Supporting Information

For

Natural alkaloid bouchardatine ameliorates metabolic disorders in high fat fed mice *via* stimulating the SIRT1-LKB1-AMPK axis

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Materials

Fetal bovine serum (10099-141, Gibco, USA), Oleic acid sodium (OAS) (O1008 ,sigma, USA), DMEM/F12 (11330032, Gibco, USA), Peridochrom TG GPO-PAP kit (A110-1, JianCheng Bio, China), NAD^+/NADH Quantification Kit (#337-100, BioVision, USA), AMP/ATP Quantification Kit (MAK-135, Sigma-Aldrich, USA), ATP determination (S0026, Beyotine, China), SIRT1 Fluorometric Drug Discovery Kit (BLM-AK555, Enzo Life Sciences, Switzerland), SRT1720 (S1129, Selleck, USA), RNAiso Plus (9018, Takara, Japan), AlexaFluor-conjugated secondary antibody (#A32727, Life Technologies, USA), protein G/A beads (#88805, Thermo, USA), LKB1 plasmid (Vigene, USA), control siRNA/SIRT1 siRNA (RiboBio, China), Lipofectamin 3000 (L3000-015, Invitrogen, USA)**,** Co-immunoprecipitation Assay Kit (#88805, Thermo, USA), DNeasy Blood and Tissue kit (#69504, Qiagen, Germany), 60% fat-high fat diet (MD12033, DietResearch, USA), ultra-sensitive insulin Elisa Kit (#90080, Crystal Chem, USA)**.**

Table S1. Primers sequences used for PCR.

Gene	Forward primer(5'-3')	Reversed primer(5'-3')
SIRT-1	GACCTCCCAGACCCTCAAGC	TGTGACACAGAGACGGCTGG
PGC-1α	CTCAGTAAGGGGCTGGTTGC	AGGGCAATCCGTCTTCATCC
PPARα	CCTGGAAAGTCCCTTATCT	GCCCTTACAGCCTTCACAT
UCP-1	CGTACCAAGCTGTGCGATGT	AAGCCACAAACCCTTTGAAAAAG
NRF-2	GGTTCAGTGACTCGGAAATGG	GAGAATGTGCTGGCTGTGCT
CPT-1b	TAGGCCTCAACACCGAACAC	TGCCTTGGCTACTTGGTACG
ACSL	TGGGGTGGAAATCATCAGCC	CATTGCTCCTTTGGGGTTGC
Dio2	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
Actin	CTGAATCTGCACCAAGCATGA	TAAAACGCAGCTCAGTAACAGTCC
Cyto C	TCGGAACCCTCTACCTATT	GGCTGTGACGATGACATTAA
18S rRNA	AACTTTCGATGGTAGTCGC	TTCCTTGGATGTGGTAGCC

Table S2 Information of Antibody

Antibody name	Dilution ratio	Cat number	Company	Location
UCP1	1:1000	ab10983	Abcam	USA
PGC-1α	1:1000	ab54481	Abcam	USA
GAPDH	1:1000	#97166	Cell Signaling Technology	USA
AMPKα	1:1000	#5831	Cell Signaling Technology	USA
pAMPKα(Thr^{172})	1:1000	#2535	Cell Signaling Technology	USA
ACC	1:1000	#3676	Cell Signaling Technology	USA
pACC(Ser^{79})	1:1000	#11818	Cell Signaling Technology	USA
FAS	1:1000	#3810	Cell Signaling Technology	USA
SCD1	1:1000	#2794	Cell Signaling Technology	USA
SREBP-1c	1:250	sc-367	Santa Cruz Biotechnology	USA
LKB1	1:250	sc-5638	Santa Cruz Biotechnology	USA
pLKB1(Ser^{431})	1:250	sc-28465	Santa Cruz Biotechnology	USA
Acetyl-Lys	1:100	sc-81623	Santa Cruz Biotechnology	USA
Lamin B	1:1000	sc-374015	Santa Cruz Biotechnology	USA
SIRT1	1:1000	A0230	ABclonal Biotechnology	USA

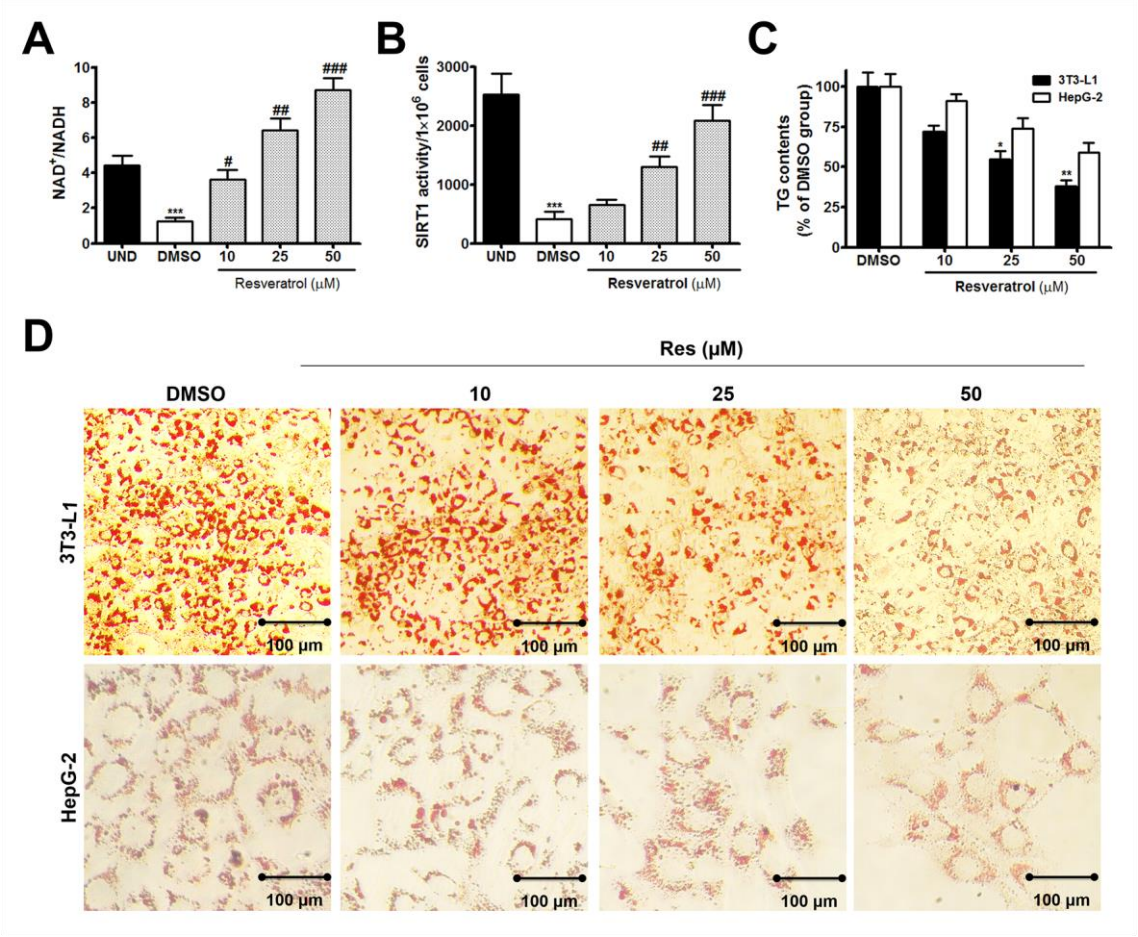

Figure S1. Effect of resveratrol (**Res**) on the TG levels and SIRT1 activity in cells. (A) Effect of Res on the ratio of NAD^+/NADH in 3T3-L1 adipocytes. *p<0.05, **p<0.01, ***p<0.001 compared with UND group; $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 compared with DMSO treatment group. (B) Effect of Res on the deacetylase activity of SIRT1 in 3T3-L1 adipocytes. *p<0.05, **p<0.01, ***p<0.001 compared with UND group; $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 compared with DMSO treatment group. (C-D) TG content analysis by TG assays and Oil-Red O staining. *p<0.05, **p<0.01, ***p<0.001 compared with DMSO group. Data are expressed as means ± standard errors from 5 independent experiments.

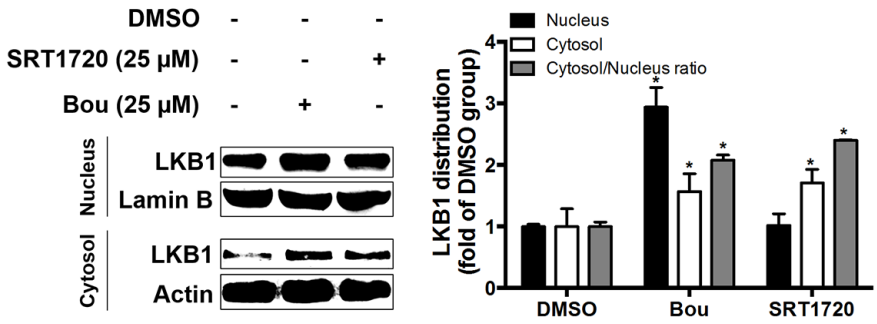

Figure S2. SRT1720 increases LKB1 translocation to cytosol in 3T3-L1 adipocytes. Confluent 3T3-L1 pre-adipocytes were exposed to adipogenic cocktail (MDI) for consecutive 9 days in the presence or absence of SRT1720 treatment. After treatment, LKB1 in cytosol and nucleus were extracted and determined by western blot, GAPDH and Lamin B were loaded as loading control. *p<0.05, compared with DMSO group.

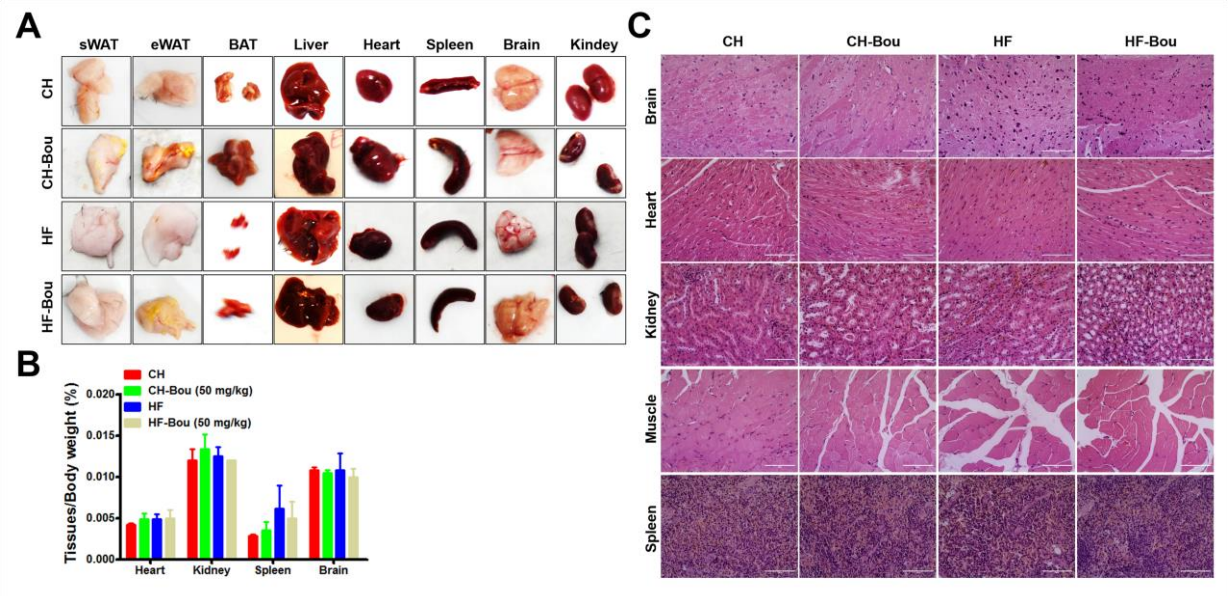

Figure S3. Toxicity analysis of bouchardatine *in vivo*. (A) Appearance of tissue was captured. (B-C) Tissue/body weight ratio. (C) Representative H&E staining from tissue sections after 5 weeks of **Bou** treatment. Original magnification, 100×. Scale bar, 100 μm.

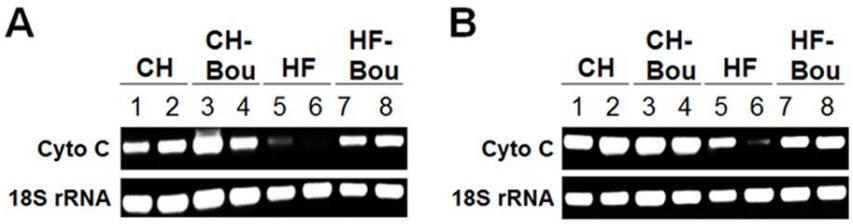

Figure S4. Expression levels of Cytochrome C gene (for mtDNA) and 18S rRNA (for nuclear DNA) in eWAT (A) and BAT (B) of mice.