

Supplemental Information

Persistent or Transient Human β Cell Dysfunction Induced by Metabolic Stress: Specific Signatures and Shared Gene Expression with Type 2 Diabetes

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Figure S1. Insulin secretion studies. Related to Figure 2.

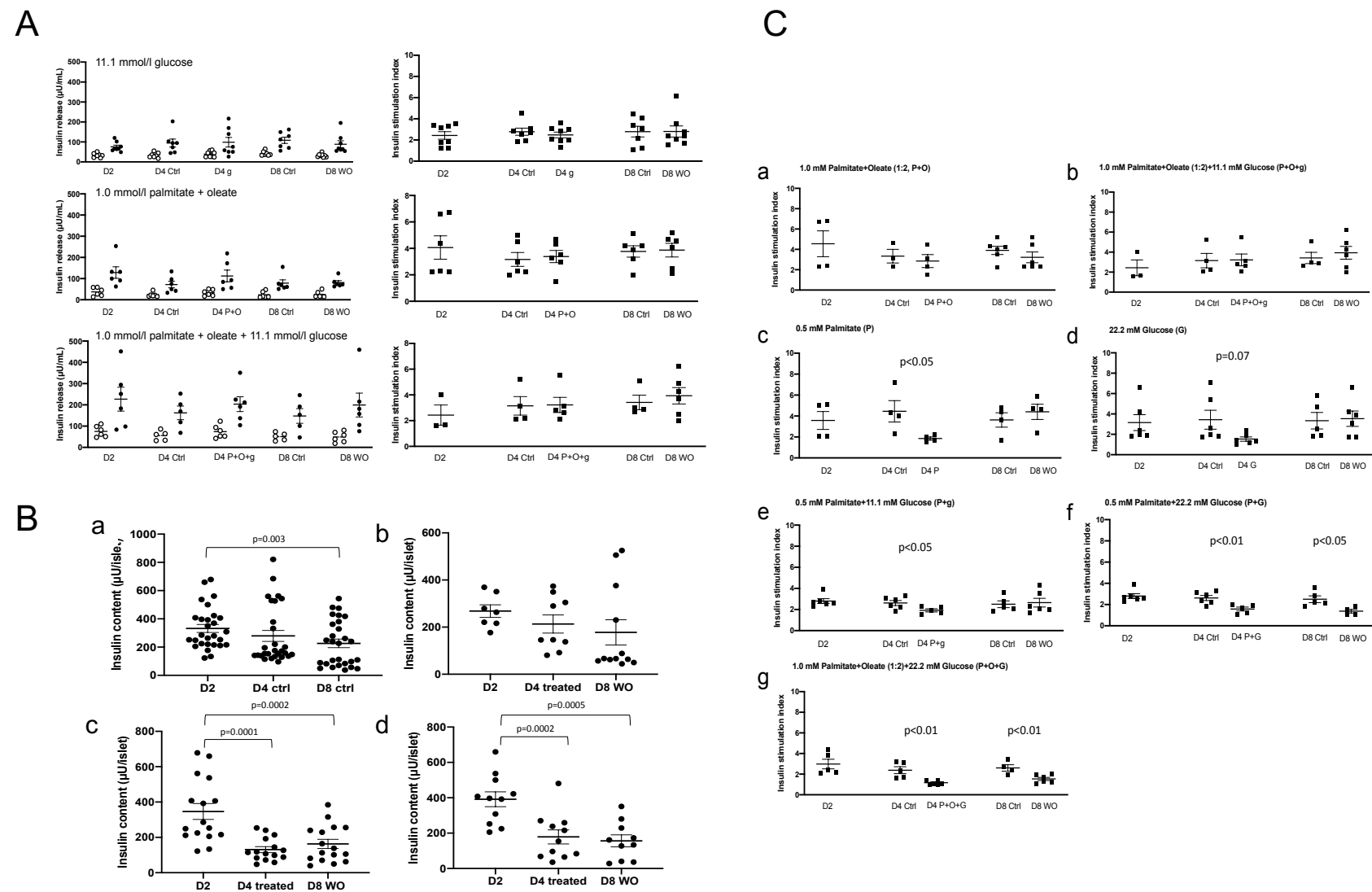


Figure S1

A: Glucose-induced insulin secretion (expressed as actual values, left panels, and stimulation index, right panels) from islets cultured in control medium (Ctrl) or incubated with 11.1 mmol/l glucose (**g**), 1.0 mmol/l palmitate + oleate, (**P+O**) and 1.0 mmol/l palmitate + oleate + 11.1 mmol/l glucose (**P+O+g**). No significant change was seen with these conditions throughout the study period. Release at 16.7 mmol/l glucose (filled dots) was significantly higher ($p < 0.05$ or less) than at 3.3 mmol/l glucose (empty dots) at any tested condition. The number of separate islet preparations and that of replicates per condition were: for **g**, 4 and 7/8 per time point (38 replicates in total); for **P+O**, 3 and 4/6 per time point (26 replicates in total); for **P+O+g**, 3 and 5/6 per time point (28 replicates in total).

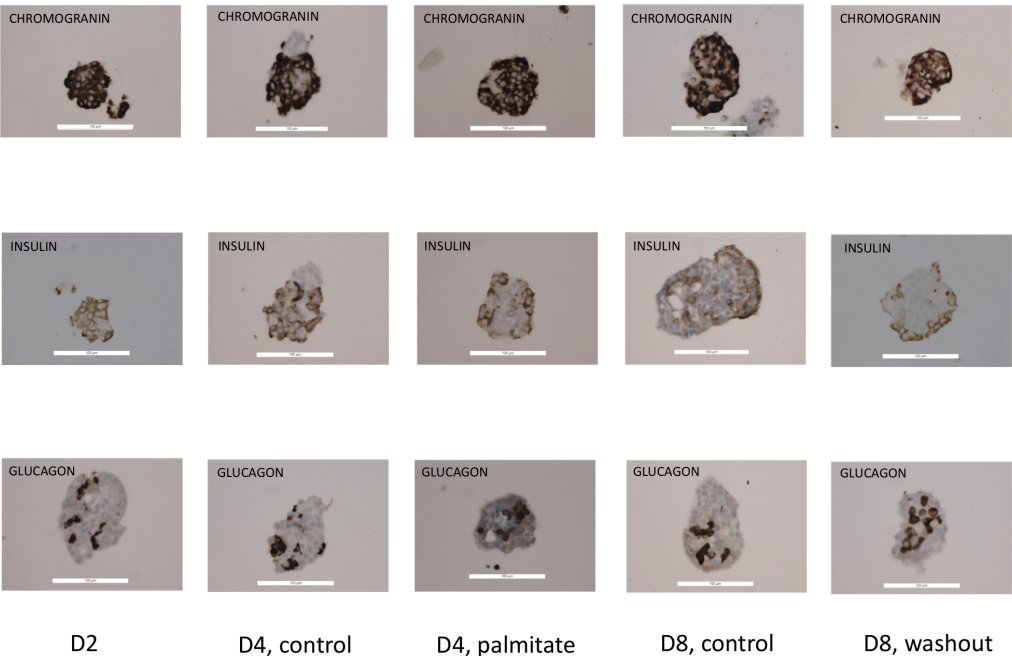
B: Insulin content at D2, D4 and D8 in control islets (a), islets exposed to stresses not affecting insulin release (b), islets exposed to stresses affecting insulin release with recovery after wash out (c) and islets exposed to stresses affecting insulin release with persistent damage after wash out (d).

C: Insulin stimulation index normalized to insulin content of control islets and islets exposed to the different metabolic stressors, showing no change (a, b), reversibility of impairment after washout (c-e) or persistence of functional damage after washout (f,g).

Bars indicate mean \pm SE.

Figure S2. Islet morphology and ultrastructure. Related to Figure 2.

A



B

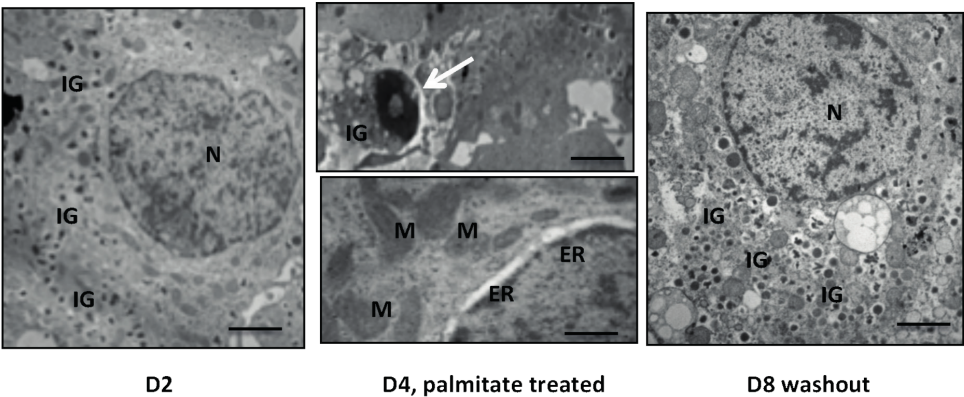
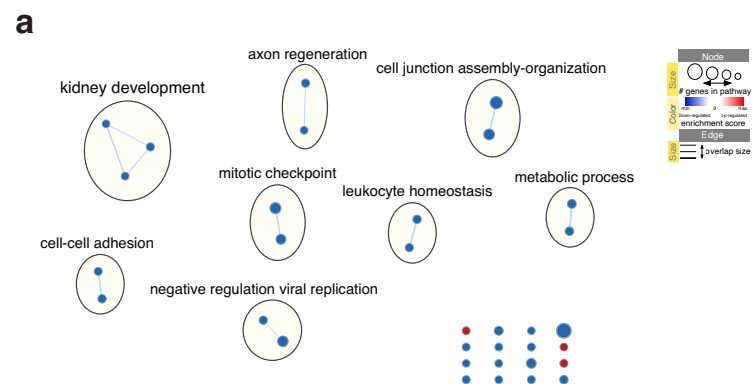


Figure S2

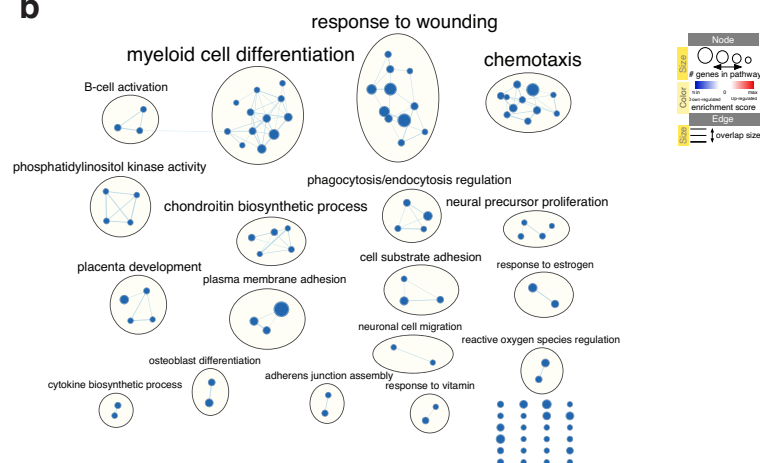
Palmitate exposure experiments. **A:** Islet architecture appeared well maintained throughout the study period (scale white bars correspond to 100 μm); **B:** Electron microscopy pictures showing a normally looking β-cell at D2, an apoptotic β-cell (white arrow) at D4 (palmitate-treated; note enlarged and round mitochondria) and a β-cell at D8 (washout). N: nucleus; IG: insulin granules; M: mitochondria; ER: endoplasmic reticulum (scale black bars correspond to 1 μm in the external panels and to 0.45 μm in the central panels)

Figure S3. Effects of high glucose culture and expression of β -cell markers in the various lipo- and/or glucotoxic conditions. Related to Figures 2 and 3

A



b



B

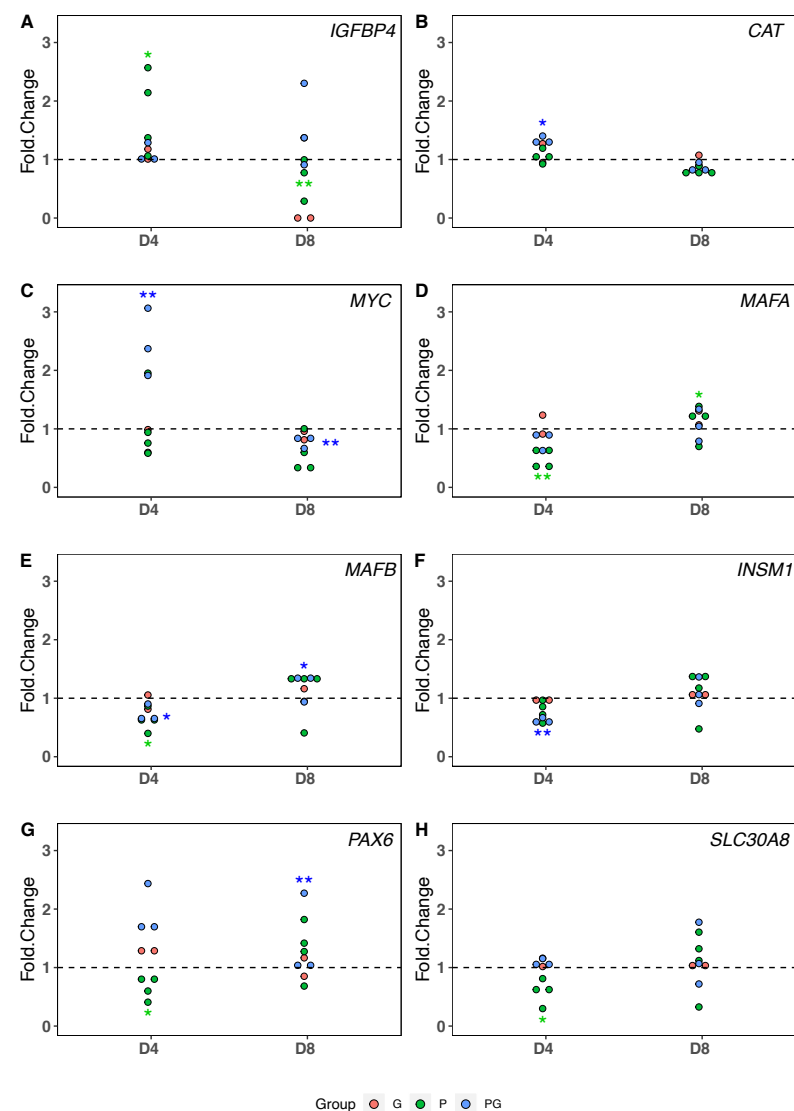


Figure S3

A: Enrichment Map analysis of the transcriptome of islets (a) exposed to 22.2 mmol/l glucose D4 vs control islets at D4 or (b) D8 22.2 mmol/l glucose washout vs D4 22.2 mmol/l glucose-treated islets. Red color indicates upregulated and blue color downregulated processes.

B: Expression of β -cell markers in human islets exposed to metabolic stresses. The markers include differentially expressed disallowed/forbidden genes (A and B), de-differentiation markers (C) and β -cell identity genes (D - H). D4 and D8 represent the RNA-seq expression levels for treatment and washout, respectively. Fold change represents the ratio of TPM expression levels at D4 relative to D4 control, and D8 washout relative to D8 control. The red symbols show high glucose exposure, green palmitate exposure and blue palmitate + high glucose exposure. FDR adjusted q value $* < 0.05$, $** < 0.01$ for D4 treatment relative to D4 control or D8 washout relative to D4 treatment.

Figure S4. Comparison of differentially expressed genes and pathways among three metabolic stress conditions at D4. Related to Figures 2 and 3.

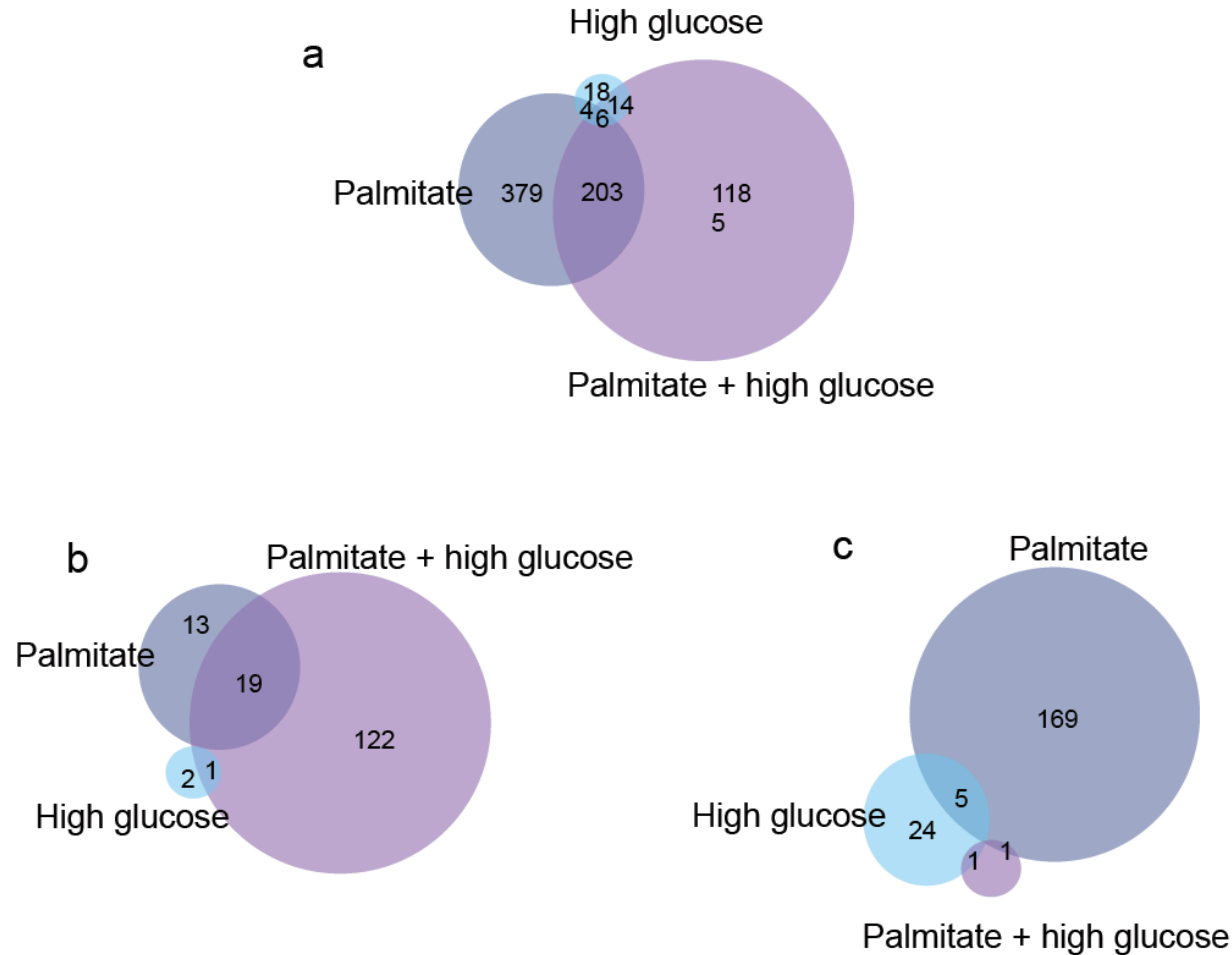


Figure S4(a) The number of differentially expressed genes from D4 treatment vs D4 control for high glucose, palmitate and palmitate + high glucose are displayed. Comparison of the similarities and differences in GO (Gene Ontology) categories enriched with (b) up- and (c) downregulated genes for D4 treatment vs D4 control. Filtering condition: adjusted $p < 0.05$

Figure S5. Validation studies. Related to Figure 3.

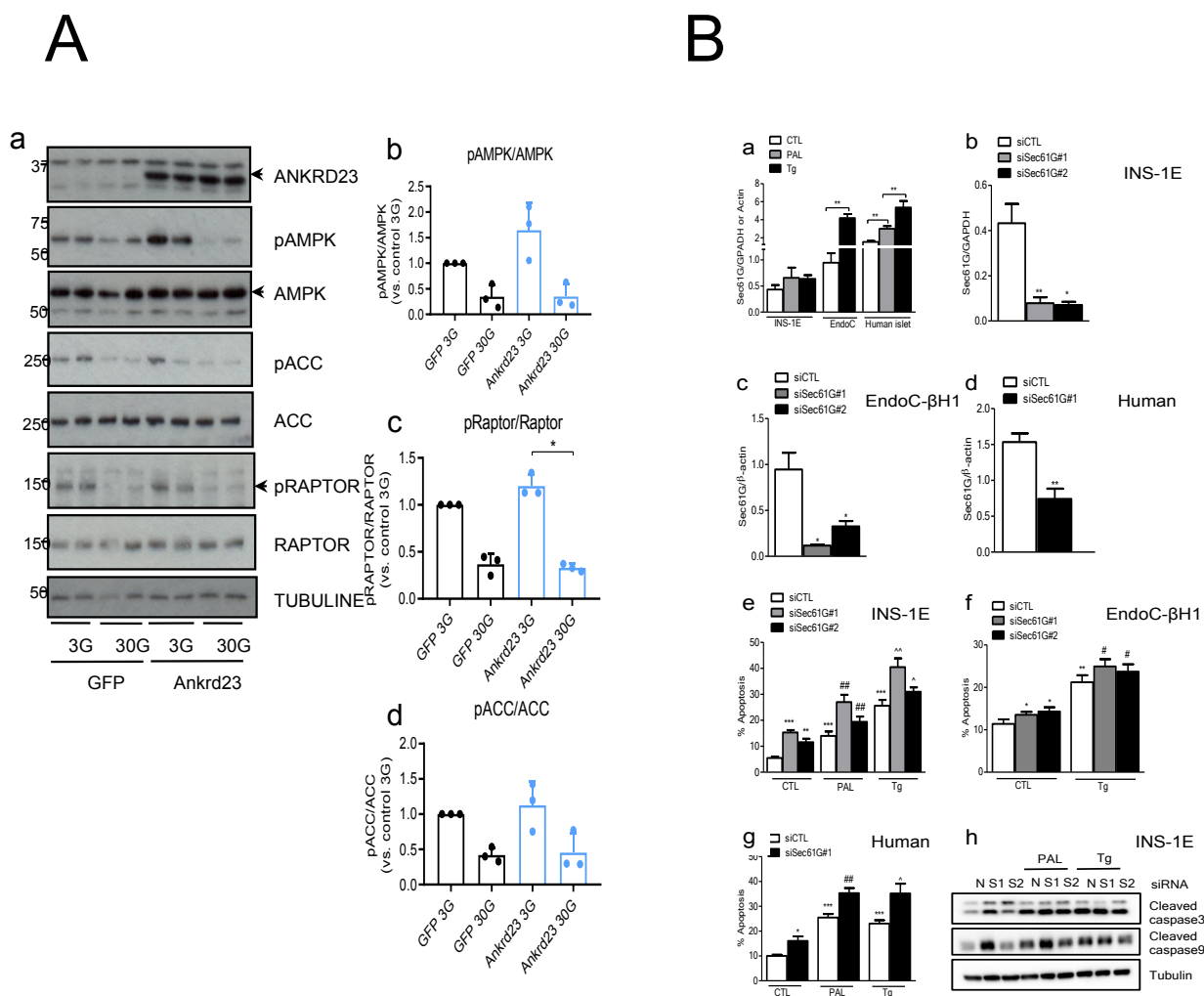


Figure S5

A: Overexpression of *ANKRD23* in the glucose-responsive pancreatic β -cell line MIN6B1 (a) associates with a tendency for an increase in the level of AMPK phosphorylated at T172 of the catalytic alpha subunit (pAMPK) under low (3 mM) glucose conditions (a,b). Similarly, phosphorylation of the downstream substrates of AMPK pRaptor and pACC (Acetyl-coA carboxylase) tended to be increased versus GFP-transfected cells (GFP) (a,c,d). As a consequence, in *ANKRD23*-transfected cells high (30 mM) glucose led to a significant change in pRaptor versus the low glucose condition.

B: The role of SEC61G. Sec61G mRNA expression in INS-1E, EndoC- β H1 and dispersed human islet cells increases after exposure to 0.5 mmol/l palmitate (PAL) or 1 μ mol/l thapsigargin (Tg) compared to control (CTL) (a). Reduced Sec61G mRNA and protein expression by RNA interference (siSec61) in INS-1E (b), EndoC- β H1 (c) and human islet (d) cells enhances PAL- or Tg-induced apoptosis (e-g) and activation of caspase 3 and 9 (h). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs CTL; # $p < 0.05$ and ## $p < 0.01$ of siSec61G treated with PAL vs CTL; ^ $p < 0.05$ and ^^ $p < 0.01$ of siSec61G treated with Tg vs CTL.

Bars indicate mean \pm SD in panels A,b-d and mean \pm SE in panels B,a-g.

Figure S6. Insulin secretion from non-diabetic and type 2 diabetic islets. Related to Figure 1.

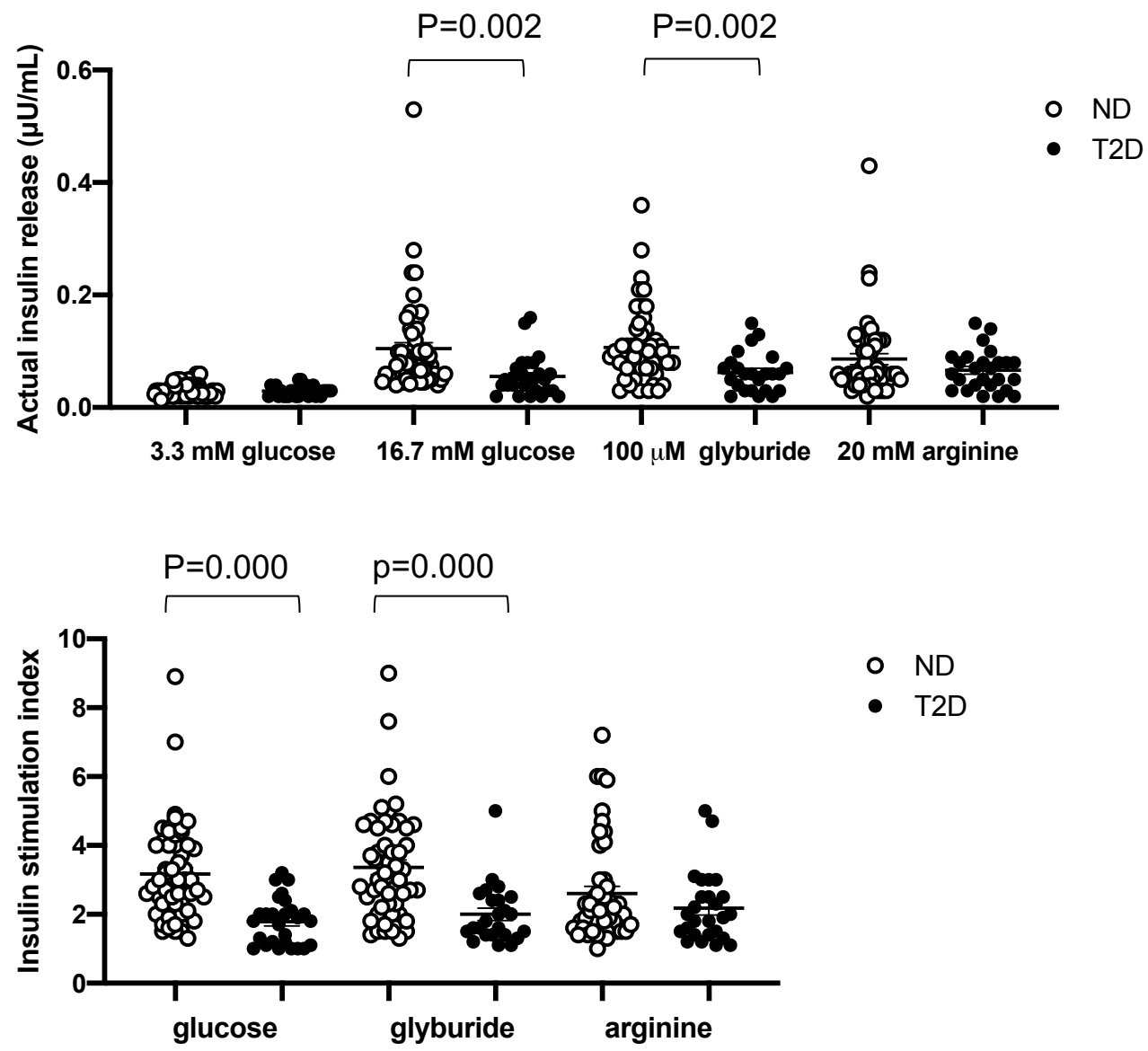


Figure S6: Insulin secretion (actual values: upper panel; insulin stimulation index: bottom panel) from non-diabetic (ND) and type 2 diabetic (T2D) islets, showing reduced release from the latter in response to acute challenge with 16.7 mmol/l glucose and 100 μmol/l glyburide. Bars indicate mean±SE.

Figure S7. RRHO maps. Related to Figure 5.

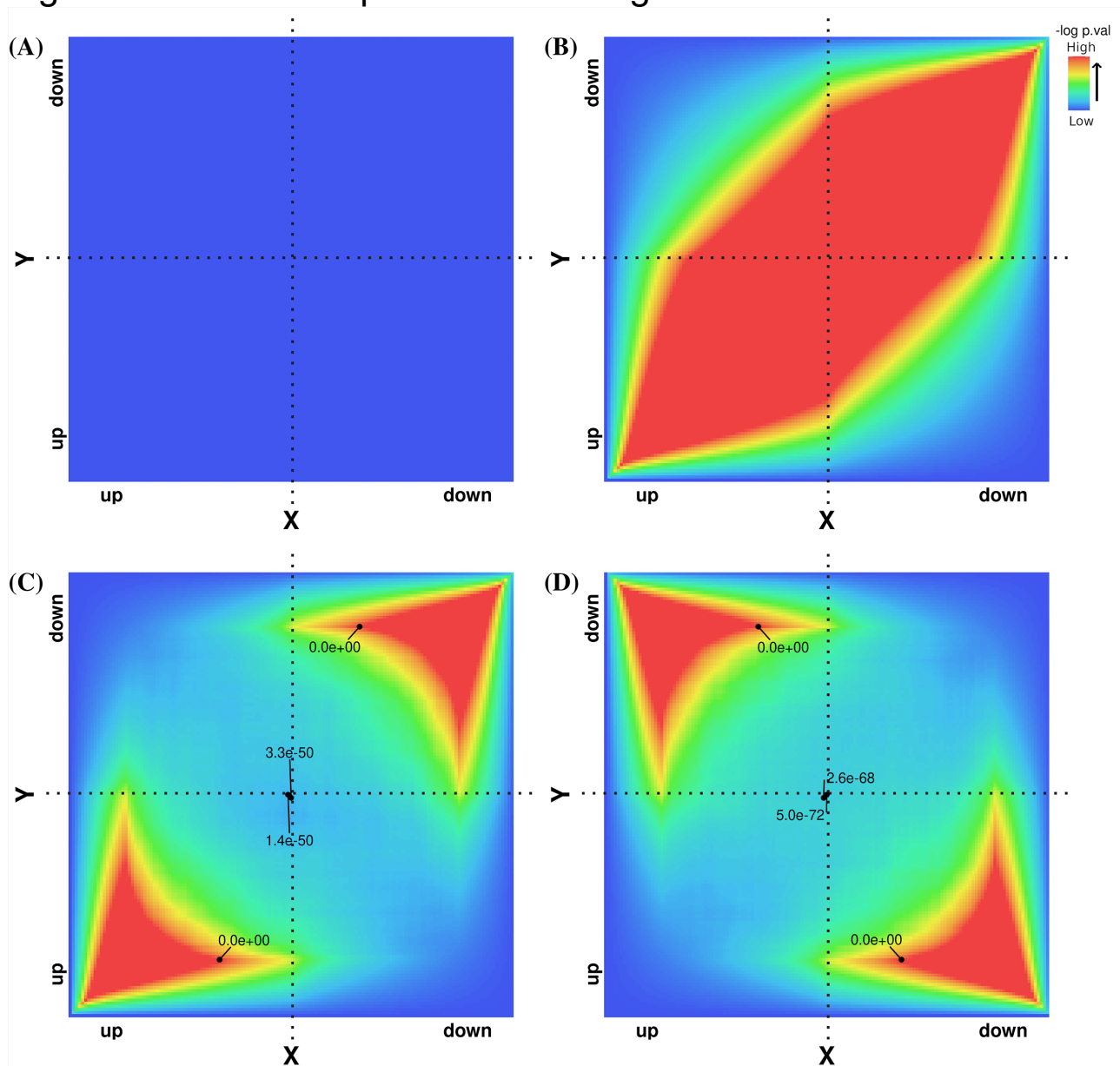


Figure S6: Theoretical rank-rank hypergeometric overlap (RRHO) maps. RRHO is a threshold-free method to compare the fold changes of two differential analyses. The genes are ranked by fold change from most down- to most upregulated in the experimental conditions. The level map colors show $-\log p$ -values for overlap, with an indication of the smallest p -value for clusters with statistically significant overlap between genes upregulated in both datasets (bottom left quadrant), downregulated in both (top right quadrant), upregulated in X and downregulated in Y differential analyses (top left quadrant) and downregulated in X and upregulated in Y (bottom right quadrant). The coordinates of minimal values are used as a reference to compute the overlapping gene sets. The scale of the p -value is not comparable between the RRHO plots. The four illustrating level maps are for (A) two random differential analyses, (B) two identical differential analyses, i.e. perfect overlap, (C) two differential analyses with the top and bottom 25% fold changes perfectly identical, and (D) two differential analyses with the top and bottom 25% fold changes perfectly identical but in opposite direction.

Table S3. LC/MS results. Related to Tables 1 and 2.

Accession	Description	Highest -10lgP	Highest Coverage (%)	Number of Peptides	Number of unique Peptides	Avg. Mass
NX_P04054-1	Phospholipase A2	68.67	14	2	2	16360
NX_P05451-1	Lithostathine-1-alpha	203.09	55	22	7	18731
NX_P48304-1	Lithostathine-1-beta	182	46	19	4	18665
NX_Q9BZQ8-1	Protein Niban	130.76	6	5	5	103135
NX_P50440-1	Glycine amidinotransferase mitochondrial	269.66	60	24	24	48455
NX_Q5BJF2-1	Transmembrane protein 97	78.35	10	2	2	20848
NX_P04004-1	Vitronectin	41.22	2	1	1	54306
NX_P00995-1	Serine peptidase inhibitor Kazal type 1	64.27	23	2	2	8507

Phospholipase A2 = PLA2G1B

Lithostatin-1-alpha = REG1A

Lithostatin-1-beta = REG1B

Protein Niban = FAM129A

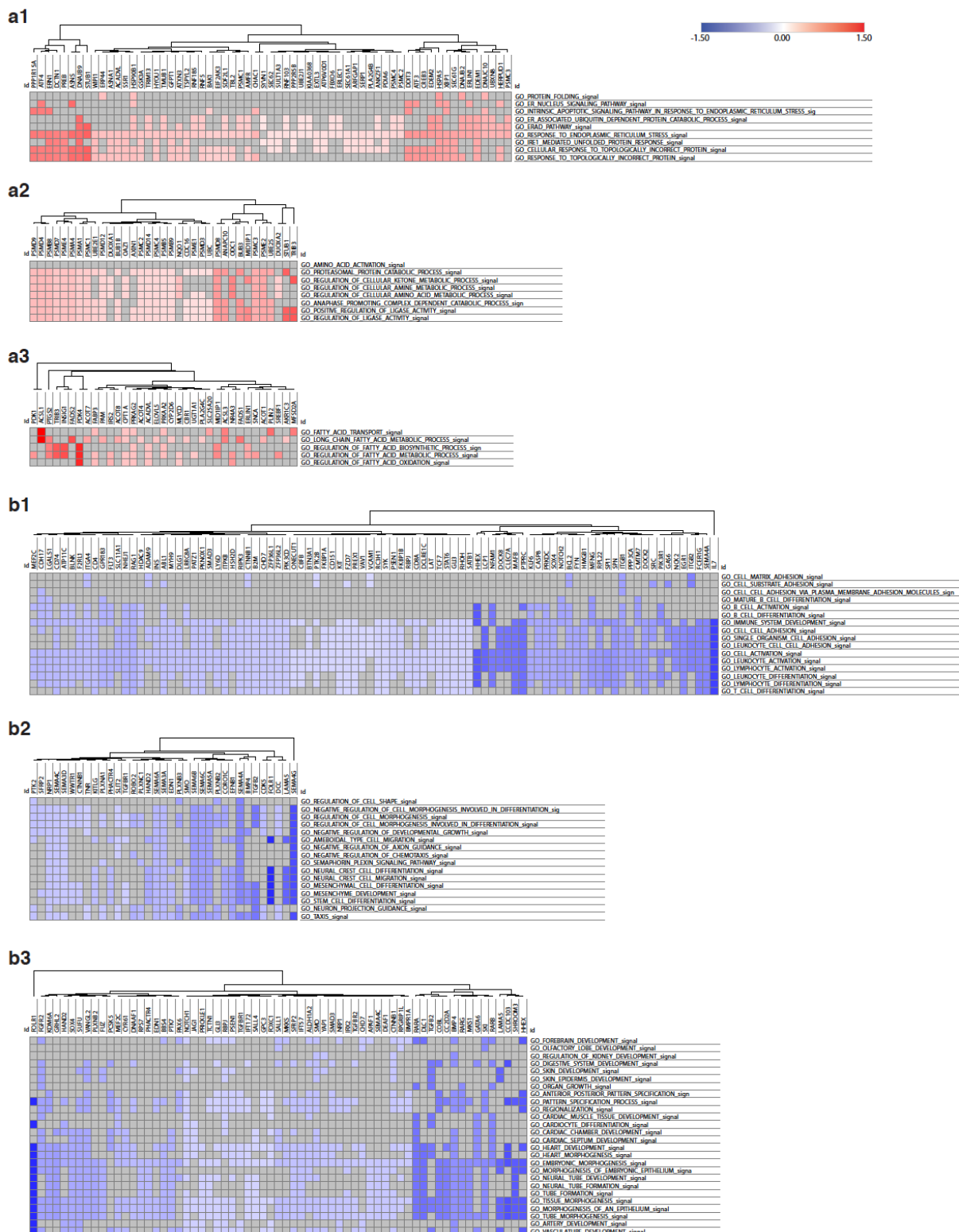
Glycine amidinotransferase mitochondrial = GATM

Transmembrane protein 97 = TMEM97

Vitronectin = VTN

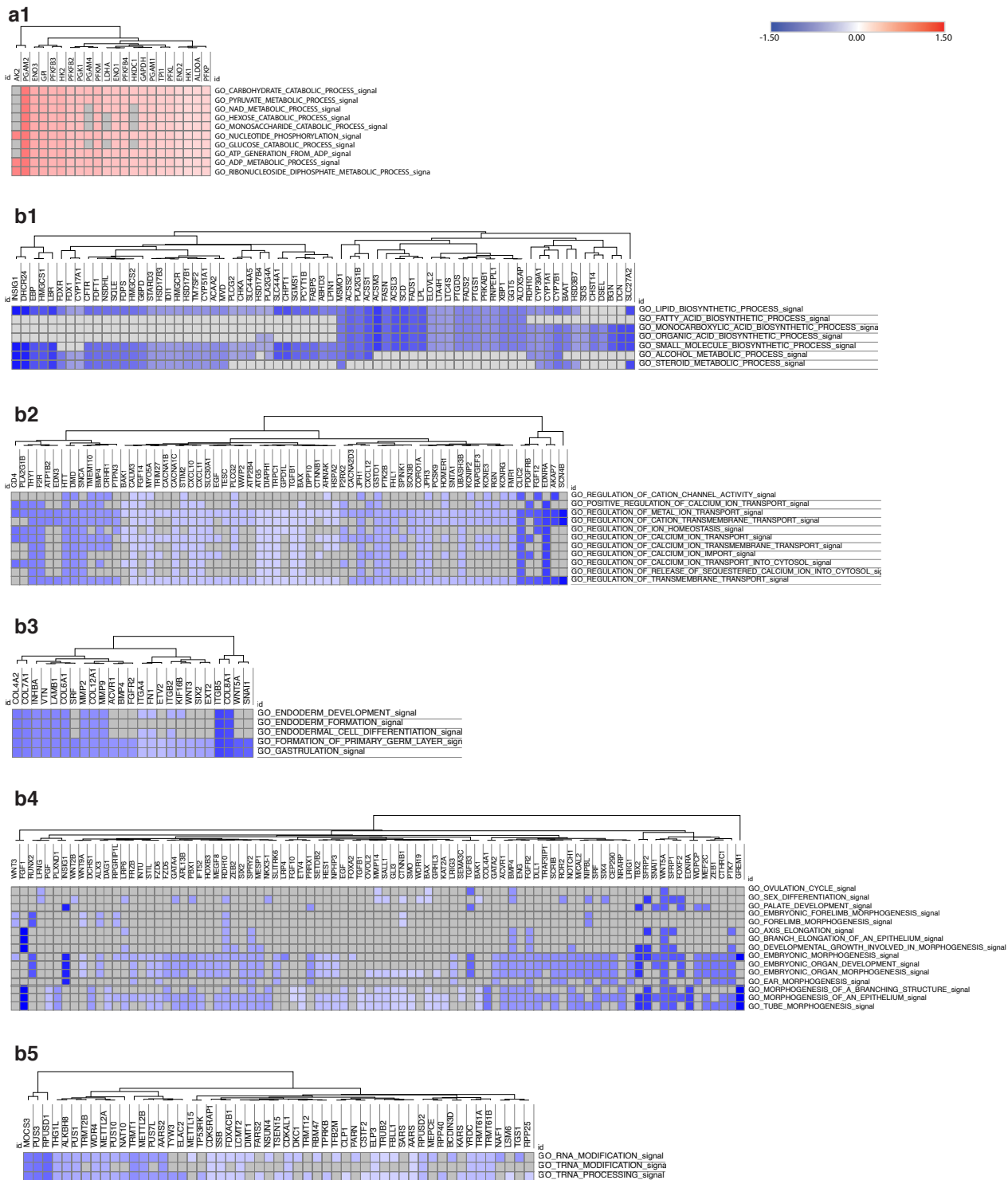
Serine peptidase inhibitor Kazal type 1 = SPINK1

Data S1A. Examples of gene-sets and individual genes at D4 palmitate-exposed vs D4 control islets. Related to Figure 3a.



Data S1A: Examples of gene-sets and individual genes up- (a) or downregulated (b) at D4 palmitate-exposed vs D4 control islets: a1. Unfolded protein response; a2. Protein catabolic process; a3. Fatty acid metabolism; b1. Cell adhesion/activation; b2. Neuronal differentiation; b3. Organ morphogenesis.

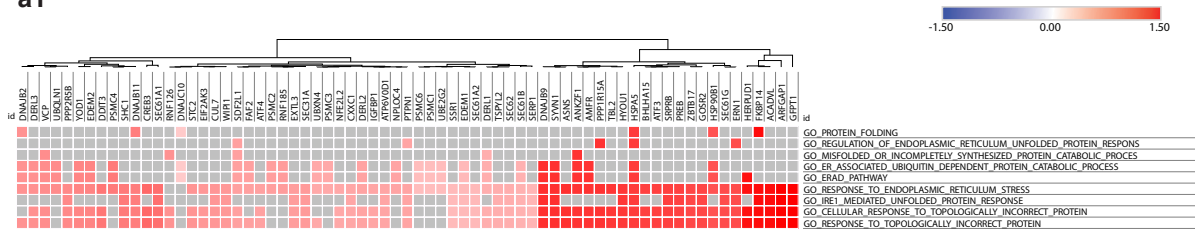
Data S1B. Examples of gene-sets and individual genes at D8 palmitate washout vs D4 palmitate-treated islets. Related to Figure 3b.



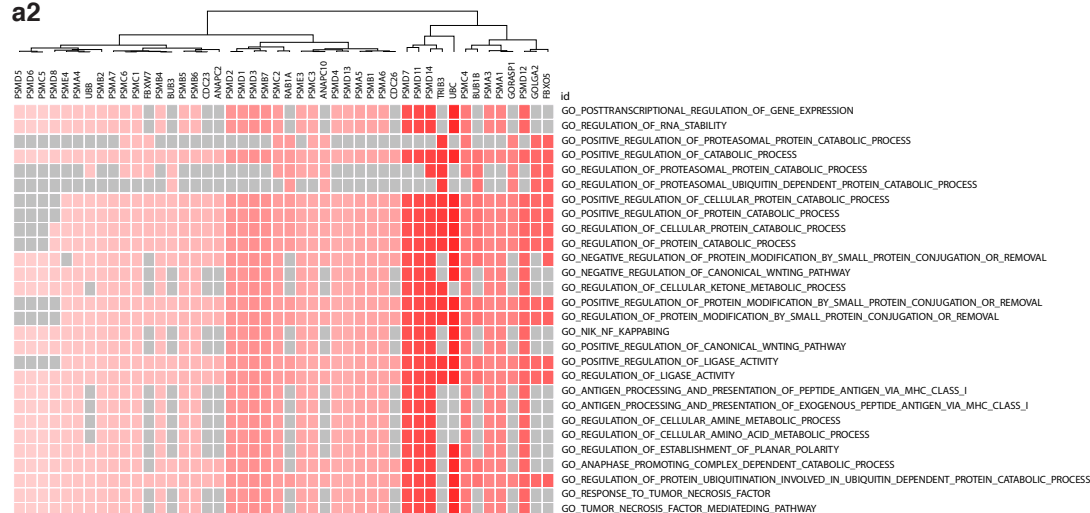
Data S1B: Examples of gene-sets and individual genes up- (a) or downregulated (b) at D8 palmitate washout vs D4 palmitate-treated islets: a1. Carbohydrate catabolic process; b1. Fatty acid metabolism; b2. Calcium transport; b3. Endoderm formation; b4. Organ morphogenesis; b5. tRNA modification.

Data S1C. Examples of gene-sets and individual genes upregulated at D4 palmitate + high glucose vs D4 control islets. Related to Figure 3c.

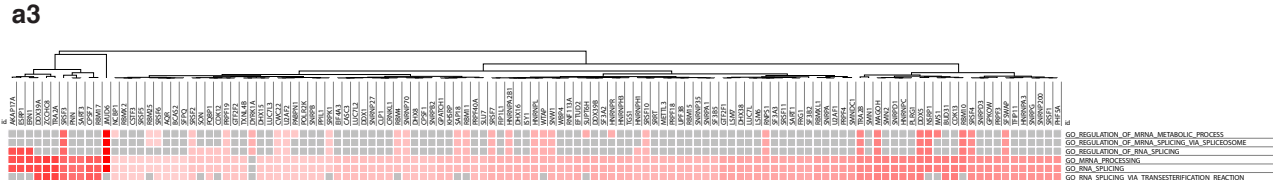
a1



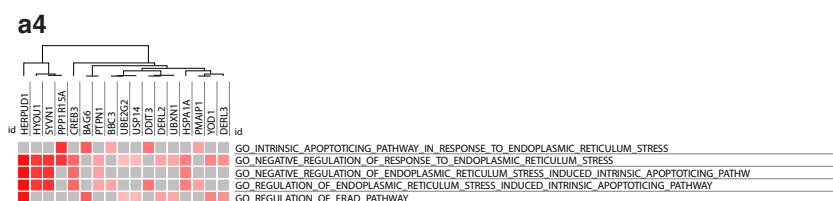
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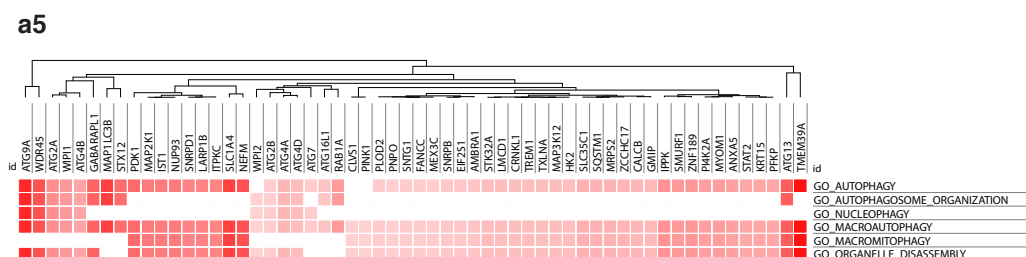
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a4



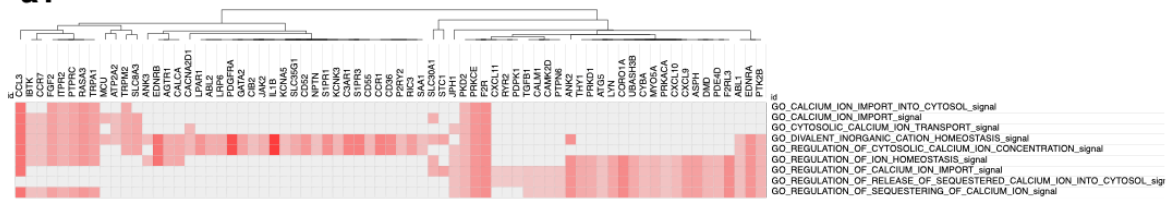
a5



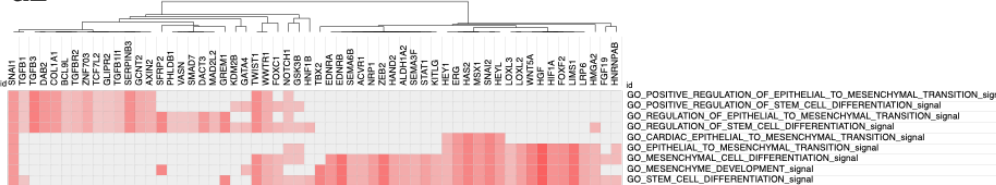
Data S1C: Examples of gene-sets and individual genes upregulated at D4 palmitate + high glucose vs D4 control islets: a1. Unfolded protein response; a2. Protein degradation; a3. mRNA splicing regulation; a4. ER stress-induced apoptosis; a5. Autophagy.

Data S1D. Examples of gene-sets and individual genes upregulated in T2D vs ND islets. Related to Figure 4.

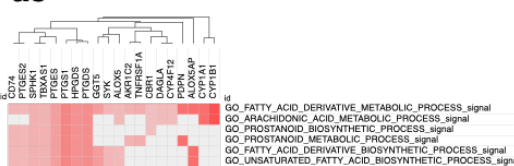
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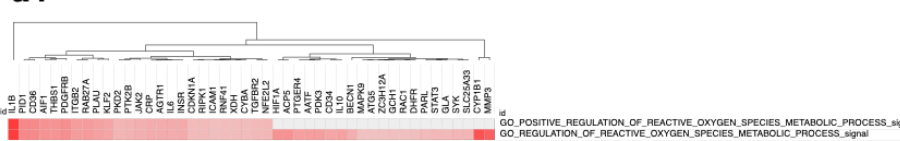
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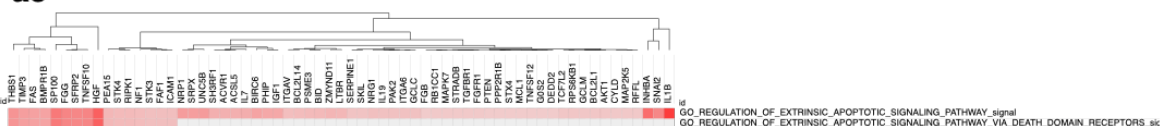
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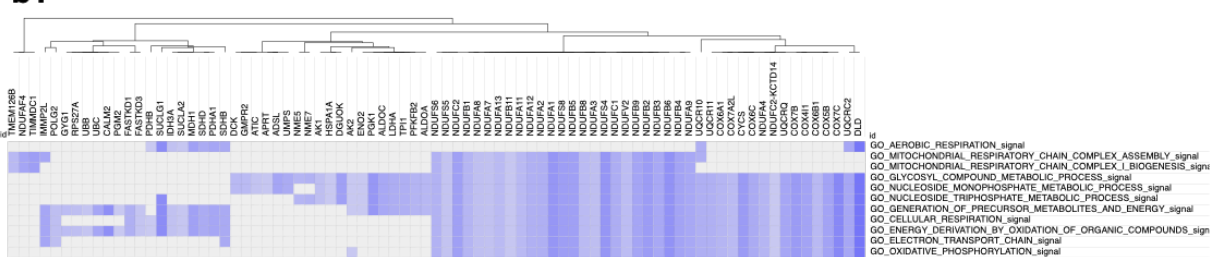
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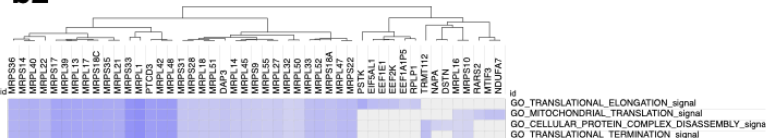
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b1

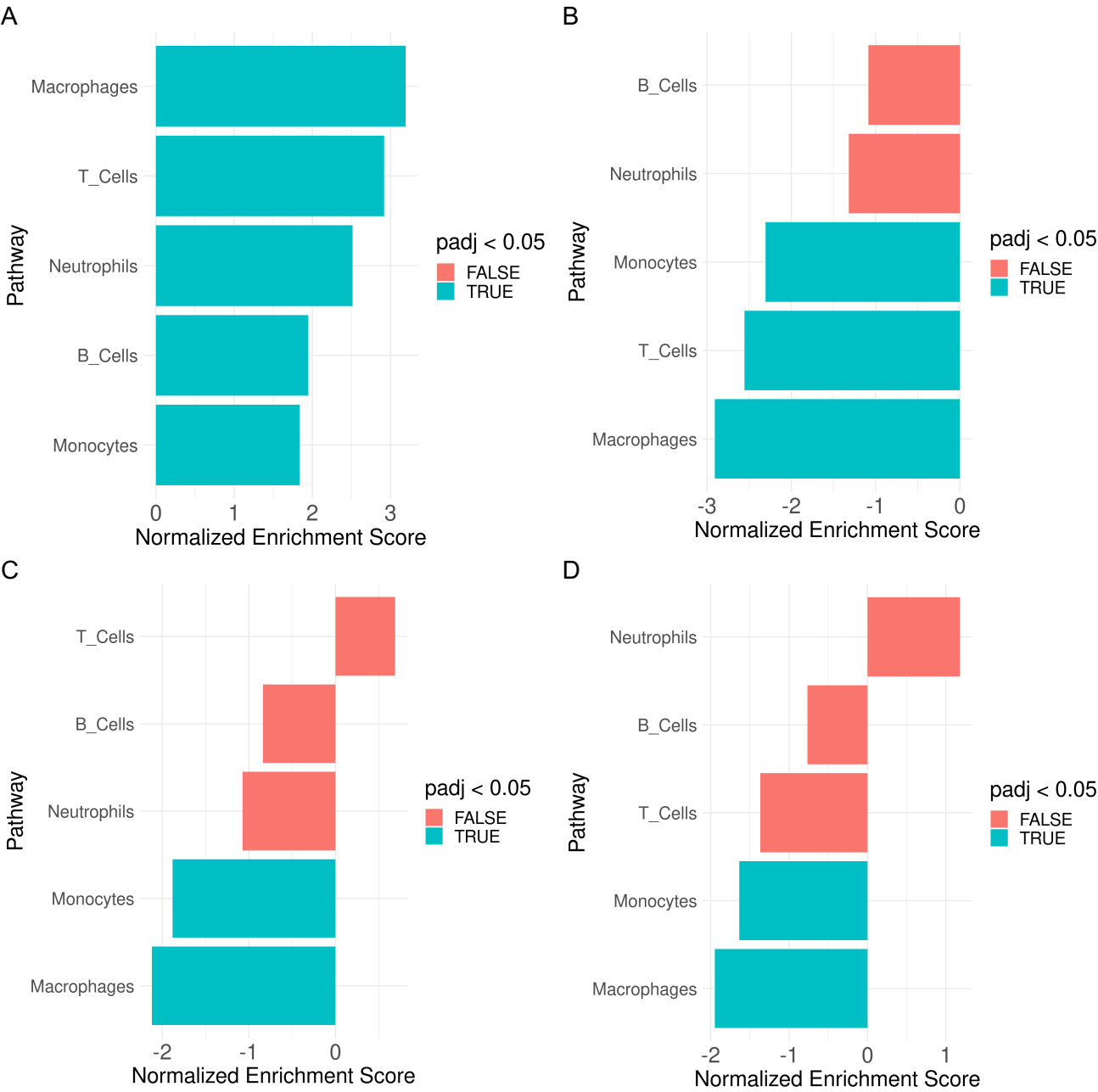


b2



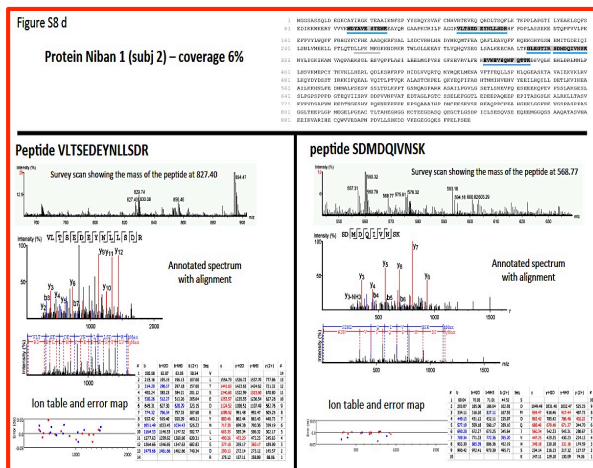
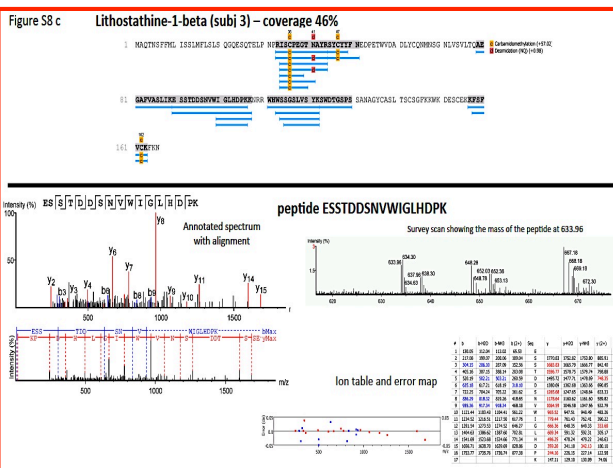
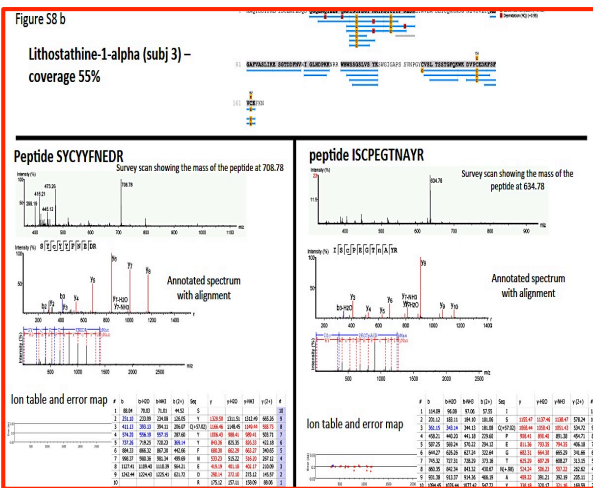
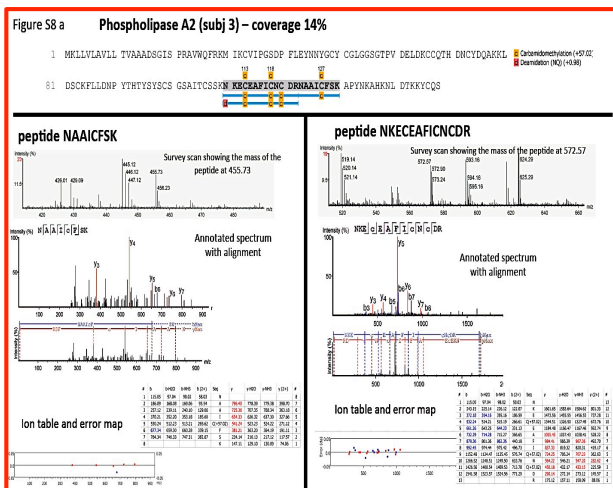
Data S1D: Examples of gene-sets and individual genes upregulated in T2D vs ND islets: a1. Intracellular calcium regulation; a2. Epithelial-mesenchymal transition; a3. Fatty acid metabolism; a4. ROS activity; a5. Extrinsic apoptotic pathway; b1. Mitochondrial respiratory chain; b2. Translation control.

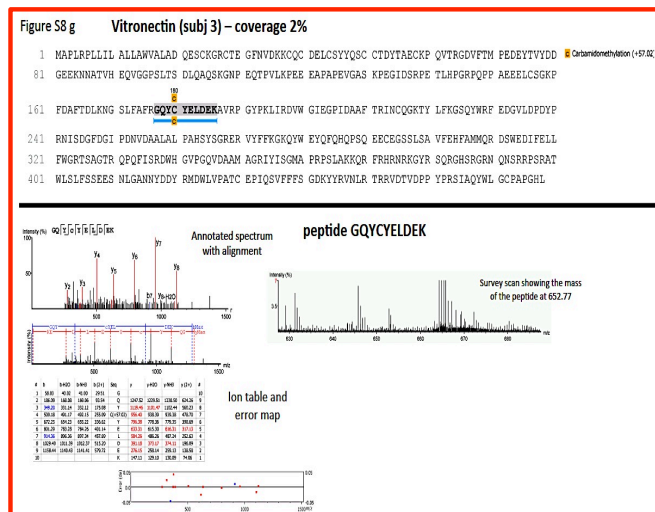
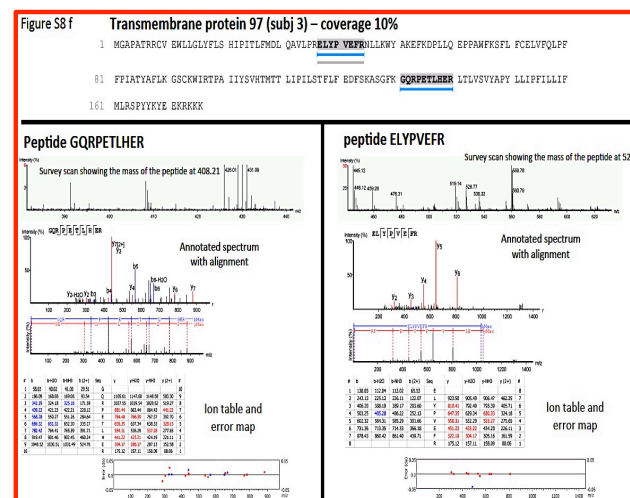
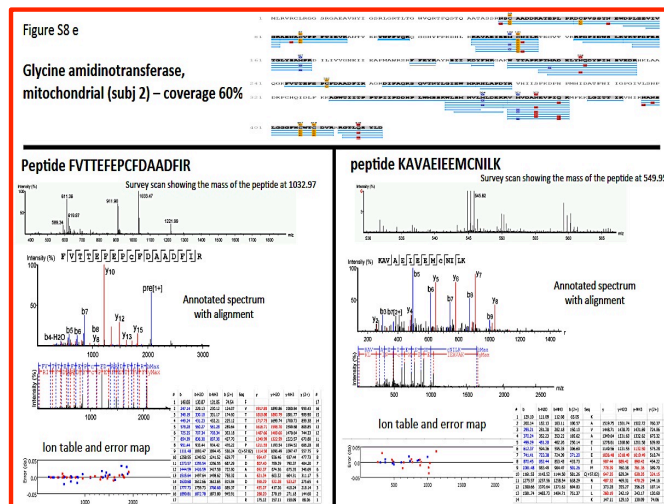
Data S1E. Gene Set Enrichment Analysis (GSEA) of immune cells.
Related to Figure 5C.



Data S1E: Gene Set Enrichment Analysis (GSEA) of immune cells in (A) T2D vs ND donor islets, (B) D4 palmitate-treated islets vs D4 control, (C) D4 high glucose-treated islets vs D4 control and (D) D4 palmitate + high glucose-treated islets vs D4 control.

Data S2. Figures of merit related to the identification of some of the reported proteins. Related to STAR methods – Proteomics experiments





Data S2: Figures of merit related to the identification of the reported proteins. Every panel includes the protein sequence coverage with highlighted all the matching peptides, the raw and annotated tandem mass spectra of the two most significant peptides, the relative survey scan zoomed on the mass region of the selected precursor mass, the error map and the ion table showing the matching fragments (blue/red). The subject number is reported in brackets after the protein name.