Supplement Material

Material and Methods

Animal Models

Six-week-old male C57BL/6, Nox2$^{-/-}$ (C57BL/6 background), Balb/c (TLR4^{wt}), Tlr4$^{Lps\text{-}d}$ (TLR4^{d}, background strain BALB/cAnPt) were obtained from Jackson Laboratories (Bar Harbor, ME). Tlr4$^{Lps\text{-}d}$ mice harbor a spontaneous mutation in the toll-like receptor gene. c-*fms*^{YFP} transgenic mice (FVB/N background) were obtained as previously described (1). All mice were randomized to exposure (see below). The Committee on Use and Care of Animals from the Ohio State University (OSU) approved all experimental procedures.

Exposures to $PM_{2.5}$ Ambient Whole-Body Inhalational Protocol

Animal exposure and the monitoring of exposure atmosphere and ambient aerosol were performed as previously described using a versatile aerosol concentration enrichment system that was modified for long-term exposures (2). Briefly, mice were exposed to concentrated $PM_{2.5}$ or filtered air (FA) in a mobile trailer at The Ohio State University in Columbus, OH (OASIS-1 chamber, "Ohio air pollution exposure system for the interrogation of systemic effects"). FA-exposed mice received an identical protocol with the exception of a high-efficiency particulate air filter (Pall Life Sciences, East Hills, NY) positioned in the inlet valve to remove $PM_{2.5}$ in the filtered air stream, as detailed described previously (3). The exposure protocol comprised of exposures for 6 h/day, 5 days/wk. The TLR4^{wt} and TLR4^{d} mice were exposed for a total duration of 20 weeks from March to July 2009. The Nox2^{wt} and Nox$^{-/-}$ mice were exposed from November 2008 – March 2009 (20 weeks) and the c-fms^{YFP} mice from October 2008 – March 2009 (23 weeks). Supplemental Table 4 shows the plasma lipid profile among the different groups after exposure.

Analysis of $PM_{2.5}$ Concentration in the Exposure Chamber

To calculate exposure mass concentrations of $PM_{2.5}$ in the exposure chambers, samples were collected on Teflon filters (PALL Life Sciences Teflo, 37 mm, 2 µm pore, Ann Arbor, MI) and weighed before and after sampling in a temperature- and humidity-controlled weighing room using an oscillating microbalance (Tapered-Element Oscillating Microbalance, model 1400, Rupprecht and Patashnick). Weight gains were used to calculate exposure concentrations.

Flow Cytometry.

Whole blood was collected to assess the expression of inflammatory monocytes and YFP positive cells in peripheral leukocytes in TLR4^{wt}, TLR4^{d} and c-fms^{YFP} mice. About 1 mL of blood was treated with red blood cell lysis buffer (Biolegend, San Diego, CA) for 5 minutes (2 times). Bone marrow cells were collected by flushing femur and tibia with 10 mL PBS. Monocytes were isolated from bone marrow with Histopaque-1077 (Sigma-Aldrich, St. Louis, MO). Isolated leukocytes from both blood and bone marrow were resuspended in flow buffer (PBS containing 5% bovine serum albumin (BSA) and 0.02% NaN_3) and washed twice. This was followed by incubation with PE-Cy7 anti-Ly6G (eBioscience, San Diego, CA), FITC anti-CD3 (Pharmingen, San Jose, CA), APC-Cy7 anti-CD45.BR (Pharmingen, San Jose, CA), PE-Cy5 anti-F4/80 (Biolegend, San Diego, CA), PE-Cy5 anti-Ly6C (Biolegend, San Diego, CA), PE anti-CD11b (Biolegend, San Diego, CA); CCR2 ((primary antibody (rabbit monoclonal) and secondary antibody, donkey polyclonal to rabbit (PE) both Abcam)), PE-Cy5 anti-CCR5 (Biolegend, San Diego, CA) and PE anti-CxCr3 (Biolegend, San Diego, CA) with 1 µg per million cells for 30 minutes. Cells were subsequently washed with flow buffer, resuspended in 1% natural buffered formaline and analyzed by flow cytometry (BD FACS LSR II™ flow cytometer, Becton Dickinson, San Jose, CA) and the data were analyzed on BD FACS Diva software (Becton

Dickinson, San Jose, CA). To detect YFP^+ monocyte infiltration in lung and epididymal fat cells were isolated as mentioned and analyzed by flow cytometry. Broncheolar alveolar lavage (BAL) cells were isolated by flushing the lung with 1 ml PBS for three times. Epididymal fat and lung tissue were digested with Collagenase II (Sigma-Aldrich, St. Louis, MO) for one hour and samples were stained for flow cytometry afterwards.

Cytokine Measurements in Tissues and Bone Marrow-Derived Monocyte Conditioned Media

Lung tissue homogenates and BMDM supernatants were analyzed for 6 different cytokines by Mouse Inflammation 6-Plex Kit from BD Bioscience (San Diego, CA). The lung lobes were snap-frozen (in liquid nitrogen) and stored at -80°C until further analysis. The snap-frozen lungs were thawed, weighed, transferred to different tubes on ice containing 1 ml of T-PER containing Complete Mini Protease Inhibitor Cocktail (Roche Diagnostics GmbH, Mannheim, Germany) tablets at a proportion of 1 tablet/10 ml of T-PER stock reagent. The lung tissues were homogenized at 4°C. Lung homogenates were centrifuged at 9,000 × g for 10 minutes at 4°C. Supernatants were transferred to clean microcentrifuge tubes, frozen on dry ice and thawed on ice. Total protein concentrations in the lung tissue homogenates were determined using a BCA kit. Lung tissue homogenates were diluted with 50% assay diluents (provided in the BD™ Cytometric Bead Array (CBA), Mouse Inflammation 6-Plex Kit, BD Bioscience, San Jose, CA) and 50% TPER reagent to a final protein concentration of 500 µg/ml.

Superoxide Measurements

NADPH oxidase derived superoxide production was measured by lucigenin chemiluminescence. Bone marrow derived $F4/80^+$ (Miltenyi Biotec, Bergisch Gladbach, Germany) monocytes were sorted by immuno magnetic bead sorting MiniMACS (Miltenyi Biotec, Bergisch Gladbach, Germany). The cell number was adjusted at $1x10^5$ cells per well. Additionally, mouse aorta without perivascular fat and perivascular fat alone were placed in chilled, modified Krebs-Hepes Buffer (pH = 7.4; initially gassed with 95% O_2, and 5% CO_2), cleaned of excessive adventitial tissue and cut into 2 to 3 mm segments. The vessel segments were placed in a microtiter plate (Berthold Technologies, Bad Wildbad, Germany) containing 300 µl of Krebs-Hepes buffer with 5 µM Lucigenin in each well were placed into an Berthold CENTRO LB 960 Luminometer (Berthold Technologies, Bad Wildbad, Germany). Scintillation counts were recorded every minute for a duration of one second. After baseline measurements for 10 min, 100 µM NADPH (Sigma-Aldrich, St. Louis, MO) was added to each well and measurements made over additional 10 minutes. Values were reported after subtracting respective background counts. Aortic tissue and perivascular fat tissue were quantified per/mg wet weight.

Myography and Intravital Microscopy

Thoracic aortas were dissected from animals and immediately immersed in physiological salt solution buffer at room temperature. In some experiments aortas were then cleaned of adhering fat and connective tissue under a microscope while as in other experiments aortas were left with adipose tissue around them. The blood vessels were cut into rings of 2 mm to 3 mm length and were mounted in a standard 5 ml organ bath (DMT 700 MO, Atlanta, GA) filled with physiological saline solution (PSS) buffer. The bath medium was maintained at 37°C with a pH of 7.4 and aerated continuously with 95% oxygen and 5% carbon dioxide. Extra care was taken to ensure that the endothelium was not damaged during the whole process of tissue preparation and mounting. Aortic rings were subject to graded doses of vasoconstrictor agonists and the endothelium dependent dilator acetylcholine as described previously (4). For intravital microscopy mice were anesthetized by i.p. injection of a mixture of 20 mg/kg xylazine (VET TEK, Blue Springs, MO) and 100 mg/kg ketamine hydrochloride (Bioniche Pharma USA LLC,

Lake Forest, IL). Cremaster muscle or mesenteric adipose tissue were stretched on an optically coherent mount. The muscle or the mesenteric adipose tissue were superfused with Ringers Lactate at 37°C and leukocyte endothelial interactions in 10-15 venules obtained using a Nikon Eclipse FN1 microscope (Nikon, Japan) with a 40x/0.80 W (muscle) and 20x/0.50 W (mesenteric tissue) water immersed objective with 2.0 mm working distance. Video images were captured by a monochrome QImaging Rolera-XR camera (Surrey, BC, Canada) at a speed of 20 FPS and digitalized to 12-bit TIF images using Metamorph software (version 7.1.2.0, Metamorph, Downingtown, PA). c-*fms*^{YFP} mice were imaged using a fluorescent filter (YFP emission 527 nm). Rolling leukocytes or YFP positive cells were counted per minute for different vessel diameters and vessel segments. All leukocytes or YFP^{+} cells per 100 µm of vessel length that were immobile for at least 30 s were interpreted as adherent cells (5). The number of rolling and adherent cells was then imputed for a theoretical 30 micron vessel, assuming a linear dependency between the vessel diameter and leukocyte adherence/rolling. Calculations were performed using OptiTest (Version 1.4.1.0).

Immunohistochemistry

Segments of thoracic aorta with perivascular fat were frozen in liquid nitrogen and embedded in Optimal Cutting Temperature compound (Tissue-Tek, Sakura Finetek USA, Inc., Torrance, CA). Rat anti-mouse F4/80 antibody (AbD Serotec, Raleigh, NC) was used to determine macrophage infiltration. Immunohistochemical 3,3'-Diaminobenzidine (DAB) staining was performed by using the primary antibodies (1:200 concentration) and a detection system using Peroxidase-conjugated AffiniPure goat anti-rat antibodies (Immunoperoxidase Secondary Detection System; Jackson ImmunoResearch Laboratories, West Grove, PA). Images were analyzed and quantified with Metamorph software (Metamorph) after digitization of the images with a color QImaging Micro Publisher 5.0 RTV camera (Surrey, BC, Canada) at 200x. Data are expressed as the percentage of the total analyzed area. For estimation of infiltrated F4/80 positive cells per area, four successive sections were collected on the same slide, and at least 10 sections from three consecutive slides per area per mouse were examined.

Total RNA extraction and quantitative RT-PCR

Pathway-focused gene expression profiling was performed using the RT^2 Profiler™ PCR Array System - Mouse Toll-Like Receptor Signaling Pathway according to manufacturer's instructions (SuperArray Bioscience Corporation, Frederick, MD). Total RNA was isolated with Absolutely RNA MiniPrep kit (Stratagene, La Jolla, CA) according to the manufacture's instructions. cDNA was synthesized using 500 nanogram of total RNA and Transcriptor™ reverse transcriptase (Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer's instructions. Real time reactions (minus primers) were prepared with a SYBR green master mix, loaded onto the array plate containing pre-loaded primer sets for 384 genes, and run on a Lightcycler 480. Expression was determined relative to 7 housekeeping genes using proprietary Superarray software. For additional confirmatory PCRs we used the following primers:
CCR2 5'-GCACTTAGACCAGGCCATGC-3' (forward) and 5'-GCTCCCTCCTTCCCTGCTTA-3' (reverse); CCR1 5'-CCATGCAGGTGACTGAGGTG-3' (forward) and 5'-TTGAAACAGCTGCCGAAGGTAC-3' (reverse); CCR5 5'-CTGGCTCTTGCAGGATGGATTT-3' (forward) and 5'-ATGGCCAGGTTGAGCAGGTAG-3' (reverse); Cx3Cr1 5'-TCTCTCCCAGCCTGTTGCTC-3' (forward) and Cx3Cr1 5'-GGAATCCAACCCCAGACCTC-3' (reverse); Hmg14 5'-GCAGAAAATGGAGAGACGGAAAACC-3' (forward) and 5'-AAGGGAGGCGGGACCACTGAC-3' (reverse); Nox2 5'-ACTCCTTGGGTCAGCACTGG-3' (forward) and 5'-GTTCCTGTCCAGTTGTCTTCG-3' (reverse); p67^{phox} 5'-CAGTCCCAAGGAGAATGGAA (forward) and 5'- TCTGCCATAGCTGGACAGTG (reverse); p47^{phox} 5'-ACCTGTCGGAGAAGGTGGT-3' (forward) and 5'-TAGGTCTGAAGGATGATGGG-3' (reverse); p22^{phox} 5'-TGCGGGACGCTTCACGCAGTGG-3' (forward) and 5'-

GGTTGGTAGGTGGCTGCTTGATGG-3' (reverse); Rac1 5'-TGGGACACAGCTGGACAAGAAGAT-3' (forward) and 5'-TCAGGATACCACTTTGCACGGACA-3' (reverse); Rps3 5'-ATCAGAGAGTTGACCGCAGTTG-3' (forward) and 5'-AATGAACCGAAGCACACCATAGC-3' (reverse). Quantitative real-time PCR was performed with a Lightcycler 480 (Roche Applied Sciences, Penzberg, Germany) using SYBR Green I Master Mix (Roche Diagnostics GmbH, Mannheim, Germany). The relative quantification values for these gene expressions were calculated from the accurate threshold cycle (C_T), which is the PCR cycle at which an increase in reporter fluorescence from SYBR green dye can first be detected above a baseline signal. The C_T values for Hmg14 and Rps3 were averaged and subtracted from the C_T values for the gene of interest in each well to calculate ΔC_T. The duplicate ΔC_T values for each sample were averaged.

***In-vitro* Experiments in Cultured Bone Marrow Derived Monocytes**

Primary bone marrow derived monocytes (BMDM) were isolated by flushing the femur and tibia by a 26-gauge needle and (PBS, 5% FBS). Afterwards, transfer the cell suspension on a 150 mm suspension culture dish (Corning Incorporated, Corning, NY). Cells are cultured in L-cell conditioned media plus D-MEM at 37°C for 5 days and harvested by trypsinization (Sigma-Aldrich, St. Louis, MO). BMDM were isolated from mice whereby TLR4 is intact and deficient as described previously. After harvesting the BMDM by trypsinization, $1x10^5$ cells were plated in a 96-well plate (Microtest, Becton Dickinson Labware, NJ) and cultured overnight. Triplet or quadruplet samples per group were either untreated, treated with 25 μM PAPC (Avanti Polar Lipids Inc., Alabaster, AL) or a mixture of 12.5 μM POVPC (Avanti Polar Lipids Inc., Alabaster, AL) and 12.5 μM PGPC (Avanti Polar Lipids Inc., Alabaster, AL) for 0.5 h (western blot) or 4 h (cytokine release or RT-PCR) at 37°C. For western blot and RT-PCR experiments cells were lysed as described previously and stored at -80°C until further procession. In experiments detecting the cytokine release cells were washed 3 times with PBS, new DMEM media with 20% FBS was added and further incubated for 48 h at 37°C. After treatment with phospholipids cells were incubated in L-cell conditioned free media for 48 h. Then supernatants were collected and stored in -80°C until further procession.

Western Blot in Lung Homogenates and Bone Marrow Derived Monocytes

Lung homogenates were received as described previously. Cells were briefly washed with ice-cold PBS. The cells were then scraped in 500 μl extraction lysis buffer containing 20 nM Tris pH 7.5, 100 mM NaCl, 0.5% NP-40, 0.5 mM EDTA, 0.5 mM PMSF and 0.5% protease inhibitor cocktail (Thermo Fisher Scientific Inc., Waltham MA). Cell debris was removed by micro centrifugation. The protein concentration of the cell lysates were determined using Bio-Rad DC (Bio-Rad Laboratories, Hercules, CA) protein assay according to the manufacturer's instructions. The supernatants were then boiled with Laemmli sample buffer for 5 minutes. Samples (25 μg of protein per lane) were separated on 8% sodium dodecyl sulfate-polyacrylamide gels at 30 mA for 3 hours. Separated proteins were transferred electrophoretically into polyvinylidene difluoride membrane (Immuno-Blot; Bio-Rad Laboratories, Hercules, CA) at 160 mA for 90 minutes. Membranes were blocked with blocking buffer phosphate-buffered saline and 0.1% Tween 20 (Acros Organics, NJ) containing 5% BSA at 4°C overnight. For detection of phosphorylated proteins, membranes were incubated with anti-phosphoserine antibody (Abcam, Cambridge, MA) overnight at 4°C. After washing, membranes were incubated with the horseradish-peroxidase conjugated anti-mouse antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, dilution of 1:500) at room temperature for 90 minutes for detection of $p47^{phox}$ membrane was stripped and reincubated with rabbit anti-$p47^{phox}$ monoclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) overnight. Prestained markers (Precision Plus Protein; Bio-Rad Laboratories, Hercules, CA) were used for molecular mass

determinations. Immunoreactive bands were detected by enhanced chemiluminescence (Super Signal West Pico; Thermo Scientific, Rockford, IL).

Liquid Chromatography Mass Spectrometry of Oxidized Phospholipids

Broncheolar alveolar lavage fluid (BALF) was isolated by flushing the lung three times with 2 ml PBS. The samples of 5 mice were pooled and further processed. Lipids were extracted three times with chloroform / methanol mixture (1:1) and combined extracts were evaporated to dryness under stream of nitrogen. Samples were stored under nitrogen atmosphere at -80^{o}C until analysis. Mass spectra were acquired in positive ion mode using Applied Biosystems 3200 QTRAP system coupled with electrospray ionization (TurbolonSpray) source. The spectrometer was first optimized by infusion of PAPC (25 nmol/ml), POVPC (5 nmol/ml) and PGPC (2 nmol/ml). All phospholipids were purchased from Avanti Polar Lipids Inc, (Alabaster, AL). The source parameters were set as follows: curtain gas (nitrogen), 10 psi; collision gas (nitrogen), medium; ion spray voltage 5000 V; temperature 550^{o}C, ion spray voltage, 5000 V; ion source gas 1 and 2, 30 and 50 psi, respectively. Optimized parameters for all phospholipids were: declustering potential, 50 V; entrance potential, 10 V and collision energy, 50 eV. For analysis of BAL extracts, samples were dissolved in mobile phase consisting of chloroform, methanol, water and trifluoroacetic acid (65:25:4:0.1, by vol). Lipids were characterized after isocratic separation on 5 μm Zorbax RX-SIL 4.6 mm x 250 mm HPLC column (Agilent Technologies, Santa Clara, CA) at 0.4 ml/min flow rate using Shimadzu LC-20AD pump interfaced to a Shimadzu CBM-20A system controller. Mass spectrometer was operated in multiple reactions monitoring (MRM) positive ionization mode. Specific monitor Q1/Q3 ion pairs were m/z 782$\rightarrow$184 for PAPC, m/z 594$\rightarrow$184 for POVPC and m/z 610$\rightarrow$184 for PGPC. Standard curves for all phospholipids were obtained in the same set of experiments by infusion of serially diluted PAPC (from 100 to 1000 ng/ml), POVPC (1 to 100 ng/ml) and PGPC (1 to 100 ng/ml). All data were acquired and processed by Analyst software (version 1.4.2, Applied Biosystems, Foster City, CA).

Endotoxin-Detection

Lipopolysaccharide (LPS) levels were analyzed in BALF and Serum by using ToxinSensor^{TM} Chromogenic LAL Endotoxin Assay Kit (Genscript, Piscataway, NJ, USA). From each sample 4 μl were used as substrate and further processed the according manufactur's instructions.

Statistical Analysis

All data are expressed as means $\pm$ standard deviation (SD) unless otherwise mentioned. Statistical tests were performed using one-way ANOVA followed by Mann Whitney test or unpaired *t* test using GraphPad Prism (version 4.1.2). The α-level was set at 0.05.

References

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3. Sun, Q, Yue, P, Deiuliis, J A, Lumeng, C N, Kampfrath, T, Mikolaj, M B, Cai, Y, Ostrowski, M C, Lu, B, Parthasarathy, S. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation 2009;119:538-546.
4. Sun, Q, Wang, A, Jin, X, Natanzon, ., Duquaine, D, Brook, R D, Aguinaldo, J G, Fayad, Z A, Fuster, V, Lippmann, M. 2005. Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. JAMA. 2005;294:3003-3010.
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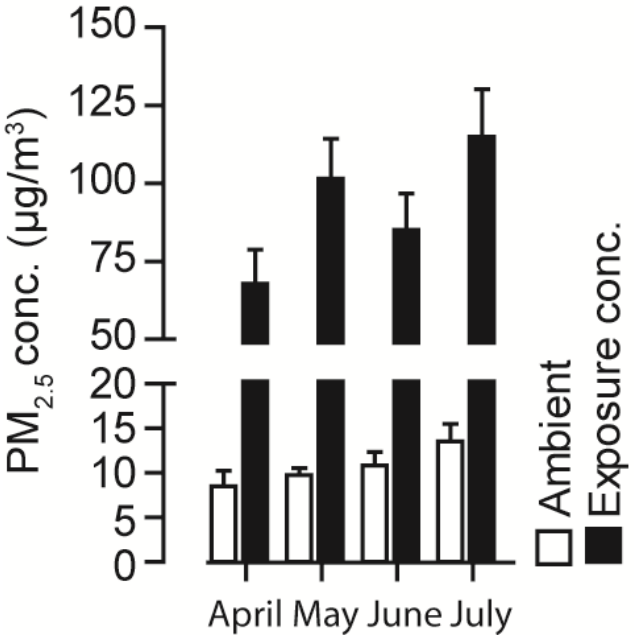
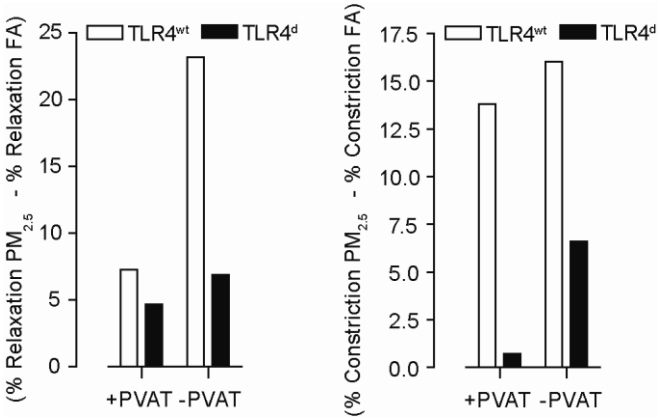
Supplementary Figure I



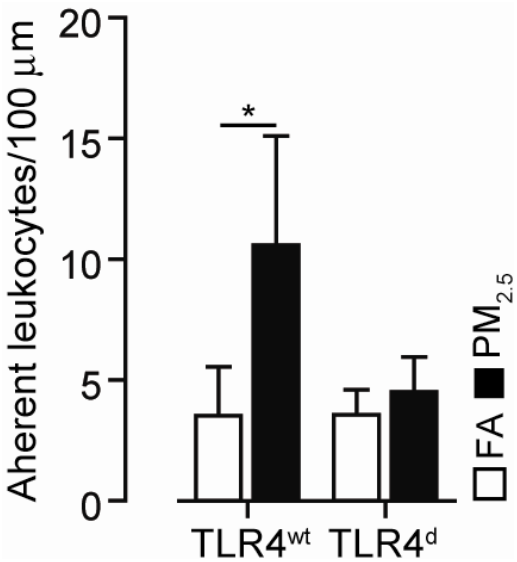
$PM_{2.5}$ concentration in the exposure chamber and particulate composition. Ambient and exposure of particulate matter ($PM_{2.5}$) concentrations during the exposure period at The Ohio State University Airport (April – July, 2009).

Supplementary Figure II



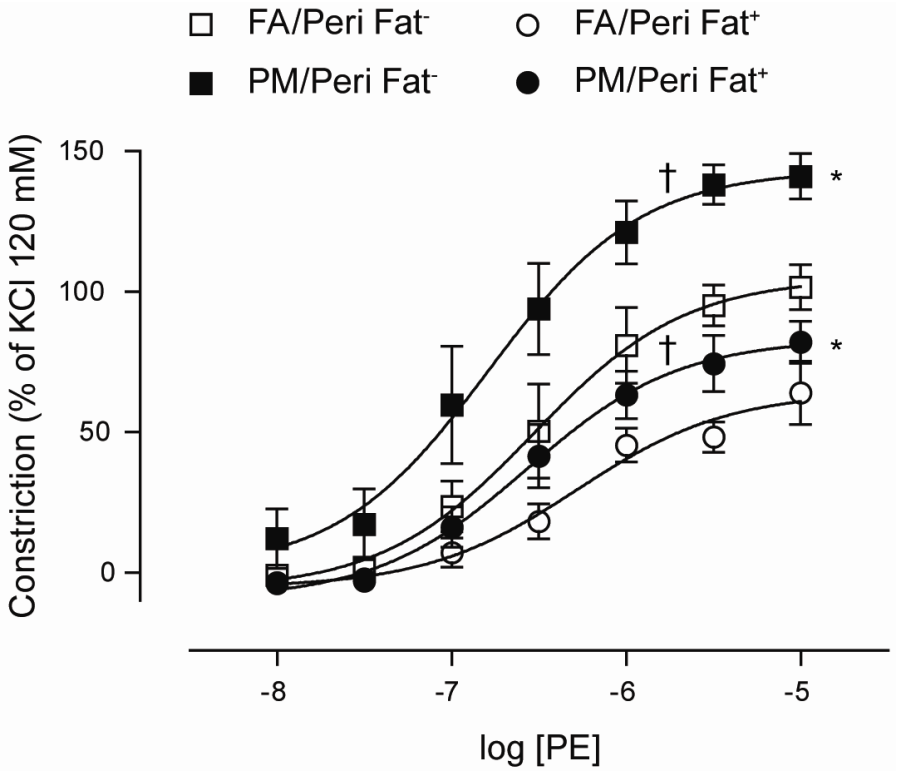
Delta change in the relaxation and constriction maxima of isolated aortic rings with and without perivascular fat (PVAT). (n=8-10/group).

Supplementary Figure III



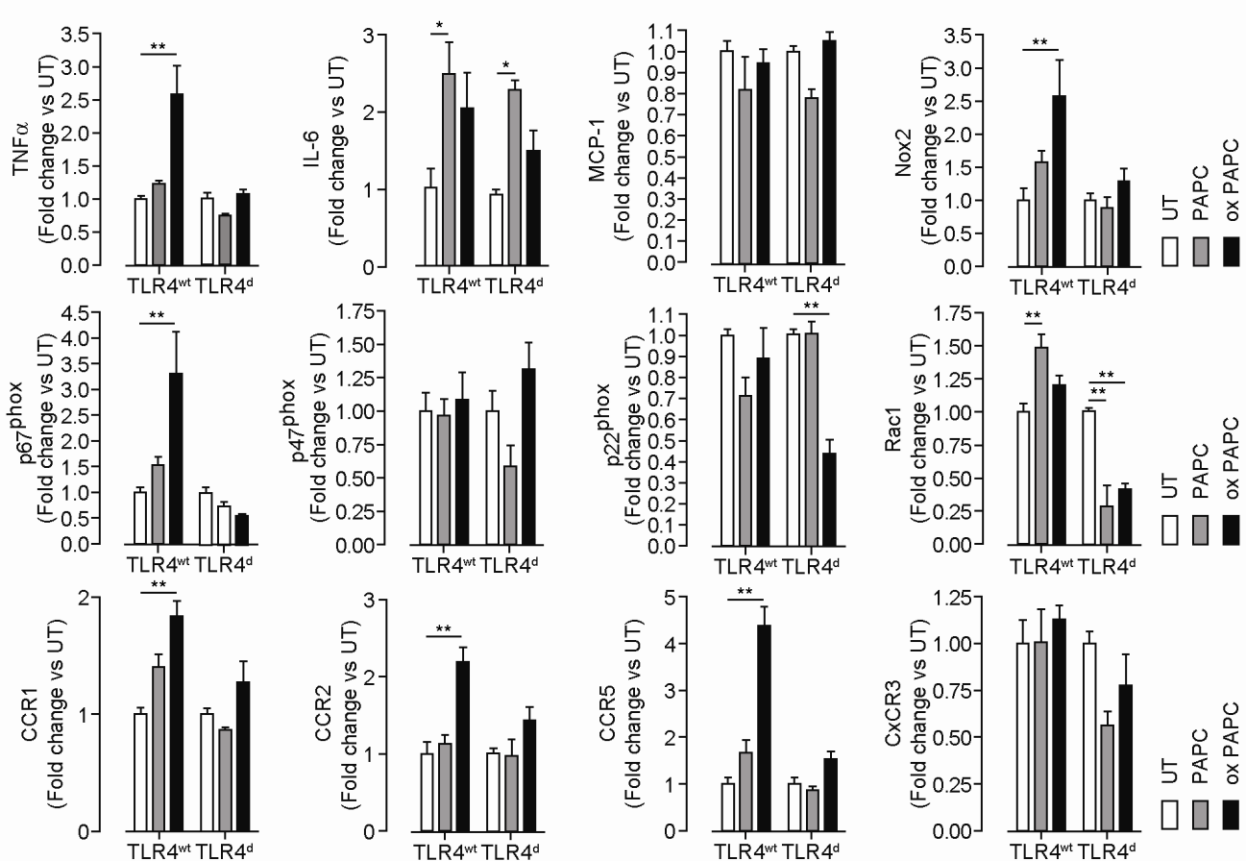
Intravital microscopic leukocyte adherence in the cremaster venular endothelium. Data are mean ± SD. (Original magnification 400x, n=5, *p<0.05).

Supplementary Figure IV



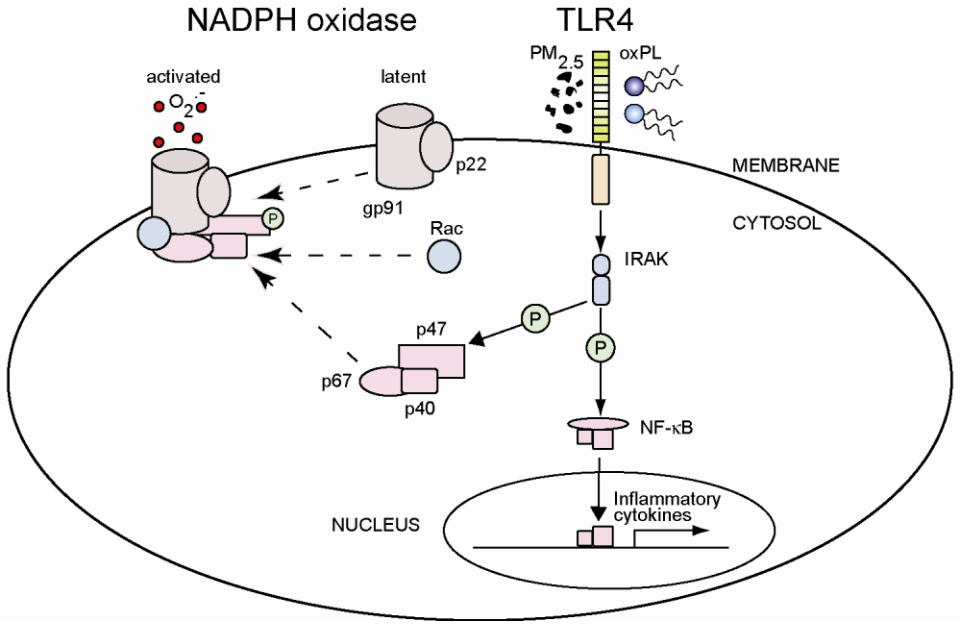
$PM_{2.5}$ impairs contractile properties of isolated thoracic aortic rings in c-*fms*^{YFP} mice. Constriction of aortic rings with and without perivascular fat in response to increasing dosages of phenylephrene. (n=4/group; *p<0.01 vs. same group FA; †logEC50 vs. same group FA).

Supplementary Figure V



TLR4 triggers inflammatory gene expression in response to oxidized phospholipid treatment in BMDM derived from $TLR4^{wt}$ and $TLR4^{d}$ mice. BMDM were treated with PAPC and oxPAPC and mRNA levels were quantified by RT-PCR to confirm the specificity of TLR4 deletion. (n=4/group; *p<0.05; **p<0.01).

Supplementary Figure VI



Hypothetical model of TLR4/ NADPH oxidase interaction in response to oxidized phospholipids and $PM_{2.5}$ upon stimulation. Preliminary data described a marked increase of oxidized phospholipids in the BAL fluid caused by $PM_{2.5}$ exposure. However, we hypothesize that oxidized phospholipids play a critical role in receptor activation for Toll-Receptor 4. In turn TLR4 signals downstream and promotes NADPH oxidase activation through phosphorylation of the NADPH oxidase subunit $p47^{phox}$ by IRAK. Although, the underlying mechanism has not been understood phosphorylated $p47^{phox}$ and other cytosolic subunits of the NADPH oxidase translocate to the membrane and form with $gp91^{phox}$ and $p22^{phox}$ an active, superoxide producing NADPH oxidase complex. A set of *in-vitro* experiments on cultured primary macrophages should elucidate the inflammatory *in-vivo* effect of particulate matter exposure. Furthermore, these experiments should generate a mechanistic link between TLR4 signaling and NADPH oxidase activation.

Supplementary Table I

Exposure time (Dates)	03/20/09 – 07/28/09
Ambient concentration ($\mu g/m^3$)	10.7 ± 2.1
FA chamber concentration ($\mu g/m^3$)	3.05 ± 1.84
PM chamber concentration ($\mu g/m^3$)	92.4 ± 7.1
PM chamber particle enrichment factor	8.8 ± 1.6
* Estimated total exposure dose in FA group, μg	36.86 ± 5.9
† Estimated total exposure dose in PM group, μg	103.65 ± 19.48

Characteristics of exposure protocol. The exposure protocol describes the time of exposure, ambient concentration and the particulate concentration within the filtered air (FA) and particulate matter ($PM_{2.5}$) chamber as well as the particle enrichment factor. Assuming a ventilation rate of 105 breaths/min and tidal volume of 0.2 mL for a 25 g mouse (20). *The baseline estimated total dose over the exposure period of 20 weeks. †The estimated total dose of ambient and exposure over the exposure period of 20 weeks. Values shown are mean ± SD.

Supplementary Table II

Vasomotor response in $TLR4^{wt}$ and $TLR4^{d}$ mice in response to $PM_{2.5}$ exposure

	Perivascular Fat	$TLR4^{wt}$ FA	$TLR4^{wt}$ $PM_{2.5}$	$TLR4^{d}$ FA	$TLR4^{d}$ $PM_{2.5}$
Max. Constrict. To PE	+	74.6 ± 2.5	88.4 ± 3.3 *	70.1 ± 3.6	69.4 ± 3.3
	-	81.9 ± 3.5	97.9 ± 3.5 *	65.8 ± 2.3	72.4 ± 3.5
logEC50	+	-7.4 ± 0.1	-7.7 ± 0.2	-7 ± 0.1	-7.5 ± 0.2
	-	-7.9 ± 0.2	-8.1 ± 0.2	-7.2 ± 0.2	-7.3 ± 0.2
Max. Relax. To Ach	+	-30.6 ± 1.7	-23.3 ± 0.7 *	-30 ± 0.7	-25.3 ± 0.5
	-	-48.9 ± 2.8	-25.7 ± 0.7 *	-44.8 ± 1	-37.9 ± 0.8 #
logEC50	+	-6.4 ± 0.1	-6 ± 0.1	-6.1 ± 0.1	-6.1 ± 0.1
	-	-6.4 ± 0.2	-6 ± 0.1	-6.1 ± 0.1	-6.1 ± 0.1

(*$p<0.01$, # $p<0.05$ vs same group FA)

Supplementary Table III

Vasomotor response in $Nox2^{wt}$ and $Nox2^{-/-}$ mice in response to $PM_{2.5}$ exposure

	$Nox2^{wt}$ FA	**$Nox2^{wt}$ $PM_{2.5}$**	**$Nox2^{-/-}$ FA**	**$Nox2^{-/-}$ $PM_{2.5}$**
Max. Constrict. To PE	102.4 ± 6.2	163.5 ± 6.4 *	118.3 ± 3.9	121.8 ± 5.4
logEC50	-6.4 ± 0.1	-6.5 ± 0.1	-6.9 ± 0.1	-6.9 ± 0.1
Max. Relax. To Ach	-30.8 ± 3.1	-23.5 ± 1.8	-33.1 ± 5.7	-24.8 ± 6.7
logEC50	-6.5 ± 0.2	-6.4 ± 0.1	-5.9 ± 0.3	-5.9 ± 0.5

(*$p<0.01$ vs same group FA)

Supplementary Table IV

Flow cytometry results – homing signals in c-*fms*^{YFP} mice

	YFP$^+$	**YFP$^+$CCR2$^+$**	**YFP$^+$CCR5$^+$**	**YFP$^+$CXCR3$^+$**
BM FA	74 ± 4.5	56.1 ± 3.7	8.5 ± 0.9	23.5 ± 2.5
BM $PM_{2.5}$	70.5 ± 6.2	63.5 ± 0.2 *	10.2 ± 0.4	23.8 ± 3.5
Spleen FA	34.8 ± 4.7	22.3 ± 4	8.3 ± 0.9	14 ± 0.3
Spleen $PM_{2.5}$	38.6 ± 5.7	26.8 ± 2.9	8.5 ± 1.8	15.6 ± 2.3

(*$p<0.05$ vs same group FA; n=3-4)

Supplementary Table V

	TLR4^{wt} FA	**TLR4^{wt} $PM_{2.5}$**	**TLR4^{d} FA**	**TLR4^{d} $PM_{2.5}$**
Cholesterol [mg/dl]	114.5 ± 9.4	113.3 ± 12.9	118.8 ± 23.4	107.3 ± 9.5
Triglyceride [mg/dl]	115.8 ± 60.5	119.8 ± 76.8	101.3 ± 13.8	122.3 ± 53.5

(n=3-4)