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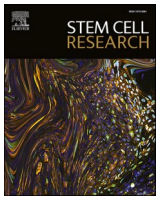

Lab Resource: Genetically-Modified Multiple Cell Lines

Three modified human IPSC lines containing mutations in the distal DEHMBA associated locus of the *SRCAP* gene

Johannes Rhode [a], Lisa Hagenau [a], Stephanie Edwards [a], Falk F.R. Buettner [b,c], Ana Tzvetkova [a,d], Lars R. Jensen [a], Andreas W. Kuss [a,*]

[a] *Human Molecular Genetics Group, Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany*
[b] *Institute of Clinical Biochemistry, Hannover Medical School, Hannover, Germany*
[c] *Proteomics, Institute of Theoretical Medicine, Faculty of Medicine, University of Augsburg, Augsburg, Germany*
[d] *Institute of Bioinformatics, University Medicine Greifswald, Greifswald, Germany*

A B S T R A C T

We modified an existing human iPSC line (MHHi001-A), using CRISPR/Cas9, to introduce heterozygous frameshift mutations in a locus of the *SRCAP* gene that is associated with the DEHMBA disease (OMIM 619595). The modified iPSCs express several stem cell markers and are able to differentiate into cells originating from all three embryonic germ layers. No additional modifications or chromosomal defects were detected. The modified cells can serve as a model for the investigation of the involvement of SRCAP in DEHMBA (Developmental delay, Hypotonia, Musculoskeletal defects, and Behavioral Abnormalities) disease and/or its molecular functions in different cell types.

1. Resource table

Unique stem cell line identifier	MHHi001-A-14 MHHi001-A-16 MHHi001-A-17
Alternative name(s) of stem cell line	**MHHi001-A-14:** UMGWi003-A-14 Phx_SRCAP_g3_1400_6/8 A-14 **MHHi001-A-16:** UMGWi003-A-16 PHX_CRISPR_JR_DIS10 A-16 **MHHi001-A-17:** UMGWi003-A-17 PHX_CRISPR_JR_DIS13 A-17
Institution	University Medicine Greifswald Department of Functional Genomics – Human Molecular Genetics
Contact information of the reported cell line distributor	Prof. Dr. Andreas 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

(*continued on next column*)

(*continued*)

Unique stem cell line identifier	MHHi001-A-14 MHHi001-A-16 MHHi001-A-17
	Ethnicity: Caucasian
Cell Source	CD34 positive human cord blood hematopoietic stem cells (CD34 + hCBHSCs)
Method of reprogramming	Non-integrating Sendai virus
Clonality	Isolation of single colonies with heterozygous genotype Tested by repeated sequencing and deconvolution with TIDE https://tide.nki.nl/(Brinkman et al., 2014) and indigo https://www.gear-genomics.com/indigo/ (Rausch et al., 2020)
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR for vector parts *KOS*, *Klf4*, *c-myc* (Primer see table) Parental cell line was free of reprogramming vectors (Haase, Göhring and Martin, 2017)
The cell culture system used	iPSC grown in mTESR1 Medium with 1 % Penicillin-Streptomycin (PAN-Biotech) on Matrigel coated plates
Type of the Genetic Modification	CRISPR/Cas9 induced heterozygous mutation

(*continued on next page*)

* Corresponding author.
E-mail address: kussa@uni-greifswald.de (A.W. Kuss).

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(*continued*)

Unique stem cell line identifier	MHHi001-A-14 MHHi001-A-16 MHHi001-A-17
Associated disease	DEHMBA: "Developmental delay, Hypotonia, Musculoskeletal defects, and Behavioral Abnormalities" OMIM: # 619,595
Gene/locus modified in the reported transgenic line	Gene: *SRCAP* MHHi001-A-14: NM_006662.3:c.9307del p.(Leu3104Trpfs*3) MHHi001-A-16/MHHi001-A-17: NM_006662.3:c.9306_9307insGG p.(Leu3104Glyfs*4)
Method of modification/user-customisable nucleases (UCN) used, the resource used for design optimisation	CRISPR/Cas9 (IDT), crRNA designed with CRISPOR https://crispor.gi.ucsc.edu/(Concordet and Haeussler, 2018)
User-customisable nuclease (UCN) delivery method	RNP
All double-stranded DNA genetic material molecules introduced into the cells	No new plasmids, for reprogramming plasmids see (Haase, Göhring and Martin, 2017)
Evidence of the absence of random integration of any plasmids or DS DNA introduced into the cells.	RT-PCR for vector parts *KOS*, *Klf4*, *c-myc* (Primer see table)Parental cell line was free of reprogramming vectors (Haase, Göhring and Martin, 2017)
Analysis of the nuclease-targeted allele status	Sanger sequencing for target region, deconvolution with TIDE and indigo
Homozygous allele status validation	N/A
Method of the off-target nuclease activity prediction and surveillance	PCR for the 5 most likely off-targets predicted by CRISPOR and ccTOP https://cctop.cos.uni-heidelberg.de:8043/(Stemmer et al., 2015)
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	2025–07-12
Cell line repository/bank	https://hpscreg.eu/cell-line/MHHi001-A-14https://hpscreg.eu/cell-line/MHHi001-A-16https://hpscreg.eu/cell-line/MHHi001-A-17
Ethical/GMO work approvals	Ethics Committee of the University of Greifswald, approval number BB047/14
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	N/A

2. Resource utility

DEHMBA, an autosomal dominant disorder, associated with intellectual disability, ADHD and ASD, is caused by heterozygous mutations in the *SRCAP* gene (Rots et al., 2021). iPSC (induced pluripotent stem cells) and thereof differentiated cells are a suitable model to understand the effects of mutations on the cellular functions.

3. Resource details

Here we present three human iPSC lines with newly introduced heterozygous frameshift mutations in the distal part of the *SRCAP* gene (Table 1. Mutations in *SRCAP* outside the so called FLHS-locus (FLHS: Floating Harbor Syndrome) lead to a disease called DEHMBA (Developmental delay, Hypotonia, Musculoskeletal defects, and Behavioral Abnormalities), which is associated with several neurological and neurodevelopmental symptoms (Rots et al., 2021).

The three iPSC were generated in two separate experiments (MHHi001-A-14 was created in an independent experiment, MHHi001-A-16 and MHHi001-A-17 were created in the same experiment), by modifying the distal part of the *SRCAP* gene. The newly introduced mutations (MHHi001-A-14: NM_006662.3:c.9307del; MHHi001-A-16/MHHi001-A-17: NM_006662.3:c.9306_9307insGG) are localized close to known DEHMBA causing mutations (NM_006662.2:c.9338_9341del; NM_006662.2:c.9364del; NM_006662.2:c.9344del) published by Rots et al. (Fig. 1 A with red line indicating the location of the newly introduced mutations; Fig. 1C dec. indicating the mutated allele deconvoluted by indigo) and were predicted as protein truncating and pathogenic by the MutationTaster tool (Schwarz et al., 2014).

The cells show an iPSC-typical morphology, with small globular cells in symmetrical round colonies (Fig. 1E). There were no mutations in the 5 most likely off-target regions for the used crRNA (Supp.A) and the karyotype (46XX), was confirmed by low coverage whole genome sequencing (Fig. 1F).

All iPSC lines tested positive for the stem cell marker OCT4 (octamer-binding transcription factor 4), SOX2 (SRY-Box Transcription Factor 2), TRA 1–60 in immunofluorescence microscopy and showed expression of the undifferentiated state markers *OCT4*, *SOX2*, *NANOG* comparable to the parental cell line as confirmed by RT-qPCR (Fig. 1B) Embryoid bodies derived from all three iPSC lines showed their ability to differentiate into cells originating from all three embryonic germ layers, which was confirmed by immunofluorescence microscopy and RT-qPCR (Fig. 1D). The endodermal marker *SOX17* (SRY-Box Transcription Factor 17) and the ectodermal marker *PAX6* (Paired Box 6) were detected in all three iPSCs by RT-qPCR. Mesodermal differentiation was tested either by RT-qPCR using a *BRACHYURY* (T-box transcription factor T) specific TaqMan Probe (MHHi001-A-16 and MHHi001-A-17) or immunofluorescence microscopy using a HAND1 (Heart- and neural crest derivatives-expressed protein 1) specific antibody (MHHi001-A-14). The latter was used to confirm the mesodermal differentiation in MHHi001-A-14, where *BRACHYURY* expression was extremely difficult to detect by RT-qPCR, which is a problem that occurs regularly with this particular marker. The endodermal marker AFP (Alpha Fetoprotein), mesodermal marker SMA (smooth muscle actin) and ectodermal marker TUJ1 (β-tubulin III) were detected in all three iPSC lines, using immunofluorescence microscopy.

All iPSC lines did not contain other mutations than the newly introduced *SRCAP* alterations and tested negative for the integration of the reprogramming vectors used to generate the parental line MHHi001-A (Supp.B). Descendance from MHHi001-A was confirmed by STR-Profiling. No mycoplasma contamination was detected by repeated PCR based testing (Supp.C).

We conclude that the IPSCs we generated can be used as a model for the investigation of the involvement of SRCAP in the aetiology of the DEHMBA disorder and/or to elucidate the molecular function of the SRCAP Protein in different cell types.

4. Materials and methods

The material/methods section is a slightly modified version of our standard iPSC culture and characterization protocol also published in (Rhode et al., 2023; Edwards et al., 2024) (Table 2).

4.1. Cell culture

iPSCs were cultured (37 °C/5% CO_2) in mTESR™1 medium (STEMCELL Technologies) supplemented with 1% Penicillin-Streptomycin (PAN-Biotech) on Matrigel (Corning) (1,5% in DMEM) coated culture ware and passaged manually.

4.2. Genetic modification

crRNA design and off-target prediction was done using CRISPOR and confirmed with ccTOP. Primers were designed using primer blast (Ye et al., 2012).

iPSCs were dissociated to single cell solution with Accutase (STEMCELL Technologies), washed with DMEM (Gibco) and passed through a

Table 1
Characterization and validation.

Classification	Output type	Result	Data
Schematic of a transgene/genetic modification	Schematic illustrating the structure and location of the introduced genetic modification	Modifications were introduced in Exon 34 of *SRCAP* downstream of the AT-Hooks in a locus known for DEHMBA causing mutations	Fig. 1 A Representation of *SRCAP* with modified locus (red line) Fig. 1 C sanger sequences and deconvolution for the modified cells
Morphology	Photography	Modified cell lines show iPSC typical morphology	Fig. 1 E Bright-Field image
Pluripotency status evidence for the described cell line	Qualitative analysis (i.e. Immunocytochemistry, western blotting)	Modified cell lines positive stained for OCT4, SOX2, TRA1-60	Fig. 1 B Immunofluorescence staining
	Quantitative analysis (i.e. Flow cytometry, RT-qPCR) of expression alongside well-known/pubished hPSC line	Modified cell lines express *NANOG*, *OCT4*, *SOX2* comparable to unmodified iPSC	Fig. 1 B
		Confirmed by RT-qPCR	Results RT-qPCR relative to parental cell line
Karyotype	Karyotype (G-banding) and higher-resolution, array-based assays (KaryoStat, SNP, etc.)	46XX	Fig. 1 F
		Confirmed with lcWGS	Results lcWGS
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site or targeted allele-specific PCR	MHHi001-A-14: Deletion of one Guanin MHHi001-A-16/MHHi001-A-17: Insertion of two Guanin	Fig. 1 C Sanger traces .ab1 files in Supplements
	Evaluation of the − (homo-/hetero-/hemi-) zygous status of introduced genomic alteration(s)	Allelic Deconvolution using TIDE, indigo and by eye confirm heterozygous state of the cell lines	Fig. 1 C Deconvoluted alleles from indigo
	Transgene-specific PCR (when applicable)	N/A	N/A
Verification of the absence of random plasmid integration events	PCR/Southern	RT-PCR for vector parts *KOS*, *Klf4*, *c-myc* (Primer see table)	Supp.B RT-PCR results
Parental and modified cell line genetic identity evidence	STR analysis, microsatellite PCR (mPCR) or specific (mutant) allele seq	Corresponding to wildtype Human Cell Line Authentication Service by STR/DNA Profiling – 2.0 By Eurofins Genomics D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, AMEL, D5S818, FGA, D19S433, vWA, TPOX, D18S51	Supplementary file submitted to archive with journal
Mutagenesis/genetic modification outcome analysis	Sequencing (genomic DNA PCR or RT-PCR product)	PCR and Sanger sequencing show heterozygous frameshift mutations mentioned above Allelic deconvolution with indigo compared to GRCh38 confirmed frameshift	Fig. 1 C
	PCR-based analyses	See above	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 top 5 off targets predicted by CRISPOR and ccTOP	Supp.A
Specific pathogen-free status	Mycoplasma	Negative PCR with MycoSPY Kit (Biontex)	Supp.C
Multilineage differentiation potential	Embryoid body formation	EB formation by known protocol Germlayer Validation using 3-Germ Layer Immunocytochemistry Kit and additional Antibody TaqMan RT-qPCR in triplicate compared to undifferentiated iPSC MHHi001-A-14 Negative for *BRACHYURY* in qPCR Positive for HAND1 instead MHHi001-A-16/MHHi001-A-17: Positive for AFP, *SOX17*, SMA, *BRACHYURY*, TUJ1, *PAX6*	Fig. 1 D
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	Ectoderm: *PAX6* RT-qPCR TUJ1 IF Endoderm: *SOX17* RT-qPCR AFP IF Mesoderm: *BRACHYURY* RT-qPCR HAND1 IF A-SMA IF	
Outcomes of gene editing experiment (OPTIONAL)	Brief description of the outcomes in terms of clones generated/establishment approach/screening outcomes	MHHi001-A-16/MHHi001-A-17 originate from the same experiment MHHi001-A-14 originates from independent experiment	N/A
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

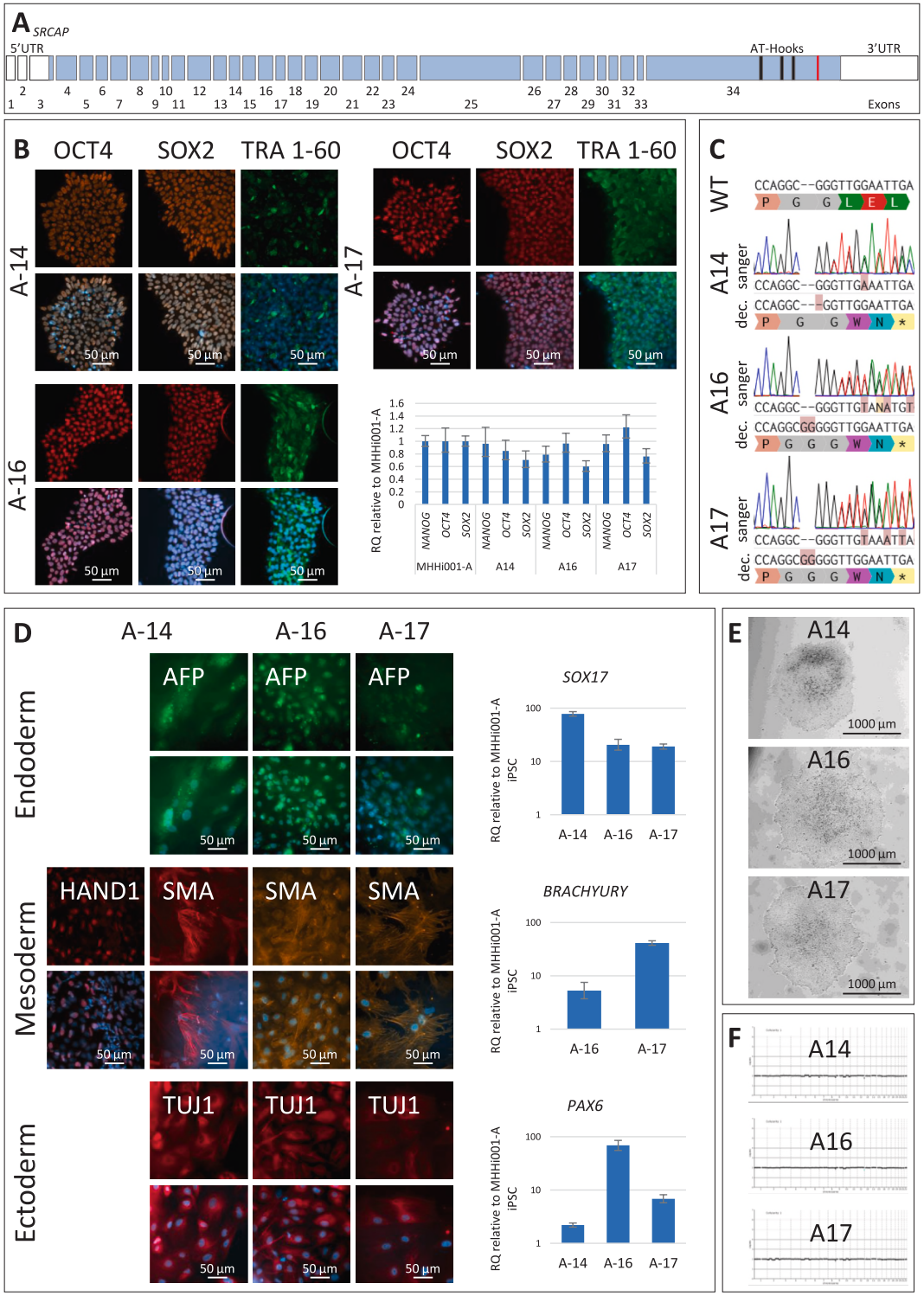

Fig. 1. .

30 µm cell strainer. Electroporation was done on a Neon transfection device (Thermo Fisher), using a 10 µl transfection kit. For each electroporation 0.55 µl crRNA (diluted to 100 µM), 0.55 µl tracrRNA (diluted to 100 µM) were mixed with 0.48 µl Nuclease-Free Duplex buffer (IDT). 0.5 µl of the former mix and 0.5 µl Cas9 (diluted to 36 µM) were used to form the RNP complex and electroporated with the following parameter settings: 1200 V/2pulses/30 ms or 1400 V/4pulses/5ms, into 1 × 10^5 cells. For further details see the original protocol (https://sfvideo.blob.core.windows.net/sitefinity/docs/default-source/user-submitted-method/crispr-cas9-rnp-delivery-ips-cell-electroporation-nepagene.pdf?sfvrsn=47980e07_8). Cells were seeded onto Matrigel-coated plates with mTESR™1 containing 1:1000 ROCK-inhibitor (BD biosciences) for 24 h, afterwards Medium without ROCK was used.

4.3. Mutant screening

DNA was extracted from individual colonies using 10 µl QuickExtract (Lucigen) (incubation 15 min/65 °C, 5 min/95 °C) and the target region

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit Anti-OCT4	1:500	Abcam ab19857 RRID: AB_445175
	Rabbit Anti-SOX2		Abcam ab97959 RRID: AB_2341193
	Mouse Anti-TRA-1–60		Abcam ab16288 RRID: AB_778563
Differentiation Markers	Rabbit anti-TUJ1	1:500	Thermo fisher A25532 RRID: AB_2651003
	Mouse IgG1 anti-AFP	1:500	Thermo fisher A25530 RRID: AB_2651004
	Mouse IgG2a anti-SMA	1:200	Thermo fisher A25531 RRID: AB_2651005
	Rabbit anti-HAND1	1:50	Thermo fisher **PA5-145103** RRID: AB_3091969
Secondary antibodies	Goat Anti-Mouse IgM mu chain (Alexa Fluor® 488)	1:500	Abcam ab98674, RRID:AB_10674182
	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed	1:500	Abcam ab150084, RRID:AB_2734147
	Alexa Fluor™ 488 goat anti-mouse IgG1	1:250	Thermo fisher A25536, RRID: AB_2651011
	Alexa Fluor™ 647 donkey anti-rabbit	1:250	Thermo fisher A25537, RRID: AB_2857990
	Alexa Fluor™ 555 goat anti-mouse IgG2a	1:250	Thermo fisher A25533, RRID: AB_2651012
	Alexa Fluor™ 594 goat anti-mouse IgG2a	1:250	Thermo fisher A25534,RRID: AB_2868398
Nuclear stain	NucBlue™ (DAPI)	1 drop in 200 µl PBS	Thermo fisher R37606
Site-specific nuclease			
Nuclease	S. pyogenes Cas9	IDT Alt-R™ S.p. Cas9 Nuclease V3	
Delivery method	Electroporation MHHi001-A-14: 1400 V 4 pulses, 5 ms MHHi001-A-16/MHHi001-A-17: 1200 V 2 pulses, 30 ms	Invitrogen™ Neon™ Transfection System, Neon™ Transfection System 10 µL Kit	

Primers and Oligonucleotides used in this study

	Target	**Forward/Reverse primer (5′-3′)**
TaqMan Assays Pluripotency marker	*OCT4*	Thermo fisher Hs04260367_gH
	SOX2	Thermo fisher Hs01053049_s1
	NANOG	Thermo fisher Hs02387400_g1
TaqMan Assays Germ layer marker	*SOX17*	Thermo fisher Hs00751752_s1
	PAX6	Thermo fisher Hs01088114_m1
	BRACHYURY	Thermo fisher Hs00610080_m1
TaqMan Assays House-Keeping Genes	*GAPDH*	Thermo fisher Hs99999905_m1
	RPS29	Thermo fisher Hs03004310_g1
Genotyping Target Region	3_SRCAP_ex34_2_fwd	AGAATCCTCCATCACCTCGG
	4_SRCAP_ex34_2_rev	CTTCAGCCTCAGACTCCTCT
Plasmid integration	147_SeV_fwd	GGATCACTAGGTGATATCGAGC
	148_SeV_rev	ACCAGACAAGAGTTTAAGAGATATGTATC
	149_SeV-KOS_fwd	ATGCACCGCTACGACGTGAGCGC
	150_SeV-KOS_rev	ACCTTGACAATCCTGATGTGG
	151_SeV-Klf4_fwd	TTCCTGCATGCCAGAGGAGCCC
	152_SeV-Klf4_rev	AATGTATCGAAGGTGCTCAA
	153_SeV-cmyc_fwd	TTACTGACTAGCAGGCTTGTCG
	154_SeV-cmyc_rev	TCCACATACAGTCCTGGATGATGATG
gDNA target	gDNA target region with PAM in brackets used for crRNA design	GGTGTCAATTCCAACCCGCC (TGG)
Bioinformatic crRNA on– and −off-target binding prediction tool	CRISPOR, ccTOP	https://crispor.gi.ucsc.edu/crispor.py https://cctop.cos.uni-heidelberg.de:8043/
Primers for top off-target mutagenesis predicted site sequencing	OT1	
	11_exon_FAT2_F	TTAGAACCACCACCCACTTCC
	12_exon_FAT2_R	TGCCTAGTCACTCCCGAGAT
	OT2	
	13_inter_TTC28_F	CCTCTGAAGGCTATGGACAAAGT
	14_inter_TTC28_R	TCTTCTAAAACCCTGCTGCTTCA
	OT3	
	15_intron_CMTM7_F	GAATGGCAAAGCTCAGGCTCA
	16_intron_CMTM7_R	TACCTTGATCCAGCCATCAGC
	OT4	
	17_ESRRG_F	CTTGAGACACAGGGATGGCT
	18_ESRRG_R	GTCAGAATGGTCCGAGTGCT
	OT5	
	21_RP4-587D13.1_F	ACACACTCACCGGGTGTTC
	22_RP4-587D13.1_R	GGGGAGTCCTTGTACAGACAAAT

analyzed by PCR on a Doppio Thermocycler (VWR) using HS Taq Mastermix (Biozym) (parameters: 5 min 95 °C, 30x (30 s 95 °C, 30 s 58 °C, 1 min 72 °C) 5 min 72 °C.) and Sanger sequencing. Colonies with mutations were passaged and sequenced repeatedly. TIDE was used for mutation detection. Allelic deconvolution was performed with Indigo and TIDE.

4.4. EB differentiation

IPSC colonies were cultivated in differentiation medium (DMEM, 20 % FBS, 20 % KnockOut™ Serum Replacement (Gibco), 1:1000 ROCK) on low adhesion plates for 14 days, with partial medium change every 3 days. EBs were used for RNA extraction or seeded onto Matrigel coated coverslips and cultivated for 3 days before immunofluorescence analysis.

4.5. Staining/microscopy

iPSCs (passage 6) were grown on coverslips coated with Matrigel, fixed for 1 h with 4% PFA, permeabilized and blocked in PBS (with 1% Triton X100, 3% BSA) for 1 h at RT, stained with primary antibodies against undifferentiated state markers over night at 4 °C and secondary antibody for 1 h at RT. Nuclear staining was done with Nucblue DAPI (Thermo fisher) in PBS for 5 min. Between steps, samples were washed 3x with PBS. Staining for germ layer markers was done with the "3-Germ Layer Immunocytochemistry Kit" (Thermo fisher) according to manuscript (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0010484_3-GermLayerImmunocytochemKit_UG.pdf), The mesodermal marker HAND1 was stained according to protocol used for the undifferentiated state markers. All samples were mounted using ProLong™ Diamond mounting medium (Thermo fisher) and analysed with a Zeiss Axioimager M2 microscope.

4.6. RNA extraction, cDNA synthesis, qPCR (passage 6)

RNA was isolated with TriFast (VWR) according to the manufacturer's guideline, followed by DNAse (Thermo Fisher) digestion. Superscript IV (Thermo Fisher) with random hexamer primers (Invitrogen) was used to generate cDNA. TaqMan assays were performed in 3 technical replicates on QuantStudio 7 Flex (Thermo Fisher). Gene expression was quantified relative to the parental cell line and normalized against housekeeping genes, using the Design&Analysis Software2.6 (Thermo Fisher).

4.7. DNA extraction/lcWGS

Cells (MHHi001-A-14: passage 10, MHHi001-A-16/MHHi001-A-17: passage 15) were lysed in 1 ml TE-buffer containing 10 % SDS, 5 µg RNase, 100 µg ProteinaseK overnight. A sodium perchlorate/chloroform/isoamylalcohol (24:1) protocol was used to isolate DNA. DNA was precipitated (ethanol), washed and diluted (TE-buffer).

Sequencing libraries were prepared with the NEBNext_Ultra_II_FS_DNA kit (NEB) and lcWGS was performed on a NextSeq550 (Illumina). Data was analyzed using using the ACE (Poell et al., 2019) and QDNAseq.hg38 (https://github.com/asntech/QDNAseq.hg38) R packages.

4.8. Mycoplasma test (passage 6, A14 in passage 17)

The MycoSPY Kit (Sysmex) was used according to protocol.

CRediT authorship contribution statement

Johannes Rhode: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Lisa Hagenau:** Writing – review & editing, Supervision, Software, Formal analysis, Data curation, Conceptualization. **Stephanie Edwards:** Resources, Methodology. **Falk F.R. Buettner:** Writing – review & editing, Resources, Conceptualization. **Ana Tzvetkova:** Writing – review & editing, Formal analysis. **Lars R. Jensen:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Andreas W. Kuss:** Writing – review & editing, Supervision, Funding acquisition, 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.

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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.2025.103847.

Data availability

Data will be made available on request.

References

Brinkman, E.K., et al., 2014. Easy quantitative assessment of genome editing by sequence trace decomposition. Nucleic Acids Res. 42 (22), e168. https://doi.org/10.1093/nar/gku936. Available at:

Concordet, J.-P., Haeussler, M., 2018. CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. Nucleic Acids Res. 46 (W1), W242–W245. https://doi.org/10.1093/nar/gky354. Available at:

Edwards, S., et al., 2024. Generation of two isogenic iPSC lines from a healthy male donor of European ancestry. Stem Cell Res. 77, 103403. https://doi.org/10.1016/j.scr.2024.103403. Available at:

Haase, A., Göhring, G., Martin, U., 2017. Generation of non-transgenic iPS cells from human cord blood CD34 + cells under animal component-free conditions. Stem Cell Res. 21, 71–73. https://doi.org/10.1016/j.scr.2017.03.022. Available at:

Poell, J.B. *et al.* (2019) 'ACE: absolute copy number estimation from low-coverage whole-genome sequencing data', *Bioinformatics*. Edited by I. Birol, 35(16), pp. 2847–2849. Available at: doi:10.1093/bioinformatics/bty1055.

Rausch, T., et al., 2020. Tracy: basecalling, alignment, assembly and deconvolution of sanger chromatogram trace files. BMC Genomics 21 (1), 230. https://doi.org/10.1186/s12864-020-6635-8. Available at:

Rhode, J., et al., 2023. 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. Stem Cell Res. 73, 103249. https://doi.org/10.1016/j.scr.2023.103249. Available at:

Rots, D., et al., 2021. Truncating SRCAP variants outside the Floating-Harbor syndrome locus cause a distinct neurodevelopmental disorder with a specific DNA methylation signature. Am. J. Hum. Genet. 108 (6), 1053–1068. https://doi.org/10.1016/j.ajhg.2021.04.008. Available at:

Schwarz, J.M., et al., 2014. MutationTaster2: mutation prediction for the deep-sequencing age. Nat. Methods 11 (4), 361–362. https://doi.org/10.1038/nmeth.2890.

Stemmer, M., et al., 2015. CCTop: an intuitive, flexible and reliable CRISPR/Cas9 target prediction tool. PLoS One 10 (4), 1–11. https://doi.org/10.1371/journal.pone.0124633. Available at:

Ye, J., et al., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinf. 13, 134. https://doi.org/10.1186/1471-2105-13-134.