Supporting information

1. Supporting Materials and Methods

1.1 Sample preparation of Immunoblot analysis of Cr metabolism−related protein.[1-3] Aliquots of iWAT were homogenized in 1 mM EDTA, 10 mM Tris, and 0.25 M sucrose (pH 7.5) with protease inhibitor cocktail (cComplete, Mini, Roche Diagnostics K.K, Tokyo, Japan). After centrifugation at 15,000 g for 10 min at 4°C, the supernatant was collected, and then added detergents to a final concentration of 1% Triton X-100, 1% NP-40, and 0.1% SDS followed by incubation for 30 min. The supernatant was centrifuged at 15,000 g, for 50 min at 4°C, and then sheared eight times with a 26G needle. The protein concentration of the obtained supernatant was determined using a Protein Assay System (Bio-Rad, Richmond, CA, USA) with bovine γ-globulin employed as a standard. Aliquots of the supernatant were treated with Laemmli sample buffer for 5 min at 100°C, and stored at -80°C until use.

1.2 H&E staining and immunostaining.[2,3] Adipose tissues were fixed in 4% paraformaldehyde for 24 h at 4°C. Then the tissues were embedded in paraffin and cut into slices for UCP1 (5-μm thick) immunostaining, and deparaffinized and rehydrated using xylene, ethanol, and water. Adipose tissue sections were stained with H&E (both from Sakura Finetek Japan, Tokyo, Japan) or UCP1 immunostained. For immunohistochemistry, slides were submerged in 0.1 M sodium citrate (pH 6.0) and heated to 100°C for 15 min in a laboratory microwave (500 W). Slides were incubated with 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase, and 2.5% normal horse serum (included in kit) for 20 min at room temperature. Slides were then incubated with anti-UCP1 antibody (1:500) for 16 h at 4°C, and with anti-rabbit IgG (included in kit) for 30 min at room temperature. Labeling was visualized with 3, 3′-diaminobenzidine (DAB) as the chromogen using ImmPress kit and Immpact DAB peroxidase substrate kit (Vector Laboratories, Burlingame, CA) according to the manufacturer's directions. Then, sections were counterstained with hematoxylin.

2. Supporting Figures

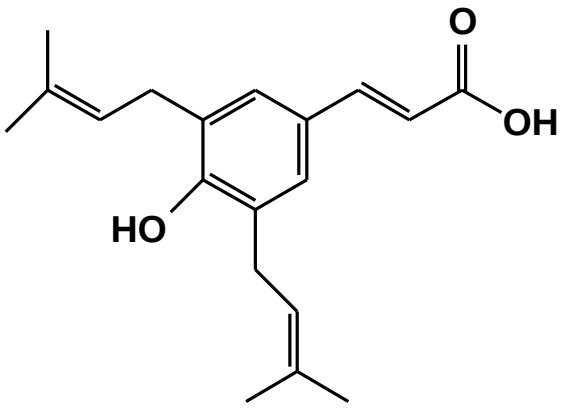

Figure S1. Chemical structure of ArtC.

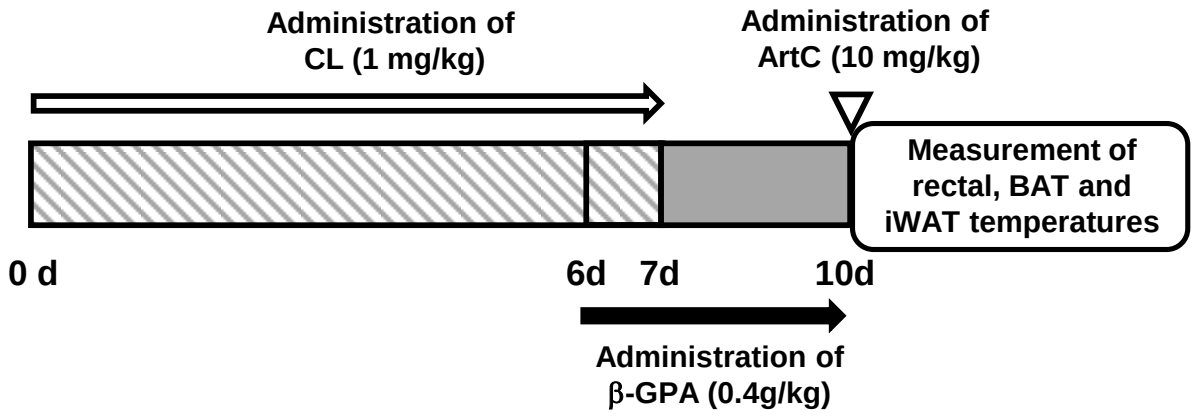

Figure S2. Experimental design of the measurement of rectal, BAT and iWAT temperatures after a single administration of ArtC and Cr metabolism inhibitor in mice.

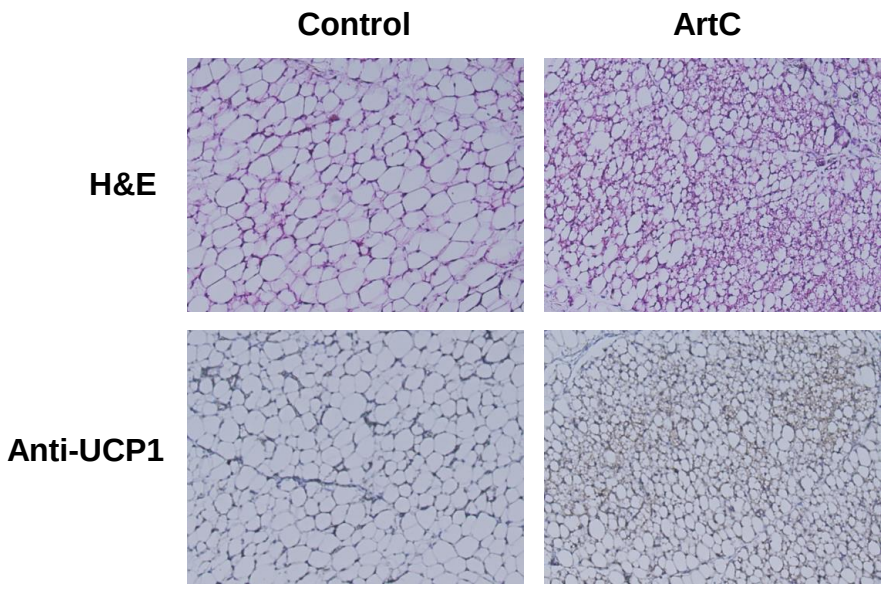

Figure S3. Representative images of H&E and immunohistochemical staining of UCP1 in sections of iWAT from mice treated for 28 days with either vehicle (control) or ArtC (10 mg/kg).

3. Supporting references

1 Lau, P.; Tuong, Z. K.; Wang, S. C.; Fitzsimmons, R. L.; Joel M. Goode, J. M.; Thomas, G. P.; Cowin, G. J.; Pearen, M. A.; Mardon, K.; Stow, J. L.; Muscat, G. E. O. Rorα deficiency and decreased adiposity are associated with induction of thermogenic gene expression in subcutaneous white adipose and brown adipose tissue. *Am. J. Physiol. Endocrinol. Metab.* **2015**, *308*, E159–E171.

2 Nishikawa, S.; Aoyama, H.; Kamiya, M.; Higuchi, J.; Kato, A.; Soga, M.; Kawai, T.; Yoshimura, K.; Kumazawa, S.; Tsuda, T. Artepillin C, a typical Brazilian propolis-derived component, induces brown-like adipocyte formation in C3H10T1/2 cells, primary inguinal white adipose tissue-derived adipocytes, and Mice. *PLoS One* **2016**, *11*, e0162512.

3 Nishikawa, S.; Kamiya, M.; Aoyama, H.; Nomura, M.; Hyodo, T.; Ozeki, A.; Lee, H.; Takahashi, T.; Imaizumi, A.; Tsuda, T. Highly dispersible and bioavailable curcumin but not native curcumin induces brown-like adipocyte formation in Mice. *Mol. Nutr. Food Res.* **2018**, *62*, 1700731.