Supplemental Materials

ADAR1 mediates macrophage activation by its non-editing activity-mediated pri-miRNA processing in abdominal aortic aneurysm

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DOI [to be added]

Materials and methods

Human Tissue Harvest

Human healthy abdominal aorta and AAA specimens were obtained from Mizzou OneHealth Biorepository and surgical operations of patients with abdominal aortic aneurysms in the Department of Surgery, School of Medicine, University of Missouri. All participants gave written informed consent before the specimens were collected. The patient information was de-identified and is included in Online Table I. All specimens were collected under a protocol approved by the Institutional Review Board of University of Missouri (IRB # 2026026). The aortic specimens were fixed overnight in formalin, embedded in paraffin, sectioned, and subsequently used for immunostaining as done in our previous publication [39].

Mice

Male ADAR1 deficient mice (B6.129(Cg)-Adartm1.1Phs/KnkMmjax), ADAR1fl/fl mice (B6.129-Adartm1Knk/Mmjax), LysM-cre mice (B6.129P2-Lyz2tm1(cre)Ifo/J), and ApoE($^{-/-}$) mice (B6;129-Apobtm2Sgy Apoetm1Unc/J) were purchased from the Jackson Laboratory (Bar Harbor, ME). We used only male mice in this study by following the ATVB Council's recommendation that identifying mechanisms of reduced AAA formation focus on males because ADAR1 deficiency reduces AAA formation[58]. All mice are in C57BL6 genetic background. Animals were housed under conventional conditions in animal care facilities and received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Missouri. Animals were randomly grouped, and all operators were blinded to the grouping. The number of animals (sample size) was determined by power calculation based on the prior experience.

Cytokines and reagents

Angiotensin II was purchased from Bachem Americas, Inc (Torrance, CA). The following antibodies used in Western bloting and immunofluorescent staining: ADAR1 (D-8) and RNase III Drosha (C-7) were obtained from Santa Cruz Biotechnology. Inducible nitric oxide synthase (iNOS) (4E5) and CD68 antibody (KP1) were purchased from Abcam. IL-1β (3A6), IκB kinase (IKK) (L570), NF-κB p65 (D14E12), phospho-NF-κB p65 (Ser536), DGCR8 (D78E4), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (IκB) (44D4) and phospho-IκB (Ser32) were from Cell Signaling Technology. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was from Proteintech. F4/80

(BM8) was from BioLegend. Nuclei were stained with 4, 6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Inc.). The secondary antibodies were from LI-COR Biosciences. M-CSF, IFNγ and TNF-α were purchased from R&D Systems and used at 10, 100 and 25 ng/mL respectively. LPS was obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA) and used at 100 ng/ml.

Angiotensin II (Ang II)-induced murine AAA model

Eight-week-old ApoE-/- (wild type or WT) and ADAR+/-;ApoE-/- with donor WT aorta transplantation, LysM-Cre+/-;ApoE-/- (WT) or ADAR1fl/fl;LysM-Cre+/-;ApoE-/- (ADAR1mφ-/-) mice were infused with phosphate buffered saline (PBS) or Ang II (1000 ng/kg/min) via osmotic minipumps (AP-2004, Alzet, CA, USA) for 28 days, as described previously [59]. Briefly, mice were anesthetized with inhaled isoflurane (5% for induction and 2% for maintenance), and the minipumps were surgically implanted into the subcutaneous space on the back of the neck of the mice. 28 days later, mice were anesthetized using 2.0% isoflurane, and hair was removed from the abdomen by using depilatory cream (Nair; Church & Dwight Co, Inc; Princeton, NJ). Mice were then laid supine on a heated table, and warmed ultrasound transmission gel was placed on the abdomen. Aortic diameters were measured using a doppler ultrasound Vevo 1100 Imaging System (VisualSonics) with a real-time microvisualization scan head in B mode. The B-Mode is a two-dimensional ultrasound image display composed of bright dots representing the ultrasound echoes. The brightness of each dot was determined by the amplitude of the returned echo signal. The abdominal aortas were then harvested for RNA, protein, and morphological or histological analyses. AAA incidence was defined by an increase of external aorta diameter by 50% or greater as compared to aortas from saline-infused mice.

Heterotopic allograft aortic transplantation

Aortic transplantation procedures were performed as described previously with modifications [60, 61]. Donor or recipient mice were anesthetized with inhaled isoflurane (5% for induction and 2% for maintenance). Carprofen was used for analgesia prior to the surgery and during the 72 hours post-operation. For donor mice, abdominal aorta from just below the left renal vein to the iliac bifurcation was identified, ligated, transected between the proximal and distal ligation, and stored in sterile saline containing heparin (100 U/mL) at 4 ^{o}C until transplantation. Recipient mice were anesthetized with inhaled isoflurane, laparotomy was performed, and the retroperitoneum exposed. The infrarenal aorta was dissected between the left renal artery and the iliac bifurcation. The aortic branches were exposed and ligated with 9-0 sutures, and the donor aorta was end-to-side anastomosed to the recipient aorta with interrupted 11-0 suture. After the distal anastomosis was completed, the distal ligature was removed, followed by removal of the proximal ligature. Fluid (1 ml of warm saline) was administered

to assure adequate volume resuscitation, and the laparotomy was closed with 4-0 Vicryl sutures. The skin incision was sealed with Vetbond tissue adhesive. One ml of warm saline was injected subcutaneously to maintain fluid homeostasis. After the surgery, the mice were kept on a Far Infrared Warming Pad (Kent Scientific) until fully recovered from anesthesia and monitored every two hours for the first day and then once daily. Two weeks after the operation, mice were infused with Ang II (1000 ng/kg/min) via osmotic minipumps (Alzet osmotic pump Model 2004, Durect Corporation) for 28 days. Animals were then anesthetized, and abdominal aorta ultrasound images were taken to measure the maximal aortic diameters followed by perfusion with PBS. The abdominal aortas were then harvested for RNA, protein, morphological or histological analyses.

Human internal mammary arteries (IMA) and peripheral blood monocytes (PBMCs).

Remnant segments of human IMAs and blood were obtained from the same patients (written informed consent) undergoing coronary artery bypass surgeries in the Department of Surgery, University of Missouri School of Medicine Hospital. Their use for research was approved by the Institutional Review Board (IRB) of the University of Missouri. To isolate human PBMCs, red blood cell lysis buffer was made by mixing together NH_4Cl (0.0155M), $KHCO_3$ (0.001M), EDTA (0.01mM) and sterilizing by passing through a 0.22 μm filter. The whole blood was mixed with the red blood cell lysis buffer at the ratio of 1: 10. The mixture was incubated at room temperature for 5 min and then spined down at 300 x g in order to remove the lysis buffer. After the PBMCs were washed twice with PBS, the cells were resuspended at a concentration of 1 x 10^7 cells/ml in a medium contained 95% FBS and 5% DMSO. PBMCs were aliquoted into cryogenic vials and stored in Cryo-Safe -1℃ (-33.8°F) freezing container which was placed in -80 ℃ freezer for 24 hours and then transferred into liquid nitrogen tank for future use. To knock down ADAR1 in PBMCs, the cells were transduced with adenoviral vector expressing either green fluorescent protein (Ad-GFP) or ADAR1 shRNA (Ad-shADAR1) by incubating the adenovirus with PBMCs in a 37℃, 5% CO2 incubator. The cells were cultured for 1 days in fresh medium and labeled with green live cell tracking dye (ab187967, Abcam). The ADAR1 knockdown efficiency was detected by western blotting to analyze ADAR1 levels. The labeled PBMCs were resuspended in 200μl sterile saline solution for injection.

Humanized abdominal aortic aneurysm model

NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice were purchased from Jackson Laboratory. Human artery–mouse chimeras were generated as published with modifications. Briefly, a segment of the abdominal aorta of NSG mouse was removed and replaced by the human remnant IMA via end-to-end anastomosis using 11-0 sutures. To preserve the arteries for transplantation, each IMA after taken

from patient was rinsed fully with saline and then placed immediately into a container with ice-cold sterile saline. The inner blood clot was flushed using saline contain 1% heparin. Branches, if any, in the IMA were ligated using 8-0 suture. The transplant surgery was performed within 1 hour after the IMA was removed from patient. Since the diameter of human IMA was two times larger than the mouse abdominal aorta, modified sleeve technique was applied to perform the end-to-end aortic transplantation. Specifically, recipient mouse was anesthetized with inhaled isoflurane (5% for induction and 2% for maintenance). The mouse was laid on a sterile platform in the supine position with all legs were tapped to the operating table. Reflexes were checked by pinching the hind feet to make sure the mouse was sufficiently anesthetized. Ophthalmic ointment was placed on the eyes to prevent drying during the procedure. Carprofen was used for analgesia prior to the surgery and during the 72 hours post-operation. Hairs on the abdomen were removed using a depilatory gel and a shaver. Abdomen was disinfected with alternating scrubs of chlorhexidine and 70% alcohol. A mid-line incision from the xiphoid to the pelvis was made with a scalpel, and the abdominal walls were retracted. The bowel was wrapped in saline-moistened gauze and was displaced very gently to the animal's right abdomen cavity. The infrarenal aorta was dissected free between the renal arteries proximally and the bifurcation distally with tweezers. All small branches of this segment were secured very carefully by using 11-0 monofilament single suture. The proximal and distal portions of the aorta was clamped with a 6-0 single silk suture. The aorta in the middle between the clamps was divided using vannas spring scissor. Then the cut ends were irrigated with heparinized saline to flush the lumen open. A 1-mm cuff with a 1-mm handle (PE-50 tubing .023x.038in, Instech Laboratories, Inc, USA) was placed on both ends of the recipient mouse aorta, and the ends were reverted over the cuff and ligated with an 8-0 silk ligature. Human IMA was grafted between the 2 ends of the aorta by sleeving the ends of the human IMA over the artery cuff and ligating them together with the 8-0 suture. The optimal overlap length between IMA and recipient aorta was 1-2 mm. The ligatures were carefully released after carefully inspecting the anastomosis. The distal clamp was releases first, which can generate a low pressure which can hold the walls together, prior to releasing the proximal high-pressure side. The graft would be perfused immediately and check for a visible pulse. The abdominal contents were then returned to the abdominal cavity, and the wound was closed with a 4-0 polyglycolic acid suture. The skin incision was sealed with Vetbond tissue adhesive. One ml of warm saline was injected subcutaneously to maintain fluid homeostasis. After the surgery, the mice were kept on a Far Infrared Warming Pad (Kent Scientific) until fully recovered from anesthesia and monitored every two hours for the first day and then once daily. Mice were then reconstituted with 1×10^7 PBMCs isolated from the same patients once every 7 days. Two weeks after the operation, mice were infused with Ang II (1000 ng/kg/min) via osmotic minipumps (Alzet osmotic pump Model 2004, Durect Corporation) for

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28 days.

Histopathology and immunofluorescent staining

Abdominal aortic tissues were fixed in 4% paraformaldehyde (PFA) and embedded in paraffin. Tissue sections (5 μm thick) were stained with hematoxylin-eosin (H&E) or Verhoeff's elastic staining (EVG) for histopathological analyses. H&E and EVG staining were performed using the commercial kits (DAKO) according to the manufacturer's protocol. For immunofluorescent staining, serial sections (10 μm) from OCT-embedded frozen tissues or primary cultured cells were fixed in cold acetone or 4% paraformaldehyde. After blocking with 1% goat serum, sections were incubated at room temperature with primary antibodies or normal IgG (negative control to validate the antibody specificity and distinguish genuine target staining from background) for 2 hours and then fluorescent dye-conjugated secondary antibodies for 1 hour. Images were acquired with a fluorescence microscope (Nikon Instruments Inc.). The images with average staining signals were selected as representative picture and used in the Figure panels.

Isolation and *in vitro* culture of PEMs and BMDMs

Mouse PEMs were obtained from the peritoneum of mice as previously described [62]. Briefly, peritoneal cells were harvested by injecting 10 ml of PBS into the peritoneal cavity. After flushing twice with cold PBS, the cells were diluted to 1×10^6 cells/ml in Dulbecco's Modified Eagle's medium (DMEM) medium supplemented with 10% heat-inactivated FBS and cultured in 12-well plates in a humidified CO_2 incubator at 37 °C for 2 hours. The non-adherent cells were removed by washing with warm PBS. The adherent cells constitute more than 90% of macrophages.

Bone marrow cells were used to generate BMDMs as previously described [62]. Bone marrow was aseptically flushed out from the tibiae and femurs of euthanized mice and depleted of red blood cells using red blood cell lysis buffer (Roche Corporation). After re-suspended in DMEM medium, the cells were placed in a cell culture dish and incubated at 37°C for 2 hours to remove adherent macrophages. The non-adherent cells were re-suspended in DMEM medium supplemented with 10% heat-inactivated FBS, 100 IU/ml penicillin, 100 ug/ml streptomycin, 2mM L-Glutamine (Thermo Fisher Scientific), and 10 ng/ml M-CSF and cultured for 7 days. Non-adherent cells were removed on day 3, and the M-CSF-conditioned medium was changed on day 5. To acquire the classically activated M1 macrophages, 100 ng/ml IFNγ and 100 ng/ml LPS were used to stimulate the macrophages for 3 hours for mRNA expression or 6 hours for protein assays. To block new protein synthesis, macrophages were treated with 30 μg/mL cycloheximide (CHX) for various times.

Reverse transcription-PCR (RT-PCR) and quantitative reverse transcription PCR (RT-qPCR)

Trizol reagent (Invitrogen) was used to extract total RNA following the manufacturer's instruction. cDNA was synthesized by the iScript cDNA synthesis kit (Bio-Rad). For miRNA cDNA synthesis, miRNA First Strand Synthesis Kit (Takara) was used. RT-PCR was performed on the Bio-Rad C1000 thermal cycler. RT-qPCR was performed on the MX3000P RT-qPCR machine using SYBR Green RT-qPCR master mix (Agilent). The primers used in this study were listed in major resource table.

Western blotting

PEMs, BMDMs, or abdominal aorta tissues were lysed in RIPA lysis buffer (1% Nonidet P-40, 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 1 mM sodium orthovanadate, and protease inhibitors) to extract total proteins. Samples were separated on SDS-polyacrylamide gels and electro-transferred onto nitrocellulose membranes (Amersham Biosciences). After blocking with 5% BSA, the membranes were incubated with various primary antibodies at 4 °C overnight. The membranes were then incubated with IRDye secondary antibodies (LI-COR Biosciences) at room temperature for 1 hour. The protein expression was detected by Odyssey CLx Imaging System (LI-COR Biosciences).

Co-immunoprecipitation (Co-IP)

The protein A/G-agarose beads (Santa Cruz, CA) were incubated with normal IgG, ADAR1, Drosha, or DGCR8 antibody at 4°C for 2 hours. Macrophages were lysed in 500 µl Co-IP lysis buffer (Pierce) on ice for 5 min, and the supernatants were incubated with antibody-conjugated beads at 4°C overnight. After washing with the Co-IP buffer, proteins were eluted from the beads and boiled in SDS loading buffer. Western blotting was performed to detect the precipitation of proteins.

Ubiquitination Assay

The protein A/G-agarose beads (Santa Cruz, CA) were incubated with IgG, Drosha, or Ubiquitin antibody at 4°C for 2 hours. Macrophages were lysed in 500 µl Co-IP lysis buffer (Pierce) on ice for 5 min, and the supernatants were incubated with antibody-conjugated beads at 4°C overnight. After washing with the Co-IP buffer, proteins were eluted from the beads and boiled in SDS loading buffer. Western blotting was performed to detect the ubiquitination of proteins.

Proximity Ligation Assay (PLA)

PLA was performed by using reagents provided in the Duo-link PLA kit (Sigma-Aldrich) according to the manufacturer instructions with minor modifications. Briefly, human or mouse control aorta or AAA

sections (5 um) were deparaffinized, re-hydrated, permeabilized with Triton 0.3% (in PBS), and then incubated with blocking solution for 45 min followed by incubation with mouse anti-Drosha (Santa Cruz) and rabbit anti-ADAR1 (Cell Signaling); or with mouse anti-Drosha (Santa Cruz) and rabbit anti-DGCR8 (Santa Cruz) at 4 °C overnight. After washing with Buffer A for three times, the sections were incubated with secondary antibodies conjugated with PLA DNA probes at 37 °C for 1 h. Following 4 ×10 min washing and a rinse at 37 °C with Buffer A, sections were incubated with ligation buffer containing oligonucleotides that can hybridize to the PLA probes to form a rolling circle DNA strand by DNA ligase, which was incubated at 37 °C for 30 min. Subsequently, the sections were washed with Buffer A at 37 °C and incubated with the amplification-detection solution containing DNA polymerase for rolling circle amplification at 37 °C for 100 min. Then, the sections were washed with Buffer B for four times followed by four times of washing with 0.01× Buffer B. Finally, the sections were mounted with mounting buffer containing DAPI under coverslips and observed with a fluorescence microscope (Keyence Corporation of America). The PLA spots were counted with ImageJ, and the mean spot number/cell was calculated for each sample. Rabbit and mouse IgG antibody were used as negative controls.

Flowcytometry and tissue digestion: Single cell suspension was prepared following the previous published methods [39, 63, 64]. Briefly, aortic tissues were digested in 1 ml collagenase cocktail: 600 µl 1X HBSS, 100 µl collagenase type I (Sigma-Aldrich, C0130, 10X stock: 6750 U/ml), 100 µl collagenase type XI (Sigma Aldrich, C7657, 10X stock: 187.5 U/ml), 100 µl hyaluronidase type I-s (Sigma-Aldrich, H1115000, 10X stock: 900 U/ml), 100 µl DNase I (Sigma-Aldrich, 11284932001, 10X stock: 900 U/ml) for 20 mins. Then cell suspension was strained through a 70-µm cell strainer and spun. Pellets from all tissues were subjected to red blood cell lysis and subsequently resuspended in flow cytometry buffer (2% FBS and 0.02% NaN3 in phenol-free DMEM) for further staining. Single-cell suspensions were incubated with Fc-receptor-blocking antibody (14-9161-73; Invitrogen) on ice for 1 hr. Cells were then stained with fluorescent dye-coagulated antibodies listed in the Major Resource Table. After being stained, cells were passed through a 70-µm filter and sorted on a BD FACSAria Fusion, BD Fortessa, or BD Canto II analyzer. Data were acquired with the FACSDiva software. Cell doublets were excluded by comparison of the side-scatter width to the forward-scatter area.

Statistical analysis

All experiments were repeated at least for three times. All data represent independent data points but not technical replicates. Data are presented as the mean ± SD. Normality of data was assessed

by the D'Agostino & Pearson normality test with alpha=0.05. For comparisons of two groups, student's unpaired two-tailed t test was used for normally distributed data, and Mann-Whitney two tailed test was used for non-normally distributed data or for groups with n less than 7. For more than 2 groups, 2-way ANOVA with Tukey post-test analysis was used for normally distributed data, and Kruskal-Wallis test with Dunn's multiple comparisons test was used for non-normally distributed data. Prism 9.0 (GraphPad Software, CA) or RStudio (Desktop 1.4.1717) was used for statistical analyses, and differences considered statistically significant when nominal $P<0.05$ or adjusted $P<0.05$ in case of multiple testing. However, the correcting for multiple testing across the entire body of the studies was not performed because both in vitro and in vivo experiments were performed, and various approaches were used in this study.

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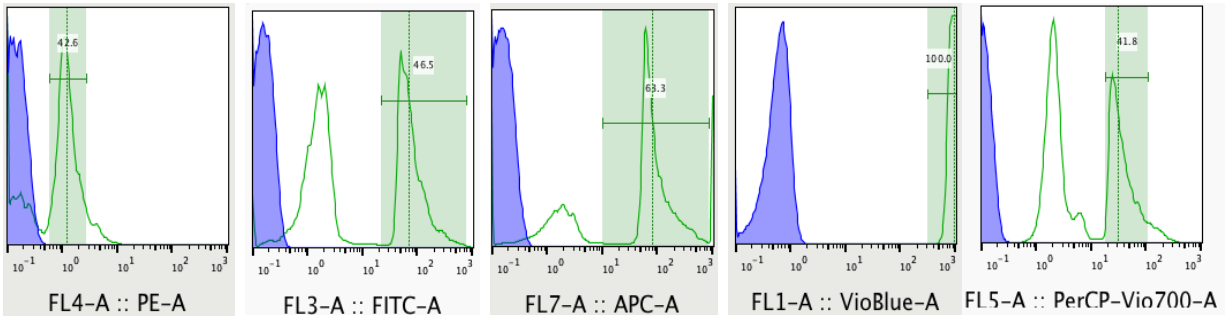
A



B

FL1-A :: VioB…	FL3-A :: FITC…	FL4-A :: PE-A	FL5-A :: PerC…	FL7-A :: APC…	HDR-SE :: H…	HDR-V :: HD…	FL8-H :: APC…	FL7-H :: APC…	FL4-H :: PE-H	FL3-H :: FITC…	FL6-H :: PE-…	FL5-H :: PerC…	HDR-CE :: H…	FL6-A :: PE-…	FL2-H :: Vio…	FL1-H :: VioB…	FL8-A :: APC…	FL2-A :: Vio…
100	0.0161	0	0	0	0	-5.0023	0.0003	0.0011	0	0.0068	0.0011	0.0012	0	0.0001	6.6295	88.1527	0	7.2807
0.5793	100	7.8848	1.5078	0	-0.3649	-73.6836	0	0.0092	5.8231	76.1334	0.14	1.1476	-0.3649	0.1909	7.493	0.5458	0	7.9216
0.6441	0.1068	0.7953	100	50.3309	-0.1787	-35.6581	5.4918	42.3054	0.5525	0.0546	11.1456	72.1566	-0.1787	15.5467	0.2582	0.5297	6.7857	0.2793
1.4441	0.0692	0	100	51.1234	-0.7922	-160.1746	14.8534	43.1538	0	0.0382	27.0398	64.3864	-0.7922	41.1344	1.0486	1.2622	18.3787	1.1566
0.8203	0.056	0	1.5492	100	-0.318	-64.5594	11.5632	84.6504	0	0.0229	0.1874	1.0523	-0.318	0.2395	0.546	0.6866	14.2508	0.6314

Figure S1: Flow cytometry analysis compensation. **A,** Single staining tubing and gating strategy on each channel. Blue color shows the negative control for each channel. Green shadow represents the gated positive cell population. **B,** Compensation matrix.

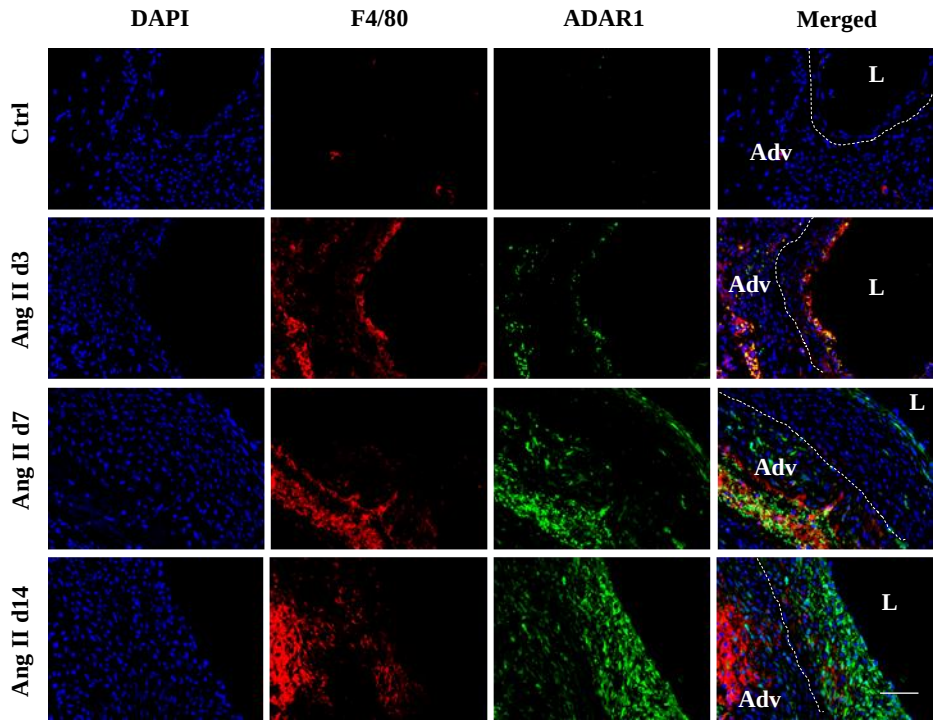


Figure S2: **ADAR1 is significantly induced in F4/80-positive macrophages in mouse AAA lesion**. ApoE-/- mice were infused with angiotensin II (Ang II, 1000ng/kg/min) for the times indicated. Frozen sections of abdominal aorta was co-immunostained with F4/80 and ADAR1 antibodies. Scale bar: 30 μm. White dash line showed the border between adventitia and media layer. Adv: Adventitia. L: Lumen.

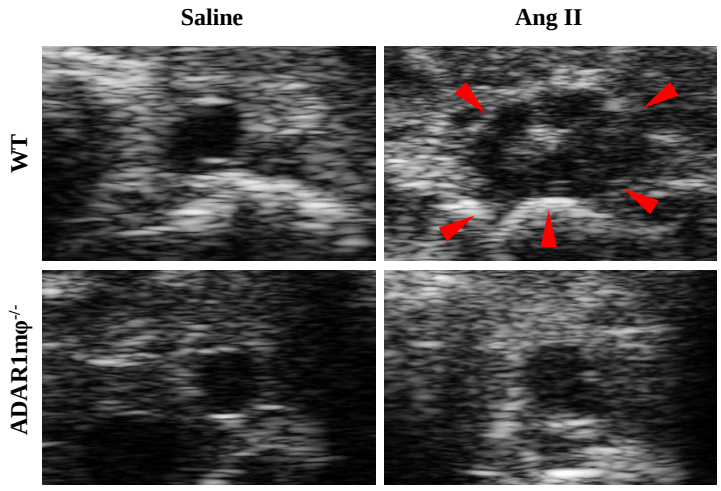


Figure S3: Representative transverse ultrasound images of abdominal aorta. Arrowhead indicates the aneurysm.

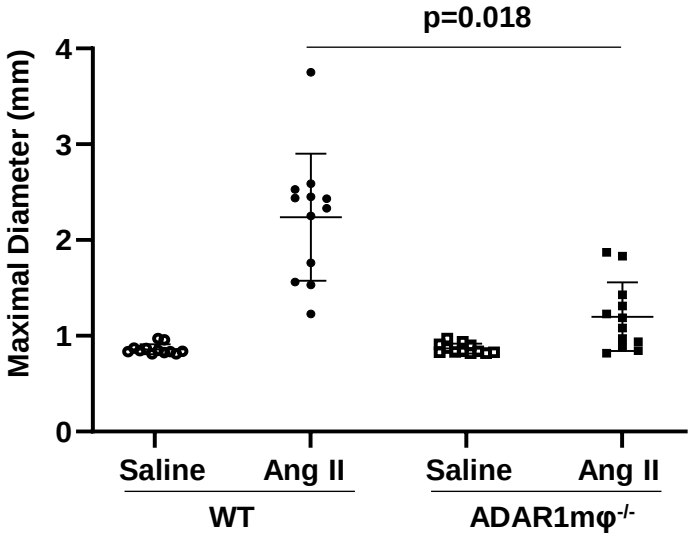


Figure S4: **Quantitative analyses of maximal aorta diameters**. ApoE-/- (WT) or ADAR1mϕ-/-;ApoE-/- mice were infused with saline or angiotensin II (Ang II, 1000ng/kg/min) for 28 days. The maximal diameters of abdominal aorta or AAA lesion were measured ex-vivo. P=0.018, ADAR1mϕ-/- vs. WT mice with Ang II infusion. Kruskal-Wallis test with Dunn multiple comparisons test was performed. n=12

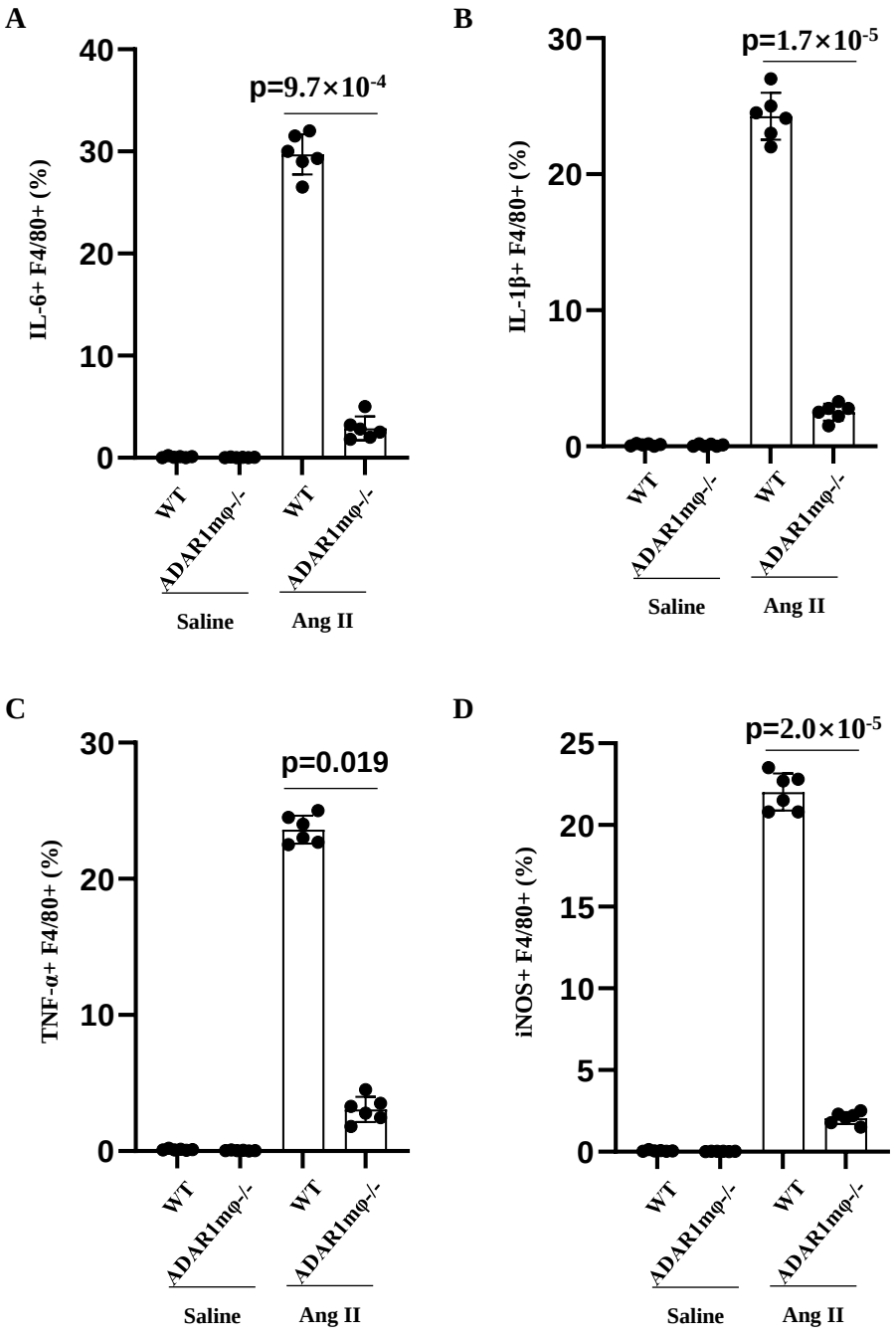


Figure S5: **Quantitative analyses the percentage of IL-6-, IL-1β-, TNF-α-, and iNOS-positive macrophages in the immuno-staining data shown in Figure 4**. p= 9.7×10^{-4} (A), 1.7×10^{-5} (B), 0.019 (C), 2.0×10^{-5} (C), ADAR1mϕ-/- vs. WT mice with Ang II infusion, n=6. Fisher exact test (2-tailed).

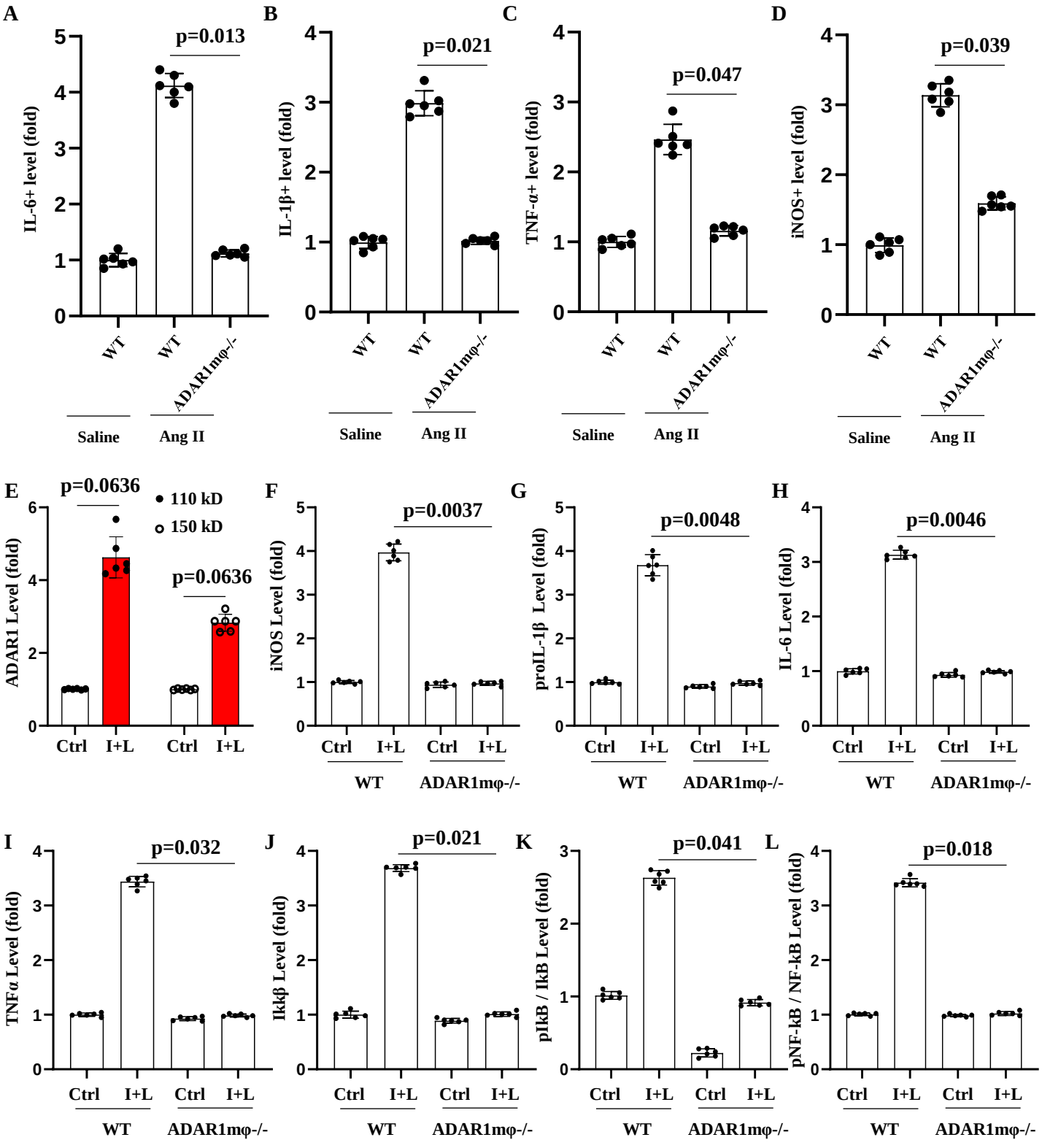


Figure S6: **Quantitative analyses the protein level showing Figure 4B (A-D), 4C (E), 4D (F-I), and 4E (J-L).** P=0.013 (A), 0.021 (B), 0.047 (C), and 0.039 (D) ADAR1mϕ-/- vs. WT mice with Ang II infusion. Kruskal-Wallis test with Dunn multiple comparisons test was performed, n=6. P=0.0636 (E), interferon γ +LPS (I+L) vs. vehicle-treated cells, Mann-Whitney test (2-sided), n=6. P=0.0037 (F), 0.0048 (G), 0.0046 (H), 0.032 (I), 0.021 (J), 0.041 (K), and 0.018 (L); ADAR1mϕ-/- vs. WT cells treated with I+L. Kruskal-Wallis test with Dunn multiple comparisons test was performed, n=6.

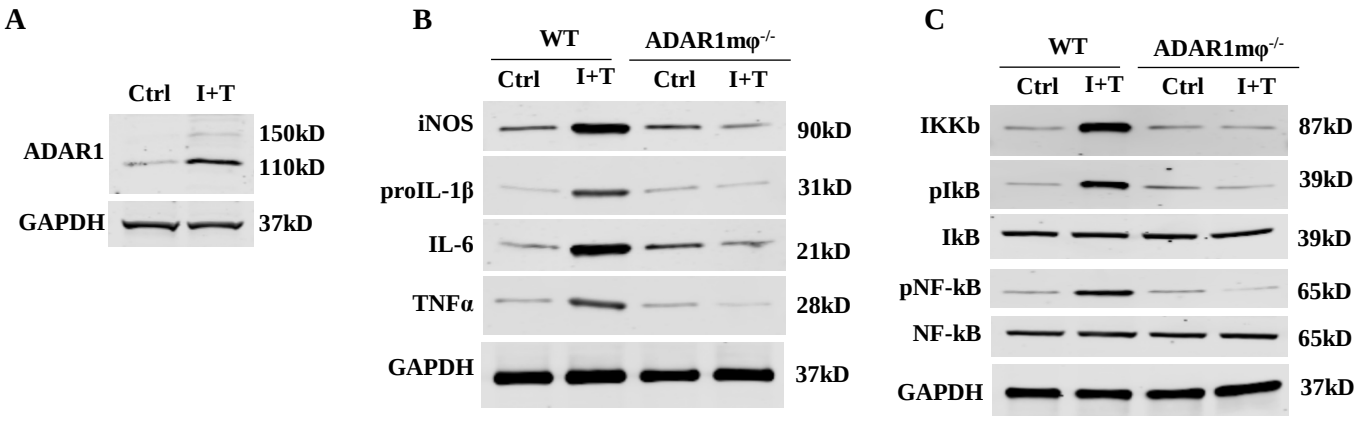


Figure S7: **A**, ADAR1 was induced in bone marrow derived macrophages (BMDMs) treated with 100 ng/mL IFNγ and 25 ng/mL TNF-α (I+T) for 8 h. ADAR1 protein expression was measured by Western blot. **B**, ADAR1 deficiency (ADAR1mϕ$^{-/-}$) significantly inhibited the I+ T-induced iNOS, IL-1β, IL-6 and TNFα production. BMDMs isolated from WT or ADAR1mϕ$^{-/-}$ mice were treated with vehicle (Ctrl) or 100 ng/mL IFNγ plus 25 ng/mL TNF-α (I+T) for 8 h to induce macrophage classical activation. The expression of iNOS and pro-inflammatory cytokines IL-1β, IL-6 and TNFα were determined at protein levels by Western blot, respectively. **C**, ADAR1 deficiency (ADAR1mϕ-/-) significantly inhibited the IFNγ+ TNF-α-induced IKKβ, pIκB, and pNF-κB expression or phosphorylation. BMDMs isolated from WT or ADAR1mϕ-/- mice were treated with vehicle (Ctrl) or 100 ng/mL IFNγ plus 25 ng/mL TNF-α (I+T) for 60 min. The total or phospho-protein levels of proteins indicated were measured by Western blot.

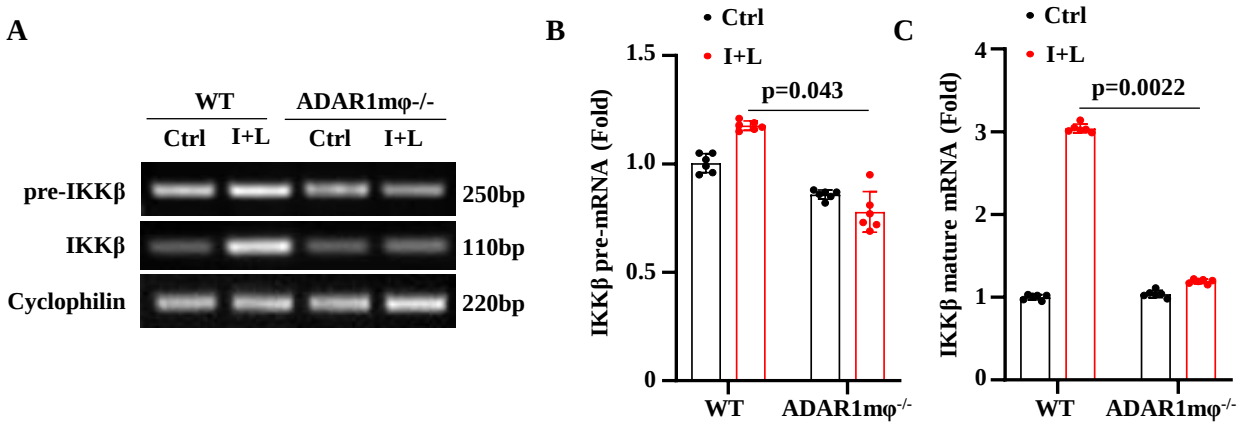


Figure S8. ADAR1 promoted IKKβ expression independent of its RNA editing function. ADAR1 deficiency (ADAR1mϕ-/-) significantly inhibited the expression of both IFNγ+LPS-induced pre- and mature IKKβ mRNA. BMDMs isolated from WT or ADAR1mϕ-/- mice were treated with vehicle (Ctrl) or 100 ng/mL IFNγ plus 100 ng/mL LPS (I+L) to induce the classical macrophage activation. The expression of pre- and mature IKKβ RNA were determined by PCR (A). IKKβ pre- and mature mRNA levels in A were quantified by normalizing to cyclophilin (B). P=0.043 (B) and 0.0022 (C); ADAR1mϕ-/- vs. WT cells with I+L-treatment, Kruskal-Wallis tests with Dunn multiple comparisons tests were performed, n=6.

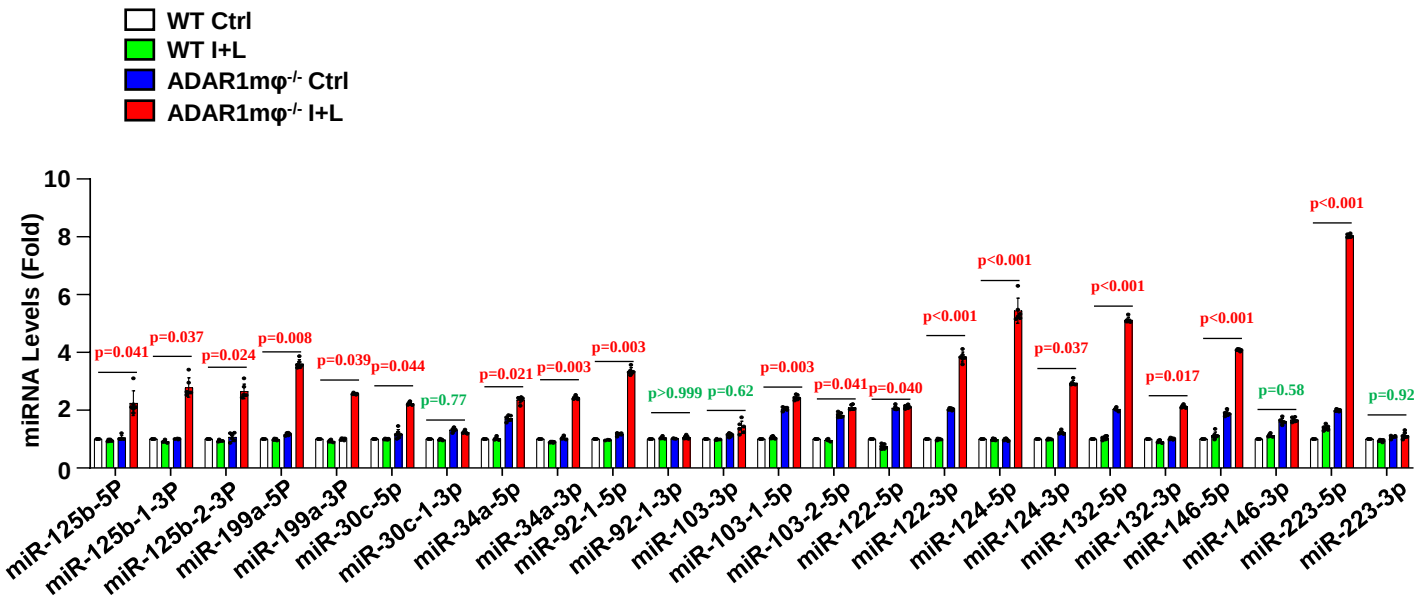


Figure S9: **ADAR1 regulated the expression of anti-inflammation microRNAs (miRNA or miR**). Bone marrow-derived macrophages (BMDMs) isolated from WT or macrophage ADAR1-deficient (ADAR1mϕ-/-) mice were treated with vehicle (Ctrl) or 100 ng/mL IFNγ plus 100 ng/mL LPS (I+L) to induce macrophage activation. The mature miRNA levels of anti-inflammatory miRNAs were assessed by RT-qPCR. ADAR1mϕ-/- significantly enhanced the IFNγ+LPS-induced production of several anti-inflammation miRNAs. Comparisons were only made within each miRNA. P= 0.041 (miR-125b-5P), 0.037 (miR-125b-1-3P), 0.024 (miR-125b-2-3P), 0.008 (miR-199a-5P), 0.039 (miR-199a-3P), 0.044 (miR-30c-5p), 0.77 (miR-30c-1-3p), 0.021 (miR-34a-5p), 0.003 (miR-34a-3p), 0.62 (miR-103-3p), 0.003 (miR-103-1-5p), 0.041 (miR-103-2-5p), 0.040 (miR-122-5p), p=0.037 (miR-124-3p), 0.017 (miR-132-3p), 0.058 (miR-146-3p), 0.92 (miR-223-3p), p>0.999 (miR-92-1-3p), P<0.001 (8.1×10^{-4} for miR-122-3p, 8.8×10^{-8} for miR-124-5p, 1.3×10^{-8} for miR-132-5p, 3×10^{-5} for miR-146-5p, and 2.2×10^{-8} for miR-223-5p); ADAR1mϕ-/- vs WT BMDMs treated with I+L, Kruskal-Wallis test with Dunn multiple comparisons test was performed, n=6.

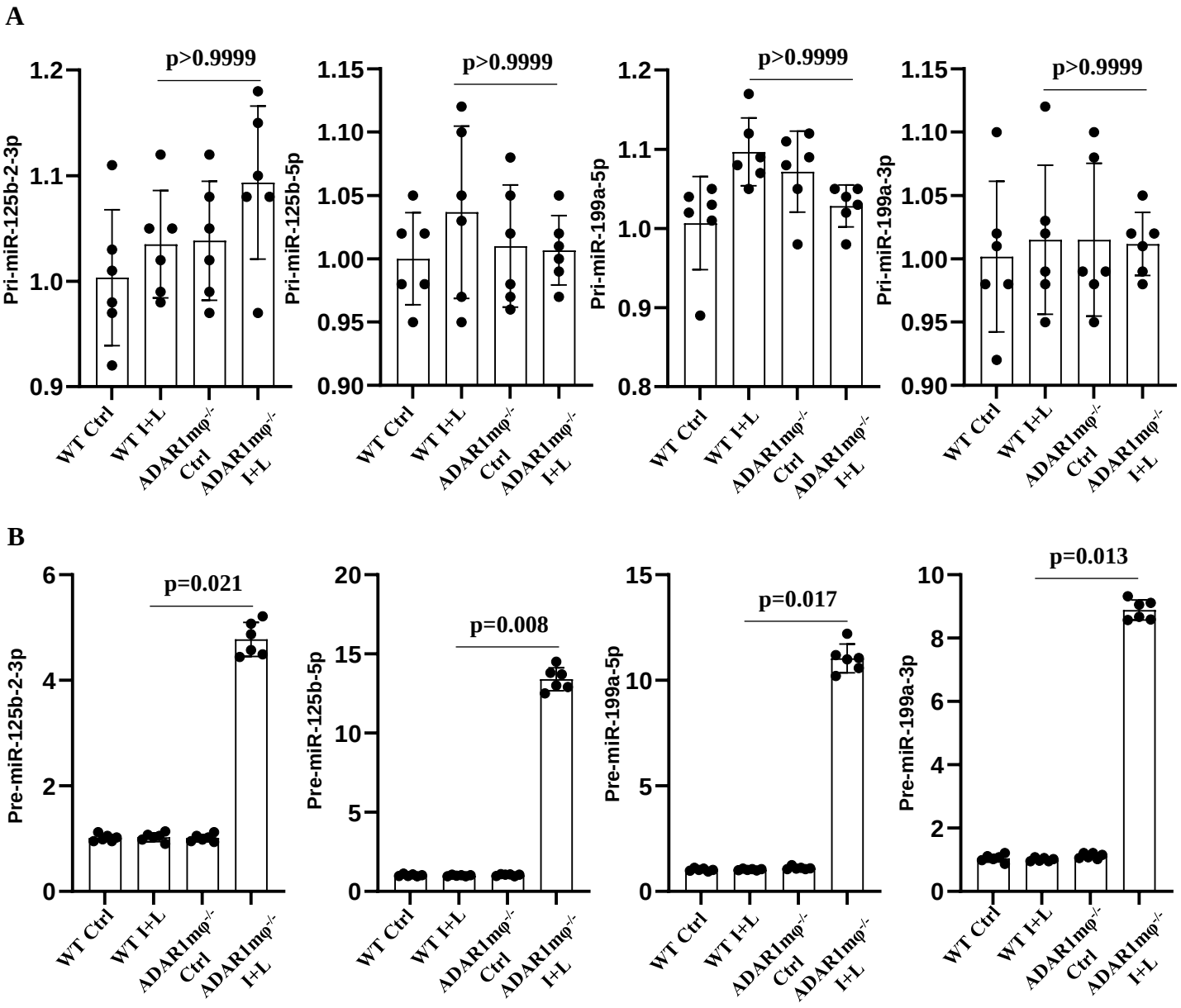


Figure S10: ADAR1 regulates pri- to pre-miRNA processing of anti-inflammatory microRNAs. BMDMs isolated from WT or macrophage ADAR1 deficient (ADAR1mϕ-/-) mice were treated with vehicle (Ctrl) or 100 ng/mL IFNγ plus 100 ng/mL LPS (I+L) to induce macrophage activation. ADAR1mϕ-/- didn't change the expression of the pri-miRNA levels **(A)** while enhanced the pre-miRNA levels (**B**) of miR125b and miR-199a in I+L-activated macrophages, as determined by RT-qPCR. P=0.021 (Pre-miR-125b-2-3p), 0.008 (Pre-miR-125b-2-5p), 0.017 (Pre-miR-199a-5p), and 0.013 (Pre-miR-199a-3p). ADAR1mϕ-/- vs. WT cell treated with I+L; Kruskal-Wallis test with Dunn multiple comparisons test was performed (n=6).

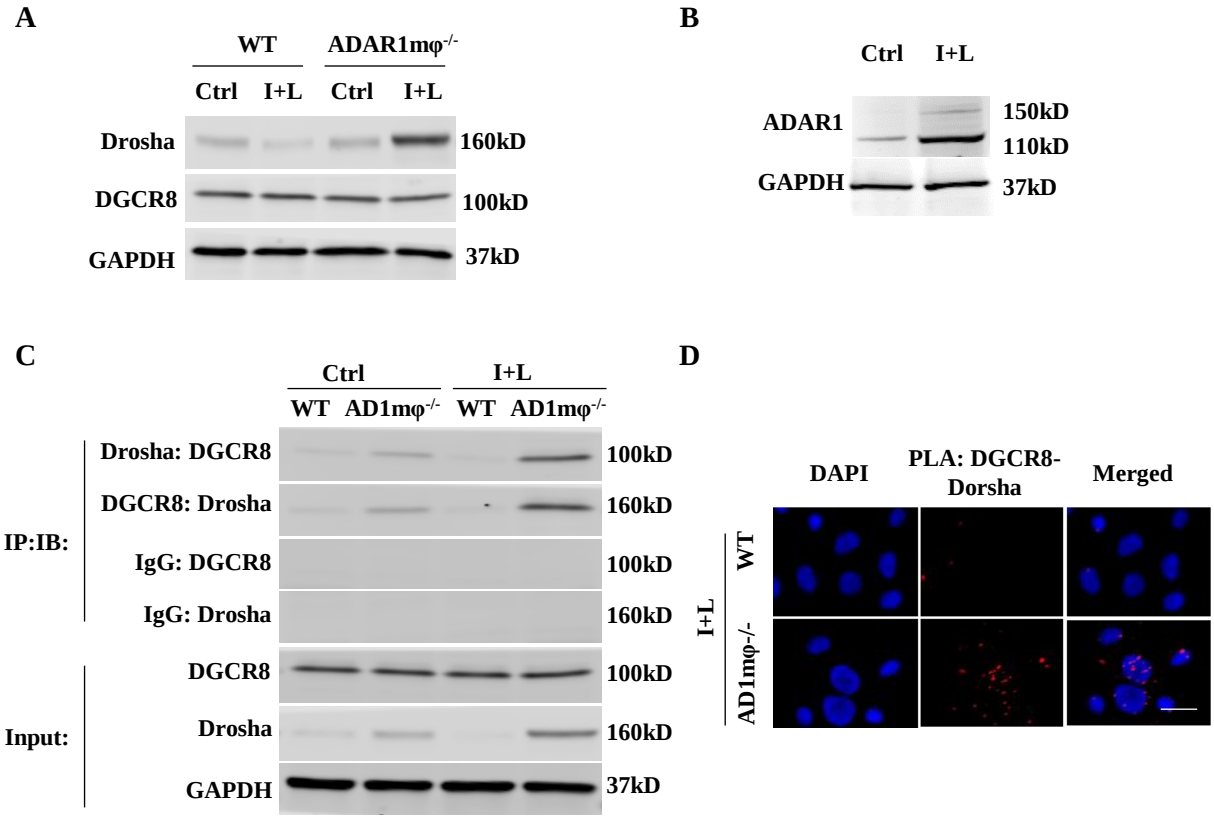


Figure S11: ADAR1mϕ-/- significantly enhanced Drosha expression and its interaction with DGCR8 in activated macrophages. **A**. BMDMs isolated from WT or macrophage ADAR1 deficient (ADAR1mϕ-/-) mice were treated with vehicle (Ctrl) or 100 ng/mL IFNγ plus 100 ng/mL LPS (I+L) to induce macrophage activation. ADAR1mϕ-/- significantly enhanced Drosha, but not DGCR8, expression in I+L-treated BMDMs, as detected by Western blot. **B**, Peritoneal macrophages (PEMs) isolated from WT mice were treated with vehicle (Ctrl) or I+L (100 ng/mL each) for 6 h to activate macrophages. ADAR1 expression was detected by Western blot. **C**, PEMs isolated from WT or ADAR1mϕ-/- (AD1mϕ-/-) mice were treated with vehicle (Ctrl) or 100 ng/mL IFNγ plus 100 ng/mL LPS (I+L) for 6 h to induce macrophage activation. Coimmunoprecipitation assay was performed: control IgG or antibodies against Drosha and DGCR8 were used for immunoprecipitation (IP); and DGCR8 and Drosha antibodies were used for immunoblotting (IB). ADAR1mϕ-/- enhanced the physical interaction between DGCR8 with Drosha in activated macrophages. **D**, In situ proximity ligation assay (PLA) was performed to confirm the DGCR8-Drosha interaction in the activated AD1mφ-/- PEMs. Nuclei were stained with DAPI. Scale bar: 10 μm.

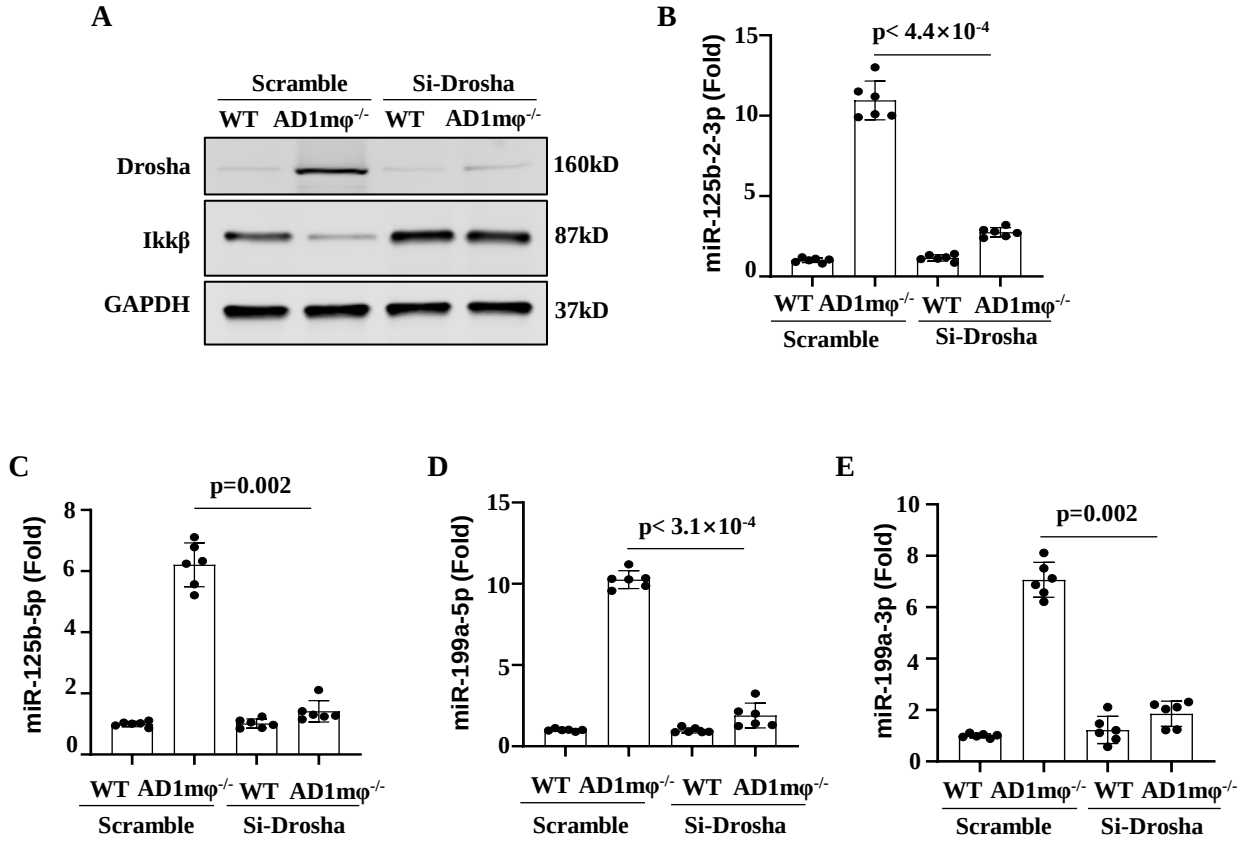


Figure S12: Knockdown of Drosha reverses the detrimental effect of increased ADAR1. BMDMs isolated from WT or ADAR1mϕ-/- (ADmϕ-/-) mice were transfected with scramble or Drosha siRNA (si-Drosha) and then treated with Interferon γ+LPS (I+L, 100 ng/mL each) for 6 h to induce macrophage activation. **A**, The protein expression of Drosha and Ikkβ were determined by Western blot. **B-E**: The mature miRNAs targeting NF-κB signaling, including miR-125b-2-3p (B), miR-125b-5p (C), miR-199a-5p (D), and miR-199a-3p (E) were assessed by RT-qPCR. P= $\mathbf{4.4\times10^{-4}}$ (B), 0.002 (C, E) and $\mathbf{3.1\times10^{-4}}$ (D); AD1mϕ-/- group with si-Drosha vs. with scramble siRNA; Kruskal-Wallis test with Dunn multiple comparisons test was performed, n=6.

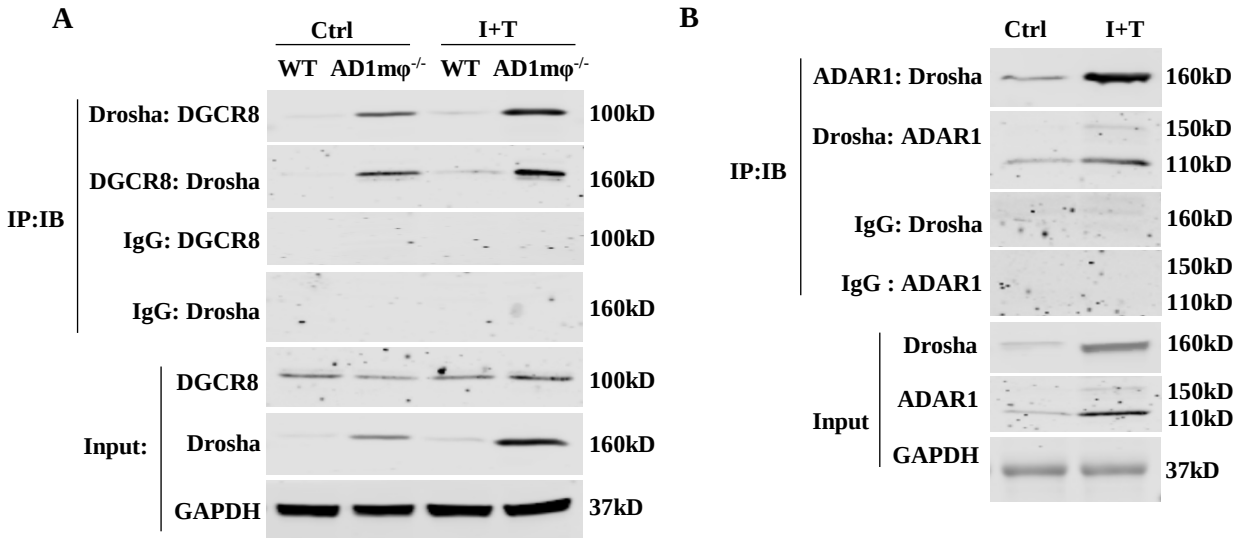


Figure S13: Drosha-DGCR8 and ADAR1-Drosha interactions in IFNγ and TNF-α-activated macrophages. A, ADAR1mϕ-/- significantly enhanced the interaction of Drosha with DGCR8 in IFNγ and TNF-α-activated macrophages. BMDMs isolated from wild type (WT) or ADAR1mϕ-/- mice (AD1mϕ-/-) were treated with vehicle (Ctrl) or 100 ng/mL IFNγ plus 25 ng/mL TNF-α (I+T) for 8 h to induce macrophage activation. IgG isotype or antibodies against Drosha and DGCR8 were used for immunoprecipitation (IP) as indicated. DGCR8 and Drosha antibodies were used for immunoblotting (IB). AD1mϕ-/- enhanced the physical interaction between DGCR8 and Drosha in classically activated BMDMs. **B,** ADAR1 interacted with Drosha in classically activated macrophages. BMDMs from WT mice were stimulated with 100 ng/mL IFNγ and 25 ng/mL TNF-α (I+T) for 8 h. IgG isotype or antibodies against Drosha and ADAR1 were used for immunoprecipitation (IP). ADAR1 and Drosha antibodies were used for immunoblotting (IB).

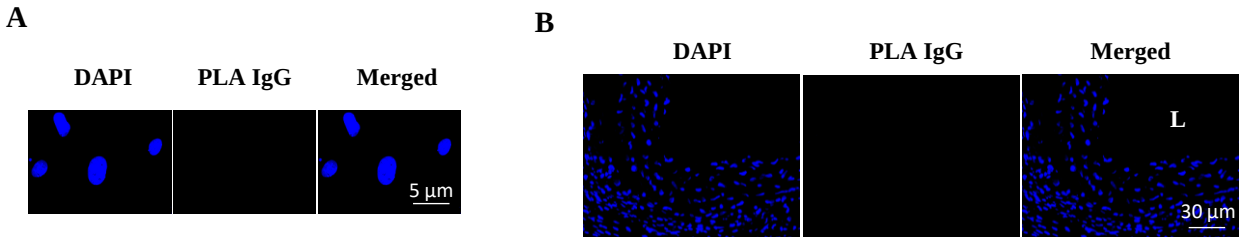


Figure S14: In situ Duolink proximity ligation assay (PLA) with IgG staining as negative controls (for Figure 5B). **A**, IgG negative control PLA was performed in BMDMs (n=6). **B**, IgG negative control PLA was performed in mouse aorta tissues (for Fig 5C, n=6). DAPI stains nuclei. L: lumen. Nuclei were stained with DAPI.

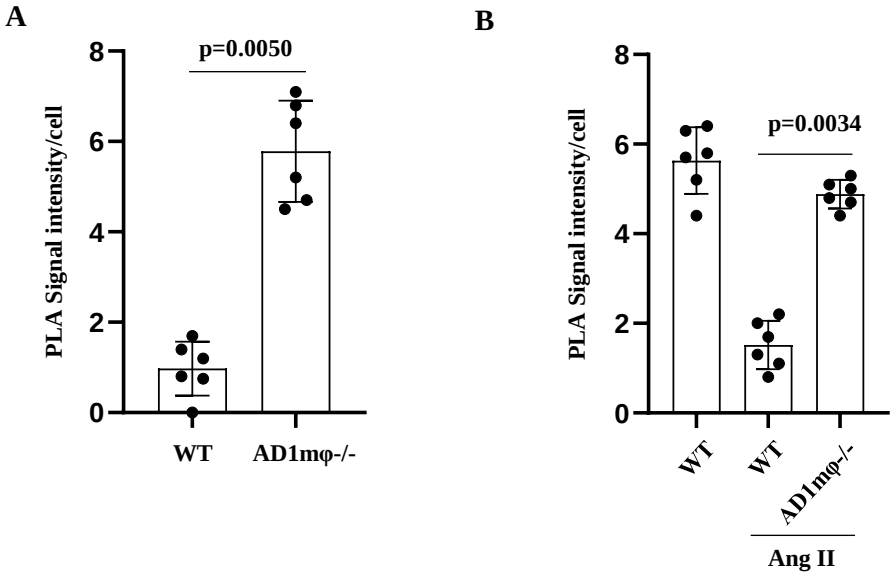


Figure S15: Quantification of PLA staining. **A,** Quantification of PLA signals in Figure 5B. P=0.0050, ADAR1mϕ-/- vs WT BMDMs, Mann-Whitney test (2-sided), n=6. **B**, Quantification of PLA signals in Figure 5C. P=0.0034, ADAR1mϕ-/- vs. WT mice infused with Ang II, Kruskal-Wallis test with Dunn multiple comparisons test was performed, n=6 .

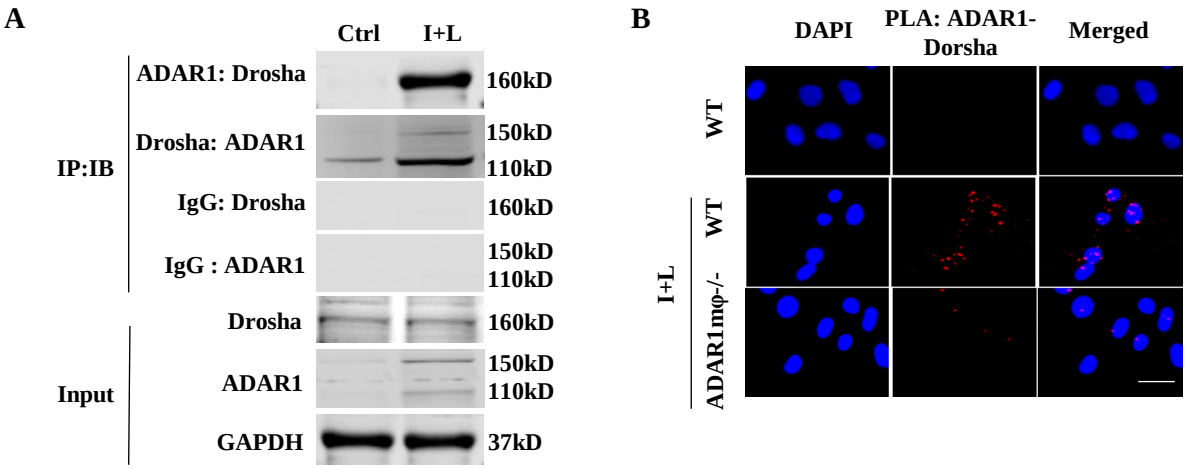


Figure S16: **ADAR1 Interacts with Drosha in IFNγ and LPS-activated peritoneal macrophages**. Peritoneal macrophages (PEMs) isolated from WT or ADAR1mϕ-/- mice were treated with vehicle (Ctrl) or 100 ng/mL IFNγ plus 100 ng/mL LPS (I+L) for 6 h to activate the macrophages. **A,** ADAR1 interaction with Drosha in PEMs was detected by co-immunoprecipitation assay. Control IgG or antibodies against Drosha and ADAR1 were used for immunoprecipitation (IP). ADAR1 and Drosha antibodies were used for immunoblotting (IB). ADAR1 interacted with Drosha in PEMS, and the interaction was enhanced in activated PEMs. **B,** ADAR1-Drosha interaction was confirmed in I+L-activated PEMs by In situ Duolink proximity ligation assay (PLA). ADAR1mϕ-/- diminished the ADAR1-Drosha interaction. DAPI stains nuclei. Scale bar=5 µm.

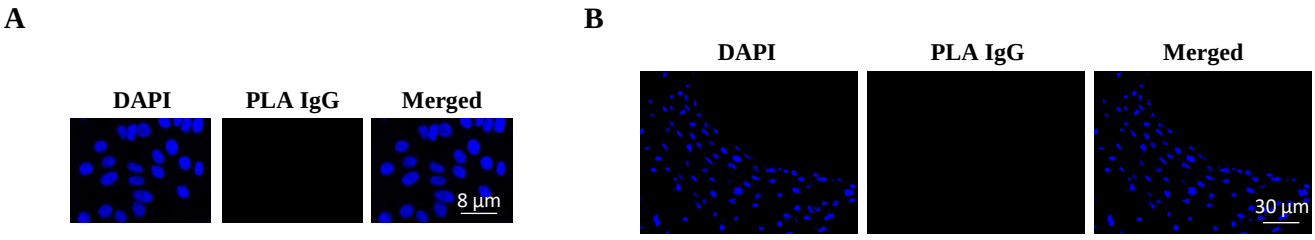


Figure S17: In situ Duolink proximity ligation assay (PLA) with IgG as negative control for Figure 6, B-C. **A**, IgG negative control PLA was performed in BMDMs (for Figure 6B). **B**, IgG negative control PLA was performed in mouse aorta tissues (for Figure 6C). DAPI stains nuclei. L: lumen. Nuclei were stained with DAPI.

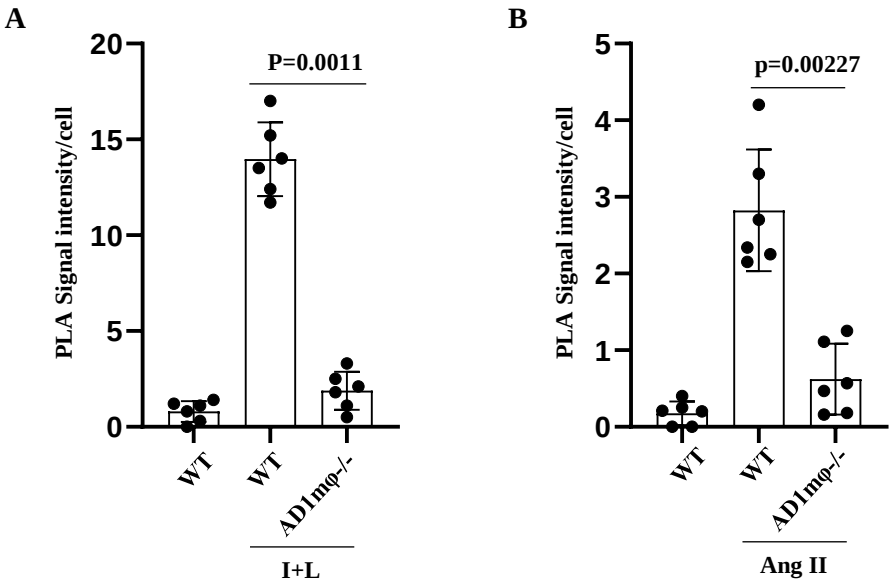


Figure S18: Quantification of PLA staining. **A,** Quantification of PLA signals in Figure 6B. P=0.0011, AD1mφ-/- vs. WT cell treated with I+L, n=6. **B,** Quantification of PLA signals in Figure 6C. P=0.00227, AD1mφ-/- vs. WT mice infused with Ang II, Kruskal-Wallis test with Dunn multiple comparisons test was performed, n=6 .

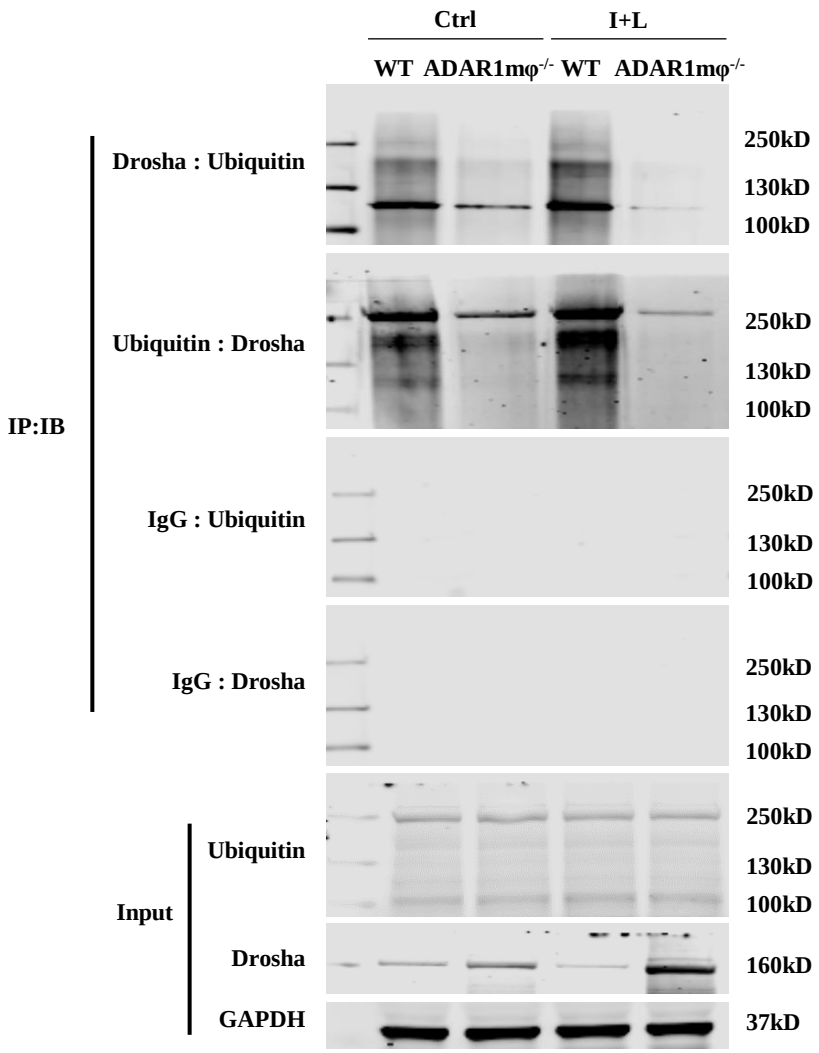


Figure S19: ADAR1 regulates Drosha ubiquitination. BMDMs isolated from WT or ADAR1mϕ-/- mice were treated with vehicle (Ctrl) or IFNγ+LPS (I+L, 100 ng/mL each) for 6 h to induce macrophage activation. Coimmunoprecipitation assays were performed to detect the Drosha ubiquitination, as indicated. IgG (Control), Drosha, or Ubiquitin antibodies were used for immunoprecipitation (IP). Immunoblotting (IB) was performed with Drosha or Ubiquitin antibodies, respectively.

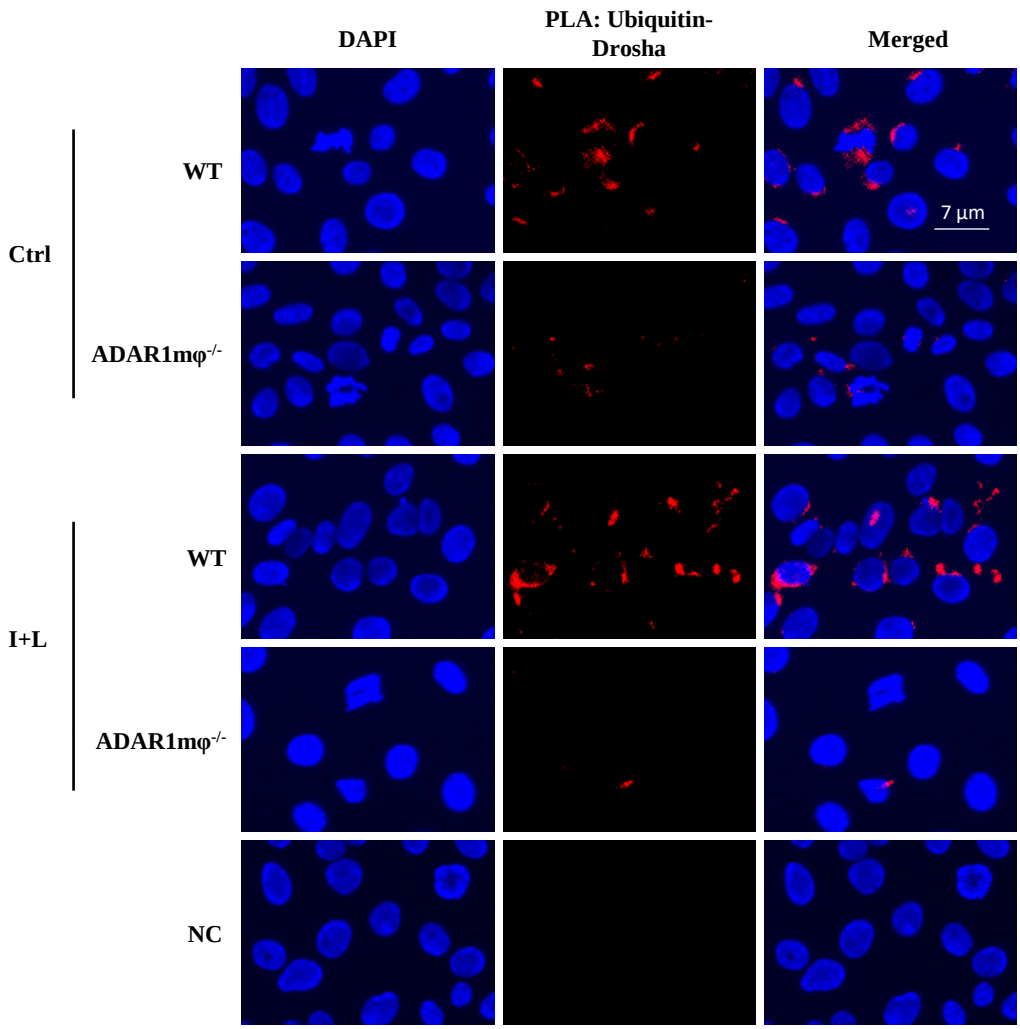


Figure S20: ADAR1 promotes Drosha ubiquitination, as detected by proximity ligation assay (PLA) assay. BMDMs isolated from WT or ADAR1mϕ-/- mice were treated with vehicle (Ctrl) or IFNγ+LPS (I+L, 100 ng/mL each) for 6 h to induce macrophage activation. In situ Duolink PLA was performed to confirm the direct binding of Drosha with ubiquitin. DAPI stains nuclei. NC: IgG negative control.

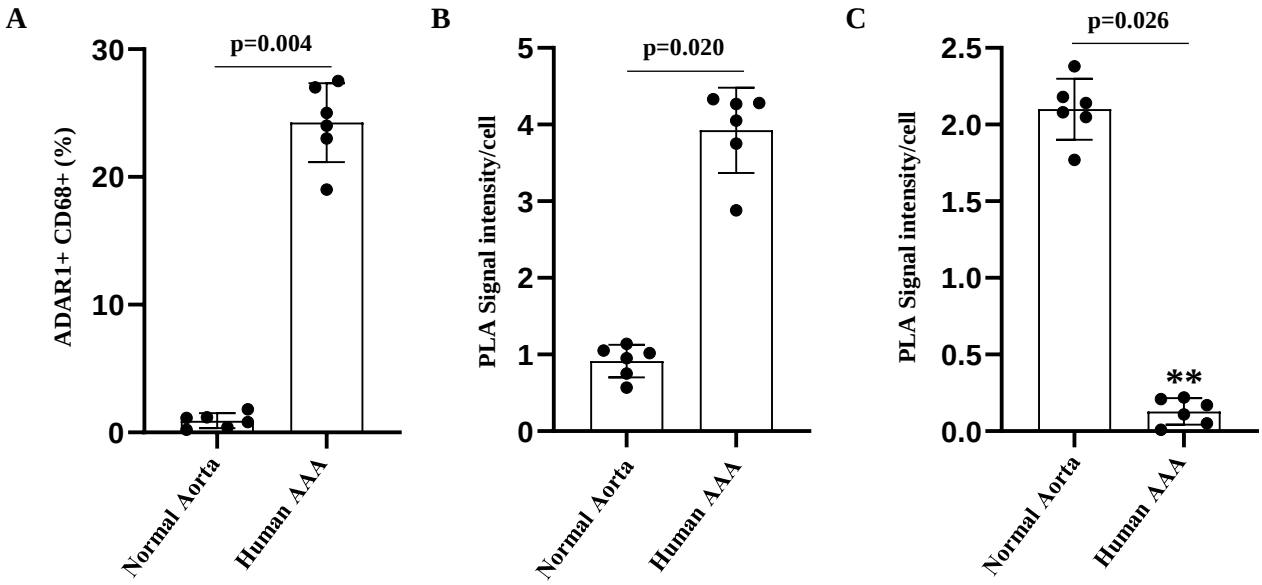


Figure S21: Quantification of immunostaining and PLA signals shown in Figure 7. **A,** Quantification of ADAR1+ macrophages in human AAA lesion shown in Figure 7A. **B,** Quantification of PLA signals for Drosha-ADAR1 interaction shown in Figure 7B. **C**, Quantification of PLA signals for Drosha-DGCR8 shown in Figure 7B. P=0.004 (A), 0.020 (B), and 0.026 (C), human AAA vs. normal aorta in each panel, respectively. Mann-Whitney test (2-sided), n=6 .

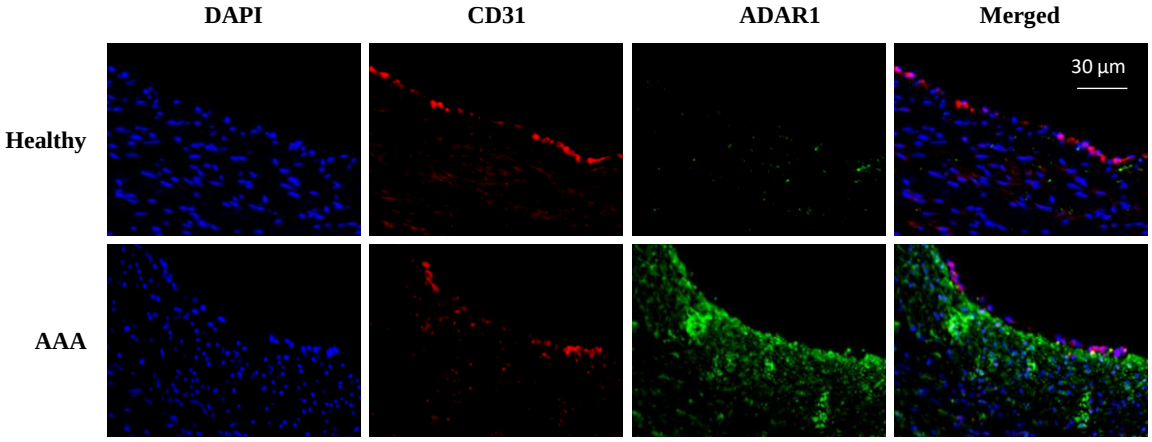


Figure S22: ADAR1 is not observed in endothelial cells of human healthy aorta or AAA lesions. Normal healthy human abdominal aorta or AAA sections were co-immunostained with CD31 and ADAR1 antibodies. Red: CD31, Green: ADAR1.

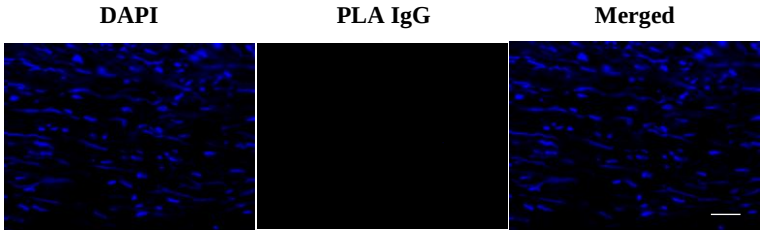


Figure S23: In situ Duolink proximity ligation assay (PLA) with IgG as negative control for Figure 7B. Scale bar: 30 µm.

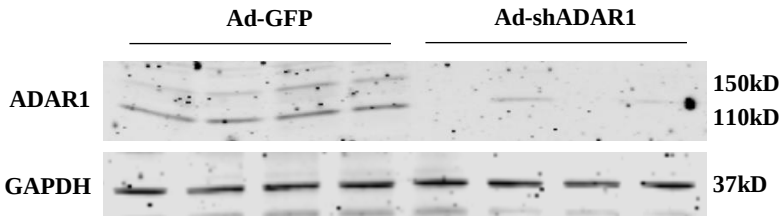


Figure S24: Ad-shRNA efficiently knocked down ADAR1 expression in human monocytes. Human PBMCs were transduced with adenoviral vector expressing either green fluorescent protein (Ad-GFP) or ADAR1 shRNA (Ad-shADAR1) by incubating the adenovirus with PBMCs in a 37℃, 5% CO2 incubator. The cells were cultured for 1 days in fresh medium and labeled with green live cell tracking dye. The ADAR1 levels were detected by Western blotting.

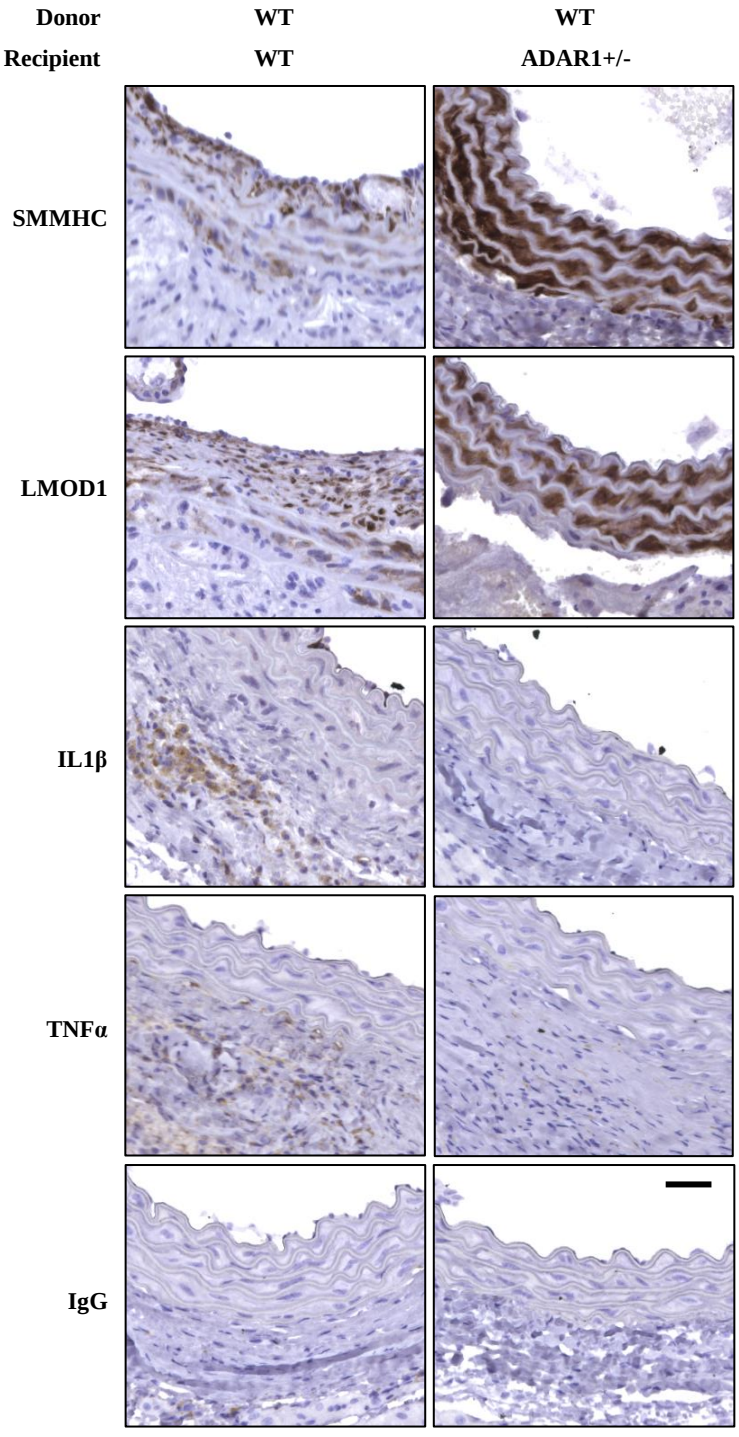


Figure S25: Proinflammatory cytokines and smooth muscle marker expression in transplanted aorta. Recipient mice were infused with Ang II (1000 ng/kg/min) for 7 days. Abdominal aorta frozen sections were immunostained with SMMHC, LMOD1, IL-1β, or TNFα antibodies, respectively**.** Scale bar: 30 μm.

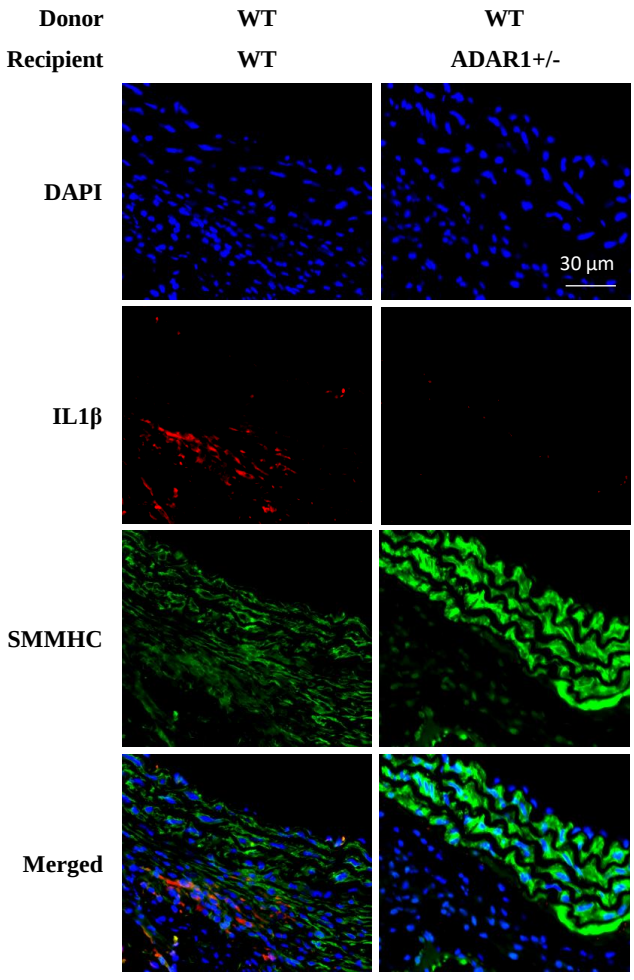


Figure S26: The correlation of proinflammatory cytokine expression in adventitia macrophage with SMC marker gene SMMHC expression in media layer in AAA lesion of transplanted aortas. Recipient mice were infused with Ang II (1000 ng/kg/min) for 7 days. Abdominal aorta frozen sections were co-immunostained with SMMHC and IL-1β antibodies**.**

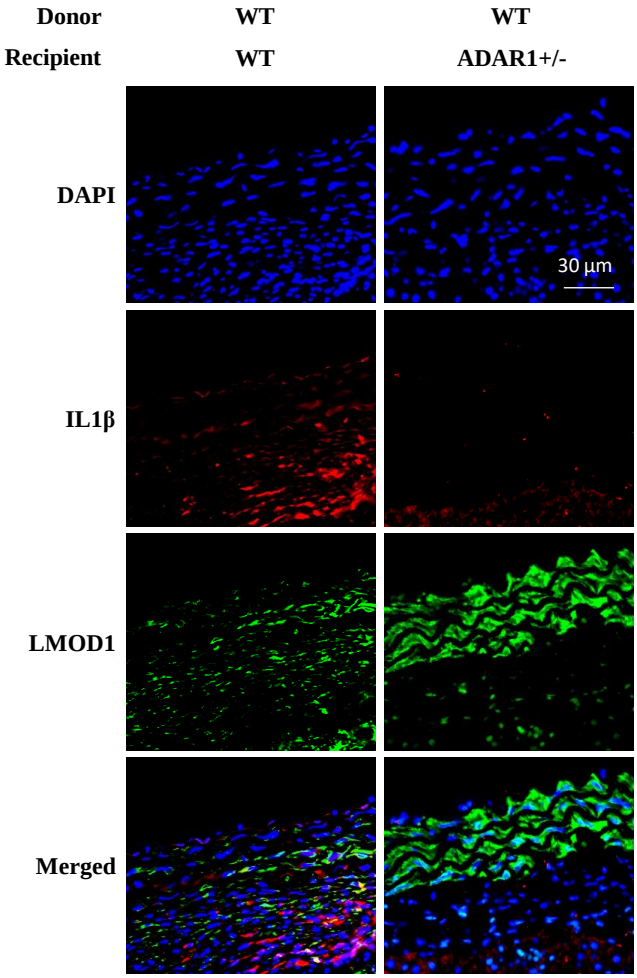


Figure S27: The correlation of proinflammatory cytokine expression in adventitia macrophage with SMC marker gene LMOD1 expression in media layer in AAA lesion of transplanted aortas. Recipient mice were infused with Ang II (1000 ng/kg/min) for 7 days. Abdominal aorta frozen sections were co-immunostained with LMOD1 and IL-1β antibodies**.**

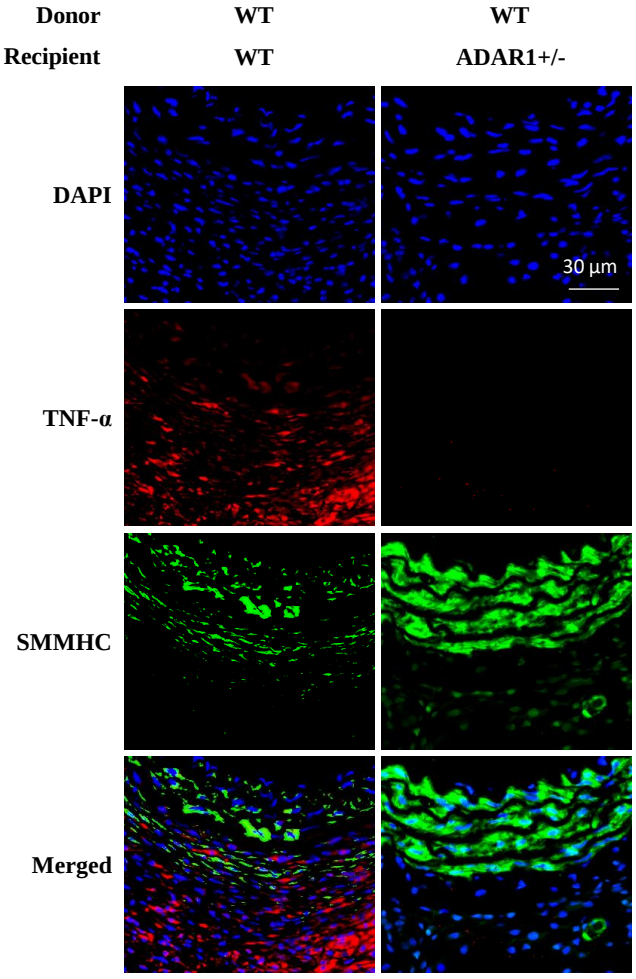


Figure S28: The correlation of proinflammatory cytokine expression in adventitia macrophage with SMC marker gene SMMHC expression in media layer in AAA lesion of transplanted aortas. Recipient mice were infused with Ang II (1000 ng/kg/min) for 7 days. Abdominal aorta frozen sections were co-immunostained with SMMHC and TNFα antibodies**.**

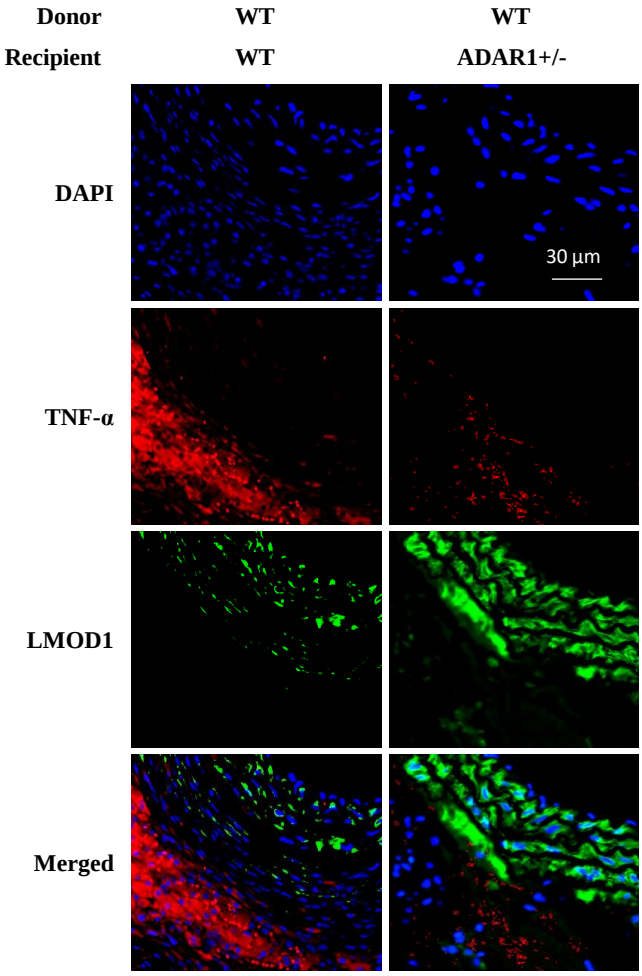


Figure S29: The correlation of proinflammatory cytokine expression in adventitia macrophage with SMC marker gene LMOD1 expression in media layer in AAA lesion of transplanted aortas. Recipient mice were infused with Ang II (1000 ng/kg/min) for 7 days. Abdominal aorta frozen sections were co-immunostained with LMOD1 and TNFα antibodies**.**

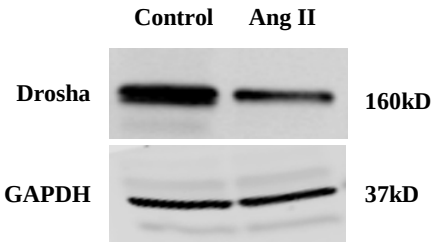


Figure S30: Drosha expression is downregulated in mouse aorta with Ang II infusion. ApoE-/- mice were infused with Ang II (1000 ng/kg/min) for 14 days. The protein expression of Drosha was determined by Western blotting.

Table S1: Healthy individual and aneurysm patients' information.

AAA or Control	Gender	Race	Age	DIAGNOSIS/PROCEDURE
Control	M	White	74	Healthy abdominal aorta near aorta-aneurysmal wall.
Control	M	n/a	77	Healthy abdominal aorta near aorta-aneurysmal wall
Control	M	White	n/a	Healthy abdominal aorta near aorta-aneurysmal wall
Control	M	White	55	Healthy abdominal aorta near aorta-aneurysmal wall
Control	M	Asian	21	Healthy abdominal aorta of an individual died from road traffic crash
Control	M	Asian	n/a	Healthy abdominal aorta of an individual died from road traffic crash
Control	M	Asian	33	Healthy abdominal aorta of an individual died from road traffic crash
Control	F	Asian	27	Healthy abdominal aorta of an individual died from road traffic crash
AAA	M	White	n/a	Aorta-aneurysmal wall, changes compatible with dissecting aneurysm; myxoid change; sclerosis; calcification
AAA	M	White	n/a	Aortic aneurysm wall; resection; vascular aneurysm with atherosclerosis and calcification
AAA	M	White	82	Aorta aneurysm repair; atherosclerosis; unorganized thrombus
AAA	F	White	n/a	Abdominal aorta-aneurysm repair. changes with aneurysm wall atherosclerosis
AAA	M	n/a	77	Dissecting aneurysm repair: consistent with aortic dissection
AAA	M	White	74	Abdominal aorta; plaque; aneurysm repair; atherosclerotic plaque
AAA	M	n/a	n/a	Abdominal aortic aneurysm; complicated atherosclerosis
AAA	M	White	n/a	Abdominal aortic aneurysm; sclerosis with focal calcification and organizing thrombus

DOI [to be added]

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
ADAR1+/-	Jackson Laboratory	C57BL6J	M	https://www.jax.org/strain/016160
APOE-/-	Jackson Laboratory	C57BL6J	M	https://www.jax.org/strain/002052
LysM-cre+/- ApoE-/-	Own Breeding Chen SY, Columbia, MO	C57BL6J	M	N/A
LysM-cre+/- ADAR1fl/fl ApoE-/-	Own Breeding Chen SY, Columbia, MO	C57BL6J	M	N/A

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent	ADAR1+/-	Jackson Laboratory	C57BL6J	M/F	https://www.jax.org/strain/016160
Parent	APOE-/-	Jackson Laboratory	C57BL6J	M/F	https://www.jax.org/strain/002052
Parent	LysM-cre	Jackson Laboratory	C57BL6J	M/F	https://www.jax.org/strain/004781
Parent	ADAR1fl/fl	Jackson Laboratory	C57BL6J	M/F	https://www.jax.org/strain/016159

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
GAPDH	Proteintech	1E6D9	WB (1:10,000)	https://www.ptglab.com/products/GAPDH-Antibody-60004-1-Ig.htm
ADAR1	Cell Signaling Technology	81284	IHC (1:100) IP (1:100) PLA (1:100)	https://www.cellsignal.com/products/primary-antibodies/adar1- e6x9r-xp-rabbit-mab/81284?site-search-type=Products&N=4294956287&Ntt=adar1&fromPage=plp
F4/80	BioLegend	123101	WB (1:1000) IHC (1:100)	https://www.biolegend.com/ja-jp/products/purified-anti-mouse-f4-80-antibody-4064
iNOS	Invitrogen	MA5-17139	WB (1:1000) IHC (1:100)	https://www.thermofisher.com/antibody/product/iNOS-Antibody-clone-4E5-Monoclonal/MA5-17139
CD68	Abcam	ab955	WB (1:1000) IHC (1:100)	https://www.abcam.com/cd68-antibody-kp1-ab955.html

DOI [to be added]

IκB kinase (IKK)	Cell Signaling Technology	2678T	WB (1:1000)	https://www.cellsignal.com/products/primary-antibodies/ikkb-l570-antibody/2678
NF-κB p65	Cell Signaling Technology	8242T	WB (1:1000)	https://www.cellsignal.com/products/primary-antibodies/nf-kb-p65-d14e12-xp-rabbit-mab/8242
phospho-NF-κB p65	Cell Signaling Technology	3031S	WB (1:1000)	https://www.cellsignal.com/products/primary-antibodies/phospho-nf-kb-p65-ser536-antibody/3031#:~:text=Phospho%2DNF%2DkappaB%20p65%20(Ser536)%20Antibody%20detects%20NF,subunit%20or%20other%20related%20proteins.
DGCR8	Cell Signaling Technology	16300	WB (1:500) IP (1:100) PLA (1:100)	https://www.cellsignal.com/products/primary-antibodies/dgcr8-d78e4-rabbit-mab/6914
α-Tubulin	Sigma Aldrich	T5168	WB (1:1000)	https://www.sigmaaldrich.com/US/en/product/sigma/t5168
ACTA2	Sigma Aldrich	A2547	IHC (1:100)	https://www.sigmaaldrich.com/US/en/product/sigma/a2547?contex t=product
IL-1β	Cell Signaling Technology	12242	WB (1:1000)	https://www.cellsignal.com/products/primary-antibodies/il-1b-3a6- mouse-mab/12242
IκB	Cell Signaling Technology	4812S	WB (1:1000)	https://www.cellsignal.com/products/primary-antibodies/ikba-44d4-rabbit-mab/4812?site-search-type=Products&N=4294956287&Ntt=44d4&fromPage=plp&_requestid=3310400
phospho-IκB	Cell Signaling Technology	2859T	WB (1:1000)	https://www.cellsignal.com/products/primary-antibodies/phospho-ikba-ser32-14d4-rabbit-mab/2859?site-search-type=Products&N=4294956287&Ntt=i%CE%BAb%CE%B1&fromPage=plp
RNase III Drosha	Santa Cruz	sc-393591	WB (1:500) IP (1:100) PLA (1:100)	https://www.scbt.com/p/rnase-iii-drosha-antibody-c-7
PerCP/Cyanine5.5- CD45	BioLegend	157207	Flow Cytometry (1:100)	https://www.biolegend.com/en-us/products/percpcyanine55-anti-mouse-cd45-antibody-19248
Brilliant Violet 421-CD11b	BioLegend	101235	Flow Cytometry (1:100)	https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-human-cd11b-antibody-7163
APC-CD31	BioLegend	160209	Flow Cytometry (1:100)	https://www.biolegend.com/en-us/products/apc-anti-mouse-cd31-pecam-1-21557
PE-Cy5-FSP	BioLegend	370003	Flow Cytometry (1:100)	https://www.biolegend.com/en-us/products/pe-anti-human-s100a4-antibody-13082
AlexaFluor700-a-SMA	R&D	IC1420N	Flow Cytometry (1:100)	https://www.rndsystems.com/products/human-alpha-smooth-muscle-actin-alexa-fluor-700-conjugated-antibody-1a4_ic1420n

DOI [to be added]

FITC-ADAR1	Invitrogen	A20181	Flow Cytometry (1:100)	https://www.thermofisher.com/order/catalog/product/A20181
IRDye® 680RD Goat anti-Rabbit IgG	IRDye	926-68071	WB (1:15,000)	https://www.licor.com/bio/reagents/irdye-680rd-goat-anti-rabbit- igg-secondary-antibody
IRDye® 680RD Goat anti-Mouse IgG	IRDye	926-68070	WB (1:15,000)	https://www.licor.com/bio/reagents/irdye-680rd-goat-anti-mouse- igg-secondary-antibody
Goat anti-Mouse IgG (H+L), Texas Red-X	Invitrogen	T-862	2 µg/mL	https://www.thermofisher.com/antibody/product/Goat-anti-Mouse- IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/T-862
Goat anti-Rabbit IgG (H+L), FITC	Invitrogen	31635	2 µg/mL	https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit- IgG-H-L-Secondary-Antibody-Polyclonal/31635
IL-6	Cell Signaling Technology	D3K2N	WB (1:1000) IHC (1:100)	https://www.cellsignal.com/products/primary-antibodies/il-6-d3k2n-rabbit-mab/12153
TNF-a	Cell Signaling Technology	3707	WB (1:1000) IHC (1:100)	https://www.cellsignal.com/products/primary-antibodies/tnf-a-antibody/3707
Ubiquitin	Abcam	Ab7780	WB (1:1000) IP (1:100)	https://www.abcam.com/ubiquitin-antibody-ab7780.html
SMMHC	Abcam	ab125884	WB (1:1000) IHC (1:100)	https://www.abcam.com/smooth-muscle-myosin-heavy-chain-11-antibody-ab125884.html
LMOD1	Proteintech	15117-1-AP	WB (1:1000) IHC (1:100)	https://www.ptglab.com/products/LMOD1-Antibody-15117-1-AP.htm
Goat Anti-Rabbit IgG H&L (HRP)	Abcam	ab205718	IHC (1:4000)	https://www.abcam.com/goat-rabbit-igg-hl-hrp-ab205718.html

DOI [to be added]

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL
Ad-shADAR1	Top strand: 5'- CGC GTC GAT ACA ATC CTC AGT GTT CTC TGT ATG TAT TCA AGA GAT ACA TAC AGA GAA CAC TGA GGA TTG TAT CTT TTT TCC AAA-3' Bottom strand: 5'-AGC CAG GAT TTG GCG GAC CTT CTC GAA CCA CAA GTT CTC TGT GGT TCG AGA AGG TCC GCC AAA TCC TGG AAA AAA GTT TTC GA-3'	The PI's Lab	N/A
siDrosha	(unknown) s65706	Thermofisher	https://www.thermofisher.com/order/genome-database/details/sirna/s65706?CID=&ICID=&subtype=sirna_silencer_select
Si-Scramble	(unknown) 12935100	Thermofisher	https://www.thermofisher.com/order/catalog/product/12935100?SID=srch-srp-12935100

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
Mouse BMDM	Chen Lab	M/F	N/A
Mouse PEM	Chen Lab	M/F	N/A
Human PBMC	Chen Lab	M/F	N/A

Primers

Name of Target	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
Pre-miR-125b-1	5'-TGCGCTCCCCTCAGTCC-3'	5'-CAGCTCCCAAGAGCCTAACC-3'
Pre-miR-125b-2	5'-GCCTAGTCCCTGAGACCCTAACT-3'	5'-GCCTAGGTCCCAAGAACCTG-3'
Pre-miR-199a-1	5'-GCCATCCCAGTGTTCAGACTAC-3'	5'-GCCTAACCAATGTGCAGACTACT-3'
Pre-miR-199a-2	5'-TGGAAGCTTCAGGAGATCCTG-3'	5'-TGCTGGCCCTTGCCC-3'
Pre-miR-223	5'-AGTGTCACGCTCCGTGTATT-3'	5'-CATGAGCCACACTTGGGGTA-3'
Pri-miR125b-1	5'GAGCTTATTCATATTATTTTCCAATTTG-3'	5'-ATGCCTACTTTTACAGAAGCCA-3'
Pri-miR125b-2	5'-TGGATAGAATGGGGTGATTCT-3'	5'TGTACATATCTCTTACAACACTTTTATACG-3'
Pri-miR199a-1	5'-CAACTTTCTCCAGATGTGAGC-3'	5'-CTAAGTATCCCAGGTAGCAGC-3'

DOI [to be added]

Pri-miR199a-2	5'-TGTCTGAGAGCAGGCGAT-3'	5'-TCTTTTGAAAGTTAAGTTCACGG-3'
Pri-miR223	5'-GCAGTGTCACGCTCCGTGTA-3'	5'-AGCCACACTTGGGGTATTTGAC-3'
Pre-IKKβ	5'-AGATGGTACGGCTGCTGCTTCA-3'	5'-CGGTCCTCTCGTCCTCGTTCAT-3'
CYP	CAA CTC CCT CAA GAT TGT CAG CAA	GGC ATG GAC TGT GGT CAT GA
GAPDH	CGA CTG TGG ACA GCT CTA AT	CCT GAG CTA CAG AAG GAA TG
mmu-miR-125b-5P	5'-ACACTCCAGCTGGGTCCCTGAGACCCT AAC-3'	
mmu-miR-125b-1-3p	5'-ACACTCCAGCTGGGACGGGTTAGGCTCTTG-3'	
mmu-miR-125b-2-3p	5'-ACACTCCAGCTGGGACAAGTCAGGTTCTTG-3'	
mmu-miR-199a-5p	5'-ACACTCCAGCTGGGCCCAGTGTTCAGACTAC-3'	
mmu-miR-199a-3p	5'-ACACTCCAGCTGGGACAGTAGTCTGCACAT-3'	
mmu-miR-30c-5p-q	5'-ACACTCCAGCTGGGTGTAAACATCCTACACT-3'	
mmu-miR-30c-1-3p-q	5'-ACACTCCAGCTGGGCTGGGAGAGGGTTGTT-3'	
mmu-miR-34a-5p-q	5'-ACACTCCAGCTGGGTGGCAGTGTCTTAGCT-3'	
mmu-miR-34a-3p-q	5'-ACACTCCAGCTGGGAATCAGCAAGTATAC-3'	
mmu-mir-92-1-5p-q	5'-ACACTCCAGCTGGGAGGTTGGGATTTGTCGC-3'	
mmu-mir-92-1-3p-q	5'-ACACTCCAGCTGGGTATTGCACTTGTCCC-3'	
mmu-miR-103-3p-q	5'-ACACTCCAGCTGGGAGCAGCATTGTACAGGG-3'	
mmu-miR-103-1-5p-q	5'-ACACTCCAGCTGGGGCTTCTTTACAGTGCT-3'	
mmu-miR-103-2-5p-q	5'-ACACTCCAGCTGGGAGCTTCTTTACAGTGCT-3'	
mmu-miR-122-5p-q	5'-ACACTCCAGCTGGGTGGAGTGTGACAATGG-3'	
mmu-miR-122-3p-q	5'-ACACTCCAGCTGGGAAACGCCATTATCACA-3'	
mmu-miR-124-5p-q	5'-ACACTCCAGCTGGGCGTGTTCACAGCGGAC-3'	
mmu-miR-124-3p-q	5'-ACACTCCAGCTGGGTAAGGCACGCGGTG-3'	
mmu-miR-132-5P-q	5'-ACACTCCAGCTGGGAACCGTGGCTTTCGAT-3'	
mmu-miR-132-3P-q	5'-ACACTCCAGCTGGGTAACAGTCTACAGCCA-3'	
mmu-mir-146-5p-q	5'-	

DOI [to be added]

	ACACTCCAGCTGGGTGAGAACTGAATTCCA-3'	
mmu-mir-146-5p-q	5'-ACACTCCAGCTGGGTGAGAACTGAATTCCA-3'	
mmu-miR-223-5P-q	5'-ACACTCCAGCTGGGCGTGTATTTGACAAGCT-3'	
mmu-miR-223-3P-q	5'-ACACTCCAGCTGGGTGTCAGTTTGTCAAAT-3'	

Other

Description	**Source / Repository**	**Persistent ID / URL**
DMEM	Corning, 17- 207-CV	https://ecatalog.corning.com/life-sciences/b2b/US/en/Media,-Sera,-and- Reagents/Classical-Media/Dulbecco's-Modification-of-Eagle's-Medium- (DMEM)/Corning%C2%AE-DMEM-(Dulbecco%E2%80%99s-Modified- Eagle%E2%80%99s-Medium)/p/17-207-CV
FBS	FBS, R&D, S11550 H	https://www.rndsystems.com/products/fetal-bovine-serum-premium- select_s11550
L-glutamine	Hyclone, SH3003 4.01	https://www.thomassci.com/Laboratory-Supplies/Tissue-Culture- Media/_/HyClone-L-Glutamine-200-mM-Solution
Hematoxylin and eosin kit	American Masterech, KTHNE PT	https://www.statlab.com/ds-h-e-stain-kitkthnept.html
Elastica van Gieson kit	Sigma Aldrich, HT25A	https://www.sigmaaldrich.com/catalog/product/sigma/ht25a?lang=en&region=US
GM-CSF	R&D; 215-GM-050	https://www.rndsystems.com/products/recombinant-human-gm-csf-protein_215-gm
DYNAREX ultrasound transmission gel	D-1243	https://www.becklee.com/1243.html
Protease Inhibitor	Sigma Aldrich, P8340	https://www.sigmaaldrich.com/catalog/product/sigma/p8340?lang=en&region=US

DOI [to be added]

Phosphatase Inhibitor	Sigma Aldrich, P0044	https://www.sigmaaldrich.com/catalog/product/sigma/ p0044?lang=en&region=US
TNF-a	R&D; 210-TA-020/CF	https://www.rndsystems.com/products/recombinant-human-tnf-alpha-protein_210-ta
Trizol	Thermo Scientific, 10296028	https://www.thermofisher.com/order/catalog/product/ 10296028#/10296028

iScript cDNA kit	Bio-Rad, 1708890	https://www.bio-rad.com/en-us/sku/1708890-iscript-cdna-synthesis-kit-25-x-20-ul- rxns?ID=1708890
SYBR Green master mix	GeneCopoeia, QP001	https://www.genecopoeia.com/product/qpcr-products/qpcr-kits-and-primers/qpcr- primers/
MyTaq™ HS Red Mix	BIO-25048	https://www.bioline.com/mytaq-hs-red-mix.html
LPS	Sigma-Aldrich; L2630-100MG	https://www.sigmaaldrich.com/US/en/product/sigma/l2630
Duolink® In Situ Red Starter Kit Mouse/Rabbit	DUO92 101-1KT	https://www.sigmaaldrich.com/US/en/product/sigma/duo92101
Osmotic minipumps	AP- 2004	https://www.alzet.com/wp-content/uploads/2019/06/Specs-2004.pdf
Angiotensin II acetate salt	400647 3.0100	https://shop.bachem.com/4006473.html
Collagenase type I	Sigma-Aldrich, C0130	https://www.sigmaaldrich.com/US/en/product/sigma/c0130
Collagenase type XI	Sigma-Aldrich, C7657	https://www.sigmaaldrich.com/US/en/product/sigma/c7657?gclid=EAIaIQobChMI hMS0y_Dg8gIVPGpvBB0xWwy4EAAYASAAEgL_P_D_BwE
Hyaluronidase type I-s	Sigma-Aldrich, H11150 00	https://www.sigmaaldrich.com/US/en/product/sial/h1115000

DOI [to be added]

CD31 magnetic beads (Dynabeads)	Invitrogen 11155D	https://www.thermofisher.com/order/catalog/product/11155D#/11155D
Fc blocker	Invitrogen; 14-9161-73	https://www.thermofisher.com/antibody/product/Fc-Receptor-Binding-Inhibitor-Antibody-Polyclonal/14-9161-73
Live Cell Labeling Kit	Abcam, ab187967	https://www.abcam.com/live-cell-labeling-kit-green-fluorescence-ex405nm-cytopainter-ab187967.html
miRNA First Strand Synthesis Kit	Takara, 638315	https://www.takarabio.com/products/gene-function/cell-biology-assays/rna-interference/mirna/microrna-quantitation
Pierce™ Co-Immunoprecipitation Kit	ThermoFisher 26149	https://www.thermofisher.com/order/catalog/product/26149
Pierce™ Peroxidase IHC Detection Kit	ThermoFisher 36000	https://www.thermofisher.com/order/catalog/product/36000?SID=srch-srp-36000

DOI [to be added]