Iron Accumulation in Macrophages Promotes the Formation of Foam Cells and Development of Atherosclerosis

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Research

Keywords: atherosclerosis, iron metabolism, Fpn1, macrophages, foam cell formation

Posted Date: September 9th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-69230/v1

Version of Record: A version of this preprint was published on November 26th, 2020. See the published version at https://doi.org/10.1186/s13578-020-00500-5.

Abstract

Background

Macrophages that accumulate in atherosclerotic plaques contribute to progression of the lesions to more advanced and complex plaques. Although iron deposition was found in human atherosclerotic plaques, clinical and pre-clinical studies showed controversial results. Several epidemiological studies did not show the positive correlation between a systemic iron status and an incidence of cardiovascular diseases, suggesting that the iron involvement occurs locally, rather than systemically.

Results

To determine the direct in vivo effect of iron accumulation in macrophages on the progression of atherosclerosis, we generated Apoe-/- mice with a macrophage-specific ferroportin (Fpn1) deficiency (Apoe-/-Fpn1LysM/LysM). Fpn1 deficiency in macrophages dramatically accelerated the progression of atherosclerosis in mice. Pathophysiological evidence showed elevated levels of reactive oxygen species, aggravated systemic inflammation, and altered plaque-lipid composition. Moreover, Fpn1 deficiency in macrophages significantly inhibited the expression of ABC transporters (ABCA1 and ABCG1) by decreasing the expression of the transcription factor LXRα, which reduced cholesterol efflux and therefore promoted foam cell formation and enhanced plaque formation. Iron chelation relieved the symptoms moderately in vivo, but drastically ex vivo.

Conclusions

Macrophage iron content in plaques is a critical factor in progression of atherosclerosis. The interaction of iron and lipid metabolism takes place in macrophage-rich atherosclerotic plaques. And we also suggest that altering intracellular iron levels in macrophages by systemic iron chelation or dietary iron restriction may be a potential supplementary strategy to limit or even regress the progression of atherosclerosis.

Full Text

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Figures

Figure 1

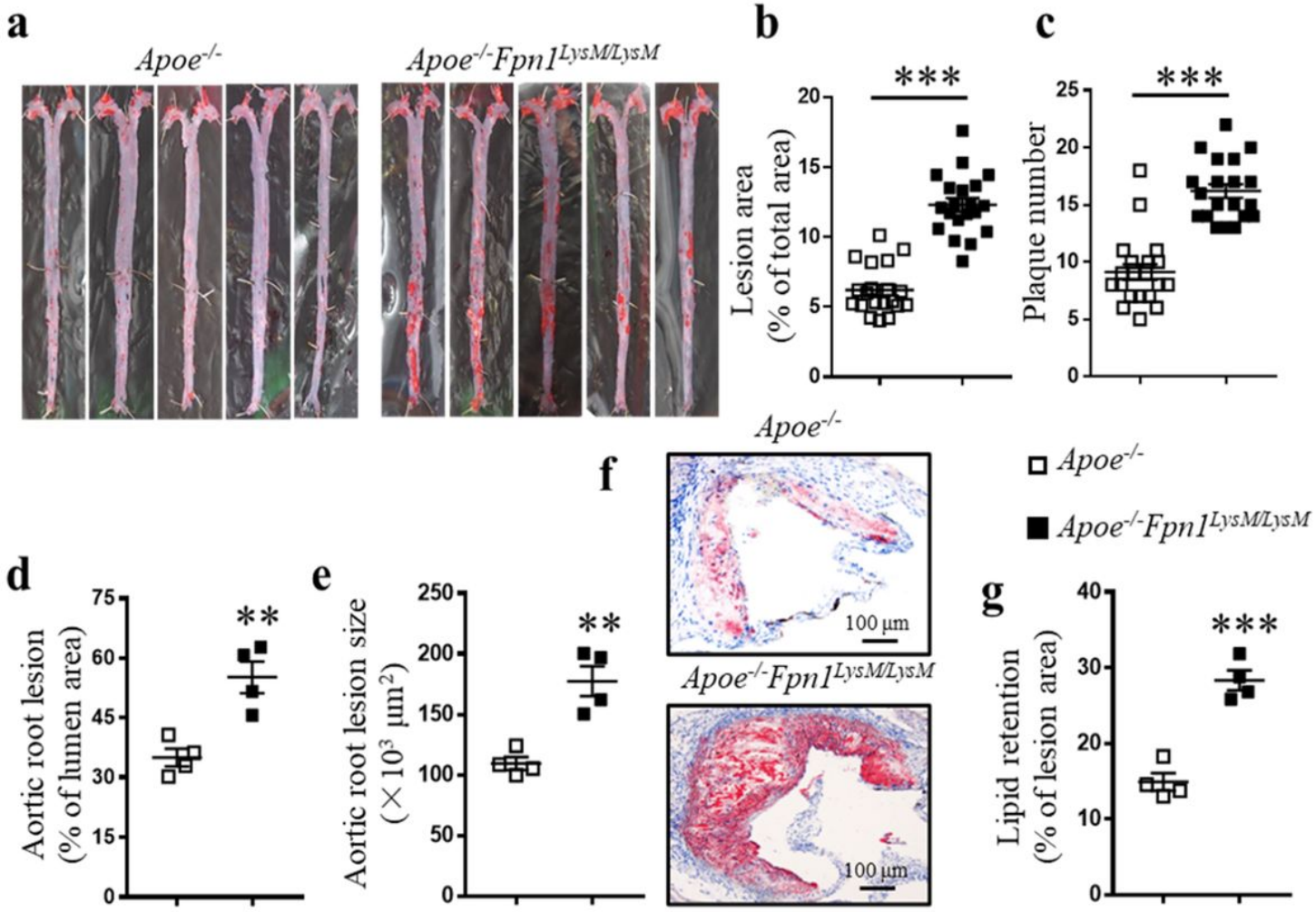

Figure 1

Macrophage-specific Fpn1 deficiency promotes atherosclerosis progression. Apoe-/- and Apoe-/-Fpn1LysM/LysM male mice were fed a Western diet for 16 weeks. (a) Representative images of Oil Red O staining of en face preparations of aortas. (b) Quantification of the aortic plaque score, n = 20. (c) Aortic plaque numbers, n = 20. (d) and (e) Determination of aortic root lesion area and size, n = 4. (f) Representative images of Oil Red O staining and (g) quantification of lipid retention in the aortic root in Apoe−/− and Apoe-/-Fpn1LysM/LysM mice, n = 4. Scale bar, 100 μm. Data are presented as the mean ± SEM. Statistical significance was determined using Student's t-test. **P < 0.01, and ***P < 0.001 vs. Apoe-/- mice.

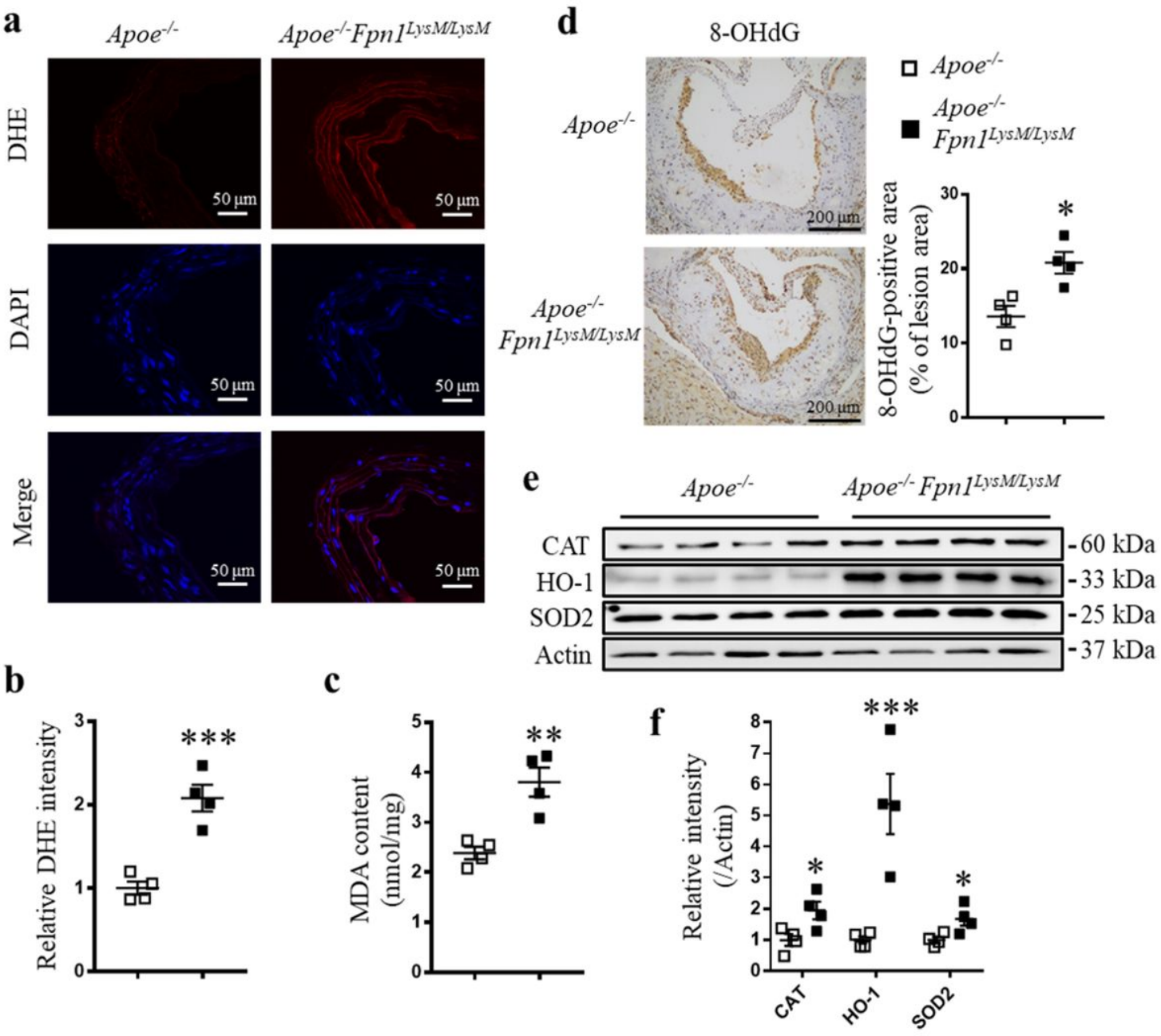

Figure 2

Macrophage-specific Fpn1 deficiency increases oxidative stress in the aorta. (a) Representative images of DHE staining in the aortas of Apoe-/- and Apoe-/-Fpn1LysM/LysM mice and (b) quantification of DHE fluorescence intensity. (c) Malondialdehyde (MDA) content in the aortas of Apoe-/- and Apoe-/-Fpn1LysM/LysM mice. (d) Representative images of IHC staining for 8-OHdG and quantification of stained areas. (e) Western blot analysis of catalase (CAT), heme oxygenase 1 (HO-1) and superoxide dismutase (SOD2) protein expression in the aortas of Apoe-/- and Apoe-/-Fpn1LysM/LysM mice and (f)

the quantification of this analysis. Data are presented as the mean ± SEM; n = 4. Statistical significance was determined using Student's t-test. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. Apoe-/- mice.

Figure 3

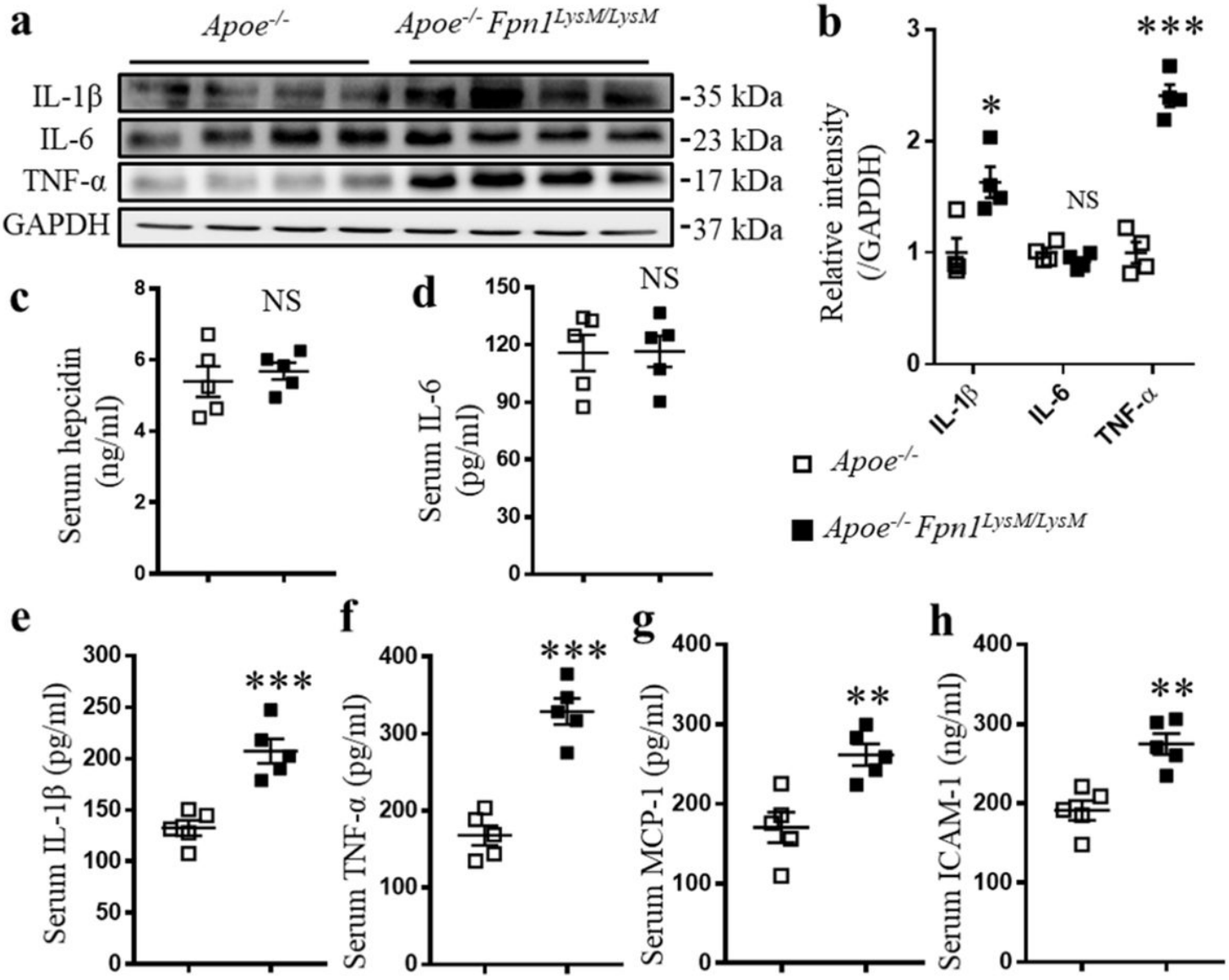

Figure 3

Macrophage-specific Fpn1 deficiency increases arterial and systemic inflammation. (a) and (b) Western blot analysis of IL-1β, IL-6 and TNF-α in the aortas of Apoe-/- and Apoe-/-Fpn1LysM/LysM mice, n = 4. Determination of the concentrations of serum hepcidin (c), IL-6 (d), IL-1β (e), TNF-α (f), MCP-1 (g), and ICAM-1 (h) in Apoe-/- and Apoe-/-Fpn1LysM/LysM mice, n = 5. Data are presented as the mean ± SEM. Statistical significance was determined using Student's t-test. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. Apoe-/- mice. NS, no significance.

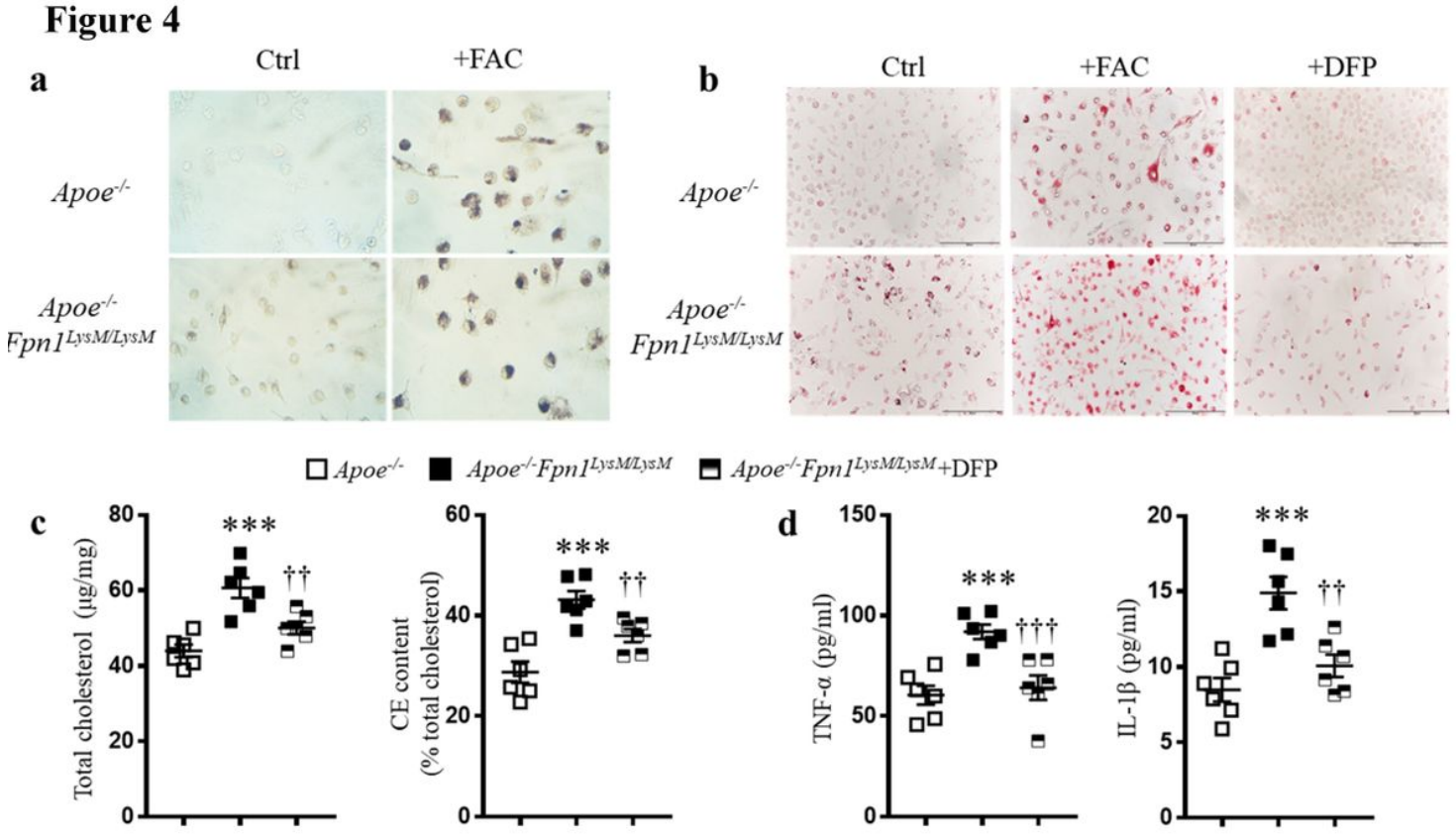

Figure 4

Macrophage-specific Fpn1 deficiency exacerbates foam cell formation and proinflammatory cytokine expression. (a) Representative images of DAB-enhanced Perls' Prussian blue staining for iron. (b) Oil red O-stained images. (c) Determination of cellular total and esterified cholesterol contents. (d) Secreted TNF-α and IL-1β levels in culture medium. Cell treatments for A-D: peritoneal cavity-derived macrophages, referred to as peritoneal macrophages, were incubated with oxLDL (50 μg/mL) in the presence or absence of DFP (50 μM) for 48 h. Data are presented as the mean ± SEM; n = 6. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparisons test. ***P < 0.001 Apoe-/-Fpn1LysM/LysM vs. Apoe-/-, ††P < 0.01 and †††P < 0.01 Apoe-/-Fpn1LysM/LysM + DFP vs. Apoe-/-Fpn1LysM/LysM. FAC: ferric ammonium citrate; DFP: deferiprone; oxLDL: oxidized low-density lipoprotein.

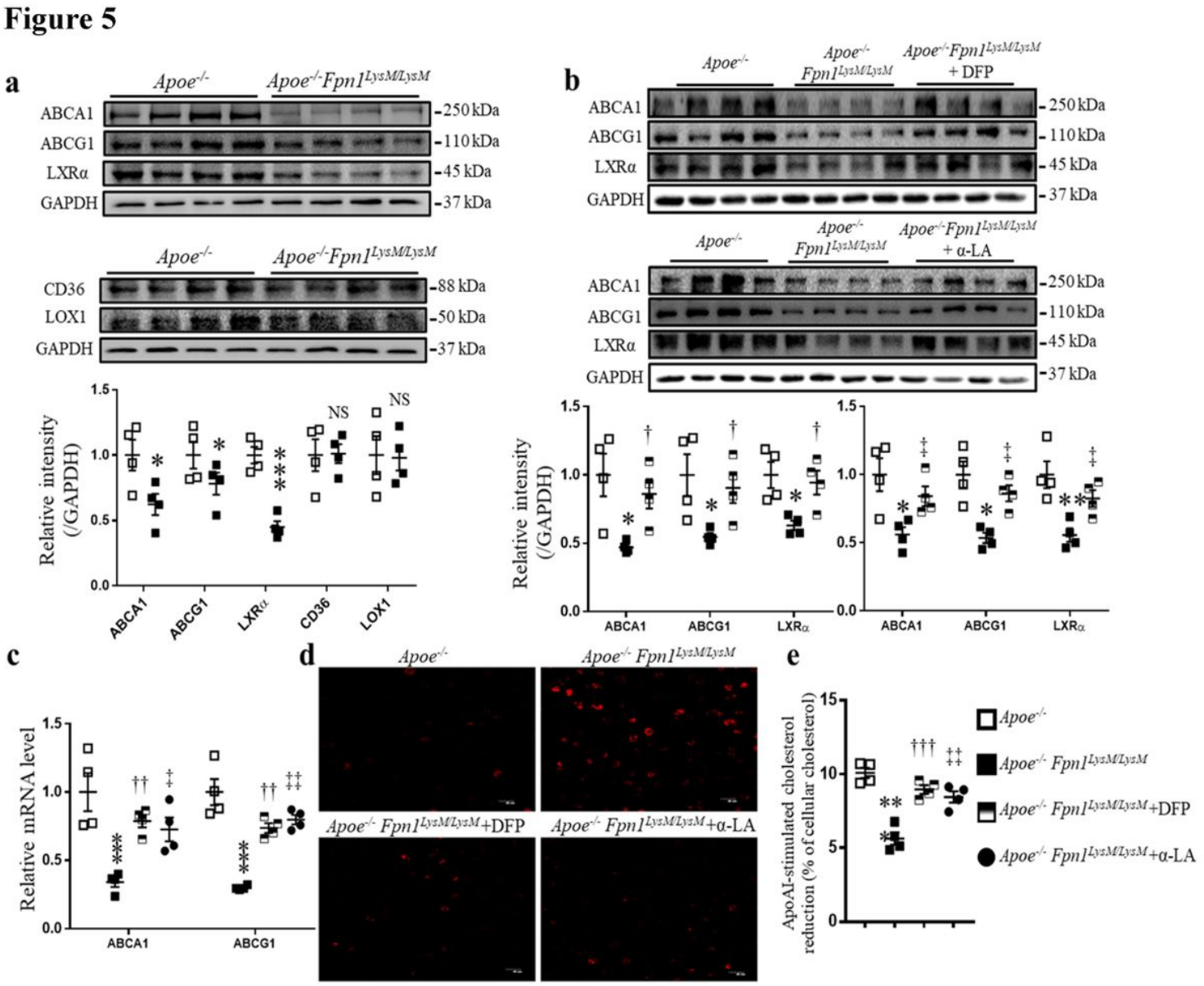

Figure 5

Macrophage-specific Fpn1 deficiency suppresses ABC transporters by downregulating LXRα expression. Peritoneal macrophages from Apoe-/- and Apoe-/-Fpn1LysM/LysM mice were collected, cultured, and treated with the iron chelator DFP or the antioxidant α-LA as indicated. (a) Protein levels of ABCA1, ABCG1, LXRα, CD36 and LOX1 revealed by Western blot analysis. (b) Protein levels of ABCA1, ABCG1, and LXRα after the macrophages were treated with DFP (50 μM) or α-LA (200 nM) for 48 h. (c) The relative mRNA levels of ABCA1 and ABCG1, as determined by qPCR. (d) Representative images of DHE staining following treatment with DFP (50 μM) or α-LA (200 nM) for 48 h. (e) ApoAI-mediated cholesterol efflux in Apoe-/-Fpn1LysM/LysM macrophages. The cholesterol-loaded macrophages were incubated with or without ApoAI (100 μg/ml) in the presence or absence of DFP (50 μM) or α-LA (200 nM) for 24 h. The results are expressed as the percentage change in the intracellular total cholesterol amount in the presence of ApoAI relative to that in ApoAI-free medium. Data are presented as the mean ± SEM; n = 4.

Statistical significance was determined using Student's t-test and one-way ANOVA followed by Tukey's multiple comparisons test. *P < 0.05, **P < 0.01, and ***P < 0.001 Apoe-/-Fpn1LysM/LysM vs. Apoe-/-; †P < 0.05, ††P < 0.01 and †††P < 0.001 Apoe-/-Fpn1LysM/LysM + DFP vs. Apoe-/-Fpn1LysM/LysM; ‡P < 0.05, ‡‡P < 0.01 Apoe-/-Fpn1LysM/LysM + α-LA vs. Apoe-/-Fpn1LysM/LysM. α-LA: alpha lipoic acid.

Figure 6

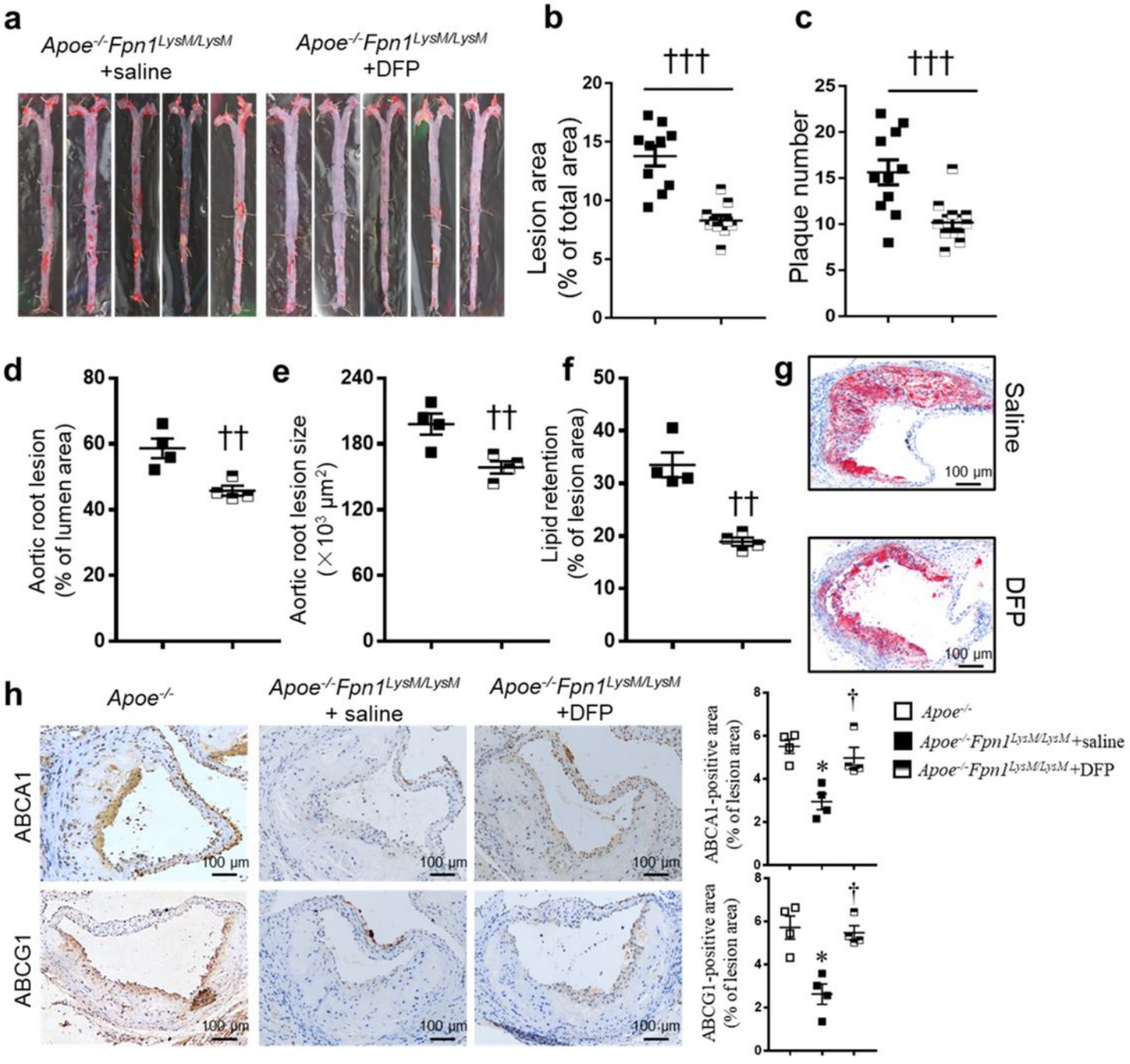

Figure 6

Iron chelation therapy prevents severe atherosclerosis in Apoe-/- Fpn1LysM/LysM mice. Eight-week-old male Apoe-/-Fpn1LysM/LysM mice were fed a Western diet and randomly divided into 2 groups: the vehicle- (saline) and iron chelator-treated (DFP, 80 mg/kg) groups. (a) Representative images of Oil Red O staining of aortas. Quantification of the aortic plaque score (b) and plaque numbers (c), n = 10. (d) and (e) Determination of aortic root lesion area and size, n = 4. (f) and (g) Representative images and quantification of Oil Red O staining of the aortic roots of Apoe-/-Fpn1LysM/LysM mice, n = 4. (h) Representative images of IHC staining for ABCA1 and ABCG1 and the quantification of the stained areas, n = 4. Scale bar, 100 μm. Data are presented as the mean ± SEM. Statistical significance was determined using Student's t-test and one-way ANOVA followed by Tukey's multiple comparisons test. *P < 0.05 Apoe-/-Fpn1LysM/LysM + saline vs. Apoe-/- mice, †P < 0.05, †††P < 0.001 Apoe-/-Fpn1LysM/LysM + DFP vs. Apoe-/-Fpn1LysM/LysM + saline.

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