SUPPLEMENTARY INFORMATION

Cell culture

HIB-1B preadipocytes were maintained in Dulbecco’s modified Eagle’s culture medium (DMEM) supplemented with 10% bovine calf serum (HyClone, Logan, UT), while HB2 preadipocytes were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA). At 80-90% confluency, cells were differentiated in DMEM with 10% cosmic calf serum (CCS) (HyClone) supplemented with 5 µg/mL insulin (Sigma, St. Louis, MO), 0.5 mM isobutylmethylxanthine (IBMX) (Sigma), 1 µM dexamethasone (Sigma), and 1 nM triiodothyronine (T3) (Sigma). After two days in this differentiation medium, cells were refed with 10% CCS in DMEM containing only insulin and T3 at the above concentrations. On the sixth day of differentiation, 1 µM isoproterenol (Sigma) was added to the culture medium for 6 hours (HIB-1B cells), or 1 mM dibutyryl adenosine 3’, 5’-cyclic monophosphate (dibutyryl-cAMP) (Sigma) for 4 hours (HB2 cells), before cells were harvested.

Retroviral transfection

BOSC23 viral packaging cells were transfected by CellPhect (Amersham Pharmacia, Piscataway, NJ) with full-length hGATA-2 or mGATA-3 incorporated into the pBabe retroviral vector. Forty-eight hours after transfection, the viral supernatant was sterile-filtered and added to DMEM (3:1 v/v) containing 4 µg/mL polybrene (Sigma). Viral supernatant was delivered to HIB-1B or HB2 preadipocytes seeded at <30% confluency. Upon confluency, infected cells
were selected 1 µg/mL puromycin (Sigma) and differentiated as described above. Infected cells
were maintained in culture medium with appropriate selection drugs. Using these methods,
pBabe-PGC-1α was used to co-express PGC-1α with GATA-2 in HIB-1B cells.

Adenoviral transfection

Immortal brown adipocytes obtained from the above procedure were seeded in 10 cm² plates at a
density of 2 x 10⁵ cells/plate and infected with AdCMV-Cre adenovirus (provided by Randy
Johnson, UCSD, San Diego, CA) at 5, 50 and 500 m.o.i. for 48 hours in primary cell culture
medium. Cells were then refed and surviving cells were harvested for genotyping by Southern
analysis. Adenoviral infection with AdCMV-Cre was repeated to improve efficacy of
recombination. Total RNA was isolated from cells treated with Cre once, twice, or not at all to
examine presence of GATA-2 gene transcripts upon targeted gene disruption by Cre
recombination. As a control, wildtype cells that did not express the floxed-GATA-2 allele were
similarly infected with adenovirus and examined by both Southern and Northern analysis. A
probe specific to a region on exon 4 of the GATA2 gene was used to detect the presence of either
intact (2.5 kb) or disrupted GATA2 (7 kb) alleles by Southern blot analysis. Finally cells were
differentiated to brown adipocytes as described above, and transcriptional profile and
morphology of these cells were examined.

Electron Microscopy

Cells were trypsinized, pelleted at 1500 rpm for 5 minutes, washed with phosphate buffered
saline at pH 7.0, and pelleted again at 1500 rpm for 5 minutes. Cells were then resuspended in
2.5% glutaraldehyde in 0.1 M potassium phosphate buffer for at least 1 hour, and washed with
0.1 M potassium phosphate buffer. Cells were postfixed in 1% OsO₄ in 0.085 M sodium cacodylate buffer, dehydrated in a graded ethanol series and propylene oxide, infiltrated with a mixture of araldite 502 (Ted Pella, Redding, CA) and propylene oxide (1:2) for 2 hours, followed by a mixture of araldite 502 and propylene oxide (1:1) overnight. Cell pellets were placed in 100% araldite 502 for 1 hour, then placed in flat bed molds with fresh 100% araldite 502 and incubated at 68°C for an additional 48 hours. Sections of 40-50 nm from each group were made with a RMC ultramicrotome (RMC, Inc., Tucson, AZ) and placed unstained onto uncoated 600-mesh copper grid for examination with a Leo EM902 energy-filtering transmission electron microscope (LEO Electron Microscopy, Inc., Thronwood, NY).

Luciferase assay

One microgram of the reporter construct was transfected into HIB-1B cells in 12-well plates along with vector or GATA-2 (0.2 μg) expression plasmids using Lipofectamine 2000 (Invitrogen) according to manufacturer’s instructions. For studies of UCP-1 promoter activity, an expression plasmid for PGC-1 (0.5 μg) was also included to activate the promoter. For both experiments, Renilla luciferase reporter (0.1 μg) was used as an internal control for transfection efficiency. Two days after transfection, cells were lysed and luciferase activity was measured using the dual luciferase kit (Promega, Madison, WI) according to the manufacturer’s instructions. Firefly luciferase activity was normalized to Renilla luciferase activity.