

Supplementary Information

Isoquercitrin from *Apocynum venetum* L produces anti-obesity effect for obese mice by targeting C-1-tetrahydrofolate synthase, carbonyl reductase, and glutathione S-transferase P and modification of AMPK/SREBP-1c/FAS/CD36 signaling pathway in mice *in vivo*

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Supplementary Tables

Table S1. Effect of Isoquercitrin on blood biochemical parameters of obese mice

Groups	ALT (U/L)	AST (U/L)	TG (mmol/L)	TC (mmol/L)	Glu (mmol/L)
ND	44.92 ± 2.56	104.10 ± 6.56	1.49 ± 0.11	4.73 ± 0.20	3.40 ± 0.22
HFD	52.27 ± 1.83 #	124.24 ± 4.51 #	2.22 ± 0.11 ##	8.70 ± 0.46 ###	7.45 ± 0.89 #
HFD + 140 mg/kg Metformin	43.05 ± 1.96 **	99.65 ± 7.22 *	1.98 ± 0.18	6.98 ± 0.63 *	4.06 ± 0.69 *
HFD + 0.1 mg/kg Isoquercitrin	58.74 ± 3.83	118.56 ± 6.87	1.71 ± 0.11 *	8.66 ± 0.41	7.06 ± 0.33
HFD + 5 mg/kg Isoquercitrin	40.69 ± 1.75 ***	102.17 ± 7.07 *	1.62 ± 0.03 **	6.42 ± 0.70 *	4.45 ± 0.37 *

ND: normal diet; HFD: high-fat diet; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TG: triglyceride; TC: total cholesterol; GLU: glucose. Numbers of mice in each group are eight. #, ## and ### represent the difference between ND group and HFD group. *, **, and *** represent the difference between HFD group and treatment groups.

Table S2 · Effect of Isoquercitrin on the weight of individual organs of obese mice

Groups	Liver	Spleen	Kidney	Heart	Epididymal Fat
ND	1.45 ± 0.04	0.09 ± 0.01	0.58 ± 0.02	0.21 ± 0.02	1.02 ± 0.15
HFD	2.4 ± 0.12 ^{###}	0.11 ± 0.01	0.68 ± 0.04 [#]	0.22 ± 0.02	2.36 ± 0.16 ^{###}
HFD + 140 mg/kg Metformin	1.88 ± 0.10 ^{**}	0.11 ± 0.03	0.57 ± 0.03 [*]	0.18 ± 0.01	1.07 ± 0.25 ^{***}
HFD + 5 mg/kg AV	1.81 ± 0.11 ^{**}	0.10 ± 0.01	0.57 ± 0.02 [*]	0.19 ± 0.01	1.62 ± 0.25 [*]
HFD + 0.1 mg/kg Isoquercitrin	2.06 ± 0.12	0.11 ± 0.01	0.66 ± 0.03	0.22 ± 0.01	1.90 ± 0.25
HFD + 0.5 mg/kg Isoquercitrin	1.60 ± 0.07 ^{***}	0.09 ± 0.00	0.60 ± 0.02	0.17 ± 0.01 [*]	1.46 ± 0.19 ^{**}
HFD + 5 mg/kg Isoquercitrin	1.56 ± 0.02 ^{***}	0.08 ± 0.00 ^{**}	0.60 ± 0.01 [*]	0.17 ± 0.01 [*]	1.47 ± 0.18 ^{**}

ND: normal diet; HFD: high-fat diet; AV: *A. venetum* L. Numbers of mice in each group are eight. # and ^{###} represent the difference between ND group and HFD group. *, ** and *** represent the difference between HFD group and treatment groups.

Table S3 · Effect of SREBP-1c and AMPK inhibitors on the weight of individual organs of obese mice

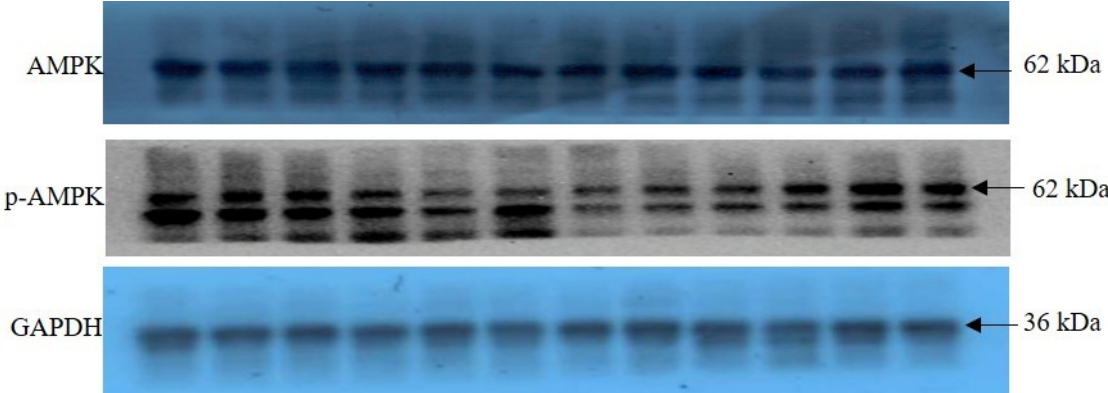
Groups	Liver	Spleen	Kidney	Heart	Epididymal Fat
ND	1.35 ± 0.04	0.09 ± 0.01	0.46 ± 0.03	0.18 ± 0.01	0.41 ± 0.07
HFD	1.49 ± 0.04 [#]	0.12 ± 0.01 [#]	0.66 ± 0.02 ^{##}	0.20 ± 0.01	0.70 ± 0.06 ^{##}
HFD + 5 mg/kg Isoquercitrin	1.36 ± 0.04 [*]	0.09 ± 0.00 [*]	0.45 ± 0.03 [*]	0.16 ± 0.01	0.47 ± 0.07 [*]
HFD + 5 mg/kg Isoquercitrin + PF429242	1.47 ± 0.4	0.13 ± 0.01	0.59 ± 0.03	0.22 ± 0.01	0.74 ± 0.06
ND	1.25 ± 0.03	0.17 ± 0.01	0.50 ± 0.03	0.21 ± 0.00	0.48 ± 0.09
HFD	2.27 ± 0.18 ^{###}	0.16 ± 0.01	0.69 ± 0.06 [#]	0.21 ± 0.01	1.49 ± .05 ^{###}
HFD + 5 mg/kg Isoquercitrin	1.12 ± 0.09 ^{***}	0.08 ± 0.00 ^{***}	0.46 ± 0.02 ^{**}	0.17 ± 0.00 ^{**}	0.62 ± .08 ^{***}
HFD + 5 mg/kg Isoquercitrin + Compound C	2.12 ± 0.09	0.12 ± 0.01	0.55 ± 0.03	0.18 ± 0.01	1.34 ± 0.16

ND: normal diet; HFD: high-fat diet. Numbers of mice in each group are six. [#] and ^{##} represent the difference between ND group and HFD group. ^{*} represents the difference between HFD group and treatment groups.

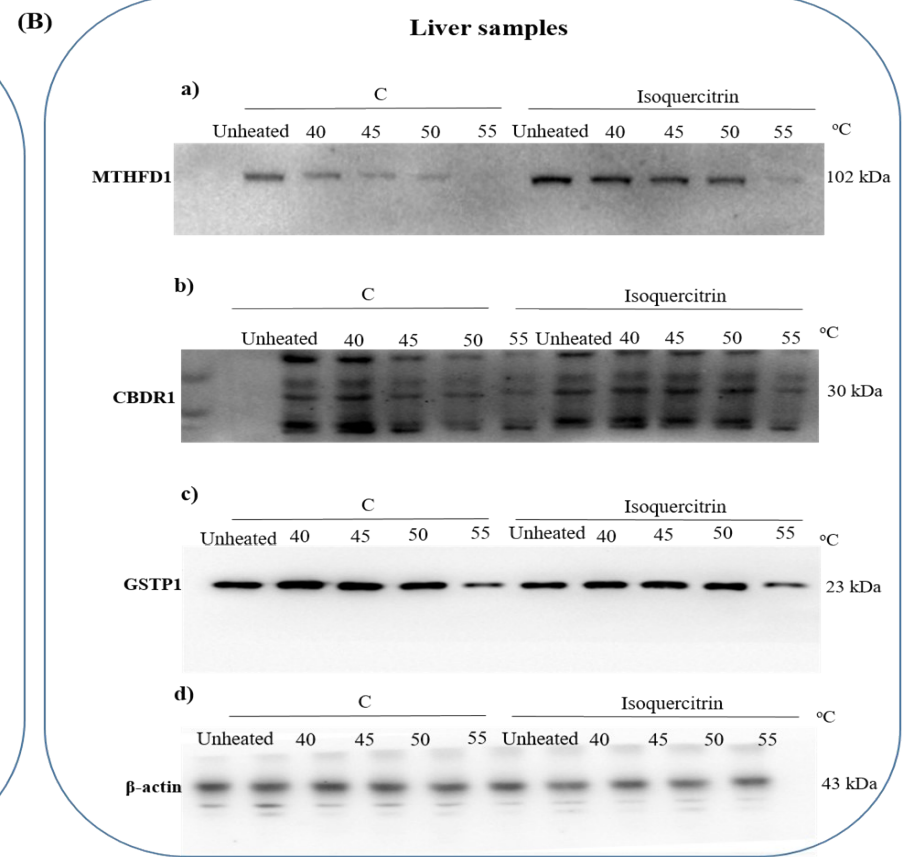
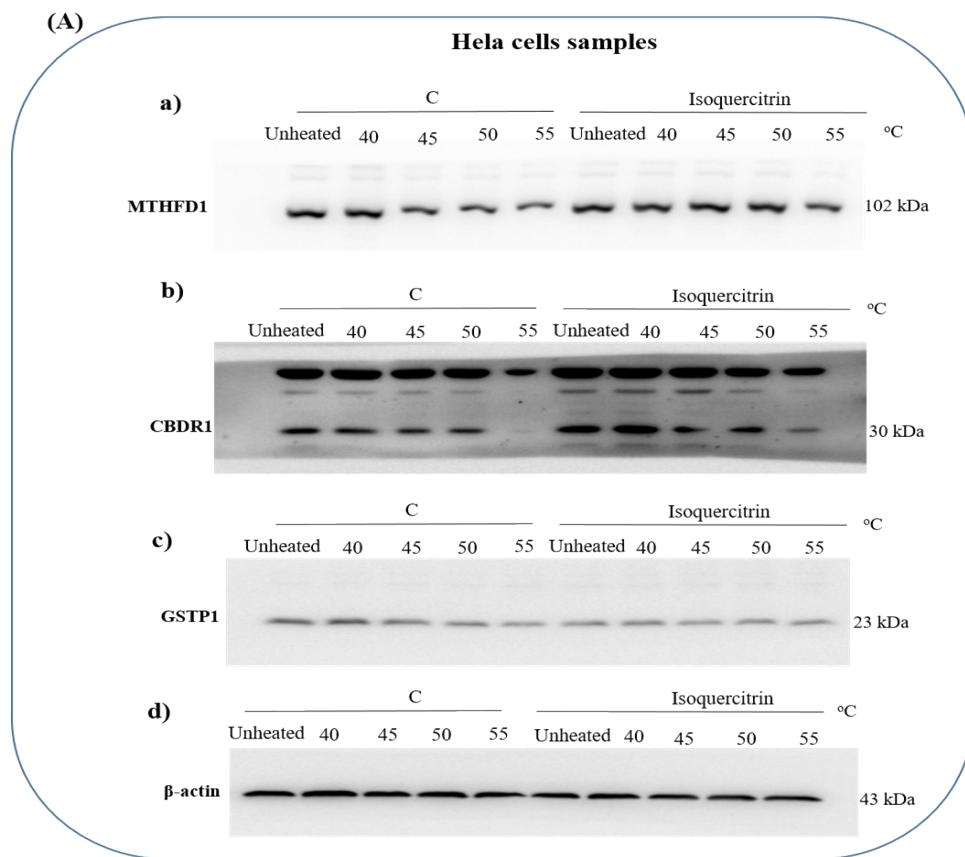
Table S4. Primers sequence in RT-PCR analysis of this study

Gene	Species	Sequences
SREBP-1C	mouse	sense: 5'-GCG CTA CCG GTC TTC TAT CA- 3'
	mouse	anti-sense: 5'-TGC TGC CAA AAG ACA AGG G-3'
FAS	mouse	sense: 5'-GAT CCT GGA ACG AGA ACA C- 3'
	mouse	anti-sense: 5'-AGA CTG TGG AAC ACG GTG GT-3'
SCD1	mouse	sense: 5'-CGA GGG TTG GTT GTT GAT CTG T- 3'
	mouse	anti-sense: 5'-ATA GCA CTG TTG GCC CTG GA-3'
CD36	mouse	sense: 5'-TCC TCT GAC ATT TGC AGG TCT ATC- 3'
	mouse	anti-sense: 5'-GTG AAT CCA GTT ATG GGT TCC AC-3'
GLUT1	mouse	sense: 5'-GTT TCA CAG CCC GCA CAG CTT GA-3'
	mouse	anti-sense: 5'-GCC CCT CCC ACG GCC AAC ATA-3'
GLUT2	mouse	sense: 5'-CAT CCA TCT TCC TCT TTG TCT G-3'
	mouse	anti-sense: 5'-GAT TTT CCT TTG GTT TCT GG-3'
GLUT4	mouse	sense: 5'-CCT GCC CGA AAG AGT CTA AAG C-3'
	mouse	anti-sense: 5'-ACT AAG AGC ACC GAG ACC AAC G-3'
18S RNA	mouse	sense: 5'-TAA CCC GTT GAA CCC CAT T-3'
	mouse	anti-sense: 5'-CCA TCC AAT CGG TAG TAG CG-3'

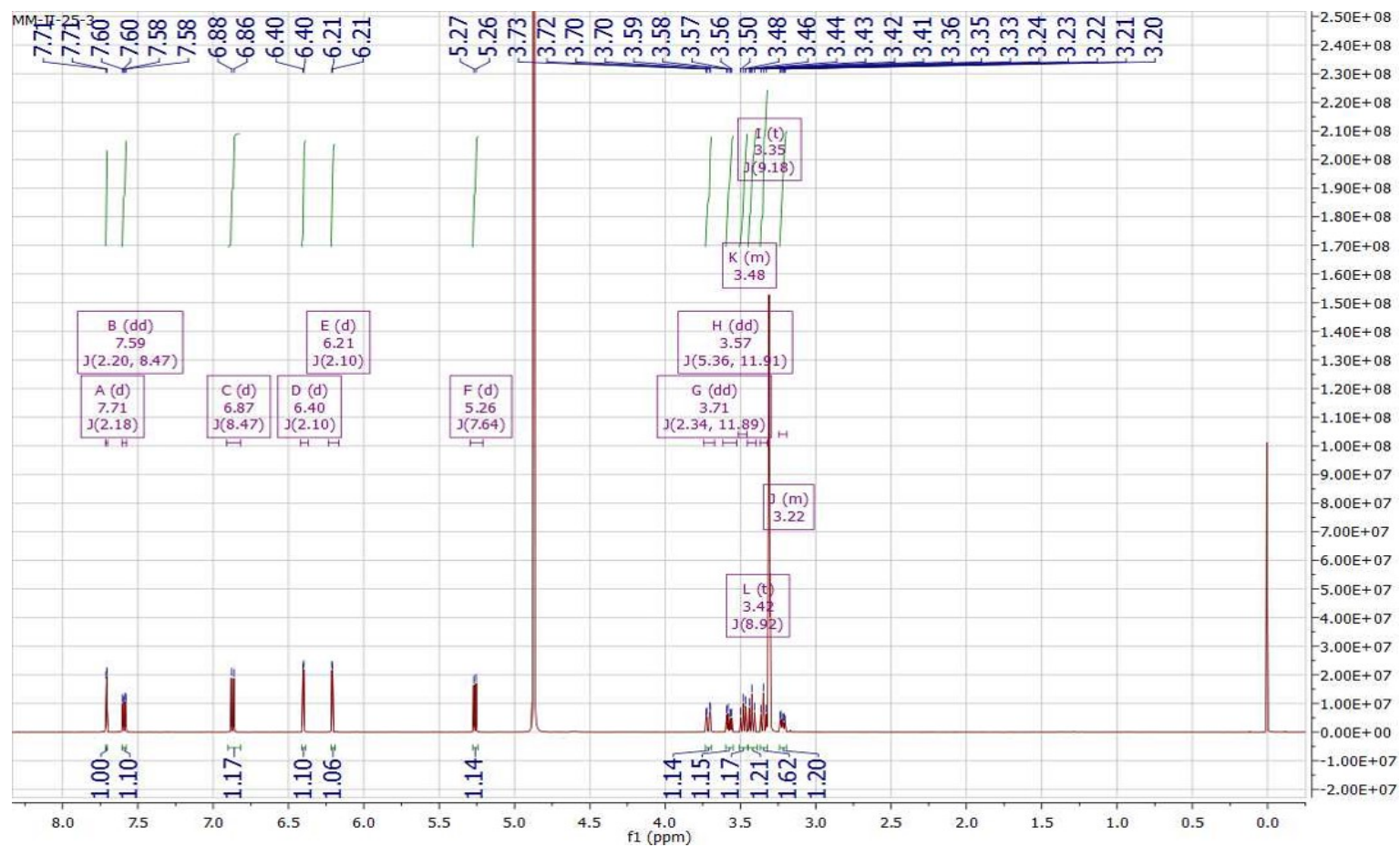
Supplementary Figures



Supplementary Figure 1. Original western blot analysis of Figure 3C. Original western blot of AMPK, phospholation-AMPK and GAPDH.



Supplementary Figure 2. Original western blot analysis of Figure 9. Original western blot results of MTHFD1 (a), CBDR1 (b), GSTP1 (c) and β -actin (d) in HeLa cells for Figure 9A, 9B and 9C, respectively. Original western blot results of MTHFD1 (a), CBDR1 (b) GSTP1 (c) and β -actin (d) in mice livers for Figure 9D, 9E and 9C, respectively



Supplementary Figure 3. The ^1H NMR spectrum of isoquercitrin from *Apocynum venetum* L