

Supplementary Information

Mapping histologic and functional maturation of human endocrine pancreas across early postnatal periods *Saunders, Feng, Hopkirk, Aamodt, Hart, & Pan et al.*

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Supplementary Figure 1 (related to Fig 1)

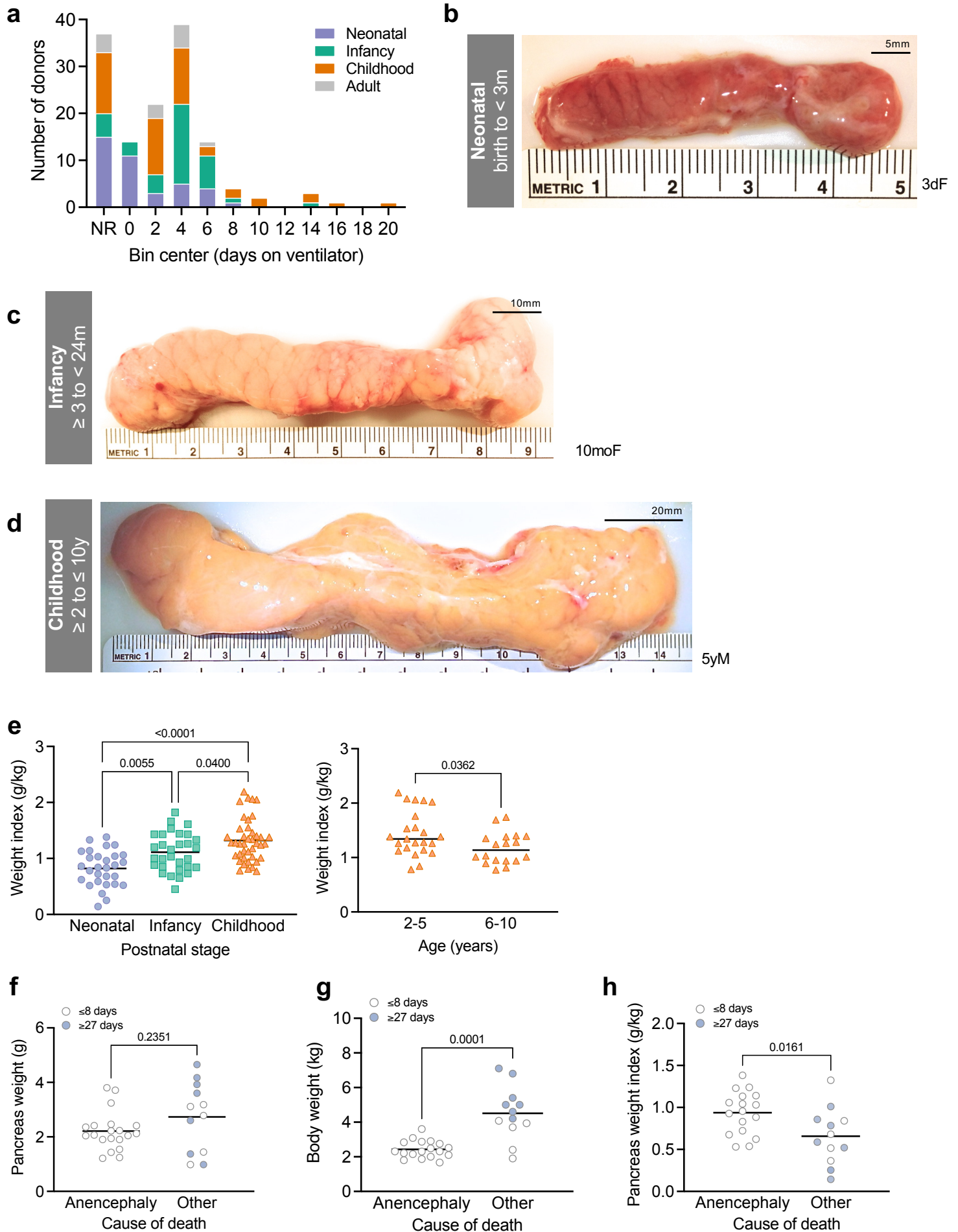


Figure S1. Pancreas size and pancreatic weight index increased with age. (a) Days on ventilator for donors by developmental stage. NR: not recorded. (b–d) Images showing gross morphology and length of select postnatal pancreata from neonatal (b), infancy (c), and childhood (d) developmental stages (HDL076, DON10, and DON7; see **Table S1** for details). (e) Weight index (ratio of pancreas weight to body weight) significantly increased from neonatal to childhood (one-way ANOVA, $F=18.63$, $p<0.001$); however, standard deviations were not significantly different among groups (Brown-Forsythe test, $F=0.06396$, $p=0.9381$). See also **Figure 1i**. (f–h) Pancreas weight (f), body weight (g) and weight index (h) of neonatal donors stratified by cause of death. Donors aged 0–8 days denoted by white circles. *P*-values shown are from Tukey’s multiple comparisons test (e) or two-tailed from Mann Whitney tests (e–h). Source data are provided as a Source Data file.

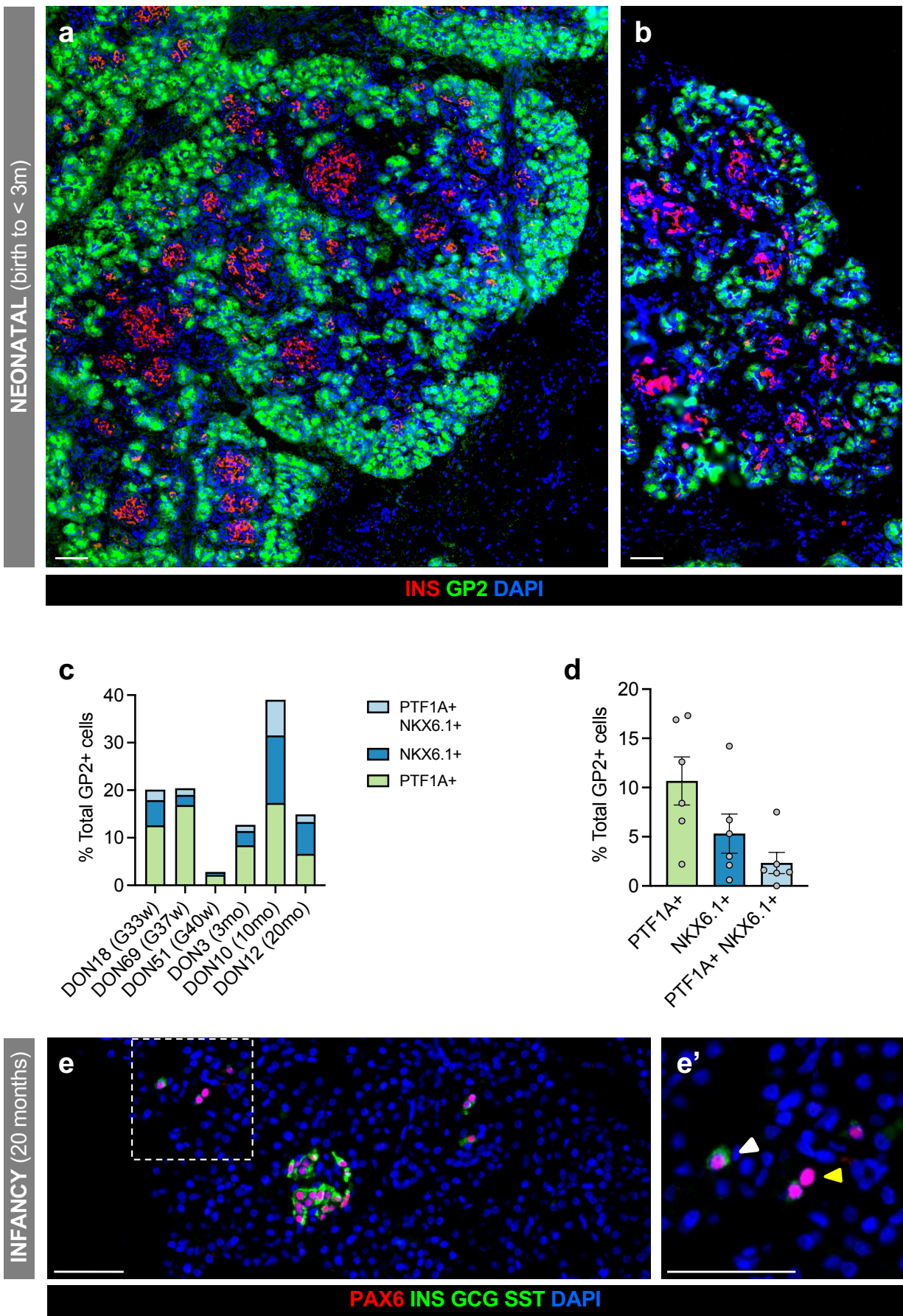


Figure S2. GP2+ multipotent progenitors, as well as proto-endocrine PAX6+ post-mitotic cells, persisted in human pancreas after birth. (a–b) Traditional IHC images of pancreatic sections from human neonatal donors born at 33 (a) and 40 (b) weeks of gestation (DON18 and DON51, see **Table S1** for details). GP2 expression was highest around periphery of pancreatic lobes. Scale bars 50µm. A: DON18, B: DON51. **(c–d)** GP2+ multipotent progenitor cells co-expressing PTF1A and NKX6.1 varied by donor ($n=6$ donors; 3 neonatal and 3 infancy) (d) with a higher percentage of GP2+ cells co-expressing PTF1A compared to NKX6.1 or both PTF1A and NKX6.1 (d). GP2+ cells counted: 2203 ± 784 per donor. **(e–e')** Newly formed endocrine cells appeared around lobe periphery during the neonatal (see **Figure 2d–e**) and infancy stages. Area within dashed line in e is magnified in e'; white arrowheads indicate PAX6+, hormone-positive cells; yellow arrowheads indicate PAX6+, hormone-negative cells. Scale bars 50µm, DON12 (see **Table S1** for details). Source data are provided as a Source Data file.

Supplementary Figure 3 (related to Fig 2)

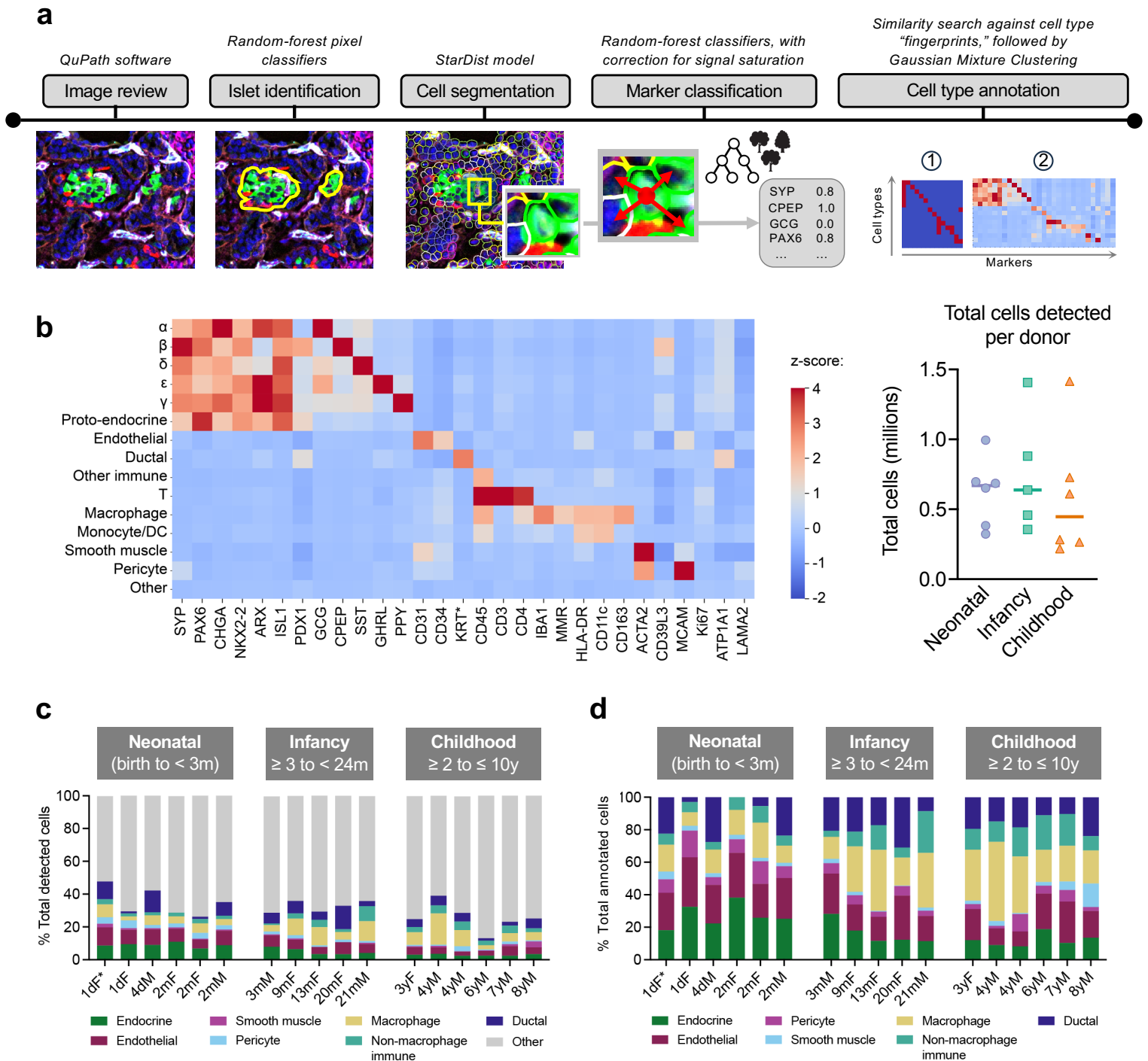


Figure S3. CO-Detection by indexing (CODEX) enables annotation of pancreatic cell types and simultaneous evaluation of multiple transcription factors. (a) Schematic of CODEX image analysis custom fingerprint pipeline for cell annotation. Images were loaded into QuPath and islets and clusters were annotated using pixel classifiers trained on manually labeled islets. Cells were then segmented with the StarDist plugin. For each marker, a random forest classifier was trained on ~60 manually annotated cells. To correct for signal saturation and spillover, marker scores were adjusted downward when neighboring cells exhibited stronger marker intensity. These marker scores were then compared to a predefined fingerprint map of cell–marker correspondences to assign preliminary cell types. Finally, a Gaussian mixture model was applied to resolve uncertain cases and refine the cell type annotations. (b) Average marker intensities for each cell type. Marker intensities per cell type were averaged within each image and then aggregated, and then z-score normalized across cell types for each marker. Mean number of cells detected per image: 0.62 million neonatal, 0.75 million infancy, and 0.59 million childhood. (c-d) Percentage of each annotated cell type by donor in comparison to the total number of detected cells by cell segmentation (c) and cells with assigned annotations (d). Of note, neonatal donor HDL027 did not have any ductal cells annotated due to no ductal marker passing QC (see Table S2B). Source data are provided as a Source Data file.

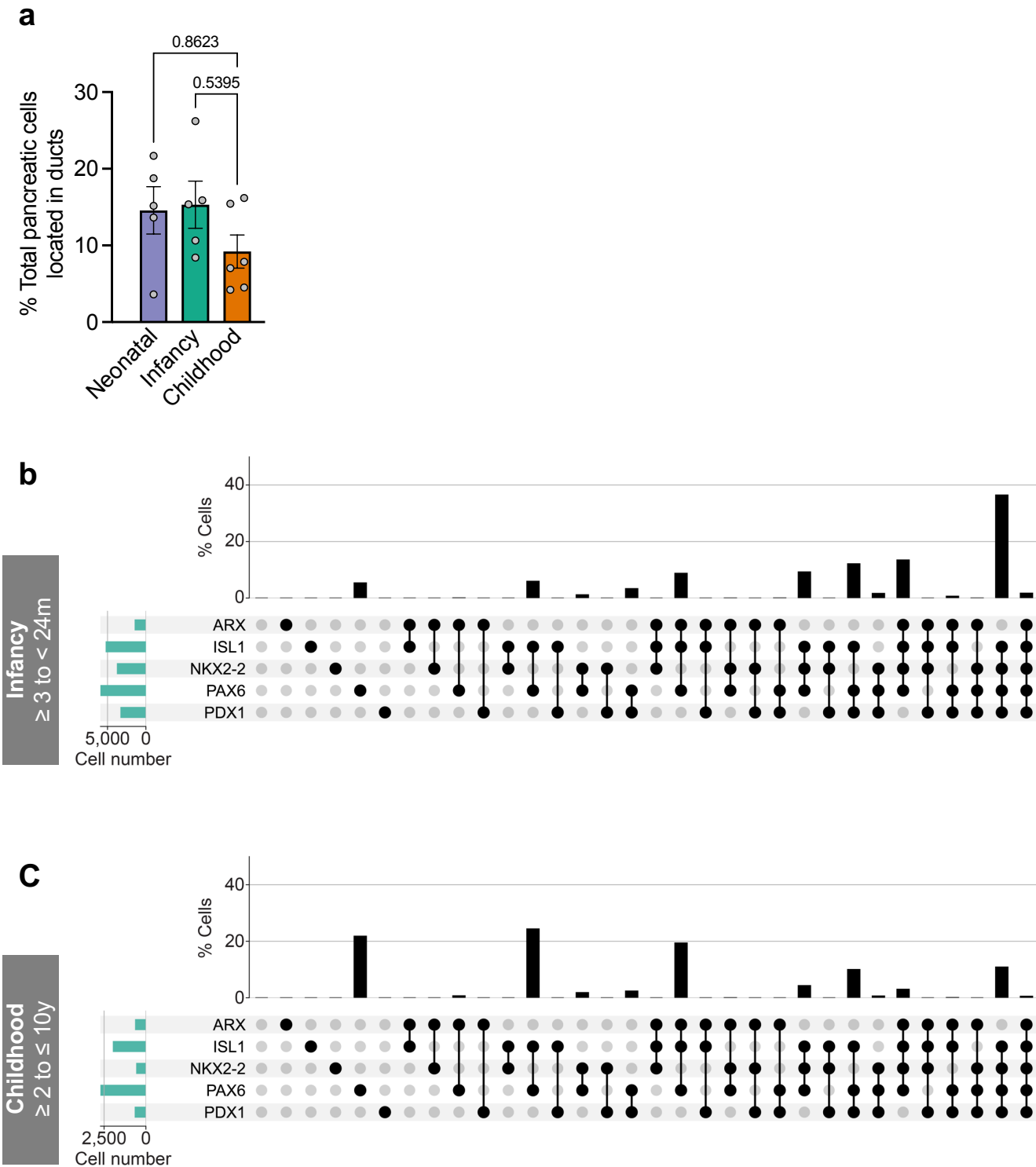


Figure S4. GP2+ multipotent progenitors, as well as proto-endocrine PAX6+ post-mitotic cells, were detected in human pancreas during infancy and childhood. (a) The percentage of all pancreatic cells located in ductal regions does not differ across developmental stages. Ductal regions are identified by random-forest pixel classifiers in QuPath software using KRT or KRT19 markers (see **Table S2B**). Adjusted *p*-values reflect Kruskal-Wallis and Wilcoxon rank-sum tests. (b-c) UpSet plot showing co-expression patterns of transcription factors among TF+ hormone-negative cells pooled across pancreata from infancy (*n*=5 donors; 15,289 total cells analyzed) (b), and childhood (*n*=6 donors; 8,566 total cells analyzed) (c) stages by analysis of CODEX images. See **Figure 2j** for neonatal plot. Source data are provided as a Source Data file.

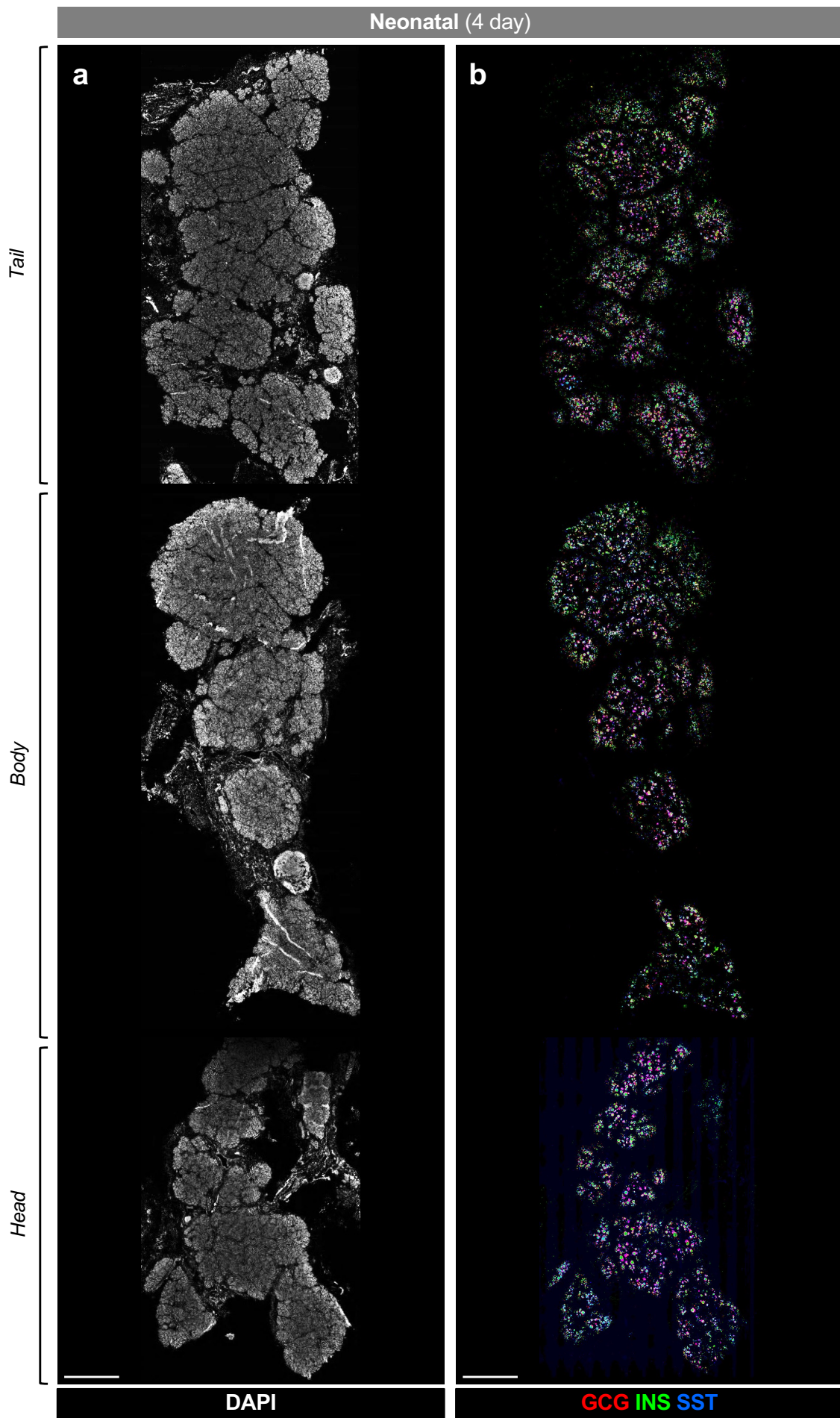


Figure S5.
Endocrine cells were distributed throughout all pancreas regions at birth. Macro view of neonatal pancreas (DON22, see **Table S1**) was generated by recombining longitudinal sections from tail, body, and head regions. Sections were counterstained with DAPI (**a**) and endocrine cells visualized by labeling for insulin (INS, green), glucagon (GCG, red), and somatostatin (SST, blue) (**b**). Scale bars, 2 mm.

Supplementary Figure 6 (related to Fig 3-4)

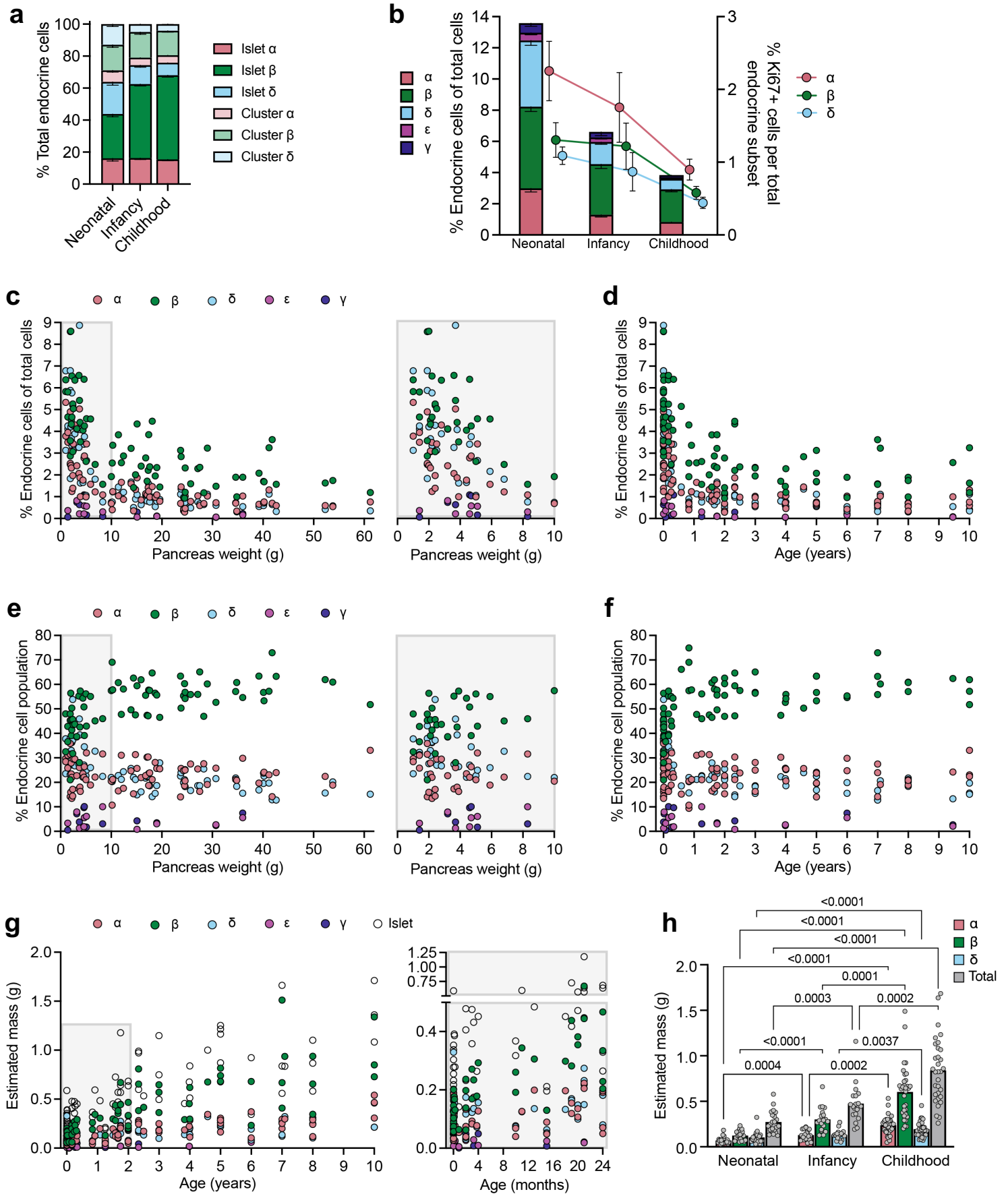


Figure S6. Relative proportions of endocrine cell populations and mass during postnatal pancreas growth.

(a) Percentage of each endocrine cell subtype (α , β , and δ cells) located in islets versus clusters at each developmental stage (neonatal, $n=6$; infancy, $n=5$; childhood, $n=6$) by CODEX image analysis. **(b)** Analysis of traditional IHC images at each developmental stage, corresponding to **Figure 4a-c**. Stacked bars represent density of endocrine cells (of total pancreatic cells), mean + SEM of β (green), α (red), δ (blue), ϵ (magenta), and γ (yellow) cells. Symbols show average proliferation frequency of α , β , and δ cells. Error bars represent $\pm$ SEM. **(c-f)** Endocrine cell density **(c-d)** and composition **(e-f)** of β (green), α (red), δ (blue), ϵ (magenta), and γ (yellow) cells expressed in relationship to pancreas weight **(c, e)** and donor age **(d, f)**; α , β , δ cells, $n=79$ donors; ϵ , γ cells, $n=12$ donors. Data between the y-axis and the vertical line $x=10$ are enlarged in panels on the right **(c, e)**. **(g-h)** Estimated mass based on pancreatic weight multiplied by percentage endocrine cell population of total cell population, expressed in relationship to age. Adjusted p -values reflect Kruskal-Wallis and Wilcoxon rank-sum tests. Source data are provided as a Source Data file.

Supplementary Figure 7 (related to Fig 3-4)

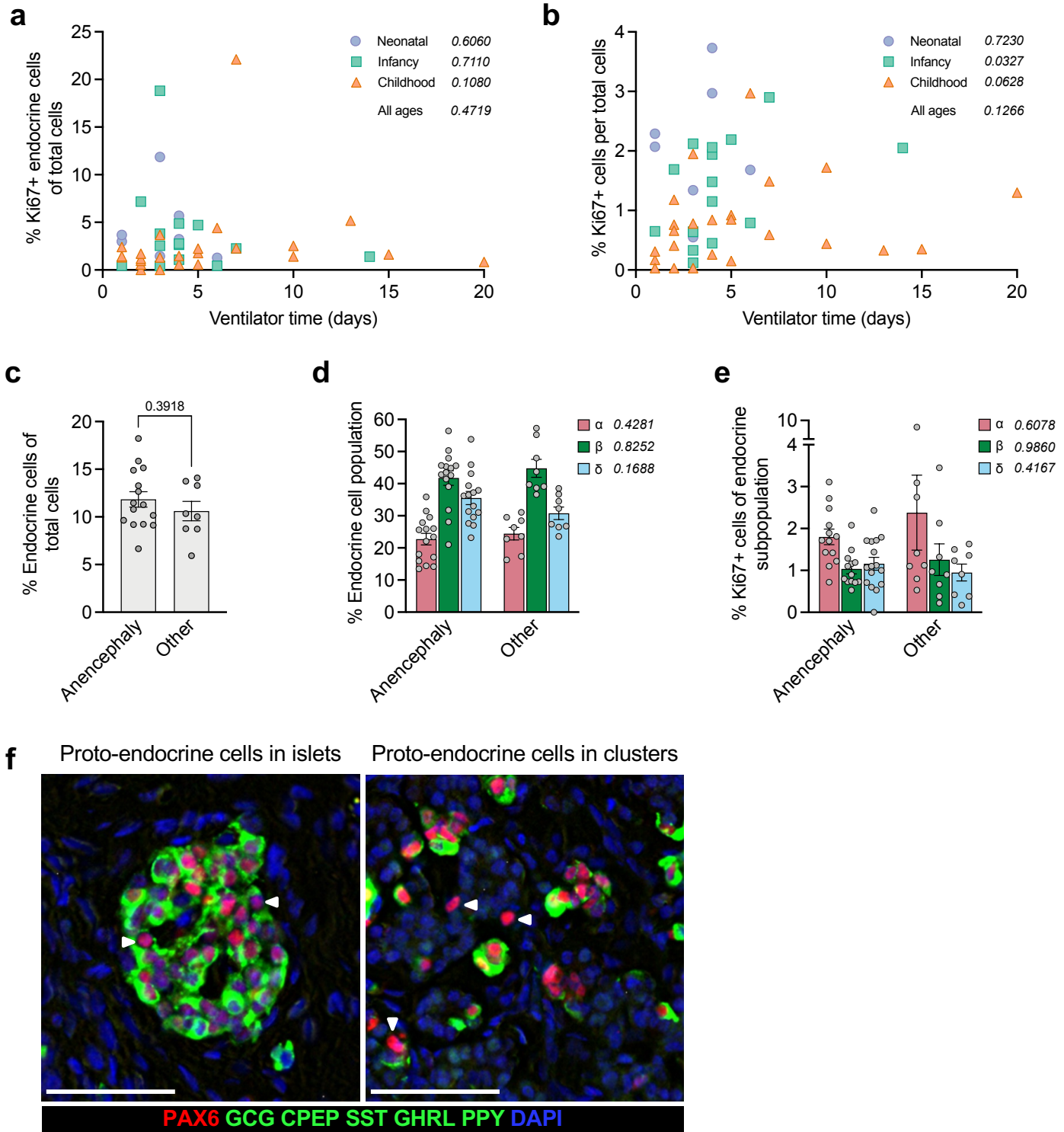


Figure S7. Relative proportions of β and δ cell populations changed during postnatal pancreas growth, but endocrine cell abundance, proportion, and proliferation were unchanged by cause of death or ventilator time prior to organ donation. (a–b) Neither total pancreatic (a) nor endocrine cell (b) proliferation varied according to ventilator time (Spearman correlation, $p > 0.05$) in the combined cohort ($n = 7$ neonatal, $n = 16$ infancy, $n = 24$ childhood donors). P -values for each age group in isolation are shown. Ventilator time was not available for all donors (see **Table S1**). **(c–e)** Endocrine cell characteristics in study cohort did not vary by cause of death. Mean $\pm$ SEM of endocrine cell density (c), islet composition (d) and endocrine cell proliferation (e) of neonatal donors stratified by cause of death; $n = 15$ anencephaly and $n = 9$ other. Adjusted p -values reflect Mann-Whitney (c) or Kruskal-Wallis and Wilcoxon rank-sum tests (d–e). **(f)** Representative image of proto-endocrine cells detected in neonatal pancreas (HDL044; see **Table S1**). Scale bars 50 μ m. Source data are provided as a Source Data file.

Supplementary Figure 8 (related to Fig 4)

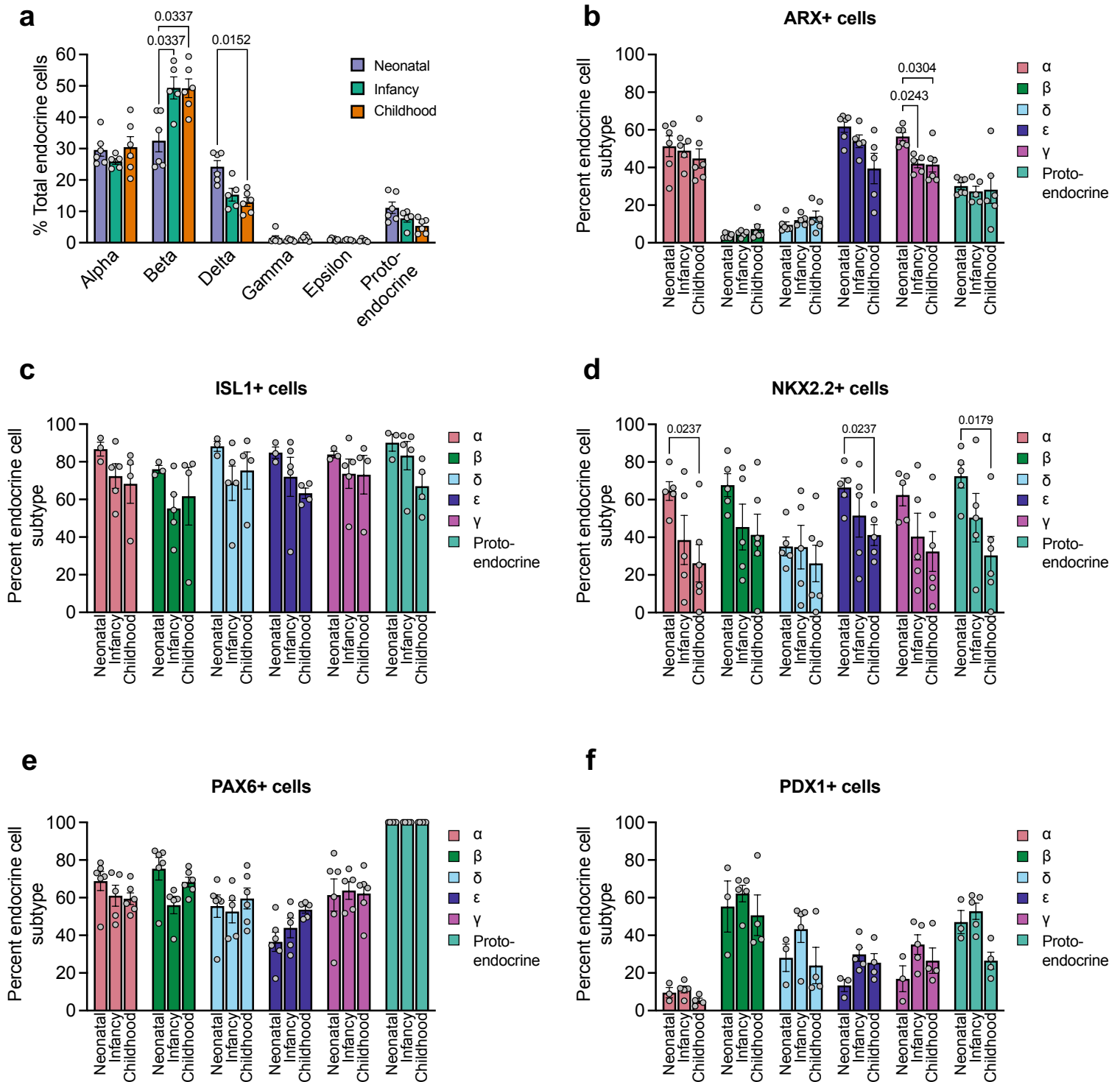


Figure S8. Transcription factor expression in endocrine cells changes during postnatal development. (a) Mean $\pm$ SEM of endocrine cell composition as percentage of total endocrine cells by analysis of CODEX images at each developmental stage (neonatal $n=6$; infancy $n=5$; childhood $n=6$ donors). (b-f) The percentage of each endocrine cell subtype in CODEX images that were positive for ARX (b), ISL1 (c), NKX2-2 (d), PAX6 (e), and PDX1 (f) across all developmental stages. Total endocrine cells for a-f were defined as PAX6+ cells with subpopulation annotations determined by cell-type specific hormone expression. Adjusted p-values (<0.05) reflect Kruskal-Wallis and Wilcoxon rank-sum tests. Source data are provided as a Source Data file.

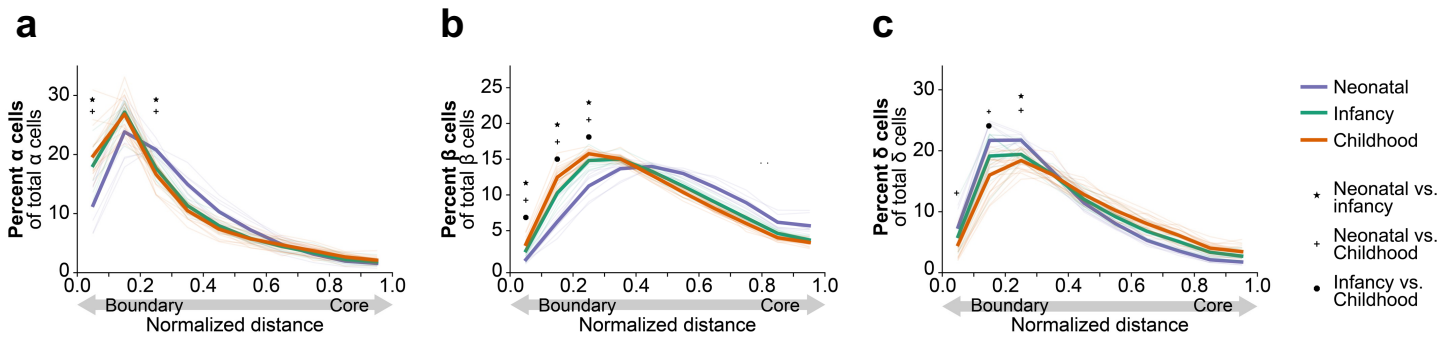


Figure S9. Spatial distributions of individual endocrine cell types within human islets change during postnatal development. (a-c) Spatial distribution of α (a), β (b), and δ (c) cells across normalized boundary-to-core positions in human islets analyzed from IHC images. Using the normalization algorithm for islet architecture analysis (see **Figure 5a** and **Methods**), each endocrine cell's position within an islet was assigned a normalized distance (0–1) and grouped into 10 equal bins from boundary to core. For each donor, the percentage of each endocrine cell type within a given bin was calculated relative to the total number of that same cell type across the entire islet (e.g., number of α cells in 0–0.1 $\div$ total number of α cells). The bin-wise distribution for each endocrine cell type per donor and averages across donors were plotted. Statistical comparisons were performed at the donor level using two-sided Mann–Whitney tests to compare developmental stages (neonatal, $n=9$; infancy, $n=11$; childhood, $n=21$ donors). Marks indicate bins with $p<0.05$ for the corresponding pairwise comparisons. Source data are provided as a Source Data file.

Supplementary Figure 10 (related to Fig 5)

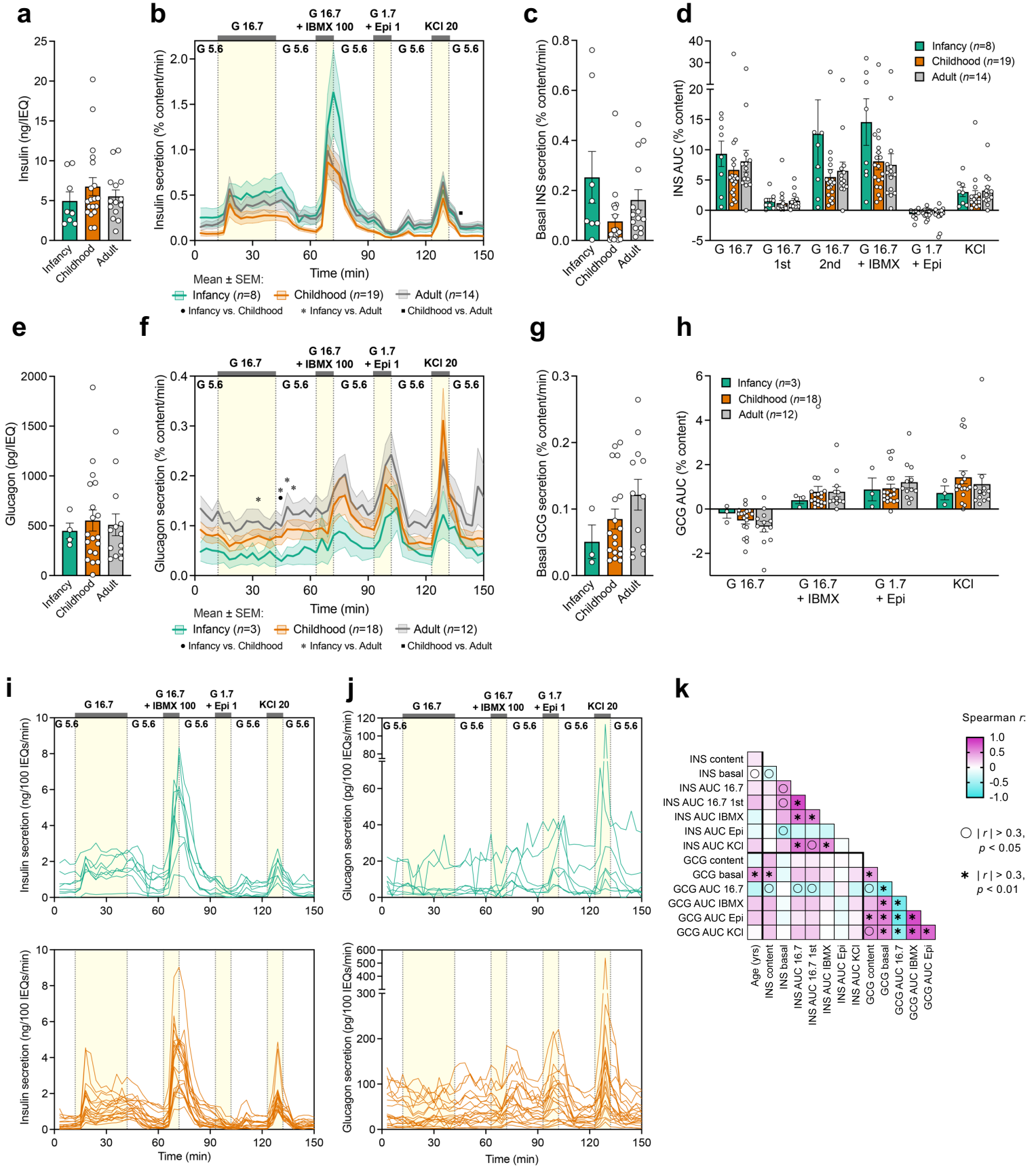


Figure S10. Normalization to islet hormone content masked functional changes in insulin and glucagon secretory responses by IEQ. (a–d) Insulin content (**a**) and dynamic insulin secretory response (**b–d**) of islets from infancy ($n=8$), childhood ($n=19$), and adult ($n=14$) donors measured by perfusion, normalized to insulin content. **(e–h)** Glucagon content (**e**) and dynamic glucagon secretory response (**f–h**) of islets from infancy ($n=3$), childhood ($n=18$), and adult ($n=12$) independent donors measured by perfusion, normalized to glucagon content. Differences in secretion trajectories were analyzed using a linear mixed-effect model (see **Table S3b**), and comparisons at each timepoint were assessed using Kruskal-Wallis and Wilcoxon rank-sum tests, controlling for FDR at 0.05. Marks in panels **b** and **f** indicate $FDR < 0.05$ for the corresponding timepoint comparisons. For glucagon, samples were excluded if glucagon fell below the limit of detection in islet extracts, and some historic samples had to be omitted due to inadequate archived material. See **Methods** for more details. Panels **d** and **h** show secretory response analyzed as area under the curve (AUC). Treatments and concentrations are shown along the top of the graph: Epi, epinephrine (μM); G, glucose (mM); IBMX, isobutylmethylxanthine (μM); KCl, potassium chloride (mM). **(i–j)** IEQ-normalized insulin (**i**) and glucagon (**j**) secretory responses of islets from individual infancy (teal, top) and childhood (pink, bottom) donors; aggregated data shown in **Figure 5e** and **5e**. **(k)** Matrix showing Spearman correlation of IEQ-normalized secretory traits from $n=28$ islet preparations from pediatric donors. Symbols denote absolute values of Spearman $r > 0.3$; $\bigcirc$, approximate $p < 0.05$; $\star$, approximate $p < 0.01$. Source data are provided as a Source Data file.

Supplementary Figure 11 (related to Fig 6)

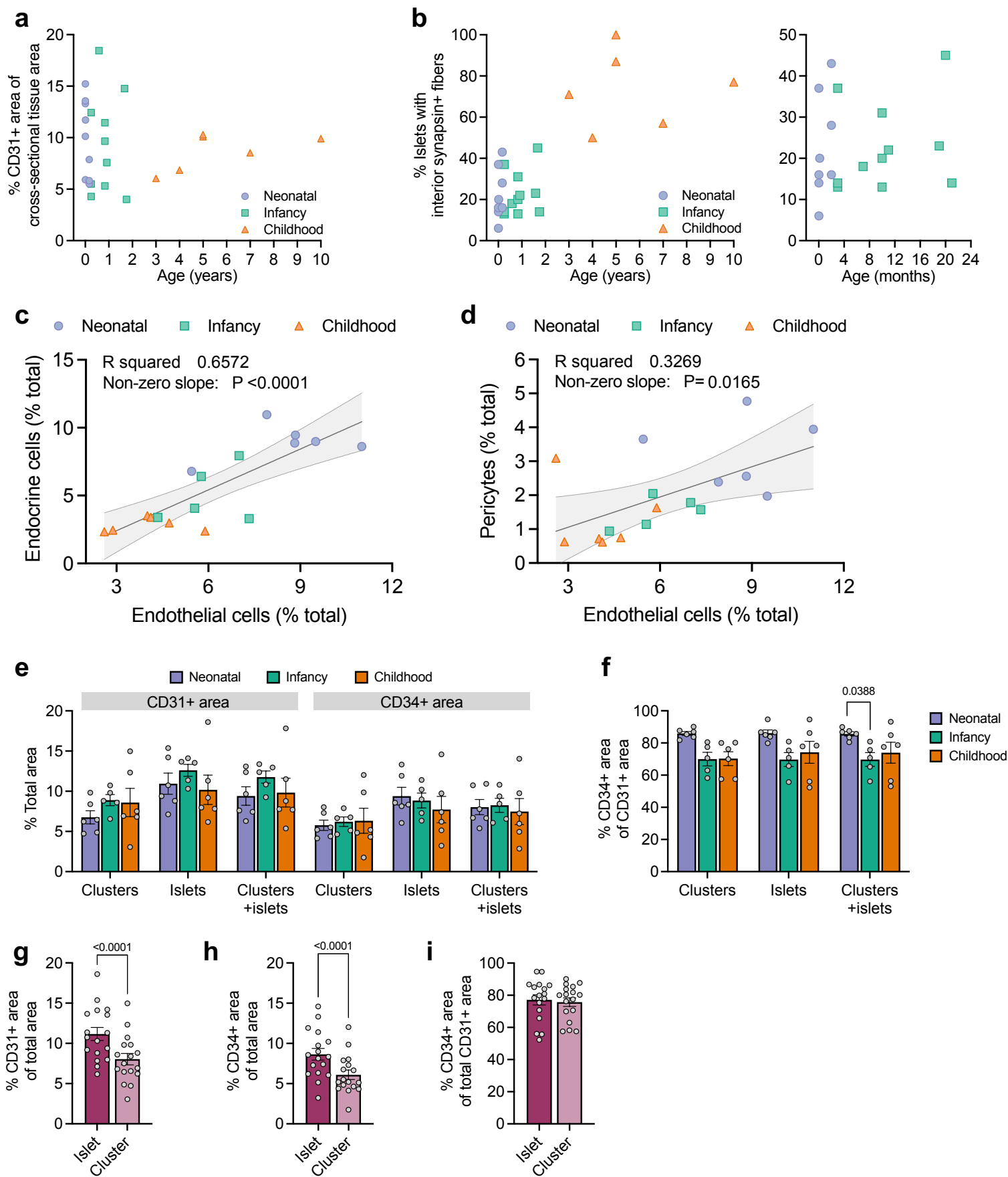


Figure S11. Islet innervation was delayed compared to islet vascularization. (a-b) Islet vascular density (**a**; defined as percentage CD31+ area of total cross-sectional tissue area) and innervation (**b**; defined as percentage of islets with interior synapsin-1/2+ fibers) depicted across stages of postnatal pancreas development based on analysis of traditional IHC images. See also **Figure 6a-h**. **(c-d)** Endothelial cell abundance in CODEX images was significantly correlated to endocrine cell (**c**) and pericyte (**d**) abundance by Spearman correlation ($r=0.7475$, $p=0.0009$ and $r=0.5956$, $p=0.0133$, respectively). Metrics for simple linear regression (95% confidence interval) are indicated on plots. **(e-i)** Endothelial (CD31+) area and area positive for stem cell biomarker CD34+ were analyzed in CODEX images across developmental stages (neonatal $n=6$, infancy $n=5$, and childhood $n=6$). There was no significant difference in CD31+ or CD34+ area as a percentage of total pancreas area (**e**) when considering clusters, islets, or both combined. However, the CD34+ area as a percentage of endothelial (CD31+) area (**f**) was higher in neonatal clusters compared to infancy. CODEX data from all ages ($n=17$) was pooled for analysis in **g-i**. Comparison of endothelial (CD31+) area (**g**) and CD34+ area (**h**) fractions between islets and clusters normalized to total tissue area showed higher CD31+ and CD34+ area in islets compared to clusters. There was no difference in CD34+ as percentage of total CD31+ area between islets and clusters (**i**). Adjusted p -values (<0.05) reflect Wilcoxon matched-pairs signed rank test (**g-i**) and Kruskal-Wallis and Wilcoxon rank-sum tests (**e-f**). Source data are provided as a Source Data file.

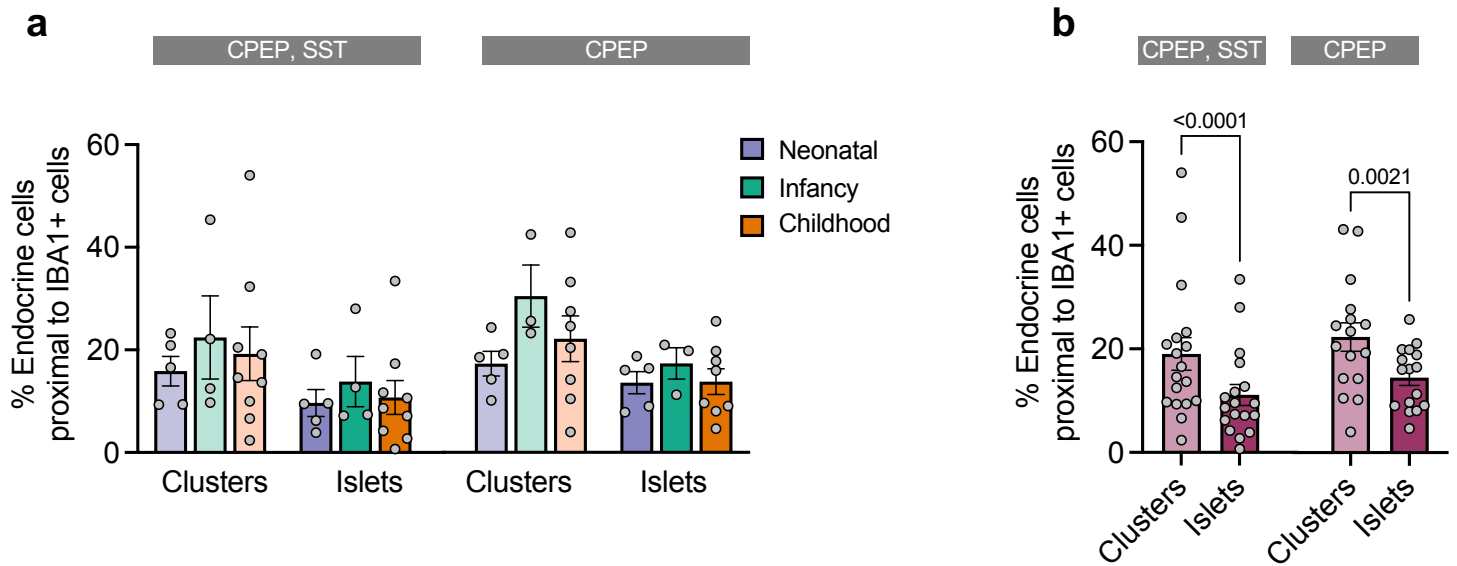


Figure S12. Macrophage proximity to islets and clusters in postnatal pancreas development. (a-b) Percentage of differentiated hormone+ endocrine cells located proximal to IBA1+ macrophages across developmental stages showed no difference stratified by islets and clusters (a). However, when all ages were combined (b) there were significantly more endocrine cells in clusters that were in proximity (<15 μ m) to macrophages compared to endocrine cells in islets. Adjusted p -values (<0.05) reflect Wilcoxon matched-pairs signed rank test (b) and Kruskal-Wallis and Wilcoxon rank-sum tests (a). Source data are provided as a Source Data file.

Supplementary Figure 13 (related to Fig 7)

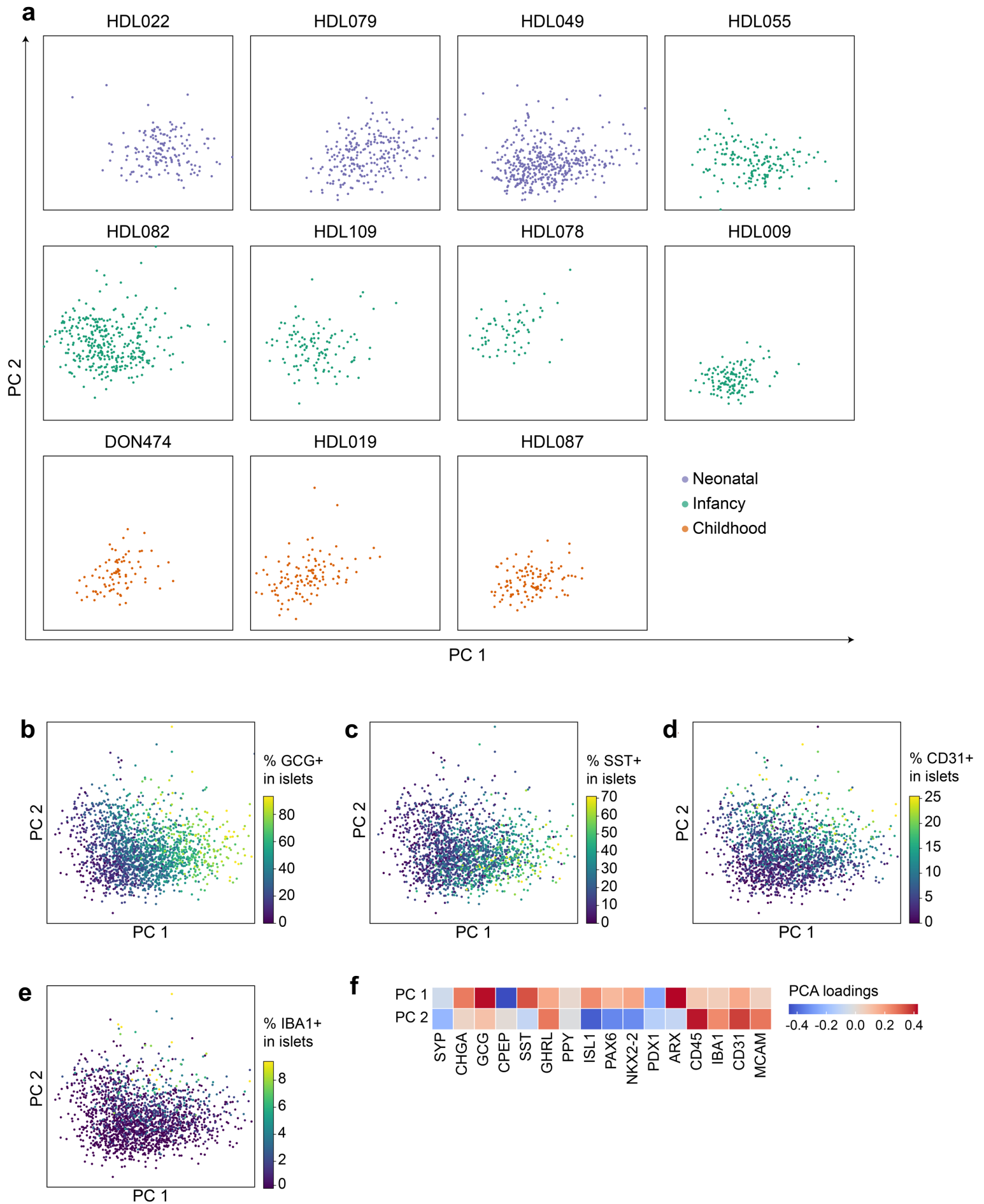


Figure S13. Principal component analysis of islet-level features across developmental stages. (a) Principal component analysis (PCA) of individual islets from 11 donors, shown separately for each donor (see **Table S1** for individual donor information). Each point represents one islet, colored by developmental stage (neonatal, $n=3$ donors, 838 total islets; infancy, $n=5$ donors, 825 total islets; childhood, $n=3$ donors, 307 total islets). PCA was performed using a combined feature set including islet morphological features and the percentage of marker-positive cells per islet. Axes indicate PC1 and PC2. (b–e) PCA plots of all islets pooled across donors, colored by the percentage of marker-positive cells within each islet by percentage of GCG+ cells (b), SST+ cells (c), CD31+ cells (d), and IBA1+ cells (e). Color scales indicate the proportion of marker-positive cells per islet. (f) Heatmap of feature loadings on PC1 and PC2. Loadings quantify the contribution of each original feature to each principal component. Source data are provided as a Source Data file.

Supplementary Figure 14 (related to Fig 7)

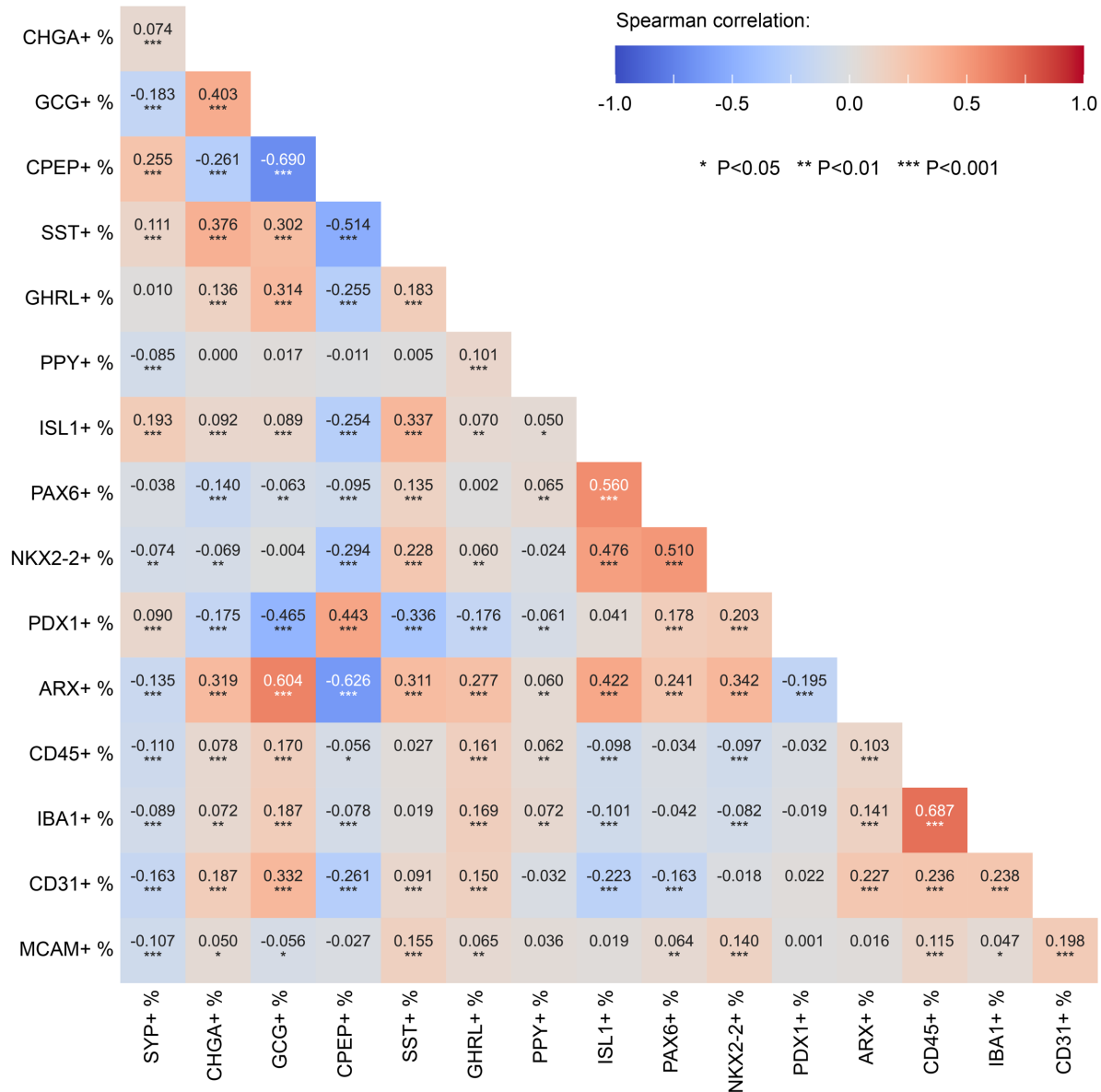


Figure S14. Correlation analysis among islet morphological and cellular composition features. Spearman correlation matrix of islet-level features, including morphological parameters and percentages of marker-positive cells per islet. Color indicates the Spearman correlation coefficient (ρ), with blue representing negative correlations and red representing positive correlations. Statistical significance is denoted by asterisks (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Correlations were computed across all islets pooled from all donors. Neonatal, $n[\text{islet}]=838$, $n[\text{donor}]=3$; infancy, $n[\text{islet}]=825$, $n[\text{donor}]=5$; and childhood, $n[\text{islet}]=307$, $n[\text{donor}]=3$. Source data are provided as a Source Data file.