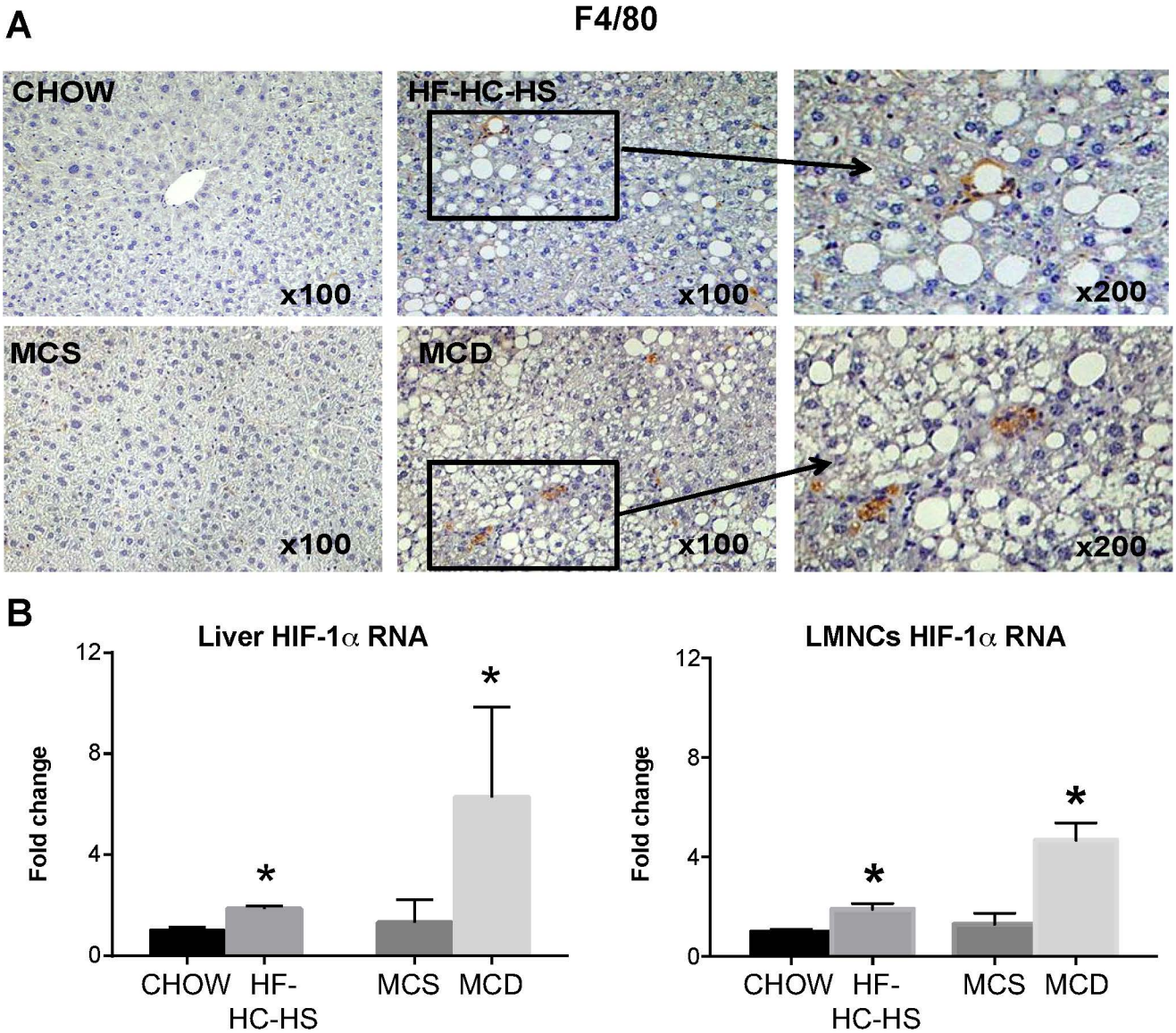
Supplementary Figure 1. HIF-1α is increased in total liver and LMNCs of MCD diet-fed mice. (A) Liver sections from MCD, MCS, and HF-HC-HS diet-fed mice were subjected to F4/80 immunohistochemistry (left and center panels are shown at 100X magnification, and right panels show the boxes in the middle panels at 200X magnification). Macrophages were increased in the liver of HF-HC-HS/MCD-diet-fed mice compared to control mice. (B) Wild-type C57BL/6J mice were fed an MCD or MCS diet for 8 weeks or HF-HC-HS diet for 24 weeks, and mRNA from either total liver (left) or liver mononuclear cells (LMNC, right) was used to measure HIF-1α expression by qPCR. Mice experiments are representative of 3-10 mice per experimental group. **P*<0.05 compared to respective controls.

Supplementary Figure 2. HIF-1α expression and autophagic flux impairment were not specific to PA. (A) Hepatic macrophages and BMDMs (B and C) were isolated from mice and stimulated with PA (A, B) or with low or high doses of LPS (C) for different time points, and HIF-1α, PAI-1 and BNIP3 and p62 mRNA levels were detected by qPCR. PCR data are shown from at least three independent experiments **P*<0.05.

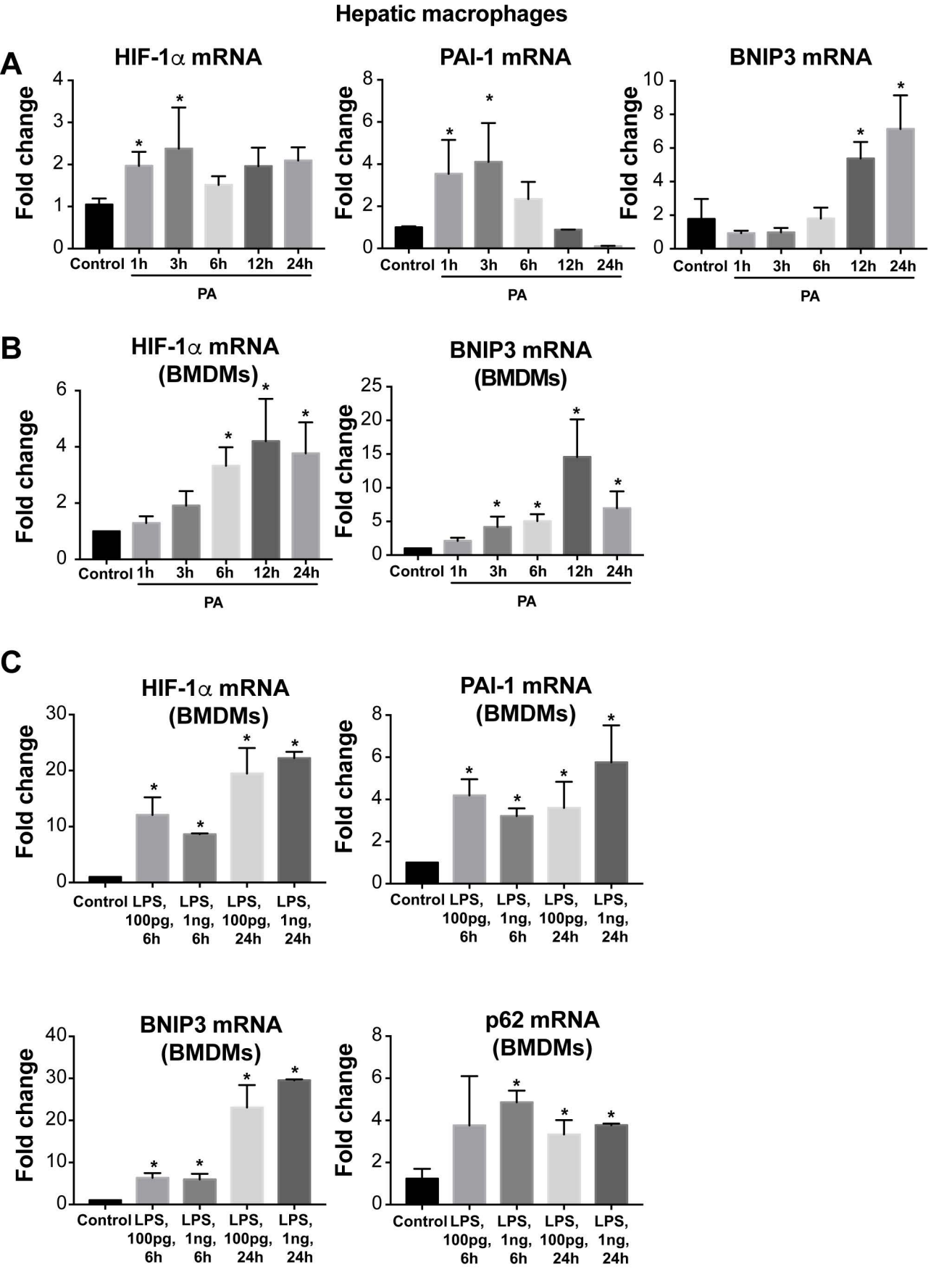
Supplementary Figure 3. PA treatment did not change the percentage of macrophages. THP-1 cells were treated with PMA (100nM) for 48h and then stimulated with PA (250μM) coupled with BSA for different time periods. Adherent cells were collected and stained with anti-CD14 antibody conjugated to PE-Cy7 (A) and anti-CD11b antibody conjugated to PE (B) and analyzed by flow cytometry. **P*<0.05 versus THP-1 unstimulated cells.

Supplementary Figure 4. Macrophage-specific HIF-1α contributes to steatosis and inflammation in non-alcoholic steatohepatitis. PA increases HIF-1α expression in macrophages and decreases autophagic flux contributing to the increase in IL-1β production. The increase in HIF-1α also contributes to the phosphorylation of NF-κB subunit p65, which is important for MCP-1 expression. Both IL-1β and MCP-1 induce liver steatosis and inflammation.

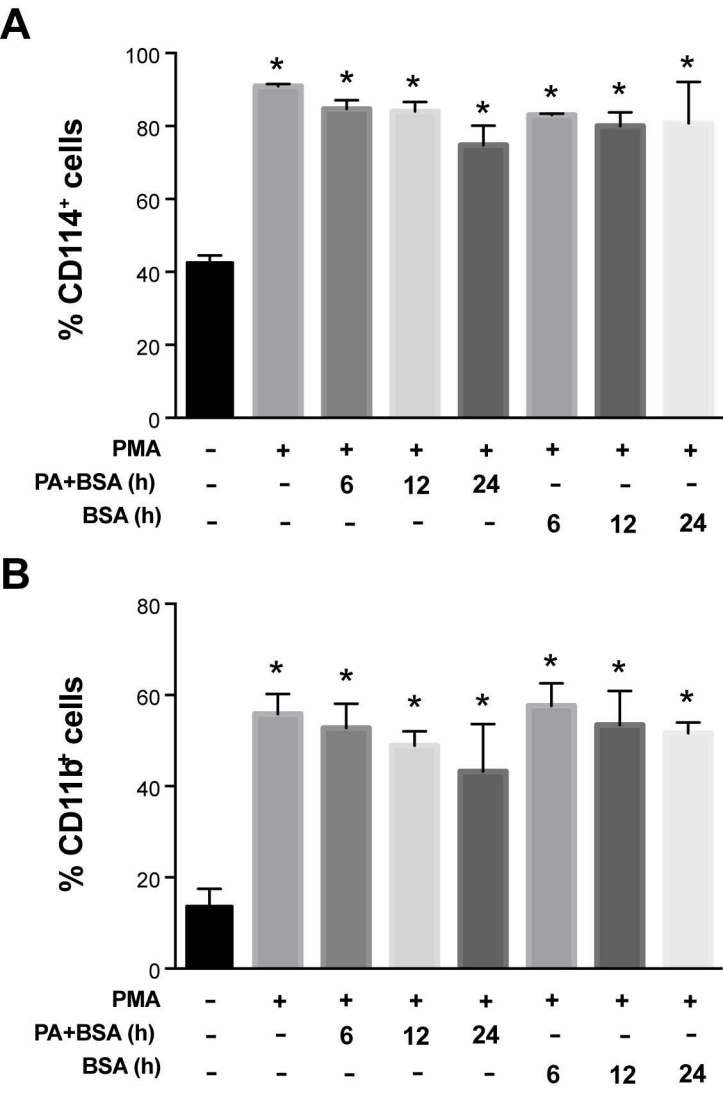
Supplementary Figure 1



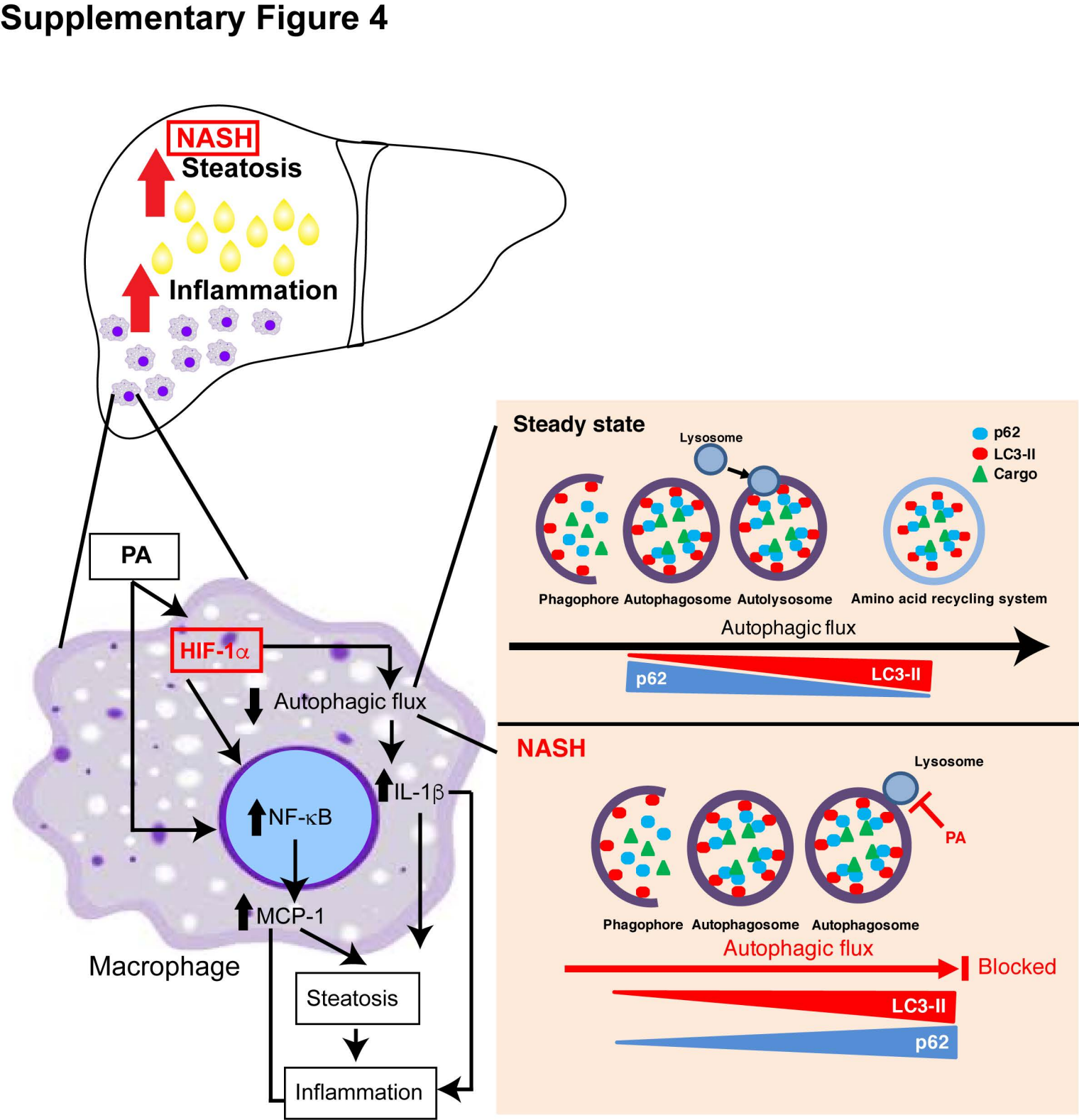
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Materials and Methods

Isolation of Hepatic Macrophages

Hepatic macrophages were isolated as described previously (1). Briefly, the livers were perfused with 0.5 mM EGTA in Hanks' Balanced Salt Solution (HBSS) for 5 minutes, followed by *in vivo* digestion with collagenase (SERVA Electrophoresis Gmbh, Heidelberg, Germany) for 5 minutes. The non-hepatocyte content was separated by Percoll gradient and centrifuged for 30 mins at 2000 rpm. The inter-cushion layer was collected, washed twice with PBS and cultured in Dulbecco's modified Eagle's medium (ThermoFisher Life Technologies, Waltham, MA) with 5% fetal bovine serum (FBS) (Gemini BioProducts, West Sacramento, CA). Non-adherent cells were removed after 3-4h and adhered hepatic macrophages were used for subsequent experiments. Depending on the experimental conditions, cells from 3-4 mice were pooled for each experiment given the limited number of hepatic macrophages available from each animal. The purity of hepatic macrophages was represented by the 40% $F4/80^{+}CD11b^{+}$ cells in the $CD45^{+}$ cell population.

Generation of bone marrow-derived macrophages and human macrophages

Tibia and femur were collected from mice, and the bone marrow cells were isolated by flushing the bone marrow with a solution of 2% FBS-PBS with a 25G needle coupled to a 10 mL syringe. The cells were passed through a 70 mm strainer, centrifuged and counted. The cells were cultivated in 15 cm Petri dishes with DMEM media supplemented with 10% FBS and 20% of L929 supernatant for 7 days at $37^{0}C$ and 5% CO_2. Additional media was added to Petri dishes on day 3, and after 7 days the cells differentiated into macrophages. Human macrophages were generated by the isolation of monocytes from PBMCs using MagniSort™ Human pan-Monocyte Enrichment Kit (Thermo Fisher) and cultivated in a 60:30:10 Medium (60% AIM V, 30% Iscove's Modified Dulbecco's Medium and 10% heat-inactivated AB-human serum) for 7 days.

In Vitro Experiments

THP-1 cells were grown in RPMI 1640 medium containing 10% FBS and treated with phorbol 12-myristate 13-acetate (PMA, 100 nM, Sigma-Aldrich, St. Louis, MO) for 48 hours to induce differentiation and attachment. PMA treatment resulted in macrophage differentiation based on

the increased percentage of $CD14^+$ (43% to 91%) and $CD11b^+$ (14% to 56%) cells. Furthermore, PA treatment did not change this effect (Supplementary Fig. 3).

Human and mouse primer sequences

The human primer sequences are as follows: HIF-1α, 5'-TCA GTT TCT GTG TCG TTG CTG CCA-3' (forward) and 5'-TAA CTT TGC TGG CCC CAG CCG-3' (reverse); BNIP3, 5'-GCC CGG GAT GCA GGA GGA GA-3' (forward) and 5'-GAG CAG CAG AGA TGG AAG GAA AAC-3' (reverse); PAI-1,5'-ACA ACC CCA CAG GAA CAG TC-3' (forward) and 5'-GAT GAA GGC GTC TTT CCC CA-3' (reverse); Glut1, 5'-TTC ACT GTC GTG TCG CTG TTT G-3' (forward) and 5'-TCA CAC TTG GGA ATC AGC CCC-3' (reverse); TNF-α, 5'-TGA CAA GCC TGT AGC CCA TG-3' (forward) and 5' AAA GTA GAC CTG CCC AGA C-3' (reverse); IL-1β, 5'-ATG GGA TAA CGA GGC TTA TGT G-3'(forward) and 5'-CAA GGC CAC AGG TAT TTT GTC-3' (reverse); IL-6, 5'-GAC AGC CAC TCA CCT CTT CA-3 (forward) and 5'-CAT CTT GGA AGG TTC AGG TTG T-3' (reverse). The mouse primer sequence of HIF-1α is 5'-CAA GAT CTC GGC GAA GCA A-3' (forward) and 5'-GGT GAG CCT CAT AAC AGA AGC TTT-3' (reverse).

The human primer sequences are as follows: HIF-1α, 5’- CAA GAT CTG GGC GAA GCA A-3’ (forward) and 5'-GGT GAC CCT CAT AAC AGA AGC TTT-3’(reverse); PAI-1, 5'-GAC CTT GCC AAG GTG ATG CTT GGC AAC-3' (forward) and 5'-GAT TGG CCT GCT AGG AAA TTA CAT TC -3' (reverse); BNIP3, 5’ CAG CCT CCG TCT CTA TTT-3’ (forward) and 5’-TTC AGC TCT GTT GGT ATC T-3’(reverse); Mcp-1 , 5’- CAG GTC CCT GTC ATG CTT CT-3’(forward) and 5’-TCT GGA CCC ATT CCT TCT TG-3’(reverse); Tnf-A, 5’ GAA GTT CCC AAA TGG CCT CC-3’ (forward) and 5’- GTG AGG GTC TGG GCC ATA GA-3’(reverse); Il-1β, 5’ TCT TTG AAG TTG ACG GAC CC-3’ (forward) and 5’-TGA GTG ATA

CTG CCT GCC TG-3'(reverse); p62/ SQSTM1, 5'-AGG ATG GGG ACT TGG TTG C-3' (forward) and 5'-TCA CAG ATC ACA TTG GGG TGC-3' (reverse).

1. Bala S, Marcos M, Kodys K, Csak T, Catalano D, Mandrekar P, Szabo G. Up-regulation of microRNA-155 in macrophages contributes to increased tumor necrosis factor {alpha} (TNF{alpha}) production via increased mRNA half-life in alcoholic liver disease. J Biol Chem 2011;286:1436-1444.