S100A11/ANXA2 belongs to a tumour suppressor/oncogene network deregulated early with steatosis and involved in inflammation and hepatocellular carcinoma development

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SUPPLEMENTARY MATERIALS AND METHODS

Mouse strains, diethylnitrosamine (DEN) treatments, diets, animals care

Mouse strains – Liver-specific PTEN knockout mice (C57BL/6J, *AlbCre/Pten*$^{flox/flox}$, LPTENKO) and *Pten*$^{flox/flox}$ (CTL, littermates of LPTENKO) were previously described[1]. Liver-specific inducible PTEN knockout mice (AlbCre-ERT2$^{Tg/+}$/Pten$^{flox/flox}$, LIPTENKO) were generated by crossing AlbCre-ERT2$^{Tg/+}$ with *Pten*$^{flox/flox}$ mice. Two-months old LIPTENKO were injected with tamoxifen to induce PTEN deletion in hepatocytes and analyzed either 9 days or 3 months post-deletion. *db/db* and control mice were obtained from Charles River Laboratories (C57BLKS/J). Liver samples from *ob/ob* and control mice (B6. V-*Lep*^{ob}/JRj) were provided by Pr. Jeanrenaud-Rohner (Geneva University).

Diethylnitrosamine treatments – For Diethylnitrosamine (DEN)-induced HCC models, 15 days-old mice (C57BL/6J) were injected intraperitoneally with 25mg/kg of DEN (Sigma-Aldrich). Animals were sacrificed 11 months post-injection. Blood/tissues samples were collected and stored at -80°C. For acute DEN treatment (tumor initiation), 2-months old male mice (C57BL/6J) were injected intraperitoneally with 100mg/kg of DEN and sacrificed 48h after.

Methionine-Choline Deficient Diet – 8 weeks-old C57BL/6J mice were fed with a standard or methionine-choline deficient diet (MCD; ssniff Spezialdiäten GmbH, Soest, Germany) (22 kJ% fat, 14KJ% protein, 64 KJ% carbohydrates), for 2 weeks. At the end of the study, mice were anesthetized by isoflurane and sacrificed by decapitation and blood and organs were collected and weighted.

Adeno-associated virus infection –Two-months old mice were injected retro-orbitally with $2x10^{11}$ genome copies of adeno-associated virus (AAV8) encoding a control shRNA or S100A11 shRNA. Ten days post-transduction, mice were either fed a MCD diet for two weeks, or treated with DEN for two days.

Animal housing – Mice were housed at 23°C in standard cages containing enrichments (*i.e,* disposable house and cocoon), with a light cycle of 07.00am - 07.00pm, free access to water and standard diet (SAFE-150 diet, SAFE, Augy, France) *ad libitum*. All animal experiments were conducted on males, during their light cycle. Animals were euthanized by isoflurane anaesthesia followed by rapid decapitation.

All experimental procedures and data are reported in compliance with the standards of the "*Animal Research Reporting of In Vivo Experiments*" (ARRIVE, https://www.nc3rs.org.uk/arrive-guidelines). All experiments were ethically approved by the Geneva Health head office and were conducted in agreement with the Swiss guidelines for animal experimentation.

Proteomic analysis by LC-MS/MS

Sample preparation – Liver tissues from Control and LPTENKO mice (4-month old, 3 mice per group) were solubilized with a dounce homogenizer in four volumes of PBS-1X and centrifuged at 1'400g at 4°C for 5 minutes. Supernatant was removed and centrifuged at 100'000g for 1hour at 4°C yielding a soluble and membrane proteome (pellet fraction). Proteomes were precipitated by adding four volumes of methanol, 1 volume of chloroform and 3 volumes of water. Proteins were then centrifuged at 20,000g for 5 minutes at 4 °C and the protein layer between the aqueous and organic layers was isolated, dried and solubilized in PBS *via* sonication. 15µg of protein were denatured with 6M urea in 50mM NH_4HCO_3, reduced with 10 mM TCEP for 30 minutes and alkylated with 25mM iodoacetamide for 30 minutes at room temperature in the dark. Samples were then diluted to 2M urea with 50mM NH_4HCO_3 and digested with trypsin for 12 hours at 37 °C (Promega; 1µL of 0.5µg/µL) in the presence of 1 mM $CaCl_2$. Finally, protein samples were acidified to a final concentration of 5% acetic acid and desalted using a self-packed C18 spin column and dried.

LC-MS/MS Analysis - Peptides were resuspended in water with 0.1% formic acid (FA) and analyzed using Proxeon EASY-nLC 1000 nano-UHPLC coupled to QExactive Plus Quadrupole-Orbitrap mass spectrometer (Thermo Scientific). The chromatography column consisted of a 30cm long, 75µm i.d. microcapillary capped by a 5µm tip and packed with ReproSil-Pur 120 C18-AQ 2.4µm beads (Dr. Maisch GmbH). LC solvents were 0.1% FA in water (Buffer A) and 0.1% FA in acetonitrile (Buffer B). Peptides were eluted into the mass spectrometer at a flow rate of 300nL/min. over a 240 minutes linear gradient (3-35% Buffer B) at 65 °C. Data was acquired in data-dependent mode (top-20, NCE 30, R = 17'500) after full MS scan (R = 70'000, m/z 400-1'300). Dynamic exclusion was set to 10 s, peptide match to prefer and isotope exclusion was enabled.

MS Data Analysis - The MaxQuant software (V1.5.2.8) was used to analyze the MS data and searched against the mouse Uniprot database and a list of 247 common laboratory contaminants (provided by MaxQuant sofware). P-values were calculated by student *t*-Tests. Adjusted P-values were calculated by the Benjamini/Hochberg method. P-values<0.05(*) were considered statistically significant. For the first search, a precursor mass tolerance of 20 ppm was used (for nonlinear mass re-calibration) and was set to 7 ppm for the main search with a fragment mass tolerance of 0.02 Da. A false discovery rate of 1% was used for peptide, protein, and site identification and the minimum peptide length was set to 6 and peptide re-quantification function was enabled. The minimal number of peptides per protein was set to two. Oxidized methionines and N-terminal acetylation were searched as variable modifications and

Sobolewski C, *et al. Gut* 2020;0:1–14. doi: 10.1136/gutjnl-2019-319019

carbamidomethylation of cysteines was searched as a fixed modification. Quantification with the MaxQuant software was performed with the label-free quantification algorithm.

Bioinformatics Analysis

Identification of cancer/HCC-related factor in the proteomic analysis. - Identification of cancer-related genes among differentially expressed proteins obtained by proteomic analysis were performed by comparing our proteomic data with cancer-related databases including TSGene database (https://bioinfo.uth.edu/TSGene/), TAG (Tumor Associated Gene) database (http://www.binfo.ncku.edu.tw/TAG/GeneDoc.php), NCG4.0 (http://ncg.kcl.ac.uk) and COSMIC (https://cancer.sanger.ac.uk/cosmic). In addition a keywords-based searching was performed with Uniprot (https://www.uniprot.org; keywords: proto-oncogenes and tumor suppressors) and GeneCards (https://www.genecards.org; keywords: cancer, tumorigenesis, metastasis, oncogene, tumor suppressor, angiogenesis). Identification of HCC-associated genes was performed by merging our proteomic analysis with a list of HCC-related genes obtained with the Metacore™ software. Only downregulated TS (fold change ≤ 0.66), upregulated ONC (fold change ≥1.5) and candidates potentially having either a TS or ONC activity (1.5≤ fold change ≤0.66) were considered and were represented in a heatmap (Log2 fold change between controls and LPTENKO mice) with a hierarchical clustering for the samples based on a Spearmann correlation, using Multiple viewer experiment (MeV) software. Considering that only 3 mice/group were used for the proteomic analysis, most deregulated candidates (ONC>1.55 and TS<0.66, Figure 1C), with a more permissive p-value (p<0.2), were selected for further validation by Western blot and qRT-PCR analyses.

Gene Ontology enrichment analysis. - In order to identify significantly overrepresented GO terms (for biological processes) DAVID 6.7 software (https://david.ncifcrf.gov/) was used to perform functional enrichment analysis of differentially expressed proteins between *Pten*$^{flox/flox}$ (control) and *AlbCre-Pten*$^{flox/flox}$ mice (LPTENKO). Enrichments were calculated by a modified Fisher‟s exact test (EASE score of P-value). GO terms with P-value < 0.05 are considered to be significantly enriched.

S100A11 interactome analysis. - The networks of proteins interacting with S100A11 were determined using the STRING (http://string-db.org) and BIOGRID (https://thebiogrid.org) databases.

Correlation analyses. - Correlation analyses between mRNA levels of S100a11-ANXA2, S100a11-HNF4A and S100a11-ALB (Supplementary Figure 10A and 11B) in human HCC (LIHC) were obtained using the GEPIA database, with a Spearman correlation coefficient (http://gepia.cancer-

pku.cn/detail.php?clicktag=correlation###).

Mutations frequencies. - The mutation frequencies of tumor suppressors and oncogenes in HCC were obtained in the cbiolPortal for Cancer Genomics (http://www.cbioportal.org/) by using three different studies *"HCC, Inserm, Nat Genet 2015"; "HCC, AMC Hepatology 2014"* and *"HCA (adenoma), Cancer Cell 2014*".

Gene Expression Omibus analysis. - The public available database "*Gene Expression Omnibus*" (http://www.ncbi.nlm.nih.gov/gds/?term=hCC) was used to determine the relative mRNA expression of tumor suppressors and oncogenes in rodents/human models of steatosis, NASH, cirrhosis and hepatocellular carcinoma. Only studies analyzed with the GEO2R algorithm were used. P-values (t-test) and adjusted p-values (Benjamini and Hochberg method) were provided by the GEO2R algorithm. For studies with different probes for the same genes, only the most significant probe was considered for the analysis.

Gene Set Enrichment Analyses (GSEA). - GSEA (version 3.0, Broad Institute, Cambridge, MA, US) was used to identify specific gene signature in patients having a high vs low S100A11 mRNA level. Patients were segregated into low and high expressing groups with an 80th percentile threshold. The enrichment score was calculated with the number of members genes ranking at the top or the bottom of the gene list with 1000 permutations (permutation type: phenotype). The Signal2Noise was used for ranking genes. Genes sets having a nominal p-value<0.05 and a FDR<0.2 were considered significant.

Survival analyses. - RNA sequencing data from patients having HCC were obtained from the human protein atlas (https://www.proteinatlas.org/) and the TCGA database (https://cancergenome.nih.gov/). Patients were segregated into low expressing and high expressing groups for selected genes (*i.e*, ANXA2, S100A11) with the best-separation threshold (80th percentile) of the human protein atlas dataset. Survival analyses were performed with a Kaplan-Meier survival curves with GraphPad™.

Exosomes database. - Expression levels of S100A11 in exosomes from healthy or HCC patient were obtained with the ExoRbase™ database (http://www.exorbase.org/). Expression values are represented in transcripts/million (TPM). The patients were arbitrary subdivided in 3 categories based on the TPM values: 0-200; 200-800 and >800.

Transcription factor binding sites. - The list of potential transcription factors binding sites within *S100A11* promoter was obtained from the TF2DNA (http://www.fiserlab.org/tf2dna_db/). Sequences of

Sobolewski C, *et al. Gut* 2020;0:1–14. doi: 10.1136/gutjnl-2019-319019

human HNF4A binding motifs were obtained with the JASPAR database (http://jaspar.genereg.net/).

For all in silico analyses performed on human samples, no ethical approval is required as this study only use public available, anonymized and previously IRB approved studies.

Biochemical analyses of triglycerides content in hepatic tissues.

Triglycerides (TG) content in hepatic tissues were extracted by hexane/isopropanol extraction and measured with a colorimetric enzymatic analysis using a commercial kit (TG Roche/Hitachi, Roche, Switzerland) as previously described [2]. TG content was normalized by the tissue weight.

Cell cultures, transfections, primary hepatocytes isolations.

Cell culture. - Human hepatoma cell lines HepG2 were purchased from ATCC (Manassas, VA); Huh-7 was kindly provided by S. Kirkland (Imperial College, London) and R.D. Beauchamp (Vanderbilt University Medical Center, Nashville, TN). Hep3B and SNU398 cells were kindly provided by Pr. Caroline Gest (Inserm U1053, Bordeaux University, Bordeaux). THLE2 and THLE3 cells were provided by Pr. Britta Skawran (Medizinische Hochschule Hannover, Institut fur Humangenetik, Hannover). Hepa-1-6 and AML12 cells were obtained from the lab of Pr. Manlio Vinciguerra (Institute for Liver and Digestive Health, University College London, UK). HepaRG cells were previously generated by Christiane. Guillouzo, Philippe Gripon and Christian Trepo [3, 4], and kindly provided by Biopredic International. Huh7, HepG2, Hep3B and Hepa1-6 cells were cultured in DMEM medium (glucose 1g/L) supplemented with 10% (v/v) fetal calf serum (FCS), 1% (v/v) antibiotic–antimycotic solution. THLE2 and THLE3 cells were cultured in BEGM media (BEGM Bullet Kit, Lonza, Basel), supplemented with 10% FBS, 70ng/ml phosphoethanolamine (Biochrom, Cambridge, UK) and 5ng/ml EGF (BEGM Bullet Kit, Lonza, Basel). SNU398 were cultured in RPMI glutamax medium supplemented with 10% (v/v) fetal calf serum, 1% (v/v) antibiotic–antimycotic solution. AML12 cells were cultured in DMEM F12 medium supplemented with 5µg/ml of insulin, 40mg/ml of dexamethasone, 10% FCS.

HepaRG cells were cultured in William's medium supplemented with 10% (v/v) FCS, 10^{-9} M insulin and 10^{-6}M hydrocortisone (Sigma). For the differentiation of HepaRG cells, a two-step protocol was performed. First, cells were maintained at confluence for 2 weeks in the growth medium and then the medium was replaced and supplemented with 2% DMSO and for two additional weeks. The medium was replaced every 2-3 days.

-Transfections. - siRNAs transient transfections were performed using the Interferin® reagent (Polyplus® transfection) following manufacturer's instruction. Briefly, 20'000 cells/cm^2 were seeded and transfected

24h post-seeding with 10nM of siRNAs against PTEN (Cell Signaling, MA, USA), S100A11 or random sequences as negative controls (Quiagen, Basel, Switzerland, Supplementary materials and Methods). Transient transfections of cells with plasmids encoding the human S100A11 (PCDNA3.1+/S100A11; PCDNA3.1+/S100A11-flag or a pEGFP-C2 encoding a GFP-S100A11 fusion protein (Addgene plasmid 107201; GFP-S100a11 was a gift from Volker Gerke & Ursula Rescher) was performed using lipofectamine 3000 (Thermo Scientific) according to manufacturer's instructions. Briefly, cells were seeded (40'000 cells/cm^2) in six well plates and transfected the day after with 500ng of plasmids.

Mouse primary hepatocytes isolation. - (MPH) were isolated as previously described[2]. Briefly, control mice were anesthetized with ketamin/xylasine and their liver was perfused through the portal vein with a collagenase-containing solution. Primary hepatocytes were then purified by Percoll density gradient centrifugation. MPH were counted and cultured on collagen-coated dishes in Williams-E-Media supplemented with 10% FBS, 50µM hydrocortisone (Sigma-Aldrich) and 5µg/ml insulin.

Western Blot analyses

Homogenized cells/tissues were lysed in ice-cold RIPA buffer (50 mM Tris-HCl, pH 6.8, 100 mM DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol). Lysates were centrifuged at 12000g for 10 min, and the supernatant were collected. The protein content was determined using a BCA (Bicinchoninic Acid) protein assay with BSA (Bovine Serum Albumin) as standard (Pierce Biotechnology). Lysates (10µg) were separated by SDS-PAGE (5-20% gradient gels) and blotted onto nitrocellulose membranes (Amersham, Dübendorf, Switzerland). Nitrocellulose membranes were blocked for 1 min at room temperature in polyvinyl alcohol and probed with primary antibodies (see table in supplementary materials and methods) overnight at 4°C. Membranes were washed 5 times with PBS-Tween and probed with Horseradish Peroxidase-conjugated secondary antibody (Biorad, Cressier, Switzerland) for 1h at RT. Detection and quantitation of blots were performed using the PXI/PXI Touch from Syngene (Synoptics group, Cambridge, UK) and quantified with ImageJ™ software

Co-immunoprecipitation assays

Huh-7 cells at 70% confluency were lysed in ice-cold co-IP lysis buffer (150mM NaCl, 1% NP40, 50mM Tris-HCl pH 8.0) supplemented with proteases (Complete, Roche) and phosphatases inhibitors (PhosSTOP, Merck). Lysates were centrifuged at 12000g for 10 min, and the supernatant were collected and incubated with 3ug of S100A11 antibody or normal rabbit IgG ON at 4°C. Then, 50ul of protein G-coupled magnetic beads were added to the lysates and incubated for 2h at 4°C. Immunoprecipitates were

then purified with a magnetic stand, resuspended in 50ul of Laemmli buffer and separated by SDS-PAGE (5-20% gradient gels). For Co-IP experiments on SNU-398 cells, cells were first transfected with a PEGFP-C2 plasmid encoding a S100A11-GFP fusion protein (Addgene plasmid 107201; GFP-S100a11 was a gift from Volker Gerke & Ursula Rescher). 24h after, cells were treated for 1h with hydrogen peroxide (1mM; Acros Organics) and then lysed in ice-cold co-IP lysis buffer.

RT-PCR analysis

mRNAs were extracted with Trizol® reagent (Life Technologies, Switzerland) according to the manufacturer's instructions. Complementary DNA (cDNA) synthesis was performed by reverse transcription using 1µg of RNA with the High-Capacity cDNA kit (Life Technologies, Switzerland) following the manufacturer's instructions. qPCR analyses were performed with the StepOnePlus® Real-Time PCR Assay System (Life Technologies, Switzerland) using gene-specific primers (Microsynth™) for SYBR green-based assay (see primers table below). Results were normalized with housekeeping genes and expressed as fold change relative to their control condition. mRNA stability was investigated by using the transcription inhibitor, actinomycin-D. Briefly, cells were treated with the transcription inhibitor actinomycin-D (5ug/ml) for 4-12h prior to RNA extraction and qRT-PCR analyses.

Cell proliferation assay.

20'000 cells/cm^2 cells (*i.e,* HepG2, Huh7, Hep3B) were seeded and transfected 12h later with siRNAs of interest. 24h post-transfection, cells were detached and re-seeded in 6 well plates at 20'000 cells/cm^2 (t=0). The number of viable cells was assessed by Trypan blue exclusion staining using a Neubauer cell counter chamber slides after 24h, 48h and 72h.

Cell Cycle analysis

Cell Cycle distribution was asssessed by flow cytometry (AccuriC6) after DNA staining of 10^6 cells with propidium iodide (Sigma, 1µg/ml) and RNAse A treatment (100µg/ml). For the acquisition (FL2A, FL2H), 10'000 events/samples were recorded and data were analyzed with FlowJo™10 software (Tree Star, Inc, Ashlanf, OR).

Transwell migration/invasion assay.

Sobolewski C, *et al. Gut* 2020;0:1–14. doi: 10.1136/gutjnl-2019-319019

Migration assays were performed with HepG2 cells in 48-microwells chambers (Neuro Probe, Gaithersburg, USA), with 8µm pore-size polyvinylpyrrolidone-free polycarbonate membranes (Neuro Probe Inc, Gaithersburg). For invasion assays, membranes were coated with matrigel (Growth factors reduced matrigel, Corning). The upper chamber wells were loaded with 70'000 cells in serum-free DMEM (1g/L glucose), while the lower chambers were filled with complete DMEM media with 10ng/ml TGFβ (Peprotech, London, UK) as a chemoatractant. After 24h of migration/invasion at 37°C, membranes were fixed in 70% ethanol and then stained with hematoxylin/eosin for 5 minutes. Quantification of migration/invasion was done by counting the number of cells in at least 3 different fields, using ImageJ™ sofware and the cell counter pluging (https://imagej.nih.gov/ij/plugins/cell-counter.html).

Analysis and quantification of apoptosis

The percentage of apoptotic/necrotic cells was investigated by counting the number of cells displaying apoptotic features, such as fragmented and condensed nuclei as previously described [5]. At least 100 cells in three random fields were counted by fluorescence microscopy (Evos® FL Cell Imaging System, Advanced Microscopy Group, Life Technology, Mill Creek, WA, USA) after staining the nuclei with Hoechst 33342 (1µg/ml Sigma) and propidium iodide (1µg/ml Sigma) for 15min at 37°C. For experiments combining S100A11 silencing (by specific siRNAs) with drugs used in clinic, HepG2 cells were treated 48h post-transfection, with 0.5µM of doxorubicin or 5µM of sorafenib for 24h. For SNU-398 cells, treatment was performed 24h post-transfection with a plasmid (PCDNA3.1+) encoding S100A11-flag (Flag tag in N-term).

Staining of lipid droplets in hepatic cancer cells

20'000 cells/cm^2 Huh7 cells were seeded and treated 12h later with oleic acid (OA)/palmitic acid (PA) (400uM, ratio 3:1) in a media containing 1g/l glucose or 4.5g/l glucose (High-glucose). 24h post stimulation with glucose and OA/PA, cells were stained with BODIPY 493/503 (Molecular probes) (1µg/ml) for 15 minutes. Lipid droplets in cells were then observed by epifluorescence (Evos® FL Cell Imaging System, Advanced Microscopy Group, Life Technology, Mill Creek, WA, USA).

Histological analyses

Histological staining. - Liver tissues were fixed in 4% paraformaldehyde overnight and then washed in PBS-1X. Then, tissues were dehydrated and embedded in paraffin. 5µm thin sections were stained with

Hematoxylin/Eosin (H&E) or trichrome Masson for morphological investigations. For neutral lipids staining, fresh livers tissues were embedded and frozen in OCT with liquid nitrogen and 2-methyl-butane. Frozen samples were stored at -80°C. 5µm thin cryosections were fixed with 4% paraformaldehyde for 15 minutes at room temperature and stained with Oil-Red-O (ORO).

Immunohistochemistry. - Immunohistochemistry staining for S100A11 was performed on human tissue microarrays (US Biomax). IHC was performed using the abcam kit (Abcam, Cambridge, UK) according to the manufacturer's instructions. Briefly, slides were deparaffinized with Xylol solution and progressively hydrated. An antigen retrieval step was then performed using a citrate buffer and a microwave. Slides were washed twice with TBS + 0.025% Triton X-100 and blocked with 10% normal serum (rabbit) and 1% BSA for 2h at room temperature. Slides were then incubated overnight with a primary antibody against S100A11 (dilution 1:200). Slides were rinsed 5x5 minutes in TBS-0.025% Triton X-100 and then incubated in TBS-0.3% H_2O_2 solution for 15 min (for blocking of endogenous peroxidases). Finally, slides were incubated with HRP-conjugated antibody (dilution 1:1000 for 1h at room temperature) and the staining was revealed with a DAB substrate. A counterstaining with hematoxylin was performed. The intensity of the immunostaining was evaluated by a qualitative scoring (unstained, -; weak staining, +; moderate staining ++ and strong staining, +++) and further validated by a pathologist blinded to clinical data and final diagnosis, Pr. Christine Sempoux (CHUV, Lausanne). For F4/80 immunostaining on liver sections from mice, the same protocol was used but the blocking was performed with Fab fragment Goat anti-mouse (IgG (H+L) (Jackson ImmunoResearch) at a dilution of 1:100 for 30 min and then with normal goat serum and 1% BSA for another 30 min. Antigen retrieval was performed with Proteinase K (Agilent) for 5 min. Anti-F4/80 antibody was used at a dilution of 1:100 and the corresponding HRP-conjugated secondary antibody (Anti-Rat) was used at a dilution of 1:2.

Detection of S100A11 in Huh7 conditioned media

Huh7 cells were seeded at 400'000 cells/well in 6 wells plates. 24h after, the media was replaced with a serum free media, supplemented with 0.5% BSA for 24h (conditioned media). The conditioned media was removed 24h after and filtered onto 22µm filters (Merck Millipore, Darmstadt, Germany). 200µl of conditioned media were used for protein precipitation with 10% TCA. Proteins were then centrifuged at 12000g for 10 min, washed twice with acetone and re-suspended in 100µl of Laemmli buffer. Finally, samples were denatured and subjected to SDS-PAGE (5-20% gradient gels) as described in the Western blot section.

Sobolewski C, *et al. Gut* 2020;0:1–14. doi: 10.1136/gutjnl-2019-319019

Kits:

Name	Provider	Catalogue number
BEGM Bullet Kit	Lonza, Basel, Switzerland	CC3170
S100A11 ELISA Kit	Aviva System Biology, San Diego, CA	OKEH07142
DAB Substrate Kit	Abcam, Cambridge, UK	ab64238

Primary antibodies:

Protein targeted	Host	Provider	Catalogue number
PTEN	Rabbit	Cell Signaling (MA, USA)	3021
AKT	Rabbit	Cell Signaling (MA, USA)	9272
pAKT (Ser473)	Rabbit	Cell Signaling (MA, USA)	9271
pAKT (Thr308)	Rabbit	Cell Signaling (MA, USA)	9275
FAS	Rabbit	Cell Signaling (MA, USA)	3189
ACC	Rabbit	Cell Signaling (MA, USA)	3662
β-Tubulin	Rabbit	Cell Signaling (MA, USA)	2128s
ERM	Goat	Santa-Cruz (Dallas, USA)	sc-6407
pACC	Rabbit	Cell Signaling (MA, USA)	3661
Selenium Binding Protein-1	Rabbit	Abcam, Cambridge, UK	ab80868
PDCD4	Rabbit	Cell Signaling (MA, USA)	9535
Galectin-1	Rabbit	Cell Signaling (MA, USA)	12936
S100A11	Rabbit	Proteintech, Manchester, UK	10237-1-AP
CD36	Rabbit	Abcam, Cambridge, UK	ab133625
ANXA2	Rabbit	Cell Signaling (MA, USA)	8235
MGLL	Rabbit	Proteintech, Manchester, UK	14986-1-AP
ENTPD5	Rabbit	Abcam, Cambridge, UK	ab92542
CREG1	Rabbit	Santa-Cruz (Dallas, USA)	sc-11728
ENDOG	Rabbit	Cell Signaling (MA, USA)	4969
PERK	Rabbit	Cell Signaling (MA, USA)	3192
phosphoEIF2α	Rabbit	Cell Signaling (MA, USA)	3597
P38MAPK	Rabbit	Cell Signaling (MA, USA)	9212

phosphoP38MAPK	Rabbit	Cell Signaling (MA, USA)	9211
MAC-3	Rat	Biolegend (CA, USA)	108502
Normal Rabbit IgG	Rabbit	Cell Signaling (MA, USA)	2729

siRNAs:

	Provider	**Catalogue number**
Human siS100A11-6	Quiagen (Basel, Switzerland)	SI03166723
Mouse S100A11-4 siRNA	Quiagen (Basel, Switzerland)	SI01409268
Human PTEN siRNA	Cell Signaling (MA, USA)	6538
Human siHNF4A-6	Quiagen (Basel, Switzerland)	SI03053785
All Stars Negative Control siRNA	Quiagen (Basel, Switzerland)	1027280

Secondary antibodies:

Protein targeted	**Host**	**Provider**	**Catalogue number**
HRP-conjugated anti-rabbit	Goat	Biorad (Cressier, Switzerland)	170-6515
HRP-conjugated anti-goat	Rabbit	Sigma (St Louis, MO)	A5420
HRP-conjugated anti-mouse	Goat	Biorad (Cressier, Switzerland)	170-6516
HRP-conjugated anti-rat	Goat	Vector	MP-7444
TrueBlot® Anti-Rabbit HRP	Mouse	Rockland (Limerick, USA)	18-8816-33

Cytokines:

Cytokines	**Provider**	**Catalogue number**
Human TNFα	Peprotech (LubioScience), London, UK	300-01A
Human Il-1β	Peprotech (LubioScience), London, UK	200-01B
Human IL-6	Peprotech (LubioScience), London, UK	200-06
Human Il-8 (CXCL8)	Peprotech (LubioScience), London, UK	200-08
Human TGF-β	Peprotech (LubioScience), London, UK	100-21

Other reagents:

Name	**Provider**	**Catalogue number**
Trizol Reagent	Invitrogen (Carlsbad, CA)	15596-018
High Capacity RNA-to-	Life technologies Europe, Switzerland	4387406

cDNA Kit		
PowerUp SYBR™ green mastermix	Life technologies Europe, Switzerland	A25778
Nitrocellulose membranes	Amersham (Dübendorf, Switzerland)	RPN303D
ECL advance	Amersham (Dübendorf, Switzerland)	RPN2135
Insulin (Humalog®)	Eli Lilly (Vernier, Switzerland)	-
Isofluorane	Rothacher & Partner (Berne, Switzerland)	ISO250
Collagenase	Sigma (St Louis, MO)	C5138
Percoll	GE Healthcare (Uppsala, Sweden)	17-0891-01
Dexamethasone Hemisuccinate	Sigma Aldrich, St Louis, MO)	D8893

Mouse Primers:

Gene	Forward	Reverse
Lgals1	5'-ATGGAGACGCCAACACCA-3'	5'-AGCTTGATGGTCAGGTCA-3'
S100a11	5'-TGCCTACAGAGACTGAGAG-3'	5'-GGATCCTTCTGGTTCTTTGTG-3'
Anxa2	5'-GTGCCTACGGGTCAGTCAAA-3'	5'-CACATTGCTGCGGTTTGTCA-3'
Fasn	5'-AAGTTGCCCGAGTCAGAGAACC-3'	5'-ATCCATAGAGCCCAGCCTTCCATC-3'
Cd36	5'-GTCTATCTACGCTGTGTTCG-3'	5'-ACAGGCTTTCCTTCTTTGC-3'
Mk2	5'-GCCCCATCAGTGTAGGTCAT-3'	5'-GCTTCAGATCTGCTCGCTCT-3'
Acc1	5'-GGACACCAGTTTTGCATTGA-3'	5'-AGTTTGGGAGGACATCGAAA-3'
Ptpn11	5'-GAAAGTGCCGCCCATGACTA-3'	5'-TCTCTGTGTTTCCCTGGAGTA-3'
Pten	5'-TCAGTGGCGGAACTTGCAA-3'	5'-TCACCTTTAGCTGGCAGACCA-3'
Sdha	5'-GGAACACTCCAAAAACAGACCT-3'	5'-CCACCACTGGGTATTGAGTAGAA-3'
Nherf	5'-TTGTGGAGGTCAATGGTG-3'	5'-TAGCTCCTCACTGGTATCG-3'
Aimp2	5'CTTCCAACCTGCATGTACCG -3'	5'-TTGAAAGGCCATCGACTGCT -3'
Endog	5'GCCATGGACGACACCTTCTA -3'	5'-GTAAGTTCGCGTCAAGCTGC -3'
Cd82	5'-TTCGGGGTGTGGATTCTTGC-3'	5'-ATTGACAGCACCGATACAGCC-3'
Pdcd4	5'-CGGTTAGAAGTGGAGTTGC-3'	5'-ACAGTTCTCCTGGTCGTC-3'
Fh	5'-AGC CAG AGC TCG AAT GAC AC-3'	5'-GACCTG CGCAAACTCTTTGG-3'
Sbp1	5'-CGC AAC AAG CTG ATA CTG CC-3'	5'-AGTGGCTGGTGTGCAAACTG-3'
Mtap	5'-AAGCAGTGTCAGTGGATGGG-3'	5'-CGGAGCGTTTCTGACCATTC-3'
Ndrg2	5'-TTTGAGCGAGGTGGTGAGAC-3'	5'-CCCATGCCTTGCAGGAAGTA-3'
Iqgap2	5'-AAAGGTACAACTCAATATTCAGGAC-3'	5'-CGAACATTTTCATCACGGCCA -3'
Entpd5	5'-ATG GTT TTG GCT TTGCCGAC-3'	5'-CTG GTGATGCCCAGAGACTG-3'
Mgll	5'-GCTGGCTCATATGTTGAAGG-3'	5'-TTGGAAGTCCGACACCAC-3'
Creg1	5'-CAATCAGTGACGGTCCTCCG-3'	5'-ATTTTCCTGCAGGTCGCTCA-3'
Tgfb	5'- GCCTGAGTGGCTGTCTTTTGA-3'	5'- GCTGAATCGAAAGCCCTGTATT-3'
18s	5'-ACATCCAAGGAAGGCAGCAG-3'	5'-TTTTCGTCACTACCTCCCCG-3'

Bax	5'-TGAAGA CAG GGG CCT TTT TG-3'	5'-AAT TCG CCG GAG ACA CTC G-3'
Bcl2	5'-TCGCAGAGATGTCCAGTCAG-3'	5'-ATCTCCCTGTTGACGCTCTC-3'
Fas	5'-TATCAAGGAGGCCCATTTTGC-3'	5'-TGTTTCCACTTCTAAACCATGCT-3'
Tp53	5'-AAGACTCCAGTGGGAACC-3'	5'-GGATCTTGAGGGTGAAATACTC-3'
Mpg	5'-TCCCGAAAGATTGGGCAAAAA-3'	5'-AAATGCACGGGCTAGGGTG-3'
Rad51	5'-AAGTTTTGGTCCACAGCCTATTT-3'	5'-CGGTGCATAAGCAACAGC C-3'
Mki67	5'-TGGTCACCATCAAGCGGAG-3'	5'-AGGCAGCTGGATACGAATGT-3'
Ccnd1	5'-AGTGCGTGCAGAAGGAGATT-3'	5'-CACAACTTCTCGGCAGTCAA-3'
Il1b	5'-GACAACTGCACTACAGGC-3'	5'-CATGGAGAATATCACTTGTTGG-3'
Tnfa	5'-AGGCTGCCCCGACTACGT-3'	5'-GACTTTCTCCTGGTATGAGATAGCAAA-3'
Il6	5'-AGT TGC CTT CTT GGG ACT GAT	5'-TCC ACG ATT TCC CAG AGA AC-3'
Fabp4	5'-CACCGAGATTTCCTTCAAACTG	5'-TTTCATAACACATTCCACCACC-3'
Fatp5	5'-TACAAGTTGGAGCCACCTG	5'-TCACCCACATACAAGATCACTG-3'
Scd1	5'-TGCTCCAAGAGATCTCCAG	5'-GGAACCAGTATGATCCCG-3'
Ptgs2	5'-CATCCCCTTCCTGCGAAGTT-3'	5'-CATGGGAGTTGGGCAGTCAT-3'
Itgam	5'-GATGCTTACCTGGGTTATGCTTCT-3'	5'-CCGAGGTGCTCCTAAAACCA-3'
Cd44	5'-ACTTTGCCTCTTGCAGTTGAG-3'	5'-TTTCTCCACATGGAATACACCTG-3'
Cd163	5'-ATGGGTGGACACAGAATGGTT-3'	5'-CAGGAGCGTTAGTGACAGCAG-3'
Egr2	5'-GCCAAGGCCGTAGACAAAATC-3'	5'-CCACTCCGTTCATCTGGTCA-3'
aSma	5'-AAAAAAAACCACGAGTAACAAATCAA-3'	5'-TCAGCGCCTCCAGTTCCT-3'
Fn1	5'-ATCTCGGAGCCATTTGTTCCT-3'	5'-CCAGGTCTACGGCAGTTGTCA-3'
Col1a1	5'-GCT CCT CTT AGG GGC CAC T-3'	5'-CCA CGT CTC ACC ATT GGG G-3'
Col1a2	5'- CACCCCAGCGAAGAACTCAT -3'	5'- TCTCCTCATCCAGGTACGCA -3'
Krt18	5'-CAG CCA GCG TCT ATG CAG G-3'	5'-CCT TCT CGG TCT GGA TTC CAC-3'
Pdgfa	5'-GAG GAA GCC GAG ATA CCC C-3'	5'-TGC TGT GGA TCT GAC TTC GAG-3'
Ace2	5'-TCC AGA CTC CGA TCA TCA AGC-3'	5'-GCT CAT GGT GTT CAG AAT TGT GT-3'
Timp2	5'-TTCCGGGAATGACATCTATGG-3'	5'-GGGCCGTGTAGATAAACTCGAT-3'
Mmp2	5'-GACATACATCTTTGCAGGAGACAAG-3'	5'-TCTGCGATGAGCTTAGGGAAA-3'
Mmp9	5'-CCTGGAACTCACACGACATCTTC-3'	5'-TGGAAACTCACACGCCAGAA-3'
Snai1	5'-CACACGCTGCCTTGTGTCT-3'	5'-GGTCAGCAAAAGCACGGTT-3'
Ccl2	5'-GTCCCTGTCATGCTTCTG-3'	5'-TTAACTGCATCTGGCTGAG-3'
Rantes (Ccl5)	5'-CTCACCATATGGCTCGGA-3'	5'-TTCCTTCGAGTGACAAACAC-3'

Human Primers:

Gene	Forward	Reverse
S100A11	5'-GCTGTCTTCCAGAAGTATGC-3'	5'-GACCATCACTGTTGGTGTC-3'
ANXA2	5'ATTGCCTTCGCCTACCAGAG-3'	5'- CAGGTGGCCAGATAAGGCTG-3'
PTEN	5'-TTATGACACCGCCAAATTTAATTG-3'	5'-TCTTCAAAAGGATATTGTGCAACTCT-3'

CDKN1A	5'- GACTCTCAGGGTCGAAAACG-3'	5'-GGATTAGGGCTTCCTCTTGG-3'
CDKN1B	5'- AATAAGGAAGCGACCTGCAA-3'	5'-GGGGAACCGTCTGAAACATT-3'
ALB	5'-CTAGAGAAGTGCTGTGCC-3'	5'-CCACGGATAGATAGTCTTCTG-3'
SERPINA1	5'-CTTCTTTAAAGGCAAATGGGAG -3'	5'-CTGGACAGCTTCTTACAGTG-3'
HNF4A	5'-CTCCTGCAGATTTAGCCG-3'	5'-CTGTCCTCATAGCTTGACC-3'
MYC	5'-TGCTCCATGAGGAGACACC-3'	5'TCGATTTCTTCCTCATCTTCTTG-3'
PCNA	5'-GGCGTGAACCTCACCAGTAT-3'	5'-AGGTATCCGCGTTATCTTCG-3'
PPP1R15A	5'-ATGATGGCATGTATGGTGAGC-3'	5'-AACCTTGCAGTGTCCTTATCAG-3'
GRP78	5'-CGTGGAGATCATCGCCAAC-3'	5'-ACATAGGACGGCGTGATGC-3'
DDIT3	5'-CAGAACCAGCAGAGGTCACA-3'	5'-AGCTGTGCCACTTTCCTTTC-3'
ATF4	5'-ATGACCGAAATGAGCTTCCTG-3'	5'-GCTGGAGAACCCATGAGGT-3'
XBP1s	5'-TGCTGAGTCCGCAGCAGGTG-3'	5'-GCTGGCAGGCTCTGGGGAAG-3'
IRE1	5'-GAAGACGTCATTGCACGTGAATT-3'	5'-AGGTCCTGAATTTACGCAGGT-3'
CycloA	5'-ATGGTCAACCCCACCGTGT-3'	5'-TCTGCTGTCTTTGGGACCTTGTC-3'

Diet	Metabolized energy	Provider	Catalog number
MCD diet	22 kJ% fat, 14KJ% protein, 64 KJ% carbohydrates	ssniff Spezialdiäten GmbH (Soest, Germany)	E15653-94

Supplementary references.

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3 Parent R, Marion MJ, Furio L, Trepo C, Petit MA. Origin and characterization of a human bipotent liver progenitor cell line. Gastroenterology 2004;**126**:1147-56.
4 Gripon P, Rumin S, Urban S, Le Seyec J, Glaise D, Cannie I*, et al.* Infection of a human hepatoma cell line by hepatitis B virus. Proceedings of the National Academy of Sciences of the United States of America 2002;**99**:15655-60.
5 Cerella C, Sobolewski C, Chateauvieux S, Henry E, Schnekenburger M, Ghelfi J*, et al.* COX-2 inhibitors block chemotherapeutic agent-induced apoptosis prior to commitment in hematopoietic cancer cells. Biochemical pharmacology 2011;**82**:1277-90.