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Lab resource: Stem cell line

# Generation of a human iPSC line from a patient with Leber congenital amaurosis caused by mutation in AIPL1

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A B S T R A C T

The human induced pluripotent stem cell (hiPSC) line, derived from dermal fibroblasts from Leber congenital amaurosis patient with homozygous mutation c.265 T > C, p.Cys89Arg in aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1) was generated by Sendai virus reprogramming. The generated hiPSC line was free of Sendai virus genes, had stable karyotype, carried the homozygous mutation, was immunopositive to pluripotency markers and able to generate all three germ layers upon embryoid body formation.

Resource table.

| | |
|---|---|
| Unique stem cell line identifier | CIPFi001-A |
| Alternative name(s) of stem cell line | LCA –FiPS4F1 |
| Institution | Research Center Principe Felipe, Eduardo Primo Yufera 3, Valencia, Spain |
| Contact information of distributor | Dunja Lukovic dlukovic@cipf.es |
| Type of cell line | iPSC |
| Origin | Human |
| Additional origin info | Age:31<br>Sex: Female<br>Ethnicity if known: Caucasian |
| Cell Source | Dermal fibroblasts |
| Clonality | Clonal |
| Method of reprogramming | Sendai virus |
| Genetic modification | N/A |
| Type of modification | N/A |
| Associated disease | Leber congenital amaurosis |
| Gene/locus | AIPL1 (NM_014336.3), Chr17: g.6337250A > G (hg19); Ex.2 c.[265 T > C];[265 T > C], p.[Cys89Arg];[Cys89Arg] |
| Method of modification | N/A |
| Name of transgene or resistance | N/A |
| Inducible/constitutive system | N/A |
| Date archived/stock date | April 2017 |

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| | |
|---|---|
| Cell line repository/bank | http://www.isciii.es/ISCIII/es/contenidos/fd-el-instituto/fd-organizacion/fd-estructura-directiva/fd-subdireccion-general-investigacion-terapia-celular-medicina-regenerativa/fd-centros-unidades/fd-banco-nacional-lineas-celulares/fd-lineas-celulares-disponibles/lineas-de-celulas-iPS.shtml |
| Ethical approval | Ethics Review Board-competent authority approval obtained by the Valencian Authority for Stem Cell Research (Approval number: S:177–15) |

## Resource utility

Human induced pluripotent stem cells (hiPSCs) hold great potential in regenerative medicine as they can be expanded indefinitely and coaxed toward any cell type of the human body including photoreceptors (Zhong et al., 2014) the affected cells in patients with blinding diseases. Leber congenital amaurosis (LCA) is the severest form of inherited retinal dystrophies characterized by loss of electroretinogram signal and severe visual impairment within the first year of life (Kumaran et al., 2017). We describe here the generation of the hiPSC line from LCA patient with homozygous mutation c.265 T > C, p.Cys89Arg in aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1), a photoreceptor specific chaperone (Ramamurthy et al., 2004). This iPSC line presents a renewable and patient specific cell source which holds the potential for in vitro disease modelling in which to perform therapeutic screening and also as a cell source for autologous replacement therapy after mutation correction.

## Resource details

The fibroblasts were derived from skin biopsy from a female patient diagnosed with Leber congenital amaurosis, expanded and reprogramed using Sendai virus (CytoTune™). Individual colonies were picked and subcultured into individual cell lines and analyzed at cellular and genetic level to confirm successful reprogramming (Table 1). The generated hiPSC line LCA-FiPS4F1 carried the mutation in aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1) (Fig. 1A) in accordance with previous genotyping results in the patient. After 30 days generated colonies displayed a typical human embryonic stem cell (hESC) colony-like morphology with refractive edges as seen by phase contrast (PC) microscopy (Fig. 1B) and the cells had high nuclear/cytoplasmic ratio. All colonies in the plastic dish were positive for alkaline phosphatase (Fig. 1C, left) and coloured colonies were detected microscopically (Fig. 1C, right). Pluripotency was assessed by flow cytometry for SSEA-4 and TRA-1-60 pluripotency markers (Fig. 1D) and immunocytochemistry to pluripotency markers OCT4, SOX2, NANOG, SSEA-4 and TRA-1-81 (Fig. 1E). The expression of endogenous pluripotency genes was detected by RT-PCR (Fig. 1F). To test the ability of the hiPSC line to generate derivatives of three germ layers in vitro, the embryoid body (EB) based assay was performed. Spontaneously differentiated cells were immunostained for endodermal markers forkhead box A2 (FOXA2, inset showing simultaneous DAPI staining) and α-fetoprotein (AFP), ectodermal markers β-III tubulin (TUJ1)) and PAX6, mesodermal markers vimentin and α-smooth muscle actin (SMA) (Fig. 1G). The genetic fingerprinting was performed and proved its genetic identity to parental fibroblasts (available with authors). The hiPSC line was karyotypically normal (Fig. 1H). The presence of mycoplasma was regularly tested and was negative (Supplementary file). The Sendai virus and exogenous reprogramming factors were cleared after 8 cell line passages (Fig. 1I).

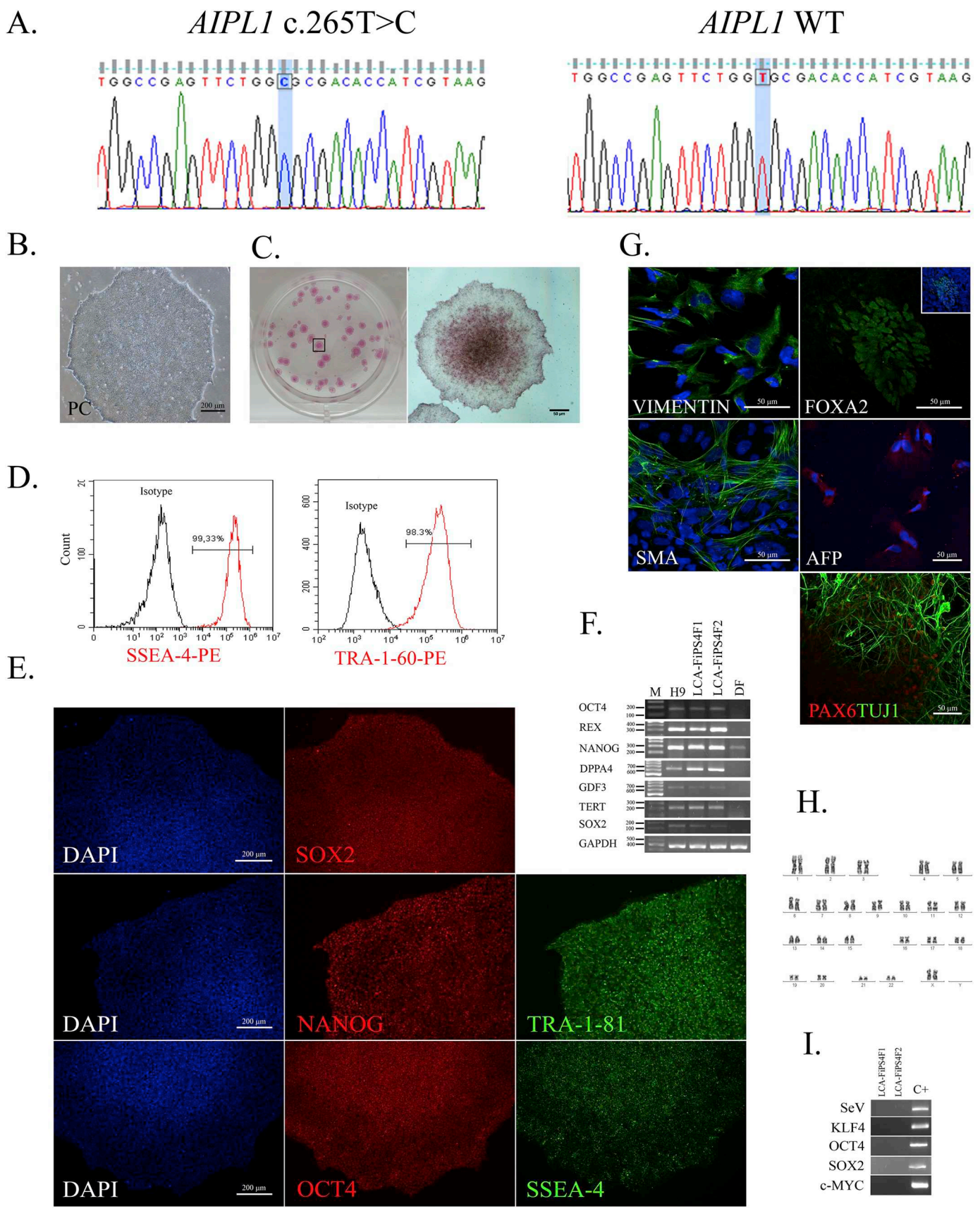
## Materials and methods

### *Reprogramming fibroblasts*

The primary fibroblasts derived from the skin biopsy were expanded for four passages in DMEM (10% FBS, 2 mM Glutamax, Penicillin-Streptomycin 1 ×) at 37 °C under 5% $CO_2$, before being reprogrammed by a Sendai virus containing four genes: Oct3/4, Sox2, Klf4 and cMyc (CytoTune™, Thermo Fisher) according to manufacturer instructions. hiPSCs were grown on irradiated (45Gy) human foreskin fibroblasts (ATCC CRL 2429) in hiPSCs medium containing Knock-out DMEM, KSR 20%, Glutamax 2 mM, non-essential amino acids 0.1 mM, β-mercaptoethanol 0.23 mM, basic FGF 10 ng/ml, penicillin/streptomycin, at 37 °C/5% $CO_2$. The hiPSCs were adapted to feeder-free cell culture on Matrigel (BD, #354277) coated plates using mTeSR1 medium. Matrigel was diluted in DMEM/F-12 according to the dilution factor specified on the certificate of analysis and incubated for 1 h/RT at 1 ml/well of a 6-

**Table 1**
Characterization and validation.

| Classification | Test | Result | Data |
|---|---|---|---|
| Morphology | Photography | Normal | Phase contrast (PC) microscopy Fig. 1 panel B |
| Phenotype | Immunocytochemisty | Positive staining/expression of pluripotency markers: OCT4, NANOG, SOX2, SSEA-4, TRA-1-81 | Fig. 1 panel E |
| | Cytometry | SSEA-4 99,33%<br>TRA-1-60 98,3% | Fig. 1 panel D |
| Genotype | Karyotype (G-banding) and resolution | 46XX, Resolution 450–500 | Fig. 1 panel H |
| Identity | Microsatellite PCR (mPCR) | Not performed | |
| | STR analysis | 10 loci analyzed, all matching | Available with authors |
| Mutation analysis (IF APPLICABLE) | Sequencing | AIPL1 Ex.2 c.265 T > C | Fig. 1 panel A |
| | Southern Blot OR WGS | No | |
| Microbiology and virology | Mycoplasma | Mycoplasma testing by luminescence. Negative. | Supplementary file |
| Differentiation potential | Embryoid body formation | Positive TUJ1 and PAX6 for ectoderm, positive SMA, VIMENTIN mesodermal staining and positive AFP and FOXA2 endodermal staining. | Fig. 1 panel G |
| Donor screening (OPTIONAL) | N/A | N/A | N/A |
| Genotype additional info (OPTIONAL) | N/A | N/A | N/A |




*(caption on next page)*

**Fig. 1.** Characterization of LCA-FiPS4F1 line.
A. DNA electropherograms showing the mutation in *AIPL1* c.265T > C in LCA-FiPS4F1 and the WT sequence in the same region of a healthy subject. B. Phase contrast micrograph of a LCA-FiPS4F1 colony cultured in feeder-free conditions. C. Alkaline phosphatase staining of LCA-FiPS4F1 colonies (left panel), higher magnification of inset in left panel showing a positively stained colony (right panel). D. Flow cytometry of surface pluripotency markers SSEA-4 and TRA-1-60. E. Immunocytochemistry for pluripotency markers OCT4, SOX2, NANOG, SSEA-4, TRA-1-81. Nuclei were counterstained with DAPI (blue). F. RT-PCR analysis of pluripotency markers. E. Immunocytochemistry for ectodermal (PAX6, TUJ1), endodermal (AFP, FOXA2, inset showing double staining with DAPI) and mesodermal (SMA, VITRONECTIN) markers. Nuclei were counterstained with DAPI (blue). H. Representative metaphase of normal human karyotype (46, XX). I. RT-PCR analysis of SeV genome and transgenes in hiPSCs and positive control (C+). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

well plate. Passages were performed using Dispase (STEMCELL Technologies, #07913), every 5–7 days at 1:6–1:10 split ratio.

*In vitro differentiation assay*

In vitro differentiation was performed by EB formation, namely the iPSC colonies were lifted manually and cultured in non-adherent conditions in mTeSR1 medium for 24 h, followed by endoderm medium (Knockout-DMEM, 10% FBS, 1% Glutamax, NEAA 1%, β-mercaptoethanol 0,23 mM, Penicillin-Streptomycin 1×) for the following 6 days. Thereafter, the EBs were seeded on glass coverslips treated with 0,1% gelatin for 2 h/RT and cultured during 2 weeks in three cell culture media: ectoderm medium (50% Neurobasal medium, 50% DMEM/F12, 1% N2 supplement, 0,5% B27 supplement, penicillin-streptomycin

**Table 2**
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry

| | Antibody | Dilution | Company Cat # and RRID |
|---|---|---|---|
| Pluripotency marker | Rabbit anti-Nanog | 1:400 | Cell Signaling Technology Cat# D73G4 |
| Pluripotency marker | Rabbit anti-Oct4 | 1:400 | Cell Signaling Technology Cat# C30A3 |
| Pluripotency marker | Rabbit anti-Sox2 | 1:400 | Cell Signaling Technology Cat# D6D9 |
| Pluripotency marker | Mouse anti-SSEA4 | 1:100 | BD Pharmigen Cat# 560073 |
| Pluripotency marker | Mouse anti TRA-1-81 | 1:100 | Milipore Cat #MAB4381 |
| Pluripotency marker | Mouse anti human SSEA4-PE | 1:800 | Stem Cell Technologies Cat #60062PE.1 |
| Isotype control | Mouse IgG3 kappa-PE | 1:6400 | Stem Cell Technologies Cat # 60073PE.1 |
| Pluripotency marker | Mouse anti human TRA- 1-60 | 1:400 | Stem Cell Technologies, Cat #60064PE.1 |
| Isotype control | PE Mouse IgM | 1:40 | BD, Pharmingen Cat #555584 |
| Ectoderm marker | Mouse anti-TUJ-1 | 1:500 | Neuromics MO15013 |
| Ectoderm marker | Rabbit anti-PAX6 | 1:500 | Covance # PRB-278P |
| Mesoderm marker | Mouse anti-SMA | 1:300 | Sigma Cat #A5228 |
| Mesoderm marker | Mouse anti-vimentin | 1:300 | Abcam Cat# ab8978 |
| Endoderm marker | Mouse anti-AFP | 1:20 | RD Cat # MAB 1368 |
| Endoderm marker | Goat anti HNF-3β/FoxA2 | 1:100 | RD Cat # 2400 |
| Secondary antibody | Anti-mouse IgG | 1:500 | Invitrogen A11001 |
| Secondary antibody | Anti-rabbit | 1:500 | Invitrogen A11002 |
| Secondary antibody | Anti-mouse IgM | 1:500 | Invitrogen A21042 |
| Secondary antibody | Anti-goat | 1:500 | Invitrogen A11055 |

Primers

| | Target | Forward/Reverse primer (5′-3′) |
|---|---|---|
| Genotyping | AIPL1 | CGGTGACTAGGTGATCTTTC/ CTTGTTCCCTCCATCTTCAC |
| Pluripotency marker (RT-PCR) | OCT4 | AAGCCCTCATTTCACCAGG<br>CTTGGAAGCTTAGCCAGGTC |
| Pluripotency marker (RT-PCR) | SOX2 | TCACATGTCCCAGCACTACC<br>CCCATTTCCCTCGTTTTTCT |
| Pluripotency marker (RT-PCR) | NANOG | CCAAATTCTCCTGCCAGTGAC<br>CACGTGGTTTCCAAACAAGAAA |
| Pluripotency marker (RT-PCR) | REX1 | CAGATCCTAAACAGCTCGCAGAAT<br>GCGTACGCAAATTAAAGTCCAGA |
| Pluripotency marker (RT-PCR) | GDF3 | CTTATGCTACGTAAAGGAGCTGGG<br>GTGCCAACCCAGGTCCCGGAAGTT |
| Pluripotency marker(RT-PCR) | DPPA4 | GGAGCCGCCTGCCCTGGAAAATTC<br>TTTTTCCTGATATTCTATTCCCAT |
| House-keeping gene (RT-PCR) | GAPDH | ATCGTGGAAGGACTCATGACCACA<br>CCCTGTTGCTGTAGCCAAATTCGT |
| Sendai virus detection (RT-PCR) | SeV | GGATCACTAGGTGATATCGAGC*<br>ACCAGACAAGAGTTTAAGGATATGTTC* |
| Sendai virus transgene detection (RT-PCR) | SOX2 | ATGCACCGCTACGACGTGAGCGC<br>AATGTATCGAAGGTGCTCAA* |
| Sendai virus transgene detection (RT-PCR) | KLF | TTCCTGCATGCCAGAGGAGCCC<br>AATGTATCGAAGGTGCTCAA* |
| Sendai virus transgene detection (RT-PCR) | c-MYC | TAACTGACTAGCAGGCTTGTCG*<br>TCCACATACAGTCCTGGATGATGATG |
| Sendai virus transgene detection (RT-PCR) | OCT3/4 | CCCGAAAGAGAAAGCGAACCAG<br>AATGTATCGAAGGTGCTCAA* |

1 ×), endoderm medium and mesoderm medium (endoderm medium, 0,5 mM ascorbic acid). The coverslips were fixed 4% PFA for 15 min and analyzed by immunofluorescence (Table 2). Confocal images were taken by Leica SP8.

*Karyotype analysis*

The karyotype was analyzed by G-banding at 400–550 band resolution, 30 metaphases analyzed (Service of Biobanco de Sistema Sanitario Público, Granada, Spain).

*Fingerprinting*

Genomic DNA from fibroblasts and hiPSCs was extracted using QIAamp DNA Blood mini kit (Qiagen) in the presence of RNAse (Roche). Fingerprinting analyses was performed using Promega kit 10 microsatellite markers (TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818 y D21S11, Amelogenin) and analyzed on Abi PRISM 3130 using GeneMapper (Thermo Fisher) by Biobanco de Sistema Sanitario Público, Granada, Spain.

*Detection of pluripotency markers and vector elimination by RT-PCR*

Total RNA was isolated with the RNeasy Mini Kit (Qiagen, Hilden, Germany), and treated with DNase I to remove any genomic DNA contamination. QuantiTect Reverse Transcription Kit (Qiagen) was used to carry out cDNA synthesis from 1 μg of total RNA according to the manufacturer's instructions. The expression level of pluripotency markers was analyzed using the primers described in Table 2. Patient's fibroblasts (DF) were used as negative control while hESC H9 (WiCell) was used as positive control. For Sendai virus elimination test patient's fibroblasts were collected at day 7 after transduction as positive control (C+). The PCR reaction was performed with MyTaq DNA Polymerase (Bioline GmbH, Luckenwalde, Germany) using Applied Biosystems Veriti Thermal Cycler with following steps: denaturation 94 °C; 15 s, annealing 50–65 °C; 30 s, extension 72 °C; 45 s, for 35 cycles. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was used as a control housekeeping gene. Thereafter, PCR products were analyzed on 2% agarose gels.

*Immunocytochemistry*

hiPSC colonies cultured in plastic dishes were washed in PBS and fixed in 4% PFA for 15 min at room temperature (RT). Fixed cells were washed twice in PBS and placed in blocking solution (3% serum, 0.5% Triton-X100 in PBS) for 1 h at RT. Cells were then incubated overnight at 4 °C with primary antibodies (Table 2). The following day, cells were washed three times in PBS and incubated with an appropriate secondary antibody at RT for 1 h. Thereafter, cells were stained with DAPI (1:1000) at RT during 5 min, washed three times in PBS and imaged at Leica DMi 8 microscope coupled with DFC/7000GT camera.

*Flow cytometry*

hiPSCs were dissociated using Accutase (Innovative Cell Technologies) for 2–4 min at RT, centrifuged at 300 rcf for 5 min and 100.000 cells resuspended in 200 μl PBS + 2% FBS. Anti-human SSEA-4, and anti-human TRA-1-60, both PE conjugated, antibodies were added and incubated for 20 min at RT. Isotype negative controls were used at the same final concentration. The cells were analyzed using a CytoFLEX flow cytometer (Beckman Coulter) and data analyzed by CytExpert 2.0 software.

*Alkaline phosphatase Staining*

Alkaline phosphatase staining was carried out using Alkaline Phosphatase Staining Kit II (Stemgent, MA).

*Mycoplasma detection*

The presence of mycoplasma was tested regularly measuring enzyme activity via luciferase (MycoAlert™ PLUS Mycoplasma Detection Kit, Lonza).

*hiPSC nomenclature*

The generated hiPSC line was named following Spanish National Stem Cell Bank recommendations. The line is registered at https://hpscreg.eu/ as CIPFi001-A line.

## Author contributions

DL designed study, performed the experiments, manuscript writing. AAC, ML, VBF performed experiments, SE coordinated patient sample, manuscript writing; MC, CA genotyped the patient and coordinated patient sample donation, manuscript writing.

## Acknowledgement

We are thankful to the patient for generously donating skin biopsy. This work was supported by Institute of Health Carlos III (ISCIII)/FEDER (European Regional Development Fund), Spain ((PI16/00409 (DL), CP10/00579 (SE), PI14/02209 (SE), Platform of Biomolecular and Bioinformatics Resources of the Institute of Health Carlos III, PRB$^2$, PT13/0001/0042; Platform for Proteomics, Genotyping and Cell lines, PRB3, PT17/0019/0024). The work was also supported by ISCIII-FEDER (PI16/00425), CIBERER 06/07/0036, IIS-FJD Biobank PT13/0010/0012, Spanish National Organization of the Blind (ONCE), the Spanish Fighting Blindness Foundation (FUNDALUCE), and the Ramon Areces Foundation. MC is supported by the Miguel Servet Program (CPII17_00006) from ISCIII.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.10.012.

## References

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Zhong, X., Gutierrez, C., Xue, T., Hampton, C., Vergara, M.N., Cao, L.H., Peters, A., Park, T.S., Zambidis, E.T., Meyer, J.S., Gamm, D.M., Yau, K.W., Canto-Soler, M.V., 2014 Jun 10. Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. Nat. Commun. 5, 4047.

| STR | Fibroblasts | LCA1-FiPS4F1 |
|---|---|---|
| **AMEL** | X | X |
| **CSF1PO** | 10 | 10 |
| **D13S317** | 12, 13 | 12, 13 |
| **D16S539** | 12 | 12 |
| **D21S11** | 28, 30 | 28, 30 |
| **D5S818** | 11 | 11 |
| **D7S820** | 8, 10 | 8, 10 |
| **TH01** | 9, 9.3 | 9, 9.3 |
| **TPOX** | 8, 9 | 8, 9 |
| **vWA** | 18, 19 | 18, 19 |

| Mycoplasma test by MycoAlert ™PLUS 24/02/2017 | |
|---|---|
| **Sample** | **Read B/Read A** |
| Positive control | 1,38 |
| Negative control | 0,51 |
| LCA-FiPS4F1 | 0,79 |