Electronic Supplementary Materials for

Calcium-dependent transcriptional changes in human pancreatic islet cells reveal functional diversity in islet cell subtypes

Ji Soo Yoon, Shugo Sasaki, Jane Velghe, Michelle Y. Y. Lee, Helena Winata, Cuilan Nian, Francis C. Lynn*

*Corresponding author (francis.lynn@ubc.ca)

Includes:

ESMMethods: Text (651 words)

ESM Methods: antibodies and primers

ESM Tables S1-S7 (separate file)

ESM Figures S1-S9

Human Islet Checklist

Sequencing

Each donor library pool was sequenced twice using the Illumina NextSeq500 with NextSeq 500/550 High Output v2 or v2.5 75-cycle kits (Illumina, USA; FC-404-2005). Libraries were sequenced using the following cycle parameters: 28 (Read 1), 50 (Read 2), 8 (i7), 0 (i5) for donors R253 and R282 libraries. Sequencing for donor R317 used 56 cycles (Read 2) as recommended by 10x Genomics (USA).

Analysis of scRNA-seq data

Sequenced data files were initially processed using Cell Ranger V.3.0.2 (10X Genomics) software. FASTQ files were generated from sequencing data using *cellranger mkfastq*, then aligned to reference human genome GRCh38 to generate gene counts using *cellranger count*. Finally, *cellranger aggr* was used to aggregate the data from all experimental conditions from one donor, from both sequencing replicates.

Following Cell Ranger pipelines, additional quality control and filtering of cells was performed using publicly available R packages. Briefly, Scater was used to remove cells with fewer than 1000 transcripts or cells with too high or too low transcripts/cell values that suggested debris or potential multiplets. Low-abundance genes or genes with a high dropout rate, and genes not expressed in at least 3 cells were also removed.

Next, the Seurat V3 package was used to filter out genes that were not expressed in at least 3 cells, cells that did not express more than 1000

genes, and cells that expressed more than 3000 genes. Seurat was also used for integration of the 3 donor datasets, clustering, visualizing, and finding DEGs.

Identifying Ca^{2+}-regulated genes

All cells were identified by their corresponding donor ID and experimental condition (Low, Positive, or Negative). Cell types and cluster identities were added to the metadata and new Seurat Objects were produced for each cell type. The dataset was parsed by cell type and three subsets were generated to contain two experimental conditions in each subset. This process was repeated in order to generate two-way comparisons for all combinations of conditions in each cell type. Final Ca^{2+}-regulated genes were identified as significantly expressed genes in either the Positive or Negative condition with an adjusted p-value less than 0.05.

RNA Velocity

RNA Velocity analysis was conducted to infer the direction of transcriptional dynamics using the scVelo Python package v0.2.1 (Theis lab, Germany; https://github.com/theislab/scvelo). [31] Count matrices of unspliced and spliced abundances were obtained from fastq files using the loompy/kallisto pipeline (loompy v3.0.6, kallisto v0.46.0, https://linnarssonlab.org/loompy/kallisto/index.html). L files across patient and condition were merged, and scVelo was ran following the pipeline for the endocrine pancreas dataset (https://scvelo.readthedocs.io/about/).

Metadata and Uniform Manifold Approximation and Projection (UMAP) embeddings were exported from our processed Seurat object (for only endocrine cells) and merged with the splicing information.

Genes with less than 20 spliced and unspliced counts were filtered out, and counts were normalized by the total initial counts in each cell. The first- and second- order moments for each cell were computed using its 30 nearest neighbours on the kNN graph in PC space (30 PCs). Velocity vectors were estimated by solving a stochastic model of transcriptional dynamics and the expected mean direction of all possible cell transitions on the kNN graph were calculated. Cosine correlations between velocity vectors and possible cell transitions were obtained in high dimensional space to generate probabilities for all potential cell transitions. The resulting matrix was used to project the velocities into lower-dimensional UMAP space for visualization.

Quantitative PCR

PCDH7+ and - cells were sorted into TRIzol. RNA was isolated, DNase-treated with the Turbo DNA-free kit (Ambion, USA; AM1907), and reverse-transcribed with SuperScript™ III (ThermoFisher, USA; 18080093). TaqMan qPCR was performed and data were normalized to *TBP*.

Fluorescence *in situ* hybridization

RNA FISH was performed on 5um-thick FFPE sections of agarose-embedded

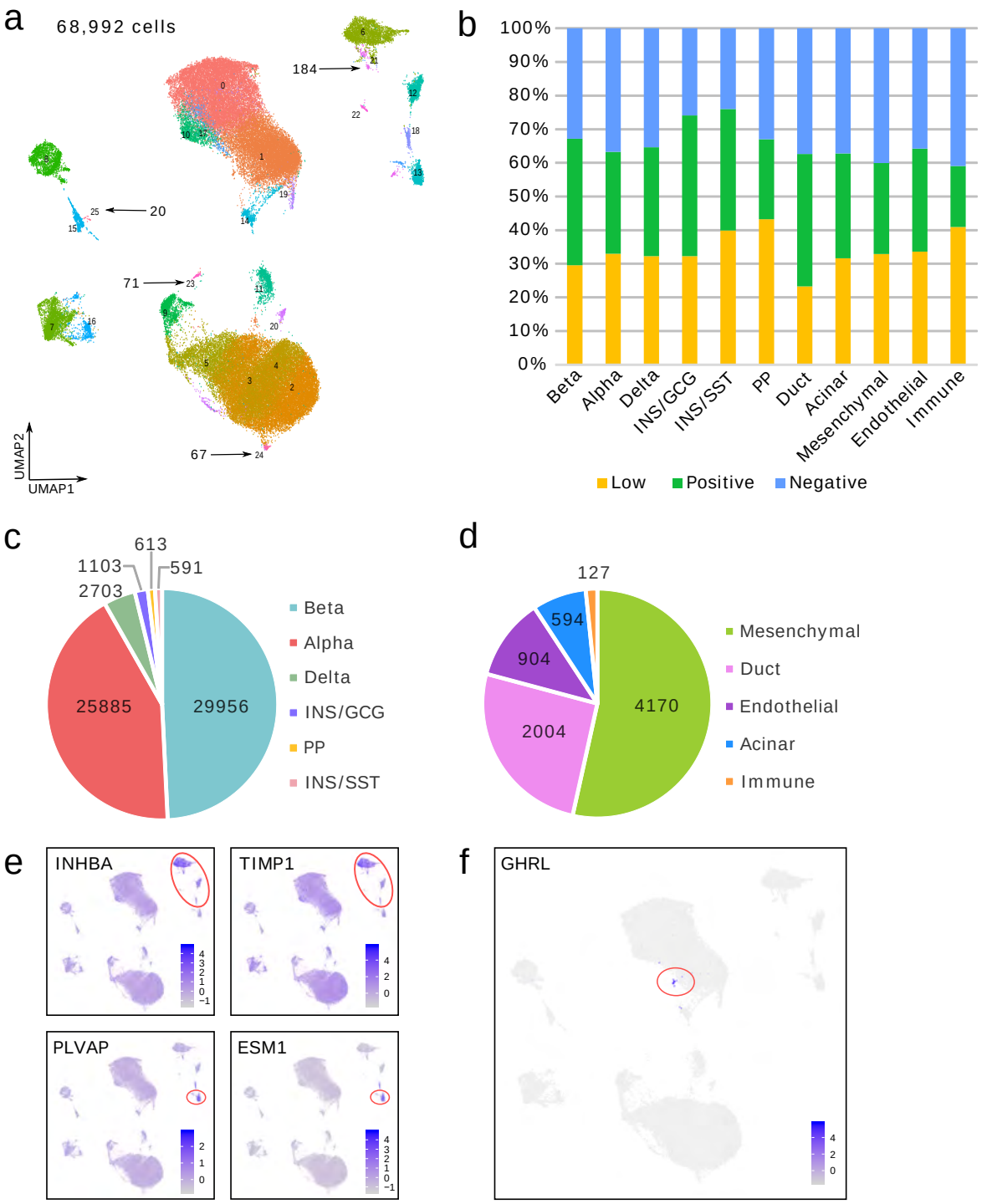
human islets using the RNAscope fluorescent multiplex v2 reagents according to manufacturer's instructions (ACDbio, USA; 323100). Probes for human *INS* (313571-C2) and *GCG* (556741-C3) were incubated with human islet sections. Opal dyes 520 and 650 (Perkin-Elmer, USA; FP1487001KT, FP1496001KT) at 1:100 dilution were used to develop *INS* and *GCG* signals, respectively, and DAPI was used as the nuclear stain. Sections were imaged using the SP8 confocal microscope (Leica, Germany).

Antibodies used for immunostaining

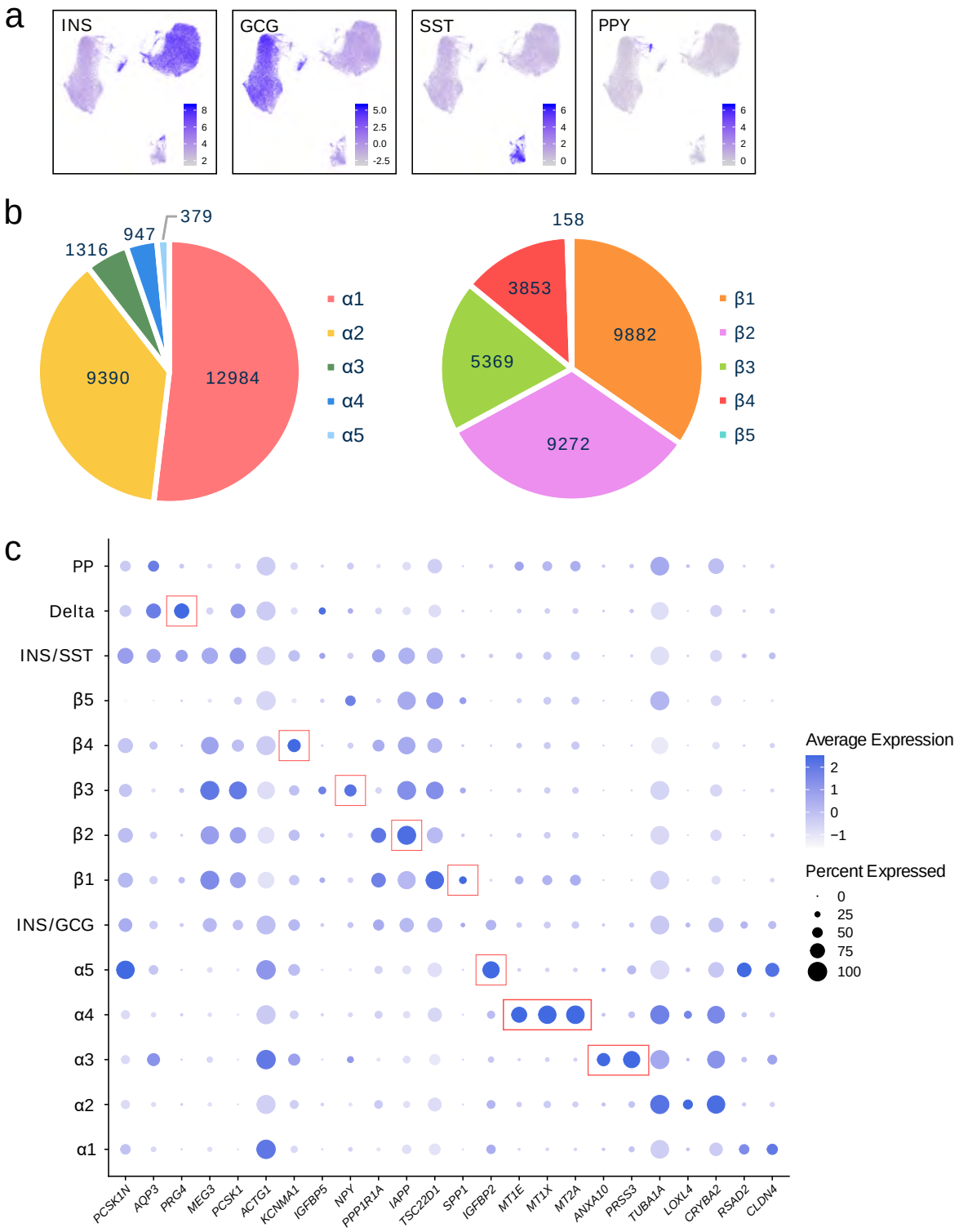
Primary or Secondary	Antibody	Dilution	Manufacturer
Primary	guinea pig anti-insulin	1:500	Dakocytomation, Denmark (A0564)
Primary	rabbit monoclonal anti-glucagon	1:100	Abcam, United Kingdom (ab92517)
Primary	mouse monoclonal anti-glucagon	1:2000	Sigma-Aldrich, USA (G2654-100UL)
Primary	rabbit polyclonal anti-IGFBP2	1:200	Thermo Fisher Scientific, USA (15699-1-AP)
Primary	rabbit monoclonal anti-Neuropeptide Y	1:400	Cell Signaling Technology, USA (11976S)
Primary	mouse monoclonal anti-PCDH7	1:100	Abcam, United Kingdom (ab139274)
Secondary	donkey anti-guinea pig FITC	1:450	Jackson ImmunoResearch, USA (706-096-148)
Secondary	donkey anti-rabbit FITC	1:450	Jackson ImmunoResearch, USA (706-166-148)
Secondary	donkey anti-mouse Cy3	1:250	Jackson ImmunoResearch, USA (715-166-150)
Secondary	donkey anti-guinea pig Cy3	1:250	Jackson ImmunoResearch, USA (711-096-152)
Secondary	goat anti-rabbit Alexa Fluor 647	1:200	Invitrogen, USA (A27040)

Primers used for qPCR

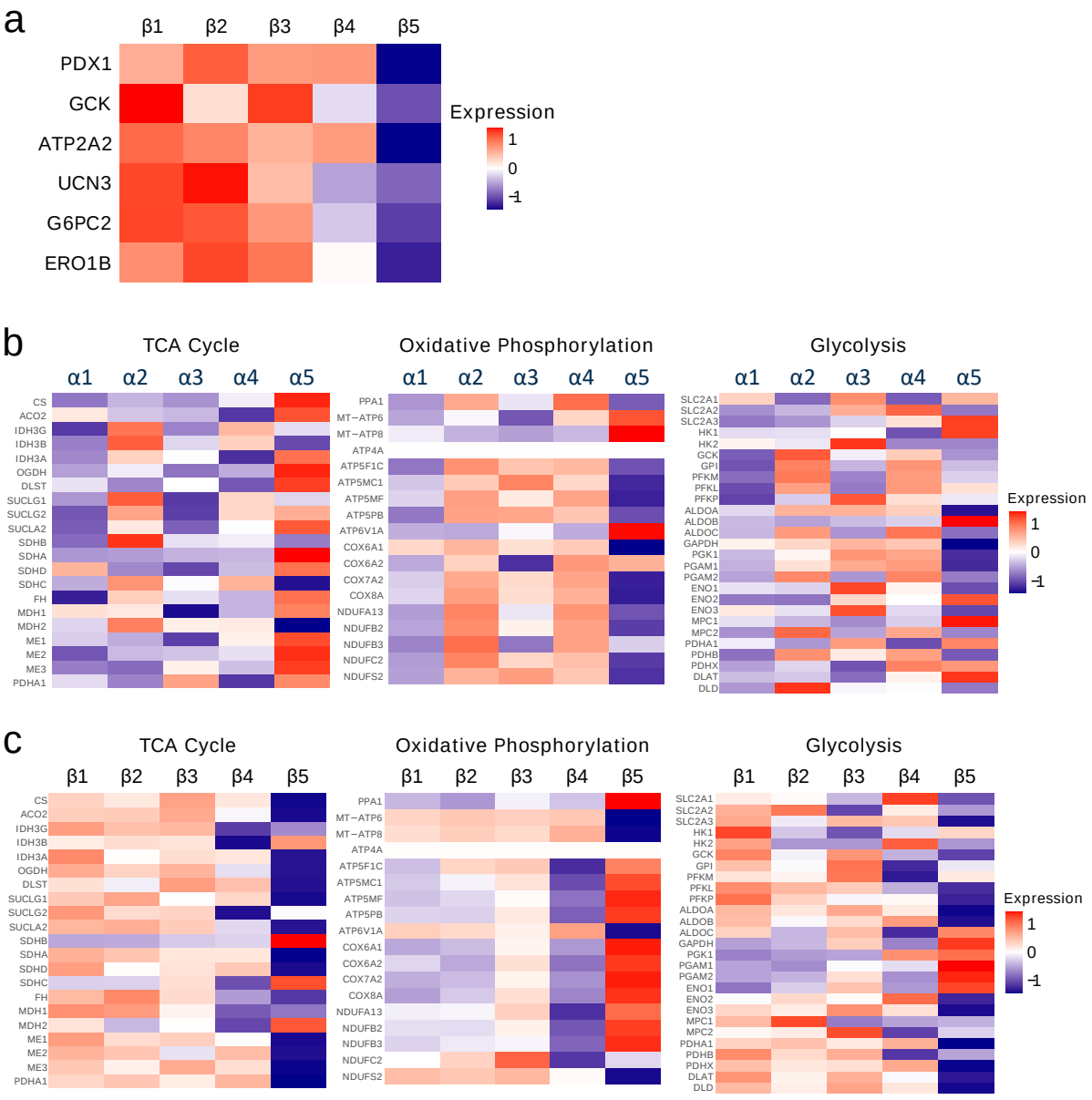
Gene	Forward Primer (5′ → 3′)	Reverse Primer (5′ → 3′)	Probe (5′ → 3′)
PCDH7	GTGGAATTCAGCCAAACACAG	ATTTGTGGGAGCAGGAGAC	ACAGCAAACAGATGCGTCTACATCCA
INS	CTAGTGTGCGGGGAACG	CACGCTTCTGCAGGGAC	CGGCGGGTCTTGGGTGTGTA
ERO1B	GGGTGAGTTGGAAGCCTTTAG	TGGACTGTGTTGGATGTGAC	ACAGACTCAGGGTTTAGGAACTGCC
NKX6-1	TCGTTTGGCCTATTCGTTGG	TGTCTCCGAGTCCTGCTTC	TGCTTCTTCCTCCACTTGGTCCG
SLC30A8	AGGTCAATTAAGAGGTGGGC	AATGAGTACGCCTATGCCAAG	CACATTGCTGGGAGTCTTGCTGTTG
TBP	GAGAGTTCTGGGATTGTACCG	ATCCTCATGATTACCGCAGC	TGGGATTATATTCGGCGTTTCGGGC



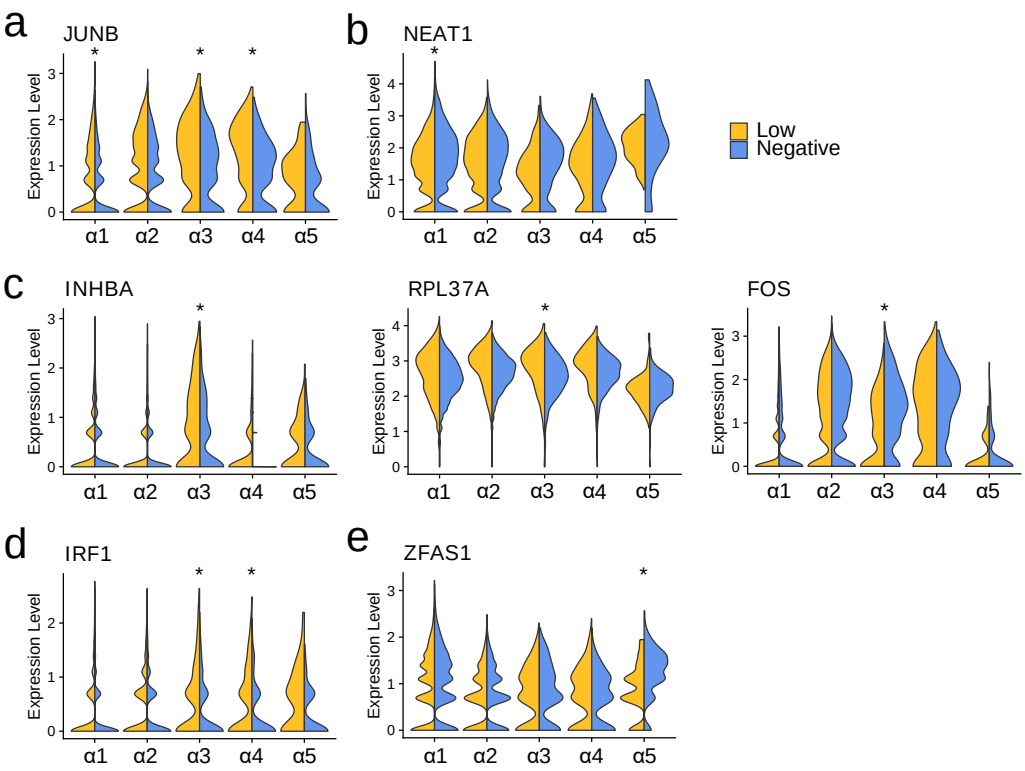
ESM Fig. 1. Human islet dataset pre- and post-removal of cells. a) UMAP plot of 68,992 human islet cells. Arrows indicate clusters that were removed due to poor integration between donors. b) Proportions of cells from Low, Positive, and Negative conditions in each cell type, post-removal of poorly integrated clusters. c) Cell numbers in each endocrine and d) non-endocrine cell type out of 68,650 cells. e) UMAP plots of mesenchymal marker genes INHBA and TIMP1, and endothelial marker genes PLVAP and ESM1. f) UMAP projection of GHRL.



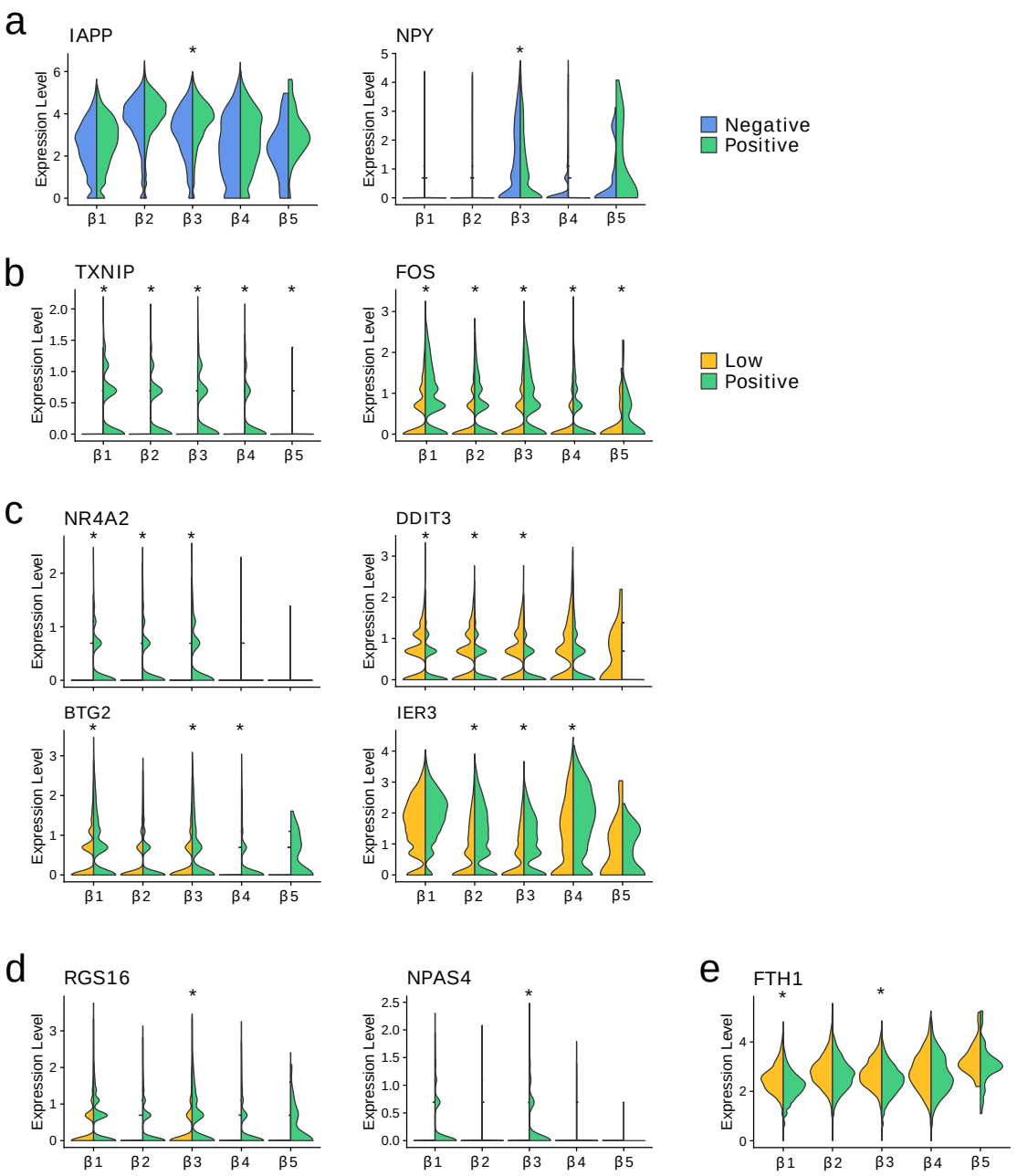
ESM Fig. 2. **α** and **β** cell clusters in the endocrine dataset. a) UMAP plots of INS, GCG, SST, and PPY expression used to identify cell types. b) Breakdown of cell numbers in each α and β cell clusters. c) Dot plot showing the average expression for candidate cluster marker genes for each cluster. Dot size shows the proportion of cells in each cluster that expresses the genes on the X-axis. Dots highlighted in red boxes are leading candidate genes for each cluster.



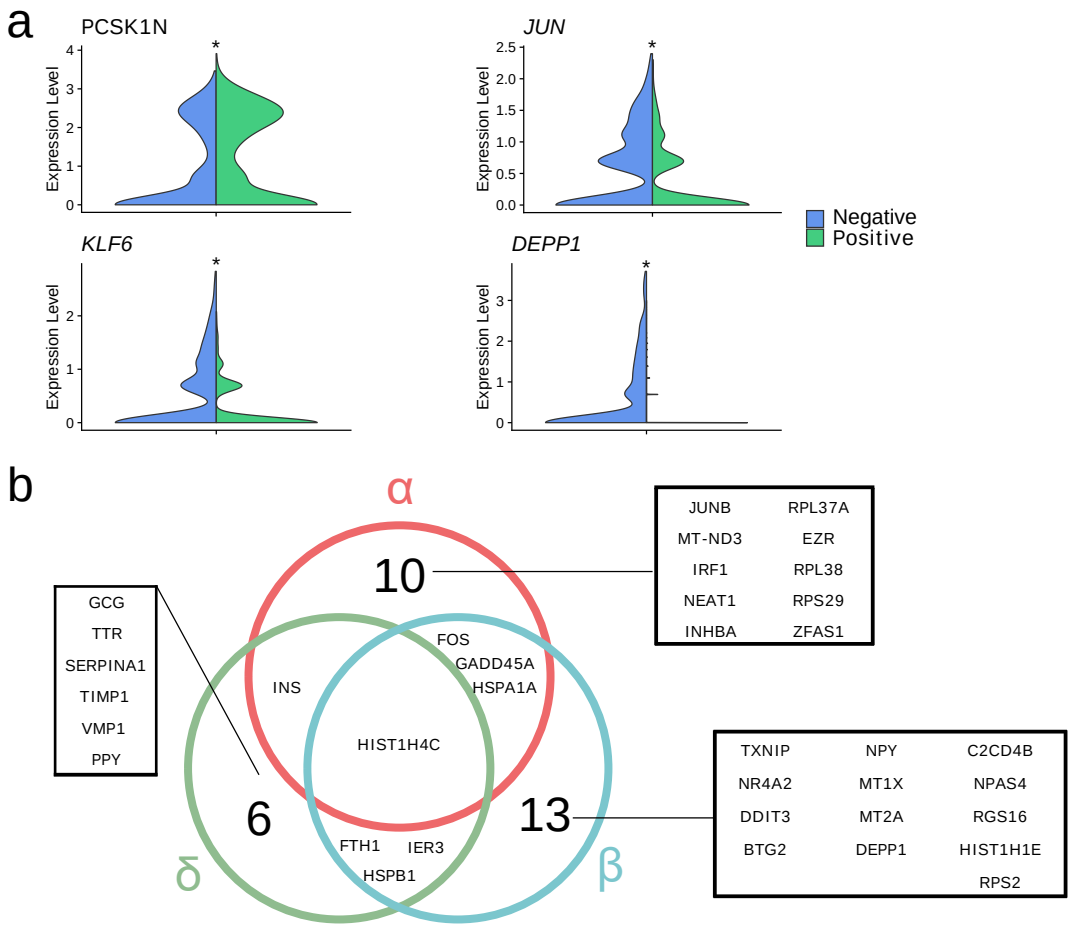
ESM Fig. 3. Maturity and metabolism gene expression profiles of **β** cell clusters. a) Heatmap of average expression per cluster of key β cell maturity genes across clusters β1- β5. b) Heatmap of average expression per cluster of genes involved in TCA cycle, oxidative phosphorylation, and glycolysis across α1-α5 and c) β1- β5. Expression shown as a range of red (high) to blue (low) comparing each cluster to all other clusters.



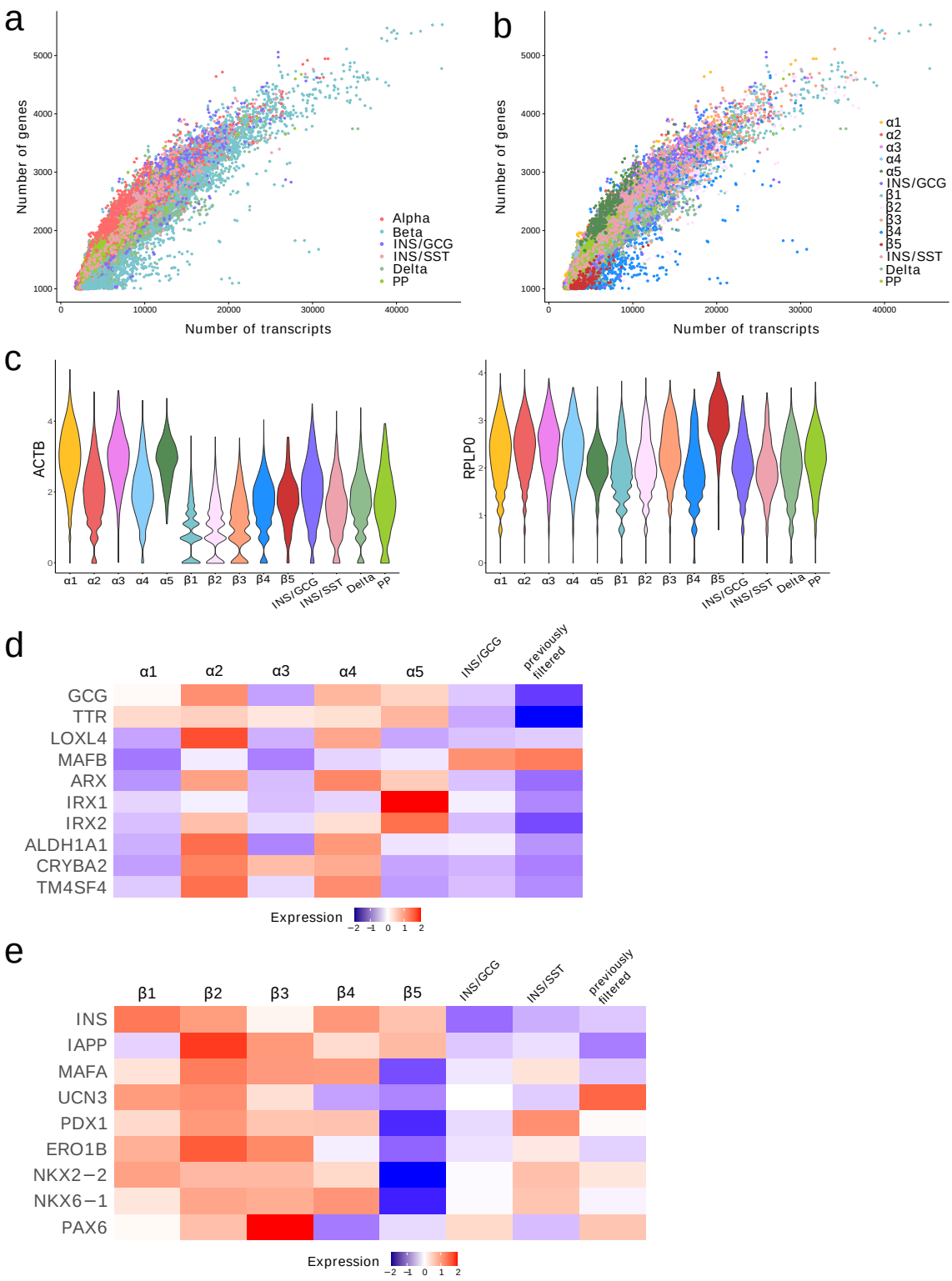
ESM Fig. 4. Comparing Low and Negative conditions in **α** cells. a) Split violin plots showing expression of genes JUNB, b) NEAT1, c) INHBA, RPL37A, FOS, d) IRF1, and e) ZFAS1 across clusters α1-α5 in the Low (yellow) and Negative (blue) conditions. *$p_{adjusted}$<0.05, Low vs Negative.



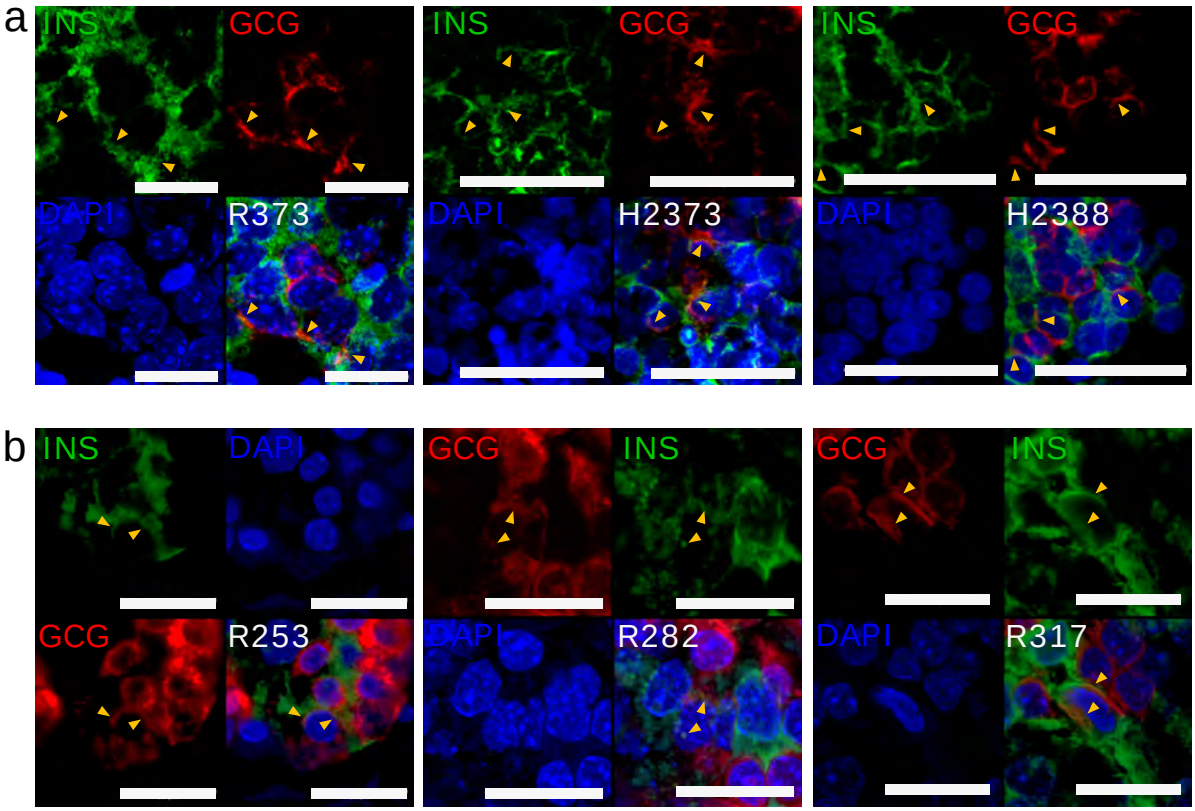
ESM Fig. 5. Calcium-regulated and glucose-regulated genes in **β** cells. a) Split violin plots showing expression of calcium-regulated genes IAPP and NPY across clusters β1- β5 in the Positive (green) and Negative (blue) conditions. *$p_{adjusted}$<0.05, Positive vs Negative. b) Split violin plots showing expression of glucose-regulated genes TXNIP, FOS, c) NR4A2, DDIT3, BTG2, IER3, d) RGS16, NPAS4, and e) FTH1 across clusters β1- β5 in the Low (yellow) and Positive (green) conditions. *$p_{adjusted}$<0.05, Low vs Positive.



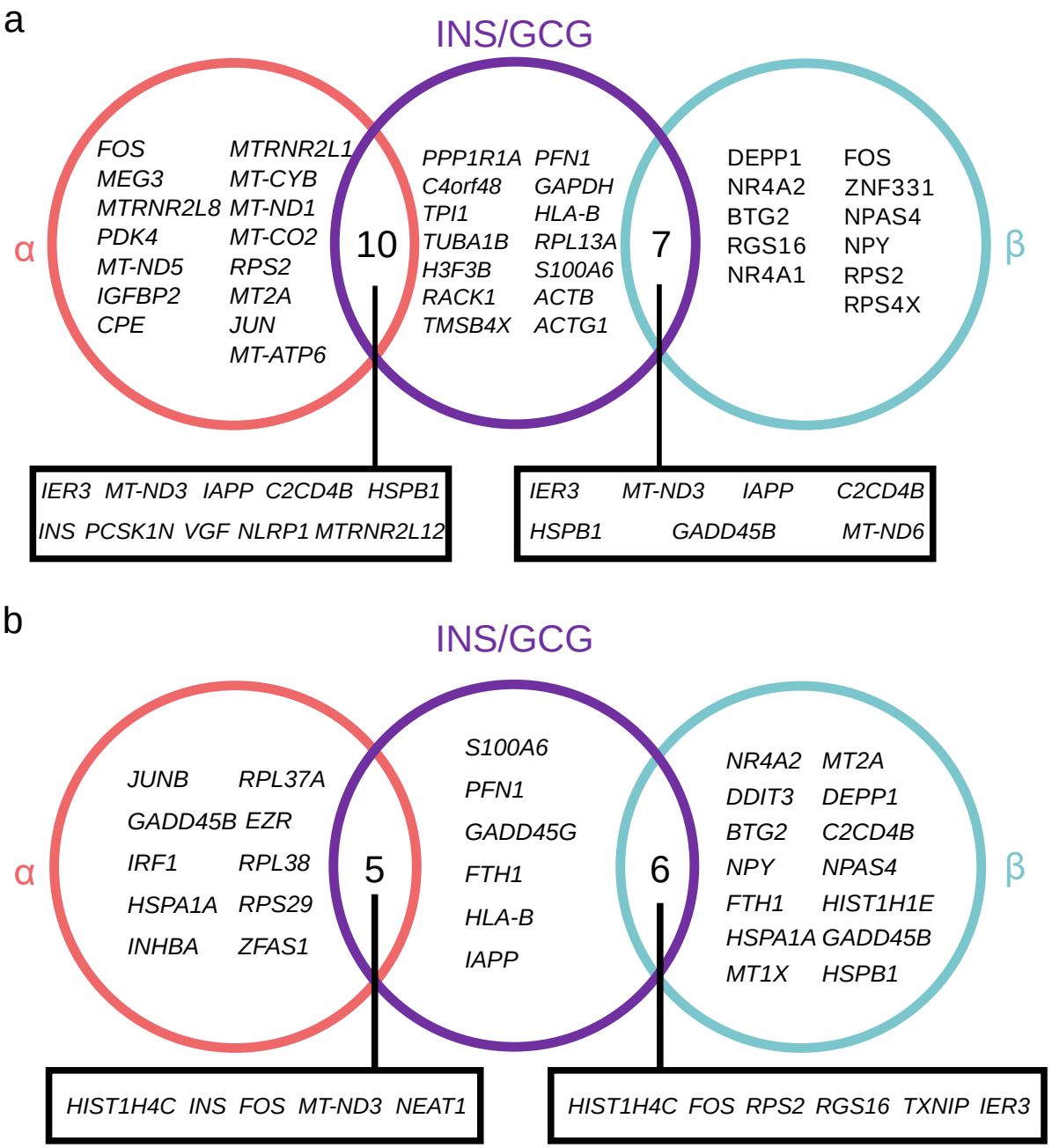
ESM Fig. 6. Calcium-regulated and glucose-regulated genes in **α**, **β**, and **δ** cells. a) Split violin plots showing expression of genes PCSK1N, JUN, KLF6, and DEPP1 in δ cells in the Positive (green) and Negative (blue) conditions. *$p_{adjusted} < 0.05$, Positive vs Negative. b) Numbers and names of all glucose-regulated genes in α, β, and δ cells.



ESM Fig. 7. Comparing INS/GCG and INS/SST cells to **α** and **β** cells. a) Scatterplot of number of genes vs. number of transcripts in each cell, coloured by cell type and b) cluster. c) Violin plots showing expression of housekeeping genes ACTB and RPLP0 across all clusters. d) Heatmap showing average expression per cluster of α cell maturity genes and e) β cell maturity genes across INS/GCG, INS/SST, α cell clusters, and β cell clusters. Average expression from 4095 cells that were filtered out during Seurat processing due to high number of genes and features are shown as comparison.



ESM Fig. 8. Histological validation of INS/GCG cells. a) Representative images of INS (green) and GCG (red) mRNA detected by RNA FISH with DAPI nuclear stain (blue) in ex vivo human islet sections of donors R373, H2373, and H2388. b) Representative images of INS (green) and GCG (red) mRNA detected by RNA FISH with DAPI nuclear stain in islets within human pancreas biopsy sections of donors R253, R282, and R317. Arrowheads indicate regions of INS and GCG mRNA co-localization. Scale bars indicate 20um in R373, R253, R282, and R317 islets; 50um in H2373 and H2388 islets. See Human Islet Checklist for more details on these donors.



ESM Fig. 9. Comparing calcium-regulated and glucose-regulated genes in INS/GCG cells with **α** and **β** cells. a) Calcium-regulated genes in INS/GCG cells vs. α and β cells. b) Glucose-regulated genes in INS/GCG cells vs. α and β cells.

Diabetologia

Checklist for reporting human islet preparations used in research

Adapted from Hart NJ, Powers AC (2018) Progress, challenges, and suggestions for using human islets to understand islet biology and human diabetes. Diabetologia https://doi.org/10.1007/s00125-018-4772-2

Islet preparation	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8[a]**
MANDATORY INFORMATION								
Unique identifier	R253	R282	R317	R366	R367	R369	H2330	H2338
Donor age (years)	57	57	54	31	58	66	49	58
Donor sex (M/F)	M	M	M	M	F	M	F	F
Donor BMI (kg/m^2)	25.5	26.4	26.4	26.2	30.5	25.6	27.2	34.4
Donor HbA_{1c} or other measure of blood glucose control	31.1	43.2	32.2	34.4	37.7	30.1	36.6	38.8
Origin/source of islets[b]	Alberta Islet Core	Alberta Islet Core	Alberta Islet Core	Alberta Islet Core	Alberta Islet Core	Alberta Islet Core	Alberta Islet Distribution Program	Alberta Islet Distribution Program
Islet isolation centre	Alberta Islet Core	Alberta Islet Core	Alberta Islet Core	Alberta Islet Core	Alberta Islet Core	Alberta Islet Core	Clinical Islet Laboratory, University of Alberta	Clinical Islet Laboratory, University of Alberta
Donor history of diabetes? Please select yes/no from drop down list	No	No	No	No	No	No	No	No
If Yes, complete the next two lines if this information is available								
Diabetes duration (years)								
Glucose-lowering therapy at time of death[c]								
RECOMMENDED INFORMATION								
Donor cause of death								

Warm ischaemia time (h)								
Cold ischaemia time (h)	14.3	9.5	21	10.5	4	12		
Estimated purity (%)	90	90	90	80	75	75	72.5	30
Estimated viability (%)							85.5	87
Total culture time (h)[d]	84	63	88	42	61	59	94	72
Glucose-stimulated insulin secretion or other functional measurement[e]	Stimulation index 34.22 (1mM glucose to 16.7mM glucose)	Stimulation index 4.06 (1mM glucose to 16.7mM glucose)	Stimulation index 19.36 (1mM glucose to 16.7mM glucose)	Stimulation index 2.45 (1mM glucose to 16.7mM glucose)	Stimulation index 7.37 (1mM glucose to 16.7mM glucose)	Stimulation index 3.88 (1mM glucose to 16.7mM glucose)		
Handpicked to purity? Please select yes/no from drop down list	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Additional notes	Used for 10x genomics scRNA-seq,and RNA FISH	Used for 10x genomics scRNA-seq and RNA FISH	Used for 10x genomics scRNA-seq and RNA FISH	Used for PCDH7 sorting, GSIS, and qPCR	Used for PCDH7 sorting, GSIS, and qPCR	Used for PCDH7 sorting, GSIS, and qPCR	Used for PCDH7 sorting, GSIS, and qPCR	Used for PCDH7 sorting, GSIS, and qPCR. Used for immunostaining and RNA FISH.

[a]If you have used more than eight islet preparations, please complete additional forms as necessary
[b]For example, IIDP, ECIT, Alberta IsletCore
[c]Please specify the therapy/therapies
[d]Time of islet culture at the isolation centre, during shipment and at the receiving laboratory
[e]Please specify the test and the results

Preps 9-13 continued on next page

Islet preparation	9	10	11	12	13	14	15	16
MANDATORY INFORMATION								
Unique identifier	H2337	H2373	H2388	R373				
Donor age (years)	24	59	39	31				
Donor sex (M/F)	M	M	M	M				
Donor BMI (kg/m^2)	24.1	25.6	35.5	27.5				
Donor HbA_{1c} or other measure of blood glucose control	31.1	32.2	39.9	24.6				
Origin/source of islets[b]	Alberta Islet Distribution Program	Alberta Islet Distribution Program	Alberta Islet Distribution Program	Alberta Islet Core				
Islet isolation centre	Clinical Islet Laboratory, University of Alberta	Clinical Islet Laboratory, University of Alberta	Clinical Islet Laboratory, University of Alberta	Alberta Islet Core				
Donor history of diabetes? Please select yes/no from drop down list	No	No	No	No				
If Yes, complete the next two lines if this information is available								
Diabetes duration (years)								
Glucose-lowering therapy at time of death[c]								
RECOMMENDED INFORMATION								
Donor cause of death								
Warm ischaemia time (h)								
Cold ischaemia time (h)				23				

Estimated purity (%)	27.5	37.5	38.8	80				
Estimated viability (%)	74	97.5	84.0					
Total culture time (h)[d]	50	74	72	54				
Glucose-stimulated insulin secretion or other functional measurement[e]				Stimulation index 4.1 (1mM glucose to 16.7mM glucose)				
Handpicked to purity? Please select yes/no from drop down list	Yes	Yes	Yes	Yes				
Additional notes	Used for PCDH7 sorting, GSIS, and qPCR	Used for RNA FISH	Used for RNA FISH	Used for RNA FISH				