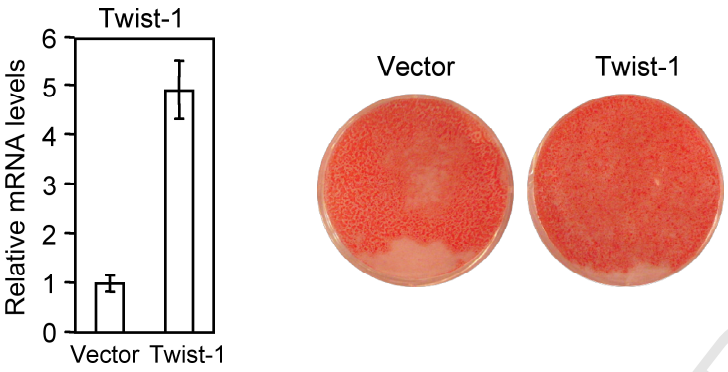
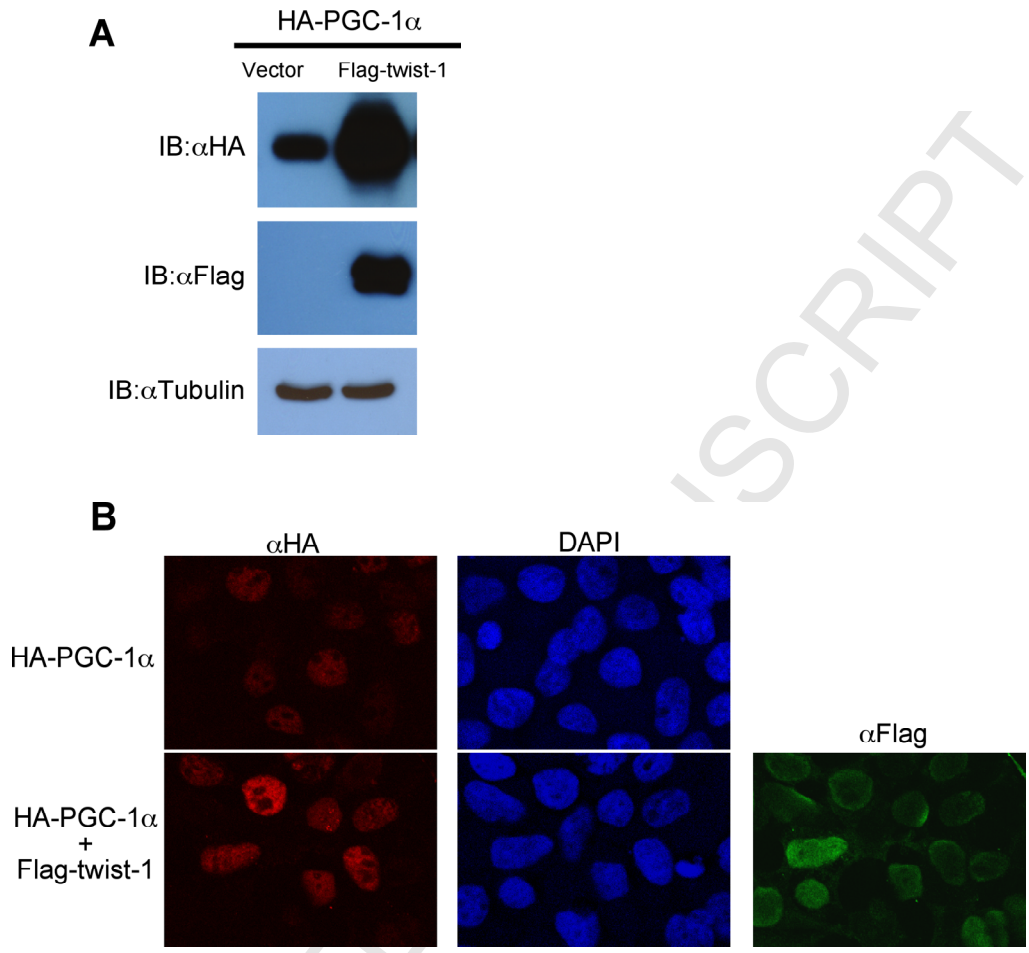
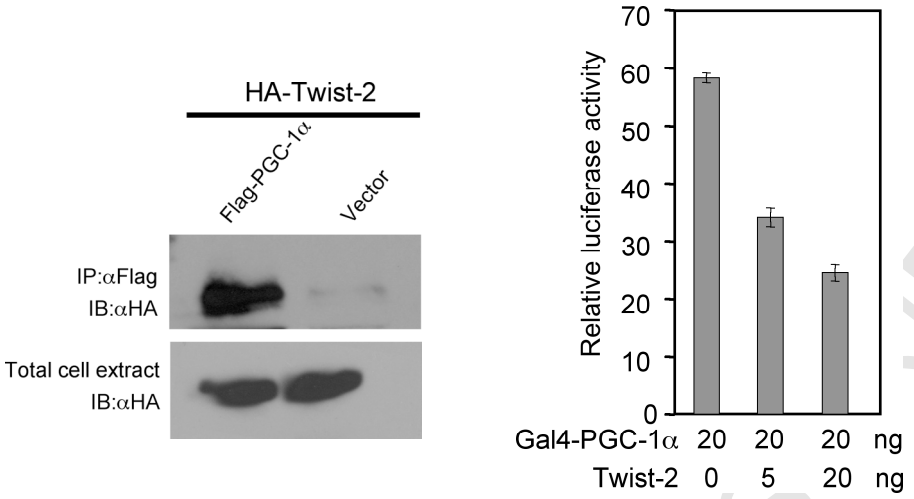


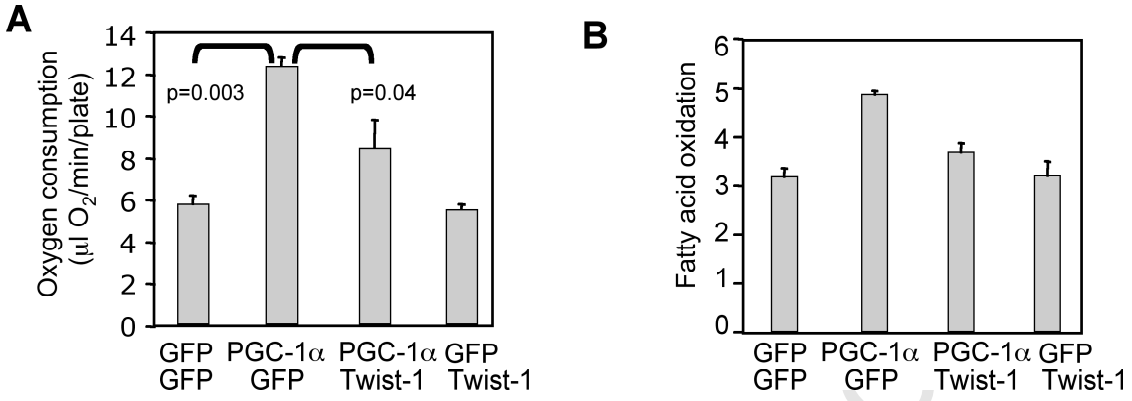
Supplemental Figure 1 Adipogenic differentiation is not affected in 3T3-L1 cells expressing twist-1. Twist-1 was stably overexpressed in 3T3-L1 preadipocytes (left panel). Differentiated adipocytes were stained with oil red O (right panel).



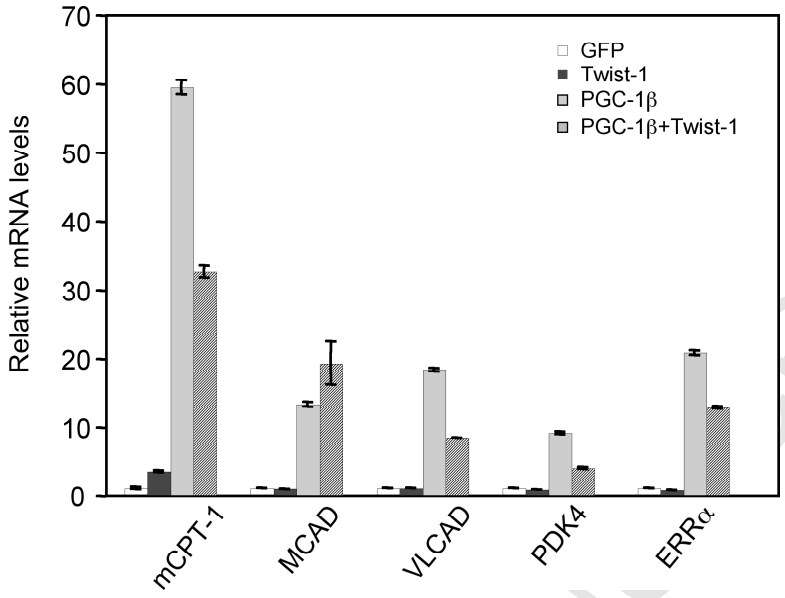
Supplemental Figure 2 Overexpression of twist-1 increases PGC-1α protein level and has no effect on its nuclear localization. HEK293 cells were transfected with HA-PGC-1α along with vector or Flag-twist-1 constructs. Western blot analysis **(A)** and immunofluorescence staining **(B)** were performed. DAPI staining showed the nuclei of the cells.



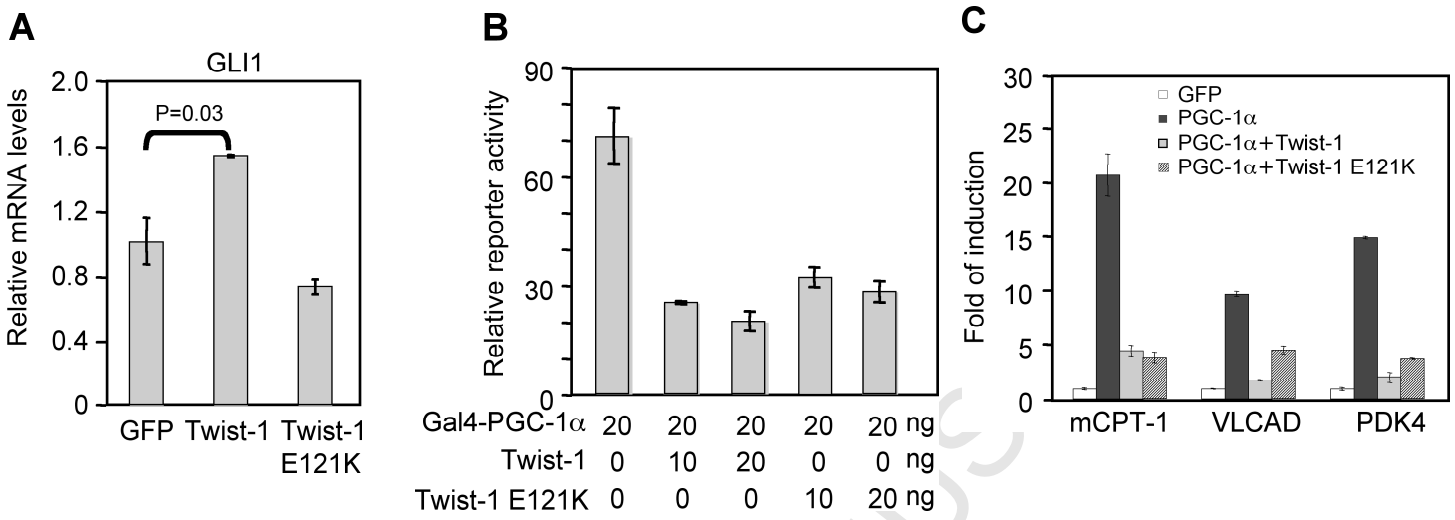
Supplemental Figure 3 Twist-2 interacts with PGC-1α and inhibits PGC-1α transcriptional activity with reporter assay .



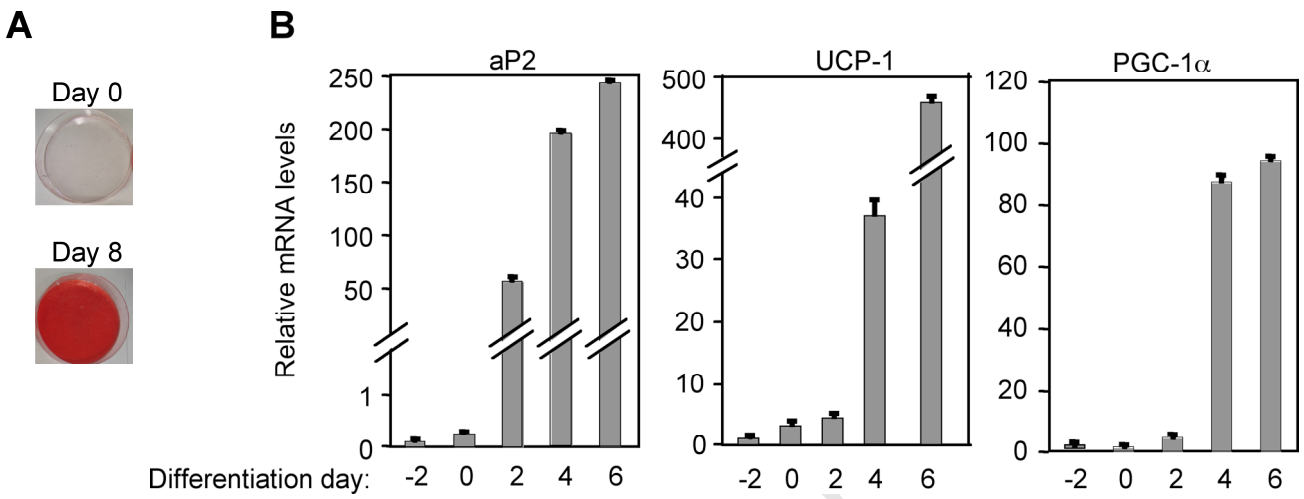
Supplemental Figure 4 Twist-1 suppresses PGC-1α-stimulated oxygen consumption and fatty acid oxidation. C2C12 myotubes were infected with adenovirus expressing indicated constructs. (**A)** Forty-eight hours after infection, cells were assayed for oxygen consumption with a Clark-type electrode. Data are average of three independent experiments. (**B)** Cells were treated with ^{3}H-oleic acid and then assayed for fatty acid oxidation.



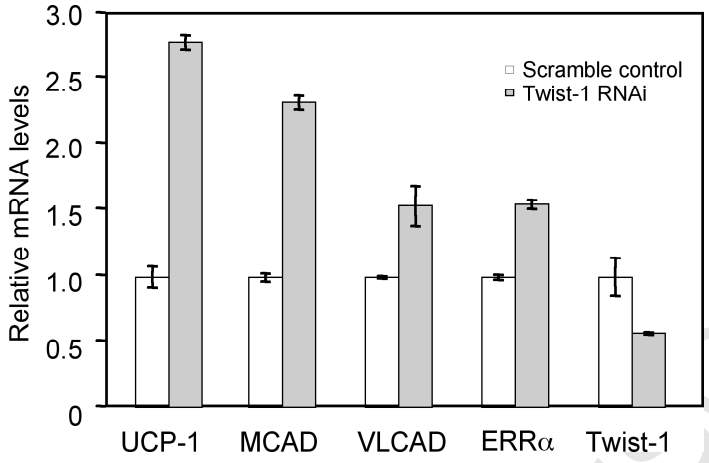
Supplemental Figure 5 Effects of twist-1 on PGC-1β-induced gene expression. C2C12 myotubes were infected with adenovirus expressing indicated constructs. Expression of PGC-1 target genes was analyzed by real-time QPCR. Compared to Figure 3A, the inhibitory effects of twist-1 on PGC-1β is much less than on PGC-1α.



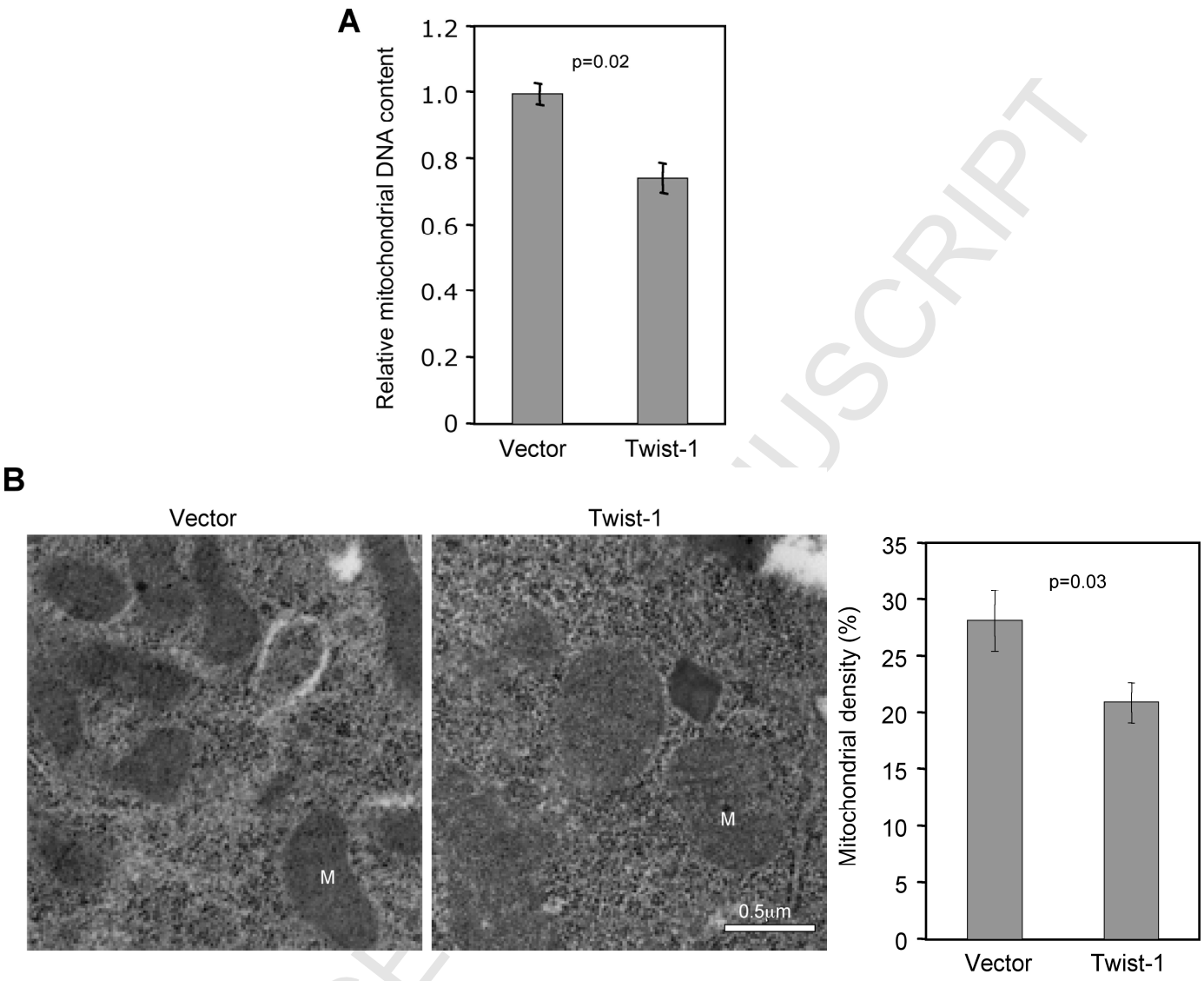
Supplemental Figure 6 The E121K twist-1 point mutation losses its ability to induce GLI1 expression yet is still capable of suppressing PGC-1α function. (A) Mouse embryonic fibroblast cells were infected with indicated adenovirus and GLI1 expression was analyzed by real-time QPCR. (**B)** Reporter assays were performed in cos-7 cells. **(C)** C2C12 myotubes were infected with adenovirus expressing PGC-1α and twist-1 constructs. Expression of PGC-1α target genes was analyzed by real-time QPCR.



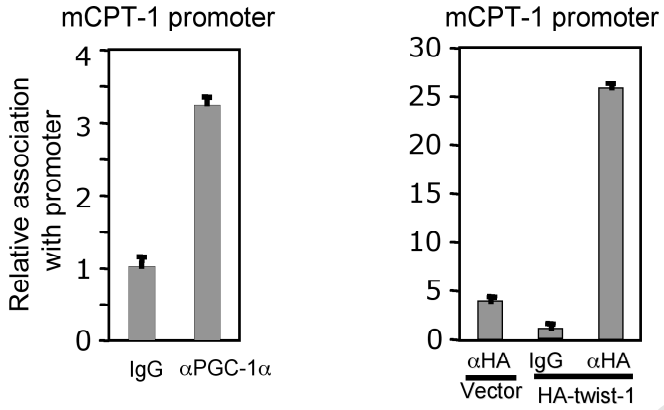
Supplemental Figure 7 Generation of immortalized preadipocyte brown fat cells (δf/f). (A) shown was oil-red O staining for lipids before and after differentiation. **(B)** Expression of general adipocyte marker (aP2) and brown fat specific markers (UCP-1 and PGC-1α) during adipogenic differentiation.



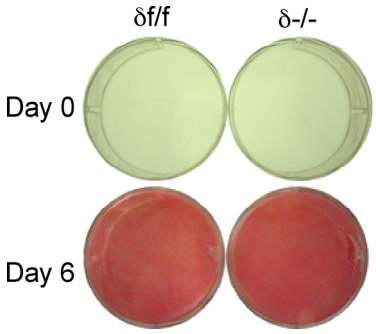
Supplemental Figure 8 Knockdown of twist-1 with a second targeting construct similarly induces expression of PGC-1α target genes. Brown fat preadiopcytes were infected with twist-1 RNAi lentivirus. Cells were then differentiated and gene expression was analyzed. Targeting sequence of twist-1 RNAi is available on Supplemental Table 1.



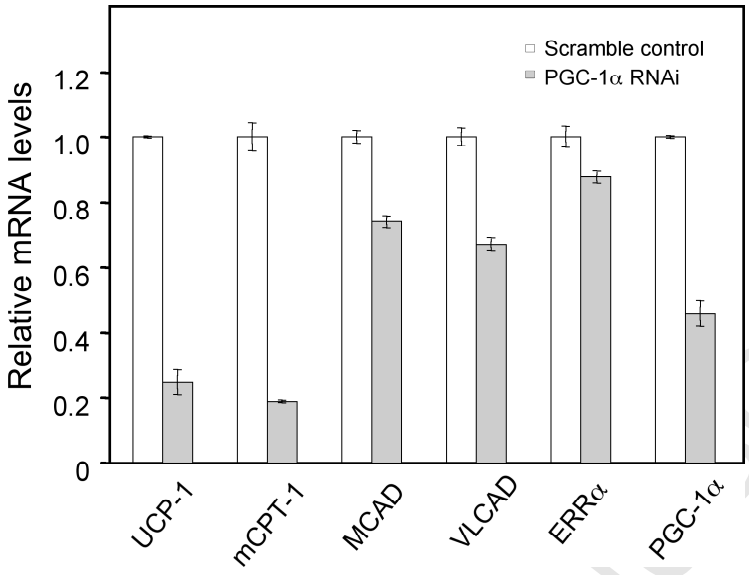
Supplemental Figure 9 Overexpression of twist-1 in brown fat cells suppresses mitochondrial biogenesis. Twist-1 was stably expressed in brown fat preadipocytes and differentiated. **(A)** Mitochondrial DNA content. The level of mitochondrial genome-encoded CoxII gene was measured by QPCR and normalized by the level of β-globin. (**B)** Left, representative electron microscopy micrographs. Right, Quantification of mitochondrial density from eleven micrographs.



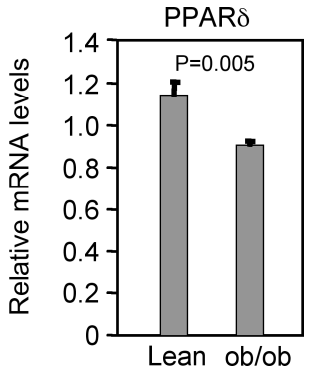
Supplemental Figure 10 Chromatin immunoprecipitation assay on mCPT-1 promoter. Both PGC-1α and twist-1 associate with the PPAR-binding element in the mCPT-1 promoter.



Supplemental Figure 11 PPARδ-deficient cells have normal adipogenesis. PPARδ was deleted in brown fat preadipocytes by cre adenovirus and cells were then differentiated and stained with oil red O.



Supplemental Figure 12 Decreased expression of UCP1 and fatty acid oxidation genes with a second PGC-1α knockdown construct. Brown fat preadiopcytes were infected with PGC-1α RNAi lentivirus. Cells were then differentiated and gene expression was analyzed. Targeting sequence of PGC-1α RNAi is available on Supplemental Table 1.



Supplemental Figure 13 PPARδ level was decreased in the brown fat of obese mice (n=3 mice per group).

Supplementary Table 1 Gene full names and sequences of primers used in this study.

Gene symbol	Gene full name	Primers used for real-time QPCR
mCPT-1	Carnitine palmitoyltransferase 1b, muscle	F: 5'GGGCACCTCTGGGAGTTTGT3' R: 5'TTGGCTCACCCACACAGTGT3'
MCAD	Acyl-coenzyme A dehydrogenase, medium chain	F: 5'GCCAAGATCTATCAGATTTATGAAGGT3' R: 5'AGCTATGATCAGCCTCTGAATTTGT3'
VLCAD	Acyl-coenzyme A dehydrogenase, very long chain	F: 5'GCCAGGGCAGAATCGAAGT3' R: 5'TGGTAAGCTGGCCTTTGAACAT3'
PDK4	Pyruvate dehydrogenase kinase, isoenzyme 4	F: 5'GCAGTAGTCCAAGATGCCTTTGA3' R: 5'AATACTGGTCGCAGAGCATCTTT3'
LCAD	Acetyl-coenzyme A dehydrogenase, long chain	F: 5'CCCTCCGCCCGATGTT3' R: 5'AAGGAGTTTCTAGACGCGCTTCT3'
ERRα	Estrogen-related receptor alpha	F: 5'AGCAAGCCCCGATGGA3' R: 5'GAGAGGCCTGGGATGCTCTT3'
FAS	Fatty acid synthase	F: 5'TGATGATTCAGGGAGTGGATATTG3' R: 5'CCGAGCCAGGGACTTCTTAGT3'
ACC2	Acetyl-coenzyme A carboxylase beta	F: 5'CCTACTATGAGGCCCAGCATGT3' R: 5'TCGGCCTCTCTTCACCAGAT3'
UCP-1	Uncoupling protein 1 (mitochondrial, proton carrier)	F: 5'GAGGTGTGGCAGTGTTCATTG3' R: 5'GGCTTGCATTCTGACCTTCA3'
aP2	Fatty acid binding protein 4, adipocyte	F: 5'GGCGTGACTTCCACAAGAGTTTA3' R: 5'GCCTCTTCCTTTGGCTCATG3'
U36b4	Ribosomal protein, large, P0	F: 5'AGATGCAGCAGATCCGCA3' R: 5'GTTCTTGCCCATCAGCACC3'
Twist-1	Twist gene homolog 1(Drosophila)	F: 5'AGATGTCATTGTTTCCAGAGAAGGA3' R: 5'TTAGTTATCCAGCTCCAGAGTCTCTAGA3'
PGC-1α	Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	F: 5'AACCACACCCACAGGATCAGA3' R: 5'TCTTCGCTTTATTGCTCCATGA3'
PPARα	Peroxisome proliferative activated receptor alpha	F: 5'ACAAGGCCTCAGGGTACCA3' R: 5'GCCGAAAGAAGCCCTTACAG3'
PPARδ	Peroxisome proliferative activated receptor delta	F: 5'GCCTCGGGCTTCCACTAC3' R: 5'AGATCCGATCGCACTTCTCA3'
PPARγ	Peroxisome proliferative activated receptor gamma	F: 5'CAAGAATACCAAAGTGCGATCAA3' R: 5'GAGCTGGGTCTTTTCAGAATAATAAG3'
GLI1	GLI-Krupped family member GLI1	F: 5'TGGTAGAGGGAACTCCAGGT3' R: 5'ACCCTGGGACCCTGACATA3'
CoxII	Cytochrome c oxidase subunit II	F: 5'GCCGACTAAATCAAGCAACA3' R: 5'CAATGGGCATAAAGCTATGG3'
β-globin	Hemoglobin, beta adult major chain	F: 5'GAAGCGATTCTAGGGAGCAG3' R: 5'GGAGCAGCGATTCTGAGTAGA3'
CPT1A	Carnitine palmitoyltransferase 1a, liver	F: 5'TCATCCATGCATACCAAAGTG3' R: 5'ACGCCACTCACGATGTTCTT3'
ACC1	Acetyl-Coenzyme A carboxylase alpha	F: 5'CATGTTGAGACGCTGGTTTG3' R: 5'GGACGCCATCTTCCTCTGT3'
Dgat1	Diacylglycerol O-acyltransferase 1	F: 5'ACGGCTACTGGGATCTGAGG3' R: 5'TCACCACACACCAATTCAGG3'
Gpam	Glycerol-3-phosphate acyltransferase, mitochondrial	F: 5'CCTCTTTTGCCACAACATCA3' R: 5'CGAGCCTCCGTCTTATGAAA3'
Mttp	Microsomal triglyceride transfer protein	F: 5'TAGGGAAGGACAACCTGGAG3' R: 5'CTATGCCCACTGGCTCGTTT3'
		Primers used for CHIP assays
UCP-1	Uncoupling protein 1	F: 5'AGTGAAGCTTGCTGTCACTC3' R: 5'GTCTGAGGAAAGGGTTGACC3'

Twist-1		F: 5'AACTGGGAAGTTCCTGTGGA3' (for distal site) R: 5'CAGCAAGCTGGTAGGTTTCA3' (for distal site) F: 5' GGTGCAGGAGTGGCTTTATT3' (for proximal site) R: 5'AAGCTCTCTTCTGAGGCCAGT3' (for proximal site)
mCPT-1	Carnitine palmitoyltransferase 1b, muscle	F: 5'ACTGTCAACCTTGAGCCCTGGAATTAG3' R: 5' TTGCATCAGTCCTAAAAATAGCTGAATGTA3'
aP2	Fatty acid binding protein 4, adipocyte	F: 5'ACATTTCACCCAGAGAGAAGGGATTGTAGT3' R: 5'AACTCTGATCCAGTAAGAAGGCAAATGTC3'
		RNAi target sequence
Twist-1		Sequence1: AAGCTGAGCAAGATTCAGACC (Yang et al., 2004) Sequence2: AGCGGGTCATGGCTAACGTGC (Yang et al., 2004)
PGC-1α		Sequence1: GGTGGATTGAAGTGGTGTAGA (Koo et al., 2004) Sequence2: CCCATTTGAGAACAAGACTAT

Supplemental Experimental Procedures

Generation and differentiation of brown fat cells. Brown fat was isolated from the newborn mice and digested by collagenase. Preadipocytes were immortalized by infection with the retroviral virus expressing SV40 T antigen and selected with G418.

To generate PPARδ knockout brown fat preadipocytes, δf/f preadipocytes were infected with adenovirus expressing cre recombinase. Cells infected with GFP adenovirus were used as controls. Deletion of the PPARδ alleles was verified by PCR genotyping.

Differentiation was performed according to a standard protocol (Tseng et al., 2004). Briefly, cells were grown to confluence in culture medium containing 20 nM insulin and 1 nM 3,3',5-triiodo-L-thyronine (differentiation medium) (Day 0). Differentiation was induced by treating confluent cells for 48 h in differentiation medium supplemented with 0.5 mM isobutylmethylxanthine, 0.5 μM dexamethasone, and 0.125 mM indomethacin. After this induction period, cells were changed back to differentiation medium. The cells start to accumulate lipid droplet at Day 1 and a fully differentiated phenotype of nearly 100% of cells could be observed at Day 5. All experiments with the brown fat cells were performed after differentiation, unless otherwise indicated.

Oil red O staining. Cells were fixed with 3.7% formaldehyde for 15 min at room temperature and then stained with 0.3% (W/V) oil red O solution in 60% isopropyl alcohol for 1 h. The excess staining was removed by washing with water.

Luciferase reporter assays. Cos-7 cells in 48-well plates were transfected with luciferase reporters, CMV-β-galactosidase plasmids and indicated expression constructs, using lipofectamine 2000 (Invitrogen). Vector plasmids were used to adjust the total amount of plasmids per well. Luciferase activity was measured 48 h after transfection and normalized with β-galactosidase activity.

Co-immunoprecipitation. To determine the interaction between twist-1 and co-activators in HEK293 cells, cells were co-transfected with indicated plasmids. Forty-eight hours after transfection, cells were lysed in buffer [250 mM NaCl, 50 mM Tris (pH 7.5), 0.5% TritonX-100, 5% glycerol]. Cell extracts were incubated with antibody beads and the beads were washed with buffer [250 mM NaCl, 50 mM Tris (pH 7.5), 0.1% TritonX-100, 5% glycerol] for four times. Immunoprecipitates were probed with indicated antibodies.

To examine the association between twist-1 and PGC-1α at physiological levels in brown fat cells, we generated a stable brown fat cell line that expresses HA-tagged twist-1 at a level close to its endogenous level. Briefly, δf/f brown fat preadipocytes were infected with HA-tagged twist-1 retrovirus for a 10% infection efficiency to ensure a single copy integration. The preadipocytes were differentiated. At day 7, nuclear extracts were isolated as described (Schreiber et al., 1989) and incubated for 2 h with agarose beads conjugated with PGC-1α antibody or IgG. The beads were washed 4 times with washing buffer [100 mM NaCl, 50mM Tris (pH 7.5), 0.1% NP-40, 3% glycerol]. Immunoprecipitates were analyzed by Western blotting.

Chromatin immunoprecipitation (CHIP) assays. Brown fat preadipocytes were differentiated for 6 days. CHIP assays were performed using the Upstate protocol with antibodies against HA (H6908, Sigma), PGC-1α (sc-13067, Santa Cruz), PPARδ (sc-7197, Santa Cruz), PPARγ (sc-7196, Santa Cruz), acetyl-Histone H3 (06-599, Upstate), or HDAC5 (H9663, Sigma). Precipitated DNA fragments were purified with QIAquick PCR Purification Kit (Qiagen). Immunoprecipitate signal was normalized with input signal; both were measured by real-time QPCR. Primer sequences were available in Supplementary Table 1.

Electron microscopy. Samples were fixed in 2.5% glutaraldehyde in PBS (pH 7.2), washed, and then post-fixed in 1% osmium tetroxide. The fixed samples were dehydrated through a graded series of ethanol to 100%, followed by two changes of propylene oxide and finally into a 50:50 (v/v) mixture of propylene oxide: epoxy resin (SPON 812/Araldite 502), and left overnight to infiltrate. Samples were processed through two changes of fresh epoxy resin and embedded, allowing the blocks to polymerize 48 hrs at 70°C. Ultrathin sections were cut on a Reichart-Jung ultramicrotome using a diamond knife. The sections (64 nm thick) were collected and mounted on copper support grids and contrasted with lead citrate and uranyl acetate, and examined on a Philips CM 10 transmission electron microscope at 80 Kv accelerating voltage.