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Lab Resource: Genetically-Modified Single Cell Line



Generation of an iPSC line IUFi004-A-13 with homozygous *NDUFS1* mutation for the study of Leigh syndrome

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A B S T R A C T

NDUFS1 is a critical component of mitochondrial respiratory chain Complex I (CI). Pathogenic variants of *NDUFS1* can cause Leigh syndrome (LS), a severe pediatric mitochondrial disorder. To model *NDUFS1*-linked LS, we generated an iPSC line with homozygous missense mutations in exon 8 using CRISPR/Cas9. The cell line demonstrated typical morphology, expression of iPSC markers, ability to differentiate into all three germ layers, and genomic integrity. This model will enable the study of LS caused by CI in an isogenic context.

Resource Table

Unique stem cell line identifier	IUFi004-A-13 https://hpscereg.eu/cell-line/IUFi004-A-13
Alternative name(s) of stem cell line Institution	DU201 University Clinic Düsseldorf (Heinrich Heine University)
Contact information of the reported cell line distributor	Alessandro Prigione: Alessandro.Prigione@med.uni-duesseldorf.de The cell line can be made available to third parties upon reasonable request and under an appropriate Material Transfer Agreement (MTA) with the hosting institution.
Type of cell line	Induced pluripotent stem cells (iPSC)
Origin	Human
Additional origin info (applicable for human ESC or iPSC)	Age: 64 Sex: female
Cell Source	iPS12-10 (IUFi004-A), Cell Applications Inc, https://www.cellapplications.com

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Method of reprogramming	/human-induced-pluripotent-stem-cells-h ipsc
Clonality	NA The desired mutation (c.691C > G) is present in both alleles (homozygous). Additional silent substitutions were intentionally introduced into only one allele to prevent Cas9 re-cutting and to enable detection of potential allelic dropout during clone validation.
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	NA
The cell culture system used	Matrigel coated plates
Type of the Genetic Modification	Induced knock-in mutation
Associated disease	Leigh Syndrome (LS)
Gene/locus modified in the reported transgenic line	<i>NDUFS1</i> 0c.691C > G (p.Leu231Val)
Method of modification / user-customisable nucleases (UCN)	CRISPR/Cas9

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(continued)

used, the resource used for design optimisation	
User-customisable nuclease (UCN) delivery method	PX458 (Cas9), plasmid, Lipofectamine™Stem
All double-stranded DNA genetic material molecules introduced into the cells	pSpCas9(BB)-2A-GFP (PX458)
Evidence of the absence of random integration of any plasmids or DS DNA introduced into the cells.	PCR from genomic DNA for Cas9 and GFP
Analysis of the nuclease-targeted allele status	Illumina Next Generation Sequencing (MiSeq)
Homozygous allele status validation	Deep Sequencing
Method of the off-target nuclease activity prediction and surveillance	https://www.rgenome.net/
Descriptive name of the transgene	NA
Eukaryotic selective agent resistance cassettes (including inducible, gene/cell type-specific)	NA
Inducible/constitutive expression system details	NA
Date archived/stock creation date	19/01/2021
Cell line repository/bank	Registered in the hpscreg database. Biosample IDs: IUFi004-A-13
Ethical/GMO work approvals	Commercially available human female iPSC12-10 (IUFi004-A) derived from reprogrammed dermal fibroblasts were purchased from Cell Applications Inc and are available for commercial research use only (RUO).
Addgene/public access repository recombinant DNA sources* disclaimers (if applicable)	pSpCas9(BB)-2A-GFP (PX458) was a gift from Feng Zhang (Addgene plasmid # 48138; https://n2t.net/addgene:48138 ; RRID:Addgene_48138)

1. Resource utility

The induced pluripotent stem cell (iPSC) line generated here can be used to study LS caused by dysfunction in mitochondrial respiratory chain Complex I (CI). The line will be useful for examining neurodevelopmental and bioenergetic impacts of CI dysfunction with the help of an isogenic control.

2. Resource details

Leigh syndrome (LS, OMIM #256000) is a severe pediatric mitochondrial disease, causing early-onset neurodegeneration and death following metabolic crisis in the first years of life (Baertling et al., 2014; Leigh, 1951). LS is typically ascribed to pathogenic variants in one of the components of the mitochondrial oxidative phosphorylation (OXPHOS) machinery. Over 100 monogenic variants have been identified to cause LS (McCormick et al., 2023). The study of LS is challenging because of the many genetic origins of the disease and the limited availability of mammalian animal models to adequately recapitulate the clinical phenotypes seen in patients (Dell'agnello et al., 2007). Induced pluripotent stem cells (iPSC) models can therefore represent a promising platform to capture the human specific conditions of LS in a controlled genetic context. To date, no isogenic cell line pair has been characterized for the study of LS harboring *NDUFS1* variants. Here, we generate an iPSC line harboring the missense mutation c.691C > G (p.Leu231Val), which has been reported to cause LS (Martín et al., 2005).

The cell line DU201 was generated by using CRISPR/Cas9 to introduce the *NDUFS1* mutation c.691C > G into healthy parental cell line iPSC12-10 (Cell Applications) (Fig. 1A). The editing strategy used a single sgRNA targeting exon 8, together with a single-stranded oligodeoxynucleotide (ssODN) donor carrying the desired substitution. Potential off-target sites were computationally assessed using Cas-OFFinder (Bae et al., 2014), which did not predict any off-target loci with fewer than

three mismatches. Following gene editing, the cell line was found to be homozygous for the mutation (Fig. 1B). Karyotype analysis demonstrated a normal 46 XX karyotype through G-banding analysis (Fig. 1C) and CNV analysis (Supplementary Fig. 3). The cell line showed typical iPSC colony morphology, with clear borders and dense colonies (Fig. 1D, scale bar = 300 µm). Undifferentiated cells were found to be positive for pluripotency marker proteins SOX2, TRA-1-60, and NANOG, as demonstrated by immunocytochemistry (ICC) (Fig. 1E, pluripotency markers in red, Hoechst in blue, scale bar = 300 µm). qPCR-based quantification of the pluripotency marker genes *OCT4*, *DNMT3B*, and *DPPA4* revealed increased expression as normalized to fibroblasts and no significant difference in expression compared to parental iPSC line iPSC12-10 (Fig. 1F). Differentiation potential was evaluated by means of embryoid body (EB) formation and ICC. Staining of the spontaneously differentiated cells revealed cells positive for endodermal, mesodermal, and ectodermal markers AFP, SMA, and PAX6, respectively (Fig. 1G, germ layer markers in red, Hoechst in blue, scale bar = 300 µm). Short tandem repeat (STR) analysis confirmed the mutant and parental cell lines share the same genetic background. The cell line DU201 was negative for mycoplasma contamination (Supplementary Fig. 1). PCR analysis for GFP and Cas9 sequences was performed using genomic DNA isolated from the original cryopreserved master stock and newly synthesized primers. No amplification of GFP or Cas9 was detected, indicating absence of stable PX458 plasmid integration (Supplementary Fig. 2) (Table 1).

3. Materials and Methods

3.1. Cell culture

iPSCs were cultured in StemMACS™ iPSCBrew XF containing 50X Supplement (Miltenyi) and MycoZap™ Plus-CL 500x (Lonza) on 6-well plates coated with 200 µg/ml Matrigel® (Corning). Cells were cultured at 37 °C, 5% CO₂, and 5% O₂. Cells were typically passaged every 4–5 days with EDTA (Invitrogen) with 10 µM ROCK inhibitor (Enzo Life Sciences) to promote survival.

3.2. Generation of homozygous *NDUFS1* mutants

Guide RNA was manually designed to target the *NDUFS1* locus and cloned into the pSpCas9(BB)-2A-GFP vector (PX458, Addgene #48138). Genome editing was performed using an sgRNA targeting the sequence 5'-CCTGTAGGTGCCCTAACCTCTAA-3' with an adjacent PAM of 5'-AGG-3', giving the full target context 5'-CCTGTAGGTGCCCTAACCTCTAAAGG-3'. The predicted SpCas9 cleavage site is located 3 bp upstream of the PAM, between 5'-CCTGTAGGTGCCCTAACCTC|TAAAGG-3'. Donor oligonucleotides used for HDR and clone screening were: 3'-GATATCTGCCCGGTAGGAGCCGTAACCTCAAGCCCTATGCCTTACTGCCCGACCTTGGGA-5' and 3'-GATATCTGCCCGGTAGGAGCCGTAACCTCTAAGCCCTATGCCTTACTGCCCGCCTTGGGA-5'

The desired edit was a C > G substitution (in red), while the remaining substitutions (underlined) were silent changes introduced to prevent re-recognition/re-cutting by the sgRNA-Cas9 complex and to provide an allele-specific sequence signature for rapid identification of allelic dropout during clone screening. Cells were co-transfected with 2 µg of plasmid and the donor oligonucleotide (IDT) using Lipofectamine™ Stem Reagent (Thermo Fisher), following the protocol described in (Ramachandran et al., 2021). EGFP-positive cells were isolated by FACS using a BD FACSAria III and expanded as single-cell clones. A total of 192 GFP⁺ single-cell clones were expanded and genotyped. One clone carrying the desired on-target knock-in genotype was selected for further characterization. Clonal lines were genotyped on an Illumina MiSeq using a V2 Nano kit, with mutation-specific primers for the first PCR and primers containing P5/P7 adapters for sequencing (Table 2). FASTQ files were analyzed using CleanFinder (Ramachandran et al., 2026). Genotyping was performed at passage 42. The master stock

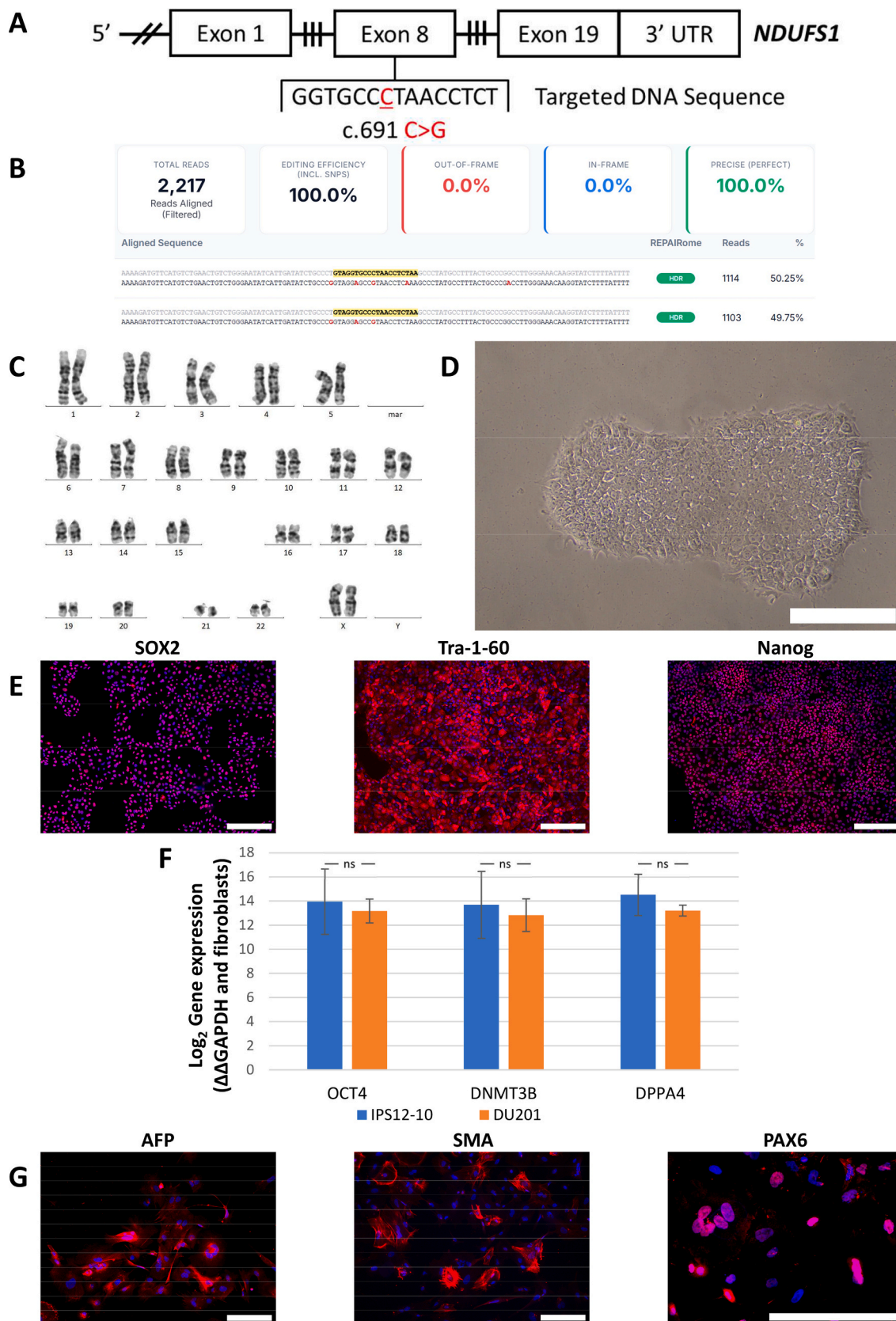


Fig. 1. Characterization of iPSC line IUFI004-A-13.

Table 1
Characterization and validation.

Classification (optional <i>italicized</i>)	Output type	Result	Data
Schematic of a transgene/genetic modification	Schematic illustrating the structure and location of the introduced genetic modification	Visual representation of the edited exon	Fig. 1 panel A
Morphology	Photography	Typical iPSC morphology	Fig. 1 panel D
Pluripotency status evidence for the described cell line	Qualitative analysis Immunocytochemistry Quantitative analysis qPCR gene expression	Confirmed expression of pluripotency markers	Fig. 1 panel E
Karyotype	Karyotype (G-banding) Karyotype (CNV analysis)	Confirmed expression of pluripotency markers compared to iPSC line IPS12 and normalized to fibroblasts 46 XX	Fig. 1 panel F Fig. 1 panel C Supplementary Fig. 3
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site or targeted allele-specific PCR Evaluation of the – homozygous status of introduced genomic alteration(s) Transgene-specific PCR (when applicable)	Amplicon Deep Sequencing of the targeted region Silent mutation present in only one allele	Fig. 1 panel B Fig. 1 panel B
Verification of the absence of random plasmid integration events	PCR GFP and Cas9	Not applicable GFP and Cas9 – Negative	Not applicable Supplementary Fig. 2
Parental and modified cell line genetic identity evidence	STR analysis	STR profile matches the parental line IPS12	STR Analysis
Mutagenesis / genetic modification outcome analysis	Sequencing (genomic DNA PCR or RT-PCR product)	<i>NDUFS1</i> homozygous 0c.691C > G (p.Leu231Val) NA	Fig. 1 panel B
Off-target nuclease activity analysis	Southern Blot or WGS No predicted offset targets		
Specific pathogen-free status	Mycoplasma	Mycoplasma testing by PCR: negative	Supplementary Fig. 1
Multilineage differentiation potential	Embryoid body formation	Spontaneous differentiation of EBs plated to coverslips were ICC positive for all three germ layers	Fig. 1 panel G
List of recommended germ layer markers	Expression demonstrated at protein level – IF with specific antibodies	Confirmed expression of germ layer markers Ectoderm: PAX6 Endoderm: AFP Mesoderm: A-SMA	Fig. 1 panel G
Outcomes of gene editing experiment	Brief description of the outcomes in terms of clones generated/establishment approach/screening outcomes	Single cell dilution, deep sequencing, fastq analysis using CleanFinder	
Donor screening	HIV 1 + 2 Hepatitis B, Hepatitis C	Refer to Cell Applications Inc	
Genotype – additional histocompatibility info	Blood group genotyping HLA tissue typing	Refer to Cell Applications Inc Refer to Cell Applications Inc	

used for downstream characterization and distribution (DU201) was cryopreserved at passage 45.

3.3. Pluripotency and spontaneous differentiation

iPSCs (passage 49 – 52) were dissociated with accutase, collected, and suspended in iPSCBrew to enable the formation of embryoid bodies (EBs). Media was exchanged every other day, and the EBs were allowed to grow for 7 days. EBs were then seeded to coverslips coated with Matrigel and cultured with spontaneous differentiation media, containing DMEM (Gibco), 20% fetal bovine serum (Gibco), 1% NEAA solution (Gibco), and 0.1 mM 2-mercaptoethanol (Gibco).

3.4. Immunocytochemistry

Coverslip mounted cells (pluripotency staining passage 48) were fixed with 4% PFA (Thermo Fisher) for 15 min. Fixed cells were then permeabilized for 10 min in a solution of 0.3% Triton-X (Merck) in PBS. Next, cells were treated with a blocking solution containing 10% donkey serum (Sigma Aldrich) and 0.05% sodium azide (Sigma-Aldrich) in PBS. Primary antibodies were diluted in blocking solution and incubated overnight at 4 °C or for 1 h at room temperature. Secondary antibodies were diluted in blocking solution and incubated overnight at 4 °C or for 1 h at room temperature (Table 2).

3.5. qPCR

RNA was extracted from cell pellets (passage 45 – 49) using RNeasy

Mini Kit (Qiagen) RNA extraction kit. RNA was used to generate cDNA using M–MLV Reverse Transcriptase (Invitrogen) with 500 µg/mL Oligo (dT)12–18 primers (Thermo Fisher). qPCR was performed with SYBR Green PCR Master Mix (Applied Biosystems) on the CFX96™ Real-Time System qPCR machine (Bio-Rad) (Table 2). qPCR testing was performed using 3 biological replicates per cell line, each with 3 technical replicates which were averaged to obtain the value of each biological replicate. Error bars represent 95% confidence intervals calculated from the biological replicates. Statistical significance was determined using a two-tailed unpaired *t*-test.

3.6. Karyotyping

Karyotyping was performed by G-banding analysis at the Institute of Human Genetics and Anthropology, Heinrich-Heine University Düsseldorf. Four metaphases were analyzed at passage 42 for IUFi004-A-13, revealing a normal female karyotype (46,XX). Low resolution karyotyping was conducted on an Illumina Infinium Global Screening GSAMD-24v3-0-EA_20034606 Array by Life&Brain. Data analysis was conducted with GenomeStudio V2.0. CNV and LOH analysis was performed via the CNV Partition 3.2 Plugin algorithm following the guidelines by (Haake and Steenpass, 2025). Copy number variations larger than 350 kb were reported, along with copy-neutral loss of heterozygosity (CN-LOH) in regions of 1 Mb or larger.

3.7. STR analysis

DNA was extracted from cell pellets (passage 47) using the DNA

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Primary antibody	Goat anti-SOX2	1:200	R&D Systems AF2018, RRID:AB_355110
Primary antibody	Mouse anti-Tra-1–60	1:200	Millipore MAB4360, RRID:AB_2119183
Primary antibody	Goat anti-Nanog	1:200	R&D Systems AF1997, RRID:AB_355097
Primary antibody	Mouse anti-AFP	1:300	Sigma-Aldrich WH0000174M1, RRID:AB_1839587
Primary antibody	Mouse anti-SMA	1:200	Agilent M0851, RRID:AB_2223500
Primary antibody	Rabbit anti-PAX6	1:200	BioLegend 901301, RRID:AB_2749901
Stain	Hoechst 33342	1:2500	Life Technologies H3570
Secondary antibody	Alexa Fluor 488 Donkey anti-Rabbit IgG	1:1000	Thermo Fisher Scientific R37118, RRID:AB_2556546
Secondary antibody	Alexa Fluor 488 Donkey anti-Goat IgG	1:1000	Thermo Fisher Scientific A-11055, RRID:AB_2534102
Secondary antibody	Alexa Fluor 568 Donkey anti-Mouse IgG	1:1000	Thermo Fisher Scientific A10037, RRID:AB_11180865
Site-specific nuclease			
Nuclease information	PX458		
Delivery method	Lipofectamine		
Selection/enrichment strategy	FACS		
Primers and Oligonucleotides used in this study			
	Target	Forward/Reverse primer (5'-3')	
Pluripotency Marker (qPCR)	<i>OCT4</i>	GTGGAGGAAGCTGACAAACA / ATTCTCCAGGTTGCCTCTCA	
Pluripotency Marker (qPCR)	<i>DNMT3B</i>	GCTCACAGGGCCCATACTT / GCAGTCCTGCAGCTCAGAGTTA	
Pluripotency Marker (qPCR)	<i>DPPA4</i>	TGGTGTCAAGTGGTGTGG / CCAGGCTTGACCAGCATGAA	
Housekeeping Gene (qPCR)	<i>GAPDH</i>	CTGGTAAAGTGGATATTGTTGCCAT / TGGAAATCATATTGGAACATGTAACCA	
gRNA	<i>NDUFS1</i>	TTAGAGGTTAGGGCACCTAC	
Plasmid absence verification	EGFP	CGACTTCTCAAGTCCGCCA / GTCCATGCCGAGAGTGATCC	
Plasmid absence verification	Cas9	CAGAGCTTCATCGAGCCGAT / CGAACAGGTGGGCATAGGTT	
<i>Mycoplasma test</i>	Myco-f1	F: CGCCTGAGTAGTACGTTCCG	
<i>Mycoplasma test</i>	Myco-f2	F: CGCCTGAGTAGTACGTTCCG	
<i>Mycoplasma test</i>	Myco-f3	F: TGCCTGAGTAGTCACTTCGC	
<i>Mycoplasma test</i>	Myco-f4	F: CGCCTGGGTAGTACATTCGC	
<i>Mycoplasma test</i>	Myco-f5	F: CGCCTGAGTAGTACTTCGC	
<i>Mycoplasma test</i>	Myco-f6	F: TGCCTGGGTAGTACATTCGC	
<i>Mycoplasma test</i>	Myco-r1	R: GCGGTGTGTACAAGACCCGA	
<i>Mycoplasma test</i>	Myco-r2	R: GCGGTGTGTACAAAACCCGA	
<i>Mycoplasma test</i>	Myco-r3	R: GCGGTGTGTACAACCCCGA	
Targeted mutation analysis	<i>NDUFS1</i>	TGACTGGAGTTCAGACGTGTGctcttcgatctCAGACAAAACAACCTGAAACCCA / ACACTCTTTCCCTACACGACGctcttcgatctGGAACAACAGGCAGAGGAAATGA	
Oligonucleotides (knock-in)	<i>NDUFS1</i>	3'-GATATCTGCCCGGTAGGAGCCGTAACCTCAAGCCCTATGCCTTTACTGCCCGACCTTGGGA-5' 3'-GATATCTGCCCGGTAGGAGCCGTAACCTCAAGCCCTATGCCTTTACTGCCCGCCTTGGGA-5'	

Flexigene kit (Qiagen) per manufacturer's instructions. STR-analysis was performed by the Institute of Forensic Medicine at the university clinic of Heinrich-Heine-University, Düsseldorf.

3.8. *Mycoplasma*

Medium was collected from iPSCs (passage 56) during cell culture and PCR was performed to detect mycoplasma DNA using primers provided by the Leibniz Institute (DSMZ) (Table 2).

CRediT authorship contribution statement

Caleb Jerred: Investigation. **Haribaskar Ramachandran:** Methodology. **Barbara Hildebrandt:** Methodology. **Annika Zink:** Formal analysis. **Nataschia Ventura:** Resources. **Andrea Rossi:** Supervision. **Alessandro Prigione:** Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nataschia Ventura, Andrea Rossi, Alessandro Prigione reports financial support was provided by German Research Foundation. Alessandro Prigione reports financial support was provided by European Commission. Alessandro Prigione reports financial support was provided by AFM-Téléthon. Alessandro Prigione and Andrea Rossi currently serve, respectively, as Editor-in-Chief and Lab Research Editor of the journal Stem Cell Research. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2026.104002>.

Data availability

No data was used for the research described in the article.

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