Supplemental Data

INHIBITORS OF SRC AND FOCAL ADHESION KINASE PROMOTE ENDOCRINE SPECIFICATION: IMPACT ON THE DERIVATION OF β-CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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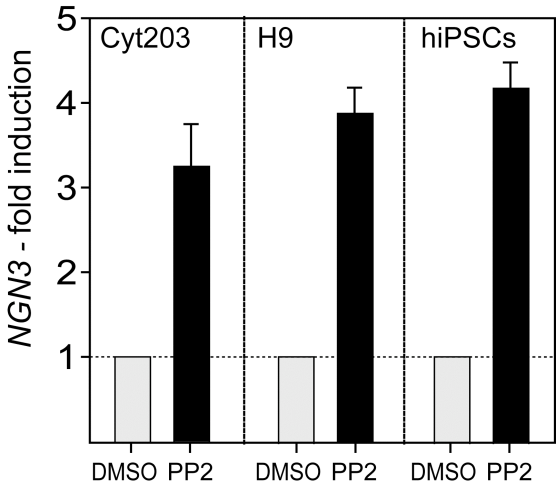
Table S1: Taqman Primers and Probes used in this study

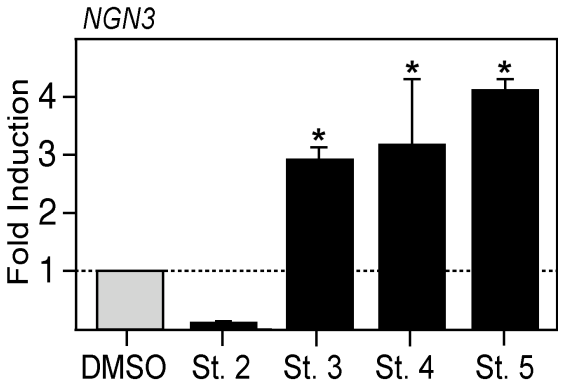
Gene	**Assay ID#**
Neurogenin 3 (Ngn3)	Hs00360700_g1
NeuroD1	Hs00159598_m1
NK2 Homeobox 2 (Nkx 2.2)	Hs00159616_m1
Insulin promoter factor 1 (Pdx1)	Hs 00426216_m1
p48 (PTF1a)	Hs00603586_g1
Insulin (Ins)	Hs 00355773_m1
Glucagon (Gcg)	Hs01031536_m1
Ghrelin (GHRL)	Hs01074053_m1
Somatostatin (Sst)	Hs00356144_m1
Pancreatic Polypeptide (PPY)	Hs00358111_g1
Paired box 4 (Pax4)	Hs00173014_m1
Paired box 6 (Pax6)	Hs01088112_m1
Cyclophilin A (CycA)	Hs99999904_m1
Aristaless-related homeobox gene (Arx)	Hs00292465_m1
POU3F4 POUclass3homeobox 4 (Brn4)	Hs00264887_s1
BHLHA15/MIST1	Hs 00703572_s1
CDKN1B (p27kip1)	Hs00153277_m1
CDKN1C (p57kip2)	Hs00175938_m1
Ribosomal protein 5/6	Hs01598518_gH
Inhibitor of DNA binding 1, Id1	Hs00357821_gl
Inhibitor of DNA binding 2, Id2	Hs00747379_ml

Figure S1: PP2 induces *NGN3* gene expression in different hESC lines and in hiPSCs



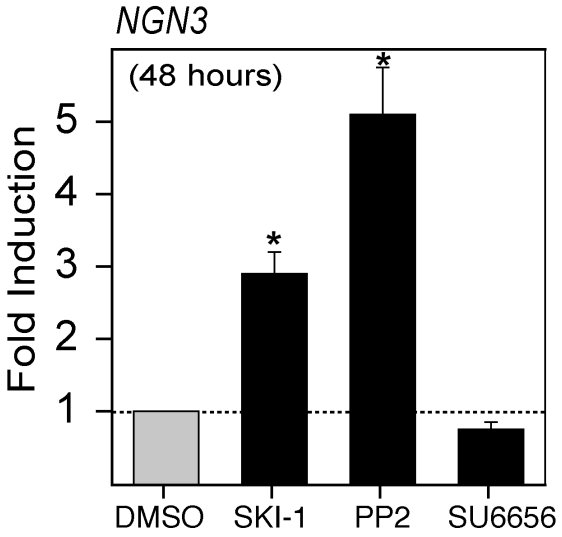
PP2 (10μM) or DMSO alone was added to CyT203, H9 or hiPSC cells at the start of stage-3 culture. PP2 was removed after 48 hours and the cultures were then harvested for Q-PCR 24 hours later. Results show the fold induction of *NGN3* relative to the DMSO treated control group. The hiPSCs used were generated from human skin fibroblasts and were reprogrammed using a non-integrating episomal vector encoding OCT4, NANOG, SOX2 and LIN28. In order to optimize the generation of endocrine progenitors culture times were altered for H9 hESCs such that these cells were incubated for 3 days under stage-1 conditions and 4 days under stage-2 conditions. Stage-3 culture remained at 3 days.

Figure S2: PP2 induces *NGN3* gene expression during stage-3, -4 and -5 culture.



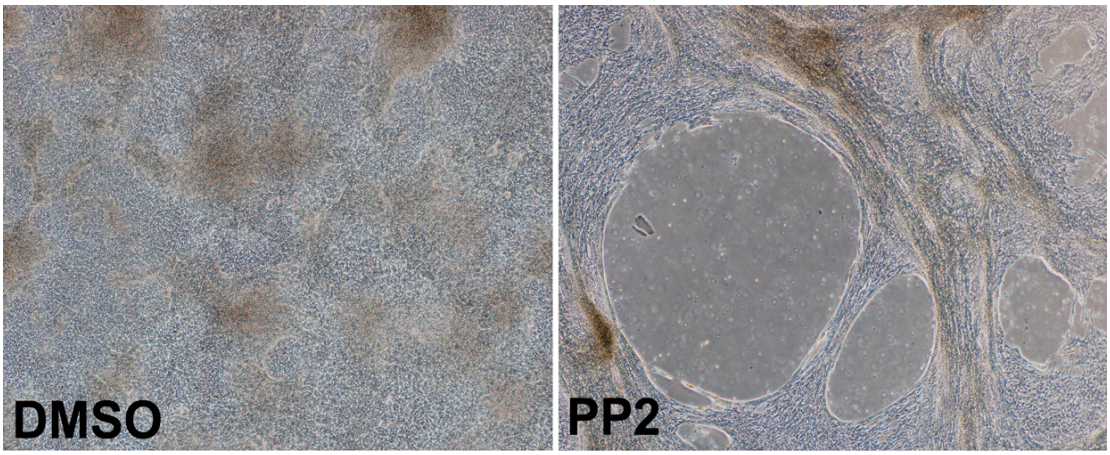
PP2 (10μM) or DMSO alone was added to CyT49 cells at the start of stage-2, -3, -4 or -5 culture for 48 hours. Cells treated during stages-2 and -3, were harvested at the end of stage-3. Cells treated during stage-4 or -5 culture were harvested at the end of stage-4 and -5 culture respectively. Q-PCR was performed to assess NGN3 expression. Results show the fold induction of *NGN3* relative to the DMSO treated control groups. *Cultures exposed to PP2 during stages-3-5 expressed significantly more NGN3 ($P<0.01$). Please note that absolute levels of NGN3 expression were significantly lower by the end of stage-5 culture (not shown).

Figure S3: Src-inhibitor SKI-1 can also induces *NGN3* gene.



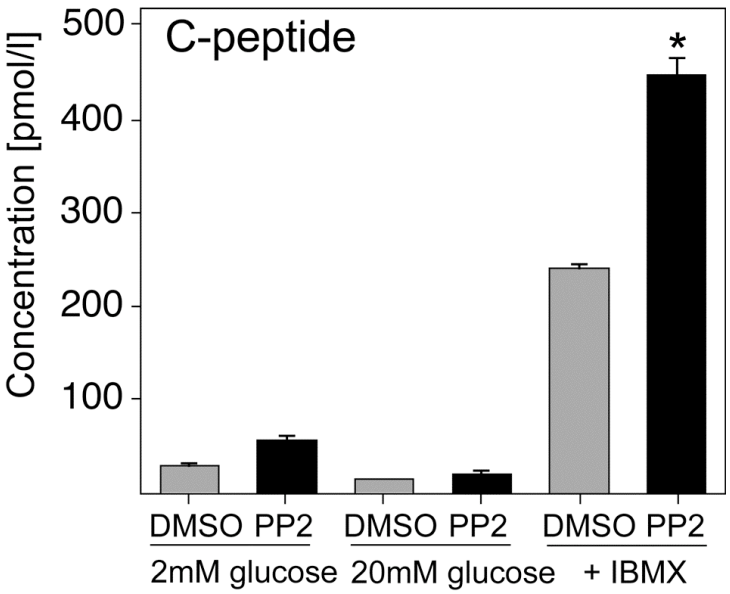
SKI-1 (2μM), SU6656 (5μM), PP2 (10μM) or DMSO alone were added to CyT49 cells at the start of stage-3 culture and the cells were harvested 48 hours later. Q-PCR was performed to assess NGN3 expression. Results show the fold induction of *NGN3* relative to the DMSO treated control groups. *Cultures exposed to PP2 or SKI-1 expressed significantly more NGN3 relative to DMSO control ($P<0.01$; $n=3$).

Figure S4: PP2 induces stage-3 monlayer cultures to retact and form cord-like structures



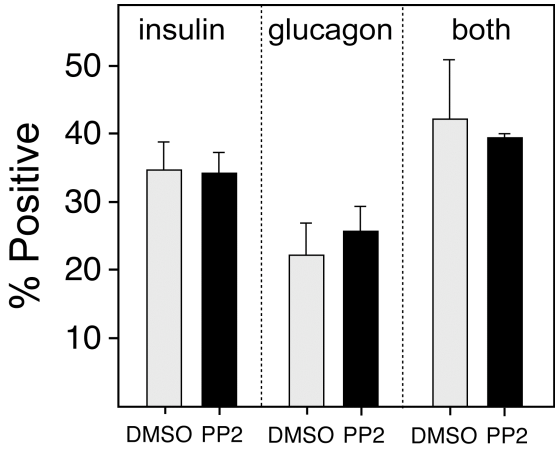
PP2 (10μM) or DMSO vehicle alone was added to CyT49 cells at the start of stage-3 culture (day 6). The inhibitor was added for 48 hours and cell monolayers were then photographed on day 9 using a 5x objective.

Figure S5: Cultures previously exposed to PP2 release more C-peptide.



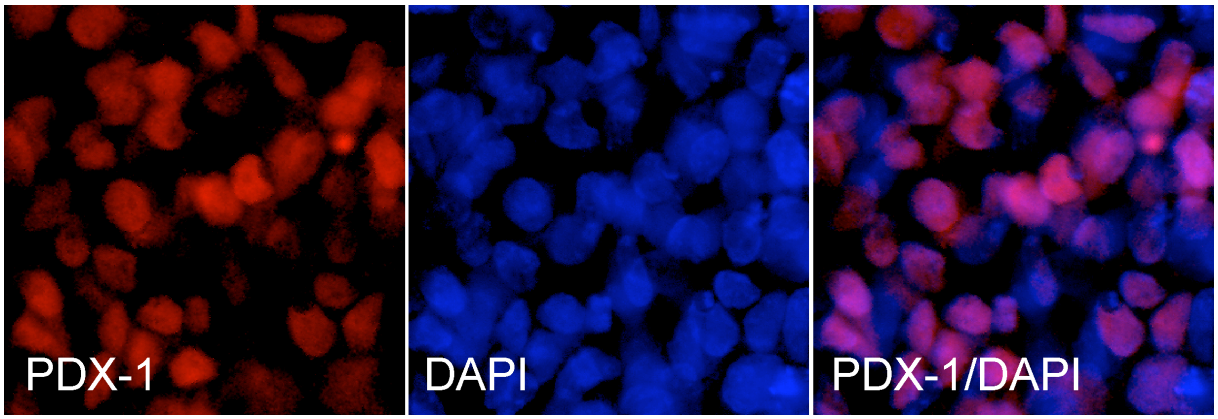
C-peptide release was measured after incubating stage-5 cultures for 1 hour in a glucose-free basal medium supplemented with 2 mM D-glucose. This was then followed by a 1 hour incubation in basal medium supplemented with 20 mM D-glucose with or without 0.5 mM IBMX. C-peptide in culture supernatants were measured using the ultrasensitive C-peptide ELISA (Alpco Diagnostics). *Cultures previously exposed to PP2 during stage-3 differentiation released significantly more C-peptide in response to IBMX ($P<0.01$).

Figure S6: Percentage of hormone-positive cells co-expressing insulin and glucagon after prior treatment with PP2 or DMSO alone.



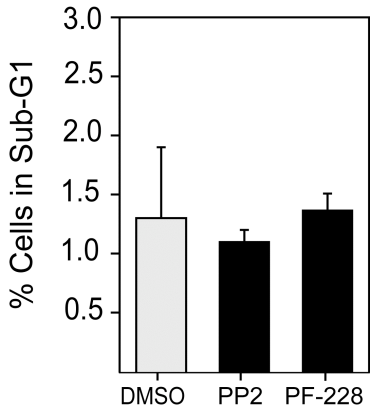
PP2 (10μM) or DMSO vehicle alone was added to CyT49 cells for 48 hours at the start of stage-3 culture (day 6). At the end of stage-5 culture (day-15) the cells were doubled stained for insulin and glucagon Results show the percentage of total glucagon and insulin-positive cells either expressing insulin or glucagon alone or expressing both hormones.

Figure S7: PDX-1 expression is confined to the nuclei of putative stage 3 pancreatic progenitors.



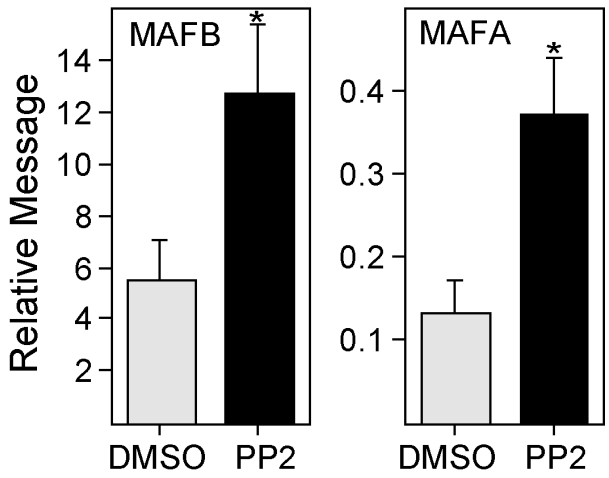
Photomicrographs (40x) showing CyT49 stage-3 cultures stained for PDX1 (red) and DAPI to mark nuclei (blue). Stage-3 cultures were differentiated in the presence of PP2 for 48 hours prior to staining. Note that the PDX-1 staining appears to be primarily confined to cell nuclei.

Figure S8: Impact of PP2 and PF-228 on the percentage of apoptotic cells in stage-3 cultures



PP2 (10μM), PF-228 (2μM) or DMSO vehicle alone was added to CyT49 cells at the start of stage-3 culture (day 6). After 48 hours the cells were harvested, fixed with 70% ethanol, and were stained with propridium iodide (10μg/ml). DNA histograms were generated using a Becton Dickinson FACScan. Results show the percentage of cells detected in the sub-G1 gate corresponding to hypodiploid or apoptotic cells (± 1 SD) (n=2).

Figure S9: Impact of prior PP2 treatment on MAFA and MAFB expression by the end of stage 5 culture



CyT49 hESCs were cultured in the presence or absence of PP2 (10μM) for the first two days of stage-3 culture. Q-PCR was then used to assess the levels of *MAFB* and *MAFA* expression induced by the end of stage-5. Consistent with increased β-cell derivation, prior exposure of PP2 significantly increased the levels of both MAFB and MAFA (*DMSO *versus* PP2; $P<0.01$). Note that the absolute levels of MAFB expression are significantly higher than that of MAFA. Results show expression relative to a standard curve generated using fetal insulin-like cell cultures.