SUPPLEMENTARY DATA

Supplementary Table 2. Antibodies used for Immunofluoresence.

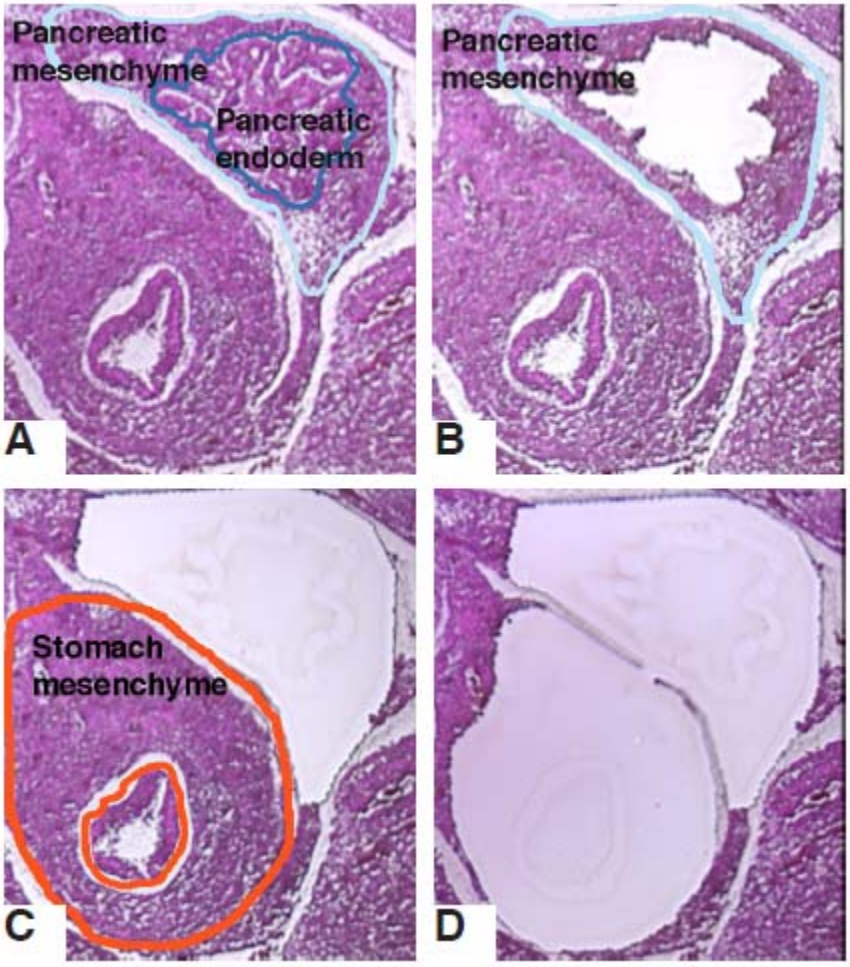
Antibody	Dilution	Source
Goat anti-PDX1	1:100	R&D Systems
Rabbit anti-HNF6	1:100	Santa Cruz Biotechnology
Mouse anti-NKX6.1	1:200	Developmental Studies Hybridoma Bank
Mouse anti-NKX2-2	1:10	Developmental Studies Hybridoma Bank
Guinea pig anti-insulin	1:500	Dako
Mouse anti-glucagon	1:500	Sigma
Rabbit anti C-peptide	1:500	Linco
Rabbit anti-somatostatin	1:300	Dako
Rabbit anti-phospho-Histone H3 (Ser10)	1:500	Millipore
Alexa-conjugated secondary antibodies	1:500	Molecular probes

Supplementary Table 3. Real-time PCR primer sequences.

Gene	Forward Primer	Reverse primer
TBP	5'-TGTGCACAGGAGCCAAGAGT	5'-ATTTTCTTGCTGCCAGTCTGG
PDX1	5'-AACTCTACCAAAGCTCACGCG	5'-GTAGGCGCCGCCTGC
INS	5'-AAGAGGCCATCAAGCAGATCA	5'-CAGGAGGCGCATCCACA
AMY2A	5'-GCCCAACCCAATCATTAACA	5'-GCAGTTGGATTTATGCTTGC
PTF1a	5'-AGAGAGTGTCCTGCTAGGGG	5'-CCAGAAGGTCATCATCTGCC
MIST1	5'-CGGATGCACAAGCTAAATAACG	5'-CCGTCAGCGATTTGATGTAGTTC
SOX9	5'-AGTACCCGCACTTGCACAAC	5'-ACTTGTAATCCGGGTGGTCCTT

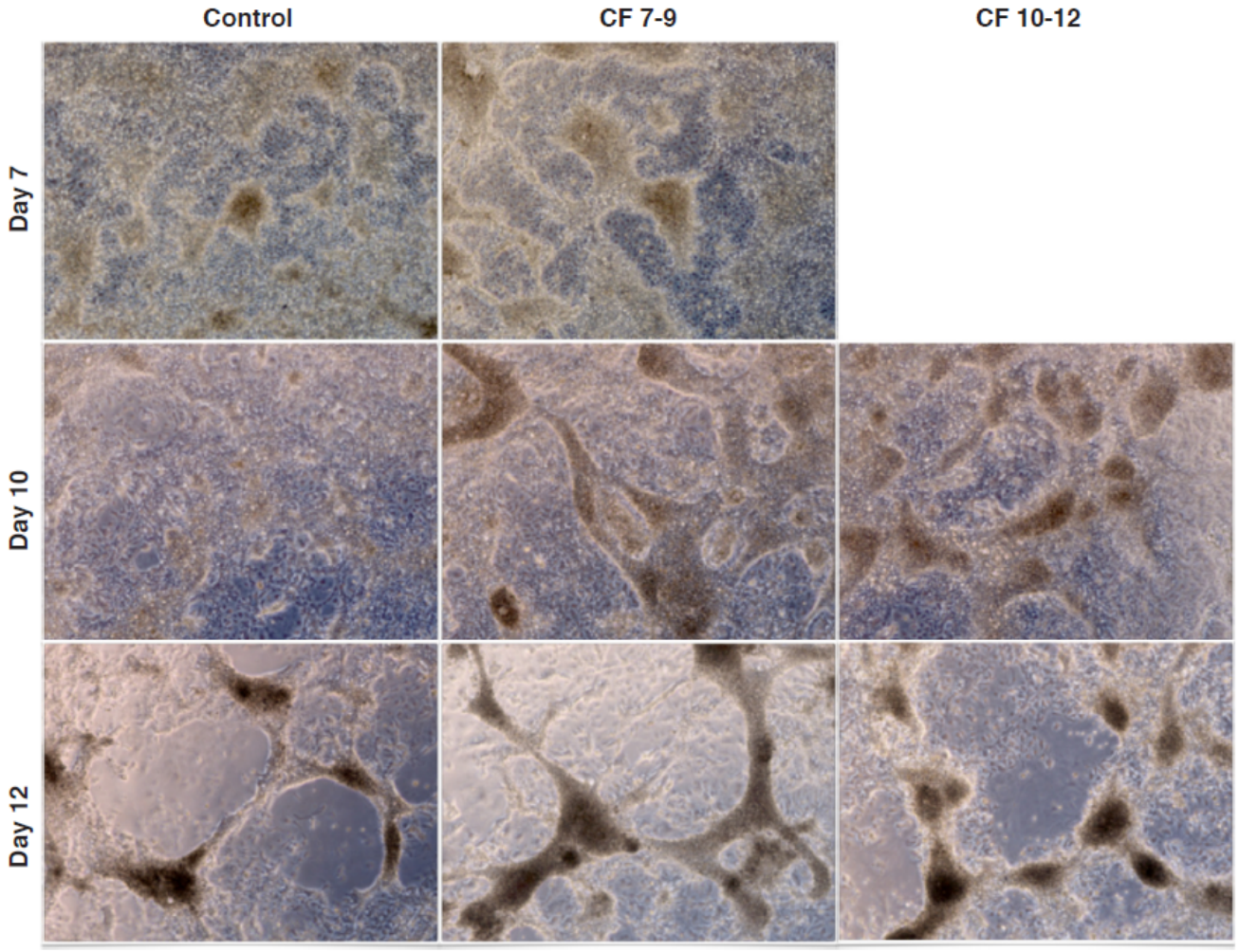
SUPPLEMENTARY DATA

Supplementary Figure 1. Isolating different mouse embryonic tissues using LCM. Representing images for isolating pancreatic endoderm, pancreatic mesenchyme, and stomach mesenchyme.

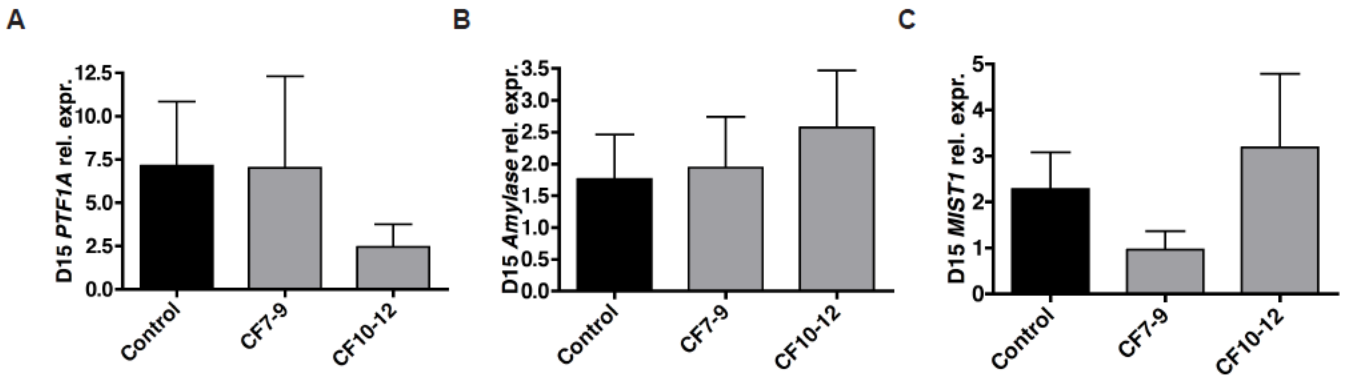


SUPPLEMENTARY DATA

Supplementary Figure 2. Morphological changes in the culture during pancreatic progenitor and endocrine precursor induction. Both control and combined factor (CF) treated samples show aggregates in culture. Highest number of cell clusters were observed in samples treated with combined factors from day 7-9 (CF 7-9).

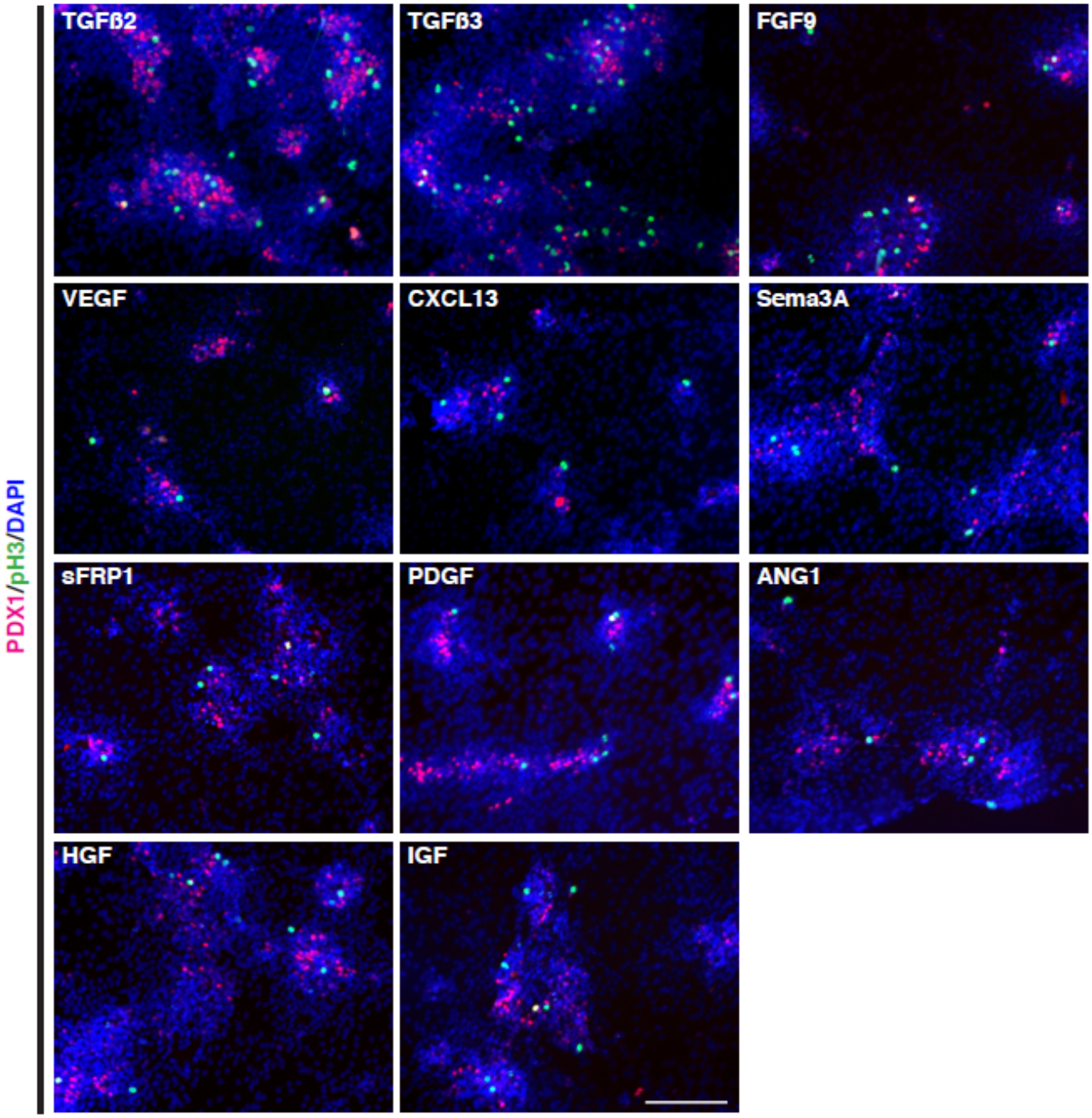


Supplementary Figure 3. Expression of exocrine markers after treatment with combined factors. A-D. Q-PCR analysis of day 15 control and combined factor-treated samples showing no significant difference in *PTF1A* (A), *AMYlASE* (B), or *MIST1* (C) expression. Statistical analysis was performed using Student's t-test (*P<0.05).



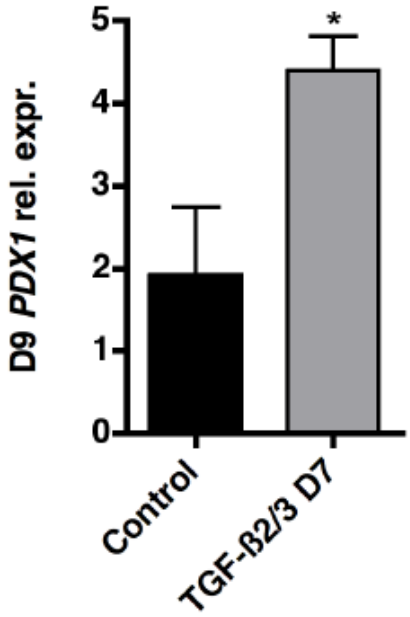
SUPPLEMENTARY DATA

Supplementary Figure 4. Induction of PDX1 expression by single mesenchymal factor. Immunostainings with PDX1 on day 9 reveal that when single mesenchymal factors are used without the standard factors R/K/N, only a small number of cells turn on this pancreatic progenitor marker. Phospho-histone H3 staining also indicate a small percentage of cells are proliferating. Most of these mitotic cells locate in the PDX1-positive cell clusters. Scale bar equals to 200um for all images.

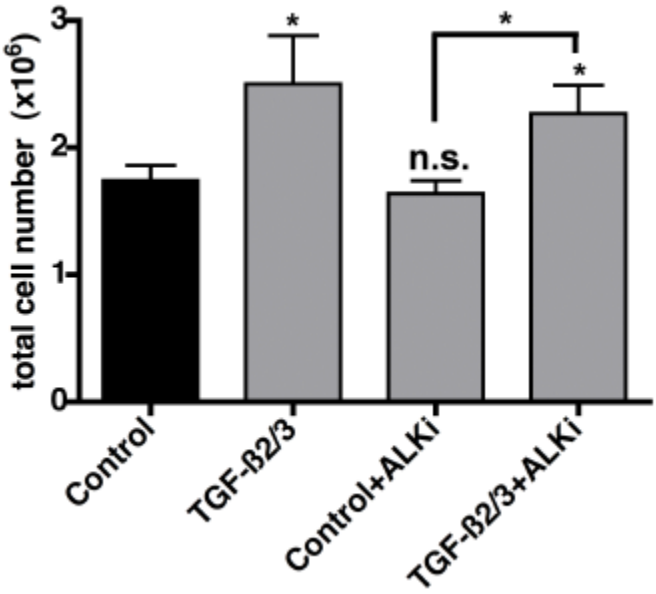


SUPPLEMENTARY DATA

Supplementary Figure 5. One day TGF-ß2/3 treatment leads to increased *PDX1* level. Q-PCR analysis shows that samples receiving TGF-ß2/3 ligands on day7 only have significantly higher levels of *PDX1* expression. N = three independent experiments. Statistical analysis was performed using Student's t-test (*P<0.05).

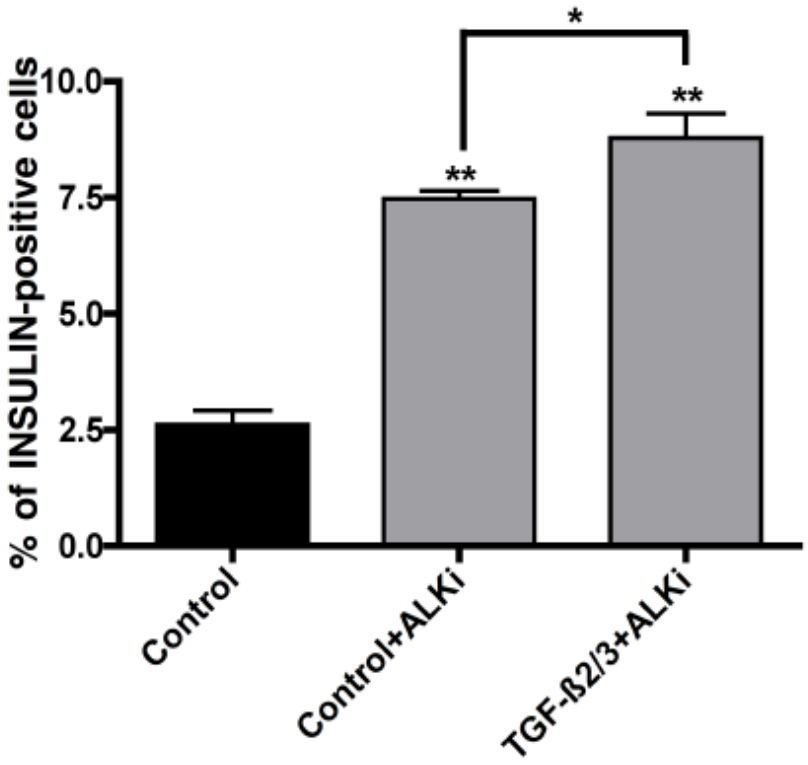


Supplementary Figure 6. TGF-ß2/3 treatment leads to increased total cell number at end of differentiation. Undifferentiated hESCs were seeded and allowed to grow for 48 hours before initiation of differentiation. Using 1 million (M) input cells, 1.74M±0.12M, 2.5M±0.38M, 1.64M±0.10M and 2.27M±0.22M cells were generated on day 15 for control, TGF-ß2/3 only, control+ALK5 inhibitor, or TGF-ß2/3-ALK5 inhibitor conditions, respectively. N = four independent experiments. Statistical analysis was performed using Student's t-test (*P<0.05).



SUPPLEMENTARY DATA

Supplementary Figure 7. Transient activation of TGF-ß pathway followed by ALK5 inhibitor increase number of INSULIN-positive cells in MEL 1 *INS*$^{GFP/w}$ cells. Quantification of GFP-positive cells on day 15 for control, control+ALK5 inhibitor, or TGF-ß2/3-ALK5 inhibitor conditions, respectively. N = three independent experiments. Comparison was made with Student's t-test (*P<0.05, **P<0.01).



SUPPLEMENTARY DATA

Supplementary Figure 8. Immunostaining on hESC transplants sections isolated from mouse kidney indicate that hESC-derived pancreatic progenitor cells give rise to mature ß-cells *in vivo.* INSULIN-positive cells co-label with Human Nuclear Antigen and PDX1 (A), PDX1 and NKX6.1 (B), but not GLUCAGON and SOMATOSTATIN (C). CK19-positive ductual cells are also found in the transplant with few Amylase-positive exocrine cells (D). Scale bar in (C) equals to 100um for all images.