



Lab resource: Stem Cell Line

Generation of a human induced pluripotent stem cell (iPSC) line from a patient with family history of diabetes carrying a C18R mutation in the *PDX1* gene



Xianming Wang^{a,b,i}, Shen Chen^c, Ingo Burtscher^{a,b}, Michael Sterr^{a,b}, Anja Hieronimus^{d,e,j}, Fausto Machicao^{f,j}, Harald Staiger^{d,g,j}, Hans-Ulrich Häring^{d,e,j}, Gabriele Lederer^k, Thomas Meitinger^{h,k}, Heiko Lickert^{a,b,i,j,*}

^a Institute of Diabetes and Regeneration Research, Helmholtz Zentrum München, 85764 Neuherberg, Germany

^b Institute of Stem Cell Research, Helmholtz Zentrum München, 85764 Neuherberg, Germany

^c iPS and Cancer Research Unit, Department of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China

^d Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Zentrum München at the University of Tübingen, 72076 Tübingen, Germany

^e Department of Internal Medicine, Division of Endocrinology, Diabetology, Vascular Disease, Nephrology and Clinical Chemistry, University of Tübingen, 72076 Tübingen, Germany

^f Institute of Experimental Genetics, Helmholtz Zentrum München, 85764 Neuherberg, Germany

^g Institute of Pharmaceutical Sciences, Department of Pharmacy and Biochemistry, Eberhard Karls University Tübingen, 72076 Tübingen, Germany

^h Institute of Human Genetics, Helmholtz Zentrum München, 85764 Neuherberg, Germany

ⁱ Technische Universität München, Ismaningerstraße 22, 81675 München, Germany

^j German Center for Diabetes Research (DZD), 85764 Neuherberg, Germany

^k Institute of Human Genetics, Technische Universität München, 81675 München, Germany

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ABSTRACT

Homozygous loss-of-function mutations in the gene coding for the homeobox transcription factor *PDX1* leads to pancreatic agenesis, whereas certain heterozygous point mutations are associated with Maturity-Onset Diabetes of the Young 4 (MODY4) and Type 2 Diabetes Mellitus (T2DM). To understand the pathomechanism of MODY4 and T2DM, we have generated iPSCs from a woman with a C18R heterozygous mutation in the transactivation domain of *PDX1*. The resulting *PDX1* C18R iPSCs generated by episomal reprogramming are integration-free, have a normal karyotype and are pluripotent *in vitro* and *in vivo*. Taken together, this iPSC line will be useful to study diabetes pathomechanisms.

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Resource table

Name of stem cell line	<i>PDX1</i> C18R iPSC1
Institution	Institute of Diabetes and Regeneration Research
Person who created resource	Xianming Wang
Contact person and email	Heiko Lickert heiko.lickert@helmholtz-muenchen.de
Date archived/stock date	April 2014
Origin	Human dermal fibroblasts
Type of resource	Induced pluripotent stem cells from a woman carrying a <i>PDX1</i> C18R mutation
Sub-type	Cell line
Key transcription factors	OCT4, SOX2, NANOG, LIN28, KLF4, and L-MYC

(continued)

Name of stem cell line	<i>PDX1</i> C18R iPSC1
Authentication	Identity and purity of cell line confirmed
Link to related literature	Not available
Information in public databases	Not available
Ethics	Informed written consent obtained

Resource details

The Tübingen Family Study for T2DM (TUF study, $N = 2500$) was screened for known rare mutations in the *MODY4* gene *PDX1*. By mass spectrometry-based genotyping, one heterozygous *PDX1* C18R carrier could be identified and recruited for full-thickness skin biopsy. From the biopsy material, the epidermal layer was separated from the dermis by dispase digestion and fibroblasts were isolated from the dermis by trypsin digestion using an established protocol (see the Materials and

* Corresponding author at: Helmholtz Zentrum München, Parkring 11, D-85748 Garching, Germany.

E-mail address: heiko.lickert@helmholtz-muenchen.de (H. Lickert).

methods section). The *PDX1* C18R fibroblasts were expanded using commercially available growth media and deep-frozen in liquid nitrogen. From this collection of dermal fibroblasts, samples were tested for the presence of viruses pathogenic to humans (HBV, HCV, HIV) as

well as for the presence of mycoplasma. All cultures were found to be negative for all of these potential contaminants. *PDX1* C18R primary fibroblasts were reprogrammed into iPSCs using nucleofection with three episomal plasmids encoding human *OCT4*, *SOX2*, *NANOG*, *LIN28*,

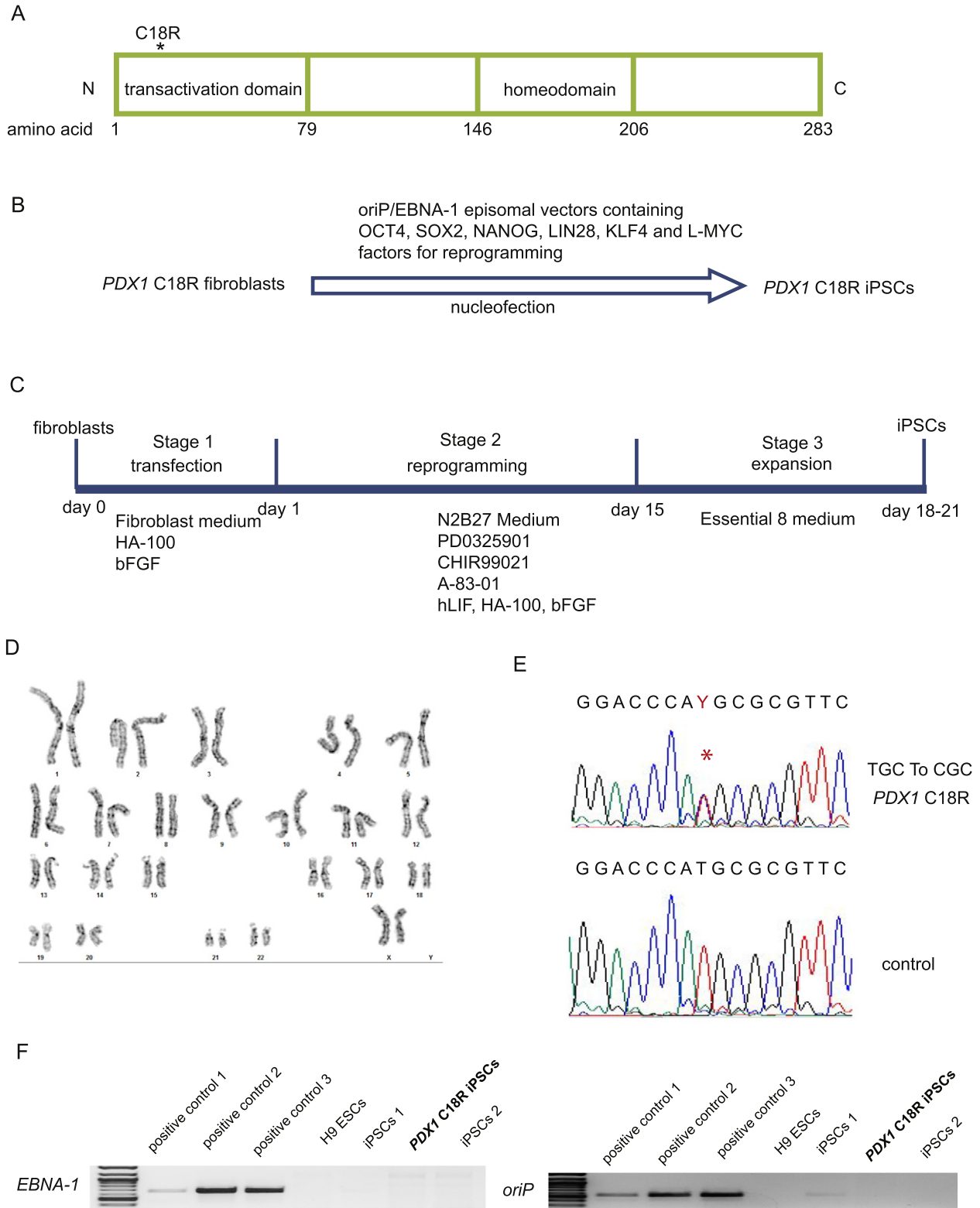


Fig. 1. Generation of *PDX1* C18R iPSCs. (A) An overview of the *PDX1* protein structure. (B) Scheme shows the reprogramming factors for the generation of the *PDX1* C18R iPSC line. (C) Reprogramming protocol of skin fibroblasts into integration-free iPSCs using episomal vectors. (D) Normal karyotype (46, XX) of one *PDX1* C18R iPSC clone. (E) Sequencing result shows heterozygous T > C mutation in *PDX1*. (F) Semi-quantitative PCR confirms that episomal vectors did not integrate into the genomic DNA of the selected *PDX1* C18R iPSC clone. H9 ESCs and two independent iPSC clones (#1 and #2) were used as negative controls, whereas transfected fibroblast at day 6 were used as positive controls.

KLF4, and *L-MYC* (Fig. 1B). The reprogramming protocol (transfection, reprogramming and expansion) is shown in Fig. 1C. 3 weeks after transfection, many colonies with human embryonic stem cell (hESC)-like morphology appeared that were hand picked by day 21, expanded and frozen down as individual iPSC lines. The analysis of one of these iPSC lines confirmed that *PDX1* C18R fibroblasts were successfully reprogrammed into iPSCs with a normal karyotype (Fig. 1D) and containing the T > C mutation causing a C18R amino acid exchange in the transactivation domain (Fig. 1A, E). Integration of the exogenous transgenes in the genome of iPSCs was excluded by semi-quantitative PCR (Fig. 1F). To confirm that this iPSC line was pluripotent, we performed immunofluorescence staining for the pluripotency transcription factors OCT4 and SOX2, as well as for the hESC surface markers SSEA-3, SSEA-4 and TRA-1-81, which were all expressed in the *PDX1* C18R iPSC line (Fig. 2A). To further demonstrate pluripotency of the selected iPSC line we injected 2×10^6 cells subcutaneously into NOD/SCID mice to generate teratomas. The histological analysis of the induced teratomas revealed that all tissues from all three germ layers were differentiated confirming pluripotency in this *in vivo* assay (Fig. 2B). Taken together, we have successfully reprogrammed *PDX1* C18R dermal fibroblasts into pluripotent iPSCs that can be used to study a point mutation in the transactivation domain of *PDX1* to study the development of diabetes.

Materials and methods

Ethics statement

The study adhered to the Declaration of Helsinki. All participants gave informed written consent, and the study protocols were approved by the local ethics board (Ethics Committee of the Eberhard Karls University Tübingen).

Study participants

The ongoing TÜF study currently includes >3000 non-related individuals at risk for type-2 diabetes mellitus (T2DM), *i.e.*, healthy subjects with a family history of T2DM, a body mass index ≥ 27 kg/m², impaired fasting glycaemia, and/or previous gestational diabetes (Stefan et al., 2005). From all TÜF participants, the medical history, smoking status, and alcohol consumption habits were queried and documented. Furthermore, all participants underwent physical examination, routine blood tests, bioelectric impedance measurement, and 5-point oral glucose tolerance tests (OGTTs) with insulin and glucose measurements. The current study population comprised 2500 individuals who spent DNA for genotyping, were not on any medication known to influence glucose tolerance, insulin sensitivity, or insulin secretion, and who had complete OGTT data sets.

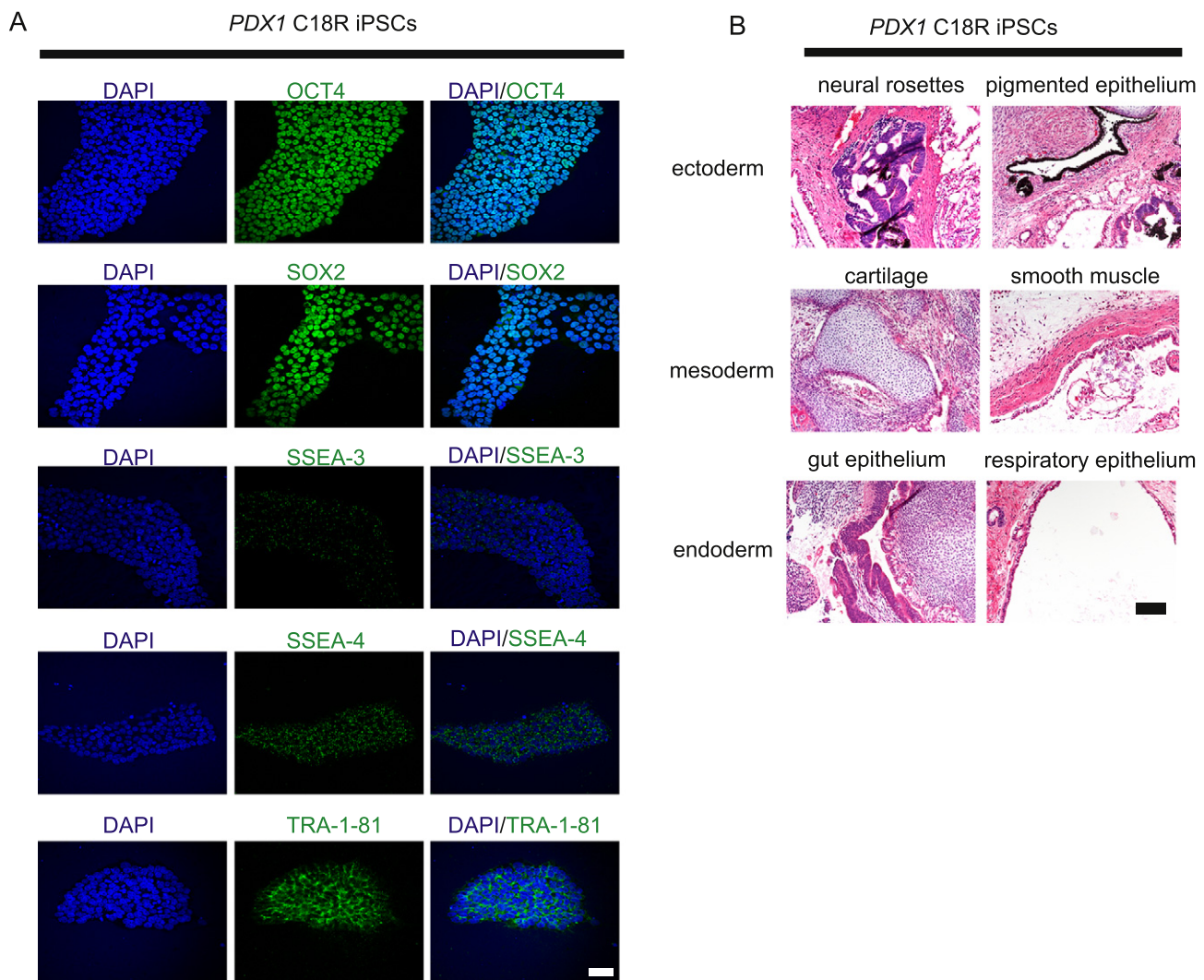


Fig. 2. *PDX1* C18R iPSCs are pluripotent and differentiate into all germ layers *in vivo*. (A) *PDX1* C18R iPSCs display typical morphological ESC-like characteristics and uniformly express several pluripotency markers. Scale bar indicates 50 μ m. (B) Hematoxylin/eosin staining of tissue sections of teratomas generated from the *PDX1* C18R iPSC clone. All germ layer-derivatives are clearly detectable *in vivo*. Scale bar indicates 100 μ m.

Using the mass spectrometry-based genotyping platform massARRAY from Sequenom (Hamburg, Germany) and the manufacturer's iPLEX software, the 2500 subjects were screened for known rare mutations in maturity onset of diabetes (MODY) genes that were described in the literature to co-segregate with MODY phenotypes in families. We identified seven individuals with heterozygous mutations in *PDX1* (MODY4) (McKinnon and Docherty, 2001). From these cases, one glucose-tolerant female *PDX1* Cys18Arg (C18R) carrier (aged 33 years; BMI 20.6 kg/m²) with history of diabetes could be recruited for full-thickness skin biopsy.

Skin biopsy, isolation and testing of dermal fibroblasts

A full-thickness skin specimen was taken by punch biopsy from the upper arm in the deltoid muscle region. After removal of adipose tissue remnants and visible blood vessels, the sample was digested overnight at 4 °C with 10 U/ml dispase II (Roche Diagnostics, Mannheim, Germany) in 50 mM Hepes pH 7.4, 150 mM NaCl. Thereafter, the digest was heated for 30 min at 37 °C under continuous shaking (1200 rpm). Using forceps, the dermis was separated from the epidermal layer, and fibroblasts were isolated from the dermis by digestion with 0.2% collagenase CLS I (Biochrom, Berlin, Germany) in DMEM, 10% BSA for 45 min at 37 °C under continuous shaking (1200 rpm). For purification of the fibroblasts, the digest was filtered through a 70 µm mesh and centrifuged. The pelleted cells were resuspended, grown for three days in DMEM, 10% FCS, and subsequently further expanded in Medium 106 supplemented with low serum growth supplement (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Samples of dermal fibroblasts were tested for the presence of viruses pathogenic to humans, *i.e.*, HBV, HCV, and HIV, with Genesig PCR-based detection kits from Primerdesign Ltd. (Chandler's Ford, UK) and for the presence of mycoplasma with a PCR test kit from PanReac AppliChem (Darmstadt, Germany) and, in parallel, by DNA staining with DAPI. All cultures were found to be negative for the tested contaminants.

iPSC generation

Primary fibroblasts from patient were reprogrammed into pluripotent stem cells by using a non-integrating Episomal iPSC Reprogramming Kit (Invitrogen, Cat. no. A14703). This kit contains mixture of three vectors which has the oriP/EBNA-1 (Epstein-Barr nuclear antigen-1) backbone that delivers six reprogramming factors: OCT4, SOX2, NANOG, LIN28, KLF4, and L-MYC (Yu et al., 2011). Human fibroblasts at 75–90% confluent were transfected using the Amaxa 4D-Nucleofector transfection system and a nucleofector kit for human dermal fibroblast (Lonza, Cat. no. VPD-1001), plated onto geltrex-coated culture dishes, incubated in supplemented fibroblast medium. This medium contained knockout DMEM/F-12 (Life technologies), 10% FBS of ESC-qualified (Life technologies), 1% MEM non-essential amino acids (Life technologies), 10 µM HA-100 (Santa Cruz) and 4 ng/ml bFGF (Life technologies). At 24 h after transfection, the medium were exchanged with N2B27 medium supplemented with 0.5 µM PD0325901 (Stemgent), 3 µM CHIR99021 (Stemgent), 0.5 µM A-83-01 (Stemgent), 10 µM HA-100 (Santa Cruz), 10 ng/ml hLIF (Life technologies) and 100 ng/ml bFGF. The basic N2B27 medium contained DMEM/F12 with HEPES (Life technologies), 1 × N2 supplement (Life technologies), 1 × B27 supplement (Life technologies), 1% MEM non-essential amino acids, 1 × Glutamax (Life technologies) and 1 × β-Mercaptoethanol (Life technologies). On day 15 after transfection, the medium was exchanged with Essential 8 medium and monitored for the emergence of iPSC colonies. Around 3 weeks after transfection, undifferentiated iPSC colonies were picked and transferred onto fresh geltrex-coated culture dishes for expansion.

iPSC characterization

DNA was extracted from iPSCs by using standard procedure. Markers for the episomal backbone were amplified by semi-quantitative PCR to

exclude transgene integration. Primers are as follows: *oriP* forward: TTCCACGAGGCTAGTGAACC. *oriP* reverse: TCGGGGTGTTAGAGACAAC; *EBNA-1* forward: ATCGTCAAAGCTGCACACAG. *EBNA-1* reverse: CCCAGGAGTCCCAGTAGTCA. For karyotype analysis, we used the cells growing in logarithmic phase. They were fed with fresh medium the night before adding colcemid for 2 h. Cells were then trypsinized, treated with hypotonic solution (0.075 M KCl) for 20 min and fixed with methanol:acetic acid (3:1). Metaphases were spread on microscope slides, and chromosomes were classified according to the International System for Human Cytogenetic Nomenclature using the standard G banding technique. At least, 20 metaphases were counted per cell line, and the final karyotype was stated if it was present in >85% of them. For teratomas, 2 × 10⁶ iPSCs were injected into the right hind leg of immunocompromised NOD/SCID mice. Tumors were excised after 8 weeks, fixed, embedded in paraffin, sectioned and stained with hematoxylin/eosin (Takahashi et al., 2007).

Mutation analysis

Genomic DNA was isolated from *PDX1* C18R and control iPSCs using standard procedure. PCR amplification with a set of primers flanking the mutation site was performed in those two samples. PCR products were sequenced using the reverse primer by Sanger sequencing on an ABI 3130 genetic analyzer (Applied Biosystems). Primers are as follows: *PDX1* forward: GGAGTGTGCAGCAAACCTCAG, *PDX1* reverse: ACGCGTGAGCTTTGGTAGAC.

Immunofluorescence imaging

Cells were fixed with 4% paraformaldehyde for 30 min and then permeabilized in PBS containing 0.2% Triton X-100. Cells were blocked with PBS containing 3% BSA, and incubated with primary antibodies overnight at 4 °C. Then secondary antibodies were incubated for 1 h at room temperature after washing with PBS. Images were acquired on a laser scanning microscope. The following antibodies and dilutions were used: goat anti-OCT3/4 (1:500, Santa Cruz), goat anti-SOX2 (1:500, Santa Cruz), rat anti-SSEA3 (1:50, Invitrogen), mouse anti-SSEA4 (1:500, cell signaling) and mouse anti-TRA-1-81 (1:50, Millipore).

Author disclosure statement

There are no competing financial interests in this study.

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