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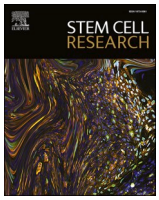

Lab Resource: Genetically-Modified Multiple Cell Lines

Generation of two iPSC lines (MHHi001-A-12 and MHHi001-A-13) carrying biallelic truncating mutations at the 3′-end of *SRCAP* using CRISPR/Cas9

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A B S T R A C T

Non-Floating Harbour Syndrome (FLHS) neurodevelopmental disorder (NDD) is a recently described disorder caused by mutations in certain regions of the *SRCAP* gene. We generated two iPSC lines that contain truncating mutation on both alleles at the 3′-end of *SRCAP* using CRISPR/Cas9 technology. Both cell lines are pluripotent, differentiate into the 3 germ layers and contain no genomic aberrations or off-target modifications. The cell lines form part of a human disease model to investigate the effects of truncating mutations in different regions of *SRCAP*.

1. Resource table

Unique stem cell line identifier	MHHi001-A-12 MHHi001-A-13
Alternative name(s) of stem cell line	Phx_SRCAP_g3_1200_4, UMGWi003-A-12 (MHHi001-A-12) Phx_SRCAP_g3_1200_7, UMGWi003-A-13 (MHHi001-A-13)
Institution	Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald
Contact information of the reported cell line distributor	Andreas W. Kuss, kussa@uni-greifswald.de
Type of cell line	iPSC
Origin	human
Additional origin info *(applicable for human ESC or iPSC)*	Age: neonate Sex: female Ethnicity: Caucasian
Cell Source	CD34+ human cord blood hematopoietic stem cells
Method of reprogramming	Non-integrating Sendai virus
Clonality	Clonal by picking of single cell colonies
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	Confirmed by RT-PCR
The cell culture system used	cells grown on Matrigel in mTESR 1 medium with antibiotics
Type of the Genetic Modification	Induced mutation, CRISPR/Cas 9
Associated disease	non-FLHS SRCAP-related neurodevelopmental disorder/DEHMBA, https://www.omim.org/entry/619595
Gene/locus	SRCAP, 16p11.2 c.[9307del];[9307-9308del] NP_006653.2:p.[(Leu3104TrpfsTer3)];[(Gly3103ValfsTer31)]

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(*continued*)

	c.[9307del];[9307-9314delinsT] NP_006653.2:p.[(Leu3104TrpfsTer3)];[(Gly3103TyrfsTer2)]
Method of modification/user-customisable nuclease (UCN) used, the resource used for design optimisation	CRISPR/Cas9 crRNA designed with CRISPOR
User-customisable nuclease (UCN) delivery method	Electroporation of RNPs
All double-stranded DNA genetic material molecules introduced into the cells	N/A
Analysis of the nuclease-targeted allele status	PCR and Sanger sequencing, deconvolution with TIDE and Indigo
Method of the off-target nuclease activity prediction and surveillance	Likely off-targets predicted with CRISPOR and ccTOP, 5 most likely regions (4 predicted by both tools) amplified with PCR and Sanger sequenced
Descriptive name of the transgene	N/A
Eukaryotic selective agent resistance cassettes (including inducible, gene/cell type-specific)	N/A
Inducible/constitutive expression system details	N/A
Date archived/stock creation date	September 2023
Cell line repository/bank	https://hpscreg.eu/cell-line/MHHi001-A-12 https://hpscreg.eu/cell-line/MHHi001-A-13
Ethical/GMO work approvals	Ethics Committee of the University of Greifswald, approval number BB 047/14
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	N/A

2. Resource utility

Truncating mutations in *SRCAP* outside of the region associated with Floating-Harbor Syndrome (FLHS) lead to a distinct neurodevelopmental disorder (non-FLHS *SRCAP*-related NDD). We describe two iPSC lines carrying truncating mutations at the distal end of *SRCAP* that mimic known patient mutations to enable further research into the newly described NDD.

3. Resource details

SRCAP is a chromatin remodeler and involved in a broad range of cellular processes, e.g. histone variant exchange, DNA damage repair and regulation of gene expression (Messina et al., 2016). Mutations at the end of exon 33 and the beginning of exon 34 are associated with FLHS (Hood et al., 2012; Kehrer et al., 2014). Non-FLHS *SRCAP*-related NDD shares many features with FLHS but lacks the characteristic short stature and facial gestalt of FLHS and shows a different methylation pattern. It is characterized by truncating mutations situated distal and proximal of the region associated with FLHS (Rots et al., 2021). The functional mechanism of both disorders is still not solved. The here described cell lines will be part of a human disease model to investigate the mechanism behind *SRCAP*-associated NDDs.

We introduced frameshift mutations in the distal part of *SRCAP* on both alleles into the wildtype iPSC line MHHi001-A using CRISPR/Cas9. The mutations lead to premature stop codons and mimic the truncation observed in the patients. Cell line MHHi001-A-12 contains a 1-bp deletion for one allele and a 2-bp deletion for the other allele (ENSG00000080603:c.[9307del];[9307-9308del]), both resulting in premature termination (NP_006653.2:p.[(Leu3104TrpfsTer3)];[(Gly3103ValfsTer31)]). MHHi001-A-13 contains a 1-bp deletion for one allele and a 7-bp deletion-insertion for the other allele (ENSG00000080603:c.[9307del];[9307-9314delinsT]), both leading to a frameshift and resulting in premature termination (NP_006653.2:p.[(Leu3104TrpfsTer3)];[(Gly3103TyrfsTer2)]). We confirmed the presence and impact of all mutations with Sanger sequencing and trace decomposition (Fig. 1H and I). Sanger sequencing confirmed that there are no off-target modifications at the top 5 predicted locations (Supplementary Fig. 1C). iPSC lines show normal morphology compared to the wildtype iPSC (Fig. 1C). Immunofluorescence (IF) staining for pluripotency markers OCT4, SOX2 and TRA 1–60 was positive for both cell lines (Fig. 1A and B). Quantitative RT-PCR for the pluripotency markers *NANOG*, *OCT4*, and *SOX2* shows that both cell lines express these markers similarly to the wildtype (Fig. 1D).

Both cell lines can differentiate into cells from 3 different germ layers when grown as embryoid bodies. Embryoid bodies from MHHi001-A-12 (Fig. 1F) and MHHi001-A-13 (Fig. 1G) express AFP (endoderm), SMA (mesoderm) and TUJ1 (ectoderm), as shown by IF. Both cell lines have a normal karyotype (46XX) as shown by whole genome low coverage sequencing (lcWGS, Fig. 1E). STR profiling (Eurofins Genomics) showed that both cell lines originate from MHHi001-A. Both cell lines are free of mycoplasma contamination (Supplementary Fig. 1B).

4. Materials and methods

4.1. Cell culture

Cells were cultured on Matrigel-coated plates (Corning) in mTESR1 Stem Cell Medium (Stemcell Technologies) containing 1 % Pen/Strep (Gibco), at 37 °C/5% CO_2. Cells were manually passaged every 7 days. Table 1.

4.2. CRISPR/Cas9

CRISPR/Cas9 was performed using the Alt-R RNP system (Alt-R S.p. Cas9 Nuclease V3, IDT). crRNAs targeting the distal end of *SRCAP* were designed with CRISPOR (Table 2). RNP transfection was performed according to the manufacturer's protocol with the Neon Transfection System (Thermo Fisher). Briefly, cells were released with Accutase (Stemcell Technologies) for 8 min, washed 2x with DMEM and 100,000 cells were diluted into buffer R. The cell suspension was mixed with RNP and Electroporation Enhancer (IDT) and electroporated for 2 pulses with 30 ms at 1200 V. Cells were seeded onto Matrigel-coated 6-well plates in mTESR1 containing ROCK inhibitor (10 μM). The following days, medium without ROCK inhibitor was used.

4.3. Screening for transfected colonies

Single colonies were picked into 12-well plates and grown to appropriate size. Single-cell derived colonies were screened for mutations with PCR and Sanger sequencing, and analysed with TIDE and Indigo. The 5 most likely off-targets (determined by combining CRISPOR and ccTOP results) were amplified and Sanger sequenced.

4.4. Embryoid body formation

iPSC from a non-confluent well were detached with a cell scraper and washed 2x with DMEM. Colonies were resuspended in EB Medium (DMEM, 10 % Knockout Serum Replacement, 10 % FBS, 10 μM ROCK inhibitor) on a non-treated plate rinsed with anti-adherence solution

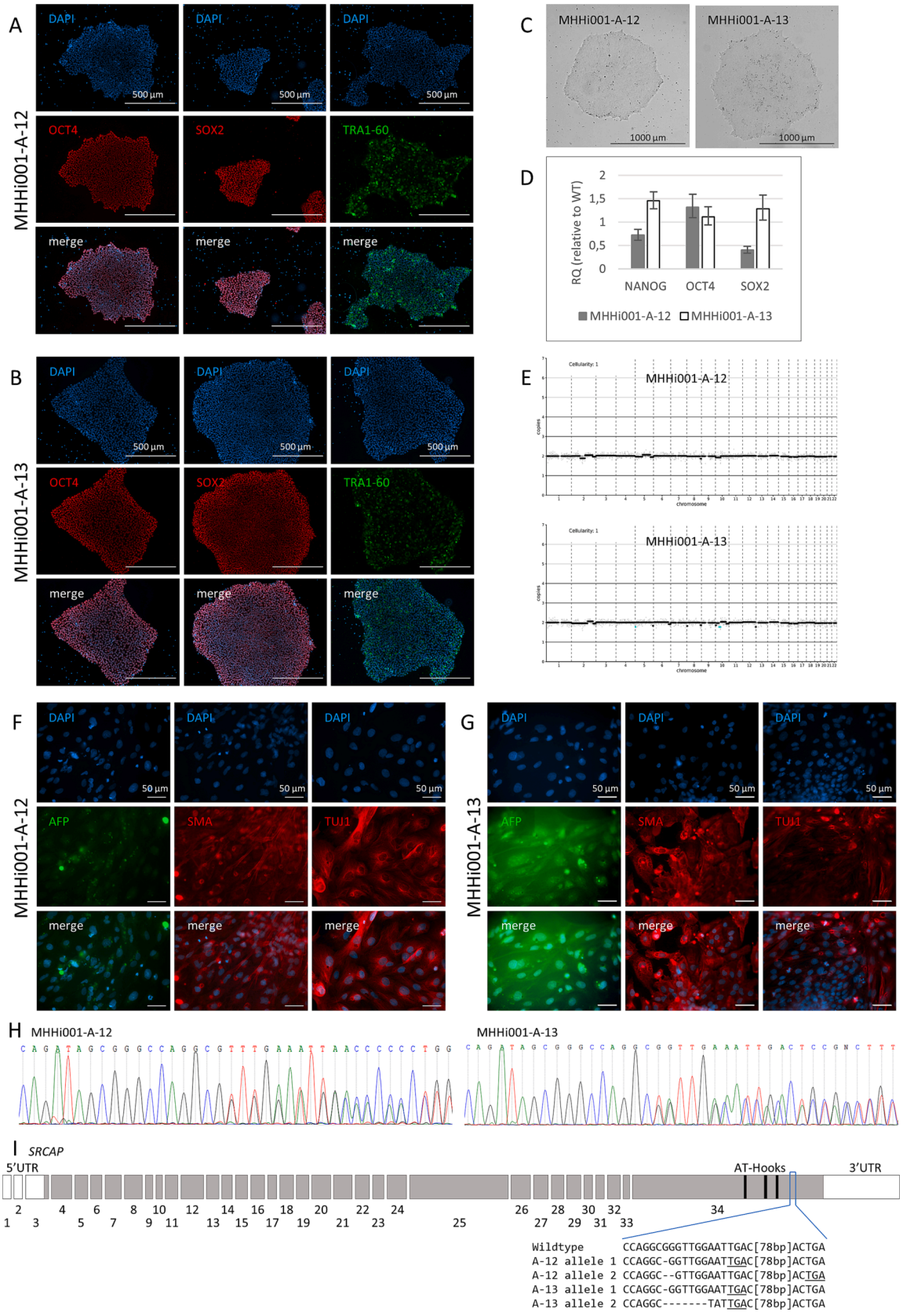
A
MHHi001-A-12
DAPI
500 µm
OCT4
SOX2
TRA1-60
merge
B
MHHi001-A-13
C
MHHi001-A-12
MHHi001-A-13
1000 µm
D
RQ (relative to WT)
2
1,5
1
0,5
0
NANOG
OCT4
SOX2
MHHi001-A-12
MHHi001-A-13
E
Cellularity: 1
copies
chromosome
F
G
50 µm
AFP
SMA
TUJ1
H
I SRCAP
5'UTR
AT-Hooks
3'UTR
Wildtype CCAGGCGGGTTGGAATTGAC[78bp]ACTGA
A-12 allele 1 CCAGGC-GGTTGGAATTGAC[78bp]ACTGA
A-12 allele 2 CCAGGC--GTTGGAATTGAC[78bp]ACTGA
A-13 allele 1 CCAGGC-GGTTGGAATTGAC[78bp]ACTGA
A-13 allele 2 CCAGGC-------TATTGAC[78bp]ACTGA

Fig. 1.

Table 1
Characterization and validation.

Classification (optional *italicized*)	Test	Result	Data
Morphology	Photography	normal iPSC colony morphology	Fig. 1C
Pluripotency status evidence for the described cell line	Qualitative analysis	Immunocytochemistry positive staining for OCT4, SOX2,TRA 1–60 MHHi001-A-12 MHHi001-A-13	Fig. 1A Fig. 1B
	Quantitative analysis	qPCR for OCT4, SOX2, NANOG; expression similar to MHHi001-A cell line	Fig. 1D
Karyotype	Low coverage sequencing	Normal karyotype (46 XX) confirmed by low coverage whole genome sequencing	Fig. 1E
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site or targeted allele-specific PCR	PCR and Sanger sequencing for target region Shows 2 overlapping traces after CRISPR/Cas9 cut site	Fig. 1H
	Evaluation of the – (homo-/hetero-/hemi-) zygous status of introduced genomic alteration(s)	Allelic deconvolution with TIDE and Indigo http://shinyapps.datacurators.nl/tide/https://www.gear-genomics.com/indigo/ MHHi001-A-12 shows deletion of 1 and 2 nucleotides MHHi001-A-13 shows deletion of 1 and 7 nucleotides Estimated allelic sequences created by Indigo aligned to MHHi001-A reference All deletions lead to frameshift mutations and truncated protein	Fig. 1I
	Transgene-specific PCR	N/A	N/A
Verification of the absence of random plasmid integration events	PCR/Southern	PCR on cDNA with primers specific for Sendai vector No integration detected	Supplementary Fig. 1A
Parental and modified cell line genetic identity evidence	STR analysis, microsatellite PCR (mPCR) or specific (mutant) allele seq	Cell Line Authentication (Eurofins Genomics) Cell lines identical to cell line of origin including markers: CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA, Amelogenin	
Mutagenesis/genetic modification outcome analysis	Sequencing (genomic DNA PCR or RT-PCR product)	PCR and Sanger sequencing for target region (see above)	Fig. 1H Fig. 1I
	PCR-based analyses	N/A	N/A
	Southern Blot or WGS; western blotting (for knock-outs, KOs)	N/A	N/A
Off-target nuclease activity analysis	PCR across top 5/10 predicted top likely off-target sites, whole genome/exome sequencing	PCR and Sanger sequencing for the 5 most likely off-targets (CRISPOR/ccTOP), no changes detected	Supplementary Fig. 1C
Specific pathogen-free status	Mycoplasma	Negative PCR Mycoplasma Test MycoSPY (Biontex)	Supplementary Fig. 1B
Multilineage differentiation potential	Embryoid body	Embryoid bodies: Immunofluorescence assay for AFP, SMA, TUJ1 MHHi001-A-12 positive MHHi001-A-13 positive	Fig. 1F Fig. 1G
Donor screening (OPTIONAL)	HIV 1+2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype – additional histocompatibility info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker	Rabbit anti-OCT4 Rabbit anti-SOX2 Mouse anti-TRA 1–60	1:500	Abcam Cat# ab19857, RRID:AB_445175 Abcam Cat# ab97959, RRID: AB_2341193 Abcam Cat# ab16288, RRID:AB_778563
Differentiation marker	Rabbit anti-TUJ1 Mouse IgG1 anti-AFPMouse IgG2a anti-SMA	1:500 1:5001:100	Thermo Fisher Scientific Cat# A25532, RRID:AB_2651003 Thermo Fisher Scientific Cat# A25530, RRID:AB_2651004 Thermo Fisher Scientific Cat# A25531, RRID:AB_2651005
Secondary antibodies	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) Goat Anti-Mouse IgM mu chain (DyLight® 488) Alexa Fluor® 647 donkey anti-rabbit Alexa Fluor® 488 goat anti-mouse IgG1Alexa Fluor® 594 goat anti-mouse IgG2a	1:500 1:500 1:500 1:5001:250	Abcam Cat# ab150084, RRID: AB_2734147 Abcam Cat# ab98674, RRID: AB_10674182 Thermo Fisher Scientific Cat# A25537, RRID:AB_2857990 Thermo Fisher Scientific Cat# A25536, RRID:AB_2651011 Thermo Fisher Scientific Cat# A25538, RRID:AB_2868398
Nuclear stain	DAPI	1 drop/200 μl wash buffer	NucBlue® Fixed Cell Stain (Thermo Fisher Scientific, Cat# R37606)
Site-specific nuclease			
Nuclease	S.pyogenes Cas9	Alt-R S.p. Cas9 Nuclease V3 (IDT, Cat# 1081058)	

(*continued on next page*)

Table 2 (*continued*)

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Delivery method	Electroporation (2 pulses, 1200 V, 30 ms)	Neon™ Transfection System Invitrogen	
Selection/enrichment strategy	Colony picking and Sanger sequencing	N/A	
Primers and Oligonucleotides used in this study			
	Target	Forward/Reverse primer (5′-3′)	
Pluripotency Markers (qPCR)	NANOG OCT4SOX2	Hs02387400_g1 (Thermo Fisher Scientific)Hs04260367_gH (Thermo Fisher Scientific)Hs01053049_s1 (Thermo Fisher Scientific)	
House-Keeping Genes (qPCR)	GAPDHRPS 29	Hs99999905_m1 (Thermo Fisher Scientific)Hs03004310_g1 (Thermo Fisher Scientific)	
Targeted mutation analysis/sequencing	target region in SRCAP gene (492 bp)	AGAATCCTCCATCACCTCGG/CTTCAGCCTCAGACTCCTCT	
Potential random integration-detecting PCRs (RT-PCR)	SeV (181 bp) KOS (582 bp)Klf4 (410 bp)c-myc (532 bp)	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC ATGCACCGCTACGACGTGAGCGC/ ACCTTGACAATCCTGATGTGG TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA TTACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG	
gRNA sequence/distributor	crRNA tracrRNA	GGTGTCAATTCCAACCCGCC IDT, Alt-R® CRISPR-Cas9 tracrRNA	
Genomic target sequence	crRNA including PAM	GGTGTCAATTCCAACCCGCCTGG chr16:30,739,341–30,739,363	
Bioinformatic gRNA on– and -off-target binding prediction tool used, specific sequence/outputs link(s)	CRISPOR, ccTOP	https://crispor.tefor.net/crispor.py?batchId = k9GRDO3jEaxLuEo1s4YR https://cctop.cos.uni-heidelberg.de:8043/result/7b7b7a9467447572a8623059a287265b8869b0a5/result.html	
Primers for top off-target mutagenesis predicted site sequencing	FAT2 (CRISPOR) (480 bp) TTC28 (CRISPOR/ccTOP) (446 bp) CMTM7 (CRISPOR/ccTOP) (303 bp) ESRRG (CRISPOR/ccTOP) (300 bp) MAGI2 (RP4-587D131) (CRISPOR/ccTOP) (304 bp)	TTAGAACCACCACCCACTTCC/TGCCTAGTCACTCCCGAGAT CCTCTGAAGGCTATGGACAAAGT/ TCTTCTAAAACCCTGCTGCTTCA GAATGGCAAAGCTCAGGCTCA/TACCTTGATCCAGCCATCAGC CTTGAGACACAGGGATGGCT/GTCAGAATGGTCCGAGTGCT ACACACTCACCGGGTGTTC/GGGGAGTCCTTGTACAGACAAAT	
ODNs/plasmids/RNA molecules used as templates for HDR-mediated site-directed mutagenesis.		N/A	

(Stemcell Technologies). After 25 days at 37 °C/5% CO_2, EBs were transferred to Matrigel-coated wells with coverslips for IF.

4.5. TaqMan assay

RNA was isolated using RNASolv (Omega Bio-Tek) according to the manufacturers protocol and treated with DNAse (Thermo Fisher). cDNA synthesis was performed using Superscript IV (Thermo Fisher) with random hexamer primers (Invitrogen). TaqMan assays were performed in triplicate on QuantStudio 7 Flex (Thermo Fisher). Gene expression was quantified relative to the wildtype cell line and normalized using housekeeping genes.

4.6. DNA Isolation/PCR

DNA was extracted by cell-lysis in a buffer (5 mM EDTA, 200 mM NaCl, 100 mM Tris, 0.2% SDS, 0.2 mg/ml Proteinase K) for 2 h at 55 °C, followed by precipitation with isopropanol.

PCR was performed using HS Taq Mastermix (Biozym) on a Sure-Cycler 8800 (Agilent) with the following parameters: 5 min 95 °C, 30x (30 s 95 °C, 30 s 58 °C, 1 min 72 °C) 5 min 72 °C.

4.7. Immunofluorescence staining

iPSC were grown on coverslips, fixed with 4 % paraformaldehyde for 1 h/RT, and permeabilized for 1 h/37 °C (PBS with 3 % BSA, 1 % Triton X-100). Incubation with primary antibody occurred overnight at 4 °C, incubation with secondary antibody 2 h/37 °C. Nuclei were stained with DAPI for 5 min and mounted using ProLong Diamond Mounting Medium (Invitrogen). Microscopy was performed on a AxioImager M2 (Zeiss).

Embryoid bodies were stained according to the manual of the 3-Germ Layer Kit (Invitrogen).

4.8. Low coverage sequencing

Sequencing libraries were prepared with NEBNext Ultra II FS DNA (NEB) and lcWGS (0.6x) was performed on a NextSeq 550 (Illumina). The data was analysed using the R-package ACE (https://rdrr.io/bioc/ACE/).

4.9. Mycoplasma test

Mycoplasma testing was performed at passage 7 with MycoSPY-Kit (Biontex) according to protocol.

4.10. STR-analysis

Cell line authentication was performed by Eurofins Genomics.

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.

Acknowledgments

The iPSC line MHHi001-A was kindly provided by the Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School, Hannover, Germany. We thank Corinna Jensen for providing outstanding technical support. We also thank the Imaging Center of the Department of Biology, University of Greifswald for access to the fluorescence microscope and technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2023.103249.

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