Supplementary information, Data S1 Materials and Methods

The measurements of whole-body energy metabolism, such as oxygen consumption, carbon dioxide production, energy absorption, and locomotor activity, followed the recommended protocols [2]. More detailed methods are provided in the Supplementary Materials.

Mice

Recipient six-week-old male C57BL/6J mice (purchased from Vital River Laboratory Animal Technology. Co. Ltd) were used for transplantation. Mice were housed 4 per cage in an Office of Laboratory Animal Welfare–certified animal facility, with a 12-hour light/12-hour dark cycle. The Institutional Animal Care and Use Committee approved all experimental plans. Age-matched C57BL/6J males fed a low fat chow (10% calories from fat; Research Diets, D12450B) or HFD (60% calories from fat; Research Diets, D12429) post BAT transplantation.

Tissue transplantation

BAT was dissected from 6-week-old C57BL/6J mice and was transplanted into the subcutaneous dorsal region of age- and sex-matched recipient C57BL/6J,, and DIO mice (the latter mice were 14 weeks old but had been fed an HFD for 8 weeks). Thereafter, the mice were fed the indicated diets.

BAT removed from the intrascapular region of six-week-old male C57BL/6J mice. After cervical dislocation of donor mice, BAT was removed, peripheral white fat was excluded, and the remaining BAT placed in sterile PBS, and transplanted into the subcutaneous region dorsal region, adjacent to the endogenous intercsapular fat pad of recipients as quickly as possible. For age and sex matched recipient mouse, 0.15g donor BAT from intrascapular region, 0.15g quadriceps muscle or 0.15g WAT from the epididymal fat pad were transplanted. Recipient mice were anesthetized by intra-peritoneal

injection with 400 mg/kg body weight avertin.

Gene expression analysis

RNA was isolated using Trizol, followed by RNeasy columns with DNase I treatment (QIAGEN). The cDNA was synthesized using random hexamers (Invitrogen) for subsequent real-time quantitative PCR analysis (ABI Prism VIIA7; Applied Biosystems Inc). PCR products were detected using Sybr Green and normalized by cyclophilin expression. Primers were designed using Primer Quest (Integrated DNA Technologies, Inc).

Metabolic assessment

For glucose tolerance tests (GTT), animals were fasted for 16 hours (17:00–9:00) with free access to drinking water. The glucose level was assessed following intraperitoneal glucose injection (1.5g/kg). Blood glucose levels were determined immediately before and 15, 30, 60, 90 and 120 min after glucose injection by using an Accu-Chek glucose monitor (Roche Diagnostics Corp). For insulin tolerance test (ITT), mice were fasted for 4 hours (9:00-13:00) and intraperitoneally injected with human insulin (0.8 units/kg Humulin R). Blood glucose levels were determined immediately before and 15, 30 and 60 min after insulin injection.

Energy intake, energy absorption and total movement measurement

Mice were housed one animal per cage, and free access food and water. Food intake and oxygen consumption measurement were performed for consecutive 3 days using a TSE lab master system as described by [1]. Energy absorption measurement was performed according to our previous report [2].

Ambulatory activity of each mouse was measured using the optical beam technique (Opto-M3; Columbus Instruments, Columbus, OH, USA) over 24 hours and expressed as the 24-hour average activity.

Total fat distribution

All mice were anesthetized with intra-abdominal injection of Avertin, and whole body fat mass was measured using non-radiotracer computerized tomography (Hitachi Aloka Latheta LCT-200).

Western blot

For western blot analysis, tissues were lysed in RIPA buffer (150 mM sodium chloride, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0), protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche). Protein concentrations were determined using a BCA assay kit (Pierce). Protein was separated by 10% SDS-PAGE, transferred to PVDF membrane (Millipore). Membranes were blocked in 5% skim milk in TBST and incubated with primary antibodies (phospho [Ser473] and total Akt, phospho [Thr202/Tyr204] and total Erk, Cell Signaling Technology, β-actin were purchased from Sigma Chemical Co), and incubated with secondary antibodies conjugated with HRP. Signals were detected with Super Signal West Pico Chemiluminescent Substrate (Pierce).

Histological analysis

Tissues were fixed overnight in 4% paraformaldehyde and embedded in paraffin wax. Sections (5μm) were dewaxed in xylene (two 5min washes) followed by incubation for 5 min in each of the following: xylene:ETOH (1:1), 100% ETOH (2X), 95% ETOH, 85% ETOH, 75% ETOH, and ddH2O. Slides

were then stained with hematoxylin and eosin, H&E.

Statistics

For all comparisons, ANOVA, ANCOVA or Student's *t* tests were performed, and *p* less than 0.05 were considered to be significant.

References

1. Chi QS, Wang DH. Thermal physiology and energetics in male desert hamsters (Phodopus roborovskii) during cold acclimation. *J Comp Physiol B* 2011; **181**:91-103.
2. Tschöp MH, Speakman JR, Arch JR, *et al.* A guide to analysis of mouse energy metabolism. *Nat Methods* 2012; **9**:57-63.