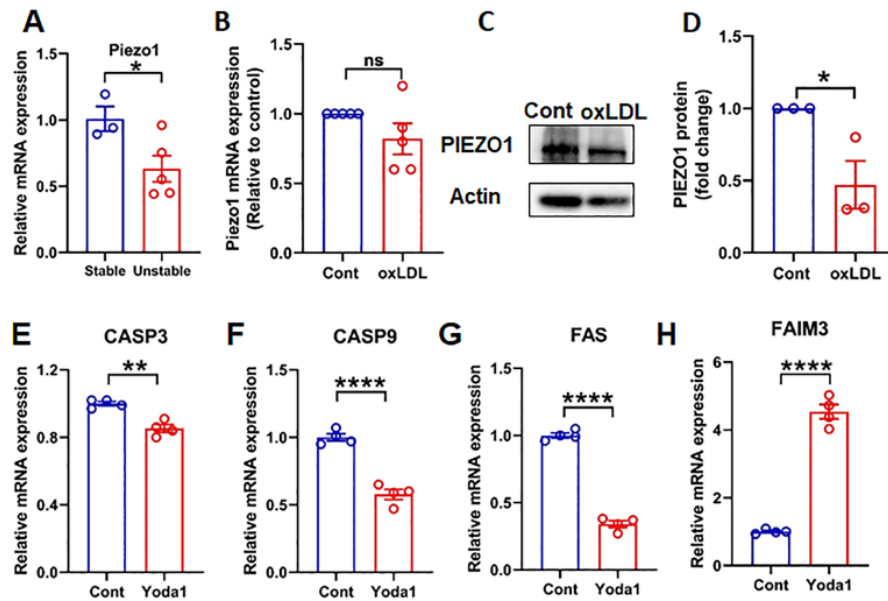
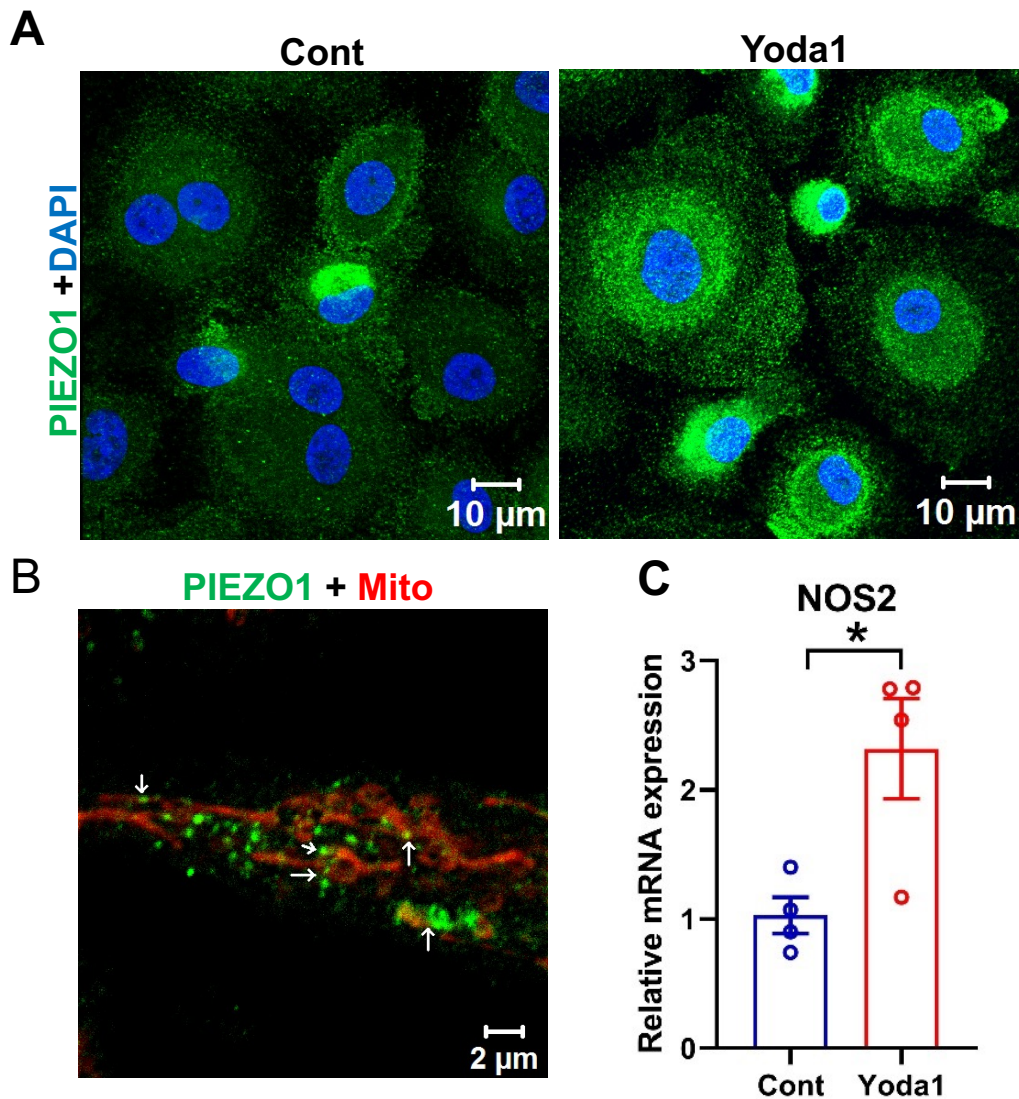


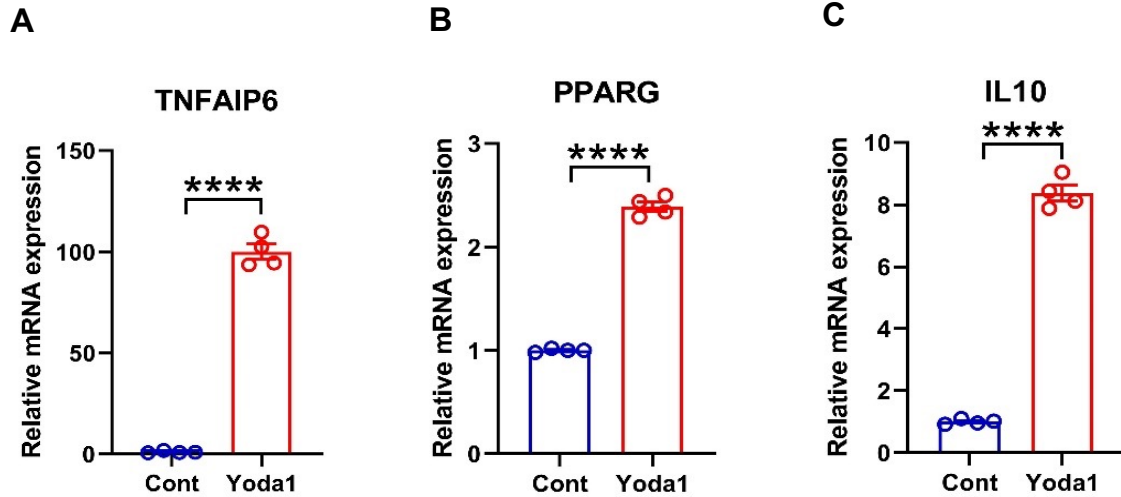
Supplementary Material



Supplementary figure 1. PIEZO1 regulation in macrophages. (A) *PIEZO1* mRNA expression is higher in stable plaque compared with unstable plaque (n=3-5). (B-D) Exposing monocyte-derived macrophages to 25 μ g/ml oxLDL for 72 h did not affect the *PIEZO1* mRNA level, but decreased *PIEZO1* protein level (n=3-5, paired test, * P <0.05). Expression levels of genes involved in apoptosis (E) *CASP3*, (F) *CASP9*, (G) *FAS*, (H) *FAIM3* are regulated in THP1 cells exposed to Yoda1 24 h compared to only vehicle treated controls (n=4). Unpaired t-test, * P <0.05, ** P <0.01, **** P <0.0001.

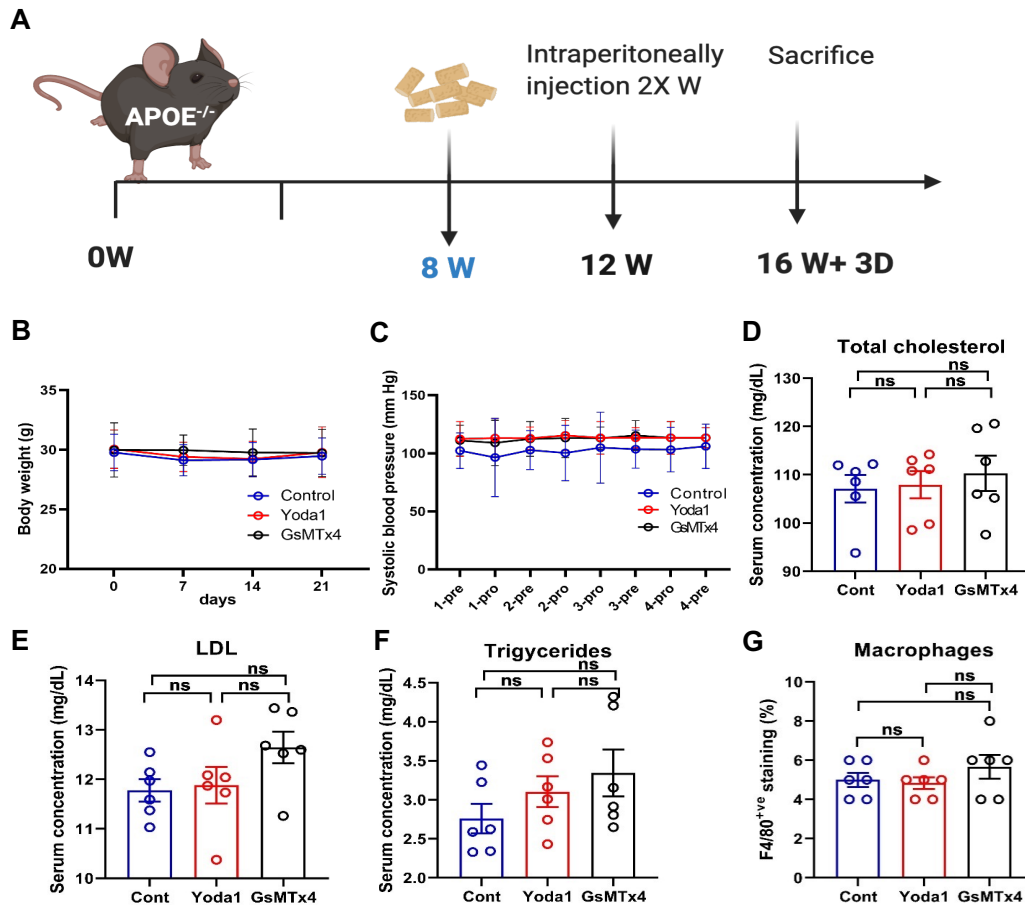


Supplementary figure 2. PIEZO1 affects macrophages phenotype and function. (A) Yoda1 increases PIEZO1 expression or re-localization in macrophages. (B) PIEZO1 (green) is distributed at the plasma membrane, cytoplasm and is also associated with the mitochondria (red) in macrophages. Arrows indicate regions of co-localization. (C) NOS2 mRNA levels is reduced in THP1 cells in response to Yoda1 exposure for 24 h compared to only vehicle treated controls (n=4, unpaired t-test, * $P < 0.05$)



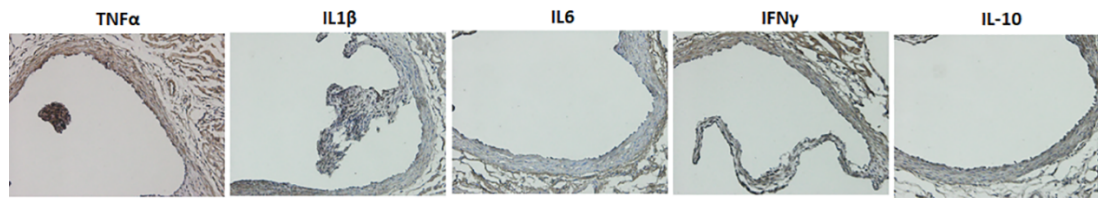
Supplementary figure 3. PIEZO1 activation casts anti-inflammation effects in macrophages.

Expression of inflammatory genes markers: **(A)** *TNFAIP6* **(B)** *PPARG* and **(C)** *IL10* in THP1 cells in response to Yoda1 exposure for 24 h compared to only vehicle treated controls (n=4, unpaired t-test, **** $P < 0.0001$).



Supplementary figure 4. Mouse systemic parameters upon PIEZO1 modulation in vivo

(A) Schematic representation of experimental plan shows 8w old *ApoE*^{-/-} mice fed with a high fat diet for 4w, then administered intraperitoneal injection of Piezo1 activator Yoda1, inhibitor GsMTx4 or saline solvent as control for 4w (twice a week), followed by sacrifice and tissue collection. Following parameters remained unchanged after treatment of *ApoE*^{-/-} mice with Yoda1, GsMTx-4 or saline control: (B) animal's body weight, (C) systolic blood pressure, (D) total serum cholesterol, (E) serum LDL and (F) serum triglyceride. (G) number of macrophages in the plaque.



Supplementary figure 5. Controls for inflammatory proteins depicted in Figure 5. Representative images of aorta (without plaque) stained with the same protocol as in Figure 5A depicting specificity of primary antibodies labeling TNF α , IL1 β , IL6, IFN γ and IL10, which indicate some basic expression of these markers on the normal vessel wall.

Online table1**Materials are used for human studies**

Phalloidin Conjugated to Alexa Flour 488	ThermoFisher	A12379
Phalloidin Conjugated to Alexa Flour 555	ThermoFisher	A30106
FLuo-4 AM	ThermoFisher	F14201
PIEZO1 antibody	abcam	Ab128245
DRP1 antibody	abcam	Ab56788
pDRP1 antibody	Sigma-Aldrich	SAB4301399
MitoSOX	ThermoFisher	M36008
MtroTracker deep red	ThermoFisher	M22426
Yoda1	Millipore Sigma	SML 1558
GsMTx-4	abcam	Ab141871
pHrodo™ Green Zymosan Bioparticles™	ThermoFisher	P35365
Lucigenin-enhanced chemiluminescence	Millipore Sigma	M8010
LysoTracker red	ThermoFisher	L7528
CellEvent Caspase-3/7 Green Detection Reagent	ThermoFisher	C10423
H2DCFDA	ThermoFisher	D399

Materials are used for animal studies

oxLDL	Yiyuan Biotechnologies	Guangzhou,China
TNF- α antibody	Bioss Inc. (Beijing, China)	bs-2081R
IL-10 antibody	Bioss Inc. (Beijing, China)	bs-0698R
IL-6 antibody	Bioss Inc. (Beijing, China)	bsm-10807m
IFN- γ antibody	Bioss Inc. (Beijing, China)	bs0480R
iNOS antibody	Bioss Inc. (Beijing, China)	bs0162R
PIEZO1 antibody	Abcam	ab128245
CD206 antibody	Santa Cruz Biotechnology	sc-34577
IL-1 β (3A6) antibody	Cell Signaling	12242S
F4/80 antibody	GeneTex	GTX101895
PE-tagged anti-F4/80 antibody	eBioscience	12-4801-80