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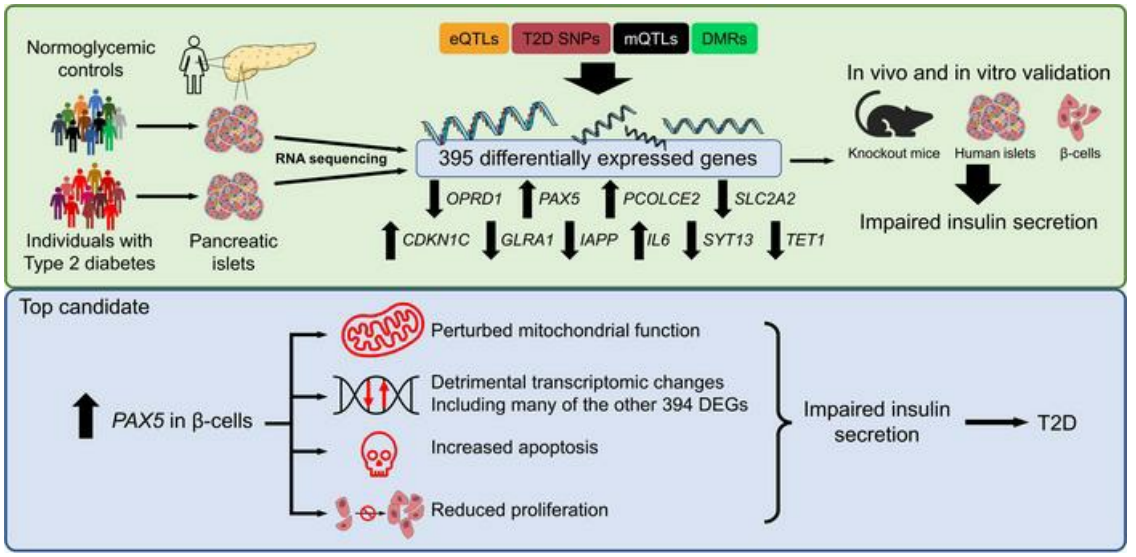
Graphical abstract



Type 2 diabetes candidate genes, including *PAX5*, cause impaired insulin secretion in human pancreatic islets

Short title

Type 2 diabetes candidates in human islets

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Abstract

Type 2 diabetes (T2D) is caused by insufficient insulin secretion from pancreatic β-cells. To identify candidates contributing to T2D pathophysiology, we studied human pancreatic islets from ~300 individuals. We found 395 differentially expressed genes (DEGs) in islets from individuals with T2D, including, to our knowledge, novel (*OPRD1*, *PAX5*, *TET1*) and previously identified (*CHL1*, *GLRA1*, *IAPP*) candidates. A third of the identified islet expression changes may predispose to diabetes, as they associated with HbA1c in individuals not previously diagnosed with T2D. Most DEGs were expressed in human β-cells based on single-cell RNA-sequencing data. Additionally, DEGs displayed alterations in open chromatin and associated with T2D-SNPs. Mouse knock-out strains demonstrated that T2D-associated candidates regulate glucose homeostasis and body composition in vivo. Functional validation showed that mimicking T2D-associated changes for *OPRD1*, *PAX5*, and *SLC2A2* impaired insulin secretion. Impairments in Pax5-overexpressing β-cells were due to severe mitochondrial dysfunction. Finally, we discovered PAX5 as a potential transcriptional regulator of many T2D-associated DEGs in human islets. Overall, we identified molecular alterations in human pancreatic islets contributing to β-cell dysfunction in T2D pathophysiology.

Introduction

Type 2 diabetes (T2D) is characterized by chronic hyperglycemia due to insufficient insulin secretion from pancreatic islets, often in combination with insulin resistance in target cells. The number of patients with T2D is increasing globally at an alarming rate, mainly due to obesity, sedentary lifestyles, and the increasing age of the world's populations. It is therefore important to identify the molecular mechanisms that underlie the disease and to better understand why pancreatic β-cells fail to adapt to an increased demand for insulin secretion, which ultimately leads to T2D.

There have been several initiatives to identify candidate genes for T2D by transcriptomic analyses in pancreatic islets from individuals with T2D and non-diabetic (ND) controls (1-9). However, most of these studies were performed in relatively small cohorts and lack replication and/or functional validation. This is especially true for single-cell (sc) RNA-sequencing of human pancreatic islets, which has been the focus in recent years, leading to limited outcomes in terms of identifying T2D disease mechanisms (10, 11). Concurrently, genome-wide association studies (GWAS) have identified hundreds of genetic signals associated with T2D (12). Importantly, since many identified risk loci are associated with impaired insulin secretion and/or processes likely affecting β-cells (13), these genetic studies convincingly demonstrate that pancreatic islet dysfunction is the key defect in T2D. Therefore, there is a clear need for well-powered transcriptomic analyses of human islets from individuals with T2D and ND controls. Moreover, studies functionally validating human islet transcriptomic data and further dissecting the molecular mechanisms that cause impaired insulin secretion are necessary to better understand the pathogenesis of T2D.

Here, we set out to perform RNA-sequencing of a larger set of human pancreatic islets from individuals with T2D and ND controls (in total 309 islet preparations), to identify previously unrecognized regulators of insulin secretion that may contribute to islet dysfunction and

development of T2D. To test whether the identified changes may influence disease development, we further tested whether a predictor of T2D (14), HbA1c levels, associated linearly with islet expression of the identified genes in individuals not diagnosed with T2D. We next investigated in which cell type(s) the identified genes were expressed. Data from ATAC-sequencing, DNA methylation analysis, GWAS, and the International Mouse Phenotyping Consortium (IMPC) were then analyzed to further dissect the regulation and function of identified genes. T2D candidate genes not identified in previous studies were then manipulated in human islets or clonal β-cells to explore whether they directly impact insulin secretion. Finally, we investigated whether the transcription factor PAX5, our main T2D candidate, is a master regulator of the identified differentially expressed genes (DEGs) in human pancreatic islets.

Results

Transcriptome analysis of human pancreatic islets identified T2D candidates

To identify regulators of insulin secretion that may contribute to T2D, we generated RNA-sequencing data from the large LUDC pancreatic islet cohort, comprising islets from a total of 309 donors (**Table S1**). From these, RNA from 283 islet samples was successfully sequenced (**Figure 1**). Before further analysis and to ensure the highest possible quality of data, we filtered our islet samples (see **Figure 1**). ND controls were defined as individuals lacking diabetes diagnosis and exhibiting HbA1c levels <42mmol/mol. Moreover, age ≥40 years was used as inclusion criterion for the ND controls since none of the individuals with T2D were below 40 years of age. The characteristics of the individuals in the *LUDC islet case-control cohort* are summarized in **Table 1** and **Figure S1A-B**. We subsequently compared RNA-sequencing data from islets from 138 ND controls versus 33 individuals with T2D (**Figure 1**) using a generalized linear model with correction for age, sex, islet purity, and days in culture (DIC). The analysis identified 395 DEGs (false discovery rate (FDR) below 5%, q<0.05, **Table S2 -** sheet A), which included 228 upregulated and 167 downregulated genes. We further applied a model in which we also added BMI as a covariate; expression of 394 of the 395 DEGs was then associated with T2D ($p = 8.6x10^{-17} – 4.5x10^{-2}$, **Table S2 -** sheet A). Additionally, we performed an analysis including only donors with islet purity ≥80% (69 ND and 18 T2D); expression of 334 of 395 DEGs was then associated with T2D ($p = 6.7x10^{-11}$ - $4.9x10^{-2}$, **Table S2 -** sheet A).

For replication, we next compared our 395 identified DEGs with published expression data from studies on human pancreatic islets from T2D case-control cohorts (1-3, 5-8). Previous bulk-expression studies identified 75 of the 395 DEGs identified here (**Figure 2A, Tables S2-S3**) (1, 2, 6, 7). Additionally, published scRNA-sequencing studies found differential expression of 28 of our 395 DEGs in islet cells from ND controls versus individuals with T2D (**Figure 2B, Tables S2-S3**) (3, 5, 8). Eight and nine of these DEGs were differentially expressed

in α- and β-cells, respectively (**Table S2 -** sheet A) and their expression in islets is presented in **Figure S1C-D**). Taken together, 94 of our 395 DEGs have been identified, while 301 have not, in any of these previous studies (**Table S2 -** sheet A). *ARG2, GLRA1, IAPP, IGFBP2, PDHX*, *PPP1R1A, PTEN, RASD2*, *SMAD9, SYT13, TBC1D4*, and *UNC5D* are DEGs previously identified by the studies included in **Figure 2A-B** (**Figure 2C**, **Table S2 -** sheet A), while e.g., *CDKN1C*, *GABRA1*, *GABRA2*, *GAD1*, *IGFBP4*, *IGFBP6*, *IL6*, *PDE7B*, *SIRT1, SLC2A5, SOCS1, SOCS6, SYT1, SYT12, SYT14,* and *TET1* are DEGs not identified by those studies (**Figure 2D**, **Table S2 -** sheet A).

To better understand in which islet cell-types our 395 DEGs were expressed, we studied their expression in fluorescence-activated cell sorted (FACS) human α- and β-cells from the *LUDC sorted α/β-cell cohort*. This cohort included 13 ND individuals, two individuals with T2D, and three individuals with prediabetes (**Table 1**). We found that α- and β-cells expressed 366 and 368 of our DEGs, respectively (**Figure 2E** and **Table S2 -** sheet A). Of these, 90 genes displayed higher expression in α- versus β-cells, while 174 had higher expression in β- versus α-cells ($q<0.05$, **Table S2 -** sheet A). This included nine of the eleven genes that we selected for functional validation (see below and **Figure 2F**). Using WebGestaltR, we found 17 enriched gene-sets ($q<0.05$), including divalent inorganic cation homeostasis and positive regulation of cell adhesion, among the DEGs with higher expression in α- versus β-cells (**Figure S1E**), while there was no enrichment among the DEGs with higher expression in β- versus α-cells. We also examined the expression of our DEGs in different islet cell types by using published human islet scRNA-sequencing data (5). We found that among our 395 DEGs, 366 (93%) were expressed in α-cells, 347 (88%) in β-cells, 331 (84%) in PP-cells, 312 (79%) in delta (δ)-cells, and 190 (48%) in epsilon (ε)-cells (**Table S4** and **Figure S2**). 97-98% of DEGs expressed in α- and β-cells in the scRNA-sequencing expression data overlapped with those expressed in α- and β-cells of the *LUDC sorted α/β-cell cohort*. We further tested if any of the 395 islet DEGs

also exhibited differential expression in the same direction in sorted α- or β-cells when comparing five donors with T2D or prediabetes versus 13 ND controls (**Table 1**). Here, 4 and 28 of our 395 DEGs showed differential expression ($p<0.05$) in sorted α- or β-cells, respectively (**Table S2 -** sheet B-C, **Figure S3A-B**). The differentially expressed genes in sorted β-cells include *BARX1*, *NEFL*, *PAX5*, and *PCOLCE2*, genes we later selected for functional follow-up (see below). It should be noted that due to modest sample size there is limited power when comparing T2D versus ND for both the sc- and sorted-RNA-sequencing data and hence one would not expect to find all 395 DEGs in these analyses. These data suggest that several DEGs in T2D islets may affect insulin secretion or other aspects of β-cell function.

Our data clearly show that individuals with T2D exhibit abundant transcriptomic alterations in pancreatic islets. However, it is not clear whether these changes predispose to T2D or whether they are a consequence of disease progression. To test if expression changes of the 395 identified DEGs potentially predispose to T2D, we studied their expression in human islets from *the LUDC islet HbA1c cohort* (**Table 1**), including individuals with a wide range in HbA1c levels, not previously diagnosed with diabetes. Interestingly, expression of 142 of 395 DEGs (36%) was linearly associated with HbA1c (**Table S2 -** sheet A). Among those genes, all had a beta-coefficient with the same directionality as that of the expression differences seen in islets from the LUDC islet case-control cohort. These data suggest that the expression changes may occur before the diagnosis of T2D and potentially contribute to development of the disease. They also indicate that the expression changes were not due to T2D treatment.

Gene-sets, including hormone secretion, are enriched among T2D-associated DEGs

We used WebGestaltR and gene ontology to discover enriched gene-sets among our 395 DEGs. We found 39, five, and six enriched gene-sets for Biological Processes, Cellular Components,

and Molecular Functions, respectively (q<0.05, **Table S5**). Interestingly, gene-sets for Growth factor binding, Regulation of lipid transport, Negative regulation of Wnt signaling, Aging, Regulation of cell-cell adhesion, Hormone secretion, Regulation of inflammatory response, and Divalent inorganic cation homeostasis were among the significantly enriched (**Figure 2G**).

T2D-associated DEGs display alterations in open chromatin and DNA methylation in human islets

Open chromatin regions are associated with active gene transcription (15). Of our 395 DEGs in islets from ND controls versus individuals with T2D, 346 (88%) were marked by at least one open chromatin region, identified by previous ATAC-sequencing of human pancreatic islets (15). From this subset, 194 and 152 DEGs were upregulated and downregulated, respectively, in islets from individuals with T2D. Top DEGs *BARX1*, *OPRD1*, *PAX5*, and *PCOLCE2* overlapped with several open chromatin regions (four, four, five and six, respectively) located both upstream and downstream of respective transcription start site (**Table S6 -** sheet A). Moreover, 24 DEGs, including *CAMK4*, *DIO2, DKK3*, *FOXP1*, *GABRA2*, *PTPRC*, *SOCS1*, and *SYNPO*, displayed alterations in open chromatin in islets from ND controls versus individuals with T2D. Of these, 23 exhibited more open chromatin and higher expression, and one (*GABRA2*) exhibited less open chromatin and reduced expression, in islets from donors with T2D **(Figure 2H**, **Table S6 -** sheet A). Together these data suggest an altered chromatin state in islets from individuals with T2D, which was associated with altered gene expression for a subset of identified DEGs.

We further tested whether the 395 DEGs showed altered DNA methylation in islets from individuals with T2D versus ND controls based on published data (16) and found 732 differentially methylated regions (DMRs) annotated to 262 DEGs (**Table S6 -** sheet B). These

included DMRs annotated to *CHL1*, *ELFN1, FAIM2*, *HHATL*, *OPRD1*, *PAX5*, *SFRP1*, and *SLC2A2,* DEGs selected for functional follow-up (see below).

SNPs associate with the identified DEGs, T2D and metabolic traits

We next assessed whether Single-Nucleotide Polymorphisms (SNPs) associated with expression of the 395 DEGs. We first explored eQTL data from human pancreatic islets from the INSPIRE study (17) and the T2D Genes portal (https://t2d.hugeamp.org/). We found 148 SNPs associated with islet expression of 120 of our 395 DEGs, including *CHL1*, *FAIM2*, *GABRA2*, *HHATL*, *PCOLCE2*, *SFRP1*, *SIRT1*, and *SMAD9* (**Table S7 -** sheet A, and **Figure 2I**).

We then used the GWAS catalogue to determine whether any of these 148 SNPs have previously been linked to T2D, or other metabolic diseases/traits (**Table S7 -** sheet A). Indeed, two eQTL SNPs associated with *FXYD2* (rs529623) and *RPL39L* (rs3887925) islet expression, have been linked to T2D risk (12) (**Table S7 -** sheet A and **Figure 2I**). Moreover, two eQTL SNPs associated with islet expression of *FOXE1* (rs7038480) and *ENTR1* (rs11145930), have been linked to blood glucose levels (12, 18-20) (**Table S7 -** sheet A and **Figure 2I**). Four eQTL SNPs associated with islet expression of *ARPC1B* (rs3843540), *COMP* (rs7260000), *DIXDC1* (rs10891295), and *HSD3B7* (rs4889599), have been linked to BMI and/or waist-to-hip ratio (18, 21) (**Table S7 -** sheet A and **Figure 2I**). Finally, six eQTL SNPs (), associated with islet expression of *ACP2* (rs11039194), *CBLC* (rs8104467), *CD5* (rs7124430), *HSD3B7* (rs4889599), *PCOLCE2* (rs6794287), and *TMED6* (rs113671952), have been linked to triglyceride or LDL cholesterol levels (18, 22-24) (**Table S7 -** sheet A and **Figure 2I**). These data demonstrate that SNPs associated with islet expression of numerous identified DEGs impact the risk for T2D and metabolic traits (**Figure 2I**).

As our islet cohort data included variables, e.g. islet purity, which were not available/used in the INSPIRE study (17), and as we used a cutoff for islet purity, we also performed an eQTL analysis adjusting for age, sex, BMI, DIC, purity, and T2D in the *LUDC islet case-control cohort*. This showed that 26 of our 395 DEGs had at least one eQTL based on q<0.05, including *BEST3*, *HSD3B7*, and *TMED6*. Moreover, 374 DEGs had eQTLs with nominal p-values (p<0.05, **Table S7 -** sheet B) and these included 47 of 148 SNPs and 117 of 123 genes identified in the INSPIRE analysis (**Table S7** – sheet A-B).

We then tested if SNPs that map to any of the 395 DEGs have been associated with T2D and/or glycemic traits (HbA1c, fasting glucose, fasting insulin, HOMA-B, disposition index (DI), or corrected insulin response (CIR)), in GWAS using the Common Metabolic Diseases Knowledge Portal (hugeamp.org, accessed August 2022). 106 DEGs were linked to 149 unique SNPs associated with these traits in GWAS (**Table S7 -** sheet C, **Figure 2I**); 131 SNPs were associated with T2D, 38 with HbA1c, ten with fasting glucose, one with fasting insulin, four with DI, and one with CIR. This included one SNP mapping to each of *BARX1*, *CHL1*, *ELFN1*, and *FAIM2*, three SNPs mapping to *SLC2A2*, and four to *SFRP1*, DEGs we subsequently selected for functional follow-up (see below).

Since genetic and epigenetic mechanisms interact and together affect biological processes, we tested whether SNPs in *cis* are associated with DNA methylation of CpG sites annotated to the 395 DEGs in human pancreatic islets, so called mQTLs (25). We found 490 SNPs associated with DNA methylation of 176 unique sites annotated to 90 DEGs (**Table S7 -** sheet D). These

included mQTLs, three each, annotated to *OPRD1* and *PAX5*, two additional DEGs subsequently selected for functional follow-up (see below).

Figure S4A-C show the overlap between DEGs that had eQTLs, DMRs, GWAS SNPs, ATAC-sec peaks, or mQTLs annotated, and SNPs in the different genetic analyses above.

Identified T2D-associated DEGs affect metabolism in vivo

To explore potential in vivo evidence for protective/susceptible functions in the development of diabetes for the 395 identified T2D DEGs, we systematically searched the International Mouse Phenotyping Consortium (IMPC, https://www.mousephenotype.org/, accessed April 15th, 2020) catalogue. The IMPC database contains knockout (KO) mouse strain entries for 168 of the 395 DEGs, with phenotype data available for 125 strains (**Figure 3A**). Most were homozygous viable (n=85), with fewer homozygous sub-viable (meaning that the number of alive homozygous pups is lower than expected, n=6), homozygous lethal but heterozygous viable (n=24), or of unknown viability (n=10). **Table S8** lists DEGs found in T2D islets with IMPC metabolic readouts for KO strains (n=125). Of the phenotyped KO strains, insulin blood (IB) measurement data were available for 36 with 16 (44%) displaying altered levels ($p<0.05$, **Figure 3**, **Table S8**). Intraperitoneal glucose tolerance test (IPGTT) data showed that fasting glucose (FG) and AUC for glucose (AUCG) were altered in 24 of 81 (30%) and 24 of 83 (29%), respectively, $p<0.05$), while the initial response to glucose challenge (IGR) was altered in 26 of 80 (33%) ($p<0.05$) (**Figure 3**, **Table S8**). With respect to body composition, DEXA phenotypic data were available for 93 KO models, and among these, 33 (35%) and 35 (38%) had altered total fat mass and total lean mass, respectively ($p<0.05$, **Figure 3**, **Table S8**).

The effects of gene KO on the studied metabolic phenotypes agrees with the direction of altered expression for several of the T2D-associated DEGs. For example, our data show that *HHATL*

expression was significantly decreased in T2D islets (**Table S2**). In line with this, IMPC data show that *Hhatl* KO mice had impaired glucose tolerance, increased total body fat mass, and decreased total body lean mass (**Figure 3B**). *SLC2A2* expression was also decreased in islets from individuals with T2D (**Table S2**), and *Slc2a2* KO mice had increased FG and IGR. Overall, these rodent in vivo findings support an important role for the identified DEGs in control of glucose homeostasis and body composition.

Functional follow-up shows that T2D-associated expression changes impair β-cell function

We then asked whether any DEGs previously unrecognized in comparable studies have a functional role in β-cells. First, we deployed a strategy to select genes for functional investigation (**Figure 4A**). We selected DEGs with >2-fold change in either direction based on mean islet expression in ND controls versus individuals with T2D, and the same directionality of expression change as the HbA1c correlation in islets from the *LUDC islet HbA1c cohort*. This generated a list of 31 genes. We further excluded DEGs that were not expressed in the sorted α- or β-cells from ND controls, unless their expression was induced in islets from individuals with T2D, nor in endocrine cell types in scRNA-sequencing data sets. Finally, we selected the top nine genes, based on lowest q-value, that to our knowledge had not previously been studied in β-cells (**Figure 4A**). In addition, we included *CHL1* and *SLC2A2*. Knockdown of *Chl1* in clonal β-cells was previously found to impair glucose-stimulated insulin secretion (GSIS) (26), and was included as a positive control. *SLC2A2,* encoding the glucose transporter GLUT2, has also been studied, but was included as it is debated whether its function is significant in human β-cells (27, 28). Of note, ten of these 11 genes have either T2D-associated DMRs (**Table S6 -** sheet B), INSPIRE eQTLs (**Table S7 -** sheet A), SNPs in *cis* associated with T2D and/or glycemic traits (**Table S7 -** sheet C), or mQTLs (**Table S7 -** sheet D), as

296 summarized in **Figure 4B**. Moreover, all 11 DEGs have nominal LUDC eQTLs ($p = 2.7x10^{-4}$
297 – $2.3x10^{-2}$, **Table S7** – sheet B).

298 To mimic the situation in individuals with T2D, selected genes that exhibited lower islet
299 expression in T2D, i.e., *CHL1*, *HHATL*, *OPRD1*, and *SLC2A2* (**Figure 4C**), were silenced by
300 siRNA transfection in human islets from ND individuals. This resulted in 50-60% reduction in
301 gene expression (**Figure 4D**). Determinations of insulin secretion showed that islets deficient
302 for *CHL1* displayed a reduction in insulin release when exposed to basal glucose (2.8mM),
303 while islets deficient for *SLC2A2* showed nominally reduced insulin secretion at the same
304 glucose concentration (p=0.062, **Figure 4E**). At stimulatory glucose (16.7mM), *OPRD1* or
305 *SLC2A2* silencing resulted in ~20 and 40% reduced insulin secretion, respectively, while islets
306 deficient for *CHL1* or *HHATL* showed nominally reduced and unaffected secretion,
307 respectively (**Figure 4E**). The changes did not translate into significant differences in the fold
308 change of insulin secretion (secretion at stimulatory glucose divided by secretion at basal
309 glucose) and occurred without differences in insulin content (**Figure S5A-B**). We also
310 measured glucagon secretion from islets where *CHL1*, *HHATL*, *OPRD1*, or *SLC2A2* had been
311 silenced and found no differences (**Figure S5C**). These data indicate that reduced islet
312 expression of *OPRD1* and *SLC2A2* may contribute to the insulin secretion defect seen in
313 individuals with T2D.

314 Selected DEGs that exhibited higher islet expression in T2D, i.e., *BARX1*, *ELFN1*, *FAIM2*,
315 *NEFL*, *PAX5*, *PCOLCE2*, and *SFRP1* (**Figure 4C**), were overexpressed by plasmid transfection
316 in 832/13 INS1 β-cells (hereafter called INS1 β-cells). We used INS1 β-cells for overexpression
317 experiments due to limited supply of human islets. Furthermore, in our hands, INS1 β-cells
318 behave more like primary mature human β-cells than the fetal human β-cell line EndoC-βH1 in
319 gene manipulation experiments (K. Bacos and J.K. Ofori, unpublished observations). Plasmid
320 transfection resulted in impaired insulin secretion at basal or stimulatory glucose levels, or

expressed as fold change, or altered insulin content, in cells overexpressing *Barx1*, *Nefl*, *Pax5,* *Pcolce2* or *Sfrp1* (**Figure S5D-G**). However, the plasmid transfection efficiency was low (20-30% of cells, **Figure S5H-J**). This together with the fact that expression was driven by the very strong cytomegalovirus promoter made us wary that the phenotypes may be due to very high overexpression overwhelming the transfected cells. We therefore proceeded to use lentiviral vectors, allowing for transgene delivery to a larger proportion of cells, with the weaker human phosphoglycerate kinase 1 promoter driving expression of HA-tagged *Barx1*, *Nefl*, *Pax5*, *Pcolce2*, or *Sfrp1*. Western blot analysis showed that transduction of INS1 β-cells with these vectors resulted in expression of proteins of expected sizes (**Figure 4F**). Overexpression of Pax5 significantly increased basal secretion and strongly reduced insulin secretion at stimulatory glucose, without influencing insulin content (**Figure 4G-H**), while Nefl or Pcolce2 overexpression caused reduced insulin secretion at basal (both) and stimulatory (Pcolce2) glucose levels (**Figure 4G**), and increased insulin content (**Figure 4H**). Overexpression of Barx1 or Sfrp1 did not alter insulin secretion or content. The changes described caused a strong reduction in fold change of insulin secretion in Pax5-overexpressing INS1 β-cells (**Figure 4I**). Finally, wells where cells were transduced with the Pax5 or Pcolce2 viruses also contained significantly less total protein than wells with cells transduced with the GFP virus (**Figure 4J**). This indicates a lower cell number and, hence, suggests that Pax5 and Pcolce2 may affect cell viability and/or proliferation and therefore potentially β-cell mass in T2D. Taken together, these functional experiments identified four, to our knowledge, previously unrecognized regulators of insulin secretion showing either lower, *OPRD1*, or higher, *NEFL*, *PAX5* and *PCOLCE2*, expression in islets from individuals with T2D (**Figure 4C-J**). Because Pax5 overexpression had the strongest effect on both insulin secretion and protein content (**Figure 4G** and **J**), we decided to further explore its impact on β-cells. First, to exclude the possibility that the HA-tag renders Pax5 pathogenic, we repeated the secretion experiments with viral vectors conferring

346 expression of untagged Pax5. The results show that also untagged Pax5 reduced GSIS in INS1
347 β-cells (**Figure S5K**).

348

349 **Overexpression of Pax5 perturbs mitochondrial function and causes β-cell loss**

350 *PAX5* encodes a transcription factor associated with leukemia (29) and has to our knowledge
351 not been studied in β-cells.. First, we used immunohistochemistry to investigate the distribution
352 of PAX5 in human islets. In accordance with the RNA-sequencing data, we found that islets
353 from individuals with T2D displayed robust PAX5 expression, with most of the staining found
354 in β-cells, while PAX5 was barely detectable in islets from ND controls (**Figure 5A**).

355 To further characterize the Pax5-induced defects in INS1 β-cells, we stimulated insulin
356 secretion with a depolarizing concentration of K^+. These experiments again showed that GSIS
357 was severely blunted by Pax5 overexpression, while K^+-stimulated secretion was increased
358 (**Figure 5B**). This indicates that secretory defects induced by Pax5 overexpression occurred
359 before depolarization of K_{ATP}-channels in the insulin secretion pathway, potentially in
360 mitochondrial metabolism. To investigate if Pax5 overexpression alters mitochondrial
361 metabolism in β-cells, we used the Seahorse XF analyzer to measure oxygen consumption rate
362 (OCR). This showed that Pax5 overexpression caused a reduction in mitochondrial respiration
363 with significantly lower glucose-stimulated respiration, both in terms of increase compared to
364 basal respiration and in absolute values, as well as lower maximal respiration (**Figure 5C-F**).
365 These respiratory defects were reflected in a reduced PercevalHR signal, demonstrating a lower
366 ATP/ADP ratio in response to glucose stimulation, in Pax5-overexpressing β-cells (**Figure 5G-**
367 **I**). To find a possible cause for these mitochondrial defects, we used Western blot to investigate
368 the protein levels of subunits of complex I-V in the electron transport chain, and of the citric
369 acid cycle enzyme citrate synthase. We found that levels of citrate synthase and Sdhb (Complex

370 II) were reduced by Pax5 overexpression, while Uqcrc2 (Complex III) was slightly increased
371 (**Figure 5J-O** and **Figure S5L-M**). Together these data support that increased Pax5 levels
372 perturb insulin secretion by negative effects on mitochondrial function.

373 In view of the data in **Figure 4J**, we hypothesized that elevated Pax5 levels may affect cell
374 number. This was supported by results from an MTT assay, which measures metabolic activity
375 and is used to investigate cell viability and number (**Figure 6A**). To investigate whether this is
376 due to increased apoptosis and/or decreased proliferation, we quantified Caspase-3/-7 activity
377 and cleaved (i.e. active) Caspase-3 as a measure of apoptotic activity, and used an 5-Ethynyl-
378 2'-deoxyuridine (EdU) incorporation assay to quantify proliferation. These analyses showed
379 that Pax5 overexpression induced a clear increase in apoptotic activity and a drop in
380 proliferation rates in INS1 β-cells (**Figure 6B-D**). Hence, Pax5 is a regulator of β-cell viability
381 and number.

382

383 In a final effort to characterize the mechanisms underlying the profound effects of Pax5 we
384 performed a global transcriptome analysis of Pax5-overexpressing INS1 β-cells. The analysis
385 revealed that 3069 genes were differentially expressed when comparing β-cells overexpressing
386 Pax5 and GFP, respectively ($q<0.05$, **Table S9**). These included 75 of the 395 T2D DEGs
387 (**Figure 6E**, **Tables S2** - sheet A and **S9**). In addition to Pax5 itself, three of the other DEGs
388 selected for functional follow-up, *Faim2*, *Pcolce2*, and *Slc2a2*, were altered in Pax5-
389 overexpressing INS1 β-cells, and in the same direction as in islets from individuals with T2D
390 (**Figure 6E**). The 3069 differentially expressed genes were enriched for many gene sets,
391 including Insulin secretion, Glucose homeostasis, Response to glucose, Positive regulation of
392 cell death and Aging (**Table S10** and **Figure 6F**). Overall, these data clearly demonstrated that
393 Pax5 overexpression leads to transcriptomic changes that can have profound effects on β-cell
394 function and survival.

PAX5 is a potential dysregulator of gene expression in T2D

As PAX5 is a transcription factor, we next used the bioinformatic prediction tool Pscan (30) to test if the 395 DEGs are enriched for genes with a PAX5 binding motif in the promoter. This would suggest that elevated PAX5 may cause dysregulation of other DEGs in T2D. Indeed, the analysis demonstrated that 196 DEGs had a putative PAX5 binding motif within the promoter, which is a significant enrichment (p=0.017, **Table S11**). Of note, the 196 genes included six of the genes we followed-up functionally (*BARX1*, *CHL1*, *FAIM2*, *HHATL*, *OPRD1*, and *PAX5* itself), and a literature search showed that ~25% of the 196 genes have been found to regulate β-cell function and/or number, or have genetic variants associated with T2D or other metabolic traits in humans (**Table S11** and **Figure 7A**). These include *IL6 (31)*, *PPP1R1A (32)*, *PTEN (33)*, *SYT13 (34)*, and *TBC1D4 (35)*. Additionally, in human islets, the expression of *PAX5* correlated with 126 (32%) of the other 394 DEGs (Spearman correlation, $p<0.05$, **Table S12**), including *BARX1*, *CDKN1C*, *CHL1*, *ELFN1*, *GABRA2*, *GAD1*, *NEFL*, *PCOLCE2*, *PDE7B*, *SFRP1*, *SLC2A2, SOCS1, SYT1*, and *SYT12*. As a final piece of evidence supporting *PAX5* as a key DEG, we used Weighted correlation network analysis (WGCNA) (36) to perform a co-expression analysis to establish if *PAX5* is part of a dysregulated gene network in T2D. This revealed a network with *PAX5* and 86 other T2D DEGs (**Table S13** and **Figure 7B**). Together, data from human islets and Pax5-overexpressing INS1 β-cells suggest that PAX5 may cause dysregulation of many identified T2D-associated DEGs.

Discussion

This study identified T2D candidate genes with altered expression in pancreatic islets from individuals with T2D versus ND controls. Their altered expression may be a contributing factor in the development of T2D, as many identified DEGs associated with HbA1c levels in a cohort of islets from individuals not previously diagnosed with T2D. Many DEGs displayed alterations in chromatin state or DNA methylation in T2D islets, and SNPs associated with DEGs impacted T2D and metabolic traits. Rodent in vivo data also supported the importance of our findings, as mouse strains deficient for identified DEGs had altered glucose homeostasis and body composition. Functional validation of top-ranked DEGs clearly supported a role for the identified expression changes in T2D, as mimicking the changes for *OPRD1*, *PAX5*, and *SLC2A2* in human islets and clonal β-cells perturbed insulin secretion. Extensive characterization of the Pax5-induced defect showed that impaired mitochondrial activity is a likely culprit. Our data further supported *PAX5* as a key T2D DEG that drives the change in expression of other DEGs. Based on the present findings, we propose the model presented in **Figure 8**, in which increased PAX5 levels contribute to impaired insulin secretion through mitochondrial dysfunction and transcriptional regulation of other genes, e.g., *SLC2A2* and *OPRD1*, in human islets.

Most identified DEGs, including *PAX5*, *OPRD1*, *PCOLCE2*, *CDKN1C*, *GABRA2*, *IL6*, *PDE7B*, *SIRT1*, *SOCS1*, *SYT1*, *SYT12*, *SYT14*, and *TET1*, represent findings not observed in previous transcriptome analyses of T2D islets (1-8). However, we also replicated nearly 100 known T2D DEGs, including *SLC2A2*, *CHL1*, *GLRA1*, *IAPP*, *PPP1R1A*, *PTEN*, and *SYT13* (1-8), supporting a robust data set. The number of identified DEGs overlapping with genes identified in more than one of the published studies was low, possibly due to limited sample size in the previous studies or varying pathophysiological and demographic profiles between individual donors, as well as differences in islet isolation and culturing protocols, analysis

platforms, and variables corrected for in the statistical analysis. For example, while we used RNA-sequencing, Solimena et al. primarily used microarray to analyze the transcriptome (6). The overlap was even smaller when comparing to scRNA-sequencing analyses, potentially explained by the small number of islet donors in sc-studies (5-12 ND donors and 3-6 donors with T2D) and/or greater number of variables adjusted for in our study ([age + sex + islet purity + DIC] versus sc-studies [sex + ethnicity]) (3, 5, 8, 11). It should also be noted that the differentially expressed genes described by the published sc-studies largely do not overlap (10) potentially due to different criteria for expression filtering, use of different statistical and bioinformatics tools, and different number of cells studied (638 (3), 1492 (8), 2209 (5)). Importantly, the DEGs identified in our analysis were enriched for gene ontology terms important for β-cell function including hormone secretion, divalent inorganic cation homeostasis, and growth factor binding. Our functional validation also showed that the expression changes may contribute to T2D pathophysiology, as knockdown of *SLC2A2* or *OPRD1*, and overexpression of Pax5, resulted in impaired GSIS. Additionally, expression of many DEGs, including *OPRD1*, *PAX5*, and *SLC2A2*, associated with HbA1c in ND individuals and individuals not diagnosed with T2D, with the direction of association indicating that the changes may predispose to disease.

PAX5, a transcription factor important for B-lymphocyte development and frequently mutated in acute B lymphoblastic leukemia (37), is barely expressed in human islets from ND individuals (38). While low islet PAX5 expression in ND individuals was confirmed in our cohort, expression a both mRNA and protein levels was upregulated in T2D, with immunostaining showing that the upregulation took place in β-cells. Interestingly, T2D and SNPs were associated with altered DNA methylation of *PAX5* in human islets, suggesting potential epigenetic regulation. PAX5, which can act as both an activator and repressor (39),

465 belongs to a transcription factor family with several members. Notably, PAX2, -4, and -6, are
466 important for pancreas development and islet function (40). In our analysis *PAX6* was highly
467 expressed in human islets, while *PAX2* and *-4* were expressed at low levels. While there were
468 no expression differences for *PAX2* or *PAX6*, *PAX4* expression was nominally reduced in islets
469 from individuals with T2D versus ND controls (p=0.004). We showed that PAX5 upregulation
470 was associated with reduced mitochondrial function, perturbed GSIS, and β-cell loss.
471 Interestingly, our analyses showed that *PAX5* may be a key T2D DEG causing dysregulation
472 of many other DEGs. This was supported by our analysis of Pax5-overexpressing INS1 β-cells,
473 which exhibited vast transcriptomic changes, including for genes selected for functional follow-
474 up (*Faim2*, *Pcolce2*, and *Slc2a2*), and for many other T2D DEGs, altered in the same direction
475 in this model as in islets from individuals with T2D. Mice heterozygous for *Pax5* knockout
476 exhibit impaired glucose tolerance, indicating that Pax5 deficiency somehow exerts negative
477 effects on glucose homeostasis in peripheral tissues. Heterozygous *Pax5* animals exhibit
478 changes in white blood cell numbers (www.IMPC.org), which could cause cytokine imbalance
479 and a resulting insulin resistance peripherally, as seen in SCID mice completely lacking mature
480 B- and T-lymphocytes (41). It should be noted that the Pax5 overexpression in INS1 β-cells
481 may be stronger than in islets of individuals with T2D, and it is therefore uncertain how strong
482 the effects of elevated PAX5 are in T2D. The proteins overexpressed by us were HA-tagged,
483 and it is possible that the tag contributes to the defect in overexpressing cells. For Pax5,
484 however, this is unlikely as overexpression of an untagged protein perturbed GSIS in a similar
485 fashion as the tagged protein.
486
487 *SCL2A2* encodes GLUT2, the main glucose transporter in rodent β-cells, as evident by the
488 impaired glucose tolerance in *Slc2a2*-KO mice (42). Its role in human islets has been questioned
489 (43), but *SLC2A2* mutations causes transient neonatal diabetes (28). Our data indicated that

GLUT2 plays an important role also in adult human β-cells, as its knockdown in human islets impaired insulin secretion. In INS1 β-cells, Pax5 overexpression downregulated *Slc2a2*, indicating that reduced Glut2-mediated glucose uptake may contribute to the perturbed mitochondrial function and secretory defect observed in this model. We also found that *PAX5* correlated negatively with *SLC2A2* expression in the *LUDC islets case-control cohort*, and PAX5 may hence directly downregulate *SLC2A2* also in human islets. *OPRD1* encodes a receptor for enkephalins, which have been shown to both inhibit and stimulate insulin secretion (44-47), through potential dose-dependent effects (45). Enkephalin is expressed in a small subset of islet cells (38) and may regulate insulin secretion via paracrine signaling. While *OPRD1* knockdown reduced GSIS in human islets by only ~20%, it should be remembered that T2D is a disease characterized by dysregulation of many genes contributing to a varying extent to cellular dysfunction. The protein encoded by *PCOLCE2* binds to procollagens (48). Increased *PCOLCE2* expression, as occurs in T2D islets, perturbed insulin secretion, but the mechanism remains unknown. *CHL1* encodes an adhesion protein, and its islet expression has been shown to be reduced in T2D (7). *Chl1* knockdown in rodent β-cells lines, including INS1 β-cells (26), has also been shown to impair GSIS. In our hands, *Chl1* was not detected in INS1 β-cells (not shown), and *CHL1* knockdown in human islets only reduced insulin secretion significantly at basal glucose. The importance of reduced *CHL1* expression in T2D therefore remains unclear. As with *SLC2A2*, we present evidence supporting that PAX5 may regulate *CHL1*, *OPRD1*, and *PCOLCE2*. While manipulation of some genes we followed-up did not alter cellular function, they may still play roles in islets *in vivo* or in T2D.

Many of the identified DEGs we did not functionally validate are highly relevant to T2D. For example, *GLRA1* belongs to the DEGs already presented by others (7) and has lower expression in β-cells from individuals with T2D (8). It encodes a glycine receptor and silencing of *Glra1*

515 in clonal β-cells reduces GSIS (49). Of note, 48h treatment of non-diabetic human islets with
516 either high glucose, palmitate, or high glucose plus palmitate, increased DNA methylation and
517 decreased expression of *GLRA1 (49-51)*. Here, we found an inverse correlation between HbA1c
518 levels and *GLRA1* expression in the *LUDC islet HbA1c cohort*. These data support that reduced
519 *GLRA1* expression in β-cells, potentially due to epigenetic alterations, may predispose to T2D.
520 In agreement with other studies (1, 5, 6), we found reduced *IAPP* expression in islets from
521 individuals with T2D. *IAPP* encodes islet amyloid polypeptide, or amylin, which is co-stored
522 and co-secreted with insulin. In T2D, it forms amyloid depots in islets and is associated with
523 cell death and pancreatic dysfunction (52). Moreover, we show that *TET1* is downregulated in
524 islets from individuals with T2D. In line with this, we previously found reduced *TET1*
525 expression in adipose tissue from individuals with T2D versus ND controls (53, 54). TET1
526 oxidizes DNA methylation to hydroxymethylation and is known to be important for β-cell
527 differentiation (55). Additionally, we identified four DEGs encoding synaptotagmins, *SYT1*,
528 *SYT12*, *SYT13* and *SYT14,* of which *SYT13* was identified by earlier studies and is differentially
529 expressed in β-cells from individuals with T2D (3, 6, 34). As Synaptotagmins are involved in
530 Ca^{2+}-triggered exocytosis (56), their differential expression may contribute to altered
531 exocytosis in T2D islets. Indeed, Synaptotagmin 1 and 13 have been shown to affect insulin
532 secretion (34, 57, 58),. *CDKN1C*, an upregulated DEG, encodes a cell cycle inhibitor and
533 demethylation of the *CDKN1C* promoter in human islets results in β-cell proliferation (59).
534 *PDE7B* encodes a cAMP phosphodiesterase, which we found upregulated in T2D islets, and
535 showed that this inhibits GSIS (60). Many other DEGs, e.g., *PTEN*, *PPP1R1A*, *IL6*, *SIRT1,* and
536 *SOCS1*, have also been shown to affect β-cell function or number (31-33, 61, 62). Of note,
537 *PPP1R1A* is differentially expressed in β-cells from individuals with T2D (8).

538 Moreover, some of our DEGs identified in islets have previously been found to be differentially
539 expressed also in other tissues from individuals with T2D versus ND controls. For example,

C1S, *CTSZ, PTGES*, *SCYL2*, *SERPING1*, and *TET1* are differentially expressed in adipose tissue (53), while *SLC39A4, MNS1*, *RAB33B*, and *ACP2* are differentially expressed in muscle cells (63) and *PPP1R1A* is differentially expressed in liver (64). Stable epigenetic modifications taking place during embryogenesis, mQTLs, eQTLs or ectopic fat accumulation may contribute to similar gene regulation in multiple tissues.

This study has some potential limitations. For example, we analyze whole islets including several cell-types. Hence, to understand in which cell types T2D-associated DEGs were expressed, we studied sorted human α- and β-cells as well as scRNA-sequencing data from human islets. This approach showed that 93% of identified DEGs were expressed in β-cells. Immunohistochemistry also showed that our main candidate, PAX5, was upregulated in human β-cells. Moreover, the bulk RNA-sequencing approach allowed us to analyze islets from a large cohort, as well as much larger numbers of cells from each islet preparation, than the scRNA-sequencing approach would. Importantly, we replicated some DEGs identified in other islet T2D case-control cohorts where they used either sc- or bulk-RNA-sequencing. It was beyond the scope of this study to follow up identified DEGs in all islet cell types and future studies should investigate gene-manipulations in cultured α-, δ- and pp-cells. For instance, *IGFBP2*, *NPAS2*, *NDUFS7*, and *TGM2* showed differential expression in α-cells from individuals with T2D (3, 5, 8) and have previously been shown to impact diabetes and islet-related phenotypes or mitochondrial function (65-68). Even though our islet cohort is among the most extensive of its kind, it would be beneficial to analyze islets from even larger donor numbers, and from individuals with other ethnic backgrounds. Unfortunately, islet supply is limited, and we had to perform the overexpression experiments in clonal β-cells. The investigated cells are thus not in their normal 3D structure, surrounded by the other islet cell types, which may influence the results. Moreover, HbA1c levels may fluctuate due to, e.g., medication and red blood cell count.

A minor subset of individuals defined as non-diabetic in our cohort based on HbA1C<42 mmol/mol may hence be prediabetic. Finally, although the IMPC in vivo data support a role for the identified DEGs in glucose homeostasis, knockout strains for many genes are lacking and based on the available data it is difficult to establish whether metabolic defects are due to islet and/or peripheral effects.

In conclusion, by studying one of the largest existing T2D case-control islet cohorts, we have identified differentially expressed T2D candidates. Collectively, our in vivo and in vitro analyses clearly support a role for these expression changes in dysregulation of insulin secretion, and thereby in T2D pathophysiology. We demonstrated that *PAX5* expression is induced in β-cells in T2D, and that this could underlie transcriptional changes to many other T2D-associated DEGs. Our data further showed that elevated PAX5 levels result in reduced glucose-induced mitochondrial activity and consequently reduced cytosolic ATP/ADP ratio and perturbed insulin secretion. Based on the strong effects on β-cell function, we propose that the identified expression changes could contribute to T2D pathophysiology and thus may be targeted in novel strategies for T2D treatment.

Methods

Full details can be found in the supplementary information.

Human islets

Human islets of Langerhans were obtained from the Human Tissue Laboratory, which is funded by the Excellence of Diabetes Research in Sweden (EXODIAB) network (www.exodiab.se/home) in collaboration with The Nordic Network for Clinical Islet Transplantation Program (www.nordicislets.org). Informed consent was obtained from pancreatic donors or their relatives and all procedures were approved by the Swedish Ethical Review Authority (Permit number 2011263). The islets were prepared from cadaver donors by use of enzymatic digestion and density gradient separation. Islet preparation purity and count were determined as described previously (69). Islet purity, measured by dithizone staining of individual islet preparations, is presented in **Table S14**.

RNA-sequencing

RNA was extracted from human pancreatic islets using AllPrep DNA/RNA kit or miRNeasy Mini Kit (Qiagen, Hilden, Germany). Sample preparation of 1 μg high quality RNA was done using TruSeq RNA Library Preparation Kit or TruSeq Stranded Total RNA Library Prep, followed by RNA-sequencing on HiSeq 2000 or NextSeq 500 (Illumina, San Diego, CA, USA), respectively.

606 **Statistical analysis**

607 The functional data were analyzed with 2-tailed paired t-tests unless stated otherwise. Other
608 analyses were performed as described in the respective section in the supplementary
609 information. Data are presented as mean ± SEM, unless stated otherwise. A p- or q-value (when
610 correction for multiple testing was required) below 0.05 was considered statistically significant.

611

612 **Study approval**

613 Written informed consent was obtained from pancreatic donors or their relatives and all
614 procedures were approved by the Swedish Ethical Review Authority (Permit number 2011263).

Author contributions:

Study design: All authors

Conducting experiments and analysing data: KB, AP, AK, EC, JKO, LBB, TR, AL, CL, SR, MN, ÅN, SG, CLL, PV, NW, UK, RP, LRC, CL

Writing – original draft: KB and CL

Writing – review and editing: All authors

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Data and materials availability: All data needed to evaluate the conclusions in the paper are presented in the paper and/or the Supplementary Materials. The human islet RNA-seq datasets are deposited in the LUDC repository (https://www.ludc.lu.se/resources/repository) under the following accession numbers: *LUDC islet case-control cohort* (accession number LUDC2022.07.111), *LUDC sorted α-/β-cell cohort* (accession number LUDC2022.07.112) and *LUDC islet HbA1c cohort* (accession number LUDC2022.07.113). Data have also been deposited at the European Genome-phenome Archive (https://www.ebi.ac.uk/ega/) under the following accession number; EGAD00001005512. The ATAC-seq dataset and mRNA

659 expression data from GFP- and Pax5-overexpressing INS1 β-cells are available in the GEO
660 repository under the accession number GSE129383 and GSE211310, respectively.

References

1. Bugliani M, Liechti R, Cheon H, Suleiman M, Marselli L, Kirkpatrick C, et al. Microarray analysis of isolated human islet transcriptome in type 2 diabetes and the role of the ubiquitin-proteasome system in pancreatic beta cell dysfunction. Mol Cell Endocrinol. 2013;367(1-2):1-10.

2. Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, Tseng YH, et al. Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell. 2005;122(3):337-49.

3. Lawlor N, George J, Bolisetty M, Kursawe R, Sun L, Sivakamasundari V, et al. Single-cell transcriptomes identify human islet cell signatures and reveal cell-type-specific expression changes in type 2 diabetes. Genome Res. 2017;27(2):208-22.

4. Olsson AH, Yang BT, Hall E, Taneera J, Salehi A, Nitert MD, et al. Decreased expression of genes involved in oxidative phosphorylation in human pancreatic islets from patients with type 2 diabetes. Eur J Endocrinol. 2011;165(4):589-95.

5. Segerstolpe A, Palasantza A, Eliasson P, Andersson EM, Andreasson AC, Sun X, et al. Single-Cell Transcriptome Profiling of Human Pancreatic Islets in Health and Type 2 Diabetes. Cell Metab. 2016;24(4):593-607.

6. Solimena M, Schulte AM, Marselli L, Ehehalt F, Richter D, Kleeberg M, et al. Systems biology of the IMIDIA biobank from organ donors and pancreatectomised patients defines a novel transcriptomic signature of islets from individuals with type 2 diabetes. Diabetologia. 2018;61(3):641-57.

682 7. Taneera J, Lang S, Sharma A, Fadista J, Zhou Y, Ahlqvist E, et al. A systems genetics
683 approach identifies genes and pathways for type 2 diabetes in human islets. Cell Metab.
684 2012;16(1):122-34.

685 8. Xin Y, Kim J, Okamoto H, Ni M, Wei Y, Adler C, et al. RNA Sequencing of Single Human
686 Islet Cells Reveals Type 2 Diabetes Genes. Cell Metab. 2016;24(4):608-15.

687 9. Wigger L, Barovic M, Brunner AD, Marzetta F, Schoniger E, Mehl F, et al. Multi-omics
688 profiling of living human pancreatic islet donors reveals heterogeneous beta cell trajectories
689 towards type 2 diabetes. Nat Metab. 2021;3(7):1017-31.

690 10. Ngara M, and Wierup N. Lessons from single-cell RNA sequencing of human islets.
691 Diabetologia. 2022.

692 11. Wang YJ, and Kaestner KH. Single-Cell RNA-Seq of the Pancreatic Islets--a Promise Not
693 yet Fulfilled? Cell Metab. 2019;29(3):539-44.

694 12. Mahajan A, Taliun D, Thurner M, Robertson NR, Torres JM, Rayner NW, et al. Fine-
695 mapping type 2 diabetes loci to single-variant resolution using high-density imputation and
696 islet-specific epigenome maps. Nat Genet. 2018;50(11):1505-13.

697 13. Rosengren AH, Braun M, Mahdi T, Andersson SA, Travers ME, Shigeto M, et al. Reduced
698 insulin exocytosis in human pancreatic beta-cells with gene variants linked to type 2 diabetes.
699 Diabetes. 2012;61(7):1726-33.

700 14. Peddinti G, Bergman M, Tuomi T, and Groop L. 1-Hour Post-OGTT Glucose Improves the
701 Early Prediction of Type 2 Diabetes by Clinical and Metabolic Markers. J Clin Endocrinol
702 Metab. 2019;104(4):1131-40.

15. Bysani M, Agren R, Davegardh C, Volkov P, Ronn T, Unneberg P, et al. ATAC-seq reveals alterations in open chromatin in pancreatic islets from subjects with type 2 diabetes. Sci Rep. 2019;9(1):7785.

16. Volkov P, Bacos K, Ofori JK, Esguerra JL, Eliasson L, Ronn T, et al. Whole-Genome Bisulfite Sequencing of Human Pancreatic Islets Reveals Novel Differentially Methylated Regions in Type 2 Diabetes Pathogenesis. Diabetes. 2017;66(4):1074-85.

17. Vinuela A, Varshney A, van de Bunt M, Prasad RB, Asplund O, Bennett A, et al. Genetic variant effects on gene expression in human pancreatic islets and their implications for T2D. Nat Commun. 2020;11(1):4912.

18. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, et al. Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. Hum Mol Genet. 2018;27(20):3641-9.

19. Liu JZ, van Sommeren S, Huang H, Ng SC, Alberts R, Takahashi A, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet. 2015;47(9):979-86.

20. Goyette P, Boucher G, Mallon D, Ellinghaus E, Jostins L, Huang H, et al. High-density mapping of the MHC identifies a shared role for HLA-DRB1*01:03 in inflammatory bowel diseases and heterozygous advantage in ulcerative colitis. Nat Genet. 2015;47(2):172-9.

21. Pulit SL, Stoneman C, Morris AP, Wood AR, Glastonbury CA, Tyrrell J, et al. Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. Hum Mol Genet. 2019;28(1):166-74.

724 22. Gazal S, Loh PR, Finucane HK, Ganna A, Schoech A, Sunyaev S, et al. Functional
725 architecture of low-frequency variants highlights strength of negative selection across coding
726 and non-coding annotations. Nat Genet. 2018;50(11):1600-7.

727 23. Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, et al. Discovery
728 and refinement of loci associated with lipid levels. Nat Genet. 2013;45(11):1274-83.

729 24. Sinnott-Armstrong N, Tanigawa Y, Amar D, Mars N, Benner C, Aguirre M, et al. Genetics
730 of 35 blood and urine biomarkers in the UK Biobank. Nat Genet. 2021;53(2):185-94.

731 25. Olsson AH, Volkov P, Bacos K, Dayeh T, Hall E, Nilsson EA, et al. Genome-wide
732 associations between genetic and epigenetic variation influence mRNA expression and insulin
733 secretion in human pancreatic islets. PLoS Genet. 2014;10(11):e1004735.

734 26. Taneera J, Dhaiban S, Hachim M, Mohammed AK, Mukhopadhyay D, Bajbouj K, et al.
735 Reduced Expression of Chl1 gene Impairs Insulin Secretion by Down-Regulating the
736 Expression of Key Molecules of beta-cell Function. Exp Clin Endocrinol Diabetes.
737 2021;129(12):864-72.

738 27. van de Bunt M, and Gloyn AL. A tale of two glucose transporters: how GLUT2 re-emerged
739 as a contender for glucose transport into the human beta cell. Diabetologia. 2012;55(9):2312-
740 5.

741 28. Sansbury FH, Flanagan SE, Houghton JA, Shuixian Shen FL, Al-Senani AM, Habeb AM,
742 et al. SLC2A2 mutations can cause neonatal diabetes, suggesting GLUT2 may have a role in
743 human insulin secretion. Diabetologia. 2012;55(9):2381-5.

744 29. Shahjahani M, Norozi F, Ahmadzadeh A, Shahrabi S, Tavakoli F, Asnafi AA, et al. The
745 role of Pax5 in leukemia: diagnosis and prognosis significance. Med Oncol. 2015;32(1):360.

746 30. Zambelli F, Pesole G, and Pavesi G. Pscan: finding over-represented transcription factor
747 binding site motifs in sequences from co-regulated or co-expressed genes. Nucleic Acids Res.
748 2009;37(Web Server issue):W247-52.

749 31. Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, et al.
750 Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from
751 L cells and alpha cells. Nat Med. 2011;17(11):1481-9.

752 32. Cataldo LR, Vishnu N, Singh T, Bertonnier-Brouty L, Bsharat S, Luan C, et al. The MafA-
753 target gene PPP1R1A regulates GLP1R-mediated amplification of glucose-stimulated insulin
754 secretion in beta-cells. Metabolism. 2021;118:154734.

755 33. Wang L, Liu Y, Yan Lu S, Nguyen KT, Schroer SA, Suzuki A, et al. Deletion of Pten in
756 pancreatic ss-cells protects against deficient ss-cell mass and function in mouse models of type
757 2 diabetes. Diabetes. 2010;59(12):3117-26.

758 34. Andersson SA, Olsson AH, Esguerra JL, Heimann E, Ladenvall C, Edlund A, et al. Reduced
759 insulin secretion correlates with decreased expression of exocytotic genes in pancreatic islets
760 from patients with type 2 diabetes. Mol Cell Endocrinol. 2012;364(1-2):36-45.

761 35. Bouzakri K, Ribaux P, Tomas A, Parnaud G, Rickenbach K, and Halban PA. Rab GTPase-
762 activating protein AS160 is a major downstream effector of protein kinase B/Akt signaling in
763 pancreatic beta-cells. Diabetes. 2008;57(5):1195-204.

764 36. Langfelder P, and Horvath S. WGCNA: an R package for weighted correlation network
765 analysis. BMC Bioinformatics. 2008;9:559.

766 37. Medvedovic J, Ebert A, Tagoh H, and Busslinger M. Pax5: a master regulator of B cell
767 development and leukemogenesis. Adv Immunol. 2011;111:179-206.

768 38. Mawla AM, and Huising MO. Navigating the Depths and Avoiding the Shallows of
769 Pancreatic Islet Cell Transcriptomes. Diabetes. 2019;68(7):1380-93.

770 39. Nutt SL, Morrison AM, Dorfler P, Rolink A, and Busslinger M. Identification of BSAP
771 (Pax-5) target genes in early B-cell development by loss- and gain-of-function experiments.
772 EMBO J. 1998;17(8):2319-33.

773 40. Panneerselvam A, Kannan A, Mariajoseph-Antony LF, and Prahalathan C. PAX proteins
774 and their role in pancreas. Diabetes Res Clin Pract. 2019;155:107792.

775 41. Bronsart LL, and Contag CH. A role of the adaptive immune system in glucose homeostasis.
776 BMJ Open Diabetes Res Care. 2016;4(1):e000136.

777 42. Guillam MT, Hummler E, Schaerer E, Yeh JI, Birnbaum MJ, Beermann F, et al. Early
778 diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. Nat
779 Genet. 1997;17(3):327-30.

780 43. McCulloch LJ, van de Bunt M, Braun M, Frayn KN, Clark A, and Gloyn AL. GLUT2
781 (SLC2A2) is not the principal glucose transporter in human pancreatic beta cells: implications
782 for understanding genetic association signals at this locus. Mol Genet Metab. 2011;104(4):648-
783 53.

784 44. Ahren B. Effects of beta-endorphin, met-enkephalin, and dynorphin A on basal and
785 stimulated insulin secretion in the mouse. Int J Pancreatol. 1989;5(2):165-78.

786 45. Green IC, Perrin D, Pedley KC, Leslie RD, and Pyke DA. Effect of enkephalins and
787 morphine on insulin secretion from isolated rat islets. Diabetologia. 1980;19(2):158-61.

788 46. Giugliano D, Quatraro A, Consoli G, Ceriello A, Torella R, and D'Onofrio F. Inhibitory
789 effect of enkephalin on insulin secretion in healthy subjects and in non insulin-dependent
790 diabetic subjects. Metabolism. 1987;36(3):286-9.

791 47. Adeghate E, and Ponery AS. The role of leucine-enkephalin on insulin and glucagon
792 secretion from pancreatic tissue fragments of normal and diabetic rats. Arch Physiol Biochem.
793 2001;109(3):223-9.

794 48. Steiglitz BM, Keene DR, and Greenspan DS. PCOLCE2 encodes a functional procollagen
795 C-proteinase enhancer (PCPE2) that is a collagen-binding protein differing in distribution of
796 expression and post-translational modification from the previously described PCPE1. J Biol
797 Chem. 2002;277(51):49820-30.

798 49. Hall E, Dekker Nitert M, Volkov P, Malmgren S, Mulder H, Bacos K, et al. The effects of
799 high glucose exposure on global gene expression and DNA methylation in human pancreatic
800 islets. Mol Cell Endocrinol. 2018;472:57-67.

801 50. Hall E, Jonsson J, Ofori JK, Volkov P, Perfilyev A, Dekker Nitert M, et al. Glucolipotoxicity
802 Alters Insulin Secretion via Epigenetic Changes in Human Islets. Diabetes. 2019;68(10):1965-
803 74.

804 51. Hall E, Volkov P, Dayeh T, Bacos K, Ronn T, Nitert MD, et al. Effects of palmitate on
805 genome-wide mRNA expression and DNA methylation patterns in human pancreatic islets.
806 BMC Med. 2014;12:103.

807 52. Gebre-Medhin S, Olofsson C, and Mulder H. Islet amyloid polypeptide in the islets of
808 Langerhans: friend or foe? Diabetologia. 2000;43(6):687-95.

809 53. Nilsson E, Jansson PA, Perfilyev A, Volkov P, Pedersen M, Svensson MK, et al. Altered
810 DNA methylation and differential expression of genes influencing metabolism and
811 inflammation in adipose tissue from subjects with type 2 diabetes. Diabetes. 2014;63(9):2962-
812 76.

813 54. Nilsson E, Vavakova M, Perfilyev A, Sall J, Jansson PA, Poulsen P, et al. Differential DNA
814 Methylation and Expression of miRNAs in Adipose Tissue From Twin Pairs Discordant for
815 Type 2 Diabetes. Diabetes. 2021;70(10):2402-18.

816 55. Li J, Wu X, Ke J, Lee M, Lan Q, Li J, et al. TET1 dioxygenase is required for FOXA2-
817 associated chromatin remodeling in pancreatic beta-cell differentiation. Nat Commun.
818 2022;13(1):3907.

819 56. Pang ZP, and Sudhof TC. Cell biology of Ca2+-triggered exocytosis. Curr Opin Cell Biol.
820 2010;22(4):496-505.

821 57. Lang J, Fukuda M, Zhang H, Mikoshiba K, and Wollheim CB. The first C2 domain of
822 synaptotagmin is required for exocytosis of insulin from pancreatic beta-cells: action of
823 synaptotagmin at low micromolar calcium. EMBO J. 1997;16(19):5837-46.

824 58. Ofori JK, Karagiannopoulos A, Barghouth M, Nagao M, Andersson ME, Salunkhe VA, et
825 al. The highly expressed calcium-insensitive synaptotagmin-11 and synaptotagmin-13
826 modulate insulin secretion. Acta Physiol (Oxf). 2022;236(1):e13857.

827 59. Ou K, Yu M, Moss NG, Wang YJ, Wang AW, Nguyen SC, et al. Targeted demethylation
828 at the CDKN1C/p57 locus induces human beta cell replication. J Clin Invest. 2019;129(1):209-
829 14.

830 60. Dayeh T, Volkov P, Salo S, Hall E, Nilsson E, Olsson AH, et al. Genome-wide DNA
831 methylation analysis of human pancreatic islets from type 2 diabetic and non-diabetic donors
832 identifies candidate genes that influence insulin secretion. PLoS Genet. 2014;10(3):e1004160.

833 61. Cottet S, Dupraz P, Hamburger F, Dolci W, Jaquet M, and Thorens B. SOCS-1 protein
834 prevents Janus Kinase/STAT-dependent inhibition of beta cell insulin gene transcription and
835 secretion in response to interferon-gamma. J Biol Chem. 2001;276(28):25862-70.

836 62. Luu L, Dai FF, Prentice KJ, Huang X, Hardy AB, Hansen JB, et al. The loss of Sirt1 in
837 mouse pancreatic beta cells impairs insulin secretion by disrupting glucose sensing.
838 Diabetologia. 2013;56(9):2010-20.

839 63. Davegardh C, Sall J, Benrick A, Broholm C, Volkov P, Perfilyev A, et al. VPS39-deficiency
840 observed in type 2 diabetes impairs muscle stem cell differentiation via altered autophagy and
841 epigenetics. Nat Commun. 2021;12(1):2431.

842 64. Nilsson E, Matte A, Perfilyev A, de Mello VD, Kakela P, Pihlajamaki J, et al. Epigenetic
843 Alterations in Human Liver From Subjects With Type 2 Diabetes in Parallel With Reduced
844 Folate Levels. J Clin Endocrinol Metab. 2015;100(11):E1491-501.

845 65. Jacovetti C, Rodriguez-Trejo A, Guay C, Sobel J, Gattesco S, Petrenko V, et al. MicroRNAs
846 modulate core-clock gene expression in pancreatic islets during early postnatal life in rats.
847 Diabetologia. 2017;60(10):2011-20.

848 66. Nuevo-Tapioles C, Santacatterina F, Stamatakis K, Nunez de Arenas C, Gomez de Cedron
849 M, Formentini L, et al. Coordinate beta-adrenergic inhibition of mitochondrial activity and
850 angiogenesis arrest tumor growth. Nat Commun. 2020;11(1):3606.

851 67. Porzio O, Massa O, Cunsolo V, Colombo C, Malaponti M, Bertuzzi F, et al. Missense
852 mutations in the TGM2 gene encoding transglutaminase 2 are found in patients with early-onset
853 type 2 diabetes. Mutation in brief no. 982. Online. Hum Mutat. 2007;28(11):1150.

854 68. Sarem Z, Bumke-Vogt C, Mahmoud AM, Assefa B, Weickert MO, Adamidou A, et al.
855 Glucagon Decreases IGF-1 Bioactivity in Humans, Independently of Insulin, by Modulating Its
856 Binding Proteins. J Clin Endocrinol Metab. 2017;102(9):3480-90.

857 69. Friberg AS, Brandhorst H, Buchwald P, Goto M, Ricordi C, Brandhorst D, et al.
858 Quantification of the islet product: presentation of a standardized current good manufacturing
859 practices compliant system with minimal variability. Transplantation. 2011;91(6):677-83.

860 70. Love MI, Huber W, and Anders S. Moderated estimation of fold change and dispersion for
861 RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.

Figures

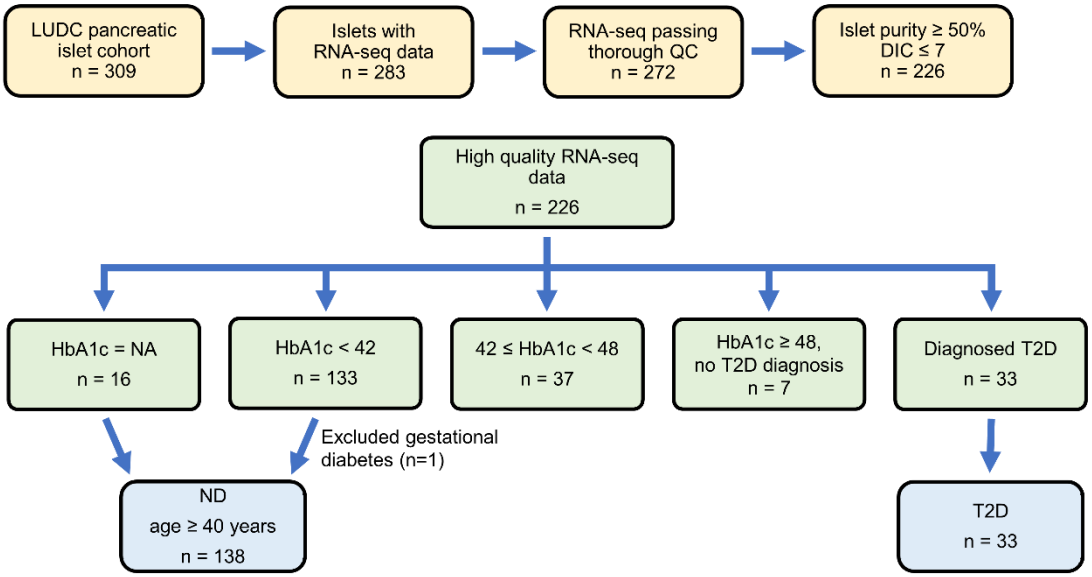

Figure 1. Workflow of RNA-sequencing sample filtering. The LUDC pancreatic islet cohort consists of islet preparations from 309 individuals. RNA-sequencing was performed on 283 of these. After quality control (QC) and other filtering, data from 171 preparations were included in the analysis to identify differentially expressed genes in islets from individuals with type 2 diabetes (T2D) versus non-diabetic controls (ND). Similarly, islet data from 176 preparations were included in the HbA1c analysis. DIC: days in culture.

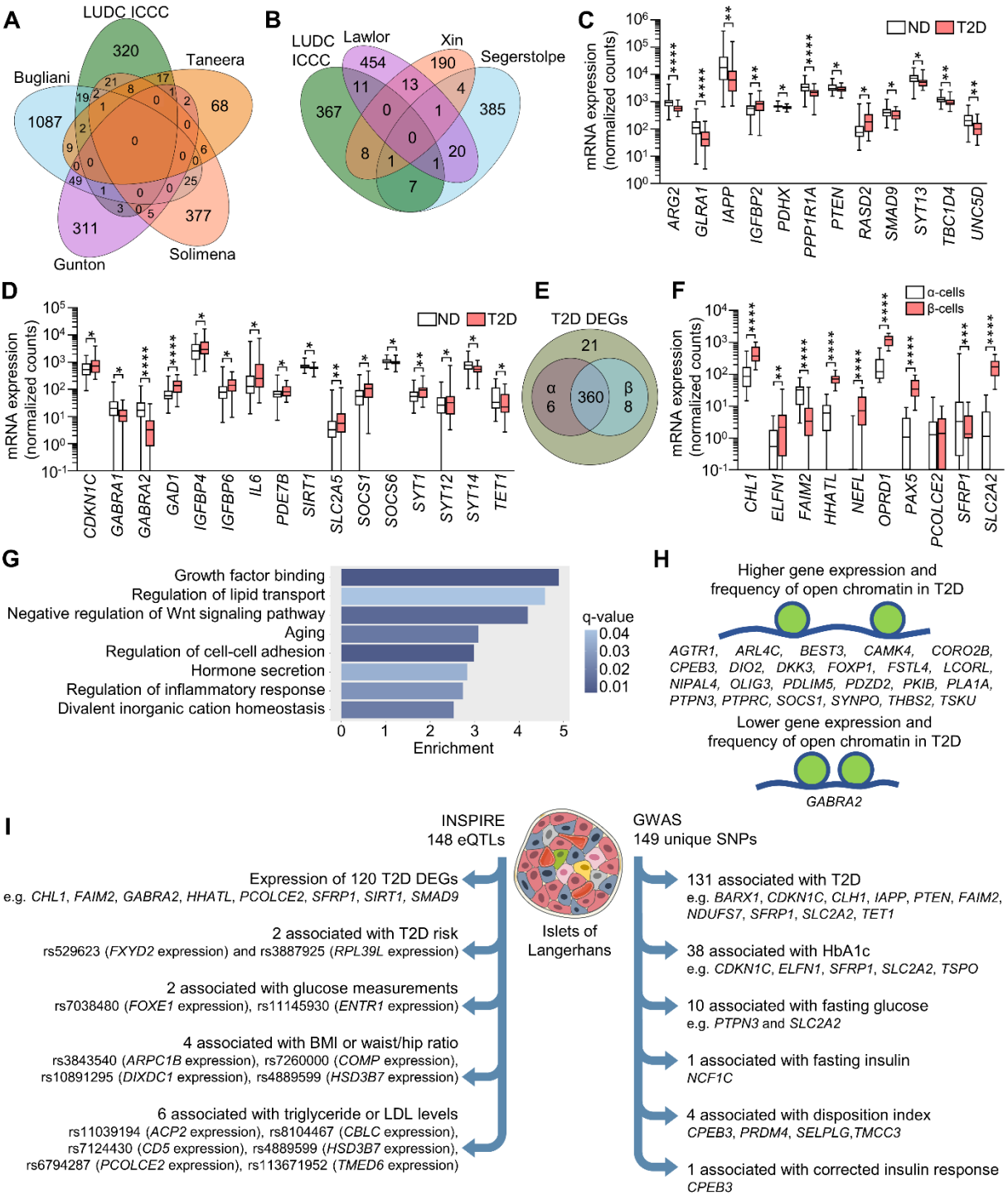

Figure 2. Characterization of the 395 identified DEGs. (**A-B**) Venn diagrams showing the overlaps between the DEGs identified in the *LUDC islet case-control cohort* (LUDC ICCC) and DEGs identified in previous bulk (**A**) and single cell (**B**) expression analyses of human pancreatic islets from T2D and ND donors. (**C-D**) mRNA expression of selected known (**C**) and, to our knowledge, novel (**D**) DEGs identified in pancreatic islets from 33 individuals with

T2D and 138 ND controls of the LUDC ICCC. *q<0.05, **q<0.01, ***q<0.001, ****q<0.0001 based on a generalized linear model as implemented in DESeq2 (70), with correction for age, sex, islet purity, and days in culture. (**E**) RNA-sequencing of sorted α- and β-cells from 16 ND individuals shows that the vast majority of the 395 identified DEGs are expressed in either or both cell types. (**F**) mRNA expression of selected genes in sorted α- and β-cells from islet preparation of 16 ND individuals. **q<0.01, ***q<0.001, ****q<0.0001 based on a generalized linear model as implemented in DESeq2 (70). (**G**) An enrichment analysis shows that T2D islet DEGs are enriched for gene ontology terms associated with β-cell function. (**H**) DEGs with altered chromatin state in islets from ND controls and individuals with T2D, as identified by ATAC-sequencing (15). (**I**) Left: 148 pancreatic islets eQTLs associate with expression of 120 DEGs as well as T2D risk and metabolic traits. Right: 149 unique SNPs annotated to 106 DEGs have been found to associate with T2D or the indicated glucose traits in GWAS. Box-and-whisker plots show the median, 25th and 75th percentile, and minimum and maximum values. The islet in Figure 2I comes from Servier Medical templates.

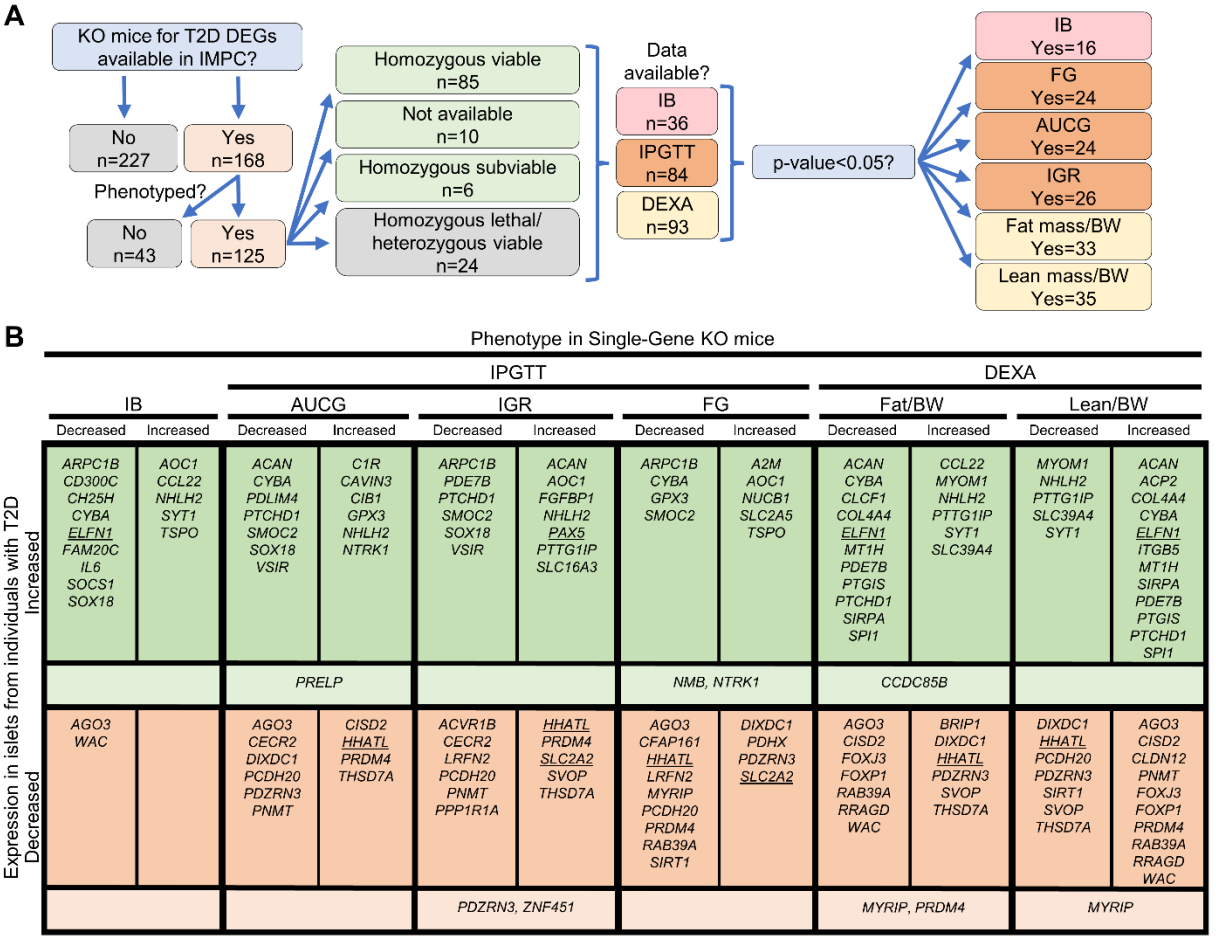

Figure 3. Mice with knockout (KO) of genes showing differential expression in pancreatic islets from individuals with T2D exhibit metabolic phenotypes. (**A**) Flow chart depicting the International Mouse Phenotyping Consortium (IMPC) data mining strategy and an overview of the findings on mice with knockout of differentially expressed genes (DEGs) identified in the *LUDC islet case-control cohort*. (**B**) Summary of IMPC phenotypic data outputs for viable KO mouse strains. Underlined genes were functionally validated in our study, while knockout mice for genes in lighter colored areas show different effects for the indicated phenotype in males and females. IB: insulin blood levels, IPGTT: intraperitoneal glucose tolerance test, AUCG: Area under glucose response curve, IGR: Initial response to glucose challenge, FG: Fasted blood glucose concentration, DEXA: Dual-energy X-ray absorptiometry, BW: Body weight.

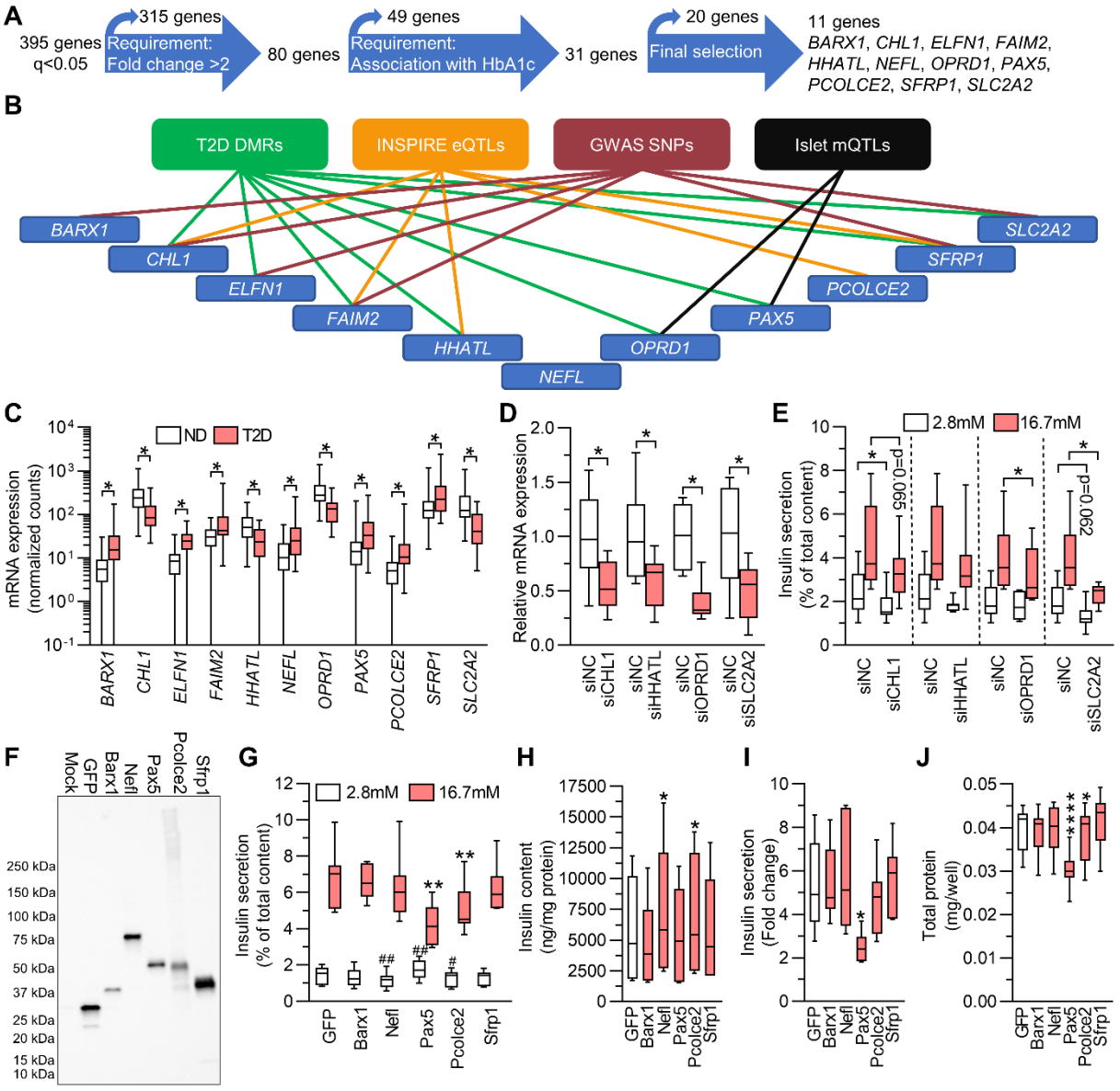

Figure 4. T2D-associated expression changes impair insulin secretion. (**A**) Flow chart showing strategy for selection of DEGs for functional follow-up. (**B**) Ten of the eleven genes selected for functional analysis have T2D DMRs, INSPIRE eQTLs, SNPs associated with T2D or glucose traits, or islet mQTLs annotated to them. (**C**) Expression of DEGs selected for functional follow-up. *q<0.0001 based on a generalized linear model as implemented in DESeq2 (70), with correction for age, sex, purity, and days in culture, on expression data on islets from 138 ND controls and 33 individuals with T2D. (**D**) qPCR quantification of siRNA-mediated knockdown of *CHL1*, *HHATL*, *OPRD1*, and *SLC2A2* in human islets (n=6-8). (**E**) Effect of knockdown of *CHL1*, *HHATL1*, *OPRD1*, or *SLC2A2* on insulin secretion from human

islets (n=6-8). (**F**) Western blot showing overexpression of GFP, Barx1, Nefl, Pax5, Pcolce2, and Sfrp1 in virally transduced INS1 β-cells. Representative image, the experiment was performed three times. (**G-J**) The effect of overexpression on insulin secretion (**G**) and insulin content (**H**) in absolute values, insulin secretion presented as fold change (**I**), and total protein (**J**) (n=7). D-E: *p<0.05 compared to siNC at the indicated glucose concentration. G: *p<0.05 and **p<0.01 compared to GFP at 16.7mM glucose, $^{\#}$p<0.05 and $^{\#\#}$p<0.01 compared to GFP at 2.8mM glucose. H-J: *p<0.05 compared to GFP. Data in D-E and G-J were analyzed by paired t-tests. Box-and-whisker plots show the median, the 25th and 75th percentile, and minimum and maximum values.

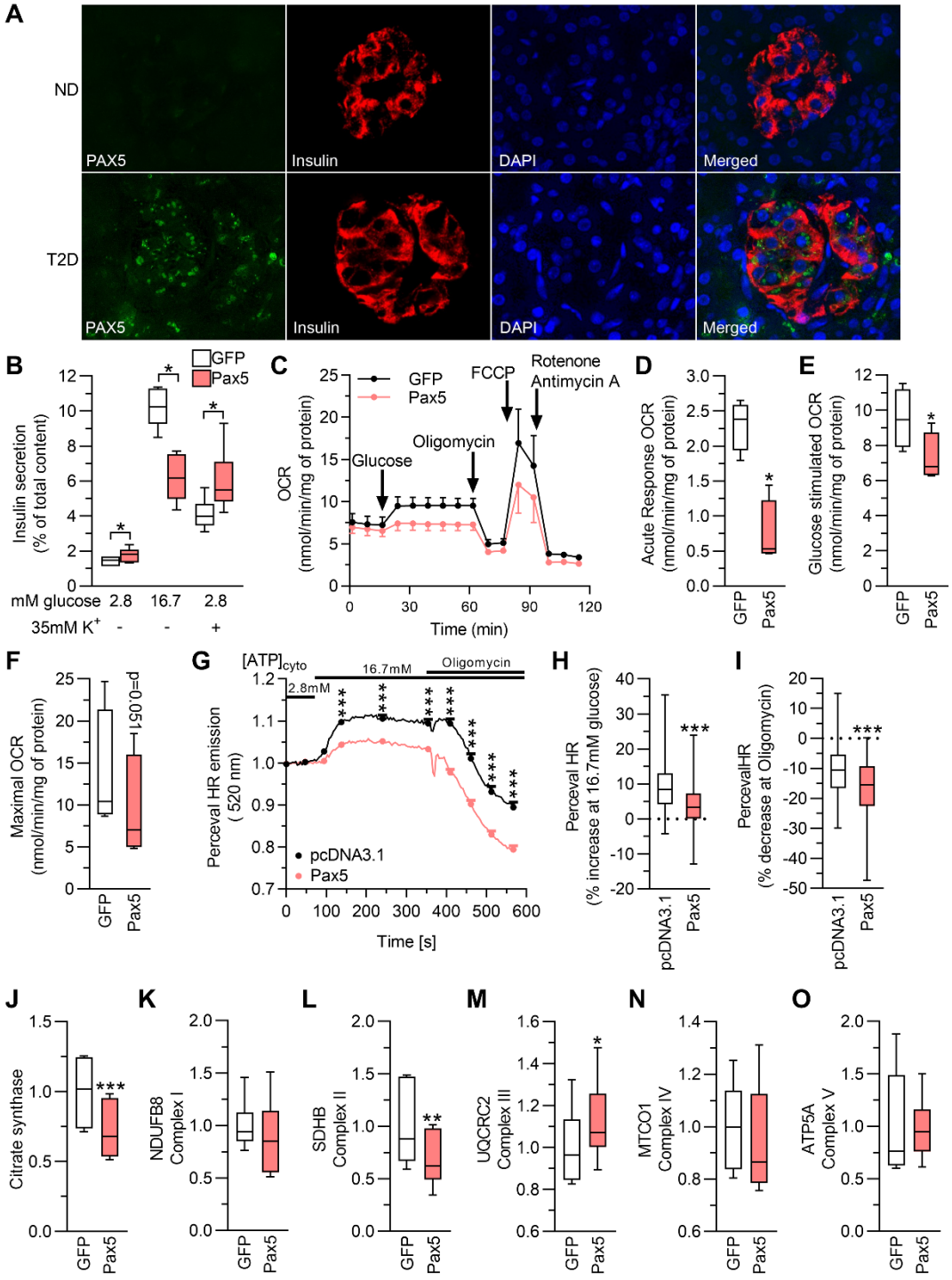

Figure 5. Increased expression of Pax5 results in perturbed mitochondrial activity. (**A**) Immunohistochemical staining of human pancreas sections (5 ND + 4 T2D) showing increased PAX5 (green) expression in T2D. Most of the expression is confined to β-cells, as evidenced by co-staining with insulin (red). Blue: nuclear stain DAPI. Representative images taken with a 40x objective are shown. (**B**) Pax5 overexpression blunts GSIS, but increases secretion

stimulated by elevated K^+ (n=6). (**C**) The oxygen consumption rate (OCR) in clonal β-cells overexpressing GFP or Pax5. OCR was measured at 2.8mM glucose (basal respiration) and then after sequential addition of 16.7mM glucose (glucose-stimulated respiration), 5μM oligomycin (inhibits ATP synthase), 4μM carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP, mitochondrial uncoupler) and 1μM rotenone/antimycin A (electron transport chain inhibitors) (n=4). (**D-F**) The respiratory response to addition of high glucose (change in respiration compared to basal glucose, **D**), glucose-stimulated respiration (respiration at high glucose, **E**), and maximal OCR (respiration after mitochondrial uncoupling, **F**) (n=4). (**G**) PercevalHR trace on INS1 β-cells stimulated with 2.8 and 16.7mM glucose, and after addition of oligomycin (n=148 (pcDNA3.1) and 213 (Pax5). (**H-I**) Pax5-overexpressing INS1 β-cells (n=213) exhibit a significantly lower increase in ATP/ADP ratio when glucose is elevated to 16.7mM (average signal between 94 and 354 seconds compared to average signal between 0 and 75 seconds, **H**), and a greater drop in ATP/ADP ratio after addition of oligomycin (average signal between 409 and 567 seconds compared to average signal between 94 and 354 seconds, **I**), when compared to pcDNA3.1-transfected cells (n=148). (**J-O**) Levels of citrate synthase and subunits of complex I-V of the electron transport chain (n=6). *p<0.05, **p<0.01, ***p<0.001. Data in B-F and J-O were analyzed by paired t-tests. Data in G-I were analyzed with unpaired t-tests. Box-and-whisker plots show the median, the 25th and 75 percentile, and minimum and maximum values.

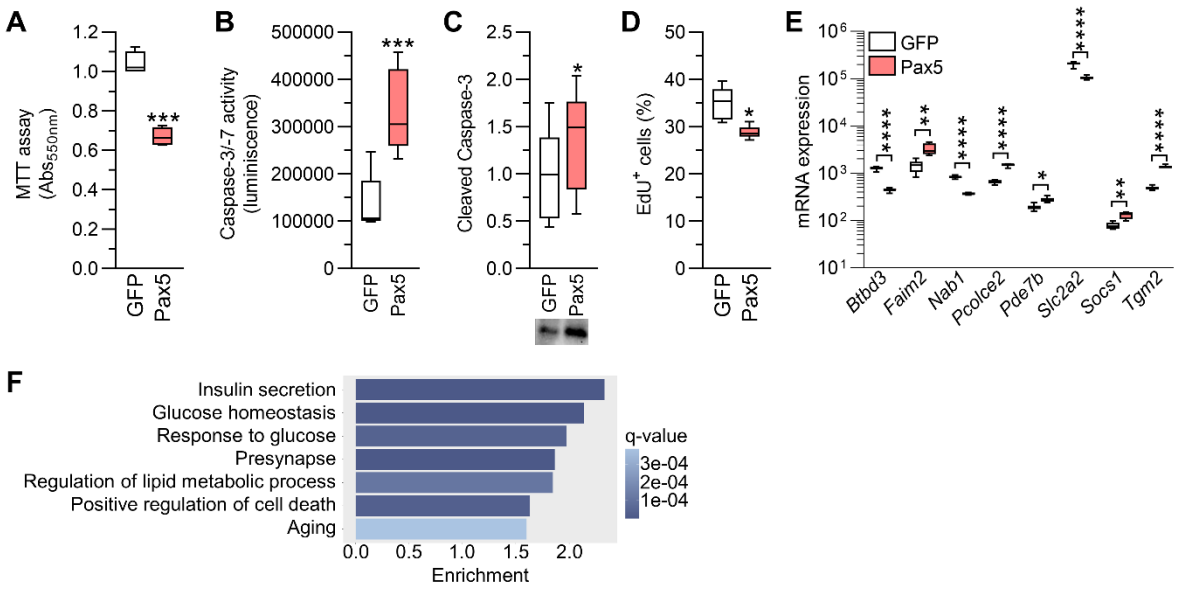

Figure 6. Elevated Pax5 in INS1 β-cells leads to cell loss and widespread transcriptomic changes affecting β-cell function. (**A**) Pax5 overexpression results in loss of INS1 β-cells, as indicated by MTT assay (n=4). (**B-C**) Pax5 overexpression in INS1 β-cells increases Caspase-3/-7 activity (n=5, **B**) and levels of cleaved (i.e., active) Caspase-3 (n=6, all samples run on one gel, **C**). (**D**) Pax5 overexpression reduces proliferation in INS1 β-cells (n=6). (**E**) Expression of *Btbd3*, *Faim2*, *Nab1*, *Pcolce2*, *Pde7b*, *Slc2a2,* and *Tgm2* is altered in Pax5 overexpressing INS1 β-cells (n=8). (**F**) Enrichment of gene ontology terms among the genes with differential expression in Pax5-overexpressing INS1 β-cells shows there are transcriptomic changes within pathways important for insulin secretion and cell number. A-E: *p<0.05, **p<0.01 ***p<0.001, ****p<0.0001, based on paired t-tests. Box-and-whisker plots show the median, the 25th and 75th percentile, and minimum and maximum values.

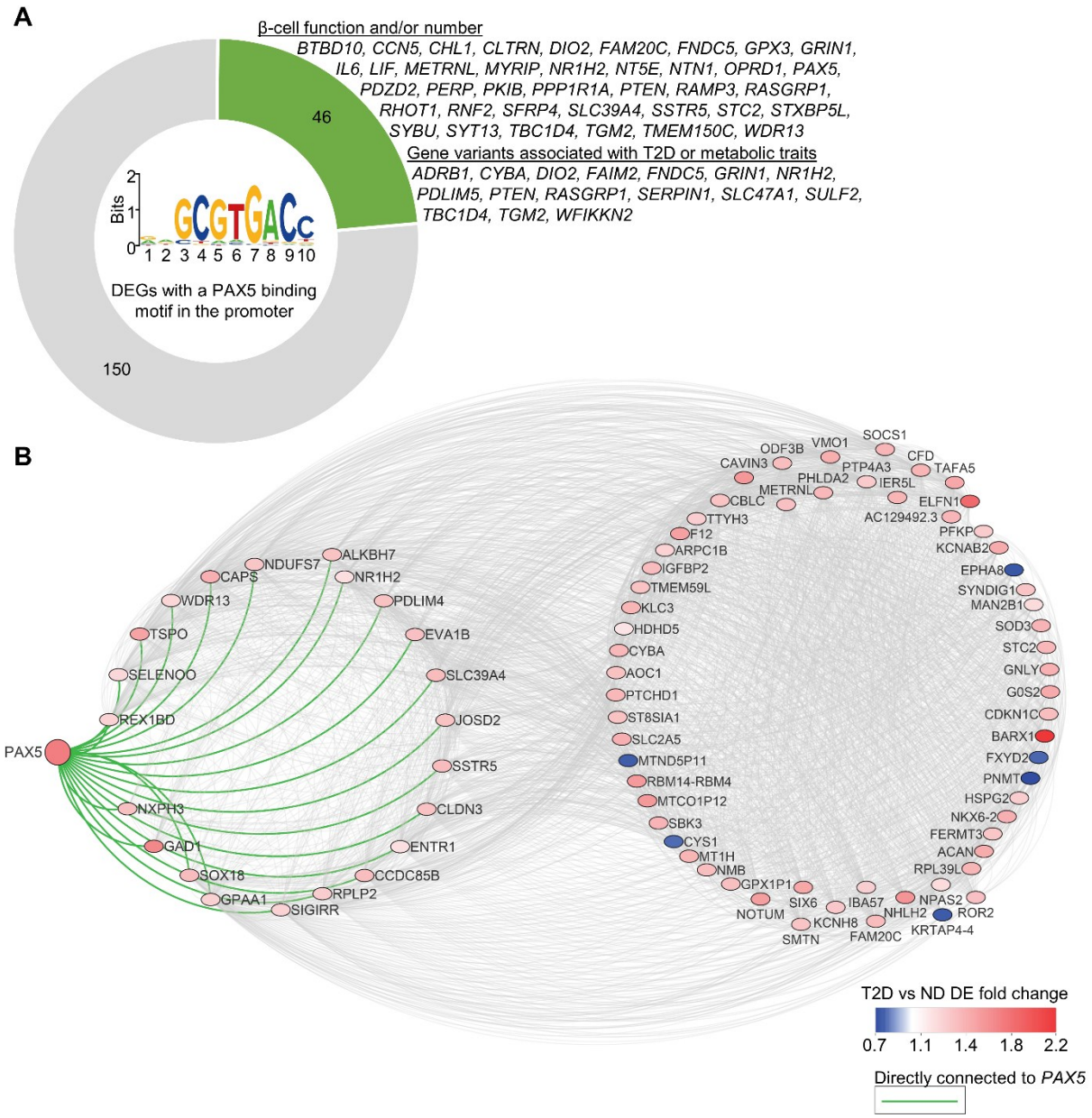

Figure 7. *PAX5* is potentially a key T2D DEG overexpressed in β-cells. (**A**) Graphic showing the number of DEGs with a PAX5 binding motif in the promoter (based on a Pscan analysis (30)), and the proportion of these that have been shown to have a regulatory role in β-cells, or have genetic variants associated with T2D or metabolic traits in humans, either in this or published studies. The PAX5 binding motif is shown in the center. (**B**) A WGCNA (36) co-expression analysis based on weighted correlations among the 395 T2D DEGs shows that *PAX5* is part of an expression cluster containing 87 DEGs, with direct connection to 22 DEGs.

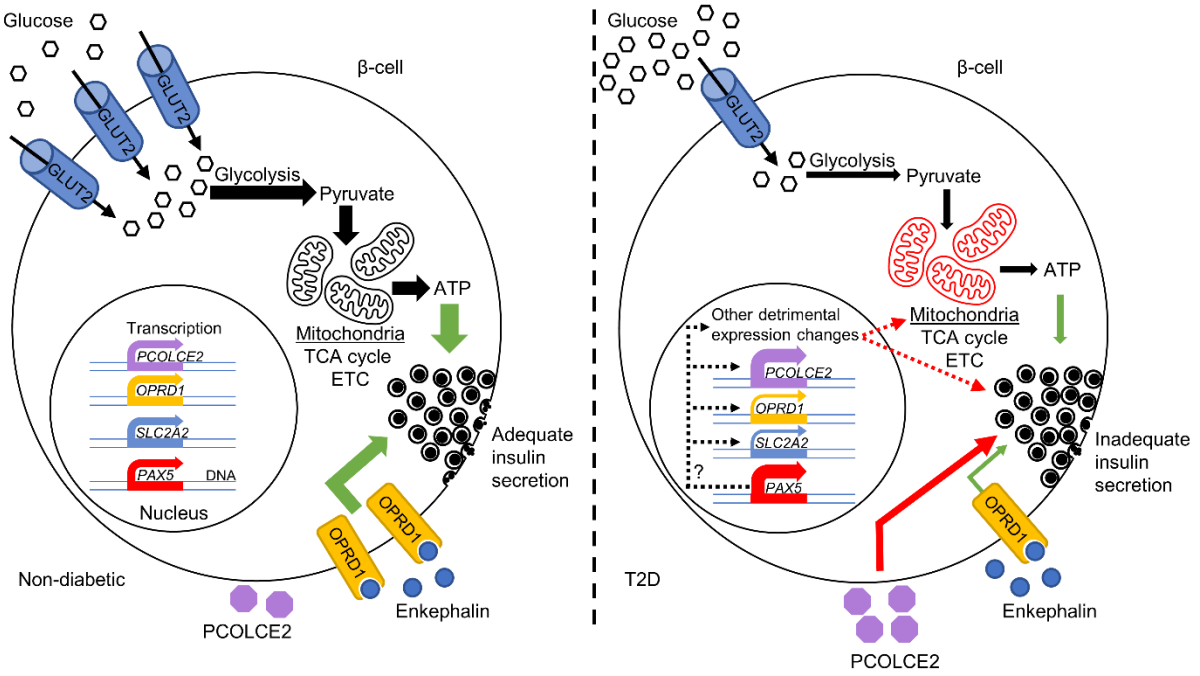

Figure 8. Schematic image presenting a model for how the T2D-associated changes may alter β-cell function. Our analysis showed that the islet expression of 395 genes is altered in T2D. These expression changes are enriched for genes affecting e.g., insulin secretion, and functional analyses show that the T2D-associated changes to *OPRD1*, *PAX5*, *PCOLCE2*, and *SLC2A2* (encoding GLUT2) impair glucose-stimulated insulin secretion. In non-diabetic individuals, β-cells highly express *SLC2A2*, leading to high levels of GLUT2 and efficient glucose uptake. This in turn, via glycolysis, tricarboxylic acid (TCA) cycle and electron transport chain (ETC) in well-functioning mitochondria, leads to ATP production, increased cytosolic ATP/ADP ratio, and the stimulation of insulin secretion. Simultaneously, our data suggest that signalling through OPRD1, an enkephalin receptor, stimulates insulin secretion. In T2D, there is dysregulation of *PAX5*, leading to greatly increased PAX5 mRNA and protein levels in β-cells, as well as a severe reduction in *SLC2A2* and *OPRD1* expression. These changes lead to diminished insulin secretion, with PAX5 overexpression causing a strong reduction in mitochondrial activity. Simultaneously *PCOLCE2* is upregulated, which via an unknown mechanism also impairs insulin secretion. Importantly, elevated *PAX5* may cause

many of the other detrimental expression changes, including reduced *SLC2A2*. Green arrows: stimulation. Red arrows: inhibition. Dashed arrows: potential effects.

Tables

Table 1. Characteristics of donors of pancreatic islets from the cohorts used in the study. ND: non-diabetic. T2D: type 2 diabetes. Data are presented as mean ± SEM (min-max).

Characteristic	LUDC islet case-control cohort*			LUDC sorted α-/β-cell cohort*	LUDC sorted α-/β-cell cohort* ND vs T2D/pre-T2D		LUDC islet HbA1c cohort*
	ND (n=138)	T2D (n=33)	p-values[†]	ND[‡] n=16	ND[‡] (n=13)	T2D and pre-T2D[‡] (n=5)	n=176
Sex (M/F)	86/52	21/12		10/6	8/5	3/2	112/64
Age (years)	60.9 ± 0.8 (40-86)	62.9 ± 1.5 (41-81)	0.23	62.5 ± 3.3 (36-81)	63.3 ± 3.9 (36-81)	63.8 ± 4.3 (54-79)	59.1 ± 0.8 (24-86)
BMI (kg/m²)	26.1 ± 0.3 (18.0-42.6)	27.9 ± 0.8 (21.6-37.0)	0.003	25.8 ± 1.0 (18.9-32.7)	25.1 ± 1.1 (18.9-32.7)	27.8 ± 1.1 (25.8-31.1)	26.6 ± 0.3 (18.0-42.6)
HbA1c (mmol/mol)	36.7 ± 0.3 (23-41)	50.8 ±1.7 (39-86)	4.9E-28	35.8 ± 1.3 (22-44)	34.2 ± 1.2 (22-40)	44.0 ± 1.3 (42-49)	38.9 ± 0.4 (22-70)[¤]
Stimulatory index[§]	7.3 ± 0.5 (1.2-37.3)	5.6 ± 0.8 (0.8-21.2)	0.15	6.8 ± 2.0 (1.0-30.7)	7.6 ± 2.6 (1.0-30.7)	3.8 ± 1.3 (0,8-7.4)	7.7 ± 0.5 (0.7-37.3)
Pancreatic islet purity (%)	76.6 ± 1.1 (50-98)[#]	78.9 ± 1.8 (53-97)[#]	0.16	82.8 ± 5.5 (37-100)	84.2 ± 5.5 (39-100)	78.8 ± 11.2 (37-97)	76.4 ± 1.0 (50-96)[#]

*There is some overlap between the ND controls and the individuals in the HbA1c and sorted α-/β-cell cohorts.

[†]Two-tailed t-tests were used to detect differences between ND and T2D.

[‡]All donors without a T2D diagnosis were included in the analysis to determine if the 395 DEGs were expressed in α- and/or β-cells. Three of these had a HbA1c between 42 and 47 mmol/mol and are therefore clinically defined as pre-diabetic. These three donors were included among the five T2D/pre-T2D cases in the ND vs T2D/pre-T2D comparison of sorted α- and β-cells

[¤]Seven individuals have an HbA1c above 48 mmol/mol.

[§]Stimulatory index is a measure of glucose-stimulated insulin secretion from human islets cultured in vitro.

[#]Islets from 14 preparations were handpicked before RNA extraction and for those the purity was set to 85%.