Using RNA sequencing to identify putative competing endogenous RNAs (ceRNAs) potentially regulating fat metabolism in bovine liver

Ruobing Liang[1,3], Bo Han[1,3], Qian Li[2], Yuwei Yuan[1], Jianguo Li[2], Dongxiao Sun[1*]

[1] Department of Animal Genetics, Breeding and Reproduction, College of Animal Science and Technology, Key Laboratory of Animal Genetics and Breeding of Ministry of Agriculture, National Engineering Laboratory of Animal Breeding, China Agricultural University, Beijing 100193, China.

[2] Department of Animal Production and Environmental Control, College of Animal Science and Technology, Hebei Agricultural University, Baoding 071001, China.

[*]Corresponding author

Email: sundx@cau.edu.cn

[3]These authors contributed equally to this work.

Supplementary Table S1: The basic statistics for lncRNA& mRNA sequencing data.

Sample name	**A_1**	**A_2**	**A_3**	**B_1**	**B_2**	**B_3**	**C_1**	**C_2**	**C_3**
Raw reads	86193692	99986546	88265716	89354306	94662560	89655958	86962770	85912968	80470996
Clean reads	83667860	97780170	85888072	87275100	92522210	86817366	84045138	83760436	78622660
clean bases(Gb)	10.46	12.22	10.74	10.91	11.57	10.85	10.51	10.47	9.83
Error rate(%)	0.04	0.04	0.03	0.03	0.04	0.03	0.04	0.04	0.04
Q20(%)	92.5	93.24	93.47	93.55	93.43	93.56	92.59	93.03	92.8
Q30(%)	86.55	87.69	88.06	88.19	88.01	88.2	86.46	87.26	86.89
GC content(%)	47.97	49.57	48.72	48.26	48.98	48.59	50.39	48.26	48.68

Q20: the proportion of bases with a phred base quality score greater than 20; Q30: the proportion of bases with a phred base quality score greater than 30; A, B, or C represents the different individual; 1, 2, or 3 represents dry period, early lactation, or peak of lactation.

Continued Table S1: The basic statistics for small RNA sequencing data.

Reads Type	**A_1**	**A_2**	**A_3**	**B_1**	**B_2**	**B_3**	**C_1**	**C_2**	**C_3**
Total reads number	10639875	11311351	12213873	15107016	13896378	10277358	13916795	12140340	12948524
Low quality	5737	6166	6581	8016	7644	9457	7498	6413	6856
Adaptor3 null	76604	84929	93449	118748	109346	79197	102389	92915	100471
Insert null	366417	494875	663170	694980	803895	306618	470220	509835	682186
5' adaptor contaminants	6908	6643	7897	7368	8417	4881	6225	7088	8314
size < 18 nt	88201	79657	100494	114330	121052	151253	73028	97281	91171
size > 31 nt	13844	14793	22805	29595	16337	27368	15774	16738	23748
polyA	547	664	537	462	728	261	442	591	420
HighQuality (18 nt <=size <= 31 nt)	10081617	10623624	11318940	14133517	12828959	9698323	13241219	11409479	12035358
Clean base(Gb)	0.50408085	0.5311812	0.565947	0.70667585	0.64144795	0.48491615	0.66206095	0.57047395	0.6017679

A, B, or C represents the different individual; 1, 2, or 3 represents dry period, early lactation, or peak of lactation.

Supplementary Table S2: Correlation coefficient (r^2) of the mRNA& lncRNA sequencing data among the three individual cows in each period

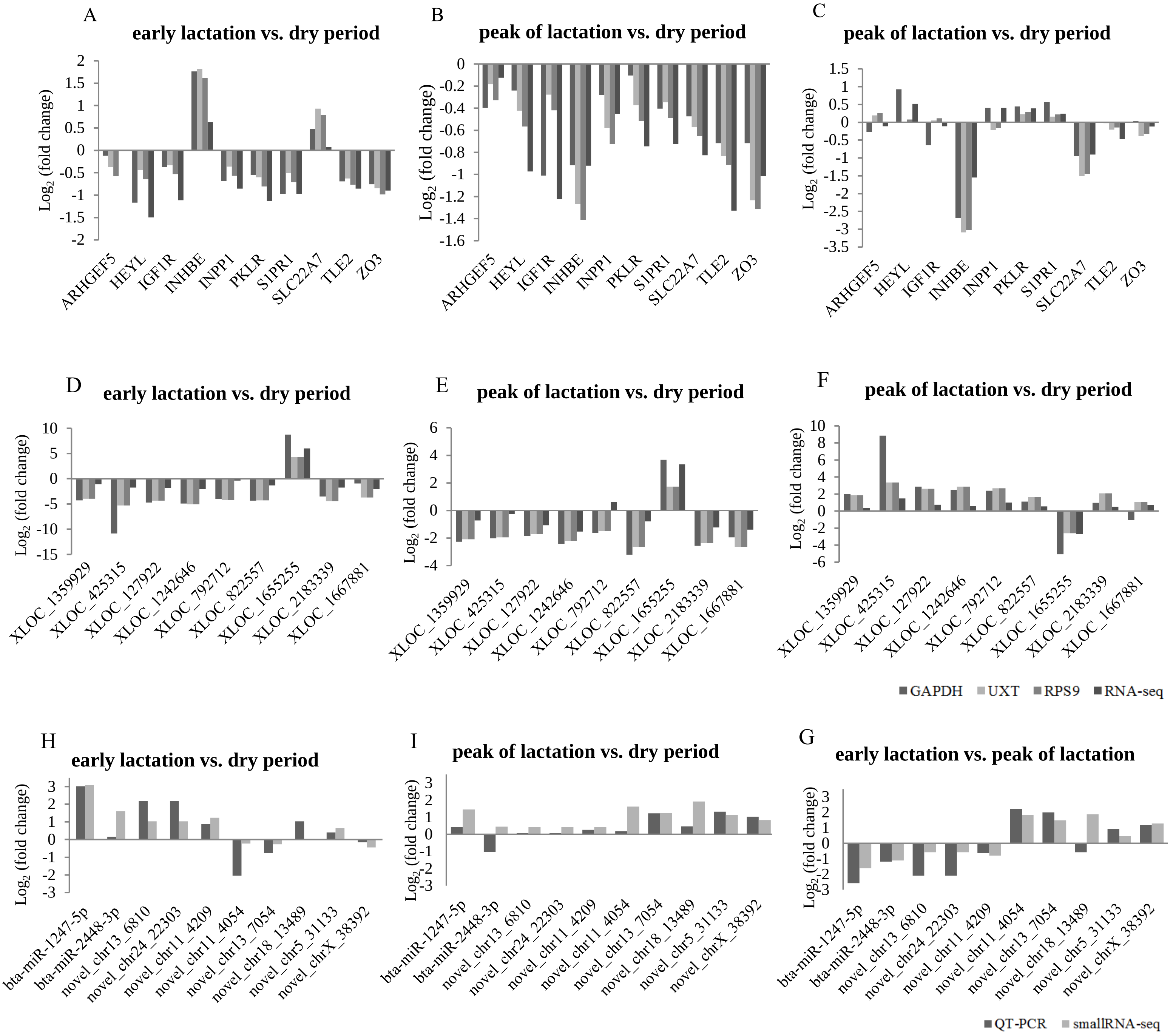
Sample	A_1	B_1	C_1	A_2	B_2	C_2	A_3	B_3	C_3
A_1	1	0.96	0.95	0.95	0.94	0.94	0.96	0.94	0.94
B_1		1	0.97	0.96	0.96	0.96	0.97	0.96	0.96
C_1			1	0.96	0.96	0.97	0.97	0.95	0.98
A_2				1	0.96	0.97	0.98	0.95	0.97
B_2					1	0.97	0.96	0.96	0.96
C_2						1	0.96	0.96	0.98
A_3							1	0.95	0.97
B_3								1	0.95
C_3									1

A, B, or C represents the different individual; 1, 2, or 3 represents dry period, early lactation, or peak of lactation.

Continued Table S2: Correlation coefficient (r^2) of the miRNA sequencing data among the three individual cows in each period

Sample	A_1	B_1	C_1	A_2	B_2	C_2	A_3	B_3	C_3
A_1	1	0.99	0.99	1.00	0.98	0.98	1.00	0.98	0.99
B_1		1	0.99	0.99	0.99	0.99	0.99	0.99	1.00
C_1			1	0.99	0.99	0.99	0.98	0.99	0.99
A_2				1	0.98	0.98	1.00	0.98	0.99
B_2					1	1.00	0.98	0.99	0.99
C_2						1	0.97	0.99	0.99
A_3							1	0.97	0.99
B_3								1	0.98
C_3									1

A, B, or C represents the different individual; 1, 2, or 3 represents dry period, early lactation, or peak of lactation.



Supplementary Figure S1. Comparison of the expression change levels of 10 mRNAs, nine lncRNAs, and 10 miRNAs randomly selected in the three periods between sequencing and qRT-PCR. A, B, C shows 10 mRNAs expression fold changes in every two periods comparison, y-axis shows the log$_2$ (ratio of mRNA levels between two periods) measured by qRT-PCR (normalized by *GAPDH*, *UXT* and *RPS9*) and RNA-seq, respectively; D, E, F shows nine lncRNAs expression fold changes in every two periods comparison, y-axis shows the log$_2$ (ratio of lncRNA levels between two periods) measured by qRT-PCR (normalized by *GAPDH*, *UXT* and *RPS9*) and RNA-seq, respectively; and G, H, I shows 10 miRNAs expression fold changes in every two periods comparison, y-axis shows the log$_2$ (ratio of miRNA levels between two periods) measured by qRT-PCR (normalized by snoRNA U6) and small RNA-seq, respectively.

Supplementary Table S7: ceRNA pairs identified during dry period, early lactation, and peak of lactation.

Comparison	lncRNA_ID	shared_miRNA	MiRNA up/down	mRNA_ID	gene_name	p_value	FDR
Early lactation vs. dry period	XLOC_822557	novel_chr21_19609,bta-miR-1247-5p	miRNA_up	ENSBTAT00000002373	ARHGEF5	8.43E-07	1.12E-05
	XLOC_1359929	novel_chr13_6810,novel_chr24_22303	miRNA_up	ENSBTAT00000028690	IGF1R	8.43E-07	1.05E-05
	XLOC_1667881	novel_chr24_22303,novel_chr13_6810	miRNA_up	ENSBTAT00000034627	CX3CL1	8.43E-07	1.01E-05
	XLOC_1359929	novel_chr24_22303,novel_chr13_6810	miRNA_up	ENSBTAT00000034627	CX3CL1	8.43E-07	8.17E-06
	XLOC_425315	novel_chr6_33592,bta-miR-2448-3p	miRNA_up	ENSBTAT00000009977	INPP1	8.43E-07	8.02E-06
	XLOC_1667881	novel_chr13_6810,novel_chr24_22303	miRNA_up	ENSBTAT00000028690	IGF1R	8.43E-07	7.50E-06
	XLOC_1667881	novel_chr13_6810,novel_chr24_22303	miRNA_up	ENSBTAT00000018241	GPR37	8.43E-07	6.64E-06
	XLOC_822557	bta-miR-1247-5p,novel_chr21_19609	miRNA_up	ENSBTAT00000003229	TNS1	8.43E-07	6.63E-06
	XLOC_1359929	novel_chr13_6810,novel_chr24_22303	miRNA_up	ENSBTAT00000018241	GPR37	8.43E-07	6.41E-06
	XLOC_822557	bta-miR-1247-5p,novel_chr21_19609	miRNA_up	ENSBTAT00000015668	COL6A1	2.53E-06	1.52E-05
	XLOC_822557	bta-miR-1247-5p,novel_chr21_19609	miRNA_up	ENSBTAT00000061325	AHDC1	8.43E-06	3.49E-05
	XLOC_127922	novel_chr6_33592,bta-miR-2448-3p	miRNA_up	ENSBTAT00000009977	INPP1	8.43E-06	3.48E-05
	XLOC_1242646	novel_chr11_4209	miRNA_up	ENSBTAT00000020610	FADS2	0.000648929	0.002117922
	XLOC_1242646	novel_chr11_4209	miRNA_up	ENSBTAT00000025788	CGN1	0.000648929	0.001241418
	XLOC_1242646	novel_chr11_4209	miRNA_up	ENSBTAT00000018649	CGN1	0.000648929	0.001238117
	XLOC_1242646	novel_chr11_4209	miRNA_up	ENSBTAT00000007861	S1PR1	0.001297859	0.001979818
Peak of lactation vs. dry period	XLOC_2183339	novel_chr11_4054,novel_chr13_7054	miRNA_up	ENSBTAT00000002076	WIPF1	8.43E-07	1.20E-05
	XLOC_2183339	novel_chr11_4054,novel_chr13_7054	miRNA_up	ENSBTAT00000001534	TLE2	2.53E-06	3.00E-05
	XLOC_2183339	novel_chr13_7054,novel_chr11_4054	miRNA_up	ENSBTAT00000009497	CASKIN2	1.77E-05	0.000123531
	XLOC_2183339	novel_chr13_7054,novel_chr11_4054	miRNA_up	ENSBTAT00000049235	DDAH1	1.77E-05	0.000122754
	XLOC_2183339	novel_chr11_4054,novel_chr13_7054	miRNA_up	ENSBTAT00000019489	ZFHX3	1.77E-05	0.000121718
	XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000011453	HEYL	0.001297859	0.004330871
	XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000018649	CGN1	0.001297859	0.004030836
	XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000025788	CGN1	0.001297859	0.003962106

XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000034627	CX3CL1	0.001297859	0.003731857
XLOC_2183339	novel_chr13_7054	miRNA_up	ENSBTAT00000018649	CGN1	0.001297859	0.003693623
XLOC_2183339	novel_chr13_7054	miRNA_up	ENSBTAT00000025788	CGN1	0.001297859	0.003691103
XLOC_2183339	novel_chr13_7054	miRNA_up	ENSBTAT00000011453	HEYL	0.001297859	0.003500123
XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000029841	SFRP2	0.001297859	0.003398686
XLOC_2183339	novel_chr13_7054	miRNA_up	ENSBTAT00000034627	CX3CL1	0.001297859	0.003397779
XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000046947	PKLR	0.001946788	0.004788902
XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000028521	HEG1	0.001946788	0.004774452
XLOC_054981	bta-miR-2887	miRNA_up	ENSBTAT00000008663	INHBE	0.001946788	0.004709402
XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000018241	GPR37	0.001946788	0.004651971
XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000063189	TPC3	0.002594874	0.005769479
XLOC_2183339	novel_chr13_7054	miRNA_up	ENSBTAT00000011517	SAMD11	0.002594874	0.005757275
XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000011517	SAMD11	0.002594874	0.00572928
XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000065036	BCOR	0.002594874	0.005700368
XLOC_1242646	novel_chr5_31133	miRNA_up	ENSBTAT00000008336	SLC22A7	0.002594874	0.005580781
XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000011846	-	0.002594874	0.005538246
XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000002076	WIPF1	0.002594874	0.005476775
XLOC_2183339	novel_chr13_7054	miRNA_up	ENSBTAT00000035742	ZFP36L1	0.002594874	0.005448703
XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000035742	ZFP36L1	0.002594874	0.005380762
XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000023515	NOS3	0.003891047	0.00686203
XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000021480	ZNF608	0.003891047	0.006847501
XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000027940	NGFR	0.003891047	0.006837597
XLOC_054981	novel_chr5_31133	miRNA_up	ENSBTAT00000008336	SLC22A7	0.003891047	0.006701767
XLOC_2183339	novel_chr13_7054	miRNA_up	ENSBTAT00000033431	NFIA	0.003891047	0.00650726
XLOC_054981	bta-miR-2887	miRNA_up	ENSBTAT00000013942	FGFR4	0.003891047	0.006473791
XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000033431	NFIA	0.003891047	0.006380171
XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000001534	TLE2	0.003891047	0.006374018
XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000015828	PLEC	0.005186378	0.00775438

	XLOC_1242646	novel_chr5_31133	miRNA_up	ENSBTAT00000028690	IGF1R	0.005186378	0.007546511
	XLOC_1242646	novel_chr5_31133	miRNA_up	ENSBTAT00000022744	HSPG2	0.005186378	0.007488562
	XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000033431	NFIA	0.00583278	0.008023816
	XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000002427	PPP2R2B	0.00583278	0.007929014
	XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000000176	LRFN3	0.00583278	0.007928703
	XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000024404	ZO3	0.006480865	0.008542226
	XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000004201	IQSEC1	0.006480865	0.008487475
	XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000017323	ARNTL	0.007771988	0.009718044
	XLOC_054981	novel_chr5_31133	miRNA_up	ENSBTAT00000022744	HSPG2	0.007771988	0.009658766
	XLOC_054981	novel_chr5_31133	miRNA_up	ENSBTAT00000028690	IGF1R	0.007771988	0.009481362
	XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000003229	TNS1	0.00777451	0.00937461
	XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000049235	DDAH1	0.009067312	0.010754686
	XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000009497	CASKIN2	0.009067312	0.010687278
	XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000019489	ZFHX3	0.009067312	0.010678777
	XLOC_1242646	novel_chr13_7054	miRNA_up	ENSBTAT00000031875	PRR12	0.01035927	0.011669388
	XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000013284	EGR1	0.01035927	0.011656396
	XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000024256	SIPA1L3	0.01035927	0.011589121
	XLOC_2183339	novel_chr13_7054	miRNA_up	ENSBTAT00000031875	PRR12	0.01035927	0.011575913
	XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000007134	GATA4	0.01035927	0.011575229
	XLOC_054981	bta-miR-2887	miRNA_up	ENSBTAT00000003229	TNS1	0.01164284	0.012824171
	XLOC_2183339	novel_chr11_4054	miRNA_up	ENSBTAT00000002375	PLXND1	0.01165039	0.0127609
	XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000019489	ZFHX3	0.01357448	0.014562026
	XLOC_054981	novel_chr18_13489	miRNA_up	ENSBTAT00000028865	TNRC18	0.0193543	0.019862799
Peak of lactation vs. early lactation	XLOC_792712	novel_chr21_19609,bta-miR-1247-5p	miRNA_down	ENSBTAT00000013221	FAM107B	8.43E-06	4.40E-05
	XLOC_1655255	novel_chrX_38392	miRNA_up	ENSBTAT00000010711	PKIA	0.000648929	0.001652694
	XLOC_1655255	novel_chrX_38392	miRNA_up	ENSBTAT00000016009	CCDC167	0.000648929	0.001636175
	XLOC_1655255	novel_chrX_38392	miRNA_up	ENSBTAT00000019772	NR0B2	0.002595717	0.005046777
	XLOC_1655255	novel_chrX_38392	miRNA_up	ENSBTAT00000022843	C20ORF82	0.003893576	0.006644547

Supplementary Table S9: Expression of lipid metabolic genes during dry period, early lactation, and peak of lactation.

Metabolic processes	Gene name	dry period (FPKM)	early lactation (FPKM)	peak of lactation (FPKM)
	SREBF1	10.61	7.39	6.55
	SREBF2	7.53	5.13	4.98
	PPARA	40.75	32.49	34.45
lipid transport	*APOA1*	185.79	1161.93	304.93
	APOA2	10523.09	7078.26	9339.80
	APOC3	1880.12	1426.49	1648.34
	APOA5	273.51	471.99	446.11
	PLTP	4.83	7.97	5.04
lipogenesis	*ME*	6.14	7.75	5.87
	SCD	41.08	22.89	79.77
	FADS2	383.45	138.22	325.31
	FASN	2.81	1.39	1.91
	ACACA	4.84	4.44	6.58
cholesterol metabolism	*CYP7A1*	123.74	364.46	327.11
	CYP8B1	156.41	122.03	137.05
	CYP27A	184.58	230.98	294.66
	NRIH3	48.83	54.86	53.11
fatty acid transport	*DBI*	488.93	771.02	717.47
	FABP1	987.53	1303.89	1089.08
	FABP3	1.66	2.95	1.09
	CD36	14.85	30.70	18.61
	SLC27A2	180.02	249.44	227.39
	LPL	5.10	3.66	3.97
	ACSL1	80.75	183.16	110.58
	OLR1	0.87	1.31	1.20
fatty acid oxidation	*EHHADH*	229.85	291.60	274.13
	ACAA1	429.31	547.26	557.52
	SCP2	720.69	878.03	1070.84
	ACOX	157.09	215.90	220.89
	CPT1A	57.72	93.99	91.95
	CPT1B	0.80	6.92	2.36
	CPT1C	0.25	0.31	0.32
	CPT2	54.03	79.21	69.61
	ACADL	74.43	88.24	82.54
	ACADM	558.43	850.58	786.05

The differentially expressed genes were marked in red.

Supplementary Table S10: Primers of the differentially expressed mRNAs, lncRNAs, and miRNAs randomly selected for qRT-PCR.

RNA	Name	Forward primer sequence(5'-3')	Reverse primer sequence(5'-3')	Amplicon (bp)	Tm (°C)
mRNA	*ARHGEF5*	GTCCGTGGGGAAAAGTGTGA	GGCATAGCCAAGGCTGAGAT	158	60
	HEYL	AGCCCTTCACAAATGCAAGC	TTCTCAAAGGCAGTGGGGAC	119	60
	IGF1R	CGCTTGTCCCAAAACCGAAG	CGATCTGCATGACCTCCCTC	137	60
	INHBE	GGACTACAGCTGACCAGTCG	GGACAGGTGGAAGGTGAGTG	186	60
	INPP1	TTGGGAATCTGGGTGGATCC	GGCACCCCTGTCTGTATGTC	146	60
	PKLR	CATCGACTCTGAGCCTGTGG	CTCCCGGATGTTAGCGATGG	178	60
	S1PR1	AGCCCTGTCTCGGACTATGT	CGCGCTGATCTTCAGCTTTC	78	60
	SLC22A7	CTGTTGGCACTGATCGGCTA	GATTCAGGCACCCACCAGAG	107	60
	TLE2	GAGTGTGGTGGAAGAGGAGC	CGCTCTTGTCCTCGTCACTT	107	60
	ZO3	CTGGTGAATATCCCTCCGGC	CTGGTGTCTGGACTCTGCTG	143	60
lncRNA	*XLOC_1359929*	CAGGCCACATGCTCGGTAA	ACTCCTGCCTCTGTCACATC	204	60
	XLOC_425315	TGCATTCGAGAATGGCACCT	TAGCGTCATGGAGGGGTGTA	106	60
	XLOC_127922	CCCATTGGACAACTCTGGCT	ACCAGAAGTTTGAAGAGTGTCCTG	177	60
	XLOC_1242646	TTCATCTTCAGCCTGGGTCG	GTTTGGGACTGCAGGTCTGT	135	60
	XLOC_792712	AAGTTCTCACCTTGGCACCTC	TAATAACAACCAGCCTCCCTCG	168	60
	XLOC_822557	AGGGCCTGATGGGCTTACTA	AACAGTCCTTCCACTGGTGC	152	60
	XLOC_1655255	TAAGACCCAGCGTCGAATGG	AGGCTCAGTTTCCCTGCATC	123	60
	XLOC_2183339	CCTGGCTGGGTTCCTCTTTT	CAGAGAAGGACCCCAAGCAG	195	60
	XLOC_1667881	AGAGTCAGGAGATGGGGCTT	GTGCTCAGCTGTGTCTGACT	126	60
Housekeeping	*GAPDH*	AGATGGTGAAGGTCGGAGTG	CCTTTCCATTGATGACGAGC	205	60
	UXT	TGTGGCCCTTGGATATGGTT	GGTTGTCGCTGAGCTCTGTG	101	60
	RPS9	CCTCGACCAAGAGCTGAAG	CCTCGACCAAGAGCTGAAG	54	60
miRNA	bta-miR-1247-5p	TGAACGTCGGGACTGGAGC	The reverse primers for miRNA validation were supplied in the box of the miRcute Plus miRNA qPCR Detection Kit (TIANGEN, Beijing,		60
	bta-miR-2448-3p	GCGGATACAACCAAACACGG			60
	novel_chr13_6810	GGTCCCCTTCAACCAGCTGT			60
	novel_chr24_22303	GGTCCCCTTCAACCAGCTGT			60

	novel_chr11_4209	GGTTCGATCCCACTTCTGACAC	China).	60
	novel_chr11_4054	ATAATAATAGCGGGGCCGGG		60
	novel_chr13_7054	TATATAGTGGGGGGGCGGC		60
	novel_chr18_13489	ATATATACGCGCGCCGGTG		60
	novel_chr5_31133	TATATACCCTGGGAGCCCGG		60
	novel_chrX_38392	TATTATATACGGCGGCGGCG		60
	U6 snoRNA (reference)	has-U6 (CD201-0145,TIANGEN)		60