Supplemental Data to:

The transcription factor Klf5 is essential for intrahepatic biliary epithelial tissue remodeling after cholestatic liver injury

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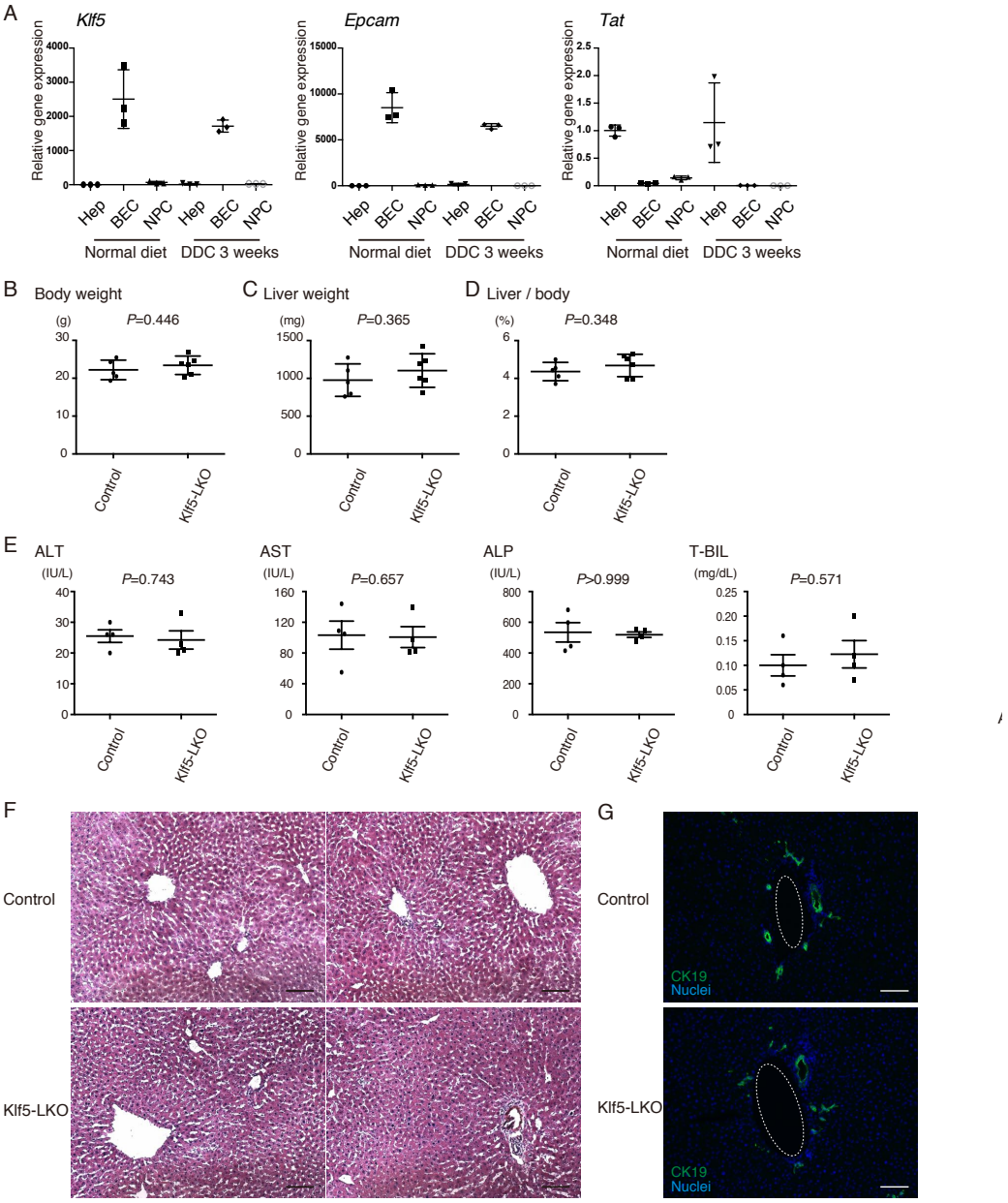
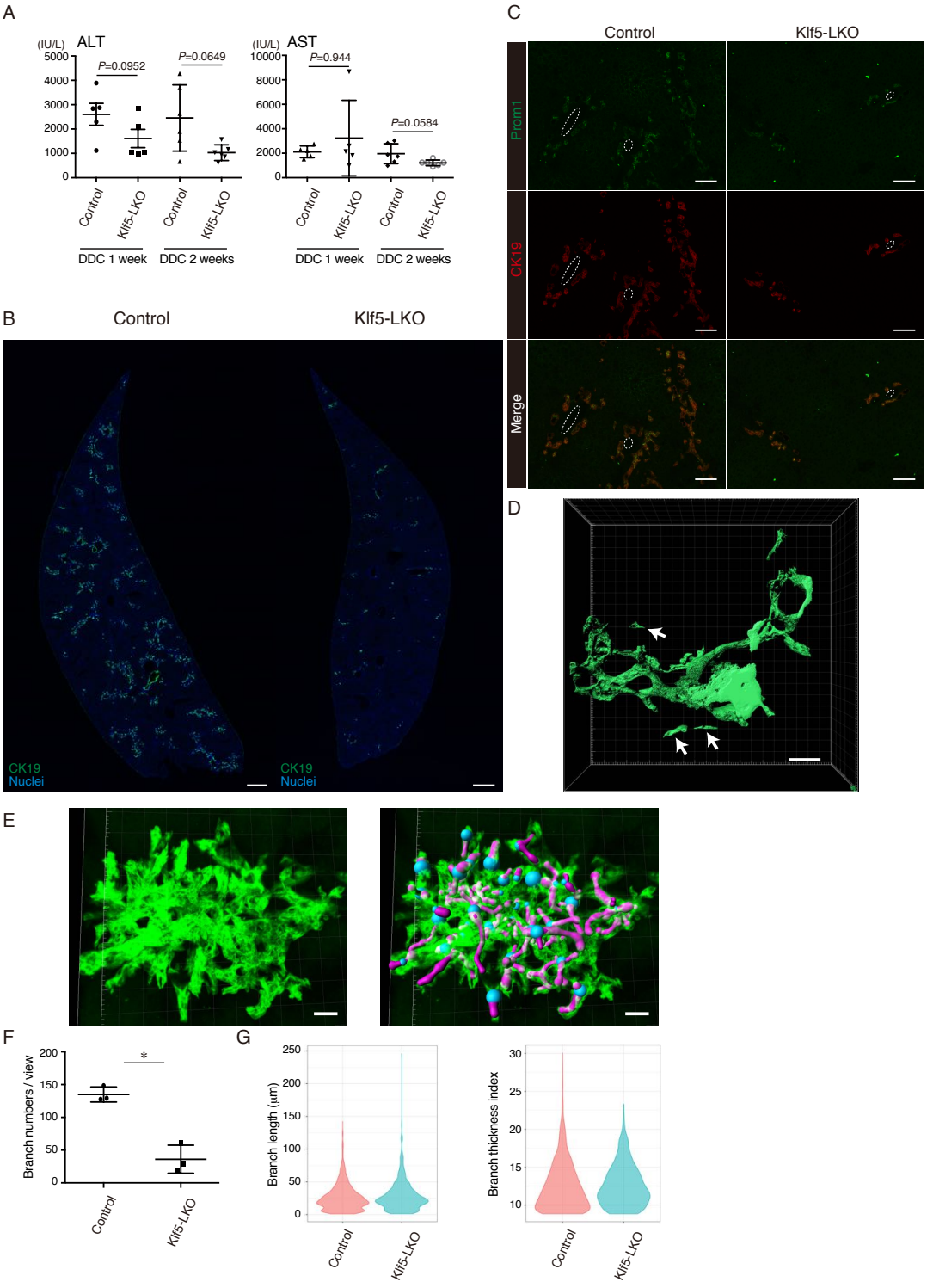
Figure S1


A
Klf5
Epcam
Tat
Relative gene expression
Hep
BEC
NPC
Normal diet
DDC 3 weeks
B Body weight
P=0.446
C Liver weight
P=0.365
D Liver / body
P=0.348
Control
Klf5-LKO
E
ALT
AST
ALP
T-BIL
P=0.743
P=0.657
P>0.999
P=0.571
F
G
CK19
Nuclei

Supplemental Figure 1. Klf5-LKO mice show normal liver development. (A) Expression level of *Klf5* in liver cell fractions collected from DDC-treated (three weeks) and non-treated (Normal diet) WT mice. The same data set used in Figure 1B for DDC injury condition was re-analyzed to compare the levels of *Klf5* expression under normal and injury conditions. Adequate cell fractionations were confirmed by analyses of a BEC marker gene *(Epcam)* and a hepatocyte marker gene *(Tat)*. (B–D) Klf5-LKO mice did not show any significant difference from control mice in terms of body weight (B), liver weight (C), and the proportion of the liver weight to the body weight (D). N = 5 and 6 for the control and the Klf5-LKO mice, respectively. P-values calculated by Student *t* test. (E) Serum biochemistry tests for hepatocyte injury markers (ALT and AST) and cholestasis markers (ALP and T-BIL), showing that no liver injury was induced in Klf5-LKO mice under physiological conditions. N = 4 mice for each group. P-values calculated by Mann Whitney *U* test. (F) H&E staining did not show any apparent difference in histology of the liver between the control and Klf5-LKO mice. Scale bar = 100 μm. (G) Immunostaining for CK19 (green) in the Klf5-LKO and control livers in normal conditions. Counterstaining for nuclei is also shown (Blue). Dashed lines show portal veins. Scale bar = 100 μm.

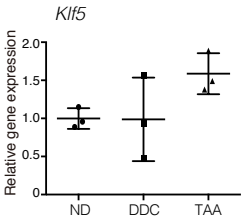
Figure S2


A
(IU/L) ALT
P=0.0952
P=0.0649
(IU/L) AST
P=0.944
P=0.0584
Control
Klf5-LKO
DDC 1 week
DDC 2 weeks
B
Control
Klf5-LKO
CK19
Nuclei
C
Control
Klf5-LKO
Prom1
CK19
Merge
D
E
F
Branch numbers / view
G
Branch length (μm)
Branch thickness index

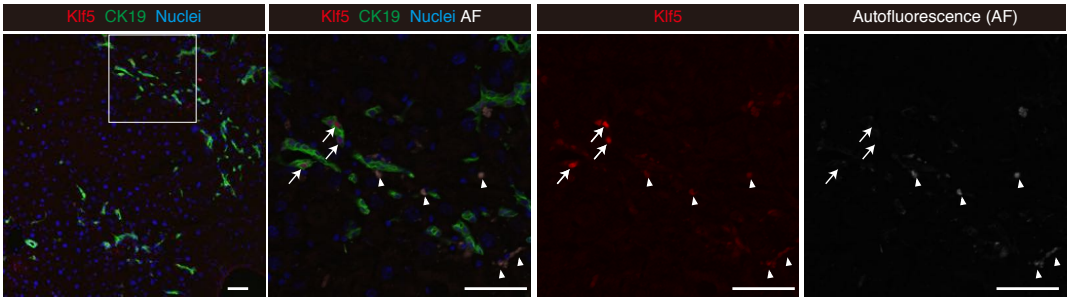
Supplemental Figure 2. DR induction upon DDC-induced liver injury is suppressed in Klf5-LKO mice. (A) Serum ALT and AST levels in the Klf5-LKO and control mice upon DDC administration for one week (N = 5 mice for each group) or two weeks (N = 6 mice for each group), showing that hepatocyte injury was not exacerbated in the Klf5-LKO mice. P-values calculated by Mann Whitney *U* test. (B) Representative images for CK19 immunostaining (green) of whole liver sections prepared form the control (left) and Klf5-LKO (right) mice treated with DDC for four weeks. Counterstaining for nuclei is also included (Blue). Scale bar = 500 μm. (C) Immunostaining for BEC markers, Prom1 (green) and CK19 (red), in the Klf5-LKO and control livers treated with DDC for four weeks. Scale bar = 100 μm. (D) A different view field for 3D immunostaining of CK19 (green) in the liver of DDC-treated Klf5-LKO mice shown in Figure 2F. The image is shown in surface mode. White arrows indicate $CK19^{+}$ cells separated from the biliary tree structure. Scale bar = 50 μm. (E) Representative images for CK19 staining (green) and traced biliary branch structure (magenta in the right panel) for quantitative analyses of 3D immunostaining data. Scale bar = 50 μm. (F) The number of biliary branches per unit view field (636.396 μm × 636.396 μm × 200 μm) in the Klf5-LKO and control livers treated with DDC for four weeks. N = 3 mice for each group and more than 5 views were analyzed per mouse. P-value was calculated by Student *t* test to be 0.00540. (G) Violin plots comparing the length (left panel) and the thickness (right panel) of the biliary branches in the Klf5-LKO and control livers treated with DDC for four weeks. “Branch thickness index” was calculated as the square root of the quotient obtained by dividing the branch volume size by the branch length.

Figure S3

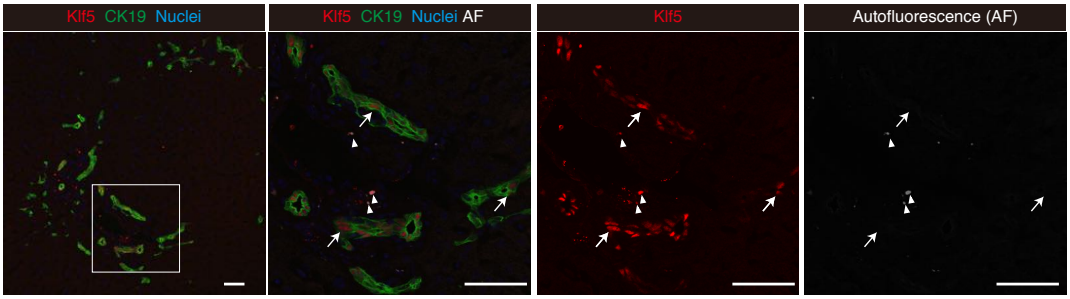
A



B



C



Supplemental Figure 3. The Klf5 expression in BECs is maintained under different types of liver injury conditions. (A) Expression levels of *Klf5* in BECs under different injury conditions. mRNA samples were prepared from BEC fractions obtained from the WT mice under physiological conditions (fed normal diet: ND), upon DDC administration for five weeks, or upon TAA administration for eight weeks, and subjected to quantitative RT-PCR analyses. P-value = 0.232 calculated by Kruskal-Wallis test. (B and C) Immunostaining for Klf5 (red) and CK19 (green) in the WT mouse liver upon TAA administration for eight weeks (B) and Abcb4 KO; $Klf5^{flox/flox}$ mouse (Abcb4 single knockout mouse) liver at eight weeks after birth (C). Counterstaining for nuclei is shown in blue. A region indicated by a white box in the leftmost panel is magnified in the other three panels. Non-specific autofluorescence signals (arrowheads) were acquired using a filter channel without any corresponding staining and are also shown in gray. Relevant signals for Klf5 are indicated by arrows. Scale bar = 50 μm.

Figure S4

A

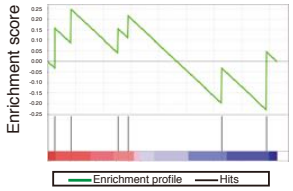
Upregulated GO gene sets	SIZE	NOM p-val	FDR q-val
GO_OSSIFICATION	14	0.00E+00	1.01E-02
GO_G_PROTEIN_COUPLED_RECEPTOR_SIGNALING_PATHWAY	14	0.00E+00	1.18E-02
GO_REGULATION_OF_PHOSPHATIDYLINOSITOL_3_KINASE_SIGNALING	9	1.15E-02	0.204
GO_RESPONSE_TO_CYTOKINE	26	4.15E-03	0.214
GO_NEGATIVE_REGULATION_OF_CELL_PROLIFERATION	25	8.06E-03	0.228
GO_REGULATION_OF_MYELOID_LEUKOCYTE_DIFFERENTIATION	7	2.72E-03	0.231
GO_NEGATIVE_REGULATION_OF_VIRAL_PROCESS	5	2.46E-03	0.236
GO_CELLULAR_RESPONSE_TO_CYTOKINE_STIMULUS	21	0.00E+00	0.240
GO_REGULATION_OF_SMOOTHENED_SIGNALING_PATHWAY	4	2.43E-03	0.240
GO_NEGATIVE_REGULATION_OF_VIRAL_GENOME_REPLICATION	4	0.00E+00	0.248
GO_REGULATION_OF_MYELOID_CELL_DIFFERENTIATION	7	2.99E-03	0.249

B

gene	baseMean	log2FC	lfcSE	padj
Notch1	193	-0.001	0.214	0.999
Notch2	700	0.091	0.167	0.925
Hes1	14419	0.158	0.210	0.890
Hey1	23	0.201	0.172	0.770
Jag1	14211	0.145	0.151	0.836

C

Enrichment plot: Wnt signaling pathway



P=0.827

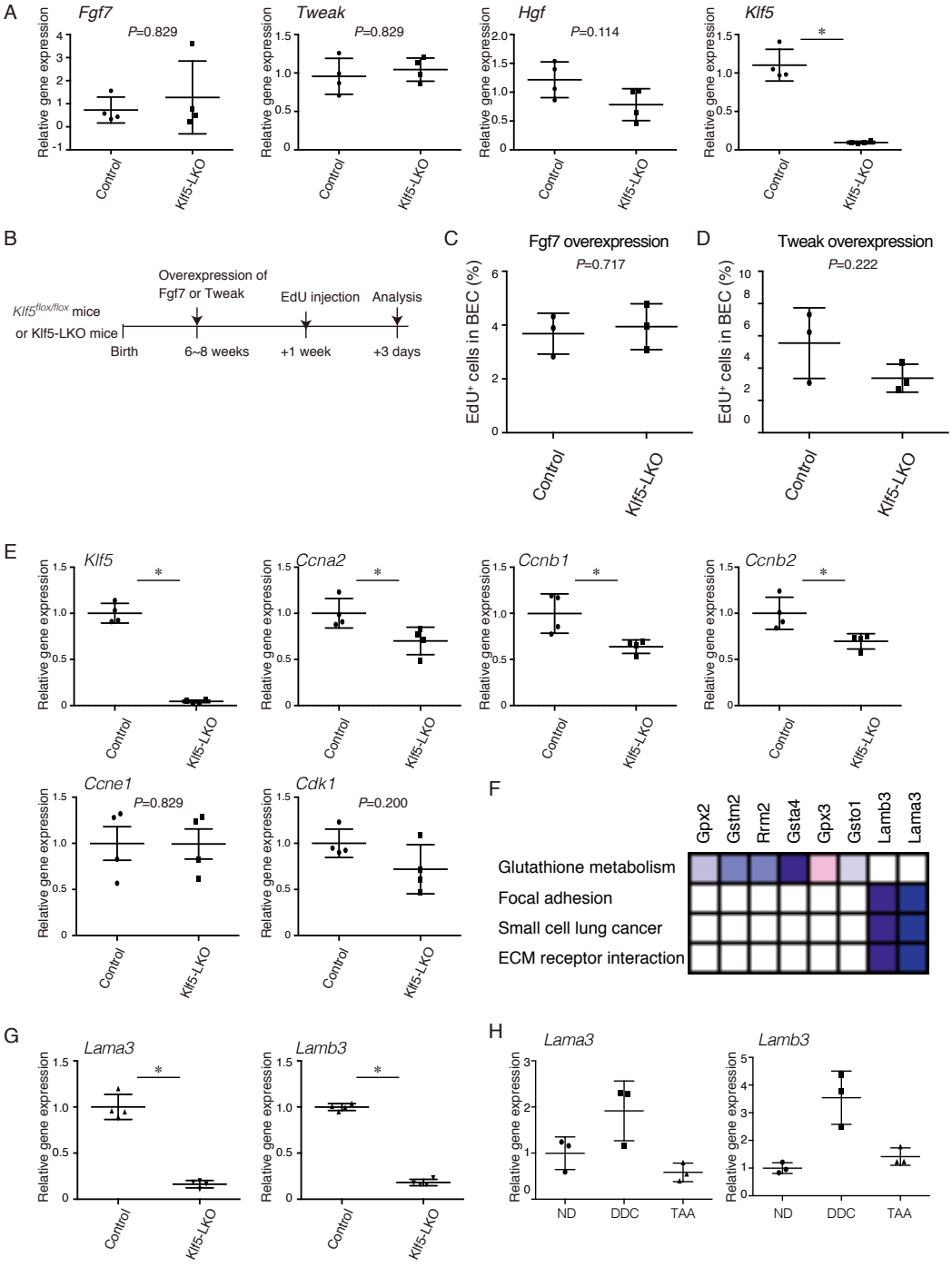
D Reactome pathways gene sets

Upregulated Reactome pathway gene sets	SIZE	NOM p-val	FDR q-val
REACTOME_CLASS_A1_RHODOPSIN_LIKE_RECEPTORS	4	2.40E-03	7.05E-02
REACTOME_GPCR_DOWNSTREAM_SIGNALING	8	0.00E+00	8.39E-02
REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS	3	4.54E-03	0.152
REACTOME_G_ALPHA_I_SIGNALLING_EVENTS	3	6.44E-03	0.185
REACTOME_G_ALPHA_Q_SIGNALLING_EVENTS	2	0.00E+00	0.216

Downregulated Reactome pathway gene sets	SIZE	NOM p-val	FDR q-val
REACTOME_CELL_CYCLE	26	1.96E-02	0.158
REACTOME_DNA_REPLICATION	14	4.05E-02	0.164
REACTOME_CYCLIN_A_B1_ASSOCIATED_EVENTS_DURING_G2_M_TRANSITION	6	2.67E-02	0.165
REACTOME_MITOTIC_PROMETAPHASE	8	3.12E-02	0.177
REACTOME_CELL_CYCLE_MITOTIC	25	2.48E-02	0.180
REACTOME_CELL_JUNCTION_ORGANIZATION	6	7.96E-03	0.201
REACTOME_REGULATION_OF_MITOTIC_CELL_CYCLE	8	3.19E-03	0.202
REACTOME_APC_C_CDH1_MEDIATED_DEGRADATION_OF_CDC20_AND_OTHER_APC_C_CDH1_TARGETED_PROTEINS_IN_LATE_MITOSIS_EARLY_G1	4	1.72E-03	0.227

Supplemental Figure 4. Effect of Klf5 deletion on pathway activations and cellular functions in the DDC-injured liver. (A) The entire list of enriched gene sets categorized in GO biological process terms that were upregulated by Klf5 deletion in BECs. The SIZE column indicates the number of genes hit in each gene set. Gene sets that meet the criteria for both NOM p-val < 0.05 and FDR q-val < 0.25 are considered to be significantly enriched and listed. (B) Expression levels of Notch signaling-related genes as revealed by RNA-seq analysis. (C) Wnt signaling pathway enrichment plot. NOM p-val = 0.817 and FDR q-val = 0.950. (D) The entire list of significantly enriched Reactome pathways gene sets upon loss of Klf5 in BECs under the DDC-induced injury condition. The SIZE column indicates the number of genes hit in each gene set. Gene sets that meet the criteria for both NOM p-val < 0.05 and FDR q-val < 0.25 are considered to be significantly enriched and listed.

Figure S5


A
Fgf7
P=0.829
Tweak
P=0.829
Hgf
P=0.114
Klf5
*
Relative gene expression
Control
Klf5-LKO
B
Klf5flox/flox mice
or Klf5-LKO mice
Birth
Overexpression of
Fgf7 or Tweak
6~8 weeks
EdU injection
+1 week
Analysis
+3 days
C
Fgf7 overexpression
P=0.717
D
Tweak overexpression
P=0.222
EdU+ cells in BEC (%)
E
Klf5
Ccna2
Ccnb1
Ccnb2
Ccne1
P=0.829
Cdk1
P=0.200
F
Gpx2
Gstm2
Rrm2
Gsta4
Gpx3
Gsto1
Lamb3
Lama3
Glutathione metabolism
Focal adhesion
Small cell lung cancer
ECM receptor interaction
G
Lama3
Lamb3
H
Lama3
Lamb3
ND
DDC
TAA

Supplemental Figure 5. Gene expression and/or functional analyses on candidate signaling pathways and molecules functioning upstream or downstream of Klf5. (A) Expression levels of cytokines and growth factors involved in DR induction. Whole liver mRNA samples prepared from the control and Klf5-LKO mice treated with DDC for one week were subjected to quantitative RT-PCR analyses. N = 4 mice. P-values calculated by Mann Whitney *U* test. (B) Experimental scheme of *in vivo* EdU incorporation assays to test whether Fgf7 or Tweak functions upstream of Klf5 in promoting BEC proliferation. (C and D) Quantification of EdU^+ BECs in livers of the control and Klf5-LKO mice where Fgf7 (C) or Tweak (D) was overexpressed. BEC fractions isolated from the livers were analyzed by flow cytometry. N = 3 mice for each group. P-values calculated by Student *t* test. (E) Expression levels of cell cycle-related genes. BEC mRNA samples prepared from the control and Klf5-LKO mice treated with DDC for two weeks were subjected to quantitative RT-PCR analyses. N = 4 mice. P-values were calculated by Mann Whitney *U* test and were as follows: 0.0286 (*Klf5*), 0.0286 (*Ccna2*), 0.0286 (*Ccnb1*), and 0.0286 (*Ccnb2*). (F) Leading edge analysis of the entire list of enriched KEGG pathway gene sets shown in Figure 5F. The range of colors in the heatmap (pink, light blue, and dark blue) corresponds to the range of gene expression values (moderate, low, and lowest, respectively). (G) Expression levels of the *Lama3* and *Lamb3* genes. BEC mRNA samples prepared from the control and Klf5-LKO mice treated with DDC for two weeks were subjected to quantitative RT-PCR analyses. N = 4 mice. P-values were calculated by Mann Whitney *U* test and were as follows: 0.0286 (*Lama3*) and 0.0286 (*Lamb3*). (H) Expression profiles of *Lama3* and *Lamb3* in BECs upon liver injury. BEC mRNA samples prepared from the WT mice under physiological conditions (fed normal diet: ND), upon DDC administration for five weeks, or upon TAA administration for eight weeks were subjected to quantitative RT-PCR analyses. P-values were calculated by Kruskal-Wallis test and were as follows: 0.0286 (*Lama3*) and 0.00360(*Lamb3*).