Supplementary Table 1. Differential expression of cholangiocyte markers in hepatoblasts and cholangiocytes

	P0 cholangiocytes vs E14.5 hepatoblasts
Cytokeratin 7	7.5
Cytokeratin 19	13
Osteopontin	44
Pkhd1 (polycystic kidney and hepatic disease 1)	6.5

Values represent fold changes.

Supplementary Table 2. Genes upregulated in cholangiocytes

Gene name	Abbreviation	Accession
Transcription factors		
B-box and SPRY domain containing	Bspry	NM_138653
grainyhead-like 2 (Drosophila)	Grhl2	NM_026496
POU domain, class 2, associating factor 1	Pou2af1	NM_011136
hairy/enhancer-of-split related with YRPW motif 1	Hey1	NM_010423
SRY-box containing gene 9	Sox9	NM_011448
SRY-box containing gene 17	Sox17	NM_011441
Kruppel-like factor 4 (gut)	Klf4	NM_010637
Kruppel-like factor 9	Klf9	NM_010638
Scleraxis	Scx	NM_198885
ets homologous factor	Ehf	NM_007914
Growth factors and chemokines		
connective tissue growth factor	Ctgf	NM_010217
chemokine (C-X3-C motif) ligand 1	Cx3cl1	NM_009142
chemokine (C-X-C motif) ligand 4	Cxcl4	NM_019932
platelet-derived growth factor, C polypeptide	Pdgfc	NM_019971
platelet-derived growth factor, D polypeptide	Pdgfd	BC030896
Proteases		
angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	Ace2	NM_027286
chitinase 3-like 3	Chi3l3	NM_009892
chitinase 3-like 4	Chi3l4	NM_145126
a disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 1	Adamts1	NM_009621
a disintegrin-like and metallopetidase with thrombospondin type 1 motif, 16	Adamts16	NM_172053
Others		
ankyrin repeat domain 1 (cardiac muscle)	Ankrd1	NM_013468
suppression of tumorigenicity 14 (colon carcinoma)	St14	NM_011176

Supplementary Table 3. Differential expression of claudins in hepatoblats and cholangiocytes

	P0 cholangiocytes vs E14.5 hepatoblasts
claudin 1	0.42
claudin 2	7.6
claudin 3	8.5
claudin 4	7.9
claudin 5	0.79
claudin 6	10
claudin 7	8.4
claudin 8	5.9
claudin 9	1.7
claudin 10	2.7
claudin 11	1.0
claudin 12	0.98
claudin 13	0.41
claudin 14	0.62
claudin 15	1.5
claudin 16	0.94
claudin 17	1.0
claudin 18	1.6
claudin 19	1.3
claudin 22	0.97
claudin 23	3.6

Values represent fold changes.

Supplementary Table 4. Possible Grhl2 targets in cholangiocytes

Gene Name		Accession	P0 cholangiocytes vs E14.5 hepatoblasts
secreted phosphoprotein 1	Spp1	NM_009263	44
chromogranin B	Chgb	NM_007694	12
PDGF-C	Pdgfc	NM_019971	11
PDGF-D	Pdgfd	BC030896	10
growth arrest specific 6	Gas6	NM_019521	8.7
doublecortin	Dcx	NM_010025	8.7
claudin 7	Cldn7	NM_016887	8.4
chromogranin A	Chga	NM_007693	6.7
plakophilin 3	Pkp3	NM_019762	6.2
RAB25	Rab25	NM_016899	6.1

Grhl2 binding sequence was searched within 1,000bp from the transcription initiation site in silico using human and mouse genome database. It was further analyzed whether those genes were suggested to increase in neonatal cholagnicoytes as compared with in hepatoblasts on the microarray data.

Fig. S1

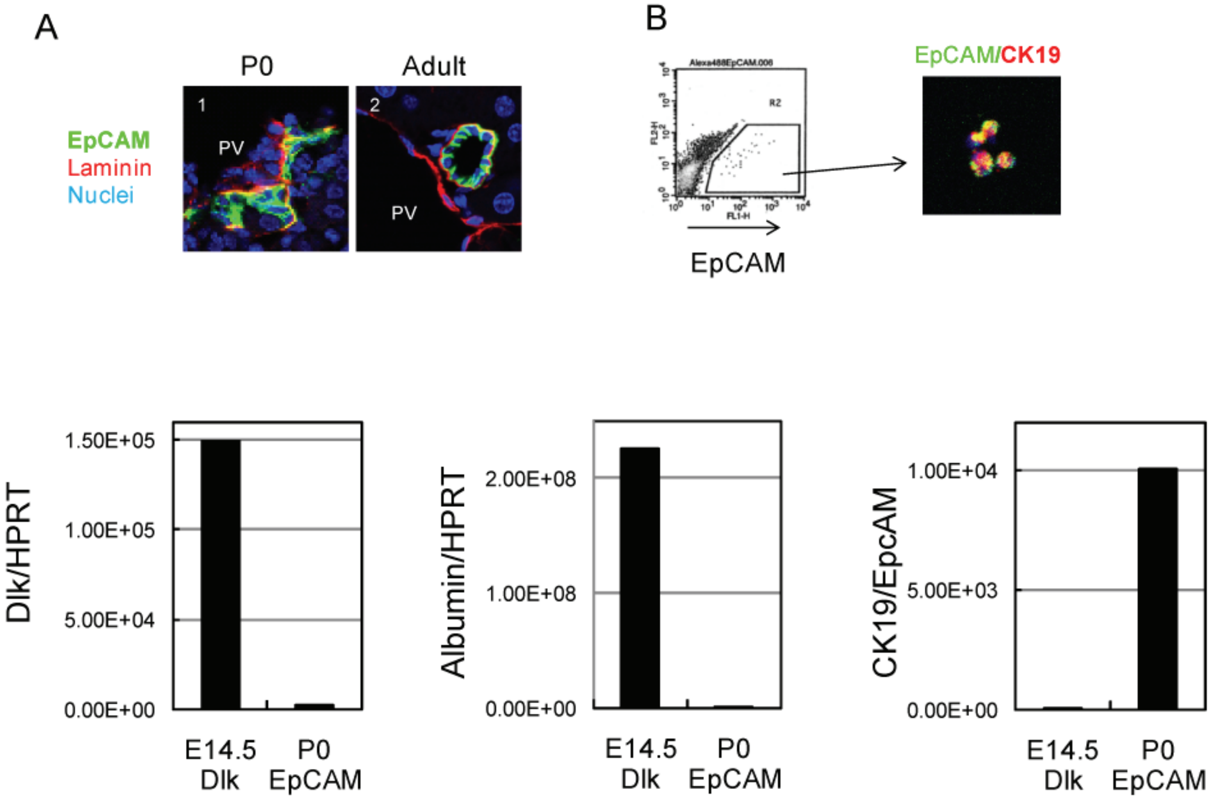

Fig. S1. Isolation and characterization of neonatal cholangiocytes

A. Expression of EpCAM in neonatal and adult livers. EpCAM is expressed in cholangiocytes forming either the ductal plate (panel 1) or bile duct tubules (panel 2).

B. Isolation of EpCAM$^+$ cholangiocytes from neonatal liver. Isolated EpCAM$^+$ cells (green) are positive for CK19 (red). P0 liver cells were stained with FITC-conjugated anti-EpCAM antibody. EpCAM$^+$ cells were isolated by FACSAria and stained with anti-CK19 antibody.

C. Expression of Dlk1 (a hepatoblast marker), albumin (a hepatoblast/hepatocyte marker), and CK19 (a cholangiocyte marker) in isolated hepatoblasts and cholangiocytes. Expression of Dlk1 and albumin are barely detected in EpCAM$^+$ cholangiocytes, whereas CK19 is strongly expressed in those cells.

Fig. S2

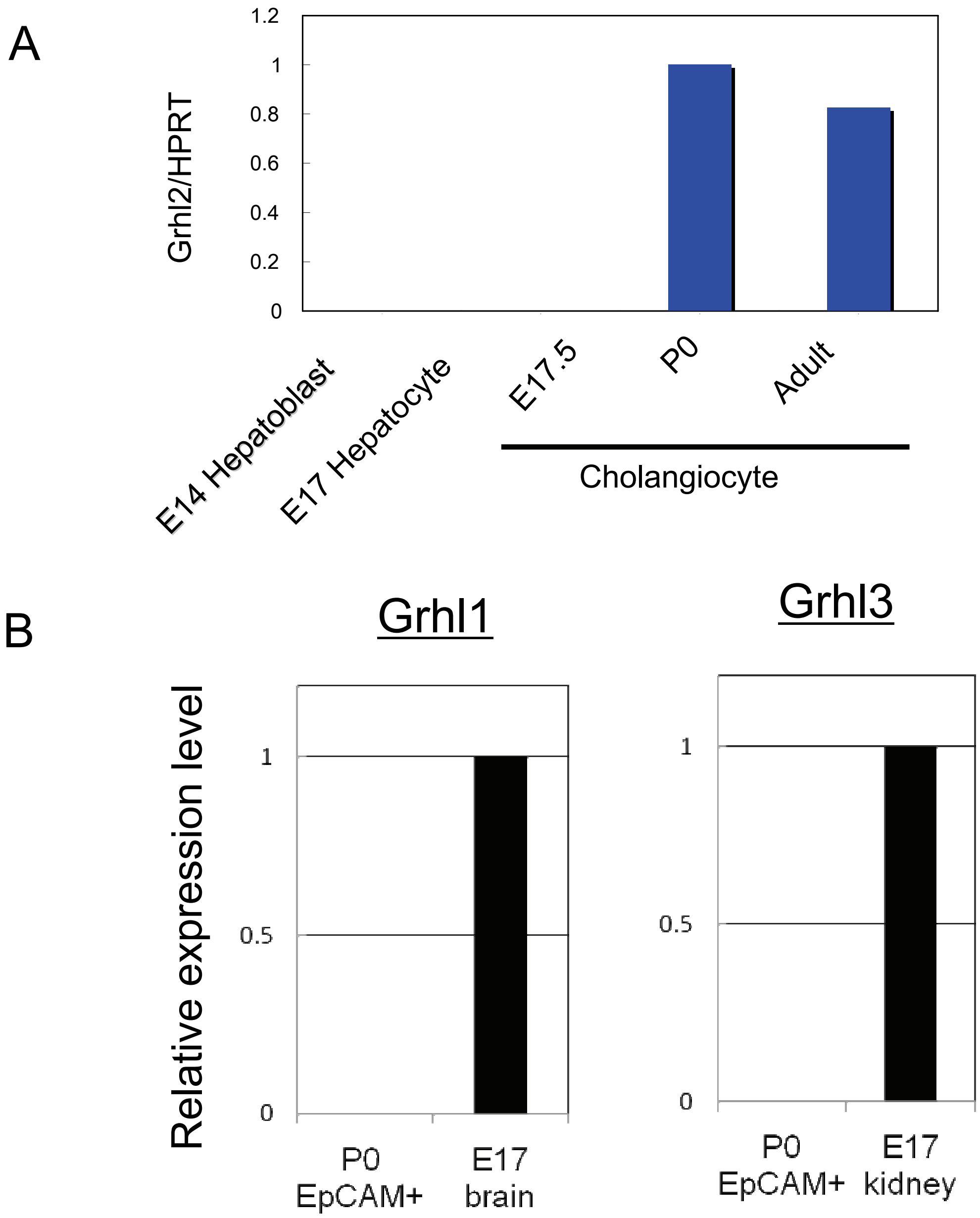

Fig. S2. Grhl2 is expressed in cholangiocytes in the liver.

A. Expression of Grhl2 mRNA in hepatoblasts and cholangiocyte in developing liver. Grhl2 is not expressed in hepatoblasts and E17.5 cholangiocytes, whereas it is detected in P0 and adult cholangiocytes.

B. Expression of Grhl1 and 3. In contrast to Grhl2, Grhl1 and 3 are not expressed in cholangiocytes.

Fig. S3

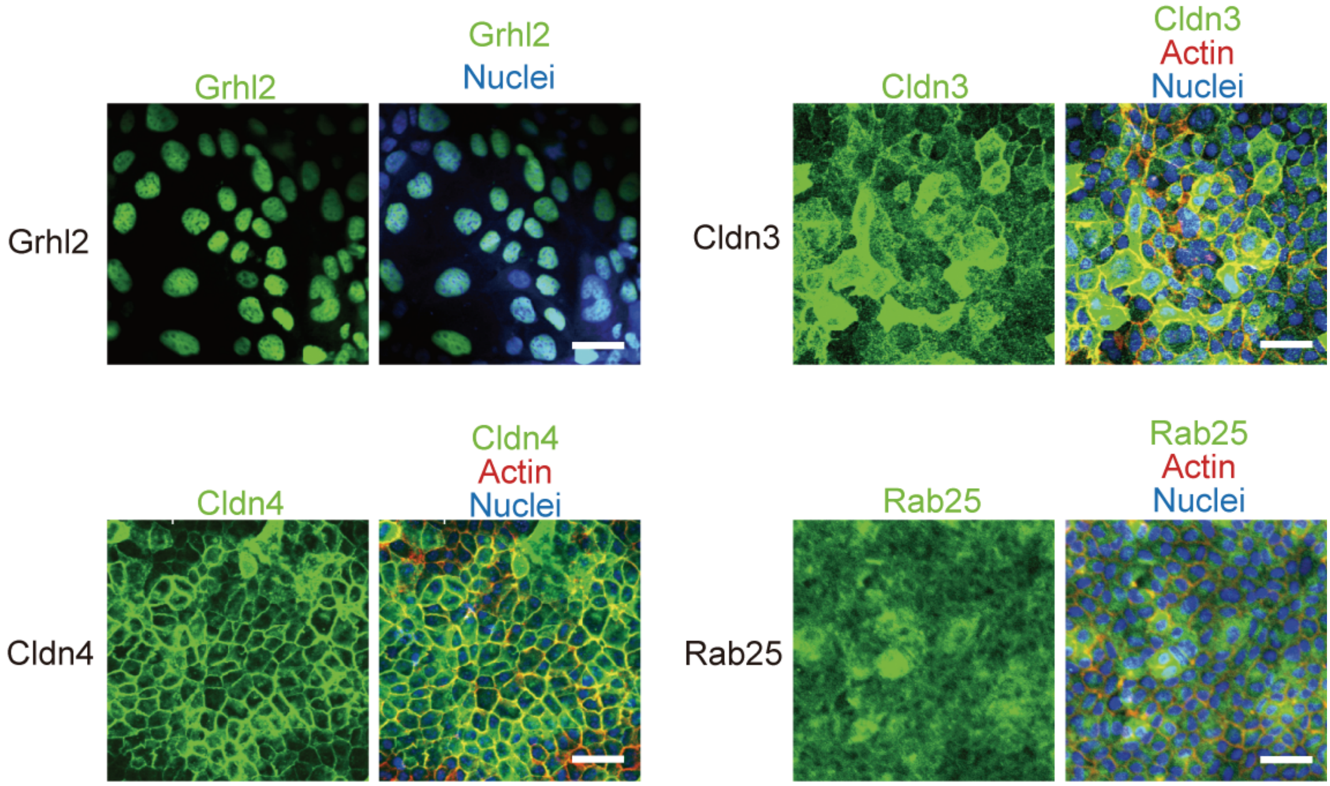

Fig. S3. Expression of transgenes introduced by retrovirus vectors in 2D cultrue of HPPL. HPPL were infected with retrovirus containing cDNA of Grhl2, Cldn3, Cldn4 or Rab25. Most of cells express each transgene. Scale bars represent 50 μm.

Fig. S4

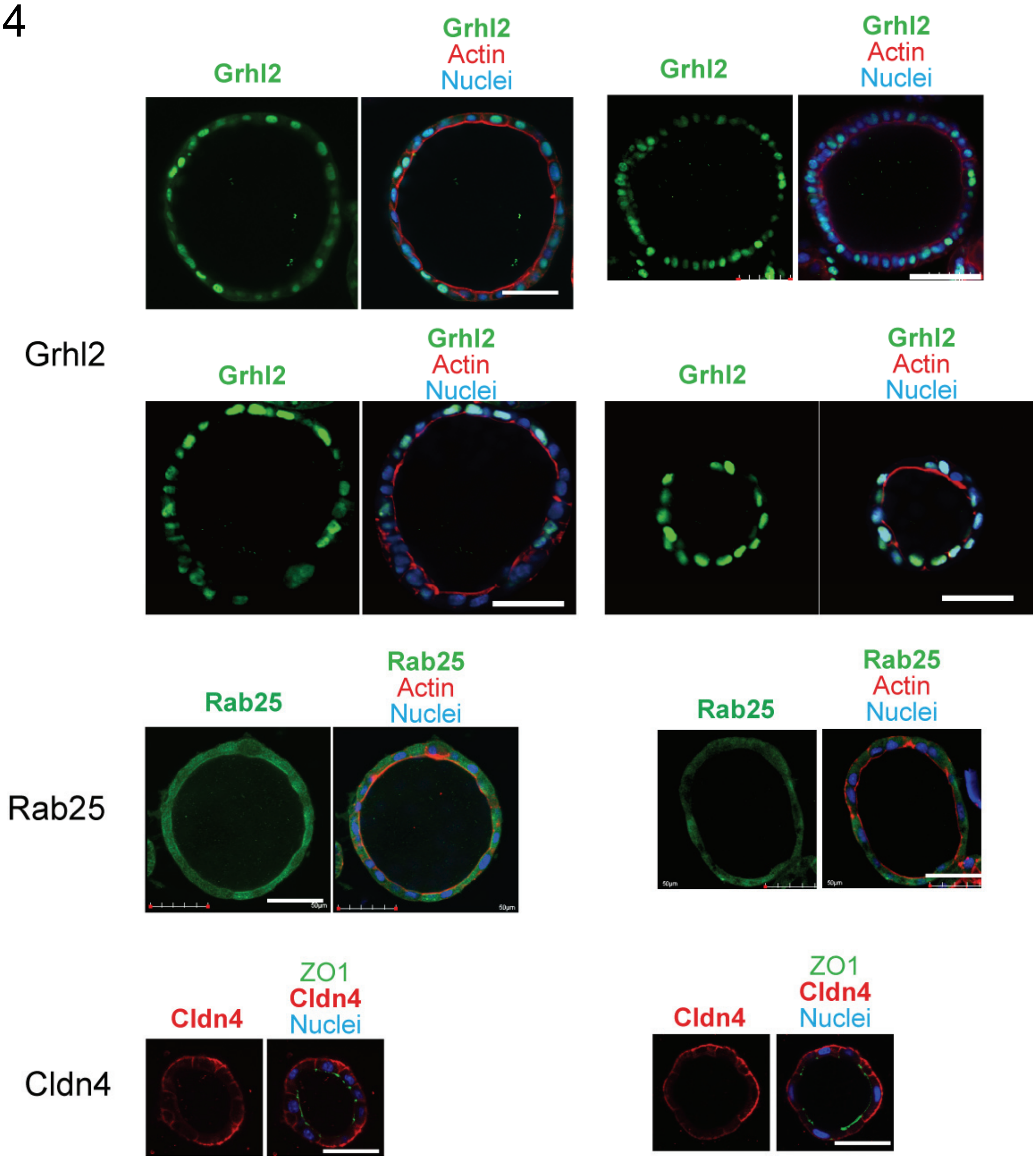

Fig. S4. Expression of transgenes introduced by retrovirus vectors in 3D cultrue of HPPL. HPPL were infected with retrovirus containing cDNA of Grhl2, Cldn4 or Rab25. Most of cells express each transgene. Scale bars represent 50 μm.

Fig. S5

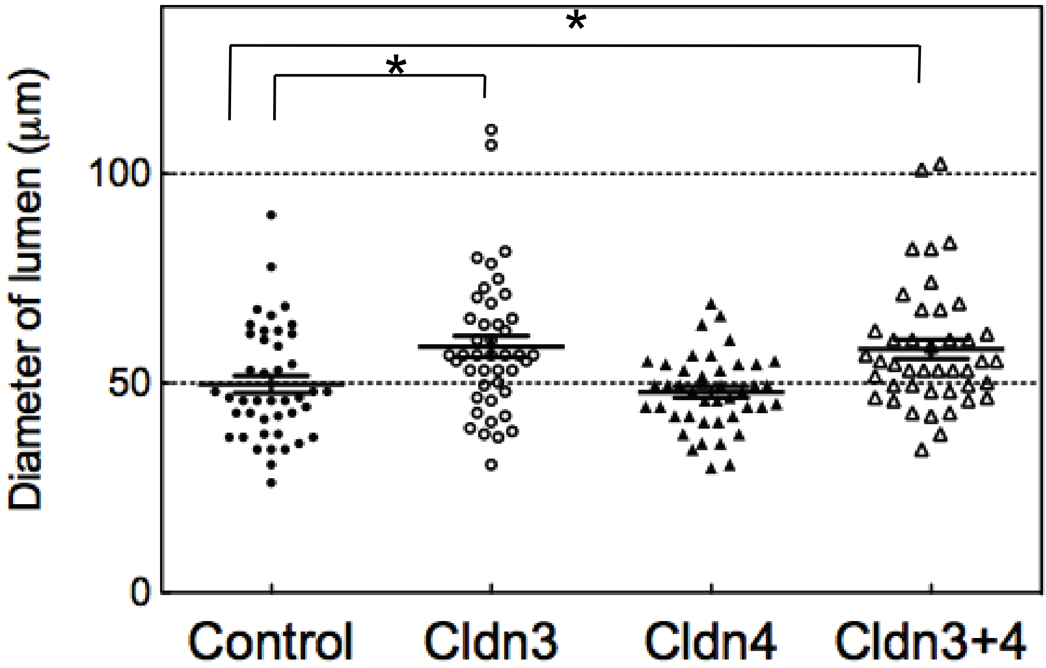

Fig. S5 Cldn4 expression does not increase the diameter of lumen. Expression of Cldn4 alone does not enlarge lumen. Furthermore, co-expression of Cldn3 and 4 does not further enhance the increase of lumen size induced by expression of Cldn3 alone. * represents $P<0.05$.

Fig. S6

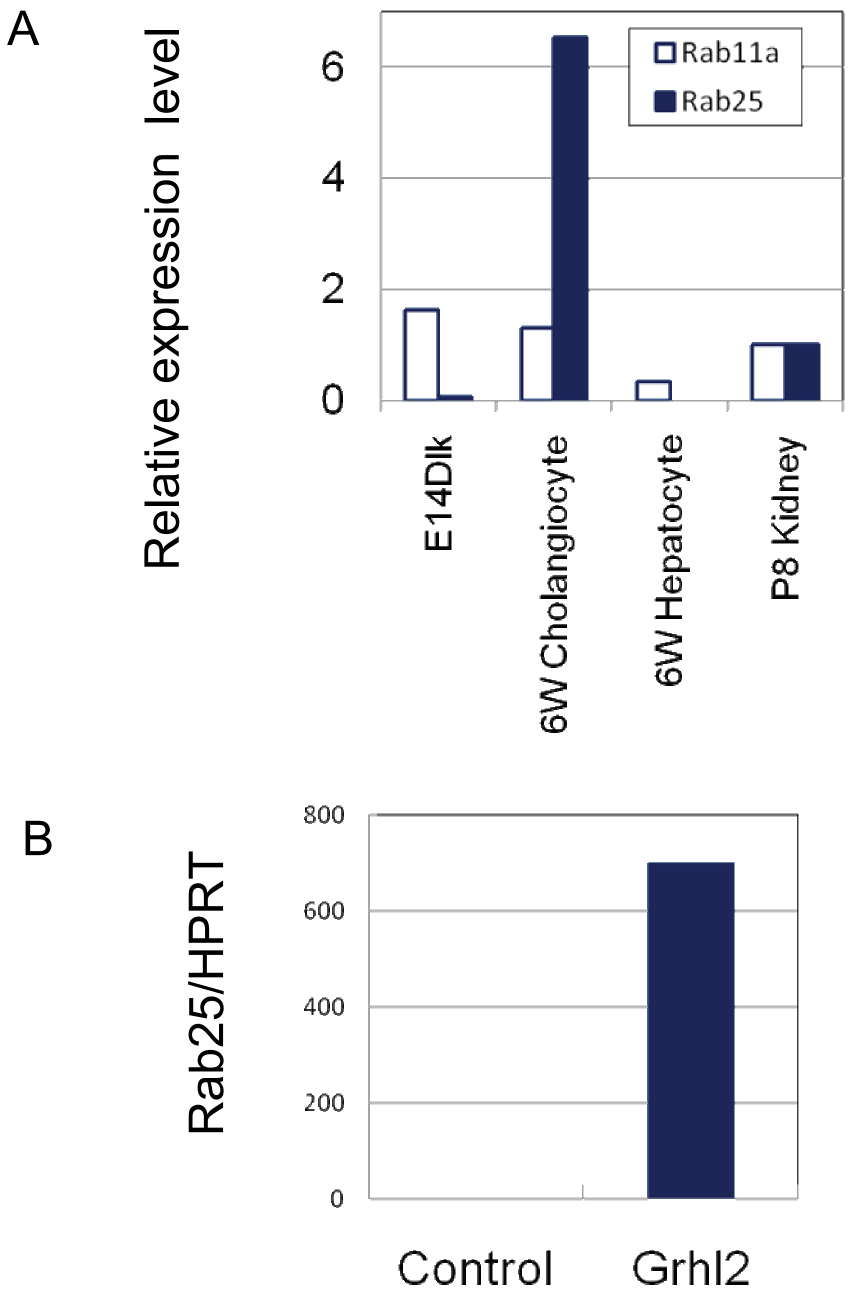

Fig. S6. Expression of Rab25 in liver epithelial cells. Rab25 is specifically expressed in cholangiocytes in the liver (A). Grhl2 remarkably increases expression of Rab25 in HPPL.

Fig. S7

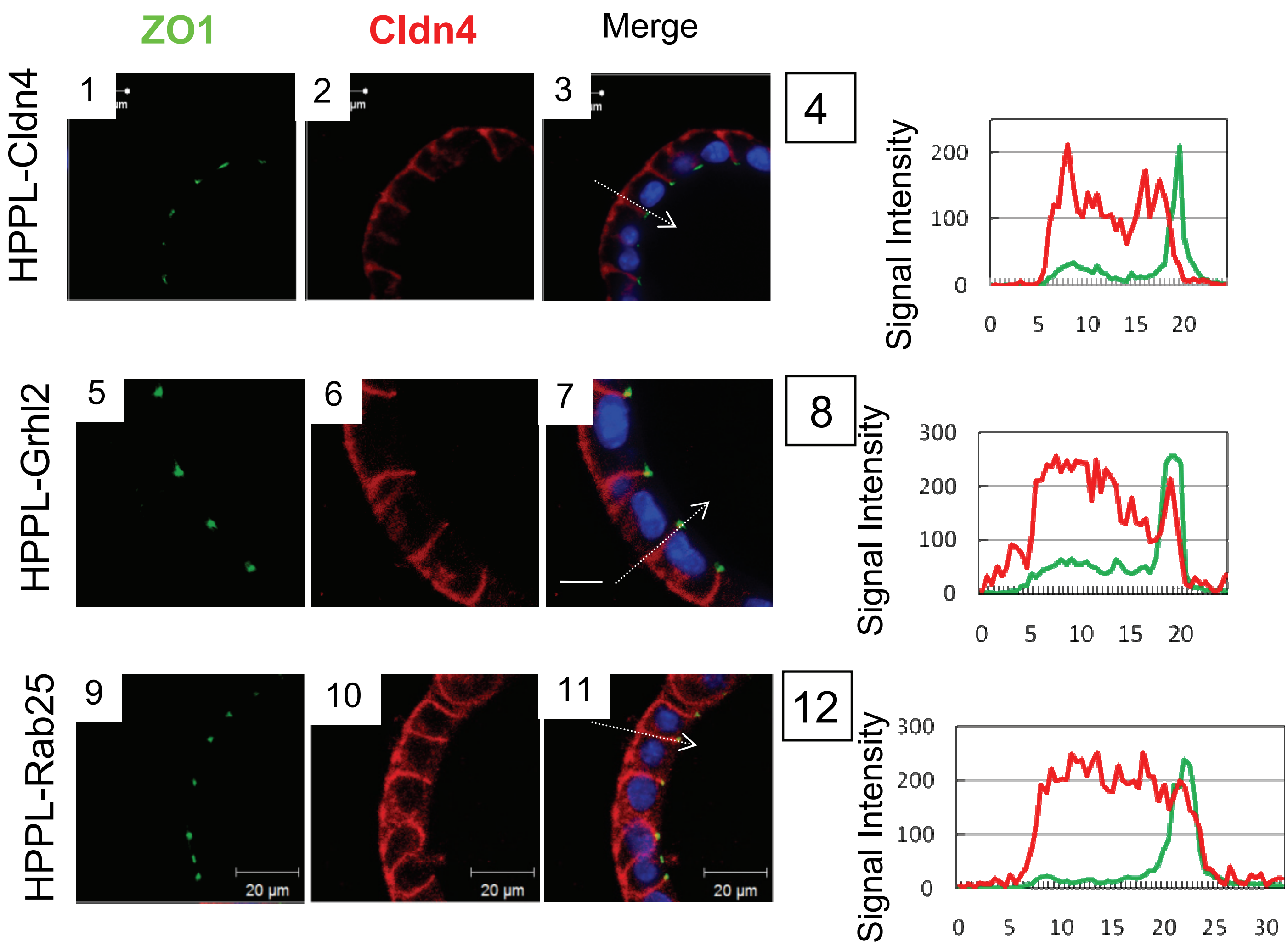

Fig. S7. Analysis for colocalization of Cldn4 and ZO1. Signal intensity along a lateral membrane is plotted in panel 4, 8, and 12. In HPPL-Cldn4, Cldn4 signal (red line) is visible from the basal tip to near the apical tip. However, it suddenly decreases at TJs, where ZO1 signal (green line) shows a peak. On the other hand, Cldn4 signal is still significantly observed at TJs in HPPL-Grhl2 and HPPL-Rab25. For quantitative analysis, the signal intensity at TJs over 30% of that at the basal was considered as localization of Cldn4 at TJs.

Fig. S8

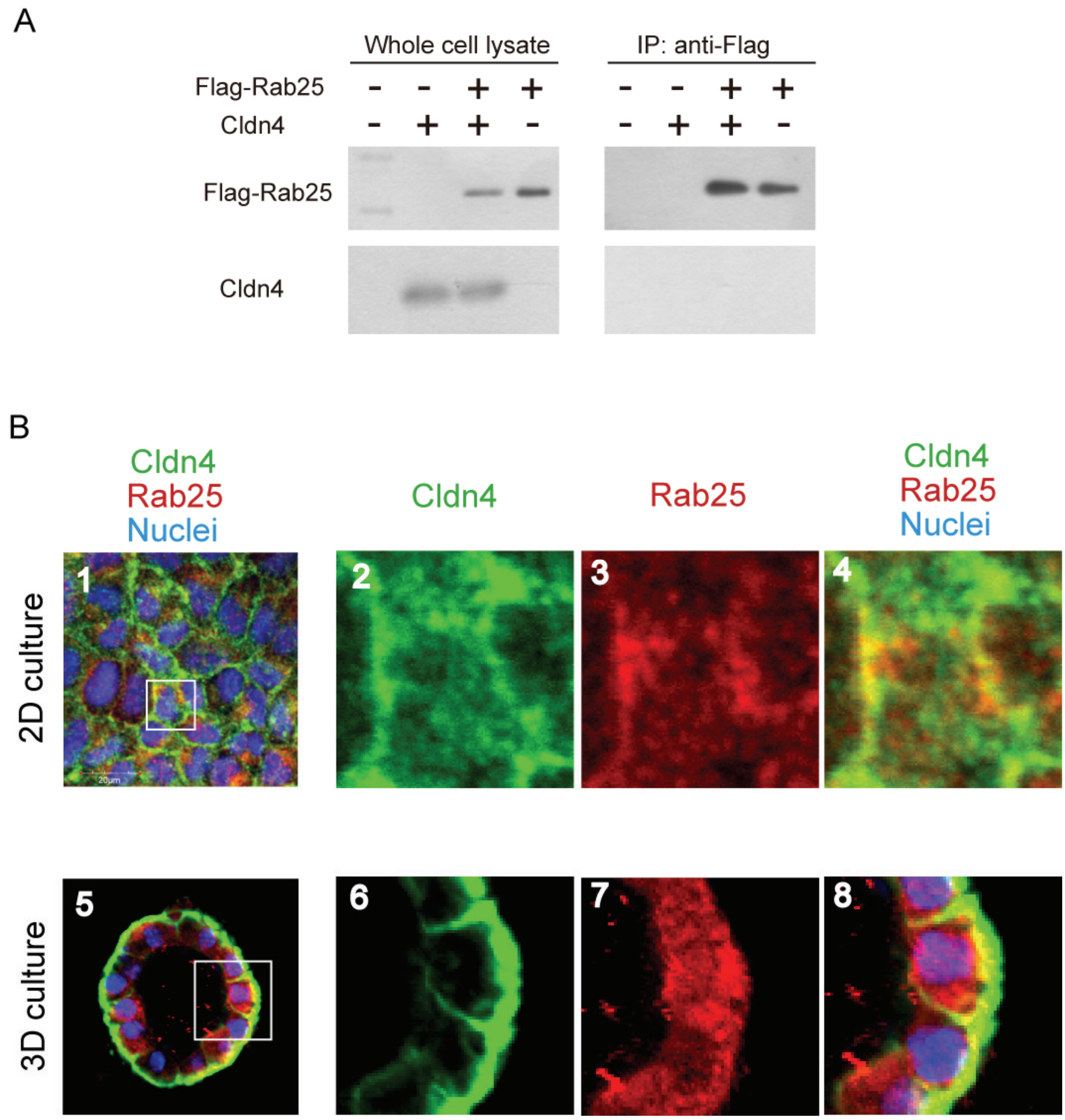

Fig. S8. Interaction between Cldn4 and Rab25.

A. No direct interaction between Cldn4 and Rab25. cDNAs of Cldn4 and Flag-tagged Rab25 were transfected to COS-7 cells. Immunoprecipitation was performed by using anti-Flag antibody and Protein G sepharose. Proteins bound to Protein G were detected with anti-Flag and anti-Cldn4 antibodies.

B. Rab25 are partly colocaized with Cldn4 and in HPPL. Cldn4 and myc-tagged Rab25 were detected with anti-Cldn4 (green) and anti-myc (red) antibodies, respectively. Yellow dots in panels 4 and 8 suggest the colocalization of Rab25 and Cldn4.