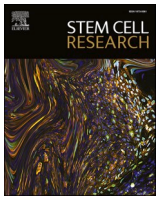
Stem Cell Research 86 (2025) 103732

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr



Lab Resource: Multiple Cell Lines

Reprogramming of two induced pluripotent stem cell clones from a patient with a novel *MT-ATP6/8* mutation (m.8570 T > C)

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A B S T R A C T

iPSC-based models are valuable for studying the mechanisms and potential treatments of mitochondrial disorders. We generated two iPSC lines from fibroblasts of a patient with a novel *MT-ATP6/8* mutation (m.8570 T > C). The infant was diagnosed with a mitochondrial disease featuring cardiac hypertrophy, brain atrophy, developmental delay, and metabolic crises with elevated lactate. Mutation heteroplasmy in blood leukocytes was 95 %. Leigh syndrome-like cranial MRI abnormalities were absent at 4 months of age. We introduced reprogramming factors by Sendai virus and assessed the pluripotency of the resulting iPSCs. As control iPSC-line, we characterized the *CRMi004-A* line from the RUCDR repository.

1. Resource Table

Unique stem cell lines identifier	*MDCi237-A* *MDCi237-B*
Alternative name(s) of stem cell lines	*Names commonly used by the researcher.* –
Institution	*Max Delbruck Center (MDC),Berlin Institute of Health (BIH)*
Contact information of distributor	*Markus Schuelke, MD markus.schuelke@charite.de*
Type of cell lines	*iPSC*
Origin	*human*
Additional origin info required	*MDCi237-A (derived from fibroblast line 11624, M, 4 y)* *MDCi237-B (derived from fibroblast line 11624, M, 4 y)*
Cell Source	*Skin fibroblasts*
Clonality	*Clonal*
Method of reprogramming	*Episomal, using Sendai virus expressing OCT3/4, SOX2, c-MYC, and KLF4*
Genetic Modification	*Yes, hereditary*
Type of Genetic Modification	*Wildtype cells derived iPSCs with a naturally occurring mtDNA mutation, no genetic modification done*
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	*PCR*
Associated disease	*Maternally Inherited Leigh Syndrome (MILS)*
Gene/locus	*MDCi237-A: Mutation m.8570 T > C in MT-ATP6*
Date archived/stock date	*MDCi237-A (18.03.2022)* *MDCi237-B (18.03.2022)*
Cell line repository/bank	*https://hpscreg.eu/cell-line/MDCi237-A* *https://hpscreg.eu/cell-line/MDCi237-B*
Ethical approval	*Ethical approval was obtained from the Institutional Review Board of Charité (Ethikkommission der Charité, Campus Virchow Klinikum) and registered under EA2/131/13*

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2. Resource utility

Patient-specific induced pluripotent stem cells (iPSCs) provide a valuable tool for generating disease models of mitochondrial DNA (mtDNA)-associated diseases, which will aid in the study of their mechanisms and potential therapeutic approaches (Inak et al., 2017;

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https://doi.org/10.1016/j.scr.2025.103732
Received 21 October 2024; Received in revised form 28 April 2025; Accepted 4 May 2025
Available online 8 May 2025

Lorenz et al., 2017). This paper, we describe two iPSC lines derived from a patient with a mitochondrial disease (see Tables 1 and 2).

3. Resource details

Human F_1F_o-ATP synthase (complex V) is a multiprotein complex consisting of 29 subunits, of which only two (*MT-ATP6*, *MT-ATP8*) are encoded by the mitochondrial DNA (mtDNA). The *MT-ATP6* gene encodes the subunit 6 (chain A) of the F_o complex, which is part of the proton channel while the *MT-ATP8* gene encodes the subunit 8, which is part of the large transmembrane domain of the F_o complex. Both genes overlap by 46 bp, making it possible for a mutation to affect both gene products. Here we describe two iPSC clones derived from a patient with clinical features consistent with mitochondrial disease, including lactic acidemia, microcephaly, frontal brain atrophy and *Corpus callosum* dysgenesis, epileptic seizures, global developmental delay, anemia, and hypertrophic cardiomyopathy.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography bright field	*Visual record of the line: normal*	Fig. 1 *panel A*
Phenotype	Qualitative analysis *(Immunocytochemistry)*	*Assess staining of pluripotency markers: OCT3/4, SOX2, Tra 1–60.*	Fig. 1 *panel A*
	Quantitative analysis *(RT-qPCR)*	*Assess mRNA rel. to GAPDH of pluripotency markers: OCT3/4, NANOG, SOX2, DPPA4, DNMT3B.*	Fig. 1 *panel B*
	Quantitative analysis *(Flow cytometry)*	Assess % of positive cells or transcripts for antigen & cell surface markers	*Supplementary file panel C-E*
Genotype	*Karyotype and resolution*	*Virtual karyotyping using Illumina OMNI-EXPRESS-8v1.6 Chip. No significant changes compared to the primary cells detected. Co-amplification and three-color detection of nine human loci Identical to profile of primary cells*	Fig. 1 *panel D*
Identity	*Microsatellite PCR (mPCR) OR STR analysis*	*N/A Co-amplification and three-color detection of nine human loci Identical to profile of primary cells*	*submitted in archive with journal*
Mutation analysis	Sequencing	*Heteroplasmy analysis by Sanger sequencing and RFLP*	Fig. 1 *panel C and Supplementary file panel B*
	Southern Blot OR WGS	*N/A*	
Microbiology and virology	Mycoplasma testing	*Mycoplasma testing by RT-qPCR. Negative*	*Supplementary file panel A*
Differentiation potential	Embryoid body formation	*Immunostaining positive for SMA and fibronectin (mesoderm), AFP and SOX17 (endoderm), and PAX6 and TUJ1 (ectoderm)*	Fig. 1 *panel E*
Donor screening	HIV 1/2, Hepatitis B, Hepatitis C	*Negative*	*not shown but available from authors*
Genotype additional info	Blood group genotyping	*N/A*	
	HLA tissue typing	*N/A*	

Two iPSC clones were generated from skin fibroblasts derived from a 10-months-old male infant with 96.2 % heteroplasmy (source fibroblast line). The cells were subsequently reprogrammed using non-integrating Sendai viruses carrying the reprogramming factors OCT3/4, SOX2, c-MYC, and KLF4 (Takahashi et al., 2007). In addition, we acquired an NIH CRM control iPSC line (CRMi004-A) from RUCDR and use this line for comparisons with our newly generated iPSC lines.

The reprogrammed lines exhibited typical embryonic stem cell-like morphology and formed well-defined, cohesive colonies (Fig. 1A, phase-contrast images). Immunofluorescence analysis confirmed the presence of the pluripotency markers SOX2, OCT3/4, and TRA-1–60 at passages 14–17 (Fig. 1A, epifluorescence images taken with a Leica DMi8 microscope). We also analyzed the mRNA expression of pluripotency the markers *POU5F1 (OCT3/4)*, *NANOG*, *DPPA4*, *SSEA4*, and *DNMT3B* by qPCR (Fig. 1B) and FACS (Supplementary Fig. S1C-E) at passages 14–25.

To evaluate the maintenance of the *MT-ATP6/8* m.8570 T > C heteroplasmy levels in the patient iPSC lines, we used a quantitative PCR-RFLP approach and subsequently confirmed the results by quantitative PCR fragment analysis using Illumina sequencing. The high level of heteroplasmy decreased after the reprogramming of iPSC Clone A (to 66.9 %) and Clone B (to 78.6 %) (Fig. 1C, RFLP quantification and Supplementary Fig. S1B, NGS quantification). Furthermore, all three lines showed a normal karyotype at passages 4–5 (Fig. 1D).

We performed an *in vitro* differentiation assessment using embryoid bodies (EBs) (Fig. 1E) and confirmed that all iPSC lines were capable of differentiating into all three germ layers. This was indicated by the presence of ectodermal markers: paired box 6 (PAX6) and class III beta-tubulin (TUJ1), mesodermal markers: smooth muscle actin (SMA) and fibronectin (FN1), and endodermal markers: alpha-fetoprotein (AFP) and SRY-box transcription factor 17 (SOX17).

4. Materials and methods

4.1. Reprogramming

Fibroblasts were tested for HIV 1/2 and Hepatitis B/C by a commercial diagnostic laboratory. The original cell lines were reprogrammed using Sendai virus (CytoTune 2.0) according to the manufacturer's protocol. A healthy human female iPSC line (*CRMi004-A*) was obtained from the NIH Center for Regenerative Medicine (RMP). After reprogramming, we used PCR to confirm the absence of the reprogramming vectors. The iPSCs were then cultured in a 6-well plate format using StemMACS iPS-Brew XF media (Miltenyi Biotec, 130–104-368) at 37 °C under hypoxic conditions (5 % O_2, 5 % CO_2).

4.2. Immunofluorescence staining for pluripotency markers

We seeded iPSCs onto GelTrex-coated (Gibco, A1413302) glass coverslips and cultured them at 37 °C in an environment of 5 % O_2 and 5 % CO_2 until they reached approximately 60 % confluence. The cells were then fixed with 4 % paraformaldehyde in phosphate-buffered saline (PBS) for 15 min, followed by rinsing with PBS. To permeabilize the cells, they were incubated with 0.1 % Triton X-100 (Merck, 108643) in PBS for 10 min with gentle rocking. After incubation with 10 % normal donkey/goat serum (Abcam, ab7475/ab7481) in PBS for 1 h, primary antibodies were diluted in 10 % serum and incubated with the cells overnight at 4 °C. After three 5-minute washes with PBS, the appropriate

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry **Antibody**	**Dilution**	**Company Cat #**	**RRID**
Pluripotency markers (IF staining)	anti-human OCT3/4 (C10), mouse	1:150	SantaCruz Biotechnology Cat #sc-5279	AB_628051
	anti-human SOX2, goat	1:200	R&D systems Cat #AF2018	AB_355110
	anti-human TRA1-60, mouse	1:200	Merck Cat #MAB4360	N/A
Pluripotency markers (FACS)	anti-SSEA4-VioBlue	1:50	Miltenyi Biotec: Cat #130–098-366	AB_2653521
	anti-OCT3/4-APC	1:50	Miltenyi Biotec: Cat #130–123-318	AB_2819472
	anti-TRA1-60-Vio488	1:50	Miltenyi Biotec: Cat #130–106-872	AB_2654228
	anti-SSEA1 (CD15) PE-Vio770	1:50	Miltenyi Biotec: Cat #130–114-012	N/A
	anti-NANOG-PE	1:50	Cell Signaling: Cat #14955S	N/A
Differentiation markers	anti-beta-Tubulin III (neuronal), mouse	1:600	SigmaAldrich: Cat #T8578	AB_1841228
	anti-PAX6, rabbit polyclonal	1:50	Invitrogen: Cat #42–1604	N/A
	anti-SMA, mouse	1:200	CellSignaling: Cat #48938S,	N/A
	anti-Fibronectin, rabbit	1:200	Abcam: Cat#ab299	AB_303474
	anti-AFP, rabbit	1:50	Proteintech: Cat #14550–1	N/A
	anti-SOX17, mouse	1:50	OriGene: Cat #CF500044	AB_2904161
Secondary antibodies	anti-mouse IgG Alexa Fluor 568, goat	1:200	Invitrogen: Cat #A11004	AB_2534072
	anti-rabbit IgG Alexa Fluor Plus 488, goat	1:200	Invitrogen: Cat #A32731	AB_2633280
	Primers **Target**	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Pluripotency markers (RT-qPCR)	POU5F1 (OCT3/4)	120 bp	F: GTGGAGGAAGCTGACAACAAR: ATTCTCCAGGTTGCCTCTCA	
	NANOG	78 bp	F: CCTGTGATTTGTGGGCCTG R: GACAGTCTCCGTGTGAGGCAT	
	SOX2	78 bp	F: GTATCAGGAGTTGTCAAGGCAGAGR: TCCTAGTCTTAAAGAGGCAGCAAAC	
	DNMT3B	93 bp	F: GCTCACAGGGCCCGATACTTR: GCAGTCCTGCAGCTCGAGTTTA	
	DPPA4	91 bp	F: TGGTGTCAGGTGGTGTGTGGR: CCAGGCTTGACCAGCATGAA	
Neuronal markers (RT-qPCR)	NES	79 bp	F: TTCCCTCAGCTTTCAGGACR: GAGCAAAGATCCAAGACGC	
	PAX6	99 bp	F: GAATTCTGCAGACCCATGCR: TCTCGTAATACCTGCCCAG	
	SOX1	84 bp	F: TTGGCATCTAGGTCTTGGCTCAR: CGGGCGCACTAACTCAGCTT	
Housekeeping genes (RT-qPCR)	GAPDH	81 bp	F: CTGGTAAAGTGGATATTGTTGCCATR: TGGAATCATATTGGAACATGTAAACC	
Quantification of the degree of heteroplasmy for the mtDNA m.8570 T > C mutation	by fluorescent restriction fragment analysis using the StyI restriction enzyme	wildtype: 66 + 95 bp mutant: 161 bp	F: TTATAACAAACCCTGAGAACCAAAA R: FAM-TGGGTGGTGATTAGTCGGTTG	
	by mtDNA PCR followed by quantitative PCR fragment analysis by Illumina sequencing. PCR primers carry Illumina adapters (in upper case letters)	445 bp	F: ACACTCTTTCCCTACACGACGCTCTT CCGATCTcacctacctccctcaccaaa R: GACTGGAGTTCAGACGTGTGCTCTT CCGATCTgatggccatggctaggttta	
Sendai virus vectors	SeV	181 bp	F: GGATCACTAGGTGATATCGAGCR: ACCAGACAAGAGTTTAAGAGATATGTATC	
	SeV-Klf4	410 bp	F: TTCCTGCATGCCAGAGGAGCCCR: AATGTATGCAAGGTGCTC	
	SeV-cMyc	532 bp	F: TAACTGACTAGCAGGCTTGTCGR: TCCACATACAGTCCTGGATGATGATG	
	SeV-KOS	528 bp	F: ATGCACCGCTACGAGTGAGCGCR: ACCTTGACAATCCTGATGTGG	

secondary antibody diluted in 10 % serum was added and incubated for 45 min at room temperature under dark conditions. A 1:1000 dilution of 4′,6-diamidino-2–2-phenylindole (DAPI, Invitrogen, D1306) was used for nuclear counterstaining. The cells were then washed three times with PBS and mounted on coverslips using Mowiol 4–88 (Roth, 0713). Fluorescence images were then captured using a Leica Thunder DMi8 microscope and processed using the Leica Application Suite (LAS) X software.

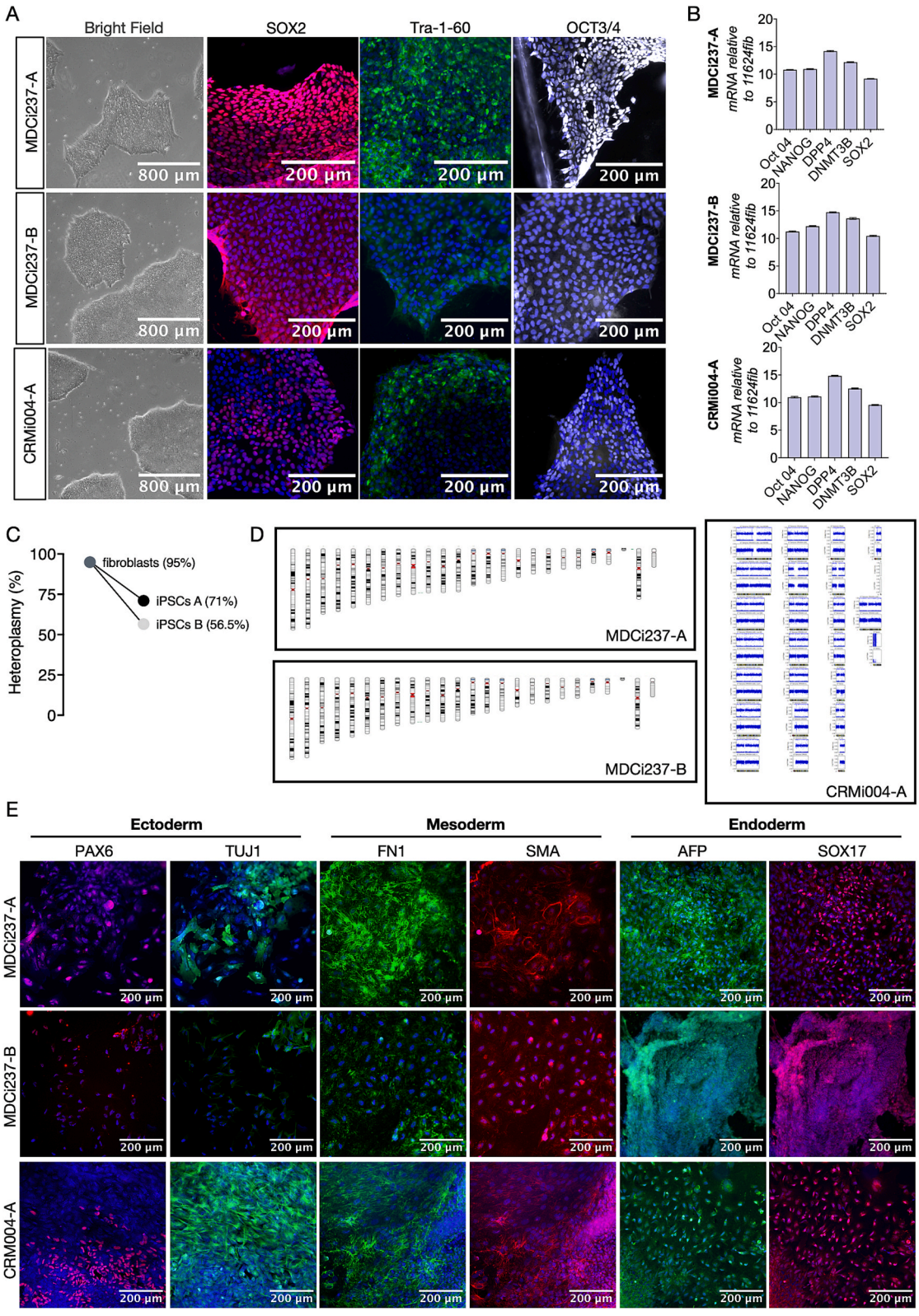

Fig. 1. Molecular and functional characterization of iPSC lines. **(A)** Representative phase-contrast images of the patient-derived iPSC and control iPSC colonies alongside immunofluorescent staining for the pluripotency markers SOX2, Tra-1–60, and OCT3/4. Nuclei were counterstained with DAPI. **(B)** Quantitative RT-qPCR analysis of pluripotency marker expression in the iPSC lines. Gene expression levels were normalized to the *GAPDH* transcript number and calculated in relation to the gene expression levels of the patient's source fibroblast line 11624. Data are depicted as mean ± SD from triplicate measurements. **(C)** Assessment of the decline of heteroplasmy levels for the mutation from before and after iPSC reprogramming. **(D)** Virtual karyotyping based on single nucleotide polymorphism (SNP) analysis. No large chromosomal insertions or deletions were detected; green indicates genomic gains, red represents genomic losses, and gray denotes regions with loss of heterozygosity. **(E)** Immunostaining of iPSC-derived embryoid bodies (EBs) confirming differentiation into all three germ layers: mesoderm (FN1, SMA), endoderm (AFP, SOX17), and ectoderm (PAX6, TUJ1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.3. Formation of embryoid bodies (EBs) and differentiation of three germ layers

To generate embryoid bodies (EBs), we detached iPSCs at approximately 80 % confluence using Dispase (ThermoFisher, 17105041). The cells were then transferred to a 6-well non-tissue culture plate (Falcon, 351146) and cultured for seven days at 37 °C under hypoxic conditions with 5 % O_2 and 5 % CO_2 in StemMACS iPS-Brew XF media (Miltenyi Biotec, 130–104-368). The EBs were then transferred to GelTrex-coated (GibCo, A1413302) glass coverslips cultured for 7 days for mesodermal and endodermal marker analysis and 14 days for ectodermal marker analysis. Culturing was performed at 37 °C with 5 % CO_2 and 21 % O_2. Staining of EBs was done according to the previously described protocol.

4.4. Quantitative real-time PCR (RT-qPCR)

Total mRNA was extracted using the NucleoSpin RNA Plus XS kit (Macherey-Nagel, 740990), and cDNA was synthesized from 1 µg of RNA using SuperScript IV reverse transcriptase (Invitrogen, 18090010). To quantify pluripotency and neuronal markers, 2 µl sample of the resulting cDNA was used for qPCR analysis. The primer sequences were those reported by Zink et al. 2021 (Zink et al., 2021). Expression levels were normalized to the housekeeping gene *GAPDH,* and plotted relation to the expression in the patient's source fibroblast line 11624. The data are based on three independent experiments.

4.5. Karyotyping and STR-analysis

DNA was isolated using the FlexiGene DNA Kit (Qiagen). Karyotype analysis was done at Life & Brain (Bonn, Germany) using BeadArray technology and iScan from Illumina. Copy number analysis was performed using CNV partition version 3.2 (Illumina). STR analysis was done at the Institute for Forensic Medicine at the University Hospital Düsseldorf.

4.6. mtDNA mutation analysis and heteroplasmy measurement

We evaluated the heteroplasmy levels of the *MT-ATP6/8* m.8570 T > C mutation after genomic DNA extraction (NucleoSpin Tissue kit, Macherey-Nagel, 740952) from the patient's source fibroblasts and iPSC lines by two methods: (i) PCR-restriction fragment length polymorphism (PCR-RFLP) analysis was performed using fluorescently labelled PCR products, in which a natural *Sty* I restriction site was abolished by the mutation. Capillary electrophoresis, coupled with laser detection of the FAM-labeled fragments, was performed on a 3500 Series Genetic Analyzer (Applied Biosystems, RRID) to determine the ratio of cleaved to uncleaved fragments. The results were normalized to a standard calibration curve. (ii) The gene stretch on the mtDNA containing the mutation was PCR amplified using gene-specific PCR primers flanked by Illumina adapters, generating a 445 bp fragment that was then Illumina sequenced (250 paired-end) and quantitatively analyzed using the IGV viewer (Robinson et al., 2023).

4.7. Test for Mycoplasma contamination

We verified the absence of *Mycoplasma* contamination in the cell lines at passages 17–27 using the qPCR-based Venor®GeM qOneStep kit (Minerva Biolabs, 11–91025).

CRediT authorship contribution statement

Anna Maria Haschke: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Sebastian Diecke:** Supervision, Methodology, Investigation. **Harald Stachelscheid:** Supervision, Methodology, Investigation. **Markus Schuelke:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2025.103732.

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