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**Supplemental information**

**Circular RNA circMYBPC1 promotes skeletal  
muscle differentiation by targeting MyHC**

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**Text S1. Sequence of circMYBPC1.**

**circMYBPC1**

ATACATCTTTGAACATAAAGGATGTGAAAGAATCATGTTTATCAATAACTGTGTACT  
AACAGATGATTCGGAGTATTATGTGACAGCTGGTGATGAGAAATGTTCCACTGAGC  
TCTTTGTGAGAGAGCCTCCAGTTATGGTGACCAAACAGCTGGAAGATACAAAAGC  
TTATTGTGGGGAGAGAGTGGAAATTAGAATGTGAAGTGTCTGAAGATGATGCCAATG  
TAAAATGGTTTAAGAATGGTGAGGAAATCATCCCTGGTCCAAAATCAAGATACCAC  
ATTAGAGTTGAAGGCAAAAAGCACATTTTGATCATAGATGGAGCAACAAAGGCTG  
ATTCTGCAGAATATTCTGTAATGACAACAGGAGGACAGTCATCTGCTAAACTTAGT  
GTGGACT

**Text S2. Online prediction of the bovine circMYBPC1 sequence revealed the presence of four miR-23a binding sites.**

Version: RNAhybrid 2.2

Command line:/vol/bioapps/bin/RNAhybrid.bin -b 4 -D -n 23 -m 398 -q

/var/bibiserv2/anonymous/rmahybrid/24/11/15/bibiserv2\_2019-05-

24\_111550\_aabZo/rmahybrid\_input\_mirna\_sequences.file -t

/var/bibiserv2/anonymous/rmahybrid/24/11/15/bibiserv2\_2019-05-

24\_111550\_aabZo/rmahybrid\_input\_target\_rna\_sequences\_.file

searching

dataset: 1

MYBPC1e of bta-miR-23a: -46.299995

Individual hits

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dataset: 1

target: circMYBPC1

length: 398

miRNA : bta-miR-23a

length: 22

mfe: -25.7 kcal/mol

p-value: undefined

position 246

target 5' A      AUC                      C                      3'

                 GGAA    AUCCCUGGU

                 CCUU    UAGGGACCG

miRNA    3' A                                      UUACACUA 5'

-----

dataset: 1

target: circMYBPC1

length: 398

miRNA : bta-miR-23a

length: 22

mfe: -23.2 kcal/mol

p-value: undefined

position 93

```
target 5' A  A   UGU  A  A  UCUU  G 3'
          UG GAAA  UCC CUG GC  UGUGA
          AC CUUU  AGG GAC CG  ACACU
miRNA  3'                UU      A 5'
```

-----  
dataset: 1

target: circMYBPC1

length: 398

miRNA : bta-miR-23a

length: 22

mfe: -20.5 kcal/mol

p-value: undefined

position 177

```
target 5' G   GAG  AAUUAG  A 3'
          GGAGA  UGG    AAUGUGA
          CCUUU  ACC    UUACACU
miRNA  3' A   AGGG  G      A 5'
```

-----  
dataset: 1

target: circMYBPC1

length: 398

miRNA : bta-miR-23a

length: 22

mfe: -20.3 kcal/mol

p-value: undefined

position 367

target 5' A C A C U A A A C U A 3'  
G G A A G U C U C U G U A G U G U G G  
C C U U U A G G G A C G U U A C A C U  
miRNA 3' A C A 5'

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**Table S3. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis.**

pathway_term	rich_factor	qvalue	gene_number
Fatty acid metabolism	0.12766	0.2072	6
Oxytocin signaling pathway	0.065359	0.300575	10
cGMP-PKG signaling pathway	0.05988	0.300575	10
Gastric acid secretion	0.081081	0.300575	6
Lysine degradation	0.092593	0.300575	5
Retrograde endocannabinoid signaling	0.067308	0.300575	7
Inflammatory mediator regulation of TRP channels	0.066667	0.300575	7
Calcium signaling pathway	0.053191	0.300575	10
Biosynthesis of unsaturated fatty acids	0.136364	0.300575	3
GnRH signaling pathway	0.068966	0.300575	6
Fatty acid elongation	0.130435	0.300575	3
Glutamatergic synapse	0.06087	0.300575	7
Pancreatic secretion	0.063158	0.358122	6
PPAR signaling pathway	0.070423	0.358122	5
Fatty acid biosynthesis	0.153846	0.521561	2
Proteoglycans in cancer	0.044776	0.534605	9
Long-term depression	0.066667	0.585082	4
Gap junction	0.05618	0.601209	5
Long-term potentiation	0.060606	0.660681	4
Fatty acid degradation	0.071429	0.660681	3

**Table S5. Primers for quantitative real time PCR.**

Name	Forward primer 5'→3'	Reverse primer 5'→3'
CircMYBPC1	AGTGTGGACTATACATCTTTGAACA	TGGTCACCATAACTGGAGGC
PCNA-bos	TCCAGAACAAGAGTATAGC	TACAACAGCATCTCCAAT
β-actin-bos	CATCCTGACCCTCAAGTA	CTCGTTGTAGAAGGTGTG
MyoG-bos	CAAATCCACTCCCTGAAA	GCATAGGAAGAGATGAACA
MyhC-bos	TGCTCATCTCACCAAGTTCC	CACTCTTCACTCTCATGGACC
MyoD-bos	ACGGCATGATGGACTACAGC	AGGCAGTCGAGGCTCGACA
bta-miR-23a	AUCACAUUGCCAGGGAUUUCCA	GCAGGGTCCGAGGTATTC
U6	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTTCAT
PCNA-mus	ACTCCGCCACCATGTTTGAGG	TCGCAGCGGTATGTGTCGAAG
β-actin-mus	CAAGAGAGGTATCCTGACCCT	TGATCTGGGTTCATCTTTTCAC
MyoG-mus	CCGTGGGCATGTAAGGTGTGT	ACTTTAGGCAGCCGCTGGTTG
Myhc-mus	CCCGCTGGCCATGAACTACCT	CGGGCCGGATAGAGTTGTCAG
MyoD-mus	CCGGAGTGGCAGAAAGTTAAG	GGCCTGTCAAGTCTATGTCCC
CyclinD-mus	CCCGCTGGCCATGAACTACCT	CGGGCCGGATAGAGTTGTCAG
CDK2-mus	AGGTCCTCCGCTCCGAGTGTC	CGCCACAACCTTCTCCCGTCAA
Pax3-mus	TCGTCTCGCCTTACCTGGAT	AGCCGCTGCGTGGGTAATTCT
Pax7-mus	GGACGACGAGGAAGGAGACAA	CGGGTTCTGATTCCACATCTG
P53-mus	TGCCATGGAGGAGTCACAGTC	ACACTCGGAGGGCTTCACTTG
Bcl2-mus	GCGTTGGCCCTTCGGAGTTTA	CACCGGACCGCTTCAGACCTC

**Table S6. Primers for vector construction.**

<b>Name</b>	<b>Primer sequence 5'→3'</b>
pCD2.1-circMYBPC1-F	<u>GGGGTACCACCACATTAGAGTTGAAGGCAAAA</u>
pCD2.1-circMYBPC1-R	<u>CGGGATCCTCTTGATTTTGGACCAGGGATG</u>
pCK-circMYBPC1-W-F	<u>CCGCTCGAGACCACATTAGAGTTGAAGGCAAAA</u>
pCK-circMYBPC1-W-R	<u>ATAAGAATGCGGCCGCTCTTGATTTTGGACCAGGGATG</u>
pCK-miR-23a 2×-F	<u>GCTCGAGTGGAAATCCCTGGCAATGTGAT</u>
	TGGAAATCCCTGGCAATGTGAT
pCK-miR-23a 2×-R	<u>GGCGGCCGCATCACATTGCCAGGGATTCCA</u>
	ATCACATTGCCAGGGATTCCA

**Notes: The nucleotide with underline is the restriction enzyme cutting site.**



Figure S1

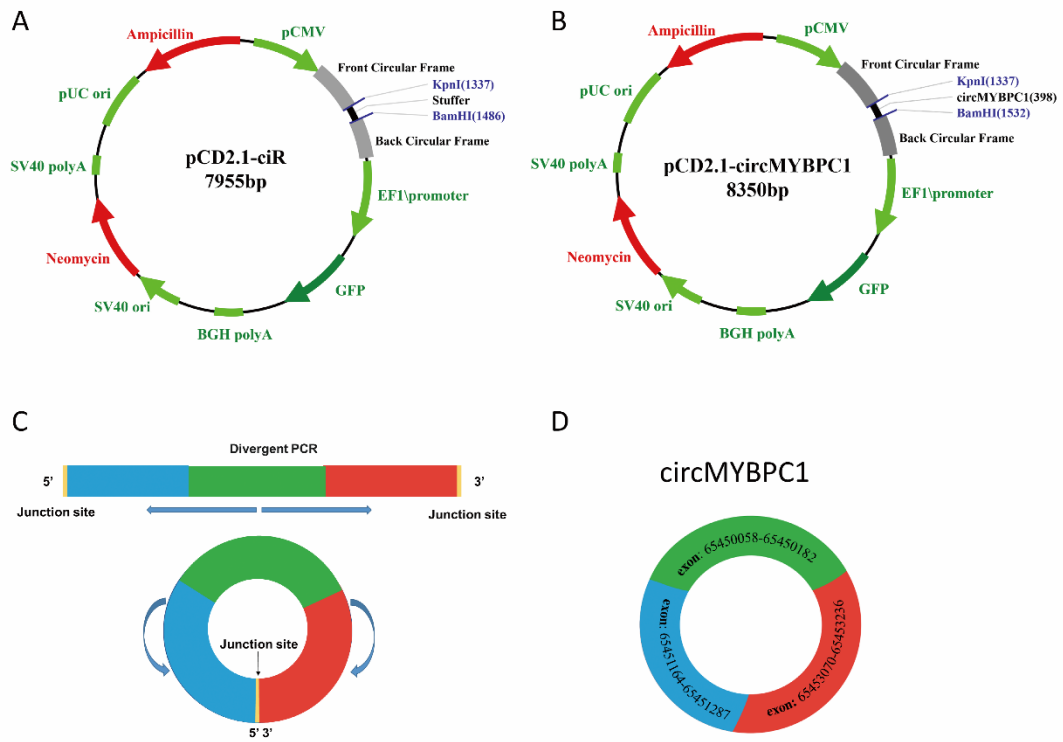
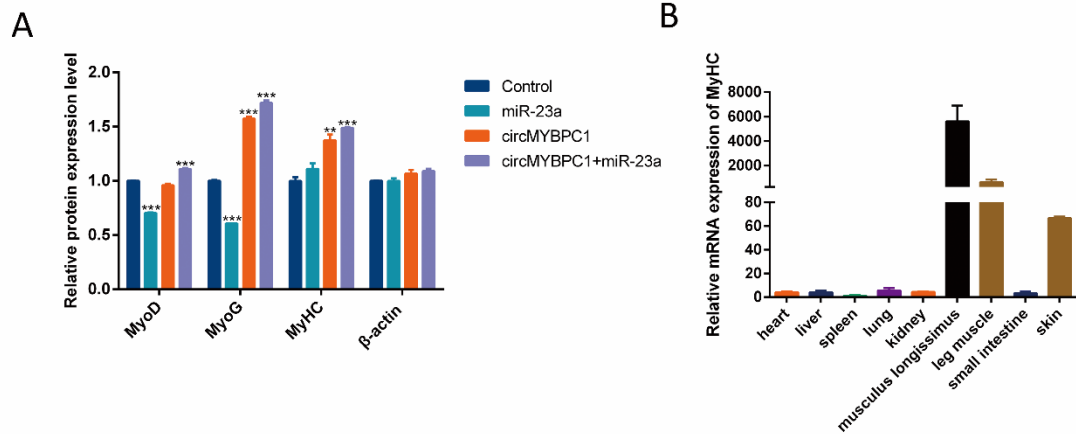


Figure S1. (A) A map of circular RNA expression vector. (B) Schematic view illustrating the design of primers for circRNAs used in qPCR. (C) Schematic view illustrating the design of primers for circRNAs used in qPCR. (D) circMYBPC1 consists of three exons.

**Figure S2**



**Figure S2. (A) Calculate the amount of gene expression in the Western blot experiment in Figure 6B. (B) qPCR results showing that MyHC were highly expressed in muscle tissues including the musculus longissimus, leg muscle.**