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Supplementary Materials for

A nanofibrous encapsulation device for safe delivery of insulin-producing cells to treat type 1 diabetes

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Materials:

Materials and methods

Stem cell culture: The nondiabetic HUES8 human embryonic stem cell (hESC) line was used to generate stem cell derived β (SC-β) cells in this study. Undifferentiated hESCs were cultured in 30-mL spinner flasks (Reprocell; ABBWVS03A) on a rotator spinning plate (Chemglass) at 60 revolutions per minute (rpm) in mTeSR1 media (StemCell Technologies; 05850). The flask remained in a humidified 5% CO_2 incubator at 37º C. The cells were passaged every 3 days by single cell dispersion with Accutase (StemCell Technologies; 07920) and counted with a Vi-Cell XR (Beckman Coulter). 18 x 10^6 viable cells were seeded for propagation in mTeSR1 with 10 μM Y27632 (Abcam; ab120129).

Static glucose stimulated insulin secretion (GSIS) of SC-β cells: Clusters were collected and washed twice in Krebs Ringer Buffer (KRB), made from 128 mM NaCl, 5 mM KCl, 2.7 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1 mM Na_2HPO_4, 1.2 mM KH_2PO_4, 5 mM $NaHCO_3$, 10 mM HEPES (Gibco; 15630-080), and 0.1% BSA. The clusters were incubated in transwells (Corning; 431752) in a 24-well plate at 2 mM glucose KRB for a 1-hour equilibration. The transwells were drained and transferred to 2 mM glucose and then 20 mM glucose for 1-hour incubations. Supernatant was collected and insulin secretion was quantified with a Human Insulin Elisa kit (ALPCO; 80-INSHU-E10.1). The cells were then single cell dispersed with TrypLE and viable cells counted on a Vi-Cell XR to normalize insulin secretion.

Dynamic GSIS of SC-β cells: A dynamic GSIS system was built with an 8-channel dispenser pump (ISMATEC; ISM931C) connected to 38.1 mm inlet and outlet two-stop tubing (ISMATEC; 070602-04i-ND) with a 275-μl cell chamber (BioRep; Peri-Chamber) in between and a dispensing nozzle (BioRep; PERI-NOZZLE) attached at the end through 101.6 mm connection tubing (BioRep; Peri-TUB-040). The system was maintained in a water bath at 37 °C. Clusters were washed twice in KRB buffer and loaded into the cell chamber between Bio-Gel P-4 polyacrylamide beads (Bio-Rad; 150-4124). The chamber was perfused with 2 mM glucose KRB for 90 minutes prior to collecting effluent for 8 minutes. Next, the clusters were challenged with 20 mM glucose KRB for 24 minutes and then with 2 mM KRB for additional 12 minutes. The flow rate is 100 μL/min with 2-4 min collections. Last, clusters within the cell chamber were lysed in 10 mM TRIS (MilliporeSigma; T6066), 0.2% Triton-X 100 solution, and 1 mM EDTA. DNA from the lysed solution and insulin secretion were quantified with Quant-iT Picogreen dsDNA (Invitrogen; P7589) and Human Insulin Elisa kits, respectively.

Oxygen consumption rate (OCR): 15-30 clusters of primary human islets or SC-β cells were placed in XF24 islet capture microplates (Agilent; 101122-100) with Seahorse media (7.4 pH RPMI-1640 (Sigma; R6504)) at 20 mM glucose. The Seahorse XFe24 flux analyzer (Agilent) measured OCR of the clusters. Measurements were normalized to DNA through Quant-iT Picogreen dsDNA assay kit (Invitrogen; P7589).

Characterizations of device: To observe the morphology of nanofibers, membrane samples were gold sputter-coated and examined by the scanning electron microscope (SEM) (LEO 1550 FESEM). ImageJ (NIH, Bethesda, USA) was applied to quantitatively characterize the fiber diameter. 10 random fibers were selected and measured in each image, and a total of 10 images were counted. To study the tensile strength of the electro-spun fibers, the tube was mounted on

the dynamic mechanical analysis instrument (DMA Q800) with a distance of ~1.5 cm between the holders. The tensile testing was conducted at a rate of 0.5 N/min at room temperature. Stress (MPa) and strain (%) were automatically calculated by the software. The Young's modulus was obtained by measuring the slope of the stress-strain curve in the elastic region between 10% and 20%. Four samples were tested for the experiment.

Generation of green fluorescent protein (GFP)/luciferase-expressing cell line: Plasmid containing enhanced green fluorescent protein (EGFP) gene (720 bp) and humanized firefly luciferase (Luc2) gene (1653 bp) was constructed by Vector Builder. 293T cell line and 4T1 cell line were received as gifts. Strain C57BL/6 Mouse Mesenchymal Stem Cells (MSCs) (MUBMX-01001) were purchased from Cyagen (Santa Clara, CA). NIT-1 cells (ATCC CRL-2055) were purchased from ATCC. 293T cells were cultured in DMEM (#2051526, Gibco) supplemented with 10% FBS and 1% P/S. 4T1 cells were cultured in RPMI 1640 media with 10% FBS and 1% P/S. Mesenchymal stem cell (MSC) lines were cultured in Mesenchymal Stem Cell Growth Medium (GUXMX-90011, Cyagen). NIT-1 cells were cultured in F-12K medium with 10% FBS and 1% P/S. Lentivirus vectors were produced by transfecting 293T cells with the designed plasmid using ViraPower Bsd Lentiviral Support Kit (K497000, ThermoFisher). Media containing lentivirus vectors were collected and stored at 4 °C. MSC single cell solutions were prepared and 3 mL media containing lentivirus vectors were used to infect MSCs in 6-well plate. After 48h of infection, lentivirus medium was discarded, and fresh MSC growth medium was added. GFP^+/$luciferase^+$ MSCs were verified under a fluorescent microscope. For the formation of MSC spheroids, about 2.5 mL solution containing 1 million MSCs were added in a non-adherent 25 mm^2 petri dish. Then the cells were cultured on an orbital shaker with a speed of 50 rpm overnight. The spheroids were imaged with a fluorescent microscope mentioned above. The GFP/luciferase 4T1 spheroids were generated in the same manner as GFP/luciferase MSC spheroids.

Purification of rodent islets: The digestion was stopped by adding 20-25 mL of cold M199 media with 10% heat-inactivated fetal bovine serum (FBS) and a slight shaking. For both mouse and rat islets, digested pancreases were washed twice in the same M199 media, filtered through a 450 mm sieve, and then suspended in a Lymphocyte Separation Medium (LSM, Corning, 25-072-CV) / M199 media gradient and centrifuged at 1750 relative centrifugal force (rcf) for 20 min at 4 °C. This gradient centrifugation step was repeated when desired for higher purity islets. Finally, the islets were collected from the gradient and further isolated by a series of gravity sedimentations, in which each supernatant was discarded after 4 min of settling.

In vitro and ex vivo GSIS of islets inside device: Islet-laden devices and the same number of free-floating islets were cultured in 2 mL RPMI 1640 complete media for 1 day or 7 days in nonadherent 25 mm^2 culture dishes. After culture, the devices or free-floating islets were incubated in pre-warmed KRB solution supplemented with 25 mM HEPES, 1 mM L-glutamax, 0.1% BSA and 2.8 mM D-glucose for 30 min at 37 °C, 5% CO_2, and then incubated for 1 h with 2.8 mM or 16.7 mM D-glucose under the same condition. The supernatant was collected and frozen for future analysis. The insulin content in the supernatant was quantified by mouse insulin ELISA kit (ALPCO) according to the manufacturer's specifications. Absorbance of reaction solution at 450 nm was measured in the Synergy plate reader (Biotek). For devices retrieved from mice, the devices were put in KRB buffer supplemented with 2.8 mM D-glucose for 30 min and incubated in KRB buffer supplemented with 2.8 mM or 16.7 mM D-glucose for 90 min. The

stimulation index (SI) was calculated as the ratio of the insulin value after high glucose (16.7 mM) stimulation divided by insulin value after low glucose (2.8 mM) solution.

Live and dead staining: Islet-laden devices and a same number of free-floating islets were cultured as described above for 1 day in nonadherent culture dish. After culture, the nanofiber skin of the device was peeled off. The islet-laden alginate inside the device and the free-floating islets were stained by calcein-AM (green, live) and ethidium homodimer (red, dead) according to the manufacture's protocol (R37601, Thermo fisher). Fluorescent microscopic images were taken using a digital inverted microscope (EVOS FL Cell Imaging System). Quantification of the percentage of live cells in islets was carried out by calculating the intensity of fluorescence using ImageJ.

Transplantation procedures in mice: The mice were anesthetized using 3% isoflurane in oxygen and maintained at the same rate throughout the procedure. The abdomens of the mice were shaved and alternately scrubbed with betadine and isopropyl alcohol to create a sterile field before being transferred to the surgical field. A ~1 cm incision was made along the midline of the abdomen and the peritoneum was exposed using blunt dissection. The peritoneum was then grasped with forceps and a ~1 cm incision was made. Two devices with 600-700 islet equivalent (IEQ) in total were then inserted into the peritoneal cavity through the incision. The incision was closed using 6-0 silk sutures (DemeTECH, FL). The skin was then closed over the incision using wound clips.

Generarion of immunosuppressed mouse model: 50 mg/ml rapamycin (LC Laboratories) stock solution in 100% ethanol was prepared and stored in -80°C. The stock solution was diluted in 10 ml mixture [5 ml of 10% poly (ethylene glycol) (PEG) 400 in deionized (DI) water and 5 ml of 10% Tween 80 in DI water] to make a final concentration of 0.05 mg/ml. Diabetic recipients were injected with 0.5 mg/kg body weight in intraperitoneal (I.P.) space daily from -1 to 14 days post-transplantation. The immunosuppressed mice were transplanted with NICE devices encapsulating SC-β cells as described previously.

Measurement of total insulin content of the pancreas: Pancreas of the engrafted mice, diabetic mice and healthy mice were collected and homogenized. The homogenized tissue was placed into acid-ethanol (1.5% HCl in 70% ethanol), cut into small pieces using scissors and digested overnight at -20 °C. Then the acid-ethanol extract solution was neutralized with pH 7.5 TRIS buffer. The samples were further diluted, and the insulin content was quantified as mentioned before.

In vivo biocompatibility analysis of the device: Blank (cell-free) devices were implanted in the I.P. space, epididymal fat pad (E.F.P.), ventral subcutaneous (S.C.) space or the dorsal S.C. space of healthy C57BL/6 mice. After 2 weeks or 1 month, the implants were harvested, fixed in 10% buffered formalin and embedded in paraffin. Cross-sections were analyzed as described in the Histological Analysis section.

Mouse total antibody analysis: C57BL/6 mice received NICE device implants encapsulating syngeneic, allogeneic and xenogeneic islets (rat islets or human SC-β cells) for a month. Blood was withdrawn from retro-orbital sinus using capillary at D0, 1w, 2w, 3w and 4w post-transplantation and collected in tubes with clotting activator (Sarstedt; Microvette 300 Z). The blood samples were centrifuged at 2000 rcf for 15 min. The serum was transferred in a new tube and stored at -80°C. After devices were retrieved from the recipients, 100 µl saline was added into the I.P. space. The I.P. fluids were collected after gently shaking the belly. The I.P. fluid was

centrifuged at 1000 rpm for 5 min to remove cellular debris. The supernatant was transferred into a new tube and store at -80°C. The alginate core inside retrieved devices was collected and degraded with 100 µl 1 mg/ml alginate lyase (Sigma; A1603) and 1X Halt Protease Inhibitor Cocktail (Thermofisher; 87786) in saline in incubator for 1 h. Then the tube was centrifuged at 1000 rpm for 5 min to separate the alginate and the encapsulated islets. The supernatant was collected and stored at -80°C. Mouse total IgG (Thermofisher; 88-50400-22) and IgM (Thermofisher; 88-50470-22) were analyzed in all the samples (serum, I.P. fluids and degraded alginate) by using ELISA kit following manufacturer's instruction.

Donor specific alloantibody analysis: BALB/c mouse spleen was harvested and placed in 6 well plates. 5 ml RPMI1640 culture medium (Fisher; 11875-119) with 10% FBS was added in each dish. The spleen was smashed using a 3 ml syringe head. The spleen cell suspension was filtered through Falcon 40 µm strainer (Corning; 431750) to get single cell solution. The cells were pelleted by centrifugation at 1000 rpm for 5 min and the supernatant was aspirated. Red blood cells (RBC) were removed by adding RBC lysis buffer (BioLegend; 420301) and cells were incubated on ice for 5 min. The reaction was stopped by adding 20 ml PBS. Cells were centrifuged at 1000 rpm for 5 min and the supernatant was discarded. T cells were isolated using EasySep Mouse T Cell Isolation Kit (Stem cell technologies; 19851) following the manufacturer's instructions. 10^5 T cells in 70 µl cell staining buffer were incubated with 30 µl samples (serum collected from recipients with allografts at D0 and 4w, and degraded alginate) at 4 °C for 30 min. Serum collected from sensitized mice with BALB/c mouse islets transplanted in the kidney capsule at 4w post-transplantation was used as a positive control. After incubation, cells were washed with PBS and centrifuged at 1000 rpm for 5 min. Supernatant was removed and cells were stained with Zombie Yellow Fixable Viability Kit (BioLegend; 423103) following the manufacturer's instructions. Cells were washed one time with 2 ml Cell Staining Buffer (BioLegend; 420201) and centrifuged into a pellet. Fc receptors were blocked by pre-incubating cells with TruStain FcX PLUS (anti-mouse CD16/32) Antibody (BioLegend; 156603) in 100 µl Cell Staining Buffer for 5 min on ice. Cells were labeled with APC anti-mouse CD3 antibody (BioLegend; 100235) and FITC anti-mouse IgG Antibody (BioLegend; 406001) or FITC anti-mouse IgM antibody (Sigma; F9259) at 1:100 dilution on ice for 15 min. Cells were washed twice with 2 ml of Cell Staining Buffer by centrifugation at 1000 rpm for 5 min. Finally, stained cells were analyzed using Attune NxT flow cytometer (Thermo Fisher). The data were analyzed by FlowJo software v10.7.

Ca^{2+} imaging: SC-β cells and human islets were retrieved from the engrafted devices as mentioned above. The SC-β cells or human islets were extracted as mentioned previously, shipped overnight, and cultured in S6 or human islet media (CMRLS with 10% FBS) for recovery of SC-β cells or human islets, respectively. The clusters were single-cell dispersed with TrypLE for 10 min and seeded in Matrigel-coated 96 well plates (Cellvis, 963-1.5H-N) overnight for attachment in their respective recovery media. Cells were washed with 2 mM glucose KRB and incubated with 20 µM Fluo-4 AM (Invitrogen; F14201) in 2 mM glucose for 45 min at 37°C in incubator. Next, the cells were washed with 2 mM glucose KRB and challenged with 2 mM glucose KRB, 20 mM glucose KRB, and 20 mM glucose 30 mM KCl KRB, sequentially. Images were taken every minute using a Leica DMI4000 fluorescence microscope and calcium flux was calculated with ImageJ.

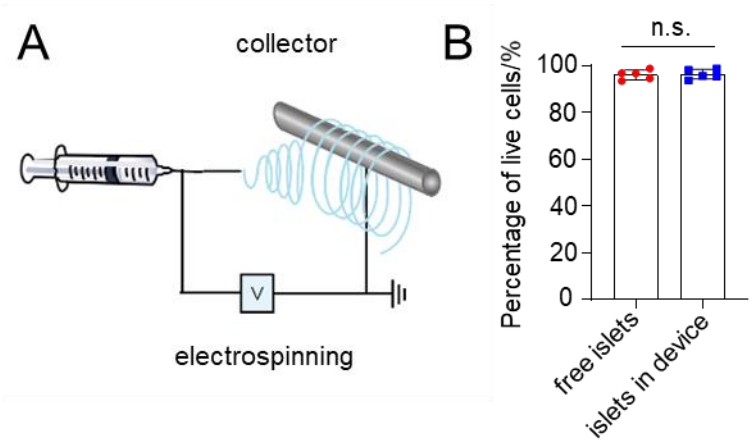


Fig. S1. In vitro analysis of NICE device. (**A**) A schematic of electrospinning used to make the device. (**B**) Quantification of live cells in free islets and encapsulated islets following 24h in vitro culture, mean ± SD (n = 5). The two-tailed Student's t-test was performed when the data consisted of two groups. n.s., non-significant.

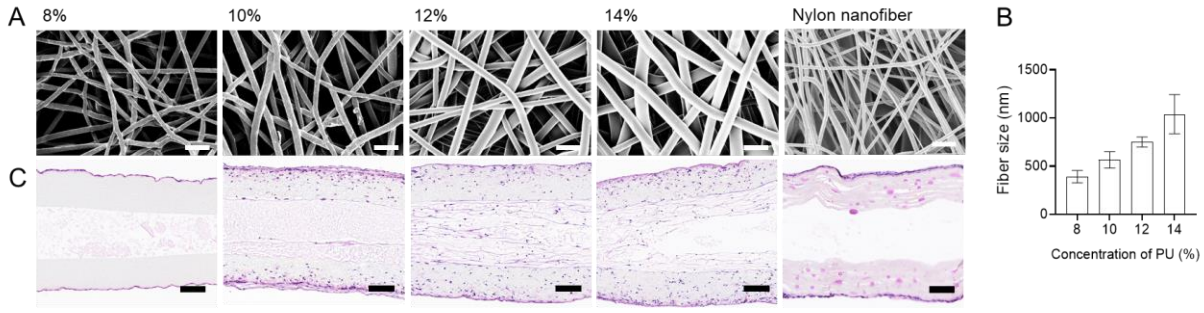


Fig. S2. **Nanofibrous devices of different fiber sizes and chemistries.** (**A**) SEM images of devices made from different concentrations of PU (from left to right: 8%, 10%, 12%, and 14%) and a different polymer (nylon). (**B**) Fiber sizes at different concentrations of PU, mean ± SD (n = 4). (**C**) H&E staining images of retrieved devices (from left to right: 8%, 10%, 12%, and 14% PU and Nylon) after one month of I.P. implantation in C57BL/6 mice. Scale bar: 100 μm (**C**) and 2 μm (**A**). SEM, scanning electron microscope; PU, polyurethane; H&E, haemotoxylin and eosin; I.P., intraperitoneal.

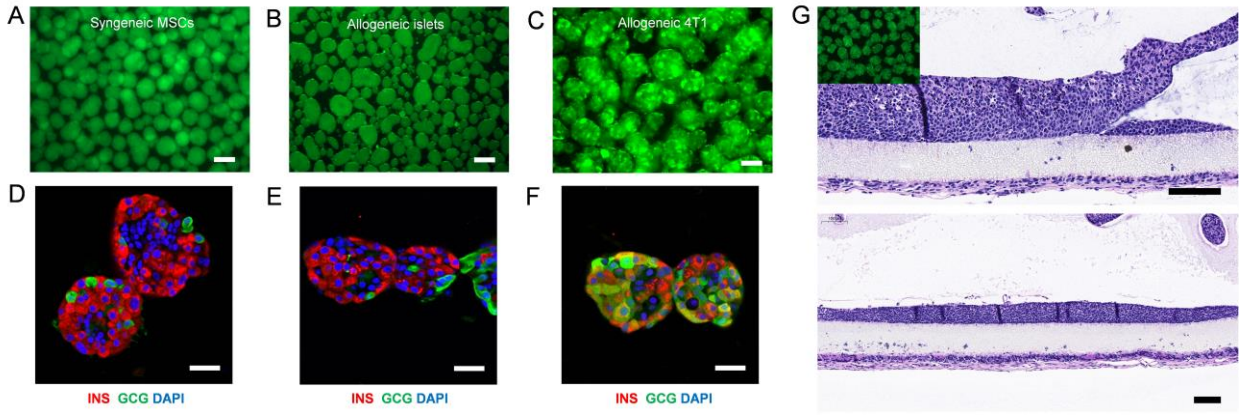


Fig. S3. **Function of NICE device in protecting allogeneic cells.** (**A**) A fluorescent image of C57BL/6 GFP/luciferase-expressing MSC spheroids. (**B**) A fluorescent image of FVB GFP/luciferase-expressing mouse islets. (**C**) A fluorescent image of BALB/c GFP/luciferase-expressing 4T1 spheroids. (**D** and **E**) Immunofluorescent staining of mouse GFP/luciferase-expressing islets encapsulated in the device for 120 days (red, INS; green, GCG; blue, DAPI). (**F**) Co-immunofluorescent staining of the mouse GFP/luciferase-expressing islets (red, insulin; green, GFP; blue, DAPI). (**G**) H&E staining of devices encapsulated with GFP/luciferase-expressing NIT-1 spheroids in healthy C57BL/6 for 2 months. The inset shows a fluorescent image of NIT-1 spheroids before transplantation. Scale bars: 250 μm (**A** and **B**), 100 μm (**C** and **G**) and 50 μm (**D**, **E** and **F**). GFP, green fluorescent protein; MSC, mesenchymal stem cell; INS, insulin; GCG, glucagon; H&E, haemotoxylin and eosin.

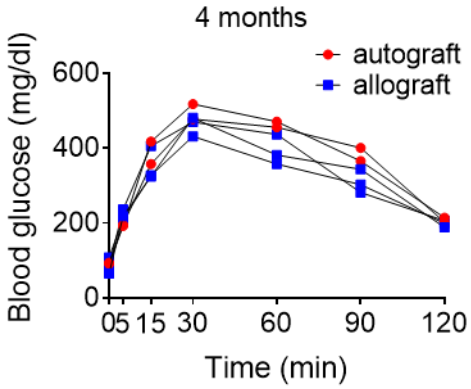


Fig. S4. Blood glucose measurement in IPGTT test of engrafted recipients at 4 months. IPGTT, intraperitoneal glucose tolerance test.

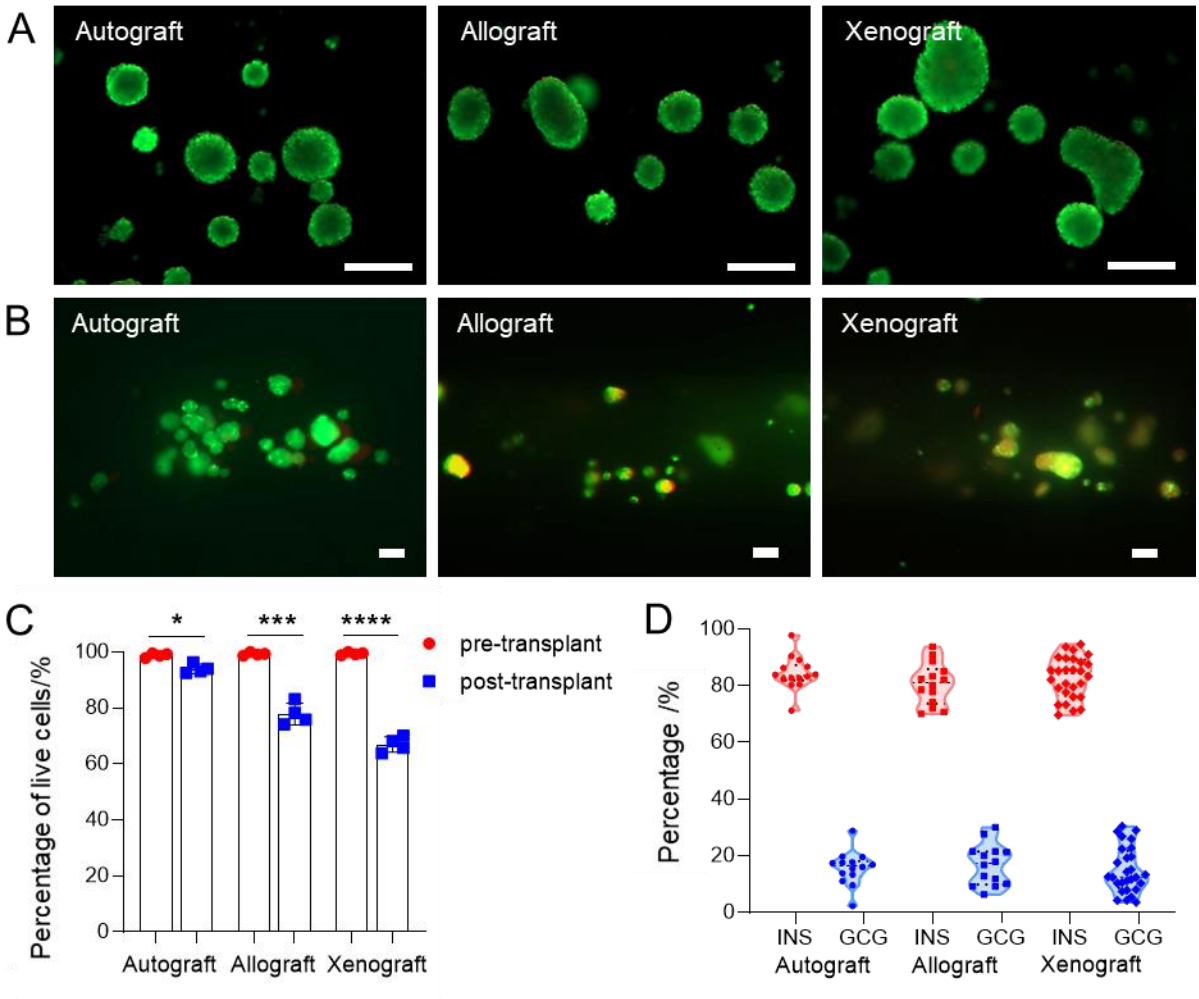


Fig. S5. **Analysis of rodent islets before and after transplantation.** Live (green) and dead (red) staining of rodent islets before transplantation (**A**) and one month after transplantation (**B**) (from left to right are syngeneic, allogeneic and xenogeneic islets). (**C**) Quantification of live cells in rodent islets before and after transplantation by calculating intensity of fluorescence in live and dead staining images (n = 4). (**D**) Percentage of hormone expression (insulin and glucagon) quantified from immunofluorescent staining images of rodent islets used in three different groups: autograft, allograft and xenograft before transplantation. One point represents one islet. The two-tailed Student's t-test was performed when the data consisted of two groups. *$P < 0.05$, ***$P < 0.001$, ****$P < 0.0001$, respectively. Scale bars: 200 μm (**A** and **B**).

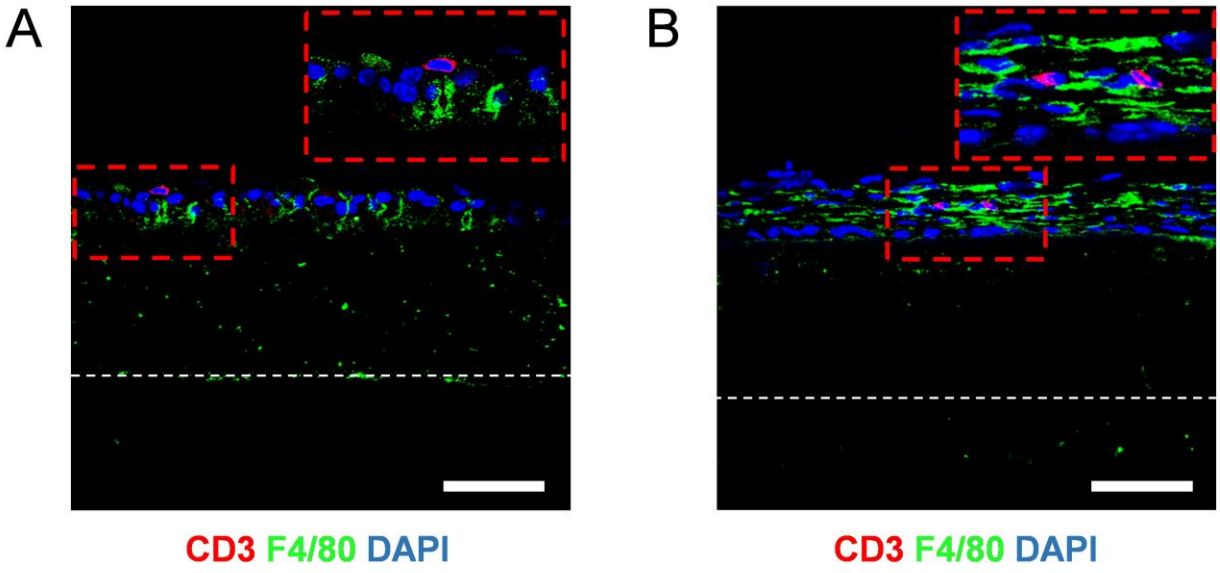


Fig. S6. **Histological analysis of fibrotic layer surrounding device.** Immunofluorescent staining of fibrotic layer around device with CD3 (red) and F4/80 (green) antibodies under two conditions: allograft (**A**) and xenograft (**B**) (higher magnifications in the top right corner). Scale bars: 50 μm (**A** and **B**). White dash lines show the boundary of the nanofibrous membrane.

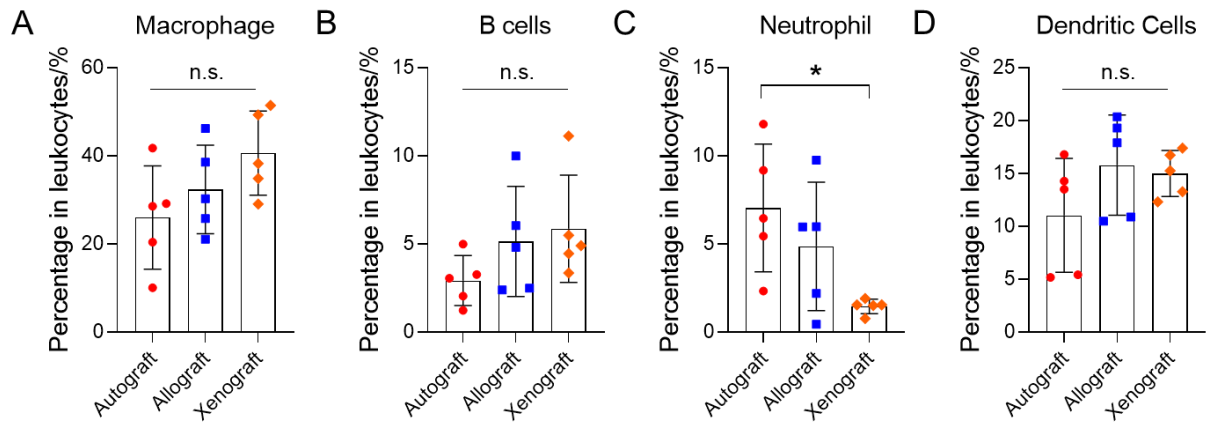


Fig. S7. **Analysis of immune cells in the fibrotic layer surrounding the NICE device in three different groups: autograft, allograft and xenograft.** Percentage of macrophages (**A**), B cells (**B**), neutrophils (**C**) and dendritic cells (**D**) in leukocytes, mean ± SD (n = 5). The one-way ANOVA followed by Tukey's test was performed for comparing the multi-group data. **P* < 0.05, n.s., non-significant.

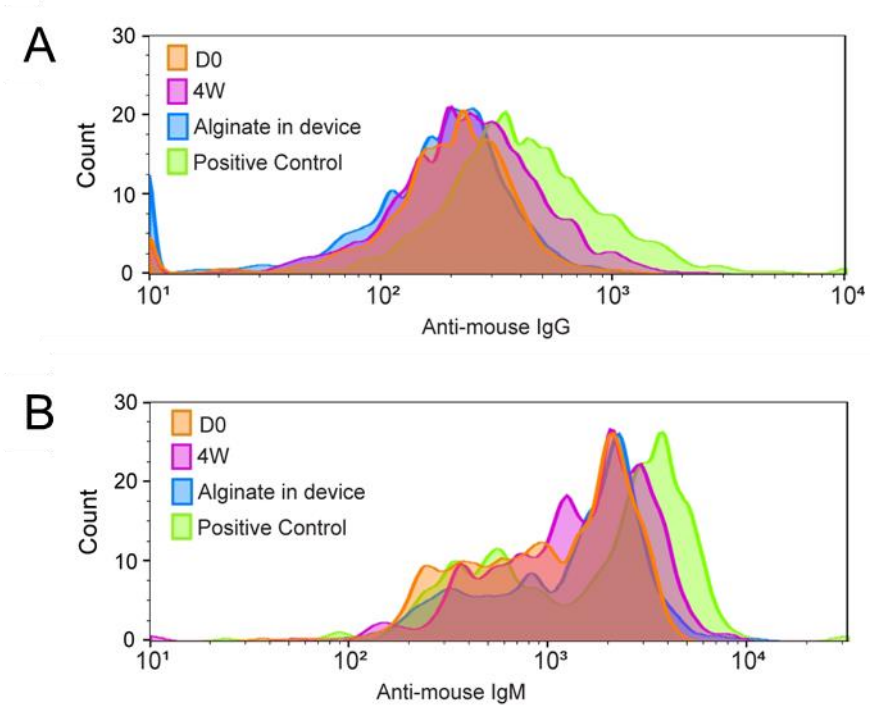


Fig. S8. **Analysis of donor specific antibodies.** Analysis of allo-antibodies IgG (**A**) and IgM (**B**) in serum extracted from C57BL/6 mice with allogeneic islets encapsulated in NICE devices at day 0 (orange curve) and day 28 (purple curve), in serum extracted from sensitized mice with allogeneic islets transplanted in kidney capsule at day 28 (green curve), and in devices retrieved from mice engrafted with encapsulated allogeneic islets at day 28 (blue curve).

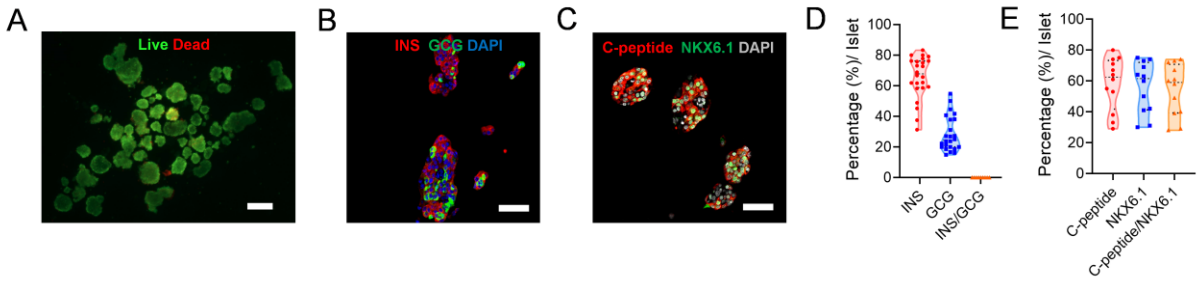


Fig. S9. **Analysis of human islets before transplantation.** (**A**) Live (green) and dead (red) staining of human islets before transplantation. (**B** and **C**) Immunofluorescent staining of human islets before transplantation. Co-immunofluorescent staining of insulin (red), glucagon (green) and DAPI (blue) (**B**). Co-immunofluorescent staining of C-peptide (red), NKX6.1 (green) and DAPI (gray) (**C**). (**D**) Percentage of hormone expression of insulin, glucagon and insulin/glucagon before transplantation. Each dot represents one islet (n = 24). (**E**) Percentage of β cell marker expression of C-peptide, NKX6.1 and C-peptide/NKX6.1 before transplantation. Each point represents one islet (n = 12). Scale bars: 200 μm (**A**) and 50 μm (**B** and **C**). INS, insulin; GCG, glucagon.

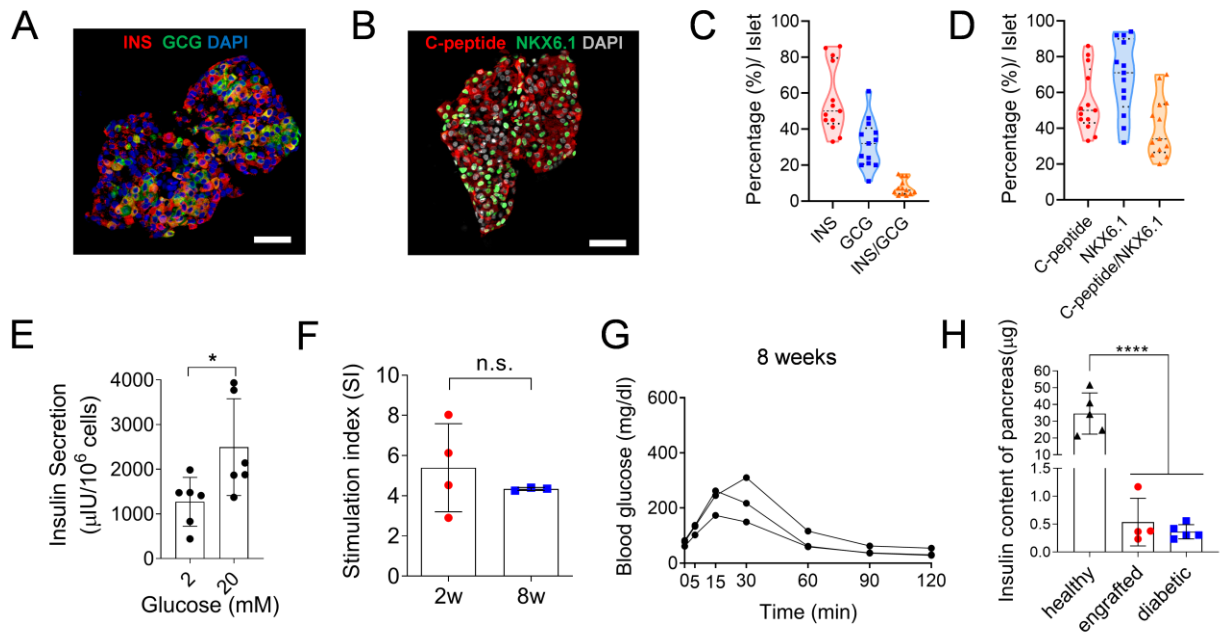
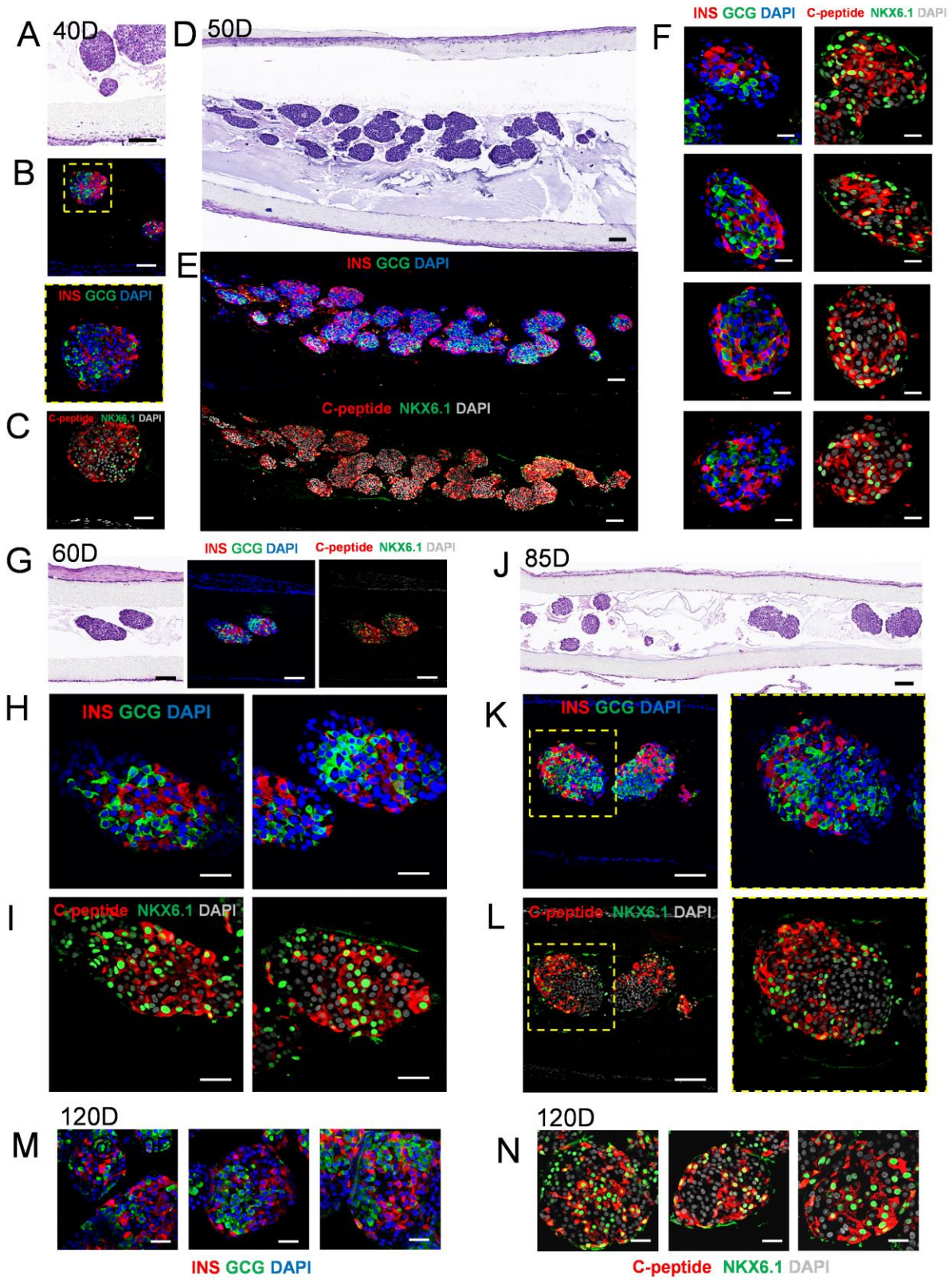


Fig. S10. **Function of NICE device in reversing diabetes using human SC-β cells in immunodeficient mice.** (**A** and **B**) Immunofluorescent staining of SC-β cells before transplantation. Co-immunofluorescent staining of insulin (red), glucagon (green) and DAPI (blue) (**A**). Co-immunofluorescent staining of C-peptide (red), NKX6.1 (green) and DAPI (gray) (**B**). (**C**) Percentage of hormone expression of insulin, glucagon and insulin/glucagon before transplantation. Each point represents one cluster (n = 13). (**D**) Percentage of β cell marker expression of C-peptide, NKX6.1 and C-peptide/NKX6.1 before transplantation. Each point represents one cluster (n = 13). (**E**) Static human insulin secretion of cells in stage 6 in a GSIS assay, mean ± SD (n = 6). (**F**) Stimulation index in IPGTT at 2 weeks and 8weeks (the ratio of C-peptide concentration at 90 mins to that at 0 mins), mean ± SD (n = 3-4). (**G**) Blood glucose measurement in IPGTT at 8 weeks (n = 3). (**H**) Measurement of total insulin content of the pancreas in different groups, mean ± SD (n = 4-5). The two-tailed Student's t-test was performed when the data consisted of two groups. The one-way ANOVA followed by Tukey's test was performed for comparing the multi-group data. *P < 0.05, ****P < 0.0001, n.s., non-significant.

Scale bars: 50 μm (**A** and **B**). SC-β, stem cell derived β cell; INS, insulin; GCG, glucagon; GSIS, glucose stimulation insulin secretion; IPGTT, intraperitoneal glucose tolerance test.


A
40D
D
50D
F
INS GCG DAPI
C-peptide NKX6.1 DAPI
B
INS GCG DAPI
E
INS GCG DAPI
C-peptide NKX6.1 DAPI
C
C-peptide NKX6.1 DAPI
G
60D
INS GCG DAPI
C-peptide NKX6.1 DAPI
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I
C-peptide NKX6.1 DAPI
L
C-peptide NKX6.1 DAPI
M
120D
INS GCG DAPI
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Fig. S11. **Explanted devices from individual recipient mice engrafted with human SC-β cells.** (**A**-**C**) H&E staining (**A**) and immunofluorescent staining (**B** and **C**) of SC-β cells from device retrieved after 40 days (higher magnification provided below). (**D**-**F**) H&E staining (**D**) and immunofluorescent staining (**E** and **F**) of SC-β cells from device retrieved after 50 days. (**F**) Higher magnification images of (**E**). (**G**) H&E staining and immunofluorescent staining of SC-β cells from device retrieved after 60 days. (**H** and **I**) Higher magnification images of (**G**). (**J**-**L**) H&E staining (**J**) and immunofluorescent staining (**K** and **L**) (higher magnification shown on the right) of SC-β cells from device retrieved after 85 days. (**M** and **N**) Immunofluorescent staining of SC-β cells from device retrieved after 120 days. Co-immunofluorescent staining of insulin (red), glucagon (green) and DAPI (blue) (**B**, **E** (top), **F** (left), **G** (middle), **H**, **K** and **M**). Co-immunofluorescent staining of C-peptide (red), NKX6.1 (green) and DAPI (gray) (**C**, **E** (bottom), **F** (right), **G** (right), **I**, **L** and **N**). Scale bars: 100 μm (**A**, **B**, **D**, **E**, **G** and **J**) and 25 μm (**C**, **F**, **H**, **I**, and **K**-**N**). SC-β, stem cell derived β cell; H&E, haemotoxylin and eosin, INS, insulin; GCG, glucagon.

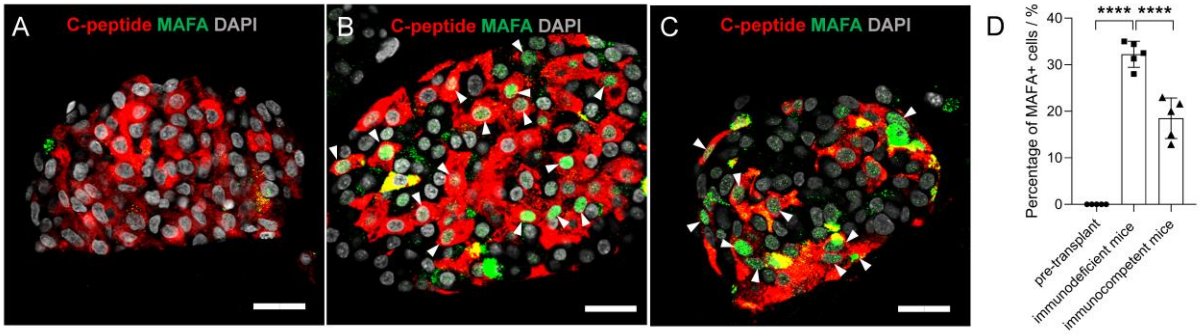

Fig. S12. **Analysis of MAFA expression in SC-β cells before and after transplantation.** Representative immunofluorescent images of human SC-β cells before transplantation (**A**) and retrieved from immunodeficient mice (**B**) and immunocompetent mice (**C**). (C-peptide, red; MAFA, green and DAPI, gray). White triangles indicate the C-peptide^{+}/MAFA^{+} β cells. (**D**)

Percentage of MAFA^{+} cells in different groups (n = 5). Scale bars: 25 μm (**A**-**C**). The one-way ANOVA followed by Tukey's test was performed for comparing the multi-group data. ****P < 0.0001.

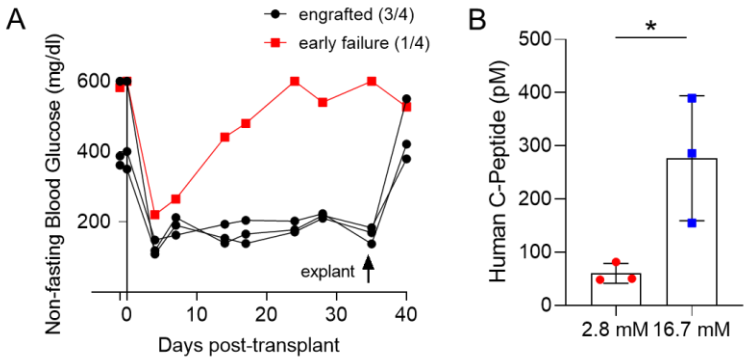

Fig. S13. **Function of NICE device in reversing diabetes using human islets in immunocompetent mice.** (**A**) Measurement of non-fasting blood glucose of diabetic mice transplanted with human islets in device (n = 4). Arrow indicates the time point when implants were retrieved from recipients. (**B**) Measurement of C-peptide concentration of retrieved devices from engrafted mice following ex vivo glucose stimulation insulin secretion (GSIS) test, mean ± SD (n = 3). The two-tailed Student's t-test was performed. *P < 0.05.

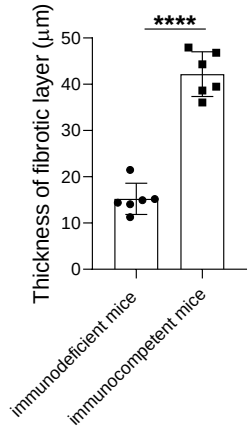

Fig. S14. **Thickness of fibrotic layer around the NICE devices with SC-β cells implanted in immunodeficient mice and immunocompetent mice.** mean ± SD (n = 6). The two-tailed Student's t-test was performed. ****$P < 0.0001$.

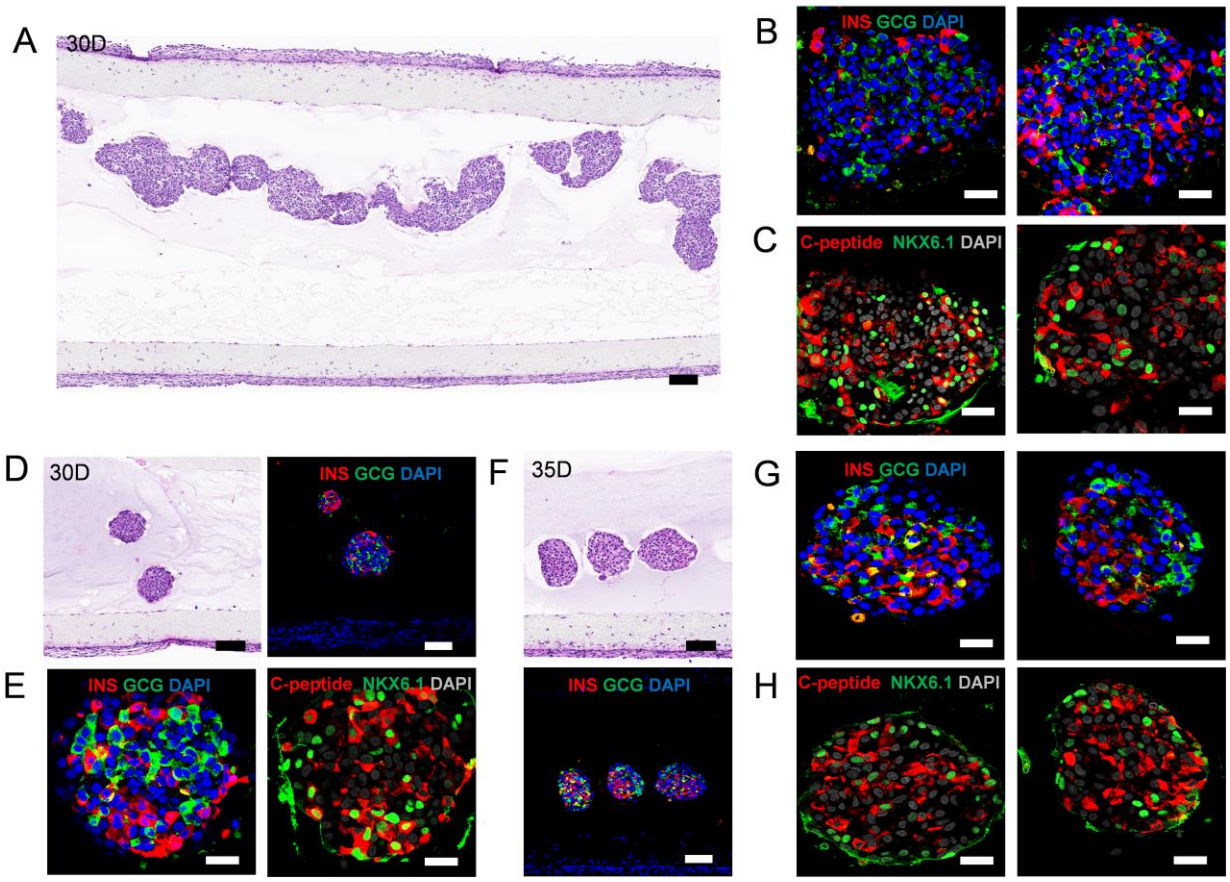

Fig. S15. **Function of NICE device in reversing diabetes using human SC-β cells in immunocompetent mice.** (**A**-**C**) H&E staining (**A**) and immunofluorescent staining (**B** and **C**) of SC-β cells from device retrieved after 30 days. (**D** and **E**) H&E staining (**D**) and immunofluorescent staining (**E**) of SC-β cells from device retrieved after 30 days. (**F**-**H**) H&E staining (**F**) and immunofluorescent staining (**G** and **H**) of SC-β cells from device retrieved after 35 days (higher magnification images on the right). Co-immunofluorescent staining of insulin (red), glucagon (green) and DAPI (blue) (**B**, **D** (right), **E** (left), **F** (bottom) and **G**). Co-immunofluorescent staining of C-peptide (red), NKX6.1 (green) and DAPI (gray) (**C**, **E** (right),

and **H**). Scale bars: 100 μm (**A**, **D** and **F**) and 25 μm (**B**, **C**, **E**, **G** and **H**). H&E, haemotoxylin and eosin; INS, insulin; GCG, glucagon.

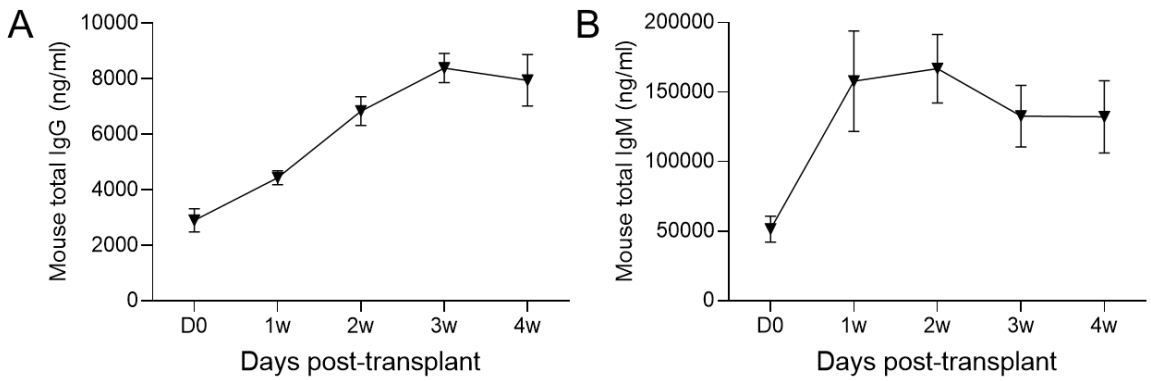

Fig. S16. **Analysis of antibodies in serum in xenografts.** Mouse total IgG concentration (**A**) and mouse total IgM concentration (**B**) in serum of recipients with discordant xenograft (human SC-β cells to mouse), before transplantation and 1w, 2w, 3w and 4 w post-transplantation, mean ± SD (n = 6).

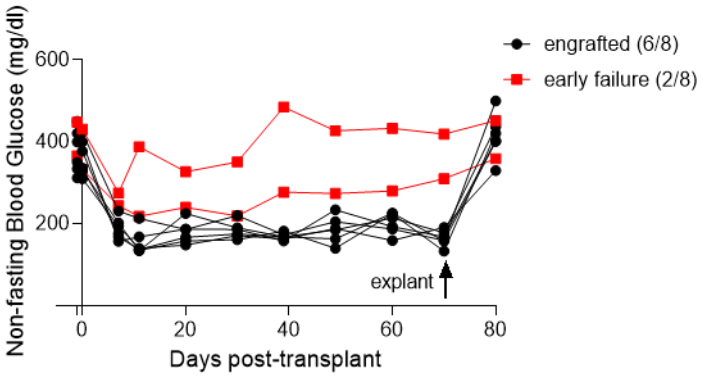

Fig. S17. **Function of NICE device in reversing diabetes using human SC-β cells in immunosuppressed immunocompetent mice.** Measurement of non-fasting blood glucose of the mice transplanted with SC-β cells in device (n = 8). Arrow indicates the time point when implants were retrieved from recipients.

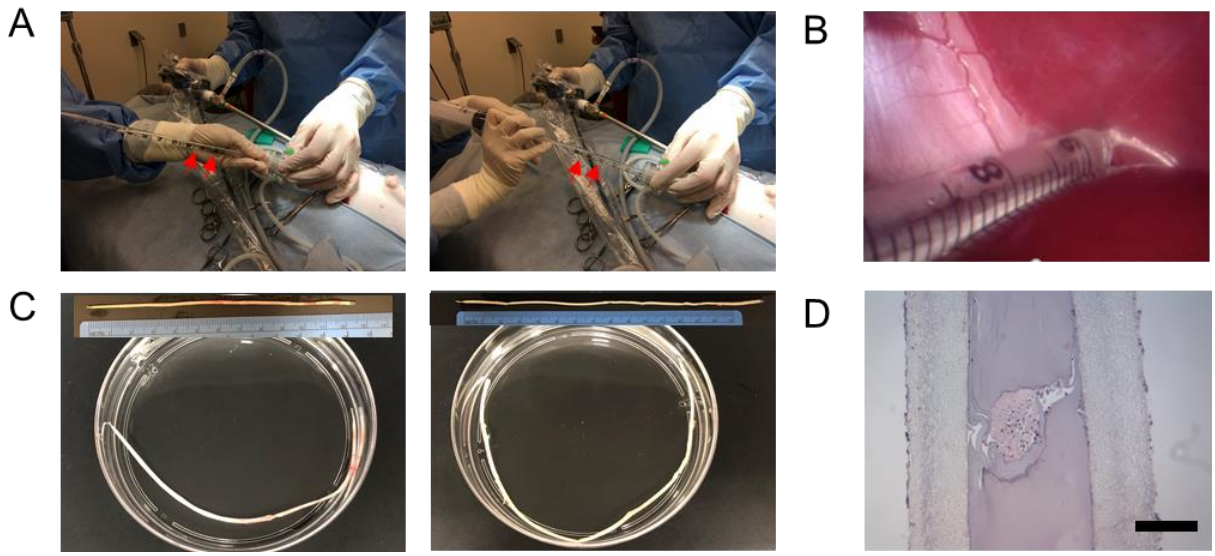

Fig. S18. **Implantation and retrieval of NICE device encapsulating human SC-β cells in dogs.** (**A**) Photographs showing the laparoscopic implantation process. Red arrows point to the device in a 10 mL pipette. (**B**) A laparoscopic image of the implantation process in the intraperitoneal cavity of dog. (**C**) Photographs of the other two retrieved devices with minimal tissue adhesion after two weeks. (**D**) H&E staining of dead SC-β cells in retrieved device. Scale bar: 100 μm (**D**). SC-β, stem cell derived β cell; H&E, haemotoxylin and eosin.

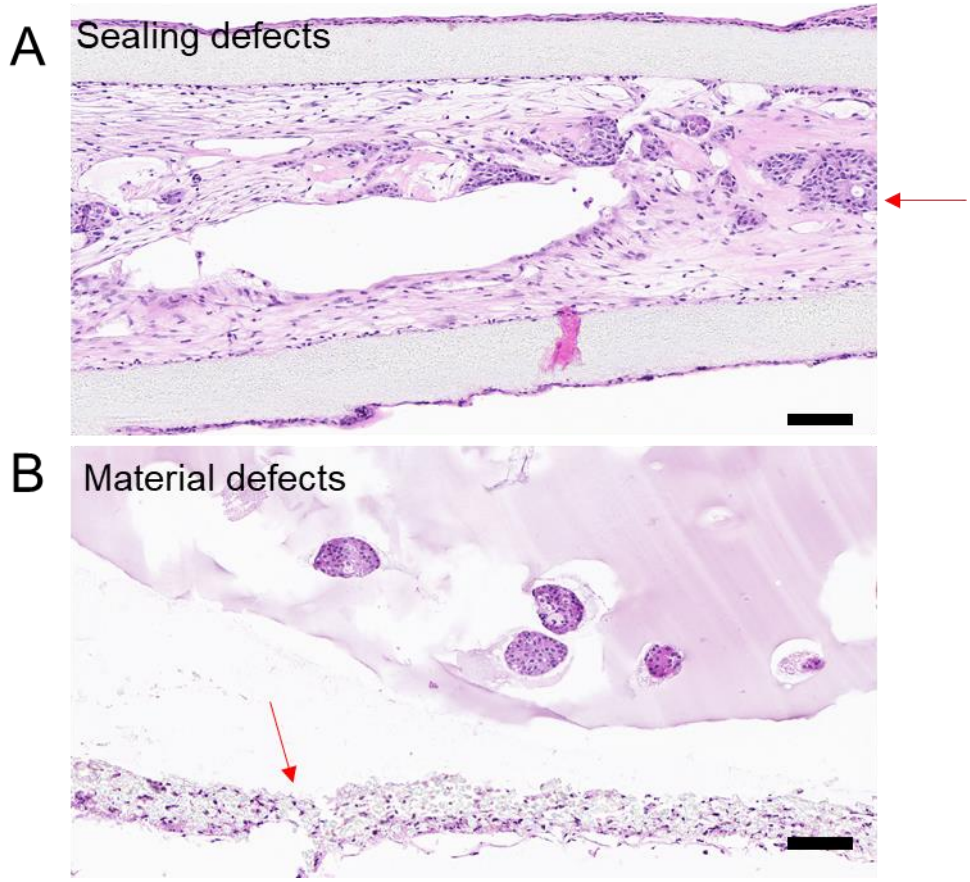

Fig. S19. **Possible reasons for early transplantation failure.** (**A**) Cells penetrating the device along the arrow direction through a sealing defect. (**B**) Cells penetrating the device through defects on the nanofiber membrane as indicated by the arrow. Scale bars: 50 μm (**A** and **B**).

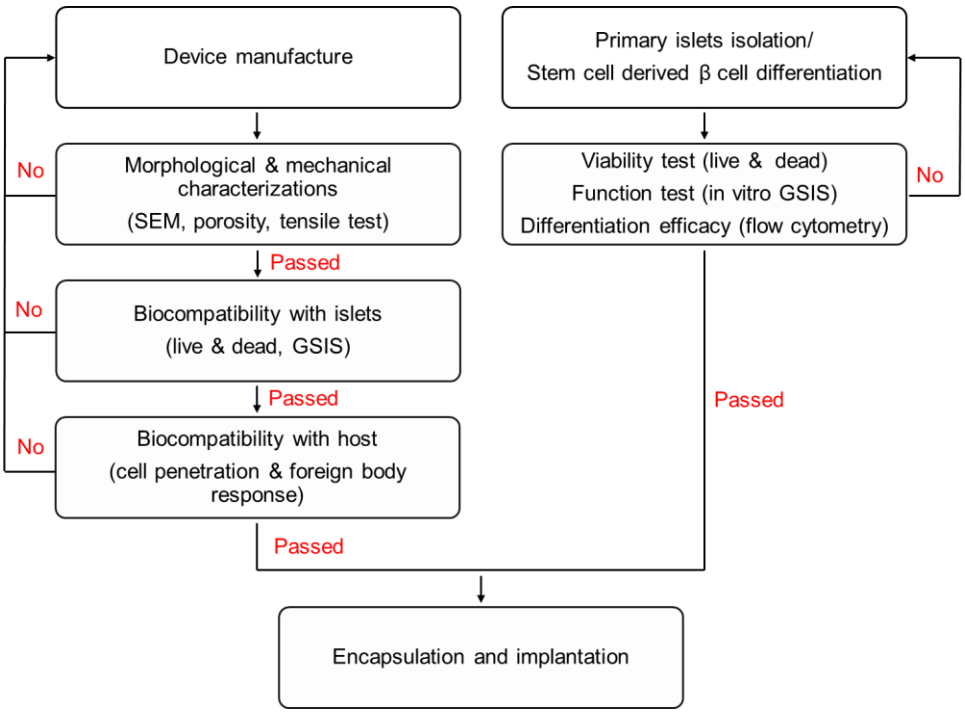

Fig. S20. Flow chart of quality control strategies.

Table S1. Stem cell differentiation protocol.

Stage	Duration (days)	Media Formulation	Added Supplements
1	3	500 mL MCDB 131 (Cellgro; 15-100-CV) supplemented with 0.22 g glucose (MilliporeSigma; G7528), 1.23 g sodium bicarbonate (MilliporeSigma; S3817), 10 g bovine serum albumin (BSA) (Proliant; 68700), 10 µL ITS-X (Invitrogen; 51500056), 5 mL GlutaMAX (Invitrogen; 35050079), 22 mg vitamin C (MilliporeSigma; A4544), and 5 mL penicillin/streptomycin (P/S) solution (Cellgro; 30-002-CI)	100 ng/mL Activin A (R&D Systems; 338-AC) + 3 µM Chir99021 (Stemgent; 04-0004-10) for 24 h
2	3	500 mL MCDB 131 supplemented with 0.22 g glucose, 0.615 g sodium bicarbonate, 10 g BSA, 10 µL ITS-X, 5 mL GlutaMAX, 22 mg vitamin C, and 5 mL P/S	50 ng/ml KGF (Peprotech; AF-100-19)

3	1	500 mL MCDB 131 supplemented with 0.22 g glucose, 0.615 g sodium bicarbonate, 10 g BSA, 2.5 mL ITS-X, 5 mL GlutaMAX, 22 mg vitamin C, and 5 mL P/S	50 ng/ml KGF + 200 nM LDN193189 (Reprocell; 040074) + 500 nM PdBU (MilliporeSigma; 524390) + 2 μM Retinoic Acid (MilliporeSigma; R2625) + 0.25 μM Sant1 (MilliporeSigma; S4572) + 10 μM Y27632
4	5	500 mL MCDB 131 supplemented with 0.22 g glucose, 0.877 g sodium bicarbonate, 10 g BSA, 2.5 mL ITS-X, 5 mL GlutaMAX, and 22 mg vitamin C	5 ng/mL Activin A + 50 ng/mL KGF + 0.1 μM Retinoic Acid + 0.25 μM SANT1 + 10 μM Y27632
5	7	500 mL MCDB 131 supplemented with 1.8 g glucose, 0.877 g sodium bicarbonate, 10 g BSA, 2.5 mL ITS-X, 5 mL GlutaMAX, 22 mg vitamin C, 5 mL P/S, and 5 mg heparin (MilliporeSigma; 9041-08-01)	10 μM ALK5i II (Enzo Life Sciences; ALX-270-445-M005) + 20 ng/mL Betacellulin (R&D Systems; 261-CE-050) + 0.1 μM Retinoic Acid + 0.25 μM SANT1 + 1 μM T3 (Biosciences; 64245) + 1 μM XXI (MilliporeSigma; 595790)
6	12 - 20	500 mL MCDB 131 supplemented with 0.23 g glucose, 10.5 g BSA, 5.2 mL GlutaMAX, 5.2 mL P/S, 5 mg heparin, 5.2 mL MEM nonessential amino acids (Corning; MT25025Cl), 84 μg ZnSO4 (MilliporeSigma; 10883), 523 μL Trace Elements A (Corning; 25-021-CI), and 523 μL Trace Elements B (Corning; 25-022-CI)	-

Table S2. List of antibodies used in analyzing SC-β cells by flow cytometry.

Type	Antibody	Dilution	Company	Part #
primary	rat anti-C-peptide	1:300	DSHB	GN-ID4-S
primary	mouse anti-glucagon	1:300	Abcam	ab82270
primary	mouse anti-NKX6.1	1:100	DSHB	F55A12-S
secondary	anti-rat-AF488	1:300	Invitrogen	A21208
secondary	anti-rat-PE	1:300	Jackson Immunoresearch	712-116-153
secondary	anti-mouse-AF594	1:300	Invitrogen	A21203
secondary	anti-mouse-AF647	1:300	Invitrogen	A31571

Table S3. List of antibodies used in analyzing composition of fibrotic layer by flow cytometry.

Antibody	Color	Dilution	Company	Part #

anti-mouse Ly-6G/Ly-6C (Gr-1)	AF488	1:100	BioLegend	108419
anti-mouse CD45	PerCP	1:100	BioLegend	103129
anti-mouse CD11c	APC	1:100	BioLegend	117309
anti-mouse/human CD45R/B220	AF700	1:100	BioLegend	103231
anti-mouse/human CD11b	APC/Cyanine7	1:100	BioLegend	101225
anti-mouse CD3	Pacific Blue	1:100	BioLegend	100213
anti-mouse F4/80	PE	1:100	BioLegend	123109
anti-mouse CD4	AF594	1:100	BioLegend	100446
anti-mouse CD8a	PE/Cyanine7	1:100	BioLegend	100721

Table S4. List of antibodies used in immunofluorescent staining.

Type	Antibody	Dilution	Company	Part #
primary	rat anti-C-peptide	1:100	DSHB	GN-ID4
primary	mouse anti-NKX6.1	1:300	DSHB	F55A12
primary	rabbit anti-alpha smooth muscle actin antibody	1:200	Abcam	ab5694
primary	rabbit anti-insulin	1:200	Abcam	ab63820
primary	mouse anti-glucagon	1:200	Sigma	G2654
primary	goat anti-GFP	1:200	Rockland	600-101-215S
primary	rabbit anti-MAFA	1:200	LifeSpan Bioscience	LP9872
primary	rabbit anti-CD3	1:100	Abcam	ab5690
primary	rat anti-F4/80	1:50	ThermoFisher	14-4801-82
secondary	anti-rat-AF555	1:400	ThermoFisher	A11055
secondary	anti-mouse-AF488	1:400	ThermoFisher	A21202
secondary	anti-rabbit-AF594	1:400	ThermoFisher	A11037
secondary	anti-goat-AF488	1:400	ThermoFisher	A11055
secondary	anti-rabbit-AF568	1:400	ThermoFisher	A10042
secondary	anti-rat-AF488	1:400	ThermoFisher	A21208

Movie S1. Device was stretched to 3 times the original length.

Movie S2. Device was bent without kink.

Movie S3. Laparoscopic video showing the implantation process of device in a dog.

Movie S4. Laparoscopic video showing the retrieval process of the device from a dog (part 1).

Movie S5. Laparoscopic video showing the retrieval process of the device from a dog (part 2).