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Lab Resource: Genetically-Modified Multiple Cell Lines

Generation of retinitis pigmentosa patient-derived hiPSC lines (IDVi007-A, IDVi007-B) carrying the RHO c.68C > A variant (p.P23H) and CRISPR/Cas9-corrected isogenic hiPSC lines (IDVi007-A-1, IDVi007-A-2, IDVi007-A-3)

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A B S T R A C T

The p.Pro23His (c.68C > A; P23H) mutation leads to autosomal dominant retinitis pigmentosa (adRP). Here, we reprogrammed adRP patient fibroblasts in human induced pluripotent stem cells (hiPSCs) using Sendai virus. We then generated two mutated hiPSC clones and three isogenic controls using CRISPR/Cas9. All five hiPSC lines express pluripotency genes and are able to differentiate into the three germ layers as well as retinal organoids. Altogether, these hiPSCs constitute unique biological tools to elucidate mechanisms of adRP linked to the RHO-P23H mutation.

Resource Table

Unique stem cell line identifiers	IDVi007-A; IDVi007-B; IDVi007-A-1; IDVi007-A-2; IDVi007-A-3
Alternative name(s) of stem cell lines	1U-RHO-P23H-01 (IDVi007-A); 1U-RHO-P23H-02 (IDVi007-B); 1U-iso-RHO-D2 (IDVi007-A-1); 1U-iso-RHO-G2 (IDVi007-A-2); 1U-iso-RHO-H6 (IDVi007-A-3)
Institution	Institut de la Vision
Contact information of the reported cell line distributor	sacha.reichman@sorbonne-universite.fr
Type of cell lines	Human iPSCs (hiPSCs)
Origin	*Human*
Additional origin info *(applicable for human ESC or iPSC)*	Age: AdultSex: XX
Cell Source	Skin fibroblasts
Method of reprogramming (if applicable)	Sendai viruses
Evidence of the reprogramming	N/A

(*continued on next column*)

Resource Table (*continued*)

Unique stem cell line identifiers	IDVi007-A; IDVi007-B; IDVi007-A-1; IDVi007-A-2; IDVi007-A-3
transgene loss (including genomic copy absence/removal/ silencing, if applicable) (if applicable)	
The cell culture system used	Vitronectin-coated culturesFeeder free conditionEnzymatic passaging
Genetic Features: Introduced and Pre-Existing	Pre-existing: p.Pro23His (P23H) patient mutation; *c.68C > A*Introduced (corrected): c.68A > C (His to Pro) and PAM modification: AGC (Ser) to TCT (Ser).
Associated disease	Class II RHO associated retinitis pigmentosaOMIM: 180380
Gene/locus modified in the reported transgenic lines	*Gene:* RHODOPIN MIM: *180380 Location: 3q22.1 Locus ID:* 6010
Method of modification / user-customizable nucleases (UCN) used, *in*	CRISPR/Cas9 system CRISPOR

(*continued on next page*)

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Resource Table (*continued*)

Unique stem cell line identifiers	IDVi007-A; IDVi007-B; IDVi007-A-1; IDVi007-A-2; IDVi007-A-3
***silico* resources used for design optimization**	
User-customizable nuclease (UCN) delivery method	Nucleofection with RNP
All double-stranded DNA genetic material molecules are administered to the cells	HDR template = CCCTAACTTCTACGTGCCC TTCTCCAATGCGACGGGTG TGGTACGCtctCcCTTCGAGTACCCACAGTACT ACCTGGCTGAGCCATGGCA GTTCTCCATG***(mutated base; silent mutation)***
Evidence of the absence of random integration of any plasmids or dsDNA introduced into the cells	*N/A*
Analysis of the resulting nuclease-targeted allele status	Genomic DNA PCR followed by Sanger sequencingPrimer Fwd = GGCACAGAAGGCCCTAACTTPrimer Rev = GGACCATGAAGAGGTCAGCC
Homozygous allele status validation	1U-RHO-P23H-01 (IDVi007-A) Heterozygosity c.68C > A1; U-RHO-P23H-02 (IDVi007-B) Heterozygosity c.68C > A; 1U-iso-RHO-D2 (IDVi007-A-1) Homozygosity c.68C > A; 1U-iso-RHO-G2 (IDVi007-A-2) Hemizygosity c.68C > A; 1U-iso-RHO-H6 (IDVi007-A-3) Hemizygosity c.68C > A
Method of the off-target nuclease activity prediction and surveillance	N/ACRISPOR analysis, Supplementary Table 2. No off-target site to verify less than 3 mismatches in exon.
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	*Date cell lines archived or deposited in a repository 01/ 2021 and 11/2024*
Cell line repository/bank	*https://hpscreg.eu/cell-line/IDVi007-A* *https://hpscreg.eu/cell-line/IDVi007-B* *https://hpscreg.eu/cell-line/IDVi007-A-1* *https://hpscreg.eu/cell-line/IDVi007-A-2* *https://hpscreg.eu/cell-line/IDVi007-A-3*
Ethics/GMO work approvals	*Approval by French regulatory agencies. IDVi007-A and IDVi007-B hiPSC clones were derived from primary Fibroblast cells of a patient in accordance with the French and American bioethics law. The fibroblast obtained from the Columbia University have been sampled and/or collected with prior consent of the donor and that no payment of any form has been provided to the donor for this sample (*Supplementary Document 1*). Handling of donor tissues adhered to the tenets of the Declaration of Helsinki of 1975 and its 1983 revision in protecting donor confidentiality.*
Disclaimers for Addgene/ repository-sourced used recombinant DNA resources (if applicable)	*N/A*

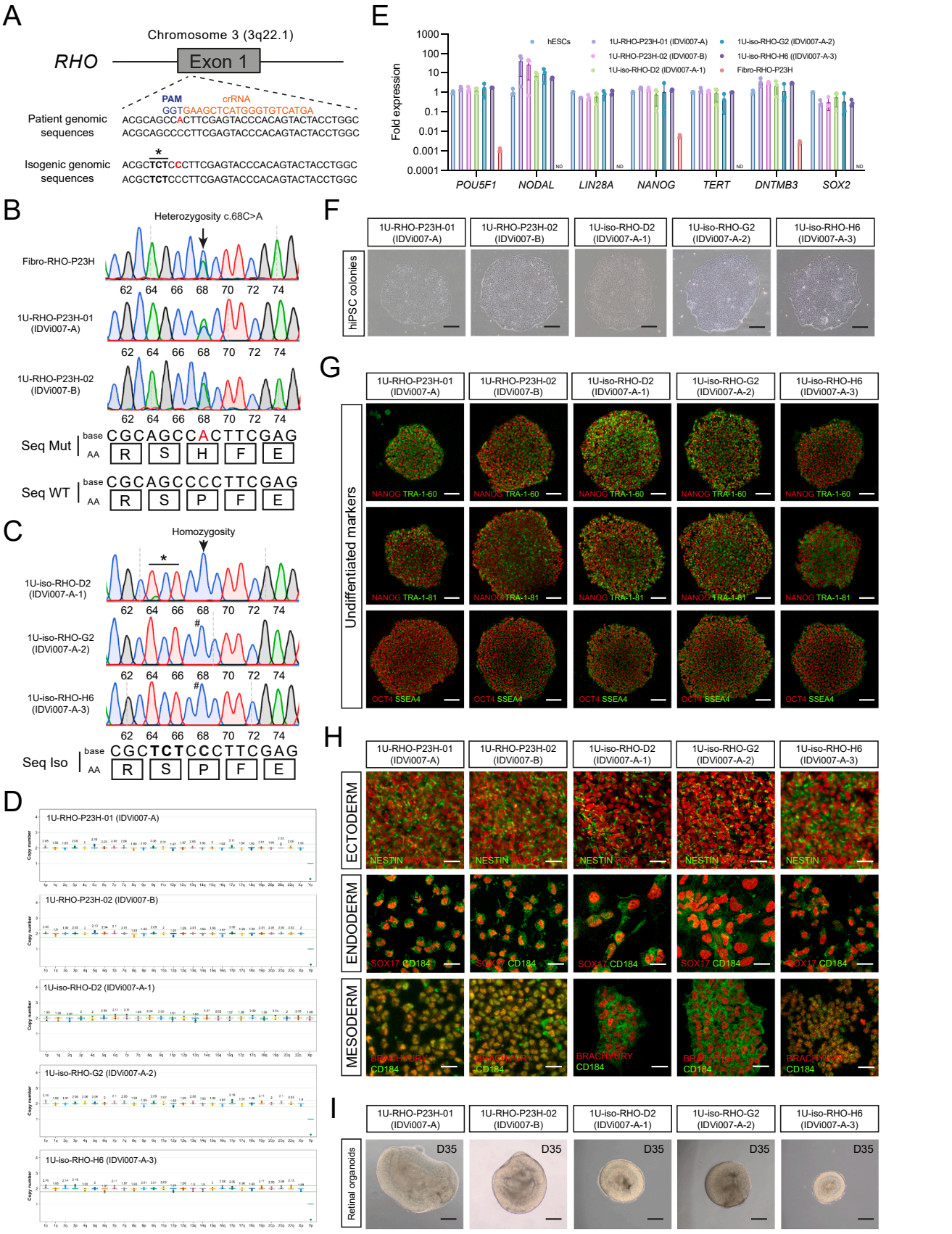
1. Resource utility

Retinitis pigmentosa (RP) is the most common inherited retinal dystrophia affecting 1:3500 people worldwide. The human induced pluripotent cell (hiPSC) lines and the isogenic control clones derived from adRP patient carrying the P23H mutation in the *RHODOPSIN* (RHO-P23H) gene constitute an excellent tool for *in vitro* disease modelling and the development of new treatments.

2. Resource details

Autosomal dominant retinitis pigmentosa (adRP) is one of the most prevalent forms of inherited retinal dystrophy and is frequently caused by pathogenic variants in the *RHODOPSIN* gene (RHO) (Wang et al., 2026). Retinitis Pigmentosa (RP) is characterized by a primary degeneration of rod photoreceptors followed by a secondary degeneration of cone photoreceptors, ultimately resulting in progressive vision loss (Hamel, 2006). In 1990, the substitution of Proline by Histidine at codon 23 of the *RHODOPSIN* gene (RHO-P23H) was the first mutation identified as causative for autosomal dominant retinitis pigmentosa (Dryja et al., 1990), and it remains the most prevalent RHO mutations in North America (Sohocki et al., 2001). RHODOPSIN is a rod photoreceptor-specific G protein-coupled receptor that plays a central role in the initiation of the phototransduction cascade, and the P23H mutation induces a Class II misfolding defect characterized by protein instability and retention within the endoplasmic reticulum, ultimately leading to photoreceptor degeneration (Athanasiou et al., 2018).

In the present study, we obtained adult female fibroblast from a patient diagnosed with adRP carrying the RHO-P23H mutation in the chromosome 3 (Fig. 1, panel A and B). The fibroblasts (Fibro-RHO-P23H) were reprogrammed in hiPSCs using Sendai virus expressing reprogramming factors OCT4, SOX2, KLF4 and c-MYC. Two hiPSC clones were characterized: the 1U-RHO-P23H-01 (IDVi007-A) and 1U-RHO-P23H-02 (IDVi007-B). The heterozygous mutation c.68C > A was verified by Sanger sequencing in the IDVi007-A and IDVi007-B hiPSCs (Fig. 1, panel B). From the 1U-RHO-P23H-01 (IDVi007-A) clone, we generated three isogenic hiPSC lines using CRISPR/Cas9 technology: the 1U-iso-RHO-D2 (IDVi007-A-1), 1U-iso-RHO-G2 (IDVi007-A-2) and 1U-iso-RHO-H6 (IDVi007-A-3) clones. A specific guide RNA (crRNA) was used to target a specific sequence to correct the p.Pro23His (*c.68C > A*) mutation (Fig. 1, panel A). A Cas9 RNP complex – the Cas9 enzyme that recognize the PAM, the crRNA and the HDR template – was nucleofected into hiPSCs. Transfected cells were then seeded at a low density (20 cell/cm^2) and each colony was picked and analysed by Sanger sequencing (Fig. 1, panel C). SNP analysis, used as a karyotyping approach, was performed to compare patient fibroblasts with the derived mutated and corrected (isogenic) hiPSCs. (Supplementary Fig. 1). For the clone 1U-RHO-P23H-01 (IDVi007-A), a 34-kb deletion (chromosome 10) was added to the three abnormalities already detected in the patient fibroblast (Supplementary Fig. 1). No additional abnormalities appeared in the derived isogenic clones from the 1U-RHO-P23H-01 (IDVi007-A) hiPSC clone, but some abnormalities can be larger (Supplementary Fig. 1). The 1U-iso-RHO-D2 (IDVi007-A-1) clone is homozygous following correction of the mutation, while retaining heterozygosity for the dbSNP rs7984 (A/G), located 94 bases upstream (5′) of the corrected c.68C > A site (Supplementary Fig. 2). However, the two other clones, 1U-iso-RHO-G2 (IDVi007-A-2) and 1U-iso-RHO-H6 (IDVi007-A-3), while homozygous after correction of the mutation, lost their heterozygosity at the dbSNP rs7984 (A/G). Therefore, these clones can be regarded as hemizygous if we consider that the maximum size of the deleted region is 1,314 bp (between two identified dbSNPs) encompassing the transcription start site (TSS) and the start codon of RHO (Supplementary Fig. 3). No recurrent genomic abnormalities were detected in mutated and isogenic hiPSC clones using the iCS-digitalTM PSC test capturing over 90% of hot spot defects (Fig. 1, panel D). Parentally between hiPSC clones was certified by Short tandem repeat (STR) analysis (Supplementary Table 1). Analysis of potential off target highlighted no site with less of four mismatch in exon or intron (CRISPOR, Supplementary Table 2). RT-qPCR revealed that hiPSCs express the specific stem genes as *POU5F1, NODAL, LIN28A, NANOG, TERT, DNTMB3 and SOX2* (Fig. 1, panel E). The morphology of the hiPSC colonies is typical of pluripotent stem cells with compact nuclei, a reduced cytoplasm volume and a rounded shape cells (Fig. 1, Panel F). Immunofluorescence assays showed that each hiPSC clones co-expressed undifferentiated markers as OCT4, SSEA4, NANOG, TRA1-60



(caption on next page)

Fig. 1. A. Schematic representation of the CRISPR crRNA, the PAM and the *RHO* exon 1 patient and isogenic genomic sequences. **B.** Electropherograms showing the heterozygous P23H-RHO mutation (arrow, base 68) in patient fibroblasts (Fibro-RHO-P23H) and in the derivate hiPSCs (1U-RHO-P23H-01 (IDVi007-A) and 1U-RHO-P23H-02 (IDVi007-B). **C.** Electropherograms showing the homozygous isogenic clone variants in *RHO* (arrow, base 68) in the isogenic clones. * Silent homozygous mutations added during the gene edition to destroy the PAM sequence. #hemizygous **D.** Genomic stability analysis of the patient-based and isogenic hiPSCs in the most common genetic abnormalities by digital qPCR analysis **E.** RT-qPCR analysis of the pluripotent markers on hESCs, 1U-RHO-P23H-01 (IDVi007-A) 1U-RHO-P23H-02 (IDVi007-B), 1U-iso-RHO-D2 (IDVi007-A-1), 1U-iso-RHO-G2 (IDVi007-A-2), 1U-iso-RHO-H6 (IDVi007-A-3) hiPSC clones, and on primary the patient fibroblast cells (Fibro-RHO-P23H). **F.** Briedfield images of the two patient-based and the three isogenic hiPSC colonies. Scale bars: 100 μm. **G.** Immunofluorescence analysis of the pluripotency markers on the patient- and isogenic-derived hiPSC lines. DAPI stains nuclei. Scale bars: 100 μm. **H.** Immunofluorescence analysis of the differentiated hiPSCs in the three germ layers. Ectoderm (NESTIN, PAX6), Endoderm (SOX17, CD184), Mesoderm (BRACHYURY, CD184). Scale bars: 25 μm. **I.** Patient and isogenic hiPSC-derived retinal organoids at 35 days (D35). Scale bar: 100 μm.

and TRA1-81 (Fig. 1, panel G). HiPSCs capability to differentiate into all three germ layers, ectoderm, endoderm and mesoderm was confirmed by an immunostaining highlighting the respectively expression of PAX6/NESTIN, SOX17/CD184 and BRACHYURY/CD184 in differentiated cells (Fig. 1, panel H). Particularly, the five hiPSCs can differentiate in retinal organoids following the differentiation protocol published by the team (Reichman et al., 2017; Reichman et al., 2014; Slembrouck-Brec et al., 2019) (Fig. 1, panel I). HiPSCs were mycoplasma free.

Thus, five new hiPSC lines have been generated and characterized; two carrying a patient based RHO-P23H mutation and three as isogenic controls (one homozygous and two hemizygous) (Table 1). These hiPSCs and their retinal deviated cells and organoids could provide new *in vitro* disease models to study the physiopathological mechanisms of adRP to find innovative treatments.

3. Materials and methods

3.1. Fibroblast reprogramming to hiPSCs

The iPSC lines were generated from RHO-P23H patient fibroblasts (from the Columbia University Medical Center, Stephen H. Tsang Lab) in the iPSC core facility of Nantes University, France. Fibroblasts were reprogrammed by Sendai viruses expressing Oct4, Sox2, Klf4 and c-Myc (CytoTuneTM-IPS 2.0 Sendai Reprogramming kit, Life Technologies). The hiPSC clones were picked and expanded on mouse embryonic fibroblasts (MEFs) feeder cells in KSR-FGF2 medium (DMEM/F12 supplemented with 0.1% β-mercaptoethanol, 20% knockout serum replacement, 10 ng/mL basic fibroblast growth factor, 2 mmol/L l-glutamine and 1% NEAA). Until passage 10 (p10), colonies were mechanically passaged with a needle. At p10, hiPSC clones were adapted to feeder-free culture conditions using the human recombinant truncated vitronectin protein (VTN-N, STEMCELL Technologies) with the mTeSR medium (STEMCELL Technologies). Feeder-free hiPSCs were passaged using the passaging Solution XF (StemMACSTM, Miltenyi Biotec).

3.2. CRISPR/Cas9 editing

RHO-P23H hiPSCs ($1x10^6$ cells) were nucleofected with the RNP complex (225 pmol of each RNA (crRNA and tracrRNA-ATTO +) and 120 pmol of Cas9 protein) and HR template (200 pmol ssODN). Twenty-four hours later, ATTO + transfected iPSCs were FACS sorted and plated at very low density (20 cell/cm^2) on Laminin-521 in presence of CloneR2 supplement (STEMCELL Technologies) for clonal selection. One week later, hiPSC clones were picked under a stereomicroscope and cultured on Ln-521 in 96 well plates. When confluent, hiPSC clones were duplicated for cryopreservation and DNA extraction. Clones were then analysed by RT-qPCR and Sanger sequencing.

3.3. Human iPSCs culture

Vitronectin coated plates were used to culture hiPSCs in mTeSR™ Plus medium (STEMCELL Technologies) at 37°C in an incubator with 5% CO2. Weekly passaging were performed at 80% confluence, without any survival factor, using Gentle Dissociation reagent buffer (STEMCELL Technologies, 1:25 dilution).

3.4. Genetic analysis

SNP analysis was performed on primary fibroblasts and on 1U-RHO-P23H-01 (IDVi007-A) and 1U-RHO-P23H-02 (IDVi007-B) between p15 to p17 and for 1U-iso-RHO-D2 (IDVi007-A-1), 1U-iso-RHO-G2 (IDVi007-A-2) and 1U-iso-RHO-H6 (IDVi007-A-3) between p34 to p35. Genomic stability was assessed by detection of recurrent genetic abnormalities on hiPSC lines at p25 (IDVi007-A and IDVi007-B hiPSCs), p32 (IDVi007-A-1), p31 (IDVi007-A-2) and p34 (IDVi007-A-3) using the iCS-digitalTM PSC test, provided as a service by Stem Genomics (https://www.stemgenomics.com/). Genomic identity was assessed on hiPSC lines at p25 (IDVi007-A and IDVi007-B hiPSCs), p35 (IDVi007-A-1), p34 (IDVi007-A-2) and p33 (IDVi007-A-3) for by Short Tandem Repeat (STR) analysis, provided as a service by Stem Genomics.

3.5. Pluripotency

HiPSCs pluripotency was analysed by immunostaining using the StemLight™ Pluripotency Antibody Kit (CellSignaling Technology, 9656S) and by RT-qPCR using the specific gene markers *POU5F1, NODAL, LIN28, NANOG, TERT, DNTMB3* and *SOX2* (Table 2).

3.6. hiPSC differentiation

The STEMdiff™ Trilineage Differentiation Kit (StemCell Technologies, 05230) was used following the manufacturer's instructions. Retinal organoids were generated following our previous established hiPSC differentiation protocol (Reichman et al., 2017; Reichman et al., 2014; Slembrouck-Brec et al., 2018).

3.7. Immunofluorescence

Human iPSCs were seeded onto 12 mm coverslip in 24-well plates or onto ibidtreat 24-well plates (Clinisciences, 82426). Cells were fixed in 4% paraformaldehyde for 10 min and then incubated for 1 h at RT with a blocking buffer containing 1X Phosphate Buffered Saline (PBS 1X), 0,2% Gelatin and 0,1% Triton to block non-specific binding sites. Cells were incubated with primary antibodies overnight at 4°C and then washed with PBS 1X containing 0,1% Tween for 5 min before incubation with secondary antibodies for 1H at RT (Table 2). There were washed three times with PBS tween and twice with PCS. Diamidino-2-phenylindole (DAPI; 1/1000 dilution) was incubated during a 5 min wash with PBS 1X Tween 0,1% to stain the nuclei. Images were acquired with the confocal microscope (Olympus FV1000 and FV1200).

3.8. RNA extraction

RNA extractions were done following the protocol of NucleoSpin RNA XS (Machery Nagel, 740902.250). Quantity and quality of the RNA were assessed using the Nanodrop.

3.9. Retrotranscription

The QuantiTeck Rev. Transcritption kit (Qiagen, 205313) was used for retrotranscription following the manufacturer's instructions.

Table 1
Characterisation and validation.

Classification	Output type	Result	Data (suggested placement)
Schematic of a transgene/genetic modification	Schematic illustrating the structure and location of the introduced genetic modification	A visual representation of edited allele and showing exon the edited Exon 5	Fig. 1 panel A
Morphology	Photographies	Typical human incuded pluripotent stem cell morphology	Fig. 1 panel F
Characterization of the pluripotent/ undifferentiated state	Analysis of specific marker expression. Analysis by immunofluorescence staining and RT-qPCR	hiPSCs express the the pluripotent markers *POU5F1, NODAL, LIN28A, NANOG, TERT, DNTMB3* and *SOX2* (RT-qPCR). hiPSCs coexpress the pluripotent markers OCT4, NANOG, SSEA4 and TRA-1–60 and TRA-1–81 (Immunostaining)	Fig. 1 panel E and G
Karyotypic characterization	SNP array methods and dPCR (iCS-digital™)	SNP analysis on the patient fibroblast and the derived mutated hiPSCs. dPCR (iCS-digital™) method analysis on patient-derived and isogenic hiPSCs: No chromosomal variation detected on 24 or 28 specific genetic sites covering respectively 90% to 93% of recurrent abnormalities.	Supplementary Fig. 1 Fig. 1 panel D
Genotyping for the desired genomic alteration/allelic status of the gene of interest	PCR across the edited site or/and targeted allele-specific PCR.	Confirmation of the heterozygous mutation c.68C > A mutation by sanger sequencing in the patient fibroblast and derived hiPSCs. Confirmation of the homozygous/hemizygous corrected mutation by sanger sequencing in the isogenic hiPSCs.	Fig. 1 panel B Fig. 1 panel C
	Evaluation of the − (homo-/hetero-/hemi-) zygous status of introduced genomic alteration (s)	Amplification of a genomic region at −25 bases from the ATG: dbSNP: rs7984. (Sanger sequencing) HOMOZYGOUS 1U-iso-RHO-D2 (IDVi007-A-1) Homozygosity c.68C and Heterozygosity dbSNP: rs7984 HEMIZYGOUS 1U-iso-RHO-G2 (IDVi007-A-2) Hemizygosity c.68C and dbSNP: rs7984 1U-iso-RHO-H6 IDVi007-A-3) Hemizygosity c.68C and dbSNP: rs7984	Supplementary Fig. 2 Supplementary Fig. 3
	Transgene-specific PCR (when applicable)	N/A	N/A
Verification of the absence of random plasmid integration events	PCR/Southern	N/A	N/A
Evidence of genetic identity for the parental and the reported genetically-modified cell lines	STR analysis, microsatellite PCR (mPCR) or specific (mutant) allele seq	DNA Profiling Fifteen short tandem repeat (STR) loci plus gender determining locus, Amelogin, were analyzed.	Fig. 1 panel B Supplementary Table 1 Including amelogenin + 8 loci (D5S818, D13S317, D7S820, D16S539, vWA, Th01, TPOX, CSF1PO)
Mutagenesis / genetic modification outcome analysis	Sequencing (genomic DNA PCR or RT-PCR product) *[mandatory]*	Sanger sequencing tracks. Fibro-RHO-P23H, IDVi007-A and IDVi007-B: Heterozygosity IDVi007-A-1, IDVi007-A-2 and IDVi007-A-3: Homozygosity	Fig. 1 panel B Fig. 1 panel C
	PCR-based analyses	N/D	N/D
	Southern Blot or WGS; western blotting (for knock-outs, KOs)	N/D	N/D
Off-target nuclease activity analysis	PCR across top 5/10 predicted top likely off-target sites, whole genome/exome sequencing *[*Optional but highly recommended if Cas editing is used*]*	No off-target site to verify in exon less than 4 mismatches (CRISPOR analysis)	Supplementary Table 2
Specific pathogen-free status	Mycoplasma	Negative Mycoplasma testing by Bioluminescence	N/A
Characterization of the differentiation potential	Directed tri-lineage differentiation	The five cell lines were able to differentiate into derivatives of all 3 germ layers and in retinal organoids.	Fig. 1 panel I Fig. 1 panel H
List of recommended germ layer markers	Expression of these markers has to be demonstrated at mRNA protein (IF) levels; at least 1 definitive marker needs to be shown per germ layer	Ectoderm: PAX6/NESTIN, Endoderm: SOX17/CD184, Mesoderm: BRACHYURY/CD184	Fig. 1 panel H
Overall outcomes of gene editing experiment (OPTIONAL)	Brief description of the outcomes in terms of clones generated / establishment approach / editing event screening outcomes	N/A.	N/A
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	N/A
Genotype − additional histocompatibility info (OPTIONAL)	Blood group genotyping	Not performed	N/A
	HLA tissue typing	Not performed	N/A

Table 2
Reagents details. RRID Requirement for antibodies: use http://antibodyregistry.org/ to retrieve RRID for antibodies and include ID in the table as shown in examples.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	**Dilution**	**Company Cat # and RRID**
Pluripotency Markers	*Rabbit anti-oct-4A*	*1:200*	*Cell Signaling Technology Cat# 2840, RRID:AB_2167691*
Pluripotency Markers	*Rabbit anti-Nanog*	*1:200*	*Cell Signaling Technology Cat# 4903, RRID:AB_10559205*
Pluripotency Markers	*Mouse anti-SSEA4*	*1:200*	*Cell Signaling Technology Cat# 4755, RRID:AB_1264259*
Pluripotency Markers	Mouse anti TRA-1-60	*1:200*	*Cell Signaling Technology Cat# 4746, RRID:AB_2119059*
Pluripotency Markers	Mouse anti TRA-1-81	*1:200*	*Cell Signaling Technology Cat# 4745, RRID:AB_2119060*
Secondary antibodies	Donkey anti-mouse 488 igG	*1:300*	*Jackson ImmunoResearch Labs Cat# 715-545-150, RRID:AB_2340846*
Secondary antibodies	Donkey anti-mouse 488 igM	*1:300*	*Jackson ImmunoResearch Labs Cat# 715-545-140, RRID:AB_2340845*
Secondary antibodies	Donkey anti-rabbit 594 IgG	*1:300*	*Jackson ImmunoResearch Labs Cat# 711-585-152, RRID:AB_2340621*
Differentiation Markers	*Rabbit anti-PAX6*	*1/1000*	*Merk Millipore Cat# AB2237*
Differentiation Markers	*Mouse anti-NESTIN*	*1/1000*	*StemCell Technologies Cat# 60091*
Differentiation Markers	*Rabbit anti-BRACHYURY*	*1/500*	*Santa Cruz Cat# sc-20109*
Differentiation Markers	*Mouse Anti-Human CD184 (CXCR4)*	*1/50*	*StemCell Technologies Cat# 60089*
Differentiation Markers	*Rabbit anti-SOX17*	*1/100*	*Abcam Cat# AF1924*
Nuclear stain	*DAPI (1mg/mL)*	*1/1000*	*Thermo Scientific Cat # 62248*
Site-specific nuclease			
Nuclease information	*Nuclease type/version*	*Alt-R® S.p. HiFi Cas9 Nuclease 3NLS, 500 μg*	
Delivery method	*Electroporation (parameters), lipofection*	*4D Nucleofector System (Core unit AAF-1002B and X unit AAF-1002)*	
Primers and Oligonucleotides used in this study			
	Target	**Forward/Reverse primer (5′-3′)**	
Pluripotency Markers (qPCR)	*POU5F1*	*POU5F1-Hs00999632_g1*	
	NODAL	*NODAL-Hs00415443_m1*	
	NANOG	*NANOG-Hs02387400_g1*	
	TERT	*TERT-Hs00972656_m1*	
	DNTMB3	*DNMT3B-Hs00171876_m1*	
	SOX2	*SOX2-Hs01053049_s1*	
House-Keeping Genes (qPCR)	*UBC*	*UBC Hs00824723_m1*	
Genotyping (desired allele/transgene presence detection)	*PCR specific for the targeted allele*	RHO_1F: GCTCAGGCCTTCGCAGCAT RHO_1R: GAGGGCTTTGGATAACATTG	
Targeted mutation analysis/sequencing	*Sequencing data from both alleles*	Fig. 1 panel B Fig. 1 panel C	
Potential random integration-detecting PCRs	*e.g. plasmid backbone, for targeting events-vector/homology arm end PCRs*	N/A	
gRNA oligonucleotide/crRNA sequence	*Provide gRNA seed genomic sequence, or full gRNA/crRNA*	crRNA = AGTACTGTGGGTACTCGAAG TGG	
Genomic target sequence(s)	*Including PAM and other sequences likely to affect UCN activity*	*RHO* exon 1 HDR template = CCCTAACTTCTACGTGCCCTTCTCCAATGCGACGGGTGTGGTACGCtctCcCTTCGA GTACCCACAGTACTACCTGGCTGAGCCATGGCAGTTCTCCATG ***(mutated base ; silent mutation)***	
Bioinformatic gRNA on- and -off-target binding prediction tool used, specific sequence/outputs link (s)	*CRISPOR*	Supplementary Table 2	
Primers for top off-target mutagenesis predicted site sequencing (for all CRISPR/Cas9, ZFN and TALENs)	*OT1 - F&R* *OT2 - F&R* …	https://crispor.gi.ucsc.edu/	
ODNs/plasmids/RNA molecules used as templates for HDR-mediated site-directed mutagenesis.	*Backbone modifications in utilized ODNS have to be noted using standard nomenclature.*	HDR template = CCCTAACTTCTACGTGCCCTTCTCCAATGCGACGGGTGTGGTACGCtctCcC TTCGAGTACCCACAGTACTACCTGGCTGAGCCATGGCAGTTCTCCATG ***(mutated base ; silent mutation)***	

3.10. Mycoplasma

Mycoplasma contamination was verified using the MycoAlert Mycoplasma Detection Kit (Lonza, LT07-318).

CRediT authorship contribution statement

Marilou Clémençon: Data curation, Formal analysis, Methodology, Validation, Writing – original draft. **Jérémy Brogard:** Data curation, Formal analysis, Methodology, Validation. **Marie Rozen:** Data curation. **Camille Hourton:** Data curation. **Rodrigo Meléndez García:** Data curation. **Stéphanie Bigou:** Conceptualization, Data curation, Validation. **Stephen H. Tsang:** Resources. **Olivier Thouvenin:** Funding acquisition. **Kate Grieve:** Funding acquisition. **Sacha Reichman:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Supervision, Validation, Visualization, Writing – original draft, 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

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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.2026.104042.

Data availability

Data will be made available on request.

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