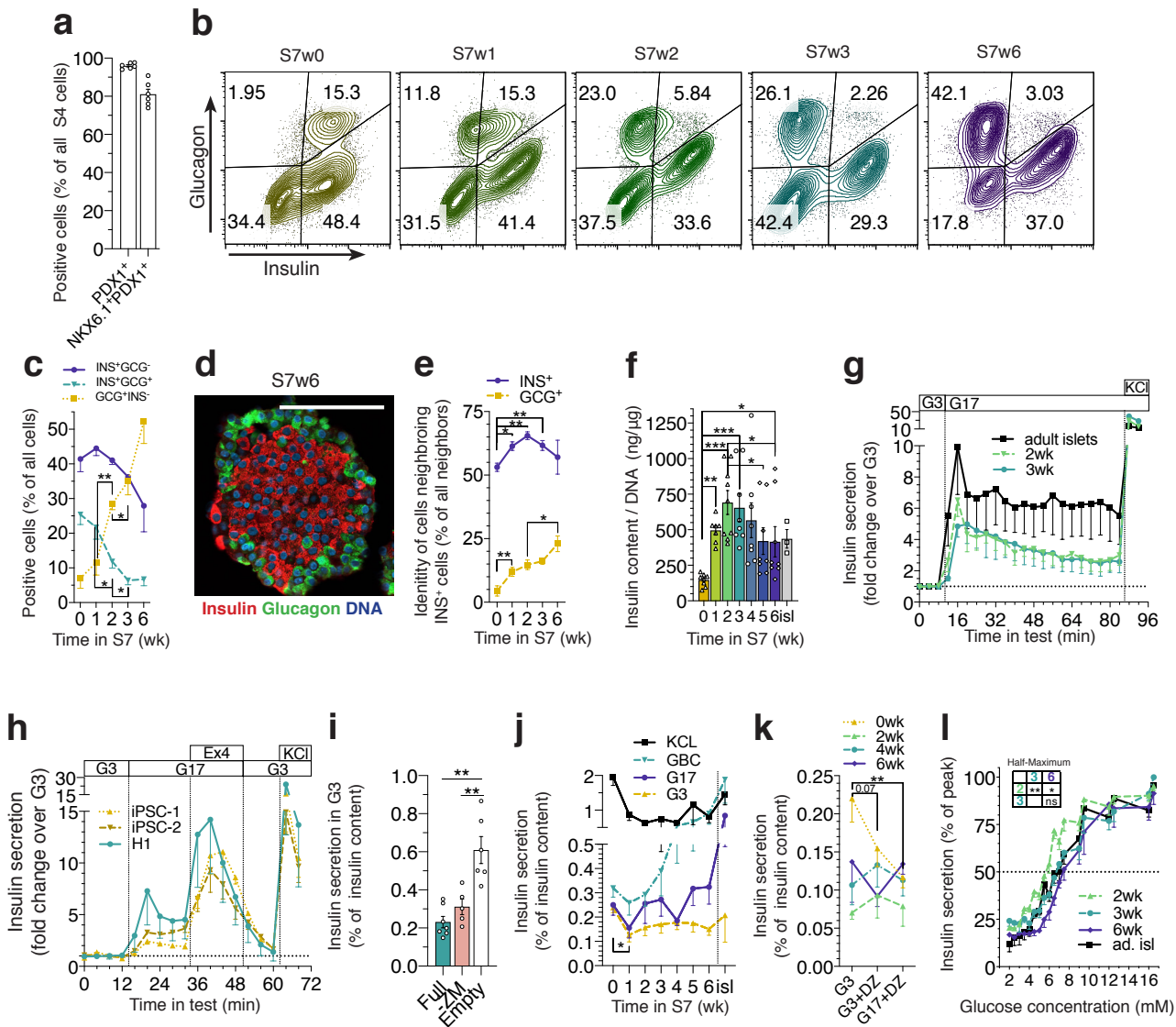

Supplementary information

Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells

In the format provided by the
authors and unedited



Supplementary Figure 1

(a) Quantification of flow cytometry for PDX1 and NKX6.1, conducted at S4 after the adherent-to-microwell dissociation.

(b) Representative flow cytometry for INS and GCG at stage 7

(c) Quantification of the populations in (a), n=3-7, Two-way ANOVA.

(d) Immunohistochemistry representing a core-mantle organized S7w6 SC-islet, the other extreme in the spectrum of cytoarchitecture at S7w6, n=2, scale bar = 100 μ m.

(e) INS⁺ and GCG⁺ nuclei within 8 μ m of INS⁺ nuclei, percentage of all nuclei within 8 μ m of INS⁺ nuclei. Quantified from INS – GCG immunostainings. n=2-9, population-wide characterizations of individual differentiations. Two-way ANOVA.

(f) Insulin content normalized to DNA content of the lysed SC-islets after static incubations (Supp.Fig.1j), One-way ANOVA with Welch's correction

(g) Insulin secretion response to 72-minute stimulation with 17mM glucose (G17). Normalized to secretion during 3mM glucose (G3) in the first 12 minutes of the test. n=3-4.

(h) Insulin secretion responses of S7w3 SC-islets to stimulation from 2.8 mM to 16.8 mM glucose (G3 to G17), 50 ng/ml exendin-4 (Ex4) and 30 mM KCl in perfusion. Comparison of two wildtype iPSC lines (n=2-3) and H1 hESC-line used in the rest of the study (n=18)

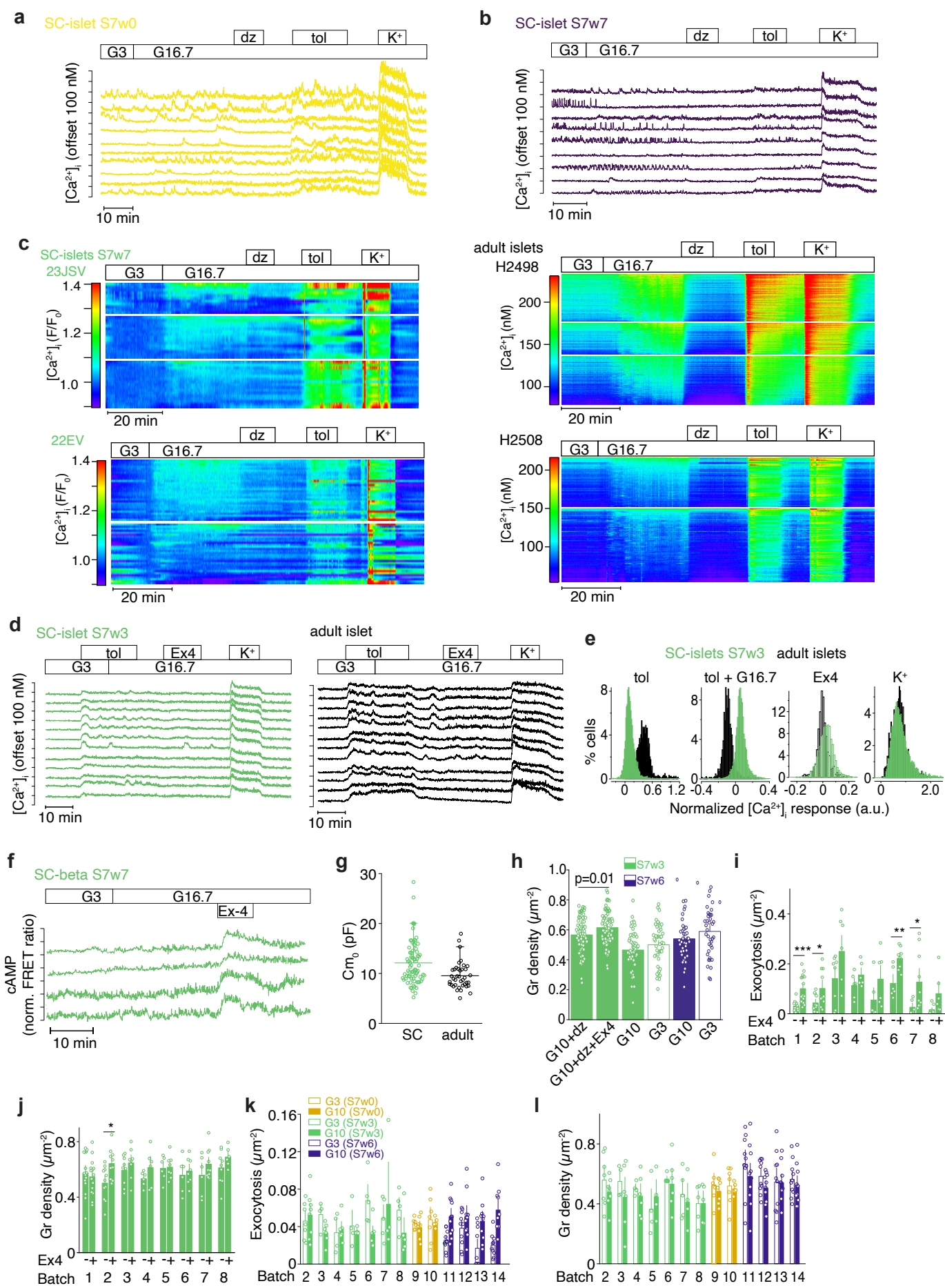
(i) Insulin secretion in G3 as percentage of total insulin content during a 30-minute incubation in a static insulin secretion assay, comparison of different media compositions used throughout S7. One-way ANOVA with Welch's correction.

(j) Insulin secretion as percentage of total insulin content during 30-min incubations in G3, G17, 0.1 μ M K_{ATP}-channel closing glibenclamide (GBC) and 30 mM KCl in a static test, n=3-8 for SC-islets, n=3 for primary islets, t-test with Welch's correction.

(k) Insulin secretion as percentage of total insulin content during 30-min incubations in G3, G3 + K_{ATP}-channel opener diazoxide 100 μ M (DZ) and G17 + DZ in a static test, n=3-7, Two-way ANOVA.

(l) Data in Figure 1l alternatively quantified as percentage of maximal secretion, plotted against the glucose concentration, statistics matrix shows significance for comparisons of the glucose concentration eliciting half-maximal secretion, n=4-6, One-way ANOVA.

All data are presented as mean $\pm$ SEM. * p < 0.05, ** p < 0.01, *** p < 0.001



Supplementary Figure 2

(a-b) Representative $[Ca^{2+}]_i$ recordings from SC-islets at S7w0 (a) and S7w7 (b) during exposure to 3 (G3) and 16.7 mM (G16.7) glucose, 250 μ M diazoxide, 1 mM tolbutamide and 30 mM K^+ .

(c) $[Ca^{2+}]_i$ recordings under similar conditions as in (a), exemplifying inter-islet and inter-preparation variability in SC- and primary islets. Top left heatmap shows all R-GECO1-expressing SC-beta cells in three different SC-islets from one SC preparation (23JSV). Bottom left is a similar recording from two SC-islets from an independent SC-preparation (22EV). The heatmaps on the right show $[Ca^{2+}]_i$ recordings with Fura-2 LR from three (top) and two (bottom) primary islets from two different human donors (H2498, H2508).

(d) $[Ca^{2+}]_i$ recordings from SC-islets at S7w2 and primary islets exposed to 1 mM tolbutamide, G16.7, 10 nM exendin-4 and 30 mM K^+ .

(e) Histograms showing the changes of $[Ca^{2+}]_i$ in response to various treatments from SC-islets (n=4079 cells) and primary islets (n=1187 cells).

(f) $[cAMP]_m$ recording from identified SC-beta cells exposed to G3, G16.7 and 10 nM Ex4.

(g) Whole cell capacitance as measure of cells size in SC- and primary islet cells. Symbols represent individual cells, and symbol color the cell preparation or donor.

(h) Docked granules measured as average granule density, normalized to footprint area, in conditions as indicated. Dots represent individual cells. $p = 0.01$, two-tailed Student's t-test.

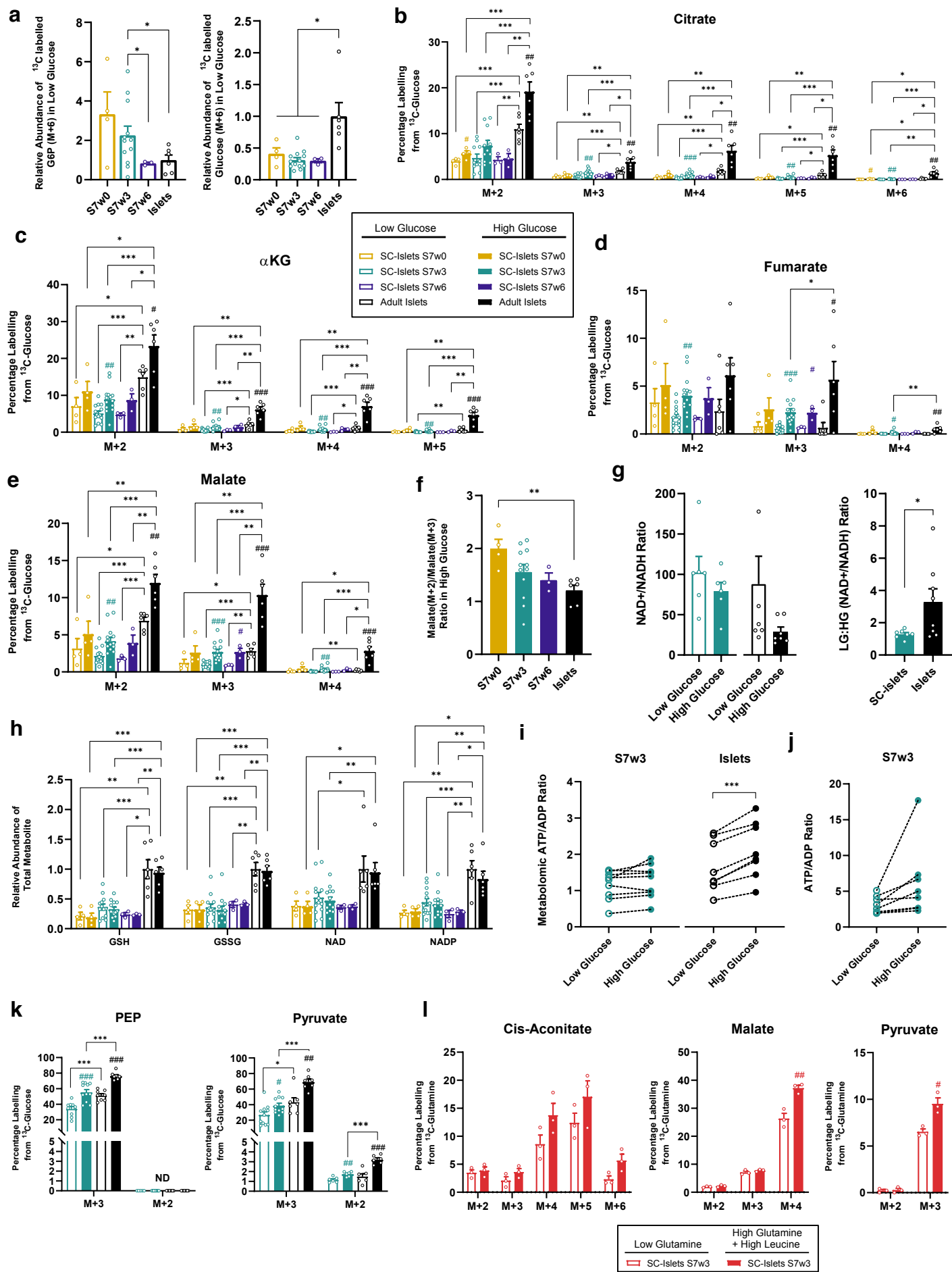
(i) Total exocytosis during 40 s of K^+ -stimulation in different SC-islet preparations. Dots represent individual cells. Two-tailed Student's t-test.

(j) Average granule density normalized to the footprint area of different SC-islet preparations. Dots represent individual cells. Two-tailed Student's t-test.

(k) Total exocytosis during 3 minutes of incubation in different concentration of glucose for different SC-islet preparation. Dots represent individual cells. Two-tailed Student's t-test.

(l) Average granule density normalized to the footprint area of different batch/preparation of cells as indicated. Dots represent individual cells.

All data presented as means $\pm$ SEM unless otherwise indicated.



Supplementary Figure 3

(a) The abundance of M+6 labelled Glucose-6-Phosphate (G6P) and M+6 labelled Glucose retained under low glucose treatment of SC-islets from week 0 to week 6 of maturation, relative to adult islets.

(b-e), The percentage of total metabolite labelling following low and high glucose exposure for each isotopologue of the TCA metabolites citrate (b), alpha-ketoglutarate (α KG) (c), fumarate (d), and malate (e).

(f) The ratio of M+2 and M+3 labelled malate under high glucose treatment in SC-islets and adult islets.

(g) Metabolic data measurements of NAD⁺/NADH ratios following low and high glucose treatment in S7w3 SC-islets and adult islets (*left panel*). A ratio of the low glucose to high glucose shift of NAD⁺/NADH ratio in S7w3 SC-islets and adult islets (*right panel*).

(h), The combined total abundance of reduced and oxidized forms of glutathione (GSH and GSSG) and the reducing agents NAD and NADP in SC-islets and adult islets.

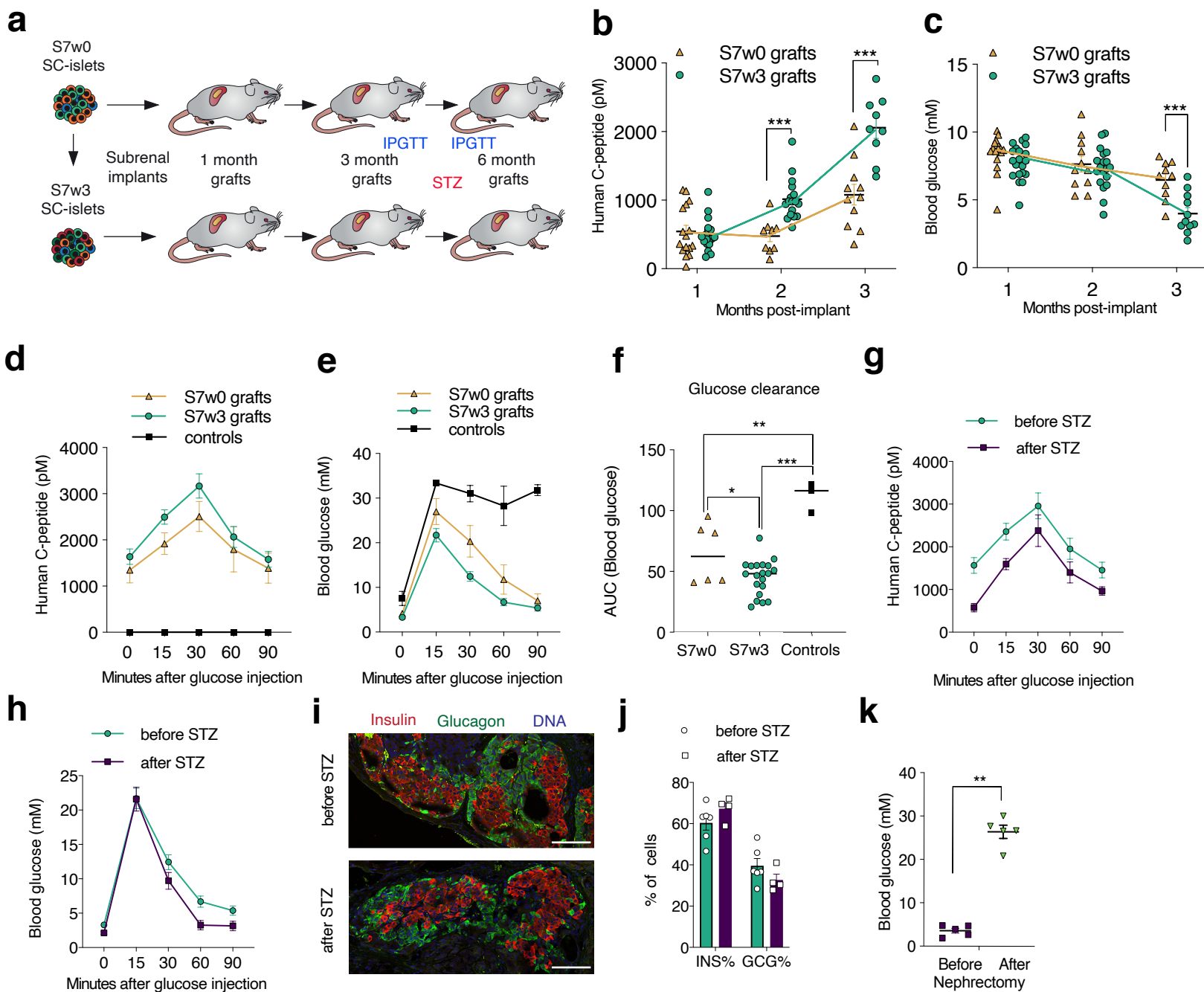
(i) Metabolic data measurements of ATP/ADP ratios in low and high glucose conditions in S7w3 SC-islets and adult islets.

(j) ATP/ADP ratio measurements under high and low glucose treatment in S7w3 SC-islets.

(k) Labelled enrichment of M+2 and M+3 isotopologues of PEP and pyruvate under low and high glucose treatment in S7w3 SC-islets and adult islets. ND = not detected.

(l) ¹³C5-glutamine labelling of S7w3 SC-islets under low (2mM ¹³C-glutamine) and high (10mM ¹³C-glutamine with 5mM leucine) conditions and resulting isotopologue patterns of cis-aconitate, malate, and pyruvate.

Error bars $\pm$ SEM with statistical significance determined by two-tailed t-tests. ‘#’ symbols indicate internal significance from low to high glucose labelling, ‘*’ symbols denote significance between SC-islet timepoints or adult islet samples at each glucose concentration. #,*p < 0.05, ##,**p < 0.01, ###,***p < 0.001. SC-islets S7w0 (n=4), S7w3 (n=6-13), S7w6 (n=3), adult islets (n=6). For glutamine labelling experiments S7w3 (n=3).



Supplementary Figure 4: Engrafted SC-islets provide effective glycemic control in mice

(a) Schematic representation of the *in vivo* experiments

(b-c) Measurements from random-fed mice engrafted with S7w0 or S7w3 SC-islets, **(b)** Human specific C-peptide levels (pM) and **(c)** Mouse blood glucose levels (mM) (S7w0: n=16 in 1m, 9-11 in 2m and 10-11 in 3m; S7w3: n=18-20 in 1m, 17-19 in 2m and 9-11 in 3m). Individual data points presented, Multiple two-tailed t-tests.

(d-e) Human C-peptide **(e)** and blood glucose **(d)** levels measured during an intraperitoneal glucose tolerance test (IPGTT) at three months post-engraftment. Mice were injected with 3 mg/kg glucose after a 6 h fast. (S7w0: n=6, S7w3: n=20, non-implanted controls n=3).

(f) Glucose clearance quantified from IPGTT blood glucose values in (d) as area under the curve (AUC). One-way ANOVA and Tukey's multiple comparisons test.

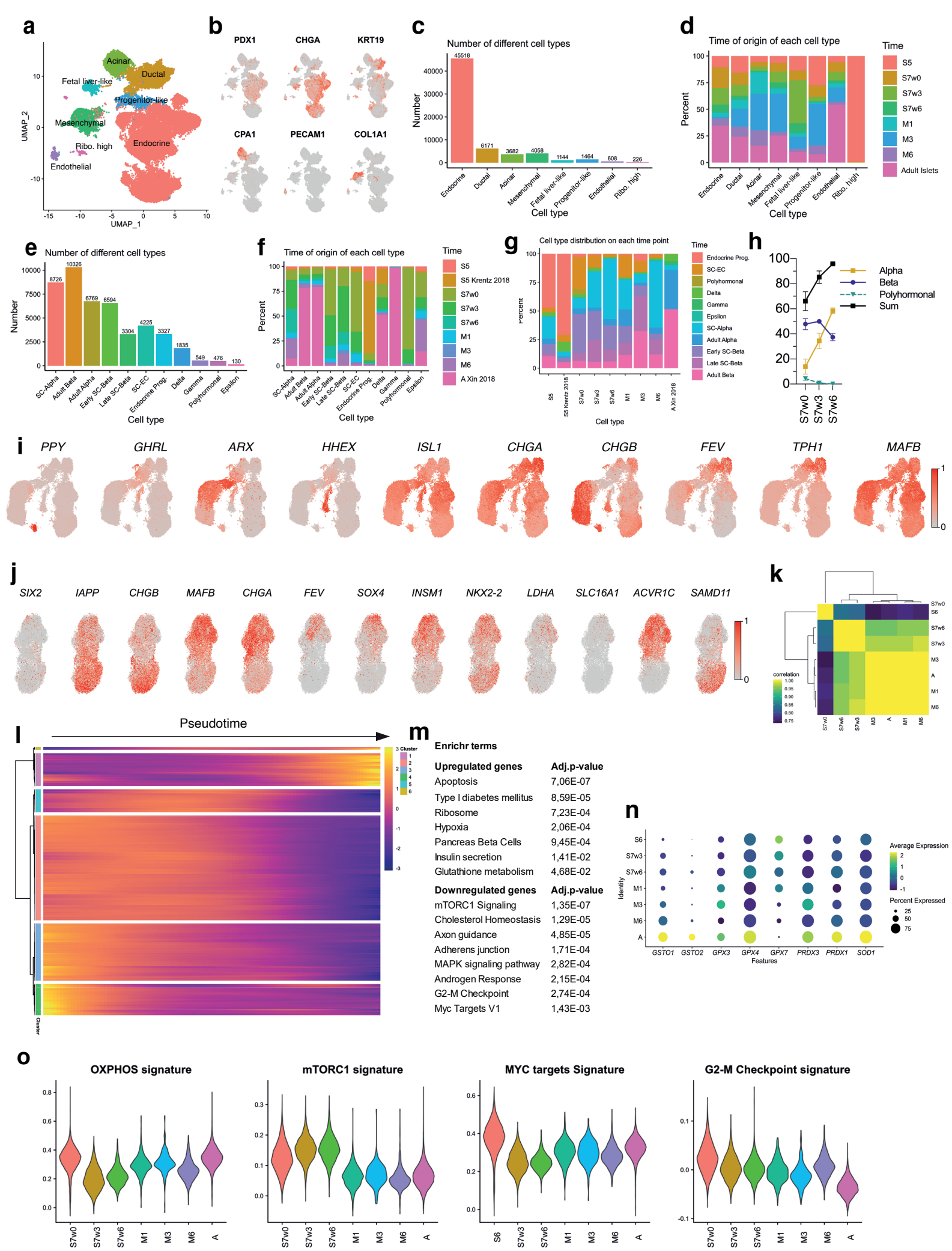
(g-h), (h-i) Human C-peptide levels (pM) **(g)** and blood glucose levels (mM) **(h)** in an IPGTT similar to the one in (d-e) in mice at 4 months post-engraftment (before streptozotocin (STZ) injection, n=10) and at 5 months post-engraftment (after STZ injection, n=9)

(i) Representative immunohistochemistry in the grafts before (n=6) and after (n=4) STZ injection. Scale bar 100µm.

(j) Quantification of INS⁺ and GCG⁺ populations before (n=6) and after (n=4) STZ injection, % of all cells positive for either INS or GCG. Dots represent graft-wide data from individual grafts.

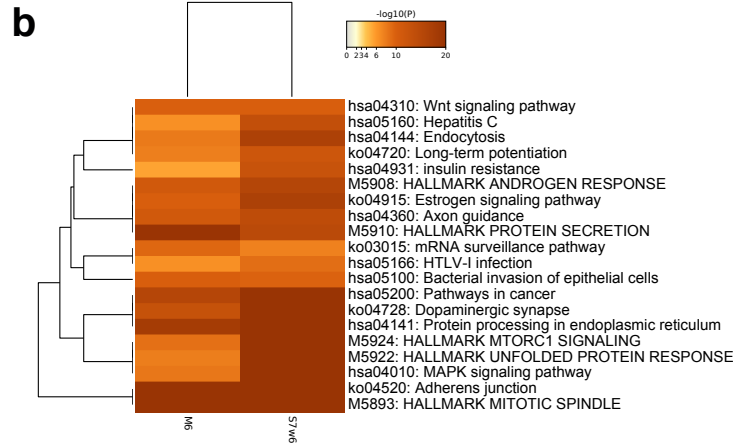
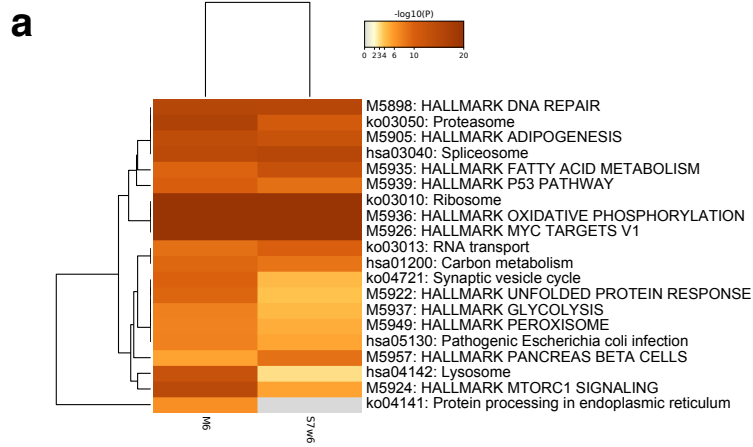
(k) Blood glucose levels from engrafted, random-fed mice before and after nephrectomy at 5-6 months post-engraftment. The mice were injected with streptozotocin (STZ) at 4 months post-engraftment. (n=4) Two-sided Mann-Whitney U-test.

All data represent mean ±SEM, unless indicated otherwise * p < 0.05, ** p < 0.01, *** p < 0.001.

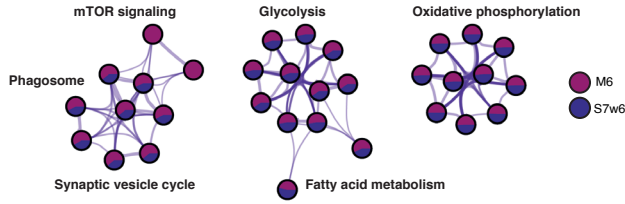


Supplementary Figure 5

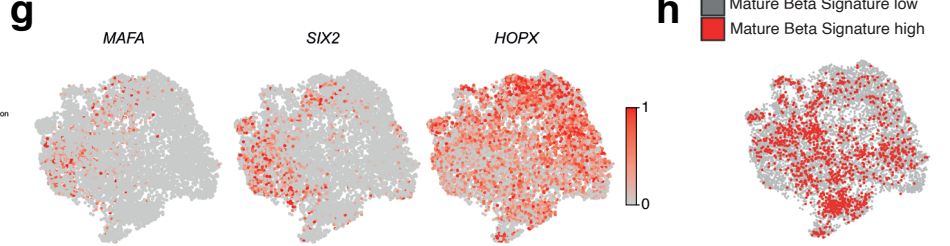
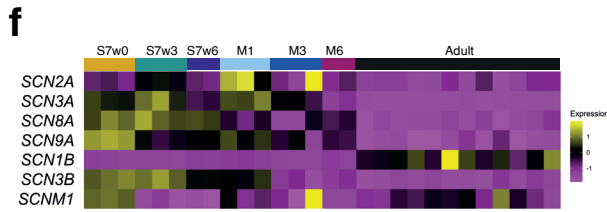
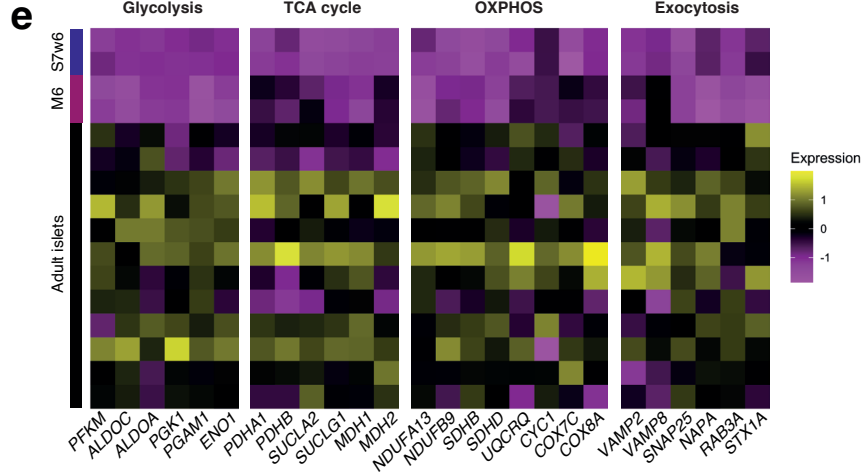
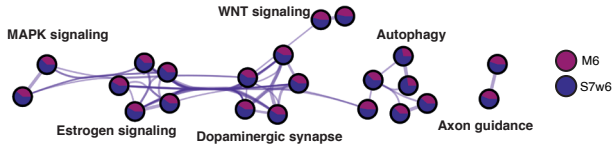
- (a)** UMAP projection of the full integrated scRNAseq dataset, containing 62 871 cells, colored by different cell types.
- (b)** Relative expression of marker genes for pancreatic (*PDX1*), endocrine (*CHGA*), ductal (*KRT19*), acinar (*CPA1*), endothelial (*PECAMI*) and mesenchymal (*COL1A1*) cells.
- (c)** Numbers of each different cell type in the full integrated scRNAseq dataset.
- (d)** Fractional contribution of each timepoint to each cell type identity in the full integrated scRNAseq dataset.
- (e)** Numbers of each different cell type in the filtered endocrine scRNAseq dataset.
- (f)** Fractional contribution of each timepoint to each cell type identity in the filtered endocrine dataset.
- (g)** Fractional contribution of each cell type identity to each timepoint in the filtered endocrine dataset
- (h)** Percentage of cells in the alpha, beta and polyhormonal clusters and the sum of these clusters in the S7 culture timepoints. Data presented as means $\pm$ SEM.
- (i)** Relative expression of marker genes for the different endocrine cell type identities.
- (j)** Relative expression of beta cell marker genes in the beta cell subpopulations.
- (k)** Pearson correlation of the average gene expression in the cells from each different time point.
- (l)** Hierarchical clustering of the top 1500 genes most differentially regulated along pseudotime (see also Supp.Table.4).
- (m)** Gene sets enriched in the upregulated and downregulated genes along pseudotime presented in (Fig.5l) Pseudotime clusters with upregulated genes 1 and 6, and cluster with downregulated genes 2,3,4 and 5 were grouped for the gene set enrichment analysis.
- (n)** Expression of glutathione metabolism-related genes across beta cells from different time of origin.
- (o)** Signatures of processes differentially regulated along pseudotime on beta cells from each different time point.



c Enriched processes in Adult beta cells upregulated genes VS SC-beta cells



d Enriched processes in Adult beta cells downregulated genes VS SC-beta cells



Supplementary Figure 6

(a-b) Gene sets enriched in the upregulated (a) and downregulated (b) genes primary adult beta cells compared to S7w6 *in vitro* and M6 *in vivo* SC-beta cells.

(c-d) Network of enriched biological pathways showing the relationships among processes that are upregulated (c) or downregulated (d) in primary adult beta cells compared to S7w6 *in vitro* and M6 *in vivo* SC-beta cells.

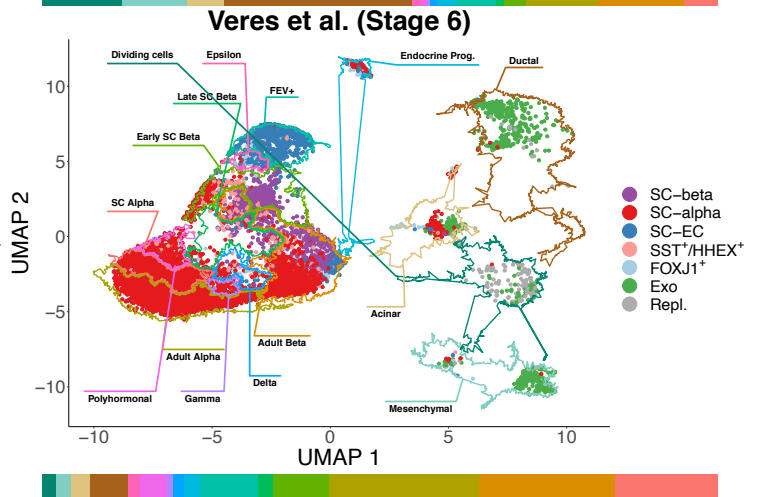
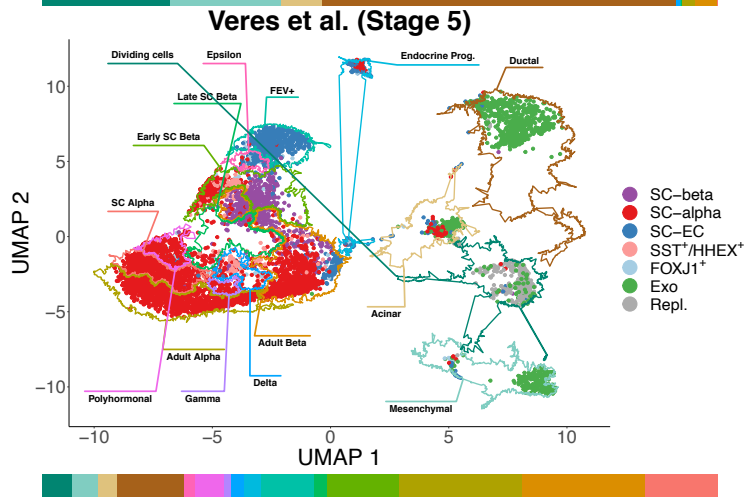
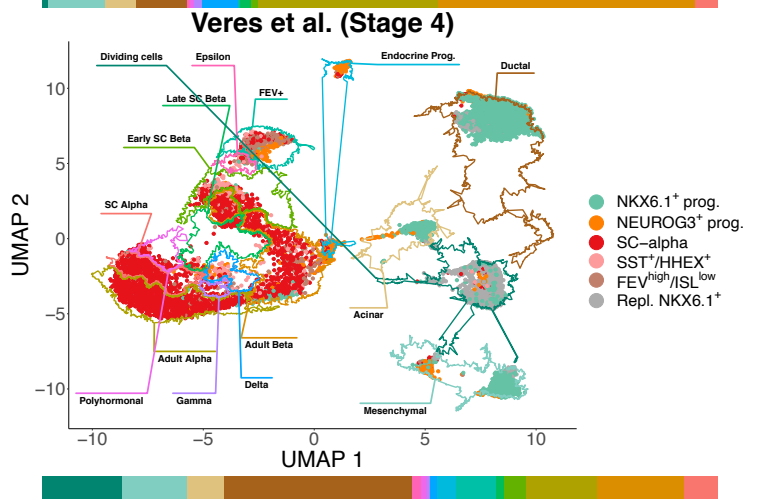
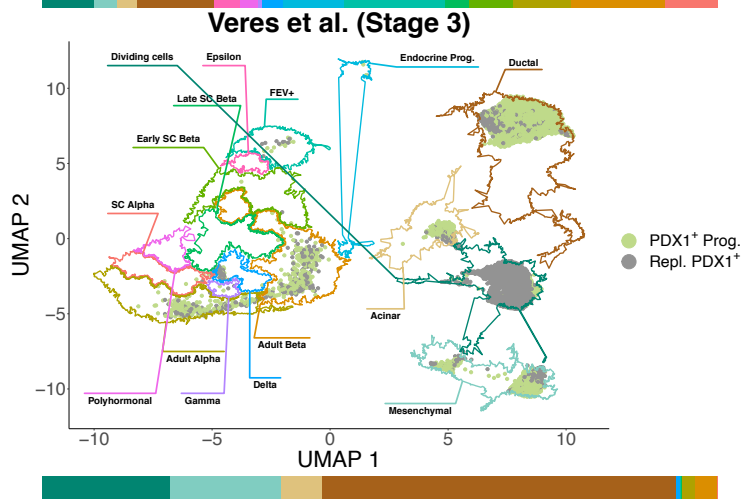
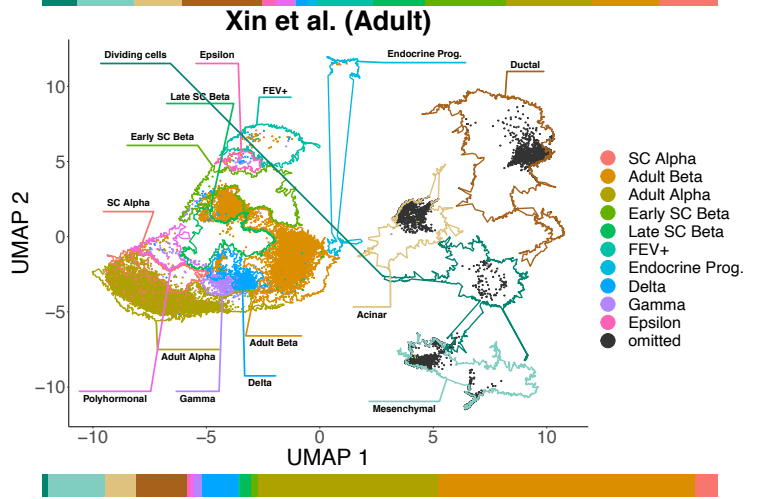
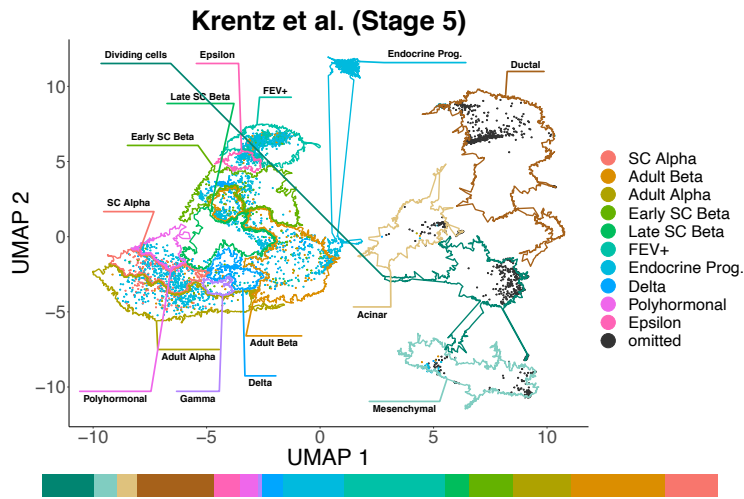
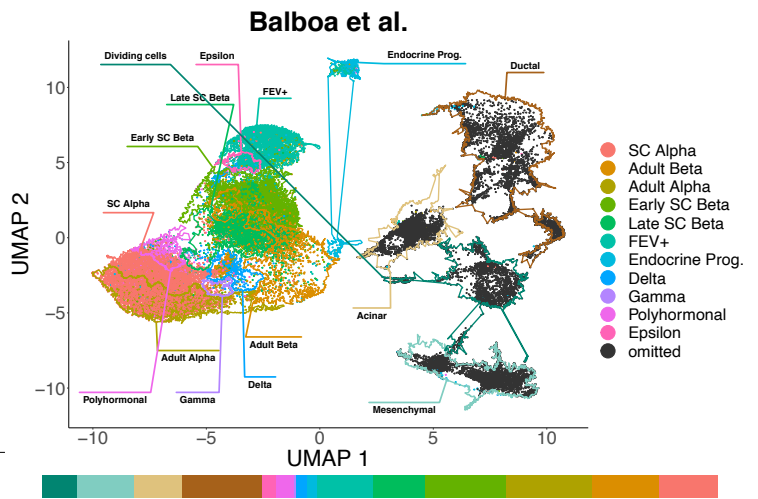
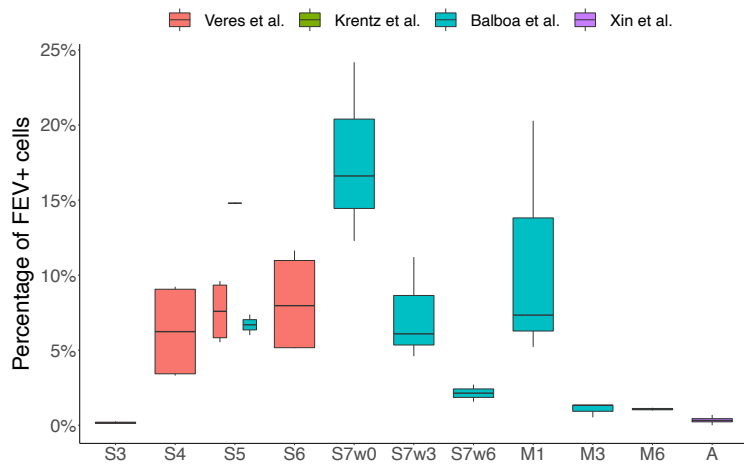
Enriched biological pathways can be represented in a network, which facilitates the understanding of relationships among biological pathways or processes,

(e) Expression of genes that are significantly upregulated in primary adult beta cells compared to S7w6 *in vitro* and M6 *in vivo* SC-beta cells, averaged by individual sample.

(f) Average expression of sodium channel subunit genes across beta cells from different time of origin.

(g) UMAP projection of *in vitro* SC-beta cells annotated by the heterogeneous expression of mature beta cell markers.

(h) UMAP projection of *in vitro* SC-beta cells annotated by high or low mature beta signature score.



Supplementary Figure 7

(a) Percentage of SC-EC cells detected in the different time points of each scRNAseq datasets included in the comparison (Veres et al., Krentz et al., Xin et al, and this study).

Boxplots represent interquartile range (IQR) showing the median, the 75th and the 25th percentile. The whiskers denote roughly 95% of the data range ($1.5 \times \text{IQR}$).

(b-h) UMAP projections indicating the similar clustering of equivalent cell types arising from the different scRNAseq datasets included in the comparison (Veres et al., Krentz et al., Xin et al, and this study). Similar cell types are coloured and labelled according to our cell type annotations. The proportions of cell types for each study are shown with bar graphs with corresponding colours for each cell type.

Supplementary table 9: differentiation medium composition

Stage	Duration (d)	Culture format	Basal medium	Additives
1	3	Matrigel coated planar	#1: MCDB131 (10372-019, Life Technologies) + 2 mM Glutmax (35050038, Life Technologies)	+ 100 ng/ml Activin A (QKine limited, UK) + CHIR (Tocris, #4423) 3.0 μ M on the 1 st day, 0.3 μ M on the 2 nd day, 0.0 μ M on the 3 rd day.
2	3	Matrigel coated planar	+ 1.5 g/l NaHCO ₃ (Sigma-Aldrich) + 5 g/l BSA fV (Sigma-Aldrich, A7030) + 5 mM glucose. Total glucose 10.44 mM	+ 50 ng/ml FGF7 (Genscript, Z03047) + 0.25 mM Vitamin C (Sigma, A4544)
3	2	Matrigel coated planar	#2: MCDB131 + 2 mM Glutmax + 2.5 g/l NaHCO ₃ + 20 g/l BSA fV + 5 mM glucose + 0.5% ITS-X (supp). Total glucose 10.42 mM	+ 50 ng/ml FGF7 + 0.25 mM Vitamin C + 0.25 μ M SANT1 (Sigma, S4572) + 1 μ M Retinoic Acid (Sigma, R2625) + 100 nM LDN (Selleckchem, S2618) + 200 nM TPB (Santa Cruz, Sc-204424)
4	4	Planar -> Aggre Well 400		+ 10 mM Nicotinamide (N0636, SIGMA) + 0.25 mM Vitamin C + 100 ng/ml EGF (AF-100-15, Peprotech) + 10 μ M ROCKi + 2 ng/ml FGF7 + 0.25 μ M SANT1 + 0.1 μ M Retinoic Acid + 200 nM LDN + 100 nM TPB + 10 ng/ml Activin A
5	4	Aggre Well 400	#3: MCDB131 + 2 mM Glutmax + 1.5 g/l NaHCO ₃ + 20 g/l BSA fV + 15 mM glucose + 0.5% ITS-X + 10 μ g/ml Heparin (H3149, Sigma-Aldrich)	+ 0.25 μ M SANT1 + 0.05 μ M retinoic acid + 100 nM LDN + 10 μ M ALK5inhII (S7233, Selleckchem) + 1 μ M GC1 (4554, Tocris) + 20 ng/mL Betacellulin (100-50, Peprotech) + 100 nM GSiXX (565789, Millipore)
6	7-8	Suspension	+ 10 μ M ZnSO ₄ (Z0251, SigmaAldrich) + 1% Penicillin-Streptomycin. Total glucose 20.13 mM	+ 100 nM LDN + 10 μ M ALK5inhII + 1 μ M GC1 + 100 nM GSiXX
7	0-42	Suspension	CMRL1066 (15-110-CVR, Corning) + 2 mM Glutmax + 20 g/l BSA fV + 0.5% ITS-X + 10 μ g/l heparin + 10 μ g/l ZnSO ₄ + 0.5 mM Sodium Pyruvate (Lonza, BE13-115E) + 1:2000 Trace elements A (25-021-CI, Cellgro) + 1:2000 Trace elements B (99-175-CI, Cellgro) + 1:2000 Lipid concentrate (11905-031, Invitrogen) + 1% Penicillin-Streptomycin. Total glucose 5.32 mM	+ 0.5 μ M ZM447439 (ZM, Selleckchem, S1103) + 10 nM Tri-iodothyronine (T3) + + 1 mM N-Acetyl-Cysteine (NAC, A9165, Sigma-Aldrich)

Supplementary table 10: Human islet characteristics

Shipment#	Source	Purity (%)	Age (y)	Sex	BMI (kg/m ²)	HbA1c (mmol/mol)
1	Uppsala	94	67	M	26.1	38
2	Uppsala	97	65	M	30.1	42
3	Uppsala	96	71	M	22.4	41
4	Uppsala	72	36	F	18.9	32
5	Uppsala	98	61	F	24.7	35
6	Uppsala	80	70	F	22.0	32
7	Alberta R389	75	65	F	24.4	37
8	Uppsala	69	51	F	28.1	35
9	Uppsala	86	51	M	24.8	37
10	Uppsala	96	54	M	41.6	39

Uppsala = Nordic Network for Islet Transplantation, Uppsala University, Sweden
Alberta = IsletCore, University of Alberta, Canada

Supplementary table 11: antibodies used

Epitope	Origin animal	Conjugate	Dilution	Supplier	Assay
PDX1	Mouse	PE	1:80	BD Biosciences #562161	FC
NKX6.1	Mouse	Red 647	1:80	BD Biosciences #563338	FC
Insulin	Rabbit	Red 647	1:160	Cell Signaling Technology Cat# 9008	FC
Insulin	Guinea pig	N/A	1:500	DAKO, A0564	IHC
Glucagon	Mouse	N/A	1:160/ 1:500	Sigma-Aldrich, #G2654	FC / IHC
Somatostatin	Rabbit	N/A	1:500	DAKO, A0566	IHC
Ki-67	Rabbit	N/A	1:500	Leica Microsystems #NCL-Ki67p	IHC
SLC18A1	Rabbit	N/A	1:500	Sigma-Aldrich, #HPA063797	IHC
LDHA	Rabbit	N/A	1:250	Cell Signaling Technology Cat#3582	IHC
Guinea pig	Goat	Red 594	1:500	Thermo-Fisher, #11076	IHC
Mouse	Donkey	Green 488	1:500	Thermo-Fisher, #21202	FC/IHC
Rabbit	Donkey	Green 488	1:500	Thermo-Fisher, #21206	IHC

FC= Flow cytometry, IHC=Immunohistochemistry