Supplementary material

Supplementary figure legends

Supplementary Figure I

KLF4 deficient macrophage show enhanced pro-inflammatory phenotype upon modified lipid stimulation.

$KLF4^{+/+}ApoE^{-/-}$ or $KLF4^{\Delta/\Delta}ApoE^{-/-}$ macrophages were treated with OxLDL for 24 hrs and expression of pro-inflammatory markers was analyzed [iNOS by nitrite assay, IL-6 and IL-1β (ELISA)] (n=5)**.** Values for the control samples were below the detection limit.

Supplementary Figure II

Myeloid KLF4 deficiency results in enhanced M1 and attenuated M2 markers in aortic vessels. M1 [Tnfα, iNOS (Nos2), ICAM1, VCAM1, IL-6, MCP-1 and IL-1β] and M2 [programmed cell death 1 ligand 2 (Pdcd1lg2), resistin-like α (Retnla, Fizz1), the mannose receptor (Mrc1), peroxisome proliferator-activated receptorγ (PPARγ), chitinase 3–like 3 (Chi3l3, Ym1), arginase-1 (Arg1), and dectin-1 (Clec7a)] macrophages markers were analyzed by qPCR in normal chow **(A)** HFD **(B)** feed animal aortic vessel samples (n=5).

Supplementary Figure III

Myeloid KLF4 deficiency results in enhanced macrophage infiltration in atherosclerotic lesion.

Representative image (A) and quantitation (B) of immunohistochemical analysis of aortic sections for macrophage (Mac3 stained) infiltration (n=4).

Supplementary Methods

Animals and Diets

To generate macrophage-specific KLF4-knockout mice, we crossed $KLF4^{Flox/Flox}$ mice with $LysM^{Cre/Cre}$ mice (The Jackson lab) and $LysM^{Cre/Cre}KLF4^{+/+}$ ($KLF4^{+/+}$) were used as control and $LysM^{Cre/Cre}KLF4^{Flox/Flox}$ ($KLF4^{\Delta/\Delta}$) as experimental animals. For *in vivo* atherogenesis studies these mice were crossed with $ApoE^{-/-}$ mice. All studies were performed with age-matched and gender-matched controls. Mice were started on HFD (Research Diets D-12108) at 8 weeks of age. Plasma lipid profile of over- night fasted animals was assed using kits from Pointe Scientific Inc (Canton, MI). All experimental procedures were approved by the IACUC of Case Western Reserve University.

Atherosclerotic lesion analysis

Mice were sacrificed and perfused with saline and formalin for 12 minutes. The heart and aorta were harvested and fixed in 10% formalin. After careful removal of excess adventitial tissue, the aortas were stained with Sudan IV (Sigma-Aldrich) for 15 minutes, and differentiated in 80% ethanol. For *enface* preparation, aortas were opened longitudinally and pinned out on plain black wax. Images of Sudan IV stained aortas were taken and analyzed with Image-Pro 6.2 software. Results were expressed as the percentage of the stained Sudan IV area to the total aorta area.

RNA isolation and qPCR

Total RNA from cultured cells or mouse tissue samples was isolated using TRIzol reagent (Invitrogen). First-strand cDNA was synthesized and subjected to qPCR with either SYBR green or Roche universal probe reagents (Universal Probe Library, Roche Applied Science) on a StepOnePlus Real-Time PCR System (Applied Biosystems). Gene expression was normalized to GAPDH, or β-actin using the ΔΔCt method.

Cell culture

Mouse peritonitis was induced by i.p. injection of 3% thioglycolate broth in 8- to 20-week-old mice as described previously[2]. Peritoneal cells were harvested at 72 hours after i.p. injection, and macrophages were enriched by quick adhesion. BMDMs were differentiated with M-CSF as described previously[7]. Cells were cultured in DMEM supplemented with 10% fetal bovine serum and antibiotics. For fluorescent LDL uptake cells were incubated with Dil-OxLDL or Dil-AcLDL (Biomedical Technologies) for 4 hrs. Cells were fixed with 3% formaldehyde/PBS and analyzed by Fluorescence microscopy or Flow cytometery. POV-PC was obtained from Cayman chemicals.

Immunohistochemistry

Aortas were paraffin embedded and 7μm serial sections were obtained. Standard immunohistochemistry procedures were used. Mac-3 antibody was obtained from BD Biosciences.

ELISA and Nitrite assay

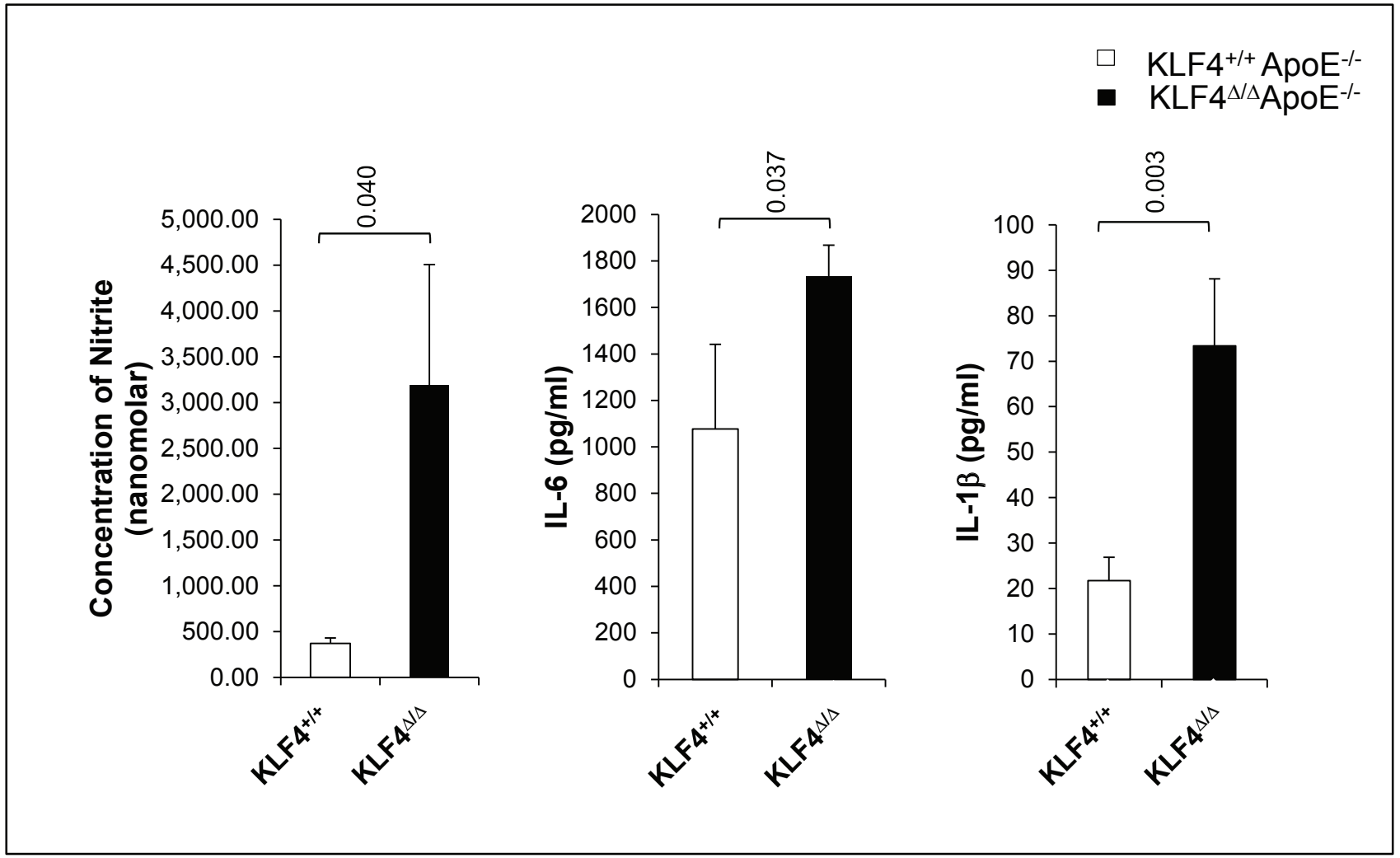
$KLF4^{+/+}ApoE^{-/-}$ or $KLF4^{\Delta/\Delta}ApoE^{-/-}$ macrophages (1.5 million cells/well in 750ul medium) were treated for 24 hrs with OxLDL in a serum free medium and cytokines in medium were analyzed using Quantikine ELISA kit (R & D systems). Nitrite concentration in medium as an indicator of iNOS activity was analyzed using a Nitric Oxide Analyzer (NOA; model 280i, Sievers Instruments, Denver, CO) using published method[1].

Statistical analysis

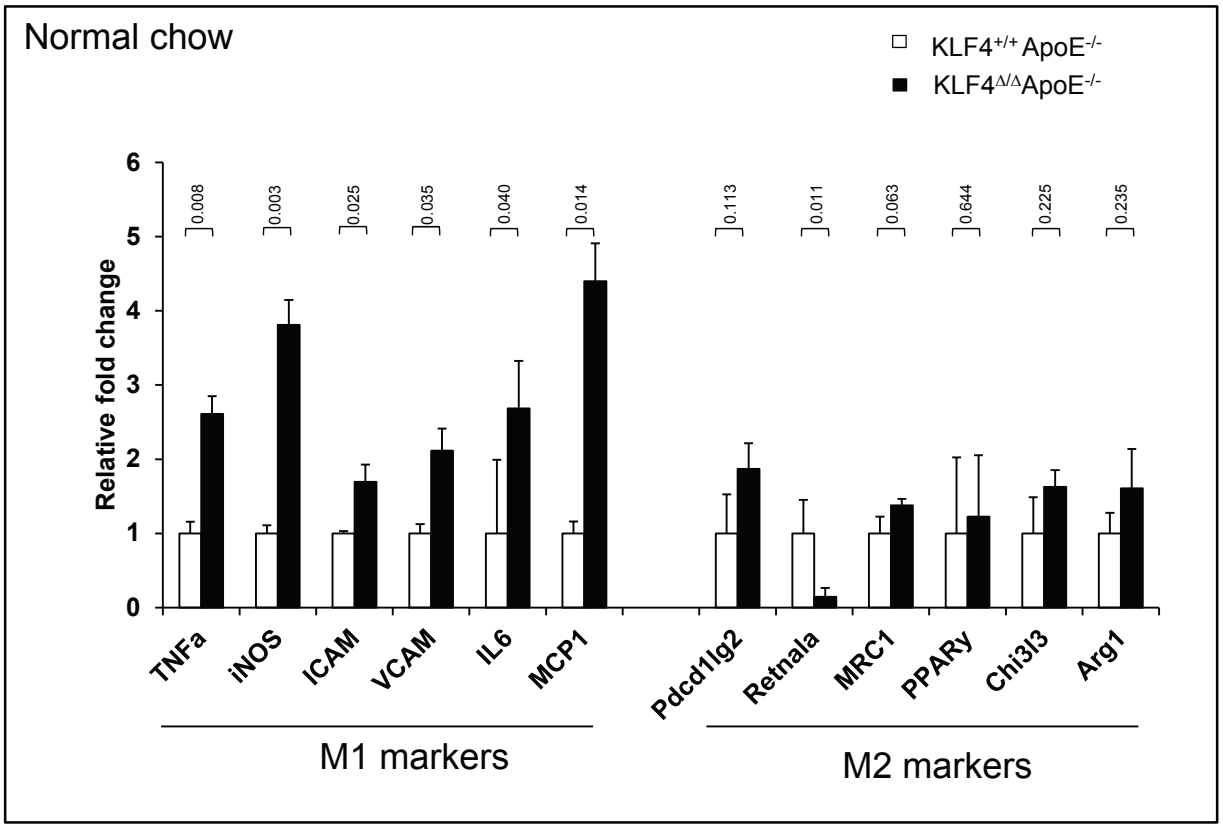
Results are presented as mean ± SEM. To analyze the difference between 2 groups, 2-tailed Student's *t* test was used. A *P* value less than 0.05 was considered as significant. Number of replicates are indicated in the figure legends.

Supplementary reference

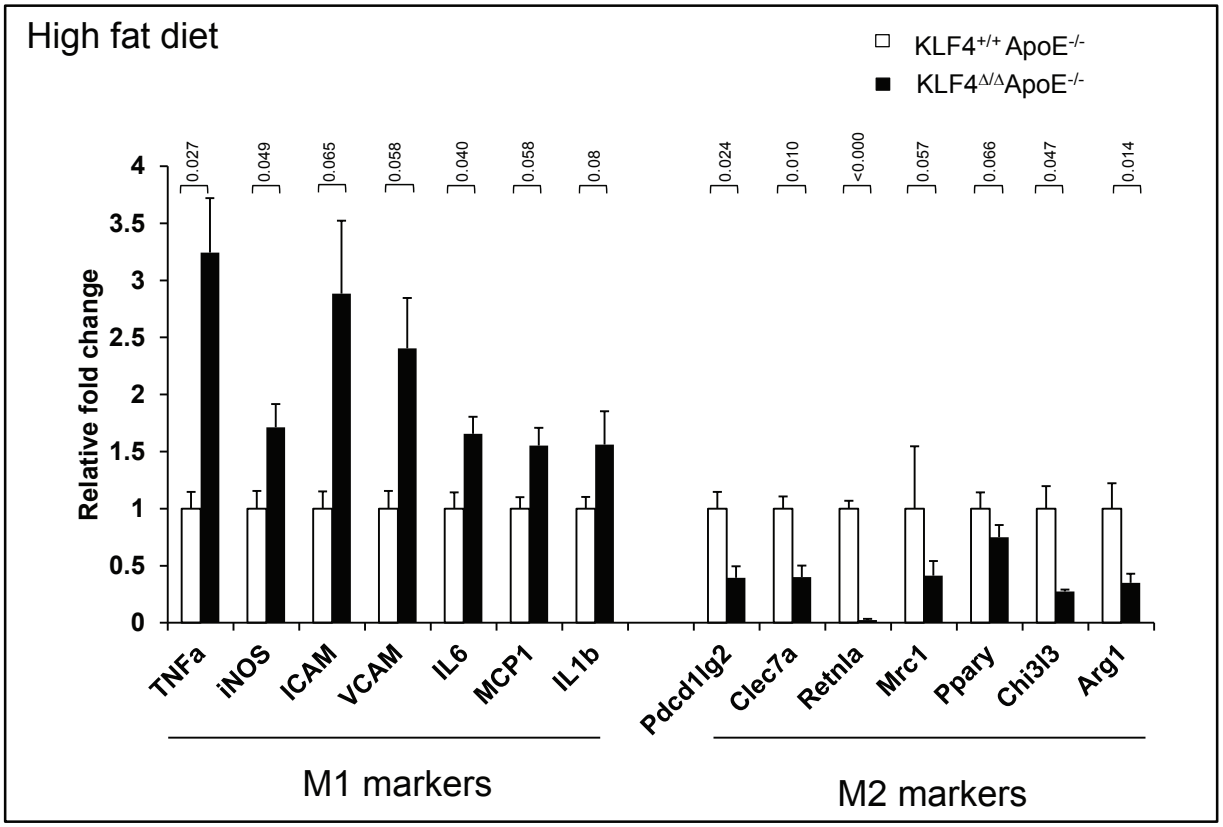
1. Hampl V, Waters CL, Archer SL. Determination of nitric oxide by the chemiluminescence reaction with ozone. Methods in Nitric oxide Research. 1996:309–318
2. Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, Hong H, Paruchuri K, Mahabeleshwar GH, Dalmas E, Venteclef N, Flask CA, Kim J, Doreian BW, Lu KQ, Kaestner KH, Hamik A, Clement K, Jain MK. Kruppel-like factor 4 regulates macrophage polarization. *J Clin Invest*. 2011;121:2736-2749



Supplementary Fig. I



Supplementary Fig. IIA



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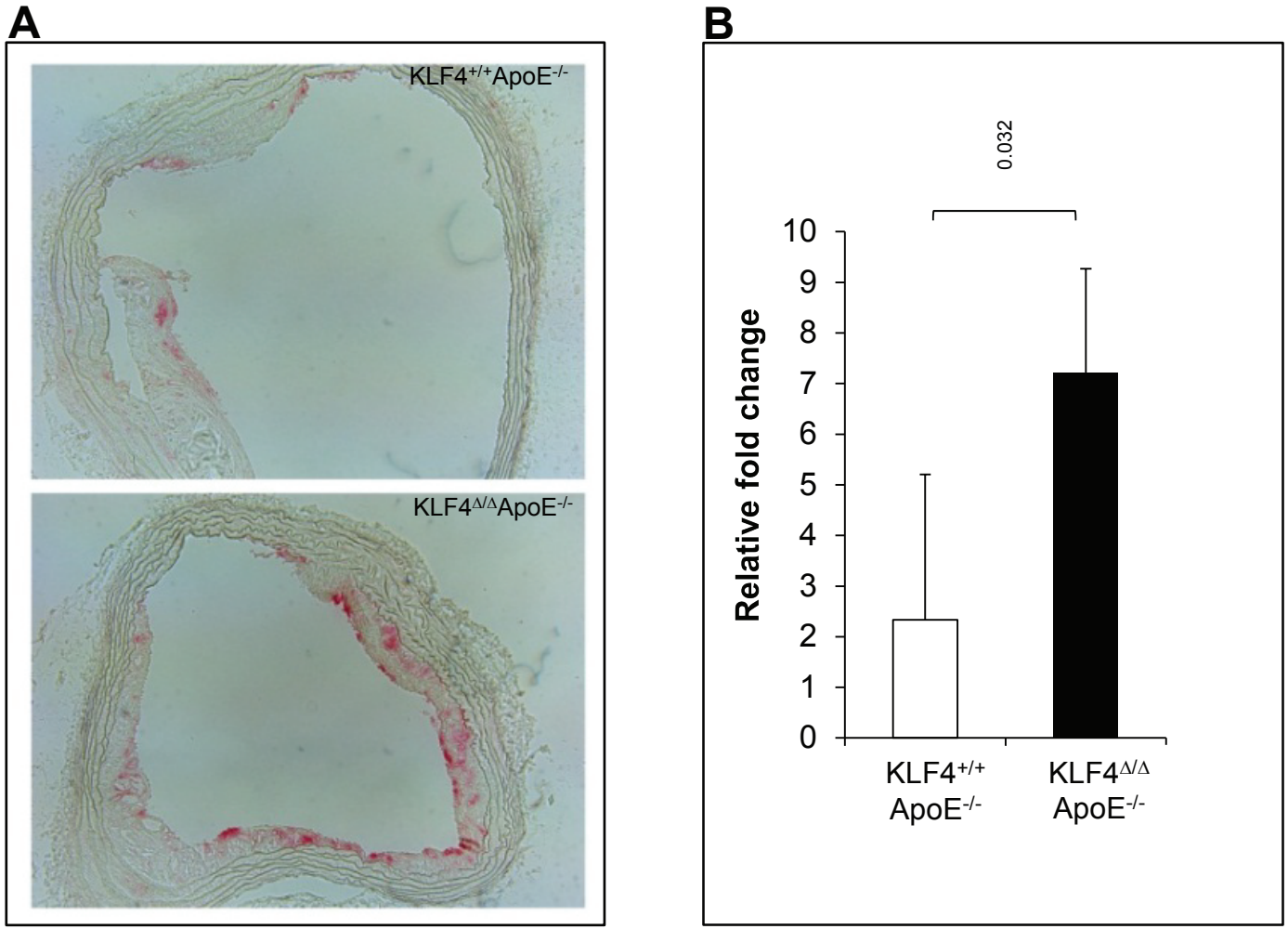

Supplementary Fig III

Supplementary table

Plasma Lipid profile: Plasma lipid profile on normal chow (n=4) and high fat diet (n=4).

Genotype	**Triglycerides**	**Total Cholesterol**	**HDL**	**LDL (calculated)**
$KLF4^{+/+}$ (Normal chow)	78.64 ±35.58	356.33 ±50.77	21.13 ±7.91	315.96 ±52.27
$KLF4^{\Delta/\Delta}$ (Normal chow)	120.91 ±39.84	381.07 ±78.11	24.30 ±6.02	330.89 ±73.4
p value	*0.091*	*0.323*	*0.296*	*0.385*
$KLF4^{+/+}$ (High Fat Diet)	88.20 ±18.83	423.22 ±13.99	35.68 ±2.65	369.37 ±11.56
$KLF4^{\Delta/\Delta}$ (High Fat Diet)	113.01 ±12.64	457.18 ±9.85	39.36 ±.72	396.13 ±8.40
p value	*0.035*	*0.013*	*0.018*	*0.015*