cavity of ApoE$^{-/-}$CCR2$^{-/-}$ mice were collected three days after intraperitoneal injection of 2 ml of 0.05% thioglycollate (BD Biosciences, Franklin Lakes, NJ, USA), and these cells were directly injected intravenously three times during angiotensin II infusion. At the beginning of the angiotensin II infusion, animals were divided into 2 groups: (i) the CCR2$^{+/+}$-inflammatory macrophage group (1x10^6 cells/ 200 μl PBS) (n=5); and (ii) the CCR2$^{-/-}$-leukocyte group (1x10^6 cells/ 200 μl PBS) (n=8). Mice were euthanized over a several day period after 4 weeks of angiotensin II infusion for this protocol.

Experimental protocol 2: To examine the effect of nanoparticle-mediated delivery of pitavastatin on plaque destabilization and rupture, animals were divided into 4 groups at the beginning of angiotensin II infusion: (i) the no treatment group (n=9); (ii) the FITC-incorporated NP group (0.1 mg PLGA/ 200 μl PBS) (n=7); (iii) the pitavastatin-only group (0.012 mg pitavastatin/ 200 μl PBS) (n=6); and (iv) the pitavastatin-incorporated NP group (0.1 mg PLGA/ 0.012 mg pitavastatin/ 200 μl PBS) (n=10). FITC-NP, pitavastatin, and pitavastatin-NP were administered intravenously via the tail vein once per week.

Experimental protocol 3: To examine the effect of nanoparticle-mediated delivery of the 7ND plasmid (a dominant negative inhibitor of MCP-1[3-6]) on plaque destabilization and rupture, animals were divided into 2 groups at the beginning of the angiotensin II infusion: (i) the FITC-incorporated NP group (1.3 mg PLGA/ 200 μl PBS) (n=12); and (ii) the 7ND plasmid-incorporated NP group (1.3 mg PLGA/ 5 μg 7ND plasmid/ 200 μl PBS) (n=10). NPs were administered by weekly intravenous injection.

Experimental Protocol 4: To examine the effect of daily oral administration of pitavastatin on plaque destabilization and rupture, animals were divided into 2 groups at the beginning of angiotensin II infusion: (i) the low pitavastatin group (lower dose: 0.1 mg/kg per day); and (ii) the high pitavastatin group (higher dose: 1.0 mg/kg per day). Pitavastatin was

administered by oral gavage every day for 4 weeks.

The no treatment group in protocol 2 was also used as the control group in protocols 1 and 4.

Histopathology

To quantify the extent of the atherosclerotic lesions in the whole aorta, the aortic arch and the thoracic aorta was opened longitudinally, stained with oil red O, and pinned on a black wax surface. The percentage of the plaque area stained by oil red O with respect to the total luminal surface area was quantified. To quantify the extent of the atherosclerotic lesions in the aortic root, approximately 3 serial cross sections (5 μm thick) of the aortic root were prepared according to the method described by Paigen et al,[7] with a slight modification. In brief, atherosclerotic lesions in the aortic sinus region were examined at 3 locations, each separated by 100 μm, with the most proximal site starting after the appearance of at least two aortic valve leaflets. Serial sections were stained with elastica van Gieson (EVG). The largest plaque of the three valve leaflets was adopted for morphological analysis. The brachiocephalic arteries were embedded in paraffin or OCT compound (Sakura Finetechnical Co. Ltd, Tokyo, Japan). Sections were cut at 3 μm for paraffin-embedded sections or 5 μm for OCT-embedded sections. Three sets of serial sections obtained at 30 μm intervals (starting from the proximal end) were stained with EVG to measure the total number of disrupted and buried fibrous caps, as previously described.[8] Disrupted and buried fibrous caps were defined as follows. A disrupted fibrous cap was defined as a visible defect in the cap accompanied by an intrusion of erythrocytes into the plaque. A buried fibrous cap was defined as an elastin layer that was overlaid with foam cells. All morphometric analyses were made on EVG-stained sections, and three vessel cross sections were quantified per mouse by computerized image analysis. Fibrous cap thickness was determined at the thinnest part of the cap by computerized image

analysis. The analysis was necessarily restricted to those plaques that had developed sufficiently to form fibrous caps, and the average thickness of three fibrous caps per section was obtained for quantitative analysis. Plaque macrophage or MCP-1 expression areas were determined by the ratio of Mac3- or MCP-1-positive areas to the intima areas of plaques.

Immunohistochemistry

Serial brachiocephalic arterial sections adjacent to those sections that were stained with EVG were deparaffinized, and endogenous peroxidase was blocked by incubation with 0.3% H_2O_2 in methanol for 5 minutes. For antigen retrieval, sections were boiled for 20 minutes in citrate buffer (pH=6.0). After blocking with 3% skim milk, sections were incubated overnight at 4°C with the following antibodies: anti-mouse macrophage antibody (Mac3; dilution 1:100, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), and anti-mouse MCP-1 antibody (dilution 1:200, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) followed by incubation with biotin-conjugated secondary antibodies. Then, the sections were washed and treated with avidin-peroxidase. The sections were developed using the DAB substrate kit (Wako Pure Chemical Industries, Osaka, Japan), and nuclei were counterstained with hematoxylin. Serial aortic root sections were also stained using anti-mouse Mac3 antibody. Multiple observers who were blinded to the experiment protocol performed the quantitative analysis. All images were captured with a Nikon microscope equipped with a digital camera (HC-2500) and analyzed using Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA, USA) and Scion Image 1.62 for Windows (Scion, Frederick, MD, USA).

Flow cytometry

Peripheral blood was drawn via a cardiac puncture, and red blood cells were lysed with

VersaLyse Lysing solution (Becton Dickinson Biosciences, San Jose, California) for 10 minutes at room temperature. Spleens were removed and triturated in HBSS at 4 °C and filtered through nylon mesh (BD Biosciences). The cell suspension was centrifuged at 300 x *g* for 5 minutes at 4 °C. Red blood cells were also lysed with VersaLyse Lysing solution. After blocking the Fc receptor with anti-CD16/32 mAb (BD Pharmingen, San Diego, California) for 5 minutes at 4°C, peripheral leukocytes were incubated with a cocktail of CA, USA against CD11b-APC (BD Pharmingen, San Diego, California), CD115-PE (BD Pharmingen, San Diego, California) and Ly-6C-FITC (eBioscience, San Diego, CA, USA), and peritoneal leukocytes were incubated with a cocktail of mAb against F4/80-APC (AbD Serotec, Oxford, UK), CD115-PE (BD Pharmingen) and Ly-6C-FITC (eBioscience) for 30 minutes at 4°C; all leukocytes were then analyzed with FACSCalibur (Becton Dickinson Biosciences, San Jose, CA, USA). For the cellular uptake of FITC-NPs, leukocytes were incubated with a cocktail of mAb against lineage cell marker (Lin: CD90/B220/CD49b/NK1.1/Ly-6G)-PE (BD Pharmingen, San Diego, CA, USA) and CD11b-APC. The leukocytes were also incubated with appropriate isotype controls (BD Pharmingen, San Diego, CA, USA). Macrophage subsets were identified as either Ly-6C^{hi}F4/80^{+}CD115^{+} or Ly-6C^{lo}F4/80^{+}CD115^{+}, as previously described.[9] Monocyte subsets were identified as either Ly-6C^{hi}CD11b^{+}CD115^{+} or Ly-6C^{lo}CD11b^{+}CD115^{+}, as previously described.[10] For the cellular uptake of FITC-NPs, neutrophils and monocytes were identified as Lin^{+}CD11b^{+} and Lin^{-}CD11b^{+}, respectively.

In vivo accumulation of inflammation-activated macrophages

Thioglycollate-elicited macrophages were labeled with PKH26 (Sigma Aldrich, St. Louis, MO, USA) *ex vivo* according to the manufacturer's protocol. 24 hours after the intravenous injection of PKH26-labeled macrophages, the brachiocephalic arteries were

fixed with 3.7% formaldehyde and embedded in OCT compound. Sections were cut at 5 μm for OCT-embedded sections and evaluated by fluorescence microscopy. Nuclei were stained with DAPI (Vector Laboratories Inc., Burlingame, California).

Splenic Monocyte/Macrophage isolation and adoptive transfer

Splenic monocytes and macrophages were obtained by negative selection using the Mouse Monocytes Enrichment Kit (StemCell Technologies, British Columbia, Canada) according to the manufacturer's protocol. In brief, monocytes and macrophages were isolated from an $ApoE^{-/-}$ mouse spleen. Cells were passed through a 100 μm nylon filter, and suspended in lysis buffer. Mouse monocytic cells were further purified from the spleens using the Purple EasySep magnet (StemCell Technologies) prior to flow cytometry analysis. These cells (0.8-1.0 $x10^6$ cells/ 200 μl PBS) were directly injected intravenously via the femoral vein once to $ApoE^{-/-}$ mice fed a high-fat diet and infused with angiotensin II. Mice were euthanized with intraperitoneal injection of pentobarbital at day 7 of angiotensin II infusion for analysis.

Preparation of PLGA nanoparticles

Poly(lactic-co-glycolic acid) (PLGA) polymer with an average molecular weight of 20,000 and a lactide-to-glycolide copolymer ratio of 75:25 (Wako Pure Chemical Industries, Osaka, Japan) was used to prepare the nanoparticles. PLGA nanoparticles incorporated with fluorescein isothiocyanate (FITC; Dojindo Laboratories, Kumamoto, Japan) (FITC-NP), pitavastatin (Kowa Pharmaceutical Co Ltd, Tokyo, Japan) (pitavastatin-NP), or 7ND plasmid (7ND-NP) were prepared by a previously reported emulsion solvent diffusion method in purified water.[11-13] PLGA was dissolved in a mixture of acetone and methanol. Then, FITC, pitavastatin, or 7ND plasmid was added to this solution. The resultant PLGA-FITC, PLGA-pitavastatin, or PLGA-7ND solution was

emulsified in polyvinyl alcohol with stirring at 400 rpm using a propeller-type agitator with 3 blades (Heidon 600G, Shinto Scientific, Tokyo, Japan). After the system was agitated for 2 hours under reduced pressure at 40°C, the entire suspension was centrifuged (20,000 x *g* for 20 minutes at -20°C). After the supernatant was removed, purified water was added and mixed with the sediment. The wet mixture was then centrifuged again to remove excess polyvinyl alcohol and the unencapsulated reagent that could not adsorb onto the surfaces of the nanoparticles. After this process was repeated, the resultant dispersion was freeze-dried under the same conditions. The FITC-, pitavastatin-, or 7ND-loaded PLGA nanoparticles contained 5.0% (w/v) FITC, 12.0% (w/v) pitavastatin, or 0.40% (w/v) 7ND, respectively. The average diameters of the PLGA-NPs were 231 nm, 159 nm, and 290 nm for the FITC-NP, pitavastatin-NP, and 7ND-NP, respectively. The surface charges (zeta potential) analyzed by Zetasizer Nano (Sysmex, Hyogo, Japan) were -16.7 mV, -4 mV, and 8.1 mV, respectively.

Cellular uptake and in vitro kinetics of the nanoparticles

Murine peritoneal macrophages were obtained from wild-type mice that were injected intraperitoneally with 2 ml of 0.05% thioglycollate 72 hours before the extraction. The cells were centrifuged at 1,000 rpm for 5 minutes at 4°C, suspended in lysis buffer and washed twice with phosphate-buffered saline (PBS) for 5 minutes at 4°C. The cell pellets were suspended in DMEM plus 10% FBS and 1% PS at an initial concentration of 5.0×10^5 cells/ml in a 35 mm culture dish. FITC-NPs were added to the dish (1.0 mg PLGA/ml) and incubated at 37°C in a 5% CO_2 environment overnight. After two washes with PBS for 5 minutes at room temperature, the cells were fixed with methanol and counterstained with propidium iodide (Vector Laboratories Inc., Burlingame, CA, USA). The intracellular uptake of FITC-NP was evaluated by fluorescence microscopy (BX50,

Olympus, Tokyo, Japan) and confocal microscopy (FV1000-D, Olympus). RAW264.7, a murine macrophage cell line, was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were seeded on 6-well-chamber slides and incubated at 37°C in a 5% CO_2 environment until they were subconfluent. The growth medium was replaced with 2 ml of an OsO_4-NP suspension medium (1.0 mg PLGA/ml), incubated for 6 hours and fixed with 2.5% glutaraldehyde. The intracellular uptake of OsO_4-NPs was observed by transmission electronic microscopy (Hitachi H7000E, Tokyo, Japan). To examine the FITC kinetics of cultured macrophages, confluent RAW264.7 cells were incubated with FITC-NP or FITC only at the indicated concentrations (1, 3, 10, 30, and 100 μM). After 2 hours of incubation, extracellular FITC-NPs or FITC was washed out, and FITC with diffuse interference contrast image was observed by confocal laser microscopy (Nikon A1R, Tokyo, Japan) at the indicated time points (days 0, 1, 4, and 7). The average fluorescent intensity of FITC was analyzed using the National Institutes of Health Image Software.

In vivo kinetics of the nanoparticles

The whole aortas of $ApoE^{-/-}$ mice that were fed with HFD and infused with angiotensin II were excised 24 hours after an intravenous injection of the FITC-NPs. The excised aortas were evaluated by stereoscopic and fluorescence microscopy (Nikon SMZ1500 equipped with Nikon HB-10103AF and appropriate fluorescence filter sets, Tokyo, Japan). The brachiocephalic arteries were fixed with 3.7% formaldehyde and embedded in OCT compound. Sections were cut at lengths of 5 μm and evaluated by fluorescence microscopy (BX50, Olympus). The nuclei were stained with propidium iodide (Vector Laboratories Inc., Burlingame, CA, USA). Serial sections were stained with hematoxylin-eosin.

In situ zymography

Gelatinase (MMP-2/gelatinase-A and MMP-9/gelatinase-B) activity was measured in unfixed frozen sections (6 μm thick) using quenched fluorescein-labeled gelatinase substrate (DQ gelatin, Invitrogen, Eugene, OR, USA).[14] The fluorescent area produced by the proteolytic digestion of quenched fluorescein-labeled gelatin was recognized as combined gelatinase activity (MMP-2 + MMP-9). The brachiocephalic artery sections were incubated at 37°C for 30 minutes according to the manufacturer's protocol. Fluorescent microscopy was used to detect gelatinase activity as green fluorescence. Negative control zymograms were incubated in the presence of 20 mM EDTA. The specific removal of essential divalent cations resulted in no detectable gelatinolytic activity.

Gelatin zymography

The RAW264.7 cells were prepared as described above. The growth medium was replaced with pitavastatin at 0.01, 0.1, or 1 μM; pitavastatin-incorporated NPs containing 0.0367, 0.367, or 3.67 μg/mL of PLGA and 0.01, 0.1, or 1 μM of pitavastatin; FITC-incorporated NPs containing 3.67 μg/mL of PLGA-NP; or the vehicle alone. When the medium was replaced, LPS was added at 25 ng/mL to each well. Twenty-four hours after the LPS stimulation, the culture supernatant was obtained. The supernatant was subjected to gelatin zymography using the Gelatin Zymo-Electrophoresis Kit (Primary Cell, Hokkaido, Japan) according to the manufacturer's directions.

Real-time quantitative RT PCR

Real-time PCR amplification was performed with the mouse cDNA with the use of the ABI PRISM 7000 Sequence Detection System (Applied Biosystems), as described previously.[15] For mouse MCP-1, the sense and antisense PCR primers were

5'-CCTGGATCGGA-ACCAAATGA-3' and 5'-CGGGTCAACTTCA-CATTCAAAG-3', respectively, and the probe oligonucleotide was 5'-AACT-GCATCTGCCCTAAGG-TCTTCAGCA-3'. For mouse CCR2, the sense and antisense primers were 5'-CCTTGGGA-ATGAGTAACTGTGTGAT-3' and 5'-ATGGA-GAGATACCTTCGGAACTTCT-3', and the probe oligonucleotide was 5'-CACTTAGACCAGGCCATGCAGGT-GACA-3'. The GAPDH probe was purchased from Applied Biosystems.

Chemotaxis assay

THP-1, the human monocyte cell line, was obtained from the German Collection of Micro-organisms and Cell Cultures (DSMZ; Brauschweig, Germany). The cells were cultured in RPMI 1640 with 10% FBS and 37°C in a 5% CO_2 environment until they were subconfluent. The growth medium was replaced with starvation medium with pitavastatin-NPs containing 0.00367 to 36.7 μg/mL of PLGA and 0.001 to 10 μM of pitavastatin, FITC-NPs containing 36.7 μg/mL of PLGA, or vehicle alone for 24 hours. The chemotactic activity of THP-1 cells in response to 10 ng/mL MCP-1 was measured in a 96-well microchemotaxis Boyden chamber (ChemoTx; Neuroprobe), as described previously.[16] Monocytes that had transmigrated through the micropore were stained with trypan blue. The number of monocytes that migrated in response to MCP-1 was counted.

Measurements of statin concentration in plasma

Pitavastatin concentrations in plasma were measured at predetermined time points by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). Briefly, the high-performance liquid chromatography (HPLC) analysis was performed using Agilent 1100 series system (Agilent Technologies, Inc, Santa Clara, CA, USA). The column temperature was maintained at 40 °C. The flow rate was 0.3 mL/min. Pre-prepared plasma

solutions were injected from the autosampler into the HPLC system. The turbo ion spray interface was operated in the positive ion mode at 4800 V and 550 °C. The analytical data were processed using Analyst software (version 1.4, Applied Biosystems, Foster City, CA, USA).

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Supplementary Figure Legends

Supplementary Figure 1. Experimental protocols for the treatments in $ApoE^{-/-}$ mice. At 16-18 weeks of age, mice began receiving the HFD. After 4 weeks of the experimental diet, all mice were infused with angiotensin II dissolved in phosphate-buffered saline (PBS) at 1.9 mg/kg per day.

Protocol 1. Animals were divided into 2 groups at the beginning of angiotensin II infusion: (i) adoptively transferred $CCR2^{+/+}$-inflammatory macrophages from $ApoE^{-/-}$ mice (1×10^6 cells/ 200 μl PBS) and (ii) adoptively transferred $CCR2^{-/-}$-leukocytes from $ApoE^{-/-}CCR2^{-/-}$ mice (1×10^6 cells/ 200 μl PBS).

Protocol 2. Animals were divided into 2 groups at the beginning of angiotensin II infusion: (i) the FITC-incorporated NP group (1.3 mg PLGA/ 200 μl PBS) and (ii) the 7ND-incorporated NP group (5 μg 7ND plasmid/ 200 μl PBS). NPs were administered by weekly intravenous injection.

Protocol 3. Animals were divided into 4 groups at the beginning of angiotensin II infusion: (i) the no treatment group, (ii) the FITC-incorporated NP group (0.1 mg PLGA/ 200 μl PBS), (iii) the pitavastatin-only group (0.012 mg pitavastatin/ 200 μl PBS), and (iv) the pitavastatin-incorporated NP group (0.1 mg PLGA/ 0.012 mg pitavastatin/ 200 μl PBS). NPs were administered by weekly intravenous injection.

Protocol 4. Animals were divided into 2 groups at the beginning of angiotensin II infusion: (i) oral daily administration of pitavastatin at a low dose (0.1 mg/kg/day) and (ii) oral daily administration of pitavastatin at a high dose (1.0 mg/kg/day). Pitavastatin was daily administered by oral gavage.

The no treatment group in protocol 2 was also used as the control group in protocols 1. and 4.

Supplementary Figure 2. Characteristics and kinetics of adoptive transferred macrophages. (**A**) Quantitative flow cytometric analysis of the number of $F4/80^+CD115^+$ macrophages in the peritoneal cavities of $ApoE^{-/-}$ or $ApoE^{-/-}CCR2^{-/-}$ mice induced by intraperitoneal injection of thioglycollate (TG). (**B**) Quantitative analysis of the mean

fluorescence intensity (MFI) of Ly-6C expression in the $F4/80^{+}CD115^{+}$ macrophages from the peritoneal cavities of the $ApoE^{-/-}$ mice. The data are reported as the mean±SEM. (**C**) Left panel: A fluorescence photomicrograph of the brachiocephalic artery of an $ApoE^{-/-}$ mouse from the no treatment group. Upper middle and right panel: PKH fluorescence photomicrographs of the brachiocephalic artery of an $ApoE^{-/-}$ mouse transferred with PKH-labeled activated macrophages. Lower middle and right panel: FITC autofluorescence photomicrographs of the brachiocephalic artery of an $ApoE^{-/-}$ mouse transferred with PKH-labeled activated macrophages. Right panel: An expanded image of the red square area in the middle panel. The nuclei were stained with DAPI. The scale bar indicates 100 μm.

Supplementary Figure 3. The adoptive transfer of splenic monocytes accelerates plaque destabilization and rupture in the brachiocephalic arteries. (A) Left panel: Representative flow cytometry dot plots of splenic leukocytes from $ApoE^{-/-}$ mice. Middle panel: The Representative flow cytometry dot plots of splenic leukocytes negatively selected with antibodies against the leukocytes other than monocytes from $ApoE^{-/-}$ mice. Right panel: The Representative histogram of Ly-6C expression on negatively selected splenic monocytes. (B) Upper panel: Photomicrographs of atherosclerotic plaques in the brachiocephalic artery stained with elastica van Gieson (EVG) in the No Treatment (N) and the Monocytes (M) groups. Arrowheads indicate disrupted/buried fibrous caps. The scale bar indicates 100 μm. Lower panel: Quantitation of the number of disrupted/buried fibrous caps and fibrous cap thickness. The data are reported as the mean±SEM. $*P<0.05$ versus the No Treatment group. There were no statistically significant differences in fibrous cap thickness between the two groups.

Supplementary Figure 4. Cellular uptake and *in vitro* kinetics of the NPs in macrophages. (**A**) Fluorescence photomicrographs of murine peritoneal macrophages incubated with FITC-NPs for 24 hours. An inset depicts a photomicrograph of macrophages incubated without FITC-NPs. (**B**) A fluorescence confocal microscopy image of RAW264.7 cells

incubated with FITC-NPs for 24 hours. (**C**) Electron microscopy image of RAW264.7 cells incubated with OsO_4-NPs for 24 hours. (**D**) Upper panel: Time course of the FITC signal retained in RAW264.7 cells after a 2-hour incubation with FITC-NPs or FITC (0.3, 1, 3, 10, 30, 100 μM) followed by a washout period. Cells were observed at 0, 24, 72 hours, and 1 week of washout. Lower panel: Quantitative analysis of relative fluorescence units (RFUs) of RAW264.7 cells incubated with FITC-NPs (green lines) or FITC (blue lines). *P<0.01 and **P<0.001 versus FITC (N = 4 per group). Data were compared using two-way ANOVA followed by Bonferroni's multiple comparison tests.

Supplementary Figure 5. Effects of daily oral administration of pitavastatin (0.1 or 1.0 mg/kg per day) on atherosclerotic plaque rupture in the brachiocephalic arteries. (**A**) Upper panel: Photomicrographs of atherosclerotic plaques stained with elastica van Gieson (EVG), Mac3 or MCP-1 in the No Treatment (N), pitavastatin 0.1 mg/kg (0.1), and pitavastatin 1.0 mg/kg (1.0) groups. Arrowheads indicate disrupted/buried fibrous caps. The scale bar indicates 100 μm. Lower panel: Quantitation of the number of disrupted/buried fibrous caps, fibrous cap thickness and Mac3- and MCP-1-positive areas. The data are reported as the mean±SEM. †P<0.05 versus the No Treatment group using one-way ANOVA followed by Dunnett's multiple comparison tests. **P<0.01 versus the No Treatment group using one-way ANOVA followed by Bonferroni's multiple comparison tests. (**B**) Upper panel: Photomicrographs of the intraluminal surface of the total aorta stained with oil red O. Lower panel: Quantitation of the percentage of the plaque area compared with the total luminal surface area. The data are reported as the mean±SEM. *P<0.05 versus the No Treatment group. (C) Upper panel: Photomicrographs of atherosclerotic plaques in the aortic root stained with EVG or Mac3. Lower panel: Quantitation of plaque size and Mac3-positive areas. The scale bar indicates 200 μm. The data are reported as the mean±SEM. †P<0.05 versus the No Treatment group using one-way ANOVA followed by Dunnett's multiple comparison tests.

Supplementary Tables

Supplementary Table 1. Body weight, heart rate, systolic blood pressure, and lipid profiles in the no treatment, $CCR2^{+/+}$ inflammatory macrophage, and $CCR2^{-/-}$ leukocyte groups.

	No Treatment (N= 9)	$CCR2^{+/+}$ Inflammatory Macrophage (N= 5)	$CCR2^{-/-}$ Leukocyte (N= 8)
Body Weight (g)	33±1	35±3	34±3
Heart Rate (beat/min)	650±20	640±40	580±40
Systolic Blood Pressure (mmHg)	120±2	112±8	115±9
Total Cholesterol (mg/dl)	660±30	710±100	720±60
Triglyceride (mg/dl)	65±9	74±16	69±5

The data are expressed as the mean±SEM. The mean values were compared using ANOVA and Bonferroni's multiple comparison tests, and there are no significant differences for any of these parameters among these groups.

Supplementary Table 2. Body weight, heart rate, systolic blood pressure, and lipid profiles in the no treatment, FITC-NP, pitavastatin, and pitavastatin-NP groups.

	No Treatment (N= 9)	FITC-NP (N= 7)	Pitava (N= 6)	Pitava-NP (N= 10)
Body Weight (g)	33±1	30±1	34±1	32±1

Heart Rate (beat/min)	650±20	630±30	650±20	590±20
Systolic Blood Pressure (mmHg)	120±0	110±10	12±10	120±0
Total Cholesterol (mg/dl)	660±30	670±50	610±50	710±40
Triglyceride (mg/dl)	65±9	60±5	53±5	70±6

The data are expressed as the mean±SEM. The mean values were compared using ANOVA and Bonferroni's multiple comparison tests, and there are no significant differences for any of these parameters compared with the No Treatment group.

Supplementary Table 3. Body weight, heart rate, systolic blood pressure, and lipid profiles in the FITC-NP and 7ND-NP groups.

	FITC-NP (N= 9)	7ND-NP (N= 10)
Body Weight (g)	28±1	25±1
Heart Rate (beat/min)	640±30	680±10
Systolic Blood Pressure (mmHg)	130±10	120±10
Total Cholesterol (mg/dl)	720±60	730±20
Triglyceride (mg/dl)	47±11	53±18

The data are expressed as the mean±SEM. The mean values were compared using the unpaired *t*-test, and there are no significant differences for any of these parameters between these 2 groups.

Supplementary Table 4. Body weight, heart rate, systolic blood pressure, and lipid profiles in the no treatment, pitavastatin 0.1 mg/kg, and pitavastatin 1.0 mg/kg groups.

	No Treatment (N= 9)	Pitavastatin 0.1 mg/kg (N= 10)	Pitavastatin 1.0 mg/kg (N= 11)
Body Weight (g)	33±1	30±1*	32±0
Heart Rate (beat/min)	650±20	610±20	630±10
Systolic Blood Pressure (mmHg)	120±0	110±0	120±0
Total Cholesterol (mg/dl)	660±30	780±20	800±50
Triglyceride (mg/dl)	65±9	82±15	43±5

The data are expressed as the mean±SEM. *P<0.05 versus the No Treatment group. The data were compared using ANOVA followed by Bonferroni's multiple comparison tests.

Supplementary Table 5. Serum biomarkers in the no treatment, $CCR2^{+/+}$ inflammatory macrophage, and $CCR2^{-/-}$ leukocyte group.

		No Treatment (N= 7)	$CCR2^{+/+}$ Inflammatory Macrophage (N= 5)	$CCR2^{-/-}$ Leukocyte (N= 7)
Apo A1	**µg/mL**	48±6	39±6	38±3
CD40	**pg/mL**	87±9	160±40	75±9
CD40 Ligand	**pg/mL**	2600±300	5700±400**	4600±700*
CRP	**µg/mL**	11±1	10±1	11±2

EGF	**pg/mL**	16±1	23±1**	21±1**
Endothelin-1	**pg/mL**	18±1	21±2	17±2
Eotaxin	**pg/mL**	320±20	330±50	380±30
Factor VII	**ng/mL**	14±1	19±1*	18±1
FGF-basic	**ng/mL**	7.0±0.6	11±1*	9.0±0.8
GCP-2	**ng/mL**	31±7	13±7	5±1**
Haptoglobin	**μg/mL**	140±20	190±30	200±20
IFN-γ	**pg/mL**	N.D.	23±8	N.D.
IgA	**μg/mL**	42±5	52±9	60±7
IL-10	**pg/mL**	430±20	N.D.	N.D.
IL-11	**pg/mL**	N.D.	490±430	85±29
IL-17	**ng/mL**	N.D.	N.D.	0.01±0.00
IL-18	**ng/mL**	18±1	30±1***	27±0***
IL-1α	**pg/mL**	260±72	160±45	94±15
IL-1β	**ng/mL**	17±1	20±1	20±1
IL-5	**ng/mL**	N.D.	0.73±0.23	0.61±0.12
IL-6	**pg/mL**	11±2	15±4	N.D.
IL-7	**ng/mL**	0.18±0.06	0.22±0.12	0.18±0.07
IP-10	**pg/mL**	68±9	230±140	54±3
LIF	**pg/mL**	1200±0	1500±100	1200±100
Lymphotactin	**pg/mL**	120±50	180±40	100±20
MCP-1	**pg/mL**	130±10	220±30**	110±10
MCP-3	**pg/mL**	400±30	700±100**	490±40
MCP-5	**pg/mL**	21±2	49±6**	37±6

M-CSF	**ng/mL**	5.2±0.3	8.2±0.4***	6.0±0.1
MDC	**pg/mL**	460±20	580±70	560±40
MIP-1α	**ng/mL**	2.4±0.3	4.1±0.2***	4.0±0.2***
MIP-1β	**pg/mL**	190±40	410±50**	280±20
MIP-1γ	**ng/mL**	50±3	67±7	52±7
MIP-2	**pg/mL**	18±4	28±3	21±2
MIP-3	**ng/mL**	2.3±0.2	3.5±0.3**	2.4±0.3
MMP-9	**ng/mL**	110±20	210±20**	140±20
MPO	**ng/mL**	110±20	200±10**	160±10*
Myoglobin	**ng/mL**	320±260	260±200	78±32
OSM	**ng/mL**	0.15±0.03	N.D.	0.03±0.01**
RANTES	**pg/mL**	0.64±0.20	N.D.	N.D.
SAP	**μg/mL**	47±2	35±1*	38±4
SCF	**pg/mL**	310±40	230±30	190±20*
SGOT	**μg/mL**	51±9	50±5	73±4
TIMP-1	**ng/mL**	4.9±0.7	5.2±0.9	4.7±0.6
Tissue Factor	**ng/mL**	8.6±0.3	11±2	7.5±1.0
TNF-α	**ng/mL**	0.11±0.02	N.D.	N.D.
TPO	**ng/mL**	110±10	150±10**	130±0*
VCAM-1	**ng/mL**	2200±100	3500±700*	2700±100
VEGF	**pg/mL**	290±40	200±10	190±10*
vWF	**ng/mL**	150±10	330±130	160±20

The data are expressed as the mean±SEM. The means were compared by means of