# Monoacylglycerol lipase reprograms hepatocytes and macrophages to promote liver regeneration


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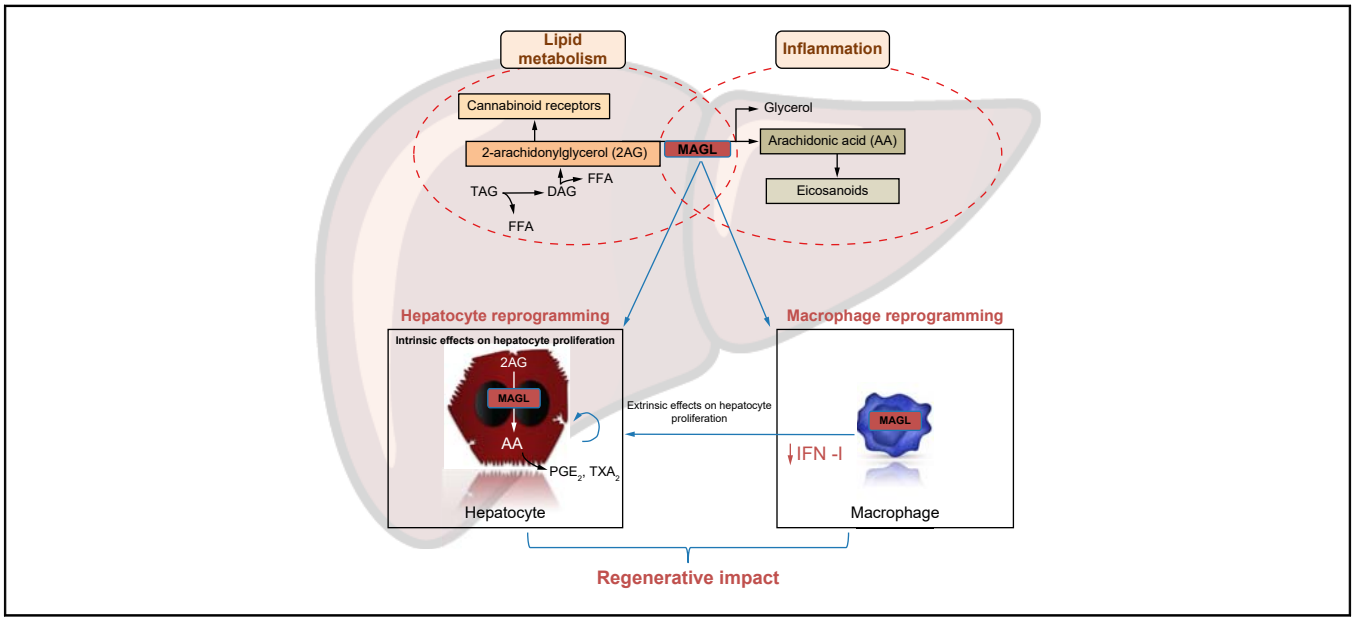
## Graphical abstract




## Highlights

- Monoacylglycerol lipase (MAGL) reduces hepatocyte proliferation in human precision-cut liver slices.
- Inhibiting MAGL specifically in hepatocytes delays liver regeneration by reducing liver 2-arachidonylglycerol production.
- Inhibiting MAGL specifically in myeloid cells delays liver regeneration by reprogramming macrophages towards the IFN-I pathway.
- Neutralising the IFN-I pathway restores liver regeneration in mice invalidated for MAGL in myeloid cells.

## Impact and Implications

By using human liver samples and mouse models of global or specific cell type invalidation, we show that the monoacylglycerol pathway plays an essential role in liver regeneration. We unveil the mechanisms by which MAGL expressed in both hepatocytes and macrophages impacts the liver regeneration process, via eicosanoid production by hepatocytes and the modulation of the macrophage interferon pathway profile that restrains hepatocyte proliferation.

https://doi.org/10.1016/j.jhepr.2023.100794

Research article

JHEP|Reports

# Monoacylglycerol lipase reprograms hepatocytes and macrophages to promote liver regeneration

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JHEP Reports **2023.** https://doi.org/10.1016/j.jhepr.2023.100794

**Background & Aims:** Liver regeneration is a repair process in which metabolic reprogramming of parenchymal and inflammatory cells plays a major role. Monoacylglycerol lipase (MAGL) is an ubiquitous enzyme at the crossroad between lipid metabolism and inflammation. It converts monoacylglycerols into free fatty acids and metabolises 2-arachidonoylglycerol into arachidonic acid, being thus the major source of pro-inflammatory prostaglandins in the liver. In this study, we investigated the role of MAGL in liver regeneration.

**Methods:** Hepatocyte proliferation was studied *in vitro* in hepatoma cell lines and *ex vivo* in precision-cut human liver slices. Liver regeneration was investigated in mice treated with a pharmacological MAGL inhibitor, MJN110, as well as in animals globally invalidated for MAGL ($MAGL^{-/-}$) and specifically invalidated in hepatocytes ($MAGL^{Hep-/-}$) or myeloid cells ($MAGL^{Mye-/-}$). Two models of liver regeneration were used: acute toxic carbon tetrachloride injection and two-thirds partial hepatectomy. $MAGL^{Mye-/-}$ liver macrophages profiling was analysed by RNA sequencing. A rescue experiment was performed by *in vivo* administration of interferon receptor antibody in $MAGL^{Mye-/-}$ mice.

**Results:** Precision-cut human liver slices from patients with chronic liver disease and human hepatocyte cell lines exposed to MJN110 showed reduced hepatocyte proliferation. Mice with global invalidation or mice treated with MJN110 showed blunted liver regeneration. Moreover, mice with specific deletion of MAGL in either hepatocytes or myeloid cells displayed delayed liver regeneration. Mechanistically, $MAGL^{Hep-/-}$ mice showed reduced liver eicosanoid production, in particular prostaglandin $E_2$ that negatively impacts on hepatocyte proliferation. MAGL inhibition in macrophages resulted in the induction of the type I interferon pathway. Importantly, neutralising the type I interferon pathway restored liver regeneration of $MAGL^{Mye-/-}$ mice.

**Conclusions:** Our data demonstrate that MAGL promotes liver regeneration by hepatocyte and macrophage reprogramming.

**Impact and Implications:** By using human liver samples and mouse models of global or specific cell type invalidation, we show that the monoacylglycerol pathway plays an essential role in liver regeneration. We unveil the mechanisms by which MAGL expressed in both hepatocytes and macrophages impacts the liver regeneration process, via eicosanoid production by hepatocytes and the modulation of the macrophage interferon pathway profile that restrains hepatocyte proliferation.

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## Introduction

Liver regeneration represents a major therapeutic issue following acute liver damage such as large surgical resections, toxic injury, or ischaemia/reperfusion lesions induced by liver transplantation. This wound healing process requires timely coordinated epithelial and immune cell interactions, allowing hepatocytes to quit their quiescent state to proliferate and the liver to recover rapidly. During regeneration, not only hepatocytes but also immune cells – particularly macrophages – undergo metabolic rewiring.[1–3] Emerging data suggest that targeting the metabolism of these cells could constitute a new therapeutic strategy to stimulate liver regeneration.[1–3] However, molecular mediators linking metabolism and liver regeneration remain poorly known.

Monoacylglycerol lipase (MAGL) is a rate-limiting enzyme in the degradation of monoacylglycerols that are hydrolysed into glycerol and free fatty acids. MAGL produces arachidonic acid from 2-arachinoylglycerol and is also the primary source for pro-inflammatory prostaglandins (PGs) in the liver. This makes this ubiquitously expressed protein a connecting hub between lipids

Keywords: Wound healing; Injury; MAGL; Proliferation.
*Received 20 October 2022; received in revised form 21 April 2023; accepted 26 April 2023; available online 16 May 2023*

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and inflammation.[4–7] We recently unraveled MAGL as a novel immunometabolic target allowing, when inhibited, to slow down liver fibrosis in the context of chronic liver injury.[8] However, the role of MAGL in the liver regeneration context had never been investigated. We thus wondered to what extent MAGL, and particularly MAGL expressed by macrophages and hepatocytes, besides its pro-fibrogenic role, might also modulate liver regeneration.

In the present study, we combined experiments in human samples and mice models to study the central role of MAGL in liver regeneration. We demonstrated that in hepatocytes MAGL induces eicosanoids production that promotes their proliferation. Using RNA sequencing (RNASeq), we showed that MAGL deficiency reprograms liver macrophages expression profile and induces type I interferon (IFN-I) pathway. Our results highlight the regenerative properties of MAGL in the liver by both an intrinsic direct lipid reprogramming of hepatocytes and an extrinsic reprogramming of macrophage expression profile.

## Materials and methods

### Generation of invalidated mice

MAGL-deficient mice ($MAGL^{-/-}$) were backcrossed for more than 10 generations on a C57BL/6J background. C57BL/6J control mice were purchased from Janvier (Le Genest-Saint-Isle, France). *Mgll-loxP/loxP* mice ($MAGL^{fl/fl}$) and MAGL-myeloid mice ($MAGL^{Mye-/-}$) were generated as previously described.[8] $MAGL^{Hep-/-}$ mice were generated by crossing $MAGL^{fl/fl}$ to *Alb-Cre* (B6.Cg-Speer6-ps1Tg(Alb-cre) 21Mgn/J Jackson Laboratory) for more than 10 generations. Animals were housed in a pathogen-free animal facility and fed *ad libitum*.

### Human liver samples

*Patients*

Liver samples were obtained from seven patients with fibrosis (F2 to F4 according to the METAVIR score system; see Table 1) undergoing liver resection or liver transplantation at the digestive surgical department of Beaujon Hospital (Clichy, France). Patients' clinical data are presented in Table 1. All patients gave written consent to participate in this study, which complied with the ethical guidelines of the 1975 Declaration of Helsinki. Fresh liver specimens were examined by a pathologist, and samples were collected at distance from the tumour (when present) and surgical margins.

*Human* ex vivo *PCLS*

Fresh liver specimens were harvested and immediately kept on ice in sterile University of Wisconsin solution (Belzer UW® Cold Storage Solution, Bridge to Life, BTLBUW-1000). Liver cores were generated from liver specimens using 8-mm-diameter biopsy punches, embedded into 5% low-gelling-temperature agarose (Sigma Aldrich) and mounted in a tissue slicer (automated vibrating blade microtome, Leica Biosystems VT1200 S) filled with HBSS supplemented with 25 mM of D-Glucose (Sigma Aldrich), 100 μg/ml streptomycin, and 1 μg/ml amphotericin B (Gibco™). Precision-cut liver slices (PCLS, 8-mm diameter, 250-μm thickness) were generated using the following slicing parameters: speed, 0.5 mm/s; thickness, 250 μm; and amplitude, 3 mm. Human PCLS were transferred on 8-μm polyethylene terephtalate tissue culture inserts (ThinCert™, Greiner Bio-One) in six-well plates and pre-incubated in William's E Medium (Gibco™) supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin, 1 μg/ml amphotericin B (Gibco™), and 25 mM D-Glucose (Sigma Aldrich). After 1-h pre-incubation, fresh culture medium containing 10 μM MJN110 or its vehicle (0.01% DMSO) was added, and PCLS were cultivated at 37 °C, 5% $CO_2$, under a continuous gentle orbital agitation during 2 days. Culture medium was renewed daily. After 48 h of culture, PCLS were washed in cold PBS and fixed in 10% formalin for 24 h for immunohistochemistry experiments.

### Experimental mouse models of liver regeneration

*Partial hepatectomy*

Ten- to 16-week-old male mice were submitted to two-thirds partial hepatectomy (PHx) as previously described.[9] Animals were sacrificed 40 h, 48 h, or 14 days after PHx.

*$CCl_4$-induced injury and* in vivo *injections*

Ten- to 16-week-old male mice received one intraperitoneally (i.p.) injection (0.6 ml/kg body weight [BW]) of carbon tetrachloride ($CCl_4$) (Sigma-Aldrich 87030) diluted 1/10 in mineral oil (Sigma-Aldrich M-5310). Control animals received mineral oil. When indicated, mice were i.p. injected with the MAGL inhibitor MJN110 (Cayman Chemical 17583), or its vehicle (Emulphor:-ethanol:PBS 1:1:18, 10 mg/kg BW), 2 h before $CCl_4$ injection and then daily until sacrifice. When indicated, anti-mouse interferon α/β receptor (IFNAR) 1 antibody (Bio Cell BE0241), or its isotype (Bio Cell BE0083), was injected i.p. the day before $CCl_4$ injection (500 μg), 1 h before $CCl_4$ injection (450 μg), and 24 h after $CCl_4$ injection (250 μg) as described in a previous study,[10] and animals were sacrificed 48 h after $CCl_4$ injection.

### Histological analysis

Immunohistochemical analysis was performed on formalin-fixed paraffin-embedded mouse or human liver sections (4 μm). To study hepatocyte proliferation and apoptosis, immunostaining was performed using anti-bromodeoxyuridine (BrdU; 1:40, Abcam ab6326), anti-phospho-histone H3 (PHH3; 1:1,000, EMD Millipore 06-570), anti-Ki67 (1:100, Dako M7240), and a cleaved caspase-3 (Asp175) (1:50, Cell Signaling Technology, Danvers,

**Table 1. Patient characteristics for precision-cut liver slices study.**

| Patient | Age (years) | Sex | Reason and type of surgery | Aetiology of fibrosis | METAVIR or SAF score |
|---|---|---|---|---|---|
| 1 | 61 | M | HCC (explanted liver) | Alcohol | A0F4 |
| 2 | 51 | M | Decompensated cirrhosis (explanted liver) | Alcohol | A0F4 |
| 3 | 74 | F | HCC (resected liver) | Metabolic | S1A4F3 |
| 4 | 68 | M | HCC (explanted liver) | Alcohol and metabolic | S0A0F4 |
| 5 | 68 | F | Decompensated cirrhosis (explanted liver) | Metabolic | S1A3F4 |
| 6 | 81 | F | CC (resected liver) | Metabolic | S1A1F2 |
| 7 | 69 | M | HCC (resected liver) | Alcohol and metabolic | S1A1F3/F4 |

CC, cholangiocarcinoma; F, female; HCC, hepatocellular carcinoma; M, male; SAF, steatosis, activity, fibrosis.

MA, USA, 9661) antibodies. Anti-rat (Vector Laboratories BA-9401) or anti-rabbit antibody (Bio-Rad STAR131B) was used as a secondary antibody. The signal was enhanced using the Vectastain Elite ABC kit (Vector Laboratories PK-6100) and revealed by the 3,3′-Diaminobenzidine kit (Dako K3468) according to the manufacturer's instructions. Images were taken at 20× magnification. Positive hepatocytes were counted blindly by two independent observers on 15 fields using ImageJ software in mice and 8 fields in human sections (4,000–8,000 hepatocytes). No staining was observed when the primary antibody was omitted.

### RNA sequencing

RNASeq was performed as previously described.[11–16] Genes were considered as expressed if their FPKM (fragments per kilobase per million mapped fragments) value was greater than 95% of the background FPKM value based on intergenic regions defined from the Mouse FAST DB v2018_1 annotations. Differential gene expression analysis was performed using DESeq2, and results were considered statistically significant for $p$ values ≤0.05 and fold changes ≥1.5. Enrichment tests were performed using WebGestaltR[17] from Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), and Reactome databases with cut-off values of $p$ <0.05 and a minimum of two overlapping genes. The sequence datasets have been deposited in the Gene Expression Omnibus with the accession number GSE 189064. For more details, see Supplementary information.

### Statistical analysis

All statistical analyses were performed with Prism software 8.4.3 (GraphPad Software, Inc., La Jolla, CA, USA) using the nonparametric Mann–Whitney $U$ test or a paired $t$ test as indicated in figure legends. Data are presented as mean ± SD, and $p$ ≤0.05 was considered statistically significant. Sample sizes were adequate to detect effects between groups, as determined by the reproducibility and variability of each experiment.

### Study approval

Experiments were performed in accordance with protocols approved by the French Ministère de l'Enseignement Supérieur de la Recherche et de l'Innovation (project authorisation using animals for scientific purposes APAFIS #30284-2021030912208867).

For western blots, reverse-transcription PCR ELISA, and *in vitro* studies, see Supplementary information.

## Results

### Inhibition of MAGL limits hepatocyte proliferation *ex vivo* in human PCLS and *in vitro* in human hepatoma cell lines

To investigate the impact of MAGL inhibition on hepatocyte proliferation, we first took advantage of the differential expression of MAGL in the human hepatoma HuH7 and HuH6 cell lines (Fig. 1A). We observed that the pharmacological MAGL inhibitor MJN110 reduces DNA synthesis in the MAGL-expressing HuH7 cell line, whereas it had no effect in the MAGL-deficient HuH6 cell line (Fig. 1B).

We then exposed PCLS from patients with chronic liver injury (Table 1) to MJN110, or its vehicle, for 48 h. The proportion of Ki67-positive hepatocytes was significantly reduced after MJN110 exposure compared with control (Fig. 1C and D). Only one PCLS coming from the non-tumoural liver with mild fibrosis of a patient with cholangiocarcinoma showed no reduced proliferation.

These results demonstrate that MAGL inhibition negatively impacts hepatocyte proliferation. We therefore further explored the role of MAGL in mouse models of liver regeneration.

### MAGL promotes liver regeneration

We investigated the impact of MAGL invalidation in experimental mouse models as induced by an acute $CCl_4$ administration (Fig. 2A) or a two-thirds PHx (Fig. 2B). We compared the regeneration kinetics of wild-type (WT) mice, global MAGL-invalidated mice ($MAGL^{-/-}$), or mice exposed to the pharmacological inhibitor MJN110 before injury. Both models of MAGL inactivation showed a significant reduction in BrdU incorporation and PHH3 hepatocyte immunostaining 48 and 72 h after toxic liver injury, compared with controls (Fig. 2A and B). Moreover, at the same time points, cyclin A was expressed at substantially lower levels in $MAGL^{-/-}$ and MJN 110-treated mice than in their control counterparts (Fig. 3A and B). Interestingly, cyclin D1 expression was already reduced 30 h after $CCl_4$ injection in MAGL-inhibited mice (Fig. 3A and B). Altogether, these results demonstrated that global invalidation delayed liver regeneration. In line with the known hepatoprotective effects of MAGL inhibitors,[8] reduced serum transaminase levels and decreased necrotic area were found in MJN110-treated and $MAGL^{-/-}$ mice compared with their respective controls after an acute $CCl_4$ injection (Fig. S1A and B). To determine whether MAGL defect impacts liver regeneration independently of its hepatoprotective effect, we performed two-thirds PHx. Survival

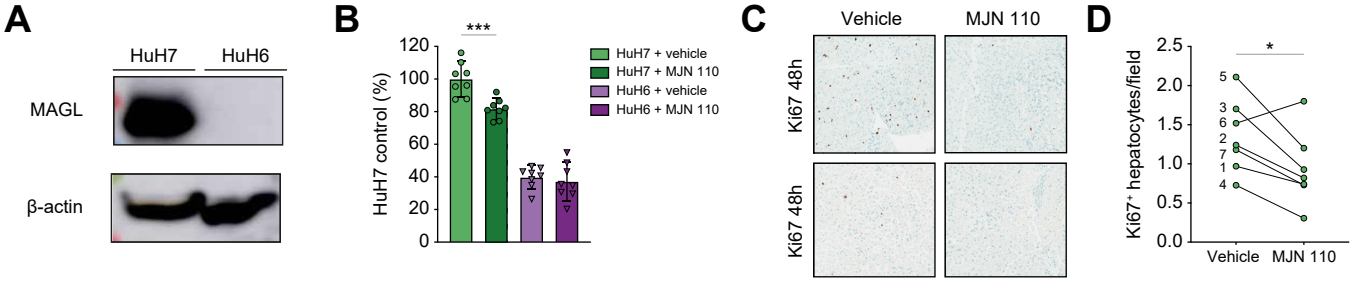


**Fig. 1. MAGL inhibition with MJN110 reduces hepatocyte proliferation in human hepatoma cell lines and PCLS from patients with chronic liver disease.** (A) Western blot of MAGL expression in human hepatocyte cell lines HuH7 and HuH6. β-actin was used as a loading control. (B) Quantification of DNA synthesis in human hepatoma cell lines exposed to MJN110 or its vehicle, expressed as a percentage of HuH7 + vehicle proliferation (representative of three experiments). ***$p$ <0.001, by two-tailed Mann–Whitney $U$ test. Human PCLS were incubated with 10 μM MJN110 or vehicle for 48 h: (C) representative images of Ki67-immunostained PCLS sections treated with MJN110 or its vehicle as indicated (magnification, 20×) and (D) quantification of Ki67-positive hepatocytes per field. *$p$ <0.05, non-parametric Wilcoxon paired test. For patient numbers, refer to Table 1. MAGL, monoacylglycerol lipase; PCLS, precision-cut liver slices.

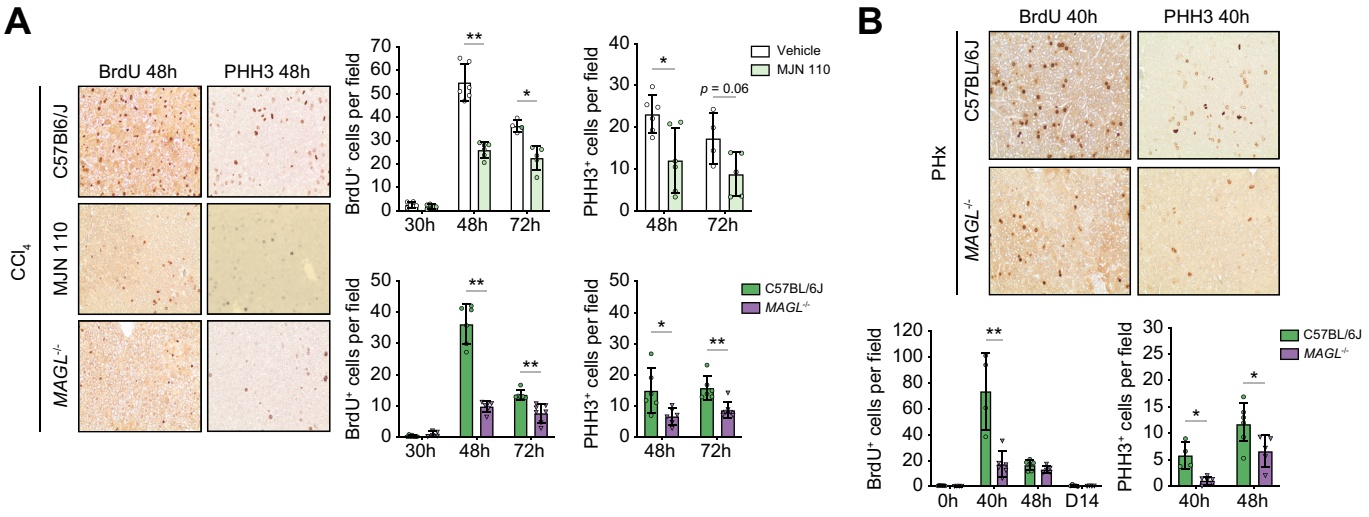


**Fig. 2. MAGL invalidation or inhibition delays liver regeneration.** Representative liver sections of BrdU- and PHH3-stained hepatocytes in C57BL/6J mice, MAGL$^{-/-}$ mice, and MJN110-treated mice (A) 48 h after $CCl_4$ injection and (B) 40 h after PHx. Corresponding quantifications of BrdU- and PHH3-stained hepatocytes are shown at indicated time points after injury (n = 5–7 mice per group). BrdU, bromodeoxyuridine; $CCl_4$, carbon tetrachloride; MAGL, monoacylglycerol lipase; PHH3, phospho-histone H3; PHx, partial hepatectomy. *$p$ >0.05, **$p$ <0.01 by two-tailed Mann-Whitney $U$ test.

was higher than 90% and independent of the genotype. In this model, the level of injury was very low, and there was no difference in either necrosis or apoptosis, as assessed by aspartate aminotransferase–alanine aminotransferase levels, necrotic area, and cleaved caspase-3-stained hepatocyte quantification between MAGL$^{-/-}$ and WT mice (Fig. S1C). We observed a delay in liver mass recovery upon MAGL global deletion 14 days after PHx at a time point where the liver weight on body weight (LW/BW) ratio of control livers is restored (Fig. S2A). A reduction in BrdU incorporation and PHH3 hepatocyte staining was observed at the peak of proliferation (*i.e.* 40 h) after surgical resection in mutated animals compared with controls (Fig. 2B). This was confirmed by a reduction in cyclin A expression in mutant mice (Fig. 3C and S2B).

## Hepatocyte-specific invalidation of MAGL impairs hepatocyte proliferation owing to a reduced eicosanoid production

We then investigated the contribution of MAGL in hepatocytes and macrophages to the liver regeneration process. We first generated mice lacking MAGL specifically in hepatocytes (MAGL$^{Hep-/-}$) by crossing MAGL$^{fl/fl}$ with transgenic mice expressing Cre recombinase under the control of the albumin promoter. The deletion efficacy was confirmed by the decrease in MAGL expression in the total liver and primary hepatocytes (Fig. S2C). We showed a reduced hepatocyte proliferation at the peak, that is, 48 h after $CCl_4$ injection (Fig. S2D). However, as for MAGL global deletion, hepatocyte MAGL deficiency protected against acute injury as shown by the reduced aspartate aminotransferase levels (Fig. S2D). To determine whether MAGL hepatocyte-specific deficiency delayed regeneration independently of liver injury, we performed PHx. We observed a delayed recovery of LW/BW ratio 14 days after surgery (Fig. S2A). Importantly, there was a reduced hepatocyte BrdU staining in MAGL$^{Hep-/-}$ animals compared with their respective MAGL$^{fl/fl}$ controls with a reduced cyclin A expression (Fig. 4A and B) 40 and 48 h after surgery. The cell-intrinsic proliferative impact of MAGL was investigated in isolated primary hepatocytes. MAGL-deficient hepatocytes showed a reduced proliferation rate as compared with control C57Bl/6J hepatocytes (Fig. 4C). Moreover, as in human hepatocytes, MJN110 reduced the proliferation of primary hepatocytes from WT C57BL/6J mice, whereas it had no impact on MAGL-invalidated hepatocytes (Fig. 4C). Taken together, these data demonstrate that *in vitro* as well as *in vivo* inhibition of MAGL in hepatocytes reduces their proliferation.

Pharmacological inhibition of MAGL leads to a decrease in arachidonic acid-derived metabolites, among which are prostaglandin $E_2$ ($PGE_2$) and thromboxane $A_2$ ($TXA_2$), two important mediators in hepatocyte growth and liver regeneration.[18–21] $PGE_2$ was constitutively reduced in total liver extracts of MAGL$^{-/-}$ and MAGL$^{Hep-/-}$ mice compared with their respective controls already at basal levels (Fig. 4D). Pharmacological inhibition of MAGL by MJN110 reduced $PGE_2$ production by control primary hepatocytes, but it had no effect on MAGL$^{-/-}$ hepatocytes (Fig. 4E). In keeping with this finding, primary hepatocytes isolated from MAGL$^{-/-}$ mice showed reduced proliferation compared with control C56BL/6J hepatocytes, and this negative impact on proliferation was overcome by exogenous addition of $PGE_2$ in the culture medium (Fig. 4F). Other metabolites of the arachidonic pathway could have also supported hepatocyte proliferation. In keeping with this hypothesis, we also found a reduced $TXA_2$ production in MAGL$^{Hep-/-}$ livers at basal levels and during regeneration as well as in invalidated hepatocytes or control hepatocytes treated with MJN110 (Fig. S2E). Altogether, these data support an intrinsic pro-regenerative impact of MAGL that is mediated via eicosanoid production.

## Macrophage MAGL deficiency impairs liver regeneration and reprograms macrophage signature towards an IFN-I pathway

To determine the contribution of MAGL from macrophages to liver repair, we used mice lacking MAGL in the myeloid lineage (MAGL$^{Mye-/-}$). The invalidation of MAGL was confirmed in bone marrow-derived macrophages (BMDM) (Fig. S2C). Impaired liver regeneration was observed in MAGL$^{Mye-/-}$ mice after $CCl_4$ acute injury (Fig. 5A and B). Importantly, in contrast to that in MAGL

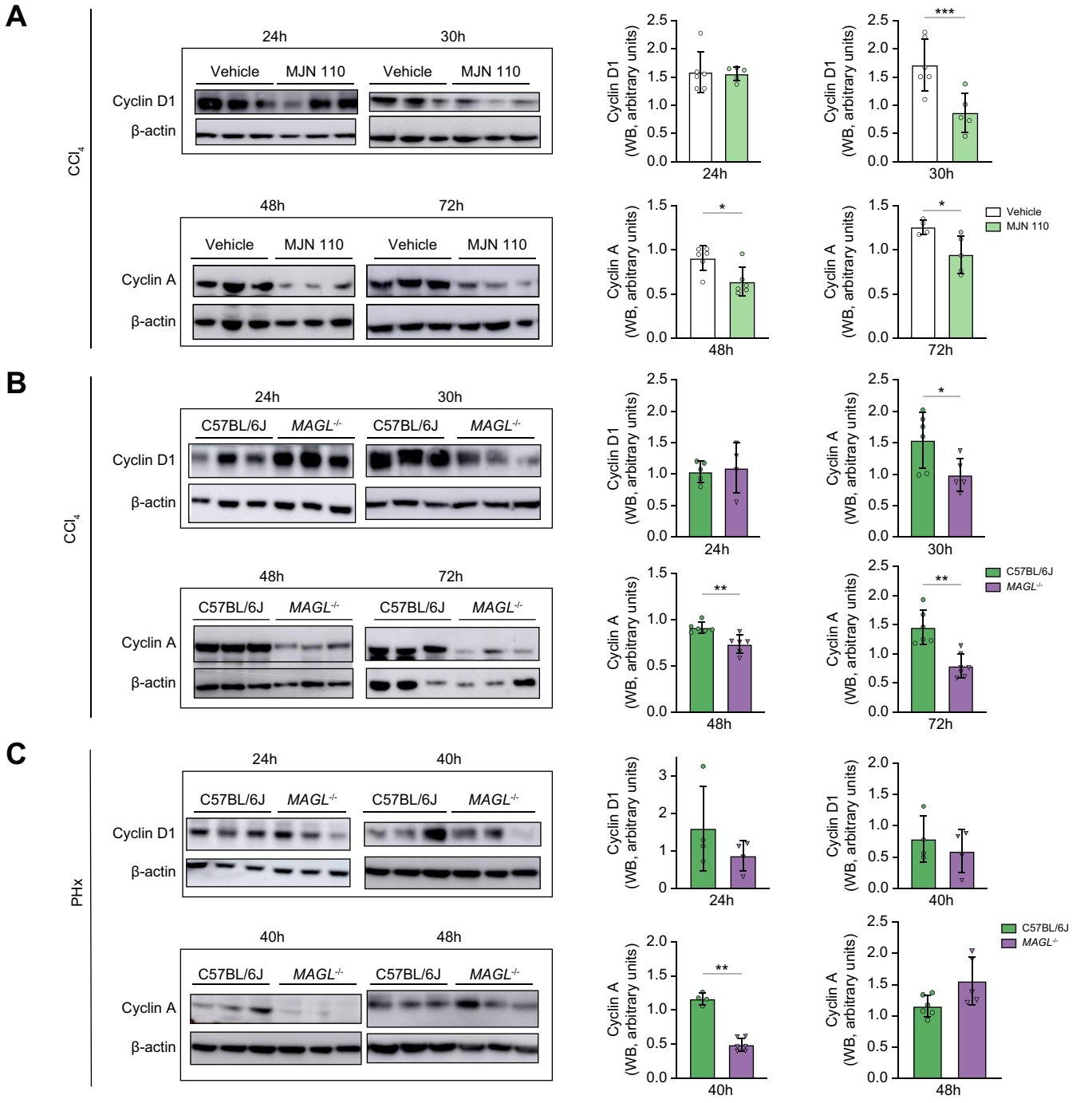


**Fig. 3. MAGL invalidation or inhibition reduces hepatocyte proliferation during liver regeneration.** Western blot analysis of liver cyclin D1 and cyclin A expression with corresponding quantifications at indicated time points in (A) C57Bl/6J mice treated with vehicle or MJN110 after $CCl_4$ injection, (B) C57Bl/6J and MAGL$^{-/-}$ mice after $CCl_4$ injection, and (C) C57Bl/6J and MAGL$^{-/-}$ mice after PHx (n = 5–7 mice per group). β-actin was used as a loading control. *$p$ <0.05, **$p$ <0.01, by two-tailed Mann–Whitney $U$ test. $CCl_4$, carbon tetrachloride; MAGL, monoacylglycerol lipase; PHH3, phospho-histone H3; PHx, partial hepatectomy; WB, western blot.

$^{Hep-/-}$ mice, there was no reduction in cell death in MAGL$^{Mye-/-}$ mice compared with their MAGL$^{fl/fl}$ control counterparts at the peak of injury (Fig. S3A). These results demonstrated that MAGL inhibition in macrophages delays regeneration independently of liver injury.

Macrophages are known to be essential in liver regeneration noteworthy by producing cytokines such as IL-6 and tumour necrosis factor α (TNF-α) in a few hours following a regeneration stimulus, allowing the hepatocytes to become responsive to growth factors.[2] Acute liver damage is accompanied by a massive recruitment of immune cells and particularly macrophages that play a major role in liver regeneration.[22] We did not observe any reduction in IL-6 or TNF-α expression in the liver of invalidated mice after an acute injury (Fig. S3B). Flow cytometry analysis of

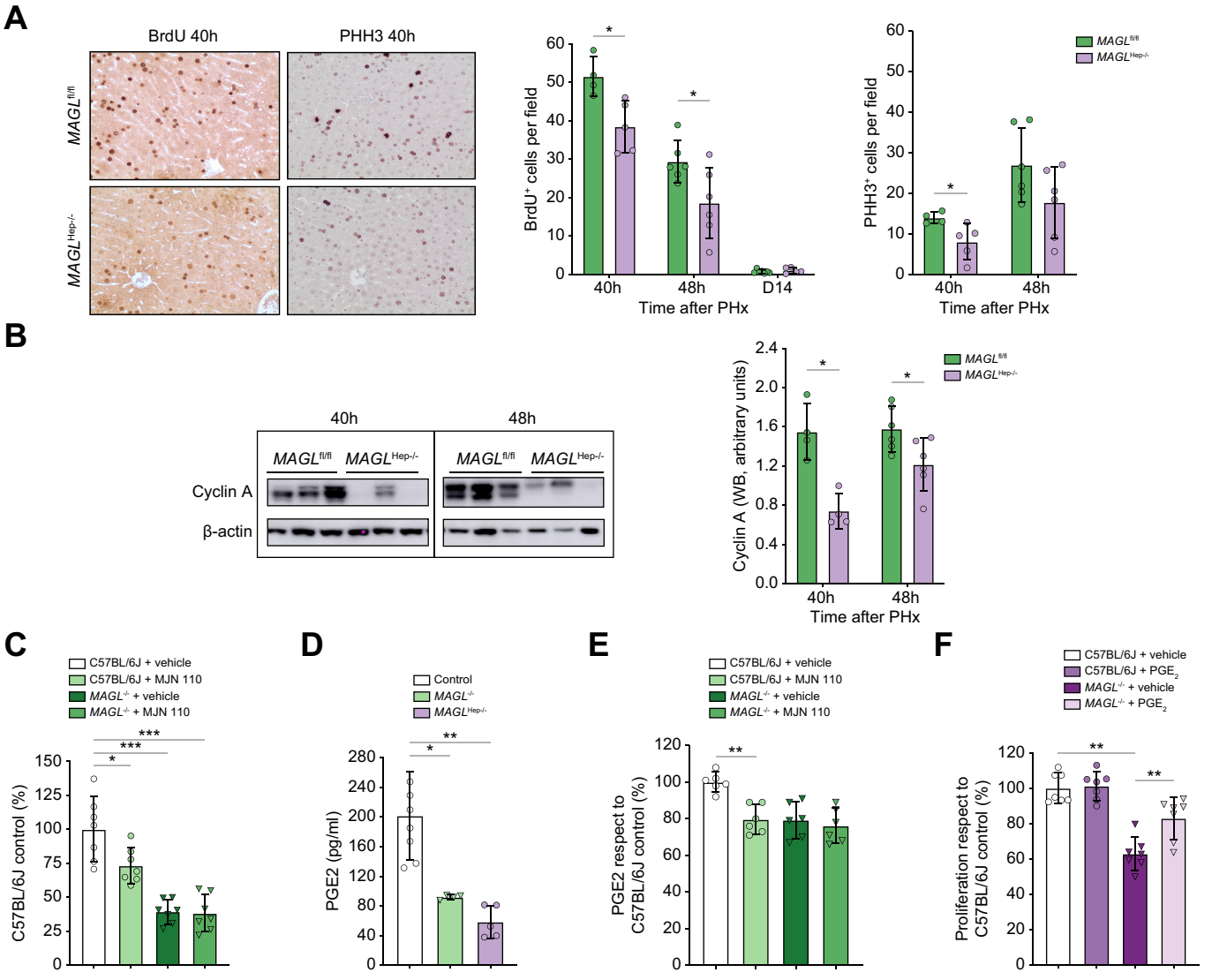


**Fig. 4. Hepatocyte-specific MAGL invalidation impacts hepatocyte proliferation *in vivo* and *in vitro* owing to a reduced eicosanoid production.** (A) Representative liver sections 40 h after PHx and respective quantification of BrdU- and PHH3-positive hepatocytes in MAGL$^{Hep-/-}$ compared with MAGL$^{fl/fl}$ controls at indicated time points (n = 4–6 mice per group). (B) WB analysis and corresponding quantification of cyclin A expression, 40 and 48 h after PHx in MAGL$^{Hep-/-}$ and MAGL$^{fl/fl}$ (n = 4–6 mice per group). β-actin was used as a control. (C) Quantification of DNA synthesis in primary hepatocytes isolated from C57BL/6J and MAGL$^{-/-}$ mice exposed to vehicle or to MJN110, expressed as a percentage of control proliferation (C57BL/6J + vehicle). Representative of three experiments. (D) $PGE_2$ quantification assayed by ELISA in the liver of MAGL$^{-/-}$ and MAGL$^{Hep-/-}$ mice and their respective controls. (E) $PGE_2$ quantification assayed by ELISA in primary hepatocytes isolated from C57BL/6J and MAGL$^{-/-}$ mice exposed to MJN110 or its vehicle for 48 h as indicated (three experiments) expressed as a percentage of C57BL/6J vehicle-treated hepatocytes. (F) Quantification of DNA synthesis in primary hepatocytes isolated from C57BL/6J and MAGL$^{-/-}$ mice exposed to vehicle or to $PGE_2$ for 48 h expressed as a percentage of control hepatocyte proliferation (C57BL/6J + vehicle). Representative of three experiments. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ ≤0.001, by two-tailed Mann–Whitney *U* test. BrdU, bromodeoxyuridine; MAGL, monoacylglycerol lipase; $PGE_2$, prostaglandin $E_2$; PHH3, phosphohistone H3; PHx, partial hepatectomy; WB, western blot.

total liver immune cells at the peak of injury revealed that the percentage of CD4$^{+}$ and CD8$^{+}$ T lymphocytes, natural killer cells, natural killer T cells, γδT cells, or macrophages (Fig. 5C) was not affected by MAGL deletion in myeloid cells, although a reduced macrophage population was observed at a later time point (Fig. S3C). Moreover, the recruitment of pro-inflammatory Ly6C$^{High}$ macrophages was similarly induced in MAGL$^{fl/fl}$ and MAGL$^{Mye-/-}$ mice at the peak of liver injury, that is, 24 h after injury as assessed by FACS (Fig. 5C) and immunohistochemistry (Fig. S3C). We measured eicosanoids in the liver in basal conditions and after injury and found no significant difference between control and MAGL$^{Mye-/-}$ mice (Fig. S3D). However, we cannot exclude from these data that a reduced production of $PGE_2$ and/or $TXA_2$ expression by macrophages might contribute to the delay in liver regeneration.

To further explore the link between MAGL inactivation in macrophages and liver regeneration, we performed an RNASeq analysis on F4/80$^{+}$CD11b$^{+}$ macrophages sorted from the liver of MAGL$^{Mye-/-}$ and MAGL$^{fl/fl}$ mice 24 h after $CCl_4$ injection (Fig. S3E). We found that 549 genes were differentially expressed with 323 upregulated and 226 downregulated in MAGL$^{Mye-/-}$ macrophages compared with MAGL$^{fl/fl}$ macrophages (Fig. 6A). As anticipated,

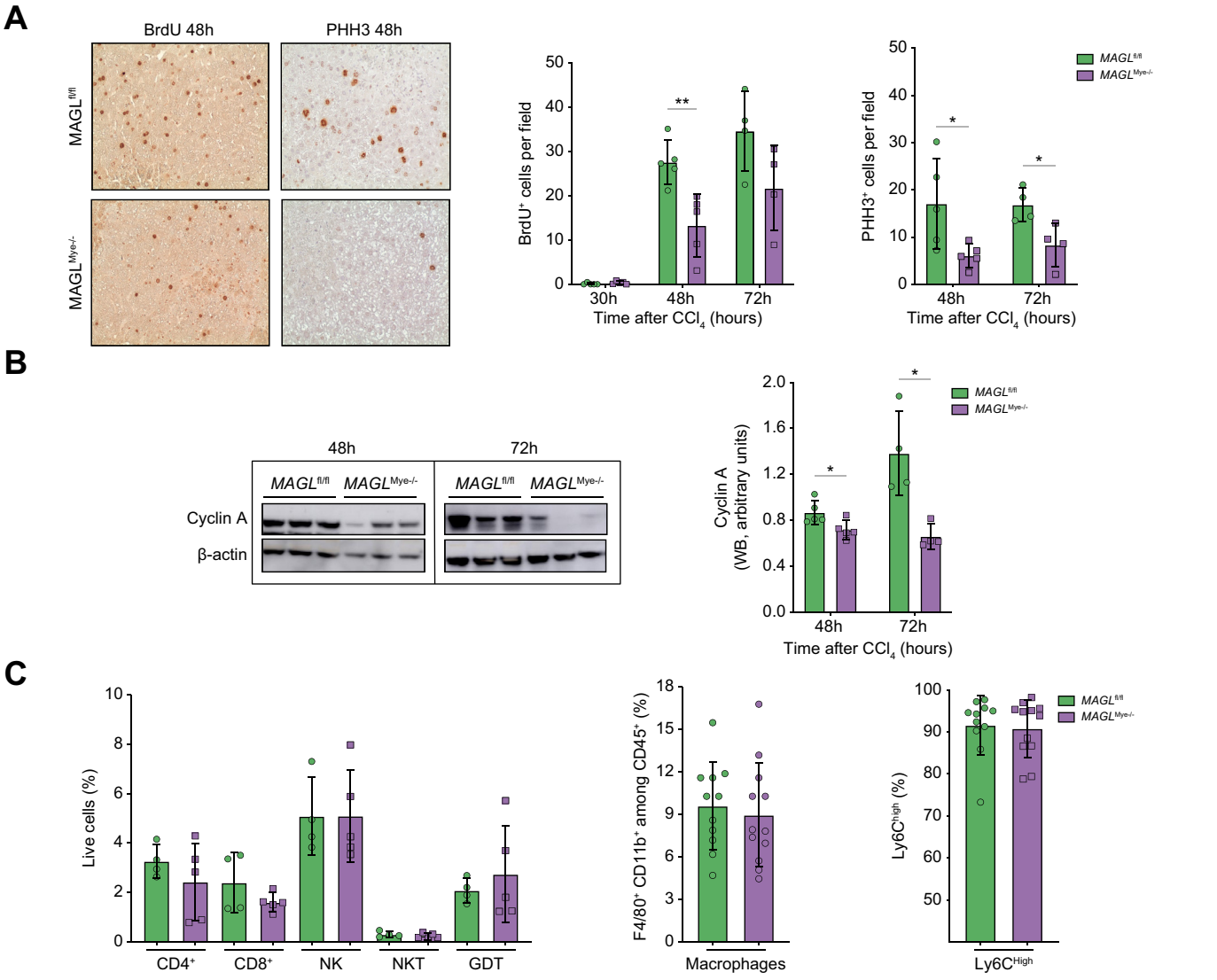


**Fig. 5. Myeloid-specific MAGL invalidation delays liver regeneration without modulating macrophage recruitment.** (A) Representative liver sections of BrdU- and PHH3-stained hepatocytes in C57BL/6J, MAGL$^{fl/fl}$, and MAGL$^{Mye-/-}$ mice 48 h after $CCl_4$ injection. Corresponding quantifications of BrdU- and PHH3-stained hepatocytes at indicated time points after injury (n = 5–7 mice per group). (B) WB analysis of liver cyclin A expression with corresponding quantifications are reported to their respective controls at indicated time points after $CCl_4$ injection (n = 5–7 mice per group). β-actin was used as a loading control. *$p$ <0.05, **$p$ <0.01, by two-tailed Mann–Whitney $U$ test. (C) Flow cytometry analysis of the frequency of LT, NK, NKT (TCRb$^+$ NK1.1$^+$), GDT, macrophages (F4/80$^+$CD11b$^+$), and Ly6C$^{High}$ macrophages sorted from MAGL$^{fl/fl}$ and MAGL$^{Mye-/-}$ mice, 24 h after $CCl_4$ injection (representative of two experiments). *$p$ <0.05, **$p$ ≤0.01, by two-tailed Mann–Whitney $U$ test. BrdU, bromodeoxyuridine; $CCl_4$, carbon tetrachloride; GDT, γδ T cells; LT, T lymphocytes; MAGL, monoacylglycerol lipase; NK, natural killer cells; NKT, natural killer T cells; PHH3, phospho-histone H3; WB, western blot.

modulation of the eicosanoid pathway was observed, with upregulation of genes upstream of MAGL such as diacylglycerol kinase, phospholipase D2, or lipin-2 in MAGL$^{Mye-/-}$ mice and downregulation of genes downstream of MAGL such as prostaglandin E receptor 2 or prostaglandin E synthase 3-like. Classical pro-inflammatory and pro-regenerative cytokines, such as IL-6 or TNF-α, were not differentially expressed in MAGL$^{Mye-/-}$ mice as compared with their WT counterparts. However, GO analysis of biological functions associated with the differentially expressed genes revealed that 5 of the 10 first Reactome deregulated pathways were related to IFN-I (Fig. 6B), a vast majority of genes being upregulated in MAGL$^{Mye-/-}$ macrophages. A gene set enrichment analysis performed on four GO terms linked to the IFN-I pathway confirmed the enrichment in this gene signature pathway (Figs. 6C and Fig. S4A). The induction of some interferon (IFN)-stimulated gene expression such as *Eif2ak2*, *IRF7*, and *IFit3* was confirmed in BMDM isolated from $CCl_4$-injected MAGL$^{Mye-/-}$ mice as compared with their MAGL$^{fl/fl}$ counterparts (Fig. S4B).

## The deleterious impact of IFN-I pathway induction on hepatocyte proliferation and liver regeneration

To determine whether the induction of the IFN-I pathway was responsible for the liver regeneration defect, we cultured primary hepatocytes in the presence of IFN-α, IFN-β, or both and observed a reduction in hepatocyte proliferation with a cumulative effect of IFN-α and IFN-β (Fig. 6D). The detrimental impact of IFN-I pathway induction was further demonstrated in an

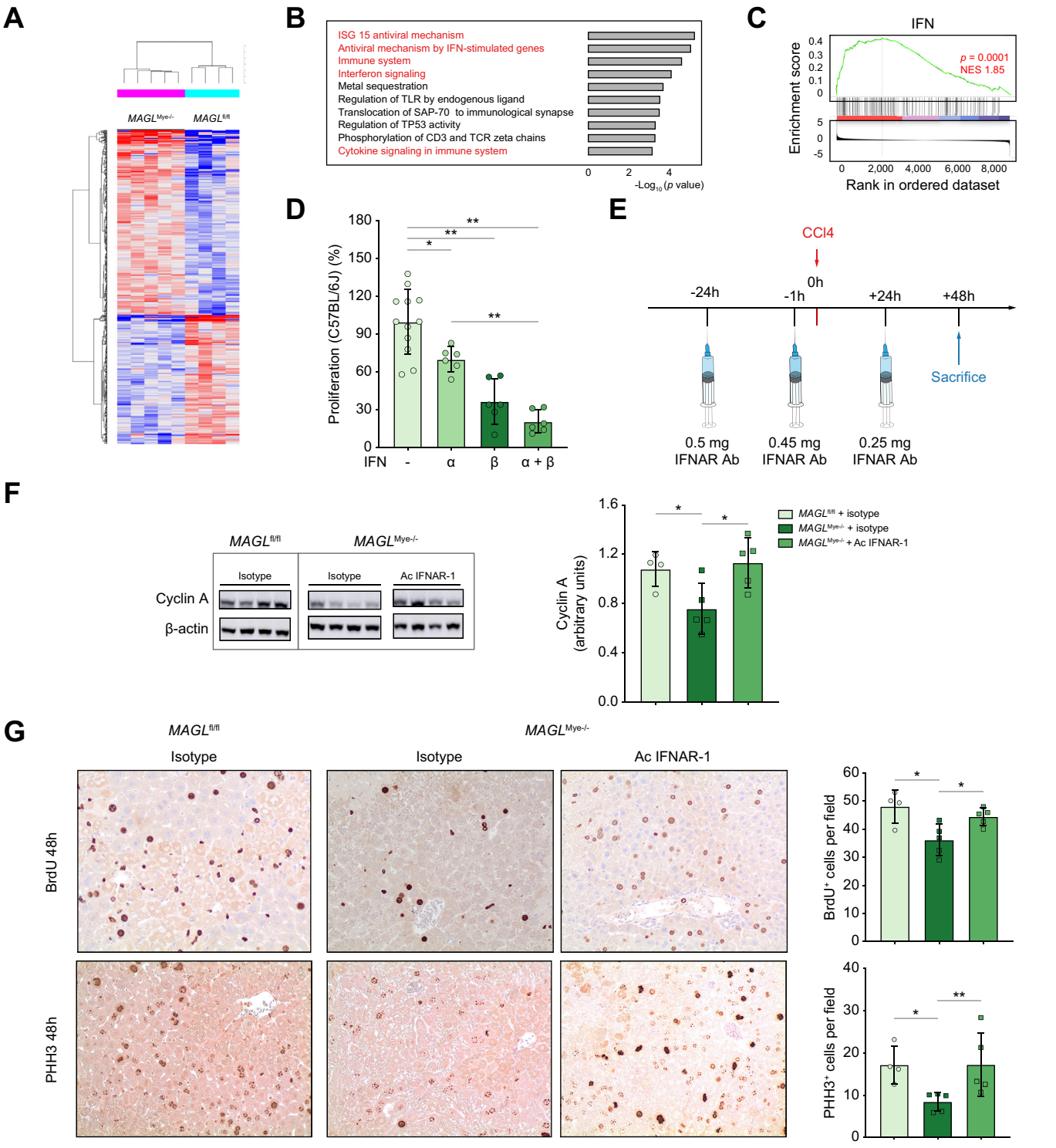


**Fig. 6. MAGL deletion reprograms macrophage signature towards IFN-I pathway profile that reduces hepatocyte proliferation.** (A) Heatmap of differentially expressed genes between liver macrophages sorted from MAGL$^{Mye-/-}$ and MAGL$^{fl/fl}$ mice 24 h after $CCl_4$ injection. (B) Top 10 Reactome pathways ordered by -$\log_{10}$ (*p* value) (Fisher's exact test). Pathways related to IFN-I are indicated in red. (C) GSEA demonstrating specific enrichment for an IFN-I signature in the gene expression profile of MAGL$^{Mye-/-}$ macrophages (custom GSEA module defined with genes from Gene Ontology GO:0032606, GO:0034340, GO:0035455, and GO:0035456). (D) Proliferation index of control C57BL/6J primary hepatocytes treated with IFN-α, IFN-β, or both, expressed in percentage of non-treated hepatocyte proliferation index (representative of two experiments). (E) Schematic of study design for mice receiving Ac IFNAR-1 antibody or isotype (24 h before, 1 h before, and 24 h after $CCl_4$ injection). Mice were harvested 48 h after $CCl_4$ injection. (F) Western blot of liver cyclin A expression and respective quantification in MAGL$^{fl/fl}$ mice exposed to isotype antibody and MAGL$^{Mye-/-}$ mice exposed to Ac IFNAR-1 or isotype 48 h after $CCl_4$ injection. β-actin was used as a loading control. (G) Representative BrdU- and PHH3-immunostained liver sections and respective quantification in MAGL$^{Mye-/-}$ mice exposed to Ac IFNAR-1 or isotype compared with their MAGL$^{fl/fl}$ counterparts, 48 h after $CCl_4$ injection. $^{*}p$ <0.05, $^{**}p$ <0.01, by two-tailed Mann–Whitney *U* test. BrdU, bromodeoxyuridine; $CCl_4$, carbon tetrachloride; GSEA, gene set-enrichment analysis; IFN, interferon; IFN-I, type I interferon; IFNAR, interferon α/β receptor; MAGL, monoacylglycerol lipase; NES, normalised enrichment score; PHH3, phospho-histone H3; TCR, T-cell receptor; TLR, Toll-like receptor.

*in vivo* rescue experiment with neutralising antibodies against IFN-I receptor compared with the isotype injected to MAGL$^{Mye-/-}$ (Fig. 6E). Importantly, IFN-I inhibition restored cyclin A expression (Fig. 6F) and hepatocyte proliferation (Fig. 6G) to the level of control mice, indicating that IFN-I induction in myeloid cells underlies the liver regeneration defect in MAGL$^{Mye-/-}$ mice.

## Discussion

Liver regeneration is an instrumental process that allows restoring tissue architecture and function following acute physical or toxic liver injury. Well-established timely orchestrated cellular and molecular responses occur to promote liver regeneration.[2] These processes involve inflammatory reactions and metabolic changes that take place not only in hepatocytes but also in environmental immune cells and particularly in macrophages.[1–3] However, the same pathways that promote liver regeneration, when chronically stimulated, may result in an imbalance driving fibrosis. This illustrates the delicate equilibrium that exists between liver regeneration and fibrosis.[23,24] Changes in lipid metabolism also contribute to fibrosis progression through the control of immune cells, hepatocyte damage, and activation of fibrogenic cells.[25] Recently, we identified MAGL as a pro-fibrogenic enzyme, and we demonstrated that MAGL inhibitors are promising anti-fibrogenic compounds that could even drive fibrosis regression.[8] Interestingly, MAGL invalidation specifically in myeloid cells was sufficient to reduce inflammation and fibrosis progression upon chronic liver injury.[8] We hypothesised that MAGL inhibition could also affect liver regeneration. Combining human data in PCLS and various models of MAGL invalidation, the present study demonstrates that inhibiting MAGL reduces hepatocyte proliferation *in vitro* and *ex vivo* and delays liver regeneration.

Inhibition of MAGL leads to an increase in 2-arachinylglycerol production,[8] which activates cannabinoid receptors 1 (CB1) and 2 (CB2). We have shown that CB1 or CB2 activation promotes liver regeneration through increased production of IL-6 from hepatic myofibroblasts for CB2[26] and activation of cell cycle proteins involved in mitotic progression such as forkhead-box M1 for CB1.[27] It is therefore most likely that the delayed liver regeneration induced by MAGL inactivation is not mediated by cannabinoid receptors.

MAGL-specific deletion in hepatocytes was associated with a defect in liver regeneration and a lower production of $PGE_2$ both *in vivo* and *in vitro*. Among metabolites of arachidonic acid, PGs, including $PGE_2$, the most abundant PG produced by the liver,[28] and prostacyclin (prostaglandin $I_2$), are known to promote the growth of hepatocytes.[19,20,29–31] Moreover, inhibition of 15-prostaglandin dehydrogenase, a PG-degrading enzyme, has been shown to potentiate tissue regeneration in multiple organs in mice, including the liver.[32] Interestingly, addition of $PGE_2$ in the culture medium of MAGL$^{Hep-/-}$ hepatocytes rescued their proliferation, suggesting a cell-intrinsic impact of $PGE_2$ on hepatocyte proliferation and liver regeneration. Among other metabolites of the arachidonic pathway that could also support hepatocyte proliferation, $TXA_2$ also promotes liver regeneration.[19,21,22] As $TXA_2$ was also downregulated in MAGL-invalidated hepatocytes, it is likely that it also contributes to the liver regeneration delay. Thus, our data uncover the beneficial pro-regenerative effect of MAGL expressed by hepatocytes on their proliferation through eiscosanoid production.

Immune cells, in particular macrophages, play a major role in the early phase of the regeneration process, particularly through their cytokine production.[2] We found that mice specifically invalidated for MAGL in the myeloid lineage also show a defective liver regeneration. Unexpectedly, intrahepatic macrophages from MAGL$^{Mye-/-}$ mice, unlike MAGL$^{Mye-/-}$ BMDMs upon lipopolysaccharide stimulation, do not express lower levels of pro-inflammatory and pro-regenerative cytokines 24 h after liver injury. However, we demonstrated that MAGL invalidation in macrophages has indeed an indirect early negative impact on hepatocyte proliferation through the induction of the IFN-I pathway, underlining a novel mechanism by which MAGL deletion reprograms macrophages. These results are in keeping with data showing reduced hepatocyte proliferation upon addition of IFN-I *in vitro* or *in vivo* during regeneration.[33,34] More importantly, liver regeneration was rescued by *in vivo* blockade of IFN-I pathway in MAGL$^{Mye-/-}$ mice, underlining its major role in the liver regeneration delay observed in the absence of MAGL in myeloid cells. Interestingly, reduced eicosanoid production has been correlated with IFN-I induction by macrophages upon viral infection.[35] Although $PGE_2$ and $TXA_2$ was not reduced in the liver of MAGL$^{Mye-/-}$ mice, liver and peritoneal MAGL-deleted macrophages are known to produce less eicosanoids.[8] Therefore, reduced $PGE_2$ production may also link the MAGL defect in macrophages and the IFN-I pathway induction observed in our model.

In conclusion, our results highlight the regenerative properties of MAGL in the liver, via both an intrinsic lipid reprogramming of hepatocytes and an extrinsic reprogramming of macrophages that turns off the IFN-I pathway and induces a macrophage-hepatocyte crosstalk. Owing to the balance between fibrogenesis and regeneration, these data illustrate that the price to pay when using MAGL inhibitors as an anti-inflammatory and anti-fibrogenic strategy is to compromise liver regeneration.

## Abbreviations

2-AG, 2-arachidonylglycerol; BMDM, bone marrow-derived macrophages; BrdU, bromodeoxyuridine; BW, body weight; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CC, cholangiocarcinoma; $CCl_4$, carbon tetrachloride; FPKM, fragments per kilobase per million mapped fragments; GO, Gene Ontology; GSEA, gene set enrichment analysis; HCC, hepatocellular carcinoma; IFN, interferon; IFN-I, type I interferon; IFNAR, interferon α/β receptor; i.p., intraperitoneally; KEGG, Kyoto Encyclopedia of Genes and Genomes; LW/BW, liver weight on body weight; MAGL, monoacylglycerol lipase; NES, normalised enrichment score; PCLS, precision-cut liver slices; PG, prostaglandin; $PGE_2$, prostaglandin $E_2$; PHH3, phospho-histone H3; PHx, partial hepatectomy; RNASeq, RNA sequencing; SAF, Steatosis, Activity, Fibrosis; TNF-α, tumour necrosis factor α; $TXA_2$, thromboxane $A_2$; WB, western blot; WT, wild-type.

## Financial support

This work was supported by grants from INSERM (France), Agence Nationale pour la Recherche (ANR 20-CE14-0038), the University de Paris Labex Inflamex, Fondation pour la Recherche Médicale (Equipe FRM EQU202203014642), Association Française pour l'Etude du Foie (AFEF), and Société Nationale Française de Gastroentérologie (SNFGE). MA was a

recipient of a doctoral fellowship from INSERM poste d'accueil. MS was a recipient of FRM fellowship.

## Conflicts of interest

The authors have declared that no conflict of interest exists regarding this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

## Authors' contributions

Study concept and design: HG, SL. Acquisition of data: MA, RAS, MM, MC, JHW, PG, AH, CC. Surgery: MS. Analysis and interpretation of data: MA, RAS, MM, MC, JHW, A Habib, VP, SL, HG. Drafting of the manuscript: MA, PG, SL, HG. Study supervision: HG. Critical revision of the manuscript: SL, MLG, CP, VP.

## Data availability statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

## Acknowledgements

The authors thank V. Fauveau, Institut Cochin, for help in surgery experiments; Olivier Thibaudeau of the Plateau de Morphologie Facility (INSERM UMR 1152, France) and Nicolas Sorhaindo of the Plateforme de Biochimie (CRI, INSERM UMR1149) for their help in the histology and liver function tests; and K. Bailly from the cytometry platform of Cochin Institute and H. Fohrer-Ting from the Centre de Recherche des Cordeliers, Paris University, for cell sorting analyses.

## Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2023.100794.

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*Author names in bold designate shared co-first authorship*

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