Myricanol rescues dexamethasone-induced muscle dysfunction via a SIRT1 dependent mechanism

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Antibody	Source	Vendor	Catalog No
Fbx32(atrogin-1)	Mouse	Abcam	ab74023
MuRF1	Mouse	Santa Cruz Biotechnology	sc-398608
SIRT1	Rabbit	Santa Cruz Biotechnology	sc-15404
p-AMPKa (Thr172)	Rabbit	Santa Cruz Biotechnology	sc-33524
ΑΜΡΚα 1/2	Rabbit	Santa Cruz Biotechnology	sc-25792
p-Akt1/2/3 (Ser 473)	Rabbit	Santa Cruz Biotechnolog	sc-7985
Akt1/2/3	Rabbit	Santa Cruz Biotechnolog	sc-8312
p-FoxO3a (Ser253)	Rabbit	Cell Signaling Technology	#9466
FoxO3a	Rabbit	Cell Signaling Technology	#2497
Tom20	Mouse	Santa Cruz Biotechnology	sc-17764
PGC-1α	Rabbit	Cell Signaling Technology	#2187
Cox2	Rabbit	Cell Signaling Technology	#12282
UCP3	Goat	Santa Cruz Biotechnology	sc-31387
Bax	Rabbit	Santa Cruz Biotechnology	sc-525
Bcl-2	Mouse	Santa Cruz Biotechnology	sc-7382
cleaved caspase-3 (Asp175)	Rabbit	Cell Signaling Technology	#9661
LC3A/B	Rabbit	Cell Signaling Technology	#4108
Beclin1	Rabbit	Santa Cruz Biotechnology	sc-11427
p62	Rabbit	Cell Signaling Technology	#23214
GAPDH	Rabbit	Santa Cruz Biotechnology	sc-25778
β-actin	Rabbit	Santa Cruz Biotechnology	sc-1616
МуНС	Mouse	R&D Systems	MAB4470

Table S1 Antibodies for immunoblotting.

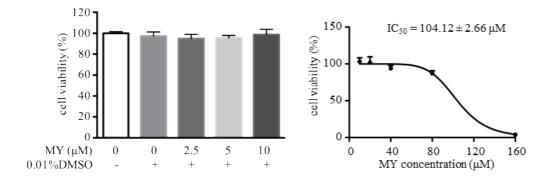


Figure S1 Cytotoxicity of MY on C2C12 myotubes. Data are shown as mean \pm S.D., n = 6.

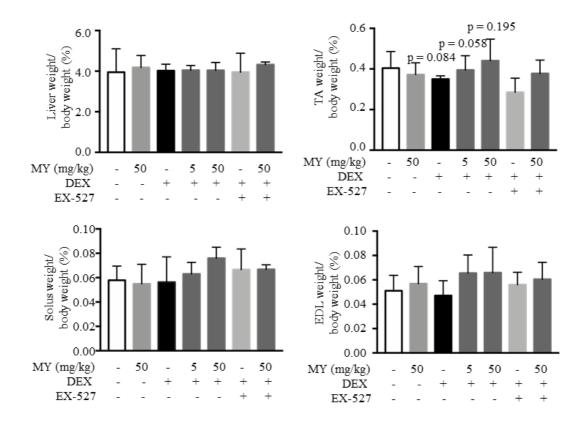


Figure S2 The ratios of liver, TA, EDL and soleus muscle to body weight. Data are shown as mean \pm S.D., n = 6.

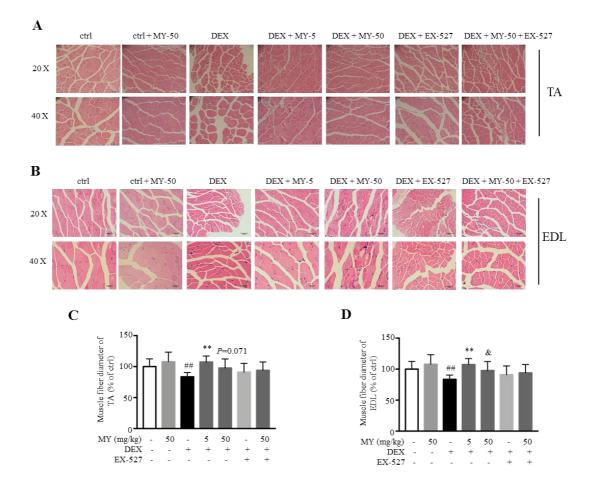


Figure S3 Representative H&E staining of myofiber cross section of TA (*A*) and EDL (*B*). Scale bar = 100 or 50 μ m on top and bottom, respectively. A microscope with a 10× or 20× objective was used to capture the images. The cross-sectional diameter of TA (*C*) and EDL (*D*) muscle fiber. Ctrl: PEG 400 solution; ctrl + MY-50: PEG 400 solution with 50 mg/Kg MY; DEX: PEG 400 solution with 25 mg/Kg dexamethasone; DEX + MY-5: DEX solution with 5 mg/Kg MY; DEX + MY-50: DEX solution with 5 mg/Kg MY; DEX + MY-50: DEX solution with 5 mg/Kg MY; DEX + MY-50: DEX solution with 50 mg/Kg MY; DEX + EX-527: DEX solution with 10 mg/Kg EX-527; DEX + MY-50 + EX-527: DEX solution with 50 mg/Kg MY and 10 mg/Kg EX-527. Data are shown as mean ± S.D., *n* = 6. ** *P* < 0.01, DEX + MY-5 vs. DEX. & *P* < 0.05, DEX + MY-50 vs. DEX. ## *P* < 0.01, control vs. DEX.

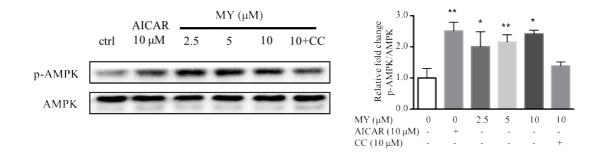


Figure S4 MY increased AMPK phosphorylation in C2C12 myotubes, and Compound C (CC, the AMPK inhibitor, 10 μ M) almost abolished the effect of MY. AICAR (the AMPK activator) was used as a positive control. Data are shown as mean \pm S.D., n = 6. * P < 0.05, ** P < 0.01, MY or AICAR vs. ctrl.

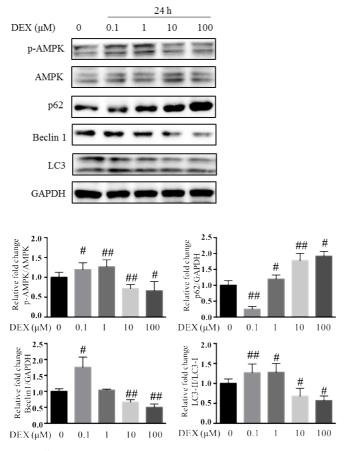


Figure S5 The expression of p-AMPK, AMPK, p62, Beclin1, LC3 in C2C12 myotubes treated with different concentrations of DEX for 24 hours. GAPDH was used as a loading control. Data are shown as mean \pm S.D., n = 6. # P < 0.05, ## P < 0.01, ctrl vs. DEX.