Supplementary Materials and Methods

Animals, cell isolation and cell culture

Male 129S1/SvImJ mice (UCP1-KO mice [1] and wildtype littermates) bred at the animal facility of Technische Universität München in Weihenstephan, aged 5 to 6 weeks, were used to prepare primary cultures of brown and brite adipocytes. Adipose tissue depots (interscapular brown adipose tissue and inguinal white adipose tissue) were dissected, carefully minced and treated with collagenase for 45 min at 37°C. The homogenate was filtered through a 250 µm nylon mesh and centrifuged at 500*g* to collect the stromal vascular fraction (SVF). The SVF cell pellet was rinsed and seeded into XF96 V3-PS cell culture microplate (Seahorse Bioscience). After reaching confluency, induction medium containing DMEM medium (Sigma D5796), 10% fetal bovine serum (FBS), 0.5 mM isobutylmethylxanthine, 125 nM indomethacin, 1 mM dexamethasone, 850 nM insulin, 1 nM T3, 1 µM rosiglitazone was added. After 2 days of induction, cells were maintained in differentiation media (10% FBS, 850 nM insulin, 1 nM T3 and 1µM rosiglitazone). Media was changed every two days. Chronic treatment of primary inguinal adipocytes with rosiglitazone robustly induces brite adipogenesis [2, 3]. Respiration was measured on day 7 of differentiation.

Respirometry

Oxygen consumption rate (OCR) was measured at 37°C using an XF96 extracellular flux analyzer (Seahorse Bioscience). At day 7 of differentiation, the medium was replaced with prewarmed, unbuffered measurement solution (DMEM basal medium (Sigma D5030) supplemented with 25 mM glucose, 2 mM sodium pyruvate, 31 mM NaCl, 2 mM GlutaMax and 15 mg/l phenol red, pH 7.4) either with or without essentially fatty acid free bovine serum albumin (BSA), and incubated at 37°C in a room air incubator for 1 h. Basal respiration was measured in untreated cells. Coupled respiration was inhibited by oligomycin treatment (5 µM). UCP1 mediated uncoupled respiration was determined after isoproterenol (0.1-1 µM) stimulation. Maximum respiratory capacity was assessed after FCCP (Sigma-Aldrich) addition (1 µM). Finally, mitochondrial respiration was blocked by antimycin A (Sigma-Aldrich) (5 µM) treatment and the residual OCR was considered non-mitochondrial respiration. For palmitate-induced stimulation of respiration, palmitate-BSA (0.2 mM) was injected instead of isoproterenol. For some experiments, cells were pretreated with 40 µM Atglistatin (ATGL inhibitor), 20 µM Hi76-0079

(HSL inhibitor) or both or 5 µg/ml cyclosporine A (CSA, PTP inhibitor) prior to bioenergetic profiling. Oxygen consumption rates were automatically calculated by the Seahorse XF-96 software. Isoproterenol induced leak respiration rates were calculated from means of 3 highest values and expressed as fold increase of basal leak respiration. Data were exported and reconstructed in GraphPad Prism 5.0 software. After completion of an assay, the microplate was saved and protein was isolated for UCP1 phenotyping. Each experiment was repeated at least 3 times with similar results.

Western blot analysis

For immunological UCP1 detection, cells were lysed in RIPA buffer (150 mM NaCl, 50 mM Tris·HCl, 1 mM EDTA, 1 % NP-40, 0.25 % Na-deoxycholat). Lysates (30µg) were resolved by 12.5% SDS-PAGE, electroblotted onto a PVDF membrane (Millipore), and probed with rabbit anti-UCP1 antibody (1:10000) (abcam, ab10983). Secondary antibodies conjugated to IRDye™ 680 (Licor Biosciences) were incubated at a dilution of 1:20000. Fluorescent images were captured by an Odyssey fluorescent imager (Licor Biosciences).

1. Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP (1997) Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* **387:** 90-94

2. Ohno H, Shinoda K, Spiegelman BM, Kajimura S (2012) PPARgamma agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. *Cell Metab* **15:** 395-404

3. Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J (2010) Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J Biol Chem* **285:** 7153-7164

Supplemental Figure Legends

Figure S1. Western blotting analysis confirming the phenotype of primary brown and brite adipocytes from UCP1 wildtype (WT) and knockout (KO) mice. Primary brown (A) and brite adipocytes (B) from UCP1 KO mice cultured in XF96 V3-PS cell culture microplate are UCP1 negative, while primary adipocytes from UCP1 WT mice are UCP1 positive. Primary brown adipocytes cultured in 6-well plates serve as positive control (+).

Figure S2. Isoproterenol (ISO) increases leak respiration in a dose dependent manner. Time course of oxygen consumption rates (OCR) of primary brite adipocytes from wildtype mice. Leak respiration is stimulated by ISO induced lipolysis. All data presented are mean values ± SEM with an average of 10–12 different wells.

Figure S3. Effects of various concentrations of BSA on ISO-induced respiration in primary brown and brite adipocytes. Time course of oxygen consumption rate (OCR) of primary brown (A) and brite (B) adipocytes from UCP1 wildtype (WT) mice in the presence of different BSA concentrations. All data presented are mean values ± SEM with an average of 10–12 different wells.