A flavonoid-rich *Smilax china* L. extract prevents obesity by upregulating the adiponectin-receptor/AMPK signalling pathway and modulating the gut microbiota in mice

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Table S1. Reagents and materials

The name of reagent	manufacturers
Haematoxylin and eosin (H&E)	Wuhan Seville Biological Technology Co., Ltd.
Oil Red O	Wuhan Seville Biological Technology Co., Ltd.
total cholesterol (TC)	Nanjing Jiancheng Bioengineering Institute
triglycerides (TG)	Nanjing Jiancheng Bioengineering Institute
lipopolysaccharide (LPS)	Nanjing Jiancheng Bioengineering Institute
adiponectin	Cloud-Clone Corp
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)	Boster biological technology
(PVDF) membrane	Millipore
Bicinchoninic acid (BCA)	Beijing Pulai gene Technology Co. LTD
Bovine serum albumin (BSA)	Beijing Pulai gene Technology Co. LTD
ACC #BM4414	Boster biological technology
CPT-1α #AO1192	Boster biological technology
AdipoR1 #66619	Proteintech
AMPK #66536	Proteintech
UCP1 #23673	Proteintech
SREBP1-c #14088	Proteintech
GAPDH #60004	Proteintech
AdipoR2 #A12777	ABclonal
p-AMPK #AF3427	AffinitY

Table S2. Ingredients formula of normal-chow (NC) and high-fat/high-sucrose (HFHS) diets (g/kg)

Diet composition	NC	HFHS
Casein	140.0	233.06
Corn starch	465.7	84.83
Dextrin	155.0	116.53
Soybean oil	40.0	29.13
Lard	-	206.84
L-cystine	1.8	3.5
Sucrose	100.0	201.52
Cholinebitartrate	2.5	2.33
Cellulose	50.0	58.26
Mineral mix AIN-93	35.0	52.44
Vitamin mix AIN-93	10.0	11.56
Total	1000	1000

Table S3. Primer Sequences

Primer Name	Forward	Reverse
Adiponectin	*5'-TCCCAATGTACCCATTCGCT-3'*	*5'-AGTCCCGGAATGTTGCAGTA-3'*
AdipoR1	*5'-CGTTGGAGAGTCATCCCGTA-3'*	*5'-ACCAAGCAGATGTGTCCAGA-3'*
AdipoR2	*5'-CTCTCTGGTCGTGCACTTTG-3'*	*5'-GACTACAGTGGTGGTGGTGA-3'*
AMPKα	*5'-CCGGACATAAAGTGGCTGTG-3'*	*5'-ATGATGTGAGGGTGCCTGAA-3'*
ACCα	*5'-TATCAGTTTCCCAGCCAGCA-3'*	*5'-ATCCTACACCACAGCCTTCA-3'*
SREBP1-c	*5'-ACACTTCTGGAGACATCGCA-3'*	*5'-CGGATGAGGTTCCAAAGCAG-3'*
FAS	*5'-CACAGCAACCAGCAATACAAA-3'*	*5'-TTCAGCAATTCTCGGGATGT-3'*
PPARα	*5'-GCATGTGAAGGCTGTAAGGG-3'*	*5'-TTGTGTGACATCCCGACAGA-3'*
CPT-1α	*5'-AGTCCTGCAACTTTGTGCTG-3'*	*5'-GGTGAGTCGACTGCCAGATA-3'*
UCP1	*5'-AAAGTCCGCCTTCAGATCCA-3'*	*5'-TGAGTCGTAGAGGCCAATCC-3'*
β-actin	*5'-GGTCATCACTATTGGCAACG-3'*	*5'-TCCATACCCAAGAAGGAAGG -3'*

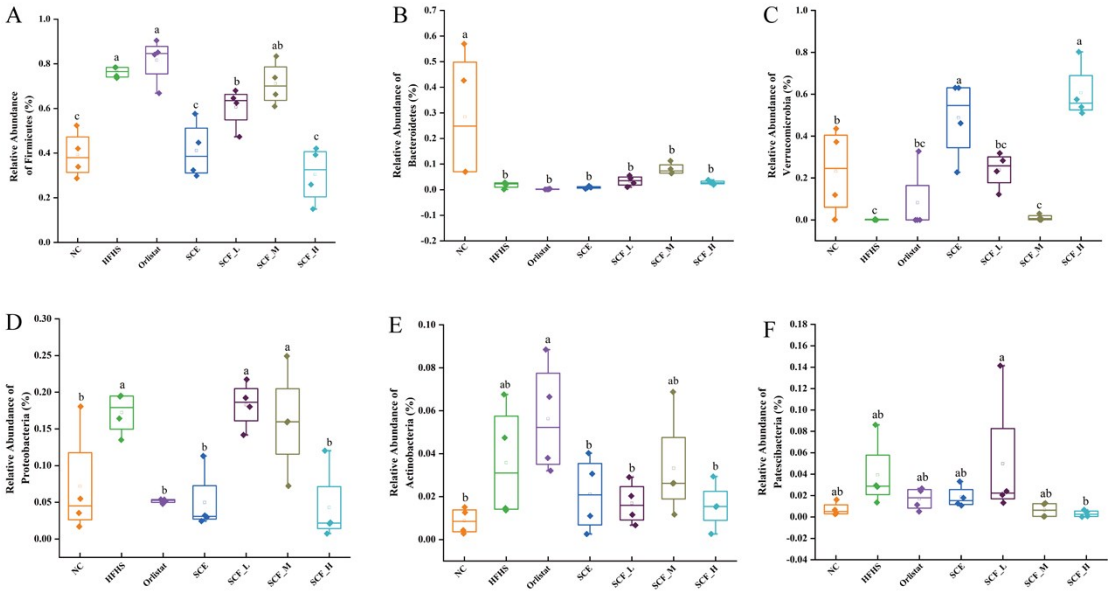

Fig. S1 SCF modulated the composition of gut microbiota at the phylum level.

(A) *Firmicutes.* (B) *Bacteroidetes.* (C) *Verrucomicrobia.* (D) *Proteobacteria.* (E) *Actinobacteria.* (F) *Patescibacteria.* (n = 4). Significance of the differences between groups was calculated using Kruskal Wallis test with Dunn's multiple comparison test. The different letters denote that the mean values are significantly different from one another ($p < 0.05$).

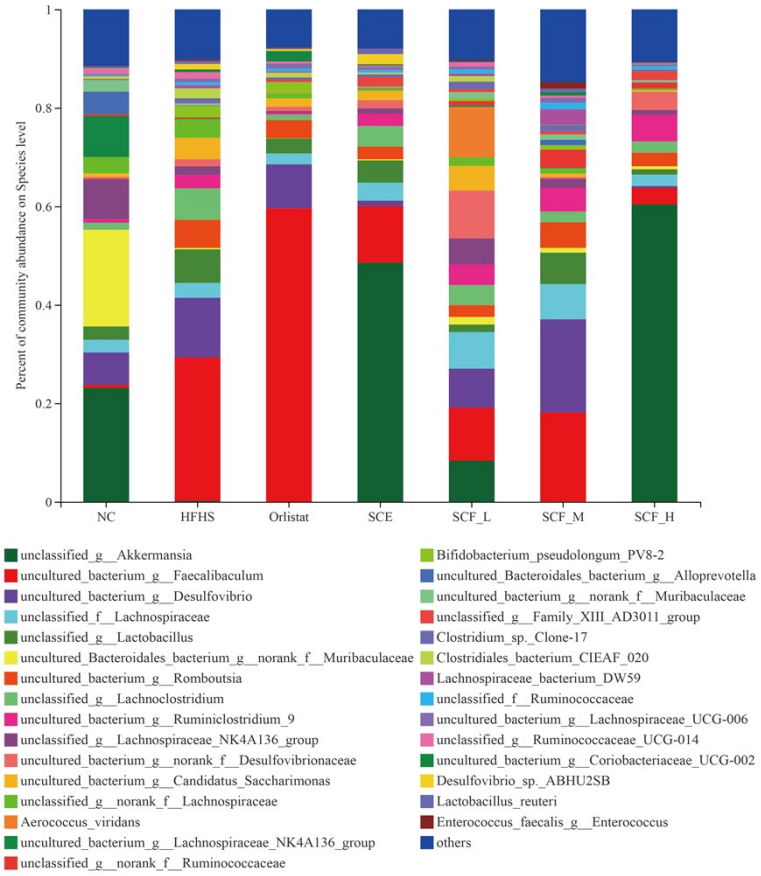

Fig. S2 SCF modulated the gut microbiota composition at the species level.

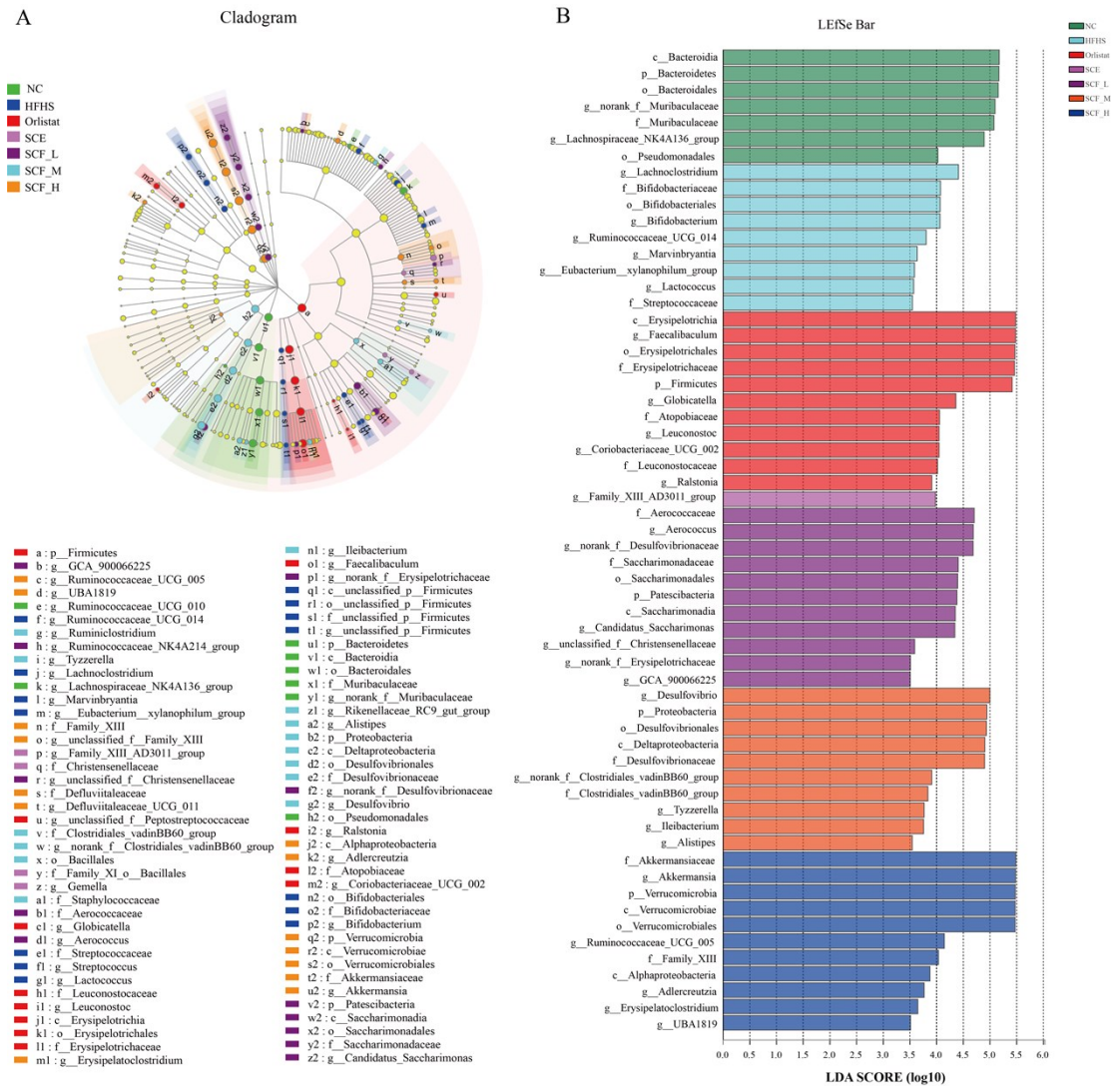

Fig. S3 Relative abundance of key-phylotypes identified in the gut microbiota of SCF-treated mice. (A) Cladogram generated from LEfSe analysis showing the relationship between taxon. The circles from the outside to the inside indicate genus, family, order, class, phylum, and kingdom. (B) LDA score (log 10).

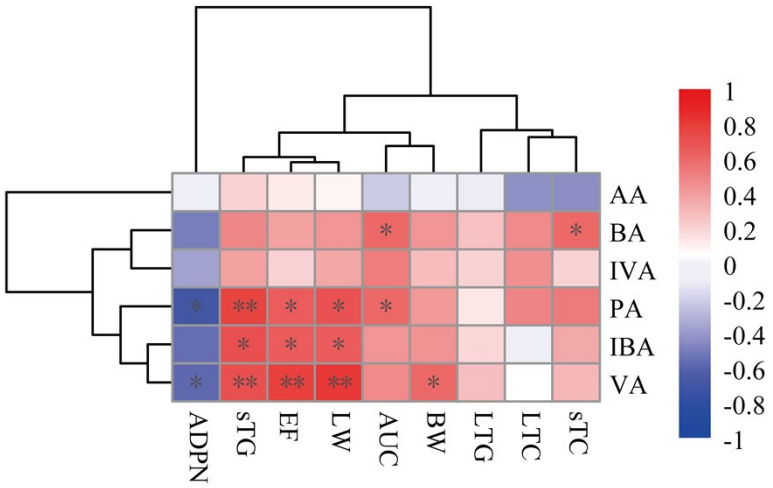

Fig. S4 The Spearman correlation of SCFAs and obesity-associated markers. Red and blue cells indicate positive and negative correlations, respectively.

ADPN: Adiponectin; BW: Body weight; EF, weight of epididymal fat; LW, liver weight; LTG: TG content in the liver; sTG: TG content in serum; AUC: areas under the glucose-concentration-time curve; LPS: lipopolysaccharide; AA: acetic acid; BA: butyric acid; IVA: isovaleric acid; PA: propionic acid; IBA: isobutyric acid; VA: valeric acid.