

***Sirt1* AS lncRNA interacts with its mRNA to inhibit muscle formation by attenuating function of**

**miR-34a**

Guo-qiang Wang #, Yu Wang #, Yan Xiong #, Xiao-Chang Chen, Mei-ling Ma, Rui Cai, Yun Gao, Yun-mei Sun, Gong-She Yang & Wei-Jun Pang \*

Laboratory of Animal Fat Deposition & Muscle Development, College of Animal Science and Technology,  
Northwest A&F University, Yangling Shaanxi 712100, China

**Running Title:** *Sirt1* AS lncRNA inhibits muscle formation

#These authors contributed equally to this work

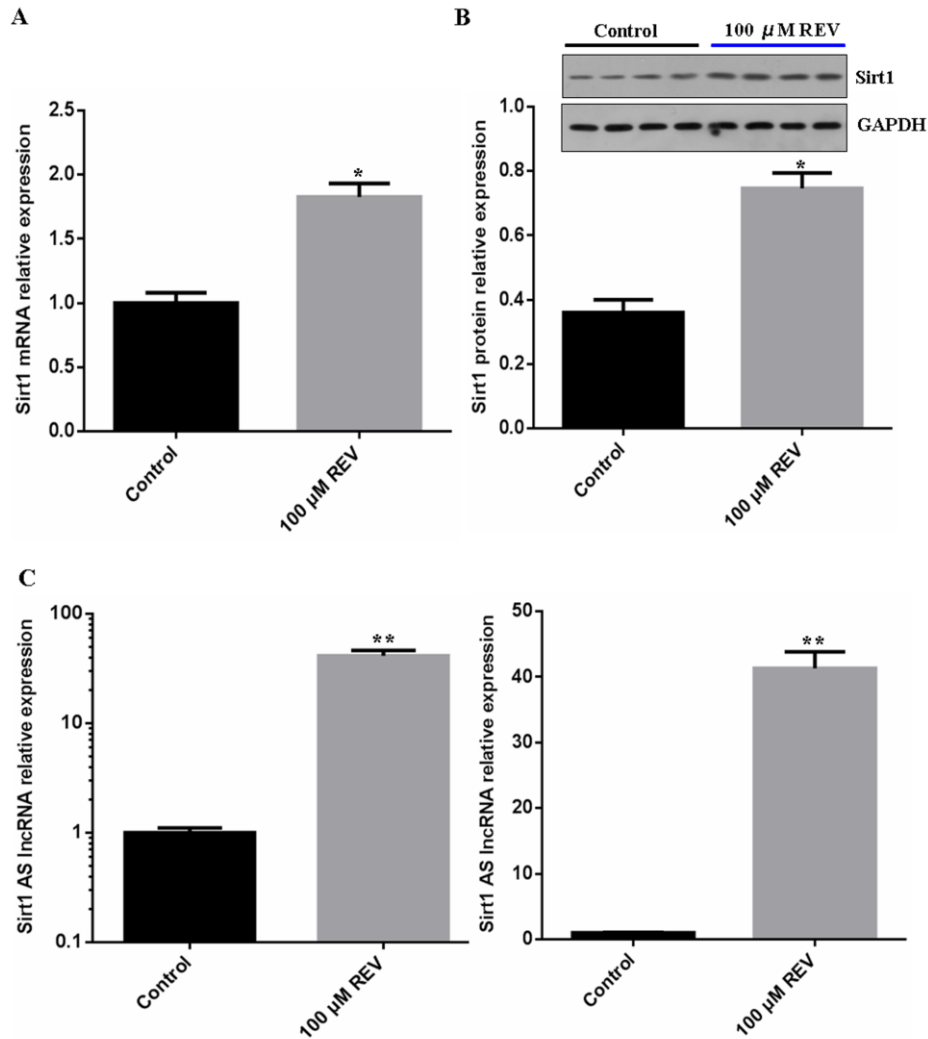
\* **Correspondence:** Wei-Jun Pang Ph.D.

**Address:** No. 22 Xinong Road, Yangling, Shaanxi Province 712100, China

**Tel:** 86-29-87091017

**Fax:** 86-29-87092430

**E-mail:** pwj1226@nwsuaf.edu.cn



**Figure S1. Resveratrol upregulates *Sirt1AS* lncRNA in myoblasts.** C2C12 myoblasts were induced with 100μM resveratrol (REV; Sigma) according to the manufacturer's instructions when the cells reached 50-60% confluence after seeding in 6-well culture plates for 12 hours. Forty-eight hours post-induction, the cells were harvested and total RNA and protein were extracted for further strand-specific RT-PCR and western blot analysis. **(A)** Expression of *Sirt1* mRNA. **(B)** Expression of Sirt1 protein. **(C)** Expression of *Sirt1AS* lncRNA. The different histogram using log (left) and non-log (right) analysis in GraphPad Prism version 6. The data were presented as means  $\pm$ SEM of 4 to 6 independent experiments. \* $P < 0.05$  and \*\* $P < 0.01$ .

**Table S1.** Nucleotide sequences of the primers used for real-time quantitative PCR.

Genes	Direction	Primer sequence
<i>Sirt1</i> AS	forward	5'-AATCCAGTCATTAACGGTCTACAA-3'
	reverse	5'-TAGGACCATTACTGCCAGAGG A-3'
miR-34a	forward	5'-CACGCATGGCAGTGTCTTAGC-3'
	reverse	5'-CAGTGCAGGGTCCGAGGTA-3'
<i>CyclinB</i>	forward	5'-AACTTCAGCCTGGGTCG-3'
	reverse	5'-CAGGGAGTCTTCACTGTAGGA-3'
<i>CyclinD</i>	forward	5'-TAGGCCCTCAGCCTCACTC-3'
	reverse	5'-CCACCCCTGGGATAAAGCAC-3'
<i>CyclinE</i>	forward	5'-CAGAGCAGCGAGCAGGAGC-3'
	reverse	5'-GCAGCTGCTTCCACACCACT-3'
<i>MyoD</i>	forward	5'-CGGCTCTCTCTGCTCCTTTG-3'
	reverse	5'-GTCGAAACACGGGTCATCA-3'
<i>MyoG</i>	forward	5'-GACCCTACAGACGCCACAA-3'
	reverse	5'-CCGTGATGCTGTCCACGAT-3'
<i>MHC</i>	forward	5'-CGCAAGAATGTTCTCAGGCT-3'
	reverse	5'-GCCAGGTTGACATTGGATTG-3'
<i>GAPDH</i>	forward	5'-TGCTGAGTATGTCGTGGAGTCT-3'
	reverse	5'-ATGCATTGCTGACAATCTTGAG-3'

**Table S2.** The primers used to construct plasmids for luciferase reporter assay.

Genes	Direction	Primer sequence
<i>Sirt1</i> AS	forward	5'-CGGGGTACCTATGCTATGAACAATGGAAG-3'
	reverse	5'-CCGCTCGAGTTGCCTGTTGAGGATTTGGT-3'
miR-34a	forward	5'-CGGGGTACCCTGCTGTACCCTGCTGCTT-3'
	reverse	5'-CCCAAGCTTTGGGCGTTGCTGACCTCT-3'
<i>Sirt1</i> 3' UTR	forward	5'-CCGCTCGAG TTGCCTGTTGAGGATTTGGT-3'
	reverse	5'-ATTTGCGGCCGCTATGCTATGAACAATGGAAG-3'

**Table S3.** Sequence of oligonucleotides used for RPA.

Genes	Direction	Primer sequence	Product length, bp
<i>Sirt1</i>	P1	5'-CTAGGTGGTGAATATGCCAAAC-3'	130
	P2	5'-TCCGAAATATGAAGAGGTGTTG-3'	
	P3	5'-GGTAGAGCCTGCATAGATCTT-3'	178
	P4	5'-ATTTATCTTATGACTTTGGGACTT-3'	
<i><math>\beta</math>-actin</i>	forward	5'-TGCTGTCCCTGTATGCCTCTG-3'	223
	reverse	5'-TTGATGTCACGCACGATTTC-3'	