Colesevelam attenuates cholestatic liver and bile duct injury in *Mdr2*$^{-/-}$ mice by modulating composition, signaling and excretion of fecal bile acids

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This work was supported by grant F3517-B20 from the Austrian Science Foundation (to MT).

Supporting Figure 1: Colesevelam does not interfere with body weight and food intake. (A) Body weight and **(B)** food intake of *Mdr2*$^{-/-}$ mice remains unaffected by colesevelam treatment.

Supporting Figure 2: Colesevelam restores liver phenotype in *Mdr2*$^{-/-}$ mice. (A) H&E staining (10x magnification) reflects complete reversion of liver and bile duct injury in *Mdr2*$^{-/-}$ mice fed with colesevelam. **(B)** Serum levels of transaminases (ALT, AST), alkaline phosphatase (AP) and bile acids (BAs) of *Mdr2*$^{-/-}$ mice fed with colesevelam reach the normal range of WT mice. **(C)** Liver to bodyweight ratio (LW/BW) is improved by colesevelam treatment. Data shown in this figure are obtained from a separate experimental series including WT mice as an additional group. # Significant difference from WT mice. * Significant difference from *Mdr2*$^{-/-}$ control group; $p < 0.05$.

Supporting Figure 3: Colesevelam treatment improves liver histology in *Mdr2*$^{-/-}$ mice. Individually displayed H&E stainings (10x magnification) show improvement of liver and bile duct injury in all mice fed with colesevelam (right panel) over the timeperiod of 8 weeks compared to untreated (chow-fed) *Mdr2*$^{-/-}$ mice (left panel).

Supporting Figure 4: Colesevelam improves liver fibrosis and alters hepatic BA transport. (A) Western blot was performed for αSMA (fibrotic marker) and tubulin (internal control), reflecting reduced levels of αSMA in the colesevelam treated *Mdr2*$^{-/-}$ mice. **(B)** Protein expression of the basolateral BA uptake system NTCP is increased while expression of the canalicular export transporter BSEP remains unaffected by colesevelam. * Significant difference from *Mdr2*$^{-/-}$ control group; $p < 0.05$.

Supporting Figure 5: Impact of colesevelam treatment on gut microbiota in *Mdr2*$^{-/-}$ mice. (A) 16s rRNA microbiome analysis showed an increase in the phylum Proteobacteria in *Mdr2*$^{-/-}$ mice fed with colesevelam compared to control mice. **(B)** Linear discriminant analysis effect size (LEfSe) method was used to compare microbiome of *Mdr2*$^{-/-}$ control and *Mdr2*$^{-/-}$ colesevelam fed mice. Green dots represent bacteria which are predominant in control animals, red dots represent bacteria predominant in colesevelam fed animals. It is indicated that the increase of Proteobacteria seen in *Mdr2*$^{-/-}$ mice fed with colesevelam is due to elevated abundance of δ-Proteobacteria. Of note, the class clostridia (exhibiting 7α dehydroxylase activity) is predominant in *Mdr2*$^{-/-}$ control mice.

Supporting Figure 6: Exendin-4 treatment increases cholangiocellular proliferation in *Mdr2*$^{-/-}$ mice. (A) CK19 IHC (10x magnification) reflects increased bile duct mass in exendin-4 treated *Mdr2*$^{-/-}$ mice. **(B)** Ki67 IHC staining (arrowheads indicate Ki67 positive cholangiocytes, 20xmagnification) reflecting increased cell proliferation in exendin-4 treated *Mdr2*$^{-/-}$ mice. * Significant difference from *Mdr2*$^{-/-}$ control group; $p < 0.05$.

Supporting Figure 7: Exendin-4 treatment does not promote reactive cholangiocyte phenotype in *Mdr2*$^{-/-}$ mice. (A) VCAM-1 (IHC, 20x magnification, corresponding enlarged inserts for better visualization of positive cells) and **(B)** OPN (IHC, 10x magnification) two markers for the reactive cholangiocyte phenotype are not increased by exendin-4 treatment in *Mdr2*$^{-/-}$ mice.

Supporting Figure 8: Exendin-4 treatment increases cholangiocellular proliferation in WT mice fed with DDC diet. A) CK19 IHC (10x magnification) reflects increased bile duct mass in exendin-4 treated WT DDC fed mice as another model of sclerosing cholangitis. **(B)** Ki67 IHC staining (20xmagnification, corresponding enlarged inserts for better visualization of positive cholangiocytes) reflecting increased cell proliferation in exendin-4 treated WT DDC fed mice. Please note that dark brown pigments (asterisks) are protoporphyrin plaques which are typical signs of DDC feeding. * Significant difference from the WT DDC group; $p < 0.05$.

Supporting Figure 9: Exendin-4 treatment does not promote reactive cholangiocyte phenotype in WT mice fed with DDC diet. (A) VCAM-1 (IHC, 20x magnification) and **(B)** OPN (IHC, 10x magnification) two markers for the reactive cholangiocyte phenotype are not increased by exendin-4 treatment in WT DDC fed mice. Please note that dark brown pigments (asterisks) are protoporphyrin plaques which are typical signs of DDC feeding