Experimental Procedures

Mouse Strains and Embryo Collection

All mice were held at the British Columbia Children's Hospital Research Institute Animal Care Facility and ethical procedures were followed according to protocols approved by the University of British Columbia Animal Care Committee. All mice were maintained on a regular chow diet *ad libitum* and housed up to 5 mice per cage on a 12-hour light/dark cycle. Timed matings were used to determine embryonic stages and the morning of vaginal plug discovery was considered embryonic day 0.5 (E0.5). Previously generated *Dpy30*$^{flox/flox}$ mice (22) were crossed to *Neurog3*-Cre driver mice (23) to obtain conditional deletion of *Dpy30* exon 4 in endocrine progenitors. In all studies, knockout mice (*Dpy30ΔN*; *Neurog3*-Cre; *Dpy30*$^{flox/flox}$) were compared to Cre-negative littermate controls (*Dpy30*$^{flox/flox}$ or *Dpy30*$^{flox/wt}$). At noon on the day of the experimentally determined time point (e.g., E14.5), embryos were harvested by hysterectomy, dissected under an Olympus dissecting microscope in ice-cold phosphate buffered saline (PBS) and tail clippings were taken for genotyping. All knockout embryos were stained for DPY30 and/or H3K4 methylation to determine recombination efficiency prior to further analysis. Embryos with insufficient loss of DPY30 or H3K4 methylation (< 80%) were not studied. For all experiments, sex-specific differences were not anticipated and embryo sex was not determined.

Pancreas and Islet Isolations

Mice were anesthetized with isoflurane inhalation and checked for toe pinch reflexes prior to euthanasia by cervical dislocation. To open the chest cavity, two lateral incisions were made in the abdomen away from the midline, followed by medial incisions up and down the midline and through the ribcage and diaphragm. The pancreas was dissected by cutting all connections to

the spleen, stomach, intestine and liver before placing the tissue directly into 4% PFA in PBS on ice, followed by fixation overnight at 4°C.

For islet isolations, mice were euthanized by decapitation and blood was drained. The common bile duct was clamped at the duodenum and a 30G needle was used for perfusion of 3 mL Collagenase XI (1000 U/mL, Sigma-Aldrich) in Hank's Balanced Salt Solution (HBSS) into the common bile duct. The pancreas was dissected and further enzymatically digested in an additional 2 mL Collagenase XI for 15 minutes in a 40°C water bath before 3 minutes of mechanical shaking. Pancreas tissue was washed twice with 25 mL HBSS with 1 mM $CaCl_2$ before filtering through a 40 or 70 μm cell strainer (depending on mouse age). Islets collected on the strainer were inverted and rinsed into a petri dish with RPMI 1640 media (11 mM D-glucose) supplemented with 10% FBS, 50 U/mL penicillin/streptomycin and 2 mM L-glutamine, and either recovered overnight at 37°C in a 5% CO_2 humidified incubator or immediately handpicked for experiments under a dissecting microscope.

Tissue Processing and Histology

Embryonic and pancreatic tissue was fixed at 4°C in 4% PFA overnight (whole embryos) or for 5 hours (dissected stomach, pancreas, spleen and intestine ≥ E15.5). All tissue was processed through a series of graded ethanol and xylene de-hydration steps before embedding in paraffin wax. Briefly, fixed embryos and tissues were washed 3x 10 minutes in PBS and processed in cassettes through 50% ethanol (embryos only), 70% ethanol, 2x 30 minutes of 95% and 100% ethanol, 2x 30 minutes of xylene, followed by 2x 1 hour in melted paraffin. Embryonic or pancreatic tissue was cut into 5 μm sagittal sections using a Microtome and mounted onto Superfrost Plus slides.

Immunostaining, Imaging and Analysis

Paraffin slides were processed through graded xylene and ethanol re-hydration steps, followed by a 10 minute heat-induced antigen retrieval (10 mM sodium citrate, pH 6.0) at 95°C and 1 hour blocking (5% FBS in PBS) at room temperature. Briefly, slides were processed through 3x 5 minutes xylene, 2x 5 minutes 100% ethanol, 5 minutes 95% ethanol, 5 minutes 70% ethanol and 10 minutes in PBS on a shaker prior to antigen retrieval. Slides were cooled for 5 minutes under cold running tap water and washed for 5 minutes each in de-ionized water and PBS on a shaker before circumscribing with a Super PAP pen (Thermo Fisher Scientific) and blocking. Primary antibodies were incubated at 4°C overnight with dilutions in blocking solution (see Table below for antibody information). The following day, slides were washed 3x 10 minutes in PBS and incubated with secondary antibodies in PBS for 1 hour at room temperature in a humidified dark chamber. Finally, slides were washed 3x 10 minutes in PBS before mounting with Prolong Gold mounting solution. Slides were imaged on a Leica TCS SP8 Confocal microscope or tiled on an Olympus Bx61 microscope and analyzed using CellProfiler software with custom pipelines.

Antibody	Host Species	Manufacturer	Catalog #	Dilution
Chromogranin A	Mouse	Santa Cruz	sc-393941	1:100
Chromogranin A	Goat	Santa Cruz	sc-1488	1:100
DPY30	Rabbit	Abcam	ab214010	1:500
DPY30	Rabbit	Bethyl	A304-296A	1:10,000
DPY30	Rabbit	Atlas Antibodies	HPA043761	1:1000
Gastrin	Goat	Santa Cruz	sc-7783	1:50
Glucagon	Rabbit	Santa Cruz	sc-13091	1:500
Glucagon	Mouse	Sigma-Aldrich	G6254	1:2000
GLP1R	Mouse	DSHB	7F38-s	4 ug/mL
GLUT2	Goat	Santa Cruz	sc-7580	1:200
H3K4me1	Rabbit	Abcam	ab8895	1:1000
H3K4me3	Rabbit	Abcam	ab8580	1:1000
H3K4me3	Rabbit	Cell Signaling	C42D8	1:1000
Insulin	Guinea Pig	Abcam	ab7842	1:1000
Insulin	Guinea Pig	Agilent	IR-002	1:4
NEUROG3	Mouse	DSHB	F25A1B3-c	1:100
Somatostatin	Goat	Santa Cruz	sc-7819	1:1000

Antibody	Host Species	Manufacturer	Catalog #	Dilution
SOX9	Rabbit	Millipore	AB5535	1:2000
DAPI		Thermo Fisher Scientific	D9542	1:5000
TOPRO-3		Thermo Fisher Scientific	T3605	1:5000
Alexa Fluor 488 anti-Rabbit	Donkey	Thermo Fisher Scientific	A-21206	1:500
Alexa Fluor 488 anti-Goat	Donkey	Thermo Fisher Scientific	A-11055	1:500
Alexa Fluor 488 anti-Mouse	Donkey	Thermo Fisher Scientific	A-21202	1:500
Alexa Fluor 546 anti-Mouse	Goat	Thermo Fisher Scientific	A-11003	1:500
Alexa Fluor 546 anti-Goat	Donkey	Thermo Fisher Scientific	A-11056	1:500
Alexa Fluor 555 anti-Guinea Pig	Goat	Thermo Fisher Scientific	A-21435	1:500
Alexa Fluor 594 anti-Rabbit	Donkey	Thermo Fisher Scientific	A-21207	1:500
Alexa Fluor 647 anti-Mouse	Donkey	Thermo Fisher Scientific	A-31571	1:500

Morphometric and Intensity analysis

Cell quantifications were determined by taking serial sections at set intervals throughout the entire embryonic (every 30-60 μm) and postnatal (every 90 μm) pancreas. At least 10 images per replicate were captured on a Leica TCS SP8 Confocal microscope. Images were analyzed using CellProfiler software to quantify relative cell fractions or H3K4me3 immunostaining intensity. The number of NEUROG3^{+} nuclei was determined manually. Total CHGA^{+} area in the P24 pancreas was determined from staining islets for CHGA on serial sections through the entire pancreas spaced 90 μm apart (50-100 total islets). Images were tiled on an Olympus Bx61 microscope and analyzed relative to total pancreatic area using CellProfiler.

Western Blot Analysis

Islets were lysed for 5 minutes at 95°C in Laemmli sample buffer (2% SDS, 10% glycerol, 60 mM Tris pH 6.8) containing 1 mM NaF, 1 mM PMSF and protease inhibitor cocktail (Roche).

The Pierce BCA kit (Thermo Fisher Scientific) was used to determine lysate total protein concentrations and 5 μg protein was re-boiled for 5 minutes with 5% β-mercaptoethanol. Proteins were separated on 4-15% acrylamide gels and transferred for 60 minutes at 12.0 V to PVDF membranes (Millipore). Membranes were blocked in 5% BSA in TBS (20 mM Tris pH 7.6, 150 mM NaCl) containing 0.1% Tween-20 (TBS-T) for 1 hour at room temperature before overnight incubation at 4°C with primary antibodies in 5% BSA/TBS-T. Primary antibodies were as follows: mouse anti-actin (DSHB, catalog # JLA20-c, 1:2500) and rabbit anti-H3K4me3 (CST, catalog # 9751, 1:1000). Membranes were washed 3x 10 minutes in TBS-T before incubated in secondary antibodies for 1 hour at room temperature. Secondary antibodies were as follows: HRP-conjugated anti-rabbit IgG (CST, catalog # 7074, 1:10,000) and HRP-conjugated anti-mouse IgG (Jackson Immunoresearch, catalog # 115-035-174 1:10,000). Membranes were washed 3x 10 minutes in TBS-T and signals were detected using ECL reagent and radiographic film. Antibodies were then stripped in mild stripping buffer (200 mM glycine 0.1% SDS, 1% Tween-20, pH 2.2) for 20 minutes at room temperature and washed 4x 10 minutes in TBS-T. The membrane was re-blocked and probed with rabbit anti-H3K4me1 (Abcam, catalog # 8895, 1:100,000), then re-stripped, blocked and probed with rabbit anti-H3 (Abcam, catalog # ab1791, 1:250,000). The resulting signals were quantified using ImageJ.

Blood Glucose and Islet Insulin Measurements

Mice were monitored after weaning at P21 for body mass and non-fasting blood glucose by a OneTouch® Ultra® 2 glucometer. For intraperitoneal glucose tolerance tests (IPGTTs), mice were fasted for 6 hours prior to IP injection of 2 g/kg 20% D-glucose (Sigma-Aldrich) in water with a 26G needle. Blood glucose measurements were obtained from the tail vein prior to injection (T_0) and 15, 30, 60 and 120 minutes after injection. For islet insulin content, isolated islets were lysed in RIPA buffer (25 mM Tris pH 7.6, 150 mM NaCl, 1% NP40, 1% sodium

deoxycholate and 0.1% SDS) for 5 minutes at 95°C. Islet insulin content was determined from the lysate by the Mouse Ultrasensitive Insulin ELISA kit (ALPCO) and a Spectramax 190 plate reader (Molecular Devices). Insulin measurements were normalized to islet lysate DNA concentration determined by the Qubit dsDNA HS Assay (Thermo Fisher Scientific).

For glucose-stimulated insulin secretion (GSIS) assays, islets from P24 control and *Dpy30ΔN* mice were isolated (as above) and allowed to recover overnight in low glucose RPMI. GSIS was performed in technical duplicates with 40 islets per mouse (n=3-5 animals). Islets were preincubated with KRBH buffer (2.8 mM glucose, 20 mM HEPES, 114 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 24 mM $NaHCO_3$ and 0.1% BSA) for 45 minutes in 5% CO_2 at 37°C. Islets were consecutively incubated for 45 minutes in KRBH buffer supplemented with 2.8 mM glucose, 16.7 mM glucose and finally 30 mM KCl. After each incubation period the conditioned media was collected. After the final incubation, the islets were lysed using RIPA buffer at 95°C for 5 minutes. Insulin concentrations were measured using the STELLUX® Chemi Rodent Insulin ELISA and normalized to DNA concentration of islet lysate determined by the Qubit dsDNA HS Assay (Thermo Fisher Scientific).

RNA Extraction and qPCR Analysis

RNA was extracted from lysed cells by pipetting in TRIzol reagent (Thermo Fisher Scientific) and combined with 1/5 the total volume of chloroform. Samples were inverted 10x before centrifugation at 12,000xg for 15 minutes at 4°C. The aqueous layer was mixed with an equal volume of ice-cold 70% ethanol and further processed with the PureLink RNA Mini Kit (Ambion). Complementary DNA (cDNA) was generated using SuperScript III Reverse Transcriptase (Thermo Fisher Scientific) and qPCR experiments were carried out using 0.25-2 μL of cDNA per reaction. Both Fast SYBR Green and TaqMan chemistry (Thermo Fisher Scientific) was used with purchased or custom designed primers (Primer3) to detect exon-intron boundaries.

Samples were loaded onto 384-well plates in triplicate and run on a ViiA 7 Real-Time PCR system (Thermo Fisher Scientific) where gene expression was normalized to β-actin and determined using the $\Delta\Delta C_t$ formula.

RNA-Sequencing Analysis

Islets from 3 control and 3 *Dpy30ΔN* male mice were isolated at P24 and 100-200 islets per mouse were handpicked into 1 mL of TRIzol reagent (Thermo Fisher Scientific). Extracted RNA was treated with TURBO DNase I (Thermo Fisher Scientific) for 20 minutes at 37°C to remove genomic DNA. For each sample at P24, mRNA was enriched from 1000 ng of total RNA using the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB) and 6 cDNA libraries were prepared with the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB) with 9 amplification cycles. Indexed libraries were analyzed for size distribution using the Agilent Bioanalyzer High Sensitivity DNA chip and quantified using the Qubit dsDNA HS Assay (Thermo Fisher Scientific). Pooled libraries were sequenced on an Illumina NextSeq500 platform for 2 × 43 nucleotide paired-end reads.

For analysis of islet RNA-seq libraries, the resulting FastQ sequencing files were combined, filtered (phred33) and trimmed to remove adapters with Trimmomatic (24). Salmon (25) was used to quasi-map, assemble and quantify transcripts using the mouse GRCm38/mm10/VM24 transcriptome. Differential gene expression analysis was performed using DEseq2 (26) in R, with the apeglm algorithm used to shrink log fold changes (51). Downregulated, upregulated and stables genes were determined (Table S1), and gene set enrichment analysis was performed on downregulated (Table S2 and S3) and upregulated (Table S4 and S5) genes with g:Profiler (27).

ChIP-Sequencing Analysis

We used published ChIP-seq data (H3K4me3, H3K4me1, H3K27ac and H3K27me3) from E13.5 NEUROG3^{HI} and adult islets (13,30) and previously published transcription factor binding data for FOXA2, MAFA, PDX1 and NEUROD1 (13), and for NKX2-2 (31), NKX6-1 (32), PAX6 (33), RFX6 (34). All data were mapped with bowtie2 to the GRCm38/mm10/VM24 genome and peaks for the transcription factors called with MACS2. Using Repitools we plotted the distribution of each histone mark at the transcriptional start sites (TSS, ± 3 kb) of the differentially expressed gene sets. We used ChromHMM (35) using a *k*-value of 10 to characterize the chromatin states of *Neurog3*^{HI} and islet cells. Chromatin states were annotated by the presence of active (H3K27ac, H3K4me3 and/or H3K4me1), repressed (H3K27me3) and bivalent (active and repressed) histone marks or unmarked histones. Using these data, to determine the chromatin state of each promoter we first, determined the percent coverage of each state at the TSS (± 2 kb) of the differentially expressed gene sets using the coverage function of bedtools2 (version 2.29.2). Chromatin states that covered < 25% of a TSS were removed. We then used the remaining states that covered > 25% of the TSS for annotation. If multiple states covered > 25% of the promoter, preference was given to the state in the following order: bivalent (H3K27me3 and H3K4me3); repressed (H3K27me3); active (H3K27ac, H3K4me3 and H3K4me1); active (H3K27ac and H3K4me3); active (H3K27ac and H3K4me1); poised (H3K4me1); and unmarked. Genes associated with each chromatin state are defined in Table S6. The number of genes in each chromatin state were calculated as a fraction of total in each gene set (downregulated, upregulated and stable genes from P24 control and *Dpy30⊿N* islets). We determined the fraction of active and repressed chromatin at downregulated, upregulated and stable genes, and conducted a Fisher's exact test to compare the levels in *Neurog3*^{HI} cells and islets.

Statistics

Data are expressed as mean ± standard deviation (SD) unless otherwise specified and all experiments were carried out at minimum in triplicate. Statistical analyses were performed using GraphPad Prism 8 Software. Statistical significance was determined using unpaired, two-tailed Student's t-tests for comparisons between two groups, and two-way ANOVA with Sidak's multiple comparisons post-hoc tests were used for repeated measures comparisons between more than two groups (unless otherwise specified), with * indicating $P < 0.05$, ** indicating $P < 0.01$, *** indicating $P < 0.001$ and **** indicating $P < 0.0001$.

Data and Resource Availability

Data generated and/or analyzed during the current study are available from the GEO accession GSE174751 or from the corresponding author upon request.