Supplementary information

Beef peptides mitigate skeletal muscle atrophy in C2C12 myotubes through protein degradation, protein synthesis, and oxidative stress pathway

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Table S1. List of primer sequences used for reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Target gene		Sequence	Length (bp)
MuRF1	F	GTGTGAGGTGCCTACTTGCTC	101
	R	GCTCAGTCTTCTGTCCTTGGA	
Atrogin-1	F	ATGCACACTGGTGCAGAGAG	168
	R	TGTAAGCACACAGGCAGGTC	
SOD1	F	ATGGGTTCCACGTCCATCAGTA	132
	R	CATTGCCCAGGTCTCCAACA	
GPx1	F	CCACCGTGTATGCCTTCTCC	105
	R	AGAGAGACGCGACATTCTCA	
CAT	F	CGAGGGTCACGAACTGTGTCA	132
	R	GGTCACCCACGATATCACCAGATAC	
MyoD	F	CCACTCCGGGACATAGACTTG	109
	R	AAAAGCGCAGGTCTGGTGAG	
Myogenin	F	AGAAGCGCAGGCTCAAGAAA	94
	R	ATCTCCACTTTAGGCAGCCG	
β-actin	F	AGACTTCGAGCAGGAGATGG	101
	R	ACCGCTCGTTGCCAATAGT	

MuRF1, muscle ring finger protein-1; Atrogin-1, muscle atrophy F-box protein 1; MyoD, myogenic differentiation 1; Myogenin, myogenic factor 4; SOD1, superoxide dismutase 1; GPx1, glutathione peroxidase 1; CAT, catalase

Table S2. List of primary antibodies used for western blot

Name of antibody	Manufacturer and catalog No.
beta-actin (13E5) Rabbit mAb	Cell Signaling: #4970S
FoxO1 (C29H4) Rabbit mAb	Cell Signaling: #2880S
mTOR Antibody #2972	Cell Signaling: #2972S
Akt (pan) (C67E7) Rabbit mAb	Cell Signaling: #4691S
4E-BP1 (53H11) Rabbit mAb	Cell Signaling: #9644T
p70 S6 Kinase (49D7) Rabbit mAb	Cell Signaling: #2708T
Phospho-FoxO1 (Ser256) Antibody	Cell Signaling: #9461S
Phospho-mTOR (Ser2448) Antibody #2971	Cell Signaling: #2971S
Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060	Cell Signaling: #4060S
Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb	Cell Signaling: #9644T
Phospho-p70 S6 Kinase (Thr389) (108D2) Rabbit mAb	Cell Signaling: #9234T

Table S3. The eluent gradient condition for liquid chromatography-mass spectrometry (LC-MS)

Time (min)	Eluent A 0.1% formic acid in double distilled water (%)	Eluent B 0.1% formic acid in acetonitrile (%)
0	95	5
1	95	5
17	55	45
24	0	100
26	0	100
26.5	95	5
27	5	5
30	5	5

(A) (B)

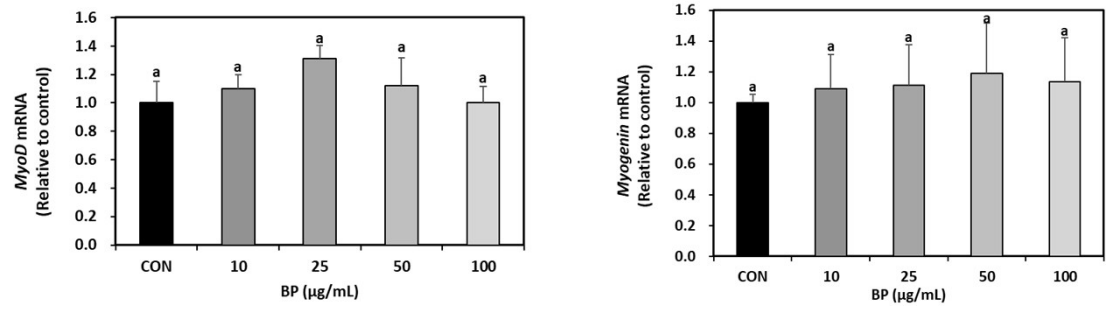
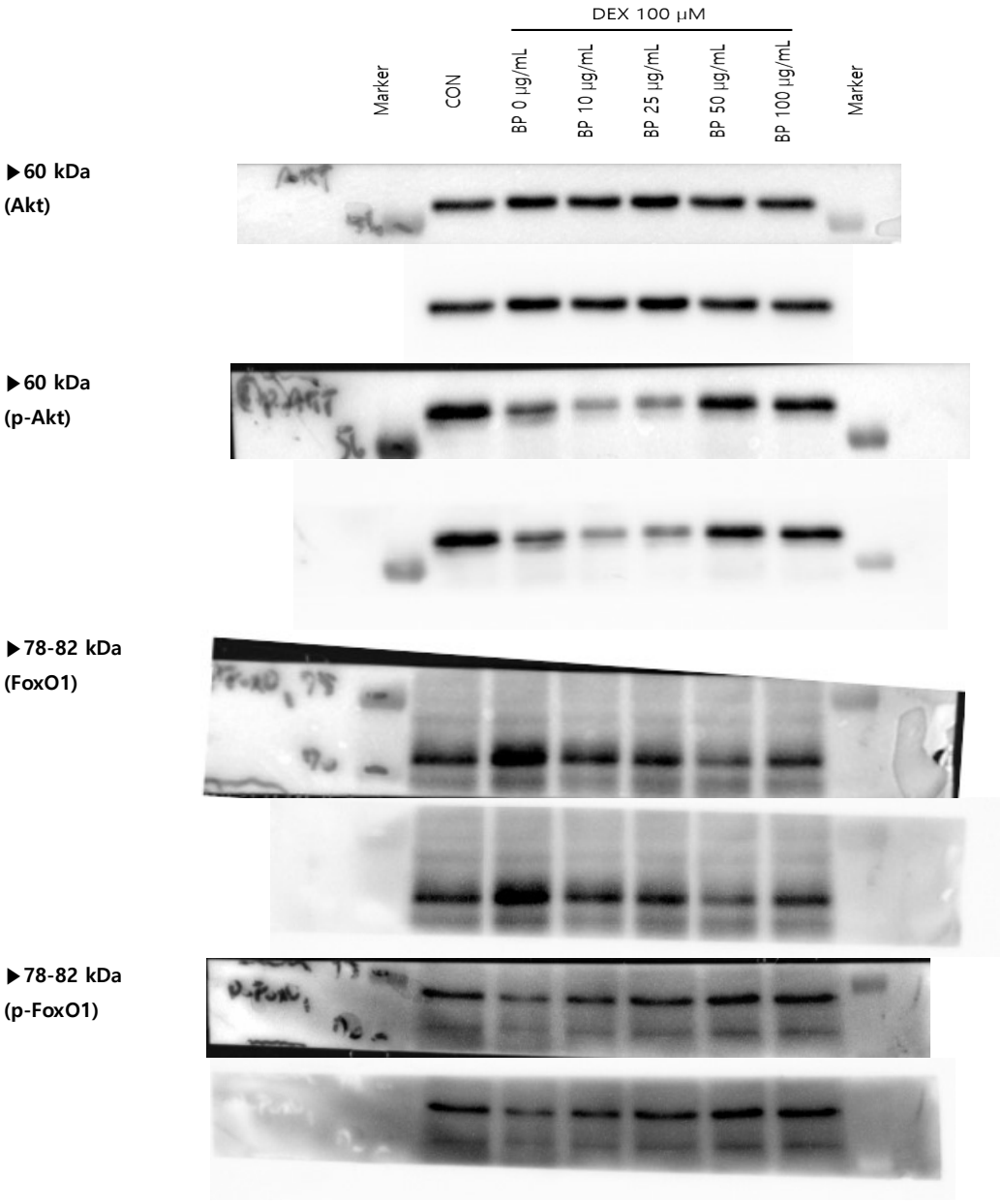
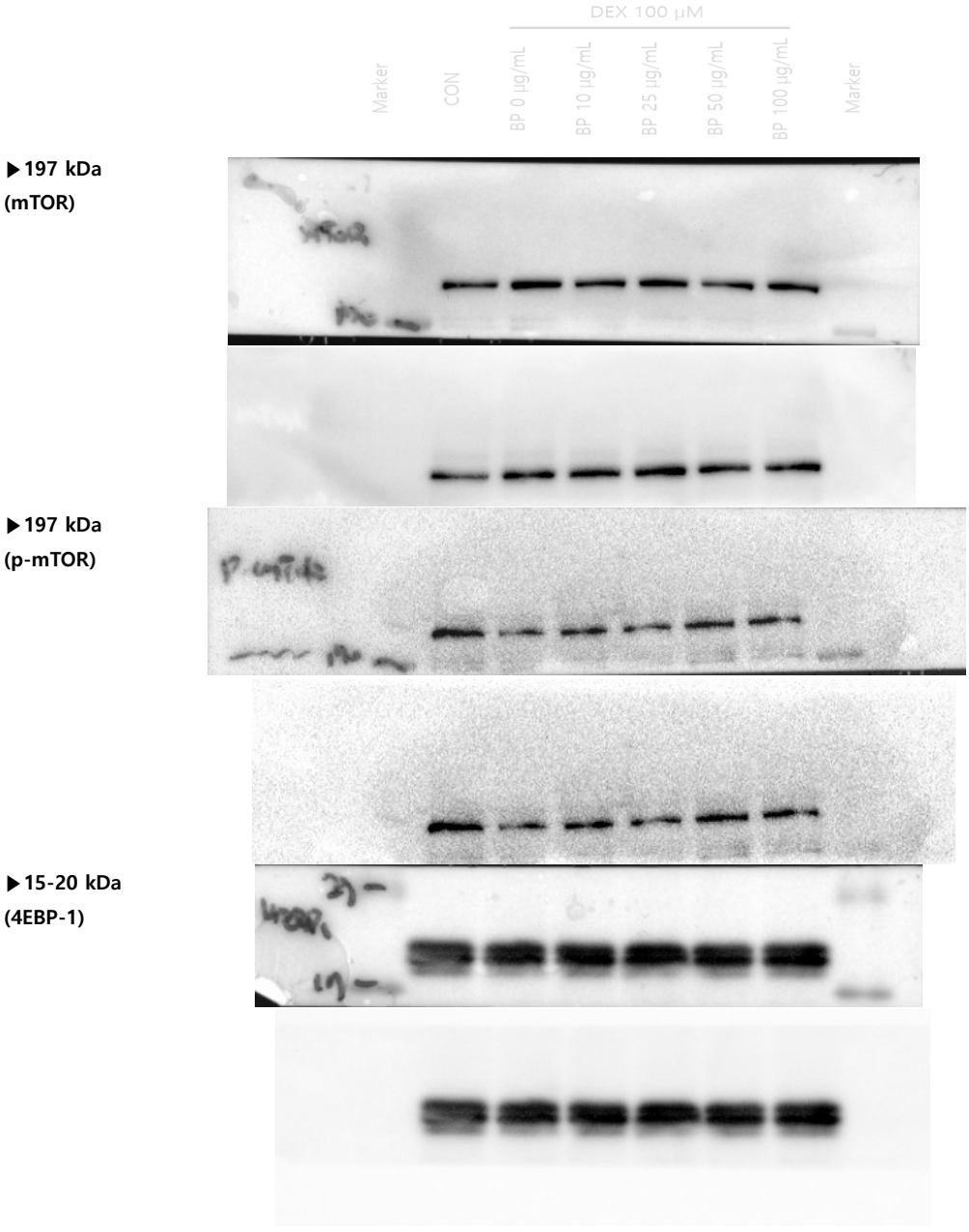


Fig. S1. Effect of beef peptides (BP) on the myogenic regulatory factors in C2C12 myotubes. On the sixth day of differentiation, C2C12 myotubes were exposed BP concentrations (10-100 μg/mL) for 24 h. The control (CON) was maintained without any treatment. A-B: Levels of mRNA for MyoD and Myogenin were measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and normalized to β-actin. $^{a-b}$ Different letters indicate significant differences ($p < 0.05$). Data are expressed as the mean ± SD.


DEX 100 μM
Marker
CON
BP 0 μg/mL
BP 10 μg/mL
BP 25 μg/mL
BP 50 μg/mL
BP 100 μg/mL
Marker
▶60 kDa
(Akt)
▶60 kDa
(p-Akt)
▶78-82 kDa
(FoxO1)
▶78-82 kDa
(p-FoxO1)

41


DEX 100 μM
Marker
CON
BP 0 μg/mL
BP 10 μg/mL
BP 25 μg/mL
BP 50 μg/mL
BP 100 μg/mL
Marker
▶197 kDa
(mTOR)
▶197 kDa
(p-mTOR)
▶15-20 kDa
(4EBP-1)

42

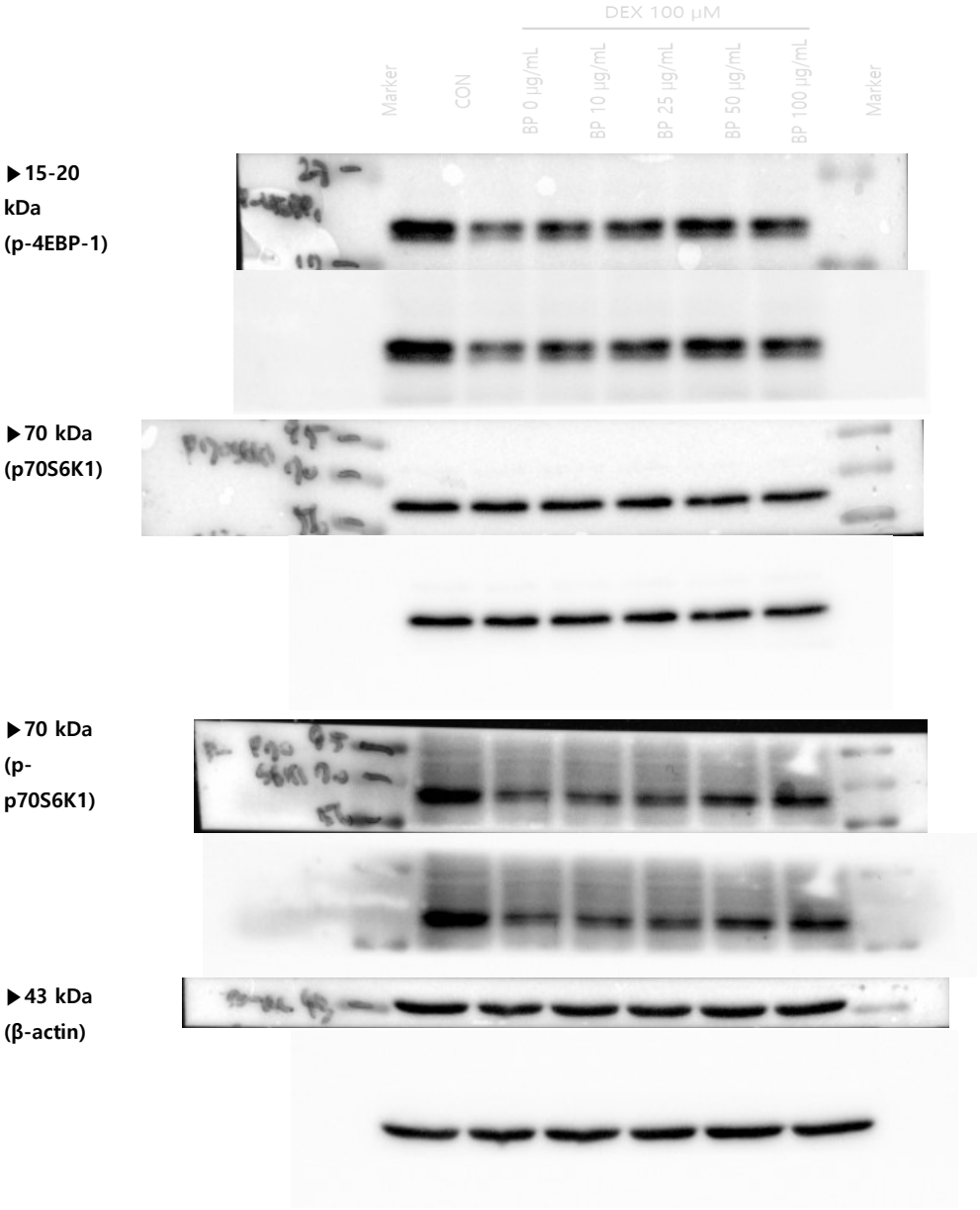


Fig. S2. Raw gel images of western blot