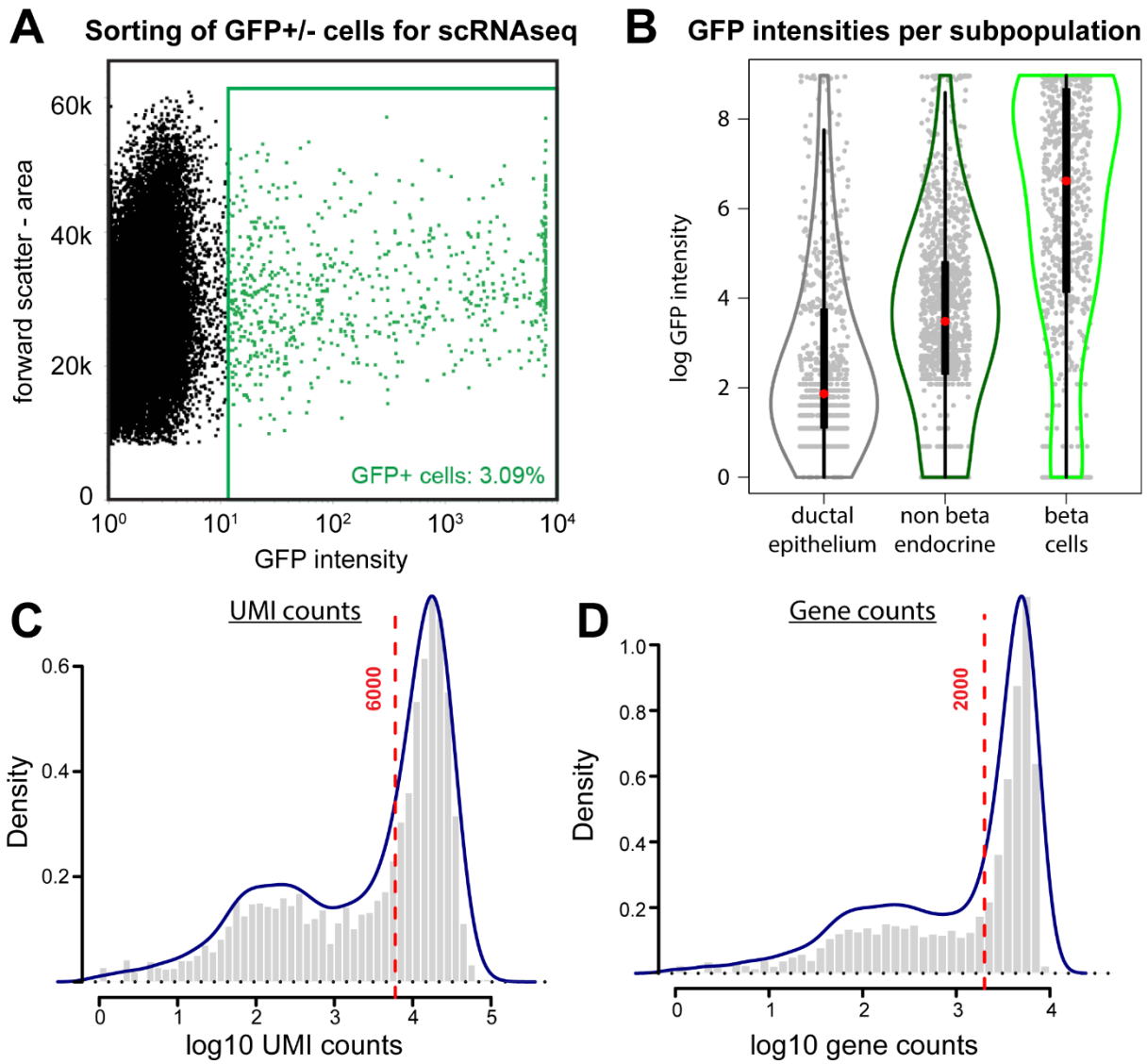
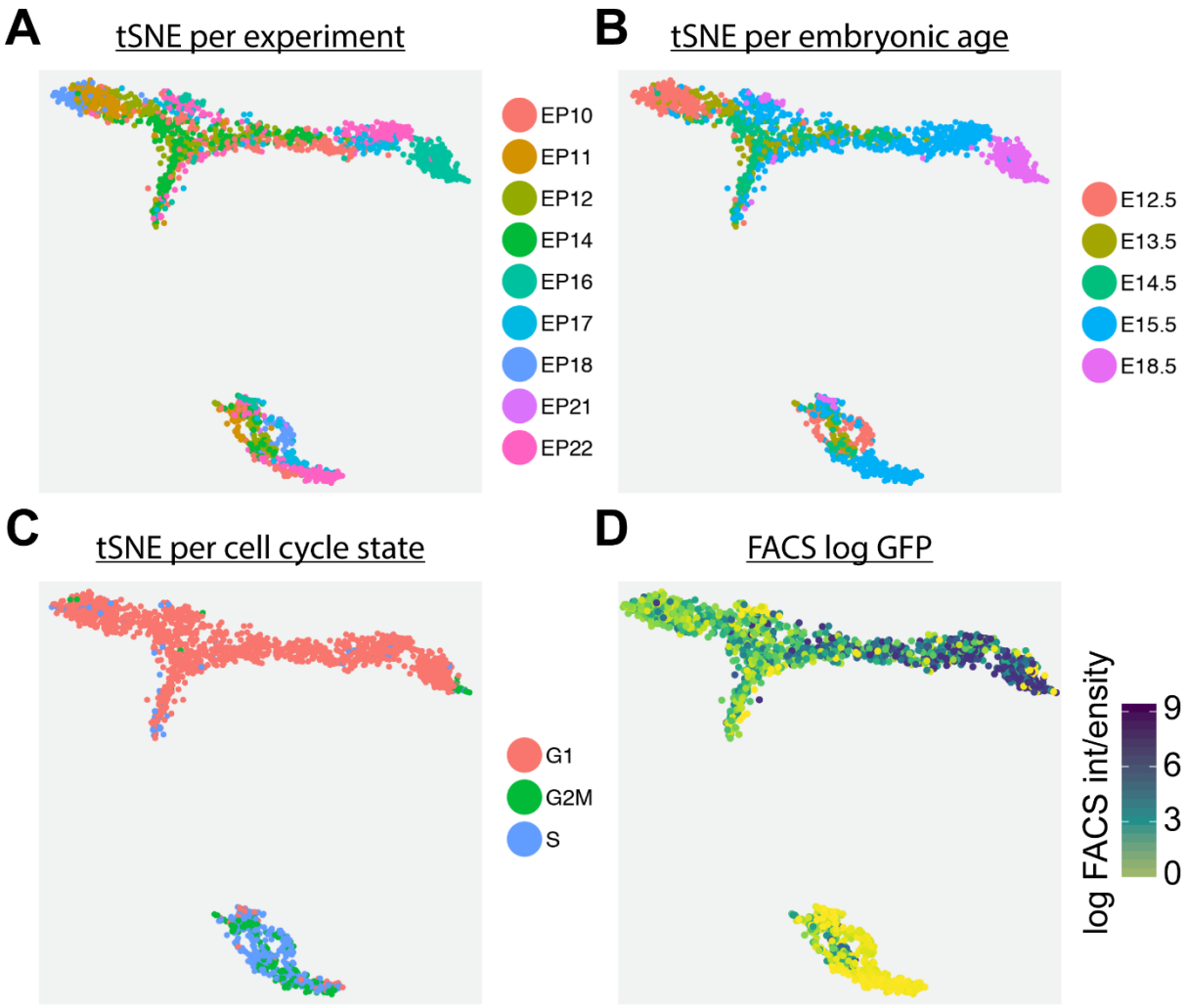


Figure S1: FACS cell acquisition and characteristics, and *in silico* quality control filtering. A) FACS plot of GFP intensity vs. forward scatter of cells isolated from E12.5 embryonic pancreas from MIP-GFP mice, indicating which cells were sorted as GFP positive cells (green box; 3.09% of all cells). Ungated cells were collected from the same plot, without GFP gating, and can thus also contain GFP positive cells. B) GFP intensity, as measured during FACS sorting, per subpopulation of the dataset (ductal epithelium = clusters 2, 3 and 6; non-beta endocrine = clusters 4, 5, 8 and 9; beta cells = clusters 1 and 7). Mean GFP intensity for non-beta endocrine cells was significantly upregulated compared to ductal epithelium ($p<0.01$) and beta cells had a significantly higher mean GFP intensity compared to non-beta endocrine cells ($p<0.01$). C) Histogram of raw data indicating the cell abundance for the number of UMIs per cell. The blue line indicates the density over the UMI counts. For quality control, cells with fewer than 6000 UMIs (red dashed line) were removed from the

dataset. D) Histogram of raw data indicating the cell abundance for the number of genes per cell. The blue line indicates the density over the number of genes. For quality control filtering, cells with fewer than 2000 expressed genes (red dashed line) were removed from the dataset.



E

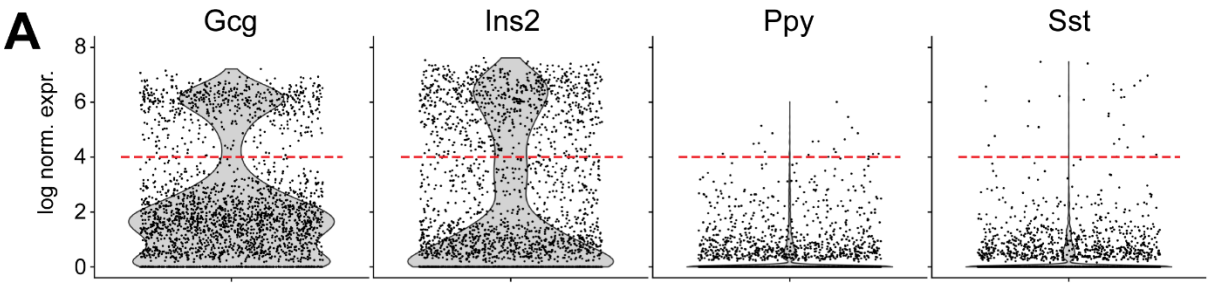
# cells	E12.5	E13.5	E14.5	E15.5	E18.5	tot/clust
clust 1	7	1	5	108	64	185
clust 2	24	34	25	107	6	196
clust 3	27	24	10	101	8	170
clust 4	41	76	77	86	9	289
clust 5	28	30	18	25	2	103
clust 6	77	71	40	48	20	256
clust 7	16	87	71	287	163	624
clust 8	330	10	7	43	22	412
clust 9	42	93	70	124	25	354
tot/E-age	592	426	323	929	319	2589

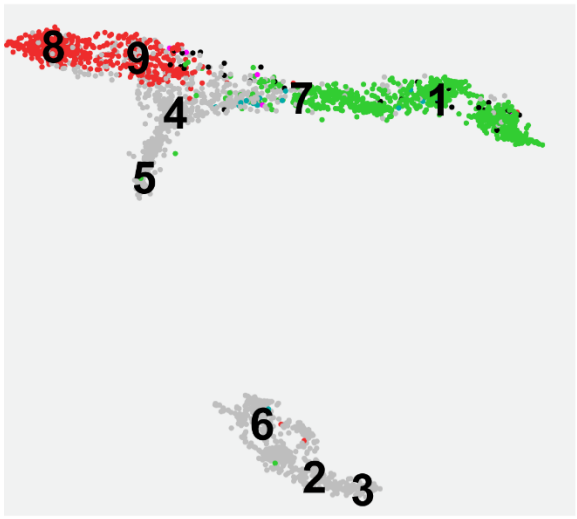
total # cells

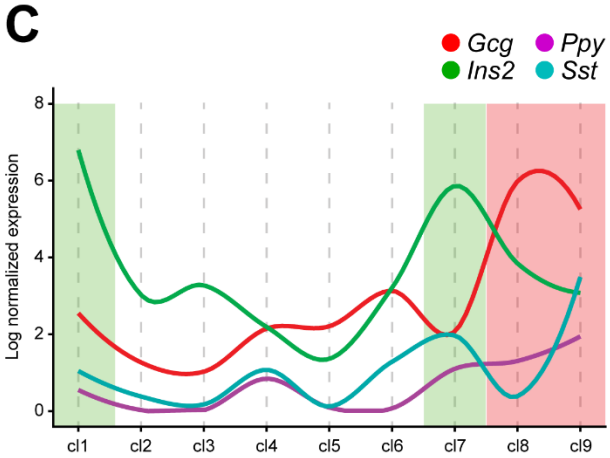
	E12.5	E13.5	E14.5	E15.5	E18.5
% of clust 1	3.8	0.5	2.7	58.4	34.6
% of clust 2	12.2	17.3	12.8	54.6	3.1
% of clust 3	15.9	14.1	5.9	59.4	4.7
% of clust 4	14.2	26.3	26.6	29.8	3.1
% of clust 5	27.2	29.1	17.5	24.3	1.9
% of clust 6	30.1	27.7	15.6	18.8	7.8
% of clust 7	2.6	13.9	11.4	46.0	26.1
% of clust 8	80.1	2.4	1.7	10.4	5.3
% of clust 9	11.9	26.3	19.8	35.0	7.1

	% of E12.5	% of E13.5	% of E14.5	% of E15.5	% of E18.5
clust 1	1.2	0.2	1.5	11.6	20.1
clust 2	4.1	8.0	7.7	11.5	1.9
clust 3	4.6	5.6	3.1	10.9	2.5
clust 4	6.9	17.8	23.8	9.3	2.8
clust 5	4.7	7.0	5.6	2.7	0.6
clust 6	13.0	16.7	12.4	5.2	6.3
clust 7	2.7	20.4	22.0	30.9	51.1
clust 8	55.7	2.3	2.2	4.6	6.9
clust 9	7.1	21.8	21.7	13.3	7.8

Figure S2: Cell type classification based on general characteristics. A) UMAP dimensional reduction, in which cells are color coded based on the experimental code. B) UMAP with color code for the embryonic age for each cell. C) UMAP with color code for cell cycle state of each cell. D) UMAP with superimposition of the log FACS GFP intensity for each cell. E) Distribution of cells from the different embryonic ages that were collected (E12.5, E13.5, E14.5, E15.5 and E18.5) over clusters. In the top left table, numbers of cells are given per embryonic age per cluster. Sums of cells per cluster are given to the right in the boxed area (tot/clust). Sums of cells per embryonic age are given on the bottom in the boxed area (tot/E-age). The total number of cells is the sum of either all the clusters, or all the embryonic ages (in red). The table in the top right indicates the percentage of cells in each cluster originating from a specific embryonic age. The table on the bottom left indicates the percentage of cells in each embryonic age allocated to a specific cluster. Color coding: light blue indicates a low percentage, dark blue indicates a high percentages.





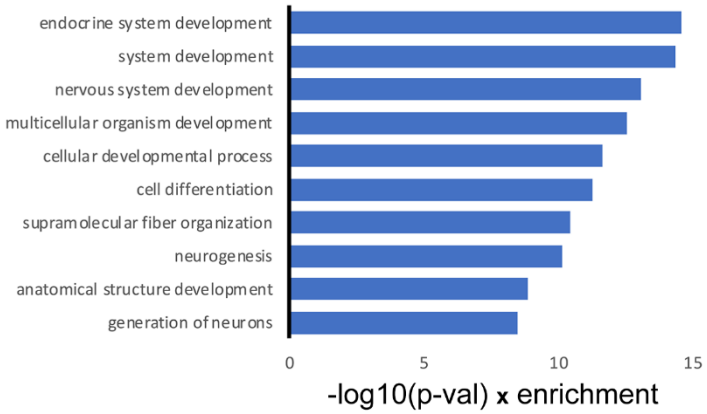


D

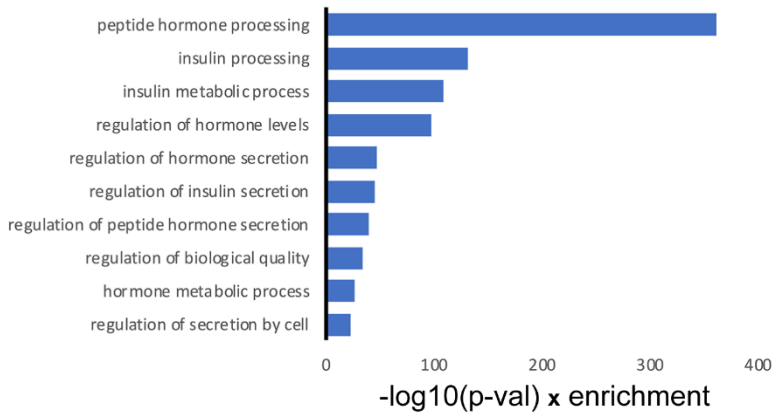
cluster	1	2	3	4	5	6	7	8	9	total
none	2	**188**	**160**	**278**	**102**	**237**	102	30	150	1249
Gcg	5	1	1			10	6	**355**	**157**	535
Ins2	**177**	6	9	8	1	8	**500**	20	15	744
Ppy				1			2		3	6
Sst		1		1		1	4		12	19
G + I	1						1	5	9	16
G + P								1	1	2
G + S										0
I + P							3		3	6
I + S							6			6
P + S				1					2	3
G + I + P								1	1	2
G + I + S										0
G + P + S										0
I + P + S									1	1
G + I + P + S										0
	185	196	170	289	103	256	624	412	354	2589

Figure S3: Classification of endocrine cells based on hormone expression. A) Violin plots of Gcg, Ins2, Ppy and Sst expression in the whole dataset. Expression is log-normalized. The red dotted line indicates the threshold for assigning cells as hormone expressing, at 4 log-normalized counts. B) UMAP plot indicating where hormone expressing cells can be found in the dataset. *Gcg* expressing cells in red, *Ins2* expressing cells in green, *Ppy* expressing cells in magenta and *Sst* expressing cells in cyan. Polyhormonal cells of any combination are indicated in black, all other cells are grey. Numbers indicate clusters.. C) Mean log-expression of all four hormones in individual clusters. Background color indicates when a cluster was identified as a hormone expression cluster (mean log expression above 4; red = *Gcg* cluster, green = *Ins2* cluster). D) Table representing the number of cells expressing a single hormone or any combination of hormones (indicated by the first letter, in the same color as for mono-hormonal cells), per cluster. The highest number of cells per cluster is printed bold. In total, more than 50% of all cells (1304/2589 cells) in the dataset are considered mono-hormonal, while 1.4% of all cells (36/2589 cells) can be considered poly-hormonal.

A



B



C

Top 10 genesets associated with cluster 5	Origin	FDR	NES
Translation	Reactome	0.00E+00	2.39
3' UTR mediated translational regulation	Reactome	0.00E+00	2.35
Influenza viral RNA transcription and replication	Reactome	0.00E+00	2.33
Ribosome	KEGG	0.00E+00	2.33
Cytosolic ribosome	GO	0.00E+00	2.32
Ribosome	GO	0.00E+00	2.32
Protein targeting to membrane	GO	0.00E+00	2.31
rRNA metabolic process	GO	0.00E+00	2.31
Peptide chain elongation	Reactome	0.00E+00	2.31
Nonsense mediated decay enhanced by the exon junction complex	Reactome	0.00E+00	2.31

D

Top 10 genesets associated with cluster 4	Origin	FDR	NES
Regulation of hormone levels	GO	5.34E-03	-2.24
Reactome regulation of insulin secretion	Reactome	3.74E-03	-2.22
Reactome integration of energy metabolism	Reactome	2.85E-03	-2.20
Transport vesicle	GO	2.40E-03	-2.19
Transport vesicle membrane	GO	1.05E-02	-2.12
KRAS signaling up	Hallmark	1.55E-02	-2.10
Vesicle membrane	GO	1.64E-02	-2.09
Intracellular vesicle	GO	2.35E-02	-2.07
Regulation of hormone secretion	GO	2.16E-02	-2.07
Regulation of peptide transport	GO	3.14E-02	-2.03

Figure S4: Pathway analysis of differential expression between the two endocrine progenitor clusters. A-B) using lists of genes that were upregulated for 1 of either cluster, gene ontology analysis was performed for biological processes in *mus musculus*. Results were ordered based on the multiplication between the -log10 p-value and the enrichment score of each GO term, to balance the influence of either factor. C-D) Gene-set enrichment analysis was performed on rank data from the differential expression analysis between the two endocrine progenitor clusters 4 and 5. Gene-sets with a false discovery rate (FDR) < 0.05 were ordered based on the normalized enrichment score (NES). The origin of the gene-set is given for reference.

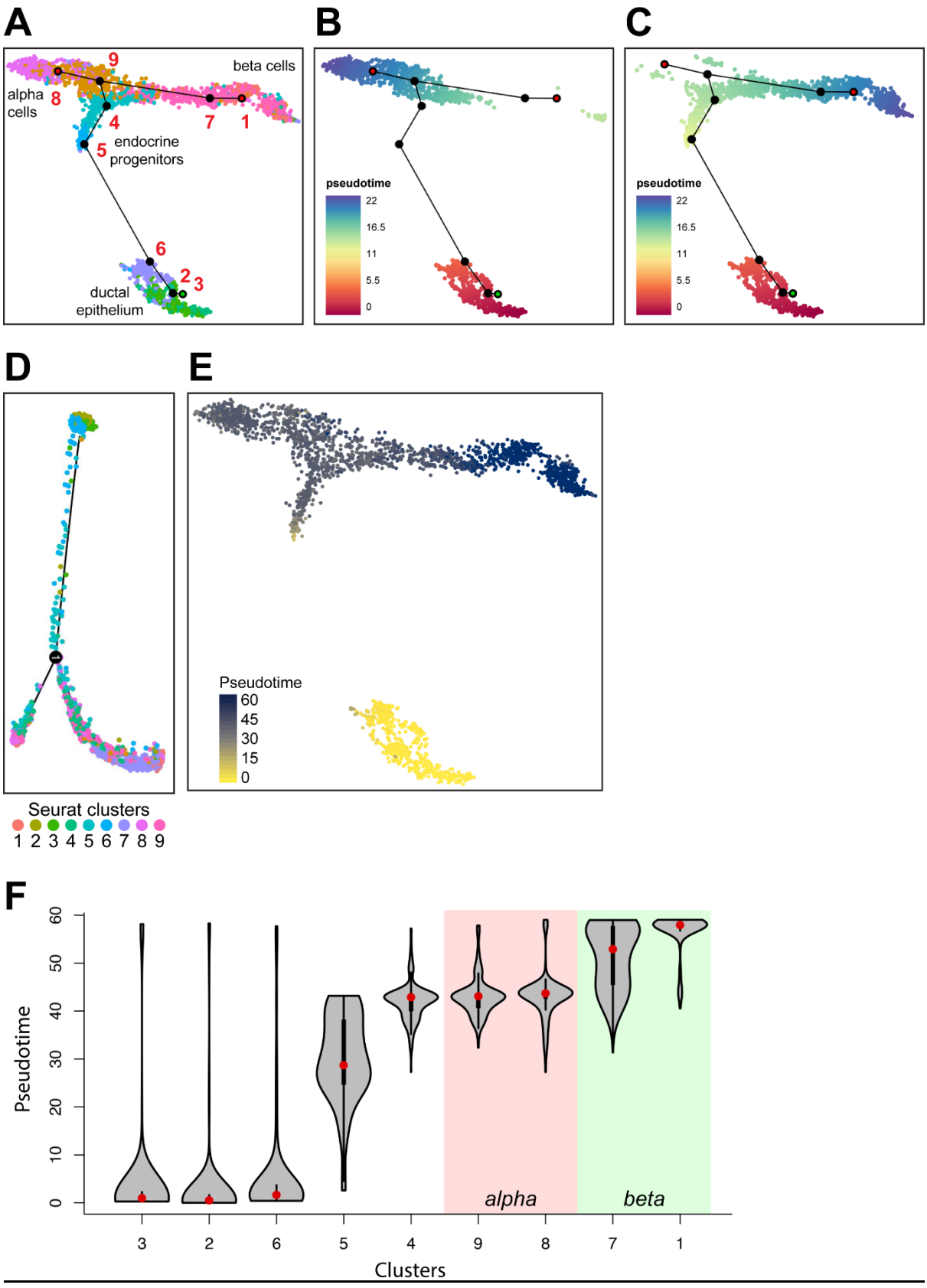


Figure S5: Details on the pseudotime analysis. A) Alternative pseudotime analysis performed using Slingshot. Trajectories are superimposed on top of the UMAP plot. Left panel: dots indicate medoids for each cluster, where a green dot (cluster 3) indicates a start-point and a red dot indicates an end-point (clusters 1 and 8). Lines between dots indicate connectivity between clusters, thus indicating progression and directionality (alpha cell trajectory: cluster 3 - 2 - 6 - 5 - 4 - 9 - 8; beta cell trajectory: 3 - 2 - 6 - 5 - 4 - 9 - 7 - 1). Middle panel: pseudotime progression for the alpha cell trajectory. Only cells are included that are designated to the alpha cell branch. Color indicates progression through pseudotime, from red via yellow to blue. Right panel: pseudotime progression for the beta cell trajectory. Only cells are included that are designated to the beta cell branch. Color indicates progression through pseudotime, from red via yellow to blue. D) Monocle-based trajectory for alpha and beta cell differentiation. Colors indicate the cluster cells originate from. E) UMAP plot of dataset. Color indicates progression through pseudotime, as calculated in monocle. F) Distribution of pseudotime, per cluster.

Table S1: Characteristics of 2589 individual cells that pass QC and after filtering non-pancreatic cell types, including the number of expressed UMIs and genes, experimental codes, embryonic age, cluster, cell cycle phase, pseudotime, and FACS characteristics

Click here to Download Table S1

Table S2: List of differentially upregulated genes per cluster. For each gene, log fold change and a bonferroni corrected p-value are given.

Click here to Download Table S2