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A Sox9/Fgf feed-forward loop maintains pancreatic organ identity

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SUMMARY

All mature pancreatic cell types arise from organ-specific multipotent progenitor cells. Although previous studies have identified cell-intrinsic and -extrinsic cues for progenitor cell expansion, it is unclear how these cues are integrated within the niche of the developing organ. Here, we present genetic evidence in mice that the transcription factor *Sox9* forms the centerpiece of a gene regulatory network that is crucial for proper organ growth and maintenance of organ identity. We show that pancreatic progenitor-specific ablation of *Sox9* during early pancreas development causes pancreas-to-liver cell fate conversion. *Sox9* deficiency results in cell-autonomous loss of the fibroblast growth factor receptor (Fgfr) 2b, which is required for transducing mesenchymal Fgf10 signals. Likewise, *Fgf10* is required to maintain expression of Sox9 and Fgfr2 in epithelial progenitors, showing that Sox9, Fgfr2 and Fgf10 form a feed-forward expression loop in the early pancreatic organ niche. Mirroring *Sox9* deficiency, perturbation of Fgfr signaling in pancreatic explants or genetic inactivation of *Fgf10* also result in hepatic cell fate conversion. Combined with previous findings that *Fgfr2b* or *Fgf10* are necessary for pancreatic progenitor cell proliferation, our results demonstrate that organ fate commitment and progenitor cell expansion are coordinately controlled by the activity of a Sox9/Fgf10/Fgfr2b feed-forward loop in the pancreatic niche. This self-promoting Sox9/Fgf10/Fgfr2b loop may regulate cell identity and organ size in a broad spectrum of developmental and regenerative contexts.

KEY WORDS: Pancreas, Sox9, Fgf10, Fgfr2b, Mesenchyme, Progenitor, Liver, Mouse

INTRODUCTION

In mammals, the dorsal and ventral pancreas, as well as the liver, hepatobiliary system, stomach and duodenum, all arise from foregut endoderm cells. Their lineage segregation into organ-committed progenitors is governed by both cell-intrinsic regulators and extrinsic mesodermally derived signals. For example, ventral pancreas and liver arise from the same bi-potential population of ventral foregut endoderm: while ventral pancreas forms by default, cardiac mesoderm-derived Fgf signaling diverts nearby endoderm cells to an hepatic fate (Bort et al., 2004; Deutsch et al., 2001; Jung et al., 1999). Similarly, the transcription factors Ptf1a and Sox17 have been shown to govern lineage segregation of foregut endoderm between ventral pancreas and duodenum (Kawaguchi et al., 2002) or ventral pancreas and biliary system (Spence et al., 2009), respectively. It remains unclear, however, whether, once allocated to a particular organ, progenitors are solely restricted to organ-specific cell fates or whether programming is still flexible and dependent on inductive signals.

Pancreas development in mice is first morphologically detectable at embryonic day (E) 8.75-9.0, when dorsal and ventral pancreatic buds emerge on opposing sides of the foregut endoderm, fusing by E12.5 to form the unified organ. Until around E12.5, buds predominantly comprise Pdx1$^+$, Ptf1a$^+$ and Sox9$^+$ pancreas-specific multipotent progenitor cells, shown by lineage tracing at the population level (Gu et al., 2002; Kawaguchi et al., 2002; Kopp et al., 2011), to give rise to all pancreatic cell types: endocrine cells and the exocrine acinar and ductal cells. As initial size of the progenitor cell pool dictates final organ size (Stanger et al., 2007), maintenance of appropriate progenitor cell numbers is vital for normal pancreas morphogenesis. Progenitor-intrinsic cues, such as Notch target genes (Apelqvist et al., 1999; Jensen et al., 2000) and the transcription factors Sox9 (Seymour et al., 2007), Pdx1 (Ahlgren et al., 1996; Offield et al., 1996) and Ptf1a (Kawaguchi et al., 2002; Krapp et al., 1998), act to maintain appropriate progenitor cell numbers and hence are crucial for pancreatic growth. Pancreas-specific *Sox9* deletion in mice causes organ hypoplasia owing to reduced proliferation of progenitors (Seymour et al., 2007), and similarly, early pancreatic growth arrest in mice lacking either *Pdx1* (Ahlgren et al., 1996; Offield et al., 1996), *Ptf1a* (Kawaguchi et al., 2002; Krapp et al., 1998), or both (Burlison et al., 2008), reveals roles for Pdx1 and Ptf1a in pancreatic epithelial expansion.

In addition to these progenitor-intrinsic cues, extrinsic cues from the surrounding pancreatic mesenchyme also promote progenitor cell proliferation. The importance of mesenchymal signals for pancreatic growth was first revealed through pancreatic explant and transplantation experiments, demonstrating reduced epithelial expansion following mesenchyme removal (Gittes et al., 1996; Golosow and Grobstein, 1962; Wessells and Cohen, 1967). As co-culture with heterotopic organ mesenchymes restored the growth of explanted pancreatic epithelia (Wessells and Cohen, 1967), it was concluded that signal(s) expressed in different types of mesenchyme stimulate pancreatic progenitor cell expansion. Subsequent studies have identified roles for several signaling molecules in pancreatic growth, including Wnt (Jonckheere et al., 2008; Landsman et al., 2011), Bmp (Ahnfelt-Rønne et al., 2010) and, most notably, Fgf10 (Bhushan et al., 2001), which is expressed

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in mesenchyme surrounding the ventral and dorsal pancreatic buds between E9.5 and E11.5. How such extrinsic mesenchymal cues are integrated with intrinsic epithelial cues at the level of the pancreatic progenitor cell, however, remains unknown.

While the liver and ventral pancreatic bud emerge in close proximity to one another from the ventral foregut, the dorsal pancreatic bud arises independently of the liver bud from the dorsal-posterior foregut region. Explant studies have shown that hepatic competence is not limited to the ventral foregut region, but that dorsal gut endoderm, which normally does not give rise to the liver, also possesses the ability to activate hepatic programs (Bossard and Zaret, 1998; Bossard and Zaret, 2000; Gualdi et al., 1996). When dorsal gut endoderm is isolated at E11.5 and cultured in the absence of gut tube mesoderm, the early hepatic marker albumin is ectopically activated (Bossard and Zaret, 1998; Gualdi et al., 1996). The observation that albumin expression is not induced in dorsal endoderm isolated at E13.5 and beyond (Bossard and Zaret, 2000) has led to the proposal that cues from the mesoderm actively repress hepatogenic gene activity in dorsal gut endoderm up until E13.5. Studies in zebrafish further suggest that hepatic competence of the fish posterior endoderm, which is thought to correspond to the dorsal gut endoderm in mice, is negatively regulated by Fgf10 signaling (Dong et al., 2007; Shin et al., 2011). To date, the identity of the mesenchymal signal(s) that suppress liver-specific genes in dorsal gut endoderm in mice has remained elusive. Moreover, it is unclear how progenitors gain competence to interpret specific mesenchymal cues and how stable relay mechanisms between epithelial progenitors and adjacent mesenchyme are established to reinforce fate commitment of dorsal gut endoderm-derived organs, such as the pancreas.

In this study, we show that in epithelial pancreatic progenitors, Sox9 cell-autonomously controls expression of the Fgf receptor, Fgfr2b, which is required for transducing mesenchymal Fgf10 signals. In turn, *Fgf10* is required to maintain progenitor expression of Sox9 and Fgfr2, showing that Sox9, Fgfr2 and Fgf10 form a feed-forward expression loop in the early pancreatic niche. Perturbation of this loop results in a pancreas-to-liver cell fate switch, showing that pancreatic progenitors are initially metastable in their organ fate commitment and require the activity of a Sox9/Fgf10/Fgfr2b feed-forward loop to repress liver-specific gene expression programs.

MATERIALS AND METHODS

Mouse strains

All mouse strains have been previously described: *Sox9^{flox}* (Kist et al., 2002), *Sox9-eGFP* (Gong et al., 2003), *Pdx1-Cre* (Gu et al., 2002), *Ptf1a^{Cre}* (Kawaguchi et al., 2002), *Rosa26-CreER* (Vooijs et al., 2001), *Pdx1^{LacZko}* (Offield et al., 1996), *Fgf10$^{+/–}$* (Min et al., 1998), *Rosa26R* (Soriano, 1999) and *Z/EG* (Novak et al., 2000). Tamoxifen (Sigma, St Louis, MO, USA) was dissolved at 20 mg/ml in corn oil (Sigma) and a single dose of 6 mg/40 g body weight administered by intraperitoneal injection. Midday on the day of vaginal plug appearance was considered E0.5. All animal experiments described herein were approved by the University of California Irvine and University of California San Diego Institutional Animal Care and Use Committees.

Histology and immunohistochemistry

Dissected E9.0-E12.5 embryos and E15.5 gut preparations were fixed in 4% paraformaldehyde in PBS, cryoembedded in Tissue-Tek OCT (Sakura Finetek USA, Torrance, CA, USA) and cut into 10 μm frozen sections. For immunofluorescence analysis, antigen retrieval was conducted in citrate buffer (pH 6.0) followed by permeabilization in 0.15% Triton X-100 in PBS. Sections were blocked in 1% normal donkey serum in PBS with 0.1% Tween-20 then incubated overnight at 4°C with primary antibodies diluted in the same buffer. The following primary antibodies were used at the given dilutions: rat anti-E-Cadherin (Sigma), 1:1000; rabbit anti-phosphohistone H3 (Upstate Biotechnology, Lake Placid, NY, USA), 1:300; rabbit anti-Sox9 (Chemicon, Temecula, CA, USA), 1:1000; goat anti-Sox9 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), 1:200; guinea-pig anti-Pdx1 (kindly provided by C. Wright, Vanderbilt University, Nashville, TN, USA), 1:10,000; rabbit anti-Ptf1a (kindly provided by B. Bréant, Université Pierre et Marie Curie-Paris 6, Paris, France), 1:1000; rabbit anti-Ptf1a (NIH Beta Cell Biology Consortium), 1:1000; mouse anti-glucagon (Sigma), 1:500; rat anti-GFP (kindly provided by C. Kioussi, Oregon State University, Corvallis, OR, USA), 1:500; rabbit anti-AFP (DAKO USA, Carpenteria, CA, USA), 1:1000; goat anti-albumin (Sigma), 1:3000; rabbit anti-α_1-antitrypsin (Sigma), 1:1000; goat anti-Hnf4α (Santa Cruz), 1:1000; and rabbit anti-Bek (Fgfr2) (Santa Cruz), 1:1000.

Staining for glucagon was conducted using the M.O.M. Kit in conjunction with AMCA Avidin D (both Vector Laboratories, Burlingame, CA, USA). Goat anti-Sox9 was detected using biotinylated horse anti-goat (1:2000) in combination with Fluorescein Avidin D (both Vector Laboratories). Simultaneous detection of AFP and either α_1-antitrypsin, Bek/Fgfr2 or phosphohistone H3 with two rabbit-raised antibodies was performed with a Zenon Rabbit IgG Labeling Kit (Invitrogen, Carlsbad, CA, USA). TUNEL was conducted using an ApopTag Fluorescein Apoptosis Detection Kit (Merck Millipore, Billerica, MA, USA). When necessary, nuclei were counterstained with Hoechst 33342 (Invitrogen).

Primary antibodies were detected with donkey-raised secondary antibodies conjugated to Cy3, Cy5, DyLight488 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) or Alexa488 (Invitrogen) at 1:1500 dilution (1:500 for Cy5).

ApoTome images were captured on a Zeiss Axio Observer Z1 microscope with Zeiss AxioVision 4.8 (both Carl Zeiss, Thornwood, NY, USA) and figures prepared with Adobe Photoshop/Illustrator CS4 (Adobe Systems, San Jose, CA, USA). Where necessary, the Cy5 channel was pseudo-colored blue.

Whole-mount X-Gal staining of embryos was performed as described previously (Seymour et al., 2004); images were acquired on a Zeiss Stemi 2000C with a Zeiss AxioCam digital camera driven by Zeiss AxioVision 3.1.

Gut explants

The foregut-midgut region was dissected from 24-25 somite (E9.5) *Sox9-eGFP* embryos and cultured on human fibronectin-coated eight-well culture slides (BD Biosciences, San Jose, CA, USA) either with 10 μM SU5402 (Calbiochem, La Jolla, CA, USA) or DMSO vehicle control. eGFP epifluorescence and bright-field images were captured on a Zeiss SteREO Discovery.V8 fluorescence stereomicroscope with Zeiss AxioVision 4.6.

Cell quantification

Cell counting was performed on every fifth section through pancreata from a minimum of three mutants and three control somite-matched littermates for embryos. For gut explants, all cells were counted in the dorsal pancreatic bud from three SU5402-treated and three control DMSO vehicle-treated explants for each of the three time-windows examined.

mRNA quantification

Total RNA was isolated and pooled from E12.5 dorsal pancreatic epithelia of *Sox9$^{fl/fl}$; Pdx1-Cre* and *Sox9$^{fl/fl}$* littermates. Each individual RNA sample was prepared from four pancreata as per the manufacturer's instructions (RNeasy Micro Kit, Qiagen, Valencia, CA, USA). RNA quality was assessed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Approximately 250 ng of total RNA was amplified and labeled with Cy3 using the QuickAmp Labeling Kit (Agilent Technologies). Four independent samples were hybridized to Agilent Whole Mouse Genome Oligo Microarray G4122A chips. Microarray data have been deposited in Gene Expression Omnibus under accession number GSE28669.

For qRT-PCR analyses, cDNA was synthesized (Superscript III cDNA Kit; Invitrogen) from total RNA pooled from dorsal pancreatic epithelia from six E12.5 *Sox9$^{fl/fl}$; Pdx1-Cre* and *Sox9$^{fl/fl}$* littermates. qRT-PCRs were

performed in triplicate using SYBR Green (Applied Biosystems, Foster City, CA, USA); for primer sequences, see supplementary material Table S1.

Statistical analyses

All data are displayed as mean±s.e.m. Statistical significance was determined using the two-tailed Student's *t*-test (Minitab 15, Minitab, PA, USA).

RESULTS

Liver differentiation in *Sox9*-deleted pancreas

In *Sox9*$^{fl/fl}$*; Pdx1-Cre* mice, *Pdx1-Cre* activation in dorsal and ventral pancreatic endoderm by E9.5 (supplementary material Fig. S1A) efficiently recombines the *Sox9*^{flox} allele in dorsal and ventral pancreatic cells by E10.5 (Fig. 2D′,F′). Although significantly hypoplastic by E11.5, dorsal and ventral pancreatic remnants arise in these mice (Fig. 1F,F′).

To identify Sox9-regulated pathways during early pancreas morphogenesis, we compared the transcriptional profiles of dorsal pancreatic epithelium in E12.5 *Sox9*$^{fl/fl}$*; Pdx1-Cre* mice with those of control (*Sox9*$^{fl/fl}$) littermates. Microarray analysis confirmed a significant 8.1-fold downregulation of *Sox9* in mutant pancreata (Fig. 1A). Surprisingly, however, we observed elevated expression of a number of genes associated with hepatic differentiation in *Sox9*-deficient pancreas, most notably α-fetoprotein (AFP) and albumin (Fig. 1A). Upregulation of these, as well as of additional liver markers, was verified by qRT-PCR analysis (Fig. 1B). Thus, *Sox9* ablation in the pancreas appears to aberrantly activate a liver developmental program.

To temporally and spatially dissect the onset of hepatic differentiation in *Sox9*-deficient pancreas, co-immunofluorescence analysis was performed for Pdx1 and the liver markers AFP and albumin. Immediately after *Sox9* ablation at E10.5, Pdx1 expression was maintained in *Sox9*-deficient progenitors and neither AFP nor albumin were detected in control or *Sox9*-deficient pancreata (Fig. 1C-D′). At E11.5, rare AFP$^+$ cells (0.4±0.1% of total pancreatic cells; *n*=3) were seen in the epithelium of control dorsal, but never ventral, pancreas (Fig. 1E,E′,I). By contrast, AFP$^+$ cells were abundant (24.0±2.0% of total pancreatic cells; *n*=5) in the *Sox9*-deficient hypoplastic dorsal pancreas in all mutant embryos examined (*n*=10; Fig. 1F,I). Paralleling AFP expression, while only scarce cells expressing albumin were evident in control pancreas (0.7±0.3% of total pancreatic cells; *n*=3), albumin$^+$ cells were readily detected in the *Sox9*-deficient dorsal pancreas (17.1±2.9% of total pancreatic cells; *n*=3) (Fig. 1G,G′,H,I). Both AFP and albumin were, however, undetectable in the *Sox9*-ablated ventral bud at E11.5 (Fig. 1F′,H′), which could be explained by the ~24-hour developmental delay of the ventral compared with the dorsal pancreas (Spooner et al., 1970). Severe hypoplasia of the *Sox9*-deficient ventral pancreas precluded its analysis in *Sox9*$^{fl/fl}$*; Pdx1-Cre* embryos at later time points.

Although some Pdx1 downregulation was apparent in E11.5 *Sox9*-deficient progenitors, the majority of pancreatic AFP$^+$ cells were Pdx1$^+$ (Fig. 1F), suggesting such AFP$^+$ hepatic-like cells to be pancreatic in origin. To assess whether the few AFP$^+$ Pdx1$^-$ cells were also derived from Pdx1$^+$ cells, we employed *Pdx1-Cre*-driven lineage tracing in conjunction with the *Z/EG* reporter allele to heritably eGFP-label *Sox9*-deleted cells and their progeny. Consistent with a pancreatic origin, lineage tracing revealed all AFP$^+$ cells, including AFP$^+$ Pdx1$^-$ cells, to be eGFP$^+$ and thus derived from *Pdx1*-expressing progenitors (supplementary material Fig. S2). Further validating this contention, immunofluorescence analysis for the proliferation marker phosphohistone H3 (pHH3) in

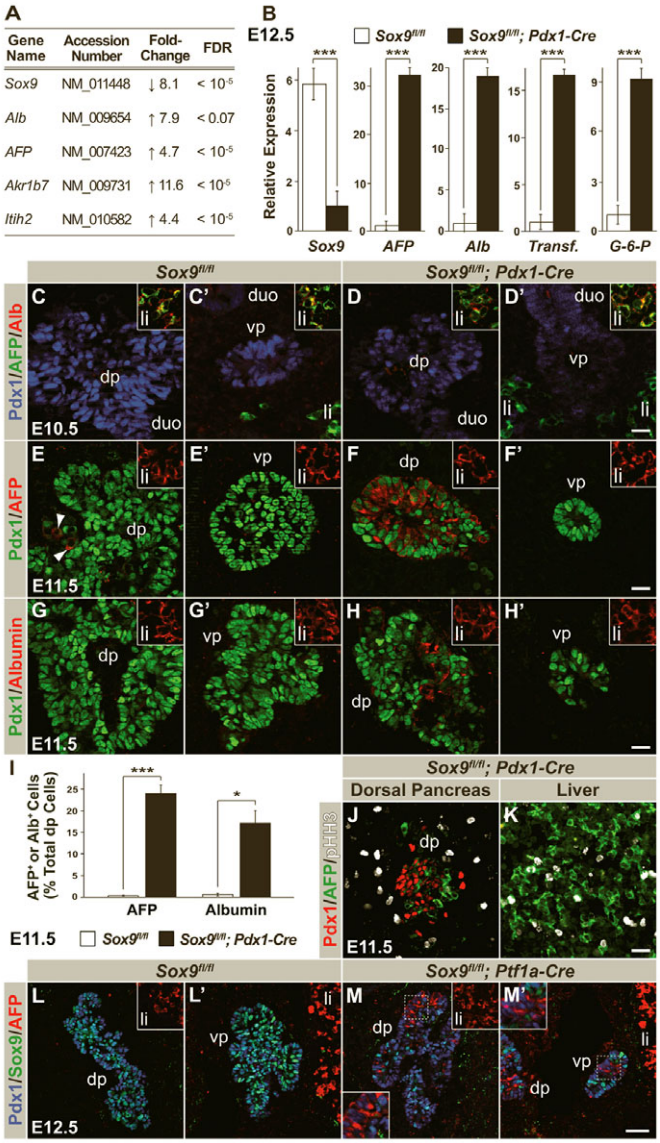

Fig. 1. *Sox9*-deleted pancreatic progenitors undergo hepatic fate conversion. (**A**,**B**) mRNA expression profiling (*n*=4) (A) and qRT-PCR (*n*=6) (B) of hepatic mRNAs in E12.5 *Sox9*$^{fl/fl}$*; Pdx1-Cre* versus control *Sox9*$^{fl/fl}$ dorsal pancreas. (**C-H′**) Immunodetection of AFP or albumin (Alb) reveals almost complete absence in E10.5 (C,C′) or E11.5 (E,E′,G,G′) control or E10.5 *Sox9*-deleted (D,D′) pancreata, but abundant expression in E11.5 *Sox9*$^{fl/fl}$*; Pdx1-Cre* dorsal (F,H), but not ventral (F′,H′), pancreas. Arrowheads in E indicate two rare AFP$^+$ cells in control dorsal pancreas. (**I**) AFP$^+$ or Alb$^+$ cells as a percentage of total pancreatic cells in E11.5 *Sox9*$^{fl/fl}$*; Pdx1-Cre* (*n*=5, AFP or 3, Alb) and control *Sox9*$^{fl/fl}$ (*n*=3) dorsal pancreata. (**J**,**K**) Phosphohistone H3 (pHH3) staining in Pdx1$^+$ progenitors and AFP$^+$ cells of dorsal pancreas (J) or AFP$^+$ hepatoblasts in liver (K) of E11.5 *Sox9*$^{fl/fl}$*; Pdx1-Cre* embryos. (**L-M′**) AFP$^+$ cells are observed in dorsal (M) and ventral (M′) pancreas of *Sox9*$^{fl/fl}$*; Ptf1a-Cre* but not control (L,L′) embryos at E12.5. Transf., transferrin; G-6-P, glucose-6-phosphatase; dp, dorsal pancreas; vp, ventral pancreas; duo, duodenum; li, liver; FDR, false discovery rate. Error bars represent s.e.m.; *P<0.05; ***P<0.001. Scale bars: 20 μm in C-H′,J,K; 50 μm in L-M′.

E11.5 *Sox9*$^{fl/fl}$*; Pdx1-Cre* dorsal pancreata (Fig. 1J) revealed similar rates of proliferation between the pancreatic AFP$^+$ cell population (3.2±2.3% pHH3$^+$ cells of total AFP$^+$ cells; *n*=62 cells) and the

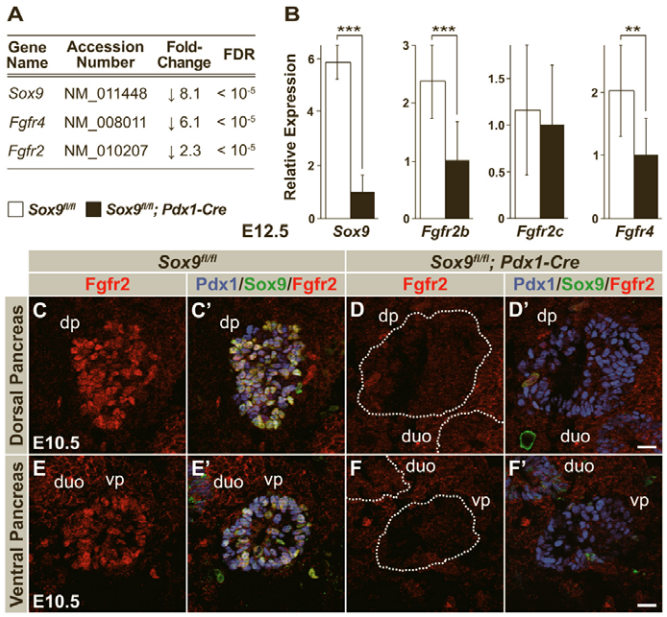

Fig. 2. Sox9 maintains Fgfr2b expression in pancreatic progenitors. (**A**,**B**) mRNA expression profiling (*n*=3) (A) and qRT-PCR (*n*=6) (B) show *Fgfr2*/*Fgfr2b* and *Fgfr4* downregulation in E12.5 *Sox9*$^{fl/fl}$; *Pdx1-Cre* versus control *Sox9*$^{fl/fl}$ dorsal pancreas. (**C-F′**) Nuclear Fgfr2 in E10.5 control dorsal (C,C′) and ventral (E,E′) progenitors is extinguished by *Sox9* ablation (D,D′,F,F′). dp, dorsal pancreas; vp, ventral pancreas; duo, duodenum; FDR, false discovery rate. Error bars represent s.e.m.; ***P*<0.01; ****P*<0.001. Scale bars: 20 μm.

Pdx1$^+$ (AFP$^-$) pancreatic progenitor population (2.9±1.4% pHH3$^+$ cells of total Pdx1$^+$ cells; *n*=933 cells). AFP$^+$ cells were extremely rare in control pancreata (four to seven cells per dorsal pancreas) and displayed proliferation rates similar to AFP$^+$ cells in *Sox9*-deficient pancreata (data not shown). This rate of proliferation of AFP$^+$ cells in the *Sox9*-deficient pancreas is much reduced in comparison with the mitotic index of endogenous AFP$^+$ hepatic bud cells in the same mutant embryos (9.5±2.1% pHH3$^+$ cells of total AFP$^+$ cells; *n*=777 cells; Fig. 1K). Thus, their relatively low proliferative index argues against increased AFP$^+$ cell numbers in *Sox9*$^{fl/fl}$*; Pdx1-Cre* pancreata being attributable to expansion of scarce endogenous pancreatic AFP$^+$ cells as a result of *Sox9* downregulation. Although conceivable that increased AFP$^+$ cell numbers might manifest from reduced apoptosis in *Sox9* mutants, TUNEL analysis refuted this hypothesis, revealing negligible AFP$^+$ cell apoptosis in both *Sox9*-deficient and control embryos at E11.5 (data not shown). Taken together, this evidence strongly supports the notion that the increased numbers of AFP$^+$ hepatic-like cells in *Sox9*$^{fl/fl}$*; Pdx1-Cre* pancreata arise via aberrant differentiation from pancreatic progenitors upon *Sox9* ablation.

To verify the induction of a bona fide hepatic program in *Sox9*-deleted pancreatic progenitors, we assayed for the expression of multiple liver markers. In addition to AFP and albumin, we detected expression of α_1-antitrypsin and Hnf4α in *Sox9*-deficient pancreas (supplementary material Fig. S3B,D). Whereas low-level Hnf4α was seen in early pancreatic epithelium (supplementary material Fig. S3C,D), AFP$^+$ cells showed high Hnf4α expression, characteristic of hepatocytes (supplementary material Fig. S3D). This induction of multiple hepatic gene products is concordant with activation of a concerted program of liver differentiation in *Sox9*-ablated pancreas. The AFP$^+$ cells occurred in small clusters predominantly at the periphery of the dorsal pancreas, in a pattern reminiscent of the first-wave glucagon$^+$ endocrine cells although AFP and glucagon expression were almost wholly exclusive (supplementary material Fig. S4).

To define the competence window through which *Sox9*-deleted pancreatic progenitors are able to adopt hepatic fates, *Sox9* was ablated using *Ptf1a-Cre,* which is activated in dorsal and ventral pancreatic endoderm at E10.5 (supplementary material Fig. S1B,C) (Kawaguchi et al., 2002), 1 day after *Pdx1-Cre*. Recapitulating the results of *Sox9* inactivation using *Pdx1-Cre*, *Ptf1a-Cre*-driven *Sox9* ablation induced a pancreatic-to-hepatic fate switch, as evidenced by AFP expression in both dorsal and ventral pancreas at E12.5 (Fig. 1M,M′). Thus, like their dorsal counterparts, *Sox9*-deficient progenitors in the ventral bud are competent to adopt hepatic fates, albeit following some temporal delay, most probably reflecting the aforementioned developmental lag of the ventral pancreas (Spooner et al., 1970).

To test the competency of later pancreatic progenitors to adopt an hepatic fate, *Sox9* was inducibly deleted via tamoxifen administration at E12.5 in *Sox9*$^{fl/fl}$ mice carrying the *Rosa26-CreER* allele (supplementary material Fig. S5A). Although *Sox9* was robustly downregulated at both mRNA and protein levels in E15.5 pancreatic epithelium (Dubois et al., 2011), no expression of AFP or albumin was evident (supplementary material Fig. S5B,C). As such, the competence window for *Sox9*-deleted pancreatic progenitors to adopt hepatic fates closes by E12.5. Together, these experiments reveal that, even after allocation to the organ niche, pancreatic progenitors remain labile in their organ fate decision.

Sox9 maintains Fgfr2b expression in pancreatic progenitors

To elucidate the mechanism underlying hepatic reprogramming of *Sox9*-ablated pancreatic progenitor cells, we re-examined the transcriptional profile of E12.5 *Sox9*-deleted pancreatic epithelium to identify potential downstream targets of Sox9. We found that *Sox9* ablation resulted in significant downregulation of both *Fgfr2* and *Fgfr4* (Fig. 2A), suggesting that Sox9 is required for maintaining their pancreatic expression. Of the two *Fgfr2* splice variants, *Fgfr2b* is expressed in early mouse pancreatic epithelium, whereas *Fgfr2c* expression is confined to the pancreatic mesenchyme (Elghazi et al., 2002; Sylvestersen et al., 2011). Concordantly, qRT-PCR analysis confirmed downregulation of *Fgfr4* and *Fgfr2b* in *Sox9*-ablated dorsal pancreatic epithelium, whereas *Fgfr2c* expression was unchanged (Fig. 2B). Significantly, Fgfr2b is the cognate receptor for Fgf10 (Ornitz et al., 1996), which is expressed by dorsal and ventral pancreatic mesenchyme between E9.5-E11.5 and is required for pancreatic progenitor cell proliferation (Bhushan et al., 2001). Moreover, *Fgf10* ablation manifests in formation of a hypoplastic pancreas, closely phenocopying *Sox9* deficiency (Bhushan et al., 2001). Likewise, *Fgfr2b*$^{-/-}$ mice exhibit pancreatic hypoplasia owing to reduced epithelial proliferation (Pulkkinen et al., 2003; Revest et al., 2001). Together, these findings indicate that *Sox9* acts in the same pathway as the *Fgf10-Fgfr2b* axis to maintain pancreatic progenitor cell proliferation.

To dissect the genetic link between Sox9 and Fgf10-Fgfr2b signaling, we characterized the pancreatic expression pattern of Fgfr2b with respect to that of Sox9. From E9.0 until E10.5, Pdx1$^+$ pancreatic cells expressing high levels of Sox9 exhibited nuclear Fgfr2b expression (Fig. 2C,C′,E,E′; Fig. 3A-D). At E10.5,

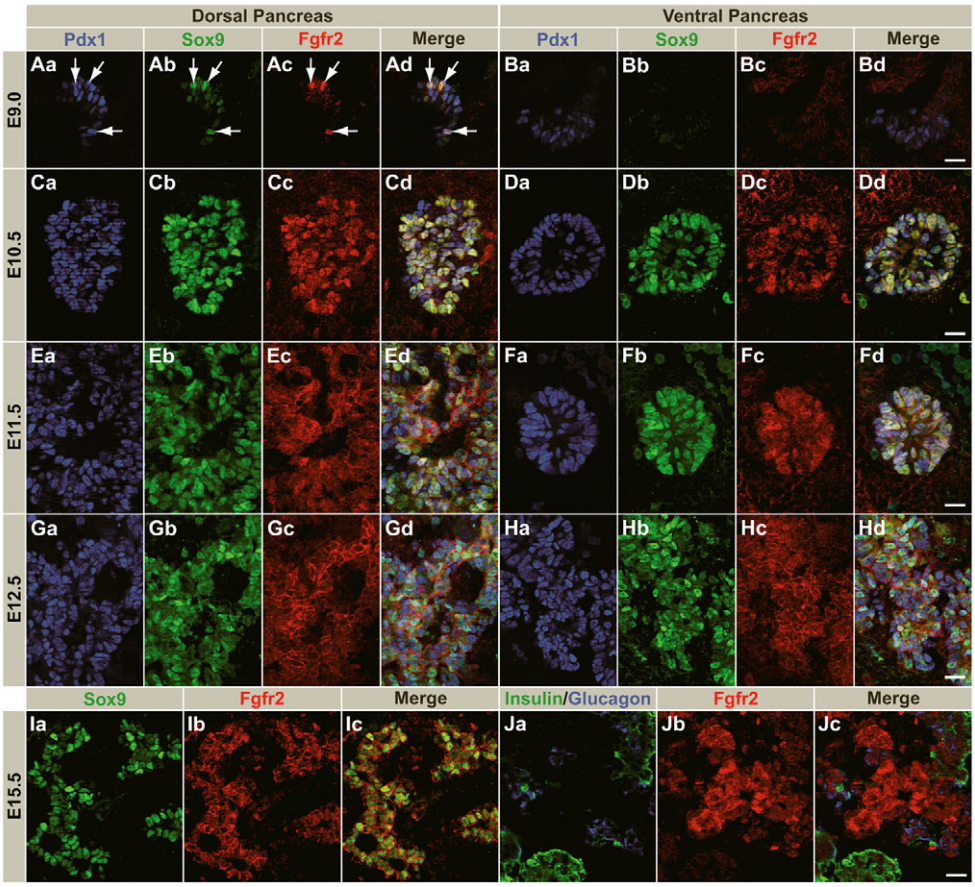

Fig. 3. Sox9 and Fgfr2b expression coincide in pancreatic progenitors. (**Aa-Bd**) Nuclear Fgfr2 expression in E9.0 dorsal pancreatic endoderm is closely correlated with that of Sox9 (arrows) (Aa-Ad); concordantly, neither Sox9 nor Fgfr2 is detectable in ventral pancreatic endoderm (Ba-Bd). (**Ca-Dd**) Nuclear Fgfr2 expression persists in pancreatic progenitors of dorsal (Ca-Cd) and ventral (Da-Dd) pancreas at E10.5. (**Ea-Hd**) Between E10.5-E12.5, Fgfr2 is redistributed from nucleus to membrane: at E11.5, Fgfr2 is localized to the membrane and nucleus (Ea-Fd), and by E12.5 is wholly membranous (Ga-Hd). (**Ia-Jc**) At E15.5, membranous Fgfr2 expression is restricted to the Sox9^{+} progenitor cords of the forming ductal tree. Scale bars: 20 μm.

93.9±0.472% of Sox9^{+} cells in the dorsal bud (*n*=491 cells) and 91.1±2.99% of Sox9^{+} cells in the ventral bud (*n*=243 cells) were Fgfr2^{+}; conversely, all Fgfr2^{+} cells in both dorsal (*n*=460 cells) and ventral (*n*=221 cells) buds expressed Sox9. Notably, evidence from previous studies has suggested such Fgf receptor nuclear localization to be associated with active Fgf signaling (Reilly and Maher, 2001). Furthermore, such close concordance between Sox9 and nuclear Fgfr2b expression has been reported previously in Sertoli cell precursors during testis development in response to active Fgf signaling (Bagheri-Fam et al., 2008; Schmahl et al., 2004). Concomitant with loss of the mesenchymal Fgf10 signaling source by E12.5 (Bhushan et al., 2001), Fgfr2b became excluded from the nucleus between E11.5 and E12.5 (Fig. 3E-H). Throughout development, Fgfr2b expression remained restricted to the Sox9^{+} domain (Fig. 3I,J).

To temporally dissect Fgfr2b downregulation following *Sox9* ablation, we assayed for Fgfr2b in *Sox9$^{fl/fl}$; Pdx1-Cre* pancreata. Fgfr2b expression was almost completely extinguished in both dorsal and ventral pancreas by E10.5 (Fig. 2D,D′,F,F′). To further determine whether Sox9 controls Fgfr2 expression cell-autonomously, we analyzed Sox9 and Fgfr2 expression in *Sox9$^{fl/fl}$; Ptf1a-Cre* pancreata, which show mosaic deletion of *Sox9* [Fig. 4; 69.8±5.5% of E-cadherin^{+} pancreatic cells expressed Sox9 in control (*n*=576 cells) versus 16.9±6.2% in *Sox9$^{fl/fl}$; Ptf1a-Cre* (*n*=368 cells) embryos]. Progenitors retaining Sox9 expression were Fgfr2^{+} (yellow arrowheads in Fig. 4B,B′,B″,D,D′,D″), while Sox9-deficient progenitors were devoid of Fgfr2 (white arrowheads in Fig. 4B-B″,D-D″). In *Sox9$^{fl/fl}$; Ptf1a-Cre* pancreata, 73.1±9.4% of Sox9^{+} cells were also Fgfr2^{+}, while 84.6±10.4% of Fgfr2^{+} cells expressed Sox9 (*n*=122 cells). Therefore, Sox9 is cell-autonomously required for maintaining Fgfr2b expression and thus, Fgf10 receptivity, of pancreatic progenitors.

Sox9 is dominant over Pdx1 in pancreatic fate maintenance

Given previous reports of reduced *Fgfr2b* expression in *Pdx1*-nullizygous pancreas (Svensson et al., 2007), we tested whether this decrease was Sox9 dependent. Although Sox9 is expressed in E10.5 *Pdx1*-deficient pancreas, varying degrees of Sox9 downregulation are evident (Fig. 5B′,Ba′,Bb′,D). In wild-type embryos, 65.5±1.65% of pancreatic epithelial cells (*n*=572 cells) displayed Sox9^{high}, 17.5±5.70% Sox9^{low} and 17.0±5.27% no Sox9 immunoreactivity. By contrast, a Sox9^{high} state was observed in only 23.3±3.29% of *Pdx1*-deficient epithelial cells (*n*=749 cells); 51.5±3.51% exhibited a Sox9^{low} state and 25.2±0.804% showed no Sox9 immunostaining. Consistent with Sox9-dependent regulation, Fgfr2b was downregulated in *Pdx1*-deficient progenitors in a pattern mirroring the loss of Sox9 (Fig. 5B-Bb″). In *Pdx1*-deficient pancreata, 89.8±1.07% of Sox9^{+} cells (*n*=560 cells) expressed Fgfr2, whereas all Fgfr2^{+} cells (*n*=503 cells) expressed Sox9 (note, Sox9^{low} cells also exhibited an Fgfr2^{low} state). This strongly suggests that loss of pancreatic Fgfr2b in *Pdx1*-null mice is Sox9 dependent. To examine whether decreased pancreatic expression of Sox9 and, thus, Fgfr2b in *Pdx1$^{-/-}$* mice manifests in adoption of hepatic fate, we assayed for AFP in E11.5 *Pdx1*-deficient pancreas. This revealed a significant upregulation of AFP^{+} cells (Fig. 5D,E), most of which were Sox9^{-} while AFP was largely excluded from Pdx1^{-} cells that retained Sox9 expression (Fig. 5D). These findings

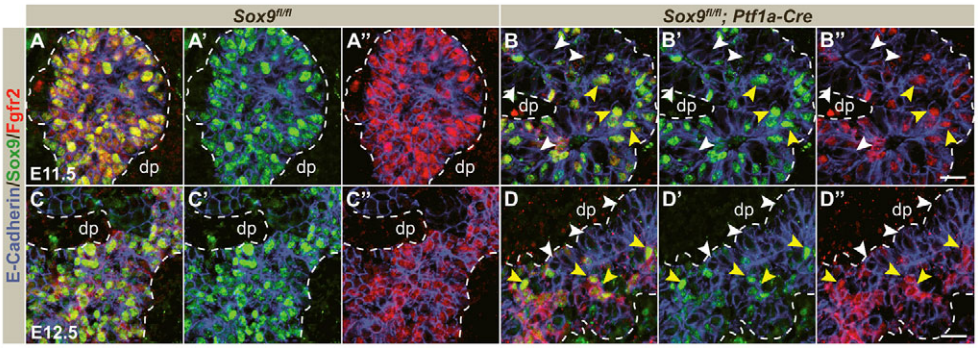

Fig. 4. Mosaic deletion reveals a cell-autonomous requirement for Sox9 in maintenance of Fgfr2 expression. (**A-A″**,**C-C″**) Nuclear Fgfr2 is expressed throughout Sox9$^+$ pancreatic progenitors in E11.5 (A-A″) and E12.5 (C-C″) control embryos. (**B-B″**,**D-D″**) In *Sox9$^{fl/fl}$; Ptf1a-Cre* mice, Fgfr2 expression is lost in *Sox9*-deleted progenitors (B-B″,D-D″; white arrowheads) but is maintained in unrecombined Sox9$^+$ cells (B-B″,D-D″; yellow arrowheads). The broken line outlines the pancreatic epithelium. dp, dorsal pancreas. Scale bars: 20 μm.

indicate that both loss of Fgfr2b expression and hepatic cell fate conversion of *Pdx1*-deficient progenitors are Sox9 dependent, inferring *Sox9* to be dominant over *Pdx1* in maintaining pancreatic fate. As Ptf1a is also co-expressed with Sox9 in pancreatic progenitors and governs early pancreas organogenesis (Kawaguchi et al., 2002), we assayed for both Fgfr2b and AFP in *Ptf1a*-nullizygous embryos. In contrast to *Pdx1*$^{-/-}$ mice, pancreatic progenitors in *Ptf1a*-null mutants retained Fgfr2b, consistent with persistence of Sox9 expression (supplementary material Fig. S6C,F,H,J). Concordantly, at E11.5, when AFP was evident in pancreata lacking either *Sox9* or *Pdx1*, AFP was undetectable in *Ptf1a*-deficient cells (supplementary material Fig. S6J). Taken together, these observations are consistent with *Sox9* being dominant over *Pdx1*, as well as *Ptf1a* in maintaining Fgfr2b expression and pancreatic identity in pancreatic progenitors.

Fgf receptor signaling maintains pancreatic fate

Consistent with Sox9 maintaining Fgfr2b expression in pancreatic progenitors, it is predicted that pharmacological perturbation of Fgfr signaling will induce their hepatic reprogramming. To test this, we assayed for hepatic fate induction in early pancreatic explants following Fgfr inhibition. Caudal foregut and midgut endoderm was microdissected from 24- to 25-somite (E9.5) *Sox9-eGFP* embryos (Fig. 6A) and grown in culture on a two-dimensional fibronectin substrate for up to 5 days. In control (DMSO vehicle-treated) explants, eGFP$^+$ dorsal pancreatic endoderm budded out of the gut tube, expanded rapidly and branched, giving rise to a readily distinguishable dorsal pancreatic bud (Fig. 6B,D,F,H,J). By days four (d4) and five (d5), the dorsal pancreas in explants closely resembled in situ E12.5 and E13.5 dorsal pancreata, respectively (Fig. 6B,H,J). Through this five-day window, the pancreatic endoderm principally comprised Sox9$^+$/Sox9-eGFP$^+$ Pdx1$^+$ cells, as well as a few glucagon$^+$ endocrine cell clusters; as in vivo, scarce AFP$^+$ cells were also evident (Fig. 6D,F,H,J). This culture system therefore closely recapitulates the in situ program, albeit with some developmental delay (~1 day per 4 days of culture), as previously reported for E11.5 dorsal pancreas explants (Percival and Slack, 1999). Inhibition of Fgf receptor activity with SU5402 for 72 hours immediately after gut dissection (d0-d3) manifested in downregulation of dorsal bud Sox9 expression, although Sox9-eGFP was retained (Fig. 6E) owing to eGFP perdurance (Corish and Tyler-Smith, 1999). Recapitulating its effects on pancreatic Sox9 expression, Fgfr abrogation for 72 hours also produced a reduction in Pdx1 expression in the dorsal pancreas (Fig. 6G). Moreover, blockade of Fgf signaling resulted in a significant induction of AFP$^+$ cells in the dorsal pancreas (Fig. 6E,G,L). These observations are consistent with a model whereby Fgfr-transduced Fgf signaling maintains Sox9 expression in pancreatic progenitors, which in turn maintains their Fgfr2b expression and, thus, Fgf receptivity. This signaling cascade therefore maintains the identify of pancreatic progenitors by blocking their adoption of an alternative hepatic program.

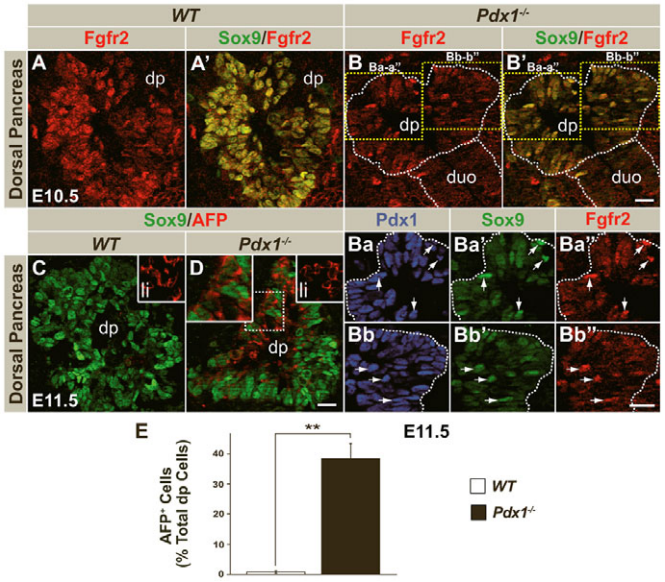

Fig. 5. Loss of Fgfr2 and hepatic fate conversion in *Pdx1*-deficient pancreas is Sox9 dependent. (**A-D**) While control E10.5 dorsal pancreatic progenitors show robust nuclear Fgfr2 expression (A,A′), in *Pdx1*-deficient dorsal progenitors, Fgfr2 is downregulated in a pattern following that of Sox9 (B-Bb″; arrows indicate Sox9$^+$ Fgfr2$^+$ cells), consistent with AFP expression in *Sox9*-deficient progenitors in E11.5 *Pdx1*$^{-/-}$ (D), compared with wild-type (*WT*) (C) mice. Immunofluorescence staining for the truncated Pdx1 protein in *Pdx1*$^{-/-}$ dorsal pancreas is shown in Ba and Bb as a reference. (**E**) AFP$^+$ cells as a percentage of total pancreatic cells (E) in E11.5 *Pdx1*$^{-/-}$ and wild-type (*WT*) (n=4) dorsal pancreata. dp, dorsal pancreas; duo, duodenum; li, liver. Error bars represent s.e.m.; $^{**}P<0.01$. Scale bars: 20 μm.

We next sought to define the competence window through which Fgf-deprived pancreatic progenitors are able to adopt a liver fate. Explants were therefore grown in the presence of SU5402 for additional overlapping 72-hour windows: either d1-d4 or d2-d5 (Fig. 6C). Mirroring our initial results, Fgf receptor inhibition for 72 hours from d1-d4 manifested in disrupted branching morphogenesis and significantly increased numbers of AFP$^+$ cells (Fig. 6H,I,L). By contrast, after SU5402 treatment over d2-d5, AFP$^+$ cells were not increased in number (Fig. 6J-L). Thus, for around 2 days after E9.5, pancreatic progenitors are competent to adopt hepatic fates when deprived of extrinsic Fgf signaling. This temporal requirement for Fgf/Fgfr signaling in pancreatic fate maintenance is concurrent with the E9.5-E11.5 expression of *Fgf10* in pancreatic mesenchyme in vivo (Bhushan et al., 2001) and also corresponds to the competence period during which removal of gut mesoderm results in spontaneous activation of liver genes in the dorsal gut endoderm (Bossard and Zaret, 1998; Bossard and Zaret, 2000; Gualdi et al., 1996). Furthermore, this time-window matches with that through which pancreatic *Sox9* ablation induces hepatic reprogramming.

Fgf10 maintains Sox9 and Fgfr2b expression in pancreatic progenitors

Pancreatic Sox9 downregulation in gut explants following pharmacological Fgfr inhibition strongly suggests that Fgf signaling is required for maintaining Sox9 and, thus, Fgfr2b expression in pancreatic progenitors. To test this hypothesis, we assayed for Sox9 and Fgfr2b in the early dorsal pancreas of 25-26 somite (E9.5) *Fgf10*-deficient mice. Our analysis revealed downregulation of both Sox9 and Fgfr2b in a corresponding pattern within Pdx1$^+$ dorsal pancreatic epithelial cells of *Fgf10*$^{-/-}$ embryos (Fig. 7A,B). Thus, while not required for Sox9 induction, mesenchymal Fgf10 is crucially required for maintaining the expression of Sox9 and, thus, Fgfr2b in pancreatic progenitors. This is consistent with pancreatic Fgf10 misexpression inducing Sox9 expression (Seymour et al., 2007). Therefore, in the early pancreas, Fgf10 participates in a feed-forward loop with Sox9 and Fgfr2b to ensure that progenitor cells in the epithelium remain receptive to Fgf10 signaling from the pancreatic mesenchyme.

An Fgf10/Fgfr2b/Sox9 feed-forward loop maintains pancreatic fate

The finding that Sox9 and Fgfr2b are downregulated in *Fgf10*-deficient pancreata would predict that pancreatic progenitors undergo hepatic reprogramming as in *Sox9*-deficient or Fgfr-inhibited pancreata. Thus, to test whether *Sox9* acts in the same pathway as *Fgf10* and *Fgfr2b* to maintain pancreatic fate, we assayed for pancreatic AFP in *Fgf10*$^{-/-}$ mice. In E11.5 *Fgf10*-nullizygous mice, both dorsal and ventral pancreatic buds were severely hypoplastic (Fig. 7C,E; data not shown) as reported previously (Bhushan et al., 2001). While Pdx1 was partly maintained in *Fgf10*-deficient dorsal pancreas, Sox9 was almost entirely extinguished; concordantly, AFP$^+$ cells were significantly upregulated (Fig. 7E,F). Intriguingly, several AFP$^+$ Sox9$^-$ cells were also observed in the dorsal pancreas of E11.5 *Fgf10*-heterozygous mutant embryos with apparently greater prevalence than the scarce AFP$^+$ cells observed in E11.5 wild-type pancreas (Fig. 7D,F). Thus, the prevalence of pancreatic AFP$^+$ cells increases with decreasing *Fgf10* gene dose (Fig. 7C-F). Taken together, our data demonstrate that pancreatic progenitors depend on an Fgf10/Fgfr2b/Sox9 feed-forward loop in the pancreatic niche to maintain their organ identity (Fig. 7G).

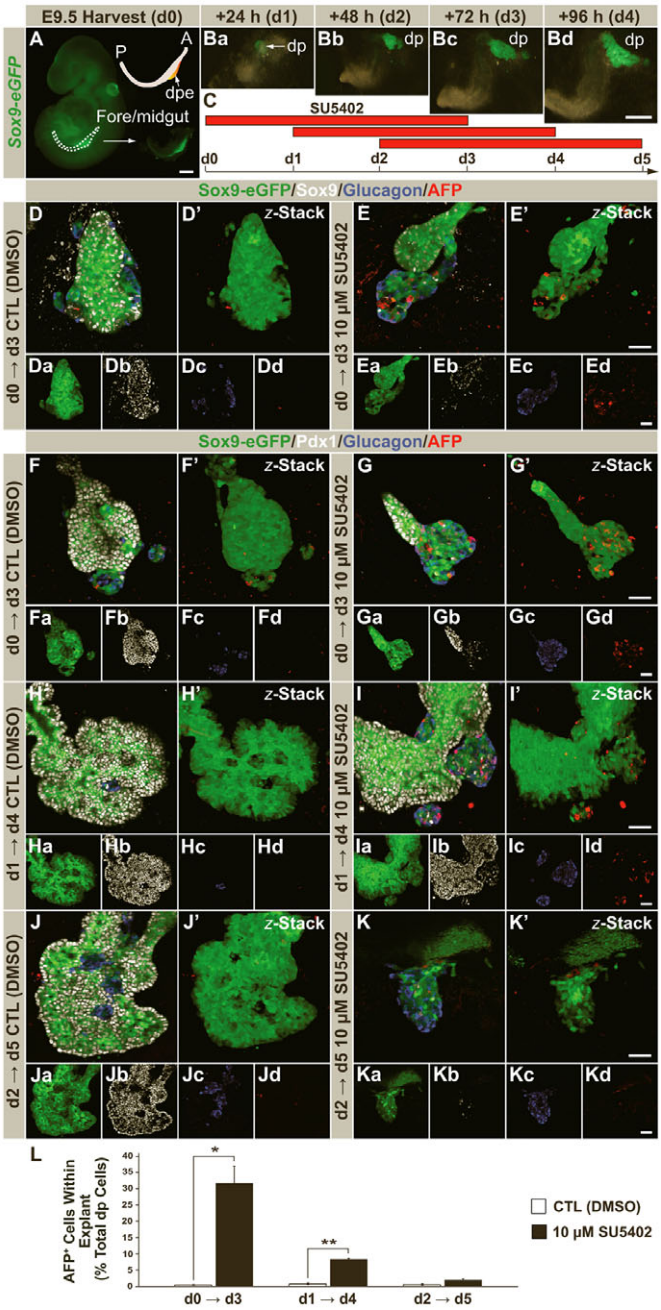

Fig. 6. Perturbed Fgfr signaling in gut explants induces hepatic fate conversion in dorsal pancreas. (**A**) Gut region microdissected from E9.5 *Sox9-eGFP* embryos for explanting. (**Ba-Bd**) In culture, the eGFP$^+$ dorsal pancreatic endoderm forms a dorsal pancreas (dp) over 96 hours (h). (**C**) Explants were cultured in 10 µM SU5402 or DMSO vehicle control for nested 72-hour windows. (**D-Dd**,**F-Fd**,**H-Hd**,**J-Jd**) Between day (d)0 and d5 in vitro, the untreated dorsal pancreas expands, branches and differentiates, as in vivo. (**E-Ed**,**G-Gd**) Inhibition of Fgf signaling from d0 to d3 results in downregulation of Sox9 (E-Ed) and Pdx1 (G-Gd), and induction of AFP expression (E-Ed,G-Gd). (**I-Id**,**K-Kd**) While AFP$^+$ cells are also apparent with SU5402 treatment at d1-d4 (I-Id), they are not detected when Fgf signaling is blocked at d2-d5 (K-Kd). For clarity, only eGFP and AFP are shown in *z*-stacks (D′,E′,F′,G′,H′,I′,J′,K′). (**L**) AFP$^+$ cells as a percentage of total dorsal pancreas cells in gut explants treated with 10 µM SU5402 or DMSO vehicle (CTL) for nested 72-hour windows (n=3); error bars represent s.e.m.; *P<0.05; **P<0.01. dpe, dorsal pancreatic endoderm; A, anterior; P, posterior. Scale bars: 250 µm in A-Bd; 50 µm in D-Kd.

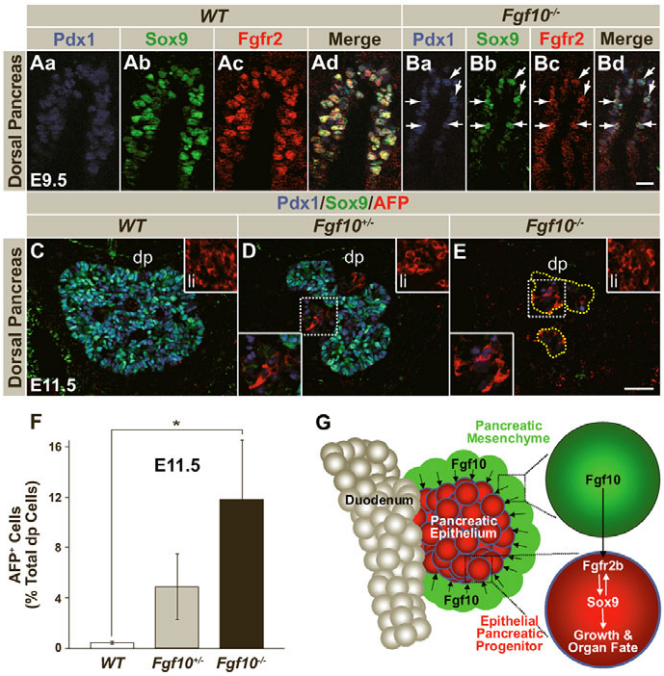

Fig. 7. Fgf10 maintains pancreatic fate through Fgfr2b and Sox9. (**Aa-Bd**) Sox9 and Fgfr2 are coordinately downregulated (arrows indicate Sox9$^+$ Fgfr2$^+$ cells) in the E9.5 dorsal pancreatic endoderm of *Fgf10*-deficient (Ba-Bd) compared with wild-type (*WT*) (Aa-Ad) mice. (**C-E**) AFP is expressed in E11.5 dorsal pancreata of *Fgf10*$^{+/-}$ (D) or *Fgf10*$^{-/-}$ (E) mice compared with wild-type pancreas (C), concordant with Sox9 downregulation (E). (**F**) AFP$^+$ cells as a percentage of total pancreatic cells in E11.5 wild-type, *Fgf10*$^{+/-}$ and *Fgf10*$^{-/-}$ (n=3) dorsal pancreata; error bars represent s.e.m.; *P<0.05. dp, dorsal pancreas; li, liver. Scale bars: 20 μm in Aa-Bd; 50 μm in C-E. (**G**) Mesenchymal Fgf10 signaling, transduced via Fgfr2b on pancreatic progenitors, maintains their Sox9 and, thus, Fgfr2b expression, to maintain Fgf10 receptivity. This Sox9/Fgfr2b/Fgf10 feed-forward loop promotes growth and maintains pancreatic fate in pancreatic progenitors.

In addition to maintaining pancreatic identity, this Fgf10/Fgfr2b/Sox9 loop between epithelial progenitors and pancreatic mesenchyme also ensures proper pancreatic growth and morphogenesis.

DISCUSSION

Our findings reveal that organ commitment of progenitors in the early pancreas is not stably programmed, but instead requires extrinsic cues from the progenitor niche (Fig. 7G). We show that maintenance of pancreatic identity depends on mesenchymal Fgf10 signaling, transduced via Fgfr2b to maintain Sox9 expression in progenitors. In turn, Sox9 is required for Fgfr2b expression and, thus, for receptivity to Fgf10. Therefore, Sox9 and Fgf10-Fgfr2b form a feed-forward loop in the pancreatic progenitor cell niche that maintains pancreatic organ identity. Pancreatic progenitors require activity of this loop to suppress their adoption of an alternative hepatic program. Our finding that *Sox9* deletion in pancreatic progenitors causes their conversion to a liver fate provides additional evidence for a previously recognized developmental plasticity of early pancreatic, liver and gut progenitors. Examples for cell fate interconversions upon loss of a single transcription factor include pancreatic-to-intestinal, liver-to-intestinal and biliary-to-pancreatic conversions in *Ptf1a*-, *Hhex*- and *Hes1*-deficient mice, respectively (Bort et al., 2006; Kawaguchi et al., 2002; Sumazaki et al., 2004). Reassignment of pancreatic progenitors to an intestinal fate has also been observed after exposure of pancreatic epithelium to splenic mesenchyme, suggesting that extrinsic signals can reverse progenitor fate commitment even after their allocation to the pancreatic anlage (Asayesh et al., 2006). Our study demonstrates that extrinsic cues are not only capable of reversing cell fate commitment, but that Fgf signals in the pancreatic niche are required to maintain organ identity by suppressing hepatic gene expression programs. It remains to be seen whether the niche micro-environment is similarly required to reinforce organ- or tissue-specific fate commitment of stem or progenitor cells in other developing organs or stem cell niches.

This pro-pancreatic role of Fgf signaling in pancreatic progenitors appears to contradict its previously identified role as an inducer of hepatic fate in mice and zebrafish (Calmont et al., 2006; Deutsch et al., 2001; Jung et al., 1999; Shin et al., 2007). Notably, the early function of Fgf as an activator of hepatic genes temporally precedes the herein-described requirement for Fgf signaling in repressing hepatic programs in dorsal and ventral pancreatic progenitors. Hepatic induction occurs in the ventral foregut around E8.25 and during this time requires Fgf signaling from cardiac mesenchyme (Jung et al., 1999). We show that Fgf signaling exerts its liver-repressive role significantly later, as evidenced by pancreas-to-liver cell fate conversion following perturbation of the Sox9/Fgf10/Fgfr2b loop between E9.5 and E11.5. Paralleling this changing influence of Fgf signaling on hepatopancreatic specification, Bmp signaling has similarly been shown to first induce hepatic fate, then, hours later, to promote adoption of pancreatic fate (Wandzioch and Zaret, 2009). Together, these studies exemplify the dynamism of cellular competence in response to extracellular signals. Our finding that Sox9 controls the expression of Fgfr2 and, thus, receptivity of epithelial progenitors to Fgf10 signaling identifies Sox9 as an important progenitor-intrinsic cue for regulating cellular competence in the developing gut tube. As Sox9 does not become expressed in the foregut endoderm until the onset of pancreatic cell specification (Seymour et al., 2007), Fgfr2-mediated signaling responses can only be elicited after Sox9 expression has been initiated. The finding that *Sox9* ablation also results in *Fgfr2* loss in the developing testes (Bagheri-Fam et al., 2008) and ventral prostate (Thomsen et al., 2008) suggests that *Sox9* might have a more global role in enabling cells to respond to Fgf signaling.

Similar to the role of *Fgf10* in mice described here, *Fgf10* is required for proper specification of the zebrafish hepatopancreatic system. *Fgf10* mutant zebrafish exhibit ectopic hepatic cells in the proximal pancreas and ectopic pancreatic cells in the liver (Dong et al., 2007). Whereas *Fgf10* is also expressed in mesenchyme surrounding the hepatic diverticulum in mouse embryos from E9.5 to E12.5 (Berg et al., 2007), Fgfr2 is not detected in hepatic progenitors during this time (data not shown). Thus, during the time-window required for repressing hepatic fate in pancreatic progenitors, the Fgf10-Fgfr2 signaling axis does not appear to have a direct influence on hepatic progenitors in mouse embryos. However, the finding that Fgf10 signaling negatively regulates hepatic competence of non-hepatic endoderm in zebrafish embryos (Shin et al., 2011) points to a conserved role for Fgf10 in repressing hepatic programs during gut development. Although the role of Sox9 and its potential regulation of Fgf receptors in the zebrafish gut tube remain to be explored, studies in other tissues or organs suggest conserved genetic interactions between Sox9 and Fgf

signaling. For example, Fgf receptor-transduced signaling promotes *Sox9* expression in embryonic zebrafish hindbrain (Esain et al., 2010) and mouse chondrocytes (Murakami et al., 2000), and *Fgfr2* ablation diminishes *Sox9* expression in both developing testes (Bagheri-Fam et al., 2008) and lung (Abler et al., 2009). Our findings now pave the way for future studies addressing whether *Sox9*, *Fgfr2b* and *Fgf10* also operate in a feed-forward loop in other tissues and organs, and whether this loop acts as a paradigm for integrating cell-extrinsic and -intrinsic cues to expand progenitors and reinforce their fate commitment in multiple developmental and regenerative contexts.

Our discovery that *Sox9*, *Fgfr2b* and *Fgf10* form a regulatory loop in the early pancreas explains why deletion of each of these genes similarly impairs progenitor proliferation and causes pancreatic hypoplasia (Bhushan et al., 2001; Pulkkinen et al., 2003; Revest et al., 2001; Seymour et al., 2007). However, the more subtle pancreatic hypoplasia of mice lacking *Fgfr2b* (Pulkkinen et al., 2003; Revest et al., 2001) compared with that in mice deficient for *Fgf10* (Bhushan et al., 2001) suggests that Fgf10 signaling might not be transduced exclusively through Fgfr2b during early pancreas organogenesis. Although Fgf10 has not been formally shown to signal through Fgfr4, our observation that Sox9 also regulates *Fgfr4* expression suggests that Fgfr2 and Fgfr4 might serve partially redundant functions in the pancreatic epithelium.

Notably, not all pancreatic progenitors convert to an hepatic fate upon perturbations of the Sox9/Fgf10/Fgfr2b loop, but pancreatic and hepatic differentiation is initiated in parallel within the organ. This observation raises the issue of why some progenitors undergo hepatic reprogramming whereas others follow a pancreatic differentiation path. Susceptibility to hepatic fate conversion may be limited to a subset of progenitors and could depend on cell cycle phasing and cell growth, as shown for cells in the *Drosophila* imaginal disc (Sustar and Schubiger, 2005). In this respect, it is interesting that hepatic cells were preferentially found in the outer layer tip cells of the developing pancreas (Fig. 7E; supplementary material Fig. S4), which have higher proliferation rates than centrally located progenitors (Zhou et al., 2007). Later in development, when tip cells have committed to an acinar fate (Zhou et al., 2007), disruption of the *Sox9*/*Fgfr2b*/*Fgf10* pathway no longer caused pancreas-to-liver conversion, suggesting that developmental plasticity of pancreatic progenitors is limited to an early time-window, when progenitors are still multipotent and highly proliferative. Comparative analysis of early and late pancreatic progenitors could provide insight into the transcriptional and epigenetic mechanisms that underlie their developmental plasticity.

Finally, our results have important implications for stably inducing pancreatic programs in directed differentiations of embryonic stem cells towards pancreas. The simultaneous induction of pancreas- and liver-specific programs in current directed differentiation protocols has hampered progress in generating insulin-producing cells (D'Amour et al., 2006; Nostro et al., 2011). Although previous strategies to prevent induction of hepatic programs have largely focused on inhibiting Bmp signaling (D'Amour et al., 2006; Nostro et al., 2011), our findings suggest that stimulating Fgf signaling in embryonic stem cell cultures beyond the time of pancreas induction could stabilize pancreatic fate commitment.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

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References

Abler, L. L., Mansour, S. L. and Sun, X. (2009). Conditional gene inactivation reveals roles for Fgf10 and Fgfr2 in establishing a normal pattern of epithelial branching in the mouse lung. *Dev. Dyn.* **238**, 1999-2013.

Ahlgren, U., Jonsson, J. and Edlund, H. (1996). The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX1-deficient mice. *Development* **122**, 1409-1416.

Ahnfelt-Rønne, J., Ravassard, P., Pardanaud-Glavieux, C., Scharfmann, R. and Serup, P. (2010). Mesenchymal bone morphogenetic protein signaling is required for normal pancreas development. *Diabetes* **59**, 1948-1956.

Apelqvist, A., Li, H., Sommer, L., Beatus, P., Anderson, D. J., Honjo, T., Hrabe de Angelis, M., Lendahl, U. and Edlund, H. (1999). Notch signalling controls pancreatic cell differentiation. *Nature* **400**, 877-881.

Asayesh, A., Sharpe, J., Watson, R. P., Hecksher-Sørensen, J., Hastie, N. D., Hill, R. E. and Ahlgren, U. (2006). Spleen versus pancreas: strict control of organ interrelationship revealed by analyses of Bapx1–/– mice. *Genes Dev.* **20**, 2208-2213.

Bagheri-Fam, S., Sim, H., Bernard, P., Jayakody, I., Taketo, M. M., Scherer, G. and Harley, V. R. (2008). Loss of Fgfr2 leads to partial XY sex reversal. *Dev. Biol.* **314**, 71-83.

Berg, T., Rountree, C. B., Lee, L., Estrada, J., Sala, F. G., Choe, A., Veltmaat, J. M., De Langhe, S., Lee, R., Tsukamoto, H. et al. (2007). Fibroblast growth factor 10 is critical for liver growth during embryogenesis and controls hepatoblast survival via beta-catenin activation. *Hepatology* **46**, 1187-1197.

Bhushan, A., Itoh, N., Kato, S., Thiery, J. P., Czernichow, P., Bellusci, S. and Scharfmann, R. (2001). Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. *Development* **128**, 5109-5117.

Bort, R., Martinez-Barbera, J. P., Beddington, R. S. and Zaret, K. S. (2004). Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. *Development* **131**, 797-806.

Bort, R., Signore, M., Tremblay, K., Martinez Barbera, J. P. and Zaret, K. S. (2006). Hex homeobox gene controls the transition of the endoderm to a pseudostratified, cell emergent epithelium for liver bud development. *Dev. Biol.* **290**, 44-56.

Bossard, P. and Zaret, K. S. (1998). GATA transcription factors as potentiators of gut endoderm differentiation. *Development* **125**, 4909-4917.

Bossard, P. and Zaret, K. S. (2000). Repressive and restrictive mesodermal interactions with gut endoderm: possible relation to Meckel's Diverticulum. *Development* **127**, 4915-4923.

Burlison, J. S., Long, Q., Fujitani, Y., Wright, C. V. and Magnuson, M. A. (2008). Pdx-1 and Ptf1a concurrently determine fate specification of pancreatic multipotent progenitor cells. *Dev. Biol.* **316**, 74-86.

Calmont, A., Wandzioch, E., Tremblay, K. D., Minowada, G., Kaestner, K. H., Martin, G. R. and Zaret, K. S. (2006). An FGF response pathway that mediates hepatic gene induction in embryonic endoderm cells. *Dev. Cell* **11**, 339-348.

Corish, P. and Tyler-Smith, C. (1999). Attenuation of green fluorescent protein half-life in mammalian cells. *Protein Eng.* **12**, 1035-1040.

D'Amour, K. A., Bang, A. G., Eliazer, S., Kelly, O. G., Agulnick, A. D., Smart, N. G., Moorman, M. A., Kroon, E., Carpenter, M. K. and Baetge, E. E. (2006). Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat. Biotechnol.* **24**, 1392-1401.

Deutsch, G., Jung, J., Zheng, M., Lóra, J. and Zaret, K. S. (2001). A bipotential precursor population for pancreas and liver within the embryonic endoderm. *Development* **128**, 871-881.

Dong, P. D., Munson, C. A., Norton, W., Crosnier, C., Pan, X., Gong, Z., Neumann, C. J. and Stainier, D. Y. (2007). Fgf10 regulates hepatopancreatic ductal system patterning and differentiation. *Nat. Genet.* **39**, 397-402.

Dubois, C. L., Shih, H. P., Seymour, P. A., Patel, N. A., Behrmann, J. M., Ngo, V. and Sander, M. (2011). Sox9-haploinsufficiency causes glucose intolerance in mice. *PLoS ONE* **6**, e23131.
Elghazi, L., Cras-Méneur, C., Czernichow, P. and Scharfmann, R. (2002). Role for FGFR2IIIb-mediated signals in controlling pancreatic endocrine progenitor cell proliferation. *Proc. Natl. Acad. Sci. USA* **99**, 3884-3889.
Esain, V., Postlethwait, J. H., Charnay, P. and Ghislain, J. (2010). FGF-receptor signalling controls neural cell diversity in the zebrafish hindbrain by regulating olig2 and sox9. *Development* **137**, 33-42.
Gittes, G. K., Galante, P. E., Hanahan, D., Rutter, W. J. and Debase, H. T. (1996). Lineage-specific morphogenesis in the developing pancreas: role of mesenchymal factors. *Development* **122**, 439-447.
Golosow, N. and Grobstein, C. (1962). Epitheliomesenchymal interaction in pancreatic morphogenesis. *Dev. Biol.* **4**, 242-255.
Gong, S., Zheng, C., Doughty, M. L., Losos, K., Didkovsky, N., Schambra, U. B., Nowak, N. J., Joyner, A., Leblanc, G., Hatten, M. E. et al. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* **425**, 917-925.
Gu, G., Dubauskaite, J. and Melton, D. A. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* **129**, 2447-2457.
Gualdi, R., Bossard, P., Zheng, M., Hamada, Y., Coleman, J. R. and Zaret, K. S. (1996). Hepatic specification of the gut endoderm in vitro: cell signaling and transcriptional control. *Genes Dev.* **10**, 1670-1682.
Jensen, J., Pedersen, E. E., Galante, P., Hald, J., Heller, R. S., Ishibashi, M., Kageyama, R., Guillemot, F., Serup, P. and Madsen, O. D. (2000). Control of endodermal endocrine development by Hes-1. *Nat. Genet.* **24**, 36-44.
Jonckheere, N., Mayes, E., Shih, H. P., Li, B., Lioubinski, O., Dai, X. and Sander, M. (2008). Analysis of mPygo2 mutant mice suggests a requirement for mesenchymal Wnt signaling in pancreatic growth and differentiation. *Dev. Biol.* **318**, 224-235.
Jung, J., Zheng, M., Goldfarb, M. and Zaret, K. S. (1999). Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* **284**, 1998-2003.
Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, R. J. and Wright, C. V. (2002). The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat. Genet.* **32**, 128-134.
Kist, R., Schrewe, H., Balling, R. and Scherer, G. (2002). Conditional inactivation of Sox9: a mouse model for campomelic dysplasia. *Genesis* **32**, 121-123.
Kopp, J. L., Dubois, C. L., Schaffer, A. E., Hao, E., Shih, H. P., Seymour, P. A., Ma, J. and Sander, M. (2011). Sox9+ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. *Development* **138**, 653-665.
Krapp, A., Knöfler, M., Ledermann, B., Bürki, K., Berney, C., Zoerkler, N., Hagenbüchle, O. and Wellauer, P. K. (1998). The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. *Genes Dev.* **12**, 3752-3763.
Landsman, L., Nijagal, A., Whitchurch, T. J., Vanderlaan, R. L., Zimmer, W. E., Mackenzie, T. C. and Hebrok, M. (2011). Pancreatic mesenchyme regulates epithelial organogenesis throughout development. *PLoS Biol.* **9**, e1001143.
Min, H., Danilenko, D. M., Scully, S. A., Bolon, B., Ring, B. D., Tarpley, J. E., DeRose, M. and Simonet, W. S. (1998). Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. *Genes Dev.* **12**, 3156-3161.
Murakami, S., Kan, M., McKeehan, W. L. and de Crombrugghe, B. (2000). Up-regulation of the chondrogenic Sox9 gene by fibroblast growth factors is mediated by the mitogen-activated protein kinase pathway. *Proc. Natl. Acad. Sci. USA* **97**, 1113-1118.
Nostro, M. C., Sarangi, F., Ogawa, S., Holtzinger, A., Corneo, B., Li, X., Micallef, S. J., Park, I. H., Basford, C., Wheeler, M. B. et al. (2011). Stage-specific signaling through TGFβ family members and WNT regulates patterning and pancreatic specification of human pluripotent stem cells. *Development* **138**, 861-871.
Novak, A., Guo, C., Yang, W., Nagy, A. and Lobe, C. G. (2000). Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. *Genesis* **28**, 147-155.
Offield, M. F., Jetton, T. L., Labosky, P. A., Ray, M., Stein, R. W., Magnuson, M. A., Hogan, B. L. and Wright, C. V. (1996). PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* **122**, 983-995.
Ornitz, D. M., Xu, J., Colvin, J. S., McEwen, D. G., MacArthur, C. A., Coulier, F., Gao, G. and Goldfarb, M. (1996). Receptor specificity of the fibroblast growth factor family. *J. Biol. Chem.* **271**, 15292-15297.
Percival, A. C. and Slack, J. M. (1999). Analysis of pancreatic development using a cell lineage label. *Exp. Cell Res.* **247**, 123-132.
Pulkkinen, M. A., Spencer-Dene, B., Dickson, C. and Otonkoski, T. (2003). The IIIb isoform of fibroblast growth factor receptor 2 is required for proper growth and branching of pancreatic ductal epithelium but not for differentiation of exocrine or endocrine cells. *Mech. Dev.* **120**, 167-175.
Reilly, J. F. and Maher, P. A. (2001). Importin beta-mediated nuclear import of fibroblast growth factor receptor: role in cell proliferation. *J. Cell Biol.* **152**, 1307-1312.
Revest, J. M., Spencer-Dene, B., Kerr, K., De Moerlooze, L., Rosewell, I. and Dickson, C. (2001). Fibroblast growth factor receptor 2-IIIb acts upstream of Shh and Fgf4 and is required for limb bud maintenance but not for the induction of Fgf8, Fgf10, Msx1, or Bmp4. *Dev. Biol.* **231**, 47-62.
Schmahl, J., Kim, Y., Colvin, J. S., Ornitz, D. M. and Capel, B. (2004). Fgf9 induces proliferation and nuclear localization of FGFR2 in Sertoli precursors during male sex determination. *Development* **131**, 3627-3636.
Seymour, P. A., Bennett, W. R. and Slack, J. M. (2004). Fission of pancreatic islets during postnatal growth of the mouse. *J. Anat.* **204**, 103-116.
Seymour, P. A., Freude, K. K., Tran, M. N., Mayes, E. E., Jensen, J., Kist, R., Scherer, G. and Sander, M. (2007). SOX9 is required for maintenance of the pancreatic progenitor cell pool. *Proc. Natl. Acad. Sci. USA* **104**, 1865-1870.
Shin, D., Shin, C. H., Tucker, J., Ober, E. A., Rentzsch, F., Poss, K. D., Hammerschmidt, M., Mullins, M. C. and Stainier, D. Y. (2007). Bmp and Fgf signaling are essential for liver specification in zebrafish. *Development* **134**, 2041-2050.
Shin, D., Lee, Y., Poss, K. D. and Stainier, D. Y. (2011). Restriction of hepatic competence by Fgf signaling. *Development* **138**, 1339-1348.
Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat. Genet.* **21**, 70-71.
Spence, J. R., Lange, A. W., Lin, S. C., Kaestner, K. H., Lowy, A. M., Kim, I., Whitsett, J. A. and Wells, J. M. (2009). Sox17 regulates organ lineage segregation of ventral foregut progenitor cells. *Dev. Cell* **17**, 62-74.
Spooner, B. S., Walther, B. T. and Rutter, W. J. (1970). The development of the dorsal and ventral mammalian pancreas in vivo and in vitro. *J. Cell Biol.* **47**, 235-246.
Stanger, B. Z., Tanaka, A. J. and Melton, D. A. (2007). Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. *Nature* **445**, 886-891.
Sumazaki, R., Shiojiri, N., Isoyama, S., Masu, M., Keino-Masu, K., Osawa, M., Nakauchi, H., Kageyama, R. and Matsui, A. (2004). Conversion of biliary system to pancreatic tissue in Hes1-deficient mice. *Nat. Genet.* **36**, 83-87.
Sustar, A. and Schubiger, G. (2005). A transient cell cycle shift in Drosophila imaginal disc cells precedes multipotency. *Cell* **120**, 383-393.
Svensson, P., Williams, C., Lundeberg, J., Rydén, P., Bergqvist, I. and Edlund, H. (2007). Gene array identification of Ipf1/Pdx1–/– regulated genes in pancreatic progenitor cells. *BMC Dev. Biol.* **7**, 129.
Sylvestersen, K. B., Herrera, P. L., Serup, P. and Rescan, C. (2011). Fgf9 signalling stimulates Spred and Sprouty expression in embryonic mouse pancreas mesenchyme. *Gene Expr. Patterns* **11**, 105-111.
Thomsen, M. K., Butler, C. M., Shen, M. M. and Swain, A. (2008). Sox9 is required for prostate development. *Dev. Biol.* **316**, 302-311.
Vooijs, M., Jonkers, J. and Berns, A. (2001). A highly efficient ligand-regulated Cre recombinase mouse line shows that LoxP recombination is position dependent. *EMBO Rep.* **2**, 292-297.
Wandzioch, E. and Zaret, K. S. (2009). Dynamic signaling network for the specification of embryonic pancreas and liver progenitors. *Science* **324**, 1707-1710.
Wessells, N. K. and Cohen, J. H. (1967). Early pancreas organogenesis: morphogenesis, tissue interactions, and mass effects. *Dev. Biol.* **15**, 237-270.
Zhou, Q., Law, A. C., Rajagopal, J., Anderson, W. J., Gray, P. A. and Melton, D. A. (2007). A multipotent progenitor domain guides pancreatic organogenesis. *Dev. Cell* **13**, 103-114.

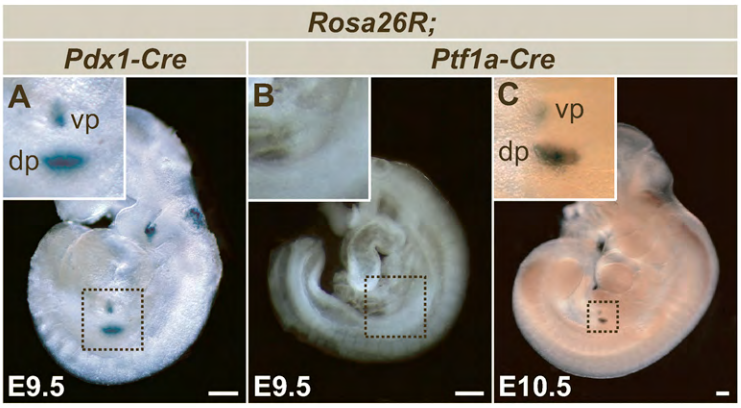

Fig. S1. *Pdx1-Cre* and *Ptf1a-Cre* are active in the pancreas by E9.5 and E10.5, respectively. (**A-C**) X-Gal staining for β-gal activity from the recombined *Rosa26R* reporter allele reveals *Pdx1-Cre* to be active in the dorsal (dp) and ventral (vp) pancreatic buds by E9.5 (A) while *Ptf1a-Cre* is not active until E10.5 (B,C). Scale bars: 250 µm.

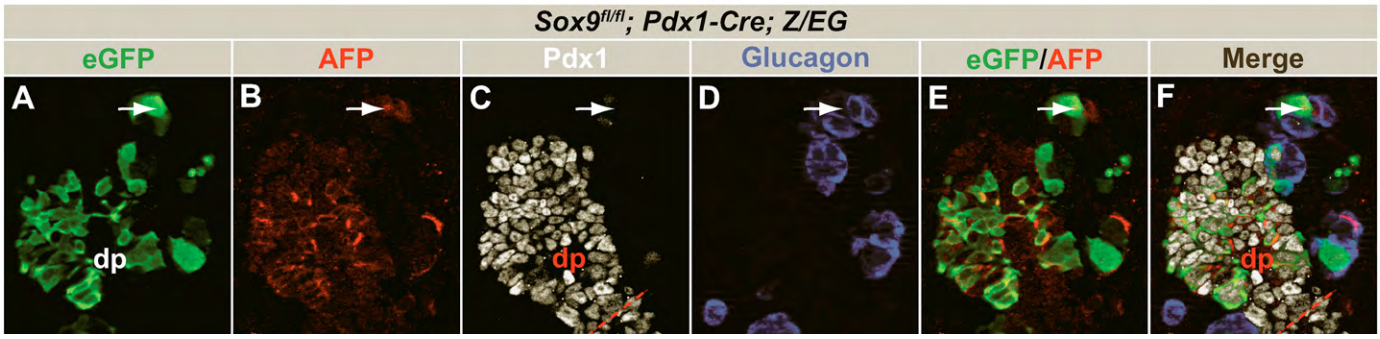

Fig. S2. AFP$^+$ cells arise from Pdx1$^+$ progenitors in *Sox9*-deficient pancreata. (**A-F**) eGFP induced by *Pdx1-Cre*-driven recombination of the *Z/EG* reporter allele (A) labels Pdx1$^+$ cells and their progeny in the dorsal pancreas (dp) (A,F). AFP$^+$ cells (B), including AFP$^+$ Pdx1$^-$ cells (arrows in A-F), are eGFP$^+$, showing their derivation from *Pdx1*-expressing pancreatic progenitors in *Sox9*-deleted pancreata (E,F). Most first-wave glucagon$^+$ endocrine cells (D) are also eGFP$^+$ and are thus derived from *Sox9*-deleted pancreatic progenitors (F). Broken line indicates the boundary between dorsal pancreas and duodenum (duo). Scale bar: 20 µm.

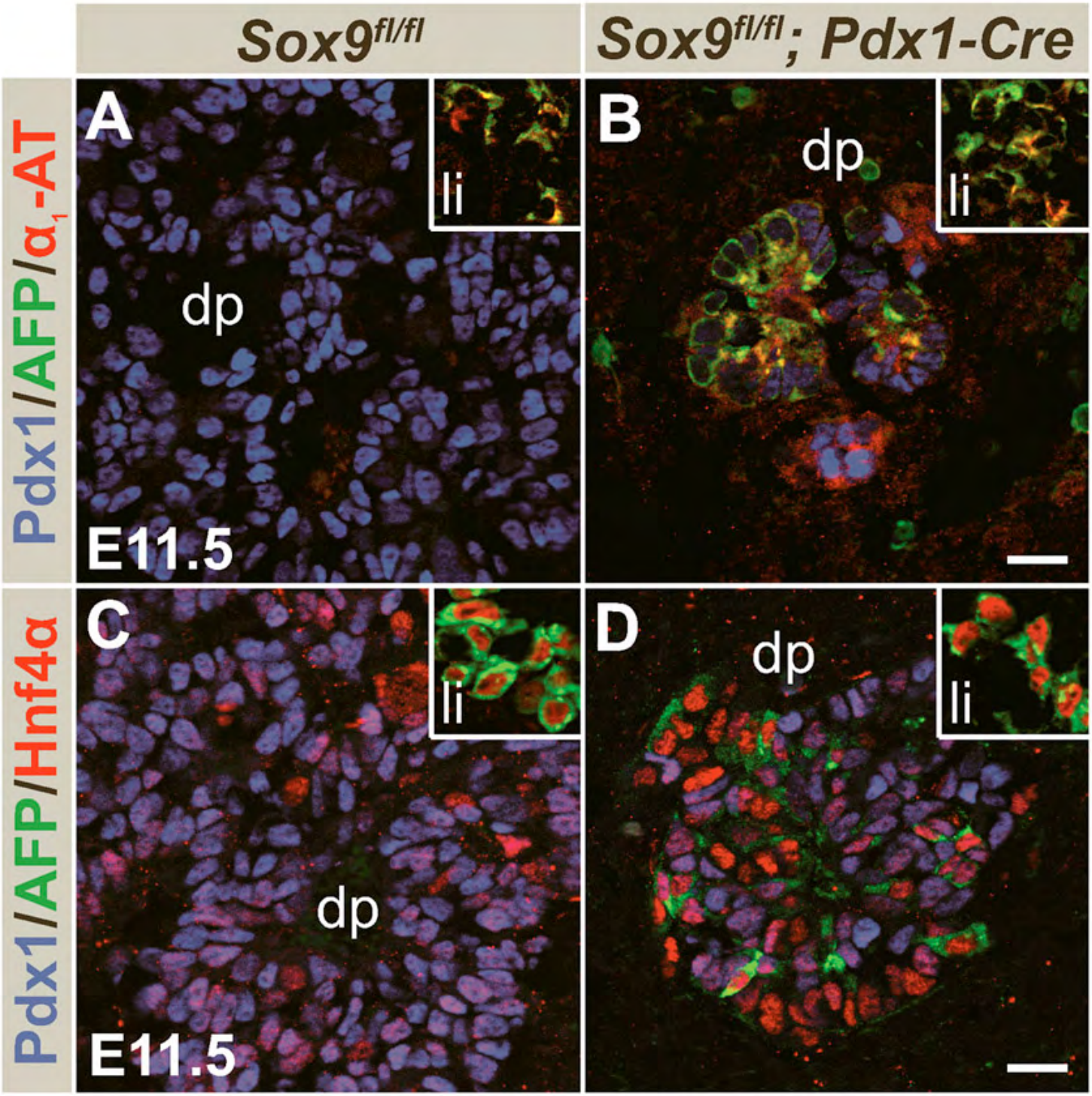

Fig. S3. *Sox9*-deleted pancreatic progenitors activate a bona fide hepatic program. (**A**,**B**) Immunodetection reveals almost complete absence of AFP or α_1-antitrypsin (α_1-AT) in the E11.5 control *Sox9$^{fl/fl}$* dorsal pancreas (dp) (A), but abundant expression in *Sox9$^{fl/fl}$; Pdx1-Cre* pancreas (B). (**C**,**D**) Although low-level Hnf4α nuclear expression is seen in Pdx1$^+$ pancreatic progenitors in E11.5 control pancreas (C), high-level Hnf4α expression is evident in AFP$^+$ cells of *Sox9$^{fl/fl}$; Pdx1-Cre* pancreas (D), similar to that seen in the liver (li) (C,D). Scale bars: 20 μm.

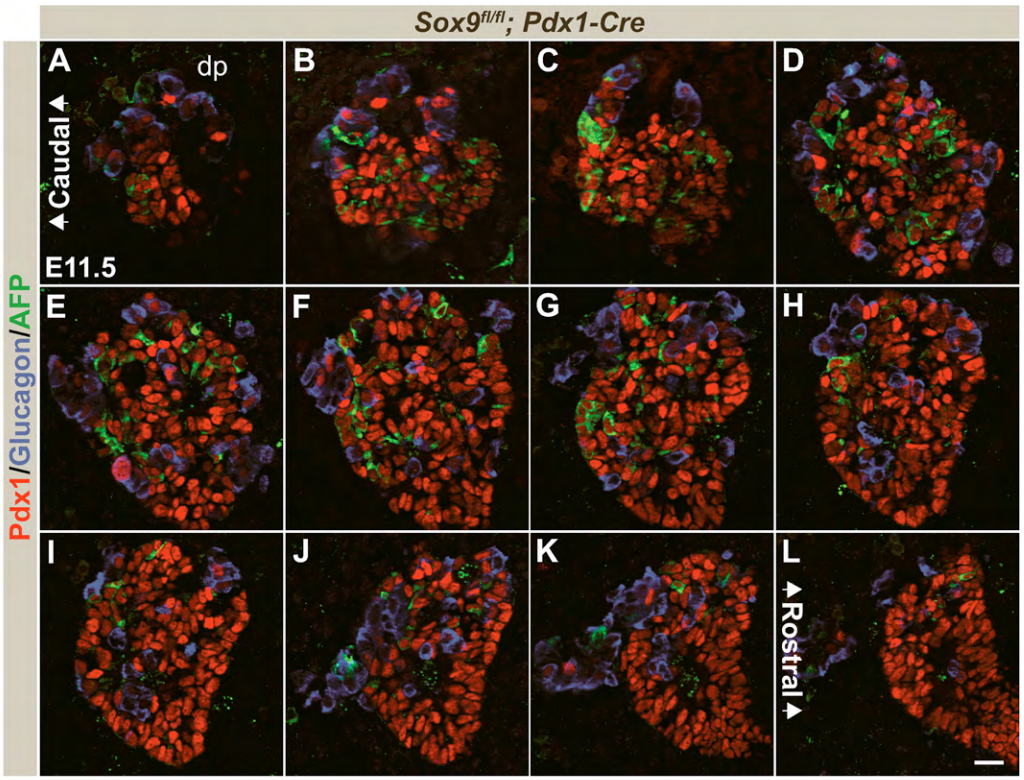

Fig. S4. Variable ratios of mutually exclusive AFP⁺ and glucagon⁺ populations. (**A-L**) Immunodetection on 12 sequentially ordered (rostral to caudal) serial sections through a single E11.5 *Sox9$^{fl/fl}$; Pdx1-Cre* dorsal pancreas (dp) shows both glucagon⁺ cells and AFP⁺ cells to occur in clusters. Glucagon and AFP expression are mutually exclusive. The ratio of glucagon⁺ cells:AFP⁺ cells:Pdx1⁺ cells varies greatly between different sections of the same *Sox9$^{fl/fl}$; Pdx1-Cre* pancreas. Scale bar: 20 µm.

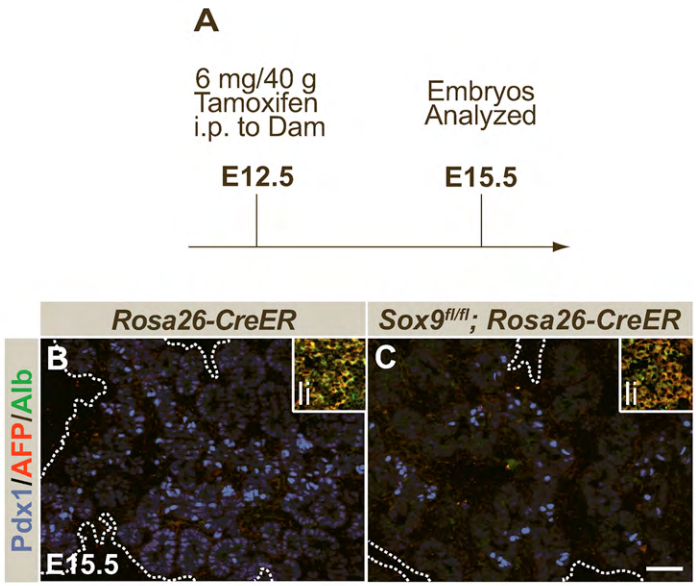

Fig. S5. Competence window for *Sox9*-deleted pancreatic progenitors to adopt hepatic fates closes by E12.5. (**A-C**) AFP⁺ or albumin⁺ cells are not detected in the E15.5 *Sox9$^{fl/fl}$; Rosa26-CreER* pancreatic epithelium (indicated by broken lines in B,C) after intraperitoneal (i.p.) tamoxifen-induced *Sox9* deletion at E12.5. li, liver. Scale bar: 50 µm.

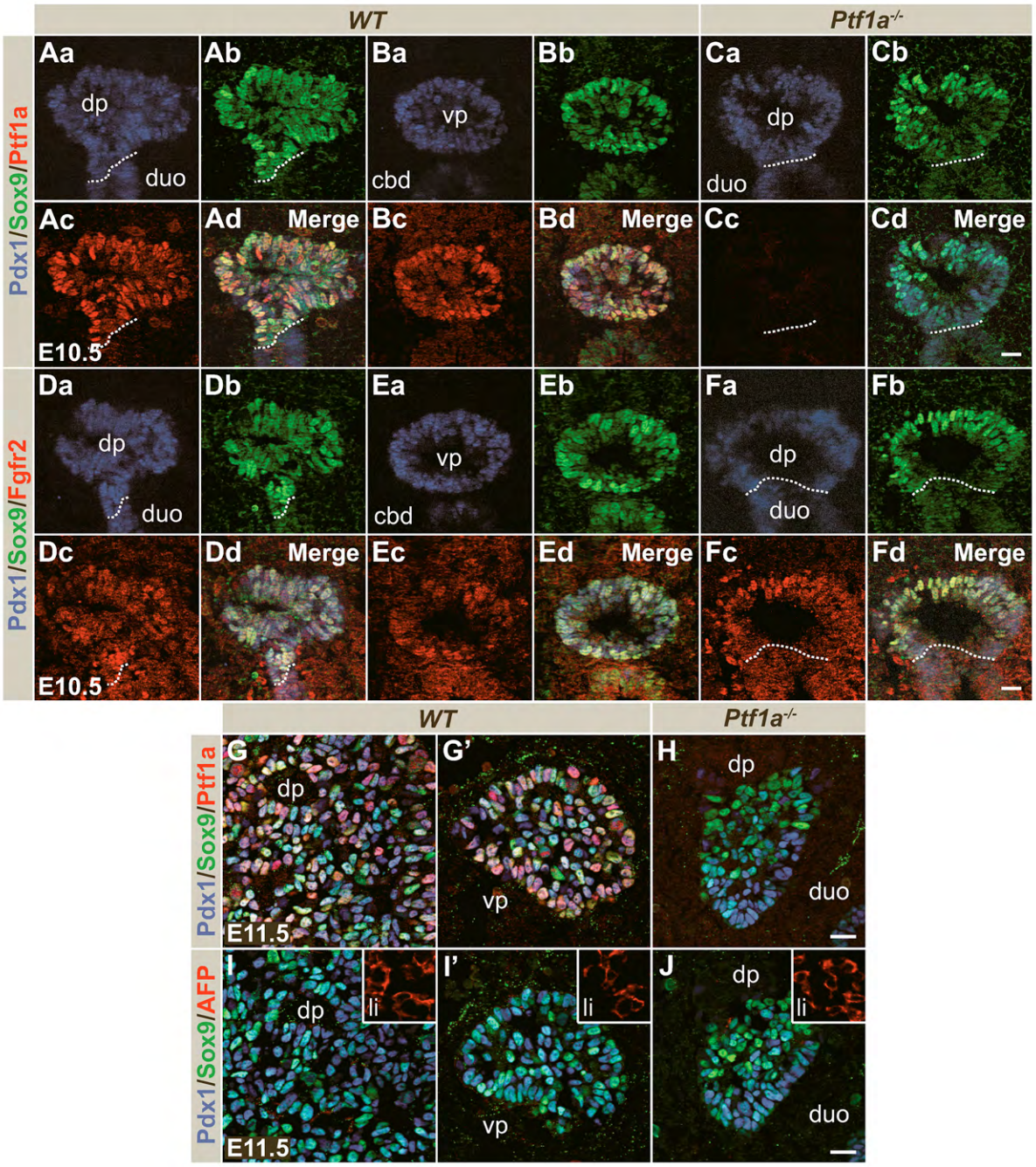

Fig. S6. *Ptf1a*-deficient pancreatic progenitors retain pancreatic identity. (**Aa-Cd**) Pancreatic progenitors in E10.5 wild-type (*WT*) dorsal (Aa-Ad) and ventral (Ba-Bd) pancreas express Pdx1, Sox9 and Ptf1a; both Pdx1 and Sox9 are expressed in dorsal pancreas of *Ptf1a*$^{-/-}$ mice (Ca-Cd). (**Da-Fd**) Like Pdx1 and Sox9, Fgfr2 expression persists in the *Ptf1a*$^{-/-}$ dorsal pancreas (Fa-Fd) as in dorsal (Da-Dd) and ventral (Ea-Ed) wild-type pancreata. (**G-J**) At E11.5, both Sox9 and Pdx1 are maintained in *Ptf1a*$^{-/-}$ dorsal pancreas (H,J), as in wild-type dorsal (G,I) and ventral (G′,I′) pancreata. AFP is not detected in E11.5 wild-type dorsal (I) and ventral (I′) pancreata or dorsal pancreas of *Ptf1a*$^{-/-}$ mice (J). *Ptf1a*$^{-/-}$ ventral pancreas is not shown as it is histologically undetectable by E11.5. Broken line demarcates the boundary between the dorsal pancreas and duodenum (Aa-Ad,Ca-Cd,Da-Dd,Fa-Fd). dp, dorsal pancreas; vp, ventral pancreas; duo, duodenum; cbd, common bile duct; li, liver. Scale bars: 20 μm.

Table S1. Sequences of primers used for mRNA quantification by qRT-PCR

Primer	Forward	Reverse
Sox9	5'-GAGCCGGATCTGAAGAGGGA-3'	5'-GCTTGACGTGTGGCTTGTTC-3'
AFP	5'-CTTCCCTCATCCTCCTGCTAC-3'	5'-ACAAACTGGGTAAAGGTGATGG-3'
Albumin	5'-TGCTTTTTCCAGGGGTGTGTT-3'	5'-TTACTTCCTGCACTAATTTGGCA-3'
Transferrin	5'-TGGGGGTTGGGTGTACGAT-3'	5'-AGCGTAGTAGTAGGTCTGTGG-3'
G-6-P	5'-CGACTCGCTATCTCCAAGTGA-3'	5'-GTTGAACCAGTCTCCGACCA-3'
Fgfr2b	5'-CCCATCCTCCAAGCTGGACTG-3'	5'-CAGAGCCAGCACTTCTGCATTG-3'
Fgfr2c	5'-CCCATCCTCCAAGCTGGACTG-3'	5'-TCTCACAGGCGCTGGCAGAAC-3'
Fgfr4	5'-TTGGCCCTGTTGAGCATCTTT-3'	5'-GCCCTCTTTGTACCAGTGACG-3'