

Supplemental Information

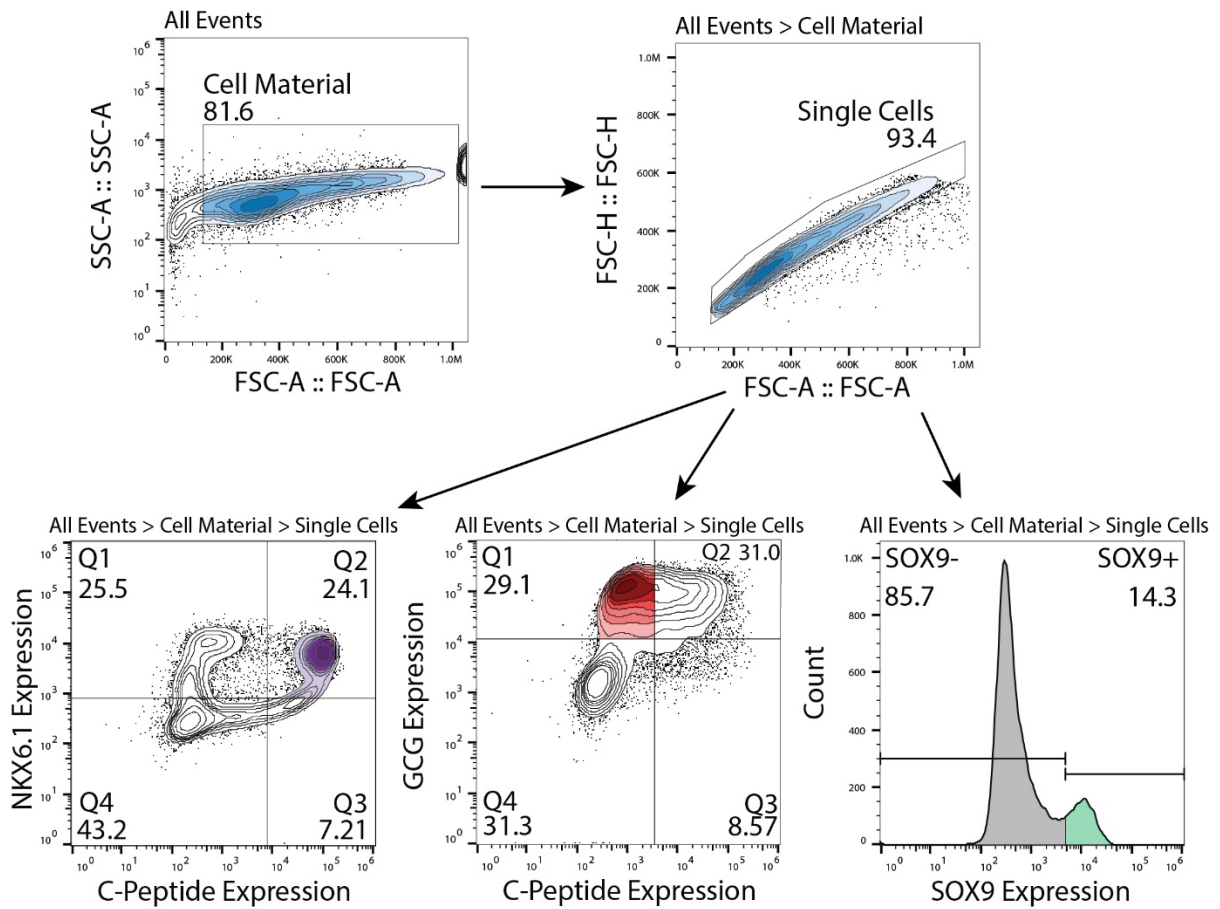


Figure S1: Flow cytometry gating strategy.

Flow cytometry data are first gated along forward and side scatter to collect cellular material. Forward scatter height is then used to eliminate doublets before protein expression is characterized using fluorescent height.

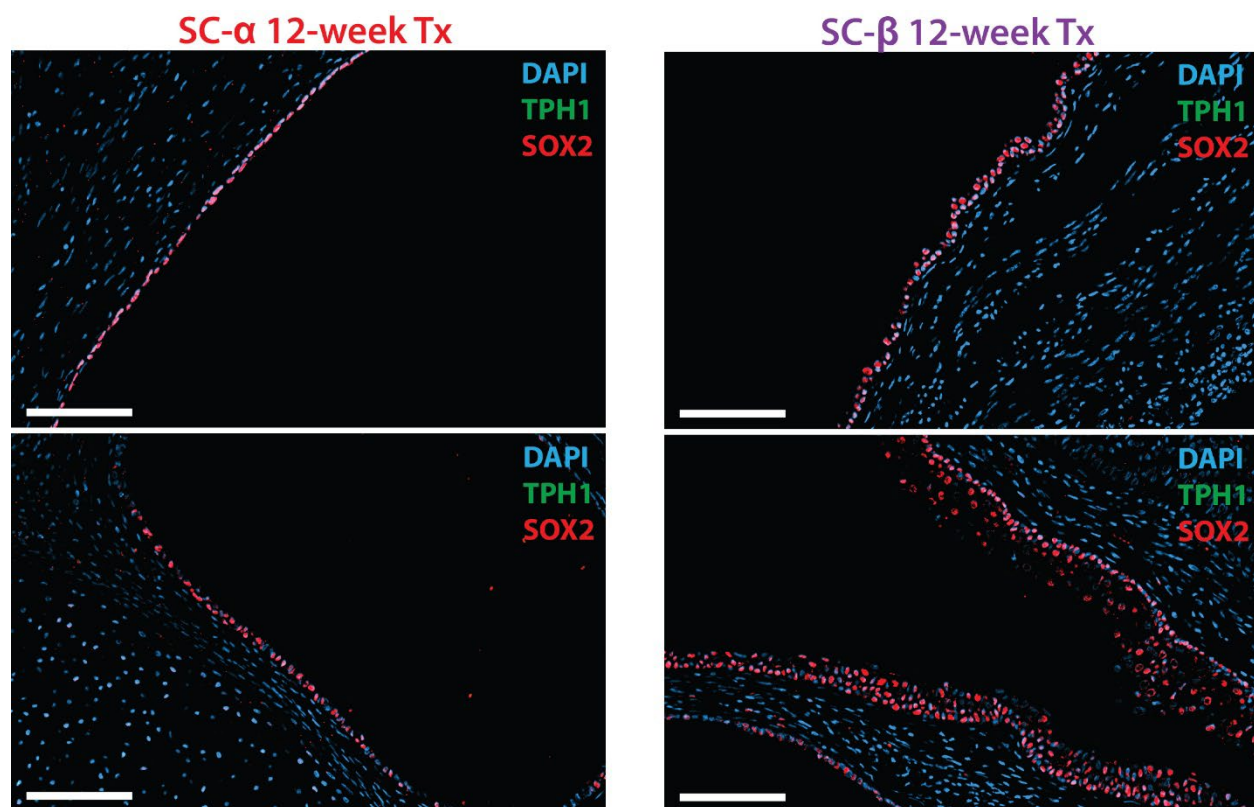


Figure S2: Enterochromaffin cells do not contribute to cystic outgrowths.

Immunofluorescent staining of TPH1 and SOX2 in outgrowths from SC-α (left) or SC-β (right) cell transplants demonstrates a lack of TPH1+ enterochromaffin cells in the outgrowth tissue. Immunofluorescent images are from unique mice transplanted with SC-α or SC-β cells. Scale bars represent 100 μm .

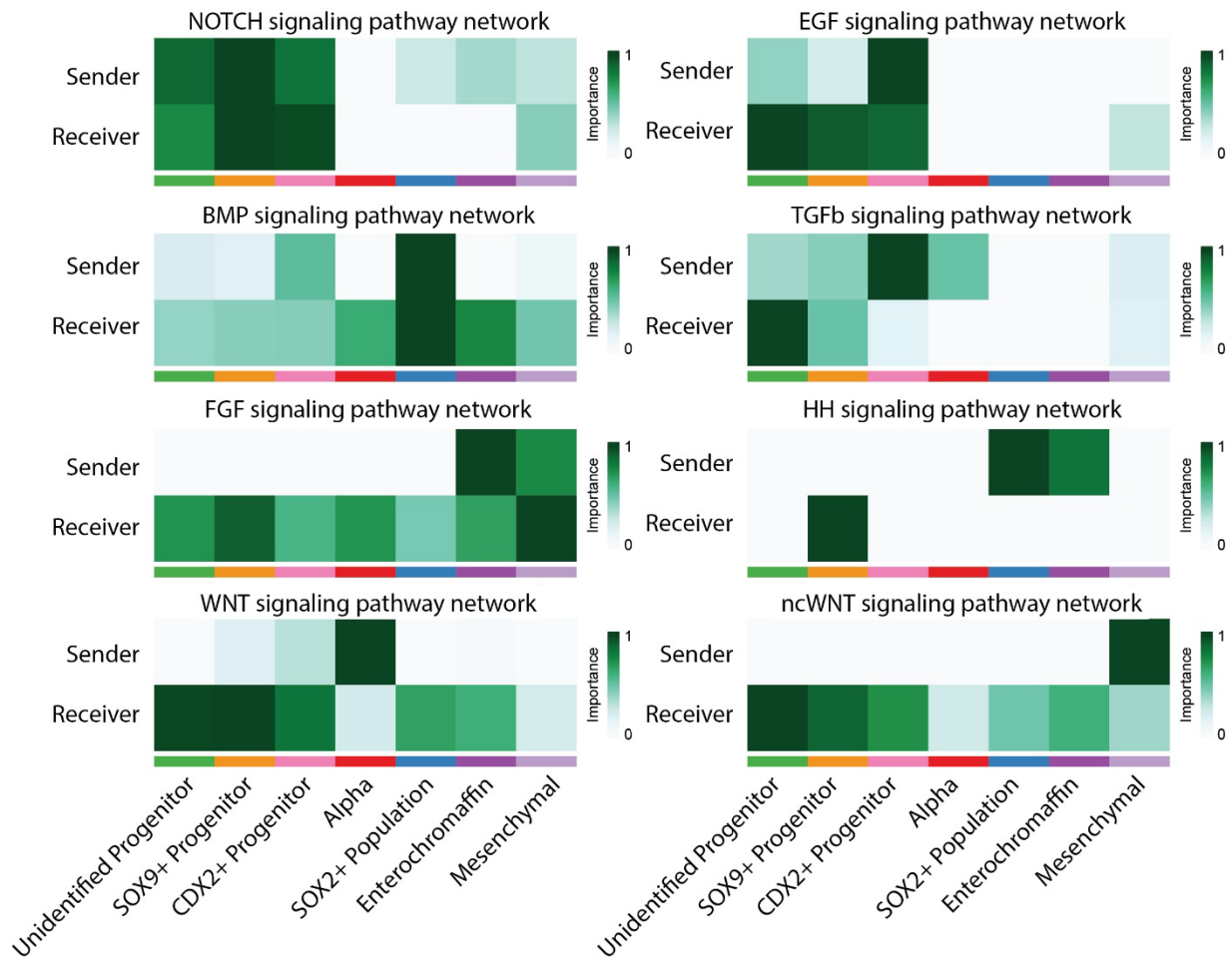


Figure S3: CellChat signaling analysis of SC- α cells.

Heatmaps of predicted sender and receiver populations for key developmental signaling pathways reveals cell-type specific signaling patterns in SC- α cells.

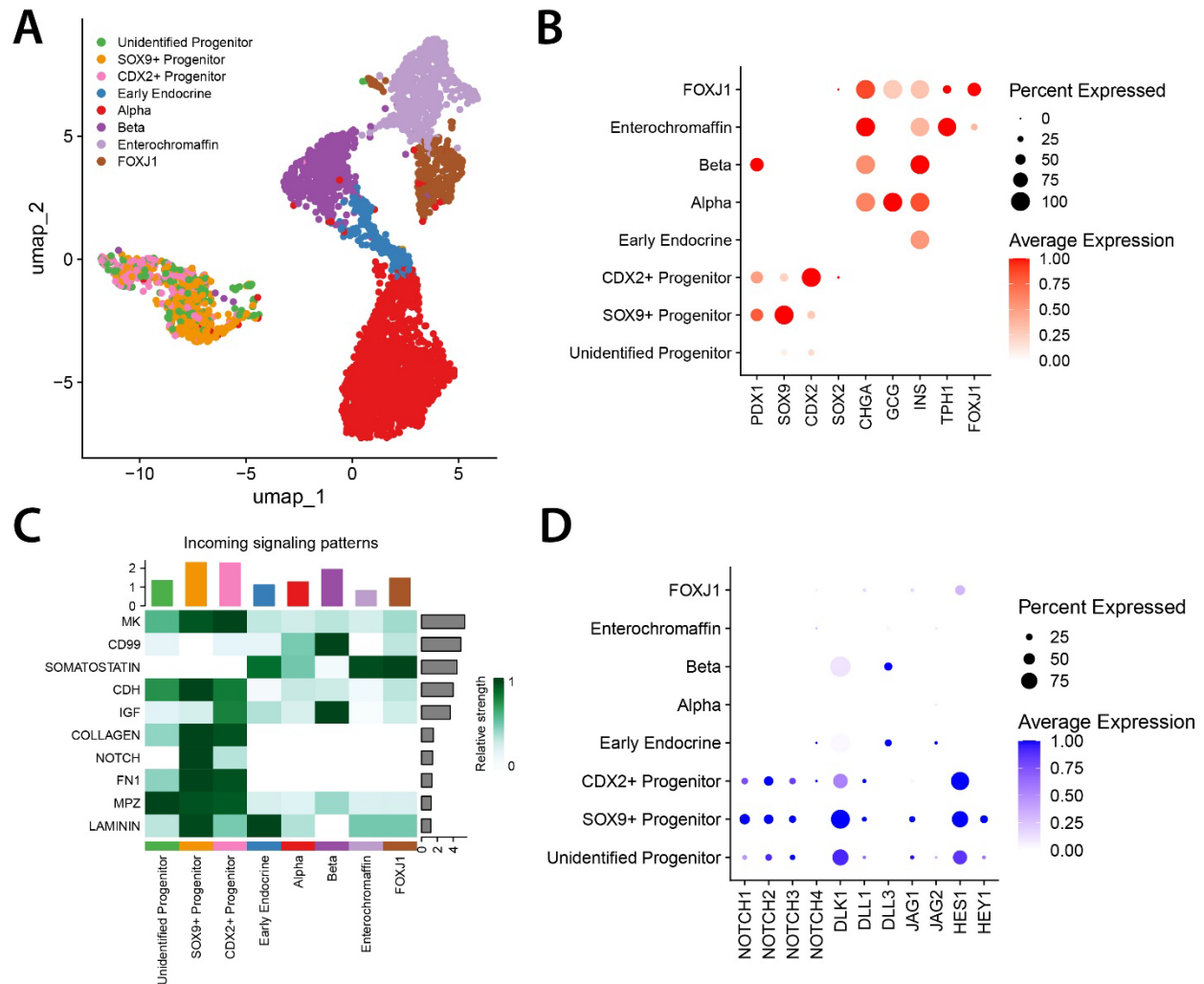


Figure S4: Single cell RNA-sequencing of SC- β cells from Veres et. al.

A) UMAP clustering reveals 8 distinct populations including SOX9+ and CDX2+ progenitor-like populations. B) Transcriptomic markers of cell populations found in SC- β differentiations. C) CellChat analysis of incoming signaling patterns reveals upregulation of cell adhesion patterns in SOX9+ and CDX2+ cell populations from SC- β differentiations. D) SOX9+ and CDX2+ cells are enriched in Notch ligands, receptors, and effectors.

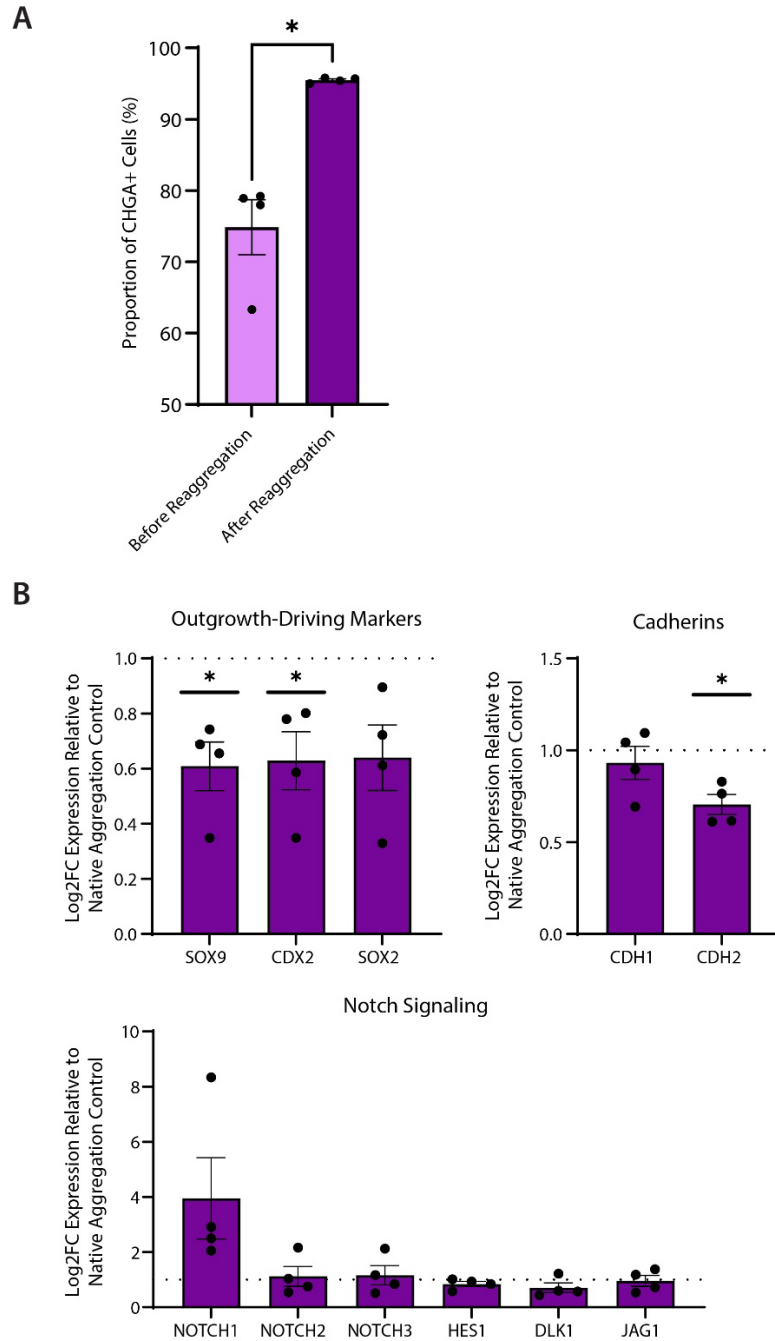


Figure S5: Reduction of off-target cells following dispersion and reaggregation.

Dispersion and reaggregation enriches for endocrine cell types and causes a reduction in outgrowth-driving cell populations. A) Flow cytometry quantification of CHGA+ endocrine cells before and after reaggregation of SC- β cells. Reaggregation enriches endocrine cells from 74.85% to 95.47%. B) Quantitative PCR for outgrowth markers, cadherins, and Notch signaling members before and after reaggregation. A decreased expression of the outgrowth-drivers and N-Cadherin (CDH2) is observed following reaggregation. Data depicted as mean $\pm$ S.E.M. Statistical significance was measured using a paired student's t-test against the control value of 1. Asterisks indicate p-values < 0.05 (*).

Table S1: Media Compositions for SC- α and SC- β Cell Differentiation

Media Component	S1 Media	S2 Media	S3 Media	BE5 Media
MCDB131 Basal Media (Corning #15-100-CV)	1 L	1 L	1 L	1 L
D-Glucose (Sigma #G7021)	0.44 g	0.44 g	0.44 g	3.6 g
Sodium Bicarbonate (Sigma #S5761)	2.46 g	1.23 g	1.23 g	1.754 g
Vitamin C (Sigma #A4544-25G)	0.044 g	0.044 g	0.044 g	0.044 g
Fatty Acid Free BSA (Proliant Biologicals #68700)	20 g	20 g	20 g	20 g
Glutagro (Corning #25-015-CI)	10 mL	10 mL	10 mL	10 mL
Penicillin-Streptomycin (Corning #30-001-CI)	10 mL	10 mL	10 mL	10 mL
ITS-X (Life Technologies #51500-056)	20 μ L	20 μ L	5 mL	5 mL
Heparin Sodium Salt (Sigma #H3149-500KU)	0 mL	0 mL	0 mL	2 KU

Table S2: Differentiation Factors for SC- α Cell Differentiation

Stage	Day	Media Composition (300 mL)	Factors	Factor Concentration	Factor Supplier
1	0	S1	Activin A CHIR99021	100 ng/mL 3 μ M	R&D #0695 R&D #4423
1	1	S1	Activin A	100 ng/mL	R&D #338-AC-01M
1	2	N/A	N/A	N/A	
2	0	S2	KGF (FGF7)	50 ng/mL	Peptrotech #100-19-1MG
2	1	N/A	N/A	N/A	
3	0	S3	Retinoic Acid	2 μ M	R&D #0695
3	1	S3	Retinoic Acid LDN 193189	2 μ M 200 nM	R&D #0695 R&D #6053
4	0	S3	LDN 193189	200 nM	R&D #6053
4	1	S3	N/A	N/A	
4	2	N/A	N/A	N/A	
4	3	S3	N/A	N/A	
4	4	N/A	N/A	N/A	
5	0	S3	RepSox (Alk5ii)	10 μ M	R&D #3742
5	1	N/A	N/A	N/A	
5	2	S3	RepSox (Alk5ii)	10 μ M	R&D #3742
5	3	N/A	N/A	N/A	
5	4	S3	RepSox (Alk5ii)	10 μ M	R&D #3742
5	5	N/A	N/A	N/A	
5	6	S3	RepSox (Alk5ii)	10 μ M	R&D #3742
6	0	S3	PDBu	500 nM	R&D #4153
6	1	N/A	N/A	N/A	
6	Even	S3	PDBu	500 nM	R&D #4153
6	Odd	N/A	N/A	N/A	
...	...				
6	28	S3	PDBu	500 nM	R&D #4153

Table S3: Differentiation Factors for SC-β Cell Differentiation

Stage	Day	Media Composition (300 mL)	Factors	Factor Concentration	Factor Supplier
1	0	S1	Activin A CHIR99021	100 ng/mL 3 μM	R&D #0695 R&D #4423
1	1	S1	Activin A	100 ng/mL	R&D #338-AC-01M
1	2	N/A	N/A	N/A	
2	0	S2	KGF (FGF7)	50 ng/mL	Peprotech #100-19-1MG
2	1	N/A	N/A	N/A	
3	0	S3	Y-27632 KGF SANT-1 PDBu Retinoic Acid LDN	10 μM 50 ng/mL 250 nM 500 nM 2 μM 200 nM	R&D #1254 Peprotech #100-19-1MG R&D #1974 R&D #4153 R&D #0695 R&D #6053
3	1	S3	Y-27632 KGF SANT-1 PDBu Retinoic Acid	10 μM 50 ng/mL 250 nM 500 nM 2 μM	R&D #1254 Peprotech #100-19-1MG R&D #1974 R&D #4153 R&D #0695
4	0	S3	Y-27632 KGF SANT-1 Activin A Retinoic Acid	10 μM 50 ng/mL 250 nM 5 ng/mL 0.1 μM	R&D #1254 Peprotech #100-19-1MG R&D #1974 R&D #0695 R&D #0695
4	1	N/A			
4	2	S3	Y-27632 KGF SANT-1 Activin A Retinoic Acid	10 μM 50 ng/mL 250 nM 5 ng/mL 0.1 μM	R&D #1254 Peprotech #100-19-1MG R&D #1974 R&D #0695 R&D #0695
4	3	N/A			
4	4	S3	Y-27632 KGF SANT-1 Activin A Retinoic Acid	10 μM 50 ng/mL 250 nM 5 ng/mL 0.1 μM	R&D #1254 Peprotech #100-19-1MG R&D #1974 R&D #0695 R&D #0695
5	0	BE5	SANT-1 Beta-Cellulin RepSox (Alk5ii)	250 nM 20 ng/mL 10 μM	R&D #1974 R&D #261-CE R&D #3742

			XXi T3 Retinoic Acid	1 μ M 1 μ M 0.1 μ M	R&D #6476 R&D #5552 R&D #0695
5	1	N/A			
5	2	BE5	SANT-1 Beta-Cellulin RepSox (Alk5ii) XXi T3 Retinoic Acid	250 nM 20 ng/mL 10 μ M 1 μ M 1 μ M 0.1 μ M	R&D #1974 R&D #261-CE R&D #3742 R&D #6476 R&D #5552 R&D #0695
5	3	N/A			
5	4	BE5	Beta-Cellulin RepSox (Alk5ii) XXi T3 Retinoic Acid	20 ng/mL 10 μ M 1 μ M 1 μ M 0.025 μ M	R&D #261-CE R&D #3742 R&D #6476 R&D #5552 R&D #0695
5	5	N/A			
5	6	BE5	Beta-Cellulin RepSox (Alk5ii) XXi T3 Retinoic Acid	20 ng/mL 10 μ M 1 μ M 1 μ M 0.025 μ M	R&D #261-CE R&D #3742 R&D #6476 R&D #5552 R&D #0695
6	0	S3	N/A	N/A	
6	1	N/A	N/A	N/A	
6	2	S3	N/A	N/A	
6	3	N/A	N/A	N/A	
6	4	S3	N/A	N/A	
6	5	N/A	N/A	N/A	
6	6	S3	N/A	N/A	

Table S4: Antibody Information for Immunofluorescence and Flow Cytometry

Target	Host Species	Supplier	Dilution (1:X)	Assay Used
<i>Primary Antibodies</i>				
SOX9	Rabbit	Abcam (AB185966)	500	IF/Flow Cytometry
CDX2	Rabbit	Abcam (AB76541)	1000	IF
SOX2	Goat	R&D (AF2018)	500 (1 ug/uL stock)	IF
TPH1	Rabbit	Invitrogen (MA5-32209)	250	IF
C-Peptide	Rat	DSHB (GN-ID4)	250	IF/Flow Cytometry
GCG	Mouse	Sigma (G2654)	250 (IF), 1000 (FC)	IF/Flow Cytometry
NKX6.1	Mouse	DSHB (F55A12-S)	100	Flow Cytometry
CHGA	Rabbit	Abcam (AB283265)	1500	Flow Cytometry
<i>Secondary Antibodies</i>				
Rabbit IgG-488	Donkey	Invitrogen (A21206)	1000	IF/Flow Cytometry
Goat IgG-594	Donkey	Invitrogen (A11058)	1000	IF
Rat IgG-488	Donkey	Invitrogen (A21208)	1000	Flow Cytometry
Mouse IgG-647	Donkey	Invitrogen (A31571)	1000	Flow Cytometry

Table S5: PCR Primers Used in This Study

Gene Target	Forward Primer (5'-3')	Reverse Primer (5'-3')
GAPDH	ATCACCATCTTCCAGGAGCGA	TTCTCCATGGTGGTGAAGACG
SOX9	AGGAAGCTCGCGGACCAGTAC	GGTGGTCCTTCTTGCTGCAC
CDX2	ACAGTCGCTACATCACCATCCG	CCTCTCCTTTGCTCTGCGGTTT
SOX2	GCTACAGCATGATGCAGGACCA	TCTGCGAGCTGGTCATGGAGTT
CDH1	GCCTCCTGAAAAGAGAGTGGAAG	TGGCAGTGTCTCTCCAAATCCG
CDH2	CCTCCAGAGTTTACTGCCATGAC	GTAGGATCTCCGCCACTGATTC
NOTCH1	GGTGAAGTCTCTGAGGAGATC	GGATTGCAGTCGTCCACGTTGA
NOTCH2	GTGCCTATGTCCATCTGGATGG	AGACACCTGAGTGCTGGCACAA
NOTCH3	TACTGGTAGCCACTGTGAGCAG	CAGTTATCACCATTGTAGCCAGG
HES1	GGAAATGACAGTGAAGCACCTCC	GAAGCGGGTCACCTCGTTCATG
DLK1	CCCCAAAATGGATTCTGCGAGG	GGTTCTCCACAGAGTCCGTGAA
JAG1	TGCTACAACCGTGCCAGTGACT	TCAGGTGTGTCGTTGGAAGCCA

Supplemental References:

Veres, A., Faust, A.L., Bushnell, H.L., Engquist, E.N., Kenty, J.H., Harb, G., Poh, Y.C., Sintov, E., Gurtler, M., Pagliuca, F.W., et al. (2019). Charting cellular identity during human in vitro beta-cell differentiation. *Nature* 569, 368-373. 10.1038/s41586-019-1168-5.