Diabetes Susceptibility Genes \( Pdx1 \) and \( Clec16a \) Function in a Pathway Regulating Mitophagy in \( \beta \)-Cells

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Mitophagy is a critical regulator of mitochondrial quality control and is necessary for elimination of dysfunctional mitochondria to maintain cellular respiration. Here, we report that the homeodomain transcription factor \( Pdx1 \), a gene associated with both type 2 diabetes and monogenic diabetes of the young, regulates mitophagy in pancreatic \( \beta \)-cells. Loss of \( Pdx1 \) leads to abnormal mitochondrial morphology and function as well as impaired mitochondrial turnover. High-throughput expression microarray and chromatin occupancy analyses reveal that \( Pdx1 \) regulates the expression of \( Clec16a \), a type 1 diabetes gene and itself a key mediator of mitophagy through regulation of the E3 ubiquitin ligase \( Nrdp1 \). Indeed, expression of \( Clec16a \) and \( Nrdp1 \) are both reduced in \( Pdx1 \) haploinsufficient islets, and reduction of \( Pdx1 \) impairs fusion of autophagosomes containing mitochondria to lysosomes during mitophagy. Importantly, restoration of \( Clec16a \) expression after \( Pdx1 \) loss of function restores mitochondrial trafficking during mitophagy and improves mitochondrial respiration and glucose-stimulated insulin release. Thus, \( Pdx1 \) orchestrates nuclear control of mitochondrial function in part by controlling mitophagy through \( Clec16a \). The novel \( Pdx1\)-\( Clec16a\)-\( Nrdp1 \) pathway we describe provides a genetic basis for the pathogenesis of mitochondrial dysfunction in multiple forms of diabetes that could be targeted for future therapies to improve \( \beta \)-cell function.

Pancreatic \( \beta \)-cells depend on mitochondrial respiration to generate the ATP required for glucose-stimulated insulin secretion (GSIS), which is required to maintain glucose homeostasis and is impaired in all forms of diabetes (1). Complex regulatory networks coordinate mitochondrial respiration, which depends on the maintenance of both mitochondrial mass and metabolic function. Mitochondrial autophagy or mitophagy is a quality-control mechanism that is necessary for the selective elimination of dysfunctional mitochondria to maintain cellular respiration (1).

Despite the importance of mitophagy to cellular function, direct transcriptional regulators of mitophagy have never been identified in mammalian cells. The helix-loop-helix transcription factor Rtg3, a regulator of the retrograde signaling pathway (RTG), is a transcriptional regulator of mitophagy in \( Saccharomyces cerevisiae \); however, no conserved mammalian ortholog of Rtg3 has been identified (2). Pancreatic and duodenal homeobox (\( Pdx1 \)) is a homeodomain transcription factor associated with type 2 diabetes (T2D) and monogenic diabetes of the young (3,4). \( Pdx1 \) is a master regulator of pancreatic \( \beta \)-cell development and adult \( \beta \)-cell function and is vital for upstream control of insulin gene transcription, endoplasmic reticulum (ER) homeostasis, \( \beta \)-cell survival, and mitochondrial respiration (5–7). While \( Pdx1 \) deficiency has been associated with impaired mitochondrial function, the pathways by which \( Pdx1 \) controls mitochondrial metabolism have not fully been elucidated.

Here, we report that \( Pdx1 \) regulates mitochondrial function through its transcriptional control of mitophagy in pancreatic \( \beta \)-cells. \( Pdx1 \) directs autophagosome-lysosome fusion during mitophagy through transcriptional regulation of C-type lectin domain family 16, member A (\( Clec16a \),...
a type 1 diabetes (T1D) gene that we recently found to be a mediator of mitophagy in pancreatic β-cells through its interaction with, and regulation of, the E3 ubiquitin ligase neuregulin receptor degradation protein (Nrdp)1 (8). Overexpression of Clec16a restores Nrdp1 expression and mitophagy and ameliorates defects in mitochondrial respiration and insulin secretion after Pdx1 deficiency.

RESEARCH DESIGN AND METHODS

Animals
Pdx1 heterozygous mice, Pdx1lacZko (courtesy of Dr. Chris Wright, Vanderbilt University), and Pdx1TTA (courtesy of Dr. Raymond MacDonald, University of Texas Southwestern Medical Center) were maintained on a C57BL/6 background and housed on a standard 12-h light/12-h dark cycle with ad libitum access to food and water. Similar results were observed between both Pdx1 heterozygous mouse models and were used interchangeably for animal studies. All animal procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Oxygen Consumption Assays
Oxygen consumption was measured on isolated islets by a Clark-type O2 electrode (Strathkelvin Instruments) or adherent cells using a XF24 Flux Analyzer (Seahorse Bioscience) as previously described (8).

Chromatin Immunoprecipitation
Chromatin isolated from three independent nondiabetic human islet donors (kindly provided by Dr. Ali Naji, University of Pennsylvania) was isolated and prepared as previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9). Immunoprecipitations were performed with anti-Pdx1 antiserum previously described (9).

Transmission Electron Microscopy
Pancreata were harvested from Pdx1+/+ and Pdx1−/− littersmates, minced into small pieces, and fixed in 4% PFA, embedded in paraffin, and sectioned. Antisera were guinea pig anti-insulin (Dako), mouse anti-Mfn2 (Abcam), and species-specific Cy2-, Cy3-, and Cy5-conjugated secondary antisera (Jackson Immunoresearch). Nuclear labeling was performed with DAPI (Molecular Probes).

Analysis of Islet cDNA Microarrays
High-throughput gene expression profiling of islets (~12,000 targets in total) from Pdx1+/+ and Pdx1−/− littersmates was previously performed as described (7). Microarray results were filtered for overlap with the mouse MitoCarta database (11) supplemented with 51 additional autophagy/mitophagy targets, including ambra1, atg10, atg12, atg13, atg14, atg16, atg2, atg3, atg4, atg5, atg7, atg9, becn1, bfl1, bnip3l, clec16a, fip200, fsi1, gabaar, gabarapl2, gate-16, ip3r, lkaa0226, lamp2, map1k3a, map1k3b, mfn1, mfn2, mtor, naf1, opa1, p150, park2, pin1, rb1c1, rnf41, rubicon, ulk1, ulk2, usp8, uvrag, vps11, vps16, vps18, vps33, vps34, vps39, wipi1, wipi2, wipi3, and wipi4. 871 of 1150 potential targets were identified on the previously performed microarrays (see all results in Supplementary Table 1). For statistical analysis of all microarray data, genes were called differentially expressed using the significance analysis of microarrays one class response package with a false discovery rate (FDR) of 20% (7).

Islet Isolation and Tissue Culture
Mouse islets were isolated after pancreatic duct cannulation and type IV collagenase digestion (Worthington). Islets were cultured in RPMI-1640 media with supplementation as previously described (12). Min6 mouse insulinoma cells were maintained in growth medium (DMEM including heat-inactivated FBS, penicillin/streptomycin, sodium pyruvate, and β-mercaptoethanol) as previously described (12).

Morphologic Analyses
Cells grown on Lab-Tek chamber slides (Nunc) were fixed with 4% PFA for 15 min at room temperature. For organelle staining assays, Min6 cells were treated with 100 nmol/L MitoTracker Deep Red FM or 50 nmol/L Lysotracker Red DND-99 (Molecular Probes) for 30 min prior to fixation. Analysis was performed with a spinning-disk confocal microscope (Perkin Elmer). Images were captured using an EM-CCD digital camera (Hamamatsu). Colocalization analysis of subcellular structures was performed using the JACoP ImageJ plugin (13). LC3 and LC3–green fluorescent protein (GFP) puncta were quantified and estimated using previously published guidelines (14).

Glucose and Insulin Measurements
Intraportaline glucose tolerance tests (2 g/kg D-glucose) were performed as previously described (9). Static incubation assays for insulin release were performed as previously described (15). Insulin concentrations were measured by ELISA (Crystal Chem).

Immunocytochemistry
After fixation and PBS washes, immunocytochemical staining was performed using the following antisera: rabbit anti-LC3 (MBL), rat anti-Lamp1 (1D4B; Developmental Studies Hybridoma Bank), mouse anti-Mfn2 (Abcam), and species-specific Cy2-, Cy3-, and Cy5-conjugated secondary antisera (Jackson Immunoresearch). Nuclear labeling was performed with DAPI (Molecular Probes).

Immunohistochemistry
Pancreata were fixed in 4% PFA, embedded in paraffin, and sectioned. Antisera were guinea pig anti-insulin (Dako), mouse anti-Mfn2 (Abcam), rabbit anti-LC3 (MBL), and species-specific Cy2-, Cy3-, and Cy5-conjugated secondary antisera (Jackson Immunoresearch). Nuclear labeling was performed with DAPI (Molecular Probes).
Figure 1—Pdx1 regulates mitophagy in pancreatic β-cells. A: Transmission EM images from 5-month-old WT and Pdx1+/− β-cells. Inset: Focused area of mitochondria on EM images. B: Quantification of mitochondrial morphology (% of total mitochondria) observed in WT and Pdx1+/− β-cells in transmission EM images (∼250 independent mitochondria scored/animal). n = 3 animals/genotype. C: Relative oxygen consumption rate (OCR) measured in isolated WT and Pdx1+/− islets (n = 3/group) of 6- to 8-week-old mice. D: LC3/Mfn2 colocalization in LC3+ puncta quantified from 5-month-old WT and Pdx1+/− β-cells. E: Representative confocal image of 5-month-old WT and Pdx1+/− β-cells stained for insulin (gray), DAPI (DNA [blue]), LC3 (autophagosomes [green]), and Mfn2 (mitochondria [red]). Data are expressed as mean ± SEM. n = 5 mice/group and ∼80 β-cells (>1,100 total LC3+ and Mfn2+ structures) were analyzed per animal. *P < 0.05.
RNA RT-PCR and Quantitative PCR of cDNA, Mitochondrial DNA, and Nuclear DNA

Total RNA was DNase treated (DNA-Free; Ambion) and reverse transcribed (ABI High Capacity Reverse Transcriptase kit; ABI) following the manufacturers’ protocols. Fluorescence-based real-time PCR was performed using the IQ Sybr Green Supermix kit (BioRad) and the IQ-5 Single Color Real-Time PCR Detection System (BioRad). Primers were designed and optimized as previously described (9). Primer sequences for mRNA targets included Nrdp1 forward 5′-AGTGGTGATGGCCTGTGAGAA-3′ and Nrdp1 reverse 5′-CCTCCACACCATGCGCAAATA-3′. Primer sequences for Pdx1, Clec16a, and hypoxanthine guanine phosphoribosyl transferase mRNA (HPRT) were previously published (7,8). Relative gene expression normalized to hypoxanthine guanine phosphoribosyl transferase. Relative mtDNA content by quantitative (q)PCR was conducted as previously described (16) after DNA isolation with the Blood/Tissue DNeasy kit (Qiagen) according to the manufacturer’s protocols.

Western Blot Analysis

Whole cell lysates (in radioimmunoprecipitation assay buffer) were generated by sonication followed by centrifugation to remove insoluble material. Lysates were resolved on 4–12% gradient Bis-Tris gels (Invitrogen) and transferred to nitrocellulose membranes as previously described (17). Primary antisera were anti-Flag (M2; Sigma), anti-Nrdp1 (Santa Cruz Biotechnology), anti-Pdx1 (253) (10), and anti-cyclophilin B (Affinity Bioreagents). Secondary antisera incubation and signal detection were performed as previously described (17). Densitometry was calculated using National Institutes of Health ImageJ software (http://imagej.nih.gov/ij/).

Viral Transductions and Transfections

Retroviral plasmids (pBABE-puro-Dest-Clec16a-Flag and pBABEpuro-LC3-GFP) were transfected into Plat-E cells using Lipofectamine 2000 transfection reagent (Invitrogen). Plasmids used for transfections included pBABE-puro-Dest-Clec16a-Flag (8) and pBABEpuro-LC3-GFP (Addgene plasmid 22405 from the laboratory of Dr. Jayanta Debnath).

Figure 2—Pdx1 loss of function leads to impaired autophagosomal-lysosomal clearance of mitochondria in Min6 β-cells. A: Left, representative confocal microscopy image of si-nontargeting (siNT)- or siPdx1-treated LC3-GFP (green)—expressing Min6 β-cells after treatment with Mitotracker (red). Nuclei demarcated by DAPI (blue). Right, quantification of LC3-GFP+ puncta colocalization with Mitotracker in siNT- or siPdx1-treated LC3-GFP—expressing Min6 β-cells. B: Left, representative confocal microscopy image of siNT- or siPdx1-treated LC3-GFP (green)—expressing Min6 β-cells after treatment with Lysotracker (red). Nuclei demarcated by DAPI (blue). Right, quantification of LC3-GFP+ puncta colocalization with Lysotracker in siNT- or siPdx1-treated LC3-GFP—expressing Min6 β-cells. Data are expressed as mean ± SEM of three independent experiments. Approximately 80–120 cells (>1,400 total LC3-GFP+, Mitotracker+, and Lysotracker+ structures) quantified per experiment. *P < 0.05.
Forty-eight hours after transfection, cell supernatant was removed and filtered before placement on target cells (Min6) for transduction. Polybrene (8 μg/mL; Sigma) supplementation was added to retroviral media during infection of target cells. Selection of transduced cells was performed with Puromycin (Millipore). Transient transfection of Min6 cells was performed using an Amaza Nucleofector as previously described (8). One nanomole of SMARTpool mouse Pdx1 and nontargeting small interfering (si)RNA was used for transient loss of function studies (Dharmacon). Nontargeting and Pdx1 siRNA sequences were published previously (18).

**Statistics**

Data are presented as means ± SEM, and error bars denote SEM. Statistical comparisons were performed using two-tailed Student t test or two-way ANOVA (Prism GraphPad). A P value <0.05 was considered significant.

**RESULTS**

To elucidate the mechanism(s) by which Pdx1 regulates mitochondrial function, we studied Pdx1 heterozygous mice (Pdx1+/−), which develop glucose intolerance and reduced GSIS due to reduced mitochondrial function (6). We evaluated mitochondrial ultrastructure by transmission electron microscopy in wild-type (WT) and Pdx1+/− islets. Pdx1+/− β-cells displayed an increased number of rounded mitochondria with disordered cristae and amorphous structure (Fig. 1A and B). Dysmorphic mitochondria were not observed by EM in non−β islet cells of Pdx1+/− mice (data not shown). As expected, Pdx1+/− mice exhibited glucose intolerance (data not shown) as well as reduced glucose-stimulated and maximal oxygen consumption [after treatment with the uncoupler carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone] in isolated islets (Fig. 1C), suggesting that Pdx1-dependent regulation of mitochondrial structure and function could contribute to impaired glucose control in Pdx1+/− mice.

Structural and functional defects in mitochondria are often observed after impairments in mitophagy (19,20). Mitophagy is activated after mitochondrial damage or depolarization, leading to recruitment of autophagosomal proteins to traffic unhealthy mitochondria into autophagosomes and, ultimately, lysosomes for degradation (21). Interestingly, reduced mitochondrial membrane potential has been observed in Pdx1-deficient β-cells (22). We observed significantly increased numbers of mitochondria in autophagosomes of Pdx1+/− β-cells, as shown by increased colocalization of the mitochondrial outer membrane protein mitofusin 2 (Mfn2) with the autophagosome marker LC3 (Fig. 1D and E). The total number of LC3+ puncta was also increased in Pdx1+/− β-cells as well as siPdx1-treated Min6 β-cells, suggesting an accumulation in autophagosomes, which is consistent with previous observations (23); however, this did not reach statistical significance (data not shown). Further, we observed an increase in mitochondria (stained with Mitotracker) targeted to LC3-GFP+ autophagosomes in siPdx1-treated Min6 β-cells (Fig. 2A). An increase in LC3-labeled mitochondria could be secondary to impaired autophagosome-lysosome fusion or to increased flux through mitophagy. To determine the cause of increased LC3-labeled mitochondria, we evaluated autophagosome-lysosome fusion in Min6 β-cells after siPdx1 treatment (or nontargeting siRNA controls). Pdx1 loss of function led to a reduced colocalization of LC3-GFP with the lysosome-specific Lysotracker dye (Fig. 2B), suggesting that Pdx1 deficiency impairs autophagosome-lysosome fusion. The evidence of increased mitochondrial targeting to autophagosomes along with reduced autophagosome-lysosome fusion induced by Pdx1 deficiency identifies Pdx1 as a novel regulator of mitophagy in pancreatic β-cells.

To identify the mechanistic basis for impaired mitophagy after Pdx1 loss of function, we used previously performed high-throughput expression profiling and chromatin occupancy analyses of Pdx1 in pancreatic islets (7,18). We performed a post hoc analysis of mitochondrial and autophagy-related targets of Pdx1 by overlapping cDNA expression microarray data from WT and Pdx1+/− islets with mitochondrial targets from the MitoCarta database (11), supplemented with ~50 additional autophagy/mitophagy related targets. Of nearly 900 transcripts analyzed, we found 4 differentially expressed transcripts with significant modulation of expression by Pdx1: Clec16a, as well as the complex I subunit Ndufs2, the aggregation suppressor Hspb7, and the NADP-dependent short-chain carboxyl reductase Dhrs4 (Table 1).

Clec16a is a recently identified gene associated with T1D in humans, with diabetogenic single nucleotide polymorphisms associated with reduced β-cell function and glucose control (8). We previously observed that Clec16a interacts with Nrdp1 and protects it from proteosomal degradation, thereby maintaining autophagosome-lysosome fusion during mitophagy. Similar to the effects of Pdx1

| Name       | Description                                                         | FC (Pdx1+/−/Pdx1+/+) | FDR    |
|------------|---------------------------------------------------------------------|----------------------|--------|
| Ndufs2     | NADH dehydrogenase (ubiquinone) Fe-S protein 2                      | −1.769               | 5.48   |
| Hspb7      | Heat shock protein family, member 7 (cardiovascular)                | −1.744               | 0      |
| Dhrs4      | Dehydrogenase/reductase (SDR family) member 4                       | −1.602               | 6.33   |
| Clec16a    | C-type lectin domain family 16, member A; KIAA0350                  | −1.583               | 6.33   |

Targets with fold change below −1.500 or above 1.500 and FDR below 20% are shown. FC, fold change (log2 adjusted).
Figure 3—Pdx1 controls expression of the Clec16a-Nrdp1 pathway. A: Top, schematic representation of the Clec16a locus. Arrows delineate the two putative Pdx1 binding sites (S1 and S2). Bottom, quantitative ChIP analysis in human islets of three independent human islet donors, expressed as fold enrichment (aPdx1 vs. input control) relative to albumin occupancy. Primers designed to amplify a known Pdx1 autoregulatory binding region (45) and albumin were used as positive and negative controls, respectively. B: Clec16a expression by qRT-PCR of RNA isolated from 3-month-old WT and Pdx1+/- islets (n = 5–7/group). C: qRT-PCR of Clec16a, Pdx1, and Nrdp1 from RNA isolated from Min6 β-cells treated with si-nontargeting (siNT) or Pdx1-specific (siPdx1) siRNA (n = 3/group). D: Nrdp1 protein expression by Western blot of 2-month-old WT and Pdx1+/- islets (n = 3/group).
deficiency, loss of Clec16a expression in pancreatic islets leads to impaired mitochondrial function, insulin secretion, and glucose homeostasis, underscoring the importance of Clec16a-regulated mitophagy to pancreatic β-cell function (8). To determine whether Pdx1 regulates expression of Clec16a, we analyzed Pdx1 occupancy of the Clec16a locus by ChIP studies in human islets. We observed two putative Pdx1 binding sites with features of transcriptional enhancers based on previously reported high-throughput ChIP-sequencing studies (18,24). We verified Pdx1 occupancy of these sites in human islets (Fig. 3A) and also observed reduced Clec16a RNA expression in Pdx1−/− islets and siPdx1-treated Min6 β-cells (Fig. 3B and C). To determine whether loss of Pdx1 affects downstream targets of Clec16a, we measured Nrdp1 protein expression and observed reduced Nrdp1 expression in both Pdx1−/− islets and siPdx1-treated Min6 β-cells (Fig. 3D and E). Nrdp1 mRNA expression was not impaired in siPdx1-treated Min6 β-cells (Fig. 3C), consistent with a post-transcriptional effect on Nrdp1 protein expression and with our previous observation that Clec16a regulates Nrdp1 degradation. Taken together, these studies indicate that Pdx1 directly modulates expression of the Clec16a-Nrdp1 pathway in pancreatic islets.

To establish that Clec16a mediates the effect of Pdx1 on mitophagy, we studied the effect of Pdx1 loss of function on mitophagy in Min6 β-cells stably overexpressing Clec16a. Overexpression of Clec16a rescued Nrdp1 protein expression after Pdx1 siRNA treatment, suggesting that Pdx1 affects Nrdp1 expression in a Clec16a-dependent manner (Fig. 4A and B). We next evaluated mitochondrial trafficking and found that restoration of Clec16a expression significantly reduced mitochondrial colocalization with autophagosomes (Fig. 4C) and increased colocalization of autophagosome and lysosome markers (Fig. 4D) in Pdx1-deficient Min6 β-cells. Overexpression of Clec16a also improved defects in mitochondrial respiration and GSIS caused by Pdx1 deficiency (Fig. 5A and B), suggesting that Pdx1 control of mitochondrial metabolism and GSIS is mediated at least in part by its regulation of Clec16a.

Pdx1 was previously demonstrated to regulate mitochondrial DNA (mtDNA) copy number in β-cells after expression of a dominant-negative form of Pdx1 (25). We also observed a reduction in mtDNA content in Pdx1−/− islets (Fig. 5C). Clec16a overexpression in Min6 β-cells, however, did not restore mtDNA content after Pdx1 deficiency (Fig. 5D), indicating that Clec16a mediates Pdx1 regulation of mitophagy but not mtDNA copy number in β-cells.

**DISCUSSION**

Here, we describe Pdx1 as a transcriptional regulator of mitophagy in pancreatic β-cells. Pdx1 deficiency leads to impaired mitochondrial ultrastructure, reduced mitochondrial function, and impaired GSIS. We identify Clec16a, a T1D gene and regulator of mitophagy, as a downstream target of Pdx1. We find that Pdx1 occupies the Clec16a locus and regulates expression of Clec16a to control downstream effects on the E3 ubiquitin ligase Nrdp1 and, thus, autophagosome-lysosome fusion during mitophagy. We demonstrate that the Pdx1-Clec16a-Nrdp1 pathway is a novel regulator of β-cell mitochondrial function and insulin release.

Pdx1 has previously been implicated in the regulation of autophagy as well as mitochondrial function in β-cells (6,23,25); however, direct transcriptional targets of Pdx1 in the regulation of mitophagy had not been described. While Pdx1 loss of function has previously been shown to increase expression of Nix (also known as Bnip3l) (26), a regulator of mitophagy in erythroid cells (27), we did not observe increased expression of Nix in Pdx1−/− islets. (See Supplementary Table 1). The other transcriptional targets we identified, Ndufs2, Hspb7, and Dhrs4, have not been implicated in the regulation of mitophagy, likely not explaining the role of Pdx1 in mitophagy that we observe. Mutations in Ndufs2, a complex I subunit protein, have been reported in patients with Leigh syndrome, a heritable mitochondrial disease resulting in cardiac and neurologic defects as well as reduced life span (28,29). Loss of Ndufs2 commonly leads to destabilization and reduced expression of complex I as well as reduced mitochondrial respiration (30,31). The small heat shock protein Hspb7 has been associated with several forms of cardiomyopathies and heart failure (32,33). Hspb7 has been reported to play a role as a suppressor of aggregated toxic polyglutamine-containing proteins, which are linked to neurodegenerative diseases and could lead to impaired mitochondrial function and apoptosis if impaired (34). Dhrs2 (Hep27) is a mitochondrial NADP-dependent short-chain dehydrogenase/reductase protein, which can regulate mitochondrial-nucleus signaling through effects on the Mdm2-p53 pathway (35). It will be of interest in the future to define the role of these genes in mitochondrial mass and function of β-cells.

Beyond its direct control of the mitochondria, Pdx1 controls several diverse functions in pancreatic β-cells that may indirectly influence mitophagy, including β-cell apoptosis, necrosis, and ER stress (7,22,26,36). Reductions in Bcl-XL and Bcl-2 expression, observed in the context of enhanced apoptosis by Pdx1 deficiency (36), induce mitophagy in HEK293T cells (37). ER stress itself affects mitochondrial membrane potential and autophagy (38,39). These diverse defects observed in a Pdx1-haplosufficient β-cell potentially contribute directly and/or indirectly to an unhealthy environment ultimately leading to enhanced mitochondrial depolarization.

Min6 β-cells (n = 3/group). For all Western blots, representative images chosen from among 3 independent experiments. Cyclophilin B expression serves as a loading control. Densitometry, shown adjacent to each Western blot, represents mean ± SEM of three independent experiments. qRT-PCR data expressed as mean ± SEM. *p < 0.05.
Figure 4—Restoration of Clec16a ameliorates defects in mitophagy due to Pdx1 deficiency. 

A: qRT-PCR of Clec16a and Pdx1 from RNA isolated from control or Clec16a-overexpressing Min6 β-cells treated with si-nontargeting (siNT) or Pdx1-specific (siPdx1) siRNA (n = 3/group). 

B: Representative Western blot image of Nrdp1 protein expression in siNT- or siPdx1-treated control or Clec16a-overexpressing Min6 β-cells (n = 3/group). Cyclphilin B expression serves as a loading control. Densitometry, shown adjacent to Western blot, represents mean ± SEM of 3 independent experiments. 

C: Top, representative confocal microscopy image of siNT- or siPdx1-treated control or Clec16a-overexpressing Min6 β-cells followed by staining for LC3 (green), Mfn2 (red), and DAPI (blue). Bottom, quantification of LC3+ puncta colocalization with Mfn2 in siNT- or siPdx1-treated control or Clec16a-overexpressing Min6 β-cells. Colocalized structures noted by white arrowheads. 

D: Top, representative confocal microscopy image of siNT- or siPdx1-treated control or Clec16a-overexpressing Min6 β-cells followed by staining for LC3 (green), Lamp1 (lysosomes [red]), and DAPI (blue). Colocalized structures noted by white arrowheads. Bottom, quantification of LC3+ puncta colocalization with Lamp1 in siNT- or siPdx1-treated control or Clec16a-overexpressing Min6 β-cells. Data expressed as mean ± SEM of 3 independent experiments. Approximately 80 cells (~1,200 total LC3+, Mfn2+, and Lamp1+ structures) quantified per immunofluorescence experiment. *P < 0.05.
and, consequently, a greater requirement for mitophagic clearance of unhealthy mitochondria. Impaired β-cell function is a common feature of both T1D and T2D, with impaired GSIS as the first observed sign of both disease processes (40,41). Both Pdx1 and Clec16a have been implicated in the regulation of GSIS in β-cells (6,8), and reduced Pdx1 expression has been observed both in the islets of pre-diabetic (nonobese diabetic) NOD mice, a model of T1D, and in islets from T2D donors, which could lead to dysregulation of the Clec16a-Nrdp1 pathway (42,43). Mitochondrial structural and functional defects have previously been reported in human islets from T2D donors and are characteristic of dysfunctional mitophagy (44); however, further study is needed to determine whether impaired mitophagy mediated by the Pdx1-Clec16a-Nrdp1 pathway occurs in the pathogenesis of T2D. Thus, the connection of T1D and T2D genes and pathways through a common mechanism provides a framework for developing Pdx1- and Clec16a-targeted translational therapies that may be useful for treatment of β-cell mitochondrial failure in all forms of diabetes.

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Author Contributions. S.A.S. conceived of, designed, and performed experiments; interpreted results; and drafted and reviewed the manuscript. A.M.F., D.N.G., and J.Y. designed and performed experiments and interpreted results. J.C.R. and B.A.K. designed and performed experiments, interpreted results, and reviewed the manuscript. D.A.S. conceived of and designed the studies, interpreted results, and edited and reviewed the manuscript. S.A.S. and D.A.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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