Supporting Information

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SI Text

Generation of Liver-Specific Heterozygous PGC-1α Mice. Liver-specific heterozygous mice were generated by mating female mice with one floxed PGC-1α allele [PGC-1α$^{(fl/+)}$] with mice transgenically expressing cre recombinase under control of the rat albumin promoter (Jackson Laboratory). Mice were on a mixed background of C57BL/129 and both PGC-1α$^{(fl/+)}$ and PGC-1α$^{(+/+,alb\text{-}cre/+)}$ littermates were used as controls. Animals were fed a regular chow diet (5008I, PharmaServ) or a high-fat diet (58% kcal fat, D12331, Research Diets Inc.), as indicated. All experiments were performed in accordance with the Animal Facility Institutional Animal Care and Use Committee regulations.

Hyperinsulemic/Euglycemic Clamp Studies. Hyperinsulemic/euglycemic clamp studies were performed as described in ref. 1. Briefly, mice were fed a diet high in fat for 4 weeks before the clamp. Fat and lean body masses were assessed by ^{1}H magnetic resonance spectroscopy (Bruker BioSpin). Seven days prior to clamp, indwelling catheters were placed into the right internal jugular vein extending to the right atrium. After an overnight fast, [3-^{3}H]-glucose (HPLC purified; Perkin-Elmer) was infused at a rate of 0.05 μCi/min for 2 h to assess the basal glucose turnover. The hyperinsulinemic-euglycemic clamp was conducted for 120 min with a primed/continuous infusion of human insulin (3.0 mU/kg/min) (Novo Nordisk) to raise plasma insulin within the physiological range. Blood samples were collected at 10- to 20-min intervals for measurement of plasma glucose, and 20% dextrose was infused to maintain plasma glucose at basal concentrations (≈6.7 mM). To estimate insulin-stimulated whole body glucose fluxes, [3-^{3}H]-glucose was infused at a rate of 0.1 μCi/min and a bolus of 2-deoxy-D-[1-^{14}C]glucose (2-[^{14}C]DG; Perkin-Elmer) was injected to estimate the rate of insulin-stimulated tissue glucose uptake, as described in ref. 2. Blood samples were collected for plasma ^{3}H and ^{14}C activities. At the end of the clamp, mice were anesthetized with pentobarbital sodium injection and tissues were frozen on liquid N_2-cooled aluminum blocks within 4 min.

For the determination of plasma ^{3}H-glucose, plasma was deproteinized with $ZnSO_4$ and $Ba(OH)_2$, dried to remove $^{3}H_2O$, and counted in scintillation fluid. Rates of basal and insulin-stimulated whole body glucose turnover were determined as the ratio of the [3-^{3}H]-glucose infusion rate [disintegrations per minute (dpm)] to the specific activity of plasma glucose (dpm per mg). Hepatic glucose production (HGP) was determined by subtracting the glucose infusion rate from the total glucose appearance rate. The plasma concentration of $^{3}H_2O$ was determined by the difference between ^{3}H counts without and with drying. Whole body glycolysis was calculated from the rate of increase in plasma $^{3}H_2O$ concentration divided by the specific activity of plasma ^{3}H-glucose, as described in ref. 3. Whole body glycogen synthesis was estimated by subtracting whole body glycolysis from whole body glucose uptake, assuming that glycolysis and glycogen synthesis account for the majority of insulin-stimulated glucose uptake (4).

For the determination of individual tissue glucose uptake, tissue samples were homogenized, and the supernatants were subjected to an ion-exchange column to separate tissue ^{14}C-2-DG-6-phosphate (2-DG-6-P) from 2-DG. Tissue glucose uptake was calculated from area under curve of plasma ^{14}C-2-DG profile and tissue ^{14}C-2-DG-6-P content, as described in ref. 3.

Creation of cDNA Expression Plasmids, shRNA Constructs, and Adenoviruses. Mouse Rev-Erbα and ALAS-1 cDNA constructs were obtained from Open Biosystems (Thermo Scientific) and cloned into pcDNA 3.1 (Invitrogen). An HA tag was inserted at the 5′ end of ALAS-1 by PCR. FLAG-tagged human Rev-Erbα (in pcDNA 3.1) was a kind gift from Mitch Lazar (Philadelphia, PA). The short hairpin oligos were ligated into the pLKO.1 lentiviral vector.

Adenoviruses were created by using the Ad-Easy adenoviral vector system, according to manufacturer's instructions. The cDNAs encoding either FLAG-Rev-Erbα or HA-ALAS-1 were subcloned into the pAd-Track-CMV vector (Stratagene). Adenoviruses expressing GFP or PGC-1α were created as described in ref. 5. For shRNA adenoviruses, each short hairpin, preceded by the human U6 promoter, was amplified from the pLKO.1 vector and ligated to the pAd-Track vector lacking the CMV promoter.

Luciferase Assays. A fragment flanking base pairs −440 to + 17 of the FGF21 promoter, including the putative RORE sites, was amplified from mouse genomic DNA and cloned into pGL3:Basic (FGF21:Luc). Mouse RORα and mouse Rev-Erbα expression constructs were obtained from Open Biosystems. pGL3:Basic containing the Bmal promoter (a kind gift from John B. Hogenesch, University of Pennsylvania) was used as a positive control for RORα and Rev-Erbα activity. HEK293 cells were cotransfected with 50 ng reporter, 250 ng RORα, and/or 50 ng Rev-Erbα, as indicated. Luciferase activity was determined 30 h post-transfection.

Primary Hepatocyte Isolation. Primary mouse hepatocytes were isolated by collagen perfusion and percoll gradient purification. Eight- to 12-week-old mice were euthanized with isofluorane immediately before the procedure. The liver was perfused with warm Hank's buffered saline supplemented with 0.4 g/L KCl, 1.0 g/L glucose, 2.1 g/L $NaHCO_3$, and 0.2 g/L EDTA (pH 7.4, 42 °C, CellGro) via the inferior vena cava. The portal vein was severed to allow drainage. Perfusion was continued with warmed Liver Digest Media (pH 7.4, 42 °C, Invitrogen). Dissected liver was manually disrupted in DMEM supplemented with 10% FBS, 4.5 g/L glucose, 2 mM sodium pyruvate, 1 μM dexamethasone (Sigma), 0.1 μM insulin (Sigma), and penicillin/streptomycin. The cell suspension was filtered (70 μM) and viable hepatocytes were isolated after resuspension of pelleted cells in plating media:PBS-buffered Percoll (Sigma) (1:1) and centrifugation for 5 min at 350 rpm. The cell pellet was washed two times with plating media before seeding (45,000 cells/cm^2) in collagen-coated plates. Two hours after seeding, media was changed to DMEM supplemented with 0.2% BSA, 4.5 g/L glucose, 2 mM sodium pyruvate, 0.1 μM dexamethasone, 1 nM insulin, and penicillin/streptomycin (maintenance media).

Chromatin Immunoprecipitation. Primary hepatocytes were isolated as described and plated at a density of 2.5×10^6 cells per 10-cm^2 plate. Cells treated with vehicle or hemin (5 plates per treatment) were washed with PBS and fixed with 1% formaldehyde for 30 min before quenching with 125 mM glycine and harvest. Pooled cell pellets were sequentially washed with 1 mL PBS before lysis in 1 mL Buffer I (0.25% Trition X-100, 10 mM EDTA, 0.5 mM EGTA, and 10 mM Hepes, pH 6.5). Pelleted nuclei were then washed with Buffer II (200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, and 10 mM Hepes, pH 6.5) before lysis

in buffer containing 500 μL 1% SDS, 10 mM EDTA, and 50 mM Tris·HCl, pH 8.0, and freezing at −80 °C. All solutions contained protease inhibitors.

Thawed lysates were sonicated on ice by using a Bioruptor (11 cycles: 30 s on, 30 s off). A 2-μL sample was used to determine average size of sheared chromatin (≈500- to 1,200-bp) and the remaining lysate was centrifuged for 10 min at maximum speed. Supernatants were collected and diluted 10× with buffer containing 1% Trition X-100, 2 mM EDTA, 150 mM NaCl, and 20 mM Tris·HCl, pH 8.1, and protease inhibitors (soluble chromatin). One hundred microliters soluble chromatin was saved to represent 10% input. Protein G-Sepharose beads were preincubated with dilution buffer containing 2 mg/mL BSA for 30 min before incubation with 1 mL soluble chromatin, 2 μg sheared herring sperm DNA, and 6 μg rabbit IgG for 2 h at 4 °C. Supernatants were collected and incubated with specific antibodies overnight at 4 °C with rotation.

Protein G-Sepharose beads, previously incubated in dilution buffer with 2 mg/mL BSA, were added to each sample with 2 μg sheared herring sperm DNA for 6 h at RT with rotation. Beads were sequentially washed for 20 min with TSE I buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·HCl, pH 8.1, and 150 mM NaCl), TSE II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris·HCl, pH 8.1, and 500 mM NaCl), and Buffer III (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, and 10 mM Tris·HCl, pH 8.1). Beads were then washed 3 times with TE before 3 × 10-min elutions with 100 μL of 1% SDS/0.1 M $NaHCO_3$. Elutes were pooled for each sample and decross-linking was performed overnight at 65 °C after addition of NaCl to a final concentration of 200 mM. PCR analysis (29 cycles) was performed on purified DNA fragments.

1. Choi CS, et al. (2007) Continuous fat oxidation in acetyl-CoA carboxylase 2 knockout mice increases total energy expenditure, reduces fat mass, and improves insulin sensitivity. *Proc Natl Acad Sci USA* 104:16480–16485.
2. Samuel VT, et al. (2006) Targeting foxo1 in mice using antisense oligonucleotide improves hepatic and peripheral insulin action. *Diabetes* 55:2042–2050.
3. Youn JH, Buchanan TA (1993) Fasting does not impair insulin-stimulated glucose uptake but alters intracellular glucose metabolism in conscious rats. *Diabetes* 42:757–763.
4. Rossetti L, Giaccari A (1990) Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. A dose-response euglycemic clamp study in normal and diabetic rats. *J Clin Invest* 85:1785–1792.
5. Yoon JC, et al. (2001) Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 413:131–138.

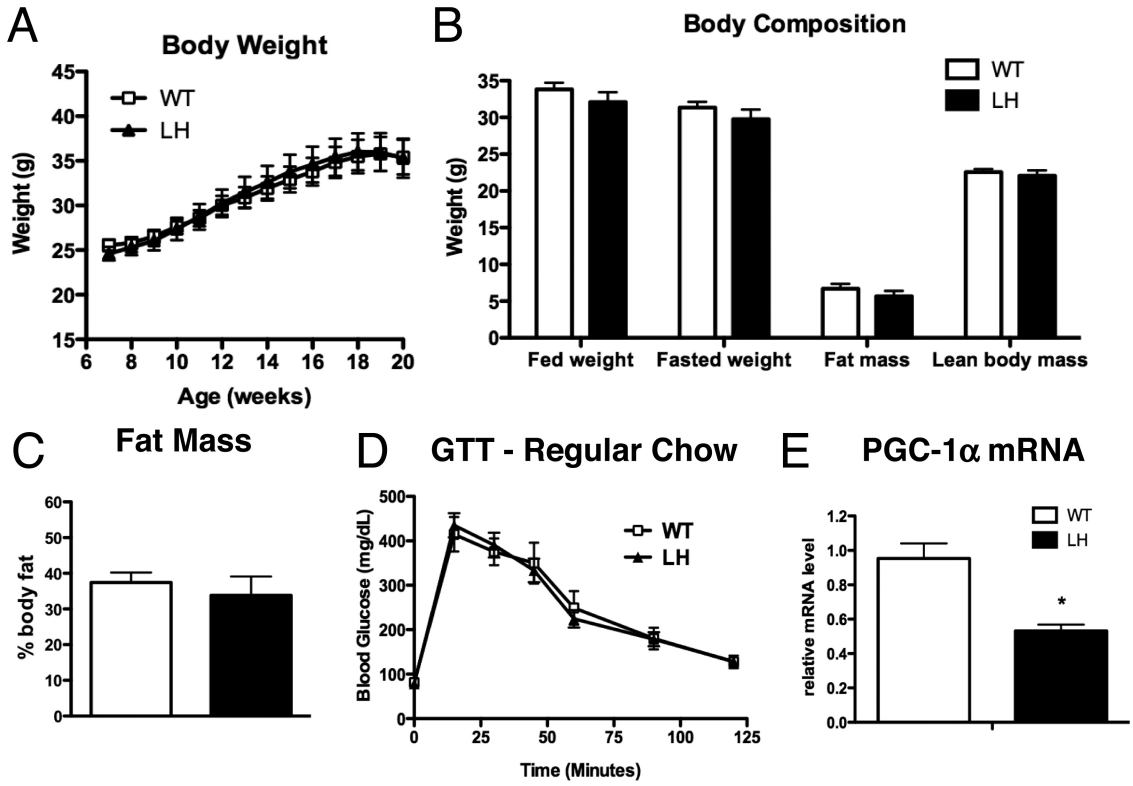

Fig. S1. LH mice exhibit normal growth and have similar body composition on a high fat diet. (*A*) Growth curve of mice fed a diet consisting of 58% fat for 13 weeks. Values are weight in grams ± SEM. ($n = 13–14$). (*B*) Mice on a high-fat diet for 4 weeks were weighed before and after a 6-h fast. Fat mass and muscle mass was determined by NMR. (*C*) Fat mass, measured by duel energy X-ray absorptiometry, in ad libitum fed mice after 16 weeks high-fat feeding. (*D*) Blood glucose levels were measured in regular chow-fed WT or LH mice after an overnight fast (time 0) and at the indicated times after an i.p. injection of glucose. Values represent means ± SEM. ($n = 6$). (*E*) Fed levels of hepatic *PGC-1α* mRNA in WT and LH mice on a HFD. Values are means ± SEM. of $n = 7–11$ mice, *, $P < 0.05$.

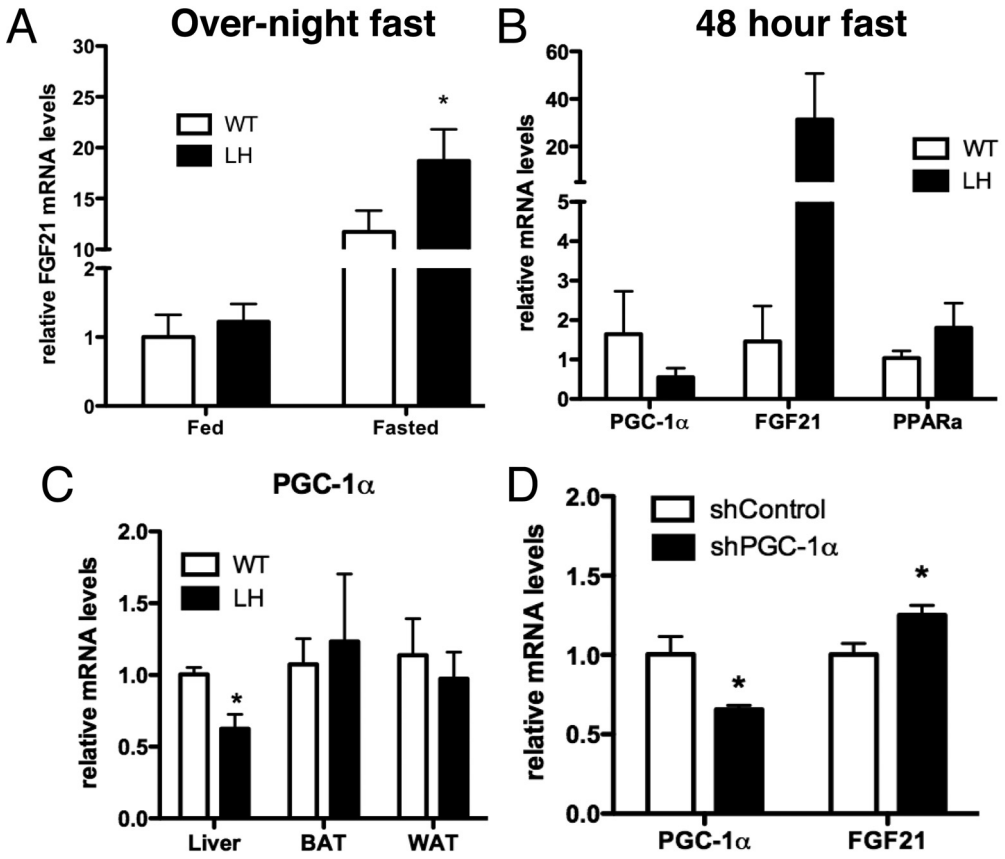

Fig. S2. Hepatic *FGF21* expression inversely correlates with *PGC-1α* in fasted chow-fed mice and primary hepatocytes. Hepatic mRNA levels were quantified by real-time PCR in WT and LH mice fed ad libitum or fasted (*A*) 16 h ($n = 12$), or (*B*) 48 h ($n = 3–4$). mRNA values for LH mice are expressed relative to corresponding WT fed (*A*) or fasted (*B*) levels (white bar) for each treatment (means ± SEM.) *, $P < 0.05$ by two-way ANOVA. (*C*) Relative *PGC-1α* mRNA expression levels in liver, white adipose tissue (WAT), and brown adipose tissue (BAT) of chow-fed WT and LH mice (means ± SEM., $n = 5–6$). (*D*) mRNA levels in primary mouse hepatocytes harvested 48 h after infection with adenoviruses expressing either shPGC-1α or control virus (shControl). Bars, means ± SD of triplicate values, representative of two individual experiments, *, $P < 0.05$.

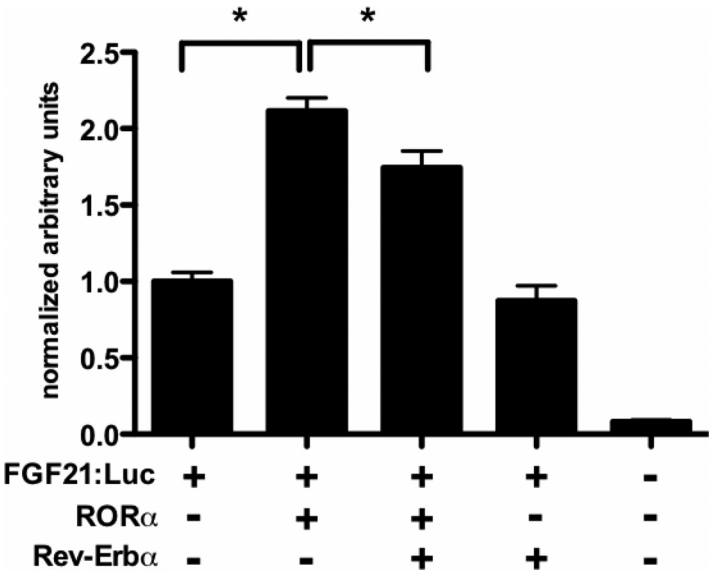

Fig. S3. Rev-Erbα attenuates RORα activation of the FGF21 promoter. RORα- and Rev-Erbα-mediated regulation of FGF21:Luc promoter transcription. Bars, means $\pm$ SD of triplicate values, normalized to basal FGF21:Luc promoter activity, representative of three individual experiments, *, $P < 0.05$.

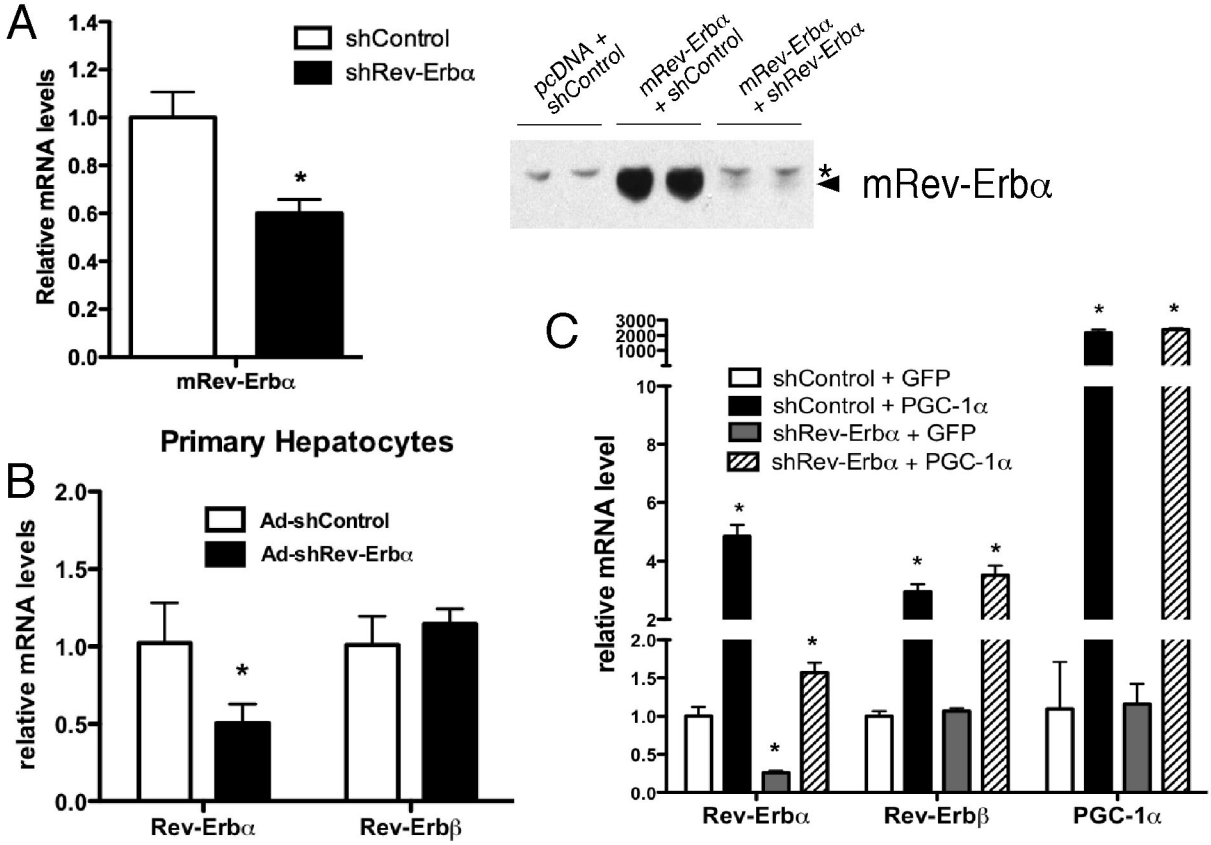

Fig. S4. shRev-rbα construct is specific and effectively reduces both mRNA and protein levels in cells. (*A*) HEK 293 cells cotransfected with expression constructs encoding mouse Rev-Erbα and shControl or shRev-Erbα. Cells were harvested for mRNA expression and Western blot analysis 48 h post-transfection. The asterisk (*) represents a nonspecific band and equal loading was also confirmed by ponceau staining before blotting with anti-Rev-Erbα antibody. Primary hepatocytes were infected with adenovirus expressing either shControl or shRev-Erbα alone (*B*) or in combination with Ad-PGC-1α or GFP (*C*), as indicated. Forty-eight hours after infection, mRNA expression was measured by quantitative PCR. Bars, means ± SD of triplicate values and are representative of at least 3–4 independent experiments. *, $P < 0.05$ represents statistical significance compared to shControl (*A*) or shControl + GFP (*B*) values.

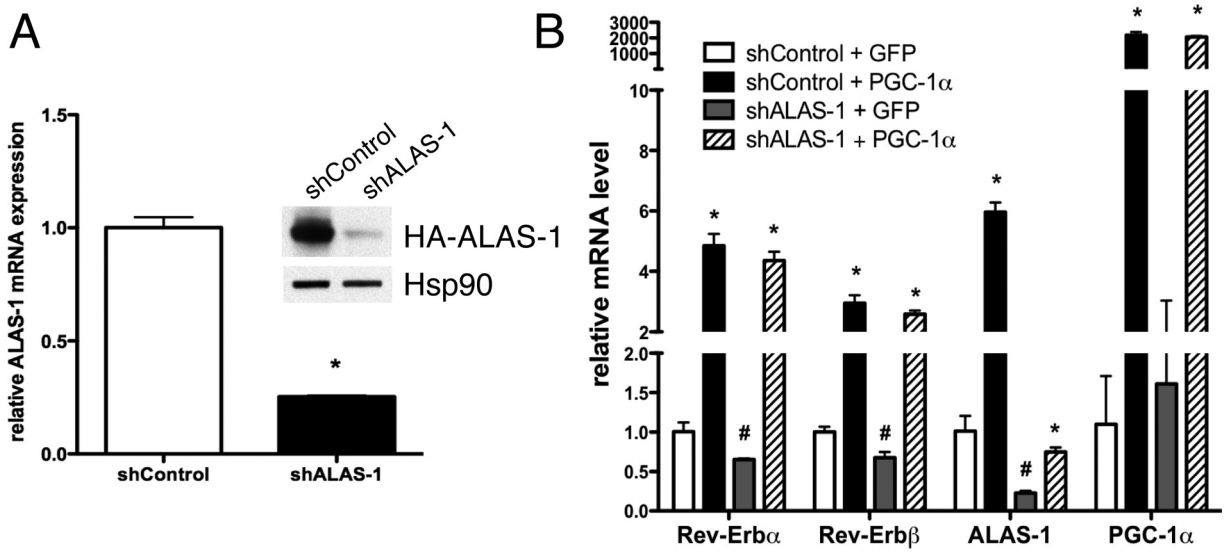

Fig. S5. The adenovirus expressing shALAS-1 effectively reduces both mRNA and protein levels in cells. (*A*) ALAS-1 mRNA and protein (*Inset*) levels in primary hepatocytes 48 h after coinfection with adenoviruses expressing HA-tagged ALAS-1 and either shControl or shALAS-1. Equal loading was confirmed by staining for Hsp90. (*B*) mRNA levels in primary hepatocytes coinfected with adenoviruses expressing either shControl or shALAS-1 and PGC-1α or GFP, as indicated, 48 h after infection. Bars, means $\pm$ SD of triplicate values and are representative of at least two independent experiments. *, $P < 0.05$ represents statistical significance between GFP and PGC-1α expressing cells for corresponding shRNA construct, #, $P < 0.05$ represents statistical significance between shControl + GFP and shALAS-1 + GFP values.

Table S1. PCR Primers

Gene	Forward primer	Reverse primer
qPCR primers		
Pgc-1α (exon 3–5)	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
Angptl3	CACGAAACCAACTACACGCTACAT	AGGGCCCCAGGGATATTG
Angptl4	TGCATCCTGGGACGAGATG	CATGGCCGAGCTGTAGCA
Angptl6	GGAGTATGGTATCATGGAGGTCATT	ACGGAACTCGGCCCAGTAG
Agt	TTCACTGCTCCAGGCTTTCGTCTA	TTCTCAGTGGCAAGAACTGGGTCA
Fgf21	AGCTCTCTATGGATCGCCTCACTT	ACACATTGTAACCGTCCTCCAGCA
Hgf	TTGGGATTCGCAGTACCCTCACAA	TAGCCAACTCGGATGTTTGGGTCA
Igf1	TGAGCTGGTGGATGCTCTTCAGTT	TCATCCACAATGCCTGTCTGAGGT
Thpo	ACACGGACCTGTGAATGGAACTCA	GTTGAATGCCAGGGAGCCTTTGTT
Glut1	CGAGGGACAGCCGATGTG	TGCCGACCCTCTTCTTTCAT
Glut4	CATGGCTGTCGCTGGTTTC	AAACCCATGCCGACAATGA
Rev-Erbα (m)	TTACCAAGCTGAATGGCATGGTGC	ATATTCTGTTGGATGCTCCGGCGA
Rev-Erbα (hm)	AGACAGTGATGTTCCTAAGCCGCA	TGAGCTTCTCGCTGAAGTCGAACA
Rev-Erbβ	TAACTGTGATGCCAACGGCAATCC	AGGACAATCTGTGCGGTCACTCTT
Bmal1	TCAAGACGACATAGGACACCT	GGACATTGGCTAAAACAACAGTG
Alas-1	GCCAGGCTGTGAAATTTACT	CTGTTGCGAATCCCTTGGAT
TBP	CCCTATCACTCCTGCCACACCAGC	GTGCAATGGTCTTTAGGTCAAGTTTACAGCC
ChIP primers		
−80 FGF21	ACTAAGGTGAAGATCCCAACCTCC	TCCCACTCCTGACGCGTGATATTT
−2667 FGF21	TTCTGGGAACCTCACAGCTCAACT	GCTGCCTTGGAATCACCCAAACTT

All primers are listed in 5′ to 3′ direction.