Supplemental Information

Single-Cell Transcriptome Profiling

Reveals β Cell Maturation in

Stem Cell-Derived Islets after Transplantation

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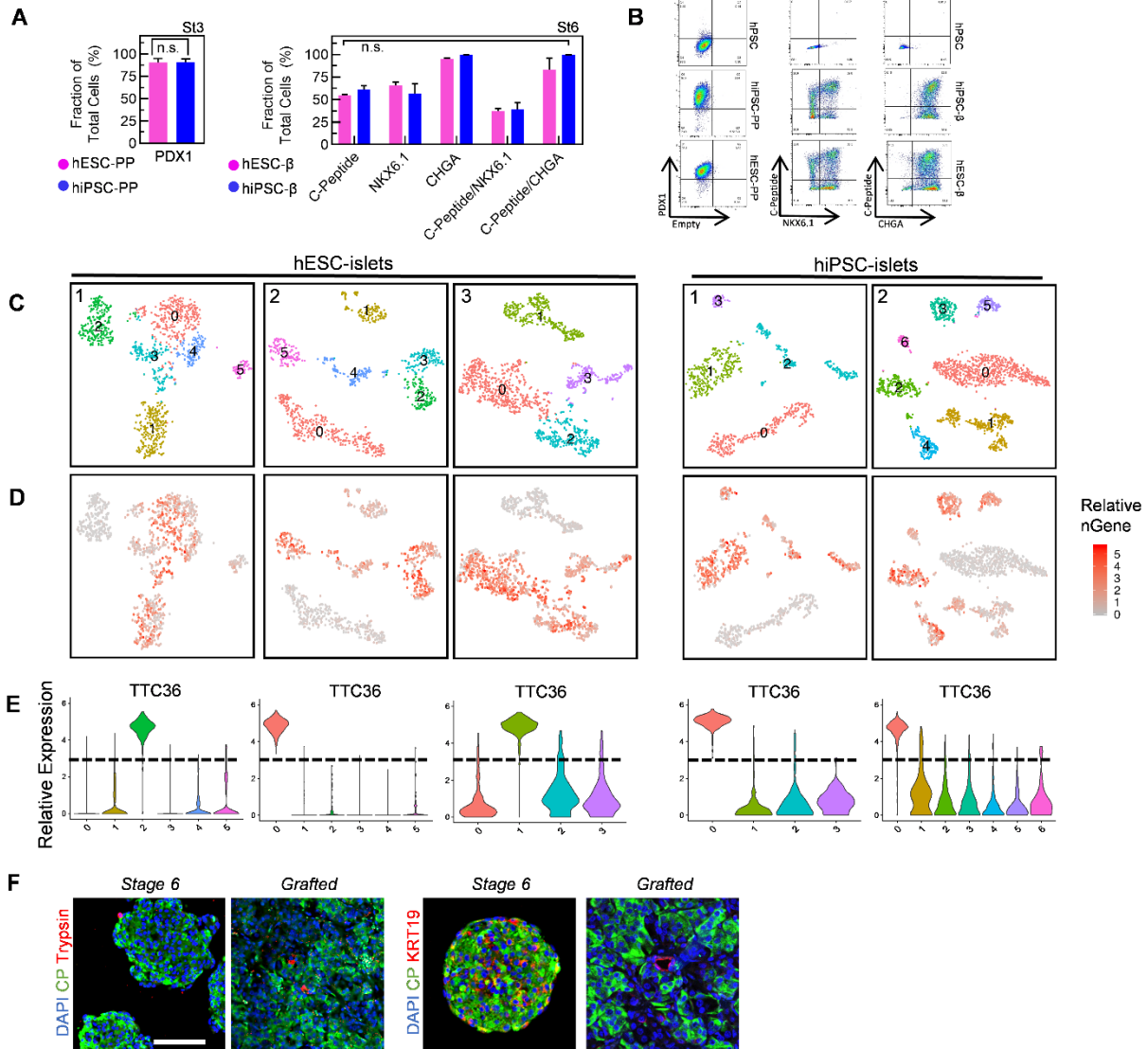Jeffrey R. Millman, jmillman@wustl.edu

Supplementary Figures

Figure S1. Kidney cell removal from grafted SC-islet datasets. Related to Figure 1. (A) Quantified fraction of cells and (B) representative flow cytometry dot plots expressing or co-expressing pancreatic progenitor (PP) and β cell (C-Peptide, NKX6.1) or endocrine (CHGA) markers for hESC-PP (*n*=4) and hiPSC-PP (*n*=4) or Stage 6 hESC-islets (*n*=4) and hiPSC-islets (*n*=4), respectively. n.s., not significant by two-way unpaired *t*-test. (C) Unsupervised tSNE projection for individual Grafted SC-islet samples, identifying different cell type clusters. (D) Feature tSNE plot displaying number of genes (nGene) per cell, represented by individual dots. (E) Violin plots representing high TTC36 expression in individual clusters for each tSNE

projection in (C). Scale: Log(Normalized read counts). (F) Immunostaining of sectioned Stage 6

and Grafted SC-islets for exocrine cell markers. Scale bar, 100 μm. Error bars represent s.e.m.
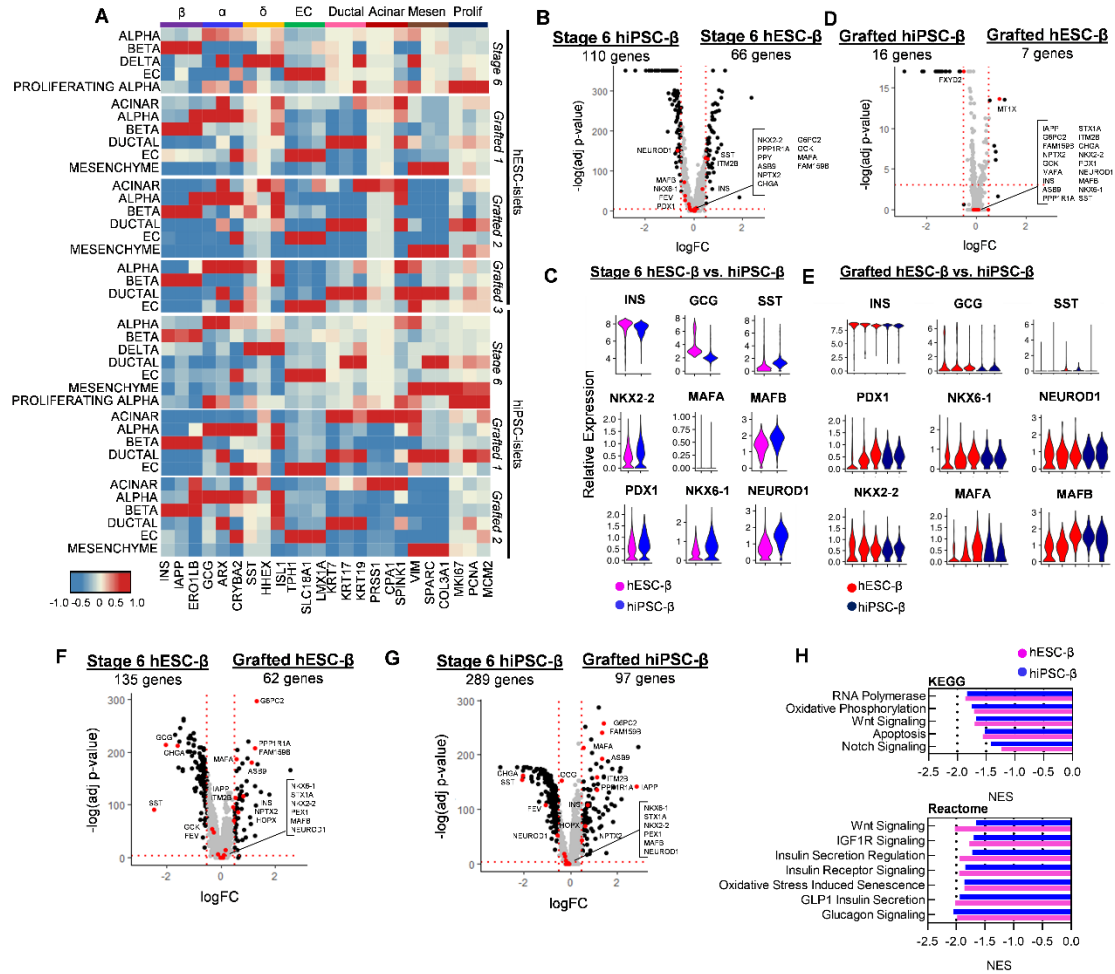
See also Table S1.

Figure S2. Identification and characterization of hiPSC-islet and hESC-islet cell types. Related to Figures 1 and 2. (A) Heat map displaying gene expression profiles of individual cell types represented in Figures 1A and 1C for cell type-specific genes (bottom x axis, gene name; top x axis, cell type-matched to genes). Each row is associated with a cell type (left y axis) and sample (right y axis). Scale: Normalized Expression (B) Volcano plot displaying fold change (FC) differences and (C) violin plots of β cell genes between Stage 6 hiPSC-β and hESC-β cells. Scale: Log(Normalized read counts). (D) Volcano plot displaying FC differences and (E) violin plots of β cell genes between Grafted hiPSC-β and hESC-β cells. Scale: Log(Normalized read counts). Volcano plot displaying FC differences between Stage 6 and Grafted (F) hESC-β and (G) hiPSC-β cells. Dashed lines are drawn to define restriction of log FC value of 0.25 and -

log of adjusted p-value 0.001. Key β cell genes are labeled and colored red. (H) KEGG and

Reactome GSEA, quantified by the normalized enrichment score (NES), for pathways

upregulated in Stage 6 hESC-β (pink) and hiPSC-β (royal blue) compared to Grafted hESC-β or

hiPSC-β cells, respectively. NES values and gene lists are available in Table S4. EC,
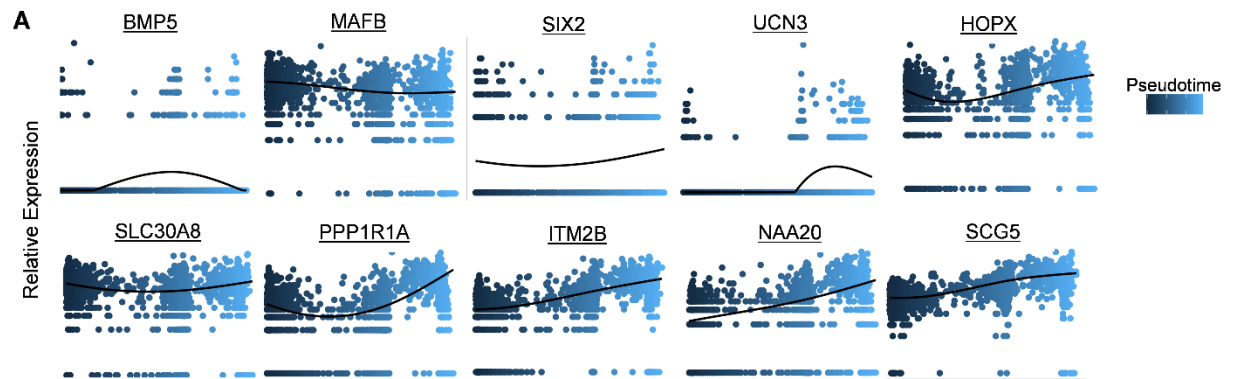
enterochromaffin. See also Tables S2 and S3.

Figure S3. Additional pseudo-time characterization of SC-β cells. Related to Figure 3. (A)
Expression of additional known and newly identified β cell maturation genes along pseudo-time.
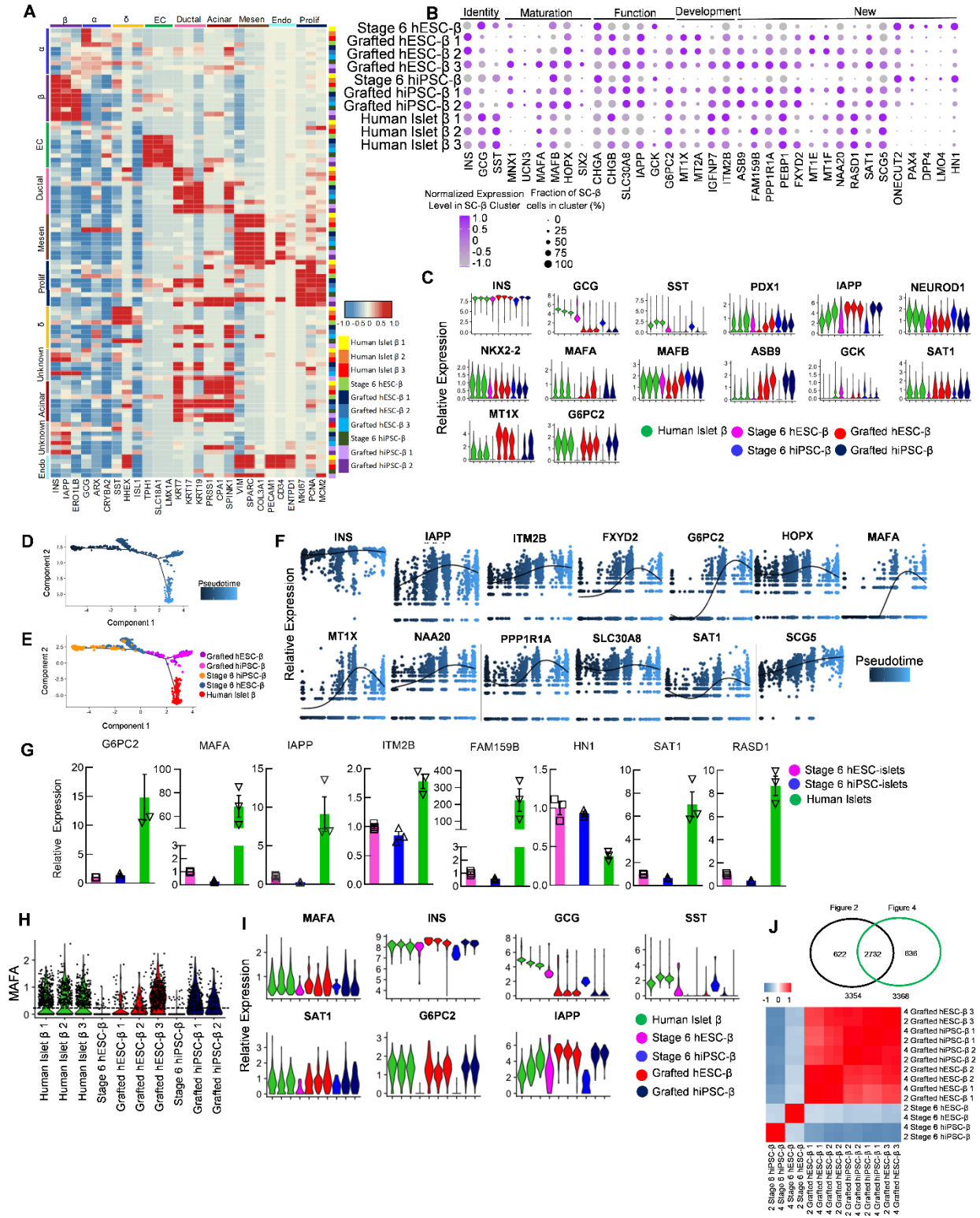Each dot represents a different cell. Scale: Relative Expression.

Figure S4. Cell cluster identification and additional characterization of primary human islets, Grafted, and Stage 6 SC-islets. Related to Figure 4. (A) Heat map displaying gene

expression profiles of individual cell types represented in Figure 4A for cell type-specific genes (bottom x axis, gene name; top x axis, cell type associated with specific gene). Each row is associated with a cell type (left y axis) and sample (right y axis and colored legend). Scale: Normalized Expression. (B) Normalized expression values for key β cell identity, maturation, functional, and newly identified genes compared between all β cell samples. Percentage of cells positive for designated gene is represented by circle size and saturation indicates low (grey) or high (purple) expression of the gene. (C) Violin Plots of key β cell identity genes compared between all β cell samples. Scale: Log(Normalized read counts). (D) Pseudo-time trajectory of Stage 6, Grafted, and Human Islet β cells with branching points. (E) Distribution of β cells from Grafted hESC-β (purple), Grafted hiPSC-β (pink), Stage 6 hiPSC-β (orange), Stage 6 hESC-β (blue), and Human Islet (red) β cells. (F) Expression of select β cell genes along SC-β cell pseudo-time. Scale: Relative Expression. (G) Real-time PCR analysis of the total Stage 6 hiPSC-islet (n=3; pink), hESC-islet (n=3; blue), and human islet (n=3; green) population measuring expression of identified β cell genes that change with maturation. (H) Violin plots of MAFA expression show individual cells in each condition for the identified β cell population from the tSNE projection in Figure 4A. Dotted line represents cut off for β cells to be considered MAFA+ for analysis in the following panel. Stage 6 hESC-β and Stage 6 hiPSC-β only had 2.09% and 1.20% MAFA+ cells, respectively, with this threshold. Scale: Log(Normalized read counts). (I) Violin plot β cell genes for MAFA+ cells in panel (H). Scale: Log(Normalized read counts). (J) Venn diagram highlighting number of β cells identified in Figure 2 and Figure 4; and correlation matrix (using top 500 variable genes) highlighting similarities between β cells identified in Figure 2 and Figure 4. Error bars represent s.e.m. See also Tables S4, S5, and S6.
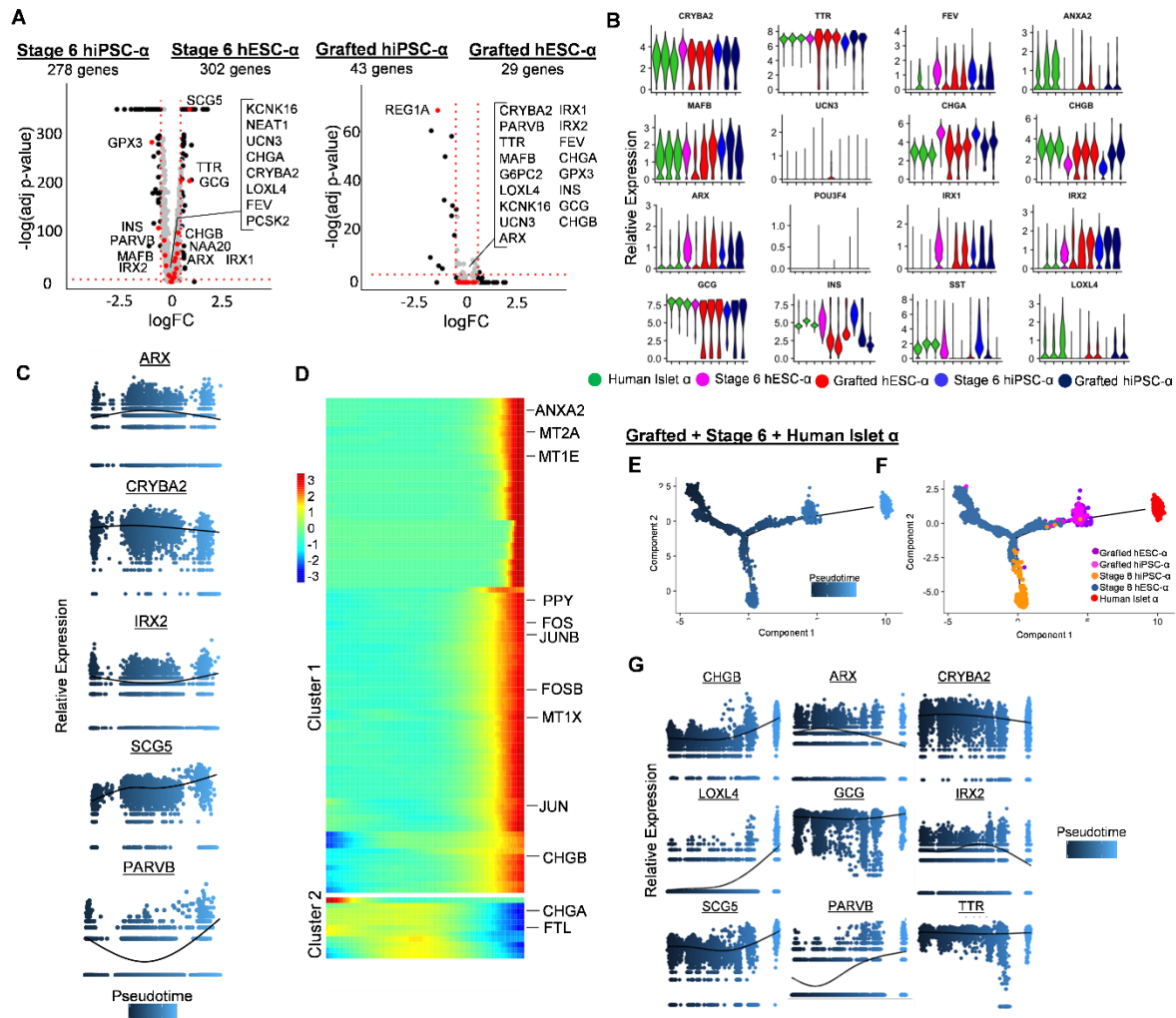
Figure S5. Additional characterization of primary human α and SC-α cells. Related to Figure 5. (A) Volcano plot displaying fold change (FC) differences of α cell genes between Stage 6 (left) and Grafted (right) hiPSC-β and hESC-β cells. Dashed lines are drawn to define restriction of log FC value of 0.25 and -log of adjusted p-value 0.001. Key α cell genes are labeled and colored red. (B) Violin Plots of key α cell identity genes compared between all α cell samples. Scale: Log(Normalized read counts). (C) Relative expression of select α cell genes along pseudo-time. Each dot represents a different cell. Scale: Relative Expression. (D) Analysis of top 100 branch-dependent genes determined based on clusters identified through pseudo-time analysis of SC-α cells, found in Table S4. Scale: Relative Expression. (E) Pseudo-time trajectory of Stage 6, Grafted, and Human Islet α cells with branching points. (F)

Distribution of α cells from Grafted hESC-α (purple), Grafted hiPSC-α (pink), Stage 6 hiPSC-α (orange), Stage 6 hESC-α (blue), and Human Islet (red) α cells. (G) Relative expression of select α cell genes along SC-α cell pseudo-time. Scale: Relative Expression. See also Tables S3, S4, and S6.
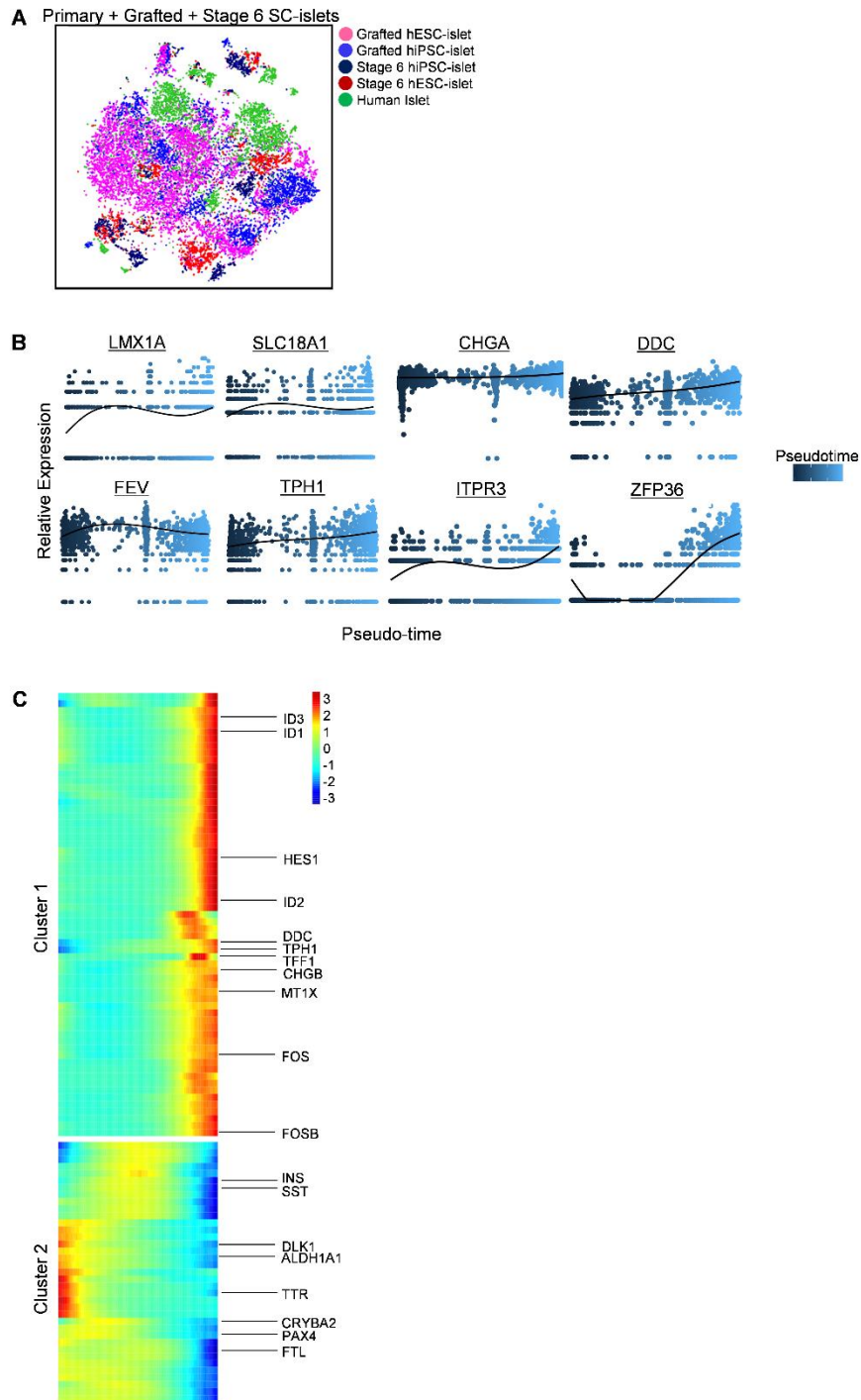
Figure S6. Additional characterization of SC-EC cells. Related to Figure 6. (A) Figure 4A

with each dot representing individual sample. (B) Expression of select EC cell genes along

pseudo-time. Each dot represents a different cell. Scale: Relative Expression. (C) Analysis of

top 100 branch-dependent genes determined based on clusters identified through pseudo-time

analysis of SC-EC cells, found in Table S4. Scale: Normalized Expression. See also Table S4.