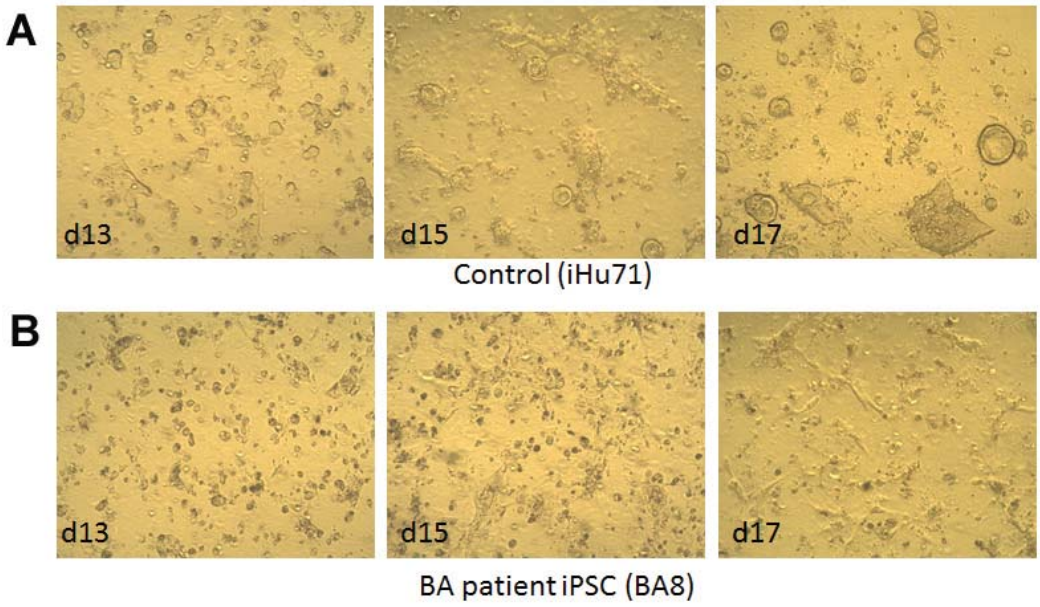
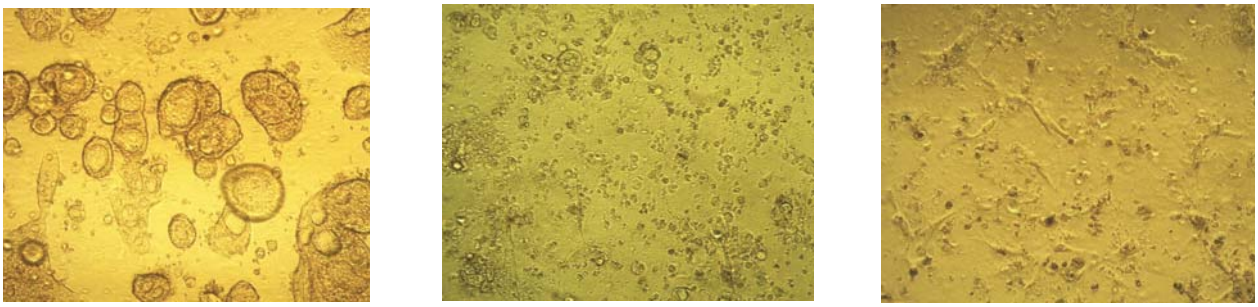


Figure S1. Early stage biliary differentiation of control and BA iPSCs. Biliary differentiation defects were observed as early as day 15 in BA patient iPSCs. The difference in biliary structure formation between control and BA iPSCs was more apparent with further maturation.



Control (iAAT2) iBA6 iBA8

Figure S2. Limited ductal structure formation of the two BA patient iPSC lines which are not shown in the figure 1B. Compared to the control iPSC, iBA6 and iBA8 (not shown in Figure 1B due to the spatial limitation) also showed significantly decreased ductal structure formation.

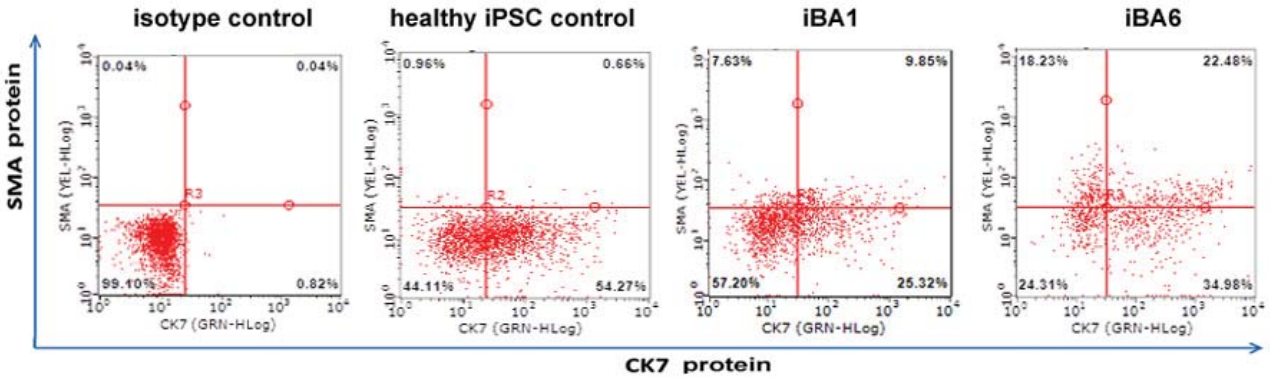


Figure S3. Isotype control for SMA and CK7 flow cytometry, and the two BA patient iPSC lines which are not shown in the figure 1D. iBA1 and iBA6 also showed similar results to other BA patient lines shown in Figure 1D.

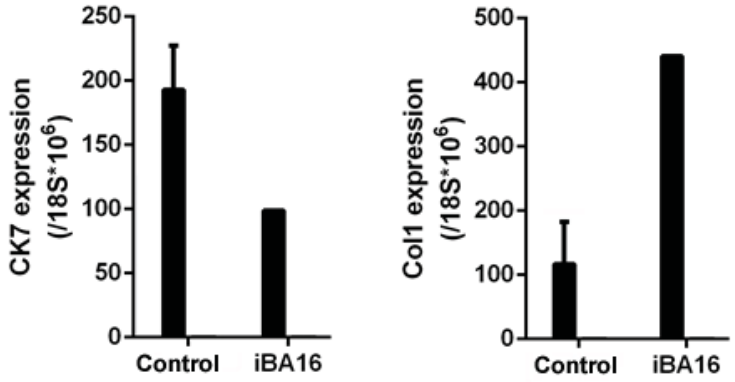


Figure S4. Biliary differentiation of a human iPSC line derived from a BA patient with anomalies (midline liver, heterotaxy syndrome with polysplenia). This line (iBA16) grows extremely poorly in regular iPSC culture conditions compared to other BA lines without anomalies, which limited sufficient repeats of various experiments. This BA-iPSC with anomalies also showed similar results to those without anomalies (i.e., reduced biliary marker CK7 and increased fibrosis marker collagen 1) after biliary differentiation.

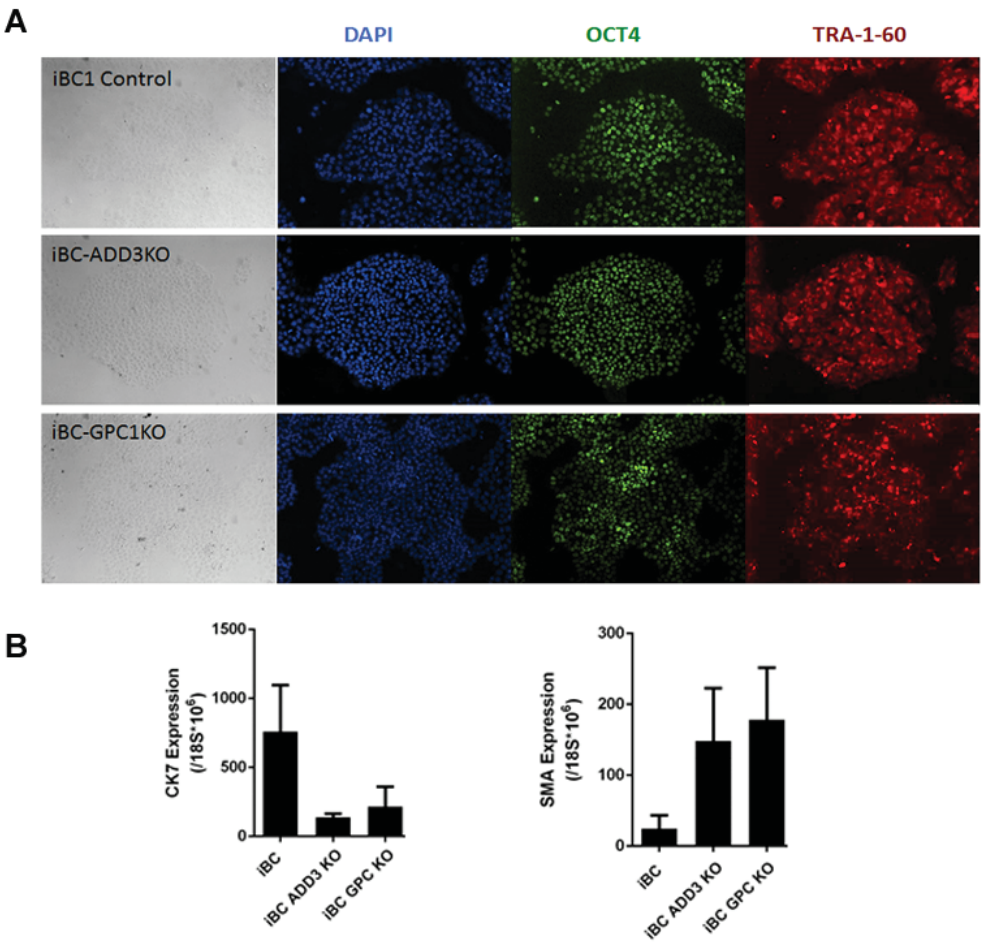


Figure S5. Biliary differentiation of another set of isogenic human iPSC lines deficient in genes implicated in BA development. (A) Expression of pluripotency markers in ADD3 and GPC1 KO iPSC lines created from another normal human iPSC (iBC1). (B) These KO iPSC lines also showed similar results (i.e., reduced biliary marker CK7 and increased fibrosis marker SMA after biliary differentiation) to another set of KO iPSCs created from iHu71 (Fig 3, 4).

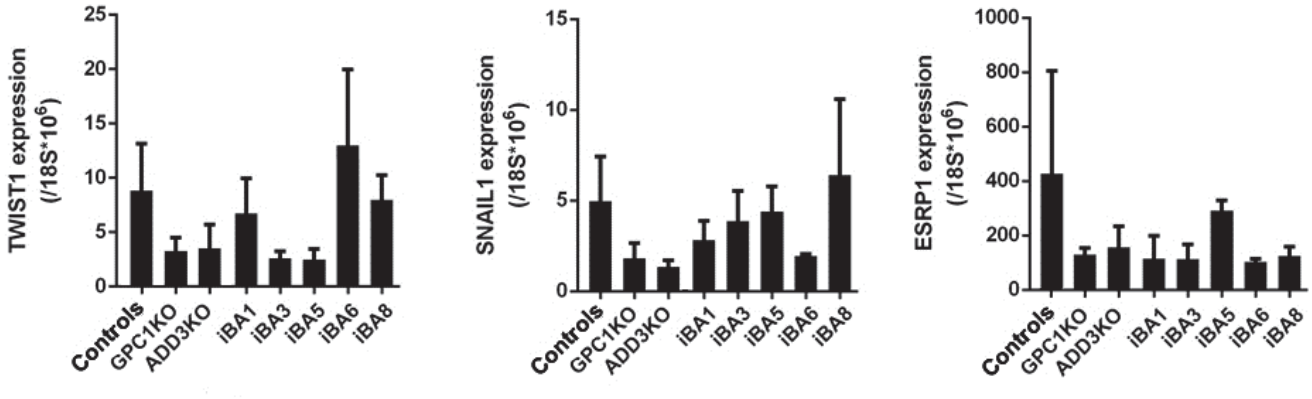


Figure S6. Variable EMT marker expressions in BA patient and KO iPSCs after biliary differentiation. Compared to controls KO iPSCs and BA patient iPSCs did not show significant EMT marker expressions after biliary differentiation and the levels were variable among the lines.

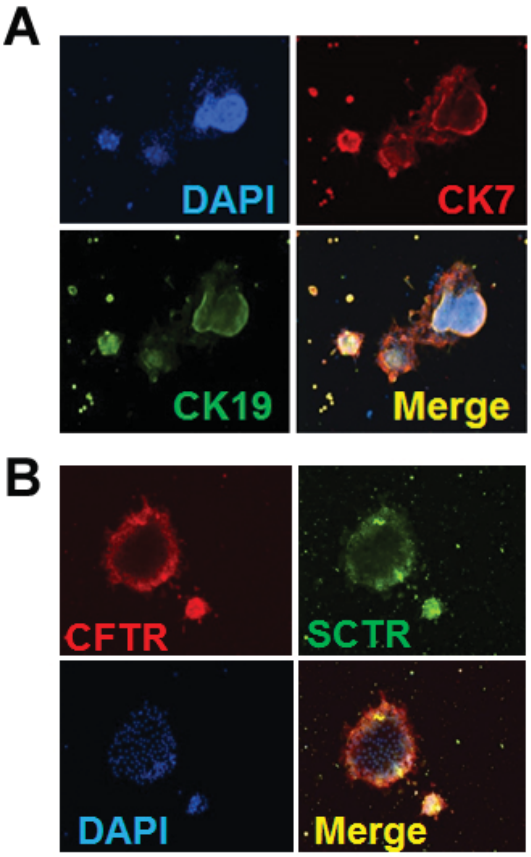


Figure S7. Biliary marker expression and morphology of human primary bile duct derived cholangiocytes. These primary cholangiocytes from human extrahepatic biliary ducts expressed multiple cholangiocyte makers such as (A) CK7 and CK19, and (B) CFTR and SCTR and formed ductal cyst-like structures, which are highly similar to the human iPSC-derived ductal cysts/structures (reference 33).