Supplementary Information for

Original article

Cordycepin promotes browning of white adipose tissue through an AMP-activated protein kinase (AMPK)-dependent pathway

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Table S1 Oligonucleotide primers used in this work.

Name	Forward (5′–3′)	Reverse (5′–3′)
CIDEA	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
UCP1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
PGC1α	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
FNDC5	ATGAAGGAGATGGGGAGGAA	GCGGCAGAAGAGAGCTATAACA
CYTOC	CCAAATCTCCACGGTCTGTTC	ATCAGGGTATCCTCTCCCCAG
FATP1	CGCTTTCTGCGTATCGTCTG	GATGCACGGGATCGTGTCT
CPT1B	ACCACTGGCCGCATGT	CTCCATGGCGTAGTAGTTGCT
β-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

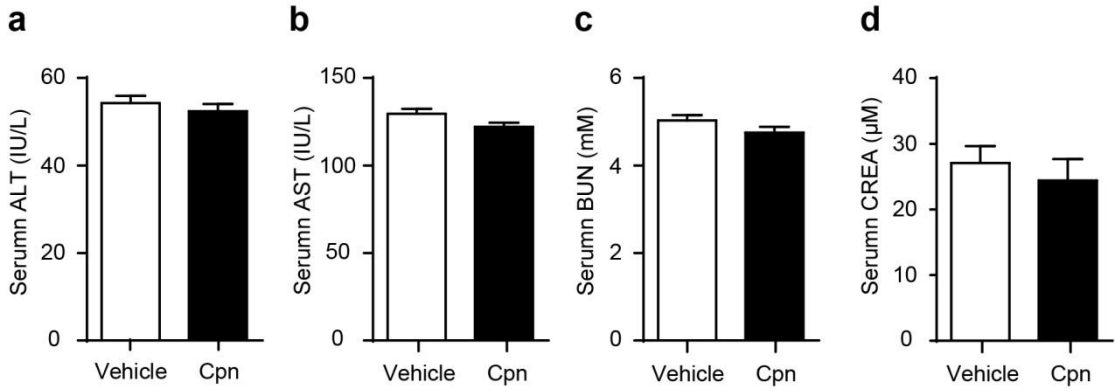

Figure S1 Cordycepin does not induce toxicity. Serum levels of (a) aspartate aminotransferase (ASAT), (b) alanine aminotransferase (ALAT), (c) urea nitrogen (BUN) and (d) creatinine (CREA) were measured after animals had been treated with cordycepin for 4 weeks (n = 7). Data are displayed as mean ± s.e.m. Significant differences compared with vehicle controls are assessed by Student's t-test. Cpn: cordycepin.

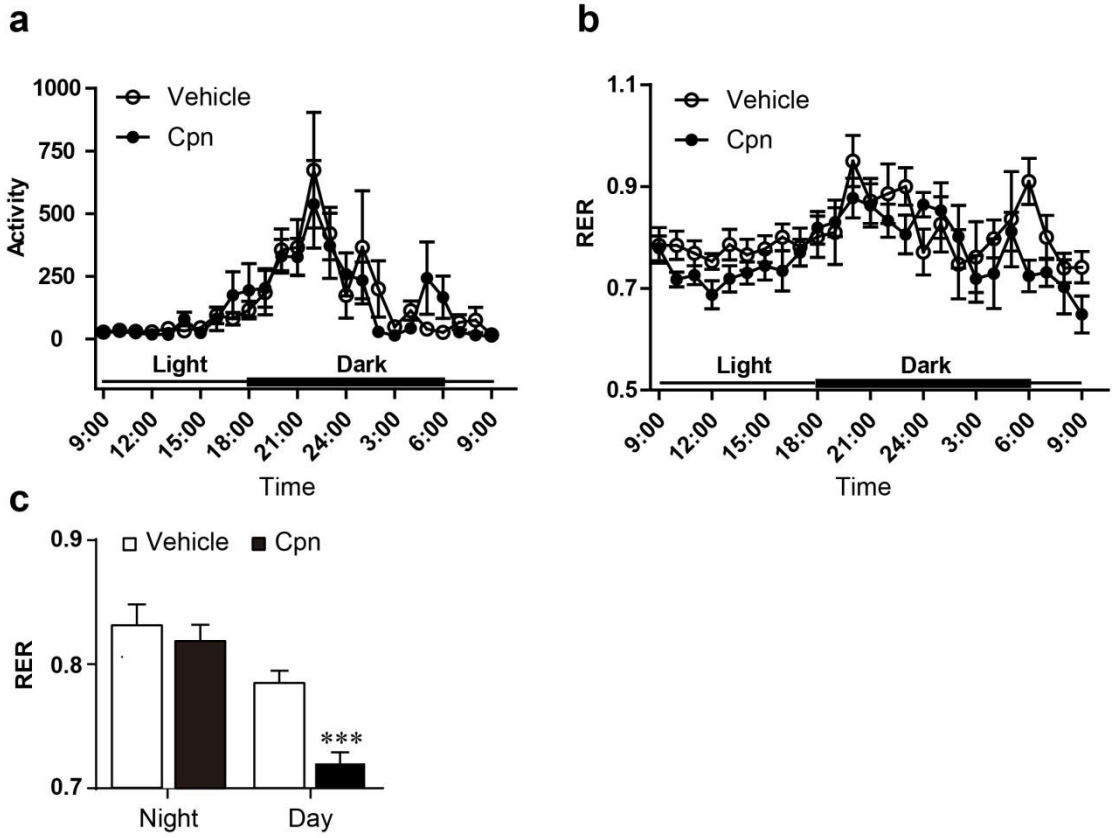

Figure S2 Cordycepin does not induce hyperactivity but decreases respiratory quotient (RQ) value in day time. (a) Spontaneous activity of mice represented by the average horizontal activities monitored over a 24-h period after 4 weeks of cordycepin treatment in mice (n = 7). (b) Respiratory exchange ratio (RER) curve. (c) Average RER of each group in a 24 h cycle. Data are displayed as mean ± s.e.m. Significant differences compared with vehicle controls are indicated by $^{***}P$ <0.001 (assessed by Student's *t*-test). Cpn: cordycepin.

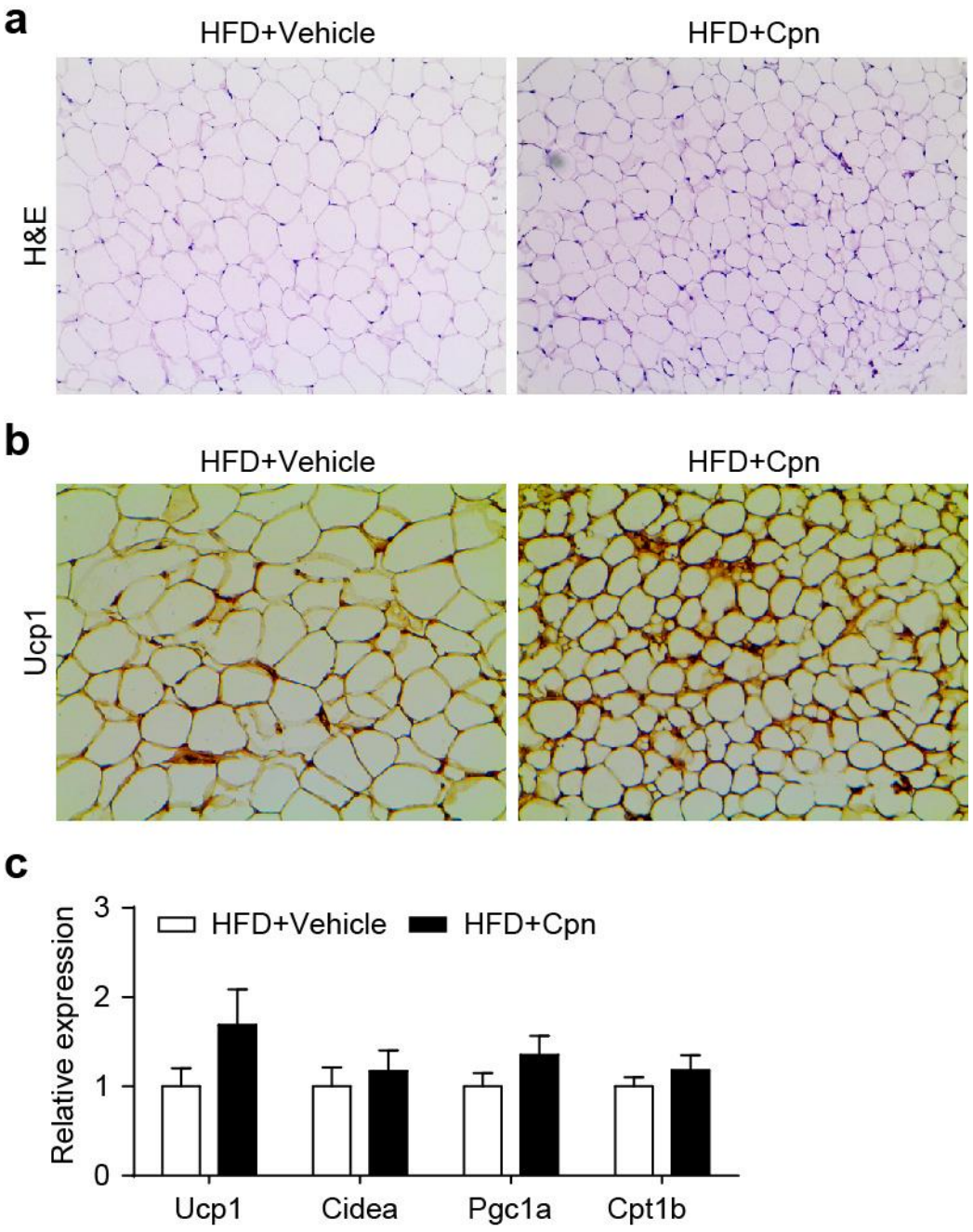

Figure S3 Cordycepin slightly promotes adipose browning in epididymal WAT. (a) Representative H&E staining image of epididymal WAT (eWAT). (b) Immunoblot analysis of UCP1. (c) Real-time PCR analysis of thermogenic gene expression. Data are displayed as mean ± s.e.m. Significant differences compared with vehicle controls are assessed by Student's *t*-test. Cpn: cordycepin.

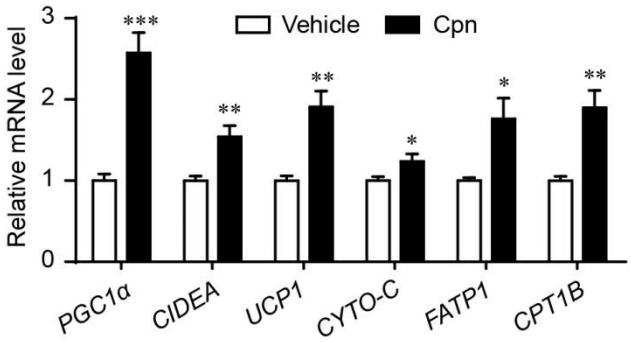

Figure S4 Cordycepin significantly increases mRNA levels of genes controlling energy expenditure and thermogenic programme in brown adipose tissue (BAT). Data are displayed as mean ± s.e.m. Significant differences compared with vehicle controls are assessed by Student's *t*-test. Cpn: cordycepin.

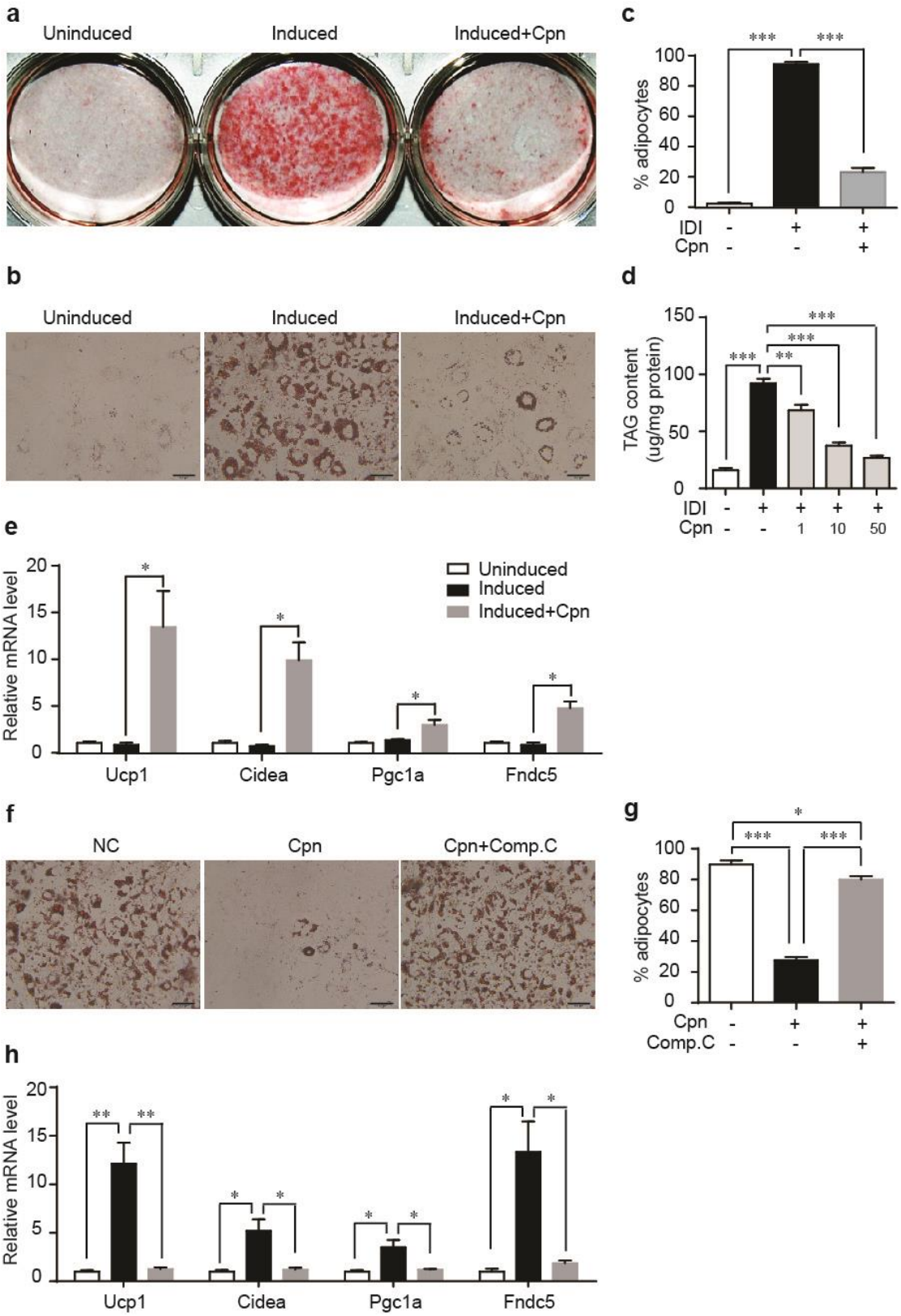

Figure S5 Cordycepin inhibits adipocyte differentiation in an AMPK-dependent manner. 3T3-L1 pre-adipocytes were differentiated into mature adipocytes in IDI

medium with or without cordycepin. (a) Gross photographs of culture plates after staining by oil red O. (b) Light microscopic analysis of cells stained with oil red O. (c) Percentages of lipid-laden adipocytes. (d) Quantitative analysis of lipid content. (e) Realtime PCR analysis of thermogenic gene expression. (f) Microscopic analysis of cells stained with oil red O under the treatment with compound C. (g) Influence of compound C on the percentage of lipid-laden adipocytes. (h) mRNA levels of thermogenic genes under the treatment by compound C. Data are displayed as mean ± s.e.m. Significant differences compared with vehicle controls are indicated by $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ (assessed by Student's *t*-test). N.S.: nonsignficance. Cpn: cordycepin; Comp.C: compound C.

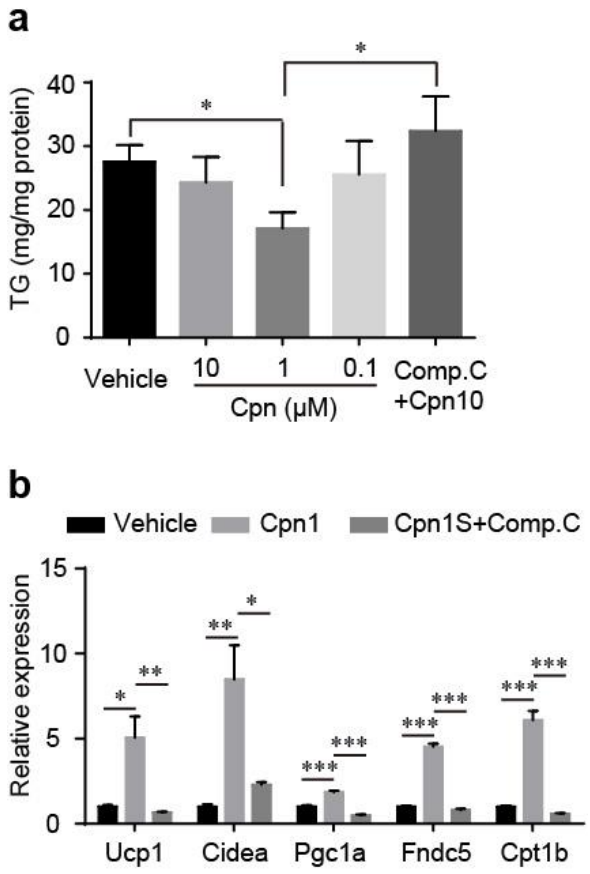

Figure S6 Cordycepin decreases lipid content (a) and regulates thermogenic genes expression (b) in an AMPK-dependent manner. Data are displayed as mean ± s.e.m. Significant differences compared with vehicle controls are indicated by $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ (assessed by Student's *t*-test). N.S.: nonsignficance. Cpn: cordycepin; Comp.C: compound C.