Enhancer signatures stratify and predict outcomes of non-functional pancreatic neuroendocrine tumors

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Most pancreatic neuroendocrine tumors (PNETs) do not produce excess hormones and are therefore considered ‘non-functional’2–4. As clinical behaviors vary widely and distant metastases are eventually lethal2,4, biological classifications might guide treatment. Using enhancer maps to infer gene regulatory programs, we find that non-functional PNETs fall into two major subtypes, with epigenomes and transcriptomes that partially resemble islet α- and β-cells. Transcription factors ARX and PDX1 specify these normal cells, respectively2,4, and 84% of 142 non-functional PNETs expressed one or the other factor, occasionally both. Among 103 cases, distant relapses occurred almost exclusively in patients with ARX+PDX1- tumors and, within this subtype, in cases with alternative lengthening of telomeres. These markedly different outcomes belied similar clinical presentations and history and, in one cohort, occurred irrespective of MEN1 mutation. This robust molecular stratification provides insight into cell lineage correlates of non-functional PNETs, accurately predicts disease course and can inform postoperative clinical decisions.

Surgery is recommended for solitary PNETs larger than 2 cm and the World Health Organization (WHO) grade is the best current tool to predict metastasis5–8. Insulinomas resemble normal pancreatic β-cells and carry a good prognosis1,2, but the lineage of most non-functional PNETs is obscure and about half the cases progress to lethal metastasis months to years after surgery1,2,9. Although 70% of PNETs carry MEN1, ATRX or DAXX gene mutations, and 15% activate mammalian target of rapamycin signaling10,11, no mutation or biological feature is sufficiently correlated with clinical outcomes to guide prognosis or therapy12–14. Alternative lengthening of telomeres (ALT) is associated with ATRX or DAXX loss and elevated risk of recurrence12,15, but is not a routine clinical test.

Cellular identities are encoded in chromatin states defined by the complement of active cis-elements, where nucleosomes bear H3K4me1/2, H3K27ac and other covalent marks16,17, ‘super’ or ‘stretch’ enhancers, which control lineage-specifying genes1,14, especially delineate cell-specific chromatin signatures19 and help nominate tumor cell origins28. Chromatin immunoprecipitation sequencing (ChIP-seq)-derived profiles of H3K27ac-marked candidate enhancers in 8 frozen, primary non-functional, pancreatic and 22 intestinal neuroendocrine (NE; carcinoid) tumors (see Supplementary Table 1) differed substantially from those of gastrointestinal carcinomas and less so from each other (Fig. 1a). Loci expressed in the two NE tumor types, such as SYP, were similarly marked in both, whereas organ-restricted loci, such as CDX2, were marked selectively (Fig. 1b). Super-enhancer profiles, distinct from those of normal islets (Fig. 1c), revealed three PNET subtypes. Considering A and B types as separate groups, 288 enhancers showed more than twofold higher H3K27ac in type A (false discovery rate (FDR) < 0.05—see Supplementary Table 2), with sites in α-cell-specific loci ARX and I RX2 showing especially large differences (Fig. 1d). Conversely, 104 regions showed notably more H3K27ac in B-type tumors, including enhancers over genes such as PDX1 and SL C17A6, which are not expressed in α-cells22,23.

Across the eight PNETs, A- and B-type tumors gave strong H3K27ac signals at ARX and I RX2 or at PDX1, respectively, whereas...
C-type tumors were variably marked at these loci and expressed low levels of ARX and PDX1 messenger RNAs (mRNAs) (see Extended Data Fig. 1a). In individual A- and B-type tumors, ARX and PDX1 met objective super-enhancer criteria\(^{19,20}\), respectively (Fig. 1c). At these and other loci, ChiP-seq for H3K4me2 (ref. 23) on the original eight and four additional PNETs (see Supplementary Table 1) revealed seven patterns to H3K27ac (see Extended Data Fig. 1a—b) of six biologically independent tumors. Each dot represents an individual site (red: FDR < 0.05). H3K27ac signals at distal regulatory elements in diverse gastrointestinal cancers. PNETs and carcinoid (small intestinal NE) tumors differ from others, reflecting distinct cell origins. Whereas intestinal NE enhancer landscapes are highly concordant, PNET s appear heterogeneous.

Knowledge of pancreatic endocrine ontogeny derives largely from studies of mouse development\(^{24}\). NEUROG3 initiates the endocrine lineage and ARX specifies α-cell fate\(^{25}\), whereas PAX4 drives β-cell differentiation\(^{26}\); absence of both factors favors the δ lineage\(^{27}\). ARX is necessary for α-cell differentiation\(^{27}\), but other transcription factors (TFs, for example NKX2.2, PAX6, PDX1) sustain β-cells\(^{28,29}\). In embryos, early α-cell specification leads to Insulin and Glucagon co-expressing bipotential precursors, followed by β-cell differentiation\(^{30}\); forced transdifferentiation between α- and β-cells also occurs through intermediate Insulin\(^{+}\) Glucagon\(^{+}\) cells\(^{31}\). In this light, we compared A- and B-type PNET-restricted enhancers (Fig. 1d) with areas of chromatin selectively open in normal α- or β-cells\(^{32}\). Type A regions were highly enriched for α-cell-specific sites (62 shared, \(P < 1.1 \times 10^{-15}\)), whereas type B-specific enhancers were enriched for β-cell-restricted sites (15 shared, \(P < 1.7 \times 10^{-10}\); see Supplementary Table 4). Comparison with RNA profiles of fractionated normal human islets\(^{33}\) also revealed enrichment of α- and β-cell-specific transcripts in A- (\(P < 1.6 \times 10^{-11}\)) and B-type (\(P < 6.2 \times 10^{-15}\)) PNETs, respectively. Differential TF expression distinguished PNETs better than Glucagon and Insulin mRNA levels, which were generally low and similar in the two subtypes (see Extended Data Fig. 1b).

From H3K27ac ChiP-seq on 13 additional frozen PNETs (validation cohort), tumors were designated as: type A if signals were high (\(>500 \text{ reads per kb per million sequence tags (RPKM)}\) at ARX and IRX2 but absent or low (\(<250 \text{ RPKM}\) at PDX1); type B if the ARX locus was largely unmarked (<250 RPKM); and type C if read counts at ARX and PDX1 were comparable. Although A or C typing was ambiguous in two PNETs, the B type was easily identified by lack of H3K27ac at ARX. Similar to the discovery set, the validation cohort readily distinguished type B from other PNETs by H3K27ac and see Extended Data Fig. 1c). Moreover, ARX and PDX1 immunostains gave the expected signals in normal human islets (see Extended Data Fig. 2a,b) and, importantly, 15 independent, additional, non-functional PNETs showed mutually exclusive, nucleus-dominant, ARX or PDX1 expression in 10 tumors (67%); 5 cases lacked or co-expressed both TFs (Fig. 3a).
H3K4me2 and mRNAs were profiled in selected tumors from the Dutch cohort using fixed-tissue chromatin immunoprecipitation sequencing (FiT-seq)\textsuperscript{36} and RNA sequencing (RNA-seq). PNETs, identified by IHC as ARX\textsuperscript{+} or PDX1\textsuperscript{+}, expressed exclusively those mRNAs and showed abundant H3K4me2 only at the corresponding super-enhancers; DP tumors (type C) expressed both ARX and PDX1 mRNAs, with less pronounced enhancer marks at both loci (Fig. 3d). Of note, no PNETs in this group expressed somatostatin (SST) (see Extended Data Fig. 3b). FiT-seq for H3K27ac and immunostaining on representative formalin-fixed, paraffin-embedded (FFPE) samples from the discovery cohort also revealed concordance (see Extended Data Fig. 3c). Thus, IHC classifies PNETs robustly, reflecting lineage-specific gene and enhancer signatures that are henceforth called ARX\textsuperscript{+}, PDX1\textsuperscript{+} and DP.

PNETs uniformly lacked H3K27ac and mRNA expression at early acting NEUROG3 and PAX4 loci (see Extended Data Fig. 4a), similar to isolated human islets, and IHC failed to detect NEUROG3 in any tumor (see Extended Data Fig. 4b). In contrast, enhancer marking and mRNA levels were high at other canonical endocrine loci (ISL1 and NEUROD1—data not shown), but not at terminal differentiation genes (for example, MAFA and FFA1; see Extended Data Fig. 4c). H3K4me2, but not H3K27ac, appeared at many such loci (Supplementary Table 6). All PNETs with mRNA data, the mature cell signature was stronger than that of progenitors (see Extended Data Fig. 5), suggesting that ARX\textsuperscript{+} and PDX1\textsuperscript{+} non-functional PNETs partially resemble mature \(\alpha\)- and beta \(\beta\)-cells, respectively.

Detailed clinical follow-up was documented on the 61 Dutch MEN1-mutant cases\textsuperscript{18} (see Supplementary Table 5). ARX\textsuperscript{+} and PDX1\textsuperscript{+} non-functional PNETs (\(n=47\)) did not differ by tumor size or grade (Fig. 4a), and the range of sizes was similar to that of insulinomas (see Extended Data Fig. 6a). With 24 months of median follow-up (longest 8 years), all relapses occurred in the liver and only in ARX\textsuperscript{+} or DN cases; no PDX1\textsuperscript{+} or DP PNETs recurred (Fig. 4b and see Extended Data Fig. 6b). A study was then made of 67 unselected cases from Massachusetts (see Supplementary Table 5), where IHC revealed roughly equal fractions of ARX\textsuperscript{+} and PDX1\textsuperscript{+} PNETs, but a larger proportion of DN tumors than the previous cohorts (Fig. 4c). Tumor size was known for 61 cases and clinical outcomes for 56 of the 60 non-functional PNETs. ARX\textsuperscript{+} and PDX1\textsuperscript{+} tumors were similar in size (see Extended Data Fig. 6c). All relapses over 66 months of median follow-up (longest >15 years) occurred at distant sites, mostly in patients with ARX\textsuperscript{+} or DN tumors (Fig. 4d and see Extended Data Fig. 6d; \(P=0.02\)); only one PDX1\textsuperscript{+} and two DP tumors relapsed. Thus, PDX1 expression correlated with favorable prognosis in two distinct PNET cohorts.

Telomere-specific FISH was used to determine ALT status, which is associated with ATRX and DAXX mutations\textsuperscript{12–14}, in 50 Dutch MEN1-related and 62 American sporadic PNETs (see Extended Data Fig. 6e). Of 27 sporadic ARX\textsuperscript{+} and DN tumors, 13 tumors (48.1\%) showed ALT compared with 14.3\% of 35 PDX1\textsuperscript{+} and DP tumors (\(P<0.005\), Fisher's exact test); similar relationships appeared in MEN1-mutant cases (see Extended Data Fig. 6f). ALT was associated with disease relapse, as expected\textsuperscript{12–14} (see Extended Data Fig. 6g), but was more informative when combined with PNET subtype: relapses occurred in every ARX\textsuperscript{+}ALT\textsuperscript{+} tumor, only 9\% of ARX\textsuperscript{+}ALT\textsuperscript{−} cases and just one PDX1\textsuperscript{+}ALT\textsuperscript{+} case (Fig. 4e).

Among the 103 total cases with clinical follow-up, 83 had data on subtype, size, ALT and WHO grade. The odds ratio (OR) for relapse of ARX\textsuperscript{+} or DN cases was higher (14.45, 95% confidence interval (CI) 1.79–116.61) than ORs for 2-cm size (1.14, CI 0.12–11.07) and even 3 cm (8.47, CI 2.11–34.02). Even excluding DN and DP cases (remaining \(n=64\)), the OR for relapse of ARX\textsuperscript{+} PNETs (10.31, CI 1.25–84.73), was higher than for tumors >2 cm (8.13, CI 0.99–66.97).

Next tissue microarrays were examined that represented 77 Dutch PNETs\textsuperscript{35}, 61 of which (79\%) had germline MEN1 mutations, including 13 insulinomas associated with hyperinsulinemia (see Supplementary Table 5). Overall, 34 of the 77 cases showed nuclear expression of ARX only (31% type A), 31 expressed only PDX1 (37\% type B) and 12 cases were either double-positive (DP) or double-negative (DN—8\% each; Fig. 3b and see Extended Data Fig. 2c). When present, the immunohistochemistry (IHC) signals for PDX1 were uniformly strong and every insulinoma, scored blindly, expressed only PDX1. Although equally unambiguous, ARX signals varied in strength across type A tumors (see Extended Data Fig. 2c), so some DN tumors may represent technical failure of IHC, whereas others may represent type A PNETs with low ARX expression. DP tumor cells largely co-express ARX and PDX1 (Fig. 3c and see Extended Data Fig. 3a), possibly representing a dual lineage. MEN1 mutations did not skew the proportions of A and B tumors (Fig. 3b).

### Fig. 2 | PNET subtypes represent distinct endocrine lineages. a. Relative H3K27ac ChIP-seq signals in all 21 PNETs (8 from the discovery set +13 additional) of types A (ARX\textsuperscript{+}), B (PDX1\textsuperscript{+}) and C (DP) at enhancers that are specific to A versus B PNETs (Fig. 1d) and \(\alpha\)- versus \(\beta\)-cells\textsuperscript{35}. b. Genomic views of the ARX (b) and PDX1 (c) loci, showing H3K27ac signals for all 21 PNETs. chr, chromosome. ChIP-seq signals are scaled by promoter-based DESeq2 normalization (see Methods).
Relapses occurred in patients with WHO grade 1 PNETs, and considering all variables by multiple logistic regression, tumor grade was a poor independent risk factor (Fig. 4f). Only ALT and especially absence of PDX1 correlated independently with relapse (Fig. 4f); PDX1⁺PNETs rarely relapsed.

In summary, enhancer profiles in overtly similar non-functional PNETs revealed superficial similarities with islet α- or β-cells, reflected in IHC delineation of ARX⁺, PDX1⁺ and fewer ARX⁺PDX2⁺ (DP) tumors. Among 103 cases followed at length after surgery, distant relapses predominated in patients with ARX⁺ tumors, occurring in only three PDX1⁺ or DP cases. This favorable association with PDX1 expression can be applied rapidly in the clinic. Specifically, patients with small PDX1⁺ tumors may be reassured and followed conservatively, whereas vigilant monitoring in patients with PDX1⁻ tumors may detect early metastases amenable to surgical or medical treatment. Consideration of ALT⁺ status, which correlates with ARX expression, adds prognostic information, but is less practical than IHC in clinical laboratories. The superior prognosis of PDX1⁺ over ARX⁺ PNETs matches the indolent and aggressive disease courses, respectively, of
metastatic insulinomas and glucagonomas45; thus, PNETs that resemble β-cells have better clinical outcomes irrespective of hormonal activity and disease extent. RNA analyses in mouse and human PNETs previously found a group with high Insulin expression46, but did not identify tumor subtypes or different clinical outcomes. Conversely, Chan et al. found enriched α-cell RNA signatures and worse prognosis in ATRX-, DAXX- or MEN1-mutant PNETs47. The new, clinically actionable differences reported among PNET types illustrate a general strategy to stratify cancers by epigenetic landscapes and cell lineage, with prognostic implications.

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Fig. 4 | Different prognosis of PNET subtypes. a, Size of all 36 primary tumors with data available for size and PNET type in the Dutch cohort (P=0.23, two-tailed Mann-Whitney U-test). Bars represent mean ± s.d. b, Kaplan–Meier analysis of disease-free survival in all 47 Dutch cases with available data, showing significant recurrence of ARX+ and DN tumors considered together (P=0.0076, two-sided log-rank test; hazard ratio (HR)=7.8, Mantel-Haenszel test; CI is indicated). No PDX1+ tumor recurred, and outcomes of ARX+ tumors (ungrouped from DN) are shown in Extended Data Fig. 6b. c, Distribution of PNET subtypes in the MGH cohort (n=67; numbers in each slice indicate independent biological samples). d, Kaplan–Meier analysis of disease-free survival in all 55 MGH cases with available data, confirming the worse prognosis associated with ARX+ and DN tumors considered together (P=0.024, two-sided log-rank test; HR=4.04, CI 1.2–13.58). Data for ARX+ and PDX1+ tumors, separated from DP and DN, are in Extended Data Fig. 6d. e, Kaplan–Meier analysis of disease-free survival in both cohorts, with cases sub-typed for both TF expression and ALT. All ARX+ and DN tumors with ALT recurred. Although PDX1+ tumors gave excellent outcomes irrespective of ALT status (the only recurrence lacked ALT), ARX+ and DN tumors without ALT had an intermediate prognosis. f, Multiple logistic regression analysis of the MGH and Dutch cohorts combined (n=83—all cases with relevant data, including 15 relapses). ARX+ and DN status are strong prognostic indicators.

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Author contributions

P.C., Y.D., C.B.E., E.S., D.C.C., B.E.B. and R.A.S. designed the study. P.C. performed the experiments. Y.D. performed the computational analyses. P.C., L.A.A.B. and V.D. analyzed immunohistochemistry data. C.B.E., M.B., E.G., H.J.W., N.S., A.F.-T. and H.W.L. coordinated ChIP- and RNA-seq efforts. K.M.A.D., E.B.C., L.A.A.B., E.H.M.M., G.D.V., M.R.V., C.F.-D.C., C.F., T.A., A.D.S., E.S., M.H.K. and D.C.C. obtained and curated tissue collections and clinical data. P.C. and K.M.A.D. analyzed clinical data. M.K.G. and C.M.H. performed and scored telomere-specific FISH for ALT. B.E.B. and R.A.S. supervised the study. Y.D., P.C. and R.A.S. wrote the first manuscript draft. K.M.A.D., V.D., M.H.K., D.C.C., B.E.B. and R.A.S. revised the paper.

Competing interests

The authors declare no competing interests. Additional information

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Telomere-specific FISH and assessment of ALT. Deparaffinized TMA slides were hydrated, steamed for 25 min in citrate buffer (Vector Laboratories), dehydrated, and hybridized with a Cy3-labeled peptide nucleic acid probe complementary to the mammalian telomere repeat sequence. An Alexa Fluor 488-labeled peptide nucleic acid probe specific to human centromeric DNA was included as a positive control. After post-hybridization washes, slides were counterstained with DAPI. ALT+ tumors were identified by telomere length heterogeneity and the presence of large, ultra-bright foci of nuclear FISH signals in ≥1% of tumor cells. Necrotic areas were excluded from consideration and two individuals blinded to the PNET subtype scored the slides, with 100% concordance.

Computational and statistical analyses. ChiP-seq reads were aligned to the reference genome (hg19) using BWA 0.7.10 (ref. 1). Reads with mapping scores <10 were discarded, and those aligned to the same position and strand were combined only once. Density signals were calculated using igvtools and visualized on the Integrated Genome Viewer41. H3K27ac and H3K4me2 peaks were called using the top 50,000 from each sample and merging those peaks using bedtools merge. Reads were acetylation signals at enhancers, estimated from ChiP-seq reads that fall within this union set and counted using featureCounts44. H3K27ac profiles were compared using DESeq2 (ref. 45). Promoter signals (<2 kb downstream and >2.5 kb upstream of transcription start sites) vary less than those at enhancers; therefore, only promoter signals were used to estimate normalization (size) factors for each library by DESeq2. When comparing libraries normalized by these size factors, only enhancers with average normalized read counts ≥100 were considered. P values were calculated using Wald’s test and FDRs using the Benjamini–Hochberg method. Super-enhancers were called by ROSE46 from H3K27ac peaks using default parameters. Public H3K27ac:ChiP-seq data, obtained from the following sources, were re-analyzed using the above methods:

- Pancreatic ductal adenocarcinoma: Gene Expression Omnibus (GEO) accessions GSM2131266 and GSM2131280.
- Gastric adenocarcinoma: GEO accessions GSM1969645 and GSM1969657.
- Colorectal cancer: GEO accessions GSM2058055 and GSM2058056.

Normal islets: ArrayExpress accession E-MTAB-118.
α-Cell- and β-cell-specific enhancers were obtained from Supplementary Table 4 in Arda et al.41. Enhancers shared with PNET types A (ARX+) and B (PDX1+) were determined by bedtools intersect, and enrichment P values were calculated using Fisher’s exact test. To cluster PNETs by enhancer signatures (see Fig. 2a and Extended Data Fig. 1c), hierarchical clustering of Pearson’s correlations on H3K27ac profiles (quantified by log2, read counts) was performed, using the shortest distance linkage.

RNA-seq reads were mapped to the hg19 reference genome using STAR 2.5.2 (ref. 42) and GENCODE v.19 annotations; reads with mapping scores <10 were discarded. To compare RNA profiles, reads were counted with featureCounts44 and compared using DESeq2. P values were calculated using Wald’s test and FDRs using the Benjamini–Hochberg method, considering only genes with an average normalized read count ≥100 and using a Cook’s cutoff of 20. RNA-seq data on normal pancreatic islets47 were obtained from GEO accessions GSM1303932 and GSM1303934. α-Cell- and β-cell-specific genes were obtained from Supplementary Table 2 in Wang et al.48. Enriched enhancers were identified using ChromHMM25. Gene expression was calculated using Fisher’s exact test (two-sided). Pancreatic endocrine cell mRNA signatures were obtained from Supplementary Table 1 in Ramond et al.14, considering populations A and B as mature, and populations C and D as progenitors (see Extended Data Fig. 5). Correlations of tumors to these Mature and Progenitor signatures were based on Spearman’s correlation of log(transcript count per million (TPM) + 1) values of the tumor and the average log(TPM + 1) values of Mature and Progenitor islet cell populations.

ALT status and PNET immunohistochemistry were compared using two-sided Fisher’s exact test. Tumor sizes between groups were compared using the two-tailed Mann–Whitney U test. Disease-free survival was analyzed using the log-rank test in Kaplan–Meier plots. An unpaired t test was used using Graphpad Prism v.7. To assess the prognostic value of different variables, we applied SPSS Statistics for multiple logistic regression analysis.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All relevant data are included in the manuscript and/or in its supplementary information files. ChiP-seq and RNA-seq data have been deposited in the National Center Biotechnology Information's GEO under GSE116356. Other original data that support the findings of this study have been uploaded as Source Data.

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Extended Data Fig. 1 | PNET subtypes are associated with distinct enhancers of lineage-restricted TFs. a, H3K27ac, H3K4me2 and mRNA data tracks at ARX and PDX1 in all eight PNETs from the discovery set and from two samples of normal islets of Langerhans (Isl). ChIP-seq signals are scaled by promoter-based DESeq2 normalization (see Methods) and mRNA read counts are normalized by total read numbers (y axis represents 0–2 fragments per million reads). b, Distributions of ARX and PDX1 mRNA levels in A- and B-type PNETs. c, Pearson’s correlations of H3K27ac signals at PNET type A/α-cell and type B/β-cell enhancers in all 21 tumors from the discovery and validation cohorts (n = 8 and n = 13 biologically independent samples, respectively).
Extended Data Fig. 2 | ARX and PDX1 immunostain in human normal islets and PNETs. a, Double immunofluorescence for PDX1 (red) and ARX (green) in normal islets (marked by dashed white outlines). Scale bar, 50 μm. The results, representing hundreds of islets, verify antibody specificity, lineage-restricted expression and cell distributions: abundant PDX1+ β-cells scattered across islets and fewer ARX+ α-cells enriched in the islet periphery. b, Top: ARX and PDX1 IHC selectively mark endocrine α- and β-cells, respectively, in normal human islets. Many exocrine and ductal cells also express PDX1, as is well known24. The results represent hundreds of normal islets from multiple individuals, which revealed no ARX+ PDX1+ DP cells. Thus, although described in rodent embryos24, such cells are absent or extremely rare in the adult human pancreas. Bottom: IHC for ARX in a representative PNET and surrounding normal cells on TMAs from the Dutch cohort. The area boxed in the left image is magnified on the right. ARX+ cells dominate in the tumor and mark invasive foci (arrows). c, Range of IHC signal strength in ARX+ PNETs (+weak, ++ moderate, +++ strong), contrasted with uniformly robust PDX1 staining. Images are examples selected from 34 ARX+ and 31 PDX+ cases (Fig. 3d). Scale bars, 50 μm.
Extended Data Fig. 3 | Additional IHC and enhancer characterization of PNETs. a, Double immunofluorescence of representative ARX+ (type A, n = 34 biologically independent samples) and PDX1+ (type B, n = 31 biologically independent samples) tumors (T) adjacent to normal islets (N), showing selective detection of ARX (green) and PDX1 (red), respectively. Lack of antibody cross-reactivity controls for ARX and PDX1 co-staining (Fig. 3c) in DP tumors. b, SST expression in normal islets (δ-cells) and absence in all 77 Dutch PNETs, including the representative DN tumor (n = 6 biologically independent samples) shown here. c, IHC results for ARX and PDX1 shown alongside H3K27ac F1T-seq data from the same samples in three of the four cases (one of each subtype) from the discovery cohort where both FFPE and frozen samples were available.
Extended Data Fig. 4 | Other endocrine-specific loci in PNETs. a, H3K27ac, H3K4me2 and mRNA data tracks from all eight PNETs in the discovery set and from two normal islet samples at loci that control early pancreas ontogeny: NEUROG3 and PAX4. Histone marks and RNA-seq data are scaled as in Extended Data Fig. 1a. b, IHC for NEUROG3 in rare normal islets (dashed outlines), showing scarce NEUROG3+ endocrine cells (arrows). Hundreds of normal islets and all 19 biologically independent PNETs represented on one TMA (one example is shown) lacked expression. c, H3K27ac, H3K4me2 and mRNA data tracks from all eight PNETs in the discovery set and from two normal islet samples at loci that control terminal endocrine cell maturation, MAFA and FFAR1. Histone marks and RNA-seq data are scaled as in Extended Data Fig. 1a. A single outlier showed strong H3K27ac and mRNA at FFAR1.
Extended Data Fig 5 | Differentiation status of PNETs. a. Correlations of mRNA profiles in individual PNETs with those of pancreatic endocrine progenitor and mature cells. x axis: Spearman’s correlations between log₂(TPM + 1) values of each tumor and the average log₂(TPM + 1) values of mature and progenitor populations.
Extended Data Fig 6 | Association of PNET subtypes with ALT status. a, c. Tumor size in all PNET subtypes in the Dutch (a) (n = 56 independent tumors) and the MGH (c) (n = 61 independent tumors) cohorts. Bars represent mean ± s.d. P values for differences in size of primary ARX+ and PDX1+ tumors determined by the two-sided Mann–Whitney U-test. b, d, Analyses of recurrence-free survival in the Dutch (b) (n = 30 cases) and MGH (d) (n = 35 cases) cohorts when ARX+ and PDX1+ tumors were considered separately, ungrouped from DP and DN tumors. P values and HRs were determined using two-sided log-rank and Mantel–Haenszel tests, respectively. e,f, Representative (e) (1 example each from 25 independent ALT+ and 87 independent ALT− cases) and aggregate (f) (n = 112 biologically independent cases) results of telomere-specific FISH in cases classified as positive or negative for ALT. The statistical test was two-sided. g, Kaplan–Meier analysis of disease-free survival in all 112 cases with ALT data from both cohorts, without consideration of PNET subtype.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection
- Standard Illumina NextSeq 500 and HiSeq 2500 processing.

Data analysis
- BWA 0.7.10, igvtools 2.3, featureCounts 1.6.2, bedtools 2.26, DESeq 2, HOMER 4.6, ROSE

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

ChIP-seq and RNA-seq data have been deposited in NCBI’s Gene Expression Omnibus and are accessible through GEO Series accession number GSE116356 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE116356).
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size          | We did not perform sample size calculation. Rather, we used the largest combined tumor sample and clinical datasets that were available to us. |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions      | Failed chip-seq tracks were excluded (IP efficiency less than 5%, as estimated by ratio of reads within peaks) and ovarian metastatic samples were excluded. |
| Replication          | All attempts of replication are described in the manuscript.                                                                    |
| Randomization        | The study described does not include any intervention. Therefore randomization of patients does not apply to this study.     |
| Blinding             | Immunohistochemical scoring was done blinded to the clinical information                                                    |

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a | Involved in the study

- Unique biological materials
- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants

Methods

n/a | Involved in the study

- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials: n/a

Antibodies

Antibodies used

H3K27ac (Active Motif 39133, lot #01613007), H3K27ac (Diagenode C15410196, lot A1723-0041D), H3K4me2 (Millipore 07-030, lot #M77948), ARX (R&D, AF7068, lot CF0M0217021), PDX1 (Abcam ab134150, lot GR97325-11), NEUROG3 (EMD Millipore AB5684, lot 2519231), SST (Agilent A056601-2). All antibodies with human reactivity.

Validation

H3K27ac (Active Motif 39133), H3K27ac (Diagenode C15410196) and H3K4me2 (Millipore 07-030) are validated for ChIP-seq application as stated on Manufacturer’s website. In addition, H3K4me2 (Millipore 07-030) is validated for FiT-seq (Nat. Med. 2016, PMID:27111282). ARX (R&D, AF7068), PDX1 (Abcam ab134150), NEUROG3 (EMD Millipore AB5684), SST (Agilent A056601-2) are all validated for immunohistochemistry as stated on the corresponding Manufacturer’s websites. We further validated the performance by analysis of the expression in normal pancreatic islets (described in the manuscript).

Human research participants

Policy information about studies involving human research participants

Population characteristics

The relevant characteristics of the participants include tumor site, MEN1 status, ALT status, size of the primary tumor at surgery,
### Recruitment

All patients that underwent surgery of a primary pNET in the hospitals involved in the study during the sample correction period and gave informed consent were included in the study.

### ChIP-seq

#### Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](https://www.ncbi.nlm.nih.gov/geo/).
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

#### Data access links

May remain private before publication.

| Files in database submission |
|-----------------------------|
| CAR_10_h3k27ac_13811.bw     |
| CAR_11_h3k27ac_8691.bw      |
| CAR_12_h3k27ac_8701.bw      |
| CAR_13_h3k27ac_13810.bw     |
| CAR_14_h3k27ac_13809.bw     |
| CAR_15_h3k27ac_13832.bw     |
| CAR_16_h3k27ac_13807.bw     |
| CAR_17_h3k27ac_13833.bw     |
| CAR_18_h3k27ac_7487.bw      |
| CAR_19_h3k27ac_7478.bw      |
| CAR_1_h3k27ac_8688.bw       |
| CAR_20_h3k27ac_13836.bw     |
| CAR_21_h3k27ac_8485.bw      |
| CAR_2_h3k27ac_13835.bw      |
| CAR_3_h3k27ac_13838.bw      |
| CAR_4_h3k27ac_13834.bw      |
| CAR_5_h3k27ac_8702.bw       |
| CAR_6_h3k27ac_7479.bw       |
| CAR_7_h3k27ac_8704.bw       |
| CAR_8_h3k27ac_8699.bw       |
| CAR_9_h3k27ac_13839.bw      |
| CAR_A_22_h3k27ac_8679.bw    |
| PNET_10_h3k4me2_16537.bw    |
| PNET_11_h3k4me2_16544.bw    |
| PNET_12_h3k4me2_16549.bw    |
| PNET_1_h3k27ac_14046.bw     |
| PNET_2_h3k27ac_8649.bw      |
| PNET_2_h3k4me2_16520.bw     |
| PNET_3_h3k27ac_8656.bw      |
| PNET_3_h3k4me2_16530.bw     |
| PNET_4_h3k27ac_9482.bw      |
| PNET_4_h3k4me2_16512.bw     |
| PNET_5_h3k27ac_14048.bw     |
| PNET_5_h3k4me2_16524.bw     |
| PNET_6_h3k27ac_8644.bw      |
| PNET_6_h3k4me2_16536.bw     |
| PNET_7_h3k27ac_8658.bw      |
| PNET_7_h3k4me2_16519.bw     |
| PNET_8_h3k27ac_8657.bw      |
| PNET_8_h3k4me2_16547.bw     |
| PNET_9_h3k4me2_16590.bw     |
| pnet_ffpe1_172_h3k4me2_16102.bw |
| pnet_ffpe2_4532_h3k4me2_16103.bw |
| pnet_ffpe3_14475_h3k4me2_16106.bw |
| pnet_ffpe5_6978_h3k4me2_16104.bw |
| pnet_ffpe6_3685_h3k4me2_16105.bw |
| pnet_ffpe7_14645_h3k4me2_16108.bw |
| pnet_ffpe8_8642_h3k4me2_16107.bw |
| CAR_10_h3k27ac_13811.bed   |
| CAR_11_h3k27ac_8691.bed     |
| CAR_12_h3k27ac_8701.bed     |
| CAR_13_h3k27ac_13810.bed    |
| CAR_14_h3k27ac_13809.bed    |
| CAR_15_h3k27ac_13832.bed    |
| CAR_16_h3k27ac_13807.bed    |
| Genome browser session (e.g. UCSC) | Carcinoid data: [https://genome.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=yotamd&hgS_otherUserSessionName=carcinoid_h3k27ac](https://genome.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=yotamd&hgS_otherUserSessionName=carcinoid_h3k27ac)  
PNET data: [https://genome-euro.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=yotamd&hgS_otherUserSessionName=pnet](https://genome-euro.ucsc.edu/cgi-bin/hgTracks?hgS_doOtherUser=submit&hgS_otherUserName=yotamd&hgS_otherUserSessionName=pnet) |
|---|---|
| **Methodology** | **Replicates** | N/A |
| **Sequencing depth** | All ChIP-seq data are paired-end. Library information is included in Suppl. Table 1. |
| **Antibodies** | For ChIP: H3K27ac (Active Motif 39133; lot #01613007), H3K27ac (Diagenode C15410196; lot #A1723-0041D), H3K4me2 (Millipore 07-030; lot #2477948). All antibodies have human reactivity and are validated for ChIP. For immunohistochemistry and immunofluorescence: ARX R&D Systems AF7068), PDX1 (Abcam ab134150), NEUROG3 (Millipore AB5684), and Somatostatin (Agilent A056601-2) antibodies were used to stain tissue sections after appropriate blocking of specimens to reduce non-specific background signals. |
| **Peak calling parameters** | Peaks were called using homer 4.6 with the command findPeaks using parameters -style histone -L 0. Super enhancers were called using ROSE (Whyte et al, Cell 2013; 153:307-319) |
| **Data quality** | We required IP efficiency of at least 5%, with at least 20,000 peaks detected at FDR of 0.001 |
| **Software** | BWA 0.7.10, igvtools 2.3, featureCounts 1.6.2, bedtools 2.26, DESeq 2, HOMER 4.6, ROSE |