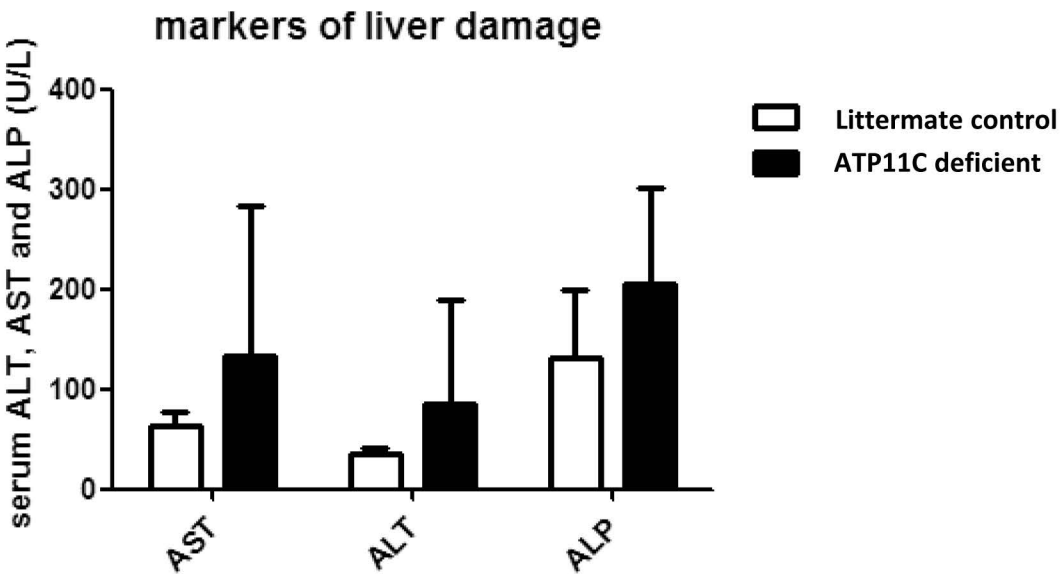
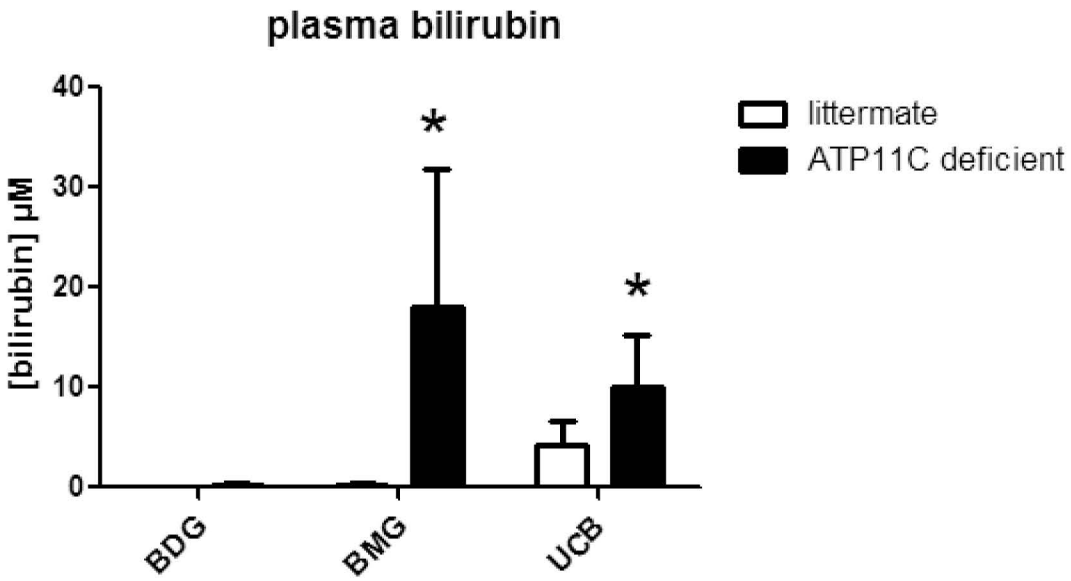
Supplemental figure 1



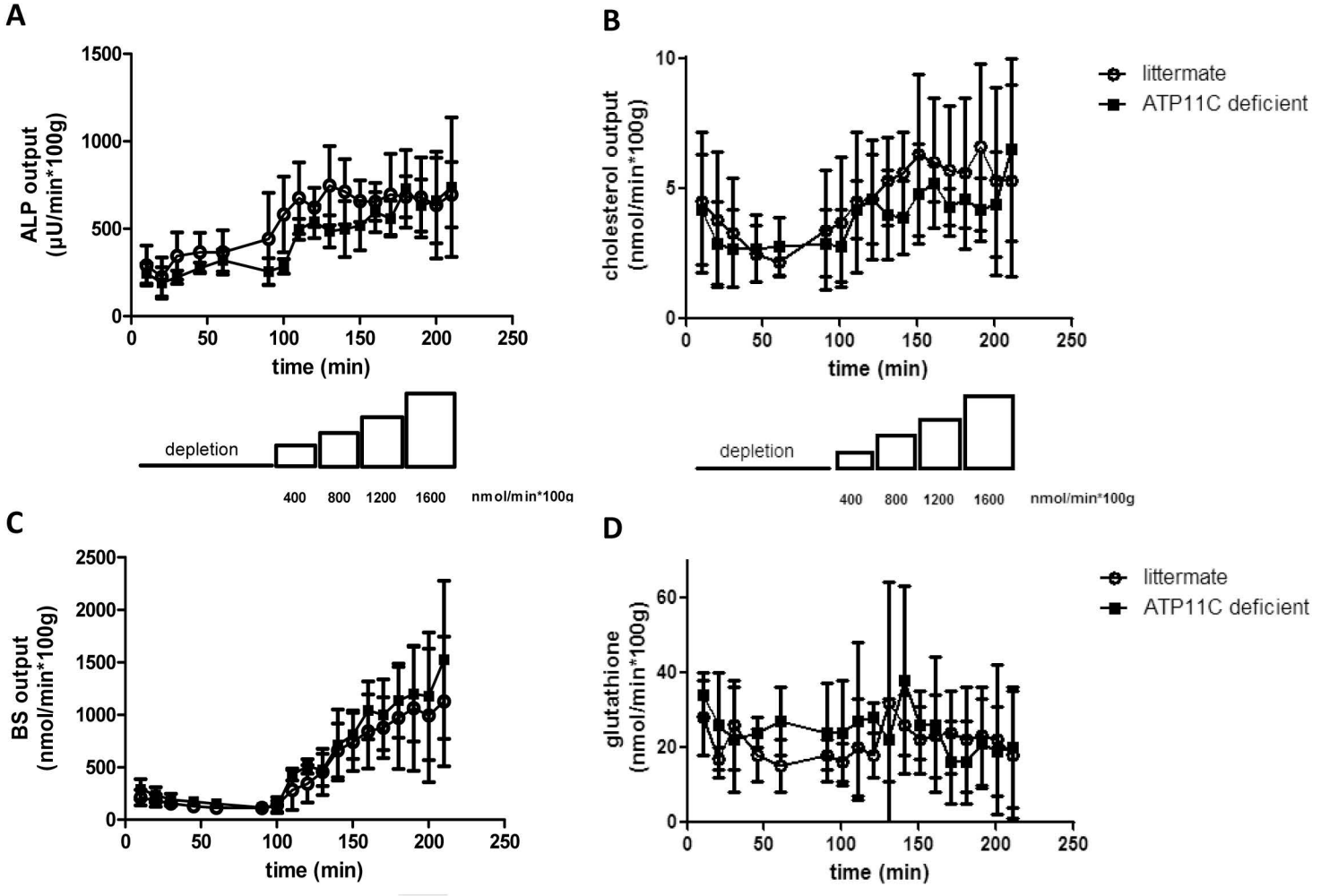
Supplemental figure 1:
Plasma levels of aspartate aminotransferase (ASAT), alkaline phosphatase (ALP) and alanine aminotransferase (ALAT) in littermate controls and ATP11C deficient mice. Data show mean values ± standard deviation for littermate controls (n=5) and ATP11C deficient mice (n = 9). Statistical significance was tested by a Student's *t*-test: *$p<0.05$.

Supplemental figure 2



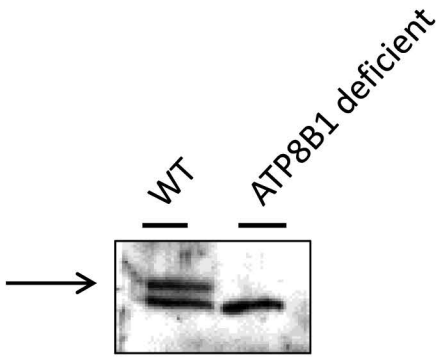
Supplemental figure 2:
Plasma bilirubin species levels in male ATP11C deficient mice (n=9) versus littermate controls (n=5). Abbreviations: BDG = bilirubin-di-glucuronide, BMG = bilirubin-mono-glucuronide and UCB = unconjugated bilirubin. Significance was tested by a Student's *t*-test: *$p<0.05$.

Supplemental figure 3



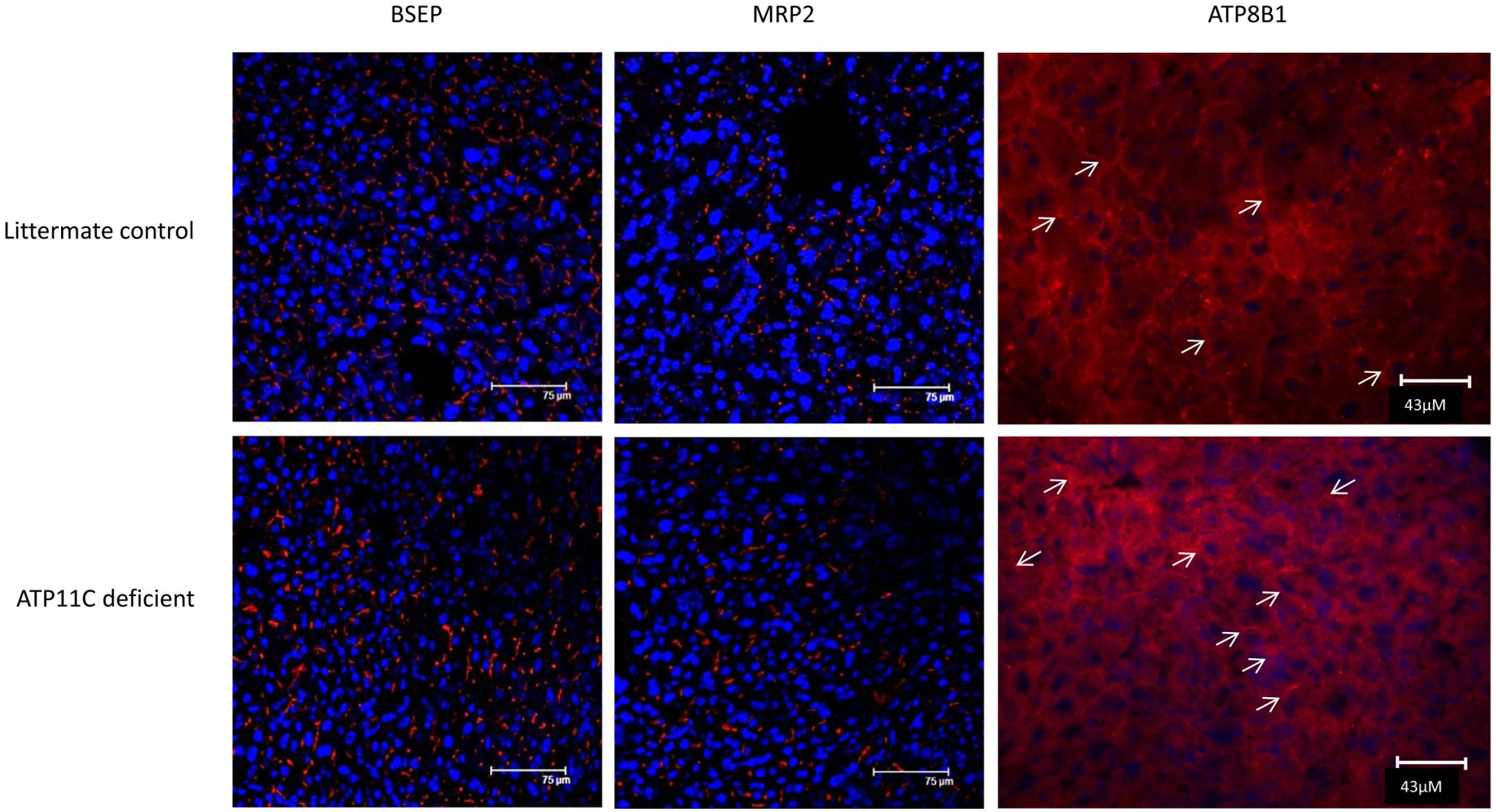
Supplemental figure 3:
ATP11C deficient mice have no defect in bile formation. Atp11c mutants (n=5) and littermate control mice (n=7) were depleted from their endogenous bile salt pool for 90 min. and taurocholate (TC) was infused via the jugular vein. Infusion rates were increased stepwise every 30 min with 400 nmol/min*100g (400-800-1200-1600 nmol/min*100g). The biliary output of (A) alkaline phosphatase (ALP), (B) cholesterol, (C) bile salts (BS) and (D) glutathione is plotted in time. Procedures were as described previously (32). Significance was tested by a Student's t-test. No significant differences were observed.

Supplemental figure 4



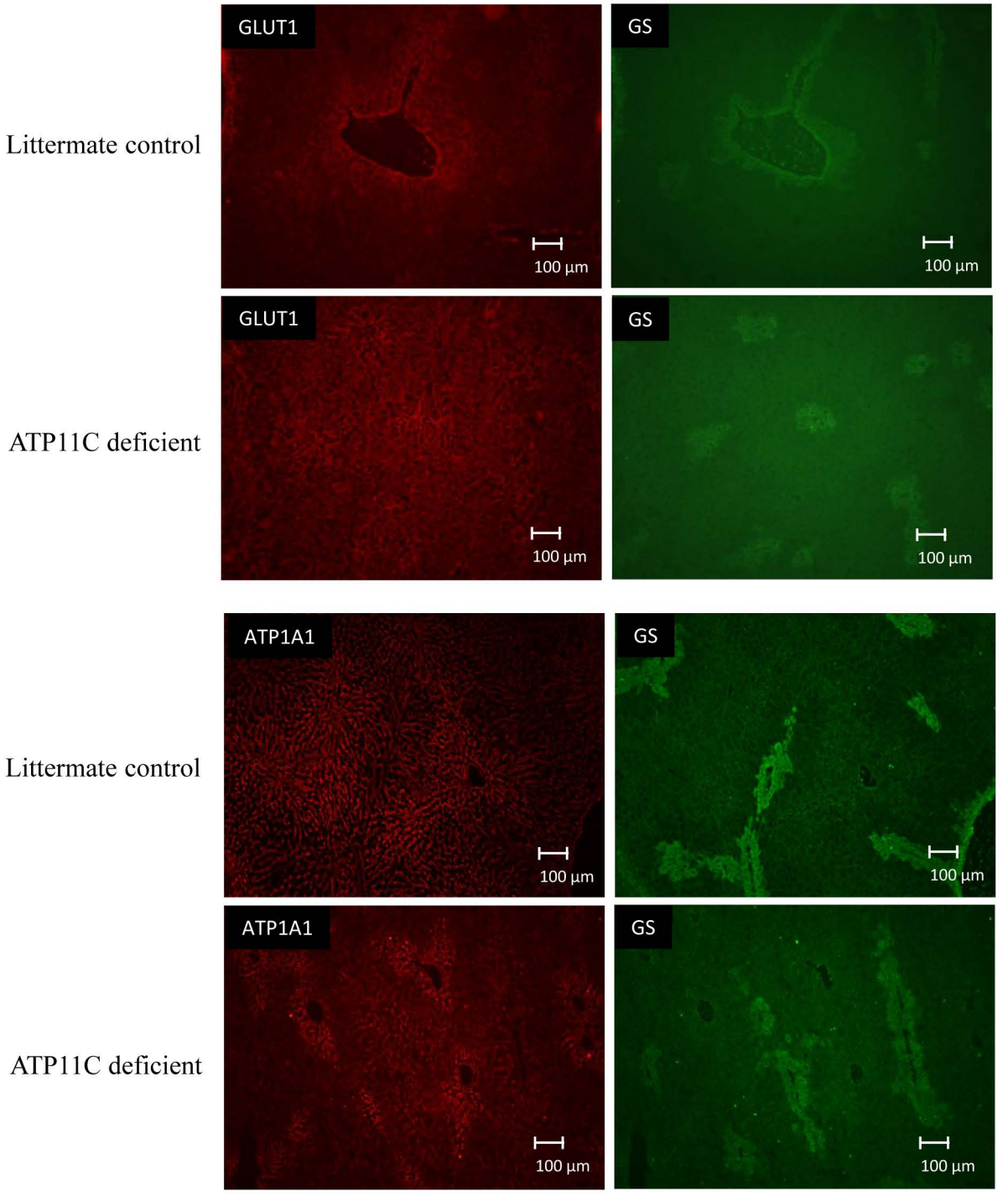
Supplemental figure 4:
Western blot analysis of ATP8B1 in liver plasma membranes from wild type and *Atp8b1* deficient mice. With the antibody used, 2 bands are observed in wild type liver whereas in *Atp8b1* deficient liver only the upper band (corresponding to ATP8B1; indicated by the arrow) has disappeared.

Supplemental figure 5A



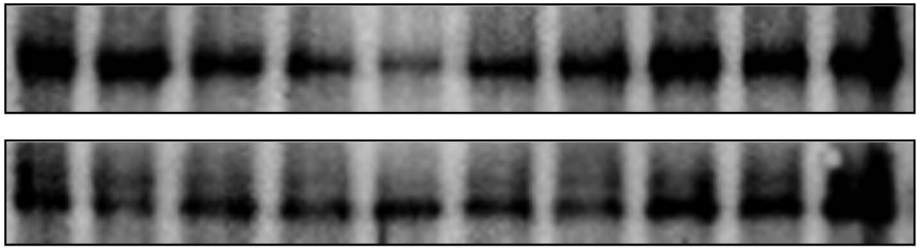
Supplemental figure 5A:
Immunofluorescent detection of BSEP and MRP2 both by confocal microscopy. ATP8B1 is detected in liver sections of littermate control and ATP11C deficient mice (canalicular staining is indicated by arrows).

Supplemental figure 5B



Supplemental figure 5B:
Immunofluorescent detection of GLUT1 and ATP1A1 in liver sections of littermate control and ATP11C deficient mice. GLUT1 immunosignal is detected in central hepatocytes of both wild type and ATP11C deficient liver. ATP1A1 is homogenously distributes in liver of both genotypes with a slight portal-to-central gradient. GS staining is included to indicate the central hepatocytes.

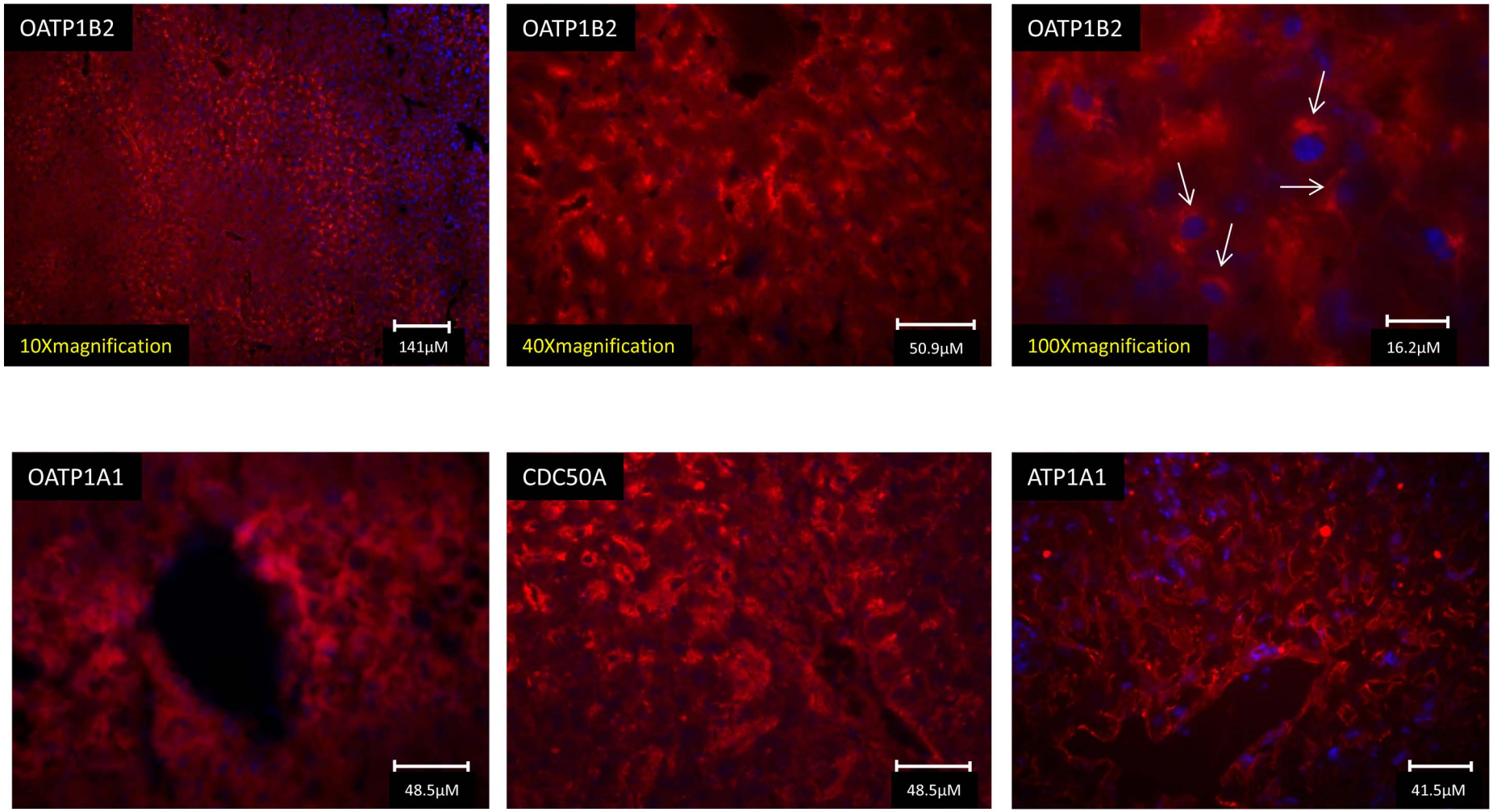
Supplemental figure 6



	% of untreated	p value
ATP8B1	101 19	0.972
ATP1A1	114 17	0.211

Supplemental figure 6:
Western blot analysis of ATP8B1 in liver plasma membranes from shControl mice and mice after AAV8-mediated delivery of shRNAs to *Cdc50a*. Data show means ± standard deviation for male ATP11C deficient (n=9) and littermate control mice (n=5). Statistical significance was tested by a Student's *t*-test: *$p<0.05$.

Supplemental figure 7



Supplemental figure 7:
Immunofluorescent detection of OATP1B2, OATP1A1, CDC50A and ATP1A1 in liver sections of ATP11C deficient mice treated with bortezomib. OATP1B2 is shown for three different magnifications; Although immunostaining was detected, no membrane localization was observed after bortezomib treatment, except for ATP1A1. Arrows indicate accumulation of OATP1B2 immunostaining in perinuclear structures.

Supporting information experimental procedures

Western blotting and protein analysis

Mouse liver plasma membrane protein lysates (60 µg protein) were separated by 8% SDS-PAGE. Proteins were transferred to PVDF membranes and blocked in phosphate-buffered saline (PBS)/ 5% milk powder/ 0.05% Tween-20. Primary antibodies were incubated for 1.5h at RT. Immune complexes were visualized with horseradish-peroxidase-conjugated immunoglobins (Biorad, Roosendaal, The Netherlands) and detected using ECL Western blot detection kit (Amersham).

Quantitative PCR analysis of gene expression

RNA was isolated using Trizol reagent (Invitrogen). cDNA was synthesized using oligo-dT / random hexamers and Superscript III reverse transcriptase (Invitrogen). Quantitative PCR was performed using a Lightcycler 2.0 with the Fast Start DNA MasterPlus SYBR Green I kit (Roche). Expression levels were normalized to the geometrical mean of *cyclophillin*, *HPRT* and *36B4*, which were the most stable as determined after Genorm (20). Primer sequences are in supplementary table 1.

Immunofluorescence

Liver cryosections were fixed in 100% acetone for 10 min at RT and blocked for 1 hr at RT in block buffer (5% normal goat serum in PBS/0.05% Tween-20 (PBS/Tw)). Primary antibodies were incubated for 1 hr at RT in block buffer. Immune complexes were visualized with goat-anti-mouse alexa 488 or goat-anti-rabbit alexa 594 (Molecular Probes) incubated in PBS/Tw for 1 hr at RT. Sections were mounted in Vectashield/DAPI (Vector Laboratories) and studied in a Leica DM-RA2 fluorescent microscope or a TCS-SP2 confocal microscope.

Supplementary table 1

Sequences of oligonucleotides used in this study

	Forward primer	Reverse primer
Cyclophilin	TCGGAGCGCAATATGAAGGT	AAAAGGAAGACGACGGAGCC
HPRT	TTGCTCGAGATGTCATGAAGGA	AGCAGGTCAGCAAAGAACTTATAG
36B4	GGACCCGAGAAGACCTCCTT	GCACATCACTCAGAATTTCAATGG
Atp11c	CGAAGAAGAAGTGCCAGGAATCCGA	GCTGCAACCACGGTCAATATGCT
Atp8b1	GTCTGGGACAGAGTCATTTC	CTTATCAGAGAAGATGTAATG
Cdc50a	GAAGAAATGAAGACAGACC	GGCAACCAGATACAATTCTAACG
Oatp1a1	CAGATAAATGGATTTGCCAG	GTCAACAAATAGTTACAGAG
Oatp1a4	ATAGCTTCAGGCGCATTTAC	TTCTCCATCATTCTGCATCG
Oatp1b2	TTCACCACAACAATGGCCTA	TTTTCCCCACAGACAGGTTC
Ntcp	CACCATGGAGTTCAGCAAGA	AGCACTGAGGGGCATGATAC
Bsep	TGGAAAGGAATGGTGATGGG	CAGAAGGCCAGTGCATAACAGA
Mrp2	AGCAGGTGTTCGTTGTGTGT	AGCCAAGTGCATAGGTAGAGAAT
Cyp7A1	CTGTCATACCACAAAGTCTTATGTCA	ATGCTTCTGTGTCCAAATGCC
Cyp8b1	TTCGACTTCAAGCTGGTCGA	CAAAGCCCCAGCGCCT
Fxr-α	TGGGCTCCGAATCCTCTTAGA	TGGTCCTCAAATAAGATCCTTGG
Fxr-β	GGGCTTAGAAAATCCAATTCAGATTA	CGTCCGGCACAAATCCTG
Shp	CAGGCACCCTTCTGGTAGATCT	TGTCTTCAAGGAGTTCAGTGATGTC
GS	CGGCCACCGCTCTGAA	ACATTTGCTTGATGCCTTTGTTC
Ki67	ACCGTGGAGTAGTTTATCTGGG	TGTTTCCAGTCCGCTTACTTCT
CK7	GCGAGGAGAGCCGGTTGTCTG	AATTGTGGGTGCCCCTGCGG
CK19	GCCACCTACCTTGCTCGGATTG	TGGCGCTCTATGCGGCACG