EASL JOURNAL OF HEPATOLOGY

Journal of Hepatology

CTAT methods

Tables for a "Complete, Transparent, Accurate and Timely account" (CTAT) are now mandatory for all revised submissions. The aim is to enhance the reproducibility of methods.

- Only include the parts relevant to your study
- Refer to the CTAT in the main text as 'Supplementary CTAT Table'
- Do not add subheadings
- Add as many rows as needed to include all information
- Only include one item per row

1.1 Antibodies

Name	Citation	Supplier	Cat no.	Clone no.
Hsc70	[1]	Stressgen	SPA-819	
Hsp27	[2]	Santa Cruz	sc-1049	
Hsp60	[3]	Enzo	ADI-SPA-805	
HSP72	[4]	Enzo	ADI-SPA-812	
Hsp90	[5]	Cell Signaling	4877	C45G5
JNK1	[6]	Cell Signaling	3708	(2C6)
JNK2	[7]	Santa Cruz	sc-827	(N-18)
phospho-c-Jun	[8]	Cell Signaling	9164 (Ser73)	
phospho-SAPK/JNK	[9]	Cell Signaling	9251	
phospho-SAPK/JNK	[10]	Cell Signalling	4668	(81E11)
Rip3	[11]	ProSci	2283	
β-actin	[12]	Sigma-Aldrich	A1978	
Goat anti-mouse IgG (H+L), HRP conjugated	[13]	Invitrogen	G21040	
Goat anti-rabbit IgG (H+L), biotinylated	[14]	Vector	BA-1000	
Goat anti-rabbit IgG (H+L), HRP conjugated	[15]	Invitrogen	G21234	
Goat anti-rat IgG (H+L), HRP conjugated	[16]	Invitrogen	A10549	

Rabbit anti-goat IgG (H+L)	[17]	Invitrogen	R21459	
Grp78	[18]	Cell Signaling	3177S	(C50B12)
MKK7	[19]	Cell Signaling	4172	
IREalpha	[20]	Cell Signaling	3294	14C10
ASK1 (D11C9)	[21]	Cell Signaling	8662	D11C9
Cytochrome P4502E1		Abcam	9140	

1.2 Organisms

Name	Citation	Supplier	Strain	Sex	Age	Overall n number
Mouse	[22]	Charles River	C57BL/6N	Males, females	2-4 Months old	30
Mouse	[23]		Lap-tTA	Males, females	2-4 Months old	13
Mouse	Generated and characterized in this paper		hHsp72	Male, females	2-4 Months old	43

1.3 Sequence based reagents

Name	Sequence	Supplier
HspA1A (HSP72) (human)	F: CTGTACCAGGGTGCCGGTGGT R: GTCCCCAAACTCACCCTGAAGTTCT	Eurofins genomics, Germany
Lap-tTA (Rat/E.coli)	F: GCTAGGTGTAGAGCAGCCTACATTG R: GTCCAGATCGAAATCGTCTAGCGCG	Eurofins genomics, Germany
mCyp1a2 (mouse) Cytochrome P450, family 1, subfamily a, polypeptide 2	F: TCTGCCAGTCTCCAGCCCCT R: AGATGGCAGTGGCCAGTAGCA	Biomers, the Serco Industrial Park West, Germany
mCyp2e1 (mouse) *Cytochrome P450, family 2, subfamily e, polypeptide 1*	F: CTGGCCGAGGGGACATTCCTGT R: AGGGAAAACCTCCGCACGTCCT	Biomers, the Serco Industrial Park West, Germany
mCyp3a11 (mouse) Cytochrome P450, family 3, subfamily a, polypeptide 11	F: TGCTGTCACAGACCCAGAGACG R: ACTCATTATCCCCACTGGGCCA	Biomers, the Serco Industrial Park West, Germany
(m+h) HSP72 (mouse+human) Heat shock protein family A (Hsp70) member 1A	F: ACGGGCGCGACCTGAACAAG R: ATCAGGATGGCCGCCTGCAC	Biomers, the Serco Industrial Park West, Germany

hHSP72 (human) *Heat shock protein family A (Hsp70) member 1A*	F: GCCAACAAGATCACCATCAC R: TTTGTACTTCTCCGCCTCCT	Eurofins genomics, Germany
mIL6 (mouse) *Interleukin 6*	F: ACAAAGCCAGAGTCCTTCAGAGAGA R: TGGTCTTGGTCCTTAGCCACTCC	Eurofins genomics, Germany
mHamp1 (mouse) *Hepcidin*	F: CTGTCTCCTGCTTCTCCTCCT R: GGCTGCAGCTCTGTAGTCTGT	Eurofins genomics, Germany
mSod2 (mouse) *Manganese superoxide dismutase 2*	F: GAACAATCTCAACGCCACCG R: GCTGAAGAGCGACCTGAGTT	Eurofins genomics, Germany
hRPLPO (human) *Large Ribosomal Protein*	F: GCAATGTTGCCAGTGTCTGT R: GCCTTGACCTTTTCAGCAAG	Eurofins genomics, Germany
mL7 (mouse) *Ribosomal protein L7*	F: GAAAGGCAAGGAGGAAGCTCATCT R: AATCTCAGTGCGGTACATCTGCCT	Eurofins genomics, Germany
Hsp27 (Hspb2) (mouse) *Hspb2 heat shock protein 2*	F: TGTCTACCTCCCGTGGTGAT R: GGTTTATTCAGCCCCACCC	Eurofins genomics, Germany
GRP78-Hspa5 (mouse) HSPA5 heat shock protein family A (Hsp70) member 5	F: GGTACCCACCAAGAAGTCTCAG R: TCAGCAAACTTCTCAGCATCAT	Eurofins genomics, Germany
Grp78 (human) HSPA5 heat shock protein family A (Hsp70) member 5	F: GCTTATGGCCTGGATAAGAGG R: CACGCTGGTCAAAGTCTTCTC	Eurofins genomics, Germany
Hsp27 (Hspb2) (human) *Hspb2 heat shock protein 2*	F: AGACGAGGTGACTGTGAGGA R: ACATAGGTGCGGCAGAACTC	Eurofins genomics, Germany
CD68 (human)	F: AGCTACATGGCGGTGGAGTA R: CGAAGGGATGCATTCTGAGC	Eurofins genomics, Germany
IL1b (human) *Interleukin 1beta*	F: GCTCGCCAGTGAAATGATGG R: GGTGGTCGGAGATTCGTAGC	Eurofins genomics, Germany
IL6 (human) *Interleukin 6*	F: TAGTGAGGAACAAGCCAGAGC R: TGGGTCAGGGGTGGTTATTG	Eurofins genomics, Germany

1.4 Biological samples

Description	Source	Identifier
Human liver biopsies	University Hospitals Ulm, Aachen, Munich and Graz	Liver specimen from healthy subjects, patients with NASH and chronic hepatitis C infection (HCV)

1.5 Software

Software name	Manufacturer	Version
Adobe Photoshop CS2	Adobe	Version CS2
Adobe Illustrator CS2	Adobe	Version CS2
Living Image®	Caliper	Version 4.3.1

Excel, Word, Powerpoint	Microsoft	Version 2010
KC4	BioTek Instruments, Inc.	
Axio Vision	Zeiss	Rel.4.8
7300 Sequenz-Detektion-Software	Applied Biosystems	SDS v1.4.1
ImageQuant LAS 400	GE Healthcare	
i-control	Tecan	
GraphPad Prism 5	GraphPad	Version 5
Endnote X7.1	Thomson Reuters	Version X7.1
Image J	Wayne Rasband	
Primer3 Input		Version 0.4.0

1.6 Other (e.g. drugs, proteins, vectors etc.)

Name of the product	Company	Catalog number
human Hsp72 construct pcDNA5/FRT/TO V5 HSPA1A	Addgene	Plasmid 19456
pBI-L Tet Vector	Clontech Laboratories	GenBank AccessionNo.:U89934.
Paracetamol Powder	Fagron	100168-0001
Palmitic acid	Sigma	P0500-10G

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1. Given Name (First Name): Alexandra K.
2. Surname (Last Name): Kiemer
3. Date: 14-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name:

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it): JHEPAT-D-17-00645

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1. Given Name (First Name): Giovanna
2. Surname (Last Name): Vella
3. Date: 15-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Pavel Strnad

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it):

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Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

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1. Given Name (First Name): Laura
2. Surname (Last Name): James
3. Date: 13-December-2017

4. Are you the corresponding author? ☑ Yes ☐ No

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it):

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Are there any relevant conflicts of interest? ☑ Yes ☐ No

If yes, please fill out the appropriate information below.

Name of Entity	Grant?	Personal Fees?	Non-Financial Support?	Other?	Comments
STTR grant to develop a point of care test of acetaminophen liver injury.	☑	☐	☐	☐	Covers a portion of my salary

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R42DK079387 awarded to Dr. James to develop a rapid assay for detection of acetaminophen protein adducts in human blood samples.

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Royalties: Funds are coming in to you or your institution due to your patent

ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name): Sonja
2. Surname (Last Name): Kessler
3. Date: 15-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Kateryna Levada

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it):

Section 2. The Work Under Consideration for Publication

Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 3. Relevant financial activities outside the submitted work.

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Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? ☐ Yes ☑ No

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Section 6. Disclosure Statement

Based on the above disclosures, this form will automatically generate a disclosure statement, which will appear in the box below.

Dr. Kessler has nothing to disclose.

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ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name): Nurdan
2. Surname (Last Name): Guldiken
3. Date:

4. Are you the corresponding author? ☐ Yes ☑ No
Corresponding Author's Name:

5. Manuscript Title
Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it)
JHEPAT-D- 17-00645

Section 2. The Work Under Consideration for Publication

Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?
Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 3. Relevant financial activities outside the submitted work.

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Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? ☐ Yes ☑ No

ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 5. Relationships not covered above

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ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name): Pavel
2. Surname (Last Name): Strnad
3. Date: 14-December-2017

4. Are you the corresponding author? ☑ Yes ☐ No

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it): JHEPAT-D-17-00645R2

Section 2. The Work Under Consideration for Publication

Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest? ☐ Yes ☑ No

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Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? ☐ Yes ☑ No

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ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name): Fa-Rong
2. Surname (Last Name): Mo
3. Date: 14-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Pavel Strnad

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it):

Section 2. The Work Under Consideration for Publication

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Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? ☐ Yes ☑ No

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Dr. Mo has nothing to disclose.

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ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name): Daniel
2. Surname (Last Name): Hartmann
3. Date: 13-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Pavel Strnad

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it):

Section 2. The Work Under Consideration for Publication

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ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name): Norbert
2. Surname (Last Name): Hüser
3. Date: 13-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Pavel Strnad

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it):

Section 2. The Work Under Consideration for Publication

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Are there any relevant conflicts of interest? ☐ Yes ☑ No

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Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? ☐ Yes ☑ No

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Dr. Hüser has nothing to disclose.

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Section 1. Identifying Information

1. Given Name (First Name): Johannes
2. Surname (Last Name): Haybaeck
3. Date: 14-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Pavel Strnad

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it): JHEPAT-D- 17-00645

Section 2. The Work Under Consideration for Publication

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Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 3. Relevant financial activities outside the submitted work.

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Are there any relevant conflicts of interest? ☐ Yes ☑ No

Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work? ☐ Yes ☑ No

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Dr. Haybaeck has nothing to disclose.

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Section 1. Identifying Information

1. Given Name (First Name): Kateryna
2. Surname (Last Name): Levada
3. Date: 18-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Pavel Strnad

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it): JHEPAT-D-17-00645R2

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ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name): Thomas
2. Surname (Last Name): Ott
3. Date: 18-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Pavel Strnad

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it):

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Dr. Ott has nothing to disclose.

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ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name) TASHA
2. Surname (Last Name) CHRISTY
3. Date

4. Are you the corresponding author? ☐ Yes ☒ No

5. Manuscript Title HSP 72 PROTECTS ---

6. Manuscript Identifying Number (if you know it)

Section 2. The Work Under Consideration for Publication

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ICMJE Form for Disclosure of Potential Conflicts of Interest

Section 1. Identifying Information

1. Given Name (First Name): xiaoji
2. Surname (Last Name): zhang
3. Date: 18-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Pavel Strnad

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it):

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Dr. zhang has nothing to disclose.

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This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

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Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

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Definitions.

Entity: government agency, foundation, commercial sponsor, academic institution, etc.
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ICMJE Form for Disclosure of Potential Conflicts of Interest

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1. Given Name (First Name): marianne
2. Surname (Last Name): ziol
3. Date: 14-December-2017

4. Are you the corresponding author? ☐ Yes ☑ No

Corresponding Author's Name: Nurdan Güldiken

5. Manuscript Title: Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

6. Manuscript Identifying Number (if you know it):

Section 2. The Work Under Consideration for Publication

Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest? ☐ Yes ☑ No

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ICMJE Form for Disclosure of Potential Conflicts of Interest

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Section 6. Disclosure Statement

Based on the above disclosures, this form will automatically generate a disclosure statement, which will appear in the box below.

Dr. ziol has nothing to disclose.

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Hsp72 protects from liver injury via attenuation of hepatocellular death, oxidative stress and JNK-signaling

Kateryna Levada, Nurdan Guldiken, Xiaoji Zhang, Giovanna Vella, Fa-Rong Mo, Laura P. James, Johannes Haybaeck , Sonja M. Kessler, Alexandra K. Kiemer, Thomas Ott, Daniel Hartmann, Norbert Hüser, Marianne Ziol, Christian Trautwein, Pavel Strnad

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Supplementary Materials and Methods

Animal experiments. For this study we used hemizygous single transgenic Lap-tTA, the newly generated Hsp72 or non-transgenic and double transgenic (i.e. containing both Lap-tTA and Hsp72) Hsp72 overexpressing mice on C57BL/6 background. Animals were housed in plastic cages and kept at a room temperature (22°C) in Animal Facility of the Ulm University (Ulm, Germany) or University Hospital in Aachen (Aachen, Germany). Both water and chow were given ad libitum. The breeding room was kept at a 12 hour day-night cycle. All treatments have been made in accordance with the German guidelines for animal research and animal welfare. For all experiments, the blood and tissue samples were collected from double transgenic mice (males) and their non-transgenic littermates.

Cell Culture Experiments. The analyzed cell populations were isolated from 2-4-month-old double transgenic mice (males and females) and their age- and sex-matched non-transgenic littermates. The isolation procedure was carried out by a specialized core facility available at the University Hospital Aachen using previously described protocols [S1]. After the isolation, cells were either stored in RNAlater (Qiagen) or immediately used for RNA preparation. Alternatively, primary hepatocytes were cultured in the HepatoZYME-SFM medium (Invitrogen, Karlsruhe, Germany) supplemented with 1% penicillin-streptomycin and L-Glutamine (Invitrogen). The cells were grown at commercially available, 60-mm collagen-coated culture dishes (BD Falcon Biocoat collagen 6-well plates) in a humidified incubator at 37°C and 5% CO_2.

To induce lipotoxic injury, hepatocytes were treated with 0.5mM palmitic acid (PA, Sigma, Munich, Germany) for 24 hours. To optimize the uptake into the hepatocytes, PA was conjugated to bovine serum albumin (BSA, Sigma) prior to the experiment as described earlier [S2]. Control solution was prepared in the same way but without addition of PA. After the treatment, the levels of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in the cell culture supernatant were measured by the Clinical Chemistry Department of the University Hospital Aachen.

To evaluate the impact of inflammatory cytokines on Hsp72 expression, mouse primary hepatocytes isolated from non-transgenic animals were treated for 6 hours with interleukin 6 (IL-6; 40 ng/ml) or interleukin 1-β (IL1-β; 20 ng/ml; all from Sigma) as described [S3]. Alternatively, the cells were treated for 6 hours with the STAT3 inducer colivelin (0,01 µM; R&D systems). Cells exposed to carrier only were used as controls.

Reverse transcription and qRT-PCR analysis. Total RNA was isolated from liver tissue samples and the isolated cells with the help of RNeasy Mini Kit (Qiagen) according to the manual. For bile ducts, a micro kit from Ambion was employed (Karlsruhe, Germany). Translation of RNA into cDNA was performed with M-MLV-Reverse-Transcriptase Kit (Promega) using oligo-dT primers. Quantitative real time PCR (qRT-PCR) was done with the 7300 Fast Real Time PCR system (Applied Biosystems, Foster City, CA) using specific primers (Supplementary Table 2). Mouse ribosomal protein (L7) and human Large Ribosomal Protein (RPLPO) were analysed as internal controls. The data were evaluated with 7300 Sequence Detection Software (Applied Biosystems).

Protein biochemistry. Total liver tissue lysates were prepared by a homogenization in a buffer containing 3% sodium dodecyl sulphate (SDS, Roth, Karlsruhe, Germany). Alternatively, protein extracts enriched in Triton X-insoluble proteins were obtained via high salt extraction [S4]. To determine the exact localization of Hsp72, subcellular fractionation was performed as described [S5]. The samples were diluted with 4x reducing Laemmli buffer and the proteins were separated via 8-10% SDS–polyacrylamide gel electrophoresis. To check for equal loading, proteins were visualized with 0.05% Coomassie Brillant Blue staining. For immunoblotting, samples were transferred to PVDF membranes and incubated with the appropriate primary and secondary antibodies (see Supplementary Table 3). To detect the amount of Hsp72 in mouse serum, dot-blots were performed as previously described [S3]. The amount of bound horseradish peroxidase that is conjugated to the secondary antibody was determined after incubation with an ECL Detection kit (GE Healthcare Europe GmbH, Freiburg, Germany).

Tissue stainings. For histological/immunohistochemical stainings, liver samples were placed overnight into 10% buffered formalin, dehydrated, embedded in paraffin and cut onto 2µm thick sections. Subsequently, hematoxylin and eosin (H&E) staining was performed or unstained sections were used for immunohistochemistry. For the latter, antigen retrieval was accomplished by boiling samples in ethylene diamine tetraacetic acid (EDTA) buffer (1 mM, pH=8) or in Antigen Unmasking Solution, Citric Acid Based (Vector Laboratories; Burlingame, CA) for 30 min. The unmasking step was followed by washing with 1x phosphate saline buffer (PBS). After that, slides were exposed overnight at +4°C with HSP72 (1:100 dilution), Grp78 (1:50) or Hsp27 (1:50). The excess primary antibodies were removed via washing with 1xPBS and incubated with a biotin-conjugated secondary

antibody (Supplementary Table 3). The specific signal was further amplified with the help of a AB reagent that couples the biotinylated antibody with a peroxidase (Vector Laboratories; Burlingame, CA). Finally, vector Nova Red (Vector Laboratories) was used as a peroxidase substrate. At the end, slides were washed with running tap water, dehydrated and mounted with Entellan (Merck, Germany).

For immunofluorescence (IF), TUNEL, DHE (Dihydroethidium) or Oil Red O (ORO) staining, livers samples were embedded in Tissue-Tek Compound (Sakura), snap frozen, cut onto 2µm thin sections and fixed with pre-cooled 100% acetone. Alternatively, primary mouse hepatocyte grown on slides covered with Poly-D-Lysine (BD, Heidelberg, Germany) were washed with 1xPBS and fixed in 10% Formalin.

ORO staining was used for visualization of lipid droplets. Briefly, samples were washed with running tap water and rinsed with 60% isopropanol (Sigma). Afterwards the slides were stained with Oil Red O diluted in methanol for 15 min at RT. Subsequently, the excess, unbound dye was removed with 60% isopropanol. Counterstaining of nuclei and other basophilic structures was done with haematoxylin (1:1 diluted with water, Roth). Finally, slides were rinsed with distilled water and mounted with aqueous Kaiser's glycerol gelatine (Merck, Germany). The extent of cell death within the slides was determined by a commercially available TUNEL assay kit (Roche Diagnostics, Penzberg, Germany) as specified in the manufacturer´s protocol. The detection of superoxide was accomplished via DHE staining using a commercially available kit (ThermoFisher, Karlsruhe, Germany) and following the manufacturer´s protocol.

Double IF staining was performed with following primary antibodies: K8/K18 (8592; [S4] and p62-Seq1 (GP62-C, Progen, Heidelberg, Germany; both diluted 1:500). Slides were exposed overnight with primary antibodies, followed by three washing steps with 1xPBS and incubation with appropriate Alexa-conjugated secondary antibodies for 30 minutes (Invitrogen).

At the end, slides were washed and mounted with ProLong Gold mounting medium containing DAPI (Invitrogen) to visualize the nuclei. Images were acquired with an Axio Imager.Z1 (Zeiss; Jena; Germany) microscope equipped with a digital camera and AxioVision Rel. 4.8 software (Zeiss).

Luciferase assays. To analyze the luciferase activity *in vivo*, mice were anesthetized with isoflurane and injected intraperitoneally with 200 µl of D-Luciferin solution (PerkinElmer,

Hamburg, Germany). Bioluminescence was measured by IVIS LUMINA XR system (Caliper, Hopkinton, MA). Luciferase activity *in vitro* was analyzed using the Lumat LB 9501 Luminometer (Berthold Technologies, Bad Wildbad, Germany) and the Luciferase assay kit (Promega, Mannheim, Germany) according to the manufacturer´s instructions.

Biochemical assays. The amount of total (GSH) and oxidized (GSSG) glutathione in liver tissue was measured with a spectrophotometric assay according to the manufacturer´s instructions (#703002, Cayman Chemical, Ann Arbor, MI).

The quantification of APAP protein adducts was performed in the lab of Prof. Laura James. After enzymatic digestion of the tissues, adducts were assessed through high pressure liquid chromatography with electrochemical detection (HPLC-EC) and normalized to protein concentration [S6, S7].

To quantify protoporhyrin IX levels, liver tissue was homogenized with 0.9 N perchloric acid/ethanol solution and the samples were centrifuged at 14000 rpm for 30 min. Protoporphyrin IX levels were determined in the supernatant based on their fluorescence (400 nm excitation and 590 nm emission) and the levels were normalized to liver tissue weight. Standard curve was generated with the help of commercially available PPIX standard (Sigma).

For caspase 3 activity assay, liver tissue was homogenized in a detergent-free lysis buffer (100µl 1M Hepes, pH=7.4; 100µl 10%CHAPS; 40µl 0.5M EDTA, pH=8; 50µl 1M DTT; 100µl 0.1M protease inhibitor; distilled water till 10 ml) and the samples were centrifuged at 12000 rpm at +4°C for 10 min. Supernatant was collected and mixed with a commercially available master mix (Biomol, Hamburg Germany). After the incubation step, the resulting absorbance was determined photometrically (excitation wavelength: 390 nm, emission wavelength: 510 nm).

Determination of parameters of lipid metabolism. A commercially available colorimetric assay (#700190, Cayman) was used to quantify the amount of β-hydroxybutyrate in the serum. The analysis was performed according to manufacturer´s recommendations. The total amount of triglycerides, free fatty acids, ceramides, phosphatidylcholine and phosphatidylethanolamine in the liver was quantified via thin-layer chromatography (TLC) method, whereas fatty acid profile was revealed by gas chromatography-mass spectrometry (GC-MS). Both methods have been described in detail as previously [S8-S11].

Statistical analysis. Statistical analysis was carried out with GraphPad Prism 5. Normality of distribution was tested with the Kolmogorov-Smirnoff-test. Based on the results, the data are shown as mean ± standard error of the mean (SEM) or medians with first and third quartiles. Two-tailed Student's t-test and the non-parametrical Mann-Whitney U-test were used for samples with and without normal distribution, respectively. A p-value < 0.05 was considered statistically significant.

Supplementary Figures

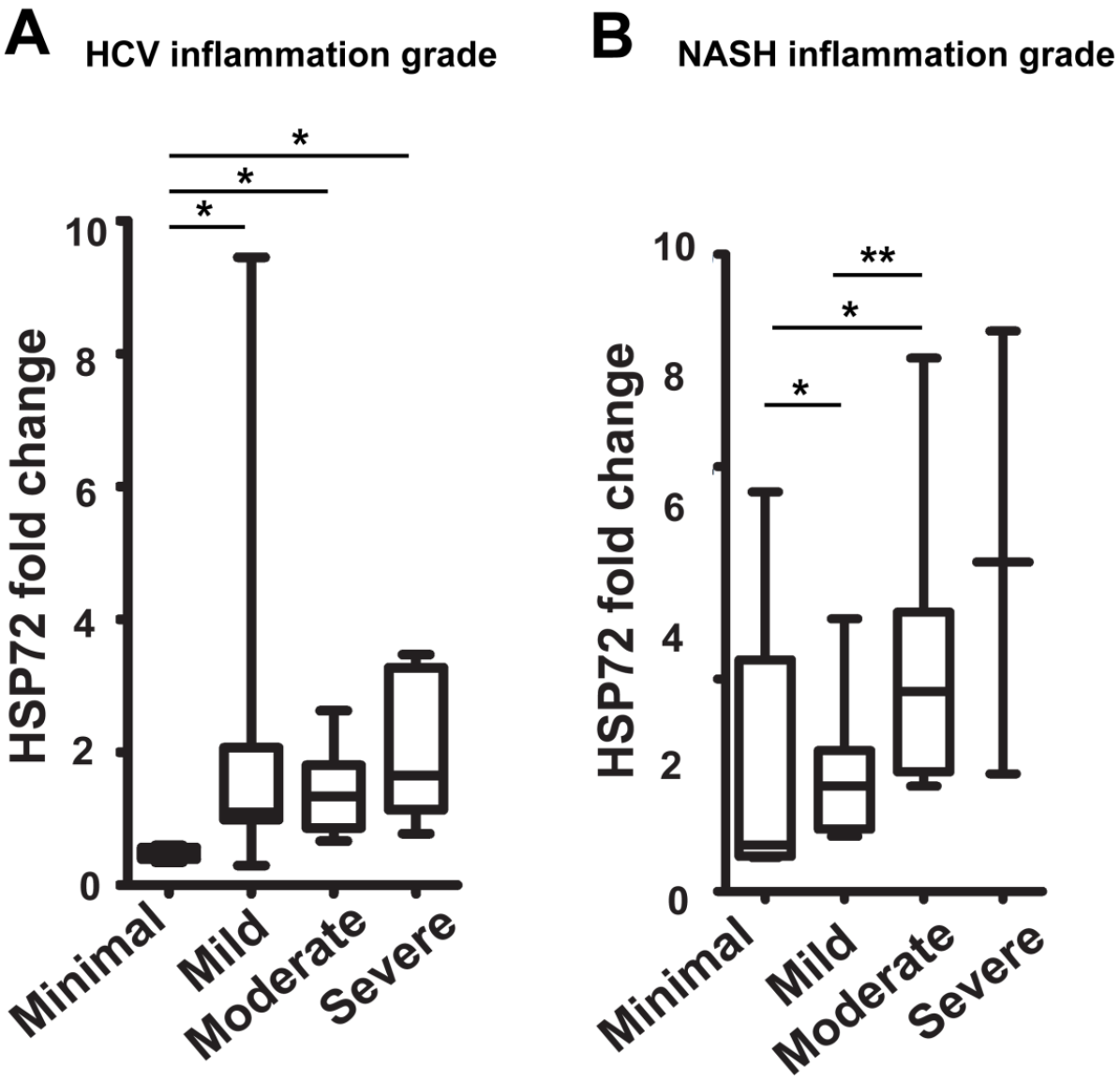

Fig. S1. Hsp72 expression correlates with the extent of hepatic inflammation. HSP72 mRNA levels were determined in patients with chronic hepatitis C infection (HCV; A) and non-alcoholic steatohepatitis (NASH; B) and the expression among patients with different inflammation grades was compared. Boxplots display medians with first and third quartiles, while whiskers indicate smallest and largest non-outlier observations. RPLPO (large ribosomal protein) gene was used as an internal control. Non-parametrical Mann-Whitney U-test was used for statistical evaluation. Asterisk and double asterisk highlight $p < 0.01$ and $p < 0.001$, respectively.

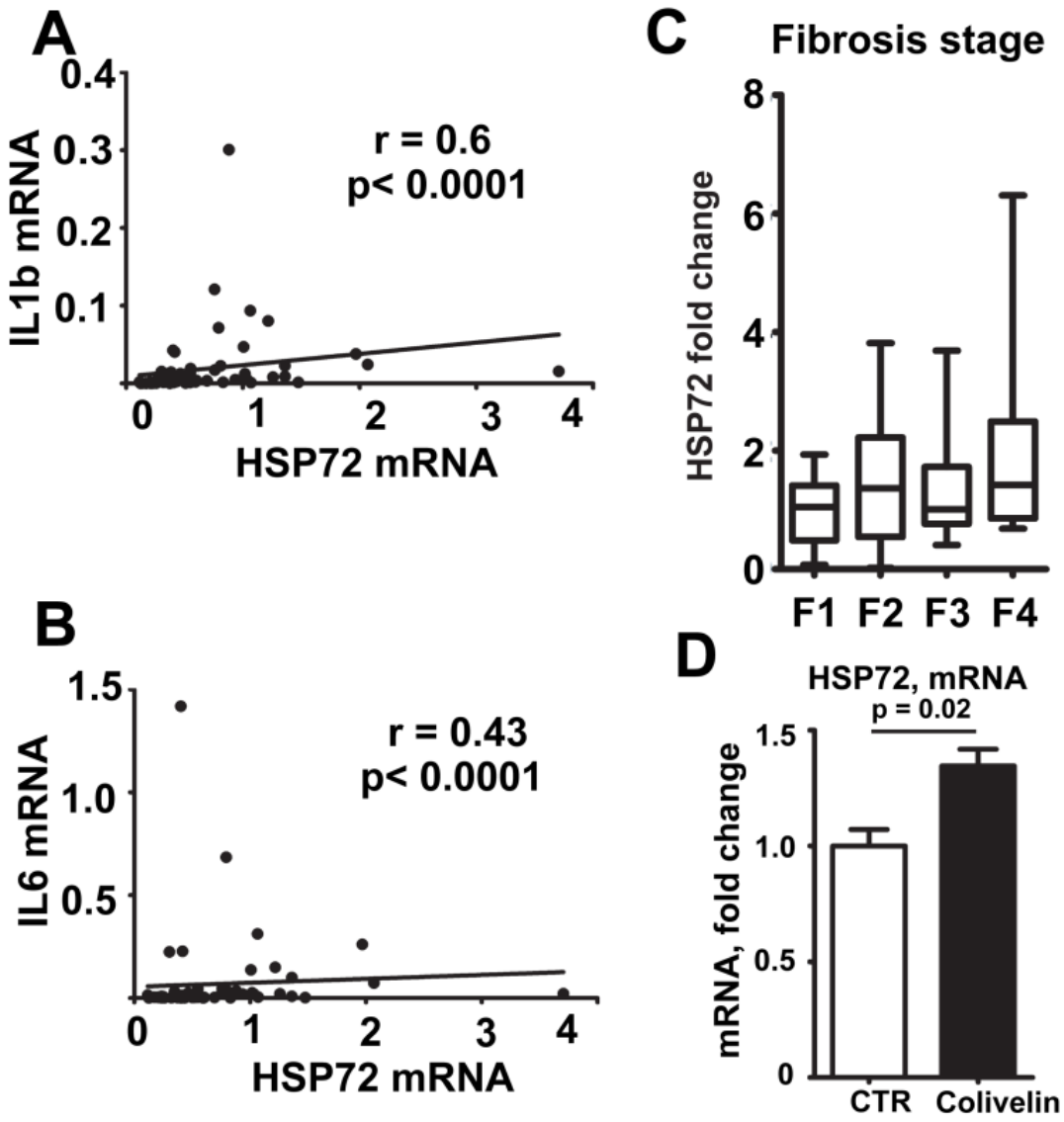

Fig. S2. Hsp72 expression correlates with the levels of inflammatory cytokines. (A,B) Hepatic mRNA levels of HSP72, interleukin 6 or interleukin 1ß were determined in liver healthy subjects (CTR), patients with non-alcoholic steatohepatitis (NASH) and patients with chronic hepatitis C infection (HCV) and compared with the help of Spearman´s correlation coefficient. (C) HSP72 mRNA levels were also compared in patients with different fibrosis stages. Boxplots display medians with first and third quartiles, while whiskers indicate smallest and largest non-outlier observations. RPLPO (large ribosomal protein) gene was used as an internal control. (D) HSP72 mRNA expression was analyzed in mouse primary hepatocytes, that were treated with carrier only (CTR) or subjected to colivelin. L7 (mouse ribosomal protein) was used as an internal control. Results are expressed as mean ± SEM (n=4 for each group). The average expression in control cells was arbitrarily set as 1 and the expression in colivelin-treated cells represents a ratio. Two-tailed Student's t-test was used for statistical evaluation.

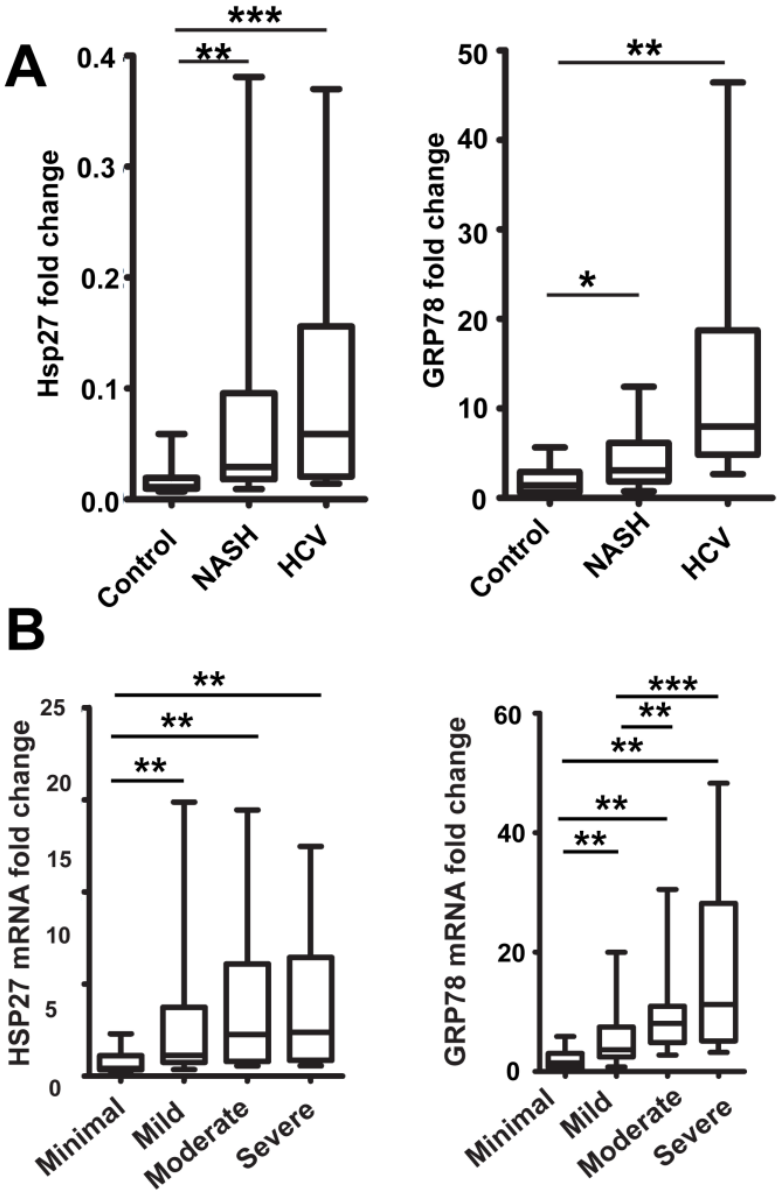

Fig. S3. Expression of Hsp27 and GRP78 is increased in human liver disease and correlates with the level of hepatic inflammation. (A) Hsp27 and GRP78 mRNA levels were determined in liver healthy subjects (Control), patients with non-alcoholic steatohepatitis (NASH) and patients with chronic hepatitis C infection (HCV). (B) mRNA levels of both heat shock proteins were also compared in patients with different inflammation grades. Human RPLPO (large ribosomal protein) gene was used as an internal control. Boxplots display medians with first and third quartiles, while whiskers indicate smallest and largest non-outlier observations. Non-parametrical Mann-Whitney U-test was used for statistical evaluation. Asterisk, double and triple asterisk highlight $p<0.01$, $p<0.001$and $p<0.0001$ respectively.

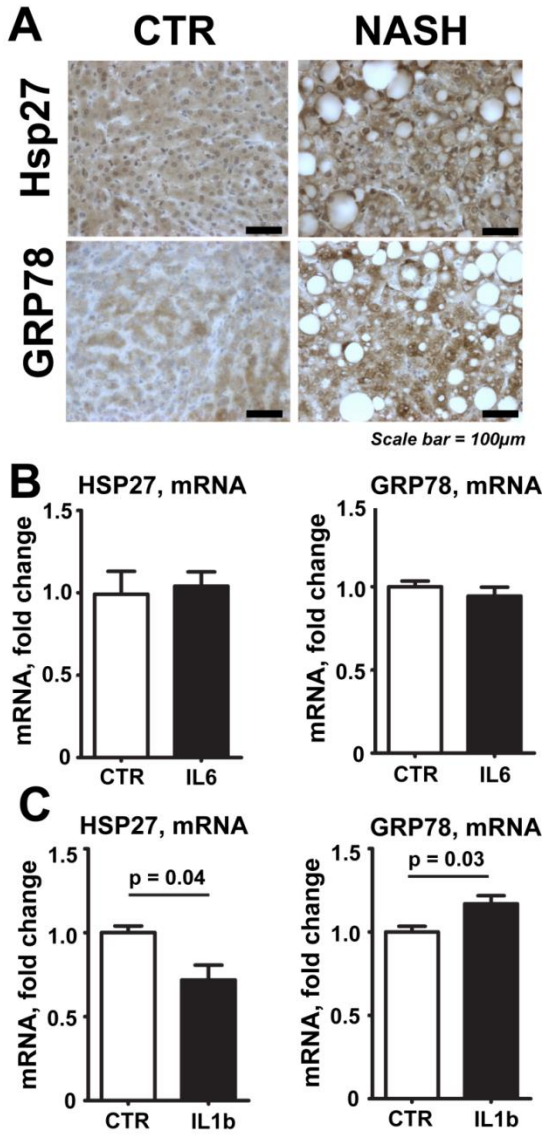

Fig. S4. Hsp27 and GRP78 in patients with non-alcoholic steatohepatitis and in response to inflammatory cytokines. (A) Hepatic Hsp27 and GRP78 distribution was assessed by immunohistochemistry in histologically inconspicuous livers (CTR) and in livers from patients with non-alcoholic steatohepatitis (NASH). Scale bar = 100 µm. (B,C) Hsp27 and GRP78 mRNA expression was analyzed in mouse primary hepatocytes, that were either treated with carrier only (CTR) or subjected to interleukin 6 (IL6; 40 ng/ml) or interleukin 1ß (IL1; 20 ng/ml) for 6 hours. L7 (mouse ribosomal protein) was used as an internal control. Results are expressed as mean ± SEM (n=4 for each group). The average expression in control cells was arbitrarily set as 1 and all other levels represent a ratio. Two-tailed Student's t-test was used for statistical evaluation.

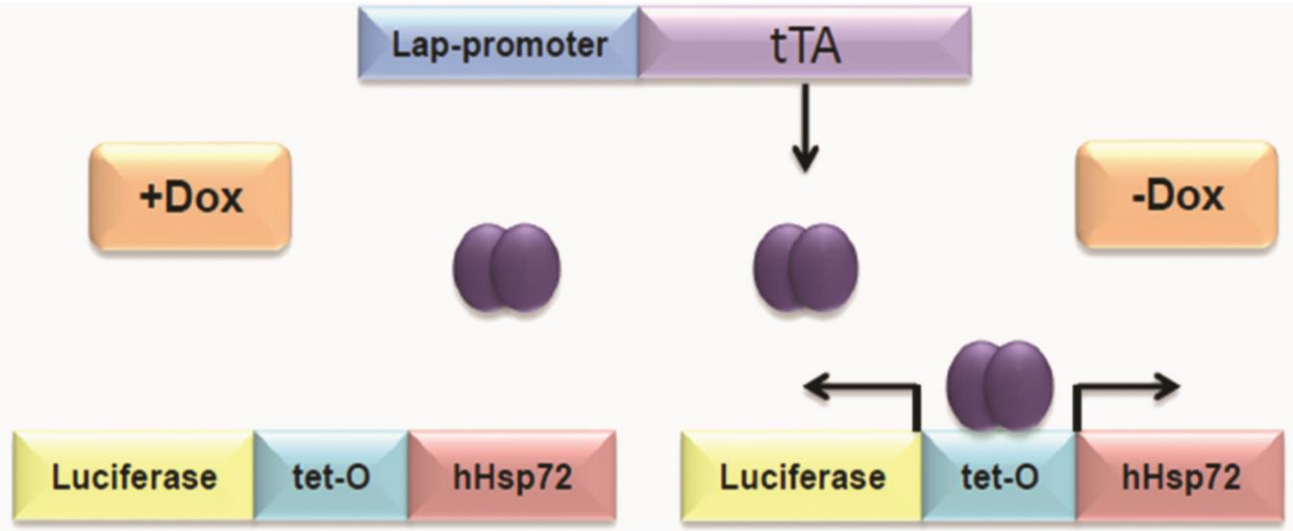

Fig. S5. Generation of Hsp72 transgenic mouse. To address the importance of Hsp72 in the liver, transgenic mice carrying a doxycycline-repressible HSP72 construct were generated. The employed vector contains a bidirectional tet-responsive promoter (tet-O) that allows simultaneous expression of human HSP72 (hHsp72; target gene) and firefly luciferase (reporter gene) in absence of doxycycline (Dox). To obtain liver-specific expression, transgenic Hsp72 overexpressing mice were crossbred with animals carrying tetracycline-responsive transactivator (tTA) under the control of the rat liver activator protein (Lap) promoter. In the absence (but not in the presence) of doxycycline, the produced tTA protein binds to a tet-O operator sequence which leads to the Hsp72/luciferase expression (tet-off system).

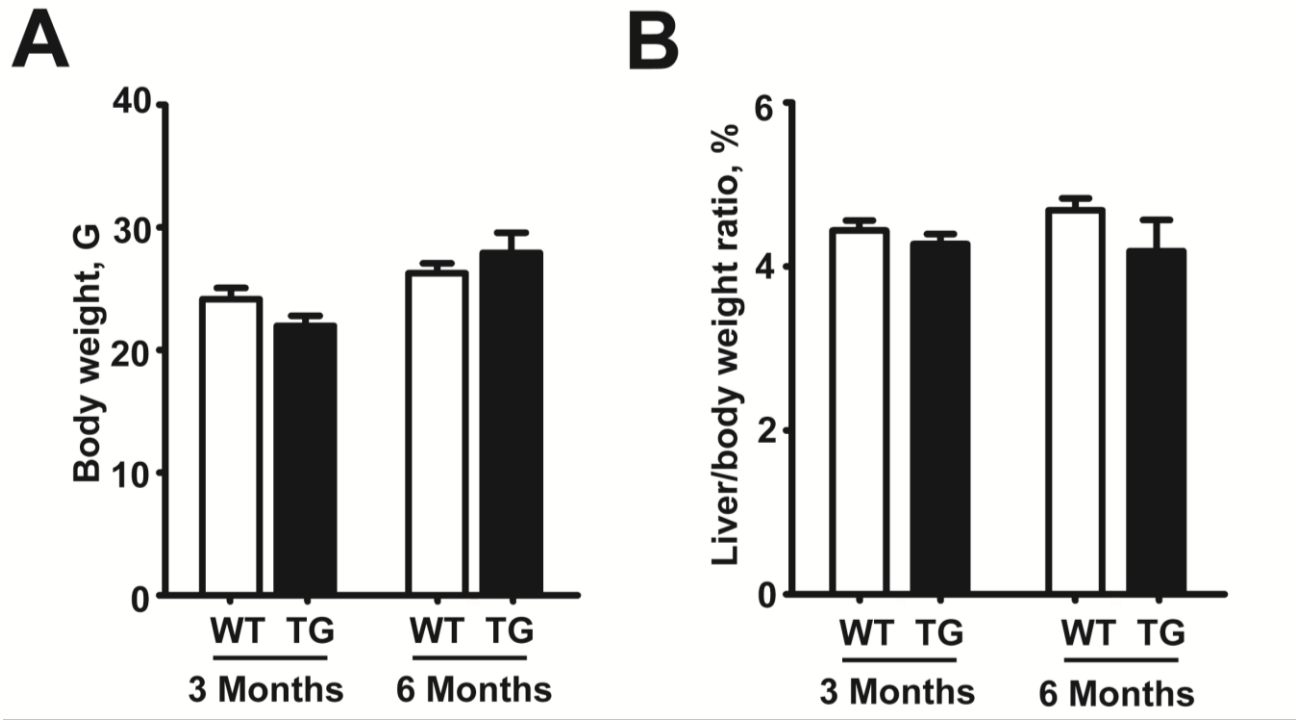

Fig. S6. Hsp72 overexpressing mice display no significant changes in body weight and a normal liver/body weight ratio. The body weight (A) of 3 or 6 months old, double transgenic (TG) and non-transgenic (WT) animals was measured. The ratio of liver to body weight (B) is shown as a percentage. Results are expressed as mean ± SEM, at least 7 samples per group were used. Two-tailed Student's t-test was used for statistical evaluation.

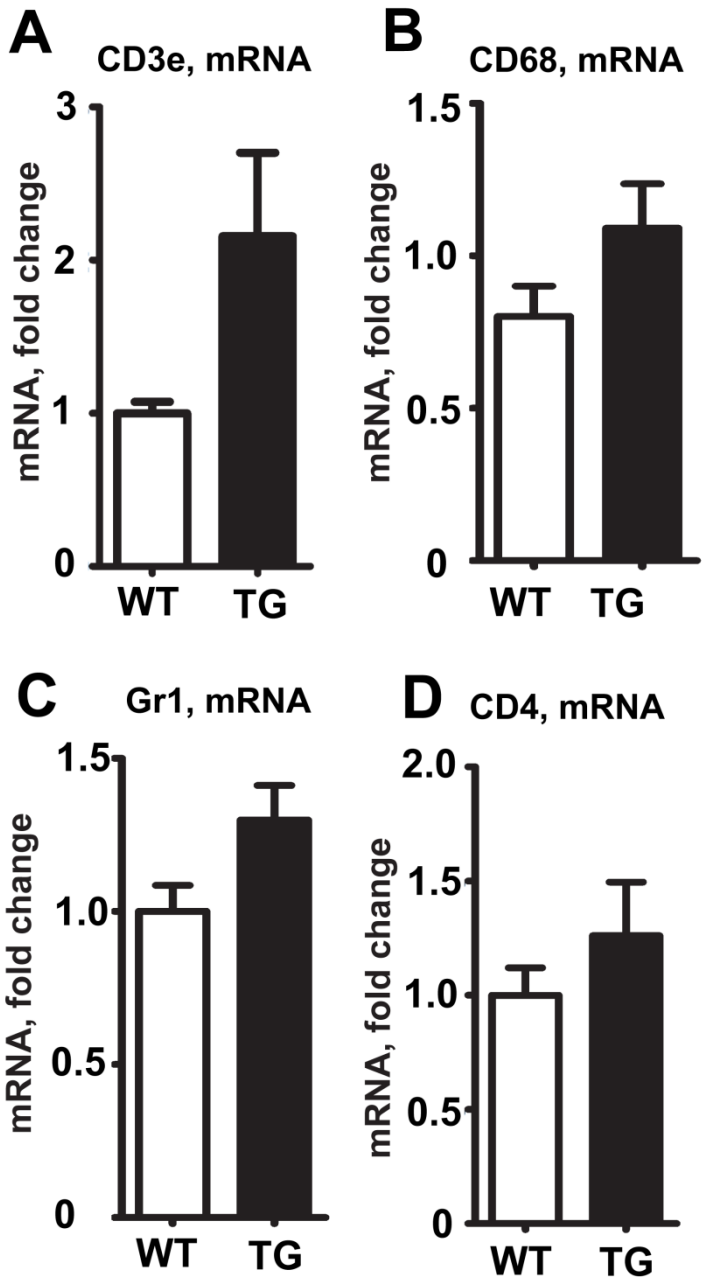

Fig. S7. Hepatic expression of inflammation-related genes in untreated non-transgenic and Hsp72-overexpressing mice. The hepatic mRNA production of the indicated inflammation-related genes was assessed in non-transgenic (WT) and double transgenic (TG) animals under basal conditions (n=5). L7 (mouse ribosomal protein) was used as an internal control and results are expressed as mean ± SEM. Two-tailed Student's t-test was used for statistical evaluation.

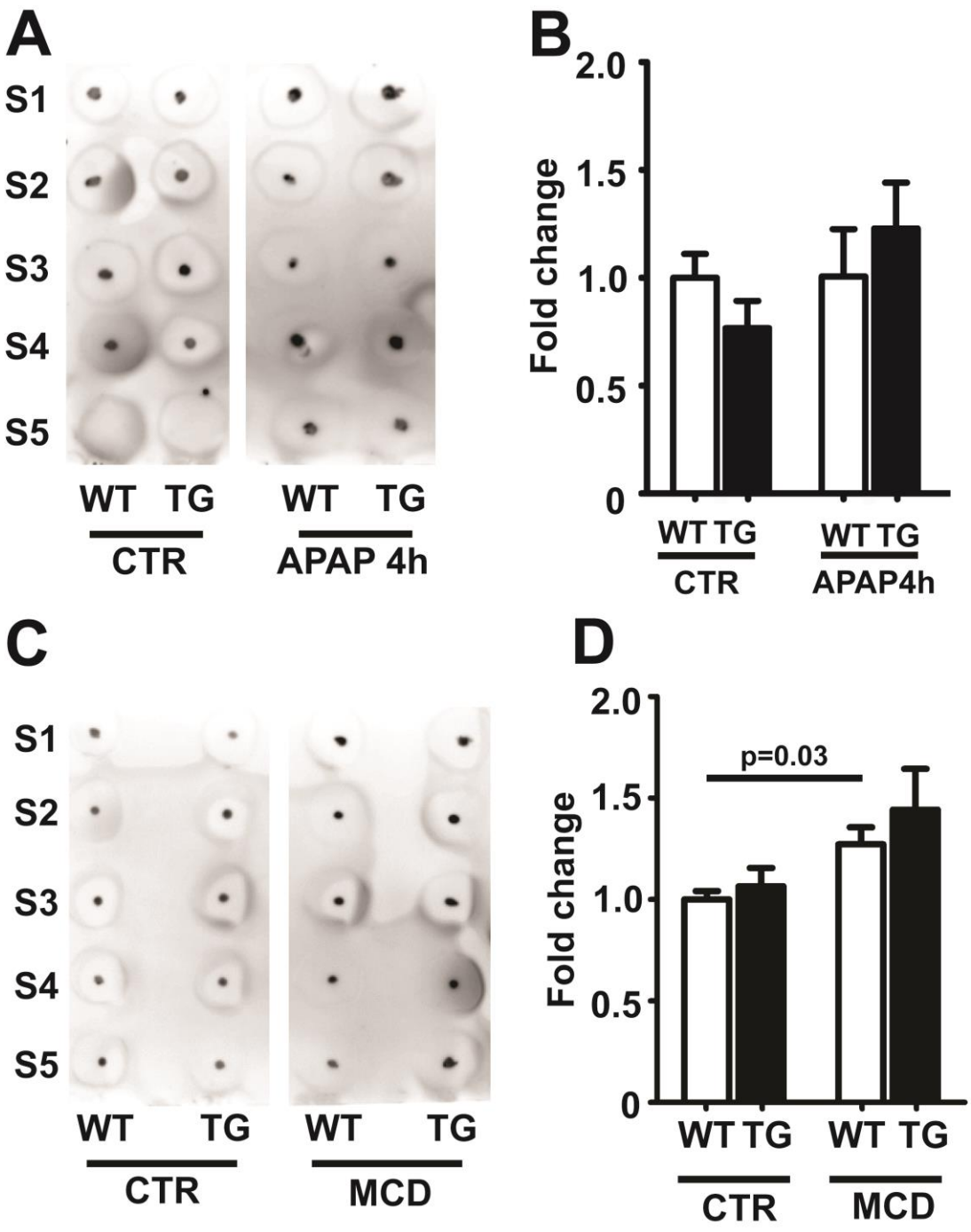

Fig. S8. Serum Hsp72 levels in non-transgenic and Hsp72-overexpressing mice. Sera from non-transgenic (WT) and double transgenic (TG) mice were assessed for its Hsp72 content via dot blots. The signal intensity in untreated mice (CTR), in mice exposed to acetaminophen for 4 hours (APAP 4h) and in animals subjected to methionine choline-deficient was quantified by ImageJ and the results are expressed as mean ± SEM. The average levels in untreated, non-transgenic animals were arbitrarily set as 1 and all other levels represent a ratio. Two-tailed Student's t-test was used for statistical evaluation.

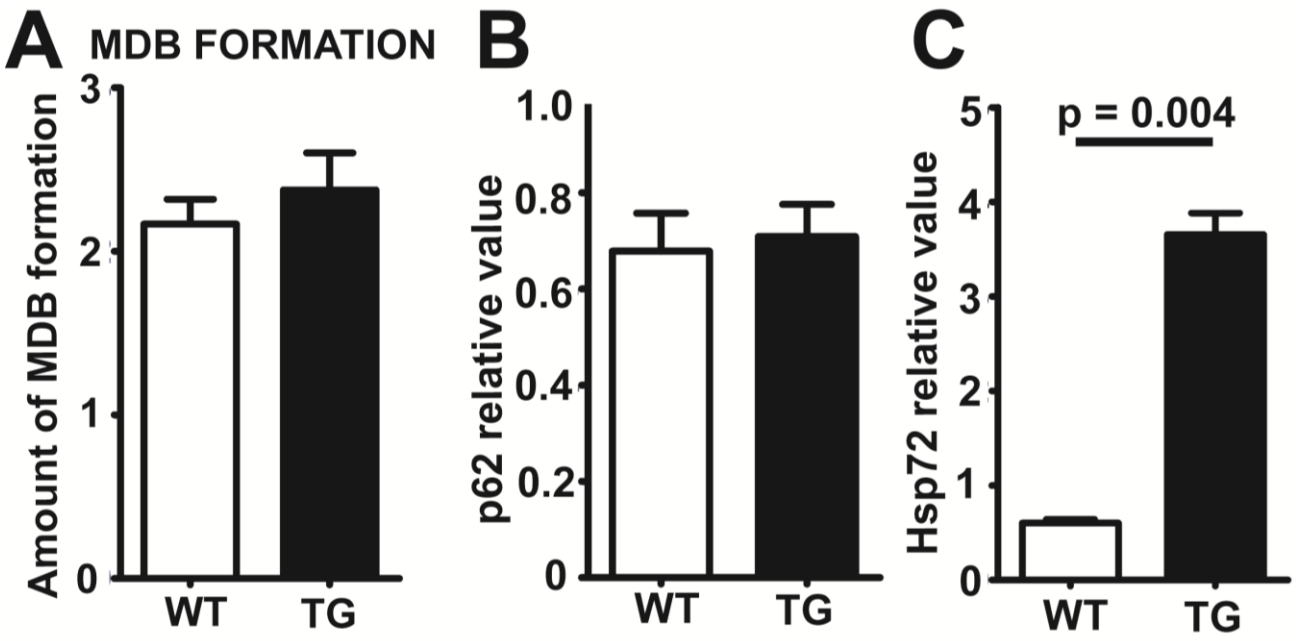

Fig. S9. Quantification of alterations seen in animals exposed to DDC. (A) The amount of Mallory-Denk bodies (MDB) was semi-quantitatively assessed in hepatic sections from DDC-exposed, non-transgenic (WT) and double transgenic (TG) mice stained with keratin 8/18 (K8/K18) and p62. (B, C) Immunoblotting against p62 and Hsp72 was carried out and the relative band intensity relative to ß-tubulin that was used as a loading control was quantified by ImageJ. Results are expressed as mean ± SEM, at least 8 mice per group were analyzed. Two-tailed Student's t-test was used for statistical evaluation.

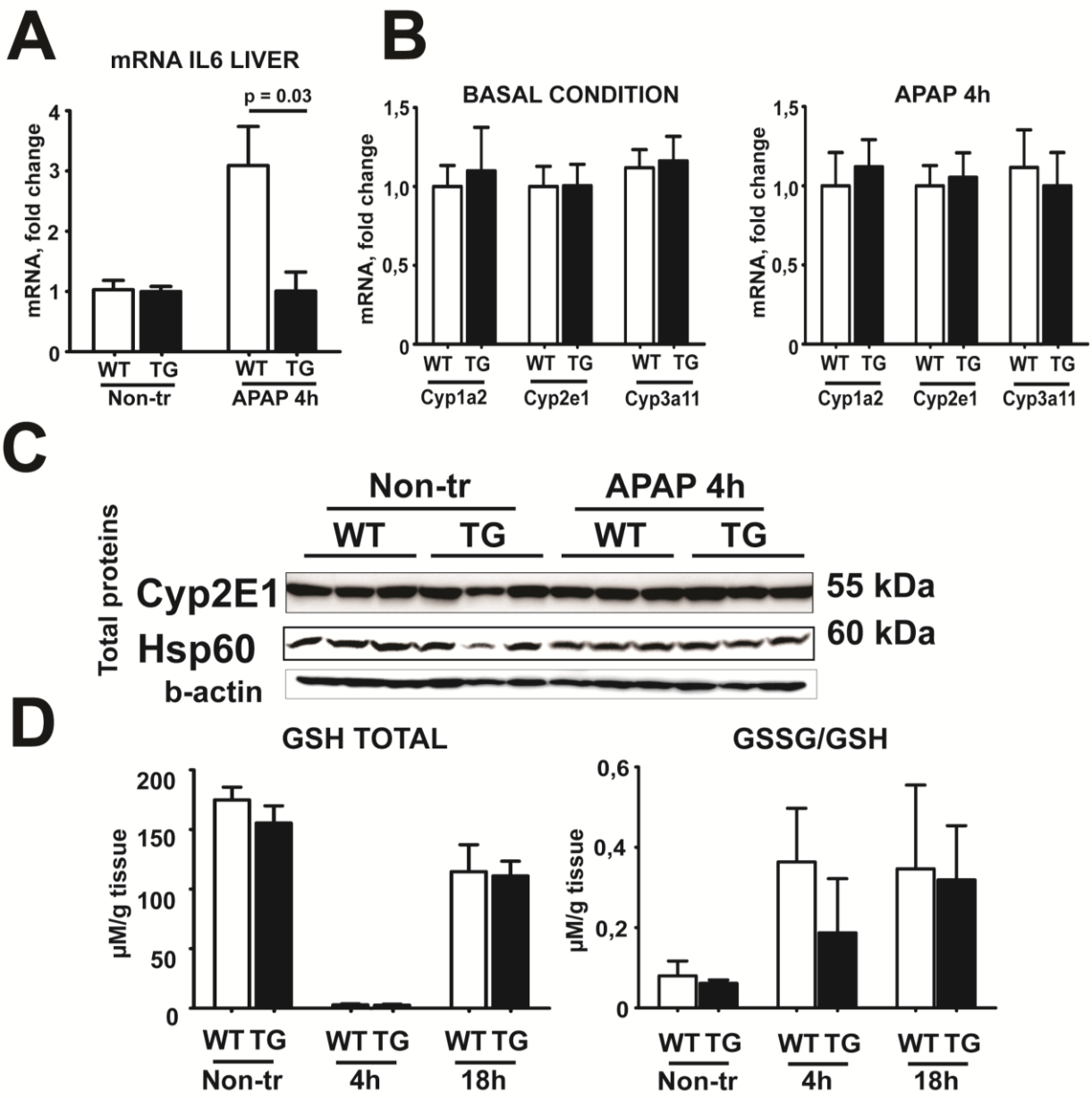

Fig. S10. Hsp72 overexpression does not affect the major parameters of APAP metabolism. The hepatic mRNA production of IL6 (A) as well as the APAP metabolism-related cytochrome genes Cyp1a2, Cyp2e1, Cyp3a11 (B) was assessed in non-transgenic (WT) and double transgenic (TG) animals under basal condition (Non-tr) and 4h after APAP administration (APAP 4h). The average mRNA expression in non-transgenic animals was arbitrarily set as 1 and the amounts in double transgenic mice represent a ratio (n=5). (C) Immunoblotting quantifies hepatic protein levels of Cyp2E1. Hsp60 and ß-actin were used as loading controls. (D) The amount of total hepatic glutathione (GSH total) and the ratio of oxidized to reduced glutathione (GSSG/GSH) was measured. Results are expressed as mean ± SEM (n = 5). Two-tailed Student's t-test was used for statistical evaluation. Cyp1a2, Cytochrome P450 1A2; Cyp2e1, Cytochrome P450 2E1; Cyp3a11, Cytochrome P450 3A11; IL6, Interleukin 6; Hsp60, Heat shock protein 60.

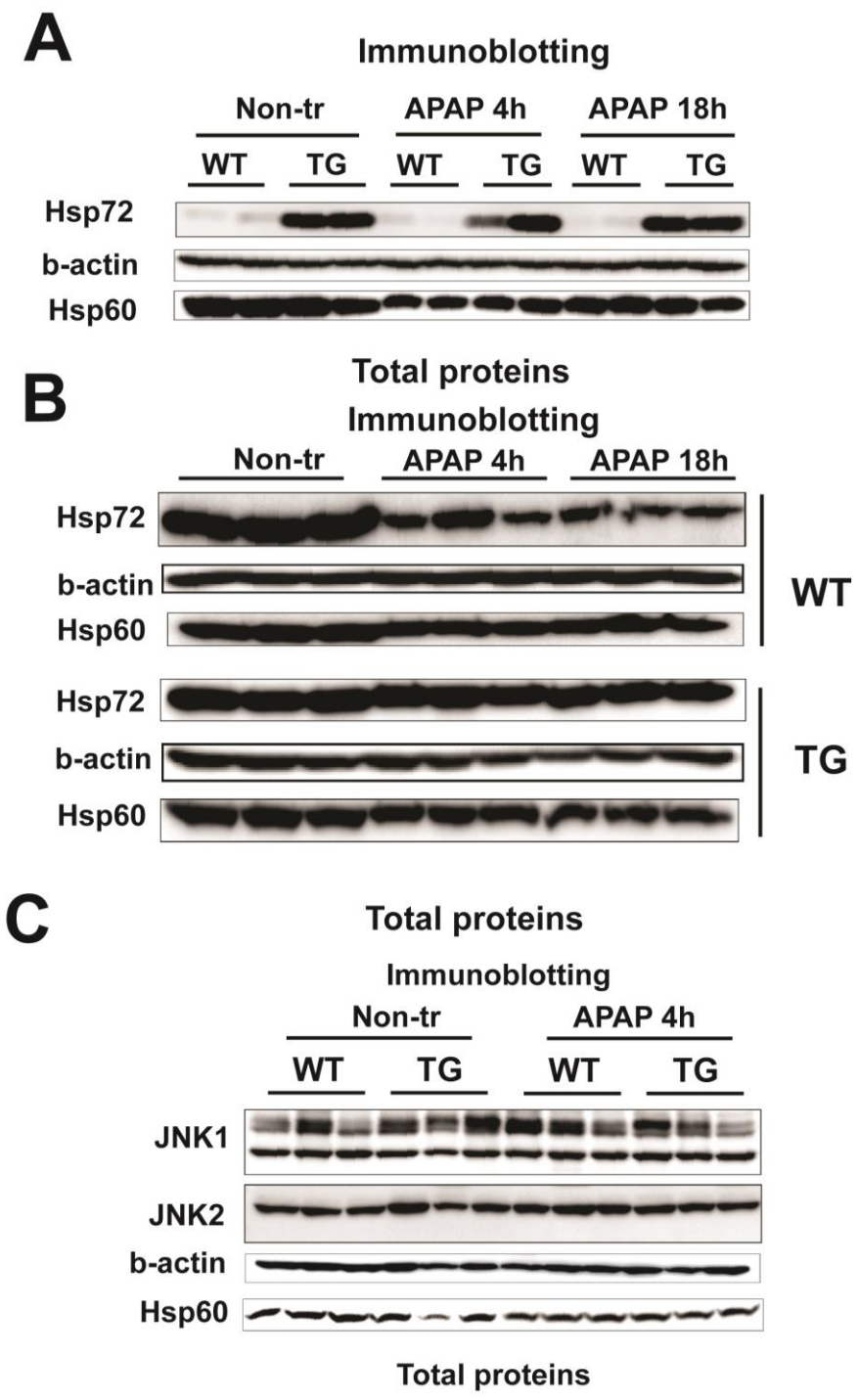

Fig. S11. Hsp72 overexpression in double transgenic animals (TG) is retained after acetaminophen (APAP) treatment. Immunoblotting depicts protein levels of Hsp72 (A, B) and JNK1/2 (C) in TG and non-transgenic (WT) mice prior (Non-tr) and 4h or 18h after APAP administration. Hsp60/ß-actin were used as a loading controls. Hsp72, Heat shock protein 72; Hsp60, Heat shock protein 60; JNK1/2, C-Jun N-Terminal Kinases 1 and 2.

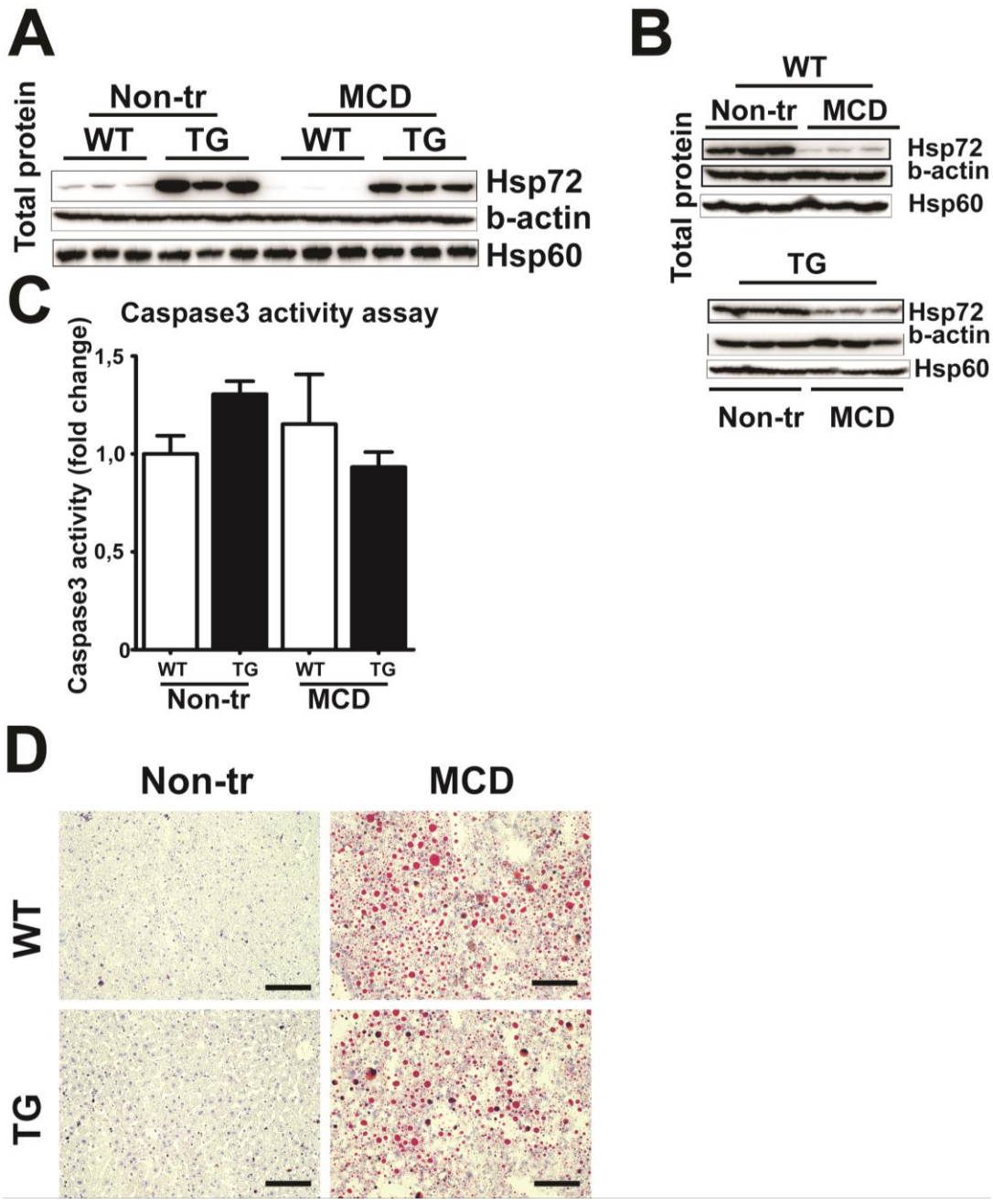

Fig. S12. Hsp72 overexpression does not affect the development of liver steatosis induced by feeding with methionine choline-deficient diet (MCD). (A,B) Immunoblotting quantifies protein levels of Hsp72 in double transgenic (TG) and non-transgenic (WT) mice prior (Non-tr) and after 8 weeks of treatment with MCD. Hsp60 and ß-actin were used as loading controls. (C) Caspase 3 activity in liver lysates was determined and expressed as mean ± SEM. The levels in non-treated, WT animals were arbitrarily set as 1 and all other levels represent a ratio. Two-tailed Student's t-test was used for statistical evaluation. (D) Oil-Red O staining was used to visualize lipid droplets in the depicted liver sections. Hsp72, Heat shock protein 72; Hsp60, Heat shock protein 60.

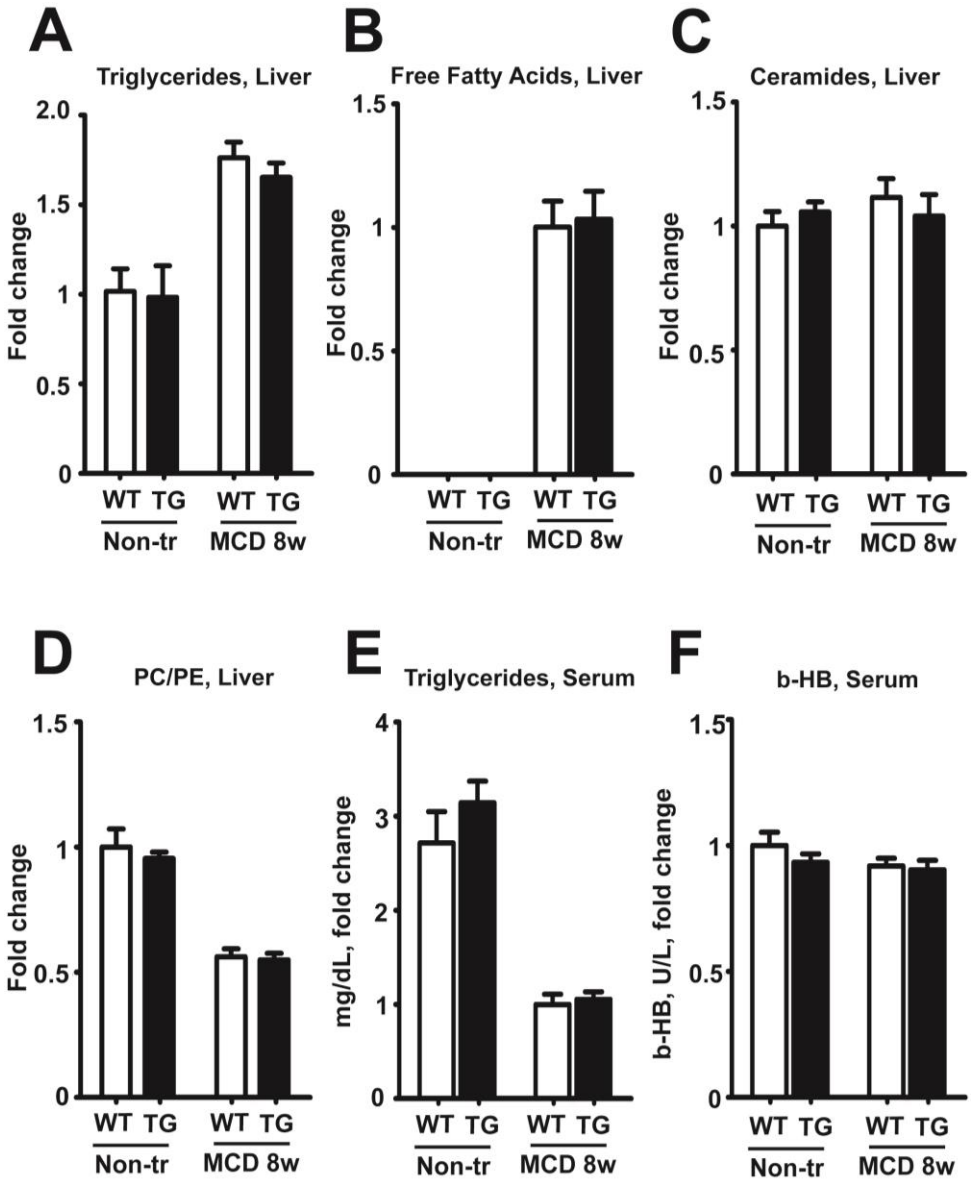

Fig. S13. Hsp72 overexpression does not affect basic parameters of lipid metabolism. The amount of triglycerides (A), free fatty acids (B), ceramides (C), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was quantified in the liver of non-transgenic (WT) and double transgenic mice (TG) kept on standard diet (Non-tr) or on MCD for 8 weeks (MCD 8w) via thin-layer chromatography (TLC). PC/PE (D) refers to a ratio between both phospholipids. Serum analysis of triglycerides (E) and beta-hydroxybutyric acid (b-HB) (F) was also performed. Data are expressed as mean ± SEM, at least 5 mice per group were analyzed. Two-tailed Student's t-test was used for statistical evaluation.

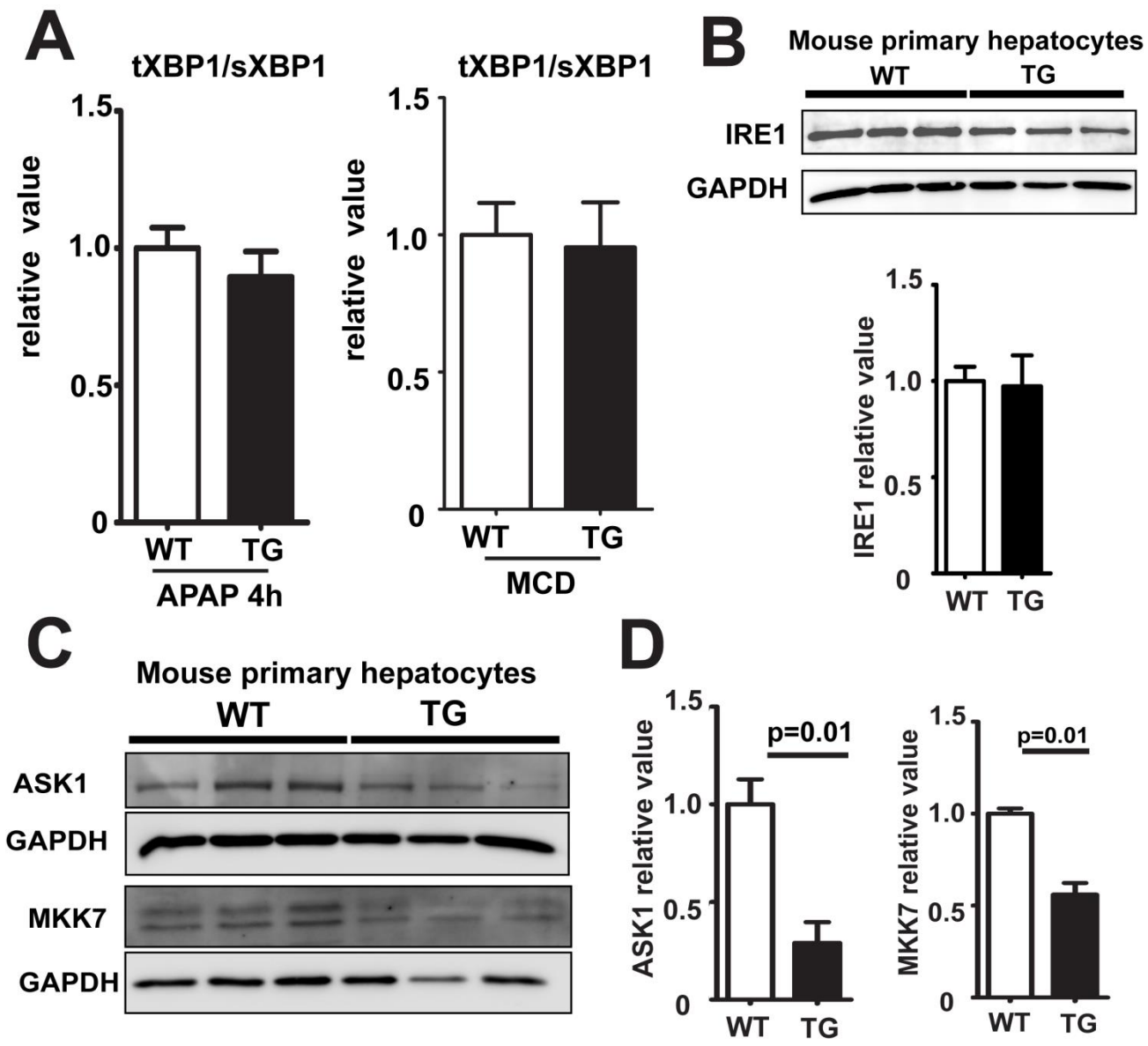

Fig. S14. Primary mouse hepatocytes from Hsp72-overexpressing animals display decreased levels of stress-inducible kinases. (A) The hepatic mRNA levels of X-box binding protein 1 (XBP1) were assessed in double transgenic (TG) and non-transgenic (WT) mice exposed to treatment with acetaminophen for 4 hours or MCD diet for 8 weeks. A ratio of total (t) versus spliced (s) isoform was analyzed. Data are expressed as mean ± SEM, at least 8 mice per group were analyzed. The levels in WT mice were arbitrarily set as 1 and the levels in TG mice represent a ratio. (B-E) Primary hepatocytes were isolated from non-treated transgenic mice (TG) and immunoblotting was used to determine the amount of highlighted proteins in total hepatocyte lysates. The signal intensity was quantified by ImageJ and the results are expressed as mean ± SEM, (n=6). GAPDH was used as loading control. Two-tailed Student's t-test was used for statistical evaluation.

Supplementary Tables

Table S1. Patient's characteristics.

Etiology	Num.	% M	Age	Inflammation stage	Fibrosis stage	ALT, U/L	AST, U/L
Control	7	57%	44.6±4.5	0.0±0.0	0.2±0,3	NA	NA
HCV	34	65%	46.8±12,5	1.4±0.5	1.7±1.0	NA	NA
NASH	23	57%	49.1±12.1	1.5±0.2	2.2±0.6	126±117	93±64

Results are expressed as mean ± SEM and list inflammation grades/fibrosis stages according to Kleiner scoring systems (NASH) and the METAVIR scoring system (HCV, controls). ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV - chronic hepatitis C infection, NA, not available, NASH - non-alcoholic steatohepatitis.

Table S2. Genotyping and RT-PCR primers

Gene	Species	5'- Sequence -3'	Accession Nr.
		Genotyping primers	
HspA1A (HSP72)	Human	F: CTGTACCAGGGTGCCGGTGGT R: GTCCCCAAACTCACCCTGAAGTTCT	
Lap-tTA	Rat/ E.Coli	F: GCTAGGTGTAGAGCAGCCTACATTG R: GTCCAGATCGAAATCGTCTAGCGCG	
		Primers for qRT-PCR	
mCyp1a2 ***Cytochrome P450, family 1, subfamily a, polypeptide 2***	Mouse	F: TCTGCCAGTCTCCAGCCCCT R: AGATGGCAGTGGCCAGTAGCA	NM_009993.3
mCyp2e1 ***Cytochrome P450, family 2, subfamily e, polypeptide 1***	Mouse	F: CTGGCCGAGGGGACATTCCTGT R: AGGGAAAACCTCCGCACGTCCT	NM_021282.2
mCyp3a11 ***Cytochrome P450, family 3, subfamily a, polypeptide 11***	Mouse	F: TGCTGTCACAGACCCAGAGACG R: ACTCATTATCCCCACTGGGCCA	NM_007818.3
(m+h) HSP72 ***Heat shock protein family A (Hsp70) member 1A***	Mouse + human	F: ACGGGCGCGACCTGAACAAG R: ATCAGGATGGCCGCCTGCAC	NM_005345.5 NM_010479.2
hHSP72 ***Heat shock protein family A (Hsp70) member 1A***	Human	F: GCCAACAAGATCACCATCAC R: TTTGTACTTCTCCGCCTCCT	NM_005345.5
mIL6 ***Interleukin 6***	Mouse	F: ACAAAGCCAGAGTCCTTCAGAGAGA R: TGGTCTTGGTCCTTAGCCACTCC	NM_031168.1
mHamp1 ***Hepcidin***	Mouse	F: CTGTCTCCTGCTTCTCCTCCT R: GGCTGCAGCTCTGTAGTCTGT	[S13]
mSod2 ***Manganese superoxide dismutase 2***	Mouse	F: GAACAATCTCAACGCCACCG R: GCTGAAGAGCGACCTGAGTT	NM_013671.3
hRPLPO ***Large Ribosomal Protein***	Human	F: GCAATGTTGCCAGTGTCTGT R: GCCTTGACCTTTTCAGCAAG	NM_001002.3
mL7 ***Ribosomal protein L7***	Mouse	F: GAAAGGCAAGGAGGAAGCTCATCT R: AATCTCAGTGCGGTACATCTGCCT	AK017074.1
Hsp27 (Hspb2) ***Hspb2 heat shock protein 2***	Mouse	F: TGTCTACCTCCCGTGGTGAT R: GGTTTATTCAGCCCCACCC	NM_001164708.1
GRP78-Hspa5 **HSPA5 heat shock protein family A (Hsp70) member 5**	Mouse	F: GGTACCCACCAAGAAGTCTCAG R: TCAGCAAACTTCTCAGCATCAT	NM_022310.3/ NM_001163434.1
Human Grp78 **HSPA5 heat shock**	Human	F: GCTTATGGCCTGGATAAGAGG R: CACGCTGGTCAAAGTCTTCTC	NM_005347.4

protein family A (Hsp70) member 5			
Hsp27 (Hspb2) ***Hspb2 heat shock protein 2***	Human	F: AGACGAGGTGACTGTGAGGA R: ACATAGGTGCGGCAGAACTC	NM_001541.3
CD68	Human	F: AGCTACATGGCGGTGGAGTA R: CGAAGGGATGCATTCTGAGC	
IL1b ***Interleukin 1beta***	Human	F: GCTCGCCAGTGAAATGATGG R: GGTGGTCGGAGATTCGTAGC	
IL6 ***Interleukin 6***	Human	F: TAGTGAGGAACAAGCCAGAGC R: TGGGTCAGGGGTGGTTATTG	

Table S3. Antibodies used for immunoblotting and immunohistochemistry

Antibody	Catalog number	Company
Cytochrome P4502E1	9140	Abcam
Hsc70	SPA-819	Stressgen
Hsp27	sc-1049	Santa Cruz
Hsp60	ADI-SPA-805	Enzo
HSP72	ADI-SPA-812	Enzo
Hsp90	4877	Cell Signaling
JNK1	3708 (2C6)	Cell Signaling
JNK2	sc-827 (N-18)	Santa Cruz
phospho-c-Jun	9164 (Ser73)	Cell Signaling
phospho-SAPK/JNK	9251	Cell Signaling
phospho-SAPK/JNK	4668 (81E11)	Cell Signalling
Rip3	2283	ProSci
β-actin	A1978	Sigma-Aldrich
Goat anti-mouse IgG (H+L), HRP conjugated	G21040	Invitrogen
Goat anti-rabbit IgG (H+L), biotinylated	BA-1000	Vector
Goat anti-rabbit IgG (H+L), HRP conjugated	G21234	Invitrogen
Goat anti-rat IgG (H+L), HRP conjugated	A10549	Invitrogen
Rabbit anti-goat IgG (H+L)	R21459	Invitrogen
Grp78	3177S	Cell Signaling

Hsp27	sc-1049	Santa Cruz
MKK7	4172	Cell Signaling
IREalpha	3294	Cell Signaling
ASK1 (D11C9)	8662	Cell Signaling

Table S4. Fatty acids profile

Fatty acid		Fold changes, TG/WT	
		Basal condition	MCD 8w
Myristoleic acid	14:0	1,18	0,58
Pentadecylic acid	15:0	1,00	
Palmitic acid	16:0	1,19	1,10
Palmitoleic acid	16:1w7cis	1,23	0,73
	16:1w7trans	1,05	0,48
Stearic acid	18:0	0,95	1,08
Oleic acid	18:1w9cis	1,16	0,91
Elaidic acid	18:1w9trans	0,10	0,65
Linoleic acid	18:2w6,9 all cis	1,09	0,84
γ-Linolenic acid	18:3w6,9,12 all cis	3,18	0,86
Arachidic acid	20:0	1,26	1,12
	20:1w7cis	1,15	0,26
Eicosenoic acid	20:1w9cis	0,69	0,69
Eicosadienoic acid	20:2w6,9 all cis	0,65	0,71
Dihomo-γ-linolenic acid	20:3w6,9,12 all cis	0,10	0,69
Arachidonic acid	20:4w6,9,12,15 all cis	1,00	1,00
Behenic acid	22:0	0,01	1,45
Adrenic acid	22:4w6,9,12,15 all cis	1,08	0,97
	22:5w3,6,9,12,15 all cis	1,94	0,68
Docosapentanoic acid w6	22:5w6,9,12,15,18 all cis	1,58	1,53
Docosahexanoic acid	22:6w3,6,9,12,15,18 all cis	1,13	0,98

Tricosylic acid	23:0	3,07	1,57
Lignoceric acid	24:0	2,20	1,11
	24:1w9cis	1,01	0,95
Cholesterol		1,01	1,04
Sum (µg fatty acids/mg tissue dry weight)		1,09	0,97
ratio sat/unsat		1,01	1,14
ratio C18/C16		0,87	0,86
sum > C22		1,21	1,15

Two-tailed Student's t-test was used for statistical evaluation.

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