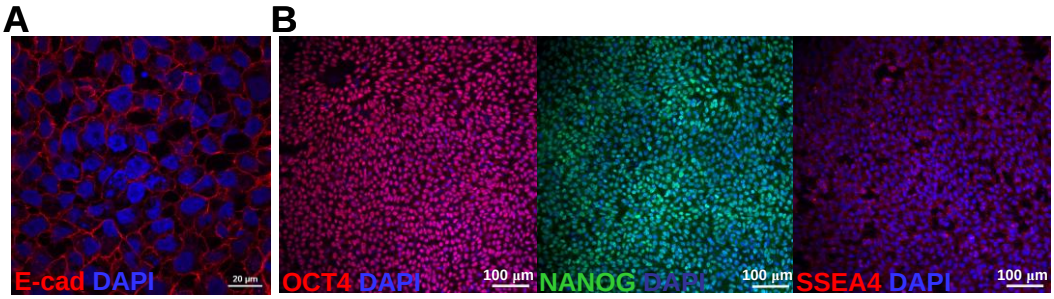
SUPPLEMENTAL MATERIAL

Detailed Methods

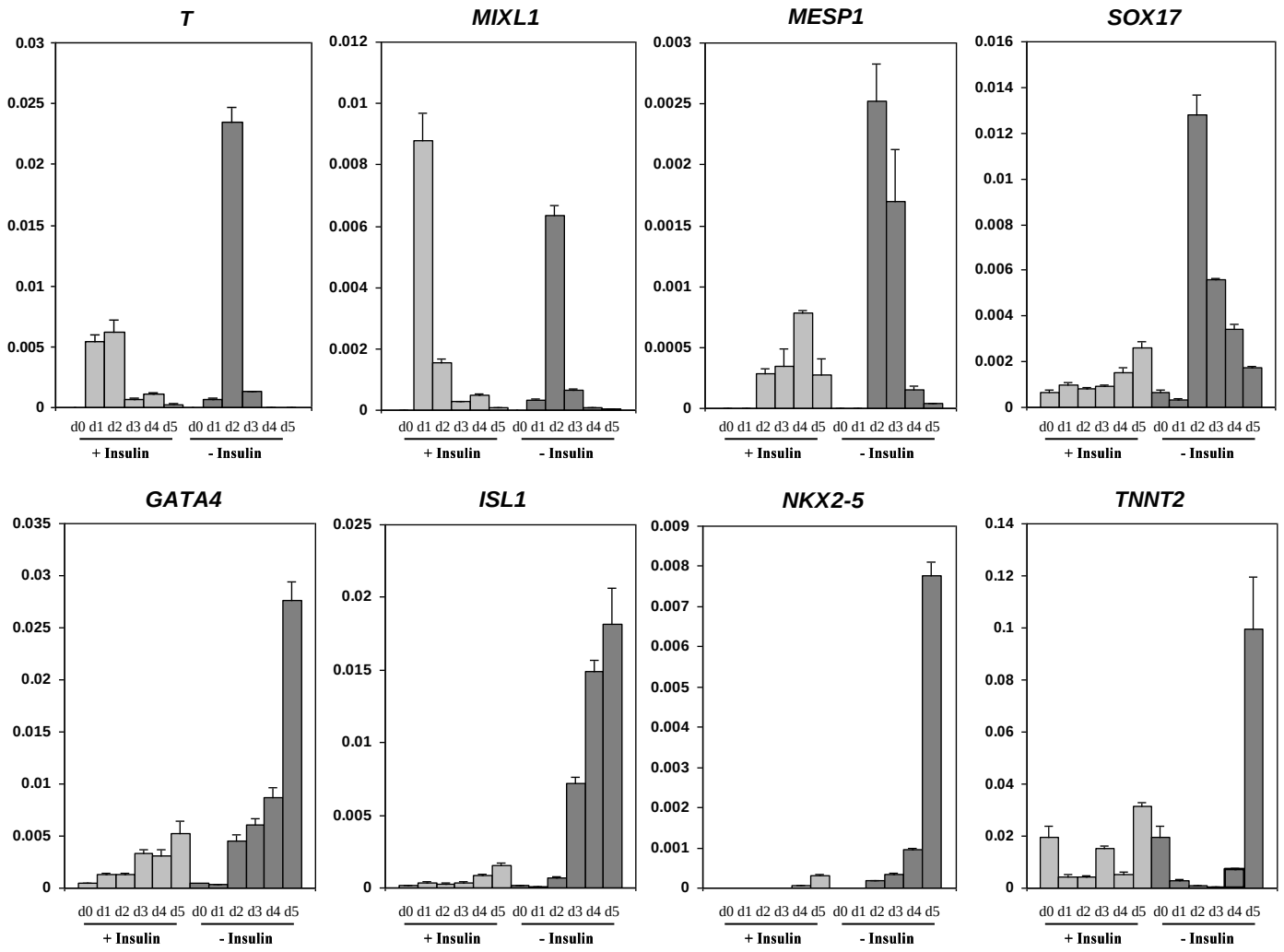
Primary antibodies used in flow cytometry
Monoclonal anti-human brachyury –APC (R&D Systems); mouse monoclonal cTnT (IgG1, Thermo Scientific, 1:200 dilution); mouse monoclonal smooth muscle actin (IgG2a, Thermo Scientific, 1:100 dilution); mouse monoclonal MLC2a (IgG2b, Synaptic Systems, Germany, 1:400 dilution); rabbit polyclonal MLC2v (IgG, ProteinTech Group, 1:200 dilution); mouse monoclonal antibody for sarcomeric myosin, MF20 (IgG2b, Developmental Studies Hybridoma Bank, Iowa City, IA, 1:20 dilution); mouse anti-human Ki-67 (IgG1, BD Biosciences, 1:100 dilution); FITC mouse anti-human CD31 (BD Biosciences Cat. No. 555445); anti-myosin, smooth muscle (rabbit IgG, Biomedical Technologies, Inc., 1:500 dilution); mouse anti-human fibroblasts (clone TE-7) (IgG1, Chemicon, 1:100 dilution).

Primary antibodies used in immunolabeling
Mouse monoclonal Oct3/4 ($IgG_{2b,}$ Santa Cruz, 1:100 dilution); rabbit polyclonal Nanog (IgG, Cosmo Bio Co Ltd, 1:100 dilution); mouse monoclonal SSEA4 (IgG_3, Abcam 1:200 dilution); goat polyclonal anti-human E-cadherin (IgG, R&D, 1:100 dilution); rabbit polyclonal N-cadherin (IgG, Santa Cruz, 1:100 dilution); rabbit polyclonal laminin (IgG, Sigma, 1:500 dilution); goat polycolonal brachyury (IgG, R&D, 1:50 dilution); mouse monoclonal cTnT (IgG_1, Thermo Scientific, 1:200 dilution); mouse monoclonal α-actinin ($IgG_{1,}$ Sigma, 1:500 dilution); mouse monoclonal smooth muscle actin (IgG_{2a}, Thermo Scientific, 1:100 dilution); mouse monoclonal MLC2a (IgG_{2b}, Synaptic Systems, Germany, 1:400 dilution); rabbit polyclonal MLC2v (IgG, ProteinTech Group, 1:200 dilution).

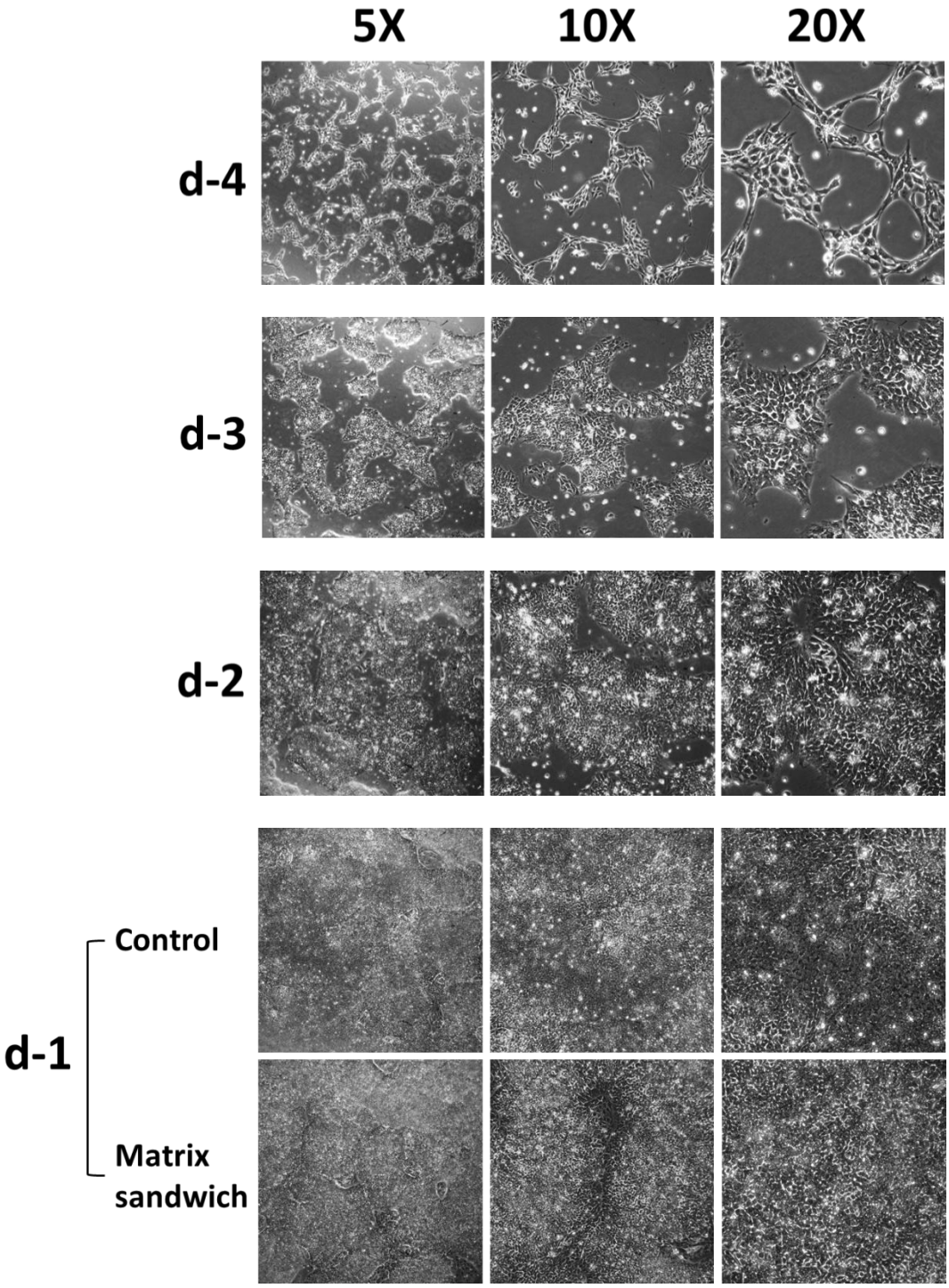
Supplemental Figures

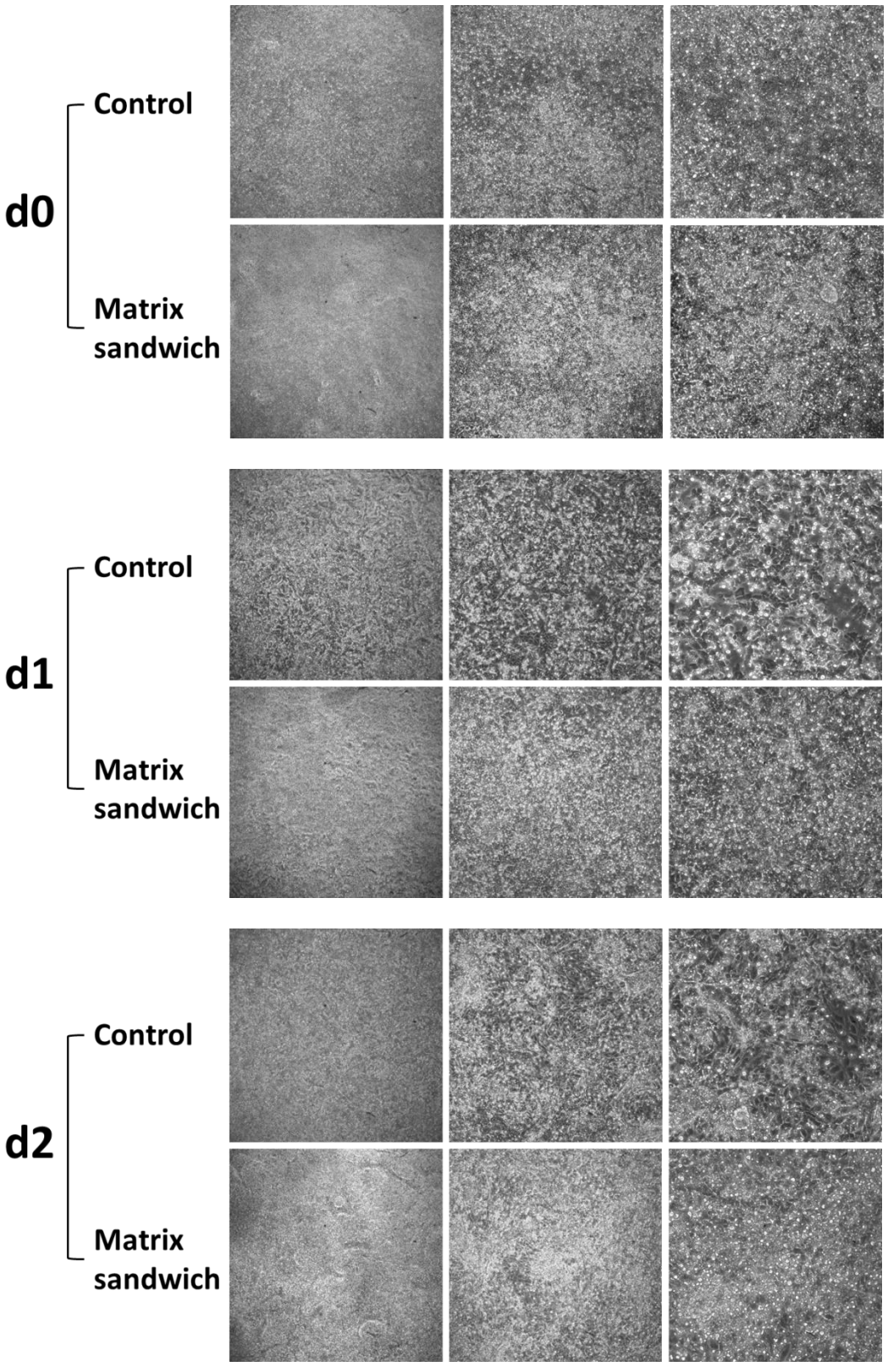


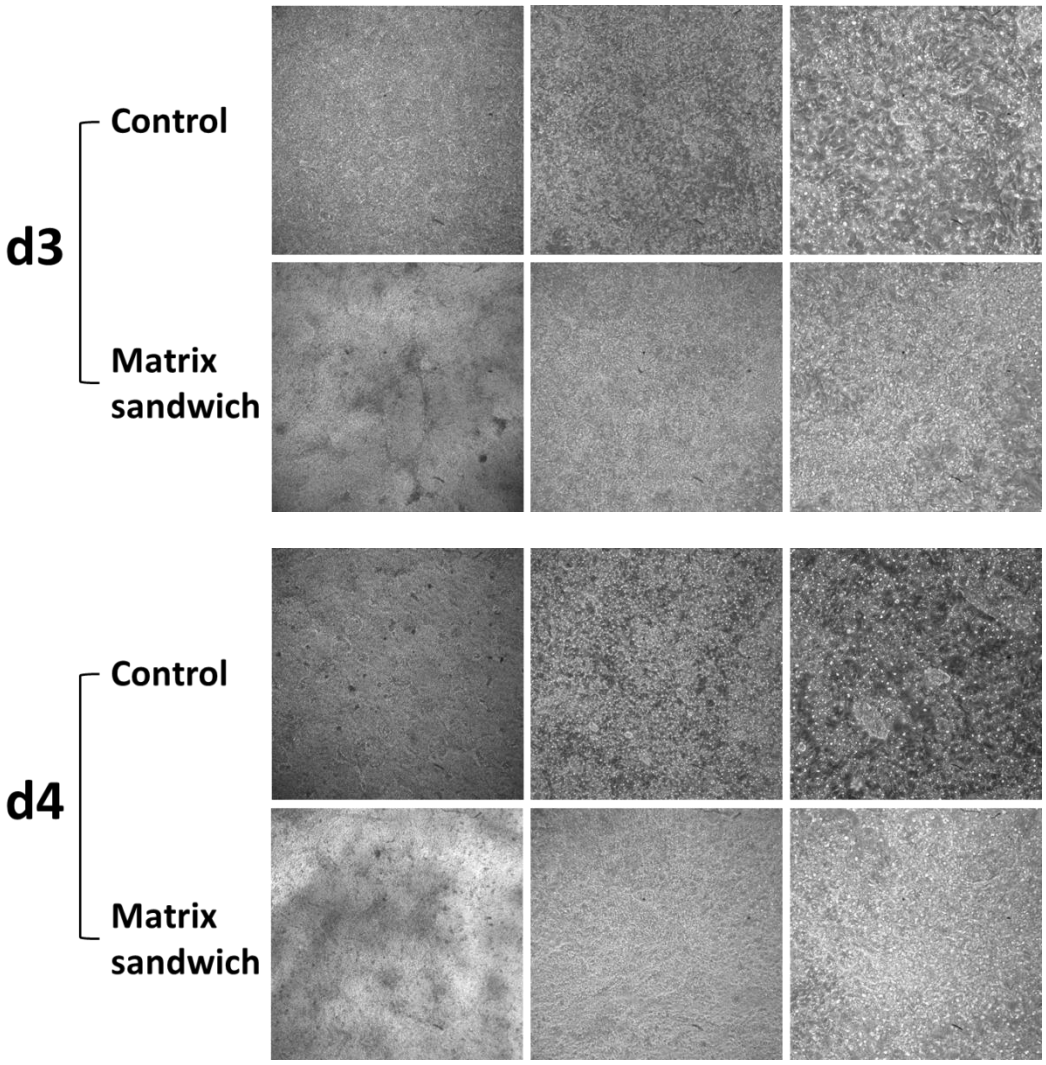
Online Figure I. Monolayer cultured human PSCs exhibit epithelial phenotype and express pluripotency markers. (**A**) Immunolabeling of monolayer cells (DF19-9-11T) cultured in mTeSR1 medium showing the adhesion junction protein E-cadherin present on the lateral surfaces of the cells. Scale bar is 20 µm. (**B**) Immunolabeling of monolayer cells (DF19-9-11T) for OCT4, NANOG and SSEA4 showing robust expression of these pluripotency markers. Scale bars are 100 µm.

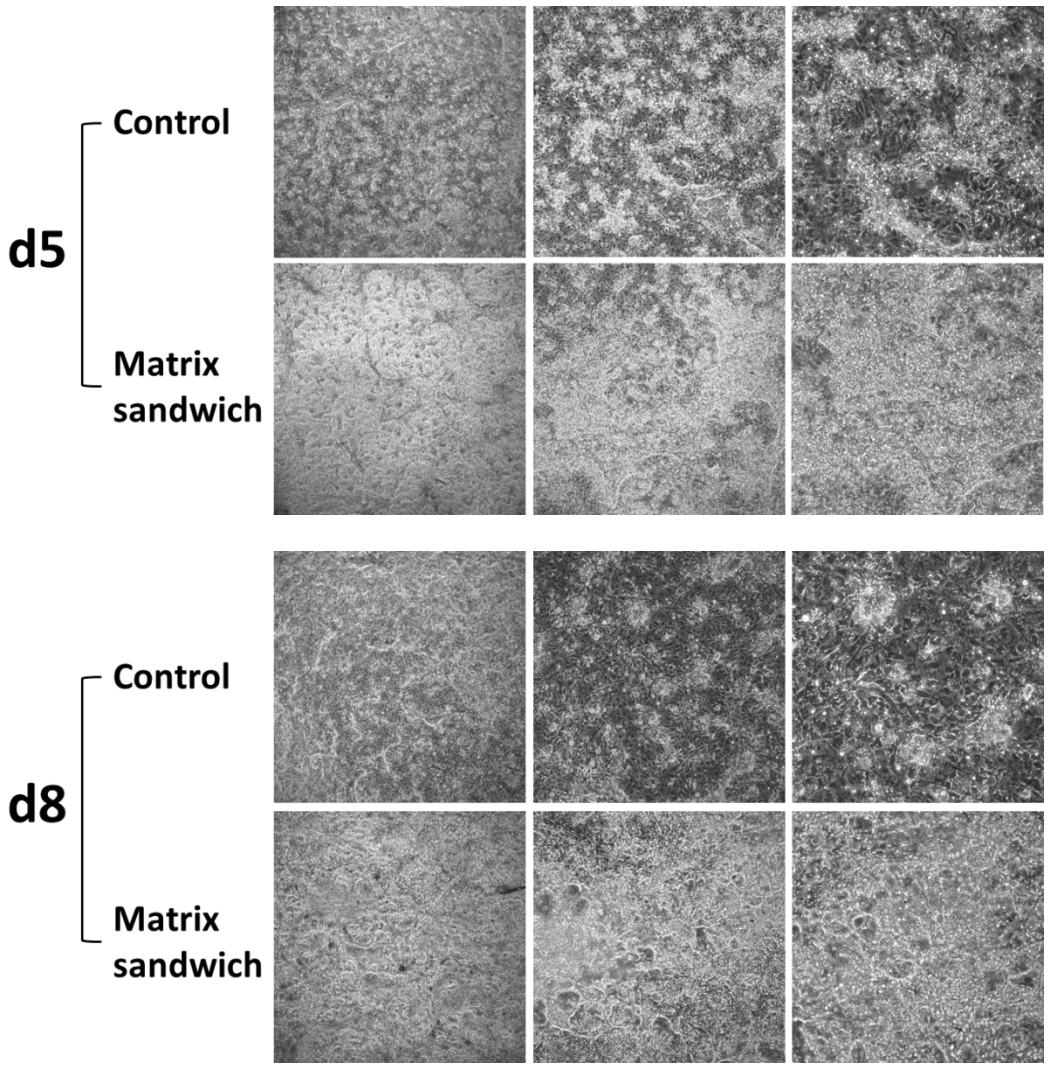


Online Figure II. Insulin inhibits cardiogenesis of human PSCs during stage 2 of the matrix sandwich protocol. Total RNA was isolated from cells at days 0-5 of the matrix sandwich protocol in the RPMI medium supplemented B27 with or without insulin. Quantitative RT-PCR for early mesoderm markers and cardiac transcription factors was performed. The relative gene expression was normalized to the endogenous control β-actin. Samples without insulin generally showed a greater expression of cardiac genes. Error bars represent SEM.

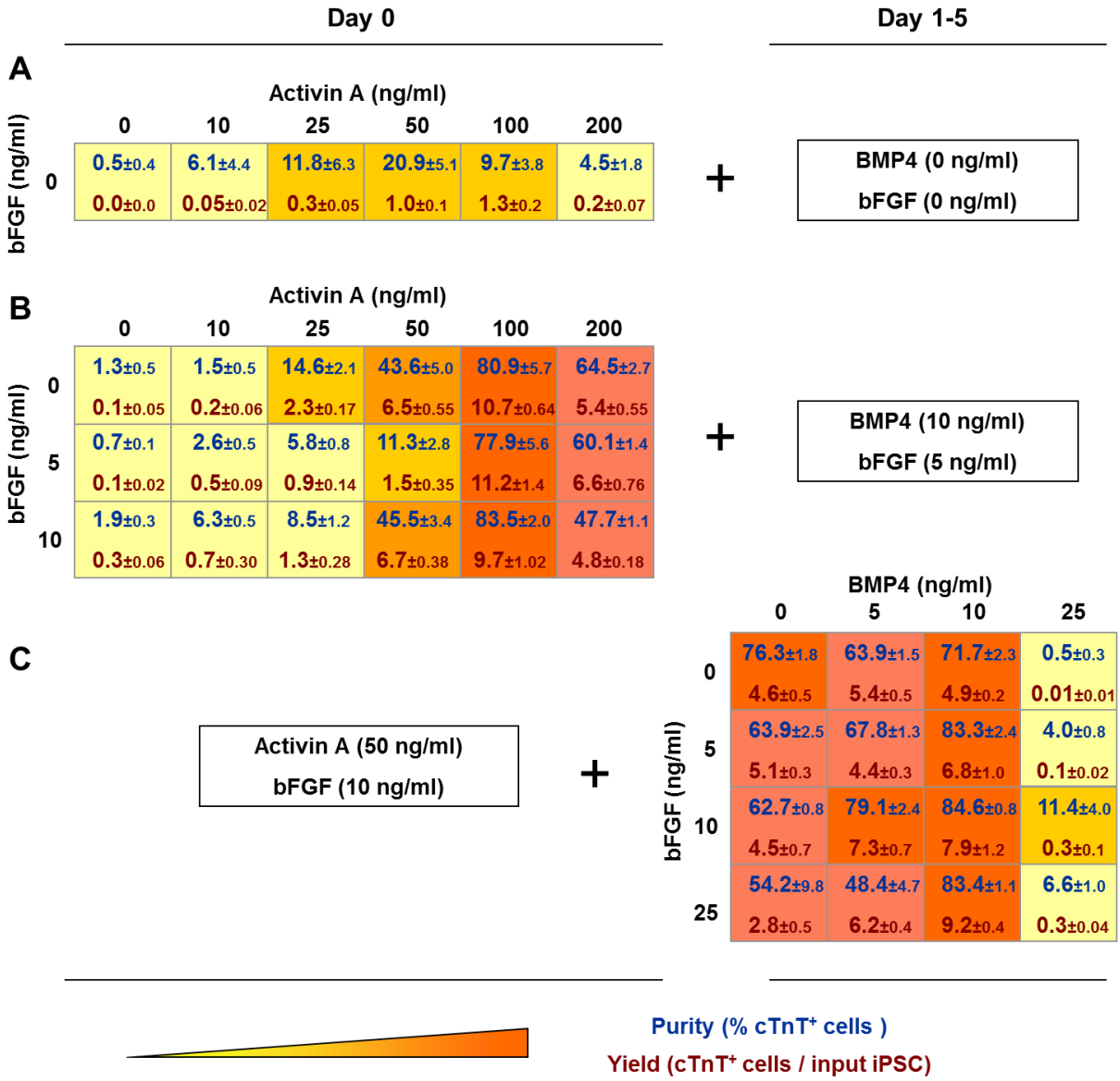

5X
10X
20X
d-4
d-3
d-2
d-1
Control
Matrix sandwich


d0
Control
Matrix sandwich
d1
Control
Matrix sandwich
d2
Control
Matrix sandwich

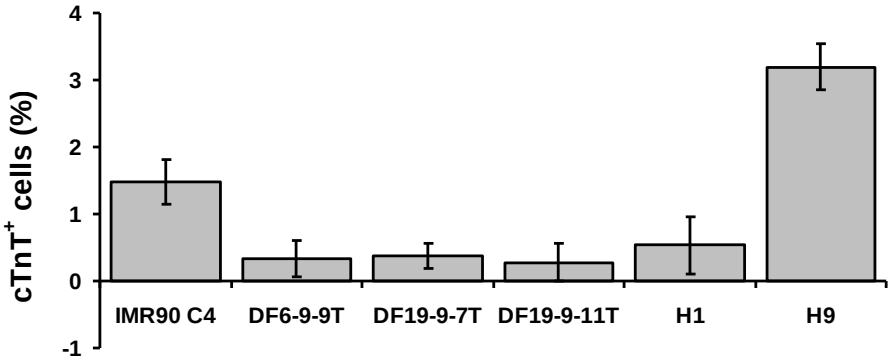

d3
Control
Matrix sandwich
d4
Control
Matrix sandwich



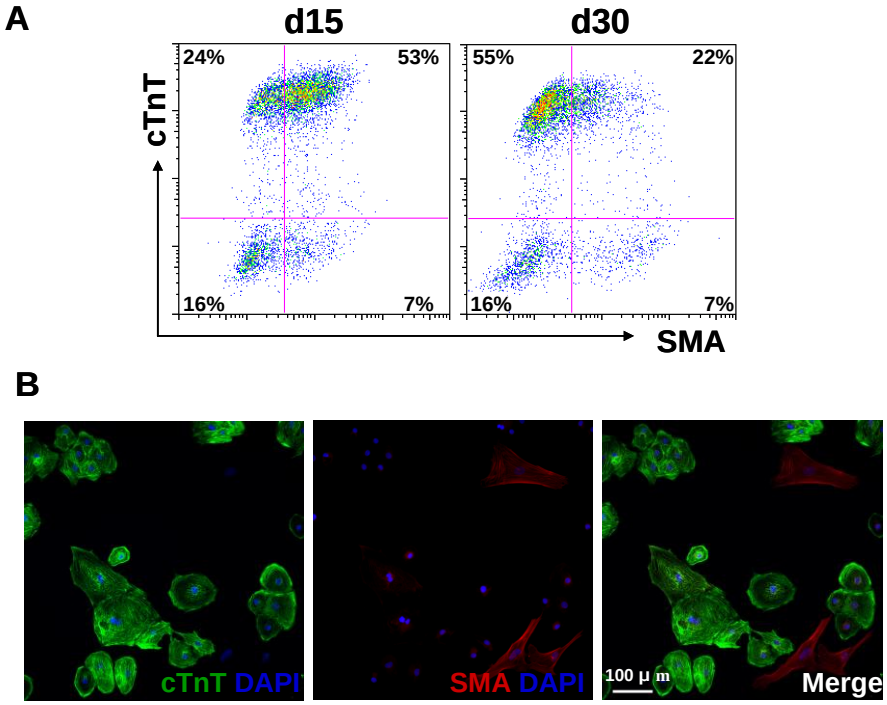
Online Figure III. Phase contrast images showing iPSCs DF19-9-11T from day -4 to day 8 of differentiation for both control (without Matrigel overlays) and matrix sandwich culture using the protocol as shown in Figure 3A. Images were taken under 5X, 10X and 20X objectives every 24 hours after single cell seeding on Matrigel coated 6-well plate, d0 images show the cells before adding Activin A.



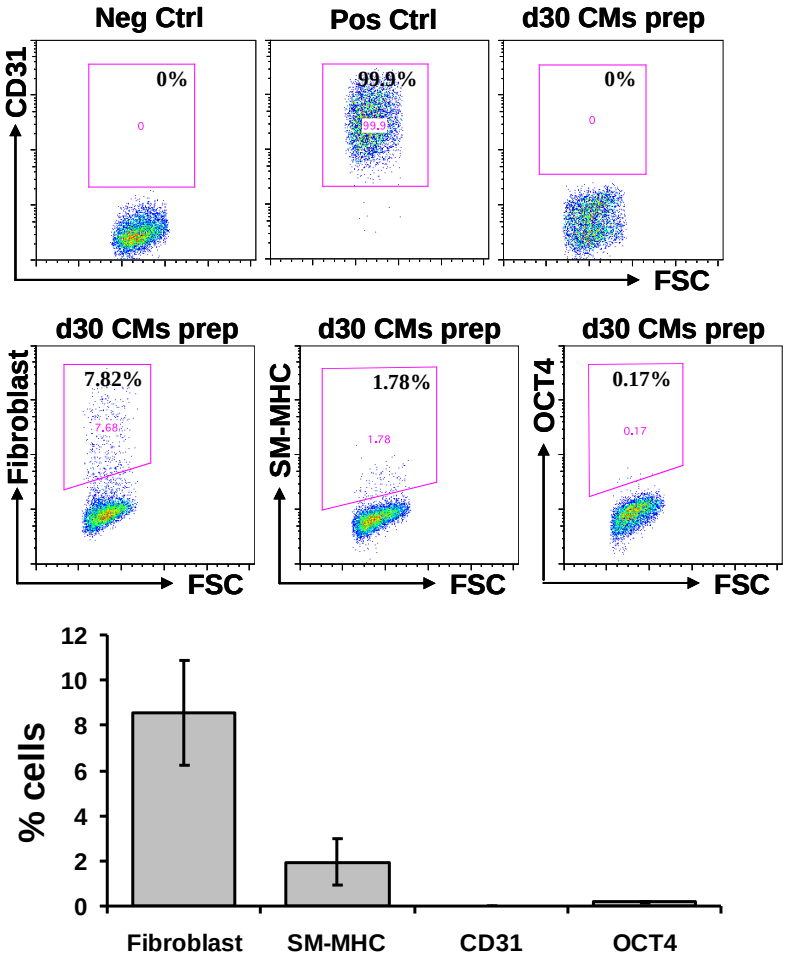
Online Figure IV. Concentration-dependent effects of Activin A, BMP4 and bFGF on cardiogenesis of human PSCs using the matrix sandwich protocol. Cardiogenesis was measured by flow cytometry of cTnT^{+} CMs at 15 days differentiation. The purity and the yield of cTnT^{+} CMs are displayed in the tables as mean ± SEM and associated with the heat map. (**A**) The effect of single growth factor, Activin A, added at day 0 of the matrix sandwich protocol. (**B**) The effect of different concentrations of Activin A and bFGF added on day 0, followed by BMP4 (10 ng/ml) and bFGF (5 ng/ml) at day 1 of the matrix sandwich protocol. (**C**) The effect of different concentrations of BMP4 and bFGF added at day 1 following 24 hour treatment with Activin A (50 ng/ml) and bFGF (10 ng/ml).



Online Figure V. Low efficiency and variable cardiac differentiation of hPSC lines using serum-based EB method. Efficiency of cardiac differentiation was measured by flow cytometry for cTnT$^+$ CMs in EB culture at 30 days of differentiation. Error bars represent SD. One-way ANOVA with Tukey test showed that the percentages of cTnT$^+$ cells fromH9 and IMR90 C4 were significantly different from the other cell lines, $P<0.05$.



Online Figure VI. Expression of smooth muscle actin (SMA) in early and late differentiated CMs using the matrix sandwich protocol. (**A**) Flow cytometry analysis comparing matrix sandwich differentiated cells (DF19-9-11T) after 15 and 30 days for expression of cTnT and SMA. (**B**) Immunofluorescence images of re-plated cells differentiated from the iPSCs (DF19-9-11T) for 30 days using the matrix sandwich protocol and labeled with antibodies to the cTnT and SMA. Scale bar is 100 µm.



Online Figure VII. Characterization of non-CMs in the matrix sandwich culture differentiated from human PSCs. Human ESCs and iPSCs were differentiated for 30 days using the matrix sandwich protocol and labeled with fibroblast (clone TE-7), smooth muscle myosin heavy chain (SM-MHC), CD31 and Oct4 antibodies for flow cytometry. CD31 labeling was performed with live cell labeling, negative control was secondary antibody only, and positive control was the endothelial cells differentiated from hESCs. The average data from iPSCs DF19-9-11T for fibroblast, SM-MHC, Oct4 and CD31 positive cells were plotted in the bar graph. Error bars represent SEM.

Supplemental Tables

Online Table I. Primers for RT-PCR and quantitative RT-PCR

Primers for RT-PCR

Genes	Sequences (5' - 3')	Size (bp)
OCT4	**F:** CAGTGCCCGAAACCCACAC **R:** GGAGACCCAGCAGCCTCAAA	161
NANOG	**F:** CAGAAGGCCTCAGCACCTAC **R:** ATTGTTCCAGGTCTGGTTGC	111
NKX2-5	**F:** GCGATTATGCAGCGTGCAATGAGT **R:** AACATAAATACGGGTGGGTGCGTG	220
TNNT2	**F:** TTCACCAAAGATCTGCTCCTCGCT **R:** TTATTACTGGTGTGGAGTGGGTGTGG	165
MYH6	**F:** GGGGACAGTGGTAAAAGCAA **R:** TCCCTGCGTTCCACTATCTT	542
ACTN2	**F:** GGCGTGCAGTACAACTACGTG **R:** AGTCAATGAGGTCAGGCCGGT	580
MYL7	**F:** GAGGAGAATGGCCAGCAGGAA **R:** GCGAACATCTGCTCCACCTCA	449
MYL2	**F:** ACATCATCACCCACGGAGAAGAGA **R:** ATTGGAACATGGCCTCTGGATGGA	164
HPPA	**F:** GAACCAGAGGGGAGAGACAGAG **R:** CCCTCAGCTTGCTTTTTAGGAG	406
PLN	**F:** ACAGCTGCCAAGGCTACCTA **R:** GCTTTTGACGTGCTTGTTGA	191
ACTB	**F:** CCTGAACCCTAAGGCCAACCG **R:** GCTCATAGCTCTTCTCCAGGG	400
SOX1	**F:** CAATGCGGGGAGGAGAAGTC **R:** CTCTGGACCAAACTGTGGCG	464
PAX6	**F:** GGCAGGTATTACGAGACTGG **R:** CCTCATCTGAATCTTCTCCG	427

SOX17	**F:** CGCACGGAATTTGAACAGTA **R:** GGATCAGGGACCTGTCACAC	180
FOXA2	**F:** GGGAGCGGTGAAGATGGA R: TCATGTTGCTCACGGAGGAGTA	326
T	**F:** CTTCCCTGAGACCCAGTTCA **R:** CAGGGTTGGGTACCTGTCAC	289
MESP1	**F:** CGCTATATCGGCCACCTGTC **R:** GGCATCCAGGTCTCCAACAG	378
GATA4	**F:** TCCAAACCAGAAAACGGAAG **R:** AAGACCAGGCTGTTCCAAGA	352
ISL1	**F:** CACAAGCGTCTCGGGATT **R:** AGTGGCAAGTCTTCCGACA	202
TNNI3	**F:** CTGCAGATTGCAAAGCAAGA **R:** CCTCCTTCTTCACCTGCTTG	379

Primers for quantitative RT-PCR

Genes	**TaqMan® Gene Expression Assay ID**	**Size (bp)**
ACTB	Hs99999903_m1	171
CDH1	Hs01023894_m1	61
CDH2	Hs00983056_m1	66
SNAIL1	Hs00195591_m1	66
SNAIL2	Hs00950344_m1	86
GSC	Hs00418279_m1	81
VIM	Hs00185584_m1	73
FN1	Hs01549976_m1	81
T	Hs00610080_m1	132
MIXL1	Hs00430824_g1	152
MESP1	Hs00251489_m1	80

SOX17	Hs00751752_s1	149
GATA4	Hs00171403_m1	68
ISL1	Hs00158126_m1	57
NKX2-5	Hs00231763_m1	64
TNNT2	Hs00165960_m1	89

Legends for Video Files

Online Movie 1 and 2. Contracting sheet of CMs differentiated from the transgene-free iPSCs (DF19-9-11T) using the matrix sandwich protocol. Movies were taken at 15 days differentiation. Movie S1 was taken with 20X magnification, and Movie S2 was with 100X magnification.

Online Movie 3. Optical mapping demonstrating reentry in human PSC-derived CM monolayers. Phase movies showing electrical reentry in ESC-H9 (*left*) and iPSC-DF19-9-11T (*right*) CM monolayers. Green represents the depolarization phase of the propagating action potential, phase zero; red represents phase 2 or the plateau phase of the action potential; finally orange and yellow represent phase 3 repolarization of the action potential.