SUPPLEMENTARY DATA

Supplementary Table 1. qPCR primer pairs used in this study

PIAS1	Forward: GCGGACAGTGCGGAACTAAA Reverse: ATGCAGGGCTTTTGTAAGAAGT
PIAS2	Forward : TTCAGCTTGGATGGTAGCTCA Reverse : TGTAATTGAAGTCGAAGGCAACG
PIAS3	Forward : TTCGCTGGCAGGAACAAGAG Reverse : GGGCGCAGCTAGACTTGAG
PIAS4	Forward : TGAGCTTCCGAGTATCAGACC Reverse : GGGCTACAGTCGAACTGCAC
CCL2	Forward : TAAAAACCTGGATCGGAACCAAA Reverse : GCATTAGCTTCAGATTTACGGGT
F4/80	Forward : CTGCACCTGTAAACGAGGCTT Reverse : GCAGACTGAGTTAGGACCACAA
IL-1β	Forward : GAAATGCCACCTTTTGACAGTG Reverse : TGGATGCTCTCATCAGGACAG
IL-6	Forward : GAACAACGATGATGCACTTGC Reverse : TCTCTGAAGGACTCTGGCTTTG
iNOS	Forward : GTTCTCAGCCCAACAATACAAGA Reverse : GTGGACGGGTCGATGTCAC
MIP2	Forward : ATCCAGAGCTTGAGTGTGACGC Reverse : AAGGCAAACTTTTTGACCGCC
SAA3	Forward : AGAGAGGCTGTTCAGAAGTTCA Reverse : AGCAGGTCGGAAGTGGTTG
TNFα	Forward : CCATTCCTGAGTTCTGCAAAGG Reverse : AAGTAGGAAGGCCTGAGATCTTATC
JNK1	Forward : AGCAGAAGCAAACGTGACAAC Reverse : GCTGCACACACTATTCCTTGAG
JNK2	Forward : AGTGACAGTAAAAGCGATGGTC Reverse : AGCACAAACAATTCCTTGGGC
18S rRNA	Forward : CGGCTACCACATCCAAGGAA Reverse : GCTGGAATTACCGCGGCT

Supplementary Table 2. siRNA used in this study

siPIAS1	UAAACAACCCGUCAAUAAUAAGGUG
si-2 (PIAS1)	AAGUGAGGUGAUAUUAAUUGGUCGG
si-3 (PIAS1)	UUAAACUGUAUCUCGUCACAGUCUG
siJNK1	GCGCGAACAGCCUGUACCAACUUUA
siJNK2	CCUCUUUCCAGGAAGGGACUAUAUU

SUPPLEMENTARY DATA

Cell culture

The 3T3-L1 preadipocytes were maintained and propagated in Dulbecco's modified Eagle medium (DMEM) with 10% calf serum. The 293A cells and Raw264.7 macrophages were maintained and propagated in DMEM with 10% fetal bovine serum (FBS).

Oil Red O staining

The adipocytes were washed with cold PBS and fixed for 10 min with 3.7% formaldehyde. Oil Red O (0.5% in isopropanol) was diluted with water (3:2), filtered through a 0.45 μm filter, and incubated with the fixed cells for 4 h at room temperature. The cells were washed with water, and stained fat droplets in the adipocytes were examined by light microscopy and photographed.

Isolation of SVF and adipocytes from adipose tissues

SVF cells and adipocytes were isolated by enzymatic digestion (collagenaseVIII; Sigma). The digested tissues were filtered through a 100 μm mesh filter to remove debris and then centrifuged. The adipocytes floated above the supernatant. The cellular pellet involving the SVF was resuspended with an ammonium chloride lysis buffer to remove red blood cells. The isolated adipocytes and SVF cells were washed twice with PBS, and subjected to further investigation.

Immunofluorescence study

iWAT or eWAT were excised, fixed with 4% paraformaldehyde overnight, embedded in paraffin and cut into sections. For immuofluorescence staining, sections were incubated with PIAS1 (Abcam) or F4/80 (Abcam) antibodies to test whether macrophages in adipose tissues expressed PIAS1. For immuofluorescence staining in adipocytes, the mature adipocytes were isolated from iWAT and eWAT as described, immediately fixed and smeared on slides, then permeabilizated before staining with PIAS1 antibody (abcam), with nile red staining to show the lipid droplets. All the fluorescence images were taken and analyzed with a Leica confocal microscope (Leica TCS SP5, Leica, Germany).

Flow cytometry staining

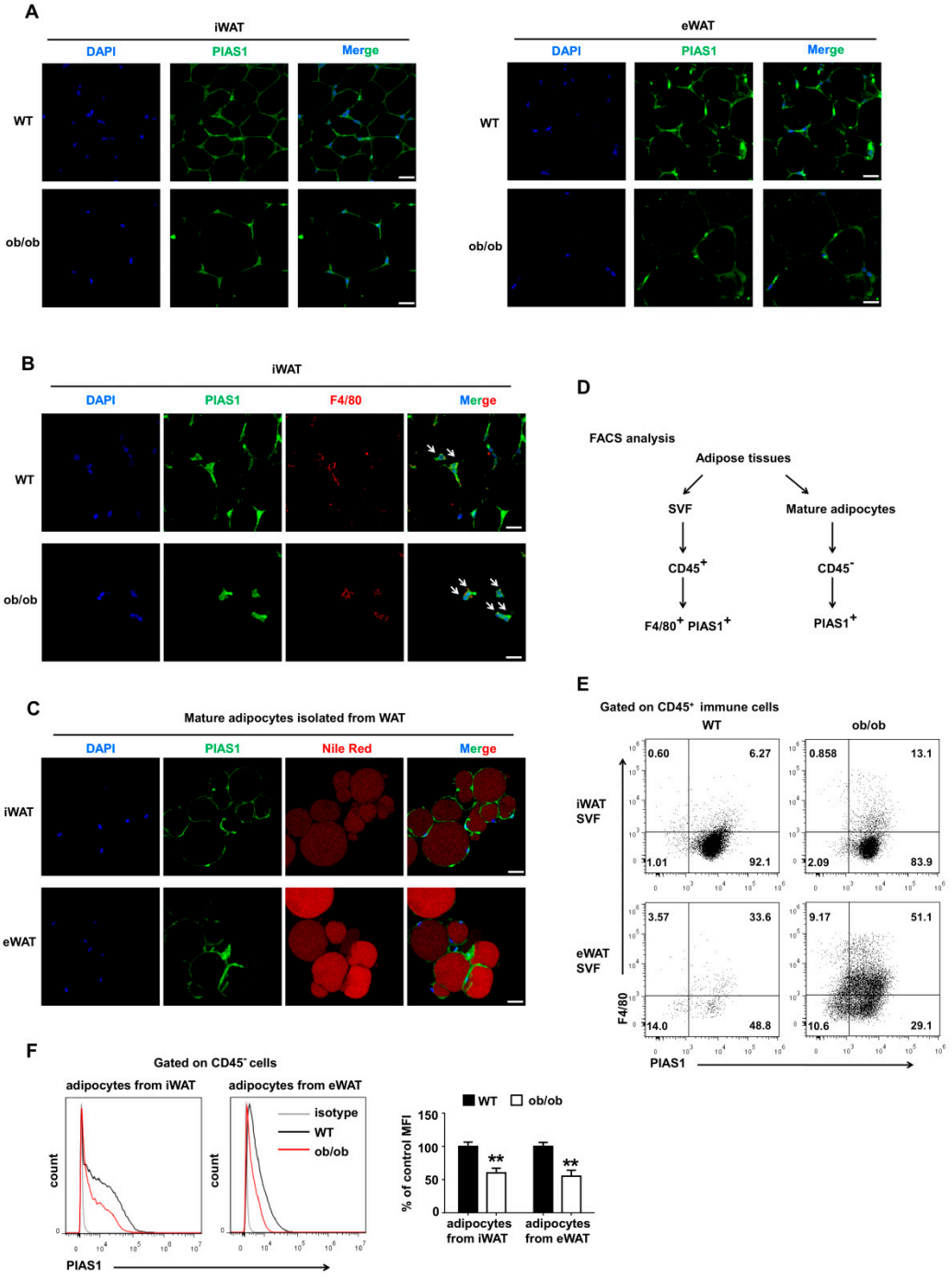
SVF cells and mature adipocytes were isolated as described. Then the cells were resuspended in PBS with 1% FBS and surface markers were stained with fluorescence-labeled antibodies. For intracellular protein staining, cells were fixed and permeabilizated by Fixation & Permeabilization fixed kit (eBioscience), and then stained with PIAS1 antibody (abcam). The data were analyzed with FlowJo software. For SVF cells, CD45, F4/80 and PIAS1 were stained. Percentages of $PIAS1^+$ cells and $PIAS1^+$ $F4/80^+$ cells were shown by gating to $CD45^+$ cells. For mature adipocytes, CD45 and PIAS1 were stained. CD45 staining was to exclude contamination of immune cells. Mean fluorescence intensity (MFI) of PIAS1 staining was shown by gating to $CD45^-$ cells.

SUPPLEMENTARY DATA

Supplementary Figure 1. Measurement of PIAS1 expression in adipocytes and macrophages from adipose tissues.

A-C. Immunofluorescence analysis of PIAS1 expression in adipocytes and macrophages. A. Representative images showed PIAS1 expression in iWAT (left) and eWAT (right) from WT or ob/ob mice. B. Representative images for PIAS1 and F4/80 staining in the iWAT from WT or ob/ob mice. White arrowheads showed the co-localization of PIAS1 and F4/80, indicating that macrophages in adipose tissues expressed PIAS1. C. Mature adipocytes were isolated from iWAT and eWAT of WT mice as described, and subjected to immunofluorescence analysis, with nile red to stain the lipid droplets. Representative images showed PIAS1 expressed in mature adipocytes. Scale bars in all the images above were 25μm. D-F. FACS analysis of PIAS1 expression in adipocytes and macrophages. D. Schematic design of FACS analysis. SVF cells and adipocytes were isolated by enzymatic digestion, and stained by indicated antibodies. E. SVF cells were stained by fluorescence-labeled antibodies for CD45, F4/80 and PIAS1. Flow cytometry representation of gated $CD45^+$ immune cells stained with F4/80 and PIAS1. Percentages of $PIAS1^+$ cells and $PIAS1^+$ $F4/80^+$ macrophages were shown. F. Mature adipocytes were stained by fluorescence-labeled antibodies against CD45 and PIAS1, with CD45 staining to exclude immune cell contamination. Flow cytometry representation of gated $CD45^-$ cells stained with PIAS1. ** $P < 0.01$

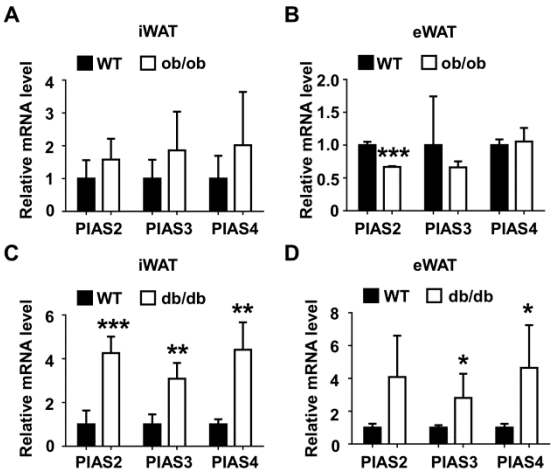
SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

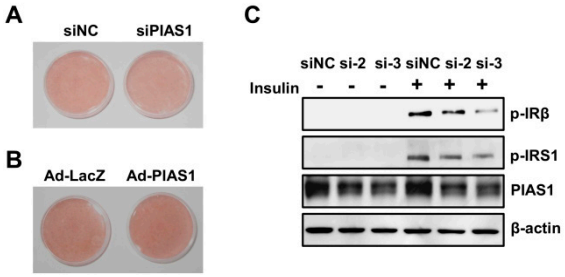
Supplementary Figure 2. The expression of *PIAS2*, *PIAS3* and *PIAS4* in WAT of ob/ob and db/db mice

A-D. qPCR analysis was used to measure PIAS2, PIAS3 and PIAS4 mRNA expression in iWAT (A) and eWAT (B) of ob/ob mice, iWAT (C) and eWAT (D) of db/db mice. Data were normalized to the mRNA level in WT mice respectively. The data shown were representative of at least three independent experiments. * $P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.



SUPPLEMENTARY DATA

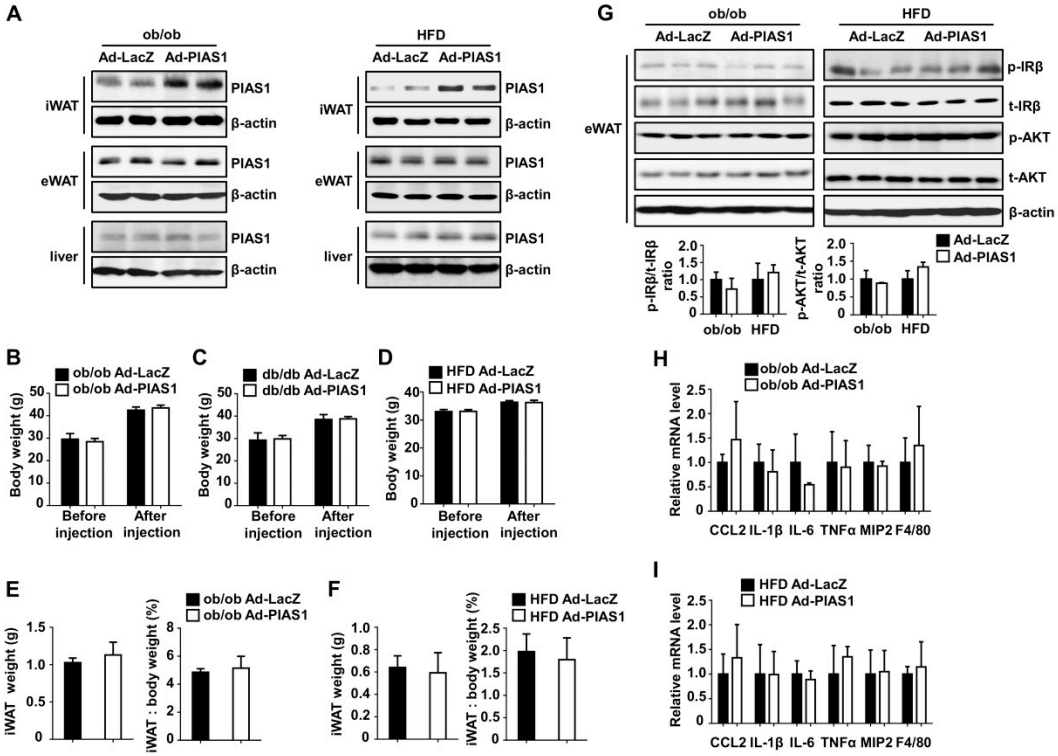
Supplementary Figure 3. The effect of PIAS1 on lipid accumulation and insulin signaling pathway
A, B. Knockdown or overexpression of PIAS1 were conducted in the 3T3-L1 adipocytes on day 5 post-induction. Oil red O staining was performed to evaluate the lipid accumulation in PIAS1-deficient cells (A) or PIAS1 overexpression cells (B) on day 8 post-induction. C. Another two sets of siPIAS1 were transfected into 3T3-L1 adipocytes on day 5 post-induction. On day 8 post-induction, cells were stimulated with or without 100 nM insulin for 5 min before harvest. The protein levels of p-IRβ, p-IRS1, PIAS1 and β-actin was detected by western blotting.



SUPPLEMENTARY DATA

Supplementary Figure 4. PIAS1 overexpression in iWAT had little effect on the body weight and iWAT development, as well as the inflammation response and insulin signaling in eWAT

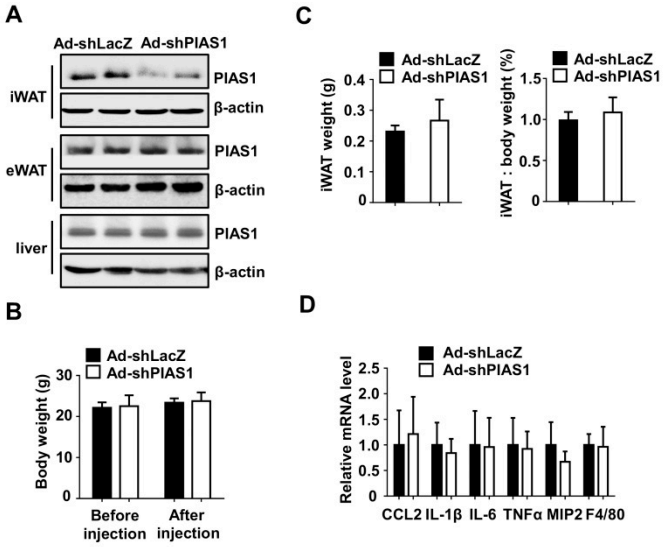
Male ob/ob, db/db, HFD mice were infected with Ad-LacZ or Ad-PIAS1 twice a week for 2 weeks via subcutaneous injection adjacent to inguinal fat pad. A. Western blotting was used to detect the protein level of PIAS1 in iWAT, eWAT and liver of ob/ob mice (left) and HFD mice (right) after adenovirus injection. B-D. The body weight of ob/ob mice (B), db/db mice (C) and HFD mice (D) were measured before and after adenovirus injection respectively (n=4-5/group). E-F. The weight of iWAT (left) and the percentage of iWAT weight relative to the body weight (right) in ob/ob mice (E) and HFD mice (F) were measured after adenovirus injection (n=3/group). The data shown were representative of at least three independent experiments. G. Insulin signaling pathway was detected in eWAT from ob/ob or HFD mice with iWAT-Ad-PIAS1 induction. H-I. qPCR analysis of inflammation genes in eWAT from ob/ob (H) or HFD mice (I) with iWAT-Ad-PIAS1 induction (n=3-4/group).



SUPPLEMENTARY DATA

Supplementary Figure 5. PIAS1 knockdown in iWAT had no effect on the body weight and iWAT development

Male C57BL/6J mice were infected with Ad-shLacZ or Ad-shPIAS1 twice a week for 2 weeks via subcutaneous injection adjacent to iWAT. A. Western blotting was used to detect the protein level of PIAS1 in iWAT, eWAT and liver after adenovirus injection. B. The body weight was measured before and after adenovirus injection respectively (n=5-6/group). C. The weight of iWAT (left) and the percentage of iWAT weight relative to the body weight were determined after adenovirus injection (n=4-5/group). The data shown were representative of at least three independent experiments. D. qPCR analysis of inflammation genes in eWAT from Ad-shLacZ treated mice (n=4/group).



SUPPLEMENTARY DATA

Supplementary Figure 6. PIAS1 regulated CCL2 expression through TNFα

A. 3T3-L1 adipocytes were treated with TNFα (20ng/ml) for 12 h, and qPCR analysis of CCL2 expression. B. PIAS1-deficient adipocytes were treated with neutralizing antibody anti-TNFα (100ng/ml) for 12 h, with qPCR analysis of CCL2 expression. ** $P < 0.01$; *** $P < 0.001$