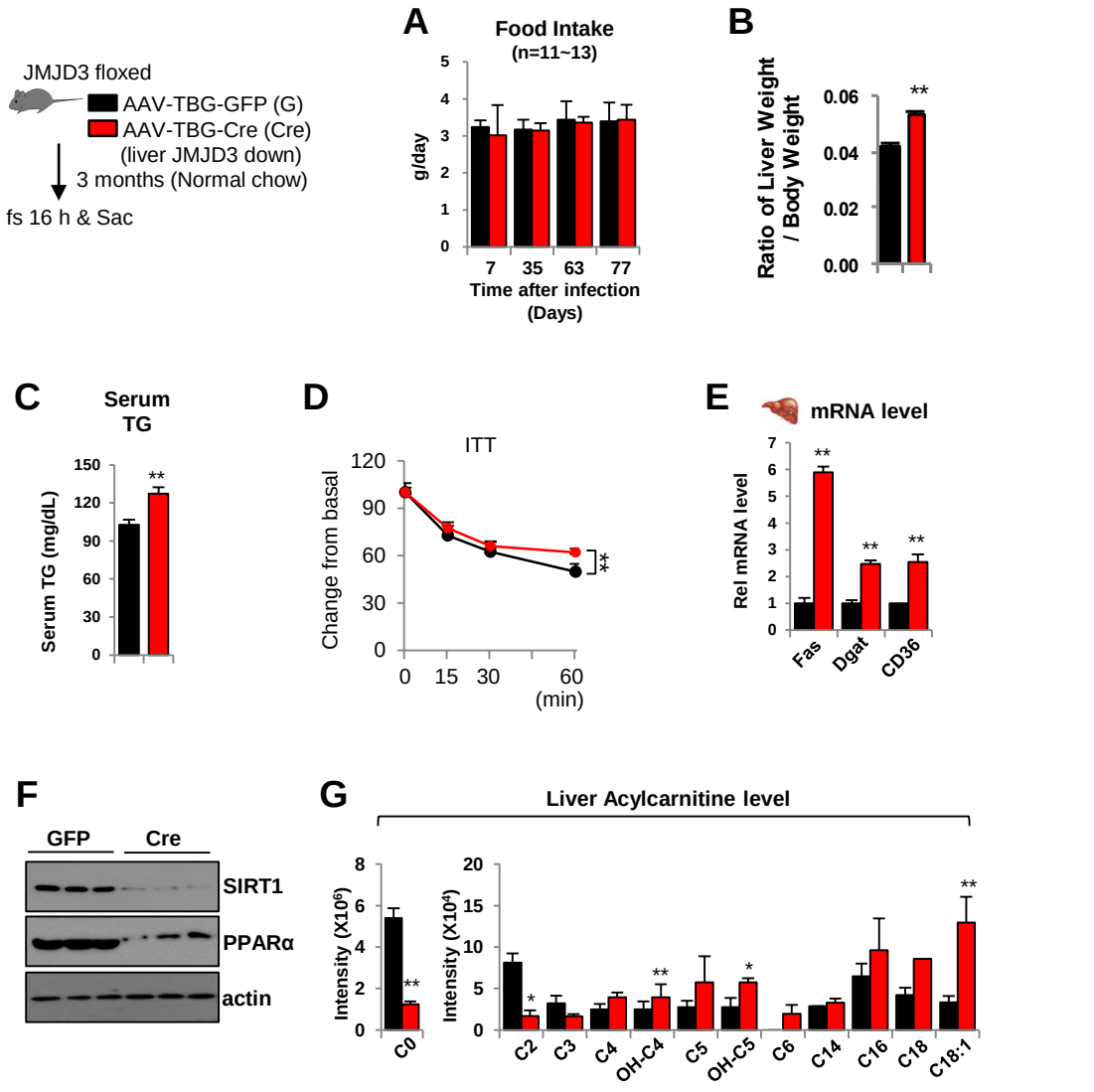


Figure S10. Downregulation of hepatic JMJD3 in mice. JMJD3 floxed mice were injected with AAV-TBG-Cre or AAV-TBG-GFP (control) for 3 months. **(A)** Food intake. **(B)** Ratio of liver weight to body weight. **(C)** Serum TG levels. **(D)** Insulin tolerance test (ITT). **(E)** Hepatic mRNA levels the indicated genes. **(F)** SIRT1 and PPARα protein levels determined by IB. **(G)** Levels of liver acylcarnitines. **(B-E, G)** Statistical significance was determined by the Student's t-test (SEM, n=6~8 mice/group, *p<0.05, **p<0.01, ns, statistically not significant).

Figure S11

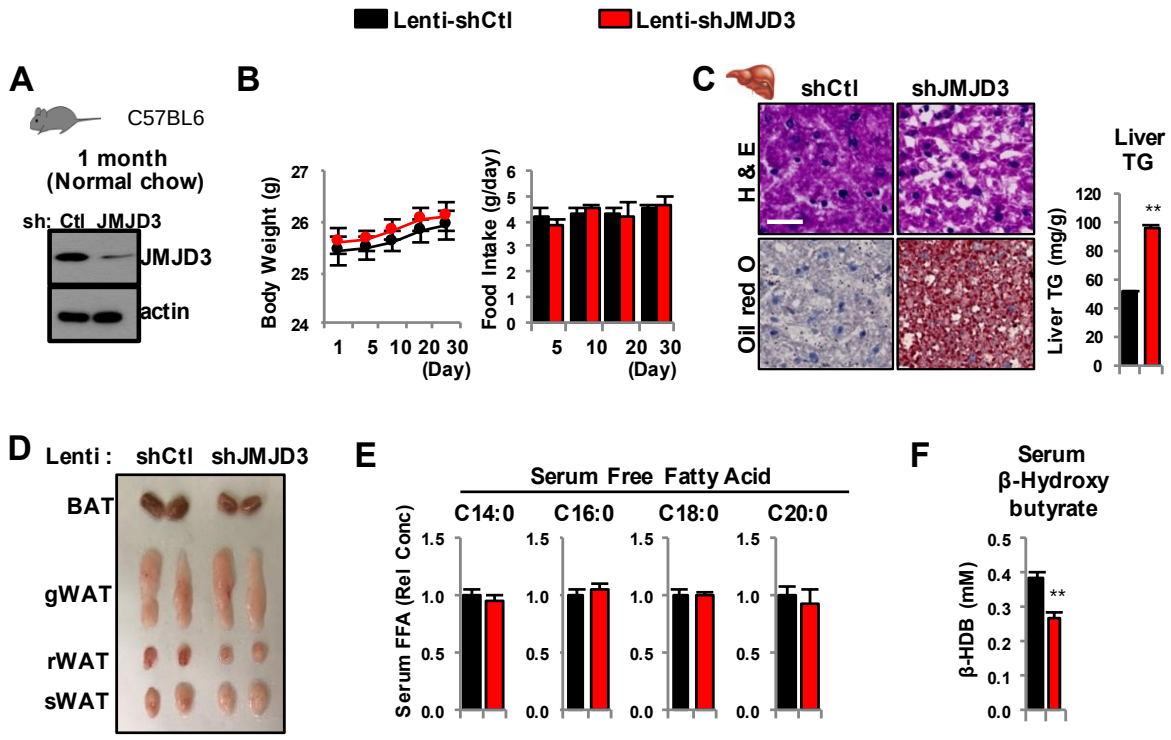


Figure S11. Downregulation of JMJD3 leads to liver steatosis without increasing body weight and adiposity. (A-F) C57BL6 mice were infected with lenti-shRNA for JMJD3 or control lenti-shCtl for 1 month. **(A)** JMJD3 protein levels determined by IB. **(B)** Body weight and food intake. **(C)** Liver sections stained with H&E and Oil Red O (left) and hepatic TG levels (right). The white bar indicates 100 μm. **(D)** Images of adipose tissues. **(E)** Levels of serum FFA levels and **(F)** serum β-hydroxybutyrate measured by GC/MS. **(C, F)** Statistical significance was determined by the Student's t-test (SEM, n=5~6 mice/group, *p<0.05, **p<0.01, ns, statistically not significant).

Figure S12

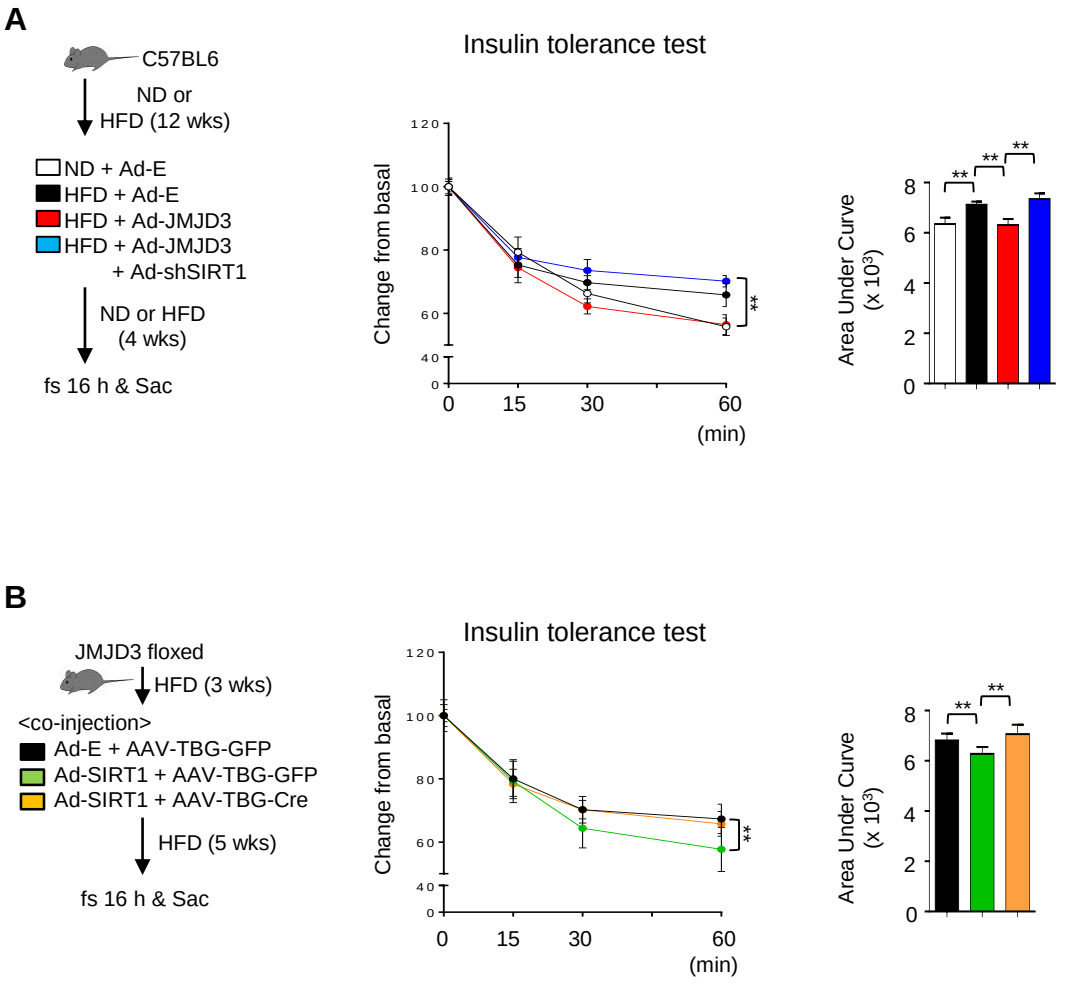


Figure S12. Improved insulin tolerance mediated by overexpression of JMJD3 or SIRT1 in diet-induced obese mice is mutually interdependent. **(A)** Mice were fed a HFD for 12 weeks or ND and injected with the indicated viruses for 4 weeks, and an insulin tolerance test (ITT) was performed. Experimental outline (left), relative changes in serum glucose levels (middle), and the area under curves (right). **(B)** Mice were fed a HFD for 3 weeks, infected with the indicated viruses for 5 weeks and fasted for 4 h and an ITT was done. Experimental outline (left), relative changes in serum glucose levels (middle), and the area under curves (right). Statistical significance was determined by one-way ANOVA with the FDR test (SEM, *P<0.05, **P<0.01).

Figure S13

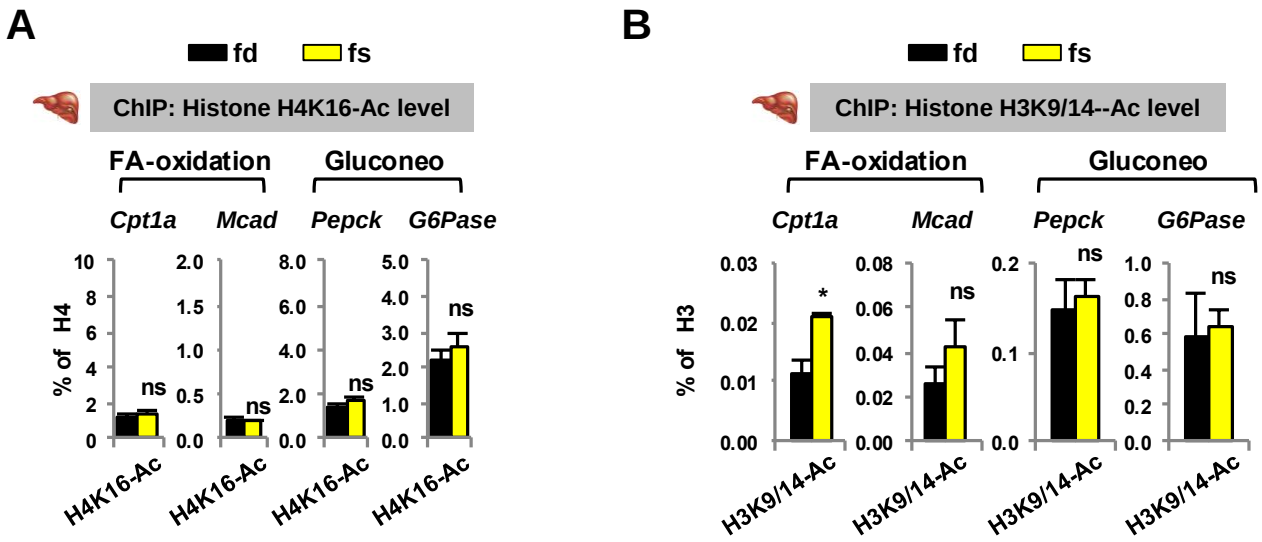


Figure S13. Effects of fasting or feeding on histone H4K16-Ac and H3K9/14-Ac levels at β-oxidation and gluconeogenic genes. Mice were fasted (fs) for 16 h or fed (fd) for 6 h after fasting, and histone H4 and H3 and histone H4K16-Ac levels and H3K9/14-Ac levels at the indicated genes were determined by liver ChIP assays. The ratios of acetylated H4K16-Ac to histone H4 **(A)** and acetylated H3K9/14-Ac to histone H3 **(B)** at the indicated genes are shown. Statistical significance was determined by the Mann-Whitney test (SEM, n=3 mice/group, *P<0.05, **P<0.01, ns, statistically not significant).

Supplemental Tables

Table S1. Biological processes potentially regulated by gene ontology analysis using DAVIS. (RNA-seq from lenti-shJMJD3 vs microarray from PPARα-KO

GO_ID	Gene Ontology Term	Gene #	p-value
GO:0055114	mitochondrion	118	3.75E-40
GO:0006629	oxidation-reduction process	59	3.60E-24
GO:0008152	lipid metabolic process	45	2.72E-20
GO:0006635	metabolic process	45	3.81E-20
GO:0006631	fatty acid beta-oxidation	17	1.49E-17
GO:0055088	fatty acid metabolic process	23	4.78E-14
GO:0033539	lipid homeostasis	10	3.07E-08
GO:0006637	long-chain fatty acid metabolism	6	1.16E-05
GO:0019433	acyl-CoA metabolic process	7	1.43E-05
GO:0032000	triglyceride catabolic process	5	3.64E-04
GO:0006810	carnitine metabolic process	4	4.18E-04
GO:0019915	triglyceride metabolic process	5	4.75E-03
GO:0001889	lipid catabolic process	7	1.26E-02

Table S2. Biological processes potentially regulated by gene ontology analysis using DAVIS. (RNA-seq from lenti-shJMJD3 vs microarray from SIRT1-LKO)

GO_ID	Gene Ontology Term	Gene #	p-Value
GO:0006637	acyl-CoA metabolic process	5	3.65E-05
GO:0051186	cofactor metabolic process	8	0.001992
GO:0006631	fatty acid metabolic process	8	0.002119
GO:0006732	coenzyme metabolic process	7	0.002706
GO:0055114	oxidation reduction	15	0.005698
GO:0044242	cellular lipid catabolic process	4	0.017083
GO:0009062	fatty acid catabolic process	3	0.020738
GO:0019395	fatty acid oxidation	3	0.022481
GO:0034440	lipid oxidation	3	0.022481
GO:0016042	lipid catabolic process	5	0.041604

Table S3. Hepatic genes potentially regulated by all of JMJD3, SIRT1 and PPARα from RNA-seq from lenti-shJMJD3 vs microarray from SIRT1-LKO *vs* microarray from PPARα-KO

Number	Gene Name	Number	Gene Name	Number	Gene Name	Number	Gene Name
1	*Ppp1r3c*	13	*Hmgcs2*	25	*Aldh3a2*	37	*Sgtb*
2	*Ugt1a9*	14	*Abcb4*	26	*Tle1*	38	*Crat*
3	*Clstn3*	15	*Pigr*	27	*Ehhadh*	39	*Dio3os*
4	*Acot1*	16	*Cyp4v3*	28	*Chpt1*	40	*Tmem98*
5	*Ppm1k*	17	*Sfxn2*	29	*Ndrg3*	41	*Slc22a5*
6	*Adck5*	18	*Btbd11*	30	*Srd5a1*	42	*Magix*
7	*Adra1a*	19	*Ppargc1a*	31	*Lipe*	43	*Acad11*
8	*Krt23*	20	*Bc031353*	32	*Kank1*	44	*Abhd6*
9	*Ube2u*	21	*Mfsd7c*	33	*C2cd2l*	45	*Hsd17b10*
10	*Crot*	22	*Acadm*	34	*Fbxo21*	46	*Pex11a*
11	*Ccbl1*	23	*Mkx*	35	*Mmd*		
12	*Cdc42ep5*	24	*Odf3b*	36	*Tmc7*		

Table S4. Primer sequences used for ChIP-qPCR assays.

No.	Gene	Forward (5'-3')	Reverse (5'-3')
1	*Jmjd3*	AGTCTTTCAGGCCACACCAC	TTTCCCTTGGCATTAGCATC
2	*Sirt1*	AACTCCTCCACCTGCCTTG	GAGAGAGCGCAAACTTCCTG
3	*Fgf21*	TGGCTGTTTCTCCTGTGTTG	AGGTTCCTGCCAAGTGTGTC
4	*Pparα*	GCCCTTGATTTCCTCATCTG	CCCTGGTATGTCCTTGGATG
6	*Cpt1*	CTGGCCCGACTTCTCTACAC	GGACAGAGTGGCTTCTGGAG
7	*Mcad*	TCAGGAAAGATTGTTGATGTTGA	AGGCACACCAAAGCCTAGAA
8	*Pepck*	AGGCCTCCCAACATTCATTA	GCACGGTTTGGAACTGACTT
9	*G6Pase*	AAACTGACCCCAGGTCCTCT	CAGCCAACAGTGTCTCCAAT

Table S5. Primer sequences used for qRT-PCR.

No.	Gene	Forward (5'-3')	Reverse (5'-3')
1	*Sirt1*	TTGACTGTGAAGCTGTACGAGGA	CAAGCGGTTCATCAGCTGG
2	*Jmjd3*	TCTGCTGTAACCCACTGCTG	AGCCAATCATCACCCTTGTC
3	*Pparα*	CGAGGTGAAAGATTCGGAAA	GGCCTTGACCTTGTTCATGT
4	*Pgc1α*	GCGCCGTGTGATTTACGTT	AAAACTTCAAAGCGGTCTCTCAA
5	*Fgf21*	CTGGGGGTCTACCAAGCATA	CACCCAGGATTTGAATGACC
6	*Fgfr1*	GATGACCTCACCGCTCTACC	GGAAGTCGCTCTTCTTGGTG
7	*Cpt1*	TCGAAACATCTACCATGCAGCA	CAGCATTCTTCGTGACGTTGG
8	*Cpt2*	TGACCGACACTTGTTTGCTC	CTGGTGGACAGGATGTTGTG
9	*Mcad*	GATCGCAATGGGTGCTTTTGATAGAA	AGCTGATTGGCAATGTCTCCAGCAAA
10	*Cytc*	GGAGGCAAGCATAAGACTGG	TCCATCAGGGTATCCTCTCC
11	*Ehhadh*	CAGCACTGGATGTGGATGAC	CATGACTGTGGCGATGGTAG
12	*Hmgcs2*	ATACCACCAACGCCTGTTATGG	CAATGTCACCACAGACCACCAG
13	*Slc27a2*	GGAGTCGTGGAGGTCTGAAG	GCGATGATGATTGATGGTTG
15	*Aldh3a2*	CTTCCTGAATTGGCTTCTGC	AGCGGTTGCATGGTAAGAAC
16	*Acox1*	ACAGCCCAACTGTGACTTCC	AGGCATGTAACCCGTAGCAC
17	*Pepck*	CTTCTCTGCCAAGGTCATCC	TTTTGGGGATGGGCAC
18	*G6Pase*	GCTGAAACTTTCAGCCACATCC	TCCAAGCGGGAAACCAAAC
19	*Dgat*	GCCAGGCGCTTCTCAA	TGGTGTGTGGTGATGCTGATC
20	*Fas*	CCTGGATAGCATTCCGAACCT	AGCACATCTCGAAGGCTACACA
21	*CD36*	GCCAAGCTATTGCGACATGA	AAGGCATTGGCTGGAAGAAC
22	*Il-1b*	AACCTGCTGGTGTGTGACGTTC	CAGCACGAGGCTTTTTTGTTGT
23	*Saa-1*	ATTTGTTCACGAGGCTTTCC	CCCGAGCATGGAAGTATTTG
24	*Cxcl10*	TTTCTGCCTCATCCTGCTG	CCTATGGCCCTCATTCTCAC
25	*36B4*	CGACTCACAGAGCAGGC	CACCGAGGCAACAGTTGG