

Supplementary Information

Extrahepatic cholangiocyte obstruction is mediated by GSH, Wnt and Notch signaling pathways in a toxic model of biliary atresia

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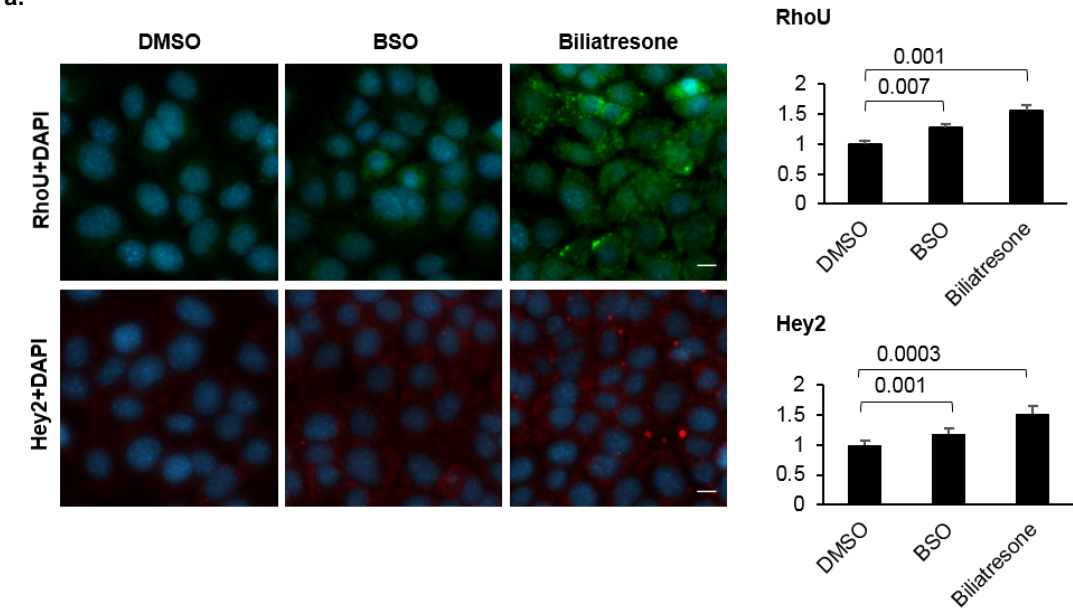
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Fig. S1

a.



b.

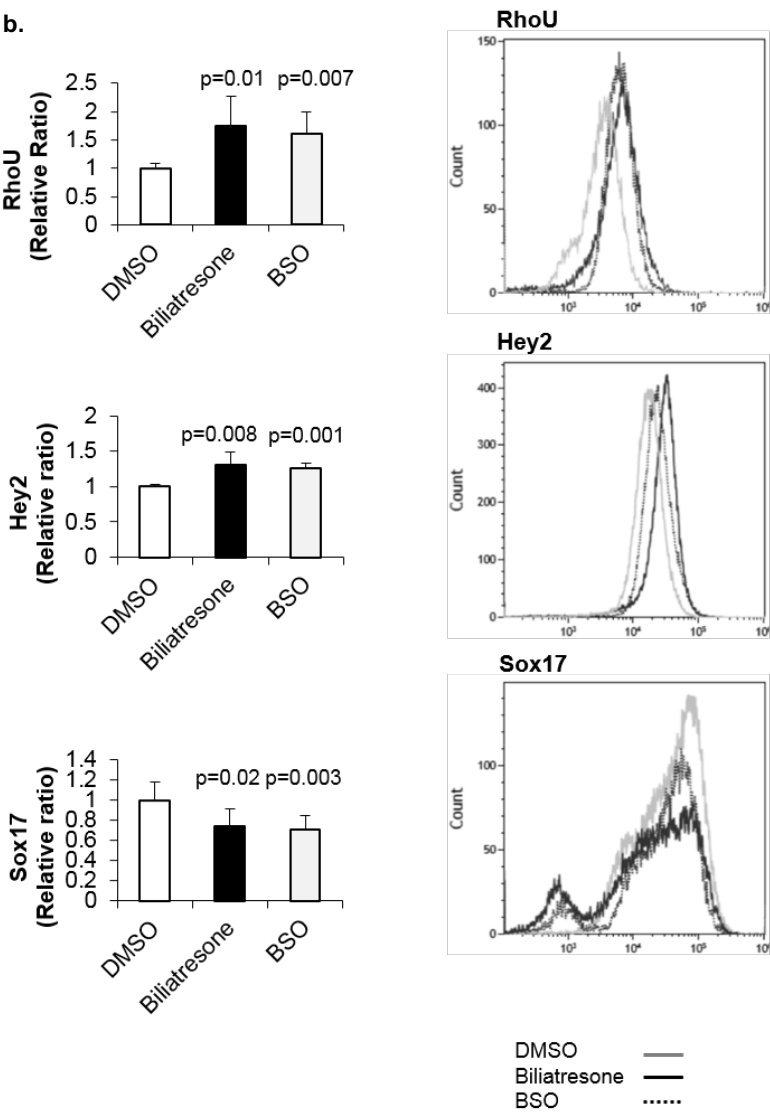


Fig. S1. Biliatresone regulates RhoU and Hey2 expression in cholangiocyte culture

a. sBEC cells were cultured in the presence or absence of 2ug/ml Biliatresone or 100uM BSO for 3h. Left: RhoU and Hey2 protein levels were evaluated by immunofluorescence. DAPI (blue), RhoU (green), Hey2 (red) Right: quantitative analysis of relative Fluorescent intensity. The images are representative of 4 experiments and values of MFI were calculated from at least 40 fields of cells for each experimental group. Bars represent mean $\pm$ s.e.m, Bar = 10 μ m.

b. RhoU/Wrch1, Hey2, and Sox17 protein levels in cholangiocytes treated with biliatresone, BSO or DMSO, as evaluated by flow cytometry. Histogram graph on the left represents mean fluorescent intensity relative to DMSO (set as 1), graphs on the right represent histograms showing the cell count (Y axis) that show a given fluorescence, and the relative fluorescence intensity detected in each condition (X-axis). Data represent mean $\pm$ s.e.m, n=5-9 independent experiments; p values represent comparison to vehicle.

Fig. S2

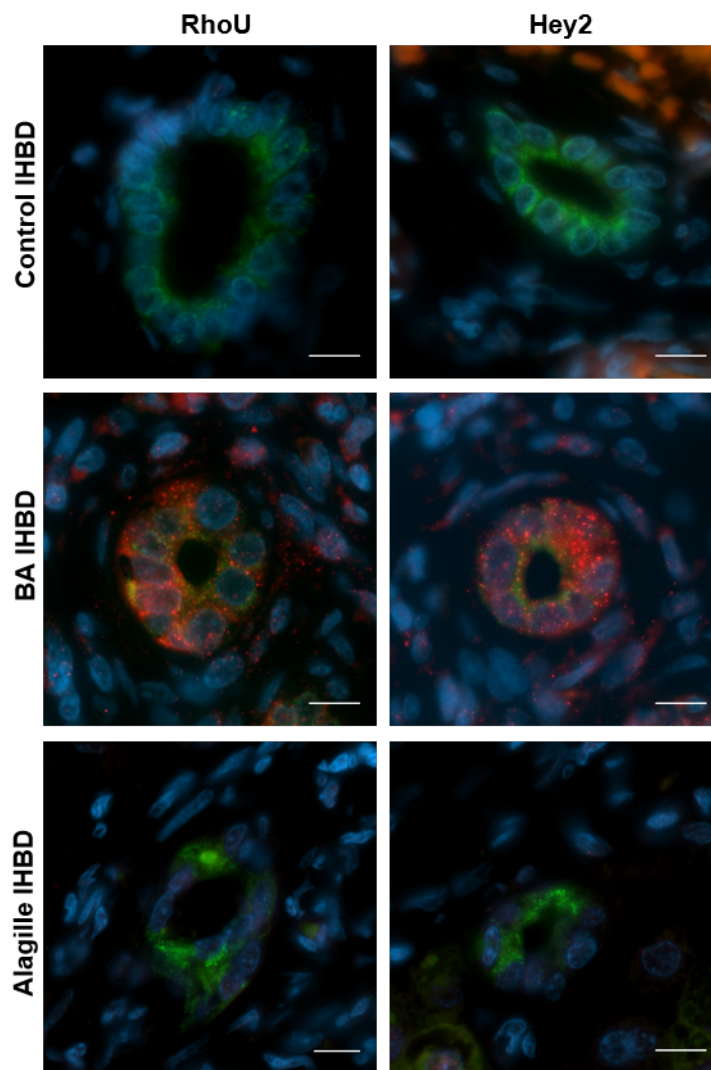


Fig. S2. RhoU/Wrch1 and Hey2 are overexpressed specifically in Intra hepatic bile ducts in liver biopsies from BA patients but not in Alagille patients or normal biopsies.

Liver biopsies from non-BA patients, BA patients and Alagille patients, were immunostained with antibodies against K19 (green) RhoU/Wrch1 (red) and DAPI (blue) stain for nuclei. The images are representative of 3 experiments. Bar = 10 μ m.