SUPPLEMENTAL MATERIAL

Cai et al.
MerTK cleavage promotes plaque necrosis and defective resolution in atherosclerosis

METHODS

Study approval. For the study examining the relationship between sol-Mer and plaque necrosis, de-identified human carotid endarterectomy (CEA) specimens were provided by Dr. M.J.A.P. Daemen from the Pathology Tissue Collection (PTC) at Academic Medical Center, Amsterdam and were used in agreement with the "Code for Proper Secondary Use of Human Tissue in the Netherlands" (http://www.fmwv.nl). For the study examining asymptomatic versus symptomatic patients, de-identified CEA specimens were provided by Dr. Bernhard Dorweiler at the Division of Vascular Surgery, University Medical Center, Johannes-Gutenberg University, Mainz, Germany, conformed with the declaration of Helsinki and were approved for use by the university ethics review board. Informed consent was obtained from each patient. All animals were cared for according to NIH and IACUC guidelines in a barrier facility at Columbia University Medical Center, New York.

Human carotid plaque studies. For the study examining the relationship between sol-Mer with plaque necrosis, alternate carotid endarterectomy lesional cross sections were flash-frozen and extracts prepared. To detect sol-Mer, the extracts were subjected to SDS-PAGE and then immunoblotted using a monoclonal antibody (R&D systems, MAB8912) specific for the ectodomain of human MerTK. Confirming previous results using cultured macrophages (1), immunoblot of N-glycanase-treated plaque extract showed a compact band that migrated at ~60 kDa, the predicted molecular weight of

non-glycosylated sol-Mer ectodomain. Equal protein amounts were loaded per lane to normalize samples. Sol-Mer was quantified by densitometric ratio analysis using b-actin (Cell Signaling Technology, 5125) as the loading control. Flanking sections were fixed and embedded for histologic morphometric analysis. Necrotic areas were defined as acellular areas in the intima beneath the fibrous cap and quantified using ImageJ as percent necrosis based on total lesion area. For the study examining asymptomatic versus symptomatic patients, specimens were classified as either asymptomatic or symptomatic, *i.e.*, transient ischemic attack or stroke within the past 6 months, as determined by preoperative assessment by a consultant neurologist. After retrieval in the operating room the excised plaques were rinsed in physiologic saline, immediately snap frozen in liquid nitrogen, and subsequently transferred to -80^{o}C for storage. Additionally, 5 ml of heparinized blood were obtained from each patient at the time of surgery, and the plasma was stored at -20^{o}C. To assay sol-Mer in these samples, the specimens were cut into small pieces, homogenized, and lysed in 4x Laemmli sample buffer. Protein concentration was assayed by a reducing agent compatible protein assay kit (Thermo Scientific, Prod#23250). Equal protein was loaded on SDS-PAGE gel, and sol-Mer was detected by immunoblotting.

Animals and diets. C57BL/6J (stock #000664) and *Ldlr*$^{-/-}$ (stock #002207) mice on the C57BL/6J background were purchased from Jackson Laboratory. *Mertk*^{CR} mice on the C57BL6/J were previously described (2). For the atherosclerosis studies, bone marrow cells from *Mertk*^{CR} mice and age-matched and sex-matched wild-type (WT) littermate control mice were transplanted into *Ldlr*$^{-/-}$ male mice as previously described (3, 4). Briefly, *Ldlr*$^{-/-}$ mice were irradiated using a cesium gamma source at a dose of

1000 rads. After 5 h, bone marrow from the femurs of either WT or *Mertk*^{CR} mice was injected i.v. into irradiated *Ldlr*$^{-/-}$ mice using 5 x 10^6 cells per mouse. Six weeks after bone marrow transplantation, the mice were started on a Western-type diet (WD; Harlan Teklad, TD88137) and continued for 16 weeks to allow the development of advanced atherosclerosis.

Atherosclerotic lesional analyses. After 16 weeks of WD feeding, mice were fasted overnight and then sacrificed using isoflurane. Body weights were measured and blood was collected by cardiac puncture. The heart, with aortic root attached, was harvested, embedded in OCT media and frozen on dry ice. Sections of the aortic root were prepared using a cryomicrotome and then stained with hematoxylin and eosin (H&E). Six sections per mouse, spaced 30 μm apart starting from the base of the aortic root, were quantified for total lesion area and necrotic area as described previously (5). Briefly, the intimal region containing lesions is demarcated and quantified using ImagePro Plus by a person blinded to the experimental groups. Acellular areas that were ≥ 3000 mm^2 were marked and quantified as necrotic areas. To assess lesional cap thickness, sections were stained with collagen using picrosirius red (PolySciences, #24901) as per the manufacturer's instructions. As described previously (4), fibrous cap thickness was assessed by averaging the thickness in micrometers of the cap in three separate regions of the lesions, *i.e.*, one measurement for each of the shoulder regions and one measurement in the middle of the lesion, and then expressed as arbitrary unit (AU) of cap thickness/ lesion area. For lesional *Col1a1* mRNA assay, lesional cross sections were extracted with RLT lysis buffer (Qiagen) to obtain RNA, which was then quantified for *Col1a1* mRNA using RT-qPCR with the following primers: forward 5'-

GCTCCTCTTAGGGGCCACT-3', reverse 5'-CCACGTCTCACCATTGGGG-3'. Plasma cholesterol and triglycerides were measured using the Cholesterol E kit (Wako, 439-17501) and L-type Triglyceride M kit (Wako, 465-09791 and 461-09891) respectively. Fasting blood glucose was measured using glucose test strips and a glucometer.

In vitro efferocytosis assay. Bone marrow-derived macrophages were incubated with PKH green-labeled apoptotic Jurkat cells were added to the macrophages at a ratio of 3:1 or 5:1 (apoptotic cells:macrophages) for 45 mins and then fixed and analyzed by fluorescence microscopy. The percentage of total BMDMs with internalized labeled apoptotic cells (efferocytosis) was quantified by analysis of fluorescence microscopy images. In Supplemental Figure 1E, some of the cells were co-incubated with recombinant mouse Mer-Fc chimera protein (rMer-Fc; R&D Systems #591-MR).

In situ efferocytosis assay. Following established procedures described previously (6), efferocytosis was determined *in situ* by counting the number of free versus macrophage-associated apoptotic cells in individual lesion sections. Frozen aortic root sections were fixed with cold acetone for 5 mins. After washing with PBS, sections were stained with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL, Roche, 12156792910) to identify apoptotic cells, followed by anti-F4/80 immunostaining to label lesional macrophages. Slides were then mounted with DAPI-containing mounting solution. As a measure of defective efferocytosis, we counted the number of free versus macrophage-associated apoptotic cells in individual lesional sections. *Free* apoptotic cells were defined as $TUNEL^+$ cells that exhibited nuclear condensation, loss of antibody F4/80 reactivity, and were not in contact with neighboring macrophages.

Macrophage-*associated* apoptotic cells were defined as cells with TUNEL+ nuclei surrounded by or in contact with neighboring F4/80^{+} macrophages.

Mouse aortic sol-Mer assay. After 16 weeks of WD feeding, mice were sacrificed, and aortic arch, brachiocephalic artery (BCA), descending aorta up to the bifurcation of the renal arteries (referred to as "aorta") were harvested and snap frozen in liquid nitrogen. Aortic tissue was then homogenized and lysed with 4x Laemmli sample buffer. The protein concentration was measured using a protein assay kit (Thermo Scientific, Prod#23250). Equal amounts of protein were subjected to SDS polyacrylamide gel electrophoresis and transferred to nitrocellulose. Sol-Mer was detected with a goat anti-MerTK antibody that is specific for the ectodomain of mouse MerTK (R&D system, AF591). For the quantification of sol-Mer released into macrophage culture medium, bone marrow-derived macrophages were incubated for 3 h in 500 µL of serum-free medium containing vehicle or 50 mg/mL oxLDL (Alfa Aesar, J65591). The media were collected, concentrated 10-fold with ultracentrifugal filters (10,000 molecular weight cut-off; Amicon, UFC501096), lysed with 4x Laemmli sample buffer, and subjected to SDS polyacrylamide gel electrophoresis and immunoblot analysis.

Assay of sol-Mer in the media of cultured macrophages. Bone marrow-derived macrophages were resuspended in 500 ml of serum-free media and then treated as indicated in the figure legends. The media were collected, concentrated 10-fold using ultracentrifugal filters (10,000 Da molecular weight cut-off; Amicon, UFC501096), lysed with 4× Laemmli sample buffer, and subjected to SDS polyacrylamide gel electrophoresis and immunoblot analysis.

Quantification of lesional MerTK by immunofluorescence microcopy. For analysis of MerTK in human carotid lesions, carotid specimens were fixed in 4% formalin overnight, decalcified in ethylenediaminetetraacetic acid buffer (EDTA, pH 7.2) for 4 days, and embedded in paraffin blocks. Sections were cut serially at 5-mm intervals and mounted on slides. Prior to staining, sections were deparaffinized in xylene and rehydrated in a graded series of ethanol. Sections were then placed in a pressure cooker for 10 mins in citrate-based solution (pH 6.0, Vector laboratories, H-3300) to expose antigens. After rinsing in phosphate buffered saline (PBS, pH 7.4) and blocking in serum-free protein blocking buffer (Dako, X0909), the sections were incubated with mouse anti-human MerTK (Abcam, ab52968) at 4^{o} C overnight, followed by incubation with Alexa fluor 594-conjugated anti-mouse secondary antibody (Thermo Scientific, A-11005) for 1 h. Slides were then mounted with DAPI-containing mounting solution (Thermo Scientific, P36935). Three imaging fields were obtained for each patient sample using a Nikon A1 confocal microscopy. In ImageJ, for each field of image, a threshold was set to reduce image background and the DAPI+ area was selected in which to measure the mean of intensity (MFI) of MerTK staining. Three fields per section for each patient were randomly chosen for imaging by confocal microscopy. In ImageJ, a threshold was set to reduce image background, using the same setting for all images. DAPI-positive areas were then analyzed for the mean of intensity (MFI) of MerTK staining within this area for each field. The MFI from the 3 fields was averaged for each patient. For mouse lesional MerTK staining, frozen sections were fixed in cold acetone for 5 mins. After washing in PBS, sections were incubated with biotin-blocking buffer (Thermo Scientific, E21390) to block endogenous biotin. Sections were then incubated with biotin-labeled anti-mouse

MerTK (R&D system, BAF591) at 4^{o} C overnight, followed by incubation with Alexa fluor 488-conjugated streptavidin (Thermo Scientific, S11223) for 1 h. Slides were mounted with DAPI-containing mounting solution. Images of every lesion for each mouse were analyzed. Image background was reduced using the same threshold setting for each image, and then the images were analyzed the MFI of MerTK in DAPI+ areas for each lesion. The MFI of all the lesions for each mouse was averaged.

Quantification of lesional cells by immunofluorescence microcopy. The number of total cells ($DAPI^{+}$), macrophages ($F4/80^{+}$(Bio-Rad, MCA771G)), smooth muscle cells (SMC, α-$actin^{+}$(Sigma, C6198)) and dendritic cells (DC, MHC-$II^{+}CD11c^{+}$(MHC- II: eBioscience, 13-5321-81; CD11c:BD Bioscience, 561044)) were quantified in immunostained aortic root section images using immunofluorescence microcopy followed by analysis using ImageJ software. The percentage of lesional cell subtypes was defined as the number of cells positive for the individual marker relative to the total number of $DAPI^{+}$ cells within the lesion. In separate sections, the number of total T cells ($CD3^{+}$(Abcam, ab16669)), $CD4^{+}$T cells ($CD3^{+}$ $CD4^{+}$(CD4: eBioscience, 14-0041-82)) and Treg cells ($Foxp3^{+}CD3^{+}$(Foxp3: eBioscience, 14-5773-82)) were counted. These were quantified as the number of positive cells relative to total cells per section including adventitia.

Identification and quantification of lipid mediators by LC-MS/MS. Aortas were collected, immediately placed in cold methanol containing deuterium-labeled standards (d_5-RvD2, d_5-LXA_4, d_4-LTB_4, d_5-LTC_4, d_5-LTD_4, d_4-PGE_2, d_8-5-HETE), and subjected to solid-phase (C_{18}) extraction as described previously (7). Methyl formate and methanol fractions were collected, the solvents were evaporated with N_2 gas, and samples were

resuspended in methanol:water (50:50) immediately prior to automated injection on a HPLC (Shimadzu) coupled to a Qtrap5500 mass spectrometer (AB Sciex). The instrument was operated in positive ion mode (cysteinyl leukotrienes) or negative ion mode (all others) and lipid mediators were identified using established scheduled multiple reaction monitoring (MRM) transitions (8). Quantification was carried out based on recovery of internal deuterium labeled standards and external calibration curves for each individual lipid mediator using authentic standards (Cayman Chemical). To generate an index of the SPM, we combined RvD1-6, 17*R*-RvD1, Mar1, Mar2, PD1, 10*S*,17*S*-diHDHA, 15*R*-LXA_4, LXB_4, RvE2 and RvE3 (see Figure 3D), while the total 5-LOX-derived SPMs displayed in Figure 3E include RvD1-6, 17*R*-RvD1**,** LXB_4, 15*R*-LXA_4, and RvE2, divided by all leukotrienes (LTB_4, LTC_4, LTD_4, LTE_4). Values for each individual lipid mediator are given in Table 1.

RvD1 treatment of Ldlr$^{-/-}$ mice. 8-10 week-old male *Ldlr$^{-/-}$* mice were placed on the WD for a total of 17 weeks. At the 12-week time point, the mice were injected i.p. 3 times per week with 500 ml of sterile PBS (vehicle control) or RvD1 (100 ng/mouse, Cayman Chemical, 10012554) for an additional 5 weeks while still on the WD. Cross-sections of murine aortic root lesion were stained with a biotinylated anti-MerTK (R&D system, BAF591) antibody at 4^{o}C overnight. Slides were gently washed with PBS, and a streptavidin secondary antibody was then added to the slides for 2 h at room temperature. The sections were counterstained with Hoechst to identify nuclei, viewed on a Nikon A1 confocal microscope, and analyzed using ImageJ software.

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Supplemental Table 1 SPMs and pro-inflammatory lipid mediators in aortic extracts from WT ® *Ldlr*$^{-/-}$ and *Mertk*^{CR} ® *Ldlr*$^{-/-}$ BMT mice.

Compound	WT à *Ldlr*$^{-/-}$ (pg/mg)	SEM (±)	*n*	Mertk^{CR} à *Ldlr*$^{-/-}$ (pg/mg)	SEM (+/-)	*n*	*P*
DHA Metabolome							
RvD1	43.8	8.7	5	65.1	19.6	8	0.431
17*R*-RvD1	292.2	63.5	8	690.8	168.9	9	0.053
RvD2	71.8	19.5	7	186.5	37.1	8	0.021
RvD3	3.2	0.5	2	12.5	3.3	6	0.181
17*R*-RvD3	-	-	-	-	-	-	-
RvD5	8.3	1.8	8	3.6	0.6	9	0.018
RvD6	2.4	0.6	8	4.3	0.7	9	0.056
Mar1	58.7	11.9	8	58.6	7.6	9	0.994
Mar2	22.9	13.7	7	58.0	27.4	8	0.294
PD1	160.3	32.3	7	247.9	28.6	9	0.062
17*R*-PD1	-	-	-	-	-	-	-
10*S*,17*S*-diHDHA	9.2	-	1	3.7	0.8	7	-
21-HDHA	0.3	0.1	8	0.1	0.0	9	0.005
17-HDHA	41.1	10.7	8	32.1	4.3	9	0.429
14-HDHA	222.9	48.7	8	183.4	23.7	9	0.461
13-HDHA	35.9	5.5	8	32.1	4.2	9	0.589
7-HDHA	11.1	1.9	8	12.9	2.0	9	0.522
4-HDHA	26.6	4.5	8	23.6	3.2	9	0.582
AA Metabolome							
LXA_4	-	-	-	-	-	-	-

15*R*-LXA_4	12.8	2.6	8	15.1	1.7	9	0.469
LXB_4	23.9	4.7	8	22.7	3.3	9	0.841
15*R*-LXB_4	-	-	-	-	-	-	-
LTB_4	163.5	26.6	8	203.0	34.8	9	0.390
6-*trans* LTB_4	79.5	10.2	8	130.9	17.7	9	0.028
6-*trans*,12-*epi* LTB_4	69.4	13.5	8	119.6	20.1	9	0.062
LTC_4	9.1	2.1	7	4.8	1.0	9	0.070
LTD_4	3.5	1.0	7	2.9	0.6	9	0.659
LTE_4	2.2	0.7	7	1.7	0.5	9	0.586
PGE_2	671.0	137.2	8	803.1	129.5	9	0.495
PGD_2	565.4	90.6	8	629.5	67.9	9	0.574
$PGF_{2\alpha}$	168.9	33.9	8	165.1	22.2	9	0.924
TxB_2	1009.7	150.1	8	858.3	93.7	9	0.394
5*S*,6*R*-diHETE	54.8	10.4	8	68.6	12.3	9	0.411
5*S*,15*S*-diHETE	81.5	15.1	8	90.2	15.8	9	0.700
15-HETE	466.0	84.8	8	433.5	50.6	9	0.740
12-HETE	5213.5	825.5	8	3722.8	461.3	9	0.125
11-HETE	587.1	93.9	8	589.6	72.7	9	0.983
5-HETE	77.5	15.1	8	69.9	9.8	9	0.672
12-HHT	278.9	48.1	8	267.0	27.7	9	0.829
EPA Metabolome							
RvE1	-	-	-	-	-	-	-
RvE2	303.8	62.6	8	412.7	38.6	9	0.149
5*S*,15*S*-diHEPE	21.7	4.5	8	16.6	1.4	8	0.299
RvE3	298.2	50.6	8	504.6	72.4	9	0.038
LXA_5	-	-	-	-	-	-	-
LXB_5	-	-	-	-	-	-	-

18-HEPE	12.2	2.1	8	11.5	1.4	9	0.794
15-HEPE	29.2	7.8	8	29.1	3.9	9	0.986
12-HEPE	1019.3	164.6	8	734.8	92.0	9	0.141
11-HEPE	24.9	3.9	8	24.3	2.7	9	0.909
5-HEPE	9.4	1.4	8	9.8	1.1	9	0.799

Compounds are displayed as pg/mg of aortic protein and expressed as mean ± SEM. The limit of detection is ~ 0.1 pg; values below this limit are indicated by "–".

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Analyses of sol-Mer and macrophage content of human carotid endarterectomy specimens. (**A**) Representative sol-Mer immunoblots and H&E-stained cross-sections of the human carotid endarterectomy specimens from 5 of the patients (P) analyzed in Figure 1A. NC, necrotic core (bar, 80 μm). The sol-Mer:b-actin densitometric ratio and % lesional necrosis are shown below each lane of the immunoblot. (**B**) Sol-Mer in plasma from asymptomatic and symptomatic patients was assayed by ELISA (n=6-8 per group. *P<0.05). (**C**) Bone marrow-derived macrophages were incubated for 45 min with PKH green-labeled apoptotic Jurkat T cells at a ratio of 1:5 in the presence of increasing doses of rMer-Fc. The percent of macrophages with internalized labeled apoptotic cells (efferocytosis) was quantified by analysis of fluorescence microscopy images (n=3 for each treatment, *, P<0.05, **P<0.01). (**D**) CD68+ macrophages were quantified in lesional sections from the asymptomatic and symptomatic subjects described in Figure 1B-C (n=6-8 per group). n.s, no significance. (**B-D**) Two-tailed Student's t test was used to evaluate significance.

Supplemental Figure 2. OxLDL- induced MerTK cleavage depends on ADAM17 activity and is absent in *Mertk*^{CR} macrophages. (A) Bone marrow-derived macrophages (BMDMs) were pre-treated with 10 mm TAPI-0 for 30 mins after which either vehicle control or 50 mg/ml oxLDL was added to the medium. After 3 h, sol-Mer from culture media and β-actin from cell lysates were detected by immunoblot. (**B**) Either WT or *Mertk*^{CR} BMDMs were treated with oxLDL for 3 h, followed by immunoblot of sol-Mer and β-actin. (**C**) After oxLDL treatment, MerTK MFI on BMDMs was

quantified by immunofluorescence microscopy (n=3 replicates for each treatment, *P<0.05) (**D**) BMDMs were incubated with 50 mg/ml oxLDL. After 3 h, PKH-green-labeled apoptotic Jurkat cells (AC) were added to the macrophages at a ratio of 3:1 (apoptotic cells:macrophages) for 45 mins. The percent of total BMDMs with internalized labeled apoptotic cells (efferocytosis) was quantified by analysis of fluorescence microscopy images (n=3 replicates for each treatment. *P<0.05). (**C-D**) Two-tailed Student's t test was used to evaluate significance.

Supplemental Figure 3. Body weight, blood glucose and plasma lipids are similar between 16-wk WD-fed WT ® *Ldlr*$^{-/-}$ and *Mertk*^{CR} ® *Ldlr*$^{-/-}$ mice. (**A-D**) Body weight, blood glucose after 5-h food withdrawal, and plasma cholesterol and triglycerides after an overnight fast in 16-week WD-fed WT ® *Ldlr*$^{-/-}$ or *Mertk*^{CR} ® *Ldlr*$^{-/-}$ mice (n=10 per group). n.s, no significance. Two-tailed Student's t test was used to evaluate significance.

Supplemental Figure 4. Blood leukocytes numbers are similar between 16-wk WD-fed WT ® *Ldlr*$^{-/-}$ and *Mertk*^{CR} ® *Ldlr*$^{-/-}$ mice. (A-D) Circulating total white blood cells, neutrophils, monocytes, and platelets in 16-week WD-fed WT ® *Ldlr*$^{-/-}$ or *Mertk*^{CR} ® *Ldlr*$^{-/-}$ mice (n=10 per group). n.s, no significance. Two-tailed Student's t test was used to evaluate significance.

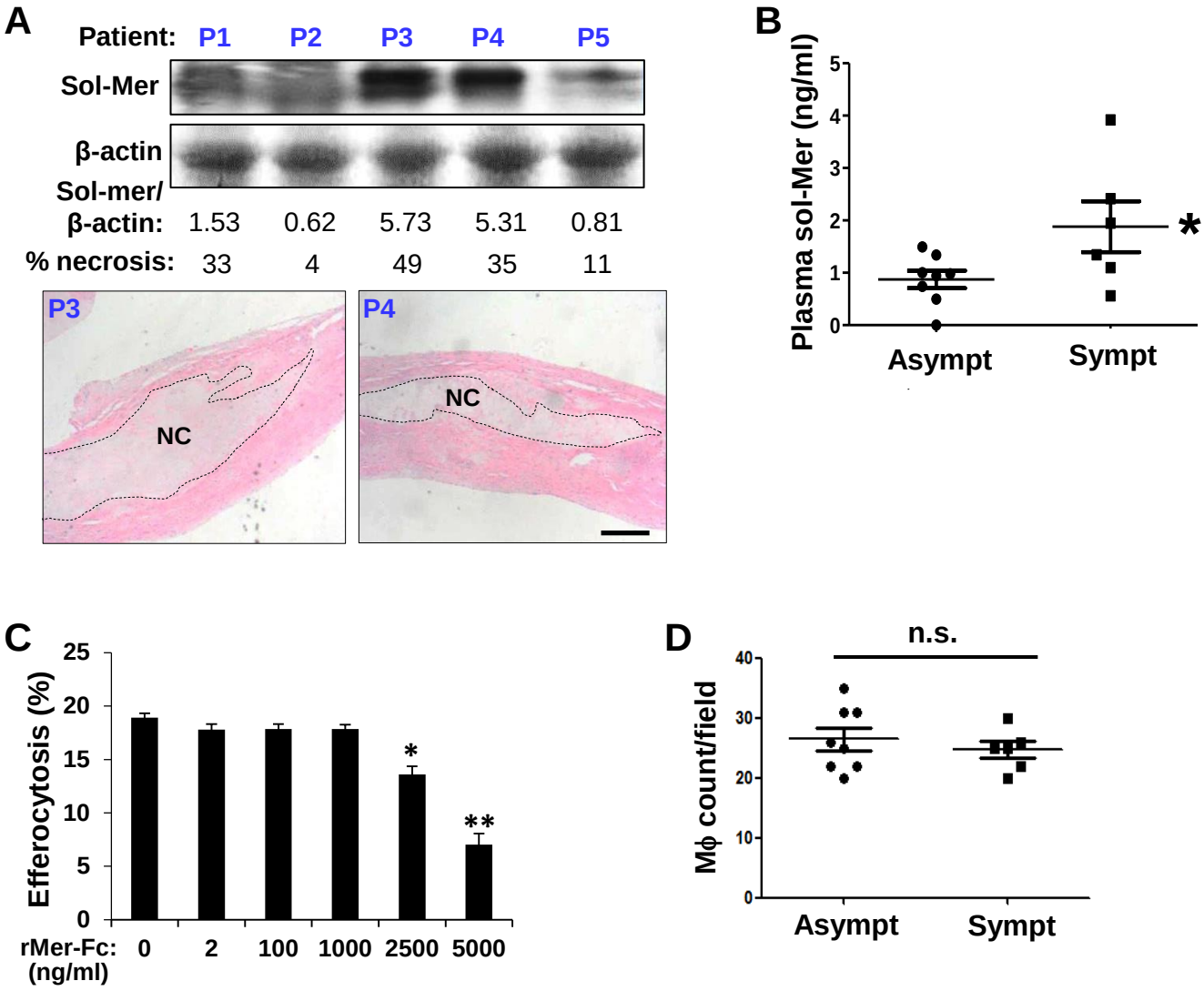
Supplemental Figure 5. Analysis of total lesion area and lesional cell populations in 16-wk WD-fed BMT mice. (**A-B**) Aortic root sections from 16-week WD-fed WT ® *Ldlr*$^{-/-}$ or *Mertk*^{CR} ® *Ldlr*$^{-/-}$ mice were quantified for total lesion area and total cell number (DAPI^{+} cells) (n=10 per group). n.s, no significance. (**C**) The sections were probed for

the indicated cell types using immunofluorescence microscopy, and cell numbers per total lesional cells, per total cells in aortic root cross sections, or as a ratio were quantified using ImageJ. The antibodies used were against F4/80 and CD206 (macrophages), α actin (smooth muscle cells), CD3 (total T cells), CD4 ($CD4^+$ T cells), and MHC-II and CD11c (dendritic cells; DC). n=10 per group. n.s, no significance. (A-C) Two-tailed Student's t test was used to evaluate significance.

Supplemental Figure 6. Plasma TGFβ1, IL10 and FPR2 expression in lesional cells are increased 16-wk WD-fed *Mertk*^{CR} ® *Ldlr*$^{-/-}$ mice. Wild-type (WT) ® *Ldlr*$^{-/-}$ or *Mertk*^{CR} ® *Ldlr*$^{-/-}$ BMT mice were placed on the WD for 16 wks. (**A**) Aortic root sections were stained with picrosirius red. Images illustrate the strategy used to measure cap thickness (bar, 40 μm). (**B**) Plasma TGFβ1 and IL-10 were assayed by ELISA (n=8-9 for per group.*, P<0.05). (**C**) Aortic root lesions were immunostained for ALX/FPR2 (red) and counterstained with the nuclear dye DAPI (blue) (bar, 30 mm). The ALX/FPR2 mean fluorescence intensity (MFI) per lesion section was quantified by image analysis of cross-sections and expressed relative to the WT value (n=8-9 per group. *P<0.05). (B-C) Two-tailed Student's t test was used to evaluate significance.

Supplemental Figure 7. RvD1 prevents LPS-induced MerTK cleavage and increases MerTK intensity in atherosclerotic lesions. (**A-B**) Macrophages were incubated with 10 nM RvD1 for 5 h and then with 50 ng/mL LPS for 2 h. The media were assayed for sol-Mer by immunoblot. The attached cells were immunostained for MerTK under non-permeabilizing conditions, and then cell-surface MerTK was quantified by fluorescence microscopy (n=3 replicates for each treatment, **P<0.01). (**C**)

Ldlr$^{-/-}$ mice were fed the WD for 12 wks and then treated with control (Veh) or 100 ng RvD1/mouse (3x/wk i.p.) for 5 additional wks, with the mice continued on WD. Aortic root lesions were immunostained for MerTK (red) and counterstained with the nuclear dye DAPI (blue) (bar, 50 mm). The MerTK mean fluorescence intensity (MFI) per lesion section was quantified by image analysis of cross-sections and expressed relative to the Veh value (n=8-10 per group). *P<0.05 (B-C) Two-tailed Student's t test was used to evaluate significance.



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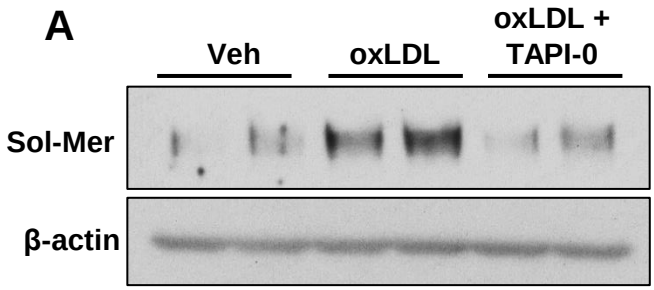
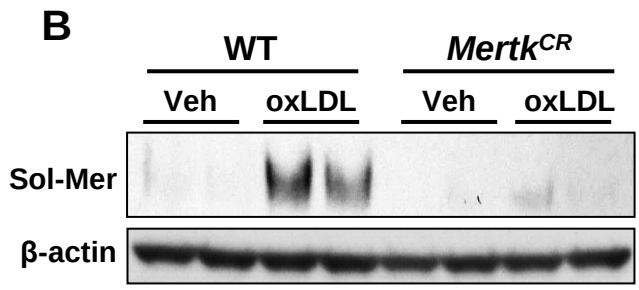
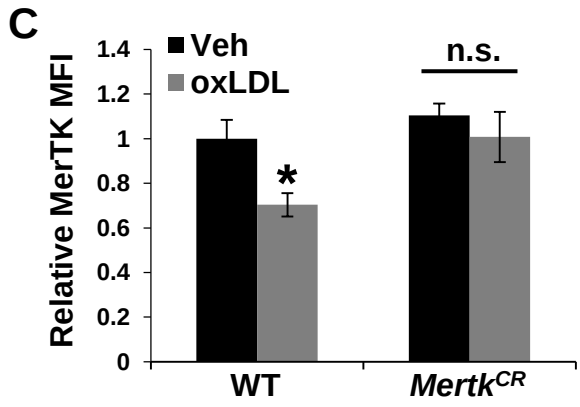
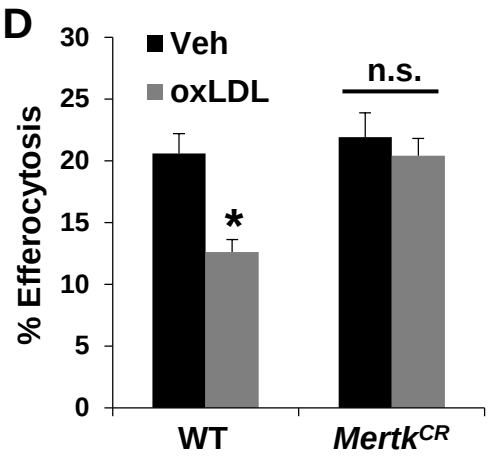
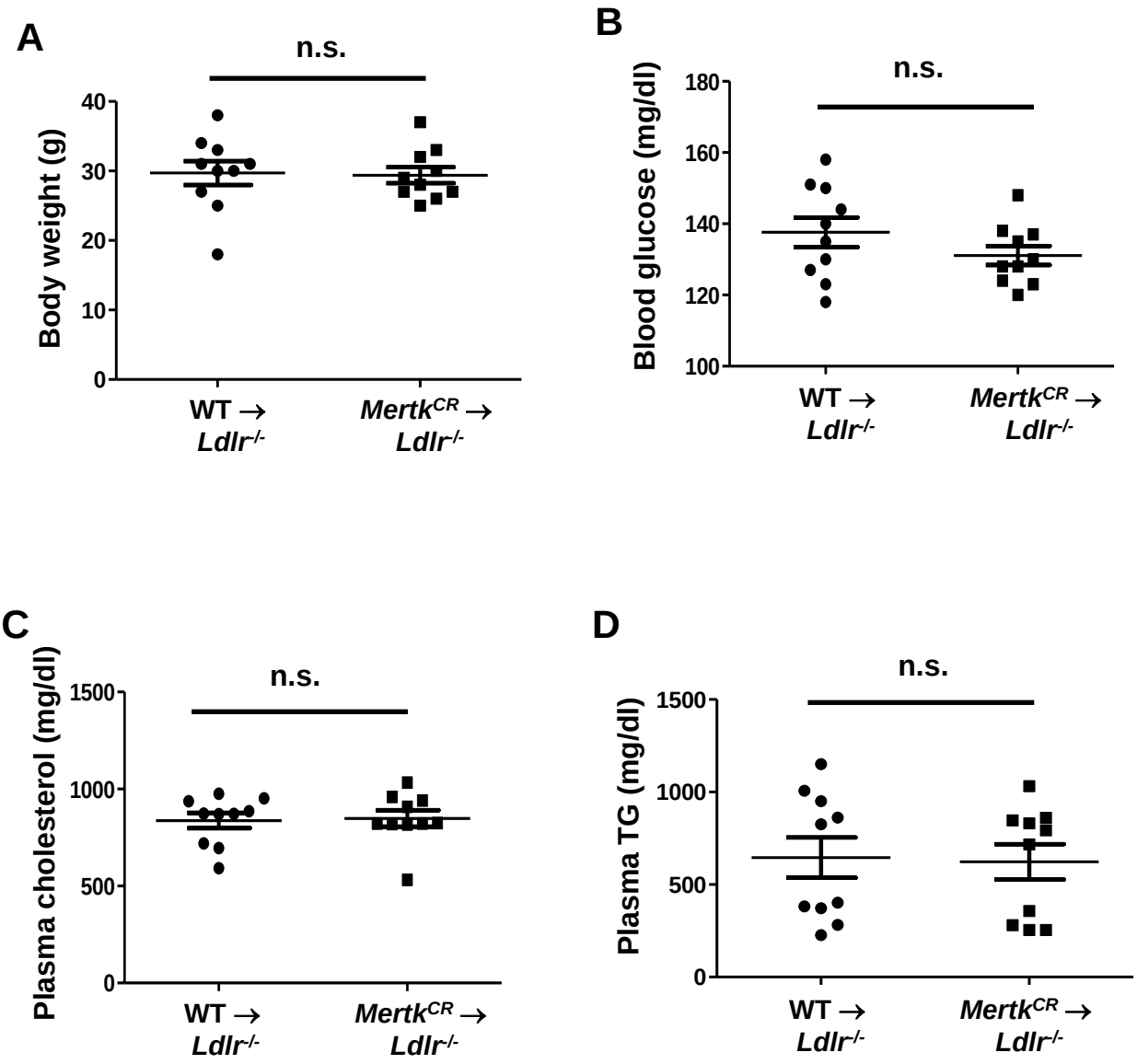




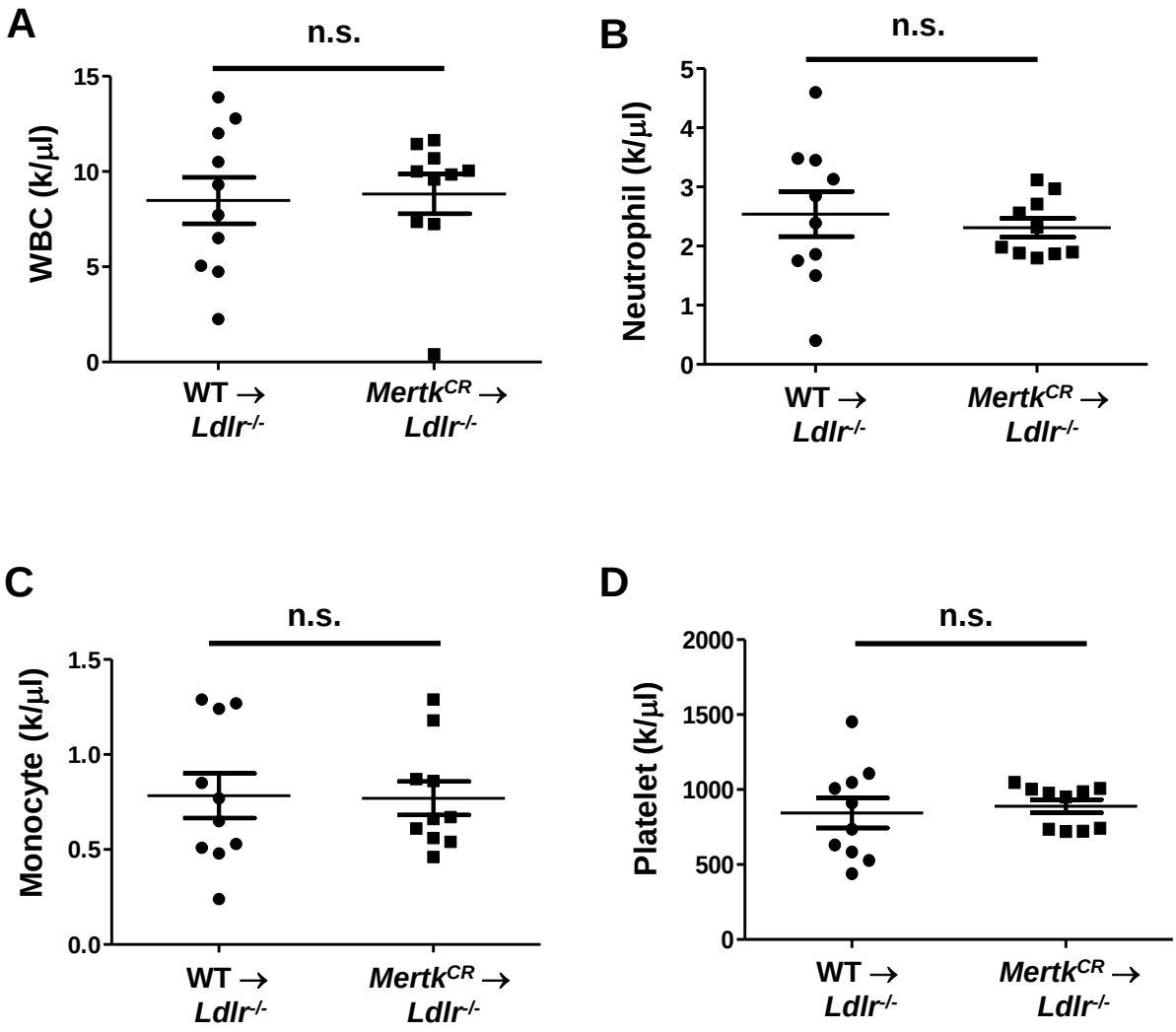




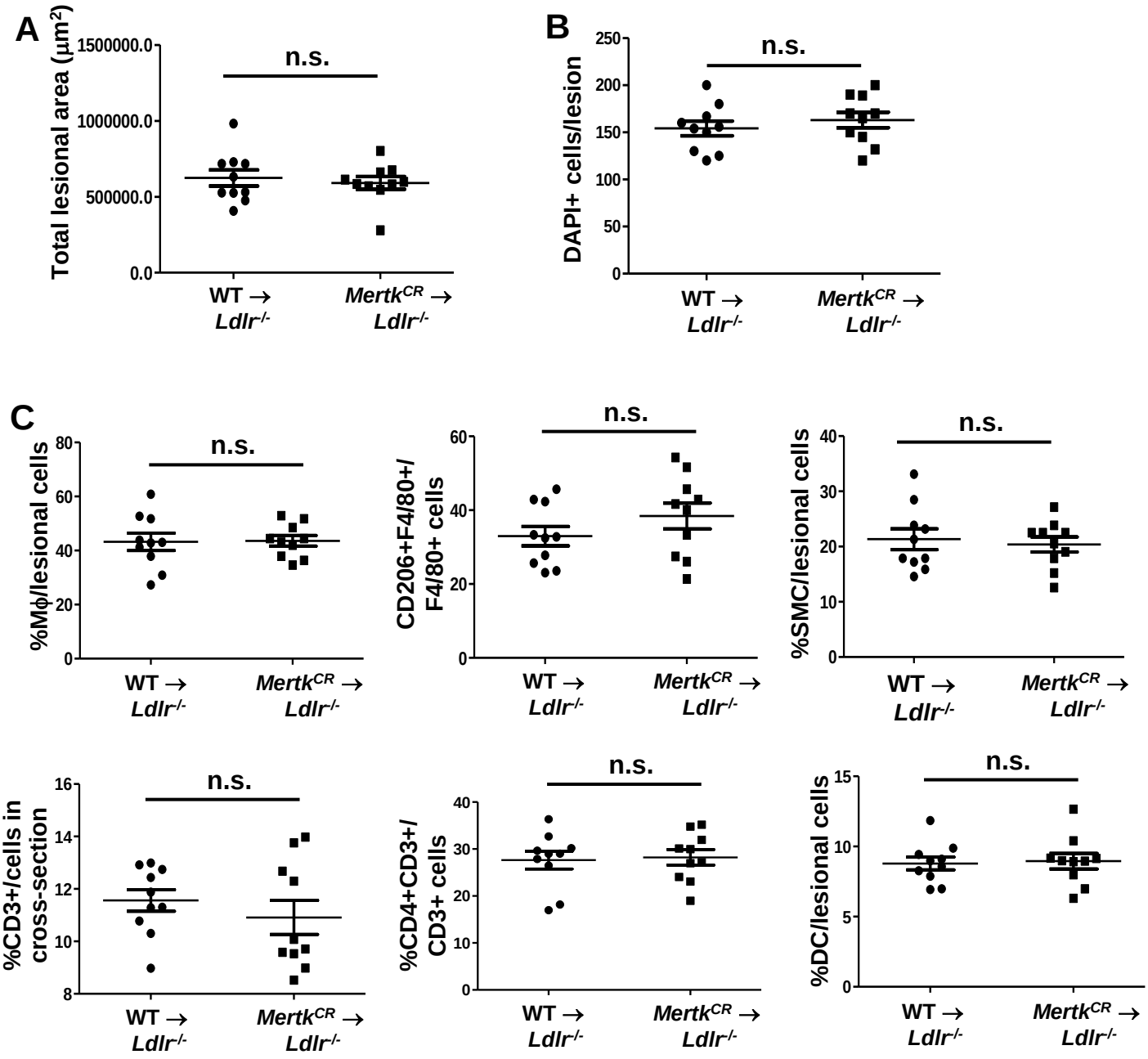
Supplemental Figure 2. OxLDL- induced MerTK cleavage depends on ADAM17 activity and is absent in *Mertk^{CR}* macrophages. (**A**) Bone marrow-derived macrophages (BMDMs) were pre-treated with 10 μm TAPI-0 for 30 mins after which either vehicle control or 50 μg/ml oxLDL was added to the medium. After 3 h, sol-Mer from culture media and β-actin from cell lysates were detected by immunoblot. (**B**) Either WT or *Mertk^{CR}* BMDMs were treated with oxLDL for 3 h, followed by immunoblot of sol-Mer and β-actin. (**C**) After oxLDL treatment, MerTK MFI on BMDMs was quantified by immunofluorescence microscopy (n=3 replicates for each treatment, *P<0.05) (**D**) BMDMs were incubated with 50 μg/ml oxLDL. After 3 h, PKH-green-labeled apoptotic Jurkat cells (AC) were added to the macrophages at a ratio of 3:1 (apoptotic cells:macrophages) for 45 mins. The percent of total BMDMs with internalized labeled apoptotic cells (efferocytosis) was quantified by analysis of fluorescence microscopy images (n=3 replicates for each treatment. *P<0.05). (C-D) Two-tailed Student's t test was used to evaluate significance.



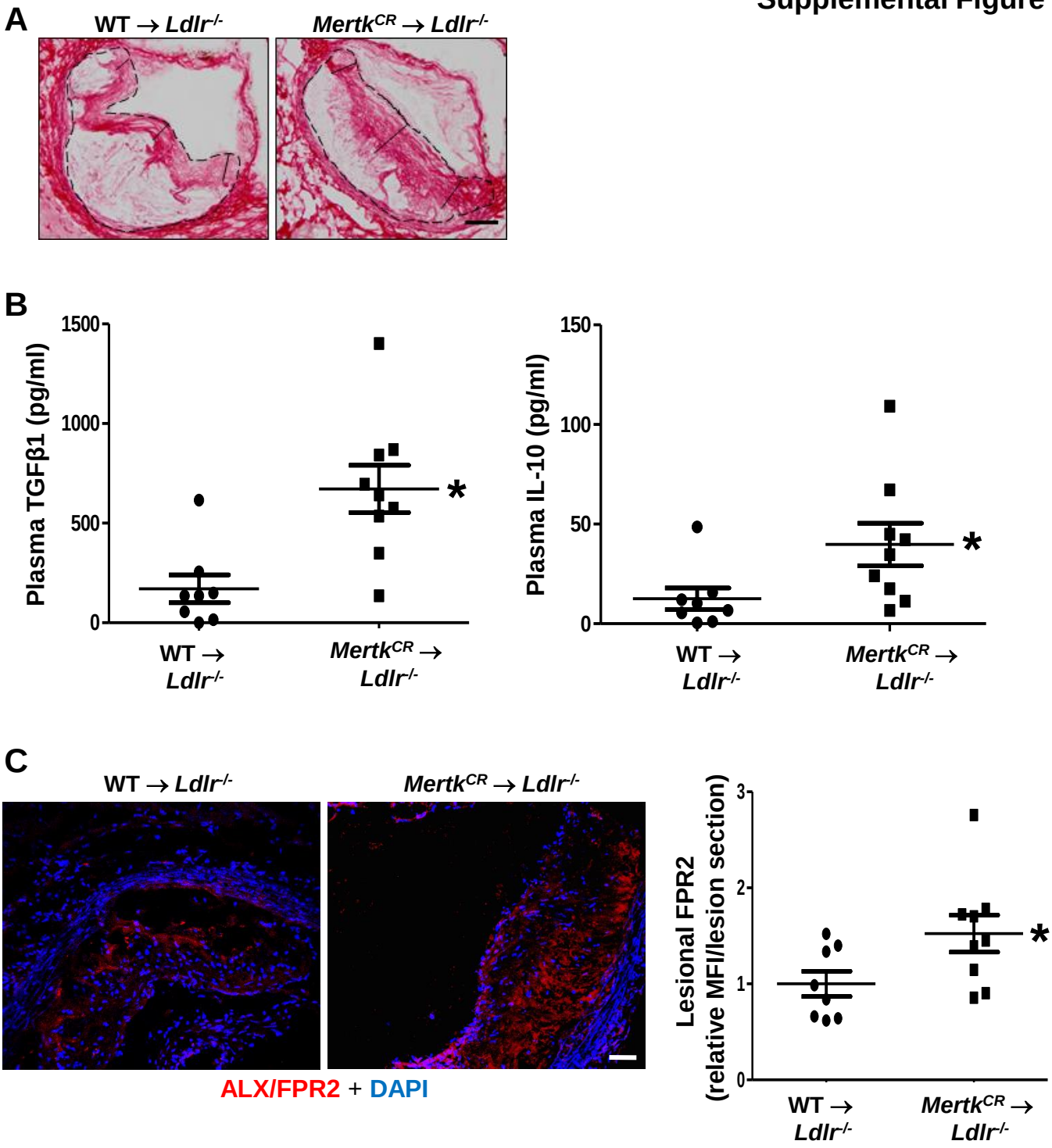
Supplemental Figure 3. Body weight, blood glucose and plasma lipids are similar between 16-wk WD-fed WT → *Ldlr*$^{-/-}$ and *Mertk*^{CR} → *Ldlr*$^{-/-}$ mice. (**A-D**) Body weight, blood glucose after 5-h food withdrawal, and plasma cholesterol and triglycerides after an overnight fast in 16-week WD-fed WT → *Ldlr*$^{-/-}$ or *Mertk*^{CR} → *Ldlr*$^{-/-}$ mice (n=10 per group). n.s, no significance. Two-tailed Student's t test was used to evaluate significance.



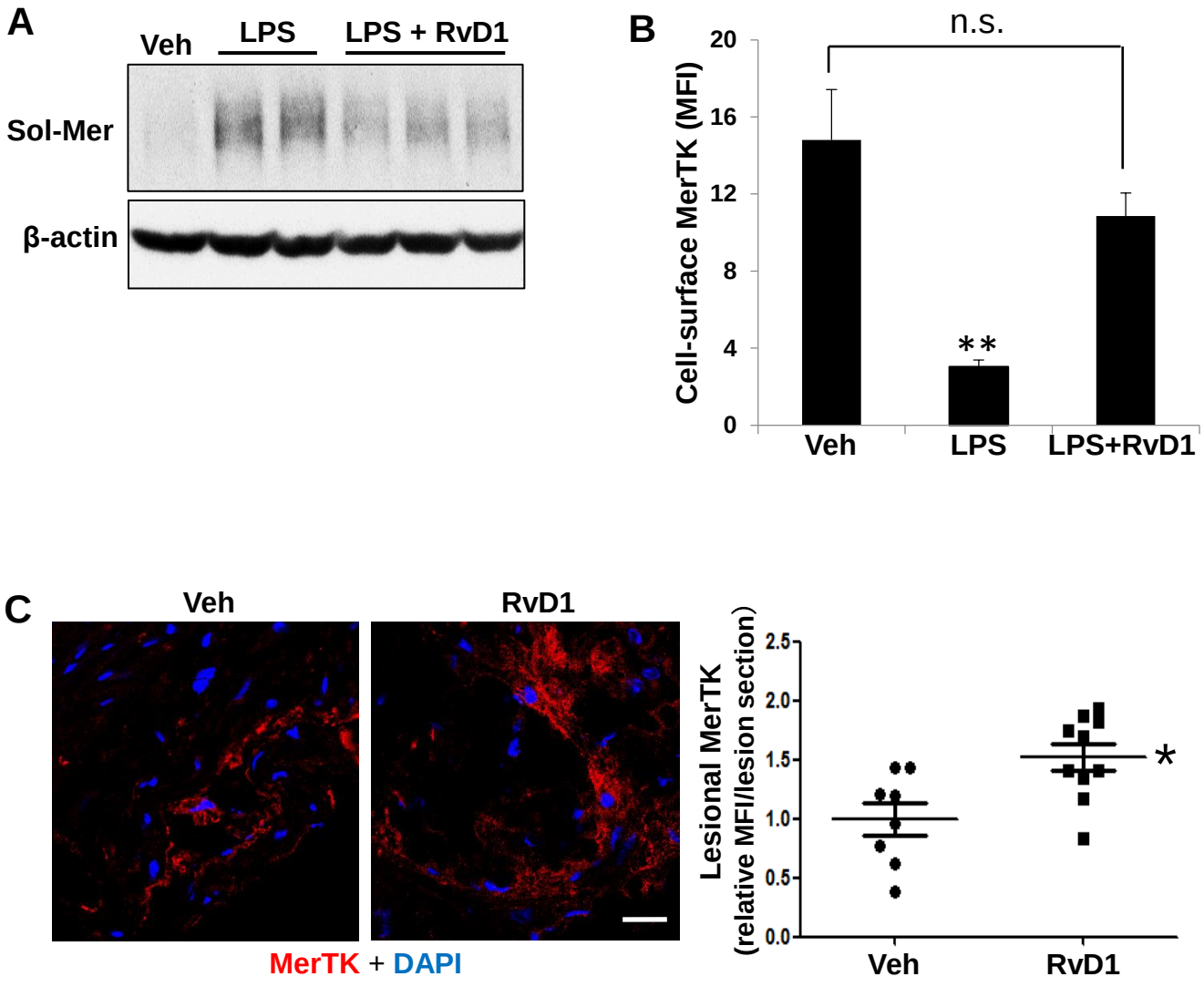
Supplemental Figure 4. Blood leukocytes numbers are similar between 16-wk WD-fed WT → *Ldlr*$^{-/-}$ and *Mertk*^{CR} → *Ldlr*$^{-/-}$ mice. (**A-D**) Circulating total white blood cells, neutrophils, monocytes, and platelets in 16-week WD-fed WT → *Ldlr*$^{-/-}$ or *Mertk*^{CR} → *Ldlr*$^{-/-}$ mice (n=10 per group). n.s, no significance. Two-tailed Student's t test was used to evaluate significance.



Supplemental Figure 5. Analysis of total lesion area and lesional cell populations in 16-wk WD-fed BMT mice. (**A-B**) Aortic root sections from 16-week WD-fed WT → *Ldlr*$^{-/-}$ or *Mertk*^{CR} → *Ldlr*$^{-/-}$ mice were quantified for total lesion area and total cell number (DAPI^{+} cells) (n=10 per group). n.s, no significance. (**C**) The sections were probed for the indicated cell types using immunofluorescence microscopy, and cell numbers per total lesional cells, per total cells in aortic root cross sections, or as a ratio were quantified using ImageJ. The antibodies used were against F4/80 and CD206 (macrophages), α actin (smooth muscle cells), CD3 (total T cells), CD4 (CD4^{+} T cells), and MHC-II and CD11c (dendritic cells; DC). n=10 per group. n.s, no significance. (A-C) Two-tailed Student's t test was used to evaluate significance.



Supplemental Figure 6. Plasma TGFβ1, IL10 and FPR2 expression in lesional cells are increased 16-wk WD-fed *Mertk^{CR} → Ldlr$^{-/-}$* mice. Wild-type (WT) → *Ldlr$^{-/-}$* or *Mertk^{CR} → Ldlr$^{-/-}$* BMT mice were placed on the WD for 16 wks. (**A**) Aortic root sections were stained with picrosirius red. Images illustrate the strategy used to measure cap thickness (bar, 40 μm). (**B**) Plasma TGFβ1 and IL-10 were assayed by ELISA (n=8-9 for per group.*, P<0.05). (**C**) Aortic root lesions were immunostained for ALX/FPR2 (red) and counterstained with the nuclear dye DAPI (blue) (bar, 30 μm). The ALX/FPR2 mean fluorescence intensity (MFI) per lesion section was quantified by image analysis of cross-sections and expressed relative to the WT value (n=8-9 per group. *P<0.05). (B-C) Two-tailed Student's t test was used to evaluate significance.



Supplemental Figure 7. RvD1 prevents LPS-induced MerTK cleavage and increases MerTK intensity in atherosclerotic lesions. (**A-B**) Macrophages were incubated with 10 nM RvD1 for 5 h and then with 50 ng/mL LPS for 2 h. The media were assayed for sol-Mer by immunoblot. The attached cells were immunostained for MerTK under non-permeabilizing conditions, and then cell-surface MerTK was quantified by fluorescence microscopy (n=3 replicates for each treatment, **P<0.01). (**C**) *Ldlr*$^{-/-}$ mice were fed the WD for 12 wks and then treated with control (Veh) or 100 ng RvD1/mouse (3x/wk i.p.) for 5 additional wks, with the mice continued on WD. Aortic root lesions were immunostained for MerTK (red) and counterstained with the nuclear dye DAPI (blue) (bar, 50 μm). The MerTK mean fluorescence intensity (MFI) per lesion section was quantified by image analysis of cross-sections and expressed relative to the Veh value (n=8-10 per group). *P<0.05 (B-C) Two-tailed Student's t test was used to evaluate significance.