Stem Cell Research 76 (2024) 103327

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Genetically-Modified Single Cell Line

Generation of human induced pluripotent stem cell line derived from Becker muscular dystrophy patient with CRISPR/Cas9-mediated correction of *DMD* gene mutation

Marta Przymuszała [a,b], Alicja Martyniak [a,b], Joanna Kwiatkowska [c], Jarosław Meyer-Szary [c], Karolina Śledzińska [d], Jolanta Wierzba [d], Józef Dulak [a,*,1], Urszula Florczyk-Soluch [a,1], Jacek Stępniewski [a,1]

[a] Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland
[b] Doctoral School of Exact and Natural Sciences, Jagiellonian University, Prof. St. Łojasiewicz 11, 30-348 Krakow, Poland
[c] Department of Paediatric Cardiology and Congenital Heart Defects, Medical University of Gdańsk, Poland
[d] Department of Paediatrics, Haematology and Oncology, Medical University of Gdańsk, Poland

A R T I C L E I N F O

Keywords:
Becker muscular dystrophy, CRISPR
Cas9 technology

A B S T R A C T

Becker muscular dystrophy (BMD) is an X-linked recessive disorder caused by in-frame deletions in the dystrophin gene (*DMD*), leading to progressive muscle degeneration and weakness. We generated a human induced pluripotent stem cell (hiPSC) line from a BMD patient. BMD hiPSCs were then engineered by CRISPR/Cas9-mediated knock-in of missing exons 3–9 of *DMD* gene. Obtained hiPSC line may be a valuable tool for investigating the mechanisms underlying BMD pathogenesis.

1. Resource Table

Unique stem cell line identifier	DMBi009-A (https://hpscreg.eu/cell-line/DMBi009-A) DMBi009-A-1 (https://hpscreg.eu/cell-line/DMBi009-A-1)
Alternative name(s) of stem cell line	BMD (DMBi009-A) repaired BMD (DMBi009-A-1)
Institution	Department of Medical Biotechnology, Jagiellonian University, Kraków, Poland
Contact information of the reported cell line distributor	Prof. Józef Dulak, jozef.dulak@uj.edu.pl
Type of cell line	hiPSC
Origin	Human
Additional origin info *(applicable for human ESC or iPSC)*	Age: 20 Sex: Male Ethnicity: Caucasian
Cell Source	Blood, 200 000 of total PBMCs BMD cell line: DMBi009-A (https://hpscreg.eu/cell-line/DMBi009-A)
Method of reprogramming	CytoTune-iPSC 2.0 Sendai Reprogramming Kit (Thermo Fisher)
Clonality	Clonal
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
The cell culture system used	Essential 8 medium (Thermo Fisher Scientific) Geltrex™ (Thermo Fisher Scientific) culture in 37 ∘C, 5 % CO2, 20 % O2; cell passage with EDTA (0,5 mM) every 3–4 days with ROCK inhibitor Y27623 (10 μM, (Med Chem Express)
Type of the Genetic Modification	*DMD* gene repair - knock-in of missing exons 3–9 of *DMD* gene
Associated disease	Becker muscular dystrophy (OMIM: 300376)
Gene/locus modified in the reported transgenic line	*DMD*, Xp21.2-p21.1 (Gene ID: 1756)
Method of modification / user-customisable nucleases (UCN) used, the resource used for design optimisation	Homology direct repair: CRISPR/Cas9

(continued on next page)

* Corresponding author.
E-mail address: jozef.dulak@uj.edu.pl (J. Dulak).
[1] Equally contributed as senior authors.

https://doi.org/10.1016/j.scr.2024.103327
Received 18 December 2023; Accepted 31 January 2024
Available online 3 February 2024
1873-5061/© 2024 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

(*continued*)

User-customisable nuclease (UCN) delivery method	Plasmid transfection (Human Stem Cell Nucleofector Kit I), B016 program on Amaxa Nucleofector 2b device (Lonza)
All double-stranded DNA genetic material molecules introduced into the cells	- Cas9 plasmid pSpCas9(BB)-2A-Puro (PX459) V2.0 with 5′sgRNA (Addgene #62988) pcDNA3.1/Hygro(+) with HDR repair template (Invitrogen #V87020)
Evidence of the absence of random integration of any plasmids or DS DNA introduced into the cells.	Confirmation of Cas9 absence - PCR-based detection of Cas9 nuclease in generated hiPSC clones.
Analysis of the nuclease-targeted allele status	Sequencing of the targeted allele
Homozygous allele status validation	Sequencing of the targeted allele
Method of the off-target nuclease activity prediction and surveillance	Top five off-target hits analyzed by PCR and Sanger sequencing
Descriptive name of the transgene	N/A
Eukaryotic selective agent resistance cassettes (including inducible, gene/cell type-specific)	Positive (puromycin)
Inducible/constitutive expression system details	N/A
Date archived/stock creation date	08.12.23
Cell line repository/bank	DMBi009-A (https://hpscreg.eu/cell-line/DMBi009-A) DMBi009-A-1 (https://hpscreg.eu/cell-line/DMBi009-A-1)
Ethical/GMO work approvals	Gdańsk Medical University Bioethical Committee approval No NKBBN/402/2020 Agreement of the Ministry of the Environment for the use of GMO/GMM (decision 41/2016)
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	- Cas9 plasmid pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene #62988) pcDNA3.1/Hygro(+) (Invitrogen #V87020)

(*continued on next column*)

(*continued*)

2. Resource utility

hiPSC line generated from a BMD patient-derived PBMCs, and modified by CRISPR/Cas9-mediated knock-in of missing exons 3–9 of *DMD* gene (isogenic repaired BMD), serves as an *in vitro* model for studying the mechanisms underlying BMD pathology. BMD and repaired isogenic BMD iPSC lines upon differentiation into cardiomyocytes (CMs) may provide a valuable tool to investigate the molecular mechanisms of dystrophic cardiomyopathy (Table 1).

Table 1
Characterization and validation.

Classification (optional italicized)	**Output type**	**Result**	**Data**
Schematic of a transgene/genetic modification	Schematic illustrating the structure and location of the introduced genetic modification	A visual representation of edited allele, showing exon structure	Fig. 1 panel A
Morphology	Photography	Normal	Fig. 1 panel C
Pluripotency status evidence for the described cell line	Qualitative analysis (Immunocytochemistry)	Assessed staining of pluripotency markers: OCT4 presence in the nuclei, SSEA4, TRA-1–60 present on the cytoplasmic membrane	Fig. 1 panel G
	Quantitative analysis (Flow cytometry)	Assess % of positive cells for cell surface markers and transcription factors: OCT3/4: 98.1 % (repaired), 99 % (BMD); NANOG: 82.6 % (repaired), 96.8 % (BMD); SSEA-4: 99.1 % (repaired), 99.8 % (BMD)	Fig. 1 panel F Supplementary Fig. 6
Karyotype	Karyotype (G-banding)	46XY, Resolution 450–500	Fig. 1 panel E
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site	DMD exons 3–9 knock-in	Fig. 1 panel B, Supplementary Fig. 1
	Evaluation of the - (homo-/hetero-/hemi-) zygous status of introduced genomic alteration(s)	N/A	N/A
	Transgene-specific PCR (when applicable)	N/A	N/A
Verification of the absence of random plasmid integration events	PCR	Absence of Cas9 was verified in PCR using primers recognizing Cas9 coding sequence.	Supplementary Fig. 3
Parental and modified cell line genetic identity evidence	STR analysis	DNA Profiling	N/A
		amelogenin, D5S818, D13S317, D7S820, D16S539, CSF1PO, TPOX, TH01, vWA, D21S11, matched with parental cell line	STR analysis excel file
Mutagenesis / genetic modification outcome analysis	Sequencing (genomic DNA PCR)	DMD exons 3–9 knock-in (331–1197 bases of the chromosome X annotated NM_004006.3 are inserted)	Supplementary Fig. 1
	PCR	Detection of correctly targeted construct status	Fig. 1 panel B
	RT-PCR	Validation of presence of missing exons 3–9 in iPSC-derived cardiomyocytes	Fig. 1 panel D
Off-target nuclease activity analysis	Sanger sequencing	Sequences of 5 selected off-target sites for 5′sgRNA1 - lack of mutations introduced by unspecific activity	Supplementary fig. 2
Specific pathogen-free status	Mycoplasma, PCR	Negative	Supplementary fig. 5
Multilineage differentiation potential	Embryoid body formation	Demonstrated ability to differentiate into derivatives of 3 germ layers: ectoderm (neurofilament H); mesoderm (GATA4); endoderm (vimentin)	Fig. 1 panel H
List of recommended germ layer markers	Expression of markers demonstrated at protein (IF) levels	Ectoderm: neurofilament H Endoderm: vimentin Mesoderm: GATA4	Fig. 1 panel H
Outcomes of gene editing experiment (OPTIONAL)	Brief description of the outcomes in terms of clones generated/establishment approach/screening outcomes	N/A	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

3. Resource Details

BMD is caused by the X-linked in-frame mutations in the dystrophin gene (*DMD*), leading to the expression of an internally truncated protein with partially preserved function (Florczyk-Soluch et al., 2021) Clinical variability ranges from asymptomatic cases to severe early-onset muscle weakness (McCormack et al., 2023). Notably, cardiomyopathy stands as a leading cause of mortality in BMD. Thus, exploring the mechanisms of BMD-related cardiomyopathy is pivotal for understanding BMD pathophysiology and future therapeutic approaches (Connuck et al., 2008).

To create a human *in vitro* BMD model, a hiPSC line was generated by Sendai reprogramming of BMD patient-derived PBMCs. *DMD* mutation was corrected using CRISPR/Cas9 technology. To precisely target and correct a mutated region within the *DMD* gene we transfected cells with Cas9 plasmid pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene #62988) containing a 5′ single guide RNA (sgRNA) designed by CHOPCHOP software and plasmid pcDNA3.1/Hygro(+) (Invitrogen #V87020), a donor template containing the desired exons 3–9, flanked by 5′ and 3′ ends that shared homology with the genomic locus (Fig. 1A).

Corrected iPSC colonies were validated by PCR analysis (BMD dystrophin DNA product: 612 bp, expected band length of 1479 bp) (Fig. 1B) that revealed the addition of exon 3 – 9 coding sequence to the exon 2 of *DMD* gene (NCBI Entry NG_012232.1, repair template introduced between nucleotide 324,471 and 324,472). Sanger sequencing further confirmed the successful repair (Supplementary Fig. 1). No off-target effect of the used sgRNAs was observed in five selected potential off-target genomic sites as confirmed by DNA sequencing (Supplementary Fig. 2). hiPSC lines were free from the random Cas9 integration (Supplementary Fig. 3).

The authenticity of the repaired cell line was confirmed by short tandem repeat (STR) analysis, demonstrating identity with the parental hiPSCs (STR analysis). Additionally, both lines were free from reprogramming Sendai vector genome expression (Supplementary Fig. 4) and showed no *Mycoplasma* contamination (Supplementary Fig. 5). Notably, in cardiomyocytes differentiated from isogenic repaired BMD hiPSCs, we detected the transcript with the presence of exons 3–9 (Fig. 1C).

The repaired isogenic BMD as well as BMD hiPSC line displayed the typical morphology of hiPSCs (Fig. 1C). Notably, in cardiomyocytes differentiated from isogenic repaired BMD hiPSCs, we detected the transcript with the presence of exons 3–9 (Fig. 1D). The normal karyotypes of the BMD and corrected cells have been confirmed by G-banding method (Fig. 1E) Both lines expressed pluripotency markers such as OCT4, SSEA4, and TRA-1–60 as confirmed by flow cytometry (Fig. 1F, Supplementary Fig. 6) and immunocytochemistry (Fig. 1 G). We assessed the iPSCs' capacity to differentiate into the three germ layers using specific markers such as vimentin, GATA4, and neurofilament H (Fig. 1H).

In conclusion, we generated and characterized isogenic repaired BMD hiPSC line derived from BMD patient. An isogenic pair, repaired BMD and BMD hiPSC lines may serve as a valuable tool for BMD research and BMD-associated cardiomyopathy.

4. Materials and Methods

4.1. Reprogramming

Sendai vectors (CytoTune-iPS 2.0 Sendai Reprogramming Kit, Thermo Fisher Scientific) were used to transduce 200,000 PBMCs in StemPro®-34 SFM medium (Thermo Fisher Scientific). After 24 h the medium was changed. Three days after transduction, the PBMCs were seeded onto Geltrex™ (Thermo Fisher Scientific) -coated wells. Starting from day 7, the cell culture was maintained in Essential 8™ (E8) medium (Thermo Fisher Scientific). Obtained colonies (around day 15) were transferred to new wells of 48 well plate.

4.2. hiPSC Culture

hiPSCs were cultured on Geltrex™-coated plates in E8 medium with daily medium changes. Passaging was performed at 70–90 % confluency using 0.5 mM EDTA. After each passage, cells were cultured in E8 medium supplemented with 10 μM Y-27632 (Med Chem Express) for 24 h.

4.3. Cardiac differentiation

The differentiation of hiPSCs into cardiomyocytes was performed as described by us previously (Martyniak et al., 2021). In brief, hiPSCs (3 × 10^4) were cultured in E8 medium until reaching 90 % confluency. On day 0, cells were stimulated for 24 h with the GSK3 inhibitor CHIR99021 (8–12 μM, Sigma-Aldrich) in RPMI-1640 medium with B-27 Supplement Minus Insulin (Thermo Fisher Scientific). On day 3, cells were treated with Wnt pathway inhibitor IWR-1 (5–7 μM) for two days. Starting from day 7, cells were cultured in RPMI-1640 medium (Sigma-Aldrich) with B-27 supplement with insulin (Thermo Fisher Scientific). To improve cardiomyocyte purity and differentiation yield, metabolic selection was performed from day 10 to day 16 in glucose-depleted RPMI medium supplemented (Thermo Fisher Scientific) with 4 mM sodium DL-lactate (Sigma-Aldrch). Subsequently, cells were harvested, reseeded on Geltrex™-coated wells in RPMI medium with B-27, and used for further analysis.

4.4. Genome Editing

Patient (hiPSCs) were subjected to genome editing through homology-directed repair (HDR).Co-nucleofection of Cas9, 5′ sgRNA, and repair template (pcDNA3.1/Hygro(+))was performed using Human Stem Cell Nucleofector™ Kit 1 (Lonza) and Amaxa Nucleofector 2B (program B16). Single-cell-derived clones were obtained after puromycin selection and genotyped via PCR flanking the DMD introduced fragment (Table 2).

4.5. Mutation Validation

Genetic modifications were confirmed through DNA sequencing of the DMD exons 3–9 locus (Genomed). Specific primers were used for PCR amplification (Table 2) and DNA was recovered using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research).

4.6. In vitro spontaneous differentiation of iPSCs

5,000 hiPSCs were seeded in U-shaped 96-well plates with Essential 6 (E6) medium (Thermo Fisher Scientific) and cultured for 7 days. Embryoid bodies were transferred to Geltrex™-coated wells for spontaneous differentiation.

4.7. Immunocytochemistry

Cells were fixed with 4 % paraformaldehyde, permeabilized with 0.1 % Triton X-100, and blocked with 3 % BSA. Primary antibodies were applied for overnight incubation at 4 °C, followed by secondary antibodies (Table 2) and Hoechst 33342 staining. Imaging was performed using a fluorescent microscope Leica DMi8.

4.8. Flow Cytometry

Cells were fixed with 4 % paraformaldehyde, permeabilized with 0.1 % Triton X-100, and blocked with 3 % BSA. Primary antibodies against pluripotency markers were applied, followed by secondary antibodies (Table 2) and Hoechst 33342 staining. Flow cytometry was conducted using the LS Fortessa flow cytometer with FACS Diva software (BD Biosciences).

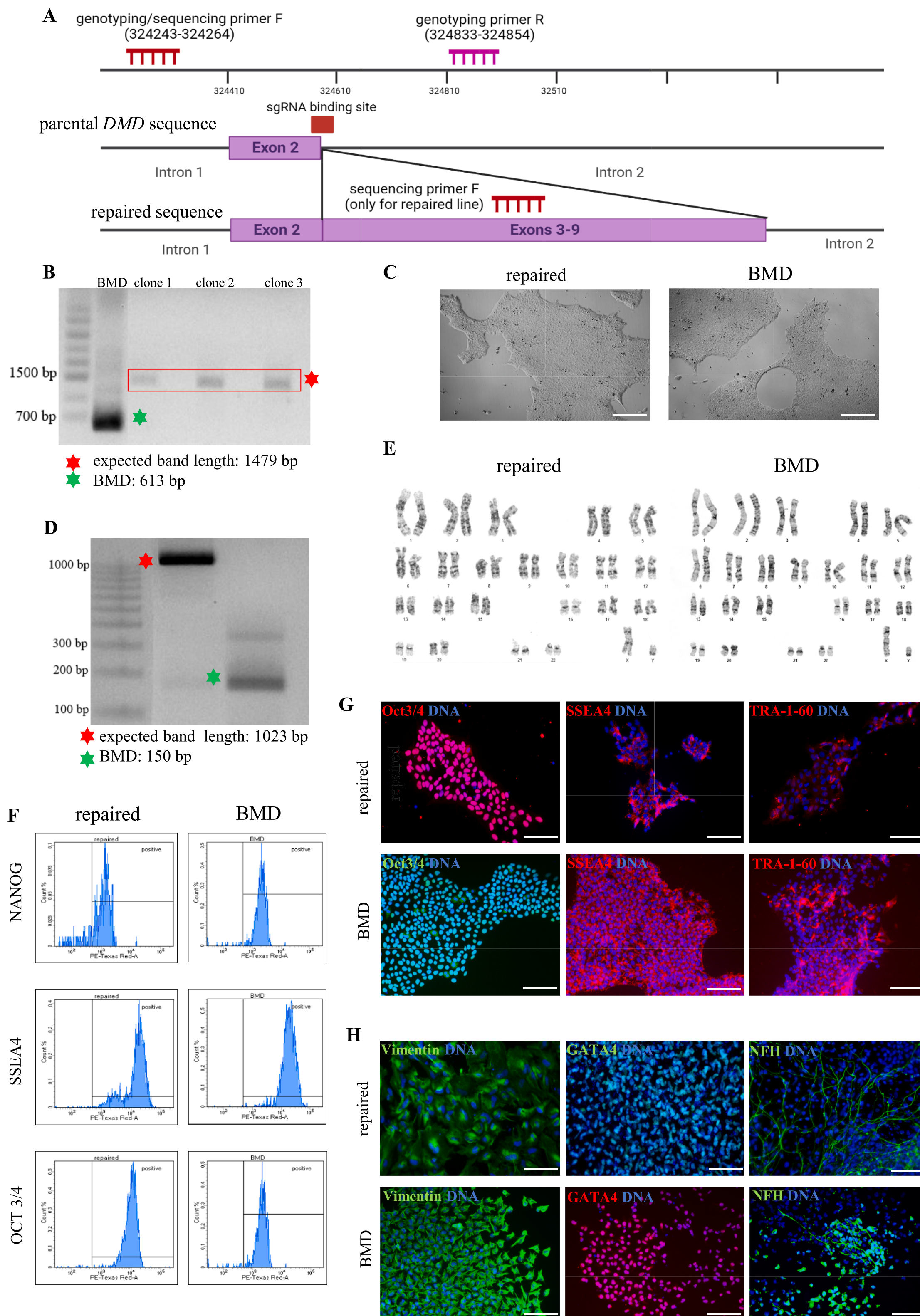

Fig. 1. Characterization of repaired and BMD hiPSC lines. (A) Gene correction strategy. The overview of our CRISPR/HDR targeting strategy, emphasizing the sgRNA binding site (in red) and the primers' strategic placement for efficient genotyping and sequencing. Additionally, contrasts the repaired sequence with the BMD-associated sequence, highlighting the specific location of the repair template insertion. (B) PCR-based genotyping of *DMD* exons 3–9 in repaired clones. (C) Morphology of the hiPSC-colonies. Scale bar:100 µm. (D) Confirmation of knock-in exons 3–9 on RNA level. (E). Karyotypic analysis of repaired and BMD hiPSC lines. (F) Flow cytometry analysis of TRA-1–60, SSEA4, OCT3/4, and NANOG. (G) Immunocytochemistry staining for pluripotency markers OCT3/4, SSEA4, and TRA-1–60. Scale bar:100 µm. (H) Immunofluorescent staining for endo-, ecto- and mesoderm markers after spontaneous differentiation. Scale bar:100 µm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry	*Antibody*	*Dilution*	*Company Cat # and RRID*
Pluripotency Markers	mouse anti-OCT3/4 goat anti-OCT3/4 mouse anti-NANOG mouse anti-SSEA-4 mouse anti-TRA1-60	1:200 IF 1:200 IF 1:100 FC 1:200 IF 1:50 FC 1:200 IF 1:50 FC 1:200 IF 1:50 FC	Santa Cruz Biotechnology Cat# sc-5279, RRID: AB_628051 Santa Cruz Biotechnology Cat# sc-8628, RRID: AB_653551 Santa Cruz Biotechnology Cat# sc-293121, RRID: AB_2665475 Santa Cruz Biotechnology Cat# sc-21704, RRID: AB_628289 Millipore Cat# MAB4360, RRID:AB_2119183
Differentiation Markers	rabbit anti-vimentin mouse anti-GATA4 rabbit anti-NFH	1:2000 1:200 1:200	Abcam Cat# ab92547,RRID:AB_10562134 Santa Cruz Biotechnology Cat# sc-25310, RRID: AB_627667 Abcam Cat# ab8135, RRID:AB_306298
Secondary antibodies	goat anti-rabbit IgG AF488 donkey anti-mouse IgG AF568 donkey anti-goat IgG AF568	1:400 IF 1:400 IF 1:500 FC 1:400 IF 1:500 FC	Thermo Fisher Scientific Cat# A-11034, RRID: AB_2576217Thermo Fisher Scientific Cat# A-11037, RRID:AB_2534095 Thermo Fisher Scientific Cat# A-11057, RRID: AB_2534104
Nuclear stain	Hoechst33342	1 μg/mL	Sigma-Aldrich
Site-specific nuclease			
Nuclease information	Cas9	Cas9 plasmids pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene #62988)	
Delivery method	Plasmid nucleofection	Human Stem Cell Nucleofector™ Kit 1 (Lonza) and Amaxa Nucleofector 2B (program B16)	
Selection/enrichment strategy	Puromycin	0,3 μg/ml, 24 h after nucleofection, for 24 h	

Primers and Oligonucleotides used in this study	**Target**	**Forward/Reverse primer (5′-3′)**
Episomal Plasmids (qPCR or RT-PCR)	e.g. OCT4 Plasmid	N/A
Pluripotency Markers	e.g. NANOG, etc.	N/A
House-Keeping Genes (PCR)	EEF2	F: TCAGCACACTGGCATAGAGG R: GACATCACCAAGGGTGTGC Supplementary Fig. 4
Potential random integration detection	Cas9 (PCR)	F: CATCGAGCAGATCAGCGAGT R: CGATCCGTGTCTCGTACAGG Supplementary Fig. 3
Demonstration of the absence of reprogramming vectors expression (RT-PCR)	Sendai Vectors	F: GGATCACTAGGTGATATCGAGC R: ACCAGACAAGAGTTTAAGAGATATGTATC Supplementary Fig. 4
Specific pathogen-free status (PCR)	Mycoplasma spp.	F: ACTCCTACGGGAGGCAGCAGTA R: TGCACCATCTGTCACTCTGTTAACCTC Supplementary Fig. 5
Genotyping (desired allele/transgene presence detection)	DMD exons 3–9	F: GGATGCCCCAAACCAGCATCAC R:CTTGGTAAAGACCAAGAGGGAC Fig. 1 panel B
Targeted mutation analysis/sequencing	Sequencing data from both alleles	F:GGATGCCCCAAACCAGCATCAC (repaired and BMD) F: CCAACAGTGAAAAGATTCTCC (only repaired)Sanger sequencing chromatograms (Supplementary Fig. 1)
Genetic modification outcome analysis	Validation of presence of missing exons 3–9	F: GCTTTGGTGGGAAGAAGTAGAGGACTGT R: GTCCAGGTTTACTTCACTCTCCAT Fig. 1 panel D
sgRNA oligonucleotide	5′ACAATTTTCTAAGGTAAGAA 3′	324459–324481 (reference ID: NG_012232.1)PAM: TGG
Genomic target sequence(s)	Including PAM and other sequences likely to affect UCN activity	N/A
Bioinformatic gRNA on- and -off-target binding prediction tool used, specific sequence/outputs link(s)	CHOPCHOP	https://chopchop.cbu.uib.no/
Primers for top off-target mutagenesis predicted site sequencing (for all CRISPR/Cas9, ZFN and TALENs)	Off-target 1: chr1:30170238 Off-target 2: chr10:63607223 Off-target 3: chr11:6338745 Off-target 4: chr12:11016229 Off-target 5: chr8:120702907	F: GAGACATGGGCACCTTGGATGG R: GGCACTATCTCACTGTGGCTATC F: GCCATACTGCATACTTGCAATC R: CAAACTACAGGTATGGCCACAGG F: ACTGACATTGTTCCTCCTAACAC R: GGTTCTATGCAGGTTTCTTCAC F: CAATCCTTCACTTAGGAGGTATG R: CCTATAACCAATATGCGAGTCC F: GATATCAACAGCATCAGGACC R: GATTGGTTATGAGACTGCTCC Supplementary Fig. 2
ODNs/plasmids/RNA molecules used as templates for HDR-mediated site-directed mutagenesis.	Backbone modifications in utilized ODNS have to be noted using standard nomenclature.	N/A

4.9. RT-PCR/PCR

Total RNA isolation was done using fenozol (A&A Biotechnology) and chloroform. Reverse transcription was done with 1 μg of RNA, oligodT primers (10 μM) and RevertAid reverse transcriptase (200 U/ μL, Thermo Fisher Scientific) and carried out in ProFlex PCR System (Thermo Fisher Scientific). Genomic DNA was isolated using Genomic Mini kit (A&A Biotechnologies). For each PCR reaction, 50 ng of cDNA, specific primers (Table 2) and KAPA2G Fast Genotyping Mix (Merck). The reaction was carried out in ProFlex PCR System (Thermo Fisher Scientific). The amplified products were electrophoretically separated in 2 % agarose gel.

4.10. STR Analysis

STR analysis was conducted on genomic DNA isolated from repaired isogenic BMD and BMD hiPSC lines by Human Genome Variation Research Group (Małopolska Centre of Biotechnology, Krakow, Poland).

4.11. Karyotype

Karyotyping was performed using the GTG-450 G-banding method (GTG-450, 15 mitoses/sample) by Kariogen cytogenic laboratory, Krakow, Poland). Karyotype analyses were performed on iPSC lines at passage numbers: 28 (BMD) and 102 (repaired).

4.12. Mycoplasma Detection

Mycoplasma contamination was verified by PCR (KAPA2G Fast Genotyping Mix (Merck)) for Mycoplasma spp. DNA (Table 2).

CRediT authorship contribution statement

Marta Przymuszała: Data curation, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft. **Alicja Martyniak:** Investigation. **Joanna Kwiatkowska:** Methodology, Validation. **Jarosław Meyer-Szary:** Methodology, Validation. **Karolina Śledzińska:** Methodology, Validation. **Jolanta Wierzba:** Methodology, Validation. **Józef Dulak:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Urszula Florczyk-Soluch:** Data curation, Formal analysis, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jacek Stępniewski:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dulak reports financial support was provided by National Science Centre Poland. 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.

Acknowledgments

This work was supported by MAESTRO grant 2018/30/A/NZ3/00412 from the National Science Centre. to JD.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2024.103327.

References

Connuck, D.M., Sleeper, L.A., Colan, S.D., Cox, G.F., Towbin, J.A., Lowe, A.M., Wilkinson, J.D., Orav, E.J., Cuniberti, L., Salbert, B.A., Lipshultz, S.E., 2008. Characteristics and outcomes of cardiomyopathy in children with Duchenne or Becker muscular dystrophy: A comparative study from the Pediatric Cardiomyopathy Registry. Am. Heart J. 155 (6), 998–1005. https://doi.org/10.1016/j.ahj.2008.01.018.

Florczyk-Soluch, U., Polak, K., Dulak, J., 2021. The multifaceted view of heart problem in Duchenne muscular dystrophy. Cell. Mol. Life Sci. 78 (14), 5447–5468. https://doi.org/10.1007/s00018-021-03862-2.

Martyniak, A., Andrysiak, K., Motais, B., Coste, S., Podkalicka, P., Ferdek, P., Stępniewski, J., Dulak, J., 2021. Generation of microRNA-378a-deficient hiPSC as a novel tool to study its role in human cardiomyocytes. J. Mol. Cell. Cardiol. 160, 128–141. https://doi.org/10.1016/j.yjmcc.2021.07.007.

McCormack, N.M., Nguyen, N.Y., Tully, C.B., Oliver, T., Fiorillo, A.A., Heier, C.R., 2023. Vamorolone improves Becker muscular dystrophy and increases dystrophin protein in bmx model mice. iScience 26 (7), 107161. https://doi.org/10.1016/j.isci.2023.107161.