Stem Cell Research 83 (2025) 103660

Contents lists available at ScienceDirect

Stem Cell Research

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

Lab Resource: Single Cell Line

Generation of a human induced pluripotent stem cell line (BIHi292-A) from PBMCs of a female patient diagnosed with Nasu-Hakola disease (NHD)/ polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL) carrying a novel heterozygous mutation in the *TREM2* gene

Ria Göttert [a], Valeria Fernandez Vallone [b], Harald Stachelscheid [b], Jakob Johannes Metzger [c,d], Cassandra Carao Caedo [c,d], Maria Buthut [a,e], Harald Prüss [a,e], Matthias Endres [a,e], Simone Schilling [a,1], Karen Gertz [a,1,*]

[a] *Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Klinik für Neurologie und Abteilung für Experimentelle Neurologie, Charitéplatz 1, 10117 Berlin, Germany*
[b] *Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Core Unit pluripotent Stem Cells and Organoids, 13353 Berlin, Germany*
[c] *Max Delbrueck Center for Molecular Medicine (MDC), Berlin, Germany*
[d] *Berlin Institute for Medical Systems Biology (BIMSB), Berlin, Germany*
[e] *German Center for Neurodegenerative Diseases (DZNE) Berlin, Berlin, Germany*

A B S T R A C T

NHD/PLOSL is an orphan disease characterized by progressive presenile dementia associated with recurrent fractures due to polycystic bone lesions. In this study, we generated the human induced pluripotent stem cell (hiPSC) line BIHi292-A from a 30-year-old women diagnosed with NHD/PLOSL, carrying two compound heterozygous frameshift mutations [c.313del (p.Ala105fs) and c.199del (p.His67fs)] in the *TREM2* (triggering receptor expressed on myeloid cells 2) gene. BIHi292-A hiPSCs are karyotypically normal, express typical markers for the undifferentiated state and have pluripotent differentiation potential. BIHi292-A cells will provide a valuable tool for investigating pathogenic mechanisms of NHD/PLOSL and TREM2-related research questions.

1. Resource Table

Unique stem cell line identifier	BIHi292-A
Alternative name(s) of stem cell line	N/A
Institution	Charité – Universitätsmedizin Berlin and Berlin Institute of Health at Charité
Contact information of distributor	cusco-user@bih-charite.de
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 30 Sex: Female Ethnicity: Caucasian
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Sendai virus (CytoTune-iPS 2.0); Oct3/4, Sox2, c-Myc, Klf4
Genetic Modification	Yes
Type of Genetic Modification	Hereditary
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	Nasu-Hakola disease (NHD)/polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL)
Gene/locus	Gene: *TREM2* Locus: 6p21.1 Mutations: c.313del (p.Ala105fs), c.199del (p.His67fs)
Date archived/stock date	October 2023
Cell line repository/bank	https://hpscreg.eu/cell-line/BIHi292-A
Ethical approval	Ethics committee of Charité – Universitätsmedizin Berlin (EA4/211/21)

* Corresponding author.
[1] Equal contribution.

https://doi.org/10.1016/j.scr.2025.103660
Received 16 November 2024; Received in revised form 3 January 2025; Accepted 12 January 2025
Available online 15 January 2025

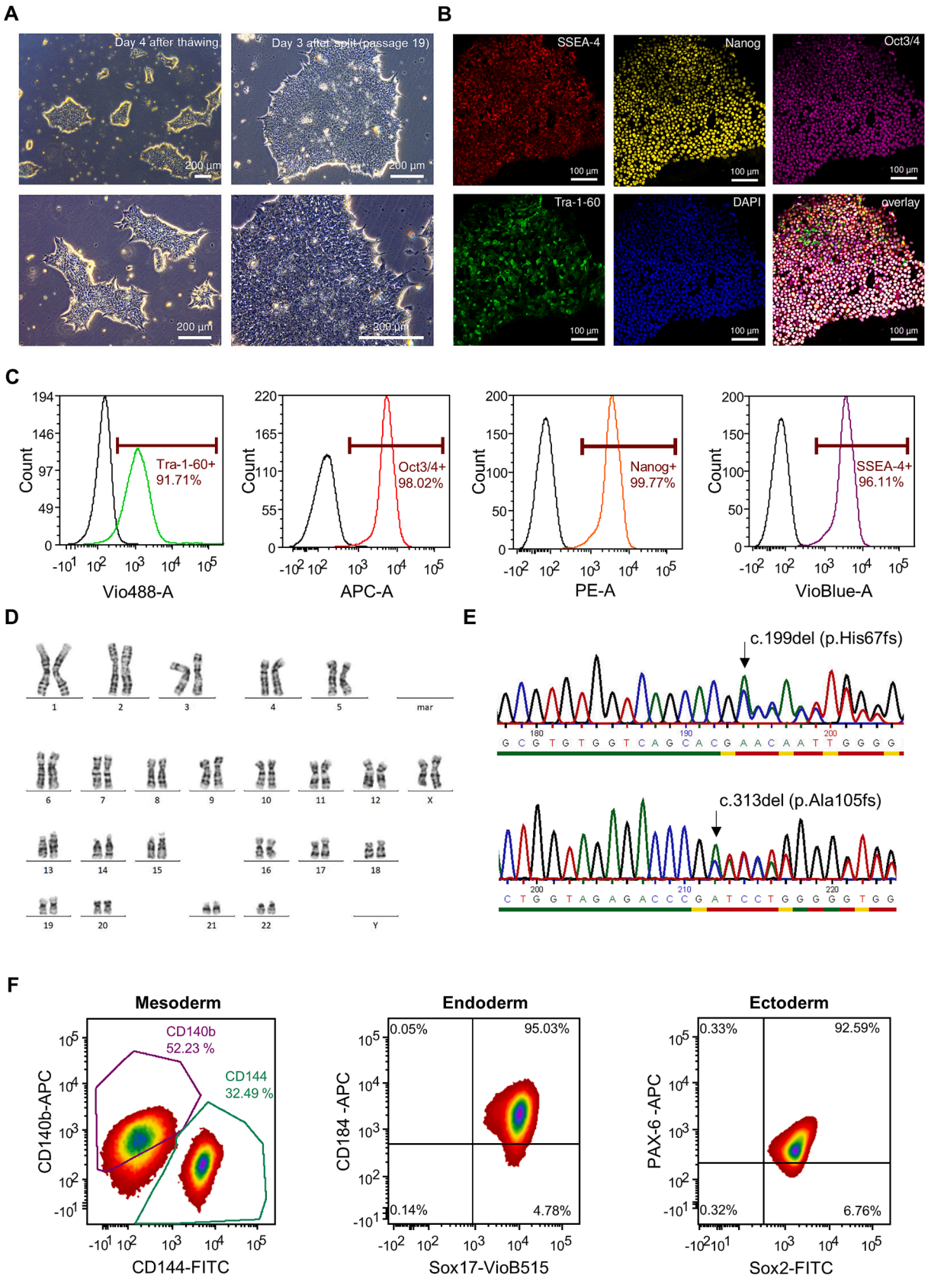

Fig. 1. Characterization of the hiPSC line BIHi292-A.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Bright field	Normal	Fig. 1 panel A
Phenotype	Qualitative analysis (Immunocytochemistry)	Cells are positive for Oct3/4, Nanog, Tra-1–60, and SSEA-4	Fig. 1 panel B
	Quantitative analysis (Flow cytometry)	Percentage (%) of cells positive for each marker: Tra-1–60: 91.7%, Oct3/4: 98%, Nanog: 99.8%, SSEA-4: 96.1%	Fig. 1 panel C
Genotype	Karyotype (G-banding) and higher resolution, array-based assay (SNP)	G-banding: normal karyotype (46,XX); SNP: compared to patients PBMCs, neither copy number variations > 2 Mb nor regions of loss of heterozygosity > 5 Mb were detected in BIHi292-A hiPSCs	G-banding: Fig. 1 panel D SNP: CNV report (Goettert, 2025)
mtDNA analysis (IF APPLICABLE)	Sanger Sequencing, NGS, Long-Read Sequencing and analysis software (Mitoverse, Mitopore, etc)	N/A	
Identity	Microsatellite PCR (mPCR) OR	N/A	
	STR analysis	Identical to cells of origin	Submitted in archive with journal
Mutation analysis	Sequencing	Two compound heterozygous mutations in *TREM2*	Fig. 1 panel E
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	qPCR, Negative	Suppl. Fig. 1B
Differentiation potential	Directed differentiation	Positive for: PAX-6 and Sox2 (Ectoderm), Sox17 and CD184 (Endoderm), CD140b and CD144 (Mesoderm)	Fig. 1 panel F
Donor screening (OPTIONAL)	HIV-1+2, hepatitis B, hepatitis C	Negative	Not shown but available with author
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

2. Resource utility

The combination of the two compound heterozygous *TREM2* mutations are disease-causing (Buthut et al., 2023). BIHi292-A hiPSCs will provide a versatile tool to study the unknown pathomechanism of NHD/PLOSL. Furthermore, BIHi292-A hiPSCs will enable research towards a better understanding of TREM2 in tissue macrophages during development, health and disease.

3. Resource details

Peripheral blood mononuclear cells (PBMCs) were isolated from HIV-1+2, hepatitis B and hepatitis C negative blood samples of the patient. PBMCs were reprogrammed using the Sendai virus reprogramming kit (CytoTune 2.0) including vectors for the human genes *Oct3/4*, *Sox2*, *c-Myc* and *Klf4*. The hiPSC line BIHi292-A derived from a single colony and shows the typical morphology of undifferentiated hiPSC when maintained in E8 medium (Fig. 1**A**). Absence of Sendai viral vectors were confirmed by PCR (**Suppl. Fig. 1A**). BIHi292-A hiPSCs express Oct3/4, SSEA-4, Nanog and Tra-1–60 as the typical markers for the undifferentiated state of hiPSCs, as shown using immunocytochemistry (Fig. 1**B**). Further flow cytometry confirmed the stemness marker expression in over 96% for Oct3/4, SSEA-4, Nanog, and Tra-1–60 expression in over 91% of BIHi292-A hiPSCs (Fig. 1**C**). G-banding karyotyping was performed on GTG (G-bands by trypsin using Giemsa) stained metaphase chromosomes and revealed a normal female karyotype 46,XX (Fig. 1**D**). Single nucleotide polymorphism analysis showed that the BIHi292-A hiPSC line did not harbor any copy number variations > 2 Mb or loss of heterozygosity regions > 5 Mb compared to the patient's PBMCs (Table 1). The results of the short tandem repeat (STR) analysis demonstrated that the genetic identity of the BIHi292-A cell line and the patient's PBMCs was identical (Table 1). In order to confirm the existence of the patient's mutations (Buthut et al., 2023), sequencing of BIHi292-A hiPSCs was done for the compound heterozygous mutations in exon 2 of the *TREM2* gene. Sanger sequencing confirmed the two *TREM2* heterozygous mutations c.313del (p.Ala105fs) and c.199del (p.His67fs) in exon 2 (Fig. 1**E**). The pluripotent differentiation potential was tested by directed differentiation into cells of the three germ layers. The differentiation test confirmed that BIHi292-A hiPSCs possess the potential to differentiate into endodermal ($CD184^+$, $Sox17^+$), mesodermal ($CD140b^+$, $CD144^+$) and ectodermal ($PAX\text{-}6^+$, $Sox2^+$) cells, as quantified by flow cytometry (Fig. 1**F**). BIHi292-A hiPSCs are tested negative for mycoplasma (**Suppl. Fig. 1B**).

In conclusion, we have generated the BIHi292-A hiPSC line from PBMCs of an NHD/PLOSL patient carrying a novel heterozygous mutation in the *TREM2* gene. BIHi292-A hiPSCs can be used to investigate the yet unknown pathomechanism of NHD/PLOSL in the brain by differentiating cells into microglia (McQuade et al., 2018; Göttert et al., 2022) or using immunocompetent brain organoids. Furthermore, potential therapeutic treatment options could be evaluated through the administration of molecules that interfere with TREM2 signaling or macrophage/microglia biology in differentiated cells. In addition, BIHi292-A hiPSCs could prove to be a valuable resource for researchers working on TREM2-related research questions.

4. Materials and methods

A detailed description of the methods for PBMC isolation and erythroblast expansion, reprogramming, test for absence of the reprogramming vector, culture of hiPSCs, immunofluorescence staining for pluripotency markers (passage no. 20), FACS staining and analysis for pluripotency and differentiation markers (passage no. 18 and 19), *in vitro* directed differentiation into the three germ layers (passage no. 19), and short tandem repeat analysis (passage no. 17) can be found in our earlier publication of the cell line BIHi002-A (Hennig et al., 2019). Information on the antibodies and primers that were used can be found in Table 2.

4.1. Mycoplasma screening

Cells were cultured without the addition of antibiotics to a confluency of 80% to 90%. Mycoplasma contamination was tested by the qPCR-based Venor®GeM qOneStep Kit. Mycoplasma are detected at 520nm by amplifying the 16S rRNA coding region in the mycoplasma genome. False-negative results caused by PCR inhibition are identified

Table 2
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	SSEA-4 Antibody, anti-human, VioBright R720	1:50	130–128-332	AB_2921840
	Nanog Antibody, anti-human, PE	1:50	130-117-377	AB_2751383
	Oct3/4 Antibody, anti-human/mouse, APC	1:10	130-123-257	AB_2819472
	Tra-1-60 Antibody, anti-human, Vio488	1:100	130-106-872	AB_2654228
	SSEA-4 Antibody, anti-human, VioBlue	1:10	130-098-366	AB_2653521
Differentiation Markers	CD140b Antibody, anti-human, APC	1:50	130–105-280	AB_2655085
	CD144 Antibody, anti-human, FITC	1:50	130-123-688	AB_2819510
	CD184 (CXCR4) Antibody, anti-human, APC	1:50	130-109-844	AB_2655771
	PAX-6 Antibody, anti-human, APC	1:50	130-123-267	AB_2819462
	Sox17 Antibody, anti-human, VioB515	1:50	130-111-031	AB_2653497
	Sox2 Antibody, anti-human/mouse, FITC	1:50	130-120-721	AB_2784458
	Primers **Target**	**Size of band**	**Forward/Reverse primer (5′-3′)**	
Sendai viral vectors (PCR)	SeV	181bp	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC	
	SeV-Klf4	410bp	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA	
	SeV-cMyc	532bp	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG	
	SeV-KOS	528b	ATGCACCGCTACGAGTGAGCGC/ ACCTTGACAATCCTGATGTGG	
House-Keeping Genes (PCR)	Hu18SRNA	152bp	GTAACCCGTTGAACCCCATT/ CCATCCAATCGGTAGTAGCG	
	Beta-Actin	128bp	CAATGTGGCCGAGGACTTTG/ CATTCTCCTTAGAGAGAAGTGG	
Sequencing	TREM2	574bp	GCACAGAGCAAGTGTTCAAAG/ CTGCACAACTATCCTTGGTAG	

by the internal amplification control, detected at 560nm.

4.2. Screening for HIV-1+2, hepatitis B and hepatitis C

Donor blood testing for viral presence was performed by an external diagnostic laboratory (Labor Berlin, Berlin, Germany).

4.3. Karyotyping

For single nucleotide polymorphism (SNP) assessment, Infinium Global Screening Array-24 Kit (Illumina) in combination with GenomeStudio V2.0.5 software was used for PBMCs and BIHi292-A (passage no. 17). For G-banded-karyotyping, the sample preparation (passage no. 18) was carried out at the BIH Stem Cell Core Facility. Samples were sent for diagnostics to the Institute of Human Genetics (Universitätsklinikum Jena, Germany).

4.4. DNA isolation, PCR and Sanger Sequencing

Genomic DNA was extracted from hiPSCs (passage no. 23) using the NucleoSpin Mini Kit (Macherey-Nagel #740952.10) according to the manufacturer's instructions. PCR was performed using Light Cycler® 480 SYBR Green I Master (Roche Diagnostics) with the listed primers (Table 2). Polymerase chain reaction conditions were as follows: pre-incubation 95°C, 10min; denaturation 95°C, 10s; annealing 62°C, 10s; elongation 72°C, 15s; 45 cycles. The PCR product was run on a 1.0% agarose gel and the amplicon (*TREM2*: 574bp) was excised. DNA was purified using NucleoSpin gel and PCR clean-up kit (Macherey-Nagel #740609) and sent for sanger sequencing to Eurofins Genomics (Köln, Germany).

CRediT authorship contribution statement

Ria Göttert: Writing – original draft, Project administration, Investigation, Data curation, Conceptualization. **Valeria Fernandez Vallone:** Visualization, Validation, Software, Methodology, Formal analysis. **Harald Stachelscheid:** Validation, Resources, Methodology. **Jakob Johannes Metzger:** Investigation. **Cassandra Carao Caedo:** Investigation. **Maria Buthut:** Resources, Investigation. **Harald Prüss:** Validation, Resources, Methodology. **Matthias Endres:** Resources, Funding acquisition. **Simone Schilling:** Writing – original draft, Project administration, Conceptualization. **Karen Gertz:** Writing – original draft, Supervision, Project administration, 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.

Acknowledgments

We thank Bettina Herrmann for excellent technical assistance and the members of the BIH Core Unit Pluripotent Stem Cells and Organoids for their technical support. This work was supported by the Deutsche Forschungsgemeinschaft (Priority Program 2395/GE 2576/6-1 to K.G.; Germany's Excellence Strategy – EXC-2049 – 390688087 to M.E.; Clinical Research Unit KFO 5023: BeCAUSE-Y, EN343/16-1 to M.E. and H.P.) and the German Center for Neurodegenerative Diseases (DZNE to M.E. and H.P.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2025.103660.

References

Buthut, M., Reber, P., Siebert, E., Eisenhut, K., Thaler, F., Finck, J., Soekadar, S.R., Prüss, H., 2023. Letter to the Editor: Novel TREM2 frameshift mutation in a 30-year-old woman with suspected frontotemporal dementia. *Neurol Sci* 44, 2581–2585.
Goettert, R., 2025. CNV report. Mendeley Data V2,. https://doi.org/10.17632/vm63bzjrgn.2.

Göttert, R., Fidzinski, P., Kraus, L., Schneider, U.C., Holtkamp, M., Endres, M., Gertz, K., Kronenberg, G., 2022. Lithium inhibits tryptophan catabolism via the inflammation-induced kynurenine pathway in human microglia. *Glia* 70, 558–571.

Hennig, A.F., Rossler, U., Boiti, F., von der Hagen, M., Gossen, M., Kornak, U., Stachelscheid, H., 2019. Generation of a human induced pluripotent stem cell line (BIHi002-A) from a patient with CLCN7-related infantile malignant autosomal recessive osteopetrosis. *Stem Cell Res* 35, 101367.

McQuade, A., Coburn, M., Tu, C.H., Hasselmann, J., Davtyan, H., Blurton-Jones, M., 2018. Development and validation of a simplified method to generate human microglia from pluripotent stem cells. *Mol Neurodegener* 13, 67.