Supplementary Material

Lack of multidrug-resistance associated protein 4 prolongs partial hepatectomyinduced hepatic steatosis.

Ajay C Donepudi¹, Gregory J. Smith¹, Oladimeji Aladelokun¹, Yoojin Lee², Steven J Toro¹, Marisa Pfohl³, Angela L Slitt³, Li Wang⁴, Ji-Young Lee², John D. Schuetz⁵, and José E Manautou¹

¹ Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT,

² Department of Nutritional Sciences, University of Connecticut, Storrs, CT;

³ Department of Biomedical Sciences, University of Rhode Island, Kingston, RI;

⁴ Department of Internal Medicine, Section of Digestive Diseases, Yale University, New

Haven, Connecticut;

⁵ Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital,

Memphis, TN.

Gene ID	Forward	Reverse
Mrp4	AGGAGCTTCAACGGTACTGG	GCCTTTGTTAAGGAGGGCTTC
Cyclin D1	GCGTACCCTGACACCAA	ACTTGAAGTAAGATACGGAGGC
Pcna	TTTGAGGCACGCCTGATCC	GGAGACGTGAGACGAGTCCAT
Lipin1	CATGCTTCGGGAAAGTCCTTCA	GGTTATTCTTTGGCGTCAACCT
Dgat1	CTGATCCTGAGTAATGCAAGGTT	TGGATGCAATAATCACGCATGG
Dgat2	CGAGACACCATAGACTACTTGCT	GCGGTTCTTCAGGGTGACTG
Lipin2	CAGTGAAGATGAGAAGACGGTTCAGGA	TTCCTTCACAGTGACGAGCACCTG
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

Supplemental table-1: Primer sequences for genes analyzed using RT-qPCR.

Fas	Ccl2	Gclc	Cidea	Cyp4a14	Hprt
Acaca	Tnf	Gstm3	Fabp4	Ehhadh	Eif3f
Ppara	IL6	Nqo1	Pparg	Hmgcs1	Actb
Acot2	Csf2ra	Nfe2l2	Fatp1	Mttp	Ppia
Srebf1	Cpt1b	Gpam	Fabp1	Lpl	Gusb
Scd1		Sod1	Cd36	Cpt1a	Gapdh

Supplemental table-2: List of genes analyzed using on multiplex platform. Total 35 genes were analyzed using multiplex platform. Housekeeping genes are tabulated in the last column of the table.



Supplemental Figure-1: Lack of Mrp4 results in persistent hepatic triglyceride accumulation following PH-surgery. Biochemical quantification of hepatic triglyceride levels in WT and Mrp4 KO mice. Data are presented as fold change compared to WT mice and $p \le 0.05$ is considered as statistically significant. An asterisk "*" denotes significant difference between WT and Mrp4 KO mice.



Supplemental Figure-2: Lipidomics analysis for determining hepatic lipid composition. WT and Mrp4 KO sham mice livers were analyzed for composition of hepatic lipid species such as acylcarnitine (Acyl Car), cholesterol ester (CE), cholesterol (Chol), diglyceride (DG), Free fatty acids (FA), ceramide (Cer), lysophosphatidylethanolamine (LPE), lysophosphatidylcholine (LysoPC), lysophosphatidylinositol (LysoPI), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM) and triglyceride (TG). Data are presented as fold change compared to WT mice and $p \le 0.05$ is considered as statistically significant. An asterisk "*" denotes significant difference between WT and Mrp4 KO mice.



Supplemental Figure-3:Hepatic expression of proinflammatory genes. WT and Mrp4 KO liver mRNA levels were analyzed for proinflammatory gene expression on quantigene 2.0 multiplex platform. Data are presented as fold change compared to WT sham mice and p≤0.05 is considered as statistically significant. An asterisk "*" denotes significance difference between WT and Mrp4 KO mice.



Supplemental Figure-4: Expression of hepatic lipid metabolism and oxidative stress genes in WT and Mrp4 KO mice following either sham or PH-surgery. Data are presented as mean \pm S.E.M. (p≤0.05). An asterisk "*" denotes significant difference between WT and Mrp4 KO mice.