Supporting Materials

Supplementary Materials and Methods

Protein isolation and western blotting

Total, mitochondrial and cytosolic proteins were isolated for western blotting, as previously described.[1,2] Lysosomal and cytosolic fractions were prepared by a kit (MilliporeSigma). Antibodies were: albumin (Abcam; #ab19194), ALIX (Cell Signaling, Beverly, MA; #2171), ATG5 (Cell Signaling; #12994), ATG7 (Cell Signaling; #2631), β-actin (MilliporeSigma; #A5441), BAX (Cell Signaling; #2772), BCL-XL (Cell Signaling; #2764), BID (kind gift of Xiao-Ming Yin, University of Indiana), BIM (Cell Signaling; #2933), caspase 3 (Cell Signaling; #9665), caspase 7 (Cell Signaling; #9492), cathepsin B (Cell Signaling; #31718), cathepsin L (Santa Cruz Biotechnology, Santa Cruz, CA; #sc-390367), cytochrome c (BD Biosciences, San Jose, CA; #556433), cytochrome oxidase (Abcam; #ab14744), IL-1R1 (Abcam; #ab106278), heat shock protein 90 (HSP90; Cell Signaling; #4875), high mobility group box 1 (HMGB1; Abcam; #ab18256), lysosomal membrane associated protein 1 (LAMP1; Cell Signaling; #3243), MCL-1 (Rockland Immunochemicals, Limerick, PA; #600-401-394), microtubule associated protein 1 light chain 3 (LC3; Cell Signaling; #2775), p53 (Cell Signaling; #32532), TNFR1 (Santa Cruz; #sc-7895), and tubulin (Cell Signaling; #2148). Western blot signals were quantitated by a FluorChem densitometer (Alpha Innotech, San Leonardo, CA).

Trypan blue staining

Trypan blue solution (Corning) diluted 1:1 with PBS was added to cells which were then incubated at 37°C for 5 min. The trypan blue solution was removed and the cells washed twice with

medium. The numbers of trypan blue positive cells per field were counted under light microscopy at a 200X magnification.

Propidium iodide staining

Cells were incubated with propidium iodide solution (Thermo Fisher Scientific) diluted 1:500 in medium at 37°C for 30 min. The solution was removed and the cells washed twice with medium. The numbers of positive-staining cells were determined under fluorescence microscopy with an excitation of 535 and emission of 617 at a 200X magnification.

ATP assay

Cellular ATP levels were measured by commercial kit (Abcam).

Cathepsin activity

Cathepsin L activity was determined in both whole cell pellets and cell culture medium by commercial kit (Abcam).

Serum alanine aminotransferase assay (ALT)

ALTs were measured by commercial kit (TECO Diagnostics, Anaheim, CA).

Terminal deoxynucleotide transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL)

TUNEL was performed on liver sections by commercial kit (Roche, Indianapolis, IN). Under light microscopy numbers of TUNEL positive cells were counted in 10 randomly selected high power fields per liver section (200X magnification).

Immunofluorescence microscopy

CD68 and LY6G immunofluorescence microscopy was performed as previously described,[3] and quantitated by ImageJ.

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

RNA extraction, reverse transcription, and qRT-PCR using the primers (Integrated DNA Technologies; Coralville, IA) in Supplemental Table 1 were performed as previously described.[4] Data were analyzed by the $2^{-\Delta\Delta CT}$ method for relative quantification and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Extracellular vesicle (EV) isolation and Kupffer cell treatment

EVs were isolated from AML12 cell medium by differential high-speed centrifugation.[5] Analysis of EV size distribution and number was performed using NanoSight LM10 with NTA2.3 (NanoSight Ltd., Minton Park, UK).[6] Kupffer cells were isolated from C57BL/6J mice as previously described,[7] and cultured with isolated EVs for 6 h.

References

1) Schattenberg JM, Wang Y, Rigoli RM, Koop DR, Czaja MJ. CYP2E1 overexpression alters hepatocyte death from menadione and fatty acids by activation of ERK1/2 signaling. HEPATOLOGY 2004;39:444-455.
2) Wang Y, Singh R, Lefkowitch JH, Rigoli RM, Czaja MJ. Tumor necrosis factor-induced toxic liver injury results from JNK2-dependent activation of caspase-8 and the mitochondrial death pathway. J Biol Chem 2006;281:15258-15267.
3) Ilyas G, Zhao E, Liu K, Lin Y, Tesfa L, Tanaka KE, et al. Macrophage autophagy limits acute toxic liver injury in mice through down regulation of interleukin-1β. J Hepatol 2016;64:118-127.

4) Fontana L, Zhao E, Amir M, Dong H, Tanaka K, Czaja MJ. Aging promotes the development of diet-induced murine steatohepatitis but not steatosis. HEPATOLOGY 2013;57:995-1004.

5) Thery C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol 2006;Chapter 3:Unit 3.22.

6) Huang MB, Gonzalez RR, Lillard J, Bond VC. Secretion modification region-derived peptide blocks exosome release and mediates cell cycle arrest in breast cancer cells. Oncotarget 2017;8:11302-11315.

7) **Ilyas G**, **Cingolani F**, Zhao E, Tanaka K, Czaja MJ. Decreased macrophage autophagy promotes liver injury and inflammation from alcohol. Alcohol Clin Exp Res 2019;43:1403-1413.

Supplemental Table S1. qRT-PCR primer sequences.

Ccl2	Forward	5′-CAGCCAGATGCAGTTAACGCCCCA-3′
	Reverse	5′-TGGGGTCAGCACAGACCTCTCTC-3′
Cd68	Forward	5′-ATTGAGGAAGGAACTGGTGTAG-3′
	Reverse	5′-CCTCTGTTCCTTGGGCTATAAG-3′
Cox2	Forward	5′-TGCACTATGGTTACAAAAGCTGG-3′
	Reverse	5′-TCAGGAAGCTCCTTATTTCCCTT-3′
Gapdh	Forward	5′-AGGTCGGTGTGAACGGATTTG-3′
	Reverse	5′-TGTAGACCATGTAGTTGAGGTCA-3′
Il1a	Forward	5′-CGCTTGAGTCGGCAAAGAAAT-3′
	Reverse	5′-CTTCCCGTTGCTTGACGTTG-3′
Il1b	Forward	5′-GCAACTGTTCCTGAACTCAACT-3′
	Reverse	5′-ATCTTTTGGGGTCCGTCAACT-3′
Il6	Forward	5′-CACATGTTCTCTGGGAAATCGTGGA-3′
	Reverse	5′-TCTCTCTGAAGGACTCTGGCTTTGT-3′
Ifng	Forward	5′-ATGAACGCTACACACTGCATC-3′
	Reverse	5′-CCATCCTTTTGCCAGTTCCTC-3′
Ly6G	Forward	5′-TGGACTCTCACAGAAGCAAAG-3′
	Reverse	5′-GCAGAGGTCTTCCTTCCAACA-3′
Mgl2	Forward	5′-TGGAGCGGGAAGAGAAAAACCAGG-3′
	Reverse	5′-TCGAAGTTGTCAGCCCTGGAGTC-3′
Nos2	Forward	5′-GTTCTCAGCCCAACAATACAAGA-3′
	Reverse	5′-GTGGACGGGTCGATGTCAC-3′
Retnla	Forward	5′-CCAATCCAGCTAACTATCCCTCC-3′
	Reverse	5′-CCAGTCAACGAGTAAGCACAG-3′

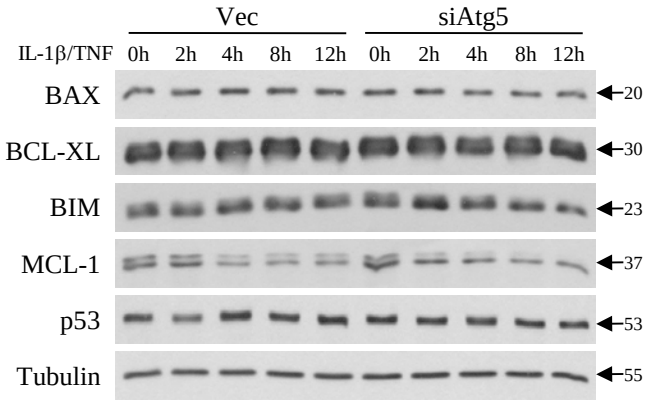

Fig. S1. AML12 cell protein levels of regulators of apoptosis are unaltered by decreased autophagy or cytokine treatment. Immunoblots are of total protein from Vec and siAtg5 cells untreated or IL-1β/TNF treated for the number of hours shown and probed with the indicated antibodies. Immunoblots are representative of three independent experiments and protein molecular weights in kD are indicated by arrows.

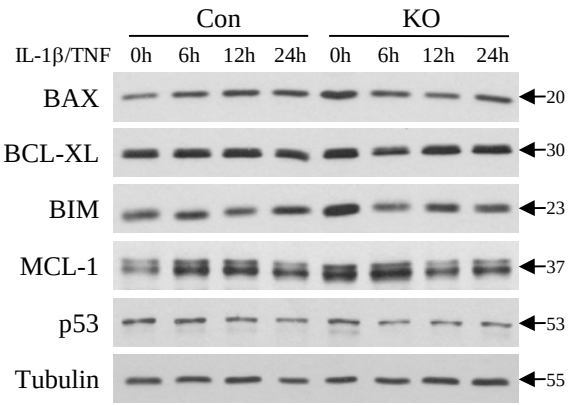

Fig. S2. Hepatic levels of apoptotic regulatory proteins are unchanged in knockout mice. Immunoblots of total protein from control (Con) and knockout (KO) mice untreated or IL-1β/TNF treated for the indicated hours. Blots were probed for the indicated antibodies. Immunoblots are representative of three independent experiments and protein molecular weights in kD are indicated by arrows.

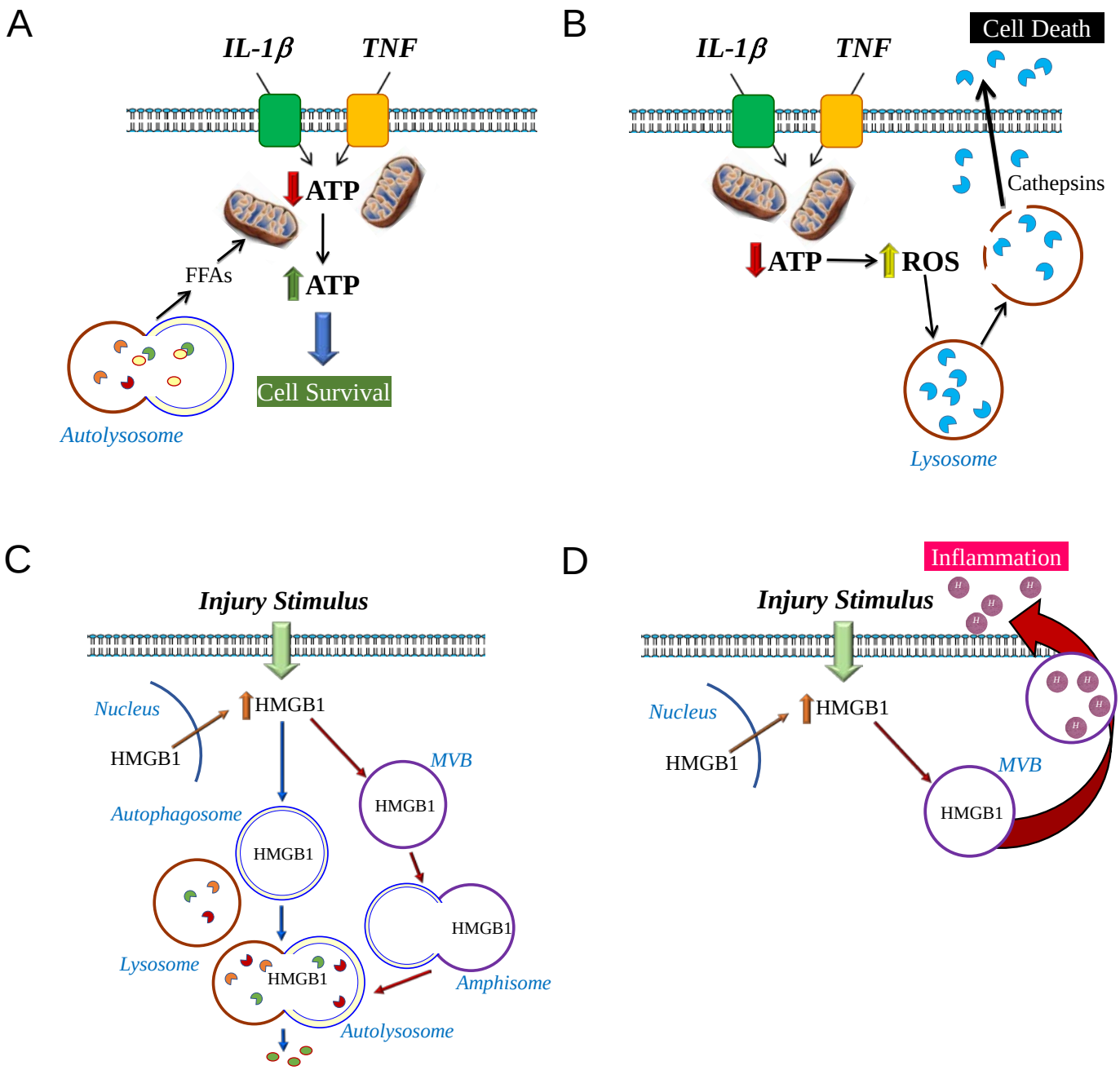

Fig. S3. Decreased hepatocyte autophagy promotes cell death (A,B) and inflammation (C,D). (A) Normal hepatocytes rely on autophagy-generated products such as free fatty acids (FFAs) to generate sufficient ATP to survive the increased metabolic demands caused by IL-1β/TNF. (B) With decreased autophagy, an inadequate supply of energy substrates causes hepatocyte ATP depletion from IL-1β/TNF. ATP depletion leads to the generation of reactive oxygen species (ROS) that trigger lysosomal permeabilization, the release into the intracellular and extracellular spaces of injurious lysosomal cathepsins, and cathepsin-dependent cell death from necrosis. (C) During hepatocellular injury cytosolic DAMPs are generated such as by the nuclear release of HMGB1. Normally these DAMPs are sequestered in an autophagosome or multivesicular body (MVB) to be inactivated by autophagic degradation. (D) With reduced levels of autophagy, DAMPs are sequestered predominantly in MVBs and secreted from the hepatocyte in exosomes. Exosomes deliver the DAMPs to hepatic macrophages to cause their activation and promote inflammation.