Supplementary materials

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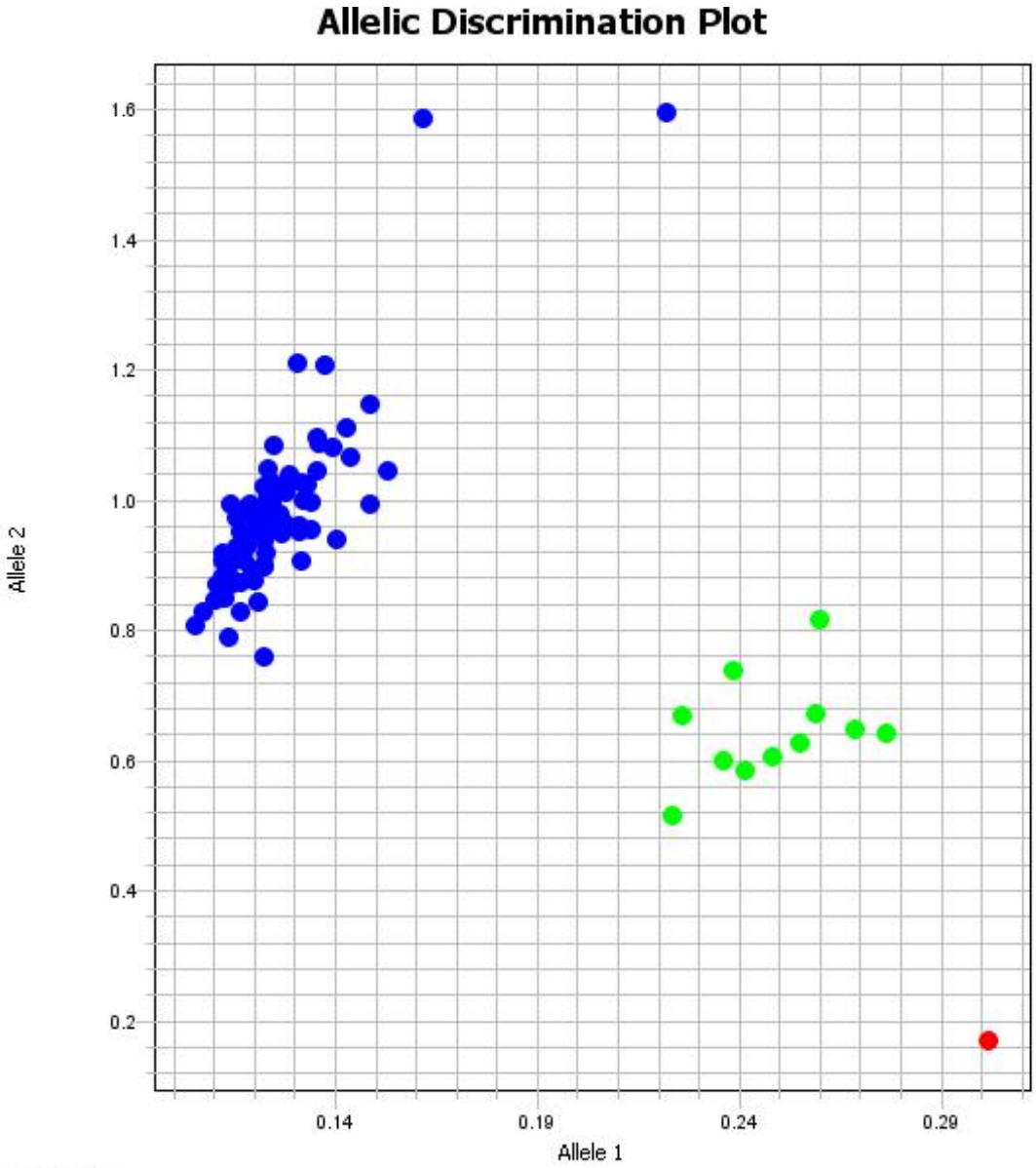

Fig. S1 Cluster plot for the KASP genotyping assay of bovine circBDP1-SNP. GG allele (red), AG allele (green), and AA allele (blue).

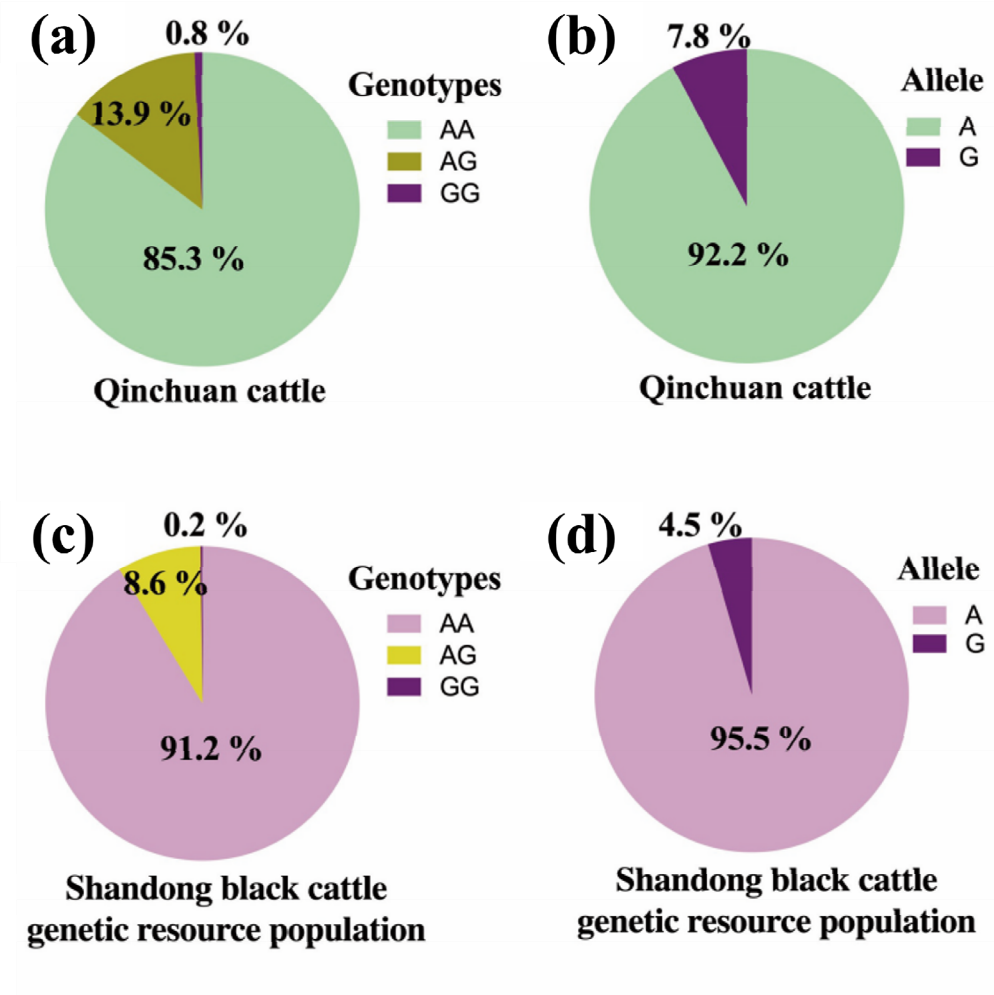

Fig. S2 Genotypic and allelic frequencies of circBDP1-SNP in two populations.

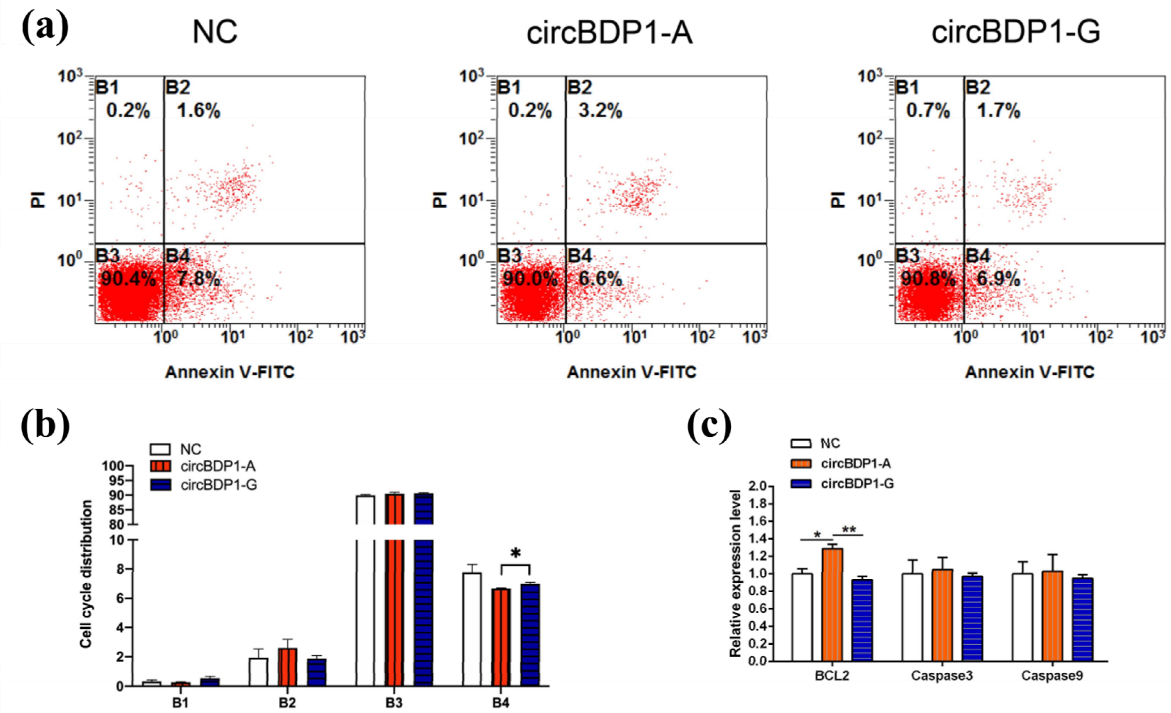

Fig. S3 The effect of circBDP1 with different alleles of circBDP1-SNP on preadipocyte apoptosis. (a, b) annexin V-PE/7-AAD dual staining followed by flow cytometry; (c) qRT-PCR.

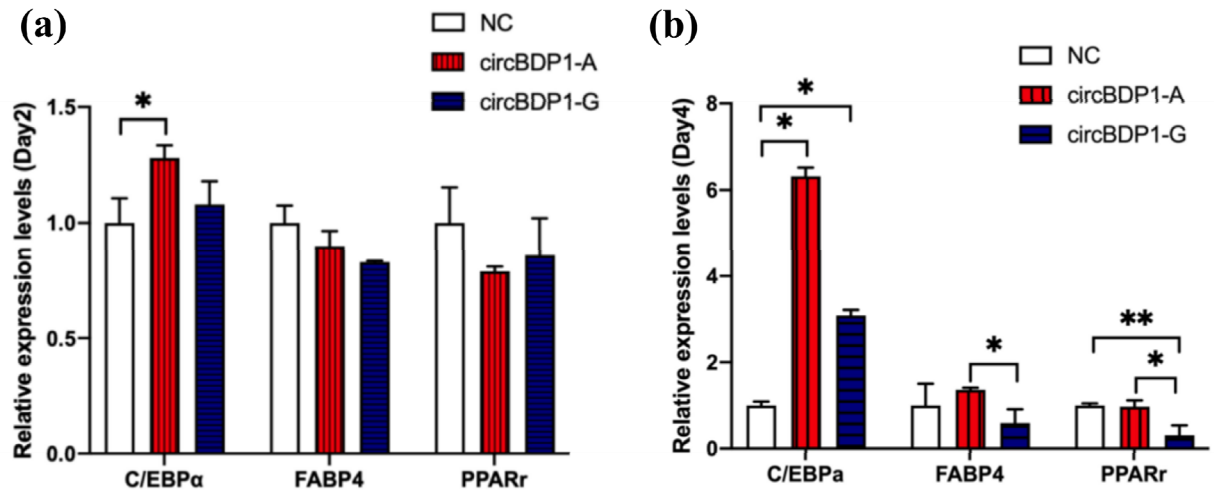

Fig. S4 The effect of circBDP1 with different alleles of circBDP1-SNP on preadipocyte differentiation.

Table S1 Primers for genetic variation screening and genotyping

Names	Primer sequences (5′–3′)	Note
F1	AGATTACTTGCTTTCTGACTGGGA	Screening and genotyping by sequencing (965 bp)
R1	TAGCTCTGTCGATCTAGTTTGGT	
F2/A	CAATGTCTTCACACTCAAAATTTTT	
F2/G	CAATGTCTTCACACTCAAAATTTTG	KASP method
R2	AAGACTCCTGAACAGCTAGAAGATCAGTC	
CyclinD-F	ATGAAGGAGACCATCCCCCT	qRT-PCR
CyclinD-R	TGAACTTCACGTCTGTGGCA	
CyclinE-F	CTGCAGCCTAAAATGCGAGC	qRT-PCR
CyclinE-R	TCCTGAACAAGCCCCATCTG	
PCNA-F	CGGACACGTTGGCACTAGTAT	qRT-PCR
PCNA-R	ACAGCATCTCCAATGTGACTG	

Table S2 Genetic parameter analysis of circBDP1-SNP

Breeds	Genotypic frequencies			Allelic frequencies		He	PIC	Ne	HWE
	AA	AG	GG	A	G				
SDBCGR	0.912 (n=561)	0.086 (n=53)	0.002 (n=1)	0.955	0.045	0.085	0.082	1.093	P>0.05
QC	0.853 (n=104)	0.139 (n=17)	0.008 (n=1)	0.922	0.078	0.144	0.133	1.168	P>0.05

SDBCGR, Shandong black cattle genetic resource population; QC, Qinchuan cattle; HWE, Hardy-Weinberg equilibrium.

Table S3 Association analysis between circBDP1-SNP genotypes and cattle carcass traits and body measurement data

Breeds / Traits	Body measurement data (Mean±SE)		*P* value
SDBCGR - male	AA (n=108)	AG (n=5)	
Gross weight (kg)	739.22±8.99	650.8±41.13	0.041
SDBCGR - female	GG (n=329)	AT (n=44)	
Cupim (kg)	1.19±0.02	1.38±0.08	0.005
Hanging tender (kg)	1.09±0.01	1.2±0.05	0.005
Chuck short rib (kg)	6.15±0.07	5.71±0.23	0.033
Rectum (kg)	1.73±0.09	2.5±0.28	0.004
Qinchuan cattle	AA (n=104)	AG (n=17)	
Body length (cm)	133.99 ± 1.29	141.29 ± 1.90	0.028

SDBCGR, Shandong black cattle genetic resource population.

Materials and methods

Sample collection and DNA extraction

The samples used for the genetic variation analysis were obtained from the Qinchuan cattle and Shandong black cattle genetic resource (SDBCGR) population. A total of 139 Qinchuan cattle samples were collected from Shaanxi Fufeng Qinchuan cattle breeding farm (Jin et al., 2018). The 615 SDBCGRs were the same as the samples used by Li et al. (2022). DNA was extracted using a high-salt method (Aljanabi and Martinez, 1997)

Genetic variation identification and genotyping

Genetic variations in circBDP1 were screened according to the information in the Ensembl database. DNA sequence information in the Ensembl and NCBI database was used to design primers for amplification of target fragments (Table S1). DNA samples from 30 to 50 cattle were mixed equally to construct a DNA pool, which was used as a template to screen genetic variations in different cattle breeds. After PCR (polymerase chain reaction) product size was detected using gel electrophoresis, the PCR products were sent to a company for sequencing to screen genetic variations.

In this study, sequencing of PCR products and Kompetitive Allele Specific PCR (KASP) methods were used to determine the genotypes of individuals. The steps for PCR amplification and sequencing of PCR products were the same as the published article (Zhang et al., 2019). The primers were listed in Table S1. The reaction system of KASP method was 10 μL, including FLU-ARMS for KASP 2x PCR Mix 5 μL, the forward and reverse primers 0.2 μL each (10 pm/μL), 20 ng DNA, and water was added up to 10 μL. Then we added 10 μL liquid paraffin oil to seal the liquid level under pressure. The reaction program was as follows: step 1: 94 °C for 10 min; step 2: 10 times of 95 °C for 15 sec, 61-55 °C (drop 1 oC per cycle) for 1 min; step 3: 26 times of 95 °C for 15 sec, 55 °C for 1 min; step 4: 4 °C preservation. The genotypes were separated according to the result in the Thermofisher ABI QuantStudio qRT-PCR instrument and QuantStudio Design & Analysis Software.

Genetic parameters and association analysis

Genotypic and allelic frequencies of different populations were calculated. Genetic parameters such as heterozygosity (He), polymorphic information content (PIC) and Hardy-Weinberg equilibrium (HWE) were calculated by MSR website (http://www.msrcall.com/). The association between genotypes and economic traits

was carried out according to the following general linear model: $Y_i = \mu + G_i + e_{ij}$ (Li et al., 2022), where Y_i is the trait measurement data of each animal; μ is the mean value of each trait; G_i is the genotype effect; e_{ij} is the random error. Different breeds, genders, and ages were analyzed separately. The association between genotypes and economic traits was analyzed using an independent samples t-test (two genotypes) in SPSS software (Jin et al., 2018).

Plasmid construction

In this study, the PCD2.1-CIR plasmid was used for circBDP1-A and circBDP1-G overexpression at General Biotechnology (Anhui, China) to detect their function in adipocyte proliferation, differentiation and apoptosis.

Cell proliferation analysis

Cell-Light EdU Apollo567 In Vitro Imaging Kit (RiboBio, Guangzhou, China) was used to determine cell proliferation according to the manufacturer's instructions. The number of positive cells stained with EdU was observed and counted using a fluorescence microscope. qRT-PCR was utilized to analyze the mRNA expression levels of marker genes for adipocyte proliferation, differentiation and apoptosis. Total cell RNA was isolated using Trizol Reagent (TaKaRa, Dalian, China). cDNA was synthesis using PrimeScript™ RT Reagent Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. qRT-PCR was conducted according to the study by (Zhang et al., 2021). The primers designed for qRT-PCR are listed in Table S1, and the other primers were referred to the previous studies (Li et al., 2016; Jin et al., 2019). Samples used for cell cycle analysis were treated with a cell cycle staining kit and analyzed by flow cytometry (Song et al., 2019).

Cell apoptosis analysis

Apoptosis was determined by annexin V-PE/7-AAD dual staining followed by flow cytometry and qRT-PCR methods. The primers used for qRT-PCR assay have been reported in a published paper (Jin et al. 2019).

Cell differentiation analysis

qRT-PCR and western blot were used to analyze cell differentiation. The primers for qRT-PCR were reported in a previous published study (Li et al. 2016). RIPA buffer (Solarbio, Beijing, China) was utilized for cell lysis and extraction of total protein. The steps for western blotting were described in a study by Song et al. (2019). Primary antibodies were purchased from Wanlei Biotechnology (Shenyang, China).

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