Supplementary Materials

Supplementary methods
Figure S1-3
Table S1-2

Supplementary methods

Hemodynamic studies

At postoperative week 4, all the mice underwent hemodynamic measurements under anesthesia with 3% isoflurane after 12 hour-fasting. A PE-50 catheter (Smiths Medical, UK) was inserted into portal vein to determine PP. The catheter was connected to a transducer, when the pressure was detected by the monitor and recorded by a multichannel physiological signal acquisition system (ALC-MPA multichannel bioinformatics analysis system, Shanghai Alcott Biotechnology Co., Ltd., China). The zero-pressure point was defined at the level of right atrium.

Biochemical analysis

After hemodynamic measurement, mice were sacrificed after hemodynamic measurements. Serum was obtained from blood samples by centrifugation at 3000 g for 15 minutes at 4°C. The serum levels of alanine aminotransferase (ALT, S03030), aspartate aminotransferase (AST, S03040), and alkaline phosphatase (ALP, G4316) were measured using commercial kits (Servicebio, Wuhan, China) following the manufacturer's instructions.

Histological, immunohistochemical (IHC), and immunofluorescence (IF) examinations

The liver sections from the right lobe were fixed by 4% paraformaldehyde and embedded in paraffin. For histological analysis, serial liver sections were subjected to hematoxylin-eosin (H&E), Masson, and Sirius Red staining, followed by random examination under light microscope by an experienced pathologist. The collagen deposition volume was calculated by ImageJ. The results were presented as the proportions of the stained areas.

For IHC staining, serial liver sections were incubated in primary antibodies at 4 ℃ overnight. Then the sections were incubated with corresponding HRP-conjugated secondary antibodies for 60 min, followed by re-staining with hematoxylin.

For IF staining, serial liver sections were incubated in primary antibodies at 4 ℃ overnight. The corresponding Alexa Fluor 488 or 594-conjugated secondary antibodies were used for detection and DAPI was added to visualized the nuclei. Fluorescent images were captured by laser-scanning confocal microscope (LSM 800, Zeiss, Germany). All the images were analyzed with ImageJ.

The primary and secondary antibodies used were listed in **Table S1**.

Cell culture and treatment

RAW264.7 cell line from Cellverse (Shanghai, China) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin (100 IU/mL)/streptomycin (100 μg/mL) solution (P/S). The culture was maintained in a humidified incubator at 37°C with 5% CO_2. Recombinant mouse Gas6 protein (rGas6) was dissolved by 10% FBS in PBS at 1000 μg/mL for storage. RAW264.7 were incubated with liposaccharide (LPS, 100 ng/mL, Sigma, US), interferon-γ (IFN-γ, 20 ng/mL, PreproTech, US), or rGas6 (10, 50, or 100 μg/mL) for 24 h for following experiments.

hLSECs purchased from Cellverse (Shanghai, China) were cultured in Endothelial Cell Medium (ECM, Sciencell, USA) supplemented with 5% fetal bovine serum (FBS), 1% endothelial cell growth supplement (ECGS), and 1% penicillin (100 IU/mL)/streptomycin (100 μg/mL) solution (P/S). The culture was maintained in a humidified incubator at 37°C with 5% CO_2.

Efferocytosis assay

Jurkat cells were stained by carboxyfluorescein succinimidyl ester (CFSE, Selleck, US) at 10 μM for 10 min to trace apoptotic cell uptake. Apoptosis in Jurkat cells were induced by irradiation with 254-nm UV lamp for 15min followed by 2-3 h incubation. The apoptotic cells (AC) were collected and added to macrophages at a 5:1 ratio and incubated at 37 °C for 45 min. The cell suspensions were subjected to flow cytometry-based measurements to assess efferocytosis.

Flow cytometry

Cells from efferocytosis assay were stained with conjugated antibody for 30 min. Flow cytometry was performed by CytoFLEX (Beckman Coulter, US) and analyzed with FlowJo. The conjugated antibody used was listed in **Table S1**.

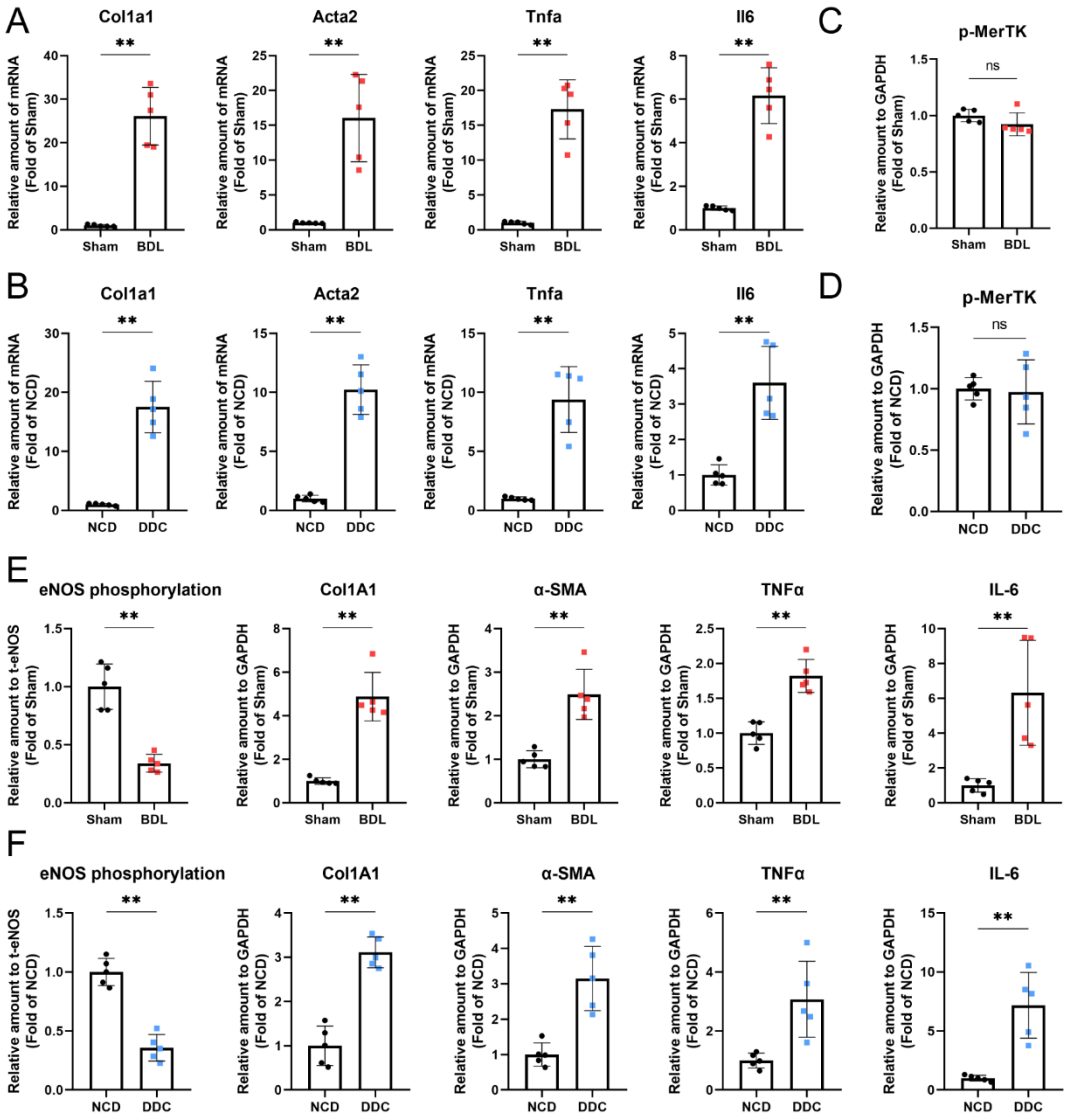
Western blotting

The protein extraction and Western blotting were performed as previously described. Briefly, liver tissues were crushed in liquid nitrogen and homogenized with RIPA buffer (Beyotime, China) to extract the protein. The extracts were centrifuged at 14000×g for 15 min at 4 ˚C. The supernatant was collected for BCA protein assay (Epizyme, China) and diluted with 5× sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample loading buffer (Beyotime, China). RAW264.7 lysates were prepared by 1× SDS-PAGE sample loading buffer (Beyotime, China). Protein extracts and cell lysates in loading buffer were further subjected to SDS-PAGE and electro-transferred onto polyvinylidene difluoride (PVDF) membranes. Subsequently, the membranes were incubated with primary antibodies at 4 ℃ overnight. Then the membranes were incubated with corresponding secondary antibodies. The blots were visualized and captured by Tanon-5200 chemiluminescent imaging system (Tanon, China). The signal intensities were measured by ImageJ. The expression levels of targeted proteins were normalized to those of glyceraldehyde-3-phosphate dehydrogenase. The primary and secondary antibodies used were listed in **Table S1**.

Quantitative real-time polymerase chain reaction (qRT-PCR)

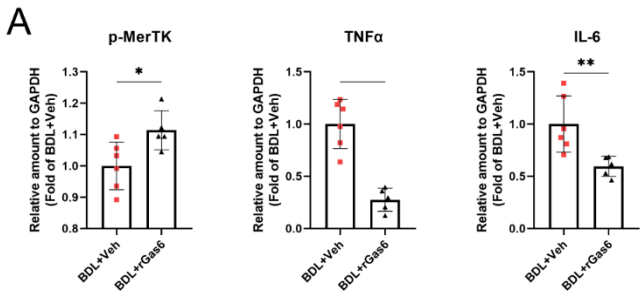
The total RNA was extracted from liver tissues and RAW264.7 by RNAiso Plus (TaKaRa, Japan). The cDNA was synthesized by RT SuperMix for qPCR (Vazyme, China). qRT-PCR was performed with ChamQ Universal SYBR qPCR Master Mix (Vazyme, China) in LightCycler® 480 Real-Time PCR System (Roche, Germany). The results from corresponding analysis software were presented as Ct values. The mRNA levels of targeted genes were normalized to GAPDH. The primer sequences used in this study were shown in **Table S2**.

Figure S1.



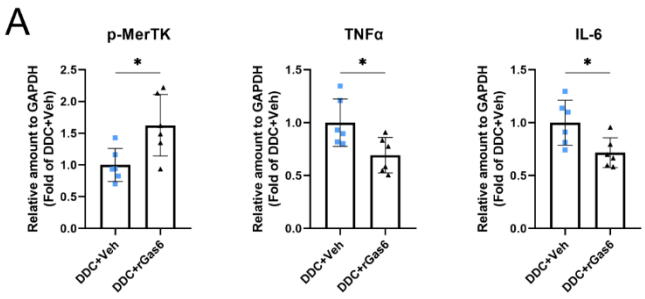
(A) Transcription levels of fibrosis genes (Col1a1 and Acta2) and pro-inflammatory genes (Tnfa and Il6) between sham and BDL mice measured by qRT-PCR. (B) Quantitative analysis of western blotting of MerTK phosphorylation between sham and BDL mice. (C) Transcription levels of fibrosis genes (Col1a1 and Acta2) and pro-inflammatory genes (Tnfa and Il6) between NCD and DDC mice measured by qRT-PCR. (D) Quantitative analysis of western blotting of MerTK phosphorylation between NCD and DDC mice. (E) Quantitative analysis of western blotting of eNOS phosphorylation, fibrosis markers, and pro-inflammatory cytokines between sham and BDL mice. (F) Quantitative analysis of western blotting of eNOS phosphorylation, fibrosis markers, and pro-inflammatory cytokines between NCD and DDC mice. * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$.

Figure S2.



(A) Quantitative analysis of western blotting of MerTK phosphorylation and inflammatory cytokines in BDL mice treated with vehicle or rGas6. * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$.

Figure S3.



(A) Quantitative analysis of western blotting of MerTK phosphorylation and inflammatory cytokines in DDC mice treated with vehicle or rGas6. * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$.

Table S1. Antibodies

Antibody	Company	Cat.No
CK19	Abcam	ab52625
CD68	Abcam	ab283654
CD163	Abcam	ab182422
phospho-eNOS (Ser1177)	Affinity	AF3247
eNOS	Proteintech	27120-1-AP
TNFα	Huabio	HA722022
IL-6	Huabio	R1412-2
Col1A1	Abclonal	A1352
Col1A1	Servicebio	GB11022
α-SMA	Proteintech	14395-1-AP
α-SMA	Servicebio	GB11044
CD206	Proteintech	60143-1-Ig
iNOS	Abways	CY5993
p-MerTK	Abways	AY0653
GAPDH	Proteintech	60004-1-Ig
CoraLite Plus 647 Anti-Mouse MHC Class II (I-A/I-E) (M5/114.15.2)	Proteintech	CL647-65122
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen	A-11001
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Invitrogen	A-11008
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Invitrogen	A-11005

Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Invitrogen	A-11012
Goat Anti-Rabbit IgG (H+L) HRP	ShareBio	SB-AB0101
Goat Anti-Mouse IgG (H+L) HRP	ShareBio	SB-AB0102

Table S2 Primer sequences used in qRT-PCR

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Mouse *Gapdh*	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Mouse *Nos2*	CAGCACAGGAAATGTTTCAGC	TAGCCAGCGTACCGGATGA
Mouse *Il6*	ACAACCACGGCCTTCCCTAC	TCTCATTTCCACGATTTCCCAG
Mouse *Tnfa*	CTCTTCTGTCTACTGAACTTCGG	AAGATGATCTGAGTGTGAGGGT
Mouse *CD206*	GAGGGAAGCGAGAGATTATGGA	GCCTGATGCCAGGTTAAAGCA
Mouse *Col1a1*	GCTCCTCTTAGGGGCCACT	ATTGGGGACCCTTAGGCCAT
Mouse *Acta2*	CCCAGACATCAGGGAGTAATGG	TCTATCGGATACTTCAGCGTCA
Human *GAPDH*	TCGGAGTCAACGGATTTGGT	TGAAGGGGTCATTGATGGCA
Human *LYVE1*	AATTTCACAGAAGCTAAGGAGGC	TCAAGGCTGTTTCAACTTGGTC
Human *VWF*	CCTTGACCTCGGACCCTTATG	GATGCCCGTTCACACCACT
Human *CD34*	ACCAGAGCTATTCCCAAAAGACC	TGCGGCGATTCATCAGGAAAT