

Functional analysis of the dairy cow mammary transcriptome between early lactation and mid-dry period

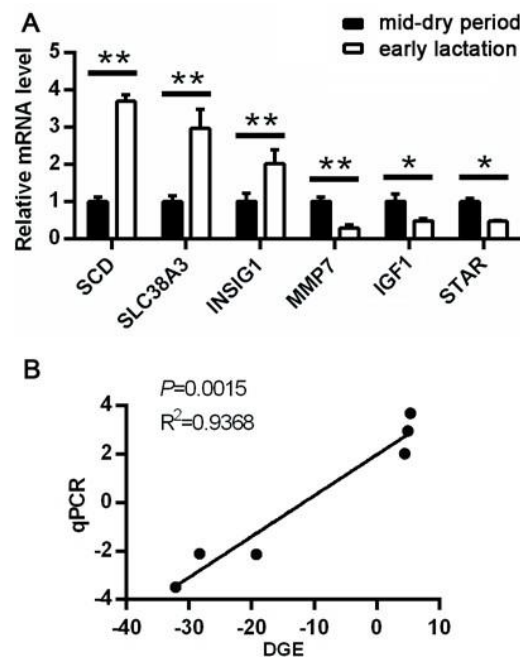
Ye Lin, He Lv, Minghui Jiang, Jinyu Zhou, Shuyuan Song and Xiaoming Hou

SUPPLEMENTARY FILE

Supplementary Fig. S1 Validation of DEGs by qPCR.

(A) Relative mRNA levels of stearoyl-CoA desaturase (*SCD*), solute carrier family 38 member 3 (*SLC38A3*), insulin induced gene 1 (*INSIG1*), matrix metalloproteinase 7 (*MMP7*), insulin like growth factor 1 (*IGF1*), and steroidogenic acute regulatory protein (*STAR*) in lactating dairy cows versus in dry cows. The RNA was extracted from mammary tissues, and mRNA levels were measured by qPCR and were normalized to the level of β -actin mRNA. Expression of *SCD*, *SLC38A3*, and *INSIG1* was up-regulated in the lactating cows compared with the dry cows. The expression of *MMP7*, *IGF1*, and *STAR* was significantly lowered in the tissues of lactating cows relative to dry cows. The qPCR results were consistent with the DGE profiling analysis, indicating that the DGE data are of high quality and are biologically relevant. Data are reported as the mean \pm SEM from three independent experiments. * $P < 0.05$, ** $P < 0.01$. (B) Correlation between DGE and qPCR gene expression. $R^2=0.9368$, $P = 0.0015$.

Supplementary Fig. S1:



Supplementary Table S1 Primer sequences for quantitative real-time PCR¹

Gene	GenBank accession number	Primer sequence (5'-3')	Product size (bp)
<i>SCD</i>	NM_173959.4	F: GGTGATGTTCCAGAGGAGGTA R: AAGGGCATAACGGAATAAGG	139
<i>SLC38A3</i>	NM_001040486.1	F: AACCTGCTGGTCATCTTTGC R: CAGTGGGTATGATGCGAAAG	121
<i>INSIG1</i>	NM_001077909.1	F: GGGGAACATAGGACGACAG R: CCTTGCCACTCTTCAGGAT	93
<i>MMP7</i>	NM_001075130.1	F: GTCAGACTCAAGCGGATGG R: GGATGATTTGGGAATAGCG	137
<i>IGF1</i>	NM_001077828.1	F: ATCAGCAGTCTTCCAACCCAA R: GTGAAGGCGAGCAAGCACA	122
<i>STAR</i>	NM_174189.3	F: GTGGAATCCCAACGTCAAG R: GTACGCTCACAAAGTCTCG	125
<i>β-ACTIN</i>	NM_173979.3	F: TGTTAGCTGCGTTACACCCT R: CTGTCACCTTACCCTTCC	163

¹*SCD*=stearoyl-CoA desaturase; *SLC38A3*=solute carrier family 38 member 3; *INSIG1*=insulin-induced gene 1; *MMP7*=matrix metalloproteinase 7; *IGF1*=insulin like growth factor 1; *STAR*=steroidogenic acute regulatory protein. F=forward; R=reverse.

Supplementary Table S2 Metabolic process enrichment of the differentially expressed genes in the mammary glands of dairy cows during early lactation versus mid-dry period

Gene symbol	GenBank accession	Gene description	SFC	P value
Up-regulated				
<i>GUK1</i>	NM_001159412.1	Guanylate kinase 1	31.63	7.48E-04
<i>ALOX15</i>	NM_174501.2	Arachidonate 15-lipoxygenase	6.20	2.20E-14
<i>SCD</i>	NM_173959.4	Stearoyl-CoA desaturase	5.34	5.47E-16
<i>LPL</i>	NM_001075120.1	Lipoprotein lipase	5.10	7.34E-15
<i>DIO2</i>	NM_001010992.5	Iodothyronine deiodinase 2	4.77	3.19E-05
<i>DNAJC12</i>	NM_174096.2	DnaJ heat shock protein family (Hsp40) member C12	4.48	2.84E-12
<i>FABP3</i>	NM_174313.2	Fatty acid binding protein 3	4.44	2.79E-12
<i>ABCG2</i>	NM_001037478.3	ATP binding cassette subfamily G member 2	4.31	8.58E-12
<i>PAH</i>	NM_001046058.2	Phenylalanine hydroxylase	4.14	1.04E-10
<i>SLC25A21</i>	NM_001015587.1	Solute carrier family 25 member 21	4.04	7.61E-09
<i>VLDLR</i>	NM_174489.2	Very low density lipoprotein receptor	3.83	2.26E-04
<i>GPAT4</i>	NM_001083669.1	Glycerol-3-phosphate acyltransferase 4	3.78	9.74E-10
<i>LRAT</i>	NM_177503.2	Lecithin retinol acyltransferase	3.73	1.86E-06
<i>GPAM</i>	NM_001012282.1	Glycerol-3-phosphate acyltransferase, mitochondrial	3.62	2.85E-09

<i>TRIB3</i>	NM_001076103.1	Tribbles pseudokinase 3	3.56	6.38E-03
<i>CTSL</i>	NM_001083686.2	Cathepsin L	3.51	1.58E-07
<i>IGFBP1</i>	NM_174554.3	Insulin like growth factor binding protein 1	3.42	1.30E-06
<i>RDH16</i>	NM_001075796.1	Retinol dehydrogenase 16	3.41	2.04E-02
<i>FBP2</i>	NM_001046164.2	Fructose-bisphosphatase 2	3.38	6.23E-06
<i>PPARGC1A</i>	NM_177945.3	PPARG coactivator 1 alpha	3.32	4.13E-05
<i>SLC28A1</i>	NM_001037458.1	Solute carrier family 28 member 1	3.31	2.71E-03
<i>SLC6A4</i>	NM_174609.2	Solute carrier family 6 member 4	3.17	1.04E-02
<i>CDC25A</i>	NM_001101100.2	Cell division cycle 25A	3.12	3.06E-02
<i>FASN</i>	NM_001012669.1	Fatty acid synthase	3.03	3.02E-07
<i>LOC777692</i>	NM_001078152.2	Uncharacterized LOC777692	2.99	3.06E-02
<i>GK</i>	NM_001075236.1	Glycerol kinase	2.99	1.65E-06
<i>GHR</i>	NM_176608.1	Growth hormone receptor	2.97	1.24E-03
<i>ACSS2</i>	NM_001105339.1	Acyl-CoA synthetase short-chain family member 2	2.97	7.38E-07
<i>IDI1</i>	NM_001075659.1	Isopentenyl-diphosphate delta isomerase 1	2.90	4.13E-06
<i>DHCR7</i>	NM_001014927.1	7-dehydrocholesterol reductase	2.83	1.77E-05
<i>CYP51A1</i>	NM_001025319.2	Cytochrome P450, family 51, subfamily A, polypeptide 1	2.82	6.85E-06
<i>GALM</i>	NM_001034795.1	Galactose mutarotase	2.81	2.18E-06
<i>BDH1</i>	NM_001034600.2	3-hydroxybutyrate dehydrogenase 1	2.80	2.96E-03
<i>ASZ1</i>	NM_174063.2	Ankyrin repeat, SAM and basic leucine zipper domain containing 1	2.75	1.44E-02
<i>CD36</i>	NM_174010.3	CD36 molecule	2.72	3.26E-06
<i>NT5E</i>	NM_174129.3	5'-nucleotidase ecto	2.69	8.16E-06
<i>XDH</i>	NM_173972.2	Xanthine dehydrogenase	2.60	7.40E-06
<i>DHCR24</i>	NM_001103276.1	24-dehydrocholesterol reductase	2.57	1.48E-03
<i>MSMO1</i>	NM_001098863.1	Methylsterol monooxygenase 1	2.54	1.54E-05
<i>PRKG2</i>	NM_001144099.1	Protein kinase, cGMP-dependent, type II	2.48	1.43E-02
<i>ALDH6A1</i>	NM_175811.2	Aldehyde dehydrogenase 6 family member A1	2.43	6.65E-04
<i>ME3</i>	NM_001075877.1	Malic enzyme 3	2.39	6.46E-03
<i>NAPEPLD</i>	NM_001015680.1	N-acyl phosphatidylethanolamine phospholipase D	2.39	4.70E-02
<i>CTPS1</i>	NM_001077858.2	CTP synthase 1	2.35	6.99E-05
<i>GCK</i>	NM_001102302.1	Glucokinase	2.35	9.31E-05
<i>SLC5A6</i>	NM_001046219.2	Solute carrier family 5 member 6	2.33	2.44E-04
<i>INSIG1</i>	NM_001077909.1	Insulin induced gene 1	2.27	9.75E-05
<i>NSDHL</i>	NM_001035482.2	NAD(P) dependent steroid dehydrogenase-like	2.26	4.76E-04
<i>ACSL1</i>	NM_001076085.1	Acyl-CoA synthetase long-chain family member 1	2.26	9.08E-05
<i>RASGEF1B</i>	NM_001083649.1	RasGEF domain family member 1B	2.21	1.34E-04
<i>DDAH1</i>	NM_001102201.2	Dimethylarginine dimethylaminohydrolase 1	2.21	1.12E-03
<i>CHPT1</i>	NM_001075507.1	Choline phosphotransferase 1	2.20	1.29E-04
<i>HMGCR</i>	NM_001105613.1	3-hydroxy-3-methylglutaryl-CoA reductase	2.12	3.94E-04
<i>SLC7A5</i>	NM_174613.2	Solute carrier family 7 member 5	2.11	3.58E-04
<i>HIF1AN</i>	NM_001083443.2	Hypoxia inducible factor 1 alpha subunit inhibitor	2.07	2.32E-02
<i>PANK3</i>	NM_001075463.1	Pantothenate kinase 3	2.07	4.98E-03

<i>UGP2</i>	NM_174212.2	UDP-glucose pyrophosphorylase 2	2.06	3.10E-04
<i>RPS27L</i>	NM_001040578.2	Ribosomal protein S27 like	2.03	9.30E-04
Down-regulated				
<i>PNMT</i>	NM_177505.3	Phenylethanolamine N-methyltransferase	-35.18	3.20E-13
<i>RSPO1</i>	NM_001105621.1	R-spondin 1	-32.54	1.49E-05
<i>MMP7</i>	NM_001075130.1	Matrix metalloproteinase 7	-32.14	1.68E-04
<i>IGF1</i>	NM_001077828.1	Insulin like growth factor 1	-32.09	1.68E-04
<i>DUT</i>	NM_001077873.2	Deoxyuridine triphosphatase	-31.70	1.09E-03
<i>MMP9</i>	NM_174744.2	Matrix metalloproteinase 9	-31.60	1.53E-03
<i>AOC1</i>	NM_001034361.2	Amine oxidase, copper containing 1	-31.44	3.08E-03
<i>S100A12</i>	NM_174651.3	S100 calcium binding protein A12	-31.30	4.41E-03
<i>LDLRAP1</i>	NM_001083668.2	Low density lipoprotein receptor adaptor protein 1	-31.29	6.38E-03
<i>PAXIP1</i>	NM_001077917.1	PAX interacting protein 1	-31.17	9.31E-03
<i>DPP4</i>	NM_174039.2	Dipeptidyl peptidase 4	-31.15	9.31E-03
<i>GSTT2</i>	NM_001075738.1	Glutathione S-transferase theta 2	-30.99	2.04E-02
<i>NPL</i>	NM_001045978.1	N-acetylneuraminase pyruvate lyase	-30.94	1.37E-02
<i>RAD9B</i>	NM_001035479.1	RAD9 checkpoint clamp component B	-30.84	3.06E-02
<i>AK4</i>	NM_001077933.2	Adenylate kinase 4	-30.79	6.52E-03
<i>DRD1</i>	NM_174042.2	Dopamine receptor D1	-30.78	6.52E-03
<i>FANCF</i>	NM_001105425.2	Fanconi anemia complementation group F	-30.76	6.52E-03
<i>ACE2</i>	NM_001024502.4	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	-30.70	6.52E-03
<i>CSF1</i>	NM_174026.1	Colony stimulating factor 1	-30.53	1.72E-02
<i>CELA1</i>	NM_174048.2	Chymotrypsin like elastase family member 1	-30.52	1.72E-02
<i>PLCXD1</i>	NM_001105044.1	Phosphatidylinositol-specific phospholipase C, X domain containing 1	-30.45	1.72E-02
<i>ASMTL</i>	NM_001035058.1	Acetylserotonin O-methyltransferase like	-30.44	1.72E-02
<i>CKMT2</i>	NM_001034656.2	Creatine kinase, mitochondrial 2	-30.31	1.72E-02
<i>B3GNT5</i>	NM_001076979.1	UDP-GlcNAc:betaGal beta-1,3-N- acetylglucosaminyltransferase 5	-30.29	2.87E-02
<i>KIN</i>	NM_001101099.1	Kin17 DNA and RNA binding protein	-30.29	2.87E-02
<i>DNAJB13</i>	NM_001034536.2	DnaJ heat shock protein family (Hsp40) member B13	-30.27	2.87E-02
<i>FADS6</i>	NM_001081722.1	Fatty acid desaturase 6	-30.21	2.87E-02
<i>PAMR1</i>	NM_001015591.1	Peptidase domain containing associated with muscle regeneration 1	-30.17	2.87E-02
<i>KIAA1456</i>	NM_001076832.1	KIAA1456 ortholog	-30.15	4.88E-02
<i>PRPSAP1</i>	NM_001076857.2	Phosphoribosyl pyrophosphate synthetase associated protein 1	-30.15	4.88E-02
<i>GPT</i>	NM_001083740.2	Glutamic--pyruvic transaminase	-30.14	2.87E-02
<i>CAPN3</i>	NM_174260.2	Calpain 3	-30.12	4.88E-02
<i>FAM20B</i>	NM_001076185.1	Glycosaminoglycan xylosylkinase	-30.12	2.87E-02
<i>LOC534967</i>	NM_001046391.1	Leukotriene-B(4) omega-hydroxylase 2-like	-30.01	4.88E-02
<i>ADAT1</i>	NM_001081620.1	Adenosine deaminase, tRNA specific 1	-29.98	4.88E-02

<i>NEU3</i>	NM_174122.3	Neuraminidase 3	-29.97	4.88E-02
<i>ACSBG1</i>	NM_001024548.1	Acyl-CoA synthetase bubblegum family member 1	-29.87	4.88E-02
<i>HP</i>	NM_001040470.2	Haptoglobin	-7.27	6.74E-23
<i>ASIP</i>	NM_206843.2	Agouti signaling protein	-6.99	3.33E-15
<i>FUT8</i>	NM_177501.2	Fucosyltransferase 8	-4.52	2.30E-06
<i>STAR</i>	NM_174189.2	Steroidogenic acute regulatory protein	-3.67	3.74E-07
<i>ITIH4</i>	NM_001015590.3	Inter-alpha-trypsin inhibitor heavy chain family member 4	-3.62	5.51E-08
<i>LOC100299600</i>	XM_002691714.2	PREDICTED: ATP-binding cassette sub-family A member 2	-3.59	1.51E-04
<i>PTGDS</i>	NM_174791.4	Prostaglandin D2 synthase	-3.48	8.40E-09
<i>SDS</i>	NM_001075662.1	Serine dehydratase	-3.47	6.28E-08
<i>RHOH</i>	NM_001078124.2	Ras homolog family member H	-3.36	7.03E-05
<i>VNN1</i>	NM_001024556.2	Vanin 1	-3.35	1.27E-07
<i>NCF4</i>	NM_001045983.1	Neutrophil cytosolic factor 4	-3.09	2.76E-04
<i>NECAB3</i>	NM_001081589.2	N-terminal EF-hand calcium binding protein 3	-3.02	2.71E-03
<i>TBXAS1</i>	NM_001046027.2	Thromboxane A synthase 1	-2.98	1.78E-04
<i>ENPEP</i>	NM_001038027.1	Glutamyl aminopeptidase	-2.82	6.10E-03
<i>VNN2</i>	NM_001163920.1	Vanin 2	-2.79	1.19E-04
<i>CTSC</i>	NM_001033617.2	Cathepsin C	-2.70	4.04E-06
<i>CRYM</i>	NM_001045914.2	Crystallin mu	-2.69	1.89E-04
<i>ADAMTS4</i>	NM_181667.1	ADAM metalloproteinase with thrombospondin type 1 motif 4	-2.67	1.07E-02
<i>PYCARD</i>	NM_174730.2	PYD and CARD domain containing	-2.62	2.36E-05
<i>GLIPR1</i>	NM_001076984.2	GLI pathogenesis related 1	-2.61	2.07E-04
<i>MND1</i>	NM_001038223.2	Meiotic nuclear divisions 1	-2.57	2.32E-02
<i>SLC6A14</i>	NM_001098461.1	Solute carrier family 6 member 14	-2.52	2.13E-04
<i>TNF</i>	NM_173966.3	Tumor necrosis factor	-2.50	4.68E-03
<i>NAAA</i>	NM_001100369.2	N-acylethanolamine acid amidase	-2.50	1.02E-04
<i>KIF22</i>	NM_001101868.2	Kinesin family member 22	-2.49	5.05E-03
<i>ALOX5AP</i>	NM_001076293.2	Arachidonate 5-lipoxygenase activating protein	-2.48	2.55E-04
<i>MOB3B</i>	NM_001046491.2	MOB kinase activator 3B	-2.47	8.80E-04
<i>CKMT1B</i>	NM_174275.2	Creatine kinase, mitochondrial 1B	-2.43	3.96E-02
<i>SLC43A2</i>	NM_001075546.1	Solute carrier family 43 member 2	-2.42	5.19E-04
<i>DHRS9</i>	NM_174733.2	Dehydrogenase/reductase 9	-2.37	8.28E-03
<i>ASRGL1</i>	NM_001077035.2	Asparaginase like 1	-2.36	1.66E-03
<i>RNASE6</i>	NM_174594.2	Ribonuclease A family member k6	-2.32	7.17E-05
<i>B3GALNT1</i>	NM_001076963.1	Beta-1,3-N-acetylgalactosaminyltransferase 1	-2.31	5.80E-04
<i>NCF1</i>	NM_174119.4	Neutrophil cytosolic factor 1	-2.30	5.16E-04
<i>RBP1</i>	NM_001025343.1	Retinol binding protein 1	-2.28	8.95E-05
<i>SOD2</i>	NM_201527.2	Superoxide dismutase 2, mitochondrial	-2.27	7.61E-05
<i>WFDC2</i>	NM_001076490.2	WAP four-disulfide core domain 2	-2.24	1.27E-04
<i>DHRS4</i>	NM_174822.3	Dehydrogenase/reductase (SDR family) member 4	-2.18	1.64E-02
<i>AAAS</i>	NM_001075269.1	Aladin WD repeat nucleoporin	-2.16	8.34E-03

<i>CHAF1A</i>	NM_001101842.2	Chromatin assembly factor 1 subunit A	-2.16	2.72E-03
<i>SNCB</i>	NM_001075360.1	Synuclein beta	-2.13	5.77E-03
<i>IKBK</i>	NM_174354.3	Inhibitor of nuclear factor kappa B kinase subunit gamma	-2.11	3.79E-03
<i>ANGPTL4</i>	NM_001046043.2	Angiotensinogen like 4	-2.10	6.44E-04
<i>ADA</i>	NM_173887.2	Adenosine deaminase	-2.07	1.18E-03
<i>SNCA</i>	NM_001034041.2	Synuclein alpha	-2.06	6.76E-03
<i>CYBB</i>	NM_174035.4	Cytochrome b-245, beta polypeptide	-2.06	4.49E-04
<i>PPIF</i>	NM_001001597.1	Peptidylprolyl isomerase F	-2.01	3.14E-03
<i>PLTP</i>	NM_001035027.2	Phospholipid transfer protein	-2.01	5.35E-04
<i>ME2</i>	NM_001076814.1	Malic enzyme 2	-2.00	8.08E-04

¹SFC=signed fold change

Supplementary Table S3 Expression changes of milk protein genes in the mammary glands of dairy cows during early lactation versus mid-dry period

Gene symbol	GenBank accession	Gene description	SFC	P value
<i>LALBA</i>	NM_174378.2	Lactalbumin alpha	8.21	6.52E-28
<i>CSN1S1</i>	NM_181029.2	Casein alpha s1	6.55	5.39E-21
<i>CSN2</i>	NM_181008.2	Casein beta	6.52	6.57E-21
<i>CSN1S2</i>	NM_174528.2	Casein alpha-S2	4.54	7.71E-13

¹SFC=signed fold change

² part of differentially expressed genes in DGE analysis

Supplementary Table S4 Expression changes of DEG associated with transport in the mammary glands of dairy cows during early lactation versus mid-dry period

Gene symbol	GenBank accession	Gene description	SFC	P value
Up-regulated				
<i>SLC34A2</i>	NM_174661.2	Solute carrier family 34 member 2	7.38	1.38E-16
<i>SLC38A3</i>	NM_001040486.1	Solute carrier family 38 member 3	4.94	1.98E-10
<i>FABP3</i>	NM_174313.2	Fatty acid binding protein 3	4.44	2.79E-12
<i>ABCG2</i>	NM_001037478.3	ATP binding cassette subfamily G member 2	4.31	8.58E-12
<i>SLC25A21</i>	NM_001015587.1	Solute carrier family 25 member 21	4.04	7.61E-09
<i>VLDLR</i>	NM_174489.2	Very low density lipoprotein receptor	3.83	2.26E-04
<i>ARL4D</i>	NM_001076135.1	ADP ribosylation factor like GTPase 4D	3.74	3.72E-07
<i>STIM1</i>	NM_001035409.1	Stromal interaction molecule 1	3.6	7.87E-04
<i>TRIB3</i>	NM_001076103.1	Tribbles pseudokinase 3	3.56	6.38E-03
<i>PPARGC1A</i>	NM_177945.3	PPARG coactivator 1 alpha	3.32	4.13E-05
<i>SLC28A1</i>	NM_001037458.1	Solute carrier family 28 member 1	3.31	2.71E-03
<i>RASEF</i>	NM_001105362.1	RAS and EF-hand domain containing	3.24	1.60E-07

<i>SLC6A4</i>	NM_174609.2	Solute carrier family 6 member 4	3.17	1.04E-02
<i>SCNN1B</i>	NM_001098075.1	Sodium channel epithelial 1 beta subunit	3.1	2.02E-02
<i>AGT</i>	NM_001114082.2	Angiotensinogen	3.02	1.43E-02
<i>RAB18</i>	NM_001075499.1	RAB18, member RAS oncogene family	2.79	2.51E-06
<i>SLC26A11</i>	NM_001014866.1	Solute carrier family 26 member 11	2.77	5.47E-03
<i>SLC31A2</i>	NM_001034556.1	Solute carrier family 31 member 2	2.77	2.53E-06
<i>MFSD2A</i>	NM_001101959.1	Major facilitator superfamily domain containing 2A	2.73	4.61E-05
<i>CD36</i>	NM_174010.3	CD36 molecule	2.72	3.26E-06
<i>KLF15</i>	NM_001082425.1	Kruppel like factor 15	2.68	1.03E-05
<i>LIN7A</i>	NM_001079602.2	Lin-7 homolog A, crumbs cell polarity complex component	2.65	1.16E-03
<i>VPS35</i>	NM_001046258.1	VPS35, retromer complex component	2.53	1.59E-03
<i>GLRB</i>	NM_174071.2	Glycine receptor beta	2.44	4.01E-02
<i>AQP3</i>	NM_001079794.1	Aquaporin 3	2.4	4.99E-05
<i>GCK</i>	NM_001102302.1	Glucokinase	2.35	9.31E-05
<i>SLC5A6</i>	NM_001046219.2	Solute carrier family 5 member 6	2.33	2.44E-04
<i>DOC2G</i>	NM_001076499.2	Double C2, gamma	2.29	8.28E-03
<i>SLC25A4</i>	NM_174658.2	Solute carrier family 25 member 4	2.29	8.70E-05
<i>SLC7A5</i>	NM_174613.2	Solute carrier family 7 member 5	2.11	3.58E-04
<i>SLC22A16</i>	NM_001076324.2	Solute carrier family 22 member 16	2.09	3.23E-04
<i>KCNJ15</i>	NM_001099018.1	Potassium voltage-gated channel subfamily J member 15	2.07	5.81E-03
Down-regulated				
<i>SLC11A1</i>	NM_174652.2	Solute carrier family 11 member 1	-32.58	9.15E-06
<i>IGF1</i>	NM_001077828.1	Insulin like growth factor 1	-32.09	1.68E-04
<i>HK3</i>	NM_001101929.1	Hexokinase 3	-31.35	3.08E-03
<i>SLC25A37</i>	NM_001034549.1	Solute carrier family 25, member 37	-31.05	1.37E-02
<i>MMGT1</i>	NM_001079630.1	Membrane magnesium transporter 1	-30.84	3.06E-02
<i>DRD1</i>	NM_174042.2	Dopamine receptor D1	-30.78	6.52E-03
<i>HBB</i>	NM_173917.2	Hemoglobin, beta	-30.5	1.05E-02
<i>CYSLTR1</i>	NM_001099726.1	Cysteinyl leukotriene receptor 1	-30.31	2.87E-02
<i>F2</i>	NM_173877.1	Coagulation factor II, thrombin	-30.26	2.87E-02
<i>VT11A</i>	NM_001079792.2	Vesicle transport through interaction with t-SNAREs 1A	-29.87	4.88E-02
<i>TRIP10</i>	NM_001081530.1	Thyroid hormone receptor interactor 10	-29.86	4.88E-02
<i>STAR</i>	NM_174189.2	Steroidogenic acute regulatory protein	-3.67	3.74E-07
<i>RAMP1</i>	NM_001083777.1	Receptor activity modifying protein 1	-3.51	1.03E-06
<i>SFXN4</i>	NM_001035060.2	Sideroflexin 4	-3.33	9.90E-04
<i>LOC525947</i>	NM_001046279.2	Serotransferrin-like	-3.15	1.36E-06
<i>NECAB3</i>	NM_001081589.2	N-terminal EF-hand calcium binding protein 3	-3.02	2.71E-03
<i>CRYM</i>	NM_001045914.2	Crystallin mu	-2.69	1.89E-04
<i>SNX20</i>	NM_001038033.2	Sorting nexin 20	-2.67	8.06E-04
<i>CNGA3</i>	NM_174279.2	Cyclic nucleotide gated channel alpha 3	-2.62	2.68E-02
<i>SLC6A14</i>	NM_001098461.1	Solute carrier family 6 member 14	-2.52	2.13E-04
<i>TNF</i>	NM_173966.3	Tumor necrosis factor	-2.5	4.68E-03
<i>UCHL1</i>	NM_001046172.2	Ubiquitin C-terminal hydrolase L1	-2.47	7.88E-04

<i>LBP</i>	NM_001038674.2	Lipopolysaccharide binding protein	-2.45	4.30E-05
<i>P2RY12</i>	NM_001001174.2	Purinergic receptor P2Y12	-2.44	5.86E-04
<i>SLC43A2</i>	NM_001075546.1	Solute carrier family 43 member 2	-2.42	5.19E-04
<i>CORO1A</i>	NM_174521.2	Coronin 1A	-2.36	1.05E-04
<i>KIF20A</i>	NM_001046288.2	Kinesin family member 20A	-2.35	1.07E-02
<i>ATP6V1C2</i>	NM_001083653.1	ATPase H ⁺ transporting V1 subunit C2	-2.34	8.24E-05
<i>SNX21</i>	NM_001103328.1	Sorting nexin family member 21	-2.32	3.69E-04
<i>AP1S2</i>	NM_001040591.2	Adaptor related protein complex 1 sigma 2 subunit	-2.29	4.01E-04
<i>SOD2</i>	NM_201527.2	Superoxide dismutase 2, mitochondrial	-2.27	7.61E-05
<i>SLC25A25</i>	NM_001038145.2	Solute carrier family 25 member 25	-2.16	3.88E-03
<i>AAAS</i>	NM_001075269.1	Aladin WD repeat nucleoporin	-2.16	8.34E-03
<i>FOLR2</i>	NM_001075325.1	Folate receptor 2	-2.08	3.65E-04
<i>CHMP4C</i>	NM_001045976.1	Charged multivesicular body protein 4C	-2.08	6.76E-03
<i>SNCA</i>	NM_001034041.2	Synuclein alpha	-2.06	6.76E-03
<i>CYBB</i>	NM_174035.4	Cytochrome b-245, beta polypeptide	-2.06	4.49E-04

¹SFC=signed fold change

² part of differentially expressed genes involved in GO biological process

Supplementary Table S5 Expression changes of DEG associated with cell proliferation in the mammary glands of dairy cows during early lactation versus mid-dry period

Gene symbol	GenBank accession	Gene description	SFC	P value
Up-regulated				
<i>PTH1H</i>	NM_174753.1	Parathyroid hormone like hormone	6.48	2.58E-19
<i>FABP3</i>	NM_174313.2	Fatty acid binding protein 3	4.44	2.79E-12
<i>GPAM</i>	NM_001012282.1	Glycerol-3-phosphate acyltransferase, mitochondrial	3.62	2.85E-09
<i>FST</i>	NM_175801.3	Follistatin	3.36	2.72E-06
<i>IL1A</i>	NM_174092.1	Interleukin 1 alpha	3.26	2.89E-06
<i>MFG8</i>	NM_176610.1	Milk fat globule-EGF factor 8 protein	3.26	5.10E-08
<i>AGT</i>	NM_001114082.2	Angiotensinogen	3.02	1.43E-02
<i>DHCR7</i>	NM_001014927.1	7-dehydrocholesterol reductase	2.83	1.77E-05
<i>DHCR24</i>	NM_001103276.1	24-dehydrocholesterol reductase	2.57	1.48E-03
<i>VIPR1</i>	NM_001081607.1	Vasoactive intestinal peptide receptor 1	2.24	1.38E-04
<i>DHPS</i>	NM_001003657.1	Deoxyhypusine synthase	2.19	4.70E-02
Down-regulated				
<i>CXCR1</i>	NM_174360.2	Chemokine (C-X-C motif) receptor 1	-32.85	2.32E-06
<i>MMP7</i>	NM_001075130.1	Matrix metalloproteinase 7	-32.14	1.68E-04
<i>IGF1</i>	NM_001077828.1	Insulin like growth factor 1	-32.09	1.68E-04
<i>CCNB1</i>	NM_001045872.1	Cyclin B1	-31.91	4.16E-04
<i>TACC3</i>	NM_001100305.2	Transforming acidic coiled-coil containing protein 3	-31.86	5.70E-04
<i>PBX3</i>	NM_001046453.1	PBX homeobox 3	-31.81	7.87E-04

<i>TNC</i>	NM_001078026.2	Tenascin C	-31.81	7.87E-04
<i>FGF1</i>	NM_174055.3	Fibroblast growth factor 1	-31.72	1.09E-03
<i>HLX</i>	NM_001101097.1	H2.0 like homeobox	-31.44	3.08E-03
<i>DPP4</i>	NM_174039.2	Dipeptidyl peptidase 4	-31.15	9.31E-03
<i>PTH1R</i>	NM_001075332.1	Parathyroid hormone 1 receptor	-31.14	9.31E-03
<i>CDKN3</i>	NM_001040582.2	Cyclin dependent kinase inhibitor 3	-31.13	9.31E-03
<i>MSX1</i>	NM_174798.2	Msh homeobox 1	-31.03	1.37E-02
<i>ETS1</i>	NM_001099106.2	ETS proto-oncogene 1, transcription factor	-30.84	3.06E-02
<i>CSF1</i>	NM_174026.1	Colony stimulating factor 1	-30.53	1.72E-02
<i>CELA1</i>	NM_174048.2	Chymotrypsin like elastase family member 1	-30.52	1.72E-02
<i>F2</i>	NM_173877.1	Coagulation factor II, thrombin	-30.26	2.87E-02
<i>ELANE</i>	NM_001105653.1	Elastase, neutrophil expressed	-30.18	2.87E-02
<i>WNT2</i>	NM_001013001.1	Wnt family member 2	-30.05	2.87E-02
<i>HILPDA</i>	NM_001105498.1	Hypoxia inducible lipid droplet associated	-30.02	4.88E-02
<i>CXCL5</i>	NM_174300.2	Chemokine (C-X-C motif) ligand 5	-3.42	6.37E-08
<i>SPN</i>	NM_001103102.2	Sialophorin	-3.39	1.48E-03
<i>CDC20</i>	NM_001082436.2	Cell division cycle 20	-2.94	3.54E-03
<i>BNIPL</i>	NM_001079621.1	BCL2 interacting protein like	-2.90	2.17E-04
<i>SERPINE1</i>	NM_174137.2	Serpin family E member 1	-2.52	6.08E-05
<i>TNF</i>	NM_173966.3	Tumor necrosis factor	-2.50	4.68E-03
<i>IL34</i>	NM_001100324.2	Interleukin 34	-2.44	4.13E-05
<i>CORO1A</i>	NM_174521.2	Coronin 1A	-2.36	1.05E-04
<i>ANGPT4</i>	NM_001076483.2	Angiopoietin 4	-2.31	3.46E-02
<i>SOD2</i>	NM_201527.2	Superoxide dismutase 2, mitochondrial	-2.27	7.61E-05
<i>IL6R</i>	NM_001110785.1	Interleukin 6 receptor	-2.26	9.22E-04
<i>CD38</i>	NM_175798.3	CD38 molecule	-2.23	5.77E-03
<i>JUNB</i>	NM_001075656.1	JunB proto-oncogene, AP-1 transcription factor subunit	-2.16	2.07E-04
<i>RARRES1</i>	NM_001075430.2	Retinoic acid receptor responder 1	-2.14	2.95E-04
<i>EFNB1</i>	NM_001080299.1	Ephrin B1	-2.11	2.50E-03
<i>ADA</i>	NM_173887.2	Adenosine deaminase	-2.07	1.18E-03
<i>DDR2</i>	NM_001083720.2	Discoidin domain receptor tyrosine kinase 2	-2.06	5.17E-03
<i>ECM1</i>	NM_001099706.1	Extracellular matrix protein 1	-2.05	2.25E-03

¹SFC=signed fold change

² part of differentially expressed genes involved in GO biological process

Supplementary File S1

High-throughput sequencing

BGI Tec (Beijing, China) performed the high-throughput sequencing. Briefly, total RNA was isolated from mammary tissue samples using Trizol reagent (Invitrogen, CA) and purified using RNeasy mini kit (Qiagen, Netherlands). Messenger mRNA from 6 µg of total RNA extracted from each cow was purified by oligo(dT) magnetic beads and reverse transcribed into cDNA, which was then digested using the restriction enzyme NlaIII. The Illumina adaptor 1 was ligated to the sticky 5' end of the digested bead-bound cDNA fragments. The bead-bound cDNA fragments with adaptor 1 were then digested by MmeI to release the tag fragment from the oligo (dT) beads. The Illumina adaptor 2 was then ligated to the 3' ends of the tags, generating tags with different adaptors at each end to form a tag library. The library was amplified by linear PCR for 15 cycles, and the resulting 105bp fragments were purified by polyacrylamide gel electrophoresis through a 6% gel, and were then submitted to Illumina HiSeq 2000 for DGE analysis.