Supporting Information

Myricanol modulates skeletal muscle-adipose crosstalk to alleviate high fat diet-induced obesity and insulin resistance

Running title: Myricanol directs myotubes-adipocytes communication

Shengnan Shen[1], Qiwen Liao[1], Tian Zhang[1], Ruile Pan[2], Ligen Lin[1,*]

[1] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Avenida da Universidade, Taipa, Macau 999078, China

[2] Institute of Medicinal Plant Development, Peking Union Medical College, Chinese Academy of Medical Science, Beijing 100193, China

*Correspondance

Dr. Ligen Lin Institute of Chinese Medical Sciences, University of Macau, Avenida da Universidade, Taipa, Macao, China. Phone: +853 88228041, Fax : +853 28841358; email: ligenl@um.edu.mo.

Table S1. Antibodies for immunoblotting.

Antibody	Source	Vendor	Catalog No.	
p-ACC (Ser79)	Rabbit	Cell Signaling Technology	#3661	RRID:AB_330337
ACC	Rabbit	Cell Signaling Technology	#3662	RRID:AB_2219400
p-AMPKα (Thr172)	Rabbit	Santa Cruz Biotechnology	sc-33524	RRID:AB_2169714
AMPKα 1/2	Rabbit	Santa Cruz Biotechnology	sc-25792	RRID:AB_2169546
CD36	Rabbit	Santa Cruz Biotechnology	sc-9154	RRID:AB_2072518
COX2	Goat	Santa Cruz Biotechnology	sc-23983	RRID:AB_2066365
PGC-1α	Rabbit	Cell Signaling Technology	#2178	RRID:AB_823600
UCP3	Goat	Santa Cruz Biotechnology	sc-31387	RRID:AB_2213920
FNDC5	Rabbit	Proteintech	23995-1-AP	
GAPDH	Rabbit	Santa Cruz Biotechnology	sc-25778	RRID:AB_10167668
p-IRS1 (Tyr632)	Rabbit	Santa Cruz Biotechnology	sc-17196	RRID:AB_669445
IRS1	Rabbit	Cell Signaling Technology	#2382	RRID:AB_330333
p-AKT1/2/3 (Ser473)	Rabbit	Santa Cruz Biotechnolog	sc-7985	RRID:AB_667741
AKT1/2/3	Rabbit	Santa Cruz Biotechnolog	sc-8312	RRID:AB_671714
p-GSK3β (Ser9)	Rabbit	Cell Signaling Technology	#9323	RRID:AB_2115201
GSK3β	Rabbit	Cell Signaling Technology	#12456	RRID:AB_2636978
UCP1	Goat	Santa Cruz Biotechnology	sc-6529	RRID:AB_2213781
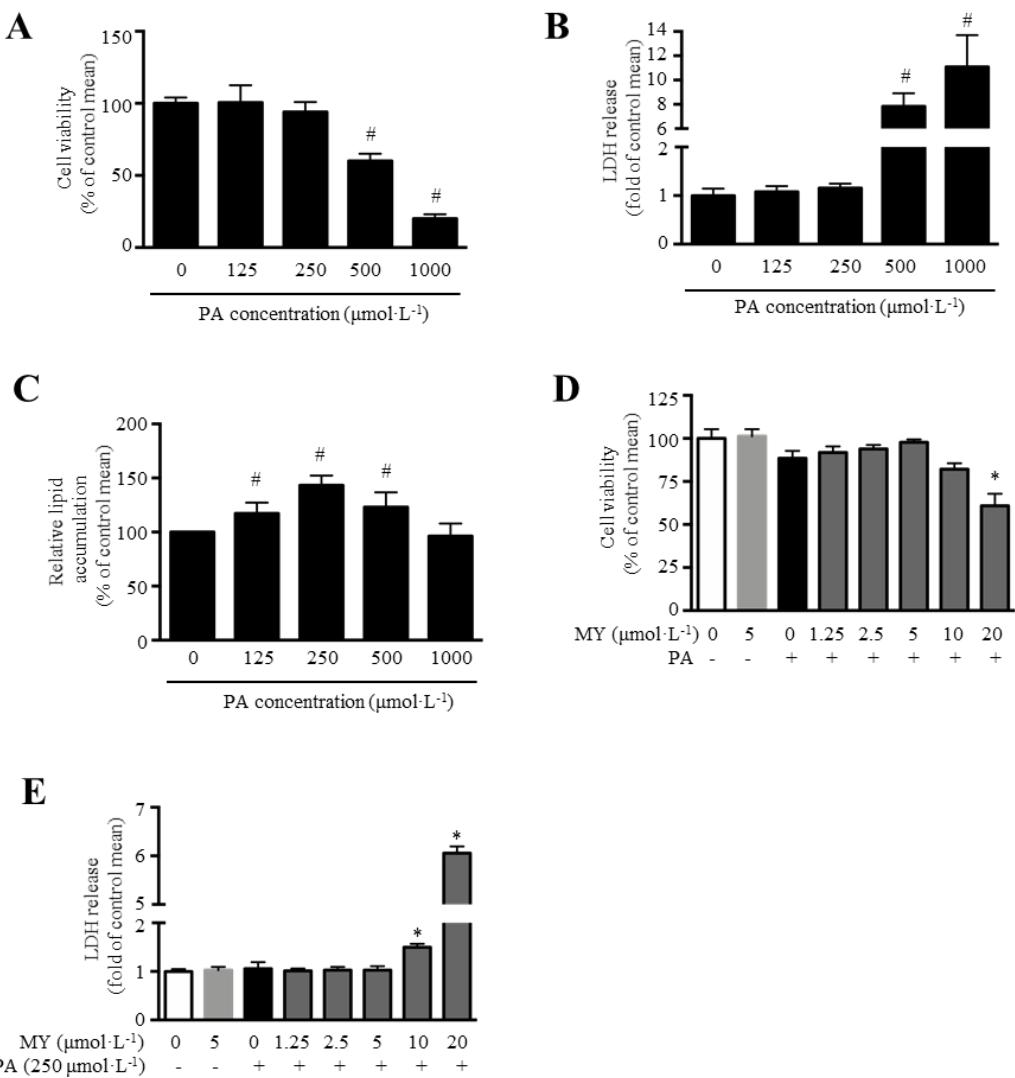
UCP1	Rabbit	Proteintech	23673-1-AP	
PPARγ	Rabbit	Santa Cruz Biotechnology	sc-7196	RRID:AB_654710
SIRT1	Rabbit	Proteintech	13161-1-AP	
β-actin	Rabbit	Santa Cruz Biotechnology	sc-1616	RRID:AB_630836
Anti-rabbit IgG		Cell Signaling Technology	#7074	RRID:AB_2099233
Anti-goat IgG		Santa Cruz Biotechnology	sc-2354	RRID:AB_628490
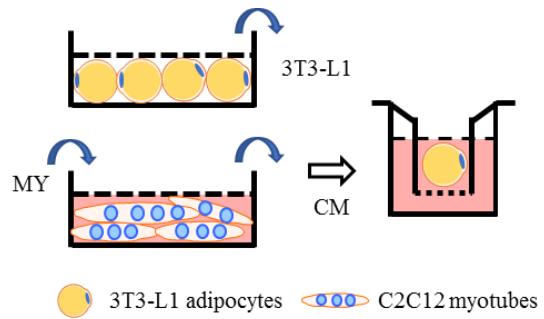
Table S2. List of oligonucleotide primer pairs used in qRT-PCR.
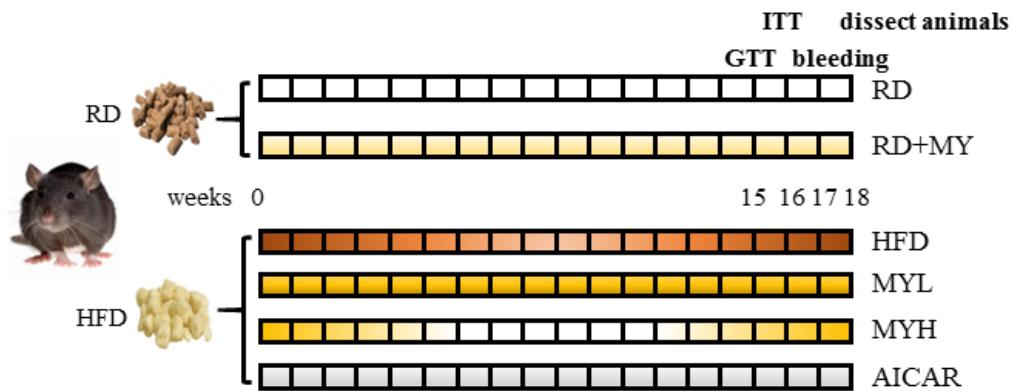
Gene	GenBank number	Forward	Reverse
18S	NR003278	AGCCTGCGGCTTAATTT GAC	CAACTAAGAACGGCC ATGCA
Ucp1	AK002759	ACTGCCACACCTCCAGT CATT	CTTTGCCTCACTCAG GATTGG
Cox7a	NM009944	CTCTGGTCCGGTCTTTT AGC	GTACTGGGAGGTCAT TGTCGG
Cidea	NM007702	TGCTCTTCTGTATCGCC CAGT	GCCGTGTTAAGGAAT CTGCTG
Ppara	NM011144	CTGAGACCCTCGGGGA AC	AAACGTCAGTTCACA GGGAAG
Cd36	NM001159558	AAATTGTACCTGGGAGT TGGCGAG	ACAGTTCCGATCACA GCCCATTCT
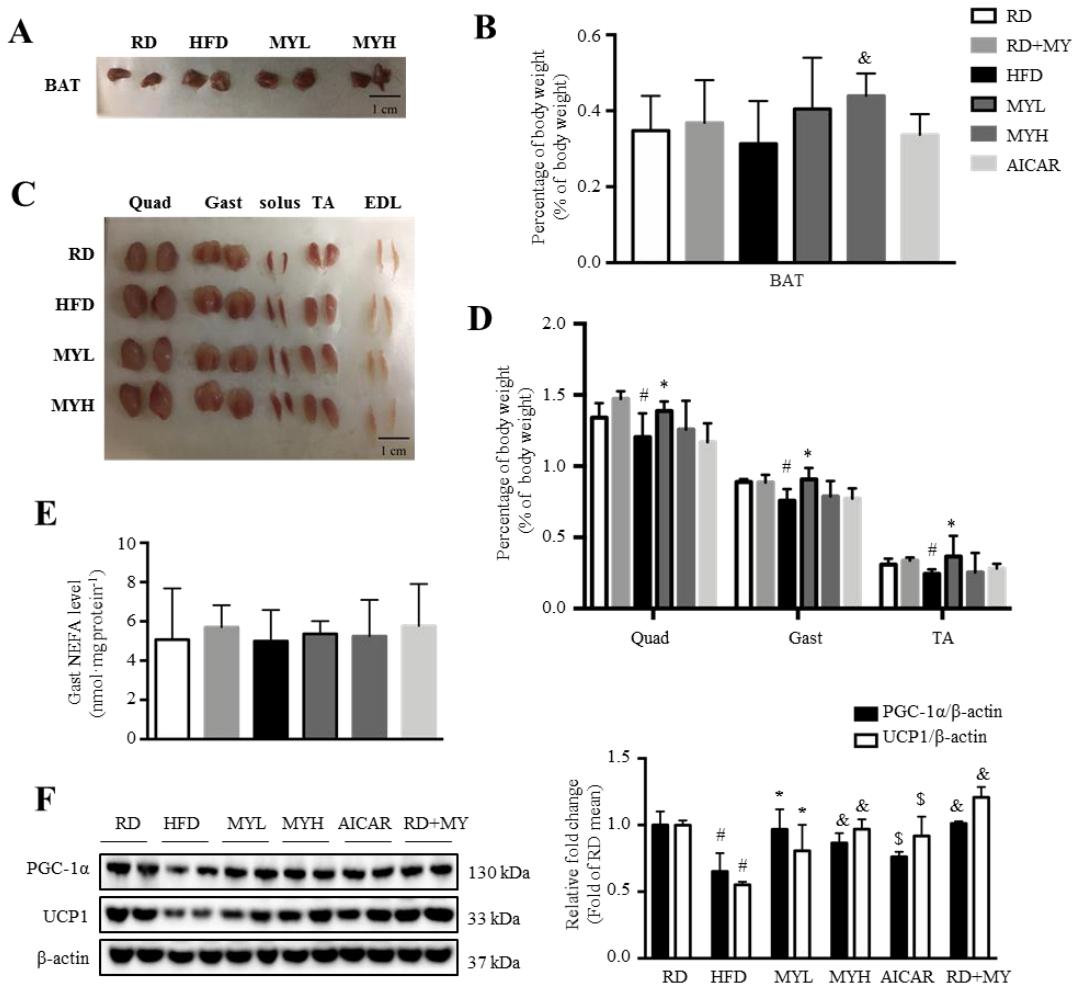
Figure S1. Cytotoxicity of PA and MY in C2C12 myotubes. Cytotoxicity of different concentrations of PA in C2C12 myotubes, assessed by MTT assay (A) and LDH release assay (B) ($n = 9$). (C) Lipid accumulation in C2C12 myotubes treated with different concentrations of PA, assessed by Nile red staining ($n = 9$). Cytotoxicity of MY in PA-treated C2C12 myotubes, assessed by MTT assay (D) and LDH release assay (E) ($n = 9$). Data are shown as mean ± S.D. #$p < 0.05$, vehicle *vs.* PA. *$p < 0.05$, MY *vs.* PA.

**Figure S2.** The experimental procedure of C2C12 myotubes and 3T3-L1 adipocytes co-culture.

**Figure S3.** The experimental procedure of HFD-fed mice model.

Figure S4. (A) Photographs of representative BAT. (B) The ratio of BAT to body weight (*n* = 6). (C) Photographs of representative skeletal muscle, including quadriceps, gastrocnemius, soleus, TA and EDL. (D) Tissue index of quadriceps, gastrocnemius, and TA muscles (*n* = 6). (E) NEFA level in Gast muscle (*n* = 6). (F) The expression levels of PGC-1α and UCP1 in BAT were determined by western blots. β-actin was used as a loading control. (*n* = 6). Data are shown as mean ± S.D. *$p < 0.05$, MYL *vs.* HFD. &$p < 0.05$, MYH *vs.* HFD or RD+MY *vs.* RD. #$p < 0.05$, RD *vs.* HFD. $$p < 0.05$, AICAR *vs.* HFD.