



Article

# CRISPR/Cas9-Mediated Knockout of miR-130b Affects Mono- and Polyunsaturated Fatty Acid Content via PPARG-PGC1 $\alpha$ Axis in Goat Mammary Epithelial Cells

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**Table S1.** The siRNA Sequences of *PGC1α* and *PPARG*.

Name	Sense sequence (5'-3')	Antisense sequence (3'-5')
siNC	ACUACCGUUGUUAUAGGUGTT	CACCUAUAACAACGGUAGUTT
siPGC1α	GCCAACACUCAGCUAAGUUTT	AACUUAGCUGAGUGUUGGCTT
siPPARG-195	CCCGAUGGUUGCAGAUUAUTT	AUAAUCUGCAACCAUCGGGTT
siPPARG-980	GCGUGCACGAGAUCAUUUATT	UAAAUGAUCUCGUGCACGCTT
siPPARG-1164	CGGCUUAGCAAUAUUUAUATT	UAUAAAUAUUGCUAAGCCGTT

**Table S2.** Primers Used for sgRNA-induced 11 Off-Target Sites Amplification.

sgRNA	Off-target site	Primer sequence (5' to 3')	Size (bp)
sgRNA1	OT1	F: GGTGGTGGTTTCGGCTTGCTT R: AACTGAGGTCTGCCTTCTTCCTTCC	771
	OT2	F: TCAGGGCTCCGTGCTAAAGAGG R: TTCACCGAAGAGTTTGTGGACAGTAAAT	828
	OT3	F: CTCAACTATCTCGCCACTGCCCAACT R: TGCCACGCAGCGTAGCCAAAA	592
	OT4	F: TGGCAAGTGGGACAAGAGGACA R: CACATGCGGCTATTTGGAAAGAAA	510
	OT5	F: GTGGCAAGAAGCCGAGGGCAAAC R: CCGAGATCCCAACCAAACTATGACAGG	423
	OT6	F: CATGTGCGTGTATCTAATTTCTATC R: ATGACAACAGCAGCCATCAGTC	892
sgRNA2	OT1	F: ATCCCGTCTGCCTGTCACCCATCC R: CCGACTTCTCCTCAGTCCGGCTTCAC	897
	OT2	F: GGGAAAGATTGAGGATGGGAGG R: GCGAAGTTGCTGTATTTGCCGTAT	853
	OT3	F: AGCTGATGGCATGAATGGGTCG R: CCTGACGAGGAGGTTCCACAGAAA	935
	OT4	F: CCCTTATCTTCTGTGGCATAAACTCC R: TTCTGCTACAAACCCGACTCCC	703
	OT5	F: GCGGGCTGTAGTCCAAGAAG R: AGCTGAGCGCCGAAGAATT	716

**Table S3.** Primers for RT-qPCR of miRNAs by S-Poly(T) Plus Method.

miRNA	Primer sequence (5' to 3')
miR-130b-3p	Reverse: GTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTT
	ATGCCC Forward: TTCGGCAGTGCAATGATGAAA
miR-130b-5p	Reverse: GTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTT
	AGTAGT Forward: CGGACTCTTCCCTGTTGC
miR-130a-3p	Reverse: GTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTT
	GCCCTT Forward: TGTGGCAGTGCAATGTATAA
miR-301b-3p	Reverse: GTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTT
	GCTTTG Forward: TCGGCAGTGCAATGATATTGT
18S rRNA	Reverse: GTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATGCAGTGATG
	GCAAAGG Forward: CAGCACATCTTGCGAGTACTC

Universal reverse  
primer

CAGTGCAGGGTCCGAGGT

**Table S4.** Characteristics of Primers for RT-qPCR of mRNA.

NCBI accession no.	Gene	Primer sequence (5' to 3')	Size (bp)
JN236219.1	<i>ACACA</i>	F: CTCCAACCTCAACCACTACGG R: GGGGAATCACAGAAGCAGCC	171
NM_001035289.2	<i>ACOX1</i>	F: CGAGTTCATTCTCAACAGTCCT R: GCATCTTCAAGTAGCCATTATCC	211
BC119914	<i>ACSL1</i>	F: GTGGGCTCCTTTGAAGAACTGT R: ATAGATGCCTTTGACCTGTTCAAAT	120
BC134532	<i>ACSS2</i>	F: GGCGAATGCCTCTACTGCTT R: GGCCAATCTTTTCTCTAATCTGCTT	100
HQ846827	<i>ADFP</i>	F: TACGATGATACAGATGAATCCAC R: CAGCATTGCGAAGCACAGAGT	202
NM_00108366.1	<i>AGPAT6</i>	F: AAGCAAGTTGCCCATCCTCA R: AAAGTGTGGCTCCAATTTTCA	101
NM_001285739.1	<i>ATGL</i>	F: GGAGCTTATCCAGGCCAATG R: TGCGGGCAGATGTCACTCT	180
X91503	<i>CD36</i>	F: GTACAGATGCAGCCTCATTTC R: TGGACCTGCAAATATCAGAGGA	81
FJ415874.1	<i>CPT1A</i>	F: AAGGACCTCTACGCCAACACG R: TTTGCGGTGGACGATGGAG	267
DQ380249.1	<i>DGAT1</i>	F: CCACTGGGACCTGAGGTGTC R: GCATCACCACACACCAATTCA	101
NM_001046597.1	<i>ELOVL5</i>	F: GTTCTGTGAGTTAGTGACGGGAGT R: GTGGTTGTTCTTGCAGGAGAT	175
NM_001046597.1	<i>ELOVL6</i>	F: GGAAGCCTTTAGTGCTCTGGTC R: ATTGTATCTCCTAGTTCGGGTGC	205
NM_174313.2	<i>FABP3</i>	F: GATGAGACCACGGCAGATG R: GTCAACTATTTCCCGCACAAAG	120
EE347846	<i>FADS1</i>	F: GGTGGACTTGGCCTGGATG R: TGACCATGAAGACAAGCCCC	101
DV895683	<i>FADS2</i>	F: GCCTGTAGGCTCAGATGTTTGTTT R: TGCCTGGCAGTAACAGAGCAC	101
DQ915966.3	<i>FASN</i>	F: GGGCTCCACCACCGTGTTC R: GCTCTGCTGGGCTGCAGCTG	226
AY515690	<i>GPAM</i>	F: ATTGACCCTTGGCACGATAG R: AACAGCACCTTCCACAAAG	188
EU273879	<i>HSL</i>	F: GGGAGCACTACAAACGCAACG R: TGAATGATCCGCTCAAAGTCG	118
NM_001286088.1	<i>INSIG1</i>	F: AGCCTCACAAAGTTCAAGCG R: ACAGTGCTGCTAATGTCAAGG	132
NM_001285738	<i>INSIG2</i>	F: ATACCCCTGCATTGACAGACAT R: ACCAAGGCCAAAACCACTTC	109
GU332719	<i>LXRA</i>	F: CATCAACCCCATCTTCGAGTT R: CAGGGCCTCCACATATGTGT	163
XM_005681211.1	<i>PPARA</i>	F: CGGTGTCCACGCATGTGA R: TCAGCCGAATCGTTCTCCTAAA	56
HQ589347.1	<i>PPARG</i>	F: CCTTACCACCGTTGACTTCT R: GATACAGGCTCCACTTTGATTGC	145
DT860044	<i>RPS9</i>	F: CCTCGACCAAGAGCTGAAG R: CCTCCAGACCTCACGTTTGTTT	64
GU947654	<i>SCD1</i>	F: CCATCGCCTGTGGAGTCAC R: GTCGGATAAATCTAGCGTAGCA	257
HM443643.1	<i>SREBP1a</i>	F: CTGCTGACCGACATAGAAGACAT R: GTAGGGCGGGTCAAACAGG	81
HM443643.1	<i>SREBP1c</i>	F: CTGCTGACCGACATAGAAGACAT	81

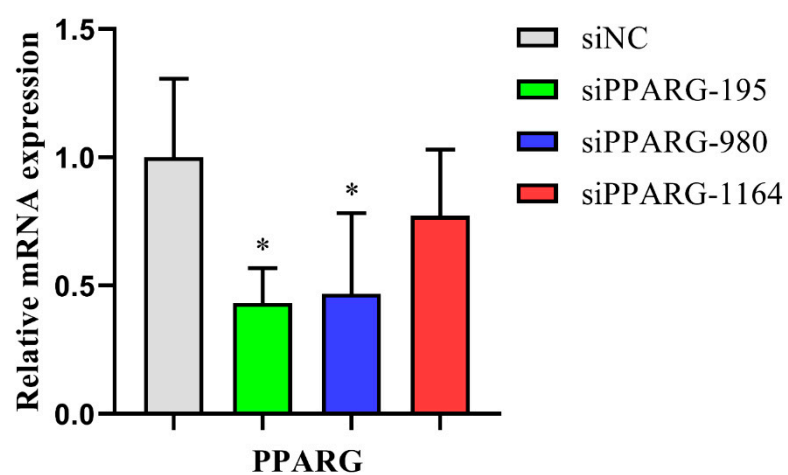
NC_030812.1	<i>SREBP2</i>	R: GTAGGGCGGGTCAAACAGG F: CGGCGTGATCGTGCTGAGCGTC R: CCGACAGGCAGGTTTGCAGGTTGG	173
HQ846826	<i>TIP47</i>	F: GGTGGAGGGTCAGGAGAAA R: TCACGGAACATGGCGAGT	123
NM_001037471	<i>UXT</i>	F: CAGCTGGCCAAATACCTTCAA R: GTGTCTGGGACCACTGTGTCAA	125
BC102076	<i>XDH</i>	F: GATCATCCACTTTTCTGCCAATG R: CCTCGTCTTGGTGCTTCCAA	100

*ACACA*, acetyl-coenzyme A carboxylase alpha; *ACOX1*, Acyl coenzyme A oxidase 1; *ACSL1*, acyl-CoA synthetase long-chain family member 1; *ACSS2*, Acyl-CoA Synthetase short-chain family member 2; *ADFP*, Adipose differentiation-related protein; *AGPAT6*, 1-acylglycerol-3-phosphate O-acyltransferase 6; *ATGL*, Adipose triglyceride lipase; *CD36*, Thrombospondin receptor; *C/EBPβ*, CCAAT enhancer binding protein beta; *CPT1A*, Carnitine palmitoyltransferase 1A; *DGAT1*, diacylglycerol acyl transferase 1; *DGAT2*, diacylglycerol acyl transferase 2; *ELOVL6*, elongase of very long chain fatty acids 6; *FADS1*, fatty acid delta-6-desaturase 1; *FADS2*, fatty acid delta-6-desaturase 2; *FASN*, fatty acid synthase; *GPAM*, Glycerol-3-phosphate acyltransferase; *HSL*, Hormone-sensitive lipase; *INSIG1*, Insulin induced protein 1; *INSIG2*, Insulin induced protein 2; *PPARA*, peroxisome proliferator-activated receptor α; *RPS9*, ribosomal protein S9; *SCAP*, SREBP cleavage activating protein; *SCD1*, stearoyl-CoA desaturase 1; *SREBP1a*, Sterol regulatory element-binding transcription protein 1a; *SREBP1c*, Sterol regulatory element-binding transcription protein 1c; *SREBP2*, Sterol regulatory element-binding transcription protein 2; *TIP47*, Tail-interacting protein 47; *UXT*, ubiquitously expressed transcript; *XDH*, Xanthine dehydrogenase.

**Table S5.** Effects of *PGC1α* Silencing on Fatty Acid Composition in miR-130b Knockout GMEC.

Fatty acid (%)	Control+siNC	Control+siPGC1α	miR-130b KO+siNC	miR-130b KO+siPGC1α
C14:0	3.72±0.10 <sup>a</sup>	3.00±0.20 <sup>b</sup>	2.98±0.10 <sup>b</sup>	2.78±0.21 <sup>b</sup>
C15:0	0.77±0.02 <sup>a</sup>	0.78±0.05 <sup>a</sup>	0.70±0.00 <sup>b</sup>	0.73±0.03 <sup>ab</sup>
C16:0	48.36±0.47 <sup>a</sup>	48.01±0.32 <sup>ab</sup>	47.47±0.31 <sup>b</sup>	47.83±0.08 <sup>ab</sup>
C16:1	1.92±0.03 <sup>a</sup>	1.98±0.08 <sup>a</sup>	1.57±0.02 <sup>c</sup>	1.67±0.02 <sup>b</sup>
C17:0	1.20±0.01 <sup>c</sup>	1.31±0.02 <sup>a</sup>	1.25±0.03 <sup>b</sup>	1.33±0.03 <sup>a</sup>
C17:1	0.47±0.01 <sup>a</sup>	0.46±0.01 <sup>ab</sup>	0.43±0.01 <sup>b</sup>	0.44±0.02 <sup>ab</sup>
C18:0	23.68±0.31 <sup>b</sup>	24.30±0.66 <sup>b</sup>	25.69±0.16 <sup>a</sup>	25.11±0.13 <sup>a</sup>
C18:1	13.23±0.17 <sup>ab</sup>	13.39±0.13 <sup>a</sup>	13.19±0.17 <sup>ab</sup>	12.98±0.24 <sup>b</sup>
C18:2	1.78±0.01 <sup>ab</sup>	1.80±0.05 <sup>a</sup>	1.72±0.05 <sup>b</sup>	1.81±0.03 <sup>a</sup>
C20:0	0.30±0.02 <sup>b</sup>	0.30±0.02 <sup>b</sup>	0.34±0.01 <sup>a</sup>	0.32±0.01 <sup>ab</sup>
C20:3	0.65±0.00 <sup>b</sup>	0.64±0.01 <sup>b</sup>	0.76±0.01 <sup>a</sup>	0.77±0.02 <sup>a</sup>
C20:4	2.47±0.10	2.47±0.12	2.43±0.09	2.62±0.03
C20:5	0.80±0.02 <sup>b</sup>	0.98±0.07 <sup>a</sup>	0.77±0.02 <sup>b</sup>	0.93±0.02 <sup>a</sup>
C22:6	0.65±0.01 <sup>b</sup>	0.59±0.02 <sup>c</sup>	0.69±0.00 <sup>a</sup>	0.67±0.02 <sup>ab</sup>
SFA*	78.03	77.69	78.44	78.10
MUFA**	15.62	15.83	15.19	15.10
PUFA***	6.35	6.48	6.37	6.80

Statistical significance is as follows: lower case letters,  $P < 0.05$ . Data are presented as mean ± SEM.



**Figure S1.** Verification of interference efficiency of siPPARG.