SUPPLEMENTAL MATERIAL

Supplemental Methods

Antibodies, siRNA, and reagents

Anti-phospho-ERK5 (T218/Y220, #3371), rabbit anti-ERK5 (#3372), Arg1 (#9819), and cleaved caspase 3 (#9664) were purchased from Cell Signaling. Antibodies made against Thbs1 (#SC-73158), C1qα (#SC-25856), PPARδ (#SC-74517), CD68 (#SC7084), MCP-1 (#SC-FL-148), and CD45 (#SC-70700) were purchased from Santa Cruz. Anti-CD3 (#D2712) and iNOS (#160862) were purchased from Thermo Scientific and Cayman Chemical, respectively. Human recombinant Thbs1 was purchased from Sigma-Aldrich (#SRP4805). Antibodies utilized for IHC were against smooth muscle actin (SMA; #M0851, DAKO), MAC3 (#550292, BD), Mfg-e8 (#D199-3, MBL), CD3 (#ab5690, Abcam), and IL-10 (#ABF13, Millipore). The ABC kit, secondary antibodies (biotinylated horseradish peroxidase conjugates), and diaminobenzidine (DAB) were purchased from Vector Laboratories (Burlingame, CA). CellTrcker^{TM} Green CMFDA was from Molecular Probes (#C7025). The recombinant human Mfg-e8 (#2767-MF, R&D Systems), Thbs1 (#SRP4805, Sigma-Aldrich), and Mfg-e8 ELISA kit (DY2805, R&D Systems) were also purchased.

Plasmids

The Thbs1-*luc* reporter (Dr. Paul Bornstein, Addgene plasmid 12409)[17] was obtained from the non-profit Addgene Plasmid Repository. The plasmid encoding Mfg-e8-2k-*luc* reporter was a kind gift from Dr. Iyoko Katoh (University of Yamanashi, Japan)[18]. Gal4-

ERK5 was created by inserting mouse ERK5 isolated from pcDNA3.1-ERK5 into BamH1 and Not1 sites of the pBIND vector. An adenovirus vector containing constitutively active (CA)-MEK5α was subcloned into the pENTR vector (Invitrogen) using specific enzyme sites, and then a recombinase reaction was performed to get a pDEST-based vector following manufacture's instruction (#K4930-00, ViraPower Adenoviral Expression System, Promega). All constructs were verified by sequencing.

Mice

All animal procedures used in this study were approved by the Animal Care and Use Committee of the University of Rochester. All animals were housed in a temperature-controlled room under a light (12 hrs)/dark (12 hrs) cycle and under pathogen-free conditions. Macrophages deficient in ERK5 were obtained from ERK5$^{fl/fl}$ mice crossed with LysM*Cre*$^{+/-}$ (C57BL/6 background) mice (ERK5-MKO). For atherosclerosis studies, these mice were crossed with LDLR$^{-/-}$ (C57BL/6J background) mice (ERK5-MKO/LDLR$^{-/-}$). At 8 weeks of age, mice were placed on a high-cholesterol diet (D01061401C, Research Diets, Inc.) for 8 or 16 weeks. We prepared apoptotic cells by culturing thymocytes from 6- to 8-week old C57BL/6J WT mice in serum-free RPMI medium for 16-18 hrs or by treating them with dexamethasone (100 nM) for 3 hrs[3]. For FACS and microscopy analyses, we labeled apoptotic thymocytes by incubating them with 2 mM CMFDA (Molecular probes) for 30 min before feeding them to macrophages.

Mouse and human macrophage culture

Bone marrow-derived macrophages (BMDMs) were obtained from bone marrow cells isolated from femurs and tibias and cultured at the density of $1x10^6$ cells/ml in DMEM (#11965118, Invitrogen) medium containing 10% FBS (v/v; Hyclone) and 10% (v/v) of the spent medium of L929 cells as the source of macrophage colony stimulating factor (M-CSF) for 5-8 days at 37˚C and 5% CO_2 in air. Peritoneal neutrophils and macrophages were obtained after i.p. injection of 2 ml autoclaved 3% (w/v) thioglycolate in H_2O (Sigma-Aldrich). After 3 days, peritoneal cells were harvested by injecting 10 ml DMEM containing 10% FBS into the peritoneal cavity, the abdomen gently massaged to dislodge cells, and cell suspension was collected. The mouse monocyte/macrophage cell line, RAW264.7 was obtained from ATCC (#TIB-71) and was propagated in DMEM containing 10% FBS at 37˚C and 5% CO_2 in air. The human monocyte/macrophage cell line, THP-1 was obtained from ATCC (#TIB-202) and was cultured in RPMI-1640 medium (#30-2001, Invitrogen) containing 10% FBS and 2-mercapto-ethanol at the final concentration of 0.05 mM at 37˚C and 5% CO_2 in air.

Promoter activity and ERK5 transcriptional activity

RAW264.7 and BMDMs were plated on 12-well plates at 5×10^4 cells/well. For a promoter assay, cells were transfected in Opti-MEM (#31985-088, Invitrogen) with Lipofectamine mixture containing the Mfg-e8-2k-*luc* or Thbs1-*luc* vector along with pRL-tk for 4 hrs. Cells were washed and fresh complete medium was added. Since pRL-*tk* contains the Renilla luciferase gene, the expression and transfection efficiencies were normalized with the Renilla luciferase activity. To detect ERK5 transcription activity, cells were transfected with Gal4-ERK5 and the Gal4-responsive luciferase reporter pG5-

luc in Opti-MEM containing Lipofectamine 2000 (#11668-019, Invitrogen) per manufacture's instruction. Opti-MEM was replaced by fresh complete culture medium 4 hrs post transfection. The cells were collected 36 hrs after transfection, and the luciferase activity was assayed with the dual luciferase kit (E1960, Promega) using a TD-20/20 luminometer (Turner Designs, CA). Transfections were performed in triplicate, and each experiment was repeated at least three times.

Western blotting

Expression levels of ERK5, C1qa, Gas6, Mfg-e8, Thbs1, Anxa1, and PPARδ in PE macrophages or BMDMs from NLC and ERK5-MKO mice were quantified by Western blotting. Macrophages were fed ACs for 8 hrs and whole cell lysates were prepared in RIPA buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1 % Nonidet P-40, 0.1% SDS, 1 mM dithiothreitol, 1:200-diluted protease inhibitor cocktail (#P8340, Sigma-aldrich), and 1 mM PMSF). Total lysates were resolved by SDS-PAGE and electrotransferred onto a Hybond enhanced chemiluminescence nitrocellulose membrane, which was then incubated with antibodies against each of the proteins to be detected in the lysate. Bound antibodies were visualized by using the enhanced chemiluminescence detection reagents (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

Real-time PCR assay

Total RNA from mouse macrophages or tissue was extracted using the TRIzol reagent according to the manufacturer's instructions. Reverse transcriptase reactions (PCR) were

performed in 20 μl mixtures containing 1 mg of total RNA according to the manufacture's protocol (Bio-Rad, #170-8890). For real-time PCR, mouse primers including Mertk (#MP202617), C1qa (#MP202861), C1qb (#MP202862), C1qc (#MP202863), gas6 (#MP205762), thbs1 (#MP216930), and Mfg-e8 (MP208190) were purchased from Origene with qSTAR qPCR primers. In addition, the following specific primers were designed using Primer Express 3.0 software (Tables 1 and 2).

Table 1. Specific primers for mouse ERK5, PPARs, Eat-me, Find-me, Don't-eat-me and GAPDH

Genes		**Sequences**
ERK5	Forward	5'-TCCTGTGTTCTCTGGCACTC-3'
	Reverse	5'-GAGAGGCTGAGAGTGAGGCT-3'
PPARα	Forward	5'-CTGCAGAGCAACCATCCAGAT-3'
	Reverse	5'-CACCTTCCTCTTCCCAAAGCTCCTTCA-3'
PPARδ	Forward	5'-TGAAGACAAACCCACGGTAA-3'
	Reverse	5'-GTGGCTGTTCCATGACTGAC-3'
CD68	Forward	5'-TTGGGAACTACACACGTGGGC-3'
	Reverse	5'-CGGATTTGA ATTTGGGCTTG-3'
CD36	Forward	5'-CCTTAAAGGAATCCCCGTGT-3'
	Reverse	5'-TGCATTTGCCAATGTCTAGC-3'
Eat-me		
Timd4	Forward	5'-TCATTGCCTGCTGTGTGGGATTTG-3'
	Reverse	5'-AGTGTTGTCTGGCCTCTTGTGTCT-3'
Tyro3	Forward	5'-ATGTCCTCATTCCAGAGCAGCAGT-3'
	Reverse	5'-CGCTTGAGGCAATGATGTCAGCTT-3'
Itgb3	Forward	5'-TTCAATGCCACCTGCCTCAACAAC-3'
	Reverse	5'-ACGCACCTTGGCCTCGATACTAAA-3'
Bai1	Forward	5'-AGGACTTTGTGGATGTCATCGGCT-3'
	Reverse	5'-TGCTGAGCACCAGATTGTCTGTCA-3'
Stab2	Forward	5'-GTGTTCTGGAAATGGGCAGTGCAT-3'
	Reverse	5'-TTGGAGCAGAACTGACACCTGGAA-3'
Rac1	Forward	5'-ATGGGACACAGCTGGACAAGAAGA-3'
	Reverse	5'-TCAGGATACCACTTTGCACGGACA-3'
Find-me receptors		
S1P1	Forward	5'-ACTTTGCGAGTGAGCTG-3'
	Reverse	5'-AGTGAGCCTTCAGTTACAGC-3'

G2A	Forward	5'-TGTGCCCAATGCTACTGAAAAAC-3'
	Reverse	5'-CGACCAGGACTATCCTGCTCTCT-3'
Find-me ligands		
CX3CL1	Forward	5'-AAGATGACCTCACGAATCCC-3'
	Reverse	5'-TGTGTCGTCTCCAGGACAAT-3'
P2Y2	Forward	5'-TTTCACCTGGCAGTTTCGGACTCT-3'
	Reverse	5'-TGGTGTAGAAGAGGAAACGCACCA-3'
Don't -find-me		
Cd47	Forward	5'-GGCGCAAAGCACCGAAGAAATGTT-3'
	Reverse	5'-CCATGGCATCGCGCTTATCCATTT-3'
Gapdh	Forward	5'-TGGCAAAGTGGAGATTGTTGCC-3'
	Reverse	5'AAGATGGTGATGGGCTTCCCG-3'

Table 2. Specific primers for human opsonins, anti-inflammatory genes and GAPDH

Genes		**Sequences**
Mfg-e8	Forward	5'-AGATGCGGGTATCAGGTGTGA-3'
	Reverse	5'-GGGGCTCAGAACATCCGTG-3'
Thbs1	Forward	5'-CCGGCGTGAAGTGTACTAGCTA-3'
	Reverse	5'-TGCACTTGGCGTTCTTGTT-3'
Anxa1	Forward	5'-ATGGCAATGGTATCAGAATTCCTCAA-3'
	Reverse	5'-GATGTCTAGTTTCCACCACACAGAG-3'
CD68	Forward	5'-GCTACATGGCGGTGGAGTACAA-3'
	Reverse	5'-ATGATGAGAGGCAGCAAGATGG-3'
GAPDH	Forward	5'-TGGCAAAGTGGAGATTGTTGCC-3'
	Reverse	5'-AAGATGGTGATGGGCTTCCCG-3'

Real-time PCR was performed using the MyiQ^{TM}2 Two-Color Real Time PCR System (Bio-Rad) and SYBR Green (Bio-Rad) to measure gene expression. The cycling program was set as follows: thermal activation for 10 min at 95 °C and 40 cycles of PCR (melting for 15 s at 95 °C, followed by annealing/extension for 1 min at 60 °C). Specific gene expression data were normalized to GAPDH gene expression.

***In vitro* apoptotic cell uptake and clearance**

To make apoptotic thymocytes (ACs), thymocytes were isolated from C57BL/6 WT mice and cultured overnight in RPMI 1640 containing 10% FBS. Apoptosis was triggered by

incubating the primary murine thymocytes with 100 nM dexamethasone for 3 hrs. The cells were then labeled with CellTracker™ Green CMFDA-FITC (#C7025, Molecular Probe). For uptake assays, we added ACs to BMDMs at a 5:1 thymocyte to macrophage ratio for 60 min. Double discrimination flow cytometry was used to distinguish internalized from externally bound ACs. Phagocytic index was calculated from images taken by a fluorescence microscope as described in our previous report[4]. Briefly, we counted over 200 cells manually per sample and applied the following formula: [(number of macrophages containing one ACs) + 2 x (number of macrophages containing two ACs) + 3 x (number of macrophages containing three ACs) + 4 x (number of macrophages containing more than four ACs)] / total number of macrophages counted]. Necrotic thymocytes (NCs) were made by incubating them at 56°C for 10 min. Cellular necrosis was confirmed by Trypan blue staining. For double feeding experiments, BMDMs plated on six-well plates were incubated with ACs (1:5) for 24 hrs and then rechallenged with CMFDA-labeled ACs. One day later, phagocytic index was determined as described above. For rescue experiments, BMDMs were cultured in DMEM containing M-CSF (10 ng/ml; Sigma-Aldrich, M9170) and 10% serum from NLC or ERK5-MKO mice. For rescue experiments with recombinant Mfg-e8 and Thbs1, BMDMs cultured in DMEM with 1% FBS and M-CSF (10 ng/ml) were treated with purified human Mfg-e8 (11 μg/ml; R&D, #2767) or Thbs1 (11 μg/ml; Sigma-Aldrich, #SRP4805) for 1 hr before feeding them ACs (1:5 ratio).

In vivo apoptotic cell uptake and clearance

For the splenic uptake and clearance assays, 6×10^7 CMFDA-labeled ACs were injected

intravenously into 8-week-old NLC or ERK5-MKO mice. The mice were sacrificed 12 hrs after injection and splenocytes were isolated. Splenic macrophages were purified using magnetic beads coated with anti-CD11b, and flow cytometry was used to determine CMFDA-labeled ACs uptake by these cells[3]. For the clearance assay, CMFDA-labeled-ACs were similarly injected into the peritoneal cavity, and 6 hrs later, cells were recovered by peritoneal lavage and counted under a fluorescence microscope[4].

FACS analysis

Cell proliferation was assessed by measuring new DNA synthesis by flow cytometry after EdU (5-ethynyl-2'-deoxyuridine) staining per manufacture's protocol (Click-iT® EdU Alexa Fluor® 488 Cell Proliferation Assay Kit, Molecular Probe; #C35002). To determine macrophage maturation, bone marrow cells (BM), blood monocytes (PB), and peritoneal macrophages (PE) were isolated from 7 or 8-week-old NLC or ERK5-MKO mice. The cells in suspension were washed in PBS and blocked by incubating them with 1 μg of FC blocks (CD16/32, eBioscience; #14-0161) for 10 min at RT. After washing them in PBS to remove free blocking antibodies, the cells were fixed in PBS containing 4% paraformaldehyde for 10 min and permeabilized by resuspending them in 1 ml of ice cold methanol for 20 min. Cells were suspended in FACS buffer and stained with PE anti-CD115 (Biolegend; #135506), APC-Cy7 anti-Gr1 (Ly6C/G, BD Biosciences; #557661), and Alexa Fluor® 647 anti-F4/80 (Biolegend; #123122), all at 1:1000 dilution, for 30 min and analyzed by flow cytometry.

Analysis of opsonin (Mfg-e8) in serum

Two mg of protein in the serum diluted in 1 ml of RIPA buffer was immunoprecipitated with 2 mg of anti-Mfg-e8 (SC-33546, Santa Cruz) for 18 hrs. Immunoprecipitates were captured by Protein A/G agarose beads (Invitrogen), washed to remove unbound protein, and bound Mfg-e8 determined by immunobloting with anti-Mfg-e8 (D199-3, MBL). Circulating levels of Mfg-e8 in serum were also quantified using a commercially available ELISA kit per manufacturer's protocol (R&D systems, DY2805).

Metabolic parameters

Mice were anesthetized and blood samples were collected from the abdominal artery. Plasma was prepared and total cholesterol, HDL, and non-HDL (LDL and VLDL) were measured enzymatically using commercially available kits (Abcam, #ab65390) according to the manufacturer's instructions. For glucose tests, overnight-fasted mice were given an i.p. glucose (2 mg/g body weight) injection, and blood was collected from tail vein before (time 0) and at indicated times after injection for glucose measurement (Glucometer Elite; Bayer).

Tissue preparation, histology, and quantification of the lesion size

ERK5-MKO/LDLR$^{-/-}$ and NLC/LDLR$^{-/-}$ female mice were fed a high-cholesterol diet for 8 or 16 weeks and sacrificed by CO_2 inhalation. The arterial tree was perfused via the left ventricle with saline containing heparin (40 USPU/mL), followed by formaldehyde (4%, pH 7.3) in PBS for 10 min. The full-length of the aorta to the iliac bifurcation was dissected out and opened along the ventral midline. En face preparations were washed in distilled water, dipped in 60% isopropyl-alcohol, and stained for 40 min with 0.16% Oil-

Red-O dissolved in 60% isopropyl-alcohol/0.2 mol/L NaOH. Stained images were captured with a digital camera mounted on a Leica stereomicroscope and analyzed using Adobe Photoshop Extended software. The aortic sinus area attached to the heart was dissected after fixation as described earlier, embedded in paraffin and sections (5 μm) were cut. Serial sections were made through the entire aortic valve area and stained with hematoxylin and eosin (H&E) and Masson's trichrome staining. The necrotic core was defined as an area free of H&E staining. The fibrosis cap was defined as the tissue between the vessel surface and the top edge of the necrotic core and the thickness of this tissue was measured at several places within each section of at least 3 consecutive serial sections. Plaque areas and necrotic core areas were also quantified. These studies were done blindly and the data was analyzed using ImageJ 1.64 (available as freeware from http://rsbweb.nih.gov/ij/).

Immunohistochemistry

To identify specific cells such as macrophages and smooth muscle cells (SMCs) and to detect specific protein expression within the plaque area, immunohistochemistry (IHC) was performed using the VECTASTAIN ABC system (Vector laboratories). Paraffin sections were blocked with 3% H_2O_2. Epitope retrieval (HIER) was performed by heating sections in the HIER buffer containing 10 mM sodium citrate and 0.05% Tween 20 (pH 6). T he samples were block with 5% normal serum (Vector laboratories) matching the host of the secondary antibody for 30 min at room temperature (RT). Primary antibodies were against Mac3 (1:500, mouse monoclonal), alpha-actin (1:500, mouse monoclonal), Mfg-e8 (1:100, mouse monoclonal), and Thbs1 (1:100, mouse

monoclonal). Secondary antibodies (goat anti-mouse or anti-rabbit) were used at 1:500 dilutions. Sections were developed by DAB substrate (Vector laboratories) and counterstained with hematoxylin.

In situ TUNEL and macrophage apoptosis assays

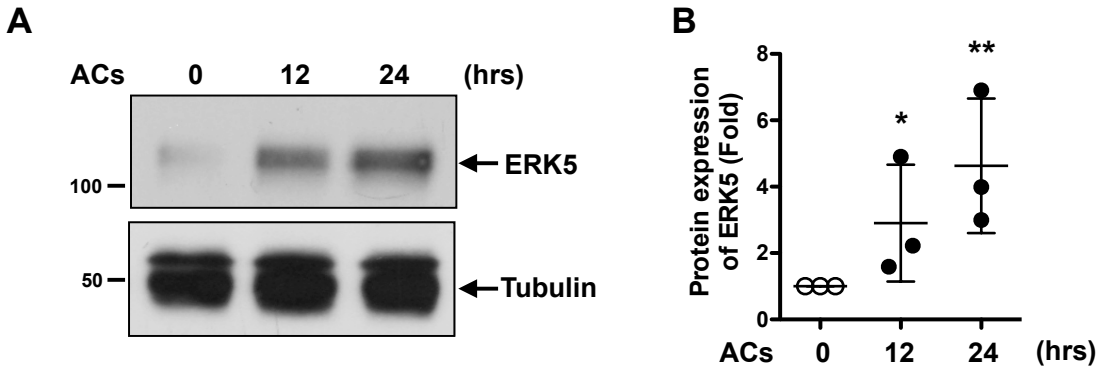
To examine apoptosis, the aortic valve area was fixed and embedded in paraffin and sectioned (5 μm). TUNEL staining was performed using ApopTag® Peroxidase *In Situ* Apoptosis Detection Kit (#S7100, Millipore) following the manufacturer's protocol. Briefly, paraffin sections were blocked for endogenous peroxidase activity (3% hydrogen peroxide in water) for 10 min at RT and treated with protein K (20 mg/ml) for 30 min. For a positive control, sections were treated with DNase I (1 mg/ml) for 10 min at RT. Terminal DNA labeling was performed at 37 °C for 1 hr with a reaction mixture containing fluorescein-conjugated nucleotides and terminal deoxynucleotidyltransferase enzyme. Slides were counterstained by eosin to identify nuclei. For each sample, a total of 200 nuclei were counted from 4 randomly selected fields and TUNEL positive cells were expressed as percent of total cells counted. Samples were examined using a 40X lens under an epi-fluorescence microscope (BX51, Olympus) equipped with a CCD camera (Spot, #22.1). To determine the active caspase 3 expressions in the section, samples were blocked with 5% Goat serum for 30 min at RT and treated with anti-caspase 3 overnight at 4 °C. Samples were then treated with biotinylated goat anti-rabbit IgG for 30 min at RT, followed by detection of biotin by DAB substrate (Vector laboratories) and counterstained with hematoxylin.

Supplemental Expended Discussion

ERK5 is not only a kinase, but also has transcriptional activity, which is critical for activating transcription factors such as Kruppel-like factor 2 (KLF2) and PPARs[5, 6]. Previously we found the importance of ERK5 kinase activity in regulating ERK5 transcriptional activity[5]. In the study, we showed that dominant negative kinase form of ERK5 inhibited ERK5 transcriptional activity. Interestingly, we also found the kinase-independent regulation of ERK5 transcriptional activity[6]. We found that ERK5 transcriptional activity was inhibited by SUMOylation, which leads to the inhibition of KLF2 and eNOS expression[6]. We investigated whether SUMOylation of ERK5 could inhibit ERK5 phosphorylation and kinase activation. In here, we did not observe any difference in ERK5 phosphorylation and kinase activity between Ubc9 overexpressed and vector transfected cells. In addition, we did not find any difference in ERK5 phosphorylation between the ERK5 wild type and K6/22R SUMOylation sites mutant[6]. These data suggested that ERK5-SUMOylation did not change ERK5 phosphorylation and kinase activation, but inhibited its transcriptional activity and subsequent KLF2 and eNOS expression. Taken together, these data support the importance of both kinase and transcriptional activity of ERK5 in regulating its downstream events.

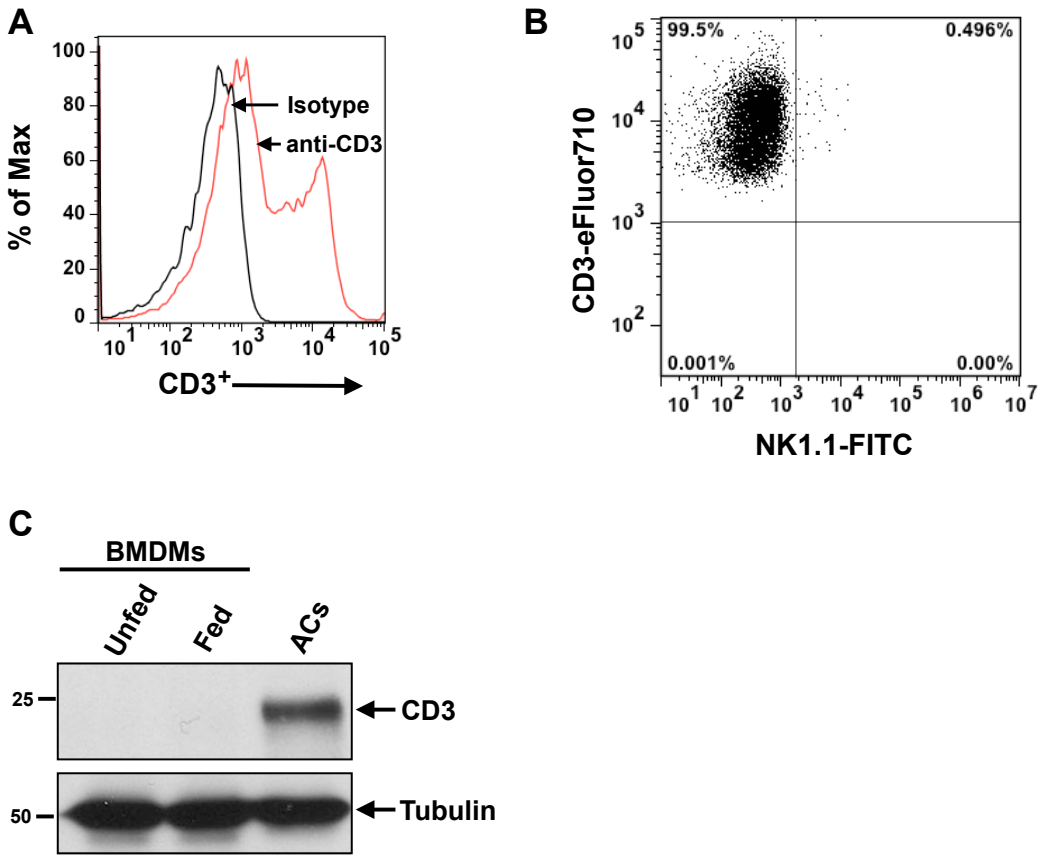
It is possible that ERK5 could phosphorylate or activate other molecules besides PPARs. The partial effect of PPARδ deletion on Thbs-1 or Mfg-e8 promoter activation as showed in Fig. 2F,G and 5E support this notion. Further investigation is necessary to clarify these issues.

Supplemental Figures and Figure Legends



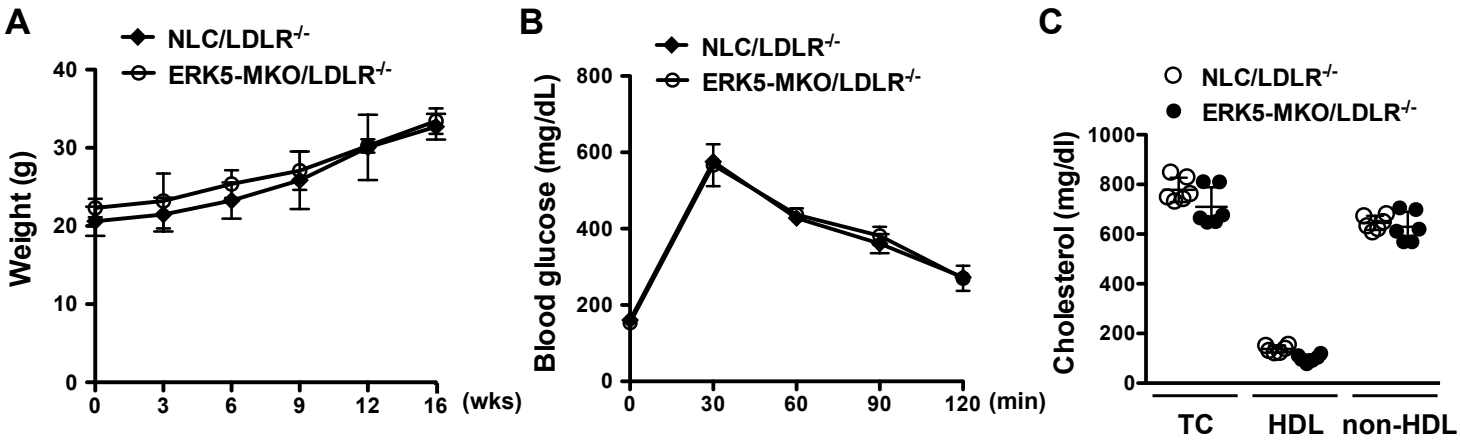
Supplementary Figure 1. ERK5 expression in macrophages fed apoptotic T-cells.

(A) Peritoneal macrophages were fed apoptotic thymocytes for 0, 12, and 24 hrs and ERK5 expression was assayed by Western blotting using anti-ERK5. (B) Quantification of ERK5 expression is shown as fold changes compared to the control (first lane) after normalization to the internal tubulin control for each point. Shown is the mean S.D., (n =3). $^{**}P<0.01$.



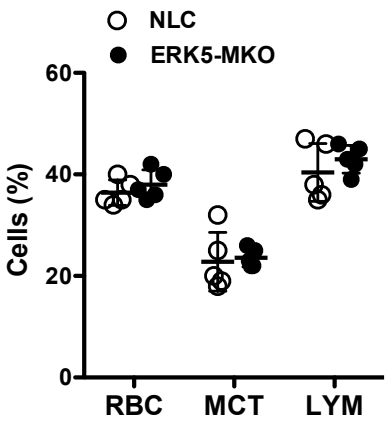
Supplementary Figure 2. Lack of a T cell antigen in macrophages fed apoptotic thymocytes.

(A) Apoptotic thymocytes were generated by serum starvation for 16-18 hrs as reported previously. Flow cytometric analysis of CD3 expression in apoptotic thymocytes. (B) Flow cytometric analysis of CD3 and NK1.1 expression in apoptotic thymocytes. The expression of NK1.1, which is a Natural killer T cell marker, is not detected. (C) BMDMs were either fed or not fed apoptotic thymocytes and macrophages or apoptotic thymocytes lysates immunoblotted for CD3, which is specifically expressed in lymphocytes. A representative experiment is shown from three independent experiments.



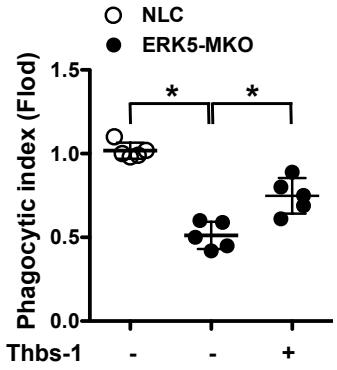
Supplementary Figure 3. Comparison of body weight, glucose tolerance, and plasma cholesterol levels between NLC/LDLR$^{-/-}$ and ERK5-EKO$^{-/-}$/LDLR$^{-/-}$ mice.

A, Body weight changes of NCL/LDLR$^{-/-}$ and ERK5-MKO/LDLR$^{-/-}$ mice fed with Western-type diet for 16 weeks show identical progression. Mean ± S.D. (n=8 per genotype). **B**, After 16-week of Western-type diet, NCL/LDLR$^{-/-}$ and ERK5-MKO/LDLR$^{-/-}$ mice were fasted overnight (16 hours) before the glucose tolerance test. Glucose was injected intra-peritoneally (2g/kg) and blood samples were taken before and at 0, 30, 60, 90, and 120 min after the injection for blood glucose measurements. means ± S.D., (n=6 per genotype). No difference was noted. **C**, The same blood cholesterol profiles were observed in NCL/LDLR$^{-/-}$ and ERK5-MKO/LDLR$^{-/-}$ mice after 16 weeks on Western-type diet. means ± S.D., (n=6 per genotype).



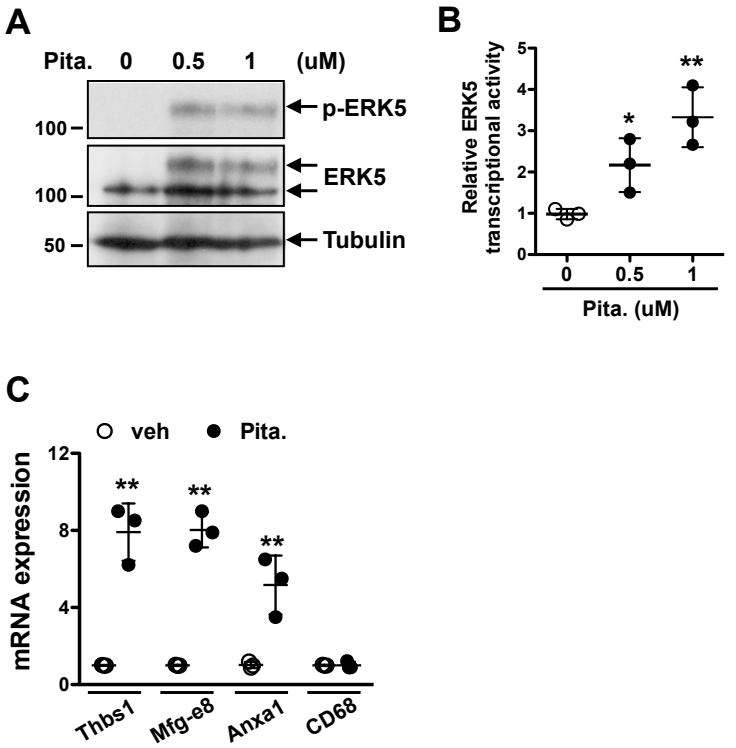
Supplementary Figure 4. Blood cell counts in macrophage ERK5 deficient mice.

Blood cell counts in NLC and ERK5-MKO mice were made. ERK5 deficiency in macrophage did not affect the number of circulating red blood cells (RBC), monocytes (MCT), or lymphocytes (LYM). n=5 per genotype.



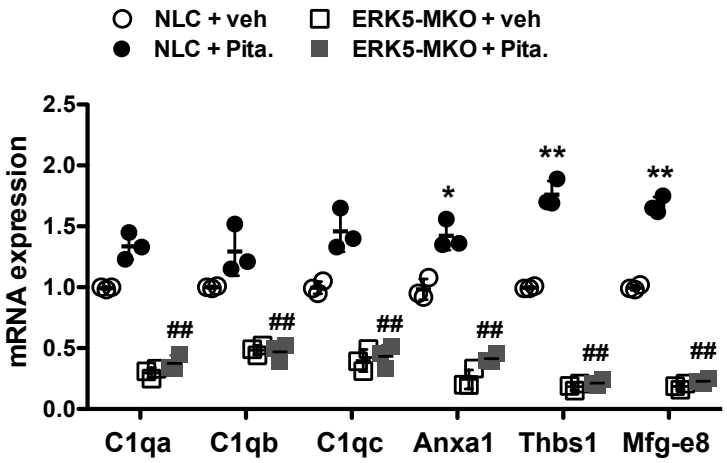
Supplementary Figure 5. Defective phagocytic capacity of ERK5-MKO macrophages was restored by purified Thbs1 protein.

NLC and ERK5-MKO BMDMs were treated with purified Thbs1 (11 ug/ml) for 24 hrs and fed CMFDA-labeled ACs at a 1:5 (BMDMs:ACs) ratio for 60 min. The phagocytic index was analyzed as described in Fig.1. means ± S.D. (n=5). *P < 0.05.



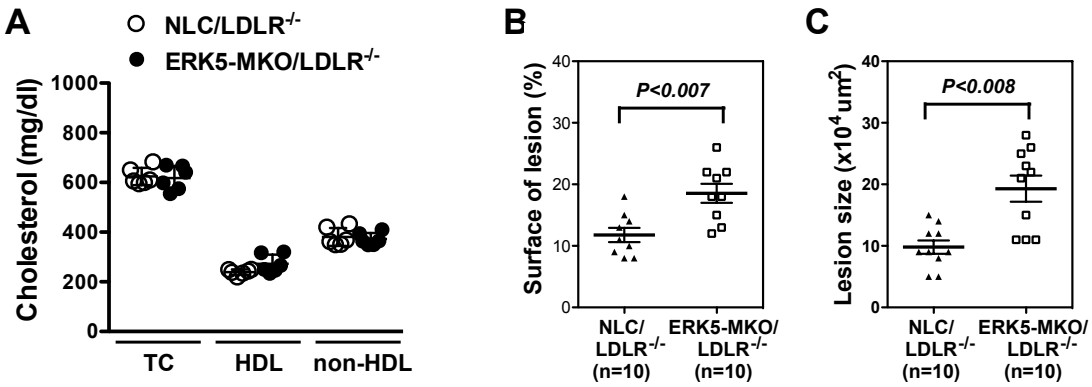
Supplementary Figure 6. Pitavastatin upregulates opsonin expression via ERK5 activation in human macrophages.

A, Pitavastatin increased ERK5 phosphorylation in THP-1 cells after 3 hrs of treatment. **B**, THP-1 cells were transfected with pBind-ERK5 and pG5-luc for 18 hrs and then ERK5 transcriptional activity was assessed after 6 hrs of pitavastatin treatment. means ± S.D. (n=3). **P < 0.01. **C**, Relative mRNA levels of indicated opsonins and a receptor in THP-1 cells after 18hrs of 1 uM pitavastatin were assessed by qPT-PCR. means ± S.D. (n=3). *P < 0.05 and **P < 0.01. Vehicle (veh); PBS.



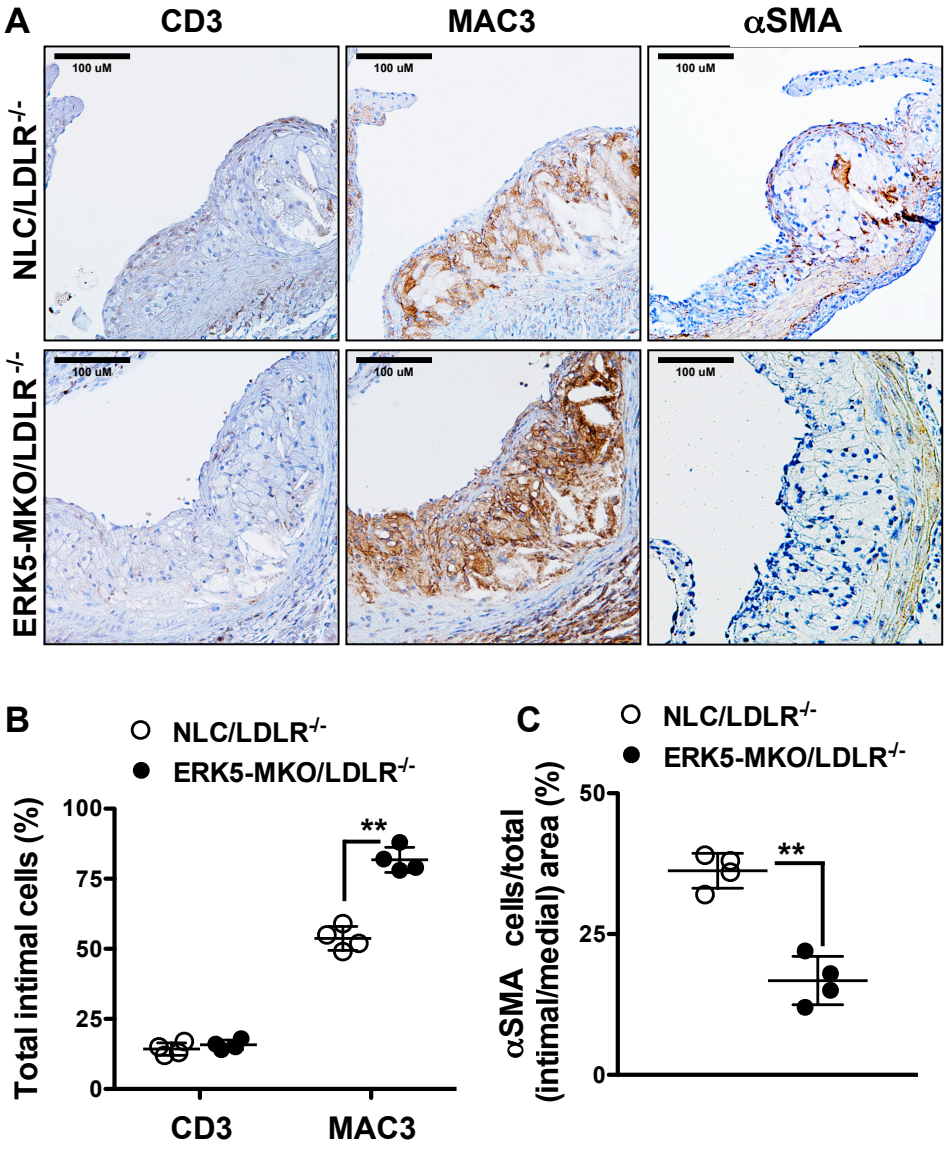
Supplementary Figure 7. Pitavastatin upregulates opsonin expression via ERK5 activation.

Relative mRNA levels of indicated opsonins and receptors in NLC and ERK5-MKO livers i.p. injected for 18hrs with 40mg/kg pitavastatin were assessed by qPT-PCR. means ± S.D. (n=3). *P < 0.05 and **P < 0.01 vs NLC livers, $^{\#}$P < 0.05 and $^{\#\#}$P < 0.01 vs NLC livers + pitavastatin.



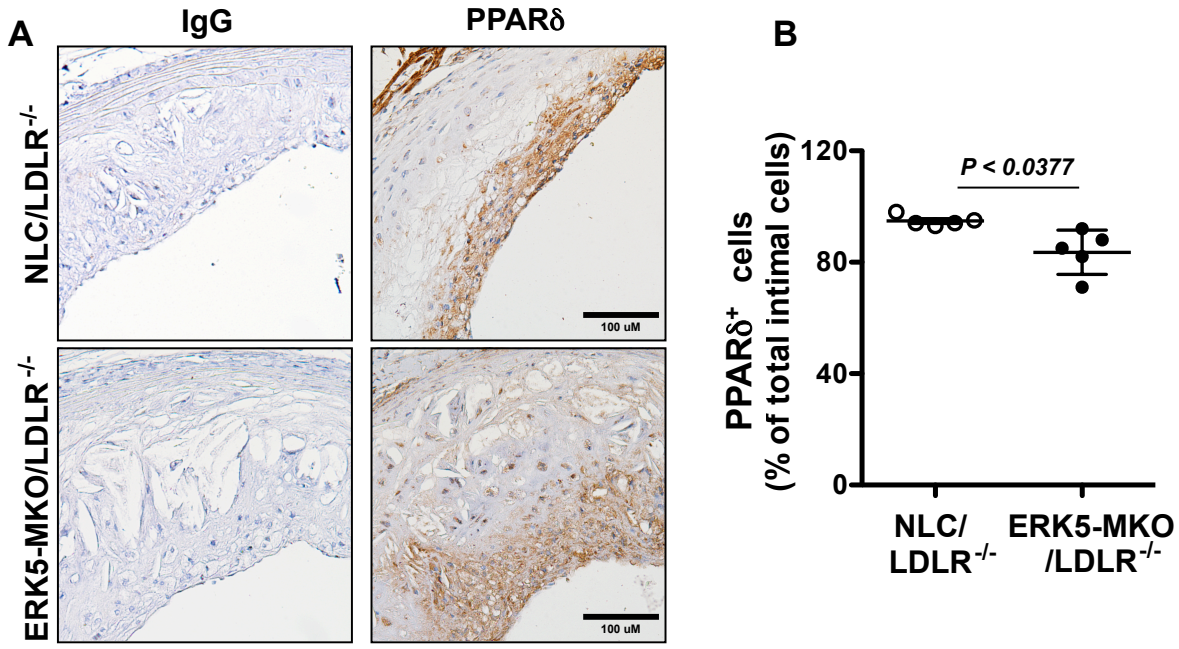
Supplementary Figure 8. Accelerated atherosclerotic plaque formation in ERK5-MKO/LDLR$^{-/-}$ mice.

A, Cholesterol profiles were determined in NCL/LDLR$^{-/-}$ or ERK5-MKO/LDLR$^{-/-}$ mice after feeding them a high cholesterol diet for 8 weeks. Mean ± S.D. (n=6 per genotype). There was no significant difference between these two groups. B,C, After 8 weeks of western-type diet, atherosclerotic lesions were quantified in NLC/LDLR$^{-/-}$ and ERK5-MKO/LDLR$^{-/-}$ mice. The quantified data are based on the whole aorta stained by Oil-red O (B) and sections from the proximal aorta stained by H&E (C) and expressed as mean percent lesion area relative to the total vessel surface area of the enface sample or the total valves area in the section. means ± S.D. (n=8 per genotype). **P < 0.01.



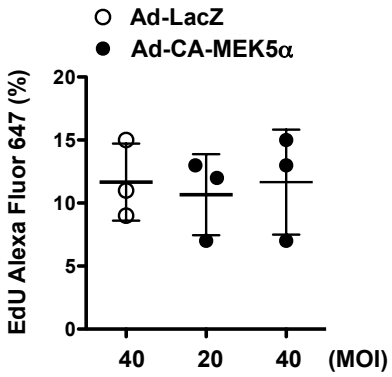
Supplementary Figure 9. Composition of immune cells in the atherosclerotic lesion area.

A,B, The area of atherosclerotic plaques in sections of the proximal aorta was stained with an antibody against CD3 (T lymphocytes) and Mac3 (macrophages) **(A)** values represent the number of CD3+ and Mac3+ cells/% of total intimal area **(B)**, and the number of aSMA+ cells/% of total (intimal/medial) area. means ± S.D. (n=4 per genotype). **P < 0.01.



Supplementary Figure 10. Expression of PPARd in the atherosclerotic lesion area.

A,B, The area of atherosclerotic plaques in sections of the proximal aorta was stained with an antibody against PPARd **(A)** and values represent the number of PPARd+ cell/mm^2 of total intimal lesion area **(B)**. means ± S.D. (*P<0.0377* compared to NLC/LDLR$^{-/-}$, n=5 per genotype).



Supplementary Figure 11. ERK5 activation and macrophage proliferation.

The BMDMs were transduced with Ad-LacZ (40 MOI) or Ad-CA-MEK5a (20 or 40 MOI) for 18 hrs. Cell proliferation was assayed by EdU (5-ethynyl-2'-deoxyuridine) incorporation for 30 min and labeled cells were quantified by flow cytometry. (n=3)

Supplemental Reference

1. Donoviel DB, Framson P, Eldridge CF, Cooke M, Kobayashi S, Bornstein P. Structural analysis and expression of the human thrombospondin gene promoter. *J Biol Chem*. 1988;263:18590-18593
2. Okuyama T, Kurata S, Tomimori Y, Fukunishi N, Sato S, Osada M, Tsukinoki K, Jin HF, Yamashita A, Ito M, Kobayashi S, Hata RI, Ikawa Y, Katoh I. P63(tp63) elicits strong trans-activation of the mfg-e8/lactadherin/ba46 gene through interactions between the ta and deltan isoforms. *Oncogene*. 2008;27:308-317
3. Scott RS, McMahon EJ, Pop SM, Reap EA, Caricchio R, Cohen PL, Earp HS, Matsushima GK. Phagocytosis and clearance of apoptotic cells is mediated by mer. *Nature*. 2001;411:207-211
4. Mukundan L, Odegaard JI, Morel CR, Heredia JE, Mwangi JW, Ricardo-Gonzalez RR, Goh YP, Eagle AR, Dunn SE, Awakuni JU, Nguyen KD, Steinman L, Michie SA, Chawla A. Ppar-delta senses and orchestrates clearance of apoptotic cells to promote tolerance. *Nat Med*. 2009;15:1266-1272
5. Woo CH, Massett MP, Shishido T, Itoh S, Ding B, McClain C, Che W, Vulapalli SR, Yan C, Abe J. Erk5 activation inhibits inflammatory responses via peroxisome proliferator-activated receptor delta (ppardelta) stimulation. *J Biol Chem*. 2006;281:32164-32174
6. Akaike M, Che W, Marmarosh NL, Ohta S, Osawa M, Ding B, Berk BC, Yan C, Abe J. The hinge-helix 1 region of peroxisome proliferator-activated receptor gamma1 (ppargamma1) mediates interaction with extracellular signal-regulated

kinase 5 and ppargamma1 transcriptional activation: Involvement in flow-induced ppargamma activation in endothelial cells. *Mol Cell Biol*. 2004;24:8691-8704