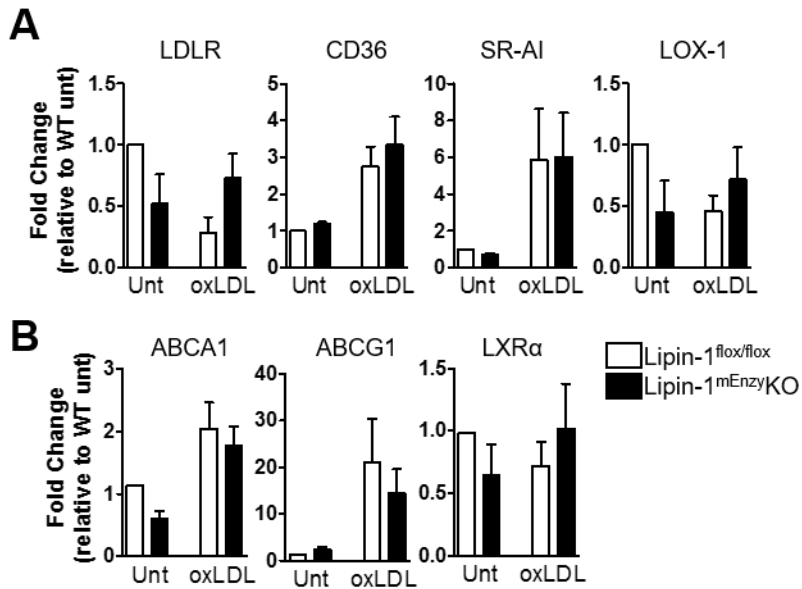
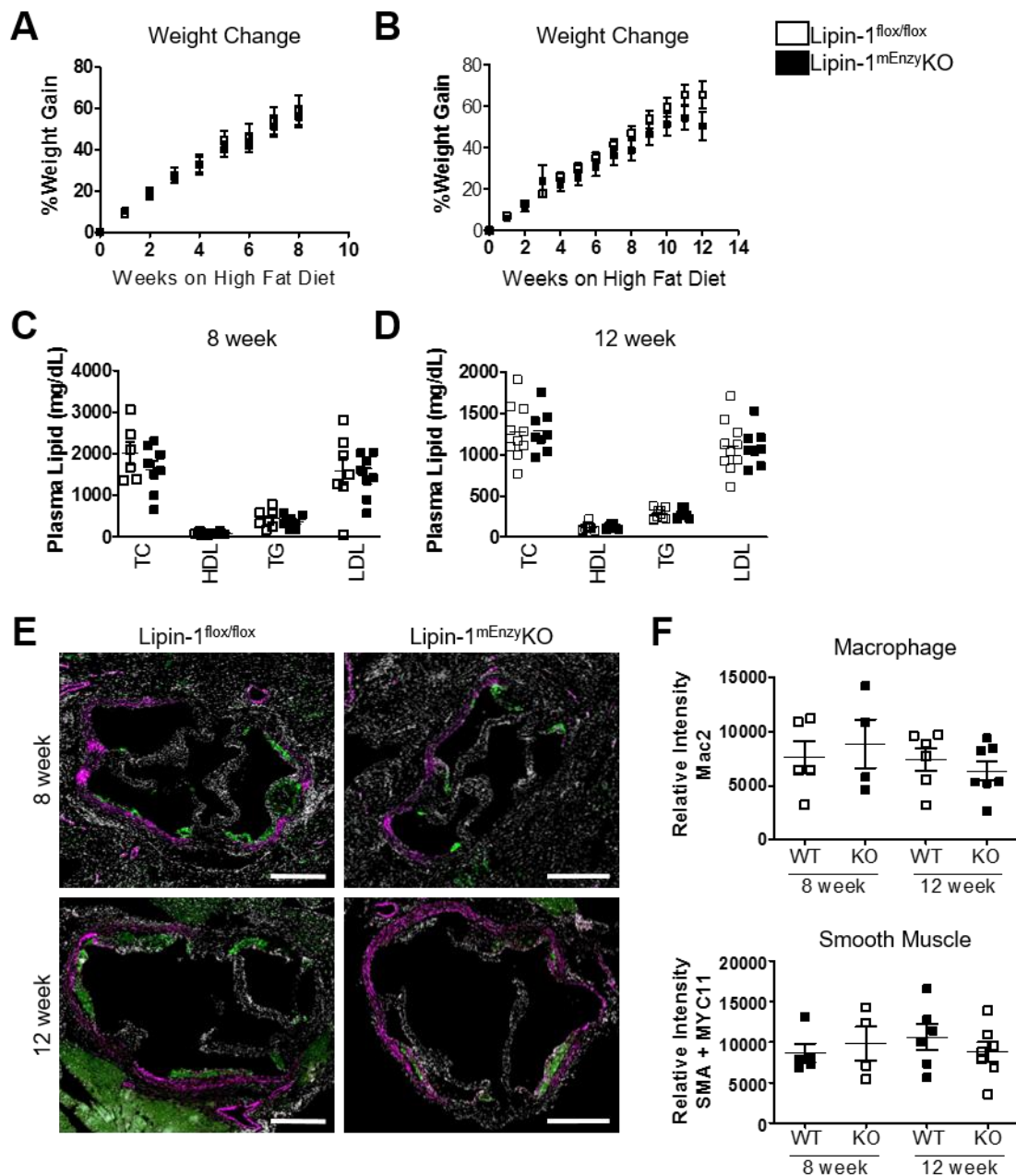


1 SUPPLEMENTAL MATERIAL

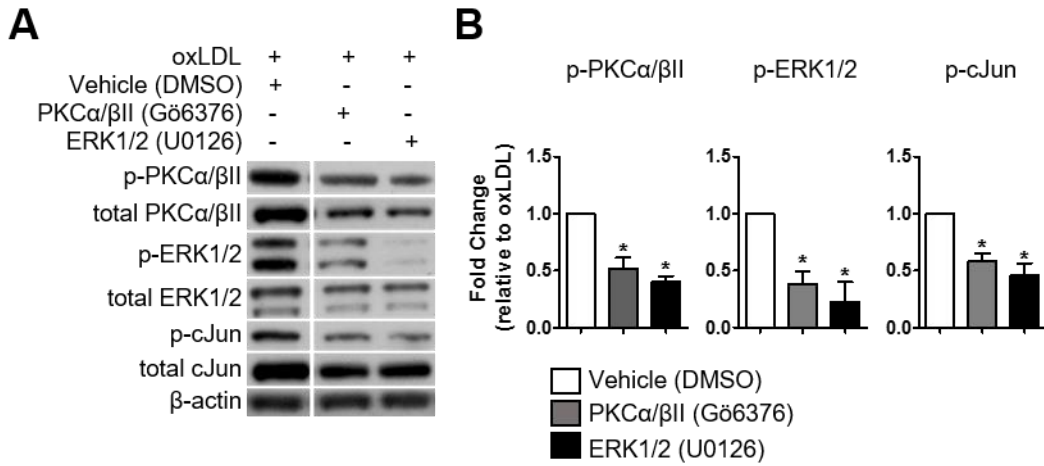


2
3 **Supplemental Figure I. Scavenger receptor and PPAR γ -regulated gene expression is not statistically**
4 **different between lipin-1^{mEnzyKO} and lipin-1^{flox/flox} control mice.** Lipin-1^{mEnzyKO} and lipin-1^{flox/flox} BMDMs
5 were either stimulated with 25 μ g/mL of oxLDL or left untreated. RNA was collected at 24 hours after
6 stimulation for qRT-PCR analysis. A) Graphical representation of scavenger receptor expression by fold
7 change analysis of oxLDL stimulated samples over untreated (0 hour) samples. Abbreviations: Low-density
8 lipoprotein receptor (LDLR), Cluster of Differentiation 36 (CD36), Scavenger receptor type A member I (SR-
9 AI), Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1). B) Graphical representation of PPAR γ -
10 regulated gene expression by fold change analysis of oxLDL stimulated samples over untreated (0 hour)
11 samples. Abbreviations: ATP-binding cassette transporter sub-family A member 1 (ABCA1), ATP-binding
12 cassette sub-family G member 1 (ABCG1), liver X receptor alpha (LXR α).

13



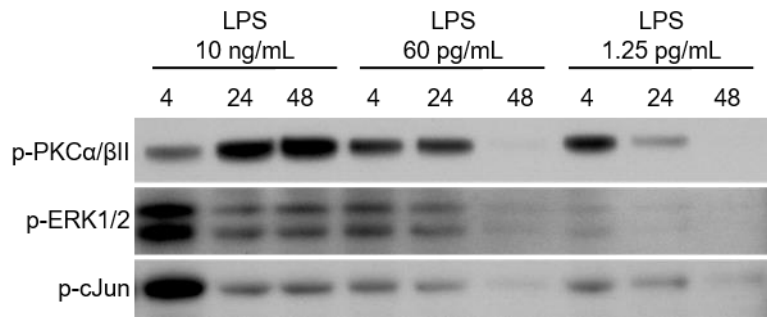
Supplemental Figure II. Loss of myeloid-associated lipin-1 reduces atherosclerosis. Lipin-1^{flox/flox} (n=18) and lipin-1^{mEnzyKO} (n=16) mice were injected with 3×10^{10} vector genomes of AAV8-PCSK9 and placed on high fat diet for 8 or 12 weeks. Graphs represent quantification by Image J analysis (A-D). A-B) Percent weight change over 8 weeks (A) and 12 weeks (B) on high fat diet of both the lipin-1^{flox/flox} and lipin-1^{mEnzyKO} mice. C-D) Plasma lipid levels in lipin-1^{flox/flox} and lipin-1^{mEnzyKO} mice at time of euthanasia. TC = total cholesterol, HDL = high-density lipoprotein, TG = triglycerides, LDL = low-density lipoprotein. E) Representative immunofluorescence images of aortic roots of lipin-1^{flox/flox} and lipin-1^{mEnzyKO} after 8 or 12 weeks of high fat diet. Staining included nuclei (DAPI = white), macrophages (Mac2 = green), smooth muscle actin (SMA = red), and smooth muscle myosin heavy chain 11 (MYC11 = blue). Scale bar = 200 μ m. F) Quantification of macrophage (Mac2) and smooth muscle (SMA + MYC11) stain in aortic roots.



27

28 **Supplemental Figure III. OxLDL stimulation of BMDMs induces activation of linked PKC α / β II-ERK1/2-**
 29 **cJun signaling cascade.** A-B) WT BMDMs were stimulated with 25 μ g/mL of oxLDL for 24 hours to induce
 30 foam cell formation prior to inhibitor addition. Inhibitors were added: 1 μ M of the PKC α / β II inhibitor Gö6376
 31 or 10 μ M of the MEK1/2 (ERK1/2) inhibitor U0126. Total cellular protein was collected at 48 hours after
 32 initial stimulation for Western blot analysis. A) Representative Western blot images of phosphorylated and
 33 total PKC α / β II, ERK1/2, and cJun. B) Graphs represent fold change analysis of p-PKC α / β II, p-ERK1/2, and
 34 p-cJun over vehicle (oxLDL only) control samples as quantified by Image J analysis. (Mean \pm SEM, n= 3-5,
 35 *p \leq 0.05).

36



37
 38 **Supplemental Figure IV. Stimulation of BMDMs with varying concentrations of LPS does not induce**
 39 **persistent activation of the PKCα/βII-ERK1/2-cJun signaling cascade.** WT BMDMs were stimulated with
 40 either 10 ng/mL (amount of LPS used in Figure 5), 60 pg/mL (50 times the maximum endotoxin present in the
 41 modLDL preparations), or 1.25 pg/mL (maximum calculated endotoxin present in the modLDL preparations) of
 42 LPS. Total cellular protein was collected at 4, 24, and 48 hours after stimulation for Western blot analysis.
 43 Representative Western blot images of phosphorylated PKCα/βII, ERK1/2, and cJun.

44

45

46 **SUPPLEMENTAL TABLE I: Quantitative Real-Time PCR Primers**

47

Gene	Forward	Reverse
Rpl13a	5' – TCC ACC CTA TGA CAA GAA – 3'	5' – GTA AGC AAA CTT TCT GGT AG – 3'
LDLR	5' – TGA CTC AGA CGA ACA AGG CTG – 3'	5' – ATC TAG GCA ATC TCG GTC TCC – 3'
CD36	5' – ATG GGC TGT GAT CGG AAC TG – 3'	5' – GTC TTC CCA ATA AGC ATG TCT CC - 3'
SR-AI	5' – AAC TGA CCA AAG ACT TAA TG – 3'	5' – CTG TAG ATT CAC GGA CTC - 3'
LOX-1	5' – AGG GGC GTT TCT TTA CAG – 3'	5' – GCA GTT TTC AGC GAA CAC – 3'
ABCA1	5' – AAA ACC GCA GAC ATC CTT CAG – 3'	5' – CAT ACC GAA ACT GCT TCA CCC – 3'
ABCG1	5' – CTT TCC TAC TCT GTA CCC GAG – 3'	5' – CGG GGC ATT CCA TTG ATA AGG – 3'
LXR α	5' – CAG TGC GTG CTC TCT GAG G – 3'	5' – GGT CGG AGA AAG ATC GTT TGT T – 3'