Mesenchymal Stem Cells Secreted-Prostaglandin E2 Ameliorates Acute Liver Failure via Attenuation Cell Death and Regulation of Macrophage Polarization

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Abstract

Background: Acute liver failure (ALF) is an acute inflammatory liver disease with high mortality. Previous preclinical and clinical trials have confirmed that mesenchymal stem cell (MSC) is a promising therapeutic approach, however the effect is not satisfied as the underlying molecular mechanisms of MSC in treating ALF remain unclear.

Methods: MSC isolated from 4- to 6-week-old C57BL/6 mice were used to treat ALF. Histological and serological parameters were analyzed to evaluate the efficacy of MSC. We explored the molecular mechanism of MSC in the treatment of ALF by detecting liver inflammatory response and hepatocyte death.

Results: In this study, we found that therapeutic potential of MSC on ALF dependent on secretion of prostaglandin E_2 (PGE_2), a bioactive lipid. MSC-derived PGE_2 inhibited TGF-β-activated kinase 1 (TAK1) signaling and NLRP3 inflammasome activation in liver macrophages to decrease the production of inflammatory cytokines. Meanwhile, macrophages in liver could be induced to anti-inflammatory (M2) macrophages by MSC-derived PGE_2 via STAT6 and mechanistic target of rapamycin (mTOR) signaling, which then to promote inflammatory resolution and limit liver injury. Finally, administrating of EP4 antagonist significantly ameliorated the therapeutic ability of MSC, which promoted liver inflammation and decreased M2 macrophages.

Conclusions: Our results indicate that PGE_2 might be a novel important mediator of MSC on treating ALF, which through inhibiting liver inflammatory response and hepatocyte death.

Introduction

Acute liver failure (ALF) is a clinical syndrome with high mortality rate, liver transplantation is the only effective way to cure ALF now[1]. Due to the shortage of organ donor resources, alternative treatments are needed urgently[2]. It has been shown that mesenchymal stem cell (MSC) not only rescued several animal models of liver injury, but that showed an effective therapeutic potential in clinical trials, which based on their ability to secrete various trophic factors[3]. Our previous study confirmed that MSC-derived PGE_2 could promote hepatocyte proliferation in ALF, however the role of MSC-derived PGE_2 on liver inflammation still remain unclear.

Several studies confirmed the cell death and hepatic inflammation in the progression of ALF[4]. Inflammatory mediators of toxic liver injury include cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1β and reactive oxygen species (ROS) accelerate liver injury[5]. Most of these inflammatory mediators were secreted by innate immune cells in liver, including monocytes, macrophages and dendritic cells (DC), which are activated by damage associated molecular patterns (DAMPs) released by damaged hepatocytes. Liver macrophages hold central functions in initiating, perpetuating and restricting inflammation. Macrophages can be classified into pro-inflammatory (M1) or anti-inflammatory (M2)

macrophages[6]. M1 cells secrete large amounts of pro-inflammatory cytokines, such as TNF and IL-1β. M2 cells secrete immunosuppressive cytokines, like IL-10, which plays vital role in resolution of inflammation. Recent studies have confirmed the role of MSC on macrophage polarization. For example, the therapeutic ability of MSC on cartilage repair and sepsis was dependent on the induction of M2 macrophages[7, 8]. In various liver disease, the therapeutic ability of MSC also rely on macrophage polarization[9, 10]. However, the mechanism of MSC on macrophage polarization remains unclear.

Sterile inflammation, which defined as an inflammatory response triggered by damage mediated by noninfectious sources, plays a major role in ALF[4]. The nucleotide-binding and oligomerization domain-like receptor 3 (NLRP3) inflammasome is the main kind of sterile inflammation, which contains three parts: NLRP3, ASC (apoptosis-associated speck-like protein) and pro-caspase 1. The inflammasome is activated by DAMPs or (pathogen associated molecular patterns) PAMPs, which contribute to maturation and secretion of IL-1β and IL-18[11]. Recent studies confirmed that inflammasome activation contributes to the progression of various liver disorders, like viral fulminant hepatitis and non-alcoholic fatty liver disease[12]. Therefore, targeting the NLRP3 inflammasome might be an effective strategy to reducing liver inflammation.

In this study, we focused on the inflammatory response in ALF. We confirmed that MSC could attenuate liver inflammation and NLRP3 inflammasome activation, meanwhile, promoted M2 macrophages to resolve inflammation thus to cure ALF.

Materials And Methods

Isolation of mouse bone marrow MSC

MSC were isolated from 4- to 6-week-old C57BL/6 mice as our previous described[13]. To deplete or overexpress COX2 in MSC to inhibit or increase the synthesis and secretion of PGE_2, the short hairpin RNA against COX2 or COX2 gene lentivirus (GeneChem, Shanghai, China) was transfected to MSCs following the manufacturer's instructions [named MSC-COX2(-) and MSC-COX2(+)].

For MSC, MSC-COX2(-) or MSC-COX2 (+) conditioned medium, MSC were maintained in serum-free medium for 48 h at 37°C in a humidified atmosphere containing 5% CO_2. Conditioned medium was harvested, clarified by centrifugation, and frozen at -80°C until use.

Animal models

Male C57BL/6J wild type mice (6~8 weeks old) were obtained from Animal Core Facility of Nanjing Medical University. Animals were bred in a pathogen-free facility. Mice were randomly divided into five groups: (1) control group (Ctrl): mice were intraperitoneally (i.p.) injected with PBS only; (2) ALF group (ALF): mice simultaneously received 600 mg/kg D-galactosamine (D-Gal) (Sigma-Aldrich, St. Louis, MO,

USA) and 100 μg/kg LPS (Sigma-Aldrich) dissolved in PBS by i.p. injection; (3-5) cell transplantation groups: mice received 600 mg/kg D-Gal and 100 μg/kg LPS via i.p. injection, and then 2×10^6 MSC, MSC-COX2(+) or MSC-COX2(-) were injected via tail vein after 6h, namely MSC group, MSC-COX2(+) group or MSC-COX2(-) group, respectively. For TAK1 inhibition, we administrated the specific TAK1 inhibitor 5z-7-ox (5mg/kg, Sigma) to mice 1h before LPS/D-Gal administration. To inhibit EP4 receptor of PGE_2, we administered EP4 specific antagonist (GW627368X, 20mg/kg, Cayman Chemical, Ann Arbor, MI, USA) 1h before LPS/D-Gal treatment and every 24h following MSC infusion. Blood and liver tissues were collected at indicated time for further analysis.

Bone marrow derived macrophage (BMDM) isolation and culture

BMDM were prepared and cultured as described[14]. In brief, BM cells were flushed from femurs and tibias of mice and dispersed mechanically. Cell suspensions were filtered through a 200-mesh filter, and the remaining cells were collected by centrifugation at 300 × g for 4 min. After centrifugation, the cells were resuspended and cultured in RIP 1640 supplemented with 10% FBS and macrophage colony-stimulating factor (M-CSF, 20 ng/mL, eBioscience) for 7 days.

For inflammasome activation, BMDMs were treated with LPS (100 ng/ml) for 4h, followed by 30 min treatment with PGE_2 (0.1 μM) or MSC, followed by stimulation with nigericin (4 μM for 1h, Cayman Chemical). EP4 antagonist were added 30min prior treatment with PGE_2 or MSC. For macrophage polarization, BMDM were stimulated with PGE_2 or MSC for 24h.

Real time quantitative PCR and western blotting

Total RNA or protein for liver tissues and cells were extracted as previous described[13]. The primer sequences and primary antibodies were listed in Supplementary Table S1 and S2.

Small interfering RNA and transfection

siRNAs specific EP4 as well as control siRNAs, were obtained from RiboBio (GuangZhou, China). The detailed sequences of siRNAs were listed in Supplementary Table S3. Transfection with siRNAs were completed using riboFECT™ CP (RiboBio) according to the manufacturer's instruction.

Immunofluorescence and HE analyses

HE staining analysis of the liver sections were conducted as described previously[13]. The images were captured by a Nikon microscope (Japan). Other sets of sections were dewaxed and subjected for

immunofluorescence labelling of NLRP3 (1:200, Adipogen Life, San Diego, USA) and F4/80 (1:200, Abcam, Cambridge, UK). Sections were incubated with the primary antibodies at the same working dilutions as noted above overnight at 4 °C, followed by incubation with goat anti-rabbit IgG H&L (Alexa Fluor® 488) (1:500, Abcam) or Goat Anti-Rat IgG H&L (1:500, Alexa Fluor® 594) for 1h. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, KeyGen BioTECH, Nanjing, China) and images were captured using fluorescence microscopy (Olympus, Japan).

Measurement of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Serum ALT and AST were determined using an automatic biochemical analyzer (Olympus, Tokyo, Japan) in the Affiliated Drum Tower Hospital of Nanjing University Medical School.

TUNEL and PI assay

Cell apoptosis and death in liver was measured using the one step TUNEL Apoptosis Assay kit and Propidium Iodide (PI) staining (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions.

IL-1β and IL-10 analysis

IL-1β levels in serum or cell supernatant and IL-10 levels in serum were detected by an enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, Thermo Scientific, Vienna, Australia).

Caspase 1 activity

Caspase-1 activity in liver tissues was measured by Caspase 1 Activity Assay Kit (Beyotime Biotechnology) according to the manufacturer's instructions.

Statistical analysis

Data were expressed as means ± SD. Differences were analyzed by student's t-test using Prism software (GraphPad). P-values less than 0.05 were considered as statistically significant.

Results

MSC protects against ALF via PGE_2

Paralyzed with our previous study[13], MSC protected against ALF through PGE_2, as revealed by plasma levels of ALT and AST (Figure 1A) and liver damage in HE staining (Figure 1B). TUNEL and PI staining confirmed the hepatocyte necrosis and apoptosis was decreased when MSC infusion, especially in MSC-COX2(+) group, however, MSC-COX2(-) failed to decrease hepatocyte necrosis and apoptosis (Figure 1C and 1D). Taken together, MSC protected hepatocyte death against ALF via PGE_2.

MSC ameliorates inflammation in ALF

Liver inflammation is associated with hepatocyte damage and death, therefore we examined the inflammatory response in liver after MSC infusion. Notably, mRNA expression levels of inflammatory cytokines and chemokines, including *CCL2*, *IL-1β*, *iNOS* and *TNF-α* were significantly inhibited in MSC group, and in MSC-COX2(+) group, the levels of these cytokines decreased more obviously. By contrast, MSC-COX2(-) group, which inhibited secretion of PGE_2 from MSC, failed to decrease these inflammatory cytokines (Figure 2A). NF-κB signaling plays a great role in inflammation[15]. We demonstrated that LPS/D-Gal induced ALF significantly activated NF-κB signaling in liver, as the increased expression of the phosphorylation of IKK-β and P65 (p-IKK-β and p-P65) (Figure 2B). MSC or MSC-COX2(+) group exhibited an ameliorated phenotype, while MSC-COX2(-) did not show this phenotype (Figure 2B). Next, we aimed to explore the mechanism of MSC on NF-κB signaling inhibition. TGF-β-activated kinase 1 (TAK1) is an intracellular hub molecule that regulates NF-κB signaling pathways which plays a key role in cell survival and death[16]. Our results demonstrated that TAK1 signaling was inhibited after MSC infusion, as the expression of the phosphorylation of TAK1 (p-TAK1), JNK (p-JNK), P38 (p-P38) and c-Jun (p-c-Jun) were significantly reduced in MSC or MSC-COX2(+) group, while these TAK1 substrates still expressed high in MSC-COX2(-) group (Figure 2C).

To confirm the role of TAK1 in ALF, we further used 5Z-7-ox, a specific TAK1 inhibitor, to inhibit TAK1 before LPS/D-Gal administration. Notably, TAK1 inhibition protected against liver damage, shown by normalized serum ALT and AST and reduced necrosis on histological analysis (Figure 2D and 2E). Treatment of 5Z-7-ox significantly suppressed TAK1 activation, reduced activation of its downstream signaling pathways, including p-JNK, p-P38, p-c-Jun and NF-κB signaling (Figure 2E). Collectively, these observations suggested that MSC-derived PGE_2 protected ALF through suppressing liver inflammation in a TAK1-NF-κB pathway.

MSC inhibits inflammasome activation of macrophages in ALF

Previous studies have confirmed the role of inflammasome activation in LPS/D-Gal induced liver injury, which accelerated liver inflammation[17]. Next we explored whether MSC infusion could inhibit NLRP3 inflammasome activation in liver. The protein levels of NLRP3, caspase1 p20 and mature-IL-1β were decreased in MSC or MSC-COX2(+) group (Figure 3A). In parallel, serum concentrations of IL-1β and the

activity of caspase 1 enzyme in liver tissues confirmed the inhibition of NLRP3 inflammasome by MSC-derived PGE_2 (Figure 3B and 3C). However, MSC-COX2(-) failed to inhibit NLRP3 inflammasome activation in liver tissues. In order to identify the cell population responsible for increased production of NLRP3 inflammasome, the double immunohistochemistry staining was performed. The results demonstrated that NLRP3 was mostly activated in macrophages in liver, MSC or MSC-COX2(+) could inhibited NLRP3 activation in liver macrophages (Figure 3D). To confirm this result, we treated mouse hepatocyte cell line AML12 or BMDM with 1μg/ml LPS for 24h *in vitro*. As showed in Figure 3E and 3F, the activation of NLRP3 and levels of IL-1β secretion was much higher in BMDM, which indicated that macrophages were more sensitive to DAMPs than hepatocytes. Collectively, our results indicated that MSC-derived PGE_2 inhibited liver macrophages inflammasome activation to protect against liver injury.

To confirm the role of MSC on NLRP3 inhibition, we treated BMDM with LPS and nigericin, a typical inducer of NLPR3. MSC or MSC-COX2(+) conditioned medium significantly inhibited NLPR3 activation and IL-1β secretion (Figure 4A and 4B), while MSC-COX2(-) conditioned medium did not show this phenomenon. In parallel, exogenous PGE_2 exhibited same effects on NLRP3 (Figure 4C and 4D). Previous studies have confirmed the role of NF-κB in NLRP3 inflammasome activation[18], thus we explored whether MSC inhibited NLRP3 through TAK1-NF-κB. Our results showed that MSC conditioned medium or PGE_2 could inhibit TAK1 and NF-κB activation in BMDM induced by LPS and nigericin (Figure 4A and 4C). Meanwhile, TAK1 inhibitor could also inhibit NLRP3 activation in BMDM (Figure 4E-F). *In vivo* study confirmed the effects of TAK1 inhibitor on NLRP3 in ALF model (Figure 4G-H). Overall, our results indicated that MSC-derived PGE_2 could inhibited NLPR3 inflammasome activation of liver macrophages through TAK1- NF-κB pathway.

MSC induces M2 macrophage polarization in ALF

Mouse models of acute liver injury have revealed the role of macrophages in accelerating damage through cytokines and chemokines releasing. However, liver macrophages are plastic and adapt their phenotype to promote tissue repair according to signals derived from the hepatic microenvironment[6]. Several studies confirmed the role of MSC on macrophage polarization in different models[19, 20]. Thus we tended to explore whether MSC could induce M2 macrophages in ALF to promote inflammation resolution. We found that genes expression associated with M2-like (*Arg1*, *Mgl1*, *Mgl2* and *Ym1*) macrophages were increased in MSC and MSC-COX2(+) group (Figure 5A), meanwhile, the serum levels of IL-10, which produced by M2 macrophages, were also increased in MSC and MSC-COX2(+) group (Figure 5B). However, MSC-COX2(-) failed to induce M2 macrophages. *In vitro* studies confirmed the MSC-derived PGE_2 on macrophage polarization, as the expression of M2 markers and numbers of CD206 positive cells of BMDM treated with MSC conditioned medium or PGE_2 were increased (Figure 5C and 5D). Taken together, our results demonstrated that MSC induced M2 macrophages to resolute inflammation in ALF through PGE_2.

Next we investigated the mechanism of MSC-derived PGE_2 on macrophage polarization. Western blotting results showed that not only the classical M2 pathway, STAT6, was activated when MSC or PGE_2 treatment, but also resulted in mTOR activation, as increased phosphorylation of the mTOR substrates mTOR, S6K, AKT and GSK-3β (Figure 6A), which correlated with previous studies[21]. In contrast, MSC-COX2(-) conditioned medium failed to activated mTOR and STAT6 pathway (Figure 6A). Treatment of BMDM with mTOR inhibitor, rapamycin (Rap) or AKT inhibitor, MK-2206, confirmed the role of mTOR signaling in MSC-derived PGE_2 on M2 macrophage polarization, as the mRNA levels of M2 markers were significantly increased after administration of Rap or MK-2206 (Figure 6B-6D). Taken together, our results indicated that MSC-derived PGE_2 induced M2 macrophages through mTOR and classical STAT6 pathway.

MSC protects against liver inflammation via PGE_2 receptor (EP) 4

Our previous studies showed that MSC-derived PGE_2 promoted hepatocyte proliferation through EP4[13], we explored whether EP4 also played a role in liver inflammation resolution. We treated mice with EP4 inhibitor (EP4i), GW627368X. The mRNA levels of inflammatory cytokines increased when EP4i treatment (Figure 7A). Western blot results confirmed that inhibition of TAK1 and NF-κB signaling by MSC were also mediated by EP4, as the expression of TAK1 and NF-κB signaling substrates was higher when EP4i administration (Figure 7B and 7C). Meanwhile, the expression of NLRP3 and the activity of caspase 1 enzyme were also higher when EP4i administration, even in MSC-COX2(+) group (Figure 7D and 7E). *In vitro* studies using EP4i or siRNA to inhibition or deplete EP4 in BMDM, the western blot and IL-1βsecretion confirmed the role of EP4 on NLRP3 inflammasome inhibition (Figure 7F,7G). Taken together, these results showed that MSC-derived PGE_2 ameliorated liver inflammation through EP4.

EP4 mediates MSC-derived PGE_2 on macrophage polarization

Next we explored that whether M2 macrophage polarization was also mediated by EP4. The mRNA levels of M2 macrophages markers and serum levels of IL-10 were also inhibited when EP4i treatment (Figure 8A and 8B). And mRNA levels of PGE_2 receptors (EP1-EP4) in BMDM confirmed the role of EP4 on M2 macrophage polarization (Figure 8C). EP4i or siRNA treatment demonstrated the role of EP4 in macrophage polarization, as the mRNA levels of M2 markers were decreased when EP4i or siRNA treatment (Figure 8E and 8G). Meanwhile, the protein levels of STAT6 and mTOR signaling substrates confirmed the role of EP4 on STAT6 and mTOR signaling (Figure 8D and 8F). Overall, these results demonstrated that EP4 played a major role in MSC-derived PGE_2 on macrophage polarization.

Discussion

Cell death and inflammation play great roles in the progression of ALF[4]. Accordingly, we speculated that reduced cell death and hepatic inflammation would be beneficial for the prevention and treatment of ALF. As described previously, MSC could regulate these important pro-inflammatory cytokines via a variety of effector mechanisms[22]. In our study, we clarified that MSC-derived PGE_2 reduced hepatocyte death and liver inflammation to attenuate ALF. Meanwhile, MSC-derived PGE_2 also modulated macrophage polarization to promote inflammation resolution in ALF.

PGE_2 is a bioactive lipid which is catalyzed by the COX2 from arachidonic acid. PGE_2 exerts a wild range of biological effects associated with inflammation and cancer[23]. Several studies confirmed that PGE_2 promoted resolution of inflammation and tissue repair. In concanavalin A (ConA) induced liver injury model, depleted COX2 in mice accelerated liver injury[24]. Hepatocyte COX2 expression protected against liver ischemia-reperfusion injury (IRI), meanwhile, in patients underwent liver transplantation, there was a significant positive correlation of plasma PGE_2 levels and graft function[25]. These results indicated that PGE_2 might have great therapeutic potentials in treating inflammatory liver diseases. In this study, we demonstrated the therapeutic role of MSC secreted PGE_2 on LPS/D-Gal induced ALF based on its anti-apoptosis and anti-inflammation ability.

We confirmed that MSC-derived PGE_2 reduced pro-inflammatory cytokines production in the liver through inhibiting TAK1-NF-κB signaling, which is important in regulating expression of pro-inflammatory cytokines. TAK1 is a central regulator of cell death and inflammation through activating downstream effectors such as NF-κB and mitogen-activated protein kinases (MAPKs)[16]. Recent studies have demonstrated the role of TAK1 in the progression of various liver diseases, like liver IRI, nonalcoholic steatohepatitis (NASH) and hepatocellular carcinoma[26-28]. In our study, we found that MSC infusion could decreased expression of TAK1 downstream substrates and NF-κB thus to alleviated liver inflammation. In parallel, administration of the TAK1 specific inhibitor, 5Z-7-ox could also protect against ALF *in vivo*.

Inflammasome activation has been identified as a major contributor to hepatocyte damage in ALF[29]. Our results showed that administration of MSC, especially MSC-COX2(+), significantly suppressed LPS/D-Gal induced inflammasome activation, while MSC-COX2(-) failed to show this phenomenon, which demonstrated the role of PGE_2 on NLRP3 inflammasome inhibition. Next, we confirmed that macrophages in liver is the main source of NLRP3, which then accelerated liver inflammatory response. Previous study has showed that TAK1 restricted NLRP3 activation and NF-κB was important for NLRP3 activation[18, 30]. We found that in mice which pretreated with 5Z-7-ox significantly inhibited NLRP3 inflammasome activation, which correlated with the *in vitro* model results, showed the role of TAK1 on NLRP3 inflammation activation. These results indicated that MSC-derived PGE_2 restricted macrophage NLRP3 activation through TAK1.

Liver macrophages hold central position in maintaining homeostasis in liver as well as in the pathogenesis of acute or chronic liver injury. We showed that MSC-derived PGE_2 ameliorated macrophage activation induced inflammatory response. Recent studies have revealed macrophages to be heterogeneous, M2 macrophages protected against exacerbated inflammation and thus limited tissue injury[6]. Several studies have showed the role of MSC on macrophage polarization[19, 20]. Our studies demonstrated the role of MSC-derived PGE_2 on M2 macrophage induction in ALF thus to limit liver injury, as the increased expression of M2 markers and plasma levels of IL-10. Next we found that MSC-derived PGE_2 not only activated STAT6 signaling in BMDM, the classical pathway on M2 macrophage polarization[31], but also enhanced the expression of mTOR signaling substrates, which has shown to control macrophage metabolism thus to shape their properties[21, 32]. Administration of the mTOR inhibitor or AKT specific inhibitor significantly inhibited ability of MSC-derived PGE_2 on M2 macrophage polarization.

PGE_2 elicits a wide range of biological effects through its receptors, EP1-EP4[23]. Our previous study found that MSC-derived PGE_2 promoted hepatocyte proliferation via EP4. In this study, we administration EP4 antagonist to mice, which significantly inhibition the effects of MSC on attenuating liver inflammation and M2 macrophage polarization. And administration of an agonist for EP4 has been found to confer protection against IRI in the liver, ischemic heart diseases and intestinal injury[33-35]. Collectively, MSC-derived PGE_2 protected against ALF through EP4.

Conclusions

In conclusion, our data confirmed that MSC attenuated ALF dependent on the secretion of PGE_2. MSC-derived PGE_2 inhibited TAK1 signaling and NLRP3 inflammasome activation in liver macrophages, meanwhile, MSC-derived PGE_2 could also induce M2 macrophages to secret anti-inflammatory cytokines, like IL-10 to promote inflammation resolution and limit liver injury through activating STAT6 and mTOR signaling in macrophages, finally to inhibit liver inflammatory response and hepatocyte apoptosis induced by LPS/D-Gal. All these phenomena were relied on the PGE_2 receptor, EP4. Inhibiting EP4 significantly alleviated therapeutic potential of MSC on ALF. This study provided a new therapeutic mechanism of MSC on ALF, which might provide a new direction to enhance functionality of MSC.

Abbreviations

MSC: mesenchymal stem cell; ALF: Acute liver failure; PGE_2: prostaglandin E_2; TAK1: TGF-β-activated kinase 1; mTOR: mechanistic target of rapamycin; TNF: tumor necrosis factor; IL: interleukin; ROS: reactive oxygen species; DAMPs: damage associated molecular patterns; DC: dendritic cell; NLRP3: nucleotide-binding and oligomerization domain-like receptor 3; ASC: apoptosis-associated speck-like protein; PAMPs: pathogen associated molecular patterns, ALT: alanine transaminase; AST: aspartate aminotransferase; BMDM: Bone marrow derived macrophage; M-CSF: macrophage colony-stimulating

factor; ConA: concanavalin A; IRI: ischemia-reperfusion injury; MAPKs: mitogen-activated protein kinases; NASH: nonalcoholic steatohepatitis;

Declarations

Ethics approval and consent to participate

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Nanjing Drum Tower Hospital (Approval No. 20160601).

Consent for publication

This manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. The materials in this manuscript is not reproduced from other articles. All the authors listed have approved the manuscript that is enclosed.

Availability of data and material

The authors confirm that all data underlying the findings are fully available.

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Authors' contributions

Yang Liu, Jinglin Wang and Haoran Ding performed experiments. Yang Liu analyzed data and wrote the manuscript. Jinglin Wang and Haoran Ding contributied in animal experiments and manuscript writing. Haozhen Ren and Xiaolei Shi designed the projectnand coordinated the execution of the experimental plan. Xiaolei Shi obtained funding, and directed the study. All authors read and approved the final manuscript

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Not applicable.

Competing Interests

The authors declare that they have no conflict of interest.

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Figures

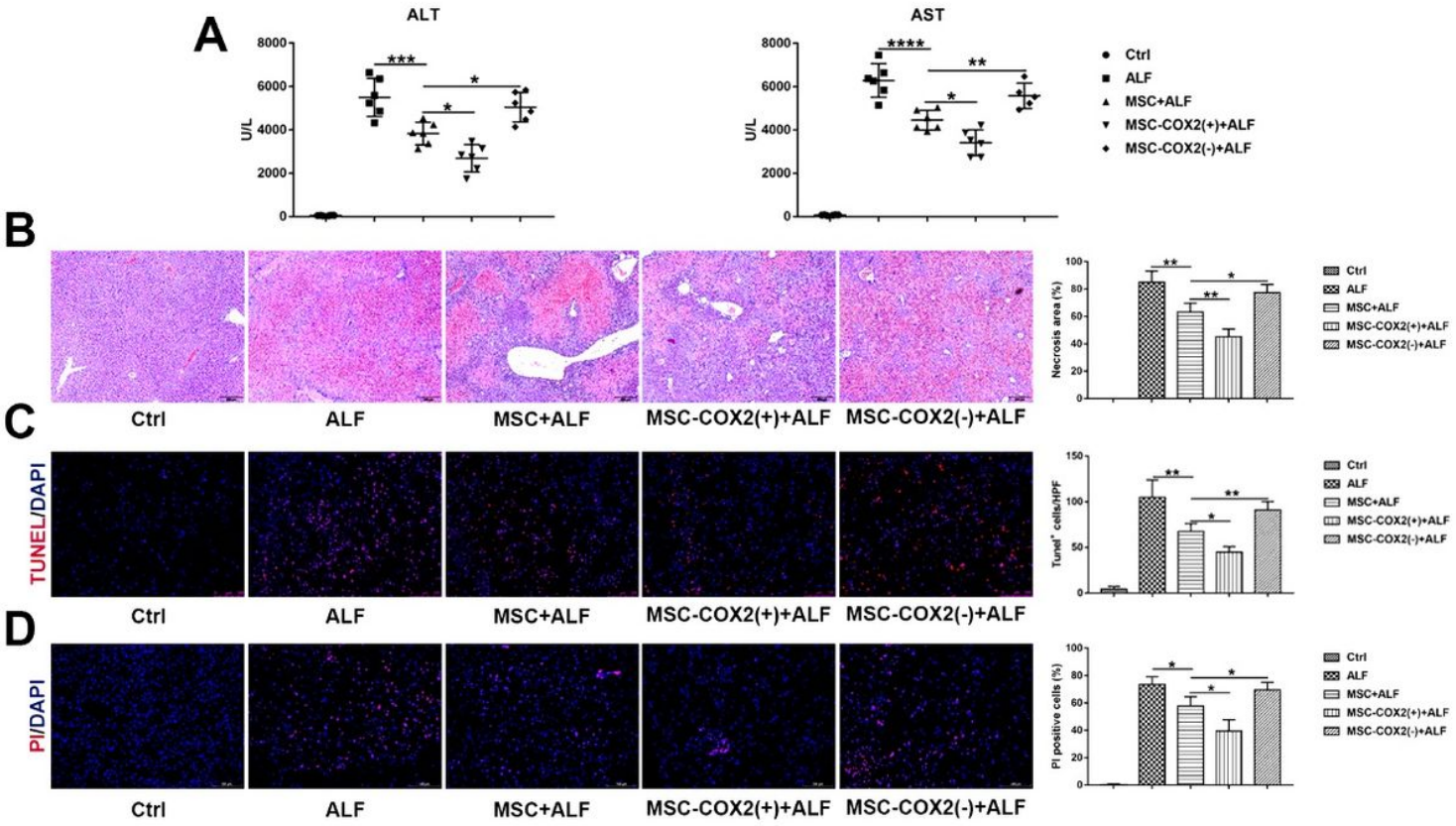

Figure 1

MSC protects LPS/D-Gal induced liver injury via PGE2. (A) Serum levels of ALT and AST in each group (n=6). (B) Representative HE stained liver sections from each group and quantitation of necrosis area in each group (n=4). (C) Representative images of TUNEL stained liver sections from each group and the number of TUNEL positive cells in each group (n=4). (D) Representative images of PI staining to indicate necrosis cells and the number of PI positive cells in each group (n=4). (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)

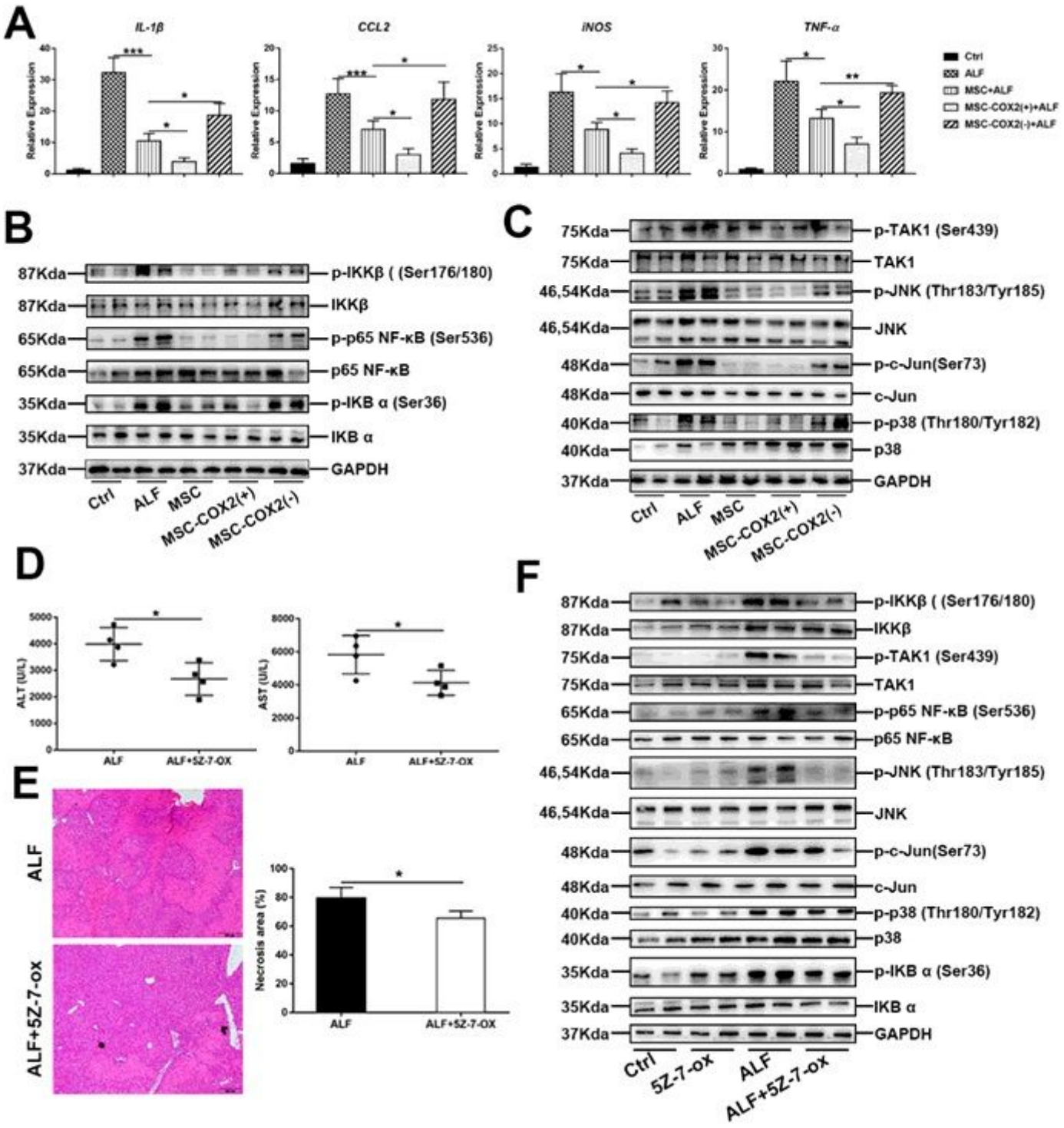

Figure 2

MSC-derived PGE2 restrains inflammatory responses in the liver during LPS/D-Gal induced ALF. (A) The mRNA levels of pro-inflammatory cytokines (IL-1β, CCL2, iNOS, TNF-α) in the liver in each group (n=4). (B) The protein expression levels of NF-κB signaling in each group. (C) The protein expression levels of TAK1 signaling in each group. (D) Serum levels of ALT and AST in mice pretreated with TAK1 inhibitor, 5Z-7-ox (n=4). (E) Representative HE stained liver sections and quantitation of necrosis area in each group (n=4). (F) Protein levels of TAK1 and NF-κB signaling in each group. (*p<0.05, **p<0.01, ***p<0.001)

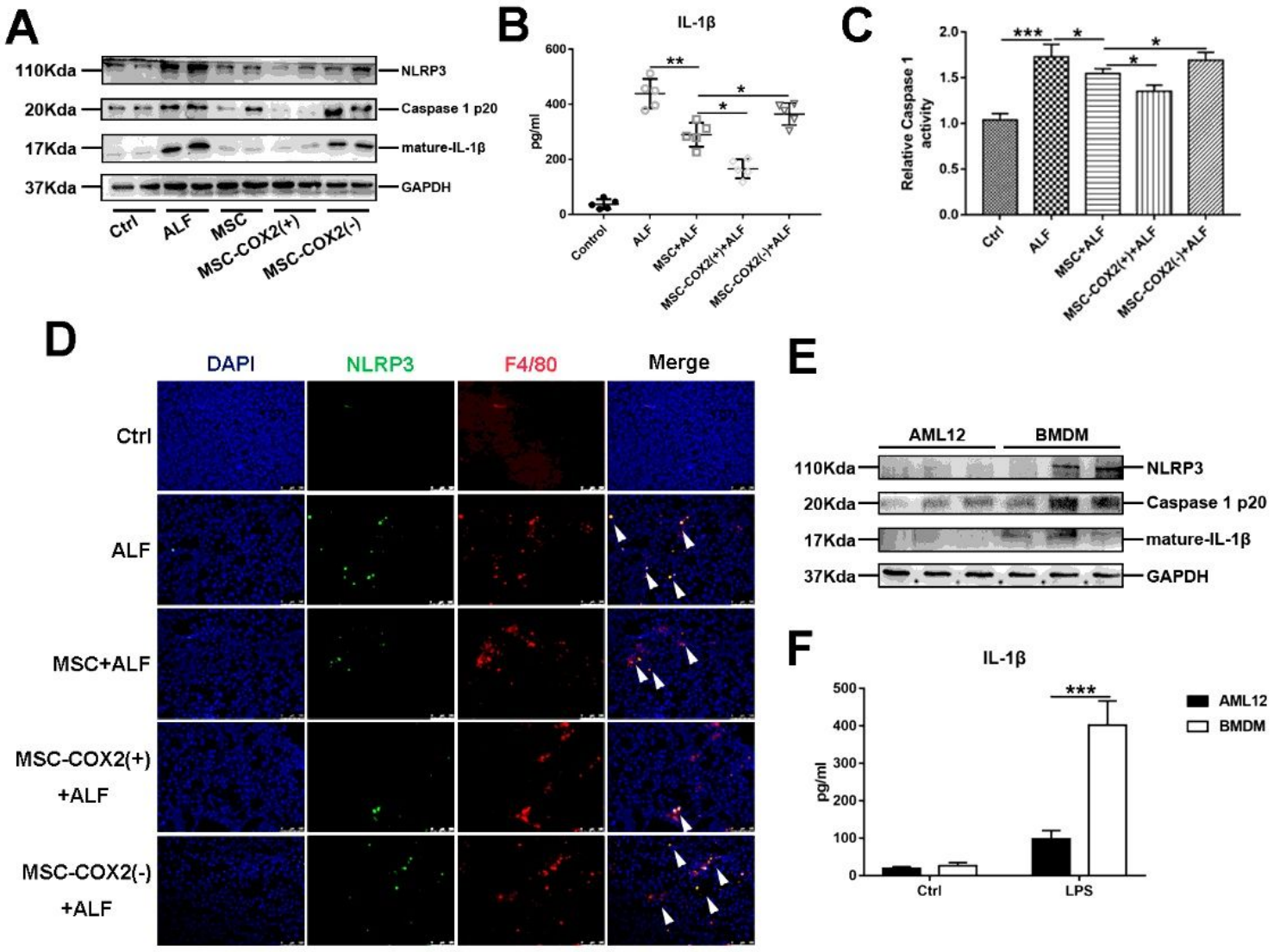

Figure 3

MSC-derived PGE2 inhibits NLPR3 inflammasome activation in liver macrophages. (A) Protein levels of NLRP3, Caspase 1 p20 and mature-IL-1β in each group. (B) Serum levels of IL-1β in each group (n=6). (C) Measurement of caspase-1 enzymatic activity in the liver of each group (n=4). (D) Representative immunofluorescence staining of co-localization of NLRP3 with F4/80 in the liver of each group. Blue: DAPI; Green: NLRP3; Red: F4/80. White arrows in the Merge images indicated co-localization. (E) Protein levels of NLRP3 inflammasome pathway in hepatocyte cell line AML12 and BMDM treated with 1μg/ml LPS for 24h. (F) The levels of IL-1β in supernatants of hepatocyte cell line AML12 and BMDM treated with 1μg/ml LPS for 24h (n=3). (*p<0.05, **p<0.01, ***p<0.001)

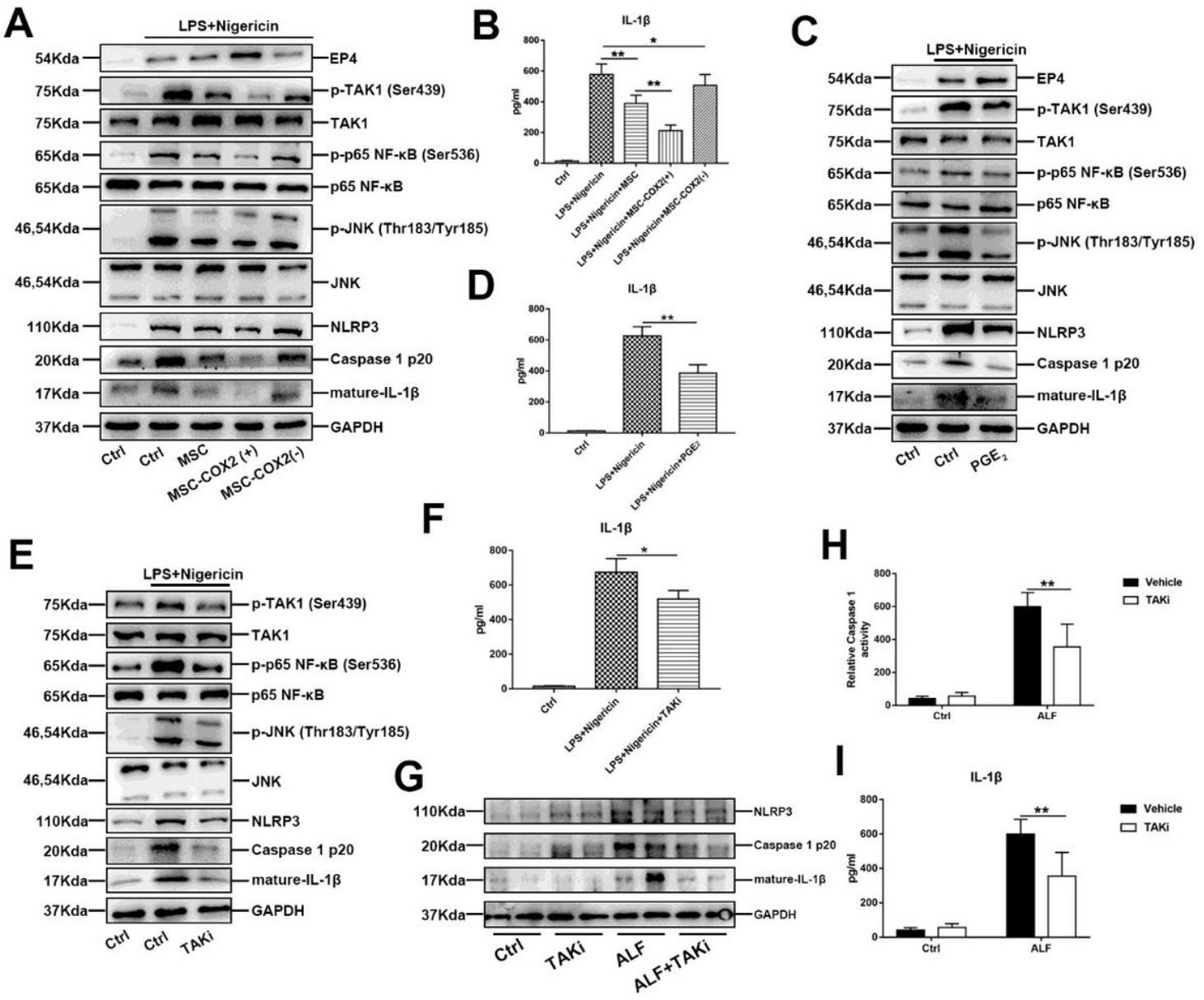

Figure 4

MSC-derived PGE2 inhibits NLRP3 inflammasome activation via TAK1. (A) The protein expression levels of NLRP3 inflammasome and TAK1 signaling in BMDM following indicated condition. (B)The levels of IL-1β in each supernatant (n=3). (C) The protein expression levels of NLRP3 inflammasome and TAK1 signaling in BMDM treated with exogenous PGE2. (D) The levels of IL-1β in cell supernatant treated with exogenous PGE2 (n=3). (E) Effects of TAK1 inhibitor, 5Z-7-ox (TAKi) on NLRP3 inflammasome activation in BMDM. (F) The levels of IL-1β in cell supernatant treated with TAKi (n=3). (G) Expression of NLRP3 inflammasome signaling in the liver pretreated with TAKi. (H) Measurement of caspase-1 enzymatic activity in the liver of each group (n=4). (I) Serum levels of IL-1β in each group (n=4). (*p<0.05, **p<0.01)

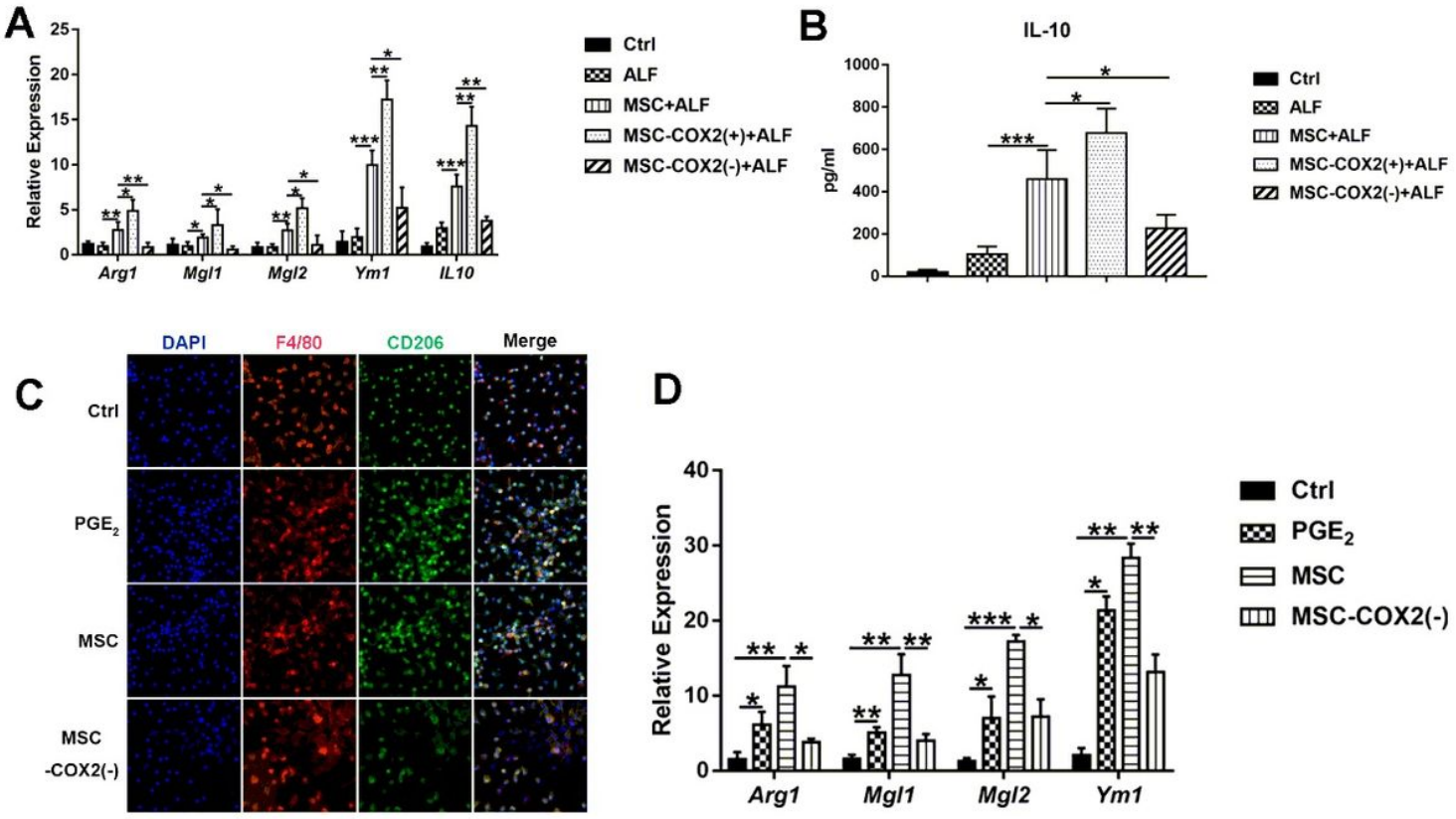

Figure 5

MSC-derived PGE2 promotes an M2 macrophage phenotype in the liver. (A) The mRNA levels of M2 markers (Arg1, Mgl1, Mgl2, Ym1 and IL-10) in the liver of each group (n=4). (B) Serum levels of IL-10 in each group (n=4). (C) Representative immunofluorescence staining of M2 marker, CD206 and F4/80 in BMDM following indicated condition. (D) The mRNA levels of M2 markers in BMDM following indicated condition (n=3). (*p<0.05, **p<0.01, ***p<0.001)

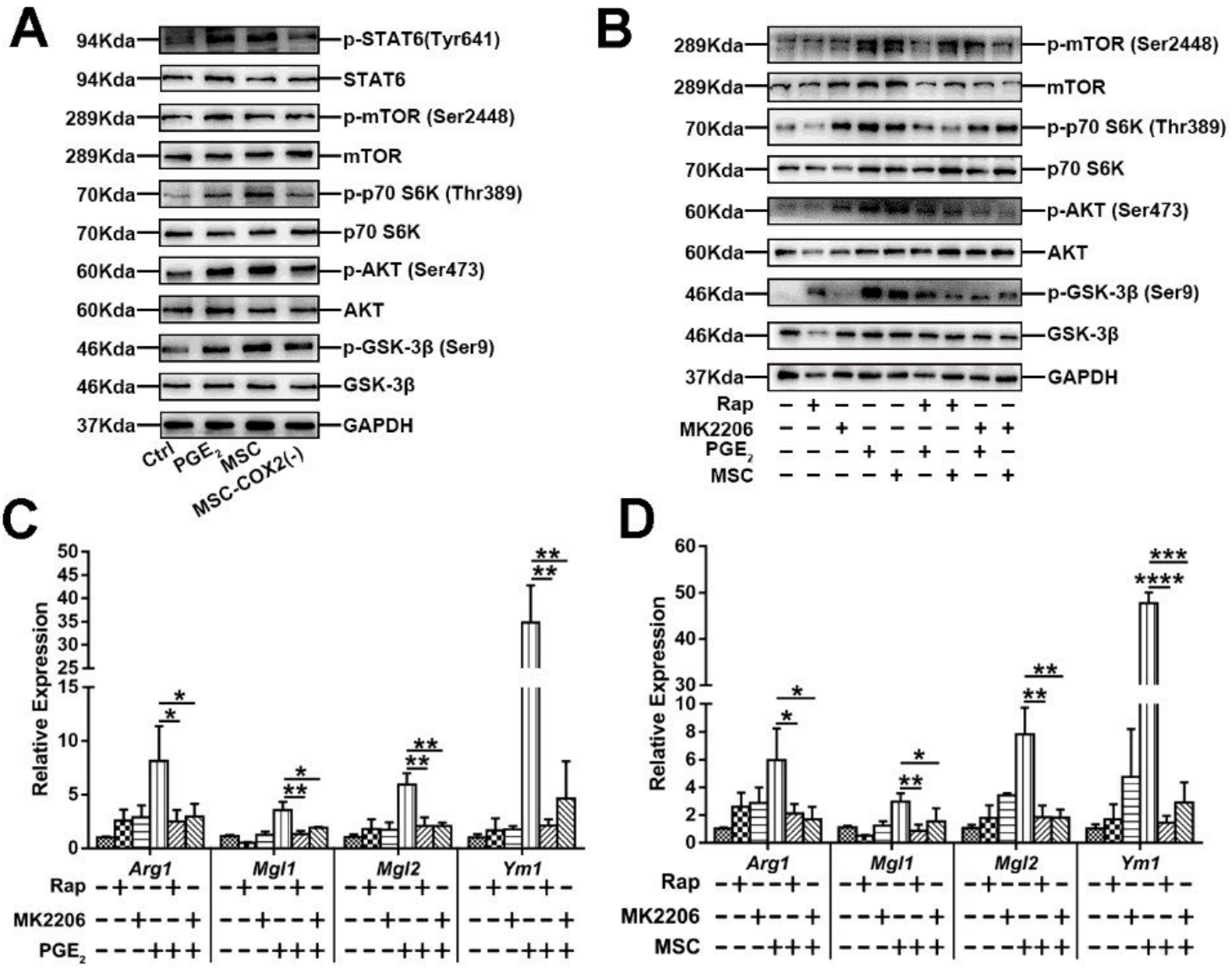

Figure 6

MSC-derived PGE2 promotes M2 macrophage through STAT6 and mTOR signaling. (A) Protein levels of STAT6 and mTOR signaling in BMDM following indicated condition. (B) Protein levels of mTOR signaling in BMDM treated with mTOR inhibitor (Rapamycin, Rap) and AKT inhibitor (MK-2206) following indicated condition. (C) The mRNA levels of M2 markers (Arg1, Mgl1, Mgl2 and Ym1) in BMDM treated with Rap following indicated condition (n=3). (D) The mRNA levels of M2 markers (Arg1, Mgl1, Mgl2 and Ym1) in BMDM treated with MK-2206 following indicated condition (n=3). (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)

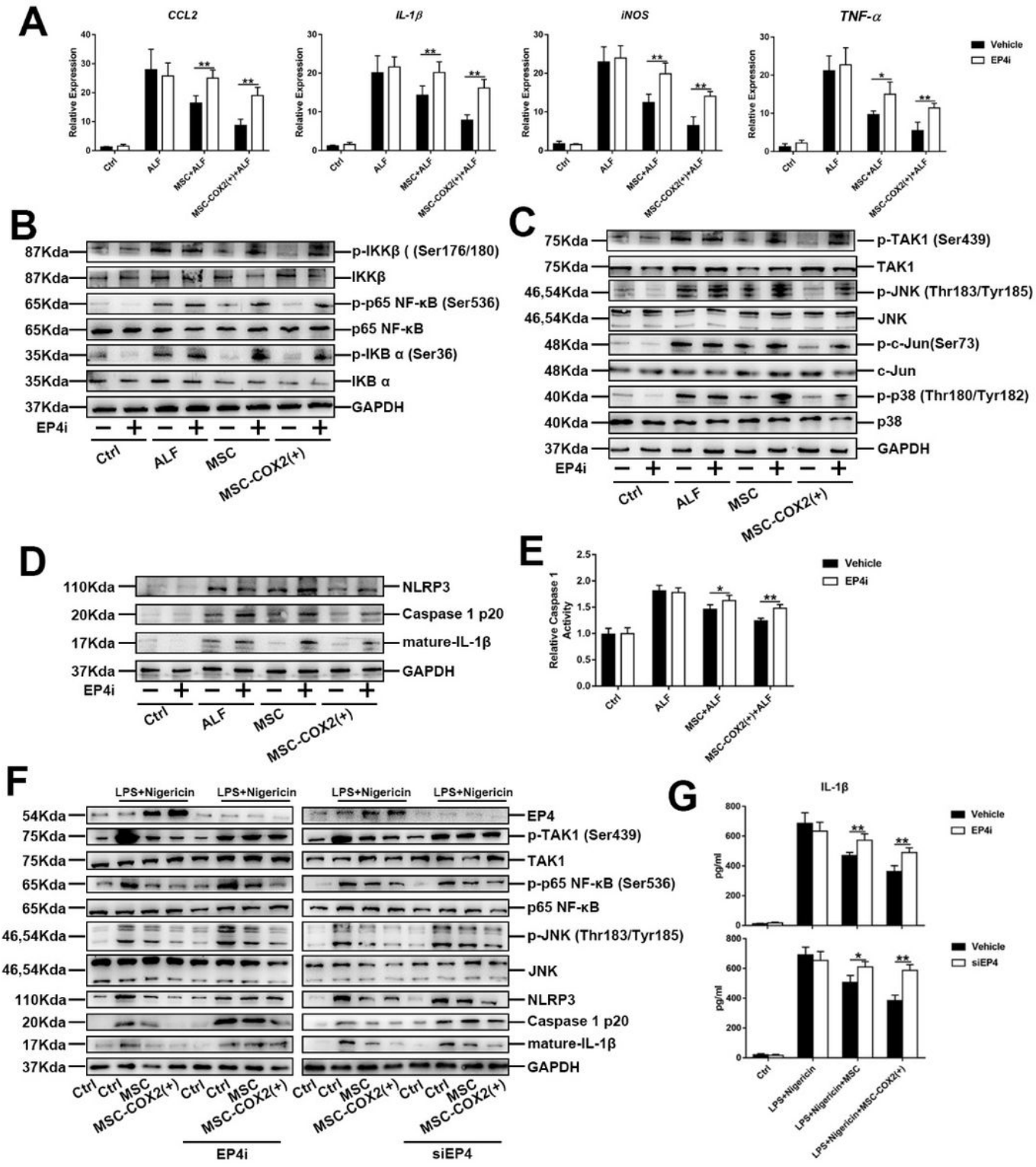

Figure 7

MSC-derived PGE2 protects against liver inflammation via EP4. (A) The mRNA levels of inflammatory cytokines (IL-1β, CCL2, iNOS, TNF-α) in the liver from each group pretreated with EP4 inhibitor (EP4i) (n=4). (B) The protein expression levels of NF-κB signaling in each group pretreated with EP4i. (C) The protein expression levels of TAK1 signaling in each group pretreated with EP4i. (D) The protein expression levels of NLRP3 inflammasome signaling in each group pretreated with EP4i. (E) Measurement of

caspase-1 enzymatic activity in the liver of each group pretreated with EP4i (n=4). (F) Protein levels of NLRP3 inflammasome and TAK1 signaling in BMDM pretreated with EP4i or knocking down of EP4 via siRNA. (G) The levels of IL-1β in supernatants of BMDM pretreated with EP4i or knocking down of EP4 via siRNA. (*p<0.05, **p<0.01)

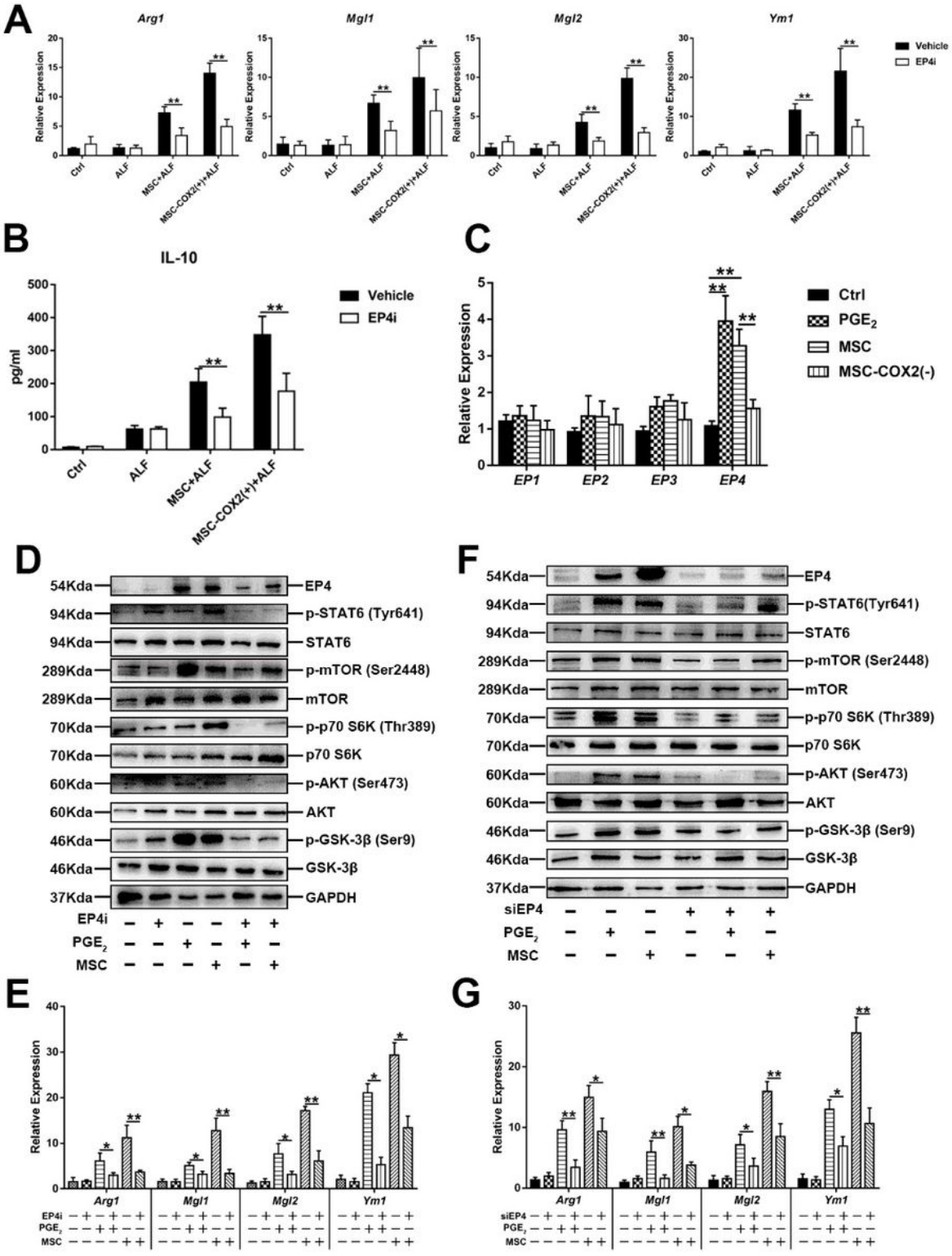

Figure 8

M2 macrophage polarization depends on EP4. (A) The mRNA levels of M2 markers (Arg1, Mgl1, Mgl2 and Ym1) in the liver of each group pretreated with EP4i (n=4). (B) Serum levels of IL-10 in each group (n=4). (C) The mRNA levels of (EP1, EP2, EP3 and EP4) in BMDM under indicated condition (n=3). (D) Protein levels of STAT6 and mTOR signaling in BMDM treated with EP4i. (E) The mRNA levels of M2 markers (Arg1, Mgl1, Mgl2 and Ym1) in BMDM treated with EP4i (n=3). (F) Protein levels of STAT6 and mTOR signaling in BMDM knocked down EP4 via siRNA. (G) The mRNA levels of M2 markers (Arg1, Mgl1, Mgl2 and Ym1) in BMDM knocked down EP4 via siRNA (n=3). (*p<0.05, **p<0.01)

Supplementary Files

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- SupplementaryMaterial.docx