6-diamidine-2'-phenylindole dihydrochloride (DAPI, Sigma). For each mouse, we counted the number of immunostained cells and primitive ductal structures in 10 randomly selected fields and present these data as the total number observed per mouse.

Immunoblot analysis

A detailed western was previously described.[4] Briefly, tissue samples were homogenized in RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 1% sodium dodecyl sulfate) containing a cocktail of inhibitors of proteases (Roche Diagnostics, Indianapolis, IN) and phosphatases (Nacalai Tesque, Kyoto, Japan). After sodium dodecyl sulfate/polyacrylamide gel electrophoresis of liver homogenates (20 or 50 μg protein) and transfer to PVDF membranes, blots were incubated with primary Abs (supplementary Table 4), followed by incubation with peroxidase-labeled secondary Ab (GE Healthcare, Uppsala, Sweden) and visualization using the ECL Western Blotting Analysis System (GE Healthcare). Images of immunoblot chemiluminescence were quantified using ImageJ software (http://rsb.info.nih.gov/ij/).

Wnt5a-blocking experiments

HPPL cells were suspended in a mixture of type I collagen and EHS gel at a

density of 4×10^4 cells/ml. Cell suspension was added to each cell culture and after incubation at 37°C for 2 h, 500 μl of DMEM/F-12 with Wnt5a inhibitors was added above and below the insert and the cells were cultured for seven days. Cells were treated with one of following compounds; 2 μg/ml Wnt5a Ab (R&D systems, Minneapolis, MN), 2 μg/ml control goat IgG (Sigma), both 2 μg/ml Wnt5a Ab plus 100 ng/ml recombinant Wnt5a (R&D systems), 100 μM Box5 (Wnt5a inhibitor, Millipore)[5], and both 100 μM Box5 plus 100 ng/ml recombinant Wnt5a.

Microarray analysis

For oligo DNA microarray analysis, total RNA samples were extracted from liver tissues of E18.5 WT or Wnt5a KO mice with Trizol reagent. We used 3D-Gene Mouse Oligo chip 24k (23,522 distinct genes, Toray Industries, Tokyo, Japan) in this assay. Total RNA was labeled with Cy3 or Cy5 using the Amino Allyl MessageAMP II aRNA Amplification Kit (Applied Biosystems). The Cy3- or Cy5-labeled aRNA pools were hybridized for 16 h. The hybridization signals obtained using 3D-Gene Detected signals for each gene, were normalized by the global normalization method (Cy3/Cy5 ratio median=1). Genes with Cy3/Cy5 normalized ratios of >2.0 or <0.5 were defined as upregulated or downregulated genes, respectively. Hierarchical cluster analysis was performed using global normalization data according to Pearson product-moment

correlation coefficient. Pathways that showed significant gene expression changes were analyzed using GenMAPP ver2.1 (MAPP finder, http://www.genmapp.org/) software. The microarray data was deposited to Gene Expression Omnibus of NCBI (http://www.ncbi.nlm.nih.gov/geo/, GEO accession number, GSE41803).

Immunocytostaining of cultured cells

Cultured cells within gels were fixed with 4% paraformaldehyde /PBS and permeabilized using 0.5% Triton X-100/PBS. After blocking with 10% donkey serum/PBS, the gels were incubated with anti-CK19 Ab followed by Alexa 488-conjugated anti-rabbit IgG Ab. Nuclei were stained with DAPI. For each analysis, addition of an appropriate immune serum provided a negative control. Immunostained bile-duct like branching structures were counted under the fluorescent microscope (Olympus, Tokyo, Japan) or confocal lasermicroscope (Olympus).

Immunocytostaining of FACS-sorted cells

Dissociated liver cells derived from WT E14.5 fetuses were incubated with FITC-conjugated anti-Dlk Ab, allophycocyanin (APC)-conjugated anti-CD45 Ab (Pharmingen) and APC-conjugated anti-Ter119 Ab (Pharmingen) for 60 min at 4°C. After washing with PBS containing 3% FCS, the stained cells were sorted directly onto slideglasses coated by poly-L-lysine by use of a MoFlo™ FACS. Sorted cells were

fixed with 4% PFA/PBS and permeabilized using 0.5% Triton X-100/PBS. After blocking with 10% donkey serum/PBS, the cells were incubated with primary Ab followed by Alexa 546-conjugated anti-rabbit IgG Ab (Invitorgen). Nuclei were stained with DAPI. For each analysis, addition of an appropriate immune serum provided a negative control.

Statistics

We used Microsoft Excel software (Microsoft, Redmond, WA, USA) to calculate standard deviations, standard error and the statistical significance of differences between samples (2-tailed Student's *t* test). P values <0.05 were considered statistically significant.

Supplementary Tables

Supplementary Table 1. Primer list for RT-PCR

Wnt5a	F	5'-ATTGTCCCCCAAGGCTTAAC-3'	Fzd6	F	5'-AGATGACATGGTTCTCTGATC-3'
	R	5'-GTCTCTCGGCTGCCTATTTG-3'		R	5'-CATGATTTGTGGTCGCTCCTG-3'
Fzd1	F	5'-AGCCGAAAAAGTATGGCTGA-3'	Fzd7	F	5'-GTTGCTACTTCATGGTGGCAG-3'
	R	5'-GCACCTTCACCAGAGGGTAG-3'		R	5'-TAGCACACTCCACTGAGTAGG-3'
Fzd2	F	5'-TATGCAATGAGCGCTTCTCAG-3'	Fzd8	F	5'-ACCAGAGCCTTGACAACCTAC-3'
	R	5'-AAGGATGTACCGATGAACAGG-3'		R	5'-ATGCCCACTACTAGGCACATG-3'
Fzd3	F	5'-ACAGAGTTCGGATTGAGATCC-3'	Fzd9	F	5'-TCTGCTATGTAGCCAGCATGG-3'
	R	5'-CAGGAGTGACTGAGCAAAGTC-3'		R	5'-TGAGCATGAAGACAGCCACAG-3'
Fzd4	F	5'-TGAGACTAGTGGATGCCGATG-3'	Fzd10	F	5'-AGTGGGCAGATGTATGTTATC-3'
	R	5'-TGCCATGTTTGAGTCATCTGC-3'		R	5'-TCAAGATAGTCTTCACAGCCG-3'
Fzd5	F	5'-ATCGCAGGTTATGCACAGTAC-3'	β-actin	F	5'-AGACTTCGAGCAGGAGATG-3'
	R	5'-AGTGCTGTTCATACAGGTAGC-3'		R	5'-ACTCATCGTACTCCTGCTTG-3'

Fzd, Frizzled; F, forward primer; R, reverse primer.

Supplementary Table 2. Primer list for quantitative RT-PCR

ALB	F	5'-AGTGTTGTGCAGAGGCTGAC-3'	MRP3	F	5'-ACCAGGAGGACCATGAAGC-3'
	R	5'-TTCTCCTTCACACCATCAAGC-3'		R	5'-TGTGGGTGCTGAGTGTGTCT-3'
β-actin	F	5'-AAGGCCAACCGTGAAAAGAT-3'	c-Myc	F	5'-CTTCTCTCCTTCCTCGGACTC-3'
	R	5'-GTGGTACGACCAGAGGCATAC-3'		R	5'-CCTCATCTTCTTGCTCTTCTTCA-3'
CK19	F	5'- TGACCTGGAGATGCAGATTG -3'	Notch1	F	5'-CTGGACCCCATGGACATC-3'
	R	5'- CCTCAGGGCAGTAATTTCCTC -3'		R	5'-AGGATGACTGCACACATTGC-3'
CPS1	F	5'-GACACCACTGCCCGAGAC-3'	Notch2	F	5'-TGCCTGTTTGACAACTTTGAGT-3'
	R	5'-CAGCAGACCTGCCACCTT-3'		R	5'-GTGGTCTGCACAGTATTTGTCAT-3'
CyclinD1	F	5'-TTTCTTTCCAGAGTCATCAAGTGT-3'	Sox9	F	5'-CAGCAAGACTCTGGGCAAG-3'
	R	5'-TGACTCCAGAAGGGCTTCAA-3'		R	5'-TCCACGAAGGGTCTCTTCTC-3'
G6Pase	F	5'-TCTGTCCCGGATCTACCTTG-3'	TAT	F	5'-GGAGGAGGTCGCTTCCTATT-3'
	R	5'-GAAAGTTTCAGCCACAGCAA-3'		R	5'-GCCACTCGTCAGAATGACATC-3'
Hes-1	F	5'-TGCCAGCTGATATAATGGAGAA-3'	TGFα	F	5'-CCTGGTGGTGGTCTCCATT-3'
	R	5'-CCATGATAGGCTTTGATGACTTT-3'		R	5'-CAGTGTTTGCGGAGCTGA-3'
HNF4α	F	5'-TCCTGCAGGCAGAGGTTC-3'	TO	F	5'-TCCAGGGAGCACTGATGATA-3'
	R	5'-CGCCATTGATCCCAGAGA-3'		R	5'-CTGGAAAGGGACCTGGAATC-3'
HNF1β	F	5'- AGAGCCCAGGCAGTCACA -3'	Wnt4	F	5'-GCGTAGCCTTCTCACAGTCC-3'
	R	5'- GGGGTTCCTGCTTATGTGC -3'		R	5'-CGCATGTGTGTCAAGATGG-3'
Jagged1	F	5'-GAGGCGTCCTCTGAAAAACA-3'	Wnt5a	F	5'- TGAAGCAGGCCGTAGGAC -3'
	R	5'-ACCCAAGCCACTGTTAAGACA-3'		R	5'- AGCCAGCACGTCTTGAGG -3'
Jagged2	F	5'-CGTCATTCCCTTTCAGTTCG-3'			
	R	5'-CCTCATCTGGAGTGGTGTCA-3'			

ALB, albumin; CPS1, carbamoyl phosphate synthetase 1; CK19, Cytokeratin 19; G6Pase, glucose-6-phosphatase; HNF4α, hepatocyte nuclear factor 4α; MRP3,

multidrug resistance-associated protein 3; TAT, tyrosine aminotransferase; TGFα, Transforming growth factor α; TO, tryptophan-2,3-oxygenase; F, forward primer; R, reverse primer.

Supplementary Table 3. List of antibodies for immunostaining

Primary antibodies	Species	Sources (Catalog#)	Dilution
Cytokeratin19	Rabbit	produced by Dr. A. Miyajima (Tokyo University)[6]	2000
Entactin	Rat	Abcam (ab44944)	500
Hes1	Rabbit	Produced by Dr. T. Sudo (Toray Industries, Kamakura, Japan)[7]	40,000
HNF1β	Rabbit	Santa Cruz (sc-22840)	250
HNF4α	Goat	Santa Cruz (sc-6556)	500
PCNA	Mouse	Cell Signaling (2586)	100
Phospho-CaMKIIα (Thr286)	Rabbit	Cell Signaling (3361)	100
Phospho-PKCaβII (Thr638/641)	Rabbit	Cell Signaling (9375)	100
p75 NTR	Rabbit	Abcam (ab8875)	100

HNF, Hepatocyte nuclear factor; CaMKII, Calcium/calmodulin-dependent kinase II; NTR, Neurotrophin receptor, PKC, Protein kinase C.

Supplementary Table 4. List of antibodies for immunoblots

Primary antibodies	Species	Sources (Catalog#)	Dilution
Albumin	Goat	Bethyl (A90-134A)	2000
AFP	Rabbit	Thermo (RB-365)	200
Phospho-β-catenin (Ser552)	Rabbit	Cell Signaling (9566)	1000
CaMKII	Rabbit	Cell signaling (3362)	1000
CaMKIIα	Mouse	Santa Cruz (sc-32288)	100
Phospho-CaMKIIα (Thr286)	Mouse	Abcam (ab2724)	1000
Cytokeratin19	Rabbit	produced by Dr Miyajima[6]	100000
p53	Rabbit	Cell Signaling (2524S)	1000
Phospho-p53 (Ser15)	Rabbit	Cell Signaling (9284)	1000
PCNA	Mouse	Cell Signaling (2586)	2000
PKCα	Rabbit	Cell Signaling (2056)	1000
Phospho-PKCaβII (Thr638/641)	Rabbit	Cell Signaling (9375)	1000
Rac1	Rabbit	Cell Signaling (2465)	1000
Phospho-Rac1 (Ser71)	Rabbit	Cell Signaling (2461)	1000
β-actin	Mouse	Sigma (A5441)	5000

AFP; α-fetoprotein; CaMKII, Calcium/calmodulin-dependent kinase II; PCNA, Proliferating cell nuclear antigen; PKC, Protein kinase C.

Supplementary Table 5. Representative genes in cDNA microarray analysis

Down-regulated genes in Wnt5a KO mice

Entrez ID	Symbol	Description	KO/WT Ratio
NM_032541	Hamp1	hepcidin antimicrobial peptide 1	0.21
NM_183257	Hamp2	hepcidin antimicrobial peptide 2	0.23
XM_130814	NP_083982.1	carboxypeptidase B1	0.25
NM_008218	Hba-a1	hemoglobin alpha, adult chain 1	0.27
NM_010544	Ihh	Indian hedgehog	0.31
NM_009606	Acta1	actin, alpha 1, skeletal muscle	0.32
-	Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2	0.34
-	Hbb-b1	hemoglobin, beta adult major chain	0.37
NM_023637	Sars2	seryl-aminoacyl-tRNA synthetase 2	0.38
NM_013465	Ahsg	alpha-2-HS-glycoprotein	0.38

Up-regulated genes in Wnt5a KO mice

Entrez ID	Symbol	Description	KO/WT Ratio
NM_134144	Cyp2c50	cytochrome P450, family 2, subfamily c, polypeptide 50	10.15
NM_009850	Cd3g	CD3 antigen, gamma polypeptide	9.90
-	IGKV7-33	Immunoglobulin Kappa light chain V gene segment	8.99
NM_010666	Krt27	keratin 27	8.95
NM_008241	Foxg1	forkhead box G1	7.49
NM_146662	Olfr365	olfactory receptor 365	7.48
-	Syce1	synaptonemal complex central element protein 1	7.48
NM_183427	Glra2	glycine receptor, alpha 2 subunit	7.43
NM_010001	Cyp2c37	cytochrome P450, family 2, subfamily c, polypeptide 37	7.36
NM_011260	Reg3g	regenerating islet-derived 3 gamma	7.05

Supplementary Figure Legends

Supplementary Figure 1. Expression analysis of Frizzled (Fzd)-family genes using densitometry. Expression levels of Fzd-family genes (Fig. 1C) were quantified using densitometry. Values represent the ratio relative to the density of Lane 1 (hepatoblasts). Lane1, 2 and 3 represent $CD45^{-}Ter119^{-}Dlk^{high}$ cells (hepatoblasts) in E14.5 Liver, $CD45^{+}Ter119^{+}$ cells (hematopoietic cells) in E14.5 Liver, and adult hepatocytes, respectively.

Supplementary Figure 2. Phenotype of E18.5 Wnt5a deficient (KO) mice. (A) Representative macroscopic view of E18.5 Wnt5a KO (left) and wild type (WT, right) embryos. (B) Representative macroscopic view of E18.5 Wnt5a KO (upper) and WT livers (lower). (C) Analysis on ratio of liver weight (left panel) and liver:body weight (right panel). Values represent the ratio relative to the mean for the livers in WT mice. Although the average liver weight in Wnt5a KO E18.5 fetal mice was significantly lower than in WT littermates, the average ratio of liver:body weight in Wnt5a KO mice was almost equal to that in littermate WT mice. Bars in dot-plot graphs represent mean ± SD of values shown. *$p < 0.001$.

Supplementary Figure 3. Expression analysis of molecules related to biliary

differentiation in Wnt5a KO livers. (A) Quantitative RT-PCR analysis of the *Notch1, Notch2, Jagged1, Jagged2, Hes1, CK19, and hepatic nuclear factor (HNF)1β* in E16.5 fetal livers. Values represent the ratio relative to the mean for the livers of WT mice. Steady-state levels of Notch1, Notch2, and Jagged1 in Wnt5a KO livers were significantly higher than in littermate WT livers. (B) Immunohistological analysis of Hes1 in E18.5 livers. Left 2 panels; immunostaining of Hes1 (green) in E18.5 livers. Right panel; number of $Hes1^+$ cells in 10 random fields examined in WT and Wnt5a KO livers. Numbers of $Hes1^+$ cells were significantly higher in Wnt5a KO livers relative to WT mice, indicating that numbers of Notch-activated cells were increased in Wnt5a KO livers. Bars in dot-plot graphs represent mean ± SD of values shown. The result is representative of 3 independent experiments. *$p < 0.01$. **$p < 0.05$. PV: portal vein. Scale bars: 50 μm.

Supplementary Figure 4. Expression analysis of proliferation markers in Wnt5a KO livers. (A) Quantitative RT-PCR analysis of the *cyclin D1, c-Myc,* and *transforming growth factor (TGF) α* in E16.5 and E18.5 fetal livers. Values represent the ratio relative to the mean for the livers of WT mice. Expression levels of these genes in Wnt5a KO livers were almost equal to that in WT livers. (B) Immunoblot analysis of Proliferating cell nuclear antigen (PCNA) in E16.5 WT and Wnt5a KO livers (left

panel). Mice 1-4 and Mice 5-8 are E16.5 WT and Wnt5a KOs, respectively. Values represent the ratio relative to the mean for the livers of WT mice (right panel). PCNA production in Wnt5a KO livers was almost equal to that in WT livers. PTC: positive control. (C) Immunohistological analysis of PCNA and CK19 in E18.5 livers. Left 2 panels; immunostaining of PCNA (green) and CK19 (red) in E18.5 livers. Right panel; number of $PCNA^{+}CK19^{+}$ cells in 10 random fields examined in WT and Wnt5a KO livers. Numbers of $PCNA^{+}CK19^{+}$ cells in Wnt5a KO livers were almost equal to those in WT mice. Bars in dot-plot graphs represent mean ± SD of values shown. The result is representative of 3 independent experiments. PV: portal vein. Scale bars: 20 μm.

Supplementary Figure 5. Expression analysis and cDNA microarray analysis of molecules in Wnt5a KO livers. (A) Quantitative RT-PCR analysis of the hepatocyte differentiation markers, albumin (ALB) and hepatocyte nuclear factor (HNF) 4α, is depicted as the ratio of ALB and HNF4α copy number in E16.5 Wnt5a KO livers relative to WT livers, respectively. Steady-state levels of ALB and HNF4α mRNA in Wnt5a KO livers were almost equal to that in WT livers. (B) Expressions of ALB, HNF4α, glucose-6-phosphatase (G6Pase), tyrosine amino transferase (TAT), and carbamoyl phosphate synthetase 1 (CPS1) in E18.5 fetal livers. Values represent the ratio relative to the mean for the livers of WT mice. Steady-state levels of the hepatic

markers in Wnt5a KO livers were almost equal to that in WT livers. Bars in dot-plot graphs represent mean ± SD of values shown. The result is representative of 3 independent experiments. (C) Hierarchical cluster analysis of up- or down-regulated genes based on cDNA microarray using liver tissues of E18.5 WT or Wnt5a KO mice (left panel). WT/KO normalized ratios >2.0 or <0.5 were defined as up- or down-regulated genes, respectively. Pathway analysis indicated the up- or down-regulated pathways in E18.5 Wnt5a KO livers (right panel).

Supplementary Figure 6. Representative views of bile duct-like cysts in three-dimensional culture of a hepatic progenitor cell line (HPPL). We categorized HPPL-derived colonies into one of three classes, (A) colonies without clear lumina, (B) small cysts (50-100 μm diameter with clear lumina) and (C) large cysts (>100 μm diameter with clear lumina). Immunocytostaining analysis showed that colonies without clear lumina produced both the hepatocytic marker ALB and the biliary marker CK19 as previously described.[8] Cells in the luminal walls of small and large cyst, in contrast, produced the biliary marker CK19 alone, indicating their differentiation to a cholangiocyte lineage. The number of cells in luminal walls judged under confocal lasermicroscope was ~20-50 cells/round and >50 cells/round in small and large cysts, respectively. Scale bars: 100 μm.

Supplementary Figure 7. Analysis of HPPL-derived cysts treated with Wnt5a inhibitors. (A) Quantitative RT-PCR analysis of *Wnt5a* in primary $CD45^{-}Ter119^{-}Dlk^{high}$ cells of E14.5 WT fetal livers (hepatoblasts, Dlk^{high}) and HPPL cultured on laminin-coated dish (HPPL). Values represent the ratio relative to the mean for the Dlk^{high} cells. *Wnt5a* is expressed in heparoblasts and HPPL. (B) Numbers of bile duct-like cysts derived from the HPPL in 5 random fields per well in cultures supplemented with either vehicle (DMSO), Wnt5a inhibitor (100 μM Box5), both Wnt5a inhibitor plus 100 ng/ml recombinant Wnt5a, or 100 ng/ml recombinant Wnt5a. Cultures with Wnt5a inhibitor resulted in a significant increase in total numbers of bile-duct like cysts derived from HPPL relative to vehicle-only controls, and blocked the effect of Wnt5a supplementation. (C) Quantitative RT-PCR analysis of the *HNF1β, Sox9, Notch1, Notch2, Jagged1, Jagged2, MRP3, HNF4α, cyclin D1, and c-Myc* in HPPL-derived cysts treated with control goat IgG or anti-Wnt5a Ab. Values represent the ratio relative to the mean for the samples of control Ab. Expression levels of HNF1β, Sox9, and Notch2 were significantly upregulated in cultured cells supplemented with anti-Wnt5a Ab relative to control Ab. (D) Immunoblot analysis of CK19, ALB, AFP, and PCNA in HPPL-derived cysts treated with anti-Wnt5a Ab. Lane 1-3 and Lane 4-6 are control IgG-supplemented controls and anti-Wnt5a Ab-supplemented samples,

respectively. Protein levels of CK19, ALB, AFP, and PCNA did not change. Bars represent mean ± SD of values. The result is representative of 3 independent experiments. *$p < 0.05$.

Supplementary Figure 8. Expression analysis of molecules in HPPL-derived cysts treated with vehicle (DMSO) or CaMKII inhibitor (KN62). Quantitative RT-PCR analysis of the *CK19*, *HNF1β, Notch1, Notch2, Jagged1, Jagged2, ALB, HNF4α, cyclin D1, c-Myc, and TGFα* in HPPL-derived cysts treated with vehicle (DMSO) or CaMKII inhibitor (KN62). Values represent the ratio relative to the mean for the samples of vehicle-only controls. Bars represent mean ± SD of values. The result is representative of 3 independent experiments.

Supplementary Figure 9. Phosphorylation of CaMKIIα and PKC in developing liver and primary hepatic stem/progenitor cells. (A) Immunoblot analysis of phosphorylated CaMKIIα (p-CaMKIIα) and phosphorylated PKC (p-PKC) in embryonic E14.5, 16.5, 18.5, postnatal P1, P7, and P14 WT livers. Homogenate of whole E14.5 embryo served as a positive control (PTC). Fluorescence of bands corresponding to of CaMKIIα and p-CAMKIIα is increased gradually during liver development. (B) Analysis of FACS-purified primary hepatic stem/progenitor cells

immunostained with anti-p-CaMKIIα or anti-p-PKC antibodies (red signals). Nuclei were counterstained with DAPI (blue signals). Non-specific rabbit IgG was used as an isotype control. Scale bars: 100 μm.

Supplementary Figure 10. Quantitative analysis of immunoblots using densitometry. (A) Immunoblot analysis of phosphorylated CaMKIIα (p-CaMKIIα) and total CaMKII protein in HPPL after Wnt5a stimulation (shown in Fig. 6A) was quantified using densitometry. Values represent the ratio relative to the density of p-CaMKIIα (red bars) or CaMKII (black bars) in HPPL at pretreatment. Wnt5a stimulation increased both CaMKII and p-CaMKIIα with the levels peaking 3 hours after Wnt5a supplementation and then decreasing to baseline levels after 12 hours. (B) Ratios of p-CaMKIIα/CaMKII quantified by densitometry increased, peaking 3 hours after Wnt5a supplementation. (C) Immunoblot analysis of phosphorylated PKC (p-PKC) and total PKCα protein in HPPL after Wnt5a stimulation (shown in Fig. 6A) was quantified using densitometry. Values represent ratios of p-PKC/PKCα relative to the value in HPPL at pretreatment. (D) Immunoblot analysis of phosphorylated Rac1 (p-Rac1) and total Rac protein in HPPL after Wnt5a stimulation (shown in Fig. 6A) was quantified using densitometry. Values represent ratios of p-Rac1/Rac1 relative to the value in HPPL at pretreatment. (E) Immunoblot analysis of p-CaMKIIα in E16.5 Wnt5a

KO and littermate WT fetal livers (shown in Fig. 6D) was quantified using densitometry. Levels of p-CaMKIIα were significantly lower in Wnt5a KO relative to WT fetal livers. Values represent the ratio relative to the mean density for WT mice. Bars in dot-plot graphs represent mean ± SD of 5 mice shown. The result is representative of 3 independent experiments. *p =0.008.

Supplementary Figure 11. Analysis of HNF1β, p75NTR, and p-CaMKII inWnt5a KO livers. (A) Immunostaining of HNF1β (green) and HNF4α (red) in E14.5 livers. Nuclei were stained with DAPI (blue). HNF1β was not detected in both E14.5 WT and Wnt5a KO livers. (B) Immunostaining of p75NTR (green) in E18.5 livers. Nuclei were stained with DAPI (blue). Cells with p75NTR were detected in E18.5 Wnt5a KO and littermate WT livers. Scale bars: 50 μm.

Supplementary Figure 12. Pancreas and kidney in Wnt5a KO mice. (A). Representative stereoscopic views of pancreas and kidney in E18.5 Wnt5a KO and littermate WT mice. White dashed lines show the margins of kidneys. Scale bars: 1 mm. (B) Representative images depicting pancreas and kidney tissues in E18.5 Wnt5a KO and littermate WT mice stained with hematoxylin and eosin.

Supplementary Figure 13. Analysis of Wnt4a, phosphorylated β-catenin, and phosphorylated p53. (A) Expression of *Wnt4* in fetal livers of E16.5 Wnt5a KO and littermate WT mice. Quantitative RT-PCR analysis of *Wnt4* is depicted as the ratio of *Wnt4* copy number in E16.5 Wnt5a KO livers relative to WT livers. Bars in dot-plot graphs represent mean ± SD of 6 mice shown. The result is representative of 3 independent experiments. *p=0.02. (B) Immunoblot analysis of phosphorylated β-catenin (p-β-catenin, activated β-catenin), phosphorylated p53 (p-p53, stabilized p53), and total p53 protein in HPPL at pretreatment (0), and then 1, 3, 6 and 12 hours after stimulation by Wnt5a. (C) Immunoblot analysis of p-β-catenin in supplementary Figure 13B was quantified using densitometry. Values represent the ratio relative to the density of p-β-catenin in HPPL at pretreatment. (D) Immunoblot analysis of p-p53 and total p53 in supplementary Figure 13B was quantified using densitometry. Values represent ratios of p-p53/p53 relative to the value in HPPL at pretreatment.

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