Organic cation transporter 3 mediates cisplatin and copper cross-resistance in hepatoma cells

SUPPLEMENTARY MATERIALS

Supplementary Table 1: Cell survival of ATP7B KO cells after long-term cisplatin exposure

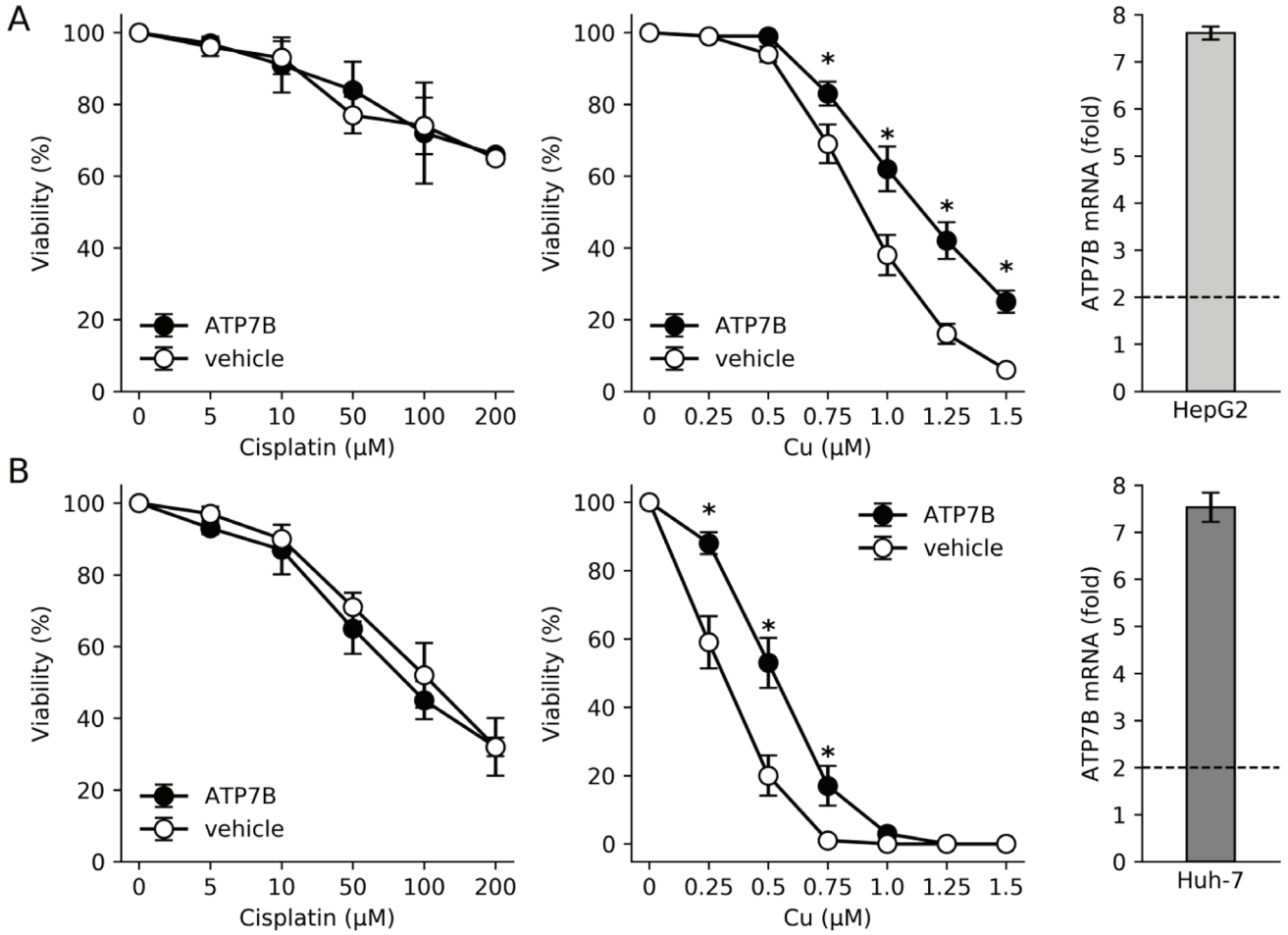
Experiment	0.1 µM	1 µM	5 µM
1	>23 d	9 d	7 d
2	>23 d	9 d	7 d
3	>23 d	21 d	ND*

*ND, not done

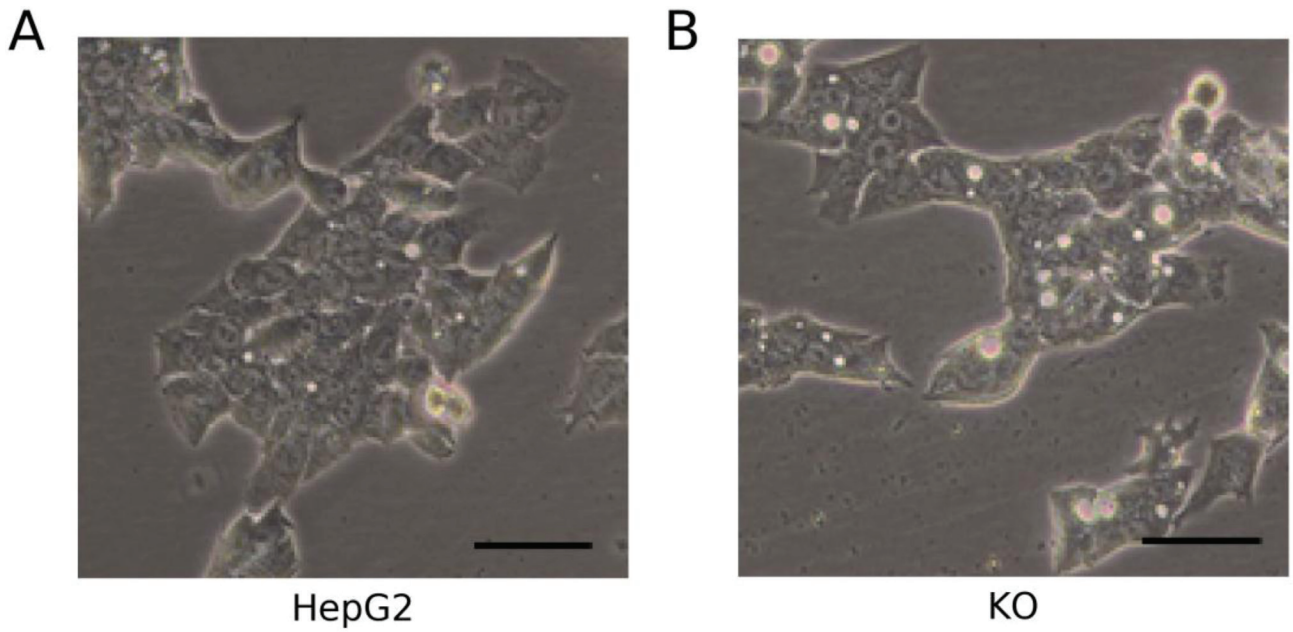
Supplementary Table 2: Primers used for RT-qPCR analysis

Gene Symbol	Synonym	Accession Number	Forward/Reverse (5′–3′)
ATP7A	ATPase, Cu++ transporting, alpha polypeptide	NM_000052	AGCAATGGCTGCTTCATCTG/ GCAGGCAGTTCATAACTCTCG
CP	Ceruloplasmin	NM_000096	CACGGCCATAGCTTCCAATAC/ CCAAATTCCAGGTGTTCTTGG
CTR1	Copper transporter 1	NM_001859	GTCCCAGGACCAAATGGAAC/ ACCACCTGGATGATGTGCAG
CTR2	Copper transporter 2	NM_001860	TGCAGGCTCAGATTCATTCC/ TGACCACCTGGATGACATGG
DMT1	Divalent metal transporter 1	NM_001174127	GGGTTGGCAATGTTTGATTG/ GCGTCCATGGTGTTCAGAAG
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	CCCACTCCTCCACCTTTGAC/ CCACCACCCTGTTGCTGTAG
GST	Glutathione S-Transferase	NM_000561	CCGTATATTTGAGCCCAAGTGC/ GCTTTGAGGGCTTGGAGAAGA
HPRT	hypoxanthine phosphoribosyltransferase 1	NM_000194	TTATGCTGAGGATTTGGAAAGG/ CCATCTCCTTCATCACATCTCG
MATE1	Multidrug and toxin extrusion member 1	NM_018242	AAGCTGGAGCTGGATGCAGTC/ CAGCAGAGGAGCAGGACGAGC
MATE2	Multidrug And Toxin Extrusion1	NM_001099646	GGAAGCTTGCTGCAGAGGAG/ GAGCACTGCAACCAGACCTG
MDR1	Multi Drug Resistance Protein 1	NM_000927	TCGTGCCCTTGTTAGACAGC/ CCAAGAAGCCCTGGACAAAG
MRP1	Multidrug Resistance Associated Protein 1	NM_004996	ACTGCACCGTCCTCACCATC/ GGAGAAATCCAGGAGTACGGC
MRP2	ATP Binding Cassette Subfamily C Member 2	NM_000392	CGAAGTGACAGAGGCTGGTG/ GCTCTGCTTCGGAAATCCAA
MT1	Metallothionein 1	NM_005952	CTCCTTGCCTCGAAATGGAC/ GCATTTGCACTCTTTGCATTTG

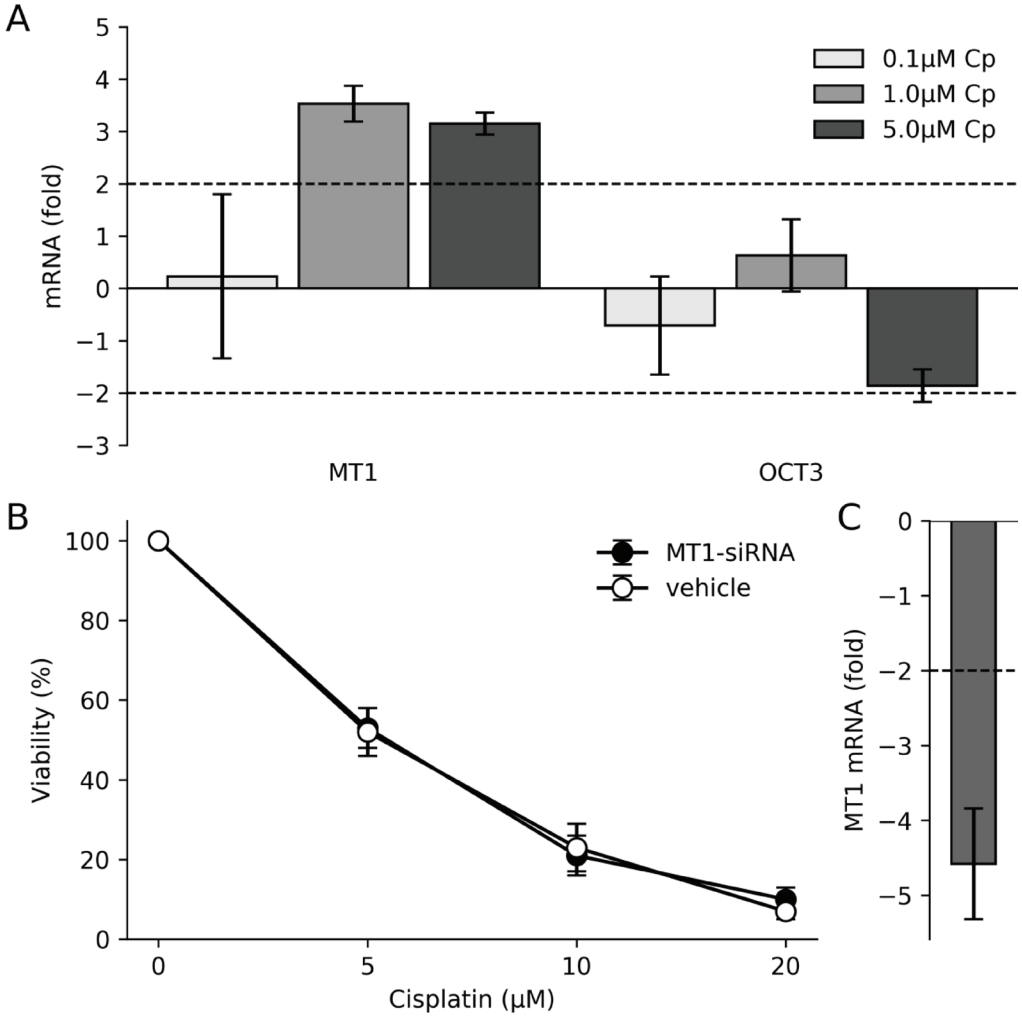
OCT1	Organic cation transporter 1	NM_001198783	CATCATAATCATGTGTGTTGGCC/ CAAACAAAATGAGGGGCAAGGCTT
OCT2	Organic cation transporter 2	NM_003058	TTCCTGGTCTACCGGCTCAC/ CAACAGCACCAGACCTCCAG
OCT3	Organic cation transporter 3	NM_001173531	GACAAGAGAAGCCCCCAACCTGAT/ CACTAAAGGAGAGCCAAAAATGTC
OCTN1	Organic carnitine transporter 1	AB007448	GTGCTGTGTGTCCCGCTGTG/ CGAGTCCTGAACAGGTCCAG
OCTN2	Organic carnitine transporter 2	AB015050	CATGCAGACAGGCTTCAGCTTC/ ATGCACACTCCTAACGTAGAG



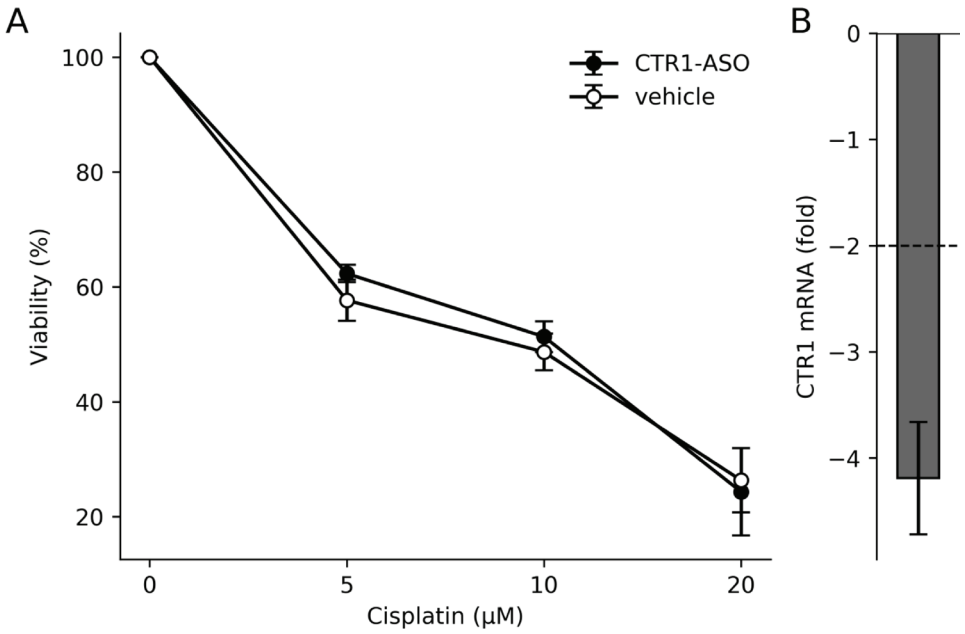
Supplementary Figure 1: Overexpression of ATP7B does not confer resistance to cisplatin in hepatoma cell lines. Cell viability of HepG2 cells (**A**) and Huh-7 cells (**B**) transduced with empty (white) or retroviral ATP7B expression vector (black) was determined. Mean/SE are given ($n = 5$) (left graphs). ATP7B mRNA expression relative to empty vector transduced cells is shown. Mean/SE are given ($n = 3$) (right bar). $^{*}P < 0.05$.



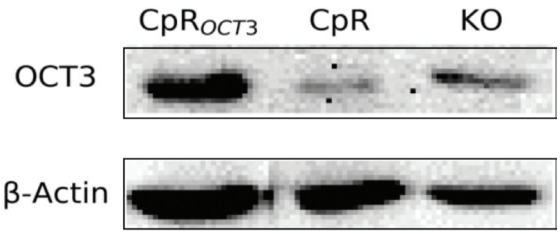
Supplementary Figure 2: Cell morphology of HepG2 and ATP7B KO. Light microscopic image of HepG2 (**A**) and ATP7B KO (**B**) cells for comparison. Scale bar, 50 µm.



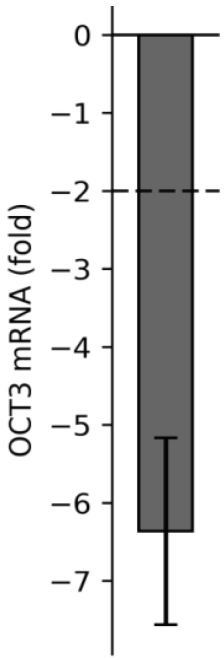
Supplementary Figure 3: Knockdown of MT1 does not affect cisplatin resistance. (**A**) mRNA expression of ATP7B KO cells after 72 h exposure to 0.1 (light grey), 1.0 (grey) and 5.0 μM (dark grey) cisplatin. Expression is shown relative to untreated cells. Mean/SE are given ($n = 3$). (**B**) Cell viability of ATP7B KO cells treated with MT1-specific siRNA (black) or vehicle (white). Mean/SE are given ($n = 3$). (**C**) mRNA expression of ATP7B KO cells after treatment with MT1-specific siRNA. Mean/SE are given ($n = 3$).



Supplementary Figure 4: Downregulation of CTR1 does not affect cisplatin sensitivity. (**A**) Cell viability of ATP7B KO cells transfected with scrambled oligonucleotide (white) or CTR1-antisense oligonucleotide (ASO) (black). Mean/SE are given ($n = 3$). (**B**) RT-qPCR analysis of ATP7B KO cells after CTR1-ASO treatment. Mean/SE are given ($n = 3$).



Supplementary Figure 5: Protein expression of OCT3. Western blot of OCT3 expression in CpR$_{OCT3}$, CpR and ATP7B KO cells. β-Actin was used for internal control. One typical experiment is shown ($n = 3$).



Supplementary Figure 6: OCT3 is downregulated after siRNA treatment. RT-qPCR analysis of ATP7B KO cells mRNA after OCT3 siRNA treatment. Mean/SE are given (n = 6).