SUPPLEMENTAL MATERIAL

Supplement Methods:

Immunofluorescence and histology: We stained the frozen sections of AAA with rabbit anti-rat C9, which cross-reacts with mouse C9 (kindly provided by Dr. BP. Morgan, University of Wales, UK)[1], and rat anti-mouse CD68, IgG_{2a} (clone: FA-1, AbD Serotec) for mononuclear phagocytes. All the primary antibodies were detected using corresponding FITC-conjugated secondary antibodies and compared with the negative controls which were stained with each non-immune isotype antibody and FITC-conjugated secondary antibody[1]. We also stained for Masson trichrome using the Accustain staining kit (HT-15) (Sigma-Aldrich), Elastin (Sigma-Aldrich) and H&E. Two independent investigators quantified immunoflourensce staining area and histological results using Image ProPlus 6.0 software in a blinded fashion.

Blood pressure measurements: Blood pressure measurements were obtained by tail-cuff plethysmography using a noninvasive BP/heart rate monitoring system (UR-5000, Ueda Electronic) as described previously [2].

Zymography: The mouse endothelial (C166, ATCC), smooth muscle (CRL-2797, ATCC) and macrophage cells (RAW264.7, ATCC) were treated with MAC (assembled by C5b6: 24 μg/ml and + for C7, C8 or C9: 24 μg/ml) for 12 hours. The proteins were isolated from the mouse AAA and MAC-treated mouse cells using M-PER mammalian protein extraction Reagent (Pierce) and denatured in protein loading buffer (Bio-Rad). The proteins were electrophoresed in 10% SDS-polyacrylamide gels containing 1 mg/ml gelatin (Sigma-Aldrich). Subsequently, SDS was removed from the gels by two washes (15 minutes) with 2.5% Triton X-100 (Sigma-Aldrich). Gels were incubated for 16 hours (37°C) in zymography buffer (50 mmol/l Tris (pH 8.0), 10 mmol/l $CaCl_2$, 0.05% Brij 35), and stained with Coomassie brilliant blue. Zymographic images were acquired using identical shutter conditions. Coomassie staining of SDS-PAGE or detection of β-actin was used to show equal protein loading [3, 4].

Reporter Assays: pGL2-AP-1-Luc and pGL2- NF-κB-Luc luciferase reporters, with AP-1 and NF-κB response elements cloned into the pGL2-Luc plasmid, respectively, were generous gifts from Drs. Zhu and Rosenfeld[5]. Mouse endothelial cells ($2x10^4$ each well) were transfected by Lipofectamine 2000 (Invitrogen) with pGL2-AP-1-Luc or pGL2- NF-κB-Luc luciferase reporter plasmid. Transfection efficiency (internal control) was determined by co-transfection with 4 fold less PRL-CMV plasmid (Promega), which expresses renilla luciferase under the control of the CMV promoter. Twenty-four hours later, cells were stimulated with MAC (assembled by C5b6: 24 μg/ml and + for C7, C8 or C9: 24 μg/ml) for 12

hrs and luciferase activity was measured (firefly and renilla) with the Dual-Glo Luciferase Assay System (PAE2940, Promega).

RNA Interference: Endothelial cells ($2.5x10^5$ per well, 6-well plates) were transiently transfected using Lipofectamine RNAiMA X (Invitrogen) 5µl/each well with 50nM of mouse p50, mouse c-jun, and siGENOME non-targeting siRNA Pool™ (Dharmacon). The sequences of these siRNA are listed in **Supplemental Table 1.** After 36 hrs incubation, cells were stimulated with MAC (C5b6: 24 µg/ml and + for C7, C8 or C9: 24 µg/ml) for an additional 12 hrs, and proteins or RNA were isolated and subjected to western blot and Zymography, or real-time RT-PCR analysis respectively.

Quantitative real-time PCR analysis: Total RNA was isolated from the cultured mouse endothelial cells C166 (ATCC) treated with C5b-9 assembled and siRNA for 12 hours with Trizol reagent (Invitrogen). Real-time PCR was performed using SYBR Green in a real-time PCR machine (iCycler; Bio-Rad). We used the primers (listed in **Supplementary Table 1**) for detecting p50, c-Jun, MMP2 and MMP9 RNA levels: To normalize expression data, 18s rRNA was used as an internal control gene.

Data were obtained with the iCycler iQ5 Systems software (Bio-Rad) and were expressed as Ct (cycle threshold). Raw fold changes in target gene expression (ΔCt) were calculated by transforming the difference in Ct values of treated vs. control: $2^{-(\text{treated Ct-untreated Ct})}$. Fold changes in target gene expression were then

normalized to 18s rRNA via the published comparative $2^{-\Delta\Delta Ct}$ method using the formula: $\Delta\Delta Ct=(Ct\ target\ gene\text{-}Ct\ 18s\ rRNA)_{treated}\text{-}(Ct\ target\ gene\text{-}Ct\ 18s\ rRNA)_{control}$ [6].

The most commonly used reference genes include β-actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 18s ribosomal RNA (18s rRNA). They are historical carryovers and were used as references for many years in Northern blots, RNase protection assays and RT-PCR assays. Their use was acceptable for these techniques where a qualitative change was being measured. This was because these RNAs are expressed at relatively high levels in all cells and made ideal positive controls if the gene of interest was switched off. A widely use of 18s rRNA as internal standard in real time PCR can be found in references [7, 8].

We continuously monitored the stability of the internal standard, which is 18s RNA used in this paper, among samples by applying the following procedures. In order to remove genomic DNA from raw RNA samples, the raw RNA concentration was determined by spectrophotometer, and 5 μg of raw RNA was added into a total 50 μl reaction system complemented with DNase I. After DNase I digestion, 1 μl of cleared RNA sample was loaded for Real-time PCR reaction, which is around 100 ng total RNA. We checked the absolute Ct value of 18s RNA of all the samples after Real-time PCR reaction. Since the RNA loading was very close (100 ng/well), we usually obtained similar Ct value of 18s RNA of different samples, which is $18\pm<1$ cycles. Here are some examples.

18s RNA Ct Value	MMP2 MAC +Control siRNA	MMP2 MAC +P50 siRNA	MMP9 MAC +c-Jun siRNA
Experiment1	18.32	17.94	18.82
Experiment2	18.18	17.80	18.56
Experiment2	18.14	17.85	18.89

The similar Ct values of 18s RNA indicates that 18s RNA abundance among different samples is stable.

Western Blotting: The proteins were isolated from the mouse AAA. The mouse endothelial cells (C166) were treated with MAC (assembled by C5b6: 24 μg/ml and + for C7, C8 or C9: 24 μg/ml) for 6 hours. Proteins were separated electrophoretically on 12% polyacrylamide gels and blotted onto PVDF membranes. Nonspecific binding was blocked by incubating the membrane for 1 hour in 20 mmol/L tris-HCl (pH 7.5) containing 0.5 mol/L sodium chloride, 0.1% Tween 20, and 5% nonfat milk. Electrophoresis and western blotting supplies were obtained from BioRad. Primary antibodies including rabbit Phospho-p65 antibody (Cell Signaling), rabbit Phospho-IKK-α/β antibody (Cell Signaling), rabbit Phospho-c-Jun antibody (Cell Signaling), rabbit Phospho-c-Fos antibody (Abcam), rabbit c-Jun antibody (Santa Cruz Biotechology), rabbit p65 monoclonal antibody (Cell Signaling), rabbit IKK-α/β antibody (Santa Cruz Biotechology), rabbit p50 antibody (Santa Cruz Biotechology), rabbit c-Fos monoclonal antibody (Cell Signaling), and mouse anti-mouse β-actin antibody (Santa Cruz

Biotechology), which were diluted in blocking buffer, were incubated with the membranes. The final concentrations for these antibodies are listed in Supplementary Table 3. IRDye 800 (Rockland) and Alexa Fluor 680 -coupled species-appropriate secondary antibodies (Invitrogen) were then applied. Immunoreactive bands were visualized with an Odyssey Image Machine, and each protein was measured using densitometry, as subsequently described. Densitometric analysis was adjusted for total cellular protein content as determined by actin protein.

Preparation of Peritoneal Macrophages for FACS analysis: We isolated peritoneal macrophages following the procedure as described in [9]. Briefly, the mice were intraperitoneally injected with 2 ml of 4% thioglycollate (BD Diagnostics). Peritoneal exudate cells were isolated from the peritoneal cavity 3 days post injection. Cells were incubated for 2 hr in DMEM culture medium containing 10%FBS, 1% Penicillin-streptomycin and then washed three times with PBS buffer. Remaining adherent cells were used as macrophage for the experiments after verifying by FACS by macrophage marker CD68.

Macrophage Migration Assay: It has been shown previously that C5b-9 assembly induces the release of growth factors[10, 11]. To determine whether these released growth factors or cytokines may induce macrophage cells migration, the conditioned media from the mouse endothelial (C166, ATCC) treated with MAC for 12 hours (assembled by C5b6: 12 μg/ml and + for C7, C8 or C9: 24 μg/ml) were used as a chemoattractant. The serum- and growth factor-starved

macrophage cells (RAW264.7) were treated without or with MAC for 12 hours (assembled by C5b6: 12 μg/ml and + for C7, C8 or C9: 24 μg/ml). To measure cell migration, Innocyte 12-well cell migration assay (Millipore's Corporate headquarters, Billerica, MA) was used following the manufacturer's instructions. Briefly, the conditioned media were added to the lower chamber, macrophage (with and without C5b-9 treatment) were added to the upper chamber of the cell migration chamber. The cells were allowed to migrate through an 5-μm pore size membrane for 24 h. Finally, we detached invaded cells and lysed cells by recommended buffers, and measured the cell density by using 480/520 nm filter set.

References

1. Wu G, Hu W, Shahsafaei A, Song W, Dobarro M, Sukhova GK, Bronson RR, Shi GP, Rother RP, Halperin JA, Qin X. Complement regulator CD59 protects against atherosclerosis by restricting the formation of complement membrane attack complex. *Circ Res.* 2009:104:550-8.

2. Chun HJ, Ali ZA, Kojima Y, Kundu RK, Sheikh AY, Agrawal R, Zheng L, Leeper NJ, Pearl NE, Patterson AJ, Anderson JP, Tsao PS, Lenardo MJ, Ashley EA, Quertermous T. Apelin signaling antagonizes Ang II effects in mouse models of atherosclerosis. *J Clin Invest.* 2008:118:3343-54.

3. Sun J, Sukhova GK, Yang M, Wolters PJ, MacFarlane LA, Libby P, Sun C, Zhang Y, Liu J, Ennis TL, Knispel R, Xiong W, Thompson RW, Baxter BT, Shi GP. Mast cells modulate the pathogenesis of elastase-induced abdominal aortic aneurysms in mice. *J Clin Invest.* 2007:117:3359-68.

4. Irwin C, Synn A, Kraiss L, Zhang Q, Griffen MM, Hunter GC. Metalloproteinase expression in venous aneurysms. *J Vasc Surg.* 2008:48:1278-85.

5. Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. *Cell.* 2004:116:511-26.

6. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 2001: 402-8.

7. Moore DF, Li H, Jeffries N, Wright V, Cooper RAJr, Elkahloun A, Gelderman MP, Zudaire E, Blevins G, Yu H, Goldin E, Baird AE. Using peripheral blood mononuclear cells to determine a gene expression profile of acute ischemic stroke: a pilot investigation. *Circulation.* 2005:111:212-21.

8. Chang L, Zhang J, Tseng YH, Xie CQ, Ilany J, Bruning JC, Sun Z, Zhu X, Cui T, Youker KA, Yang Q, Day SM, Kahn CR, Chen YE. Rad GTPase deficiency leads to cardiac hypertrophy. *Circulation.* 2007:116:2976-83.

9. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Forster I, Akira S. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity.* 1999:10:39-49.

10. Benzaquen LR, Nicholson-Weller A, Halperin JA. Terminal complement proteins C5b-9 release basic fibroblast growth factor and platelet-derived growth factor from endothelial cells. *J Exp Med.* 1994:179:985-92.

11. Nicholson-Weller A, Halperin JA. Membrane signaling by complement C5b-9, the membrane attack complex. *Immunol Res.*1993:244-57.

Supplemental Table 1: The sequences of si RNAs targeted to mouse p-50 and c-jun

siRNAs	Sequence
siGENOME Non-Targeting siRNA #1	UAGCGACUAAACACAUCAA
siGENOME Non-Targeting siRNA #2	UAAGGCUAUGAAGAGAUAC
siGENOME Non-Targeting siRNA #3	AUGUAUUGGCCUGUAUUAG
siGENOME Non-Targeting siRNA #4	AUGAACGUGAAUUGCUCAA
ON-TARGET p-50 siRNA#1	CUGCAAAGGUUAUCGUUCA
ON-TARGET p-50 siRNA#2	GGAAACGCCAGAAGCUUAU
ON-TARGET p-50 siRNA#3	GGAUUUCGAUUCCGCUAUG
ON-TARGET p-50 siRNA#4	UAAAUGGGAAACCGUAUGA
ON-TARGET c-jun siRNA#1	CCAAGAACGUGACCGACGA
ON-TARGET c-jun siRNA#2	GCAGAGAGGAAGCGCAUGA
ON-TARGET c-jun siRNA#3	GAAACGACCUUCUACGACG
ON-TARGET c-jun siRNA#4	GAACAGGUGGCACAGCUUA

Supplemental Table 2. Primers for real-time PCR

Gene	Forward primer	Reverse primer	References
18S	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA	[1]
MMP-2	CCTTAAAAGTATGGAGCGACGTCA	AGCGTTCCCATACTTTACGCG	[2]
MMP-9	ACCCTGTGTGTTCCCGTTCAT	GATACTGGATGCCGTCTATGTCGT	[2]
c-JUN	ACGGCCAACATGCTCAGG	TGTTTGCAACTGCTGCGTTAG	[3]
P50	ATGATCCCTACGGAACTGGGCAAA	TGGGCCATCTGTTGACAGTGGTAT	Designed by us

References

1. Furuhashi M, Tuncman G, Gorgun CZ, Makowski L, Atsumi G, Vaillancourt E, Kono K, Babaev VR, Fazio S, Linton MF, Sulsky R, Robl JA, Parker RA, Hotamisligil GS. Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein aP2. *Nature.* 2007;447:959-65.

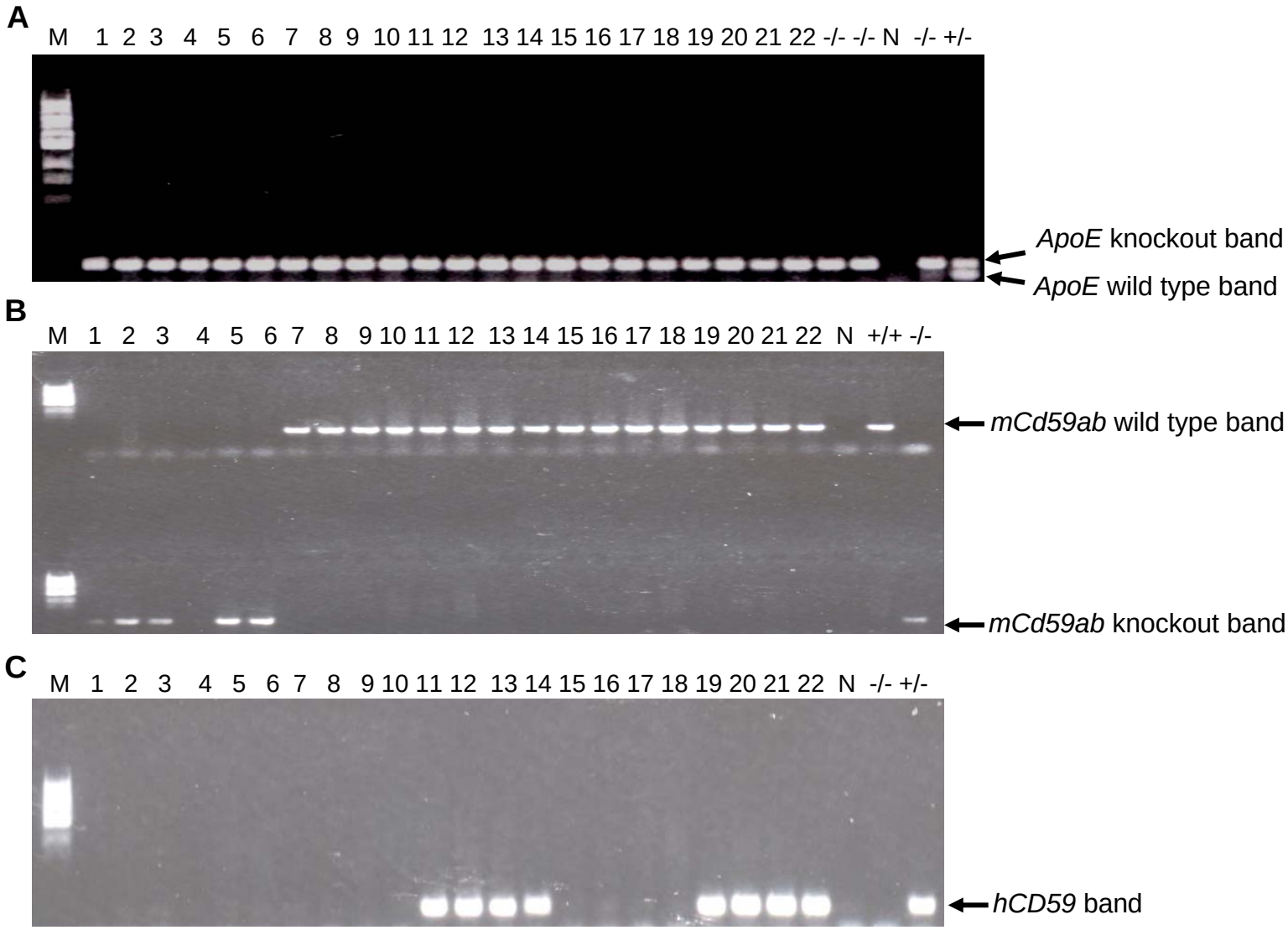
2. Oviedo-Orta E, Bermudez-Fajardo A, Karanam S, Benbow U, Newby AC. Comparison of MMP-2 and MMP-9 secretion from T helper 0, 1 and 2 lymphocytes alone and in coculture with macrophages. *Immunology.* 2008;124:42-50.

3. Holloway G, Coulson BS. Rotavirus activates JNK and p38 signaling pathways in intestinal cells, leading to AP-1-driven transcriptional responses and enhanced virus replication. *J Virol.* 2006;80:10624-33.

Supplemental Table 3: The final concentration for primary antibodies.

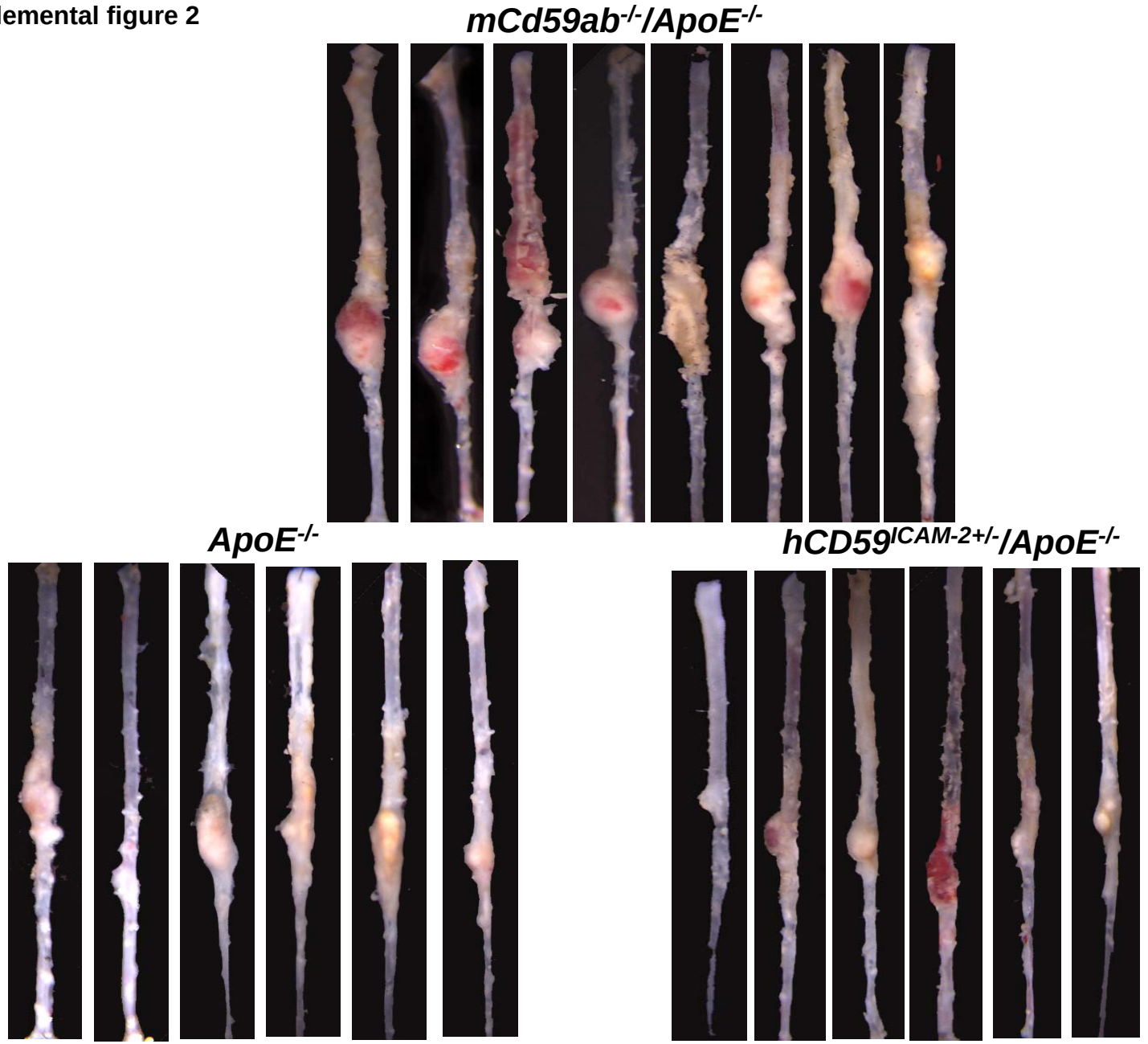
Antibody name	Final concentration for Western blot
p-p65	1μg/ml
p65	0.2μg/ml
IKK-α/β	1μg/ml
p-IKK-α/β	2μg/ml
c-Jun	0.5μg/ml
p-c-Jun	1μg/ml
p-c-Fos	1μg/ml
c-Fos	1μg/ml
p50	0.5μg/ml
β-Actin	0.2μg/ml

Supplementary Figure 1



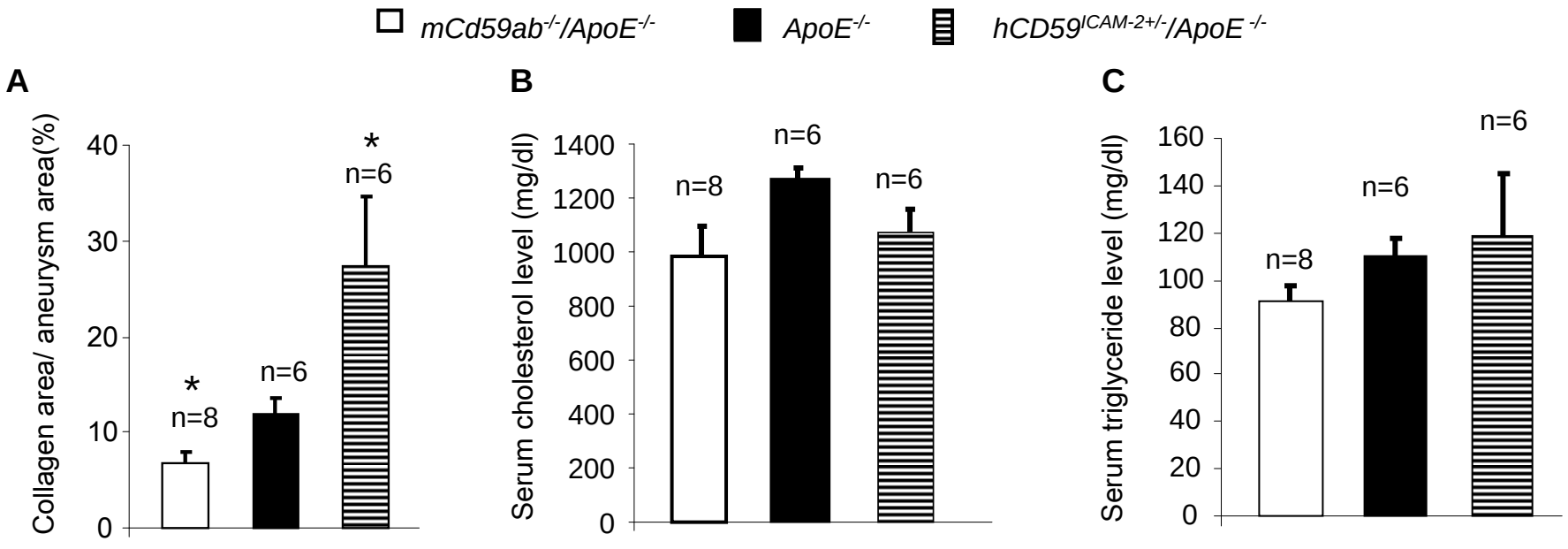
Supplementary Figure 1: Generation of *mCd59ab*$^{-/-}$/*ApoE*$^{-/-}$, *ApoE*$^{-/-}$ and *hCd59*$^{lCAM\text{-}2+/-}$/*ApoE*$^{-/-}$ mice. *mCd59ab*$^{-/-}$/*ApoE*$^{-/-}$ or *hCD59*$^{lCAM\text{-}2+/-}$/*ApoE*$^{-/-}$ mice were generated by crossing *mCd59ab*$^{-/-}$ or *hCD59*$^{lCAM\text{-}2+/-}$ with *ApoE*$^{-/-}$ to identify the experimental mice with PCR method. (**A-C**) Genotyping for determining *ApoE*$^{-/-}$ (A), *mCd59ab*$^{-/-}$ (B) and *hCD59*$^{lCAM\text{-}2+/-}$ (C) respectively. 1, 2, 3, 5, and 6 DNAs were determined as *mCd59ab*$^{-/-}$/*Apoe*$^{-/-}$ genotyping. 7, 8, 9, 10, 15, 16, 17, and 18 DNAs were determined as the *ApoE*$^{-/-}$ genotyping. 11, 12, 13, 14, 19, 20, 21, and 22 DNAs were determined as *hCD59*$^{lCAM\text{-}2+/-}$/*ApoE*$^{-/-}$ genotyping. N: negative control.

Supplemental figure 2



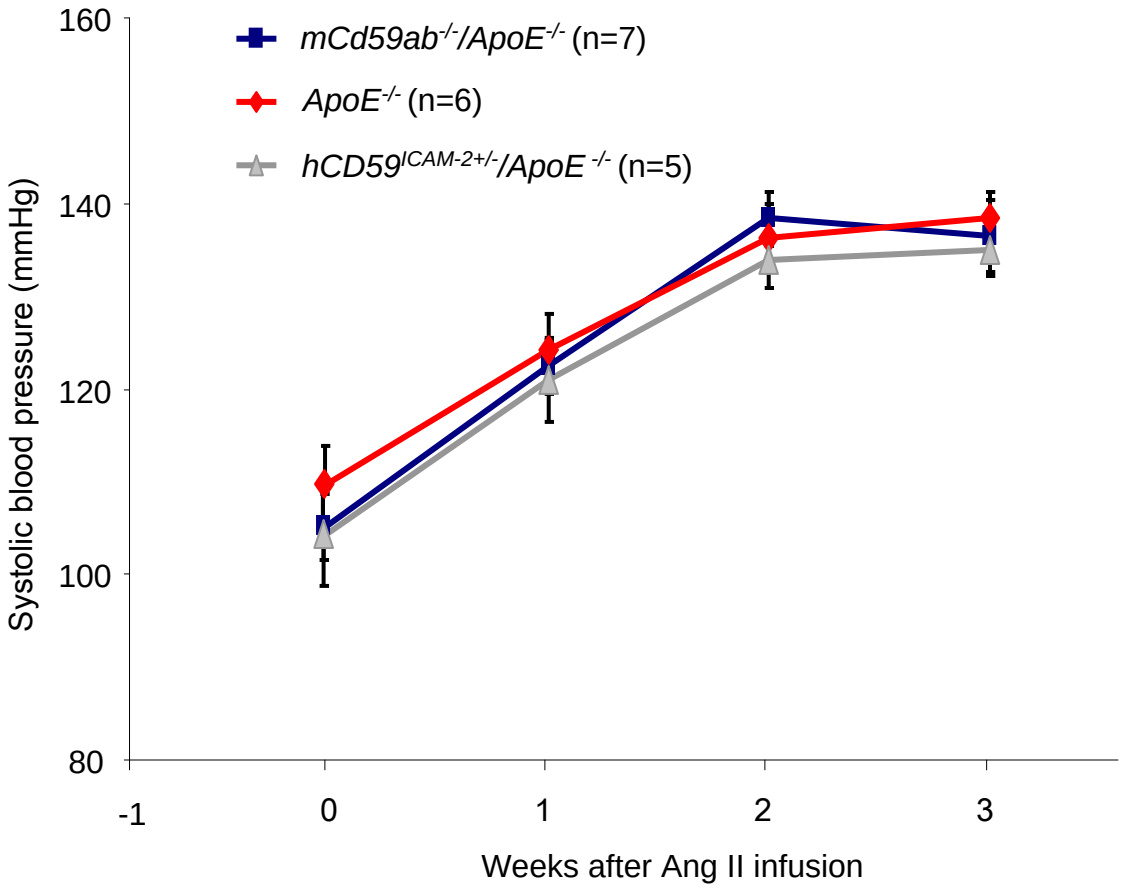
Supplemental figure 2. The aneurysm lesions of *mCd59ab*$^{-/-}$*/ApoE*$^{-/-}$, *ApoE*$^{-/-}$ and *hCD59*$^{lCAM\text{-}2+/-}$*/ApoE*$^{-/-}$ mice.

Supplemental Figure 3



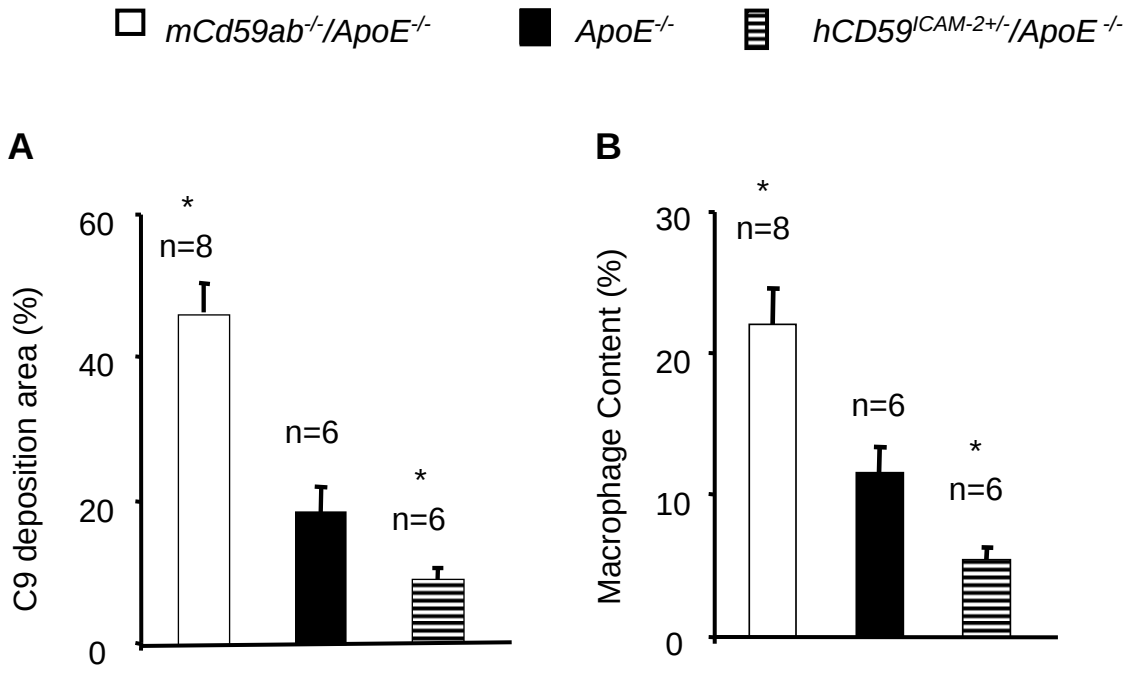
Supplementary figure 3: **A.** The collagen content of the aneurysm lesions. **B** and **C.** The levels of serum cholesterol and triglyceride among three groups. The data are displayed by mean ± sem. Statistical significance ($P < 0.01$ *vs ApoE*$^{-/-}$) is indicated by an asterisk.

Supplemental figure 4



Supplementary figure 4: **There were no significant differences in the level of systolic blood pressure among three experimental groups**. Two to three-month-old mice (both genders) were infused with 1000 ng/kg/min Ang II and simultaneously fed a high fat diet. Three *mCd59ab*$^{-/-}$*/ApoE*$^{-/-}$ spontaneously died between 2 and 3 weeks after Ang II infusion. Data are mean $\pm$ SEM of weekly measurement.

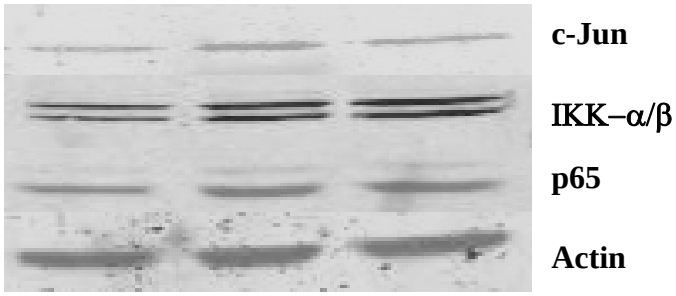
Supplemental Figure 5



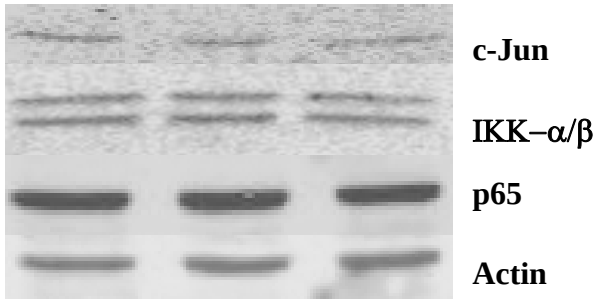
Supplementary figure 5: **A.** Levels of C9 deposition (percentage of positive area *vs* lesion area) detected in AAA lesions. **B.** Percentages of stained macrophage areas in lesion areas. The data are displayed by mean ± sem. Statistical significance ($P < 0.01$ *vs* *ApoE*$^{-/-}$) is indicated by an asterisk.

Supplemental figure 6

A

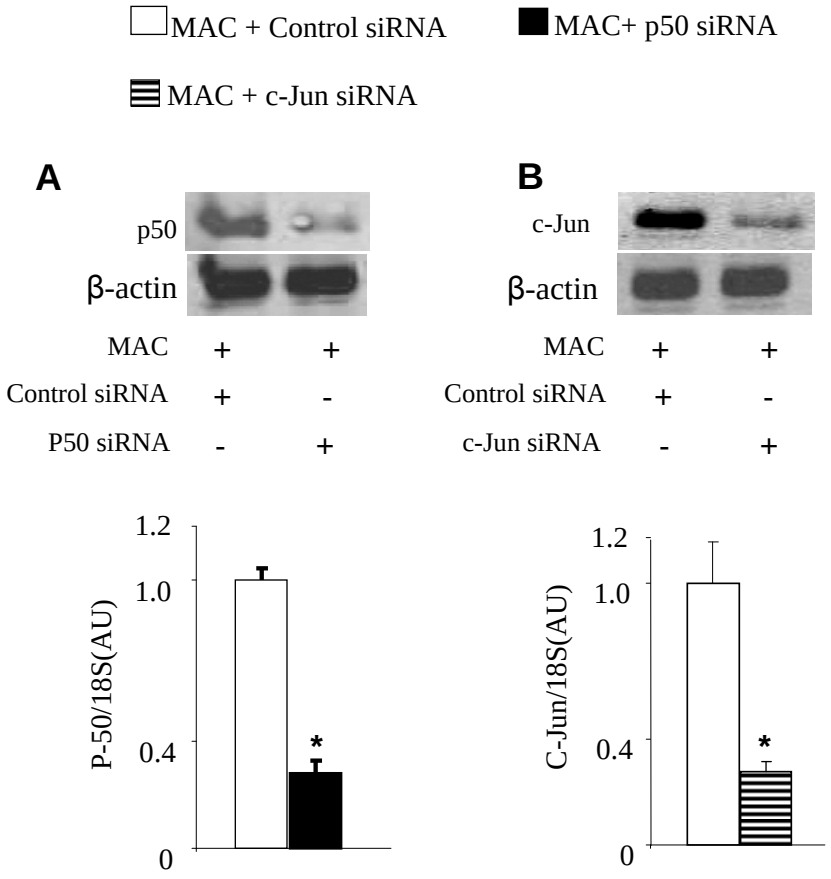


B



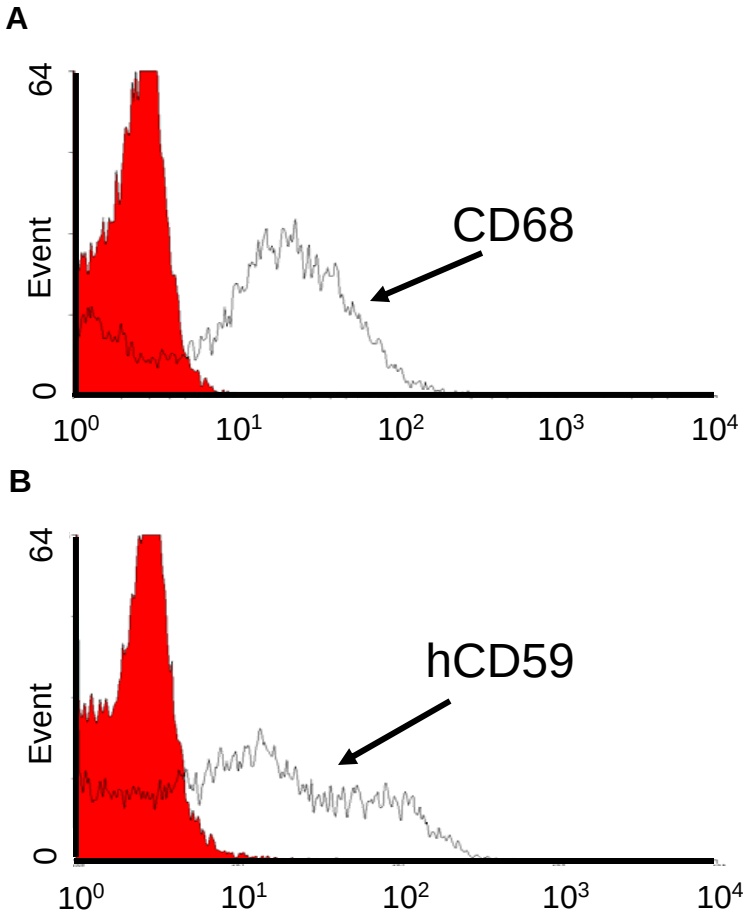
Supplemental figure 6: The total protein expression was not affected by MAC. (**A**) Total protein expression of c-Jun, P65, and IKK α/β levels among three groups on Ang II infusion and HFD for 28 days in vivo (western blot). (**B**) Total protein expression of c-Jun, p65, and IKK-α/β levels in mouse endothelial cell line (western blot).

Supplemental Figure 7



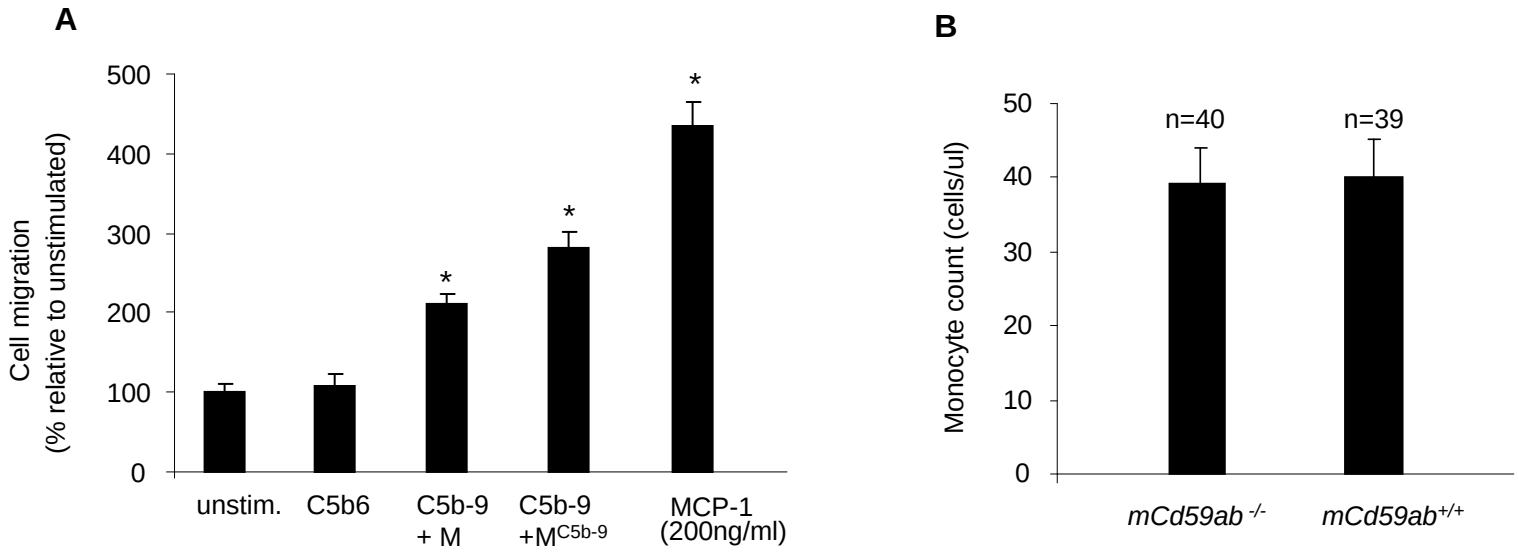
Supplemental figure 7: Validation of p50 (A) and c-Jun siRNA efficacy (B) in mouse endothelial cells. Top panel: Protein level determined by Western blot and RT-PCR; and bottom panel: Quantatitive RT-PCR. Fold changes in the p50 or c-Jun levels with specific siRNAs treatment compared to these treated with control siRNA. Results are mean ± sem from four independent experiments per group. *$P < 0.05$ *vs* control siRNA. Specific primers for p-50, c-Jun or 18S were listed in Supplemental table 2.

Supplemental Figure 8



Supplemental figure 8: hCD59 is expressed in the macrophages of *ThCD59*$^{ICAM\text{-}2+/-}$ mice. (**A**) Confirmation of peritoneal macrophage population by FACS analysis. Red curve: goat anti-rat IgG-FITC secondary antibody staining only and black curve: rat anti-mouse CD68 plus goat anti-rat IgG-FITC secondary antibody. (**B**) hCD59 is present in the macrophages of *ThCD59*$^{ICAM\text{-}2+/-}$ mice. Red curve: FITC secondary antibody and black curve: anti-human CD59 antibody (Bric 229) plus FITC secondary antibody.

Supplemental Figure 9



Supplemental figure 9: (A) The cultural supernatant from MAC-treated endothelial cells promotes the macrophage migration. Unstim: culture medium as the negative control to assess the migration of macrophage; C5b6: the supernatant from the mouse endothelial cells treated with C5b6 alone as the chemoattractant was used to assess the migration of macrophages; C5b-9 + M: the supernatant from the mouse endothelial cells treated with C5b9 as the chemoattractant was used to assess the migration of macrophages; C5b-9 + M^{C5b-9}: the supernatant from the mouse endothelial cells treated with C5b9 as the chemoattractant was used to assess the migration of the MAC-treated macrophages; and MCP-1 (200ng/ml) as the postive chemoattractants to assess the migration of macrophages. (**B**) **The deficiency of CD59 does not result in the reduced number of monocyte count in the circulation**. The CDC counts of the mouse were performed in the department of pathology at Boston Children's Hospital. Data are mean $\pm$ sem of four experimental results. Statistical significance ($P < 0.01$ *vs* C5b6 alone) is indicated by an asterisk.