

SUPPLEMENTARY RESEARCH DESIGN AND MATERIALS

Reagents and Solutions RPMI-1640, HBSS, and FBS were purchased from Life Technologies Inc. (Burlington, ON, Canada). Penicillin/Streptomycin and D-PBS were from Multicell Wisent Inc. (Saint-Jean-Baptiste, QC, Canada). FA-free BSA was from Equitech-Bio (Kerrville, TX, USA). Collagenase type XI, Histopaque-1119/1077, glucose, harmine, sodium oleate and palmitate were from Sigma Aldrich (Saint-Louis, MI, USA). NAC and Conoidin A were from Cayman Chemical (Ann Arbor, MI, USA). 10058-F4/1-RH was from Tocris (Bristol, United Kingdom). Accutase was from Innovative Cell Technologies Inc. (San Diego, CA, USA).

Animals and islet isolation All procedures were approved by the Institutional Committee for the Protection of Animals at the Centre Hospitalier de l'Université de Montréal. Male Wistar rats weighing 250–300 g (~2 months old) (Charles River, Saint-Constant, QC, Canada) were group housed (2 animals per cages) under controlled temperature on a 12 h light-dark cycle with free access to water and standard laboratory chow. Islet isolation was performed by collagenase type XI digestion of the pancreas as described previously (1). In brief, 10 ml of collagenase [0.65mg/ml in Hanks buffered saline solution (HBSS)] was injected into the bile duct. The perfused pancreas was dissected and placed in water bath for 10-13 min at 37 °C. Then, 25 ml of cold HBSS+BSA (1%) was added to the samples prior to mechanical digestion by hand shaking, followed by centrifugation at 339xg at 4°C. Samples were then mesh screen filtered and washed twice with HBSS+BSA solution. Pellets were resuspended in 10 ml of Histopaque-1119. Second (Histopaque-1077) and third layer (HBSS+BSA) were respectively added to form a 3-layer gradient. Samples were centrifuged at 339 x g for 30 min at 4 °C. The interphase between the upper and the middle

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layers of the gradient was harvested and washed once with HBSS+BSA. Islets were hand-picked under the microscope prior to culture.

Beta-cell proliferation Following treatment islets were washed once with D-PBS + 2 mM EDTA solution and digested by enzymatic disaggregation for 10 min at 37 °C using Accutase. The reaction was stopped using islet culture medium and the islet cell suspension was washed once with PBS and dead cells labeled using the LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (405 nm; Thermofisher Scientific, Waltham, MA). Sample fixation, permeabilization and EdU detection were performed using the Click-iT™ Plus EdU Alexa Fluor™ 488 Flow Cytometry Assay Kit (BD Biosciences, San Jose, CA) according to the manufacturer's instructions. Fluorophore-coupled anti-C-peptide antibody and dilution are listed in Supplementary Table 1. Dead-cell stain, EdU and C-peptide labelled cells were detected on an LSRII flow cytometer with FACSDiva™ Software (BD Biosciences) using the 405-, 488- and 640-nm lasers coupled with 525/50-, 530/30- and 670/14-nm BP filters. A minimum of 10,000 C-peptide⁺ cells were counted in each sample. Results were presented using FlowJo v10.7 software (Ashland, OR; <https://www.flowjo.com/solutions/flowjo>).

ROS determination Following treatment for 24 h islets were dissociated into single cells and then incubated for an additional 1.5 h in the corresponding treatment conditions. 2.5 μM of CellROX™ Green Reagent fluorogenic probe (Thermofisher Scientific) was added for the final 30 min. Cells were washed twice with PBS and 5 μM of SYTOX™ Red (Thermofisher Scientific) was added to label dead cells. CellROX™ fluorescence intensity was determined on a LSRII flow cytometer (BD Biosciences) using 488- and 640-nm lasers

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coupled with 530/30- and 670/14-nm BP filters. Data were analyzed using FlowJo software. At least 30,000 live cells were counted in each sample.

Quantitative PCR Following treatment RNA was extracted from 150-200 islets using the RNeasy Micro kit (Qiagen, Valencia, CA). RNA was quantified by spectrophotometry with a NanoDrop 2000 (Life Technologies Inc.), and 1µg of total RNA was reverse transcribed using M-MLV reverse transcriptase (Thermo Fisher Scientific). Real-time PCR was performed using the QuantiNova SYBR Green RT-PCR Kit (Qiagen). Primer sequences are listed in Supplementary Table 1.

Immunostaining of islet sections Following treatment islets were embedded in optimal cutting temperature (OCT) compound, frozen, sectioned at 8 µm and mounted on Superfrost Plus slides (Life Technologies Inc., Burlington, ON, Canada). Sections were fixed for 10 min in formalin (10%) and incubated with primary antibody (see Supplementary Table 1 for antibody list and dilutions) in PBS for 1 h using a Ventana Benchmark XT autostainer platform (Roche Diagnostics, QC, Canada). Sections were then treated manually with a serum-free protein blocking solution (DAKO, Agilent, Santa Clara, CA, USA) for 20 min and incubated for 50 min at RT with secondary antibody in PBS containing 1% bovine serum albumin (BSA, Sigma-Aldrich, St-Louis, MO, USA, see Supplementary Table 1) and counterstained with DAPI (1/1000 in PBS). To quench tissue autofluorescence, sections were incubated for 15 min at RT with a 0.1% solution of Sudan Black B (Research organics, Cleveland, OH, USA) in 70% ethanol. Sections were then mounted with Fluoromount™ Aqueous Mounting Medium (Sigma-Aldrich) and kept at 4°C overnight and scanned with an Aperio VERSA 200 scan system (Leica Biosystems,

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ON, Canada). At least 1,500 beta cells were manually counted per condition. The experimenter was blind to group assignments.

Preprocessing of droplet-based scRNA-seq data Demultiplexing of binary base call (BCL) files, alignment to the *Rattus norvegicus* genome (version *Rnor_6.0*), read filtering, barcode and unique molecular identifier (UMI) counting were performed using the Cell Ranger analysis pipeline (v2.0) provided by 10x Genomics. High quality barcodes were selected on the basis of the overall distribution of total UMI counts per cell using the standard Cell Ranger cell detection algorithm. All further analyses were run with R (version 4.1.0) using the Seurat R package (v4.0, <https://satijalab.org/seurat/>). We excluded genes expressed in less than 4 cells from the analysis; as well as cells that had a high fraction of counts from mitochondrial genes (20% or more), that expressed fewer than 200 genes, or that had more than 4,000 genes. Based on these criteria, 2,241 cells (from 54,811 cells) were discarded, resulting in 52,570 cells for further analysis. We did not see bias in the number of cells excluded based in the culture condition.

Embedding, clustering and cell type annotation Sample Raw counts were normalized separately using SCTransform (2) and integrated using Seurat (3). The normalized and log-transformed expression values were used for downstream analysis. PCA was performed on the normalized expression using top 3,000 most variable genes across all samples. A neighborhood graph was built with n-neighbors set to 20 and 30 calculated PCs as inputs. This neighborhood graph was used as input for clustering via the Leiden algorithm (with a resolution parameter of 0.4) (4) and the identified clusters were visualized using Uniform Manifold Approximation and Projection (UMAP) (5). Annotation of cell types was performed using a manually curated list of previously characterized cell type markers (e.g.,

Ins2, Gcg, Sst and Ppy for Beta, Alpha, Delta, and PP-cells respectively). Re-clustering of the Non-beta (12,660 cells) and Beta Cell subpopulations (39,910 cells), identified in the first round of clustering, was performed by extracting the matrix of raw counts of transcripts followed by a second round of normalization, sample dataset integration, dimension reduction and clustering via the Leiden algorithm (with a resolution parameter of 0.2 for non-beta cells and 0.4 for beta cells).

Differentially expressed genes and pathway analysis Pairwise differential gene expression comparisons were made across experimental treatment groups and/or cell type subpopulation. Scaled normalized expression values were used as input to Seurat's FindMarkers function implementing the MAST test (6) to identify DEGs. Genes were declared significantly differentially expressed at a False Discovery Rate (FDR) cut-off of 5% and absolute log₂ fold change > log₂(1.2). MsigDB's Hallmark and C5 Gene Ontology (GO) gene sets were used for Fisher Enrichment Analysis followed by an Enrichment Map (7) analysis to aggregate and organize overlapping GO gene-sets into networks making functional interpretation easier. Gene sets were declared significant at an FDR of 5%. The CytoTRACE function from the R package CytoTRACE (8) was used to assign a differentiation state score to each cell based on expression of a set of genes.

Trajectory inference The Slingshot (9) R/Bioconductor package was used to reconstruct lineages and infer pseudotime. The goal of Slingshot is to use clusters of cells to uncover global structure and convert this structure into smooth lineages represented by a smooth curve and a one-dimensional variable, called pseudotime. A single lineage represents a path in a cellular trajectory (from a start point to end point) and the cells belonging to the lineage are then ordered by the pseudotime calculated by Slingshot. The Condiments

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R/Bioconductor package (10), which implements a statistical workflow for modeling cell trajectories across multiple experimental conditions was used to refit a slingshot trajectory for each experimental condition and to map the Cell weights, the probability that a cell belongs to a particular lineage in the trajectory, to each experimental condition.

To identify genes whose expression patterns along the trajectory are differentially modulated between the experiment's conditions, for every gene in the dataset we fit a negative binomial generalized additive model (NB-GAM), using the gam function as implemented in the R's package mgcv (doi: 10.1201/9781315370279), to smooth each gene's expression in each lineage and generate separate smoothers for each condition based on the pseudotime inferred by slingshot. We tested whether the smoothers are significantly different between conditions by comparing smooths in factor-smooth interactions. The resulting p-values were adjusted for multiple testing and significance set at FDR level set to 5%. Significant Differences between pairs of smooths were visualized using plot_diff function as implemented in R's package itsadug.

SUPPLEMENTARY REFERENCES

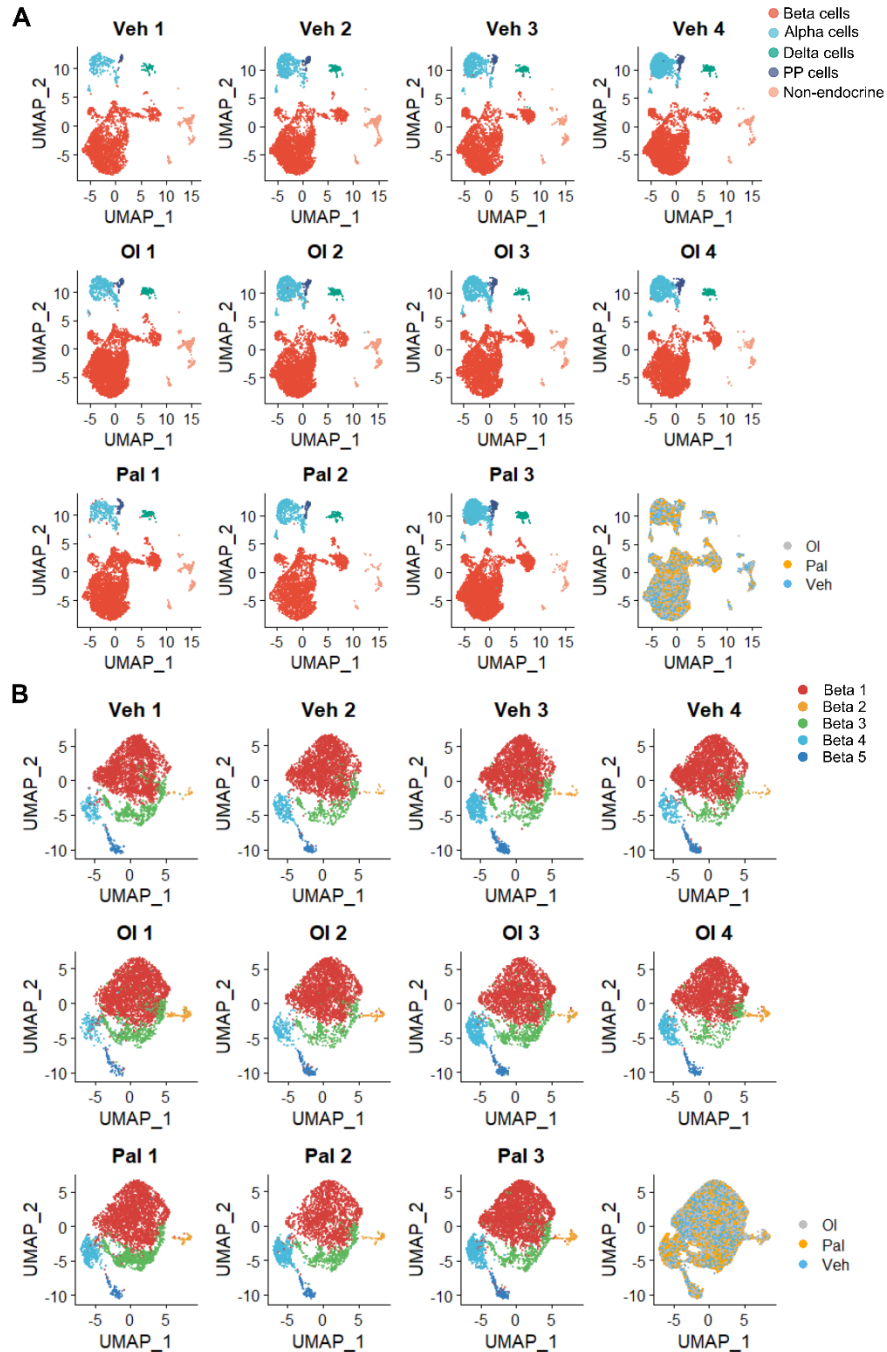
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SUPPLEMENTARY FIGURES

Supplementary Figure 1

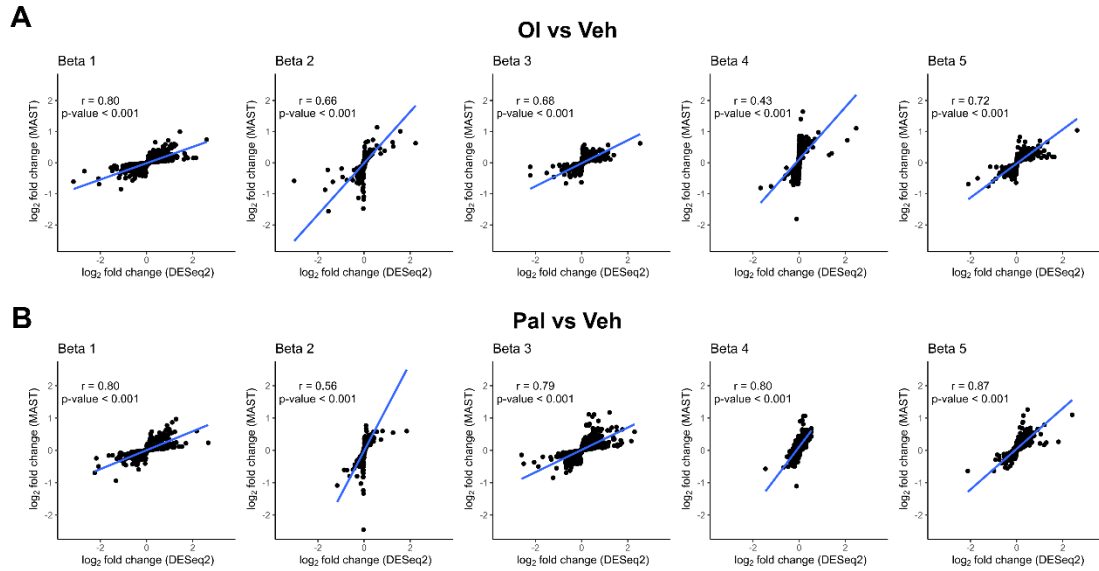


Supplementary Figure 1: scRNA-seq cell distribution across biological replicates and treatment conditions.

(A) UMAP plot total cells for each biological replicate (1-4) and condition (Veh, Pal and OI) showing the cell distribution among endocrine (alpha, beta, delta and PP cells) and non-endocrine cell types.

(B) UMAP plot of beta cells for each biological replicate (1-4) and condition (Veh, Pal and OI) showing the cell distribution among the five beta-cell subpopulations (Beta 1-5).

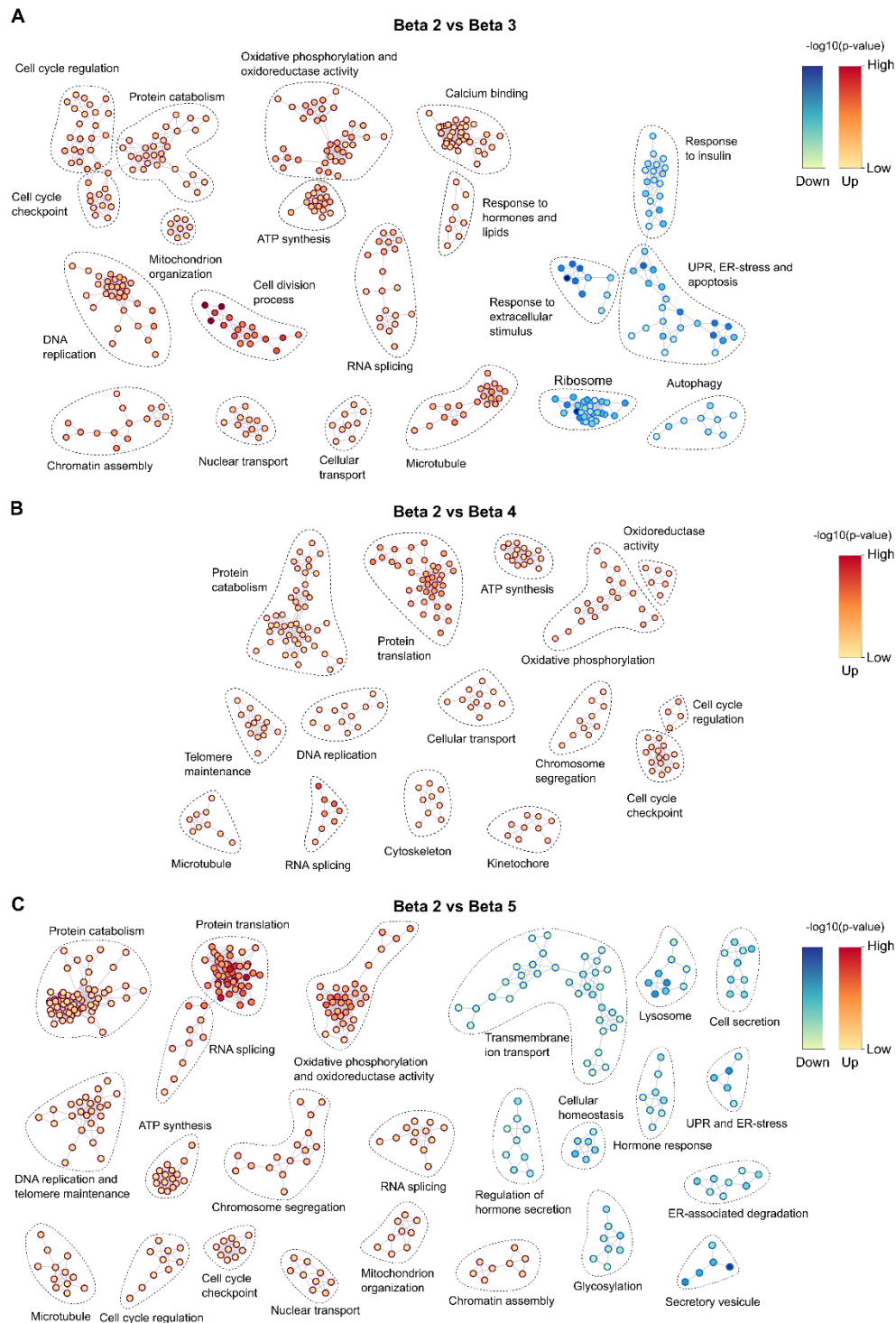
Supplementary Figure 2



Supplementary Figure 2. Comparison of methods for identification of differentially expressed genes.

(A, B) Correlation between differentially expressed genes identified in the Beta 1-5 subpopulations using MAST or DESeq2 expressed as Log2 fold change of OI (A) or Pal (B) vs Veh.

Supplementary Figure 3



Supplementary Figure 4

SUPPLEMENTARY TABLES

Supplementary Table 1

Flow cytometry antibodies

Antibody	Dilution/Concentration	Company	Cat #
Alexa Fluor® Mouse anti-C-peptide	1:25	BD Biosciences	565831
PE Mouse Anti-Glucagon	1:25	BD Biosciences	565860

RT-PCR primers

	Forward sequence	Reverse sequence
Cyclophilin-A	GCCATTATGGCGTGTGAAGTC	CTTGCTGCAGACATGGTCAAC
Myc	GAGGTGGAAAACCCGACAGT	AAATAGGGCTGCACCGAGTC
Cdk1	GGAACAGAGAGGGTCCGTTG	CCACACCATAAGTCCCTTCTCC

Immunostaining antibodies

Primary antibody	Dilution/Concentration	Company	Species	Cat #
Recombinant Anti-c-Myc antibody [Y69]	1:50	Abcam	Rabbit	ab32072
Insulin Monoclonal Antibody(INS05 (2D11-H5))	1:200	Thermofisher	Mouse	MA5-12037

Secondary antibody	Dilution/Concentration	Company	Species	Cat #
Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 546	1:250	Thermofisher	Goat	A-11035
Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	1:250	Thermofisher	Goat	A-11029

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Supplementary Table 2

Number of cells per biological replicate (scRNA-sequencing)

	Veh1	Veh2	Veh3	Veh4	Ol1	Ol2	Ol3	Ol4	Pal1	Pal2	Pal3	Total
Beta cells	2580	3414	2832	4026	4067	3511	3691	3465	4195	2994	5135	39910
Alpha cells	253	538	603	1446	442	694	794	906	285	489	1371	7821
Delta cells	63	127	112	192	137	125	161	143	78	88	193	1419
PP cells	22	72	92	208	54	71	116	184	58	60	220	1157
Non-endocrine	195	300	191	152	334	226	290	115	237	106	117	2263
Total	3113	4451	3830	6024	5034	4627	5052	4813	4853	3737	7036	52570