Supplemental Methods

Chromatin immunoprecipitation studies

E12.5 dissected WT pancreata were fixed in 1% formaldehyde at RT for 10 min and quenched with 125 mM glycine. Nuclei were lysed in RIPA buffer, flash-freezed in liquid nitrogen and kept at -80º C. Chromatin pools (n=150) were sonicated (15x 10sec ON / 1min30 OFF, Bioruptor UCD-200TM-EX) to obtain fragments of ~500bp, and then incubated overnight at 4º C with 4 µg of Hnf1b or rabbit IgG antibodies (both from Santa-Cruz).

The Hnf1b antibody (H85, Santa Cruz) was raised against a non-conserved domain between the dimerization domain and the POU specific domain of HNF1b and it does not cross react with HNF1a. It was extensively validated by immunoprecipitation, immunofluorescence and western blot techniques using WT and mutant tissues. Antibody specificity was further determined by the formation of specific supershifts on gel shif binding assays using either kidney extracts or extracts from transfected cells overexpressing Hnf1b or Hnf1a proteins. Furthermore, the ability of the antibody to react with endogenous Hnf1b protein in cross-linked chromatin was analyzed by ChIP on embryonic kidneys, assaying two well-known targets genes: Ksp-Cadherin and Wnt9 (first intron) (see Heliot and Cereghini, 2012; Heliot et al, 2013; Lokmane et al, 2010).

Chromatin-antibody complexes were immunoprecipitated with Protein-A agarose (Roche). Eluted chromatin was decrosslinked at 65ºC and purified by phenol/chloroform extraction. Immunoprecipitated chromatin and input (0.01% dilution) were analyzed by qPCR. These data were normalized to a reference DNA (pool of diluted inputs) and then expressed as fold enrichment relative to the values obtained with the immunoprecipitated chromatin using the non- immune IgG serum. A total of 4 targets were analyzed in duplicate per ChIP experiment, with at least 3 independent experiments performed for each of these targets.

Figure S1. No phenotypic difference between control and heterozygous pancreata. Haematoxylin/Eosin staining of control and heterozygous *Pdx1-Cre;Hnf1b*$^{+/Flox}$ pancreata at E16.5, showing no morphological difference in acinar, endocrine and ductal cells. Scale bars: 50 µm.

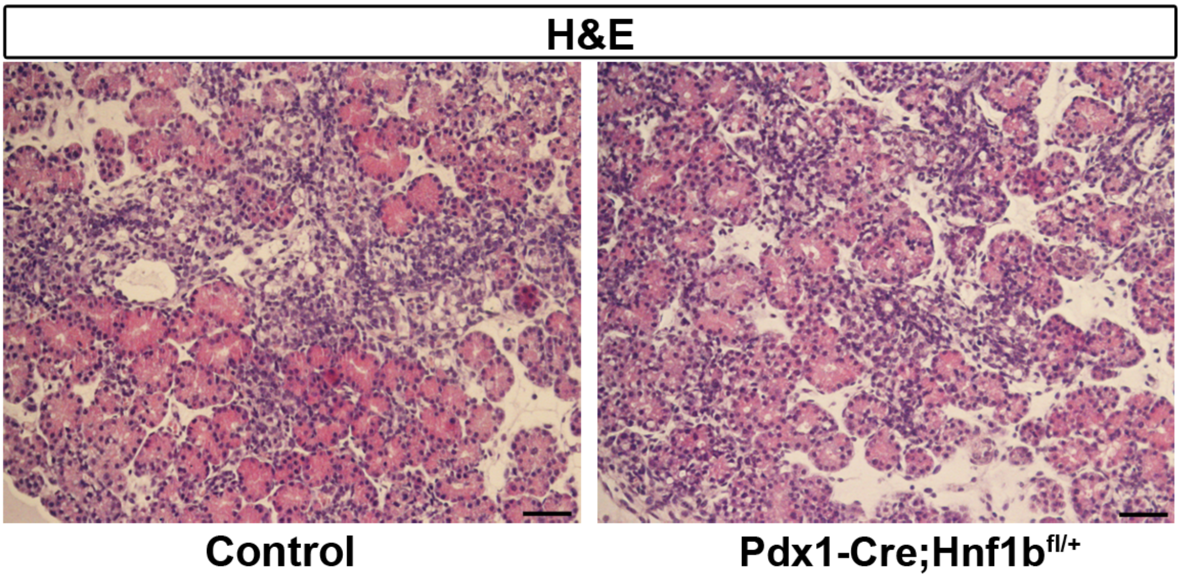

Figure S2. Pancreatic hypoplasia and morphogenesis defects in *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E9.5) embryos.
(A, B) Digestive tracts of controls and *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E9.5) mutants at E18.5 (C-F) Haematoxylin/Eosin staining of control and mutant pancreata at E16.5 and 18.5. Note the dramatic reduction in acinar cells in mutants (D, F) and cystic ducts (asterisks in F). (G) Pancreas weight of control, heterozygous (*Sox9-CreER^{T2};Hnf1b$^{Flox/+}$*) and mutant (*Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$*) pancreata at E16.5 (TM at E9.5) (Control n=4, Heterozygous n=6, Mutant n=3), showing 40% decrease in mutants compared to controls. (H) qRT-PCR analysis of WT *Hnf1b* transcripts at E14.5 showing 70% decrease in *Hnf1b* expression in mutant pancreas (Control n=4, Mutant n=3). (I, J) Efficient *Hnf1b* inactivation in *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$;R26R$^{+/YFP}$* (TM E9.5) mutant pancreas at E11.5 shown by Hnf1b (red) and GFP (green) immunostainings. Note the high number of GFP+ cells with almost no Hnf1b+ cells in the mutant pancreas. Nuclei were stained in blue with DAPI. Scale bars: 200 µm in A-B; 50 µm in C-F and I-J'.

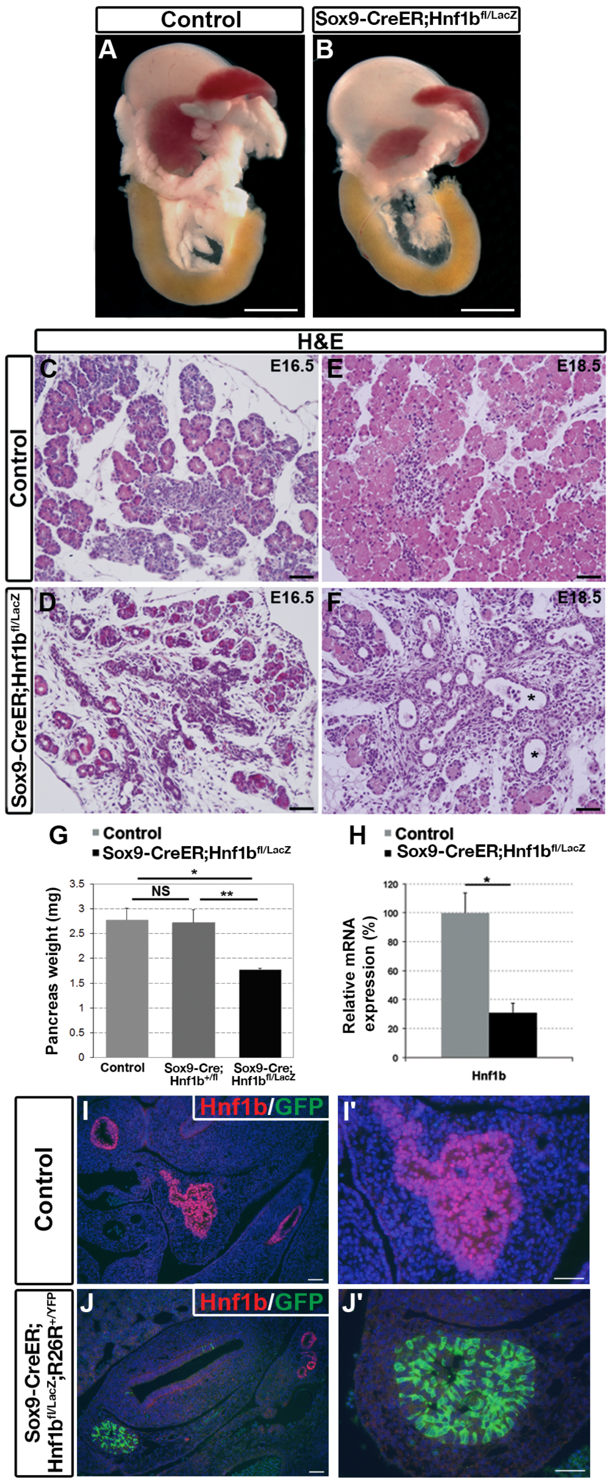

Figure S3. Lack of endocrine precursors in *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E9.5) mutants.
(A, B) Amylase (AMY, green) and NGN3 (red) immunostainings in control and *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E9.5) mutant pancreata at E16.5. Note the absence of Ngn3+ endocrine precursor cells in the mutant section (B). (C, D) Immunostainings of Insulin (INS, green) and Glucagon (GLUC, red) in control and *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E9.5) mutant pancreata at E18.5. (E) qRT-PCR analysis of *Ngn3, Glucagon, Insulin, Somatostatin* and *Amylase* expression in controls and mutants at E16.5 (Control n=10, Mutant n=3). Scale bars: 50 µm.

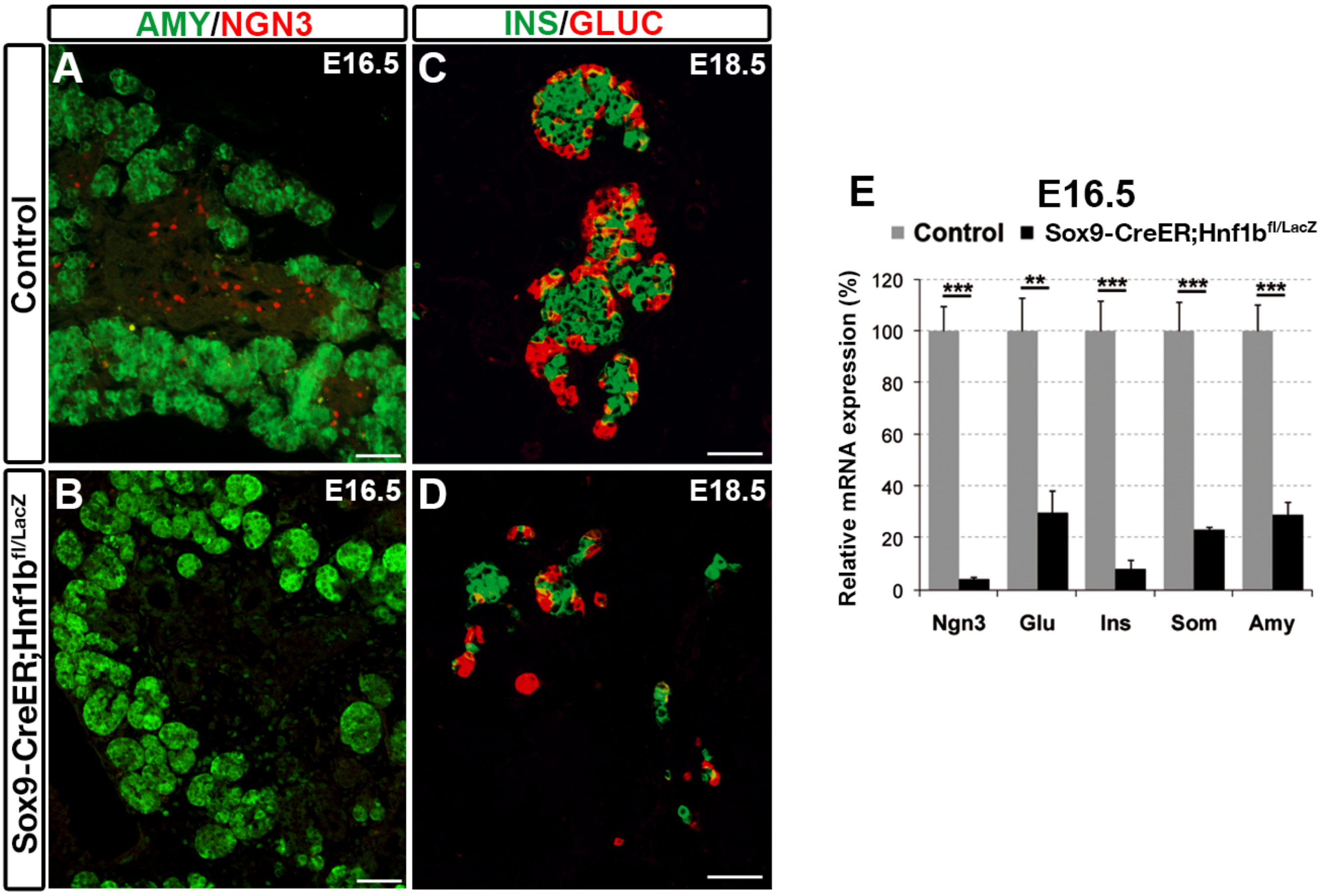

Figure S4. Cystic ducts with polarity defects in *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E9.5) mutants.
(A, B) Sox9 immunohistochemistry of control and *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E9.5) mutant pancreata at E18.5. (C, D) Mucin1 (MUC1, green) and β-catenin (red) coimmunostainings in control and *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E9.5) mutant pancreata at E18.5. Note the polarity defects in epithelial cells lining the cysts, evidenced by a multilayered epithelium and a discontinuity in MUC1 expression at the apical region of ductal cells. Asterisks indicate cystic ducts in mutants (B, D). Scale bars: 50 µm.

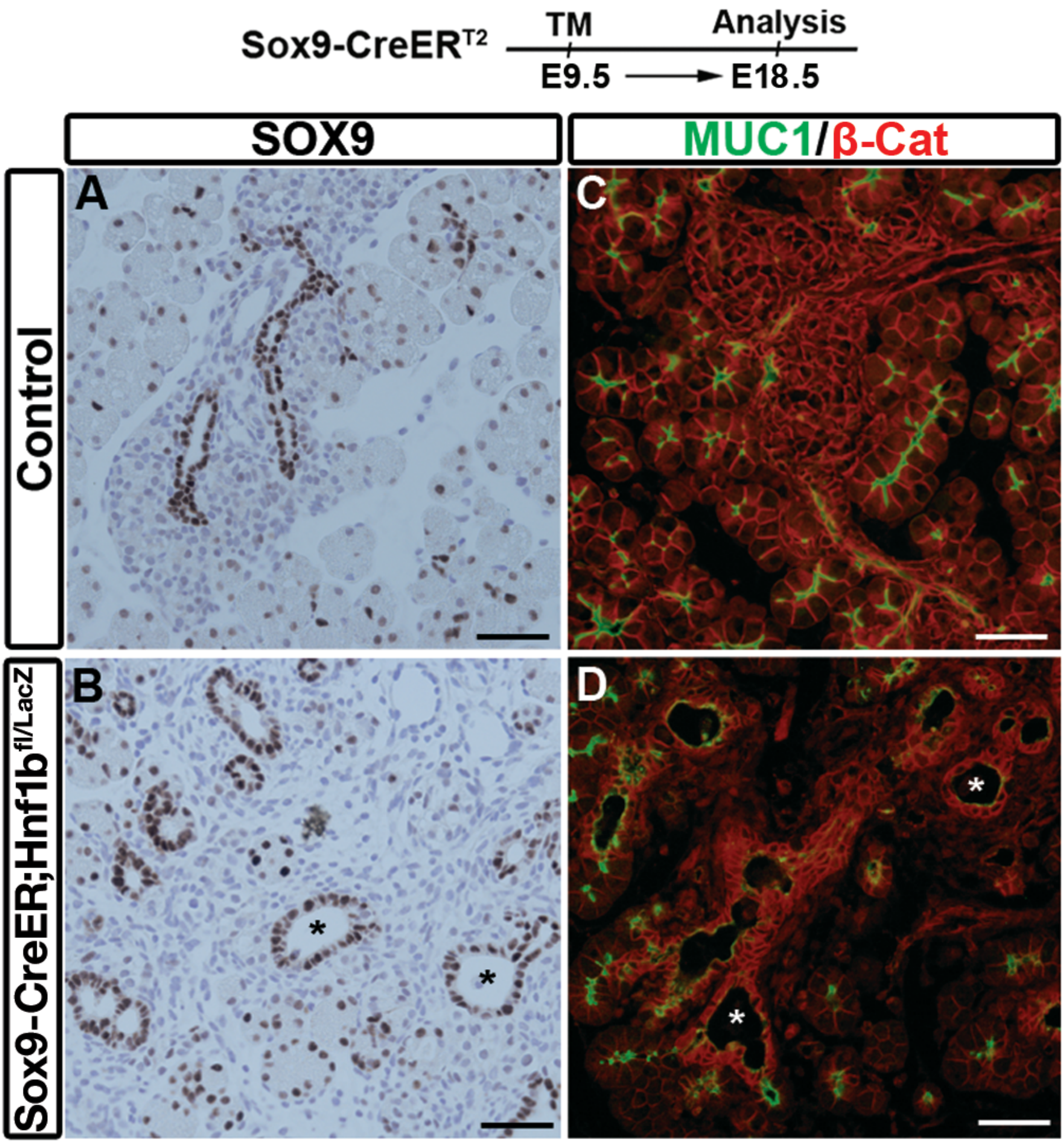

Figure S5. Exocrine defects in *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E12.5) mutants.
(A) qRT-PCR analysis of the acinar markers *Ptf1a*, *Nr5a2*, *Mist1* and *Amylase* in control and *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E12.5) mutant pancreata (Control n=8, Mutant n=7). (B-C) Amylase (AMY, green) staining showing a moderate loss of acinar cells in mutants. (D, E) MUC1 (green) and β-catenin (red) coimmunostainings showing cystic ducts and loss of polarity. Nuclei were stained in blue with DAPI. (I) qRT-PCR analysis of Notch pathway genes in control and *Sox9-CreER^{T2};Hnf1b$^{Flox/LacZ}$* (TM E12.5) at E16.5 (Control n=8, Mutant n=7), showing an upregulation of *Notch2, Hey2* and *Heyl* at the onset of acinar differentiation.

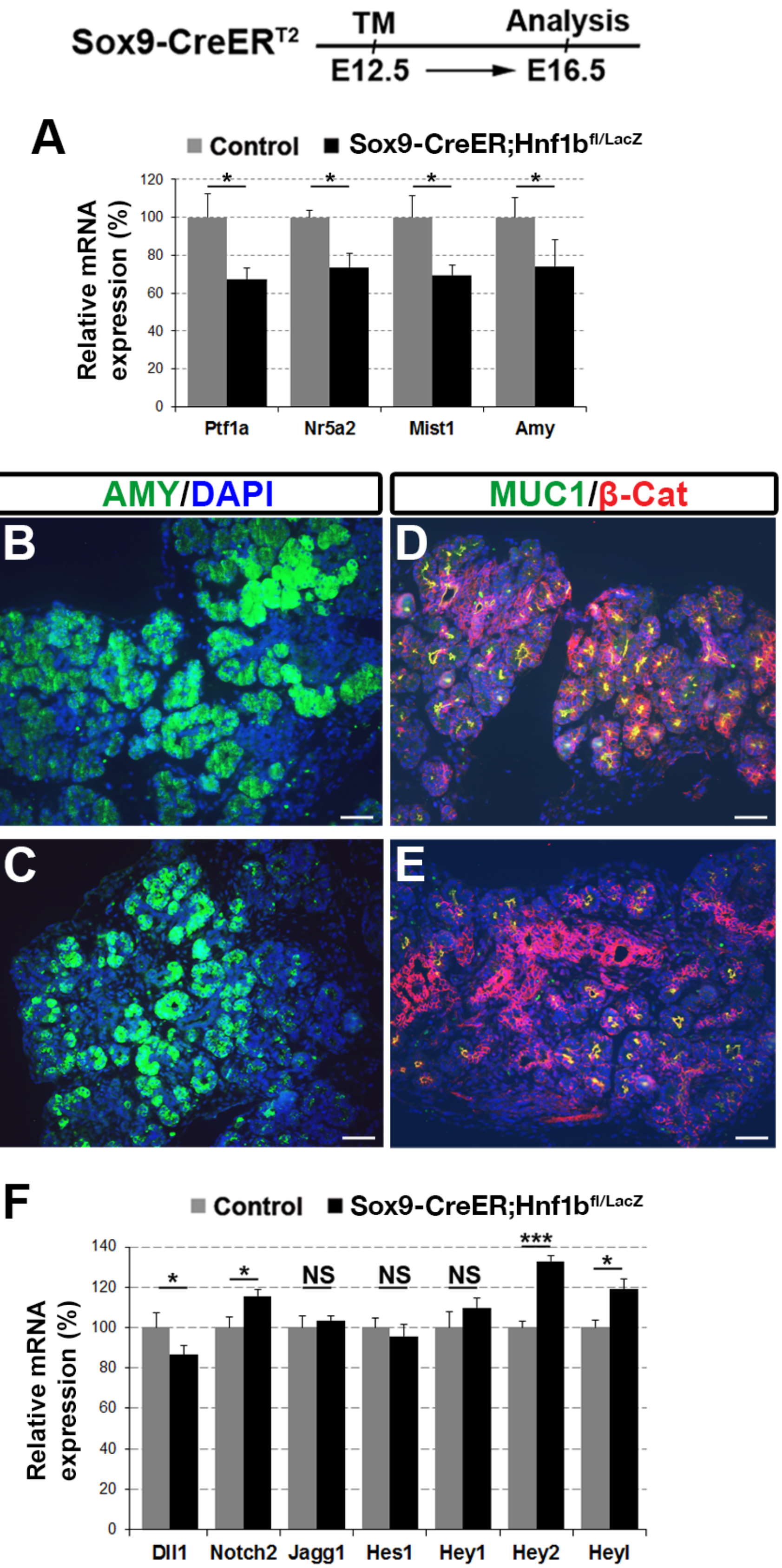

Figure S6. Regulatory sequences in *Ngn3* promoter.

Three regions upstream *Ngn3* TSS are represented: one distal (-5000 bp; -4740 bp), one intermediate (-4100 bp, -3200 bp) and one proximal (-900 bp; -170 bp). Putative binding sites for Hnf1b, Pdx1, Sox9 and FoxA2 were identified with the JASPAR database. Binding sites for Hnf6 were identified previously (Jacquemin et al., 2000). Sox9 binding sites could correspond to the ones previously described in the human promoter (-4061 bp; -3328 bp and –3306 bp; -400 bp and -385 bp; -161 bp upstream TSS) (Lynn et al., 2007). Intermediate regulatory sequences include the cluster 1 characterized in human (Lee et al., 2001). We identified 2 novel Hnf1b binding sites in the distal and the proximal regions, characterized by ChIP experiments (see Fig. 7). Hnf1b binding sites could correspond to putative ones in the human promoter identified by JASPAR database (-5043 bp, -3824 bp; -3783 bp; -445 bp).

-5000 ggtggccccaaatggtcgtcagaacccagagtctggagagggccagagttcagaccctgtgggctcctttccaggggaggt
HNF1B
ccaggcagccagccataaggtttattttgagcccttctccaagcgctcacttggcttgagcgcttcataaaagggtaggtatc
PDX1 SOX9 FOXA2 FOXA2
aacatggtgaccattagaggtggcctttcatctcctctgacttcccttgtttgtttttggacagagagaatcacgaagtctgtttattttcccaac… -4740

SOX9
-4100 taaagacctcgggtgctccagagcgtcccagcccctcttctccctttgttcgaggactccctgggccgaagctccctcgtgcgctctgtggggtg
catatggcgccctctcacctacccaagaaaaggaaccaggcactcattcaagagcgcacaagctcccagggcccccagagggggggagtcac
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HNF6
cgggggcagggcccctgggggggaggcccgctcagtgccaaatccatgtgtcagcttctaggacaggtgtgcccaggggccaggggccagg
FOXA2
tctccccccccctccggatttatcacggcaaaggtaatattgtgctaactatgagtaaacagtcattgtgaagaccaaggagagtttatcagg
caaactagttgtgggggaggcttaacaataaagttgctcgccttaggagcaggtgatggctggctacgcagactcccgccagagatgttttcc
HNF1B FOXA2 SOX9
PDX1
aaagcatcccgctttacaatctcttgtaattatttattaaacggaatctatttattattattttagcaaacactggggacaggtggggctttcttttg
SOX9
tcgtctccttttgtttgaagggctgtttgaagtggccagtctcggtcccggcagcctc… -3200

HNF6
-900 tttccctatcacctgcctctcgggtcaggccttcccgatagcatccatagtggggcggggcgtgatgagatgccccctctgcactctctctac
SOX9
aaccaccctcgcctccggaatagaacccaatgtctcggatgaggactatggtgggggttttcaaggtctggtctgggggctggagggttgg
HNF1B SOX9
atcccaaggtgatattgaacctggccaagcaatagtttctgagtagaaaggacttgagcagggaccgtctctggtcactctgtcctctttccc
PDX1
aggatggagtcagtctgtgaaacatggttgcacacacatttcctgacccaacccatagtggcggagagctggatagcactttgaactaatgg
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catgtgtaatactcaaagaagaggccttccttatatatatataggcacccccaaacctccttcatgctaccaagaaagggtctggacacatgc
caaaaagaaagaggaaaaggcaaagctctccccagcggccggacgggactcttctggctgggcgaggctctttgaggaaccgagagttgc
SOX9
tgggactgagcccgcgacgggggaggcgtggagtgggggaacaaacagagtgctgctcccctccccccgacccctgccctttgtccgga… -170

Table S1 : Primary and Secondary antibodies

Primary antibodies

Antigen	Host	Dilution	Source
Hnf1b	rabbit	1/50	Santa Cruz
GFP	chicken	1/500	Aves Labs
P-Histone H3	mouse	1/300	Millipore
Pdx1	rabbit	1/1200	C. Wright,Vanderbilt University, USA
E-cadherin	mouse	1/100	BD Transd. Lab.
Ngn3	guinea pig	1/1000	M. Sander, University of California-San Diego, USA
CPA1	rabbit	1/500	Biogenesis
Insulin	rabbit	1/1000	ImmunoStar
Glucagon	mouse	1/1000	Sigma
Amylase	rabbit	1/300	Sigma
Sox9	rabbit	1/300	Chemicon
MUC1	Armenian hamster	1/100	Neomarkers
beta-Catenin	mouse	1/100	BD Transd. Lab.
acetylated alpha-Tubulin	mouse	1/300	Sigma
Hnf6	guinea pig	1/5000	P. Jacquemin & F. lemaigre, De Duve Institute, Belgium
pan-Cytokeratin	mouse	1/100	Sigma
Ezrin	rabbit	1/300	S. Louvet, UMR7622 CNRS UPMC, France
Dystroglycan	rabbit	1/200	Novus Biologicals
Laminin	rabbit	1/50	Sigma
AQP1	rabbit	1/100	Interchim
PKCz	rabbit	1/500	Sigma

Secondary antibodies

Conjugation	Species	Dilution	Source
Cy3	rabbit	1/500	Jackson
Alexa Fluor 488	rabbit	1/500	Invitrogen
Alexa Fluor 488	mouse	1/500	Invitrogen
Alexa Fluor 488	chicken	1/500	Jackson
FITC	Armenian hamster	1/500	Jackson
Biotin	rabbit	1/1000	Vector
Streptavidin-Alexa 594	-	1/500	Jackson

Table S2. Oligonucleotide sequences for qRT-PCR and ChIP experiments.

qRT-PCR gene expression

Name	**Forward Sequence (5'→3')**	**Reverse Sequence (5'→3')**
Cyclophilin A	CAGGTCCTGGCATCTTGTCC	TTGCTGGTCTTGCCATTCCT
Hnf1b	GGCCTACGACCGGCAAAAGA	GGGAGACCCCTCGTTGCAAA
Sox9	AAGCCGACTCCCCACATTCCTC	CGCCCCTCTCGCTTCAGATCAA
Hnf6	CAAATCACCATCTCCCAGCAG	CAGACTCCTCCTCCTGGCATT
Ptf1a	TTCCTGAAGCACCTTTGACAGA	ACGGAGTTTCCTGGACAGAGT
Pdx1	CCAGATCTGCCTCTAGGACTCTTT	CAGTTTGGAGCCCAGGTTGT
Muc1	CTCTGGAAGACCCCAGCTCCAA	CCACGGAGCCTGACCTGAACT
Spp1	CCCTCCCGGTGAAAGTGACTGA	GCACCAGCCATGTGGCTATAGG
Mist1	TGGGCCTCCAGATCTCACCAA	CGTCACATGTCAGGTTTCTCTGCT
Nr5a2	CTGCTGGACTACACG GTTTGC	CTGCCTGCTTGCTGATTGC
Ck19	ACCCTCCCGAGATTACAACC	TCTGAAGTCATCTGCAGCCA
Pkhd1	TGCTCCTCAGGCAGGCAATCG	ACCTGTACCCTGGGGTGGCTT
Kif12	ACGAGGCTTCTATGTGGAACAG	GAGGTACCTGCTGAGAAGTTGG
Cys1	AGAGGAGCTCATGGCGAGCATT	GCCTGTGGCACAGATGCCAAGA
Glis3	TGGGAAGCCTCAGTTCCAGGTC	GCACTGAGGCCCAAAGCCAA
Bicc1	ACTCGGTGGAAGGCTGCAATGA	AGTCGCCAGCGTTTCCAGAATG
Pkd1	GCTGCATGCCAGTTCTTTTG	TTTTAAAGTGCAGAAGCCCCA
Pkd2	CATGTCTCGATGTGCCAAAGA	ATGGAGAACATTATGGTGAAGCC
Ngn3	TTCGCCCACAACTACATCTG	TTGGGAGACTGGGGAGTAGA
Glucagon	CCAAGAGGAACCGGAACAAC	CCTTCAGCATGCCTCTCAAAT
Insulin	ATCCACAATGCCACGCTTCT	AAACCCACCCAGGCTTTTGT
Somatostatin	TCCGTCAGTTTCTGCAGAAGTCTC	GTACTTGGCCAGTTCCTGTTTCCC
Amylase	CTGGGTTGATATTGCCAAGG	TGCACCTTGTCACCATGTCT
Notch1	AACACCGCCCGTGGATTCAT	ACATGTGGCACCCTCGAAGC
Notch2	CCTGCCAGGTTTTGAAGGGA	GGGCAGTCGTCGATATTCCG
Dll1	GCCCTCCATACAGACTCTCCC	AGGCGGCTGATGAGTCTTTCT
Jag1	TGCCCTCCAGGACATAGTGG	ACTCTCCCCATGGTGATGCA
Rbpj	GTTTTGGCGAGAGTTTGTGGAAGAT	TGGAGGCCGCTCACCAAACT
Lfng	CTCGCGCCACAAGGAGATGAC	CCGAGGAGCAGTTGGTGAGCA
Hes1	CAAAGACGGCCTCTGAGCAC	CCTTCGCCTCTTCTCCATGAT
Hes5	CTCCGCTCGCTAATCGCCTC	TCTCCACCGCCACGGTACTT
Hey1	TCACCTGAAAATGCTGCACAC	CGTGCGCGTCAAAATAACCT
Hey2	AGCGCCCTTGTGAGGAAACGA	TGTAGCGTGCCCAGGGTAATTG
Heyl	CAGCCCTTCGCAGATGCAA	CCAATCGTCGCAATTCAGAAAG
Fgfr2b	TGATGGGCTGCCCTACCTCAA	CCCCAGCATCCATCTCCGTCA
Fgfr4	CAGGCCTTCCACGGGGAGAAT	CACGGTCCGAGGGTACCACA

qPCR ChIP

Name *	# of HNF1B binding sites	Forward Sequence (5'→3')	Reverse Sequence (5'→3')	Amplicon genome location
Ngn3-700bp	1	TGGAGGGTTGGATCCCAAGGTG	CAGAGACGGTCCCTGCTCAAGT	chr10:61,595,110-61,595,193
Ngn3-3300bp	1	GGGGACAGGTGGGGCTTTCTT	GGGACCGAGACTGGCCACTT	chr10:61,592,554-61,592,627
Ngn3-4900bp	1	GGCCCCAAATGGTCGTCAGAAC	CAAGCCAAGTGAGCGCTTGGA	chr10:61,590,840-61,590,975
Hnf6+4950bp	2	CTTGCAGCTTGGTTGATTGA	CGGCAGTACCAGACACTTGA	chr9:74714652-74714752
Cys1-4500bp	4	TGATGGGAGTGTCCCGTGCAA	CATGGCTGGCTGTGTGCAGAA	chr12:25,371,128-25,371,227
Pkhd1-70bp	1	TCCTGTTGGACTGGAACTCA	AGCCCTTTCTTTGGGTCTCT	chr1:20,608,118-20,608,248
Glis3+120100bp	2	CAACAAGAAGCCCTTTTGGA	CATGTCAGAGATGAGGGAGGT	chr19:28,634,413-28,634,533
Fgfr4+280bp	2	AGCGCACACAGGGCCTTT	GCCCCGGTGGGCAATAAGT	chr13:55,254,476-55,254,555
Bicc1+2857	1	CCCCCAGGACAGTCTCTCTAAAA	TGGCCTTCAAGTCTTCAGAGTG	chr10:71,156,782-71,156,857

** Primer names indicate the approximate position of the HNF1B-binding site/s amplified, relative to the TSS of the respective gene.*