APPENDIX

Parasite-induced ER stress response in hepatocytes facilitates
***Plasmodium* liver stage infection**

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Contents

Supplementary Methods

Cells. Huh7 cells, a human hepatoma cell line, were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 1% Penicillin/Streptomycin solution, 1% HEPES, 1% Non-essential amino acids, and 1% Glutamine in a 5% CO_2 humidified incubator at 37°C. Hepa 1.6 cells, a mouse hepatoma cell line, and the 293A cell line (Invitrogen) were cultured in DMEM medium supplemented with the same components as listed above and were maintained in a 5% CO_2 humidified incubator at 37°C. All components were purchased from Gibco/Invitrogen. For isolation of murine primary hepatocytes and culture conditions please check supplemental information. Cell viability was assessed by measuring Alamar Blue fluorescence (Invitrogen, UK), using the manufacturer's protocol.

Mice bearing an inducible, conditional disruption of the Xbp1 gene in the liver generation and genotyping. Liver Xbp1Δ mice were generated as previously described [1]. Briefly, Xbp1^{flox} mice harboring loxP sites in the first and second intron of the Xbp1 gene were crossed with Mx1-cre mice that express interferon-dependent Cre recombinase. 6 weeks old mice were intraperitoneally injected 2 or 3 times with 250 µg of poly(I:C) each time with 2 days intervals to induce Cre expression. Mice were used for experiments at least 2-3 weeks after the final poly(I:C) injection. All experiments with XBP1 mice were performed at Columbia University under the guidelines of the Columbia University Animal Facility. Mouse genotyping was performed using DNA from mouse tail blood extracted with DNease Blood & Tissue Kit (Qiagen). PCR genotyping was performed with primers for the floxed allele (*flox*), *Cre* and *Il2* (PCR control) primers with the following cycle: 94°C for 3 minutes, 94°C for 30 seconds/56°C for 30 seconds/72°C for 30 seconds (35 cycles), 72°C 5 minutes. Primers specific sequences were:

ACTTGCACCAACACTTGCCATTTC and CAAGGTGGTTCACTGCCTGTAATG for XBP1 *flox*, GCGGTCTGGCAGTAAAAACTATC and GTGAAACAGCATTGCTGTCACTT for *Cre*, CTAGGCCACAGAATTGAAAGATCT and GTAGGTGGAAATTCTAGCATCATCC for *Il2*. Lack of XBP1 protein in Xbp1Δ livers compared to control mice (CTRL) was confirmed by Western blot (Figure 3C). Sex-matched littermate Xbp1$^{flox/+;cre}$ mice also injected with poly(I:C) were used as controls.

Adenovirus *in vivo* and *ex vivo* transduction. Mice were infected with 1x10^8 GFPu (GFP positive units) / g of body weight for each adenovirus (Ad-US/Ad-CREBHi) by tail-vein injection 48h prior to sporozoite infection. All mice were then infected with 5x10^4 *Plasmodium berghei* sporozoites and 42-44 hours later livers were collected and liver infection was determined by expression of PbA 18S rRNA. Primary hepatocytes were infected with the recombinant adenoviruses at 10 GFPu per cell 24 hours prior to sporozoite infection. *Plasmodium* infection was determined at 44 hours by expression of PbA 18S rRNA. Amplification and purification of high-titer recombinant adenoviruses was performed as previously described [2]. Viral titer was determined by infecting HEK-293A cells with serially diluted viral stock and counting GFP positive cells 24 hours after infection by FACS. CREB-H protein determination by western blot was performed using a rabbit polyclonal antibody raised against bacterially produced mouse CREB-H amino acids 1-232, kindly provided by Dr. Ann-Hwee Lee, Weill Cornell Medical College.

Histopathology analysis. Liver tissues were harvested from noninfected mice 8h after tunicamycin injection and from infected mice 42 h after infection. Tissues were fixed

over-night in 4% paraformaldehyde solution for paraffin embedding and hematoxylin-eosin (HE) staining.

Analysis of proteomics screen. Samples were prepared by FASP method [3] and then peptides were analysed in a LTQ Orbitrap XL machine. The raw data from the mass spectrometer were processed with MaxQuant software [4] and with label free quantification. Proteins with at least two values from three replicates were considered for further analysis. The missing values were then imputed with the minimum value for each sample. The data was then normalized with the TMM method [5]. We performed the analysis between 12 hours data set (GFP positive versus GFP negative) with R package "limma" [6]. This statistical method was employed to capture the mean-variance relation in the data and determine whether a gene was differentially expressed (DE) between two groups of samples. We considered as statistically significant the DE proteins analysed by the previous method with a corrected p-value ≤ 0.05. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium [1] via the PRIDE partner repository with the dataset identifier PXD002269.

A

original magnification, 200x
➤ parasitophorous vacuole
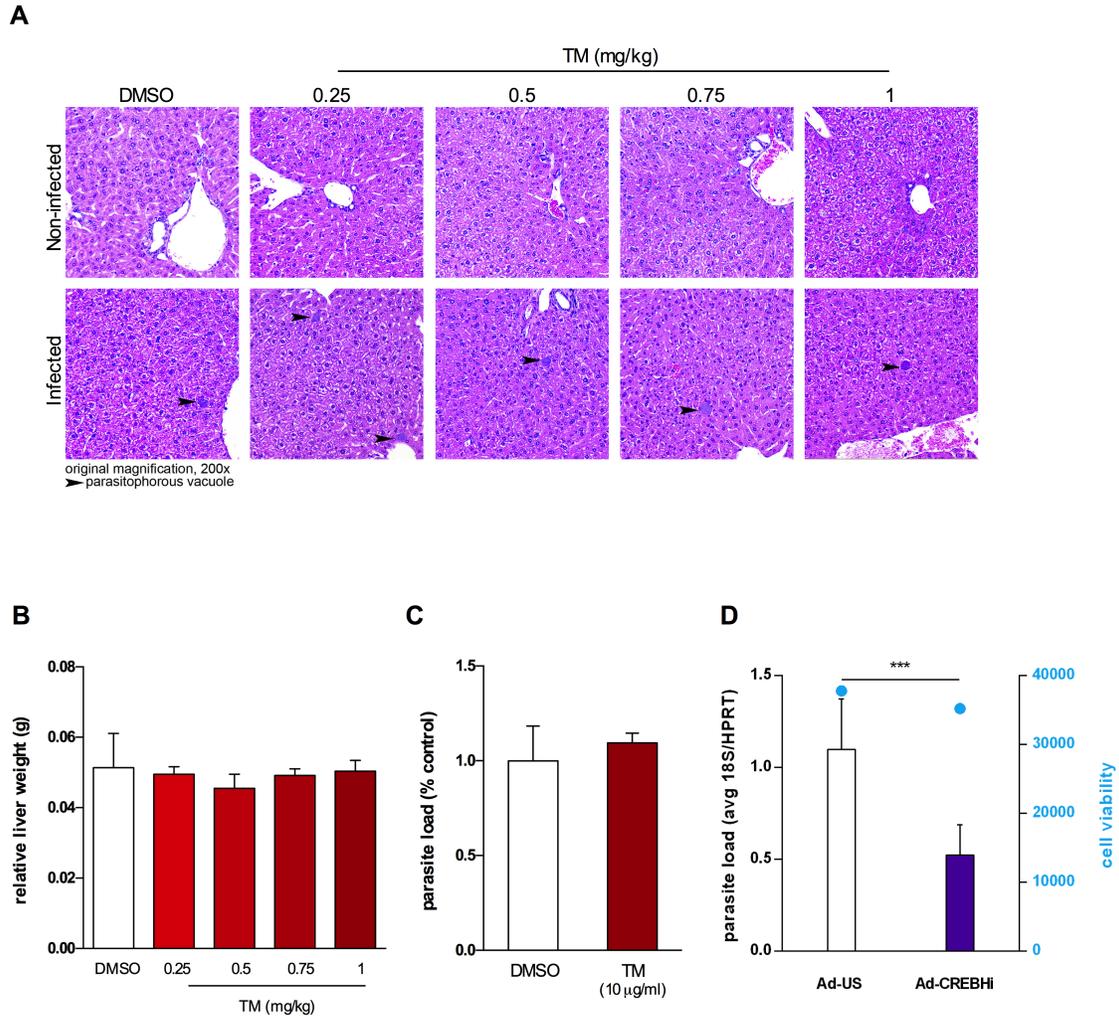
B

C

D

Figure S1

A. Liver sections of infected and non-infected mice, injected with different doses of DMSO and TM. No important histomorphological changes were seen in liver, in all of the conditions tested; discrete foci of hepatocellular necrosis was seen exclusively in liver of infected mice injected with both test compound and vehicle. Liver samples from all mice (n=5/group) were analysed and scored but only representative histological slices are presented. H&E staining; original magnification*: 200x.*

B. Liver weight normalized to body weight in DMSO and TM treated mice. Results are expressed as means ± s.d. (n=5 mice per group, 2 independent experiments).

C. Sporozoites infectivity is not affected by pre-treatment with TM. Quantification of infectivity of *P. berghei* sporozoites pre-incubated for 30 minutes with $10\mu g$ / ml of DMSO or TM on hepatoma cells. Infection determined by luminescence at 24 h after sporozoite invasion. Results are expressed as means ± s.d. (n=2 independent experiments).

D. Determination of cell viability upon adenoviral transduction. *P. berghei* infection quantified on mouse primary hepatocytes infected with adenovirus-expressing short hairpin RNA for *Crebh* (Ad-CREBH RNAi) and control adenovirus (Ad-US) by qRT-PCR for parasite 18S ribosomal RNA at 44 h after sporozoite delivery, normalized with hypoxanthineguanine phosphoribosyltransferase (*Hprt*) expression. Cell viability measured by Alamar Blue assay (fluorescence intensity, blue dots). Results are expressed as means ± s.d, *** p <0,001, t test. (n=3).

Table S1

Real-time primers used for mRNA quantification.

Target	Forward Sequence	Reverse Sequence
P. berghei r*18S*	AAGCATTAAATAAAGCGAATACATCCTTAC	GGAGATTGGTTTTGACGTTTATGTG
mouse *Hprt*	TTTGCTGACCTGCTGGATTAC	CAAGACATTCTTTCCAGTTAAAGTTG
mouse *Gapdh*	AACTTTGGCATTGTGGAAGC	ACACATTGGGGGTAGGAACA
mouse *Atf4*	CGGCCTAAGCCATGGCGCTC	AGCCCCCAAACCCGACTGGT
mouse *Chop*	GGTGGCAGCGACAGAGCCAG	GCACGTGGACCAGGTTCTGCTT
mouse *Atf3*	CCAGGTCTCTGCCTCAGAAG	TCCAGGGGTCTGTTGTTGAC
mouse *Herpud1*	TGGACCTATTCCGCAGCGACG	CCACCCGACGTGGTGCAGGT
mouse *Xbp1s*	GAGTCCGCAGCAGGTG	GTGTCAGAGTCCATGGGA
mouse *Dnajb9*	CGTCGGGGCGCACAGGTTATT	TCGCTCTGAGGCAGACTTTGGC
mouse *Crebh*	AGCGGGGACTTGCAACAGCA	GAGAGGCAGCTGGGTGGGGA

Supplementary References

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4. Cox J, Mann M (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol* **26:** 1367-1372

5. Robinson MD, Oshlack A (2010) A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol **11**: R25

6. Smyth GK (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol **3**: Article3