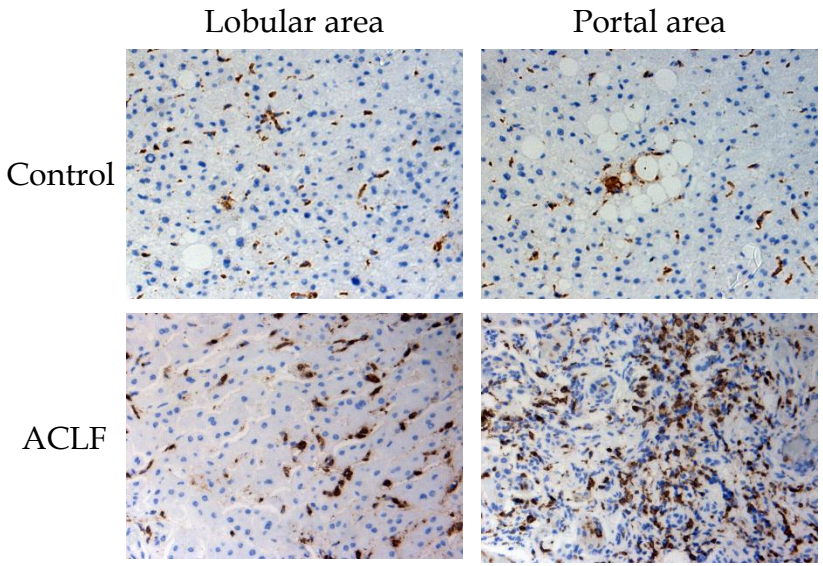
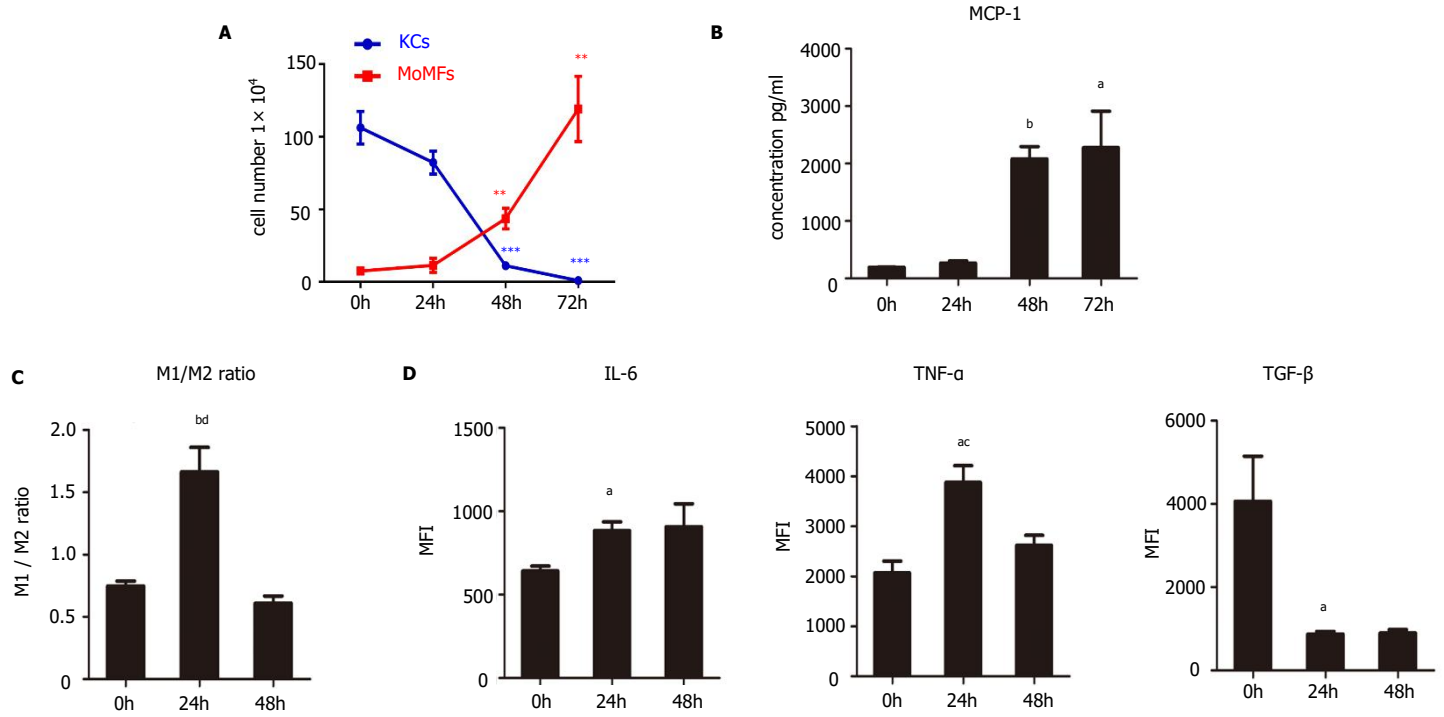
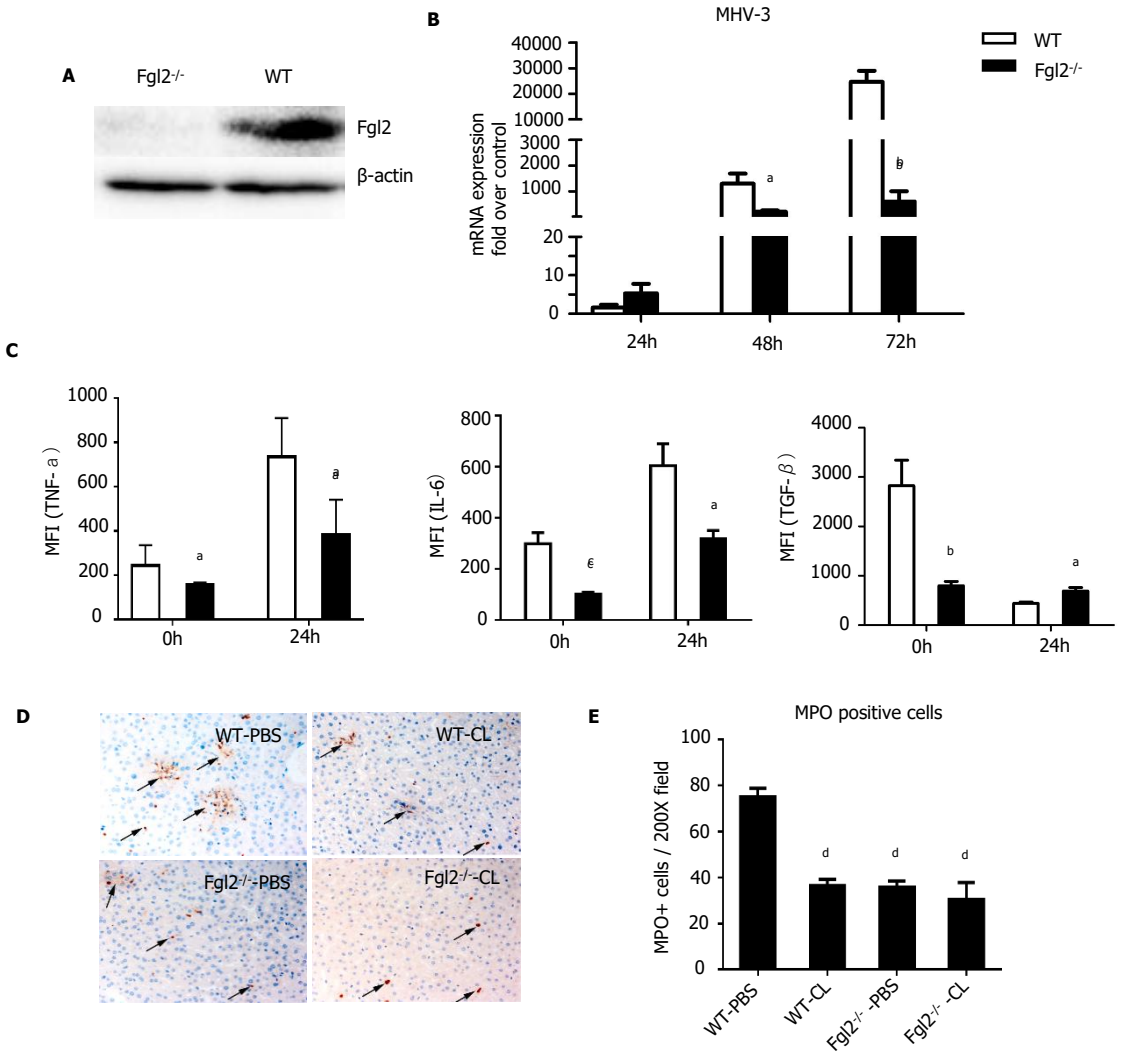


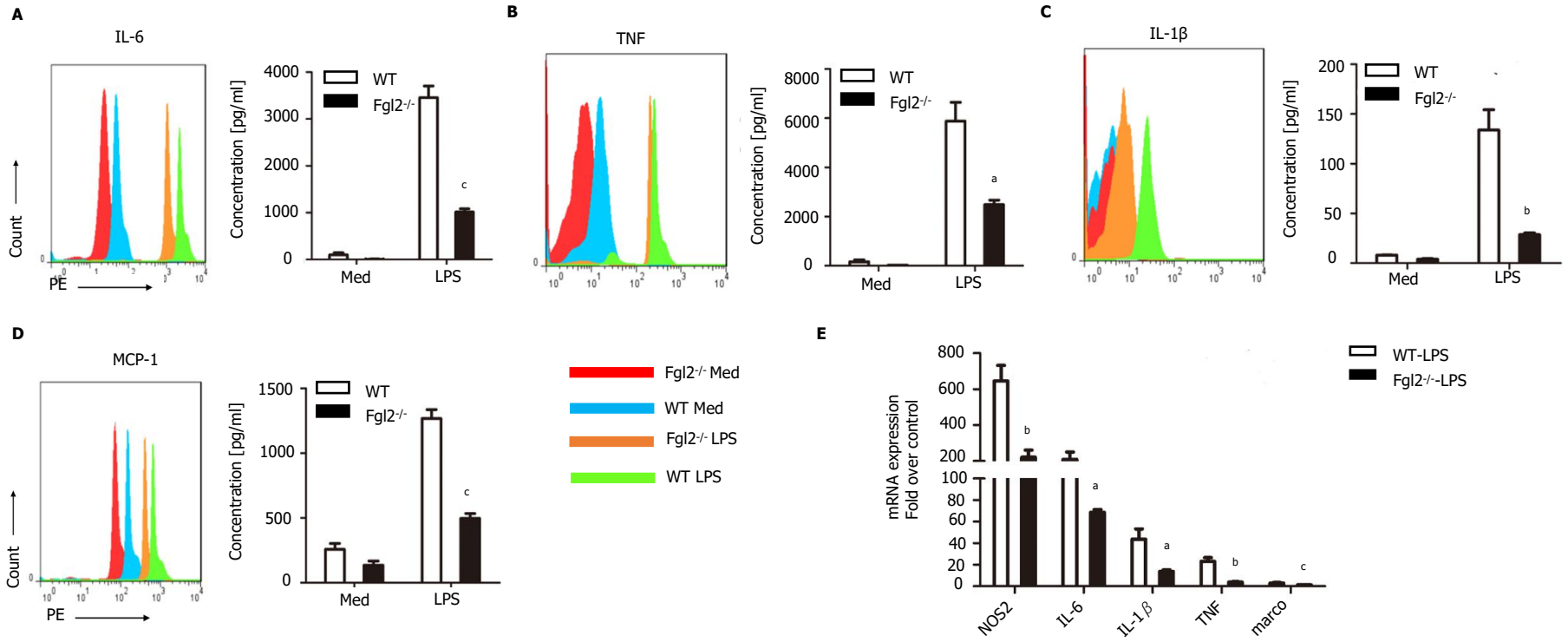
Supplemental Figure 1. Immunochemistry against CD68 on liver sections from lobular and periportal area in ACLF patients and health individuals.



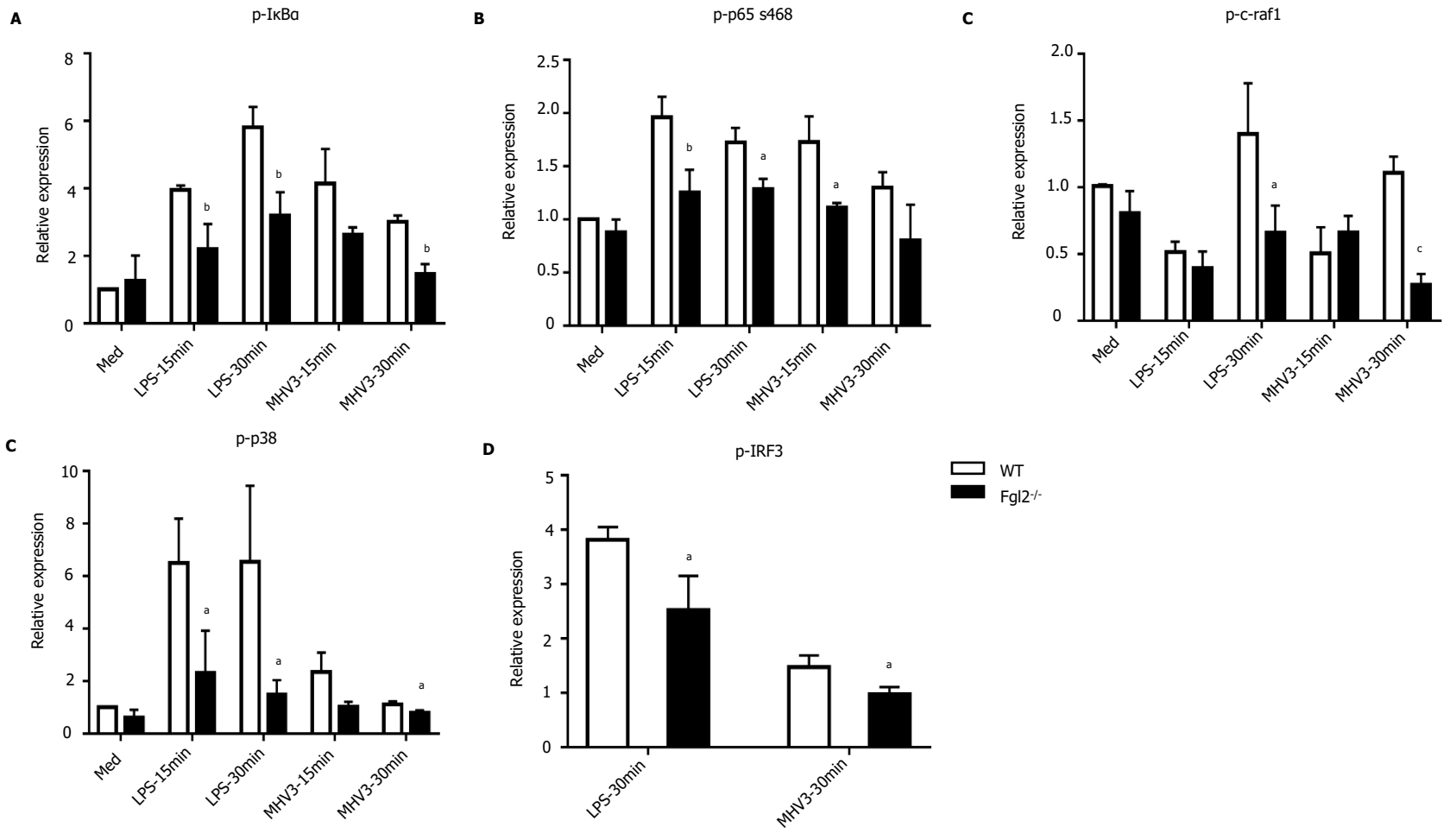
Supplemental Figure 2. A: Time-course calculation of hepatic KCs and MoMFs following MHV-3 infection; B: Serum level of MCP-1 post MHV-3 infection; C: M1/M2 KCs ratio before and after viral infection; D: MFI of IL-6, TNF-α, TGF-β expression on KCs 0h, 24h and 48 h post MHV-3 infection. All data are presented as mean ± SD (n = 5). $^{a}P < 0.05$ and $^{b}P < 0.001$ vs oh group, $^{c}P < 0.05$ and $^{d}P < 0.001$ vs oh group, $^{b}P < 0.001$ vs 48h group; $^{c}P < 0.01$ vs 0h group; $^{d}P < 0.01$ vs 48h group. These experiments were repeated at least three times.



Supplemental Figure 3. Expression of TNF-a, IL-6, TGF-β on KCs in wild type and Fgl2-/- mice. A: Immunoblotting against Fgl2 in BMDM lysates from WT mice and Fgl2-/- mice after LPS stimulation; B: Semi-quantitative PCR of MHV-3 DNA level in liver of WT and Fgl2-/- mice at 48 hour post infection); C: MFI of TNF-α, IL-6, TGF-β on KCs from WT and Fgl2-/- mice at 0 and 24 hours post MHV-3 infection; D: Representative images of MPO+ cells on liver sections from PBS and clodronate liposomes-treated mice at 48 hour post MHV-3 infection; E: Average number of MPO+ cells counted in 5 random fields in each mice. Data are presented as mean ± SD (n = 5). $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ vs WT group, $^{d}P < 0.05$ vs WT-PBS group. These experiments were repeated at least three times.



Supplemental Figure 4. Fgl2 deficiency impaired M1 polarization. A-D: Supernatant concentration of pro-inflammatory cytokines A: IL-6; B:TNF-α; C: IL-1β; D: chemokine MCP-1 in culture supernatant of PEM from WT and Fgl2-/- mice with (Med) and without LPS treatment; E: Relative mRNA levels of M1 markers in PEM in response LPS stimulation. Data are presented as mean ± SD ($n > 3$). $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ vs WT group. These experiments were repeated at least three times.



Supplemental Figure 5. Densitometry analysis for the relative protein level of A: p-IκBα; B: NF-κB p65 phosphorylated at s468; C: p-p38 and D: p-IRF3 compared to β-actinin response to either LPS stimulation or MHV-3 infection. All data are presented as mean ± SD (n = 3). $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ vs WT group.

Supporting material

Cytometric Bead Array for cytokines measurement

Cytometric Bead Array (CBA) allows for simultaneous measurement of multiple analytes in single sample (46). Hepatic cytokines were measure by following manufacturer's instructions (BD Bioscience). Cytokines analyzed were TNF-α, IL-6, IL-1β, MCP-1, IL-12 and IL-10. After flow cytometry, the concentrations of cytokines were calculated based on standard curve using CBA analysis software.

Nitric oxide assay

NO-derived nitrite (NO_2^-) was measured using Greiss reagent system (Promega Corporation, USA) according to the two-step assay protocol. Briefly, nitrate from supernatant of liver homogenates were converted into nitrite using nitrate reductase. Nitrite were then converted into a deep purple azo compound that can be recorded at 540 nm. Following luminescence, production of NO_2^- was quantitated according to Nitrite Standard curve by following instructions.

Phagocytosis

Single cell suspension of BMDMs were incubated in ice bath for 10 min followed by adding FITC-labeled E.coli suspension using phagotest reagent kit (Glycotope Biotechnology GmbH, Germany). The mixture was moved to a warm water bath at 37.0°C for 5 to 30 min for phagocytosis and removed on ice again to stop the reaction. Cells washed and re-suspended with PBS were subjected to flow cytometry. Mean fluorescence intensity of FITC were calculated using BD FACS DIVA software.

Quantitative Real-time PCR

RNA was extracted from Cell pellets using Tri-reagent (Thermo) according to the manufacturer's instructions. cDNA was synthesized with reverse transcription kit (Toyobo, Japan). qRT-PCR was performed using SYBR green Real-time PCR master kit (Toyobo, Japan). Relative expression levels of target genes were calculated by normalized to levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with the $2^{-\triangle\triangle Ct}$ methodology.

Western Blot

Cells were lysed in RIPA buffer For Western blotting. Briefly, equal amounts of protein (20 μg) were

USA). Autoradiograms densities were evaluated using Image-J software.

Antibodies purchased from either Cell Signaling Technology (CST) or Abcam were used with suggested dilutions. Antibodies were: Anti-MyD88 (1:1000, CST), anti-TRIF (1:1000, CST), anti-phospho-IkBα (1:1000, CST), anti-phospho-s468 p65 (1:1000, CST), anti-phospho-s276 p65 (1:1000, Abcam) and anti-p65 (1:1000, CST), anti-acetyl p65 (lys310) (1:1000, CST), anti-phospho-ERK 1/2 (1:2000, CST), anti-ERK 1/2(1:1000, CST), anti-phospho-JNK (1:2000, CST), anti-JNK (1:1000, CST), anti-phospho-c-raf1 (1:1000, CST), anti-phospho-P38 (1:1000, CST), anti-P38 (1:1000), anti-phospho-IRF3 (1:1000), anti-IRF3 (1:1000, CST), anti-IRF7 (1:1000), anti-phospho-Y550 BTK (1:1000, abcam), anti-phospho-Y223 BTK (1:1000, abcam), anti-BTK (1:1000, abcam), anti-β-actin (1:2000, CST).

Immunohistochemistry and immunofluorescence

Liver tissue were fixed with 4% paraformaldehyde and embedded in paraffin. Tissues were sectioned consecutively and imposed to hematoxylin - eosin staining. For immunostaining, continuous sections were incubated with primary antibodies overnight at 4℃. Sections were washed with PBS and followed by incubation with fluorescent labelled or HRP linked secondary antibody for 90 min. Images were taken under Nikon microscope. Primary antibodies were: anti-human CD68 (Abcam, 1:1000), anti-human S1009A (Abcam, 1:500), anti-human Fgl2 (Abnova, 1:200), anti-mouse F4/80 (Abcam, 1:1000), anti-mouse MPO (Abcam, 1:500), anti-mouse Fgl2 (Abnova, 1:500). Omission of the primary antibody was used as a control.

Antibodies for Flow cytometry

Antibodies used were: anti-CD45 (clone I3/2.3, Biolegend), anti-Ly6G (clone 1A8, Biolegend), anti-F4/80 (clone BM8, Biolegend), anti-CD11b (clone M1/70, Biolegend), anti-Ly6C (clone HK 1.4, Biolegend), anti-iNOS/NOS2 (clone 961-1144, BD Bioscience), anti-CD206 (clone C068C2, Biolegend), anti-IL6 (clone MP5-20F3, Biolegend),anti-TNFα(clone MP6-XT22, Biolegend)，anti-TGFβ(clone TW7-16B4, Biolegend)，anti-fgl2 (clone 6D9, Abnova), anti-MHC II (clone 28-8-6, Biolegend), anti-CD80 (clone 16-10A1, Biolegend),anti-CD86 (clone GL-1, Biolegend), anti-MHC I (clone 15-5-5, Biolegend).