Islet transplantation in the subcutaneous space achieves long-term euglycaemia in preclinical models of type 1 diabetes

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The intrahepatic milieu is inhospitable to intraportal islet allografts, limiting their applicability for the treatment of type 1 diabetes. Although the subcutaneous space represents an alternate, safe and easily accessible site for pancreatic islet transplantation, lack of neovascularization and the resulting hypoxic cell death have largely limited the longevity of graft survival and function and pose a barrier to the widespread adoption of islet transplantation in the clinic. Here we report the successful subcutaneous transplantation of pancreatic islets admixed with a device-free islet viability matrix, resulting in long-term euglycaemia in diverse immune-competent and immuno-incompetent animal models. We validate sustained normoglycaemia afforded by our transplantation methodology using murine, porcine and human pancreatic islets, and also demonstrate its efficacy in a non-human primate model of syngeneic islet transplantation. Transplantation of the islet-islet viability matrix mixture in the subcutaneous space represents a simple, safe and reproducible method, paving the way for a new therapeutic paradigm for type 1 diabetes.

Pancreatic islet transplantation is the most effective β-cell replacement therapy available to achieve precise glycaemic control in patients with type 1 diabetes. Current clinical islet transplantation is based on intraportal infusion of pancreatic islets, which is fraught with complications, including haemorrhage, portal vein thrombosis, inflammatory response and amyloidosis, ultimately resulting in graft loss. Past attempts at utilization of alternative sites have been largely unsuccessful, necessitating surgical preparation of vascular beds, or are dependent on a bio-engineered module with limited long-term follow-up (reviewed in ref. ). Interest in the subcutaneous space has garnered recent attention on transplantation of allogeneic stem-cell-derived islets, since this would facilitate monitoring for teratoma formation and allow simple, complete excision of the graft if needed. Nevertheless, while the skin is a highly attractive site for islet transplantation owing to its safety profile and ease of access, paucity of nutrients and oxygen inevitably results in islet graft failure.

The milieu in which islets are engrafted is critically important for their survival. Indeed, the importance of collagen I in the peri-islet extracellular environment in the healthy mammalian pancreas has been elucidated in past reports. Building on this, attempts at restoration of the peri-islet cyto-architecture with oligomeric or encapsulation-based collagen matrices have demonstrated a pro-survival effect on mouse islet function both in vitro and in vivo. Here, we report a previously undescribed mixture of human collagen I, L-glutamine, fetal bovine serum, sodium bicarbonate and medium 199 which, when admixed with murine, porcine or human islets, promotes uniform survival of the islets subcutaneously. The preparation of this compound, termed islet viability matrix (IVM), is described in Extended Data Fig. 1 (visual protocol in Supplementary Video 1).

In the immuno-incompetent murine model, subcutaneous transplantation of 800 syngeneic islets without IVM into diabetic B6 SCID or WT B6 hosts uniformly failed to reverse diabetes, with most experiencing primary non-function (Fig. 1a). In contrast, even a 50% reduction in islet mass, when bedaubed with IVM, consistently rendered the recipients normoglycaemic within 24 hour post transplantation (Fig. 1a). Excision of islet-bearing skin invariably resulted in recurrence of diabetes. Histological comparison of tissue containing IVM-treated (IVM+) versus control (IVM-) islets demonstrated abundant healthy β-cells in the former cohort, in contrast to loss of islet morphology and viability in the latter (Extended Data Fig. 2). These observations were consistent across xenogeneic transplantation of porcine islets (Fig. 1a and Extended Data Fig. 2).

We next assessed whether human islets would undergo similar survival when engrafted subcutaneously in diabetic immuno-incompetent hosts. In contrast to human pancreatic islets transplanted without IVM, those engrafted with IVM uniformly reversed diabetes, as corroborated by measurements of blood glucose (Fig. 1b) and human C-peptide (Fig. 1c). Excision of islet-bearing skin in the long-term recipients of human islets led to prompt recurrence of hyperglycaemia. The IVM+ islets exhibited persistent collagen I in the islet extracellular environment and demonstrated preserved cellular morphology (Fig. 1d). Subsequently, we evaluated the...
The loss of islet POD 49 in the IVM to the subcutaneous grafts, excision of the retroperitoneal island glycaemia across various species of diabetic mice (Fig. 3a). Similar to the subcutaneous grafts, excision of the retroperitoneal island containing pancreatic islets confirmed healthy cellular morphology and function (Fig. 3b). Collectively, these data confirm that IVM supports islet viability and insulin release in vivo, building on previous work where the addition of extracellular matrix components such as collagen induced islet adhesion and inhibit inflammation. Additionally, we compared human islet graft survival with IVM in the subcutaneous space with traditional islet transplant sites used in mice, namely the kidney capsule and portal vein, using a range of islet mass in genetically identical animals. Comparative analysis showed that the subcutaneous islet–IVM mixture consistently rendered the hosts normoglycaemic at an earlier time post transplantation (Extended Data Fig. 4).

To delineate whether IVM induces early changes in expression of the primary glucose receptor Glut2 (SLC2A2) and insulin, we cultured human islets in the presence or absence of IVM and sampled a subset of the islets at days 0, 3 and 7. The expression of SLC2A2 and INSULIN were quantified at each time point between the IVM+ and IVM− islets. Although the SLC2A2 and INSULIN expression levels were sustained and higher in the IVM+ group represents excision of the islet-bearing skin at the times of elective retrieval and not due to graft destabilization. SCID, severe combined immune deficiency. B. Metabolic homeostasis, as evaluated by glucose measurement in B6/SCID animals transplanted with human islets ± IVM, showed that IVM+ islets consistently rendered the recipients normoglycaemic. C. Human C-peptide levels were measured in the serum of these recipients and are shown in the violin plot. Each dot represents C-peptide measured from an individual recipient mouse. The difference in C-peptide levels was statistically significant (**P<10⁻¹⁰ based on one-sided Mann–Whitney U-test). D. As a representative example, in B6/SCID mice at POD 7 in the IVM+ cohort and POD 49 in the IVM− cohort, an excisional biopsy was performed showcasing fragmented insulin+ cells in the former group, in contrast to preserved islet architecture and integrated collagen in the latter. Images show the results from H&E staining and IHC (red, insulin; green, glucagon).
To further examine the kinetics of gene expression with islet endocrine function in vivo, we divided age-matched, streptozotocin (STZ)-treated NSG mice into two groups; in one group, human IVMc islets were subcutaneously engrafted while the other received IVMc islets. Glucose measurements at 3, 6, 24 and 48 h post-plantation revealed that animals in the IVMc group achieved glucose homeostasis as early as 6 h post-implantation (Fig. 4a). RNA analysis from human islet grafts excised at POD 2 from mice in both cohorts substantiated a significant increase in SLC2A2 expression (Extended Data Fig. 5b,c). These insights suggesting enhanced islet cell survival and replication in the presence of the islet-IVMc mixture were further supported by increased bromodeoxyuridine (BrdU) incorporation in the proportionality for BrdU incorporation in the presence of IVM (Extended Data Fig. 5b,c).

Next, to probe the presence of a pro-β-cell-survival RNA signature in the IVMc population, we performed immunohistochemistry (IHC) on sections from the human-into-B6/SCID islet transplant model. Utilizing antibodies targeting specific epitopes for Bcl-2, von Willebrand factor (VWF), vascular endothelial growth factor (VEGF) and glucagon-like peptide 1 (GLP-1), we observed that human IVMc islets had significantly higher proportion and intensity for stained epitopes compared to IVMc− at PODs 1 and 10 (Extended Data Fig. 6). The Bcl-2 family of proteins is important in inhibiting mitochondria-dependent extrinsic and intrinsic cell death pathways, whereas GLP-1 and GLP1R promote β-cell function and insulin secretion. Therefore, we sought further evidence for whether an anti-apoptotic mechanism might underlie the IVM-mediated success of subcutaneous islet transplantation, using transplant-islet-specific exosomes (TISEs) as a marker for tissue health status.

Using human-into-mouse islet transplants, we compared intra-exosomal cargoes in recipients of IVMc and IVMc− transplants at different time points during the first 10 days post-engraftment, and found upregulation of markers associated with cellular regeneration and insulin regulation in the former. Specifically, anti-inflammatory and pro-cell survival were upregulated in islet exosomes from the IVMc group (Extended Data Fig. 7a,b). Furthermore, immunoblot analyses of circulating TISEs showed insulin expression almost exclusively in the IVMc− exosomes, as well as an increase in GLP-1, GLP1R, Bcl-2 and Bcl-xL in the IVMc group, compared to IVMc− (Extended Data Fig. 7c). In accordance with the IHC and immunoblot data, further gene set analyses revealed upregulation of pro-angiogenic and proliferative pathways in the IVMc− TISEs, whereas apoptosis and mTOR/MAPK/PI3K-Akt pathways were downregulated (Extended Data Fig. 7).

Lastly, in light of the translational potential of IVM, we developed a preclinical model of subcutaneous islet autotransplantation in a cynomolgus monkey (Fig. 4c; details in Methods). After induction of diabetes by a ~90% pancreatectomy, autologous islets were transplanted subcutaneously. Grafting islets with IVM below the skin rendered the monkey normoglycaemic, and a partial excisional skin biopsy the recipient was treated with STZ to eliminate native islets. The animal had become insulin dependent by POD 820, probably due to a concordant and progressive increase in body weight, or possibly secondary to reduced islet mass due to a previous excisional skin biopsy. Moreover, at euthanasia, the monkey islets demonstrated secondary to reduced islet mass due to a previous excisional skin biopsy. Additionally, the monkey islets demonstrated secondary to reduced islet mass due to a previous excisional skin biopsy.

In another cynomolgus monkey, following subtotal pancreatectomy the recipient was treated with STZ to eliminate native islets in the residual pancreatic head. The isolated islets were cultured...
glucagon production. Establishment of this new method augments the utility of allogeneic pancreatic islet transplantation and future stem-cell-derived and xenograft islet grafts, as well as related cellular therapies in tissue engineering and reparative medicine.

Methods

Composition and preparation of IVM. Composition. The composition of IVM comprised 10× M199 (Sigma Life Science, catalogue no. M0650-100ML), l-glutamine (Mediatech, catalogue no. 25-005-CI), fetal bovine serum (HyClone Laboratories, catalogue no. SH30071.03), 7.5% sodium bicarbonate, NaHCO₃ (Life Technologies, catalogue no. 11879010), 1× Hepes, 100 U ml⁻¹ penicillin, 100 μg ml⁻¹ streptomycin, 250 μg ml⁻¹ amphotericin B (Mediatech, catalogue no. SH30071.03), 7.5% sodium bicarbonate, NaHCO₃ (Life Technologies, catalogue no. 11879010), 1× Hepes, 100 U ml⁻¹ penicillin, 100 μg ml⁻¹ streptomycin, 250 μg ml⁻¹ amphotericin B (Mediatech, catalogue no. 25-005-CI), fetal bovine serum (HyClone Laboratories, catalogue no. SH30071.03), 0.1 M HEPES (Sigma Life Science, catalogue no. H2140-500ML), 250 μg ml⁻¹ gentamicin, 100 μg ml⁻¹ streptomycin, 0.25% bovine serum albumin (Sigma Life Science, catalogue no. A5440-25G), and 1× HEPES, 100 U ml⁻¹ penicillin, 0.25% bovine serum albumin (Sigma Life Science, catalogue no. A5440-25G), and 1× HEPES, 100 U ml⁻¹ penicillin.

Preparation. Extended Data Fig. 1 shows the concentration of each ingredient used to create 1 ml of IVM. Each ingredient and the final product were required to be kept on ice at all times, to prevent solidification at higher temperatures. A tutorial for how to make the IVM is provided in Supplementary Video 1.

Murine islet transplantation models. Animals. Immune-competent (Balb/cBy) (stock no. 000651) and C57BL/6J, B6 (stock no. 006641), and immune-incompetent (B6.Cg-Ptdc−/−Sじ/J (B6/scid (stock no. 001913), CBySmn.Cg-Ptdc−/−/J (Balb/c/ scid (stock no. 001803), NU/J (Balb/c/nude (stock no 002019), B6.Cg-Foxn1−/−J (B6/nude (stock no. 000819) and NOD-Scid IL2Rgammam−/− (NOD (stock no. 0055577) male mice aged 8–12 weeks, used as islet donors and recipients, were obtained from the Jackson Laboratory. Intermediate controls were not utilized because these experiments involved transplantation of murine, porcine and human islets into diabetic recipients.

Animals were housed in conditions appropriate to minimization of stress, including a 12/12-h light/dark cycle, ~50% humidity and 20–21 °C.

Diabetes induction. Recipients were rendered diabetic by a single intraperitoneal injection of STZ (SICOR Pharmaceuticals) at a dose of 200 mg kg⁻¹. Five days after STZ administration, animals with three consecutive (daily) non-fasting blood
Glucose levels >350 mg dL⁻¹ (Contour Blood Glucose Monitoring System, Bayer HealthCare) were used as islet recipients. The protocol and all animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Pennsylvania (protocol nos. 805662, 800932 and 805005), and in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the US National Institutes of Health (NIH).

Islet isolation. Mouse pancreatic islet isolation was performed by collagenase P (Roche Diagnostics) digestion and density gradient separation. Islets were maintained in a suspension of RPMI-1640 medium.

Islet transplantation. Recipient mice were anaesthetized by inhalation of 2–5% isoflurane (Isoflurane USP, Clipper Distribution).

1 In the subcutaneous transplantation model, a small skin incision (0.3–0.5 cm) was established over the lower abdomen to create right and left lower-quadrant subcutaneous pockets in which 400 fresh islets (hand-picked) were injected immediately, in either a suspension of 360 µl of RPMI-1640 (islets alone, IA) or admixed in 360 µl of IVM, into right and left subcutaneous pockets separately.

2 In the retroperitoneal transplantation model, the right lower abdominal cavity was opened and 400 manually picked fresh islets, either in a suspension of 180 µl of RPMI-1640 (islets alone) or admixed in 180 µl of IVM, were injected with a 25-gauge butterfly needle underneath the peritoneal layer in the right posterior retroperitoneal space.

Of note, regardless of the number of isolated islets (range, 200–800), the volume of IVM (360 µl for subcutaneous and 180 µl for retroperitoneal) in which they were suspended remained the same, largely due to anatomical space constraints in the animal.

Immunosuppressive regimen following allogeneic or xenogeneic transplantation in mice. The immunosuppression regimen was based on targeting both T and B-cells to promote the survival of islet allograft tolerance. Briefly, maintenance immunosuppression consisted of rapamycin (0.5 mg kg⁻¹ intraperitoneally four times daily), starting on the day of islet transplantation for a duration of 7 d. To target the B-cell compartment we used 10F4, a monoclonal antibody against mouse BLYS (100 µg intraperitoneally 2 d before transplantation, in two doses 24 h apart); 10F4 (eliminates primary B-cells) was provided by Human Genome Sciences, courtesy of M. Cancro (Pathology and Laboratory Medicine, University of Pennsylvania). 10F4 was also given again starting on day 10, in a tapering dose, from 50 µg per week in week 2 down to 5 µg per week starting from week 8. Immunosuppression was discontinued at day 66.

Porcine islet transplantation models. Islet isolation. Porcine islets were obtained from B. Herrig’s laboratory at the University of Minnesota. Islets were incubated in CMRL 1066 medium (Mediatech, catalogue no. 98-304-CV) containing 5.5 mM D-glucose, 0.05% human albumin (Telesis Biotherapeutics), 10 µl ml⁻¹ heparin (Sagent Pharmaceuticals), 100 µg ml⁻¹ penicillin/streptomycin and 2 mM L-glutamine.
Anaesthesia was initiated with midazolam (1 mg kg$^{-1}$) and maintained with ...300 mg dl$^{-1}$ after a period of 1 week, islet grafts were considered to have failed and the recipient was considered to have primary non-function of the islet transplant.

Glucose tolerance testing. An intraperitoneal glucose tolerance test was performed on animals with long-term normoglycaemia (>100 d$^{-1}$). Blood glucose levels were analysed using the Contour Blood Glucose Monitoring System (Bayer HealthCare).

Excision of islet-bearing sites. To confirm that the islet grafts were the sole source of maintenance of normoglycaemia, a cohort of long-term normoglycaemic islet recipients underwent excision of islet-bearing sites (skin or retroperitoneum). This uniformly led to prompt recurrence of diabetes, within 24 h. In recipients of autotransplanted monkeys and a needle connected to a syringe containing the isolated islets, plus IVM, was inserted into the subcutaneous space. The total volume of inoculum was slowly infused into the subcutaneous compartment and the needle point was then swabbed with antiseptic. Finally, the animal was left to recover in an incubator and monitored thereafter. According to the advice of our IACUC veterinarians, we did not treat the monkeys with exocrine extracts during the post-transplantation period.

**Islet graft function.** According to IACUC recommendations, after transplantation blood glucose levels in autotransplanted monkeys were monitored twice daily for the first 2 months, then every 3 months, thereafter. Recipients with non-fasting glucose concentrations <200 mg dl$^{-1}$ were considered to have achieved normoglycaemia requiring no daily exogenous insulin administration. When two consecutive daily non-fasting glucose levels >300 mg dl$^{-1}$ were recorded after a period of primary graft function, islet grafts were considered to have failed, mandating treatment with exogenous insulin twice daily. Towards POD 400, as a result of frequent blood sampling, the tail tip developed a wound. The IACUC veterinarian’s recommendation was to not sample the animal for a period of several weeks to months, giving sufficient time for the tail to heal completely. Any gaps in daily blood glucose measurements during the 3-year follow-up were based on the uniform recommendation of the veterinary staff to comply with ethical handling of the animal with proper care.

Biopsies of islet-bearing skin were performed under general anaesthesia. Tissue samples (1–2 cm$^2$) were processed for standard haematoxylin and eosin (H&E) and IHC staining.

**Tracking the clinical course of the monkeys.** For monkey no. 212077 (body weight 3.5 kg) the excised pancreas weighed 6g, yielding 84,000 islet equivalents (IEQs). The volume of the islet tissue pellet was 0.3 ml, which was added to 16.7 ml of IVM yielding an inoculum containing 5,000IEQ ml$^{-1}$ of IVM. The entire inoculum (327,292 IEQ kg$^{-1}$ body weight) was infused into the subcutaneous space. It is important to note that the pre-planned glucose level in this monkey ranged from 30 to 110 mg dl$^{-1}$. The time from subtotal pancreatectomy to subcutaneous islet autotransplantation was 5h.

For monkey no. 210069, in addition to the protocol detailed above, this animal received STZ (50 mg kg$^{-1}$) after subtotal pancreatectomy; to eliminate residual native islets in the pancreatic head remnant. The excised pancreas weighed 5g, yielding 46,123IEQs that were cultured in vitro overnight. During this period the monkey had free access to water and food, but several blood glucose determinations revealed a level of 200 mg dl$^{-1}$ indicating the requirement for exogenous insulin treatment. The islets were then removed from culture. The volume of the islet tissue pellet was 0.2 ml, which was added to 9.8 ml of IVM yielding an inoculum containing ~5,000IEQ ml$^{-1}$ IVM. The entire inoculum (11,827 IEQ kg$^{-1}$ body weight) was transplanted subcutaneously into the left lower quadrant of the abdomen. Of note, the time from subtotal pancreatectomy to subcutaneous islet autotransplantation was 20h. In our attempts to eliminate residual islets in the remnant of the pancreatic head, persistent STZ administration may have led to the loss of islet cells transplanted under the skin 20h after STZ administration.

BrdU incorporation assay. In vivo BrdU labelling was achieved by dilution of drinking water with (1 mg ml$^{-1}$ BrdU; Sigma-Aldrich, no. B9285) for 3d, as described in ref. 15. Briefly, islet-bearing skin sections containing human islets were harvested, fixed overnight with 4% paraformaldehyde and processed for paraffin embedding. Histological analysis of slides was performed using BrdU, Ki67, insulin and glucagon. Slides were incubated for immunostaining as follows: sections were incubated in blocking reagent (1% bovine serum albumin in PBS) for 30 min, followed by overnight incubation with the appropriate primary antibodies in blocking reagent at 4°C. Slides were washed three times in PBS for 3–5 min each, followed by incubation for 45 min at room temperature with the
appropriate secondary antibodies in blocking reagent. Slides were washed again in PBS (three times for 5 min each) and mounted. Images were captured on a Keyence All-in-One Fluorescence Microscope using the x40/0.95 numerical aperture objective (Nikon) and positive nuclei were counted, blinded from at least 20 islet β cells per mouse from four mice per group. The protocol was approved by the IACUC of the University of Pennsylvania.

RNA isolation for real-time (RT)–PCR analysis. Islets from 18-week-old mice fasted overnight were isolated using the standard collagenase procedure15. Islet purity was assessed by Dithiozone staining and was determined to be >90% endocrine tissue. Total RNA from islets was isolated in Trizol (Invitrogen) followed by the use of the RNeasy mini kit or RNeasy FFPE kit (both Qiagen) according to the manufacturer’s instructions. Islet RNA was reverse transcribed using either 1 μg of Oligo(dT) primer or random hexamer, SuperScript II Reverse Transcriptase and the appropriate reverse primers (Invitrogen). PCR reactions were assembled using the Brilliant SYBR Green QPCR Master Mix and performed with the SYBR Green (with dissociation curve) programme on the Mx3000P qPCR System (Stratagene). All reactions were performed in triplicate with reference dye normalization, and median cycle threshold values were used for analysis. The primers used were as follows:

| Primer   | Sequence                                    |
|----------|---------------------------------------------|
| hSLC2A2 F | ATCCAAACCTGGAAAGAACCC                     |
| hSLC2A2 R | CTATGTCGCACTCTACACAA                     |
| hINSULIN F | AGGGCTATCAACGAGCATGCT                     |
| hINSULIN R | CAGTTGCTTCTAATTCCTAA                     |
| hPDX1 F   | CTTCTGTGCGGTTGATAGGT                     |
| hPDX1 R   | ATACCTCCACTGCCAAGAGG                     |
| hVEGF F   | CTACCTCACCATTGCGAAGT                     |
| hVEGF R   | GCAGATGCTGCGTGCATAGA                     |

IHC analyses. Islet-bearing skin or native pancreatic tissue biopsy samples were fixed in either Bouin's or formalin solution. The tissues were processed for routine histology and stained with H&E. For IHC analysis, serial paraffin sections were prepared and stained using the following antibodies: anti-insulin and glucagon (DakoCytomation), anti-bovine and human collagen I, anti-human CD2, anti-human VWF, anti-human VEGF, anti-human GLP-1 (Abcam) and anti-human K67 (ThermoScientific). The anti-collagen antibodies were species specific, with minimal to no cross-reactivity with mouse collagen: anti-human collagen antibody (Sigma, no. C-2456) and anti-bovine collagen antibody (Novus Biologicals, no. NB100-64253).

Immunofluorescence analyses were performed using the DakoCytomation Cell Pathway kit and were assembled using the appropriate primary antibody and HRP-coupled secondary antibody (Santa Cruz Biotechnologies) according to the manufacturer's protocol and detected by chemiluminescence using Phospho-Imager (Amersham Imager 680, GE Healthcare).

Library preparation and sequencing. Exosomal RNA samples were assayed for quantity and quality with an Agilent 2100 Bioanalyzer and the Agilent RNA 6000 Pico Kit (Agilent Technologies, no. 5067–1513). Libraries were prepared using the QiAseq miRNA Library Kit (Qiagen, catalogue no. 331502) per standard protocol R. The individual samples were prepared in triplicate. Libraries were assembled and quantified and quantified using the High Sensitivity DNA Kit for the Agilent 2100 Bioanalyzer (Agilent Technologies, no. 5067–4626). Samples were multiplexed for sequencing, then 100-base-pair (bp) single-read sequencing of a multiplexed pool of samples was carried out on an Illumina HiSeq 4000 sequencer. Illuminus bcl2fastq v2.0.0.422 software was used to convert bcl to demultiplexed fastq files.

Building chimeric Bowtie libraries and transcript quantification. We built three Bowtie libraries (Bowtie v1.2.5) consisting of mouse and human miRNA hairpins, trans RNA sequences, and isoform sequences. The miRNA reads from above were aligned to each separately using the command bowtie -q -k 4 --best --sam --norc. The use of miRNA, trNA and RefSeq was quantified using the Bowtie output files with a custom R script, with the libraries 'Rsamtools' and 'GenomicAlignments' to process the Bowtie BAM files. Simple filters indicating reads that had been filtered out and retained only those with the best Bowtie stratum (X/SAM tag), then any reads with a top-stratum match to both human and mouse were discarded. UMI reduction was performed by binning unique combinations of reference sequence name (that is, miRNA, trNA or RefSeq ID), start position and UMI. Overall raw expression was calculated as the number of unique reads aligning to each sequence. Raw read counts were also converted to counts per million by the addition of a pseudo-count of 1 to all detected transcripts, normalizing to total counts then taking log2.

Statistics and reproducibility. All statistical analyses were performed using R v3.5.3 (https://www.r-project.org). Data are expressed as mean ± s.d. unless indicated otherwise. The significance of differences between two independent groups was calculated by either Wilcoxon rank-sum Mann–Whitney U-test or Student's two-sample t-test, as indicated in the individual figure legends. For representative IHC images, experiments were performed five times independently using distinct biological isolates. The IHC distributions obtained from QuPath were compared between the IVM+ and IVM groups using the Kruskal–Wallis H-test. Cell culture experiments were repeated in independent biological triplicate to ensure the reproducibility of observations. Differences were considered significant at P < 0.05 after Benjamini–Hochberg correction for multiple hypothesis testing. The initial pathway analyses were carried out using multicos (https://cran.r-project.org/web/packages/multicos/index.html), and subsequent gene set enrichment analysis was performed using the WEB-based Gene Set Analysis Toolkit (Webgestalt; http://www.webgestalt.org)16. A filter of false discovery rate <0.05 was used to obtain selected pathways with the highest enrichment.

Unless stated otherwise in the figure legends, each experiment was repeated a minimum of five times per sample. Representative histology images shown in
Extended Data Fig. 1 | The constituents and their respective concentrations needed to create 1.0 mL of Islet Viability Matrix (IVM). A visual protocol to make the IVM is described in Supplementary Video 1.

| Ingredients                          | Concentration in 1ml IVM | Approximate Ratio |
|--------------------------------------|--------------------------|-------------------|
| 10x Medium (M) 199                   | 91 µl                    | 23                |
| L-glutamine                          | 8 µl                     | 2                 |
| Fetal Bovine Serum (FBS)             | 100 µl                   | 25                |
| NaHCO₃ (7.5%)                        | 23 µl                    | 6                 |
| Type 1 Collagen                      | 778 µl                   | 195               |
Extended Data Fig. 2 | Syngeneic and xenogeneic islet transplantation in the subcutaneous space. a and c Murine or porcine islet grafts were transplanted with IVM in immunoincompetent diabetic mice, following which non-fasting blood glucose level returned to physiological ranges (<200mg/dl) and remained stable long term. Hyperglycemia promptly resumed upon removal of the grafts (indicated by downward arrows in a and c). Additionally, we established the presence of viable and functional transplanted islets from donor mice b and pigs d in the subcutaneous space by histologic examination and staining for insulin (red) and glucagon (green).
Extended Data Fig. 3 | Intraperitoneal glucose tolerance test (GTT) in non-diabetic, normal/healthy mice, compared with immunoincompetent mice with long term survival (>6 months) with subcutaneous islet-IVM grafts. In each set of experiments, GTT kinetics were evaluated in controls (n = 5) and B6 recipients of a, mouse islets (n = 5) and b, porcine islets (n = 5). GTT was also performed in B6 nude and B6 SCID recipients of c, human islets (n = 5 in each group). Islet grafts in the subcutaneous tissue promptly restore normoglycemia upon glucose challenge. Mean glycemic values for each experimental group are plotted, and the error bars represent the standard deviation. There were no statistically significant differences in glucose regulation between healthy/normal mice and the IVM+ islet transplant groups.
### Extended Data Table 4

| Recipient   | N/sample size | Number of Islets | Transplant Site* | Graft‡ | NG$_{24}$ | NG$_{48}$ | NG$_{168}$ |
|-------------|---------------|------------------|------------------|--------|-----------|-----------|------------|
| NSG         | 8             | 200              | KC               | IA     | 0         | 0         | 25         |
| NSG         | 6             | 200              | PV               | IA     | 0         | 0         | 17         |
| NSG         | 6             | 200              | SC               | IVM    | 17        | 33        | 67         |
| NSG         | 10            | 400              | KC               | IA     | 60        | 60        | 80         |
| NSG         | 6             | 400              | PV               | IA     | 33        | 50        | 67         |
| NSG         | 8             | 400              | SC               | IVM    | 88        | 100       | 100        |
| B6/Nude     | 10            | 500              | KC               | IA     | 60        | 70        | 100        |
| B6/Nude     | 5             | 500              | PV               | IA     | 60        | 60        | 80         |
| B6/Nude     | 10            | 500              | SC               | IVM    | 80        | 80        | 100        |

*KC = Kidney Capsule; PV = Portal Vein; SC = Subcutaneous Space.
‡IA = islets alone; IVM = islets implanted subcutaneously with IVM.
NG$_x$ = % of animals normoglycemic within $x$ hours
NSG = non-obese diabetic SCID gamma mouse

Extended Data Fig. 4 | Table comparing the human islet graft survival with IVM in the subcutaneous space, with traditional islet transplant sites used in mice, viz., the kidney capsule and portal vein. Of note, the same protocol for islet isolation and preparation was followed and N animals were used to for each particular set of transplant experiments. Hand-picked, healthy human islets transplanted in the subcutaneous space with IVM uniformly resulted in normoglycemia, whereas the same number of islets transplanted in the kidney capsule and the portal vein in the genetically identical animals resulted in a delayed restoration of normoglycemia.
Extended Data Fig. 5 | SLC2A2 (Glut2) and INSULIN levels were upregulated at day 7 in islet culture with IVM (n=4 animals in each group). a, All gene expressions were normalized to TBP and expressed as mean ± SEM. * denotes $p < 0.05$ based on a one-sided Student’s t-test. Each dot represents an individual data point. No p-value correction for multiple hypothesis adjustment was done. The p-values corresponding to SLC2A2 at days 3 and 7 were 0.006 and 0.01 respectively; $p=0.008$ for INSULIN at day 7. b, Human islets transplanted subcutaneously in immune-incompetent diabetic mice with and without IVM (n=4 mice in both groups) were excised on POD7, and the grafts were immunoassayed for insulin (green), BrdU (red) and counterstained for nuclear DNA with DAPI (blue). Yellow arrows point to β-cells with DNA replication as indicated by BrdU incorporation. One hundred insulin$^+$ cells were counted per section in the IVM$^+$ group, whereas $<20$ insulin$^+$ cells could be counted in the IVM$^-$ cohort. The red structures represent nuclei, albeit some nuclei appear en face or out of plane. c, Quantification of DNA replication rate in IVM$^+$ and IVM$^-$ cohorts (n=100 cells in the IVM$^+$ group and 18 cells in the IVM$^-$ group) shows increased replication in the IVM$^+$ group (** indicates one-sided Student’s t-test $p=4x10^{-5}$).
Extended Data Fig. 6 | Based on human-into-mouse islet transplant model, immunohistochemical profiling of islets ± IVM for markers of angiogenesis (VEGF), anti-apoptosis (Bcl-2, GLP-1) and endothelial cells (VWF) highlights the increased intensity and stained area of these epitopes in the IVM+ group. The proportion and intensity of staining, quantified by QuPath using five automated regions of interest (ROIs) per sectioned image, is plotted below each section (* denotes \( p < 0.05 \) and ** denotes \( p < 5 \times 10^{-5} \) based on the one-way Kruskal-Wallis H Test; arrow in the GLP-1 IVM− section denotes staining artifact). The particular significant \( p \)-values were — VEGF at POD 1 (\( p=0.009 \)), Bcl-2 at POD1 (\( p=7 \times 10^{-8} \)) and POD10 (\( p=1 \times 10^{-9} \)), GLP-1 at POD1 (\( p=10^{-8} \)) and POD 10 (\( p=4 \times 10^{-8} \)) and VWF at POD 10 (\( p=2 \times 10^{-8} \)).
Extended Data Fig. 7 | Circulating human TISEs isolated at 6 hr, 12 hr, and on PODs 1, 3, and 10 from the IVM⁺ and IVM⁻ cohorts were sequenced. Differential expression across the five time points analyzed using one-sided Mann-Whitney U, as summarized in the Volcano Plot a. The x-axis is log₂ ratio of gene expression levels between the two cohorts; the y-axis is the Benjamin Hochberg-adjusted p-value based on −log₁₀. The colored dots represent the differentially expressed genes (blue = lower expression; red = higher expression in the IVM⁺ cohort) based on p < 0.05 and two-fold expression difference. Gene Set enrichment Analysis identified the major pathways (FDR ≤ 0.05) that were either upregulated or downregulated in the IVM⁺ group b. TISEs from IVM⁺ group showed higher expression of β-cell-specific proteins and anti-apoptotic markers as part of its intraexosomal cargo by Western blot analysis c. TSG101 protein is shown as a canonical exosome marker.
Extended Data Fig. 8 | Morphological and immunohistochemical analysis of autologous cynomolgus monkey islets, implanted in the subcutaneous space, was performed at the time of euthanasia (animal ID# 212077, POD 918). We found abundant healthy islet cell clusters, exhibiting vivid expression of key markers such as Insulin, Glucagon, Bcl-2, GLP-1, Ki67, VEGF, VWF and Collagen. Due to IACUC regulations and ethical care guidelines for nonhuman primate research, subcutaneous autologous islet transplants without IVM as a control could not be performed in a cynomolgus monkey.
Extended Data Fig. 9 | For cynomolgus monkey ID# 210069, the animal’s blood glucose just prior to pancreatectomy was 72 mg/dl; blood glucose monitoring post-transplantation demonstrated persistent hyperglycemia in the animal, which required management by exogenous insulin therapy. a. Failure to achieve normoglycemia in this monkey can be attributed partly to the suboptimal islet yield and transplantation of a relatively low mass of islets (11,827 IEQs/kg body weight), as well as the infusion of streptozotocin which likely led to the destruction of both native remnant islets as well as subcutaneously transplanted islets. b. In view of the persistent state of insulin dependency and per the recommendation of the IACUC veterinarian, the monkey was subjected to euthanasia on POD 250. During this course, an excisional biopsy of the islet bearing skin was performed on POD 46 and at the time of euthanasia. Both histologic assessments revealed abundant well granulated islet β-cells as well as glucagon-positive α-cells.
Extended Data Fig. 10 | Characteristics of human islet donors used in the described experiments.

| Donor age (years) | Gender | Purity | Viability | GSIR \( ^{\text{r}} \) | DNA content per islet (ng) | Experiments* |
|-------------------|--------|--------|-----------|----------------|--------------------------|--------------|
| 42                | M      | 90     | 88        | 2.1            | 4.5                      | Fig. 1       |
| 53                | F      | 90     | 91        | 1.4            | 10.1                     | Fig. 1       |
| 56                | M      | 90     | 91        | 1.2            | 2.4                      | Fig. 1       |
| 40                | M      | 90     | 89        | 1.1            | 2.3                      | Fig. 1       |
| 51                | M      | 95     | 77        | 1.3            | 8                        | Fig. 1       |
| 51                | M      | 90     | 73        | 1.4            | 4.2                      | Fig. 1       |
| 50                | F      | 90     | 93        | 1              | 13.4                     | Fig. 1       |
| 53                | M      | 90     | 88        | 1.05           | 2.5                      | Fig. 1       |
| 19                | M      | 70     | 95        | 1.19           | 4.8                      | Fig. 1       |
| 15                | M      | 75     | 93        | 1.1            | 3.2                      | Fig. 1       |
| 50                | F      | 85     | 96        | 2.8            | 4.2                      | Fig. 1       |
| 51                | F      | 90     | 96        | 1.63           | 2.6                      | Fig. 1       |
| 16                | M      | 70     | 91        | 3.53           | 4.4                      | Fig. 1,3     |
| 51                | F      | 80     | 94        | 2.8            | 6.1                      | Fig. 1,3     |
| 39                | F      | 96     | 94        | 1.31           | 3.4                      | Fig. 1,3     |
| 24                | M      | 90     | 92        | 0.6            | 5.2                      | Figs. 1, 4   |
| 13                | M      | 95     | 91        | 0.78           | 4.5                      | Figs. 1, 4   |
| 35                | M      | 90     | 94        | 1.16           | 5.2                      | Figs. 1, 4   |
| 55                | M      | 85     | 91        | 1.8            | 1.8                      | Figs. 1, 4   |
| 40                | M      | 80     | 93        | 2.4            | 1.3                      | ED Figs. 2-5 |
| 44                | F      | 90     | 95        | 1.6            | 1.4                      | ED Figs. 2-5 |
| 17                | F      | 80     | 94        | 3.3            | 1.24                     | ED Figs. 2-5 |
| 35                | F      | 85     | 94        | 1.9            | 6.9                      | ED Figs. 2-5 |
| 20                | M      | 80     | 92        | 1.6            | 2.24                     | ED Figs. 2-5 |
| 41                | F      | 90     | 97        | 2.55           | 7.3                      | ED Table 1   |
| 29                | M      | 90     | 93        | 2              | 5.5                      | ED Table 1   |
| 21                | M      | 90     | 95        | 1.5            | 1.9                      | ED Table 1   |

\( ^{\text{r}} \) GSIR = Glucose-Stimulated Insulin Release

*ED = Extended Data
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) and variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever possible.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

Exosomal RNA samples were assayed for quantity and quality with an Agilent 2100 Bioanalyzer instrument using the Agilent RNA 6000 Pico Kit (Agilent Technologies, Part number 5067-1513). Libraries were prepared using QIAseq miRNA Library Kit (QIAGEN, cat #331502) as per standard protocol in the kit’s sample prep guide. Libraries were assayed for overall quality and quantified using High Sensitivity DNA Kit of Agilent 2100 Bioanalyzer (Agilent Technologies, Part number 5067-4626). Samples were multiplexed for sequencing. 150bp single-read sequencing of multiplexed pool of samples was carried out on an Illumina HiSeq 4000 sequencer. Illumina’s bcl2fastq version v2.20.0.422 software was used to convert bcl to demultiplexed fastq files.

Data analysis

We build three bowtie libraries (bowtie v1.2.3) that consisted of mouse and human (1) miRNA hairpins, (2) tRNAs, and (3) RefSeq sequences. The miRNA reads from above were aligned to each separately using the command 'bowtie -q -k 4 --best --sam-noarc'. Expression of miRNA, tRNA, and RefSeq were quantified using the bowtie output files using a custom R script that used libraries 'Rsamtools', and 'GenomicAlignments' to process the bowtie BAM files. Simple species filtering and UMI reduction was performed as follows. Alignments were filtered to retain only those with the best bowtie stratum (XA BAM tag). Then any reads which had a top-stratum match to both human and mouse were discarded. UMI reduction was performed by binning unique combinations of reference sequence name, i.e., miRNA, tRNA, or RefSeq id, start position, and UMI. Overall raw expression for a reference sequence was calculated as the number of unique reads aligning to each sequence. Raw read counts were also converted to CPM by adding a pseudocount of 1 to all detected transcripts, normalizing to total counts, then taking log2.

The proportion and the intensity of the stained epitopes were evaluated using QuPath v0.1.2. Prior to running the image analysis algorithm on a particular slide, automated image masking was performed per the developer’s recommended steps (https://github.com/quipath/quipath/wiki). The accuracy of this automated ROI annotation was manually checked by an independent pathologist. Subsequently, QuPath’s algorithm analyzed all ROIs in the inclusion annotation, excluding any tissue artifacts. Each section image had ≥5 ROIs of sufficiently high quality, and using each ROI as the unit of analysis, a Kruskal–Wallis H Test was performed to compare the output distributions for the proportion and intensity of staining between the IVM+ and IVM− groups.

All statistical analyses were performed using R v3.5.3 (https://www.r-project.org). Data are expressed as mean ± standard deviation.
unless indicated otherwise. The significance of differences between two independent groups was calculated by the Wilcoxon Rank Sum/Mann-Whitney U Test, or the Student’s two-sample t-test, as indicated in the figure legends. For representative IHC images, experiments were performed five independent times using distinct biological isolates. The IHC distributions obtained from QuPath were compared between the IV- and IV+ groups using the Kruskal-Wallis H Test. Cell culture experiments were repeated in independent biological triplicates to ensure reproducibility of the observations. Differences were considered significant at P<0.05 after Benjamini-Hochberg correction for multiple hypothesis testing. The initial pathway analyses were carried out using multicross [https://cran.r-project.org/web/packages/multicross/index.html], and subsequent gene set enrichment analysis performed using the Web-based Gene Set Analysis Toolkit [WebGestalt; http://www.webgestalt.org].

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Raw and processed exosome sequencing data have been submitted to GEO (Accession number GSE145593). Any additional information that supports the data within this paper and findings of this study are available from the corresponding authors upon request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-fat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

All animal models except the nonhuman primate were conducted on n=5. No sample size calculation was formally performed; instead the samples were chosen based on previous researcher experience and providing the fullest degree of confidence in the findings deduced from each experiment. We experimented on two nonhuman primates as a pilot trial for the proof-of-concept and feasibility of this method. Due to the ethical issues involved in NHP experiments, we limited it to two animals.

Data exclusions

None

Replication

All experiments were successfully replicated with biological replicate experiments across multiple mouse species and in several mice within each strain, unless indicated otherwise (for example in Figure 2 where two graft-failures have been denoted in the replicates and only 3 out of 5 transplants were successful). The replicates are indicated in each corresponding figure and table.

Randomization

This is not a clinical trial and a randomization procedure was not applicable to the mouse transplant experiments because the animals in the IV- and IV+ group were age- and strain-matched. Further, experiments were conducted at a similar time of the day to limit circadian fluctuations.

Blinding

Investigators were not blinded to mouse genotype during experiments to ensure age and sex matching.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | Antibodies           |
| ☒   | Eukaryotic cell lines|
| ☒   | Palaeontology        |
| ☒   | Animals and other organisms|
| ☒   | Human research participants|
| ☒   | Clinical data        |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | ChIP-seq              |
| ☒   | Flow cytometry        |
| ☒   | MRI-based neuroimaging|
Antibodies

Antibody — Vendor — Catalog Number [Clone, where applicable] — Lot Number — Dilution

Polyclonal guinea pig anti-insulin — Dako — A0564 — 10095957 — 1:250
Polyclonal rabbit anti-Gluconag — Proteintech Group, Inc — 15954-1-AP — 00024282 — 1:500
Monoclonal mouse anti-Collagen I — Sigma — C2456 [COL-1] — 08847A784V — 1:75
Monoclonal rabbit anti-Bcl-2 — abcam — ab137174 [E17] — GR3737704-3 — 1:100
Monoclonal mouse anti-VWF — abcam — ab201336 — [E2013540/W635] — GR3195786-9 — 1:100
Monoclonal rabbit anti-VEGF — abcam — ab27670 [SP28] — GR3214987-1 — RTU
Monoclonal rabbit anti-GLP-1 — abcam — ab108443 [EPR4042-1] — GR3159063-3 — 1:100
Monoclonal rabbit anti-Ki67 — Thermo Fisher Scientific — RM-9106-R4 (SP1) — 9106RR802C — RTU
Cy2™ AffiniPure Donkey Anti-Rabbit IgG [H+L] — Jackson ImmunoResearch Laboratories, Inc — 711-225-152 — 136061 — 1:400
Cy2™ AffiniPure Donkey Anti-Mouse IgG [H+L] — Jackson ImmunoResearch Laboratories, Inc — 715-225-151 — 137363 — 1:400
Cy3™ AffiniPure Donkey Anti-Guinea Pig IgG [H+L] — Jackson ImmunoResearch Laboratories, Inc — 706-165-148 — 119998 — 1:400
Cy3™ AffiniPure Donkey Anti-Rabbit IgG Jackson ImmunoResearch Laboratories, Inc — 711-175-152 — 139802 — 1:200
10F4 — Human Genome Sciences — Not Applicable — Not Applicable [company was bought by GSK and product no longer exists for commercial purchase] — RTU
Coat anti rabbit HRP conjugated antibody — Vector lab — MP-7451 — ZE1015 — RTU
Coat anti mouse HRP conjugated antibody — Vector lab — MP-7452 — ZE0620 — RTU

RTU unconjugated HLA allele-specific anti-HLA A2 monoclonal IgG antibody [Catalogue # 0791HA] was purchased from One Lambda [West Hills, CA, USA], for donor HLA class I specific exosome isolation from recipient mouse plasma total pool of exosomes. For the exosome analysis, antibodies to insulin (15848-1-AP; used at a dilution of 1:200) and TSG 101(288283-1-AP; used at a dilution of 1:500) were purchased from Proteintech Lab. Antibodies to GLP-1 (sc-390774; used at a dilution of 1:200), GLP-1 (sc-57166; used at a dilution of 1:200), Bcl-2 (sc-3782; used at a dilution of 1:200), and Bcl-X (sc-56021; used at a dilution of 1:200) were purchased from Santa Cruz Biotechnologies, Inc. Secondary antibodies conjugated to HRP [ready-to-use antibody; anti-mouse conjugates were purchased from Vector Lab: MP7451 and MP7452, respectively).

Validation

All commercially available antibodies were validated by the respective companies and manufacturers. In each experiment, we had appropriate positive and negative controls as described in the manuscript.

Animals and other organisms

Policy information about studies involving animals, ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Immune competent (Balb/cBy) — Stock Number:000651, and C57Bl/6J, B6 — Stock Number:000664, and Immune Incompetent (B6.Cg-Prkdcscid/SjL) (B6/scid — Stock Number:001913), CBySnr.Cg-Prkdcscid/J (Balb/c/scid — Stock Number:001803), NOD/LtJ (Balb/c/nude — Stock No: 002019), B6.Cg-Foxn1nu/J (B6/nude — Stock Number:000819) and NOD-scid IL-2Rgammamut/Nog (Nog — Stock Number:005557) male mice aged 8-12 weeks, used as islel donors and recipients, were obtained from the Jackson Laboratory, Bar Harbor, ME. Littermate controls were not utilized as these experiments involved transplantation of mouse, porcine and human islets into diabetic recipients. Animals were housed in conditions to minimize stress, including a 12-hour light/12-hour dark cycle, “50% humidity, and a 20-21°C temperature.

Two male cynomolgus monkeys [Macaca fascicularis] were obtained from Spring Scientific Perkasie, PA, for autologous islet transplantation. Monkey 1 (ID #211077) had an initial body weight of 3.5 kg and age 4 years, whereas monkey 2 (ID #210069) had an initial body weight of 3.9 kg and age 4 years.

Wild animals

No wild animals were used in the study.

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

The protocol and all animal studies [murine, porcine and nonhuman primate] were approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Pennsylvania (Protocol Numbers: 805662, 800932 and 805005), and in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the U.S. National Institutes of Health.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics

The demographic information of deceased organ donors is described in Extended Data Table 2. The minimum donor age was 13, and maximum was 56.

Recruitment

The recovery of pancreata from deceased organ donors was overseen under the auspices of the local/regional organ procurement agency [Gift of Life, Philadelphia, PA, U.S.A.]. The staff of the organ procurement agency obtained consent for organ recovery from the next of kin/donor’s family members without any intervention from the investigators utilizing the organs for research, in accordance with the U.S. Federal Mandate.

Ethics oversight

The human islets were Institutional Review Board (IRB)-exempt because IRB is responsible for alive human beings and not for
postmortem tissues. As such, the entire protocol/set of experiments using the human islets in mouse fell under the IACUC at the University of Pennsylvania.

Note that full information on the approval of the study protocol must also be provided in the manuscript.