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Generation of a healthy heavy smoker patient-derived induced pluripotent stem cell line UHOMi007-A from peripheral blood mononuclear cells

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Abstract

Human pluripotent stem cells (hiPSC) represent a unique opportunity to model lung development and chronic bronchial diseases. We generated a hiPSC line from a highly characterized healthy heavy smoker male donor free from emphysema or tobacco related disease. Peripheral blood mononuclear cells (PBMCs) were reprogrammed using integration-free Sendai virus. The cell line had normal karyotype, expressed pluripotency hallmarks, and differentiated into the three primary germ layers. The reported UHOMi007-A iPSC line may be used as a control to model lung development, study human chronic bronchial diseases and drug testing.

Conflict of interest statement

Declaration of competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

Resource Table:	
Unique stem cell line identifier	UHOMi007-A
Alternative name(s) of stem cell line	SMOK1
Institution	Institute for Regenerative Medicine & Biotherapy (IRMB)
Contact information of distributor	John De Vos john.devos@inserm.fr
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 51 Sex: male Ethnicity if known: Caucasian
Cell Source	Peripheral Blood Mononuclear Cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Sendai virus method with the CTS™ CytoTune™-iPS 2.0 Sendai Reprogramming Kit
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	Negative by PCR
Associated disease	Healthy patient
Gene/locus	N/A
Date archived/stock date	30.01.2020
Cell line repository/bank	hPSCreg website: https://hpscereg.eu/cell-line/UHOMi007-A
Ethical approval	The study was approved by the regional scientific ethical committee (CPP Sud Med IV) under the number ID-RCB: INVECCO ClinicalTrials.gov Identifier: NCT03181204, Promoter University Hospital of Montpellier) and informed consent was obtained from the patient.

2. Resource utility

UHOMi007-A iPSC line was generated from a healthy heavy smoker donor. This cell line is used as a control with other cell lines derived from patients with severe **chronic obstructive pulmonary disease** (COPD) disease (Ahmed et al., 2021), for lung disease modelling. This cell line represents a unique model to investigate mechanisms of susceptibility to environmental exposure.

3. Resource details

Peripheral blood mononuclear cells (PBMCs) were isolated from a healthy heavy smoker patient (>50 pack years). Clinical examination was normal, the patient didn't have any comorbidity and thoracic computed tomography (CT) scan highlighted normal parenchyma and airways structure, especially no emphysema. PBMCs were reprogrammed into UHOMi007-A cell line using CytoTune-iPSC 2.0 Sendai Reprogramming kit.

The cell line had a normal morphology (Fig 1, panel A, scale bar: 50 μ m). We confirmed the absence of exogenous reprogramming vector by PCR using Sendai virus vector (SeV) specific primers (Fig. 1B). The transgenes and viral sequences were absent after about 15 passages in UHOMi007-A iPSC line but present in positive controls consisting of fibroblasts freshly transduced, at 6 days post transduction (i.e.; positive control) with the Sendai virus (Fig. 1, panel B). Karyotype analysis revealed normal number and structure of chromosomes (Fig. 1, panel C).

Expression of pluripotency markers were assessed by immunocytochemistry, using antibodies against human SOX2, OCT3/4, NANOG and SSEA-4 (Fig. 1, D scale bar 50 μ m). Expression of pluripotency genes was evidenced by q-RT-PCR, using previously published UHOMi002-A healthy cell line (Hy03 iPSC line) as positive control, for SOX2, NANOG and OCT4 (Fig. 1, panel F) (Fieldes et al., 2020).

We generated embryoid body (EB) from UHOMi007-A cell line, highlighting the ability of the iPSC line to differentiate into the three germ layers *in vitro*. Immunofluorescence analysis of the EB showed that they expressed ectodermal (β -III-tubulin), mesodermal (vimentin) (Fig. 1, panel E). Endodermal protein CXCR4 was assessed by flow cytometry (Fig. 1, panel G), with at least 89 % expression of the surface marker. The gene expression of ectoderm (ALDH1A1, PAX6, DCX), mesoderm (GATA2, IGF2, BMP4) and endoderm (COL1A1, SOX7) markers were also assessed by q-RT-PCR in comparison to the control cell line Hy03 (Fig. 1, panel H).

Furthermore, the identity of the UHOMi007-A cell line was confirmed by performing short tandem repeats (STR) DNA fingerprinting, which demonstrated a 100 % match to the PBMCs parental sample. Finally, iPSC line had a negative status for Mycoplasma contamination (Supplemental Table 1).

4. Materials and methods

4.1. Reprogramming of PBMCs to iPSCs

PBMCs were isolated by Ficoll gradient centrifugation (Ficoll-Paque PLUS GE Healthcare)(Vissers et al., 1988) and washed with PBS plus foetal bovine serum (FBS) 10 %.

PBMCs were cultured in StemSpan with Erythroid Expansion Medium (SSEM, StemCell Technologies), according to the manufacturer's instructions. The four reprogramming factors OCT3/4, SOX2, KLF4, and C-MYC were transiently expressed in the erythroid progenitor cells using the integration-free Sendai virus transduction method (see Table 1).

The expansion and reprogramming of erythroid progenitor cells (EPC) from PBMCs was performed following the previously described method (Fieldes et al., 2020). Briefly, PBMCs were amplified and differentiated into EPC in during 7 days. 500 000 EPC were then transduced by Sendai-virus with the CytoTune®-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific) in the StemSpan SFEM II medium with the supplement (STEMCELL Technologies), using the following MOI: 5/5/3 respectively for KOS, hc-Myc and hKlf4. Transfected cells were culture in StemSpan SFEM II complemented media for 24 h. The day after, virus clearance was performed by centrifugation (10 min, 200 rpm) and the supernatant was discarded. Cells were cultured for 48 h extra hours in the same media, in the presence of 10 µM of Y-27632 dihydrochloride (ROCK inhibitor, Tocris) and butyrate sodium at 0,5 mM (Stemcell Technologies). The day after, cells were transferred on plates coated with Matrigel and cultures for 3 days in SFEM II media without complement in presence of ROCK inhibitor. Cells were finally cultured E8 medium (Thermo Fisher Scientific). Colonies were mechanically picked and isolated based on morphologic features for further clonal expansion.

4.2. Cell culture of iPSC cell lines

Smoker cell line was cultured on feeder free Geltrex matrix (Thermo Fisher Scientific) in E8 medium (Thermo Fisher Scientific). The cells were cultivated at 37 °C in normoxic conditions/ 5 % CO₂ and passaged by mechanical dissociation. They were then adapted by passaging them using enzyme free EDTA dissociation reagent (Versene Solution, Thermo Fisher Scientific) every week into clumps or single cells, using 1:10 split ratio.

4.3. Generation and culture of embryoid bodies

iPS cells were dissociated into clumps and cultured in low-adhesion dishes (Falcon) in E8 medium. EB were cultured in differentiation medium composed of RPMI-1640 medium (Gibco) with 10 % FBS (Gibco) and 1 % Penicillin-streptomycin. The medium was changed every other day during 30 days. Afterward, the EB were plated on Geltrex-coated dishes and cultured for an extra week. For the generation of the endoderm layer, EB cells were dissociated and plated at high density on plates coated with Geltrex (70 k.cm²), and cultured in the presence of B27 supplement (50x, Thermo Fisher), minus vitamin at 1 %, Activin A (Peprotech) at 100 ng/mL, 3 µM of CHIR-99021 (Sigma) and Y-27632 for 3 days.

4.4. Karyotyping

Karyotyping was performed at mechanic passage 20. Karyotyping was performed using a service provider using the KaryoStat™ assay. Cells were prepared according to the Genomic DNA Purification Kit (Catalog #: K0512) and quantified using the Qubit™ dsDNA BR Assay Kit (Catalog #: Q32850). GeneChip® Preparation 250 ng total gDNA was used to prepare the GeneChip® for KaryoStat™ according to the manual.

4.5. Immunofluorescence labelling

Cells were tested after passage 20 and fixed in 4 % PFA for 20 min at room temperature (RT) and washed 3 times in phosphate-buffered saline (PBS, Gibco). Permeabilization was performed using with 0.5 % Triton X-100 (Sigma Aldrich) for 15 min, and blocking step using 10 % of normal donkey serum (Sigma Aldrich) for 1 h at RT. Primary antibodies incubation was performed overnight at 4 °C in PBS BSA 1 % 0.1 % Triton X-100 (listed in Table 2). Cells were washed 2 times in PBS and incubated for one hour at RT in secondary antibodies solution used at the dilution of 1:2000. Nuclei were counterstained with DAPI (Sigma Aldrich). Zeiss Axioimager Z1 Apotome and Confocal Microscopy Zeiss LMS700 were used to capture images.

4.6. Flow cytometry analysis

Cells were tested after passage 20 and harvested into single cell suspension using dissociation reagent (Versene Solution). Cells were then incubated 15 min at room temperature with a Live Cell/Dead Cell staining (Violet Zombie, Biolegend, 1:1000), washed twice in PBS and then incubated with the conjugated primary CXCR antibody or the control isotype for 30 min at 4° C. Acquisition was performed on flow cytometry using Beckman Coulter Gallios. Analysis was performed with Kaluza software.

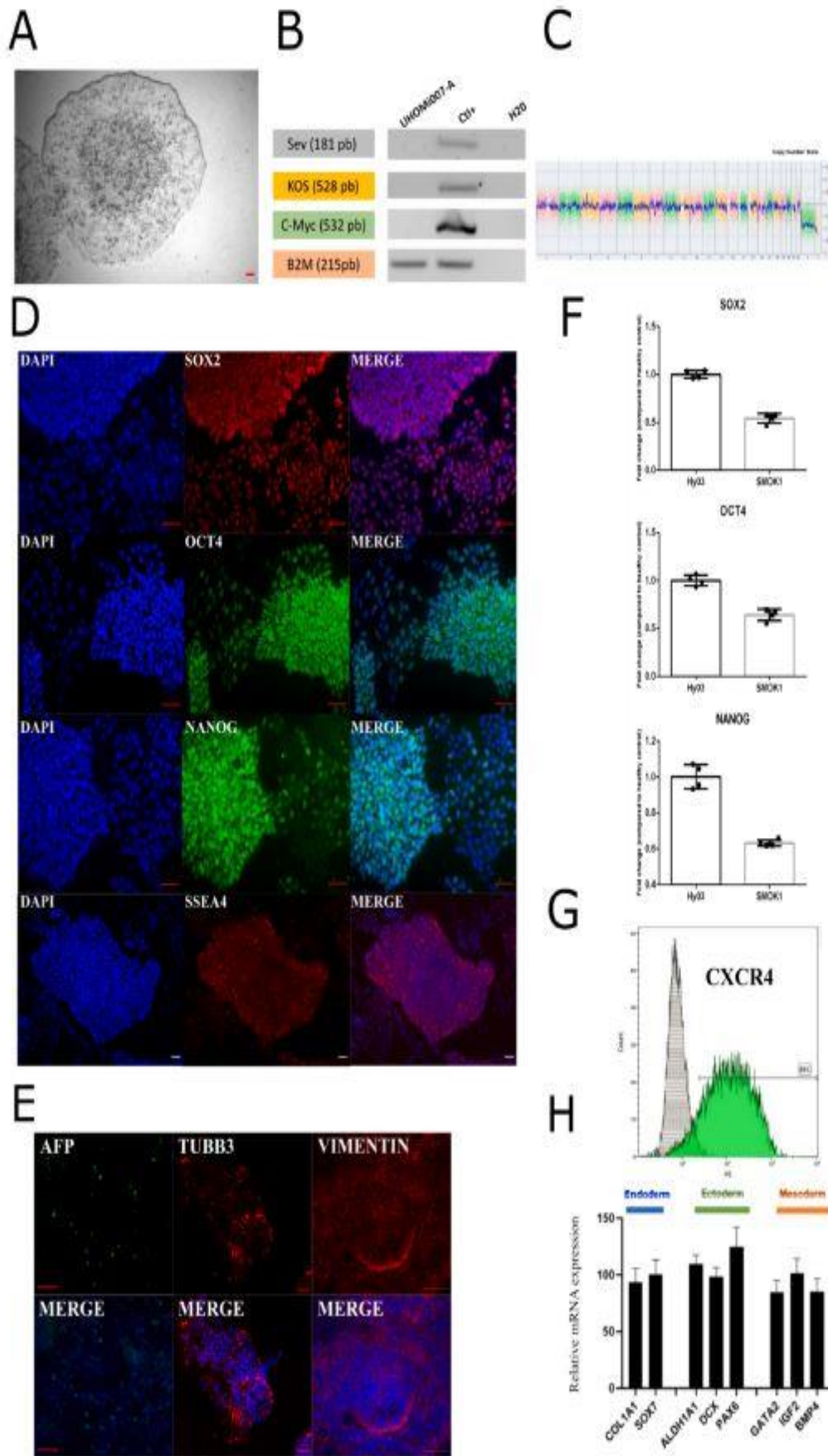


Fig. 1. Characterization of the UHOM007-A hiPSC line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis	Expression of pluripotency markers: SOX2, OCT4, NANOG and SSEA-4	Fig. 1 panel D
	Immunocytochemistry	Expression of SOX2, NANOG and OCT4	Fig. 1 panel F
Genotype	Quantitative analysis		
	RT-qPCR		
Genotype	Karyostat and resolution	46XY, G & R banding Resolution 400-500	Fig. 1 panel C, Supplemental Data 2
Identity	Microsatellite PCR (mPCR) OR	DNA Profiling not performed	N/A
	STR analysis	STR: specific 16 loci tested, 16/16 matched between iPS cell line and patient derived PBMCs	Supplemental Table 3, 4 and 5
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplemental Table 1
Differentiation potential	e.g. Embryoid body formation OR Teratoma formation OR Scorecard OR Directed differentiation	Proof of three germ layers formation by embryoid body and directed differentiation for endoderm	Fig. 1
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA (RT PCR) or protein (IF) levels, at least 2 markers need to be shown per germ layer	RT-qPCR	Fig. 1 Panel H
		Immunofluorescence	Fig. 1 Panel D, E
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Endoderm: PAX6, DCX, ALDH1A1	
		Endoderm: SOX7, COL1A1	
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	Mesoderm: GATA2/IGF2/BMP4	
		Flow cytometry	Fig. 1
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	Endoderm: alpha fetoprotein (AFP)	Panel G
		Endoderm: TUBB3/TUJ1	
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	Mesoderm: VIMENTIN	Fig. 1
		Flow cytometry	Panel G
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Endoderm: C-X-C Motif Chemokine Receptor (CXCR4): 89 %	
		Negative	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	N/A	N/A
		N/A	N/A

4.7. RT-qPCR analysis

Around passage 20, RNA isolation from the UHOMi007-A cell line was performed using the RNeasy mini kit (ref 74106, Qiagen) in accordance with the manufacturer's instruction. Reverse transcription (RT) was carried out on 1 µg of total RNA on cells using SuperScript™ First-Strand Synthesis System (ref 11904–018, Invitrogen), superscript II RT, oligo-dT primer, dNTP mixture, MgCl₂, 0.1 M DTT and RNase inhibitor. RT Quantitative PCR (RT-qPCR) was then performed using the LightCycler® 480 SYBR Green I Master (04707516001, Roche) with 2 µl of the RT reaction product (1:20 dilution) and 0, 5 µl of each primer diluted at 10 µM. Primers were listed in Table 2. Fold change was calculated using the ddCt method and gene expression levels were normalised to the expression of the housekeeping gene GAPDH.

4.8. Test for absence of the reprogramming vector

After passage 15, iPSC line was tested for absence of the Sendai reprogramming vectors by performing a RT-PCR, which detects the Sendai virus (SeV) genome and the transgenes. Total RNA was extracted from fibroblasts, and the UHOMi007-A iPSC line as described above (RT-q PCR analysis). cDNA were obtained following reverse transcription using the Superscript III First-strand synthesis system (Invitrogen, Thermo Fisher Scientific), with a Master Cycle gradient device (Eppendorf). Expression of transgenes was assessed using Sendai-virus-specific primers (Table2) and conventional PCR. B2M was used as housekeeping gene (Primers are listed in Table 2). The PCR was performed on a Master Cycle gradient device (Eppendorf) according to this following program: DNA denaturation at

95 °C during 5 min, followed by 40 cycles at 95 °C during 60 s, 55 °C for 60 s and 72 °C for 20 s, final elongation for 5 min at 72 °C. Products were analysed on 2 % agarose gel, ran at 130 Velectric field.

4.9. Short Tandem Repeat analysis (STR)

Genomic DNA was isolated using Qiamp DNA mini kit (Qiagen) as described below on cells after passage 10. The DNA was sent to the service provider Eurofins for STR DNA fingerprinting. STR analysis of generated iPSC and parental PBMCs was performed to detect 16 loci (matching of 16/16 loci: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, AMEL, D5S818, FGA, D19S433, vWA, TPOX and D18S51; Supplemental Tables 3,4 and 5).

4.10. Mycoplasma testing

Cell culture supernatants were tested around passage 20 using bioluminescence MycoAlert® Detection Kit (Lonza). The absence of mycoplasma contamination was subsequently confirmed according to the manufacturer's instructions. A ratio < 0.9 means that the sample was negative for Mycoplasma.

CRedit authorship contribution statement

Engi Ahmed: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization.

Mathieu Fieldès: Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization.

Chloé Bourguignon: Writing – review & editing, Validation.

Joffrey Mianné: Writing – review & editing, Validation.

Aurélie Petit: Writing – review & editing.

Nasri Amal: .

Florent Foisset: Writing – review & editing.

Carine Bourdais: Writing – review & editing.

Isabelle Vachier: Writing – review & editing, Resources, Project administration, Funding acquisition.

Said Assou: Writing – review & editing, Software, Formal analysis, Data curation.

John De Vos: Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition.

Arnaud Bourdin: Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition.

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry				
	Antibody	Dilution	Company Cat #	REFID
Pluripotency Markers	Rabbit polyclonal anti SOX2	1:50	Santa Cruz Technology	RRID: AB_2255358
	Rabbit Polyclonal anti OCT4	1:400	Coat# sc-20083 Santa Cruz Technology Coat#	RRID: AB_2167703
	Rabbit polyclonal anti NANOG	1:250	Abcam Coat# ab90892	RRID: AB_2150714
	PerCP Mouse monoclonal SSEA-4	1:250	BD pharmingen Coat# 561565	RRID: AB_10894210
Differentiation Markers	Mouse IgG3 PerCP Dsypc Control (Clone 133316) antibody	1:250	RRID Coat# CG07C	RRID: AB_1207029
	Polyclonal goat anti Vimentin	1:50	Santa Cruz Biotechnology Coat# sc-7557	RRID: AB_793928
	Mouse anti β III-tubulin	1:1000	Covance Research Products Inc. Coat# MMS-435P	RRID: AB_2313773
	Mouse anti-CXCR4 (P1)	1:200	BD Biosciences	RRID: 557145
Secondary antibodies	Mouse anti AIF	1:200	Sigma-Aldrich	RRID: AB_256392
	donkey anti mouse Alexa fluor 555	1:2000	Thermo Fisher Scientific Coat# A-21570	RRID: AB_2536780
	donkey anti rabbit Alexa fluor 488	1:2000	Thermo Fisher Scientific Coat# A-21206	RRID: AB_2535792
	Mouse IgG2a, (PE)	1:200	BD Biosciences	RRID: 556653
Primers				
	Target	Size of band	Forward/Reverse primer (5'-3')	

Transgene from SeV Vector (PCR)	SeV	181 bp	Forward: GGA TCA CTA GGT GAT ATC GAG C* Reverse: ACC AGA CAA GAG TTT AAG AGA TAT GTA TC*
	KOS	528 bp	Forward: ATG CAC GGC TAC GAC GTG AGC GC Reverse: ACC TTG ACA ATC GTG ATG TGG Forward: TAA CTG ACT AGC AGG CTT GTC G*
	c-MYC	532 bp	Reverse: TCC AGA TAC AGT CCT GGA TGA TGA TG
House-Keeping Gene	B2M (PCR)	215 bp	Forward: ACTCACTTCATCCAGCAGAG Reverse: CACCGCAGGCATACCTATCT
Pluripotency genes (q-RT-PCR)	OCT4	196bp	Forward: GGG CTC TCC GAT GCA TTC AAA C Reverse: CAC CTT GGC TTC AAC CAG TTG C
	SOX2	118pb	Forward: GGCAATTAAGGGCAGACTGCC Reverse: TTACTCTCCTCTTTTGGAGCCCTCC

Table 2 (continued)

Antibodies used for immunocytochemistry/flow-cytometry				
Antibody	Dilution	Company Cat #	EBID	
			Forward: TGA TTT GTG GGC CTG AAG AAA Reverse: GAG GCA TCT CAG CAG AAG ACA	
Differentiation markers (q- RT-PCR)	ALDH1A1	89 pb	Forward: CTGCTGGCGAACAATGGAGT Reverse: CGCAATGTTTTGATGGAGCCT	
	CD133	140 pb	Forward: GAGGGCCAAAGACGAGACATC Reverse: CAGATCAAGTCATGTCACAAAC	
	PAX5	120 pb	Forward: AAGGATAACGATACCAAGGGTGT Reverse: GGTCTGACCGTTCAACATC	
	CD34	94 pb	Forward: TGCTTAGGGAGTGGCTA Reverse: GAA CAGACATAGCTTTTCCCTTC	
	GATA2	89 pb	Forward: CTGCTGGGACGGCCCTGTG Reverse: GTTCCGAGCTGGATCCCTT	
	BMP4	135 pb	Forward: GCACCTGGCTTGAGTATGCTG Reverse: TGCTGAGGTTAAGAGGAAACG	
	IGF2	121 pb	Forward: AGAGTACTGTGCTAGCCG Reverse: TGCTTCAGGGTGCATATTCG	
	SOX7	143 pb	Forward: TCGACCCCTGGATCAACT Reverse: CTGGAGGACCGGAAATGTC	
House -keeping Genes	GAPDH (qRT-PCR)	64 pb	Forward: GAC CTG ACC TGC CGT CTA GAA A Reverse: CTTGCT TCA GCA CCT TCT TCA	

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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