Supplemental figure legends and figures

FINAL MANUSCRIPT CELLMET-D-06-00034R1

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Title: Hypomorphic Mutation in *PGC1*β causes mitochondrial dysfunction and liver insulin resistance

Vianna_1S

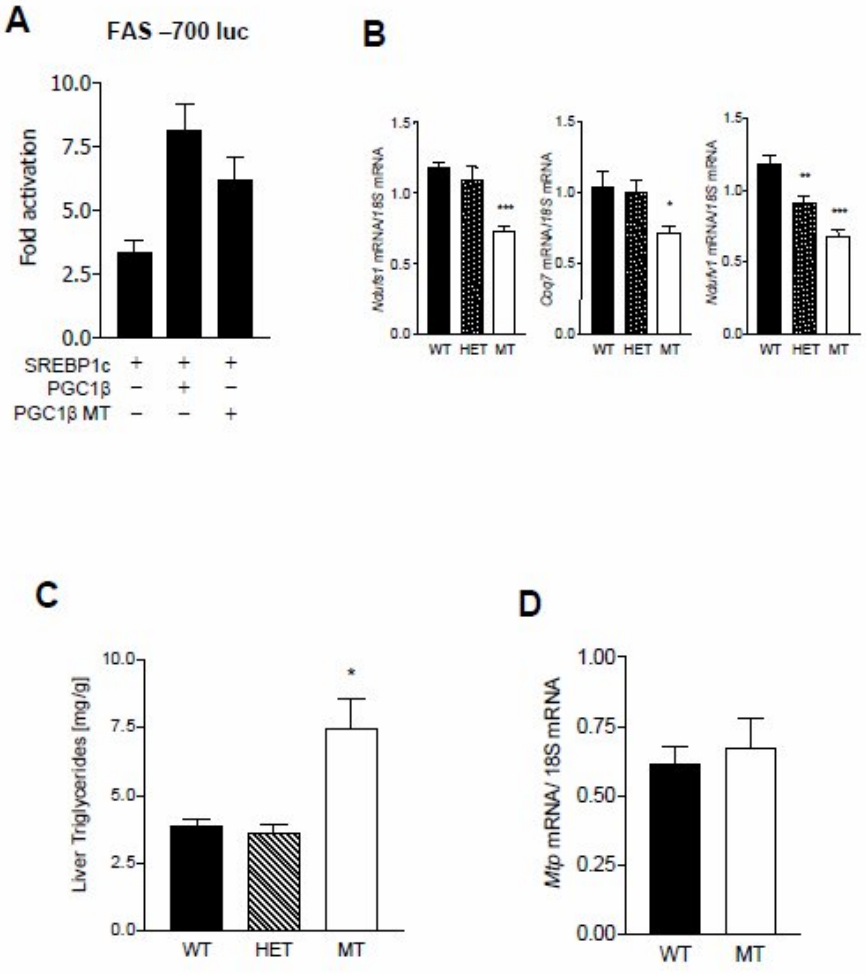

Figure 1S. SREBP1c Coactivation, Gene expression, liver triglycerides, and MTP gene expression

(A) Reduced SREBP1c coactivation by PGC1β mutant protein. Mouse H2.35 hepatoma cells were transiently cotransfected with FAS –700 luc reporter plasmid and with pCATCH-PGC1β or pCATCH-PGC1β mutant. pCMV-βGal was added for transfection efficiency normalization. Cells were harvested 48 hours after cotransfection and assayed for luciferase and β-galactosidase assays. The results represent the means ± SEM of at least three independent experiments. (B)

Gene expression in heterozygous mice versus wild-type and homozygous mice. Quantitative PCR was performed on liver tissue from heterozygous mice to investigate the expression levels of genes found to be markedly abnormal in the liver of homozygous mice (see Figure 3). The mRNA content of specific genes was normalized to 18S mRNA content. Black bars represent WT; shaded bars represent HETs, white bars represent MT. Results are expressed as mean ± SEM (n=6-10). (C) Increased triglycerides in the liver of homozygous PGC1β mice. Triglycerides were isolated from the liver of fed wild-type, heterozygous and homozygous PGC1β mice and measured with a commercially available kit. Results are expressed as mean ± SEM (n=6). (D) Unaltered *Mtp* gene expression in liver. *Mtp* gene expression in liver tissue of wild-type and homozygous PGC1β mice was analyzed by q-PCR. The mRNA content was normalized to 18S mRNA content. Black bars represent WT; white bars represent MT. Results are expressed as mean ± SEM (n=6). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Vianna_2S

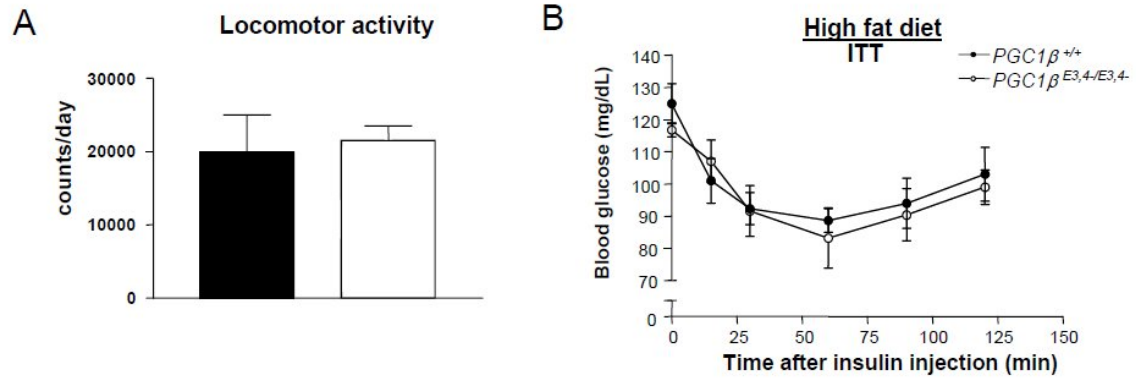

Figure 2S. Locomotor activity and high fat fed mice-insulin tolerance test.

(A) 24h locomotor activity measured with CLAMS. (B) Intraperitoneal insulin tolerance test (ITT). ITT was performed in high fat fed male *Pgc1β* $^{+/+}$ and *Pgc1β* $^{E3,4-/E3,4-}$ mice. Following 4 hours without food, mice were injected with insulin 1.5 U/Kg of body weight. Bars represent *Pgc1β* $^{+/+}$ (black) and *Pgc1β* $^{E3,4-/E3,4-}$ (white) mice. Results are expressed as mean ± SEM (n=5-6).

Vianna_3S

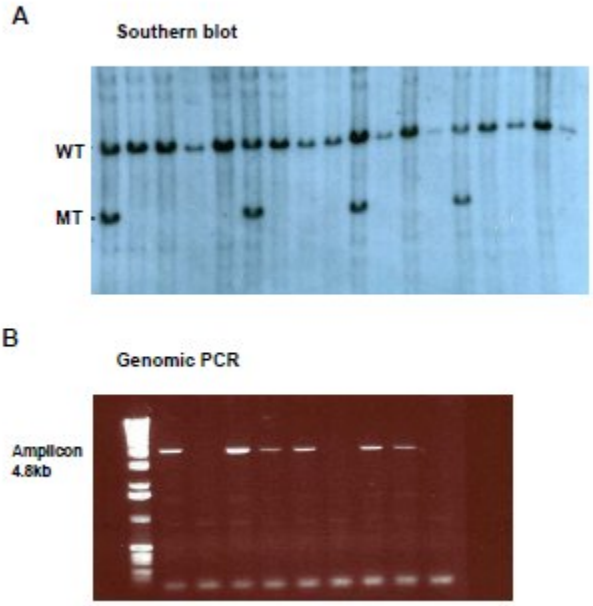

Figure 3S. Screening of 129sv embryonic stem cell (ES) clones.

(A) Southern blot analysis of ES clones. Clones that underwent 3' end homologous recombination contain both, wild-type (WT) and mutant DNA fragments (MT). (B) Genomic PCR analysis of ES clones. A 4.8kb amplicon is obtained using as template DNA of clones that underwent 5' end homologous recombination.

Figure 4S

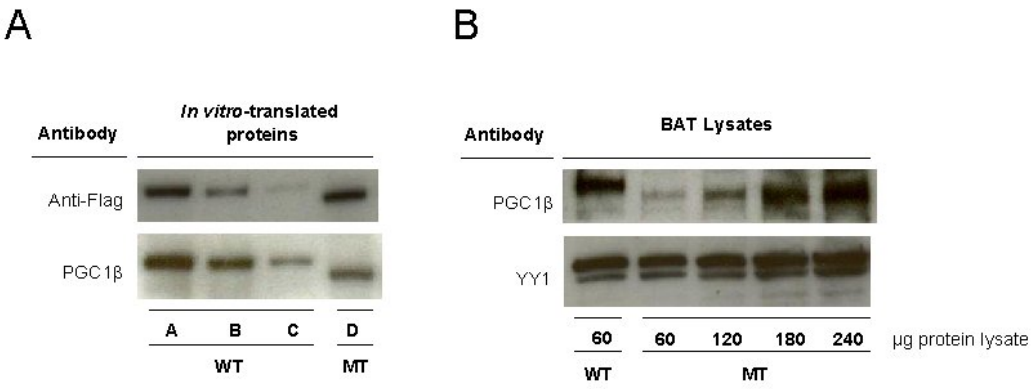

Figure 4S. Western blot titration curves with *in vitro*-translated proteins and BAT lysates. (A) Decreasing amounts of *in vitro* translated FLAG-tagged wild-type and FLAG-tagged mutant PGC1β protein (lacking amino acids AA 85 to AA 195) were loaded (WT protein: lanes A = 10 µl, B = 5 µl, and C = 2.5 µl; MT protein: lane D = 10 µl). Anti-FLAG antibody was used to determine the amount of *in vitro* translated protein loaded. Based upon the signal detected by anti-FLAG antibody, the MT sample in lane D contains the same amount of PGC1β protein as the WT sample in lane A. However, based upon the signal detected by the PGC1β antibody, the MT signal in lane D was intermediate between the WT signal detected in lanes B (50% WT PGC1β protein compared with lane A) and C (25% of WT PGC1β protein compared with lane A). Based upon this (and other studies not shown), we estimate that the PGC1β antibody detects the MT protein with an efficiency of about 25-50%. (B) The expression of the wild-type and mutant PGC1β was assessed in BAT lysates. BAT lysate (60 µg) from WT mice and increasing amounts of BAT protein lysates from MT mice (60, 120, 180, and 240 µg; 2-, 3-, and 4-fold, respectively) were loaded on the gel. As indicated in the figure, 3- to 4-times greater amounts of BAT lysate from MT mice were required to generate a PGC1β signal similar to that seen in BAT lysate from WT mice. Given the reduced efficiency of the PGC1β antibody for detection of MT versus WT protein observed in panel A, this indicates that PGC1β protein is not reduced in MT mice.. All western blots were performed using the same antibody dilutions. Antiserum, raised against the N-terminal (AA 1-350) of the mouse PGC1β was used as the primary antibody. The specific signal for PGC1β was detected at ~160 kD in *Pgc1β* $^{+/+}$ BAT lysate. The PGC1β MT signal was detected at ~150 kD in *Pgc1β* $^{E3,4-/E3,4-}$ BAT lysate. Rabbit polyclonal antiserum against YY1 was used as loading control. Mouse monoclonal Anti-FLAG M2 antibody (Sigma) was used to detect the in vitro translated proteins.