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# Generation of a patient-specific induced pluripotent stem cell line carrying the DES p.R406W mutation, an isogenic control and a DES p.R406W knock-in line

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## ABSTRACT

Mutations in the *DES* gene, which encodes the intermediate filament desmin, lead to desminopathy, a rare disease characterized by skeletal muscle weakness and different forms of cardiomyopathies associated with cardiac conduction defects and arrhythmias. We generated human induced pluripotent stem cells (hiPSC) from a patient carrying the DES p.R406W mutation, and employed CRISPR/Cas9 to rectify the mutation in the patient's hiPSC line and introduced the mutation in an hiPSC line from a control individual unrelated to the patient. These hiPSC lines represent useful models for delving into the mechanisms of desminopathy and developing new therapeutic approaches.

## 1. Resource Table:

Unique stem cell line identifier	1. ITXi013-A ITXi013-B ITXi006-A-1
Alternative name(s) of stem cell line	PT-R406W (ITXi013-A) IC-R406 (ITXi013-B) IM-R406W (ITXi006-A-1)
Institution	L'institut du thorax (INSERM UMR1087, CNRS UMR6291, Nantes, France)
Contact information of the reported cell line distributor	Flavien Charpentier, Flavien. charpentier@univ-nantes.fr
Type of cell line	Induced pluripotent stem cells (iPSCs)
Origin	Human
Additional origin info (applicable for human ESC or iPSC)	Age: 22 Sex: Female Ethnicity: European
Cell Source	Peripheral blood mononuclear cells (PBMCs)
Method of reprogramming	Integration-free Sendai virus expressing human <i>OCT4</i> , <i>SOX2</i> , <i>KLF4</i> , and <i>c-MYC</i>
Clonality	Clonal

(continued on next column)

(continued)

Unique stem cell line identifier	1. ITXi013-A ITXi013-B ITXi006-A-1
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-qPCR
The cell culture system used	StemMACS™ iPS Brew XF Medium on Matrigel® hESC-Qualified Matrix
Type of the Genetic Modification	PT-R406W: spontaneous/naturally occurred mutation IC-R406: mutation correction of PT-R406W hiPSCs by CRISPR/Cas9 IM-R406W: induced mutation by CRISPR/Cas9 in control hiPSCs
Associated disease	Cardiomyopathy associated with desmin ( <i>DES</i> ) mutation OMIM: 601419 / 125660
Gene/locus modified in the reported transgenic line	PT-R406W and IM-R406W: <i>DES</i> (GRCh38 2q35: 219418377–219426734) exon 6, NM_001927.4(DES):c.1216C > T (p.R406W)

IC-R406: *DES* (GRCh38 2q35: 219418377–219426734) exon 6,

(continued on next page)

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(continued)

Unique stem cell line identifier	1. ITXi013-A ITXi013-B ITXi006-A-1
	NM_001927.4(DES):c.1216 T > C (p.R406W)
Method of modification / user-customisable nucleases (UCN) used, the resource used for design optimisation	HiFi-CRISPR/Cas9 endonuclease
User-customisable nuclease (UCN) delivery method	Electroporation of Cas9-RNP
All double-stranded DNA genetic material molecules introduced into the cells	Single-stranded oligo DNA nucleotide (ssODN) CRISPR/Cas9
Evidence of the absence of random integration of any plasmids or DS DNA introduced into the cells.	Whole genome sequencing (WGS)
Analysis of the nuclease-targeted allele status	WGS
Homozygous allele status validation	Sanger sequencing and WGS
Method of the off-target nuclease activity prediction and surveillance	WGS
Descriptive name of the transgene	N/A
Eukaryotic selective agent resistance cassettes (including inducible, gene/cell type-specific)	N/A
Inducible/constitutive expression system details	N/A
Date archived/stock creation date	2020/01/03
Cell line repository/bank	PT-R406W: <a href="https://hpscereg.eu/cell-line/ITXi013-AIC-R406">https://hpscereg.eu/cell-line/ITXi013-AIC-R406</a> : <a href="https://hpscereg.eu/cell-line/ITXi013-B">https://hpscereg.eu/cell-line/ITXi013-B</a> IM-R406W: <a href="https://hpscereg.eu/cell-line/ITXi006-A-1">https://hpscereg.eu/cell-line/ITXi006-A-1</a>
Ethical/GMO work approvals	A signed informed consent has been received. Authorization has been obtained from competent ethics review board (CPP Ouest II n° 2010-33) and approval authority (MESR n°DC2011-1399) to generate these lines.
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	N/A

## 2. Resource utility

The patient generated hiPSC line harbouring the desmin gene (*DES*) p.R406W mutation, along with the isogenic control and isogenic mutant lines are a useful resource for investigating the function of the desmin protein in derived cardiomyocytes. Desmin mutations have previously been linked to cardiac and skeletal disorders.

## 3. Resource Details

Desmin is a type III intermediate filament that is encoded by *DES* and is found in smooth, skeletal and cardiac muscle cells. Desmin is important for interconnecting essential organelles to one another as well as to the cell membrane in order to provide support and maintain the structural integrity of the cells. Mutations in *DES* have been shown to cause skeletal and/or cardiac myopathies (Capetanaki et al., 2007). Histology experiments that have been performed on patient skeletal and cardiac muscle samples most often reveal dense cytoplasmic aggregates (Tsikitis et al., 2018). *In vitro* studies have shown that mutations in *DES* impact the formation of proper filaments (Bär et al., 2005).

A nine-year old female patient presented with sudden cardiac death (SCD) and a persistent ST-segment depression on her electrocardiogram

(ECG). Genetic testing revealed a *de novo* mutation in *DES* at c.1216C > T (p.R406W), while the patients parents were negative for the mutation (Fig. 1A). The mutation (c.1216C > T) is located in exon 6 that codes for the highly conserved segment 2B of the desmin filament (Fig. 1B) and has been previously reported in patient cases with cardiac disorders (Brodehl et al., 2018).

At the age of 22, the patient's peripheral blood mononuclear cells (PBMCs) were collected and hiPSC lines were generated using the Sendai virus method. We generated an isogenic control line (IC-R406) from the patients hiPSC line (PT-R406W) as well as an isogenic mutant line (IM-R406W) from the WT8288 (ITXi006-A) line that was published previously (Girardeau et al., 2022). Both isogenic lines were created using the CRISPR/Cas9 technology as previously published (Caillaud et al., 2022).

The four hiPSC lines were cultured on Matrigel® coated plates in StemMACS™ iPS-Brew XF media. Mycoplasma contamination assessment was performed by Eurofins genomics and no contamination has been identified in any of the lines (Supplementary Table 1). All hiPSC clones showed typical colony morphology that was assessed by bright-field microscopy (Fig. 1C, left panel). Expression of pluripotency markers OCT3/4 and TRA-1-60 was evaluated by immunofluorescence experiments (Fig. 1C). Pluripotency status of hiPSCs was likewise quantitatively assessed by RT-qPCR using *SOX2*, *NANOG* and *POU5F1* markers (Fig. 1D). The absence of Sendai virus persistence following reprogramming of PT-R406W clone was shown (Fig. 1E). Each cell line was differentiated into endoderm, mesoderm, and ectoderm using the Miltenyi Biotec Trilineage differentiation kit. The ability of PT-R406W, IC-R406 and IM-R406W hiPSCs to be able to differentiate into derivatives of the three germ layers was validated by RT-qPCR with *PAX6* as the ectoderm marker, *FOXA2* as the endoderm marker, and *HAND1* as the marker for the mesoderm layer (Fig. 1F). Furthermore, no major chromosomal aberrations were found between (1) the control (WT8288) and the CRISPR/Cas9 generated isogenic mutant as well as (2) the patient cells, the patient hiPSC line and the CRISPR/Cas9 generated isogenic control clone (Supplementary Fig. 1) Finally, relatedness between clones was validated from the WGS data using Somalier tool (Supplementary Table 2). Detailed information for all lines is provided in Tables 1 and 2.

## 4. Materials and methods

### 4.1. HiPSC Generation and Maintenance

After Sendai virus reprogramming and CRISPR/Cas9 based editing, all hiPSC lines were maintained at 37 °C, 5 % CO<sub>2</sub>, 21 % O<sub>2</sub> in StemMACS™ iPS Brew XF Medium (Miltenyi Biotec) and on Matrigel® hESC-Qualified Matrix (0.05 mg/mL, Corning). At 80 % confluency, cells were passaged as clusters using Gentle Cell Dissociation Reagent (STEMCELL Technologies).

### 4.2. CRISPR/Cas9 Genome Editing

The gRNA, gRNA\_KI\_DES, was first duplexed with ATTO™ 488-tagged transactivating RNA and then complexed to the Alt-R® HiFi Cas9 Nuclease according to IDT recommendations. The complex and a specific ssODN donor (either *DES\_Donor\_Mut* or *DES\_Donor\_c.1216C-T*) was delivered into hiPSCs using the P3 Primary Cell 4D-Nucleofector™ Kit L with the Amaxa nucleofector (CA137, Lonza). hiPSCs double positive for ATTO™488 and Alexa Fluor®660, and negative for DAPI, were single-cell FACS-sorted 48 h post-transfection (BD FACSMelody™ cell sorter, BD). Cells were maintained in iPS Brew until they reached 80 % confluency and then used for cloning, amplification and duplication for banking and genotyping.

### 4.3. DNA Extraction, PCR, Sanger Sequencing and WGS

The genomic DNA was isolated using the NucleoSpin Tissue

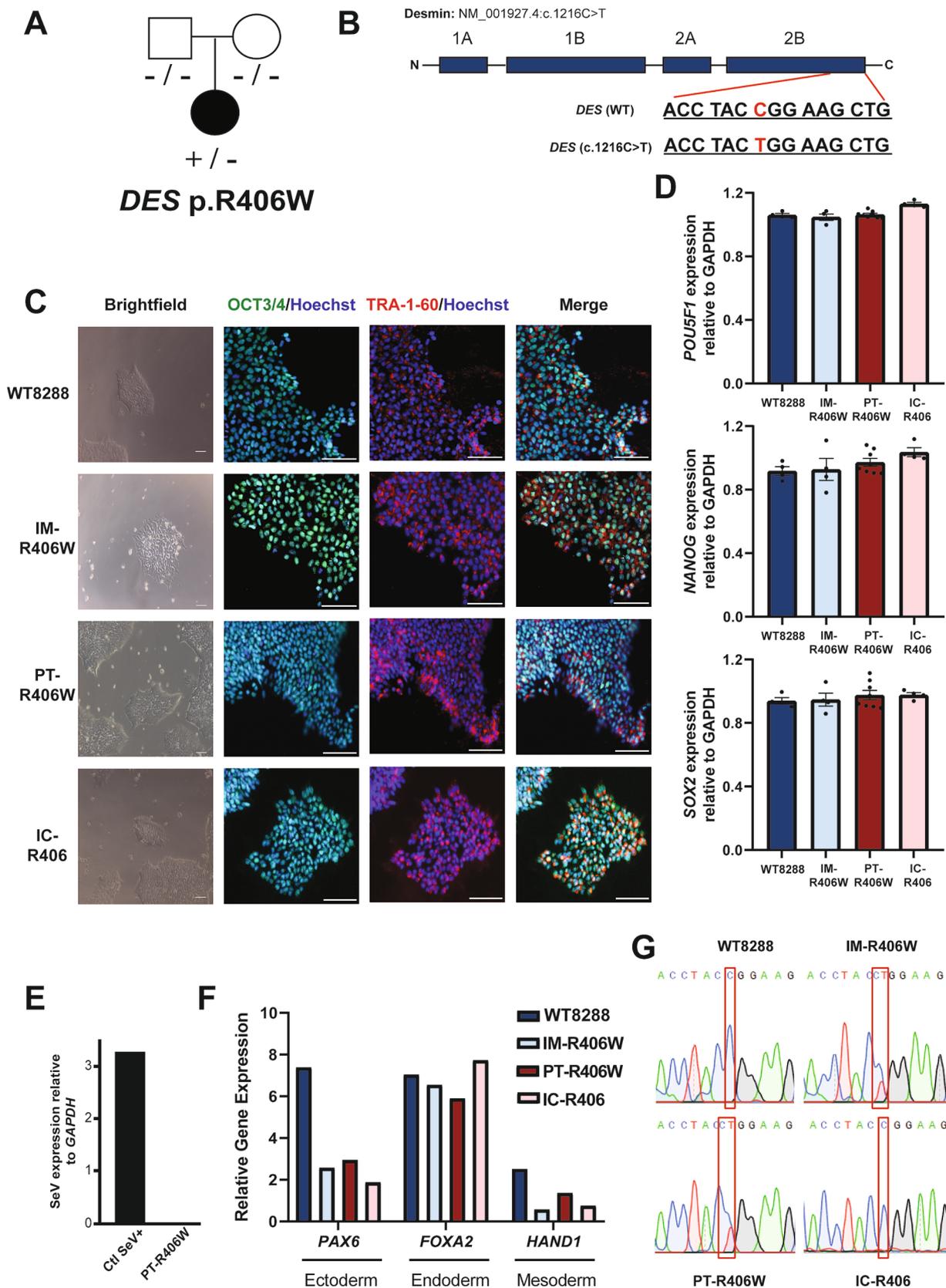


Fig. 1.

**Table 1**  
Characterization and validation.

Classification	Output type	Result	Data
Schematic of a transgene/genetic modification	Schematic representation	Visual representation of the desmin protein structure and the location of the mutation on segment 2B. The c.1216 C > T or c.1216 T > C transition causes a change in amino acids p.R406 to R406W and vice versa.	Fig. 1B
Morphology	Photography	Normal	Brightfield images Fig. 1C Scale bar = 100 $\mu$ m
Pluripotency status evidence for the described cell line	Qualitative analysis (Immunofluorescence)	Positive expression of pluripotency markers: OCT3/4 and TRA-1-60	Fig. 1C Scale bar = 100 $\mu$ m
	Quantitative analysis (RT-qPCR)	Stable expression of pluripotency markers throughout cell lines: <i>SOX2</i> , <i>POU5F1</i> , and <i>NANOG</i>	Fig. 1D
Karyotype	WGS, copy number variations (CNV)	No evidence for CNVs	Supplementary Fig. 1
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR, Sanger sequencing and WGS (BAM files)	Presence of desired genomic alteration in <i>DES</i> c.1216C > T or c.1216 T > C	Fig. 1G
	PCR, Sanger sequencing and WGS (BAM files)	Successful knock-in of desired nucleotide in the desmin gene	Fig. 1G
	Transgene-specific PCR (when applicable)	N/A	N/A
Verification of the absence of random plasmid integration events	N/A	N/A	N/A
Parental and modified cell line genetic identity evidence	WGS (relatedness based on common variants along the genome)	Edited cell lines matched (100 %) their parental cell line	Supplementary Table 2
		N/A	N/A
Mutagenesis / genetic modification outcome analysis	PCR, Sanger sequencing	Sanger sequencing of alteration: <i>DES</i> (GRCh38 2q35: 219418377–219426734) exon 6, c.1216C > T (p.R406W) and <i>DES</i> (GRCh38 2q35: 219418377–219426734) exon 6, c.1216 T > C (p.R406)	Fig. 1G
		PCR-based analyses	N/A
		Southern Blot or WGS; western blotting (for knock-outs, KOs)	N/A
Off-target nuclease activity analysis	WGS	N/A	N/A
Specific pathogen-free status	Mycoplasma	No contamination was detected in any of the cell lines	Supplementary Table 1
Multilineage differentiation potential	Directed trilineage differentiation	Miltenyi Biotec Trilineage differentiation kit and RNA analysis by RT-qPCR. Cell lines were positive for: Mesoderm: <i>HAND1</i> Endoderm: <i>FOXA2</i> Ectoderm: <i>PAX6</i>	Fig. 1F
List of recommended germ layer markers	N/A	N/A	N/A
Outcomes of gene editing experiment (OPTIONAL)	N/A	N/A	N/A
Donor screening (OPTIONAL)	N/A	N/A	N/A
Genotype - additional	N/A	N/A	N/A
histocompatibility info (OPTIONAL)	N/A	N/A	N/A

Purification Kit (MACHEREY-NAGEL). The DNA fragment of *DES* containing the knock-in was amplified by PCR using 10 ng/ $\mu$ l of genomic DNA with specific forward and reverse primers (Table 2). The PCR products were visualized on 1.5 % agarose gel and sequenced by Eurofins Genomics.

To visualize any large CNVs that would have occurred in our cell lines, CRAM files were filtered for primary reads having a mapping quality greater than 30 and were reindexed. The resulting BAM files were processed with indexcov (<https://pubmed.ncbi.nlm.nih.gov/29048539/>). The relatedness between cell lines was validated using Somalier (<https://pubmed.ncbi.nlm.nih.gov/32664994/>). Analysis was processed using Nextflow (<https://pubmed.ncbi.nlm.nih.gov/28398311/>) and our local pipeline (<https://github.com/lindenb/gazoduc-nf/>).

#### 4.4. Trilineage Differentiation

HiPSC lines were differentiated into the 3 germ layers using the STEMdiff™ Trilineage Differentiation Kit (Miltenyi Biotec) according

to the manufacturer's instructions. RNA was extracted using NucleoSpin RNA kit (MACHEREYNAGEL). The three differentiation-markers-Taqman™ probes that were used are listed in Table 2.

#### 4.5. RT-qPCR

RNA was extracted from samples as described above and reverse transcribed using High-Capacity cDNA Reverse Transcription kit (Applied Biosystems™). PCR amplification was performed using FAM-labeled TaqMan probes (Applied Biosystems) for pluripotency and trilineage differentiation (Table 2). Data was normalized to ACTB or GAPDH.

#### 4.6. Immunofluorescence

Cells were washed three times using PBS and fixed with 4 % paraformaldehyde (Sigma-Aldrich) for 20 mins at room temperature (RT). Cells were washed another three times with PBS and permeabilized with 0.5 % Triton X-100 (Sigma-Aldrich) for 15 mins at RT and then blocked

**Table 2**  
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Mouse anti-TRA-1-60	1:200	Thermo Fisher Scientific Cat# 14-8863-82, RRID: AB_891610
	Rat anti-OCT3/4	1:200	Thermo Fisher Scientific Cat# 14-5841-82, RRID: AB_914301
Nuclear Stain	Hoechst 33,342	1:500	Thermo Fisher Scientific Cat# 62,249
Secondary antibodies	Goat anti-rat 488 nm	1:1000	Invitrogen Cat# A11006, RRID: AB_2534074
	Donkey anti-mouse 568 nm	1:1000	Invitrogen Cat# A10037, RRID: AB_2534013
<b>Site-specific nuclease</b>			
Nuclease information	Alt-R® S.p. HiFi Cas9 Nuclease V3	Integrated DNA Technologies (IDT) Cat# 1,081,061	
Delivery method	Nucleofection	P3 Primary Cell 4D-Nucleofector™ X Kit L, Lonza, Cat# V4XP-3024	
Selection/enrichment strategy	FACS sorting using transactivator-associated fluorescence	Transactivator: Alt-R® CRISPR-Cas9 tracrRNA ATTO™ 488, IDT ssODN: Single-stranded DNA oligo Alexa Fluor® 660, IDT	
<b>Primers and Oligonucleotides used in this study</b>			
	<b>Target</b>	<b>Forward/Reverse primer (5'-3')</b>	
Differentiation markers (qPCR)	<i>PAX6</i>	Thermo Fisher Scientific TaqMan®probe ID: Hs01088114_m1	
	<i>FOXA2</i>	Thermo Fisher Scientific TaqMan®probe ID: Hs00232764_m1	
	<i>HAND1</i>	Thermo Fisher Scientific TaqMan®probe ID: Hs02330376_s1	
Pluripotency Markers (qPCR)	<i>SOX2</i>	Thermo Fisher Scientific TaqMan®probe ID: Hs01053049_s1	
	<i>POU5F1</i>	Thermo Fisher Scientific TaqMan®probe ID: Hs04260367_gH	
	<i>NANOG</i>	Thermo Fisher Scientific TaqMan®probe ID: Hs02387400_g1	
House-Keeping Genes (qPCR)	<i>ACTB</i>	Thermo Fisher Scientific TaqMan®probe ID: Hs99999903_m1	
	<i>GAPDH</i>	Forward: 5'-AAT CCC ATC ACC ATC TTC CA-3' Reverse: 5'TGG ACT CCA CGA CGT ACT CA-3'	
Primers for PCR and Sanger sequencing	Desmin	Forward 1: 5'-GAA ATC CGG CAC CTC AAG-3' Reverse 1: 5'-CAG GTG GCC TTG GTT AAT TC-3' Forward 2: 5'-CAG TGG CTA CCA GGA CAA CA-3' Reverse 2: 5'-CCT GGG GAC AGA AAT GGA C-3'	
Potential random integration-detecting PCRs	N/A	N/A	
Sendai Virus Detection (primer)	SeV	Forward: 5'-GGA TCA CTA GGT GAT ATC GAG C-3' Reverse: 5'-ACC AGA CAA GAG TTT AAG AGA TAT GTA TC-3'	
gRNA oligonucleotide	gRNA_KI_DES	5'-ATG TGG AGA TTG CCA CCT AC-3'	
Genomic target sequence(s)	N/A	N/A	
Bioinformatic gRNA on- and off-target binding prediction tool used, specific sequence/ outputs link(s)	IDT	<a href="https://eu.idtdna.com/site/order/designtool/index/CRISPR_CUSTOM">https://eu.idtdna.com/site/order/designtool/index/CRISPR_CUSTOM</a>	
Primers for top off-target mutagenesis predicted site sequencing (for all CRISPR/Cas9, ZFN and TALENs)	N/A	N/A	
ssODN guides for HDR-mediated mutagenesis	DES_Donor_Mut (induction of the mutation)	5'-AGGACCTGCTCAACGTGAAGATGGCCCTGGATGTGGAGATT GCCACCTACTGGAAGCTGCTGGAGGGAGAGAGAGCCGGTGAGGGGCCAGGCAGGAGCC-3'	
	DES_Donor_c.1216C-T (correction of the mutation)	5'- AGGACCTGCTCAACGTGAAGATGGCCCTGGATGTGGAGATTG CCACCTACCGGAAGCTGCTGGAGGGAGAGAGAGCCGGTGAGGGGCCAGGCAGGAGCC-3'	

with 3 % BSA at RT for 30 mins. Primary antibodies in 1 % BSA were applied to cells and incubated for 2 h at RT. Following three washing steps with PBS, cells were incubated for 1 h at RT with secondary antibodies and then washed again three times with PBS and kept in 0.5 % paraformaldehyde at 4 °C. The next day, cells were imaged using Eclipse Ti2 fluorescence microscope (Nikon) using the Nikon Standard software. Antibody information is listed in [Table 2](#).

#### 4.7. Mycoplasma Detection

Mycoplasma contamination assessment was performed by Eurofins genomics ([Supplementary Table 1](#)).

#### CRedit authorship contribution statement

**Michelle Geryk:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Robin Canac:** Data curation, Investigation, Methodology, Writing – review & editing. **Virginie Forest:** Investigation, Methodology, Writing – review & editing. **Pierre Lindenbaum:** Data curation, Investigation, Methodology, Writing – review & editing. **Aurore Girardeau:** Investigation, Methodology, Writing – review & editing. **Manon Baudic:** Investigation, Methodology, Writing – review & editing. **Estelle Baron:** Investigation, Methodology, Writing – review & editing. **Anne Bibonne:**

Investigation, Methodology. **Caroline Chariou:** Investigation, Methodology. **Florence Kyndt:** Data curation, Investigation. **Richard Redon:** Funding acquisition, Validation, Writing – review & editing. **Jean-Jacques Schott:** Supervision, Writing – review & editing. **Jean-Baptiste Gourraud:** Conceptualization, Data curation, Funding acquisition, Supervision, Writing – review & editing. **Julien Barc:** Supervision, Writing – review & editing. **Flavien Charpentier:** Conceptualization, Funding acquisition, Project administration, Validation, Writing – review & editing.

#### 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.2024.103396>.

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