SUPPLEMENTAL MATERIALS

The coronary artery disease risk-associated *Plpp3* gene and its product lipid phosphate phosphatase 3 regulate experimental atherosclerosis

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Major Resources Tables

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex
Mice	The Jackson Laboratory	Ldlr-/- B6.129S7-Ldlrtm1Her/J Stock No: 002207	Both
Mice	The Jackson Laboratory	MX1-Cre B6.Cg-Tg(Mx1-cre)1Cgn/J Stock No: 003556	Male
Mice	The Jackson Laboratory	LysM-Cre B6.129P2-Lyz2tm1(cre)Ifo/J Stock No: 004781	Male
Mice	The Jackson Laboratory	B6.Cg-Tg(Tagln-cre)1Her/J Stock No: 017491	Male
Pig	The Francis Owen Blood Research Laboratory at the University of North Carolina at Chapel Hill	Spotted Poland/China and Yorkshire crosses; Male and female pigs from the following two genotypes were used: normocholesterolemic or heterozygous familial hypercholesterolemic	Both

Animal breeding

	Species	Vendor or Source	Background Strain	Other Information
Parent - Male	Mice	The Jackson Laboratory; Bar Harbor, ME	Ldlr-/-	Plpp3fl/fl animals (fl/fl) with or without the MX1-Cre or SMC-22 Cre transgene were crossed to Ldlr-/- mice[8].
Parent - Female	Mice	The Jackson Laboratory; Bar Harbor, ME	Ldlr-/-	Plpp3fl/fl animals (fl/fl) with or without the MX1-Cre or SMC-22 Cre transgene were crossed to Ldlr-/- mice[8].
Parent - Male	Mice	The Jackson Laboratory; Bar Harbor, ME	C57BL/6	To simulate LDL receptor deficiency, mice lacking Lpar49

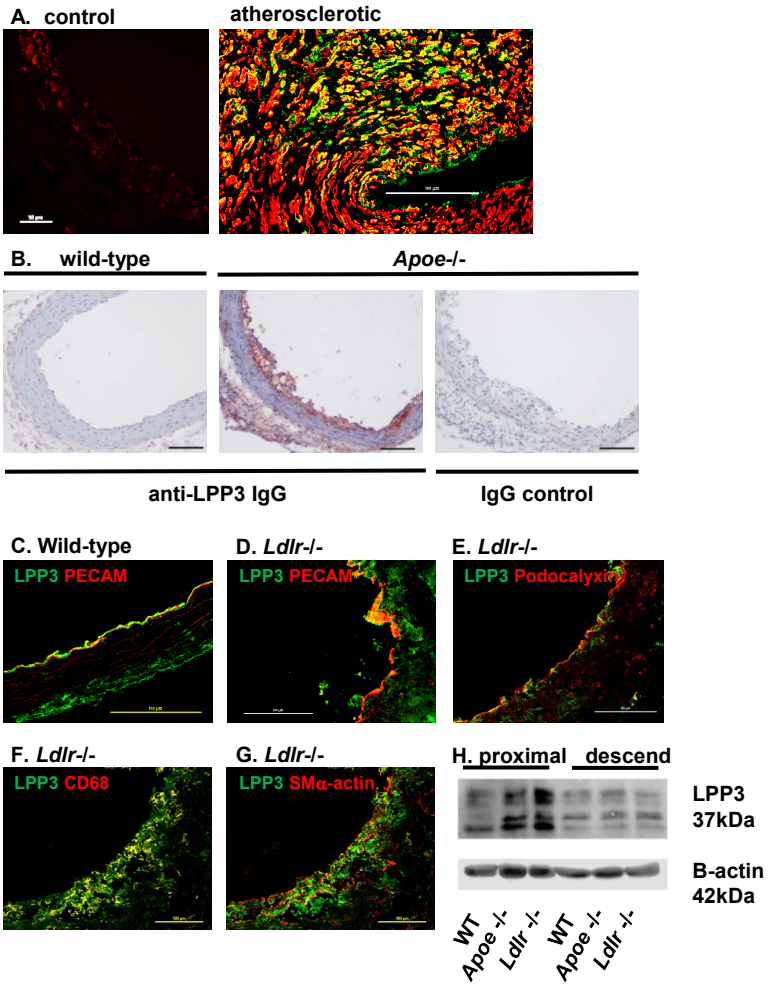
				were backcrossed to C57BL/6 background, and then bred to generate Lpar4Y/+ and Lpar4 Y/- mice[9].
Parent - Female	Mice	The Jackson Laboratory; Bar Harbor, ME	C57BL/6J	To simulate LDL receptor deficiency, mice lacking Lpar49 were backcrossed to the C57BL/6J background, and then bred to generate Lpar4Y/+ and Lpar4 Y/- mice[9].

Antibodies

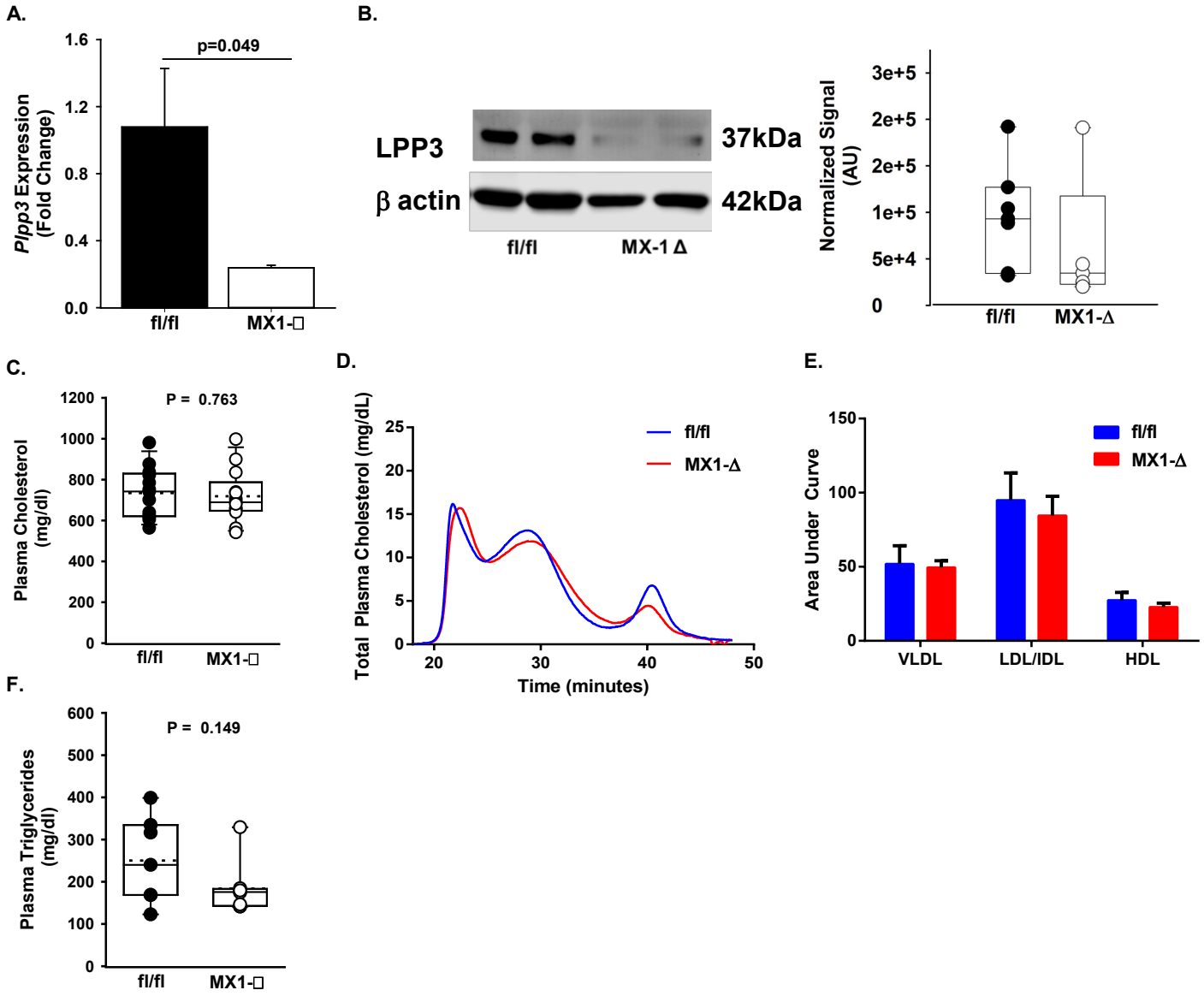
Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)
LPP3 (PPAP2B)	A peptide corresponding to residues 2–17 (QNYKYDKA-IVPESKNG) of the sequence of human PAP2b was synthesized. Rabbits were immunized, and antibody titers were determined by enzyme-linked immunosorbent assay using the individual peptide antigen as the solid phase[12].	n/a	1µg/mL	
Smooth muscle alpha-actin	Abcam	AB5694	2µg/mL	
CD68	Abcam	AB53444	10µg/mL	
PECAM1	BD Biosciences	553370	5µg/mL	
Podocalyxin	R&D Syatems	AF1556	2µg/mL	

Cultured Cells

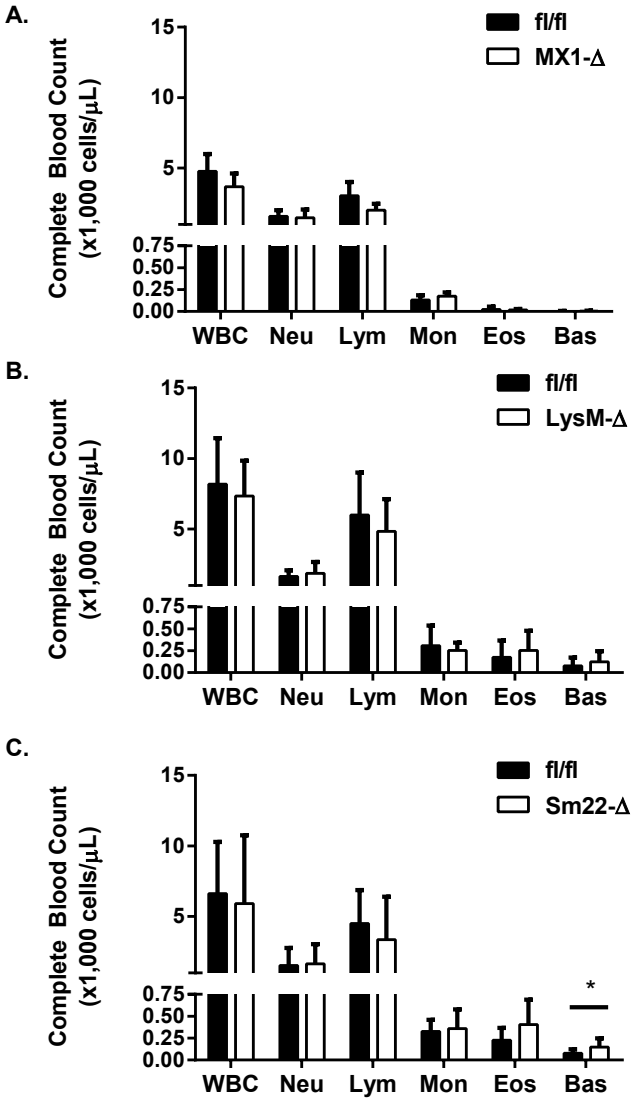
Name	Vendor or Source	Sex (F, M, or unknown)
Bone marrow-derived macrophages (BMDM)	Femur and tibia.	Both
SMC	Aorta	Both
SMC-derived foam cells	Primary human coronary artery SMC (CloneticsTM, Lonza, Allendale, NJ)	unknown



Supplemental Figure 1. Upregulation of LPP3 expression during experimental atherosclerosis. A) Immunofluorescence staining of LPP3 (green) and SMC α-actin (red) in cross-sections of healthy (left) and atherosclerotic (right) porcine coronary arteries. Pigs are the result of crosses between Spotted Poland/China and Yorkshire animals. The heterozygous familial hypercholesterolemic pigs have a recessive inheritance pattern as the result of a mutation in the LDL receptor that results in an $arginine_{94}$ to $cysteine_{94}$ mutation, located in the region that corresponds to exon 4 in the human ligand binding domain. The FH pigs are normocholesterolemic at baseline and only exhibit hypercholesterolemia when fed a high fat diet. Scale bar = 100μm **B**) Immunohistochemical staining with anti-LPP3 IgG (left and middle) or control rabbit anti-IgG (right) of sections of aortic root from C57BL/6J wildtype mice and $Apoe^{-/-}$ mice that were fed Western diet for 12 weeks. Scale bar = 300μm. **C**) Immunofluorescence staining of LPP3 (green) and PECAM (red) in cross-sections of aortic root from C57BL/6J mice and **C**) Ldlr-/- mice fed Western diet for 12 weeks. Co-localization of LPP3 expression (green) in atherosclerotic $Ldlr^{-/-}$ aortic root sections with **E**) podocalyxin (red), **F**) CD68 (red), and **G**) SMC α-actin (red). Scale bar = 100μm. **H**) Immunoblot of LPP3 and β-actin tissue lysates from the proximal and descending aorta of WT, $Apoe^{-/-}$, and $Ldlr^{-/-}$ mice fed Western diet for 12 weeks.



Supplemental Figure 2. Plasma Characterization in mice with MX1-mediated global reductions of *Plpp3*. A) *Plpp3* gene expression (fold change; mean ± SD) determined by qRT-PCR analysis of RNA from bone marrow cells from fl/fl and MX1-Δ mice (n=3-5/group). **B)** Quantification of LPP3 expression normalized to β-actin in proximal aortas from fl/fl and MX1-Δ mice on the *Ldlr*$^{-/-}$ background after 12 weeks on Western diet (right panel; data displayed as arbitrary relative units; median and IQR), as determined by immunoblotting (left panel). **C)** Total plasma cholesterol (mg/dl; median and IQR) from fl/fl and MX1-Δ mice on the *Ldlr*$^{-/-}$ background 12 weeks after Western diet. **D)** Representative plasma cholesterol content of fast protein liquid chromatography (FPLC) fractionated lipoproteins. **E)** Area under the curve analysis of plasma cholesterol distribution determined by FPLC (N=4/genotype; mean ± SD). **F)** Total plasma triglycerides (mg/dL; mean ± SEM) (n=16/group).



Supplemental Figure 3. Complete Blood Counts. Complete blood counts performed on whole blood from **A)** fl/fl and MX1-Δ; **B)** fl/fl and LysM-Δ; and **C)** fl/fl and SM22-Δ mice on the *Ldlr*$^{-/-}$ background fed Western diet for 12 weeks.*P<0.05