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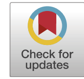
Lab Resource: Single Cell Line



Establishment of an induced pluripotent cell line (ABCRIi001-A) from an elderly female for ageing research

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ABSTRACT

Human induced pluripotent stem cells (hiPSCs) hold promises to model and understand human diseases, including those associated with ageing. Here, we describe ABCRIi001-A, a hiPSC line generated from peripheral blood mononuclear cells (PBMCs) of a 79-year-old female enrolled in a study for development of an ageing score (ALFA Score). PBMCs were reprogrammed using three Sendai virus-based reprogramming vectors (hKOS, hc-Myc, and hKlf4). ABCRIi001-A showed normal morphology and karyotype, viral clearance, absence of genomic aberrations, and their pluripotency was confirmed by expression of pluripotency-related markers and their ability to differentiate into the three germ layers. ABCRIi001-A is valuable for ageing-related studies.

Resource table

Unique stem cell line identifier	ABCRIi001-A
Alternative name(s) of stem cell line	hiPSC_ALFAS037_C9
Institution	Universidade do Algarve, Algarve Biomedical Center Research Institute (ABC-RI), Faro, Portugal
Contact information of distributor	jebraganca@ualg.pt
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 79 Sex: Female Ethnicity if known: Caucasian/Portuguese
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Clonality	Clonal
Method of reprogramming	Sendai virus-based reprogramming vectors expressing the four Yamanaka factors, hOct3/4, hSox2, hKlf4, and hc-Myc
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-qPCR
Associated disease	N/A
Gene/locus	N/A
Date archived/stock date	July 2024
Cell line repository/bank	https://hpscreg.eu/cell-line/ABCRIi001-A

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Resource table (*continued*)

Ethical approval	The study was approved by the Ethics and Health Committee of the Administração Regional de Saúde do Algarve (nr. 6/2022) and the Health Ethics Committee of the Algarve Biomedical Center. The participant signed a written consent before blood collection.

1. Resource utility

The ABCRIi001-A iPS cell line was derived from a 79-year-old female enrolled in a study aiming to develop a score (ALFA Score) for the assessment of ageing status. This cell line offers an in vitro model to study ageing and age-related diseases, underlying molecular mechanisms, and therapeutic strategies.

2. Resource details

The ABCRIi001-A induced pluripotent stem cell (iPSC) line was obtained by reprogramming peripheral blood mononuclear cells (PBMCs) collected from a 79-year-old female. The donor was enrolled in a multidisciplinary study aiming at developing a novel ageing score (ALFA Score) to assess individual and/or population ageing status, and to predict disease risk and management. This iPS cell line provides a useful model for ageing research.

The PBMCs were reprogrammed into iPSCs using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific), including reprogramming vectors based on a modified form of Sendai virus (SeV) to ensure the delivery and expression of key genetic factors, namely the four Yamanaka factors, Oct3/4, Sox2, Klf4, and c-Myc (Takahashi and Yamanaka, 2006). Clonal iPSCs were established and clone 9 (ABCRIi001-A) was further characterised (Table 1). This cell line exhibited typical human pluripotent stem cell-like morphology (Fig. 1A). After clearance of the SeV reprogramming vectors (Fig. 1B), G-banding of chromosomes showed that the iPSC line presented a normal karyotype (46, XX) at passage 26 (Fig. 1C). Short tandem repeat (STR) analysis of 16 loci confirmed that iPSCs were genetically identical to parental PBMCs (archived with journal). Regular mycoplasma testing revealed the absence of contamination in the iPS cell culture (Fig. 1D). Flow cytometry analysis quantified the positive expression of surface pluripotency markers SSEA4 (99.4 %) and TRA-1–60 (99.5 %), and intracellular marker OCT4 (99.6 %) (Fig. 1E). Expression of the pluripotency markers SSEA4, NANOG, OCT4, and SOX2 was further confirmed by immunocytochemistry analysis (Fig. 1F). ABCRIi001-A colonies also stained positive for alkaline phosphatase (AP) (Fig. 1G). The differentiation potential of ABCRIi001-A was confirmed by spontaneous embryoid body formation and subsequent immunofluorescence staining of ectoderm (TUBB3), mesoderm (SMA), and endoderm (AFP) markers (Fig. 1H).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography Bright field	*Normal*	Fig. 1*A*
Phenotype	Qualitative analysis *Immunocytochemistry*	*Positive pluripotency markers: SSEA4, NANOG, OCT4, SOX2; and Alkaline Phosphatase (AP)*	Fig. 1*F and G*
	Quantitative analysis *RT-qPCR* *Flow cytometry*	*Sendai reprogramming vectors clearance* *Positive pluripotency markers: SSEA4 (99.4 %), TRA-1–60 (99.5 %), OCT4 (99.6 %)*	Fig. 1*B* Fig. 1*E*
Genotype	Karyotype (G-banding) and resolution	*46XX, Resolution 400–500*	Fig. 1*C*
***mtDNA analysis* (IF APPLICABLE)**	Sanger Sequencing, NGS, Long-Read Sequencing and analysis software	*N/A*	*N/A*
Identity	STR analysis	*16 loci tested, all matched*	*Available with the authors*
Mutation analysis (IF APPLICABLE)	Sequencing Southern Blot OR WGS	*N/A* *N/A*	*N/A* *N/A*
Microbiology and virology	Mycoplasma	*Mycoplasma testing PCR: Negative*	Fig. 1*D*
Differentiation potential	Embryoid body formation and spontaneous differentiation	*Expression of differentiation markers: Ectoderm – Tubulin β3* (TUBB3/TUJ1) Endoderm – Alpha Feto Protein (AFP) Mesoderm – α-Smooth Muscle Actin (SMA)	Fig. 1*H*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	*N/A*	*N/A*
Genotype additional info (OPTIONAL)	Blood group genotyping HLA tissue typing	*N/A* *N/A*	*N/A* *N/A*

3. Materials and methods

3.1. PBMC isolation and reprogramming

Four mL of blood were collected in BD Vacutainer® CPT™ Tubes (BD Biosciences). Samples were centrifuged within 2 h of blood drawing and PBMCs were isolated according to the manufacturer's instructions. Four days before transduction, PBMCs were seeded (1.5×10^6 cells) and maintained in PBMC medium (StemPro™-34 SFM medium (Life Technologies) with 2 mM Glutamine, 0.5 % Penicillin/Streptomycin/Amphotericin B, 100 ng/mL SCF, 100 ng/mL FLT-3, 20 ng/mL IL-3, and 20 ng/mL IL-6). Next, 3.5×10^5cells were transduced using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific), and a MOI of 5-5-3, for hKOS, hc-Myc, and hKlf4, respectively. After 24 h, the viruses were removed and cells were plated in PBMC medium. Two days later, transduced PBMCs were plated onto mitotically inactivated human foreskin fibroblasts (HFFs) and maintained without the cytokines for 3 days. Cells were then transitioned to the iPSC medium mTeSR™1 (STEMCELL Technologies) by replacing half of the PBMC medium with iPSCs medium for 3 days, and afterwards by changing the full volume to iPSCs medium. Twelve days after transduction, individual colonies with stem cell-like morphology were manually picked and transferred onto HFF plates. After the first passage, iPSC colonies were transitioned to feeder-free conditions, i.e., onto plates coated with Geltrex™ LDEV-Free hESC-Qualified Reduced Growth Factor Basement Membrane Matrix (Life Technologies).

3.2. iPSC culture

Manual picking of colonies was performed for up to the first 5 passages, after which cells were passaged at a ratio of 1:4 every 5–7 days, using ReLeSR™ (STEMCELL Technologies). iPSC clones were cultured and expanded on Geltrex™-coated 6-well plates with mTeSR™1 medium (STEMCELL Technologies) in a humidified atmosphere with 5 % CO_2 at 37 °C.

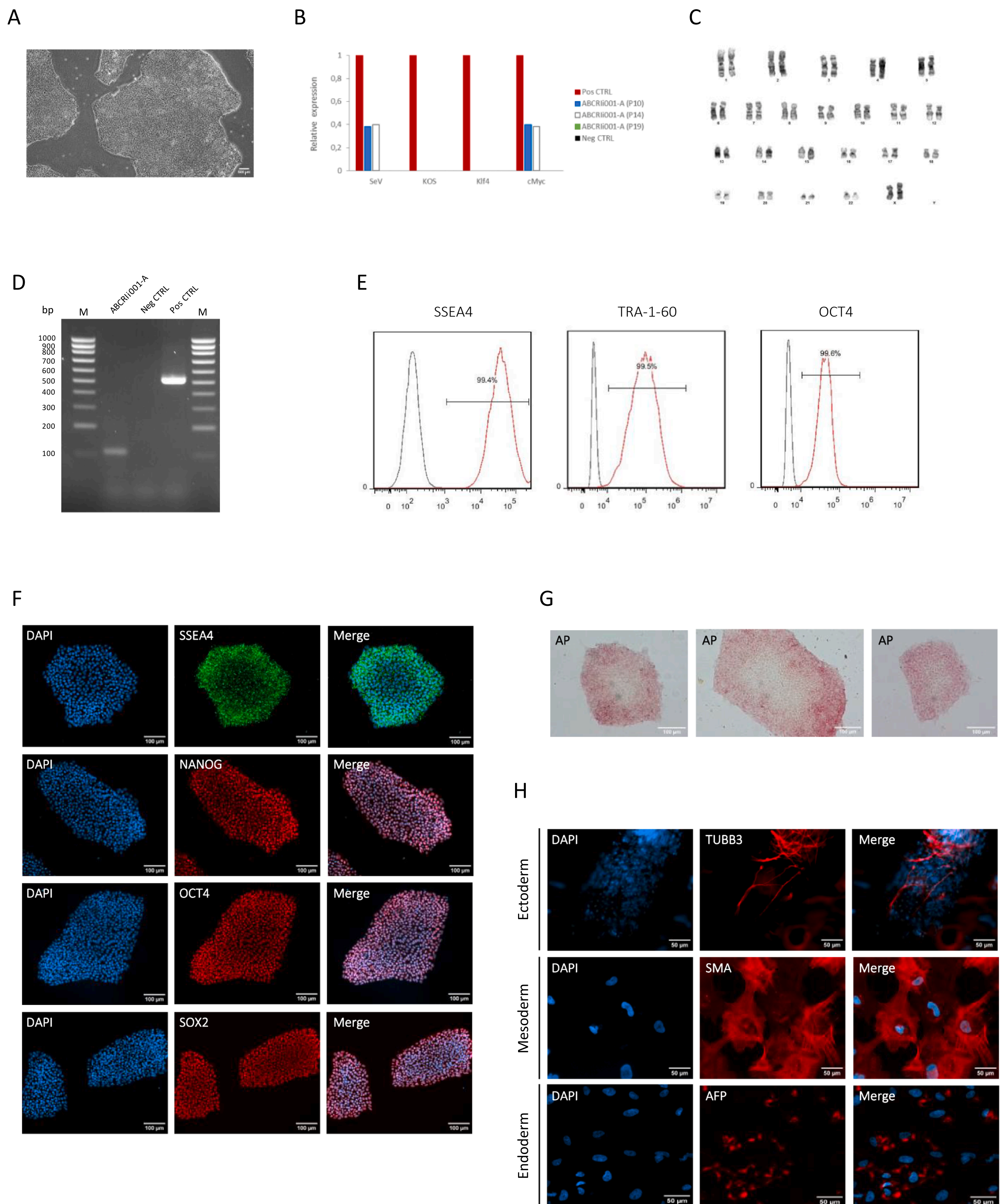

Fig.1. Characterization of the ABCRIi001-A iPC line.

3.3. RT-qPCR for detection of reprogramming vectors clearance

Total RNA was isolated from iPSCs at passages 0 (positive control), 10, 14, and 19, using the RNeasy® Plus Mini Kit (Qiagen), and cDNA was synthesized with SuperScript™ VILO™ cDNA Synthesis Kit (Thermo Fisher Scientific) using 1 µg of total RNA as template. The presence of CytoTune™ 2.0 Sendai reprogramming vectors was detected by qPCR with pre-validated TaqMan™ assays (Table 2) using the

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	*PE anti-human TRA-1–60-R, Mouse IgM, κ*	*1:50*	*BioLegend Cat# 330610*	*RRID: AB_2119065*
	PE anti-human SSEA4, Mouse IgG3,κ	*1:50*	*BioLegend Cat# 330406*	*RRID: AB_1089206*
	Alexa Fluor(R) 488 anti-OCT4 (OCT3), Mouse IgG2b, κ	*1:50*	*BioLegend Cat# 653706*	*RRID: AB_2562251*
	Mouse anti-SSEA4	*1:100*	*BD Pharmigen Cat# 560073*	*RRID: AB_1645601*
	Rabbit anti-NANOG	*1:400*	*Cell Signaling Technology Cat# 4903*	*RRID: AB_10559205*
	Rabbit anti-OCT4	*1:400*	*Cell Signaling Technology Cat# 2840*	*RRID: AB_2167691*
	Rabbit anti-SOX2	*1:400*	*Cell Signaling Technology Cat# 3579*	*RRID: AB_2195767*
Differentiation Markers	*Mouse anti-Tubulin beta 3 (TUBB3)*	*1:500*	*BioLegend Cat# 801201*	*RRID: AB_2313773*
	Mouse Anti-Actin, alpha-Smooth Muscle	*1:300*	*Sigma-Aldrich Cat# A5228*	*RRID: AB_262054*
	Mouse anti-alpha-1-Fetoprotein	*1:75*	*BioLegend Cat# 847102*	*RRID: AB_2629665*
Secondary antibodies	*Donkey anti-Mouse IgG (H + L), Alexa Fluor™ 488*	*1:500*	*Thermo Fisher Scientific Cat# A-21202*	*RRID: AB_141607*
	Donkey anti-Rabbit IgG (H + L), Alexa Fluor™ 594	*1:500*	*Thermo Fisher Scientific Cat# A-21207*	*RRID: AB_141637*
	Goat anti-Mouse IgG (H + L), Alexa Fluor™ Plus 594	*1:500*	*Thermo Fisher Scientific Cat# A32742*	*RRID: AB_2762825*

Primers

	Target	Size of band	Forward/Reverse primer (5′-3′)
Mycoplasma (PCR)	*M. alkalescens, M. arginini, M. arthridis, M. bovirhinis, M. canadense, M. fermentans, M. gallinaceum, M. gallinarum, M. gallopavonis, M. gatae, M. iowae, M. meleagridis, M. mycoides, M. orale, M. pneumoniae, M. pullorum, M. salivarium, M. synoviae, M. verecundum*	*504–519 bp*	*YGCCTGRGTAGTAYRYWCGC/ GCGGTGTGTACAARMCCCGA*

Gene Expression Assays

	Target	Amplicon Length	Assay ID (Company, Cat#)
Sendai reprogramming vectors (qPCR)	*SeV*	*59 bp*	*Mr04269880_mr (Thermo Fisher Scientific, Cat# 4453320)*
	KOS	*80 bp*	*Mr04421257_mr (Thermo Fisher Scientific, Cat# 4453320)*
	Klf4	*67 bp*	*Mr04421256_mr (Thermo Fisher Scientific, Cat# 4453320)*
	cMyc	*89 bp*	*Mr04269876_mr (Thermo Fisher Scientific, Cat# 4453320)*

TaqMan™ Fast Advanced Master Mix (Thermo Fisher Scientific).

3.4. Karyotype analysis

iPSCs at passage 26, were treated with 0.1 μg/mL KaryoMAX™ Colcemid™ Solution (Life Technologies) for 1 h at 37 °C. Cells were dissociated with TrypLE™ Select Enzyme (Life Technologies) and metaphases were prepared by incubation of cells with 75 mM KCl for 20 min at 37 °C, and fixation with methanol: acetic acid (3:1). Genetic integrity of iPSCs was verified by G-banding of chromosomes at Genomed (Lisbon, Portugal). At least 30 metaphases were analysed at 400–500 band resolution.

3.5. STR analysis

Genomic DNA from PBMCs and iPSCs was extracted using the DNeasy® Blood & Tissue Kit (Qiagen). Cell line authentication was performed by StabVida (Lisbon, Portugal) with the Powerplex® 16 loci kit (Promega) using an ABI 3730 XL DNA Analyzer (Thermo Fisher Scientific). Data was analysed with GeneMarker® HID v1.75 (Promega).

3.6. Mycoplasma testing

The iPSC cultures were regularly screened for mycoplasma contamination by PCR using the primers described in Table 2 (Wirth et al., 1994). Cell culture medium incubated for at least 48 h was used as template in the PCR reaction. The amplified products were analysed by agarose gel electrophoresis and visualized with iBright™ CL1500 Imaging System (Thermo Fisher Scientific).

3.7. Flow cytometry

iPSCs at passage 21, were dissociated into single cell suspension with TrypLE™ Select Enzyme (Life Technologies), and fixed with ice-cold methanol for 20 min at −20 °C. After washing with 1 %BSA in PBS, fixed cells were incubated with the directly labelled antibodies diluted in 1 % BSA in PBS (Table 2) for 30 min at 4 °C, protect from light. Cells were then analysed by flow cytometry for quantification of pluripotency markers using CytoFLEX Flow Cytometer Violet-Blue-Red (V-B-R) Series and the CytExpert software (Beckman Coulter).

3.8. Immunocytochemistry

iPSCs were cultured onto glass coverslips, and fixed with 4 % paraformaldehyde for 15 min at room temperature (RT). Fixed cells were washed 3 times with 0.1 % Triton-X in PBS (washing buffer) and incubated in PBS with 0.1 % Triton™ X-100 and 2 % BSA (blocking buffer) for 1 h at RT. Cells were then incubated with the primary antibodies (Table 2) diluted in blocking buffer, overnight at 4 °C. After washing 3 times, cells were incubated with secondary antibodies (Table 2) for 1 h at RT. Cells were washed 3 more times and slides were mounted with Fluoromount-G™ Mounting Medium, with DAPI (Thermo Fisher Scientific). Images were acquired using an Axio Imager.Z2 fluorescence microscope (Zeiss) and processed with Fiji software (Schindelin et al., 2012).

3.9. Alkaline phosphatase staining

iPSCs were cultured onto glass coverslips and alkaline phosphatase (AP) was detected with StemAb™ Alkaline Phosphatase Staining Kit II (REPROCELL) according to the manufacturer's instructions. Slides were mounted with Fluoromount-G™ Mounting Medium (Thermo Fisher Scientific), and images were acquired using an Axio Imager.Z2 microscope (Zeiss). Data was analysed with Fiji software (Schindelin et al., 2012).

3.10. Embryoid body formation and differentiation

iPSCs at passage 24 were scrapped and cultured for 72 h in non-adherent conditions in mTeSR™1 medium (STEMCELL Technologies) containing 0.4 % polyvinyl alcohol to form embryoid bodies (EBs). Suspension EBs were then transferred and plated onto coverslips for spontaneous differentiation with DMEM culture medium supplemented with 10 % FBS, 1 % GlutaMAX™ (Life Technologies), 1 % Penicillin/Streptomycin/Amphotericin B, 1 % MEM Non-Essential Amino Acids, and 1X β-Mercaptoethanol. Cultures were maintained at 37 °C in a humidified atmosphere with 5 % CO_2, and the culture medium was changed every other day for 21 days. Immunofluorescence staining of ectoderm (TUBB3), mesoderm (SMA), and endoderm (AFP) markers was performed as described in the Immunocytochemistry section with the antibodies listed in Table 2.

CRediT authorship contribution statement

Filipa Esteves: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **David Brito:** Resources, Methodology. **Ana Teresa Rajado:** Methodology, Investigation. **Nádia Silva:** Resources, Methodology, Investigation. **Joana Apolónio:** Resources, Methodology, Investigation. **Vânia Palma Roberto:** Resources, Methodology, Investigation. **Raquel P. Andrade:** . **Sofia Calado:** . **Maria Leonor Faleiro:** . **Carlos Matos:** . **Nuno Marques:** . **Ana Marreiros:** . **Hipólito Nzwalo:** . **Sandra Pais:** . **Isabel Palmeirim:** . **Sónia Simãoa:** . **Natércia Joaquim:** . **Rui Miranda:** . **António Pêgas:** . **Daniela Marques Raposo:** . **Ana Sardo:** . **Inês Araújo:** Funding acquisition. **Clévio Nóbrega:** Funding acquisition. **Pedro Castelo-Branco:** Project administration, Funding acquisition. **José Bragança:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jose Braganca reports financial support was provided by Regional Development and Coordinating Commission of the Algarve. 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.

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