Supplemental methods:

Generation and genotyping of MPKCδKO/ APoE$^{-/-}$ mice.

The PKCδ flox/flox mice were backcrossed with C57/BL6J mice more than 6 generations. The PKCδ flox/flox mice were crossed with lysozyme M Cre mice and ApoE$^{-/-}$ mice to generate macrophage specific knockout mice. Lysozyme M Cre and ApoE$^{-/-}$ mice, also on C57/BL6J background, were purchased from Jackson Laboratory (Bar Harbor, ME). The genotyping of Lysozyme M Cre and ApoE$^{-/-}$ mice followed the Jackson Laboratory protocol. The following primers were used for PKCδ flox/flox genotyping: 5' CTGCTGGGTAACTTAACAACAAGACC-3' and 5' CTGCTAAATAACATGATGTTCGGTCC-3'. Male mice were fed with HFD (Research Diet Inc, New Brunswick, NJ) in which 60% calories was from fat or atherogenic diet (TD.02028, Harland Laboratory, Indianapolis, IN) starting at 6 weeks of age.

Isolation of PBMC from Zucker diabetic fatty rats and control rats and in situ PKC assay

The PBMC of Zucker diabetic fatty rats or control rats (ZL) were isolated using HISTOPAQUE 1083 (Sigma, Saint Louis, MO). In situ PKC assay was performed as described previously(1). Briefly, the cells were incubated with PKC assay buffer (137 mM NaCl, 5.4 mM KCl, 0.3 mM Na2HPO4, 0.4 mM KH2PO4, 5.5 mM glucose, 10 mM MgCl2, 25 mM β-glycerophosphate, 5 mM EGTA, 2.5 mM CaCl2, 20 mM HEPES, 50 mg/ml digitonin, 100 μM ATP mixed with [γ-32P]ATP (<1500 cpm/pmol), and 100 μM PKC-specific peptide substrate (RKRTLRRL)) for 15 min at room temperature. The kinase reaction was stopped by adding 5% (wt/vol; final concentration) TCA. Then the reaction solution was spotted on 2 × 2-cm phosphocellulose squares of Whatman P-81, and washed three times with 0.75% phosphoric acid. The Whatman paper was put in scintillation vials and P32 was counted with scintillation counter.

Measure PKCδ expression in the circulating leukocytes by flow cytometry in ZL and ZDF rats

Blood was collected from rat tail with heparin filled capillary tubes. After fixation with BD Perm/Fixation buffer, the cells were stained with anti-CD19, anti-CD3, anti-CD11b and monocolonal anti-PKCδ (Cell signaling techonology). The mean fluorescence of PKCδ was determined by flow cytometry.

Culture bone marrow macrophages

The cells in mouse tibiae and femurs were flushed out with ice-cold PBS. The red blood cells were lysed with 1XACK buffer and other cells were precipitated by centrifuge. The cell pellets were resuspended and filtered through a 70μm cell strainer. After centrifuge, the cell pellets were resuspended with DMEM containing 10% FBS and 20% conditioned L929 medium and cells were cultured on petri dish.

Western blotting

Protein samples were separated by electrophoresis in a 10% Tris-HCl polyacrylamide gel and transferred to a PVDF membrane, which was blocked with 5% non-fat dry milk in Tris-buffered saline-0.1% Tween-20 and incubated with primary antibody in 4°C overnight. Detection was carried out using an ECL Plus Western Blotting Detection kit (Thermo Scientific, Rockford, IL). Quantitative densitometry was performed using ImageJ. Anti-PKCδ, p-Akt, Akt, p-FoxO3a,

FoxO3a, Bim, P85, phosho-motif[LXRXX(pS/pT)], Na,K ATPase, p-IRS2 and IRS2 antibodies were purchased from Cell Signaling Technology. GAPDH antibody was obtained from Abcam.

Migration

The macrophage migration was determined by Transwell assay. The macrophages were starved for 8 hours and then 10^5 macrophages were plated in the insert of transwell with 5 μm pore. Vehicle, MCP-1, PMA or PMA+MCP-1 were added to the indicated wells to determine their effects on migration. After 16 hours, non-migrated cells were scrubbed and the cells migrated through membrane were stained and counted.

Phagocytosis

Macrophages were treated with vehicle or PMA (100nM) for 10 minutes. Then FITC conjugated IgG coated latex beads were added to the medium. After 90 minutes, the cells were washed and incubated with trypan blue buffer for 2 minutes to quench FITC fluorescence from beads which simply bound to cell surface. The cells were washed and harvested and the fluorescence from beads which were uptaken by macrophages was measured by flow cytometry.

Monocyte-macrophage differentiation

PBMC in the blood were isolated by Ficoll density gradient separation. Then PBMC were cultured in DMEM containing FBS and GM-CSF. After 6 days, the cells attached to the bottom of dishes were harvested and stained with anti-CD36, anti-Cd11b and PI. Then the fluorescence of CD36 in PI^-CD11b^+ cells was measured by flow cytometry.

Glucose and insulin tolerance tests

Mice were fasted for 6 hours and then glucose (2mg/g body weight) or insulin (0.75U/Kg body weight) was administered to mice via intraperitoneal injection as described previously(2). Blood was sampled from tail vein for glucose determination at 0, 30, 60,120 minutes after glucose or insulin injection.

Quantification of atherosclerotic lesion size in aorta

The aorta was harvested and stained for 5 minutes in 0.5% Sudan IV, and destained for 5 minutes in 70% ethanol. The stained aortas were placed on a glass slide and photographed with a QColor3 Digital Camera mounted on an Olympus stereo microscope. Two pictures were taken on each aorta because the length of aorta was over the maxi field of camera. The two parts of aorta was merged into one intact aorta with Photoshop software. Lesion areas were measured by Photoshop and ImageJ as reported previously[2].

Immunohistochemistry

The aorta root or abdominal aorta which is between left and right renal artery, were dissected and embedded in OCT medium and frozen on dry ice. The aortic plaque was sectioned with a 7μm thickness. Six sections, each 100μm apart, were mounted on one slide. The slides were fixed in acetone in -20ºC for 10 minutes and then blocked with avidin and biotin. Then the slides were incubated with anti-Ki67 antibody at $4^{\circ}C$ overnight. After washing, the slides were incubated with biotin-conjugated anti-rabbit antibody and followed by avidin-conjugated Dylight 549.

Then the slides were incubated with anti-Mac2 antibody at 4°C overnight and followed by FITC-conjugated anti-rat second antibody.

Aorta single cell preparation and flow cytometry analysis

Aorta single cells were prepared using previous methods(3). The aorta was dissected and cut into small pieces. The aorta pieces were digested with 125U/ml collagenase type XI, 60U/ml hyaluronidase type I-s, 60U/ml DNase1, and 450U/ml collagenase type I (all enzymes were obtained from Sigma-Aldrich) in PBS containing 20mM Hepes at 37°C for 1 h. A cell suspension was obtained by mashing the aorta through a 70-μm strainer. The cells were incubated with indicated antibody on ice for 30 mins and then stained with PI for 10 mins. After washing, immunofluorescence was detected by flow cytometry. Antibodies used were as follows: CD45, CD115, Ly6G, F4/80, CD19, TCR and CD11b were purchased from Biolegend.

Trichrome staining

Collagen and necrosis area in the plaque of abdominal aorta was stained using Trichrome Stain kit (Sigma-Aldrich Inc., St. Louis, MO).

Infusion of BrdU and measurement BrdU positive cells in aorta by flow cytometry:

BrdU was delivered by an Alzet osmotic pump (1003D). Mice are anesthetized by intramuscular (IM) injection of ketamine (80-100mg/kg)/xylazine (5-10 mg/kg) prior to the procedure. The flank dorsal surface of the animal was clipped and the skin was wiped with Betadine. A 5mm incision was made into the skin and a pocket was created by separation with sterile scissors. The osmotic pump was slipped through the incision and into the subcutaneous cavity. A single surgical staple was used to close the skin. After 3 days, the aorta was dissected and digested into single cells. The cells were incubated with Alexa488-Cd11b for 30 minutes on ice. The cells were stained with BrdU antibody using APC BrdU flow kit (BD Bioscience, San Jose, CA). Then the fluorescence was determined by flow cytometry.

Apoptosis assay of cultured BM macrophages

Apoptosis in macrophages were induced by stimulation with thapsigargin (0.25 μM) plus OxLDL (50 μg/ml) or withdrawing growth factor for 16 hours. Then the cells were stained with Annexin V and PI and the apoptotic cells were detected by flow cytometry.

EDU incorporation

The cells were grown in 5% FBS and EDU incorporation was performed following the instruction of Click-iT Plus Edu Alexa Fluor 647 flow cytometry kit (Thermo Fisher Scientific).

Apoptosis assay of spleen macrophages

The spleen was homogenized into single cell. The cells were incubated with anti-CD45 and anti F4/80. After washing, the cells were incubated with FITC conjugated annxin V (BD Bioscience, San Jose, CA) and PI. The $CD45^+F4/80^+$annexin V^+PI^+cells were determined by flow cytometry.

Detecting p-Akt and PKCδ in splenocytes using flow cytometry

The spleen was homogenized into single cell and stained with LIVE/DEAD Fixable Violet Dead Cell Stain Kit (Life Technologies, Carlsbad, CA). Then the cells were fixed with Cytofix cytoperm fixation/permeabilization kit (BD Biosciense, San Jose, CA) after incubation with anti-CD45, anti-CD19, anti-TCRβ and anti-F4/80. After washing with perm/wash buffer, the cells were incubated with anti-p-Akt (1:400) or anti-PKCδ (1:1000) at 4°C overnight. The next day, the cells were incubated with Alex647 conjugated anti-rabbit antibody and the fluorescence was determined by flow cytometry.

Lentivirus transduction

Lentivirus with Akt ShRNA or control RNA were purchased from Antibodies-online Inc. Lentivirus containing PKCβ or PKCδ and inducible lentivirus with PKCδ were obtained from Vigene Bioscience. The confluent cells were incubated with lentivirus for 24 hours. Then the cells were passaged and purimycin was added into the medium to kill the cells which were not transduced with lentivirus.

Cell cycle analysis

Spleen was minced into single cells and filtered through a 70μm cell strainer. The red blood cells were lysed by 1XACK buffer. The splenocytes were fixed with 75% ethanol in 4°C overnight. After washing, the cells were stained with PI and cell cycle was determined by flow cytometry.

Real-time PCR analysis

1μg mRNA was used to generate cDNA using high capacity cDNA reverse transcription kit (Applied Biosystems, Grand Island, NY). Gene expression level was normalized to the expression level of 36B4. PCR primers were: PKCδ, 5'- CAAGAAGAACAACGGCAAGG-3' and 5'- GACGGTTCATGGTTGGAAAC-3'; 36B4, 5'- GCTCCAAGCAGATGCAGCA-3' and 5'- CCGGATGTGAGGCAGCAG-3'; F4/80, 5'-ACCACAATACCTACATGCACC-3', and 5'-AAGCAGGCGAGGAAAAGATAG-3'; IL2, 5'- CGGCATGTTCTGGATTTGAC-3' and 5'-ACCACAGTTGCTGACTCATC-3'; CXCL9, 5'- CTTTTCCTTTTGGGCATCAT-3' and 5'-GCATCGTGCATTCCTTATCA-3'; CCL5, 5'-GGGTACCATGAAGATCTCTGC-3' and 5'-TCTAGGGAGAGGTAGGCAAAG-3'.

Reference List

1. Igarashi,M., Wakasaki,H., Takahara,N., Ishii,H., Jiang,Z.Y., Yamauchi,T., Kuboki,K., Meier,M., Rhodes,C.J., and King,G.L. 1999. Glucose or diabetes activates p38 mitogen-activated protein kinase via different pathways. *J Clin Invest* **103**:185-195.

2. Li,Q., Park,K., Li,C., Rask-Madsen,C., Mima,A., Qi,W., Mizutani,K., Huang,P., and King,G.L. 2013. Induction of vascular insulin resistance and endothelin-1 expression and acceleration of atherosclerosis by the overexpression of protein kinase C-beta isoform in the endothelium. *Circ Res* **113**:418-427.

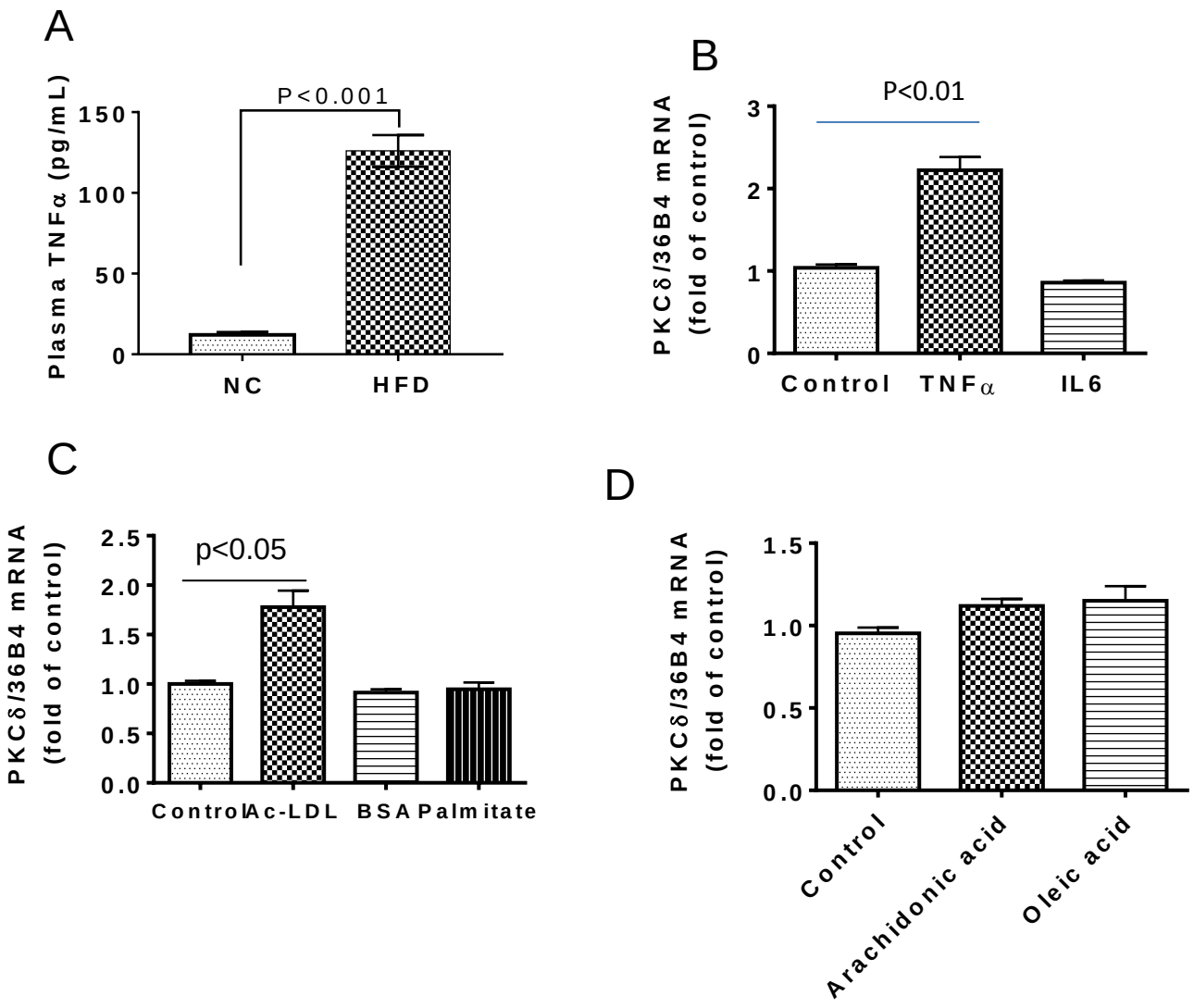
3. Galkina,E., Kadl,A., Sanders,J., Varughese,D., Sarembock,I.J., and Ley,K. 2006. Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent. *J Exp. Med.* **203**:1273-1282.

Supplemental Table I. Characterization of Zucker Diabetic Fatty (ZDF) and Lean (ZL) rats

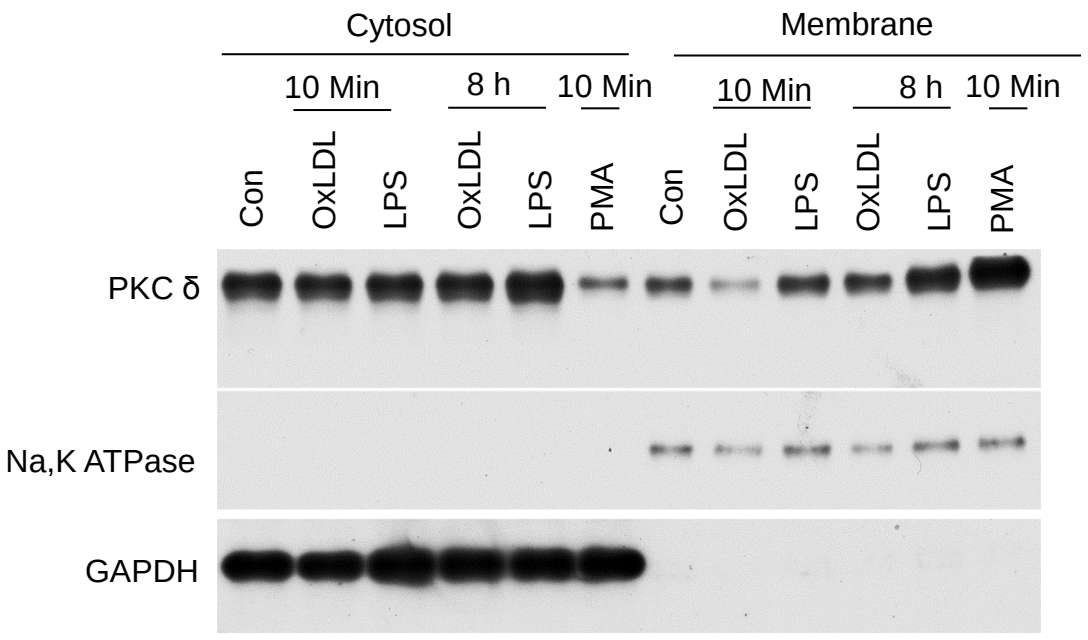
	ZL	ZDF	
	Lean control	Diabetic fatty	P value
Number	6	6	
Age (weeks)	19	19	
Body weight (g)	401±11	410±40	
Random blood glucose (mg/dL)	96.5±20.6	476.0±96.0	p<0.001
Insulin (ng/mL)	2.40±0.60	1.91±0.78	
FFA (mg/L)	0.39±0.09	0.47±0.14	
TG (mg/dL)	45.3±9.3	219.0±44.0	p<0.001

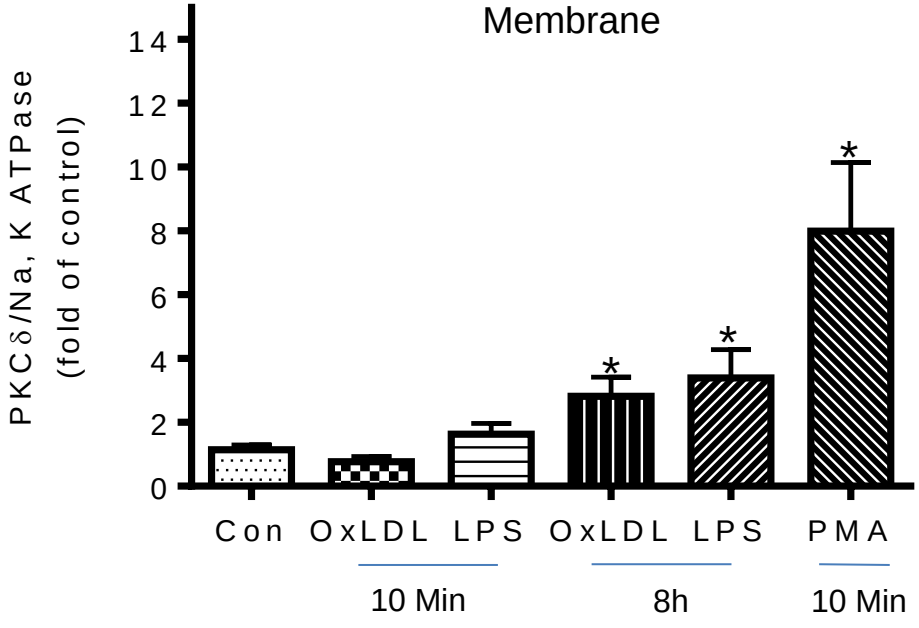
Data was presented as means±SD. FFA, free fatty acid; TG, triglyceride.

Supplemental Figures and Legends

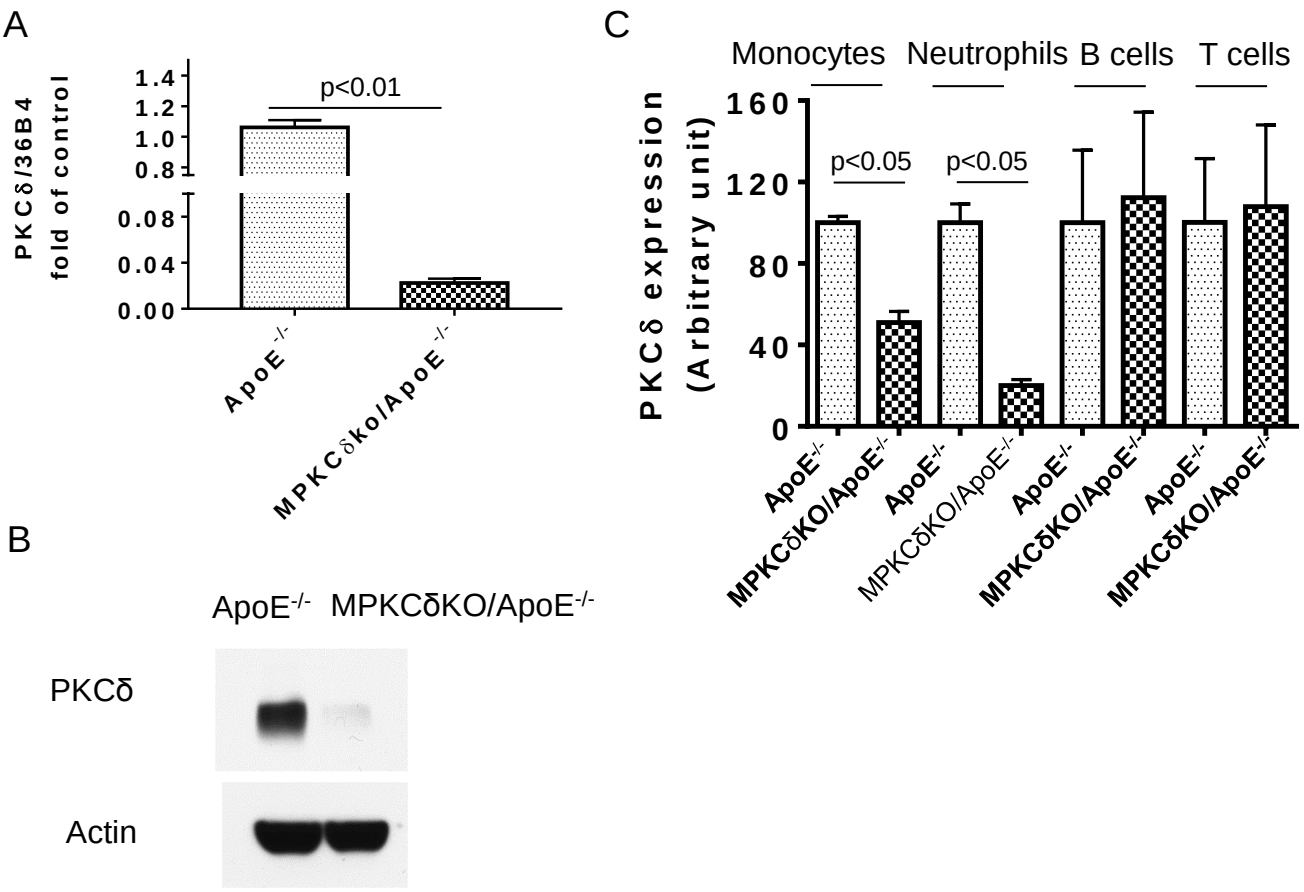


Supplemental Figure I. TNFα level in plasma of ApoE$^{-/-}$ mice fed with normal chow or HFD and PKCδ gene expression in bone marrow macrophages. A. TNFα level in plasma of ApoE$^{-/-}$ mice fed with normal chow or HFD for 16 weeks (n=6 per group). **B**. BM macrophages were stimulated with TNFα (10 ng/ml) or IL6(20 ng/ml) for 4 h and PKCδ gene was measured by Real-time PCR and normalized by 36B4 (n=3-6 per group). **C**. BM macrophages were stimulated with Ac-LDL(200 ug/ml) or palmitate (0.5 mM) for 4 h and PKCδ gene was measured by Real-time PCR and normalized by 36B4 (n=4 per group). **D**. BM macrophages were stimulated with arachidonic acid (0.1 mM) or oleic acid (0.3 mM) for 4 h and PKCδ gene was measured by Real-time PCR and normalized by 36B4 (n=3-4 per group).

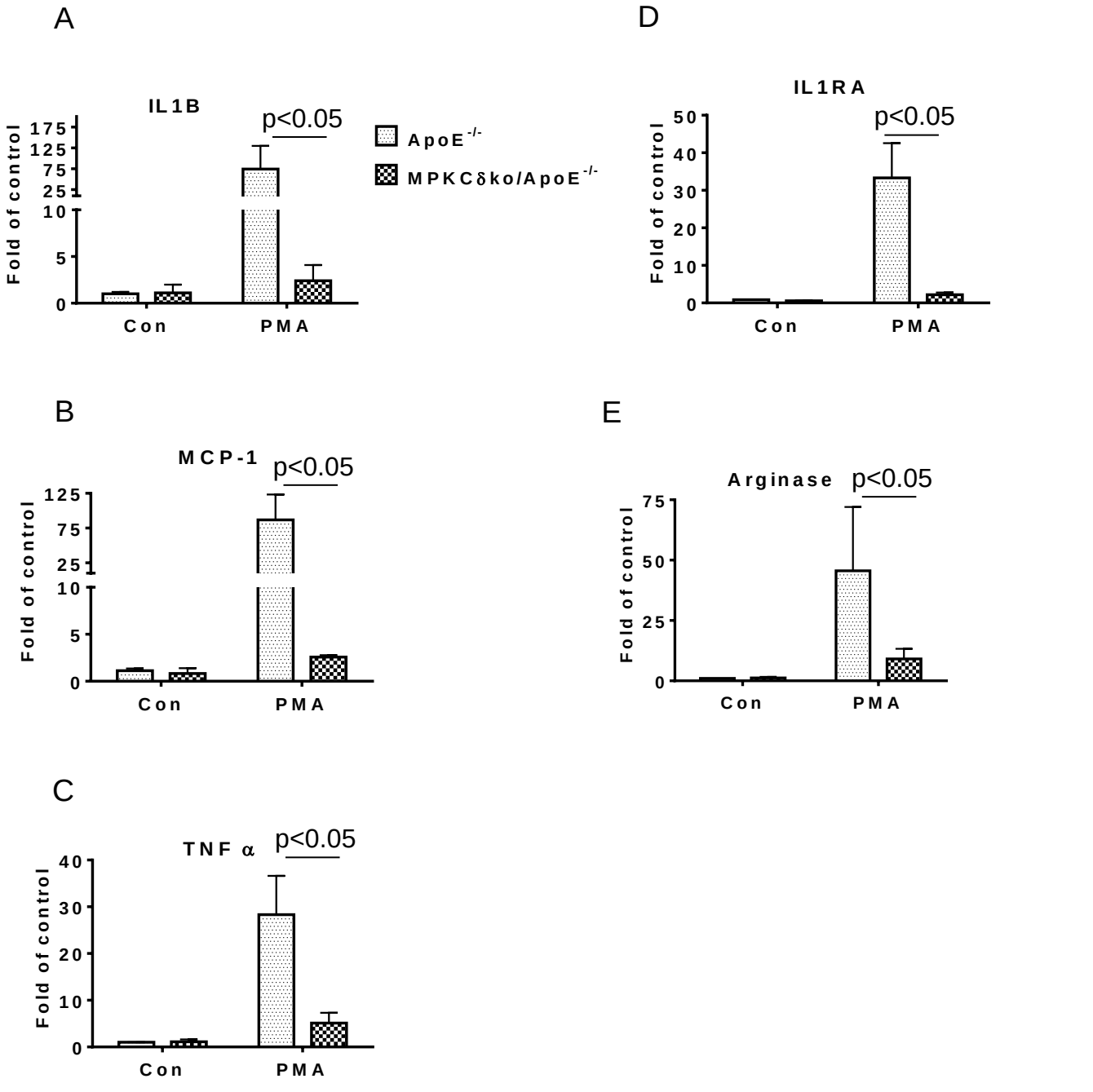




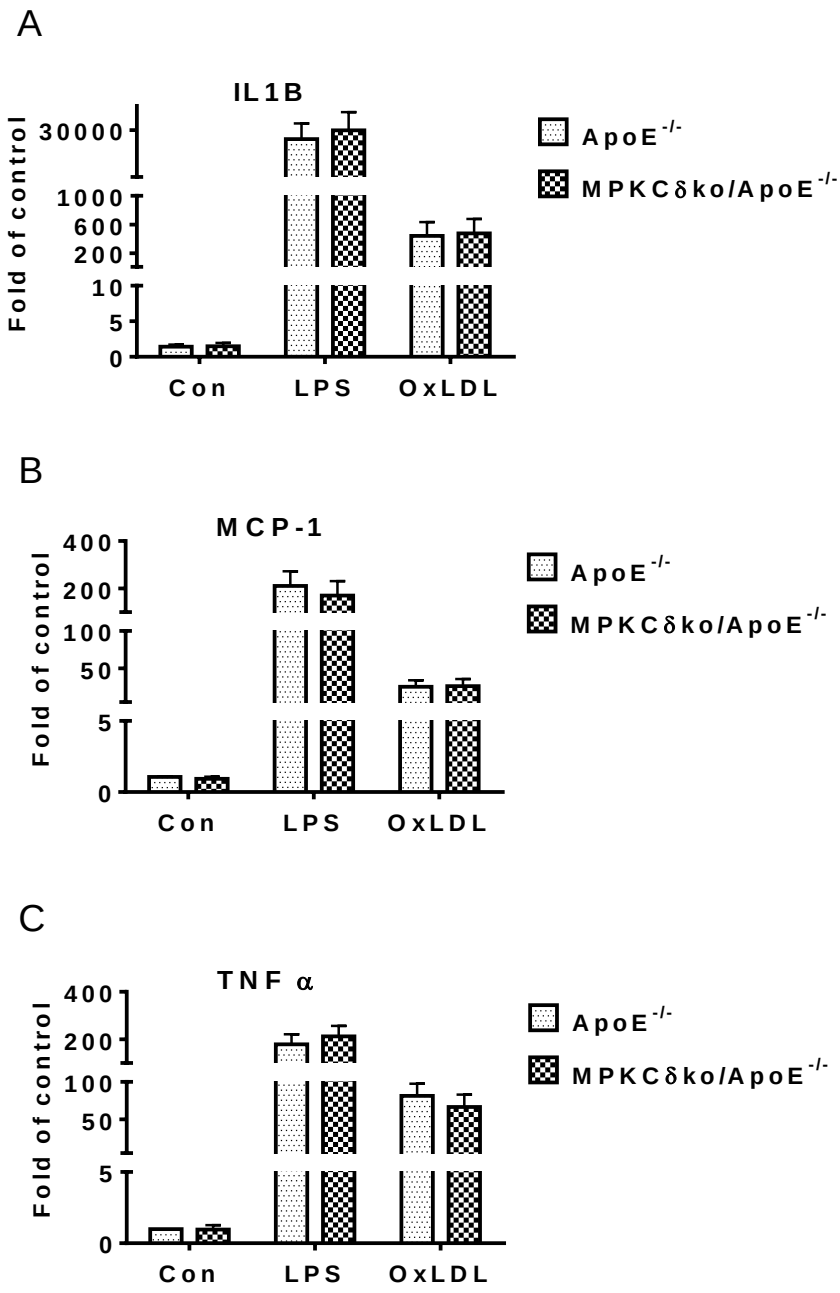
Supplemental Figure II. PKCδ translocation to membrane. Cellular membrane and cytosolic protein were separated and the translocation of PKCδ from cytosol to membrane was determined by Western blotting after treatment with OxLDL (50 μg/ml), LPS (100 ng/ml) or PMA(100 nM) at indicated times. n=4-7 per group. * $p<0.05$, vs control (Con).



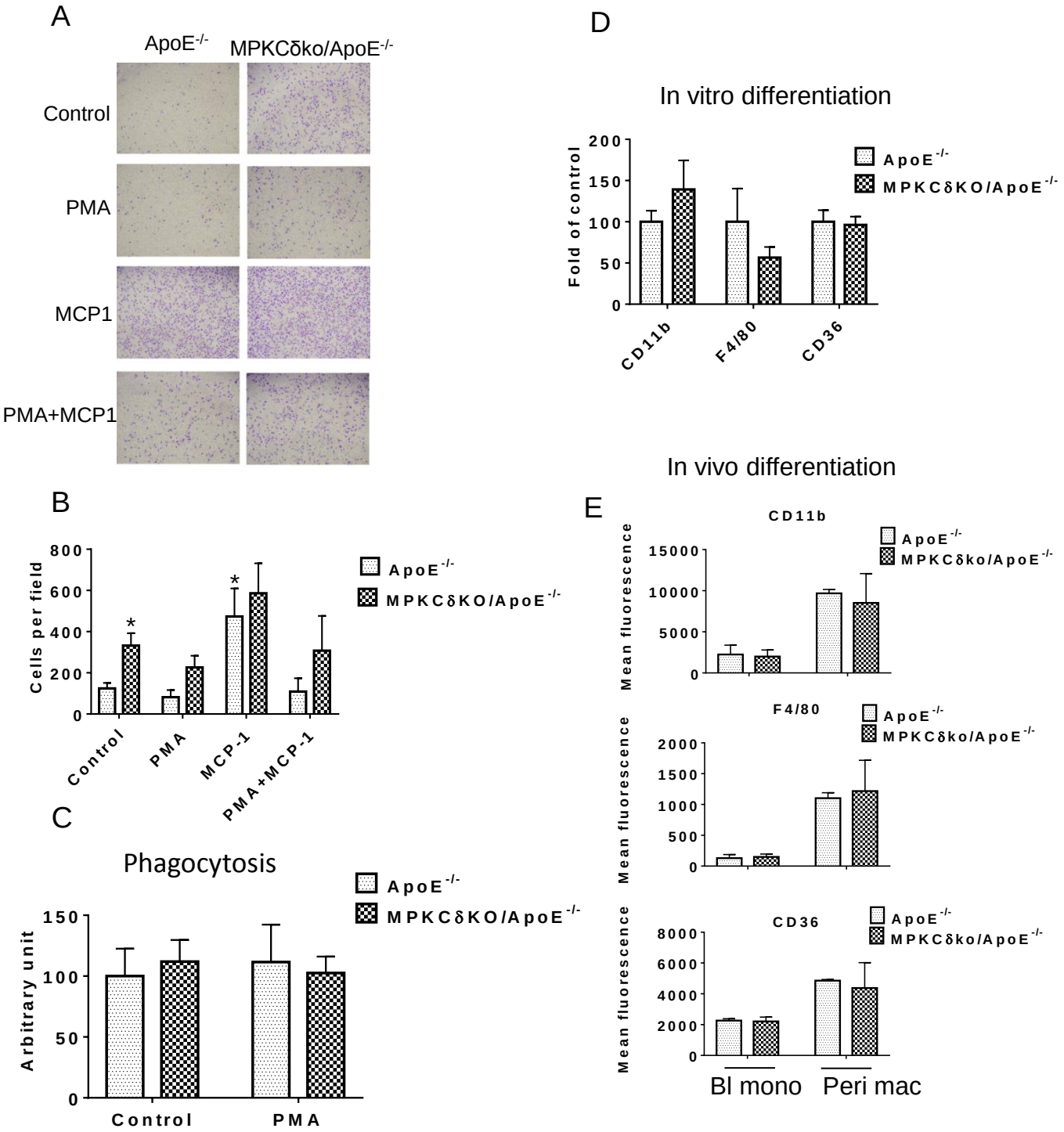
Supplemental Figure III. PKCδ expression in ApoE$^{-/-}$ and MPKCδKO/ApoE$^{-/-}$ mice. **A-B.** The expression of PKCδ in cultured bone marrow macrophages was determined by qRT-PCR (A, n=6 per group, ** p<0.01 vs ApoE$^{-/-}$) or Western blotting (B). **C.** PKCδ expression in blood monocytes, neutrophiles, B cells and T cells was determined by flow cytometry (n=3 per group).



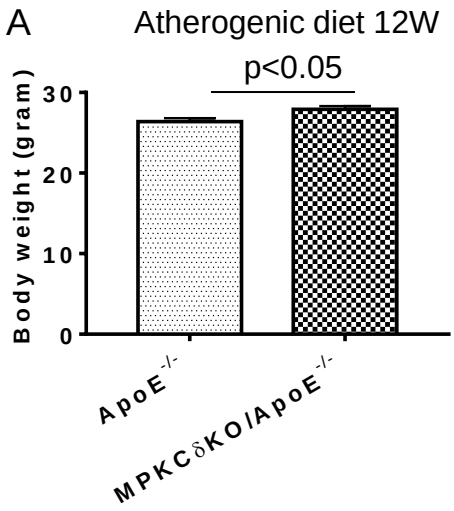
Supplemental Figure IV. Effect of PKCδ isoform deletion on the production of pro-inflammatory and anti-inflammatory genes. Bone marrow macrophages were treated with 100 nM PMA for 4 hours and gene expression of IL1B (A), MCP-1 (B), TNFα (C), IL1RA (D) and arginase (E) were determined by RT PCR and normalized by 36B4 (n=3-5 for each group).

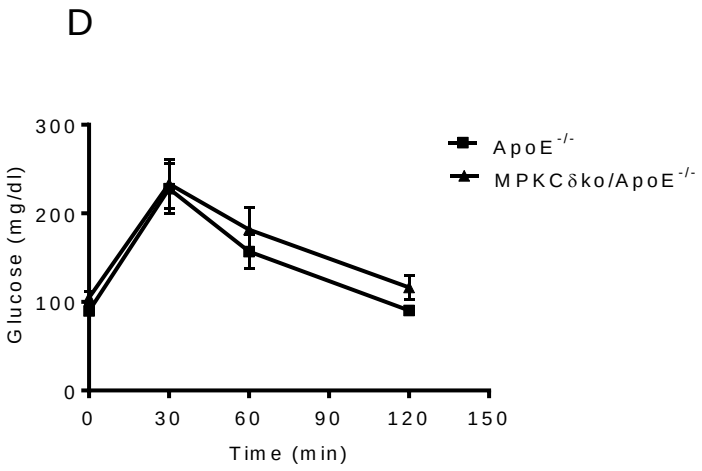


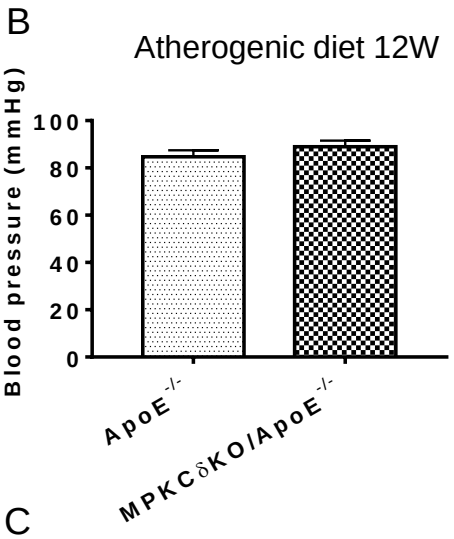
Supplemental Figure V. Effect of PKCδ isoform deletion on the production of pro-inflammatory genes after LPS or oxLDL stimulation. BM macrophages were treated with 100 nM LPS or 50 μg/ml oxLDL for 4 hours and gene expression of IL1B (A), MCP-1 (B), TNFα were determined by RT PCR and normalized by 36B4 (n=5-10 per group).

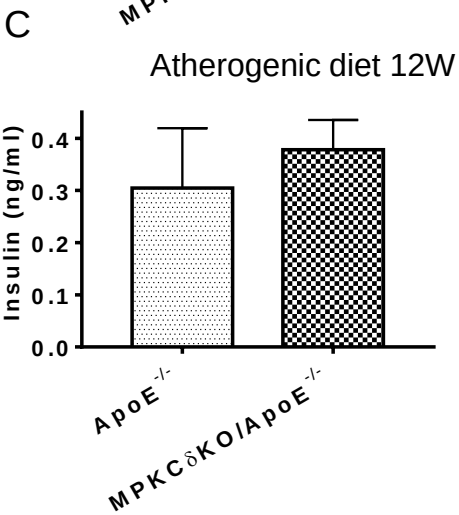


Supplemental Figure VI. Effect of PKCδ isoform deletion on macrophage migration, phagocytosis and monocyte-macrophage differentiation. (A-B), Macrophage migration was investigated by transwell assay. Macrophages were treated with vehicle, PMA (100nM), MCP-1 (200ng/ml) or PMA (100nM) plus MCP-1 (200ng/ml). ($ApoE^{-/-}$ control n=7; $ApoE^{-/-}$ PMA n=3; $ApoE^{-/-}$ MCP-1 n=8; $ApoE^{-/-}$ PMA+MCP-1 n=3; MPKCδko/$ApoE^{-/-}$ control n=8; MPKCδko/$ApoE^{-/-}$ PMA n=3; MPKCδko/$ApoE^{-/-}$ MCP-1 n=7; MPKCδko/$ApoE^{-/-}$ PMA+MCP-1 n=3, * vs $ApoE^{-/-}$ control). C, Macrophages were treated with or without PMA (100nM) for 10 minutes and phagocytosis was determined by uptake of FITC-conjugated IgG coated beads. The intensity of fluorescence of beads in macrophages was measured by flow cytometry. (n=3 per group). D. Monocyte-macrophage differentiation. PBMC was isolated from the blood of two groups of mice and was cultured in DMEM containing 10% FBS and GM-CSF (5ng/ml) for 6 days. (n=3-8per group). E. Differentiation of peritoneal macrophages from blood monocytes in vivo. The mice were intraperitoneally injected with thioglycollate broth. After 3 days, the mean fluorescence of CD11b, F4/80 and CD36 in blood monocytes ($CD45^{+}CD115^{+}$) or peritoneal macrophages ($CD45^{+}CD115^{+}$) were determined by flow cytometry (n=3 per group, Bl mono=blood monocytes, Peri mac=peritoneal macrophages).

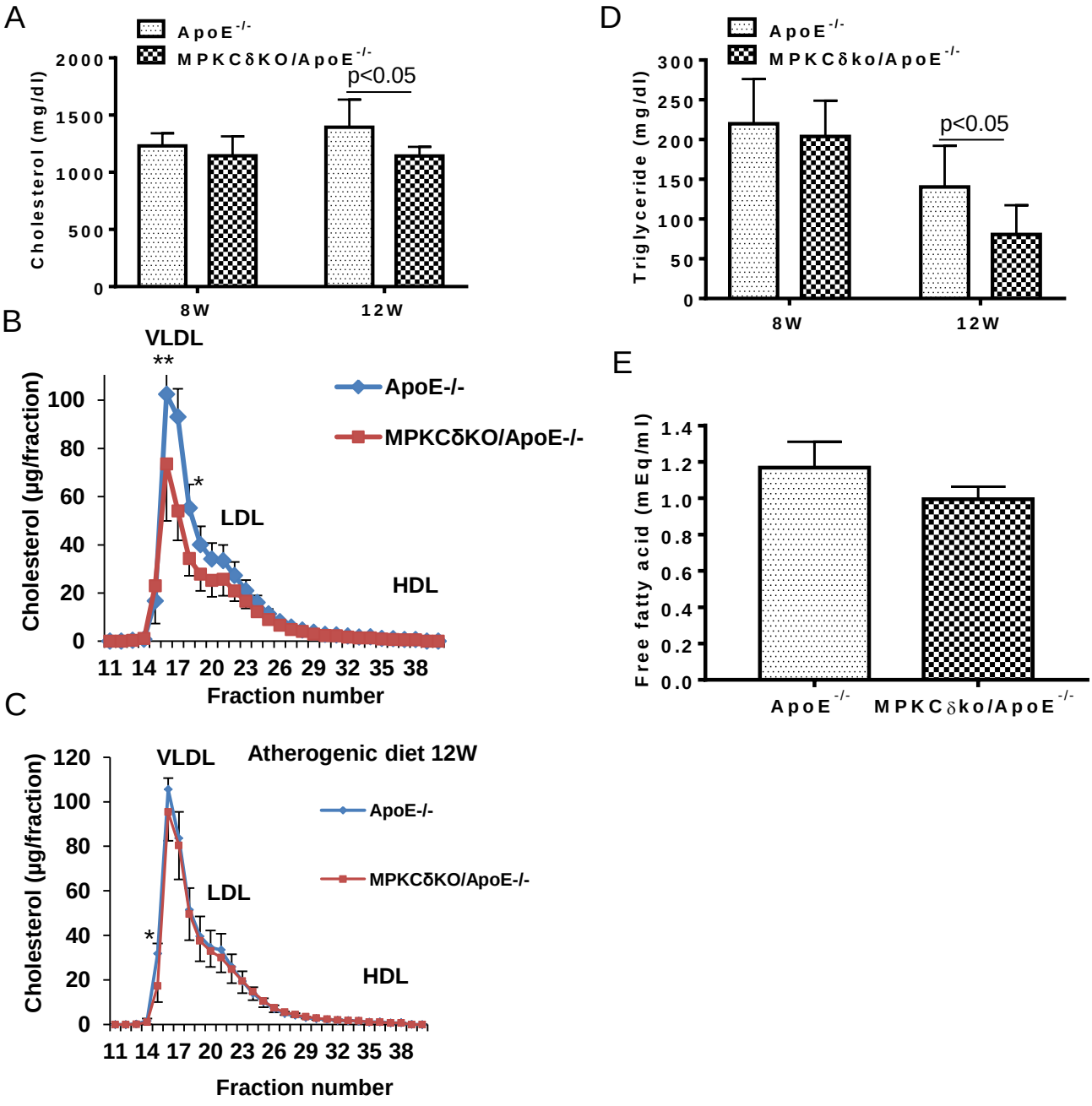




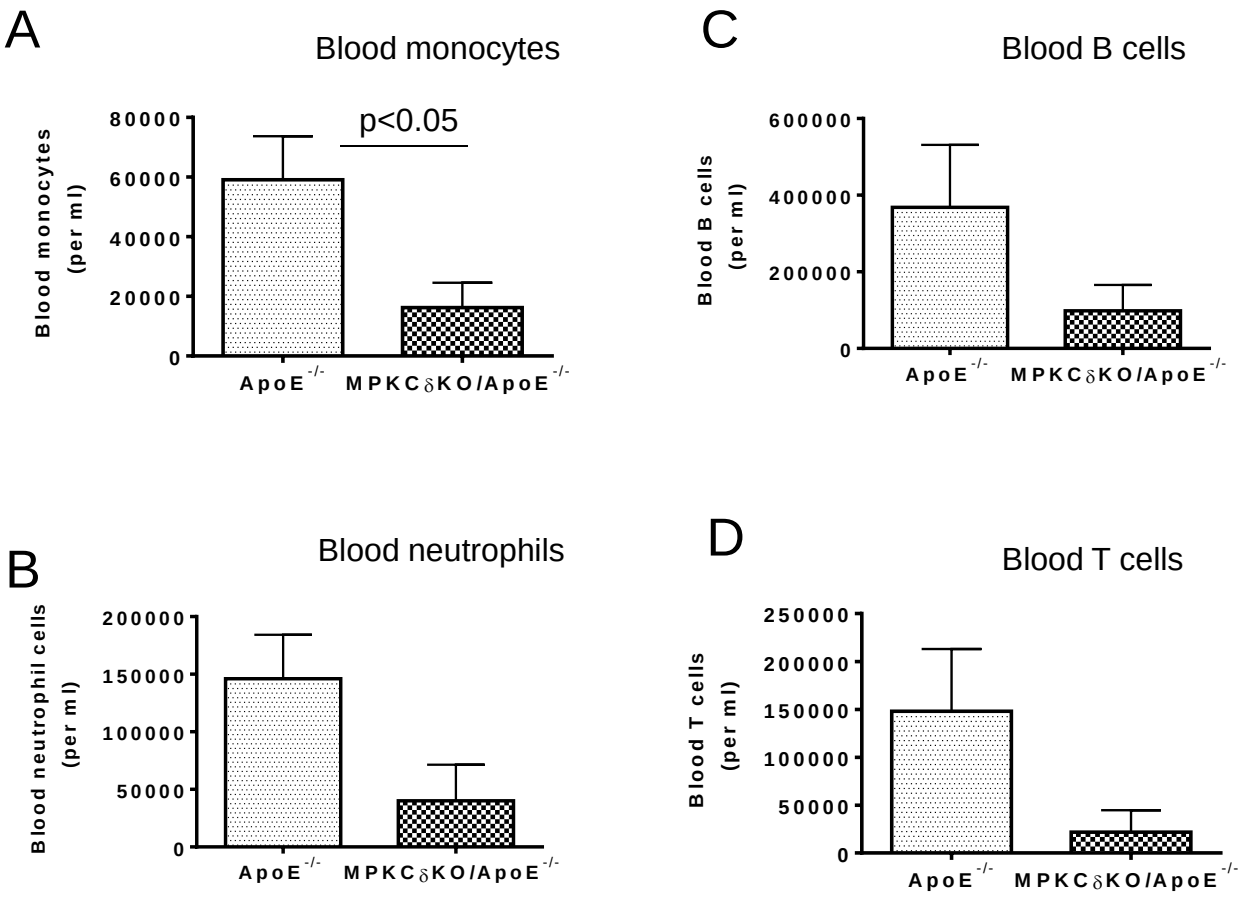




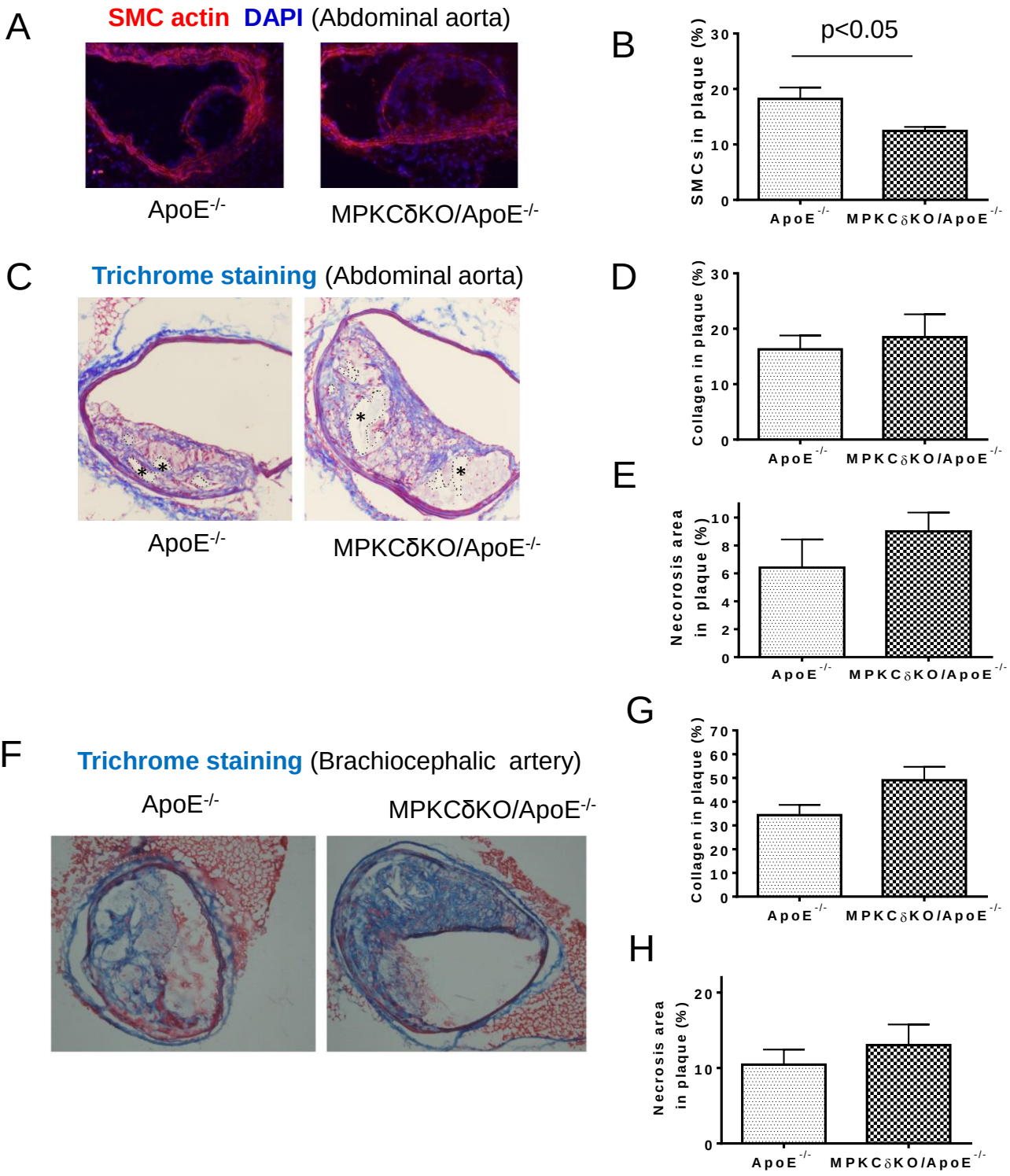
Supplemental Figure VII. Physiological characterization of MPKCδko/ApoE$^{-/-}$ and ApoE$^{-/-}$ mice after 12 weeks of AD feeding. A. Body weight of two groups of mice (ApoE$^{-/-}$ n=18, MPKCδko/ApoE$^{-/-}$ n=21).**B.** Mean blood pressure was measured by tail vein plethysmography (n=7 for each group). **C.** Fasting insulin level (ApoE$^{-/-}$ n=8, MPKCδko/ApoE$^{-/-}$ n=6). **D.** IPGTT (ApoE$^{-/-}$ n=4, MPKCδko/ApoE$^{-/-}$ n=7).



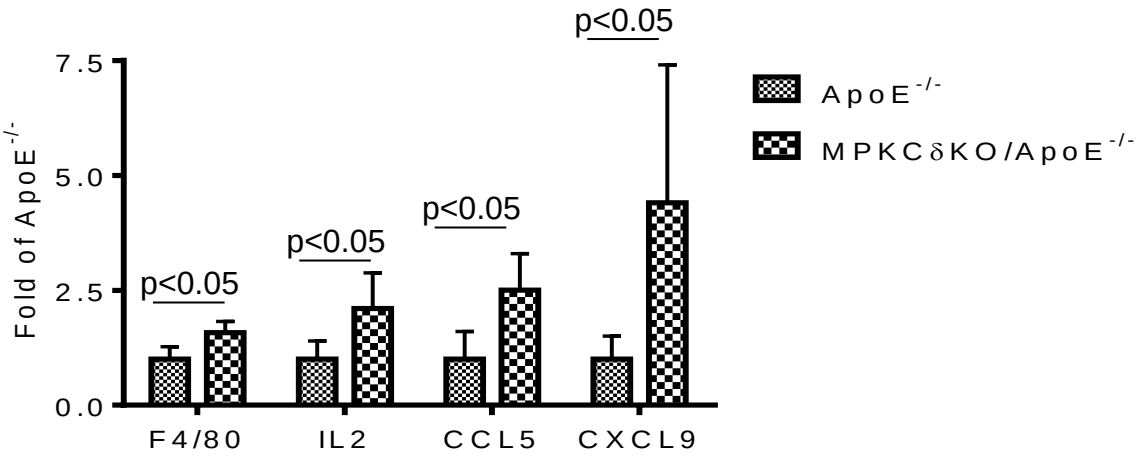
Supplemental Figure VIII. Characterization of plasma lipids in ApoE$^{-/-}$ and MPKCδKO/ApoE$^{-/-}$ mice. A. Fasting plasma cholesterol level after 8 weeks or 12 weeks of AD feeding (AD 8 weeks, n=4 for each group; AD 12 weeks ApoE$^{-/-}$ n=7, MPKCδKO/ApoE$^{-/-}$ n=10, * p<0.05 vs. ApoE$^{-/-}$). **B.** Cholesterol concentration in FPLC fractions of plasma after 8 weeks of AD feeding (n=4 for each group, * p<0.05 vs. ApoE$^{-/-}$). **C.** Cholesterol concentration in FPLC fractions of plasma after 12 weeks of AD feeding (ApoE$^{-/-}$, MPKCδKO/ApoE$^{-/-}$ n=4, *p<0.05 vs. ApoE$^{-/-}$). **D.** Fasting plasma triglyceride level after 8 or 12 weeks of AD feeding (AD 8 weeks, n=4 for each group; AD 12 weeks, ApoE$^{-/-}$ n=7, MPKCδKO/ApoE$^{-/-}$ n=10). **E.** Fasting plasma free fatty acid level after 12 weeks of AD feeding (ApoE$^{-/-}$ n=7, MPKCδKO/ApoE$^{-/-}$ n=8).



Supplemental Figure IX. Circulating leukocytes in mice fed with atherogenic diet. Blood monocytes **(A)**, neutrophils **(B)**, B cells **(C)** and T cells **(D)** in mice fed with AD for 12 weeks were determined by flow cytometry ($ApoE^{-/-}$, n=5; $MPKC\delta KO/ApoE^{-/-}$, n=5).

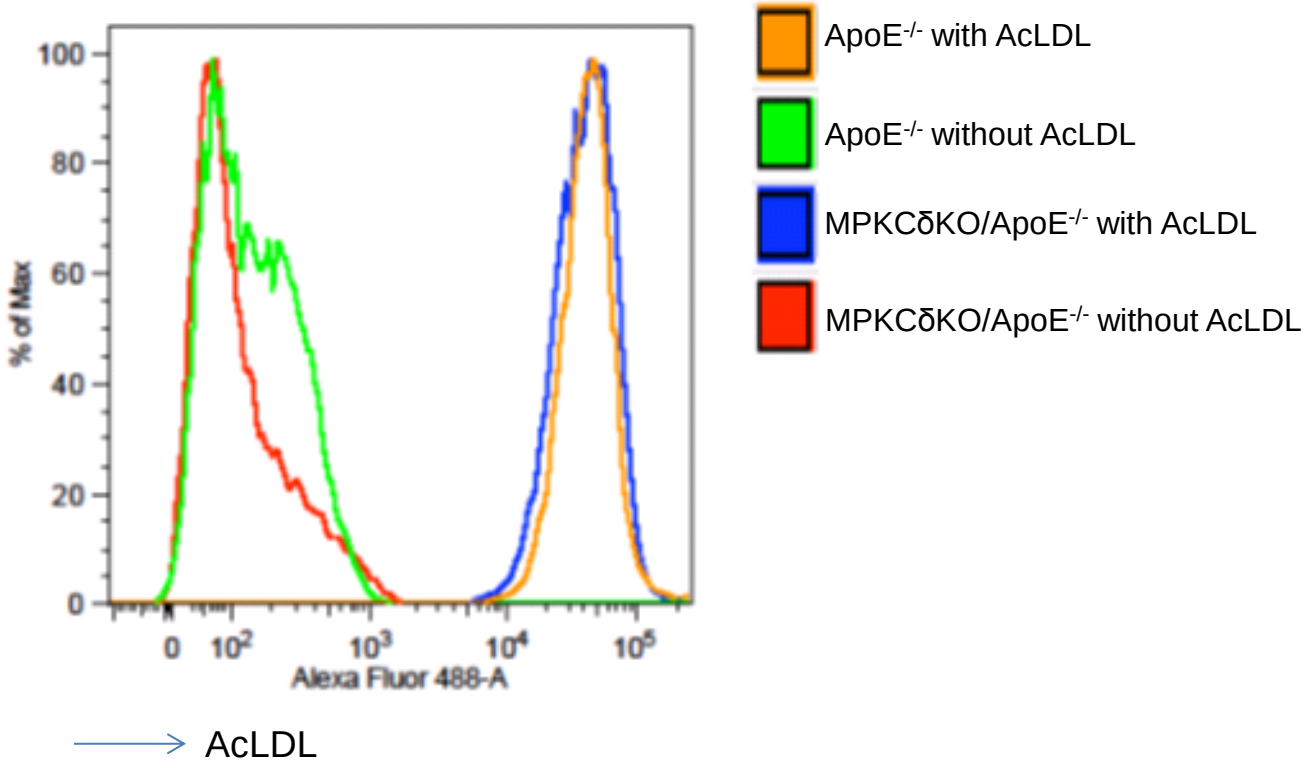


Supplemental Figure X. Complexity of atherosclerotic plaques. A-E, Smooth muscle cells α-actin staining of the abdominal aorta of Apo E$^{-/-}$ mice and MPKCδKO/ApoE$^{-/-}$ mice. **A.** Representative images and **B.** Mean values of quantitative analysis (n=5 for each group). **(C-E).** Collagen staining and necrosis area of the abdominal aorta of ApoE$^{-/-}$ mice and MPKCδKO/ApoE$^{-/-}$ mice. **C.** Representative images. *indicated necrosis area. **D.** Mean values of quantitative analysis of collagen content; **E.** Necrosis area (n=5 for each group). **F-H**, Collagen content and necrosis area in brachiocephalic artery. F, Trichrome staining of brachiocephalic artery. Collagen content (G) and necrosis area (H) in the plaque of brachiocephalic artery (ApoE$^{-/-}$, n=5; MPKCδKO/ApoE$^{-/-}$, n=6)



Supplemental Figure XI. Analysis of the expression of inflammatory cytokine mRNA levels in the aorta. The expression of inflammatory mRNA levels in the aorta of two groups of mice were determined by qRT-PCR and normalized by 36B4 (ApoE$^{-/-}$ n=4, MPKCδKO/ApoE$^{-/-}$ n=6).

A



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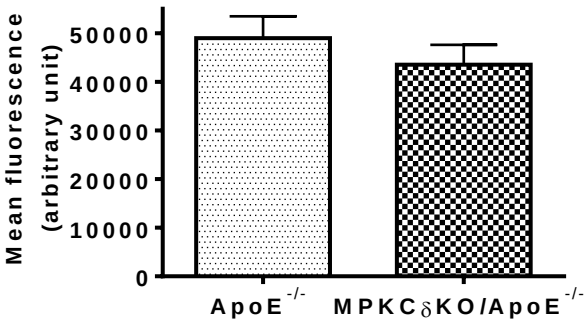
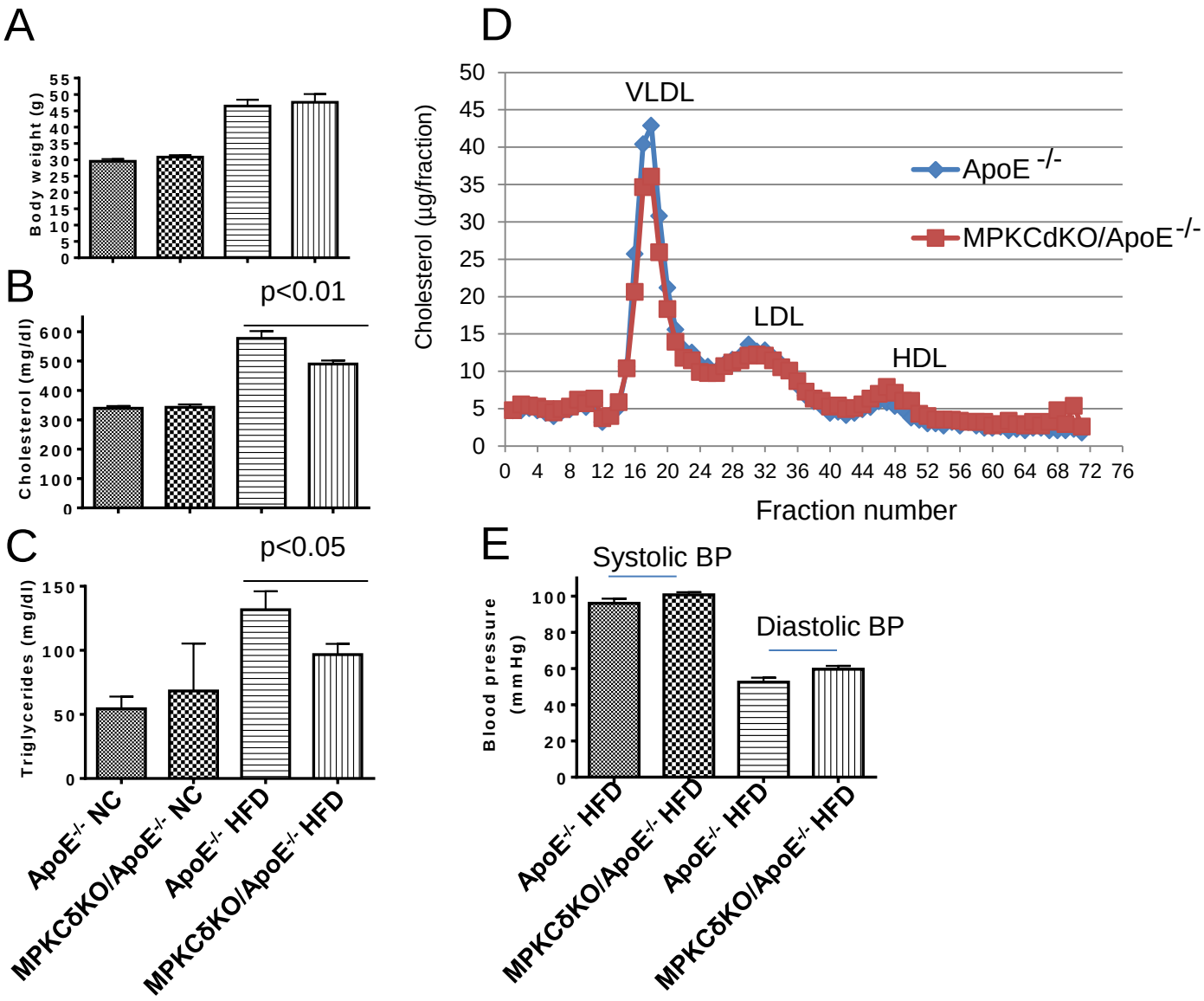
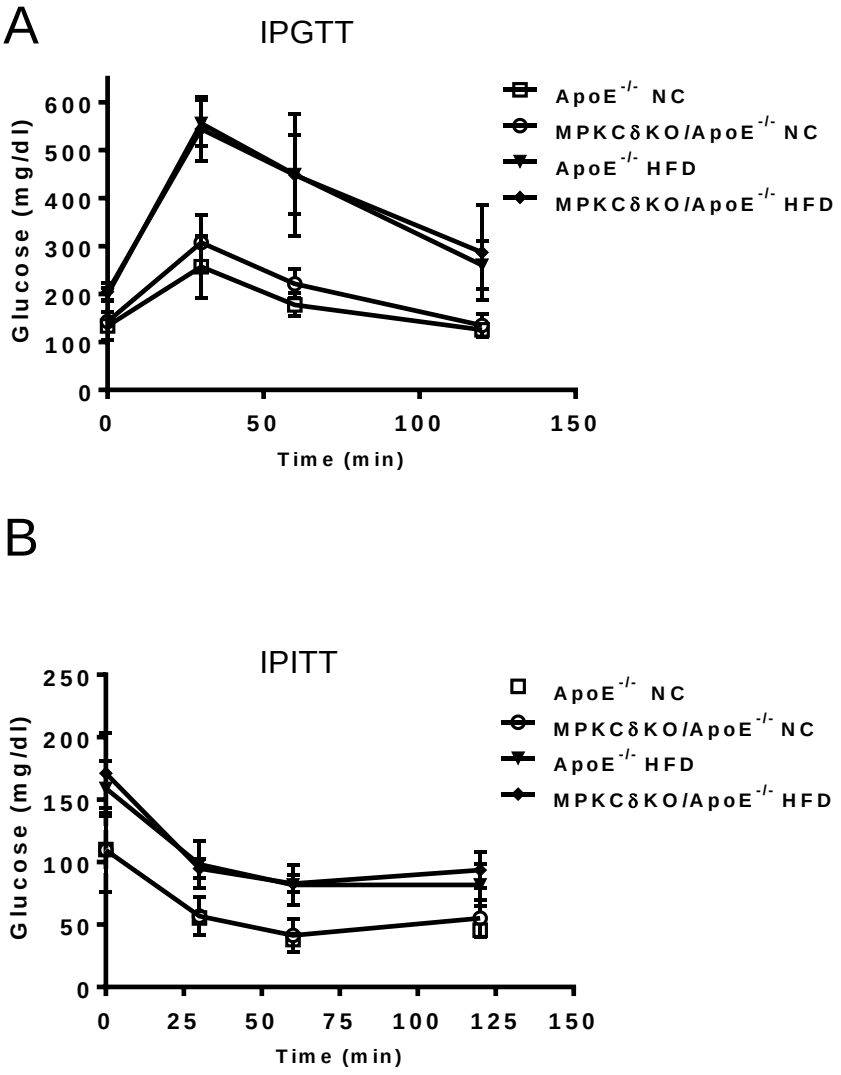


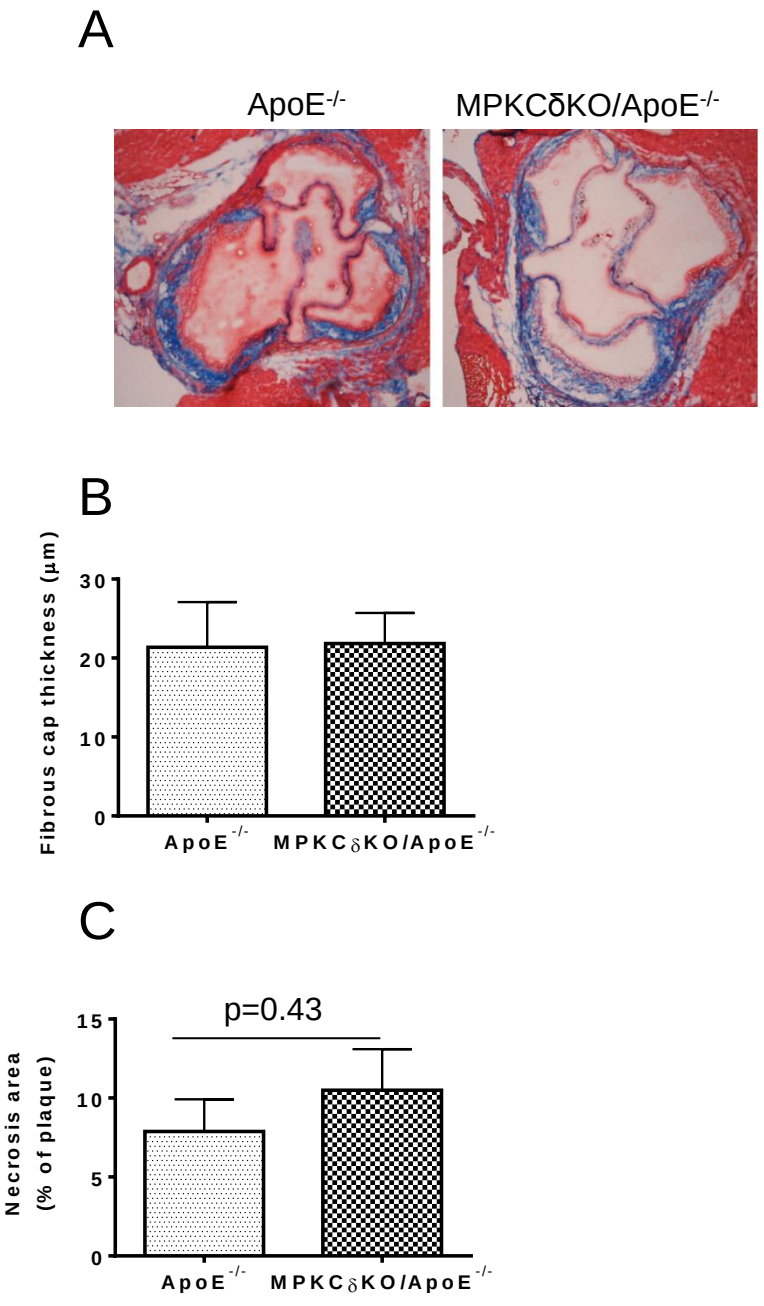
Supplemental Figure XII. Lipid uptake in the macrophages. Peritoneal macrophages were incubated with Alexa488 conjugated AcLDL and the immunofluorescence intensity was determined by flow cytometry (n=4 for each group).



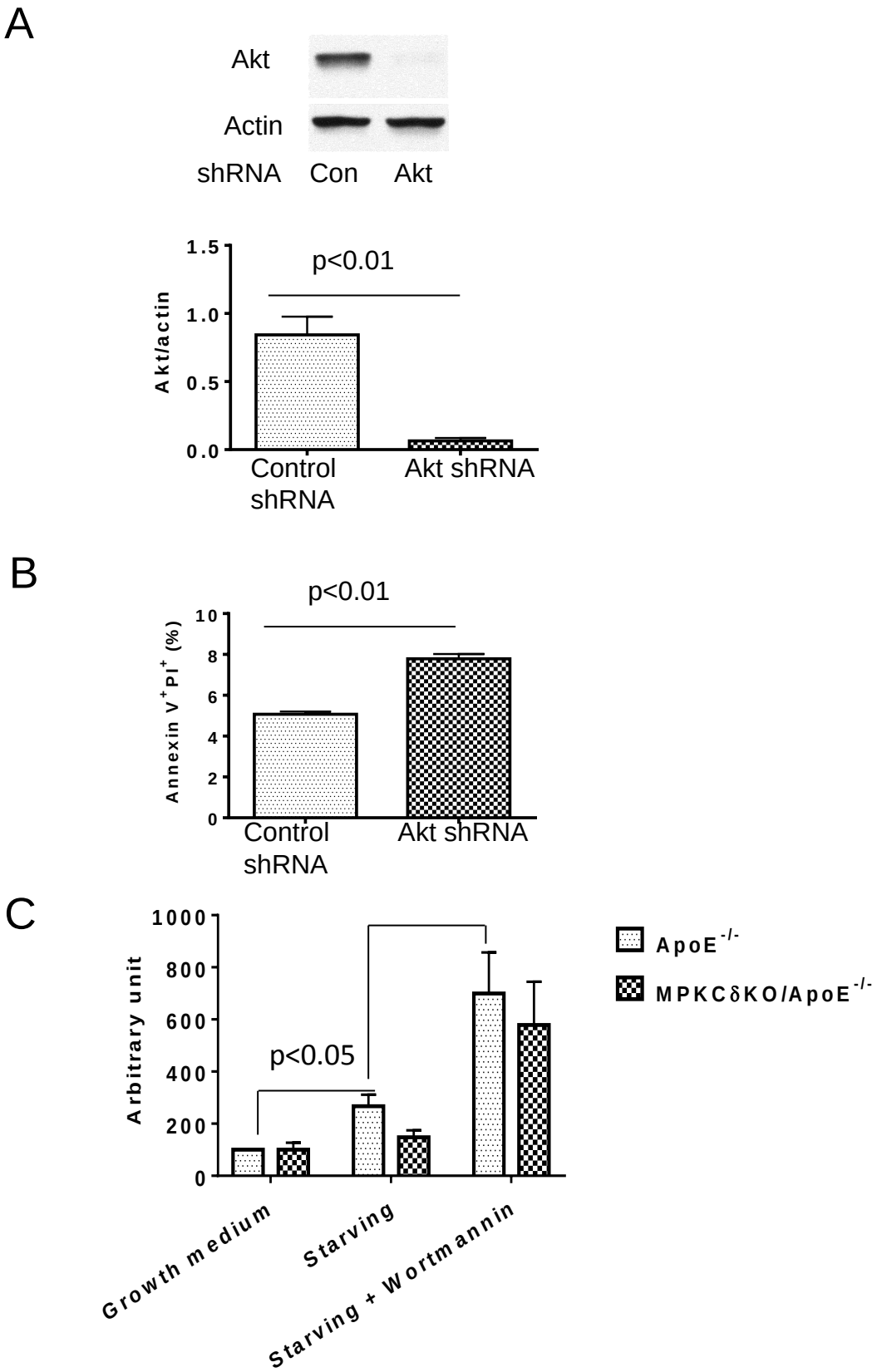
Supplemental Figure XIII. Physiological characterization of MPKCδKO/ApoE$^{-/-}$ mice. **A.** Body weight (ApoE$^{-/-}$ NC n=5, MPKCδKO/ApoE$^{-/-}$ NC n=5, ApoE$^{-/-}$ HFD n=5, MPKCδKO/ApoE$^{-/-}$ HFD n=7). **B.** Fasting plasma cholesterol level. (ApoE$^{-/-}$ NC n=3, MPKCδKO/ApoE$^{-/-}$ NC n=3, ApoE$^{-/-}$ HFD n=5, MPKCδKO/ApoE$^{-/-}$ HFD n=6). **C.** Fasting plasma triglycerides. (ApoE$^{-/-}$ NC n=3, MPKCδKO/ApoE$^{-/-}$ NC n=3, ApoE$^{-/-}$ HFD n=5, MPKCδKO/ApoE$^{-/-}$ HFD n=6). **D.** Cholesterol concentration in FPLC fractions of plasma (n=4 for each group). **E.** Blood pressure was measured by tail vein plethysmography (n=5 for each group). BP (blood pressure).



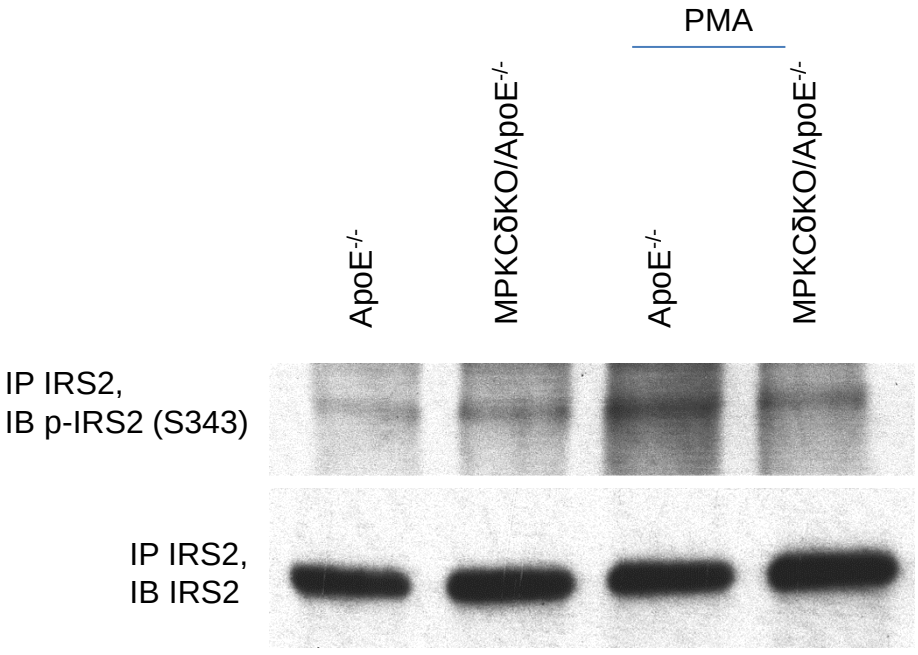
Supplemental Figure XIV. Glucose and insulin tolerance tests. The mice were fasted for 6 hours and then the mice were injected with glucose at 2 g/Kg body weight (**A**, IPGTT) or insulin at 0.75 mIU/Kg body weight (**B**, IPITT). ($ApoE^{-/-}$ NC n=5, $MPKC\delta KO/ApoE^{-/-}$ NC n=5, $ApoE^{-/-}$ HFD n=5, $MPKC\delta KO/ApoE^{-/-}$ HFD n=7).

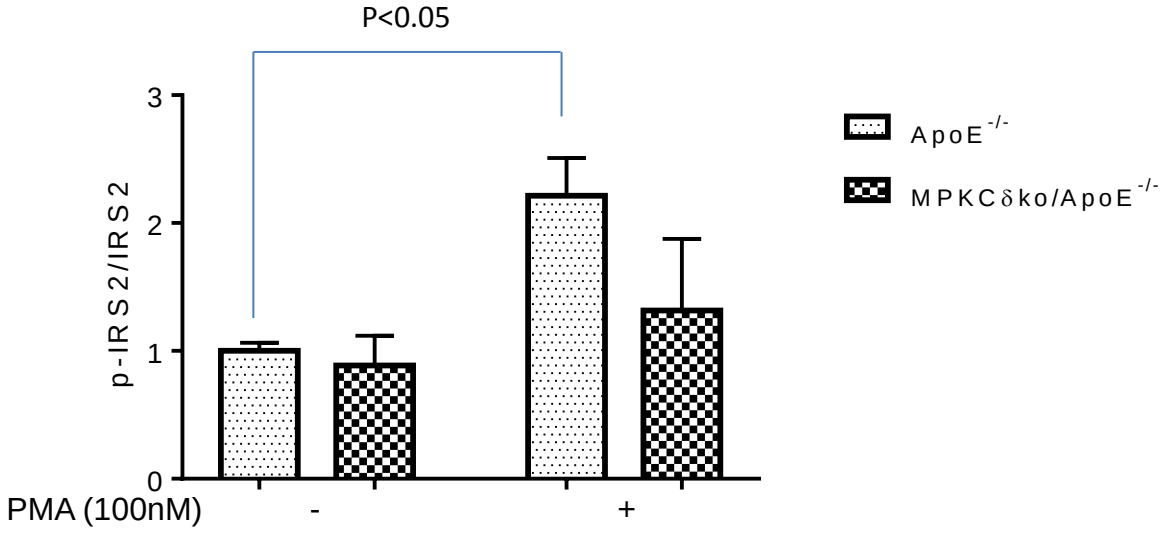


Supplemental Figure XV. Fibrosis cap and necrosis area in the plaque of aorta sinus of two groups of mice. A. Representative images of trichrome staining of aorta sinus. B**.** Fibrosis cap of the plaque in aorta sinus (n=9 per group). C. Necrosis area in the plaque of aorta sinus (n=9 per group).

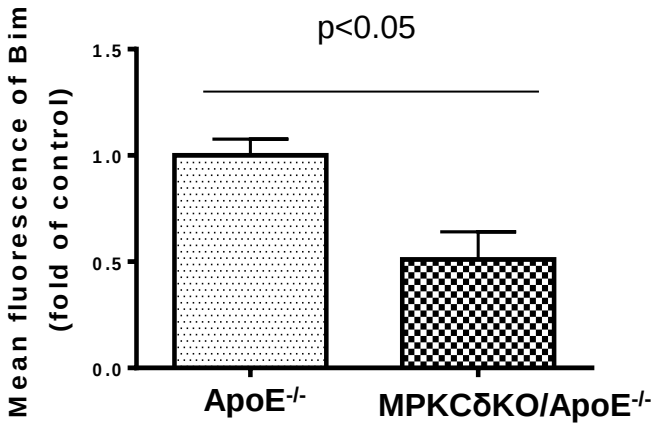


Supplemental Figure XVI. Inhibition of Akt induced macrophage apoptosis. A. Akt was knocked down in macrophage cell line Raw 264.7 cells by lentivirus mediated Akt shRNA transduction. (n=3 per group). **B**. Raw 264.7 cells transduced by control ShRNA or Akt ShRNA was stained with Annexin V and PI. The apoptotic dells were determined by flow cytometry. (n=3 per group). **C**. Bone marrow macrophages apoptosis was induced by withdrawing growth factor or withdrawing growth factor plus wortmannin. (n=4 per group).

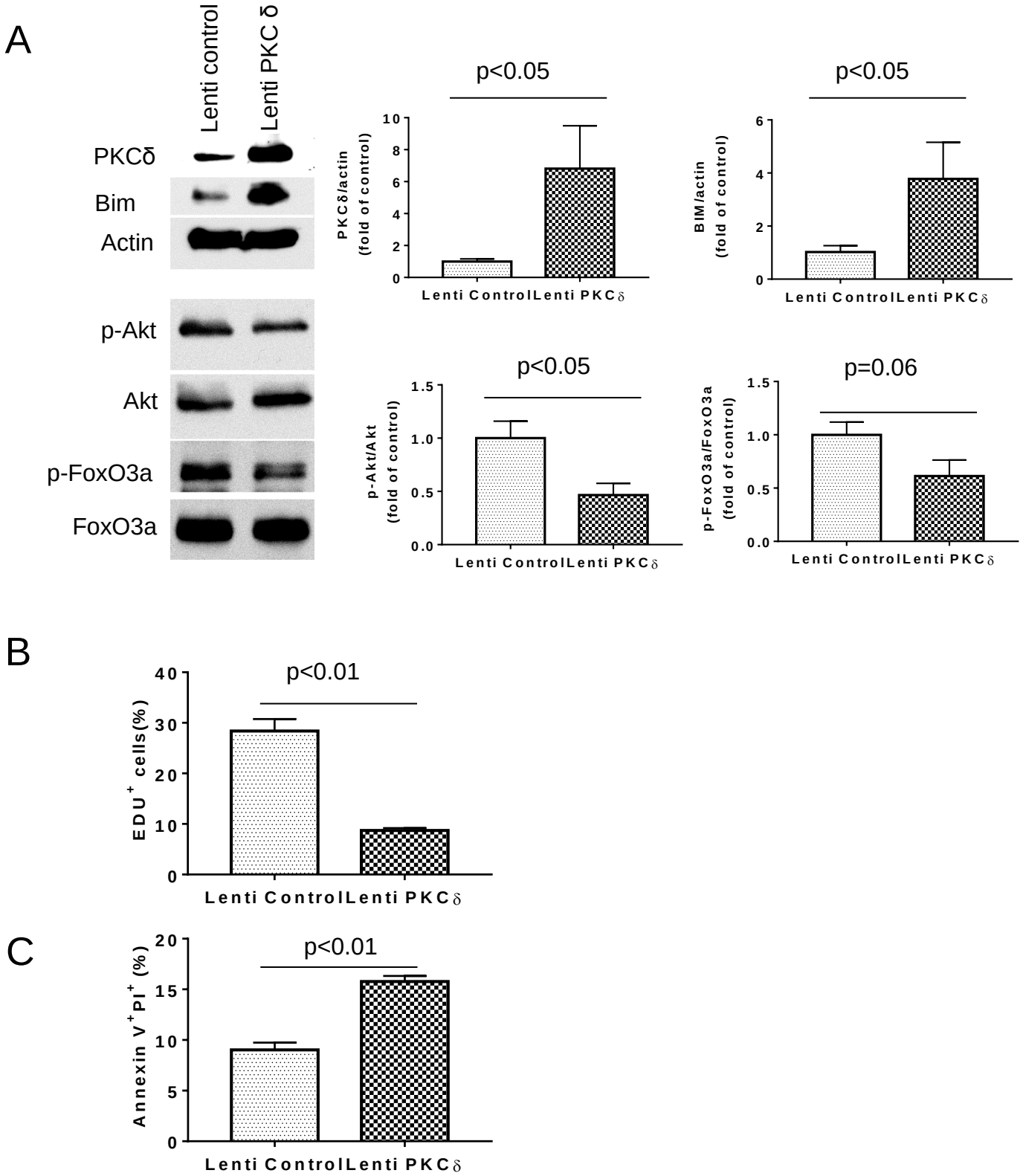




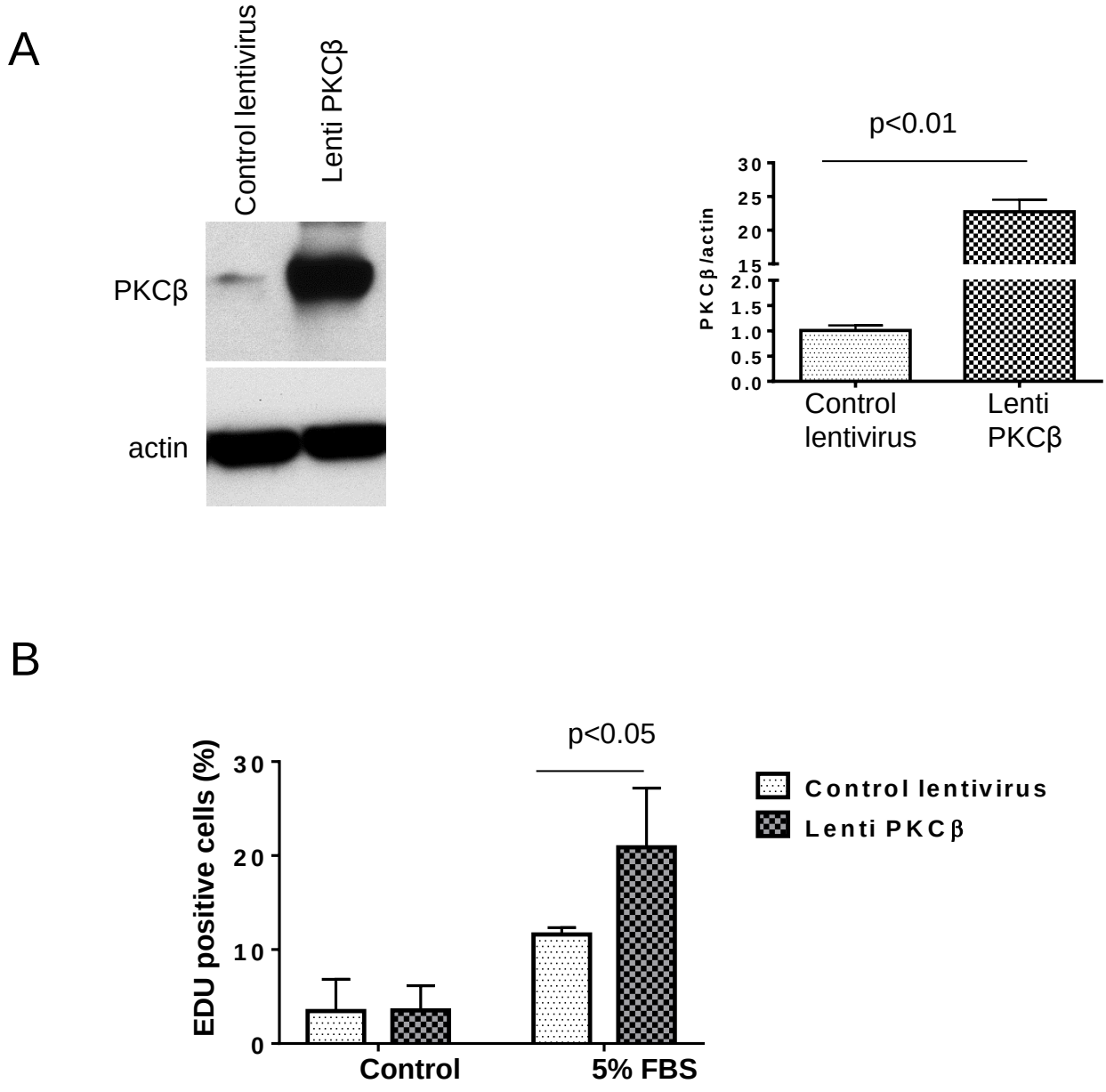
Supplemental Figure XVII. IRS2 phosphorylation. Bone marrow macrophages were stimulated with PMA (100nM) for 10 minutes and IRS2 was immunoprecipitated with anti-IRS2 antibody. IRS2 phosphorylation at Ser343 was determined by Western blotting and normalized by IRS2 (n=4-6 per group).



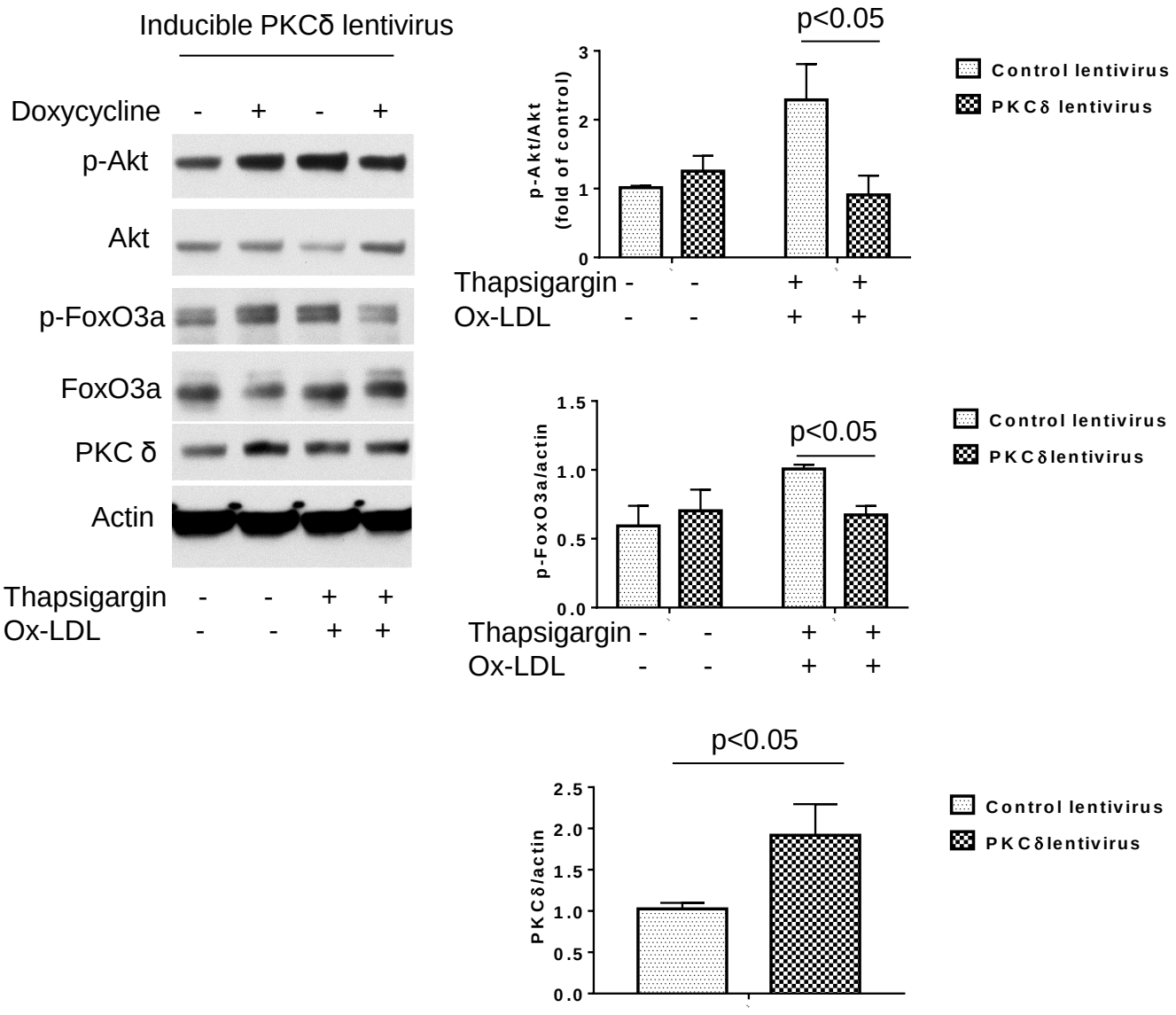
Supplemental Figure XVIII. Bim expression in the blood monocytes . Bim expression in the blood monocytes was determined by flow cytometry (n=4 per group).



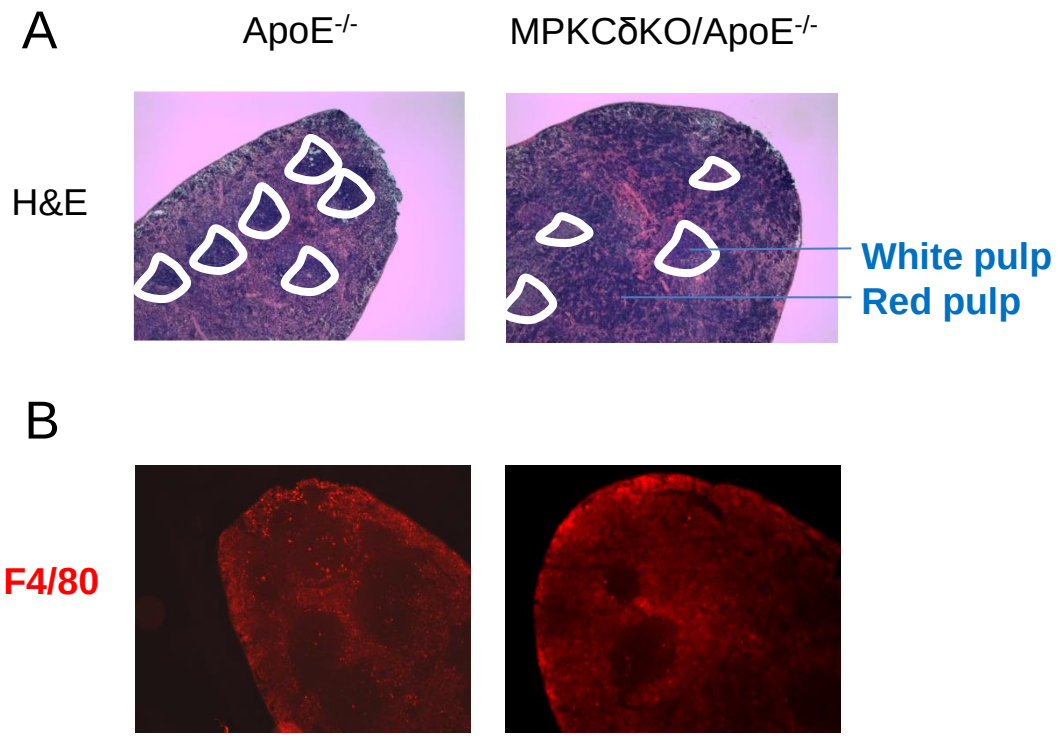
Supplemental Figure XIX. Overexpression of PKCδ in Raw264.7 cells. **A**. PKCδ was overexpressed in Raw 264.7 cells by lentivirus mediated transduction and the expression of PKCδ, Bim, p-Akt/Akt and p-FoxO3a/FoxO3a were determined by western blotting (n=4-6 per group). B. Cells were grown in DMEM containing 5% FBS and proliferation was determined by EDU incorporation. EDU positive cells were measured by flow cytometry (n=3 per group). C. The cells were starved for 24 hours and cell apoptosis was measured by Annexin V and PI double staining. The apoptotic cells were detected by flow cytometry (n=3 per group).



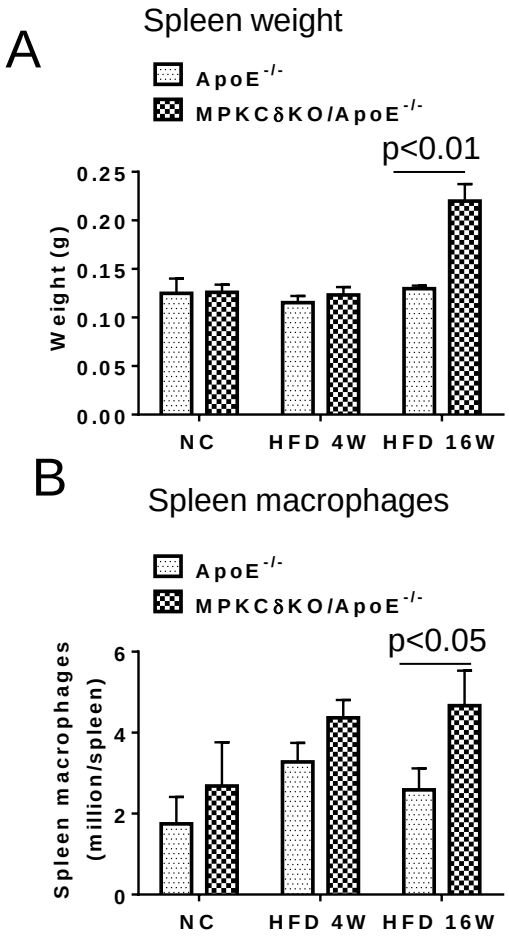
Supplemental Figure XX. Overexpression of PKCβ promoted macrophages proliferation. **A**. PKCβ was overexpressed in Raw 264.7 cells by lentivirus mediated transduction and the expression of PKCβ was determined by western blotting. (n=3-4 per group). B. Cell proliferation in PKCβ transduced Raw 264.7 cells was determined by EDU incorporation. EDU positive cells were measured by flow cytometry (n=4 per group).



Supplemental Figure XXI. Akt and FoxO3a phosphorylation in PKCδ deficient macrophages reconstituted with PKCδ. PKCδ deficient macrophages were transduced with inducible lentivirus which contained PKCδ open reading frames. The expression of PKCδ was induced by doxycycline (100 ng/ml). The cells were treated with thapsigargin (0.25 μM) plus Oxldl (50 μg/ml) for 3 hours. Akt and FoxO3a phosphorylation were determined by western blotting and normalized by actin (n=6 per group).

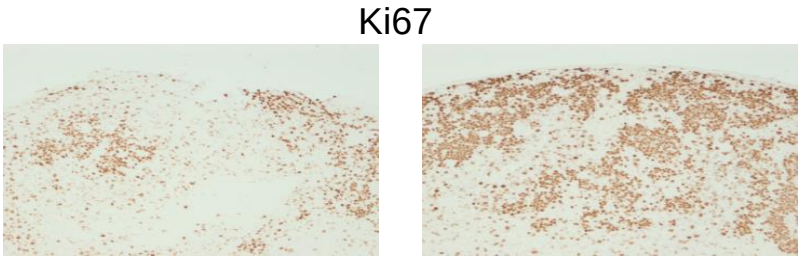


Supplemental Figure XXII. Hematoxylin and eosin (H&E) staining **(A**, white circle indicated white pulp**)** and immunohistologic staining with anti F4/80 antibodies of spleen from ApoE$^{-/-}$ and MPKCδKO/ApoE$^{-/-}$ mice **(B)**.

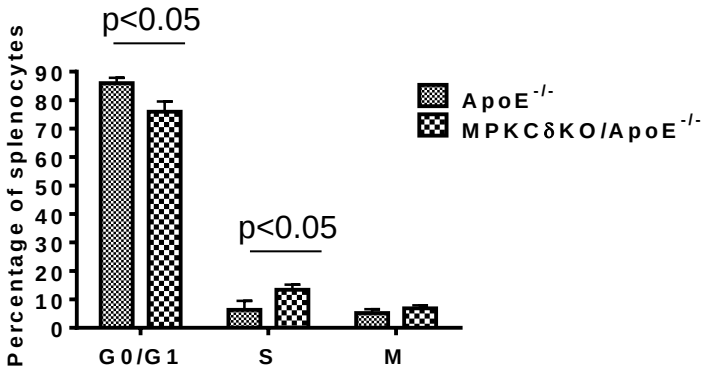


Supplemental Figure XXIII. Analysis of spleen after normal chow (NC) or HFD feeding. A. Spleen weight. ($ApoE^{-/-}$ NC, n=6; $MPKC\delta KO/ApoE^{-/-}$ NC, n=5 ; $ApoE^{-/-}$ HFD 4W, n=7; $MPKC\delta KO/ApoE^{-/-}$ HFD 4W, n=7; $ApoE^{-/-}$ HFD 16W, n=10; $MPKC\delta KO/ApoE^{-/-}$ HFD 16W, n=10). **B.** Macrophage numbers in spleen. The spleen was homogenized into single cells and macrophage numbers were determined by flow cytometry. ($ApoE^{-/-}$ NC, n=6; $MPKC\delta KO/ApoE^{-/-}$ NC, n=5 ; $ApoE^{-/-}$ HFD 4W, n=7; $MPKC\delta KO/ApoE^{-/-}$ HFD 4W, n=7; $ApoE^{-/-}$ HFD 16W, n=7; $MPKC\delta KO/ApoE^{-/-}$ HFD 16W, n=8).

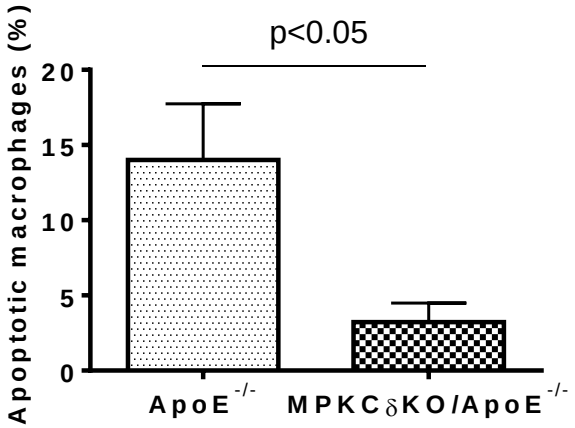
A



B



Supplemental Figure XXIV. Analysis of cell proliferation in the spleen. **A.** Spleen was stained with anti-Ki67 antibody. **B.** Cell cycle analysis. Splenocytes were stained with PI and their distribution in cell cycle were determined by flow cytometry (n=3 per group).



Supplemental Figure XXV. Analysis of macrophage apoptosis in the spleen after HFD feeding. The spleen was homogenized into single cells and apoptotic macrophages (CD45+F4/80+Annexin V+PI+) were determined by flow cytometry (ApoE$^{-/-}$ n=3, MPKCδKO/ApoE$^{-/-}$ n=4).