Supplemental Data

Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins

Dohoon Kim, Chun-Hyung Kim, Jungil Moon, Young-Gie Chung, Mi-Yoon Chang, Baek-Soo Han, Sanghyeok Ko, Eungi Yang, Kwang Yul Cha , Robert Lanza, Kwang-Soo Kim

Supplemental Experimental Procedures

Cell Culture

Human newborn fibroblasts (HNF) were purchased from ATCC (CCL-117) and cultured in Dulbecco's modified Minimal Essential Medium (DMEM, Invitrogen, Carlsbad, CA), supplemented with 2mM L-glutamine (Invitrogen, Grand island, NY). 1mM β-mercaptoethanol, 1x non-essential amino acids (NEAA; Invitrogen, Carlsbad, CA), 15% fetal bovine serum (FBS, Hyclone, Thermo Scientific, Logan UT), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen, Grand island, NY). Reprogramming experiments were started when the cultures reached 10-20% confluence. Cultures were maintained at 37°C and 5% CO_2, and the media changed every other day. For mouse embryonic fibroblast (MEF) isolation, uteri were isolated from 13.5-day-pregnant CD1 mice and washed with phosphate-buffered saline (PBS). The head and visceral tissues were removed, and the remaining bodies washed with fresh PBS, transferred into a 0.1 mM trypsin/1 mM EDTA solution, and incubated for 20 min. After incubation, MEF culture medium (DMEM containing 15% defined FBS) was added and pipetted up and down to dissociate cells. MEFs were used as feeders at passages one to three.

Induced pluripotent stem (iPS) cells were generated and maintained in human ES media 3 (DMEM (Invitrogen, Carlsbad, CA), supplemented with 2mM L-glutamine (Invitrogen), 1mM β-mercaptoethanol, 1x non-essential amino acids (NEAA; Invitrogen, Carlsbad, CA), 20% knock-out serum replacement (KSR, Invitrogen, Carlsbad, CA), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen)). hES and hiPS cells were maintained on feeder layers consisting of mitomycin C (10μg/ml media, Sigma-Aldrich, St. Lake, MO)-treated MEF cells. For picking and passaging, hiPS cells were washed once with ES media 3, incubated with 0.1 % collagenase type IV solution (StemCell Tech., Vancouver, BC, Canada) for 10 min, and then mechanically disrupted. An appropriate volume of medium was added and hiPS cells were transferred onto MEF feeder cells on a new dish. The cultures were split at a 1:1 ratio until passage 3, and 1:5 thereafter. For feeder-free culture of iPS cells, the plates were coated with gelatin (StemCell Tech).

Plasmid Construction

Human cDNAs for Oct4, Sox2, Klf4, and c-Myc were amplified by RT-PCR from human ES poly(A^+)RNA using the following primers: 5'-GGA TCC GAA TTC ATG GCG GGA CAC CTG GCT TCGG-3' and 5'-AAA AAA GTC GAC GCG GCG TCT GCG TCT GCG GCG TCT GCG GTT TGA ATG CAT GGG AGA GCC-3' for human Oct4, 5'-GGA TCC GAA TTC ATG TAC AAC ATG ATG GAG ACG G-3' and 5'-AAA AAA CTC GAG GCG GCG TCT GCG TCT GCG GCG TCT GCG CAT GTG CGA CAG GGG CAG TG-3' for human Sox2, 5'-GGA TCC GAA TTC ATG GCT GTC AGC GAC GCG CTG C-3' and 5'-AAA AAA CTC GAG GCG GCG TCT GCG TCT GCG GCG TCT GCG AAA GTG CCT CTT CAT GTG TAA GGC -3' for human Klf4, and 5'-GGA TCC GAA TTC ATG CCC CTC AAC GTT AGC TTC AC-3' and 5'-AAA AAA CTC GAG GCG GCG TCT GCG TCT GCG GCG TCT GCG CGC ACA AGA GTT CCG TAG CTG TTC-3' for human c-Myc (underlines indicate the 9R coding sequences). Amplified PCR products were digested with Eco RI and XhoI (or Sal I) and then cloned into pCDNA3.1/myc-His A (Invitrogen), resulting in

plasmid pCMV cDNA-9R-myc (Fig.1). RFP and RFP-9R DNAs were amplified by PCR using the following primers: 5'- AAT GAC CTC GAG ATG GCC TCC TCC GAG AAC GTC AT-3' and 5'-GAA AAC CTC GAG TTA CAG GAA CAG GTG GTG GCG GCC CTC-3' for RFP, and 5'- AAT GAC CTC GAG ATG GCC TCC TCC GAG AAC GTC AT-3' and 5'-GAA AAC CTC GAG GCG GCG TCT GCG TCT GCG GCG TCT GCG CAG GAA CAG GTG GTG GCG GCC CTC-3' for RFP-9R, digested with XhoI, and subcloned into pCDNA3.1/myc-His A (Invitrogen) All plasmids that were used in transfection experiments were prepared using the Qiagen EndoFree Plasmid Medi Kit. For construction of retrovirus vectors, the 4 factors cDNAs were cloned into the retroviral expression vector pCL (Orbigen, San Diego, CA, USA). Retroviruses were prepared using the 293GPG retroviral producer cell line as described in Ory et al. (Ory et al., 1996). The correct cDNA insertions into the vector were confirmed by restriction enzyme digestion and sequence analyses.

Preparation of protein extracts

HEK 293 cells were maintained in DMEM supplemented with 10% fetal bovine serum containing 100 units/ml penicillin and 100 μg/ml streptomycin. Using Lipofectamine^{TM} (Invitrogen), 2 μg of plasmid DNA was transfected per 4 x 10^5 cells. To establish cells stably expressing all 4 factors, 0.1% of transfected HEK 293 cells were seeded and cultured in the presence of 500 μg/ml neomycin (G418 Sulfate, Clontech, Palo Alto, CA). Individual cells were isolated from neomycin-resistant colonies. The expression of 4 factors from neomycin-resistant stable clones was determined by western blot analysis (Fig. S2). To prepare cell extracts, cells were washed in PBS and sedimented at 400 X g followed by suspension in 1 volume of cold cell lysis buffer (100 mM HEPES, pH 8.2, 50 mM NaCl, 5 mM MgCl2, 1 mM dithiothreitol, and protease inhibitors), and incubated for 30–45 min on ice. Cells were sonicated on ice using a Labsonic-M pulse sonicator fitted with a 3-mm-diameter probe until all cells and nuclei were lysed as judged by microscopy.

Lysates were sedimented at 15,000g for 15 min at 4°C to pellet the coarse material. The supernatant was filtered through a 0.2 μm membrane, aliquoted, frozen quickly in dry ice methanol, and stored at -80°C.

Retroviral infection, protein transduction and hiPS generation

For viral transduction, HNF cells cultured *in vitro* were incubated with viral supernatant containing polybrene (hexadimethrine bromide; 1 μg/ml; Sigma) for 2-4 h. After infection, cells were incubated five days in normal culture medium (DMEM with 15% defined-FBS). At d6 post infection, cells were replated on gelatin-coated plates. Virus-infected HNF cells were cultured in DMEM with 15% defined-FBS for 24 hrs. For protein transduction, HNF cells cultured *in vitro* were incubated with the 4 protein factors for 8 hrs per week up to 6 weeks with ES media1 (DMEM supplemented with 2mM L-glutamine, 1mM β-mercaptoethanol, 1x non-essential amino acids, 20% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin and 1500 U/ml LIF). After 6 weeks, cells were dissociated and transferred onto MEF. The cells were then cultured with ES media 2 (1:3 = ES media 1 :ES media 3)) for a week, and thereafter, with ES media 3 until hiPS formation.

RT-PCR analysis for marker genes

Cells were treated with Trizol reagent (Invitrogen) to extract total RNA. Five micrograms of total RNA were used for reverse transcription reaction with SuperScript II (Invitrogen) and oligo-dT primer, according to the manufacturer's instructions. Real-time PCR analyses were performed in triplicate using SYBR green I using a DNA engine Opticon^{TM} (MJ Research, Waltham, MA) to analyze mRNA expression levels. Primer sets used to detect mRNA are shown in Table S1. Amplifications were performed in $25\ \mu l$ containing $0.5\ \mu M$ of each primer, 0.5 X SYBR Green I (Molecular Probes, OR), and $2\ \mu l$ of cDNA. Fifty PCR cycles were performed with a temperature profile consisting of 95^{o}C for 30 sec, 55^{o}C for 30 sec, 72^{o}C for 30 sec, and 79^{o}C for 5 sec. The dissociation curve of each PCR product

was determined to ensure that the observed fluorescent signals were only from specific PCR products. After each PCR cycle, the fluorescent signals were detected at 79^{o}C to melt primer dimers (Tms of all primer dimers used in this study were < 76^{o}C). A standard curve was constructed using plasmid DNAs containing the GAPDH gene (from 10^4 to 10^9 molecules). The fluorescent signals from specific PCR products were normalized against that of the β-actin gene, and then relative values were calculated by setting the normalized value of control as 1. All reactions were repeated twice in triplicate using independently prepared cDNAs. Primer sequences used qRT-PCR are listed in Table S1.

Bisulfite genomic sequencing

Genomic DNA was isolated with the DNeasy Tissue Kit (Qiagen). Bisulfite treatment was carried out using the EpiTect Kit (Qiagen) following the manufacturer's instruction. The promoter regions of the human Nanog and Oct4 genes were amplified by PCR using primers published previously (Deb-Rinker et al., 2005; Freberg et al., 2007) (Table S2). The resulting amplified PCR products were gel-purified, subcloned into the pGEM-T Easy vector (Promega) and sequenced.

Karyotyping and DNA Fingerprinting analysis

Standard G-band chromosome analysis was performed in the Cell Line Genetics (Madison, WI). In order to confirm the origin of p-hiPS clones, regions of highly variable numbers of tandem repeats (VNTR) were amplified by PCR from genomic DNA (Park et al., 2008). Amplified PCR products were electrophoresed onto 7% acrylamide gels. Primer sequences used in this study are listed in Table S3.

Western blotting.

Cells were lysed with RIPA buffer consisting of 50mM Tris (pH7.5), 150mM NaCl, 1% NP-40, 0.5% deoxycholic acid, and 0.1% SDS), supplemented with a protease

inhibitor cocktail (Roche) and mixed with an equal volume of sodium dodecyl sulfate (SDS)-sample buffer consisting of 125mM Tris (pH 6.8), 2% SDS, 15% glycerol, 5% β-mercaptoethanol, 0.05% bromophenol blue. Samples were subjected to 10% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to a nitrocellulose membrane (Hybond-ECL, Amersham). After blocking, the membrane was incubated with 1:3000-dilution of an anti-myc antibody (Roche), followed by reaction with 1:300-dilution of a horseradish peroxidase-conjugated anti-mouse immunoglobulin G (IgG) antibody (Amersham). Detection was achieved using an enhanced-chemiluminescent substrate (Amersham).

Alkaline phosphatase staining and immunocytochemistry

Alkaline phosphatase (AP) staining was performed using the Alkaline phosphatase staining kit II (Vector Vector Laboratories, Burlingame, CA). For immunocytochemistry, cells were fixed with 4% paraformaldehyde for 20 min at room temperature. After washing with PBS, cells were treated with PBS containing 10% normal goat serum and 0.1% Triton X-100 for 45 min at room temperature. Primary antibodies used in this study were myc tag (polyclonal, 1:500, Upstate, Lake Placid, NY), SSEA3 (monoclonal, 1:300, R&D Systems, Minneapolis, MN), SSEA4 (monoclonal, 1:300, Chemicon, Temecula, CA), Oct4 (monoclonal, 1:300, SantaCruz Biotech., Santa Cruz, CA), TRA-1-60 (monoclonal, 1:300, Chemicon, Temecula, CA),, smooth muscle actin (SMA; monoclonal, 1:400, Dako, Glostrop, Denmark), anti-bIII tubulin (Tuj1; monoclonal, 1:500, Covance, Richmond, CA), Desmin (polyclonal, 1:500, DAKO), hepatocyte necrosis factor (HNF 3β ; monoclonal, 1:1000, Chemicon, Temecula, CA), alpha-fectoprotein (AFP; monoclonal, 1:50, SantaCruz Biotech., Santa Cruz, CA) tyrosine hydroxylase (TH; polyclonal,1:1,000, Pel-Freez, Rogers, AR), nestin (monoclonal, 1:1,000, BD Sciences, Franklin Lakes, NJ). For detection of primary antibodies, fluorescence-labeled (Alexa fluor 488 or 568; Molecular Probes, Eugene, OR) secondary antibodies were used according to the specifications of the manufacturer. Cells

were mounted using Vectashield containing 4',6-diamidino-2-phenylindole (DAPI; Vector Lab.) and analyzed by fluorescent microscopy.

In vitro differentiation of iPS cells

Briefly, p-hiPS cells were dissociated and EBs were allowed to form for eight days after plating p-hiPS cells on bacterial dishes in ES media 3 without bFGF. EBs were allowed to attach to tissue culture dishes (one day) and neural precursors were selected by incubation in serum-free ITSFn (Insulin-Transferrin-Selenium-Fibronectin; (Okabe et al., 1996) medium for a month. Thereafter, various other cell types were differentiated from EBs.

Teratoma formation

p-hiPS cells (clone 1 and 2) were suspended in DMEM containing 10% FBS. SCID mice were anesthetized with diethyl ether and the cell suspension injected under the kidney capsule. Six to eight weeks after the injection, tumors were surgically dissected. Samples were weighed, fixed in PBS containing 4% formaldehyde, and embedded in paraffin. Sections were stained with hematoxylin and eosin.

Microarray analysis.

Total RNAs were prepared from human fibroblasts, H9, and hiPS lines using TRIzol reagent (Invitrogen, Carlsbad, CA) and hybridized to Affymetrix Human Expression Array U133 Plus 2.0 analyzing over 47,000 human transcripts at the Harvard Partners Center for Genomics and Genetics. Detailed procedure is described in the website at http://www.hpcgg.org/Microarrays/overview.jsp.

References for SOM

Deb-Rinker, P., Ly, D., Jezierski, A., Sikorska, M., and Walker, P.R. (2005). Sequential DNA methylation of the Nanog and Oct-4 upstream regions in human NT2 cells during neuronal differentiation. J Biol Chem *280*, 6257-6260.

Freberg, C.T., Dahl, J.A., Timoskainen, S., and Collas, P. (2007). Epigenetic reprogramming of OCT4 and NANOG regulatory regions by embryonal carcinoma cell extract. Mol Biol Cell *18*, 1543-1553.

Okabe, S., Forsberg-Nilsson, K., Spiro, A.C., Segal, M., and McKay, R.D. (1996). Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro. Mech Dev *59*, 89-102.

Ory, D.S., Neugeboren, B.A., and Mulligan, R.C. (1996). A stable human-derived packaging cell line for production of high titer retrovirus/vesicular stomatitis virus G pseudotypes. Proc Natl Acad Sci U S A *93*, 11400-11406.

Park, I.H., Zhao, R., West, J.A., Yabuuchi, A., Huo, H., Ince, T.A., Lerou, P.H., Lensch, M.W., and Daley, G.Q. (2008). Reprogramming of human somatic cells to pluripotency with defined factors. Nature *451*, 141-146.

Figure S1: Fluorescence microscopy analysis of COS-7 and HNF cells treated with dsRED and dsRED-9R proteins. A, COS-7 cells and **B,** HNF cells were treated with HEK 293 cells extract expressing RFP (upper panel) and RFP fused with 9 repeat arginines (RFP-9R; lower panel) for 8 hours and then analyzed by fluorescence microscopy at the wavelength of 594nm.

Figure S2: Stable expression of reprogramming factors in HEK293 cells. A, Schematic representation of the mammalian expression vector of the 4 reprogramming factors. The respective cDNA of Klf4, c-Myc, Oct4, and Sox2 was connected with 9R and the myc tagging peptide at the C-terminus. **B,** HEK293 cells were transfected with pCMV hKlf4-9R-myc, pCMV hOct4-9R-myc, pCMV hSox2-9R-myc, and pCMV hc-Myc-9R-myc vectors, respectively and were grown in the presence of G-418 to select stably transformed cells. Cell lysates (50μg) of individual stable cell lines were subjected to SDS-PAGE followed by Western blotting using anti-myc antibodies.

Figure S3: Protocols for direct protein delivery to generate p-hiPS cells from HNF cells. These two protocols were initially attempted to generate p-hiPS cells from HNF cells (see text for details). After many experiments, neither protocol was able to generate reprogrammed cells, which led us to use the repeated cycle protocol in Fig. 1.

Figure S4: Generation and characterization of the rv-hiPS01 cell line derived from HNFs using the conventional retroviral transduction method. Multiple p-hiPS lines were generated from HNFs by retroviral transduction of the same four reprogramming factors. Here we show the analysis of one of these clones (rv-hiPS01). **A,** Immunofluorescence staining of the rv-hiPS01 clone shows

expression of hES markers including TRA-1-60, Oct-4, and SSEA-4. **B,** Embryoid body (EB)-mediated differentiation of rv-hiPS 01 cells. EBs were made by suspension culture of rv-hiPS cells at day 8. Phase contrast images show all three germ cells differentiated from EBs at day 24 including neural cells (ectodermal), endothelial-like cells (mesodermal), and endoderm-like cells (endoderm). All other criteria including epigenetic status, gene expression, and teratoma formation demonstrated that this rv-hiPS01 is an authentic hiPS line (data not shown).

Figure S5: A, Gene expression profiles were compared by Affymetrix microarray analysis and are given as heat maps with the corresponding scale as minimum and maximum fold differences. **B,** The global gene-expression patterns were compared between human iPS cell lines (p-hiPS02, and rv-hiPS) and HNF, and between human iPS cell lines and hES cells (H9) using GeneSpring GX7.3.1. The red lines indicate the diagonal and 5-fold changes between the paired samples.

Figure S6: Expression of endogenous and exogenous (transgene) hES marker genes in HNF, H9, protein-induced iPS (p-hiPS01 and p-hiPS02), and retrovirus-induced iPS (rv-hiPS01) cells. RT-PCR was performed using primer sets that can amplify total, endogenous, or exogenous genes. β-Actin was used as an amplification control. Exogenous transgene mRNAs were detected only in hips-rv01, but not in HNF, H9, and p-hiPS01 and 02. These results confirm that there is no transgene contamination in protein-induced hiPS cells.

Figure S7: DNA fingerprinting analysis. Genomic DNAs were prepared from HNF, H9, p-hiPS01, p-hiPS02, rv-hiPS01, and HEK293 cells and analyzed by genomic PCR using primer sets containing highly variable tandem repeats (Table S3). Amplified PCR products were subjected to 7% acrylamide gel electrophoresis. The DNA finger printing patterns of p-hiPS01, p-hiPS02, and rv-hiPS01 were identical to that of HNFs, but not those of H9 and HEK293 cells.

Figure S8: Teratoma formation in immunodeficiency mice by p-hiPS cells. A, EBs were made by suspension culture of both p-hiPS lines at day 8 (Far left of the top row panels). **B,** Hematoxylin and eosin staining were additionally performed for teratoma derived from both p-hiPS cell lines (01 and 02). Tumors were well-developed from a single injection site after cells were transplanted under the kidney capsules of SCID mice. The resulting teratomas contained various tissues representing ectoderm, mesoderm and endoderm differentiation (top row panel (p-hiPS01) and bottom row panel (p-hiPS02)). Ectoderm: neural tissue and pigmented neural epithelium (rosette); mesoderm: muscle; endoderm: gut-like epithelium and intestinal-like epithelium. **C**, Standard G-band chromosome analysis was performed for HNF, p-hiPS01, and p-hiPS02.

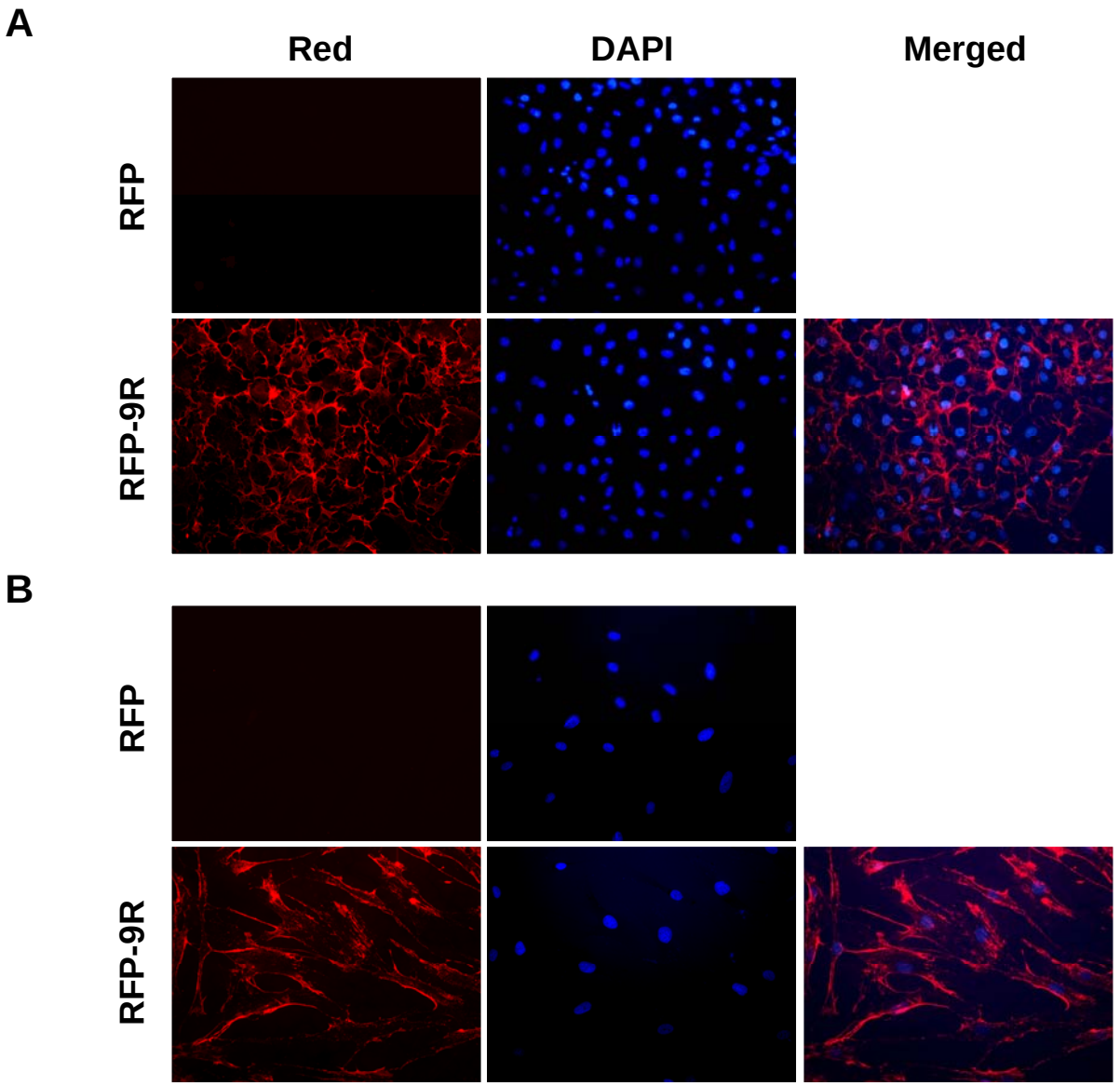
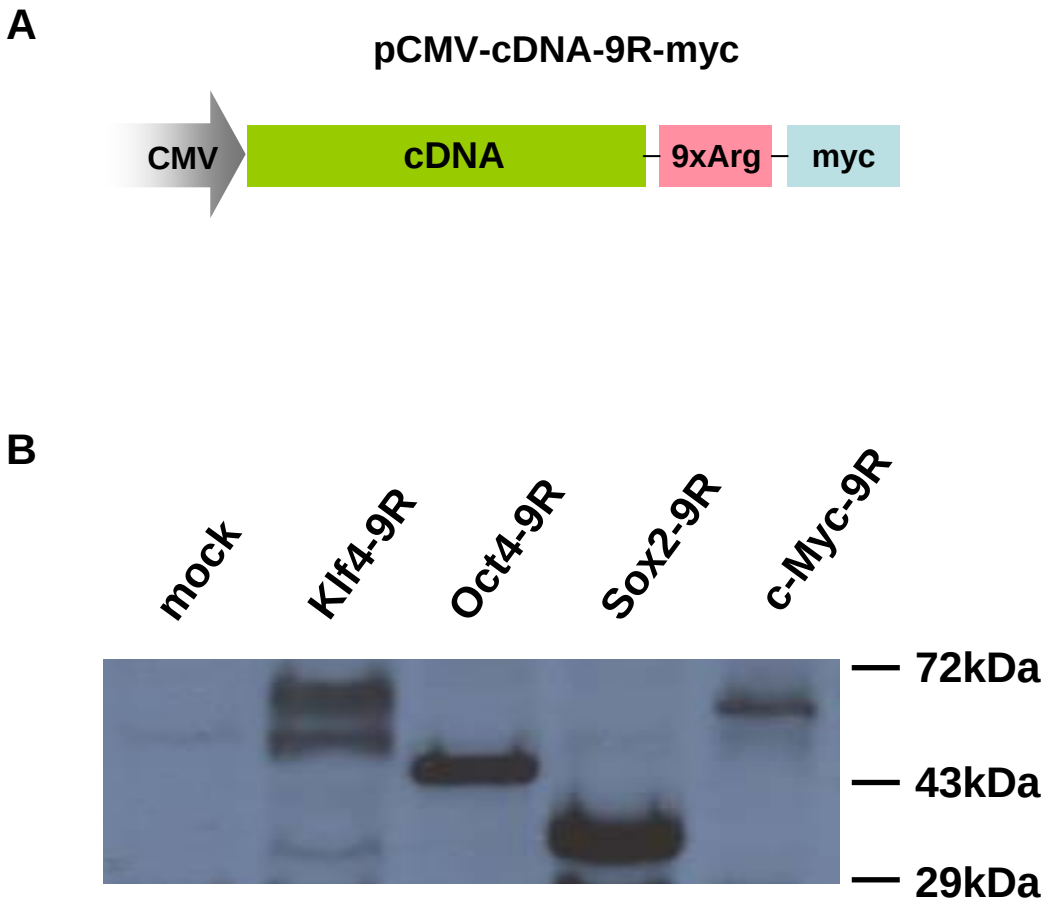


Figure S1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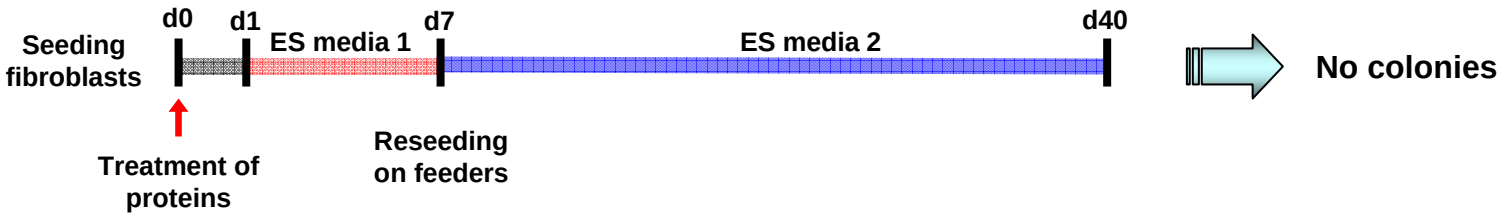
43kDa
29kDa

Figure S2

Protocol 1



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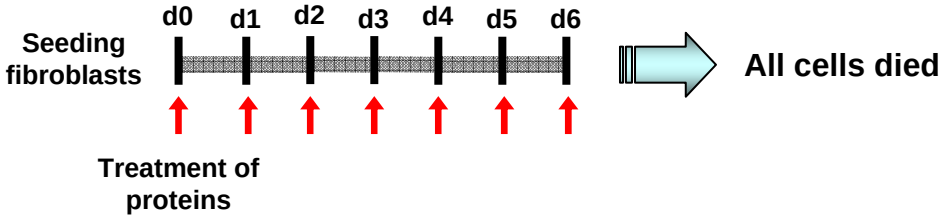
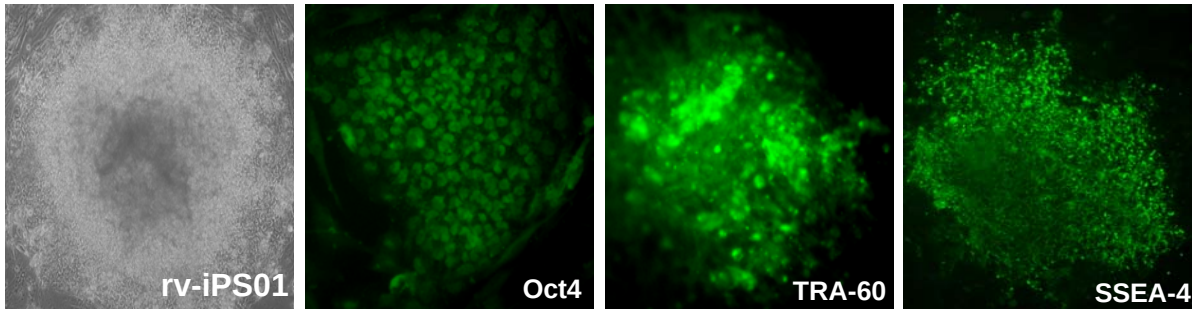


Figure S3

A



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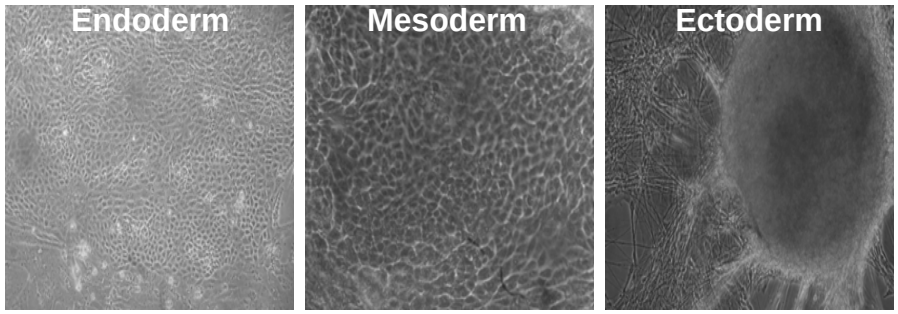
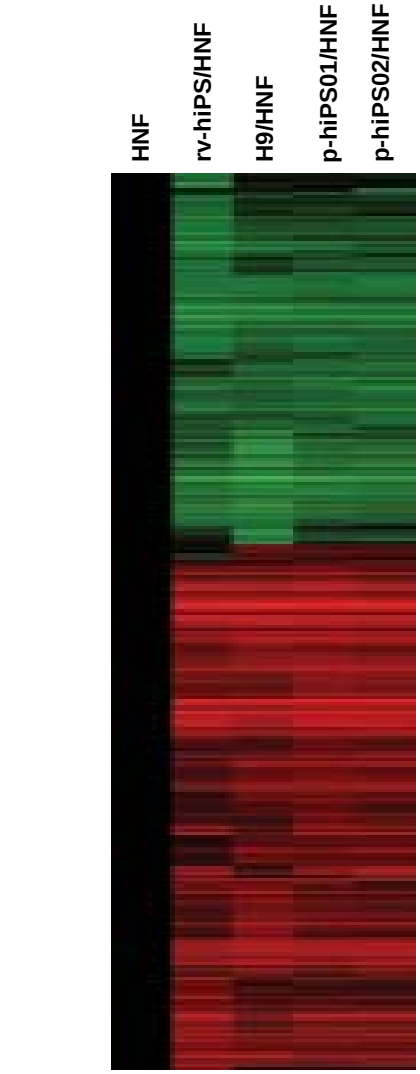


Figure S4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H9/HNF
p-hiPS01/HNF
p-hiPS02/HNF
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HNF
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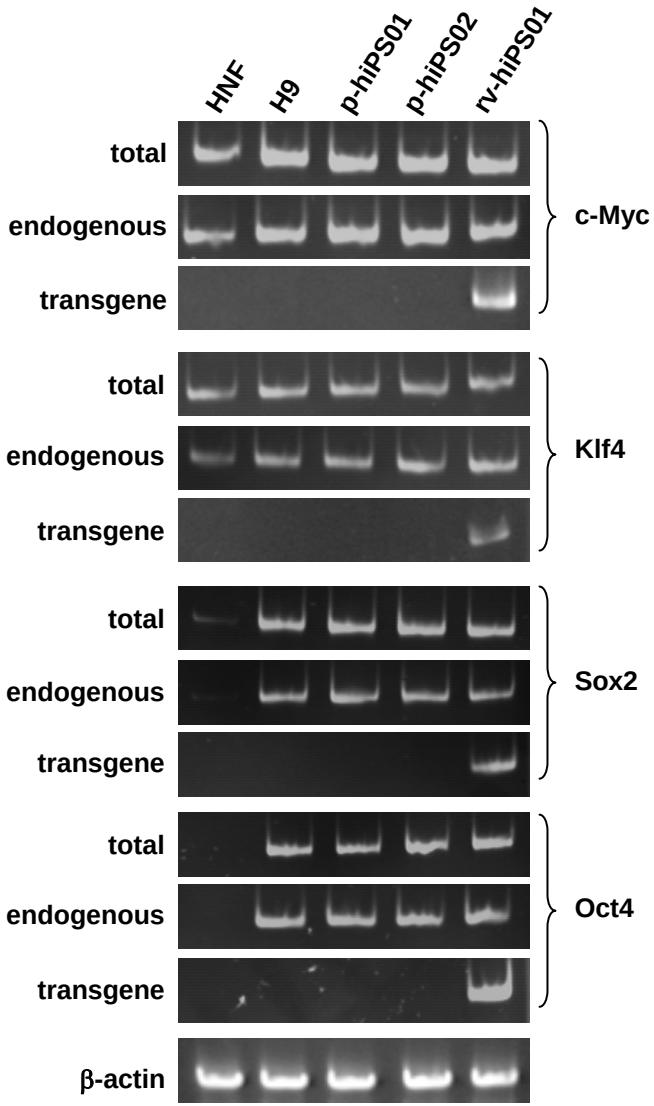
rv-hiPS
H9

Figure S5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H9
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Oct4
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Figure S6

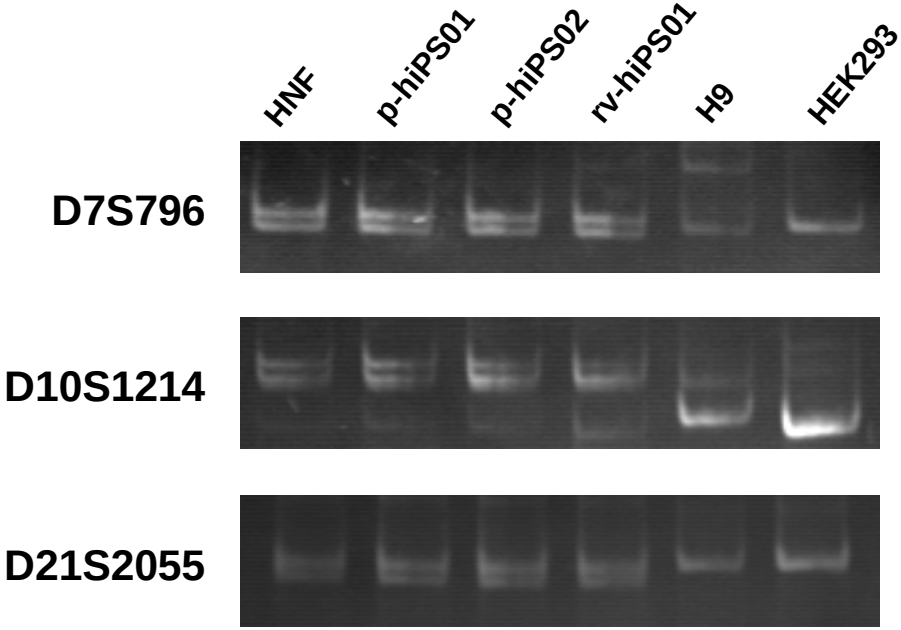
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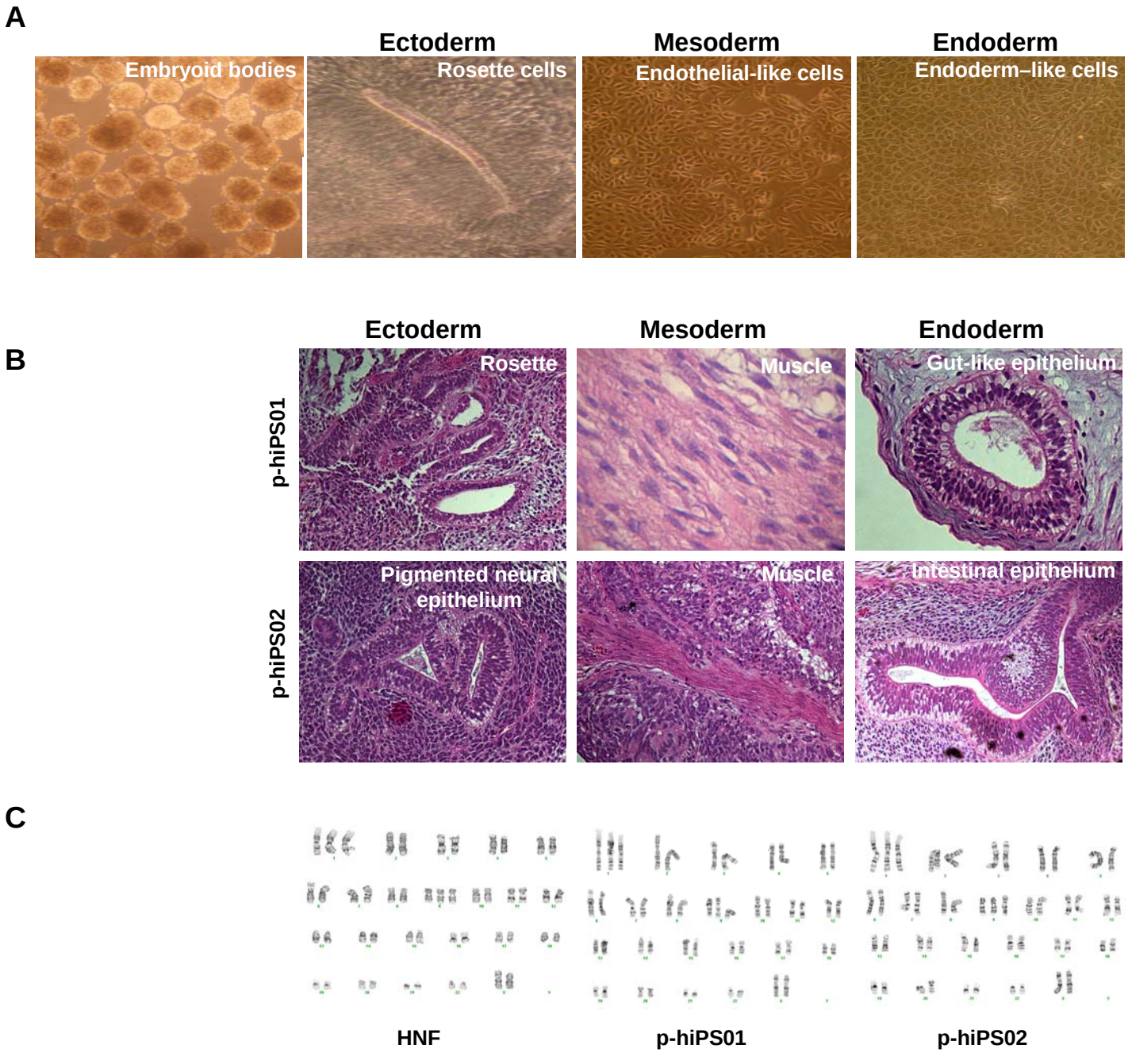
Figure S7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Mesoderm
Endoderm
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Rosette cells
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Mesoderm
Endoderm
p-hiPS01
Rosette
Muscle
Gut-like epithelium
p-hiPS02
Pigmented neural epithelium
Muscle
Intestinal epithelium
C
HNF
p-hiPS01
p-hiPS02

Figure S8

Table S1: Primer sequences for quantitative RT- PCR

Gene	Sense primer (5’ to 3’)	Antiense primer (5’ to 3’)
c-Myc	ACTCTGAGGAGGAACAAGAA	TGGAGACGTGGCACCTCTT
Klf4	TCTCAAGGCACACCTGCGAA	TAGTGCCTGGTCAGTTCATC
Oct4	GACAGGGGGAGGGGAGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCCAAAC
Sox2	AGCTACAGCATGATGCAGGA	GGTCATGGAGTTGTACTGCA
Nanog	TGAACCTCAGCTACAAACAG	TGGTGGTAGGAAGAGTAAAG
hTERT	TGTGCACCAACATCTACAAG	GCGTTCTTGGCTTTCAGGAT
Rex1	TCGCTGAGCTGAAACAAATG	CCCTTCTTGAAGGTTTACAC
Gdf3	AAATGTTTGTGTTGCGGTCA	TCTGGCACAGGTGTCTTCAG
β-actin	CTGGCACCACACCTTCTACAATG	AATGTCACGCACGATTTCCCGC
GAPDH	CAAAGTTGTCATGGATGACC	CCATGGAGAAGGCTGGGG

Table S2: Primer sequences for bisulfite sequencing

Gene	Sense primer (5’ to 3’)	Antiense primer (5’ to 3’)
Oct4-2	TTAGGAAAATGGGTAGTAGGGATTT	TACCCAAAAAACAAATAAATTATAAAACCT
Oct4-3	ATTTGTTTTTTGGGTAGTTAAAGGT	CCAACTATCTTCATCTTAATAACATCC
Oct4-4	GGATGTTATTAAGATGAAGATAGTTGG	CCTAAACTCCCCTTCAAAATCTATT
OCT4-7	TAGTTGGGATGTGTAGAGTTTGAGA	TAAACCAAAACAATCCTTCTACTCC
Nanog-1	AGAGATAGGAGGGTAAGTTTTTTTT	ACTCCCACACAAACTAACTTTTATTC
Nanog-2	GAGTTAAAGAGTTTTGTTTTTAAAAATTAT	TCCCAAATCTAATAATTTATCATATCTTTC
Nanog-3	TTAATTTATTGGGATTATAGGGGTG	AACAACAAAACCTAAAAACAAACC

Table S3: Primer sequencing for fingerprinting

	Sense primer (5’ to 3’)	Antiense primer (5’ to 3’)
D7S796	TTTTGGTATTGGCCATCCTA	GAAAGGAACAGAGAGACAGGG
D10S1214	ATTGCCCCAAAACTTTTTTG	TTGAAGACCAGTCTGGGAAG
D21S2055	AACAGAACCAATAGGCTATCTATC	TACAGTAAATCACTTGGTAGGAGA

Table S4: Primer sequencing for RT-PCR

	Sense primer (5’ to 3’)	Antiense primer (5’ to 3’)
Endogenous Oct4	GCACTGTACTCCTCGGTCCCTTTCCC	CTTCCCTCCAACCAGTTGCCCCAAAC
Transgene Oct4	GCACTGTACTCCTCGGTCCCTTTCCC	AGGCCTGTCGACAAGCGGCCGCCTC
Endogenous Klf4	CAGTGCCAAAAATGCGACCGAGC	GACCATGATTGTAGTGCTTTCTGGC
Transgene Klf4	CAGTGCCAAAAATGCGACCGAGC	AGGCCTGTCGACAAGCGGCCGCCTC
Endogenous Sox2	GCCCCCAGCAGACTTCACATG	CGCGGTTTTTGCGTGAGTGTGGATGGG
Transgene Sox2	GCCCCCAGCAGACTTCACATG	AGGCCTGTCGACAAGCGGCCGCCTC
Endogenous c-Myc	GCCACAGCATACATCCTGTCCGTCCA AGC	CCAAAGTCCAATTTGAGGCAGTTTAC
Transgene c-Myc	GCCACAGCATACATCCTGTCCGTCCA AGC	AGGCCTGTCGACAAGCGGCCGCCTC