Supplemental Figure 1. Characterization of Peritoneal Lavage Cells before and after 3 days of ex vivo culture

Analysis of peritoneal lavage cells after isolation from a wild type mouse reveals about 50% macrophage content (Left Panel). After 3 days of ex vivo culture, peritoneal macrophages are about 90% of the cells present (Right Panel). Representative of 3 independent experiments.
Supplemental Figure 2. Cytochalasin D Inhibits engulfment of apoptotic thymocytes and Methyl-β-Cyclodextran (MβCD) increases Annexin V positivity of apoptotic thymocytes

A. Engulfment of apoptotic thymocytes by primary peritoneal macrophages in the presence of 1µM Cytochalasin D or DMSO vehicle control (mean ± SD). Representative of at least 3 independent experiments.

B. Jurkats were UV-irradiated and then incubated for 3 hours. They were then treated for 1 hour with MβCD or vehicle, washed extensively, and analyzed for apoptosis by Annexin V and 7AAD staining. Representative of 2 independent experiments
Supplemental Figure 3. ABCA1 Upregulation in response to apoptotic cells is not impaired in the presence of the LXR antagonist GSK-1440233A.

Primary peritoneal macrophages were treated with 2 million apoptotic cells for the indicated time period in the presence of DMSO vehicle control or 1µM GSK-1440233A (GSK). (Left Panel) ABCA1 levels relative to HPRT were measured by RT-PCR, with each time point normalized to vehicle or drug treatment alone (without apoptotic Jurkat cells) for the same duration. (Right Panel) To better understand any differences in the kinetics of ABCA1 upregulation over time, the fold upregulation of ABCA1 calculated in the left panel was then set as a percentage of the maximum ABCA1 upregulation in the experiment. Data are from two independent experiments (mean ± SEM) conducted along with Figure 1C.
Supplemental Figure 4. Measurement of mRNA levels in knockout and transgenic mouse models

C. RT-PCR measurement of murine BAI1 levels relative to HPRT in peritoneal macrophages isolated from wild type and BAI1−/− mice (mean ± SD).

D. RT-PCR measurement of murine Timd4 levels relative to HPRT in peritoneal macrophages isolated from wild type and Timd4−/− mice (mean ± SD).

E. RT-PCR measurement of murine Elmo1 levels relative to HPRT in peritoneal macrophages isolated from wild type and Elmo1−/− mice (mean ± SD).

F. RT-PCR measurement of murine Rac1 levels relative to HPRT in peritoneal macrophages isolated from wild type and Rac1fl/fl LysM-Cre+ mice (mean ± SD).

G. RT-PCR measurement of human BAI1 levels relative to HPRT in peritoneal macrophages isolated from wild type and wild type BAI1 Tg mice (mean ± SD).
Supplemental Figure 5. Serum lipid levels in wild type and BAI-/- mice after 30 weeks on Western Diet

A. Fluorescent images of aortic root sections from BAI1-/- or wild type littermate controls after 30 weeks on Western diet. DAPI staining of nuclei is depicted in blue and the TUNEL staining of apoptotic nuclei in red (arrow). Graph represents the number of TUNEL+ nuclei as a percentage of DAPI+ nuclei automatically counted with CellProfiler software. Scale bar = 100µm. *p<0.05 (t-test)

B-G. Analysis of serum levels of total cholesterol, HDL, LDL, triglycerides, the ratio of HDL to total cholesterol, and the ratio of HDL to LDL in wild type mice compared to BAI1-/- mice after 30 weeks on Western diet.

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Supplemental Figure 6. LDLR−/− and BA11−/−LDLR−/− mice have similar levels of atherosclerosis.

A. Plaque area in aortic root sections stained with H&E were measured using ImageJ software and each mouse was normalized to set the wild type average to 100%

B. Necrotic core area, as characterized by acellular areas by H&E within the atherosclerotic plaques in aortic root sections were measured using ImageJ software and each mouse was normalized to set the wild type average to 100%

C. The percentage of atherosclerotic plaque in individual mice in the aortic root that was necrotic, as measured using ImageJ software to analyze H&E stained sections.

D. Aortic roots were stained with Oil Red O (ORO) and the positive area was quantified using ImageJ software. Scale bar = 200µm