

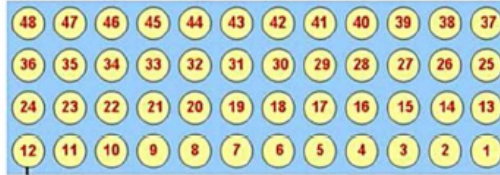
SUPPLEMENTARY DATA

Supplementary Figure 1. Immunoprecipitation of endogenous NT-PGC-1 α ²⁵⁴ protein from brown adipose tissue of FL-PGC-1 α ^{-/-} mice with a specific PGC-1 α antibody. *A:* Peptide microarray analysis for PGC-1 α antibody profiling. A rabbit polyclonal PGC-1 α antibody raised against a GST fusion of PGC-1 α (1-200aa) and a monoclonal PGC-1 α antibody raised against a GST fusion of PGC-1 α (1-120aa) were epitope-mapped using a series of overlapping 15-aa-long peptides corresponding to aa 1-200 of mouse PGC-1 α (JPT Peptide Technologies). The 15-mer peptides were spotted onto glass slides and hybridized with each antibody to identify the specific sequences recognized by each antibody. *B:* Expression of endogenous NT-PGC-1 α ²⁵⁴ protein in FL-PGC-1 α ^{-/-} mice. BAT lysates from WT and FL-PGC-1 α ^{-/-} mice were immunoblotted with a monoclonal PGC-1 α antibody (left panel) or immunoprecipitated with a rabbit PGC-1 α antibody followed by immunoblotting with a monoclonal PGC-1 α antibody (right panel). NSB represents non-specific bands.

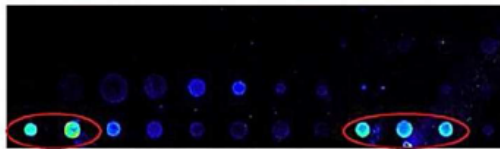
A. Specificity of PGC-1 α antibody

NT-PGC-1 α

1 MAWDMCSQDS VWSIDIECAAL VGEDQPLCPD LPELDLSELD VNDLDTDSFL GGLKWCSDQS
 61 EIISNQYNNE PANIFEKIDE ENEANLLAVL TETLDSLPLVD EDGLPSFDAL TDGAVTTDNE
 121 ASPSSMPDGT PPPQEAEEPS LLKKLLLPAPA NTQLSYNECS GLSTQNHAAN HTHRIRTNPA
 181 IVKTENSWSN KAKSICQQQK PQRPCSELL KYLTTNDPP HTKPTENRNS SRDKCASKKK
 241 SHTQPQSQHA QAKPTTSLP LTPESPFL



JPT's RepliTope™ peptide microarray



Polyclonal PGC-1 α antibody

Identified peptide sequences

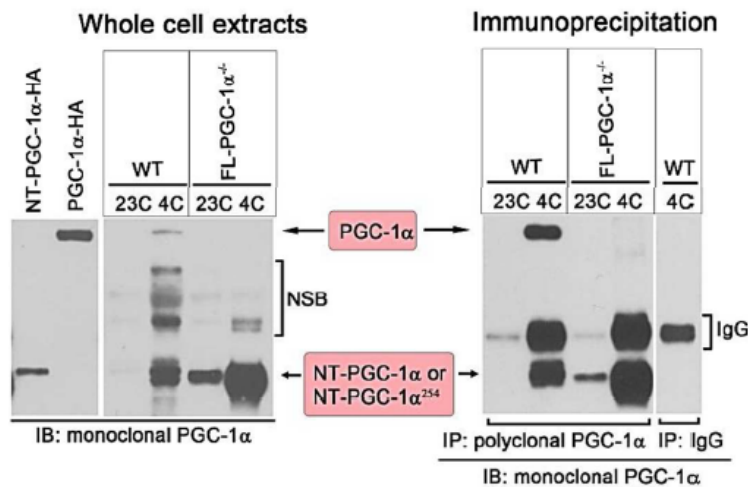
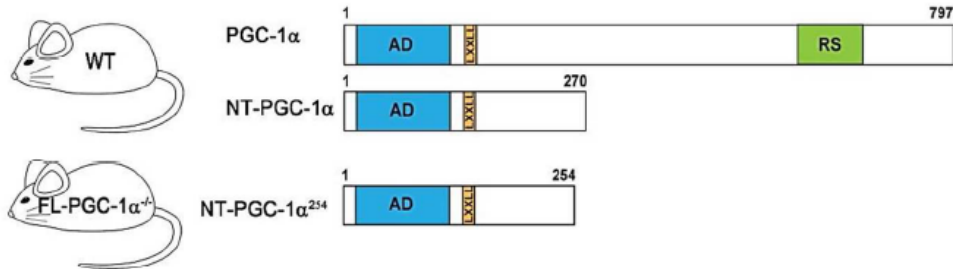
- 2 MCSQDSVWSIDIECAA
- 3 DSVWSIDIECAALVGE
- 4 SDIECAALVGEDQPL
- 11 VNDLDTDSFLGGLKW
- 12 DTDSFLGGLKWCSDQ



Monoclonal PGC-1 α antibody

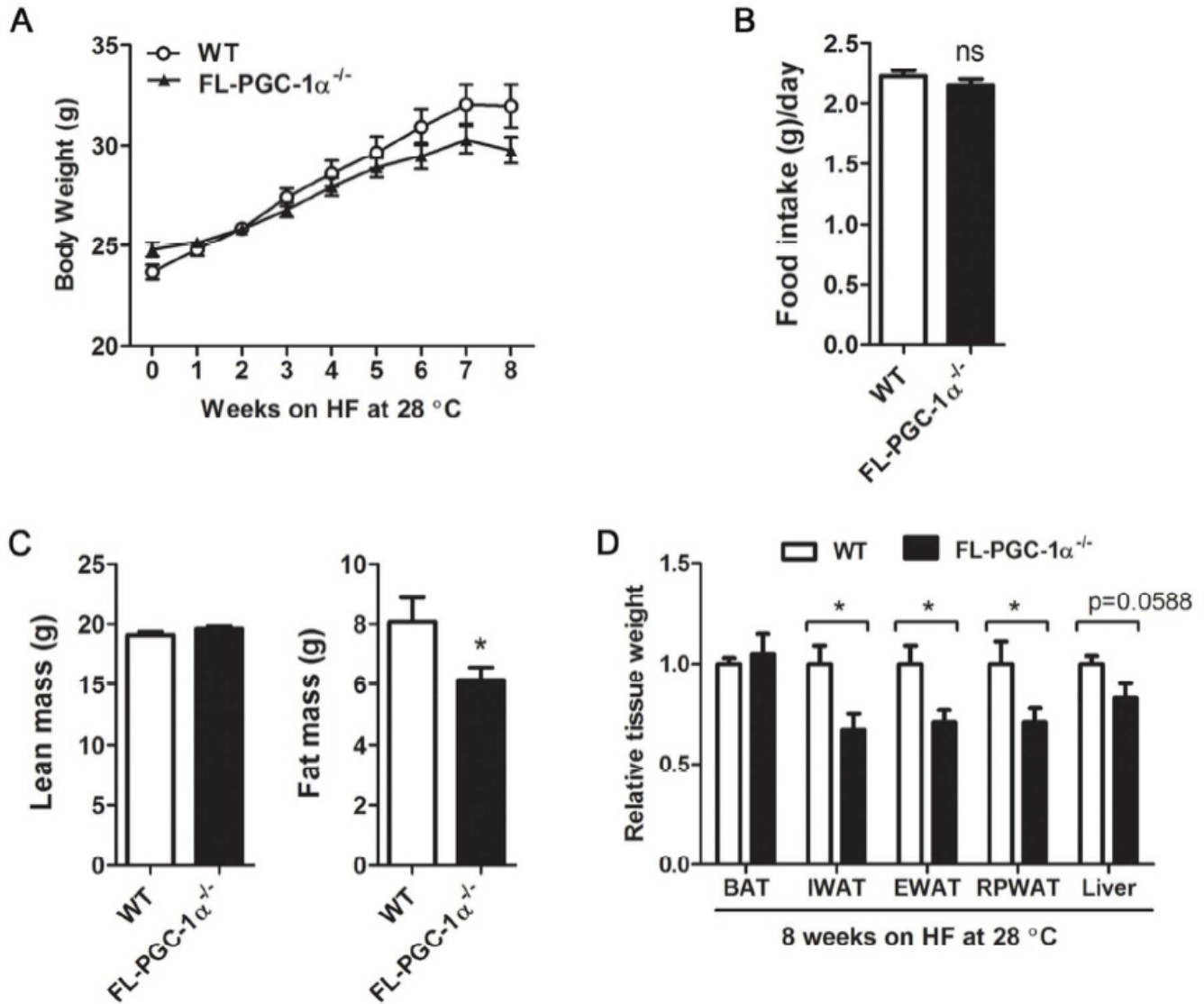
- 21 ENEANLLAVLTETLD
- 22 NLLAVLTETLDSLPLV

B. Immunoprecipitation of endogenous PGC-1 α /NT-PGC-1 α /NT-PGC-1 α ²⁵⁴ protein by PGC-1 α antibody



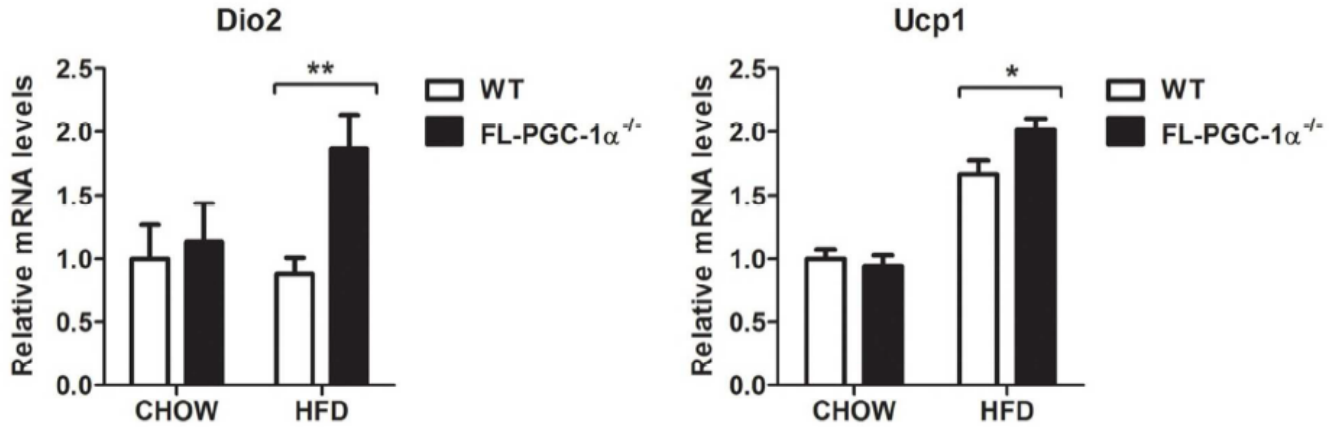
SUPPLEMENTARY DATA

Supplementary Figure 2. Attenuation of body fat accumulation in FL-PGC-1 $\alpha^{-/-}$ mice on HFD at thermoneutrality. *A:* Body weights of WT and FL-PGC-1 $\alpha^{-/-}$ male mice (n=12 per group) at 28°C during the 8 weeks of HFD. *B:* Average food intake of WT and FL-PGC-1 $\alpha^{-/-}$ mice on HFD at 28°C. *C:* Body composition of WT and FL-PGC-1 $\alpha^{-/-}$ mice determined by NMR after 8 weeks of HFD-feeding at 28°C. *D:* Relative weights of BAT, IWAT, EWAT, RPWAT fat pads and livers from WT and FL-PGC-1 $\alpha^{-/-}$ mice. All data are presented as the mean \pm SEM. * P < 0.05 determined by Student's *t* test.



SUPPLEMENTARY DATA

Supplementary Figure 3. Transcript levels of DIO2 and UCP1 in BAT from WT and FL-PGC-1 $\alpha^{-/-}$ mice fed either chow or HFD. Quantitative real-time PCR analysis of DIO2 and UCP1 in BAT from WT and FL-PGC-1 $\alpha^{-/-}$ mice (n=8 per group) fed either chow or HFD for 16 weeks. All data are presented as the mean \pm SEM. * P < 0.05, ** P < 0.01 determined by Student's *t* test.



Supplementary Table 1. Multiple regression model for energy expenditure (kJ/h) in WT and FL-PGC-1 $\alpha^{-/-}$ mice fed HFD. *Estimated change in mean EE per unit change in the independent variable. SE: standard error of the coefficient estimate. Lean mass, fat mass, activity, and genotype are significant independent determinants of EE.

Independent variable	Coefficient* \pm SE	<i>P</i>
Intercept	0.5785 \pm 0.095	< 0.0001
Lean mass (g)	0.0489 \pm 0.006	< 0.0001
Fat mass (g)	0.0181 \pm 0.004	< 0.0001
Activity (counts)	0.0003 \pm 5.975e-6	< 0.0001
Genotype	0.0156 \pm 0.007	0.0241