Supplementary Table 1. The oligonucleotide sequences used for knocking-down

siRNA	Sequence (5′→3′)
mouse_circAtxn10 siRNA #1, forward	AAGAAAACCAAGACAAACUA
mouse_circAtxn10 siRNA #1, reverse	AAGUUUGUCUUGGUUUUCUU
mouse_circAtxn10 siRNA #2, forward	AGACAAACUUUUCGATGUGA
mouse_circAtxn10 siRNA #2, reverse	CCACAUCGAAAAGUUUGUCU
mouse_siChrna1, forward	GAGCUAAGCCUACCUCUGU
mouse_siChrna1, reverse	ACAGAGGUAGGCUUAGCUC

siRNA, small interfering RNA.

Supplementary Table 2. The primers used for mRNA amplification in qPCR

Primers	Sequences (5′→3′)
Acta1, forward	GACCTCACT GACTACCTGATGAAA
Acta1, reverse	CAGACTCCATACCGATAA AGGAAG
Myogenin forward	AGTACATTGAGCGCCTACAG
Myogenin reverse	ACCCACCCT GACAGACAATC
MCK, forward	AGCAGCTCATTGATGACCAC
MCK, forward	TCAAACTTGGGGTGCTTGCT
MyoD, forward	TGCTCTGA TGG CATGATGGA
MyoD, reverse	CACTATGCTGGACAGGCAGT
Atxn10, forward	TAGAAGCTCACCAAAAGCACCCG
Atxn10, reverse	CGAGAAGCTCAGTGTTGG ACGTC
Chrna1, forward	GAGTCCAATAACGCCGCTGA
Chrna1, reverse	AATACAGCCGTGTGAGCAGA
Gapdh, forward	GCATGGCCTTCCGTGTTCCT
Gapdh, reverse	CCCTGTTGCTGTAGCCGTATTCAT
Malat1, forward	ATCCTAACGACTAGCATTGG
Malat1, reverse	GCTCCTCAGTCCTTCCTA

Supplementary Table 3. The primers used for circRNA amplification in PCR

Primer	Sequence (5′→3′)
circAtxn10, forward	GAAGGCGTGGACCCAAACAATG
circAtxn10, reverse	AGG GTGACATCGAACACATGACTG
circRhbdd1, forward	AGAACCAGACGTTGACAGCCAAG
circRhbdd1, reverse	ATGTCTACACAGCGGGCCTGA
circLdlrad3, forward	ATACAGTGGATGCCACT GGCG
circLdlrad3, reverse	GAGCTTCATCTGCGATGGTCAG
circWdr78, forward	TT GAGGCAGCATATCCTTTCCCTG
circWdr78, reverse	AACTGAGTCGATGTCAGAGGA CC
circCep70, forward	CCCATTGATGCTTTCCCATTCTGC
circCep70, reverse	CTAAACAGGGTTTGCCAGC
circSfmbt2, forward	TCACGGATGAGGAAGTC TGTCC
circSfmbt2, reverse	GCTGTGCCATGCAGATTCTTTG
circPrdm5, forward	TGCAGGCTTGACACTGAGGTAC
circPrdm5, reverse	ACGGACTGAAGATGCACATTCG
circEvc2, forward	AGATGAGGAAGGCTGCGATGAAG
circEvc2, reverse	TGGTT GCCATCCTGACTTCTGAG
circLdlr, forward	AACCCGTGAGTCGATTGGCAC
circLdlr, reverse	ATGCTGCTGGCCAAGGACATG
circFam114a2, forward	TCCACTGGCTCACAGTTCTCATC
circFam114a2, reverse	GAGAAGGCAGAGACTTCTCTTGGG
circPoli, forward	TCACTTGGAAGCTGCTGGAGTC
circPoli, reverse	AATCGAGAGAGTCGTCAGTGCC
circNcoa3, forward	ATTTGCAGAGATGAGCTCTGCC
circNcoa3, reverse	AATGATGGGCAGTCATGGTCC

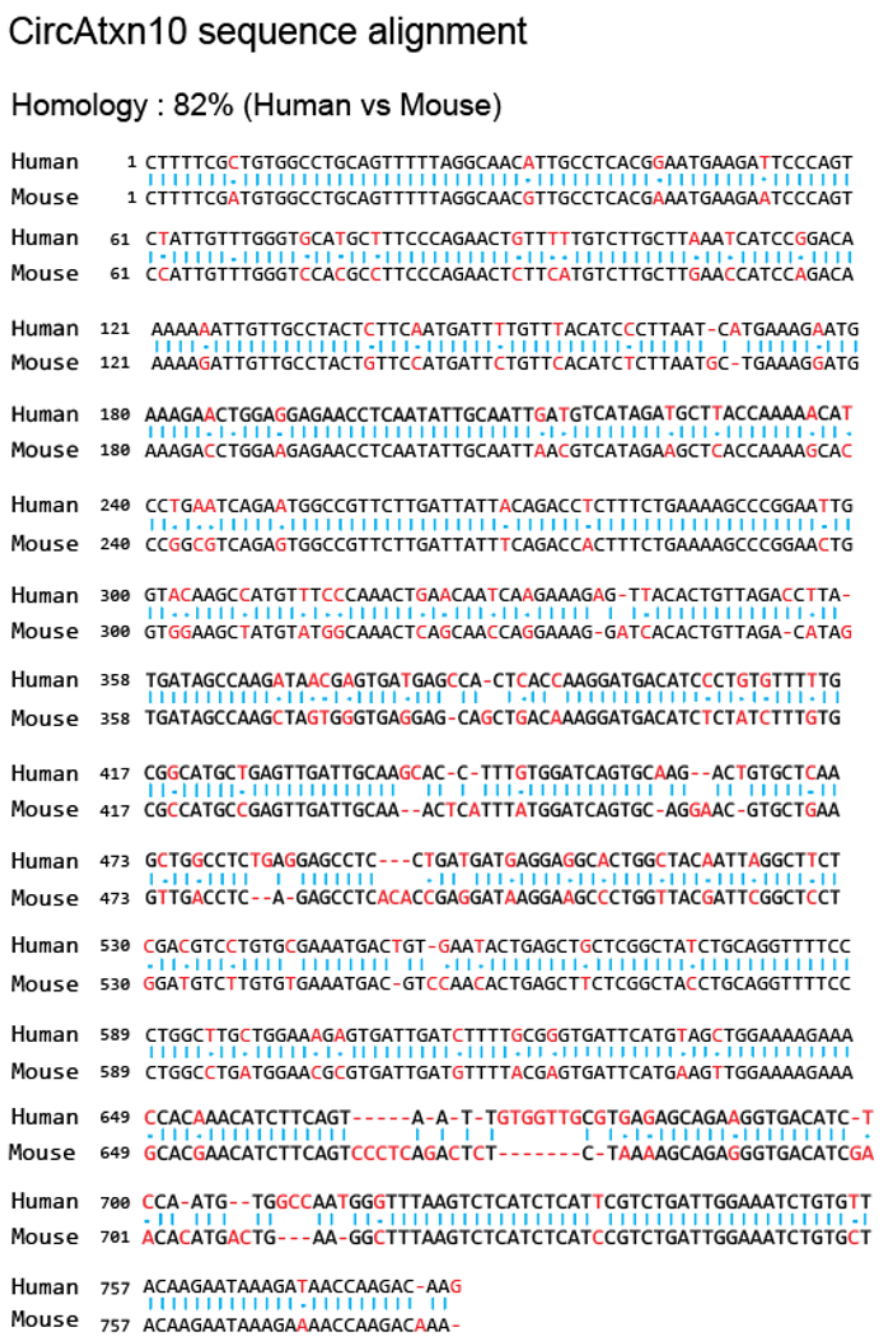
circRNA, circular RNA.

Supplementary Table 4. The oligonucleotide sequences used for cloning of circAtxn10 overexpression vector

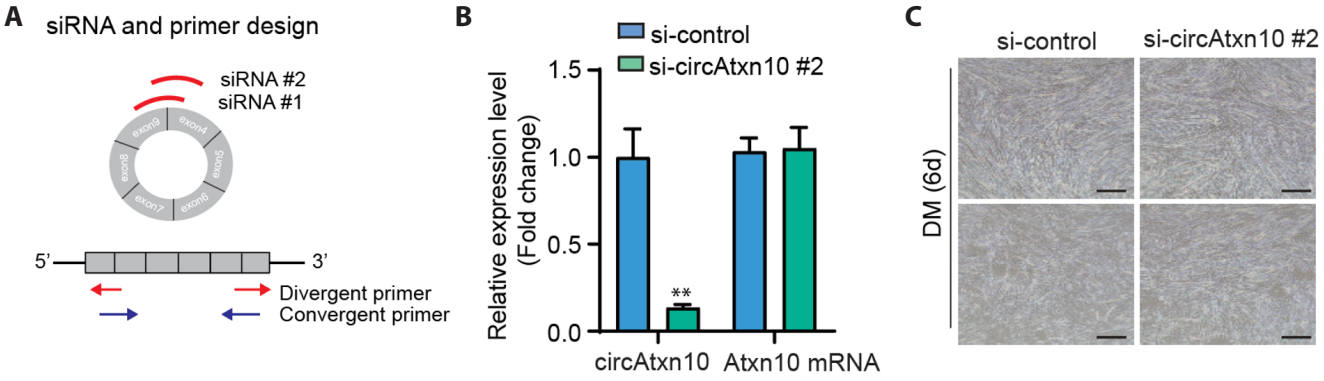
Primer	Sequence (5′→3′)	Amplicon size (bp)
circAtxn10_Zkscan, forward	TACCGAGCTCGGATCCcttttcgatgtgg	794
circAtxn10_Zkscan, reverse	TTGCTTCTTACCTCCGCGGtttgtcttggtttt	

Supplementary Table 5. The oligonucleotide sequences used for cloning of luciferase reporter construct

Primer	Sequence (5′→3′)	Amplicon size (bp)
circAtxn10_SacII, forward	CTAGAGCGAATTCCCGCGGcttttcgatgtggc	817
circAtxn10_BamHI, reverse	ATCGGTCGACGGATCCtttgtcttggttttctttattcttgtagcacag	
Chrna1 3-UTR_SacII, forward	CTAGAGCGAATTCCCGCGGCTTCAGAAGAACAGTCGGGTGCTCTTACCC	662
Chrna1 3-UTR_BamHI, reverse	ATCGGTCGACGGATCCCTTTCTGAGAAACTGCCAATTGTTTCCACTTTAAAG TGCAAACTATTTcacatcAAAGAGCCCAGCACTGGTG	



Supplementary Fig. 1. Sequence alignment of CircAtxn10. The sequence homology between human and mouse is 82%. Nucleotide differences are highlighted in red.



Supplementary Fig. 2. Knockdown of circAtaxn10 impairs myocyte differentiation. (A) Schematic illustration of siRNA design and primer positions targeting circAtxn10. Two independent siRNAs were designed to target the back-splicing junction of circAtxn10. Divergent primers were then used to verify the expression of circAtxn10, while convergent primers were used to confirm the expression of linear Atxn10 mRNA. (B) qRT-PCR analysis confirming efficient knockdown of circAtxn10 by si-circAtxn10 #2 in C2C12 cells. Expression levels of linear Atxn10 mRNA remained unchanged. Data are presented as mean ± SEM (n = 3). **p < 0.01 vs. control. (C) Representative images of myotube formation in C2C12 cells differentiated for 6 days after transfection with control siRNA or si-circAtxn10 #2. Knockdown of circAtxn10 resulted in impaired myogenic differentiation. siRNA, small interfering RNA; DM, differentiation medium.