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LPCAT3 deficiency in hematopoietic cells alters cholesterol and phospholipid homeostasis and promotes atherosclerosis

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ABSTRACT

Background and aims: LPCAT3 plays a major role in phospholipid metabolism in the liver and intestine. However, the impact of LPCAT3 on hematopoietic cell and macrophage functions has yet to be described. Our aim was to understand the functions of LPCAT3 in macrophages and to investigate whether LPCAT3 deficiency in hematopoietic cells may affect atherosclerosis development.

Methods: Mice with constitutive Lpcat3 deficiency (*Lpcat3*$^{-/-}$) were generated. We used fetal hematopoietic liver cells to generate WT and *Lpcat3*$^{-/-}$ macrophages *in vitro* and to perform hematopoietic cell transplantation in recipient *Ldlr*$^{-/-}$ mice.

Results: *Lpcat3*-deficient macrophages displayed major reductions in the arachidonate content of phosphatidylcholines, phosphatidylethanolamines and, unexpectedly, plasmalogens. These changes were associated with altered cholesterol homeostasis, including an increase in the ratio of free to esterified cholesterol and a reduction in cholesterol efflux in *Lpcat3*$^{-/-}$ macrophages. This correlated with the inhibition of some LXR-regulated pathways, related to altered cellular availability of the arachidonic acid. Indeed, LPCAT3 deficiency was associated with decreased *Abca1*, *Abcg1* and *ApoE* mRNA levels in fetal liver cells derived macrophages. *In vivo*, these changes translated into a significant increase in atherosclerotic lesions in *Ldlr*$^{-/-}$ mice with hematopoietic LPCAT3 deficiency.

Conclusions: This study identifies LPCAT3 as a key factor in the control of phospholipid homeostasis and arachidonate availability in myeloid cells and underlines a new role for LPCAT3 in plasmalogen metabolism. Moreover, our work strengthens the link between phospholipid and sterol metabolism in hematopoietic cells, with significant consequences on nuclear receptor-regulated pathways and atherosclerosis development.

1. Introduction

While *de novo* synthesis of phospholipids (PLs) is thought to occur via the Kennedy pathway, PLs are continuously remodeled in cells through deacylation and reacylation reactions in a process called the Lands cycle [1]. In this metabolic pathway, the turnover of fatty acids at the sn-2 position is mediated by the opposite actions of phospholipases A2 and lyso-PL acyltransferases (LPLAT), as well as CoA synthetases. The different LPLATs differ in their tissue distribution and in their substrate preferences for both acyl donors

Abbreviations: LPCAT3, lysophosphatidylcholine acyltransferase 3; PUFA, polyunsaturated fatty acids; FA, fatty acids; LDLR, low density lipoprotein receptor; ABC, ATP binding cassette transporter; PCs, phosphatidylcholines; PEs, phosphatidylethanolamines; LPCs, lysophosphatidylcholines; pPE, plasmalogen phosphatidylethanolamine; CE, Cholesteryl ester; TGs, Triglycerides; WT, wild type; WTD, western-type diet; AA, arachidonic acid; LPLATs, lyso-PL acyltransferases; GWAS, genome-wide association study.

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and acceptors. Their combined action is therefore critical to maintain fatty acid homeostasis within the PLs in different tissues and cells [1–3]. LPLATs affect both the PUFA content of phospholipids and the availability of free fatty acids such as arachidonic acid (AA) used for eicosanoid synthesis. We have recently demonstrated that lysophosphatidylcholine acyl transferase 3 (LPCAT3), a member of the LPLAT family, is regulated by liver X receptors (LXRs) in human macrophages and acts as a key determinant of AA availability for eicosanoid synthesis [4]. Indeed, the treatment of primary human macrophages with LXR agonists results in the enrichment of PLs (mainly phosphatidylcholines) with AA and increased eicosanoid secretion in an LPCAT3-dependent manner [4]. LPCAT3 is highly expressed in the intestine and liver and also shows moderate but significant levels of expression in macrophages [4–6]. *In vitro* and *in vivo* characterization studies have revealed that LPCAT3 uses polyunsaturated fatty acids (PUFAs) (mainly AA) and lysophosphatidylcholines as preferential substrates [5]. A recent GWAS found a significant correlation between a genetic variation at the LPCAT3 locus and the fatty acid composition of red blood cells, thus demonstrating that LPCAT3 is a determinant of PL composition *in vivo* in humans [7]. *Lpcat3*$^{-/-}$ mice present neonatal mortality due to major intestinal malabsorption of dietary lipids [5,8–10]. Moreover, LPCAT3 deficiency in the liver results in a significant decrease in VLDL secretion with impaired ability of LXR agonists to stimulate VLDL secretion [5]. It appeared that AA depletion alters the properties of biological membranes, notably their ability to handle neutral lipids such as triglycerides (TGs) [5,6]. LPCAT3 therefore appears to be a molecular tool regulated by nuclear receptors such as LXRs and peroxisomes proliferator-activated receptors (PPARs) that allows the dynamic modulation of cell membrane composition and properties in response to metabolic changes [4,11,12]. While initial studies in *Lpcat3*$^{-/-}$ mice were mainly focused on the organs expressing highest LPCAT3 levels, *i.e.* the liver and the intestine, the impact of LPCAT3 deficiency on the properties and functions of immune cells, in particular the macrophages, has yet to be described.

To decipher the functions of LPCAT3 in macrophages and hematopoietic cells *in vivo* and *in vitro,* we generated germline *Lpcat3* knock-out mice. We showed not only that LPCAT3 in macrophages is required for glycerophospholipid homeostasis but also that it had a specific and previously unrecognized impact on plasmalogens. Additionally, *Lpcat3* deficiency in macrophages and hematopoietic cells alters LXR-dependent pathways, cholesterol homeostasis and inhibits cholesterol efflux. Finally, using hematopoietic cell transplantation, we showed that *Lpcat3* deficiency in hematopoietic cells increases atherosclerosis development in recipient *Ldlr* $^{-/-}$ mice.

2. Materials and methods

A detailed Materials and methods section is available in the Supplementary data.

2.1. Generation of Lpcat3-deficient mice

Lpcat3 mutant ES cell clones from C57BL/6 N mice (JM8A1.N3) were purchased from the European Conditional Mouse Mutagenesis Program (EUCOMM). *Lpcat3*$^{-/-}$ mice were generated at the Institut Clinique de la Souris (Strasbourg, France).

2.2. Fetal-liver-cell-derived macrophages

WT and *Lpcat3*$^{-/-}$ fetuses were generated by crossbreeding *Lpcat3*$^{+/-}$ mice. At 12–14 days of gestation, pregnant females were euthanized and the fetuses were collected. Fetal livers were then isolated to generate macrophages, and the tail of each fetus was removed for genotyping. Livers were placed in RPMI and dissociated by gentle pipetting. According to genotype, fetal liver cells were plated at a concentration of 0.3×10^6 cells per ml and were cultured in complete medium (RPMI supplemented with 10% FBS and 1% penicillin/streptomycin) in the presence of MCSF at 20 ng/ml. The medium was changed after 3 days and non-adherent cells were removed. Mature macrophages, adherent to the dishes, were harvested after 7 days.

2.3. Hematopoietic cell transplantation

Eight-week-old *Ldlr*$^{-/-}$ mice were lethally irradiated with 1000 rads (11 Gy) before transplantation. Recipient mice were injected with 2×10^6 fetal liver cells through the tail vein.

3. Results

3.1. LPCAT3 is required for glycerophospholipid homeostasis in macrophages in vitro

We generated germline *Lpcat3*-deficient mice from embryonic stem cells (Fig. 1A and B). As previously reported, while *Lpcat3*$^{-/-}$ and *Lpcat3*$^{+/-}$ mice were born at the expected frequency, *Lpcat3*$^{-/-}$ mice did not survive beyond one day [5,8]. Therefore, we used hematopoietic fetal liver cells collected from day 14 embryos to generate WT and *Lpcat3*$^{-/-}$ macrophages. As shown in Fig. 1C, *Lpcat3*$^{-/-}$ macrophages did not display significant levels of *Lpcat3* expression at the mRNA level. To characterize the impact of *Lpcat3* deficiency on macrophages, we first performed an extensive analysis of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) species. As already observed in the intestine and the liver, we found that *Lpcat3* deficiency selectively decreased the proportion of arachidonate containing PE and PCs, such as 36:4 and 38:4 PCs as well as 36:4, 38:4 and 38:5 PEs (approx. 50% relative decrease) (Fig. 1D and E). This was compensated by an increase in PE species containing 22:4 fatty acid, such as 40:4 PEs. Total amounts of PCs, LPCs and PEs did not differ between WT and *Lpcat3*$^{-/-}$ macrophages. Unexpectedly, we observed a major impact of LPCAT3 on plasmalogen homeostasis thus uncovering a previously unrecognized function for LPCAT3. As shown in Fig. 1D and E, plasmenylethanolamines display a dramatically reduced AA and EPA content (60% decreases in their relative and absolute abundance), thus underlining that LPCAT3 specificity extends beyond AA to its omega 3 counterpart EPA. As observed for PCs and PEs, this was compensated by an increase in 22:4-containing molecules, consequently the total amounts of plasmalogens were similar between the two genotypes (WT: 4.14 ± 1.45 nmol/mg prot; *Lpcat3*$^{-/-}$: 4.76 ± 0.90 nmol/mg prot). The LPLATs responsible for the incorporation of adrenic acid into PLs remained to be identified, but public databases (Immgen) indicate that several other LPLATS are expressed at significant levels in mouse macrophages (Supplementary Fig. 1).

3.2. Lpcat3$^{-/-}$ macrophages display altered cholesterol homeostasis

Cholesterol homeostasis is tightly related to the lipid composition of cell membranes, in particular to their glycerophospholipid content; therefore, we analyzed the cholesterol composition of macrophages differentiated from WT or *Lpcat3*$^{-/-}$ hematopoietic fetal liver cells. As shown in Fig. 2A, we observed a non-significant trend towards an increased cholesterol content in *Lpcat3*$^{-/-}$ macrophages in basal conditions and after incubation with acetylated LDLs (ac-LDLs) as compared with WT macrophages. Interestingly the ratio of free to esterified cholesterol was increased (approx. 50% increase, $p < 0.01$) in *Lpcat3*$^{-/-}$ macrophages under control or Ac-

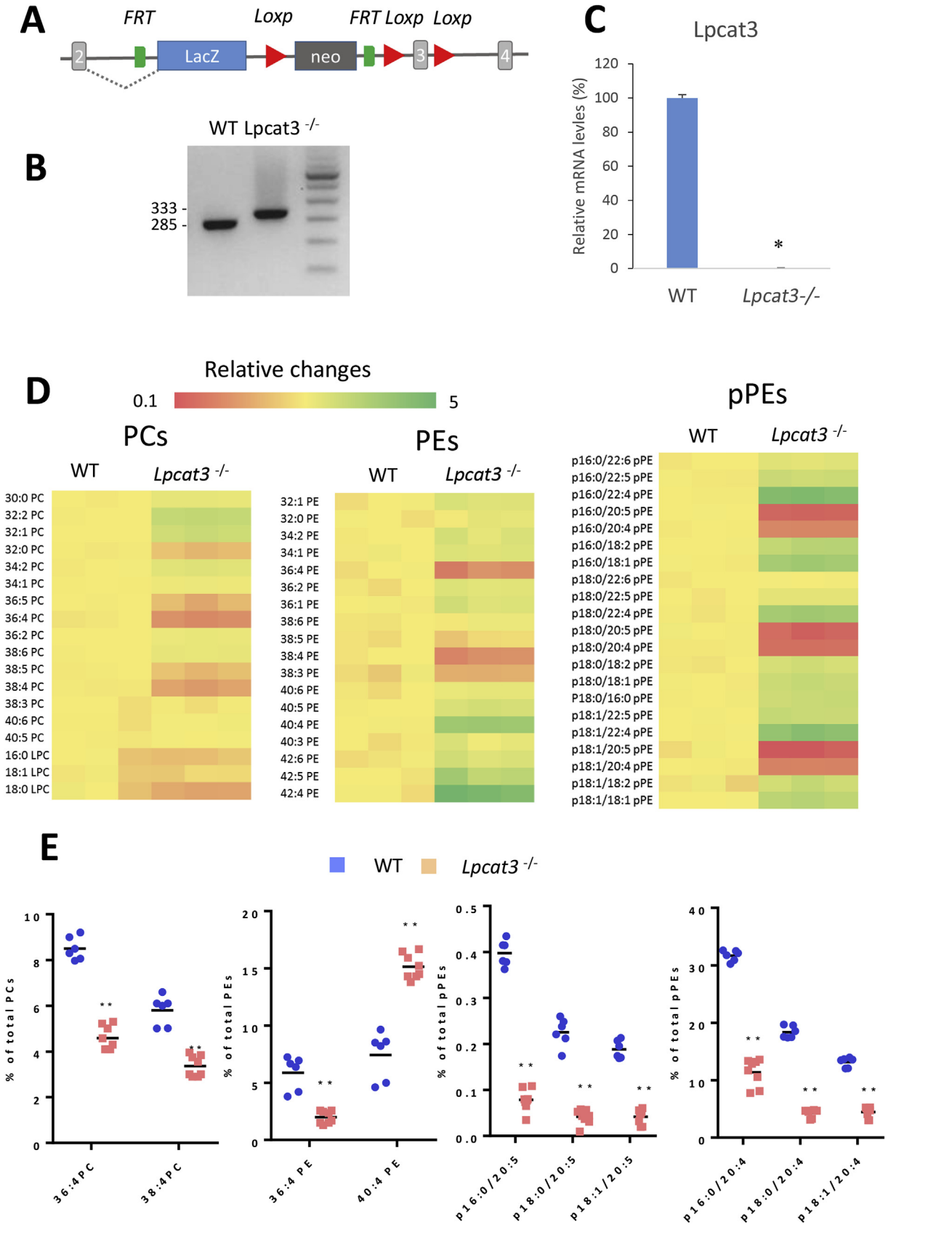

Fig. 1. Generation of *Lpcat3*$^{-/-}$ mice and lipidomic characterization of *Lpcat3*$^{-/-}$ macrophages.
(A) Lpcat3 targeting vector. A gene-trap LacZ cassette is located downstream of exon 2 of the *Lpcat3* gene. (B) Genotyping of WT and *Lpcat3*$^{-/-}$ mouse embryos. (C) Relative *Lpcat3* mRNA levels in WT and *Lpcat3*$^{-/-}$ fetal liver derived macrophages. (D) Heatmap of PCs, PEs and pPEs molecules in WT and *Lpcat3*$^{-/-}$ macrophages. Data are expressed as % of total phospholipid subclasses and are normalized as 1 in the WT group. n = 3 independent mice in each group. (E) Amount of selected PCs, PEs and pPEs molecules in WT and *Lpcat3*$^{-/-}$ macrophages. n = 3 independent mice in each group; values are means ± S.D. Data are representative of independent experiments including a total of 7 mice in each group (*$p < 0.05$ *vs.* WT Mann–Whitney test).

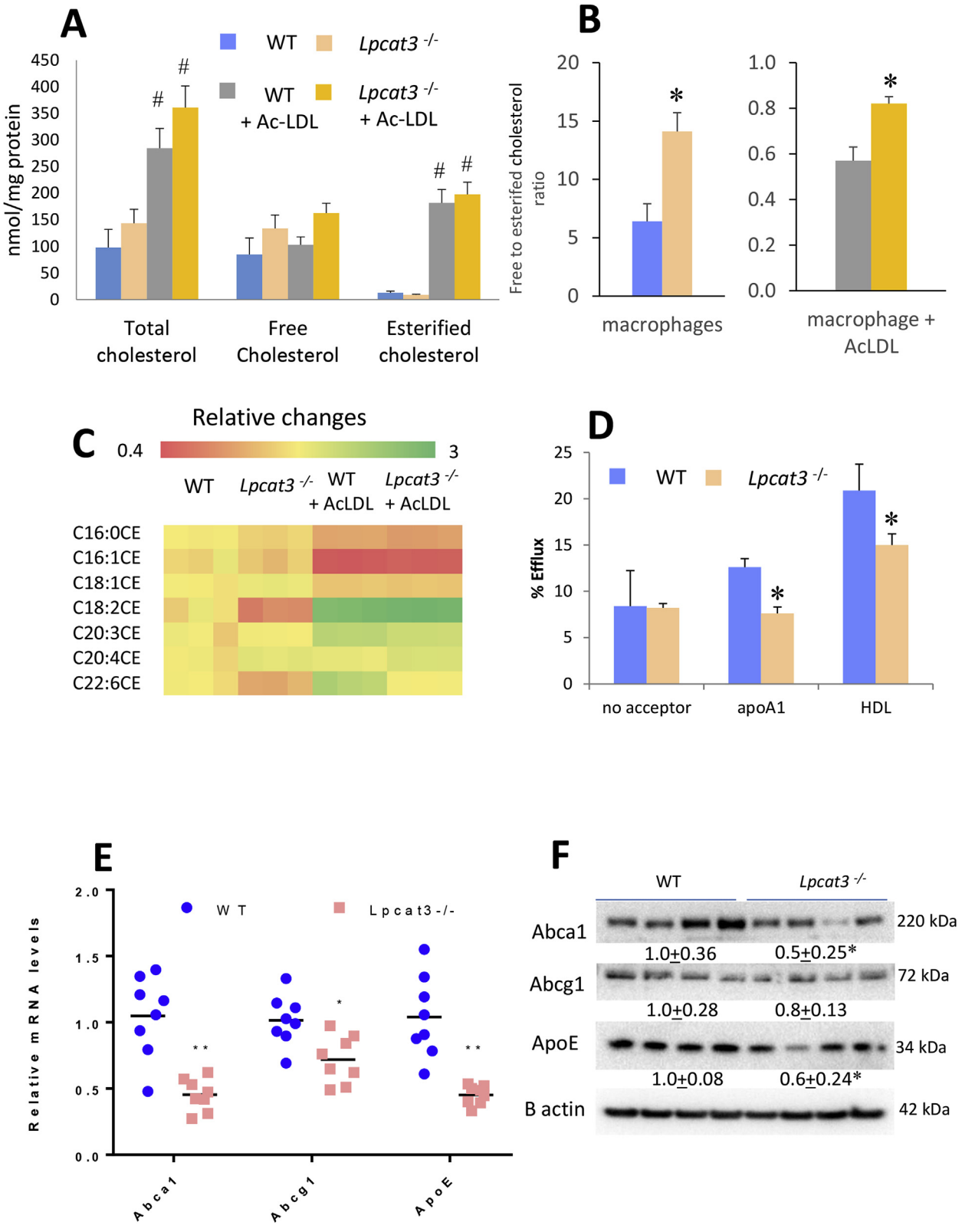

Fig. 2. Alteration of cholesterol homeostasis in *Lpcat3*$^{-/-}$ macrophages.
(A) Total cholesterol, free cholesterol and esterified cholesterol content. (B) Ratio of free to esterified cholesterol. (C) Heatmap of CEs in WT and *Lpcat3*$^{-/-}$ macrophages loaded or not with acetylated LDL (Ac-LDL). Data are expressed as % of cholesteryl esters and are normalized as 1 in the WT group without Ac-LDL. (D) Cholesterol efflux with lipid-free ApoA-I or HDL was assessed in [^{3}H] cholesterol-acetylated LDL loaded macrophages. (E) *Abca1, Abcg1 and ApoE* mRNA levels n = 8 independent mice in each group. (F) ABCA1, ABCG1 and APOE protein levels, n = 4 independent mice in each group (*$p < 0.05$ *vs.* WT **$p < 0.01$ Mann–Whitney test).

LDL conditions (Fig. 2B). The qualitative analysis of cholesterol esters revealed significant changes, with a relative increase in C20:4- and C18:1-CE molecules at the expense of C18:2- and C22:6-CE (Fig. 2C). Since macrophages are dependent on cholesterol efflux to maintain their cellular cholesterol homeostasis, we measured cholesterol efflux from WT or *Lpcat3*$^{-/-}$ macrophages in the presence of exogenous acceptors such as apoA1 or HDLs. As shown in Fig. 2D, we observed that cholesterol efflux was significantly decreased in *Lpcat3*$^{-/-}$macrophages as compared to WT macrophages when either apoA1 or HDL were used as extracellular cholesterol acceptors. Analysis of major genes involved in this pathway revealed a significant decrease in the mRNA levels of *Abca1*, *Abcg1* and *ApoE* (Fig. 2E). A significant reduction of Abca1 (in cell lysate) and ApoE (assessed in the extracellular medium) but not of Abcg1 was also observed at the protein level as assessed by Western blot analysis (Fig. 2F).

3.3. LPCAT3 deficiency alters eicosanoid secretion after acute LPS stimulation

We have previously observed that the transient inhibition of LPCAT3 in human macrophages by using a siRNA approach does not alter the global inflammatory response after LPS stimulation but selectively affects the secretion of eicosanoids derived from arachidonic acid such as prostaglandin E2 (PGE2) and thromboxane B2 (TxB2). In order to confirm these observations in our murine model and in the context of a constitutive *Lpcat3* deficiency, we stimulated WT and *Lpcat3*$^{-/-}$ macrophages with LPS (100 ng/ml) and analyzed the expression of key genes of the inflammatory response at 4 h and 24 h post LPS stimulation. In parallel, the secretion of cytokines and AA-derived mediators was determined in the extracellular medium. As shown in Fig. 3A, we did not observe any significant differences between WT and *Lpcat3*$^{-/-}$ macrophages regarding the mRNA levels of major genes involved in the inflammatory response, such as *Tnfa*, *Cox2*, *Inos*, *Il10,* at either 4 h or 24 h after LPS stimulation, while a moderate decrease was observed for Ilβ at 4 h in the *Lpcat3*$^{-/-}$ group. Likewise, no significant differences in the release of Il1β, Il10 or Mcp1 cytokines were observed (Fig. 3B). By contrast, the secretion of AA-derived eicosanoids, such as TxB2 or PGE2, was significantly decreased in *Lpcat3*$^{-/-}$ macrophages as compared to WT mouse macrophages (Fig. 3C). Treatment with low doses of LPS (0.1 ng and 1 ng/ml) did not reveal significant differences between WT and *Lpcat3*$^{-/-}$ macrophages regarding the inflammatory response (Supplementary Fig. 2A). Because *Lpcat3*$^{-/-}$ macrophages exhibit a relative increase in their free cholesterol content, a potential inducer of ER stress, we measured the expression of ER stress markers such as *Chop* and *Atf3*. No differences between WT and *Lpcat3*$^{-/-}$ were observed in basal conditions or after Ac-LDL loading of macrophages (Supplementary Fig. 2B). Interestingly, level of hydroxyeicosatetraenoic acids (HETEs) tended to lower in *Lpcat3*$^{-/-}$ macrophages, with a nearly significant decrease of 15-S-HETE ($p = 0.055$) (Supplementary Fig. 2C).

3.4. LPCAT3 deficiency alters AA homeostasis and LXR activity in macrophages

In order to gain further insights into the mechanisms involved in the reduction of cholesterol ABC transporters and ApoE, we investigated whether LPCAT3 deficiency may alter the levels of non-esterified fatty acids which are potent LXR antagonists. As shown in Fig. 4A, while levels of free arachidonate remained unchanged, C22:4 n-6, which is a direct elongation product of AA, was increased by more than 3-fold in *Lpcat3*$^{-/-}$ cells arguing for an increased AA bioavailability. To test whether increased AA availability may account for the observed phenotype, we measured the impact of AA treatment on WT and *Lpcat3*$^{-/-}$ macrophages. As shown in Fig. 4B, AA loading at 25 μM induced a decrease in *Abca1*, *Abcg1* and *ApoE* mRNA levels, with an approx. 1.5–2 fold reduction of expression for the three genes. Interestingly, the impact of AA treatment was less pronounced in *Lpcat3*$^{-/-}$ cells with significant reduction observed for *Abca1* and *Abcg1* only. Consequently, there were no differences between WT and *Lpcat3*$^{-/-}$ macrophages after AA treatment regarding *Abca1*, *Abcg1* and *ApoE* mRNA levels (Fig. 4B). Similar results were also observed by using C22:4 n-6 at 25 μM (Fig. 4C). Finally, we directly measured LXR activity in WT and *Lpcat3*$^{-/-}$ macrophages by using a LXR-responsive luciferase reporter plasmid (Fig. 4D). We observed that LXR activity was significantly lower in *Lpcat3*$^{-/-}$ cells as compared to WT cells. Moreover, AA treatment at 50 μM for 6 h significantly inhibited LXR activity in WT but not *Lpcat3*$^{-/-}$ macrophages (Fig. 4D).

3.5. LPCAT3 deficiency in hematopoietic cells promotes atherosclerosis

As cholesterol accumulation in macrophages is a hallmark of the early steps of atherosclerosis, we investigated whether LPCAT3 deficiency in hematopoietic cells would alter the development of atherosclerosis in a hypercholesterolemic mouse model. For this purpose, recipient *Ldlr*$^{-/-}$ mice were irradiated and then injected with hematopoietic cells harvested from fetal WT or *Lpcat3*$^{-/-}$ livers. After 4 weeks of recovery, the mice were fed with a western type diet (WTD). We did not observe any differences in weight gain during the diet (data not shown). The two groups of mice did not display any significant differences in their plasma lipid profile (Supplementary Fig. 3) or total peripheral blood leukocyte counts after 12 weeks of diet (Supplementary Fig. 4). However, at 12 weeks of the WTD, *Lpcat3*$^{-/-}$ > *Ldlr*$^{-/-}$ presented significantly higher monocyte counts with a relative increase in Ly-6C^{high} monocyte subsets (Fig. 5A and Supplementary Fig. 4). Analysis of atherosclerotic lesions after 12 weeks revealed significantly greater lesion size in mice reconstituted with *Lpcat3*$^{-/-}$ fetal liver hematopoietic cells. This was observed in aortic roots and in aortic arches (Fig. 5B and D and Supplementary Fig. 5A). Mac3-immunostaining in atherosclerotic lesions revealed increased macrophage infiltration in the aortic valves of the *Lpcat3*$^{-/-}$ > *Ldlr*$^{-/-}$ group (Fig. 5C), however, no changes in the necrotic core area were observed in trichrome stained sections (91 ± 58 μm^2 *vs.* 95 ± 41 μm^2). Targeted lipidomic analysis of atheroma plaques from aortic valves revealed significant alteration of cholesteryl esters (CEs) composition with highly significant increases in the proportion of C20:4 and C22:4 n-6 CE in the plaques from the *Lpcat3*$^{-/-}$ group (Supplementary Fig. 5B). However, no significant increase in free to esterified cholesterol ration was observed (Supplementary Fig. 5C).

4. Discussion

LPLATs, which are involved in the Land cycle, play a critical role in maintaining fatty acid homeostasis within PLs in different tissues and cells [1]. LPCAT3 has recently been the subject of particular interest because of its special properties. Indeed, this enzyme, essential for incorporating AA into glycerophospholipids, is dynamically regulated by nuclear receptors such as PPARs and LXRs [4,12] and its deficiency in the liver or intestine in the mouse results in major modifications in the properties of cell membranes by altering their capacity to handle neutral lipids such as TGs. While the role of LPCAT3 in the intestine and liver has been elucidated in recent studies, the function of LPCAT3 in hematopoietic cells has yet to be determined. Due to a relatively low level of expression in macrophages as compared with the liver or intestine, we

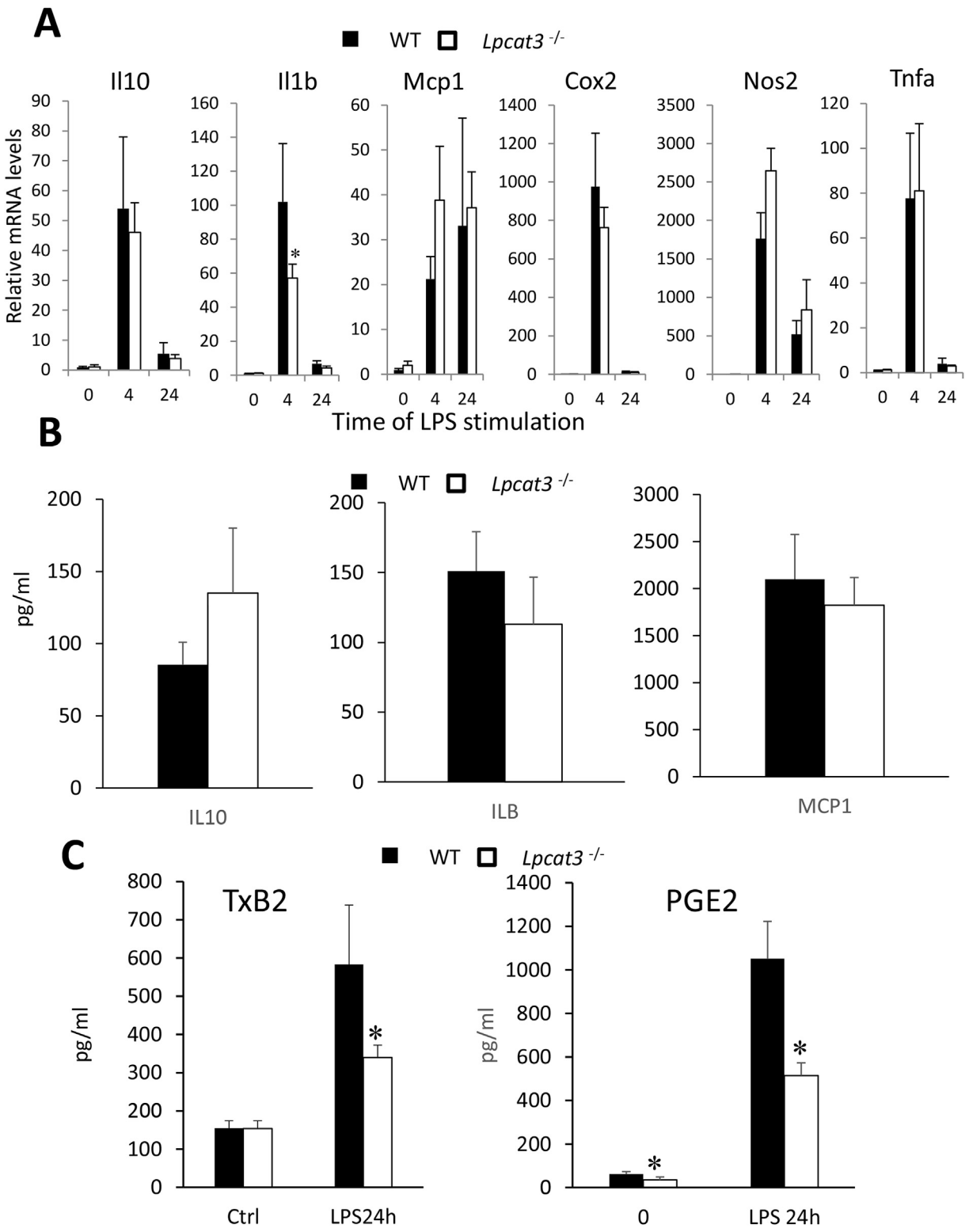

Fig. 3. Impact of *Lpcat3* deficiency on the inflammatory response.
(A) Relative mRNA levels of inflammatory response genes 4 h and 24 h after LPS stimulation (100 ng/ml) of fetal liver derived murine macrophages. (B) Cytokine release 24 h after LPS stimulation (100 ng/ml). (C) Eicosanoid release under basal and LPS-stimulated conditions. Each point represents the mean ± SD of 4 determinations (*$p < 0.05$ *vs.* WT same treatment Mann-Whitney test).

considered the possibility that LPCAT3 deficiency might not have a marked effect on phospholipid metabolism in these cells. The present study clearly demonstrated that LPCAT3 plays a key role in PL metabolism in macrophages. The observed changes in the PL profile of *Lpcat3*$^{-/-}$ macrophages are similar to those found in the liver and intestine of *Lpcat3*$^{-/-}$ mice with a decrease in AA-containing PCs and PEs, which was compensated by an increase in the relative abundance of C22:4 n-6 (adrenic acid)-containing

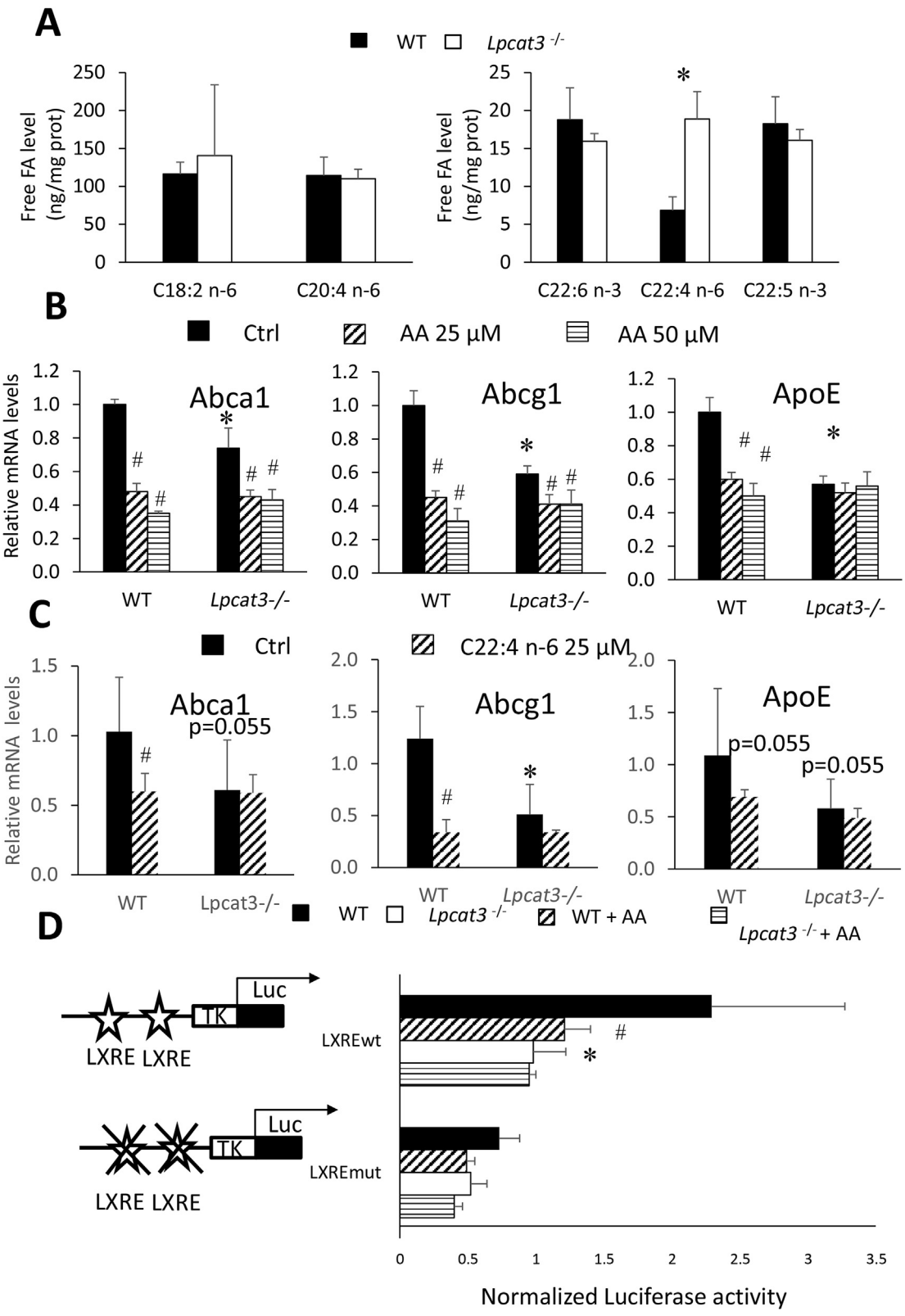

Fig. 4. *Lpcat3* deficiency affects AA homeostasis and LXR activity in macrophages.
(A) Cellular levels of non-esterified fatty acids in WT and *Lpcat3*$^{-/-}$ macrophages (n = 4 independent mice in each group; *$p < 0.05$ *vs.* WT Mann-Whitney test). (B and C) Impact of AA and adrenic acid treatment on mRNA levels of selected genes in WT and *Lpcat3*$^{-/-}$ macrophages. Macrophages were treated with AA/BSA or adrenic acid/BSA complexes at the indicated concentrations for 24 h (n = 4 independent points in each group; *$p < 0.05$ *vs.* WT same experimental conditions, #$p < 0.05$ *vs.* same genotype, control conditions, Mann-Whitney test). (C) LXR-responsive luciferase activity in WT and *Lpcat3*$^{-/-}$ macrophages treated or not with AA at 50 µM. Macrophages were transfected with an LXR-responsive luciferase reporter plasmid containing two DR4 LXRE (LXREWT) or with a control plasmid containing two mutated LXRE (LXREmut) (n = 3 independent experiments; *$p < 0.05$ *vs.* WT same experimental conditions, #$p < 0.05$ *vs.* same genotype, control conditions, Mann-Whitney test).

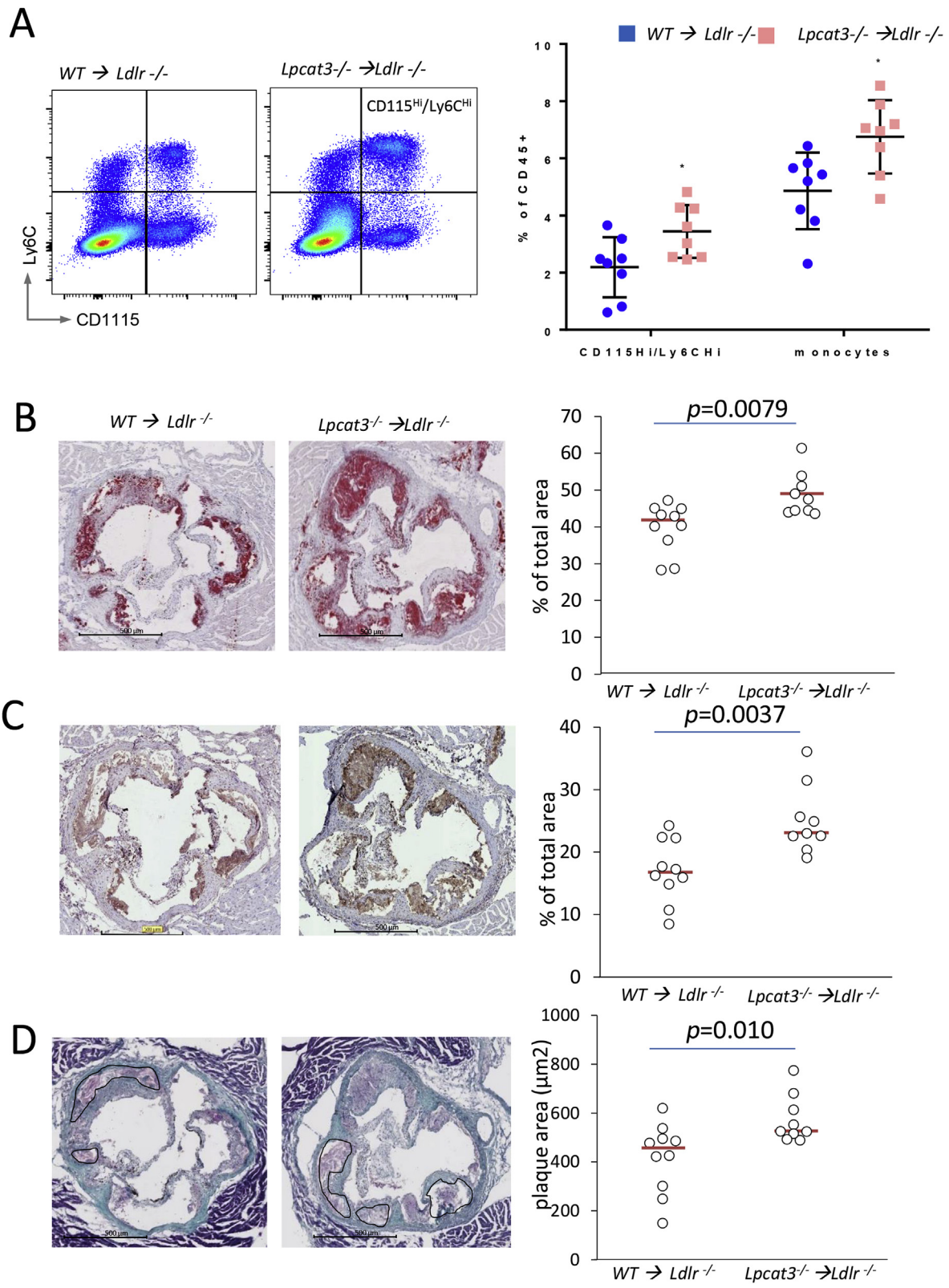

Fig. 5. Hematopoietic *Lpcat3* deficiency promotes atherosclerosis in recipient *Ldlr*$^{-/-}$ mice.
(A) Percentage of CD115 + and CD115+/Ly6C^{high} amongst CD45 $^{+}$ cells assessed by flow cytometry (gating strategy is shown on the first panel). (B) Oil red O staining of aortic valves, (C) macrophage immunostaining and (D) Masson Trichrome staining of aortic valves in *Ldlr*$^{-/-}$ recipient mice fed a Western-type diet for 12 weeks (n = 10 WT and n = 9 *Lpcat3*$^{-/-}$; Mann-Whitney test). Median is indicated with the red horizontal bar; necrotic cores are indicated with a continuous line. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

molecules. To our knowledge, this study is the first to demonstrate that LPCAT3 is involved in plasmalogen metabolism, with an even greater impact than on diacylglycerophospholipids. Thus, while AA-containing molecules represented 66% of plasmenylethanolamines in WT macrophages their relative proportion dropped to just 25% in *Lpcat3*$^{-/-}$ macrophages. Interestingly, little is known about the specific roles of plasmalogens in macrophages. Nevertheless, it appears that plasmalogen metabolism is dynamically regulated during monocyte to macrophage differentiation and that plasmalogens represent an important reservoir of arachidonate for activated cells [13,14]. Once we had established the role of LPCAT3 in PL metabolism in macrophages, we next wanted to evaluate the consequences of LPCAT3 deficiency on macrophage functions and properties. In a previous work from our laboratory, inhibition of LPCAT3 with siRNAs in primary human and mouse macrophages did not promote significant changes in the overall inflammatory response after LPS stimulation [4]. However, we did find a significant reduction in the secretion of AA-derived eicosanoids, probably in relation to a decrease in the pool of AA available within the PLs [4]. In the present study, we observed that there were no major differences in the acute LPS response between WT and *Lpcat3*$^{-/-}$ macrophages, at least for key genes of the inflammatory response (*Cox2*, *Nos2*, *Il1β*, *Tnfa*) even with very low doses of LPS stimulation. In agreement with our previous observations, we also found a decrease in the secretion of specific eicosanoids, such as PGE2 or thromboxane, in *Lpcat3*$^{-/-}$ macrophages after LPS stimulation. Under physiological conditions (macrophages loaded with ac-LDL but without ACAT inhibitors), there was no difference in the expression of ER stress markers, despite an increased ratio of free to esterified cholesterol in *Lpcat3*$^{-/-}$ macrophages. The impact of LPCAT3 on unfolded protein response under more severe stress conditions (saturated fatty acid loading or ACAT inhibitor) remains however to be assessed. Nevertheless, it is worth noting that chronic loss of function of *Lpcat3* in mouse liver did not promote significant activation of ER stress pathways [5].

Importantly, we found that *Lpcat3*$^{-/-}$ macrophages exhibited significant changes in cholesterol homeostasis including an increase in the ratio of free to esterified cholesterol and inhibition of cholesterol efflux pathways. It is likely that alterations in the intracellular bioavailability of long-chain polyunsaturated fatty acids contribute to these changes. Indeed, these molecules are natural LXR inhibitors [15,16], which would account for the reduction in expression levels of LXR target genes, namely those involved in cholesterol efflux in *Lpcat3*$^{-/-}$ macrophages. We did not detect an increase in the intracellular levels of free arachidonate. However, the enrichment of cholesteryl esters with arachidonate and the accumulation of adrenic acid (C22:4 n-6), a direct elongation product of AA, in cholesteryl esters, phospholipid and as free fatty acid are in line with the increased availability of arachidonate for metabolic pathways besides incorporation into PL. The efficacy of these alternative metabolic routes (adrenic acid synthesis and cholesterol esterification) probably explains why free arachidonate does not accumulate significantly in *Lpcat3*$^{-/-}$ cells. Interestingly, it has previously been reported that adrenic acid displays the highest LXR inhibitory activity among all polyunsaturated fatty acids (approx. twice as effective as arachidonic acid) [17]. Accordingly, we found that LXR activity measured by using an LXR-responsive luciferase reporter vector was decreased in *Lpcat3*$^{-/-}$ macrophages and was inhibited by AA loading of macrophages. Moreover, *Abca1, Abcg1* and *ApoE* mRNA levels were significantly decreased after incubation of macrophages with AA. Similarly to our observations, Shridas et al. have demonstrated that increased AA availability in link with Group X sPhospholipase A2 (GX sPLA2) overexpression inhibited LXR activity, decreased ABC transporter expression and cholesterol efflux in macrophages [18]. Reciprocally, Schneider et al. previously observed that genetic deletion of fatty-acid synthase in macrophages results in activation of LXRs, probably by decreasing cellular levels of fatty acid-derived LXR antagonists [19]. Consequently, *Fasn*$^{-/-}$ macrophages displayed increased cholesterol efflux and *Fasn* deficiency was protective against atherosclerosis development in *ApoE*$^{-/-}$ mice. We propose that a similar mechanism would contribute to observed phenotypes, except that LPCAT3 deficiency would increase the levels of FA-derived LXR antagonists in macrophages. We cannot exclude that other molecular mechanisms could also account for the reduced cholesterol efflux capacity of *Lpcat3* deficient macrophages. Indeed, qualitative changes in PL composition (including plasmalogens) induced by *Lpcat3* deficiency may alter the biophysical properties of cell membranes in macrophages and therefore the activity of ABC transporters. Indeed, recent observations in these animals indicated that AA depletion of cellular membranes reduced their dynamics as well as mobility of their lipid components [5,6], which could theoretically contribute to a reduction in cholesterol efflux. Unfortunately, this hypothesis was difficult to explore in the present study because of the concomitant decreased expression of cholesterol transporters that occurs at the transcriptional level in *Lpcat3*$^{-/-}$ macrophages. Nevertheless, characterization of the biophysical changes in macrophage cell membranes induced by LPCAT3 deficiency and their potential impact on cholesterol trafficking will require further investigations.

Altered cholesterol homeostasis in *Lpcat3*$^{-/-}$ hematopoietic cells associated with decreased levels of LXR target such as Abca1, Abcg1 and ApoE may explain the observed increase in atherosclerosis in *Ldlr*$^{-/-}$ mice with hematopoietic *Lpcat3* deficiency. Indeed, cholesterol accumulation in macrophages is a hallmark of atherosclerosis and numerous studies have shown that genetic inactivation of cholesterol efflux pathways in macrophages and hematopoietic cells promotes monocytosis, foam cell formation and atherosclerosis development in hypercholesterolemic mouse models [20–25]. Whether higher amount of Ly-6Ch monocytes or defective cholesterol efflux in differentiated macrophages play a prominent role in our model remains to be addressed. Similarly, LXR deletion in macrophages reduces Abca1, Abcg1 and ApoE expression and accelerates atherogenesis in *ApoE*$^{-/-}$ or *Ldlr*$^{-/-}$ mice [26] while activation of LXR pathways in macrophages is strongly protective [27]. Whether higher amount of Ly-6Ch monocytes and subsequent increased aortic macrophage infiltration or defective cholesterol efflux in differentiated macrophages play a prominent role in our model remains to be addressed.

In conclusion, our study underscores the importance of intracellular fatty acid metabolism in the control of immune cell functions as recently underlined by recent studies and strongly suggests that FA metabolism in hematopoietic cells could be a potential target in the field of cardio-metabolic diseases [19,28,29].

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Authors contributions

C.T. and D.M. conceived and performed experiments, wrote the manuscript. A.J., C.M., M.I., V.B, T.B., N.L., D.P., L.M., J.L., JP.P. performed experiments. T.G. and R.Q. provided expertise and feedback. L.L. provided expertise and feedback and secured funding.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2018.05.023.

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