Supplemental Information

Trans-palmitoleic acid promotes adipose thermogenesis to reduce obesity via hypothalamic FFAR1 signaling
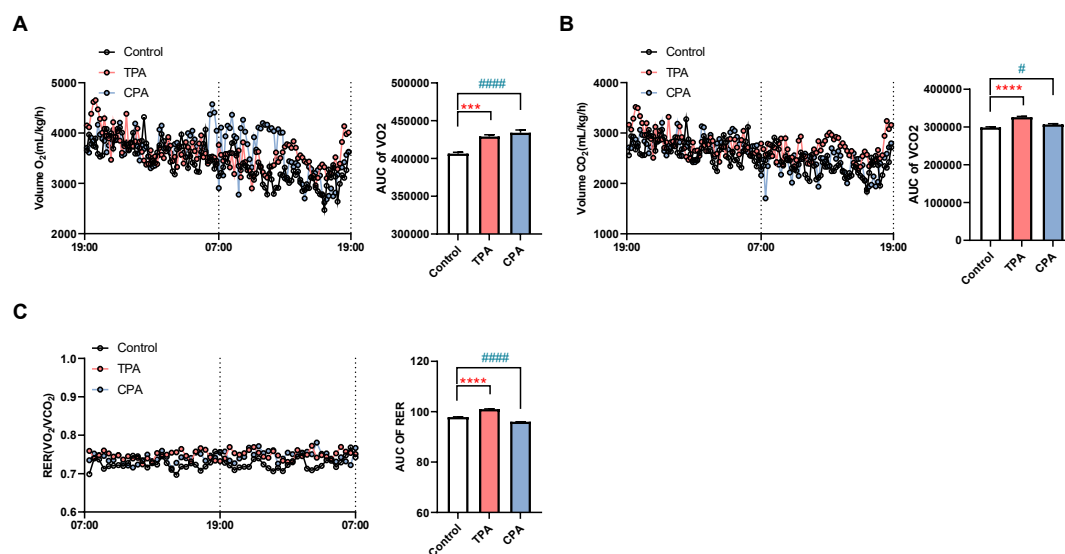

Fig. S1 Feeding C16 unsaturated FAs promote energy expenditure to reduce diet-induced obesity

C57BL/6J male mice were fed a HFD (60 kcal% fat) supplemented with 0 (Control), 5mg/kg/BW TPA (red), CPA (blue) and PA (purple) daily for 3 months. (A) Time-course of oxygen consumption, and AUC of oxygen consumption, n=4; (B) Time-course of CO2 production, and AUC of CO2 production, n=4; (C) Time-course of RER, and AUC of RER, n=4; Data are mean ± SEM. ****$P < 0.0001$ TPA versus control; #$P < 0.05$ and ####$P < 0.0001$ CPA versus control; Significant differences between mean values were determined by one-way ANOVA with the post hoc Bonferroni's multiple comparisons test.
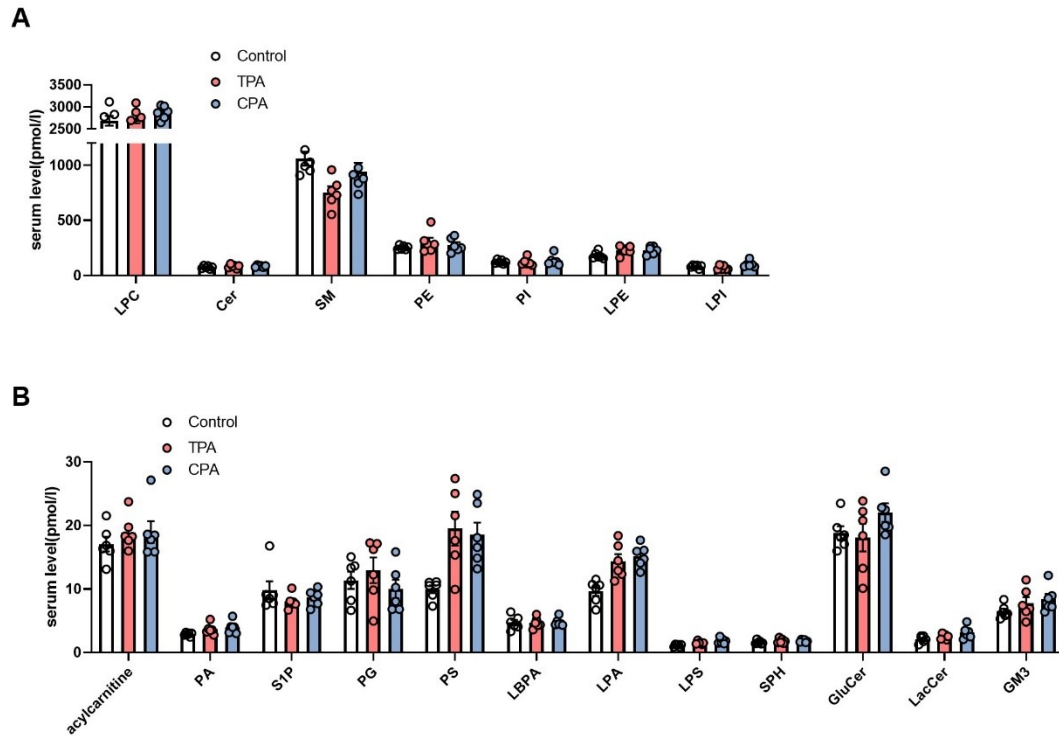
Fig. S2 Feeding C16 FAs do not influence other lipids serum levels.

C57BL/6J male mice were fed a HFD (60 kcal% fat) supplemented with 0 (Control), 5mg/kg/BW TPA (red), CPA (blue) and PA (purple) daily for 3 months. (A) The serum level of lipids, n=4; (B) The serum level of lipids, n=4; Data are mean ± SEM. Significant differences between mean values were determined by one-way ANOVA with the post hoc Bonferroni's multiple comparisons test.
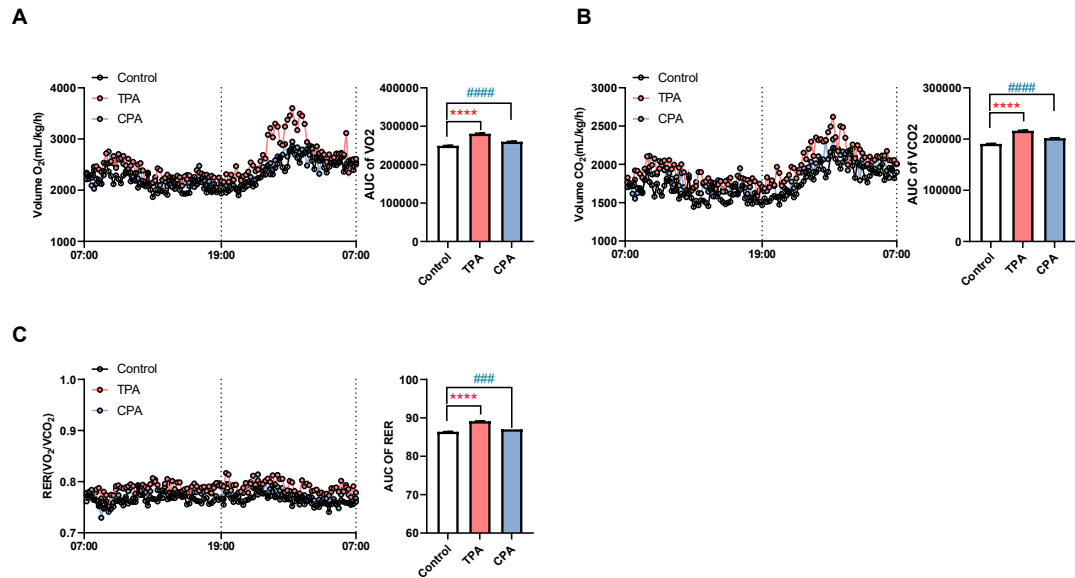
Fig. S3 Injecting free C16 unsaturated FAs promote energy expenditure to reduce diet-induced obesity

C57BL/6J male mice were fed with an HFD (60 kcal% fat) and then were injected with 0 (Control), 0.01mmol/kg/BW TPA (red), CPA (blue) and PA (purple) once every third day for continuous 3 months; n=9. (A) Time-course of oxygen consumption, and AUC of oxygen consumption; (B) Time-course of CO_2 production, and AUC of CO_2 production; (C) Time-course of RER, and AUC of RER; Data are mean ± SEM. ****P<0.0001 TPA versus control; ###P<0.001 and ####P<0.0001 CPA versus control; Significant differences between mean values were determined by one-way ANOVA with the post hoc Bonferroni's multiple comparisons test.
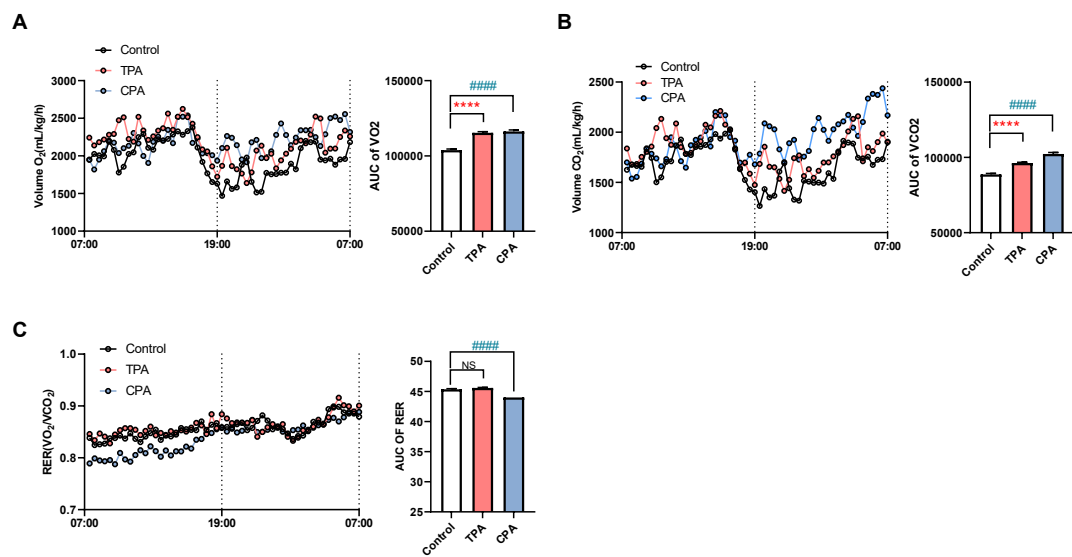
Fig. S4 Injecting free C16 unsaturated FAs increase energy expenditure and reduce obesity in ob/ob mice.

The leptin-deficient (ob/ob) mouse male mice were fed a chow diet and were injected with 0 (Control), 0.01mmol/kg/BW TPA (red), CPA (blue) and PA (purple) once every third day for 2 months; n=6. (A) Time-course of oxygen consumption, and AUC of oxygen consumption; (B) Time-course of CO2 production, and AUC of CO2 production; (C) Time-course of RER, and AUC of RER; Data are mean ± SEM. ****P＜0.0001 TPA versus control; ####P＜0.0001 CPA versus control; Significant differences between mean values were determined by one-way ANOVA with the post hoc Bonferroni's multiple comparisons test.
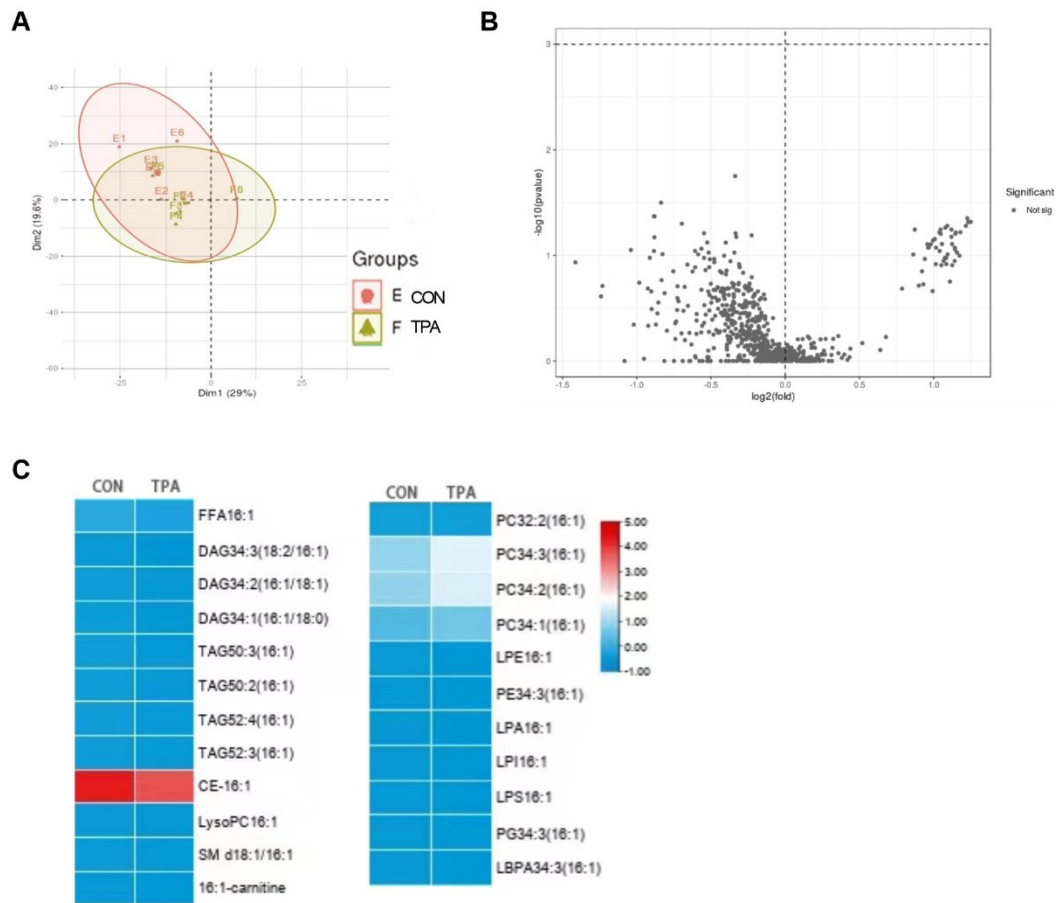
Fig. S5 TPA treatment does not influence serum lipid metabolites profiles.

Lipid profiling of mice serum by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS); (A) Principal component analysis comparing TPA group with control group; (B) Volcano plot showing the log2 fold change of lipid metabolite plotted against the -log10 p value of a one-sided t test. Significance is considered for Benjamini-Hochberg corrected p values < 0.05 and log2 fold change > 0.58; (C) Heatmap of the product spectrum of C16 fatty acids;
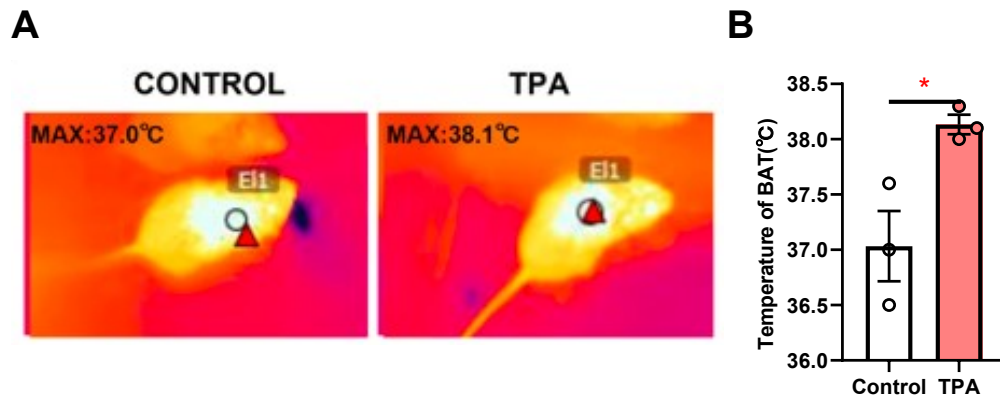
Fig. S6 The infrared pictures of the interscapular area

WT mice injected intracerebroventricular (ICV) with TPA or vehicle for 2 weeks, n =4

(A) Representative infrared pictures of the interscapular area in WT mice after ICV injections for 2 weeks; (B) The temperature of BAT after ICV injections of TPA or vehicle for 2 weeks; Data are mean ± SEM.*P< 0.05 TPA versus control; Significant differences between 2 mean values were determined by a two-tailed Student's t-test.

A

Donor vector and CRISPR/Cas9 system

Mouse GPR40 gene locus

~1.4kb   4175bp

HR3   1   Ffar3-201 E1

ATG   TAG

Wild type allele

HR2   HR3   1   Ffar3-201 E1

Targeted allele

HR2   HR3   1   Ffar3-201 E1

Cre/loxp excision

HR2   Ffar3-201 E1

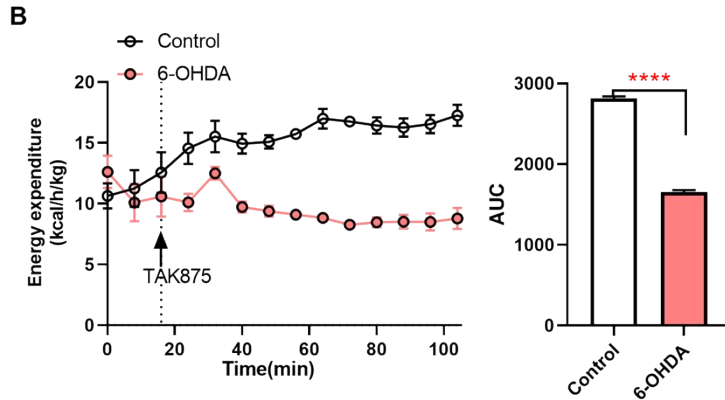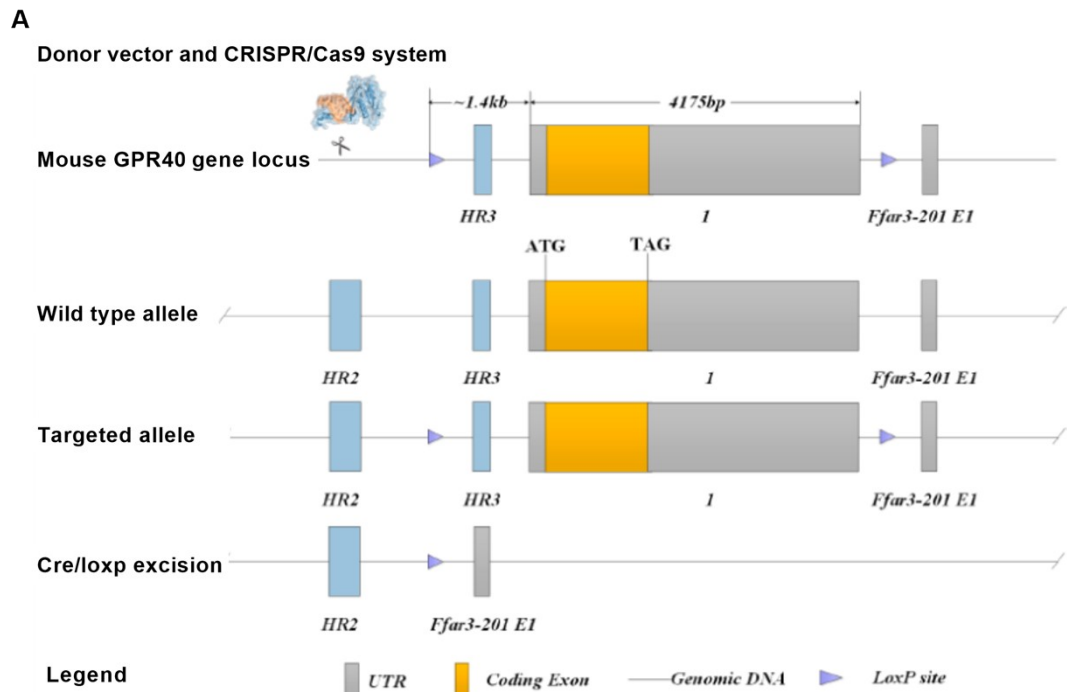Legend   UTR   Coding Exon   Genomic DNA   LoxP site

B

Fig. S7 Hypothalamic FFAR1 signaling mediates TPA-induced adipose thermogenesis

(A) Mouse *Ffar1* gene locus and targeting construct. When mating with cre expression allele, sequence between two LoxP sites can be deleted. (B) Time-course of energy expenditure after injected with TAK875 in WT mice, subsequent to intraperitoneal

administration of either vehicle or 6-OHDA two days prior, n =5; Data are mean ±

SEM. ****P< 0.0001; Significant differences between 2 mean values were determined

by a two-tailed Student's t-test.