MATERIAL AND METHODS

Cell culture. THP-1 cells (AmericanTypeCultureCollection) were culture data starting density of $2x10^5$ per mL in RPMI 1640 (Cambrex,Germany) supplemented with 10% SFB (GILBCO),100U/mLpenicillin and 100 mg/mL streptomycin.Cells were cultured at 37°C, 100% humidity and 5% CO_2. THP1 cells were treated with PD 153035 in 1 or 10 µM for 2 hours. Untreated THP1 cells incubated with DMSO were used of control. (n=3).

THP1cells chemotaxis. THP1 cells migration was measured by the Boyden blindwell chamber technique with a 96-well multiwell chamber (Neuro Probe, Inc., Bethesda, MD, USA) and a polycarbonate membrane filter with pores of 5 µm in diameter (Nucleopore, Pleasanton, CA, USA). The wells in the microplate were filled with 29 µL of chemotatic agent RANTES (100ng/ml) in RPMI. A polycarbonate filter (5 µm pores size) was positioned on the loaded microplate and secured in plate with corner pins. THP1 cells ($1x10^6$ cells/ ml in 25 µL) treated with PD (1 or 10 µM) or vehicle (DMSO) were applied to the top compartment. The chamber was incubated for 2 hours at 37°C, in a humidified 5% CO_2 atmosphere. After this period, the chamber was centrifuged 200 g for 5 minutes at 20°C. The cells were collected and the number of migrated cells to the bottom compartment was counted in a Neubauer chamber.

Glucose uptake in isolated soleus muscle. Soleus muscles from control mice were isolated and incubated in the presence of PD153035 (100 nM) for 4 h. In the basal state and 30 min after insulin treatment, glucose uptake was measured, as previously described (31; 33).

Isolation of the Stroma Vascular Fraction and adipocyte fraction of adipose tissue

Retroperitoneal, mesenteric and epididymal fat pads were excised and isolation of the stroma vascular fraction and adipocyte fraction of adipose tissue was performed. To ensure proper isolation, adipocyte fractions were examined by microscopy before and after plating on plastic to detect adherent cells. Samples were digested until adipocyte fractions were free of adherent cells by these 2 quality-control methods to ensure recovery of the majority of the SVF population. The whole cell lysate were normalized by protein, and then treated with Laemmli sample buffer containing 100mM DTT and heated in a boiling water bath for 5min, after which they were subjected to SDS-PAGE.

Arginase assay.

Briefly, tissues were homogenized in 0.1% Triton X-100. Tris-HCl was added to 12.5-mM and $MnCl_2$ added to 1-mM final concentration. Arginase was activated by heating for 10 minutes at 56°C, and L-arginine substrate was added to 250-mM final concentration. Reactions were incubated at 37°C for 30 minutes and stopped by the addition of H_2SO_4/H_3PO_4. After addition of α-isonitrosopropiophenone and heating for 30 minutes at 95°C, urea production was measured by absorbance at 540 nm and normalized to total protein content.

RESULTS

Effect of PD153035 on insulin-induced glucose uptake in isolated soleus muscle of mice

PD153035 treatment for 4 h (100 nM) did not change basal or insulin-stimulated glucose uptake in isolated soleus muscle of control mice, suggesting no direct effect of this drug on glucose transport in muscle (Figure S4D).

Effect of PD153035 on liver and muscle weight and cytokine expression in High-fat diet-fed mice

The results are presented in table 1 and in Figure S7A-D, and showed that there is an increase in muscle and liver mass in HFD group, and also an increase in TNF-α and IL-6 in these tissues. This increase in tissue mass and in cytokine expression is reduced by PD153035 treatment for 14 days.

Table 1. Liver and muscle weights after PD153035 treatment in HFD-fed mice.

Groups (n=6)	Liver (g)	Soleus (mg)	EDL (mg)
Control	1.05 ± .22	9.48 ± 1.09	11.76 ± 0.98
HFD	2.56 ± 0.31*	11.74 ± 1.23*	14.93 ± 1.13*
HFPD14 days	1.91± 0.21*#	9.91 ± 1.82*	13.67 ± 1.62*

*$p < 0.05$, versus control group. #$p < 0.05$, versus control HFD.

Figure S1. Effect of different doses of PD153035 in HFD-fed mice mice. Representative blots show the levels of EGFR tyrosine phosphorylation in the liver (A), muscle (B) and retroperitoneal adipose tissue (C) in HFD mice after different doses (1, 10 and 30 mg/Kg/day) of PD153035 during 14 days (upper panels). **P*<0.05, versus HFD plus vehicle group. IB, immunoblot; IP, immunoprecipitate. HFD: high-fat diet.

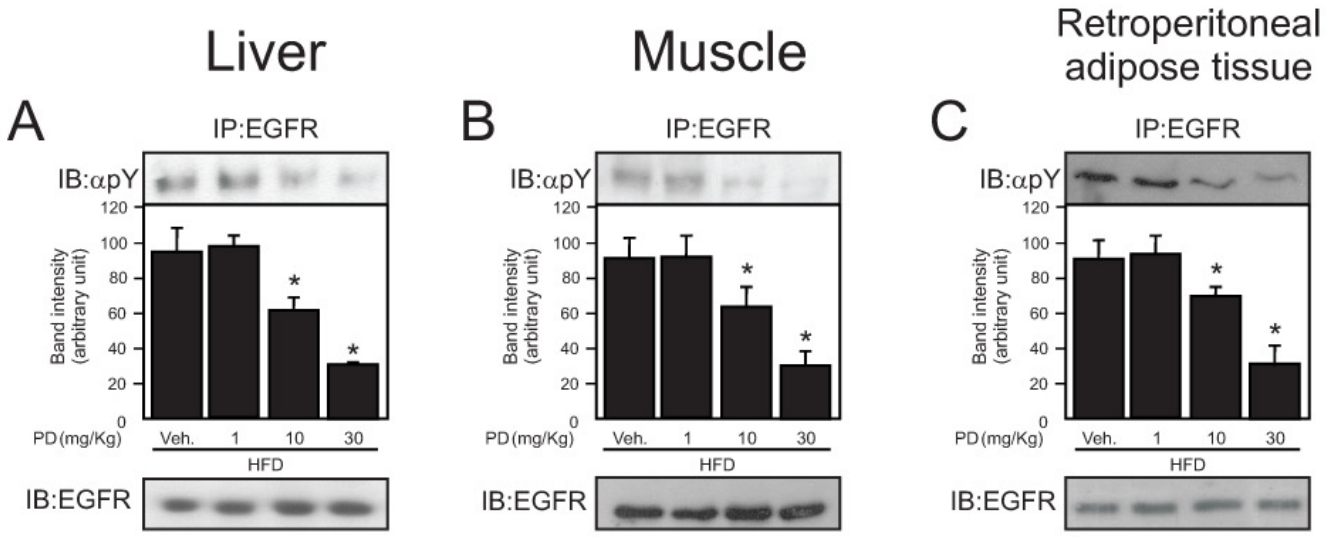

Figure S2. Effect of PD153035 on blood glucose. (A) Fasting blood glucose 14 days after different doses (1, 10 and 30 mg/Kg/day) of PD153035 treatment in HFD-fed mice. *P<0.05, versus HFD plus vehicle group. HFD: high-fat diet.

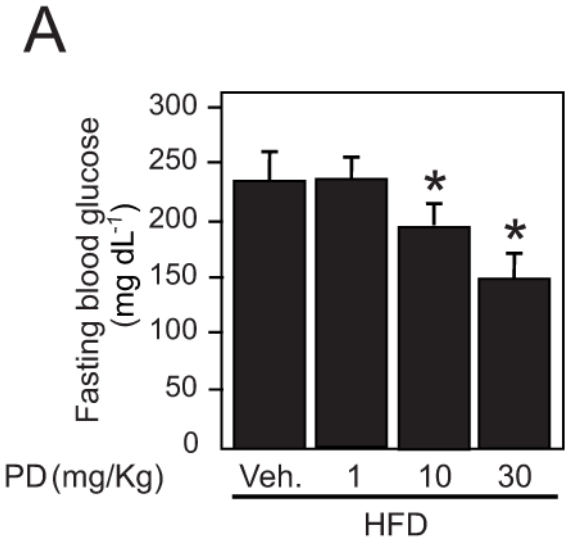

Figure S3. Effects of different doses of PD153035 in Akt phosphorylation in HFD-fed mice. Representative blots show the levels of insulin-induced Akt phosphorylation in the liver (**A**), muscle (**B**), epididymal (**C**), retroperitoneal (**D**) and mesenteric fat pads (**E**) in HFD-fed mice 14 days after different doses of PD153035. Data are presented as means +/- S.E.M from 6-8 mice per group. **P*<0.05, versus vehicle plus insulin group. IB, immunoblot; IP, immunoprecipitate. HFD: high-fat diet.

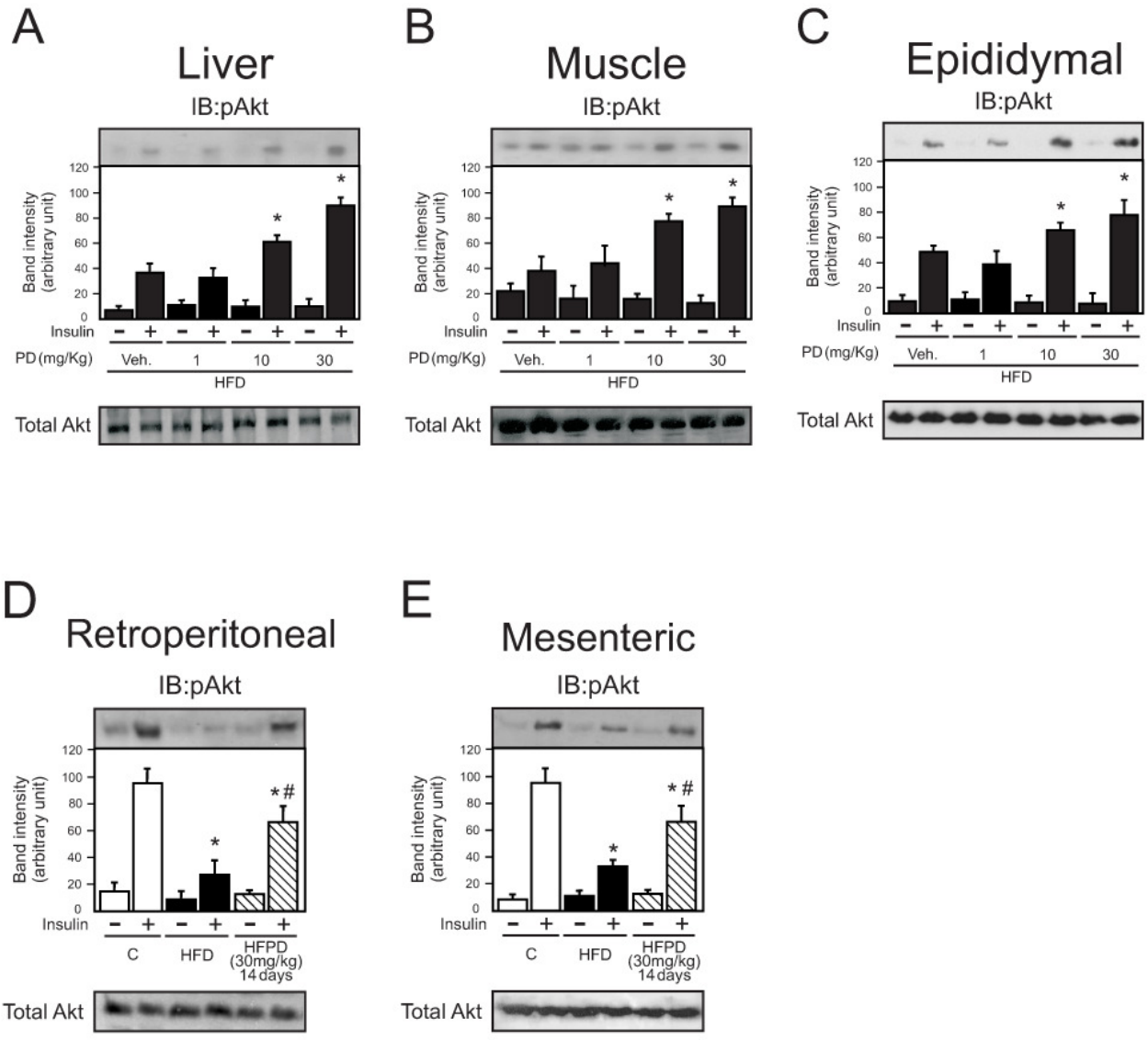

Figure S4. Effect of PD153035 in lean mice. Representative blots show the levels of insulin-induced Akt phosphorylation in the liver (**A**), muscle (**B**) and adipose tissue (epididymal) (**C**) in lean mice after PD153035 treatment during 14 days (upper panels). (**D**) Insulin-induced glucose uptake in isolated soleus muscles from control mice in the presence of PD153035 (100 nM) for 4 h. **P*<0.05, versus respective control group without insulin stimulus, n=6.

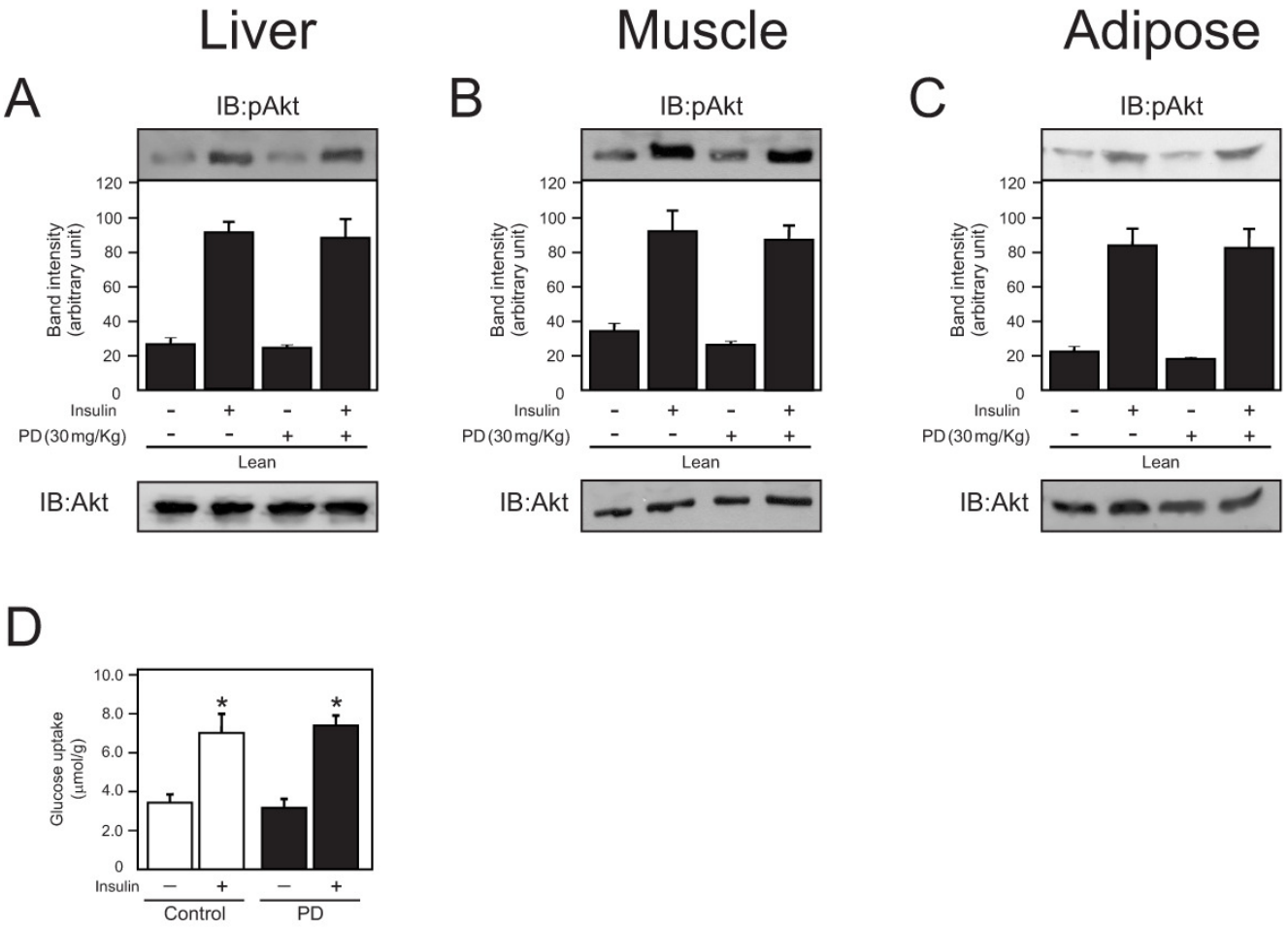

Figure S5. Effects of PD153035 an adipocyte morphology in epididymal and mesenteric adipose tissue. (**A** and **B**) Histological sections of epididymal and mesenteric fat pads from control, HFD, and HFD plus PD after 14 days, 50µm scale bar for all pictures (**C and D**) Quantification of adipocyte size. About 100 cells were measured in each group, and the average adipocyte was calculated. Data are presented as means +/- S.E.M from 6 mice per group, **P*<0.05, versus control group and # *P*<0.05, versus HFD. HFD: high-fat diet; HFPD14: high-fat diet treated with PD153035 for 14 days.

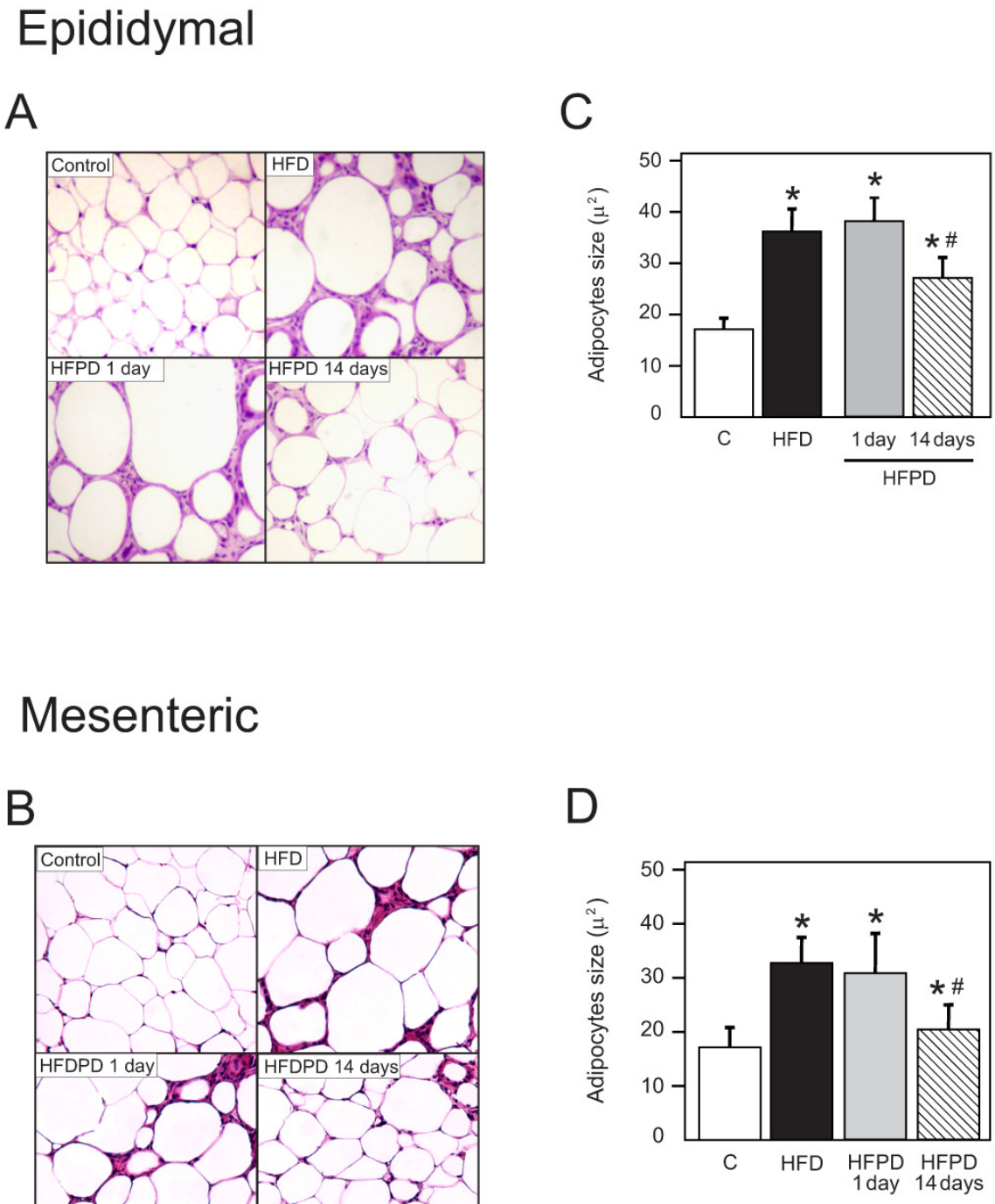

Figure S6. Effects of PD153035 on macrophage infiltration in adipocyte tissue. (**A** and **B**) Representative immunohistochemical staining of epididymal and mesenteric white adipose tissue using the specific macrophage marker F4/80+ (**C** and **D**) F4/80-positive cells (+ cells/total cells) of all above groups. Data are presented as means +/- S.E.M from 6 mice per group, **P*<0.05, versus control group and # *P*<0.05, versus HFD. * *P*<0.05 versus control; #*P*<0.05 versus HFD. Values represent the average of 5 different assays: HFD: high-fat diet; HFPD14: high-fat diet treated with PD153035 for 14 days.

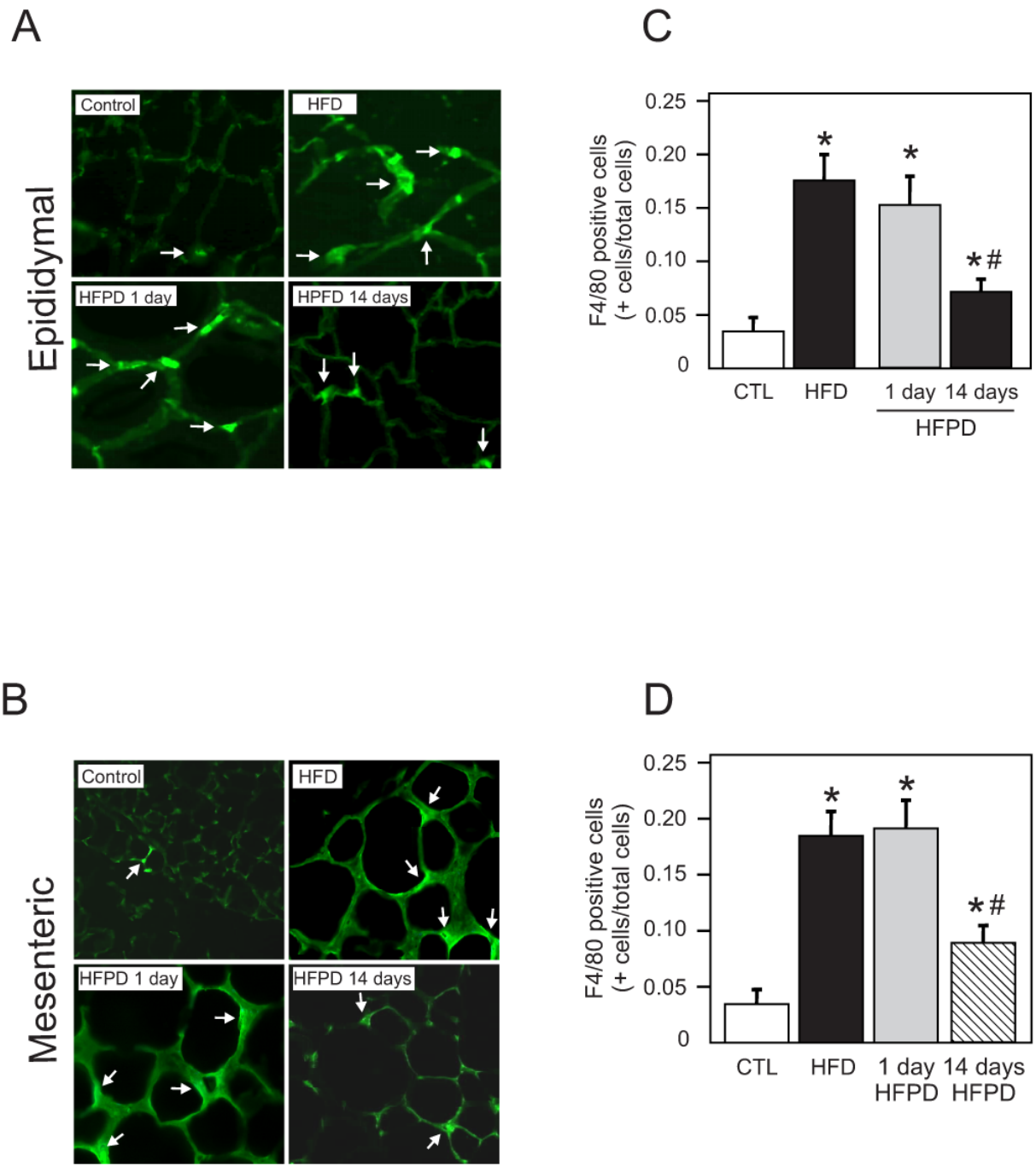

Figure S7. Effect of PD153035 on protein levels of TNF-α and IL-6 in the liver and muscle of HFD-fed mice. Representative blots show the tissue levels of TNF alpha and IL-6 in the hepatic tissue and in the gastrocnemius muscle of HFD-fed mice 14 days after PD 153035 treatment (30 mg/Kg/day) (**A-D**). Data are presented as means +/- S.E.M of 6-8 mice per group. **P*<0.05 versus control group; #*P*<0.05 versus HFD group. HFD: high-fat diet; HFPD14: high-fat diet treated with PD153035 for 14 days.

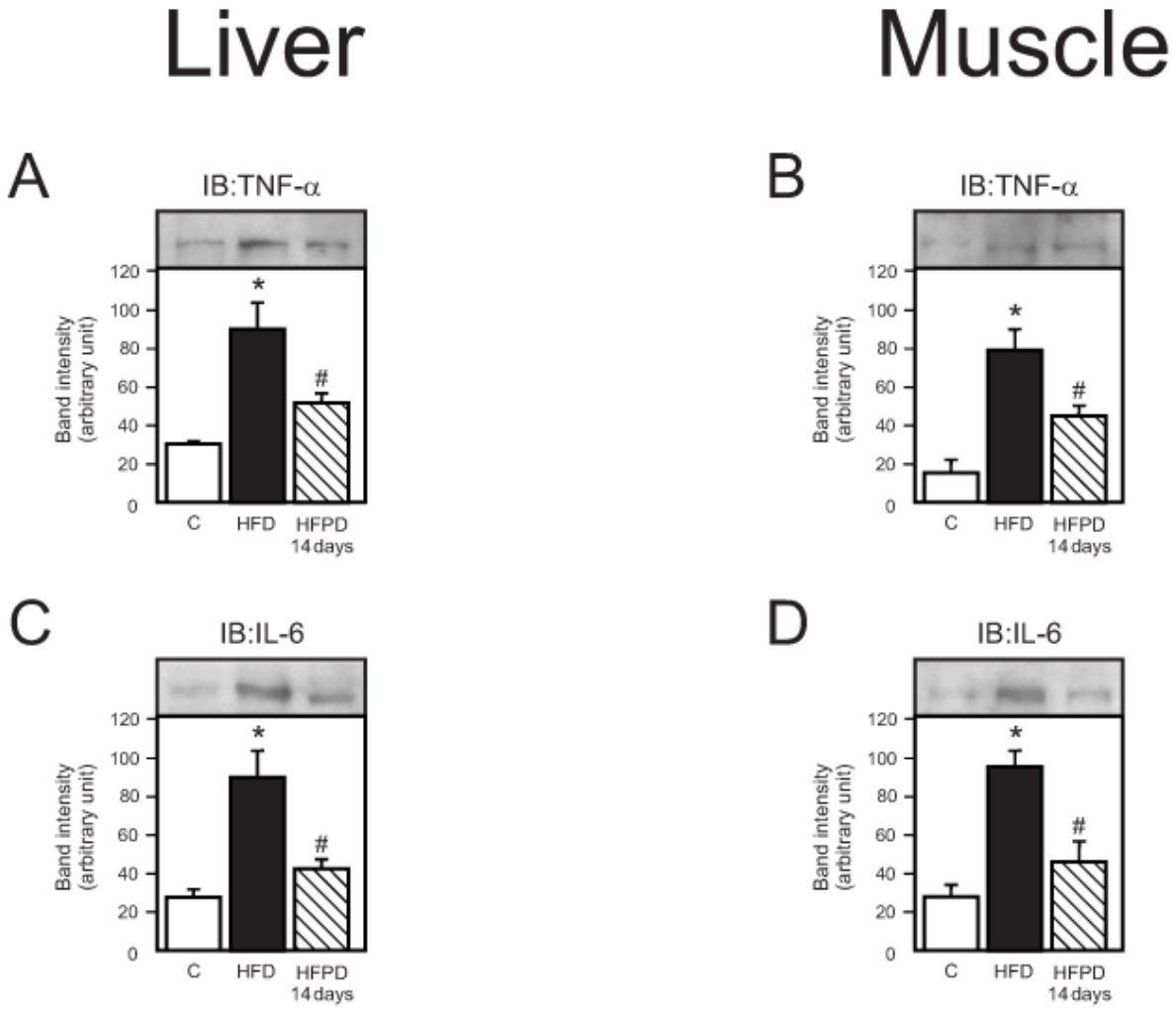

Figure S8. Effect of PD153035 on tissue protein levels of TNF-α, IL-6 and iNOS, and arginase activity in adipocytes and stromal vascular fraction from epididymal adipose tissue. Representative blots show the tissue levels of TNF alpha, IL-6, iNOS, EGRF tyrosine phosphorylation, EGRF, Caveolin and Cd68 protein expression in adipocytes (**A-D**) and TNF alpha, IL-6, iNOS, EGRF tyrosine phosphorylation, EGRF, Cd68 and actin protein expression in the stromal vascular fraction (**F-J**). (**K**) Arginase activity of adipocytes and stromal vascular fraction from control mice, HFD mice and HFD+PD 1 and 14 days. Serum levels of adiponectin (**L**), TNF-α (**M**), leptin (**N**) and IL-6 (**O**) and MCP-1 (**P**), MCP-2 (**Q**) and MCP-3 (**R**) protein expression were obtained using ELISA assay. Data are presented as means +/- S.E.M of 6-8 mice per group. **P*<0.05 versus control group; #*P*<0.05 versus HFD group. HFD: high-fat diet;

HFPD1: high-fat diet treated with PD153035 for 1 day; HFPD14: high-fat diet treated with PD153035 for 14 days.