# Supporting Information

## The natural dihydrochalcone phloretin reduces lipid accumulation via downregulation of sbp-1/SREBP pathway in HepG2 cells and Caenorhabditis elegans

*Qi Gu* [a, #], *Litao Wang* [a, #], *Mingyue Xu* [a], *Wanmei Zhou* [a], *Guosheng Liu* [a], *Haiting Tian* [a], *Thomas Efferth* [c], *Chenlu Wang* [a, *], *Yujie Fu* [a,b *]

[a] The College of Forestry, Beijing Forestry University, 100083, Beijing, PR China.

[b] Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, 150040, Harbin, PR China.

[c] Department of Pharmaceutical Biology, Institute of Pharmaceutical and Biomedical Sciences, Johannes Gutenberg University, Mainz, Germany.

[*] Corresponding author.

Tel: +010 62336778.

E-mail address: yujie_fu@163.com (Y.-J. Fu).

## Establishment of a metabolomics assay for *C. elegans*

An in-house quality control sample was prepared by mixing an equal amount of each constituent sample. Extracted *C. elegans* samples were analyzed using a Thermo ScientificTM Q Exactive TM system (Waltham, MA, USA). The chromatographic column was performed with a Accuity UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm) maintained at 40 ℃, inject 1 μL in positive and negative mode respectively. The mobile phase was the aqueous solution with 0.1% formic acid (A) and acetonitrile solution (B). The flow rate was 0.35 mL/min. Post time was set as 5 min for system balance. The optimized elution gradient was as follows: 0 to 1 min, 3 to 3.3% B; from 1 to 3 min, 3 to 50% B; from 3 to 5 min, 50–60% B; from 5 to 8 min, 60 to 92 % B; from 8 to 14 min, 92% B; from 14 to 15 min, 92 to 3% B; and from 15 to 18 min, 3% B for equilibrating the systems. Mass spectrometry was operated in both positive and negative ion modes.

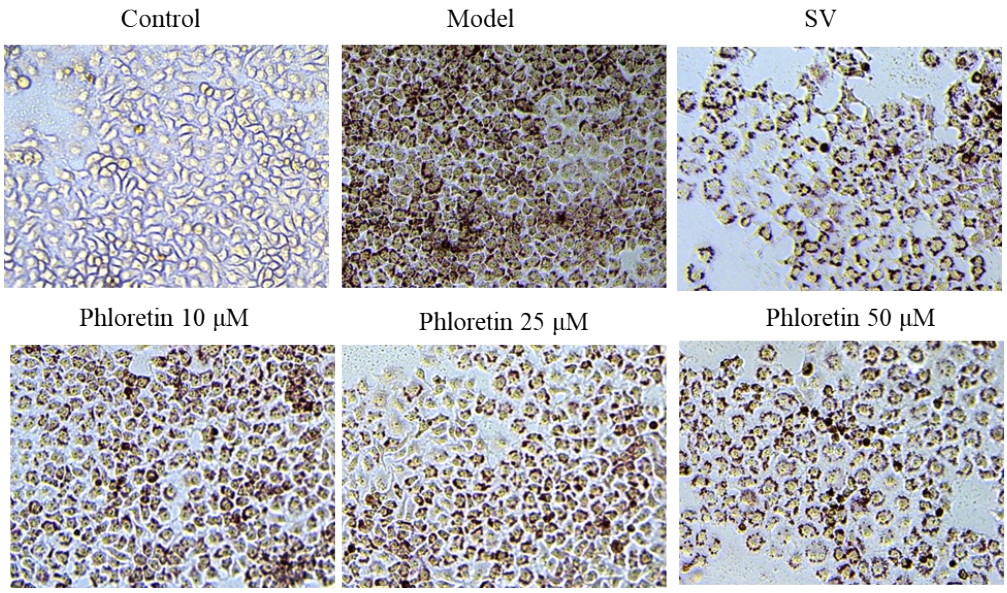


Figure S1. Oil Red O staining of lipid droplets in HepG2 cells treated with phloretin. Representative bright-field micrographs showing intracellular lipid accumulation (red droplets).

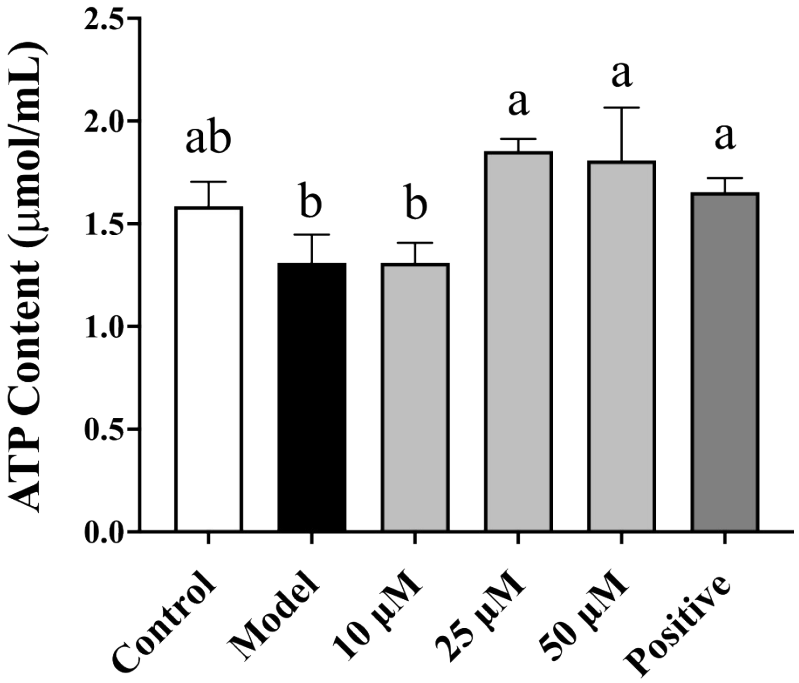


Figure S2. Effects of phloretin on intracellular ATP levels in HepG2 cells. Quantitative analysis showing ATP concentration changes. Values represent mean ± SEM (n = 3 independent replicates). Bars labeled with different letters indicate statistically significant differences ($p < 0.05$ by ANOVA/Tukey's test).

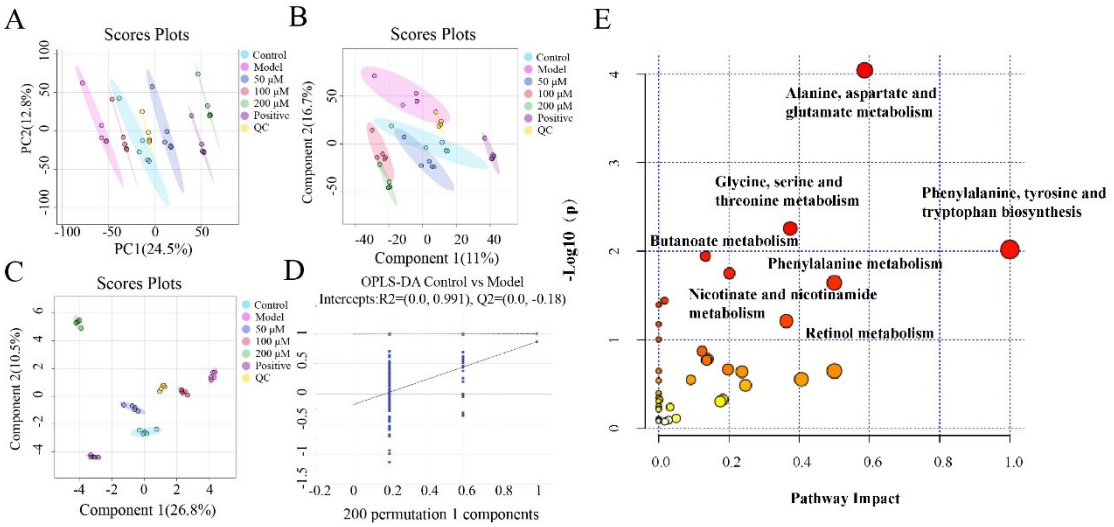


Figure S3. The results of metabolomics analysis. A-B, Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). C-D, The result of OPLS-DA analysis. E, KEGG pathway analysis of differential metabolites.

**Table S1.** (Primer sequence of HepG2 cell)

| GENE | Forward (5′-3′) | Reverse (5′-3′) |
|---|---|---|
| GAPDH | GTCAAGGCTGAGAACGGGAA | AAATGAGCCCCAGCCTTCTC |
| SREBP | GTGAAGACCAGTCTCCCACG | TCCCCATCCACGAAGAAACG |
| ACC | AGGAGCTGTCTATTCGGGGT | GGTCGCTCAGCCTGTACTTT |
| PPARα | GCTTCGCAAACTTGGACCTG | GCTACCAGCATCCCGTCTTT |
| SCD | CCACTTGCTGCAGGACGATA | TAGAGGGGCATCGTCTCCAA |
| FASN | GTCTTGAACTCCTTGGCGGA | GAGCGGGTGGTTCTGAGAAA |
| AKT1 | CAGGATGTGGACCAACGTGA | AAGGTGCGTTCGATGACAGT |
| MTOR | CAGCTAAGTCCACCCTGGTG | GTGAAGGCAGAAGGTCGGAA |
| FOXO4 | TCTACGAGTGGATGGTCCGT | ATGAACTTGCTGTGCAGGGA |

**Table S2.** (Primer sequence of *C. elegans*.)

| GENE | Forward (5′-3′) | Reverse (5′-3′) |
|---|---|---|
| *act-1* | ACGAGGTTGCCGCTCTTGTTG | ACGAGTCCTTCTGTCCCATACCG |
| *daf-2* | GCTCTCGGAACAACCACTGA | GACAAGTCGAAGCCGTCTCA |
| *age-1* | TCTCTGGGCTCGTACGAAGA | GGCACGATTCACCAACACAC |
| *akt-1* | CAAAGCCTAAGGAAGGACAACC | CATGAATCCAACGCTGACGAAC |
| *skn-1* | AGTGCTTCTCTTCGGTAGCCG | TGTTGGACGATGGTGAACTGA |
| *daf-16* | TCGTCGTCTCGTGTTTCTCCA | TTCCATAGGCACCCGGTAGTG |
| *sod-3* | TCTCCAACCAGCGCTGAAAT | TTTGCACAGGTGGCGATCTT |
| *sbp-1* | GGCGGCGAAGATTGTGATTC | GGCGGCGAAGATTGTGATTC |

| | | |
|---|---|---|
| *mdt-15* | CAAAACCAGACAAGCGGTGG | CAATTCAGACGAGGCGGAGT |
| *nhr-49* | CTGCAACGGGTGTAAGGGAT | GACACGCGCATCGAAAGTTT |
| *fat-5* | GGATGGCAGCCATACGATCA | CTCCGACTGCCGCAATAGAT |
| *fat-6* | AAGATTGAGAAGGACGGCGG | TTCATTTGGATCCACGGCGA |
| *fat-7* | TCGTTGCCATCACAAGTGGA | GAGTTTGCCTCCATGCTCCT |

**Table S3.** (Identification of differential metabolic compounds in KEGG pathway using UPLC-Q-Exactive Orbitrap-MS method)

| Name | RT [min] | Formula | Calc. MW | m/z | Fragments | Reference Ion |
|---|---|---|---|---|---|---|
| 4-acetamidobutyric acid | 2.305 | C6 H11 N O3 | 145.073 4 | 354.163 4 | 296.07184,223.06178,166.084 9 | [2M+ACN+Na]+ 1 |
| 5-Aminopentanoic acid | 1.776 | C5 H11 N O2 | 117.078 | 140.067 1 | 118.08549, 58.06529 | [M+Na]+1 |
| Betaine | 1.1 | C5 H12 N O2 | 117.078 1 | 118.085 4 | 101.05921,58.06531 | [M+H]+1 |
| Choline | 1.076 | C5 H14 N O | 103.099 1 | 104.106 4 | 60.08096，58.06533 | [M+H]+1 |
| Dopamine | 5.262 | C8 H11 N O2 | 153.079 2 | 348.192 5 | 269.13345,154.08517 | [2M+ACN+H]+1 |
| L-(+)-Alanine | 1.078 | C3 H7 N O2 | 89.0473 8 | 90.0546 5 | 90.0547 | [M+H]+1 |
| Leucine | 1.607 | C6 H13 N O2 | 131.093 6 | 132.100 8 | 132.10081,86.0961 | [M+H]+1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| L-Histidine | 1.117 | C6 H9 N3 O2 | 155.0682 | 156.0755 | 156.07459,110.07059 | [M+H]+1 |
| L-Phenylalanine | 1.934 | C9 H11 N O2 | 165.0776 | 166.0849 | 166.08496, 102.0459 | [M+H]+1 |
| L-Threonine | 1.082 | C4 H9 N O3 | 119.0576 | 120.0646 | 120.07988, 97.00709, 78.99681 | [M+H]+1 |
| L-Tyrosine | 1.285 | C9 H11 N O3 | 181.0725 | 182.0797 | 165.03025, 91.05385 | [M+H]+1 |
| Succinic acid | 3.878 | C4 H6 O4 | 118.0271 | 119.0343 | 119.03428,118.06422 | [M+H]+1 |
| 1,2-dihexadecanoyl-sn-glycero-3-phosphoserine | 13.192 | C38 H74 N O10 P | 735.5031 | 734.4958 | 734.49707,733.49335 | [M-H]-1 |
| 1-myristoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine | 18.784 | C37 H72 N O8 P | 689.4996 | 690.5069 | 690.50714,689.50543 | [M+H]+1 |
| Arachidonic acid | 14.519 | C20 H32 O2 | 304.2357 | 303.2284 | 303.22882，231.44618 | [M-H]-1 |
| Ceramide | 1.259 | C42 H81 N O3 | 647.6218 | 646.6146 | 628.6028 | [M-H]-1 |
| Choline | 1.076 | C5 H14 N O | 103.0991 | 104.1064 | 60.08096，58.06533 | [M+H]+1 |
| LysoPC | 4.815 | C30 | 571.364 | 286.689 | 286.2985 | [M+2H]+2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | H54 N O7 P | 4 | 5 | | |
| Palmitic Acid | 9.274 | C16 H32 O2 | 273.264 | 274.271 2 | 256.26082,71.08537 | [M+H]+1 |
| TxB2 | 7.085 | C20 H34 O6 | 370.236 5 | 371.244 | 371.244 | [M+H]+1 |