

**Leptin signaling promotes milk fat synthesis via PI3K/AKT/mTOR/SREBP1 in mammary
gland of dairy cows**

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SUPPLEMENTARY FILE

Material and methods

Transfection

For leptin or OB-Rb knockdown, transient transfection was performed using siRNA-mate (G04003, GenePharma, Shanghai, China) according to the manufacturer's instructions. Mammary epithelial cells were plated in 6-well plates at 1×10^6 cells/well, ~50% confluent cells were transfected with 20 μ M of bovine specific small interfering RNA (siRNA, GenePharma, Shanghai, China), and scrambled siRNA as negative control. The siRNA sequences used are shown in Table S1. To detect the effect of leptin on OB-Rb expression and milk fat synthesis, cells were transfected with OB-Rb or SREBP1 RNAi and with or without the addition of 200 ng/mL leptin for 24 h. To detect the effect of fatty acid synthesis precursors on leptin and OB-Rb expression, were changed to serum-free medium (1% BSA) with 8 mM acetate and 1 mM BHB before transfection, then transfected with leptin RNAi. All experiments were performed in triplicate. After 24 h of transfection, cells were harvested for quantitative real-time PCR (qRT-PCR). After 48 h of transfection, cells were harvested for western blot analysis and TAG content assays.

Quantitative real-time PCR

The expression levels of leptin, OB-Rb and DEGs randomly screened in transcriptome sequencing were determined by qRT-PCR using gene-specific primers (Table S2). qRT-PCR was performed in the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Gland Island, NY) with 10 μ L ChamQ Universal SYBR qPCR Master Mix (Q711-02, Vazyme, Nanjing, China), 100 ng cDNA and 10 pM primers. Thermal cycling conditions were: pre-denaturalization at 95°C for 30 s, followed by 10 s denaturation at 95°C and 30 s annealing and extension at 60°C, repeated 40 cycles. Each gene was amplified in a separate reaction and each reaction was performed in triplicate. The relative expression of target genes was normalized to β -actin and calculated by the $2^{-\Delta\Delta CT}$ method.

Western blot

The tissues or cells were lysed at 4°C with 1% SDS lysis containing phenylmethylsulfonyl fluoride (PMSF, 0.5 mM, ST506, Beyotime), and divided in 10% SDS-PAGE and transferred to nitrocellulose membrane. Membranes were blocked in 5% skim milk and incubated with primary antibodies Leptin (1:1000 dilution), OB-Rb (1:1000 dilution), AKT (1:1000 dilution, A11016, ABclonal), p-AKT-S473 (1:1000 dilution, AP0140, ABclonal), p-AKT-T308 (1:1000 dilution, AP1259, ABclonal), mTOR (1:1000 dilution, A11355, ABclonal), p-mTOR (1:1000 dilution, AP0115, ABclonal), ACC (1:1000 dilution, 3662S, Cell Signaling Technology, Danvers, MA), p-ACC (1:1000 dilution, 11818T, Cell Signaling Technology), FASN (1:1000 dilution, 3189S, Cell Signaling Technology), SREBP1 (1:200 dilution, sc-17755, Santa Cruz Biotechnology, CA), DGAT1 (1:1000 dilution, A6857, ABclonal) and β -actin (1:10,000 dilution, AC006, ABclonal) at 4°C overnight. After washing, membranes were incubated with anti-rabbit IgG HRP-linked secondary antibody (1:10,000 dilution; ABclonal) at 37°C for 1 h. β -actin was used as an internal control. The blots were then visualized using enhanced chemiluminescent detection reagent (ECL, 180-506, Tanon, Shanghai, China), and then quantified via ImageJ (Media Cybernetics Inc., Warrendale, PA).

Supplementary Figures

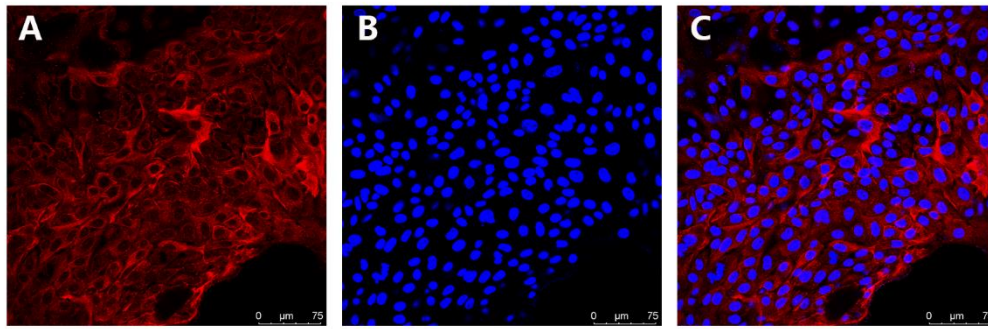


Fig. S1. Cytokeratin 18 expression in cultured mammary epithelial cells of dairy cows detected by laser confocal microscope. (A) Alexa Fluor 647-labeled cytokeratin 18 (red). (B) DAPI-labeled nucleus (blue). (C) Merge of (A) and (B). scale bar = 75 μm .

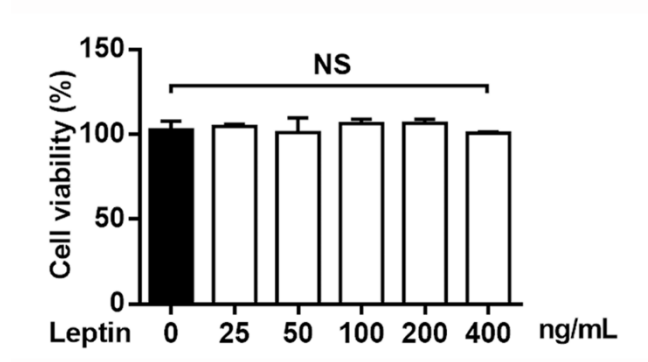


Fig. S2. The bovine mammary epithelial cells were treated with indicated concentrations of Leptin for 24 h, and cell viability was assessed with the MTT assay. Values are expressed as the mean \pm SD (n = 3); * $P < 0.05$; ** $P < 0.01$. NS = no significance.



Fig. S3. The expression of OB-Rb in mammary epithelial cells of dairy cows detected by western blot. (A) Transfection efficiency of GFP-labeled scramble RNA transfected cells. scale bar = 500 μ m. (B) Western blot analyses show the levels of OB-Rb in OB-Rb-knockdown bovine mammary epithelial cells. (C) Quantification of protein levels from the western blot in panel A. Values are expressed as the mean \pm SD (n = 3); * P < 0.05; ** P < 0.01.

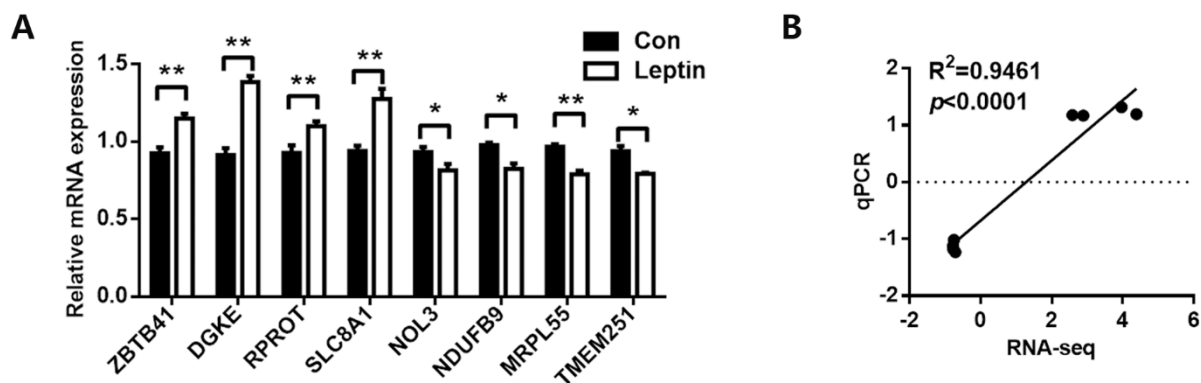


Fig. S4. Relative expression of the selected DEGs mRNA levels in Leptin-treated cells were detected by quantitative RT-PCR. Values are expressed as the mean \pm SD (n = 3); * P < 0.05; ** P < 0.01. (A) Quantitative analysis of up- and down-regulated genes. (B) Correlation analysis of RNA-seq and qPCR data. Values are expressed as the mean \pm SD (n = 3); * P < 0.05; ** P < 0.01.



Fig. S5. The expression of Leptin in mammary epithelial cells of dairy cows detected by western blot. (A) Transfection efficiency of GFP-labeled scramble RNA transfected cells. scale bar = 500 μ m. (B) Western blot analyses show the levels of Leptin in Leptin-knockdown bovine mammary epithelial cells. (C) Quantification of protein levels from the western blot in panel A. Values are expressed as the mean \pm SD (n = 3); * P < 0.05; ** P < 0.01.

Supplemented data Tables

Table S1. siRNA sequence.

Site	Sequence (5'-3')
Negative control	UUCUCCGAACGUGUCACGUTT
OB-Rb-siRNA1-879	GCUAGUAGACAGUGUGCUUTT
OB-Rb-siRNA2-965	GGAGUGCCCUUCUUACCUUTT
OB-Rb-siRNA3-1027	GCGGUUGGGUCUAACAUUUTT
Leptin-siRNA1-74	CCUGUGGCUUUGGCCCUAUTT
Leptin-siRNA2-265	CCAAGAUGGACCAGACAUUTT
Leptin-siRNA3-441	GGCGUUGUCCUGGAAGCUUTT

Table S2. Primer sequences for quantitative real-time PCR.

Gene	GenBank ID	Primer sequences (5' to 3')
<i>Leptin</i>	NM_173928.2	Forward: 5'-CAGAGGGTCACTGGTTTGGGA-3' Reverse: 5'-CTGTTGGTAGATCGCCAATGT-3'
<i>OB-Rb</i>	NM_001012285.2	Forward: 5'-CACGGGCTATCAGCAAAGTA-3' Reverse: 5'-GACAGCATCCAGGAAAGAAT-3'
<i>β-actin</i>	NM_173979.3	Forward: 5'-TGTTAGCTGCGTTACACCCT-3' Reverse: 5'-CTGTCACCTTCACCGTTCC-3'
<i>ZBTB41</i>	XM_003587103.4	Forward: 5'-GCGACATTTAAACGGAAGGAC-3' Reverse: 5'-GGGTCATCGGGAGACTTTATG-3'
<i>SLC8A1</i>	NM_176632.2	Forward: 5'-TCCTTTCAGTAATCGAGGTGTG-3' Reverse: 5'-CTTGATCTTCCTTGTCTCCCC-3'
<i>RPTOR</i>	NM_001192130.3	Forward: 5'-CATCCAGAAGGGTATCACAAGG-3' Reverse: 5'-TTCTCCCAGTCCCAAAAGC-3'
<i>DGKE</i>	NM_001192930.3	Forward: 5'-GTTCTGAATATCGGCTACTGGG-3' Reverse: 5'-GAAAGACCCATATACTCCGACG-3'
<i>NOL3</i>	NM_001083688.2	Forward: 5'-CTGACTCAGGAACCACGTG-3' Reverse: 5'-GGTTCAGCCCCTTCAGAG-3'
<i>NDUFB9</i>	NM_175783.4	Forward: 5'-GAAAAGGACATGGTGAAGGC-3' Reverse: 5'-AGACTCAGGGAAGATGTACGG-3'
<i>TMEM251</i>	NM_001077132.1	Forward: 5'-CAAAGCCACCCGATTATTCAG-3' Reverse: 5'-TGGTCCCGAAAATCTTCCTG-3'
<i>MRPL55</i>	NM_001303490.1	Forward: 5'-CCATCCACATCCGTTATCCAG-3' Reverse: 5'-GCTCCTCCTTCTTCTTTCAG-3'

Table S3. Expression changes of DEGs.

Gene_ID	Gene name	Gene description	Log2FC ¹	P-value
Up-regulate				
ENSBTAG00000050242	<i>Uncharacterized</i>	-	20.13	4.98E-06
ENSBTAG00000052987	<i>LOC112445144</i>	melanoma-associated antigen D4-like	1.11	5.68E-06
ENSBTAG00000015808	<i>ZNF609</i>	zinc finger protein 609	4.04	1.57E-05
ENSBTAG00000052980	<i>lncRNA</i>	-	2.95	1.57E-04
ENSBTAG00000015103	<i>IQCC</i>	IQ motif containing C	1.82	2.02E-04
ENSBTAG00000049828	<i>Uncharacterized</i>	-	5.52	2.37E-03
ENSBTAG00000051001	<i>Uncharacterized</i>	-	5.45	4.34E-03
ENSBTAG00000002037	<i>ATXN7L1</i>	ataxin 7 like 1	2.51	4.71E-03
ENSBTAG00000021835	<i>PRICKLE2</i>	prickle planar cell polarity protein 2	2.83	6.28E-03
ENSBTAG00000053749	<i>Uncharacterized</i>	-	1.23	7.39E-03
ENSBTAG00000054576	<i>Uncharacterized</i>	-	3.14	8.56E-03
ENSBTAG00000054137	<i>Uncharacterized</i>	-	5.01	9.64E-03
ENSBTAG00000012694	<i>UHMK1</i>	U2AF homology motif kinase 1	2.57	1.00E-02
ENSBTAG00000018697	<i>WDR44</i>	WD repeat domain 44	0.69	1.07E-02
ENSBTAG00000051822	<i>Uncharacterized</i>	-	1.25	1.15E-02
ENSBTAG00000046333	<i>C6H4orf54</i>	chromosome 6 C4orf54 homolog	2.92	1.17E-02
ENSBTAG00000049661	<i>Uncharacterized</i>	-	1.28	1.20E-02
ENSBTAG00000025071	<i>TENM2</i>	teneurin transmembrane protein 2	0.98	1.25E-02
ENSBTAG00000010131	<i>CBFA2T2</i>	CBFA2/RUNX1 partner transcriptional co-repressor 2	1.67	1.33E-02
ENSBTAG00000000321	<i>TBL2</i>	transducin beta like 2	0.65	1.37E-02
ENSBTAG00000052993	<i>Uncharacterized</i>	-	1.97	1.40E-02
ENSBTAG00000017074	<i>ANO8</i>	anoctamin 8	0.60	1.41E-02

ENSBTAG00000004813	<i>SUSD1</i>	sushi domain containing 1	1.31	1.45E-02
ENSBTAG000000047036	<i>GPR39</i>	G protein-coupled receptor 39	0.54	1.46E-02
ENSBTAG000000010389	<i>STBD1</i>	starch binding domain 1	0.52	1.53E-02
ENSBTAG000000051364	<i>Uncharacterized</i>	-	0.52	1.66E-02
ENSBTAG000000018199	<i>ZBTB8A</i>	zinc finger and BTB domain containing 8A kelch like family member	1.10	1.68E-02
ENSBTAG000000050549	<i>KLHL11</i>	11 Entrezgene);Acc:538695]	1.55	1.68E-02
ENSBTAG000000002081	<i>BMPRI1B</i>	bone morphogenetic protein receptor type 1B	1.17	1.70E-02
ENSBTAG000000045727	<i>EPPK1</i>	epiplakin 1	0.59	1.79E-02
ENSBTAG000000012762	<i>C7H5orf24</i>	chromosome 7 C5orf24 homolog	1.60	1.80E-02
ENSBTAG000000054862	<i>Uncharacterized</i>	-	4.16	1.82E-02
ENSBTAG000000003906	<i>SUPT7L</i>	SPT7 like, STAGA complex subunit gamma	0.62	1.84E-02
ENSBTAG000000052276	<i>Uncharacterized</i>	-	1.91	1.86E-02
ENSBTAG000000009523	<i>SLC9A6</i>	solute carrier family 9 member A6	1.12	1.86E-02
ENSBTAG000000019174	<i>ZNF710</i>	zinc finger protein 710	0.57	1.87E-02
ENSBTAG000000005108	<i>SLIT2</i>	slit guidance ligand 2	0.98	1.89E-02
ENSBTAG000000053268	<i>Uncharacterized</i>	-	0.53	1.91E-02
ENSBTAG000000015887	<i>FOXJ3</i>	forkhead box J3	1.27	1.92E-02
ENSBTAG000000032031	<i>ZBTB12</i>	zinc finger and BTB domain containing 12	0.75	1.96E-02
ENSBTAG000000011971	<i>NRP2</i>	neuropilin 2	1.50	1.98E-02
ENSBTAG000000021741	<i>RPS6KA2</i>	ribosomal protein S6 kinase A2	0.79	1.98E-02
ENSBTAG000000018658	<i>TNRC6C</i>	trinucleotide repeat containing adaptor 6C	1.13	2.00E-02

ENSBTAG00000013486	<i>C5H12orf66</i>	chromosome 5 C12orf66 homolog	2.53	2.05E-02
ENSBTAG00000012555	<i>RBM15</i>	RNA binding motif protein 15	1.27	2.08E-02
ENSBTAG00000010402	<i>MYH9</i>	myosin heavy chain 9	0.53	2.09E-02
ENSBTAG00000019953	<i>CYBB</i>	cytochrome b-245 beta chain	1.56	2.20E-02
ENSBTAG00000020270	<i>NFKB1</i>	nuclear factor kappa B subunit 1	0.47	2.26E-02
ENSBTAG00000022922	<i>Uncharacterized</i>	-	4.89	2.28E-02
ENSBTAG00000020489	<i>SEMA6A</i>	semaphorin 6A regulatory associated	0.95	2.34E-02
ENSBTAG00000002883	<i>RPTOR</i>	protein of MTOR complex 1	0.64	2.36E-02
ENSBTAG000000055046	<i>Uncharacterized</i>	-	2.20	2.39E-02
ENSBTAG000000048672	<i>Uncharacterized</i>	-	0.99	2.39E-02
ENSBTAG000000052099	<i>Uncharacterized</i>	-	2.60	2.45E-02
ENSBTAG000000010360	<i>LRIG1</i>	leucine rich repeats and immunoglobulin like domains 1	0.56	2.47E-02
ENSBTAG000000001586	<i>OTUD7B</i>	OTU deubiquitinase 7B centrobin, centriole	0.95	2.50E-02
ENSBTAG000000047756	<i>CNTROB</i>	duplication and spindle assembly protein	0.37	2.52E-02
ENSBTAG000000009755	<i>GOLGA3</i>	golgin A3	0.59	2.57E-02
ENSBTAG000000044195	<i>SDK2</i>	sidekick cell adhesion molecule 2	0.57	2.60E-02
ENSBTAG000000015905	<i>ARHGAP32</i>	Rho GTPase activating protein 32	1.62	2.64E-02
ENSBTAG000000002920	<i>KAT6B</i>	lysine acetyltransferase 6B	0.87	2.67E-02
ENSBTAG000000010951	<i>NHLRC3</i>	NHL repeat containing 3	1.17	2.67E-02
ENSBTAG000000011894	<i>C22H3orf62</i>	chromosome 22 C3orf62 homolog	2.26	2.70E-02

ENSBTAG00000052421	<i>Uncharacterized</i>	-	1.16	2.88E-02
ENSBTAG00000026371	<i>SNX29</i>	sorting nexin 29	1.40	2.89E-02
ENSBTAG00000002349	<i>STX1B</i>	syntaxin 1B	2.13	2.90E-02
ENSBTAG00000014170	<i>MAP7D3</i>	MAP7 domain containing 3	0.89	2.96E-02
ENSBTAG00000055301	<i>Uncharacterized</i>	-	1.79	2.97E-02
ENSBTAG00000003062	<i>FAM160B1</i>	family with sequence similarity 160 member B1	1.67	3.01E-02
ENSBTAG00000018984	<i>PIK3CD</i>	bisphosphate 3-kinase catalytic subunit delta	0.46	3.08E-02
ENSBTAG00000040019	<i>KRT6C</i>	keratin 6C	2.30	3.14E-02
ENSBTAG00000022799	<i>NOTCH1</i>	notch receptor 1	0.87	3.14E-02
ENSBTAG00000051182	<i>Uncharacterized</i>	-	1.02	3.16E-02
ENSBTAG00000005514	<i>TRIO</i>	trio Rho guanine nucleotide exchange factor	0.70	3.23E-02
ENSBTAG00000051567	<i>CDKN2B</i>	cyclin dependent kinase inhibitor 2B	0.80	3.27E-02
ENSBTAG00000014126	<i>UNKL</i>	unk like zinc finger	0.83	3.34E-02
ENSBTAG00000018284	<i>KBTBD11</i>	kelch repeat and BTB domain containing 11	3.08	3.34E-02
ENSBTAG00000020469	<i>TMED8</i>	trafficking protein family member 8	2.51	3.37E-02
ENSBTAG00000012412	<i>URB1</i>	URB1 ribosome biogenesis 1 homolog (<i>S. cerevisiae</i>)	0.52	3.37E-02
ENSBTAG00000018093	<i>KMT2A</i>	lysine methyltransferase 2A	0.79	3.39E-02
ENSBTAG00000000421	<i>EEA1</i>	early endosome antigen 1	0.92	3.39E-02
ENSBTAG00000048699	<i>Uncharacterized</i>	-	0.45	3.40E-02
ENSBTAG00000011101	<i>EML6</i>	EMAP like 6	1.18	3.45E-02
ENSBTAG00000052094	<i>Uncharacterized</i>	-	2.12	3.49E-02
ENSBTAG00000026909	<i>Uncharacterized</i>	-	0.47	3.54E-02

ENSBTAG00000020426	<i>TFAP2B</i>	transcription factor AP-2 beta	0.63	3.54E-02
ENSBTAG00000020570	<i>PRDM8</i>	PR/SET domain 8	0.63	3.59E-02
ENSBTAG00000008932	<i>SHISA7</i>	shisa family member 7	4.43	3.59E-02
ENSBTAG00000013163	<i>ADAM33</i>	ADAM metallopeptidase domain 33	0.44	3.62E-02
ENSBTAG00000022360	<i>SOX5</i>	SRY-box transcription factor 5	4.44	3.64E-02
ENSBTAG00000048547	<i>Uncharacterized</i>	-	0.99	3.65E-02
ENSBTAG00000011197	<i>IPMK</i>	inositol polyphosphate multikinase	1.72	3.70E-02
ENSBTAG000000051942	<i>Uncharacterized</i>	-	0.72	3.74E-02
ENSBTAG00000004449	<i>DGKE</i>	diacylglycerol kinase epsilon	2.07	3.75E-02
ENSBTAG00000017429	<i>PREPL</i>	prolyl endopeptidase like	1.34	3.75E-02
ENSBTAG00000016498	<i>PHACTR2</i>	phosphatase and actin regulator 2	1.31	3.77E-02
ENSBTAG00000009267	<i>UHRF1BP1</i>	UHRF1 binding protein 1	1.36	3.77E-02
ENSBTAG000000051915	<i>C6H4orf36</i>	chromosome 6 C4orf36 homolog	4.50	3.78E-02
ENSBTAG00000002291	<i>ZBTB41</i>	zinc finger and BTB domain containing 41 tumor necrosis factor	1.65	3.81E-02
ENSBTAG00000049392	<i>LOC104968444</i>	receptor superfamily member 10B-like	1.12	3.84E-02
ENSBTAG00000004975	<i>CCDC191</i>	coiled-coil domain containing 191	0.84	3.85E-02
ENSBTAG00000018424	<i>ACKR3</i>	atypical chemokine receptor 3	0.51	3.88E-02
ENSBTAG00000007186	<i>ARHGAP39</i>	Rho GTPase activating protein 39	0.54	3.89E-02
ENSBTAG000000053731	<i>Uncharacterized</i>	-	0.96	3.91E-02
ENSBTAG00000014920	<i>SEMA3E</i>	semaphorin 3E	1.88	3.93E-02

ENSBTAG00000018449	<i>NUMA1</i>	nuclear mitotic apparatus protein 1	0.35	3.95E-02
ENSBTAG00000021151	<i>MYH10</i>	myosin heavy chain 10	0.58	3.97E-02
ENSBTAG00000009358	<i>MTSS2</i>	MTSS I-BAR domain containing 2	0.40	4.01E-02
ENSBTAG00000019256	<i>GLA</i>	galactosidase alpha	1.00	4.01E-02
ENSBTAG00000019807	<i>COL27A1</i>	collagen type XXVII alpha 1 chain	0.42	4.02E-02
ENSBTAG00000004364	<i>THNSL2</i>	threonine synthase like 2	0.90	4.06E-02
ENSBTAG00000009736	<i>CDC42BPG</i>	CDC42 binding protein kinase gamma	0.44	4.07E-02
ENSBTAG00000022890	<i>MBP</i>	myelin basic protein	1.65	4.09E-02
ENSBTAG00000006511	<i>MTF1</i>	metal regulatory transcription factor 1	0.79	4.13E-02
ENSBTAG00000007052	<i>BDKRB1</i>	bradykinin receptor B1	2.89	4.14E-02
ENSBTAG00000013439	<i>ARHGEF26</i>	Rho guanine nucleotide exchange factor 26	1.20	4.15E-02
ENSBTAG00000021945	<i>NID2</i>	nidogen 2	0.48	4.16E-02
ENSBTAG00000044121	<i>NUBPL</i>	nucleotide binding protein like	1.04	4.18E-02
ENSBTAG00000051572	<i>ZNF646</i>	zinc finger protein 646	0.44	4.18E-02
ENSBTAG00000006261	<i>GPR17</i>	G protein-coupled receptor 17	3.70	4.22E-02
ENSBTAG00000038674	<i>ZNF677</i>	zinc finger protein 677	1.10	4.24E-02
ENSBTAG00000007379	<i>ALK</i>	ALK receptor tyrosine kinase	4.42	4.28E-02
ENSBTAG00000046765	<i>POLR2A</i>	RNA polymerase II subunit A	0.62	4.29E-02
ENSBTAG00000033515	<i>Uncharacterized</i>	-	0.69	4.33E-02
ENSBTAG00000013412	<i>NFAT5</i>	nuclear factor of activated T cells 5	1.87	4.33E-02
ENSBTAG00000021237	<i>DST</i>	dystonin	0.60	4.34E-02

ENSBTAG00000002528	<i>EIF3A</i>	eukaryotic translation initiation factor 3 subunit A	0.46	4.35E-02
ENSBTAG00000004948	<i>LRATD1</i>	LRAT domain containing 1	0.73	4.41E-02
ENSBTAG00000009159	<i>PLXNA1</i>	plexin A1	0.63	4.43E-02
ENSBTAG00000020379	<i>AREL1</i>	apoptosis resistant E3 ubiquitin protein ligase 1	0.46	4.43E-02
ENSBTAG00000014079	<i>GIGYF2</i>	GRB10 interacting GYF protein 2	0.46	4.49E-02
ENSBTAG00000015801	<i>EFNB1</i>	ephrin B1	0.47	4.50E-02
ENSBTAG00000013861	<i>SLC8A1</i>	solute carrier family 8 member A1	1.33	4.52E-02
ENSBTAG00000015151	<i>FOXN2</i>	forkhead box N2	1.90	4.54E-02
ENSBTAG00000016931	<i>E2F7</i>	E2F transcription factor 7	1.15	4.56E-02
ENSBTAG00000011011	<i>SSH2</i>	slingshot protein phosphatase 2	1.47	4.57E-02
ENSBTAG00000006167	<i>BTBD8</i>	BTB (POZ) domain containing 8	0.89	4.58E-02
ENSBTAG00000052789	<i>Uncharacterized</i>	- phosphoinositide kinase,	0.87	4.59E-02
ENSBTAG00000002177	<i>PIKFYVE</i>	FYVE-type zinc finger containing	1.56	4.61E-02
ENSBTAG00000020126	<i>MYO10</i>	myosin X	0.64	4.63E-02
ENSBTAG00000009760	<i>LOC515736</i>	uncharacterized LOC515736	0.47	4.64E-02
ENSBTAG00000018324	<i>AGO3</i>	argonaute RISC catalytic component 3	1.08	4.65E-02
ENSBTAG00000031891	<i>NLGN1</i>	neuroligin 1	1.95	4.65E-02
ENSBTAG00000012867	<i>TXLNA</i>	taxilin alpha	0.81	4.65E-02
ENSBTAG00000014734	<i>CHD4</i>	chromodomain helicase DNA binding protein 4	0.32	4.66E-02
ENSBTAG00000048551	<i>Uncharacterized</i>	-	4.97	4.68E-02
ENSBTAG00000043964	<i>ARL5B</i>	ADP ribosylation factor like GTPase 5B	1.98	4.69E-02

ENSBTAG00000008097	<i>WNT2</i>	Wnt family member 2	2.47	4.71E-02
ENSBTAG000000054541	<i>Uncharacterized</i>	-	0.96	4.74E-02
ENSBTAG000000017090	<i>SMCR8</i>	SMCR8-C9orf72 complex subunit	1.19	4.75E-02
ENSBTAG00000000939	<i>KIF16B</i>	kinesin family member 16B	0.53	4.77E-02
ENSBTAG00000006471	<i>OSBPL11</i>	oxysterol binding protein like 11	1.00	4.81E-02
ENSBTAG000000055174	<i>Uncharacterized</i>	-	1.21	4.81E-02
ENSBTAG000000020638	<i>TIMP3</i>	TIMP metallopeptidase inhibitor 3	0.60	4.82E-02
ENSBTAG000000017440	<i>METTL16</i>	methyltransferase like 16	0.66	4.83E-02
ENSBTAG000000002329	<i>ASAP2</i>	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2	1.17	4.83E-02
ENSBTAG000000048521	<i>Uncharacterized</i>	-	1.64	4.87E-02
ENSBTAG000000048887	<i>Uncharacterized</i>	-	3.70	4.87E-02
ENSBTAG000000008814	<i>ADGRA2</i>	adhesion G protein-coupled receptor A2	0.51	4.87E-02
ENSBTAG000000008665	<i>DIXDC1</i>	DIX domain containing 1	1.40	4.87E-02
ENSBTAG000000050748	<i>Uncharacterized</i>	-	1.38	4.90E-02
ENSBTAG000000015026	<i>ABL2</i>	ABL proto-oncogene 2, non-receptor tyrosine kinase	0.39	4.92E-02
ENSBTAG000000016707	<i>FKBP9</i>	FKBP prolyl isomerase 9	0.70	4.93E-02
ENSBTAG000000013205	<i>ILIRAP</i>	interleukin 1 receptor accessory protein	1.01	4.94E-02
ENSBTAG000000003669	<i>BNC2</i>	basonuclin 2	1.77	4.95E-02
ENSBTAG000000006995	<i>SPTBN1</i>	spectrin beta, non-erythrocytic 1	0.56	4.96E-02
ENSBTAG000000006878	<i>TAF1</i>	TATA-box binding protein associated factor 1	0.61	4.97E-02

ENSBTAG00000007779	<i>GTF3C3</i>	general transcription factor IIIC subunit 3	0.68	4.97E-02
Down-regulate				
ENSBTAG00000006194	<i>FOSL1</i>	FOS like 1, AP-1 transcription factor subunit	-0.75	1.43E-03
ENSBTAG00000003937	<i>LOC784160</i>	40S ribosomal	-1.88	2.66E-03
ENSBTAG00000014540	<i>PERM1</i>	PPARGC1 and ESRR induced regulator, muscle 1	-0.91	6.01E-03
ENSBTAG00000019051	<i>NAT14</i>	N-acetyltransferase 14 (putative)	-0.73	6.53E-03
ENSBTAG00000007554	<i>IFI6</i>	interferon alpha inducible protein 6	-0.66	6.86E-03
ENSBTAG00000009461	<i>STYXL1</i>	serine/threonine/tyrosine interacting like 1	-2.96	7.56E-03
ENSBTAG000000037972	<i>SLC25A26</i>	solute carrier family 25 member 26	-0.65	7.89E-03
ENSBTAG00000019891	<i>MRPL40</i>	mitochondrial ribosomal protein L40	-0.83	8.35E-03
ENSBTAG000000031205	<i>Uncharacterized</i>	-	-5.65	8.93E-03
ENSBTAG000000049723	<i>LOC112441457</i>	density-regulated protein	-0.55	9.06E-03
ENSBTAG00000011451	<i>RTN1</i>	reticulum 1	-1.56	1.01E-02
ENSBTAG000000023186	<i>Uncharacterized</i>	-	-5.66	1.12E-02
ENSBTAG000000051079	<i>TMSB4</i>	Bos taurus thymosin beta 4, X-linked (TMSB4), mRNA.	-1.22	1.36E-02
ENSBTAG00000009587	<i>NRG3</i>	neuregulin 3	-1.09	1.42E-02
ENSBTAG00000019280	<i>CRYBB1</i>	crystallin beta B1	-0.84	1.54E-02
ENSBTAG000000039462	<i>PCLAF</i>	PCNA clamp associated factor	-0.47	1.60E-02
ENSBTAG00000019106	<i>EIF1B</i>	eukaryotic translation initiation factor 1B	-0.43	1.60E-02
ENSBTAG000000031885	<i>TMEM251</i>	transmembrane protein 251	-0.60	1.64E-02

ENSBTAG00000020923	<i>GDF1</i>	growth differentiation factor 1	-3.49	1.65E-02
ENSBTAG00000016936	<i>MISP3</i>	MISP family member 3	-0.72	1.66E-02
ENSBTAG00000031217	<i>MYL6B</i>	myosin light chain 6B	-1.18	1.80E-02
ENSBTAG00000050979	<i>bta-mir-12053</i>	bta-mir-12053	-1.06	1.81E-02
ENSBTAG00000049554	<i>C6orf52</i>	chromosome 6 open reading frame 52	-0.61	1.86E-02
ENSBTAG00000046725	<i>TNNC2</i>	troponin C2, fast skeletal type	-1.50	1.87E-02
ENSBTAG00000021219	<i>SEPTIN1</i>	septin 1	-0.72	1.88E-02
ENSBTAG00000006894	<i>NOS2</i>	nitric oxide synthase 2	-1.35	1.96E-02
ENSBTAG00000050733	<i>ATP6AP1L</i>	ATPase H ⁺ transporting accessory protein 1 like	-1.37	2.01E-02
ENSBTAG00000026886	<i>ATP5MPL</i>	ATP synthase membrane subunit 6.8PL	-0.77	2.12E-02
ENSBTAG00000008122	<i>GNG5</i>	G protein subunit gamma 5	-0.59	2.14E-02
ENSBTAG00000005390	<i>GMFG</i>	glia maturation factor gamma	-2.37	2.15E-02
ENSBTAG00000053346	<i>SPSB2</i>	splA/ryanodine receptor domain and SOCS box containing 2	-0.72	2.16E-02
ENSBTAG00000034496		26S proteasome complex subunit SEM1	-1.06	2.18E-02
ENSBTAG00000027426	<i>Uncharacterized</i>	-	-0.87	2.22E-02
ENSBTAG00000003519	<i>NOL3</i>	nucleolar protein 3	-0.65	2.26E-02
ENSBTAG00000003322	<i>TLL7</i>	tubulin tyrosine ligase like 7	-0.89	2.32E-02
ENSBTAG00000005208	<i>CLEC16A</i>	C-type lectin domain containing 16A	-0.84	2.37E-02
ENSBTAG00000004871	<i>NDUFV2</i>	NADH:ubiquinone oxidoreductase core subunit V2	-0.60	2.38E-02
ENSBTAG00000031432	<i>LYRM4</i>	LYR motif containing 4	-0.79	2.40E-02

ENSBTAG00000012954	<i>ERAS</i>	ES cell expressed Ras	-2.69	2.42E-02
ENSBTAG00000018404	<i>PRKG1</i>	protein kinase cGMP- dependent 1	-0.91	2.43E-02
ENSBTAG00000015618	<i>GDF15</i>	growth differentiation factor 15	-0.60	2.52E-02
ENSBTAG00000002190	<i>DOCK8</i>	dedicator of cytokinesis 8	-0.61	2.53E-02
ENSBTAG00000003066	<i>NSA2</i>	NSA2 ribosome biogenesis homolog (<i>S. cerevisiae</i>)	-0.93	2.53E-02
ENSBTAG00000013326	<i>ANAPC10</i>	anaphase promoting complex subunit 10	-0.59	2.60E-02
ENSBTAG00000031737	<i>TMEM102</i>	transmembrane protein 102	-0.78	2.63E-02
ENSBTAG00000001615	<i>RUNDC3A</i>	RUN domain containing 3A	-0.68	2.66E-02
ENSBTAG00000014060	<i>LSM6</i>	LSM6 homolog, U6 small nuclear RNA and mRNA degradation associated	-0.64	2.73E-02
ENSBTAG00000015820	<i>UXT</i>	ubiquitously expressed prefoldin like chaperone	-0.64	2.76E-02
ENSBTAG00000014971	<i>SEC61G</i>	SEC61 translocon subunit gamma	-0.65	2.81E-02
ENSBTAG00000027506	<i>SNRPB2</i>	small nuclear ribonucleoprotein polypeptide B2	-0.46	2.82E-02
ENSBTAG00000018363	<i>RBM48</i>	RNA binding motif protein 48	-0.42	2.85E-02
ENSBTAG00000013105	<i>SYT3</i>	synaptotagmin 3	-2.90	2.86E-02
ENSBTAG00000012760	<i>NDUFB3</i>	NADH:ubiquinone oxidoreductase subunit B3	-0.60	2.90E-02
ENSBTAG00000002256	<i>YEATS4</i>	YEATS domain containing 4	-0.44	2.92E-02
ENSBTAG00000001637	<i>FUNDC1</i>	FUN14 domain containing 1	-0.44	2.95E-02
ENSBTAG00000000619	<i>KRT74</i>	keratin 74	-4.20	2.95E-02

ENSBTAG00000000141	<i>NFU1</i>	NFU1 iron-sulfur cluster scaffold	-0.61	2.97E-02
ENSBTAG00000003712	<i>FAM89B</i>	family with sequence similarity 89 member B	-0.70	3.07E-02
ENSBTAG00000027610	<i>RPL36A</i>	60S ribosomal protein L36a	-9.27	3.23E-02
ENSBTAG00000052325	<i>Uncharacterized</i>	-	-0.82	3.23E-02
ENSBTAG00000037555	<i>FAM183A</i>	family with sequence similarity 183 member A	-4.35	3.29E-02
ENSBTAG00000051779	<i>PFDN1</i>	prefoldin subunit 1	-0.62	3.35E-02
ENSBTAG00000014130	<i>MGC148714</i>	cytochrome c oxidase subunit 6C	-0.63	3.38E-02
ENSBTAG00000046193	<i>Uncharacterized</i>	-	-0.94	3.45E-02
ENSBTAG00000044633	<i>bta-mir-2469</i>	bta-mir-2469	-0.62	3.46E-02
ENSBTAG00000054726	<i>MNF1</i>	mitochondrial nucleoid factor 1	-0.66	3.48E-02
ENSBTAG00000011517	<i>CCDC106</i>	coiled-coil domain containing 106	-1.07	3.56E-02
ENSBTAG00000020405	<i>NDUFB9</i>	NADH:ubiquinone oxidoreductase subunit B9	-0.63	3.57E-02
ENSBTAG00000015943	<i>MICU2</i>	mitochondrial calcium uptake 2	-0.39	3.58E-02
ENSBTAG00000000560	<i>LOC507271</i>	60S ribosomal protein L4 pseudogene	-1.06	3.60E-02
ENSBTAG00000013152	<i>NIPSNAP1</i>	nipsnap homolog 1	-0.91	3.60E-02
ENSBTAG00000023513	<i>DNAJC19</i>	DnaJ heat shock protein family (Hsp40) member C19	-0.53	3.62E-02
ENSBTAG00000022032	<i>CHCHD10</i>	coiled-coil-helix-coiled-coil-helix domain containing 10	-0.73	3.62E-02
ENSBTAG00000021965	<i>SUB1</i>	SUB1 regulator of transcription	-0.43	3.65E-02

ENSBTAG00000000856	<i>FBXL6</i>	F-box and leucine rich repeat protein 6	-0.44	3.66E-02
ENSBTAG00000003861	<i>GPATCH11</i>	G-patch domain containing 11	-0.73	3.68E-02
ENSBTAG00000044198	<i>THAP7</i>	THAP domain containing 7	-0.57	3.72E-02
ENSBTAG00000010235	<i>NOP9</i>	NOP9 nucleolar protein	-0.38	3.79E-02
ENSBTAG00000012658	<i>TMA16</i>	translation machinery associated 16 homolog	-0.47	3.83E-02
ENSBTAG00000054362	<i>Uncharacterized</i>	-	-4.19	3.86E-02
ENSBTAG00000021319	<i>MFF</i>	mitochondrial fission factor	-0.32	3.88E-02
ENSBTAG00000012898	<i>RPS27L</i>	ribosomal protein S27 like	-0.58	3.89E-02
ENSBTAG00000013444	<i>ETV2</i>	ETS variant transcription factor 2	-0.73	3.91E-02
ENSBTAG00000008584	<i>CLN8</i>	CLN8 transmembrane ER and ERGIC protein	-0.48	3.91E-02
ENSBTAG00000037781	<i>FKBPL</i>	FKBP prolyl isomerase like	-0.58	3.93E-02
ENSBTAG00000002463	<i>NDUFB5</i>	NADH: ubiquinone oxidoreductase subunit B5	-0.44	3.98E-02
ENSBTAG00000019667	<i>RNF187</i>	ring finger protein 187	-0.61	3.99E-02
ENSBTAG00000000981	<i>KLHDC8B</i>	kelch domain containing 8B	-0.54	4.02E-02
ENSBTAG00000009032	<i>TMEM80</i>	transmembrane protein 80	-0.85	4.03E-02
ENSBTAG00000003535	<i>DPM3</i>	dolichyl-phosphate mannosyltransferase subunit 3, regulatory	-0.69	4.06E-02
ENSBTAG00000039440	<i>LOC101903567</i>	cytochrome c oxidase subunit 7C, mitochondrial	-0.72	4.10E-02
ENSBTAG00000054678	<i>DUSP15</i>	dual specificity phosphatase 15	-0.60	4.13E-02
ENSBTAG00000000295	<i>CLHC1</i>	clathrin heavy chain linker domain containing 1	-0.54	4.13E-02
ENSBTAG00000027442	<i>NFIB</i>	nuclear factor I B	-0.48	4.13E-02

ENSBTAG00000020524	<i>UPK3BL1</i>	uroplakin 3B-like	-0.56	4.16E-02
ENSBTAG00000003407	<i>SF3B6</i>	splicing factor 3b subunit 6	-0.48	4.19E-02
ENSBTAG00000002500	<i>FBXL15</i>	F-box and leucine rich repeat protein 15	-0.53	4.19E-02
ENSBTAG00000046394	<i>RPL23A</i>	ribosomal protein L23a	-0.78	4.20E-02
ENSBTAG00000055252	<i>UBE2E1</i>	ubiquitin conjugating enzyme E2 E1	-0.36	4.20E-02
ENSBTAG00000011694	<i>TIMM23</i>	translocase of inner mitochondrial membrane 23 homolog (yeast)	-0.39	4.21E-02
ENSBTAG00000044038	<i>TEN1</i>	TEN1 subunit of CST complex	-0.90	4.22E-02
ENSBTAG00000001710	<i>CMTM8</i>	CKLF like MARVEL transmembrane domain containing 8	-0.54	4.26E-02
ENSBTAG00000046943	<i>OCM</i>	oncomodulin	-0.67	4.30E-02
ENSBTAG00000040308	<i>RPS13</i>	ribosomal protein S13	-0.57	4.30E-02
ENSBTAG00000007389	<i>IFI35</i>	interferon induced protein 35	-0.61	4.31E-02
ENSBTAG00000000979	<i>SMIM19</i>	small integral membrane protein 19	-0.40	4.32E-02
ENSBTAG00000014024	<i>POMP</i>	proteasome maturation protein	-0.52	4.34E-02
ENSBTAG00000007812	<i>NDUFA13</i>	NADH:ubiquinone oxidoreductase subunit A13	-0.72	4.35E-02
ENSBTAG00000021779	<i>MGST2</i>	microsomal glutathione S- transferase 2	-0.36	4.36E-02
ENSBTAG00000018593	<i>PSENEN</i>	presenilin enhancer, gamma-secretase subunit	-0.56	4.37E-02
ENSBTAG00000046569	<i>CISD3</i>	CDGSH iron sulfur domain	-0.69	4.38E-02

ENSBTAG00000010577	<i>PAICS</i>	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	-0.32	4.39E-02
ENSBTAG00000012796	<i>ZNF428</i>	zinc finger protein 428	-0.51	4.40E-02
ENSBTAG00000020721	<i>MRPL55</i>	mitochondrial ribosomal protein L55	-0.77	4.43E-02
ENSBTAG00000019500	<i>CNIH1</i>	cornichon family AMPA receptor auxiliary protein 1	-0.31	4.46E-02
ENSBTAG00000019387	<i>NIFK</i>	nucleolar protein interacting with the FHA domain of MKI67	-0.38	4.47E-02
ENSBTAG00000010904	<i>PAGR1</i>	PAXIP1 associated glutamate rich protein 1	-0.83	4.47E-02
ENSBTAG00000022275	<i>LOC100337136</i>	60S ribosomal protein L10	-0.83	4.47E-02
ENSBTAG00000053905	<i>Uncharacterized</i>	-	-0.59	4.51E-02
ENSBTAG00000021069	<i>PBK</i>	PDZ binding kinase	-0.33	4.54E-02
ENSBTAG00000014646	<i>MZT2B</i>	mitotic spindle organizing protein 2B	-0.63	4.55E-02
ENSBTAG00000039582	<i>NDUFC1</i>	NADH:ubiquinone oxidoreductase subunit C1	-0.60	4.57E-02
ENSBTAG00000010321	<i>TTC1</i>	tetratricopeptide repeat domain 1	-0.37	4.59E-02
ENSBTAG00000012621	<i>RTN4RL2</i>	reticulon 4 receptor like 2	-0.60	4.64E-02
ENSBTAG00000009517	<i>DBI</i>	diazepam binding inhibitor, acyl-CoA binding protein	-0.52	4.64E-02
ENSBTAG00000016590	<i>NME4</i>	NME/NM23 nucleoside diphosphate kinase 4	-1.12	4.66E-02
ENSBTAG00000013461	<i>RPL24</i>	ribosomal protein L24	-0.60	4.66E-02
ENSBTAG00000020684	<i>LOC100300624</i>	actin related protein 2/3 complex, subunit 1B	-0.79	4.66E-02

ENSBTAG00000009211	<i>UBE2S</i>	ubiquitin conjugating enzyme E2 S	-0.51	4.70E-02
ENSBTAG000000052310	<i>EIF4EBP3</i>	eukaryotic translation initiation factor 4E binding protein 3	-1.19	4.71E-02
ENSBTAG000000031246	<i>CCNI2</i>	cyclin I family member 2	-0.57	4.73E-02
ENSBTAG000000001107	<i>CACYBP</i>	calcyclin binding protein	-0.43	4.73E-02
ENSBTAG000000031059	<i>LRRC61</i>	leucine rich repeat containing 61	-0.62	4.76E-02
ENSBTAG000000007363	<i>LSM3</i>	LSM3 homolog, U6 small nuclear RNA and mRNA degradation associated	-0.53	4.76E-02
ENSBTAG000000020504	<i>CDK2AP1</i>	cyclin dependent kinase 2 associated protein 1	-0.46	4.76E-02
ENSBTAG000000054329	<i>Uncharacterized</i>	-	-0.87	4.82E-02
ENSBTAG000000046644	<i>HRAS</i>	HRas proto-oncogene, GTPase	-0.62	4.82E-02
ENSBTAG000000055165	<i>SNORA73</i>	Small nucleolar RNA SNORA73 family	-0.48	4.82E-02
ENSBTAG000000003205	<i>RPL35</i>	ribosomal protein L35	-0.61	4.82E-02
ENSBTAG000000044079	<i>SMIM4</i>	small integral membrane protein 4	-0.58	4.85E-02
ENSBTAG000000047376	<i>PIN4</i>	peptidylprolyl cis/trans isomerase, NIMA- interacting 4	-0.63	4.85E-02
ENSBTAG000000013226	<i>HUS1</i>	HUS1 checkpoint clamp component	-0.48	4.86E-02
ENSBTAG000000016326	<i>C3H1orf52</i>	chromosome 3 C1orf52 homolog	-0.45	4.86E-02
ENSBTAG000000010584	<i>AP2S1</i>	adaptor related protein complex 2 subunit sigma 1	-0.57	4.88E-02
ENSBTAG000000010232	<i>NDUFS5</i>	NADH:ubiquinone oxidoreductase subunit S5	-0.60	4.90E-02

ENSBTAG00000012186	<i>DKKLI</i>	dickkopf like acrosomal protein 1	-0.55	4.90E-02
ENSBTAG00000001727	<i>PRXL2B</i>	peroxiredoxin like 2B	-0.63	4.93E-02
ENSBTAG000000036101	<i>KYATI</i>	kynurenine aminotransferase 1	-0.44	4.97E-02
ENSBTAG000000015156	<i>NDUFBI</i>	NADH:ubiquinone oxidoreductase subunit B1	-0.62	4.98E-02

¹FC, Fold change (Leptin groups / Control groups)