Supplemental Figure1

L-Theanine promotes adipose tissue browning and thermogenic capacity in mice. Results related to Fig.1.

The 8-week-old C57BL6 NCD-fed male mice were administered L-Theanine intraperitoneally (100 mg/kg per mouse, once a day) or saline as a control for 7 days.

A, Body weight gain after 7 days of treatment with saline or L-Theanine (n=6). B, Quantification of Western blotting was done for Fig.1F by using ImageJ and shown. C, mRNA expression of indicated genes in eWAT. D, mRNA expression of indicated genes in BAT. E, BAT Tissue lysates were subjected to Western blotting by using the indicated antibodies. Hsp90 serves as an internal control. F, Quantification of Western blotting was done for E by using ImageJ and shown. All groups were normalized to and compared with saline group. All values are represented as means with error bars representing S.D. *p < 0.05, **p <0.01, ***p < 0.001. n=6 for each group.

Supplemental Figure2

The effect of L-Theanine on the expression of thermogenic and energy expenditure genes in primary iWAT adipocytes.

Fractionated and differentiated primary iWAT adipocytes were treated with L-Theanine at a final concentration of 50μM or with saline as a control for 48 hours.

A, Thermogenic and energy expenditure genes' expression profile after L-Theanine treatment. B, The representative western blotting result of the protein expression level. Hsp90 serves as an internal control. C, Quantification of Western blotting for B. All groups were normalized to and compared with saline group. All values are represented as means with error bars representing S.D. *p < 0.05, **p <0.01, ***p < 0.001 as compared with saline group. n=5 for each group.

Supplemental Figure3

Knockdown of Prdm16 in the adipocytes derived from C3H10T1/2 cells blunts the role of L-Theanine in promoting UCP1 protein expression.

Cells were treated as described in Fig. 4D and Quantification of Western blotting results in Fig. 4D was shown here. All values are represented as means with error bars representing S.D. **p <0.01, ***p < 0.001 as compared with shLacZ+saline group. n=3 for each group.

Supplemental Figure4

Knockdown of Prdm16 by injecting adenovirus harboring shPrdm16 into iWAT of the mice.

8 weeks-old male WT mice were injected subcutaneously in iWAT with adenovirus of shLacZ or shPrdm16. Five days later, the stromal vascular fraction (SVF) and mature adipocytes fraction (MA) from iWAT was isolated. RT-qPCR was performed to examine the expression level of Prdm16 in SVF and in MA. All groups were normalized to and compared with shLacZ group. All values are represented as means with error bars representing S.D. **p <0.01 as compared with shLacZ group. n=6 for each group.

Supplemental Figure5

The effect of knocking down Prdm16 in iWAT on eWAT genes expression and on iWAT protein expression.

A, Mice were treated as in Fig. 5 and then the mRNA expression of indicated genes in eWAT was examined. n=6 for each group. B, Quantification of Western blotting was done for Fig.5E by using ImageJ and shown. n=3 for each group. All values are represented as means with error bars representing S.D. *p < 0.05.

Supplemental Figure6

Knockdown of AMPKα blunts the activation of AMPK sinaling.

Quantification of Western blotting results for Fig.6B by using ImageJ. All values are

represented as means with error bars representing S.D. *p < 0.05. n=3 for each group.

Supplemental Figure7

L-Theanine promotes adipose tissue browning and enhanced energy expenditure in HFD-fed mice.

Diet induced-obese C57BL/6 mice were generated by 16 weeks of high fat diet feeding starting at 6 weeks of age. Intraperitoneal administration of saline or L-Theanine (100 mg/kg) was performed once daily from the 7th week of HFD for consecutive10 weeks. A, Food intake of the mice was monitored. B, Quantification of Western blotting results for Fig.8K by using ImageJ. n=3. C, The mRNA expression of indicated genes in eWAT. D, The mRNA expression of indicated genes in BAT. E, H&E staining of eWAT and BAT. Scale bar, 40μm. F to G Metabolic cage studies and OCR test of iWAT were performed at the 9th week (9 weeks of HFD feeding with L-Theanine treatment in the last 3 weeks). F, Basal oxygen consumption rate (OCR) of iWAT tissues in mice was measured by Clark-type electrode oxygraph and shown. G. Energy expenditure was evaluated by measurement of oxygen consumption (VO2) as shown, and heat production of the mice as shown in H. The above parameters were measured in mice during a 12-h light/12-h dark cycle placed in a metabolic cage. Energy expenditure expressed as Kcal/h per animal. I, RER were calculated using equations described in Methods. All groups were normalized to and compared with saline group. All values are represented as means with error bars representing S.D. *p < 0.05, **p <0.01, ***p < 0.001. n=6 for each group.

Supplemental Figure8

Proposed working model for this article

L-Theanine induces the AMPKα activation in white adipocytes, which is essential for WAT browning. AMPKα activation elevates the intracellular level of α-KG in

adipocytes, which facilitates demethylation of the Prdm16 promoter and increases Prdm16 expression in WAT, thereby increasing energy expenditure through thermogenesis and ameliorating diet-induced obesity.