

Supplemental Table 1. Plasma lipid levels and blood cell counts in mice after 4 weeks exposure to Ang II.

	ApoE ^{-/-} xFpr2 ^{+/+}	ApoE ^{-/-} xFpr2 ^{-/-}	p
Lipids	Mean ± SEM	Mean ± SEM	
Cholesterol (mmol/L)	9.2 ± 0.4	7.9 ± 0.6	0.161
Triglycerides (mmol/L)	1.2 ± 0.2	0.9 ± 0.8	0.160
Total leukocytes (10⁹/L)	5.4 ± 0.5	6.1 ± 0.9	0.782
Lymphocytes (10 ⁹ /L)	3.9 ± 0.3	4.3 ± 0.7	0.713
Monocytes (10 ⁹ /L)	0.1 ± 0.02	0.2 ± 0.04	0.741
Neutrophils (10 ⁹ /L)	1.3 ± 0.2	1.6 ± 0.2	0.923
	N= 5	N=6	

Supplemental Table 2: Plasma lipid levels and blood cell counts mice after 4 weeks exposure to Ang II in the second experimental groups.

	ApoE ^{-/-} xFpr2 ^{+/+}	ApoE ^{-/-} xFpr2 ^{-/-}	ApoE ^{-/-} x12/15 LO ^{-/-}	p
Lipids	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Cholesterol (mmol/L)	9.7 ± 0.9	9.1 ± 0.6	9.0 ± 0.5	0.051
Triglycerides (mmol/L)	2.2 ± 0.4	1.6 ± 0.4	2.3 ± 0.2	0.287
Total leukocytes (10⁹/L)	6.1 ± 0.6	6.3 ± 1.1	6.9 ± 0.6	0.305
Lymphocytes (10 ⁹ /L)	4.3 ± 0.5	4.6 ± 0.9	4.5 ± 0.5	0.762
Monocytes (10 ⁹ /L)	0.2 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	0.174
Neutrophils (10 ⁹ /L)	1.7 ± 0.1	1.5 ± 0.3	2.1 ± 0.2	0.111
	N= 6	N=5	N=9	

Supplementary Material and Methods

Mouse Model of Abdominal Aortic Aneurysm

ApoE^{-/-}xFpr2^{+/+}, ApoE^{-/-}xFpr2^{-/-} or ApoE^{-/-}x12/15LO^{-/-} mice were anesthetized with isoflurane. Osmotic pumps (Aztec Mod #2004) containing Ang II (1µg/kg/min, Sigma # A9525) were implanted dorsally and the skin was closed with 5-0 Vicryl®. Subcutaneous injections of 0.1% buprenorphine were performed at pump implantation and once daily the following two days. Using an ultrasound scanner (Vevo 2100, FUJIFILM, Visual Sonics, Toronto, Canada), the inner diameter of the supra renal segment of the abdominal aorta was assessed one day prior to pump implantation and either every week or every other week for the next four weeks. After abdominal fur removal and under anesthesia, the ultrasound probe (MS700) was placed transversally to identify the renal artery. The suprarenal aortic diameter was measured (inner edge to inner edge) at three locations towards the thoracic artery. If aneurysmal lesions or dilatations were observed, three measurements were taken at the largest diameter. Pulse-wave Doppler registration of systolic flow was performed with the probe in longitudinal position.

After the last ultrasound examination (4 weeks), mice were put to death in a CO₂ chamber, blood was withdrawn and spleen and aorta were collected for mRNA analysis. Leukocytes counts were measured by ABC Vetpack®. Cholesterol and triglycerides were measured using kits from Randox following manufacturer's protocol and expressed as mmol/l.

The aorta was collected from the aortic root until the bifurcation of the iliac arteries in either RNA^{later}® or zinc-formaldehyde. After measurement of the maximal aortic diameter from external edge to external edge, aortic segments were used for the experiments described below. At least 6 mice were used per group.

Collagen quantification

Supra-renal abdominal aortae were used for collagen quantification using Sirius red (Histolab® #HL27150). Imaging of the slides with polarized light allowed identifying thick (orange) and thin (green) collagen fibers, respectively, which were quantified using Qwin (Leica®). The results are expressed as either positive area (µm²) or as the ratio orange/green, as previously described (19).

Elastin quantification

Supra-renal abdominal aortae were used for elastin quantification using Van Gieson elastin stain (Sigma HT25A Elastin staining kit) and imaging with 20x LEICA DMRB optical microscope. The results are expressed as elastin breaks per optical field as previously described by Aikawa *et al.* (Circulation. 2009;119:1785) and quantifications of 3 optical fields were averaged for each section.

Real-Time PCR

Total RNA was isolated using QIAzol (Qiagen) and concentrations were measured spectrophotometrically using Nanodrop 1000 (Thermo Fisher Scientific). Reverse-transcription was performed with a High capacity kit® (Life Technologies, USA). Real time PCR was performed on a 7900HT Fast Real-Time PCR system (Perkin-Elmer Applied Biosystems) using the following TaqMan Assay-on-Demand from Applied Biosystems: MMP-2: Mm00439498_m1; MMP-9: Mm00442991_m1; Timp 1: Mm01341361_m1; Timp 2: Mm00441825_m1). Results are expressed

as $2^{-\Delta CT}$ obtained by comparing the threshold cycle (CT) for the gene of interest with that obtained using hypoxanthine phosphoribosyl-transferase (HPRT; Mm01545399_m1) as housekeeping gene.

Immunohistochemistry

5 μm sections from paraffin embedded abdominal aortic segments were prepared. After blocking of endogenous peroxidases, the sections were exposed to primary antibodies against neutrophils (Ly6-g cat #551459, BD Pharmingen) or macrophages (MAC2 cat #CL8942AP, Cedarlane labs) to determine the lesion composition. Secondary biotinylated antibodies were used with biotin-streptavidin and DAB to identify the positive areas. All sections were counterstained with hematoxylin and eosin. Stained and unstained areas were identified and quantified with Qwin[®] (Leica) and results are expressed as per cent positive area of the total vessel area.

Isolation of bone marrow derived murine neutrophils

Isolation of bone marrow was performed as previously described (33, 34) using a single percoll layer gradient centrifugation. In brief, after being put to death in CO₂ chambers, the femur and tibia were harvested from ApoE^{-/-}xFpr2^{+/+} and ApoE^{-/-}xFpr2^{-/-} mice and the bone marrow was flushed with a 21G needle syringe using Hanks Balanced Salt Solution (HBSS) containing 20mM HEPES (pH 7.4) and 0.5% FCS. Cells were centrifuged at 400G for 5 min before being suspended in 5ml of 0.2 % NaCl for 30 seconds, and restored with 5ml 1.6% NaCl. The cells were then flushed through a 70 μm cell strainer in a 50mL conical tube before being centrifuged and suspended in 5 mL HBSS. The cell suspension was carefully layered on top of 5mL of 62.5% Percoll and centrifuged at 1000G for 30 min, without acceleration or break. The pellet containing neutrophils was then transferred to another tube of 5mL HBSS and centrifuged at 400G again before the pellet was resuspended in RPMI media, supplemented with 1% glucose, 0,1% FCS, 100 IU/ml penicillin G, and 100 μmol / mL streptomycin. The cells were counted by scil Vet ABC hematology analyzer (Gurney, USA) and yielded a population of at least 65% neutrophils.

Protein extraction and Western blot

Bone marrow-derived neutrophils were treated with ATL (100nM) for 20 min either before or after UV-C radiation from laminar flow cabinets for 10 min. Neutrophils were lysed with RIPA buffer (Sigma) and Halt Phosphatase Inhibitor Cocktail (ThermoFisher Scientific) and scraped. Protein extraction was performed by vortexing the samples with RIPA for 40 minutes, sonication for 5min and centrifugation for 20min at 13 000g. The supernatant was used for immunoblot analysis and the protein concentration was measured by BCA assay (Biorad). Equal amounts of total protein were separated on 4-15% SDS-polyacrylamide gels (Biorad) under reducing conditions and electroblotted onto a PVDF membrane (Millipore). The blots were blocked with 5% nonfat milk in TBS and 0.1% Tween 20 (TBST) for 1 h at room temperature and incubated overnight at 4°C with anti-Vinculin antibody (1:10,000 abcam) and anti-phospho-p38 (1:750, Cell Signaling Technologies #9211), in 5% nonfat milk and 5% BSA, respectively, in TBST. After washing in TBST for three times, the membranes were incubated with near-infrared fluorescence dye 800CW labelled donkey-anti-rabbit secondary IgG (LI-COR) diluted 1:12000 in TBST and 0.01% SDS for 1 hour at room temperature. After washing in TBST for five times, fluorescent signals were detected on the CLX Odyssey imaging system (LI-COR). Afterwards, the same membranes were incubated with a p38 antibody (1:750, Cell Signaling Technologies #9228) in 5% nonfat milk in TBST overnight at 4°C.

Detection of signals was performed as described above, with a goat-anti-mouse IRDye 680RD conjugated secondary antibody (LI-COR). Fluorescent signals were quantified with the Image Studio Software (LI-COR).

Human abdominal aneurysmal tissue and microarray Analysis

Tissue RNA was extracted using Qiazol Lysis Reagent (Qiagen) and purified using the RNeasy minikit (Qiagen). RNA concentration was measured using Nanodrop ND -1000 (Thermo Scientific, Waltham, MA, USA) and the quality verified using a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). From the Affymetrix HTA 2.0 Genechip arrays dataset, only data from three specific genes were extracted; FPR2/ALX (probe TC19000788.hg.1), CEACAM8/CD66b (probe TC19001576.hg.1) and CEACAM3/CD66d (probe TC19000585.hg.1). Raw CEL intensity files were normalized through Guanine Cytosine Count Normalization, Signal Space Transformation and Robust Multi-array Average by use of the Affymetrix Expression Console software (Thermo Fisher, Waltham, MA, USA). Aneurysmal samples not used for gene expression studies were fixed in paraformaldehyde, paraffin embedded and used for immunohistochemistry as described above.

Statistical analysis

Data are expressed as mean \pm standard error of the mean (SEM). Student's t-test or Mann-Whitney was performed when 2 groups were compared. A one way ANOVA was performed for multiple comparisons with one variable. Differences in murine aneurysms size were determined using a two-way ANOVA with genotype and time as variables. All ANOVA analyses were performed for non-repeated measures with a Holm-Sidak *post hoc* test for multiple comparison testing. The proportion of aortic dissections in was compared using Fisher's exact test. Statistical analyses of experimental data were performed using SigmaPlot version 12.5 (Systat Software Inc). Differential gene expression in microarray data was determined with Bioconductor tool limma (35) using the empirical Bayes moderation of standard errors for the microarray linear model, applied in R environment. Pearson correlation coefficient was used for normalized gene expression values to examine gene to gene correlations. A $p < 0.05$ was considered significant.