Additional file 1
Supplemental information

ISL1 controls pancreatic alpha cell fate and beta cell maturation

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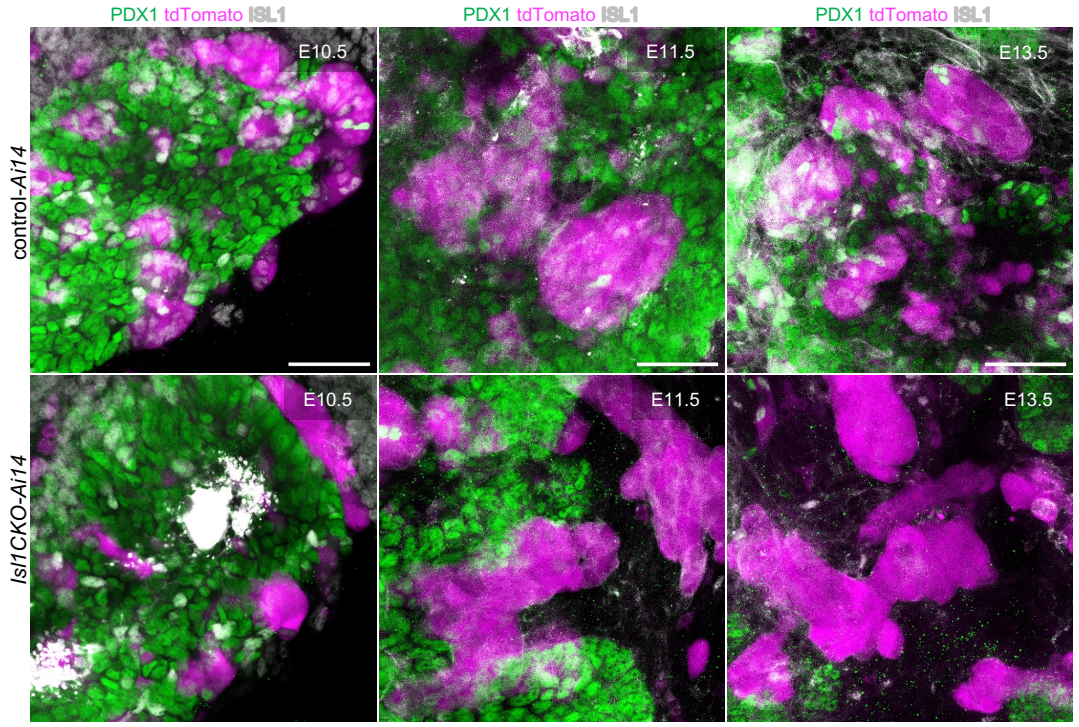


Fig. S1 Efficient deletion of ISL1 in the *Isl1CKO* developing pancreas. Representative whole-mount immunolabeling of the pancreas of tdTomato reporter control-*Ai14* and *Isl1CKO-Ai14* embryos during the primary transitions (E10.5 and E11.5) and the secondary transition (E13.5) shows ISL1 expression in the Neurod1^{Cre} positive domain visualized by tdTomato expression. The pancreatic epithelium is delineated by the expression of PDX1. Scale bars: 50 μm.

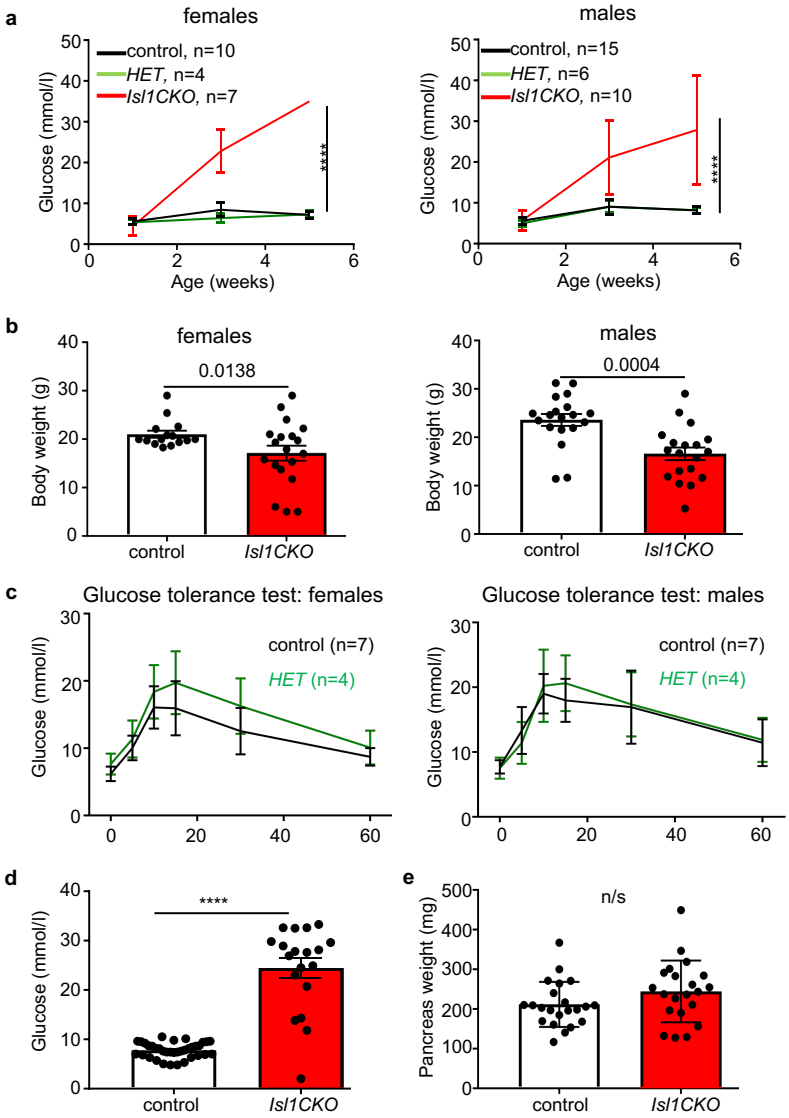


Fig. S2 Diabetic phenotype of *Isl1CKO*. **a** The average blood glucose levels over time (from 1 week to 5 weeks of age) in females and males mice fed *ad libitum.* The 5 weeks of age female mice had blood glucose unmeasurable (above 35 mmol/l), the 5 week of age males show a high variability with 7 mice with blood glucose unmeasurable, and 3 with 0.8, 7.9 and 31.9 mmol/l. Data are presented as mean ± SD, analyzed by two-way ANOVA (****$P < 0.0001$). **b** The average body weight of adult female and male mice. Data are presented as mean ± SEM, Student's *t* test. **c** Glucose tolerance test plotted using glucose vs time in heterozygous (*Neurod1*^{Cre}*/Isl1flox*$^{+/-}$), and control mice. Data are presented as mean ± SD, analyzed by two-way Anova with Bonferroni post-hoc analysis for glucose vs time. **d** Blood glucose concentration in adults fed *ad libitum*, 6-8 weeks of age. Only measurable levels of glucose (< 35 mmol/l) are shown for *Isl1CKO*. Data are presented as mean ± SEM, Student's *t* test (****$P < 0.0001$). **e** The weight of pancreas of adult mice. Total pancreas weights of 244 ± 78 mg (n = 22) in adult *Isl1CKO* mutants compared to those of controls (211 ± 57 mg, n = 21). Data are presented as mean ± SEM, Student's *t* test.

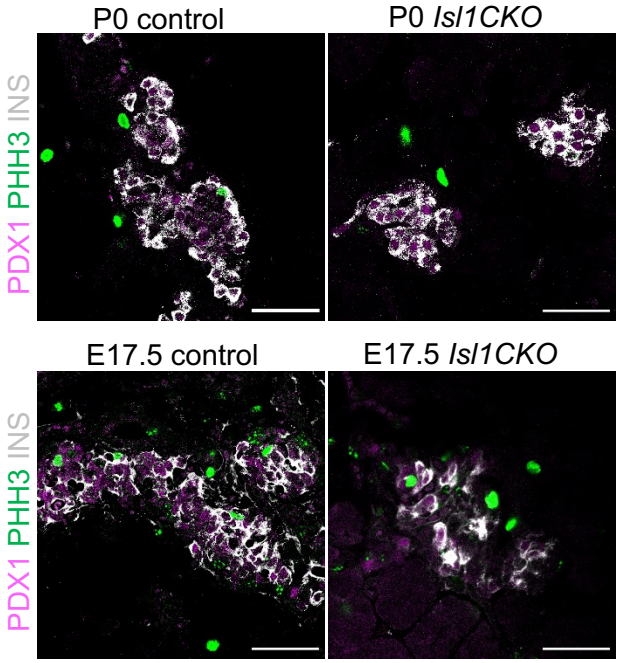
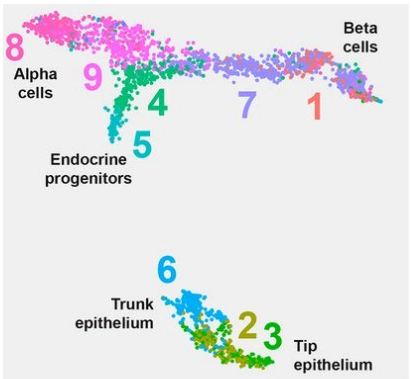
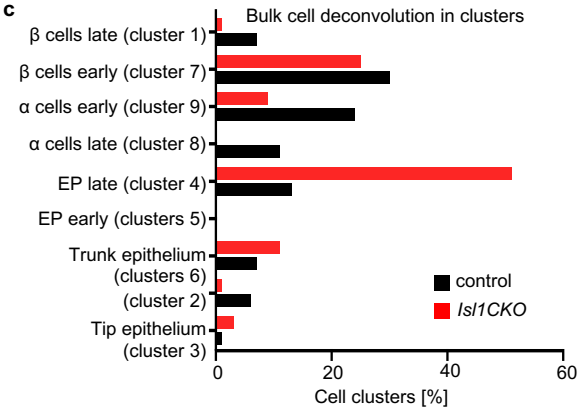


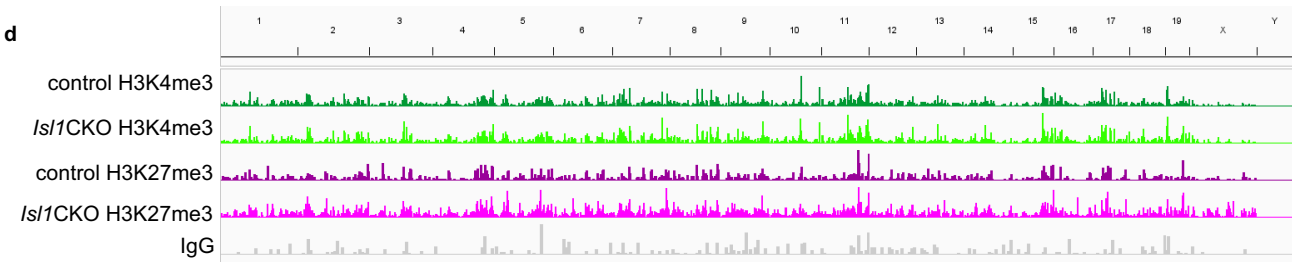
Fig. S3 Immunolabeling for the marker of cellular mitosis, phosphorylated histone H3 (pHH3). Representative sections from the control and *Isl1CKO* pancreas immunostained for insulin (INS) and PDX1 (marker of β cells) at P0 and E17.5. Scale bars: 50 μm.

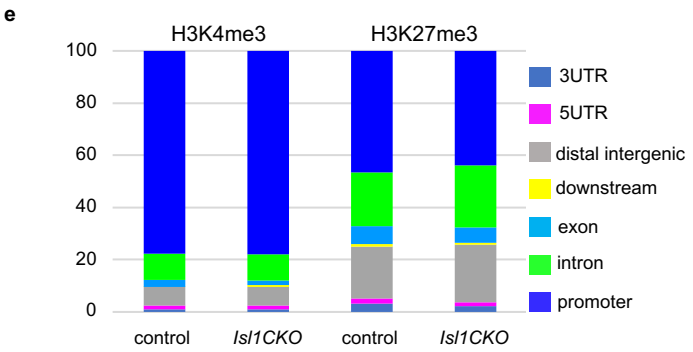
a

source	term_name	term_id	-log10 (p value)
downregulated genes			
REAC	Incretin synthesis, secretion, and inactivation	REAC:R-MMU-400508	7.01
REAC	Synthesis, secretion, and inactivation of Glucagon-like Peptide-1 (GLP-1)	REAC:R-MMU-381771	5.49
REAC	Synthesis, secretion, and inactivation of Glucose-dependent Insulinotropic Polypeptide (GIP)	REAC:R-MMU-400511	4.35
GO:MF	glucagon receptor binding	GO:0031769	2.87
GO:BP	cell-cell signaling	GO:0007267	2.68
GO:BP	peptide hormone secretion	GO:0030072	2.65
GO:BP	peptide secretion	GO:0002790	2.51
GO:BP	cell migration	GO:0016477	2.26
upregulated genes			
REAC	Post-translational protein phosphorylation	REAC:R-MMU-8957275	3.56
GO:BP	regulation of localization	GO:0032879	3.50
REAC	Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	REAC:R-MMU-381426	3.34
KEGG	Fatty acid elongation	KEGG:00062	2.88
GO:MF	phosphatidylcholine-sterol O-acyltransferase activator activity	GO:0060228	2.31
GO:MF	identical protein binding	GO:0042802	2.31
KEGG	Fatty acid metabolism	KEGG:01212	2.26









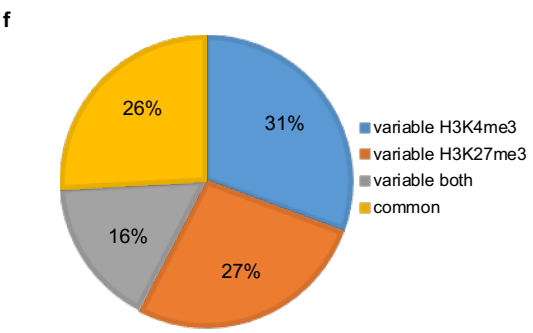


Fig. S4 H3K4me3 and H3K27me3 CUT&Tag-seq analyses of E14.5 pancreatic endocrine cells. **a** The functional enrichment analysis of the differentially expressed genes in *Isl1CKO* was performed using g: Profiler (Gene Ontology: MF, molecular function; BP, biological processes). **b** A UMAP overview of nine cell clusters of different pancreatic cell types used as a reference for the cell type deconvolution analysis (van Gurp et al., 2019). **c** The deconvolved cell cluster proportions in E14.5 endocrine population from our bulk RNA-seq data of *Isl1CKO* and control. **d** The UCSC browser view of whole genome showing H3K4me3 and H3K27me3 peaks in control and *Isl1CKO* endocrine cells at E14.5 based on CUT&Tag-seq analyses. The mapped read counts distributed across all chromosomes are in comparable read depth for control and *Isl1CKO* samples. **e** Bar plot showing percentage of H3K4me3 and H3K27me3 peaks at promoter regions (± 3 kb from TSS), gene body regions, and intergenic regions. **f** Pie chart illustrating the proportion of differentially expressed genes that differentially exhibited one or both H3K4me3 and H3K27me3 marks at their promoter regions from pairwise comparison of *Isl1CKO* and control pancreatic endocrine cells at E14.5.

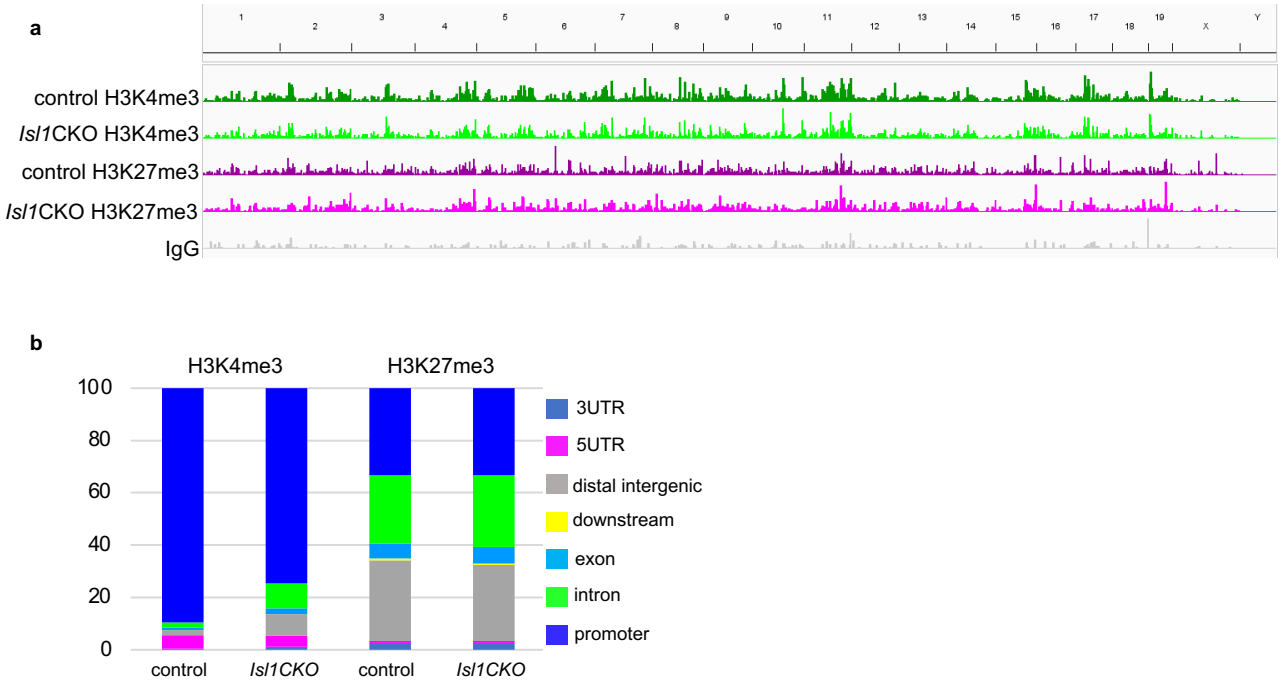


Fig. S5 H3K4me3 and H3K27me3 CUT&Tag-seq analyses of P9 pancreatic endocrine cells. **a** The UCSC browser view of whole genome showing H3K4me3 and H3K27me3 peaks in control and *Isl1CKO* endocrine cells at P9 based on CUT&Tag-seq analyses. The mapped read counts distributed across all chromosomes are in comparable read depth for control and *Isl1CKO* samples. **b** Bar plot showing percentage of H3K4me3 and H3K27me3 peaks at promoter regions (± 3 kb from TSS), gene body regions, and intergenic regions of endocrine cells of the P9 pancreas.

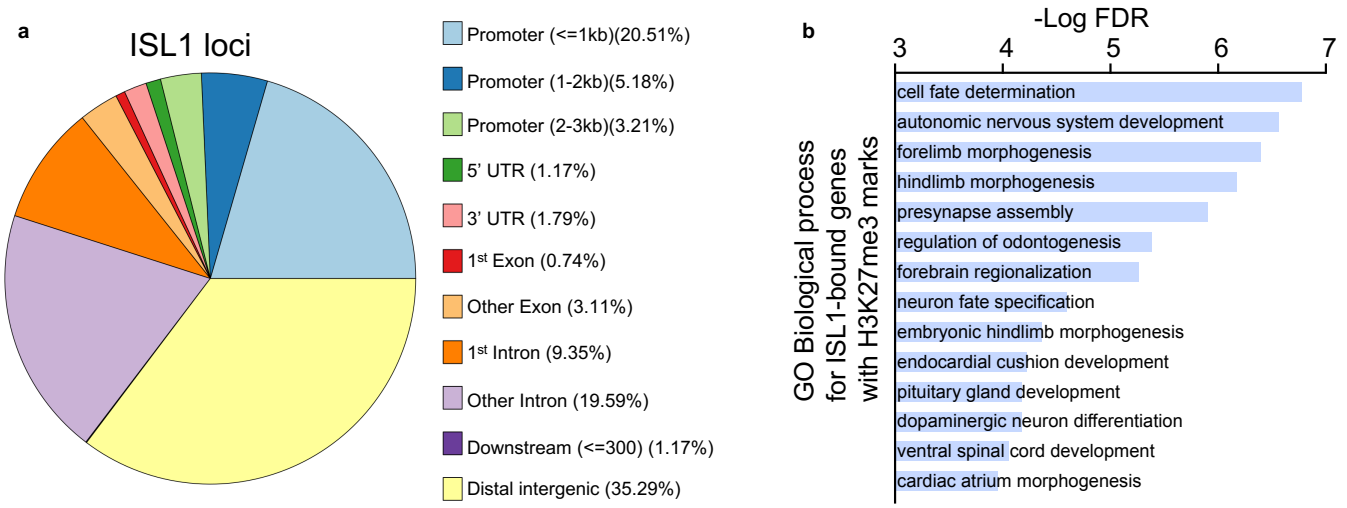


Fig. S6 ISL1 binding CUT&Tag-seq analyses of E14.5 pancreatic endocrine cells. **a** Pia chart showing genomic distribution of ISL1 loci in E14.5 pancreatic endocrine cells. **b** The most enriched Gene Ontology (GO) biological processes for genes bound by ISL1 and with *Isl1CKO*-specific H3K27me3 depositions.

control-*Ai14*

(Isl1$^{f/+}$; Neurod1^{Cre}; tdTomatoAi14)

Isl1CKO-Ai14

(Isl1$^{f/f}$; Neurod1^{Cre}; tdTomatoAi14)

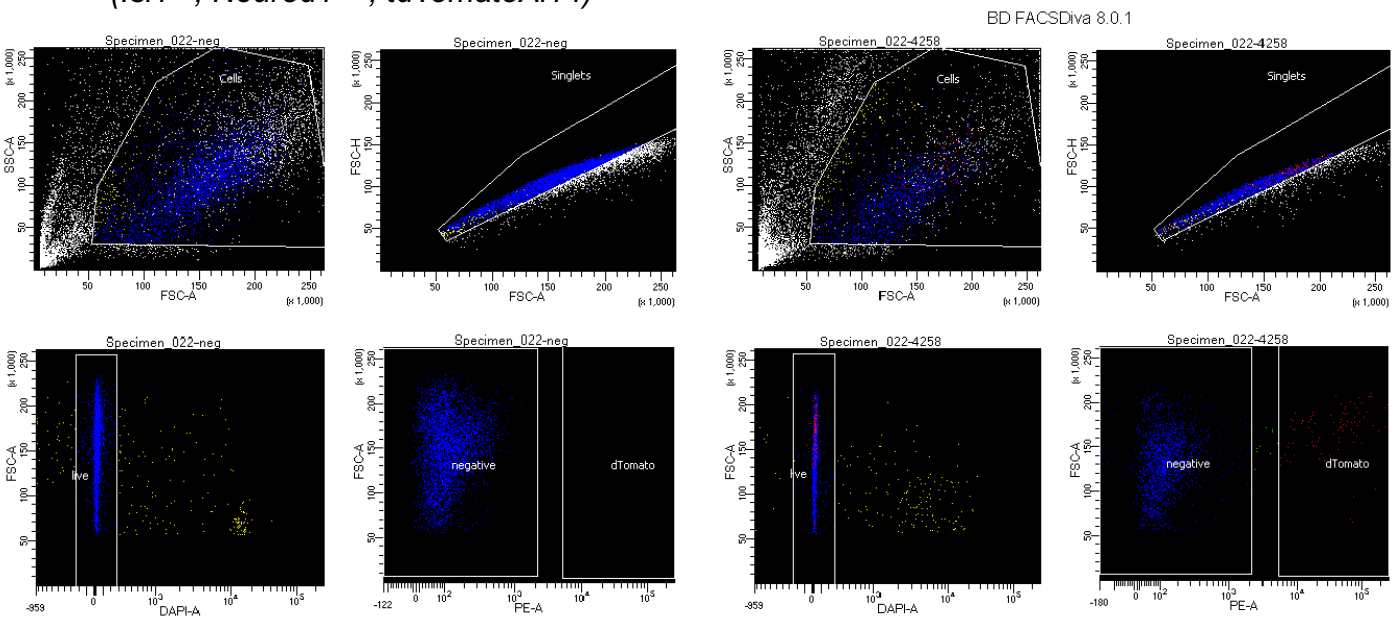


Fig. S7 Gating strategy used to isolate tdTomato^{+} cells. Representative example to show gating to purify live and individual tdTomato^{+} cells for RNA-seq and CUT&Tag-seq.

Supplementary Table S1. Primer sequences for genotyping

Gene	Primer sequence
Isl1^{flox}_F	5′ CTCTGGAACATCCCACATTG 3′
Isl1^{flox}_R	5′ GATGCAACCCCTGTTCCTAC 3′
Neurod1/cre_F	5′ CCATTTTGCAGTGGACTCCT 3′
Neurod1/cre_R	5′ ACGGACAGAAGCATTTTCCA 3′
WT- TomatoAi14_F	5′ AAGGGAGCTGCAGTGGAGTA 3′
WT- TomatoAi14_R	5′ CCGAAAATCTGTGGGAAGTC 3′
Transgene-TomatoAi14_F	5′ CTGTTCCTGTACGGCATGG 3′
Transgene-TomatoAi14_R	5′ GGCATTAAAGCAGCGTATCC 3′

Supplementary Table S2. Primer sequences for RT-qPCR

Gene	Forward	Reverse
Arx	5′ TTTTCTAGGAGCAGCGGTGT 3′	5′ GGGCCATAGTGGAAAAGAGC 3′
Fev	5′ AAACATCTCTGCCGTTCCCC 3′	5′ TGGGAGCTTTAATGGGGCTG 3′
Gcg	5′ CAGAAGAAGTCGCCATTGCC 3′	5′ AAGTCCCTGGTGGCAAGATT 3′
Ghrl	5′ CCAGAGGACAGAGGACAAGC 3′	5′ ACATCGAAGGGAGCATTGAA 3′
Ins1	5′ GACCAGCTATAATCAGAGACCATC 3′	5′ GTAGGAAGTGCACCAACAGG 3′
Ins2	5′ GGCTTCTTCTACACACCCAT 3′	5′ CCAAGGTCTGAAGGTCACCT 3′
Mafb	5′ GCAGGTATAAACGCGTCCAG 3′	5′ TGAATGAGCTGCGTCTTCTC 3′
Ngn3	5′ AGTGCTCAGTTCCAATTCCAC 3′	5′ CGGCTTCTTCGCTTTTTGCTG 3′
Peg10	5′ CCCTCATCCTTCGTGGCATC 3′	5′ CTCGTGGTTGGCGTCTTTTG 3′
Pou3f4	5′ CTGCCTCGAATCCCTACAGC 3′	5′ CTGCAAGTAGTCACTTTGGAGAA 3′
Ppy	5′ CAGGCGACTATGCGACACC 3′	5′ CAGGGAATCAAGCCAACTGG 3′
Sst	5′ ACCGGGAAACAGGAACTGG 3′	5′ TTGCTGGGTTCGAGTTGGC 3′
Hprt1	5′ GCTTGCTGGTGAAAAGGACCTCTCGAAG 3′	5′ CCTGAAGTACTCATTATAGTCAAGGGCAT 3′

Supplementary Table S3. List of antibodies

primary antibody	host species	company	catalog number	dilution
Alpha-Amylase	rabbit	Sigma-Aldrich	A8273	1:2500
Cre Recombinase	rabbit	BioLegend	908001	1:500
GLP1	rabbit	Abcam	ab111125	1:10 000
Glucagon	mouse IgG1	Abcam	ab10988	1:400
Glucagon	goat	BIO-RAD	4660-1140	1:500
Histone H3K4me3	rabbit	Active Motif	39159	1:100
Histone H3K27me3	rabbit	Active Motif	39155	1:100
Insulin	rabbit	Cell Signaling	C27C9	1:400
Insulin	guinea pig	Abcam	ab7842	1:50
*Islet1/2	mouse IgG2b	Developmental Hybridoma Bank	39.4D5	1:200
Ki67	rabbit	Cell Signaling	9129	1:400
NeuroD1	goat	Santa Cruz Biotechnology	sc-1084	1:100
NGN3	mouse	DSHB	F25A1B3	1:3
NKX6.1	mouse	DSHB	F64A6B4	1:3
Pax6	rabbit	BioLegend	901301	1:100
PDX1	rabbit	Abcam	ab47267	1:2 000
PHH3	rabbit	Merck	06-570	1:100
TUBB3	mouse	BioLegend	801202	1:500

secondary antibody	host species	company	catalog number	dilution
Alexa Fluor® **405**-conjugated AffiniPure Donkey Anti-**Goat** IgG (H+L)	donkey	Jackson ImmunoResearch	705-475-147	1:500
Alexa Fluor® **488**-conjugated AffiniPure Donkey Anti-**Rabbit** IgG (H+L)	donkey	Jackson ImmunoResearch	711-545-152	1:500
Alexa Fluor® **647**-conjugated AffiniPure Donkey Anti-**mouse** IgG	donkey	Jackson ImmunoResearch	715-605-151	1:500
Alexa Fluor® **594** AffiniPure Goat Anti-**Rabbit** IgG (H+L)	goat	Jackson ImmunoResearch	111-585-144	1:500
Alexa Fluor® **488** AffiniPure Goat Anti-**Mouse** IgG (H+L)	goat	Jackson ImmunoResearch	115-545-146	1:500
DyLight **405**-conjugated AffiniPure Goat Anti-**Guinea Pig** IgG (H+L)	goat	Jackson ImmunoResearch	106-475-003	1:500

*Monoclonal antibody 39.4D5 was deposited to the DSHB by Jessell, T.M. / Brenner-Morton, S. (DSHB Hybridoma Product 39.4D5).

Legends for Movies

Video S1. P9 control tdTomato GLP1 INS. Microdissected pancreas of tdTomato reporter control-*Ai14* mice was cleared (CUBIC protocol), immunolabeled, imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). Video shows the distribution and formation of islets in the anatomical microenvironment of the pancreas at P9; tdTomato^{+} endocrine cell population (magenta), β cells with expression of insulin (white), and α cells expressing glucagon-like peptide-1 (green).

Video S2. P9 ISL1CKO tdTomato GLP1 INS. Microdissected pancreas of tdTomato reporter *Isl1CKO-Ai14* mice were cleared (CUBIC protocol), immunolabeled, imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). Video shows the distribution and formation of islets in the anatomical microenvironment of the pancreas; tdTomato^{+} endocrine cell population (magenta), β cells with expression of insulin (white), and α cells expressing glucagon-like peptide-1 (green).

Video S3. P9 control tdTomato GLP1 Tubulin. Microdissected pancreas of tdTomato reporter control-*Ai14* mice were cleared (CUBIC protocol), immunolabeled, imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). LFSM video shows the distribution and formation of islets in the anatomical microenvironment of the pancreas at P9; tdTomato^{+} endocrine cells (magenta), α cells expressing glucagon-like peptide-1 (green), and neuronal fibers labeled by anti-tubulin (white fibers).

Video S4. P9 Isl1CKO tdTomato GLP1 Tubulin. Microdissected pancreas of tdTomato reporter *Isl1CKO-Ai14* mice were cleared (CUBIC protocol), immunolabeled, imaged, and reconstructed in 3D using light-sheet fluorescence microscopy (LFSM). LFSM video shows the distribution and formation of islets in the anatomical microenvironment of the pancreas at P9; tdTomato^{+} endocrine cells (magenta), α cells expressing glucagon-like peptide-1 (green) and neuronal fibers labeled by anti-tubulin (white fibers).