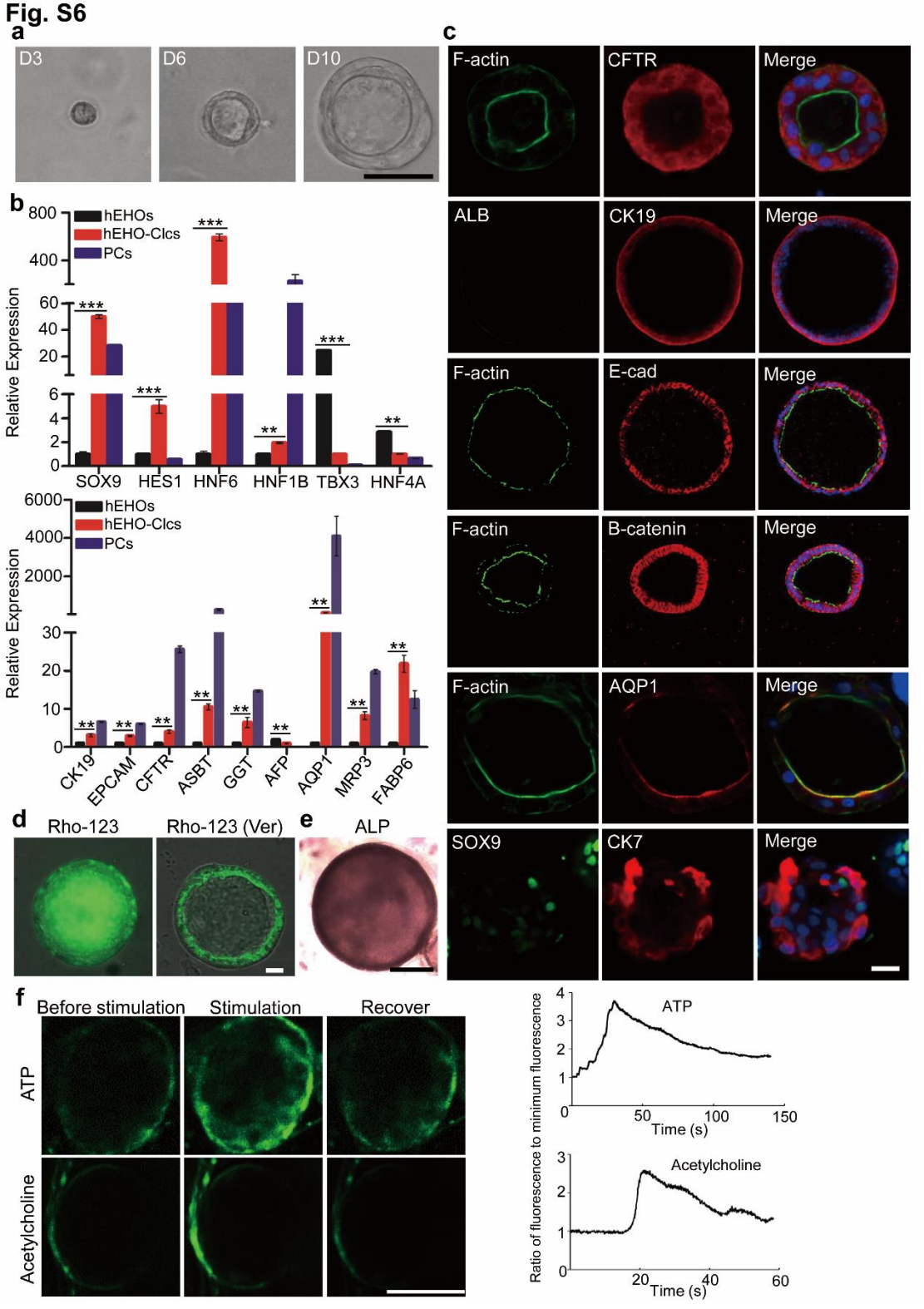
Fig. S6


a
D3
D6
D10
b
hEHOs
hEHO-Clcs
PCs
Relative Expression
SOX9
HES1
HNF6
HNF1B
TBX3
HNF4A
CK19
EPCAM
CFTR
ASBT
GGT
AFP
AQP1
MRP3
FABP6
c
F-actin
CFTR
Merge
ALB
CK19
E-cad
B-catenin
AQP1
SOX9
CK7
d
Rho-123
Rho-123 (Ver)
e
ALP
f
Before stimulation
Stimulation
Recover
ATP
Acetylcholine
Ratio of fluorescence to minimum fluorescence
Time (s)

Supplementary information, Fig. S6 Cells of hEHOs could differentiate into cholangiocytes in vitro. (**a**) Representative phase-contrast morphology of hEHO-Clcs at day 3, 6, and 10 of the culture. (**b**) Gene expression profile of hEHO-Clcs compared with undifferentiated hEHOs and PCs. The lowest expression levels were normalized to 1. Results are presented as mean ± SD from 3 replicates from three independent repeated experiments. (**c**) hEHO-Clcs were examined for localization of epithelial polarity and cholangiocyte markers. (**d**) Rhodamine 123 (Rho-123) can be transported into the central lumen of hEHO-Clcs (left panel) and the MDR inhibitor Verapamil (Ver) blocks luminal accumulation of Rhodamine 123 (Right panel). (**e)** Staining of hEHO-Clcs for ALP activity. (**f**) Analyzing the response of hEHO-Clcs to ATP and acetylcholine by calcium indicator Fluo-4-based fluorescence intensity measurements. ** $P < 0.01$; *** $P < 0.001$. Scale bar: 100 μm (**a**, **e** and **f**), 50 μm (**c**), 33 μm (**d**). hEHO-Clcs, hEHO-derived cholangiocytes; PCs, primary cholangiocytes; MDR, multidrug resistance; ALP, alkaline phosphatase.