Stem Cell Reports, Volume *17*

Supplemental Information

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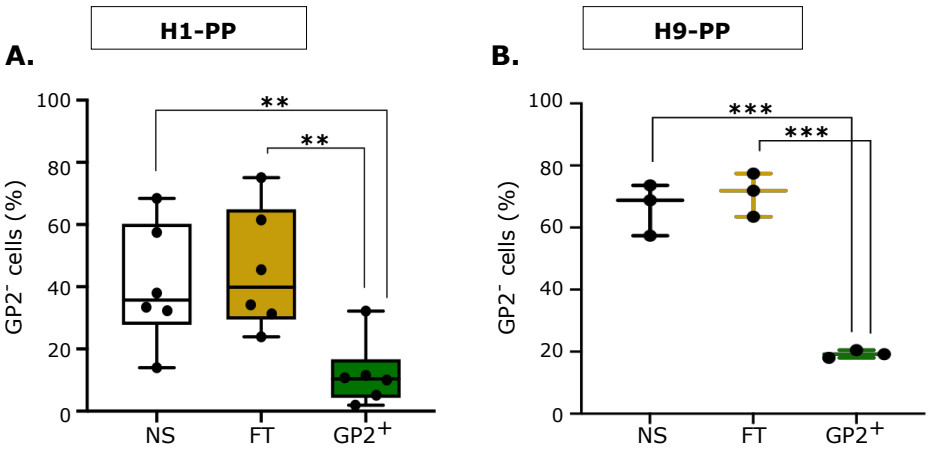
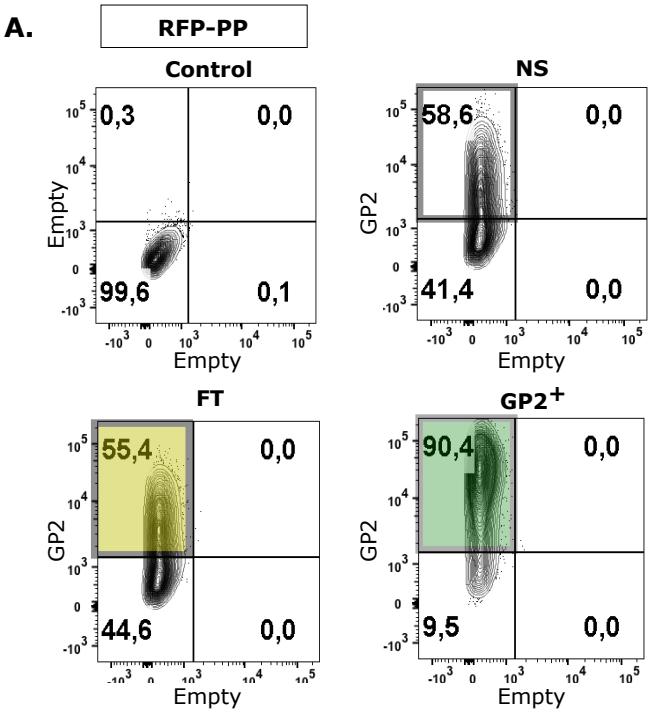
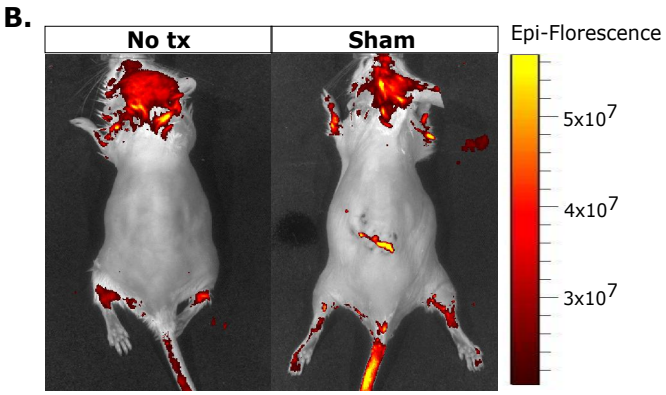
Supplementary Figure 1



Supplementary Figure 1. *Related to Figure 1*. (A-B) Frequency of GP2 negative (GP2-) cells in NS, FT and $GP2^+$ cell populations after MACS in H1 (A) and H9 (B)- derived cells. (A, n=6 independent experiments; B, n=3 independent experiments; One-way ANOVA analysis with Tukey's multiple comparisons; ** $P < 0.01$, *** $P < 0.0001$, error bars represent S.E.M.).

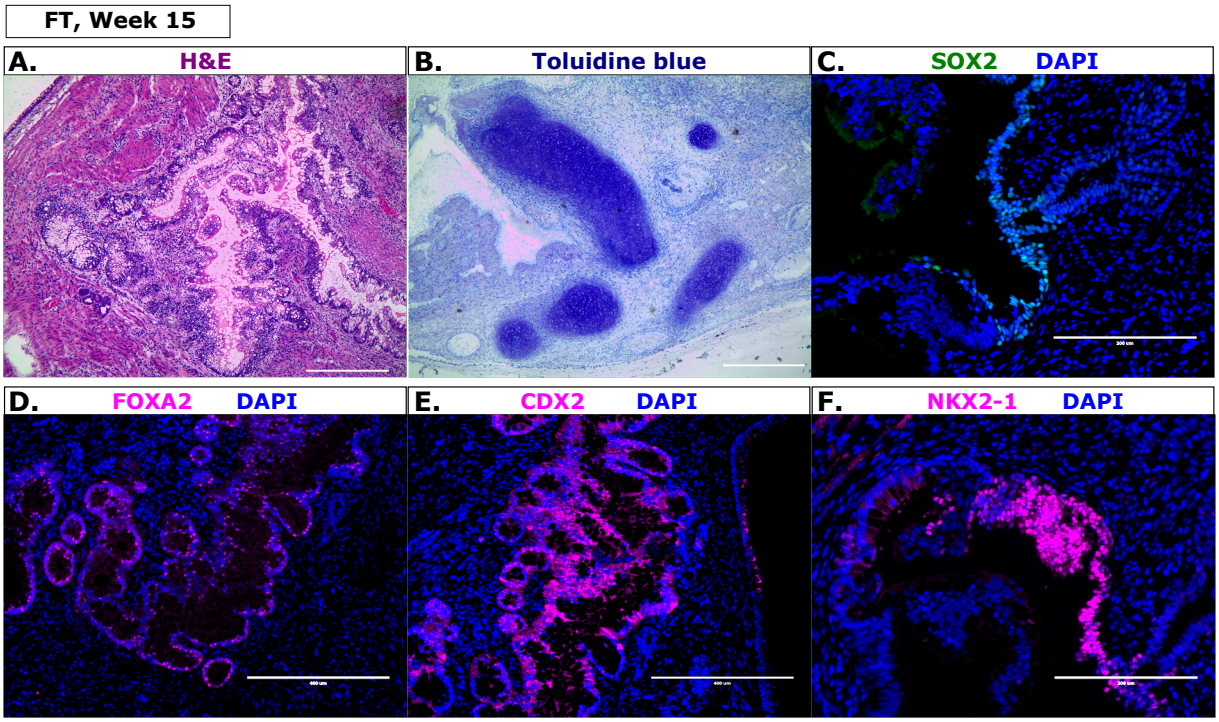
Supplementary Figure 2





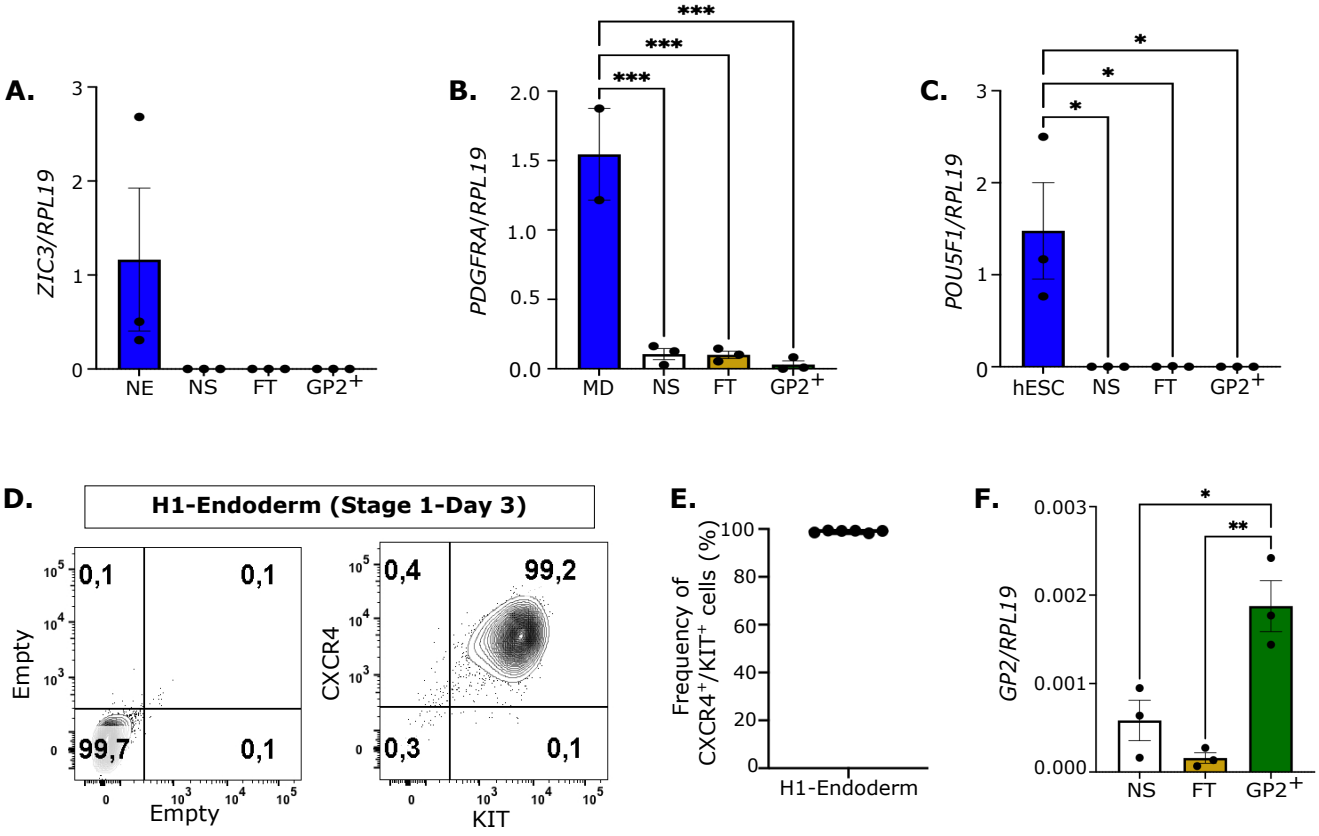
Supplemental Figure 2. *Related to Figure 2.* (A) Flow cytometry plot quantifying GP2 expression in RFP-H1-derived PPs at stage 4. (B) Live imaging of mice without transplantation (No Tx), or transplantation of an empty collagen hydrogel (sham) at week 1 post-transplantation.

Supplementary Figure 3



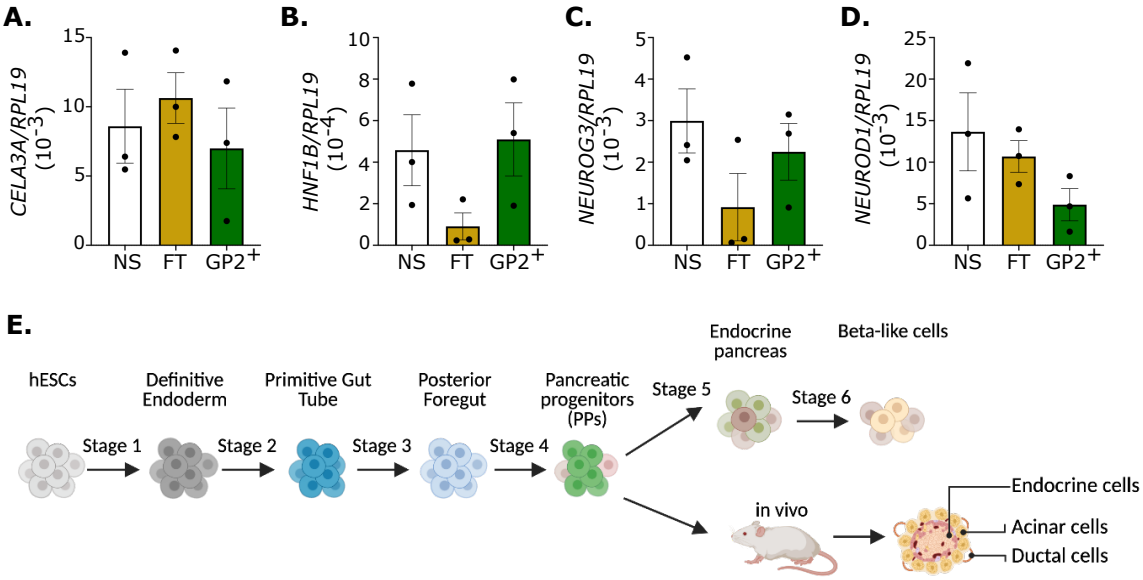
Supplemental Figure 3. *Related to Figure 2***.** (A-F) Representative images of FT-derived outgrowths stained with H&E (A), toluidine blue (B), and immunostained using SOX2 (C), FOXA2 (D), CDX2 (E), and NKX2-1 (F) antibodies at week 15 post-transplantation. DAPI marks cell nuclei. (A, scale bar is 100μm; C & F, scale bar is 200μm; B, D, E, scale bar is 400μm).

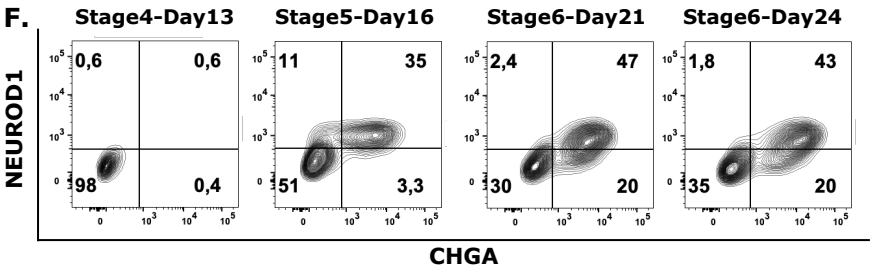
Supplementary Figure 4

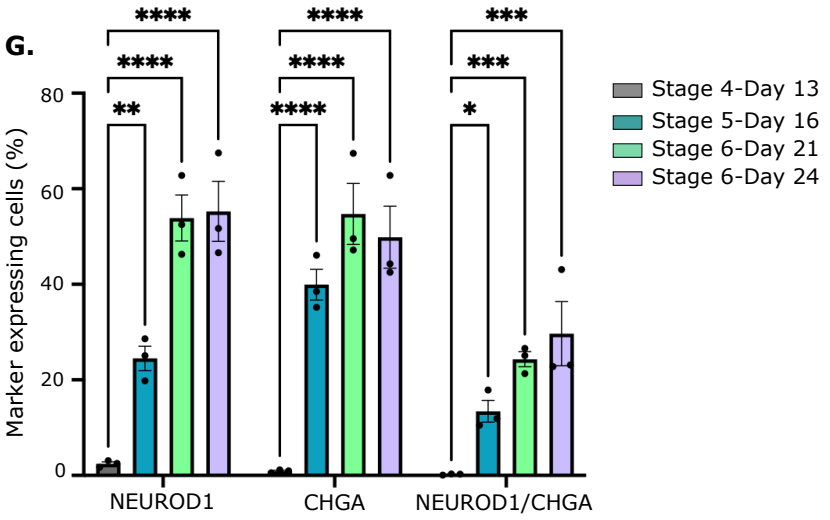


Supplementary Figure 4. *Related to Figure 3*. (A-C) RT-PCR quantification of *ZIC3* (neurectoderm marker), *PDGFRA* (mesoderm marker) and *POU5F1* (pluripotency marker) levels in NS, FT and $GP2^+$ cell fractions after MACS normalized to *RPL19* housekeeping gene. HESC-derived NE (neuroectoderm), MD (mesoderm), and undifferentiated hESCs are used as a positive control (NE, NS, FT, $GP2^+$, hESC, n=3 independent experiments; MD, n=2 independent experiments; One-way ANOVA analysis with Tukey's multiple comparisons; * $P< 0.05$, *** $P<0.001$). (D, E) Representative flow cytometry plot and quantification of CXCR4 and KIT expression in hESC-derived endodermal cells, indicating robust differentiation efficacy (~ 100%). (F) RT-PCR quantification of *GP2* levels normalized to *RPL19* housekeeping gene in NS, FT, and $GP2^+$ fractions after MACS confirming enrichment of GP2+ cells (n=3 independent experiments; One-way ANOVA analysis with Tukey's multiple comparisons; * $P< 0.05$, ** $P<0.01$).

Supplementary Figure 5







Supplementary Figure 5. *Related to Figure 3*. (A-D) RT-PCR quantification of *CELA3A* (acinar marker), *HNFB1* (ductal marker), *NEUROG3* and *NEUROD1* (endocrine markers) normalized to *RPL19* housekeeping gene in NS, FT and $GP2^+$ fractions after MACS. (E) Schematic of hESC differentiation to PP (Stage 4, Days 8-13), which can be transplanted *in vivo* to generate endocrine pancreas, acinar and ductal cells or differentiated in vitro to endocrine pancreatic cells (Stage 5, Days 13-16), and Beta-like cells (Stage 6, Days 16-24). (F) Representative flow cytometry plots of NEUROD1 and Chromogranin (CHGA) expression, which indicates commitment to the endocrine pancreas. (G) Quantification of the frequency of endocrine-committed cells at various differentiation stages. (n=3 independent experiments; One-way ANOVA with Tukey's multiple comparisons; * $P<0.05$, ** $P<0.01$, *** <0.001, **** $P<0.0001$).

Supplementary Tables:

Supplementary Table 1. List of antibodies used for immunostaining (I) and/or flow cytometry (F). *Related to Figures 1-5.*

Antigen	Species	Vendor	Dilution
CDX2	Rabbit	Abcam	1:300 (I), 1:2000 (F)
Chromogranin A (CHGA)	Rabbit	Abcam	1:100 (I), 1:1000 (F)
CD117 (KIT)-PE conjugated	Mouse	Invitrogen	1:100 (F)
CD184 (CXCR4)-APC conjugated	Mouse	BD Biosciences	1:200 (F)
Cytokeratin 19 (KRT19)	Rabbit	Abcam	1:800 (I)
C-peptide	Rat	Developmental Studies Hybridoma Bank	1:1000 (I)
FOXA2	Rabbit	Abcam	1:500 (I)
Glucagon (GCG)	Mouse	Sigma-Aldrich	1:500 (I)
GP2	Human	MBL	1:800 (F)
KI67	Rabbit	Abcam	1:1000 (I, F)
KU80	Rabbit	Cell Signaling	1:450 (I)
NKX2-1	Rabbit	Abcam	1: 250 (I)
NKX6-1	Mouse	Developmental Studies Hybridoma Bank	1:1000 (F)
NEUROD1-AF657 conjugated	Mouse	BD Bioscience	1:3 (F)
PDX1	Goat	Abcam	1:10000 (I)
PDX1	Goat	R&D Systems	1:100 (F)
SLC18A1	Rabbit	Sigma	1:1000 (I)
Somatostatin (SST)	Rabbit	Abcam	1:500 (I)
SOX2	Rabbit	Cell signaling	1:400 (I, F)
Trypsin (PRSS1)	Sheep	R&D Systems	1:300 (I)
IgG-AF488	Goat	Jackson Immuno Research Labs	1:400 (F)
IgG-AF488	Mouse	Jackson Immuno Research Labs	1:800 (I)
IgG-AF488	Rabbit	Jackson Immuno Research Labs	1:800 (F)

IgG-Cy5	Mouse	Jackson Immuno Research Labs	1:800 (I)
IgG-Cy5	Rabbit	Jackson Immuno Research Labs	1:800 (I)
IgG-Cy5	Rat	Jackson Immuno Research Labs	1:800 (I)
IgG-AF647	Mouse	Jackson Immuno Research Labs	1:400 (F), 1:800 (I)
IgG-AF549	Mouse	Jackson Immuno Research Labs	1:800 (I)
IgG-AF549	Rabbit	Jackson Immuno Research Labs	1:800 (I)
IgG-R-Phycoerythrin-conjugated	Mouse	Jackson Immuno Research Labs	1:800 (F)

Supplementary Table 2. List of QPCR primers. *Related to Figures 3 and 4.*

Gene	***Forward primer (5' to 3')***	***Reverse primer (3' to 5')***
CDX2	*AGC CAA CCT GGA CTT CCT GTC ATT*	*ACA CAG ACC AAC AAC CCA AAC AGC*
CELA3A	*ATG ACA TGC CCC TCA TCA AGC TCT*	*ATG TAG CAG GGT GTC TTG TTG GGA*
GP2	*AAC CCT TCC GAA GCA CAG AG*	*GGA CAC AGG TCT CCG ACA TC*
HNF1B	*AGA GTA ACA TGC CAG CTT CCT CCT GTG*	*TAT CAA ACA GCC AGT TTC CCT CCT GCC*
MKI67	*AAT TTG CTT CTG GCC TTC CC*	*GAC CCC GCT CCT TTT GAT AGT*
NEUROD1	*TCC CAT GTC TTC CAC GTT AAG CCT*	*CAT CAA AGG AAG GGC TGG TGC AAT*
NEUROG3	*GCG CAA TCG AAT GCA CAA CCT CAA*	*TTC GAG TCA GCG CCA AGA TGT AGT T*
NKX2-1	*GCC AAA CTG CTG GAC GTC TTT CTT*	*CCT TGA GAT TGG ATG CGC TTG GTT*
POU5F1	*ATG CAT TCA AAC TGA GGT GCC TGC*	*CCA CCC TTT GTG TTC CCA ATT CCT*
PDGFRA	*GGCAGTACCCCATGTCTGAAG*	*CGTCACAAAAAGGCCGCTG*
RPL19	*CTCGATGCCGGAAAAACACC*	*TTCTCTGGCATTCGGGCATT*
SOX2	*GGA TAA GTA CAC GCT GCC CG*	*ATG TGC GCG TAA CTG TCC AT*
TOP2A	*GCT GCC CCA AAA GGA ACT AA*	*GGC GAT TCT TGG TTT TGG CA*
ZIC3	*TAT CAG TCT CGC GCT CAC*	*TGT CTT TTG CGG TTT ATC TTC CTG*