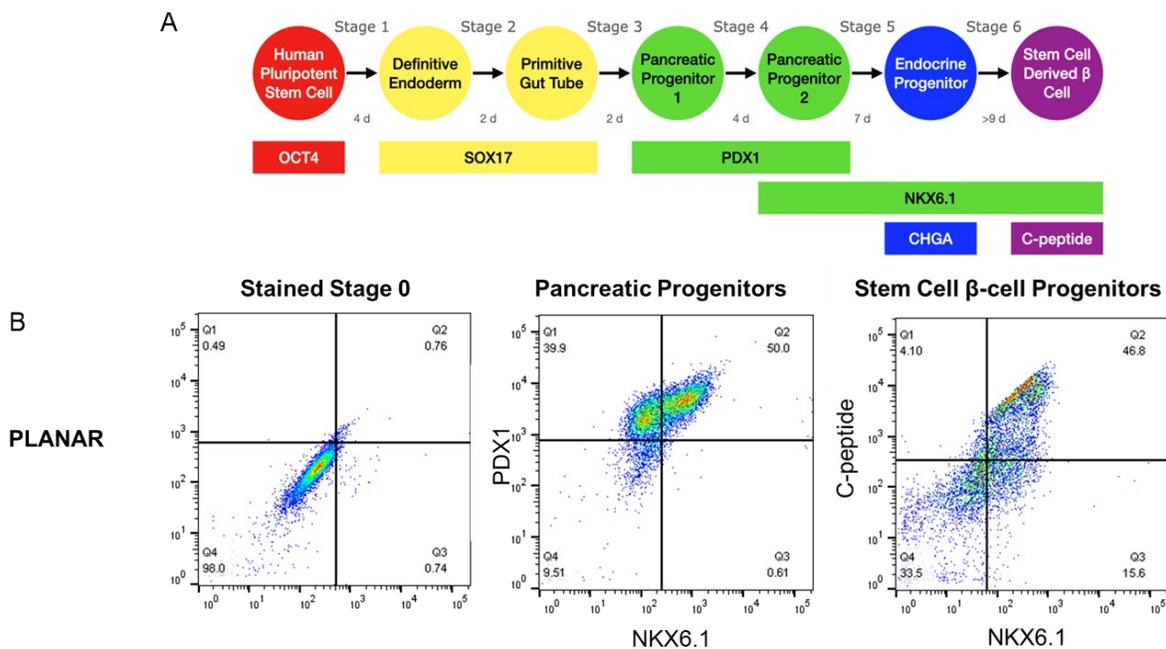
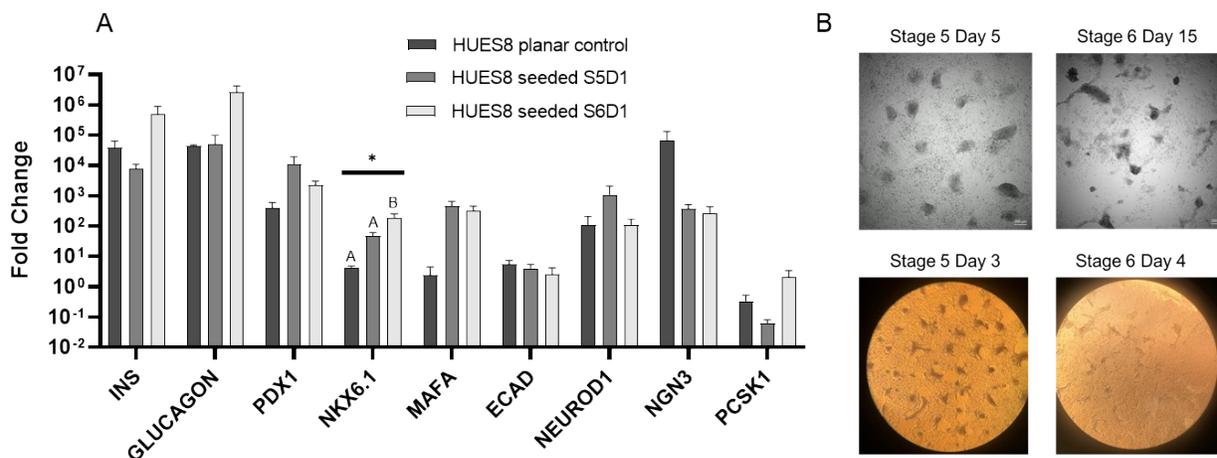


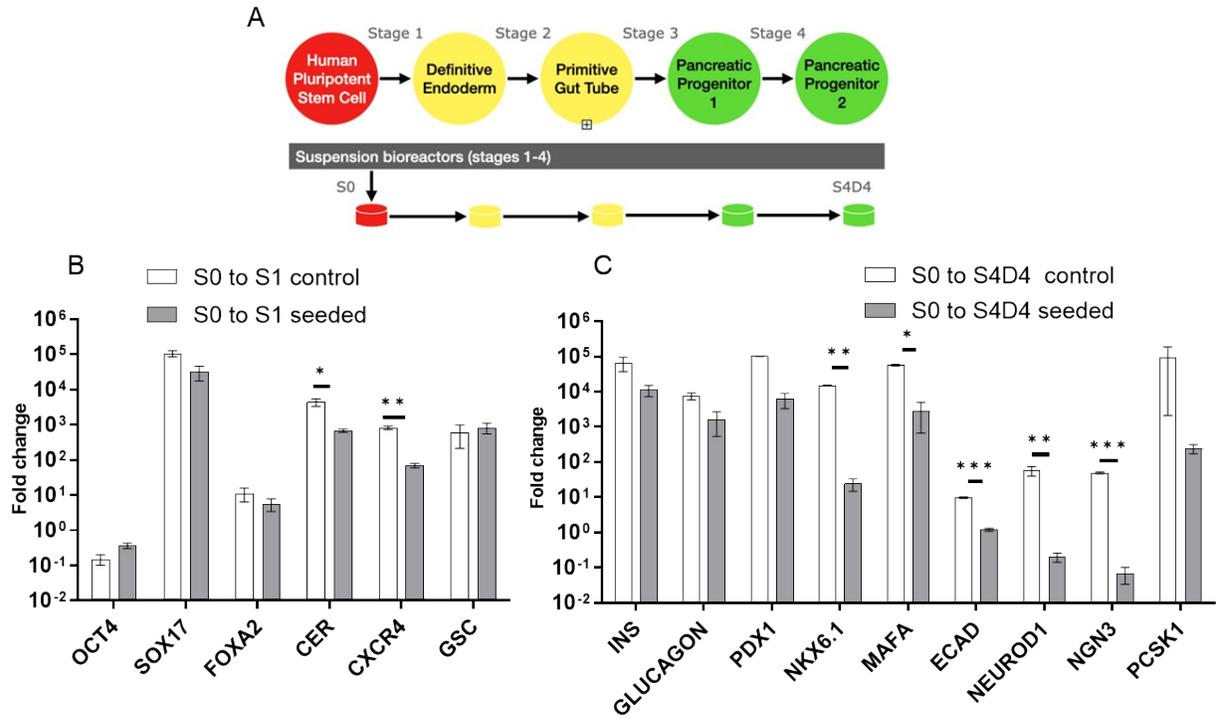
Supplementary Information



Supplementary Figure 1. (A) Schematic of the stages of differentiation and corresponding markers assessed by flow cytometry. (B) Representative flow cytometry plots from different stages in planar culture. Pancreatic progenitors were evaluated at the conclusion of stage 4 and stem cell β -cells progenitors at maturation in stage 6.



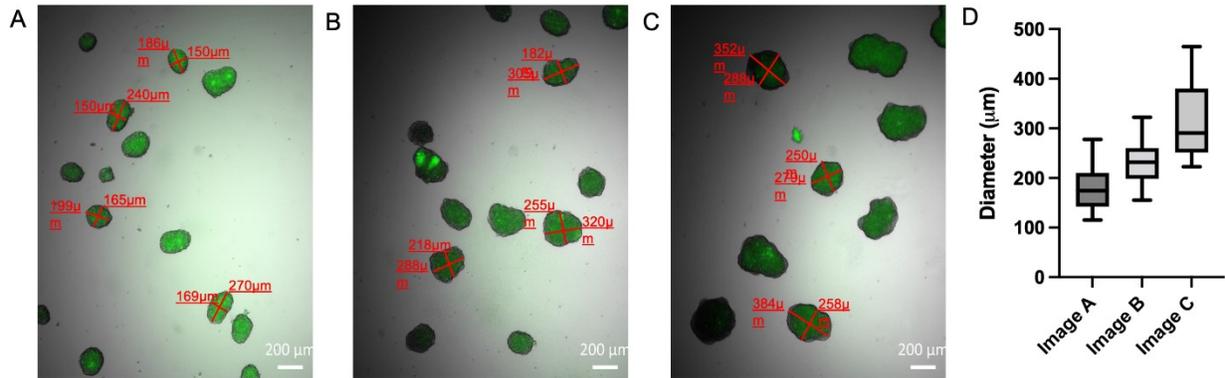
Supplementary Figure 2. (A) Gene expression analysis of maturation markers of cells seeded onto scaffolds at S5D1 and S6D1 compared to planar culture. The HUES8 cell line was used in planar and scaffold culture. Statistical significance was determined based on unpaired T-test for each gene pair $*P < 0.05$. ($n=3$ planar, $n=5$ S5D1, $n=4$ S6D1) (B) Brightfield images of HUES8-derived cells at the endocrine progenitor and stem cell-derived β -cell stages of differentiation (Scale bar indicates 200 μ m).



Supplementary Figure 3. Early Stage (S0) Cluster Seeding on Scaffolds: (A) Schematic of seeding timing and analysis. (B) Clusters were cultured in suspension, or seeded onto scaffolds prior to stage 1, and cultured to completion of stage 1 definitive endoderm. (n=2 for suspension, n=3 for scaffold culture) (C) Clusters were cultured in suspension, or seeded onto scaffolds prior to stage 1, and cultured to completion of stage 4 pancreatic progenitor. (n=2 for suspension, n=3 for scaffold culture) Statistical significance was determined based on unpaired T-test for each gene pair *P<0.05, **P<0.01, ***P<0.001.

Supplementary Table 1. qRT-PCR primers used during early stages of β -cell differentiation

Gene	Function
OCT4	Marker of pluripotency
SOX17	Sex determining region Y-box 17; expressed by definitive endoderm and pancreatic endocrine cells ³⁹
FOXA2	Forkhead box A2; involved in early pancreatic progenitor formation ³⁹
CER	Cerberus; expressed by definitive endoderm cells
CXCR4	Chemokine [CXC motif] receptor 4; expressed by definitive endoderm cells ³⁹
GSC	Goosecoid homeobox protein involved in morphogenesis; expressed by definitive endoderm and pancreatic endocrine cells ³⁹



Supplementary Figure 4. (A, B, C) Representative images from three separate batches of reagggregated cell clusters, imaged 3-4 days after cells were initially dissociated for reagggregation. (D) Average diameter measured from each image. Measurements of 13, 11, and 9 clusters were taken from images A, B, and C, respectively. Two perpendicular measurements were taken from each cluster. The overall average length from 66 measurements was $234\mu\text{m}$ with a standard deviation of $74.7\mu\text{m}$.