Genome-scale in vivo CRISPR screen identifies RNLS as a target for beta cell protection in type 1 diabetes

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Type 1 diabetes (T1D) is caused by the autoimmune destruction of pancreatic beta cells. Pluripotent stem cells can now be differentiated into beta cells, thus raising the prospect of a cell replacement therapy for T1D. However, autoimmunity would rapidly destroy newly transplanted beta cells. Using a genome-scale CRISPR screen in a mouse model for T1D, we show that deleting RNLS, a genome-wide association study candidate gene for T1D, made beta cells resistant to autoimmune killing. Structure-based modelling identified the U.S. Food and Drug Administration–approved drug pargyline as a potential RNLS inhibitor. Oral pargyline treatment protected transplanted beta cells in diabetic mice, thus leading to disease reversal. Furthermore, pargyline prevented or delayed diabetes onset in several mouse models for T1D. Our results identify RNLS as a modifier of beta cell vulnerability and as a potential therapeutic target to avert beta cell loss in T1D.
Notably, one of these genes was Rnls, the candidate gene for a region in the human genome associated both with the overall risk of T1D and with the age of diabetes onset by GWAS. Based on its previous association with human autoimmune diabetes, we prioritized Rnls for validation.

**Rnls deletion protects beta cells against autoimmune killing.** We generated an Rnls mutant NIT-1 cell line (Rnlsmut) by using the Rnls gRNA identified in the screen (Extended Data Fig. 2). NIT-1 cells were also engineered to carry a luciferase reporter for longitudinal monitoring of beta cell survival and proliferation in the absence of autoimmunity, we also monitored beta cell transplants in NOD-scid mice that did not receive diabetic NOD mice. As anticipated, autoimmunity decreased the size and function of Rnlsmut islets. Two weeks later, we injected graft recipients with splenocytes from diabetic NOD mice to induce autoimmune beta cell killing. To control for the effects of gene disruption in the absence of autoimmunity, we also followed islet grafts in NOD-scid recipients that did not receive splenocytes from diabetic mice. As anticipated, autoimmunity decreased the size and insulin expression in Ctrl grafts (Fig. 2j–l). In contrast, Rnlsmut islets survived autoimmune and maintained insulin expression. These results show that targeting Rnls in primary beta cells was protective in a pathophysiologically relevant model of autoimmune diabetes. Notably, we found that Rnls targeting did not affect the ability of islet cells to secrete insulin (Extended Data Fig. 2).

**Rnls mutation diminishes immune recognition of beta cells.** We proceeded to ask if Rnls deficiency had a direct effect on immune recognition. The expression of major histocompatibility complex (MHC) class I and class II molecules on the surface of Rnlsmut NIT-1 cells was comparable to that of Ctrl cells (Fig. 3a–d). Rnls mutant cells showed that only approximately 75% of the cells carried mutant Rnls alleles, many of which also carried a wild-type (WT) copy of the gene (Extended Data Fig. 2), indicating that a partial loss of function may be sufficient for protection.
mutation did not significantly affect the response of beta-cell-reactive (BCD2.5 T cell receptor (TCR) transgenic) CD8+ T cells cocultured with antigen–presenting cells and NIT-1 beta cells. However, Rnls−mut NIT-1 cells elicited a significantly weaker response from polyclonal beta-cell-reactive CD8+ T cells isolated from diabetic NOD mice (Fig. 3g,h). Because Rnls deficiency diminished the response of autoreactive cytotoxic T cells, we asked if Rnls−mut NIT-1 cells would also be protected against T cell alloreactivity. To test this, we transplanted Rnls−mut and Ctrl NIT-1 cells into opposite flanks of MHC-mismatched C57BL/6 mice. Both beta cell grafts were rapidly destroyed by the strong allogeneic response of host immune cells (Extended Data Fig. 3), showing that Rnls deficiency did not affect allo-rejection. These data suggest that Rnls−mut beta cells are not impervious to immune detection or killing but that they are less prone to stimulating autoreactive CD8+ T cells.

Rnls mutation confers endoplasmic reticulum (ER) stress resistance. A growing body of evidence supports a role for ER stress in the demise of beta cells in diabetes. The unfolded protein response (UPR) triggered by ER stress has been implicated in beta cell apoptosis in both T1D and T2D. Notably, ER stress was proposed to contribute not only directly but also indirectly to beta cell death in T1D, owing to its ability to increase the presentation of autoantigens and neoantigens, for example, by affecting post-translational modifications and antigen processing. We speculated that Rnls mutation may affect the cellular response to ER stress and thereby...
diminish the stimulation of diabetogenic CD8+ T cells. To test this notion, we challenged NIT-1 cells with the ER stressor thapsigargin (TG). Ctrl cells were highly sensitive to TG treatment, with concentrations >50 nM killing most cells. Remarkably, Rnls mutant cells withstood even a 20-fold greater concentration of TG (Fig. 4a). We obtained similar results with the alternative ER stressor tunicamycin (Fig. 4b). Furthermore, Rnls mutation made cells resistant to the apoptotic effect of the inflammatory cytokines interleukin-1β (IL-1β) and interferon-γ (IFN-γ) implicated in beta cell stress and killing in T1D [1,2] (Fig. 4c). Of note, Rnlsmut NIT-1 cells remained sensitive to mitomycin C and streptozotocin (STZ), which cause ER-stress-independent cell death (Extended Data Fig. 4). These data indicate that Rnls deficiency does not prevent all forms of cell death and that its protective effect is limited to specific sources of cellular stress, including inflammatory cytokines associated with T1D [1,2]. To ascertain that ER stress resistance was a direct effect of Rnls mutation and not caused by an off-target effect of the Rnls gRNA, we generated additional cell lines wherein Rnls exons 2–4 or exon 5 were deleted by using different sets of gRNAs. These alternative Rnls-deficient beta cell lines were again protected against ER-stress-induced cell death (Extended Data Fig. 4), confirming that ER stress resistance was a direct result of Rnls deletion.

Rnls overexpression sensitizes beta cells to ER stress and autoimmunity. To further evaluate the role of Rnls in modifying the sensitivity of beta cells to ER stress and autoimmunity, we overexpressed Rnls in NIT-1 beta cells by using a lentiviral transgene. While Rnls overexpression alone only marginally increased sensitivity to TG-induced killing (Extended Data Fig. 4), it significantly accelerated the autoimmune killing of beta cells implanted into diabetic

Fig. 3 | Rnls deficiency diminishes immune recognition of beta cells. a–d. Representative flow cytometry data (a,c) and summary data (b,d) for MHC class I (a,b, mean fluorescent intensity) and MHC class II (c,d, expressed as the percentage of MHC class II+ cells) expression in Ctrl and Rnlsmut cells. Data are representative of three independent experiments. e,f. BDC2.5 TCR transgenic CD4+ T cells were cocultured with NIT-1 cells and irradiated splenocytes from NOD-scid mice. IFN-γ expression in CD4+ T cells was measured at 24 h by flow cytometry. Representative (e) and combined data (f) from technical triplicates is shown. Data are representative of five independent experiments. NS, not significant. g,h. ELISPOT measurement for the activation of polyclonal CD8+ T cells from a diabetic NOD mouse after stimulation with control or Rnlsmut NIT-1 cells. Wells without NIT-1 cells or with phorbol myristate acetate (PMA) and ionomycin were used as negative and positive controls, respectively. Representative ELISPOT images (g) and representative data from one of three independent experiments (h) are shown. Data were compared by one-way analysis of variance (ANOVA) with Tukey’s multiple comparison test. ***P = 0.0001. All data show the mean ± s.e.m.
mice (Extended Data Fig. 5). We proceeded to re-introduce Rnls into Rnlsmut cells by using a transgene that carried a synonymous mutation within the gRNA target site to prevent CRISPR–Cas9 targeting. Rnls re-expression restored the proliferative capabilities of Rnlsmut cells to ER stress (Extended Data Fig. 4) and accelerated their autoimmune killing in diabetic NOD mice (Extended Data Fig. 5). Collectively, the data show that Rnls expression modulates the vulnerability of beta cells to ER stress and autoimmunity.

**Rnls modifies the cellular response to ER stress.** To understand how Rnls deficiency increases ER stress resistance, we measured the UPR, which mediates the cellular adaptation to ER stress. We found that the activation of the critical ER stress sensors inositol-requiring protein 1α (IRE1α)31, PKR-like ER kinase (PERK)24 and cyclic AMP-dependent transcription factor ATF-6 alpha (ATF6) (ref. 23) was diminished in Rnlsmut cells after TG treatment (Fig. 4d and Extended Data Fig. 6). Downstream of these UPR triggers, the phosphorylation of eukaryotic initiation factor-2α (eIF2α), protein levels of cyclic AMP-dependent transcription factor ATF-4 (ATF4) and splicing of X-box-binding protein 1 (XBP1) were markedly reduced (Fig. 4d,e and Extended Data Fig. 6). The expression of Chop and Txnip, both implicated in ER-stress-induced apoptosis26–28, was also diminished (Fig. 4f and Extended Data Fig. 6). The data suggested that Rnls deficiency increased the threshold of the ER stress that triggers the UPR. This would explain how Rnls mutation inhibits the proapoptotic effect of stimuli that cause cellular stress. The protective effect of Rnls deletion was not limited to ER stress because Rnlsmut cells also better withstood oxidative stress compared to Ctrl NIT-1 cells (Extended Data Fig. 6). Consistent with this finding, Rnls deficiency increased the expression of a key regulator of the oxidative stress response, nuclear factor erythroid 2-related factor 2 (NRF2) (ref. 29) (Fig. 4d and Extended Data Fig. 6). We conclude that Rnls deficiency increases the ability of beta cells to withstand the cellular stress involved in their destruction during T1D.

**The Food and Drug Administration (FDA)-approved drug paragline reproduces the effects of Rnls deletion.** RNLS is a flavoprotein oxidase whose cellular function has not yet been elucidated49. Its proposed substrates are 2- and 6-dihydroNAD(P)31, isoforms of β-NAD(P)H, although whether these are physiologically relevant is unknown. However, the crystal structure of human RNLS was solved several years ago32. The enzyme uses a flavin adenine dinucleotide (FAD) cofactor for catalysis and is structurally related to other flavoprotein oxidases including monoamine oxidases.
RNLS deletion confers ER stress resistance to human stem cell-derived beta cells. We identified RNLS by using the NOD mouse model, whose relevance to human T1D has repeatedly been questioned. Notably, RNLS had already been implicated in human T1D by GWAS, suggesting that the effects of RNLS mutation on beta cell vulnerability may be conserved in humans. To test this, we generated clonal RNLS knockout human iPSCs by CRISPR–Cas9 gene targeting (Extended Data Fig. 10). RNLS deficiency did not affect stem cell differentiation into beta cells, according to our published protocol (Fig. 6a–c) and did not impair insulin secretion (Extended Data Fig. 10). Significantly, RNLS knockout human stem/beta cells were resistant to TG-induced apoptosis (Fig. 6d,e), reproducing the phenotype of RNLS mutant mouse beta cells.

Pargyline mimics the effect of RNLS deletion in human stem/beta cells. We showed that the FDA-approved drug pargyline binds human recombinant RNLS and that it confers protection to mouse beta cells in the setting of autoimmunity. We extended these findings by testing if pargyline would replicate the protective effects of RNLS deletion in human stem/beta cells. We found that pargyline decreased ER-stress-induced cell death in both induced pluripotent and embryonic stem/beta cells (Fig. 6f,g) after TG treatment. Collectively, the data indicate that pargyline mimics the protective effects of RNLS deletion in both mouse and human beta cells.

Discussion

A beta cell replacement therapy for T1D has become a realistic prospect. Advances in stem/beta cell differentiation now allow the manufacture of billions of patient-derived beta cells for transplantation. The critical hurdle to this therapeutic strategy is the susceptibility of beta cells to autoimmunity that can only be abrogated by the use of broad immunosuppression. In this study, we have described an unbiased and genome-wide search for genes whose suppression would protect beta cells against autoimmunity. We identified a small number of mutations that allowed beta cells to survive in a host with autoimmune diabetes. Although we performed this screen in a mouse model, one of the few candidates that emerged from our stringent experimental system was RNLS, a gene that had already been associated with human T1D by GWAS. This supportive evidence from human genetic studies led us to extensively validate the protective effects of RNLS mutation in both mouse and human cells. Collectively, our data show that RNLS is a modifier of beta cell vulnerability in T1D.

First, this finding may explain why genome variants in the RNLS locus impact the overall risk and age of onset of T1D. How disease-associated variants modify RNLS function or expression is unknown and lies beyond the scope of the present study. Nevertheless, in light of our results, exploring how this candidate T1D risk gene is regulated seems warranted.

Second, the data underscore the central role of beta cell ER stress in promoting islet autoimmunity. The ER and oxidative stress resistance afforded by RNLS deficiency was correlated with protection against ER stress. A beta cell replacement therapy for T1D has become a realistic prospect. Advances in stem/beta cell differentiation now allow the manufacture of billions of patient-derived beta cells for transplantation. The critical hurdle to this therapeutic strategy is the susceptibility of beta cells to autoimmunity that can only be abrogated by the use of broad immunosuppression. In this study, we have described an unbiased and genome-wide search for genes whose suppression would protect beta cells against autoimmunity. We identified a small number of mutations that allowed beta cells to survive in a host with autoimmune diabetes. Although we performed this screen in a mouse model, one of the few candidates that emerged from our stringent experimental system was RNLS, a gene that had already been associated with human T1D by GWAS. This supportive evidence from human genetic studies led us to extensively validate the protective effects of RNLS mutation in both mouse and human cells. Collectively, our data show that RNLS is a modifier of beta cell vulnerability in T1D.

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against autoimmunity, consistent with a growing body of literature that implicates beta cell ER stress in T1D\(^1\). We propose that \textit{RNLS} may associate with the risk of T1D owing to its role in modulating the stress resistance of pancreatic beta cells that, in turn, modifies their susceptibility to autoimmune targeting. The detailed mechanism by which \textit{RNLS} impacts on the cellular response to ER and oxidative stress remains obscure. This mechanism will certainly prove challenging to elucidate given that even the biochemical function of \textit{RNLS} is yet to be understood. Notwithstanding, our finding that \textit{RNLS} deficiency renders beta cells resistant to cellular stress provides a probable albeit speculative explanation for their protection from autoimmunity, consistent with the emerging notion that beta cell stress is central to T1D pathogenesis. Of note, \textit{RNLS} may have enzyme-independent properties as an extracellular receptor ligand in other tissues\(^3\). The \textit{RNLS} protein was reported to be protective in this role\(^4\), which
seems to be distinct from the enzymatic function underlying the results presented in this study.

Notably, our discovery that RNLS deficiency endows beta cells with the ability to resist autoimmunity suggests a genetic engineering solution to beta cell replacement in T1D that would interfere neither with the identity of the beta cell nor with immunity and immune surveillance. We envisage that RNLS deletion could be a safe and effective modification in stem/beta cells to overcome autoimmunity in patients with T1D. Because this approach targets beta cells, it may be ideally suited in combination with an immune therapy such as teplizumab, which targets T lymphocytes and was recently shown to delay disease progression. Conceivably, RNLS...
deletion could also be combined with other protective candidates identified in this screen, once these have been validated, to provide even more robust protection against autoimmunity.

Finally, we have identified an FDA-approved drug that replicates the protective effect of RNLS deletion. Its apparent efficacy in protecting beta cells and preventing diabetes onset in mice, together with its favourable safety profile, should make pargyline—and other MAO inhibitors predicted to target RNLS—worthy of further evaluation for the prevention or treatment of T1D.

In sum, our unbiased screen for therapeutic targets in a mouse model for T1D converged with human GWAS data to identify RNLS as a modifier of beta cell vulnerability. Our discovery that an MAO inhibitor predicted to target RNLS—worthy of further evaluation for the prevention or treatment of T1D.

Methods

Misc. NOD, NOD-scid (NOD.CB 17-Prkdc<scid>)(J) and C57BL/6J mice were purchased from The Jackson Laboratory. Animals were housed in pathogen-free facilities at the Joslin Diabetes Center and all experimental procedures were approved and performed in accordance with institutional guidelines and regulations.

CRISPR GeCKO-A library screen. The mouse GeCKO v2 A lentiviral pooled library was obtained from Addgene (catalogue no. 1000000052), targeting 19,050 genes with 3 gRNAs per gene and was prepared as described previously, WT NIT-1 cells (catalogue no. CRL-2055; ATCC) were infected with GeCKO-A CRISPR lentiviral library at an MOI of 0.3, and then selected by puromycin (2 μg/mL) at day 3 post-infection. Ten mutant NIT-1 cells were transplanted subcutaneously into 8-week-old female WT NOD mice; 10% of diabetic NOD splenocytes in 200 μL sterile PBS were injected intravenously at the same time to induce autoimmunity. NOD-scid mice with subcutaneously transplanted mutant NIT-1 cells but without diabetic NOD splenocyte injection were used as Controls (non-autoimmune group). Diabetic NOD splenocytes were isolated from spontaneously diabetic female NOD mice as described previously. In brief, the spleen was mechanically disaggregated into a single-cell suspension. Red blood cells were lysed using a hypotonic buffer and cells were washed in PBS and counted before injection. We terminated the screen at 8 weeks post-injection and the remaining grafts were retrieved from both the autoimmune and non-autoimmune groups of mice. Genomic DNA was extracted from the grafts (Quick-gDNA MiniPrep Kit; Zymo Research), the next-generation sequencing (NGS) libraries were prepared as described previously and subjected to NGS analysis (Novogene). The gRNA sequences from the NGS data were extracted using standard bioinformatics methods; the distribution of gRNAs was calculated as counts per million (CPM).

Cell lines. NIT-1 and 293FT (catalogue no. R70007) cell lines were obtained from ATCC and Thermo Fisher Scientific, respectively. Cells were maintained in DMEM (catalogue no. 10310309; Gibco), supplemented with 10% FCS (Gibco), glutagro and penicillin/streptomycin (Corning) in a 37 °C incubator with 5% CO2. To generate control and RNLS knockout human iPSCs, we used a non-autoimmune group of mice. Genomic DNA was extracted from the grafts (Quick-gDNA MiniPrep Kit; Zymo Research), the next-generation sequencing (NGS) libraries were prepared as described previously and subjected to NGS analysis (Novogene). The gRNA sequences from the NGS data were extracted using standard bioinformatics methods; the distribution of gRNAs was calculated as counts per million (CPM).

To generate Citransplating RNLS−cells, Rnls mutant NIT-1 cells were transduced with lentivirus carrying a CRISPR-immune, EF1α promoter–driven full-length mouse Rnls (Citransplating RNLS−) carrying a synonymous mutation in the Rnls gRNA target site. The modified gRNA targeting site sequence used in Citransplating Rnls was 5′-TATATATGGCCGTAGGCA-3′. The Rnls-deficient NIT-1 cell lines (Rnls Δ exons 2−4 and Rnls Δ exon 5) were generated following previously published protocols. Two gRNAs were designed to target the 5′- and 3′-end of Rnls exons 2−4 or exon 5 genomic DNA sequences. The gRNA sequences for exons 2−4 were 5′-GGCTCTGGAAGAAGCTGTGGC-3′ and 5′-GGGAAGCATCCATCCATGGC-3′; the gRNA sequences for exon 5 were 5′-GGGGAGGGAGGTAGGATAC-3′ and 5′-TGCTGTAGGTTTGAAGTATG-3′. The Lenti-multi-CRISPR plasmid (catalogue no. 85402; Addgene) was used to express two single gRNA cassettes for the deletion of exons 2−4 or exon 5 of Rnls. The two gRNA cassettes were amplified by a Phusion High-Fidelity PCR Kit (Thermo Fisher Scientific): 40 cycles of 98 °C, 15 s; 60 °C, 15 s; 72 °C, 30 s. The PCR products were digested with BsdI (Invitrogen) and subcloned into the pSpCas9(9)Bb-2A-Puro (PX459) V2.0 vector (catalogue no. 62988; Addgene). NIT-1 cells were then transfected with these plasmids by polyethyleneimine (Thermo Fisher Scientific), followed by puromycin selection. All plasmid sequences were verified by Sanger sequencing before transduction and transfection.

Preparation and transplantation of primary islets. Islets were prepared and purified as described previously from 8-week-old female NOD-scid mice. Briefly, the pancreas was perfused with collagenase type V/cold Hank's balanced salt solution (HBSS) and was immediately removed by surgical dissection. The pancreatic tissue was digested in a 37 °C water bath for 15−17 min. The digested tissue was then washed with cold HBSS three times, followed by Histopaque gradient separation. Islets were handpicked under a dissection microscope. Purified NOD-scid islets were disrupted into small clusters by gentle pipetting and then cultured in a low-attachment plate in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco) supplemented with 10% FCS and penicillin/streptomycin for viral infection and reaggregation. Lentivirus encoding an NT or Rnls gRNA together with Cas9 endonuclease under the control of RIP was added to the culture media for overnight infection. The next day, islets were washed with culture media twice and approximately 300 islets were transplanted under each kidney capsule of 8-week-old female NOD-scid mice. Gift recipients were left to recover from surgery for 2 weeks, then mice were randomly assigned to non-autoimmune and autoimmune groups. Mice in the autoimmune group were injected intravenously with 107 splenocytes purified from spontaneously diabetic female NOD mice. Splenocytes were prepared as described earlier (see ‘CRISPR GeCKO-A library screen’ section). At day 25 post-splenocyte injection, islet grafts were retrieved for gene expression analysis by quantitative PCR with reverse transcription (RT−qPCR).

RT−qPCR. Cells or islet grafts were treated with TRizol (Thermo Fisher Scientific) for RNA extraction according to the manufacturer’s protocol. Purified RNA was reverse-transcribed into complementary DNA (cDNA) using the SuperScript IV First-Strand Synthesis System (Invitrogen). Inst) (MM01259683_g1), Gg (MM01269055_m1) and Hprt (MM0302475_m1) probes for TaqMan assays were purchased from Thermo Fisher Scientific. Gene expression levels of Chop and Tenip were analysed by SYBR Green PowerUp RT−qPCR assays (Applied Biosystems). The primer sequences used for Chop were: forward, 5′-CCACACACCTGAAGCCAGAA-3′; reverse, 5′-GGGTAAGGGCGGAGAAGATCTA-3′. The primer sequences used for Tenip were: forward, 5′-TCAAGGCCCCGTTGGACACT-3′; reverse, 5′-GAACCTGTGGCATTAGATCG-3′. All RT−qPCR assays were performed using a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems).

Cell viability assay. Cells were seeded in a 96-well white plate (50,000 cells per well) for overnight culture with or without TG, tunicamycin, STZ (Sigma-Aldrich), mitomycin C (Thermo Fisher Scientific), murine recombinant IL-1β (Biolegend) and IFN-γ (PeproTech), or hydroxyl peroxide (Thermo Fisher Scientific) at the indicated concentrations. Cell viability was assessed after 24 h using the CellTiter-Glo Luminescence Cell Viability Assay (Promega Corporation).
Isole cell ER stress assay. The 8-week-old female NOD-scid mice were given drinking water with or without pargyline (5 mg·ml$^{-1}$) ad libitum for 1 week. Isolets were prepared and purified as described earlier. Purified NOD-scid islets were cultured in a low-attachment plate in full culture media with or without pargyline (2 mg·ml$^{-1}$) with or without 1 μM of Tg for 5 h. Isolet protein samples were collected as described later (see ‘Western blotting’). Then, 20 μg denatured islet proteins was used for SDS–polyacrylamide gel electrophoresis (PAGE). Cleaved caspase-3 (catalogue no. 9664S; Cell Signaling Technology) and GAPDH (catalogue no. 2118; Cell Signaling Technology) were blotted to detect apoptotic pathway activation.

In vivo bioluminescence imaging. NIT-1 cells line were engineered to constitutively express the firefly luciferase gene (lacZ) driven by the EF1α promoter via lentiviral delivery, except in Extended Data Fig. 1, where a CMV-lacZ construct was used. Mice transplanted with luciferase-expressing cells were injected with β-luciferase intraperitoneally at a concentration of 15 mg·ml$^{-1}$ for bioluminescence imaging, and data was recorded using an IVIS Spectrum imaging system (PerkinElmer).

Xbp1 splicing assay. Cells were treated with either dimethylsulfoxide or Tg at 1 μM for 5 h. RNA was extracted by TRIzol and reverse-transcribed into cDNA as described for RT–qPCR. Spliced (s) and unspliced (u) Xbp1 cDNA were amplified by PCR using Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific) at 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min. The PCR products of Xbp1 were sequenced using the Sanger method.

Western blotting. Cell lysates were collected on ice in radioimmunoprecipitation assay (RIPA) buffer containing protease and phosphatase inhibitors (Complete Proteinase Inhibitor Cocktail; Sigma-Aldrich; Pierce Phosphatase Inhibitor; Thermo Fisher Scientific). Protein concentrations were measured by Pierce BCA Protein Assay (Thermo Fisher Scientific); 40 μg of denatured cell lysate protein were used for SDS–PAGE electrophoresis (4–20% TGX gel; Bio-Rad Laboratories). The following primary antibodies were used: PERK (catalogue no. 3192; Cell Signaling Technology); phospho-PERK (Thr980, catalogue no. 11815S; Cell Signaling Technology); ATF4 (catalogue no. 11815S; Cell Signaling Technology); spliced (s) and unspliced (u) Xbp1 (catalogue no. 2103; Cell Signaling Technology); phospho-eIF2α (Ser51, catalogue no. 3192; Cell Signaling Technology); phospho-PERK (Thr980, catalogue no. DY999; R&D Systems) was added to each well and incubated for 15–30 min. After washing four times with washing buffer and three more times with PBS, substrate reagent (catalogue no. 557630; BD Biosciences) was added to each well for 1 h. After washing four times with washing buffer and three more times with PBS, substrate reagent (catalogue no. DY999; R&D Systems) was added to each well and incubated for 15–30 min. The reaction was stopped by adding 1x PBS 0.1% Tween 20 washing buffer three times. Then, 2 μg·ml$^{-1}$ of detection antibody (biotinylated mouse IFN-γ ELISPOT, catalogue no. 31-18118A; BD Biosciences) was added to each well and the plate was incubated for 2 h at room temperature. Plates were washed three times with washing buffer and horseradish peroxidase-conjugated streptavidin (catalogue no. 557630; BD Biosciences) was added to each well for 1 h. After washing four times with washing buffer and three more times with PBS, substrate reagent (catalogue no. DY999; R&D Systems) was added to each well and incubated for 15–30 min. The reaction was stopped by adding 1x PBS 0.1% Tween 20 washing buffer three times.

iPSC cultures and generation of RNLS knockout iPSCs. iPSC maintenance and differentiation was carried out as described previously. iPSCs from an individual with TID were obtained from stocks maintained by the Melton Lab. RNLS exon 2 was targeted for deletion using a dual gRNA strategy in undifferentiated iPSCs. Deletion of the targeted region was verified by PCR and sequencing.

Flow cytometry analyses of iPSC-beta cells. Differenitized clusters, sampled from the expansion culture (1–2 ml), were dissociated using TrypLE Express (catalogue no. 12604013; Gibco) at 37°C. Mechanically disrupted to form single cells, fixed and stained as described previously.

Apoposis assay for iPSC- and HUES8-beta cells. Differenitized clusters were treated for 24 h with Tg (5 μM) and stained at room temperature for 30 min using a 1:100 dilution of PE antihuman CD90 (clone 3EB3, catalogue no. 559596; BD Biosciences) and Annexin V-FITC (catalogue no. ab176750; Abcam). For pargyline treatment, stem/beta cells were treated with 5 μM pargyline for 24 h and the drug was kept at 37°C during TG challenge for another 24 h.
structures based on FAD suggested that these inhibitors, for instance, pargyline, may inhibit human RNLS also. The model of full-length human RNLS in complex with pargyline hydrochloride (catalogue no. P8013; Sigma-Aldrich) at concentrations of 0, 0.1, 1, 10, 25, 50 and 100 mM for 20 min at 4 °C before adding SYPRO Orange dye (catalogue no. S6650; Invitrogen) to measure thermal denaturation. The thermal shift assay was performed using the QuantStudio 6 Flex Real-Time PCR system with an initial temperature hold at 25 °C for 2 min, followed by a temperature ramp up to 95 °C at a rate of 1 °C per and a final temperature hold at 95 °C for 2 min. Results were collected at 0.25 °C increments. The melting temperature of RNLS in the presence and absence of pargyline was calculated by the first derivative of the fluorescence emission as a function of temperature (dF/dT).

Oral pargyline treatment studies. Nine-week-old female NOD mice were injected intraperitoneally with cyclophosphamide (200 μg·kg⁻¹ of body weight; catalogue no. C0768; Sigma-Aldrich) for diabetes induction. Diabetic NOD mice (blood glucose >450 mg·dl⁻¹) identified 10–14 d later were randomly assigned to the Ctrl (normal water) or pargyline treatment groups (5 μg·ml⁻¹ pargyline hydrochloride in drinking water). Treatment was started one week before beta cell transplantation. NIT-1 beta cells carrying a luciferase reporter were pretreated with 5 μM of RNLS dissolved with 10 μM of RNLS dissolved with pargyline hydrochloride (catalogue no. P8013; Sigma-Aldrich) at concentrations of 0, 0.1, 1, 10, 25, 50 and 100 mM for 20 min at 4 °C before adding SYPRO Orange dye (catalogue no. S6650; Invitrogen) to measure thermal denaturation. The thermal shift assay was performed using the QuantStudio 6 Flex Real-Time PCR system with an initial temperature hold at 25 °C for 2 min, followed by a temperature ramp up to 95 °C at a rate of 1 °C per and a final temperature hold at 95 °C for 2 min. Results were collected at 0.25 °C increments. The melting temperature of RNLS in the presence and absence of pargyline was calculated by the first derivative of the fluorescence emission as a function of temperature (dF/dT).

Glucose-stimulated insulin secretion and insulin enzyme-linked immunosorbent assay (ELISA). Primary islets were isolated from 8-week-old ICR male C57BL/6J mice were either injected intraperitoneally with a single high-dose of either cyclophosphamide (200 μg·kg⁻¹ of body weight) or anti-CD1 blocking antibody (250 μg per mouse; clone RMPI1-41; Bio X Cell). Alternatively, disease was induced in 10-week-old NOD-scid mice by injection of 10⁶ spleenocytes from overtly diabetic NOD mice; cell recipients were treated with or without pargyline-infused water (25 μg·ml⁻¹). Blood glucose was monitored every 1–2 d and graft bioluminescence was measured every 2–3 d. For preventative studies, 10-week-old NOD mice were pretreated with or without pargyline-infused water for 1 week (25 μg·ml⁻¹), then disease was induced with a single injection of pargyline (200 μg·kg⁻¹ of body weight) or anti-CD-PD 1 blocking antibody (250 μg per mouse; clone RMPI1-41; Bio X Cell). Alternatively, disease was induced in 10-week-old NOD-scid mice by injection of 10⁶ spleenocytes from overtly diabetic NOD mice; cell recipients were treated with or without pargyline-infused water (25 μg·ml⁻¹). Blood glucose was monitored every 1–2 d and graft bioluminescence was measured every 2–3 d. For preventative studies, the STZ-induced diabetic mouse study, 8-week-old male C57BL/6J mice were either injected intraperitoneally with a single high-dose of STZ (150 mg·kg⁻¹) or with 5 low doses (50 mg·kg⁻¹) on consecutive days. For high-dose STZ, mice were given drinking water with or without pargyline (25 μg·ml⁻¹) ad libitum 3 d after STZ injection. For multi-low-dose STZ, mice were pretreated with pargyline 1 week (25 μg·ml⁻¹). Blood glucose was monitored every day in the first week, then every 3–4 d in the second week and every week thereafter for experiments that were monitored for longer than 2 weeks.

Immunofluorescence staining. Pancreatic islets were isolated from Ctrl and pargyline-treated mice. Pancreatic sections were stained with anti-insulin (catalogue no. A0564; Dako), anti-CD3 (catalogue no. MCA8241; R&D Systems). Images of individual islets were taken with a Zeiss LSM 710 NLO confocal microscope.

Statistical analyses. Statistical analyses were performed by unpaired or paired tests as indicated using Prism v.8.0.2 (GraphPad Software). All data are presented as the mean ± s.e.m. P < 0.05 was considered statistically significant. Sufficient sample size was estimated without the use of a power calculation. No samples were excluded from the analysis. No randomization was used for the animal experiments.
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**Author contributions**

E.P.C., Y.I., W.Z., J.L. and B.K. performed the mouse experiments, analysed the data and edited the manuscript. N.C.L. performed all human cell experiments, analysed the data and edited the manuscript. J.H.-L. performed the islet transplantations. S.H. and Y.I. performed the structural analyses under the supervision of C.A.S. D.A.M. supervised the research with human cells, interpreted the data and edited the manuscript. P.Y. and S.K. conceived the project, designed and supervised the experimental work, analysed and interpreted data, and wrote the manuscript. All authors edited the manuscript.

**Competing interests**

P.Y. and S.K. have filed patent applications related to the work described in this manuscript. D.A.M. is a scientific founder and a board observer of Semma Therapeutics. The authors declare that they have no other competing interests.

**Additional information**

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Extended Data Fig. 1 | Autoimmune killing of NIT-1 cells in NOD mice can be visualized by bioluminescence imaging. a, b. Bioluminescence imaging of 10^7 NIT-1 cells transplanted subcutaneously into NOD.scid mice. Transplanted cells were engineered to carry a CMV-luciferase2 (Luc2) reporter. Some recipient mice were also injected intravenously with 10^7 splenocytes isolated from spontaneously diabetic (DM) NOD mice to cause beta cell killing. Images were taken at day 1 (a) and 15 (b) post-injection.
Extended Data Fig. 2 | Generation of Rnls-mutant beta cells by CRISPR-Cas9 targeting. a, T7 endonuclease I assay. Genomic DNA from NIT-1 wild-type (WT) and Rnls<sup>mut</sup> cells was tested for CRISPR-Cas9 gene editing events. Cleavage at heteroduplex mismatch sites by T7 endonuclease I digestion was analyzed by agarose gel electrophoresis. DNA from Rnls<sup>mut</sup> cells segregated into multiple digested fragments, indicating efficient mutation of the targeted region in the Rnls gene.

b, Genomic DNA from Rnls<sup>mut</sup> cells was sequenced to identify individual mutations. The Rnls gRNA targeting site is labelled in red. The frequency of the wild-type allele and of the most abundant mutations and their predicted consequence (frameshift / in-frame deletion) are shown. These frequencies indicate that 75% of the cells are predicted to carry at least one deleterious mutant allele.

c, Islets (≈1700) were purified from 8-week old CD1 mice, dispersed and transduced with lentivirus encoding a non-targeting (NT) or Rnls-targeting gRNA together with the Cas9 endonuclease driven by the rat insulin promoter. 72 h later, islets were stimulated sequentially with 2.8 mM glucose, 16.8 mM glucose and finally 30 mM KCl to induce insulin secretion. Islet genomic DNA was quantified for normalization of ELISA insulin measurements to DNA content. n=5 technical replicates per condition and genotype. Data show mean ± SEM. Note that islet dispersion necessary for lentiviral transduction decreased the overall responsiveness of purified islets compared to intact islets. Insulin secretion by Rnls mutant islet cells was not significantly different from that of control (NT) islets.

d, Growth curves for NIT-1 WT, control (NT gRNA) and Rnls<sup>mut</sup> cells seeded in 96-well plates at 50,000 cells/well over one week. Culture media were refreshed in every 2 days. Cell growth was measured on days 0, 3 and 7 using the CellTiter-Glo luminescence Cell Viability Assay (Promega). Growth rates were not significantly different as calculated by one-way ANOVA with Dunnett’s multiple comparisons test. n=3 technical replicates per genotype. Data show mean ± SEM.
Extended Data Fig. 3 | See next page for caption.
**Extended Data Fig. 3 | Rnls mutation prevents autoimmune killing but not allo-rejection of NIT-1 beta cells.** Control and Rnls<sup>−/−</sup> NIT-1 cells (10<sup>7</sup>) carrying a luciferase reporter were implanted on opposing flanks of NOD.scid (a) or C57BL/6 mice (b, c). a, Graft bioluminescence was measured on days 0 and 57 after transplantation of NIT-1 cells together with diabetogenic NOD splenocytes (as in Fig. 2). b, c, Graft bioluminescence was measured on days 0, 4 and 7 after transplantation. Representative bioluminescence images (b) and relative luminescence of grafts over time (c) are shown (n=3). Data represent mean ± SEM. Both control and mutant grafts were destroyed by allo-rejection within a week.
Extended Data Fig. 4 | Rnls expression modulates the sensitivity to ER stress-induced cell death but not to ER stress-unrelated apoptosis. a. Viability of Rnls\textsuperscript{mut} and control NIT-1 cells at 6 h and 24 h after treatment with 40 mM streptozotocin (STZ) (n=3 technical replicates). b. NIT-1 cell viability 48 h after mitomycin C (MMC) at the indicated concentration (n=3 technical replicates). ***P < 0.0001, calculated by two-way ANOVA with Sidak’s multiple comparisons test.

c-f, Rnls knockout NIT-1 cell lines were generated by deleting either exons 2-4 or exon 5. Deletion efficiency was confirmed by qPCR of genomic DNA. Rnls ΔEx2/4 cells showed ~60% deletion of exons 2-4 genomic DNA qPCR (c) while Rnls ΔEx5 cells showed ~87% deletion of exon 5 (d). Cell viability of Rnls deficient cells was measured 72 h after thapsigargin (TG, e) and tunicamycin (TC, f) treatment. ***P < 0.0001, calculated by unpaired t-test (c,d) and two-way ANOVA with Sidak’s multiple comparisons test (e,f).

g-h, Overexpression of Rnls in WT NIT-1 cells increased sensitivity to low dose-TG-induced killing (g). n=4 technical replicates per group. ***P < 0.0001, calculated by two-way ANOVA with Sidak’s multiple comparisons test. CRISPR-immune Rnls (CiRnls) expressed in Rnls\textsuperscript{mut} cells restored sensitivity to TG-induced killing (h). n=4 technical replicates per group. ***P < 0.001, *P = 0.0138, ***P = 0.0002, 0.0005 for 0.05 and 0.25 TG(μM) respectively, calculated by two-way ANOVA with Sidak’s multiple comparisons test.

*Comparison of control vs. Rnls\textsuperscript{mut} cells; #comparison of Rnls\textsuperscript{mut} vs. Rnls\textsuperscript{mut} + CiRnls cells. All data represent mean ± SEM.
Extended Data Fig. 5 | Rnls overexpression increases sensitivity to autoimmune killing in vivo. a-c, Control (WT) and Rnls overexpressing (RnlsOE) NIT-1 cells carrying a luciferase reporter were implanted on opposing flanks of NOD.scid mice. Some graft recipients were also injected intravenously with splenocytes from diabetic NOD mice (DM NOD splenocytes). Graft bioluminescence was imaged on days 0, 2, 3 and 7 (a). The relative luminescence of RnlsOE and control grafts over time, normalized to day 0, is shown in (b). Data for all mice analyzed on day 3 is shown in (c). RnlsOE graft were more sensitive to autoimmune killing as evidenced by more rapid loss of luminescence. By day 7, both control and RnlsOE grafts were killed to ~90% (data not shown), resulting in a similar relative luminescence level. n=6 mice (each with two grafts). Data represent mean ± SEM, **P < 0.0022, calculated by two-sided Mann-Whitney test.
d,e, Rnlsmut NIT-1 cells and Rnlsmut cells expressing the CRISPR-immune Rnls transgene (CiRnls), all carrying a luciferase reporter, were implanted on opposing flanks of NOD.scid mice. Graft recipients were also injected intravenously with splenocytes from diabetic (DM) NOD mice. Graft bioluminescence was imaged on days 0, 2, 3 and 5 post-injection (d). Relative luminescence of paired grafts over time normalized to day 0 is shown in (e). n=5 mice. Data represent mean ± SEM.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Rnls deficiency diminishes the UPR following ER stress and protects against oxidative stress. a, Quantification of Western blot data shown in Fig. 4e. Images were obtained and quantified using a C-DiGit scanner and the Image Studio software (LI-COR Biosciences). n=3 per group. Data show mean ± SEM, *# P < 0.05, **### P < 0.01, ****### P < 0.001, calculated by one-way ANOVA with Dunnett’s multiple comparisons test. *Comparison to control cells without TG treatment; #comparison to control cells with 5-hour TG treatment. b, Control (Ctrl) and Rnls mutant NIT-1 cells were cultured overnight with or without hydrogen peroxide (H₂O₂) at the indicated concentrations. Cell viability was assessed using the CellTiter-Glo luminescence Cell Viability Assay. Data show mean ± SEM of triplicate cultures and are representative of three independent experiments. **** P<0.0001, calculated by two-way ANOVA with Sidak’s multiple comparison test.
Extended Data Fig. 7 | Pargyline treatment preserves insulin expression in NOD mice with long-duration diabetes. Pancreases were isolated from control and pargyline-treated diabetic NOD mice described in Fig. 6 that were euthanised at day 20 post beta cell-transplantation. Pancreatic sections were stained with anti-insulin (DAKO, #A0564), anti-CD3 (Bio-rad, #MCA500), and DNA dye Hoechst 33342 (Invitrogen, #H3570). Goat anti-guinea pig Alexa Flour 488 and donkey anti-rat Alexa Flour 594 secondary antibodies (Thermo Fisher Scientific, #A11073 and #A21209) were used to detect insulin and CD3 antibodies, respectively. a, Representative images of individual islets, taken with a Zeiss LSM710NLO confocal microscope. b, Representative pancreas section from a pargyline (PG)-treated animal scanned using a Thermo Fisher Scientific EVOS FL Auto imaging system. Five islets were identified on the section: islets #1-4 showed many insulin-expressing cells, islet #5 had no remaining insulin-expressing cells. No significant insulin staining was detectable in the pancreas of untreated mice (not shown). c, Plasma insulin levels at day 20 post-transplantation in diabetic mice with a NIT-1 beta cell graft that were treated or not with PG. Data show mean ± SEM of n=5 mice per group and are representative of two independent experiments, * P = 0.0367, calculated by two-sided unpaired t-test.
Extended Data Fig. 8 | Pargyline treatment does not prevent beta cell destruction after allo-transplantation and has no glucose-lowering effect on its own. a, b, Wild-type NIT-1 cells (10⁷) carrying a luciferase reporter were implanted into C57BL/6 mice that were treated or not with oral pargyline via addition to the drinking water. Graft bioluminescence was measured on days 1, 2, 3 and 4 after transplantation. Representative bioluminescence images (a) and relative luminescence of grafts over time (b) are shown. Data show mean ± SEM for n=3 mice per group. c, Pargyline did not decrease hyperglycemia in C57BL/6 mice rendered diabetic by streptozotocin (STZ) injection (150mg/kg). Data show mean ± SEM for n=5 mice (control) and n=9 (pargyline).
Extended Data Fig. 9 | Pargyline prevents or delays diabetes in multiple mouse models for T1D. 

**a**, Diabetes frequency after cyclophosphamide injection of NOD mice fed with control water (Ctrl, n=40) or water containing pargyline (PG, n=39). 

**b**, Day of disease onset in mice that developed diabetes after cyclophosphamide injection (Ctrl n=19, PG n=11). 

**c**, Diabetes frequency in NOD mice injected with blocking anti-PD-1 antibody with (n=6) or without (n=5) oral PG treatment (as in **a**). 

**d**, Diabetes frequency in NOD.scid mice transplanted with splenocytes (10^7 cells) from diabetic NOD mice and treated with or without PG (n=10 per group). 

**e,f**, Diabetes frequency (n=10 per group) and day of disease onset (ctrl n=9, PG n=8) in C57BL/6 mice treated with multiple low doses of streptozotocin. Kaplan-Meier survival curves were compared by Log-rank test (**a, c, d and e**). Time of disease onset is shown as mean ± SEM and was compared by Mann-Whitney test (**b, f**). 

**g**, Insulin and T cell marker staining in pancreas sections from NOD mice two weeks after anti-PD-1 injection, with or without PG treatment.
Extended Data Fig. 10 | Design, genotyping and phenotyping of RNLS deletion in human SC-beta cells. a, RNLS dual-gRNA design for the generation of RNLS knockout (KO) human induced pluripotent stem cells (SC). b, Genotyping of SC clones. CRISPR targeted clones were genotyped by PCR that was repeated for confirmation for all mutant clones, and individual mutations were verified by sequencing. Clone 1 was used as RNLS KO in this study and carried a 112bp deletion on both alleles. c, Glucose stimulated insulin secretion by SC-beta cells differentiated from WT or RNLS KO isogenic SC clones. Data in c show mean insulin secretion from four independent SC-beta cell batches, each measured in triplicate, following stimulation with 2.8mM glucose, 20mM glucose, or 30mM potassium chloride (KCl). Data show mean ± SEM for n=4 technical replicates per condition and genotype.
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