Stem Cell Reports, Volume *12*

Supplemental Information

Macroencapsulated Human iPSC-Derived Pancreatic Progenitors Protect against STZ-Induced Hyperglycemia in Mice

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STEM-CELL-REPORTS-D-18-00175R2

Title: Macroencapsulated Human iPSC-derived pancreatic progenitors protect against STZ-induced hyperglycemia in mice.

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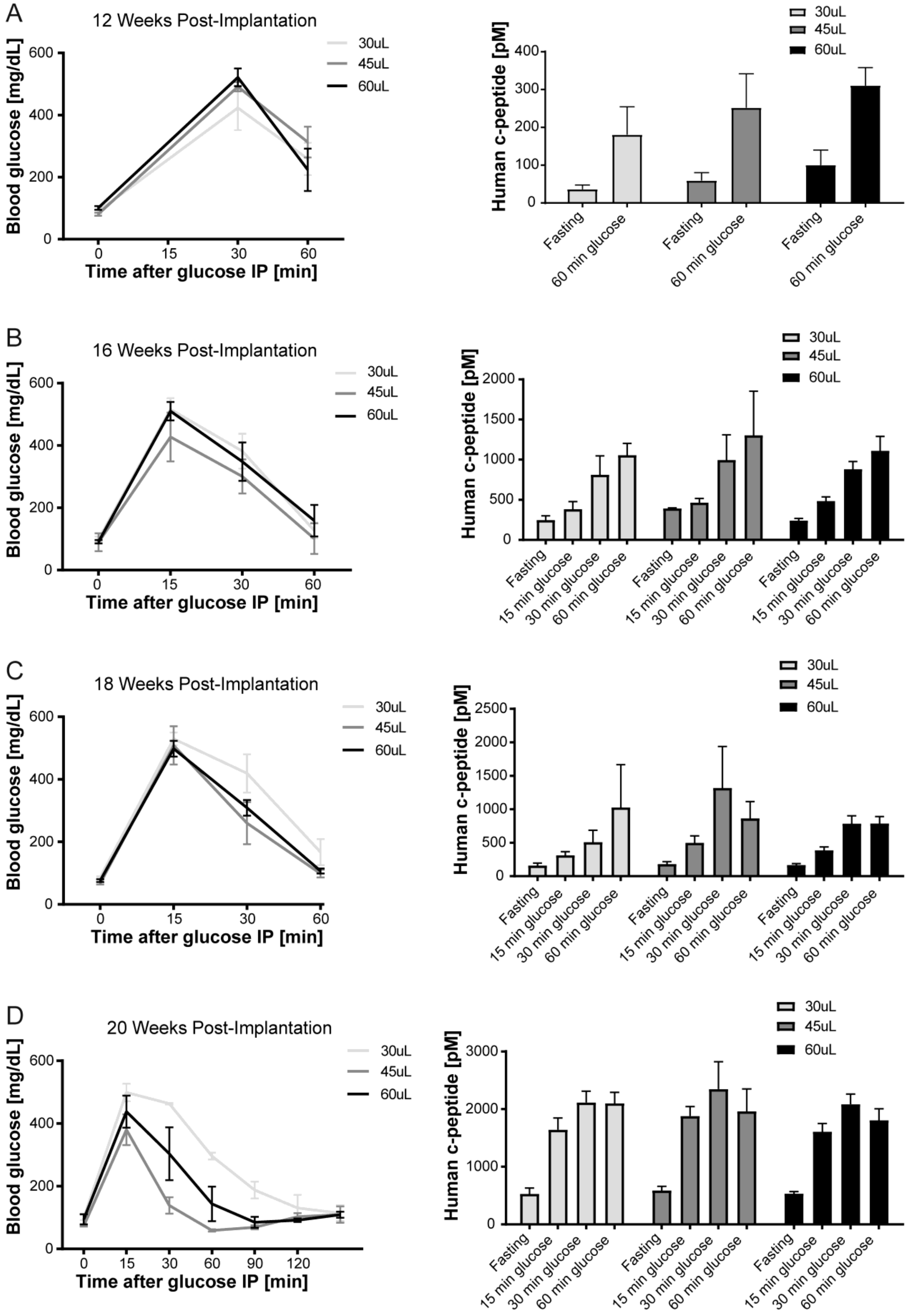
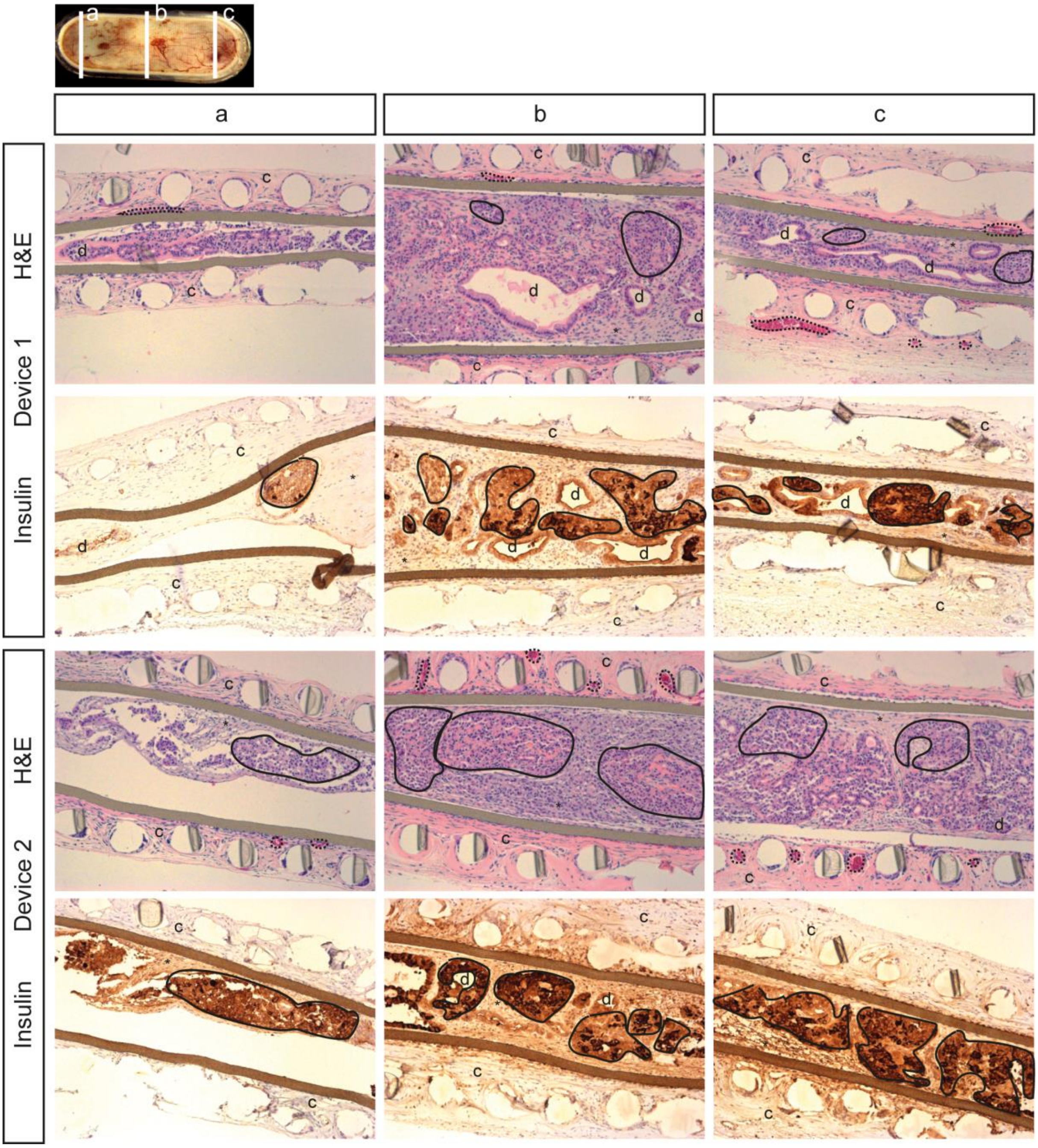
Supplemental Information

Figure S1: Analyses of cell density loading effect on maturation of C-peptide producing cells. Related to Figure 3



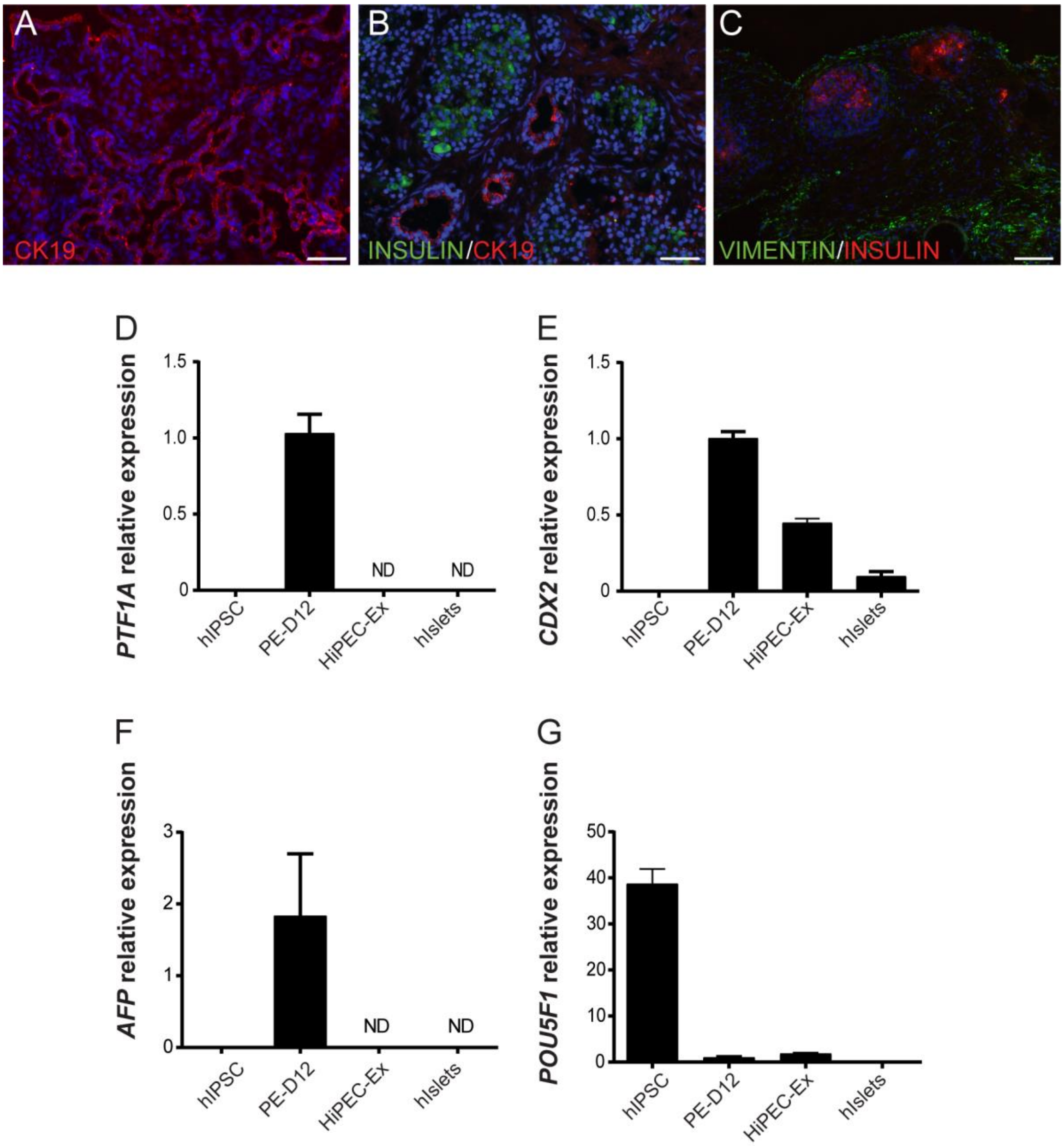
Analyses of blood glucose levels and serum levels of human C-peptide in mice implanted with 3 different cell densities (30 µl, 45 µl, and 60 µl) of LHiPEC-1. Typically, three volumes of aggregates: 30 µl (1-2*10^6 cells), 45 µl (3-5*10^6 cells) and 60 µl (5-8*10^6 cells) were loaded in the flat-sheet macro-encapsulation device and implanted subcutaneously SCID-beige mice. We assessed glucose-stimulated insulin secretion specifically of implanted cells by analyzing human C-peptide levels in sera collected from mice during a glucose challenge performed at 12, 16, 18 and 20 weeks post-implantation. Mice implanted with either 30 µl, 45 µl, or 60 µl LHiPSC-1-derived pancreatic endoderm cells (LHiPEC) were analyzed at 12 weeks (A), 16 weeks (B), 18 weeks (C), and 20 weeks (D) post-engraftment for blood glucose levels and for serum levels of human C-peptide at fasting, 15 min, 30 min, 60 min, 90 min and 120 min after intraperitoneal glucose administration. Average of blood glucose levels in response to intraperitoneal glucose tolerance test are shown for the indicated concentrations (n=3-6 mice per group). Error bars indicate SEM. Although the differences between the 3 concentrations tested were not significant when considering the levels of C-peptide secreted, there was a tendency for better glucose clearance as well as augmented C-peptide levels using a volume of 45 µl of aggregates. This concentration of cells was thus used in the following implantation experiments.

Figure S2: Morphological and immunohistochemistry analyses of post-implantation graft samples. Related to Figure 4A



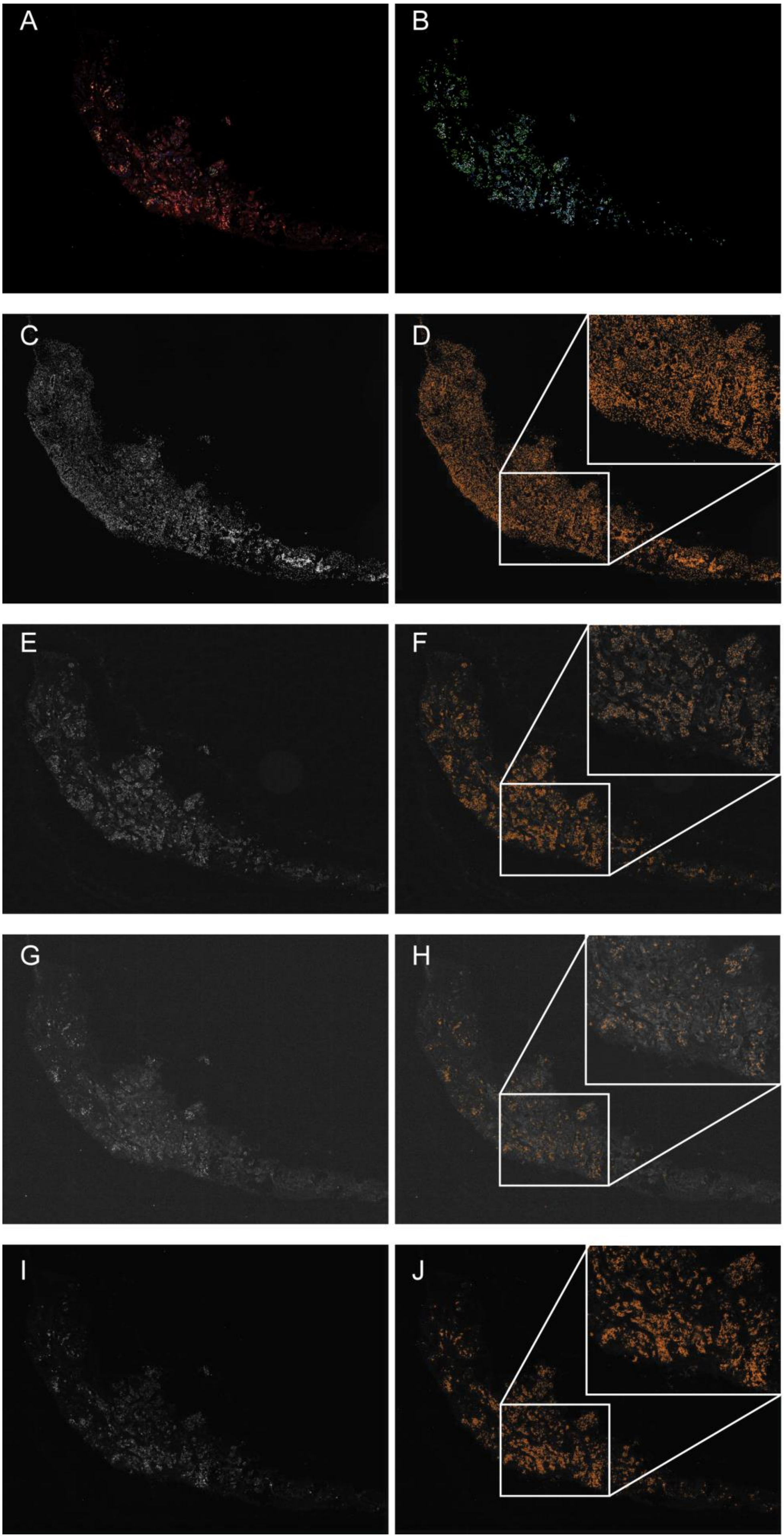
Two HiPEC-1-derived grafts (devices) were explanted at 32 weeks post-implantation and analyzed for Hematoxylin and Eosine (H&E) staining or Insulin staining on serial sections. a, b, c indicate 3 different section angles of the devices (a-c: sides, b: center) to obtain a good morphological representation of the whole tissue.

Figure S3: Characterization of off-target tissues in explanted grafts. Related to Figure 4.



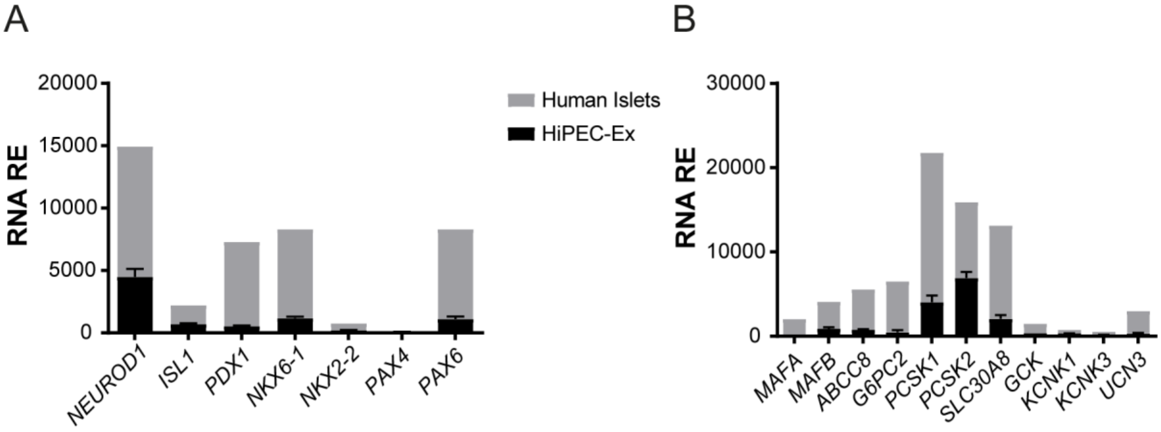
(A-C) Representative images from HiPEC-1-derived grafts after explantation. (A-B) Immunostaining for the ductal marker CK19 (A-B) in red and insulin (B) in green. (C) Immunostaining for the mesenchymal cell marker vimentin (green) and insulin (red). (D-G) RT-qPCR for the following markers: *PTF1A* (acinar, D), *CDX2* (intestinal, E), *AFP* (hepatic, F) and *POU5F1* (pluripotency, G), and on hiPSC (n=2), PE-D12 (n=6), HiPEC-Ex (n=4) and human islets (n=2). Error bars represent SEM.

Figure S4: High content image quantification of cell composition in graft explants. Related to Figures 4E, 5A and Experimental procedures.


A
B
C
D
E
F
G
H
I
J

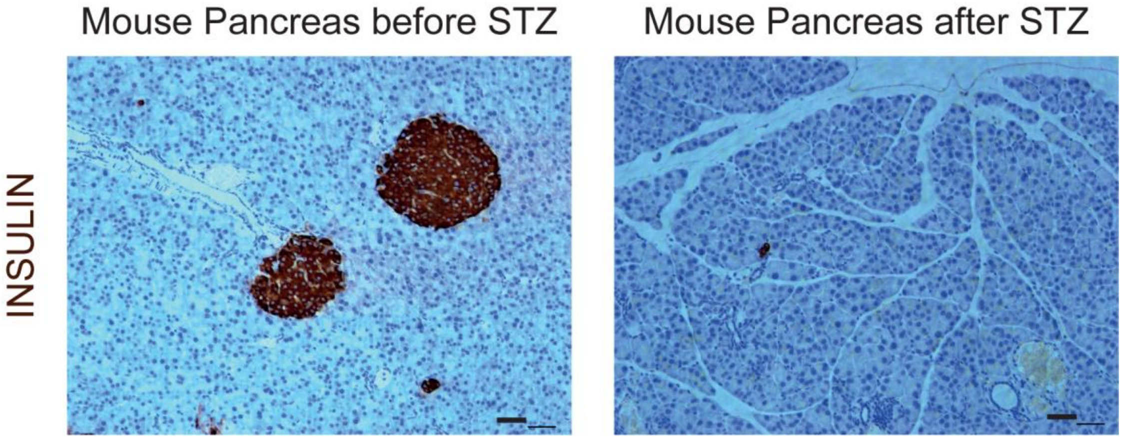
(A) Example of a composite image from the Slide Scanner. Red cytoplasmic staining is INSULIN, blue (nuclear) is MAFA and green (nuclear) is NKX6-1. DAPI is not shown. (B) Composite image after analysis with MetaExpress which represents each cell which was considered as positive for INSULIN and/or MAFA and/or NKX6-1. Red=NKX6-1; Green=MAFA; Blue=INS; Yellow=MAFA/NKX6-1; Cyan=MAFA/INS; Magenta=NKX6-1/INS. (C-J) Separate channel for each marker (C: DAPI; E: MAFA; G: NKX6-1; I: INS) and respective example of positivity thresholding (D, F, H, J).

Figure S5: Gene expression analyses of HiPEC-derived graft tissues. Related to Figures 4F and 5B.



(A-B) RNA expression analyses by Nanostring of important indicated markers of beta-cell maturation as compared to human islets controls (n=2). Error bars indicate SD.

Figure S6: Depletion of mouse beta-cells following STZ treatment. Related to Figure 7.



Immunohistochemistry for insulin after deletion of mouse beta-cells by STZ in animals implanted with LHiPEC

Table S1: Nanostring combo6980 code-set with sequences. Related to Experimental procedures and Figures 4E and S5.

Accession	Hugo gene	Postion	Target sequence
NM_001100.3	ACTA1	45-145	GGCGACCAGGGCCCGAGCCGAGAGTAGCAGTTGTAGCTACCCGCCCAGAAACTAGACACAATGTGCGACGAAGACGAGACCACCGCCCTCGTGTGCGACA
NM_001613.1	ACTA2	645-745	ATTCCTTCGTTACTACTGCTGAGCGTGAGATTGTCCGGGACATCAAGGAGAAACTGTGTTATGTAGCTCTGGACTTTGAAAATGAGATGGCCACTGCCGC
NM_001101.2	ACTB	1010-1110	TGCAGAAGGAGATCACTGCCCTGGCACCCAGCACAATGAAGATCAAGATCATTGCTCCTCCTGAGCGCAAGTACTCCGTGTGGATCGGCGGCTCCATCCT
NM_001134.1	AFP	395-495	AGTGAAGAGGGAAGACATAACTGTTTTCTTGCACACAAAAAGCCCACTCCAGCATCGATCCCACTTTTCCAAGTTCCAGAACCTGTCACAAGCTGTGAAG
NM_000700.1	ANXA1	515-615	GAAATCAGAGACATTAACAGGGTCTACAGAGAGGAACTGAAGAGAGATCTGGCCAAAGACATAACCTCAGACACATCTGGAGATTTTCGGAACGCTTTGC
NM_139058.2	ARX	2621-2721	TGCACTCAGCGTGGTATGGTAAAAGTTTGTCCTCCCGTAGATTCTTACTGTGTTGTAGATACGGTAGGGTTCTAGACAAATATTTATGTACTCAAGCCC
NM_001200.2	BMP2	1515-1615	TTAGGATAAGCAGGTCTTTGCACCAAGATGAACACAGCTGGTCACAGATAAGGCCATTGCTAGTAACTTTTGGCCATGATGGAAAAGGGCATCCTCTCCA
NM_001202.2	BMP4	490-590	ACCGAATGCTGATGGTCGTTTTATTATGCCAAGTCCTGCTAGGAGGCGCGAGCCATGCTAGTTTGATACCTGAGACGGGGAAGAAAAAAGTCGCCGAGAT
NM_001719.1	BMP7	525-625	GCTTCGTCAACCTCGTGGAACATGACAAGGAATTCTTCCACCCACGCTACCACCATCGAGAGTTCCGGTTTGATCTTTCCAAGATCCCAGAAGGGGAAGC
NM_004701.2	CCNB2	980-1080	AGGTTGATGTTGAACAGCACACTTTAGCCAAGTATTTGATGGAGCTGACTCTCATCGACTATGATATGGTGCATTATCATCCTTCTAAGGTAGCAGCAGC
NM_053056.2	CCND1	690-790	TTGAACACTTCCTCTCCAAAATGCCAGAGGCGGAGGAGAACAAACAGATCATCCGCAAACACGCGCAGACCTTCGTTGCCCTCTGTGCCACAGATGTGAA
NM_001769.2	CD9	405-505	ATATTCGCCATTGAAATAGCTGCGGCCATCTGGGGATATTCCCACAAGGATGAGGTGATTAAGGAAGTCCAGGAGTTTTACAAGGACACCTACAACAAGC
NM_004360.2	CDH1	1230-1330	CGATAATCCTCCGATCTTCAATCCCACCACGTACAAGGGTCAGGTGCCTGAGAACGAGGCTAACGTCGTAATCACCACACTGAAAGTGACTGATGCTGAT
NM_001792.3	CDH2	941-1041	GGTCATCCCTCCAATCAACTTGCCAGAAAACTCCAGGGGACCTTTTCCTCAAGAGCTTGTCAGGATCAGGTCTGATAGAGATAAAAACCTTTCACTGCGG
NM_001265.2	CDX2	1812-1912	CTTCTCTGGGCTGAATGTATGTCAGTGCTATAAATGCCAGAGCCAACCTGGACTTCCTGTCATTTTCACAATCTTGGGGCTGATGAAGAAGGGGGTGGGG
NM_001275.3	CHGA	292-392	CTGCGCCGGGCAAGTCACTGCGCTCCCTGTGAACAGCCCTATGAATAAAGGGGATACCGAGGTGATGAAATGCATCGTTGAGGTCATCTCCGACACACTT
NM_000090.3	COL3A1	180-280	TTGGCACAACAGGAAGCTGTTGAAGGAGGATGTTCCCATCTTGGTCAGTCCTATGCGGATAGAGATGTCTGGAAGCCAGAACCATGCCAAATATGTGTCT
NM_001868.2	CPA1	690-790	ACCGCCATTCTCGACACCTTGGACATCTTCCTGGAGATCGTCACCAACCCTGATGGCTTTGCCTTCACGCACAGCACGAATCGCATGTGGCGCAAGACTC
NM_001901.2	CTGF	1100-1200	ACCACCCTGCCGGTGGAGTTCAAGTGCCCTGACGGCGAGGTCATGAAGAAGAACATGATGTTCATCAAGACCTGTGCCTGCCATTACAACTGTCCCGGAG
NM_199168.2	CXCL12	505-605	GGGCCTGAGGTTTGCCAGCATTTAGACCCTGCATTTATAGCATACGGTATGATATTGCAGCTTATATTCATCCATGCCCTGTACCTGTGCACGTTGGAAC
NM_003467.2	CXCR4	1335-1435	ATTGATGTGTGTCTAGGCAGGACCTGTGGCCAAGTTCTTAGTTGCTGTATGTCTCGTGGTAGGACTGTAGAAAAGGGAACTGAACATTCCAGAGCGTGTA
NM_001554.3	CYR61	1390-1490	AAGGGAGAAGAGTGTCAGAATCAGAATCATGGAGAAAATGGGCGGGGGTGGTGTGGGTGATGGGACTCATTGTAGAAAGGAAGCCTTGCTCATTCTTGAG
NM_014420.2	DKK4	640-740	CCAGAAGAGGGCATAAAGACACTGCTCAAGCTCCAGAAATCTTCCAGCGTTGCGACTGTGGCCCTGGACTACTGTGTCGAAGCCAATTGACCAGCAATCG
NM_005618.3	DLL1	2580-2680	ACCAGTCGGTGTACGTCATATCCGAGGAGAAGGATGAGTGCGTCATAGCAACTGAGGTGTAAAATGGAAGTGAGATGGCAAGACTCCCGTTTCTCTTAAA
NM_005221.5	DLX5	575-675	CCGAGCCCGAGGTGAGAATGGTGAATGGCAAACCAAAGAAAGTTCGTAAACCCAGGACTATTTATTCCAGCTTTCAGCTGGCCGCATTACAGAGAAGGTT
NM_001935.3	DPP4	2700-2800	CAGCAGTCAGCTCAGATCTCCAAAGCCCTGGTCGATGTTGGAGTGGATTTCCAGGCAATGTGGTATACTGATGAAGACCATGGAATAGCTAGCAGCACAG
NM_199286.2	DPPA3	8-108	GAGGCTCTGTCATCAGTTTCTGCTACGTTTCAAAGATCCTGGAGAAGCCTAGTGTTGTGTCAAGACGCCGATGGACCCATCACAGTTTAATCCAACCTAC
NM_018189.3	DPPA4	270-370	CCCCAGACCTCAGAAGAAGATACCAATCCCTCCATTACCTTCTAAACTGCCACCTGTTAATCTGATTCACCGGGACATTCTGCGGGCCTGGTGCCAACAA
NM_001955.2	EDN1	770-870	TTTCATGATCCCAAGCTGAAAGGCAATCCCTCCAGAGAGCGTTATGTGACCCACAACCGAGCACATTGGTGACAGACCTTCGGGGCCTGTCTGAAGCCAT
NM_004428.2	EFNA1	650-750	TGCTGCCCCACGCCTCTTCCCACTTGCCTGGACTGTGCTGCTCCTTCCACTTCTGCTGCTGCAAACCCCGTGAAGGTGTATGCCACACCTGGCCTTAAAG
NM_005442.2	EOMES	1670-1770	ATCCCATGCCCTGGGGTATTACCCAGACCCAACCTTTCCTGCAATGGCAGGGTGGGGAGGTCGAGGTTCTTACCAGAGGAAGATGGCAGCTGGACTACCA
NM_001430.3	EPAS1	4246-4346	TGCACTGAGCTATGTGACTCGGATGGTCTTTCACACGGCACATTTGGACATTTCCAGAACTACCATGAGATGGTTTAGACGGGAATTCATGCAAATGAGG
NM_000503.4	EYA1	1469-1569	ACAGCACAATCCACAGCCCATCAACACCCATTAAAGATTCAGATTCTGATCGATTGCGTCGAGGTTCAGATGGGAAATCACGTGGACGGGGCCGAAGAAA
NM_001993.3	F3	1030-1130	GAGCTGGAAGGAGAACTCCCCACTGAATGTTTCATAAAGGAAGCACTGTTGGAGCTACTGCAAATGCTATATTGCACTGTGACCGAGAACTTTTAAGAGG
NM_002006.4	FGF2	620-720	GTCCGGGAGAAGAGCGACCCTCACATCAAGCTACAACTTCAAGCAGAAGAGAGAGGAGTTGTGTCTATCAAAGGAGTGTGTGCTAACCGTTACCTGGCTA
NM_002007.2	FGF4	688-788	GGCGTGGTGAGCATCTTCGGCGTGGCCAGCCGGTTCTTCGTGGCCATGAGCAGCAAGGGCAAGCTCTATGGCTCGCCCTTCTTCACCGATGAGTGCACGT
NM_004496.2	FOXA1	2465-2565	TGATACATTCTCAAGAGTTGCTTGACCGAAAGTTACAAGGACCCCAACCCCTTTGTCCTCTCTACCCACAGATGGCCCTGGGAATCAATTCCTCAGGAAT
NM_021784.4	FOXA2	1774-1874	CCACACAGATACCCCACGTTCTATATAAGGAGGAAAACGGGAAAGAATATAAAGTTAAAAAAAAGCCTCCGGTTTCCACTACTGTGTAGACTCCTGCTTC
NM_003468.2	FZD5	2265-2365	GAGAGAGGGAAGAGGGGCGTTTTCGAGGAAGAACCTGTCCCAGGTCTTCTCCAAGGGGCCCAGCTCACGTGTATTCTATTTTGCGTTTCTTACTGCCTTC
NM_002052.3	GATA4	2140-2240	GACTTCTTTCCCAAGATGTCCTTGTCCCCTGCGTTCCCCACTGTGGCCTAGACCGTGGGTTTTGCATTGTGTTTCTAGCACCGAGGATCTGAGAACAAGC
NM_005257.3	GATA6	2130-2230	GACAGTGGCGACTGCGCTGACAGAACGTGATTCTCGTGCCTTTATTTTGAAAGAGATGTTTTTCCCAAGAGGCTTGCTGAAAGAGTGAGAGAAGATGGAA
NM_002054.2	GCG	295-395	TGGACTCCAGGCGTGCCCAAGATTTTGTGCAGTGGTTGATGAATACCAAGAGGAACAGGAATAACATTGCCAAACGTCACGATGAATTTGAGAGACATGC
NM_000162.3	GCK	2110-2210	CCACCTTTCTCGCTGGAATCAATTTCCCAGAAGGGAGTTGCTCACTCAGGACTTTGATGCATTTCCACACTGTCAGAGCTGTTGGCCTCGCCTGGGCCCA
NM_001134944	GHRL	337-437	CCAGCCGACAAGTGATCGCCCACAAGCCTTACTCACCTCTCTCTAAGTTTAGAAGCGCTCATCTGGCTTTTCGCTTGCTTCTGCAGCAACTCCCACGACT
NM_173849.2	GSC	909-1009	TCGGACAGCTGACGGCCGCGGGACACTTGCCCGTATTACTTACCTAACTCGAAGGACTTGCACAGACAGACGATGCTACTTTCTTGCACACGCGCTGCCT
NM_004821.2	HAND1	1230-1330	TTTGGAGCGAATTTAGAACCTCAGCCCTATCTCCATTTCCCTATCTGGCTCTTTCTCTCTTGTCCCTCCATATGATCCGCCCCGACGCCGTCTTCTCTAA
NM_003865.2	HESX1	480-580	CAGCTCATCAGGGAAAGATGGTAACTTATGTCTACATGTCCCAAATCCTCCCAGTGGGATTTCATTCCCTAGCGTGGTGGATCACCCAATGCCAGAAGAA
NM_012258.3	HEY1	585-685	AATGCCTGGCAGAAGTTGCGCGTTATCTGAGCATCATTGAAGGACTAGATGCCTCTGACCCGCTTCGAGTTCGACTGGTTTCGCATCTCAACAACTACGC
NM_002729.4	HHEX	1479-1579	GCACTATCACTTAGTACCTGTTTGACCAAGGTGTTAAGGGGATAGTACCTCCCAATTCAAGCAGAGAAACTGACCTGACTAAAGTTAATCGCAGATGAAC
NM_000458.1	HNF1B	2000-2100	ACGTCCTGCTGGCACCTCAGACAATCCACTCTCAGGAGCGCAGCCCGAAGCCCAGTTTCCCTTCTATGCAGTATTGCCACAATGCCTCTCCCACGATGTC
NM_178849.1	HNF4A	2970-3070	TCTCCCTGGATCAGAACAGGAGCTCTTAACTACAGTGGCTGAATAGCTTCTCCAAAGGCTCCCTGTGTTCTCACCGTGATCAAGTTGAGGGGCTTCCGGC
NM_019102.2	HOXA5	895-995	TGAGCAGTATTAGCGGATCCCGCGTAGTGTCAGTACTAAGGTGACTTTCTGAAACTCCCTTGTGTTCCTTCTGTGAAGAAGCCCTGTTCTCGTTGCCCTA
NM_014620.4	HOXC4	1058-1158	AGCGCCGCCAGCAAGCAACCCATAGTCTACCCATGGATGAAAAAAATTCACGTTAGCACGGTGAACCCCAATTATAACGGAGGGGAACCCAAGCGCTCGA
NM_002165.2	ID1	345-445	CTGCCCCAGAACCGCAAGGTGAGCAAGGTGGAGATTCTCCAGCACGTCATCGACTACATCAGGGACCTTCAGTTGGAGCTGAACTCGGAATCCGAAGTTG
NM_002166.4	ID2	505-605	CGGATATCAGCATCCTGTCCTTGCAGGCTTCTGAATTCCCTTCTGAGTTAATGTCAAATGACAGCAAAGCACTGTGTGGCTGAATAAGCGGTGTTCATGA
NM_003897.2	IER3	1040-1140	TCAACTCCGTCTGTCTACTGTGTGAGACTTCGGCGGACCATTAGGAATGAGATCCGTGAGATCCTTCCATCTTCTTGAAGTCGCCTTTAGGGTGGCTACG
NM_000599.3	IGFBP5	3320-3420	AATGGGTTGCAAAATAGAAATGAGCTTAATCCAGGCCGCAGAGCCAGGGAAGGTGAGTAACTTTAGGAGGGTGCTAGACTTTAGAAGCCAGATAGGAAGA
NM_000584.2	IL8	25-125	ACAGCAGAGCACACAAGCTTCTAGGACAAGAGCCAGGAAGAAACCACCGGAAGGAACCATCTCACTGTGTGTAAACATGACTTCCAAGCTGGCCGTGGCT
NM_000207.2	INS	120-220	CAGCCGCAGCCTTTGTGAACCAACACCTGTGCGGCTCACACCTGGTGGAAGCTCTCTACCTAGTGTGCGGGGAACGAGGCTTCTTCTACACACCCAAGAC
NM_002196.2	INSM1	1980-2080	AAGCCTCCCCTTGGCGGGGAGAAGCTTTTTTTCTTGCTAGTATTCGCTGTGTTCATGGTCTAGAAATGCGGTCTGGTCTCGCCTCGCCTACCAATCTCTG
NM_002202.2	ISL1	1375-1475	CTTACAGGCTAACCCAGTGGAAGTACAAAGTTACCAGCCACCTTGGAAAGTACTGAGCGACTTCGCCTTGCAGAGTGACATAGATCAGCCTGCTTTTCAG
NM_002253.2	KDR	1420-1520	CAATCACACAATTAAAGCGGGGCATGTACTGACGATTATGGAAGTGAGTGAAAGAGACACAGGAAATTACACTGTCATCCTTACCAATCCCATTTCAAAG
NM_004235.4	KLF4	1980-2080	CGAGCATTTTCCAGGTCGGACCACCTCGCCTTACACATGAAGAGGCATTTTTAAATCCCAGACAGTGGATATGACCCACACTGCCAGAAGAGAATTCAGT
NM_005556.3	KRT7	1425-1525	GGGAACCATGGGCAGCAATGCCCTGAGCTTCTCCAGCAGTGCGGGTCCTGGGCTCCTGAAGGCTTATTCCATCCGGACCGCATCCGCCAGTCGCAGGAGT
NM_201589.2	MAFA	28-128	AGCTGCCCAGCAGCCCGCTGGCCATCGAGTACGTCAACGACTTCGACCTGATGAAGTTCGAGGTGAAGAAGGAGCCTCCCGAGGCCGAGCGCTTCTGCCA
NM_005461.3	MAFB	1655-1755	GGCGGCGAGGCATAGTCCCGAGAAGTCACCAAGGCCATCTGGAGACTCCTGGCTTTCTGAACTTTGCGCGTTAAGCCGGGACAGCTGCTTTGCTGCCCGG
NM_00104000	MEOX1	1710-1810	TCCTCCATGGGATGGATTTAAGCTCTTGCTGTGTGTTCTACAAATGCTGTTATTGTGGGAGGAAATGCTAGGTTTTTGTGTGTGGACTGCCCAGACCTCA
NM_000902.2	MME	5059-5159	GGATTGTAGGTGCAAGCTGTCCAGAGAAAAGAGTCCTTGTTCCAGCCCTATTCTGCCACTCCTGACAGGGTGACCTTGGGTATTTGCAATATTCCTTTGG
NM_004535.2	MYT1	3240-3340	GAGTTTCCTCACCCACCGGAGTTTGTCAGGCTGTCCCAGAGCAACCTTTGCTGGAAAGAAGGGAAAACTGTCAGGGGATGAGGTCCTCAGTCCAAAGTTC
NM_024865.2	NANOG	1100-1200	CTACTCCATGAACATGCAACCTGAAGACGTGTGAAGATGAGTGAAACTGATATTACTCAATTTCAGTCTGGACACTGGCTGAATCCTTCCTCTCCCCTCC
NM_002500.2	NEUROD1	1275-1375	GTGCCCAGCTCAATGCCATATTTCATGATTAGAGGCACGCCAGTTTCACCATTTCCGGGAAACGAACCCACTGTGCTTACAGTGACTGTCGTGTTTACAA
NM_020999.2	NEUROG3	1025-1125	CGCGCGAAGTGGGCATTGCAAAGTGCGCTCATTTTAGGCCTCCTCTCTGCCACCACCCCATAATCTCATTCAAAGAATACTAGAATGGTAGCACTACCCG
NM_002509.2	NKX2-2	85-185	CAGGCCGCCCGGCTTCCTGCGTCCATTTCCGCGTGCTTTCAAAGAAGACAGAGAGAGGCACTGGGTTGGGCTTCATTTTTTTCCTCCCCATCCCCAGTTT
NM_004387.2	NKX2-5	1046-1146	AACTTCGTGAACTTCGGCGTCGGGGACTTGAATGCGGTTCAGAGCCCCGGGATTCCGCAGAGCAACTCGGGAGTGTCCACGCTGCATGGTATCCGAGCCT
NM_006168.2	NKX6-1	660-760	CTGGCCTGTACCCCTCATCAAGGATCCATTTTGTTGGACAAAGACGGGAAGAGAAAACACACGAGACCCACTTTTTCCGGACAGCAGATCTTCGCCCTGG
NM_018055.3	NODAL	320-420	CTCACTTGCCATTGAGATTTTCCACCAGCCAAAGCCCGACACAGAGCAGGCTTCAGACAGCTGCTTAGAGCGGTTTCAGATGGACCTATTCACTGTCACT
NM_000905.2	NPY	270-370	AGAGATATGGAAAACGATCCAGCCCAGAGACACTGATTTCAGACCTCTTGATGAGAGAAAGCACAGAAAATGTTCCCAGAACTCGGCTTGAAGACCCTGC
NM_004498.1	ONECUT1	1755-1855	AACACCACTGGATCTCACACCTTCAATCCATGACCATCCTCGCTGTGCTTGGCTGTTTAGTGGTTTGGAGCATAGTGATTTTGAGCCATTGAGCGGACAT
NM_004852.2	ONECUT2	13270-13370	GACATGAGTGTACCCAGCCCTTTTTTTAATCACAGGCAATGCATGGGTCTGGCTGGTTACACTTTGCCAAGAAGACTTGTCTTATGAAACCCAAGGTATA
NM_014562.2	OTX1	1240-1340	GAAACTCAACTTCAACTCCCCCGACTGTCTGGACTATAAGGACCAAGCCTCATGGCGGTTCCAGGTCTTGTGAGCCCAGGAATGAAAGAGGAGAAGAAAC
NM_021728.2	OTX2	0-100	GAGAGCGGGACCGGCCTCAGCTCCAACACAGCCTCCACTGTGATTAAAAATAAAAATTGCTAGAGCAGCCCTCACTCGCCACATCTACTTTGATAGCTGG
NM_006193.2	PAX4	1283-1383	CTGCCCATTGTCCTTACCGTCCTGCCCATACAGACTGTGGCTCCTTCCTCCTTCCTGTGATTGCTCCCTCCTGTGTGGACGTTGCCTGGCCCTGCCTCGA
NM_001604.4	PAX6	315-415	CTTTAACTAGGGGCGCGCAGATGTGTGAGGCCTTTTATTGTGAGAGTGGACAGACATCCGAGATTTCAGAGCCCCATATTCGAGCCCCGTGGAATCCCGC
NM_000439.3	PCSK1	2273-2373	AGTGCAAAGCTCAACATCCCTTATGAAAACTTCTACGAAGCCCTGGAAAAGCTGAACAAACCTTCCCAGCTTAAAGACTCTGAAGACAGTCTGTATAATG
NM_006206.3	PDGFRA	1925-2025	TAGTGCTTGGTCGGGTCTTGGGGTCTGGAGCGTTTGGGAAGGTGGTTGAAGGAACAGCCTATGGATTAAGCCGGTCCCAACCTGTCATGAAAGTTGCAGT
NM_000209.3	PDX1	1860-1960	TGACCTCTTTCTTTCTCCTCCTCCTCCTTCTACCTCCCCTTCTCATCCCTCCTCTTCCTCTTCTCTAGCTGCACACTTCACTACTGCACATCTTATAACT
NM_000937.2	POLR2A	3775-3875	TTCCAAGAAGCCAAAGACTCCTTCGCTTACTGTCTTCCTGTTGGGCCAGTCCGCTCGAGATGCTGAGAGAGCCAAGGATATTCTGTGCCGTCTGGAGCAT
NM_002701.4	POU5F1	1225-1325	AAGTTCTTCATTCACTAAGGAAGGAATTGGGAACACAAAGGGTGGGGGCAGGGGAGTTTGGGGCAACTGGTTGGAGGGAAGGTGAAGTTCAATGATGCTC
NM_004792.2	PPIG	1250-1350	CTTCCAGATCCAGATCAAGGGATCGTTTCAGACGTAGTGAGACTCCTCCACATTGGAGGCAAGAGATGCAGAGAGCTCAAAGAATGAGGGTATCAAGTGG
NM_002763.3	PROX1	1920-2020	TCTCCTTGTCGCTCATAAAGTCCGAGTGCGGCGATCTTCAAGATATGTCTGAAATATCACCTTATTCGGGAAGTGCAATGCAGGAAGGATTGTCACCCAA
NM_153362.1	PRSS35	195-295	CACCCCTGGGTGGACCCTCATTGATGGATCTGAAATGGAATGGGATTTTATGTGGCACTTGAGAAAGGTACCCCGGATTGTCAGTGAAAGGACTTTCCAT
NM_178161.2	PTF1A	926-1026	CAACAGCAAATCTTCCTTCAACAACATAGAAAACGAACCACCATTTGAGTTTGTGTCCTGAGAAGTCCCAGACTCGGCTGAAGATCTGATTATGTCTCTG
NM_173560.2	RFX6	660-760	AGCAGTGCATATTACCACTCCGTTTATTCTGGAAAGGGCTTGACAAGGTTTTCTGGAAGCAAGCTAAAGAATGAGGGTGGCTTCACTCGTAAATATTCGC
NM_003015.2	SFRP5	1295-1395	CAGGGGTCCTGGGGTCCCTGGGATGTTGGGCTTCCTCTCTCAGGAGCAGGGCTTCTTCATCTGGGTGAAGACCTCAGGGTCTCAGAAAGTAGGCAGGGGA
NM_006516.2	SLC2A1	2500-2600	AGGCTCCATTAGGATTTGCCCCTTCCCATCTCTTCCTACCCAACCACTCAAATTAATCTTTCTTTACCTGAGACCAGTTGGGAGCACTGGAGTGCAGGGA
NM_001098484	SLC4A4	666-766	GAGATGATTGTTGACCATCAGATTGAGACAGGCCTATTGAAACCTGAACTTAAGGATAAGGTGACCTATACTTTGCTCCGGAAGCACCGGCATCAAACCA
NM_022454.3	SOX17	1374-1474	AGCCACGGGGCCATTTCCTCGGTGGTGTCCGACGCCAGCTCCGCGGTATATTACTGCAACTATCCTGACGTGTGACAGGTCCCTGATCCGCCCCAGCCTG
NM_003106.2	SOX2	151-251	CTTAAGCCTTTCCAAAAAATAATAATAACAATCATCGGCGGCGGCAGGATCGGCCAGAGGAGGAGGGAAGCGCTTTTTTTGATCCTGATTCCAGTTTGCC
NM_000346.2	SOX9	2135-2235	CAGTGGCCAGGCCAACCTTGGCTAAATGGAGCAGCGAAATCAACGAGAAACTGGACTTTTTAAACCCTCTTCAGAGCAAGCGTGGAGGATGATGGAGAAT
NM_000582.2	SPP1	760-860	CGCCTTCTGATTGGGACAGCCGTGGGAAGGACAGTTATGAAACGAGTCAGCTGGATGACCAGAGTGCTGAAACCCACAGCCACAAGCAGTCCAGATTATA
NM_001048.3	SST	285-385	AGCTGCTGTCTGAACCCAACCAGACGGAGAATGATGCCCTGGAACCTGAAGATCTGTCCCAGGCTGCTGAGCAGGATGAAATGAGGCTTGAGCTGCAGAG
NM_004099.4	STOM	420-520	TGACAATTAGCGTGGATGGTGTGGTCTATTACCGCGTTCAGAATGCAACCCTGGCTGTGGCAAATATCACCAACGCTGACTCAGCAACCCGTCTTTTGGC
NM_003179.2	SYP	2265-2365	TAGTGCCTGTGATCGTGTGTTGCCATTTTGTCTGGCTGTGGCCCCTCCTTCTCCCCTCCAGACCCCTACCCTTTCCCAAACCCTTCGGTATTGTTCAAAG
NM_003181.2	T	1836-1936	TTTTTGTCGTGGCAGCCAGTGGTGACTGGATTGACCTACTAGGTACCCAGTGGCAGTCTCAGGTTAAGAAGGAAATGCAGCCTCAGTAACTTCCTTTTCA
NM_003194.3	TBP	25-125	CGCCGGCTGTTTAACTTCGCTTCCGCTGGCCCATAGTGATCTTTGCAGTGACCCAGCAGCATCACTGTTTCTTGGCGTGTGAAGATAACCCAAGGAATTG
NM_003212.2	TDGF1	1567-1667	AAGGAAAGAAAACATCTTTAAGGGGAGGAACCAGAGTGCTGAAGGAATGGAAGTCCATCTGCGTGTGTGCAGGGAGACTGGGTAGGAAAGAGGAAGCAAA
NM_003220.2	TFAP2A	1155-1255	AGGGACTTTGGGTACGTGTGCGAAACCGAATTTCCTGCCAAAGCAGTAGCTGAATTTCTCAACCGACAACATTCCGATCCCAATGAGCAAGTGACAAGAA
NM_003577.2	UTF1	328-428	AGCAGGTGCGCCGCACCCCCGCGCAGTGCCGCCGCCGCTACAAGTTCCTTAAAGACAAGTTCGCGAGGCGCACGGCCAGCCGCCCGGGCCCTTCGACGA
NM_033131.2	WNT3A	1810-1910	TCTCTCCGCGGGTGGGACTCTTCCCTGGGAACCGCCCTCCTGATTAAGGCGTGGCTTCTGCAGGAATCCCGGCTCCAGAGCAGGAAATTCAGCCCACCAG

Table S2: List of antibodies used in this study with references and dilution. Related to Experimental procedures.

Polyclonal rabbit anti-MAFA (ab26405)	Abcam	1/1000
Rat Mouse IgG1 anti-NKX6-1 (F55A12)	Dev Studies Hyb Bank	1/500
Polyclonal Guinea Pig anti-INSULIN (A0564)	DAKO	1/100
Polyclonal Goat anti-PPY (ABIN769045)	Antibodies-online	1/200
Polyclonal Rabbit anti-SOMATOSTATIN (A0566)	DAKO	1/500
Polyclonal Rabbit IgG anti-human C-PEPTIDE (AB14181)	Abcam	1/1000
Goat anti-GHRELIN (SC-10368)	Santa Cruz	1/50
Polyclonal Rabbit anti-GLUCAGON (SAB4501137)	Sigma	1/100
Monoclonal Mouse Anti-VIMENTIN (ab8978)	Abcam	1/500
Monoclonal Mouse Anti-CYTOKERATIN 19 (M08888)	DAKO	1/200
Donkey anti-rabbit568 (A10042)	Life Technologies	1/1000
Donkey anti-rabbit488 (A21206)	Life Technologies	1/1000
Donkey anti-guinea pig647 (AP193SA6)	Millipore	1/1000
Donkey anti-goat488 (A11055)	Life Technologies	1/1000
Goat anti-guinea pig568 (A11075)	Life Technologies	1/1000
Goat anti-mouse488 (A11001)	Life Technologies	1/1000
Donkey anti-mouse568 (A10037)	Life Technologies	1/1000
Donkey anti-guinea pig Fluorescein (706-545-148)	Jackson	1/800

Table S3: Differentiation media for PE differentiation. Related to Experimental procedures and Supplemental Experimental procedures.

Stage	Days	Base Media	Growth Factors	
			Small scale	**Large scale**
Stage1	**Day0**	RPMI (Thermofisher, 31870-025), 0.2% FBS (Thermofisher 10270-106), 1x GlutaMAX (Thermofisher, 35050038), 1% v/v pen/strep (PS), 1:5000 Insulin-Transferrin-Selenium (ITS) (Thermofisher, 41400-04550)	**100** ng/mL recombinant mouse Wnt3A (Bio-Techne 1324-WN), 100ng/ml Activin and 10 uM Y-27632	**50** ng/mL recombinant mouse Wnt3A (Bio-Techne 1324-WN), 100ng/ml Activin and 10 uM Y-27632
	Day1	RPMI, 0.2% FBS, 1x GlutaMAX, 1% v/v PS, 1:5000 ITS	Activin A 100ng/ml, and **10 uM Y-27632**	Activin A 100ng/ml
Stage2	**Day2**	RPMI, 0.2% FBS, 1x GlutaMAX, 1% v/v PS, 1:1000 ITS	25 ng/mL recombinant human KGF (Bio-Techne, 251-KG) and 2.5 uM TGF-β RI Kinase inhibitor IV (EMD Bioscience, 616454), and **10 uM Y-27632**	25 ng/mL recombinant human KGF (Bio-Techne, 251-KG) and 2.5 uM TGF-β RI Kinase inhibitor IV (EMD Bioscience, 616454)
	Day3	RPMI, 0.2% FBS, 1x GlutaMAX, 1% v/v PS, 1:1000 ITS	25 ng/mL recombinant human KGF and 10 uM Y-27632	25 ng/mL recombinant human KGF and 10 uM Y-27632
	Day4	RPMI, 0.2% FBS, 1x GlutaMAX, 1% v/v PS, 1:1000 ITS	25 ng/mL recombinant human KGF	25 ng/mL recombinant human KGF
Stage3	**Day5-Day7**	DMEM high glucose GlutaMAX (Thermofisher, 61965026), 1% v/v PS, 0.5x B27 (Thermofisher, 17504-044)	50ng/ml Noggin (Bio-Techne, 3344-NG), 30ng/ml Heregulin (Peprotech, PEPR100-03), 0.25 uM KAAD-Cyclopamine (Toronto Research Chemicals, K171000) and 0.3 nM TTNBP (Sigma, T3757)	50ng/ml Noggin (Bio-Techne, 3344-NG), 30ng/ml Heregulin (Peprotech, PEPR100-03), 0.25 uM KAAD-Cyclopamine (Toronto Research Chemicals, K171000) and 0.3 nM TTNBP (Sigma, T3757)
Stage4	**Day8-Day12**	DMEM high glucose Glutamax (Thermofisher, 61965026), 1% v/v penicillin/streptomycin, 0.5x B27	50ng/ml Noggin, 30ng/ml Heregulin, 50ng/mL EGF (Bio-Techne, 236-EG) 50ng/mL KGF and **10 uM Y-27632**	50ng/ml Noggin, 30ng/ml Heregulin, 50ng/mL EGF (Bio-Techne, 236-EG) 50ng/mL KGF

Supplemental Experimental Procedures. Related to Experimental procedures

Cell culture and Differentiation:

During expansion, hiPSC were maintained in DMEM/F12/Glutamax medium (Thermofisher, 313331028) supplemented with 20% KnockOut serum replacement (Thermofisher, 10828-028), 1mM nonessential amino acids (Thermofisher 11140-035), penicillin/streptomycin, 10ng/mL recombinant human FGF2 (Bio Techne 233 FB) and 10ng/mL Activin A (Bio Techne, 338-AC). Cells were passaged by dissociation with Accutase (Thermofisher; A1110501) and seeded at 40,000 cells/cm2 for a 4 days passage or 60,000 cells/cm2 for a 3 days passage. On the day of plating, the medium was supplemented with 10 uM Y27632 (Abcam, 120129). A standardized plating volume of 0.2mL/cm2 was used for different tissue culture T flasks and cell factories (2, 5 and 10 chamber CellSTACK). Medium was replaced daily and the volume of media used was increased for each additional day of feeding. Feeding volumes were adapted to cells confluence: 0.27 ml/cm2 for the second day, 0.35 ml/cm2 for the third day and 0.43 ml/cm2 for the fourth day.

For egging, hiPSC were aggregated to form spherical clusters at a concentration of $1x10^6$ cells/ml in hiPSC media supplemented with 10mM Y27632. For the small-scale setup, $5.5*10^6$ cells were seeded per well of ultra low adherent 6 well plates (Corning, 734-1482) and were incubated on orbital rotators set at 95 rpm (Biolabo). For the large scale, $500x10^6$ cells were seeded per 2L Roller Bottles (Corning, 25382-462) and were incubated on FlexiRoll Digigal Cell Roller (Argos, H5300) set at 31 rpm.

Differentiation media for PE were supplemented as described in Table S3.

HiPEC Freezing protocol: The program used on the Controlled-Rate Freezer (Planer plc Kryo 560-16) was as follows: Start temp 0°C, , -0.2°C/minutes to -0.9°C, hold 10 minutes, Manual seed, hold 10 minutes, -0.2°C/minutes to -40°C, -25°C /minutes to -150°C. Cryovials were then transferred to liquid N2. Large Scaled HiPEC runs were typically giving 80-100 cryovials.

Encapsulation and Implantation: Hydrophilized PTFE membranes with 0.4µm nominal pore size (Millipore) were used as porous material. The loading port was cut and the device sealed using ultraviolet curing adhesive (Loctite 3310). The resultant loaded devices were placed in S4 medium and incubated at 37°C and 8% CO2 until implantation, typically by the next day.

Gene expression analyses: Total RNA was isolated from aggregates or from human islets using the Agencourt RNAdvance Tissue Lysis kit (Beckman Coulter, A332646) and RNA was quantified using Quant-iT RiboGreen RNA Assay Kit (ThermoFisher, R11490). RNA integrity was verified on the AATi Fragment Analyzer using the Standard Sensitivity RNA Analysis Kit (Advanced Analytical Technologies, DNF-473). Total RNA from explanted tissue was extracted using DirectZol (Zymoresearch, R2071) according to the manufacturer's instructions. The NanoString nCounter gene expression assay was performed using 100 ng RNA per reaction and the Combo_6980 Code set according to the manufacturer's instructions (Nanostring; Seattle, WA). The code set included 109 human genes and detail of the sequences are provided in Table S1. The raw count data were normalized to the count data from internal control sequences (''spikes'') [1], followed by normalization with four different housekeeping genes (*ACTB*, *POLR2A*, *PPIG*, and *TBP*) applying geometric means of the spike-normalized counts using the nSolver software according to manufacturer's instructions (Nanostring). The average and standard deviations of the fully normalized counts were calculated for 2 biological replicates.

Ex vivo tissue immunohistochemistry: Briefly, section were deparaffinized in Toluol for 10min and rehydrated in water. Antigen retrieval was performed in 10mM citrate buffer pH 6.0 for 20min at 95 °C. After 10 min wash in PBS, endogenous peroxidases were quenched with H2O2 3% for 10 min at room temperature and blocking buffer (3% BSA , 5% rabbit serum in PBS) was applied for 1h at RT. Anti-human insulin diluted 1/100 was incubated in blocking buffer overnight at 4 °C. Biotinylated anti-guinea-pig IgG (Vector Laboratories) was applied 1/200 for 1 h at RT for DAB revelation. Counterstain was performed with hematoxylin.

Human islets preparation: Culture medium: RPMI 1640 medium supplemented with 5.55 mM glucose, 10% (v/v) FCS (Thermofisher 10270-106), 10 mM HEPES pH 7.3, 1 mM sodium pyruvate, 50 µM β-mercaptoethanol, 1% v/v penicillin/streptomycin.

Immunofluorescence and High content image quantification: For cryosectioning, D12 aggregates or explants were rinsed with PBS followed by overnight fixation in 4% PFA at 4 °C. PFA was then washed with PBS and samples were incubated overnight at 4 °C in 15% sucrose solution. The samples were next overlaid with 7.5% gelatine solution, flash frozen using Isopentane at -70°C and stored at –80 °C. Gelatin blocks were sectioned at -28°C in 4µM thick sections using a microtome. For immunofluorescence, slides with sections were blocked for 1h in 20% donkey serum and then incubated with primary antibodies mix overnight at 4°C. The next day, slides were washed in PBS Triton and incubated for 1-2h with secondary antibodies mix at room temperature. After a DAPI counterstained the sections were mounted using Aqua/Polymount (Polyscience) or 90% glycerol and stored at 4°C until analyzed. For high content image quantification, the threshold of positive staining was corrected manually for each image. Once all pictures were thresholded, the objects were segmented with an iterative process involving morphological operators, watershed separation of touching objects and filtering. Objects between 20 and 150 micrometer square were considered as nuclei and stored whereas object bigger than 150 micrometer square were fed into the next iteration where the erosion filter size was increase by two pixels. After nuclei segmentation, positive objects in other fluorescent channels were filtered to remove artifacts below 20 micrometer square and co-localized with nuclei objects. Nuclei were considered positive for a specific channel if, at least, one pixel co-localized. This co-localization method, whereas not perfect for cytoplasmic dyes, was sensitive enough to include statistically sufficient number of positive cells. If a nucleus positive for one marker was also positive for another it was then considered a double positive and similarly for the triple positive ones.

Calcium signaling analyses: KRBH contains (in mM): 140 NaCl, 3.6 KCl, 0.5 NaH2PO4, 0.5 MgSO4, 1.5 CaCl2, 10 Hepes, 5 NaHCO3, pH 7.4, and 1 mM glucose. Image acquisition: Cells were excited at 430 nm through a BP436/20 filter. The two emission images were acquired with BP480/40 and BP535/30 emission filters. Fluorescence ratios were calculated in MetaFluor 7.0 (Meta Imaging Series) and analyzed in Excel (Microsoft) and GraphPad Prism 5 (GraphPad).

FACS antibodies: PDX1-Alexa fluor 488 1/40 (BD Biosciences, 562274), PAX6-PerCP-Cy5.5 1/50 (BD Biosciences, 562388), CHGA-PC7 (polyclonal) 1/20 (Abcam, ab8204), NKX6-1- Alexa fluor 647 1/161 (BD Biosciences, 563338), CDX2: L-L APC-Cy5.5 1/50 (Abcam, ab157524), AFP: L-L PE-TxRed 1/50 (Abcam, ab8202).

Taqman probes: *POU5F1* (OCT4) (ABI_Hs04260367_gH), *CDX2* (ABI_Hs01078080_m1), *AFP* (ABI_Hs00173490_m1), *PTF1A* (ABI_Hs00603586_g1), *GAPDH* (ABI_Hs02758991_g1) and *MAFA* (Roche, UPL probe 39 # 04687973001, UPL MAFA F: agcgagaagtgccaactcc, UPL MAFA R:ttgtacaggtcccgctcttt)

Supplemental References.

1. Geiss, G.K., et al., *Direct multiplexed measurement of gene expression with color-coded probe pairs.* Nat Biotechnol, 2008. **26**(3): p. 317-25.