Supplemental Methods:

Immunohistochemical staining procedures

Staining for alkaline phosphatase activity was performed in tissue that was fixed with 4% paraformaldehyde for four hours at 4°C prior to being paraffin wax imbedded. Paraffin sections were dehydrated and dewaxed as described previously [1]. Slides were stained with a biotin conjugate of the duct binding lectin *Dolichos biflorus* agglutinin (Vector Laboratories; B-1035) to mark ducts. Endogenous phosphatases were inactivated via incubation at 65°C for 30 minutes in NTM buffer containing 100mM NaCl, 100mM Tris, and 5 mM $MgCl_2$. Subsequently, slides were stained with BM Purple AP substrate (Roche) overnight and then counterstained with nuclear fast red (Vector Laboratories; H-3403) to provide contrast. For Alcian Blue staining, rehydrated paraffin sections were stained for 30 minutes at room temperature in a 3% solution of Alcian Blue (8GX 10g/l) diluted in acetic acid. Slides were then washed for 2 minutes in water, and counterstained with nuclear fast red.

Antibodies

The following primary antibodies were used at the indicated dilution: mouse AE1/AE3 (ID Labs, Canada; BP715) rabbit alpha-1-antitrypsin 1:1000 (Biogenix, Inc; AR015-5R), mouse β-catenin 1:100 (BD; 610154), mouse CD10 1:100 (Novocastra, Clone 56C6; Org8941), mouse CD56 1:100 (Invitrogen, clone 123C3; 07-5603), mouse chromogranin A 1:50 (ID Labs, Canada clone LK2H10; BP705), chymotrypsin 1:2,000 (Biodesign, Inc; A50142H), mouse CK19 1:1000 (Hybridoma Bank), goat clusterin-α 1:100 (Santa Cruz Biotech, C18; SC6419) rabbit cyclin D1 1:100 (Lab Vision Corp.; RM-9104-R7), mouse E-cadherin 1:200 (BD, 610181), rabbit estrogen

receptor alpha 1:200 (Labvision/Neomarkers clone SP1; RM9101) rabbit Hes11:10,000 (kindly provided by T. Sudo, Toray Industries, Inc., Kanagawa, Japan), neuron specific enolase (NSE, Cell Marque Corp, clone e27; CMC720), rabbit Pdx-1 1:1,000 (kindly provided by Dr. Michael German), rabbit Patched 1:100 (Abcam, ab27529), rabbit progesterone receptor (Ventana, clone 1E2; 790-4296), hamster mucin 1 1:500 (Neomarkers; HM-1630), goat Shh 1:50 (R&D, AF445), mouse Synaptophysin 1:200 (Biogenex, MU363-UC), mouse vimentin 1:100 (Dako, MO725 clone V9).

The following secondary antibodies were used for immunofluorescence: anti rabbit Alexa 555; 1:200 (Molecular Probes; A-21428), anti rabbit Alexa 555 1:200 (Molecular Probes; A -11034), anti hamster-Cy3, 1:200 (Jackson; 127-165-099), and anti mouse Alexa 488, 1:200 (Molecular Probes; A-11029). For immunohistochemistry, a biotinylated anti-goat (Vector; BA-9500) was used at a dilution of 1:200. Staining for diaminobenzidine (DAB) was performed with the ABC Elite immunoperoxidase system (Vector Laboratories; PK-6100).

Immunofluorescently stained slides were mounted with Vectashield hard mount media containing the nuclear stain, DAPI (Vector). Immunohistochemically stained slides were mounted with Permount reagent. Bright field images were acquired using a Zeiss Axio Imager D1 scope; fluorescent images were captured using a Leica DMIRE2 SP2 confocal microscope, gross morphology images were acquired using a Nikon D70 digital SLR.

Morphometric quantification of Pdx1^{+} cell number

At e12.5, the whole pancreatic bud was sectioned and aliquoted as described previously [2] in order to obtain representative results. In order to quantify the number of Pdx1^{+} cells present in 7 control, 5 *PdxCre^{early}; β-cat^{active}*, and 5 *Ptf1aCre; β-cat^{active}* intact e12.5 embryos were paraffin

imbedded, cut into 6μm sagittal sections, and aliquotted onto slides. Following immunofluorescence staining for E-cadherin (to mark the epithelium) and Pdx1, the number of Pdx1+ cells within the pancreatic epithelium was counted on 1 section every 96μm until the entire pancreatic bud was evaluated.

Quantitative PCR analysis

RNA isolation, cDNA preparation, and qPCR was performed as described previously[3]. RNA expression of Wnt and Hh pathway target genes was normalized based upon comparison to Cyclophilin or glucoronidase (GUS) expression.

qPCR primer sequences

Axin2	F	GCCAATGGCCAAGTGTCTCT
	R	GCGTCATCTCCTTGGGCA
Cdk4	F	CCCACCTCTCCTTACGAGGTT
	R	AGAAGACAGATACACCTGCCCTTTA
E-cadherin	F	GAGCGTGCCCCAGTATCGT
	R	GGCTGCCTTCAGGTTTTCATC
c-jun	F	TCCAAGTGCCGGAAAAGG
	R	GAGTTTTGCGCTTTCAAGGTTT
c-Myc	F	CCTAGTGCTGCATGAGGAGACA
	R	CCTCATCTTCTTGCTCTTCTTCAGA
CyclinD1	F	CGGCCCGAGGAGCTG
	R	GGCCAGGTTCCACTTGAGC

Cyclophilin	F	TCACAGAATTATTCCAGGATTCATG
	R	TGCCGCCAGTGCCATT
Gli1	F	GCCACACAAGTGCACGTTTG
	R	AAGGTGCGTCTTGAGGTTTTCA
GUS	F	ACGGGATTGTGGTCATCGA
	R	TCGTTGCCAAAACTCTGAGGTA
Hes1	F	AAACCAAAGACGGCCTCTGA
	R	TCTTGCCCTTCGCCTCTTC
Ihh	F	TCCAAGGCCCACGTGC
	R	GCAGCCACCTGTCTTGGC
p21	F	TCCACAGCGATATCCAGACA
	R	GGACATCACCAGGATTGGAC
Ptc	F	CCCTAACAAAAATTCAACCAAACCT
	R	GCATATACTTCCTGGATAAACCTTGAC
Shh	F	CAAAGCTCACATCCACTGTTCTG
	R	GAAACAGCCGCCGGATTT
Tcf1	F	GCTGCCATCAACCAGATCCT
	R	AGTTCATAGTACTTGGCCTGCTCTTC

Western blotting

Tissue lysates were prepared via homogenization in RIPA buffer (50 mM Tris-HCl at pH 7.4, 1% [v/v] NP40, 0.1% [w/v] SDS, 0.25% [w/v] Na-deoxycholate, 1 mM EDTA, phosphatase [1 mM Na-orthovanadate, 40 mM NaF, 10 mM glycerophosphate, 5 mM pyrophosphate], protease inhibitor [Complete—Roche]). Lysates were electrophoresed in 15% SDS-PAGE gels,

transferred to PVDF membrane (Bio-Rad), and processed for immunoblotting with antibodies against ERK1/2, phospho-ERK1/2 (p44/42), AKT, phospho-AKT (Ser473), phospho-PDK1, phospho-s6K (p70), and phospo-EGFR (1:1000 dilution; Cell Signaling Technology) or GAPDH (1:3000 dilution). HRP-conjugated secondary antibody was used at 1:1000 dilution and detected by ECL (Amersham Biosciences).

References

1. Kim SK, Hebrok M, Melton DA. Notochord to endoderm signaling is required for pancreas development. Development 1997b;124:4243-4252.
2. Kawahira H, Ma NH, Tzanakakis ES, McMahon AP, Chuang PT, Hebrok M. Combined activities of hedgehog signaling inhibitors regulate pancreas development. Development 2003;130:4871-9.
3. Cano DA, Sekine S, Hebrok M. Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis. Gastroenterology 2006;131:1856-69.