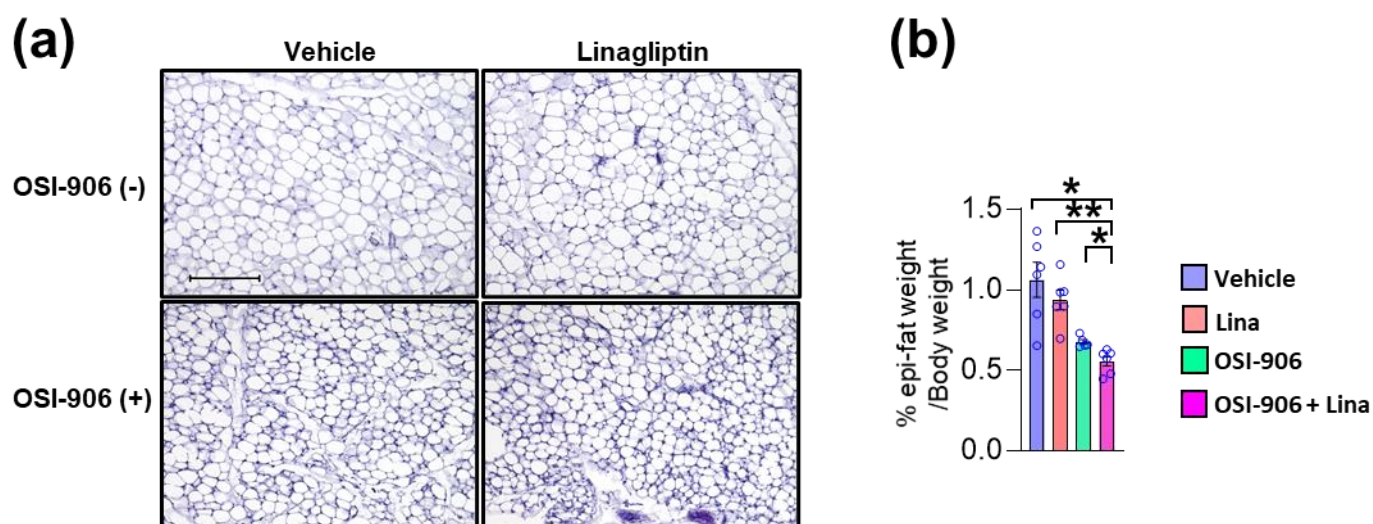


## Supplementary Materials

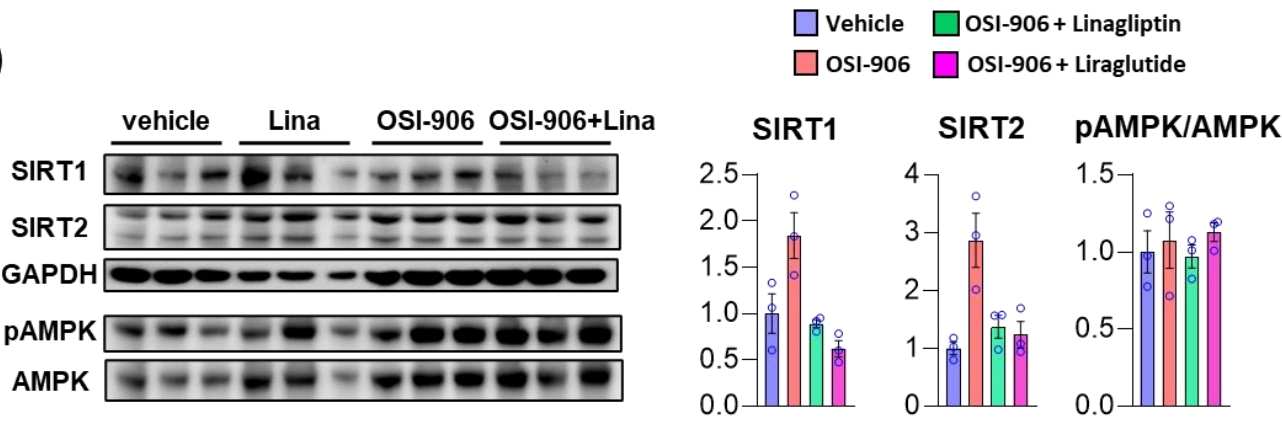
### Supplementary Figure 1



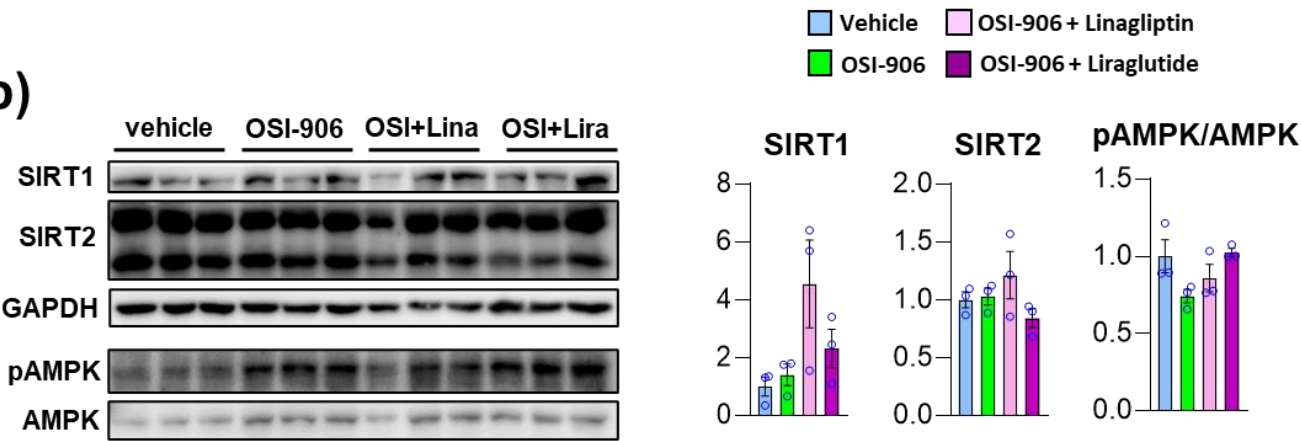
**Supplementary Figure 1.** Linagliptin did not improve lipoatrophy evoked by OSI-906. **(a)** Hematoxylin and eosin-stained sections of epididymal fat on day 7. Scale bar = 200  $\mu$ m. **(b)** Ratio of epididymal fat weight to body weight on day 7. Data represent the mean  $\pm$  SEM. \* $P$  < 0.05, \*\* $P$  < 0.01 ( $n$  = 5-6 per group) by ANOVA with an additional Tukey-Kramer post-hoc test.

Supplementary Figure 2

(a)



(b)

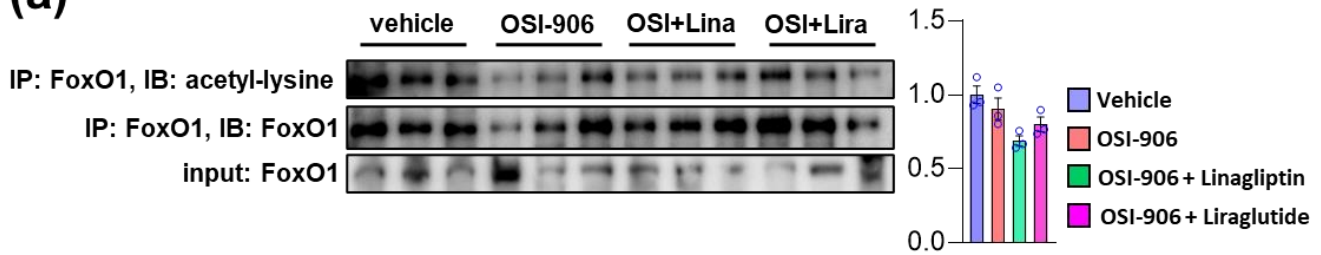


**Supplementary Figure 2.** Sirtuin expression and phosphorylation in AMPK in the liver and AML-12 cells.

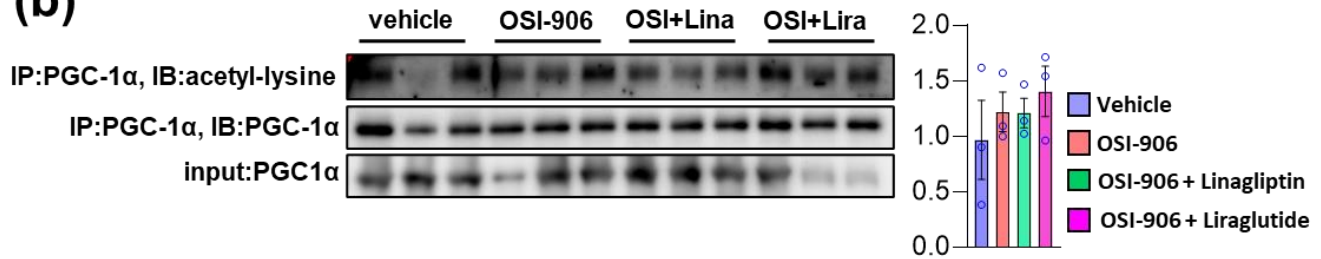
(a) Immunoblotting for SIRT1, SIRT2, and phosphorylation in AMPK in the liver. Densitometry of each protein was described in right graphs ( $n = 3$  per group). (b) Immunoblotting for SIRT1, SIRT2, and phosphorylation in AMPK in AML-12 cells treated with 10 nM linagliptin or 100 nM liraglutide in the presence of 200 nM OSI-906 are shown. Cells were serum-starved overnight and then incubated with OSI-906 for 4 hours before the treatment with linagliptin or liraglutide. Then, the cells were incubated for 24 hours with the indicated drugs. Densitometry of each protein was described in right graphs ( $n = 3$  per group). Data represent the mean  $\pm$  SEM.

## Supplementary Figure 3

(a)



(b)



**Supplementary Figure 3.** Sirtuin deacetylase activity on FoxO1 and PGC-1α in the liver.

(a) Immunoblotting for acetylated lysine after immunoprecipitation with FoxO1 in the liver. Densitometry of each protein was described in right graphs ( $n = 3$  per group). (b) Immunoblotting for acetylated lysine after immunoprecipitation with PGC-1α in the liver. Densitometry of each protein was described in right graphs ( $n = 3$  per group). Data represent the mean  $\pm$  SEM.

**Supplementary Table 1.** Proteins with hepatic expressions that were significantly altered by OSI-906 administration and restored by treatment with linagliptin

Protein expressions that were upregulated in OSI-906-treated liver and downregulated by linagliptin treatment

Protein Description	OSI-906 vs. vehicle		OSI-906 vs. OSI-906 + Lina	
	FC	ANOVA ( <i>P</i> )	FC	ANOVA ( <i>P</i> )
Cytochrome P450 2B10	4.51	0.0091	3.44	0.0272
Perilipin-2	3.60	0.0007	2.19	0.0073
Aspartate aminotransferase, cytoplasmic	2.58	0.0000	1.38	0.0265
Phosphoacetylglucosamine mutase	1.35	0.0296	1.31	0.0387
Bifunctional ATP-dependent dihydroxyacetone kinase/FAD-AMP Lyase (cyclizing)	1.92	<0.0001	1.28	0.0101
Fibronectin	1.21	0.0489	1.22	0.0389

Proteins expressions that were downregulated in OSI-906-treated liver and upregulated by linagliptin treatment

Protein Description	OSI-906 vs. vehicle		OSI-906 vs. OSI-906 + Lina	
	FC	ANOVA ( <i>P</i> )	FC	ANOVA ( <i>P</i> )
Major urinary protein 20	3.52	0.0038	2.23	0.0440
Lanosterol 14- $\alpha$ demethylase	2.30	0.0065	1.89	0.0147
Isopentenyl-diphosphate Delta-isomerase 1	1.67	0.0375	1.74	0.0041
ORM1-like protein 2	2.34	0.0013	1.57	0.0106
Protein YIPF5	1.69	0.0034	1.51	0.0370
Sterol-4- $\alpha$ -carboxylate 3-dehydrogenase, decarboxylating	1.38	0.0396	1.50	0.0026
Aldose reductase	1.32	0.0467	1.50	0.0125
Fetuin-B	1.67	0.0014	1.46	0.0292
Murinoglobulin-2	2.18	0.0002	1.42	0.0146
60S ribosomal protein L6	1.56	0.0009	1.38	0.0196
7-Dehydrocholesterol reductase	1.44	0.0437	1.37	0.0422
Pyrethroid hydrolase Ces2a	1.50	0.0026	1.33	0.0137
RNA-binding protein with multiple splicing 2	1.74	0.0018	1.32	0.0416
Thymosin beta-4	1.47	0.0001	1.27	0.0261
Vimentin	1.36	0.0368	1.27	0.0370
Carboxylesterase 1D	1.58	0.0011	1.23	0.0284
UDP-glucuronosyltransferase 2B17	1.32	0.0141	1.22	0.0194
60S ribosomal protein L19	1.50	<0.0001	1.22	0.0395
Cysteine desulfurase, mitochondrial	1.25	0.0042	1.21	0.0293

FC, fold change

**Supplementary Table 2.** Top upregulated or downregulated proteins in OSI-906-treated liver compared with vehicle-treated liver as revealed by a proteomic analysis

Upregulated in OSI-906-treated liver, compared with vehicle-treated liver

Protein Description	FC	ANOVA ( <i>P</i> )
Steroid 17- $\alpha$ -hydroxylase/17,20 lyase	36.6895	0.005451
Cytochrome P450 2B10	4.512313	0.00908
Nicotinamide N-methyltransferase	4.330706	1.25E-05
Apolipoprotein A-V	3.775928	0.00259
Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-1	3.627437	0.006191
Perilipin-2	3.603212	0.000742
BRISC complex subunit Abro1	2.799342	0.004814
Acyl-CoA synthetase short-chain family member 3, mitochondrial	2.787295	0.00223
Aspartate aminotransferase, cytoplasmic	2.576786	1.33E-05
Apolipoprotein A-IV	2.535404	0.004353

Downregulated in OSI-906-treated liver, compared with vehicle-treated liver

Protein Description	FC	ANOVA ( <i>P</i> )
Seminal vesicle secretory protein 4	22.56498	0.030458
Major urinary protein 17	4.820739	0.047282
Major urinary protein 20	3.52338	0.003799
Complement component C8 alpha chain	3.003397	0.002165
Developmentally-regulated GTP-binding protein 1	2.89517	0.047867
Alpha-1-antitrypsin 1-5	2.817766	0.000491
Cytochrome P450 4A12A	2.681725	0.032885
Bile salt sulfotransferase 2	2.597675	0.001371
3 beta-hydroxysteroid dehydrogenase type 5	2.5856	0.00529

FC, fold change

**Supplementary Table 3.** Top upregulated or downregulated proteins in OSI-906 + Lina-treated liver compared with OSI-906-treated liver as revealed by a proteomic analysis

Upregulated in OSI-906 + Lina-treated liver, compared with OSI-906-treated liver

Protein Description	FC	ANOVA ( <i>P</i> )
Major urinary protein 20	2.232176	0.044048
Lanosterol 14- $\alpha$ demethylase	1.892745	0.01474
Isopentenyl-diphosphate Delta-isomerase 1	1.743062	0.004141
ORM1-like protein 2	1.569106	0.010644
Protein YIPF5	1.509277	0.037018
Sterol-4- $\alpha$ -carboxylate 3-dehydrogenase, decarboxylating	1.50342	0.002579
Aldose reductase	1.501844	0.012548
Vacuolar protein-sorting-associated protein 25	1.499779	0.018167
Ubiquinone biosynthesis protein COQ7 homolog	1.49701	0.005744
60S ribosomal protein L37	1.478085	0.048925
Fetuin-B	1.464528	0.029177
Transgelin-2	1.453406	0.026038
Murinoglobulin-2	1.42467	0.014641
60S ribosomal protein L6	1.3833	0.019583

Downregulated in OSI-906 + Lina-treated liver, compared with OSI-906-treated liver

Protein Description	FC	ANOVA ( <i>P</i> )
Cytochrome P450 2B10	3.442558	0.027156
Perilipin-2	2.189518	0.007271
E3 ubiquitin-protein ligase UBR4	1.592003	0.03672
Sulfotransferase 1 family member D1	1.485781	0.041096
Histone H1.5	1.423912	0.029306
Aspartate aminotransferase, cytoplasmic	1.382278	0.026541

FC, fold change

**Supplementary Table 4.** Top upregulated or downregulated phosphopeptides in OSI-906-treated liver compared with vehicle-treated liver as revealed by a phosphoproteomic analysis

Upregulated phosphopeptide in OSI-906-treated liver compared with vehicle-treated liver

Uniprot ID	Phospho site	Protein Description	FC
Q3UHX2	S60	28 kDa heat- and acid-stable phosphoprotein	Infinity
Q8K2K6	S293	Arf-GAP domain and FG repeat-containing protein 1	Infinity
Q8K019	S396	Bcl-2-associated transcription factor 1	Infinity
O35218	S419, S420	Cleavage and polyadenylation specificity factor subunit 2	Infinity
Q5NCI0	S437	Up-regulator of cell proliferation	365.43
P57016	S62	Ladinin-1	197.61
Q3UPL0	S526, S530	Protein transport protein Sec31A	121.93
Q9CZ44	S74	NSFL1 cofactor p47	66.80
Q02819	S85	Nucleobindin-1	64.62
Q9Z131	S424	SH3 domain-binding protein 5	62.64
Q64511	S1387	DNA topoisomerase 2-beta	54.83
Q63918	S363	Serum deprivation-response protein	47.72
Q9CW07	S81	Protein phosphatase 1 regulatory subunit 3G	38.60
Q8K3J9	Y386	G-protein coupled receptor family C group 5 member C	31.32
Q4FJU9	S129	Transmembrane protein 40	30.37
E9Q557	S2620	Desmoplakin	25.28
P53996	S49	Cellular nucleic acid-binding protein	20.91
P60904	S10	DnaJ homolog subfamily C member 5	19.47
P16546	S1031	Spectrin alpha chain, non-erythrocytic 1	16.66
Q9Z1X4	S482	Interleukin enhancer-binding factor 3	15.77

FC, fold change

**Supplementary Table 5.** Top upregulated or downregulated phosphopeptides in OSI-906-treated liver compared with vehicle-treated liver as revealed by a phosphoproteomic analysis

Downregulated phosphopeptide in OSI-906-treated liver compared with vehicle-treated liver

Uniprot ID	Phosphosite	Protein Description	FC
Q6PAM0	S183	5'-AMP-activated protein kinase subunit beta-2	Infinity
Q8BP67	S86	60S ribosomal protein L24	Infinity
P28474	S247	Alcohol dehydrogenase class-3	Infinity
Q6PEE2	S72	CBP80/20-dependent translation initiation factor	Infinity
O88735	T243	Ensconsin	Infinity
Q6P9Q6	S1159	FK506-binding protein 15	Infinity
Q6ZQ58	S824	La-related protein 1	Infinity
O08638	S1954	Myosin-11	Infinity
Q8CES0	S39	N-alpha-acetyltransferase 30	Infinity
Q8K3K8	T210	Optineurin	Infinity
A2AJ88	S377	Patatin-like phospholipase domain-containing protein 7	Infinity
Q3U1F9	S351	Phosphoprotein associated with glycosphingolipid-enriched microdomains 1	Infinity
P60335	S190	Poly(rC)-binding protein 1	Infinity
Q8K1L5	S82	Protein phosphatase 1 regulatory subunit 11	Infinity
Q3TSG4	S385	RNA demethylase ALKBH5	Infinity
O89032	S812	SH3 and PX domain-containing protein 2A	Infinity
Q1HFZ0	S723	tRNA (cytosine(34)-C(5))-methyltransferase	Infinity
Q6P4T2	S225	U5 small nuclear ribonucleoprotein 200 kDa helicase	Infinity
Q9CQU5	S216	ZW10 interactor	Infinity
P56212	S62	cAMP-regulated phosphoprotein 19	784.37

FC, fold change



**Supplementary Table 6.** Top upregulated or downregulated phosphopeptides in OSI-906 + Lina-treated liver compared with OSI-906-treated liver as revealed by a phosphoproteomic analysis.

Upregulated phosphopeptide in OSI-906 + Lina-treated liver compared with OSI-906-treated liver

Uniprot ID	Phosphosite	Protein Description	FC
Q99J36	S8	THUMP domain-containing protein 1	Infinity
Q8K3K8	T210	Optineurin	Infinity
Q8VCB3	S8	Glycogen [starch] synthase, liver	Infinity
P14869	S304	60S acidic ribosomal protein P0	1302.87
P11352	S201	Glutathione peroxidase 1	255.83
Q8K2C9	S114	Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3	147.00
O88622	S311	Poly(ADP-ribose) glycohydrolase	104.31
C0HKD8	T267	Microfibrillar-associated protein 1	42.10
P24788	S270	Cyclin-dependent kinase 11B	37.66
Q1HFZ0	S723	tRNA (cytosine(34)-C(5))-methyltransferase	24.68
Q3UIR3	S4	E3 ubiquitin-protein ligase DTX3L	22.55
Q3UPL0	S526	Protein transport protein Sec31A	15.80
Q8R0W0	S3222	Epiplakin	12.02
Q5XG73	S184	Acyl-CoA-binding domain-containing protein 5	11.86
P07901	S194	Heat shock protein HSP 90- $\alpha$	10.32
Q91YI0	S5	Argininosuccinate lyase	9.05
Q61586	S687	Glycerol-3-phosphate acyltransferase 1, mitochondrial	7.97
Q9D0F9	S117	Phosphoglucomutase-1	7.46
Q8K2C9	S114	Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3	7.31
Q3THG9	S409	Alanyl-tRNA editing protein Aarsd1	6.72

FC, fold change

**Supplementary Table 7.** Top upregulated or downregulated phosphopeptides in OSI-906 + Lina-treated liver compared with OSI-906-treated liver as revealed by a phosphoproteomic analysis.

Downregulated phosphopeptide in OSI-906 + Lina-treated liver compared with OSI-906-treated liver

Uniprot ID	Phosphosite	Protein Description	FC
Q7TN98	S99	Cytoplasmic polyadenylation element-binding protein 4	Infinity
Q91YR1	S143	Twinfilin-1	Infinity
Q3TLH4	S189, S191	Protein PRRC2C	Infinity
Q8BWW4	S578	La-related protein 4	Infinity
P59242	S134	Cingulin	Infinity
P29699	S138	Alpha-2-HS-glycoprotein	Infinity
D3Z6Q9	S443	Bridging integrator 2	Infinity
P51954	S1071	Serine/threonine-protein kinase Nek1	Infinity
P11679	S336	Keratin, type II cytoskeletal 8	1286.64
Q8CI51	S228	PDZ and LIM domain protein 5	68.39
Q8K2C9	S114	Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3	64.81
Q9JLV1	S360	BAG family molecular chaperone regulator 3	22.55
P51859	T200	Hepatoma-derived growth factor	13.39
P47955	S101, S104	60S acidic ribosomal protein P1	11.59
Q99K28	S339	ADP-ribosylation factor GTPase-activating protein 2	7.58
P70302	S519	Stromal interaction molecule 1	7.40
P27546	S901	Microtubule-associated protein 4	5.84
Q8BI08	S2	Protein MAL2	4.72
Q9JIX8	S825	Apoptotic chromatin condensation inducer in the nucleus	2.56
Q6ZQ58	T626	La-related protein 1	2.28

FC, fold change

**Supplementary Table 8.** Top upstream regulators as revealed by a proteomic analysis

OSI-906 vs. Vehicle

Top Upstream Regulators	$-\text{LOG}_{10}(P\text{-value})$	Predicted Activation
PPARA	9.436518915	Inhibited
bezafibrate	6.28567024	Inhibited
XBP1	6.262012674	Activated
pirinixic acid	5.747146969	Inhibited
ciprofibrate	5.329754147	Inhibited

OSI-906 + Lina vs. OSI-906

Top Upstream Regulators	$-\text{LOG}_{10}(P\text{-value})$	Predicted Activation
SREBF2	9.436518915	Inhibited
SH3TC2	6.28567024	
lysophosphatidylcholine	6.262012674	Inhibited
AGN194204	5.747146969	
NFE2L2	5.329754147	

**Supplementary Table 9.** Primer sequence for real-time PCR

Gene Name	Forward	Reverse
Ccl2	5'-TTAAAAACCTGGATCGGAACCAA-3'	5'-GCATTAGCTTCAGATTACGGGT-3'
Cd36	5'-CCTTAAAGGAATCCCCGTGT-3'	5'-TGCATTTGCCAATGTCTAGC-3'
Cyp2b10	5'-CAATGTTTAGTGGAGGAACTGCG-3'	5'-CACTGGAAGAGGAACGTGGG-3'
Fas	5'-CCTGGATAGCATTCGGAACCT-3'	5'-AGCACATCTCGAAGGCTACACA-3'
Gck	5'-GCGGAGATGCTCTTTGAC-3'	5'-GTCCCACGATGTTGTTCC-3'
G6pase	5'-TCTGTCCCGGATCTACCTTG-3'	5'-GAAAGTTTCAGCCACAGCAA-3'
ICAM-1	5'-AGATCACATTCACGGTGCTG-3'	5'-CTTCAGAGGAGGAAACAGG-3'
Il-6	5'-TAGTCCTTCCTACCCCAATTTCC-3'	5'-TTGGTCCTTAGCCACTCCTTC-3'
iNOS	5'-CCCTTCCGAAGTTTCTGGCAGCAGC-3'	5'-GGCTGTCAGAGAGCCTCGTGGCTTTGG-3'
NNMT	5'-AGGAACCAGGAGCCTTTGACT-3'	5'-CCTGAGGGCAGTGCGATAGG-3'
PAI-1	5'-GACACCCTCAGCATGTTTCATC-3'	5'-AGGGTTGCACTAAACATGTCAG-3'
Pepck	5'-CTGCATAACGGTCTGGACTTC-3'	5'-CAGCAACTGCCCCGTACTCC-3'
Perilipin 2	5'-GTGGAAAGGACCAAGTCTGTG-3'	5'-GACTCCAGCCGTTCATAGTTG-3'
Pgc1a	5'-AACCACACCCACAGGATCAGA-3'	5'-TCTTCGCTTTATTGCTCCATGA-3'
Ppara	5'-ACGATGCTGTCCTCCTTGATG-3'	5'-GTGTGATAAAGCCATTGCCGT-3'
Scd1	5'-CCGGAGACCCCTTAGATCGA-3'	5'-TAGCCTGTAAAAGATTTCTGCAAACC-3'
Socs3	5'-GGGTGGCAAAGAAAAGGAG-3'	5'-GTTGAGCGTCAAGACCCAGT-3'
Srebp1c	5'-GGCACTAAGTGCCCTCAACCT-3'	5'-GCCACATAGATCTCTGCCAGTGT-3'
Tbp	5'-GGGGAGCTGTGATGTGAAGT-3'	5'-CCAGGAAATAATTCTGGCTCA-3'
Tnf	5'-CCCTCACACTCAGATCATCTTCT-3'	5'-GCTACGACGTGGGCTACAG-3'