

Supplementary information

Supplementary figure legends:

Supplementary figure 1: Bile flow, plasma bile salt levels, biliary phospholipid output and cholesterol saturation index of bile in WT mice.

(A) Biliary phospholipid output in WT C57BL/6J mice (in nmol/min/100g body weight). Gall bladders of the mice were cannulated one hour after treatment, standardized 10-min bile samples were collected and data was plotted in time. (B) Hepatic bile flow in WT C57BL/6J mice. (C) Plasma bile salt levels of the same mice. (D+E) Hepatic mRNA expression levels of (D) *Cyp7a1* and (E) *Shp* of the same mice. (F) Molar cholesterol % of bile, (G) cholesterol saturation index of bile (calculated using critical tables for cholesterol saturation (Carey MC. Critical tables for calculating the cholesterol saturation of native bile. *J Lipid Res.* 1978;19(8):945-55) and (H) phospholipid to bile salt ratio in bile. White squares/bars indicate the vehicle group and black squares/ dark grey bars indicate the Myrcludex B group. Differences between groups were analyzed using Mann-Whitney U. Asterisk indicates $p < 0.05$. $n = 8$ mice/group.

Supplementary figure 2: Bile flow in *Abcg8*^{-/-} mice

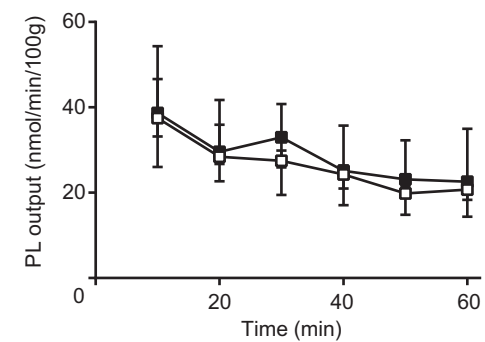
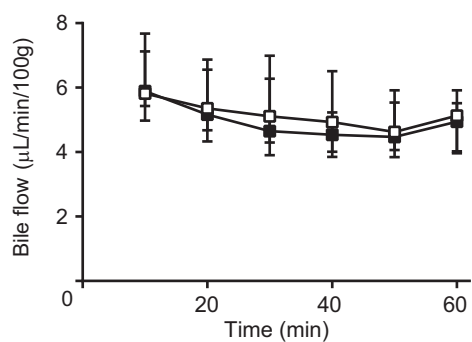
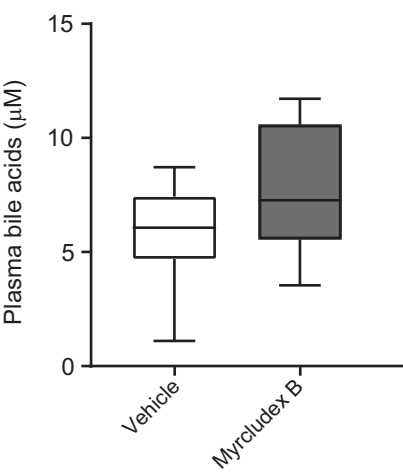
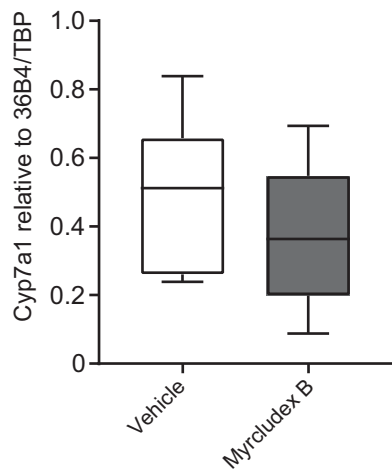
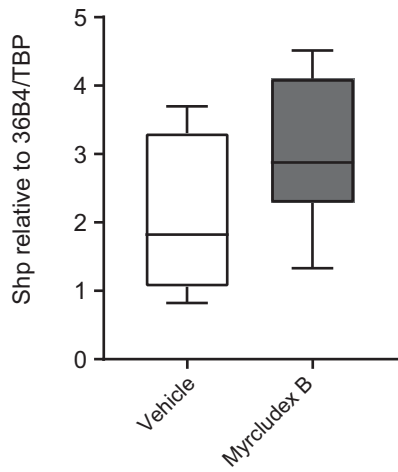
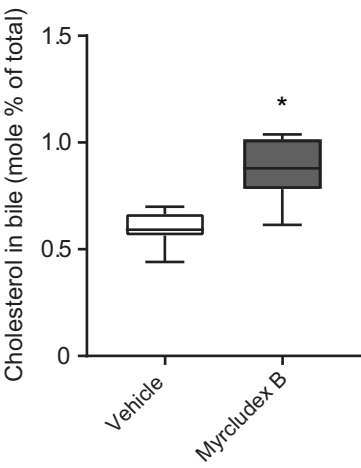
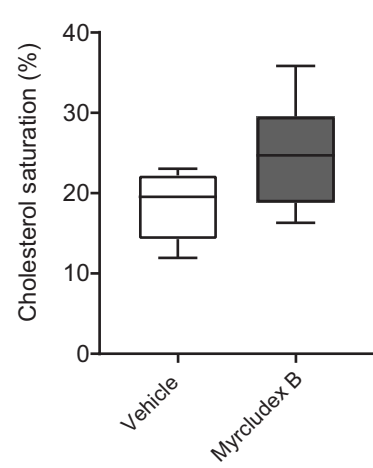
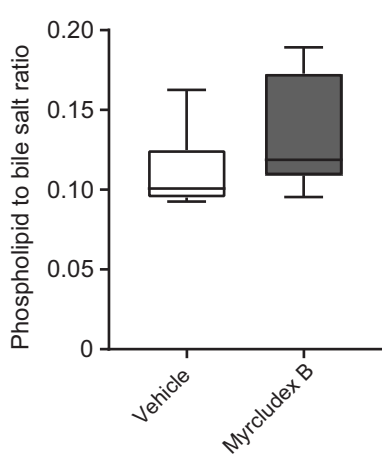
(A) Hepatic bile flow in *Abcg8*^{-/-} mice. White squares indicate the vehicle group and black squares indicate the Myrcludex B group. Differences between groups were analyzed using Mann-Whitney U. Asterisk indicates $p < 0.05$. $n = 8$ mice/group.

Supplementary figure 3: Hepatic cholesterol ester content and HMG CoA reductase expression levels after NTCP inhibition

(A) Total cholesterol (TC), cholesterol esters (CE) and free cholesterol was measured in livers of vehicle and Myrcludex B treated mice (n=4 mice/group). (B) HMG CoA reductase mRNA levels in livers of the same mice (n=8 mice/group). White bars indicate the vehicle group and dark grey bars indicate the Myrcludex B group. Differences between groups were analyzed using Mann-Whitney U. Asterisk indicates $p < 0.05$.

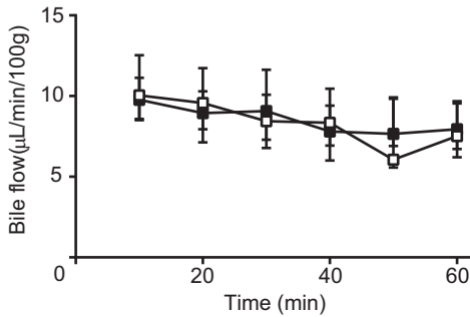
Supplementary figure 4: Bile flow in *Sr-bl*^{-/-} mice

(A) Hepatic bile flow in *Sr-bl*^{-/-} mice. White squares indicate the vehicle group and black squares indicate the Myrcludex B group. Differences between groups were analyzed using Mann-Whitney U. Asterisk indicates $p < 0.05$. n=8 mice/group.

A**B****C****D****E****F****G****H**

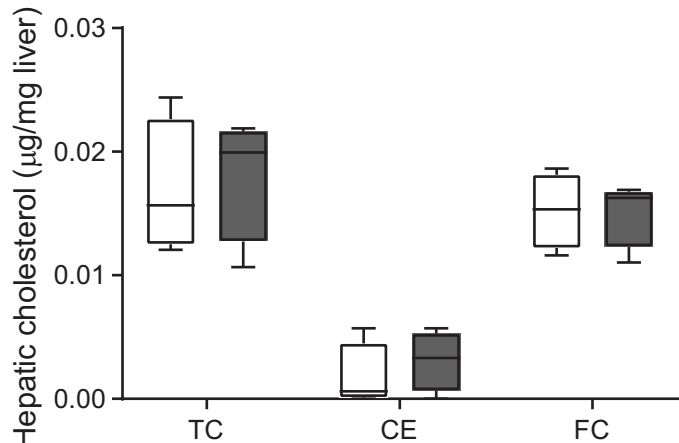
Supplemental figure 2:

A



Supplemental Figure 3

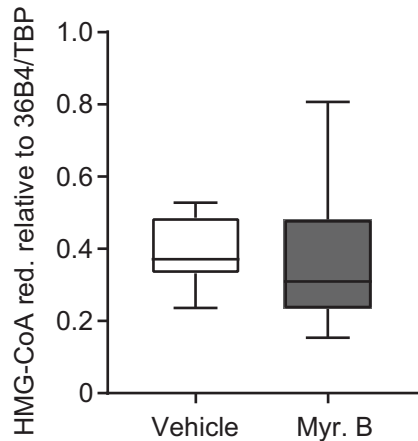
A



□ Vehicle

■ Myrcludex B

B



Supplemental figure 4

A

