Supplementary Materials file

Pharmacological inhibition of lysine-specific demethylase 1A reduces atherosclerotic lesion formation in apolipoprotein E-deficient mice by a mechanism involving decreased oxidative stress and inflammation; evidence for potential implications in human atherosclerosis

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Parameter	Values (n = 25)
Gender: Female (F)/Male (M) (N, %)	9/16 (36%/64%)
Age (years)	64.2 ± 8.3
Body-mass index (BMI) (kg/m2)	26.1 ± 3.6
Current smoker (N, %)	16 (64%)
Former smoker (N, %)	4 (16%)
Alcohol consumption (N, %)	1 (4%)
Diabetes (N, %)	8 (32%)
Hypertension (N, %)	21 (84%)
Other cardiac diseases	
Coronary artery disease (CAD) (N, %)	9 (36%)
Peripheral artery disease (PAD) (N, %)	8 (32%)
Glucose (mg/dl)	110.8 ± 33.6
TC (mg/dl)	175.1 ± 59.4
CHOL LDL (mg/dl)	116.8 ± 50.2
CHOL HDL (mg/dl)	43.5 ± 11.3
TG (mg/dl)	120 ± 46.2
Medication	
Aspirin (N, %)	23 (92 %)
Insulin (N, %)	1 (4%)
Metformin (N, %)	7 (28%)
Sulphonylurea (N, %)	2 (8 %)
Statin (N, %)	24 (96 %)

Table S1. Clinical characteristics of the patients. TC: total cholesterol; CHOL LDL: cholesterol low-density lipoprotein fraction; CHOL HDL: cholesterol high-density lipoprotein fraction; TG: triglycerides.

Gene	GeneBank® Accession Number	Sequences of oligonucleotide primers
Mouse Nox1	NM_172203.2	S: 5'-CATCCAGTCTCCAAACATGACA-3' A: 5'-GCTACAGTGGCAATCACTCCAG-3'
Mouse Nox2	FJ168469.1	S: 5'-ACTCCTTGGGTCAGCACTGG-3' A: 5'-GTTCCTGTCCAGTTGTCTTCG-3'
Mouse Nox4	AF276957.1	S: 5'-TGAACTACAGTGAAGATTTCCTTGAAC-3' A: 5'-GACACCCGTCAGACCAGGAA-3'
Mouse p22phox	NM_007806.3	S: 5'-TGGCCTGATTCTCATCACTGG-3' A: 5'-GGGACAACTCCACAGAAACTC-3'
Mouse MCP-1	NM_011333.3	S: 5'-ACTGAAGCCAGCTCTCTCTTCCTC-3' A: 5'-TTCCTTCTTGGGGTCAGCACAGAC-3'
Mouse TNFα	NM_013693.3	S: 5'-GGTGCCTATGTCTCAGCCTCT-3' A: 5'-CATCGGCTGGCACCACTAGTT-3'
Mouse NOS2	NM_010927.4	S: 5'-CAGAGGACCCAGAGACAAGC-3' A: 5'-TGCTGAAACATTTCCTGTGC-3'
Mouse β-Actin	NM_007393.5	S: 5'-CGTGAAAAGATGACCCAGATCA-3' A: 5'-TGGTACGACCAGAGGCATACAG-3'
Human Nox1	NM_013955	S: 5'-CACAAGAAAAATCCTTGGGTCAA-3' A: 5'-GACAGCAGATTGCGACACACA-3'
Human Nox2	KU178009.1	S: 5'-TCACTTCCTCCACCAAAACC-3' A: 5'-CACCTTCTGTTGAGATCGCC-3'
Human Nox4	NM_016931	S: 5'-TGGCTGCCCATCTGGTGAATG-3' A: 5'-CAGCAGCCCTCCTGAAACATGC-3'
Human Nox5	NM_024505	S: 5'-CAGGCACCAGAAAAGAAAGCAT-3' A: 5'-ATGTTGTCTTGGACACCTTCGA-3'
Human p22phox	NM_000101.4	S: 5'-GTTTGTGTGCCTGCTGGAGT-3' A: 5'-TGGGCGGCTGCTTGATGGT-3'
Human β-Actin	NM_001101	S: 5'-CTGGCACCCAGCACAATG-3' A: 5'- GCCGATCCACACGGAGTACT-3'

Table S2. Sequences of sense (S) and antisense (A) oligonucleotide primers used in real-time PCR assays.

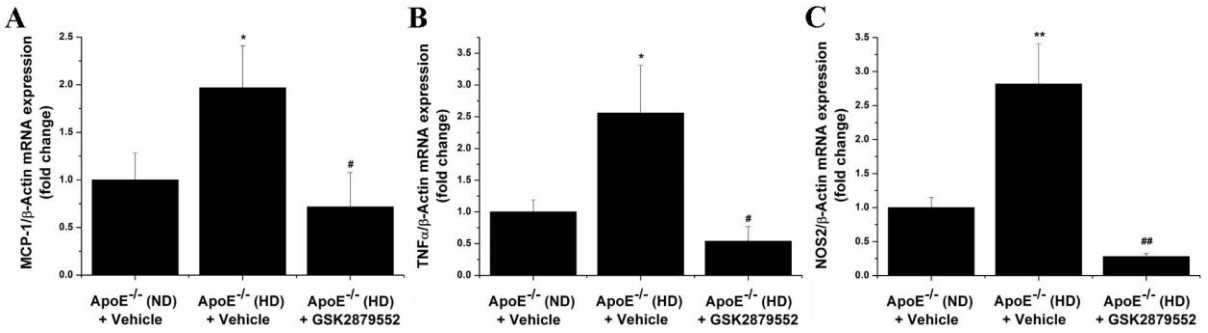

Figure S1. LSD1 blockade suppresses the up-regulation of (A) MCP-1, (B) TNFα, and (C) NOS2 transcript levels in the atherosclerotic aorta of ApoE-/- (HD) mice. n=3, **P* < 0.05, ***P* < 0.01. *P*-values were taken in relation to vehicle-treated ApoE-/- (ND) condition. # *P* < 0.05, ## *P* < 0.01. *P*-values were taken in relation to vehicle-treated ApoE-/- (HD) condition.

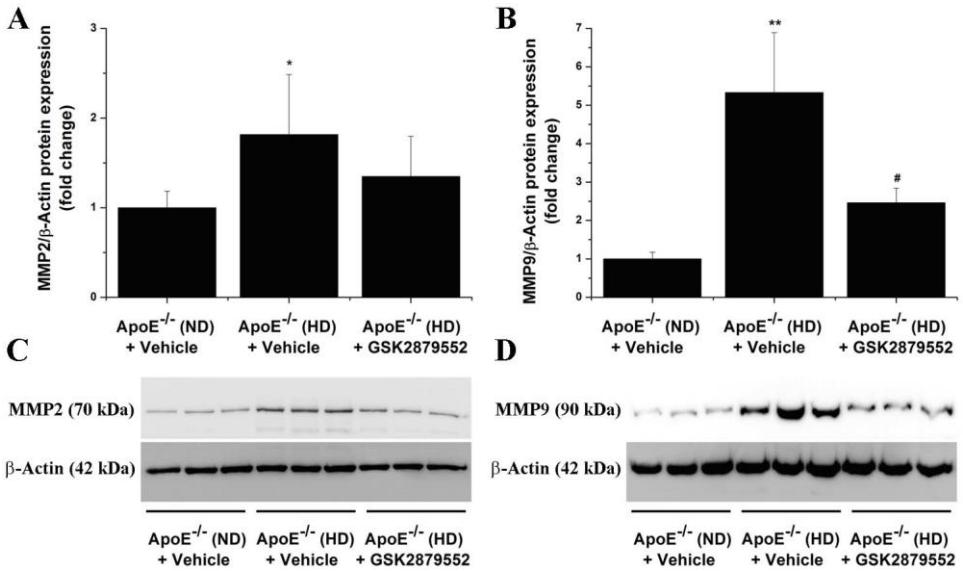

Figure S2. (A, B) LSD1 mediates the up-regulation of MMP9, but not MMP2 protein levels in the atherosclerotic aorta of ApoE-/- mice. (C, D) Representative immunoblots depicting the changes in MMP2 and MMP9 protein expression levels function of experimental condition. n=3-6, **P* < 0.05, ***P* < 0.01. *P*-values were taken in relation to vehicle-treated ApoE-/- (ND) condition. # *P* < 0.05. *P*-value was taken in relation to vehicle-treated ApoE-/- (HD) condition.

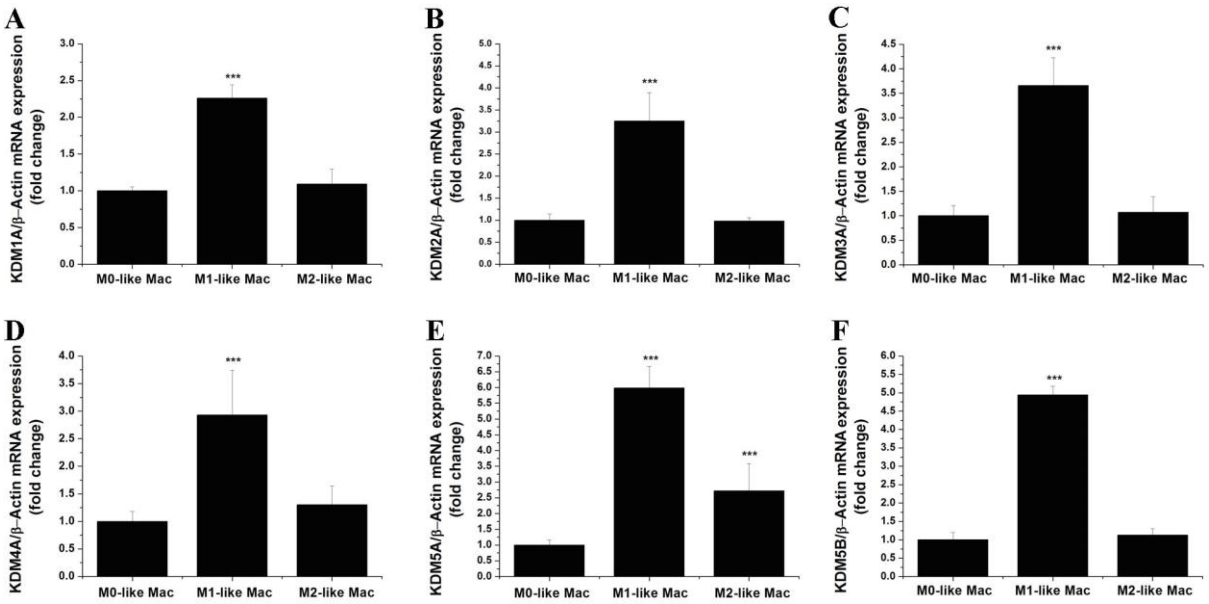

Figure S3. Elevated mRNA expression levels of (A) LSD1/KDM1A, (B) KDM2A, (C) KDM3A, (D) KDM4A, (E) KDM5A, and (F) KDM5B subtypes are associated with a pro-inflammatory macrophage phenotype in vitro. n=4, ***$P < 0.001$. *P*-values were taken in relation to resting macrophage phenotype (M0-like Mac) condition.

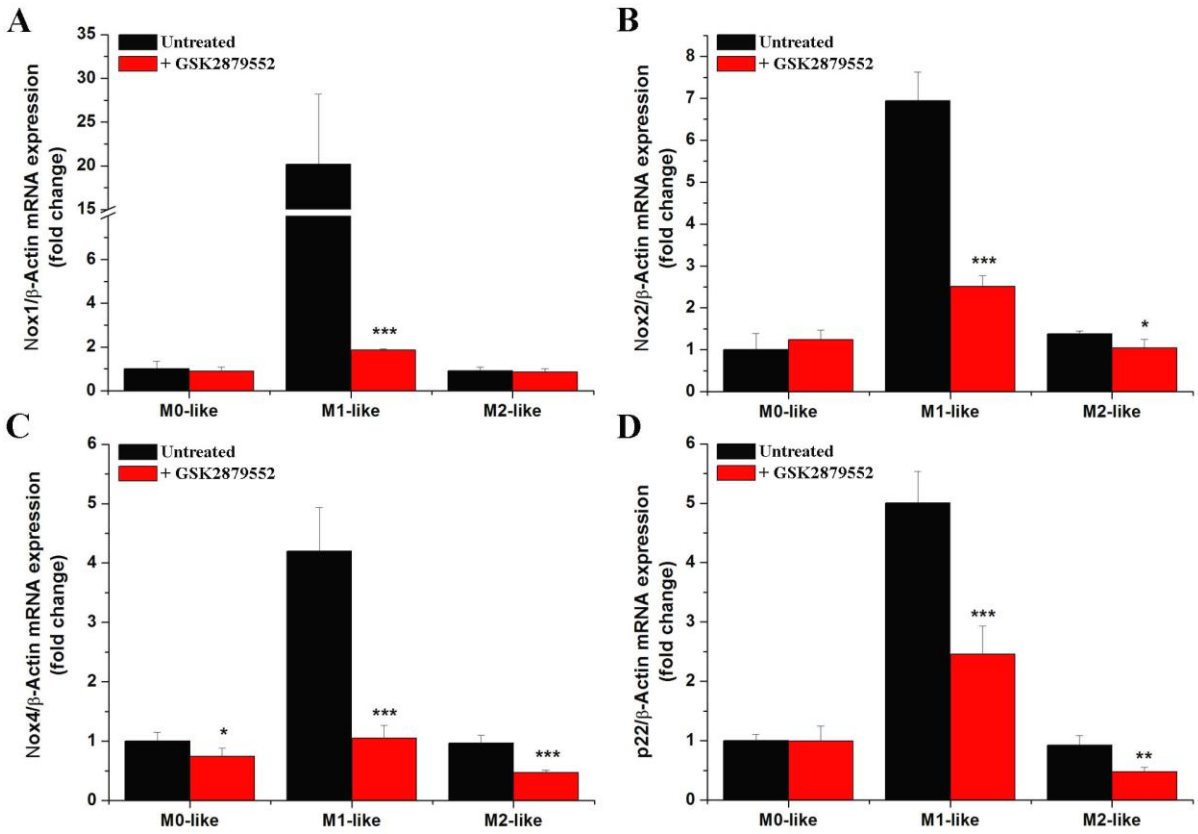

Figure S4. GSK2879552-induced pharmacological inhibition of LSD1 function suppresses the up-regulation of (A) Nox1, (B) Nox2, (C) Nox4, and (D) p22phox mRNA levels in cultured pro-inflammatory macrophages (M1-like Mac). n=4, **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *P*-values were taken in relation to untreated conditions.

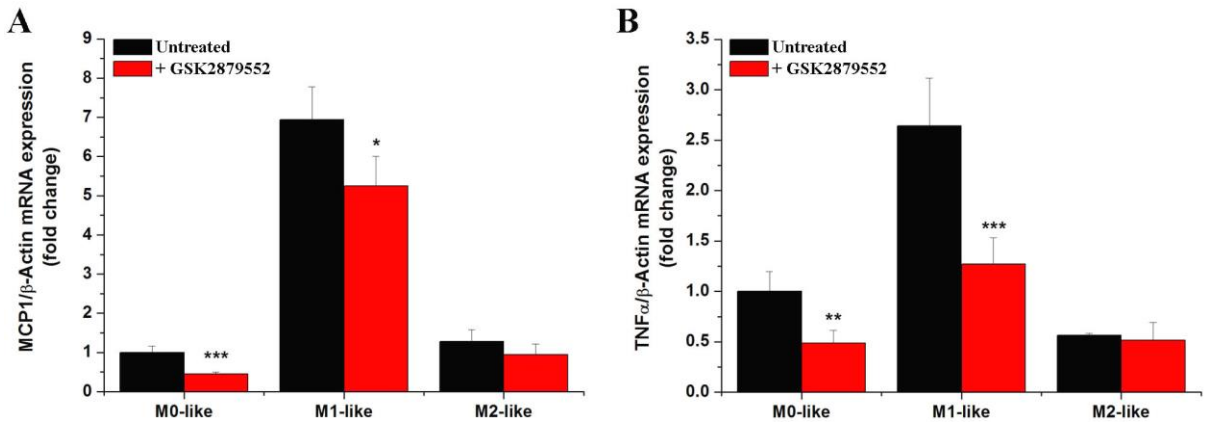

Figure S5. LSD1 blockade reduces the up-regulation of (A) MCP-1 and (B) TNFα transcript levels in cultured pro-inflammatory macrophages (M1-like Mac). n=4, **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *P*-values were taken in relation to untreated conditions.