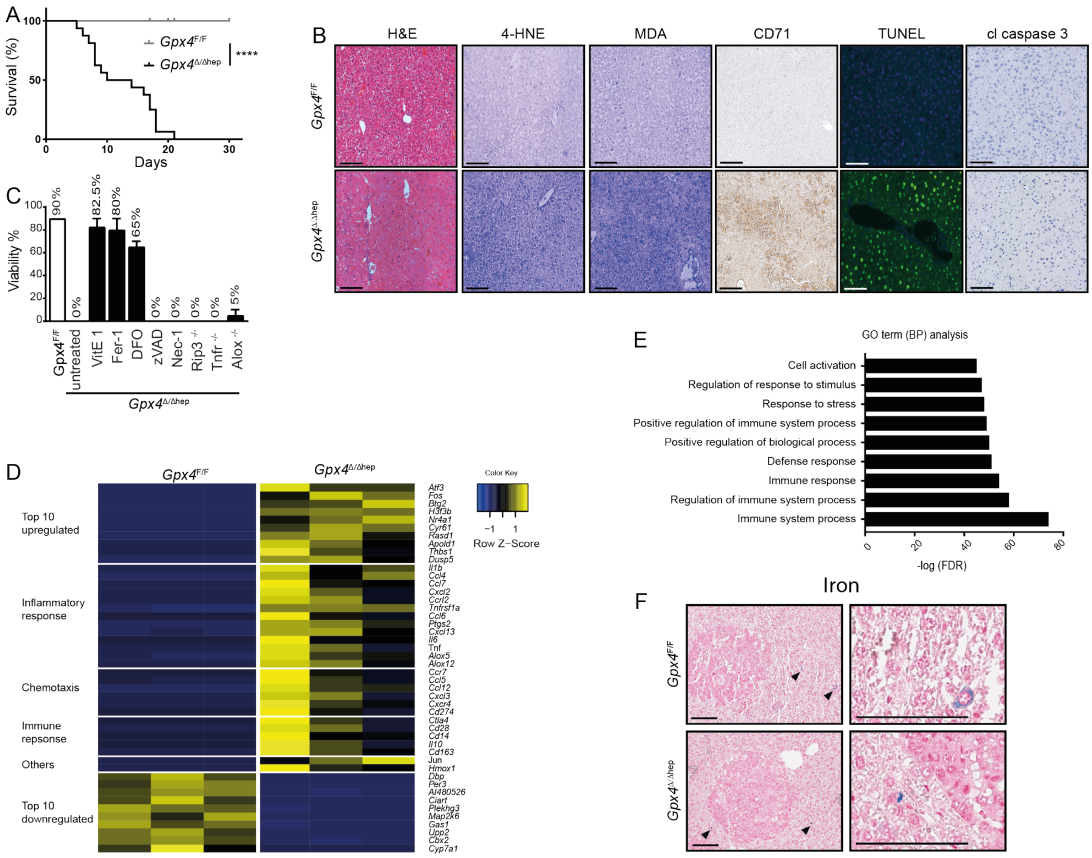


Supplemental figure 1: *Gpx4* deletion in hepatocytes causes ferroptosis and inflammation.

A, Survival of *Gpx4*$^{F/F}$ and *Gpx4*$^{\Delta/\Delta hep}$ mice placed on a vitamin E-depleted diet (n=9) ****p≤0,0001 by log-rank (Mantel-Cox) test.

B, Representative H&E, 4-HNE, MDA, TUNEL and cleaved caspase 3 staining of livers from *Gpx4*$^{F/F}$ and *Gpx4*$^{\Delta/\Delta hep}$ mice fed with vitamin E-depleted diet.

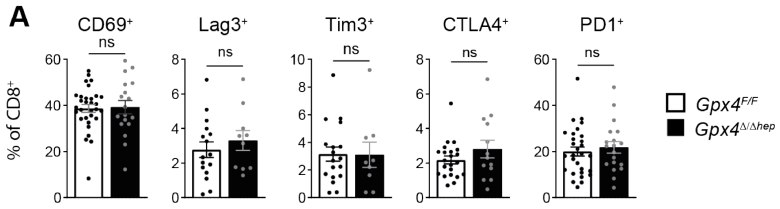
C, Viability of primary hepatocytes from *Gpx4*$^{F/F}$, *Gpx4*$^{\Delta/\Delta hep}$, *Gpx4*$^{\Delta/\Delta hep}$/*Rip3*$^{-/-}$, *Gpx4*$^{\Delta/\Delta hep}$/*Tnfr1*$^{-/-}$ and *Gpx4*$^{\Delta/\Delta hep}$/*Alox*$^{-/-}$ mice cultured for 24 hours *ex vivo* left either untreated or in the presence of vitamin E (VitE), ferrostatin-1 (Fer-1), deferoxamine (DFO), zVAD or necrostatin-1 (Nec-1), Data are ± SD (n=2).

D, Heatmap of the most differentially expressed genes in livers from *Gpx4*$^{F/F}$ and *Gpx4*$^{\Delta/\Delta hep}$ mice fed with vitamin E-depleted diet (n=3).

E, GOterm biological process analysis of *Gpx4*$^{F/F}$ and *Gpx4*$^{\Delta/\Delta hep}$ mice fed with vitamin E-depleted diet (n=3).

F, Representative images with zoomed in of iron staining of the liver of HCC bearing *Gpx4*$^{F/F}$ and *Gpx4*$^{\Delta/\Delta hep}$ mice 25 days after HTVI. Scale bars = 100µm.

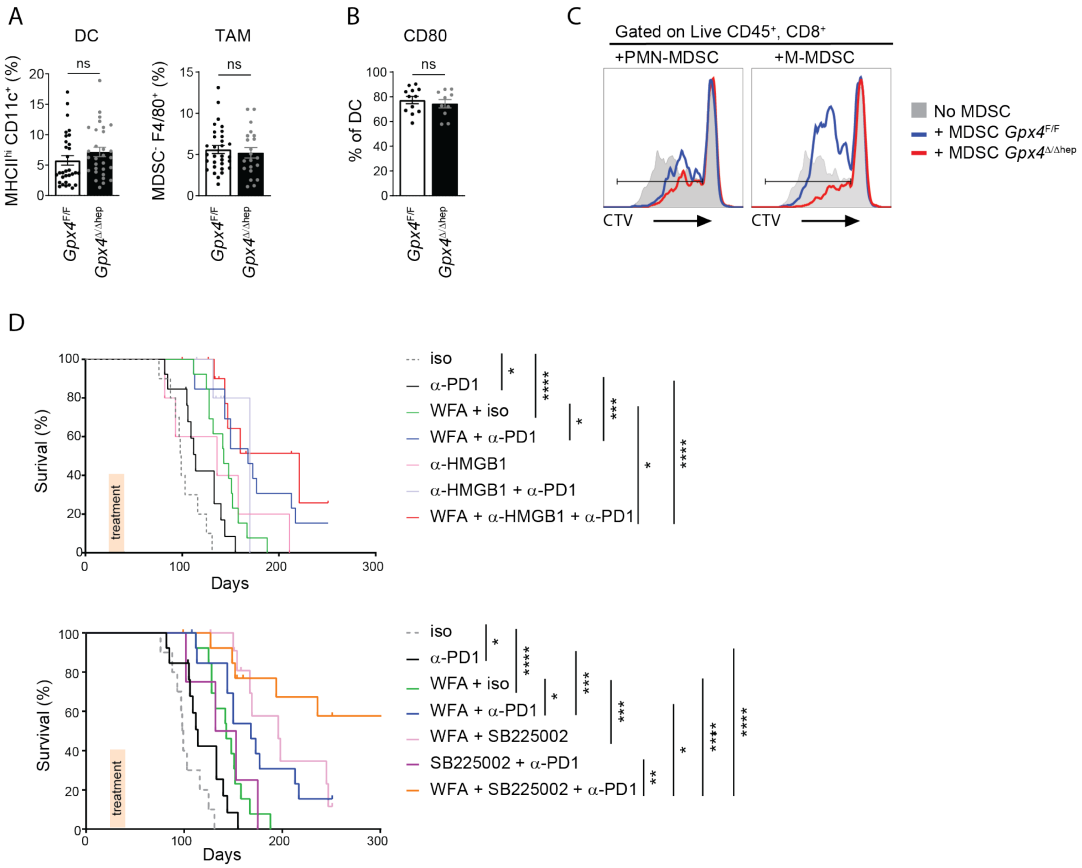
Conche C, *et al. Gut* 2023;0:1–9. doi: 10.1136/gutjnl-2022-327909



Supplemental figure 2

A, Flow cytometry analysis of the indicated surface markers on CD8 T cells from liver infiltrates of *Gpx4*$^{F/F}$ and *Gpx4*$^{\Delta/\Delta hep}$ of tumor bearing mice (n≥7). Data are mean ±SEM, n.s not significant by t test.

Conche C, *et al. Gut* 2023;0:1–9. doi: 10.1136/gutjnl-2022-327909



Supplemental figure 3

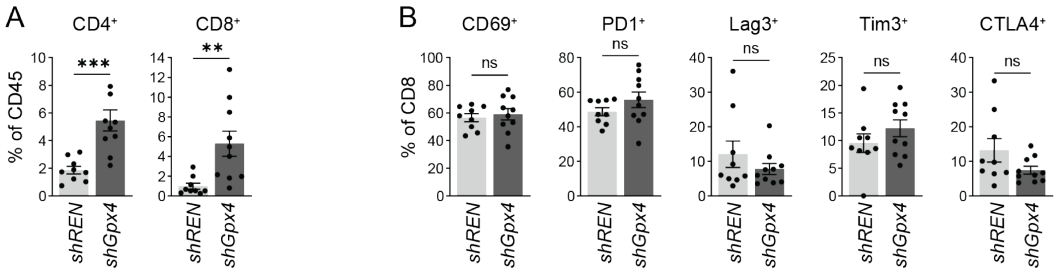
A, Quantification by flow cytometry analysis of DC and TAM liver infiltrates of *Gpx4*$^{F/F}$ and *Gpx4*$^{\Delta/\Delta hep}$ tumor bearing mice (n≥21). Data are mean ±SEM, n.s, not significant, **p≤0,01, by t test.

B, Quantification by flow cytometry analysis of activation marker CD80 expression on DC of *Gpx4*$^{F/F}$ and *Gpx4*$^{\Delta/\Delta hep}$ tumor bearing mice (n≥10).

C, Representative Celltrace violet dilution profile of WT CD8 T cell proliferation without MDSC (solid grey) or incubated with tumor infiltrating MDSC from *Gpx4*$^{F/F}$ (Blue) and *Gpx4*$^{\Delta/\Delta hep}$ (red) mice.

D, Survival curves presented in figure 3H and separated into two graphs depending on according to MDSC blockade: α-HMGB1 vs SB225002.

A-D, Data are mean ±SEM, n.s, not significant, *p≤0,5, **p≤0,01, ***p≤0,001 and ****p≤0,0001by t test (**A-B**) or by log-rank (Mantel-Cox) test (**D**).



Supplemental figure 4

A, Quantification of the percentages of CD4 and CD8 T cells in immune infiltrates of *shREN* and *shGpx4* s.c. APTKA tumors analyzed by flow cytometry (n≥9).

B, Percentages of cells expressing the indicated surface markers on CD8 T cells in immune infiltrates of *shREN* and *shGpx4* s.c. APTKA tumors analyzed by flow cytometry (n≥9).

Methods

Mice

Gpx4$^{F/F}$ [1], *Tnfr1*$^{-/-}$(Jackson Laboratory, JAX:003242), *Rip3*$^{-/-}$[2], Alox12/15$^{-/-}$[3] and *Rage*$^{-/-}$ [4] mice were crossed to *Alb-Cre* mice (JAX:003574; Jackson Laboratory). FvB mice from Charles River and C57BL/6J mice Janvier were used. Mice were kept in a temperature-controlled room with 12 h light and 12 h dark diurnal cycle. They were housed in filter-topped cages and were standard laboratory chow and water ad libitum. All animal procedures were performed in accordance with institutional guidelines. All animal experiments were reviewed and approved by the Regierungspräsidium Darmstadt, Germany. No statistical method was used to predetermine sample size. Livers were photographed and sampled. Counting of tumors was done in a blinded fashion. Vitamin E–deficient diet was purchased from Ssniff (E15791-147).

Isolation of primary murine hepatocytes

Mice were anaesthetized with ketamine/rompune (100 mg/200 mg per kg body weight). Afterwards a cannula was inserted in the vena cava inferior for perfusion of the liver. The isolation procedure was adapted from [5]. Briefly, perfusion was performed with 37°C warm HBSS without Ca2+ and Mg2+ (supplemented with 15 mM HEPES, 2.5 mM EDTA, 1 g/l glucose, 100 U/ml penicillin and 100 µg/ml streptomycin, 1% non-essential amino acids) using a roller pump (10 ml/min) for 10 min. Thereafter, the liver was perfused with HBSS with Ca2+ and Mg2+ (supplemented with 15 mM HEPES, 5 mM CaCl2, 0.13 mg/ml collagenase IV (Sigma-Aldrich, C5138, Darmstadt, Germany), 100 U/ml penicillin, and 100 µg/ml streptomycin for additional 10 min. The liver was carefully removed from the abdominal cavity, placed in a Petri dish on ice in Williams E medium (supplemented with

10% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin) and opened with forceps. Liver cells were resuspended and put over a 100 µm cell strainer. Cells were washed and seeded in Williams Medium E (supplemented with 10% FCS, 2 mM L-Alanyl-L-Glutamin (Biochrom), 100 U/ml penicillin, and 100 µg/ml streptomycin) on collagen coated plates (Roche collagen rat tail).

For HMGB1 and CXL10 release, hepatocyte supernatants were collected after 4h.

For inhibitor treatment in Figure S1A adherent hepatocytes were washed after 4 h with PBS and fresh Williams Medium E (supplemented with 10% FCS, 2 mM L-Alanyl-L-Glutamin (Biochrom), 100 U/ml penicillin, and 100 µg/ml streptomycin). Vitamin E (T3251; Sigma), Necrostatin-1 (480065; Calbiochem), Ferrostatin-1(341494; Calbiochem), Deferroxamin (deferoxamine mesylate salt) (D9533; Sigma), Z-VAD (OMe)-FMK (Caspase Inhibitor-1) (627610; Calbiochem) were dissolved in DMSO, EtoH or PBS according to manufacturer suggestions and used in the described concentrations. Then inhibitors were dissolved in medium and changed after 4 hours and every 24 hours. For quantification of cell death, cells were trypsinized and stained with trypan blue followed by counting with a haemocytometer using standard protocol. Cells stained blue were considered as dead cells.

HCC cell lines.

HCC cell lines were generated as previously described [6]. Briefly, HTVI with $Nras^{G12V}$ and myristoylated-AKT (see Liver Tumor mouse model with Hydrodynamic tail vein injections) oncogenes in *p19*$^{-/-}$ mice given that *p19* deletion is required for HCC tumor cells to growth in vitro. Briefly, HCC nodules were digested and put in culture on agarose coated plates. Then cells were cultured in Advanced DMEM supplemented with 10% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin, 2mM Glutamax.

Organoid cell culture

APTK and APTKA organoids were established as previously described [7]. Organoids were expanded by manual disruption and seeded in Matrigel BME or Ultra-Matrix (Corning) with advanced DMEM/F12 supplemented with N2, B27, L-NAC and appropriate antibiotics. Gpx4 shRNA was cloned in RT3REN vector as described previously [8]. Rtta3-hygro retroviral vector was a kind gift from Lars Zender and RT3REN (Addgene plasmid # 111166) a kind gift from Johannes Zuber. TGCTGTTGACAGTGAGCGCAGGAATTATAATGCTTATCTATAGTGAAGCCACAGAT GTATAGATAAGCATTATAATTCCTATGCCTACTGCCTCGGA sequence was used for REN shRNA while TGCTGTTGACAGTGAGCGAAGGAAGTAATCAAGAAATCAATAGTGAAGCCACAGA TGTATTGATTTCTTGATTACTTCCTGTGCCTACTGCCTCGGA was used for shRNA targeting *Gpx4*. For transplantation organoids were mechanically disturbed and incubated for 5 to 10 min with accutase to generate single cell suspension.

Tumor mouse models

Liver Tumor mouse model with Hydrodynamic tail vein injections. For transposon-mediated intrahepatic gene transfer, mice received a 5:1 molar ratio of transposon- to transposase encoding vectors (30 μg total DNA). One transposon vector contains transposable elements encoding oncogenic $Nras^{G12V}$ and myristoylated-AKT with CAGGS promoter (Caggs), inverted repeats and direct repeats (IR/DR) and internal ribosome entry site (IRES) while the other vector encodes fror sleeping beauty (SB) transposase as depicted in (Figure 4A). DNA for hydrodynamic tail vein injection was prepared using Qiagen EndoFreeMaxi Kit

(Qiagen, Hilden, Germany) and dissolved in 0.9% NaCl solution to a final volume of 10% of animal's body weight. Animals (5-7 weeks old) were injected within 10 s with 25 μg transposon plasmids and 5 μg transposase. Transposon-based vector pCAGGs-IRES was used. For the survival study, human endpoint was reached when mice were lethargic or with reduced activity for 3 consecutive days without improvement. Some mice developed lesion on the ear, paws and mostly on the tails as described previously [9]. Following our animal experimentation protocol, mice with lesion at eminent risk of rupture or bleeding were euthanized. Any mouse that was sacrificed due to those lesions or for any health reason unrelated to liver tumor was censured at the time of sacrifice.

Subcutaneous tumor transplantation

Organoids or HCC cells were injected in 10-20% Matrigel into the right flank. Once all tumors reached a measurable size of 3-4mm, approximately 7 days after transplantation for organoids and 9 days for HCC cells treatment of the mice was initiated. For organoids encoding for shRNA, first shRNA expression was induced in vitro for several passages by adding 0,5μg/ml of doxycycline to the medium. Then, one day after s.c. transplantation, the drinking water was supplemented with 0,5μg/ml doxycycline and 3% sucrose. Tumor volumes were calculated with ½(width^2 x length).

For Liver metastasis model with intrasplenic injection [10], 50 000 single cell organoids or 15.000 HCC cells were injected into the spleen and treatment was initiated 5 days after transplantation. Due to the time required for the surgery, a limited number of mice could be injected. Therefore, only 2 treatment groups could be assessed by cohort. Mice that haven't developed any liver metastasis were considered as miss-injected and therefore excluded from analysis.

In vivo treatments

For treatment therapies, treatments were given for 16 days and initiated at different time point according to the tumor models. 250μg of α-PD-1 (CD279) monoclonal antibody (clone RMP1-14, BioXCell) or IgG2a isotype control was administered i.p. every 3 days for six injections. 50μg of α-HMBG1, obtained from Dr. Huan Yang, was injected i.p every other day for the tumor model and daily for the vitamin E depleted diet. Withaferin A (Sigma-Aldrich) and SB225002 (SelleckChem) were injected i.p. daily at 2,5mg/kg and 1mg/kg respectively. WFA, SB225002, αPD1 and αHMGB1 treatments were administrated at days 25 after HTVI, day 5 after intra-splenic injections, when s.c tumors were palpable, e.g. 7 days and when the orthotopic tumors reach ~2/3 of the colon lumen. For depletion of CD8 cells, mice were treated after HTVI weekly with 150μg of depleting α-CD8 or Rat IgG2b, κ antibodies for 3 consecutive days.

Histology and immunochemistry

For histological analysis tissue was fixed in 4% PFA or Formaline and paraffin embedded. After deparaffinization and rehydration of the tissue haematoxylin and eosin (H&E) staining was done according to standard protocols. Tumor number, size and tumor-to-liver ratio quantification was performed manually on H&E stained liver sections scanned on a Aperio CS2 (Leica). To determine cell proliferation, mice were injected i.p. with 75 mg/kg BrdU (Sigma-Aldrich) 90 min before sacrifice. For MDA (ab6463; Abcam), 8-OHdG (ab10802; Abcam) and 4-HNE (ab46545; Abcam) staining, paraffin sections were incubated in proteinase K for up to 40 minutes at 37C° for antigen retrieval. Alkaline phosphatase reaction was used as a color reaction with nuclear fast red (Sigma) as a nuclear counter stain. For Nras staining (sc-31; Santa Cruz) paraffin sections were incubated in proteinase K for up to 40

minutes at 37C° for antigen retrival and vector mouse on mouse Kit tumor (MOM PK-2200; Vector) used for staining. For CD71 (H68.4; Invitrogen) immunohistochemistry, slides were subjected to heat-induced antigen retrieval in Tris-EDTA (pH:9.0) solution for 15 min. Slides were blocked and treated with Transferrin Receptor primary antibody (H68.4; Invitrogen) using MOM kit according to the manufacturer`s instructions. Detection was performed with the DAB substrate kit (SK-4100; Vector). For α-F4/80 (ab6640; Abcam), α-GPx4 (ab125066; Abcam), α-BrdU (MCA2060; AbD Serotec), α-cleaved caspase 3 (9661S; Cell Signaling Technology), α-CD3 (IS503: DAKO), α-CD8 (98941; cell signaling), α-GR-1 (Ly6G) (11-5931-82; eBioscience), α-p-H2AX (9718; Cell Signaling Technologies) and Prussian Blue iron stain (Merck; 10498401000, paraffin sections were stained using a Leica Bond Max following standards immunochemistry staining protocols. 4-HNE, MDA, p-H2AXa, CD71 and 8-OHdG stained sections were scanned using Aperio CS2 (Leica). Quantification of 4-HNE, MDA, and CD71 was done using a membrane algorithm whereas quantification of 8-OHdG and p-H2AX was done using a nuclear staining algorithm (Aperio eSlide manager software) on manually marked tumors. For CD3, Gr-1, F4-80 and cleaved caspase 3, non-overlapping images within the tumor area were acquired with 20x objective and positive cells were counted manually. Histology allowed for the individual analysis of the multiple tumors generated by HTVI (see Figure 1 C) therefore n represented the number of tumors analyzed from at least 3 mice per group (Figure 1D, 2B, 3A, 3G)

Immunofluorescence

Terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling (TUNEL) assay was performed with the ApoAlert DNA Fragmentation Assay Kit (Clontech, 630107). Briefly, tissues were treated with 0,9% NaCl for 5 min, then with with 4% PFA for 5min

followed by an incubation for 30min with proteinase K for 5min. After being blocked for 10-15 min, sections were incubated with terminal deoxynucleotidyl transferase enzyme mix for 1h and then mounted in medium containing 4,6 diamidino-2-phenylindole. For CD8 and PD-L1 staining, Section were stained overnight with αPD-L1 (13684; Cell Signaling Technologies) or α-CD8 (361003; Synaptic Systems) after antigen retrieval with EDTA or Citrate Buffer respectively for 10min and followed by a AF594-coupled secondary antibody. Image acquisition was performed using a Zeiss Axio Imager M2 with a 20×/0.5 EC Plan Neofluar or a 40×/0.95 korr Apochromat objective. The images were captured on a Zeiss microscope and positive cells were quantified manually. Immunofluorescence allowed for the individual analysis of the multiple tumors generated by HDTVI (see Figure 1 C) therefore n represented the number of tumors analyzed from at least 3 mice per group (Figure 1D, 2B, 2D, 2F, 2G).

RNA sequencing

RNA sequencing was done with the help of the "Transcriptome and Genome Analysis Laboratory (TAL), University of Göttingen, Germany. RNA was extracted via Trizol extraction protocol. Quality control was done by Agilent Bioanalyzer 2100. As starting material for the library preparation, 0.5µg of total RNA was used. The libraries were generated according to the TruSeq mRNA Sample Preparation Kits v2 Kit from Illumina (Cat. N°RS−122-2002). The fluorometric based QuantiFluor™ dsDNA System from Promega (Mannheim, Germany) was used for accurate quantitation of cDNA libraries. The size of final cDNA libraries was determined by using the Fragment Analyzer from Advanced Bioanalytical. cDNA libraries were amplified and sequenced by using the cBot and

HiSeq4000 from Illumina (SR; 1×50bp; ca. 30 Mio reads per sample). Sequence images were transformed to *bcl* files using Illumina software BaseCaller, which were demultiplexed to *fastq* files with *bcl2fastq* and quality checks were done via *fastqc*. Sequences were aligned to the genome reference (mm9) using the STAR alignment software (version 2.3.0e) allowing for 2 mismatches within 50 bases. Subsequently, filtering of unique hits and counting were conducted with SAMtools (version 0.1.18) and HTSeq (version 0.6.1p1). Read counts were analyzed in the R/Bioconductor environment (version 3.2, www.bioconductor.org) using the DESeq2 package (version 1.8.1). Candidate genes were filtered to a minimum of 2-fold change and FDR-corrected p-value < 0.05. Gene ontology enrichment analysis: the gene ontology analysis was carried out by using DAVID/EASE 6.7 [11].

Quantitative PCR

RNA isolation was performed using RNeasy Mini Kit (Qiagen) according to the manufacturers' instructions. For the RNA isolation from tumors the tumor pieces were shock frozen after harvest. The concentration and purity of RNA was determined and 1 µg RNA was used for cDNA synthesis using Superscript II reverse transcriptase kit (Invitrogen). First, 1 µg RNA, 2.5 µM OligodT and 0.5mM dNTP-mix were incubated at 65°C for 5 minutes for denaturation. The mixture was cooled down on ice for 2 minutes and mixed with 1X reaction buffer, 5 mM DTT, 0.5 U RNaseOUT (Invitrogen) and 10 U Superscript II reverse transcriptase and incubated 60 minutes at 50°C. For quantitative PCR analysis a reaction was performed with SYBR-Green MasterMix (Roche) on a StepOnePlus Real Time PCR system (Applied Biosystems). Expression levels were normalized based on the levels of the housekeeping gene cyclophilin. In the following table are the primer used for the real time PCR.

Gene	**forward-primer**	**reverse-primer**
Ccl2	GAA GGA ATG GGT CCA GAC AT	ACG GGT CAA CTT CAC ATT CA
Ccl4	GCC CTC TCT CTC CTC TTG CT	GTC TGC CTC TTT TGG TCA GG
Ccl5	TGC CCA CGT CAA GGA GTA TTT	TTC TCT GGG TTG GCA CAC A
Cxcl1	ACT GCA CCC AAA CCG AAG TC	TGG GGA CAC CTT TTA GCA TCT T
Cxcl2	CTC TCA AGG GCG GTC AAA AAG TT	TCA GAC AGC GAG GCA CAT CAG GTA
Cxcl10	GAC GGT CCG CTG CAA CTG	CTT CCC TAT GGC CCT CAT TCT
Cxcl13	CAT CAT GAG GTG GTG CAA AG	GGG TCA CAG TGC AAA GGA AT
Il1b	GTG GCT GTG GAG AAG CTG TG	GAA GGT CCA CGG GAA AGA CAC
Il6	ATG GTA CTC CAG AAG ACC AGA GGA	GTA TGA ACA ACG ATG ATG CAC TTG
Tnfa	GCC TAT GTC TCA GCC TCT TCT	AAC TGA TGA GAG GGA GGC CAT T

Flow cytometry

For flow cytometry analysis of the primary and subcutenous HCC immune infiltrates, livers were collected minced and digested twice with 1mg/ml of Collagenase IV (Sigma) for a total of 1h. For analysis of immune cells in organoids tumors, digestion was performed with BD Tissue Dissociation Reagent (661563) for 30min at 37C. The remaining organ pieces were

mashed and cell suspensions were filtered through a 70µm cell strainer. Digestion was stopped by addition of EDTA. Immune cells were enriched thanks to a 30%/40%/75% Percoll gradient with a 1700rpm 17min centrifugation. Tumor immune filtrates were then washed and stained for 15 to 20 min on ice, after a pre-stain of 5 min with α-CD16/CD32 (14-0161-82; eBioscience), with Fixable Viability Dye-eF780 (65-0865-18; eBioscience), α-CD45-FITC (11-0451-85; eBioscience), α-MHCII-PercP-Cy5.5 (107626, Biolegend), α-CD80-APC (104714; Biolegend), α-CD11b-BV650 (56340; BD), α-CD11c-BV785 (563735; BD), α-Ly6G-BV605 (563005; BD), α-Ly6C-AF700 (128024; Biolegend), α-F4/80-PE (12-4801-82, eBioscience), or with Fixable Viability Dye-eF780 (65-0865-18; ebioscience), α-CD45-BV786 (564225; BD), α-CD4-BuV496 (564667; BD) or α-CD4-AF700 (100429; Biolegend), α-CD8-BV605 (100744; Biolegend), α-CD69-FITC (557392; BD), α-PD1-APC (17-9985-82; eBioscience), α-TIM-3-PECy7 (119715; Biolegend), α-CTLA4-PerCP-Cy5.5 (106315; Biolegend) and α-Lag3-BV421 (125221, Biolegend).

For cytokines expression, immune cells were stimulated *ex vivo* with 20ng/ml PMA and 1µg/ml Ionomycin for 3h in RPMI, 10% FBS, 1x Non-Essential Amino Acid, 1mM Sodium Pyruvate, 10mM Hepes, 50µM β-Mercaptoethanol, 100 U/ml penicillin, and 100 µg/ml streptomycin and 2mM Glutamax containing Brefeldin A (Biolegend) then stain as previously described with α-CD16/CD32 (14-0161-82; eBioscience)Fixable Viability Dye-eF780 (65-0865-18; eBioscience), α-CD8 PeCy7 (Biolegend; 100722) and α-CD4-eFluor450 (65-0865-18; eBioscience, 48-0042-82). After 20min fixation in fixation/permeabilization solution (Cytofix/Cytoperm BD) and cells were stained overnight with α-IFNγ-PerCP-Cy5.5 (45-7311-82; eBioscience) in Perm/Wash buffer (BD). Samples were then fixed with 1% PFA and acquired on a Fortessa (BD) or Aurora (Cytek) and analyzed with FlowJo. For best

visualization of positive and negative fractions all gates were performed on dot plots rather than histograms. Cell doublets were excluded through FSC-A and SCC-A vs H dot plots and then debris were excluded by size exclusion with FSC-A vs SCC-A dot plots. Then, immune cells were gated as $CD45^+$, $Dead^-$. All the subsequent cells described below are therefore $Doublet^-$ (in FSC & SSC), $Debris^-$, $Dead^-$, $CD45^+$. Exhaustion, activation markers and cytokines were quantified as percentage of positive cells on gated $CD8^+$ cells. DCs were $CD11c^+$ $MHCII^{hi}$. The identification of MSDC by surface markers is still ill define [12]. A clear consensus was found on the expression of CD11b, Ly6C and Ly6G for the MDSCs while TAM were defined as $F4/80^{hi}$ with low to negative expression of Ly6C/G. Expression of Ly6C/G and F4/80 isn't mutually exclusive (data not shown). Our gating strategy was set-up to unsure that the Ly6C/G, F4/80 DP cells weren't counted twice e.g. as MDSC and as TAM. Those cells were considered as MDSC with our flow gating strategy. First cells were gated as $CD11b^+$ $CD11c^-$ and then M-MDSC were gated as $Ly6C^{hi}$, $Ly6G^{lo}$ and PMN-MDSC as $Ly6C^{int}$, $Ly6G^{hi}$. Then the M-MDSC and PMN-MDSC negative fraction of $CD11b^+$ $CD11c^-$ cells were gated for $F4/80^+$ to identified TAM.

Immunosuppressive assay.

Immune infiltrates from liver of tumor bearing mice were isolated as described in the flow cytometry method, stained with Fixable Viability Dye-eF780 (65-0865-18; ebioscience), α-CD45 FITC (11-0451-85; eBioscience), α-Ly6G-PeCy7 (25-5931-81; eBioscience) and α-Ly6C-AF700 (128024; Biolegend) and sorted by flow cytometry as $CD45^+$ $Ly6G^{hi}$ $Ly6C^{lo}$ for PMN-MDSCs and as $CD45^+$ $Ly6G^{lo}$, $Ly6C^{hi}$ for M-MDSCs. T cell were isolated from spleen of unchallenged WT mice by negative magnetic selection with MoJoSort kit (Biolegend; 480024) according to manufacturer's instructions with magnetic columns (Miltenyi) and then

stained with 5μM CelltraceViolet (Thermofischer; C34571) for 10min. T cells +/- MDSCs were stimulated for 3 days with 1ug/ml of plate-bound α-CD3 and α-CD28 at a ratio of 4/1. Then cells were stained for CD4 and CD8. CD8 T cell were then gate as described above as $Doublet^-$ (in FSC & SSC), $Debris^-$, $Dead^-$, CTV^- $CD8^+$. Proliferative, e.g., divided CD8 T cells were determined according to Celltrace $violet^{lo}$ staining intensity as shown in supplemental figure 3B. The immunosuppressive effect of the MDSC was quantified as the ratio of the percentages of proliferative T cells incubated with MDSC to of the percentages of proliferative T cells without any MDSC.

ELISA

The HMGB1 (IBL International) and Cxcl10 (R&D) ELISAs were performed according to the manufacturers' instructions using supernatants of cultured primary hepatocytes for 4 hours. The color reactions were detected spectrophotometrically using a plate reader at 450 nm with a correction at 540nm.

Statistical analysis

Statistical parameters including the minimal value of n per experiment, dispersion and precision measures (mean ± SEM) and statistical significance and the tests used are reported in the figures and figure legends. GraphPad PRISM software was used for statistical analysis. Samples with obvious technical or biological issue as well as prism-identified outliers were excluded from the analysis. Data were judged to be statistically significant based on t-test when comparing two samples, based on one-way ANOVA when comparing multiple samples with Dunnett's multiple or Šídák's multiple comparison test of the relevant groups which are indicated on the graphs. Unpaired tests were used unless the data were generated from

multiple independent experiments with known experimental bias, where a paired test was performed as for figures 2H, 3B, 3C, supplemental 2A and 3A. Log-rank (Mantel-Cox) test was performed for statistical analysis of the survivals. The p values were represented in the figures and indicated in the legends as n.s., not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplemental References

1 Seiler A, Schneider M, Förster H, Roth S, Wirth EK, Culmsee C*, et al.* Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. Cell Metab 2008;**8**:237-48.
2 Newton K, Sun X, Dixit VM. Kinase RIP3 is dispensable for normal NF-kappa Bs, signaling by the B-cell and T-cell receptors, tumor necrosis factor receptor 1, and Toll-like receptors 2 and 4. Mol Cell Biol 2004;**24**:1464-9.
3 Sun D, Funk CD. Disruption of 12/15-lipoxygenase expression in peritoneal macrophages. Enhanced utilization of the 5-lipoxygenase pathway and diminished oxidation of low density lipoprotein. J Biol Chem 1996;**271**:24055-62.
4 Liliensiek B, Weigand MA, Bierhaus A, Nicklas W, Kasper M, Hofer S*, et al.* Receptor for advanced glycation end products (RAGE) regulates sepsis but not the adaptive immune response. J Clin Invest 2004;**113**:1641-50.
5 Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S*, et al.* Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. Arch Toxicol 2013;**87**:1315-530.
6 Seehawer M, Heinzmann F, D'Artista L, Harbig J, Roux PF, Hoenicke L*, et al.* Author Correction: Necroptosis microenvironment directs lineage commitment in liver cancer. Nature 2018;**564**:E9.
7 Varga J, Nicolas A, Petrocelli V, Pesic M, Mahmoud A, Michels BE*, et al.* AKT-dependent NOTCH3 activation drives tumor progression in a model of mesenchymal colorectal cancer. J Exp Med 2020;**217**.
8 Fellmann C, Hoffmann T, Sridhar V, Hopfgartner B, Muhar M, Roth M*, et al.* An optimized microRNA backbone for effective single-copy RNAi. Cell Rep 2013;**5**:1704-13.
9 Carlson CM, Frandsen JL, Kirchhof N, McIvor RS, Largaespada DA. Somatic integration of an oncogene-harboring Sleeping Beauty transposon models liver tumor development in the mouse. Proc Natl Acad Sci U S A 2005;**102**:17059-64.
10 Soares KC, Foley K, Olino K, Leubner A, Mayo SC, Jain A*, et al.* A preclinical murine model of hepatic metastases. J Vis Exp 2014:51677.
11 Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;**4**:44-57.

12 Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF*, et al.* Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun 2016;**7**:12150.