



**HAL**  
open science

## Generation of human induced pluripotent stem cell lines from five patients with Myofibrillar myopathy carrying different heterozygous mutations in the DES gene

Pierre Joanne, Yeranuhi Hovhannisyan, Alexandre Simon, Gaëlle Revet, Romain Diot, Gabriel Friob, Denisa Calin, Zhenlin Li, Anthony Béhin, Karim Wahbi, et al.

### ► To cite this version:

Pierre Joanne, Yeranuhi Hovhannisyan, Alexandre Simon, Gaëlle Revet, Romain Diot, et al.. Generation of human induced pluripotent stem cell lines from five patients with Myofibrillar myopathy carrying different heterozygous mutations in the DES gene. *Stem Cell Research*, 2024, 76, pp.103338. 10.1016/j.scr.2024.103338 . hal-04466294

**HAL Id: hal-04466294**

**<https://hal.sorbonne-universite.fr/hal-04466294>**

Submitted on 19 Feb 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Lab Resource: Multiple Cell Lines



## Generation of human induced pluripotent stem cell lines from five patients with Myofibrillar myopathy carrying different heterozygous mutations in the *DES* gene

Pierre Joanne<sup>a,\*</sup>, Yeranuhi Hovhannisyan<sup>a</sup>, Alexandre Simon<sup>a</sup>, Gaëlle Revet<sup>a</sup>, Romain Diot<sup>b</sup>, Gabriel Friob<sup>a</sup>, Denisa Calin<sup>a</sup>, Zhenlin Li<sup>a</sup>, Anthony Béhin<sup>c</sup>, Karim Wahbi<sup>d,e</sup>, Gérard Tachdjian<sup>b</sup>, Onnik Agbulut<sup>a,\*</sup>

<sup>a</sup> Sorbonne Université, Institut de Biologie Paris-Seine (IBPS), CNRS UMR 8256, Inserm ERL U1164, Biological Adaptation and Ageing, Paris, France

<sup>b</sup> Assistance Publique-Hôpitaux de Paris, Université Paris Saclay, Hôpital Antoine Béchère, Service d'Histologie, Embryologie et Cytogénétique, Clamart, France

<sup>c</sup> Sorbonne Université, Assistance Publique-Hôpitaux de Paris, Hôpital Pitié-Salpêtrière, Inserm UMR\_S 974, Paris, France

<sup>d</sup> AP-HP, Cochin Hospital, Cardiology Department, Paris, France

<sup>e</sup> Université de Paris, Paris, Paris Cardiovascular Research Center (PARCC), INSERM Unit 970, Paris, France

### ABSTRACT

Myofibrillar myopathy (MFM) is a rare genetic disorder characterized by muscular dystrophy that is often associated with cardiac disease. This disease is caused by mutations in several genes, among them *DES* (encoding desmin) is the most frequently affected. Peripheral blood mononuclear cells from 5 different MFM patients with different *DES* mutations were reprogrammed into induced pluripotent stem cells (iPSC) using non-integrative vectors. For each patient, one iPSC clone was selected and demonstrated pluripotency hallmarks without genomic abnormalities. SNP profiles were identical to the cells of origin and all the clones have the capacity to differentiate into all three germ layers.

### Resource Table:

(continued)

Unique stem cell lines identifier	INSRMi012-C INSRMi013-A INSRMi019-A INSRMi020-A INSRMi021-A		Age: 73 Sex: Male Ethnicity if known: NA
Alternative name(s) of stem cell lines	PC173T19 PC179c1 PC129K8 PC130K2c PC177 3c14		For Patient PC129k8 (line INSRMi019-A) Age: 70 Sex: Male Ethnicity if known: NA
Institution	Sorbonne Université, UMR8256, INSERM ERL U1164, Team CARTHER		For Patient PC130k2c (line INSRMi020-A) Age: 56 Sex: Male Ethnicity if known: NA
Contact information of distributor	Dr. Pierre JOANNE (pierre.joanne@sorbonne-universite.fr)		For Patient PC177 3c14 (line INSRMi021-A) Age: 57 Sex: Female Ethnicity if known: NA
Type of cell lines	iPSC		
Origin	Human		
Additional origin info required for human ESC or iPSC	For Patient PC173T19 (line INSRMi012-C) Age: 35 Sex: Male Ethnicity if known: NA	Cell Source Clonality Method of reprogramming	PBMC Clonal Non-integrative reprogramming using episomally or Sendai-delivered factors
	For Patient PC179c1 (line INSRMi013-A)	Genetic Modification	No

(continued on next column)

(continued on next page)

\* Corresponding authors.

E-mail addresses: [pierre.joanne@sorbonne-universite.fr](mailto:pierre.joanne@sorbonne-universite.fr) (P. Joanne), [onnik.agbulut@sorbonne-universite.fr](mailto:onnik.agbulut@sorbonne-universite.fr) (O. Agbulut).

<https://doi.org/10.1016/j.scr.2024.103338>

Received 15 January 2024; Accepted 4 February 2024

Available online 8 February 2024

1873-5061/© 2024 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

(continued)

Type of Genetic Modification	Spontaneous mutations are carried by the patients of origin (see Gene/locus row of this table)
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	PCR
Associated disease	Myofibrillar myopathy associated with cardiac disease: Patient PC173T19 (line INSRMi012-C): Severe atrioventricular block (type 3), Ventricular fibrillation, Atrial fibrillation, Cardiomyopathy biventricular Patient PC179c1 (line INSRMi013-A): Moderate atrioventricular block (type 1), Ventricular tachycardia, Cardiomyopathy (LVEF 45 %) Patient PC129k8 (line INSRMi019-A): Severe atrioventricular block (type 2/1) Patient PC130k2c (line INSRMi020-A): Severe atrioventricular block (type 3) Patient PC177 3c14 (line INSRMi021-A): Severe atrioventricular block (type 3), Atrial fibrillation, Ventricular tachycardia, Cardiomyopathy biventricular
Gene/locus	Mutations in the desmin gene (location 2Q35): Patient PC173T19 (line INSRMi012-C): $DES^{D214-E245Del}$ Patient PC179c1 (line INSRMi013-A): $DES^{S46Y}$ Patient PC129k8 (line INSRMi019-A): $DES^{P419H}$ Patient PC130k2c (line INSRMi020-A): $DES^{E439K}$ Patient PC177 3c14 (line INSRMi021-A): $DES^{E245D}$
Date archived/stock date	2023–12–28
Cell line repository/bank	<a href="https://hpscereg.eu/user/cellline/edit/INSRMi012-C">https://hpscereg.eu/user/cellline/edit/INSRMi012-C</a> <a href="https://hpscereg.eu/user/cellline/edit/INSRMi013-A">https://hpscereg.eu/user/cellline/edit/INSRMi013-A</a> <a href="https://hpscereg.eu/user/cellline/edit/INSRMi019-A">https://hpscereg.eu/user/cellline/edit/INSRMi019-A</a> <a href="https://hpscereg.eu/user/cellline/edit/INSRMi020-A">https://hpscereg.eu/user/cellline/edit/INSRMi020-A</a> <a href="https://hpscereg.eu/user/cellline/edit/INSRMi021-A">https://hpscereg.eu/user/cellline/edit/INSRMi021-A</a>
Ethical approval	Approval number from the French « Ministère de l'enseignement supérieur, de la recherche et de l'innovation »: AC-2019–3388

## 1. Resource utility

These iPSC lines, generated from five different patients affected by MFM associated with dilated cardiomyopathy (DCM) with five different *DES* mutations, can be used to study cellular and molecular mechanisms of the MFM. They also can be used as a cell model to develop new therapeutic approaches, especially based on drug-screening.

## 2. Resource details

Desmin is the specific intermediate filament of muscle cells, within which it forms a three-dimensional network connecting the various cellular components to each other, including organelles and contractile apparatus (Brodehl et al., 2018). Mutations in the gene encoding this protein is known to cause desmin-related myofibrillar myopathy (MFM1: OMIM:601419), a heterogeneous disease affecting primarily skeletal muscles (weakness) (Joanne et al., 2013). However, approximately 75 % of patients also present signs of cardiac disease (van Spaendonck-Zwarts et al., 2010) that mainly evolve toward dilated cardiomyopathy and heart failure. At the cell level, mutations of desmin

cause several disorganizations of the muscle cell that lead to contractile and metabolic dysfunctions (Hovhannisyann et al., 2023; Winter et al., 2016).

Here we described the generation of human induced pluripotent stem cells from the peripheral blood mononuclear cells (PBMC) of 5 patients affected by MFM associated with a cardiac dysfunction. These five patients have different mutations in *DES* even if they present similar cardiac symptoms (see Table 1.). These mutations ( $DES^{D214-E245Del}$ ,  $DES^{S46Y}$ ,  $DES^{P419H}$ ,  $DES^{E439K}$ ,  $DES^{E245D}$ ) have been previously described (Wahbi et al., 2012) and are linked to the development of dilated cardiomyopathy. Five different clones (INSRMi012-C from the patient PC173T19 with the mutation  $DES^{D214-E245Del}$ , INSRMi013-A from the patient PC179c1 with the mutation  $DES^{S46Y}$ , INSRMi019-A from the patient PC129k8 with the mutation  $DES^{P419H}$ , INSRMi020-A from the patient PC130k2c with the mutation  $DES^{E439K}$  and INSRMi021-A from the patient PC177 3c14 with the mutation  $DES^{E245D}$ ) were derived and are described here (Table 1). In details, PBMC of these patients were reprogrammed using non-integrative strategy (Phenocell SAS, France) and clones for each patient were manually isolated and amplified until passage 20 where they were frozen in liquid nitrogen with CTS PSC Cryomedium (Thermo Fisher Scientific, France). After thawing on Matrigel, these clones were passaged at least one time before analysis. Colonies of all clones demonstrate normal morphology in culture with a high nucleus/cytoplasm ratio (Fig. 1A, scale bar = 100  $\mu$ m) and express pluripotency markers as shown by immunocytochemistry (Fig. 1B, scale bar = 100  $\mu$ m) and cytometry (Fig. 1C and Supplemental Data A). The absence of residual reprogramming plasmids was confirmed by PCR. Genomic integrity, gender and identity were confirmed for each clone by G-banding karyotyping (Fig. 1E) and by SNP analysis through the comparison of the iPSCs genotype with that of parental PBMCs to confirm their origin (see Table 1 and Supplemental Data C and D). Moreover, each clone was tested for its capacity to differentiate into the three germ layer lineages using the STEMdiff™ Trilineage Differentiation Kit (Stem Cell Technologies, France). As shown in Fig. 1D, each clone expresses endodermic, mesodermic and ectodermic markers after specific differentiation (expresses as fold change compared to undifferentiated condition) further confirming their pluripotency. The presence of the specific mutations (Table 1) was assessed by sequencing the genomic DNA of each clone and reveal that the expected mutation was correctly observed (Supplemental Data B). Finally, absence of mycoplasma was confirmed using the Mycoplasma check service of Eurofins Genomics.

## 3. Materials and methods

### 3.1. Generation and culture of iPSCs

Patient PBMC were reprogrammed by a non-integrative method using a commercial episome-based kit (Cat. #A15960, ThermoFischer Scientific) or sendai-based kit (#A16517, ThermoFischer Scientific). After manually isolated, clones are cultured and amplified under 37°C, 5 % CO<sub>2</sub>, 20 % O<sub>2</sub> on Matrigel® (hESC-qualified, Corning) coated dishes with daily replacement of mTeSR™1 (Stem Cell Technologies) and passaged in clumps (1:30 ratio) at approximately 80 % confluency using ReLeSR (Stem Cell Technologies).

### 3.2. Immunocytochemistry

Cells cultured on coverslip coated with Matrigel were fixed with 4 % paraformaldehyde for 5 min and permeabilization were performed with cold methanol (-20 °C) for 5 min. After incubation with 5 % bovine serum albumin for one hour cells were incubated with primary antibodies (see Table 2) overnight at 4 °C. After 45 min incubation with secondary antibodies (see Table 2), nuclei were counterstained with Hoescht 33,342 (Life Technologies). Then, slides were mounted with homemade medium containing Mowiol. Images were taken on an

**Table 1**  
Characterization and validation.

Classification	Test	Result	Data
<b>Morphology</b>	Photography Bright field	Normal for all INSRMi012-C: passage 24 INSRMi013-A: passage 21 INSRMi019-A: passage 20 INSRMi020-A: passage 20 INSRMi021-A: passage 21	<a href="#">Fig. 1A</a>
<b>Phenotype</b>	Qualitative analysis by immunocytochemistry	Normal staining of Nanog (green) and Tra-1-80 (red) markers INSRMi012-C: passage 24 INSRMi013-A: passage 21 INSRMi019-A: passage 20 INSRMi020-A: passage 20 INSRMi021-A: passage 21	<a href="#">Fig. 1B</a>
	Quantitative analysis by flow cytometry	Assess more than 90 % positive cells for Nanog, Oct3/4 and Sox. staining of Nanog and Tra-1-80 markers INSRMi012-C: passage 24 INSRMi013-A: passage 21 INSRMi019-A: passage 20 INSRMi020-A: passage 20 INSRMi021-A: passage 21	<a href="#">Fig. 1C and Supplemental Data A</a>
<b>Genotype</b>	Karyotype (G-banding) and resolution	Normal karyotype (46, XY or 46,XX) Resolution 450–500 INSRMi012-C: passage 24 INSRMi013-A: passage 21 INSRMi019-A: passage 23 INSRMi020-A: passage 20 INSRMi021-A: passage 23	<a href="#">Fig. 1E</a>
<b>Identity</b>	Microsatellite PCR (mPCR) OR STR analysis	Not performed SNP Analysis INSRMi012-C: passage 23 INSRMi013-A: passage 24 INSRMi019-A: passage 19 INSRMi020-A: passage 19 INSRMi021-A: passage 20	<a href="#">Supplemental Data C and D</a> Original data submitted in archive with journal

**Table 1 (continued)**

Classification	Test	Result	Data
<b>Mutation analysis (IF APPLICABLE)</b>	Sequencing	Heterozygous mutations	<a href="#">Supplemental Data B</a>
<b>Microbiology and virology</b>	Southern Blot OR WGS Mycoplasma	Not performed Mycoplasma testing by RT-PCR	Submitted as <a href="#">supplementary files</a> <a href="#">Fig. 1D</a>
<b>Differentiation potential</b>	Directed differentiation	Expression of genes from cells induced for differentiation toward endoderm, mesoderm or ectoderm germ layers: FOXA2, EOMES, Brachyury, MIXL1, LHX2 and MAP2 INSRMi012-C: passage 24 INSRMi013-A: passage 21 INSRMi019-A: passage 20 INSRMi020-A: passage 20 INSRMi021-A: passage 21	
<b>Donor screening (OPTIONAL)</b>	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	
<b>Genotype additional info (OPTIONAL)</b>	Blood group genotyping HLA tissue typing	Not performed Not performed	

inverted epifluorescence microscope (DMi8, Leica).

### 3.3. Cytometry

Cells were detached with accutase (10 min of incubation at 37 °C). Then, cells were fixed with a 1 % formaldehyde solution for 20 min and permeabilized using 0.1 % TritonX-100. Then, cells were labelled fluorescent-coupled antibodies (see [Table 2](#)) for 15 min, centrifuged (300 g, 5 min), washed in 0.5 % BSA and resuspended in the same buffer before analysis on a MACSQuant10 cytometer (Miltenyi Biotec). Analysis was performed using the FlowJo™ v.10.6.1.

### 3.4. Karyotyping

At least 2 passages after thawing cells were karyotyped by Cell Guidance systems (UK) and at least 10 metaphases were counted.

### 3.5. Differentiation assay

STEMdiff Trilineage Differentiation Kit (Cat. # 05230, StemCell Technologies) was used according to the manufacturer's instructions, at a density of 400,000 cells/well for the ectoderm and endoderm conditions and 100,000 cells/well for the mesoderm condition. Differentiated cells were then collected for RNA isolation using TRIzol. RevertAid First Strand cDNA Synthesis Kit was used to generate cDNA using random hexamer primers and expression of lineages markers ([Table 2](#)) was performed in SYBR Green Master Mix (Roche) on a LightCycler480 (Roche).

### 3.6. Genomic DNA extraction

Genomic DNA was extracted using the Genelute Mammalian

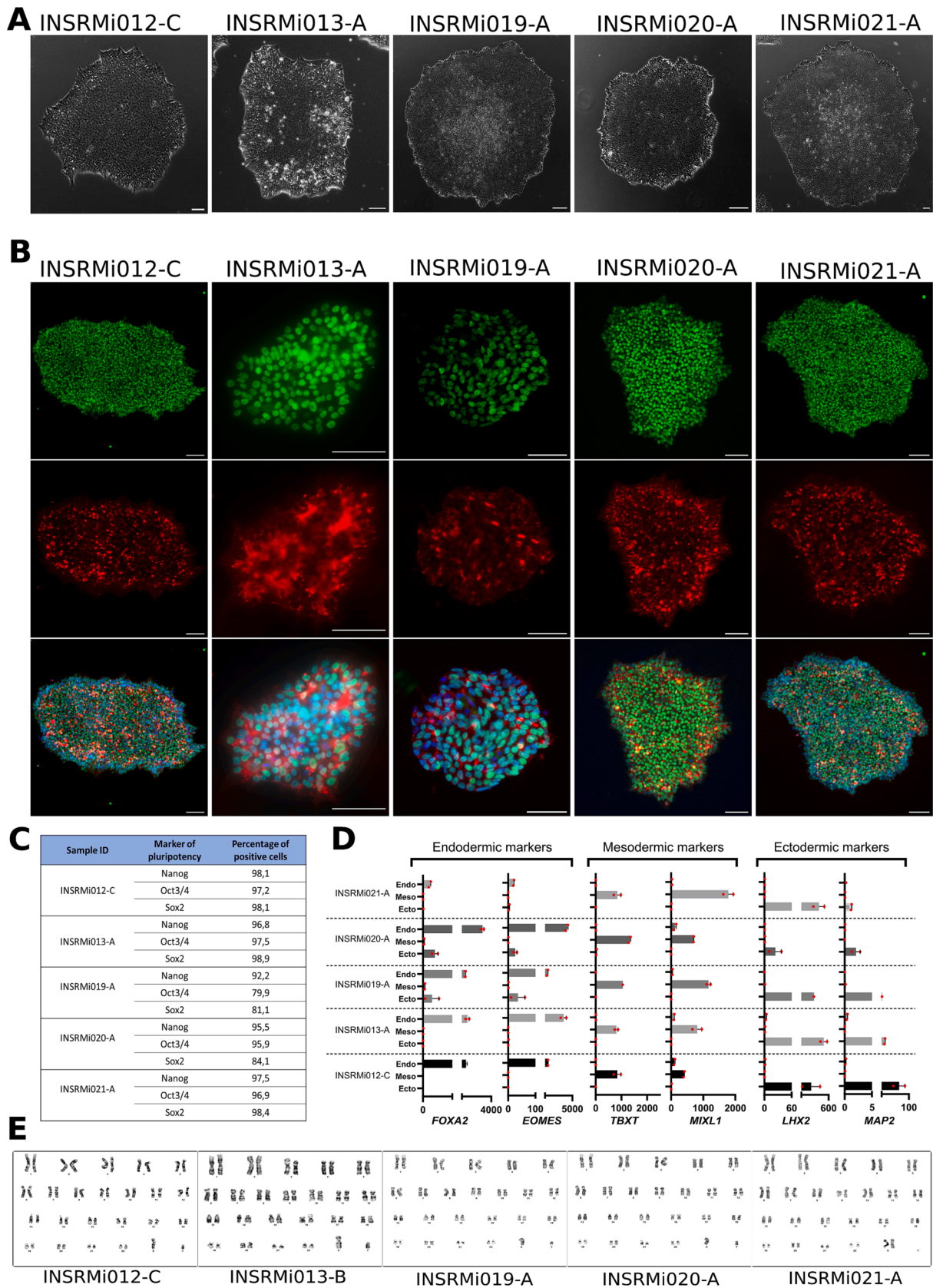


Fig. 1.

**Table 2**  
Reagents details.

	Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat #	RRID
Pluripotency Markers	Rabbit (IgG1) anti Nanog clone D73G4	1:200	Cell Signaling #4903	RRID: AB_10559205
Pluripotency Markers	Mouse monoclonal (IgM) anti Tra-1-81	1:100	Merck #MAB4381	RRID: AB_177638
Pluripotency Markers	PE Mouse anti-Human Nanog (clone N31-355)	1:10	BD Biosciences Human Pluripotent Stem Cell Transcription Factor Analysis Kit #51-9006414	RRID: AB_2722505
Pluripotency Markers	PerCP-Cy <sup>TM</sup> 5.5 Mouse anti-Oct3/4 (clone 40/oct-3)	1:10	BD Biosciences Human Pluripotent Stem Cell Transcription Factor Analysis Kit #51-9006267	RRID: AB_2722505
Pluripotency Markers	Alexa Fluor® 647 Mouse anti-Sox2	1:10	BD Biosciences Human Pluripotent Stem Cell Transcription Factor Analysis Kit #51-9006407	RRID: AB_2722505
Isotype control antibodies	PE Mouse IgG1, κ Isotype Control	1:10	BD Biosciences Human Pluripotent Stem Cell Transcription Factor Analysis Kit #51-9006406	RRID: AB_2722505
Isotype control antibodies	PerCP-Cy <sup>TM</sup> 5.5 Mouse IgG1, κ Isotype Control	1:10	BD Biosciences Human Pluripotent Stem Cell Transcription Factor Analysis Kit #51-9006272	RRID: AB_2722505
Isotype control antibodies	Alexa Fluor® 647 Mouse IgG2a, κ Isotype Control	1:10	BD Biosciences Human Pluripotent Stem Cell Transcription Factor Analysis Kit #51-9006410	RRID: AB_2576217
Secondary antibodies	Goat anti-Rabbit IgG (H + L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	1:1000	ThermoFisher Scientific #A-11034	RRID: AB_2535807
Secondary antibodies	Goat anti-Mouse IgM (Heavy Chain) Secondary Antibody, Alexa Fluor 647	1:1000	ThermoFisher Scientific #A-21238	

**Table 2 (continued)**

Antibodies used for immunocytochemistry/flow-cytometry			
Antibody	Dilution	Company Cat #	RRID
Primers			
Target	Size of band	Forward/Reverse primer (5'-3')	
Differentiation markers	FOXA2	138 bp	AGCCCGAGGGCTACTCC/ACGACATGTCATGGAGCCC
Differentiation markers	EOMES	90 bp	TACCCAGACCCAACCTTTC/ GGAGGTCCATGGTAGTCCAG
Differentiation markers	BRACHYURY	121 bp	CCAGTGGCAGTCTCAGGTTA/CTACTGCAGGTGTGAGCAAG
Differentiation markers	MIXL1	95 bp	AGTCCAGGATCCAGGTATGGT/GATAATCTCCGGCCTAGCCA
Differentiation markers	LHX2	199 bp	GGGTCCTCCAGGTCTGGTTC/GTGGGGCTAGTCAAGTCTGTG
Differentiation markers	MAP2	157 bp	GGCCAACCTTCTCCAGTGAAC/TGCTGGAACCTCAGCAGGTAA
House-Keeping Genes (qPCR)	NUBP1	88 bp	CAGAGGGGCTTATGTATCAGG/GATTTCTCTATAGCCGTGTCC
Targeted mutation analysis/sequencing	PCR/Sequencing primer for DESD214-E245Del and E245D		TATCACCCGCAACTGTCTGT/CGTCGTTGTTCTTGTGGCT
Targeted mutation analysis/sequencing	PCR/Sequencing primer for DESS46Y		CCCGCCGCTCCTCCGTG/GAAAAGAGAGTCTGGGGTGC
Targeted mutation analysis/sequencing	PCR/Sequencing primer for DESP419H		GGAGGTTTGTCTCTCCCTTT/CTCTACCTAAACTCCCCACCT
Targeted mutation analysis/sequencing	PCR/Sequencing primer for DESE439K		ACCCTGTTACAGAAACCAGCC/AACCATGTGCTAGCCAGAGAAT

Genomic DNA kit (Sigma) and was amplified using specific primers (Table 2). After migration into an 0.1 % agarose gel, bands were purified using the NucleoSpin Gel and PCR clean-up kit (Macherey Nagel) following the manufacturer's instructions.

### 3.7. Sequencing

Genomic DNA was sequenced by Eurofins Genomics using the primers indicated in Table 2.

### 3.8. SNP analysis

Genomic DNA (at least 500 ng) was analysed for SNP by Integragen (Evry, France) using Illumina-Infinium Core24 technology. Results were analyzed using Genome Studio by comparing the SNP profile of the clone to the profile of the corresponding genomic DNA extracted from PBMC of the patient to verify cell identity and genomic integrity.

### 3.9. Mycoplasma checking

Supernatant medium (500 µL) from 80 to 90 % confluency iPSC were boiled at 95 °C for 10 min, centrifuge at 10,000 g to pellet cellular debris and analyzed by eurofins genomics. Reports were deposited as archive with the manuscript.

### Credit authorship contribution statement

**Pierre Joanne:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

**Yeranuhi Hovhannisyan:** Data curation, Investigation, Methodology, Writing – review & editing, Formal analysis, Validation, Visualization. **Alexandre Simon:** Data curation. **Gaëlle Revet:** Data curation. **Romain Diot:** Data curation. **Gabriel Friob:** Data curation. **Denisa Calin:** Data curation, Formal analysis. **Zhenlin Li:** Data curation, Formal analysis, Methodology. **Anthony Béhin:** Resources. **Karim Wahbi:** Resources. **Gérard Tachdjian:** Data curation, Formal analysis, Investigation. **Onnik Agbulut:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing – review & editing, Funding acquisition, Project administration, Visualization.

#### 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.

#### Acknowledgements

This research was partly supported by Sorbonne Université, France, and CNRS France, and INSERM, France. OA and PJ are supported by the AFM-Téléthon, France (Association Française contre les Myopathies) (contract number: 22142 & 28597). YH was funded by a Ph.D. fellowship from the AFM-Téléthon, France during this study. We thank Dr Brigitte Onteniente and Phenocell SAS (Grasse, France) for iPSC generation from MFM1 patients; Stéphanie Bigou and iPS cell culture facility of the Paris Brain Institute who has received funding from the program “Investissements d’avenir” ANR-10- IAIHU-06 for the clonal

selection of iPSC; Dr. Nathalie Lefort (Institut Imagine, France) for her assistance concerning the analysis of SNP profiles and all personnel from the IBPS photon microscopy core facility for helpful advice and technical assistance during flow cytometry.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scr.2024.103338>.

#### References

- Brodehl, A., Gaertner-Rommel, A., Milting, H., 2018. Molecular insights into cardiomyopathies associated with desmin (DES) mutations. *Biophys. Rev.* 10, 983–1006.
- Hovhannisyan, Y., Li, Z., Callon, D., Suspène, R., Batoumeni, V., Canette, A., Blanc, J., Hocini, H., Lefebvre, C., El-Jahrani, N., et al. (2023). Critical contribution of mitochondria in the development of cardiomyopathy linked to desmin mutation.
- Joanne, P., Chourbagi, O., Hourdé, C., Ferry, A., Butler-Browne, G., Vicart, P., Dumonceaux, J., Agbulut, O., 2013. Viral-mediated expression of desmin mutants to create mouse models of myofibrillar myopathy. *Skelet. Muscle* 3, 4.
- van Spaendonck-Zwarts, K., van Hessem, L., Jongbloed, J.D.H., de Walle, H.E.K., Capetanaki, Y., van der Kooi, A.J., van Langen, I.M., van den Berg, M.P., van Tintelen, J.P., 2010. Desmin-related myopathy: a review and meta-analysis. *Clin. Genet.*
- Wahbi, K., Béhin, A., Charron, P., Dunand, M., Richard, P., Meune, C., Vicart, P., Laforêt, P., Stojkovic, T., Bécan, H.M., et al., 2012. High cardiovascular morbidity and mortality in myofibrillar myopathies due to DES gene mutations: a 10-year longitudinal study. *Neuromuscul. Disord.* 22, 211–218.
- Winter, L., Wittig, I., Peeva, V., Eggers, B., Heidler, J., Chevessier, F., Kley, R.A., Barkovits, K., Strecker, V., Berwanger, C., et al., 2016. Mutant desmin substantially perturbs mitochondrial morphology, function and maintenance in skeletal muscle tissue. *Acta Neuropathol.* 132, 453–473.