Supplementary Data: Overexpression or Knock-down Vector Construction of CFL1 and CFL2

For overexpression of the bovine CFL1 and CFL2 gene, we designed and synthesized CFL1 and CFL2 overexpression primers containing *Kpn*I and *Hin*dIII (TaKaRa, Dalian, China) restriction sites, the coding sequences of CFL1 and CFL2 were subcloned into the pAdTrack-CMV (Miaoling bio, Wuhan, China) plasmid vector to construct the recombinant shuttle vectors pAdTrack/CMV-CFL1 and pAdTrack/CMV-CFL2. Then, this vector was homologously recombined with plasmid pAdEasy-1 to generate an adenoviral plasmid in BJ5183 (Miaoling bio, Wuhan, China) cells. The adenoviral plasmids linearized by *Pac*I (TaKaRa, Dalian, China) were transfected into 293A cells to generate the adenovirus pAdEasy-1/pAdtrack-CMV-CFL1 (CFL1-CMV) and pAdEasy-1/pAdtrack-CMV-CFL2 (CFL2-CMV).

For interference with the bovine CFL1 and CFL2 gene, the BlockiT shRNA interference system was used to design and synthesize primers for CFL1 gene and CFL2 gene. All primer sequences containing *Bam*HI and *Hin*dIII (TaKaRa, Dalian, China) restriction sites. The oligonucleotides were cloned into the pENTR/CMV-GFP/U6 vector (Miaoling bio, Wuhan, China), and then recombined with the adenovirus backbone vector pAD/PL-DEST (Miaoling bio, Wuhan, China) to produce recombinant vectors pAD/PL-DEST/CMV-GFP/U6-shCFL1-1 (shCFL1-1), pAD/PL-DEST/CMV-GFP/U6-shCFL1-2 (shCFL1-2), pAD/PL-DEST/CMV-GFP/U6-shCFL2-1 (shCFL2-1) and pAD/PL-DEST/CMV-GFP/U6-shCFL2-2 (shCFL2-2).

Supplementary Data: Protein Extraction and Western Blot

Total proteins were extracted from cells using radio immunoprecipitation assay (RIPA) lysis buffer containing 1 mM phenylmethylsulfnoyl fluoride (PMSF) (Solarbio, Beijing, China). Proteins were measured and adjusted by using the BCA protein assay kit (MultiScience, Hangzhou, China) and denatured with 5×SDS loading buffer (Beyotime, Shanghai, China) at 98 °C for 10 min. The primary antibodies including anti-CFL1 (ab131519), anti-CFL2 (ab14134) and anti-GAPDH (ab9485) were purchased from Abcam (Cambridge, UK). Anti-immune rabbit IgG-HRP (LK2001) was purchased from Sungene Bio (Tianjin, China) as secondary antibody, antibody-reacting bands were detected using ECL luminous fluid (Solarbio, Beijing, China).

Table S1. Primer information for vector construction.

Name	Primer (Contains Protective Bases and Restriction Sites) (5′-3′)
CFL1-CMV-F	CGGggtaccATGGCCTCCGGTGTGGCTGTCT
CFL1-CMV-R	CCCaagcttTCAcatcatcaccatcaccatCAAAGGCTTGCCCTCCAG
CFL2-CMV-F	CGGggtaccATGGCTTCTGGAGTTAC
CFL2-CMV-R	CCCaagcttTCAcatcatcaccatcaccatTAAGGGTTTTCCTTC
shCFL1-1F	gatccCCTCTATGATGCAACCTACTTCAAGAGAGTAGGTTGCATCATAGAGGttttta
shCFL1-1R	agcttaaaaaaCCTCTATGATGCAACCTACTCTCTTGAAGTAGGTTGCATCATAGAGGg
shCFL1-2F	gatccGGATCAAGCATGAATTACAAGCAAATTCAAGAGATTTGCTTGTAATTCATGCTTGATCCttttta
shCFL1-2R	agcttaaaaaaGGATCAAGCATGAATTACAAGCAAATCTCTTGAATTTGCTTGTAATTCATGCTTGATCCg
shCFL2-1F	gatccCTGAAAGTGCACCGTTAAATTCAAGAGATTTAACGGTGCACTTTCAGttttta
shCFL2-1R	agcttaaaaaaCTGAAAGTGCACCGTTAAATCTCTTGAATTTAACGGTGCACTTTCAGg
shCFL2-2F	gatccGCTCTAAAGATGCCATTAATTCAAGAGATTAATGGCATCTTTAGAGCttttta
shCFL2-2R	agcttaaaaaaGCTCTAAAGATGCCATTAATCTCTTGAATTAATGGCATCTTTAGAGCg
shRNA-NC-F	gatccTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCGGAGAAttttta
shRNA-NC-R	agcttaaaaaaTTCTCCGAACGTGTCACGTTCTCTTGAAACGTGACACGTTCGGAGAAg

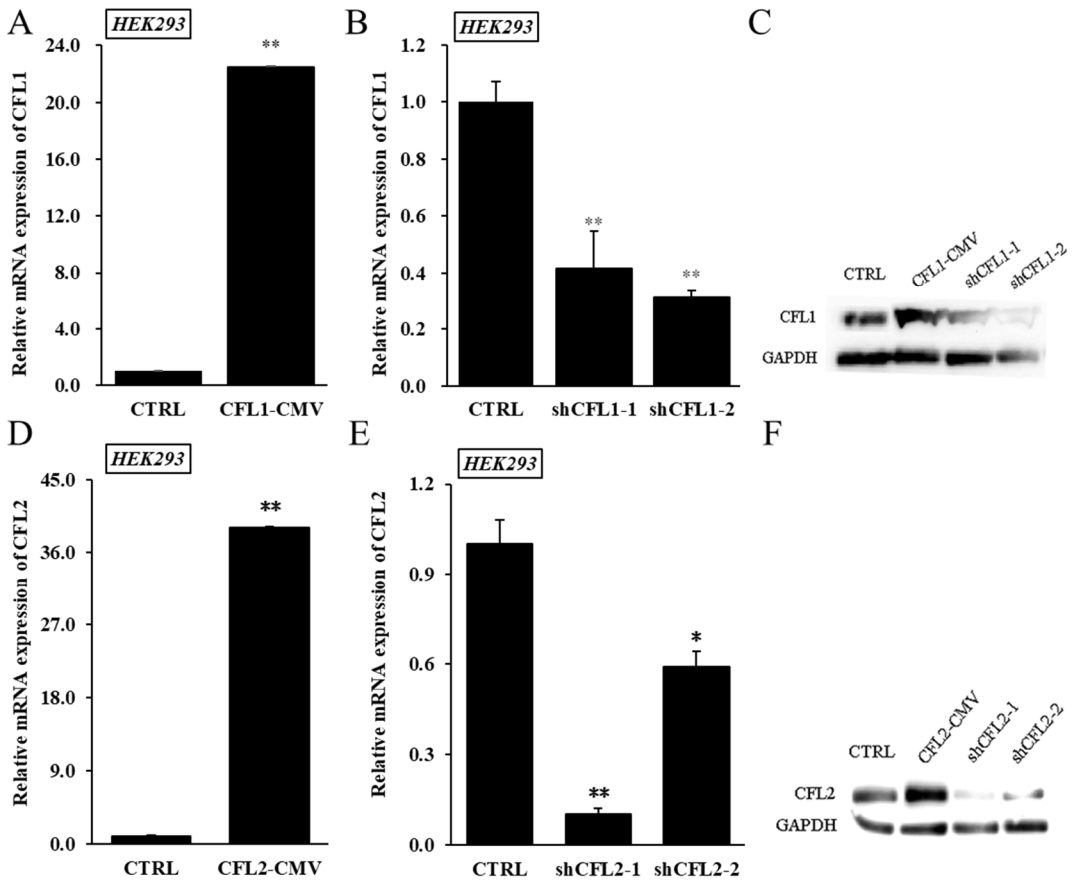

Figure S1. The efficiency detection of recombinant vectors by qRT-PCR and western blot. Expression efficiency of CFL1 and CFL2 was detected by qRT-PCR (A-B, C-D) and western blot (C, F) in HEK293 cells after transfected with adenovirus overexpression (CFL1-CMV, CFL2-CMV) and interference (shCFL1-1 and shCFL1-2, shCFL2-1 and shCFL2-2) vectors for 48h.

Supplementary Data: The stability data expression of GAPDH

Table S2. Stability data expression of GAPDH in eight tissues of Qinchuan cattle at three growth stages

Sample Name	Fetal Bovine	Calf	Adult Cattle
Heart	20.27	19.08	20.03
	20.29	19.18	20.08
	20.05	19.06	19.88
Liver	19.58	19.44	19.93
	19.65	19.52	19.51
	19.77	19.52	19.74
Spleen	20.07	20.89	20.51
	20.63	20.81	20.47
	20.29	20.95	20.21
Lung	20.63	20.18	20.2
	20.49	20.47	20.05
	20.63	19.89	20.23
Kidney	18.92	19.78	19.86

	18.51	19.19	19.53
	18.81	19.49	19.77
Stomach	20.16	19.44	20.31
	20.63	19.47	20.61
	20.04	19.47	20.44
Small Intestine	19.12	20.15	20.48
	19.13	20.04	20.87
	18.98	20.21	20.96
Muscle	18.49	17.44	17.76
	18.35	17.64	17.48
	18.46	17.44	17.76