SUPPLEMENTARY DATA

In vitro differentiation optimization

The effect of various basal media formulations were examined during stages 3 – 4 of the differentiation protocol. For these studies, conditions were as described for the final protocol above, but no PKC activator was added during stage 4. All media was obtained from Invitrogen: Dulbecco's Modified Eagle Medium - High Glucose (DMEM HG; #10569044), F10 Nutrient Mixture (F10; #12390-035), Medium 199 (M199; #12350-039), Iscove's Modified Dulbecco's Medium (IMDM; #31980-030), Advanced RPMI 1640 (Adv RPMI; #12633-012), F12 Nutrient Mixture (F12; #31765-035), CMRL Medium-1066 (CMRL; #11530-037), Modified IMEM (IMEM; #A10488), Standard RPMI 1640 (RPMI; #22400) and DMEM/F-12 - High Glucose (DMEMF12; #11330057). All experiments were performed in triplicate, with the exception of DMEM-HG, F10 and RPMI, which were performed in duplicate. The role of HEPES in the development of pancreatic progenitor cells was examined by adding HEPES (Invitrogen) to DMEM-HG during stages 3 – 4 of the differentiation protocol at a final concentration of 0, 5, 10 or 15 mM (n = 2 per concentration).

The effect of PKC activators was also tested at stage 4, with or without Noggin and Alk5i (n = 2 for all conditions). Two different PKC activators were examined: TPB ((2S,5S)-(E,E)-8-(5-(4-(Trifluoromethyl)phenyl)-2,4-pentadienoylamino)benzolactam) and PBDu (Phorbol-12,13-dibutyrate), (EMD Chemicals Inc). For the initial experiments with and without Noggin / ALK5i, both PKC activators were used at a concentration of 20 nM. The optimal concentration of TPB was then further examined in a dose titration study (0, 31.2, 62.5, 125, 250 and 500 nM).

The *in vivo* development of hESCs was also examined following differentiation with or without ALK5i included in the S4 media (i.e. Basal S4 media with Noggin/TPB +/- ALK5i). Similarly, *in vivo* maturation of cells was also examined following differentiation in either "Basal S4 Media" (i.e. DMEM-HG media, no additional factors; n = 4) or "Basal S4 + 3 factors" (i.e. DMEM-HG + ALK5i, Noggin and TPB; n = 5). In both studies, cells were transplanted under the kidney capsule of SCID-beige mice and human C-peptide was assessed monthly in random fed plasma samples.

The high fraction of pancreatic endoderm generated using the optimized protocol with the H1 line (Fig 2A) is also highly dependent on the quality of small molecules used along with the cell density of cultures at the end of stage 1. Reagents such as RA are highly labile and should be used with minimal exposure to light or air when preparing aliquots. Furthermore, at end of stage 1 an approximate cell density of ~5-6x10^5 cells/cm^2 generated highly uniform pancreatic endoderm at stage 4 (Supp Fig 4B).

Flow Cytometry

hESC-derived cells were released into single-cell suspension by incubation in TrypLE Express (Invitrogen No. 12604) at 37^{o}C for 3-5 minutes. Cells were then washed twice in staining buffer (PBS containing 0.2% BSA) (BD Biosciences, No. 554657). For surface marker staining, 1x10^5 to 1x10^6 cells were re-suspended in 100 µl blocking buffer (0.5% human gamma-globulin diluted 1:4 in staining buffer). Directly conjugated primary antibodies (CD184/CXCR4 APC (Allophycocyanin, BD No. 555976)) were added to the cells at a final dilution of 1:20 and incubated for 30 min at 4^{o}C. Stained cells were washed twice in BD staining buffer, re-suspended in 200 µl staining buffer, followed by incubation in 15 µl of 7AAD for live/dead discrimination prior to analysis on the BD FACS Canto II.

For intracellular antibody staining, cells were first incubated with Green Fluorescent LIVE/DEAD cell dye (Invitrogen, No.L23101) for 20 min at 4^{o}C to allow for live/dead cell discrimination during analysis, followed by a single wash in cold PBS. Cells were fixed in 250 µl of Cytofix/Cytoperm Buffer (BD, No. 554722) for 20 min at 4^{o}C followed by two washes in BD Perm/Wash Buffer Solution (BD, No. 554723). Cells were re-suspended in 100 µl staining/blocking solution consisting of Perm

wash buffer with 2% normal goat serum (or appropriate species of the secondary antibody). Cells were then incubated for 30 min at 4°C with primary antibodies at empirically pre-determined dilutions followed by two washes in Perm/Wash buffer. Lastly, cells were incubated with the appropriate secondary antibodies for 30 min at 4°C followed by two washes prior to analysis on the BD FACS Canto II. The following concentrations of primary antibodies were used: rabbit anti-insulin (1:100; Cell Signaling C27C9; Danvers, MA), or mouse anti-insulin (1:100; Abcam, Cambridge, MA), mouse anti-glucagon (1:1250; G2654 Sigma-Aldrich, St Louis, MO), rabbit anti-synaptophysin (1:100; Dako A0010; Carpinteria, CA), rabbit anti-chromogranin A (1:800; Dako), mouse anti-NKX6.1 (1:50; DSHB, University of Iowa); mouse anti-CDX2 (1:250; Invitrogen); goat anti-NeuroD (1:500; R&D Systems), mouse anti-PDX1 (1:20; BD No. 562161), mouse NKX2.2 (1:100; DSHB, University of Iowa, Cat#74.5A5), mouse Oct3/4 (1:20; BD Cat# 560329) and goat SOX17 (1:20; R&D Systems, Cat# IC1924A). The antibodies for pancreas markers were all tested for specificity using human islets or where appropriate undifferentiated H1 cells as a positive control. For secondary antibodies, goat anti-mouse Alexa 647 (1:500; Invitrogen), goat anti-rabbit PE (1:200; Invitrogen) or donkey anti-goat Alexa 647 (1:800; Invitrogen) were added and incubated for 30 min at 4°C followed by a final wash in Perm Wash buffer. Cells were analyzed on BD FACS Canto II using the BD FACS Diva Software with at least 30,000 events being acquired.

High Content Image Analysis

For quantitative analysis of expression of intracellular markers, cells grown on 6-well culture plates were rinsed with PBS (-/-) to remove residual medium and fixed in 4% PFA for 15 min at room temperature. Cells were then permeabilized in 0.5% Triton X-100 for 20 min at RT followed by blocking for 30 min in serum from the same species as the secondary antibody. Primary antibodies were added at pre-determined dilutions followed by overnight incubation at 4°C (Supplementary Table 2; all antibodies were commercially available except for rabbit NKX6.1, which was custom-made by LifeSpan (Biosciences, Seattle WA). Cells were then washed at least twice in PBS and secondary antibodies diluted in serum were added and incubated for 45 minutes at 4°C. Cells were washed and incubated in Hoechst 33342 at 10 µg/ml in PBS for nuclear counterstain. Images were acquired and analyzed using the IN Cell Analyzer 1000 (INCA1000; GE Healthcare, Piscataway, NJ) and using the Developer Software (GE Healthcare). A list of primary and secondary antibodies used is provided in Supplementary Table 2. For each marker, at least $5x10^5$ cells representing 100 randomly selected fields in a well of a 6-well plate were analyzed. A minimum of two wells was used per marker and averages representing three independent experiments were reported.

Quantitative RT-PCR

Cultured Cells

Gene expression was analyzed at various stages of differentiation. Total RNA was extracted with the RNeasy Mini Kit (Qiagen; Valencia, CA) and reverse-transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. cDNA (100-120 ng) was amplified by PCR using Taqman Universal Master Mix and Taqman Gene Expression Assays which were pre-loaded onto custom Taqman Arrays (Applied Biosystems). Data were analyzed using Sequence Detection Software (Applied Biosystems) and normalized to undifferentiated hESCs using the ΔΔCt method. In our experience not all ES cells in culture are undifferentiated. We routinely find that 0.5-1% of cells are spontaneously differentiating. Thus, not all of the genes are "off", but rather are expressed at very low levels, making undifferentiated hESCs a suitable control for gene expression. A list of primers (Applied Biosystems) is provided in Supplementary Table 3.

Engrafted Cells

A small piece of the engrafted cells (n = 3), harvested 34 weeks after transplantation, was dissected away from the kidney prior to fixation, immediately immersed in RNAlater (Qiagen) and stored at 4°C. The tissue was homogenized in Buffer RLT (Qiagen) using a rotor-type tissue homogenizer. RNA extraction was then performed using the RNeasy kit (Qiagen) with on-column DNase digestion as described in the manufacturer's instructions. cDNA synthesis was performed using the RT2 first strand cDNA synthesis kit and mRNA levels measured using a custom designed PCR array (primers provided in Supplementary Table 4; SA Biosciences, Frederick, MD) run on a StepOne Plus Real Time PCR machine (Applied Biosystems). Data analysis was performed using the online RT2 Profiler PCR Array Data Analysis Tool (SA Biosciences). Gene expression is presented as the fold change relative to adult human islets (n = 3).

In Vivo Analysis of Transplanted Cells

All metabolic analyses were performed in conscious, restrained mice on the indicated days. Routine blood glucose measurements were taken following a 4 h morning fast. For monthly meal challenges, animals were fasted overnight (16 h) prior to initial blood glucose testing and blood collection; mice were then provided with normal chow for a 45 min feeding period, after which blood glucose was assessed and blood collected again. All other metabolic tests were performed following a 4 h morning fast and the Linbit insulin pellet was explanted from STZ-treated mice at least one week prior to the procedure, so that there was no contribution from exogenous insulin. For the glucose tolerance test (GTT) at 12 and 25 weeks post-transplant, 1.5 g/kg of glucose (30% solution; Vétoquinol, Lavaltrie, QC) prepared in water was delivered by i.p. injection. In addition, a 2 g/kg glucose challenge was provided by i.p. injection at 32 weeks and by oral gavage at 34 weeks post-transplant. For all tests, blood glucose was tested via saphenous vein, and saphenous blood samples were collected at the indicated time points using heparinized microhematocrit tubes. Plasma was stored at -30°C and later assayed using a human and/or mouse C-Peptide ELISA (Alpco Diagnostics; Salem, NH).

For nude rats, plasma was collected following an i.p. glucose injection (2 g/kg) at the indicated times post-transplant for human C-peptide measurements. At 14-weeks post-transplant, an i.p. GTT (2 g/kg glucose) was performed; blood glucose and human C-peptide was measured in the plasma at the indicated times following the glucose challenge.

Immunofluorescent Image Quantification

To quantify the hESC-derived endocrine cells, pre-transplant clusters (n = 3) and post-transplant kidney graft sections from mice at 1 month (n = 4), 3 months (n = 3) and 8 months (n = 4) post-transplant were co-immunostained for synaptophysin (endocrine marker) with either insulin and glucagon, or somatostatin and pancreatic polypeptide. Whole slide fluorescence scanning was performed using the ImageXpress$^{MICRO^{TM}}$ Imaging System (Molecular Devices Corporation, Sunnyvale, CA). Individual images were stitched together to recreate the entire engrafted kidney section and then quantified using MetaXpress software. The endocrine fraction was determined as the number of synaptophysin-positive cells relative to the total number of hESC-derived cells (defined as cells within the engrafted region of the kidney). Within the endocrine compartment, the number of synaptophysin-positive cells that co-expressed each of the four main pancreatic hormones was determined (e.g. number of insulin/synaptophysin co-positive cells relative to total number of synaptophysin-positive cells). Finally, insulin and glucagon co-expressing cells were assessed by measuring the area of insulin-positive, glucagon-positive and insulin/glucagon co-positive immunostaining relative to the total insulin and/or glucagon-positive area.

Endogenous pancreatic β-cell area was also assessed in healthy control mice (n = 3) and compared to mice at 8 (n = 4) and 34 (n = 4) weeks following STZ treatment. Pancreas sections were immunostained for insulin and glucagon. After whole slide fluorescence scanning, pancreas images were stitched together as described above. Insulin-positive area was expressed relative to the whole pancreas area; at least two sections were quantified per animal, separated by at least 200 μM.

Statistical Analysis

All statistics were performed using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA). For long-term meal challenge tracking (Fig 3C-D, Supplementary Fig 8A), 1-way repeated-measures ANOVA (SNK post-hoc) was used to compare human C-peptide: a) fasted levels over time and b) fed levels over time. Paired t-test was used to compare fed vs fasted C-peptide levels at each time point (Fig 3C and Supplementary Fig 8B). AUC for the glucose and C-peptide GTT responses was compared by unpaired t-test for 25 vs 12 weeks post-transplant (Fig 3E, F). Glucose-stimulated human C-peptide secretion was assessed by 1-way repeated measures ANOVA with SNK post-hoc test (Fig 3H, J). Endogenous pancreatic β-cell area was compared by 1-way ANOVA with SNK post-hoc test (Supplementary Fig 8C).

Supplementary Table 1. Teratomous elements identified within engrafted hESC-derived tissue.

Cohort	Animal #	Graft Stage	Teratomous Elements
1	4	6 months	Immature teratoma
	5	8 months	Mature bone and cartilage
	11	8 months	None
	12	8 months	Mature bone and cartilage
	14	8 months	None
	15	8 months	None
2	4	1 month	None
	9	1 month	Focal mature cartilage
	28	1 month	None
	24	1 month	None
	29	3 months	None
	2	3 months	None
	20	3 months	Bone marrow
	27	3 months	None
	5	8 months	Focal mature cartilage
	10	8 months	Mature bone
	30	8 months	Mature bone
	13	8 months	None
3	31	17 weeks	Mature bone, mesenchyme and glandular tissue
	34	17 weeks	None

Supplementary Table 2. Details of antibodies used for high content image analysis of *in vitro* cultures.

PRIMARY ANTIBODY						SECONDARY ANTIBODY				
Antigen	**HOST**	**Dilution**	**Manufacturer**	**Cat. No.**	**Block**	**2^{o} Antibody**		**Dilution**	**Manufacturer**	**Cat. No.**
PDX1	rabbit	1:750	AbCam	ab47267	5.0% Donkey	Alexa Fluor-	donkey-anti-rabbit	1:500	Molecular Probes	488-A21206
PDX1	goat	1:50	Santa Cruz	SC-14664	4.0% Chicken	Alexa Fluor-	chicken-anti-goat	1:100	Molecular Probes	488-A21467 647-A21469
NKX6.1	mouse	1:500	University of Iowa	F55A12	5.0% Donkey	Alexa Fluor-	donkey-anti-mouse	1:500	Molecular Probes	488-A21202 647-A31571
NKX6.1	rabbit	1:250	LifeSpan		5.0% Donkey	Alexa Fluor-	donkey-anti-rabbit	1:250	Molecular Probes	488-A21206 647-A31573
CDX2	mouse	1:500	Invitrogen	39-7800	5.0% Donkey	Alexa Fluor-	donkey-anti-mouse	1:250	Molecular Probes	647-A31571
NEUROD1	goat	1:250	R&D	AF2746	5.0% Donkey	Alexa Fluor-	donkey-anti-goat	1:250	Molecular Probes	647-A21447
hISLET1	mouse	1:500	Abcam	Ab86472	5.0% Donkey	Alexa Fluor-	donkey-anti-mouse	1:500	Molecular Probes	488-A21202 647-A31571
NKX2.2	goat	1:250	Santa Cruz	sc15015	5.0% Donkey	Alexa Fluor-	donkey-anti-goat	1:250	Molecular Probes	647-A21447

SUPPLEMENTARY DATA

Supplementary Table 3. Applied Biosystems qRT-PCR primers.

Category	GENE NAME	CATALOGUE NUMBER
Endocrine Markers	*CHGA*	Hs00154441_m1
	INS	Hs00355773_m1
	GCG	Hs00174967_m1
	SST	Hs00175082_m1
	IAPP	Hs00356144_m1
Pancreas Transcription Factors	*ARX*	Hs00292465_m1
	HNF4a	Hs00604435_m1
	ISL1	Hs00158126_m1
	MAFB	Hs00534343_s1
	NEUROD1	Hs00159598_m1
	NGN3	Hs00360700_g1
	NKX6.1	Hs00232355_m1
	NKX2.2	Hs00159616_m1
	PAX4	Hs00173014_m1
	PAX6	Hs00240871_m1
	PDX1	Hs00236830_m1
	PTF1a	Hs00603586_g1
Non-pancreatic Lineage Markers	*AFP*	Hs00173490_m1
	ALB	Hs00609411_m1
	CDX1	Hs00230919_m1
	HAND1	Hs00231848_m1
	MUC1	Hs00159357_m1
	NKX2.1	Hs00163037_m1
	PECAM	Hs00169777_m1
	POU5F1	Hs01895061_u1
	ZIC1	Hs00602749_m1
Pluripotency Markers	*NANOG*	Hs02387400_g1
	OCT4	Hs01895061_u1
	TDGF1	Hs02339499_g1
	ZFP42	Hs00399279_m1

Supplementary Table 4. SABiosciences qRT-PCR primers.

GENE CATEGORY	GENE NAME	OFFICIAL FULL NAME	GenBank #
Pancreas Hormones	*INS*	Insulin	NM_000207
	IAPP	Islet amyloid polypeptide	NM_000415
	GCG	Glucagon	NM_002054
	PPY	Pancreatic polypeptide	NM_002722
	SST	Somatostatin	NM_001048
	GHRL	Ghrelin/obestatinpreprohormone	NM_016362
Transcription Factors	*ARX*	Aristaless related homeobox	NM_139058
	FOXO1	Forkhead box O1	NM_002015
	ISL1	ISL LIM homeobox 1	NM_002202
	MAFA	V-mafmusculoaponeuroticfibrosarcoma oncogene homolog A (avian)	NM_201589
	MAFB	V-mafmusculoaponeuroticfibrosarcoma oncogene homolog B (avian)	NM_005461
	NEUROD1	Neurogenic differentiation 1	NM_002500
	NEUROG3	Neurogenin 3	NM_020999
	NKX2.2	NK2 homeobox 2	NM_002509
	NKX6.1	NK6 homeobox 1	NM_006168
	PAX4	Paired box 4	NM_006193
	PAX6	Paired box 6	NM_000280
	PDX1	Pancreatic and duodenal homeobox 1	NM_000209
	PTF1A	Pancreas specific transcription factor, 1a	NM_178161
	SOX9	SRY (sex determining region Y)-box 9	NM_000346
Maturation Markers	*ABCC8* (SUR1)	ATP-binding cassette, sub-family C (CFTR/MRP), member 8	NM_000352
	KCNJ11 (KIR6.2)	Potassium inwardly-rectifying channel, subfamily J, member 11	NM_000525
	GCK	Glucokinase (hexokinase 4)	NM_000162
	SLC2A1 (GLUT1)	Solute carrier family 2 (facilitated glucose transporter), member 1	NM_006516
	PCSK1	Proproteinconvertasesubtilisin/kexin type 1	NM_000439
	PCSK2	Proproteinconvertasesubtilisin/kexin type 2	NM_002594
	SLC30A8 (ZNT8)	Solute carrier family 30 (zinc transporter), member 8	NM_173851
Other Tissues	*RUNX2*	Runt-related transcription factor (BONE)	NM_004348
	PECAM	Platelet/endothelial cell adhesion molecule (ENDOTHELIAL)	NM_000442
	MYF5	Myogenic factor 5 (MUSCLE)	NM_005593
	MYOD1	Myogenic differentiation 1 (MUSCLE)	NM_002478
	ALB	Albumin (Liver)	NM_000477
	CDX2	Caudal type homeobox 2	NM_001265

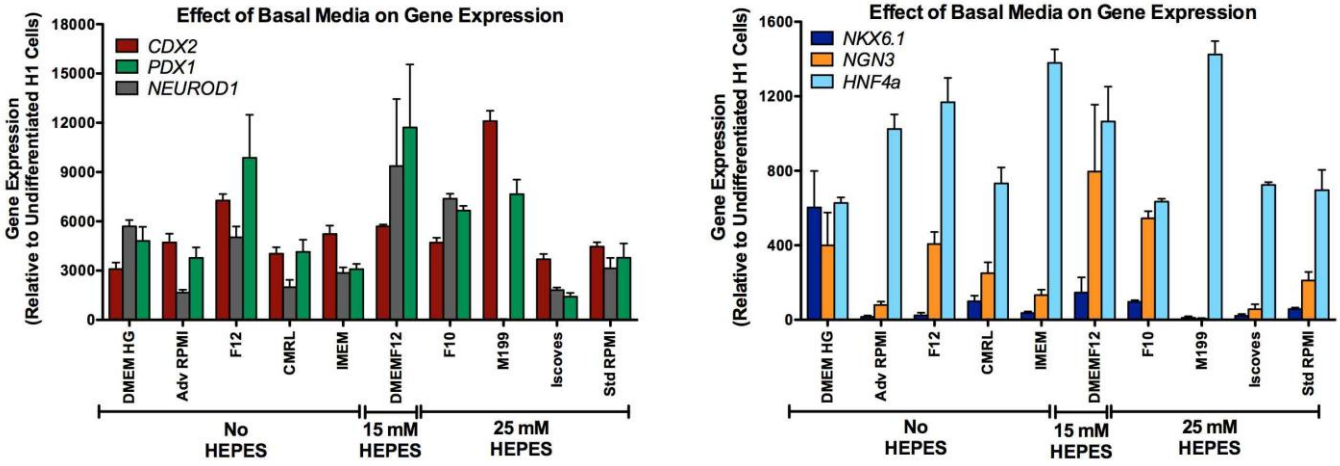
	OLIG2	Oligodendrocyte lineage transcription factor 2 (NEURAL)	NM_005806
Housekeeping Genes	*B2M*	Beta-2-microglobulin	NM_004048
	HPRT1	Hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)	NM_000194
	RPL13A	Ribosomal protein L13a	NM_012423
	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046
	ACTB	Actin, beta	NM_001101

Supplementary Table 5. Details of primary antibodies used for immunofluorescent staining.

ANTIGEN	**SPECIES**	**SOURCE**	**DILUTION**
Amylase	Rabbit	Sigma Aldrich	1:500
ARX	Rabbit	Dr. P Collombat, Inserm, University of Nice; Nice, France	1:500
Chromogranin	Sheep	Enzo Life Sciences; Plymouth Meeting, PA	1:200
CK19	Mouse	Dako; Denmark	1:200
C-Peptide	Guinea Pig	AbCam	1:100
Glucagon	Mouse	Sigma Aldrich	1:1000
Glucagon	Rabbit	Thermo Scientific; Waltham, MA	1:100
IAPP (Amylin)	Rabbit	AbCam	1:200
Insulin	Guinea Pig	Millipore; Billerica, MA	1:1000
Insulin	Rabbit	Cell Signaling Technologies, Danvers, MA	1:100
Pancreatic Polypeptide	Guinea Pig	Millipore	1:500
PC1/3	Rabbit	Dr. Lakshmi Devi, Mount Sinai School of Medicine; New York, NY	1:500
PC2	Rabbit	ABR Affinity Bioreagents; Golden, CO	1:500
PCNA	Mouse	BD Transduction Laboratory; Franklin Lakes, NJ	1:100
PDX1	Rabbit	Dr. Joel Habener, Harvard Medical School, Boston, MA	1:1000
MAFA	Rabbit	Lifespan Biosciences; Seattle, WA	1:1000
MAFB	Rabbit	Sigma	1:500
NKX2.2	Mouse	Developmental Studies Hybridoma Bank; University of Iowa, Iowa City, IA	1:100
NKX6.1	Rabbit	Lifespan Biosciences; Seattle, WA	1:4000
Somatostatin	Mouse	GeneTex, Inc.; Irvine, CA	1:50
Synaptophysin	Rabbit	Novus Biologicals; Littleton, CO	1:50
Trypsin	Sheep	R&D Systems; Minneapolis, MN	1:100
Zinc Transporter 8	Mouse	Mellitech; Grenoble, France	1:200

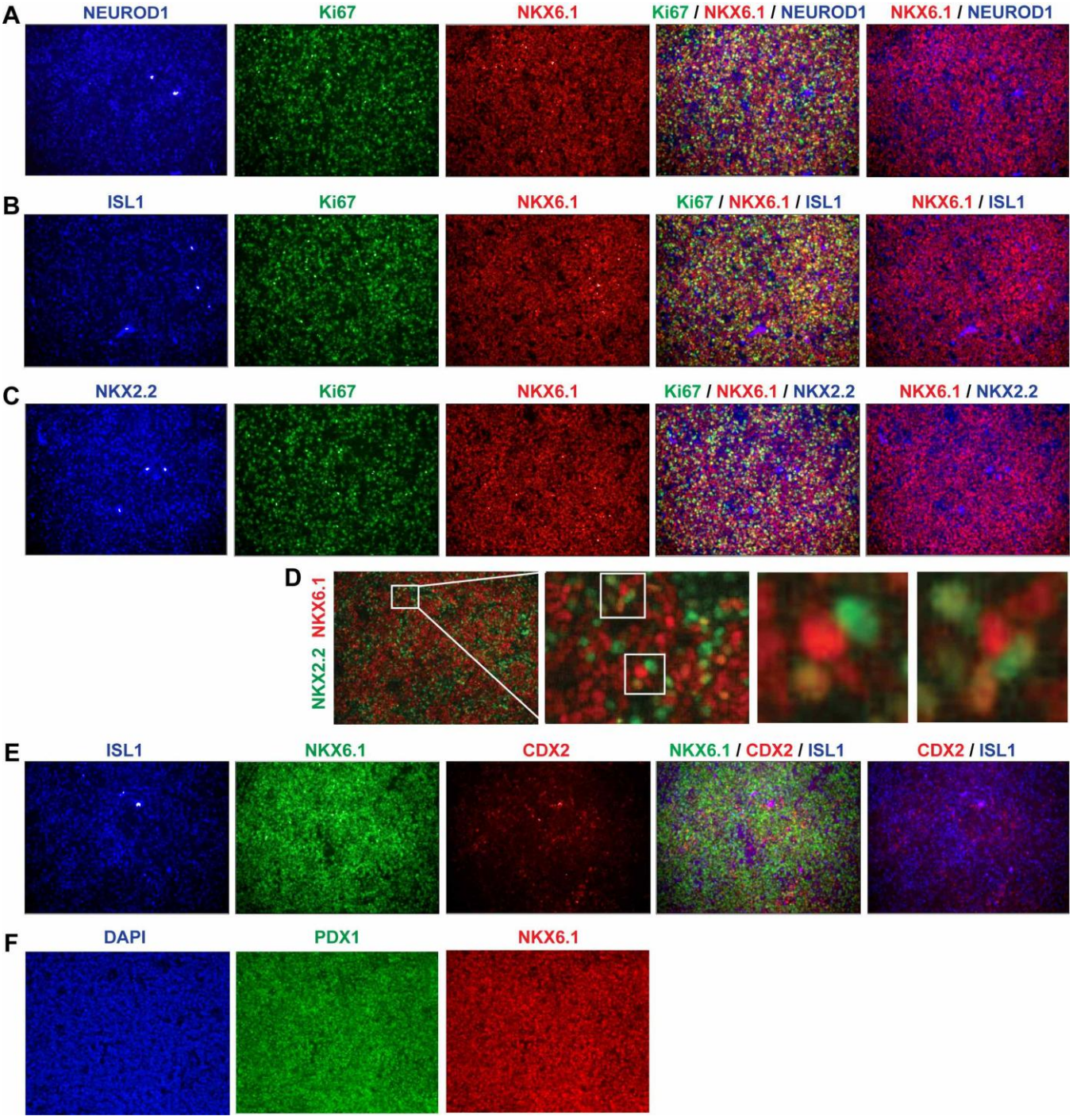
SUPPLEMENTARY DATA

Supplementary Figure 1. Optimization of the *in vitro* differentiation protocol *(related to Figure 1A)*. Gene expression of pancreatic endocrine lineage (*PDX1*, *NeuroD*, *NKX6.1*, *NGN3* and *HNF4* α) and intestinal lineage (*CDX2*) markers to assess the optimal basal media formulation during stage 4. Data are presented as mean ± SD.



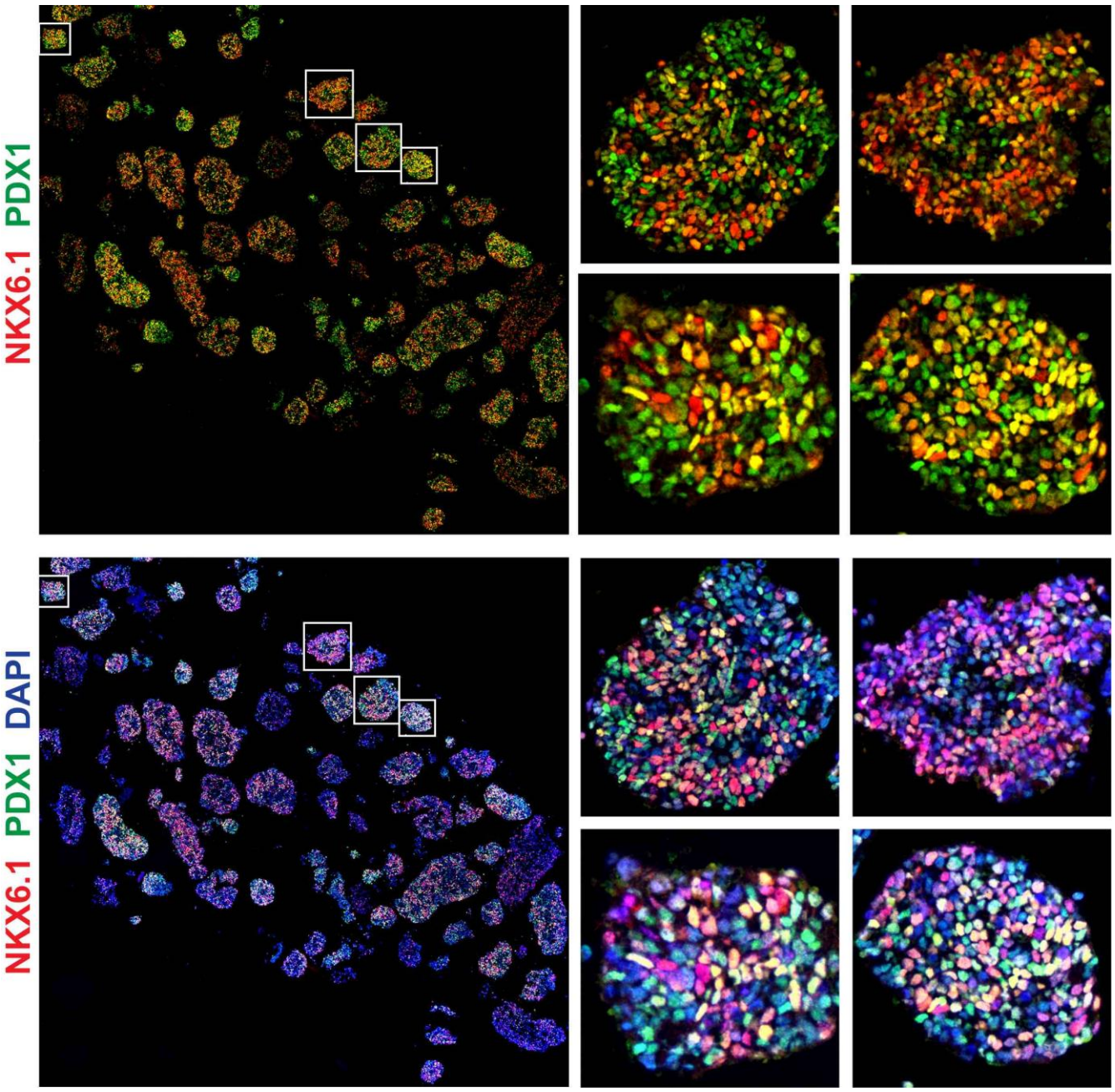
SUPPLEMENTARY DATA

Supplementary Figure 2. High content image quantification *(related to Figure 2F).* A - F) Representative images of high content quantification for various combinations of markers in monolayer cultures at stage 4, days 4-5. Panel D shows that the majority of cells express either NKX2.2 or NKX6.1, but that rare cells co-express both transcription factors.



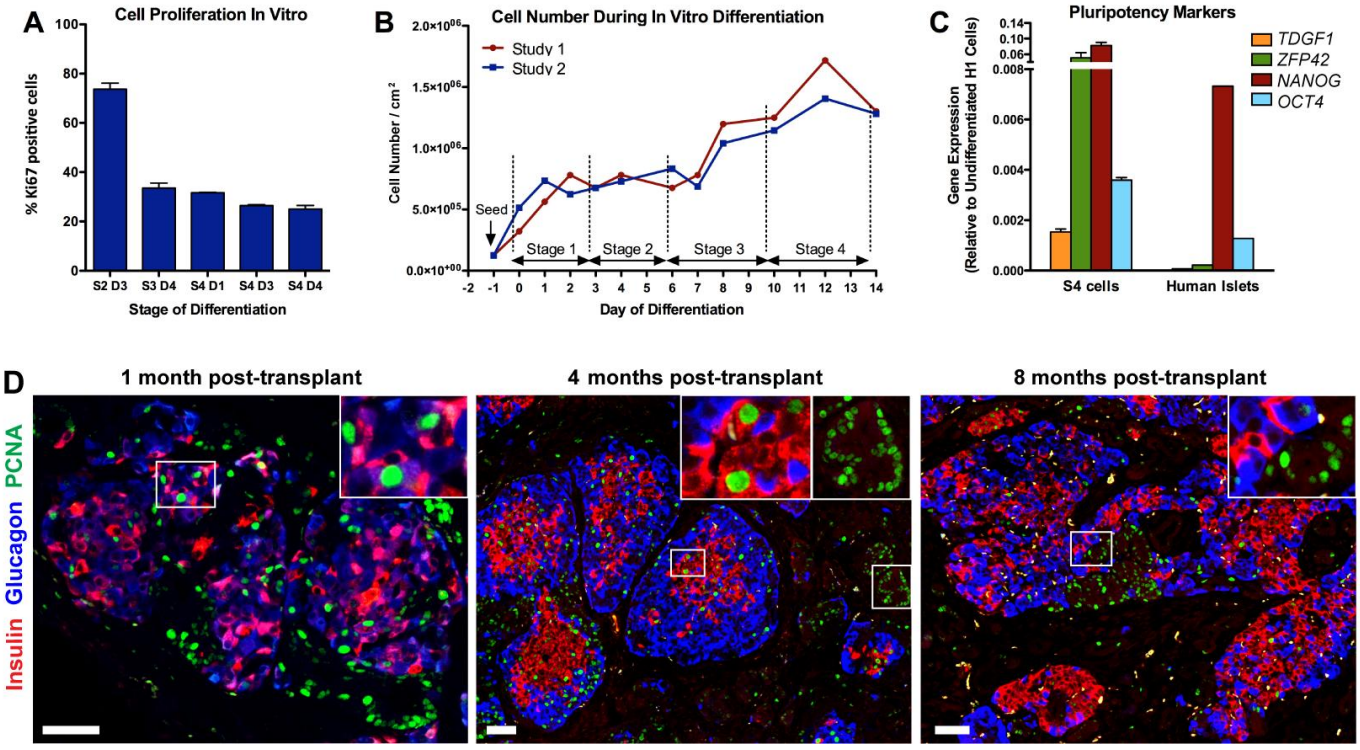
SUPPLEMENTARY DATA

Supplementary Figure 3. PDX1 and NKX6.1 co-expression in stage 4 suspension clusters *(related to Figure 2).* Representative immunofluorescent staining for PDX1 (green) and NKX6.1 (red) with or without DAPI (blue). Magnified islets illustrate the heterogeneity in co-expression of PDX1 and NKX6.1.



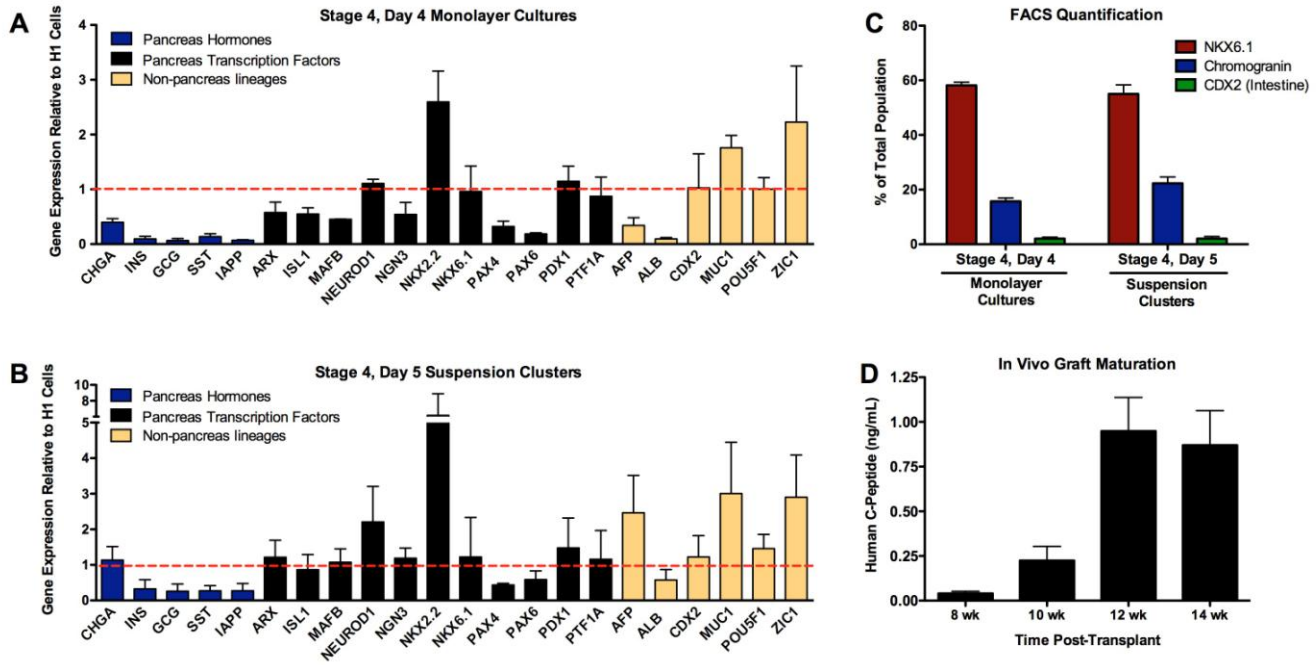
SUPPLEMENTARY DATA

Supplementary Figure 4. hESC expansion during *in vitro* and *in vivo* differentiation *(related to Figures 2 and 4).* A) Proportion of cells expressing Ki67 (proliferation marker) and B) number of cells/cm^2 throughout the *in vitro* differentiation. C) Gene expression of pluripotency markers in S4 cells and adult human islets relative to undifferentiated H1 cells. Data are presented as mean ± SEM. D) Representative images of insulin, glucagon and PCNA (proliferating cell nuclear antigen) expression in engrafted cells at 1, 4 and 8 months following transplantation under the kidney capsule. Scale bars = 50 μm.



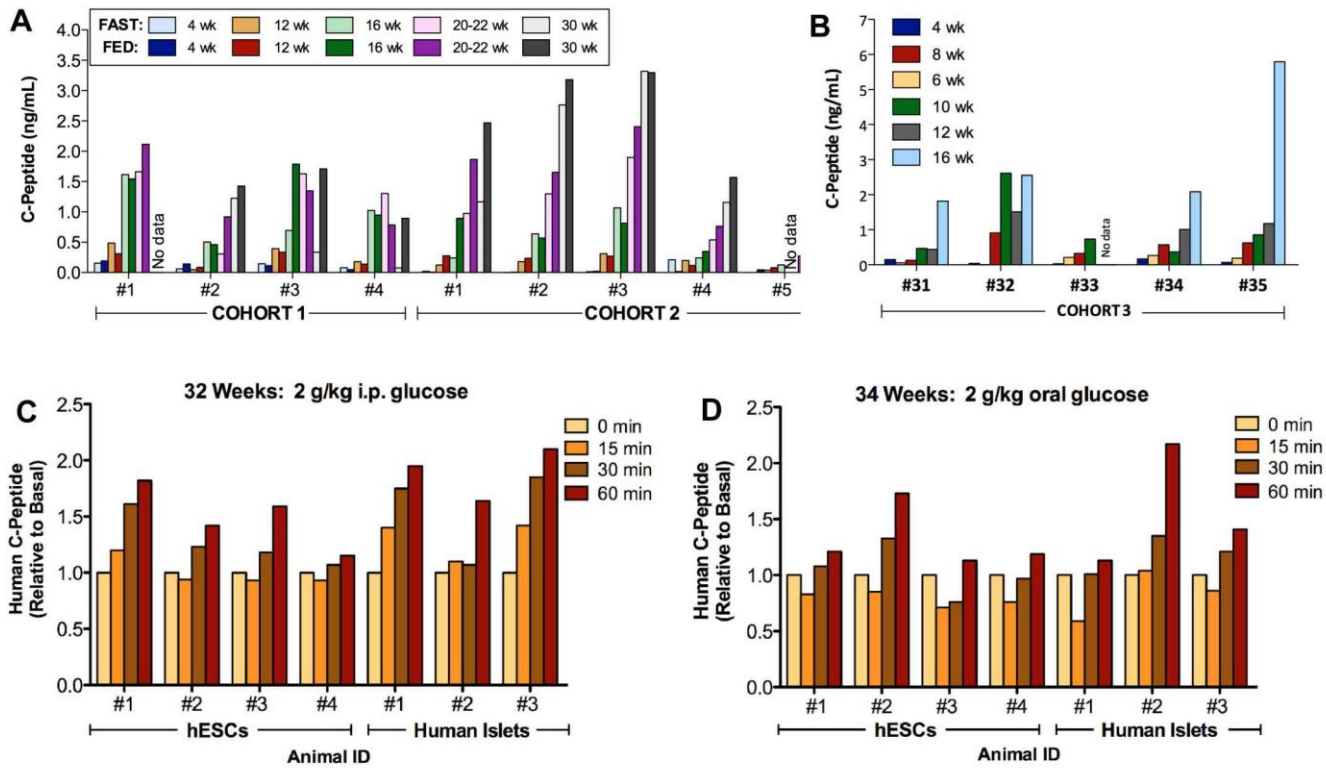
SUPPLEMENTARY DATA

Supplementary Figure 5. Confirmation of the *in vitro* differentiation protocol efficiency in ESI-49 cells. A-B) qRT-PCR for expression of numerous genes, including pancreas hormones (blue bars), pancreas transcription factors (black bars) and other lineages (yellow bars) in differentiated stage 4, day 4-5 ESI-49 monolayer cultures (A) and suspension clusters (B). All data are expressed relative to levels in differentiated H1 cells at stage 4, day 4 (H1 cell levels indicated by dotted red line). C) FACS quantification of the proportion of cells expressing NKX6.1 (red), chromogranin (blue) or CDX2 (green) in differentiated monolayer and suspension cultures. D) Human C-peptide levels (ng/mL) in plasma of SCID-beige mice at 8, 10, 12 and 14 weeks following transplant of differentiated ESI-49 cells.



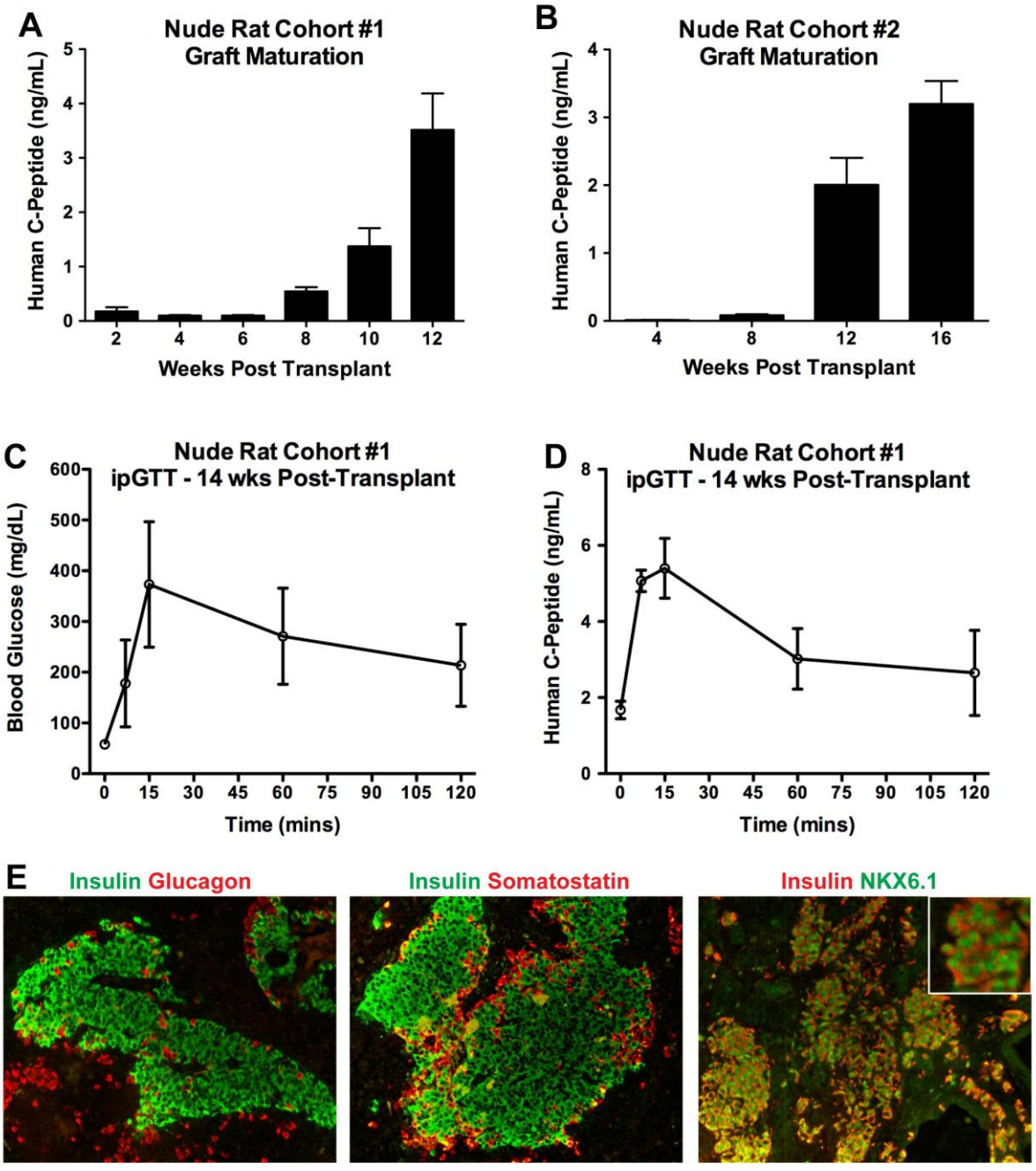
SUPPLEMENTARY DATA

Supplementary Figure 6. Human C-peptide levels in individual mice during various metabolic tests *(related to Figure 3)*. Circulating human C-peptide (ng/mL) during: A) monthly meal challenges from cohorts 1 and 2, and B) random fed blood sampling from cohort 3. Human C-peptide levels at 0, 15, 30 and 60 minutes following an C) i.p. or D) oral glucose challenge (2 g/kg), expressed relative to basal levels. GTTs were performed in mice with engrafted hESCs at 32 and 34 weeks post-transplant and compared to mice with engrafted human islets.



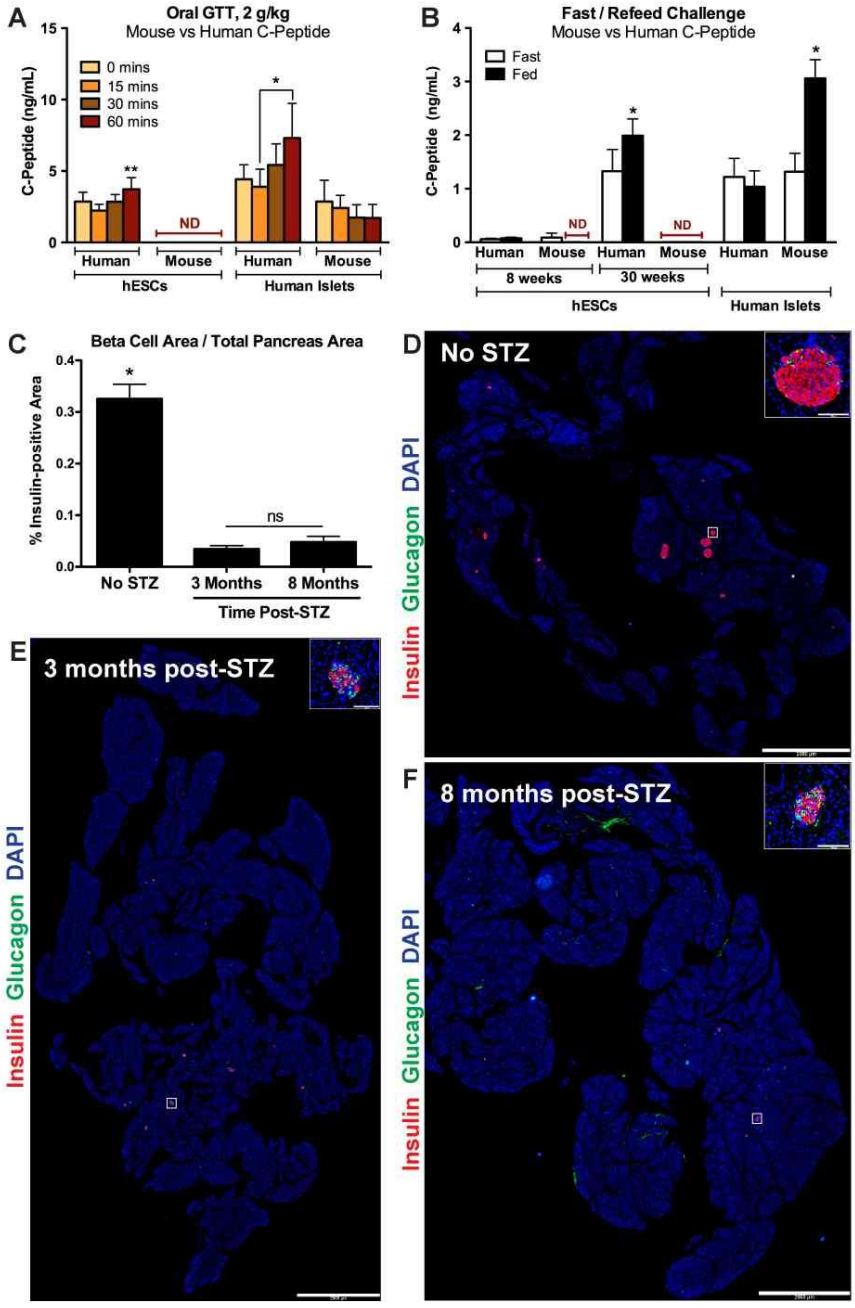
SUPPLEMENTARY DATA

Supplementary Figure 7. Maturation of hESC-derived progenitor cells in nude rats. A-B) Glucose-stimulated plasma human C-peptide levels (ng/mL) at various time points following transplantation of hESC-derived progenitor cells in nude rats from 2 independent cohorts. C) Blood glucose levels (mg/dL) and D) human C-peptide secretion following an intraperitoneal glucose tolerance test (ipGTT) at 14 weeks post-transplant in nude rats from Cohort #1. All data are presented as mean ± SEM. E) Representative images of engrafted hESC-derived cells under the kidney capsule of nude rats in Cohort #2 showing insulin co-immunostaining with glucagon, somatostatin or NKX6.1.



SUPPLEMENTARY DATA

Supplementary Figure 8. Contribution of the endogenous pancreas to glucose homeostasis *(related to Figure 3).* Human and mouse C-peptide levels (ng/mL) during: A) an oral GTT at 34 weeks post-transplant, and B) fast/re-feed challenges at 8 and 30 weeks post-transplant. Mice transplanted with hESC-derived cells were STZ-treated prior to transplant, but human islet recipients were not treated with STZ. ND = non-detectable. C) Quantification of relative β-cell area in the endogenous pancreas (insulin-positive area / total pancreas area) from healthy control mice (no STZ) and mice at 3 and 8 months post-STZ treatment. D-F) Representative images of whole pancreas sections (scale bar = 200 μm) immunostained with insulin (red), glucagon (green) and DAPI (blue) from healthy controls (D) and mice at 3 (E) and 8 (F) months post-STZ; magnified islets are shown in the top right corner (scale bar = 100 μm). All data are presented as mean ± SEM. For the oral GTT (A): * $p < 0.05$, 60 vs 15 min; ** $p < 0.05$, 60 vs 0, 15 and 30 mins (1-way ANOVA, repeated measures). For the fast/re-feed challenge (B): * $p < 0.05$, 30 wk fast vs 30 wk fed (paired 2-tailed t-test). For β-cell area (C): * $p < 0.05$, No STZ vs 3 and 8 months (1-way ANOVA), ns = not significant.



SUPPLEMENTARY DATA

Supplementary Figure 9. Graft composition at 8 months post-transplant. A – B) Gene expression of markers for various tissues, including bone, endothelial cells, muscle, neural, pancreas, liver and intestine in engrafted cells at 8 months post-transplant. Data are expressed relative to adult human islets and presented in panel A as mean ± SEM and panel B as individual animals. Mouse #10 (red symbols) had confirmed presence of mature bone within the graft by pathology. Representative H&E images are provided for: C) a healthy graft containing only pancreas tissue (islets and ducts) and D) a graft containing pancreas tissue as well as a region of mature bone.