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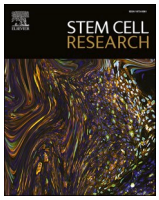

Characterization of two human induced pluripotent stem cell lines derived from Batten disease patient fibroblasts harbouring CLN5 mutations

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A B S T R A C T

The neuronal ceroid lipofuscinoses (NCLs) are a group of common inherited neurodegenerative disorders of childhood. All forms of NCLs are life-limiting with no curative treatments. Most of the 13 NCL genes encode proteins residing in endolysosomal pathways, such as CLN5, a potential lysosomal enzyme. Two induced pluripotent stem cell lines (hiPSCs) were generated from skin fibroblasts of CLN5 disease patients via non-integrating Sendai virus reprogramming. They demonstrate typical stem cell morphology, express pluripotency markers, exhibit trilineage differentiation potential and also successfully differentiate into neurons. These hiPSCs represent a potential resource to model CLN5 disease in a human context and investigate potential therapies.

Resource Table:

Unique stem cell lines identifier	UCLi021-A UCLi022-A
Alternative name(s) of stem cell lines	CLN5$^{c.335G>A;619T>C}$ or 484Pb (UCLi021-A) CLN5$^{c.1072\text{-}1073delTT}$ or 546 Pa (UCLi022-A)
Institution	University College London (UCL)
Contact information of distributor	Prof. Paul GISSEN. p.gissen@ucl.ac.uk.
Type of cell lines	iPSC
Origin	human
Additional origin info required	UCLi021-A: Female, 7 years old. White British. UCLi022-A: Male, 9 years old, Pakistani.
Cell Source	Human dermal fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune iPS 2.0 Sendai Reprogramming Kit, ThermoFisher.
Genetic Modification	UCLi021-A: NO, UCLi022-A: YES
Type of Genetic Modification	UCLi022-A: 60 % abnormal 46, XY, add (1)(q42) + 30 % normal karyotype 46, XY
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-qPCR

(*continued on next column*)

(*continued*)

Unique stem cell lines identifier	UCLi021-A UCLi022-A
Associated disease	Batten disease (CLN5 mutation); CLN5 disease
Gene/locus	UCLi021-A: CLN5:c.[335G > A];[619 T > C] compound heterozygous. UCLi022-A: CLN5:c.[1072-1073delTT];[1072-1073delTT] homozygous.
Date archived/stock date	2018 archived
Cell line repository/bank	https://hpscreg.eu/cell-line/ UCLi021 https://hpscreg.eu/cell-line/ UCLi022-A
Ethical approval	London - Hampstead Research Ethics Committee, REC reference: 13/LO/0171

1. Resource utility

Human neural cells are not easily available. Patient-derived induced pluripotent stem cells (hiPSC) can be differentiated into neurons and therefore represent an invaluable resource for disease modelling studies and development of drugs; here targeting CLN5 disease in patient cells.

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2. Resource details

The neuronal ceroid lipofuscinoses (NCLs), also known as Batten disease, are a group of inherited lysosomal storage disorders. Symptoms include epileptic seizures, visual failure, motor and cognitive decline. NCLs are caused by mutations in 13 genes and are characterized by lysosomal accumulation of autofluorescent ceroid-lipopigments containing subunit c of the mitochondrial ATP synthase or sphingolipid activator proteins A and D (Anderson et al., 2013; Gardner et al., 2021).

Typical CLN5 disease is a late-infantile-onset NCL (onset of symptoms at 4–7 years old), characterized by mutations in the *CLN5* gene. First described in Finland, it is found across the world. Juvenile and adult onset patients exist. The gene encodes a ubiquitous soluble lysosomal glycoprotein, highly expressed in the cerebral cortex and cerebellum, especially in neurons and microglia whose structure is known (Luebben et al., 2022). It is proposed to be a lysosomal enzyme involved in maintaining lysosome homeostasis, displaying cysteine *S*-palmitoyl thioesterase activity in vitro, confirmed by a reduced thioesterase activity in $Cln5^{-/-}$ mouse neuronal progenitor cells (Luebben et al., 2022). Its natural substrates are unknown. To date, there is no approved treatment or cure (Basak et al., 2021, Mole et al., 2019).

We report the generation of two CLN5 disease patient human induced pluripotent cell lines, $CLN5^{c.335G>A;619T>C}$ and $CLN5^{c.1072\text{-}1073delTT}$, from dermal fibroblasts (Table 1). These were reprogrammed using non-integrating Sendai virus to deliver the 4 Yamanaka factors and both cell lines were cleared of Sendai viruses by passage 10 (Supplementary Fig. 1A). hiPSC $CLN5^{c.335G>A;619T>C}$ harbours two missense mutations in *trans*, p.(Arg112His) for c. 335G > A in exon 2 and p.(Trp207Arg) for c. 619 T > C in exon 3. hiPSC $CLN5^{c.1072\text{-}1073delTT}$ carries a homozygous two-base-pair deletion (c.1072-1073delTT) in exon 4 that causes a frameshift and a new stop codon (p.(Leu358Alafs*4)), as shown by Sanger sequencing (Fig. 1.A). Both generated hiPSC lines show a typical human embryonic stem cell (ES) morphology (Fig. 1.B).

hiPSC $CLN5^{c.335G>A;619T>C}$ showed normal human karyotype 46, XX, while 60 % of $CLN5^{c.1072\text{-}1073delTT}$ showed an abnormal 46, XY, add(1)(q42) and 30 % a normal karyotype 46, XY (Supplementary Fig. 1. B). SNPs analysis confirmed > 99.92 % identity between the iPSC lines and their original human dermal fibroblasts (Supplementary Fig. 1.E). Their pluripotency potential was confirmed by positivity for the markers OCT4, NANOG, TRA1-81 and SOX2 by immunofluorescence staining (Fig. 1.C) and quantitative real-time PCR. They displayed a similar range of stem cell gene expression as the hESC and hiPSC controls, while SH-SY5Y, a control neuronal cell line, had no or little expression (Fig. 1.D). Differentiation potential into the derivatives of all three germ layers was confirmed by directed differentiation, resulting in the presence of positive cells for endoderm (SOX17 and AFP), ectoderm (TUJ1) and mesoderm (SMA and NCAM/CD56) markers, as confirmed by immunofluorescence staining (Fig. 1.E-F). Cell lines were screened monthly for mycoplasma (Supplementary Fig. 1.C).

As CLN5 disease is a neurogenerative disease, differentiations towards cortical and dopaminergic neurons were performed using established protocols. hiPSCs $CLN5^{c.335G>A;619T>C}$ and $CLN5^{c.1072\text{-}1073delTT}$ were able to form both types of neurons as demonstrated by positive staining for the neuronal marker β-tubulin (Tuj1), deep layer cortical marker chicken ovalbumin upstream promoter transcription factor-interacting protein 2 (CTiP2), and the dopaminergic marker tyrosine hydroxylase (TH) by immunofluorescence (Fig. 1.F and Supplementary Fig1.D).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	$CLN5^{c.335G>A;619T>C}$ p18 and $CLN5^{c.1072\text{-}1073delTT}$ p14: Normal morphology. Scale bar 200 μm.	Fig. 1 panel B
Phenotype	Qualitative analysis	$CLN5^{c.335G>A;619T>C}$ p18 and $CLN5^{c.1072\text{-}1073delTT}$ p14 positive for Oct3/4, Nanog, Sox2, and Tra-1–81. Scale bar 100 μm.	Fig. 1 panel C
	Quantitative analysis	$CLN5^{c.335G>A;619T>C}$ p20 and $CLN5^{c.1072\text{-}1073delTT}$ p15 have similar level of mRNA as stem cells controls hESC H1 and control iPS for Oct3/4, Klf4, Sox2, and c-Myc and have higher mRNA level than the differentiated neuronal cell line SH-SY5Y.	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	$CLN5^{c.335G>A;619T>C}$ p19: 46, XX $CLN5^{c.1072\text{-}1073delTT}$ p16: 60 % 46, XY, add(1)(q42) and 30 % 46, XY Resolution 375–400 bands per haploid chromosome set.	Supplementary Fig. 1 panel B
Identity	Microsatellite PCR (mPCR) OR STR analysis	Genotyping DNA arrays performed. SNP analysis and report provided. Identical genotypes (>99.92 %) for > 560000 SNPs, indicating iPSCs and fibroblasts are from the same individual.	Supplementary Fig. 1 panel E and SNPs reports submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	$CLN5^{c.335G>A;619T>C}$ p17: 2 heterozygous missense mutations, c.335G > A (exon 2) and c.619 T > C (exon 3). $CLN5^{c.1072\text{-}1073delTT}$ p12: homozygous 2 base pair deletion (c.1072-1073delTT) (exon 4), as shown by Sanger sequencing.	Fig. 1 panel A
	Southern Blot OR WGS	Not performed.	
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence: Negative.	Supplementary Fig. 1 panel C
Differentiation potential	Directed Differentiation	$CLN5^{c.335G>A;619T>C}$ p21 $CLN5^{c.1072\text{-}1073delTT}$ p16: positive staining for ectoderm, endoderm and mesoderm markers by immunofluorescence staining. Scale bar 100 μm.	Fig. 1 panel E and F
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**	Antibodies for: Ectoderm: TUJ1 Endoderm: SOX17, AFP Mesoderm: A-SMA, NCAM.	Immunofluorescence
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	NA	
Genotype additional info (OPTIONAL)	Blood group genotyping	NA	
	HLA tissue typing	NA	

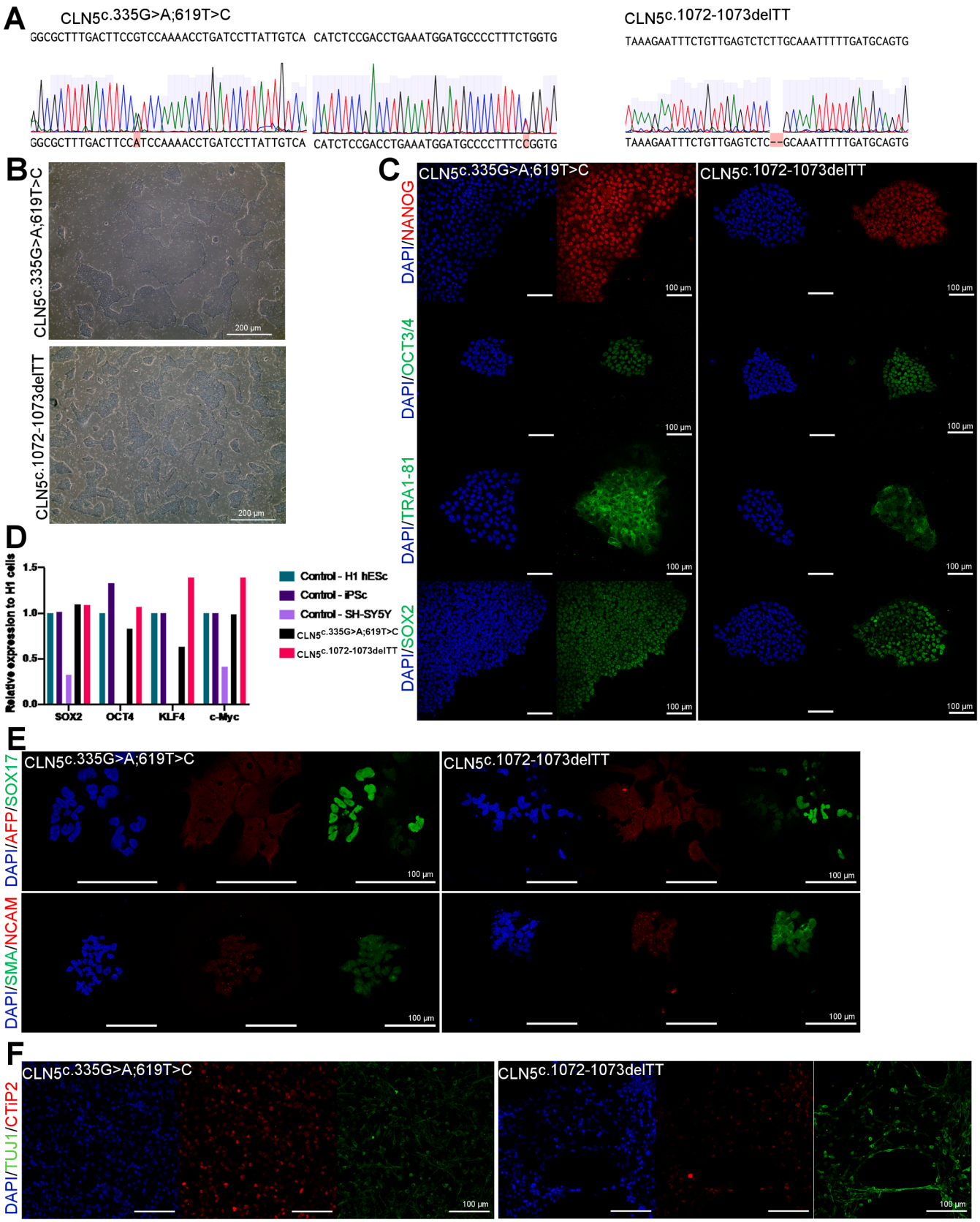
A
CLN5c.335G>A;619T>C
GGCGCTTTGACTTCCGTCCAAAACCTGATCCTTATTGTCA CATCTCCGACCTGAAATGGATGCCCCTTTCTGGTG
GGCGCTTTGACTTCCATCCAAAACCTGATCCTTATTGTCA CATCTCCGACCTGAAATGGATGCCCCTTTCCGGTG
CLN5c.1072-1073delTT
TAAAGAATTTCTGTTGAGTCTCTTGCAAATTTTTGATGCAGTG
TAAAGAATTTCTGTTGAGTCTC--GCAAATTTTTGATGCAGTG
B
CLN5c.335G>A;619T>C
CLN5c.1072-1073delTT
200 µm
C
CLN5c.335G>A;619T>C
CLN5c.1072-1073delTT
DAPI/NANOG
DAPI/OCT3/4
DAPI/TRA1-81
DAPI/SOX2
100 µm
D
Relative expression to H1 cells
1.5
1.0
0.5
0.0
SOX2
OCT4
KLF4
c-Myc
Control - H1 hESc
Control - iPSc
Control - SH-SY5Y
CLN5c.335G>A;619T>C
CLN5c.1072-1073delTT
E
CLN5c.335G>A;619T>C
CLN5c.1072-1073delTT
DAPI/AFP/SOX17
DAPI/SMA/NCAM
100 µm
F
CLN5c.335G>A;619T>C
CLN5c.1072-1073delTT
DAPI/TUJ1/CTiP2
100 µm

Fig. 1.

3. Materials and methods

3.1. Reprogramming of dermal fibroblasts and cell culture

Human dermal fibroblasts from two CLN5 disease patient biopsies were reprogrammed at Cambridge Biomedical Research Centre using the CytoTune iPS 2.0 Sendai Reprogramming Kit (ThermoFisher). hiPSCs and H1 ES cells were cultured on human recombinant laminin 521 (5ug/ml, BioLamina) coated dishes in mTeSR Plus culture media (Stemcell Technologies) and passaged as clumps using 0.5 mM EDTA. SH-SY5Y were grown in DMEM, high glucose, GlutaMax, Sodium Pyruvate (Thermofisher) with 10 % Foetal Bovine Serum (Sigma) and 1 % Penicillin/Streptomycin (Thermofisher). Cells were cultured at 37C in a 5 % CO_2/air incubator.

3.2. Mutation detection

DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). Primers in Table 2 were used to amplify the mutations containing regions. The products were purified using the QIAquick PCR Purification Kit (Qiagen) and sent for Sanger sequencing.

3.3. RNA extraction and RT-qPCR

RNA (hiPSCs passage 12–15) was extracted using the RNeasy Plus Mini Kit (Qiagen) and cDNA was synthesized using the ImProm-II Reverse Transcription System (Promega). RT-qPCR was performed using the MESA Blue qPCR 2x MasterMix Plus (Eurogentec) on Biorad CFX, see primers in Table 2.

3.4. Fluorescent immunocytochemistry

hiPSCs (passages 9–15) and iPS-derived neurons (day 40–45) were fixed with methanol or 4 % paraformaldehyde, permeabilized in 0.1 % Triton-X and blocked one hour with 5 % goat serum in PBS, followed by overnight incubation at 4C with primary antibodies, and incubation of secondary antibodies for two hours at room temperature, see Table 2. DAPI was used for nuclear DNA staining. Images were acquired using a Leica TCS SPE3 confocal microscope.

3.5. Karyotyping and genetic evidence of identity

Chromosomal G-band analysis was performed by TDL Pathology laboratory, London.

Genotyping DNA array was performed instead of STR analysis. Isolation of DNA, genotyping, quality control (QC), and imputation were performed using standard procedures. hiPSCs (passages 9–15) and human dermal fibroblasts (HF) were genotyped by University College London Genomics (Institute of Child Health, University College London, London, United Kingdom) on the Illumina Infinium Global Screening Array v3.0 + Multi-Disease Array (Illumina). Genome Studio software (Illumina) was used to visualize the data and confirm the identity between the iPSC clones and original human dermal fibroblasts comparing $>$ 560000 SNPs.

3.6. Tri-lineages directed differentiation

hiPSCs were differentiated in vitro into the three embryonic germ layers using the STEMdiff Trilineage Differentiation Kit (Stemcell technologies).

3.7. Neuronal Differentiation

Neuronal induction starts on day 0. Media was changed every two days unless otherwise stated.

For cortical differentiation, hiPSCs were seeded on laminin-521 in

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Mouse anti-SOX2	1:50	R&D Systems Cat# MAB2018	AB_358009
	Mouse anti-OCT3/4	1:50	Santa Cruz Biotechnology Cat# sc-5279	AB_628051
	Mouse anti-TRA-1–81	1:50	Santa Cruz Biotechnology Cat# sc-21706	AB_628386
	Rabbit anti-NANOG	1:100	ReproCELL Cat# RCAB004P-F	AB_1560380
Trilineage Differentiation markers	Goat anti-SOX17	1:100	R&D Systems Cat# AF1924	AB_355060
	Mouse anti-AFP	1:200	Sigma-Aldrich Cat# A8452	AB_258392
	Rabbit anti-SMA	1:100	Abcam Cat# ab5694 Abcam Cat# ab200698	AB_2223021
	Mouse anti-NCAM	1:100		AB_2890933
Neuronal Differentiation markers	Rat anti- CTiP2	1:100	Abcam Cat# ab18465	AB_2064130
	Mouse anti-TH	1:100	R&D Systems Cat# MAB7566	AB_2923064
	Rabbit anti-TUJ1	1:100	BioLegend Cat# 802,001	AB_2564645
Secondary antibodies	Alexa Fluor 488-conjugated Goat anti-Mouse	1:500	ThermoFisher Scientific Cat# A-11029	AB_2534088
	Alexa Fluor 546-conjugated Goat anti-Mouse	1:500	ThermoFisher Scientific Cat# A-11030	AB_2534089
	Alexa Fluor 488-conjugated Donkey anti-Goat	1:500	ThermoFisher Scientific Cat# A-11055	AB_2534102
	Alexa Fluor 488-conjugated Goat anti-RabbitAlexa Fluor	1:500	ThermoFisher Scientific Cat# A-11034ThermoFisher	AB_2576217
	546-conjugated Goat anti-Rabbit	1:500	Scientific Cat# A-11010	AB_2534077

	Primers		
	Target	**Size of band**	**Forward/Reverse primer (5′-3′)**
Sendai virus vectors (qPCR, passage 10)	SeV genome	181 bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS transgene	528 bp	ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG
	Klf4 transgene	410 bp	TTCCTGCATGCCAGAGGAGCCC/ AATGTA TCGAAGGTGCTCAA
	c-Myc transgene	532 bp	TAACTGACTAGCAGGCTTGTCG/ TCCACA TACAGTCCTGGATGATGATG
House-Keeping gene (PCR)	GAPDH	452 bp	ACCACAGTCCATGCCATCAC/ TCCACCACCCTGTTGCTGTA
Pluripotency Markers (qPCR, passage 12–15)	OCT4	164 bp	CCTCACTTCACTGCACTGTA/ CAGGTTTTCTTTCCCTAGCT
	SOX2	141 bp	ATGTCCCAGCACTACCAGAG/ GCACCCCTCCCATTTCCC
	KLF4	172 bp	GGTCGGACCACCTCGCCTTACAC/ CTCAGTTGGGAACTTGACCA
	C-MYC	190 bp	CTGAAGAGGACTTGTTGCGGAAAC/ TCTCAAGACTCAGCCAAGGTTGTG
House-Keeping gene (qPCR)	PBDG	159 bp	GGAGCCATGTCTGGTAACGG/ CCACGCGAATCACTCTCATCT
Targeted mutation analysis / Sanger sequencing	Exon 2		TATGGTCCGACCTTTGAAT/ TGAATCTGAAACCAGCTCCTG
	Exon 3		TGATGTGCCACATTTTCTCC/ TCACTGGAAGGAAAATGGGACAT
	Exon 4		GGGCCAACAGGAAACAAGACTC/ TCCAATCACCAGCATCTGTTTTT

neuronal induction media (1:1 Neurobasal and DMEM/F12 media, B27, N2, L-glutamine, 100 nM LDN193189 and 10uM SB432541). At day 11, cells were passaged using Accutase in neuronal induction medium with 0.5uM cAMP and without LND193189 or SB432541 and changed every other day. Cells were passaged at day 16 and 25. From day 25, the media was supplemented with 20 ng/ml BDNF and 10 ng/ml GDNF.

For dopaminergic differentiation, hiPSCs were seeded on laminin-111 in neuronal induction medium (1:1 Neurobasal and DMEM/F12 media, N2, B27 without vitamin A, 100 nM LDN193189, 10uM SB432541, 0.9uM CHIR99021 and 600 ng/ml sonic hedgehog C24II). At day 4, B27 and N2 were reduced by 50 %. At day 9, 100 ng/ml FGF8b and 1ug/ml Heparin were added. At day 11, cells were passaged with Accutase in Neurobasal medium, B27 without vitamin A, 2 mM L-glutamine, 100 ng/ml FGF8b, 1ug/ml Heparin, 20 ng/ml BDNF, 10 ng/ml GDNF and 0.2 mM ascorbic acid. From day 16, FGF8b and Heparin were removed, and 10uM DAPT added. From day 35, cells were seeded on poly-L-ornithine and laminin-521. 10uM Y27632 was added to media when passaging or thawing.

4. Mycoplasma testing

hiPSCs were checked monthly from passage 10 onwards: 100 μL of supernatant was analysed using the MycoAlert Detection Kit (Lonza).

CRediT authorship contribution statement

Marisa Ofrim: Formal analysis, Investigation, Visualization, Writing – original draft. **Daniel Little:** Investigation, Methodology, Visualization, Writing – original draft. **Mina Nazari:** Investigation. **Christopher J. Minnis:** Investigation, Methodology, Validation, Visualization, Writing – original draft. **Michael J. Devine:** Resources, Writing – review & editing. **Sara E. Mole:** Funding acquisition, Resources, Writing – review & editing. **Paul Gissen:** Conceptualization, Funding acquisition, Resources, Supervision, Validation, Writing – review & editing. **Maëlle Lorvellec:** Conceptualization, Formal analysis, Supervision, Validation, Visualization, Writing – review & editing.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103291.

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