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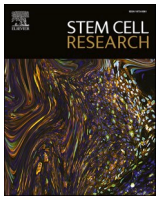

Lab Resource: Multiple Cell Lines

Generation and characterization of six human induced pluripotent stem cell lines (hiPSCs) from three individuals with SSADH Deficiency and CRISPR-corrected isogenic controls

Wardiya Afshar-Saber [a,*], Cidi Chen [a,b], Nicole A. Teaney [a], Kristina Kim [a,b], Ziqin Yang [a], Federico M. Gasparoli [c], Darius Ebrahimi-Fakhari [a], Elizabeth D. Buttermore [a,b], Ivy Pin-Fang Chen [a,b], Phillip L. Pearl [a], Mustafa Sahin [a,*]

[a] FM Kirby Neurobiology Center, Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA
[b] Human Neuron Core, Rosamund Stone Zander Translational Neuroscience Center, Boston Children's Hospital, Boston, MA, USA
[c] Department of Cell Biology, Harvard Medical School, Boston, MA, USA

A B S T R A C T

Succinic Semialdehyde Dehydrogenase Deficiency (SSADHD) is an ultra-rare autosomal recessive neurometabolic disorder caused by *ALDH5A1* mutations presenting with autism and epilepsy. Here, we report the generation and characterization of human induced pluripotent stem cells (hiPSCs) derived from fibroblasts of three unrelated SSADHD patients – one female and two males with the CRISPR-corrected isogenic controls. These individuals are clinically diagnosed and are being followed in a longitudinal clinical study.

Resource Table

Unique stem cell lines identifier	BCHi007-A (HNDS0005-01 #B) BCHi007-A-1 (HNDS0005-01 #B2 +/+) BCHi009-A (HNDS0002-01 #D) BCHi009-A-1 (HNDS0002-01 #D CC26 +/+) BCHi011-A (HNDS0003-01 #F) BCHi011-A-1 (HNDS0003-01 #F CC39 +/+)
Alternative name(s) of stem cell lines	HNDS0005-01 #B HNDS0005-01 #B2 +/+ HNDS0002-01 #D HNDS0002-01 #D CC26 +/+ HNDS0003-01 #F HNDS0003-01 #F CC39 +/+
Institution	Boston Children's Hospital
Contact information of distributor	wardiya.afsharsaber@childrens.harvard.edu; mustafa.sahin@childrens.harvard.edu
Type of cell lines	iPSCs
Origin	Human
Additional origin info required for human ESC or iPSC	BCHi007-A (HNDS0005-01 #B), Age: 21, Sex: F, Ethnicity: White; BCHi009-A (HNDS0002-01 #D), Age: 20, Sex: M, Ethnicity: White; BCHi011-A (HNDS0003-01 #F), Age: 4, Sex: M, Ethnicity: White.
Cell Source	Fibroblasts
Clonality	Clonal cell
Method of reprogramming	Sendai Virus, non-integrating (OCT4, SOX2, KLF4 and hc-MYC)
Genetic Modification	Yes

(*continued on next page*)

* Corresponding authors.
E-mail addresses: wardiya.afsharsaber@childrens.harvard.edu (W. Afshar-Saber), mustafa.sahin@childrens.harvard.edu (M. Sahin).

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Resource Table (*continued*)

Type of Genetic Modification	Gene correction
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	N/A
Associated disease	Succinic semialdehyde dehydrogenase deficiency (SSADHD; OMIM #271980)
Gene/locus	*ALDH5A1* (Gene ID: 7915; RefSeq: NM_170740)
Method of modification/site-specific nuclease used	CRISPR/Cas9
Site-specific nuclease (SSN) delivery method	Ribonucleoprotein (RNP)
All genetic material introduced into cells	Single-stranded oligodeoxynucleotides (ssODN)
Analysis of the nuclease-targeted allele status	Sanger sequencing PCR product
Method of the off-target nuclease activity surveillance	N/A
Name of transgene	N/A
Eukaryotic selective agent resistance (including inducible/gene expressing cell-specific)	N/A
Inducible/constitutive system details	N/A
Date archived/stock date	January 2024
Cell line repository/bank	hPSCreg BCHi007-A (HNDS0005-01 #B) BCHi007-A-1 (HNDS0005-01 #B2 +/+) BCHi009-A (HNDS0002-01 #D) BCHi009-A-1 (HNDS0002-01 #D CC26 +/+) BCHi011-A (HNDS0003-01 #F) BCHi011-A-1 (HNDS0003-01 #F CC39 +/+)
Ethical approval	Human Subjects ethics committee Boston Children's Hospital Institutional Review Board (IRB) approved the protocol (IRB-P00016119) to study hiPSC lines at the Boston Children's Hospital (Boston, MA, USA)
Addgene/public access repository recombinant DNA sources' disclaimers	N/A

1. Resource utility

The generated hiPSC lines are the first patient-derived disease model of Succinic Semialdehyde Dehydrogenase Deficiency (SSADHD) and isogenic controls. Thus, they provide a valuable resource to investigate SSADHD disease mechanisms and therapeutic interventions.

2. Resource details

iPSC technology is a promising tool to decipher the mechanisms responsible for SSADHD and is complimentary to existing *in vivo* mice models (Hogema, 2001). We previously reported the first human *in vitro*

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	Normal	Fig. 1
Phenotype	Qualitative analysis (Immunocytochemistry)	Positive for SSEA4, OCT4, Nanog, and TRA-1–60	Fig. 1
Genotype	Karyotype (G-banding) and resolution	HNDS0005-01 #B (46XX, resolution 425–450 at p16) HNDS0005-01 #B2 +/+ (46XX, resolution 400–475 at p31) HNDS0002-01 #D (46XY, resolution 500–550 at p9) HNDS0002-01 #D CC26 +/+ (46XY, resolution 425–500 at p17) HNDS0002-03 #A (46XY, resolution 425–450 at p14) HNDS0003-01 #F (46XY, resolution 425–475 at p14) HNDS0003-01 #F CC39 +/+ (46XY, resolution 425–450 at p25)	Fig. 1
Identity	STR analysis	Performed using the PowerPlex 16 HS System by PromegaTM. Results are reported as 13 CODIS STR markers, Amelogenin for gender determination and two low-stutter, highly discriminating pentanucleotide STR markers.	Submitted in archive with journal
Mutation analysis	Sanger Sequencing	HNDS0005-01 #B homozygote: c.1226G > A/c.1226G > A; p.Gly409Asp HNDS0005-01 #B2 +/+ homozygote: c.1226G/c.1226G HNDS0002-01 #D homozygote: c.612G > A/c.612G > A; p.Trp204Ter HNDS0002-01 #D CC26 +/+ homozygote: c.612G/c.612G HNDS0003-01 #F compound heterozygote: exon 4c.612G > A; pTrp204* and exon 9c.1273C > T; p.Arg245* (R412X) HNDS0003-01 #F CC39 +/+ homozygote exon 4c.612G and exon 9c.1273C	Fig. 1
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Cultures were routinely tested for mycoplasma by PCR. Negative	Available with the authors
Differentiation potential	Directed differentiation	Ectoderm: Nestin and PAX6. Endoderm: FOXA2 and SOX17 Mesoderm: BRACHYURY and NCAM.	Fig. 1
List of recommended germ layer markers	Immunocytochemistry	Ectoderm: Nestin and PAX6. Endoderm: FOXA2 and SOX17 Mesoderm: BRACHYURY and NCAM.	Fig. 1
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

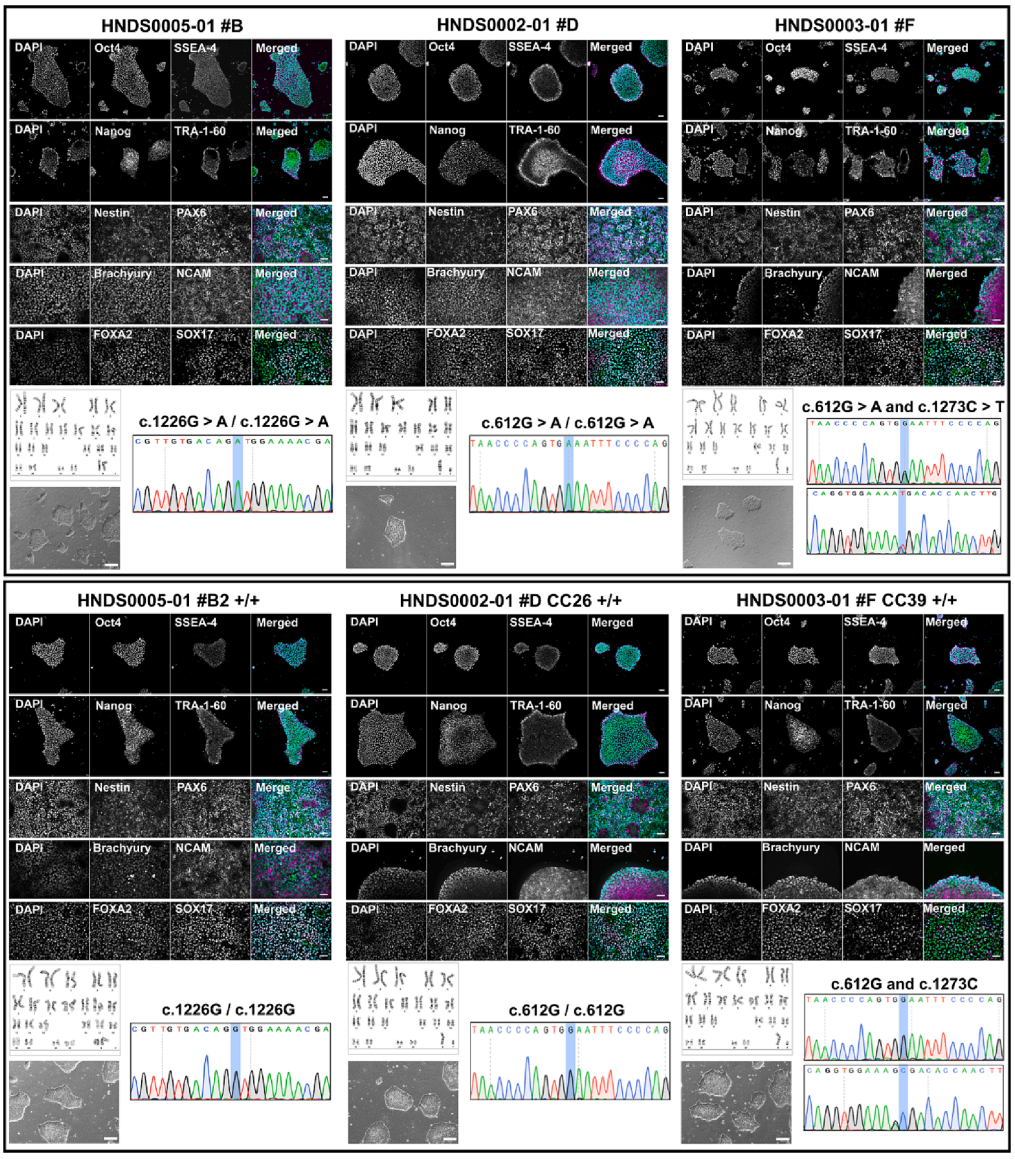

Fig. 1. Generation and characterization of six human induced pluripotent stem cell lines (hiPSCs) from three individuals with SSADH Deficiency and CRISPR-corrected isogenic controls.

model of SSADHD using iPSC-derived GABAergic and excitatory neurons (Afshar-Saber, 2024). Using this approach, we identified that the loss of SSADH caused neuron subtype-specific metabolic and gene expression change. Additionally, functional characterization of this model showed altered GHB and GABA metabolism and altered network activity. Finally, CRISPR correction or mRNA expression rescued metabolic and functional alteration (Afshar-Saber, 2024).

Fibroblasts from one female (*HNDS0005-01 #B*) and two males (*HNDS0002-01 #D* and *HNDS0003-01 #F*) with SSADHD carrying pathogenic variants in *ALDH5A1* were reprogrammed using non-integrating Sendai virus (Table 1). In this study, *HNDS0005-01 #B* hiPSCs were derived from an individual with SSADHD due to homozygote pathogenic variant in the catalytic domain c.1226G > A; p. Gly409Asp, *HNDS0002-01 #D* from an individual with SSADHD due to homozygote pathogenic variants in the NAD^+ domain c.612G > A, p. Trp204Ter, and *HNDS0003-01 #F* hiPSCs were derived from an individual with SSADHD due to compound heterozygote pathogenic variants in the NAD^+ and catalytic domain exon 4c.612G > A; pTrp204* exon 9c.1273C > T; p.Arg245* (R412X).

We carried out targeted mutation analysis on genomic DNA at mutated loci in iPSCs (Fig. 1), which matched with respective patient's clinical diagnosis and fibroblasts. Additionally, we used CRISPR-Cas9 for bi-allelic correction using the gRNA listed in Table 2 and generated the lines: *HNDS0002-01 #D CC26*$^{+/+}$ c.612G/c.612G, *HNDS0003-01 #F* CC39$^{+/+}$ exon 4c.612G, exon 9c.1273C and *HNDS0005-01 #B2*$^{+/+}$ c.1226G/c.1226G.

All iPSCs exhibited typical morphology, were karyotypically normal (Fig. 1), and expressed pluripotency markers at protein levels (Oct4, SSEA-4, Nanog, TRA-1–60) (Fig. 1). Differentiation potential was assessed via immunocytochemistry as a functional readout for pluripotency using markers for three germ layers: ectoderm (Nestin in green and PAX6 in magenta), mesoderm (Brachyury in green and NCAM in magenta) and endoderm (FOXA2 in green and SOX17 in magenta), scale bar 50 μm (Fig. 1). We confirmed the identity of these CRISPR corrected iPSCs using STR profiling, which matched with the hiPSC-derived from patients (Table 2, archived with journal).

3. Materials and methods

3.1. iPSC derivation

Skin punch biopsies were collected and derived as described in (Chen, 2021). iPSCs were generated from fibroblasts at the Harvard Stem Cell Institute iPS core, using the CytoTune-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher #A16517) as described in (Chen, 2021). The iPSCs were subsequently cultured in mTeSR™ Plus complete medium (StemCell #100–0276) on vitronectin VTN-N recombinant human protein, truncated (Gibco #A14700) or Geltrex for the CRISPR corrected lines (ThermoFisher #A1413302) and passaged every 5–7 days using Gentle Cell Dissociation Reagent (StemCell #07174).

3.2. Generation of isogenic lines

The lines BCHi007-A (HNDS0005-01 #B), BCHi009-A (HNDS0002-01 #D), and BCHi011-A (HNDS0003-01 #F) were corrected using CRISPR-Cas9 induced homology directed repair respectively to *BCHi007-A-1 (HNDS0005-01 #B2$^{+/+}$), BCHi009-A-1 (HNDS0002-01 #D CC26$^{+/+}$), and BCHi011-A-1 (HNDS0003-01 #F CC39$^{+/+}$)*. Delivery of the RNP complex and ssODN as well as Cas9 protein (Alt-R® S.p. Cas9 Nuclease V3 or HiFi Cas9 Nuclease V3, IDT #1081061 and #1081059) was achieved through nucleofection (2×10^5cells, 1000–1400 V, 20 ms, 1

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers	Mouse anti-SSEA4	1:250	anti-SSEA4 (Invitrogen 41–4000)	RRID:AB_2533506
	Rabbit anti-Oct-4A		anti-Oct-4A (Cell Signaling Technology 2840S)	RRID:AB_2167691
	Mouse anti-TRA-1–60		anti-TRA-1–60 (Invitrogen 41–1000)	RRID:AB_2533494
	Rabbit anti-Nanog		anti-Nanog (abcam 109250)	RRID:AB_10863442
Differentiation Markers	Endoderm: Mouse anti-FOXA2 and Rabbit anti-SOX17	1:250	anti-FOXA2 (abcam ab60721) anti-SOX17 (abcam ab224637)	RRID:AB_941632 RRID:AB_2801385
	Mesoderm: Mouse anti-Brachyury and Rabbit anti-NCAM		anti-Brachyury (Invitrogen 14–9770-82)anti-NCAM1(abcam ab313779)	RRID:AB_2573016RRID: AB_3095734
	Ectoderm:Mouse anti-Nestin and Rabbit anti-PAX6		anti-nestin(abcam 6320)anti-PAX6 (abcam 195045)	RRID:AB_308832RRID:AB_2750924
Secondary antibodies	Alexa Fluor™ 488 Goat anti-Mouse IgG (H + L)	1:500	Alexa Fluor™ 488 Goat anti-Mouse IgG (H + L)(Invitrogen A11029)	RRID:AB_2534088
	Alexa Fluor™ 647Goat anti-Rabbit IgG (H + L)		Alexa Fluor™ 647Goat anti-Rabbit IgG (H + L)(Invitrogen A21244)	RRID:AB_2535812
	Alexa Fluor™ 568 Goat anti-mouse IgG (H + L)		Alexa Fluor™ 568 Goat anti-mouse IgG (H + L)(Invitrogen A11031)	RRID:AB_144696
	Alexa Fluor™ 488Goat anti-Rabbit IgG (H + L)		Alexa Fluor™ 488Goat anti-Rabbit IgG (H + L)(Invitrogen A11008)	RRID:AB_143165
Imaging parameters				
Microscope	Wavelength	Filters sets		
Yokogawa CSU-W1	405 nm laser	dichroic mirror Semrock Di01-T405/488/568/647 and emission filter Chroma ET455/50 m		
spinning disk confocal	488 nm laser	dichroic mirror Semrock Di01-T405/488/568/647 and emission filter Chroma ET525/50 m		
installed on a Nikon Ti-E	561 nm laser	dichroic mirror Semrock Di01-T405/488/568/647 and emission filter Chroma ET605/52 m		
	640 nm laser	dichroic mirror Semrock Di01-T405/488/568/647 and emission filter Chroma ET705/72 m		
Gene editing strategy				
Line	Variant	Mutation	sgRNA	ssODN
HNDS0005-01#B to HNDS0005-01#B2 +/+	c.1226G > A; p. Gly409Asp	c.1226G > A/ c.1226G > A; to c.1226G/ c.1226G	sgRNA1: GGTGCCACCGTTGTGACAGA (TGG) sgRNA2: TCGTTTTCCATCTGTCACAA (CGG)	CATTCTAAAAGATTGTATCATGTGGA AAGCTTTTTTTCTTCCTCATTACACA GGTGGAGAAACAGGTGAATGATGCCGTTTC TAAAGGTGCCACAGTTGTGACAG GTGGAAAACGACACCAACTTGGAAAAAATTTCTTT GAGCCTACCCTGCTGTGCAATGTCACCCAGGACATGCTG
HNDS0002-01#D to HNDS0002-01#D CC26 +/+	c.612G > A, p. Trp204Ter	c.612G > A/ c.612G > A to c.612G/c.612G	sgRNA1: TTCCGGGTGATCATGGCACT (GGG) sgRNA2: GGCACTGGGGAAATTtCACT (GGG)	AGGAGGTGGTCCTTCCTCTCACATACT TCCTCTGCTCTTCTAACCCCAG TGGAATTTCCCCAGcGCCATGATCACCCGGAA GGTGGGGGCCGCCCTGGCAGCCG
HNDS0003-01#F HNDS0003-01#F CC39 +/+	exon 4c.612G > A; pTrp204* exon 9c.1273C > T; p. Arg245*	c.612G > A/ c.1273C > T to c.612G/c.1273C	sgRNA1: TTCCGGGTGATCATGGCACT (GGG) sgRNA2: GGCACTGGGGAAATTtCACT (GGG)	AGGAGGTGGTCCTTCCTCTCACATACTTCCTCTGC TCTTCTAACCCCAGTGGAATTTCCCCAGcGCCATG ATCACCCGGAAGGTGGGGGCCGCCCTGGCAGCCG
Primers				
Targeted sequencing of ALDH5A1	ALDH5A1	c.612 mutation	C612_F1 CAGGTGTTCTGAGAGCTCACCTGC612_R1 TGCATCAGGAGGCGTAAGCAAAG	
Targeted sequencing of ALDH5A1	ALDH5A1	c.1226 and c.1273	C1273_F1 AAATCCAAGCAAACGGCTGAGCC1273_R1 AGCAGCCCAGGAATCTCTTTCAG	
Mycoplasma	Myco280_CReM Myco279_CReM MGSO-5 GPO-3		5′- ACACCATGGGAGYTGGTAAT-3′ 5′-CTTCWTCGACTTYCAGACCCAAGGCAT-3′ 5′-TGCACCATCTGTCACTCYGTTAACCTC-3′ 5′- GGGAGCAAACAGGATTAGA-TACCCT-3′	

pulse). After nucleofection, cells were seeded into a 24-well plate coated with rhLaminin-521 (Thermo Fisher Scientific # A29248) in StemFlex media (Stemcell technologies) supplemented with small molecule HDR enhancer NU7026 (20 μM) (S2893 selleckchem), Alt-R HDR enhancer V1 or V2 (DT#1081073, #10007921). The genomic editing efficiency was determined by genomic DNA isolation and Amplitaq PCR (ThermoFisher CAT#4398881, 95 °C for 10 min, then 40 cycles of 95 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 60 sec, followed by 72 °C for 2 min) and amplification of the target region with forward primer and reverse primers list in Table 1. The PCR product was submitted for validation by Sanger sequencing to confirm the mutation site. Cells from the highest efficiency well were singlized using 0.75X TrypLE Select (Invitrogen #12563029) and 1000–4000 cells were seeded onto 10 cm plates for single clone generation. Single clones were picked into 96-well plates and upon reaching 70–80 % confluency, cells were harvested by adding 30 μl of 1X TrypLE Select and incubated at 37 °C for 5 min. Cells were then resuspended and washed with an additional 60 μl of StemFlex using a multichannel pipette. From this cell mix, 60 μl were transferred into PCR array tubes, spined for 1 min, removed 50 μl of supernatant (leave 10 μl) and heat up 6 min 65 °C, and then 2 min at 95 °C. The genotype of clonal lines was determined by Terra direct PCR (Takara Bio, 95 °C for 3 min, then 35 cycles of 95 °C for 15 sec, 60 °C for 15 sec, and 72 °C for 60 sec, followed by 72 °C for 2 min) using 5/10 μl of the crude cell extract (65 °C for 6 min and 95 °C for 2 min heated) from a 96-well and sequencing with the primers described in Table 2.

3.3. iPSCs maintenance and characterizations

iPSCs were cultured with mTeSR™ Plus Basal Medium and mTeSR™ Plus 5X Supplement (STEMCELL Technologies #100–0276) in plates coated with Cultrex™ (R&D Systems® #3434-001-02). Media was changed every other day and iPSCs were passaged with Gentle Cell Dissociation Reagent after reaching 70 % confluency. iPSCs were tested for mycosplasma using the primers in Table 2 (results available upon request), submitted for G-band karyotyping (WiCell) every ten passages (Fig. 1). Pluripotency of iPSCs was characterized for SSEA4, OCT4, Nanog, and TRA-1–60 using immunofluorescent as described (Afshar-Saber, 2024). Additionally, we used the STEMdiff™ Trilineage Differentiation Kit according to the manufacturer's recommendations (StemCell #05230) to functionally validate the ability of the iPSCs to differentiate to the three germ layers: ectoderm, mesoderm, and endoderm, and fixed samples with 4 % PFA for immunocytochemistry using the antibodies listed in Table 2.

3.4. Imaging

We imaged the stained hiPSCs with a Yokogawa CSU-W1 spinning disk confocal installed on a Nikon Ti-E microscope, the Hamamatsu Orca-Fusion BT camera with a Nikon Plan Apo 20 × 0.75NA DIC M N2 objective (Afshar-Saber, 2024). The laser used are listed in Table 2. We used the Nikon NIS Elements software for acquisition, and maximum projections of the images were created from the z-stacked images. Acquired images were handled using Fiji software (Schindelin, 2012).

4. Mycoplasma

Cultures were routinely tested for mycoplasma by PCR. Media supernatants (with no antibiotics) were collected, centrifuged, and resuspended in a saline buffer. Ten microliters of each sample were used for a PCR with the sets of primers in Table 2. Only negative samples were used in the study.

5. STR analysis and cell line identity testing

STR analysis was performed by WiCell using the PowerPlex 16 HS System by Promega™. Results are reported as 13 CODIS STR markers, amelogenin for gender determination and two low-stutter, highly discriminating pentanucleotide STR markers and was used to confirm matching identity between hiPSC-derived from patients and CRISPR corrected lines.

CRediT authorship contribution statement

Wardiya Afshar-Saber: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Project administration. **Cidi Chen:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing. **Nicole A. Teaney:** Investigation, Writing – review & editing. **Kristina Kim:** Investigation. **Ziqin Yang:** Investigation. **Federico M. Gasparoli:** Data curation, Investigation, Visualization. **Darius Ebrahimi-Fakhari:** Project administration, Resources. **Elizabeth D. Buttermore:** Project administration, Resources. **Ivy Pin-Fang Chen:** Project administration, Resources, Supervision. **Phillip L. Pearl:** Funding acquisition, Project administration, Resources. **Mustafa Sahin:** Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Darius Ebrahimi-Fakhari reports a relationship with National Institutes of Health that includes: funding grants. 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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