ESM METHODS

In Situ Hybridization

Tissue was harvested and immediately fixed in 4% (wt./vol.) PFA in PBS. Samples were embedded in RNAse-free Tissue-Tek OCT as described above. RNAseZap (Life Technologies) was utilized to clean cryostat and all equipment used to obtain 20 µm sections, which were stored immediately at -80°C. Slides were removed from freezer and dried at room temperature for 20 minutes. Slides were then fixed in 4% PFA in PBS for 20 minutes; slides were then washed and treated for 10 minutes with 5µg/ml proteinase K (Roche; Laval QC, Canada) in 50mM Tris, 5mM EDTA. Slides were then post fixed for 15 minutes in 4% PFA and acetylated for 10 minutes at RT. Slides were the washed in PBS and prehybridized for 2 hours at 65°C in 50% (vol./vol.) deionized formamide, 5X SSC, 5X Denhardt’s, 400 µg/ml salmon sperm DNA (all Life Technologies), 250 µg/ml extracted yeast tRNA, 50 µg/ml Heparin (Sigma), 2.5 mM EDTA, 0.1% (vol./vol.) Tween-20, and 0.25% (wt./vol.) CHAPS (Fisher). Probes were synthesized from PCR amplified DNA generated from mouse Sox4 or pro-glucagon coding sequences and digoxigenin labeled (DIG-11-UTP; Roche) using the Maxiscript in vitro transcription kit, T7 or SP6 RNA polymerase and the manufacturer’s protocol (Life Technologies). Probes were diluted to 1ng/µL in prehybridization buffer and hybridized overnight at 65°C. The next day slides were briefly washed in 5x SSC, followed by 3x 20 minute washes in 0.2x SSC at 65°C. Slides were briefly washed with PBS, and permeabilized and blocked with 0.1% (vol./vol.) Triton X-100, 1% (vol./vol.) goat serum in PBS for 2 hours at room temperature. Sections were incubated overnight at 4°C with 1:2500 alkaline phosphatase conjugated sheep anti-DIG antibody (Roche; Fab fragments) and then washed extensively
in PBS and then alkaline phosphatase buffer (100mM Tris pH 9.5, 50mM MgCl$_2$, 100mM NaCl, 0.1% (vol./vol.) Tween; 10 minutes) prior to alkaline phosphatase staining using NBT/BCIP ready-to-use tablets (Roche). Imaging was carried out in the CFRI Diabetes Group Imaging Core using an Olympus BX61.

**TUNEL Staining**

*In situ* Cell Death Detection Kit, Fluorescein (Roche) used according to manufacturer’s recommended protocol. Fixed cryosectioned tissue were permeabilized in 0.2% (vol./vol.) Triton X-100 in PBS for 10 minutes and then washed in PBS twice for 5 minutes each. Sections then incubated in TUNEL reaction mixture for 1 hour in the dark at 37°C in a pre-warmed humidified chamber. Slides then washed 2 times in PBS for 5 minutes each and followed by immunostaining or mounted for microscope evaluation. Positive control sections created by incubation in DNaseI recombinant for 10 minutes (3U/mL in 50mM Tris-HCl pH 7.5) prior to labeling reaction.

**EdU Staining**

EdU labeling was performed by IP injection of 1mg EdU, 12-hours prior to sacrifice. Tissues was embedded and sectioned then permeabilized in 0.3% (vol./vol.) Triton X-100 in PBS for 15min/RT. Sections were then washed in PBS and incubated in 100mM Tris pH 8.5, 1mM CuSO$_4$, 30μM Alexa 594-conjugated Azide (Life Technologies), 100mM Ascorbate for 1 hour in the dark [3]. Sections were then washed with PBS and immunostained.
Gene targeting in mPAC-L20 cells

Transcriptional Activator Like Effector Nucleases (TALEN)s were generated in house using the protocol and TALE toolbox (pTALEN_v2) ([1]). Guanine binding was encoded by the repeat-variable diresidue Asn-His (NH) as described [2]. TALEN binding sites flanked the stop codon of the Sox4 gene with the forward TALEN designed to bind to the sequence: 5’-TCTCTAACCTGGTCTTCACC-3’ and the reverse TALEN to the sequence: 5’-TGGCCCACCTTCTCCCAGGC-3’. This creates a doubled stranded break which can be resolved through homology-directed repair with our targeting vector (pSox4HA-2AGLoxPP)designed with 600bp homology arms and a hemagglutinin (HA) included at the C-terminus of Sox4 to replace the stop codon. Homologous recombination with the vector inserts a porcine teschovirus 2A peptide fused in frame with Sox4HA on its N-terminal side and eGFP on its C-terminal side, which also included a stop codon. Selection for positively targeted clones was facilitated by a LoxP flanked PGK-Puro cassette that was situated downstream of the eGFP but upstream of the 3’ homology arm.

Gene targeting was carried out by co-transfecting 1.5μg of each TALEN with 3μg of intact pSox4HA-2AGLoxPP in a 1 well of a 6-well plate with 12μl of Lipofectamine 2000 (Life Technologies) in 1ml total volume overnight. The next day media was changed and 48 hours following the transfection, selection was initiated with 3μg/ml puromycin. After 7 days of selection, clones were picked, plated in a 96-well plate and then 96-well plates were replica plated prior to genotyping. Cells were lysed in QuickExtract DNA extraction solution using the manufacturer’s protocol (Epicentre Biotechnologies; Madison WI, USA) and then genotyping carried out using PCR across the homology arms. Sequencing across homology arms confirmed in-frame knock-in.
Approximately 23% of screened clones were properly targeted. The PGK-Puro cassette was excised by infecting the correctly targeted cells with an adenovirus expressing Cre recombinase as described above (Vectorbiolabs; Philadelphia PA, USA). All experiments reported here were carried out using mPAC-L20-Sox4HA-2AGFP clone A8.

**Human Embryonic Stem Cell Culture**

CyT49 human embryonic stem cells (hESCs) [4] were cultured on mitomycin C treated mouse embryonic fibroblasts using a growth media of DMEM/F12 (Cellgro; Manassas, VA, USA) containing 10% Xeno-free KnockOut Serum Replacement (Life Technologies), 1% non-essential amino acids, 1% GlutaMAX, 1% penicillin/streptomycin (pen/strep) (Life Technologies) and 0.1 mM 2-mercaptoethanol (Sigma) supplemented with 10 ng/mL heregulin-1β (Peprotech; Quebec, Canada) and 10 ng/mL activinA (R&D) that was changed daily. hESCs were passaged twice-weekly and plated at a density of 0.5x10^6 or 1x10^6 per 35-mm or 60-mm dish, respectively. Cells were differentiated using a modified protocol and adherent cell cultures [5]. Briefly, to form definitive endoderm cells were cultured for three days in RPMI (Hyclone), 1% GlutaMAX (Life Technologies), 0.5% penicillin/streptomycin (Life Technologies). For the first 24 hours media was supplemented with 100 ng/mL activin A (e-bioscience), 25 ng/mL Wnt3a (R&D), 1:5000 insulin-transferrin-selenium (ITS) (Gibco), followed by 48 hours with 100 ng/mL activin A, 1:5000 ITS and 0.2% dFBS (Life Technologies). To induce primitive gut tube, cells were cultured in RPMI with 0.5% pen/strep, 1% GlutaMAX supplemented for the first 24 hours with 2.5 μM TGF-β RI kinase inhibitor IV (EMD Bioscience; Billerica, MA, USA), 25 ng/mL KGF (R&D Systems; Minneapolis, MN, USA), 1:1000 ITS, 0.2% dFBS followed by 48 hour culture in the
same media without TGF-β RI kinase inhibitor IV. For formation of posterior foregut cells were cultured for 72 hours in DMEM-High Glucose (Hyclone) with 0.5x B27 (Life Technologies), 1% GlutaMAX, 0.5% pen/strep and supplemented with 0.25 μM KAAD-Cyclopamine (Toronto Research Chemicals; Toronto, ON, Canada), 2 nM TTNPB (Sigma), and 50 ng/mL Noggin (R&D Systems). Finally, to produce pancreatic progenitors cells were cultured for 72 hours in DMEM-HG with 0.5x B27, 1% GlutaMAX, 0.5% pen/strep supplemented with 50 ng/mL Noggin, 50 ng/mL KGF, and 50 ng/mL EGF (R&D Systems).

**Fluorescence Activated Cell Sorting**

Mouse E17.5 embryos were harvested and immediately dissected on ice to obtain pancreases that were placed in 2mL of 0.25% Trypsin for 20 minutes with periodic agitation to homogenize the tissue. 1mL of cold FBS and 2mL of cold PBS were added and mixed to stop digestion. The solution was filtered through a 40μm nylon filter and centrifuged at 200g for 5 minutes at 4C. Supernatant was aspirated and the cell pellet was resuspended in 2mL of cold PBS by pipette to wash the cells. Cells were centrifuged again at 200g for 5 minutes at 4C and supernatant aspirated. Cells were resuspended in 350μL of 2% (vol./vol.) FBS in PBS and placed on ice for sorting by BD FACS Aria II.

**ESM References**


