Supplementary Figure 1. Cholangiocyte spheroid formation and polarity development.

Cholangiocytes were dispersed and plated in 3D culture, then fixed and stained at the days noted. By 7 days, individual cholangiocytes have proliferated and organized to form polarized spheroids. Immunostains for F-actin (green) and β1-integrin (red). DAPI nuclear stain (blue). Scale bars 10 μm.

Supplementary Figure 2. Biliatresone does not interfere with GSH assays.

GSH at the concentrations noted was assayed in the presence and absence of biliatresone (2 μg/ml). Data are mean ± SD. There were 3 repeats for each concentration, with no significant difference detected between groups.

Supplementary Figure 3. Extrahepatic obstruction precedes significant liver damage in biliatresone-exposed lambs.

Two lambs with minimal symptoms of biliary atresia during a large outbreak in Australia in 2013 were necropsied at 1 and 2 weeks, and the extrahepatic biliary trees and livers stained with H and E and examined histologically. Size bar, 100 μm.

Supplementary Figure 4. Spheroid abnormalities are not associated with changes in cholangiocyte proliferation or apoptosis.

(a) Cholangiocytes were cultured in 3D to form spheroids. They were treated with DMSO for 24 h, biliatresone for 24 h and then stained for Ki-67 (red). DAPI nuclear stain (blue). Scale bar, 10 μm. Representative of 3 independent experiments each with duplicate wells of spheroids. (b) Neonatal bile ducts were treated with DMSO or biliatresone for 24 h and then embedded in paraffin and sectioned. Slices were stained with a TUNEL kit. DNase positive control was done on DMSO-treated ducts by adding

DNase prior to staining. Scale bar 100 μm. Representative of 3 independent experiments.

Supplementary Figure 5. The effects of biliatresone are reversible.

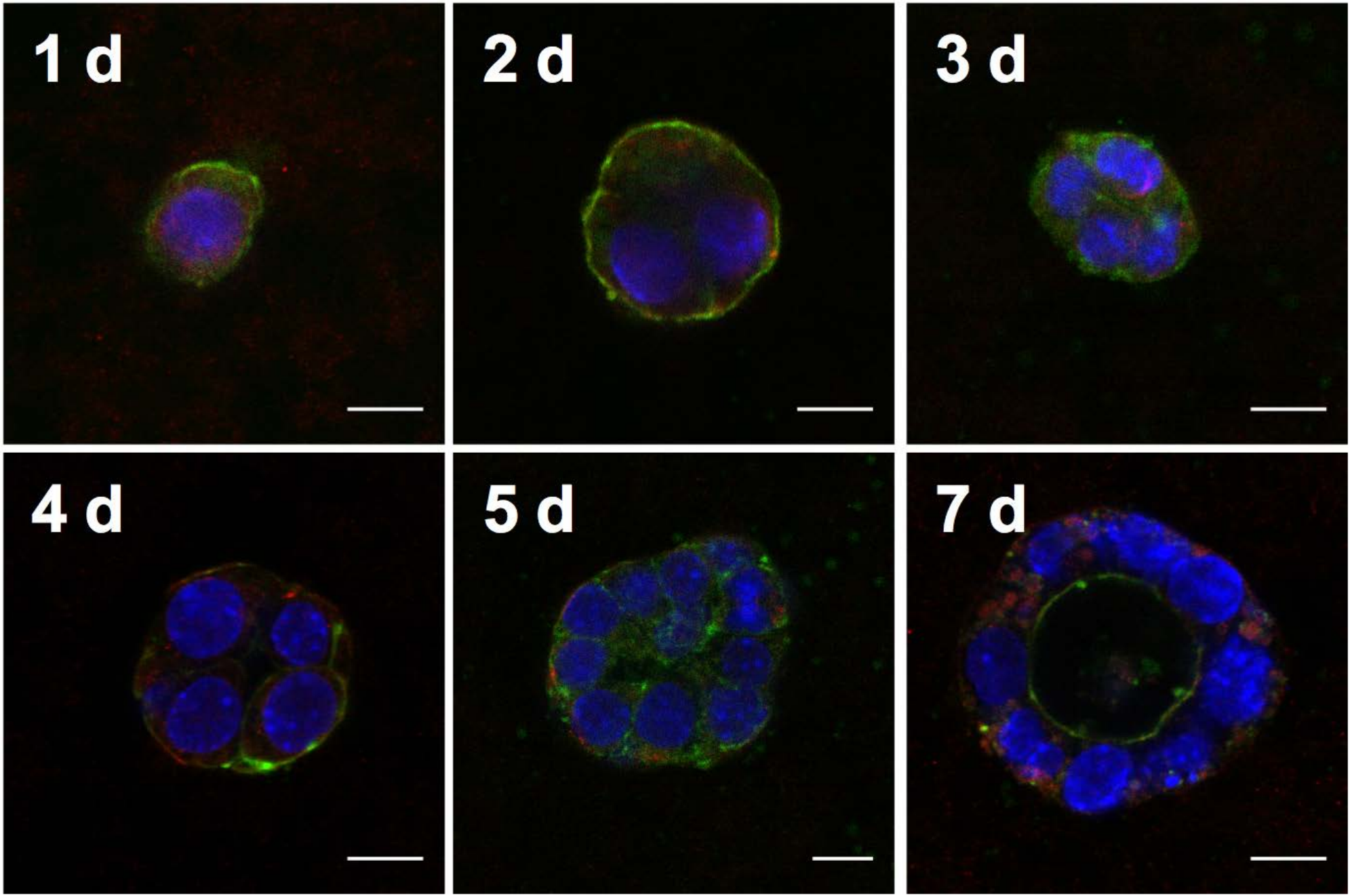
Cholangiocytes were cultured in 3D to form spheroids. They were treated with a) DMSO for 24 h, b) biliatresone for 24 h, c) biliatresone for 24 h followed by a 24 h washout period, or d) biliatresone for 48 h, then stained for F-actin (green) and β1 integrin (red). DAPI nuclear stain (blue). Scale bar, 10 μm. Representative of 3 independent experiments each with duplicate wells of spheroids.

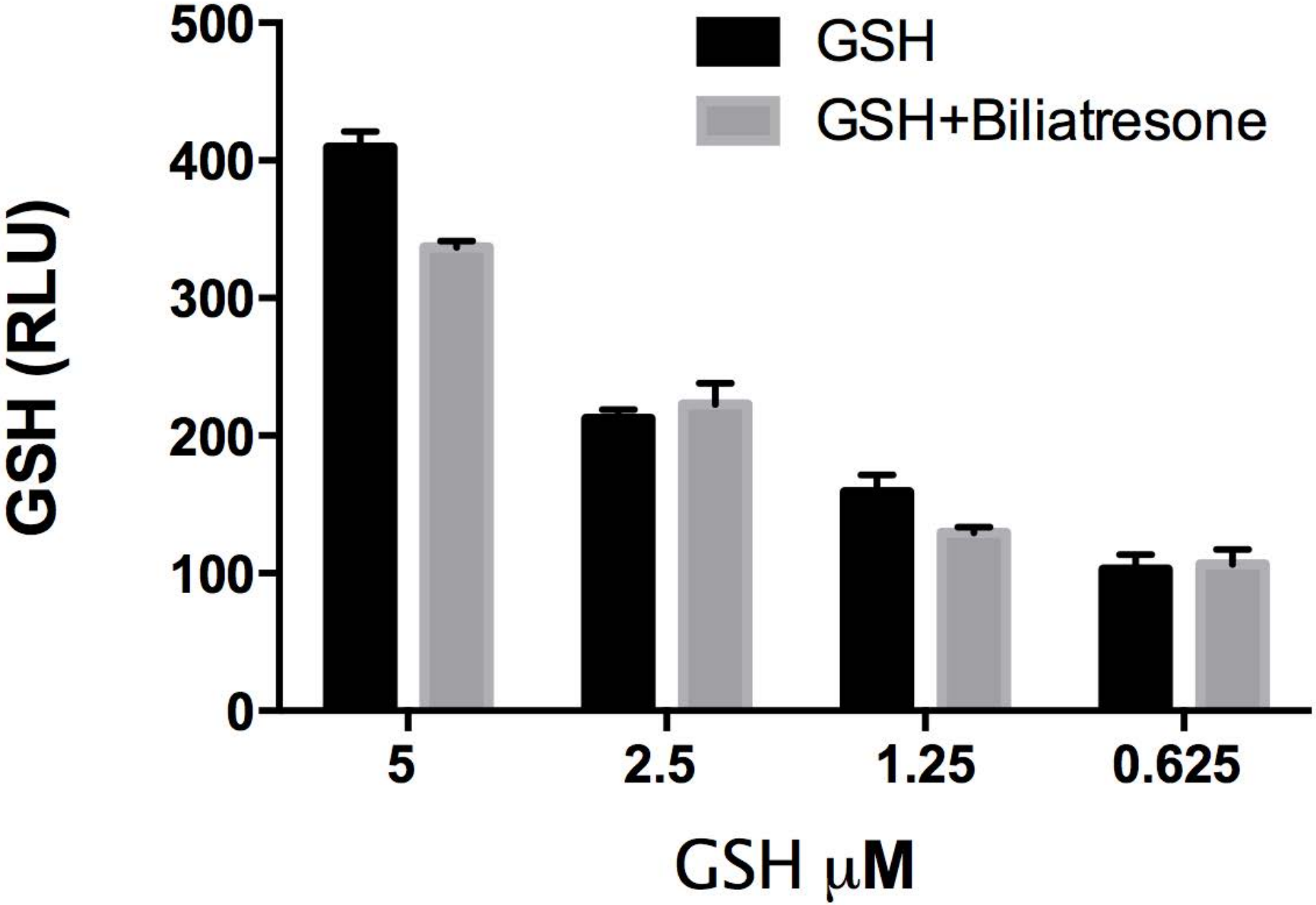
Supplementary Figure 6. Glutathione levels are lower in extrahepatic bile ducts compared to liver parenchyma but similar in adult EHBD and neonatal EHBD.

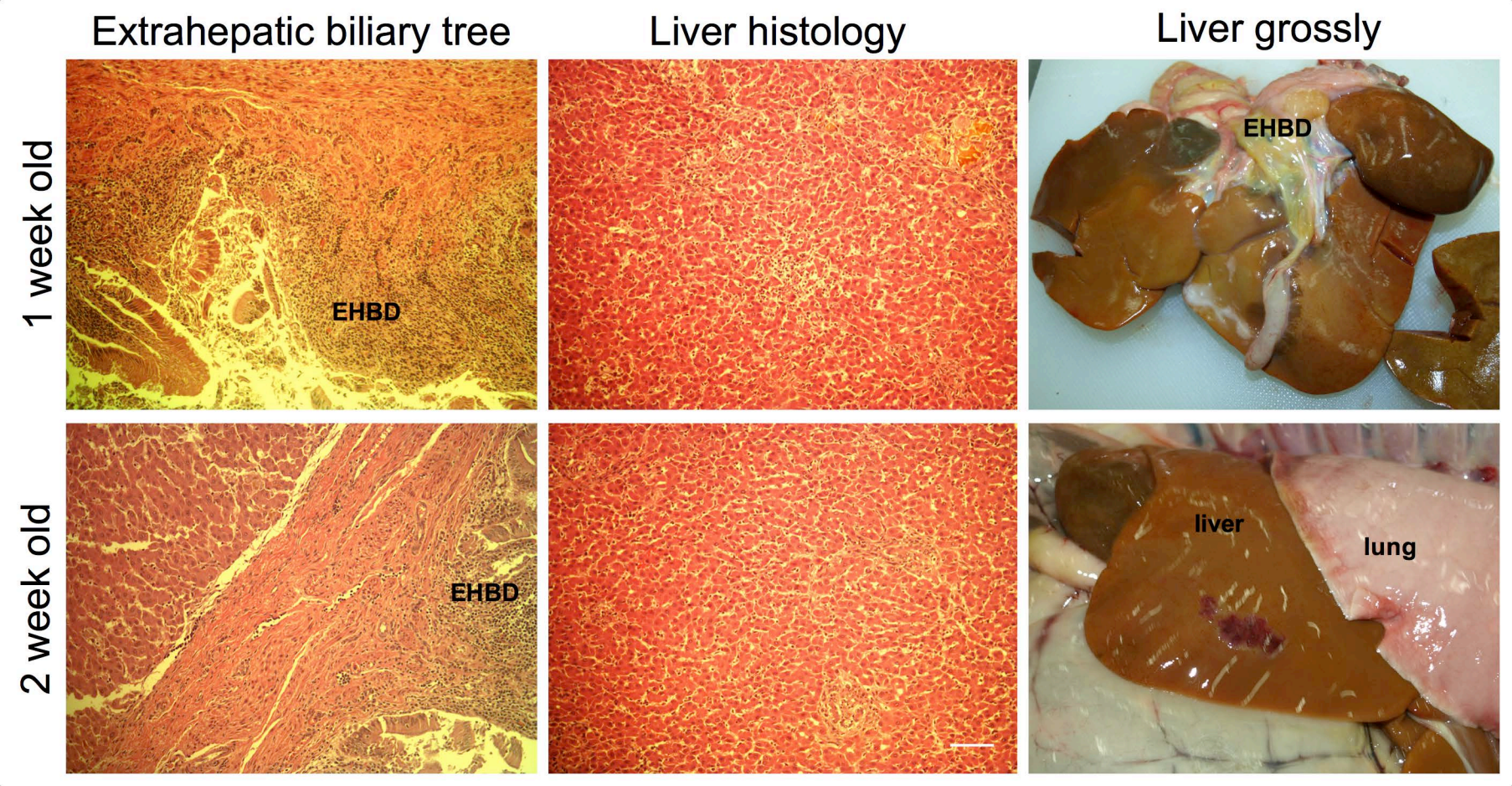
Reduced (GSH) and total (GSH plus GSSG) glutathione were measured in EHBDs isolated from adult (a) and neonatal (1-3 days old) (b) mice. Equal weights of tissue were used for each measurement. (c) Adult and neonatal EHBD; data extrapolated from panels a and b. Values are mean ± SD for 3 independent experiments. *, $p<0.050$.

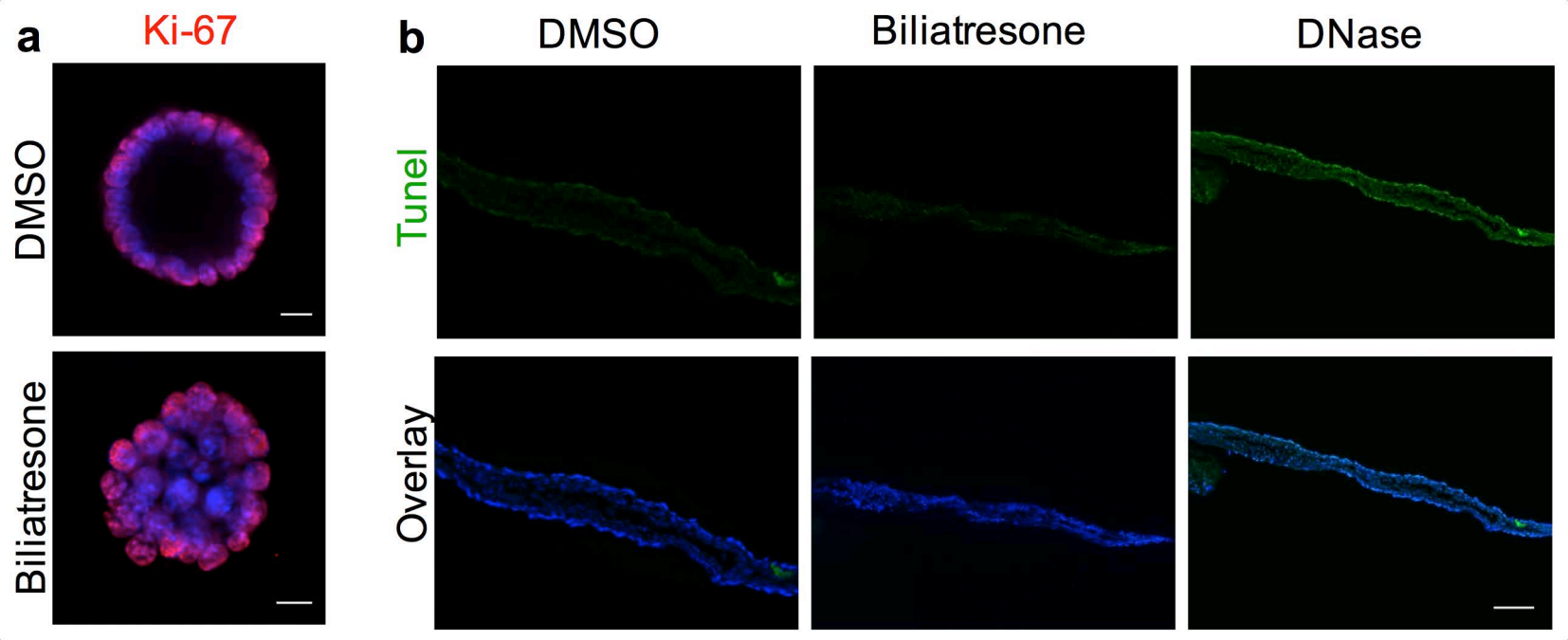
Supplementary Figure 7. *Sox17* was efficiently silenced by S*ox17* siRNA.

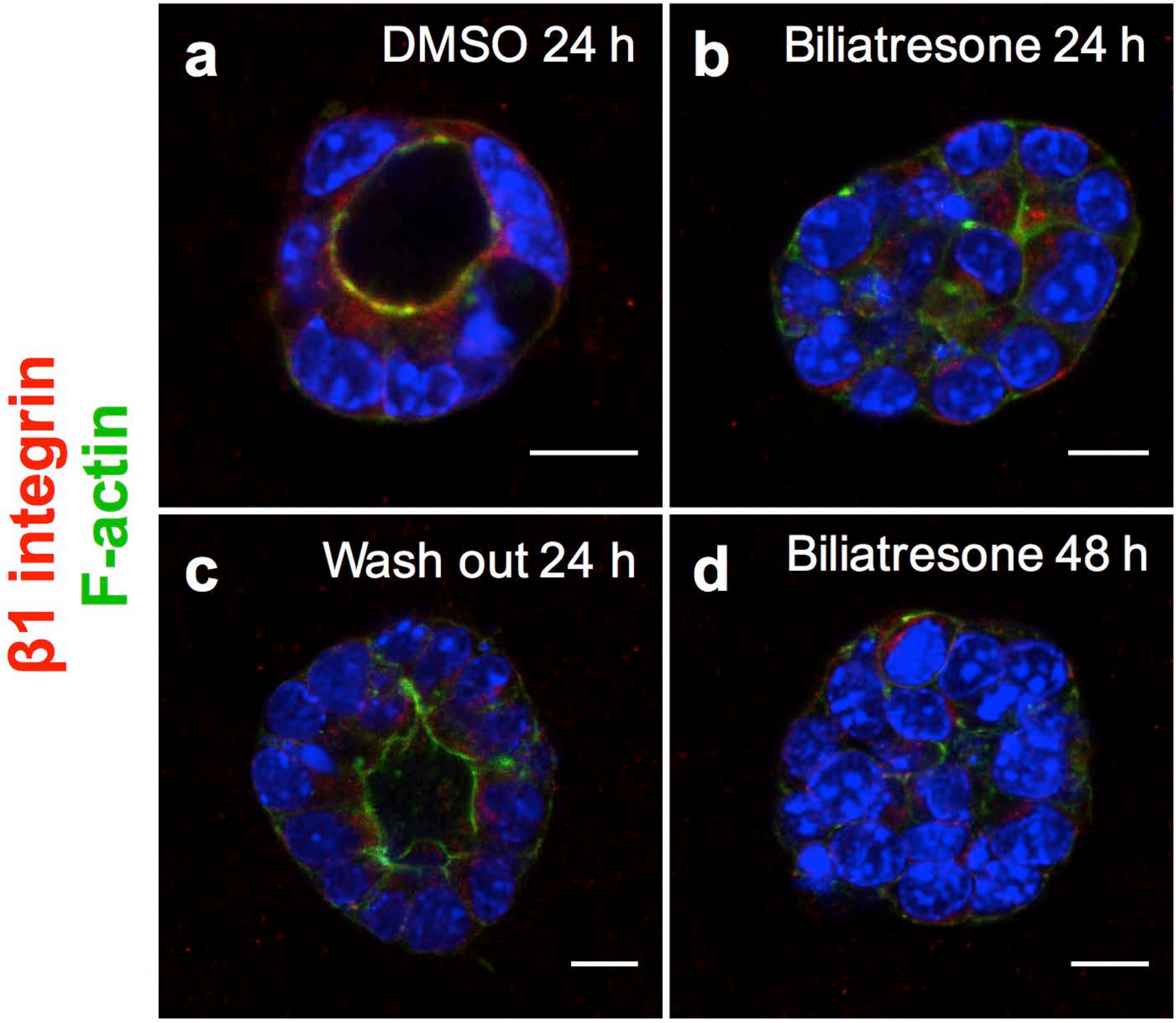
(a) *Sox17* mRNA levels of cholangiocytes in 2D culture treated with *Sox17* siRNA or scrambled RNA. Mean ± SD. 3 repeats, * $p<0.05$. (b) Immunofluorescence of cholangiocytes in 2D treated with scrambled siRNA on the left and *Sox17* siRNA on the right, stained for SOX17 (green). DAPI nuclear stain (blue). Scale bar, 50 μm. Representative of 3 independent experiments each with duplicates.

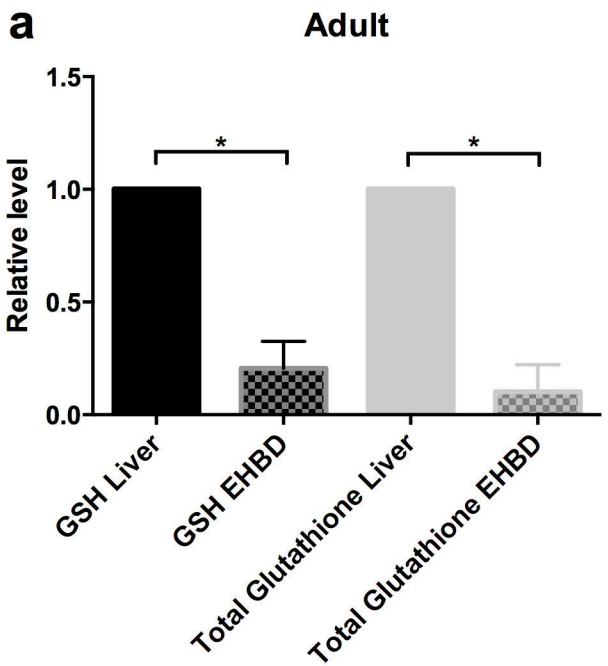

1 d
2 d
3 d
4 d
5 d
7 d
DAPI
F-Actin
β1-integrin

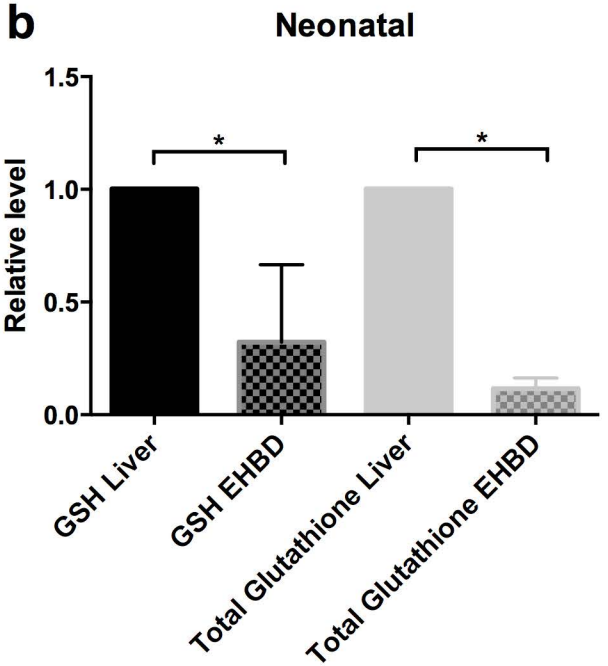

GSH
GSH+Biliatresone
GSH (RLU)
500
400
300
200
100
0
5
2.5
1.25
0.625
GSH μM

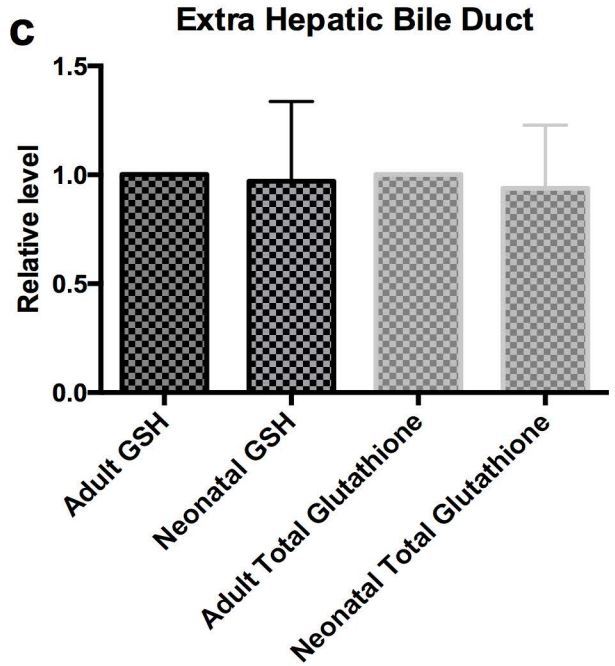

Extrahepatic biliary tree
Liver histology
Liver grossly
1 week old
2 week old
EHBD
EHBD
EHBD
liver
lung

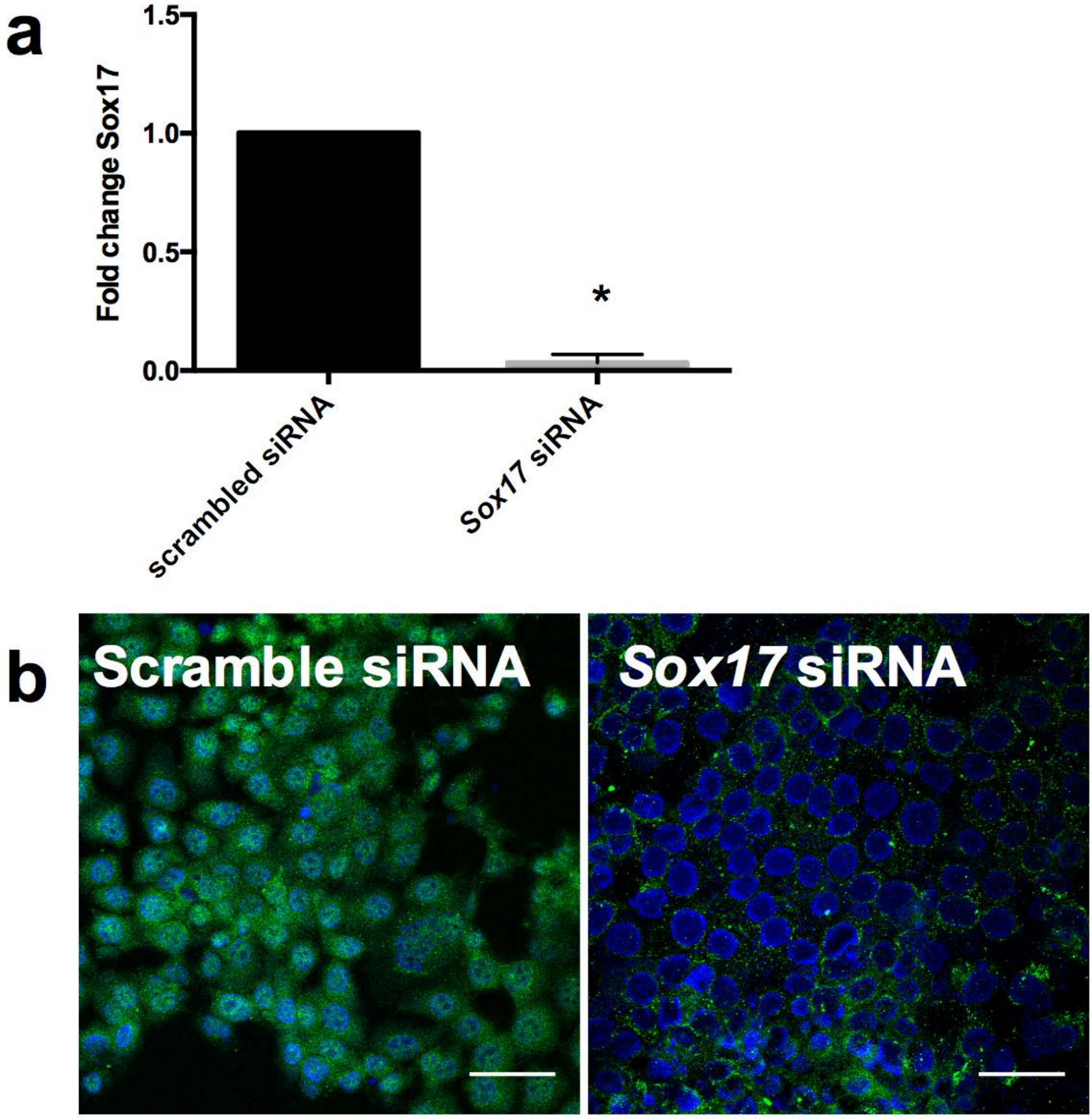

a
Ki-67
DMSO
Biliatresone
b
DMSO
Biliatresone
DNase
Tunel
Overlay


β1 integrin
F-actin
a
DMSO 24 h
b
Biliatresone 24 h
c
Wash out 24 h
d
Biliatresone 48 h


a
Adult
Relative level
1.5
1.0
0.5
0.0
*
*
GSH Liver
GSH EHBD
Total Glutathione Liver
Total Glutathione EHBD


b
Neonatal
Relative level
1.5
1.0
0.5
0.0
*
*
GSH Liver
GSH EHBD
Total Glutathione Liver
Total Glutathione EHBD


c
Extra Hepatic Bile Duct
Relative level
1.5
1.0
0.5
0.0
Adult GSH
Neonatal GSH
Adult Total Glutathione
Neonatal Total Glutathione


a
1.5
1.0
0.5
0.0
Fold change Sox17
*
scrambled siRNA
Sox17 siRNA
b
Scramble siRNA
Sox17 siRNA