

SUPPLEMENTARY DATA

Cell culture and induction. 3T3-L1 cells obtained from ATCC were cultured in DMEM supplemented with 10% FCS, 1% streptomycin and penicillin, at 37°C in a 5% CO₂ incubator. For differentiation, 3T3-L1 preadipocytes were allowed to reach confluence and induced with DMEM supplemented with 10% FCS, 0.5 mM 3-isobutyl-1-methyl-xanthine, 1 μM dexamethasone (Sigma-Aldrich, St.Louis, MO, USA), and 10 μg/mL insulin. After 2 days of induction, cells were maintained in DMEM containing 10% FCS and 10 μg/ml insulin. The medium was changed every 2 days before experiments. For bone marrow-derived macrophages, bone marrow cells from femurs and tibias of 8-week-old Pdc4^{-/-} or WT mice were cultured in complete RPMI 1640 supplemented with 50 ng/ml GM-CSF. Half of the medium was replaced every 2 days. At day 8, the adherent cells were collected as macrophages. For peritoneal macrophages, cell were harvested from peritoneal cavity of 8-week-old Pdc4^{-/-} or WT mice and placed in DMEM supplemented with 10% FCS at 37°C in a humidified atmosphere of 5% CO₂. After 2 h of incubation, the non-adherent cells were removed by three washes with DMEM, the adherent cells were harvested as macrophages.

Cell treatment. 3T3-L1 adipocytes or macrophages from Pdc4^{-/-} or WT mice were treated with 50 μg/ml oxLDL (Union-Biology, Beijing, China) or 500 μM PA combined with FFA-free, low-endotoxin BSA (Sigma, St.Louis, MO, USA) for indicated time, respectively. Cells were collected for western-blot, RNA-binding protein immunoprecipitation assay or Oil red O staining.

Oil red O staining and lipid quantification. Cells were washed with PBS and fixed with 4% paraformaldehyde, then Oil red O solution was added for 1 h of incubation at room temperature. After removal of Oil red O solution and wash with distilled water, equal volumes of 100% isopropanol were added to elute Oil red O. The solution containing the Oil red O stain was collected, and absorbance was measured at 500 nm.

Gene knockdown with siRNAs. Peritoneal macrophages were transfected with small interfering RNA (siRNA) for mouse LXR- α using GenePORTER® 2 Transfection Reagent (Genlantis, San Diego, CA, USA), according to the manufacturer's instructions. After 48 h, the cells were ready for oxLDL treatment. The LXR- α siRNAs was designed and synthesized by GenePharma (Shanghai, China), LXR- α siRNAs used in this experiment included: Sense-GCCUGAUGUUUCUCCUGAUTT, anti sense-AUCAGGAGAAACAUCAGGCTT and Sense-GUGCAGGAGAUUGUU GACUTT, anti sense-AGUCAACAAUCUCCUGCACTT.

Measurements of oxidative stress in liver tissues. Liver tissues from Pdc4^{-/-} or WT mice fed on 24-week HFD were used for measurements of GSH/GSSG ratio and 4-hydroxy-trans-2-nonenal (4-HNE) levels. For GSH/GSSG ratio, the supernatants from 20% liver homogenates were prepared, reduced (GSH) and oxidized (GSSG) glutathione concentrations were examined respectively, according to the manufactory's protocols using a commercial GSH/GSSG kit (njcbio, Nanjing, China). For 4-HNE assay, the supernatants from 20% liver homogenates were measured using an ELISA kit (CUSABIO,Wuhan, China) according to the manufactory's instruction.

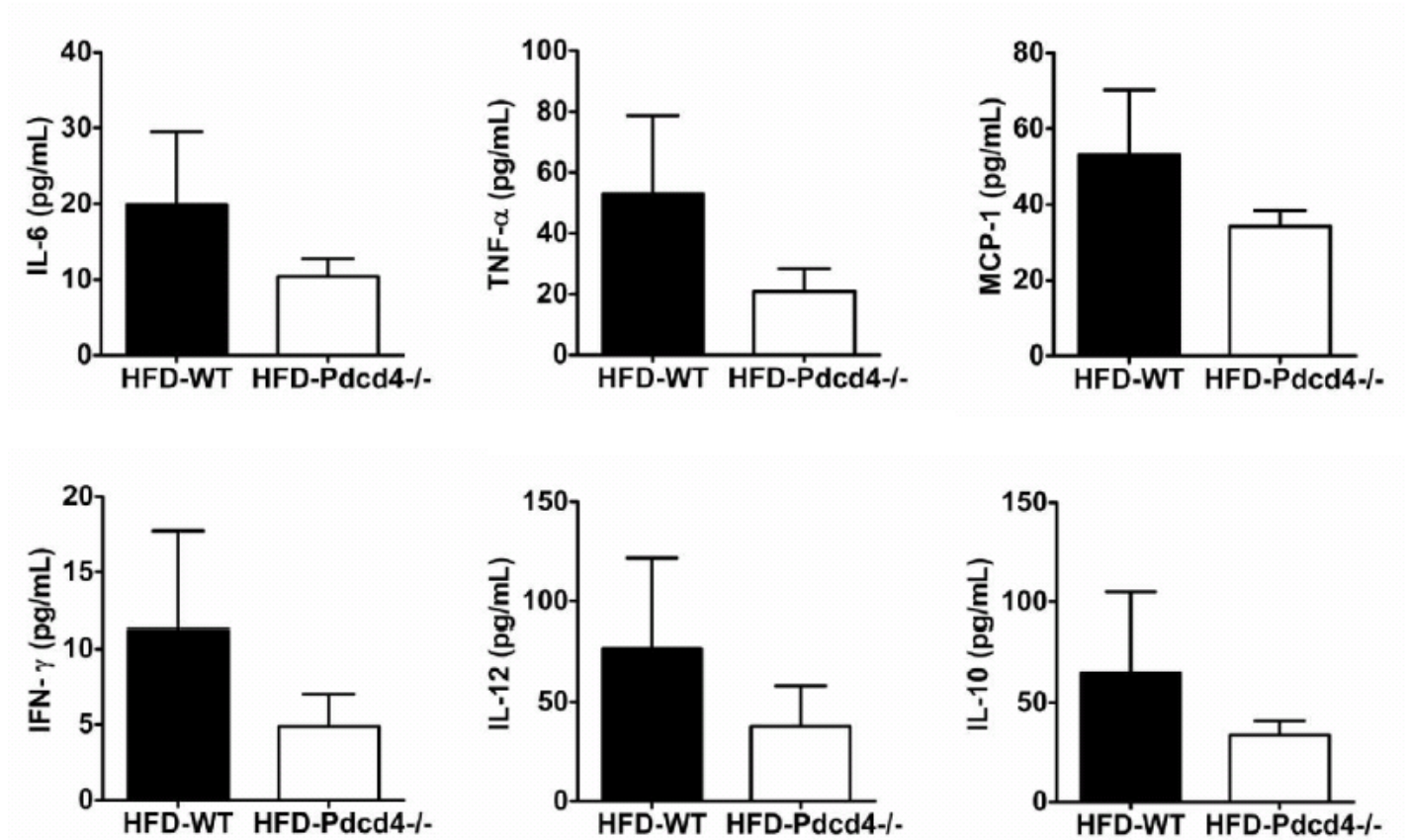
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Supplementary Table 1. qRT-PCR primer pairs used in this study.

Adiponectin	Sense: CCTGTTCTCTTAATCCTGCCCA Anti-sense: ATCTCCTTTCTCTCCCTTCTCTCCA
Leptin	Sense: TCAAGCAGTGCCTATCCAGAAAGTC Anti-sense: GGGTGAAGCCCAGGAATGAAGTC
C/EBP α	Sense: TTGAAGCACAATCGATCCATCC Anti-sense: GCACACTGCCATTGCACAAG
PPAR γ	Sense: GGAGCCTAAGTTTGAGTTTGCTGTG Anti-sense: TGCAGCAGGTTGTCTTGGATG
LXR- α	Sense: CTCTGGAGGCTGCTGGGATTAG Anti-sense: TTCCTGGAGCCCTGGACATTAC
ABCA1	Sense: AGCCCGGAGATTCTTGTGGA Anti-sense: CACTGCCAAGGCACCTGAAC
ABCG1	Sense: AGGGACACGATTCGCCTTT Anti-sense: GTCCACCCAACACCCATTCT
SREBP-1c	Sense: GGGTCAAAACCAGCCTCCCAAG Anti-sense: CCAGTCCCCGTCCACAAAGAAA
FAS	Sense: CTGAGGACTTCCCAAACGG Anti-sense: TGGCCTGATGAAACGACAC
SCD1	Sense: ATGTCTGACCTGAAAGCCGAGAA Anti-sense: GAGCACCAGAGTGTATCGCAAGAA
UCP1	Sense: ACTGCCACACCTCCAGTCATT Anti-sense: CTTTGCCTCACTCAGGATTGG
PGC-1 α	Sense: CCAGCCTCTTTGCCAGAT Anti-sense: GTCGCTACACCACTTCAATCCA
PEPCK	Sense: ATCTTTGGTGGCCGTAGACCT Anti-sense: GCCAGTGGGCCAGGTATTT
G6P	Sense: TTACCAAGACTCCCAGGACTG Anti-sense: GAGCTGTTGCTGTAGTAGTCG
GLUT4	Sense: ACTCTTGCCACACAGGCTCT Anti-sense: AATGGAGACTGATGCGCTCT
PDCD4	Sense: AAACAACCTCCGTGATCTTTGTCCA Anti-sense: TCAGGTTTAAGACGGCCTCCA
18S rRNA	Sense: GCCTGAGAAACGGCTACCACAT Anti-sense: CCGCTCCCAAGATCCAACACTACG
Primers for UCP1, PGC-1 α , PEPCK, G6P and GLUT4 are synthesized based on the references	

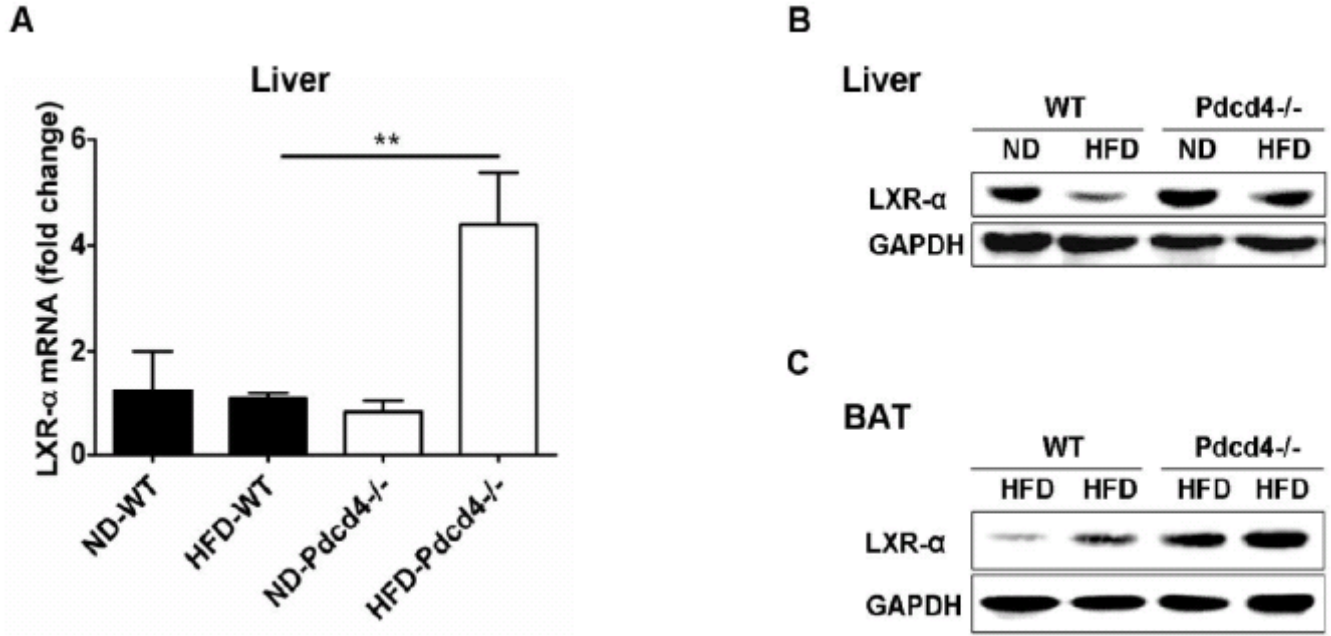
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Supplementary Figure 1. Serum inflammation detection. Inflammation detection was performed by cytometric bead array immunoassay on serum from WT or *Pdcd4*^{-/-} mice fed on 24-week HFD (n=5/group).



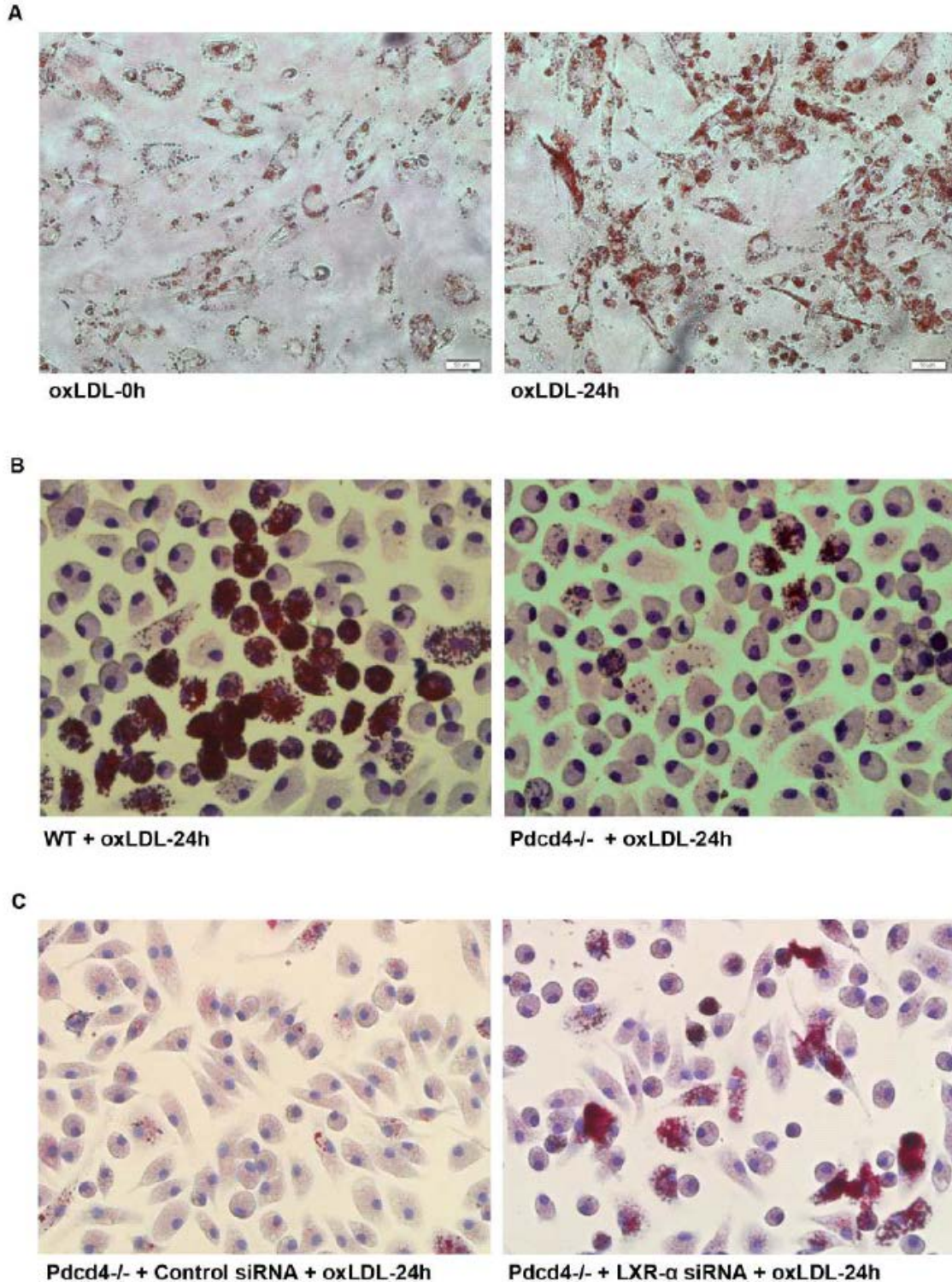
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Supplementary Figure 2. Expression of LXR- α in liver and BAT. Liver tissues and BAT were collected from *Pdcd4*^{-/-} and WT mice fed on ND or HFD for 24 weeks. *A*: mRNA levels of LXR- α in liver tissues (n=4-9 mice/group). *B-C*: Representative western-blot of LXR- α in liver tissues (*B*) and BAT (*C*) (n=4 mice/group). Data are presented as means \pm SEM, **P<0.01.



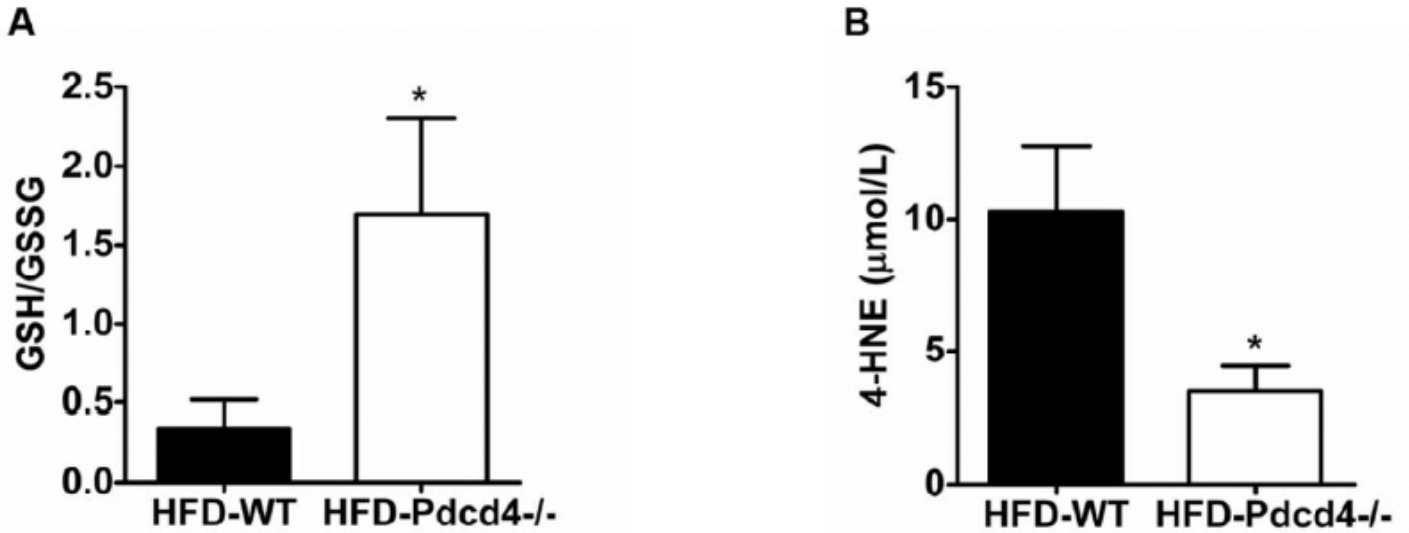
SUPPLEMENTARY DATA

Supplementary Figure 3. Lipid accumulation in oxLDL-treated adipocytes or macrophages. *A*: Representative Oil red O staining in 3T3-L1 adipocytes treated with oxLDL (50 $\mu\text{g/ml}$) for indicated time. *B*: Representative Oil red O staining in WT or *Pdcd4*^{-/-} macrophages treated with oxLDL (50 $\mu\text{g/ml}$) for 24h. *C*: Representative Oil red O in *Pdcd4*^{-/-} macrophages which were transfected with control or LXR- α siRNA and then treated with oxLDL (50 $\mu\text{g/ml}$) for 24h (n=3 mice/genotype).

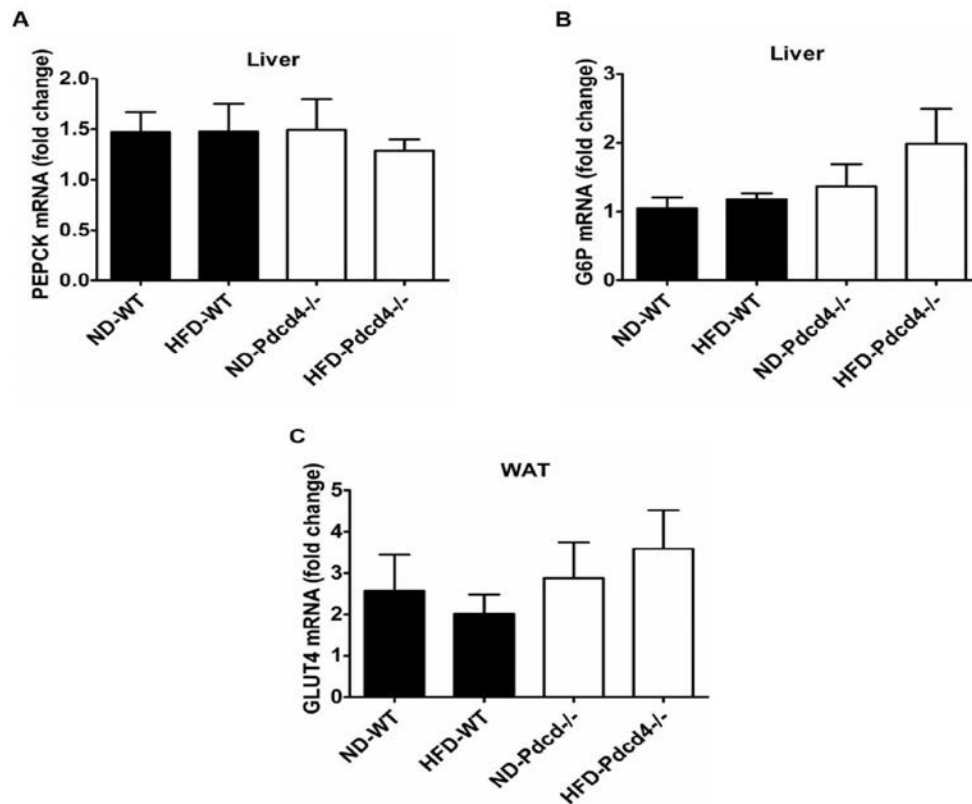


SUPPLEMENTARY DATA

Supplementary Figure 4. Hepatic oxidative stress measurements. Liver tissues were collected from *Pdcd4*^{-/-} and WT mice fed on HFD for 24 weeks, the supernatants from 20% liver homogenates were used for measurements of GSH/GSSG ratio (A) and 4-HNE levels (B). Data are presented as means±SEM, *P<0.05.



Supplementary Figure 5. Expression of glucose metabolism-related genes in liver and WAT. mRNA levels of phosphoenolpyruvate carboxykinase (PEPCK) (A), glucose-6-phosphatase (G6P) (B) in liver tissues and insulin-sensitive glucose transporter 4 (GLUT4) (C) in WAT from *Pdcd4*^{-/-} or WT mice fed on 24-week HFD (n=4-9 mice/group).



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Supplementary Figure 6. Expression of PDCD4 in WAT, liver and macrophages. *A-C*: mRNA (*A-B*) and protein (*C*) levels of PDCD4 in WAT and liver tissues from mice fed on 24-week ND or HFD. *D*: Protein levels of PDCD4 in WT macrophage treated with oxLDL (50 μ g/ml) or PA (500 μ M) for indicated time.

