Supplementary file

Group V secreted phospholipase A_2 plays a protective role against aortic dissection

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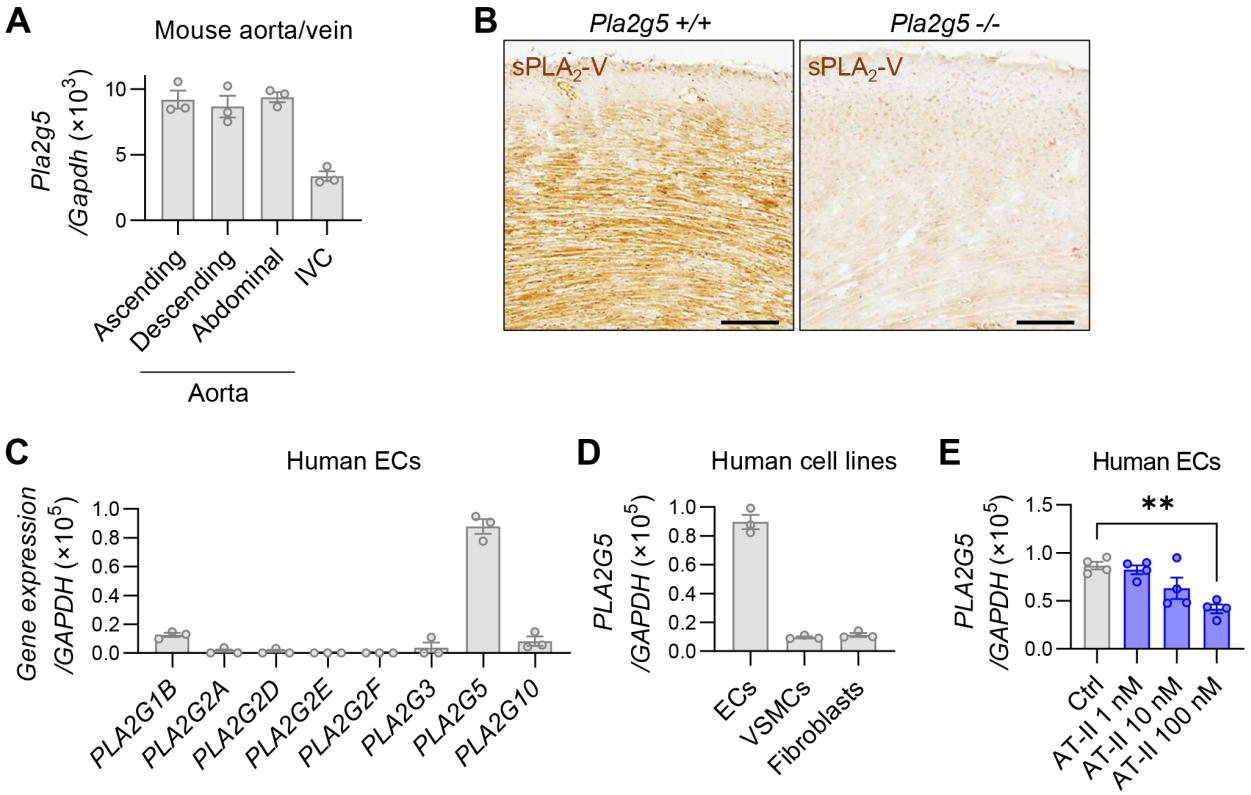

Figure S1.

Expression of sPLA$_2$-V in mouse aorta, vein and heart, and human ECs. Related to Figure 1.

(**A**) Expression of *Pla2g5* in various aortas (thoracic ascending, thoracic descending, and abdominal aortas) and inferior vena cava (IVC) in mice (n=3). (**B**)Immunohistochemistry of sPLA$_2$-V in the heart of *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice. Scale bars, 100 μm. (**C**) Expression of sPLA$_2$ mRNAs relative to *GAPDH* in human umbilical vein ECs (HUVECs) (n = 3). (**D**) Expression of *PLA2G5* in cultured human ECs (HUVECs), VSMCs (HA-VSMCs), and fibroblasts (WI-38) (n = 3). (**E**) Expression of *PLA2G5* in HUVECs treated for 24 hours with or without AT-II (1-100 nM) (n = 4). **P < 0.01 by one-way ANOVA followed by Tukey's multiple comparisons test. Data are presented as mean ± SEM of the indicated number (n) of biological replicates.

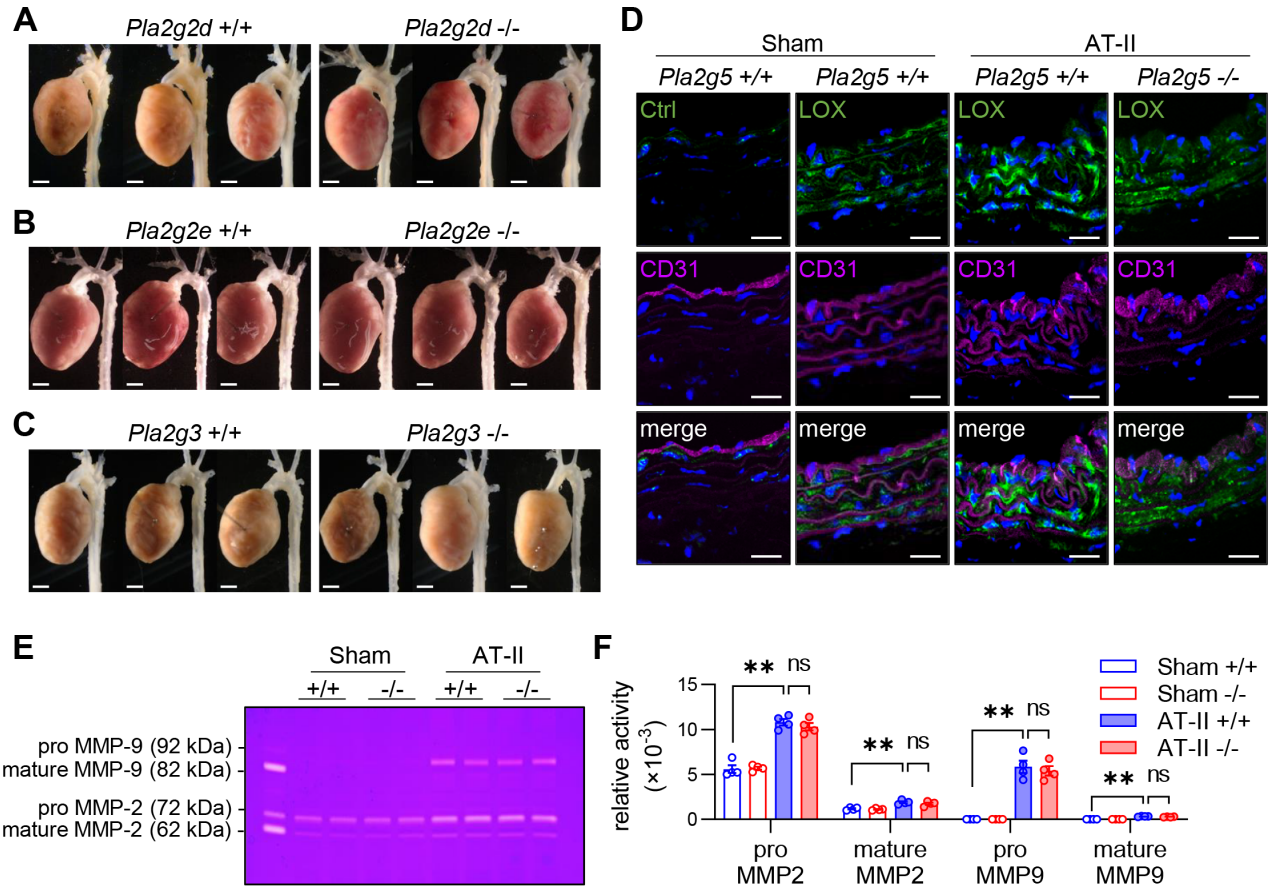

Figure S2.

Thoracic aortic dissection in various sPLA$_2$ knockout mice and LOX expression and MMP activities in the aorta of *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice after AT-II infusion. Related to Figure 2, 3.

(**A-C**) Representative photos of the thoracic aortas of *Pla2g2d*$^{-/-}$ (**A**), *Pla2g2e*$^{-/-}$ (**B**), *Pla2g3*$^{-/-}$ (**C**), and their littermate wild-type mice after 7 days of AT-II infusion. Scale bars, 1 mm. (**D**) Immunofluorescence of the aortas of *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice with or without AT-II infusion for 48 hours using control IgG (Ctrl), anti-LOX antibody (RRID: AB_776074), which is different from that used in Figure 3g (RRID: AB_2630340), and anti-CD31 antibody with DAPI (blue). Scale bars, 20 μm. (**E**) Gelatin zymography and (**F**) densitometry analysis of MMP-2 and MMP-9 activities (both pro- and mature forms) in the aortas of *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice with or without AT-II infusion for 48 hours (n = 4). **P < 0.01, and ns = not significant by two-way ANOVA followed by Tukey's multiple comparisons test. Data are represented as mean ± SEM of the indicated number (n) of biological replicates.

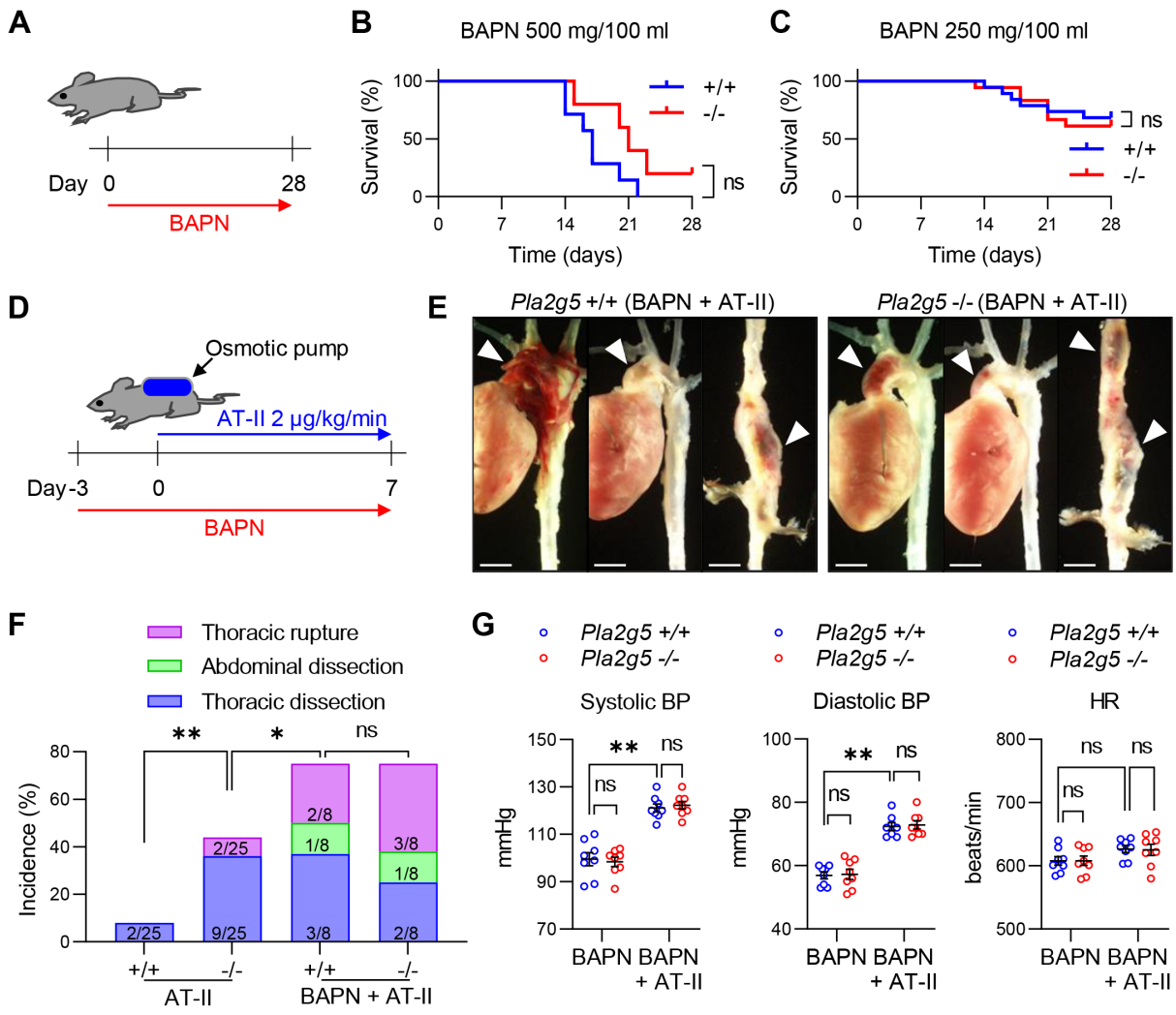

Figure S3.

BAPN-induced dissection in the aorta of *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice after AT-II infusion. Related to Figure 3.

(**A**) Schematic representation of the BAPN administration procedure. BAPN was dissolved in drinking water *ad libitum*. (**B**, **C**) Survival curves of *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice over 4 weeks of BAPN administration at a dose of 500 mg/100 ml (**B**) or 250 mg/100 ml (**C**). Survival curves were calculated by the Kaplan-Meier method and compared by the log-rank test. ns = not significant. (**D**) Schematic representation of the AT-II infusion procedure in the presence of BAPN administration. (**E**) Representative photos of the thoracic aortas of *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice after 7 days of AT-II infusion with BAPN administration (250 mg/100 ml). Arrowheads indicate aortic dissection with intramural hematoma. Scale bars, 1 mm. (**F**) Incidence of aortic dissection or rupture in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice within 7 days of AT-II infusion with or without BAPN administration. *P < 0.05, **P < 0.01, and ns = not significant by Fisher's exact test. (**G**) Systolic blood pressure (BP), diastolic BP, and heart rate (HR) of BAPN treated *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice with or without AT-II infusion for 2 days (n = 8). **P < 0.01, and ns = not significant by two-way ANOVA followed by Tukey's multiple comparisons test. Data are represented as mean ± SEM of the indicated number (n) of biological replicates.

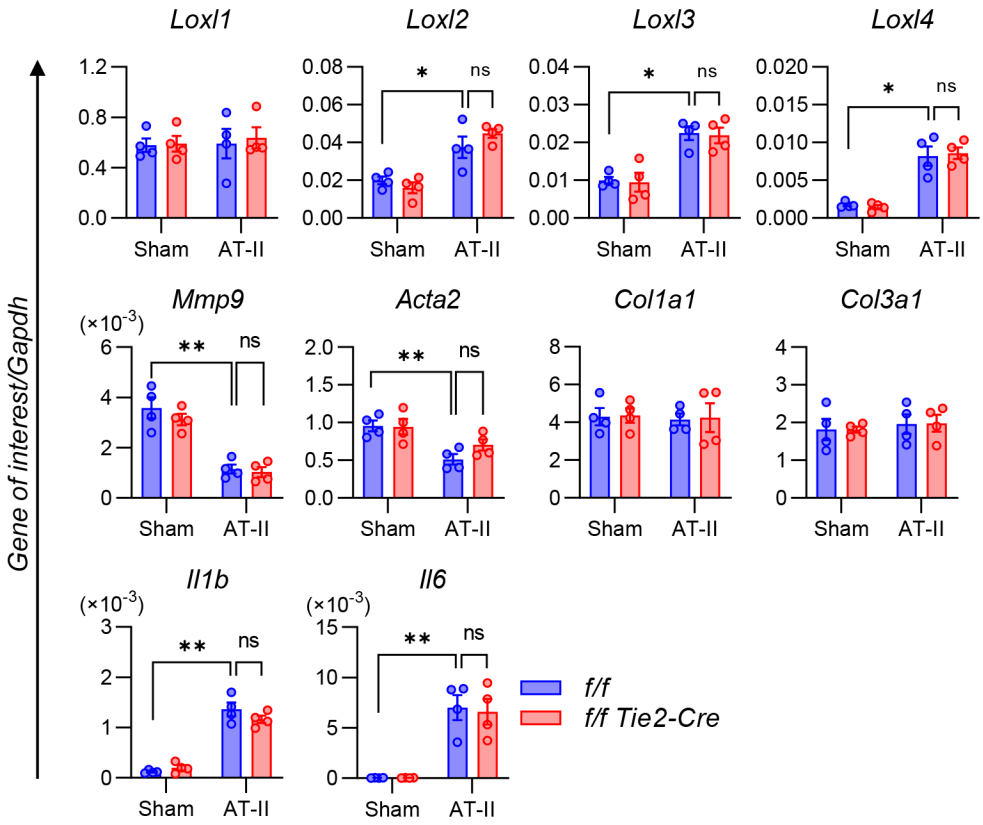

Figure S4.

Quantitative RT-PCR analysis using the aorta of EC-specific *Pla2g5*-null mice. Related to Figure 4.

mRNA expression of LOX family members, vascular remodeling markers, and pro-inflammatory cytokines in the aortas of control and EC-specific *Pla2g5*-null mice with or without AT-II infusion for 48 hours (n = 4). *P < 0.05, **P < 0.01, and ns = not significant by two-way ANOVA followed by Tukey's multiple comparisons test. Data are represented as mean ± SEM of the indicated number (n) of biological replicates.

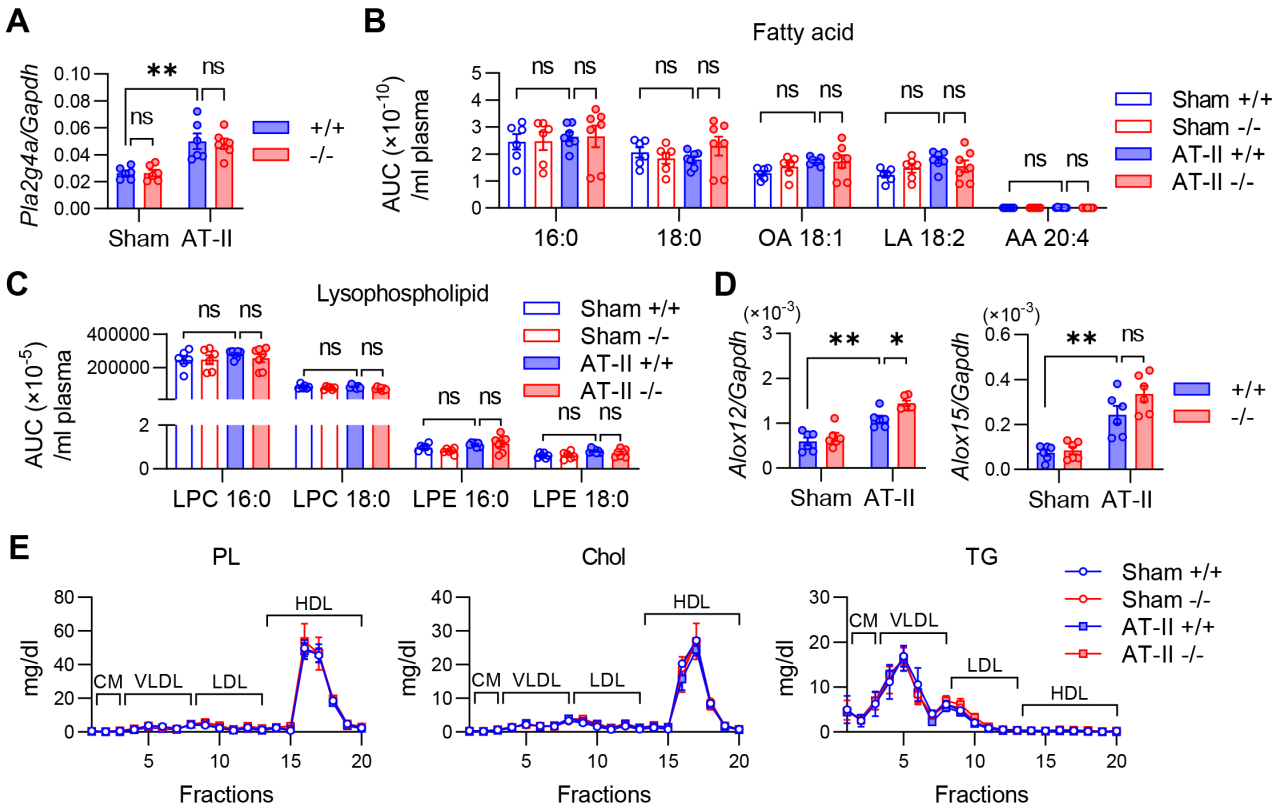

Figure S5.

Aortic expression of cPLA$_2$α and 12/15-lipoxygenases, plasma levels of lipids, and evaluation of plasma lipoproteins in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice. Related to Figure 5.

(**A**) Aortic expression of *Pla2g4a* in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice (n = 6). (**B**, **C**) Plasma levels of fatty acids (**B**) and lysophospholipids (**C**) in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice as assessed by LC-ESI-MS/MS (n = 6-7). (**D**) Aortic expression of *Alox12* and *Alox15* in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice (n = 6). (**E**) High-performance liquid chromatography (HPLC) profiles of phospholipids (PL), cholesterol (Chol), and triglyceride (TG) in plasma lipoproteins in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice (n = 3). The mice were treated for 12 hours with or without AT-II infusion. *P <0.05, **P <0.01, and ns = not significant by two-way ANOVA followed by Tukey's multiple comparisons test (**A-D**). Data are represented as mean ± SEM of the indicated number (n) of biological replicates.

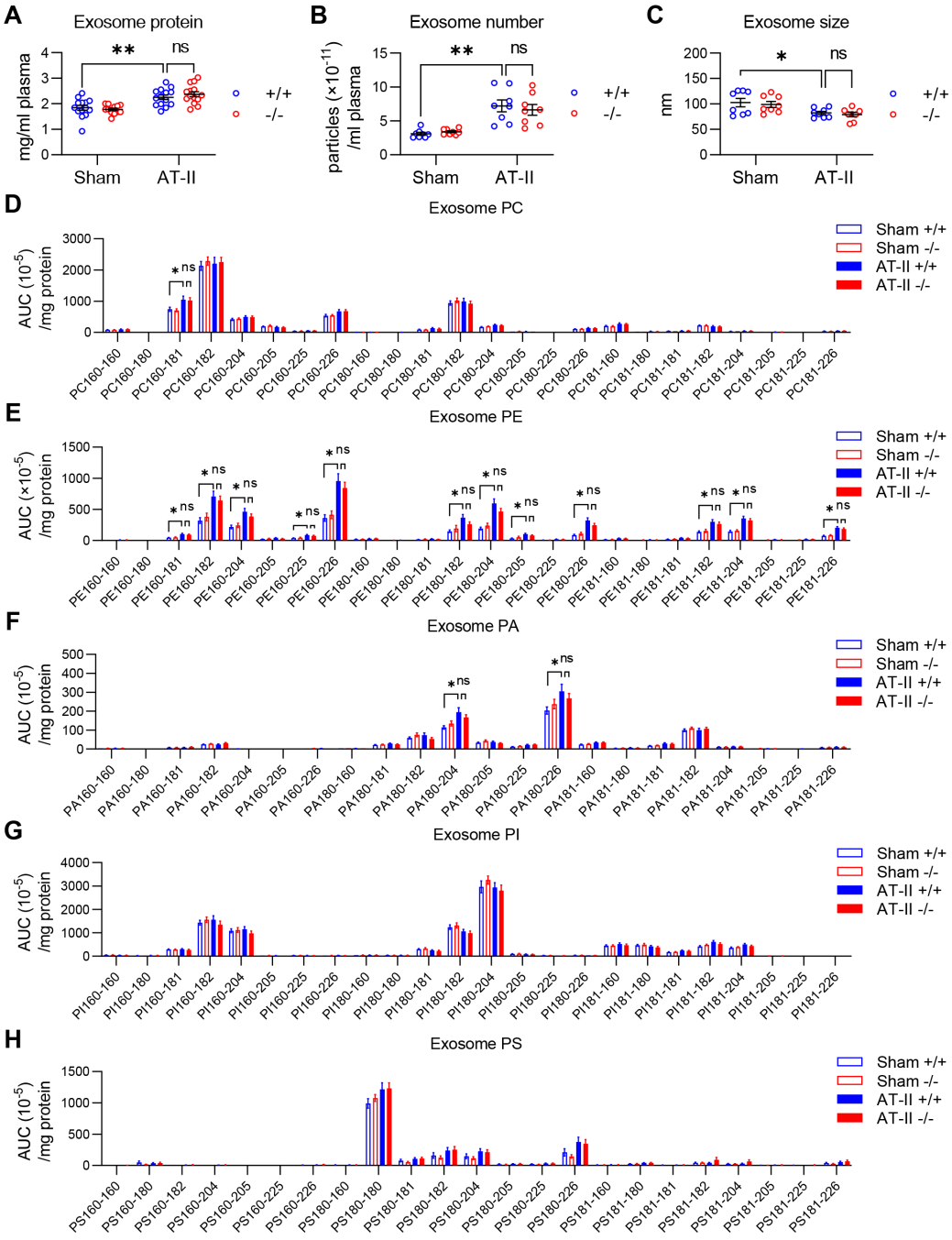

Figure S6.

Analyses of plasma exosomes in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice. Related to Figure 5.

(**A-C**) Protein contents (n = 12-14) (**A**), particle numbers (n = 8) (**B**), and particle sizes (n = 8) (**C**) of plasma exosomes in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice. (**D-H**) Phosphatidylcholine (PC) (**D**), phosphatidylethanolamine (PE) (**E**), phosphatidic acid (PA) (**F**), phosphatidylinositol (PI) (**G**) and phosphatidylserine (PS) (**H**) in plasma exosomes from *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice were assessed by LC-ESI-MS/MS (n = 12-14). The mice were treated for 12 hours with or without AT-II infusion. *P <0.05 and ns = not significant by two-way ANOVA followed by Tukey's multiple comparisons test. Data are represented as mean ± SEM of the indicated number (n) of biological replicates.

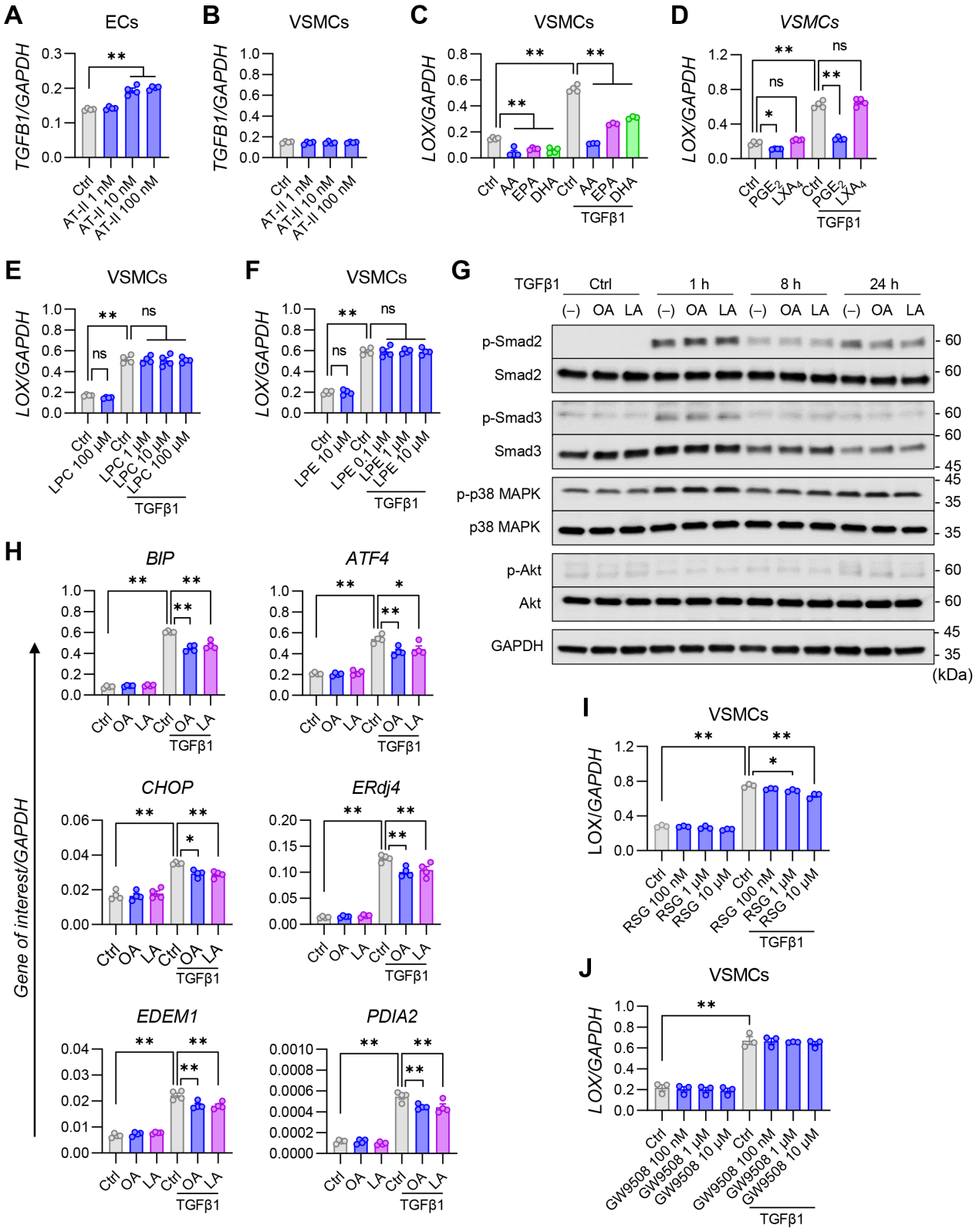

Figure S7.

Effects of fatty acids, lysophospholipids, and lipid mediators on *LOX* expression and TGFβ1 signaling in ECs and VSMCs. Related to Figure 6.

(**A**, **B**) Expression of *TGFB1* relative to *GAPDH* in ECs (**A**) or VSMCs (**B**) treated for 24 hours with or without AT-II (1-100 nM) (n = 4). (**C**-**F**) Effect of AA, EPA, or DHA (**C**), PGE_2 or LXA_4 (**D**), LPC (18:0) (**E**), and LPE (18:0) (**F**) on the expression of *LOX* in VSMCs treated for 24 hours with or without TGFβ1 (n = 4). (**G**) Immunoblot of TGFβ1 signaling proteins in VSMCs treated with TGFβ1 with or without (-) OA (100 μM) or LA (100 μM). (**H**) Expression of mRNAs for various ER stress markers, including *BIP*, *ATF4*, *CHOP*, *ERdj4*, *EDEM1*, *PDIA2*, in VSMCs treated for 24 hours with or without TGFβ1 in the presence or absence of OA (100 μM) or LA (100 μM) (n = 4). (**I**, **J**) Effects of RSG (PPARγ agonist) (**I**) or GW9508 (GPR40/120 agonist) (**J**) on the expression of LOX relative to GAPDH in VSMCs treated for 24 hours with or without TGFβ1 (n = 3). *P <0.05, **P <0.01, and ns = not significant by one-way ANOVA followed by Tukey's multiple comparisons test. Data are represented as mean ± SEM of the indicated number (n) of biological replicates.

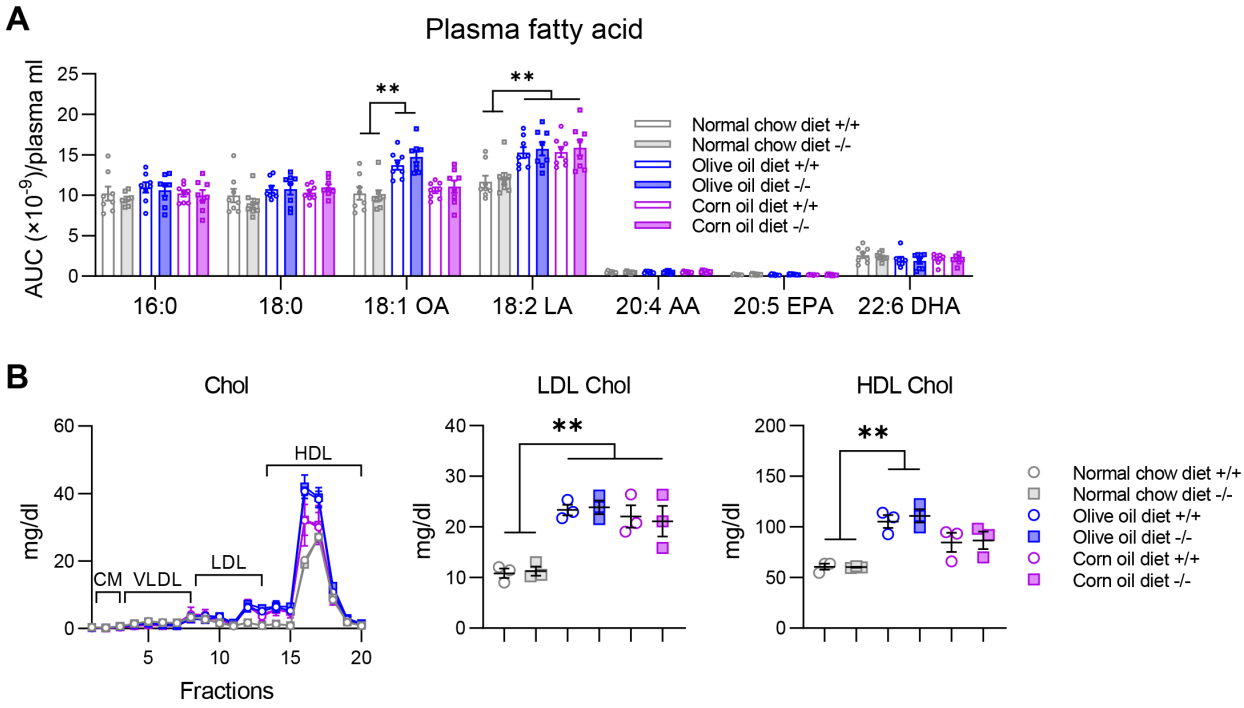

Figure S8.

Plasma levels of fatty acids and lipoproteins in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice fed a normal chow, olive oil-rich, or corn oil-rich diet. Related to Figure 7.

(**A**) LC-ESI-MS/MS of plasma fatty acids in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice (n = 8). (**B**) High-performance liquid chromatography (HPLC) profiles of cholesterol (Chol) in plasma lipoproteins in *Pla2g5*$^{+/+}$ and *Pla2g5*$^{-/-}$ mice (n = 3). The mice were fed a normal chow, olive oil-, or corn oil-rich diet. *P <0.05, **P <0.01, and ns = not significant by two-way ANOVA followed by Tukey's multiple comparisons test. Data are represented as mean ± SEM of the indicated number (n) of biological replicates.

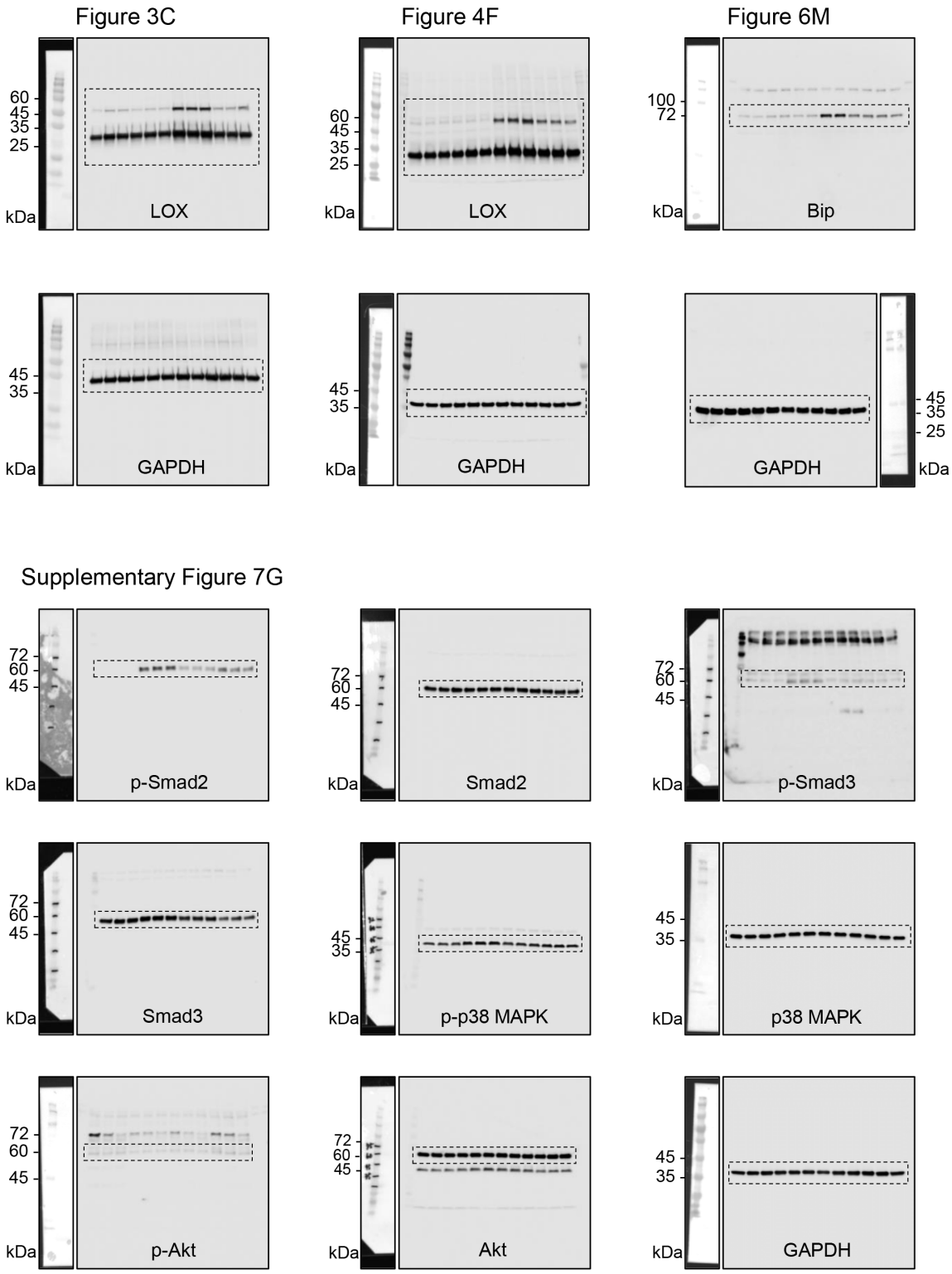

Figure S9.

Full representation of immunoblots shown in individual figures.

Molecular weight markers are indicated on the left or right margins.

Table S1.

Compositions of western diet, olive oil-rich diet, and corn oil-rich diet.

	Western diet		Olive oil diet		Corn oil diet	
	(g %)	(kcal %)	(g %)	(kcal %)	(g %)	(kcal %)
Protein	20	17	24	21	24	21
Carbohydrate	50	43	45	39	45	39
Fat	21	41	20	40	20	40
Total		100		100		100
kcal/g	4.7		4.61		4.61	
Ingredient	(g)	(kcal)	(g)	(kcal)	(g)	(kcal)
Casein	195	780	200	800	200	800
DL-Methionine	3	12	3	12	3	12
Corn Starch	50	200	125	500	125	500
Maltodextrin	100	400				
Sucrose	341	1364	249	996	249	996
Cellulose, BW200	50	0	50	0	50	0
Mineral Mix S10001	35	0	35	0	35	0
Vitamin Mix V10001	10	40	10	40	10	40
Choline Bitartrate	2	0	2	0	2	0
Milk Fat	200	1800	0	0	0	0
Oils						
Soybean Oil	0	0	10	90	10	90
Olive Oil	0	0	162.7	1464	0	0
Corn Oil	10	90	0	0	162.7	1464
Total	1001.54	4686	846.7	3902	846.7	3902

Table S2.

PCR primers used in this study.

Accession numbers for TaqMan probes (Applied Biosystems) are indicated.

Mouse genes	Assay no.
Pla2g1b	Mm00478249_m1
Pla2g2d	Mm00478250_m1
Pla2g2e	Mm00478870_m1
Pla2g2f	Mm00478872_m1
Pla2g3	Mm01191142_m1
Pla2g5	Mm00448162_m1
Pla2g10	Mm00449532_m1
Tgfb1	Mm01178820_m1
Acta2	Mm00725412_s1
Col1a1	Mm00801666_g1
Col3a1	Mm01254476_m1
Lox	Mm00495386_m1
Loxl1	Mm01145738_m1
Loxl2	Mm00804740_m1
Loxl3	Mm01184865_m1
Loxl4	Mm00446385_m1
Il1b	Mm00434228_m1
Il6	Mm00446190_m1
Tnf	Mm00443258_m1
Mmp2	Mm00439506_m1
Mmp9	Mm00442991_m1
Gata3	Mm00484683_m1
Pla2g4a	Mm00447040_m1
Alox12	Mm00545833_m1
Alox15	Mm00507789_ml
Gapdh	Mm99999915_g1

Human genes	Assay no.
PLA2G1B	Hs00386701_m1
PLA2G2A	Hs00179898_m1
PLA2G2D	Hs00173860_m1
PLA2G2E	Hs00173897_m1
PLA2G2F	Hs00224482_m1
PLA2G3	Hs00210447_m1
PLA2G5	Hs00173472_m1
PLA2G10	Hs00358567_m1
LOX	Hs00942480_m1
GATA3	Hs00231122_m1
BIP	Hs00607129_gH
ATF4	Hs00909569_g1
CHOP	Hs00358796_g1
ERdj4	Hs01052402_m1
EDEM1	Hs00976004_m1
PDIA2	Hs00429010_m1
GAPDH	Hs99999905_m1

Table S3.

A list of representative multiple reaction monitoring (MRM) transitions used in LC-ESI-MS/MS.

Quantification was performed based on the peak area of the MRM transition and calibration curve obtained with an authentic standard for each compound. DP; Declustering potential, EP; Entrance potential, CE; Collision energy, CXP; Collision cell exit potential.

Molecular species	Parent ion (*m/z*)	Product ion (*m/z*)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)	Authentic standard sources
Fatty acids							
16:0	255.2	255.2	-105	-10	-5	-14	Cayman Chemicals
18:0	283.3	283.3	-105	-10	-5	-14	Cayman Chemicals
18:1 (OA)	281.2	281.2	-105	-10	-5	-14	Cayman Chemicals
18:2 (LA)	279.0	279.0	-105	-10	-5	-14	Cayman Chemicals
20:0	311.3	311.3	-105	-10	-5	-14	Cayman Chemicals
20:1	309.3	309.3	-105	-10	-5	-14	Cayman Chemicals
20:2	307.3	307.3	-105	-10	-5	-14	Cayman Chemicals
20:4 (AA)	303.2	259.1	-105	-10	-18	-14	Cayman Chemicals
20:5 (EPA)	301.2	257.2	-80	-10	-16	-14	Cayman Chemicals
22:5	329.2	329.2	-90	-10	-20	-10	Cayman Chemicals
22:6 (DHA)	327.2	283.2	-90	-10	-16	-14	Cayman Chemicals
Lysophospholipids							
LPC (16:0)	540.4	480.4	-80	-10	-24	-15	Avanti Polar Lipids
LPC (18:0)	568.4	508.4	-80	-10	-24	-15	Avanti Polar Lipids
LPC (18:1)	566.4	506.4	-80	-10	-24	-15	Avanti Polar Lipids
LPE (16:0)	452.3	196.0	-130	-10	-36	-15	Avanti Polar Lipids
LPE (18:0)	480.3	196.0	-130	-10	-36	-15	Avanti Polar Lipids
LPE (18:1)	478.3	196.0	-130	-10	-36	-15	Avanti Polar Lipids
LPA (16:0)	409.1	152.8	-100	-10	-28	-11	Avanti Polar Lipids
LPA (18:0)	437.2	152.8	-110	-10	-32	-9	Avanti Polar Lipids
LPI (18:0)	599.2	241.0	-95	-10	-48	-11	Avanti Polar Lipids
LPS (18:0)	524.3	152.8	-100	-10	-28	-13	Avanti Polar Lipids
Lipid mediators							
PGE_2	351.2	271.2	-75	-10	-24	-14	Cayman Chemicals
PGD_2	351.2	271.2	-75	-10	-24	-14	Cayman Chemicals
TXB_2	369.2	195.1	-90	-10	-20	-10	Cayman Chemicals
6-keto-$PGF_{1\alpha}$	369.2	163.1	-100	-10	-36	-11	Cayman Chemicals

12-HHT	279.1	179.0	-85	-10	-18	-13	Cayman Chemicals
LTB_4	335.2	194.9	-95	-10	-22	-13	Cayman Chemicals
LTD_4	495.3	177.0	-90	-10	-28	-10	Cayman Chemicals
LXA_4	351.2	217.2	-75	-10	-28	-14	Cayman Chemicals
12-HETE	319.2	179.1	-85	-10	-20	-13	Cayman Chemicals
15-HETE	319.2	219.1	-90	-10	-15	-10	Cayman Chemicals
9*S*-HODE	295.1	170.9	-90	-10	-26	-10	Cayman Chemicals
13*S*-HODE	295.1	170.9	-95	-10	-26	-14	Cayman Chemicals
RvE1	349.2	195.1	-90	-10	-20	-10	Cayman Chemicals
RvD1	375.2	215.1	-65	-10	-26	-14	Cayman Chemicals
RvD2	375.2	174.8	-85	-10	-30	-13	Cayman Chemicals
Internal standards							
d5-EPA	306.1	262.0	-95	-10	-18	-5	Cayman Chemicals
LPC (17:0)	554.4	494.3	-75	-10	-24	-13	Avanti Polar Lipids
d4-PGE_2	355.1	275.1	-75	-10	-24	-13	Cayman Chemicals