**Supplementary Experimental Procedures**

*Operations*

The kidney capsule surgery involved a small incision through the muscle layer above the left kidney. The kidney was then externalized, and a small nick was made in the capsule using a needle. A glass probe was used to create a pocket in the capsule into which cells were pipetted using a P200 pipette. The opening was sealed by cauterizing the capsule, the kidney was gently pushed back into the body, and the incision was closed.

The gonadal fat pad graft preparation and operation was performed as previously described by Kroon et al. (1). A sterile hole punch was used to create 6 mm diameter x 2 mm thick discs from Vetspon® absorbable hemostatic gelatin sponge (Gelfoam; 07-849-4024; Patterson Veterinary, Greeley, CO). The discs were immersed in Dulbecco's Phosphate Buffered Saline (PBS-/-; D8537; Sigma-Aldrich, Oakville, ON), transferred to microfuge tubes, and centrifuged for 20 minutes at 10K rcf to remove air bubbles. Cells were then pipetted onto the disc in a round bottom polystyrene tube lid and overlaid with 10-15 μL of Corning™ Matrigel™ hESC-qualified matrix (08-774-552; Fisher Scientific, Waltham, MA). An incision was made in the left lower abdomen to externalize the gonadal fat pad. The cell construct was then surrounded with the fat pad and fixed into place with a small amount of GLUture® topical tissue adhesive (07-867-9063; Patterson Veterinary, Greeley, CO). The fat pad was then put back into the body and the incision was closed.

Each TheraCyte™ device was immersed in 95% ethanol for 5 seconds, then soaked in three consecutive 20 minute PBS +/+ (14040133; Life Technologies, Carlsbad, CA) baths. Five million PECs were aspirated into a 1 mL syringe (309628; BD Biosciences, San Jose, CA) through a 20G angiocath and gently dispersed into the TheraCyte™ device. The devices were sealed with Type

A medical-grade silicone adhesive (MED-1137; NuSil Technology LLC, Carpinteria, CA). Each loaded and sealed device was placed in one well of a 6-well plate with 5 mL of media and transferred to the incubator for an hour before implant. Subcutaneous implantation of the TheraCyte™ devices involved making an incision in the right flank and blunt dissection was performed under the skin to create a pocket for the device. The device was then rinsed with PBS-/-, inserted under the skin, and the incision was closed.

*Assays*

Plasma human insulin, glucagon, and GLP-1 levels were measured using a multiplex assay (K15160C-2; Meso Scale Discovery, Rockville, MD) at a dilution of 3:20. Concentrations were interpolated using the MESO QuickPlex SQ 120 Imager (Meso Scale Discovery, Rockville, MD) and MSD DISCOVERY WORKBENCH software. Plasma human C-peptide levels were determined using an ultrasensitive human C-peptide ELISA (10-1141-01; Mercodia, Uppsala, Sweden) at a dilution ranging from 1:5 to 3:50. The concentration of human C-peptide was interpolated from absorbance values using an asymmetric sigmoidal, 5PL, curve. Any plasma samples below the limit of detection were multiply imputed (see Statistics).

*Statistics*

Missing values were multiply imputed using Amelia (2) with an m parameter of the greatest percentage of missing values for that experiment or 10, whichever was higher (e.g. samples above or below a limit of detection), using priors of the limit of detection. For each test type, a linear mixed effects model was fitted with time and weeks treated as categorical. If no result is reported, the posterior probability did not exceed 95%. All Bayesian models were created in Stan computational framework accessed with the brms package (3,4). Models were assessed and parameters adjusted to improve fit and/or address issues, including treating individual animals as

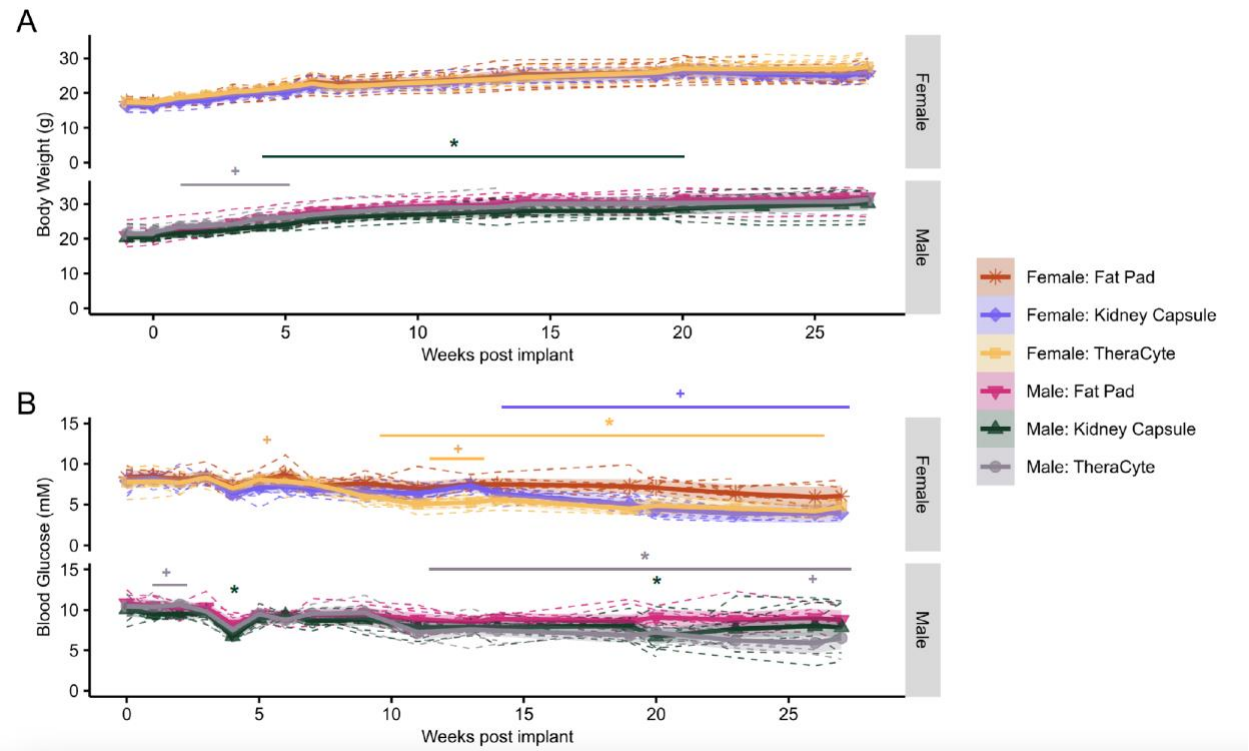
random variables contributing to Time and Weeks, and adjusting the response distribution and link function. Strength of evidence was assessed as per convention (5), and all reported results had evidence at the strong level or higher. Evidence at the strong level indicates that it is $10^{3/2}$ (~31) times more likely that the hypothesis being tested is true compared to not true; between 31 and 100 times more likely indicates it is very strong; over 100 times more likely means it is decisive. In related figures, points and lines are the fitted values of the posterior distribution with shading indicating the 95% credible interval. Related figures were generated using ggplot2 (6,7). Absolute differences in blood glucose or plasma metabolites were examined rather than percent differences as the absolute differences are more relevant to translation. Code and data for analyses are available at https://github.com/caraee/TJKV06.

**References**


1. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol. 2008;26(4):443–52.
2. Honaker J, King G, Blackwell M. Amelia II: A Program for Missing Data. J Stat Softw. 2011 Dec 12;45(7):1–47.
3. Bürkner PC. brms: An R Package for Bayesian Multilevel Models Using Stan. J Stat Softw. 2017 Aug 29;80:1–28.
4. Bürkner PC. Advanced Bayesian Multilevel Modeling with the R Package brms. R J. 2017 May 31;10(1):395–411.
5. Jeffreys H. Theory of Probability Harold Jeffreys (Third edition). Oxford, UK: Oxford University Press; 1961.
6. Wickham H. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag; 2016. (Use R!).
7. Iannone R, Cheng J, Schloerke B. gt: Easily Create Presentation-Ready Display Tables. R package version 0.3.0. [Internet]. 2021. Available from: https://cran.r-project.org/package=gt

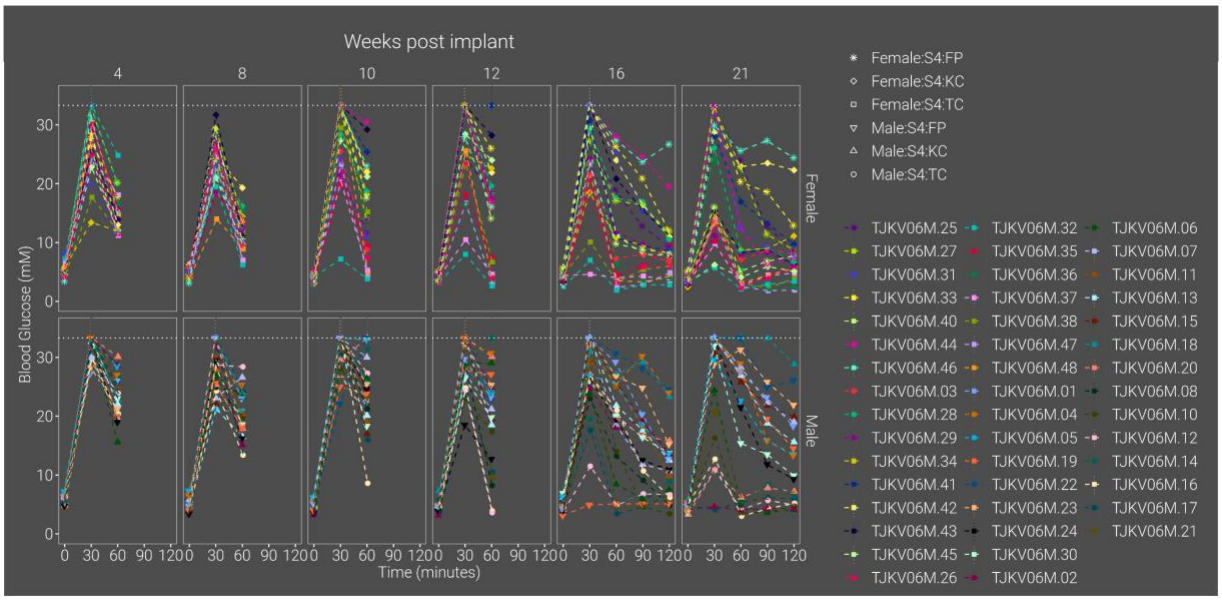
**Supplementary Table 1.** Antibody information for immunofluorescent staining

| Antigen | Species | Source | Catalog # | Dilution |
|---|---|---|---|---|
| Cytokeratin 19 (CK19) | Mouse | Dako, Denmark | M0888 | 1:100 |
| Glucagon (GCG) | Mouse | Sigma-Aldrich | G2654 | 1:1000 |
| Insulin (INS) | Mouse | Cell Signaling | 8138BF | 1:200 |
| Insulin (INS) | Rabbit | Cell Signaling | 3014 | 1:200 |
| MAFA | Rabbit | Betalogics (J&J) | LP9872 | 1:1000 |
| NKX6.1 | Rabbit | Betalogics (J&J) | LP9878 | 1:1000 |
| PDX1 | Rabbit | Gifted from Joel Habner | | 1:1000 |
| Somatostatin (SST) | Rabbit | Sigma-Aldrich | HPA019472 | 1:500 |
| SOX9 | Rabbit | Millipore-Sigma | AB5535 | 1:1000 |
| Synaptophysin (SYN) | Rabbit | Novus Biologicals | NB120-16659 | 1:50 |
| Trypsin 1/2/3 (TRYP) | Sheep | R&D Systems | AF3586 | 1:25 |

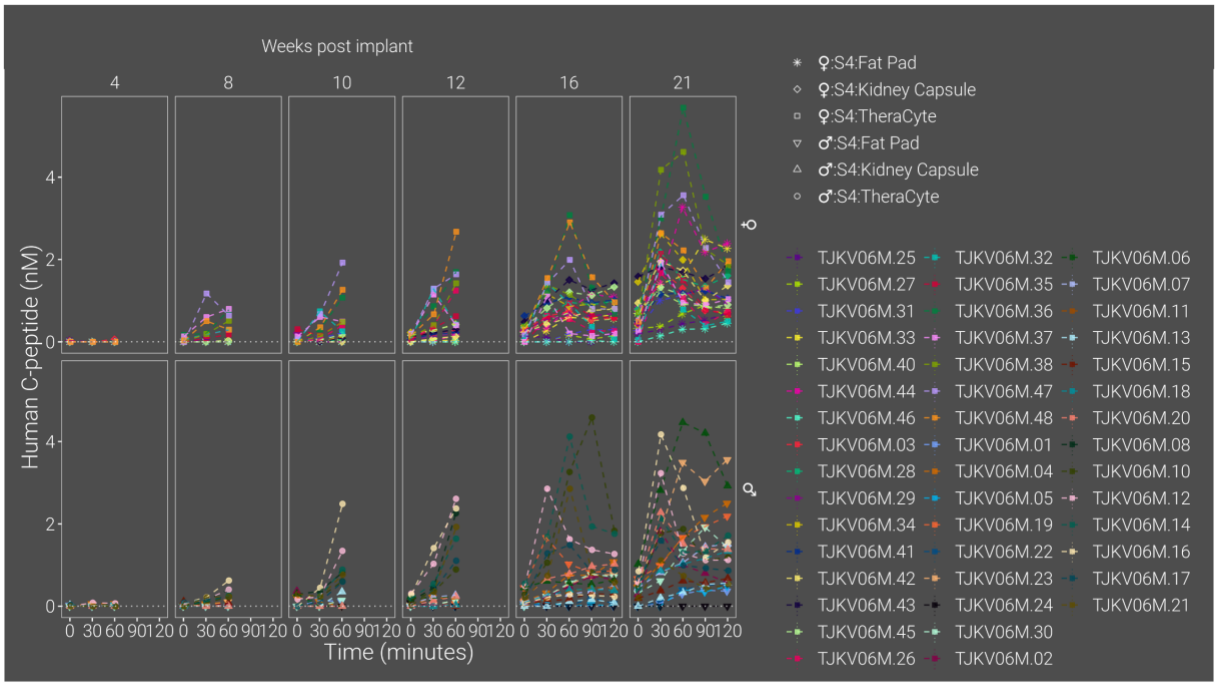



**Supplementary Figure 1. hESC-derived PECs controlled glycemic set point earlier in TheraCyte™ devices compared to under the kidney capsule or in the gonadal fat pad in both sexes.** Body weight (A) and blood glucose (B) levels were measured following a 4-hour fast in female (top) and male (bottom) SCID-beige mice that received hESC-derived PECs either under the kidney capsule (n = 7-8 males, green; n = 8 females, purple), in the gonadal fat pad (n = 7 males, pink; n = 6-7 females, red), or subcutaneously within TheraCyte™ devices (n = 6-7 males, grey; n = 8 females, yellow). Data are presented as point estimates ± credibility interval line graphs with solid lines and points representing the point estimates, dashed lines representing the individual data, and shading representing the credibility interval: $^{+}$strong or higher level of evidence versus kidney capsule group and *strong or higher level of evidence versus fat pad group; Bayesian approach with multilevel regression modelling.

**A)**

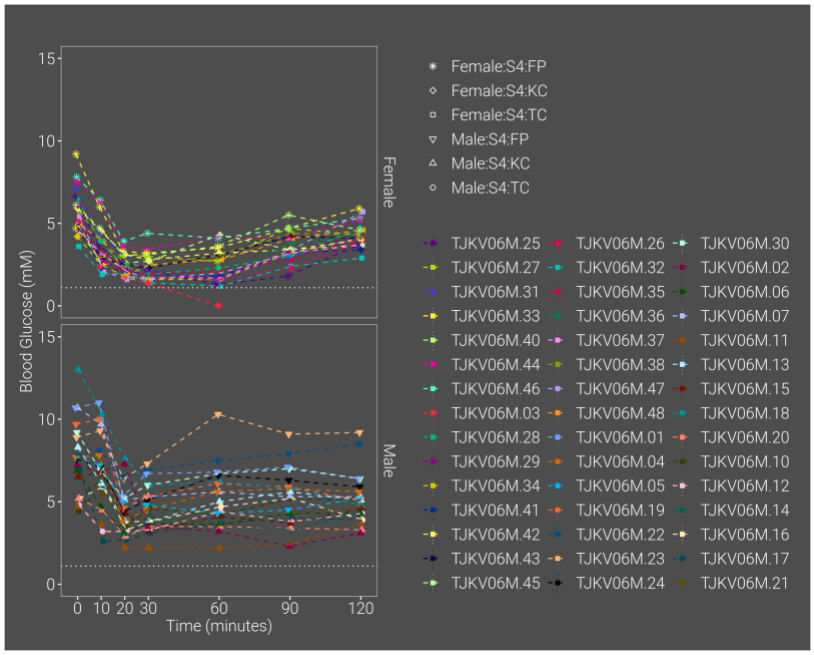



**B)**




**Supplementary Figure 2.** Individual data from Figure 1 for blood glucose (A) and human C-peptide (B) following an intraperitoneal glucose tolerance test. Data are presented as line graphs with individual data points. Dashed, coloured lines represent data from individual mice in the current study denoted as TJKV06. Mice implanted with hESC-derived PECs in the gonadal fat pad (FP) are represented by asterisks in females and inverted triangles in males; under the kidney capsule (KC) are represented by diamonds in females and triangles in males; and in TheraCyte™ (TC) devices are represented by squares in females and circles in males. Dashed white line in (A) indicates the upper limit of detection of the glucometer whereas in (B), it indicates the lower limit of detection of the human C-peptide assay.

**A)**

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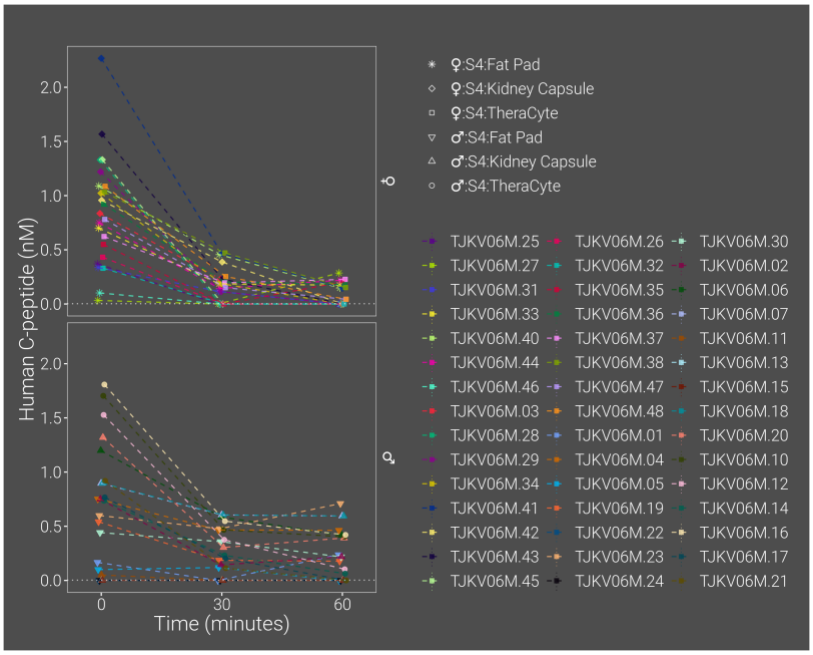
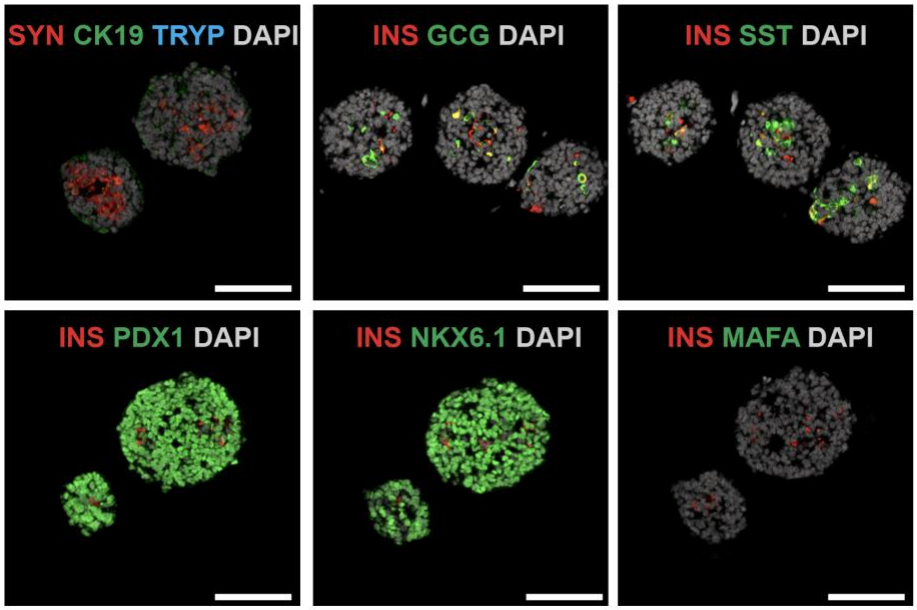
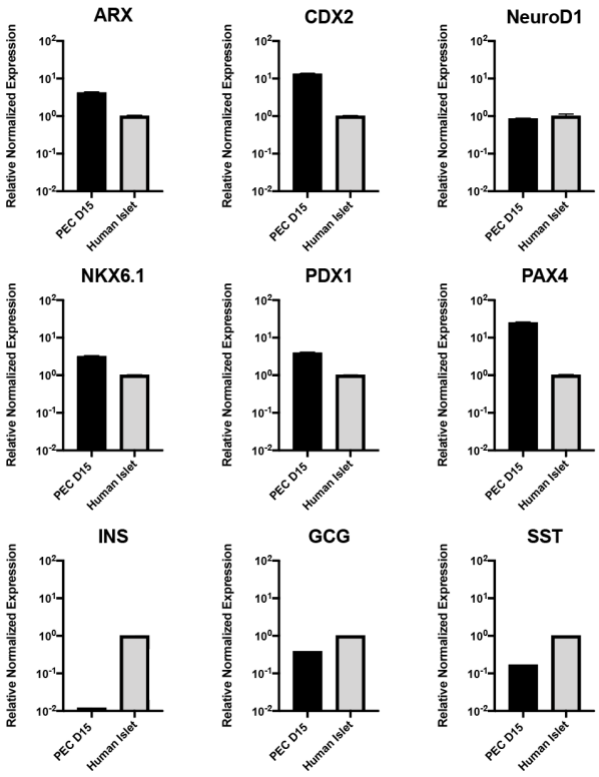



**Supplementary Figure 3.** Individual data from Figure 2 for blood glucose (A) and human C-peptide (B) following an intraperitoneal insulin tolerance test. Data are presented as line graphs with individual data points. Dashed, coloured lines represent data from individual mice in the current study denoted as TJKV06. Mice implanted with hESC-derived PECs in the gonadal fat pad (FP) are represented by asterisks in females and inverted triangles in males; under the kidney capsule (KC) are represented by diamonds in females and triangles in males; and in TheraCyte™ (TC) devices are represented by squares in females and circles in males. Dashed white line in (A) indicates the lower limit of detection of the glucometer whereas in (B), it indicates the lower limit of detection of the human C-peptide assay.

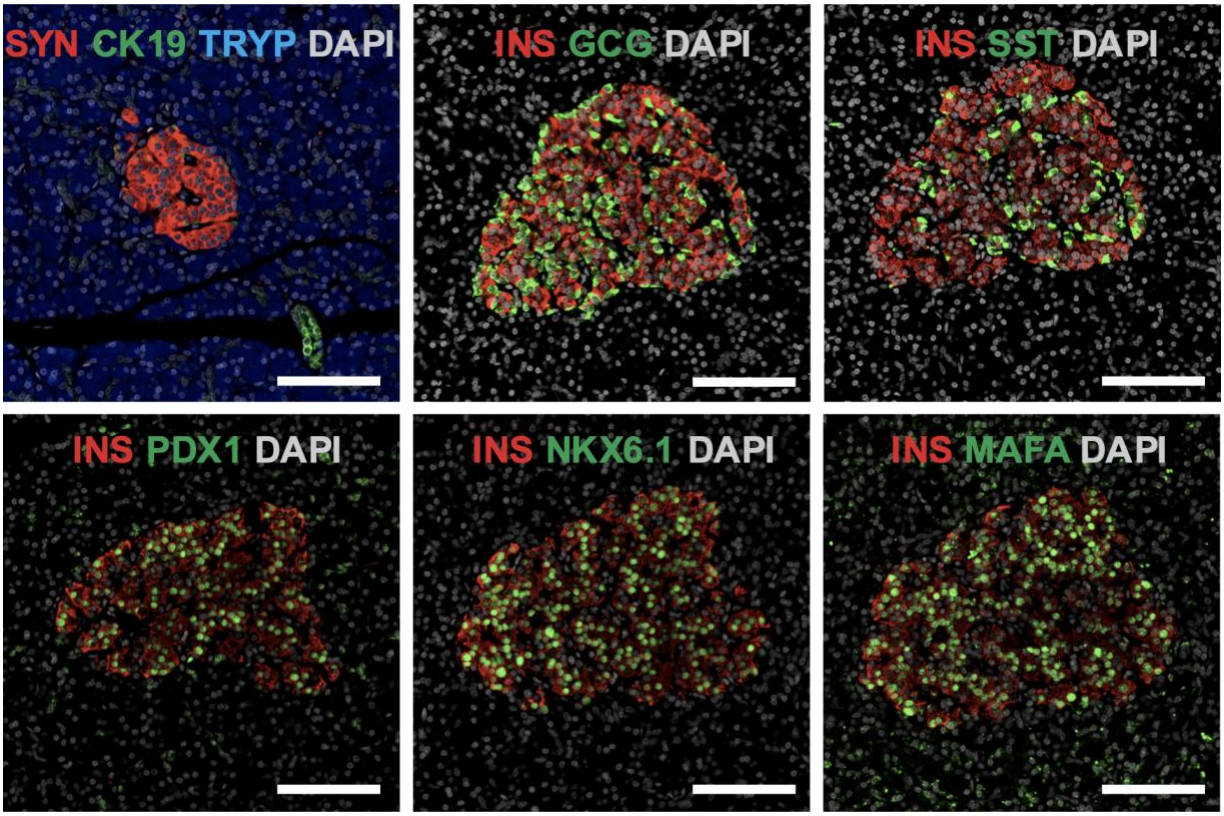
**A)**



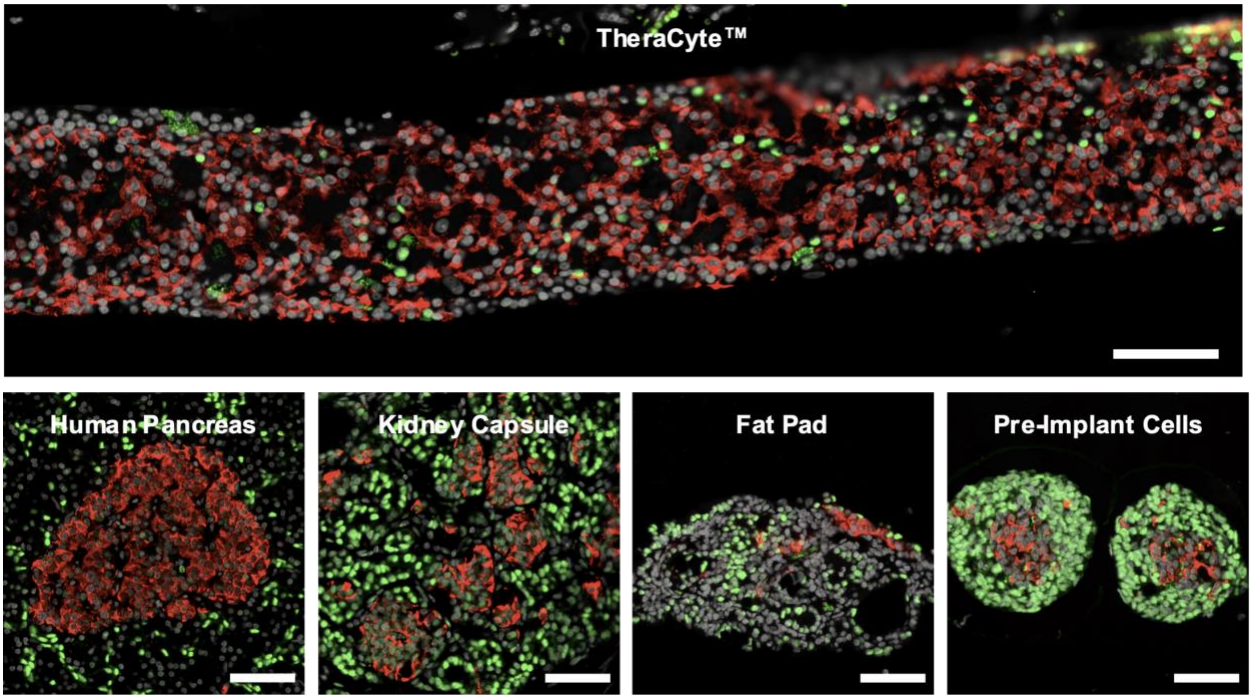

**B)**




**Supplementary Figure 4.** Immunofluorescent staining (A) and qPCR analysis (B) of hESC-derived PECs prior to implantation. Stained for synaptophysin (SYN) and insulin (INS) in red; cytokeratin-19 (CK19), glucagon (GCG), somatostatin (SST), PDX1, NKX6.1, and MAFA in green; trypsin (TRYP) in blue; nuclear marker DAPI in white; scale bar = 200 μm. Expression levels were normalized to human islets.




**Supplementary Figure 5.** Immunofluorescent staining of adult human pancreas sections as positive control for primary antibodies. Stained for synaptophysin (SYN) and insulin (INS) in red; cytokeratin-19 (CK19), glucagon (GCG), somatostatin (SST), PDX1, NKX6.1, and MAFA in green; trypsin (TRYP) in blue; nuclear marker DAPI in white; scale bar = 200 μm.




**Supplementary Figure 6.** Immunofluorescent staining of human pancreas, hESC-derived PECs prior to implantation, and hESC-derived grafts at 28 weeks post-implant for the nuclear ductal cell marker SOX9. Stained for insulin (INS) in red, SOX9 in green, and the nuclear marker DAPI in white; scale bar = 200 μm.