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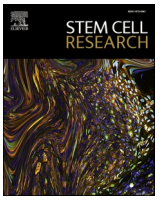

Lab Resource: Multiple Cell Lines

Generation of ten human induced pluripotent stem cell lines (hiPSCs) from patients with and without Chemotherapy-Induced Peripheral Neuropathy (CIPN) and Post Chemotherapy Cognitive Impairment (PCCI)

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A B S T R A C T

Chemotherapy-induced peripheral neuropathy (CIPN) and post-chemotherapy cognitive impairment (PCCI) represent major side effects of chemotherapy. Using Sendai virus vectors, ten hiPSC lines from patients who had undergone chemotherapy and did or did not develop CIPN and PCCI were generated. Each line was characterized to confirm markers of the undifferentiated state, differentiation potential, genomic integrity, identity verification and reprogramming vector removal. These lines serve as a valuable resource to create two disease models for 1) CIPN (hiPSC lines from five patients with CIPN vs. five without CIPN) and 2) PCCI (hiPSC lines from four patients with PCCI vs. five without PCCI).

Resource Table

Unique stem cell lines identifier	BIHi263-A https://hpscreg.eu/cell-line/BIHi263-A BIHi264-A https://hpscreg.eu/cell-line/BIHi264-A BIHi265-A https://hpscreg.eu/cell-line/BIHi265-A BIHi271-A https://hpscreg.eu/cell-line/BIHi271-A BIHi272-A https://hpscreg.eu/cell-line/BIHi272-A BIHi273-A https://hpscreg.eu/cell-line/BIHi273-A BIHi291-A https://hpscreg.eu/cell-line/BIHi291-A BIHi294-A https://hpscreg.eu/cell-line/BIHi294-A BIHi296-B https://hpscreg.eu/cell-line/BIHi296-B BIHi300-A https://hpscreg.eu/cell-line/BIHi300-A
Alternative name(s) of stem cell lines	N/A

(continued on next page)

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(*continued*)

Institution	Charité – Universitätsmedizin Berlin, Berlin Institute of Health (BIH) Berlin, Germany
Contact information of distributor	BIH Core Unit pluripotent Stem Cell and Organoids: Harald Stachelscheid (cusco@bih-charite.de)
Type of cell lines	hiPSC
Origin	Human
Additional origin info required	Age: 31–63 Sex: Female Ethnicity: Caucasian
Cell Source	Peripheral blood mononuclear cells
Clonality	Clonal
Method of reprogramming	Sendai virus (Oct3/4, Sox2, c-Myc, Klf4)
Genetic Modification	No
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR demonstrated absence of reprogramming vector
Associated diseases	CIPN, PCCI
Gene/locus	N/A
Date archived/stock date	2023
Cell line repository/bank	BIH Core Unit pluripotent Stem Cell and Organoids
Ethical approval	Approved by the Ethics Committee of Charité – Universitätsmedizin Berlin (EA04/069/14)

1. Resource utility

The ten hiPSC lines generated can be used to develop two different patient-specific, in-vitro disease models for CIPN and PCCI (Huehnchen et al., 2022; Schinke et al., 2021; Scholz et al., 2022). They aim to elucidate the biological underpinnings of both diseases as well as the differential susceptibility of patients to neurotoxic sequelae.

1.1. Resource details

Peripheral blood mononuclear cells (PBMCs) from ten breast cancer patients (all female, 31–63 years) who had undergone paclitaxel chemotherapy and neurological phenotyping were selected for reprogramming. Selection was based on the change of pre-chemotherapy baseline and post-chemotherapy follow-up scores for Total Neuropathy Score-reduced (TNSr), Chemotherapy-induced Peripheral Neuropathy 20 (CIPN20) and Rey–Osterrieth complex figure test (ROCF) (mean time interval 28 weeks). Based on the ΔTNSr and ΔCIPN20, five patients who developed CIPN vs. five patients who did not develop CIPN were selected (age ± SD: CIPN: age 53.8 ± 5.76 years, no-CIPN: 44.0 ± 11.0 years, $p = 0.116$; ΔTNSr ± SD: CIPN: 7.25 ± 4.57 points, no-CIPN: −0.800 ± 3.27 points, $p = 0.0176$; ΔCIPN20 ± SD: CIPN: 20.8 ± 4.35 points, no-CIPN: 6.80 ± 6.06 points, $p = 0.00624$; all unpaired *t*-test, Table 1). Based on the change in ROCF test scores(ΔROCF), the patients can be grouped into four patients with PCCI and five patients without PCCI (age ± SD: PCCI: 42.0 ± 10.6 years, no-PCCI: 51.6 ± 4.93 years, p

Table 1
Summary of hiPSC Cell lines grouped by CIPN.

	Disease	iPSC line name	Sex	Ethnicity	Genotype of locus	Age (years)	ΔTNRr (points)	ΔCIPN20 (points)
	CIPN	BIHi264-A	Female	Caucasian	N/A	50	14	27
	CIPN	BIHi265-A	Female	Caucasian	N/A	56	4	17
	CIPN	BIHi271-A	Female	Caucasian	N/A	50	6	20
	CIPN	BIHi273-A	Female	Caucasian	N/A	63	20 (follow-up only)	N/A
	CIPN	BIHi296-B	Female	Caucasian	N/A	50	5	19
Mean with SD						53.8 ± 5.76	7.25 ± 4.57	20.8 ± 4.35
	No CIPN	BIHi263-A	Female	Caucasian	N/A	31	−5	3
	No CIPN	BIHi272-A	Female	Caucasian	N/A	35	−2	0
	No CIPN	BIHi291-A	Female	Caucasian	N/A	45	−2	8
	No CIPN	BIHi294-A	Female	Caucasian	N/A	57	2	7
	No CIPN	BIHi300-A	Female	Caucasian	N/A	52	3	16
Mean with SD						44.0 ± 11.0	−0.800 ± 3.27	6.80 ± 6.06

Table 2
Summary of hiPSC Cell lines grouped by PCCI.

	Disease	**iPSC line name**	**Sex**	**Ethnicity**	**Genotype of locus**	**Age (years)**	**ΔROCF (points)**
	PCCI	BIHi263-A	Female	Caucasian	N/A	31	1
	PCCI	BIHi271-A	Female	Caucasian	N/A	50	−1
	PCCI	BIHi272-A	Female	Caucasian	N/A	35	−1
	PCCI	BIHi300-A	Female	Caucasian	N/A	52	0.5
Mean with SD						42.0 ± 10.6	−0.125 ± 1.03
	No PCCI	BIHi264-A	Female	Caucasian	N/A	50	4
	No PCCI	BIHi265-A	Female	Caucasian	N/A	56	2.5
	No PCCI	BIHi291-A	Female	Caucasian	N/A	45	6.5
	No PCCI	BIHi294-A	Female	Caucasian	N/A	57	6
	No PCCI	BIHi296-B	Female	Caucasian	N/A	50	7
Mean with SD						51.6 ± 4.93	5.20 ± 1.89

Table 3
Overview of all hiPSC Cell Lines with CIPN and/or PCCI.

iPSC line name	Sex	Ethnicity	Genotype of locus	Age (years)	Disease (CIPN and/or PCCI)
BIHi263-A	Female	Caucasian	N/A	31	PCCI
BIHi264-A	Female	Caucasian	N/A	50	CIPN
BIHi265-A	Female	Caucasian	N/A	56	CIPN
BIHi271-A	Female	Caucasian	N/A	50	CIPN, PCCI
BIHi272-A	Female	Caucasian	N/A	35	PCCI
BIHi273-A	Female	Caucasian	N/A	63	CIPN
BIHi291-A	Female	Caucasian	N/A	45	N/A
BIHi294-A	Female	Caucasian	N/A	57	N/A
BIHi296-B	Female	Caucasian	N/A	50	CIPN
BIHi300-A	Female	Caucasian	N/A	52	PCCI

= 0.111; ΔROCF ± SD: PCCI: −0.125 ± 1.03 points, no-PCCI: 5.20 ± 1.89 points, p = 0.00153, all unpaired *t*-test, Table 2). For one patient, corresponding to hiPSC line BIHi273-A, no data on ROCF test results was available. For an overview of all cell lines refer to Table 3.

After selection, PBMCs were reprogrammed using the CytoTune iPS 2.0 Sendai Reprograming Kit to deliver the genes Klf4, c-Myc, Sox2 and Oct3/4 and a single colony was selected for each hiPSC line. RT-PCR was used to confirm the absence of Sendai viral vectors (Supplemental Table 1) before expansion into a master bank. Quality and characterization assessments were conducted for all lines (Table 4). Fig. 1 shows data for line BIHi294-A, as an example. Data for all other lines can be found in the supplemental information. Flow cytometry confirmed the expression of markers for undifferentiated hiPSCs, Oct3/4 (> 95 %), Nanog (> 98 %), Tra-1–60 (> 81 %), and SSEA4 (> 90 %, Fig. 1A, Supplemental Fig. 1, Supplemental Table 2). Unstained cells were used as negative gating controls for flow cytometry. Immunofluorescence staining with Oct3/4, Tra-1-60, SSEA4 and Nanog (Fig. 1B, Supplemental Fig. 2) and PluriTest analysis (Supplemental Table 2) were exemplarily conducted for three lines and confirmed the undifferentiated state. Pluripotent differentiation potential was validated by the StemMACS Trilineage Differentiation Kit (MACS Miltenyi Biotec). The differentiated cells were quantified by flow cytometry to confirm expression of the mesodermal markers CD140b (>10 %) and CD144 (>4%); the ectodermal markers Sox2/Pax6 (>16 %) and the endodermal markers CD184/Sox17 (>55 %) (Fig. 1C, Supplemental Table 3). All generated cell lines showed the typical morphology of undifferentiated iPSCs (Fig. 1D and Supplemental Fig. 3). G-banding showed normal female karyotypes (46, XX) for all cell lines (Fig. 1E, Supplemental Fig. 4) and SNP array copy number variation (CNV) analysis found no CNVs compared to cells of origin (Supplemental Table 4). All hiPSC lines showed unique short tandem repeat (STR) for the 10 genomic loci analysed that were identical to the cells of origin (archived with journal). All lines tested negative for mycoplasma using Minerva Venor®GeM qOneStep (Supplemental Table 5) and donors tested negative for HIV 1 + 2, Hepatitis B, and Hepatitis C (Central Laboratory of Charité – Universitätsmedizin Berlin).

2. Materials and Methods

In depth method description for PBMC isolation, erythroblast expansion, reprogramming, PluriTest, directed differentiation into the three germ layers and short tandem repeat analysis can be found in the earlier publications (Cernoch et al., 2021; Hennig et al., 2019).

2.1. Culture of hiPSC

Essential 8 (E8) medium was used to culture hiPSCs in Geltrex (680 mg/ml) coated 6 well plates with daily whole media changes (2 ml/day). Cells were clump passaged every 3–5 days using 0.5 mM EDTA at a ratio of 1:6 without ROCK inhibitor and incubated at 37 °C, 5 % CO_2, 5 % O_2. Mycoplasma screening was conducted regularly using the qPCR-based Venor®GeM qOneStep Kit. Cultures were regularly tested for the presence of contaminants by maintaining them without antibiotics over 7 days and inspected under light microscope for fungi, bacteria or yeast growth.

Table 4
Characterization and validation.

Classification	Test	Result	Data
Morphology	Phase contrast microscopy	Normal hiPSC morphology	Fig. 1A, Suppl.Fig. 1
	Quantitative analysis by flow cytometry	Positive staining of Oct3/4 (> 95 %), Nanog (> 98 %), Tra-1–60 (> 81 %), and SSEA4 (> 90 %) across all cell lines confirm the undifferentiated state.	Fig. 1G, Suppl.Fig. 1
	PluriTest	Pluripotency and novelty scores confirm the iPSC identity. Exemplarily performed for lines BIHi263-A, BIHi264-A, BIHi265-A.	Suppl.Table.2
Genotype	Karyotype (G-banding) and resolution	All lines showed normal female karyotype 46XX resolution minimum of 200 bands per haploid chromosome set	Fig. 1C, Suppl.Fig.3
	CNV using SNP array	All lines showed no significant changes compared to cells of origin	Suppl.Table.4
Identity	Microsatellite PCR (mPCR) STR analysis	N/A All 10 STR Loci are unique in the cohort and match between donor PBMCs and iPSC lines	N/A Submitted in archive with journal
Microbiology and virology	Mycoplasma (qPCR)	Negative	Suppl.Table.5
Clearance of reprogramming vectors	SeV, SeV-cMyc, SeV-Klf4, SeV-KOS (RT-PCR)	No detectable Sendai virus genes, vectors and transgenes	Not shown but available with the author
Differentiation potential	Directed differentiation and quantitative analysis	Positive germ layer-specific marker expression by flow cytometry: Ectoderm: PAX6/SOX2 Endoderm: SOX17/CD184 Mesoderm: CD140b/CD144	Suppl.Table.3
Donor screening	HIV 1 + 2, Hepatitis B, Hepatitis C	Negative	Not shown, available with author

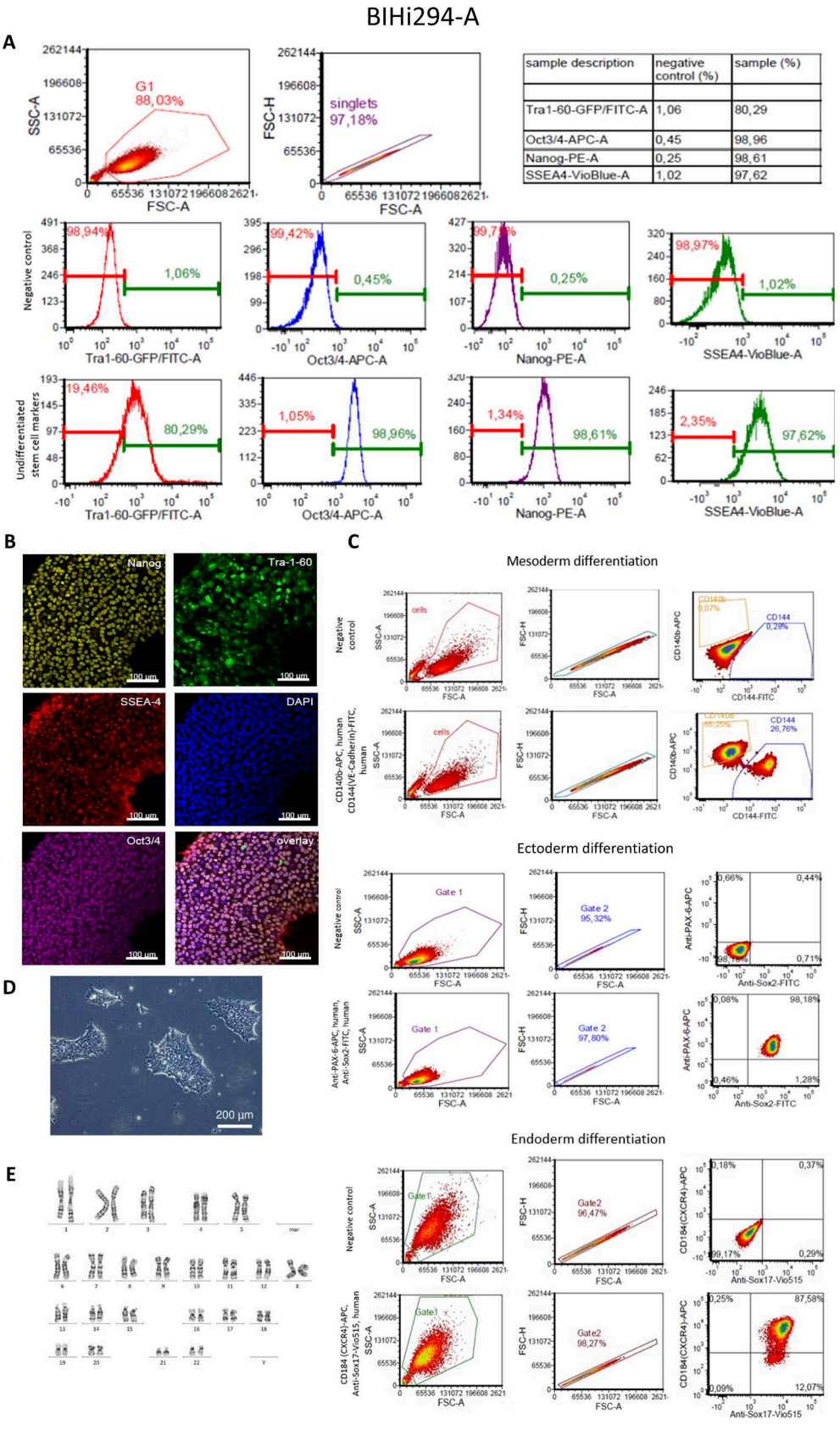
BIHi294-A
A
sample description | negative control (%) | sample (%)
Tra1-60-GFP/FITC-A | 1,06 | 80,29
Oct3/4-APC-A | 0,45 | 98,96
Nanog-PE-A | 0,25 | 98,61
SSEA4-VioBlue-A | 1,02 | 97,62
G1 88,03%
singlets 97,18%
Negative control
Undifferentiated stem cell markers
98,94%
1,06%
99,42%
0,45%
99,75%
0,25%
98,97%
1,02%
19,46%
80,29%
1,05%
98,96%
1,34%
98,61%
2,35%
97,62%
B
Nanog
Tra-1-60
SSEA-4
DAPI
Oct3/4
overlay
100 µm
C
Mesoderm differentiation
Ectoderm differentiation
Endoderm differentiation
Gate 1
Gate 2
95,32%
97,80%
Gate2
96,47%
98,27%
D
200 µm
E

Fig. 1.

Table 5
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat #**	**RRID**
Pluripotency Markers Immunocytochemistry	SSEA-4 Antibody, anti-human, VioBlue-A, REAfinity™	1:20	Miltenyi Biotec, 130–098-366	RRID: AB_2653521
	Oct3/4 Antibody, anti-human/mouse, APC, REAfinity™	1:10/ 1:100	Miltenyi Biotec, 130–123-257	RRID: AB_2819457
	Tra1-60 Antibody, anti-human, Vio488, REAfinity™	1:100/ 1:700	Miltenyi Biotec, 130–106-872	RRID: AB_2654228
	Nanog (D73G4) XP Rabbit mAb (PE Conjugate)	1:100	Cell Signaling, 14,955	RRID: AB_2798659
Pluripotency Markers Flow Cytometry	Anti-Nanog-PE	1:100	Cell Signaling Cat# 14,955	RRID: N/A
	Anti-Oct3/4-APC	1:20	Miltenyi Biotec Cat# 130–105–555	RRID: AB_2653087
	Anti-Tra1-60-Vio488	1:700	Miltenyi Biotec Cat# 130–106–872	RRID: AB_2654228
Differentiation Markers Flow Cytometry	CD140b-APC	1:11	Miltenyi Biotec Cat# 130–105–280	RRID: AB_2655085
	CD144 (VE-Cadherin)-FITC	1:11	Miltenyi Biotec Cat# 130–100–742	RRID: AB_2655151
	CD184 (CXCR4)-APC	1:11	Miltenyi Biotec Cat# 130–109–844	RRID: AB_2655771
	Anti-PAX-6-APC	1:11	Miltenyi Biotec Cat# 130–107–776	RRID: AB_2653169
	Anti-Sox17-Vio515	1:50	Miltenyi Biotec Cat# 130–111–031	RRID: AB_2653497
	Anti-Sox2-PE	1:11	Miltenyi Biotec Cat# 130–104–994	RRID: AB_2653501
	Primers			
	Target	**Forward/Reverse primer (5′-3′)**		
Sendai virus vectors (PCR)	SeV	GGATCACTAGGTGATATCGAGC/ ACCAGACAAGAGTTTAAGAGATATGTATC		
	SeV-Klf4	TTCCTGCATGCCAGAGGAGCCC/ AATGTATCGAAGGTGCTCAA		
	SeV-cMyc	TAACTGACTAGCAGGCTTGTCG/ TCCACATACAGTCCTGGATGATGATG		
	SeV-KOS	ATGCACCGCTACGAGTGAGCGC/		
House-Keeping Genes (PCR)	Hu18SRNA	GTAACCCGTTGAACCCCATT/ CCATCCAATCGGTAGTAGCG		

2.2. Immunofluorescence staining of undifferentiated hiPSC markers

hiPSCs were fixed with Roti®Histofix 4 % (Roth) reagent at room temperature for 10 min. Following fixation, a 30-minute step of blocking and permeabilization was carried out using a buffer consisting of 1 % BSA and 0.2 % Saponin in PBS with Mg2 + and Ca2 + . The cells were then exposed to fluorophore-conjugated antibodies (Oct3/4-APC, Nanog-PE, SSEA4-PerCpVio700, and Tra-1–60-Vio488) diluted in the same permeabilization/blocking buffer for 1 h at room temperature (Table 5). Nuclei were counterstained with Hoechst 33,342 (1 μg/mL). Microscopic examination was conducted using the Opera Phenix high content imaging system (Perkin Elmer).

2.3. Flow cytometry for pluripotency and differentiation markers

Single cells were collected using TrypLE. Surface marker staining was conducted on $2x10^5$ hiPSCs for 10 min at 4 °C in 100 μL of surface staining solution (Table 5). Cells were then washed twice with PBS, fixed and permeabilized using the FoxP3 Staining Buffer Set (Miltenyi Biotec). Intracellular markers were stained for 30 min at 4 °C in 100 μL of intracellular permeabilization solution (Table 5). Analysis was performed with MACSQuant® VYB system (Miltenyi Biotec).

2.4. Absence of the reprogramming vector RT-PCR

RNA isolation by RNeasy Plus Mini Kit (Qiagen) and cDNA synthesis by CytoTune 2.0 TaqMan Reverse Transcription Reagents (Applied Biosystems) were performed. RT-PCR was used to test for the absence of the Sendai virus (SeV) genome and transgene using the primers listed in Table 5 and the following temperature program: 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, finally 72 °C for 10 min. Internal controls of 18S ribosomal RNA was present and PCR products were analysed by 2 % agarose gel electrophoresis.

2.5. Karyotype analysis and SNP array

G banding was performed by quantifying GTG stained metaphase chromosomes (resolution > 200 bands/haploid chromosome set) by the Institut für Humangenetik at Universitätsklinikum Jena or by the Praxis für Humangenetik Berlin-Mitte. For each line, a composite karyotype from 20 metaphase plates with passage number (p) tested are reported in Supplemental Fig. 3. Additionally, copy number variation (CNV) analysis was performed using SNP array data. For this DNA of all lines were quantified by Illumina Infinium Global Screening Array-24 BeadChip or OMNI-EXPRESS-8v1.6 Chip and analysed using Illumina GenomeStudio V2.0.5 software.

CRediT authorship contribution statement

Karyn Lewis: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Data curation. **Valeria Fernandez Vallone:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Investigation, Data curation. **Adam Dordevic:** Investigation. **Johannes Kern:** Investigation. **Harald Stachelscheid:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology. **Matthias Endres:** Funding acquisition. **Wolfgang Boehmerle:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Petra Huehnchen:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Christian Schinke:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, 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.2025.103674.

Data availability

All relevant data can be found in the Supplementary Material.

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