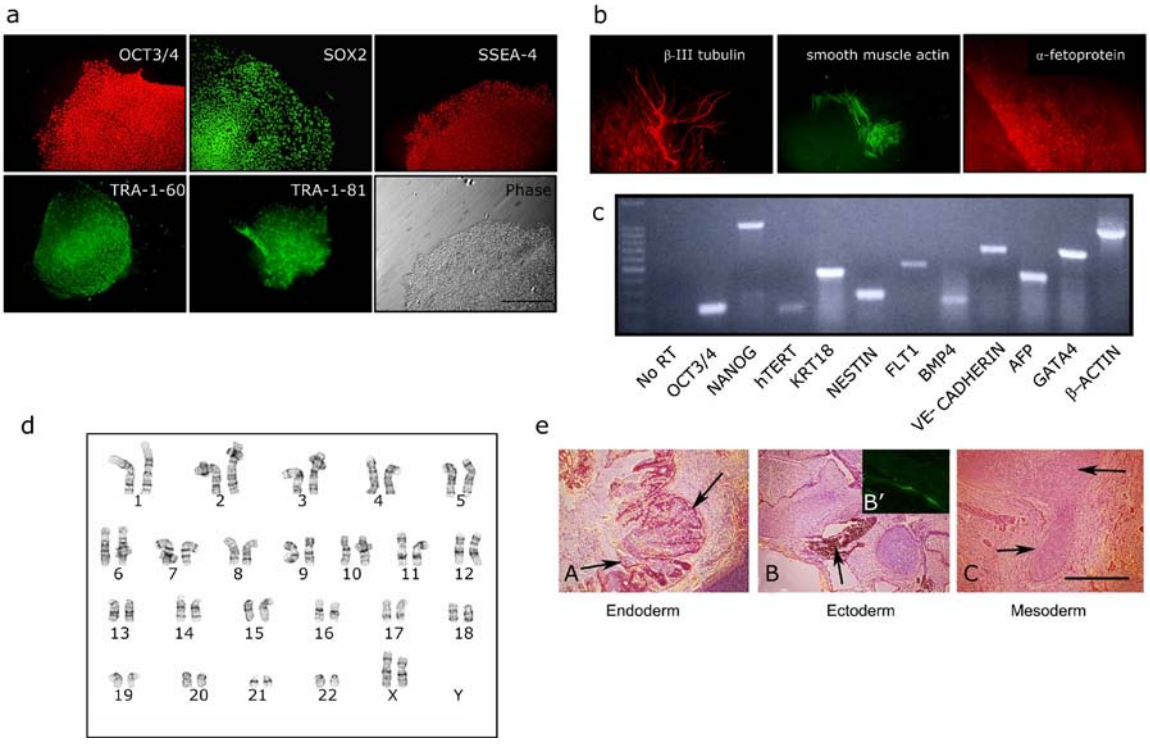
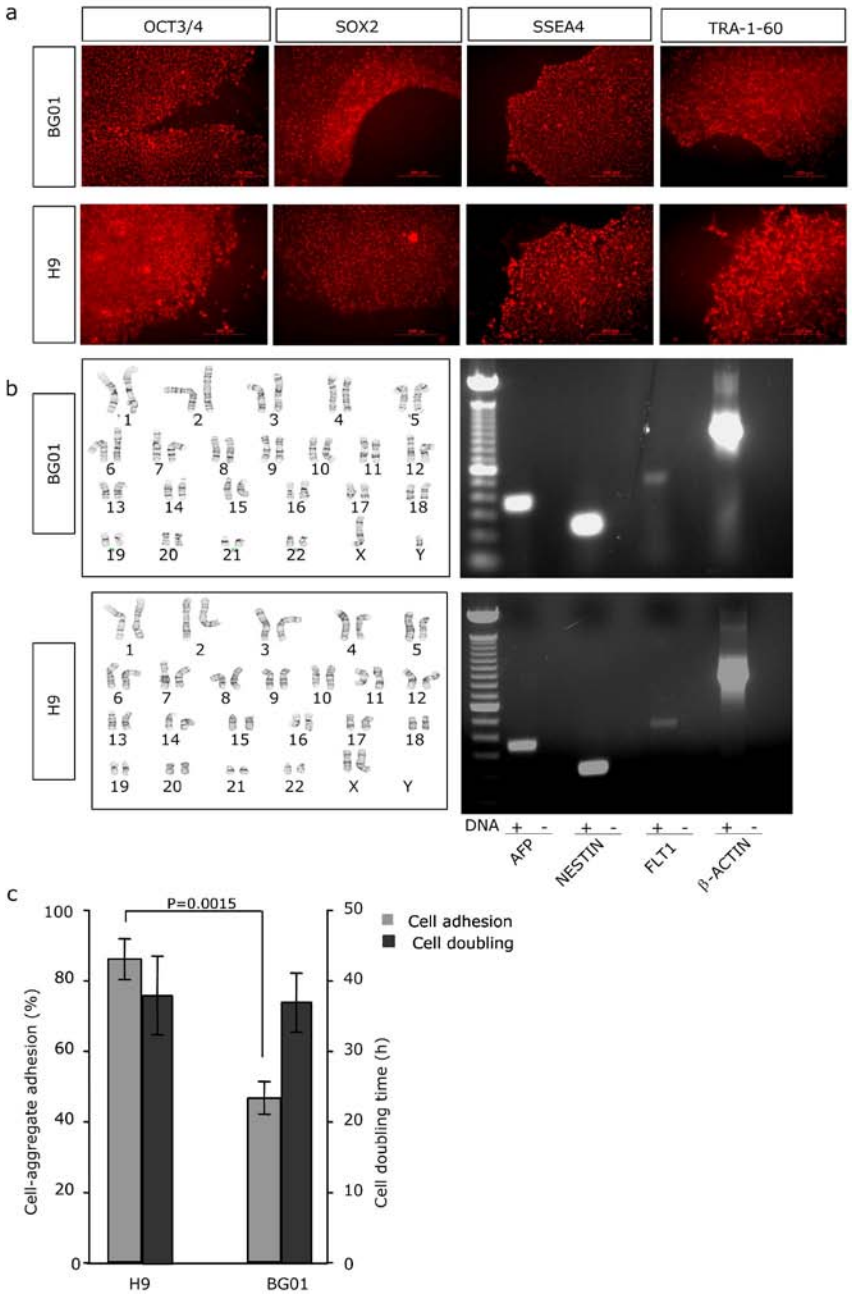
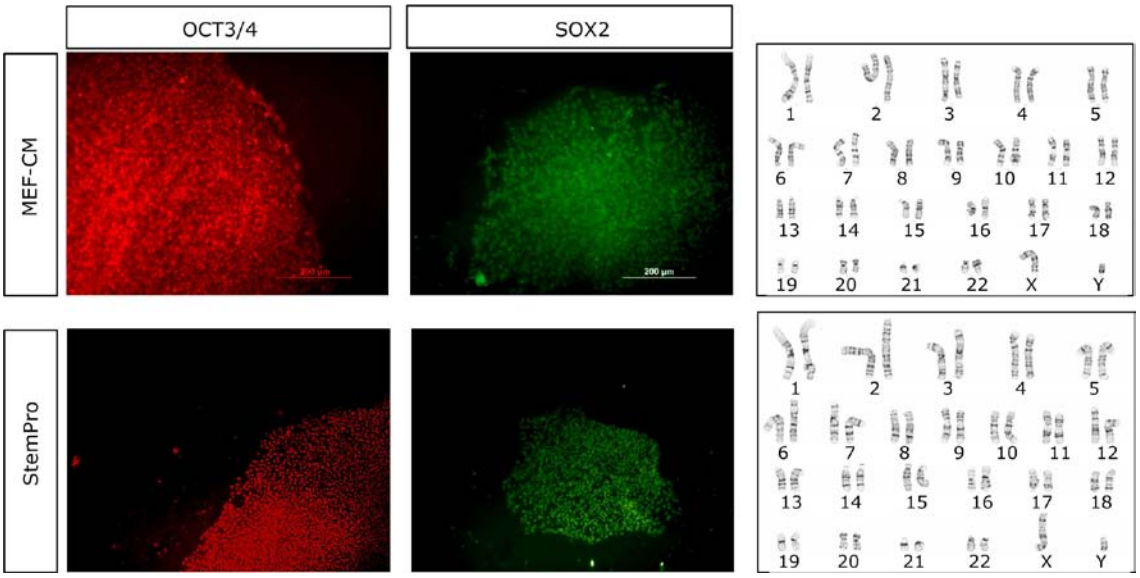


Supplementary Figure 1. **Long-term culture of H9 hES cells on PMEDSAH and Matrigel in MEF-CM**. (a) Fluorescence micrographs of colonies of H9 cells cultured on PMEDSAH in MEF-CM showing expression of hES cell markers: OCT3/4, SOX2, SSEA-4 TRA-1-60 and TRA-1-81; and a phase-contrast image. (b) Micrographs illustrating immunoreactivity for β-III tubulin (ectoderm), smooth muscle actin (mesoderm) and α-fetoprotein (endoderm) demonstrating that H9 cells remained capable of tri-linage differentiation. Scale bar indicates 200 μm. (c) RT-PCR analysis of expression of hES cell markers (*OCT3/4*, *NANOG*, *hTERT*) from undifferentiated cell colonies and from RNAs isolated from embryoid bodies illustrating the expression of genes characteristic of: ectoderm (*KRT-18*, *NESTIN*), mesoderm (*BRACHURY*, *FLT-1*, *BMP-4*, *VE-CADHERIN*) and endoderm (*AFP*, *GATA-4*). Negative control (Lane 1: no template) and positive control (*β-ACTIN*). (d) Representative karyotype of H9 cells cultured on Matrigel. (e) Sections of teratomas induced by injecting hES cells, cultured on Matrigel, in immunosuppressed mice. H & E stained paraffin sections indicating the presence of endoderm (goblet-like cells: arrow A), pigmented ectoderm (arrows B; and immunohistochemical localization of β-III tubulin in neuronal cells: arrow B') and mesodermal derivatives (cartilage, connective tissue and muscle: arrow C). Scale bar indicates 200 μm.



Supplementary Figure 2. **Characterization of BG01 and H9 hES cells on PMEDSAH in hCCM**. (a) Fluorescence micrographs of colonies illustrating expression of hES cell markers: OCT3/4, SOX2, SSEA-4 and TRA-1-60. (b) Representative chromosomal spreads and RT-PCR analysis of RNAs from embryoid bodies illustrating expression of genes characteristic of: endoderm (*AFP*), ectoderm (*NESTIN*) and mesoderm (*FLT-1*). *β-ACTIN* was used as positive control and for each primer set tested, a reaction lacking RNA was assessed in parallel as a negative control. (c) Comparison of percentage (mean ± standard error) of cell-aggregate adhesion and cell population-doubling time of both cell lines cultured on PMEDSAH in hCCM.



Supplementary Figure 3. Expression of hES cell markers (OCT3/4 and SOX2) and normal karyotype of BG01 hES cells cultured on PMEDSAH in MEF-CM and StemPro at passages 5 and 3, respectively. Tri-lineage differentiation capability of BG01 hES cells was confirmed via EB formation.

Supplementary Table 1. Microarray analysis showing fold change in gene expression of hES cells cultured on PMEDSAH compared to cells grown on Matrigel. Genes corresponding to ES cell markers and those found exclusively in hES cells are shown.

Symbol	Name	UniGene	Fold Change
ABHD9	Abhydrolase domain containing 9	Hs.156457	0.083
C2orf56	Chromosome 2 open reading frame 56	Hs.433466	-0.164
CLDN6	Claudin 6	Hs.533779	0.076
CXorf15	Chromosome X open reading frame 15	Hs.555961	-0.079
CYP26A1	Cytochrome P450, family 26, subfamily A, polypeptide 1	Hs.150595	0.088
DBNDD1	Dysbindin (dystrobrevin binding protein 1) domain containing 1	Hs.301394	-0.107
DCC1	Defective in sister chromatid cohesion homolog 1	Hs.315167	-0.039
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	Hs.515840	-0.172
DTYMK	Deoxythymidylate kinase (thymidylate kinase)	Hs.471873	-0.159
EPHA1	EPH receptor A1	Hs.89839	0.161
ETV4	Ets variant gene 4 (E1A enhancer binding protein, E1AF)	Hs.434059	0.082
FGF2	Fibroblast growth factor 2 (basic)	Hs.284244	0.010
GDF3	Growth differentiation factor 3	Hs.86232	-0.170
GPR23	G protein-coupled receptor 23	Hs.522701	0.243
HESX1	HESX homeobox 1	Hs.171980	-0.849
L1TD1	LINE-1 type transposase domain containing 1	Hs.562195	-0.099
LIN28	Lin-28 homolog (C. elegans)	Hs.86154	-0.019
MCM10	Minichromosome maintenance complex component 10	Hs.198363	0.218
MYBL2	V-myb myeloblastosis viral oncogene homolog (avian)-like 2	Hs.179718	-0.353
NANOG	Nanog homeobox	NM_024865	0.036
NCAPH	Non-SMC condensin I complex, subunit H	Hs.308045	-0.124
ORC1L	Origin recognition complex, subunit 1-like (yeast)	Hs.17908	-0.453
ORC2L	Origin recognition complex, subunit 2-like (yeast)	Hs.444870	-0.239
PODXL	Podocalyxin-like	Hs.16426	0.315
POU5F1	POU class 5 homeobox 1	Hs.249184	-0.027
PRDM14	PR domain containing 14	Hs.287532	-0.715
PWP2	PWP2 periodic tryptophan protein homolog (yeast)	Hs.449076	0.047
RRP9	RRP9, small subunit (SSU) processome component	Hs.153768	-0.179
SLC5A6	Solute carrier family 5 6	Hs.435735	0.108
SOX2	SRY (sex determining region Y)-box 2	Hs.518438	0.079
TDGF1	Teratocarcinoma-derived growth factor 1	Hs.385870	0.220
WSCD1	WSC domain containing 1	Hs.370166	-0.218
ZIC3	Zic family member 3 heterotaxy 1	Hs.111227	-0.594

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Supplementary Table 2. List of primers used in RT-PCR and qPCR

RT-PCR			
Gene	**Forward primer**	**Reverse primer**	**Product (bp)**
OCT3/4	ctgcagtgtgggtttcgggca	cttgctgcagaagtgggtggagga	168
NANOG	cggcttcctcctcttcctctatac	atcgatttcactcatcttcacacgtc	953
hTERT	cggaagagtgtctggagcaa	ggatgaagcggagtctgga	144
KRT18	tctgtggagaacgacatcca	ctgtacgtctcagctctgtga	378
NESTIN	cagctggcgcacctcaagatg	agggaagttgggctcaggactgg	209
BMP4	tgagcctttccagcaagttt	cttccccgtctcaggtatca	182
VE-CADHERIN	acgggatgaccaagtacagc	acacactttgggctggtagg	593
FLT-1	atcagagatcaggaagcacc	ggaacttcatctgggtccat	451
AFP	ccatgtacatgagcactgttg	ctccaataactcctggtatcc	357
GATA-4	ctccttcaggcagtgagagc	gagatgcagtgtgctcgtgc	574
ß-ACTIN	atctggcaccacaccttctacaatgagctgcg	cgtcatactcctgcttgctgatccacatctgc	835

qPCR			
Gene	**Forward primer**	**Reverse primer**	**Product (bp)**
SOX-2	gagagaaagaaagggagagaag	gagagaggcaaactggaatc	140
NANOG	tcctcctcttcctctatactaac	cccacaaatcaggcatag	112
OCT3/4	agtcagtgaacagggaatgg	tcgggattcaagaacctcg	131
ß-ACTIN	gccgaggactttgattgc	gtgtggacttgggagagg	143

SUPPLEMENTARY METHODS

Polymer synthesis

All polymer coatings were prepared on tissue culture polystyrene (TCPS) dishes (35 mm; Becton Dickinson and Co, Franklin Lakes, NJ). All monomers were purchased from Sigma Aldrich (St. Louis, MO) except carboxybetaine methacrylate (CBMA) which was synthesized and characterized as previously described[1]. Graft-polymerization was carried out using a 0.2 M solution of methacrylate monomers in a 4:1 mixture of water and ethanol[2]. The TCPS dishes were activated using a UV-ozone cleaner (Jelight Co. Inc, Irvine, CA) for 40 min. Surface-activated dishes were immersed into the monomer solution at 80 ^{o}C for 2.5 h. Polymer-coated dishes were allowed to cool to 50^{o}C and were rinsed with a warm saline solution (1% NaCl in water, at 50 ^{o}C). Polymer-coated dishes were then rinsed overnight in saline solution at 50 ^{o}C. Dishes were cleaned by ultra-sonication in DI-water and dried under a stream of nitrogen gas. Polymer-coated dishes were stored at room temperature prior to cell culture. For Fourier transform infrared spectroscopy and ellipsometry, a gold-coated Si substrate coated with poly-*p*-xylylene was included in each graft-polymerization reaction[3].

Characterization of polymer coatings

Elemental analysis of the polymer coatings was conducted using X-ray photoelectron spectroscopy (Axis Ultra XPS, Kratos Analyticals, UK) equipped with a monochromatized Al Kα X-ray source. Spectra were referenced to an unfunctionalized aliphatic carbon at 285.0 eV. Presence of polymer coatings was confirmed using Fourier transform infrared (FTIR) spectroscopy (Nicolet 6700 spectrometer) using the grazing angle accessory (SAGA) with an angle of 85^{o}. Coating thickness was recorded at a wavelength of 532 nm using EP^{3}-SW imaging ellipsometer (Nanofilm Technology GmbH, Germany). Four-zone nulling was performed at an angle of incidence of 65^{o} and an anisotropic Cauchy parameterization model was used for curve fitting. Nanoindentation was performed by CSM instruments Inc. (Needham, MA) using a CSM NanoHardness tester equipped with a conospherical diamond tip (with a radius of 20 μm) in the load-control mode. A typical nanoindentation experiment involved engaging the

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tip under loading rate of 1.00 mN/min, indenting to a maximum load of 0.5 mN, and then withdrawing the tip with the same rate as for the loading. For each hydrogel coating, load-displacement data was acquired for 5 indentations. Reduced modulus (E_r) was calculated using the unloading portions of these load-displacement curves according to a method developed by Gerberich et al[4].

Preparation of polymer-coated dishes before cell culture

Before cell seeding, all polymer-coated dishes were sterilized with UV-light overnight (12 h), washed twice with sterile phosphate buffer saline (PBS) and equilibrated with culture medium for at least 48 h at 37 ^{o}C in 5% CO_2 atmosphere.

Matrigel preparation

Matrigel (BD BioSciences, San Jose, CA) was diluted 1:20 in cold Dulbecco's modified Eagle's medium/F12 (DMEM/F12; GIBCO, Carlsbad, CA), was applied to the dishes, and the coating was allowed to polymerize overnight at 4 ^{o}C or for 2 h at room temperature[5].

Cell culture media preparation

Culture medium for hES cells grown on irradiated MEFs was composed of standard DMEM/F12 supplemented with 20% KnockOut serum replacement (GIBCO), 0.1 mM β-mercaptoethanol, 1 mM L-glutamine, 1% non-essential amino acids and 4 ng/ml human recombinant basic fibroblast growth factor (Invitrogen; Carlsbad, CA). MEF-CM was prepared as described previously[6]. Irradiated MEFs (8×10^6 cells) were seeded onto gelatin-coated culture dishes in medium composed of high glucose DMEM, 10% fetal bovine serum (FBS; GIBCO), 1% non-essential amino acids, and 200 mM L-glutamine. After 24 h, MEF culture medium was replaced with the hES cell culture medium described above (60 ml). This medium was left in contact with MEFs and was collected as MEF-CM after 24 h of conditioning. Media was changed daily and MEF-CM was collected for 3 days. The MEF-CM was frozen at -2 ^{o}C and was supplemented with 0.1 mM β-mercaptoethanol, 2 mM L-glutamine, and 4 ng/ml bFGF prior to use. Human-cell-conditioned-medium (hCCM, GlobalStem, Inc., Rockville, MD), mTeSR^{TM}1

(STEMCELL Technologies, Vancouver, BC) and StemPro®hESC SFM (Invitrogen, Carlsbad, CA) were prepared according to manufacturers' protocols, with the exception that defined medium was prepared with DMEM/F12 plus L-glutamine and 15 mM HEPES (GIBCO). Media were pre-equilibrated at 37 ^{o}C in 5% CO_2 atmosphere before use.

Cell culture

Two federally-approved hES cell lines: BG01 (NIH code: BG01; BresaGen, Inc., Athens, GA), and H9 (NIH code: WA09; WiCell, Madison, WI) were used and cultured at 37 ^{o}C with 5% CO_2. Human ES cell colonies were observed every 48 h using a Leica stereomicroscope and differentiated cells were removed mechanically using a sterile pulled-glass pipette. Cell culture medium was replaced every 48 h.

Cell culture media transition and passaging

Before mechanical harvesting of hES cells growing on MEFs, cell culture medium was replaced by either MEF-CM or hCCM. Similarly, a transition from hCCM to defined media (mTeSR or StemPro) was performed. Cultures were passaged depending on size and density of colonies every 7-10 days. Undifferentiated hES cell colonies were mechanically passaged by cutting small aggregates of cells using a sterile pulled-glass pipette, when colonies were large, beginning to merge, and had centers that were dense and brighter compared to their edges. 50-100 hES cell-aggregates were transferred to each polymer- or Matrigel-coated dishes.

Cell-aggregate adhesion assay

The cell-aggregate adhesion assay was performed at each passage and an average value was reported. Number of adherent colonies and number of floating embryoid bodies (EBs) were counted 48 h after cell seeding. Percentage of cell-aggregate adhesion was calculated as follows:

Cell-aggregate adhesion (%) = (number of adhered colonies)×(100)/(number of adhered colonies + number of EBs floating). Statistics were performed using an unpaired t-test.

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Cell population-doubling time

Cell population-doubling time was calculated as time required for the area of a colony to increase two-fold[7]. Colony area was calculated by using the surface area equation of an ellipse ($\pi \times a \times b/4$, where a and b are the horizontal and longitudinal diameters). ImageJ software (http://rsb.nih.gov/ij) was used to measure colony diameters. Colony area (n=15) was calculated at five time-points (for 5 days after cell seeding), and cell population-doubling time was estimated as an exponential function. Unpaired t-test was used to calculate p values.

Immunocytochemistry

Cells were fixed in 2% paraformaldehyde for 30 min at room temperature and then permeabilized with 0.1% Triton X-100 for 10 min. Primary antibodies were diluted in 1% normal serum and incubated overnight at 4 ^{o}C and detected by respective secondary antibodies. Samples were imaged and captured using a Leica DM IRB inverted microscope with an Olympus DP-30 CCD camera (Microscopy and Image Analysis Laboratory, University of Michigan, Ann Arbor). Throughout this study, hES cells were characterized every fifth passage for their expression of the hES cell markers: OCT3/4, SOX2, SSEA4, TRA-1-60 and TRA-1-81. ImageJ software was used to count the number of OCT3/4 or SOX2 positive cells and number of cell nuclei (Hoechst) present in each colony. The percentage of cells expressing each marker was calculated for colonies cultured on PMEDSAH- and Matrigel- coated plates. Data were averaged and compared using unpaired t-test to calculate p values.

Cytogenetic analysis

Karyotype analysis was performed at Cell Line Genetics (Madison, WI) by GTL-banding on at least 20 cells per analysis per substrate.

Evaluation of pluripotency

Undifferentiated H9 hES cells (5×10^6 cells) growing on PMEDSAH or Matrigel in MEF-CM at passage 25 were injected subcutaneously into CB17 SCID mice (Charles River Laboratories, Wilmington, MA) to induce teratomas. When tumors became

palpable after 3-6 weeks, mice were euthanized; tumors were harvested, and processed for histological analysis at the Center for Organogenesis Morphology Core (University of Michigan).

Pluripotency was also evaluated by EB formation from undifferentiated hES cells. Colonies were cultured in suspension in hES cell culture medium lacking bFGF for 10 days to promote differentiation. Alternatively, hES cells were allowed to overgrow in MEF-CM without bFGF for 10 days.

Extraction and purification of total RNA

Cells were manually scraped from dishes and pelleted by centrifugation at 800 g. Pellets were then disrupted by vigorous pipetting in 1000 µl of Trizol Reagent (Invitrogen, Carlsbad, CA). Chloroform (200 µl) was added to this solution followed by centrifugation (~13,000 g). Aqueous phase containing RNA was separated and additionally purified using the RNeasy Mini-Kit (Qiagen, Valencia, CA) following the manufacturer's RNA Clean-up protocol with the optional On-column DNAse treatment. RNA quality was checked using RNA 6,000 Nano assays performed on the Bioanalyzer 2100 Lab-on-a-Chip system (Agilent Technologies, Palo Alto, CA).

Reverse-transcription PCR (RT-PCR) analysis

Total RNA was reverse transcribed using SuperScript™ One-Step RT-PCR with platinum® Taq (Invitrogen). In a single reaction (50 µl), 1 µg of total RNA and 20 pmol of forward (f) and reverse (r) primers were used (**Supplementary Table 2** online). The cDNA synthesis and pre-denaturation were carried out in the first cycle at 48 ºC for 45 min, followed by a second cycle at 94 ºC for 2 min. The PCR amplification was performed for 35 cycles at 94 ºC for 15 sec, 5 ºC for 30 sec, and 72 ºC for 1 min. The final extension cycle was run at 72 ºC for 8 min. Finally, 10µl of PCR reaction products were loaded onto a 1.0% agarose gel and size-fractionated.

Microarray analysis

Total RNA (10 µg) from hES cells grown on each substrate was hybridized to Affymetrix Human Genome U133 Plus 2.0 microarray (Affymetrix; Santa Clara, CA) following the

manufacturer's instructions. Data analysis was performed using a Robust Multi-array average algorithm that converted the plot of perfect match probe intensities into an expression value for each gene[8]. Based on a variance of 0.05, probe-sets that did not appear to be differentially expressed in any sample were filtered and removed. Differentially expressed genes were detected by fitting a linear model to each probe-set and selecting those with a multiplicity-adjusted p-value (FDR) of 0.05 or less[9, 10].

Quantitative Real-time PCR (qPCR) analysis

Total RNA was reverse transcribed using the MultiScribe™ Reverse Transcriptase System (Applied Biosystems; Foster city, CA). The ABI 7300 PCR and Detection System (Applied Biosystems) with SYBR® Green PCR Master Mix (Applied Biosystems) were used to conduct real-time PCR in triplicate for each sample. Primers used are listed in **Supplementary Table 2** online. Human *β-ACTIN* was amplified as an internal standard. Relative quantification of *NANOG, OCT3/4* and *SOX2* gene expression was performed using the Comparative C_T Method[11].

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