Examination of methionine stimulation of gene expression in dairy cow mammary epithelial cells using RNA-sequencing

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Supplementary File

Supplementary Table S1 Primer sequences for quantitative real-time PCR[1]

Gene	GenBank ID	Primer sequence (5'-3')	Product size (bp)
MCOLN2	NM_001192734.1	F: GGATCGTCTTAGGACCGTATC R: GTGAGCAAAGGTCGCATACA	107
JAZF1	NM_001206257.3	F: ACGAGGAGGAGGTGGATTA R: CTGAGGATGGCTTCAGAGC	85
RNF24	NM_001077052.2	F: TGCTGCTCCCTTTGTCTCA R: AGGCTCATCCATTCCTCTTG	122
LIFR	NM_001192263.3	F: TGGGTCAGACAGAATCAACA R: AATGCCAGGAAAGTATCACA	117
KLHL28	NM_001099030.1	F: GGAGCGTTACGATCCAAGTA R: CCATTATGTCCACCCACCAC	120
SLC12A5	NM_001206380.1	F: CGTATCACCGCAGAGGTCG R: AACGCTGCTCCATCACCAA	91
MAP6D1	NM_001076354.1	F: TGGCGACCACATCATACAGA R: GGTTGTGATGACTTTGGCG	95
PPIL6	NM_001046537.2	F: CAAGAATTTGCATGGCATCA R: GCATCACCCAGGAGCTTATC	116
NOSTRIN	NM_001046257.2	F: TGGACGAGAATAGCTTGAAAC R: TACTACAGGGATGGTTGGGT	108
SEC14L5	NM_001191259.3	F: CGAGGTCACCGTGGAGATTC R: CCTGCTTGGCATGGTACAGAC	101
TMC2	NM_001113766.2	F: ATGGCTAAGAAATGGGTCAA R: ATGCCACTGAAGAACCAAAG	115

FAM71A	NM_001099391.2	F: CTCCCACTGGACTTCTACAACC R: CCCTGCTGCTGACTCTTCAT	93
β-ACTIN	NM_173979.3	F: TGCGGCATTCACGAAACTAC R: ACAGCACCGTGTTGGCGTA	85

[1]*MCOLN2* = mucolipin 2; *JAZF1* = JAZF zinc finger 1; *RNF24* = ring finger protein 24; *LIFR* = LIF receptor alpha; *KLHL28* = kelch like family member 28; *SLC12A5* = solute carrier family 12 member 5; *MAP6D1* = MAP6 domain containing 1; *PPIL6* = peptidylprolyl isomerase like 6; *NOSTRIN* = nitric oxide synthase trafficking; *SEC14L5* = SEC14 like lipid binding 5; *TMC2* = transmembrane channel like 2; *FAM71A* = family with sequence similarity 71 member A; *β-ACTIN* = actin beta; F = forward; R = reverse.

Supplementary materials and methods

Immunofluorescence

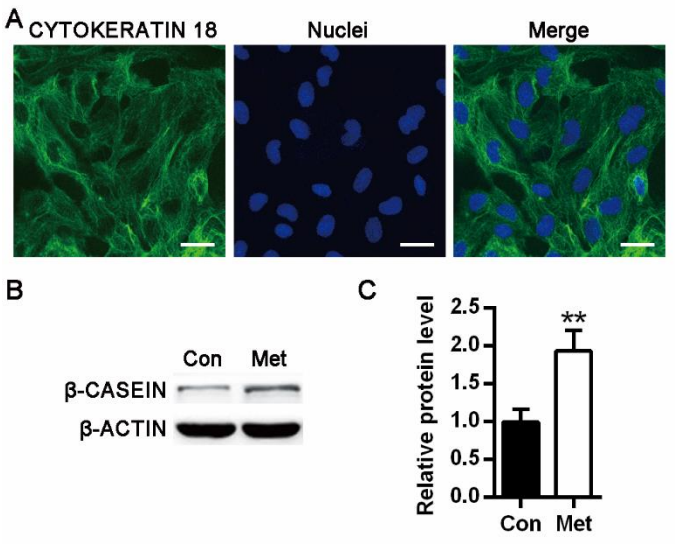
Boving mammary epithelial cells were fixed in ice-cold methanol for 10 min. Cells were blocked with 10% normal rabbit serum (ZSGB-BIO, Beijing, China) in PBS at room temperature for 1 h and then treated with CYTOKERATIN 18 antibody (1:200 dilution; Santa Cruz, CA) at 4℃ overnight. After three washes with PBS, cells were incubated with FITC-conjugated AffiniPure rabbit anti-Goat IgG (1:200 dilution; ZSGB-BIO) for 1 h at room temperature. The nuclei were stained with 4', 6-diamidino-2-phenylindole for 10 min. After three washes, the cells were mounted with DABCO (Sigma-Aldrich, St Louis, MO) and assessed by confocal microscopy (TCS SP2, Leica Microsystems, Wetzlar, GmbH, Germany).

Western blot analysis

To detect the expression of β-CASEIN, mammary epithelial cells were washed twice with PBS and lysed with RIPA buffer (Beyotime Biotechnology, Shanghai, China) containing 0.5mM PMSF (Beyotime Biotechnology) on ice. Total protein were separated by 10% SDS-PAGE and transferred onto nitrocellulose membranes. Western blot analysis was performed with different primary antibodies as follows: β-CASEIN (1:500 dilution; Bioss Antibodies, Beijing, China), β-ACTIN (1:1000 dilution; Cell Signaling Technology, Danvers, MA). After incubated with secondary antibodies, the protein bands were detected with a chemiluminescence system and analyzed on Image-Pro Plus 6.0. The expression level of β-CASEIN was normalized with β-ACTIN. The experiments were carried out in triplicate. Data are shown as the mean ± SEM and were analyzed using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). A two-tailed unpaired *t*-test was used to detect the effect of methionine on β-CASEIN expression. Significance was assigned at $P < 0.05$.

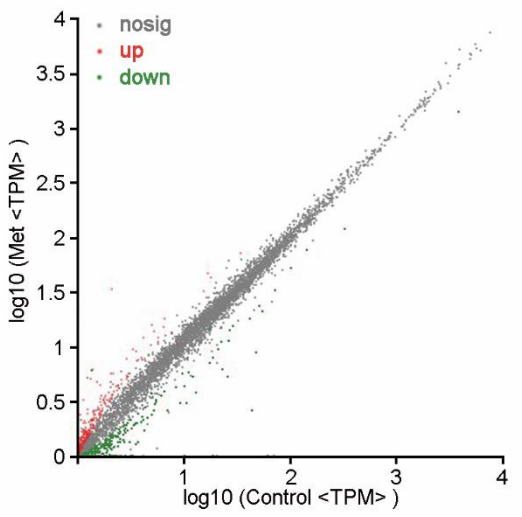
Supplementary Fig. S1. Establishment of a cell model with a high capacity for milk protein synthesis. (A) Immunofluorescence staining of CYTOKERATIN 18 (FITC) in bovine mammary epithelial cells. nuclei were stained with 4',6-diamidino-2-phenylindole (blue). The merged image shows the overlap between CYTOKERATIN 18 and nuclei. Scale bas = 30 μm. (B) Western blot analysis showing the levels of β-CASEIN and β-ACTIN (loading control) in methionine-stimulated cells (Met) and control cells (Con). (C) Quantification of β-CASEIN expression from the western blot in panel B. Results are shown as the mean ± SEM from three independent experiments. $^{**}P < 0.01$.

Supplementary Fig. S1.



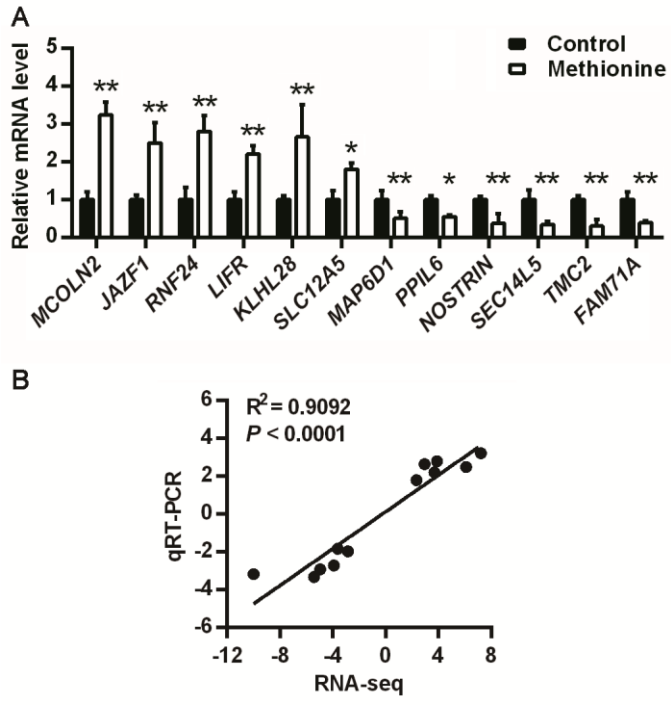
Supplementary Fig. S2. Comparison of DEGs between methionine-stimulated cells (Met) and control cells. Each library was normalized to the number of transcripts per million clean tags (TPM). Red dots represent upregulated transcripts. Green dots represent downregulated transcripts. Gray dots represent transcripts that did not change significantly.

Supplementary Fig. S2.



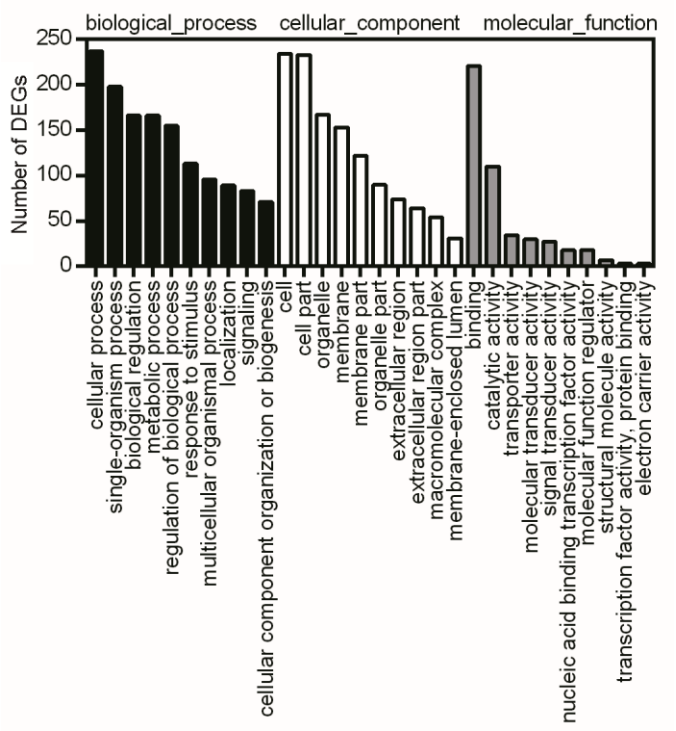
Supplementary Fig. S3. Validation of RNA-seq results by qRT-PCR. (A) qRT-PCR analysis of 12 randomly selected DEGs. The RNA was extracted from bovine mammary epithelial cells, and mRNA levels were measured by qRT-PCR and normalized to the level of *β-ACTIN* mRNA. Data are reported as the mean ± SEM from three independent experiments. **P* < 0.05, ***P* < 0.01. (B) Correlation between RNA-seq and qRT-PCR analysis.

Supplementary Fig. S3.



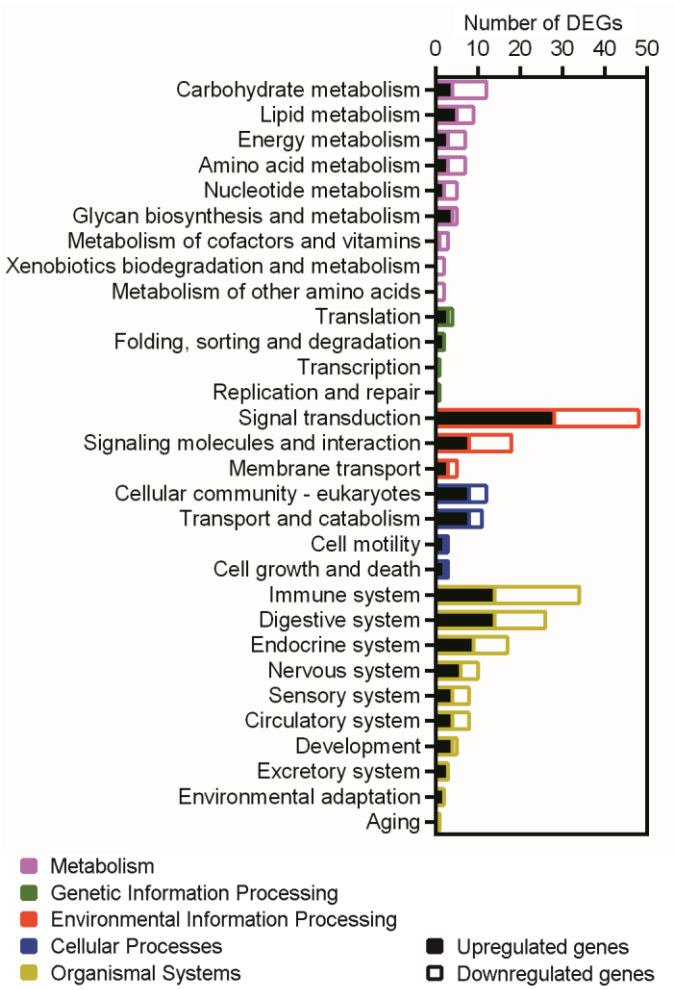
Supplementary Fig. S4. GO analysis of DEGs between the methionine stimulated bovine mammary epithelial cells and control cells. The DEGs are grouped into three main categories: biological process, cellular component, and molecular function. The *y* axis denotes the number of DEGs in a category.

Supplementary Fig. S4.



Supplementary Fig. S5. KEGG analysis of DEGs between methionine-stimulated cells and control cells. The *x* axis shows the number of DEGs within each term

Supplementary Fig. S5.


Number of DEGs
0
10
20
30
40
50
Carbohydrate metabolism
Lipid metabolism
Energy metabolism
Amino acid metabolism
Nucleotide metabolism
Glycan biosynthesis and metabolism
Metabolism of cofactors and vitamins
Xenobiotics biodegradation and metabolism
Metabolism of other amino acids
Translation
Folding, sorting and degradation
Transcription
Replication and repair
Signal transduction
Signaling molecules and interaction
Membrane transport
Cellular community - eukaryotes
Transport and catabolism
Cell motility
Cell growth and death
Immune system
Digestive system
Endocrine system
Nervous system
Sensory system
Circulatory system
Development
Excretory system
Environmental adaptation
Aging
Metabolism
Genetic Information Processing
Environmental Information Processing
Cellular Processes
Organismal Systems
Upregulated genes
Downregulated genes