SUPPLEMENTAL MATERIAL

Major Resources Tables

Supplementary Table I.

Patient clinical information (n=10)

Patient	Gender	Age	Smoking status	Diameter (mm)	HL	HTN	CAD
ID1	male	65	Yes	75	No	No	No
ID2	male	76	No	45	Yes	Yes	Yes
ID3	male	67	No	61	No	No	No
ID4	male	68	Yes	63	No	No	No
ID5	male	52	Yes	40	No	No	No
ID6	male	70	Yes	64	No	No	No
ID7	male	66	Yes	53	No	No	No
ID8	male	70	Yes	50	No	No	No
ID9	male	62	Yes	79	Yes	Yes	No
ID10	male	56	No	54	No	No	No

HL = Hyperlipidemia
HTN = Hypertension
CAD = coronary artery disease

Supplementary Table II.

Primer sequences for quantitative real-time PCR (from 5' to 3')

Gene		Sequence 5'- 3'
TNF-a	Forward	GAGGCCAAGCCCTGGTATG
(human)	Reverse	CGGGCCGATTGATCTCAGC
TNF-a	Forward	ACTCCAGGCGGTGCCTATGT
(mouse)	Reverse	GTGAGGGTCTGGGCCATAGAA
IL-6	Forward	CCCTGAGAAAGGAGACATGTAAC
(human)	Reverse	CTCTTTGCTGCTTTCACACATG
IL-6	Forward	TGATGGATGCTACCAAACTGGA
(mouse)	Reverse	TGTGACTCCAGCTTATCTCTTGG
IL-18	Forward	TCTTCATTGACCAAGGAAATCGG
(human)	Reverse	TCCGGGGTGCATTATCTCTAC
IL-18	Forward	TGACCAAGTTCTCTTCGTTGACAA
(mouse)	Reverse	CACAGCCAGTCCTCTTACTTCAC
IL-1b	Forward	CCTGAGCTCGCCAGTGAAA
(human)	Reverse	TCCTGGAAGGAGCACTTCATCT
IL-1b	Forward	TGCCACCTTTTGACAGTGATG
(mouse)	Reverse	GTGCTGCTGCGAGATTTGAA
GAPDH	Forward	GCACCGTCAAGGCTGAGAAC
(human)	Reverse	TGGTGAAGACGCCAGTGGA
GAPDH	Forward	GTGTTCCTACCCCCAATGTGT
(mouse)	Reverse	ATTGTCATACCAGGAAATGAGCTT
miR-146a-5p	Forward	CGCGTGAGAACTGAATTCCA
(human/mouse)	Reverse	AGTGCAGGGTCCGAGGTATT
U6	Forward	CTCGCTTCGGCAGCACA
(human/mouse)	Reverse	AACGCTTCACGAATTTGCGT
TRAF6	Forward	TTTGCTCTTATGGATTGTCCCC
(human)	Reverse	CATTGATGCAGCACAGTTGTC
TRAF6	Forward	AGTGCCCAGTTGACAATGAAA
(mouse)	Reverse	CACTTTACCGTCAGGGAAAGAAT
RCAN1	Forward	TTGTGTGGCAAACGATGATGT
(mouse)	Reverse	CCCAGGAACTCGGTCTTGT
SIAH2	Forward	CCAATGCCGCCAGAAGTTAAG
(mouse)	Reverse	CAGGGAAACAGAACTGCCGA

Supplementary Table III.

Antibodies for Western blots

Antibody	Vendor or Source	Catalog #	Dilute Proportion
anti-NLRP3 (human)	Proteintech	191771-1-AP	1:1000
anti-NLRP3 (mouse)	CST	15101S	1:1000
anti-GSDMD (human/mouse)	HuaBio	HA721144	1:1000
anti-GSDMD (mouse)	Abclonal	A18281	1:1000
Anti-Caspase1 p10 (mouse)	Invitrogen	PA5-105049	1:1000
anti-Caspase 1(mouse)	Abcam	Ab179515	1:1000
anti-TRAF6	Abclonal	A0973	1:1000
anti-GAPDH	Proteintech	60004-1-Ig	1:5000

Supplementary Table IV.

Antibodies for immunofluorescence staining

Antibody	Vendor or Source	Catalog #	Dilute Proportion
Anti-Caspase1 p10 (human and mouse)	Invitrogen	PA5-105049	1:200
Anti-NLRP3 (human and mouse)	Abclonal	A5652	1:100
Anti-GSDMD (mouse and human)	Abclonal	A18281	1:200
Anti-SMC a-actin (mouse)	Proteinthch	67735-1-Ig	1:400
Anti-CD68 (mouse)	Abcam	Ab955	1:100
Anti-CD80 (mouse)	Abcam	Ab254579	1:50
Anti-CD206 (mouse)	Abcam	Ab64693	1:500

Supplementary Figure legends

Supplementary Figure 1 PTE enhances the number of SMCs in the aortic wall. Representative immunofluorescence staining with antibodies against α-actin (red colour) and nuclear counterstaining with DAPI (blue colour) in PPE-infused C57BL/6 mice. Scale bar=100 μm and 50 um. PPE, porcine pancreatic elastase; PTE, pterostilbene. SMCs, smooth muscle cells.

Supplementary Figure 2 PTE reduces the levels of the proinflammatory cytokines in mouse aortas and plasma. (A-D) PCR analysis of TNF-α, IL-6, IL-1β, and IL-18 in aortas from PPE-infused C57BL/6 mice. $^{*}p < 0.05$; n = 7 to 11 mice/group (parametric unpaired t test). (E-H) PCR analysis of TNF-α, IL-6, IL-1β and IL-18 expression in the aortas of Ang II-infused ApoE-/- mice. $^{*}p < 0.05$; n=9 to 10 mice/group (parametric unpaired t test). (I-L) Plasma concentrations of TNF-α, IL-6, IL-1β, and IL-18 in Ang II-infused ApoE-/- mice in the two groups. $^{*}p < 0.05$; n=6 to 9 mice/group (parametric unpaired t test). PPE, porcine pancreatic elastase; PTE, pterostilbene.

Supplementary Figure 3 Oral PTE administration does not alter systolic blood pressure or body weight gain in mice. (A) Systolic blood pressure on day 28 after Ang II infusion. p=0.2025, n=7 to 9 mice/group (parametric unpaired t test). ns for not significant vs. vehicle group. (B) No difference in body weight at baseline (p=0.4984) and at 28 days (p=0.1850) following treatment with vehicle or PTE, n=7 to 10 mice/group (two-way ANOVA). ns for not significant vs. vehicle group. (C-F)

The level of blood lipids in mice after treatment with vehicle or PTE. $^{***}p < 0.001$, $^{*}p < 0.05$; n = 6 to 9 mice/group (parametric unpaired t test). All data are means±SD. CHOL, cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; PTE, pterostilbene; TG, triglyceride.

Supplementary Figure 4 Macrophage pyroptosis is activated in human and murine AAA tissues. (A and B) Western blot analysis of pyroptosis-related proteins in human AAA and normal aortas (NA) from organ donor samples. $^{**}p<0.01$ and $^{*}p<0.05$; n=8 to 9 per group (parametric paired t test). (C) PCR analysis of inflammatory cytokines in human NA and AAA samples. $^{***}p<0.001$ and $^{*}p<0.05$; n=9 to 13 per group (parametric paired t test). (D) Representative photographs showing the macroscopic features of the aortas at baseline (day 0) and 14 days after PPE infusion in C57BL/6 mice. (E and F) Representative images and quantification of immunofluorescence staining with antibodies against pyroptosis-related proteins (NLRP3, GSDMD and Caspase1 p10) conjugated with Alexa Fluor 488 (green colour) and antibodies against α-actin and CD68 conjugated with Alexa Fluor 594 (red colour); the nucleus was counterstained with DAPI (blue colour) in PPE-infused C57BL/6 mice. Scale bar=200 μm. (G and H) Aortic sections were prepared from human AAAs, representative images and quantification of immunofluorescence stained with antibodies against α-actin or CD68 (macrophages) conjugated with Alexa Fluor 595 (red), and antibodies against related pyroptosis proteins conjugated (NLRP3, GSDMD and Caspase1 p10) with Alexa Fluor 488 (green), and the nucleus

was counterstained with DAPI (blue). Scale bar=200 μm. AAA, abdominal aortic aneurysm; NA, normal aorta; PPE, porcine pancreatic elastase.

Supplementary Figure 5 The level of macrophage markers was decreased in PTE-treated mouse aortas compared with vehicle-treated mouse aortas. Aortic sections were prepared from PPE-induced AAAs, stained with antibodies against CD68 (macrophages) and CD80 (M1 macrophage marker) conjugated with Alexa Fluor 594 (red), and the nucleus was counterstained with DAPI (blue). Scale bar=100 μm. PTE, pterostilbene.

Supplementary Figure 6 PTE protects against LPS-induced ROS stress in macrophage. Raw 264.7 cells were pretreated with or without PTE (2 μM, 4 μM or 6 μM) for 24 h, followed by exposure to LPS (1 μg/mL). After 6 h, ROS levels were measured using DCFH-DA. Scale bar=200 μm. LPS, lipopolysaccharide; PTE, pterostilbene.

Supplementary Figure 7 PTE inhibits macrophage pyroptosis in vivo. Western blot analysis of pyroptosis-related proteins in the peritoneal macrophages of Ang II-infused ApoE-/- mice treated with PTE (4 μM, 24 h) or vehicle and then stimulated with LPS (1 μg/mL, 24 h) and ATP (5 mM, 2 h). $^{**}p<0.01$ and $^{*}P<0.05$ vs. vehicle group; n=3 per group (parametric unpaired t test). ATP, adenosine triphosphate; LPS, lipopolysaccharide; PTE, pterostilbene.

Supplementary Figure 8 PTE suppresses macrophage pyroptosis and exerts the strongest effect among the four polyphenols. (A-E) Western blot analysis of pyroptosis-related proteins in RAW 264.7 macrophages that were pretreated with vehicle or PTE (4 μM, 24 h), RSV (10 μM, 24 h), OXY (10 μM, 24 h), or RHA (10 μM, 24 h) and then stimulated with LPS (1 μg/mL, 24 h) and ATP (5 mM, 2 h). $^{\#\#}p<0.01$ and $^{\#}p<0.05$ vs. NC group; $^{**}p<0.01$ and $^{*}p<0.05$ vs. LPS group; n=3 per group (parametric unpaired t test). (F-H) PCR analysis of IL-1β, IL-6, and TNF-α in Raw 264.7 cells treated as described in (a). $^{\#\#\#}p<0.001$ vs. NC group; $^{***}p<0.001$, $^{**}p<0.01$ and $^{*}p<0.05$ vs. LPS group. n=3 to 6 per group (parametric unpaired t test). ATP, adenosine triphosphate; LPS, lipopolysaccharide; OXY, oxyresveratrol; PTE, pterostilbene. RHA, rhapontigenin; RSV, resveratrol.

Supplementary Figure 9 MiR-146a-5p inhibitor weakened the inhibitory effect of PTE on the uptake of YO-PRO-1 dye. Raw 264.7 macrophages that were pretreated with the miR-146a-5p inhibitor (50 nM) or NC inhibitor (50 nM) for 48 h, treated with PTE (4 μM, 24 h) and then stimulated with LPS (1 μg/mL, 24 h) and ATP (5 mM, 2 h). and then cells were stained with YO-PRO-1 dye (green). Hoechst (blue) was used to stain nucleus. Raw 264.7 macrophages treated with 0.1% Triton were used to be the positive control group. Scale bar=100 μm. ATP, adenosine triphosphate; LPS, lipopolysaccharide; PTE, pterostilbene.

Supplementary Figure 10 MiR-146a-5p inhibitor weakened the inhibitory effect of PTE on macrophage pyroptosis. Raw 264.7 macrophages that were pretreated

with the miR-146a-5p inhibitor (50 nM) or NC inhibitor (50 nM) for 48 h, treated with PTE (4 μM, 24 h) and then stimulated with LPS (1 μg/mL, 24 h) and ATP (5 mM, 2 h), pyroptosis levels were measured using Calcein AM (green)/ PI (red) double-stained. Raw 264.7 macrophages treated with 0.1% Triton were used to be the positive control group. Scale bar=100 μm. ATP, adenosine triphosphate; LPS, lipopolysaccharide; PI, propidium iodide; PTE, pterostilbene.

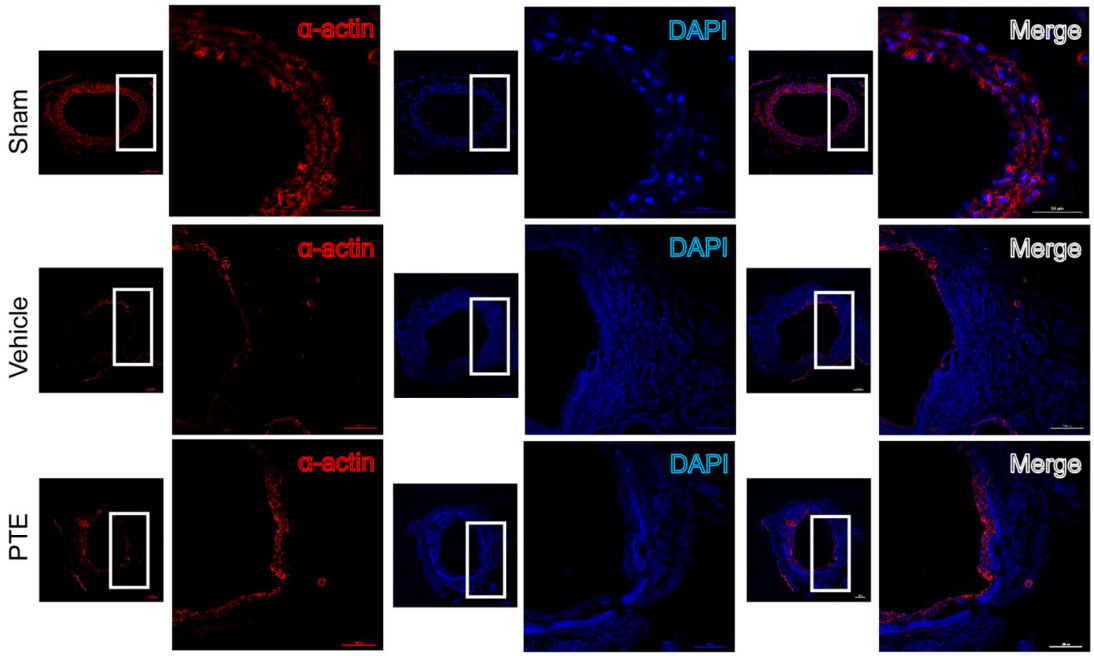
Supplementary Figure 11 (A) Schematic view of the generation of miR-146a-5p$^{-/-}$ mice by CRISPR/Cas-mediated genome engineering. (B) Genotyping of heterozygous miR-146a-5p-knockout mice by PCR analysis. (C) Relative miR-146a-5p expression in macrophages of normal mice and macrophage miR-146a-5p-knockout mice (3 months, male; n=4 mice per genotype). The data were analysed using parametric unpaired t tests, $^{***}p<0.001$. KO, miR-146a-5p-knockout mice.

Supplementary Figure 12 qPCR analysis of RCAN1 and SIAH2 in Raw 264.7 macrophages. (A and B) Raw 264.7 macrophages were pretreated with vehicle or PTE (4 μM, 24 h) and then stimulated with LPS (1 μg/mL, 24 h) and ATP (5 mM, 2 h), $^{**}p<0.01$ and $^{*}p<0.05$; n=8-12 per group (parametric unpaired t test). (C and D) Raw 264.7 macrophages were pretreated with the miR-146a-5p inhibitor (50 nM) or NC inhibitor (50 nM) for 48 h, treated with PTE (4 μM, 24 h) and then stimulated with LPS (1 μg/mL, 24 h) and ATP (5 mM, 2 h), ns for not significant, n=7-8 per group (parametric unpaired t test). ATP, adenosine triphosphate; LPS,

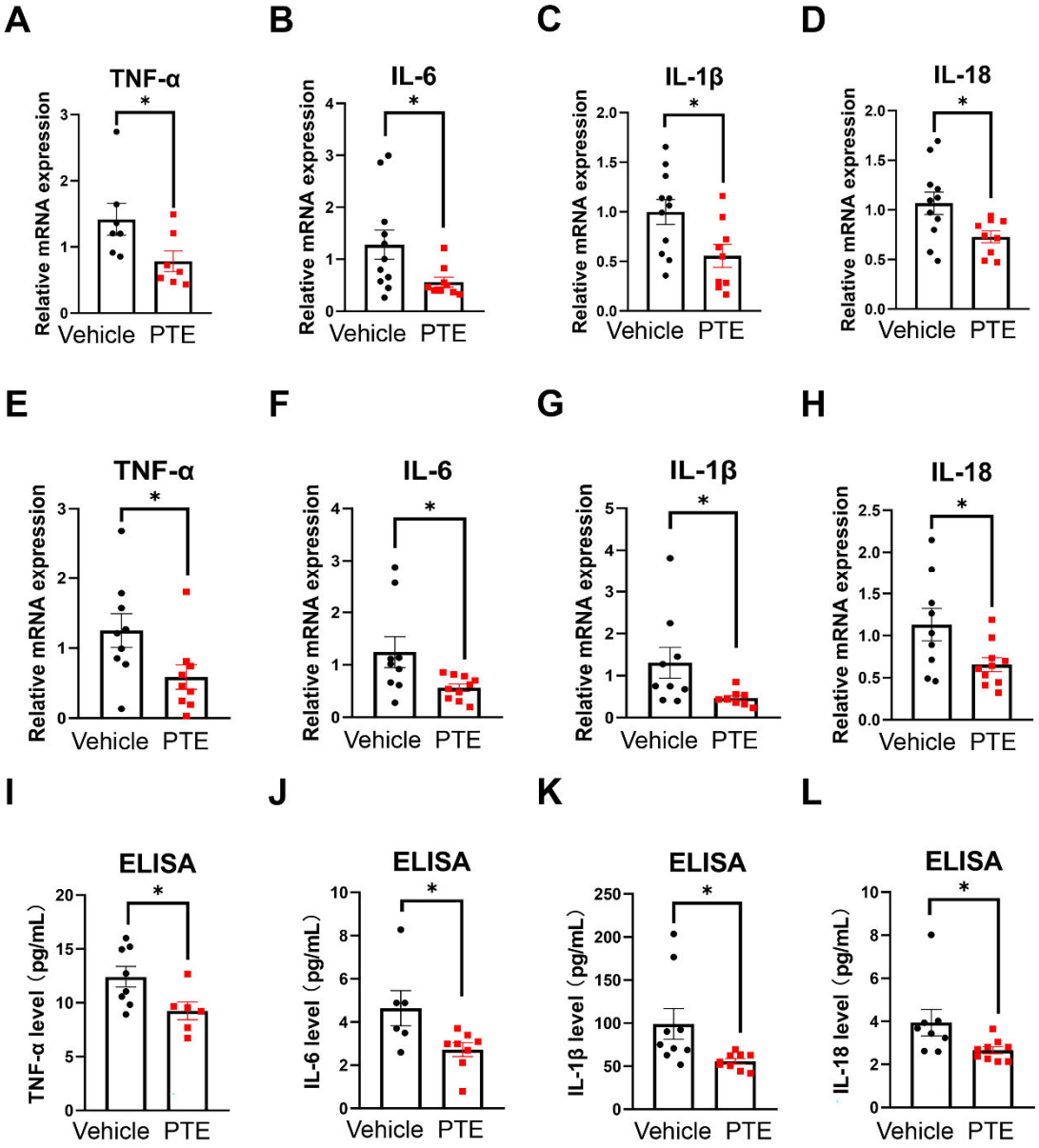
lipopolysaccharide; PTE, pterostilbene.

Supplementary Figure 13 qPCR analysis of TRAF6, TNF-α, IL-6, IL-1β, and IL-18 expression in mouse peritoneal macrophages and mouse aortas. (A-D) Peritoneal macrophages from PPE-infused C57BL/6 mice after transfection with AAV carrying a TRAF6 overexpression plasmid (AAV-TRAF6) or a control plasmid (AAV-GFP) and then stimulated with LPS (100 ng/mL, 24 h), $^{*}p < 0.05$; n = 8 per group (parametric unpaired t test). (E-I) Mouse aortas from PPE-infused C57BL/6 mice after transfection with AAV carrying a TRAF6 overexpression plasmid (AAV-TRAF6) or a control plasmid (AAV-GFP), $^{*}p<0.05$; n=6-8 per group (parametric unpaired t test). AAV, adeno-associated virus; LPS, lipopolysaccharide; PPE, porcine pancreatic elastase.

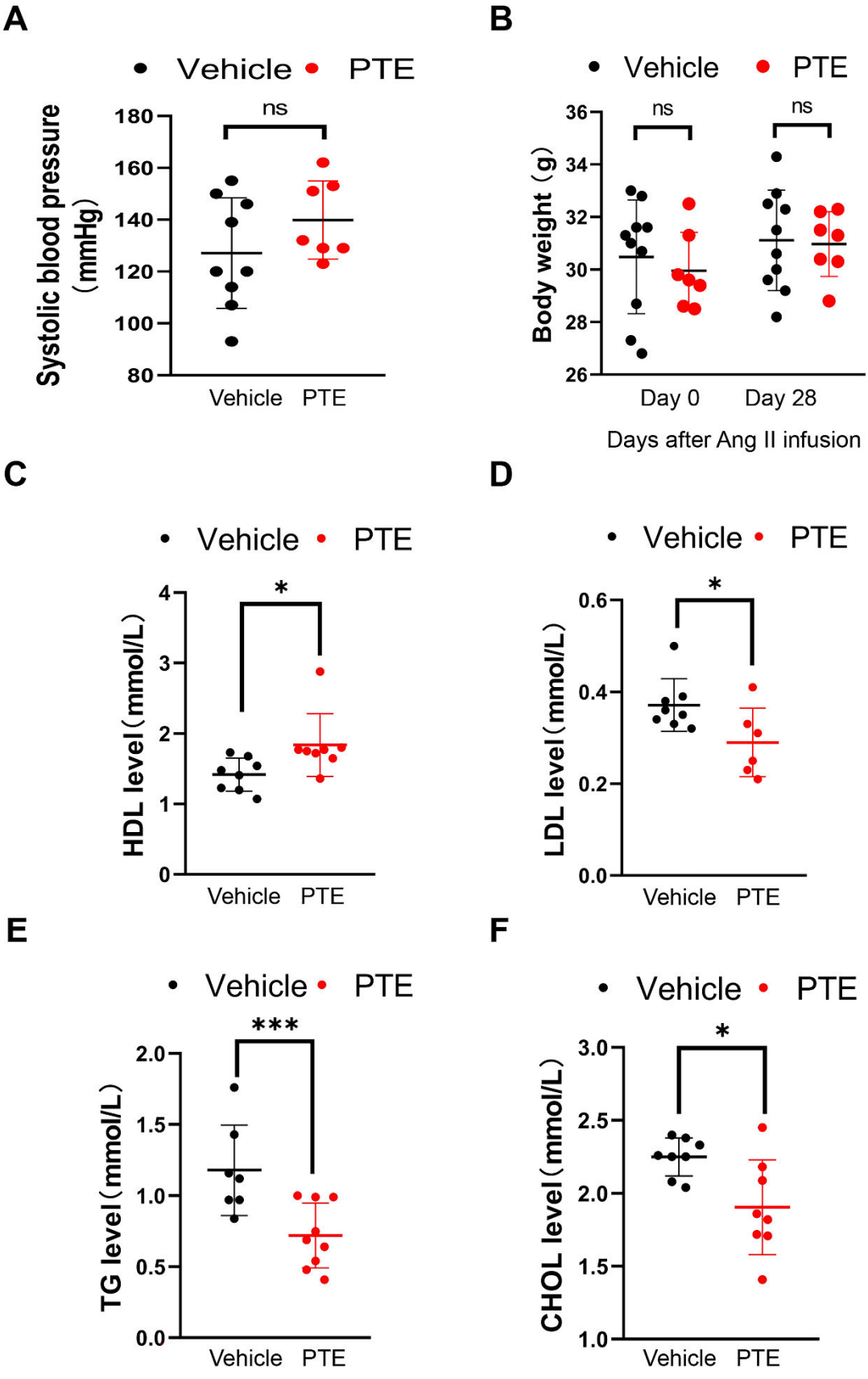
Supplementary Figure 1



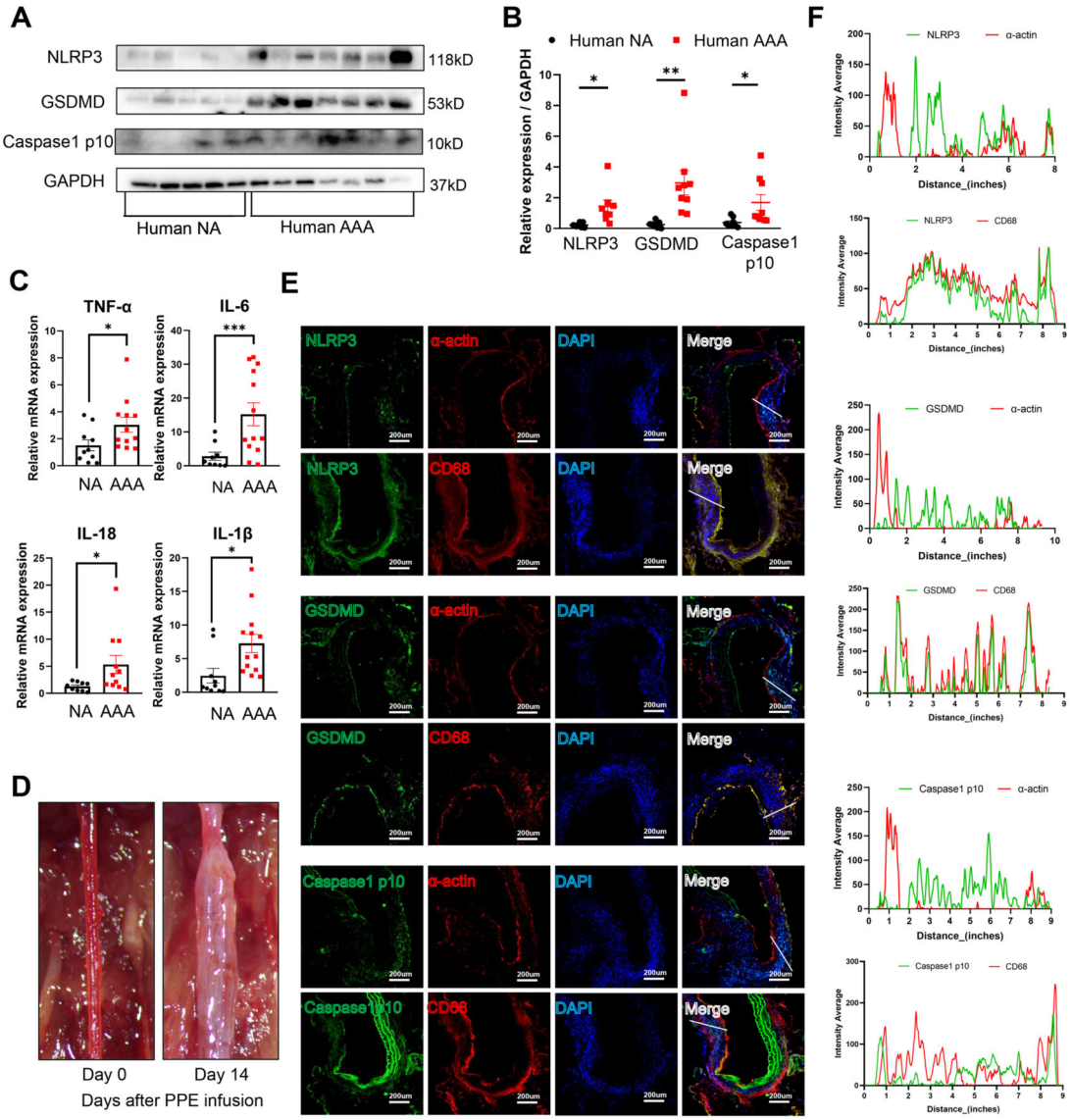
Supplementary Figure 2



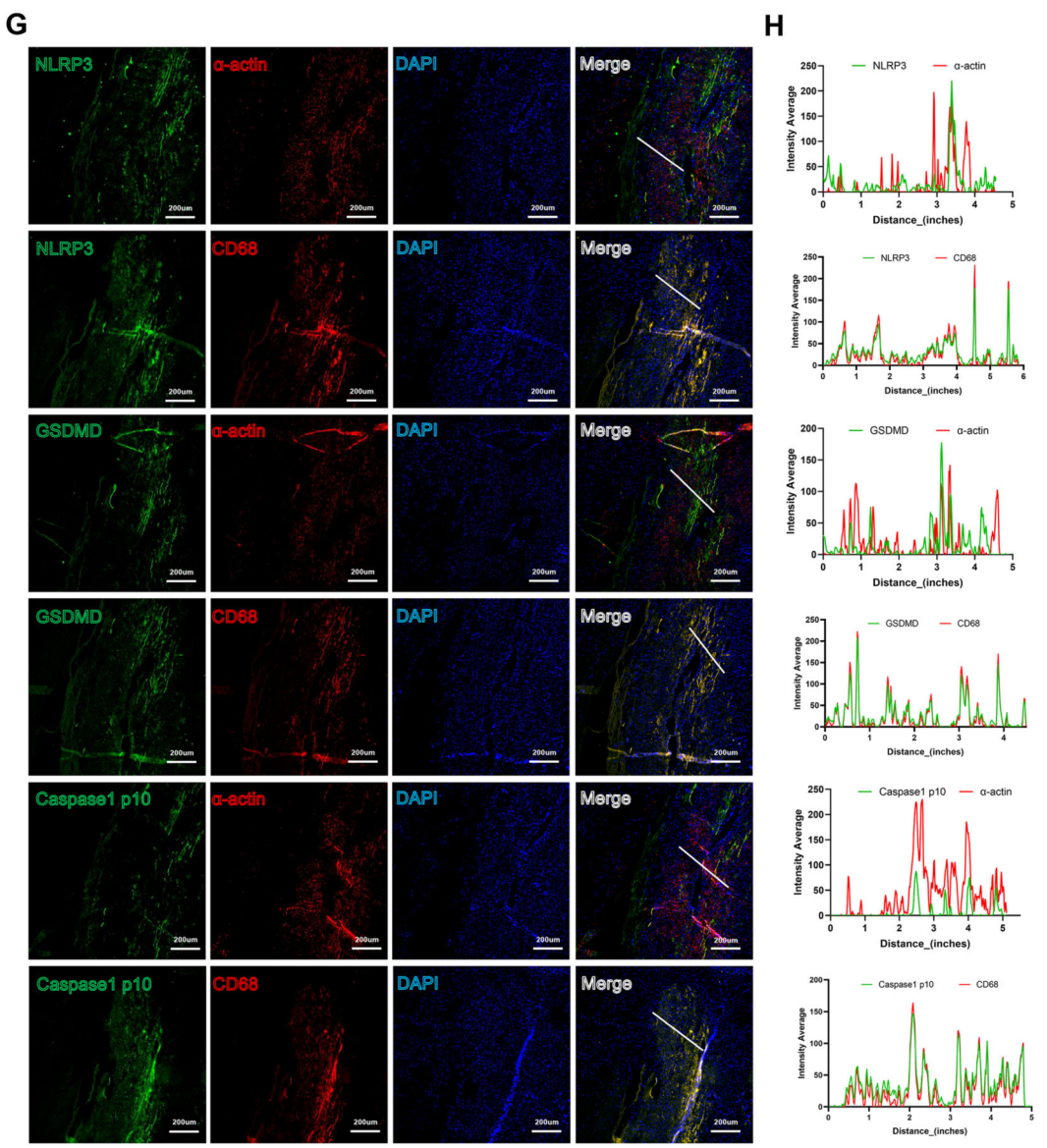
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 4 Continued



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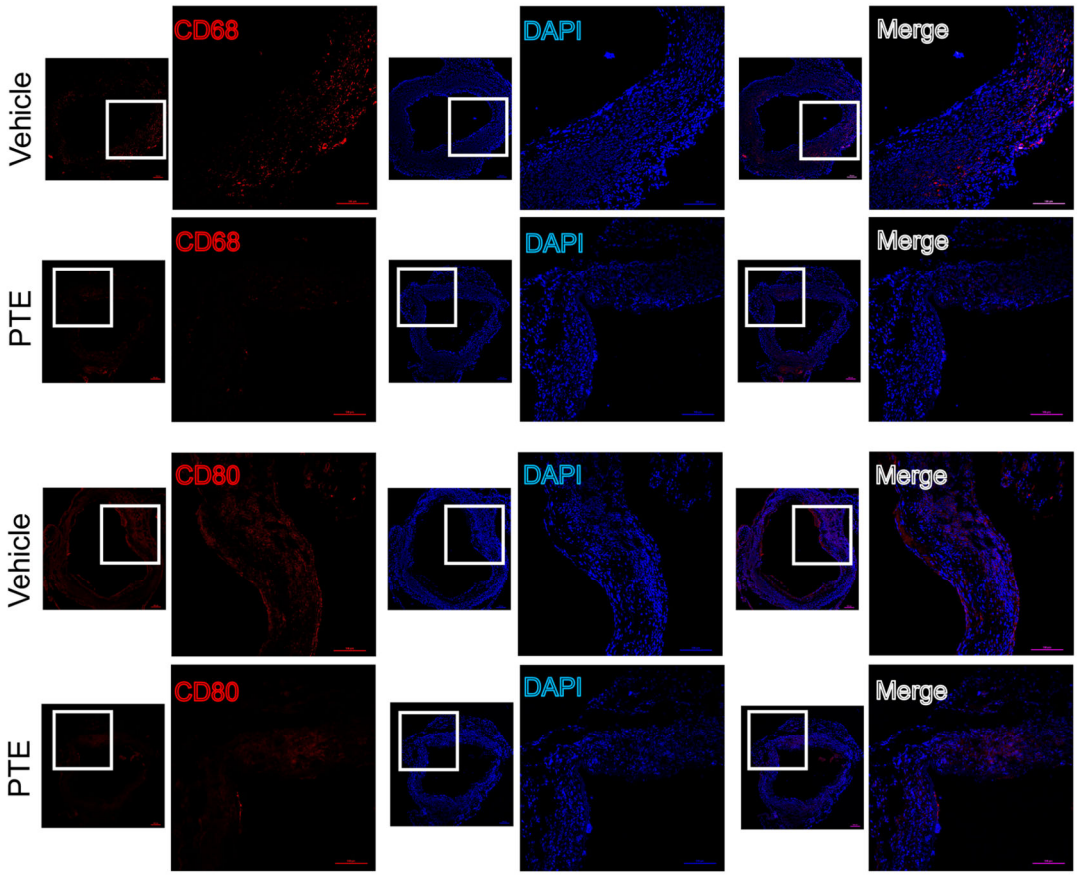

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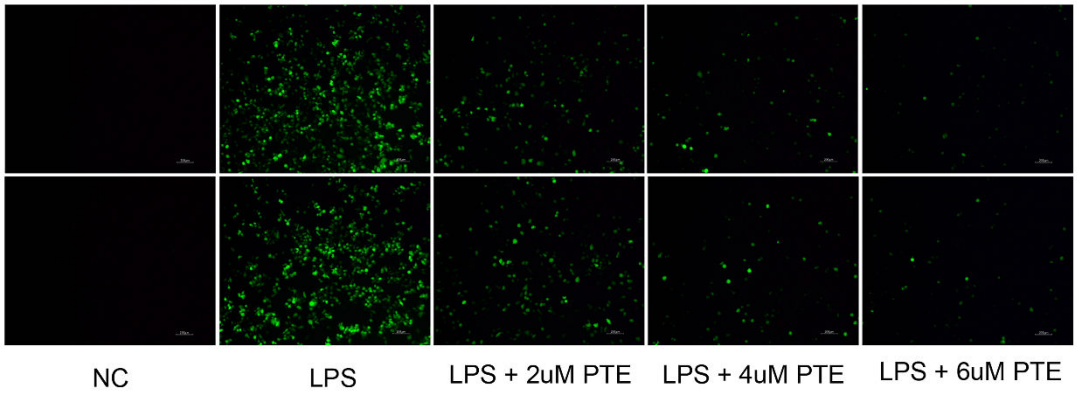

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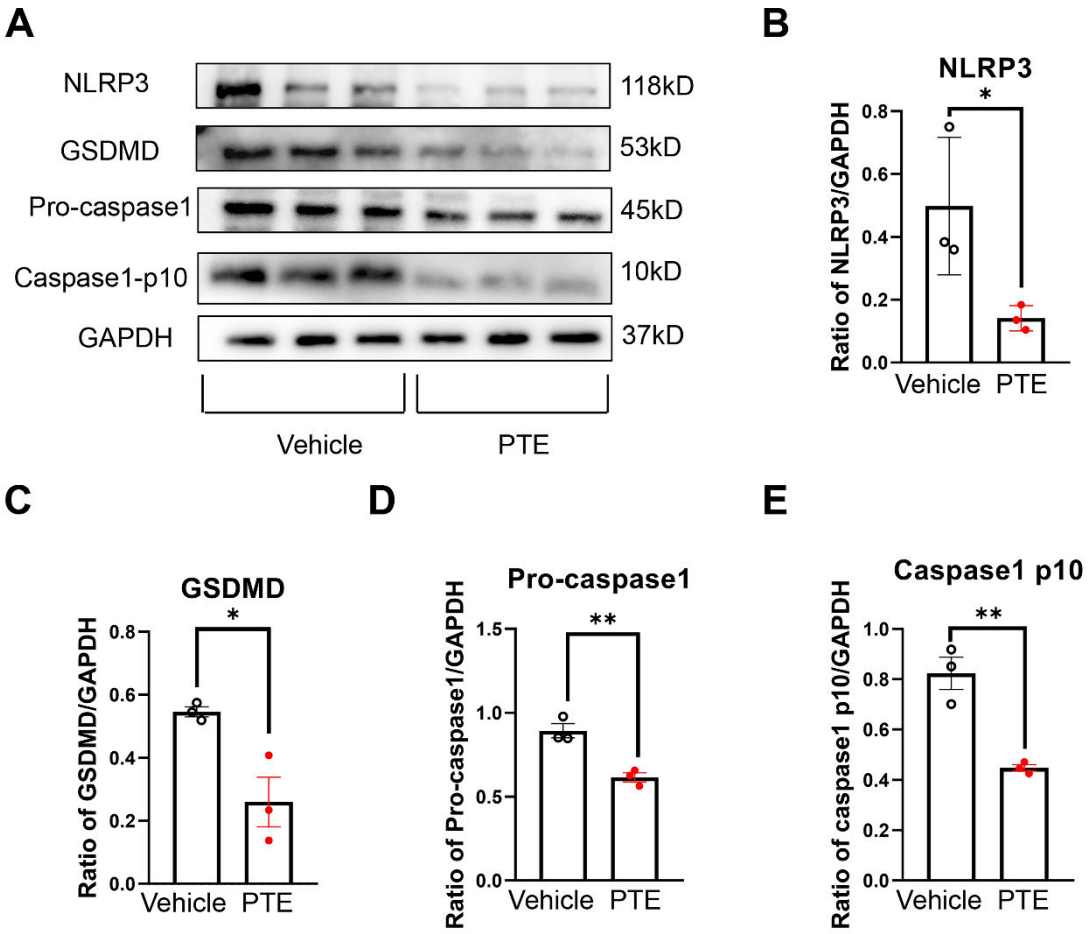
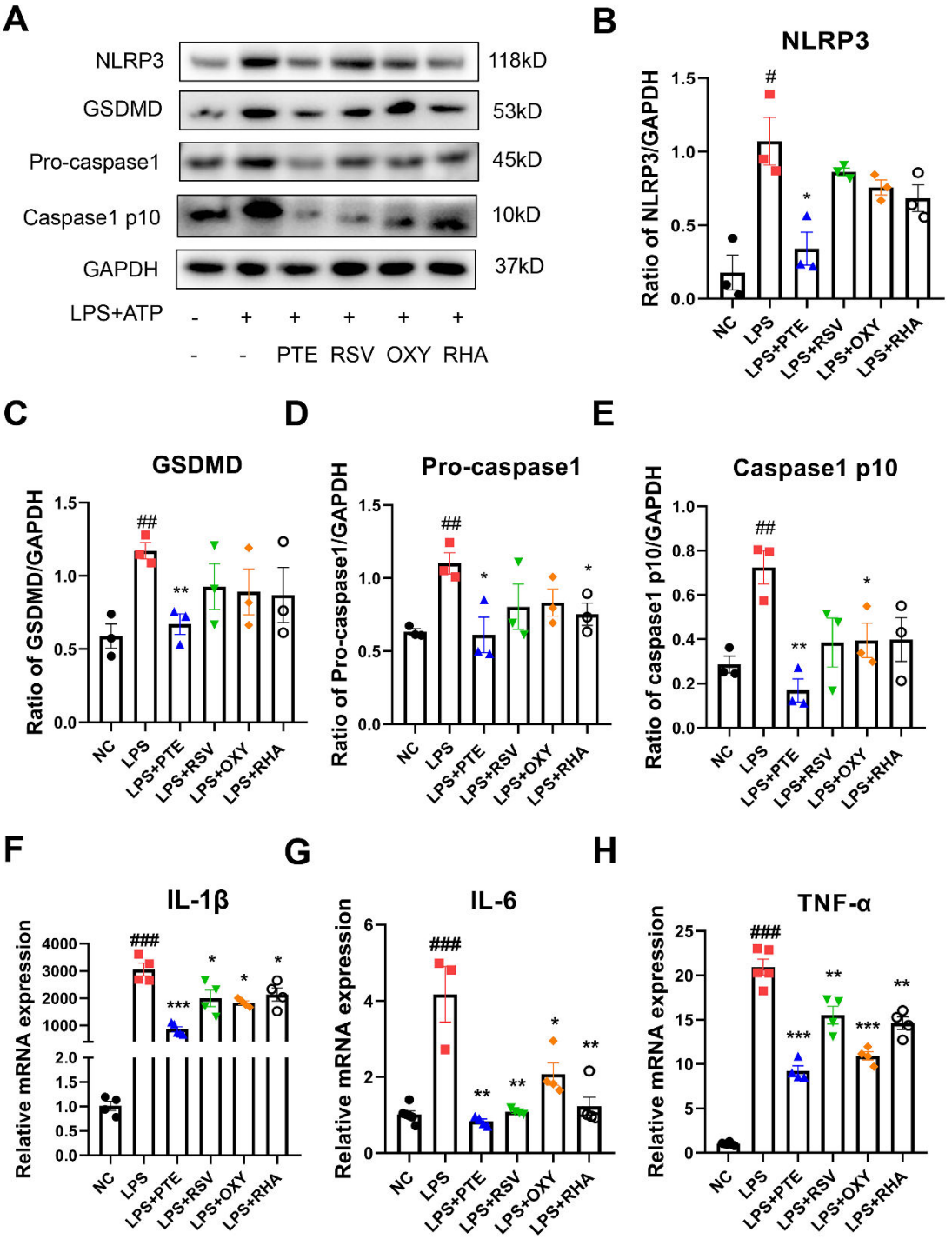
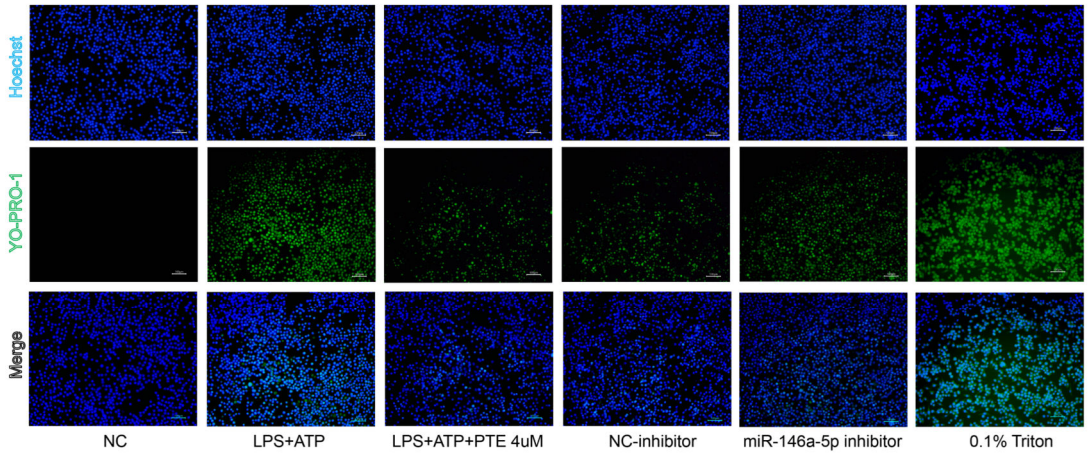


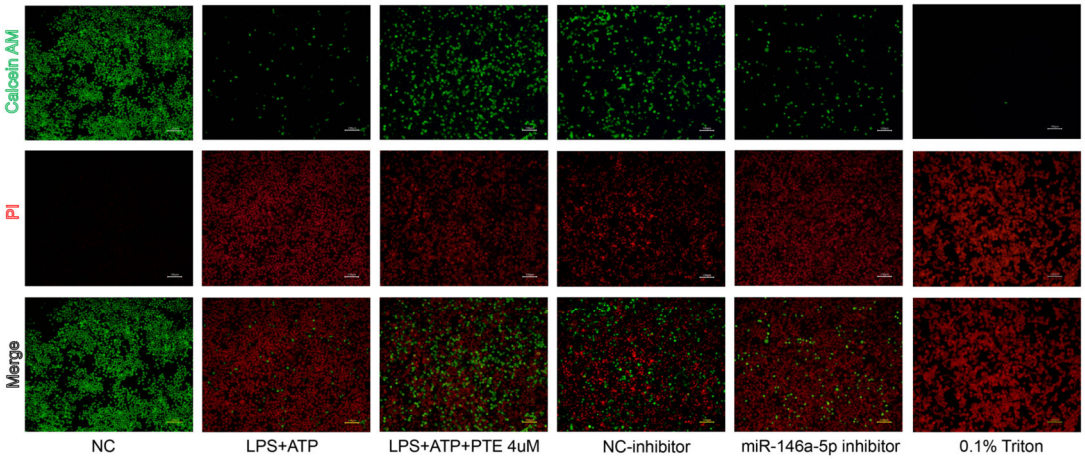
Supplementary Figure 8



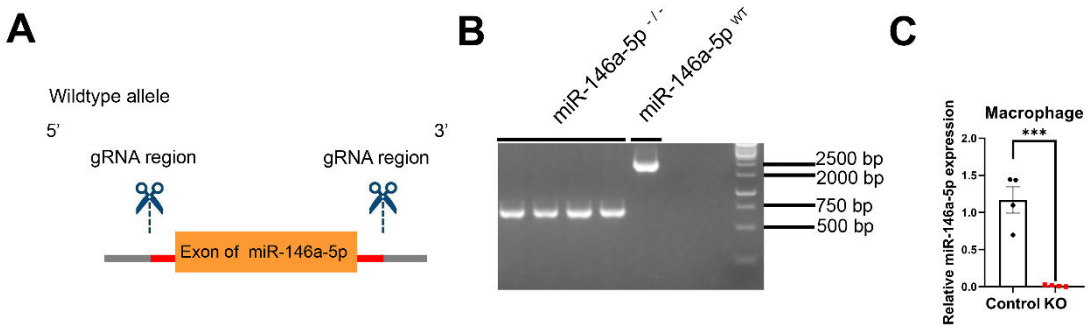
Supplementary Figure 9



Supplementary Figure 10



Supplementary Figure 11



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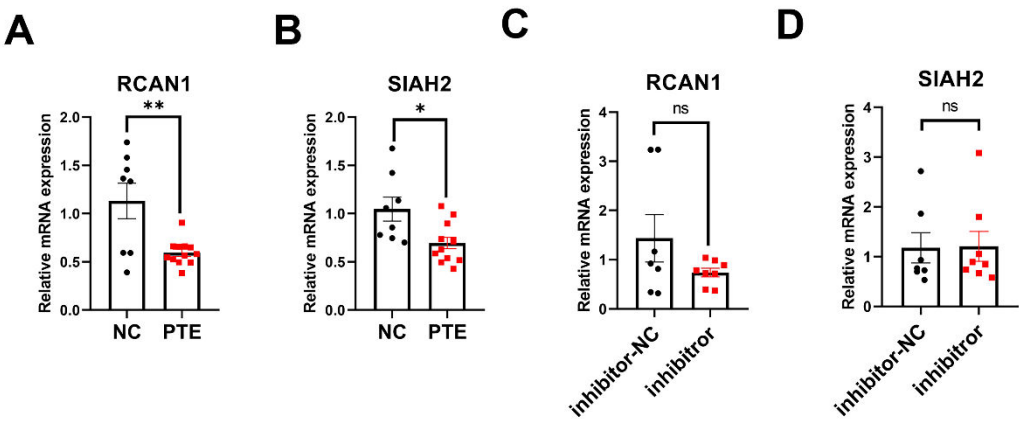

Supplementary Figure 13