SUPPORTING INFORMATION

SLU7 prevents oxidative stress-mediated HNF4α degradation preserving hepatic differentiation and protecting from liver damage

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MATERIAL AND METHODS

ANIMAL MODELS

SLU7 haploinsufficient mice (C57BL/6NTac-Slu7tm1a(KOMP)Wtsi/Wtsi) were generated by the KOMP/EUCOMM targeted embryonic stem cells resource and were obtained from European Mouse Mutant Archive (EMMA). Mice were maintained on a consistent inbred C57BL/6N background. 14-weeks-old SLU7 haplo-insufficient male mice (*Slu7*$^{+/-}$) and their corresponding wild type littermates (*Slu7*$^{+/+}$) were maintained under fed conditions with regular chow diet or fasted overnight when experimentation required.

For the acute model of liver damage mice were fasted overnight and then intraperitoneal injected with a single dose of 300 mg/kg of acetaminophen (APAP) (Sigma-Aldrich, St Louis, MO, USA) dissolved in warm phosphate-buffered saline (PBS) (Gibco-Life Technology, Madrid, Spain) as described(1). Controls received the equivalent volume of PBS. Three hours after the injection, mice were allowed to eat *ad libitum* and were euthanized when indicated.

For the chronic model of liver damage, mice received an intraperitoneal injection twice a week for 6.5 weeks with CCl_4 (Sigma-Aldrich) at 0.6 µL/g dissolved in corn oil(2).

Controls received the equivalent volume of corn oil. Mice were sacrificed 24h after the last administration.

For both models, blood was collected at indicated time points and at sacrifice livers were removed to be snap frozen in liquid nitrogen or formalin-fixed and paraffin-embedded.

For adeno-associated virus (AAV) experiments, mice were intravenously injected with 10^{11} pfu of control (AAV-Ren) or SLU7 expressing (AAV-SLU7) AAVs(3) two weeks before CCl_4 chronic treatment.

Experiments were performed with a minimum of 5 animals per group and were repeated at least twice.

CELL LINES, TRANSFECTIONS AND TREATMENTS

Human HCC cell lines PLC/PRF/5 and HepG2 were obtained from the ATCC and were grown in DMEM (Gibco-Life Technology, Madrid, Spain) supplemented with 5% (PLC/PRF/5) or 10% (HepG2 and Hep3B) fetal bovine serum (FBS), glutamine and antibiotics. The human HCC cell line HepaRG was obtained from BioPredic (Rennes, France). Differentiated HepaRG cells were obtained following described culture conditions (4). All cells were grown in a humidified atmosphere at 37°C and 5% CO_2.

Transfections with siRNAs were performed using Lipofectamine RNAiMAX reagent (Invitrogen, Grand Island, NY, USA) following the manufacturer's instructions. All siRNAs were obtained from Sigma-Aldrich and were used at 75 nM individually or at 37.5 nM when combinated. Silencing was confirmed by qPCR and Western blot. Sequence of siRNAs will be provided upon request.

Where indicated, cells were treated with 10 µM of the proteasome inhibitor MG-132 (Calbiochem, CA, USA 474790) for the last 12 h; with 10 µg/mL of the protein synthesis inhibitor cycloheximide (CHX) (Sigma-Aldrich, C-7698) for the last 4, 12 or 24 h; or with 10 mM of the antioxidant N-acetylcysteine (NAC) (Sigma-Aldrich, A8199) added to the culture medium 4 h after transfection with siRNAs. The same volume of vehicle (DMSO for MG-132 and CHX, and water for NAC) was added to control cells.

RNA ISOLATION AND PCR

Cell lines lysates and liver tissue samples were homogenated and subjected to total RNA extraction using the automated Maxwell RSC Instrument with simplyRNA tissue kit (Promega, Madison, WI, USA). Reverse transcription was performed as described (2). Real-time PCRs were performed with the iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) in a CFX96 Real-Time System (Bio-Rad) as previously described (5). Gene expression was normalized relative to that of the housekeeping gene *Rplp0* as described (6). Sequence of primers used in the study will be provided upon request.

PROTEIN EXTRACTION AND WESTERN BLOT ANALYSIS

For protein extraction, mouse or human liver tissue samples were lysed in urea buffer (Urea, Tiourea, Chaps, Dithiotreitol (DTT), a cocktail of phosphatases (1mM sodium orthovanadate, 10mM sodium fluoride, 100mM β-glycerophosphate) and proteases inhibitors (Roche, Basel, Switzerland)). The homogenates were sonicated and cleared by centrifugation at 75000 rpm for 20 min at 4°C. Cell lines were lysed in RIPA buffer (5M NaCl, 1M Tris, 0,5% Deoxycholate, 20% SDS, 1% Tritón X-100 and proteases-phosphatases inhibitors cocktail) for 20 min at 4°C under constant rotation, sonicated and centrifuged at 12,000 rpm for 20 min at 4°C. Protein extracts were subjected to Western blot analysis as reported (6). Antibodies used are described in Table S1.

SUBCELLULAR FRACTIONATION

PLC/PRF/5 siGL-transfected cells were subjected to subcellular fractionation to separate cytoplasm, nucleoplasm and chromatin-bound fraction for subsequent Western blot analysis. The NE-PER Nuclear and Cytoplasmic Extraction Reagents Kit (Thermo Fisher Scientific, Waltham, MA, USA #78835) was used following the manufacturer's instructions. Next, nuclear extract was incubated in NP40 buffer (15 mM Tris HCl pH 7.4, 1 mM EDTA, 250 mM NaCl, 1 mM $MgCl_2$, 10% Glycerol, 0.1% NP40 and protease-phosphatase inhibitors cocktail) for 30 min at 4°C under constant rotation. The nucleoplasm and the chromatin bound fraction were separated by centrifugation at 2500 rpm for 10 min at 4°C. Chromatin bound fraction was treated with 250 U/µL benzonase (Sigma-Aldrich) in a buffer containing 20 mM Tris HCl pH 7.4, 60 mM NaCl,

1.5 mM $MgCl_2$, 0.1% NP40 and protease-phosphatase inhibitors cocktail for 30 min at 4°C and then centrifuged at 13000 rpm for 10 min at 4°C to get the supernatant.

IMMUNOFLUORESCENCE

PLC/PRF/5 cells were cultured on glass coverslips, transfected with siGL or siSLU7 for 48h and treated with 500 µM sodium arsenite (Sigma-Aldrich S7400) for the last 1 h. Cells were washed twice with PBS, fixed in 4% formaldehyde for 10 min and quenched with 50 mM NH_4Cl in PBS for 10 min. After three washes with PBS, cells were permeabilized with 0,1% Triton X-100 in PBS for 5 min at 4°C, and excess binding sites were blocked with SuperBlock Blocking Buffer in PBS (Thermo Fisher Scientific, #37517) for 1 h at room temperature. Incubation with the primary antibody was carried out overnight at 4°C, followed by incubation with secondary antibody for 1 h at room temperature. The antibodies used are described in Table S1. Cells were then washed three times with PBS-BSA 1% and coverslips were mounted onto glass slides using Vectashield containing DAPI (Vector Laboratories, Burlingame, CA, USA). Images were captured using the Zeiss Axio ImagerM1 automated microscope (Zeiss, Oberkochen, Germany).

COIMMUNOPRECIPITATION ASSAY

To coimmunoprecipitate endogenous proteins with SLU7 in PLC/PRF/5 cells, cytoplasmic and nuclear soluble protein complexes were isolated as described in the subcellular fractionation section. Frozen liver samples were lysed in ice-cold lysis buffer containing 20 mM Tris HCl pH 8, 137 mM NaCl, 1% Nonidet P-40, 2 mM EDTA and protease-phosphatase inhibitors cocktail for 30 min at 4°C under constant rotation. Lysates were cleared by centrifugation at 12,000 rpm for 20 min at 4°C. Protein concentration was measured using the BCA assay (Pierce Technologies, Rockford, IL, USA) and 800-100 µg of proteins were incubated with 25 µL of Protein G dynabeads (Invitrogen, #10003D) for 2 h at 4°C under constant rotation. Precleared samples were incubated with 5 µg of primary antibody or the corresponding control IgG, previously coupled to protein G dynabeads (2 h under constant rotation at room temperature), overnight at 4°C under constant rotation. The immunocomplexes were washed three times with cold PBS with proteases and phosphatases inhibitors and eluted in loading buffer (50 mM Tris pH 6.8,

100 mM β-mercaptoethanol, 2% SDS, 10% glycerol, and 0.01% bromophenol Blue) at 95°C for 5 min. Pelleted beads were discarded and the supernatants were subjected to Western blot analysis. The antibodies used are described in Table S1.

MASS SPECTROMETRY ANALYSIS

Soluble protein lysates of PLC/PRF/5 were prepared following the subcellular fractionation protocol described (7) and discarding the chromatin fraction. The antibody used to immunoprecipitate human SLU7 and the pre-immune IgG are described in Table S1. Eluted samples were subjected to LC-ESI-MS/MS TRIPLE-TOF analysis as previously described (8). According to the analysis, proteins with at least 5 peptide sequences identified in SLU7 and none in the IgG immunoprecipitated were considered for cross-matching with two stress granule components databases: MSGP (https://msgp.pt/index/) (9) and RNA Granule Database (http://rnagranuledb.lunenfeld.ca/) (10).

SERUM BIOCHEMISTRY

Collected blood was conserved at 4°C overnight and was centrifuged at maximum rpm for 15 minutes to separate serum from blood clot. The supernatant (serum) was collected and diluted 1/10 with physiological serum for the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) on a Roche/Hitachi Cobas system.

HISTOLOGICAL DETERMINATIONS

Sections from formalin-fixed and paraffin-embedded liver tissue were stained with Haematoxylin-Eosin (H&E) to visualize liver morphology and with Pico-Sirius Red to determine collagen deposition as described (2). Periodic acid-Schiff (PAS) staining was performed for liver glycogen detection as described (3).

Immunohistochemical detections in liver sections were carried out as described (2) using the antibodies listed in Table S1.

ROS DETECTION

Estimation of intracellular reactive oxygen species (ROS) in PLC/PRF/5 cell line and in freshly prepared liver extracts (40 mg) homogenized in ice-cold 40mM Tris-HCl buffer (pH 7.4) (1:10 w/v) and incubated with 10μM DCFDA in Tris-HCl buffer at 37°C for 30 min was performed as previously described (5).

ANALYSIS OF APAP-GLUCURONIDE

APAP-Glucuronide (APAP-Gluc) was measured in the serum of APAP-treated mice by liquid chromatography dual mass spectrometry (LC-MS/MS) in an Acquity UPLC (Waters) equipped with an Acquity HSS T3 C18 2.1x100mm 1.8 μm column (Waters), which was coupled to a Xevo TQ MS triple-quadrupole mass spectrometer (Waters) using an electrospray ionization source in negative ionization mode. The protocol was adapted from a Waters' application note (11).

Supporting Table S1. Antibodies used in the study

Antibody name	Catalog number	Manufacturer	Technique	Dilution/ Quantity
SLU7	NBP2-20403	Novus Biologicals (Centennial, USA)	Western blot	1:1000
SLU7	612604	BD Bioscience (Franklin Lakes, USA)	Coimmunoprecipitation	5μg
SLU7	Sc-10829	Santa Cruz Biotechnology (Santa Cruz, USA)	Immunohistochemistry	1:500
CYP2E1	Ab28146	Abcam (Cambridge, UK)	Western blot	1:1000
G3BP1	Ab181150	Abcam	Western blot	1:1000
			Immunocytochemistry	1:700
			Coimmunoprecipitation	5μg
USP10	Ab72486	Abcam	Western blot	1:1000
HISTONE 3	Ab1012	Abcam	Western blot	1:1000
3'NITROTYROSINE	Sc-32757	Santa Cruz	Western blot	1:500
HK2	Sc-28889	Santa Cruz	Western blot	1:500
PKCα	Sc-8393(H-7)	Santa Cruz	Western blot	1:500
PKCδ	Sc-937 (C-20)	Santa Cruz	Western blot	1:1000
HNF4α P1 isoforms	K9218-00	R&D Systems (Minneapolis,USA)	Western blot	1:1000
HNF4α P2 isoforms	H6939-00	R&D Systems	Immunohistochemistry	1:400
HNF4α	Sc-374229	Santa Cruz	Coimmunoprecipitation	5μg
PKM2	3198	Cell Signalling (Danvers, USA)	Western blot	1:1000
p-GSK3β	9336	Cell Signalling	Western blot	1:1000
ɣH2AX	2577	Cell Signalling	Western blot	1:1000
TP53	9282S	Cell Signalling	Western blot	1:1000
p-SAPK/JNK (T183/Y185)	9251S	Cell Signalling	Western blot	1:1000
α-SMA	A2547	Sigma-Aldrich	Immunohistochemistry	1:1000
GAPDH	2118	Cell Signalling	Western blot	1:5000
ACTIN	A2066	Sigma-Aldrich	Western blot	1:5000
goat anti-rabbit IgG HRP-linked	A0545	Sigma-Aldrich	Western blot	1:6000
goat anti-mouse IgG HRP-linked	A0168	Sigma-Aldrich	Western blot	1:6000
m-IgGκ BP-HRP	Sc-516102	Santa Cruz	Western blot	1:6000
Alexa Fluor 594 donkey anti-rabbit IgG	A21207	Invitrogen	Immunocytochemistry	1:1000
IgG mouse	Sc-2025	Santa Cruz	Coimmunoprecipitation	5μg
IgG rabbit	Sc-2027	Santa Cruz	Coimmunoprecipitation	5μg

Supporting Table S2. SLU7 interactome

	Protein AC	Description / Name
1	sp|O75165	DnaJ homolog subfamily C member 13 OS=Homo sapiens OX=9606 GN=DNAJC13 PE=1 SV=5
2	sp|Q92900	Regulator of nonsense transcripts 1 OS=Homo sapiens OX=9606 GN=UPF1 PE=1 SV=2
3	sp|P42704	Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens OX=9606 GN=LRPPRC PE=1 SV=3
4	sp|Q04637	Eukaryotic translation initiation factor 4 gamma 1 OS=Homo sapiens OX=9606 GN=EIF4G1 PE=1 SV=4
5	sp|Q9UDY2	Tight junction protein ZO-2 OS=Homo sapiens OX=9606 GN=TJP2 PE=1 SV=2
6	sp|Q10570	Cleavage and polyadenylation specificity factor subunit 1 OS=Homo sapiens OX=9606 GN=CPSF1 PE=1 SV=2
7	sp|Q08211	ATP-dependent RNA helicase A OS=Homo sapiens OX=9606 GN=DHX9 PE=1 SV=4
8	sp|Q7L2E3	ATP-dependent RNA helicase DHX30 OS=Homo sapiens OX=9606 GN= DHX30 PE=1 SV=1
9	sp|Q9NZI8	Insulin-like growth factor 2 mRNA-binding protein 1 OS=Homo sapiens OX=9606 GN=IGF2BP1 PE=1 SV=2
10	sp|P26599	Polypyrimidine tract-binding protein 1 OS=Homo sapiens OX=9606 GN=PTBP1 PE=1 SV=1
11	sp|P11940	Polyadenylate-binding protein 1 OS=Homo sapiens OX=9606 GN=PABPC1 PE=1 SV=2
12	sp|Q92499	ATP-dependent RNA helicase DDX1 OS=Homo sapiens OX=9606 GN=DDX1 PE=1 SV=2
13	sp|Q13310	Polyadenylate-binding protein 4 OS=Homo sapiens OX=9606 GN=PABPC4 PE=1 SV=1
14	sp|P14866	Heterogeneous nuclear ribonucleoprotein L OS=Homo sapiens OX=9606 GN=HNRNPL PE=1 SV=2
15	sp|Q14152	Eukaryotic translation initiation factor 3 subunit A OS=Homo sapiens OX=9606 GN=EIF3A PE=1 SV=1
16	sp|Q9Y6M1	Insulin-like growth factor 2 mRNA-binding protein 2 OS=Homo sapiens OX=9606 GN=IGF2BP2 PE=1 SV=2
17	sp|O00425	Insulin-like growth factor 2 mRNA-binding protein 3 OS=Homo sapiens OX=9606 GN=IGF2BP3 PE=1 SV=2
18	sp|Q9HCE1	Putative helicase MOV-10 OS=Homo sapiens OX=9606 GN=MOV10 PE=1 SV=2
19	sp|P61978	Heterogeneous nuclear ribonucleoprotein K OS=Homo sapiens OX=9606 GN=HNRNPK PE=1 SV=1
20	sp|Q12906	Interleukin enhancer-binding factor 3 OS=Homo sapiens OX=9606 GN=ILF3 PE=1 SV=3
21	sp|P43243	Matrin-3 OS=Homo sapiens OX=9606 GN=MATR3 PE=1 SV=2
22	sp|Q96PU8	Protein quaking OS=Homo sapiens OX=9606 GN=QKI PE=1 SV=1
23	sp|Q07157	Tight junction protein ZO-1 OS=Homo sapiens OX=9606 GN=TJP1 PE=1 SV=3
24	sp|Q8WWM7	Ataxin-2-like protein OS=Homo sapiens OX=9606 GN=ATXN2L PE=1 SV=2
25	sp|P17844	Probable ATP-dependent RNA helicase DDX5 OS=Homo sapiens OX=9606 GN=DDX5 PE=1 SV=1
26	sp|P23246	Splicing factor, proline- and glutamine-rich OS=Homo sapiens OX=9606 GN=SFPQ PE=1 SV=2
27	sp|Q92841	Probable ATP-dependent RNA helicase DDX17 OS=Homo sapiens OX=9606 GN= DDX17 PE=1 SV=2
28	sp|Q13283	Ras GTPase-activating protein-binding protein 1 OS=Homo sapiens OX=9606 GN=G3BP1 PE=1 SV=1
29	sp|O60506	Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens OX=9606 GN=SYNCRIP PE=1 SV=2
30	sp|O75643	U5 small nuclear ribonucleoprotein 200 kDa helicase OS=Homo sapiens OX=9606 GN=SNRNP200 PE=1 SV=2
31	sp|P51114	Fragile X mental retardation syndrome-related protein 1 OS=Homo sapiens OX=9606 GN=FXR1 PE=1 SV=3
32	sp|O00571	ATP-dependent RNA helicase DDX3X OS=Homo sapiens OX=9606 GN=DDX3X PE=1 SV=3
33	sp|Q9H361	Polyadenylate-binding protein 3 OS=Homo sapiens OX=9606 GN=PABPC3 PE=1 SV=2
34	sp|Q9BUJ2	Heterogeneous nuclear ribonucleoprotein U-like protein 1 OS=Homo sapiens OX=9606 GN=HNRNPUL1 PE=1 SV=2
35	sp|P67809	Nuclease-sensitive element-binding protein 1 OS=Homo sapiens OX=9606 GN=YBX1 PE=1 SV=3
36	sp|Q15233	Non-POU domain-containing octamer-binding protein OS=Homo sapiens OX=9606 GN=NONO PE=1 SV=4
37	sp|P22626	Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens OX=9606 GN=HNRNPA2B1 PE=1 SV=2
38	sp|Q96PK6	RNA-binding protein 14 OS=Homo sapiens OX=9606 GN=RBM14 PE=1 SV=2
39	sp|Q8IX12	Cell division cycle and apoptosis regulator protein 1 OS=Homo sapiens OX=9606 GN=CCAR1 PE=1 SV=2
40	sp|Q14444	Caprin-1 OS=Homo sapiens OX=9606 GN=CAPRIN1 PE=1 SV=2
41	sp|Q58FF7	Putative heat shock protein HSP 90-beta-3 OS=Homo sapiens OX=9606 GN=HSP90AB3P PE=5 SV=1
42	sp|Q9P2I0	Cleavage and polyadenylation specificity factor subunit 2 OS=Homo sapiens OX=9606 GN=CPSF2 PE=1 SV=2
43	sp|P52272	Heterogeneous nuclear ribonucleoprotein M OS=Homo sapiens OX=9606 GN=HNRNPM PE=1 SV=3
44	sp|Q96HS1	Serine/threonine-protein phosphatase PGAM5, mitochondrial OS=Homo sapiens OX=9606 GN=PGAM5 PE=1 SV=2
45	sp|Q15637	Splicing factor 1 OS=Homo sapiens OX=9606 GN=SF1 PE=1 SV=4
46	sp|Q07666	KH domain-containing, RNA-binding, signal transduction-associated protein 1 OS=Homo sapiens OX=9606 GN=KHDRBS1 PE=1 SV=1
47	sp|P51991	Heterogeneous nuclear ribonucleoprotein A3 OS=Homo sapiens OX=9606 GN=HNRNPA3 PE=1 SV=2
48	sp|Q6PKG0	La-related protein 1 OS=Homo sapiens OX=9606 GN=LARP1 PE=1 SV=2
49	sp|Q7Z417	Nuclear fragile X mental retardation-interacting protein 2 OS=Homo sapiens OX=9606 GN=NUFIP2 PE=1 SV=1
50	sp|Q9UN86	Ras GTPase-activating protein-binding protein 2 OS=Homo sapiens OX=9606 GN=G3BP2 PE=1 SV=2
51	sp|P62140	Serine/threonine-protein phosphatase PP1-beta catalytic subunit OS=Homo sapiens OX=9606 GN=PPP1CB PE=1 SV=3
52	sp|O43390	Heterogeneous nuclear ribonucleoprotein R OS=Homo sapiens OX=9606 GN=HNRNPR PE=1 SV=1
53	sp|Q9P2E9	Ribosome-binding protein 1 OS=Homo sapiens OX=9606 GN=RRBP1 PE=1 SV=5
54	sp|Q9Y262	Eukaryotic translation initiation factor 3 subunit L OS=Homo sapiens OX=9606 GN=EIF3L PE=1 SV=1
55	sp|P60228	Eukaryotic translation initiation factor 3 subunit E OS=Homo sapiens OX=9606 GN=EIF3E PE=1 SV=1
56	sp|Q9Y3I0	tRNA-splicing ligase RtcB homolog OS=Homo sapiens OX=9606 GN=RTCB PE=1 SV=1
57	sp|Q9H0D6	5'-3' exoribonuclease 2 OS=Homo sapiens OX=9606 GN=XRN2 PE=1 SV=1
58	sp|P49750	YLP motif-containing protein 1 OS=Homo sapiens OX=9606 GN=YLPM1 PE=1 SV=4
59	sp|P09651	Heterogeneous nuclear ribonucleoprotein A1 OS=Homo sapiens OX=9606 GN=HNRNPA1 PE=1 SV=5
60	sp|Q9NR30	Nucleolar RNA helicase 2 OS=Homo sapiens OX=9606 GN=DDX21 PE=1 SV=5
61	sp|P63244	Receptor of activated protein C kinase 1 OS=Homo sapiens OX=9606 GN=RACK1 PE=1 SV=3
62	sp|Q13137	Calcium-binding and coiled-coil domain-containing protein 2 OS=Homo sapiens OX=9606 GN=CALCOCO2 PE=1 SV=1
63	sp|Q9NZB2	Constitutive coactivator of PPAR-gamma-like protein 1 OS=Homo sapiens OX=9606 GN=FAM120A PE=1 SV=2
64	sp|Q14694	Ubiquitin carboxyl-terminal hydrolase 10 OS=Homo sapiens OX=9606 GN=USP10 PE=1 SV=2
65	sp|Q8IY67	Ribonucleoprotein PTB-binding 1 OS=Homo sapiens OX=9606 GN=RAVER1 PE=1 SV=1
66	sp|P42167	Lamina-associated polypeptide 2, isoforms beta/gamma OS=Homo sapiens OX=9606 GN=TMPO PE=1 SV=2
67	sp|Q9Y2W1	Thyroid hormone receptor-associated protein 3 OS=Homo sapiens OX=9606 GN=THRAP3 PE=1 SV=2
68	sp|P31943	Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens OX=9606 GN=HNRNPH1 PE=1 SV=4
69	sp|P60842	Eukaryotic initiation factor 4A-I OS=Homo sapiens OX=9606 GN=EIF4A1 PE=1 SV=1

70	sp|Q07955	Serine/arginine-rich splicing factor 1 OS=Homo sapiens OX=9606 GN=SRSF1 PE=1 SV=2
71	sp|O00303	Eukaryotic translation initiation factor 3 subunit F OS=Homo sapiens OX=9606 GN=EIF3F PE=1 SV=1
72	sp|P36873	Serine/threonine-protein phosphatase PP1-gamma catalytic subunitOS=Homo sapiensOX=9606GN=PPP1CC PE=1SV=1
73	sp|O75533	Splicing factor 3B subunit 1 OS=Homo sapiens OX=9606 GN=SF3B1 PE=1 SV=3
74	sp|Q9Y224	RNA transcription, translation and transport factor protein OS=Homo sapiens OX=9606 GN=RTRAF PE=1 SV=1
75	sp|Q9UKF6	Cleavage and polyadenylation specificity factor subunit 3 OS=Homo sapiens OX=9606 GN=CPSF3 PE=1 SV=1
76	sp|Q14240	Eukaryotic initiation factor 4A-II OS=Homo sapiens OX=9606 GN=EIF4A2 PE=1 SV=2
77	sp|Q8WVV9	Heterogeneous nuclear ribonucleoprotein L-like OS=Homo sapiens OX=9606 GN=HNRNPLL PE=1 SV=1
78	sp|Q9C0J8	pre-mRNA 3~ end processing protein WDR33 OS=Homo sapiens OX=9606 GN=WDR33 PE=1 SV=2
79	sp|P38159	RNA-binding motif protein, X chromosome OS=Homo sapiens OX=9606 GN=RBMX PE=1 SV=3
80	sp|Q12905	Interleukin enhancer-binding factor 2 OS=Homo sapiens OX=9606 GN=ILF2 PE=1 SV=2
81	sp|A5A3E0	POTE ankyrin domain family member F OS=Homo sapiens OX=9606 GN=POTEF PE=1 SV=2
82	sp|B5ME19	Eukaryotic translation initiation factor 3 subunit C-like protein OS=Homo sapiens OX=9606 GN=EIF3CL PE=3 SV=1
83	sp|Q53EP0	Fibronectin type III domain-containing protein 3B OS=Homo sapiens OX=9606 GN=FNDC3B PE=1 SV=2
84	sp|Q8N684	Cleavage and polyadenylation specificity factor subunit 7 OS=Homo sapiens OX=9606 GN=CPSF7 PE=1 SV=1
85	sp|P32969	60S ribosomal protein L9 OS=Homo sapiens OX=9606 GN=RPL9 PE=1 SV=1
86	sp|P51116	Fragile X mental retardation syndrome-related protein 2 OS=Homo sapiens OX=9606 GN=FXR2 PE=1 SV=2
87	sp|P26196	Probable ATP-dependent RNA helicase DDX6 OS=Homo sapiens OX=9606 GN=DDX6 PE=1 SV=2
88	sp|Q9UMS4	Pre-mRNA-processing factor 19 OS=Homo sapiens OX=9606 GN=PRPF19 PE=1 SV=1
89	sp|P38919	Eukaryotic initiation factor 4A-III OS=Homo sapiens OX=9606 GN=EIF4A3 PE=1 SV=4
90	sp|O75534	Cold shock domain-containing protein E1 OS=Homo sapiens OX=9606 GN=CSDE1 PE=1 SV=2
91	sp|Q00839	Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens OX=9606 GN=HNRNPU PE=1 SV=6
92	sp|P12956	X-ray repair cross-complementing protein 6 OS=Homo sapiens OX=9606 GN=XRCC6 PE=1 SV=2
93	sp|Q07065	Cytoskeleton-associated protein 4 OS=Homo sapiens OX=9606 GN=CKAP4 PE=1 SV=2
94	sp|P62277	40S ribosomal protein S13 OS=Homo sapiens OX=9606 GN=RPS13 PE=1 SV=2
95	sp|Q9NVI7	ATPase family AAA domain-containing protein 3A OS=Homo sapiens OX=9606 GN=ATAD3A PE=1 SV=2
96	sp|Q15393	Splicing factor 3B subunit 3 OS=Homo sapiens OX=9606 GN=SF3B3 PE=1 SV=4
97	sp|P26368	Splicing factor U2AF 65 kDa subunit OS=Homo sapiens OX=9606 GN=U2AF2 PE=1 SV=4
98	sp|Q16630	Cleavage and polyadenylation specificity factor subunit 6 OS=Homo sapiens OX=9606 GN=CPSF6 PE=1 SV=2
99	sp|Q6P2Q9	Pre-mRNA-processing-splicing factor 8 OS=Homo sapiens OX=9606 GN=PRPF8 PE=1 SV=2
100	sp|P0DN76	Splicing factor U2AF 35 kDa subunit-like protein OS=Homo sapiens OX=9606 GN=U2AF1L5 PE=1 SV=1
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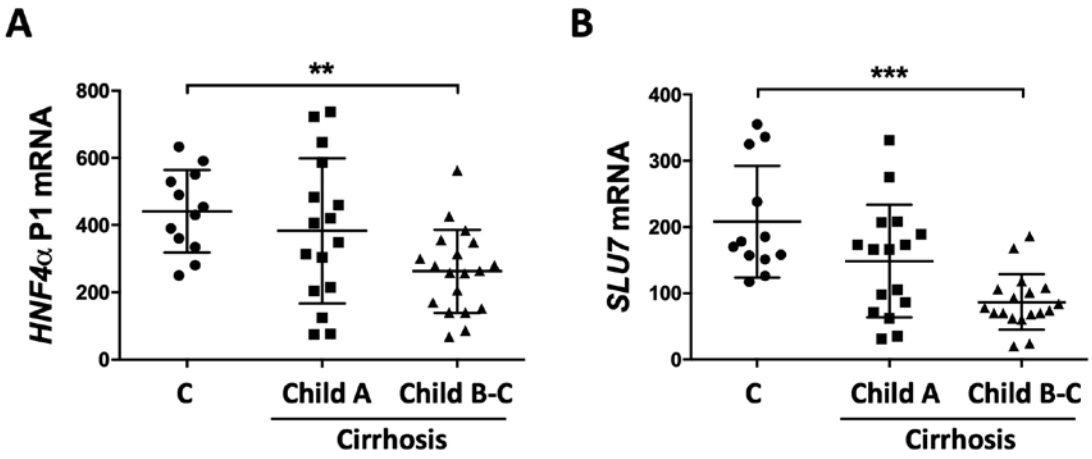
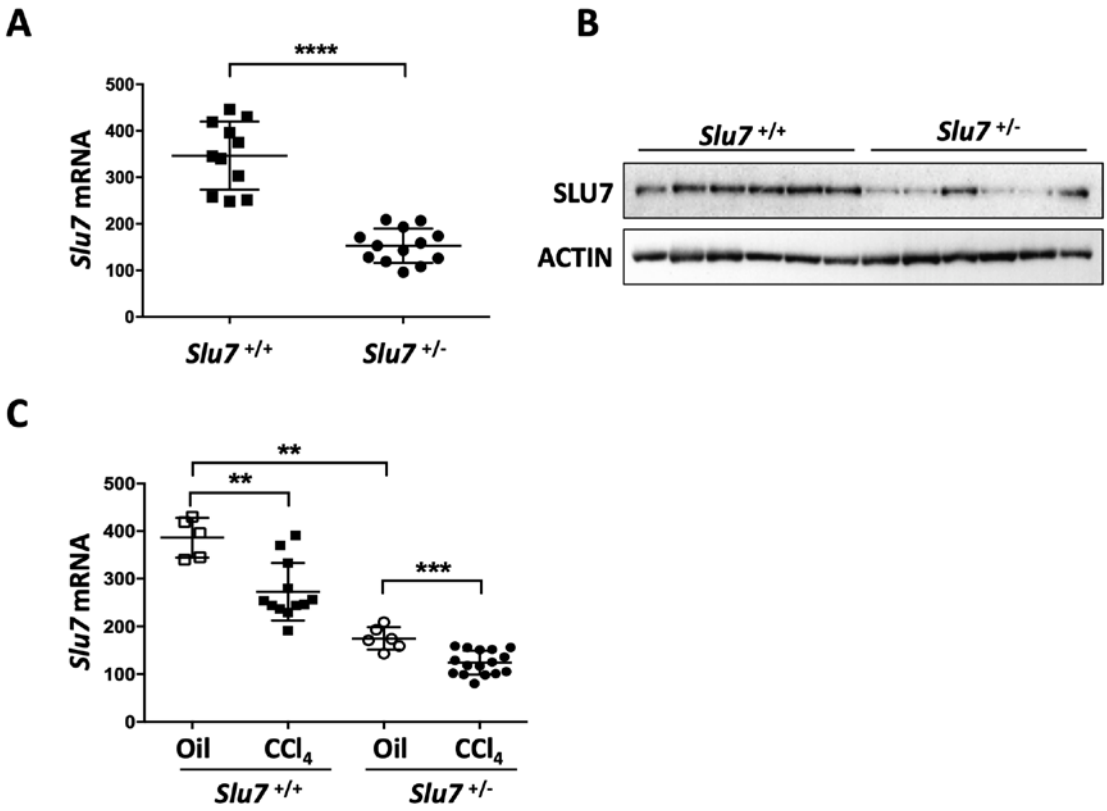
SUPPORTING FIGURES



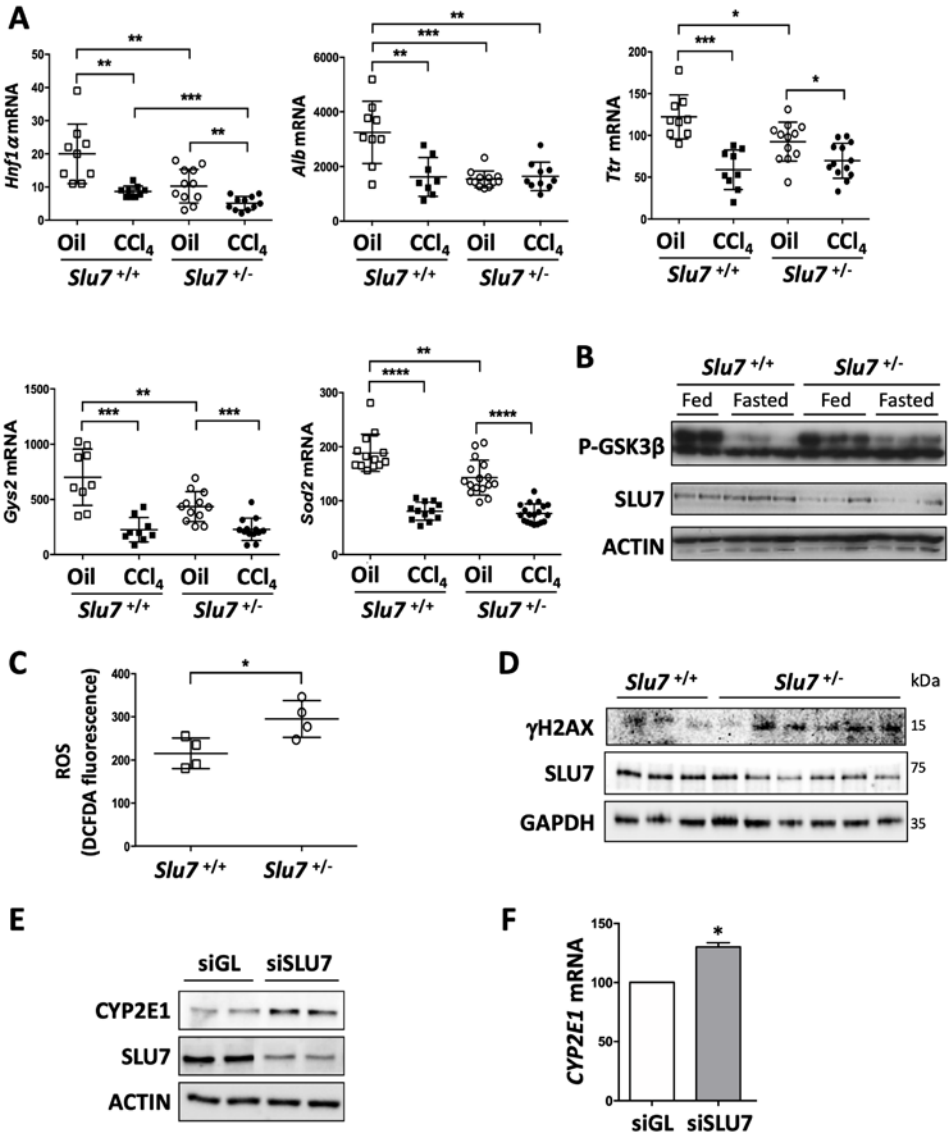
Supporting Figure 1

FIG. S1. A-B. The mRNA of *HNF4α* isoforms expressed from P1 promoter (**A**) and SLU7 (**B**) was analyzed by real time PCR in the liver of controls (n=12) and patients with Child-Pugh A (n=16) or Child-Pugh B-C (n=19) cirrhosis.



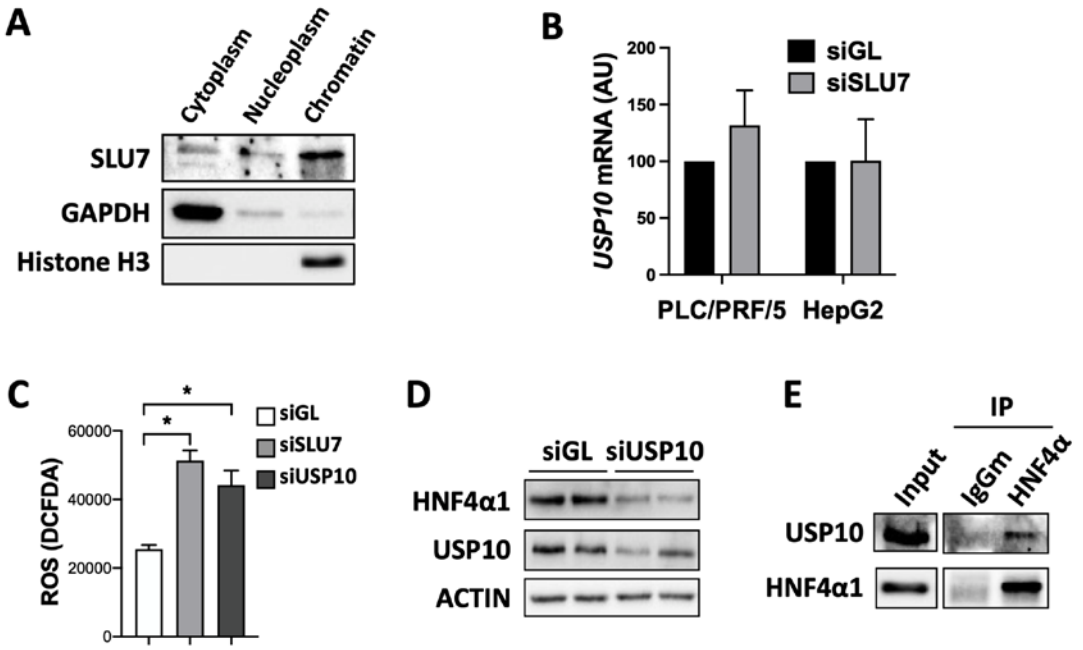
Supporting Figure 2

FIG. S2. A-B. *Slu7* mRNA **(A)** and protein **(B)** levels in the liver of *Slu7* haploinsufficient mice (*Slu7* $^{+/-}$) and their control littermates (*Slu7* $^{+/+}$). **C.** *Slu7* mRNA levels in the liver of *Slu7* $^{+/+}$ and *Slu7* $^{+/-}$ mice intraperitoneally injected with vehicle (Oil) or CCl_4 twice a week for 6.5 weeks.



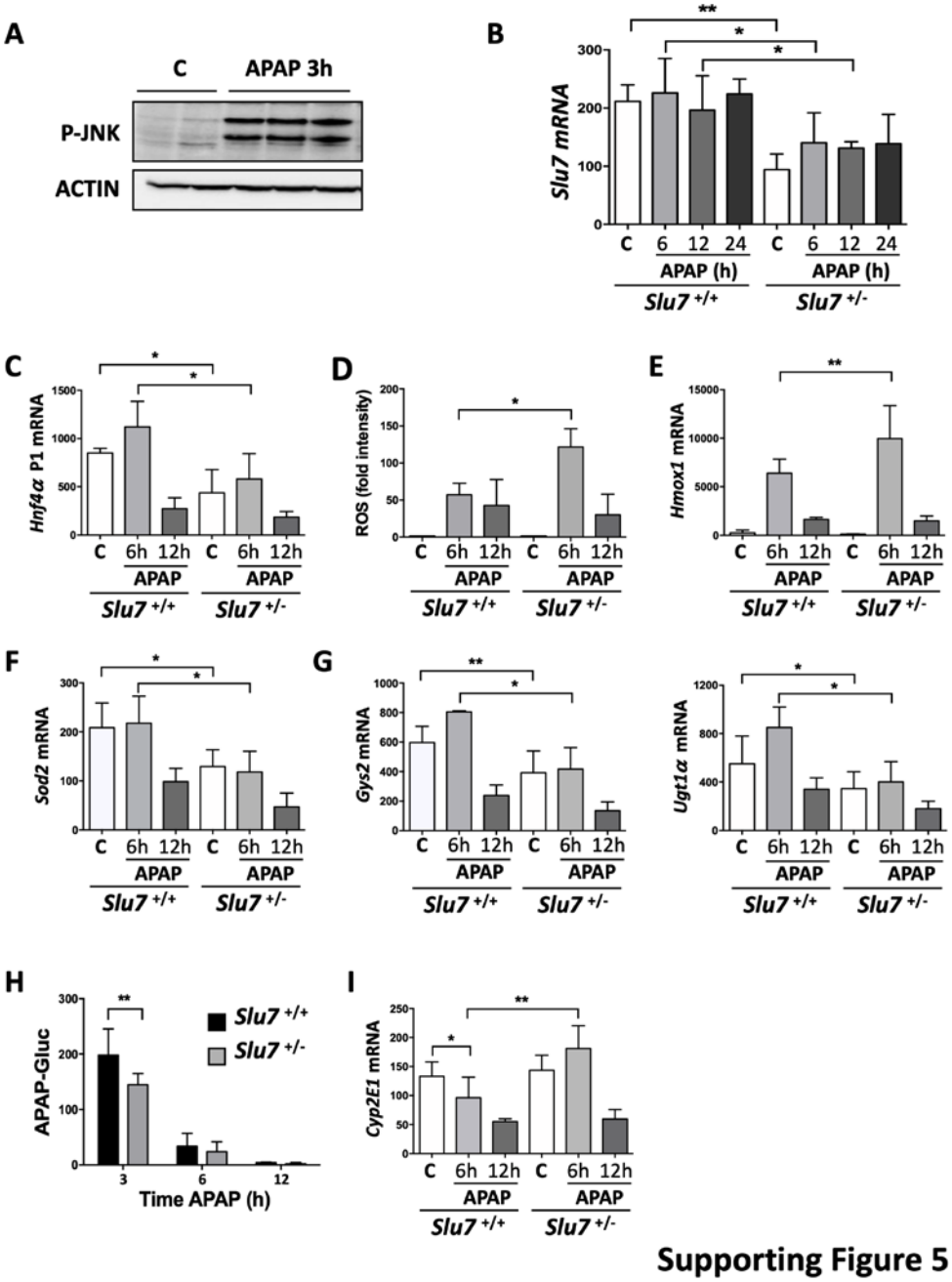
Supporting Figure 3

FIG. S3. A. RT-PCR analysis of the expression of the hepato-specific genes transcription factor *Hnf1α*, the serum proteins albumin (*Alb*) and transthyretin (*Ttr*), the enzymes glycogen synthase 2 (*Gys2*) and antioxidant superoxide dismutase 2 (*Sod2*) in the liver of *Slu7* $^{+/+}$ and *Slu7* $^{+/-}$ mice intraperitoneally injected with vehicle (Oil) or CCl_4 twice a week for 6.5 weeks. Data are expressed as mean ± SEM. **B.** Western blot analysis of proteins P-GSK3β, SLU7 and ACTIN, as loading control, in the liver of *Slu7* $^{+/+}$ and *Slu7* $^{+/-}$ mice fasted overnight or refed for 4 hours. **C.** Levels of reactive oxygen species (ROS) measured as 2', 7'-dichlorofluorescin diacetate (DCFDA) fluorescence in the liver of *Slu7* $^{+/+}$ and *Slu7* $^{+/-}$ mice. **D.** Western blot analysis of γH2AX, SLU7 and GAPDH, as loading control, in the liver of *Slu7* $^{+/+}$ and *Slu7* $^{+/-}$ mice. The samples are the same as in FIG. 3A. **E.** Western blot analysis of CYP2E1, SLU7 and ACTIN, as loading control, in HepRG cells 72 hours after transfection with siGL or siSLU7 siRNAs. **F.** RT-PCR analysis of *CYP2E1* in HepG2 cells 72 hours after transfection with siGL or siSLU7 siRNAs. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.



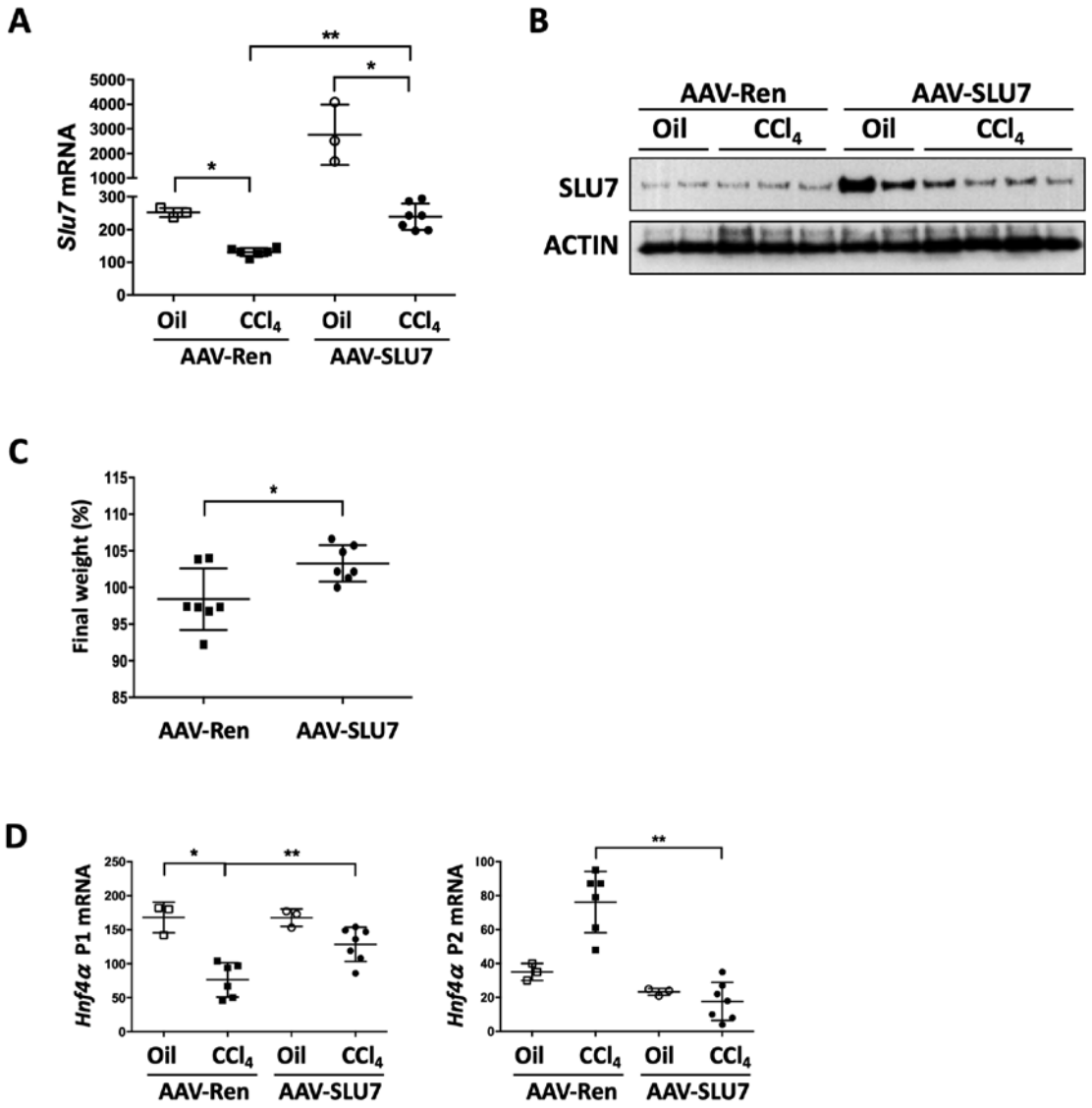
Supporting Figure 4

FIG. S4. A. Western blot analysis of SLU7 expression in different cellular compartments after cell fractionating. The expression of GAPDH and histone H3 is shown as control. **B.** *USP10* mRNA levels measured by real-time PCR in PLC/PRF/5 and HepG2 cells 48 hours after transfection with control siGL or siSLU7. Data are expressed as mean ± SEM of 4 independent experiments performed with biological duplicates. **C.** Quantification of reactive oxygen species (ROS) by 2',7'-dichlorofluorescin diacetate (DCFDA) assay in PLC/PRF/5 cells 48 hours after transfection with siGL, siSLU7 or siUSP10. **D.** Western blot expression of HNF4α1 in HepG2 cells 48 hours after transfection with control siGL or siUSP10. The expression of USP10 and ACTIN is shown as control. **E.** USP10 and HNF4α1 proteins in HNF4α1 immunoprecipitated (IP) from PLC/PRF/5 cells. Input and IP with control mouse (m) IgG are shown as control.



Supporting Figure 5

FIG. S5. A. Western blot analysis of P-JNK expression in the liver of wild type mice 3 hours after treatment with acetamoniphen (APAP). **B.** *Slu7* mRNA levels measured by real-time PCR in the liver of *Slu7* $^{+/+}$ and *Slu7* $^{+/-}$ mice intraperitoneally injected with vehicle PBS (C) or APAP, 6, 12 or 24 hours before. **C.** mRNA levels of *Hnf4*α P1 isoforms measured by real-time PCR in the liver of *Slu7* $^{+/+}$ and *Slu7* $^{+/-}$ mice 6 or 12 hours after APAP or vehicle (C) intraperitoneal injection. **D.** Levels of reactive oxygen species (ROS) measured as fold change in the intensity of 2', 7' –dichlorofluorescin diacetate (DCFDA) fluorescence in the liver of *Slu7* $^{+/+}$ and *Slu7* $^{+/-}$ mice 6 or 12 hours after APAP or vehicle intraperitoneal injection referred to control mice without APAP. **E-G.** mRNA levels of *Hmox1* **(E),** *Sod2* **(F),** *Gys2* and *Ugt1*α **(G)** measured as described in C. **H.** Serum levels of APAP-glucuronide (APAP-Gluc) conjugate in APAP-treated *Slu7* $^{+/+}$ and *Slu7*$^{+/-}$ mice. **I.** *Cyp2E1* mRNA measured as described in C. Data are expressed as mean ± SEM. **P* < 0.05, ***P* < 0.01.



Supporting Figure 6

FIG. S6. A. *Slu7* mRNA levels in the liver of wild type mice injected with a control adenoassociated virus (AAV-Ren) or an adenoassociated virus expressing SLU7 in the liver (AAV-SLU7) that 15 days later were intraperitoneally injected with vehicle (Oil) or CCl_4 twice a week for 6.5 weeks. **B.** SLU7 protein levels in the liver of wild type mice treated as described in A. **C.** Body weight of the AAV-Ren and AAV-SLU7 mice after the treatment with CCl_4 as described in A. The weight is expressed as the percentage with respect the weight recorded at the onset of CCl_4-treatment. **D.** Real time PCR analysis of *Hnf4*α-P1 and *Hnf4*α-P2 isoforms expression in the samples described in A.

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