ANOVA and Bonferroni's multiple comparison tests. **P*<0.05 versus the No Treatment group, ***P*<0.01 versus the No Treatment group, ****P*<0.001 versus the No Treatment group. Multiplex immunoassay was performed using the Luminex LabMAP instruments by Charles River Inc. Apo A1 (Apolipoprotein A1), CD (Cluster of Differentiation), CRP (C Reactive Protein), EGF (Epidermal Growth Factor), FGF-9 (Fibroblast Growth Factor-9), FGF-basic (Fibroblast Growth Factor-basic), GCP-2 (Granulocyte Chemotactic Protein-2), GM-CSF (Granulocyte Macrophage-Colony Stimulating Factor), GST-α (Glutathione S-Transferase alpha), IFN-γ (Interferon-gamma), IgA (Immunoglobulin A), IL (Interleukin), IP-10 (Inducible Protein-10), KC/GROα (Melanoma Growth Stimulatory Activity Protein), LIF (Leukemia Inhibitory Factor), MCP (Monocyte Chemoattractant Protein), M-CSF (Macrophage Colony-Stimulating Factor), MDC (Macrophage-Derived Chemokine), MIP (Macrophage Inflammatory Protein), MMP-9 (Matrix Metalloproteinase-9), MPO (Myeloperoxidase), OSM (Oncostatin M), RANTES (Regulation Upon Activation, Normal T-Cell Expressed and Secreted), SAP (Serum Amyloid P), SCF (Stem Cell Factor), SGOT (Serum Glutamic-Oxaloacetic Transaminase), TIMP-1 (Tissue Inhibitor of Metalloproteinase Type-1), TNF-α (Tumor Necrosis Factor-alpha), TPO (Thrombopoietin), VCAM-1 (Vascular Cell Adhesion Molecule-1), VEGF (Vascular Endothelial Cell Growth Factor), vWF (von Willebrand Factor). N.D. (Not Detected).

Supplementary Table 6. Serum biomarkers in the FITC-NP and pitavastatin-NP groups.

		FITC-NP (N= 6)	Pitava-NP (N= 9)
Apo A1	**μg/mL**	45±2	46±2

CD40	**pg/mL**	110±10	90±11
CD40 Ligand	**pg/mL**	1900±100	1400±100*
CRP	**μg/mL**	7.6±0.8	7.5±0.8
EGF	**pg/mL**	26±3	24±1
Endothelin-1	**pg/mL**	24±2	24±1
Eotaxin	**pg/mL**	370±10	420±20
Factor VII	**ng/mL**	28±2	28±1
FGF-basic	**ng/mL**	17±2	17±0
GCP-2	**ng/mL**	39±5	35±4
Haptoglobin	**μg/mL**	150±10	150±10
IgA	**μg/mL**	44±12	32±3
IL-10	**pg/mL**	N.D.	N.D.
IL-11	**pg/mL**	120±60	61±9
IL-18	**ng/mL**	18±1	16±1
IL-1α	**pg/mL**	440±130	200±40
IL-1β	**ng/mL**	7.9±0.3	7.8±0.6
IL-4	**pg/mL**	71±28	59±6
IL-5	**ng/mL**	0.80±0.12	1.1±0.2
IL-6	**pg/mL**	N.D.	12±4
IL-7	**ng/mL**	0.082±0.018	N.D.
IP-10	**pg/mL**	40±3	47±7
LIF	**pg/mL**	1900±100	1900±100
Lymphotactin	**pg/mL**	80±9	82±7
MCP-1	**pg/mL**	130±10	110±0*

MCP-3	**pg/mL**	380±30	320±20
MCP-5	**pg/mL**	28±4	30±2
M-CSF	**ng/mL**	7.3±0.1	7.5±0.2
MDC	**pg/mL**	650±40	840±70
MIP-1α	**ng/mL**	3.3±0.2	3.2±0.1
MIP-1β	**pg/mL**	200±30	180±10
MIP-1γ	**ng/mL**	54±4	45±3
MIP-2	**pg/mL**	28±2	22±2
MIP-3	**ng/mL**	2.0±0.1	2.1±0.1
MMP-9	**ng/mL**	130±10	120±10
MPO	**ng/mL**	140±20	120±10
Myoglobin	**ng/mL**	240±60	360±150
OSM	**ng/mL**	0.05±0.01	N.D.
SAP	**μg/mL**	32±2	30±2
SCF	**pg/mL**	280±10	240±10*
TIMP-1	**ng/mL**	5.0±0.7	4.3±0.5
Tissue Factor	**ng/mL**	14±1	12±0
TPO	**ng/mL**	30±3	32±2
VCAM-1	**ng/mL**	2600±100	2500±200
VEGF	**pg/mL**	200±20	150±10*
vWF	**ng/mL**	180±10	150±10*

The data are expressed as the mean±SEM. The mean values were compared using an unpaired *t*-test. *$P<0.05$ versus the FITC-NP group.

Supplementary Table 7. Plasma concentration of pitavastatin in the pitavastatin and pitavastatin-NP groups.

	2 hours	6 hours	24 hours
Pitavastatin (ng/mL)	1.3±0.2	N.D.	N.D.
Pitavastatin-NP (ng/mL)	2.5±0.2*	N.D.	N.D.

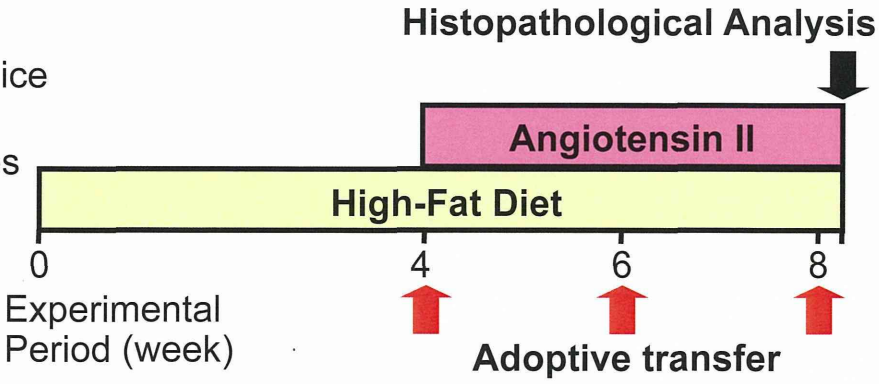
The data are expressed as the mean±SEM. The mean values were compared using an unpaired *t*-test. *$P<0.05$ versus the Pitavastatin group.

Experiment Protocol 1

ApoE$^{-/-}$mice or ApoE$^{-/-}$CCR2$^{-/-}$mice
Thioglycollate-induced peritoneal macrophages

↓

ApoE$^{-/-}$mice
18 weeks of age

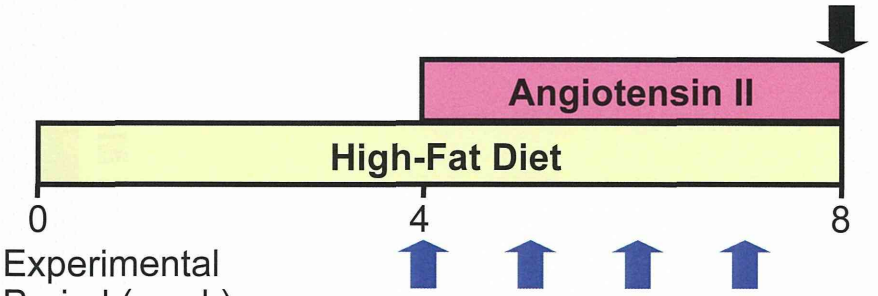


Treatment Group

1. CCR2$^{+/+}$-Inflammatory Macrophage (1x10^6 cells/ 200 μL PBS)
2. CCR2$^{-/-}$-Leukocyte (1x10^6 cells/ 200 μL PBS)

Experiment Protocol 2

ApoE$^{-/-}$mice
16 weeks of age

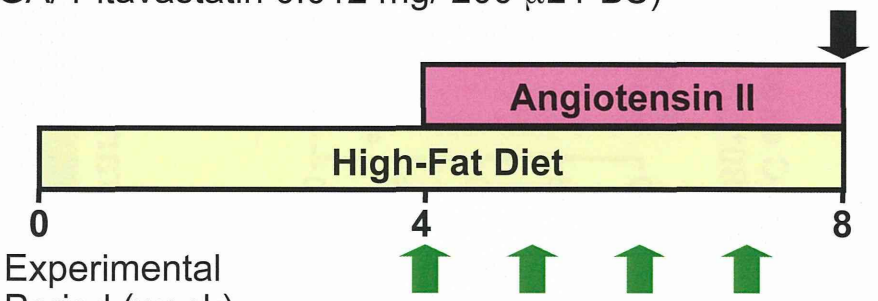


Treatment Group

1. No treatment
2. FITC-NP (0.1 mg PLGA/ 200 μL PBS)
3. Pitavastatin (Pitavastatin 0.012 mg/ 200 μL PBS)
4. Pitavastatin-NP (0.1 mg PLGA/ Pitavastatin 0.012 mg/ 200 μL PBS)

Experiment Protocol 3

ApoE$^{-/-}$mice
16 weeks of age

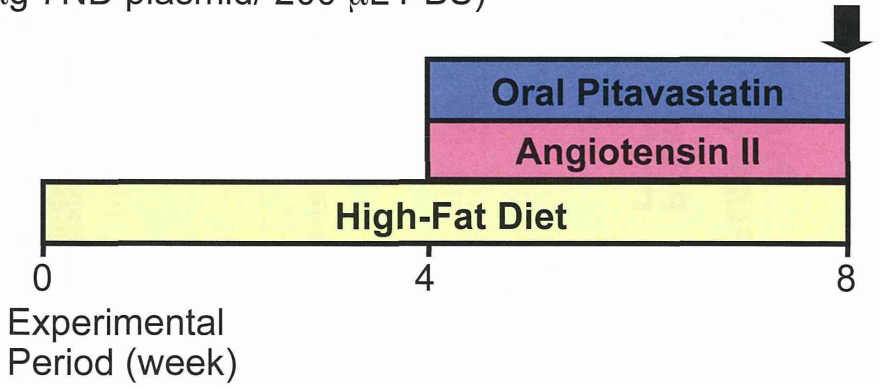


Treatment Group

1. FITC-NP (1.3 mg PLGA/ 200 μL PBS)
2. 7ND-NP (1.3 mg PLGA/ 5 μg 7ND plasmid/ 200 μL PBS)

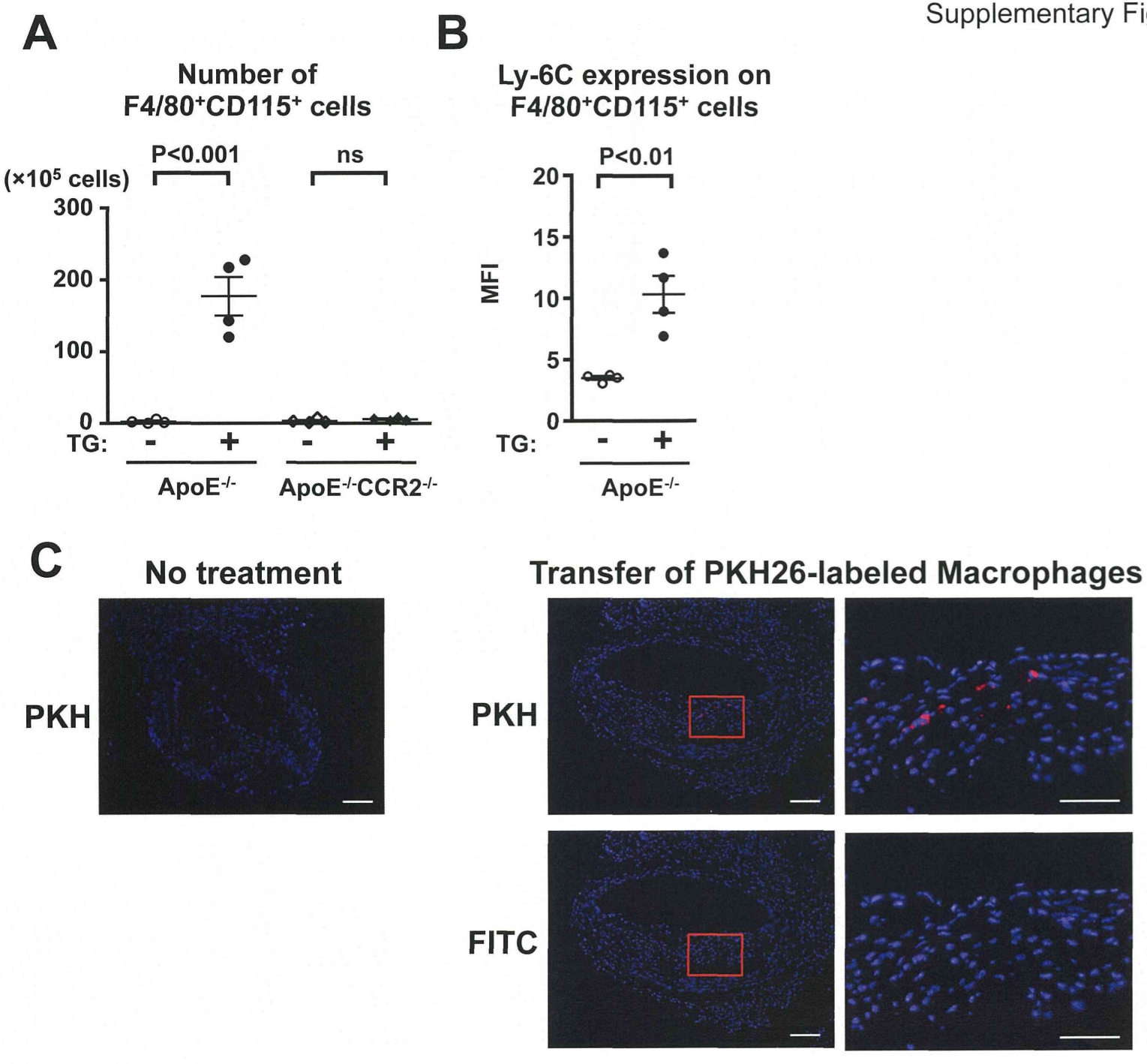
Experiment Protocol 4

ApoE$^{-/-}$mice
16 weeks of age

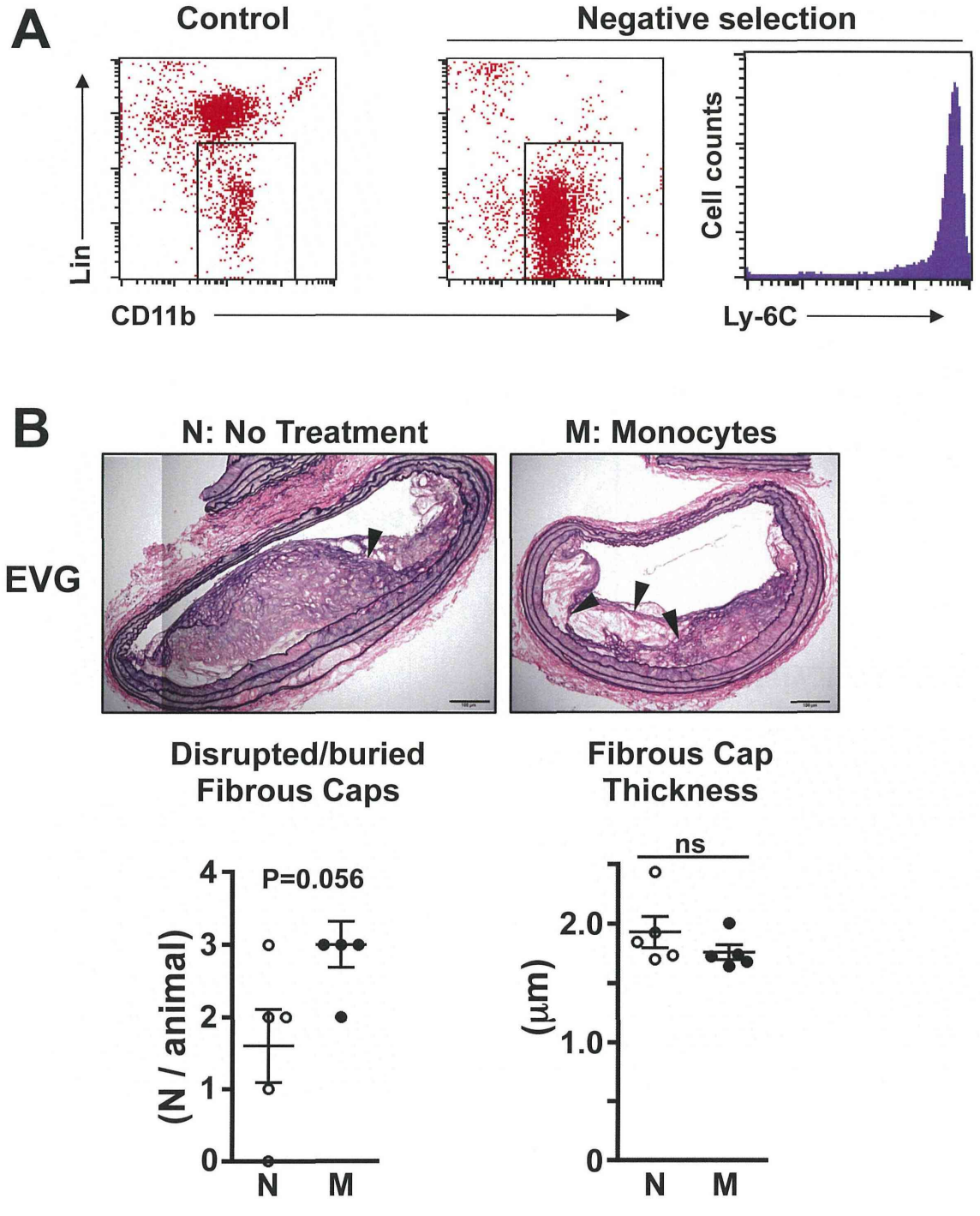


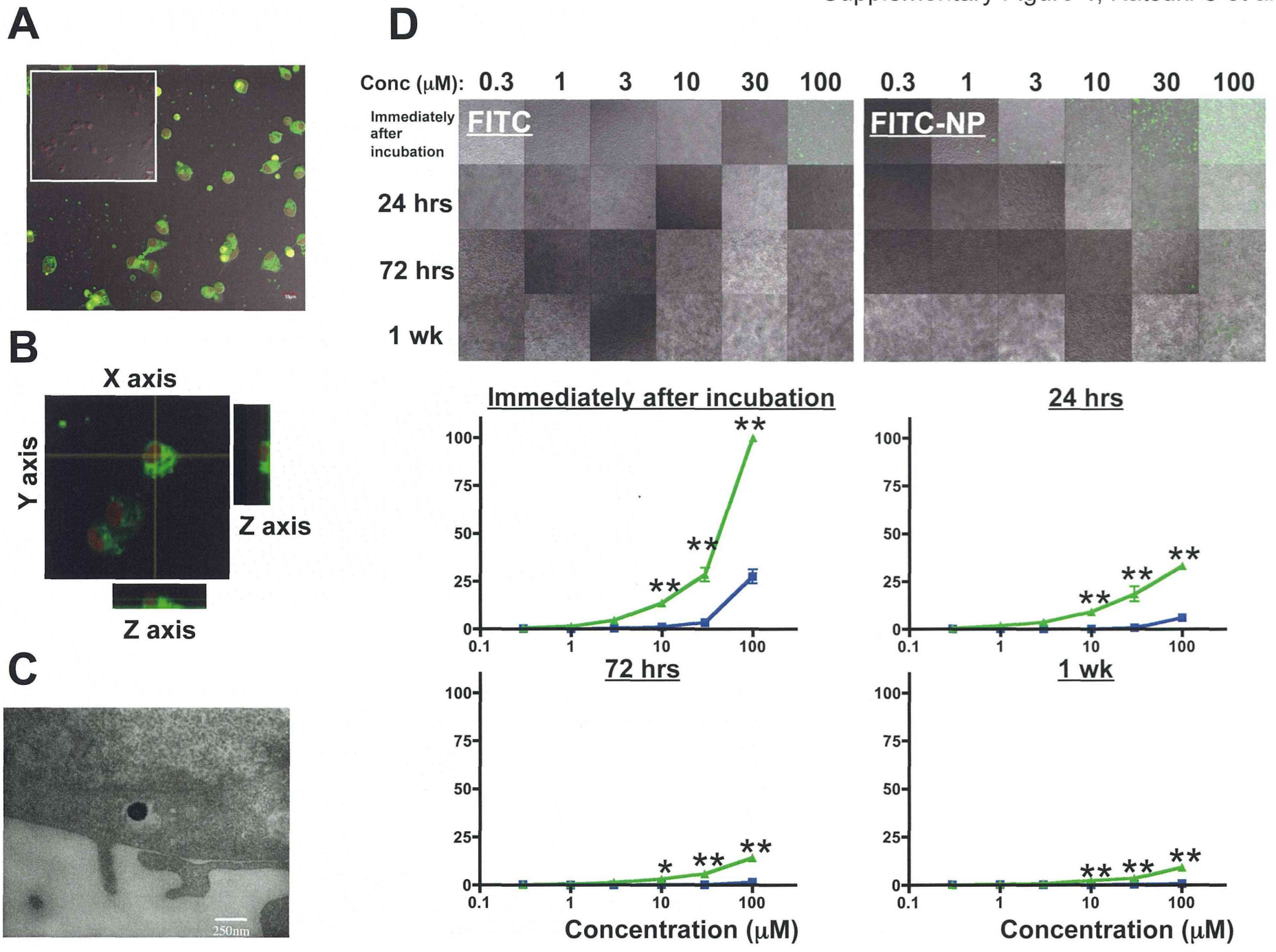
Treatment Group

1. Pitavastatin (Lower dose: 0.1 mg/kg/day)
2. Pitavastatin (Higher dose: 1.0 mg/kg/day)

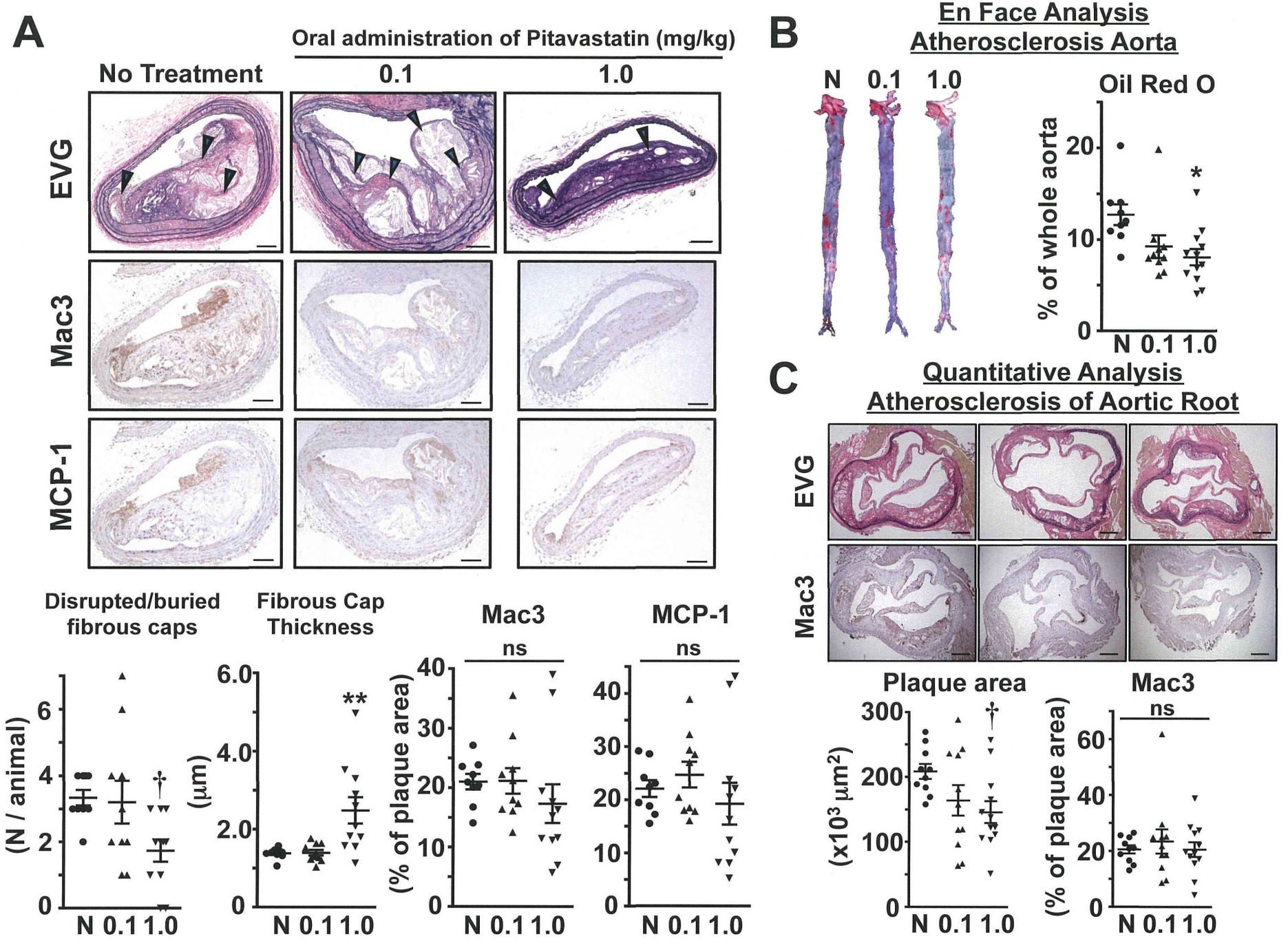

A
Number of
F4/80+CD115+ cells
P<0.001
ns
(×10⁵ cells)
300
200
100
0
TG: - + - +
ApoE-/-
ApoE-/-CCR2-/-
B
Ly-6C expression on
F4/80+CD115+ cells
P<0.01
MFI
20
15
10
5
0
TG: - +
ApoE-/-
C
No treatment
Transfer of PKH26-labeled Macrophages
PKH
PKH
FITC

Supplementary Figure 3, Katsuki S et al




A
B
X axis
Y axis
Z axis
Z axis
C
250nm
D
Conc (μM): 0.3 1 3 10 30 100 0.3 1 3 10 30 100
Immediately after incubation
24 hrs
72 hrs
1 wk
FITC
FITC-NP
Immediately after incubation
24 hrs
72 hrs
1 wk
Concentration (μM)
Concentration (μM)

Supplementary Figure 5, Katsuki S et al



REVIEW

Mouse models of plaque rupture

Tetsuya Matoba[a], *Kei Sato*[b], *and Kensuke Egashira*[a,b]

Purpose of review
Atherosclerotic plaque destabilization and rupture is an important pathological condition that may account for approximately 70% of acute myocardial infarction cases. To analyse the mechanisms by which an atherosclerotic plaque destabilizes and ruptures and examine the effects of novel therapeutic approaches, several groups have developed mouse models of plaque rupture.

Recent findings
Findings from intracoronary imaging modalities support the role of rupture-prone 'vulnerable plaques' characterized by pathological studies as precursors of plaque rupture and acute myocardial infarction. Atherosclerotic plaques in the brachiocephalic arteries of apolipoprotein E (ApoE)-deficient mice fed a high-fat diet demonstrate several key histological features of ruptured human plaques. Angiotensin II infusion accelerates plaque destabilization and rupture, which has enabled researchers to analyse the role of pathophysiological and genetic factors that accelerate plaque destabilization and rupture and qualitatively examine the effects of experimental therapies. The plaque rupture model in the brachiocephalic arteries of ApoE-deficient mice is disputed due to dissimilarities from human plaques regarding the incidence of thrombotic occlusion and computer-simulated mechanical stress in the plaque.

Summary
Although no mouse model examined completely simulates the entire process of plaque rupture, the brachiocephalic artery in ApoE-deficient mice fed a high-fat diet, with or without angiotensin II infusion, is a practically feasible model for plaque rupture.

Keywords
angiotensin II, atherosclerosis, hypercholesterolaemia, mouse, plaque rupture

INTRODUCTION

Acute myocardial infarction (AMI) is a significant cause of mortality and morbidity worldwide, causing sudden cardiac death or complications including heart failure and arrhythmia. Earlier pathological studies in cases of sudden coronary death identified plaque ruptures in more than 60% of the cases studied and suggested that plaque rupture and subsequent thrombotic occlusion was a central mechanism of AMI and sudden coronary death. By contrast, plaque erosion and calcified nodules were other less-frequent morphologies in the coronary arteries of this population [1]. These findings led to the concept of vulnerable plaques that are prone to rupture and cause AMI [2].

Intracoronary imaging modalities including intravascular ultrasound (IVUS) and optical coherence tomography (OCT) provide valuable information regarding lesion morphology during coronary interventions [3▪]. Clinical studies using these imaging modalities have identified ruptured plaques and thin-cap fibroatheromas (TCFAs) more frequently in AMI cases than in cases of stable coronary artery disease, supporting the cause–effect relationship between plaque rupture and AMI and the roles of TCFAs and vulnerable plaques as precursors to plaque rupture. Understanding the molecular and cellular mechanisms of plaque destabilization to form a vulnerable plaque and induce plaque rupture and developing new therapeutics for vulnerable plaques are, therefore, quite important issues for vascular biology and clinical cardiovascular medicine. However, basic research on plaque destabilization and rupture is hampered by the lack

[a]Department of Cardiovascular Medicine, Kyushu University Hospital and [b]Department of Cardiovascular Research, Development, and Translational Medicine, Kyushu University Graduate School of Medical Science, Fukuoka, Japan

Correspondence to Kensuke Egashira, MD, PhD, Department of Cardiovascular Research, Development, and Translational Medicine, Graduate School of Medical Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel: +81 92 642 6919; fax: +81 92 642 6720; e-mail: egashira@cardiol.med.kyushu-u.ac.jp

Curr Opin Lipidol 2013, 24:419–425
DOI:10.1097/MOL.0b013e3283646e4d