Monogenic Diabetes and Integrated Stress Response Genes Display Altered Gene Expression in Type 1 Diabetes

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Tweet: @ufdiabetes investigators demonstrate dysregulated expression of monogenic diabetes-associated genes in human type 1 diabetes pancreas, highlighting an activated integrated stress response as a feature and perhaps driver of the disease.

ABSTRACT

Type 1 diabetes has a multifactorial autoimmune etiology, involving environmental prompts and polygenic predisposition. We hypothesized that pancreata from individuals with and at risk for type 1 diabetes would exhibit dysregulated expression of genes associated with monogenic forms of diabetes caused by non-redundant single-gene mutations. Employing a "monogenetic transcriptomic strategy," we measured the expression of these genes in human type 1 diabetes, autoantibody positive (autoantibody+), and control pancreas tissues using RTqPCR in accordance with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines. Gene and protein expression were visualized *in situ* using immunofluorescence, RNAScope, and confocal microscopy. Two-dozen monogenic diabetes genes showed altered expression in human pancreata from individuals with type 1 diabetes versus unaffected controls. Six of these genes also saw dysregulation in pancreata from autoantibody+ persons at increased-risk for type 1 diabetes. As a subset of these genes are related to cellular stress responses, we measured integrated stress response (ISR) genes and identified 20 with altered expression in type 1 diabetes pancreata, including three of the four eIF2α-dependent kinases. Equally intriguing, we observed significant repression of the three arms of the ISR in autoantibody+ pancreata. Collectively, these efforts suggest monogenic diabetes and ISR genes are dysregulated early in the type 1 diabetes disease process and likely contribute to the disorder's pathogenesis.

Keywords: gene expression, monogenics, Type 1, pancreas, stress Response

Type 1 diabetes is widely considered a multifactorial disorder, polygenic in etiology with environmental factors thought to contribute toward pathogenesis, resulting in autoimmune destruction of insulin producing pancreatic β-cells (1; 2). In contrast, monogenic diabetes comprises an expanding group of rare heterogeneous, single gene disorders with a collective prevalence of ~1-5% of all diabetes cases, depending on age of onset, geography and ethnicity (3-6). Monogenic forms of diabetes distinguish critical proteins within human β-cell development and biology where no sufficient compensatory proteins or pathways exist in the presence of a sufficiently deleterious mutation, reflecting the critical nature of the protein and a lack of "redundancy" at that point within the affected pathway. Most forms of monogenic diabetes result, through a variety of mechanisms, in a reduced ability to process or secrete insulin, with some variants associated with insulin resistance (7).

We therefore studied genes associated with monogenic forms of diabetes with the rationale being a relevance to disease pathology. Traditionally, these non-redundant forms of diabetes have been classified based on age of onset: (Maturity Onset Diabetes of the Young (8-10) (MODY); neonatal diabetes mellitus *(11; 12)* (NDM) *which* includes transient (TNDM1 and TNDM2) and permanent NDM (PNDM) or as syndromic. However, to provide a physiological reference point, we have addressed the biological heterogeneity of monogenic diabetes genes, by separating our studied genes into four physiological groups: immune, β-cell function, β-cell development and endoplasmic reticulum (ER) function/stress.

We therefore hypothesized that a phenotypic assessment of gene expression levels for the ever-expanding cohort of genes linked to monogenic diabetes could be enlightening in our understanding of multifactorial/polygenic type 1 diabetes disease etiology and pathogenesis. In particular, we have sought to address the question of the importance of genes causative in

monogenic diabetes using real time qPCR (RTqPCR), immunofluorescence (IF), and *in situ* hybridization (ISH) studies on human pancreatic tissues from unaffected control, type 1 diabetes, autoantibody positive (autoantibody+, high risk for type 1 diabetes), and type 2 diabetes organ donors from the Network for Pancreatic Organ donors with Diabetes (nPOD) repository.

Research Design and Methods

Donors The JDRF nPOD program (www.jdrfnpod.com) recovers transplant-quality pancreata from organ donors as previously described (13). All procedures were approved by the University of Florida Institutional Review Board and the United Network for Organ Sharing (UNOS) according to federal guidelines, with informed consent obtained from each donor's legal representative. For each donor, a medical chart review was performed in addition to assays for type 1 diabetes-associated autoantibodies and C-peptide (14), with type 1 diabetes diagnosed according to the guidelines established by the ADA (15). Patient #, autoantibody+ status, age, disease duration, gender, ethnicity, C-peptide, HbA1c, BMI, cause of death, and hiRES HLA were obtained from nPOD records (Table S1). Cause of death was validated via an independent medical chart review by a medical expert.

Sample processing and RNA extraction Pancreata were recovered, placed in transport media on ice, and shipped via organ courier to the University of Florida where tissues were processed by a licensed Pathology Assistant as previously described (13). Tissue from pancreas was preserved as flash frozen or in RNAlater (Qiagen, Valencia, CA) an average of 16h from cross clamp. Total RNA was isolated following homogenization in Qiagen RNeasy Plus Mini Kit isolation buffer as per the manufacturer's instructions including treatment with DNase 1. RNA concentrations were determined using a Nanodrop 2000C (Thermo Scientific, Waltham, MA) and when necessary,

integrity was verified by visualization of ribosomal RNA by gel electrophoresis and ethidium bromide staining.

RTqPCR, gene stability ranking and qPCR minimum guidelines All samples were confirmed to be free of DNA contamination in controls with an intron/exon primer pair and without reverse transcriptase. cDNA was produced with SuperScript II (Invitrogen, Carlsbad, CA) using oligo dT priming and subsequently utilized for RTqPCR using Thermo Luminaris Color HiGreen Fluorescein qPCR Master Mix (Thermo Scientific). 0.5-1 µg of total RNA was used for each 20 µl cDNA reaction which was then diluted to 200 µl and 2 µl of diluted cDNA employed for each 25 µl RT-qPCR reaction containing 600 nM of each primer pair. Individual RT-qPCR reactions were carried out in duplicate in a Biorad MyiQ. All samples were standardized for experimental design, nucleic acid isolation, measurement of total RNA concentration, reverse transcription, primer design/target specificity (16) and all RTqPCR parameters (17) in accordance with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines.

The MIQE guidelines (Table S2) were implemented to ensure the reliability/integrity of scientific data and to provide for experimental transparency/consistency amongst laboratories (17). Table S2 illustrates a checklist for MIQE guidelines, which were followed in this publication. A fundamental axiom of MIQE is normalization of RTqPCR data by utilizing multiple reference genes (RGs) to address intra- and inter-kinetic variations in qPCR studies (18; 19). The following genes were identified as potential RGs based on the lowest standard deviation (SD) and coefficient of variation (CoV) of un-normalized C_q values across all unaffected and type 1 diabetes pancreata: ASNS (asparagine synthetase), GLP1R (Glucagon-like peptide 1 receptor), MAFB (V-maf musculoaponeurotic fibrosarcoma oncogene homolog B), NKX6-1 (NK6 homeobox 1), NRP1

(Neuropilin 1), PFKM (phosphofructokinase, muscle), PPIA (cyclophilin A), and GCG (glucagon). To compare and rank potential reference genes with the lowest variation and highest stability across the control and type 1 diabetes pancreata, four algorithms were evaluated simultaneously using the Web-based tool, RefFinder (20) (https://github.com/fulxie/RefFinder) which incorporates four well accepted algorithms: geNorm (21), Normfinder (22), BestKeeper (23), and the comparative delta-Ct method (24) as well as its own comprehensive assessment (20). RefFinder requires an equal number of data points for all genes evaluated, therefore, only 45 donors were included in this analysis. RefFinder identified three human pancreas specific reference genes (RGs) demonstrating the most stable expression in human pancreata, PPIA (SD=2.02; CoV=0.06), MAFB (SD=1.89; CoV=0.07) and ASNS (SD=2.15; CoV=0.07). We then utilized the geNorm algorithm (21) to generate separate normalization factors derived from the geometric mean of these RGs: PPIA, ASNS and MAFB for unaffected and the type 1 diabetes cohorts, respectively. The normalization factors were applied to the analysis of all monogenic diabetes and ISR genes to report quantitative changes in gene expression between unaffected and type 1 diabetes pancreata, with the fold difference (FD) reported as the ratio of the means (type 1 diabetes/Unaffected). PPIA (cyclophilin A) also served as the inter-run calibrator. Using the interquartile range outlier test (25; 26), only extreme outliers defined as values below Q_1-3(Q_3-Q_1) or above Q_3+3(Q_3-Q_1) (where Q1 and Q3 are the first and third data quartile) were identified and excluded from statistical analyses (http://www.itl.nist.gov/div898/handbook/prc/section1/prc16.htm).

Primer design The respective monogenic diabetes genes analyzed in this study were selected based on review of the National Center for Monogenic Diabetes at the University of Chicago, Kolver Diabetes Center (http://monogenicdiabetes.uchicago.edu), the Institute of Biomedical and

Clinical Science, University of Exeter, Medical School (http://medicine.exeter.ac.uk/research/biomedicalclinical/moleculargenetics-monogenic/) and OMIM (https://www.omim.org). We would like to point out, however, that this may not be an all-inclusive set of monogenic diabetes genes. Human gene symbols, based on HGNC (http://www.genenames.org) were used throughout this study with gene names, chromosomal location, NCBI accession numbers, common protein names, OMIM #, chromosomal location, genomic coordinates, MODY number, Neonatal Diabetes Mellitus classification and appropriate references provided in Table S3. We utilized the public Primer-Blast software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/ (16)) which incorporates the Primer3 program (27) for primer design, genome-wide BLAST analysis along with the Needleman-Wunsch global alignment algorithm (28) to identify internal homology between primers and any unwanted targets in the human genome. This satisfies the requirements for primer specificity compared to both the human transcriptome and genome. Primers were designed, when possible, as exonic primers spanning an intron, with comparable GC content and an optimal T_m of 60 °C (Table S4).

Immunofluorescence (IF) Immunofluorescence staining was completed using the PerkinElmer Opal 4-Color IHC Kit (Perkin-Elmer NEL810001KT, Waltham, MA) as per the manufacturer's instructions. After deparaffinization and rehydration, all slides were subjected to a microwaved treated antigen retrieval step using kit's appropriate AR buffer. Slides were then incubated in Antibody Diluent / blocking solution followed by incubation with primary antibody in Antibody Diluent / blocking solution. Slides were stained with the following antibodies: insulin (Dako, A0564, RRID:AB_10013624) ; somatostatin (Dako A0566, RRID:AB_2688022); glucagon (Abcam ab10988, RRID:AB_297642); STAT5 (LifeSpan BioSciences LS‑B5540,

RRID:AB_10915294); GLIS3 (Sigma/Aldrich HPA056426, RRID:AB_2683128); GATA4 (Santa Cruz sc-25310, RRID:AB_627667); WFS1 (LifeSpan BioSciences LS‑B14378); and EIF2AK3/PERK (Proteintech 24390-1-AP). For detection and visualization, slides were subjected to the Polymer HRP Ms + Rb and the TSA Plus Fluorescent system (Perkin-Elmer NEL703001KT, NEL741001KT, NEL744001KT, NEL745001KT Waltham, MA). Coumarin (Maxima Excitation 402 nm; Emission 443 nm) was assigned to insulin; Fluorescein (Maxima Excitation 494 nm; Emission 517 nm) was assigned to somatostatin; gene of interest (STAT5, WSF1, GATA4, GLIS3 and EIF2K3) to CY3 (Maxima Excitation 550 nm; Emission 570 nm); and glucagon to CY5 (Maxima Excitation 648 nm; Emission 667 nm). For each panel, negative and positive control slides were also stained to determine the exposure time and image processing necessary to provide optimal visualization of antibody signal. All images were captured and processed using an IX73 Olympus florescence microscope using cellSense software.

RNAscope coupled with immunofluorescence Fluorescent-labeled RNA probes (STAT5B, Cat. Nb. PN 501151; GLIS3, Cat. Nb. PN 525701-C3; GATA4, Cat. Nb. PN 579821; WFS1, Cat. Nb. PN 436081; EI2AK3, custom reagent (targeting 330-1487 of NM_001313915.1); PPP1R15A, Cat. Nb. PN 311141; PSEN1, Cat. Nb. PN 502001; COL6A2, Cat. Nb. PN 482611; ERN1/IRE1, Cat. Nb. PN 497331-C2; Hs-PPIB (positive control) Cat. Nb. 313901, DapB (negative control) Cat. Nb. 310043) were purchased from ACD (Advanced Cell Diagnostics, Inc.; Newark, CA). RNA staining was performed with an RNAscope® Multiplex Fluorescent Reagent Kit v2 (PN 323100) according to the manufacturer's instructions: Slides were dewaxed 2x 5min in Xylene and 2x 2min in 100% EtOH then air dried. Antigen retrieval was performed for 15min using RNAscope® 1X Target Retrieval Reagents heated up to 98°C. Slides were dipped in ddH_2O then 100% EtOH for 2min and air dried. RNAscope® Protease III was applied to the dry slides and incubated for 30min

at 40°C in the oven then rinsed with RNAscope® 1X Wash Buffer. After incubation with RNA probes for 30min at 40°C, probes were amplified using RNAscope® Multiplex FL v2 AMP 1 for 30min, then RNAscope® Multiplex FL v2 AMP 2 for 30min, RNAscope® Multiplex FL v2 AMP 3 for 15min and RNAscope® Multiplex FL v2 HRP-C1 for 15min. All amplification steps were performed at 40°C and slides washed between steps in RNAscope® 1X Wash Buffer 2x 2min. Signal was developed using TSA® Plus Cyanine 3 (NEL744001KT) 1:1500 for 30min at 40°C and blocked with HRP Blocker for 15min at 40°C. Slides were washed in TBS and incubated overnight at 4°C with primary antibodies (mouse anti-CD99, Biolegend Cat. Nb. 318002, clone HCD99, RRID:AB_604112, 1:200 and rabbit anti-Cytokeratin 19, Cell Signaling Technologies Cat. Nb. 13092, clone D7F7W, 1:100). The next day, slides were washed in TBS and incubated for 2h at room temperature with secondary antibodies (goat anti-rabbit Alexa Fluor 488, Thermo Fisher Cat. Nb. A-11008, and goat anti-mouse Alexa Fluor 647, Thermo Fisher Cat. Nb. A-21235). Images were acquired on a Leica DMI 6000 widefield microscope.

Confocal microscopy Samples were imaged on a Nikon A1plus confocal microscope using a 20X (NA0.75) air objective (Nikon Instruments, MellvilleNY). Excitation and emission for each target were as follows: insulin Ex441/Em450; somatostatin Ex488/Em525; WFS1/STAT5B Ex561/Em595; glucagon Ex647/Em700. 3D Z-stacks were constructed with a 2.5 μm step size and compressed to a 2D maximum intensity projection for display. All images for a given gene and condition (i.e. WFS1 healthy control) are displayed with identical lookup table values. Imaging parameters (laser power, pixel dwell, pinhole size, gain, offset) remained constant for all samples. All raw images of single islets were denoised in Nikon Elements (Nikon Instruments, Mellville, NY) for display.

Statistical analysis For each patient population, a two-tailed Wilcoxon rank-sum test comparing unaffected versus disease patients was performed for each gene. The Storey method was used to control the false discovery rate (FDR); the adjusted p-values (q-values) were estimated using the qvalue R package (http://github.com/jdstorey/qvalue) with lambda=0. Q-values $\leq$ 0.024 were considered to be statistically significant. R (v3.6.1) was used for all calculations. GraphPad Prism v8.0 (La Jolla, CA) was used for all graphical presentations.

Results

Of relevance to the quality of human donor pancreatic RNA for gene expression analysis (29-32), normalized expression levels for ASNS, GCG, GLP1R, NRP1, and PFKM were not significantly different across donors grouped when correlated with cause of death (head trauma, anoxia or cerebrovascular/stroke), ICU or organ transport times. A summary of metadata appears in Table S1 where subsets of unaffected controls and T1D are analyzed in experiments reported below.

Some Monogenic diabetes genes in type 1 diabetes, autoantibody positive and type 2 diabetes donor pancreata are differentially expressed.

Overall 24 of the 45 monogenic diabetes genes showed an increased expression in T1D pancreas (Table 1). We then examined these 24 monogenic diabetes genes differentially expressed in the type 1 diabetes pancreas in organs from a population of autoantibody+ donors (n=20-24) considered at risk (33) for type 1 diabetes, as well as in type 2 diabetes pancreata (n=20). Of note, the vast majority of autoantibody+ donors were seropositive for only a single autoantibody (constituting Pre-Stage 1 type 1 diabetes), with five seropositive for two autoantibodies (considered potentially Stage 1-2 type 1 diabetes (34), dysglycemia unknown). We identified five genes (*BSCL2*, *DUT-N*, *EIF2AK3*, *ITCH* and *MNX1*; Fold Difference (FD) range = 1.58-3.41) displaying increased expression in autoantibody+ pancreata compared to unaffected controls and a single gene, *HNF4A*, which was highly repressed in autoantibody+ pancreata (FD = 0.08 vs controls) but only marginally induced in the type 1 diabetes group (FD = 1.78). In contrast, five monogenic diabetes genes (*DUT-N*, *EIF2AK3*, *GLIS3*, *ITCH* and *NR0B2*) were significantly induced in type 2 diabetes pancreata compared to unaffected donor organs (Table 1).

The remaining 21 of the 45 monogenic diabetes genes were either not expressed differentially or not amplified in control or T1D pancreata as shown in Table S5, whereas four genes (ABCC8, GCK, NKX2.2, and RFX6) demonstrated a trend towards repression but did not achieve statistical significance. In addition, we have previously shown that INS, IAPP and the INS-IGF2 read through mRNA levels are dramatically inhibited in type 1 diabetes pancreata (35). To address whether altered expression of these monogenic diabetes genes is indeed pancreas specific, we examined the expression of 19 genes altered in type 1 diabetes pancreata by RTqPCR using total RNA isolated from 10 control and 10 type 1 diabetes human spleens, and found no genes differentially expressed in this organ (Table S6).

Classification of monogenic diabetes genes into physiological groups reveals broad differential expression in type 1 diabetes pancreata.

The monogenic diabetes genes were separated into four physiological classifications: 1) immune, 2) β-cell function, 3) β-cell development and 4) ER function/stress (Table S7), with knowledge that certain genes associate with multiple categories (Table S7).

Of the seven monogenic diabetes genes examined from the immune physiological group (Table S7), normalized RTqPCR revealed significant differences in *LRBA, SIRT1, STAT1, STAT3* and *STAT5B* expression in type 1 diabetes versus unaffected pancreata, with FDs ranging from 2.75 to 3.89 (Table 1). For STAT5B a significant 3.59 FD was noted (Fig 1a). Widefield IF for STAT5B, insulin (INS), glucagon (GCG) and somatostatin (SST) with IF for islet (CD99) and exocrine cell markers (KRT19) and ISH for STAT5 (Fig. 1b), along with STAT5B confocal ISH-IF (Fig. 1c) in unaffected and type 1 diabetes human pancreata, reveals cytosolic expression of STAT5B most appreciably within the islets. These studies suggest that STAT5B co-localizes with

glucagon and insulin in unaffected α- and β-cells, respectively (Fig. 1b and c). To demonstrate the uniformity of these results, we have included images of additional type 1 diabetes and control donor pancreata (Figs. S1 and S2). Most strikingly, we observed a number of islet cells that display only STAT5B expression with limited to no expression of either INS, GCG or SST in both unaffected and type 1 diabetes islets (Figs. 1c, S1 and S2).

We similarly observed significantly altered expression for numerous monogenic diabetes genes in the β-cell function group (Table 1), including *DUT-N, DUT-M, GATA4, PLAGL1* and *NR0B2*, with the FDs ranging from 1.88-4.35 for type 1 diabetes compared to unaffected control pancreas. GATA4 expression FD 4.35 (Fig 2a) was also examined by IF and ISH-IF revealing primarily nuclear expression in both islets and the exocrine pancreas (Figs. 2b, S3 and S4).

RTqPCR analysis of genes belonging to the β-cell development group (Table S7) identified altered expression in the type 1 diabetes pancreas for *GATA6, GLIS3, HNF1B, MNX1* and *TRMT10A* (Table 1), with FDs ranging from 2.25-4.01 relative to control donor pancreata. Examination of GLIS3 FD 4.01 (Fig 3a) by IF and ISH-IF demonstrated expression in both islets and the exocrine pancreas in unaffected controls (Fig. 3b). Close examination, however, reveals an obvious decrease in islet specific expression of GLIS3 in the type 1 diabetes pancreas with no obvious change in the exocrine region (Figs. 3b, S5 and S6, yellow outlines). In addition, the combined ISH-IF (Fig. 3b, right) is consistent with decreased *GLIS3* mRNA in the type 1 diabetes islet and demonstrates that exocrine expression is most likely specific to ductal epithelial cells based on *GLIS3* mRNA colocalization (ISH) with IF for KRT19, a type 1 keratin specific to ductal cells (36).

The final set of monogenic diabetes genes examined are grouped based on their association with endoplasmic reticulum-ER function/stress (Table S7), with altered expression in type 1

diabetes pancreata observed for *BSCL2, CEL, ITCH* and *WFS1* (FD range from 2.2-4.41, Table 1). WFS1 (Wolframin, regulator of ER calcium homeostasis) expression FD of 4.41 (Fig 4a) was also evaluated by IF to evaluate WFS1 (37) protein localization in the human pancreas (Figs. 4b, S7 and S8). Consistent with the IF data, ISH-IF identified *WFS1* mRNA in the islet along with scattered expression in exocrine regions of the pancreas from type 1 diabetes and control donors (Fig. 4b, right). These data noted islet specific cytosolic expression with apparent colocalization involving both INS and GCG in unaffected control pancreas. Most strikingly, we consistently observed non-hormone expressing WFS1+ cells in the vast majority of islets from both control and type 1 diabetes pancreas. To confirm this observation, we utilized confocal microscopy using sections of unaffected control and type 1 diabetes pancreata co-stained for WFS1 (Fig. 4c) in conjunction with INS, GCG and SST. The combined z stacks for the overlay and each separate channel illustrate individual cells (insets and yellow arrows) that only express WFS1, having no co-registration with endocrine hormones. The overlay panel (Fig. 4b) for the type 1 diabetes pancreas 6180 also revealed a potential islet exclusively expressing cells positive only for WFS1 (green only islet, single INS positive cell in the lower middle). The existence of islets/cells exclusively positive for WFS1 is reproduced in other islets from control and type 1 diabetes patient pancreata (Figs. S7 and S8). To be clear, WFS1 only positive cells are also evident in islets that display hormone positivity as well (Figs. 4b, S7, and S8).

Deeper analysis of the ER function and stress pathway reveals that the apex of the integrated stress response (ISR) is activated in type 1 diabetes pancreata

EIF2AK3/PERK gene expression revealed a significantly increased FD of 8.3 (Fig 5a). Using IF and ISH-IF in unaffected control and type 1 diabetes pancreata (Figs. 5b, S9 and S10), we noted EIF2AK3/PERK localization in both islets and the exocrine pancreas. The localization

in control islets is consistently higher when compared with the exocrine tissue (Figs. 5b, S9), whereas EIF2AK3/PERK localization in the type 1 diabetes pancreas was more uniform in intensity in both the endocrine and exocrine tissue (Figs. 5b, S10). We then examined 30 ISR-associated genes, identifying 20 that were significantly induced in type 1 diabetes versus control pancreas (Table 2), with FDs ranging from 2.4-18.11. The ISR contains three arms where ATF6 (ARM1), IRE1α (ERN1; ARM2) and each of the eIF2α kinases: PERK, HRI, PKR and GCN2 (ARM3) constitute the apex of each arm. RTqPCR data for genes at the apex of ARMs 1-3 of the ISR (Fig. 5c) demonstrate that all three ARMs are activated, including three of the four eIF2αkinases [EIF2AK3/PERK (Fig 5a), EIF2AK2/PKR and EIF2AK4/GCN2 (Fig 5c)]. These data therefore strongly implicate that the ISR is chronically activated in T1D pancreata, with disease duration for our T1D donor samples spanning 7 months to 57 years (Table S1)

To evaluate a potential role for the ISR in pre-type 1 diabetes, we tested the expression levels of all 20 ISR genes showing altered expression in type 1 diabetes pancreata within our autoantibody+ cohort (Table 2). We identified eight ISR-linked genes that showed significantly altered expression in pancreata from autoantibody+ donors with four of these being induced (*DNAJB11*, *EIF2AK3*, *FEN1*, and *NELFA*, Table 2). The remaining four dysregulated ISR genes showed a significant level of repression in autoantibody+ donor tissues associated with a remarkable inhibition at the apex of all three arms of the ISR (Table 2, Fig 5d).

Discussion

As a complement to traditional genetic linkage strategies, we utilized a transcriptomic analysis to test a novel hypothesis that genes classically associated with monogenic forms of diabetes (3-6) would be differentially expressed in human pancreas from individuals with type 1 diabetes or those

at increased risk for the disease. Studies were conducted on tissues from donors that do not carry monogenic diabetes associated mutations, with features consistent with T1D or T2D [HLA, Age of onset, BMI, HbA1c and c-peptide (Table S1 and Fig S11)]. In support of this strategy, our data demonstrate that 24 of the 45 genes associated with monogenic diabetes displayed altered expression in pancreata from type 1 diabetes versus control donors, and six of these genes were also dysregulated in autoantibody+ organ donors, considered to have high risk (single autoantibody+) or pre-type 1 diabetes (≥2 autoantibody+). The uniformity of the repressed *HNF4A* expression across the entire autoantibody+ cohort further advances the notion that even a single autoantibody may have relevant prognostic value.

The type 2 diabetes pancreas also demonstrated altered expression of five monogenic diabetes genes relative to unaffected control tissue. Not surprisingly, these genes fell primarily within the ER function/stress physiological group while expression of monogenic diabetes genes associated with all four physiological groups (i.e., immune, β-cell function, β-cell development, and ER function/stress) were altered in type 1 diabetes. Hence, genes exhibiting altered expression likely point toward mechanisms underlying type 1 diabetes and type 2 diabetes pathogenesis, respectively.

Localization (IF) studies on select dysregulated monogenic diabetes and ISR genes from each physiological study group pinpointed genes that are expressed to a greater extent in the islets (e.g., *STAT5B* and *WFS1*) while others were expressed in both islet and exocrine regions of the pancreas (e.g., *GATA4*, *GLIS3* and *PERK*). Hence, these data highlight a potential role for ISR activation within the exocrine pancreas in type 1 diabetes pathogenesis, in line with our previous studies demonstrating reduced pancreas mass (38; 39) and volume (40) as well as low serum levels of trypsinogen in recent-onset type 1 diabetes patients and pre-type 1 diabetes subjects (41). We

also identified non-hormone expressing cells in human islets that were positive for either the transcription factor STAT5B or an ER calcium channel/regulator, WFS1. Although both of these proteins were sometimes co-registered with GCG, INS or SST, identifying α-, β- and δ-cells, respectively, in control and type 1 diabetes pancreata, there are cells scattered across the pancreas that express these proteins and are clearly hormone negative. We might speculate that these results represent a possible stem cell population, a state of dedifferentiation, cells exhausted of hormone at the time of death or a previously undetected islet cell type. We are currently attempting to identify unique surface markers that could facilitate cell sorting and single cell RNA-sequencing analysis to further characterize these cells

We believe the power of our monogenetic strategy has been borne out in identifying a plethora of noteworthy genes coupled with their unique pancreatic localization, as well as the potential functional implications of their dysregulated expression. In addition to monogenic diabetes genes, we extended our studies to ISR-linked genes with 20 out of 30 ISR genes studied displaying altered expression in type 1 diabetes pancreata. These results included three of four eIF2α-dependent kinases (42), thus attendant with ISR (43) activation in type 1 diabetes pathogenesis strongly implicating an overarching, chronic stress response (44; 45). Interestingly, we uncovered numerous additional loci with altered expression that are central to the regulation and function of each arm of the ISR pathway in type 1 diabetes pancreata, suggesting a global activation of the ISR in the type 1 diabetes pancreas, not simply extrapolated from the study of a single gene. The ISR can be induced by intrinsic stress associated, for example, with the accumulation of unfolded proteins in the ER mediated by PERK, or through extrinsic stressors including oxidative challenge (HRI), viral infection (PKR) or amino acid deprivation (GCN2) (42). While such stress responses may lead to activation of the UPR and/or ISR in type 2 diabetes (46-

50), a similar argument for the ISR in type 1 diabetes (47; 51-54) has been hypothesized, but not directly demonstrated. Indeed, prior reports have implicated ER stress and the UPR as potentially contributing toward inflammation and β-cell death in type 1 diabetes, but to our knowledge, this represents a first report demonstrating comprehensive activation of all three arms of the ISR in human type 1 diabetes pancreas tissue.

Of note, our studies are restricted due to the inherent limitations of our organ donor study group such that more mechanistic studies are not easily implemented in archival tissue. To this end, future studies in human islets or live human pancreas organ slices could more directly address pathway-specific and physiologically relevant mechanisms. From a diagnostic standpoint, our data are all derived from organ donor pancreata which precludes any diagnostic assay in live patients, however we are beginning studies to address some of these markers in peripheral blood. We thus believe our data afford the diabetes community a rich opportunity to investigate potential therapeutics targeting numerous metabolic and pancreas intrinsic signaling pathways. Our observations in the type 1 diabetes pancreas taken in the context of a lifelong disease may be best described by the title of a review by Rutkowski and Kaufman entitled, “That which does not kill me makes me stronger: adapting to chronic ER Stress” (45).

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Data Availability All the data presented in this manuscript will be made available upon request

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Duality of interest The authors declare that no conflicts of interest exist pertaining to the work reported herein.

Contribution statement CHW researched the data and wrote the manuscript; DEB, HH, JJL and SE researched the data and reviewed/edited the manuscript; JRM contributed to the study design, analyzed the data and reviewed/edited the manuscript; DRM, IK, and LMJ researched the data and reviewed/edited the manuscript; ALP, HK, RO, DAS, AH, and BB contributed to discussion and reviewed/edited the manuscript; HSN and MAA conceived of the study and wrote the manuscript.

Guarantor Statement. As guarantor of this work, Clive Wasserfall had full access to all of the data and takes full responsibility for the integrity of the data and accuracy of its analysis.

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FIGURE LEGENDS

Figure 1. Monogenic diabetes genes were sorted into 4 physiological groups: **Immune, β-cell Function, β-cell Development and ER Function/Stress** (Table S7). **a.**) Scatter plot of gene expression data for *STAT5B,* a gene representing an **Immune** monogenic diabetes gene, studied using RTqPCR in human organ donor pancreata. Cq values from the Unaffected (control) and type 1 diabetes cohorts were independently normalized using the geometric mean of three pancreas-specific reference genes and the fold difference (FD) is calculated based on the ratio of the means (Methods). See Methods for statistical analysis [*p*-values and *q*-values (estimation of false discovery rates)]. N/D refers to N # of samples yielding no data, out of the total (D). **b.**) Wide Field immunofluorescence (IF) of STAT5B (**green**) and overlay with insulin (**INS**), glucagon (**GCG**) and somatostatin (**SST**) from a control and type 1 diabetes pancreas. Final panel in each row shows combined STAT5B *in situ* hybridization (ISH, RNAscope (green dots)) coupled with IF for CD99 and KRT19. Magnification bars are 40μm. **c.**) Confocal imaging on a Nikon A1plus confocal microscope of STAT5B (**green**), insulin (**INS**), glucagon (**GCG**), and somatostatin (**SST**), showing an overlay of an islet from an unaffected and type 1 diabetes pancreas (left panels). 3D Z-stacks were constructed with a 2.5 μm step size and compressed to a 2D maximum intensity projection for display. Individual channels in black and white identify cells positive only for STAT5B and negative for the other endocrine hormones, illustrated with yellow arrows (overlay) and with insets in the respective black and white channels.

Figure 2. **a.**) Scatter plot of RTqPCR data depicting expression levels for *GATA4* a monogenic diabetes gene in the category ***β-cell Function*** (Table S7). The fold difference (FD) is calculated based on the ratio of the means (Methods). See Methods for statistical analysis [*p*-values and *q*-

values (estimation of false discovery rates)]. N/D refers to N # of samples yielding no data, out of the total (D). **b.**) Wide Field immunofluorescence (IF) of GATA4 (**green**) and overlay with insulin (**INS**), glucagon (**GCG**) and somatostatin (**SST**) from a control and type 1 diabetes pancreas. Final panel in each row shows combined GATA4 *in situ* hybridization (ISH, RNAscope (green dots)) coupled with IF for CD99 and KRT19. Magnification bars are 40µm.

Figure 3. Scatter plot of RTqPCR data depicting expression levels for *GLIS3* a monogenic diabetes gene in the category ***β-cell Development*** (Table S7). The fold difference (FD) is calculated based on the ratio of the means (Methods). See Methods for statistical analysis [*p*-values and *q*-values (estimation of false discovery rates)]. N/D refers to N # of samples yielding no data, out of the total (D). **b.**) Wide Field immunofluorescence (IF) of GLIS3 (**green**) and overlay with insulin (**INS**), glucagon (**GCG**) and somatostatin (**SST**) from a control and type 1 diabetes pancreas. Yellow outlines depict the islets to illustrate the significant reduction of GLIS3 in the T1D islet. Final panel in each row shows combined GLIS3 *in situ* hybridization (ISH, RNAscope (green dots)) coupled with IF for CD99 and KRT19. Magnification bars are 40µm.

Figure 4. **a.**) Scatter plot of RTqPCR data depicting expression levels for *WFS1* a monogenic diabetes gene in the category ***ER Function/Stress*** (Table S7). The fold difference (FD) is calculated based on the ratio of the means (Methods). See Methods for statistical analysis [*p*-values and *q*-values (estimation of false discovery rates)]. N/D refers to N # of samples yielding no data, out of the total (D). **b.**) Wide Field immunofluorescence (IF) of WFS1 (**green**) and overlay with insulin (**INS**), glucagon (**GCG**) and somatostatin (**SST**) from a control and type 1 diabetes pancreas. Final panel in each row shows combined GATA4 *in situ* hybridization (ISH, RNAscope

(green dots)) coupled with IF for CD99 and KRT19. Magnification bars are 40μm. **c.**) Confocal imaging on a Nikon A1plus confocal microscope of WFS1 (**green**), insulin (**INS**), glucagon (**GCG**), and somatostatin (**SST**), showing an overlay of an islet from an unaffected and type 1 diabetes pancreas (left panels). 3D Z-stacks were constructed with a 2.5 μm step size and compressed to a 2D maximum intensity projection for display. Individual channels in black and white identify cells positive only for WFS1 and negative for the other endocrine hormones, illustrated with yellow arrows (overlay) and with insets in the respective black and white channels.

Figure 5. **a.**) Scatter plot of RTqPCR data depicting expression levels for EIF2AK3/PERK a representative gene in Arm3 of the Integrated Stress Response (ISR) (see Graphical Abstract). The fold difference (FD) is calculated based on the ratio of the means (Methods). See Methods for statistical analysis [*p*-values and *q*-values (estimation of false discovery rates)]. N/D refers to N # of samples yielding no data, out of the total (D). **b.**) Wide Field immunofluorescence (IF) of PERK (**green**) and overlay with insulin (**INS**), glucagon (**GCG**) and somatostatin (**SST**) from a control and type 1 diabetes pancreas. Final panel in each row shows combined PERK *in situ* hybridization (ISH, RNAscope (green dots)) coupled with IF for CD99 and KRT19. Magnification bars are 40μm. **c.**) Scatter plots of RTqPCR data depicting expression levels for ISR genes from the apex of each ISR ARM 1-3 in type 1 diabetes versus unaffected control pancreata: ATF6, ERN1/IRE1α, EIF2AK2/PKR, EIF2AK3/PERK, EIF2AK4/GCN2. The fold difference (FD) is calculated based on the ratio of the means. See Methods for statistical analysis [*p*-values and *q*-values (estimation of false discovery rates)]. N/D refers to N # of samples yielding no data, out of the total (D). **d.**) Scatter plot of RTqPCR data depicting expression levels for genes significantly repressed in AAB+

(autoantibody positive) pancreata in each of the three Arms of the ISR: MBTPS1/S1P, MBTPS2/S2P, ERN1/IRE1α, EIF2AK2/PKR.

TABLE LEGENDS

Table 1. Tabulated fold differences (FD), p-values and q-values (estimation of false discovery rates) for the monogenic diabetes genes showing altered expression in type 1 diabetes (left), AAB+ (autoantibody positive) (middle) and type 2 diabetes (right) pancreata. Cq values from the Unaffected (control) and type 1 diabetes cohorts were independently normalized using the geometric mean of three pancreas-specific reference genes and the FD calculated based on the ratio of the means (Methods). See Methods for statistical analysis (*p*-values and *q*-values). *n* refers to the number of independent pancreata in each cohort and the ***bold italics*** denote those genes showing altered expression (shaded rows) relative to control donors: Type 1 diabetes: 24 altered genes out of a total of 45 monogenic diabetes genes; AAB+: 6 altered genes; and type 2 diabetes: 5 altered genes. * denotes genes where either the p-value (<0.05) and/or q values (≤ 0.024) approached significance.

Table 2. Tabulated fold differences (FD), p-values and q-values for the ISR genes showing altered expression in type 1 diabetes (left), and autoantibody+ (right) pancreata. *n* refers to the number of independent pancreata in each cohort and the ***bold italics*** denote those genes showing altered expression. 20 out of 30 ISR genes tested showed differential expression in type 1 diabetes pancreata. Eight of the 20 genes altered in type 1 diabetes showed differential expression in the autoantibody+ cohort (4 genes were induced and 4 repressed).

Figure 1

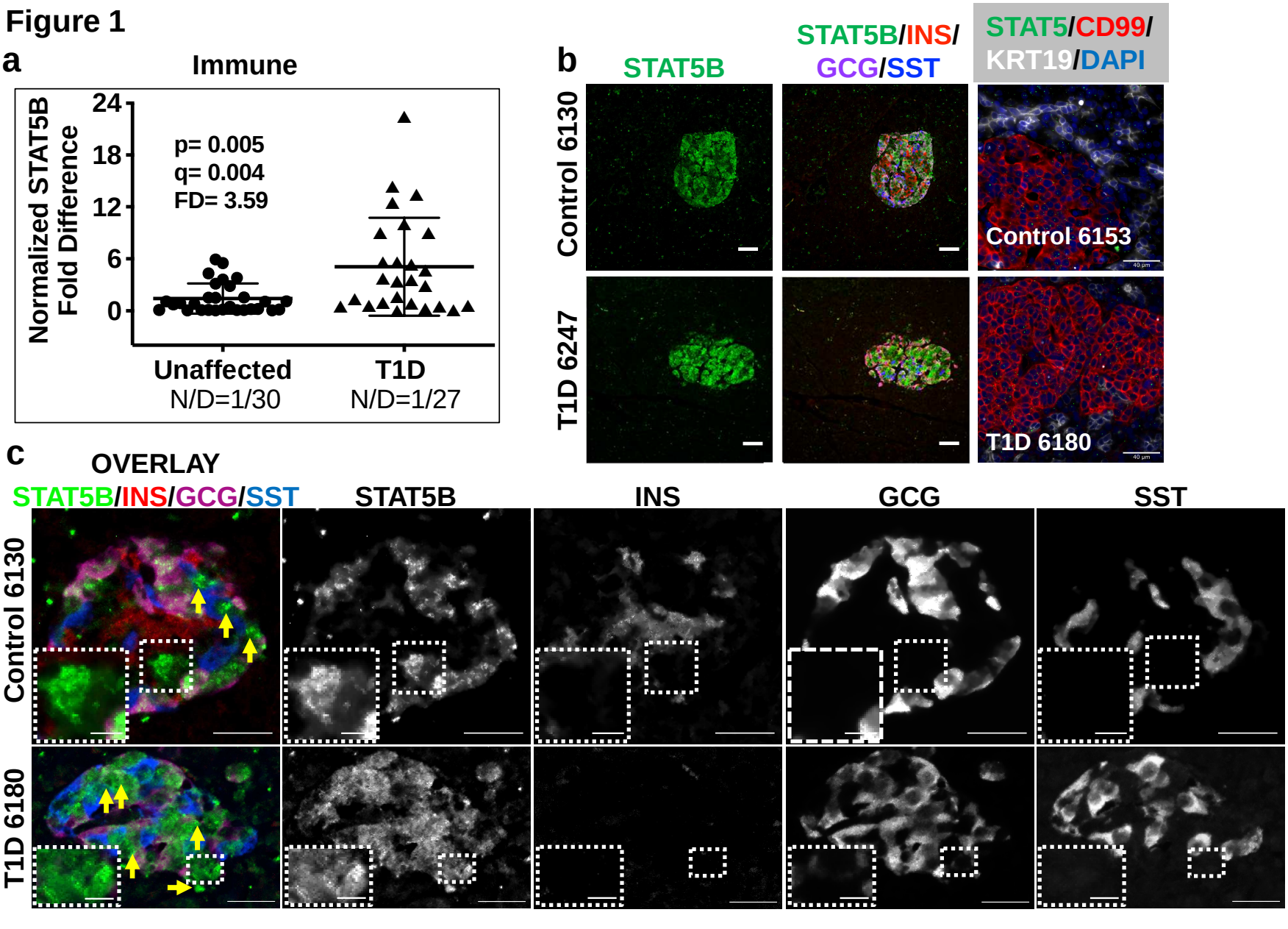
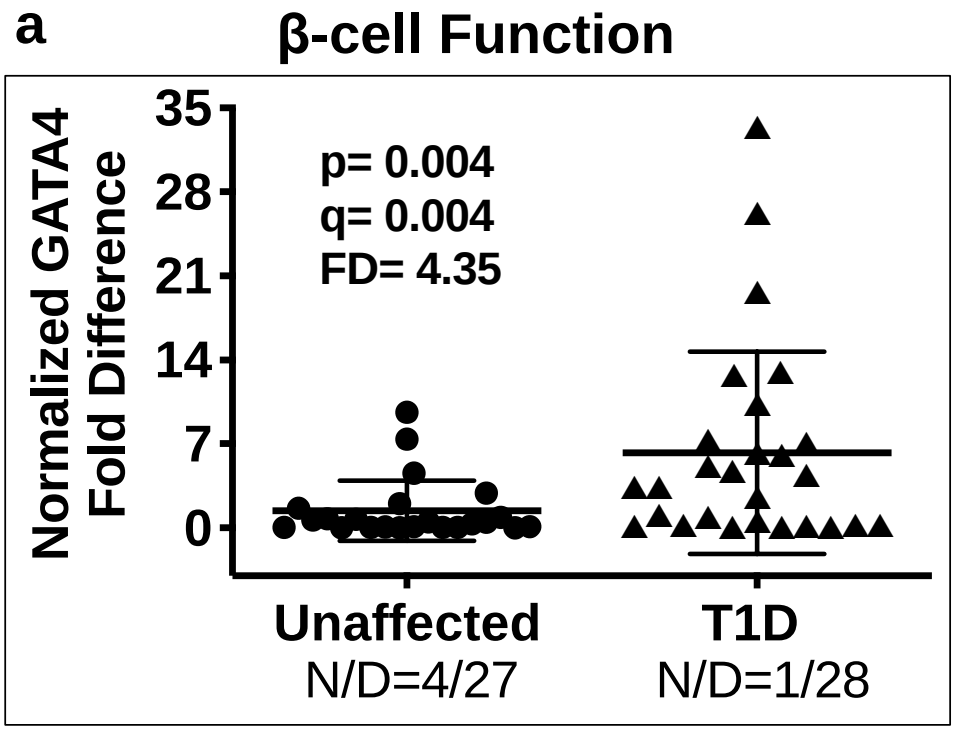

a
Immune
Normalized STAT5B Fold Difference
24
18
12
6
0
p= 0.005
q= 0.004
FD= 3.59
Unaffected
N/D=1/30
T1D
N/D=1/27
b
STAT5B
STAT5B/INS/
GCG/SST
STAT5/CD99/
KRT19/DAPI
Control 6130
T1D 6247
Control 6153
T1D 6180
c
OVERLAY
STAT5B/INS/GCG/SST
STAT5B
INS
GCG
SST
Control 6130
T1D 6180

Figure 2


a
β-cell Function
Normalized GATA4
Fold Difference
35
28
21
14
7
0
p= 0.004
q= 0.004
FD= 4.35
Unaffected
N/D=4/27
T1D
N/D=1/28

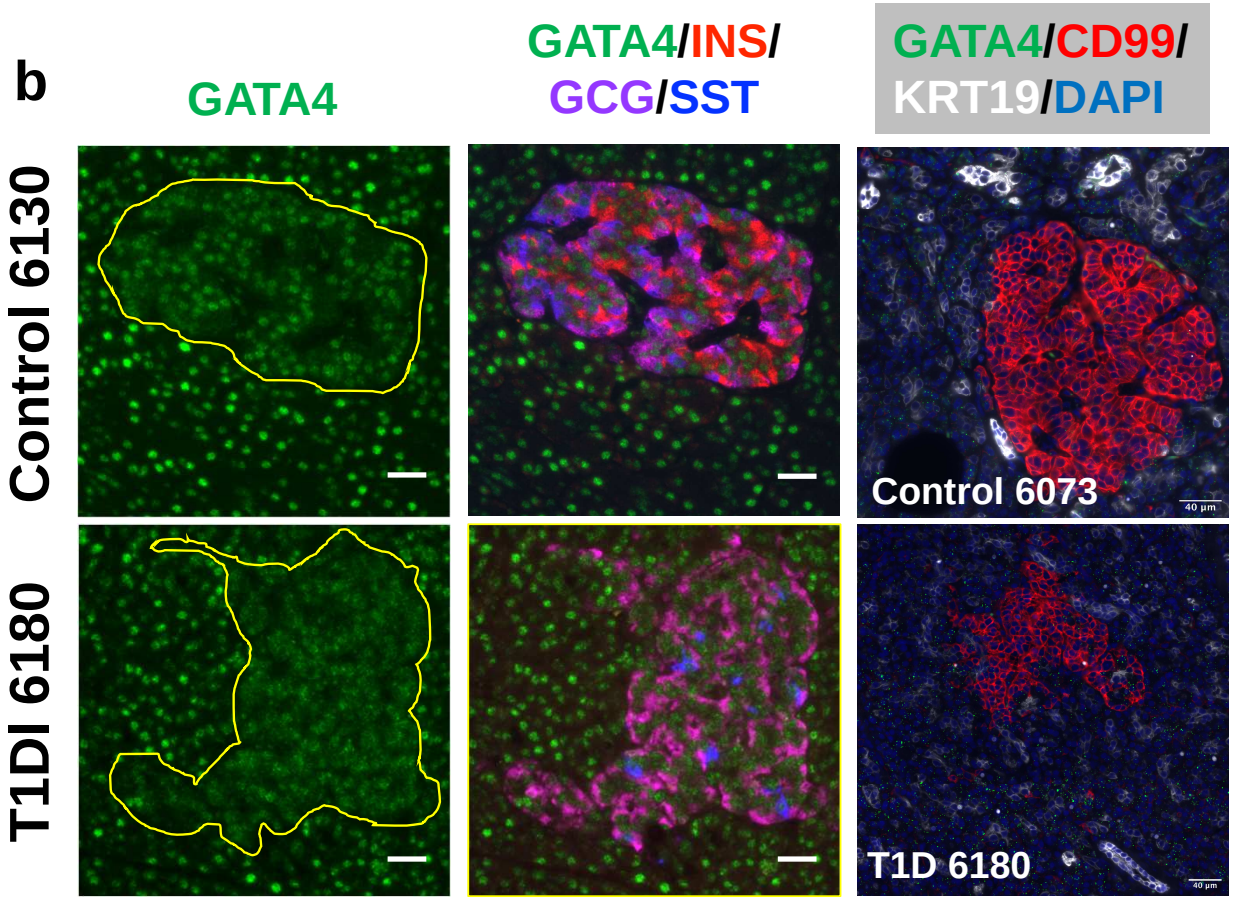

b
GATA4
GATA4/INS/
GCG/SST
GATA4/CD99/
KRT19/DAPI
Control 6130
T1DI 6180
Control 6073
T1D 6180

Figure 3

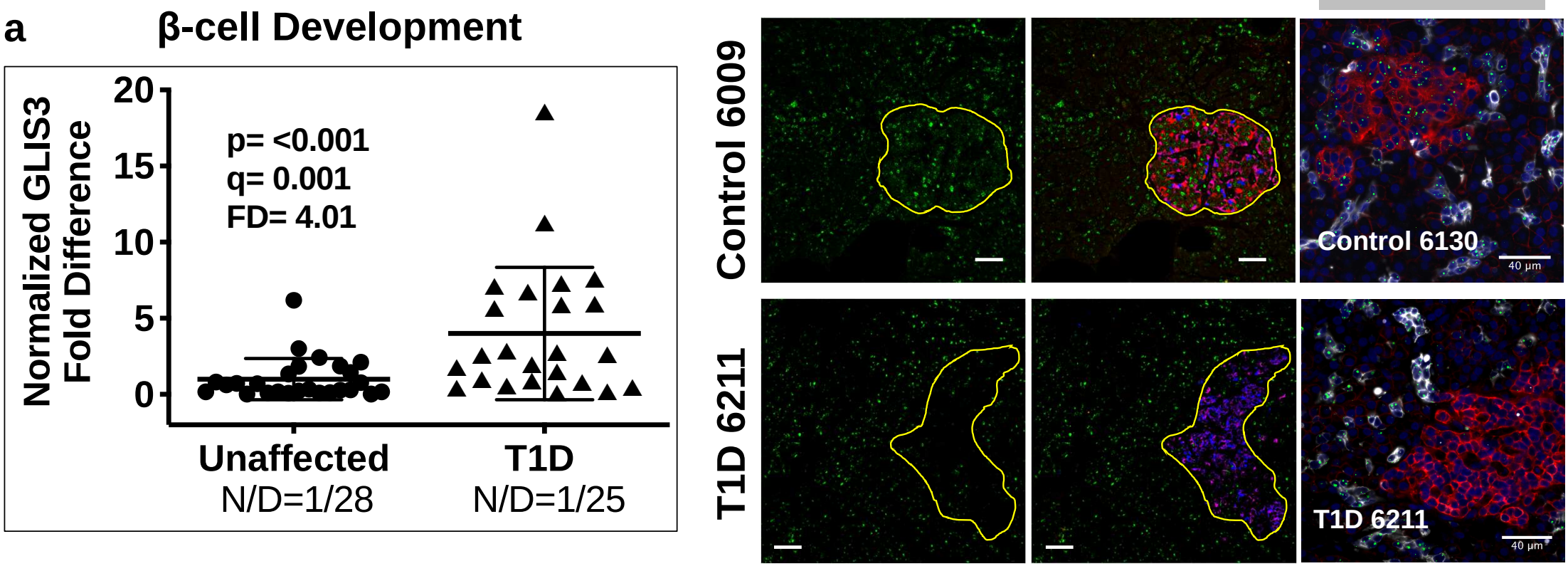

a
β-cell Development
Normalized GLIS3
Fold Difference
20
15
10
5
0
p= <0.001
q= 0.001
FD= 4.01
Unaffected
N/D=1/28
T1D
N/D=1/25
b
GLIS3
GLIS3/INS/
GCG/SST
GLIS3/CD99/
KRT19/DAPI
Control 6009
T1D 6211
Control 6130
T1D 6211
40 µm
40 µm

Figure 4

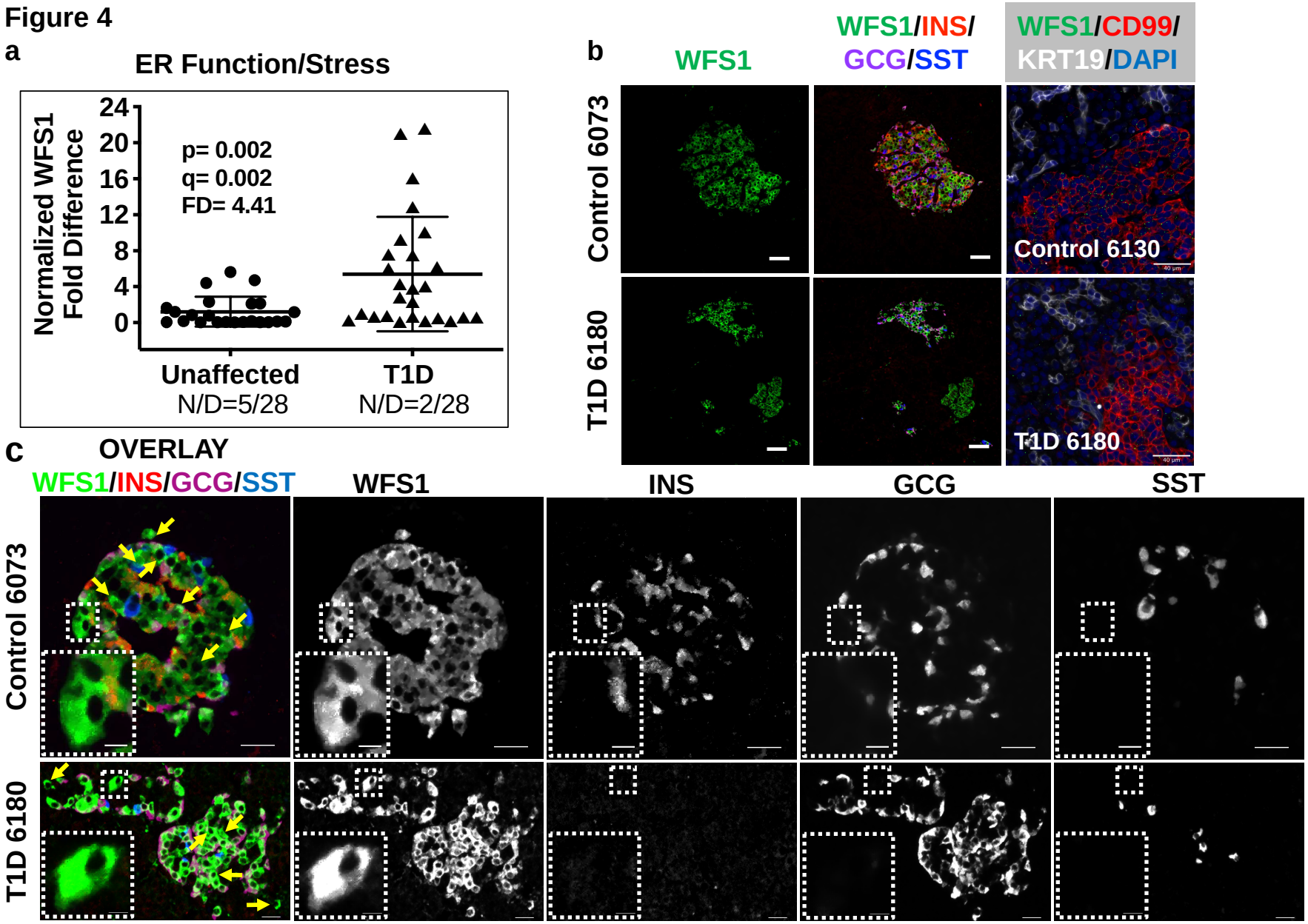
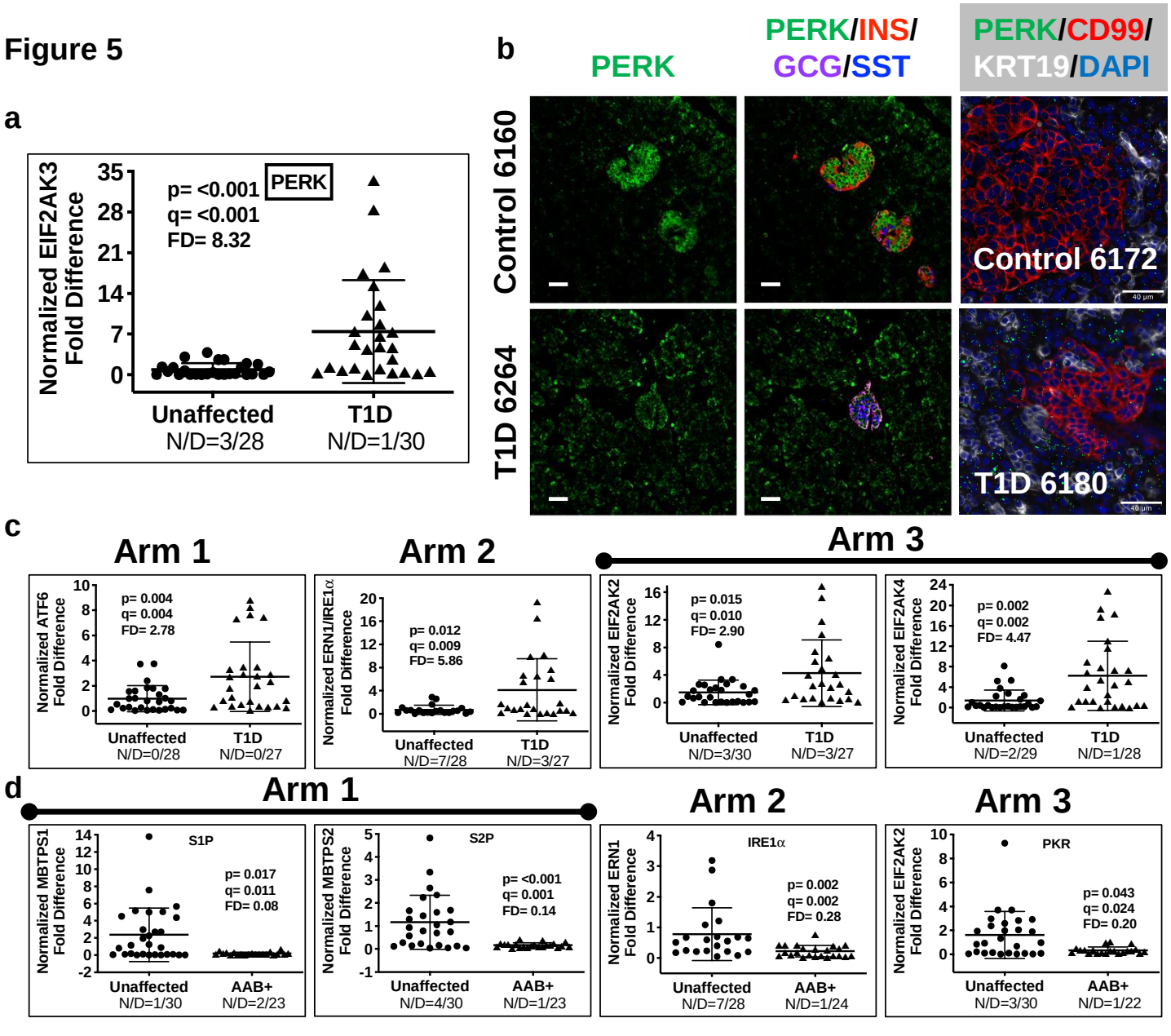
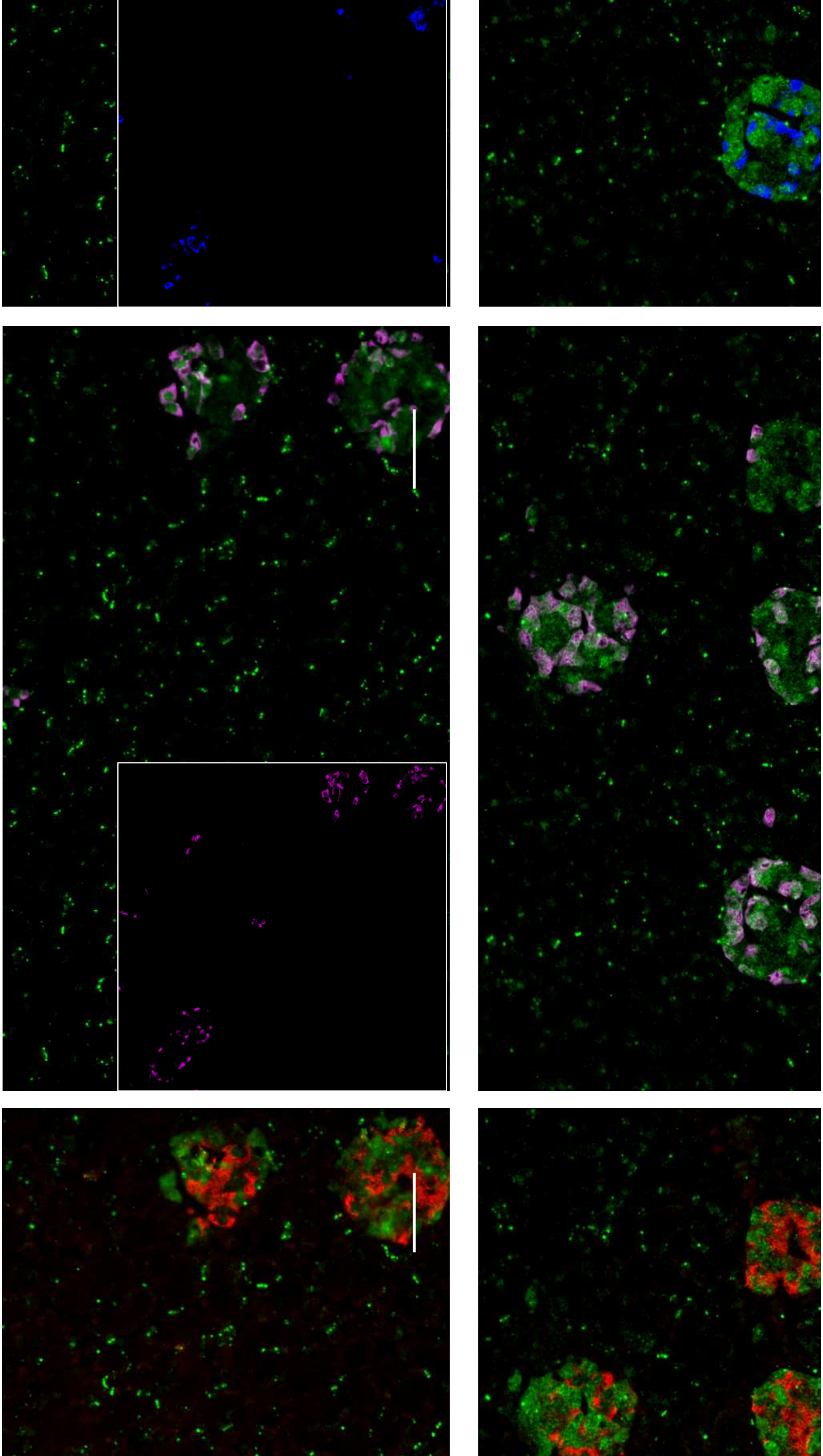
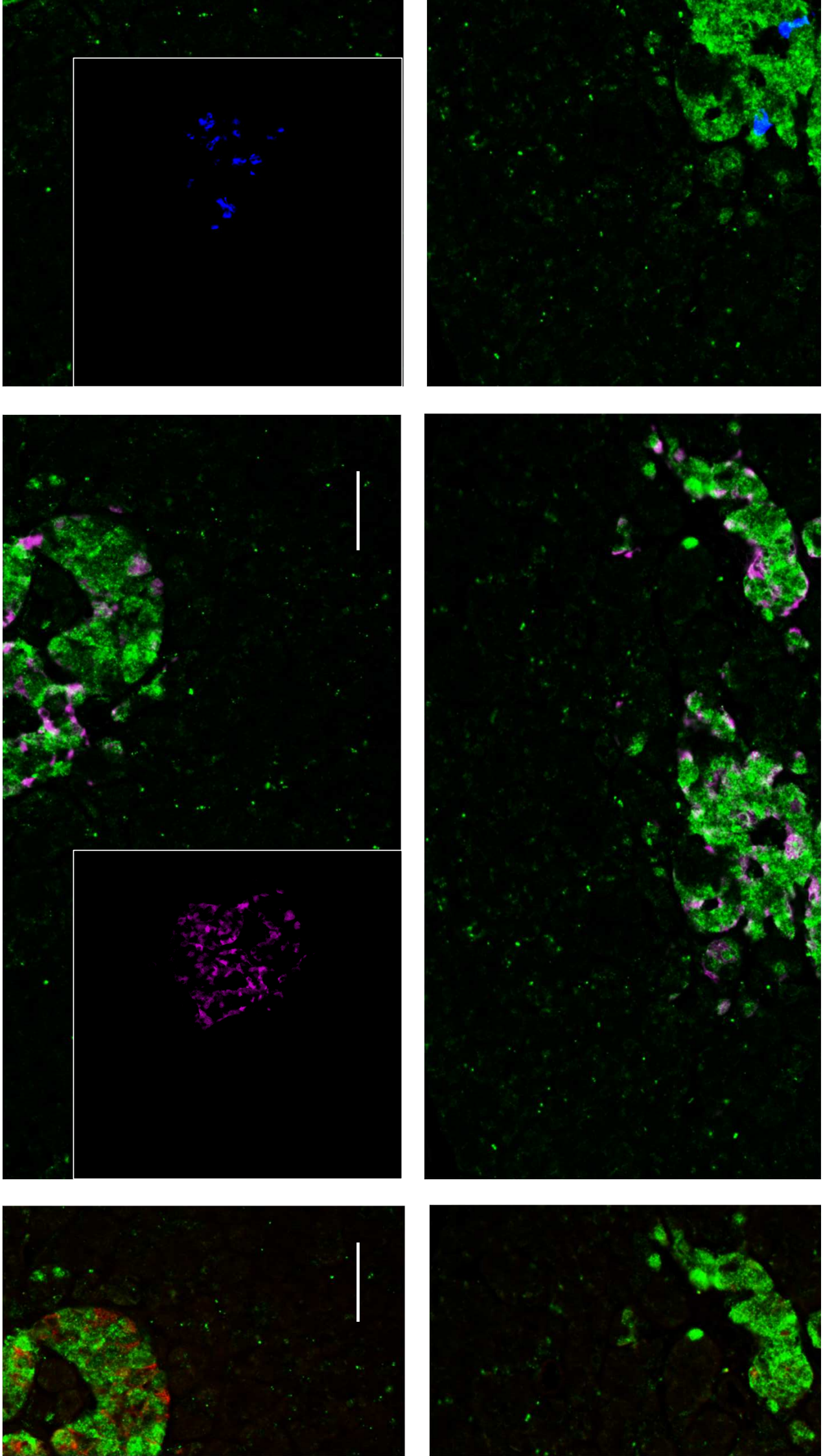
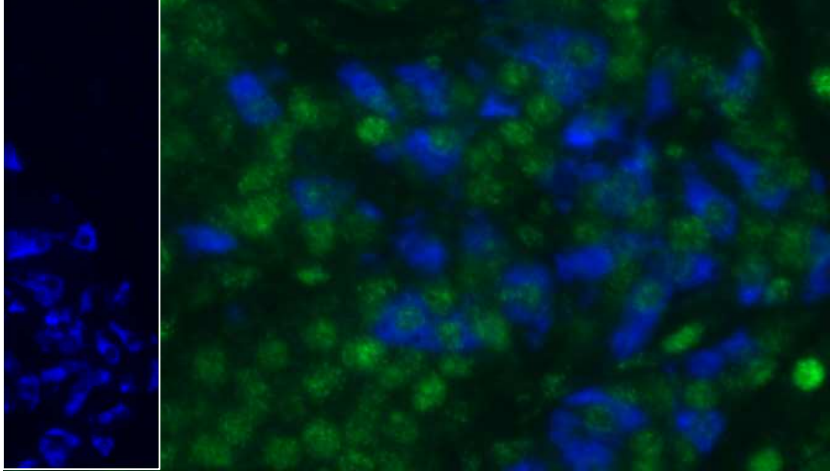

a
ER Function/Stress
Normalized WFS1 Fold Difference
24
20
16
12
8
4
0
p= 0.002
q= 0.002
FD= 4.41
Unaffected
N/D=5/28
T1D
N/D=2/28
b
WFS1
WFS1/INS/ GCG/SST
WFS1/CD99/ KRT19/DAPI
Control 6073
T1D 6180
Control 6130
T1D 6180
c
OVERLAY
WFS1/INS/GCG/SST
WFS1
INS
GCG
SST
Control 6073
T1D 6180

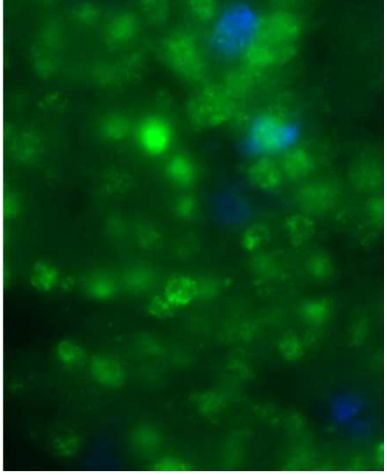
Figure 5

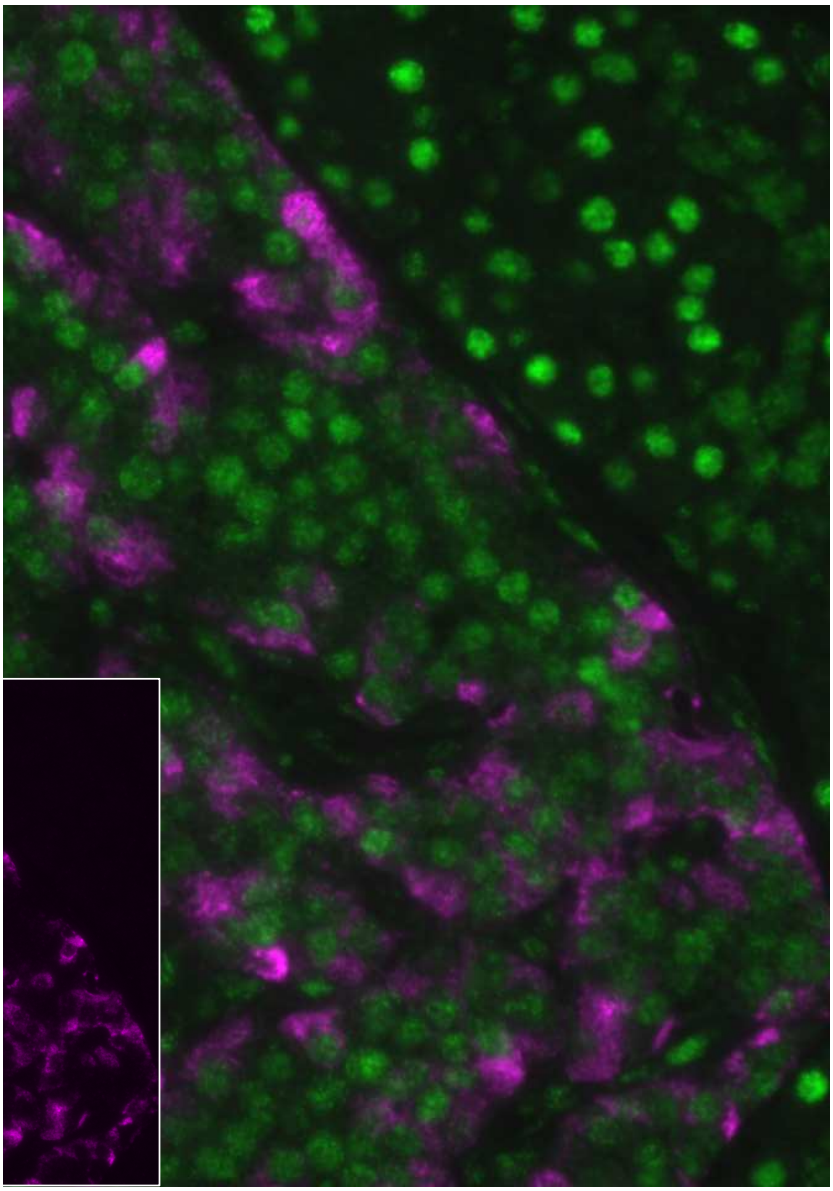

a
Normalized EIF2AK3 Fold Difference
35
28
21
14
7
0
p= <0.001
q= <0.001
FD= 8.32
PERK
Unaffected
N/D=3/28
T1D
N/D=1/30
b
PERK
PERK/INS/
GCG/SST
PERK/CD99/
KRT19/DAPI
Control 6160
T1D 6264
Control 6172
T1D 6180
c
Arm 1
Arm 2
Arm 3
Normalized ATF6 Fold Difference
p= 0.004
q= 0.004
FD= 2.78
Unaffected
N/D=0/28
T1D
N/D=0/27
Normalized ERN1/IRE1α Fold Difference
p= 0.012
q= 0.009
FD= 5.86
Unaffected
N/D=7/28
T1D
N/D=3/27
Normalized EIF2AK2 Fold Difference
p= 0.015
q= 0.010
FD= 2.90
Unaffected
N/D=3/30
T1D
N/D=3/27
Normalized EIF2AK4 Fold Difference
p= 0.002
q= 0.002
FD= 4.47
Unaffected
N/D=2/29
T1D
N/D=1/28
d
Arm 1
Arm 2
Arm 3
Normalized MBTPS1 Fold Difference
S1P
p= 0.017
q= 0.011
FD= 0.08
Unaffected
N/D=1/30
AAB+
N/D=2/23
Normalized MBTPS2 Fold Difference
S2P
p= <0.001
q= 0.001
FD= 0.14
Unaffected
N/D=4/30
AAB+
N/D=1/23
Normalized ERN1 Fold Difference
IRE1α
p= 0.002
q= 0.002
FD= 0.28
Unaffected
N/D=7/28
AAB+
N/D=1/24
Normalized EIF2AK2 Fold Difference
PKR
p= 0.043
q= 0.024
FD= 0.20
Unaffected
N/D=3/30
AAB+
N/D=1/22

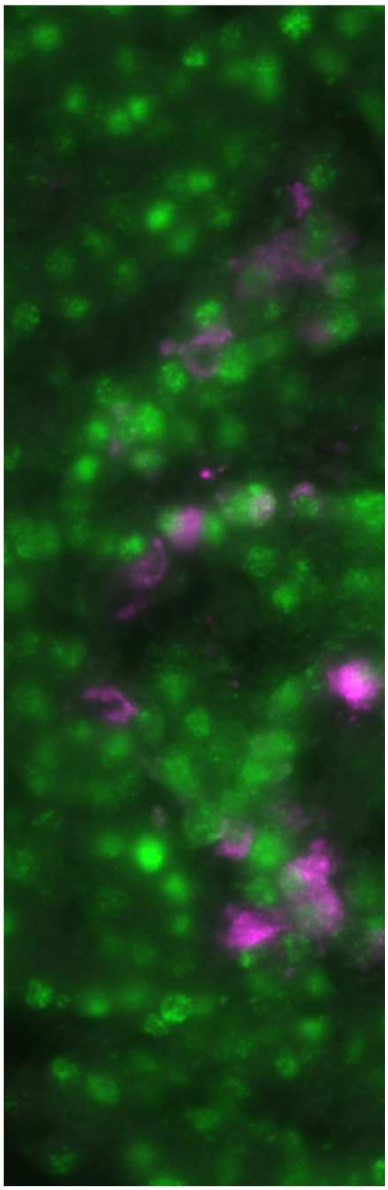


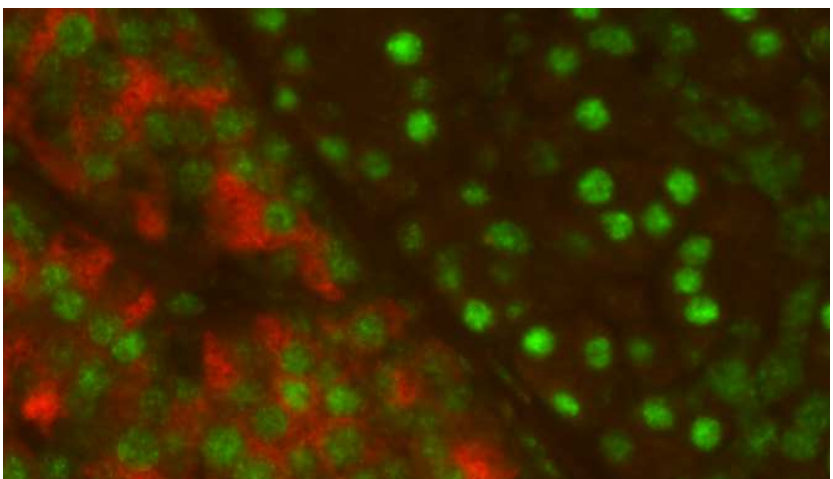


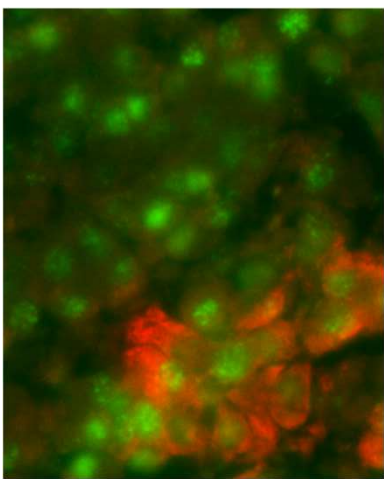


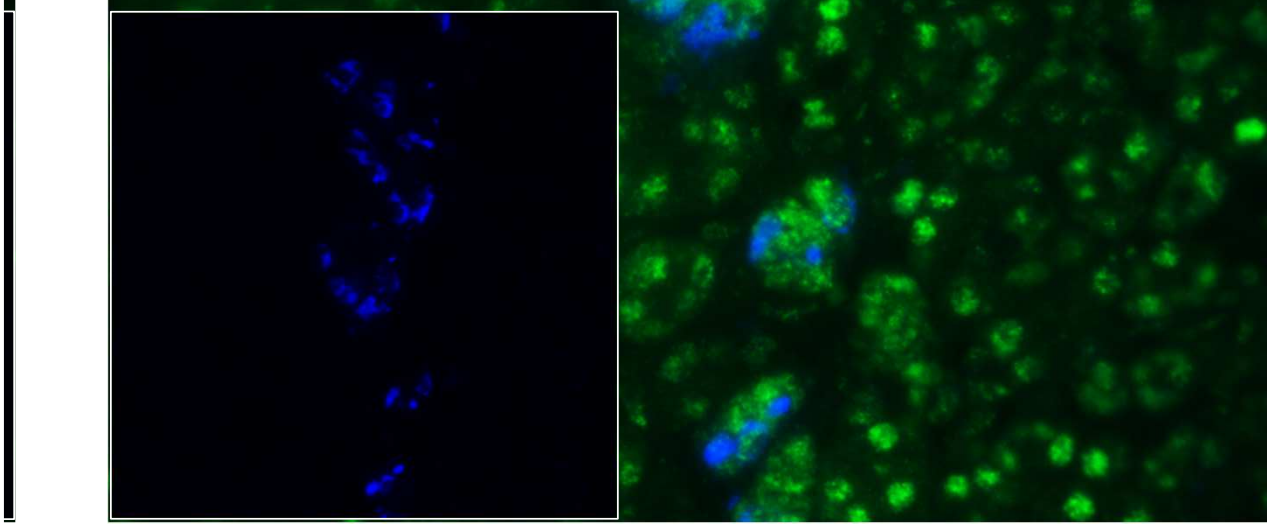


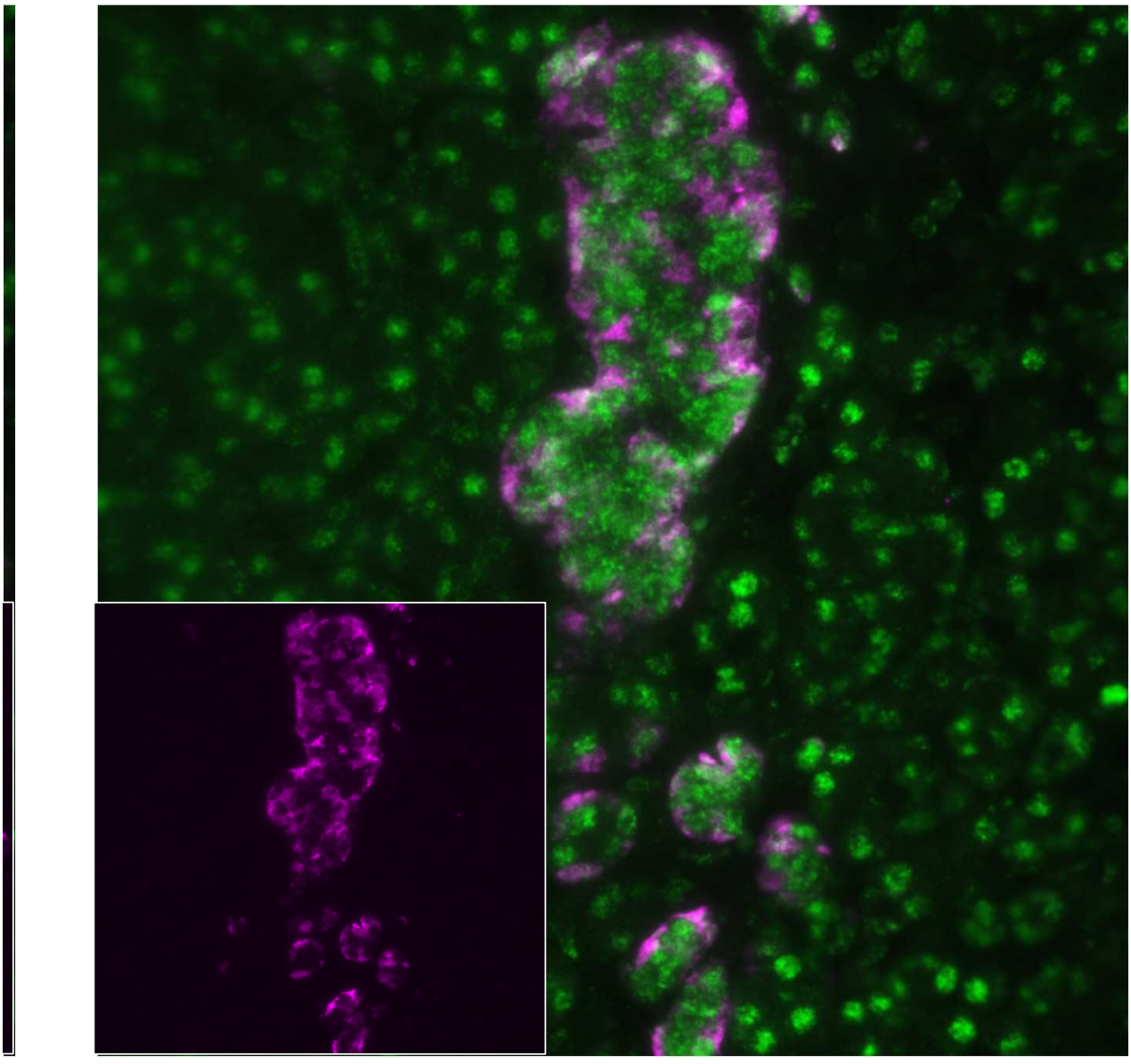


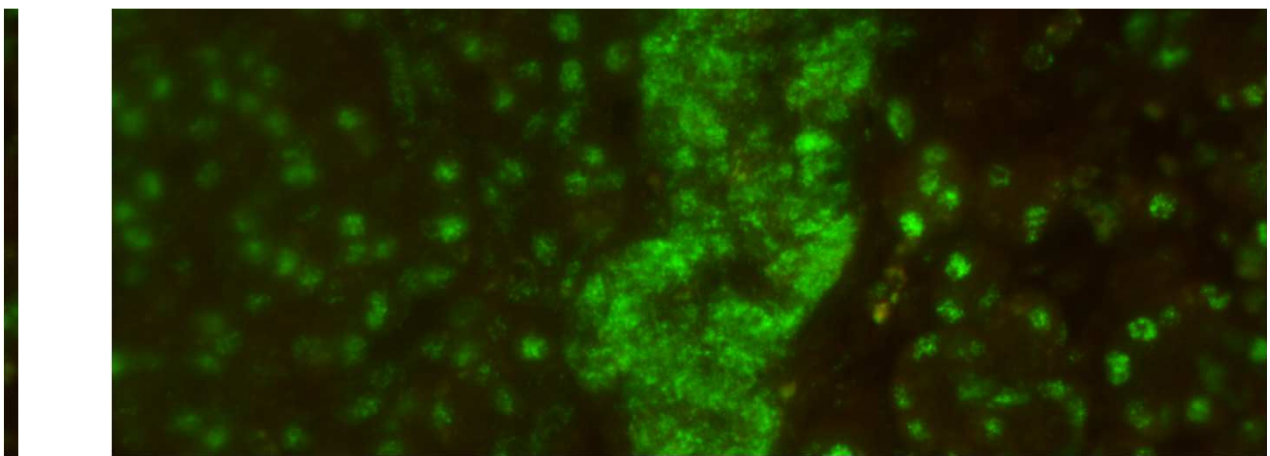


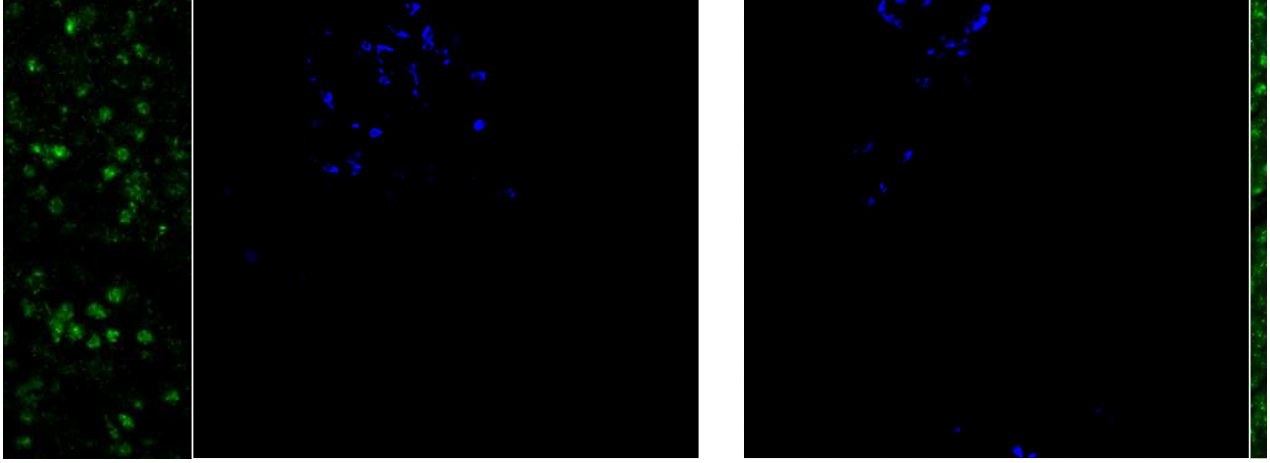


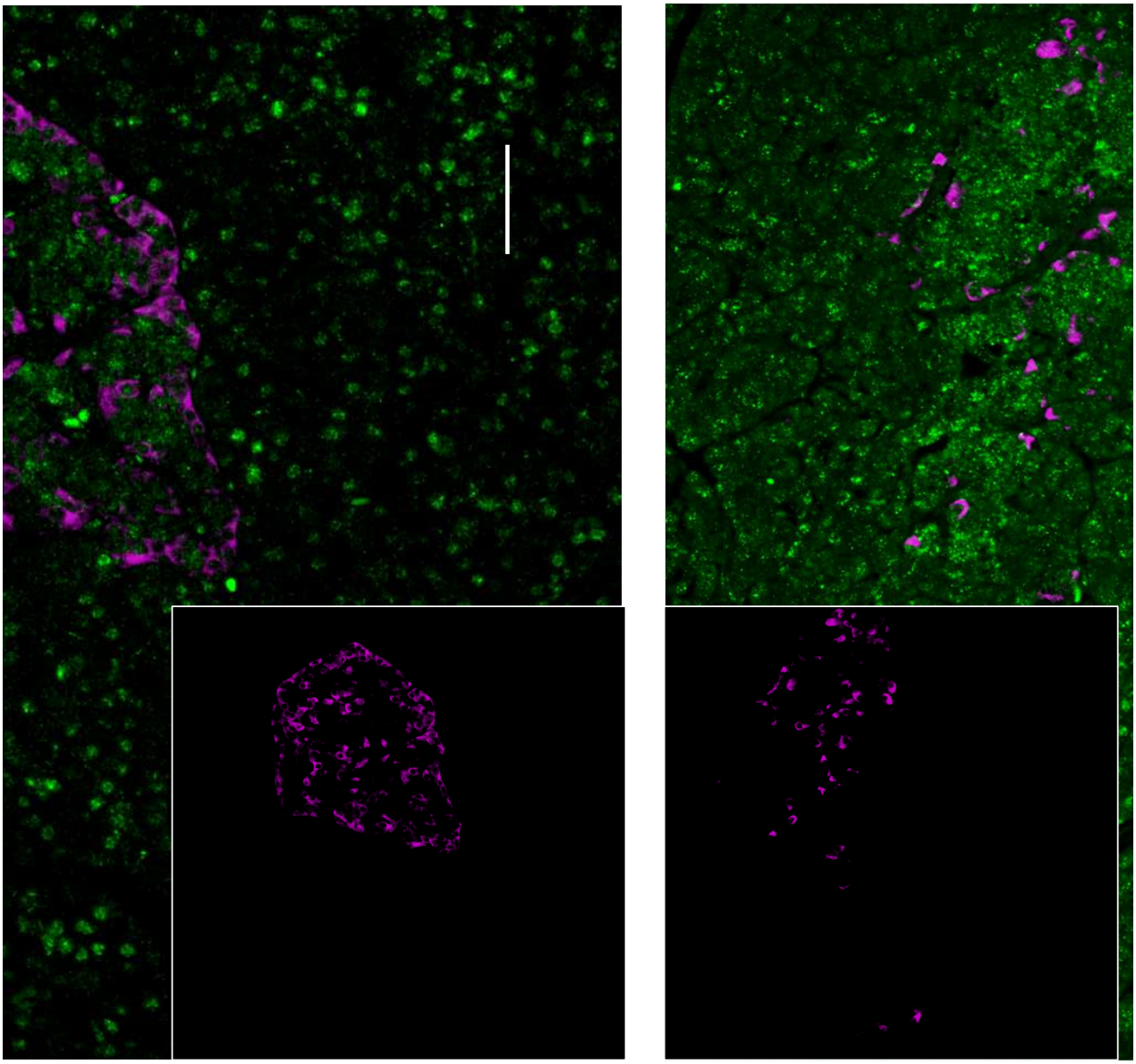


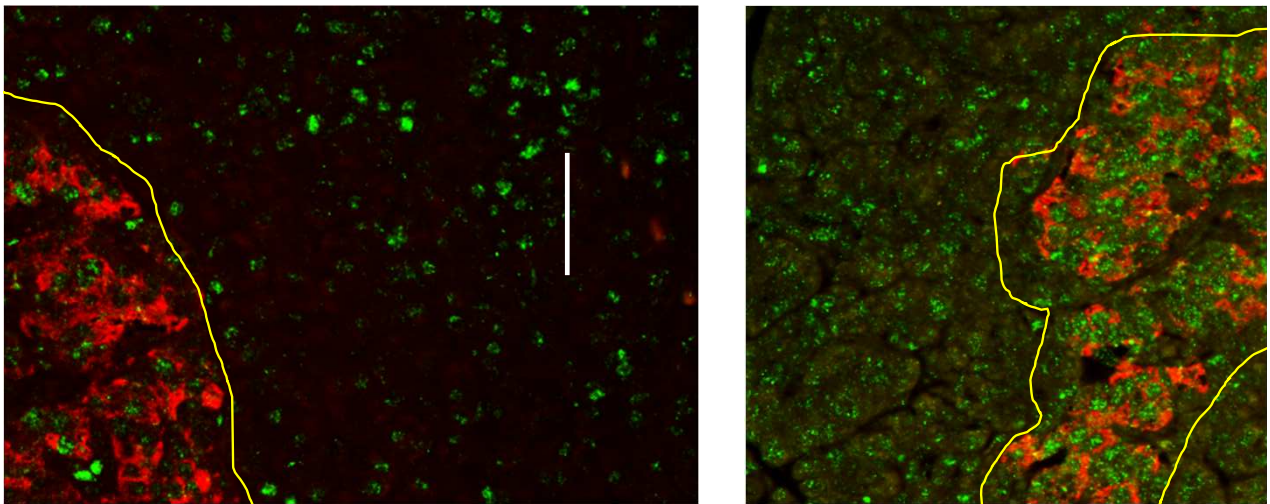


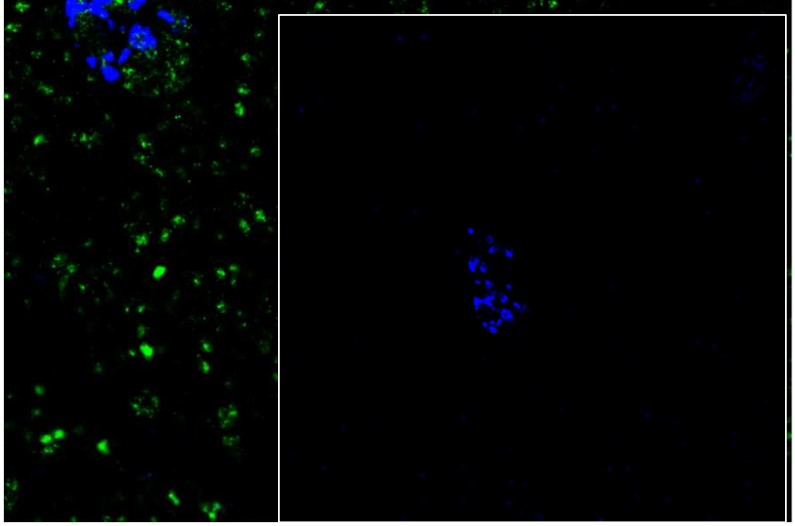


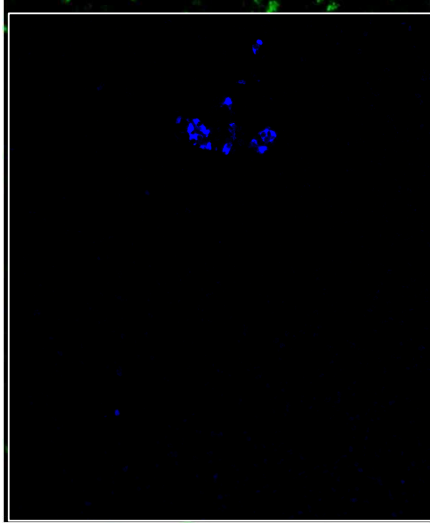


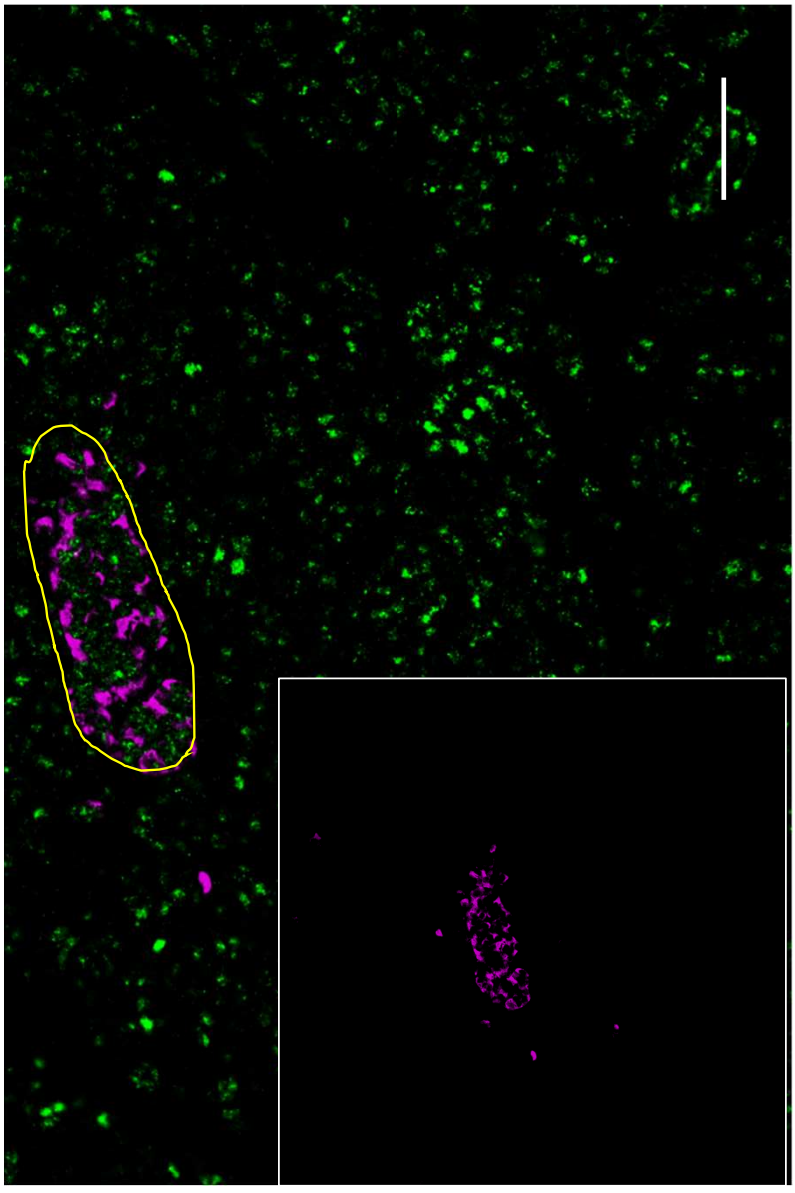


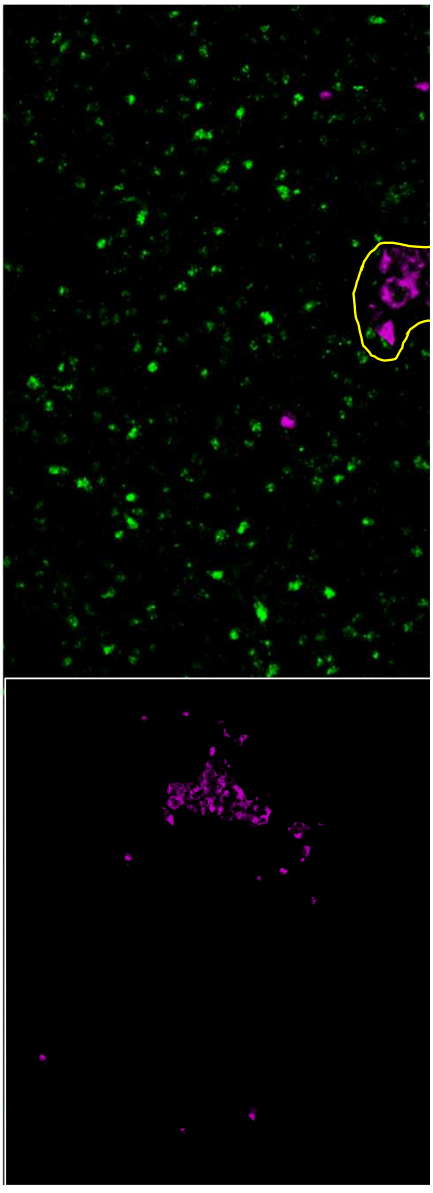


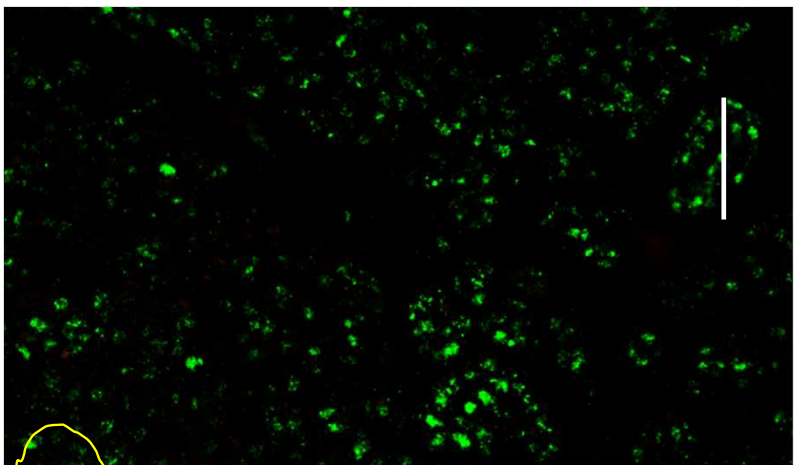


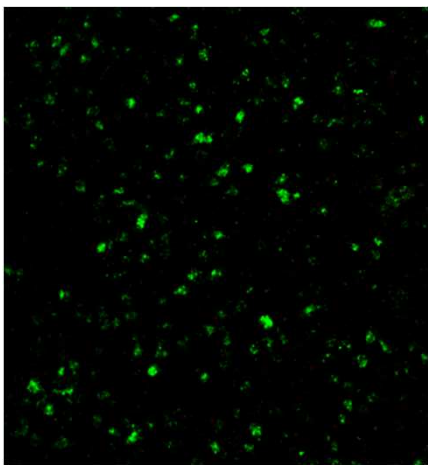


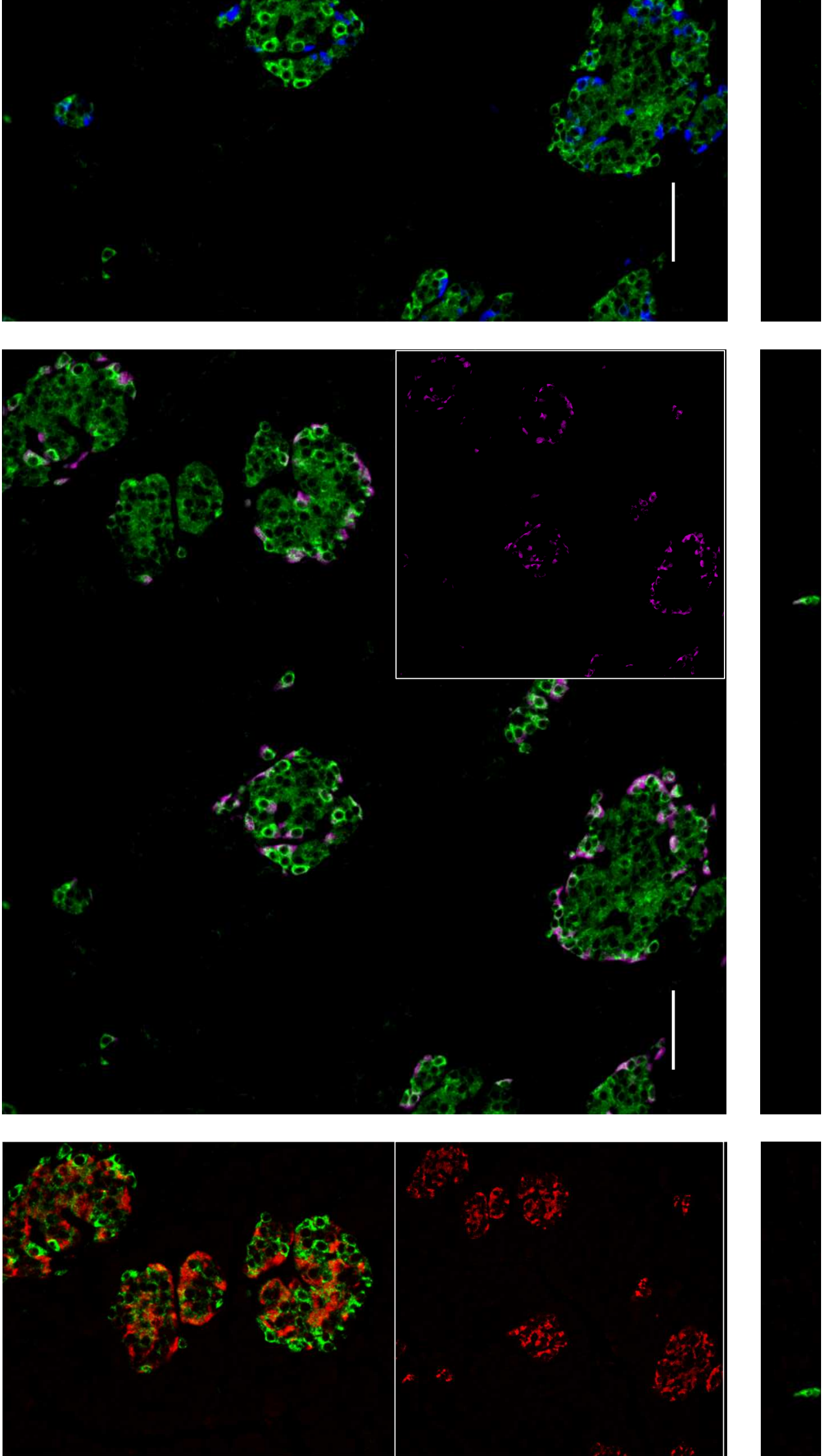


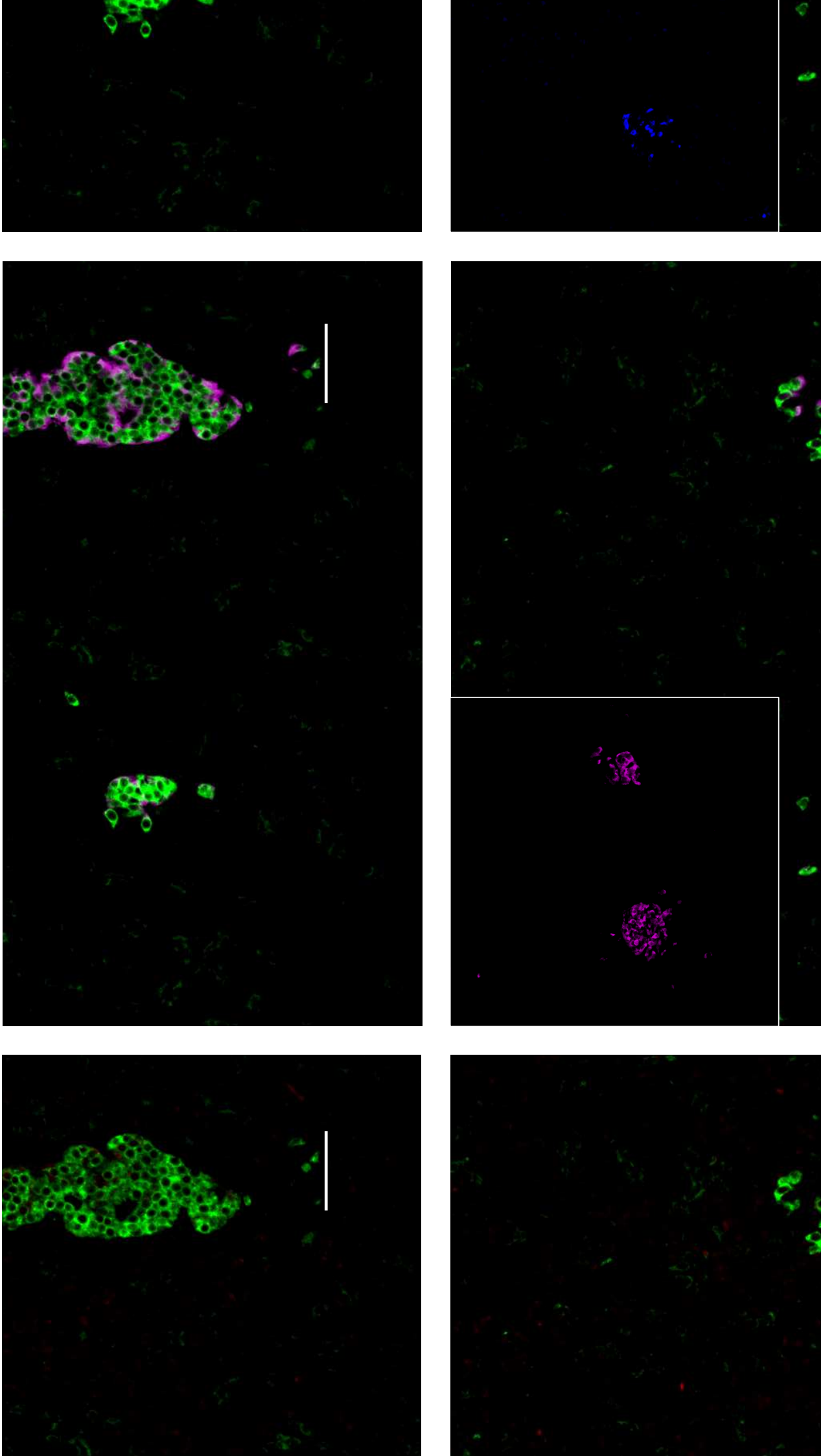


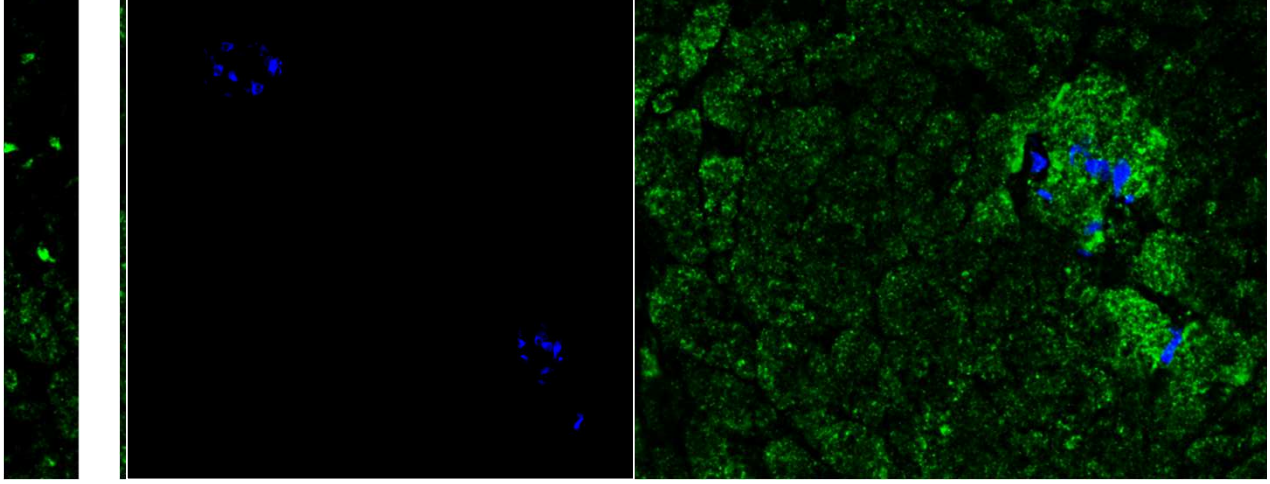


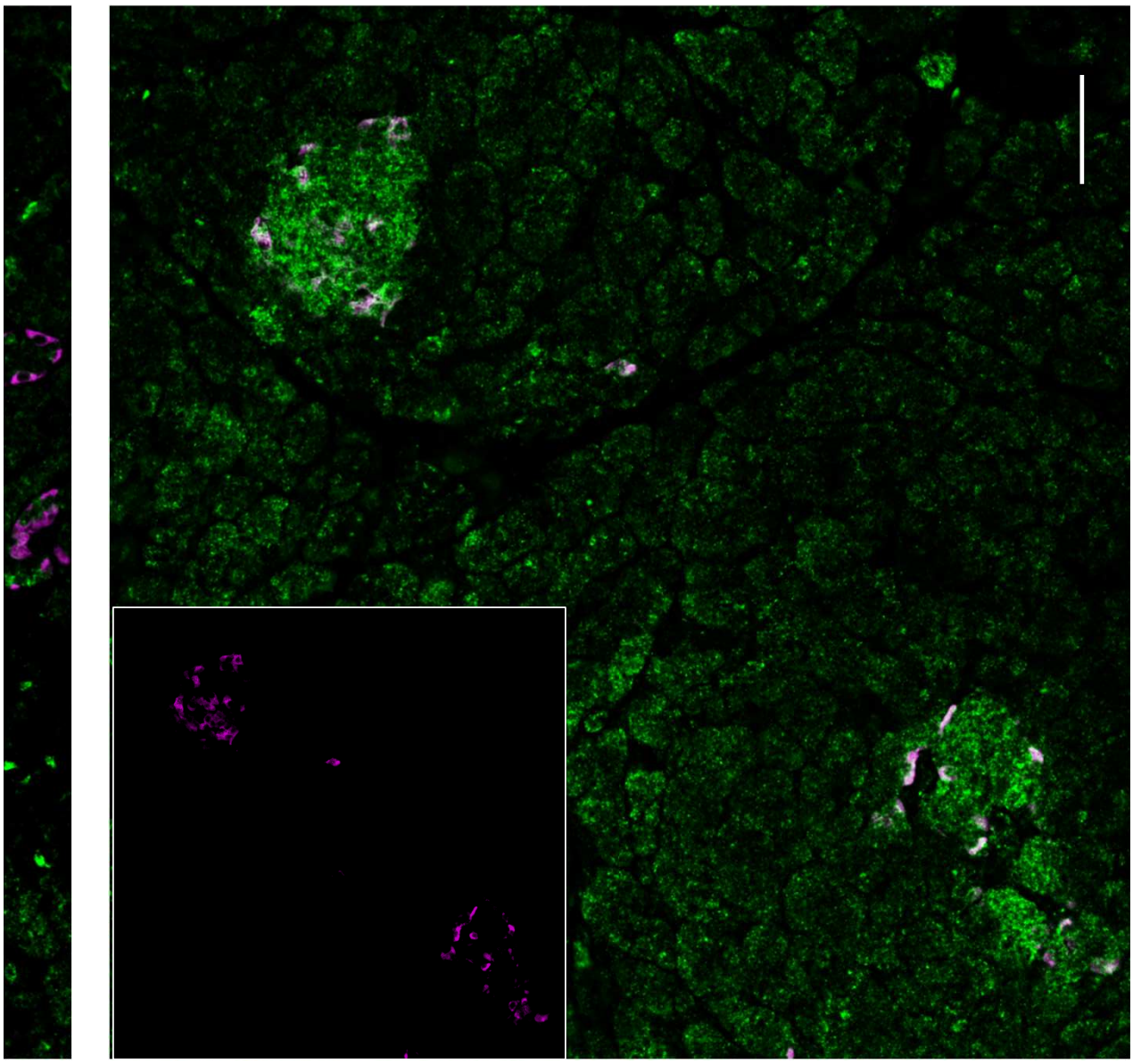


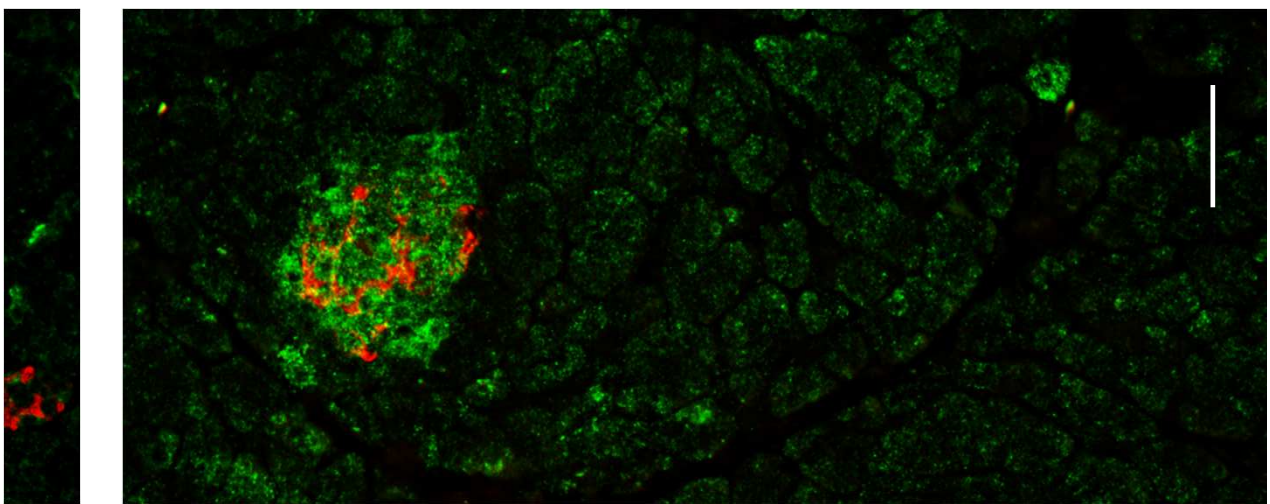


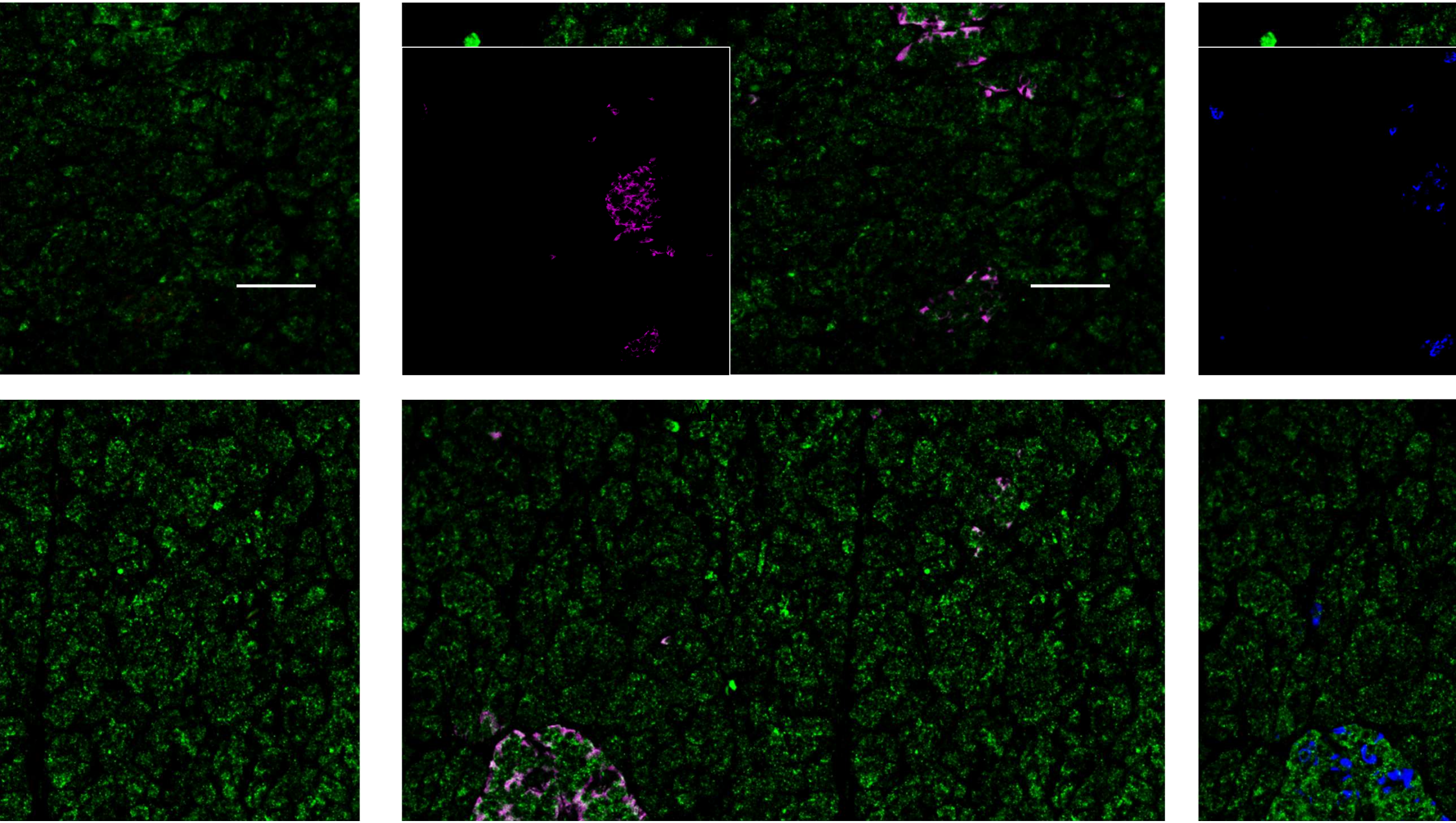












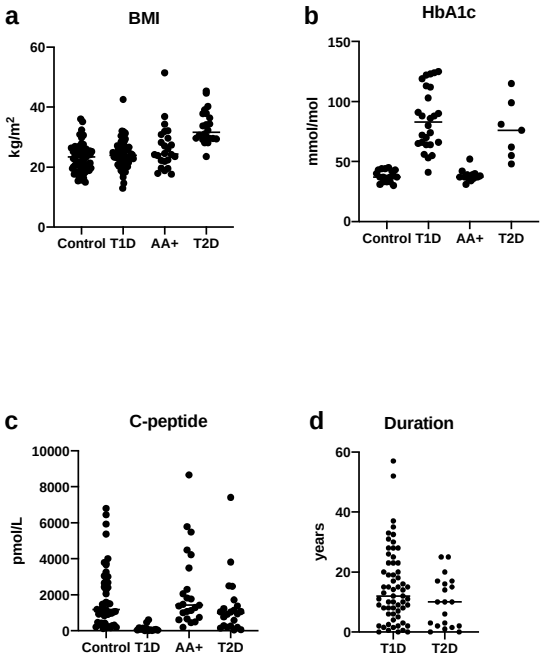


Figure S11. Donor metadata for the Unaffected/Control, T1D AAB+ and T2D. a) Body Mass Index (BMI kg/m^2); b) Hemoglobin A1c (HbA1c mmol/mol); c) C-peptide pmol/L and d) Disease duration, years.

Monogenic Diabetes Genes									
	T1D (n=25-30)			AAB+ (n=20-24)			T2D (n=18-22)		
Gene	**FD**	**p-Value**	**q-Value**	**FD**	**p-Value**	**q-Value**	**FD**	**p-Value**	**q-Value**
APPL1	1.97	0.056*	0.029*	1.08	0.223	0.078	1.01	0.491	0.141
BSCL2	2.14	0.021	0.012	***1.70***	***0.013***	***0.009***	1.31	0.234	0.080
CEL	2.52	0.040	0.023	0.90	0.560	0.158	2.27	0.257	0.085
DUT-M	2.87	0.001	0.001	1.16	0.657	0.174	5.91	0.178	0.067
DUT-N	4.07	0.004	0.003	***2.29***	***<0.001***	***0.001***	***3.11***	***<0.001***	***<0.001***
EIF2AK3	8.32	<0.001	<0.001	***3.41***	***<0.001***	***<0.001***	***4.03***	***0.004***	***0.004***
GATA4	4.35	0.004	0.004	0.71	0.431	0.127	1.37	0.242	0.081
GATA6	2.99	<0.001	0.001	0.91	0.243	0.081	1.84	0.208	0.076
GLIS3	4.01	<0.001	0.001	1.05	0.435	0.127	***3.13***	***0.052****	***0.027****
HNF1A	1.54	0.042	0.024	0.54	0.812	0.203	1.00	0.385	0.116
HNF1B	3.44	<0.001	0.001	0.70	0.582	0.161	1.66	0.097	0.045
HNF4A	1.78	0.072*	0.037*	***0.08***	***0.006***	***0.005***	1.53	0.953	0.231
ITCH	2.81	0.003	0.003	***2.54***	***<0.001***	***<0.001***	***3.41***	***<0.001***	***0.001***
KLF11	2.12	0.021	0.012	0.42	0.243	0.081	1.25	0.670	0.174
LRBA	3.89	0.002	0.002	0.36	0.580	0.161	1.44	0.366	0.112
MNX1	2.51	0.003	0.003	***1.58***	***0.004***	***0.003***	1.11	0.359	0.111
NR0B2	1.88	0.037	0.022	1.04	0.497	0.141	***2.41***	***0.002***	***0.002***
PLAGL1	2.87	0.001	0.001	1.14	0.624	0.168	1.56	0.155	0.062
SIRT1	3.05	0.013	0.009	0.28	0.154	0.062	1.52	0.649	0.173
STAT1	2.89	0.050*	0.027*	0.80	0.961	0.232	2.35	0.287	0.093
STAT3	2.75	0.012	0.009	1.38	0.097	0.045*	1.46	0.160	0.063
STAT5B	3.59	0.005	0.004	1.28	0.116	0.051	2.03	0.216	0.078
TRMT10A	2.25	0.018	0.011	0.65	0.442	0.128	0.69	0.219	0.078
WFS1	4.41	0.002	0.002	0.89	0.767	0.193	1.35	0.207	0.076

Integrated Stress Response Genes						
	T1D (n=25-30)			AAB+ (n=20-24)		
Gene	FD	p-Value	q-Value	FD	p-Value	q-Value
ALS2	***12.36***	***<0.001***	***<0.001***	0.60	0.703	0.179
ATF4	1.27	0.306	0.097	na		
ATF6	***2.78***	***0.004***	***0.004***	0.85	0.869	0.216
BLOC1S1	1.48	0.379	0.115	na		
COL6A2	***8.25***	***<0.001***	***<0.001***	0.50	0.598	0.163
DDIT3/CHOP	1.83	0.073	0.037	na		
DNAJB11	***4.58***	***<0.001***	***<0.001***	***4.35***	***<0.001***	***<0.001***
DNAJB9	***5.61***	***<0.001***	***<0.001***	0.45	0.905	0.223
DNAJC3	1.81	0.225	0.078	na		
EDEM1	***18.11***	***<0.001***	***<0.001***	1.20	0.113	0.050
EIF2AK1	2.12	0.142	0.058	na		
EIF2AK2	***2.90***	***0.015***	***0.010***	***0.20***	***0.043***	***0.024***
EIF2AK3	***8.32***	***<0.001***	***<0.001***	***3.41***	***<0.001***	***<0.001***
EIF2AK4	***4.47***	***0.002***	***0.002***	0.79	0.170	0.065
EIF2S1	2.14	0.082	0.039	na		
ERN1/IRE1a	***5.86***	***0.012***	***0.009***	***0.28***	***0.002***	***0.002***
FEN1	***8.37***	***<0.001***	***<0.001***	***2.47***	***0.001***	***0.001***
HSP90B1	***4.07***	***0.002***	***0.002***	0.56	0.358	0.111
HSPA5	2.54	0.122	0.051	na		
MBTPS1	***3.12***	***0.026***	***0.015***	***0.08***	***0.017***	***0.011***
MBTPS2	2.77	0.159	0.063	***0.14***	***<0.001***	***0.001***
MSMO1	***2.40***	***0.045***	***0.025***	0.40	0.291	0.093
MVK	***6.43***	***<0.001***	***<0.001***	0.97	0.204	0.076
NELFA	***7.48***	***<0.001***	***<0.001***	***1.65***	***0.017***	***0.011***
PDIA4	***8.38***	***<0.001***	***<0.001***	1.26	0.075	0.037
PPP1R15A	***9.48***	***<0.001***	***<0.001***	0.47	0.689	0.176
TMBIM6	***4.42***	***<0.001***	***<0.001***	0.76	0.689	0.176
VCP	***12.44***	***<0.001***	***<0.001***	1.05	0.078	0.038
XBP1s	3.81	0.138	0.057	na		
XBP1u	1.48	0.361	0.111	na		

Controls Unaffected											
nPOD ID	**AutoAb**	**Age (years)**	**Duration (years)**	**Age at Onset (years)**	**Sex**	**Ethnicity**	**C-Peptide (pmol/L)**	**HbA1c (mmol/mol)/ %**	**BMI (kg/m2)**	**Cause of Death**	**HiRes HLA**
6004	No serum	33			male	Caucasian			30.9	head trauma	A*23:01/24:02 B*44:02/52:01 C*05:01/12:02 DRB1*04:01/15:02 DQA1*01:03/03:01 DQB1*03:01/06:01 DPA1*01:03/02:01 DPB1*04:02/13:01
6005	neg	5			female	Caucasian			15.7	cerebrovascular/stroke	A*02:01/24:02 B*37:01/55:01 C*03:03/06:02 DRB1*01:01/12:01 DQA1*01:01/05:01 DQB1*03:01/05:01 DPA1*01:03/01:03 DPB1*04:01/04:02
6007	no serum	9			male	African Am			20	anoxia	A*30:02/30:02 B*35:01/35:01 C*04:01/04:01 DRB1*11:01/11:02 DQA1*01:02/05:01 DQB1*03:01/06:02 DPA1*02:01/03:01 DPB1*01:01/04:02
6008	no serum	50			female	Caucasian			24.2	head trauma	A*02:05/26:01 B*38:01/58:01 C*07:01/12:03 DRB1*04:02/13:02 DQA1*01:02/03:01 DQB1*03:02/06:09 DPA1*01:03/01:03 DPB1*03:01/04:01
6009	negative	45			male	Caucasian	3746.92		30.6	anoxia	A*11:01/29:02 B*50:01/57:01 C*06:02/06:02 DRB1*07:01/15:01 DQA1*01:02/02:01 DQB1*03:03/06:02 DPA1*01:03/01:03 DPB1*02:01/04:01
6010	no serum	47			female	Caucasian			19.7	cerebrovascular/stroke	A* 02:01/02:01 B* 07:02/57:01 C* 06:02/07:02 DRB1* 07:01/15:01 DQA1* 01:02/02:01 DQB1* 03:03/06:02 DPA1* 01:03/01:03 DPB1* 02:01/04:01
6011	no serum	46			female	African Am			26.3	cerebrovascular/stroke	A*30:01/68:01 B*42:02/58:02 C*06:02/17:01 DRB1*08:04/13:03 DQA1*04:01/05:01 DQB1*03:01/03:01 DPA1*02:01/02:01 DPB1*01:01/17:01
6012	negative	68			female	Caucasian	983.07		23.7	cerebrovascular/stroke	A*30:01/68:01 B*42:02/58:02 C*06:02/17:01

											DRB1*08:04/13:03 DQA1*04:01/05:01 DQB1*03:01/03:01 DPA1*02:01/02:01 DPB1*01:01/17:01
6013	negative	65			male	Caucasian	926.8		24.2	cerebrovascular/stroke	A*01:01/01:01 B*07:02/44:03 C*02:02/07:02 DRB1*01:02/13:01 DQA1*01:01/01:03 DQB1*05:01/06:03 D PA1*01:03/01:03 DPB1*04:01/04:01
6017	negative	59			female	Caucasian	3273.59		24.8	cerebrovascular/stroke	A*02:01/11:01 B*35:01/40:01 C*03:04/04:01 DRB1*01:03/11:01 DQA1*01:01/05:01 DQB1*03:01/05:01 DPA1*01:03/01:03 DPB1*02:01/04:01
6020	negative	60			male	Caucasian	933.42		29.8	cerebrovascular/stroke	A*03:01/23:01 B*07:02/44:03 C*04:01/07:02 DRB1*07:01/13:01 DQA1*01:03/02:01 DQB1*02:01/06:03 DPA1*01:03/01:03 DPB1*04:01/04:01
6024	negative	21			male	Caucasian	1165.12		27.8	head trauma	A*03:01/23:01 B*07:02/44:03 C*04:01/07:02 DRB1*07:01/13:01 DQA1*01:03/02:01 DQB1*02:01/06:03 DPA1*01:03/01:03 DPB1*04:01/04:01
6030	negative	30.1			male	Caucasian	840.74		27.1	head trauma	A*02:01/31:01 B*07:02/44:03 C*07:02/16:01 DRB1*07:01/15:01 DQA1*01:02/02:01 DQB1*02:01/06:02 DPA1*01:03/02:02 DPB1*01:01/03:01
6034	negative	32			female	Caucasian	1042.65		25.2	head trauma	A*03:01/03:01 B*07:02/15:01 C*01:02/07:02 DRB1*01:01/08:01 DQA1*01:01/04:01 DQB1*04:02/05:01 DPA1*01:03/01:03 DPB1*03:01/03:01
6047	negative	7.8			male	Caucasian	215.15	37/5.5	23.9	anoxia	A*02:01/03:01 B*07:02/51:01 C*01:02/07:02 DRB1*14:01/15:01 DQA1*01:01/01:02 DQB1*05:03/06:02 DPA1*01:03/01:03 DPB1*04:01/04:02
6048	negative	30			male	Caucasian	5928.21		20.6	cerebrovascular/stroke	A*03:01/31:01 B*07:02/47:01 C*06:02/07:02 DRB1*04:01/15:01

											DQA1*01:02/03:01 DQB1*03:01/06:02 DPA1*01:03/01:03 DPB1*04:01/04:01
6055	negative	27			male	Caucasian	195.29		22.7	anoxia	A*03:01/03:01 B*07:02/15:01 C*01:02/07:02 DRB1*01:01/08:01 DQA1*01:01/04:01 DQB1*04:02/05:01 DPA1*01:03/01:03 DPB1*03:01/03:01
6057	negative	22			male	Caucasian	5372.13		26	head trauma	A*02:01/31:01 B*40:01/44:02 C*03:04/05:01 DRB1*01:01/04:04 DQA1*01:01/03:01 DQB1*03:02/05:01 DPA1*01:03:01:03 DPB1*04:02/06:01
6058	negative	27			male	Hispanic	3008.79		19.1	head trauma	A*02:01/02:06 B*39:01/39:02 C*07:02/07:02 DRB1*08:02/16:xx DQA1*04:01/05:01 DQB1*03:01/04:02 DPA1*01:03/01:03 DPB1*04:02/04:02
6073	negative	19.2			male	Caucasian	228.39		36	anoxia	A*29/01, B*37/44, DR*13/16, DQ*05/06
6075	negative	16			male	African Am	973.14		14.9	anoxia	A*23:01/33:03 B*15:03/58:01 C*02:02/07:01 DRB1*07:01/11:01 DQA1*02:01/05:01 DQB1*02:01/03:01 DPA1*02:01/02:01 DPB1*11:01/13:01
6096	negative	16			female	African Am	983.07		18.8	head trauma	A*02:01/'30:02 B*'14:02/'42:01 C*'08:02/'17:01 DRB1*'03:02/'15:03 DQA1*'01:02/'04:01 DQB1*'04:02/'06:02 DPA1*'02:02/'03:01 DPB1*'01:01/'04:02
9098	negative	17.8			male	Caucasian	466.71	30/4.9	22.8	head trauma	A*01:01/32:01 B*08:01/27:05 C*01:02/07:01 DRB1*03:01/08:01 DQA1*04:01/05:01 DQB1*02:01/04:02 DPA1*01:03/02:01 DPB1*01:01/04:01
6102	negative	45.1			female	Caucasian	182.05	43/6.1	35.1	cerebrovascular/stroke	A*03:01/31:01 B*08:01/44:02 C*05:01/07:01 DRB1*03:01/04:01 DQA1*03:01/05:01 DQB1*02:01/03:01 DPA1*01:03/02:01 DPB1*01:01/02:01

6103	negative	1.5			male	Caucasian	324.38	43/6.1	16.8	anoxia	A*02:01/80:01 B*40:02/44:02 C*04:01/05:01 DRB1*11:01/13:01 DQA1*01:03/05:01 DQB1*03:01/06:03 DPA1*01:03/02:01 DPB1*04:01/10:01
6104	negative	41			male	Caucasian	6802.05		20.5	anoxia	A*29:02/68:01 B*39:06/44:03 C*12:03/16:01 DRB1*07:01/13:01 DQA1*01:01/02:01 DQB1*02:01/05:01 DPA1*01:03/02:02 DPB1*01:01/03:01
6106	negative	2.9			male	Caucasian	2436.16		17.4	anoxia	A*31:01/32:01 B*27:05/52:01 C*01:02/15:02 DRB1*01:01/04:07 DQA1*01:01/03:01 DQB1*03:02/05:01 DPA1*01:03/01:03 DPB1*04:01/04:02
6117	negative	0.33			male	Caucasian	1082.37		18.4	head trauma	A*01:01/11:01 B*18:01/57:01 C*06:02/07:01 DRB1*07:01/07:01 DQA1*02:01/02:01 DQB1*02:01/03:03 DPA1*01:03/01:03 DPB1*02:01/04:01
6125	negative	0.42			male	Caucasian	3051.82		18.9	other: meningitis	A*24:02/24:02 B*07:02/40:01 C*03:04/07:02 DRB1*04:04/15:01 DQA1*01:02/03:01 DQB1*03:02/06:03 DPA1*01:03/01:03 DPB1*03:01/06:01
6126	negative	25.2			male	Hispanic	291.28		25.1	head trauma	A*29:02/33:01 B*14:02/44:03 C*08:02/16:01 DRB1*03:01/07:01 DQA1*02:01/05:01 DQB1*02:01/02:01 DPA1*01:03/01:03 DPB1*04:01/04:01
6129	negative	42.9			female	Caucasian	168.81	33/5.2	23.4	anoxia	A*03:01/23:01 B*07:02/14:02 C*07:02/08:02 DRB1*03:01/15:01 DQA1*01:02/05:01 DQB1*02:01/06:02 DPA1*01:03/02:01 DPB1*01:01/04:01
6130	negative	5.2			male	Caucasian	1588.8		18.5	head trauma	A* 01:01, 03:01 DRB1* 03:01, 15:01 DQA1* 01:02, 05:01 DQB1* 02:01, 06:02
6131	negative	24.2			male	Caucasian	334.31		24.8	anoxia	A*01:01/11:01 B*08:01/18:01 C*05:01/07:01 DRB1*03:01/03:01 DQA1*05:01/05:01 DQB1*02:01/02:01

											DPA1*01:03/02:01 DPB1*01:01/02:02
6134	negative	26.7			male	Caucasian	1188.29		20.1	anoxia	A*03:01/29:02 B*37:01/44:03 C*06:02/16:01 DRB1*07:01/10:01 DQA1*01:01/02:01 DQB1*02:01/05:01 DPA1*01:03/02:01 DPB1*04:01/11:01
6137	negative	8.9			female	Hispanic	4015.03	44/6.2	24.2	cerebrovascular/stroke	A*24:02/33:01 B*14:02/44:02 C*02:02/08:02 DRB1*03:01/07:01 DQA1*02:01/05:01 DQB1*02:01/02:01 DPA1*01:03/01:03 DPB1*02:01/04:01
6140	negative	38			male	Caucasian	3674.1	42/6	21.7	cerebrovascular/stroke	A*01:01/03:01 B*35:01/35:01 C*04:01/04:01 DRB1*01:01/03:01 DQA1*01:01/05:01 DQB1*02:01/05:01 DPA1*01:03/01:03 DPB1*04:01/04:02
6144	negative	7.5			female	Hispanic	420.37		16.3	respiratory distress/failure	A*02:01/02:01 B*40:05/44:03 C*03:02/16:01 DRB1*07:01/08:02 DQA1*02:01/04:01 DQB1*02:02/04:02 DPA1*01:03/02:02 DPB1*01:01/04:02
6153	negative	15.2			male	Hispanic	2773.78	37/5.5	20.5	head trauma	A*24:02/26:01 B*35:05/58:01 C*03:02/04:01 DRB1*04:04/13:02 DQA1*01:02/03:01 DQB1*03:02/06:09 DPA1*01:03/01:03 DPB1*04:01/04:02
6160	negative	22.1			male	Caucasian	132.4	33/5.2	23.9	head trauma	not listed
6165	negative	45.8			female	Caucasian	1472.95	38/5.6	25	cerebrovascular/stroke	A*01:01, 02:01 DRB1*13:01, 15:01 DQA1*01:02, 01:03 DQB1*06:02, 06:03
6168	no serum	51			male	Hispanic		44/6.2	25.2	cerebrovascular/stroke	A*02:01, 24:02 DRB*01:03, 04:04 DQA1*01:01, 03:01 DQB1*03:02, 05:01
6172	negative	19.2			female	Caucasian	2654.62	36/5.4	32.4	cerebrovascular/stroke	A*03:01 , 68:02 DRB1*01:01 , 13:03 DQA1*01:01 , 05:01 DQB1*03:01 , 05:01
6174	negative	20.9			male	Caucasian	993		19.5	cerebrovascular/stroke	A*02:05 , 26:01 DRB1*03:01 , 07:01 DQA1*02:01 , 05:01 DQB1*02:01 , 02:02
6178	negative	24.5			female	Caucasian	1506.05	31/5.0	27.5	anoxia	A*02:01 , 24:02 DRB1*04:01 , 15:01 DQA1*01:02 , 03:01 DQB1*03:01 , 06:02

6179	negative	20			female	Caucasian	906.94		20.7	head trauma	A*02:01, 24:02 DRB*03:01, 04:04 DQA1*03:01, 05:01 DQB1*02:01, 03:02
6183	no serum	0.3			male	African Am			15.4	birth defect	A*02:01, 30:01 DRB1*01:02, 03:02 DQA1*01:01, 04:01 DQB1*04:02, 05:01
6217	negative	0.58			male	Caucasian	433.61		17.6	anoxia	A*03:01, 03:01 DRB*04:07, 08:01 DQA1*03:01, 04:01 DQB1*03:01, 04:02
6227	negative	17			female	Caucasian	910.25		26.4	cerebrovascular/stroke	A*02:01, 03:01 DRB1*04:01, 13:02 DQA1*01:02, 03:01 DQB1*03:02, 06:04
6229	negative	31			female	Caucasian	2062.13	37/5.5	26.9	head trauma	A*02:01, 24:02 DRB1*01:01, 13:02 DQA1*01:02, 01:02 DQB1*05:04, 06:04
6232	negative	14			female	Caucasian	6454.5		20.8	head trauma	A*02:01, 24:02 DRB1*15:01, 15:01 DQA1*01:02, 01:02 DQB1*06:02, 06:02
6233	negative	14			male	Caucasian	2403.06	37/5.5	21.9	anoxia	A*01:01, 26:01 DRB1*01:01, 13:01 DQA1*01:01, 01:03 DQB1*05:01, 06:03
6234	negative	20			female	Caucasian	2280.59	40/5.8	25.6	head trauma	A*02:01, 11:01 DRB1*11:01, 15:01 DQA1*01:02, 05:01 DQB1*03:01, 06:02
6235	negative	30			male	Caucasian	2681.1		25.4	head trauma	A*24:02, 68:01 DRB1*03:01, 04:07 DQA1*03:01, 05:01 DQB1*02:01, 03:02
6271	negative	17			male	Caucasian	3796.57		24.4	head trauma	A*02:01,02:06 DRB1*07:01,15:02 DQA1*01:01,02:01 DQB1*02:02,05:01
6278	negative	12			female	African Am	1502.74	45/6.3	21.3	anoxia	A*23:01,68:02 DRB1*11:04,12:01 DQA1*03:01,05:01 DQB1*03:01,05:02
6316	negative	6			male	Caucasian	1515.98		18.4	anoxia	A*24:02, 31:01 DRB1*04:04,13:01 DQA1*03:01,03:01 DQB1*03:02,03:03
6318	negative	10			female	Caucasian	1287.59	33/5.2	17.6	head trauma	A*03:01, 03:01 DRB1*04:04,11:01 DQA1*03:01,05:01 DQB1*03:01,03:02
Type 1 Diabetes											
nPOD ID	**AutoAb**	**Age (years)**	**Duration (years)**	**Age at Onset (years)**	**Sex**	**Ethnicity**	**C-Peptide (pmol/L)**	**HbA1c (mmol/mol)/ %**	**BMI (kg/m2)**	**Cause of Death**	**HiRes HLA**
6025	mIAA+	23.8	19	4.80	male	Caucasian			26.6	anoxia	A*03:05/29:02 B*13:02/44:03 C*06:02/16:01 DRB1*07:01/07:01 DQA1*02:01/02:01 DQB1*02:01/02:01

											DPA1*01:03/02:02 DPB1*01:01/04:01
6026	mIAA+	22.4	9	13.40	male	Caucasian			24.1	head trauma	A* 01:01/24:02 B*08:01/18:01 C*07:01/12:03 DRB1*03:01/13:02 DQA1*01:02/05:01 DQB1*02:01/06:04 DPA1*01:03/01:03 DPB1*02:01/04:01
6031	mIAA+	39	35	4.00	male	Caucasian			24.5	cerebrovascular/stroke	A*03:01/30:01 B*07:02/13:02 C*06:02/07:02 DRB1*01:01/07:01 DQA1*01:01/02:01 DQB1*02:01/05:01 DPA1*01:03/02:01 DPB1*02:01/17:01
6032	mIAA+	33.8	no info		male	Caucasian			29.4	anoxia	A*02:01/02:01 B*40:01/44:05 C*02:02/03:04 DRB1*03:01/04:01 DQA1*03:01/05:01 DQB1*02:01/03:02 DPA1*01:03/01:03 DPB1*03:01/04:02
6035	mIAA+	32.1	28	4.10	male	Caucasian			27.1	cerebrovascular/stroke	A*02:01/02:01 B*08:01/15:01 C*03:04/07:01 DRB1*03:01/04:01 DQA1*03:01/05:01 DQB1*02:01/03:02 DPA1*01:03/02:01 DPB1*01:01/04:01
6039	GADA+ IA-2A+ ZnT8A+ mIAA+	28.7	12	16.70	female	Caucasian			23.4	head trauma	A* 01:01/02:01 B* 08:01/27:05 C* 01:02/07:01 DRB1* 03:01/04:01 DQA1* 03:01/05:01 DQB1* 02:01/03:02 DPA1* 01:03/02:02 DPB1* 04:01/05:01
6040	mIAA+	50	20	30.00	female	Caucasian		56/7.3	31.6	cerebrovascular/stroke	A*11:01/30:02 B*15:01/18:01 C*03:03/05:01 DRB1*01:02/03:01 DQA1*01:01/05:01 DQB1*02:01/05:01 DPA1*01:03/01:03 DPB1*02:01/02:02
6041	negative	26.3	23	3.30	male	Caucasian			28.4	cerebrovascular/stroke	A*02:01/02:01 B*35:01/40:01 C*03:04/04:01 DRB1*01:01/04:01 DQA1*01:01/03:01 DQB1*03:02/05:01 DPA1*01:03/02:01 DPB1*01:01/16:01
6045	ZnT8A+ mIAA+	26.4	8	18.40	male	Caucasian			23.1	head trauma	A*01:01/03:01 B*08:01/14:02 C*07:01/08:02 DRB1*03:01/13:02 DQA1*01:02/05:01 DQB1*02:01/06:09

											DPA1*01:03/01:03 DPB1*02:01/04:02
6049	GADA+ mIAA+	15	10	5.00	female	African Am			20.8	anoxia	A*32:01/33:03 B*35:01/40:01 C*03:04/04:01 DRB1*04:04/09:01 DQA1*03:01/03:01 DQB1*02:01/03:02 DPA1*01:03/02:01 DPB1*04:01/17:01
6051	mIAA+	20.3	13	7.30	male	Caucasian			21.5	head trauma	A*01:01/68:02 B*08:01/27:02 C*02:02/07:01 DRB1*03:01/16:01 DQA1*01:02/05:01 DQB1*02:01/05:02 DPA1*01:03/02:01 DPB1*01:01/02:01
6054	mIAA+	35.1	30	5.10	female	Caucasian			30.4	cerebrovascular/stroke	A*01:01/02:01 B*08:01/44:02 C*05:01/07:01 DRB1*03:01/04:01 DQA1*03:01/05:01 DQB1*02:01/03:02 DPA1*01:03/02:01 DPB1*01:01/02:01
6062	no serum avail	10.7	6	4.70	male	African Am		112/12.4	21.9	cerebral edema	A*02:01/24:02 B*14:01/45:01 C*02:10/16:01 DRB1*01:02/07:01 DQA1*01:01/03:01 DQB1*02:01/05:01 DPA1*01:03/02:01 DPB1*01:01/18:01
6063	mIAA+	4.4	3	1.40	male	Caucasian			23.8	anoxia	A*02:01/24:02 B*08:01/15:01 C*03:04/07:01 DRB1*03:01/04:01 DQA1*03:01/05:01 DQB1*02:01/03:02 DPA1*01:03/01:03 DPB1*02:01/04:01
6064	GADA+ IA-2A+ mIAA+ ZnT8A+	19.6	9	10.60	female	Caucasian			22.6	anoxia	A*02:01/29:02 B*15:01/44:03 C*03:04/16:01 DRB1*01:01/04:01 DQA1*01:01/03:01 DQB1*03:02/05:01 DPA1*01:03/01:03 DPB1*03:01/04:01
6067	negative	32.6	8	24.60	female	Hispanic			26.8	anoxia	A*24:02/30:02 B*18:01/39:01 C*05:01/07:02 DRB1*03:01/04:07 DQA1*03:01/05:01 DQB1*02:01/03:02 DPA1*01:03/01:03 DPB1*04:01/04:02
6070	IA-2A+ mIAA+	22.6	7	15.60	female	Caucasian			21.6	anoxia	A*02:01/02:05 B*38:01/58:01 C*07:01/12:03 DRB1*10:01/16:01 DQA1*01:01/01:02 DQB1*05:01/05:02

											DPA1*01:03/02:01 DPB1*13:01/14:01
6076	GADA+ mIAA+	25.8	14	11.80	male	Caucasian		66/8.2	18.8	anoxia	A*03:01/30:02 B*18:01/40:01 C*03:04/05:01 DRB1*03:01/04:04 DQA1*03:01/05:01 DQB1*02:01/03:02 DPA1*01:03/01:03 DPB1*02:02/06:01
6081	negative	31.4	15	16.40	male	Hispanic	79.44		28	cerebrovascular/stroke	A* 01:01/24:02 B*07:02/39:01 C*07:02/07:02 DRB1*04:07/08:02 DQA1*03:01/04:01 DQB1*03:01/04:02 DPA1*01:03/01:03 DPB1*03:01/04:02
6113	mIAA+	13.1	1.58	11.52	female	Caucasian			24.8	head trauma	A*02:01/03:01 B*08:01/44:02 C*07:01/07:04 DRB1*01:01/03:01 DQA1*01:01/05:01 DQB1*02:01/05:01 DPA1*01:03/02:01 DPB1*04:01/14:01
6141	GADA+ IA-2A+ ZnT8A+ mIAA+	36.7	28	8.70	male	Caucasian			26	cerebrovascular/stroke	A*01:01/01:01 B*08:01/15:01 C*01:02/03:03 DRB1*03:01/04:01 DQA1*03:01/05:01 DQB1*02:01/03:02 DPA1*01:03/01:03 DPB1*02:01/04:01
6180	GADA+ IA-2A+ ZnT8A+ mIAA+	27.1	11	16.10	male	Caucasian			25.9	head trauma	A*01:01 , 02:01 DRB1*01:01 , 03:01 DQA1*01:01 , 05:01 DQB1*02:01 , 05:01
6196	GADA+ mIAA+	26.5	15	11.50	female	African Am	158.88		26.6	anoxia	A*03:01 , 30:02 DRB1*12:01 , 13:03 DQA1*01:01 , 05:01 DQB1*02:01 , 05:01
6205	mIAA+	40.9	33	7.90	female	Caucasian	46.34		22.6	anoxia	A*01:01 , 11:01 DRB1*04:01 , 16:01 DQA1*01:02 , 03:01 DQB1*03:01 , 05:02
6207	IA-2A+ ZnT8A+ mIAA+	16.7	10	6.70	female	African Am			24.4	cerebrovascular/stroke	A*23:01 , 24:02 DRB1*09:01 , 13:02 DQA1*01:02 , 03:01 DQB1*02:02 , 06:04
6211	GADA+ IA-2A+ ZnT8A+ mIAA+	24	4	20.00	female	African Am		91/10.5	24.4	anoxia	A*02:01 , 03:01 DRB1*04:05 , 12:01 DQA1*03:01 , 05:01 DQB1*03:01 , 03:02
6215	GADA+ mIAA+	34	15	19.00	male	Caucasian		64/8.0	26.6	anoxia	A*02:01, 11:01 DRB1*01:03, 04:01 DQA1*01:01, 03:01 DQB1*03:02, 05:01
6223	mIAA+	61	52	9.00	male	Caucasian			23.4	cerebrovascular/stroke	A*01:01, 29:02 DRB1*03:01, 04:01 DQA1*03:01, 05:01 DQB1*02:01, 03:02
6224	negative	21	1.5	19.50	female	Caucasian			22.8	anoxia	A*02:01, 11:01 DRB1*01:01, 04:04 DQA1*01:01, 03:01 DQB1*03:02, 05:01

6236	GADA+ mIAA+	25	11	14.00	male	Caucasian		103/11.6	20.1	anoxia	A*24:02, 24:02 DRB1*03:01, 08:01 DQA1*04:01, 05:01 DQB1*02:01, 04:02
6237	GADA+ mIAA+	18	12	6.00	female	Caucasian			26	head trauma	A*02:01, 29:01 DRB1*04:01, 13:03 DQA1*03:01, 05:01 DQB1*03:01, 03:02
6241	mIAA+	33	31	2.00	male	Caucasian			18.4	cerebrovascular/stroke	A*02:01, 03:01 DRB1*03:01, 04:01 DQA1*03:01, 05:01 DQB1*02:01, 03:02
6242	IA-2A+ mIAA+	39	19	20.00	male	Caucasian			19.5	head trauma	A*02:01, 02:01 DRB1*01:01, 04:01 DQA1*01:01, 03:01 DQB1*03:02, 05:01
6244	mIAA+	34	28	6.00	male	Caucasian	16.55	41/5.9	23.8	head trauma	A*01:01, 02:06 DRB1*03:01, 04:01 DQA1*03:01, 05:01 DQB1*02:01, 03:02
6247	mIAA+	24	0.6	23.40	male	Caucasian	155.57		24.3	head trauma	A*24:02 , 68:01 DRB1*04:04 , 11:01 DQA1*03:01 , 05:01 DQB1*03:01 , 03:02
6258	mIAA+	39	37	2.00	female	Caucasian		64/8.0	28.7	head trauma	A*02:01 , 02:01 DRB1*04:01 , 04:01 DQA1*03:01 , 03:01 DQB1*03:02 , 03:02
6261	GADA+ mIAA+	16	14.16	1.84	male	Caucasian		55/7.2	20.7	anoxia	A*03:01 , 30:01 DRB1*01:01 , 04:01 DQA1*01:01 , 03:01 DQB1*03:02 , 05:01
6262	GADA+ IA-2A+ mIAA+	44	8	36.00	male	African Am			21.5	anoxia	A*01:02,11:01 DRB1*04:01,04:05 DQA1*03:01,03:01 DQB1*03:02,03:02
6264	negative	12	9	3.00	female	Caucasian		74/8.9	22	DKA	A*23:01 , 32:01 DRB1*03:01 , 04:04 DQA1*03:01 , 05:01 DQB1*02:01 , 03:02
6265	GADA+ mIAA+	11	8	3.00	male	Caucasian	19.86		12.9	cerebrovascular/stroke	A*03:01 , 32:01 DRB1*03:01 , 04:01 DQA1*03:01 , 05:01 DQB1*02:01 , 03:02
6266	GADA+ IA-2A+ mIAA+ ZnT8A+	30	23	7.00	male	Caucasian		119/13.0	27.1	anoxia	A*01:01,03:01 DRB1*03:01,04:04 DQA1*03:01,05:01 DQB1*02:01,03:02
6298	mIAA+	29	26	3.00	male	African Am		122/13.3	24.3	anoxia	A*24:02, 74:01 DRB*04:05,13:03 DQA1*02:01,03:01 DQB1*02:02,02:02
6299	mIAA+	32	23	9.00	male	Caucasian			31.8	anoxia	A*01:01, 11:01 DRB*03:01,04:01 DQA1*03:01,05:01 DQB1*02:01,03:02
6302	negative	38.5	32.5	6.00	male	African Am	56.27	66/8.2	20.5	anoxia	A*24:02, 66:02 DRB*03:01,04:01 DQA1*03:01,05:01 DQB1*02:01,03:02
6307	GADA+ mIAA+	45	10	35.00	female	Caucasian			19.5	anoxia	A*01:01, 24:02 DRB1*03:01,03:01 DQA1*05:01,05:01 DQB1*02:01,02:01

6319	GADA+ mIAA+	52	25	27.00	male	Caucasian		70/8.6	25.5	anoxia	A*24:02, 25:01 DRB1*03:01,04:01 DQA1*03:01,05:01 DQB1*02:01,03:02
6321	IA-2A+ ZnT8A+ mIAA+	27	16	11.00	female	Caucasian		65/8.1	20.3	anoxia	A*02:01, 02:01 DRB1*04:01,11:01 DQA1*03:01,05:01 DQB1*03:02,03:02
6322	mIAA+	22	17	5.00	male	Caucasian			23.6	anoxia	A*02:01, 31:01 DRB1*03:01,03:01 DQA1*05:01,05:01 DQB1*02:01,02:01
6324	GADA+ mIAA+	29	2	27.00	male	Hispanic		88/10.2	26.2	anoxia	A*11:01, 24:02 DRB1*11:04,15:02 DQA1*01:03,05:01 DQB1*03:01,06:01
6325	GADA+ IA-2A+ mIAA+	20	6	14.00	female	African Am	46.34		31.2	anoxia	A*02:01,66:01 DRB1*04:01,15:03 DQA1*01:02,03:01 DQB1*03:01,06:02
6327	mIAA+	71.2	57	14.20	male	Hispanic			23.2	cerebrovascular/stroke	A*32:01,68:01 DRB1*03:01,03:01 DQA1*05:01,05:01
6328	GADA+ mIAA+	39	20	19.00	male	Hispanic		72/8.7	24	anoxia	A*29:02,68:01 DRB1*03:01,04:05 DQA1*03:01,05:01 DQB1*02:01,03:02
6330	IA-2A+ mIAA+	22	18	4.00	male	Caucasian			22.6	anoxia	A*01:01, 03:01 DRB1*03:01,04:01 DQA1*03:01,05:01 DQB1*02:01,03:02
6337	mIAA+	20.6	5	15.60	female	Caucasian		113/12.5	17.9	cerebrovascular/stroke	A*01:01, 33:01 DRB1*01:02,03:01 DQA1*01:01,05:01 DQB1*02:01,05:01
6341	mIAA+	26	15	11.00	male	Caucasian		125/13.6	21.8	cerebrovascular/stroke	A*26:01,29:02 DRB1*03:01,03:01 DQA1*05:01,05:01 DQB1*02:01,02:01
6360	mIAA+	4.8	2.5	2.30	female	Caucasian		88/10.2	26.1	anoxia	A*31:01,68:01 DRB1*03:01,04:04 DQA1*03:01,05:01 DQB1*02:01,03:02
6362	GADA+	24.9	0		male	Caucasian	125.78	86/10.0	28.5	head trauma	A*03:01,11:01 DRB1*01:03,03:01 DQA1*01:01,05:01 DQB1*02:01,05:01
6371	GADA+ IA-2A+ mIAA+ ZnT8A+	12.5	2	10.50	female	Caucasian	36.41	80/9.5	16.6	cerebral edema	A*01:01,68:02 DRB1*03:01,13:02 DQA1*01:02,05:01 DQB1*02:01,06:04
6380	negative	11.6	0		female	African Am	72.82	124/13.5	14.6	DKA, cerebral edema	A*33:03,68:02 DRB1*03:01,13:02 DQA1*01:02,05:01 DQB1*02:01,06:04

6396	negative	17.1	2	15.10	female	Caucasian	19.86	123/13.4	22.6	DKA, cerebral edema	A*23:01,24:02 DRB1*03:01,07:01 DQA1*02:01,05:01 DQB1*02:01,02:02
6399	GADA+ IA-2A+ ZnT8A+	17.4	0		male	Caucasian	466.71	90/10.4	32	anoxia	A*02:01,03:01 DRB1*09:01,15:01 DQA1*01:02,03:02 DQB1*03:03,06:02
6405	GADA+ IA-2A+ ZnT8A+	29.1	0.6	28.50	female	Hispanic	609.04	53/7.0	42.5	cerebrovascular/stroke	A*30:02,31:01 B*18:01,40:02 DRB1*03:01,04:07 DQA1*05:01,03:01 DQB1*02:01,03:02
Aab+											
nPOD ID	**AutoAb**	**Age (years)**	**Duration (years)**	**Age at Onset (years)**	**Sex**	**Ethnicity**	**C-Peptide (pmol/L)**	**HbA1c (mmol/mol)/ %**	**BMI (kg/m2)**	**Cause of Death**	**HiRes HLA**
6044	GADA+	41.4			male	hispanic	4485.05		27.4	no info	A*01:01/33:01 B*14:02/37:01 C*06:02/08:02 DRB1*01:02/15:01 DQA1*01:01/01:02 DQB1*05:01/06:02 DPA1*01:03/01:03 DPB1*04:01/04:02
6090	GADA+	2.2			male	Hispanic	1767.54		18.8	head trauma	A*02:05/24:02 B*07:02/40:05 C*03:04/07:02 DRB1*04:04/15:01 DQA1*01:02/03:01 DQB1*03:02/06:02 DPA1*01:03/01:03 DPB1*03:01/04:02
6101	GADA+	64.8			male	Caucasian	8665.58		34.3	stroke	A*02:01/32:01 B*44:02/51:01 C*01:02/05:01 DRB1*13:02/15:01 DQA1*01:02/01:02 DQB1*06:02/06:04 DPA1*01:03/01:03 DPB1*03:01/04:01
6123	GADA+	23.2			female	Caucasian	665.31	36/5.4	17.6	head trauma	A*02:01/24:02 B*35:01/51:01 C*01:02/03:03 DRB1*08:01/11:01 DQA1*04:01/05:01 DQB1*03:01/04:02 DPA1*01:03/01:03 DPB1*04:01/04:01
6151	GADA+	30			male	Caucasian	1817.19		24.2	anoxia	A*02:01 , 24:02 DRB1*01:01 , 07:01 DQA1*01:01 , 02:01 DQB1*02:02 , 05:01
6154	GADA+	48.5			female	Caucasian	BD		24.5	head trauma	A*02:01/03:01 B*07:02/07:02 C*07:02/07:02 DRB1*09:01/15:01 DQA1*01:02/03:01 DQB1*03:03/06:03 DPA1*01:03/01:03 DPB1*02:01/04:01

6170	GADA+	34.5			female	African Am	1419.99	52/6.9	36.9	anoxia	A*29:02 , 74:01 DRB1*04:01 , 13:03 DQA1*02:01 , 03:01 DQB1*02:02 , 03:01
6181	GADA+	31.9			male	Caucasian	198.6		21.9	head trauma	A*03:01 , 11:01 DRB1*01:01 , 04:01 DQA1*01:01 , 03:01 DQB1*03:02 , 05:01
6184	GADA+	47.6			female	Hispanic	1132.02		27	head trauma	A*02:06 , 68:03 DRB1*04:07 , 04:07 DQA1*03:01 , 03:01 DQB1*03:02 , 03:02
6197	GADA+ IA-2A+	22			male	African Am	5785.88	37/5.5	28.2	head trauma	A*02:02 , 24:02 DRB1*03:02 , 07:01 DQA1*02:01 , 04:01 DQB1*02:02 , 04:02
6267	GADA+ IA-2A+	23			female	Caucasian	5491.29	31/5.0	23.5	anoxia	A*01:01,11:01 DRB1*04:01,04:04 DQA1*03:01,03:01 DQB1*03:02,03:02
6301	GADA+	26			male	African Am	1297.52	37/5.5	32.1	head trauma	A*23:01, 23:01 DRB*11:01,13:04 DQA1*01:02,05:01 DQB1*03:19,06:02
6303	GADA+	22			male	Caucasian	1002.93	36/5.4	31.9	head trauma	A*01:01, 11:01 DRB*03:01,07:01 DQA1*02:01,05:01 DQB1*02:01,02:02
6310	GADA+	28			female	Hispanic	3488.74		22.4	anoxia	A*03:01, 30:01 DRB1*07:01,11:02 DQA1*02:01,05:01 DQB1*02:02,03:19
6314	GADA+	21			male	Caucasian	493.19		23.8	head trauma	A*02:05, 24:02 DRB1*01:03,04:01 DQA1*01:01,03:01 DQB1*03:01,05:01
6347	mIAA+	8.5			male	Caucasian	1079.06		19.5	head trauma	A*02:01,32:01 DRB1*01:01,15:01 DQA1*01:01,01:02 DQB1*05:01,06:02
6388	GADA+ mIAA+	25.2			female	Hispanic	456.78	39/5.7	26	anoxia	A*02:01,02:01 DRB1*01:02,04:07 DQA1*01:01,03:01 DQB1*03:01,05:01
6397	GADA+	21.16			female	Caucasian	4226.87	42/6.0	29.6	head trauma	A*02:01,02:01 DRB1*13:01,15:01 DQA1*01:02,01:03 DQB1*06:02,06:03
6400	GADA+	25.15			male	Hispanic	1380.27	37/5.5	22.2	head trauma	A*26:01,31:01 DRB1*04:07,13:04 DQA1*03:01,05:05 DQB1*03:02,03:19
6421	GADA+	6.73			male	Hispanic	609.04	38/5.6	17.9	head trauma	A*02:01,03:01 B*15:01,27:05 DRB1*01:01,01:01 DQA1*01:01,01:01 DQB1*05:01,05:01
6424	GADA+ mIAA+	17.7			male	Caucasian	2307.07	40/5.8	51.4	head trauma	A*30:01,68:01 B*08:01,35:03 DRB1*03:01,04:01 DQA1*05:01,03:01 DQB1*02:01,03:02

6429	GADA+ mIAA+	22.1			male	African Am	744.75	37/5.5	19.6	head trauma	A*01:01,02:01 B*44:02,81:01 DRB1*01:03,03:01 DQA1*01:01,05:01 DQB1*05:01,02:01
6433	GADA+	24			male	Hispanic	1469.64	34/5.3	30.8	head trauma	A*30:02,68:01 B*08:01,45:01 DRB1*04:05,12:01 DQA1*03:01,05:05 DQB1*03:02,03:01
6437	GADA+	27.4			male	Caucasian	2062.13	39/5.7	24.2	head trauma	A*01:01,23:01 B*08:01,18:01 DRB1*03:01,11:04 DQA1*05:01,05:01 DQB1*02:01,03:01
Type 2 Diabetes											
nPOD ID	**AutoAb**	**Age (years)**	**Duration (years)**	**Age at Onset (years)**	**Sex**	**Ethnicity**	**C-Peptide (pmol/L)**	**HbA1c (mmol/mol)/ %**	**BMI (kg/m2)**	**Cause of Death**	**HiRes HLA**
6028	negative	33.2	17	16	male	African Am	7414.4		30.2	head trauma	A*02:01/02:01 B*44:02/45:01 C*05:01/16:01 DRB1*13:01/14:01 DQA1*01:01/01:03 DQB1*05:03/06:03 DPA1*01:03/02:01 DPB1*01:01/04:01
6114	negative	42.8	2	40	male	Caucasian	191.98	62/7.8	31	anoxia	A*03:01/31:01 B*07:02/15:01 C*03:03/07:02 DRB1*07:01/15:01 DQA1*01:02/02:01 DQB1*03:03/06:02 DPA1*01:03/01:03 DPB1*04:01/04:01
6124	negative	62.3	3	59	male	Caucasian	943.35		33.7	stroke	A*29:02/29:02 B*27:05/45:01 C*02:02/06:02 DRB1*04:01/15:01 DQA1*01:02/03:01 DQB1*03:01/06:02 DPA1*01:03/01:03 DPB1*04:01/04:01
6132	negative	55.8	0	55	female	Hispanic	264.8	76/9.1	44.6	anoxia	A*01:01/02:01 B*08:01/56:01 C*01:02/07:01 DRB1*03:01/04:04 DQA1*03:01/05:01 DQB1*02:01/03:02 DPA1*01:03/02:01 DPB1*01:01/04:01
6133	negative	45.8	20	25	female	Caucasian	278.04		40.2	anoxia	no info
6139	negative	37.2	1.5	35	female	Hispanic	198.6		45.4	anoxia	A*02:01/30:02 B*40:01/57:03 C*03:04/07:01 DRB1*04:04/15:03 DQA1*01:02/03:01 DQB1*03:02/06:02 DPA1*01:03/01:03 DPB1*02:01/06:01

6142	mIAA+	29.8	14	16	female	Hispanic	62.89		34.4	infectious disease	A*24:02/80:01 B*39:06/58:01 C*07:01/07:02 DRB1*01:02/14:06 DQA1*01:01/05:01 DQB1*03:01/05:01 DPA1*01:03/01:03 DPB1*02:01/04:02
6149	GADA+	39.3	16	23	female	African Am	3823.05		29.1	stroke	A*30:02/66:02 B*07:02/53:01 C*04:01/15:05 DRB1*09:01/15:03 DQA1*01:02/03:01 DQB1*02:02/06:02 DPA1*01:03/03:01 DPB1*04:02/18:01
6188	negative	36.1	0	36	male	Hispanic	1141.95	55/7.2	30.6	anoxia	A*02:02 , 66:01 DRB1*12:01 , 14:01 DQA1*01:02 , 05:01 DQB1*03:01 , 06:04
6249	mIAA+	45	15	30	female	Asian	1380.27		32.3	stroke	A*02:01 , 24:02 DRB1*04:05 , 04:05 DQA1*03:01 , 03:01 DQB1*04:02 , 04:02
6252	negative	20			male	Caucasian	46.34	115/12.7	37.8	head trauma	A*02:06 , 24:02 DRB1*08:02 , 14:02 DQA1*04:01 , 05:01 DQB1*03:01 , 04:02
6255	negative	55	6	49	male	Caucasian	920.18		29.4	stroke	A*02:01 , 11:01 DRB1*11:01 , 12:01 DQA1*05:01 , 05:01 DQB1*03:01 , 03:01
6272	negative	57	10	47	female	African Am	2499.05		29.6	anoxia	A*23:01,33:03 DRB1*04:05,13:02 DQA1*01:02,03:01 DQB1*03:02,06:04
6273	negative	45	2	43	female	African Am	1049.27		39.1	anoxia	A*23:01,33:03 DRB1*04:05,13:02 DQA1*01:02,03:01 DQB1*03:02,06:04
6277	negative	48	10	38	male	African Am	155.57		29.5	stroke	A*23:01,23:01 DRB1*11:02,13:04 DQA1*03:01,05:01 DQB1*03:19,03:19
6280	mIAA+	47	10	37	male	African Am	1228.01		28.1	stroke	A*03:01,68:01 DRB1*04:04,07:01 DQA1*02:01,03:01 DQB1*02:02,03:02
6283	mIAA+	56	17	39	female	Caucasian	585.87	81/9.6	28.1	stroke	A*01:01,02:01 DRB1*07:01,13:01 DQA1*01:03,02:01 DQB1*02:02,06:03
6297	mIAA+	60	3	47	male	Caucasian	1069.13		29.5	anoxia	A*32:01, 68:01 DRB*01:01,13:01 DQA1*01:01,01:03 DQB1*05:01,06:03
6300	GADA+	67	>10		male	Hispanic	1055.89	48/6.5	23.5	anoxia	A*02:01, 02:06 DRB*04:05,04:07 DQA1*03:01,03:01 DQB1*03:02,03:02
6304	negative	52	25	27	female	Hispanic	774.54		37.9	stroke	A*30:02,68:02 DRB1*03:02,11:02

											DQA1*04:01,05:01 DQB1*03:19,04:02
6308	negative	13	1	12	female	Caucasian	1721.2		34.1	other	A*01:01, 24:02 DRB1*03:01,03:01 DQA1*05:01,05:01 DQB1*02:01,02:01
6329	mIAA+	49	25	24	female	Hispanic	2469.26	99/11.2	36.4	anoxia	A*24:02, 68:03 DRB1*04:11,08:02 DQA1*03:01,04:01 DQB1*03:02,04:02

Table S1. Subject Metadata.

TABLE S2 MIQE Checklist

ITEM TO CHECK	IMPORTANCE	CHECKLIST	METHOD DETAILS
EXPERIMENTAL DESIGN			
Definition of experimental and control groups	**E**	✓	
Number within each group	**E**	✓	>20
Assay carried out by core lab or investigator's lab?	D	✓	Investigator's lab
Acknowledgement of authors' contributions	D	✓	
SAMPLE			
Description	**E**	✓	
Volume/mass of sample processed	D		
Microdissection or macrodissection	**E**	**Macro**	
Processing procedure	**E**	✓	
If frozen - how and how quickly?	**E**	**snap**	
If fixed - with what, how quickly?	**E**	**N/A**	
Sample storage conditions and duration (especially for FFPE samples)	**E**	✓	Frozen in RNAlater
NUCLEIC ACID EXTRACTION			
Procedure and/or instrumentation	**E**	✓	
Name of kit and details of any modifications	**E**	✓	Qiagen RNeasy Plus Mini Kit #74134
Source of additional reagents used	D	✓	Worthington DNase I LS006344
Details of DNase or RNAse treatment	**E**	✓	
Contamination assessment (DNA or RNA)	**E**	✓	primer sets that span exon/intron boundaries in RNA only qPCR reaction
Nucleic acid quantification	**E**	✓	
Instrument and method	**E**	✓	Nanodrop 2000C
Purity (A260/A280)	D	✓	
Yield	D	✓	
RNA integrity method/instrument	**E**		
RIN/RQI or Cq of 3' and 5' transcripts	**E**		
Electrophoresis traces	D		
Inhibition testing (Cq dilutions, spike or other)	**E**	✓	
REVERSE TRANSCRIPTION			
Complete reaction conditions	**E**	✓	65° 5 m, 42° 2 m, 42° 50 m, 70° 15 m, 37° 20 m
Amount of RNA and reaction volume	**E**	✓	1 ug RNA 20 ul reaction
Priming oligonucleotide (if using GSP) and concentration	**E**	✓	oligo d(T) 0.5 ug/ul stock
Reverse transcriptase and concentration	**E**	✓	Invitrogen SuperScript II # 100004925
Temperature and time	**E**	✓	
Manufacturer of reagents and catalogue numbers	D	✓	NEB Rnase H # M0297L, Invitrogen RnaseOUT # 100000840, Invitrogen First Strand 5x buffer # Y02321
Cqs with and without RT	D	✓	
Storage conditions of cDNA	D	✓	negative 20 C
qPCR TARGET INFORMATION			
If multiplex, efficiency and LOD of each assay.	**E**	**N/A**	
Sequence accession number	**E**	✓	Table S2
Location of amplicon	D	✓	Table S3
Amplicon length	**E**	✓	Table S3
In silico specificity screen (BLAST, etc)	**E**	✓	PrimerBlast
Pseudogenes, retropseudogenes or other homologs?	D		
Sequence alignment	D	✓	PrimerBlast
Secondary structure analysis of amplicon	D	✓	PrimerBlast
Location of each primer by exon or intron (if applicable)	**E**	✓	
What splice variants are targeted?	**E**	✓	when possible all
qPCR OLIGONUCLEOTIDES			
Primer sequences	**E**	✓	Table S3
RTPrimerDB Identification Number	D	**N/A**	
Probe sequences	D	**N/A**	
Location and identity of any modifications	**E**	**No mod**	
Manufacturer of oligonucleotides	D	✓	Invitrogen
Purification method	D	✓	manufacturer's protocol
qPCR PROTOCOL			
Complete reaction conditions	**E**	✓	
Reaction volume and amount of cDNA/DNA	**E**	✓	25 ul 10 ng
Primer, (probe), Mg++ and dNTP concentrations	**E**	✓	600 nM
Polymerase identity and concentration	**E**	✓	Thermo Luminaris Color HiGreen Fluorescein qPCR Master Mix
Buffer/kit identity and manufacturer	**E**	✓	
Exact chemical constitution of the buffer	D	**Manufacturer**	
Additives (SYBR Green I, DMSO, etc.)	**E**	**SYBR**	
Manufacturer of plates/tubes and catalog number	D	✓	BioRad iCycler iQ PCR plates 2239441
Complete thermocycling parameters	**E**	✓	50° 2 m, 95° 10 m, 95° 15 s, 60° 1 m 40 cycles
Reaction setup (manual/robotic)	D	**Manual**	
Manufacturer of qPCR instrument	**E**	**Biorad MyiQ**	
qPCR VALIDATION			
Evidence of optimisation (from gradients)	D	✓	
Specificity (gel, sequence, melt, or digest)	**E**	**Melt**	
For SYBR Green I, Cq of the NTC	**E**	✓	
Standard curves with slope and y-intercept	**E**	**N/A**	
PCR efficiency calculated from slope	**E**	**N/A**	
Confidence interval for PCR efficiency or standard error	D	**N/A**	
r2 of standard curve	**E**	**N/A**	
Linear dynamic range	**E**	✓	
Cq variation at lower limit	**E**	✓	
Confidence intervals throughout range	D	✓	
Evidence for limit of detection	**E**	✓	
If multiplex, efficiency and LOD of each assay.	**E**	**N/A**	
DATA ANALYSIS			
qPCR analysis program (source, version)	**E**	✓	RefFinder, Prism 6
Cq method determination	**E**	✓	BioRad iCycler iQ
Outlier identification and disposition	**E**	**No**	
Results of NTCs	**E**	✓	
Justification of number and choice of reference genes	**E**	✓	RefFinder, Δ Cq, NormFinder, BestKeeper, geNorm
Description of normalisation method	**E**	✓	geNorm
Number and concordance of biological replicates	D		
Number and stage (RT or qPCR) of technical replicates	**E**	✓	2 replicates
Repeatability (intra-assay variation)	E	✓	
Reproducibility (inter-assay variation, %CV)	D	✓	
Power analysis	D		
Statistical methods for result significance	**E**	✓	one way ANOVA, student's t-test, linear regression
Software (source, version)	E	✓	Graphpad Prism 8
Cq or raw data submission using RDML	**D**	**Raw only**	

Gene OMIM # Cytogenetic location Genomic coordinates	**Protein/** ***Mode of inheritance***	***MODY***	***Neonatal Diabetes Mellitus (NDM)***	***TNDM***	***PNDM***	***NDM autoimmunity***	***Syndromic***	***Reference***
ABCC8 600509 11p15.1 11:17,392,884-17,476,869	-ATP-binding cassette transporter sub-family C member 8 ***-Spontaneous, dominant or recessive***	*12*	*X*		*X*		*familial hyperinsulinemic hypoglycemia-1 (HHF1)*	***N Engl J Med*** *355: 456-466, 2006.* ***Diabetologia****. 55(1):123-7, 2012.*
APPL1 604299 3p14.3 3:57,227,736-57,273,470	-Adaptor Protein, Phosphotyrosine Interaction, PH domain, and leucine zipper containing 1 ***-Dominant***	*14*						Am J Hum Genet. 97(1):177-85, 2015.
BLK 191305 8p23.1 8:11,493,990-11,564,598	-B lymphocyte kinase ***-Dominant***	*11*						***PNAS*** *106(34):14460-5, 2009.* ***Diabetologia.*** *56(3):492-6, 2013.* ***PLOS ONE*** *12(1):1-15, 2017*
BSCL2 606158 11q12.3 11:62,690,261-62,709,618	-Seipin ***-Recessive***		*X*				*Congenital Generalized Lipodystrophy Type 2*	***Nat Genet.*** *28(4):365-70, 2001.*
CEL 114840 9q34.13 9:133,061,977-133,071,862	-Carboxyl-ester lipase ***-Dominant***	*8*						***Nat Genet.*** *38(1):54-62, 2006.* Hum Genet. 127(1):55-64, 2010.
DNAJC3 601184 13q32.1 13:95,677,138-95,794,988	-Inhibitor of Protein Kinase, Interferon-Inducible Double-Stranded RNA-Dependent Kinase (PKR) PRKRI ***-Recessive***						*Diabetes Mellitus and Multisystemic Neurodegeneration*	Diabetes 54: 1074-1081, 2005. Am. J. Hum. Genet. 95: 689-697, 2014.
EIF2AK3 604032 2p11.2 2:88,556,739-88,627,575	-Eukaryotic translation initiation factor 2-alpha kinase 3 (PERK) ***-Recessive***		*X*		*X*		*Wolcott-Rallison syndrome*	Nat Genet. 25(4):406-9, 2000.
FOXP3 300292 Xp11.23 X:49,250,435-49,266,504	-forkhead box P3 ***-X-linked, recessive***		*X*		*X*	*X*	*Immune dysregulation, IPEX Syndrome, X-linked- IDDMX locus*	***Nat Genet****. 27(1):18-20, 2001.*
GATA4 600576 8p23.1 8:11,676,918-11,760,001	-Transcription factor GATA-4 ***-Dominant***		*X*		*X*		*Permanent neonatal diabetes with pancreatic agenesis and congenital heart defects*	***Diabet Med.*** *27(10):1195-200, 2010.*
GATA6 601656 18q11.2 18:22,169,436-22,202,527	-GATA-binding factor 6 ***-Dominant***		*X*		*X*		*pancreatic agenesis insulin-treated neonatal diabetes and exocrine pancreatic insufficiency*	***Nat Genet****. 44(1):20-2, 2011.* ***Diabetes****. 62(3):993-7, 2013.*
GCK 138079 7p13 7:44,143,212-44,189,438	-Glucokinase ***-Recessive***	*2*			*X*			***Nature.*** *23;356(6371):721-2, 1992.*
GLIS3 610192 9p24.2 9:3,824,126-4,310,693*****	-GLIS Family Zinc Finger 3 ***-Recessive***		*X*		*X*		*Diabetes mellitus, neonatal, with congenital hypothyroidism; NDH syndrome*	***Nat Genet****. 38(6):682-7, 2006.*
HNF1A 142410 12q24.31	-Hepatocyte nuclear factor 1-alpha ***-Dominant***	*3*						***Nature****. 384(6608):455-8, 1996.* ***Diabetes.*** *46(4):720-5, 1997.*

12:120,978,514 -121,002,511								
HNF1B 189907 17q12 17:37,686,430-37,745,077	-Hepatocyte nuclear factor 1-beta ***-Dominant***	*5*	*X*				*Renal cysts and diabetes syndrome pancreatic agenesis*	***Nat Genet.*** *17(4):384-5, 1997.*
HNF4A 600281 20q13.12 20:44,355,800-44,432,844	-Hepatocyte nuclear factor 4-alpha ***-Dominant***	*1*						***Nature.*** *384(6608):458-60, 1996.*
IER3IP1 609382 18q21.1 18:47,155,018-47,176,373	-Immediate early response 3-interacting protein 1 ***-Recessive***		*X*		*X*		*Microcephaly, epilepsy, and diabetes syndrome; MEDS*	***Am J Hum Genet.*** *89(2):265-76, 2011.*
IL2RA 147730 10p15.1 10:6,010,693-6,062,369**	-Interleukin-2 receptor alpha chain ***-Susceptibility locus***		*X*			*X*	*Immunodeficiency 41 lymphoproliferation and autoimmunity; IMD41*	J Allergy Clin Immunol 119: 482-487, 2007
INS 176730 11p15.5 11:2,159,778-2,161,208***	-Insulin ***-Spontaneous or dominant***	*10*	*X*	*X*	*X*			***Diabetes.*** *57(4):1034-42, 2008*
ITCH 606409 20q11.22 20:34,363,234-34,511,772	-E3 Ubiquitin Protein Ligase ***-Recessive***					*X*	*Autoimmune disease, multisystem, with facial dysmorphism; ADMFD*	***Am J Hum Genet.*** *86(3): 447–453, 2010.*
KCNJ11 600937 11p15.1 11:17,385,245-17,389,330	-inward-rectifying ATP-sensitive K^+ channel ***-Spontaneous or dominant***	*13*	*X*	*X*	*X*		*Hyperinsulinemic Hypoglycemia*	***PLoS One.*** *;7(6): e37423, 2012*
KLF11 603301 2p25.1 2:10,043,554-10,054,835	-Krueppel-like factor 11 ***-Dominant***	*7*						J. Biol. Chem. 286, 28414–28424, 2011.
LRBA 606453 4q31.3 4:150,264,514-151,015,724	-Lipopolysaccharide-responsive and beige-like anchor protein ***-Recessive***		*X*			*X*	*common variable immunodeficiency-8 with autoimmunity; CVID8*	***J Clin Endocrinol Metab.*** *101(3):898-904, 2016.*
MNX1 142994 7q36.3 7:157,004,852-157,010,652	-motor neuron and pancreas homeobox 1 ***-Recessive***		*X*		*X*		*Neonatal Diabetes; Currarino Syndrome*	***Diabetes Metab.*** *39(3):276-80, 2013.* ***Cell Metab.*** *19(1):146-54, 2014.*
NEUROD1 601724 2q31.3 2:181,676,105-181,680,664	-Neurogenic differentiation 1 ***-Recessive***	*6*	*X*		*X*			***Diabetes*** *59(9):2326-31, 2010.*
NEUROG3 604882 10q22.1 10:69,571,439-69,573,453	-Neurogenin-3 ***-Recessive***		*X*		*X*		*childhood-onset diabetes; Permanent Neonatal Diabetes and Enteric Anendocrinosis; DIAR4*	***Diabetes*** *60(4):1349-1353, 2011.*
NKX2-2 604612 20p11.22 20:21,511,009-21,514,025	-Homeobox protein Nkx-2.2 ***-Recessive***		*X*		*X*		*Neonatal Diabetes*	***Cell Metabolism*** *19:146-154, 2014.*
NR0B2 604630 1p36.11	-Nuclear Receptor Subfamily 0 Group B Member 2 (SHP)		*X*				*Diabetes associated with mild to moderate obesity*	***PNAS*** *98:575-580, 2001.*

1:26,911,483-26,914,075	***-Dominant (obesity not diabetes)***							
PAX4 167413 7q32.1 7:127,610,291-127,618,191	-Paired box gene 4 ***-Dominant***	*9*						***Acta Diabetol.*** *53(2):205-16, 2016.*
PAX6 607108 11p13 11:31,784,791-31,817,960	-Paired box protein Pax-6 ***-Recessive***						*Aniridia and Glucose Intolerance*	***Diabetes*** *51:224–230, 2002.*
PCBD1 126090 10q22.1 10:70,882,279-70,888,785	-pterin-4 α-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 α ***-Recessive***		*X*		*X*		*Early-onset non-autoimmune diabetes with features similar to dominantly inherited HNF1A-diabetes*	***Diabetes*** *63(10):3557-64, 2014.*
PDX1 600733 13q12.2 13:27,919,981-27,926,313	-Pancreatic duodenal homeobox 1 ***-Recessive***	*4*	*X*		*X*		*Permanent neonatal diabetes +/- pancreatic agenesis*	***Diabet Med*** *30(5): e197-200, 2013.*
PLAGL1 603044 6q24.2 6:143,940,299-144,064,598	-Pleomorphic adenoma gene-like 1 ***-Variable (imprinting)***		*X*	*X*			*transient neonatal diabetes mellitus.*	***Hum. Molec. Genet.*** *9: 453-460, 2000.* ***Hum. Genet.*** *110: 139-144, 2002.*
PTF1A 607194 10p12.2 10:23,192,530-23,194,251	-Pancreas transcription factor 1 subunit alpha ***-Recessive***		*X*		*X*		*cerebellar and pancreatic agenesis*	***Nat Genet.*** *36(12):1301-5, 2004.*
RFX6 612659 6q22.1 6:116,877,212-116,932,162	-Regulatory factor X, 6 ***-Recessive***		*X*		*X*		*Permanent neonatal diabetes; pancreatic hypoplasia, intestinal atresia, gallbladder aplasia or hypoplasia: Mitchell-Riley Syndrome*	***Nature.*** *463(7282):775-80, 2010.*
SIRT1 604479 10q21.3 10:67,884,668-67,918,389	-NAD-dependent deacetylase sirtuin-1 ***--Dominant***					*X*	*Late onset 7,12, 15 and 27 yo T1D diagnosis*	***Cell Metabolism*** *17: 448–455, 2013.*
SLC19A2 603941 1q24.2 1:169,463,908-169,485,969	-solute carrier family 19 member 2 (Thiamine transporter 1) ***-Recessive***		*X*		*X*		*Thiamine responsive megaloblastic anaemia, diabetes and deafness (TRMA) syndrome*	***Pediatric Diabetes*** *13(4):314-21, 2012.* ***Nat Genet*** *22: 300-304, 1999*
SLC2A2 138160 3q26.2 3:170,996,340-171,026,978	-solute carrier family 2 member 2 (GLUT2) ***-Recessive***		*X*	*X*			*Fanconi-Bickel Syndrome*	***Nat Genet.*** *17(3):324-6, 1997.*
STAT1 600555 2q32.2 2:190,969,035-191,014,249	-Signal transducer and activator of transcription 1 ***-Dominant***					*X*	*Immunodeficiency 31A,C; Gain-of-function mutations in STAT1 can cause an IPEX-like phenotype*	J Allergy Clin Immunol. 131(6):1611-23, 2013
STAT3 102582 17q21.2 17:42,313,323-42,388,504	-Signal transducer and activator of transcription 3 ***-Dominant***		*X*		*X*	*X*	*Infantile-Onset Multisystem Autoimmune Disease 1 (ADMIO1)*	***Blood*** *125:591-59, 2015.* ***Nat Genet.*** *46(8):812-4, 2014.*
STAT5B 604260 17q21.2 17:42,199,176-42,276,459	-Signal transducer and activator of transcription 5					*X*	*Growth hormone insensitivity with immunodeficiency*	***Ann N Y Acad Sci.*** *1079:198-204, 2006* ***Diabetes*** *55(10):2705-12, 2006*

								J Biol Chem. 279):11553-61, 2004
TRMT10A 616013 4q23 4:99,546,706-99,564,056	-tRNA methyltransferase homolog gene ***-Recessive***		*X*				*Microcephaly, short stature, and impaired glucose metabolism 1*	*PLOS Genetics 9:1-15, 2013.*
WFS1 606201 4p16.1 4:6,260,367-6,303,264	-Wolframin ***-Recessive***						*Wolfram syndrome (Diabetes insipidus, diabetes mellitus, optic atrophy and deafness, DIDMOAD)*	*Nat Genet. 20(2):143-8, 1998.*
ZFP57 612192 6p22.1 6:29,672,391-29,681,149	-Zinc finger protein 57 homolog ***-Recessive***		*X*	*X*			*Transient neonatal diabetes*	*Nat Genet. 40(8):949-5, 2008.*

Table S3

The annotation of this table was derived from data associated with the University of Exeter Molecular Genetics Laboratory (http://www.diabetesgenes.org/sites/default/files/tngs_genes_v4.pdf) and the Online Mendelian Inheritance in Man® (OMIM®) site (https://www.omim.org/). Genomic coordinates are derived from the reference genome GRCh38.

* The GLIS3 gene associates with the markers: rs10758593; rs7020673; and rs6476839 (Immunobase https://www.immunobase.org/disease/T1D/).

** The IL2RA gene associates with the markers:
rs61839660; rs12251307; rs2104286; rs41295121; rs7090530; and rs10795791.

*** The INS gene associates with the markers:
rs72853903; rs689; rs7111341; and rs7928968.

GENE	*FORWARD*	*REVERSE*	*ACCESSION #*
ABCC8	ATGCTGCTCTTCGTCCTGGT	CAAACTTGATGGTCTTGGTGATG	NM_001287174.1
ALS2	TCTGACTGAAGATGGTGAGGTC	ATTCTCTCCCCACATGTACGC	NM_020919.3
APPL1	TTTGTTCTTCGGACATCAAGCG	GTTTTGCCAGTCCAACAGAATCA	NM_012096.2
ASNS	TGAGATAGAAACTGTGAAGAACAACCT	TAGCAGCCAGTAAATCGGGG	NM_133436.3
ATF4	TCTAAAAGAGAGGGCGGATTCC	CTACAGCACTCTATGTACAAGCACA	NM_182810.2
ATF6	GTTATTCAGTCTCGTCTCCTCGG	CAGTCCATTTTCAGTCTTGTTCCTA	NM_007348.3
BLK*	ATGGTGTTGGAAGTTGCTCGT AACACCACTGAAGCATTGCC	TGAAGACAACCAGGGGCG	NM_001715.2
BLOC1S1	CCTCAATGTGGGTGTGGCC	CGAGCCCAGTTCTCCACATC	NM_001487.3
BSCL2	TATATGCCGACAGTCAGCCAC	CCATACATCAGCACCCGATCA	NM_001122955.3
CEL	GACGCCAATCTGCCAGGTAA	CGGATGAGGCCCTTGTTGTAG	NM_001807.4
COL6A2	AGCCTACGGAGAGTGCTACAA	CTCCAAATTCACCCTTCTCTCCTTT	NM_001849.3
DDIT3/CHOP	CCTGGAAATGAAGAGGAAGAATCAA	TCTGACTGGAATCTGGAGAGTGA	NM_001195057.1
DNAJB9	TCGGAGGGTGCAGGATATTAGA	TTGATTTGGCGCTCTGATGC	NM_012328.2
DNAJB11	GGCTCCCCAACTTTGACAAC	AGCTGTTTGATACCTTCTCTCGC	NM_016306.5
DNAJC3	TCAGTGAAGTTCGGGAATGTCTT	TAGCATCTGTGTATCTGCCATCT	NM_006260.4
DUT-M	TTACGTCTCTGCTTCGCTCAG	CGCCTTAGGAAGCTCGCC	NM_001025248.1
DUT-N	GTCTCCTCGCTCGCCTTC	CGCTTACTGGGTGAAATGGC	NM_001948.3
EDEM1	ATATGGTGCCCTCCCTGAGA	GGATTCTTGGTTGCCTGGTAGA	NM_014674.2
EIF2AK1	CGAGTCTGCCTGTCCTTATGTT	TCGGTGCACAATTCCCATGT	NM_014413.3
EIF2AK2	AATTGGCCGCTAAACTTGCA	GATGATTCAGAAGCGAGTGTGC	NM_001135652.2
EIF2AK3	CACACAGGACAAGTAGGGACC	GTTAAGGTCCTGACTCTCTCCATC	NM_004836.6
EIF2AK4	CTCGGCATCAAGTTACAGGTCTT	CCTTCGTTTGATGAAAGCCACAA	NM_001013703.3
EIF2B1	GACTCACGCCTACTCCAGAG	GGCCATTTTCTTACCTGACAAATCA	NM_001414.3
EIF2S1	AGAGACCTGGATATGGTGCCTA	AGCCACTTCAATATCTGCTCGA	NM_004094.4
ERN1/IRE1α	TCCAGTTCTTCCAGGACGTGA	TGATGTTCTCCCGCCAGTC	NM_001433.4
FEN1	CTACCGAGGACATGGACTGC	CCACAAACTGTTCCTGGTTCAG	NM_004111.5
FOXO3	TAGGGTCCTGAGAACTTCTGAGTTC	CCAGGGTCTGTAAAACTGCAAA	NM_001455.3
FOXP3	TTCATCTGTGGCATCATCCGAC	GAGCGTGGCGTAGGTGAAA	NM_001114377.1
GATA4	CTCTACATGAAGCTCCACGGG	TGAAGGAGCTGCTGGTGTCTTA	NM_001308093.1
GATA6	AGCAAGATGAACGGCCTCA	TGGTAGTTGTGGTGTGACAGT	NM_005257.5
GCK	AACAATGTCGTGGGGCTTCT	TGATGGTCTTCGTAGTAGCAGGA	NM_000162.3
GLIS3	TGGACACCAAACCTTATGCTTGT	CTGGACCGCAACTTTTTCCTT	NM_001042413.1
HNF1A	CTCATCACCGACACCACCAA	GCTGGAGGACACTGTGGG	NM_000545.6
HNF1B	TGTCAGGAGTGCGCTACAG	GGGAGAGGCTGTGGATATTCG	NM_001304286.1
HNF4A	CTCGTTGAGTGGGCCAAGTA	GTAGTCATTGCCTAGGAGCAGC	NM_001287182.1
HSP90B1	GTGAAAAGCGGCCCGAC	CAAGTCCCCTTCGATCGCAG	NM_003299.2
HSPA5	TTTTAATGATGCCCAACGCCA	CAGCTGCCGTAGGCTCG	NM_005347.4
IER3IP1	GAGAGAAATCGCTTGGACTTCG	TGGCACTCTCATCACGGTTC	NM_016097.4
IL2RA	GGGGACTGCTCACGTTCAT	GGATCTCTGGCGGGTCATC	NM_001308243.1
ITCH	ATCATGTGGTTTTGGCAGTTTGTTA	AGAATTTCTGTGGTCCATTGCTCC	NM_001324197.1
KCNJ11	CAGCATCCACTCCTTCTCGTC	GGGCACTCCTCAGTCACCAT	NM_001166290.1
KLF11	CCCATCTTCGCACTCACACA	TGACAGCTCATCCGAACGAG	NM_001177718.1
LRBA	TGCTCATCAAGGTGCTGTACAAG	TTATCCCAGAAGCCACAGACG	NM_006726.4
MAFB	AAGAGAGCAACGAGGAAAGGAG	GAGCAGGCAATAAAACTGATGAGA	NM_005461.4
MBTPS1	TGGACGCCTTCAACTATGCC	GCTGTTAATTCCCACACCTTGTC	NM_003791.3
MBTPS2	TCCCAGTTAGAGCATACAAACGA	TTGCTTCAACTGCTTTCCGG	NM_015884.3
MNX1	GGAGCACCAGTTCAAGCTCA	AATCTTCACCTGGGTCTCGG	NM_005515.3
MSMO1	GCTTTGGTTGTGCAGTCATTGA	GGTCACCCATGCCCAAAGAA	NM_001017369.2
MVK	ACACCAAAGTCCCTCGCAAT	TCTGGGAACTTGAGCAGCC	NM_001301182.1
NEUROD1*	CACTATCCTGCAGCGACACT ACTGCTCAGGACCTACTAACAAC	CGGAAATGGTGAAACTGGCG ACACTCGTCTGTCCAGCTTG	NM_002500.4
NEUROG3*	CCGGTAGAAAGGATGACGCC	TGCCAACTCGCTCTTAGGC	NM_020999.3
NELFA	AGAACCTGTCCCTCACGAGA	GGCACGGGTTCTCTCGG	NM_005663.4
NFE2L2	CTCCACAGAAGACCCCAACC	AAGTAGCAGGTGAGGGCATG	NM_006164.5

NKX2.2	TACTCCCTGCACGGTCTGG	CCGCTTTCGCTTCTTGCC	NM_002509.3
NR0B2	TTCAACCCCGATGTGCCAG	GATAGGGCGAAAGAAGAGGTCC	NM_021969.2
PAX4	CACGGCTCAGGTCACCAG	CCAGACCCTCACCGTGTC	NM_006193.2
PAX6	GTTGGTATCCGGGGACTTCG	TCTCTCAAACTCTTTCTCCAGGG	NM_001310159.1
PCBD1	TTCAAAGACTTCAACAGGGCCT	GGTGGTCCAGTTTCTCAGCC	NM_000281.3
PDIA4	CCTTATGACTACAACGGCCCA	TCAGGTTGTTAGCGGCATCC	NM_004911.4
PDX1	GATGAAGTCTACCAAAGCTCACG	ACATGACAGCCAGCTCCAC	NM_000209.3
PLAGL1	CCCACGACCCCAACAAAATG	CCAGGTGCCTCTTATAGCCC	NM_001317157.1
PPIA	GTGGTATAAAAGGGGCGGGAG	TGCTGTCTTTGGGACCTTGT	NM_001300981.1
PPP1R15A	GCTCTTATCGGTTCCCATCCC	ATGTGTCTGGGCGGCTG	NM_014330.3
PSEN1	CCAGAGGAAAGGGGAGTAAAACT	GGTTGTGTTCCAGTCTCCACT	NM_000021.3
PTF1A	CGACCCTGATTATGGCCTCC	GATCTTCAGCCGAGTCTGGG	NM_178161.2
RFX6	ATTTCAGGCAGCACAGACACT	TGACCTTCCATTTTGTTTGCTGG	NM_173560.3
SIRT1	ACTTCGCAACTATACCCAGAACA	TGTTGCAAAGGAACCATGACAC	NM_001314049.1
SLC2A2	AGGGGAGCACTTGGCACTT	GGTATCTGGGGCTTTCTGGAC	NM_000340.1
SLC19A2	AGCCAGACCGTCTCCTTGTA	ACAGCAACAGCACCCAGTAA	NM_001319667.1
STAT1	GTGATCTCCAACGTCAGCCA	TGGCGTTAGGACCAAGAAGC	NM_139266.2
STAT3	GGAAAGTATTGTCGGCCAGAGA	TTCAGCACCTTCACCATTATTTCCA	NM_213662.1
STAT5B	TGCTTGGAAGTTTGATTCTCAGG	CTTCACGTATCCATCAACAGCTT	NM_012448.3
TMBIM6	TGGTCACTCATTTCATTCAGGCT	ATCAGCCAAATCATCAATATCAGGG	NM_003217.2
TP53	ATTTCACCCTTCAGATCCGTGG	TTATGGCGGGAGGTAGACTGA	NM_001126115.1
TRMT10A	AGCACCCTTCGCCTTATTATTGA	TGTCAAGTAAAACTGCACAGGATG	NM_152292.4
VCP	AGGGGAGCCTATCAAACGAGA	TCTAGGAGGCTTCACACCAATTG	NM_001354927.1
WFS1	TGCGTCTGAAGGTGGTCAAG	GTGGGGATGATGGTGGACAG	NM_006005.3
XBP1s	GTGAGTCCGCAGCAGGTG	GGGTCCTTCTGGGTAGACCT	NM_001079539.1
XBP1u**	CGCACCTGAGCCCCGA	CTAAATCTACCACTTGCTGTTCC	NM_005080.3
ZFP57	GTGAAGAAGAAGCCAGTCACCTT	GATGGACAAACTCTCTCCACTGT	NM_001109809.2

Table S4. Primer sequences

*PRIMER SET(S) DID NOT AMPLIFY
**THIS PRIMER SET DETECTS BOTH SPLICED AND UNSPLICED MESSAGES

Monogenic Diabetes Genes			
	T1D (n=25-30)		
Gene	***Fold Difference***	***p-Value***	***q-Value***
ABCC8	0.45	0.667	0.174
BLK	ND		
DNAJC3	1.85	0.221	0.078
FOXP3	ND		
GCK	0.51	0.625	0.168
IER3IP1	1.53	0.266	0.087
IL2RA	ND		
INS*			
KCNJ11	1.49	0.409	0.122
NEUROD1	ND		
NEUROG3	ND		
NKX2.2	0.41	0.121	0.051
PAX4	ND		
PAX6	1.30	0.594	0.163
PCBD1	1.51	0.166	0.064
PDX1	1.45	0.099	0.045
PTF1A	1.55	0.103	0.046
RFX6	0.87	0.923	0.226
SLC19A2	2.13	0.118	0.051
SLC2A2	2.38	0.098	0.045
ZFP57	0.45		

Table S5. Monogenic diabetes genes which did not display altered gene expression.
ND = no amplification; *Insulin (INS) data published previously: Cell Metab. 2017 Sep 5;26(3):568-575.e3. doi: 10.1016/j.cmet.2017.08.013. Note all values above 1 did not reach statistical significance ($p < 0.05$).

	HUMAN SPLEEN GENE EXPRESSION			
	FOLD DIFFERENCE	***p Value***	***CONTROL***	***T1D***
EIF2AK3	1.27	1.0	0/10	0/10
STAT5B	1.06	0.836	0/10	0/10
WFS1	1.35	0.780	0/10	0/10
GATA4	ND			
GLIS3	0.96	0.618	0/10	0/10
LRBA	1.42	1.0	0/10	0/10
STAT1	1.13	1.0	0/10	0/10
HNF1B	0.71	0.714	1/10	1/10
SIRT1	1.52	0.893	0/10	0/10
GATA6	1.99	0.308	0/8	0/10
ITCH	1.14	0.893	0/10	0/10
STAT3	0.95	0.671	0/10	0/10
MNX1	ND			
PLAGL1	1.05	0.836	0/10	0/10
CEL	0.889	0.826	0/10	0/9
TRMT10A	1.36	0.950	0/10	0/10
BSCL2	0.95	0.950	0/10	0/10
APPL1	1.05	0.836	0/10	0/10
KLF11	1.94	0.702	0/9	0/10

Table S6. Normalized RTqPCR data comparing control to T1D spleen. GATA4 and MNX1 did not amplify. RefFinder (http://150.216.56.64/referencegene.php?type=reference) was utilized to determine spleen reference genes, CALM1 and MAFB. Normalization factors were calculated using the geometric mean of relative quantities for both control and T1D samples. Control and T1D columns indicate non-amplified samples/n.

Physiological Grouping of Monogenic Diabetes Genes							
Immune		**β -cell Function**		**β-cell Development**		**ER Function/Stress**	
FOXP3	XR	ABCC8	AR/AD	GATA4	AD	BSCL2	AR
IL2RA		APPL1	AD	GATA6	AD	***CEL***	***AD***
LRBA	AR	BLK	AD	GLIS3	AR	DNAJC3	AR
SIRT1	***AR***	***CEL***	***AD***	***HNF1B***	***AD***	***DUT-N***	***AR***
STAT1	***AD***	***DUT-N***	***AR***	MNX1	AR	***DUT-M***	***AR***
STAT3	***AD***	***DUT-M***	***AR***	NEUROD1	AR	EIF2AK3	AR
STAT5B	AR	GCK	AR	NEUROG3	AR	***GATA4***	***AD***
		GATA4	*AD*	NKX2-2	AR	***GATA6***	***AD***
		HNF1A	AD	PAX4	AD	***GLIS3***	***AR***
		HNF1B	***AD***	PAX6	AR	***HNF4A***	***AD***
		HNF4A	***AD***	PDX1	AR	IER3IP1	AR
		INS	AD	PTF1A	AR	ITCH	AR
		KCNJ11	AD	RFX6	AR	***SIRT1***	***AD***
		KLF11	AD	***SIRT1***	***AR***	***STAT1***	***AD***
		NR0B2	AD	***TRMT10A***	***AR***	***STAT3***	***AD***
		PCBD1	AR			WFS1	AR
		PLAGL1	IMP				
		SLC19A2	AR				
		SLC2A2	AR				
		TRMT10A	***AR***				
		ZFP57	IMP				

Table S7. Monogenic diabetes genes sorted into 4 physiological groups: Immune, b-cell Function, b-cell Development and ER Function/Stress. Bold and Italics indicates inclusion in another Group. The following denotes the genetic inheritance pattern for each monogenic diabetes gene: Autosomal Recessive (AR); Autosomal Dominant (AD); Imprinted (IMP); X-Linked Recessive (XR).