

1 **Effect of *INSIG1* on the milk fat synthesis of buffalo mammary epithelial cells**

2

3 Xinyang Fan, Lihua Qiu, Xiaohong Teng Yongyun Zhang, and Yongwang Miao

4

5 SUPPLEMENTARY FILE

6

7

8

**Table S1.** Primer pairs used in PCR for amplification of CDS

Name of fragment	Sequence (5' to 3')	Annealing temperature (°C)
pLVX-INSIG1-CDS	ctcgagATGCCCAGACTGGACGACCA ggatccATCACTGTGTGGCTTTTCGG	55
pEGFP-INSIG1-CDS	ctcgagATGCCCAGACTGGACGACCA aagcttATCACTGTGTGGCTTTTCGG	55

9

The lowercase letters indicates the restriction sites

10

11

**Table S2.** Oligonucleotide sequences of shRNAs for buffalo INSIG1

Name of shRNA	Sequence (5' to 3')
shRNA103	ccggGCCAATAACGTTTCAGCTCTCCCTCGAGGGAGAGCTGAACGTTATTGGCTTTTTG aattcAAAAAGCCAATAACGTTTCAGCTCTCCCTCGAGGGAGAGCTGAACGTTATTGGC
shRNA547	ccggGGTGTACAACGGAGTCTATCACTCGAGTGATAGACTCCGTTGTACACCTTTTTG aattcAAAAAGGTGTACAACGGAGTCTATCACTCGAGTGATAGACTCCGTTGTACACC
shRNA681	ccggGGGAAACATAGGACGACAGCTCTCGAGAGCTGTCGTCCTATGTTTCCCTTTTTG aattcAAAAAGGGAAACATAGGACGACAGCTCTCGAGAGCTGTCGTCCTATGTTTCCC

12

Three shRNAs (numbers stand for their position in CDS) were designed, and each shRNA was added with restriction sites *EcoRI* and *AgeI* (lowercase letters).

13

14

15

**Table S3.** Primers used for RT-qPCR.

Gene (Accession number)	Primers (5' to 3')	Product length (bp)
<i>INSIG1</i> (JX853922)	F: ACGTTCAGCTCTCCTTGACATT R: CTGTCGTCCTATGTTTCCCAC	239
<i>INSIG2</i> (JX534502)	F: ATTGACAGGCATCTAGGA R: AGCCAGTGTGAGAGACAAC	217
<i>SREBP</i> (NM_001113302)	F: GCACCGAGGCCAAGTTGAATAA R: CAGGTCCTTCAGCGATTTGCTT	146
<i>PPARG</i> (XM_006077448)	F: GCTCCAAGAGTACCAAAGTG R: GTCCTCCTGAAGAAACCCTT	204
<i>SCD</i> (NM_001290915)	F: CGTGCCGTGGTATCTGTGG R: AAAGGTGTGGTGGTAGTTGTGG	217
<i>GPAM</i> (XM_006043939)	F: CTACGGATGTGTCAGAGTGGAT R: CACTGGGTCTTGAGGGAAGTATAG	141
<i>DGAT2</i> (XM_006045187)	F: GTCCTGTCTTTCCTCGTGCT R: CCTCCTGCCACCTTTCTT	141
<i>AGPAT6</i> (NM_001290846)	F: AACCTGCATCAATAATACATCA R: GGTAGGTCACCATTCGGTA	242
<i>ACTB</i> (NM_001290932)	F: TCTTGGTCACCTCGTCGTC R: GGC GCGATGATCTTGAT	196

16

Annealing temperature for all primers in this table is 55 °C.

17

18 **Supplementary Figure legends:**

19

20 **Figure S1:**

21 The full image of the western blot described in figure 1.

22

23 **Figure S2:**

24 Photomicrographs of the process obtained buffalo epithelial cells and morphology of epithelial  
25 cells. A. Fibroblast cells grew from mammary tissue; B. symbiosis of fibroblast and epithelial  
26 cells; C. epithelial cells form island-like structures; D. epithelial cells form cobblestone-like  
27 structure.

28

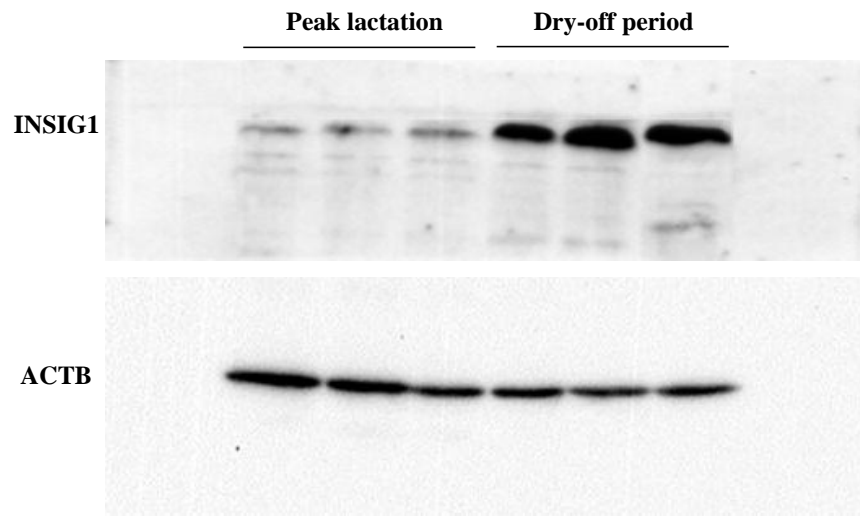
29 **Figure S3:**

30 Images of buffalo mammary epithelial cells incubated with anti-cytokeratin 18  
31 monoclonal antibody (A×200, B×400).

32

33 **Figure S1:**

34

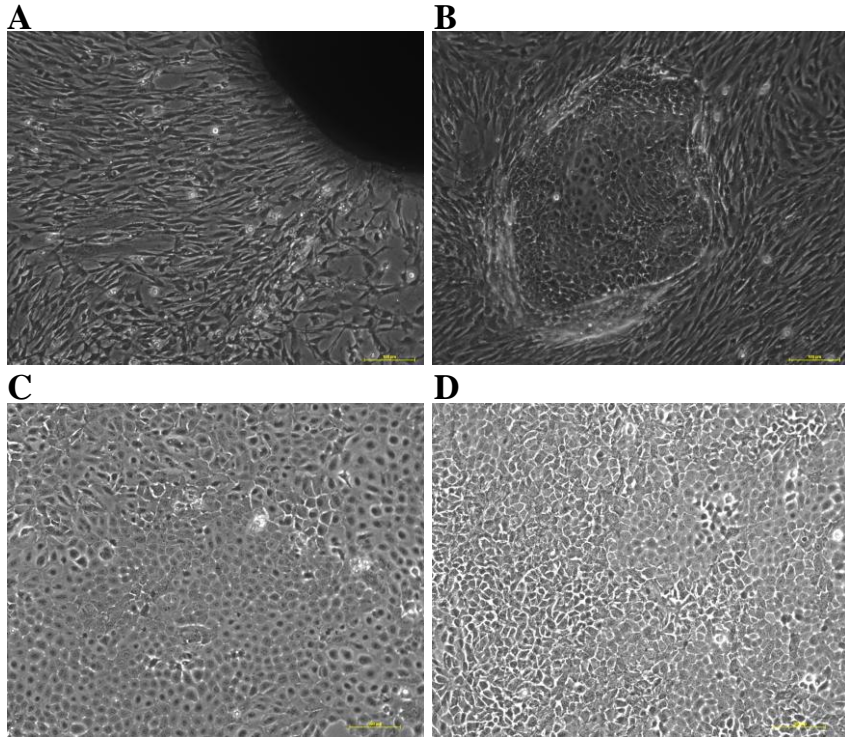


35

36 **Figure S2:**

37

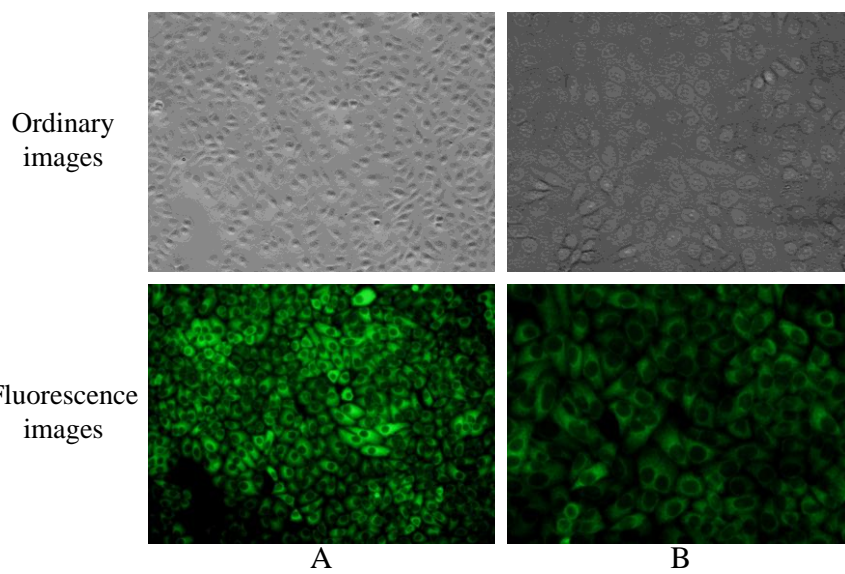
38



39

40

41 **Figure S3:**



42