SUPPLEMENTARY FILE 1

Characterization of Gene Expression Related to Milk Fat Synthesis in the Mammary Tissue of Lactating Yaks

SUPPLEMENTARY MATERIALS AND METHODS

1. RNA preparation and RT-qPCR

Table S1. Gene symbols, their primer sequences, and RT-qPCR results for expression analysis of 40 genes related to yak milk fat synthesis.

Gene	Acc#[1]	Primer sequence (5' -> 3')	Slope	Eff	AT[2] (℃)	Amp[3] (bp)	*P* value[4]	SEM[5]
ABCA1	DQ059505	F: CGGCGGCTTCTCTTGTATAGC R: TTCAAGCGTGAGCTGAAACG	3.46	1.94	57.9	101	0.474	1.4278
ABCG2	DQ825760	F: GAGCCATAGGTTTCCACTGTGA R: CCACAGCAGAAGAATCTCCATT	3.37	1.98	61.0	83	0.003	1.7501
ACACA	AJ132890	F: CATCTTGTCCGAAACGTCGAT R: CCCTTCGAACATACACCTCCA	3.37	1.98	59.9	101	0.015	1.7781
ACSL1	BC119914	F: GTGGGCTCCTTTGAAGAACTGT R: ATAGATGCCTTTGACCTGTTCAAAT	3.41	1.96	62.4	120	0.012	1.3994
ACSS1	AB046741	F :CCGATCAGGTCCTGGTAGTGA R: CTCGGCCCATGACAATCTTC	3.34	1.99	59.3	90	0.047	1.3086
ACSS2	BC134532	F: GGCGAATGCCTCTACTGCTT R: GGCCAATCTTTTCTCTAATCTGCTT	3.32	2.00	59.3	100	0.01	1.5872
AGPAT6	DY208485	F: AAGCAAGTTGCCCATCCTCA R: AAACTGTGGCTCCAATTTCGA	3.47	1.94	57.6	101	0.053	1.8244
BDH1	CR455522	F; CCCACCACCAGTCTGAGCAT R: CCCACTACTCTGCACCCCAA	3.43	1.96	58.5	101	0.005	1.6065
BTN1A1	M35551	F: AGGACGGACTGGGCAATTG R: GAACCCATTCTCGGGAGTCAT	3.43	1.96	57.1	81	0.005	1.7972
CD36	X91503	F: GTACAGATGCAGCCTCATTTCC R: TGGACCTGCAAATATCAGAGGA	3.43	1.96	57.9	81	0.031	1.5974
CERS2	BC103330	F: TGACGTCAAGCGAAAGGATTT R: TCCCTGCTCGGACGTAATTG	3.47	1.94	56.1	101	0.052	1.0925
DGAT1	NM_174693	F: CCACTGGGACCTGAGGTGTC R: GCATCACCACACACCAATTCA	3.47	1.94	57.6	101	0.604	0.9017
DGAT2	BT030532.1	F: CATGTACACATTCTGCACCGATT R: TGACCTCCTGCCACCTTTCT	3.46	1.94	57.1	100	0.136	1.1024

FABP3	DN518905	F: GAACTCGACTCCCAGCTTGAA R: AAGCCTACCACAATCATCGAAG	3.37	1.98	59.3	102	0.005	2.6757
FADS1	EE347846	F: GGTGGACTTGGCCTGGATG R: TGACCATGAAGACAAGCCCC	3.36	1.99	58.5	101	0.949	0.5711
FADS2	DV895683	F: AAAGGGTGCCTCTGCCAACT R: ACACGTGCAGCATGTTCACA	3.30	2.01	57.0	101	0.921	0.9259
GPAM	AY515690	F: GCAGGTTTATCCAGTATGGCATT R: GGACTGATATCTTCCTGATCATCTTG	3.40	1.97	61.0	63	0.022	2.315
INSIG1	NM_ 001077909.1	F: AAAGTTAGCAGTCGCGTCGTC R: TTGTGTGGCTCTCCAAGGTGA	3.39	1.97	59.3	120	0.981	0.8366
INSIG2	XM_614207	F: TCCAGTGTGATGCGGTGTGTA R: TGGATAGTGCAGCCAGTGTGA	3.39	1.97	57.0	109	0.437	0.547
LPIN1	DV797268	F: TGGCCACCAGAATAAAGCATG R: GCTGACGCTGGACAACAGG	3.38	1.98	57.0	101	0.003	1.3334
LPL	BC118091	F: ACACAGCTGAGGACACTTGCC' R: GCCATGGATCACCACAAAGG	3.34	1.97	57.6	101	0.01	2.0775
NAAA	AW656293	F: ATTTACCACGGCCGGAATCT R: CCTGTGTAGGCAATCTGCCC	3.47	1.94	59.3	101	0.288	0.7619
OSBP	EH174150	F: GTGAGCAGGTGAGCCACCAT R: GGTATTTGCCGCGAAACTTG	3.49	1.94	57.9	111	0.586	0.7526
OSBPL2	BC102883	F: AGAAGTGCATCGGCTTGGAG R: CGGGAGGCTCTGTGAATTAGG	3.43	1.96	57.1	124	0.564	0.9261
OSBPL10	DT840936	F: GGAGGGAAAGTCAGCATCACC R: CTGTGACCCTGTGGACCTT	3.43	1.95	58.5	101	0.033	0.7118
OXCT1	CN441025	F: CAATGCTAGGAGCCATGCAG R: CACTAGATCCATAGCCCCTCCC	3.43	1.96	58.5	101	0.021	0.7154
PLIN1	DV814745	F: GATCGCCTCTGAGCTGAAGG R: AGAGCGGCCCCTAGGATTT	3.45	1.95	57.1	108	0.497	0.9368
PLIN2	BC102211	F: TGGTCTCCTCGGCTTACATCA R: TCATGCCCTTCTCTGCCATC	3.43	1.96	59.3	81	0.017	1.7191
PPARG	NM_181024	F: CCAAATATCGGTGGGAGTCG R: ACAGCGAAGGGCTCACTCTC	3.35	1.99	57.1	101	0.862	1.3375
PPARGC1A	AB106107	F: AAAAGCCACAAAGACGTCCG R: TCTGCTGCTGTTCCGGTTCT	3.37	1.98	61.0	101	0.025	0.8943
PPARGC1B	CX951189	F: GCCTCCTTCAGTAAGCTGTCAA R: GGCCCCGCTATACTGACTATGA	3.45	1.95	61.6	103	0.408	0.9179
SCD	AY241933	F: TCCTGTTGTTGTGCTTCATCC R: GGCATAACGGAATAAGGTGGC	3.35	1.99	57.9	101	0.002	1.7704
SGPL1	EH163381	F: GGCTTATGGAGATTTCGCATG R: CCCCCATTGAATAGGGAACAA	3.43	1.96	61.0	111	0.049	0.8188
SPHK2	BC116169	F: CCTCTCAGAGCCACAGACCAA R: ACCATGTCAGCAAGACGCTG	3.49	1.93	57.6	103	0.249	1.6143
SPTLC1	BC105250	F: GGCACATTTGATGTGCACTTG R: CTGGCTATGGTGGCAAATCC	3.44	1.95	62.4	101	0.016	1.0314

SPTLC2	DN518712	F: GCAGTATCAAGCGTTTCTCTGGTAT R: TTGGTGTAGTTGTGGTAGGATTTCC	3.48	1.94	59.9	103	0.097	0.6215
THRSP	AY656814	F: CTACCTTCCTCTGAGCACCAGTTC R: ACACACTGACCAGGTGACAGACA	3.41	1.96	59.9	151	0.033	1.1793
UGCG	BC123602	F: TGACCGAGGTTGGAGGTTTG R: TGGTCCACCTGATCATTCTGG	3.44	1.96	57.9	101	0.201	0.4754
VLDLR	AJ609502	F: GCCCAGAACAGTGCCATATGA R: TTTTCACCATCACACCGCC	3.39	1.97	59.3	103	0.127	0.8168
XDH	BC102076	F: GATCATCCACTTTTCTGCCAATG R: CCTCGTCTTGGTGCTTCCAA	3.42	1.96	59.3	100	0.009	1.4733

[1]Acc# indicates the accession number in GenBank (www.ncbi.nlm.nih.gov).

[2]AT indicates annealing temperature in PCR.

[3]Amp indicates PCR amplicon size.

[4]*P* value indicates the significance with time (d) in GLIMMIX analysis using SAS (v 9.4, SAS Institute Inc.).

[5]SEM indicates the standard error of the mean.

Table S2. Sequencing results of amplification of 40 genes related to yak milk fat synthesis through BLAST

Gene name	Match acc.#	Best hit in NCBI	Score
ABCA1	NM_001024693.1	Bos taurus ATP-binding cassette, sub-family A (ABC1), member 1 (ABCA1), mRNA	180
ABCG2	BT030709.1	Bos taurus ATP-binding cassette, sub-family G (WHITE), member 2 (ABCG2), mRNA, complete cds	154
ACACA	XM_010815746.1	PREDICTED: Bos taurus acetyl-CoA carboxylase alpha (ACACA), transcript variant X8, mRNA	183
ACSL1	XM_005906554.1	PREDICTED: Bos mutus acyl-CoA synthetase long-chain family member 1 (ACSL1), mRNA	224
ACSS1	XM_010836608.1	PREDICTED: Bison bison bison acyl-CoA synthetase short-chain family member 1 (ACSS1), mRNA	69.9
ACSS2	XM_010854888.1	PREDICTED: Bison bison bison acyl-CoA synthetase short-chain family member 2 (ACSS2), mRNA	161
AGPAT6	NM_001083669.1	Bos taurus 1-acylglycerol-3-phosphate O-acyltransferase 6 (AGPAT6), mRNA	187
BDH1	XM_010834383.1	PREDICTED: Bison bison bison 3-hydroxybutyrate dehydrogenase, type 1 (BDH1), mRNA	111
BTN1A1	NM_174508.2	Bos taurus butyrophilin, subfamily 1, member A1 (BTN1A1), mRNA	69.9
CD36	XM_010804155.1	PREDICTED: Bos taurus CD36 molecule (thrombospondin receptor) (CD36), transcript variant X6, mRNA	104
CERS2(LASS2)	NM_001034667.1	Bos taurus ceramide synthase 2 (CERS2), mRNA	111
DGAT1	NM_174693.2	Bos taurus diacylglycerol O-acyltransferase 1 (DGAT1), mRNA	69.9
FABP3	XM_010836889.1	PREDICTED: Bison bison bison fatty acid binding protein 3, muscle and heart (FABP3), mRNA	156
FADS1	XM_005901286.1	PREDICTED: Bos mutus fatty acid desaturase 1 (FADS1), mRNA	187
FADS2	XM_010854037.1	PREDICTED: Bison bison bison fatty acid desaturase 2 (FADS2), mRNA	81.8
GPAM	XM_005225720.2	PREDICTED: Bos taurus glycerol-3-phosphate acyltransferase, mitochondrial (GPAM), transcript variant X1, mRNA	44.1
INSIG1	NM_001077909.1	Bos taurus insulin induced gene 1 (INSIG1), mRNA	89.8

INSIG2	XM_010826529.1	PREDICTED: Bos taurus insulin induced gene 2 (INSIG2), transcript variant X1, mRNA	174
LPIN1	XM_005903204.1	PREDICTED: Bos mutus lipin 1 (LPIN1), transcript variant X2, mRNA	119
LPL	NM_001075120.1	Bos taurus lipoprotein lipase (LPL), mRNA	172
NAAA (ASAHL)	NM_001100369.2	Bos taurus N-acylethanolamine acid amidase (NAAA), mRNA	182
OSBP	NM_001205970.1	Bos taurus oxysterol binding protein (OSBP), mRNA	191
OSBPL2	NM_001035020.2	Bos taurus oxysterol binding protein-like 2 (OSBPL2), mRNA	200
OSBPL10	XM_005894168.1	PREDICTED: Bos mutus oxysterol binding protein-like 10 (OSBPL10), partial mRNA	97.6
OXCT1	KJ472110.1	Bubalus bubalis 3-oxoacid CoA transferase 1 (OXCT1) mRNA, complete cds	176
PLIN1(PLIN)	XM_010841036.1	PREDICTED: Bison bison bison perilipin 1 (PLIN1), transcript variant X5, mRNA	193
PLIN2 (ADFP)	XM_005902268.1	PREDICTED: Bos mutus perilipin 2 (PLIN2), transcript variant X2, mRNA	54
PPARGC1A	XM_010839915.1	PREDICTED: Bison bison bison peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGC1A), transcript variant X2, mRNA	187
PPARGC1B	XM_005895350.1	PREDICTED: Bos mutus peroxisome proliferator-activated receptor gamma, coactivator 1 beta (PPARGC1B), mRNA	102
SCD	XM_010859526.1	REDICTED: Bison bison bison stearoyl-CoA desaturase (delta-9-desaturase) (SCD), mRNA	99
SGPL1	XM_005226397.2	PREDICTED: Bos taurus sphingosine-1-phosphate lyase 1 (SGPL1), transcript variant X4, mRNA	139
SPHK2	XM_005195460.2	PREDICTED: Bos taurus sphingosine kinase 2 (SPHK2), transcript variant X11, mRNA	119
SPTLC1	XM_010835958.1	PREDICTED: Bison bison bison serine palmitoyltransferase, long chain base subunit 1 (SPTLC1), mRNA	187
SPTLC2	XM_006052887.1	PREDICTED: Bubalus bubalis serine palmitoyltransferase, long chain base subunit 2 (SPTLC2), mRNA	185
THRSP	XM_005908067.1	PREDICTED: Bos mutus thyroid hormone responsive (THRSP), mRNA	268
UGCG	XM_010843578.1	PREDICTED: Bison bison bison UDP-glucose ceramide glucosyltransferase (UGCG), mRNA	189

VLDLR	XM_010860735.1	PREDICTED: Bison bison bison very low density lipoprotein receptor (VLDLR), mRNA	187
XDH	XM_010829804.1	PREDICTED: Bison bison bison xanthine dehydrogenase (XDH), transcript variant X2, mRNA	97.6

* *PPARG* and *DGAT2* were not sequenced

2. Milk yields and Analysis of milk fat composition

1) Milk yields during yaks' lactation periods

Table S3.Milk yields in lactating yaks

Milk Yield (kg/day)	15	30	60	90	120	180	210
yak #1	0.40	0.50	0.60	0.80	1.20	0.80	0.30
yak #2	0.45	0.60	0.70	1.10	1.80	0.37	0.30
yak #3	0.50	0.70	0.80	1.20	0.90	0.29	0.20
yak #4	0.30	0.47	0.30	0.90	1.10	0.50	0.45

2) Analysis of milk fat composition

Milk fat profile included FA methyl ester analysis using a 6890A-5975C GC-MS system (Agilent, Santa Clara, USA) with an HP88 capillary column (60 m×250 μm×0.2 μm, Agilent, Santa Clara, USA).

Chromatographic conditions : The injector temperature was at 280℃. The temperature of the GC oven was set to 120℃, increased at 8℃ /min to 145℃, held for 15 min, increased at 3℃/min to 220℃, and held for 3 min. The carrier gas was nitrogen, with flow rate of 1 ml/min. The sample was injected using a split / splitless model and a split ratio of 30:1.

Mass spectrometer conditions were as follows: electron energy (EI), 70eV; interface temperature, 280℃; ion source temperature, 200℃; quadrupole temperature, 100℃; mass scan range, 35-500 AMU; solvent delay, 4.0 min.

Measured FA concentrations were also analyzed by GLIMMIX procedure of SAS (v 9.2, SAS Institute Inc., Cary, USA) (Table S3).

46 **Table S4**. Fat composition analysis of yak milk fat using GC-MS

% (g,targeted FA/g, analyzed FA)	Day relative to parturition													
FA	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**15**	**30**	**60**	**120**	**180**	***P***	**SEM**
C10:0	1.6	1.8	2.2	2.4	2.3	2.1	2.1	1.9	1.8	1.5	1.6	1.6	0.002	0.191
C10:1n6	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.002	0.018
C12:0	1.6	1.7	2.0	2.2	2.0	1.9	1.8	1.8	1.7	1.6	1.7	1.7	0.097	0.190
C13:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.668	0.022
C14:0	8.8	9.2	9.3	9.7	9.2	8.7	8.7	8.7	8.7	8.2	8.4	8.6	0.231	0.425
C14:1n2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.321	0.024
C15:0	1.3	1.2	1.2	1.2	1.2	1.3	1.2	1.2	1.2	1.3	1.4	1.4	0.601	0.082
C15:1n2	0.4	0.4	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.4	0.5	0.5	0.018	0.040
C15:1n2	0.9	0.8	0.8	0.8	0.8	0.9	0.9	1.0	1.0	1.1	1.2	1.2	<0.0	0.079
C16:0	32.5	34.0	35.9	36.9	34.9	32.6	33.9	32.3	33.5	30.4	30.3	33.7	0.043	1.365
C16:1n2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.125	0.029
C16:1n7	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.043	0.029
C16:1n9	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.015	0.037
C16:1n14	1.1	1.0	0.8	0.8	0.8	0.9	0.8	0.8	0.9	1.0	1.1	1.3	0.014	0.126
C17:0	0.9	0.7	0.6	0.6	0.6	0.7	0.6	0.6	0.5	0.6	0.7	0.7	0.104	0.066
C17:0 (methyl ester)	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.673	0.039
C17:1n3	0.5	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.4	0.4	0.4	0.447	0.048
C17:1n7	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.008	0.033
C18:0	17.6	17.1	17.4	17.5	17.7	18.3	18.5	18.7	18.4	19.1	19.4	17.0	0.626	1.044
C18:1n5	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.3	0.3	0.2	0.000	0.037
C18:1n7	0.7	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.6	0.6	0.5	0.281	0.054
C18:1n9	22.7	23.1	20.6	18.6	20.5	21.4	20.1	20.1	18.6	22.5	22.1	22.9	0.020	1.117
C18:1n10	3.9	3.8	3.6	3.8	4.5	5.1	5.4	6.6	7.1	5.5	4.4	3.1	0.000	0.819
C18:2n cis6,cis9	0.2	0.2	0.2	0.2	0.3	0.4	0.4	0.4	0.4	0.3	0.5	0.4	0.005	0.063
C18:2n trans6,trans9	1.3	1.1	1.0	1.0	1.0	1.0	0.9	0.9	0.8	0.9	0.9	0.6	0.002	0.120
C18:2n-3,9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.010	0.009
C18:2n-7,9	0.9	0.8	0.8	0.8	1.0	1.2	1.2	1.5	1.8	1.7	1.4	1.2	0.000	0.206
C18:3n-3,6,9	0.7	0.6	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.7	0.9	0.7	0.026	0.073
C19:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.764	0.024
C20:0	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.4	0.5	0.026	0.061
C20:4n-6,9,12,15	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.151	0.013
C20:5n-3,6,9,12,15	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.146	0.016
C22:0	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.001	0.021
Milk yield (kg/day)								0.5	0.6	0.7	1.2	0.5	0.000	0.103
Fat % (g/g, milk)								5.6	5.3	5.5	4.9	7.1	0.478	0.866
Fat amount (100g/day)								2.5	2.9	4.0	5.9	3.1	0.003	5.618

Synthesized FA (mmoles/day)		17.6	20.2	26.6	40.2	21.4	0.005	4.040
Imported FA (mmoles/day)		45.1	51.8	75.9	110.0	54.7	0.004	11.224
Synthesized / Imported		0.4	0.4	0.3	0.4	0.4	0.053	0.025
Δ9 desaturase in 16C		0.2	0.4	0.3	0.3	0.2	0.212	0.095
Δ9 desaturase in 18C		51.9	50.5	54.3	53.6	57.0	0.001	1.024

[1]FA name denotes the chemical composition of the fatty acid. The value indicates the FA concentration (%, g/g milk).

[2]*P* value was estimated by the GLIMMIX procedure of SAS (v 9.4, SAS Institute Inc.) with time.

[3]SEM indicates the standard error of the mean.

[4]Synthesized indicates the % FAs synthesized, which was calculated as the sum of FAs with C10, C12, and C14/sum of all FAs except C16, and synthesized FA was *de novo* synthesized intracellularly.

[5]Imported indicates the % FAs imported from the blood, which was calculated as the sum of FAs with C17-22 included C15 /sum of all FA s except C16.

[6]Syn/Imp indicates the ratio of synthesized FAs (mmoles/day) and imported FAs (mmoles/day).

[7]Desaturation index was calculated by (C16:1, cis9) /(sum of C16:0 and C16:1) and (C18:1, cis9) /sum of (C18:0, and C18:1) each.

SUPPLEMENTARY RESULTS AND DISCUSSIONS

Table S5. The Comparison of gene expression between yaks and dairy cows in milk fat synthesis. Gene symbol, description, overall %RNA abundance* among genes investigated, and Pearson analysis of gene expression with time between yaks and dairy cow.

		% mRNA abundance*		Pearson analysis	
		Yak	Cow	Correlation Value (*r*)	*P* value
		% mRNA			
FA import into cells					
LPL	Lipoprotein lipase	7.24	9.56	0.69	0.20
CD36	CD36 molecule	**0.76**	**4.66**	0.80	0.10
VLDLR	Very low density lipoprotein receptor	0.21	0.09	0.58	0.31
Xenobiotic and Cholesterol transport					
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	0.92	0.07	0.21	0.74
ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2	**4.84**	**8.54**	0.78	0.12
Acetate and FA activation and intra-cellular transport					
ACSS1	Acyl-CoA synthetase short-chain family member 1	0.85	0.33	N.A.	
ACSS2	Acyl-CoA synthetase short-chain family member 2	**2.01**	**0.59**	N.A.	
ACSL1	Acyl-CoA synthetase long-chain family member 1	1.14	0.89	0.76	0.13
FABP3	Fatty acid binding protein 3, muscle and heart	**31.80**	**15.49**	0.71	0.18
Fatty acid synthesis and desaturation					
ACACA	Acetyl-CoA carboxylase alpha	**6.71**	**0.91**	0.80	0.11
FADS1	Fatty acid desaturase 1	0.04	0.20	0.53	0.36
FADS2	Fatty acid desaturase 2	0.08	0.01	0.48	0.41
SCD	Stearoyl-CoA desaturase (delta-9-desaturase)	6.88	23.14	0.80	0.03
Lipid Droplet formation					
PLIN2	Perilipin 2	**2.51**	**9.56**	0.08	0.90
BTN1A1	Butyrophilin, subfamily 1, member A1	**2.75**	**4.78**	0.69	0.09
XDH	Xanthine dehydrogenase	**2.40**	**7.39**	0.60	0.29
PLIN1	Perilipin 1	0.03	0.01	N.A.	
Ketone body utilization					
BDH1	3-hydroxybutyrate dehydrogenase, type 1	**1.32**	**0.02**	N.A.	
OXCT1	3-oxoacid CoA transferase 1	0.19	0.07	0.95	0.01

TAG synthesis					
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	**18.43**	**2.31**	0.56	0.33
AGPAT6	1-acylglycerol-3-phosphate O-acyltransferase 6	**3.79**	**1.28**	0.77	0.12
DGAT1	Diacylglycerol O-acyltransferase 1	0.02	0.14	**-0.52**	0.37
DGAT2	Diacylglycerol O-acyltransferase 2	0.16	0.01	0.18	0.78
LPIN1	Lipin 1	**2.52**	**0.13**	0.76	0.14
Regulation of transcription					
INSIG1	Insulin induced gene 1	0.06	0.35	**-0.62**	0.27
INSIG2	Insulin induced gene 2	0.05	0.09	**-0.31**	0.61
THRSP	Thyroid hormone responsive	0.48	0.01	0.74	0.15
PPARG	Peroxisome proliferator-activated receptor gamma	0.04	0.01	-0.39	0.51
PPARGC1A	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	0.28	0.04	0.31	0.61
PPARGC1B	Peroxisome proliferator-activated receptor gamma, coactivator 1 beta	0.03	0.01	-0.29	0.64
Sphingolipid synthesis					
SPTLC1	Serine palmitoyltransferase, long chain base subunit 1	0.28	0.15	**-0.26**	0.67
SPTLC2	Serine palmitoyltransferase, long chain base subunit 2	0.13	0.15	0.38	0.53
CERS2	Ceramide synthase 2	0.44	0.61	0.64	0.24
SPHK2	Sphingosine kinase 2	0.02	0.09	0.30	0.62
NAAA	N-acylethanolamine acid amidase	0.03	0.05	0.10	0.88
SGPL1	Sphingosine-1-phosphate lyase 1	0.17	0.06	0.62	0.27
UGCG	UDP-glucose ceramide glucosyltransferase	0.08	0.18	0.81	0.10
OSBP	Oxysterol binding protein	0.13	0.12	0.19	0.76
OSBPL2	Oxysterol binding protein-like 2	0.13	0.17	0.83	0.08
OSBPL10	Oxysterol binding protein-like 10	0.03	0.06	**-0.66**	0.23

* %mRNA abundance is calculated relative expression quantity of a specific gene X 100/relative expression quantity of all genes measured.

First analysis was accomplished using the % mRNA abundance of all investigated genes in yaks and dairy cows. Second analysis was conducted the Pearson's correlation analysis by relatives expression profile (i.e. Log_2 (RQ)) with time (-15, 15, 30, 60, and 240 d) between yaks and dairy cows.

Table S6. **The Correlation of %RNA abundance pattern between each yak.**

		yak_1	yak_2	yak_3	yak_4
Total (yak_1~4)	***r*** *(Correlation value)*	0.651	0.976	0.769	0.922
	P*(Significance)*	0.000	0.000	0.000	0.000

FA uptake by mammary cells

Mammary cells take up FA sources through active transport using albumin-bound FAs or lipoprotein or through passive transport to produce milk fat (Abumrad *et al*, 1998). LCFAs are anchored to the epithelium membrane and TAGs are hydrolyzed to FAs by *LPL* which is a known critical factor for producing milk fat. According to previous research, *LPL* significantly influences the milk fat percentage because of its roles as an enzyme that catalyzes hydrolysis and as a regulator of the milk fat synthesis process; specifically, the expression of key regulator genes such as *SREBF1*, *FASN*, *LIPE*, *PPARG*, and *FFAR3* was changed when *LPL* was knocked out during lactation (Crepaldi *et al*., 2013; Zhao *et al*., 2014). Additionally, *LPL* is up-regulated to 60-70-fold during early parturition in bovine mammary tissue (Bionaz and Loor, 2008) compared to 1.25-fold in mice mammary tissue (Michael *et al*., 2007). And *LPL* was up-regulated to 6-fold in lactating yak mammary tissue (Fig. S1). The expression pattern of *LPL* was very similar to that of bovine, although *LPL* expression in yak mammary tissue was up-regulated during the first month of lactation, which is earlier than dairy cows mammary tissue which *LPL* in lactating cows was up-regulated during the first 2 months of lactation. The relative % mRNA abundance of *LPL* was 7% of the total genes measured similar to that in bovine mammary tissue (Table S5).

VLDL receptor (*VLDLR*), known as a transmembrane protein of lipoprotein receptor-

anchored apo-B48 protein, primary interacts with *LPL* to transport LCFAs during lactation (Tomkin and Owens, 2012). *VLDLR* was up-regulated somewhat during early parturition but was not effective for lactation in yak mammary tissue (P = 0.127) ; similarly, *VLDLR* up-regulation in dairy cow was not significant with time (Bionaz and Loor, 2008). The relative % mRNA abundance of *VLDLR* was 0.2% of the total genes expressed, and *VLDLR* and *LPL* were strongly correlated (r = 0.768, P = 0.044). Of note, *VLDLR* and *LPL* are known to interact with each other to transport LCFAs into cells.

CD36, which is a known membrane protein primarily involved in the uptake of fat, largely LCFAs, from plasma through passive diffusion into the epithelial membrane or into milk fat globules (Joost *et al*, 1999). The *CD36* expression pattern in yak mammary tissue is similar to that in bovine mammary tissue, in which *CD36* expression was also up-regulated 2-fold during early parturition and decreased during late lactation, changing with time (P = 0.031). The relative % mRNA abundance of *CD36* was low, at 0.7% of the total genes measured against that of dairy cows (4%) (Bionaz and Loor, 2008) (Table S5). However, *CD36* expression increased in yak mammary tissue during early parturition and continued to increase with time (> 4-fold) (Fig. S1). In our study, the expression of *FABP3* and *ACSL1*, which are related to FA activation and transport into cells, strongly correlated with *CD36* (r = 0.955, P = 0.001 with *FABP3* and r = 0.949, P = 0.001 with *ACSL1*) in lactating yak mammary tissue.

Activation and intracellular channeling of FAs

LCFAs that are taken up from blood plasma undergo esterization into fatty acyl-CoAs to synthesize TAGs in the endoplasmic reticulum. *ACSL* is a critical enzyme required to activate fatty acyl-CoA production from LCFAs inside the plasma membrane, and *ACSL1* mRNA is predominant among the *ACSL* isoforms within each specific gene family (Bionaz and Loor, 2008). Our data have shown that *ACSL1* was up-regulated during the lactation period (> 4-fold) (Fig. S1) and that the relative % mRNA abundance of *ACSL1* was 1% of the total genes in lactating yak mammary tissue (Table S5). For *de novo* FA synthesis, carbon intermediates are also activated by *ACSS* members into acetyl-CoA. *ACSS2* is more predominantly found in the cytoplasm and has higher affinity to acetate than *ACSS1*. *ACSS1* can be primarily found in inner mitochondria, and has affinity to acetate and propionate. The relative % mRNA abundance of *ACSS2* in yak was 2% of the total genes measured, slightly higher than that of dairy cows, whereas the relative % mRNA abundance of *ACSS1* was 0.8% of the total genes measured (Table S5). *ACSS2* was up-regulated 5-fold in yak mammary tissue during lactation. *ACSS1*, which is primarily found in mitochondria, was also up-regulated to activate FA into acetyl-CoA (> 4% fold). The expression of *ACSL1*, *ACSS1*, and *ACSS2*, which are involved in FA activation in yak mammary tissue, coincided with that of dairy cow mammary tissues and was significant with time ($P < 0.05$ in all three genes, Table S1).

FABP is known to express FA-binding proteins for intracellular transport of LCFAs

and their acyl-CoA esters in various cell types. *FABP3* is the most abundant protein of *FABP* families in mammary tissue (Bionaz and Loor, 2008, Boehmer *et al*, 1987). In a recent study, *FABP3* up-regulated *SREBP* and *PPAR*, which are critical regulators of milk fat synthesis; oleic acid, stearic acid, and palmitic acid also increased lipid droplet accumulation by affecting the expression of *FABP3* in cow mammary epithelial cells (Liang *et al*., 2014). Additionally, some genes related to *FABP*, such as *ANXA9* and *FABP4*, in cows have SNPs and influence the low milk fat and high milk protein composition of milk (Kuliq *et al*., 2013). According to these results, *FABP3* plays an important role in not only the regulation in milk fat synthesis but also the transportation of milk fat for both LCFAs and SCFAs. Our results have shown that *FABP3* was the most abundant transcript (> 31%) among all measured transcripts (Table S4). In addition, *FABP3* was up-regulated 8-fold during lactation; this level was the highest expression level observed of all the investigated milk fat-related genes (Fig. S1). *FABP3* expression in mice does not increase during lactation (Michael *et al*., 2007) but increased 80-fold in dairy cows. Moreover, the relative % mRNA abundance of *FABP3* was shown to be 16% of the measured genes in lactating cow mammary tissue (Bionaz and Loor, 2008). *FABP3* also interacts with *ACSL1* and *CD36* proteins to esterize to LCFA-CoA. *FABP3*, *CD36*, and *ACSL1* were up-regulated with a similar expression pattern that was vigorously expressed during lactation, as these proteins interact to stimulate LCFAs uptake and transport from blood as mentioned earlier (r = 0.955 in *CD36* and 0.949 in *ACSL1*).

ABC family proteins present in mammary tissue play a key role in transporting cholesterol and metabolites such as vitamins during the lactation (Kessler *et al*., 2014). In previous results, the expression of *ABC* transporter family members, namely, *ABCA1*, *ABCA7*, *ABCG1*, *ABCG2*, and *ABCG5* was investigated in lactating and non-lactating cow mammary tissues (Farke *et al*., 2008). The findings demonstrate that *ABCA1* enhances expression during the dry period while *ABCA7* and *ABCG2* expression are significantly decreased during this period. In contrast, ABC transporters involved in lipid and cholesterol transport shows different mRNA expression between lactation and the dry period. We investigated the expression pattern of *ABCA1* and *ABCG2* in lactating yak. *ABCA1* is a transporter protein that plays a role in the directed movement of cholesterol into, out of or within a cell, or between cells in the mammary tissue. In our study, *ABCA1* was constantly expressed with time (P = 0.474) (Fig. S2), and the relative % mRNA abundance of *ABCA1* accounted for < 1% of the total genes measured. (Table S5) (Bionaz and Loor, 2008). In contrast, the expression of *ABCG2* in lactating yak significantly increased 6-fold with time (P = 0.003). *ABCG2* functions as a mediator of the transport of substance such as riboflavin and other nutrients into milk (Antonius *et al*., 2007). The relative % mRNA abundance of *ABCG2* was high at 4.8% of the total genes measured.

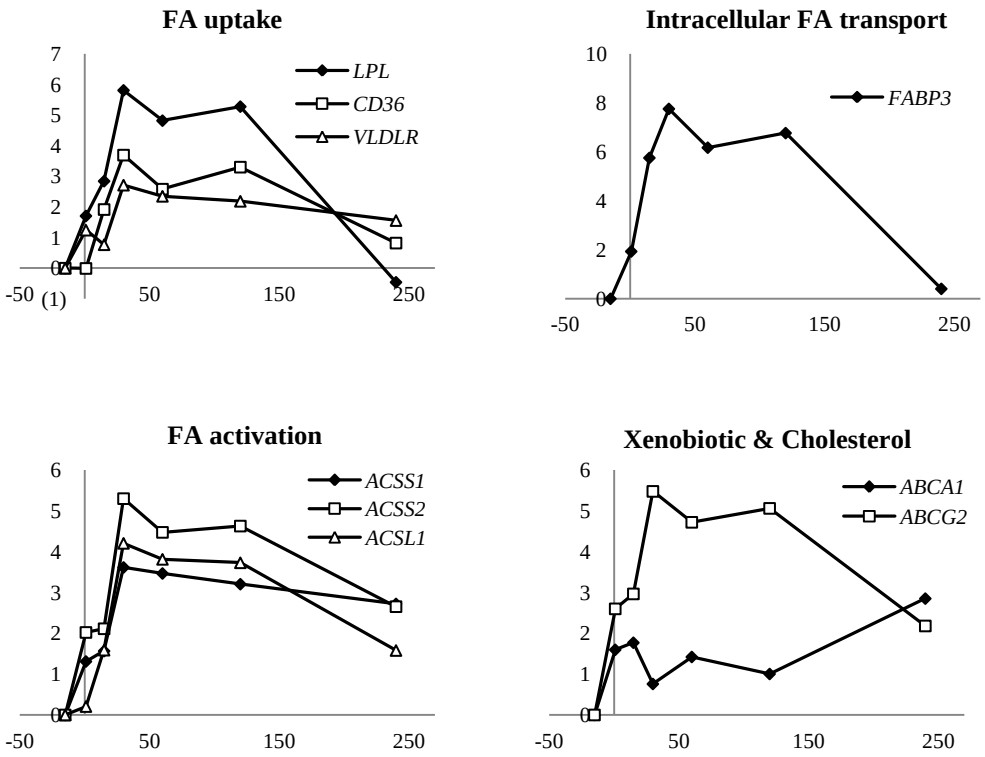


Fig. S1 Expression of genes involved in FA uptake, activation, intracellular trafficking, and xenobiotic and cholesterol transport. X-axis indicates the days relative to parturition. Y-axis indicates the relative expression levels calculated by $\log_2$ using the fold change relative to -15 d. Statistical value with time, $P < 0.05$ for all genes measured except *VLDLR* and *ABCA1*. SEM was calculated (Table S1).

198 ***De novo FA synthesis and desaturation for TAG synthesis***

199 To produce TAGs which are secreted into milk, SCFAs and palmitate are synthesized
200 from acetate and produced malonyl-CoA by *ACACA* (Mao *et al*, 2012). In our study,
201 *ACACA* had a 5-fold increase in expression by 30 d (Fig. S2), and the relative %
202 mRNA abundance of *ACACA* accounted for 6.7 % of the total genes measured during
203 lactation. The relative % mRNA abundance of *ACACA* accounted for 6.7% of the total
204 genes in yak measured during lactation. The *ACACA* relative % mRNA abundance in
205 yak was much greater than that of dairy cows (< 1 %, Bionaz and Loor, 2008)
206 (Table S5).

207

208 *SCD* activity in the mammary tissue is important for determining the relative
209 proportions of saturated and monounsaturated fatty acids in milk (Garnsworthy *et al*,
210 2010), and the primary role of *SCD* is to catalyze the insertion of a cis double bond at
211 the delta-9 position into fatty acyl-CoA substrates including palmitoyl-CoA and
212 stearoyl-CoA (Wang *et al*, 2005). In our study, positive expression of *SCD* increased
213 7-fold during early lactation (Fig. S2), and the relative % mRNA abundance of *SCD*
214 was 6.8% of all genes measured in yak mammary tissue (Table S5). This result is
215 significantly different from that of dairy cow, in which *SCD* had the highest increase
216 in the relative % mRNA abundance (increased up to 23%), although the patterns of
217 *SCD* expression were similar in yaks and dairy cows. We also surveyed *SCD* activity
218 by analyzing the 16:0 and 18:0 FA composition. Desaturated oleic acid (C18:1, n-9)
219 was compared to stearic acid (C18:0), and desaturated oleic acid composed

220 approximately 53% of total 18-carbon FA in the yak milk and increased with time
221 (P = 0.001, Fig. 1(c)). This result is less than 10% in dairy cows' milk (approximately
222 63% in bovine cow, Bionaz and Loor, 2008). Palmitic acid (16:0) content did not
223 change with time in yak (Fig. 1(c)). *SCD* had comparatively lower expression and
224 functional activities in lactating yaks than in dairy cow.
225 *FADS1* and *FADS2*, which are members of *FADS* gene family are related to the
226 desaturation of LCFA in delta-5 and delta-6, respectively (Rodriguez-Cruz *et al*., 2005;
227 Lattka *et al*., 2009). In yak mammary tissue, lactation only slightly affects *FADS1* and
228 *FADS2* expression with time (*FADS1* P = 0.949, *FADS2* P = 0.921). Relative *FADS1*
229 and *FADS2* % mRNA abundances in lactating yak measured below 1% of all
230 measured genes, similar to those in dairy cows.
231
232 ***Formation of TAG and milk lipid droplets***
233 Intracellular FAs can be incorporated to TAGs by sequential enzyme activities.
234 Glycerol 3-phosphate produced from a carbon source in mammary cells is
235 transformed to acyl-glycerol phosphate by *GPAM*, and synthesized acyl-glycerol
236 phosphate is added to acyl-CoA to produce diacyl-glycerol 3-phosphate by *AGPAT6*.
237 *LPIN1* catalyzes the conversion of phosphatidic acid to diacylglycerol during TAG
238 biosynthesis and can produced TAG by *DGAT* in l metabolism (KEGG,
239 http://www.genome.jp). These process can be accomplished sequentially to synthesize
240 TAGs for excretion of milk fat. According to a recent study, *AGPAT6* has an important
241 role in milk fat production. The genotype of high milk fat concentration is additively

associated with increased expression of *AGPAT6*, and the *AGPAT6* isoform plays key regulatory roles in TAG synthesis (Littlejohn, 2014). *LPIN1* is also known to be a transcription regulator that acts as an inducible transcriptional coactivator in conjunction with PGC-1a and PPARA (Reue and Zhang, 2008; Thering *et al*., 2009). In our study, the expression of *AGPAT6*, *GPAM* and *LPIN1* strongly correlated (r = 0.977, P = 0.001 between *AGPAT6* and *GPAM*, r = 0.989, P = 0.001 between *AGPAT6* and *LPIN1*) and their gene expression patterns nearly coincided in lactating yak mammary gland (Fig. S2). *GPAM*, *AGPAT6*, and *LPIN1* were largely up-regulated during lactation (P of all three genes < 0.05, Table S1), and their relative % mRNA abundances accounted for 18.3, 3.8, and 2.5%, respectively, of the total transcribed genes (Table S5). The yak *GPAM* gene, which is related to triacylglycerol synthesis, is highly expressed during lactation in contrast to the *GPAM* gene of bovine (Bionaz and Loor, 2008).

DGAT1 and *DGAT2* are responsible for the final step of TAG synthesis (Cases *et al*, 2001; Orland *et al*, 2005). In contrast to *DGAT1*, *DGAT2* essentially required for synthesis and storage of intracellular TAGs. A recent study revealed that a polymorphism of *DGAT1* influences the milk fat percentage and fatty acid profile in Holstein cattle (Tabaran *et al.*, 2015). The relative % mRNA abundances of *DGAT1* and *DGAT2* were very low (< 1 %, Table 1) and were only slightly up-regulated with time during lactation (P = 0.604 and 0.132, respectively, Fig. S2). *DGAT1* and *DGAT2* were not effective for TAG synthesis in lactating yak. *DGAT2*, which correlates well

with the enzyme genes *GPAM*, *AGPAT6*, and *LPIN1* of previous TAG synthesis steps (Supplementary File 2), directly involved to the final step of TAG synthesis in lactating yak mammary tissue. In contrast, *DGAT1* had a negative relationship with the expression of *GPAM*, *AGPAT6*, and *LPIN1* and was known that is not essential for life.

Synthesized TAG is incorporated into milk fat globules in the ER membrane, transported to the membrane, and finally released into milk. Three genes related to milk lipid droplet formation, namely, *BTN1A1*, *XDH*, and *PLIN2*, were investigated in this study. The expression patterns of these genes were similar and had strong positive correlations ($r > 0.8$, $P < 0.05$ in all correlations). All three genes were up-regulated 5-fold at 30 d in all samples (Fig. S2). Their relative % mRNA abundances compared to all measured genes were also similar (2.7% for *BTN1A1*; 2.4% for *XDH*; 2.5% for *PLIN2*). The expression patterns of these genes are proportionally less expressed compared to those of dairy cows (Table S5).

PLIN1 is located in the periphery of intracellular lipid droplets and coats lipid storage droplets to protect them from breakdown by hormone-sensitive lipase (Bionaz and Loor, 2008; Sun *et al*, 2013). However, our study demonstrated that *PLIN1* did not effectively change during lactation ($P = 0.497$, Fig. S2) and that the relative % mRNA abundance of *PLIN1* compared to all measured genes was low (0.11%, Table S5).

BDH1 and *OXCT1* are located in mitochondria and catalyze FA production using

286 β-hydroxybutyrate taken up from blood (Maurer *et al*. 2011). These ketone bodies
287 (i.e., acetone, acetoacetate, and β-hydroxybutyrate), as small molecules, can also be
288 used as minor energy sources through the TCA cycle, depending on the dietary
289 situation or precursor for milk FAs synthesis;

290 *BDH1* is assumed to catalyze β-hydroxybutyrate to acetoacetate, and then *OXCT1*
291 uses acetoacetate to produce acetoacetyl-CoA (Bionaz and Loor, 2008). We found that
292 *BDH1* and *OXCT1* were largely up-regulated to 5-fold and 2-fold, respectively, in
293 lactating yaks. In a recent study, *BDH1* was shown to have strong correlations
294 between *de novo* milk fat synthesis and milk yield in lactating buffalo mammary
295 epithelial cells.
296

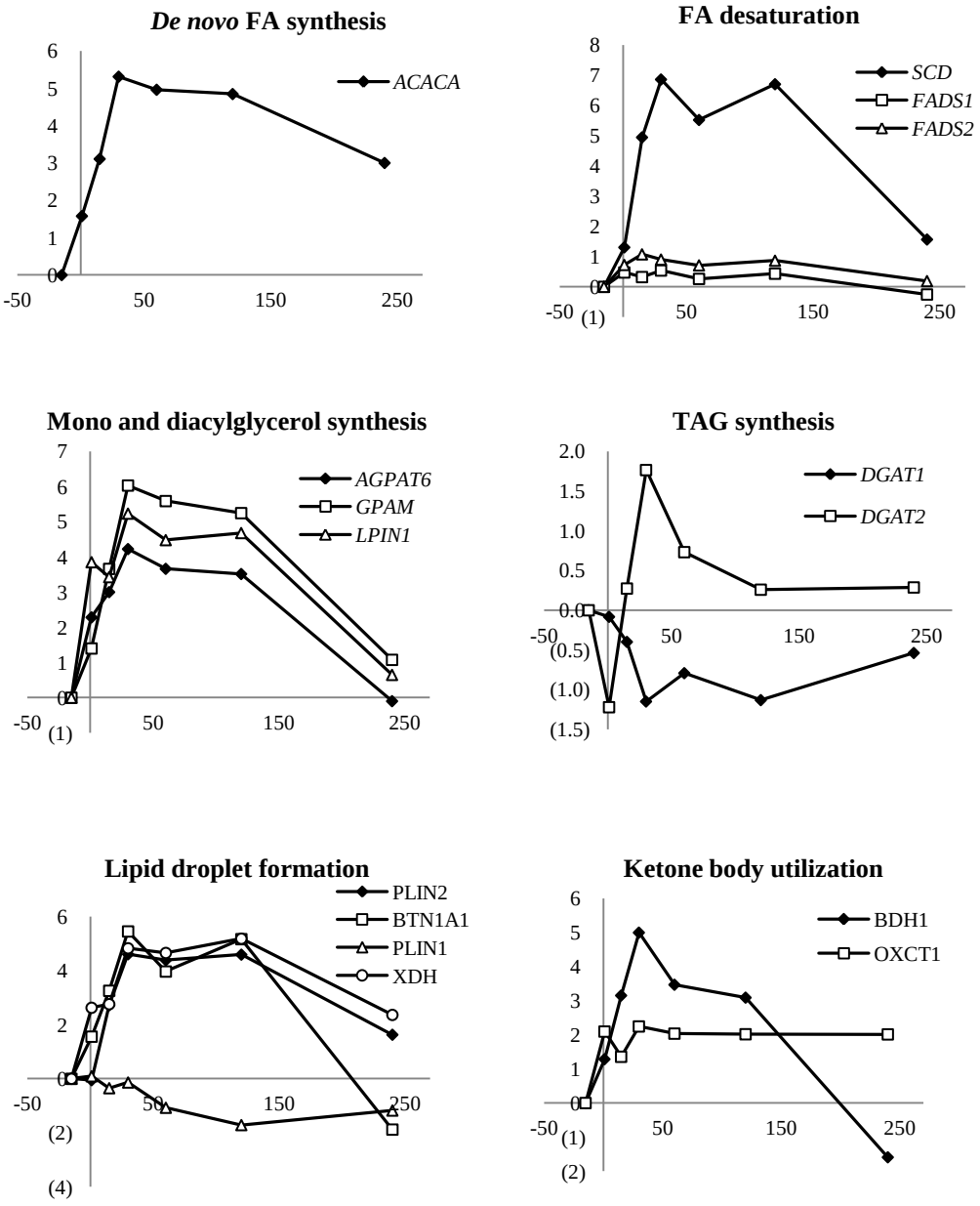


Fig. S2 Expression of genes involved in *de novo* FA synthesis, LCFA desaturation, TAG synthesis, lipid droplet formation, and BHBA utilization. X-axis indicates the days relative to parturition. Y-axis indicates the relative expression levels calculated by $\log_2$ using the fold change relative to -15d. Statistical effect with time, $P < 0.05$ for all genes investigated except for *FADS1*, *FADS2*, *DGAT1*, and *DGAT2*. SEM was calculated (Table S1).

Transcription factors and nuclear receptors during lactation

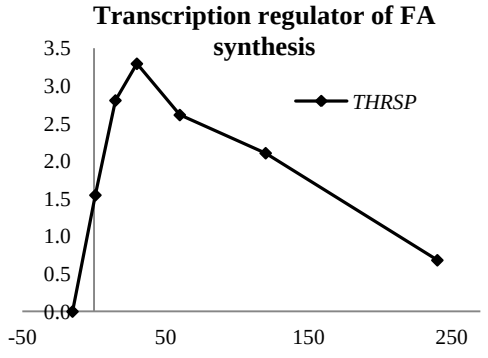
A large body of evidence supports the suggestion that *SREBP1* is pivotal in the regulation of milk fat synthesis in mice and cows (Horton *et al*., 2002; Andersen *et al*., 2007; Rudolph *et al*., 2007; Bionaz and Loor, 2008). *SREBF1* and *2* are retained as inactive precursors in the ER membrane and are transported to the Golgi for proteolytic cleavage by SCAP before entering the nucleus and participating in the activation of sterol responsive element-containing genes (e.g., *ACACA*, *FASN*, and others). The transport step to the Golgi is blocked by sterols via the sterol-sensing protein SCAP. SCAP is essential for the movement of *SREBP* isoforms from the ER to the Golgi, essentially acting as a gatekeeper for the movement of inactive *SREBP1* and *2*. *INSIG1* and *2* are translated to proteins that interact with *SCAP* in oxysterol-dependent and independent fashion and regulate the responsiveness of *SREBP1* and *2* processing via *SCAP*, thus altering rates of lipogenesis. If *INSIG* gene is not translated to protein in ER, bound SREBP protein is processed by SCAP, and can move to nucleus and make related milk fat proteins stimulate to transcribe (Yabe *et al*, 2002). In contrast to dairy cow, the expression of *INSIG 1* and *2* did not effectively change with time ($P > 0.4$ for *INSIG 1* and *2*) in lactating yaks. The relative % mRNA abundances of *INSIG 1* and *INSIG 2* were as low as 0.1 % of the total genes (Table S5).

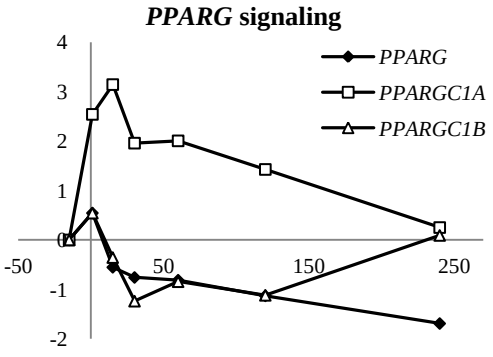
Thyroid hormone responsive spot 14 (*THRSP*, Spot, S14) is a nuclear protein that regulates of TAG biosynthesis process. Recently, *THRSP* expression was investigated in dairy cow mammary tissue. According to Cui *et al*.(2015), dairy cow milk high fat

correlated to high *THRSP* mRNA and protein expression levels. Bovine mammary epithelial cells with over-expressed *THRSP* displayed increased TAG levels and enhanced *PPARG* and *SREBP1* expression. Overall, increased mammary expression of *THRSP* can be used as a marker of high fat and has an effect on *PPARG* and *SREBP1*, which are regulators of milk fat synthesis. Our results revealed that *THRSP* was highly up- regulated to 30d during lactation in yak mammary tissues and decreased during the dry period (Fig. S3). The expression levels of other regulator genes (i.e., *INSIG1 and 2*, *PPARG*, and *PPARGC1B)* did not meaningfully change with time ($P > 0.05$, Table S1 and Fig. S3).

PPARG is located inside the nucleus and affects FA transport-related genes such as *LPL*, *CD36*, and *ACSL1* and lipid droplet formation genes. In yak mammary tissue, the relative % mRNA abundance of *PPARG* was 0.16% of the total genes measured (Table S5). Furthermore, *PPARG* expression did not change with time ($P = 0.826$, Fig. S3). The pattern of yak *PPARG* expression differed from the expression of *PPARG* in dairy cows, which was up-regulated during early parturition (Bionaz and Loor, 2008).

The relative % mRNA abundances of *PPARGC1a* and *PPARGC1b*, as co-activators of *PPARG*, were 0.37 and 0.09% of the total genes measured, respectively (Table S5). The expression of *PPARG1a* was meaningfully up-regulated 3-fold at 30 d ($P = 0.025$, Fig. S3). However, *PPARG1b* expression did not change with time, although it decreased during lactation; this expression pattern is also shown in bovine mammary tissue.





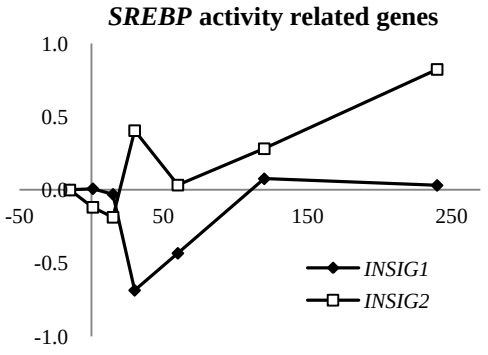


Fig. S3 Regulation of transcription during yak mammary milk fat synthesis. X-axis indicates the days relative to parturition. Y-axis indicates the relative expression level calculated by $\log_2$ using the fold change relative to -15 d. *SREBP1* and *2* expression was not investigated. *P* values of *THRSP* and *PPARGC1A* expression level were significant ($P < 0.05$) with time. SEM was calculated (Table S1).

Sphingolipid synthesis genes

Ceramide is a sphingolipid that is usually found in the cell membrane. This sphingolipid acts as intermediate in metabolic processes such as cell signaling, programmed cell death, and cell proliferation. Sphingolipids and cholesterol play pivotal roles in milk fat globule membrane formation. Mammary tissue *de novo* synthesizes sphingolipids from palmitoyl-CoA, leading to ceramide formation and incorporation into sphingomyelin in sphingolipid metabolism (KEGG, www.genome.jp).

According to a recent study, *SPTLC1, 2,* and *3* were investigated and found to play a critical roles in the synthesis of ceramide. The heterodimer formed with *SPTLC 1, 2,* or *3* constitutes the catalytic core. Their functions were verified by knocking down *SPTLC1, 2,* and *3*. *SPTLC1, 2,* and *3*. The silencing of *SPTLC1, 2,* and *3* strongly affected the expression of genes entirely involved in lipid metabolism (Wanida *et al*., 2012). Our study results showed that *SPTLC1* and *2* were up-regulated in lactating yak mammary tissue and that *SPTLC1* expression was significantly increased 3-fold (P = 0.016 in *SPTLC1* and P = 0.097 in *SPTLC2*). The relative % mRNA abundances of *SPTLC1* and *2* was 0.29% and 0.14% of the total genes measured, respectively.

CERS is a key enzyme in production of the intermediate of ceramide, dihydroceramide, and sequentially reacts to *SPTLC*. We found that *CERS2* and *SPTLC1 and 2* had positive correlations (r = 0.610, P = 0.145 with *SPTLC1* and r = 0.810, P = 0.027 with *SPTLC2*). *CERS2* was the most abundant gene related to sphingolipid synthesis (0.44% of the total genes measured, Table S5) and was up-

386 regulated 3-fold at 30 d (Fig. S4). *SPHK2* is an important enzyme that transforms
387 sphingomyelin to sphingomyelin-1-phospate. This reaction sequentially occurs after
388 the production of ceramide by *CERS*. *SPHK2* had a strong negative correlation with
389 *CERS2* (r = -0.868, P=0.011). *SPHK2* was down regulated while *CERS2* was highly
390 expressed in lactating yak mammary tissue. This pattern also appeared in dairy cow
391 mammary tissue and *SPHK* negatively interacts with *CERS2* (Bionaz and Loor, 2008).
392 OSBP is a protein that transports ceramide from the ER to the Golgi and that acts as a
393 sterol sensor function to integrate the cellular sterol status with sphingomyelin
394 metabolism. The relative % mRNA abundance of *OSBP*, *OSBPL2* and *OSBPL10* were
395 0.14, 0.15 and 0.06 %, respectively, of the total genes estimated (Table S5). In
396 addition, *OSBP* and *OSBPL2* relative % mRNA abundances were not significant with
397 time. *OSBPL10* transports ceremide from the ER to plasma membranes and plays a
398 role in negative regulation of lipid biosynthesis (Perttila *et al*, 2009).
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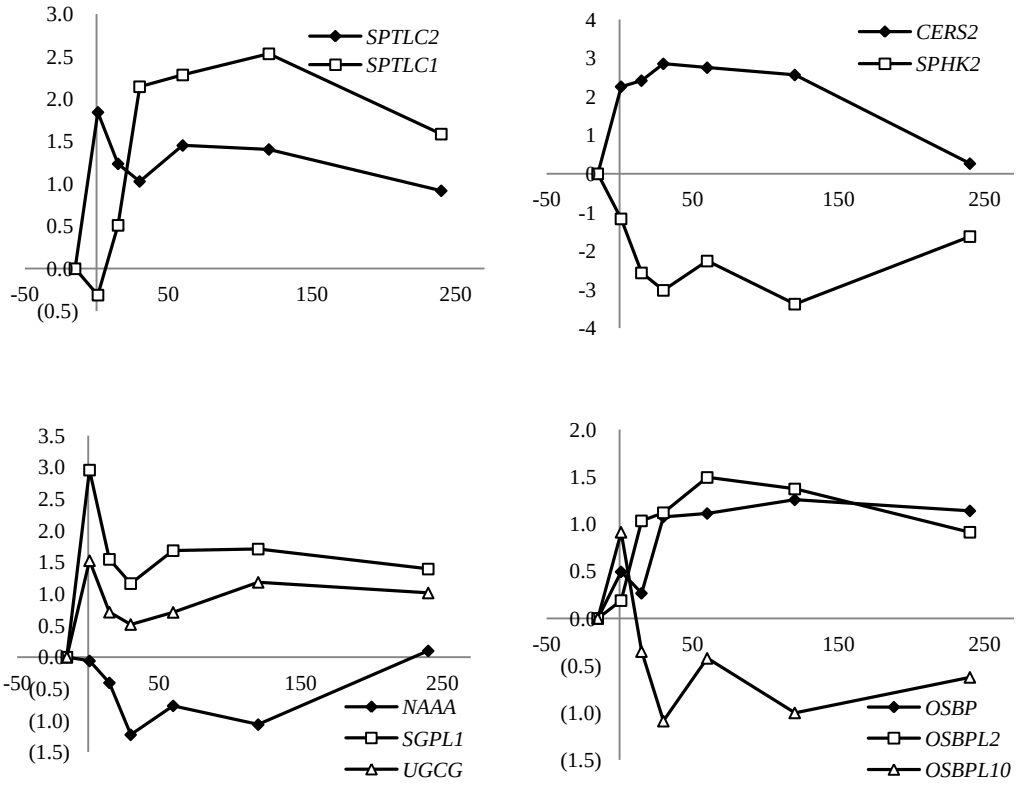

Fig. S4 Expression of genes involved in sphingolipid synthesis in yak mammary tissue. X-axis indicates the days relative to parturition. Y-axis indicates the relative expression levels calculated by $\log_2$ using the fold change relative to -15 d. *P* value of the timeline expression of *SPTLC1*, *SGPL1*, and *OSBPL10* was below 0.05. SEM was calculated (Table S1).

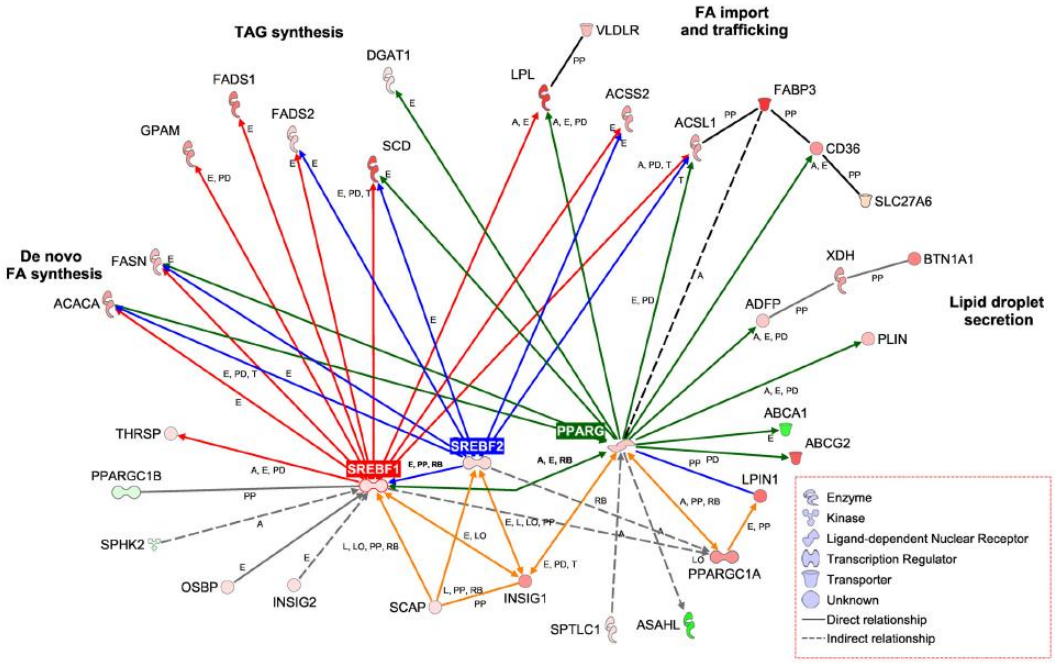

Fig. S5 Networks among genes involved in milk fat synthesis (Bionaz and Loor, 2008). Networks were developed with Ingenuity Pathway Analysis (Ingenuity Systems, http://www.ingenuity.com). Red, blue, and green edges denote genes whose transcription is under the control of *SREBF1*, *SREBF2*, and *PPARG*, respectively. Highlighted in orange is the network encompassing *PPARG*, *PPARGC1A*, *LPIN1*, *INSIG1*, and *SCAP* which controls expression function of SREBF proteins. Letters along the edges denote effects on activity (A), expression(E), localization(LO), proteolysis (L), RNA binding (RB), protein-DNA binding (PD), and protein-protein binding (PP). Genes are grouped based on their primary function during milk fat synthesis.

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