Endosomal Actin Remodeling by Coronin-1A Controls Lipoprotein Uptake and Degradation in Macrophages

Maarit Hölttä-Vuori, Saara Vainio, Maria Kauppi, Miranda Van Eck, Eija Jokitalo, Elina Ikonen

Rationale: The actin cytoskeleton has been implicated in the processing of atherogenic lipoproteins in macrophages. However, the functional role of actin and the regulatory proteins involved are unknown.

Objective: Coronin-1A (Coro1A) was identified as a differentially expressed transcript in wild-type versus Niemann-Pick type C1 deficient macrophages exposed to acetylated low-density lipoproteins (AcLDL). We investigated whether Coro1A plays a role in the uptake or processing of modified lipoproteins in macrophages and if this is related to its actin regulatory functions.

Methods and Results: In wild-type primary macrophages, filamentous actin transiently decorated AcLDL containing endosomes that also recruited Coro1A. This dynamic association of F-actin with endosomes was disturbed in Coro1A deficient macrophages. In Coro1A knockout macrophages the uptake of AcLDL was increased, rate of AcLDL delivery to lysosomes enhanced, and lipoprotein-derived cholesteryl ester hydrolysis accelerated. Overexpression of wild-type Coro1A normalized AcLDL uptake in Coro1A knockout macrophages while a Coro1A actin binding mutant did not. Furthermore, the effects of macrophage Coro1A silencing on endosomal actin association and AcLDL delivery to lysosomes resembled those of cofilin silencing.

Conclusions: Coro1A controls actin association with endocytic organelles, thereby negatively regulating endosomal delivery, degradation of modified lipoproteins and cholesterol deposition in macrophages. (Circ Res. 2012;110:00-00.)

Key Words: macrophage ■ lipoprotein endocytosis ■ actin cytoskeleton

Macrophages scavenge cholesterol-rich lipoproteins in the arterial intima, a process of key importance in atherogenesis. The molecular mechanisms controlling macrophage foam cell formation are not fully understood but the processing of atherogenic lipoproteins requires an intact actin cytoskeleton and lipoprotein-derived cholesterol enhances actin polymerization.

The coronin family of proteins was originally identified in Dictyostelium where deletion of coronin affected actin-dependent processes, such as phagocytosis, cell motility, and cytokinesis. Mammalian Coronin-1A (Coro1A) belongs to Type I coronins and is expressed in hematopoietic cells. There is an increasing appreciation on the role of Type I coronins in coordinating Arp2/3 complex and ADF/cofilin proteins in leading edge actin dynamics, but their role in regulating actin elsewhere than at the cell cortex is not well understood. Intriguingly, Coro1A was reported to be dispensable for actin-dependent functions in macrophages.

To identify novel proteins involved in macrophage cholesterol processing, we compared transcripts of mouse peritoneal macrophages in untreated and acetylated low-density lipoproteins (AcLDL)-loaded conditions using microarrays. One of the mRNAs upregulated on AcLDL loading in wild-type (wt) but not Niemann-Pick C1 deficient macrophages was Coro1A (S.V. and E. I., unpublished data). We therefore studied whether Coro1A plays a role in the uptake or processing of modified lipoproteins in macrophages and if this is related to actin regulatory functions of Coro1A.

Methods

C57BL/6 Coro1A knockout mouse strain was from Genentech. Experiments were conducted in unelicited peritoneal macrophages, bone-marrow derived macrophages (plasmid transfections), or Raw264.7 macrophages (siRNA transfections). An expanded methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

Results and Discussion

We found that Coro1A protein was upregulated in AcLDL loaded and reduced in lipoprotein-deprived macrophages (Figure 1A). The protein was also downregulated in Niemann-Pick C1-deficient macrophages, where lipoprotein-derived cholesterol accumulates in lysosomes and its levels...
failed to respond to AcLDL in Niemann-Pick C1 silenced macrophages (Figure 1A). Overexpression of Coro1A alleviated cholesterol accumulation in Niemann-Pick C1-deficient cells (Online Figure I, available at http://circres.ahajournals.org), suggesting that Coro1A function somehow counteracts lysosomal cholesterol deposition. Interestingly, lipids can regulate Coro1A gene transcription and in the liver Coro1C transcript responds to sterol-regulatory element binding proteins.

To investigate the functional role of Coro1A in AcLDL processing, we incubated wt and Coro1A knockout (Cor/−/−) mouse macrophages with 50 μg/mL of AcLDL. In accordance with previous studies on Cor/−/− macrophage spreading, we observed no differences in the morphological response of wt and Cor/−/− macrophages to AcLDL (Online Figure IIA). However, fluorescent 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate-labeled AcLDL (Dil-AcLDL) uptake was increased in Cor/−/− cells (Figure 1B). Labeling of cells with [3H]cholesterol oleate-AcLDL ([3H]AcLDL) on ice was similar in wt and Cor/−/− macrophages, indicating normal binding to scavenger receptors (Figure 1C). Instead, on endocytosis at 37°C, the amount of [3H]AcLDL taken up by Cor/−/− macrophages exceeded that of wt. These results suggest that Coro1A deficiency increases the uptake of modified lipoproteins by macrophages, a pathway used, eg, by e.g AcLDL. The internalization of oxidized LDL, which occurs via different mechanism, was unaffected (Online Figure IIB). The increased AcLDL uptake resulted in increased cholesterol loading of Cor/−/− macrophages (Figure 1D). However, cholesterol efflux was not impaired as Cor/−/− macrophages were able to clear the excess sterol to apolipoprotein AI (Figure 1D) and [3H]cholesterol efflux to BSA, apolipoprotein AI, or HDL was not altered in Cor/−/− macrophages (not shown).

By immunofluorescence and immunoelectron microscopy, we detected a fraction of the endogenous Coro1A protein in Dil-AcLDL organelles and endosomal profiles (Figure 2A, B). When assessing the time-dependent delivery of AcLDL to dextran-labeled lysosomes, we observed that a fraction of the endogenous Coro1A protein in Dil-AcLDL organelles and endosomal profiles (Figure 2A, B). When assessing the time-dependent delivery of AcLDL to dextran-labeled lysosomes, we observed that a fraction of the endogenous Coro1A protein in Dil-AcLDL organelles and endosomal profiles (Figure 2A, B). When assessing the time-dependent delivery of AcLDL to dextran-labeled lysosomes, we observed that a fraction of the endogenous Coro1A protein in Dil-AcLDL organelles and endosomal profiles (Figure 2A, B).
DiI-AcLDL was found in lysosomes already at 15 minutes (Figure 2C), in agreement with studies reporting rapid macropinosome-lysosome fusion in macrophages.14 Remarkably, the lysosome colocalizing fraction of DiI-AcLDL was larger in Coro1A/H11002/H11002 macrophages (Figure 2C). This was paralleled by a more rapid hydrolysis of AcLDL-derived [3H]cholesteryl esters (Figure 2D), indicating accelerated delivery of AcLDL to lysosomes in the absence of Coro1A. In mycobacterium-infected macrophages, Coro1A was required for activation of calcineurin to block phagosome-lysosome fusion, and lack of Coro1A or administration of the calcineurin inhibitor FK506 facilitated lysosomal delivery and killing of the bacteria.6 We therefore tested if FK506 mimics the effects of Coro1A deficiency on AcLDL processing. This was not the case (Online Figure III), leading us to investigate if Coro1A function in lipoprotein endocytosis involves actin regulation.

When added 5 minutes after AcLDL internalization, actin perturbing compounds enhanced DiI-AcLDL delivery to lysosomes in wt macrophages, an effect similar to Coro1A deficiency (Figure 3A, 2C). To directly visualize actin dynamics, we studied living cells expressing Lifeact-GFP on DiI-AcLDL loading. We observed that actin increasingly assembled onto the DiI-AcLDL organelles until 15 to 20 minutes postuptake and then started to disappear, showing a clear reduction by 30 minutes (Figure 3B, and Online Video). To quantify this, we stained DiI-AcLDL labeled wt and Coro1A/H11002/H11002 macrophages with phalloidin and analyzed the area of DiI-AcLDL containing endosomes covered by F-actin. After 5 minutes incubation, the DiI-AcLDL organelles in wt cells were largely devoid of actin coating both in wt and Coro1A/H11002/H11002 macrophages (Figure 3C). After 15 minutes of labeling, the DiI-AcLDL organelles in wt cells were heavily decorated with F-actin, and this was less pronounced in Coro1A/H11002/H11002 macrophages (Figure 3C). After 50 minutes chase, the F-actin coating on DiI-AcLDL organelles in wt cells became significantly reduced, and this reduction was inhibited in Coro1A/H11002/H11002 macrophages (Figure 3C). Together, these results reveal a temporally controlled F-actin recruitment and disassembly on AcLDL transporting endosomes in macrophages. If actin is disrupted at early stages postinternalization, AcLDL delivery to lysosomes is accelerated. Importantly, this endosomal actin association is physiologically controlled by Coro1A.

To assess the Coro1A actin regulatory activity in AcLDL processing more directly, we investigated if Coro1A protein mutated at actin-binding residues15,16 Coro1A-K10A,R12A was able to normalize the AcLDL uptake in Coro1A/H11002/H11002 macrophages. We found that on expression of GFP-wtCoro1A, but not GFP-Coro1A–K10A,R12A, AcLDL uptake was reduced.
to the level of wt cells (Figure 4A). Cofilin is a key regulator of F-actin dynamics and has synergy with coronins in the disassembly of aged actin filaments. We silenced Coro1A or cofilin with siRNAs (Figure 4B) and found that, similar to Coro1A silencing, cofilin depletion resulted in enhanced delivery of AcLDL to lysosomes (Figure 4C). In addition, although DiI-AcLDL organelles were heavily decorated with F-actin after 15 minutes AcLDL loading on both siRNA treatments (not shown), the depletion of either Coro1A or cofilin resulted in impaired removal of F-actin from endosomes after 50 minutes chase (Figure 4D), similarly as observed in Coro1A/macrophages (Figure 3C). These results argue that Coro1A affects AcLDL endocytic processing via its actin regulatory functions.
In summary, this study demonstrates a role for Coro1A in controlling actin association with endocytic organelles, thereby negatively regulating endo-lysosomal delivery, degradation of modified lipoproteins, and cholesterol deposition in macrophages. The results provide direct evidence for Coro1A as a regulator of F-actin dynamics and actin-dependent functions in macrophages and identify Coro1A as the first actin regulator involved in macrophage cholesterol processing. Coro1A may be part of an F-actin–based inhibitory system that helps to prevent overloading of macrophage lysosomes under situations of high intake, such as during scavenging of modified lipoproteins.
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Disclosures
None.

References
5. Chan KT, Creed SJ, Bear JE. Unraveling the enigma: progress towards understanding the coronin family of actin regulatory proteins involved are unknown. Coro1A is an actin-binding protein expressed in hematopoietic cells. Previous studies have failed to find a role for Coro1A in actin-mediated processes in macrophages. We discovered that in macrophages, Coro1A regulates endosomal F-actin dynamics, controlling the uptake and processing of atherogenic lipoproteins. Our study reveals a temporally controlled actin assembly/disassembly on endosomes that deliver modified lipoproteins for degradation and identifies Coro1A as the first actin regulator involved in macrophage cholesterol processing. These findings are of key importance for understanding the regulatory mechanisms of foam cell formation.

Novelty and Significance
What Is Known?
• Coronin-1A (Coro1A) is an actin-binding protein abundantly expressed in macrophages.
• Actin is involved in macrophage cholesterol processing.

What New Information Does This Article Contribute?
• In macrophages, Coro1A controls actin association on endosomes that deliver modified lipoproteins for degradation.
• Perturbed endosomal actin dynamics due to Coro1A deficiency lead to increased uptake and lysosomal delivery of modified lipoproteins.
• Coro1A is the first identified actin-binding protein involved in macrophage cholesterol processing.

Macrophage cholesterol processing is sensitive to actin-perturbing drugs but the precise function of actin and the regulatory proteins involved are unknown. Coro1A is an actin-binding protein expressed in hematopoietic cells. Previous studies have failed to find a role for Coro1A in actin-mediated processes in macrophages. We discovered that in macrophages, Coro1A regulates endosomal F-actin dynamics, controlling the uptake and processing of atherogenic lipoproteins. Our study reveals a temporally controlled actin assembly/disassembly on endosomes that deliver modified lipoproteins for degradation and identifies Coro1A as the first actin regulator involved in macrophage cholesterol processing. These findings are of key importance for understanding the regulatory mechanisms of foam cell formation.
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Detailed methods

cDNA constructs and siRNAs

The murine Coro1A cDNA was cloned by RT-PCR from wt Balb/C peritoneal macrophage mRNA with the primers 5'-TGCACAGTTATGGTGTGGGA-3' (forward) and 5'-TAGGCCTCTATGATGGAATG-3' (reverse). The PCR product was ligated into the BamHI sites of the Bluescript SK(-) plasmid (Stratagene). The construct was verified by sequencing. Subsequently, Coro1A was subcloned into the BamHI sites of EGFP-C1 (Clontech) and pGAT-4 (kindly provided by Dr. Johan Peränen) vectors, for expression in mammalian cells and bacteria, respectively. The K10A, R12A mutant Coro1A was generated by using Phusion Site Directed Mutagenesis Kit (Finnzymes) according to manufacturer’s instructions, and mutations were verified by sequencing. Lifeact-GFP has been described1. The siRNAs against murine Coro1A were 5'-GGUUGUGACAAUGUGAUCCtt-3' (sense) and 5'-GGAUCACAUUGUCACAACCtt-3' (antisense) and against murine NPC1 5'-CCAGGUUCUGGACUUACAGtt-3' (sense) and 5'-CUGUAAGUCACAACCUGtt-3' (antisense) (Sigma-Aldrich). Murine Cofilin Silencer select siRNA (Ambion) was 5'-GCGGUGCUCUUUUGCCUGAtt-3' (sense) and UCAGGCAAAAGAGCACCGCtt (antisense). Control siRNA against firefly luciferase2 (GL2) has been described.

Cell culture and transfection

C57BL/6 Coro1A knockout mouse strain3 was a courtesy of Genentech and maintained by heterozygous breeding. The expression level of other Coronin family members was not altered by Coro1A deletion4 and our unpublished observations. Balb/C NPC1 null mouse strain5 was maintained as described6, and cultured in DMEM with aforementioned supplements plus 10-20 ng/ml of recombinant macrophage-colony stimulating factor (M-CSF, Sigma-Aldrich) for 6-8 days prior to experiments. Raw264.7 macrophages (used in RNAi experiments) were isolated from femura of the mice as described6, and cultured in DMEM with aforementioned supplements plus 10-20 ng/ml of recombinant macrophage-colony stimulating factor (M-CSF, Sigma-Aldrich) for 6-8 days prior to experiments. Raw264.7 macrophages (used in RNAi experiments) were cultured under the same conditions as peritoneal macrophages. Transfection of bone marrow-derived macrophages was carried out using Lipofectamine 2000 (Invitrogen) and Raw264.7 macrophages by electroporation with Nucleofector I electroporation device (Amaxa), according to manufacturer’s instructions. NPC1 patient fibroblasts (GM3123) were cultured and transfected as described before9. Cell culture media and reagents were from Sigma-Aldrich and Lonza.

Antibodies and reagents

The Full-length murine Coro1A protein was expressed as a His6-GST fusion protein in Escherichia coli JM109(DE3) and the insoluble protein purified from cell debris on preparative SDS-polyacrylamide gels. New Zealand White rabbits were immunized with the purified protein, and the polyclonal antiserum used as 1:400-1:1000 dilution for immunofluorescence and as 1:2000-1:5000 dilution for immunoblotting (Online Figure IV). The following antibodies were also used: anti-NPC1 (Novus Biologicals, 1:2000 for immunoblotting), anti-Cofilin10 (courtesy of Pekka Lappalainen, 1:2000 for immunoblotting), and anti-Lamp1 (H4A1) (Developmental Studies Hybridoma Bank, 1:100 for immunofluorescence). Oregon-green phalloidin, FITC-conjugated dextran (MW 10 000), Alexa 488 and 568 –conjugated anti-IgG secondary antibodies and Dil-AcLDL were from Invitrogen. Non-fluorescent AcLDL was prepared and labeled with [3H]cholesterol (Perkin Elmer) or [3H]cholesteryl oleate (Amersham Biosciences) as described11. LDL was oxidized by incubating fresh LDL with 5 µM CuSO4 overnight at 37°C and oxidized LDL (OxLDL) labeled with [3H]cholesterol as above. ApoA-I was provided by Peter Lerch (Swiss Red
Cross, Bern, Switzerland), or obtained from Calbiochem. Other reagents were from Sigma-Aldrich.

**AcLDL uptake**

For analysis of fluorescent AcLDL uptake, wt or Cor bone marrow macrophages were incubated with 50 µg/ml DiI-AcLDL (pool of 10 µg/ml DiI-AcLDL+40 µg/ml unlabeled AcLDL) in serum-free medium for 15 min at 37°C. The cells were then fixed and imaged with a TCS SP2 confocal microscope (Leica) with a 63 x HCX PL APO CS objective with 2 airy units pinhole, without changing intensity settings for the laser and photomultiplier. Integrated fluorescence intensity of individual cells was determined with ImageJ software. For radiolabeled AcLDL uptake, wt or Cor peritoneal macrophages were incubated with 50 µg/ml of [³H]cholesterol or [³H]cholesteryl oleate–labeled AcLDL for 30 min on ice or 1 h at 37°C, and the amount of radioactivity taken up by cells was determined by scintillation counting.

**Biochemical lipid analysis, protein determination and Western blotting**

For determination of total cellular cholesterol and cholesteryl esters, peritoneal macrophages isolated from wt and Cor mice were incubated with 1) normal growth medium (10% serum) 2) serum-free medium supplemented with 100 µg/ml AcLDL overnight, or 3) loaded with AcLDL as in 2), followed by incubation in the presence of Apo-AI (10 µg/ml) for 18 h. Cellular lipids were extracted with chloroform-methanol as described, and analyzed by high-performance thin-layer chromatography using hexane/diethyl ether/ acetic acid (80:20:1 vol/vol). Lipids were visualized by dipping the plate into CuSO₄(3%)/H₃PO₄(8%) and heating for ~5 min at 180° C, the plate was imaged, and the amount of lipid analyzed by densitometric scanning. To analyze the effect of lipoprotein availability on Coro1A expression in peritoneal and Raw264.7 macrophages, cells were cultured in normal or lipoprotein-deficient serum (LPDS) containing medium for 48 h, or incubated with or without AcLDL (50 µg/ml) for 4 h prior to collecting to 1% Nonidet-P40 supplemented with protease inhibitors (chymostatin, leupeptin, antipain and pepstatin, at 25 µg/ml each). Protein determinations were performed using Bio-Rad protein assay, and immunoblotting of equal protein amounts with the indicated antibodies was performed as described.

**Cholesterol efflux**

Cholesterol efflux was analyzed from bone marrow-derived macrophages labeled for 24 h with [³H]cholesterol in DMEM/0.2% BSA, then incubated for 24 h with DMEM/0.2% fatty acid-free BSA alone or supplemented with either 10 µg/ml Apo-AI or 50 µg/ml human high density lipoprotein (HDL). Radioactivity in the cells and medium was determined by liquid scintillation counting.

**Immunofluorescence and immunoelectron microscopy**

Cells were fixed with 4% paraformaldehyde for 20 min at room temperature, quenched with 50 mM NHCl for 10 min and permeabilized with 0.1% Triton X-100 for 4 min at room temperature. Primary and secondary antibodies were diluted in 5% FBS and incubated at 37°C for 3 h or 30 min, respectively. Filipin staining was performed as described and when present, also used for permeabilization. Filipin stained cells were imaged with an IX70 inverted microscope (Olympus), equipped with a Polychrome IV monochromator (TILL Photonics) with appropriate filters. For immuno-electron microscopy, pre-embedding immunolabeling using nano-gold conjugated secondary antibodies and silver enhancement for detection was performed as described.
Lysosomal delivery of AcLDL

Macrophages were labeled overnight with 1 mg/ml FITC-dextran, and chased for 2-3 h to ensure labeling of terminal endocytic compartments. The cells were then incubated with 50 µg/ml Dil-AcLDL (pool of 10 µg/ml Dil-AcLDL+ 40 µg/ml unlabeled AcLDL) in serum-free medium at 37°C, with or without chase as indicated. The cells were fixed, imaged with a TCS SP2 confocal microscope (Leica) with a 63 x HCX PL APO CS objective and the fraction of co-localizing fluorescent signals was determined from single focal planes after background subtraction by using Overlap Coefficient function according to Manders (Image Pro Plus 5.1 software).

AcLDL-derived cholesteryl ester hydrolysis

Wt or Cor−/− peritoneal macrophages were incubated with 50 µg/ml of [3H]cholesteryl oleate in serum-free medium for 30 min at 37°C in the presence of 5 µg/ml ACAT inhibitor (PKF 058-035, Novartis), then collected or further incubated for 1-4 h in serum-free medium + ACAT inhibitor before collecting. The lipids were extracted as above and the percentage of radioactivity remaining in cholesteryl ester analyzed by thin layer chromatography and liquid scintillation counting.

Lifeact imaging

Raw264.7 macrophages transfected 2 days with Lifeact-GFP cDNA were incubated with 50 µg/ml of Dil-AcLDL for 30 min on ice, rinsed briefly, then transferred to CO2-independent medium at 37°C and imaged live from 5 min onwards with a TCS SP2 confocal microscope (Leica) using a 63 x HCX APO LU-V-I dipping objective. Images were at taken at 30 s intervals over a 30 min time period.

F-actin association with endosomes

Macrophages were labeled with 50 µg/ml of Dil-AcLDL (or alternatively with a pool of 10 µg/ml Dil-AcLDL+40 µg/ml unlabeled AcLDL) for 5-15 min in serum-free medium at 37°C, with or without a 50 min chase as indicated. The cells were then fixed with cytoskeleton preserving fixation and stained with Oregon green phalloidin as described13. The cells were imaged with a TCS SP2 confocal microscope (Leica) with a 63 x HCX PL APO CS objective. Masks of Dil-AcLDL stained areas and phalloidin stained areas were generated by intensity thresholding using Image Pro Plus 5.1 software from confocal micrographs representing a single focal plane, and the percentage of Dil-AcLDL area occupied by phalloidin staining was analyzed.

Statistical analyses

The results are expressed as mean of individual values +/- s.e.m. Statistical significance was determined by two-tailed Student’s t-test in Microsoft Excel.
Figure I Coro1A overexpression reduces cholesterol accumulation in cells lacking functional NPC1 protein. NPC1 patient fibroblasts were transfected with GFP-Coro1A cDNA, fixed at 48 h post-transfection and stained with filipin and anti-Lamp1. The cells were imaged with IX70 inverted microscope (Olympus), equipped with a Polychrome IV monochromator (TILL Photonics), and filipin intensity in Lamp1 positive organelles of GFP-Coro1A expressing (Coro1A) and non-expressing (Control) cells was quantified using Image Pro Plus 5.1 software as described. The bars represent fluorescence intensity values (N=of cells 21-22, *p<0.05). Scale bar, 20 µm.
Online Figure II

Figure II (A) Morphological response of peritoneal wt or Cor−/− macrophages to AcLDL loading. Cells were incubated −/+ 50 μg/ml AcLDL for 15 min, then fixed and stained with filipin. Scale bar, 20 μm. Bars: cell area analyzed from fluorescence micrographs, N=120-188 cells. (B) Uptake of OxLDL in peritoneal wt or Cor−/− macrophages. Cells were incubated with 50 μg/ml of [³H]cholesterol–labeled OxLDL for 1 h at 37°C and the amount of radioactivity taken up by cells was determined by scintillation counting. Bars: OxLDL uptake, % of wt (N=8 samples from 2 experiments).
**Online Figure III**

**A**

![Graph showing [3H]AcLDL uptake](image)

**B**

![Graph showing Dil AcLDL in lysosomes](image)

**Figure III** Effects of calcineurin inhibitor FK506 on AcLDL uptake and delivery to lysosomes in primary macrophages. (A) Peritoneal wt macrophages were pretreated overnight with ethanol (0.1%, vehicle control) or 1 µM FK506 in normal growth medium. After pretreatment, cells were incubated with 50 µg/ml of [3H]AcLDL for 1 h at 37°C in the presence of the compounds, and the radioactivity taken up by cells was analyzed. The bars represent [3H]AcLDL uptake in FK506 treated cells as percent of uptake in control cells (N of samples=11-12) (B) Peritoneal wt macrophages were labeled overnight with FITC-dextran, chased for 2-3 h, and further incubated with 50 µg/ml of AcLDL for 15 min at 37°C in the presence of ethanol (0.1%, vehicle control) or 1 µM FK506. The cells were fixed and the co-localization of fluorescent tracers was analyzed as described in Methods. The bars represent Pearson’s correlation coefficients between fluorescent signals (N of cells=39-48).
Online Figure IV

Figure IV Anti-Coro1A antibody immunoblotting and immunofluorescence. (A) Left panel: Bone-marrow derived wt (+) or Coro1A−/− (-) macrophages were lysed and indicated amounts of protein lysates analyzed by Western blotting with anti-Coro1A antiserum (1:3000 dilution). Right panel: Ponceau S staining of the same blot. (B) Peritoneal wt or Coro1A−/− macrophages were fixed, permeabilized with Triton X-100, stained with anti-Coro1A antiserum (1:1000 dilution), and imaged with TCS SP2 confocal microscope (Leica) with 63x HCX PL APO CS objective, using constant laser settings. Scale bar, 20 μm.
Supplemental references: