Supplementary Table 1: Genomic targets and primers for the droplet digital PCR testing of the 20p.11.2 deletions.

GRCh37 genomic coordinates	**Forward**	**Reverse**
chr20:17930867-17930972	TTGTGTGAAAGCTGACAAAATG	CAAGTCTTTTTATCCAAACACAGC
chr20:18794590-18794685	GCTTCCCAGTGTTTCAGGAC	TGGTGATGTCCAGCTGAAAG
chr20:19956252-19956341	ACTTCGGGTGCTTAGTGCAG	GGATGGTCTGCAGCATGTC
chr20:21687223-21687326	GCTGCGTGAGCAAGATCC	CTTGACCACGTTGGGAGTG
chr20:22262921-22263030	AAGGACATGAATGACAAGATC	GAGCTTGATGGAACAATCACC
chr20:22381210-22381300	TTTGCTCCTTTTGACTGCTG	ACCCATGTGTTCATCCACTG
chr20:22441027-22441122	TGAGTGGCAAACACCTGAAC	CAAGGCCTCTTGAGGTATGC
chr20:22550245-22550351	GCCTGGAAATTTGTCTGAGC	TTGCAATGTCTGTGCAGGTC
chr20:22564830-22564927	TTTAAACTGCCATGCACTCG	CTCGGGCTCTGCATAGTAGC
chr20:23370596-23370687	GCTTTGGTTCTTGATTTCAGC	TCAATGGCTTTCTGGTACTGC
chr20:24565487-24565591	TGCAGAGCGACTACTCAAGC	AGAAGCAGCAGAGCATGGAG

Supplemental Table 2: Table describing all public genomic datasets used in this study.
ES – embryonic stem, DE – definitive endoderm, GT – gut tube, PP – pancreatic progenitor.

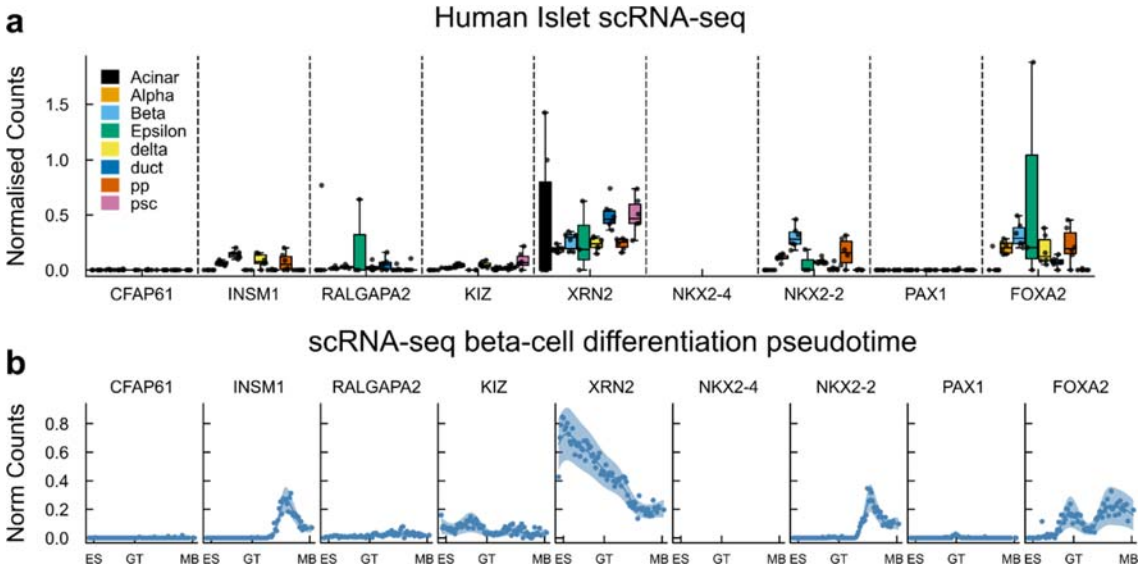
Accession	DOI	Description	Cell	Target
GSE149148	doi.org/10.7554/eLife.59067	ATAC-seq, ChIP-seq for TFs over pancreatic differentiation	ES, DE, GT, PP1, PP2	FOXA2, ATAC
E-MTAB-1919	doi.org/10.1038/ng.2870	ChIP-seq for TFs in islets	Islets	CTCF, FOXA2, H2AZ, H3K27ac, MAFB, NKX2_2, NKX6_1, PDX1
E-MTAB-1990, E-MTAB-3061	doi.org/10.1038/ncb3160	ChIP-seq for TFs liver buds	LiverBud,	FOXA2
GSE148368	doi.org/10.1038/s41467-021-26950-0	ChIP-seq for TFs in pancreatic and liver differentiation	GT, HP	FOXA2
GSE160472	doi.org/10.1038/s41588-021-00823-0	Single nuclei ATAC-seq in islets	Islets	ATAC
GSE101207	doi.org/10.1016/j.celrep.2019.02.043	Single cell RNA-seq in islets	Islets	
GSE143783	doi.org/10.1038/s42255-020-00314-2	Single cell RNA-seq of beta-like cell differentiation, abundances projected on pseudotime available in ref's Supplementary Table 4	ES cell to beta-like cell differentiation	

Supplementary Table 3: Summary of clinical features and genetic findings in individuals with 20p11.2 deletions.

Genetic coordinates relate to GRCh37. * denotes the total genomic region disrupted in this patient which includes an inverted region (details provided in the main text). ACTH, Adrenocorticotropic hormone; BOHB, Beta-hydroxybutyrate; EEG, Electroencephalogram; FFA, Free fatty acids; FSH, Follicle stimulating hormone; FT4, Free thyroxine (T4); GA, Gestational age; GH, Growth hormone; HI, Hyperinsulinism; IGF-1, Insulin-like growth factor 1; IGFBP3, Insulin-like growth factor binding protein 3; LH, Luteinizing hormone; MRI, Magnetic resonance imaging; NA, Not available; PRL, Prolactin; T3, Triiodothyronine; TSH, Thyroid stimulation hormone; ↔, at average range; ↑, above average range; ↓, below average range

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**
Coordinates of 20p11.2 deletions	Chr20:20158646–24080787	Chr20:19434987–22528253	Chr20:19507014–22525896*	Chr20:16400000–24400000	Ch20:18200000–22600000
Del includes *FOXA2*	Yes	No	No	Yes	No
Sex	Male	Female	Female	Female	Male
Age at latest follow-up	7 years	12 years	3 years	4 years	7 years
Birth weight (centile), GA	4.2 kg (99th) 38 weeks	3.7kg (63rd) 41 weeks	4.5 kg (99th) 40 weeks	2.8 kg (15th) 39 weeks	4.0 kg (73rd) 40 weeks
Age at Presentation of HI	Hypoglycemia at birth resolved, HI at 6 months	12 weeks	52 weeks	2 days	1 day
Critical sample during hypoglycemia					
Glucose, mmol/L (Insulin, pmol/L)	2.3 (117)	2.3 (582)	3.0 (14)	<0.3 (209)	1.4 (53)
Other biochemistry during hypoglycemia	NA	BOHB 0.26 nmol/L ↓ FFA 0.34 mmol/L ↓ Cortisol 684 nmol/L ↑ GH 16.7 µg/L ↔	NA	BOHB 0.33mmol/L ↓ FFA 0.98mmol/l ↓ GH 1.2ug/l ↓	BOHB <0.05 mmol/L ↓ FFA 0.10 mmol/L ↓ Cortisol 879 nmol/L ↑ GH 38.2µg/L ↑
Treatment for HI					
Diazoxide-responsive (initial dose)	No (11 mg/kg/d) 80% pancreatectomy aged 3.3 years, histology of diffuse HI	Yes (10 mg/kg/d)	Yes (10 mg/kg/d)	Yes (9mg/kg/d)	Yes (4.2 mg/kg/d)
Current treatment	Frequent feeds	Diazoxide (6.2mg/kg/d)	Diazoxide (6mg/kg/d)	Diazoxide (7mg/kg/d)	None - diazoxide stopped at 6 months
Other features					
Pituitary hormone examination (normal range)	GH deficiency diagnosed at 7 years of age (on GH treatment. <u>At 7 years of age</u> Cortisol ↔ TSH ↔ FT4 ↔ Brain MRI: Hypoplastic AP and ectopic PP, Chiari 1 malformation. <u>At 4 years of age</u> TSH 2.3 mU/L ↔ (0.49–4.9) FT4 8.7 pmol/L ↔ (6.1–12.9) IGF-1 33 ng/mL ↔ (22–208)	No pituitary dysfunction identified. <u>At 7 years of age</u> No growth delay (height 75th centile). No pubertal delay. TSH 2.15 mU/L ↔ (0.1–5.5) FT4 17.8 pmol/L ↔ (10–22) Brain MRI: Normal	No pituitary dysfunction identified. <u>At 3 years of age</u> TSH 0.74 mUI/ml ↔ (0.27-4.2) FT4 16.34 pmol/L (11.58-12.88) T3 1.63 ng/ml ↔ (0.8-2.0) Cortisol 248 nmol/L ↔ (132-530) ACTH 3.3 pmol/L ↔ (0-10.12) PRL 15.09 mcg/L ↔ (4.79 –23.3) IGF-1 63.6 ng/ml ↔ (29-118) IGFBP3 2.16 ug/ml ↔ (0.9-4.7) Height 10–25th centile (appropriate for parental heights)	No pituitary dysfunction identfied. Pituitary MRI and GH stimulation test planned. <u>At 4 years of age</u> TSH 2.54 mU/L ↔ (0.7–4.17) FT4 11.5 pmol/L ↔ (10.3–17) IGF-1 6.7 nmol/L ↔ (6–24) Height at 2nd centile (–1.9 SDS) <u>At 2 years of age</u> IGF-1 6.3 nmol/L ↔ (3–17) IGFBP3 1.9 mg/L ↔ (1.2–3.7) <u>At 2 months of age</u> Cortisol (morning) 406 nmol/L ↔	No pituitary dysfunction identified. <u>At 7 years of age</u> TSH 1.49 mU/L ↔ (0.50–3.80) FT4 15.9 pmol/L ↔ (10.8–22.9) IGF-1 31.5 nmol/L ↑ (7.1–26.8) Height at 75th centile. <u>At 4.5 years of age</u> LH <0.1 U/L ↔ (<2.6) FSH 0.3 U/L ↔ (0.2–3.0) PRL 465 mU/L ↔ (82–967) <u>At birth</u> GH 12.9 ug/L ↔ Cortisol 111 nmol/L ↔
Structural defects	Cryptorchidism, micro-penis, left lacrimal duct stenosis, inguinal hernia	Mild left ventricular hypertrophy in infancy (HI-related, resolved)	None	Horseshoe Kidney, ventricular septal defect	Anal stenosis, patent ductus artery (resolved)
Dysmorphology	Right preauricular pit, telecantus, wide & flat nasal bridge, clinodactyly (3rd toe), uneven folds between upper legs	Long eyelashes, synophrysis, and mildly dysmorphic facial features (possibly diazoxide-related)	Subtle features: long face, epicanthus, eversion of lower eyelid, retro-micrognathia, thin lips, short columella	None	Hypertelorism

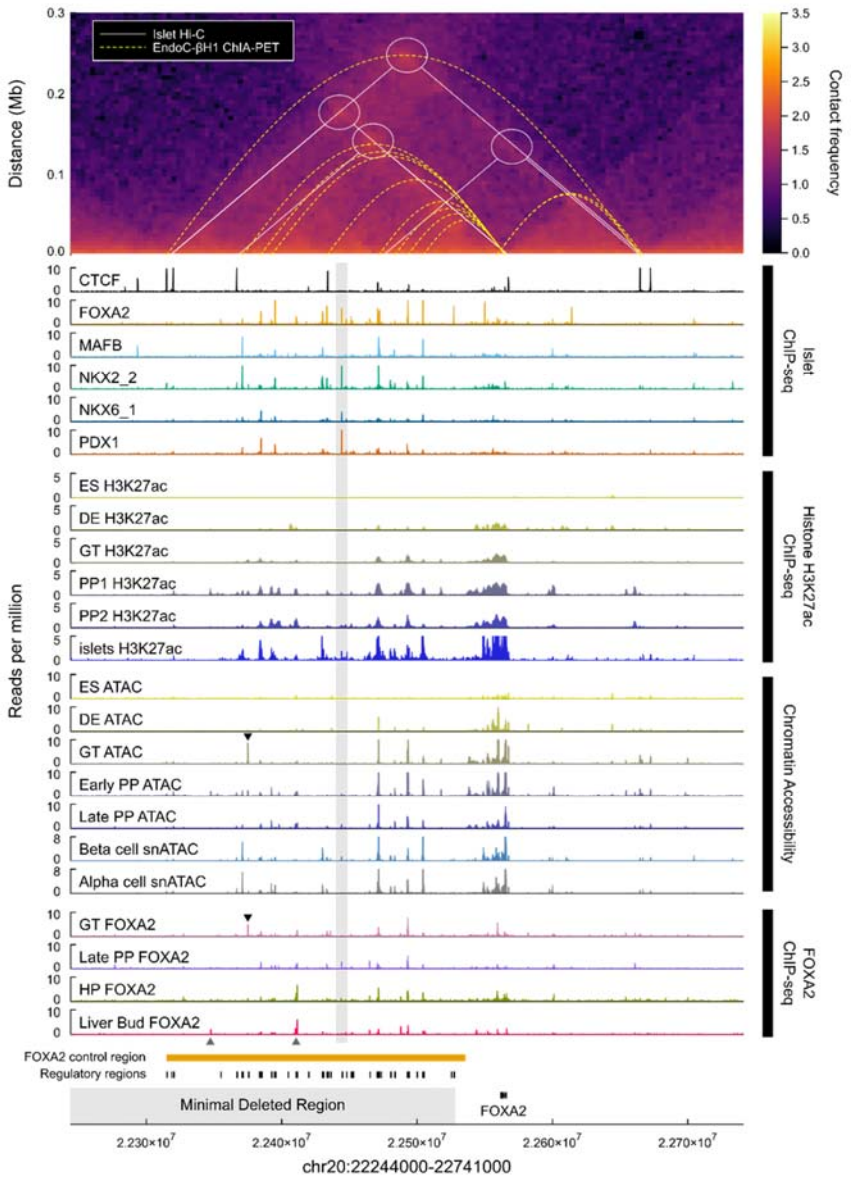
Development and neurology	Mild motor delay	Speech delay, mild motor delay, mild learning difficulties, epilepsy (focal features on EEG)	Developmental delay	No concerns with development	Developmental delay and learning difficulties



Supplementary Figure 1: Expression of genes partially or fully deleted by minimal deleted region and FOXA2.

a. Human islet single-cell RNA-seq (scRNA-seq) (GSE101207 [1]) expression data of the 8 genes whose sequence is disrupted and FOXA2 shown for reference. Data points give mean normalised counts of 6 independent donors for scRNA-seq cell type clusters defined in original publication (pp – pancreatic polypeptide, psc= pancreatic stellate cell). Boxplot central lines give the median, boxes span interquartile range, and whiskers extend to the furthest point within 1.5x IQR of the box. Order of cell types (left to right) matches order of legend (top to bottom).

b. scRNA-seq expression over pancreatic cell differentiation from embryonic stem (ES) cells to maturing beta-cells (MB), mapped onto a beta-cell differentiation pseudotime [2] that includes gut tube stage (GT). scRNA-seq data-points on pseudotime shown with Gaussian process regression, line and shaded region mark posterior Gaussian process median and 95% confidence intervals.



Supplementary Figure 2: Regulatory activity of FOXA2 control region.

Human islet Hi-C contact frequencies, transcription factor binding in multiple cell types and chromatin accessibility show regulatory activity of FOXA2 control region (orange bar).

Top: Heatmap gives contact frequencies of human islet Hi-C (5 kb bins) [3], white lines and circles describe CTCF-CTCF chromatin loops called in same study. Yellow dotted lines mark EndoC-βH1 RNA Pol II ChIA-PET enhancer-promoter loops (GSM3333915 [4]), highlighting contact between individual transcription factor binding sites and *FOXA2* promoter.

Bottom: human islet chromatin immunoprecipitation followed by sequencing (ChIP-seq) data [5]; H3K27ac ChIP-seq over pancreatic cell differentiation [6] and in human islets [5], ES – embryonic stem, DE – definitive endoderm, GT – gut tube, PP – pancreatic progenitor; chromatin accessibility data over pancreatic cell differentiation [6]; single nuclei assay for transposase-accessible chromatin sequencing (snATAC) of human islets showing alpha and beta cell clusters [7], (beta_1 and alpha_1 clusters from original study shown); FOXA2 ChIP-seq from *in vitro* differentiation gut tube (GT), pancreatic progenitors (PP), and hepatic progenitors (HP) [8], and *in vivo* in liver bud [9].

Individual regulatory regions shown strong contact with FOXA2 promoter by Hi-C and RNA Pol II ChIA-PET, activity of regulatory regions varies by factor and cell type. Beta-cell specific regulatory region, marked by grey vertical bar. FOXA2 ChIP-seq shows varied binding of FOXA2 at its own control region across differentiation and between pancreas and liver. Black down triangle (▼) marks gut-tube specific regulatory region by chromatin accessibility and FOXA2 binding. Grey up triangle (▲) marks liver dominant binding of FOXA2.

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