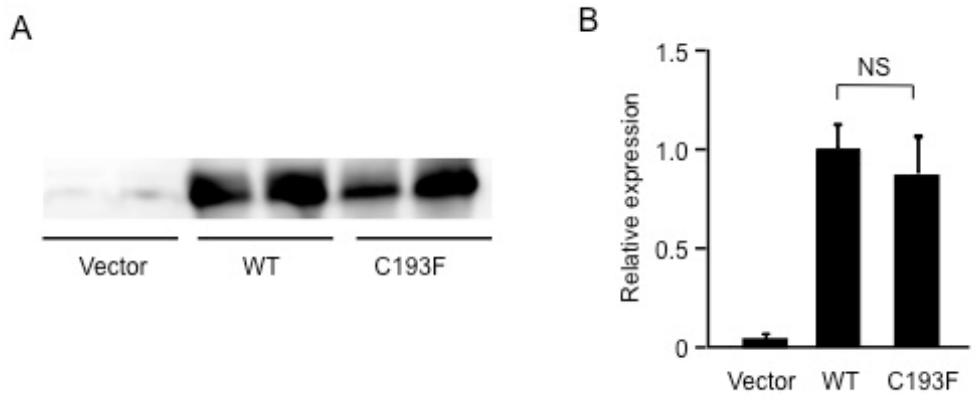
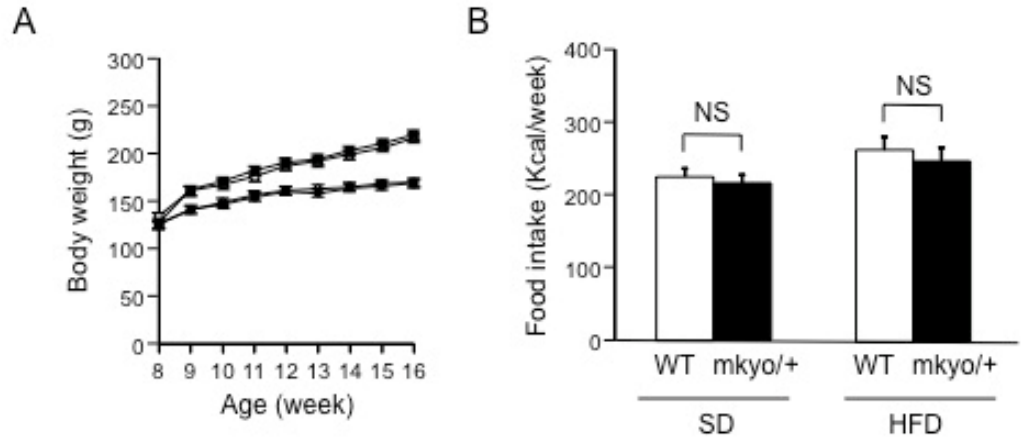
SUPPLEMENTARY DATA

Supplementary Figure 1.
Western blot analyses for effect of C197F mutation on PPARγ2 protein expression. (A) Representative blot performed with anti-FLAG mouse antibody. (B) Quantification of WT PPARγ2 and C197F mutant protein expression. Values of WT PPARγ2 are defined as 1.0. Vector, pTARGET empty vector; WT, pTARGET-3xFLAG-WT PPARγ2 vector; C193F, pTARGET-3xFLAG-C193F mutant PPARγ2 vector. Values are means ± SEM of 3 independent experiments. NS, not significant (Student's *t*-test).



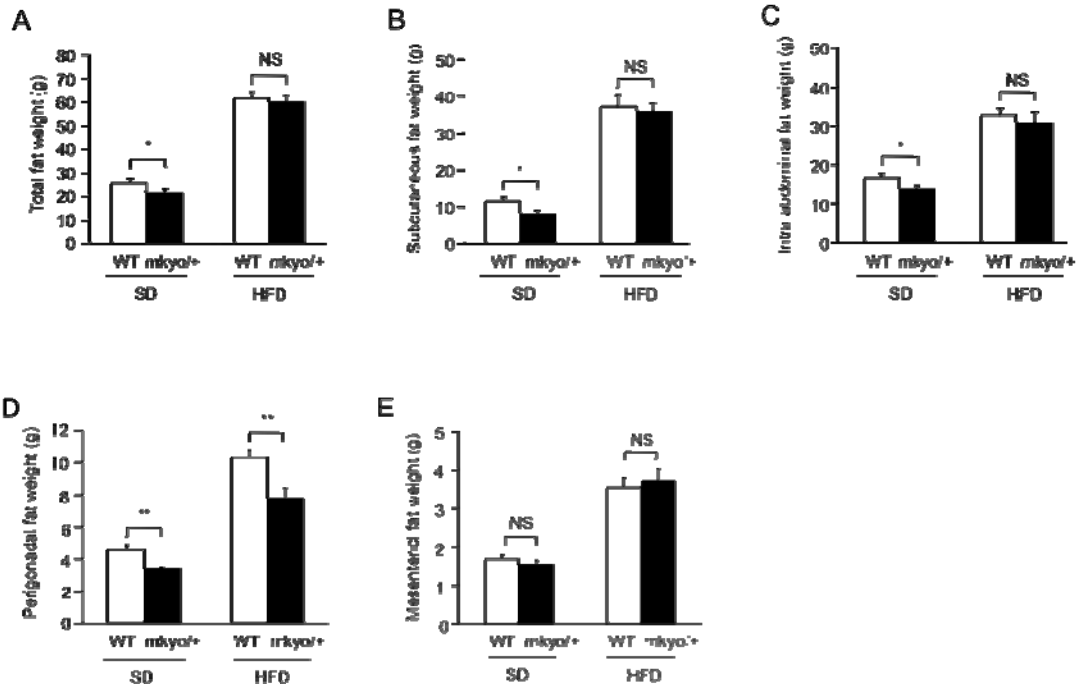
SUPPLEMENTARY DATA

Supplementary Figure 2.
Effect of HFD on body weight on body weight and food intake in female *Pparg*^{mkyo}/+ rats. (A) Body weight in female *Pparg*^{mkyo}/+ rats and their WT littermates. HFD was started at 8 weeks old. ○, WT rats under SD; ●, *Pparg*^{mkyo}/+ rats under SD; □, WT rats under HFD; ■, *Pparg*^{mkyo}/+ rats under HFD. Values are means ± SEM (n = 6 per group). (B) Food intake in female *Pparg*^{mkyo}/+ rats and their WT littermates under SD or HFD. Values are means ± SEM (n = 6 per group). NS, not significant (Student's *t*-test).



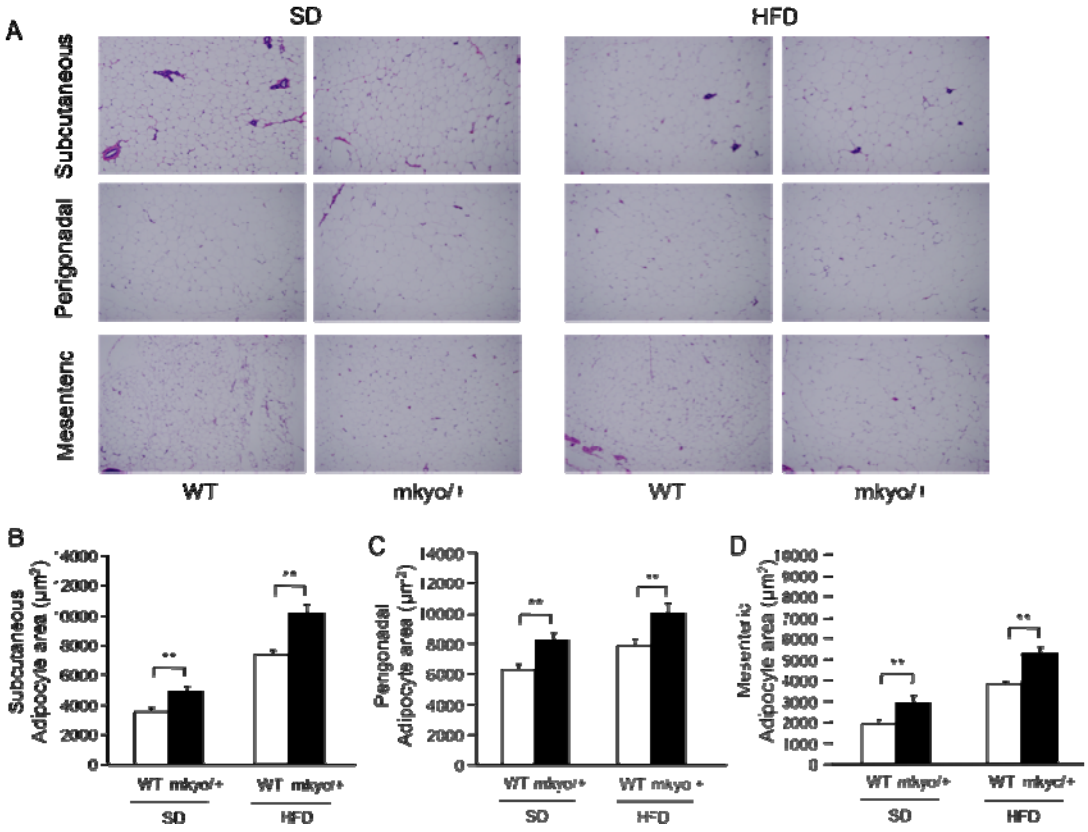
SUPPLEMENTARY DATA

Supplementary Figure 3.
Site-specific fat weight in female *Pparg*^{mkyo}/+ rats and their WT littermates under SD or HFD. (A, B, C) Weight of total fat (A), subcutaneous fat (B) and intra abdominal fat (C) measured by whole body CT scan. (D, E) Weight of perigonadal fat (D) and mesenteric fat (E) measured directly. Values are means ± SEM (n = 6 per group). ***P* < 0.01, NS, not significant (Student's *t*-test).



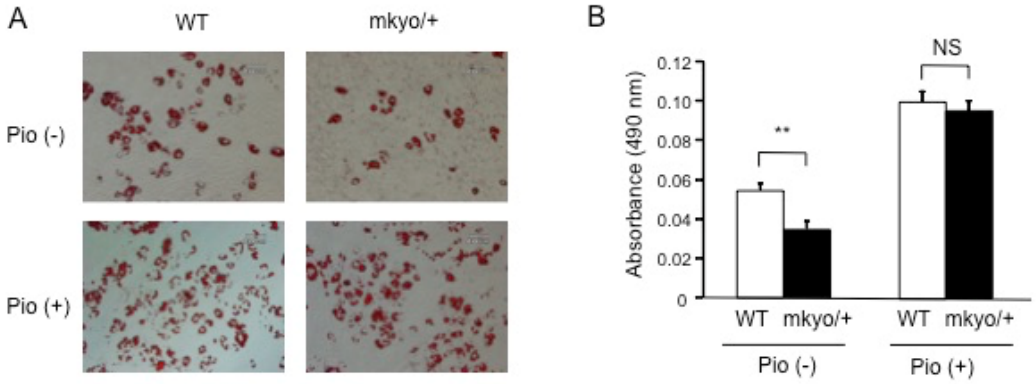
SUPPLEMENTARY DATA

Supplementary Figure 4.
Site-specific adipocyte mean size in female *Pparg*^{mkyo}/+ rats and their WT littermates under SD or HFD. (A) Histological images of subcutaneous (inguinal), perigonadal and mesenteric fats. For histological examination, hematoxylin and eosin sataining was used. Original magnification of x100 is shown. (B, C, D) Mean cross-sectional area of adipocyte in subcutaneous (inguinal) (B), perigonadal (C) and mesenteric fats (D). Values are means ± SEM (n = 6 per group). $^{**}P < 0.01$, NS, not significant (Student's *t*-test).



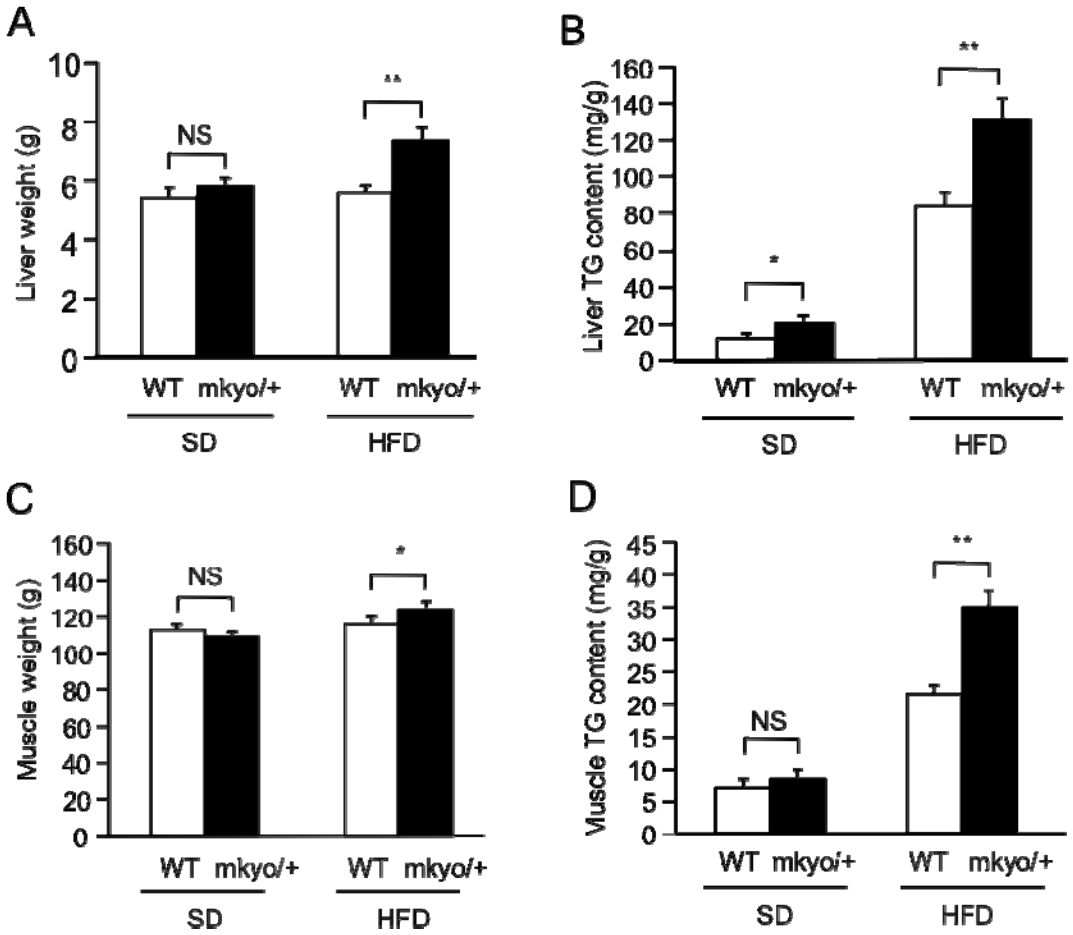
SUPPLEMENTARY DATA

Supplementary Figure 5.
Adipocyte differentiation in rat dermal fibroblasts (RDFs) from WT and *Pparg*^{mkyo}/+ rats. RDFs were obtained from abdominal skin from WT and *Pparg*^{mkyo}/+ rats at 6 weeks of age. Adipocyte differentiation was initiated after 2 days at confluence in 10% FBS/DMEM with 10 μg/ml insulin, 0.5 mM IBMX, and 0.25μM Dexamethasone for 2 days. Then, cells were maintained in 10% FBS/DMEM containing 10μg/ml insulin, with or without 5 μM pioglitazone for 14 days. (A) Micrographs of RDFs stained with Oil Red O. Original magnification of x40 is shown. (B) Absorbance values at 490 mm of extracted Oil Red O from RDFs. Values are means ± SEM of 3 independent experiments. ***P* < 0.01, NS, not significant (Student's *t*-test).



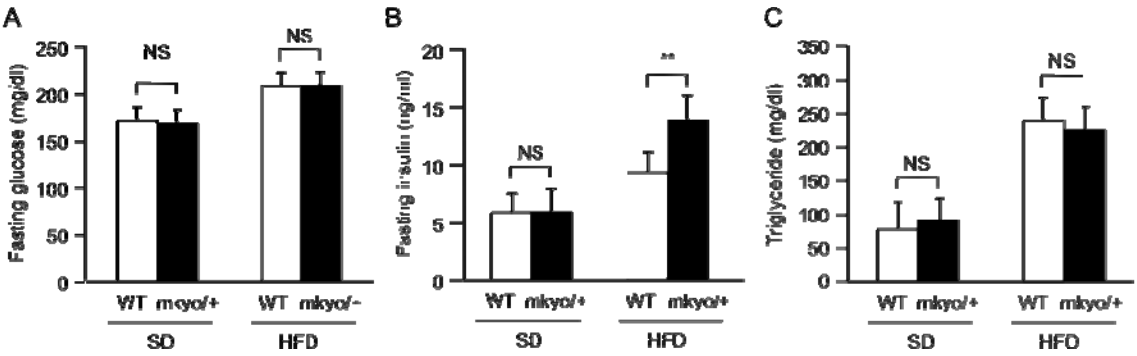
SUPPLEMENTARY DATA

Supplementary Figure 6.
Phenotypes of liver and skeletal muscle in female *Pparg*^{mkyo}/+ rats and their WT littermates under SD or HFD. (A) Liver weight, (B) liver triglyceride (TG) content, (C) total muscle weight measured by whole body CT scan and (D) TG content in gastrocnemius muscle. Values are means ± SEM (n = 6 per group). **P* < 0.05, ***P* < 0.01, NS, not significant (Student's *t*-test).



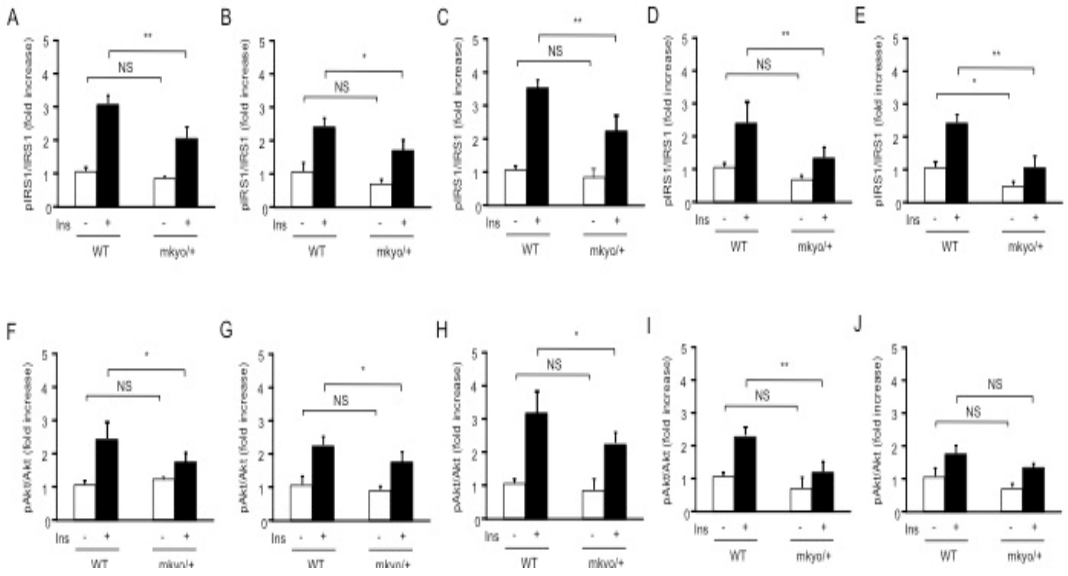
SUPPLEMENTARY DATA

Supplementary Figure 7.
Glucose and lipid metabolism in female *Pparg*^{mkyo}/+ rats and their WT littermates under SD or HFD. (A) Fasting plasma glucose, (B) fasting plasma insulin concentrations and (C) fasting plasma triglyceride. Values are means ± SEM (n = 6 per group). ***P* < 0.01, NS, not significant (Student's *t*-test).



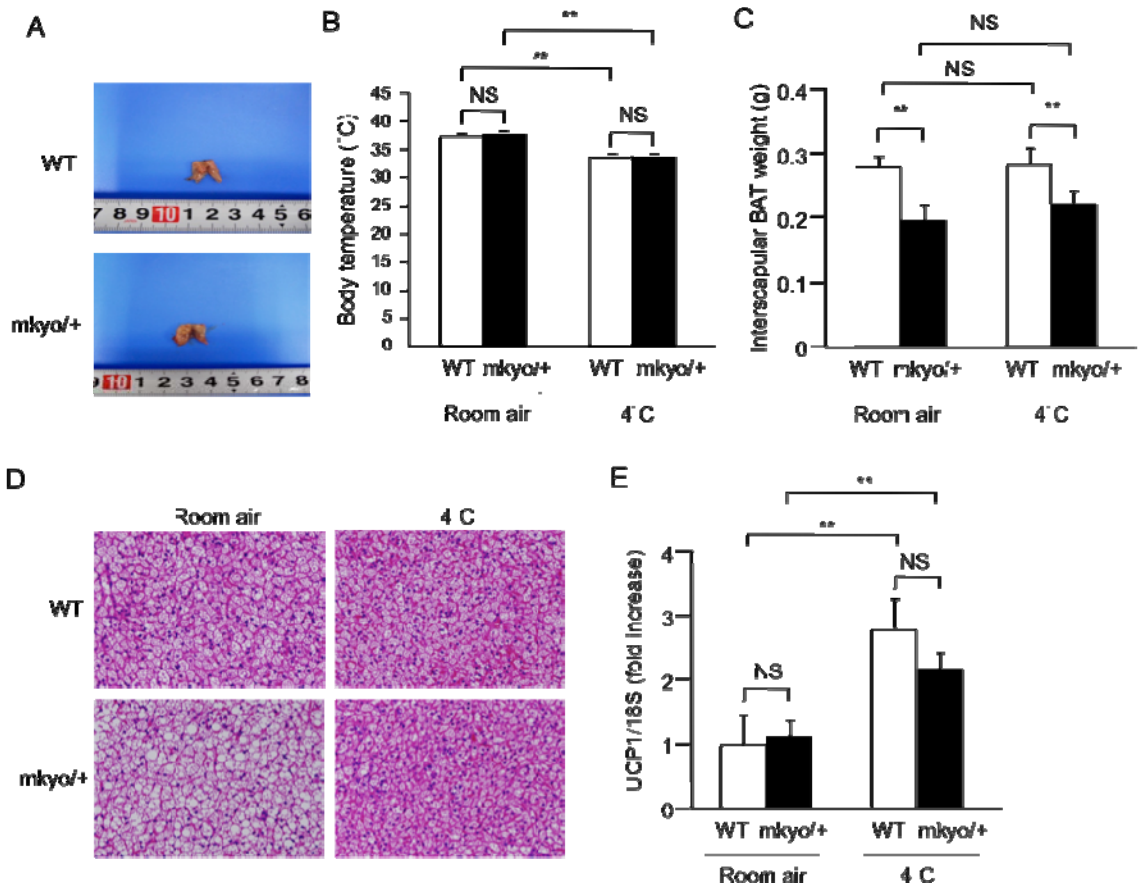
SUPPLEMENTARY DATA

Supplementary Figure 8.
Insulin induced phosphorylation of IRS1 and Akt in various tissues in male *Pparg*^{mkyo}/+ rats and their WT littermates under HFD. Tissues were sampled 30 min after insulin intraperitoneal injection (0.75 IU/kg). Western blot analyses for phospho-IRS1, IRS1, phospho-Akt and Akt were performed in subcutaneous (inguinal) (A, F), epididymal (B, G) and mesenteric fats (C, H), liver (D, I) and gastrocnemius muscle (E, J). (A, B, C, D, E) phospho-IRS1/IRS1 ratio. (F. G. H. H. J) phopho-Akt/Akt ratio. Values are means ± SEM (n = 4 per group). **P* < 0.05, ***P* < 0.01, NS, not significant (Student's *t*-test).



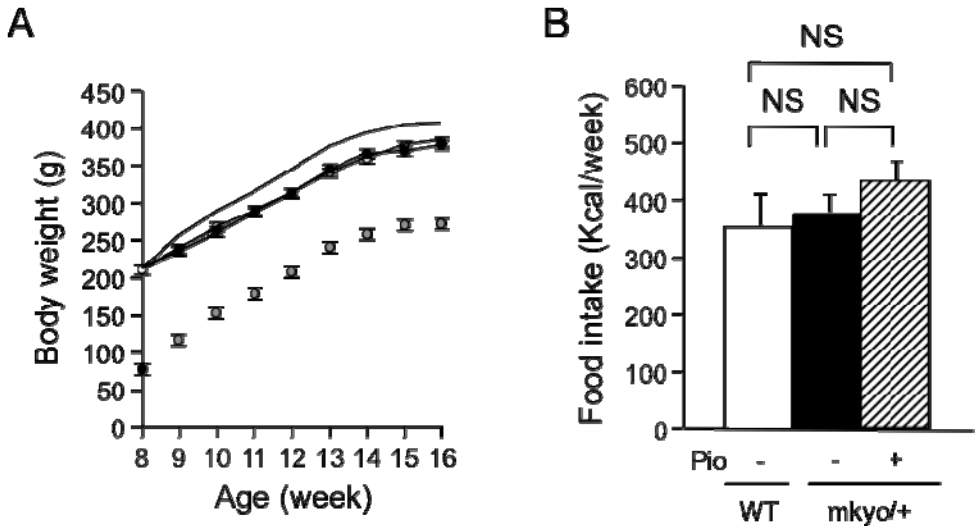
SUPPLEMENTARY DATA

Supplementary Figure 9.
Phenotypes of brown adipose tissue in *Pparg*^{mkyo}/+ rats. (A) Macroscopic images of the interscapular BAT in a 16 weeks old male *Pparg*^{mkyo}/+ rat and its WT littermate. (B) Body temperature before and after 24 h exposure of 4ºC or room temperature in 16 weeks old male *Pparg*^{mkyo}/+ rats and their WT littermates. (C) Interscapular BAT weight after 24 h exposure of 4ºC or room temperature in *Pparg*^{mkyo}/+ rats and their WT littermates. (D) Histological images of the interscapular BAT after 24 h exposure of 4ºC or room temperature in *Pparg*^{mkyo}/+ rats and their WT littermates. Hematoxylin and eosin staining was used. Original magnification of x200 is shown. (E) *Ucp1* mRNA expression in interscaplur BAT after 24 h exposure of 4ºC or room temperature in *Pparg*^{mkyo}/+ rats and their WT littermates. Quantitative RT-PCR was performed with SYBR Green (Applied Biosystems) using gene specific primers (rat *Ucp1* forward; 5'-AGACATCATCACCTTCCC-3', reverse; 5'-CAGCTGTTCAAAGCACAC-3'). *Ucp1* mRNA expression levels were normalized by 18S. The fold change is displayed as relative to WT rats under room temperature. (B, C, E) Values are mean ± SEM (n = 4 per group). $^{**}P < 0.01$, NS, not significant (Student's t-test).



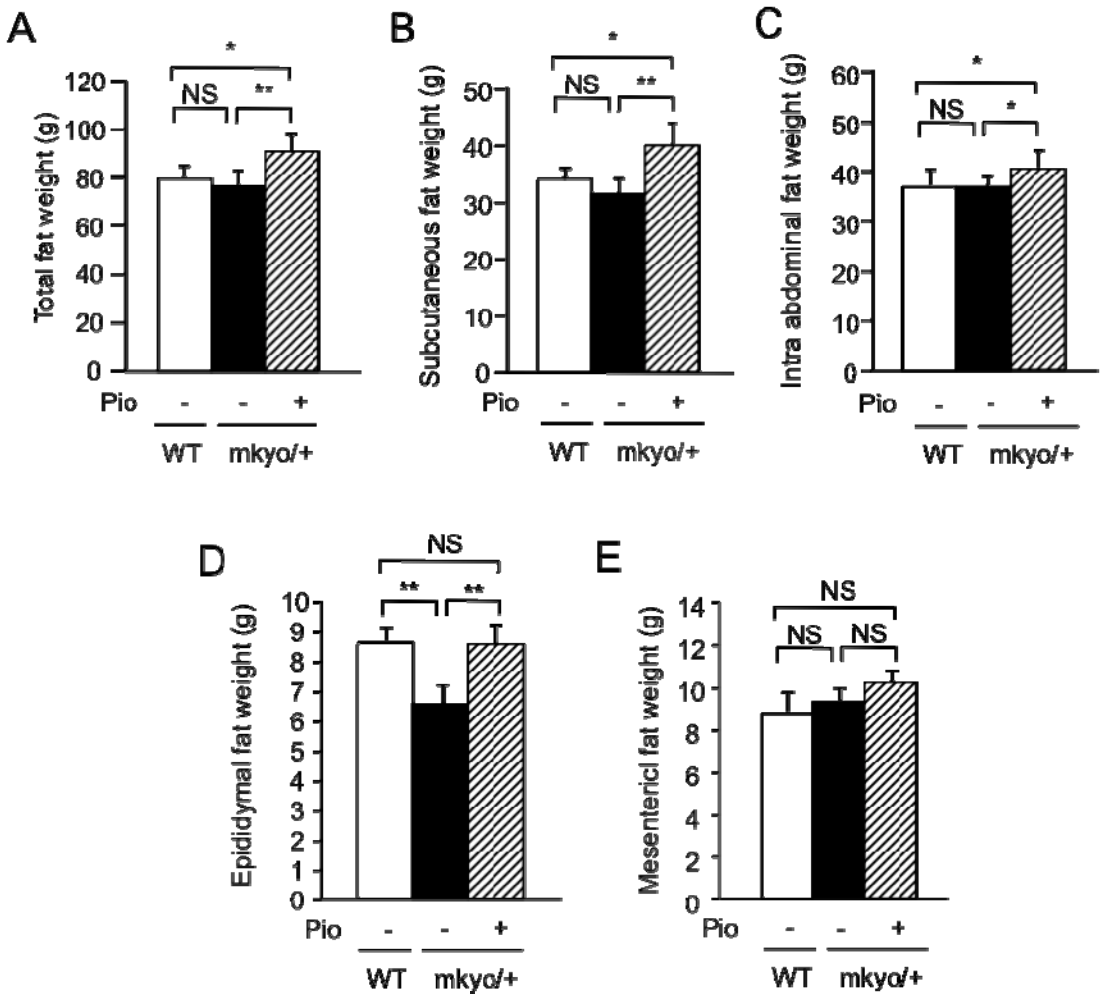
SUPPLEMENTARY DATA

Supplementary Figure 10.
Effect of pioglitazone treatment on body weight and food intake in male *Pparg*^{mkyo}/+ rats under HFD. (A) Body weight in male *Pparg*^{mkyo}/+ rats. HFD and pioglitazone treatment was started at 8 weeks old. Open circle, WT rats with vehicle treatment; black circle, *Pparg*^{mkyo}/+ rats with vehicle treatment; gray circle, *Pparg*^{mkyo}/+ rats with pioglitazone treatment. Values are means ± SEM (n = 5 per group). (B) Food intake in male *Pparg*^{mkyo}/+ rats with or without pioglitazone treatment under HFD. Values are means ± SEM (n = 5 per group). NS, not significant (Student's *t*-test).



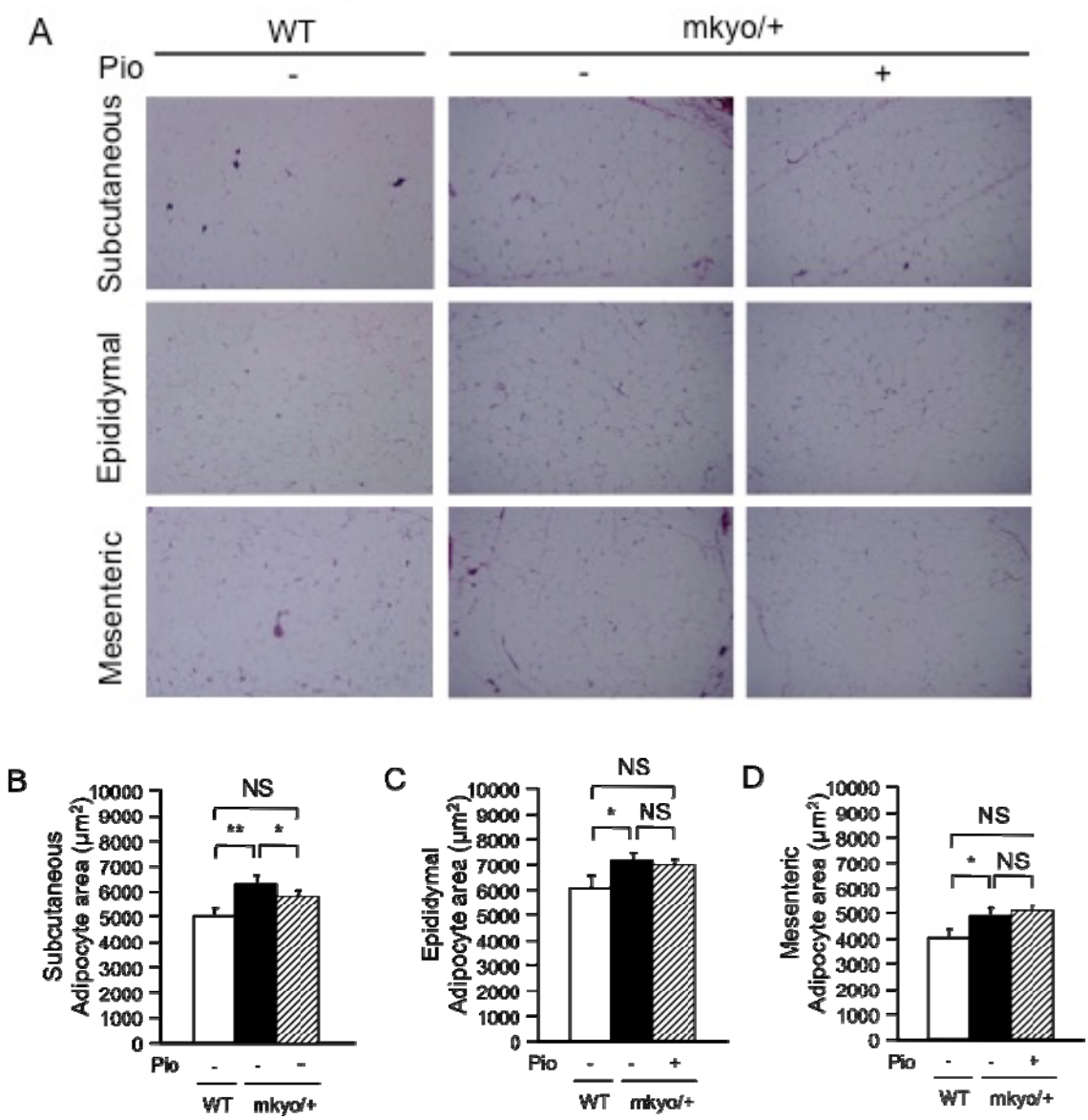
SUPPLEMENTARY DATA

Supplementary Figure 11.
Effect of pioglitazone treatment on site-specific fat weight in male *Pparg*^{mkyo}/+ rats under HFD. (A, B, C) Weight of total fat (A), subcutaneous fat (B) and intra abdominal fat (C) measured by whole body CT scan. (D, E) Weight of epididymal fat (D) and mesenteric fat (E) measured directly. Values are means ± SEM (n = 5 per group). **P* < 0.05, ***P* < 0.01, NS, not significant (Student's *t*-test).



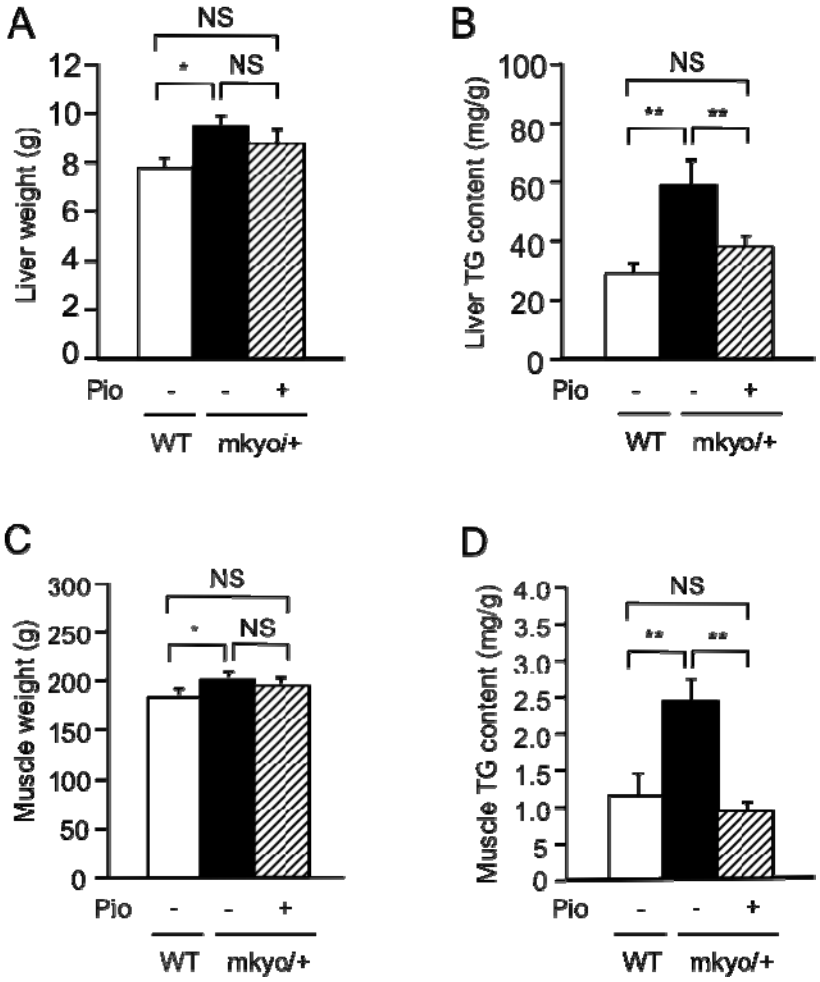
SUPPLEMENTARY DATA

Supplementary Figure 12.
Effect of pioglitazone treatment on site-specific adipocyte mean size in male *Pparg*^{mkyo}/+ rats under HFD. (A) Histological images of subcutaneous (inguinal), epididymal and mesenteric fats. For histological examination, hematoxylin and eosin sataining was used. Original magnification of x100 is shown. (B, C, D) Mean cross-sectional area of adipocyte in subcutaneous (inguinal) (B), epididymal (C) and mesenteric fats (D). Values are means ± SEM (n = 5 per group). **P* < 0.05, ***P* < 0.01, NS, not significant (Student's *t*-test).



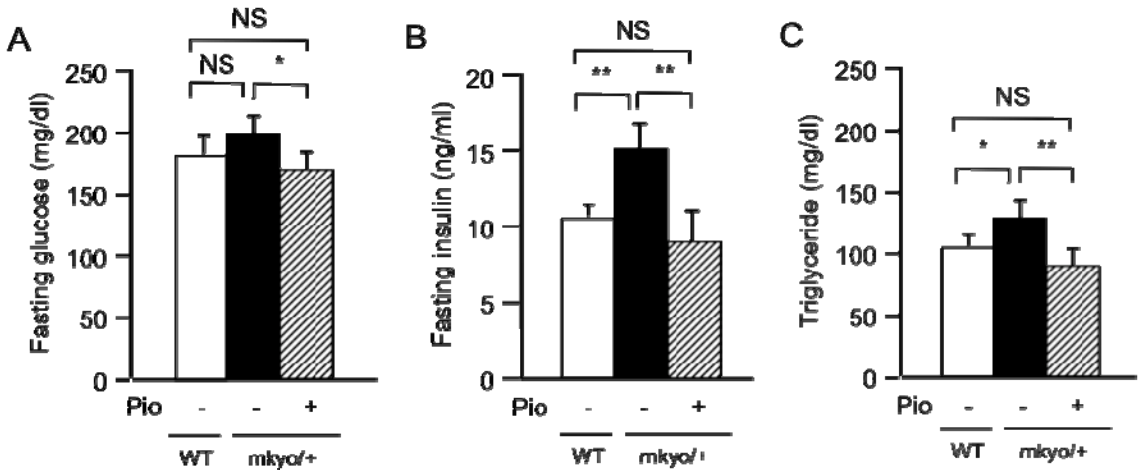
SUPPLEMENTARY DATA

Supplementary Figure 13.
Effect of pioglitazone treatment on liver and skeletal muscle in male *Pparg*^{mkyo}/+ rats under HFD. (A) Liver weight, (B) liver triglyceride (TG) content, (C) total muscle weight measured by whole body CT scan and (D) TG content in gastrocnemius muscle. Values are means ± SEM (n = 5 per group). **P* < 0.05, ***P* < 0.01, NS, not significant (Student's *t*-test).



SUPPLEMENTARY DATA

Supplementary Figure 14.
Effect of pioglitazone treatment on glucose and lipid metabolism in male *Pparg*^{mkyo}/+ rats under HFD. (A) Fasting plasma glucose, (B) fasting plasma insulin concentrations and (C) fasting plasma triglyceride. Values are means ± SEM (n = 5 per group). **P* < 0.05, ***P* < 0.01, NS, not significant (Student's *t*-test).



SUPPLEMENTARY DATA

Supplementary Figure 15.
Adipoq, *Cd36*, *Fabp4* and *Plin1* mRNA expressions in fat tissues in male *Pparg*^{mkyo}/+ rats and their WT littermates under SD or HFD. Quantitative RT-PCR was performed with the TaqMan ready-made primer and probe sets (Applied Biosystems). (A, B, C) *Adipoq*, (D, E, F) *Cd36*, (G, H, I) *Cd36* and (J, K, L) *Plin1* mRNA expressions in (A, D, G, J) subcutaneous, (B, E, H, K) epididymal, and (C, F, I, L) mesenteric fats. *Pparg*^{mkyo}/+ rats under HFD were treated with pioglitazone or vehicle. mRNA expression levels were normalized by 18S. The fold change is displayed as relative to WT rats under SD. Values are means ± SEM (n = 5 per group). *$P < 0.05$, **$P < 0.01$, NS, not significant (Student's t-test).

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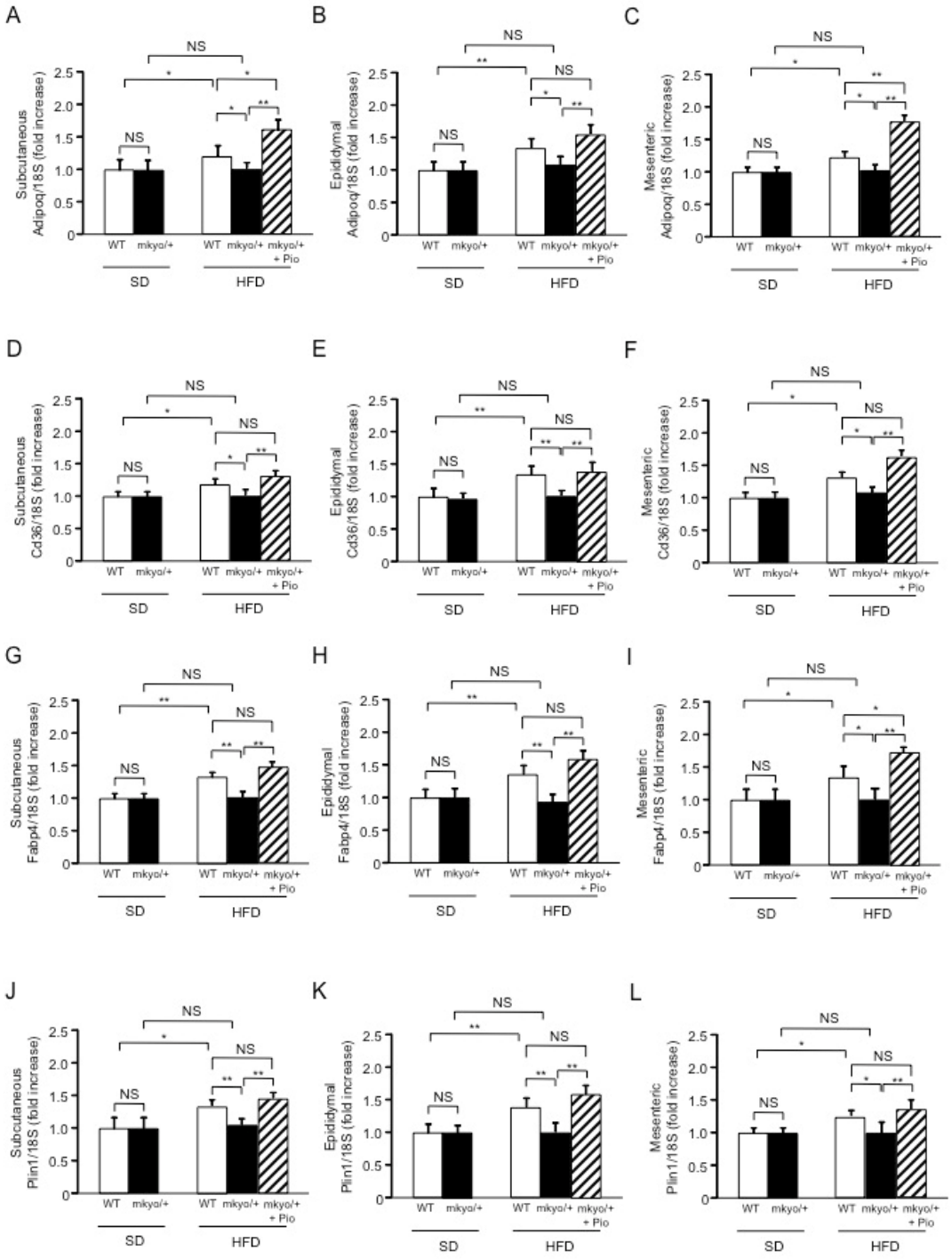

SUPPLEMENTARY DATA

Supplementary Figure 16.

Pparg, *Fsp27* and *Cd36* mRNA expressions in liver and gastrocnemius muscle in male *Pparg*^{mkyo}/+ rats and their WT littermates under SD or HFD. Quantitative RT-PCR was performed with the TaqMan ready-made primer and probe sets (Applied Biosystems). (A, D) *Pparg*, (B, E) *Fsp27* and (C, F) *Cd36* mRNA expressions in (A, B, C) liver and (D, E, F) gastrocnemius muscle. *Pparg*^{mkyo}/+ rats under HFD were treated with pioglitazone or vehicle. mRNA expression levels were normalized by 18S. The fold change is displayed as relative to WT rats under SD. Values are means ± SEM (n = 5 per group). **P* < 0.05, ***P* < 0.01, NS, not significant (Student's *t*-test).