Supplemental Material

ONLINE FIGURES

Online Figure I. IL-17A promote ox-LDL-induced macrophage apoptosis
Mouse primary macrophages from C57BL/6 mice were pre-stimulated with ox-LDL for 12 hours and then IL-17A was added for 24 hours. Apoptosis rate of cells with PI staining determined by flow cytometry. Quantification of apoptotic cells on the right. Data are mean±SEM from 3 representative experiments. * P<0.05, **P<0.01.

Online Figure II. 4-phenyl butyric acid (PBA) can alleviate the development of atherosclerosis
ApoE−/− mice, 8 weeks old, were fed a high-fat diet for 4 weeks and then treated for 5 weeks with PBA (100mg/kg/time), or normal saline (control). Oil-red O staining for lipid content and plaque size (original magnification ×40). Quantification of staining from control and PBA–treated mice (n=5/group) expressed as percentage of total lesion area. Data are mean±SEM. * P<0.05.
Online Figure III. Effective concentration and time of aP2 inhibitor (BMS309403)
Mouse primary macrophages were stimulated with aP2 inhibitor at various concentrations. The protein level of aP2 was analyzed by Western blot. Results are representative of 3 independent experiments.

Online Figure IV. IL-17A promotes macrophages secreting TNF-α, IL-6 and IL-1β
Mice macrophage cell line (RAW264.7) and mouse primary macrophages were cultured in vitro and stimulated with IL-17A (20 and 50 ng/ml) for 12 or 24 h. The levels of TNF-α, IL-6 and IL-1β in cultured supernatant were detected by ELISA. Data are mean ±SEM from 3 independent experiments. (A) RAW264.7 cells; (B) Primary macrophages. * P<0.05, **P<0.01, ***P<0.001.
Online Table I: Body weight and plasma lipid levels after IL-17A or IL-17A and PBA treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=10)</th>
<th>IL-17A (n=10)</th>
<th>IL-17A+PBA (n=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>25.52±1.93</td>
<td>28.30±1.00</td>
<td>28.10±0.39</td>
<td>0.27</td>
</tr>
<tr>
<td>TCH (mmol/l)</td>
<td>42.02±0.67</td>
<td>39.51±2.41</td>
<td>38.27±1.68</td>
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<td>TG (mmol/l)</td>
<td>2.25±0.26</td>
<td>2.07±0.16</td>
<td>2.02±0.18</td>
<td>0.72</td>
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<tr>
<td>HDL (mmol/l)</td>
<td>11.04±0.34</td>
<td>10.47±0.68</td>
<td>10.84±0.70</td>
<td>0.80</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>29.02±0.47</td>
<td>27.62±1.66</td>
<td>26.49±1.50</td>
<td>0.49</td>
</tr>
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</table>

Values are expressed as means ± SEM.
TCH, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Online Table II: Body weight and plasma lipid levels after IL-17A or IL-17A and aP2 inhibitor treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=5)</th>
<th>IL-17A (n=5)</th>
<th>IL-17A+ aP2 inhibitor (n=5)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>27.75±2.10</td>
<td>28.03±1.77</td>
<td>29.52±1.85</td>
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<tr>
<td>TCH (mmol/l)</td>
<td>35.56±2.65</td>
<td>31.29±2.58</td>
<td>23.41±2.50</td>
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<td>TG (mmol/l)</td>
<td>2.41±0.18</td>
<td>2.32±0.22</td>
<td>2.14±0.31</td>
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<tr>
<td>HDL (mmol/l)</td>
<td>10.69±0.38</td>
<td>10.51±0.87</td>
<td>10.01±0.92</td>
<td>0.81</td>
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<tr>
<td>LDL (mmol/l)</td>
<td>29.36±0.55</td>
<td>26.84±1.89</td>
<td>24.86±2.08</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM.
TCH, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
ONLINE METHODS

Animals
Male C57BL/6 wild-mice and ApoE-/- mice were purchased from Beijing University. Mice were 6–8 weeks old (21–25g) and were housed at a constant temperature (24°C), under a 12-h dark/12-h light cycle room in the Animal Care Facility of Shandong University Medical School according to institutional guidelines. All animal studies were approved by the Animal Care and Utilization Committee of Shandong University, China.

In Vivo Application of Exogenous IL-17A, 4-phenyl butyric acid (PBA) and aP2 inhibitor
ApoE-/- mice (n=10 for each group) were fed a high-cholesterol diet from 8 weeks of age. Four weeks later, two groups for treatment once a week for 5 weeks: intraperitoneal injection with exogenous recombinant IL-17A, 0.02% of recombinant mouse IL-17A in normal saline (2μg/mouse/time, PMC0175, Invitrogen, Clasbad, CA, USA); and normal saline containing 0.02% albumin as a control. The third group (n=10), mice were pretreated before IL-17A with the chemical chaperone PBA (P21005, Sigma-Aldrich, St. Louis, MO, USA) (100 mg/kg/time) twice a week for 5 weeks, at the same time mice also received IL-17A treatment (2μg/mouse/time) once a week for 5 weeks. The fourth group (n=7) fed with a high-cholesterol diet from 8 weeks of age and two weeks later, mice were treated by oral gavage with vehicle including 10% 1-methyl-2-pyrrolidone and 5% cremophor EL with ethanol in 100μl of water or 20mg/kg/d of the aP2 inhibitor BMS309403 dissolved in the vehicle for 6 weeks. And mice also received IL-17A treatment (2μg/mouse/time) once a week for 5 weeks after four weeks high-cholesterol diet.

Lipid Profile
Total plasma cholesterol and triglyceride levels were determined by an automated enzymatic technique, and low-density lipoprotein and high-density lipoprotein levels were detected with use of an automated chemically modified technique (Roche Modular DPP System, Roche, Basel, Switzerland).

Histopathology and Immunohistochemistry
After mice were killed, aortic root vessels were perfused with phosphate buffered saline (PBS), then 4% paraformaldehyde. Aortic root vessels were removed and fixed in 4% paraformaldehyde overnight, then embedded in OCT compound. Serial cryosections of 6 μm were cut along the aortic root specimens and were stained with Oil-red O. Corresponding sections on separate slides were stained for macrophages with a rat anti-mouse macrophage-specific antibody (Moma-2, AbD Serotec, UK) or primary antibodies for P-PERK (sc-32577, Santa Cruz Biotechnology, Santa Cruz, CA, USA), p-eIF2α (119A11, Cell Signaling Technology, Danvers, MA, USA), FABP4(aP2) (ab-13979, Abcam, Hong Kong), CHOP (sc-575, Santa Cruz Biotechnology, CA, USA), or caspase 12 (ab-62463, Abcam, Inc. Cambridge, MA, USA). Histological stains were detected by use of an Olympus microscope (IX71), and the area of plaque was measured by use of ImagePro-Plus 6.0 and dp2-bsw (Olympus, Tokyo, Japan).

TUNEL and Macrophage Apoptosis Assay
Apoptotic cells in the intimal area of atherosclerotic lesions were labeled after proteinase K treatment by TUNEL with use of the in situ cell death detection kit Fluorescein (Roche Diagnostics, Basel, Switzerland). For in vitro analysis, macrophages were assayed for early to
mid-stage apoptosis by staining with Alexa Fluor 488-conjugated Annexin V and for late-stage apoptosis by co-staining with propidium iodide (PI) according to the manufacturer’s directions, then were detected by flow cytometry with use of Cytomics FC500 (Beckman Coulter, Brea, CA, USA). Hoechst staining for nuclei was viewed at room temperature by use of an Olympus IX-71 inverted fluorescent microscope.

**Isolation of Mouse Peritoneal Macrophages and Culture in Vitro**

Peritoneal macrophages from male C57BL/6 mice were harvested 3 days after intraperitoneal injection of starch broth by peritoneal lavage. All macrophages were cultivated in 6-well flat-bottom plates with Dulbecco's modified Eagle's medium (DMEM, Gibco, Invitrogen, Clasbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin and incubated in humidified 5% CO₂ at 37°C for 2 h to allow macrophage adherence. The nonadherent cells were removed by 3 washes with DMEM. The adherent cells were harvested, and purity of macrophages was detected by flow cytometry after staining with PECy5-conjugated anti-F4/80Ab (BM8; eBioscience, San Diego, CA, USA). Purified macrophages (purity >95%) were incubated as described in figure legends on the day of the experiment.

**Induction and culture of Human Monocyte-derived Macrophages and THP-1-derived macrophages**

Peripheral blood mononuclear cells (PBMCs) from healthy volunteer blood at Shandong University Medical School were isolated by Ficoll density gradient centrifugation and washed twice with serum-free RPMI 1640 medium (Gibco, Invitrogen, Clasbad, CA, USA). PBMCs were then plated at 3 ml/well in 6-well cell culture plates, 3 hours later un-adherent cells were removed and adherent cells cultured with RPMI 1640 complete medium (10% FBS, 1% L-glutamine, 1% sodium pyruvate, 1% non-essential amino acids, 1% penicillin/streptomycin and 50ng/ml rhGM-CSF) (catalog: 300-03, PeproTech, Suzhou, JS, P.R. China). Half of the medium was replaced every 2 days for culture for 7 days. On the day of the experiment, the cells were washed 3 times in warm PBS and incubated as described in the figure legends. THP-1 cell lines were cultivated in 6-well flat-bottom plates with DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin and PMA(50ng/ml) for 48 hours, then the cells were washed 3 times in warm PBS and incubated as described in the figure legends.  

**Western Blot Analysis**

Extraction and detection of protein was as described. A 50-μg quantity of protein was separated by SDS-PAGE and transferred to PVDF membranes (Millipore, Billerica, MA, USA), which were blocked with 1% bovine serum albumin in TBST containing 0.1% Tween-20 for 1 hour, then incubated overnight at 4°C with antibodies for p-PERK, PERK, p-eIF2α, eIF2α, CHOP, caspase3, p-p38, p38, p-ERK, ERK, p-JNK, JNK, p-IkB, IκB (all 1:1000; Cell Signaling Technology, Danvers, MA, USA), aP2 (ab-13979, 1:1000; Abcam, Inc. Hong Kong), or β-actin (1:2000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), then horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit IgG or murine anti-mice IgG) for 1 hour at room temperature. After a washing, signals were visualized by use of SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL, USA). Western blot was performed at least 3 times for each sample.
RT-PCR Analysis
Total RNA in primary macrophages was isolated by the Trizol reagent method (Invitrogen, Clasbad, CA, USA) and underwent reverse transcription and semi-RT-PCR. The primers for aP2 were sense, 5’-ATG CCTTTGTGGGAACCTG-3’ and antisense, 5’-CCTGTCGTCTGCCTGATT-3’ (230-bp product length).

Enzyme-linked Immunosorbent Assay (ELISA)
Cytokines in culture supernatants quantified using mouse TNF-α, IL-6 and IL-1β ELISA kits (eBioscience, San Diego, CA, USA) according to the manufacturer's instructions.

Statistical Analysis
All analysis involved SPSS v11.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as mean±SEM. Nonparametric ANOVA and unpaired t tests were used to compare continuous data. A p< 0.05 was considered statistically significant.

REFERENCES