Supplemental Figures:

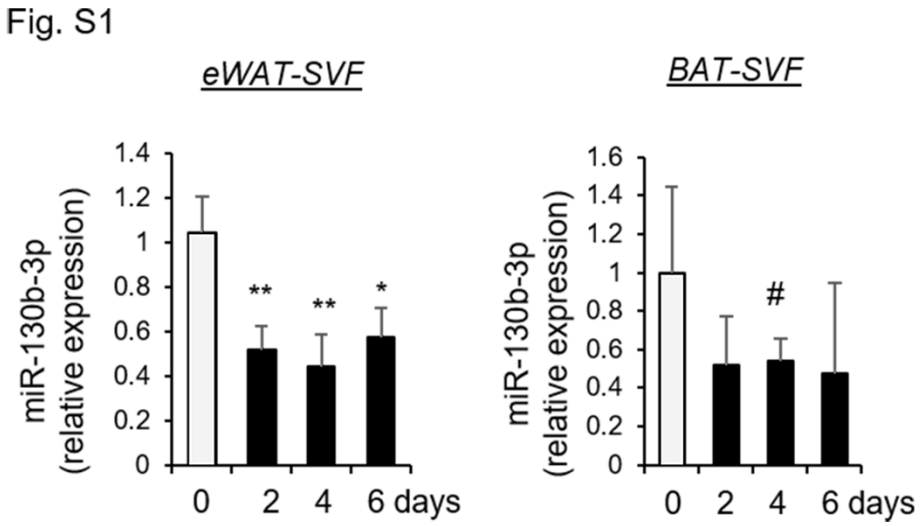

Fig. S1. Decreased mR-130b-3p in eWAT and BAT SVF cells following beige differentiation. Stromal vascular fraction cells were isolated from epididymal white fat (eWAT-SVF) or brown fat from WT mice (3 months age) followed by induction of beige adipogenesis *in vitro*. Before (day 0) and at 2, 4, 6 days after differentiation, fold change of mRNA levels of miR-130b-3p relative to day 0 are shown. n=3-4 in each group, Mean±SD, * $P<0.05$; ** $P<0.01$; # $P<0.1$ compared to day 0 undifferentiated cells.

Fig. S2

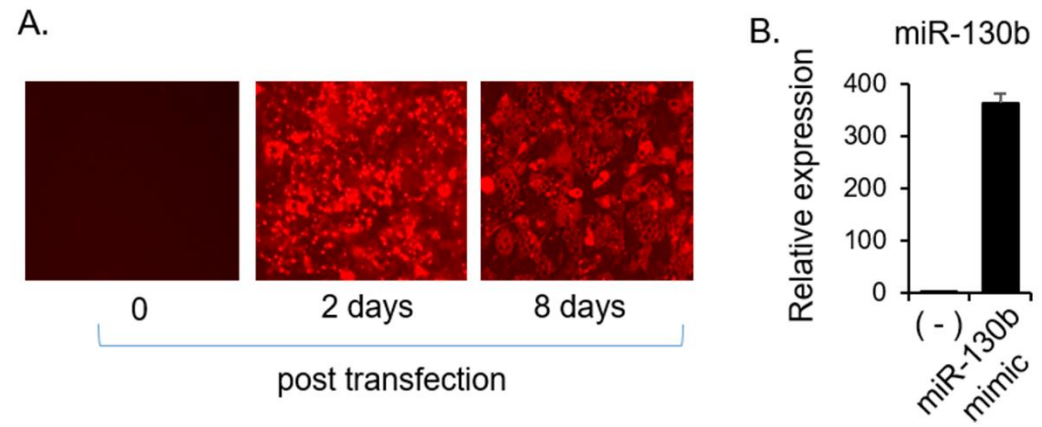

Fig. S2. Transfection of iWAT progenitor cells. The stroma vascular fraction cells isolated from iWAT of mice were transfected with miR-130b-3p (Cy3 labeled). (A) Fluorescent imaging of cells at 0, 2, and 8 days after transfection with Cy3 labeled-miR-130b. (B) Levels of miR-130b-3p at 8 days after transfection was determined by real-time PCR and normalized to house keep gene sno234.

Fig. S3

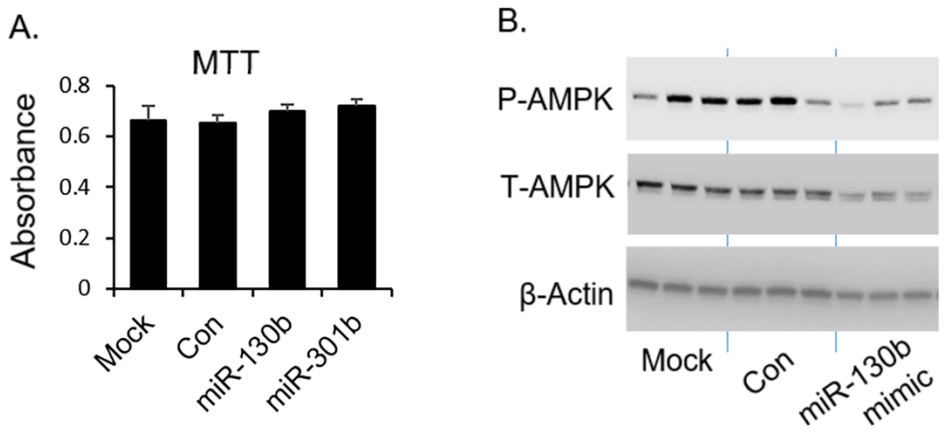

Fig. S3. Cell viability assay of iWAT progenitor cells following transfection. A). The stroma vascular fraction (SVF) cells isolated from iWAT of mice were transfected with miR-130b-3p, or miR-301b-3p mimic, or scrambled negative control (Con), or mock control (transfection reagent only). 2 days after transfection, MTT assay was performed. B). iWAT SVF cells were transfected with miR-130b mimic, negative control miRNA (Con), or transfection reagent only (Mock). Beige adipogenic differentiation was induced at 2 days after transfection. At 6 days after differentiation, P-AMPK and T-AMPK were measured by Western blot analysis.

Fig.S4

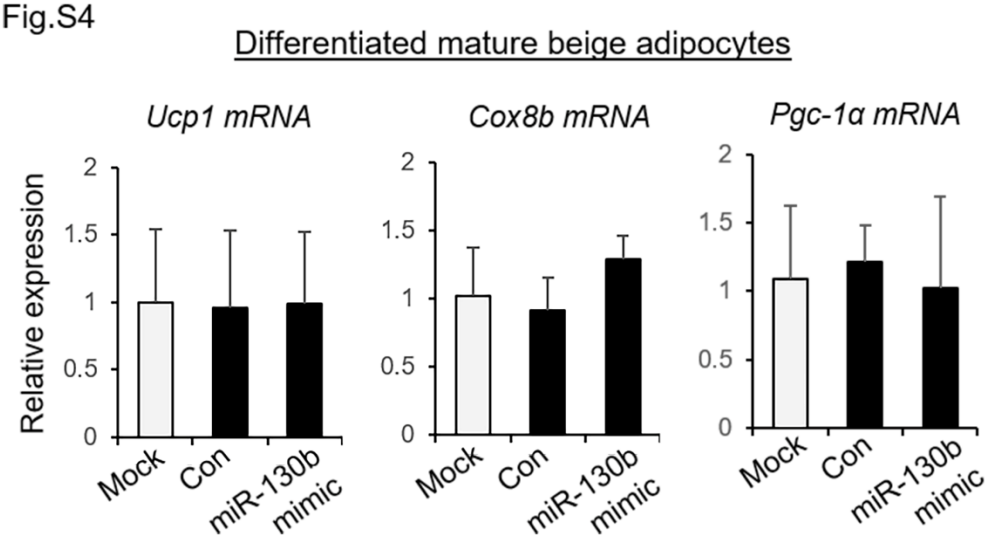

Fig. S4. Role of miR-130b/301b in mature beige adipocytes. Progenitor cells were isolated from inguinal white fat from WT mice at the time of weaning and induced for beige adipocyte differentiation for 6 days. Differentiated beige adipocytes were transfected with miR-130b-3p mimics, or negative control miRNA mimic, or transfection reagent only (mock). Two days after transfection, mRNA expression of Ucp1, Cox8b, and Pgc-1a were measured by real-time PCR, n=3 in each group.

Fig.S5

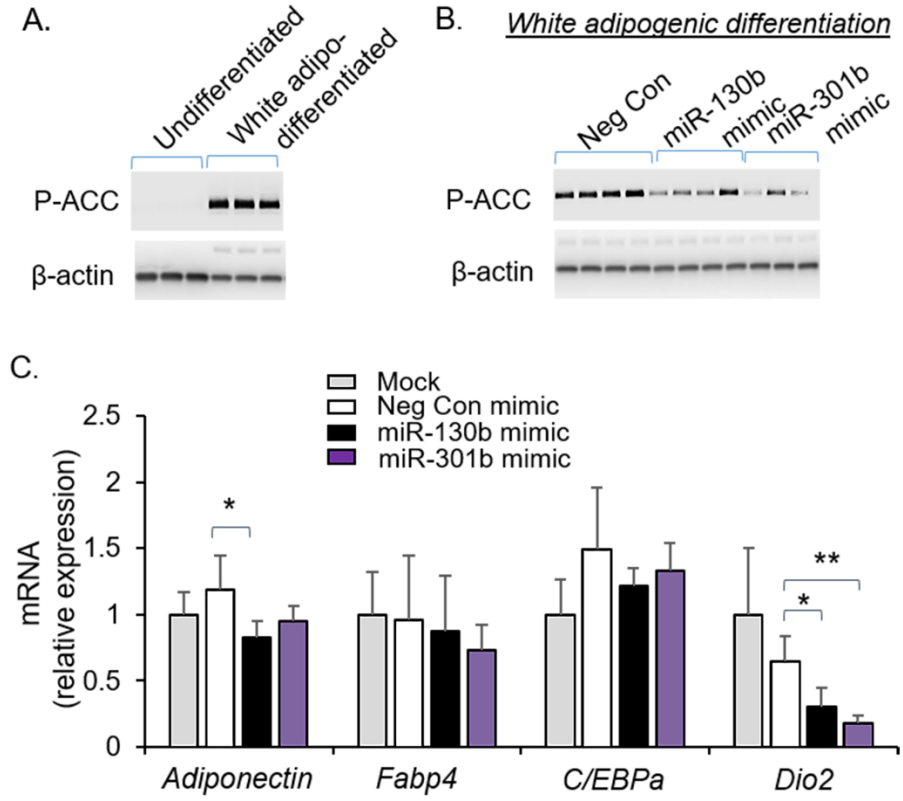

Fig. S5. Role of miR-130b/301b in white adipogenic differentiation of progenitor cells isolated from fat tissues. Progenitor cells were isolated from inguinal white fat from WT mice at the time of weaning followed by induction of white adipocyte differentiation. (A) Blots showing protein levels of P-ACC; (B) iWAT SVF cells were transfected with miR-130b-3p mimics, miR-301-3p mimics, or negative control, or transfection reagent only (mock). White adipogenic differentiation was induced at 1 day after transfection. At 3 days after white differentiation, level of ACC phosphorylation was measured by WB. (C) At 7 days after differentiation, mRNA levels of *adiponectin, Fabp4, C/EBPa, and Dio2* (type 2 deiodinase) were measured by real-time PCR and normalized to TBP. * $P<0.05$, ** $P<0.01$ compared to negative control group (n=4).

Research Design and Methods:

Luciferase reporter assay:

Luciferase assays were performed on progenitor cells isolated from mouse iWAT tissue. The Firefly/Renilla dual luciferase reporter vector containing mouse AMPKα1 3'UTR region (position 1728-2226) was made commercially (creative biogene, NY, USA). The luciferase reporter plasmid (100 ng) and microRNA mimics (miR-130b, 301b, or 148a, 50 nM) were co-transfected into iWAT progenitors (undifferentiated) with Lipofectamine 3000 (ThermoFisher Scientific, MA, USA). Two days after transfection, the activities of firefly and renilla luciferases were measured by Dual-Glo™ Luciferase Assay System (Promega) according to manufacturer's protocol.

Histology:

Tissues were fixed in 4% paraformaldehyde for 24 hours and embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed on the tissue sections (Histology lab, OUHSC). The number of adipocytes were counted using ImageJ software. Immunohistochemical staining of UCP1 was performed on the paraffin sections using the anti-UCP1 antibody (1:500, Invitrogen) and HRP/DAB (ABC) detection IHC kit (Abcam, Boston, MA).

RNA extraction and real-time PCR:

Murine tissues or cells were homogenized and lysed in QIAzol for total RNA extraction using the miRNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The concentrations of isolated total RNA were determined by Nanodrop Spectrophotometers. For miRNA measurement, reverse transcription was performed using the TaqMan MicroRNA Reverse

Transcription Kit or advanced miRNA cDNA Synthesis Kit according to the Applied Biosystems protocol (Life Technologies, Grand Island, NY). All qPCR reactions for miRNAs were performed using TaqMan Universal Master Mix II, no UNG (Life Technologies, Grand Island, NY). Relative expression of individual miRNA was calculated using the 2-ΔΔCT method, where Sno234 served as the endogenous control small non-coding RNA. Absolute concentrations of miRNAs were quantified using the standard curve for each microRNA. For measurement of *Ucp1, Cox8b, Pgc-1α, Prdm16, Adiponectin, Fabp4, C/EBPα,* and *Dio2* mRNA expression, reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific) and real-time PCR was performed using SYBR Green Master Mix (ThermoFisher Scientific) and specificity was determined by performing a melting curve. TBP (TATA box binding protein) was used as housekeeping gene reference. The primers are: *Ucp1* F: GAGGTCGTGAAGGTCAGAAT; *Ucp1* R: CTGTGGTGGCTATAACTCTGTAA. *Cox8b* F: GAACCATGAAGCCAACGACT; *Cox8b* R: GCGAAGTTCACAGTGGTTCC. *Pgc1α* F: CACCAAACCCACAGAAAACAG; *Pgc1α* R: GTACAACTCAGATTGCTCGGG. *Prdm16* F: CAGCACGGTGAAGCCATTC; *Prdm16* R: GCGTGCATCCGCTTGTG. *Fabp4* F: TGAAATCACCGCAGACGACA; *Fabp4* R: ACACATTCCACCACCAGCTT. *C/EBPα* F: TTACAACAGGCCAGGTTTCC; *C/EBPα* R: CTCTGGGATGGATCGATTGT. *Dio2* F: CAGTGTGGTGCACGTCTCCA; *Dio2* R: TGAACCAAAGTTGACCACCA. *Perilipin A* F: CTTTCTCGACACACCATGGAAACC; *Perilipin A* R: CCACGTTATCCGTAACACCCTTCA. *PPARγ2* F: TCGCTGATGCACTGCCTATG; *PPARγ2* R: GAGAGGTCCACAGAGCTGATT.

Western Blot Analysis:

Adipose tissue samples were lysed and homogenized in radioimmunoprecipitation assay (RIPA) protein lysis buffer containing a protease and phosphatase inhibitor cocktail (Pierce Biotechnology, Rockford, IL). Protein concentrations were measured by BCA assay (Pierce, Rockford, IL). Twenty µg of protein lysate was treated with Laemmli sample buffer containing dithiothreitol (DTT), subjected to sodium dodecyl sulfated polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane followed by incubation with antibodies specific for UCP-1 (Abcam, Cambridge, MA), T-AMPKα1, P(S97)-ACC, PGC-1α (Abcam, Cambridge, MA), or β-actin (Cell Signaling Technology, Danvers, MA). The proteins of interest were detected by enhanced chemiluminescence (Pierce, Rockford, IL) and analyzed by imaging densitometry with Image Lab Software (Bio-Rad, Hercules, CA).

Oil Red O staining:

Oil Red O staining was performed and quantified according to protocol previously described (30). Briefly, differentiated adipocytes were washed with PBS, fixed in 4% paraformaldehyde for 15 minutes, and stained with freshly prepared Oil Red O for 15 minutes. The cells were photographed and visualized using a light microscope. Oil Red O staining intensity of four equal areas were quantified using histogram analysis in Photoshop.

Measurement of oxygen consumption rate (OCR)

Oxygen consumption rates (OCR) in differentiated cells were measured by using an XFe96 extracellular flux analyzer (Agilent Seahorse). The oxygen consumption rate was measured over time and after stress treatment (Seahorse XF cell mito stress test kit, Agilent) using oligomycin (Olig, ATP synthase inhibitor, 1 µM), uncoupler FCCP (carbonyl cyanide p-trifluoromethoxy-

phenylhydrazone, 1μM), and Antimycin A & Rotenone (mitochondrial respiration inhibitor, 0.5 μM). Indices of Baseline OCR, Proton leak, Maximal respiration, ATP production were calculated according to altered OCR following the treatment.

HOMA-IR assessment:

The levels of insulin in the serum of mice after 4 hours fasting were measured using mouse insulin ELISA kit (Mercodia, NC, USA) according manufacture's protocol. Blood glucose levels were measured using the Glucose Assay Kit (Abcam, MA, USA). HOMA-IR was calculated based on the equation: HOMA1-IR = (FPI × FPG)/22.5, where FPI is fasting plasma insulin concentration (mU/l) and FPG is fasting plasma glucose (mmol/l).

Glucose tolerance test:

After HFD feeding for 10 weeks, mice were fasted overnight in fresh cages with *ad libitum* water supply. Body weight and fasting glucose were measured, followed by intraperitoneal injection of D-glucose (1.5 mg glucose/g body weight) and an i.p. glucose tolerance test was performed. Blood glucose levels were measured in the tail vein using blood glucose meter (True Metrix Pro, Mckesson) at 15, 30, 60, and 90 min after injection.