Leukocyte RhoA exchange factor Arhgef1 mediates vascular inflammation and atherosclerosis

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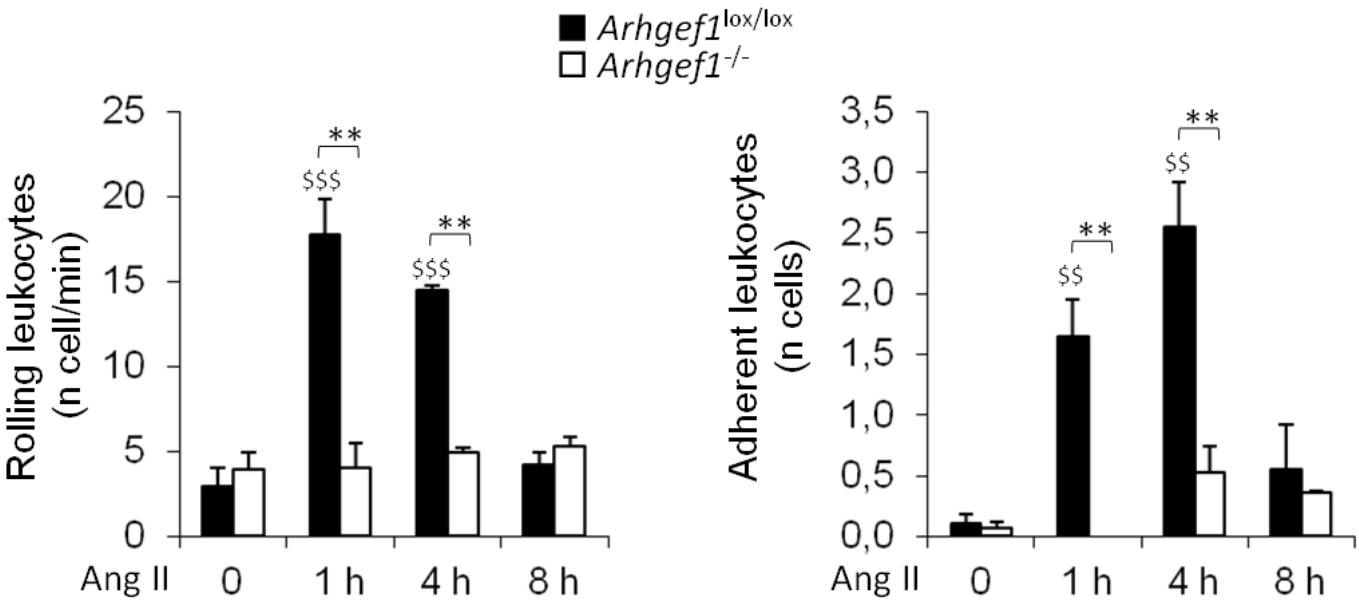
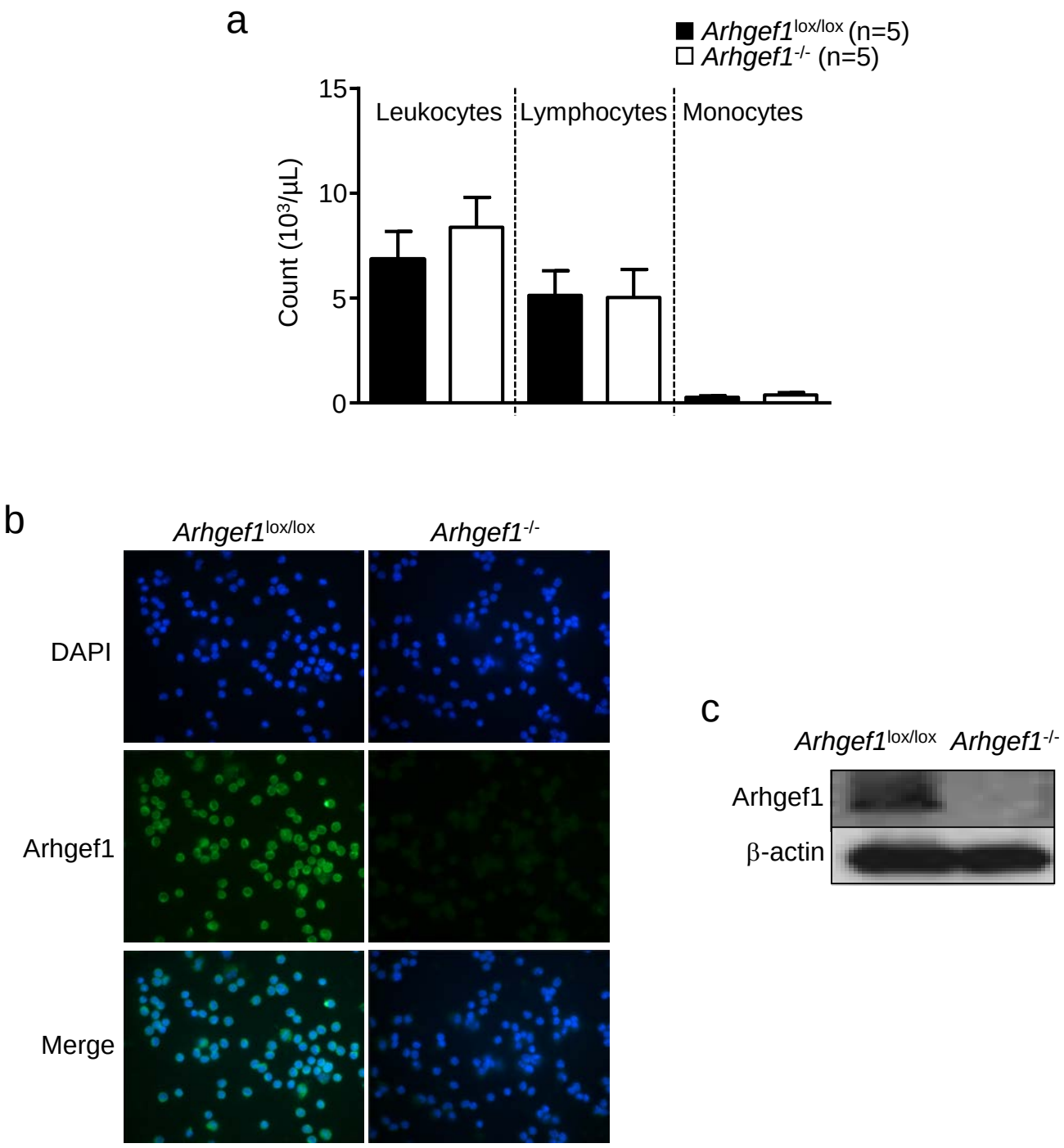
Supplemental Figures

Supplemental Figure 1



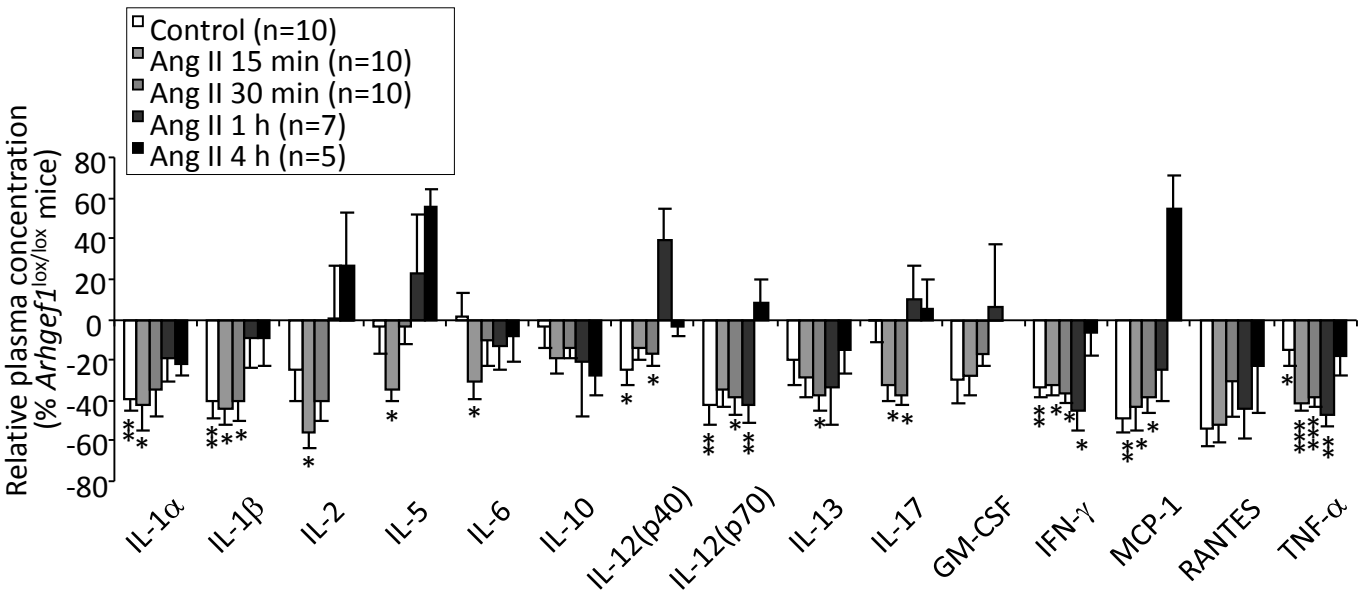
Supplemental Figure 1. Deletion of the RhoA exchange factor Arhgef1 inhibits Ang II-induced leukocyte rolling and adhesion. Time-dependent *in vivo* effect of Ang II (0.1 pmol) on leukocyte rolling and adhesion in mesenteric vessels of *Arhgef1*$^{lox/lox}$ and *Arhgef1*$^{-/-}$ mice (n=5 mice).

Supplemental Figure 2



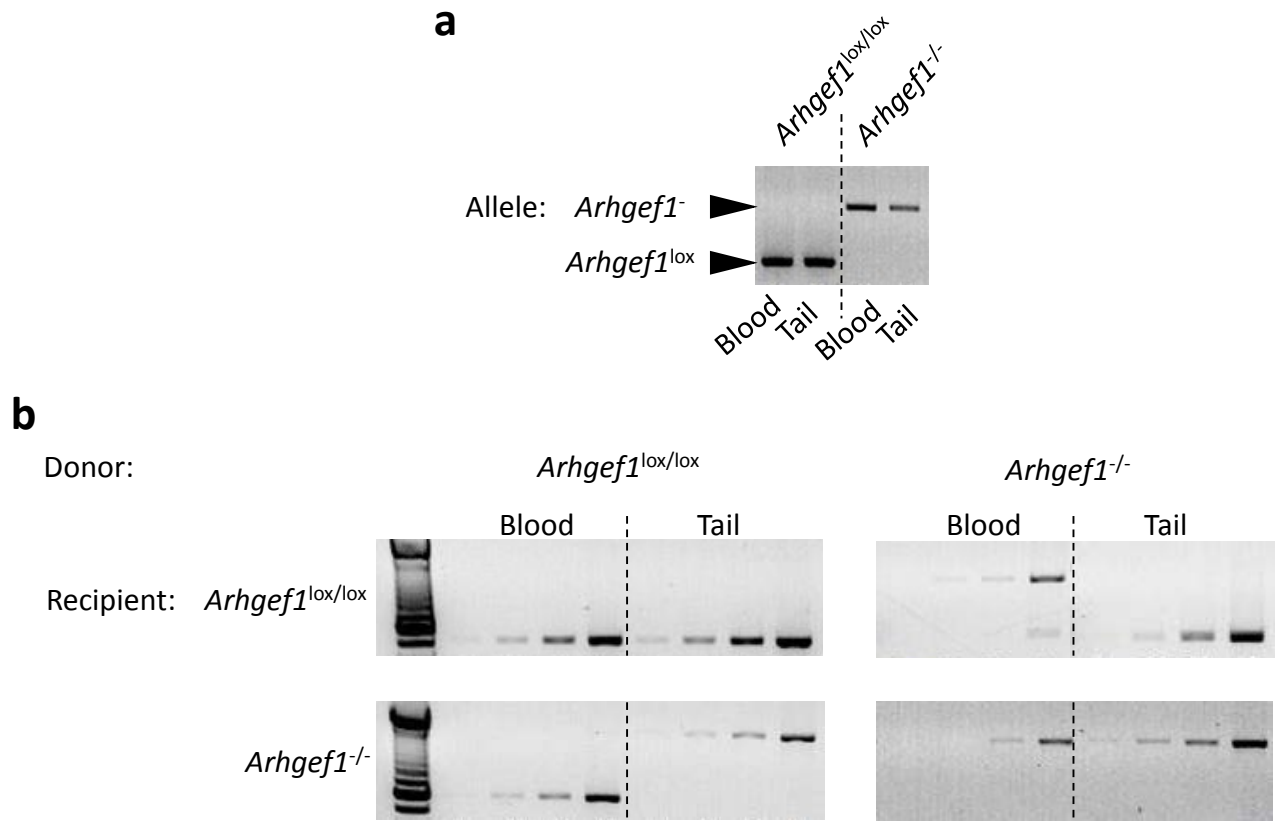
Supplemental Figure 2. Complete peripheral blood count analysis and Arhgef1 expression in peripheral blood mononuclear cells from *Arhgef1*$^{lox/lox}$ and *Arhgef1*$^{-/-}$ mice. (**a**) Leukocyte, lymphocyte and monocyte counts obtained in EDTA-anticoagulated peripheral blood samples collected by retro-orbital sinus puncture by automated analyzer (Hemavet Hematology Analyzer 950FS). (**b**) Representative immunofluorescent labeling of Arhgef1 (Anti-Lsc M-19 antibody; sc 8491; Santa Cruz Biotechnology, followed by FITC-conjugated anti-goat antibody) (n=3). (c) Representative Western blot analysis of Arhgef1 and β-actin expression (Anti-Lsc M-19 antibody; sc 8491; Santa Cruz Biotechnology).

Supplemental Figure 3



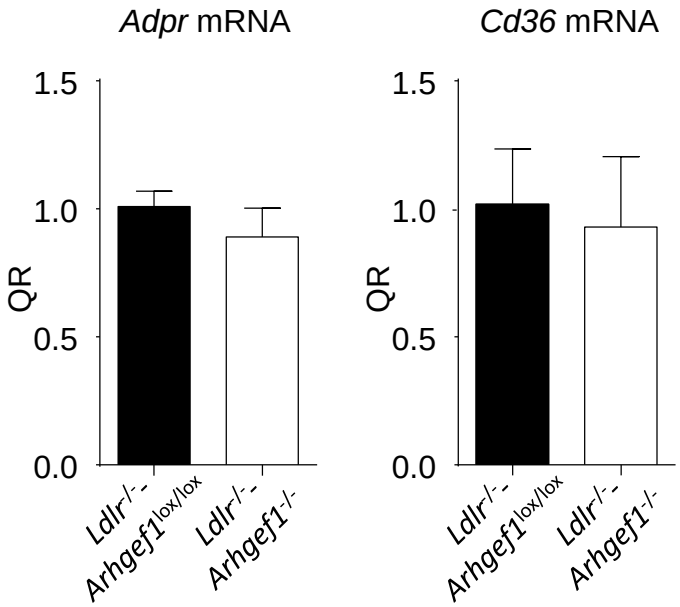
Supplemental Figure 3. Multiplex mouse plasma cytokine levels in *Arhgef1*$^{-/-}$ mice under control condition and 15 min, 30 min, 1 h and 4 h after Ang II injection (30 pmol). EDTA-anticoagulated peripheral blood samples were collected by retro-orbital sinus puncture in isoflurane-anesthetized mice, and plasmas were separated after centrifugations and stored at -80°C until analysis. The Bio-Plex Pro™ Mouse Cytokine Assay (Bio-Rad Laboratories) was used to quantify mouse cytokines/chemokines. The assays were performed according to the manufacturer's protocol by Luminex xMAP Technology - BioPlex 200 system (BioRad). Data was collected using BioPlex Manager 6.0 software. Cytokine levels were determined in pg/mL and the relative plasma concentration in *Arhgef1*$^{-/-}$ mice was expressed as a percentage of its value in the same condition in *Arhgef1*$^{lox/lox}$ *mice* (**P*<0.05, ***P*<0.01, ****P*<0.001 *Arhgef1*$^{lox/lox}$ vs *Arhgef1*$^{-/-}$ in same the condition).

Supplemental Figure 4



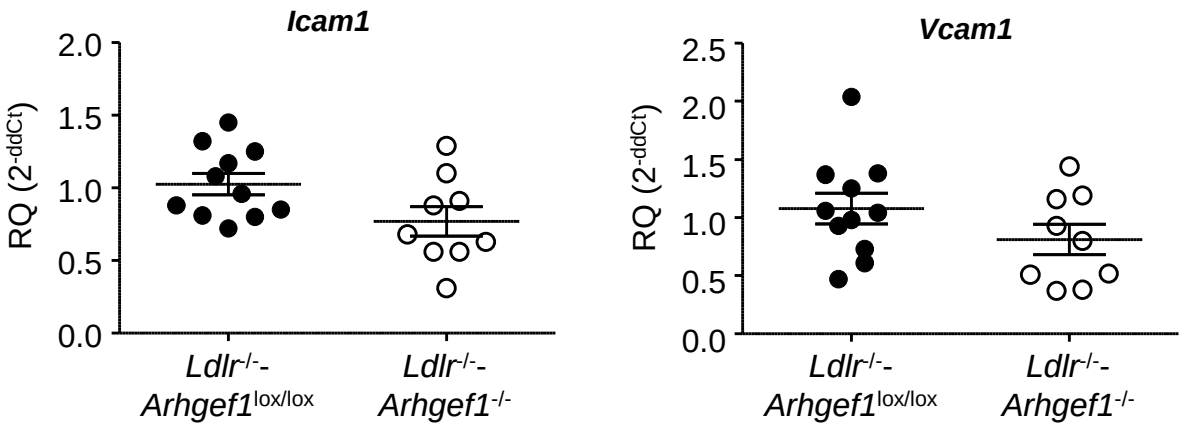
Supplemental Figure 4. PCR analysis of *Arhgef1* expression in blood and tail DNA samples from chimeric mice. a. Control PCR experiment in blood and tail DNA sample from *Arhgef1*$^{lox/lox}$ and *Arhgef1*$^{-/-}$ mice. A common antisense primer (A: 5' TCC-TAA-GCT-CAA-TGG-AGC-CTC 3') and a sense allele-specific primer were used (B: 5' AGT-CCC-GGA-TTC-ATA-TTG-AAG 3' and C: 5' GAG-GTA-AGC-ACT-GTT-TGC-AG 3') to amplify a 550 bp (A-B) and a 1500 bp (C-B) from the lox and the deleted alleles, respectively. **b.** Typical PCR analysis of *Arhgef1* expression in blood and tail DNA samples from chimeric mice, irradiated *Arhgef1*$^{lox/lox}$ and *Arhgef1*$^{-/-}$ recipient mice transplanted with BM from *Arhgef1*$^{lox/lox}$ and *Arhgef1*$^{-/-}$ donor mice.

Supplemental Figure 5



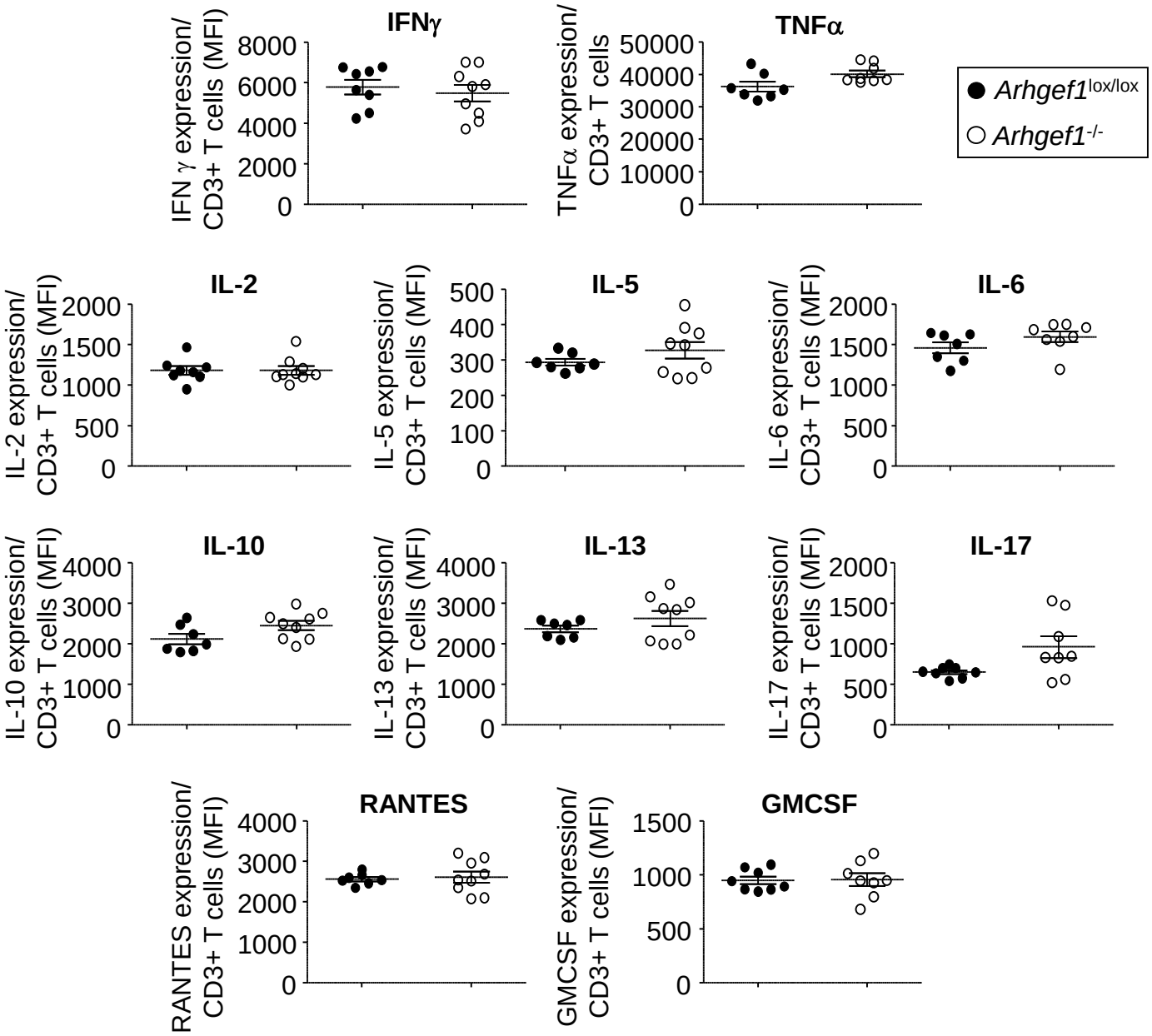
Supplemental Figure 5. Adipose differentiation-related protein (*Adpr*) and *Cd36* mRNA levels in *Ldlr*$^{-/-}$-*Arhgef1*$^{lox/lox}$ and *Ldlr*$^{-/-}$-*Arhgef1*$^{-/-}$ mice peritoneal macrophages incubated for 48 hours in 10% FCS RPMI supplemented with 100µg/ml LDLox (Abd serotec). Total RNA from foam cells was purified using Trizol (Life technology, les Ulis, France) according to the manufacturer's instructions. 500 ng of were reverse-transcribed and real-time quantitative PCR was performed using the TaqMan 7900 Sequence Detection System (Applied Biosystems, Warrington, UK). Primers used to assess *Adpr* and *Cd36* mRNA expression were designed using the Primer Express 3.1 software. Primer sequences used were as follows: *Adpr,* forward, GTAGACCAGTACTTCCCTCTCACTCA; *Adpr,* reverse, GGCTTCTGAACCATATCAAATCCT; *Cd36*, forward, GAGCAACTGGTGGATGGTTT, *Cd36*, reverse, GCAGAATCAAGGGAGAGCAC. Levels of mRNA expression were normalized to the ribosomal protein *36B4* mRNA expression (*36B4*, forward, AGATGCAGCAGATCCGCAT; *36B4*, reverse, GTTCTTGCCCATCAGCACC).

Supplemental Figure 6



Supplemental Figure 6. *Icam1* and *Vcam1* mRNA levels in atherosclerotic aortas from *Ldlr*$^{-/-}$-*Arhgef1*$^{lox/lox}$ and *Ldlr*$^{-/-}$-*Arhgef1*$^{-/-}$ mice. No significant difference between *Ldlr*$^{-/-}$-*Arhgef1*$^{lox/lox}$ and *Ldlr*$^{-/-}$-*Arhgef1*$^{-/-}$ mice (Mann-Whitney test). Primer sequences used were as follows: *Icam1*, forward, CGCTACCATCACCGTGTATTC; *Icam1*, reverse, GCTCAGTATCTCCTCCCCA; *Vcam1*, forward, TGGGAGAGACAAAGCAGAAG, *Vcam1*, reverse, ACGTCAGAACAACCGAATCC. Levels of mRNA expression were normalized to the ribosomal protein *36B4* mRNA expression (*36B4*, forward, AGATGCAGCAGATCCGCAT; *36B4*, reverse, GTTCTTGCCCATCAGCACC).

Supplemental Figure 7



Supplemental Figure 7. Cytokine production by T-cell from *Arhgef1*$^{lox/lox}$ and *Arhgef1*$^{-/-}$ mice. Spleen were removed and crushed using a potter then the cells were filtered through a 40 µm cell strainer. After red blood lysis, T cells were sorted using a Pan T cell isolation kit (Miltenyi). Then, 10^6 T cells were placed in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin and stimulated 5 hours with a cell stimulation cocktail (Life technologies) at 37°C, 5% CO2. Surface-specific staining was made with CD3-BUV 395, CD4-BV421 or CD4-APC (BD biosciences) together with a cell viability dye ZOMBIE NIR (Biolegend). The cells were then fixed and permeabilized with a fixation/permeabilisation kit (ebiosciences) and stained with intracellular markers: IL-17-BV421, IL-5-APC, IL-13-Pecy7, RANTES-PE, IFN-γ-PE, IL-2-BV421, IL10-PE, GM-CSF-BV421 and TNFα-PE-cy7 (BD Biosciences). Flow cytometry analysis was then performed with BD LSR FORTESSA X20 (BD Biosciences) and data were analyzed using FlowJo vX. (No significant difference between *Arhgef1*$^{lox/lox}$ and *Arhgef1*$^{-/-}$ mice; Mann-Whitney test).