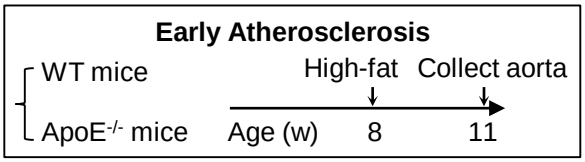
Supplemental Figure I

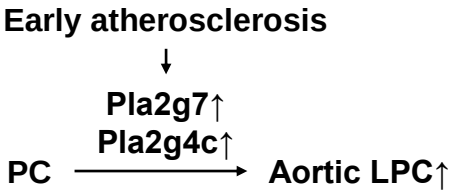
A



B

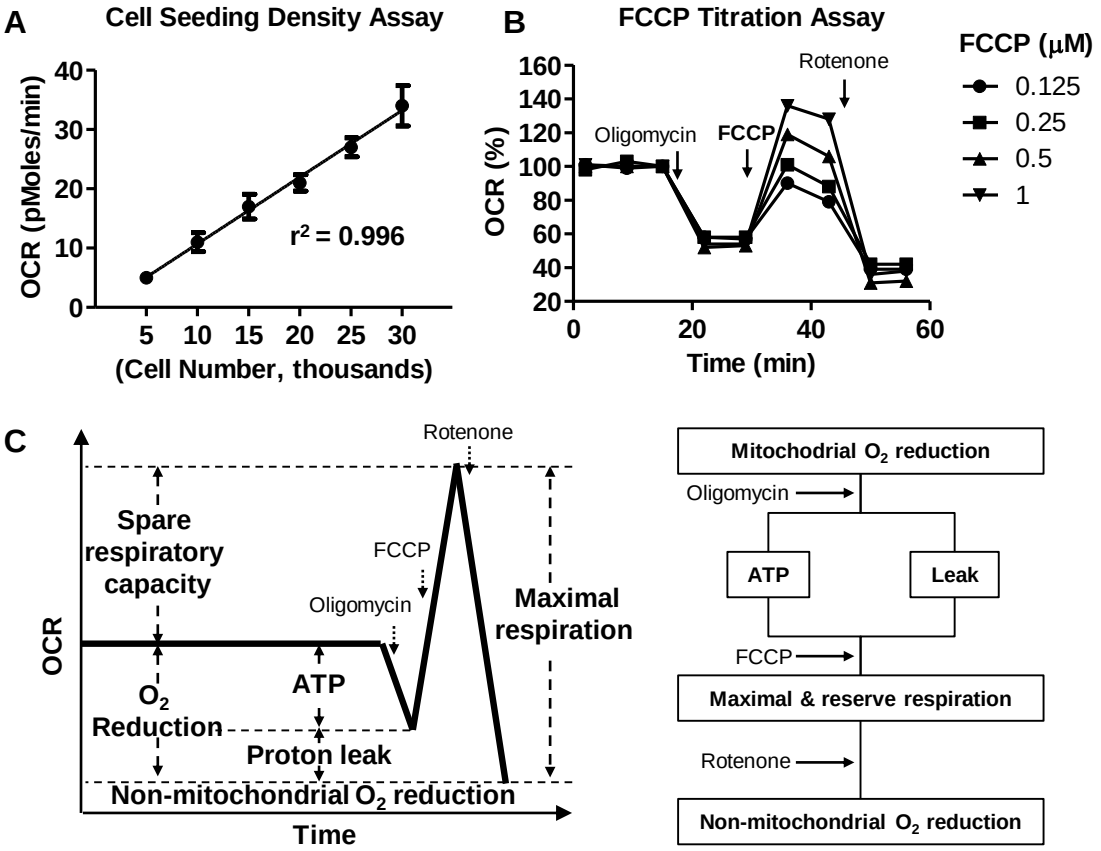
Name	Fold change (ApoE$^{-/-}$ / WT)	*P* value
Palmitate (16:0)	0.9604	0.5733
Stearate (18:0)	0.9354	0.3378
Oleate (18:1n9)	1.1360	0.2185

C



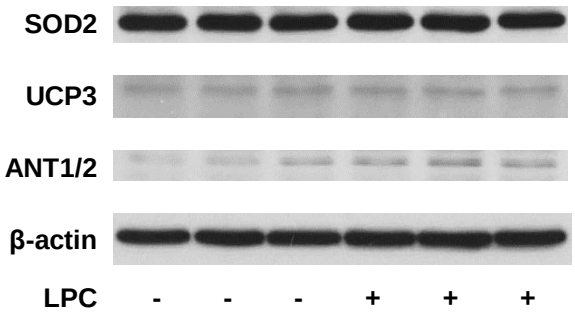
Supplemental figure I. Aortic LPC species are induced during early atherogenesis in mice. A. Experiment design. Wild-type (WT) mice and apolipoprotein E knockout (ApoE$^{-/-}$) mice were fed with high-fat diet for 3 weeks (w) starting from 8 week. Aortas were collected from these mice for metabolomics and microarray analysis. **B. Long-chain fatty acids were not significantly induced in the aortas of mice during early atherosclerosis.** Relative expression fold changes and statistic *P* values of three aortic long chain fatty acid between WT and ApoE$^{-/-}$ were shown (n=8 per group). **C. Schematic representation of how aortic LPC species are induced during early atherosclerosis.** During early atherosclerosis, LPC could be generated from phosphatidylcholine (PC) due to increased Pla2g7 and Pla2g4c enzyme expression in the aorta. WT, wild-type; ApoE$^{-/-}$, apolipoprotein E deficient; PC: phosphatidylcholine.

Supplemental Figure II



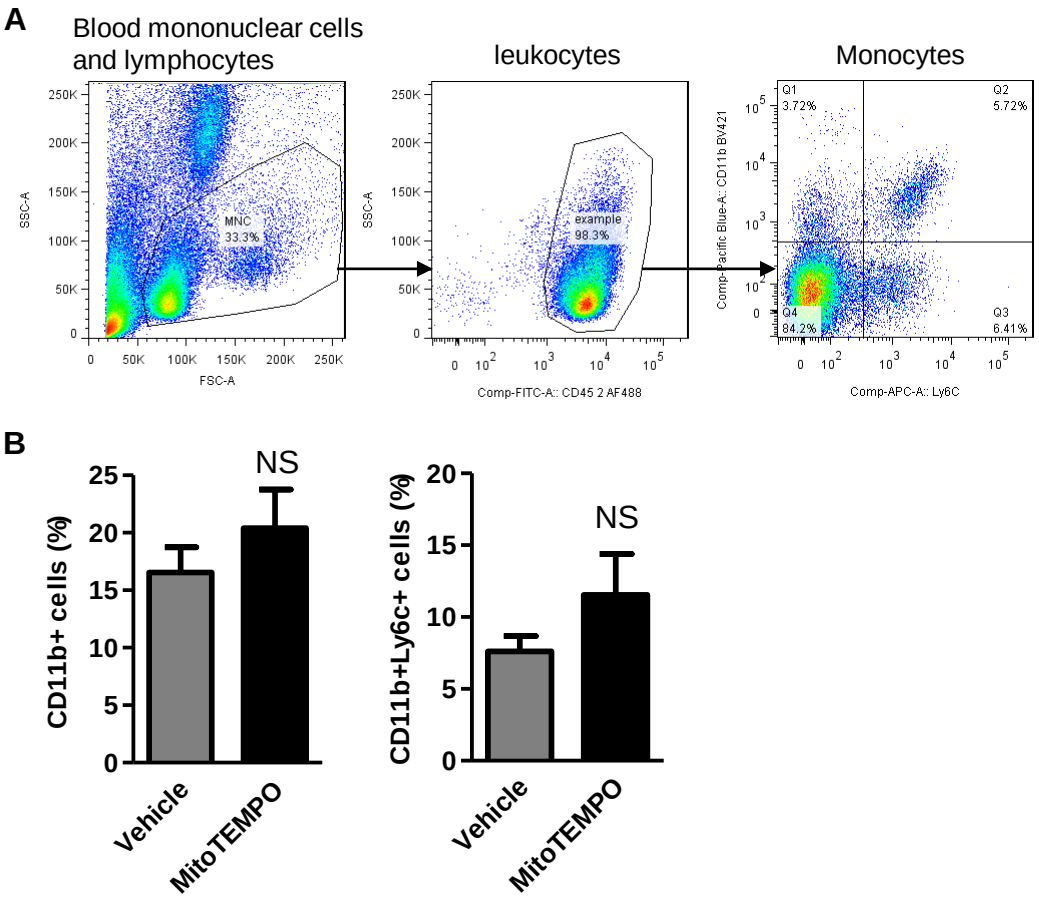
Supplemental Figure II. Mitochondrial function profiling by Seahorse XF96 analyzer in human aortic endothelial cells (HAECs). A. Linear correlation between cell seeding density and OCR. Varying densities of HAECs (5,000 cells /well to 30,000 cells /well) were seeded into 96-well plate and oxygen consumption rate (OCR) was measured by Seahorse XF96 Analyzer (n=6 per group). **B. FCCP dose-dependently increased OCR.** After three basal measurements of OCR, XF Mito Stress Test was applied by sequential adding of Oligomycin (1 μM), FCCP, and Rotenone (1 μM). Different dose of FCCP (0.125 μM, 0.25 μM, 0.5 μM, and 1 μM) was added in each group (n=22-24 per group). **C. Schematic representation of how the 6 mitochondrial parameters are determined by Seahorse XF96 analyzer.** For all panels, values represent mean ± SEM.

Supplemental Figure III



Supplemental Figure III. The effects of LPC on the expression of mitochondrial antioxidant SOD2 and uncoupling proteins including UCP3 and ANT in human aortic endothelial cells (HAECs). HAEC were treated with LPC (10 μM) or vehicle control for 18 hours before proteins were collected for Western blot analysis. SOD2, superoxide dismutase 2; UCP3, uncoupling protein 3; ANT1/2, Adenine nucleotide translocator 1/2.

Supplemental Figure IV



Supplemental Figure IV. MitoTEMPO does not significantly affect monocyte in the blood. At eight weeks of age, mini-pumps containing saline or MitoTEMPO (1500 μg/kg/day) were implanted in ApoE$^{-/-}$ mice before they were fed with a high fat diet for three weeks. Flow cytometry analyses were carried out afterwards to determine the monocyte numbers in the blood. **A. Gating strategy of blood monocyte by flow cytometry. B. MitoTEMPO did not affect blood monocyte numbers.** Percentages of CD11b^{+} total monocytes (Q1+Q2 in panel B) and CD11b^{+}Ly6c^{+} inflammatory monocytes (Q2 in panel B) in CD45^{+} blood leukocyte cell population between the two groups were shown (n=7-9 mice per group). Values represent mean ± SEM. NS, not significant.

Supplemental Table I

Gene Symbol	ApoE$^{-/-}$ vs WT FC	*P* value
Pla2g7 (Lp-PLA$_2$)	1.94	0.012
Pla2g4c	1.42	0.016
Pla2g5	1.32	0.075
Pla2g3	1.06	0.699
Pla2g1b	1.05	0.653
Pla2g2d	1.04	0.872
Pla2g2e	1.04	0.801
Pla2g4b	1.02	0.858
Pla2g4d	1.02	0.903
Pla2g2f	1.01	0.957
Pla2g4f	1	0.991
Pla2g12a	1	0.984
Pla2g10	0.98	0.886
Pla2g6	0.97	0.721
Pla2g2c	0.89	0.434
Pla2g12b	0.89	0.284
Pla2g2a	0.88	0.392
Pla2g4e	0.86	0.075
Pla2g4a	0.83	0.073
Housekeeping genes: Actb	0.97	0.463
Housekeeping genes: Gapdh	0.97	0.761
Housekeeping genes: Nono	1.06	0.296

Supplemental Table I. LPC processing enzyme Pla2g4c and Pla2g7 are significantly induced in the aortas during early atherosclerosis. Fold change (FC) and statistic *P* value of expression changes of phospholipase A2 superfamily members and three housekeeping genes (*Actb, Gapdh, and Nono*) between apolipoprotein E knockout (ApoE$^{-/-}$) and wild-type (WT) mice fed with 3 weeks of high fat diet were shown (n=5). LP-PLA$_2$, lipoprotein-associated phospholipase A2. ApoE$^{-/-}$, apolipoprotein E knockout mice; WT, wild-type mice; FC, fold change. PLA2 family enzymes that were significantly induced were highlighted.

Supplemental Table II

Gene Name	Fold change (LPC vs control)	Fold change (LPC + MT vs control)
PLAT	6.9653	1.9204
NOS3	3.5738	2.2601
TEK	2.7487	1.6439
PTGIS	2.3309	1.4531
THBD	1.792	1.4466
BCL2L1	1.6958	1.9524
ACE	1.6474	1.3946
COL18A1	1.5245	1.8763
VEGFA	1.512	1.756
CDH5	1.4943	1.5694
CASP3	1.449	1.1829
SERPINE1	1.449	1.5977
PTGS2	1.4457	1.1202
KDR	1.4453	1.3329
FLT1	1.4402	1.3048
HMOX1	1.4375	1.531
IL6	1.4236	1.043
KIT	1.4021	1.8298
PROCR	1.3998	1.3774
IL7	1.3771	1.2878
FAS	1.3546	1.1762
ADAM17	1.3173	1.1737
TIMP1	1.3151	1.3525
VWF	1.3127	1.2311
ICAM1	1.3005	0.9859
TGFB1	1.2946	1.2359
ITGA5	1.2933	1.2661
PTK2	1.2757	1.3421
CFLAR	1.249	1.4001
MMP2	1.2393	1.0168
ITGB3	1.2388	1.1837
BAX	1.234	1.3792
F2R	1.224	1.1042
FGF2	1.2057	1.2843
HIF1A	1.1875	1.2849
PECAM1	1.1809	1.1546
ITGB1	1.1714	1.204
SPHK1	1.1669	1.3976
ENG	1.1666	0.6155
FN1	1.1474	1.1297
PLAU	1.1415	0.9469
ITGAV	1.1275	1.0781
ANXA5	1.0928	1.1302
TNFSF10	1.0798	1.2652
MMP1	1.068	0.6514
TFPI	1.0469	1.2842
PGF	1.0161	1.0273
CASP1	1.0134	0.8812
EDN1	1.0085	1.0771
OCLN	0.9929	1.0649
CAV1	0.9839	0.7808
SOD1	0.9183	1.3463
THBS1	0.9016	0.8662
VCAM1	0.8992	0.9438
SELE	0.735	0.692
CCL2	0.7212	0.771
F3	0.5965	0.4462

Supplemental Table II. LPC-induced mtROS contribute to endothelial activation. HAECs were treated with vehicle, LPC (10 μM), LPC (10 μM) plus MitoTEMPO (1μM) for 6 hours and RNAs were collected for Human EC Biology PCR array (QIAGEN) analysis. Fold changes of gene expression between LPC versus control and LPC plus MitoTEMPO (MT) versus control were shown. Pooled samples (n=3) in each group. The PCR array was repeated once with similar results.

Supplemental Table III

Gene	PMID (AP-1 target)	PMID (ROS target)
ICAM1	8834767	8958136
PLAT	19088796	21468253
IL6	11098847	10564193
MMP2	12371906	11223425
PTGS2	10825375	20826536
CASP3	N.F.	9840939
PTGIS	N.F.	N.F.
TEK	N.F.	N.F.
F2R	N.F.	25994290
FAS	N.F.	12504821
NOS3	20371606	12569550
ACE	12433834	18227481
ADAM17	N.F.	19846666
THBD	20472895	23844043

Supplemental Table III. LPC contributes to endothelial activation by upregulating AP-1 and ROS. The genes that were induced by LPC but inhibited by mtROS inhibitor (Figure 5A) were also found to be AP-1 and ROS targets in previous studies. Pubmed IDs (PMIDs) which reported the relationship between these genes and AP-1/ROS were shown in the table. N.F., not found.