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 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<0.001 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.
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.