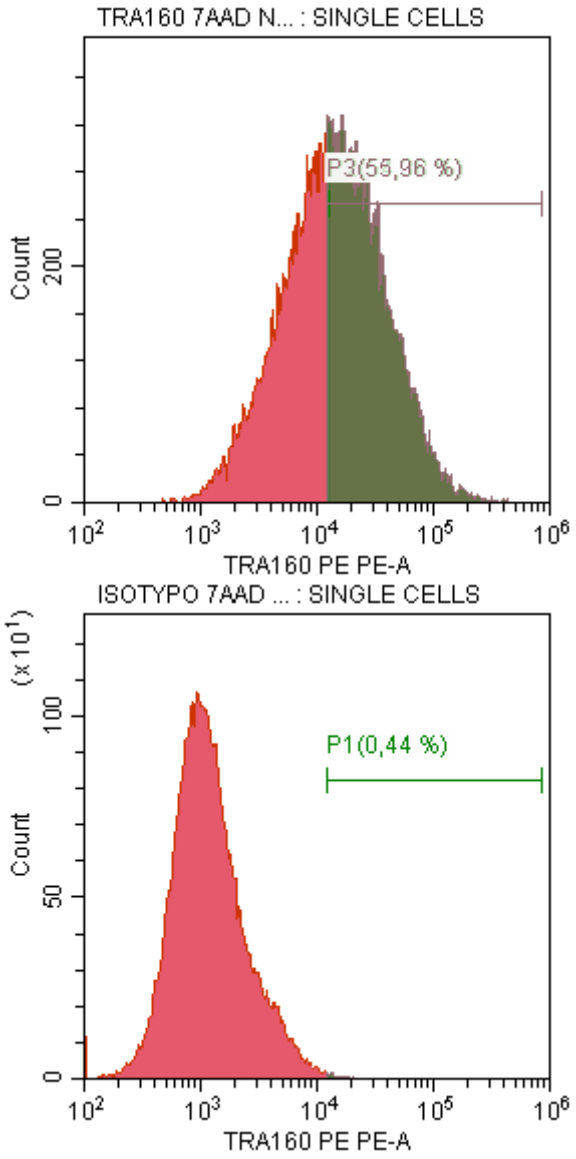
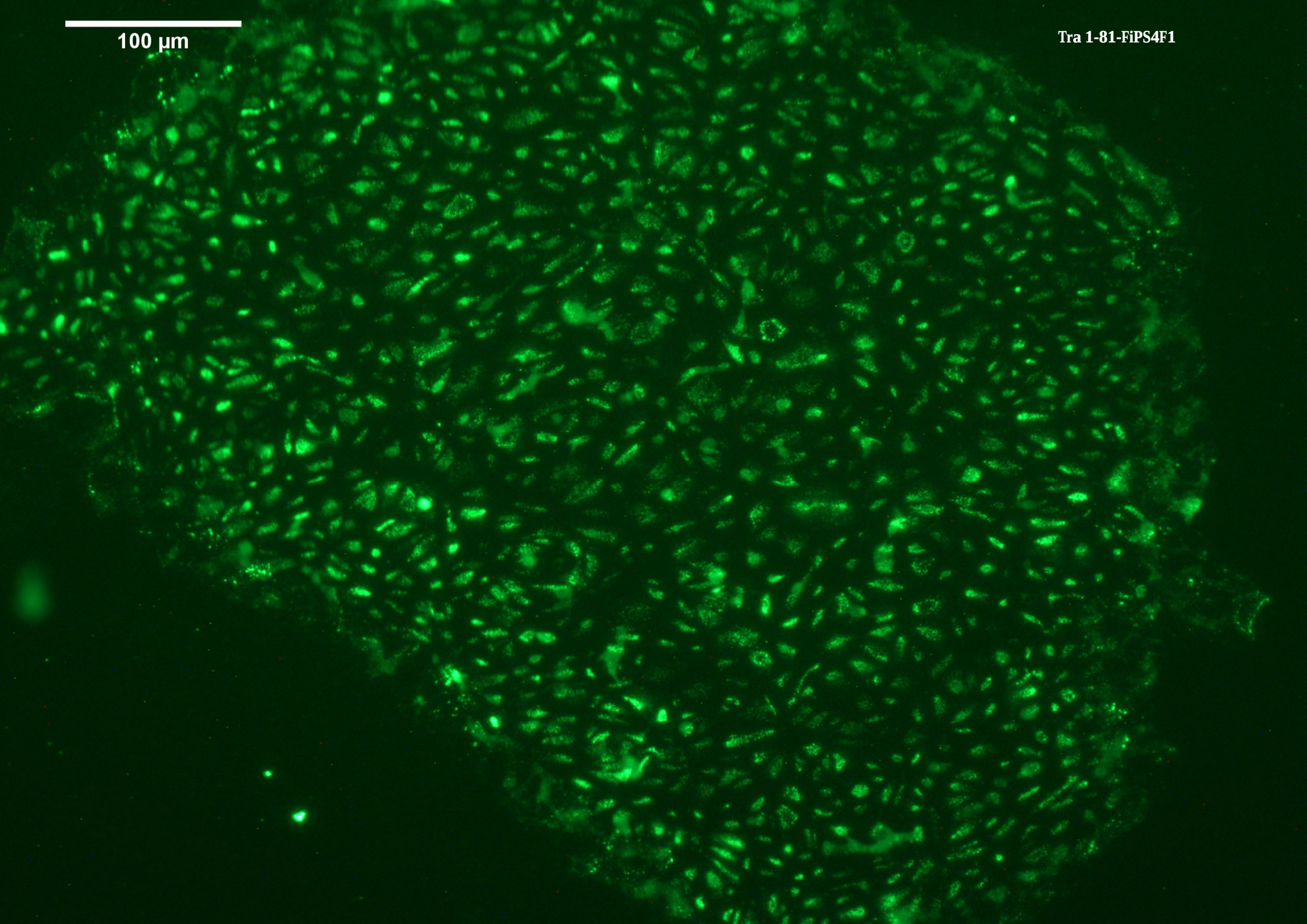
Citometría pluripotencia ESi063-A
Marcador TRA1-60 (ISOTYPO como control negativo)



	Fibroblasts	ARSFiPS4F1-Clon 1	ARSFiPS4F1-Clon 10
D19S572	119/129	119/129	119/129
D2S159	174/178	174/178	174/178
D14S972	199/199	199/199	199/199
D8S601	223/225	223/225	223/225
D9S1853	252/252	252/252	252/252

Fingerprint ARS-FiPS4F1

	HDF11 FIBROS	ARS-FiPS4F1
AMEL	X, Y	X, Y
CSF1PO	11, 13	11, 13
D13S317	11, 12	11, 12
D16S539	11, 13	11, 13
D21S11	28, 29	28, 29
D5S818	11	11
D7S820	10, 12	10, 12
TH01	6, 7	6, 7
TPOX	8, 11	8, 11
vWA	17, 19	17, 19


100 µm
Tra 1-81-FiPS4F1

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Lab Resource: Stem Cell Line

Generation of a human iPSC line from a patient with autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) caused by mutation in SACSIN gene

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A B S T R A C T

The human iPSC cell line, ARS-FiPS4F1 (ESi063-A), derived from dermal fibroblast from the patient autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) caused by mutations on the gene *SACSIN,* was generated by non-integrative reprogramming technology using OCT3/4, SOX2, CMYC and KLF4 reprogramming factors. The pluripotency was assessed by immunocytochemistry and RT-PCR. Differentiation capacity was verified *in vitro*. This iPSC line can be further differentiated toward affected cells to better understand molecular mechanisms of disease and pathophysiology.

Resource table.

Unique stem cell line identifier	ESi063-A
Alternative name(s) of stem cell line	ARS-FiPS4F1
Institution	Research Center Principe Felipe, Eduardo Primo Yufera 3, Valencia, Spain
Contact information of distributor	Slaven Erceg, serceg@cipf.es
Type of cell line	iPSC
Origin	Human
Additional origin info	Sex: male Age: 14
Cell Source	Dermal fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus
Genetic Modification	No
Type of Modification	n/a
Associated disease	Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)
Gene/locus	Gene: SACSIN gene (SACS) Locus: 13q12.12 Mutations: c.9938delC (p.G3313Qfs*11) and c.11374C > T (p.R3792*) mutation in compound heterozygosity
Method of modification	n/a
Name of transgene or resistance	n/a
Inducible/constitutive system	n/a
Date archived/stock date	n/a
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-

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	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

The generation of human induced pluripotent stem cells (hiPSC) from the ARSACS patients permits the development of disease specific stem cells that can be further differentiated toward affected cells to better understand molecular mechanisms of disease and pathophysiology.

Resource details

Skin punch biopsy was taken from a 14- year-old patient who was diagnosed with autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) harbouring the c.9938delC (p.G3313Qfs*11) and c.11374C > T (p.R3792*) mutations in compound heterocigosis in the *SACS* gene (NM_014363.5) and primary fibroblast cell line was established. The generation of the human induced pluripotent stem cell (hiPSC) line, ARS-FiPS4F1 (registered as ESi063-A at www.hPSCreg.com), was carried out using non-integrative Sendai virus containing the human reprogramming factors, Oct3/4, Sox2, C-Myc, and Klf4 (Takahashi et al., 2007), following instructions by manufacturer. After 30 days generated colonies displayed a typical ES-like morphology (polygonal shape; refractive edges, high nuclear/cytoplasmic ratio) and growth behaviour. DNA sequencing analysis of ARS-FiPS4F1 confirmed the *SACS* mutations in each allele (Fig. 1A). The clearance of the virus and the exogenous reprogramming factor genes were confirmed by RT-PCR after twelve cell culture passages (Fig. 1B). The genetic fingerprinting was performed with ARS-FiPS4F1 hiPSC line and proved its genetic identity to parental fibroblasts (available with the author). The selected line showed normal karyotype (46, XY) at low passages (passage 9) (Fig. 1C) and medium passage number (passage 30). Genetic and functional assays were performed to determine the quality of the ARS-FiPS4F1 line. Pluripotency was assessed by immunocytochemistry to pluripotency markers OCT-4, SOX2, NANOG and SSEA-4 and flow cytometry for SSEA-4 pluripotency marker (Fig. 1D). The alkaline phosphatase is known to be more active in hiPSCs and the colorimetric assay depicting its activity confirmed that the selected hiPSC colonies are indeed pluripotent (Fig. 1D). The expression of endogenous plutipotency genes was detected by RT-PCR (Fig. 1E). To test the ability of the hiPSC line to generate derivates of three germ layers *in vitro*, the hiPSCs were differentiated into the three germ layers using an embryoid body based assay. Spontaneous differentiated cells were immunostained for differentiation markers such as TUJ1 for ectoderm, SMA for mesoderm and positive FOXA2 for endoderm (Fig. 1F). The mycoplasma was regularly checked without positive results.

Materials and methods

Reprogramming patient's fibroblasts

The hiPSCs were derived from patient's fibroblasts using Sendai virus (Cyto Tune- iPS 2.0 reprogramming Kit, Life Technologies) according to manufacturer instructions. hiPSCs were grown on irradiated (45Gy) human foreskin fibroblasts (ATCC CRL 2429) in hiPSCs medium containing KO 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. Cells were mechanically passaged every 6–8 days.

In vitro differentiation assay

For *in vitro* differentiation assay the colonies from a fully confluent 6-well plate were cut mechanically and cultured in suspension to form embryoid bodies in hiPSCs media without bGFG. After 7 days in suspension, embryoid bodies were transferred into 0.1% gelatin-coated plates and cultured for additional 7–10 days to allow spontaneous differentiation. Then, the cells were fixed and immunostained to detect cells from the three germ layers.

Karyotype analysis

The hiPSCs were adapted to feeder-free cell culture on Matrigel (BD, #354277) coated plates using mTeSR1 medium. Passages were performed using Dispase (STEMCELL Technologies, #07913), every 5–7 days. 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

gDNA from fibroblasts and hiPSCs was extracted using QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) in the presence of RNAse (Roche). Fingerprinting analyses was performed using 5 microsatellite markers (D19S572, D2S159, D14S972, D8S601, D9S1853) and analyzed on Abi PRISM 3130 using GeneMapper (Thermo Fisher) by Biobanco de Sistema Sanitario Público, Granada, Spain.

Mutation screening

Genomic DNA from fibroblasts and hiPSCs was isolated using the QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany). To detect the *SACS* c.9938delC variant, the used primers were: Forward: 5′- GCAGA ACATCTCCTTCAGGA -3′, and Reverse: 5′- CCGCTATGTAAGCATTGG AAA-3′, and to investigate the SACS c.11374C > T change, the used primers were: Forward: 5'-TGTTAACCTGGATCCTCCTC -3′, and Reverse: 5′- GAACAACTGGTGAAATGTGC -3′.

Detection of pluripotency markers 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, Hilden, Germany) was used to carry out cDNA synthesis from 1 μg of total RNA according to the manufacturer's instructions. The PCR reaction was performed with MyTaq DNA Polymerase (Bioline GmbH, Germany) using Applied Biosystems Veriti Thermal Cycler. The expression level of pluripotency markers was analyzed using the primers described in Table 2. Fibroblasts and hESC H9 (WiCell) were used as negative and positive control, respectively.

Immunocytochemistry

Cells 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. 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 visualized on Leica DM600 fluorescent microscope equipped with Leica DC500 camera. Samples grown on coverslips were mounted using Vectashield.

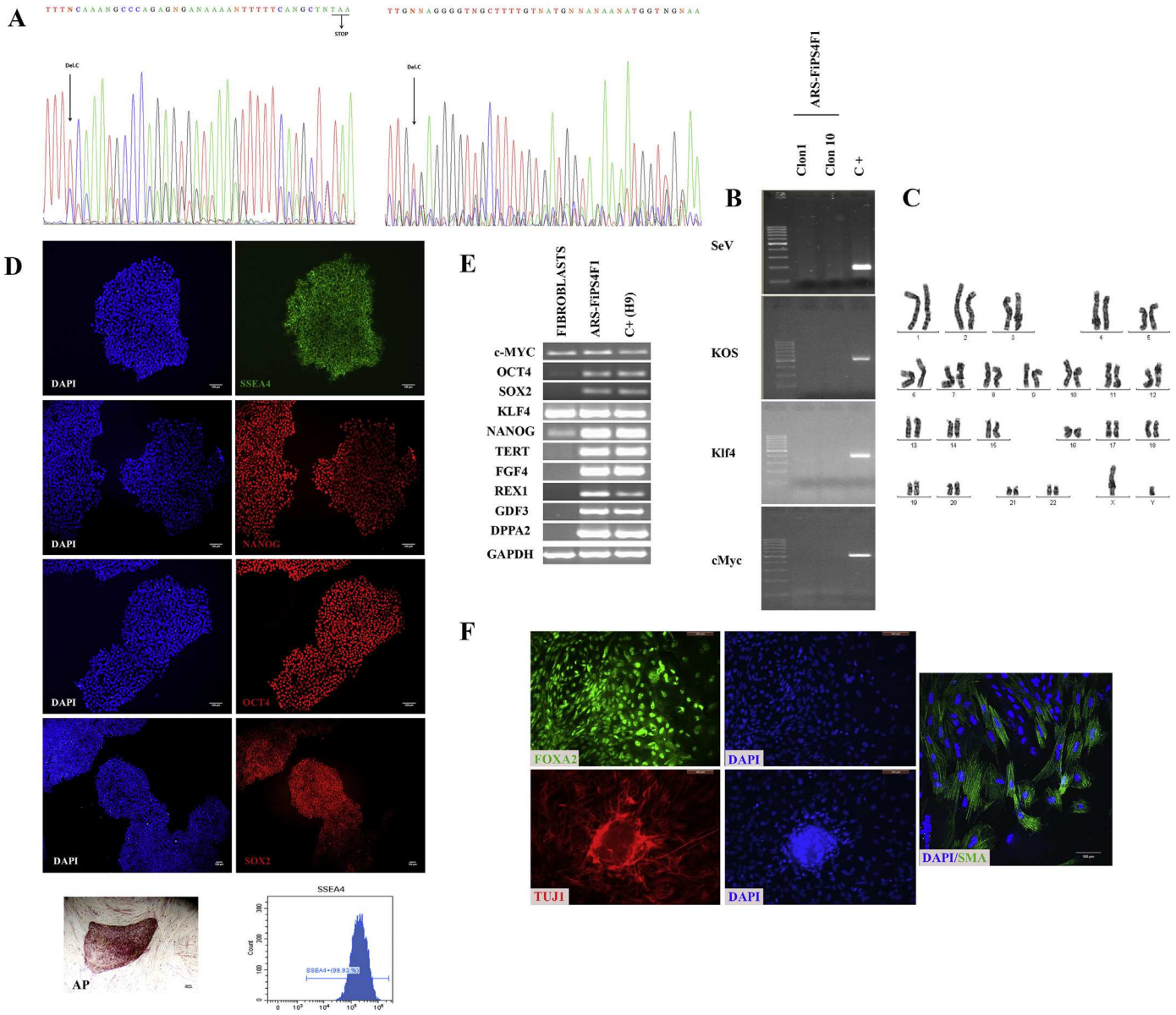

Fig. 1. Characterization of ARS-FiPS4F1 line.

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Shown by immunocytochemistry
Phenotype	Immunocytochemisty	Positive staining/expression of pluripotency markers: Oct4, Nanog, Sox2, SSEA4	Fig. 1 panel D
	Cytometry	SSEA4 99%	Fig. 1 panel D
Genotype	Karyotype (G-banding) and resolution	46XY, Resolution 450–500	Fig. 1 panel C
Identity	Microsatellite PCR (mPCR)	N/A	N/A
	STR analysis	10 loci analyzed, all matching	Available with authors
Mutation analysis (IF APPLICABLE)	Sequencing	Compound heterozygous	Fig. 1 panel A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Mycoplasma testing by luminescence. Negative	Supplementary Fig.1
Differentiation potential	Embryoid body formation	Positive TUJ1 and TUBB ectodermal staining, positive SMA mesodermal staining and positive AFP endodermal staining.	Fig. 1 panel F
Donor screening (OPTIONAL)	N/A	N/A	N/A
Genotype additional info (OPTIONAL)	N/A	N/A	N/A
	N/A	N/A	N/A

Table 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-Nanog	1:400	Cell Signaling Technology Cat# D73G4
Pluripotency Markers	Rabbit anti-Oct4	1:400	Cell Signaling Technology Cat# C30A3
Pluripotency Markers	Rabbit anti-Sox2	1:400	Cell Signaling Technology Cat# D6D9
Pluripotency Markers	Rabbit anti-SSEA4	1:100	BD Pharmigen Cat# 560073
Pluripotency Markers	Mouse anti human SSEA4-PE	1:800	STEMCELL Technologies Cat #60062PE.1
Differentiation Markers	Mouse anti-SMA	1:200	Abcam Cat# ab11570
Differentiation Markers	Mouse anti-FoxA2	1:100	R&D Cat# AF2400
Differentiation Markers	Mouse anti-BTubulin (Tuj1)	1:500	Neuromics Cat# MO15013

Primers		
	Target	Forward/Reverse primer (5′-3′)
Pluripotency Markers (qPCR)	OCT4	AAGCCCTCATTTCACCAGG CTTGGAAGCTTAGCCAGGTC
Pluripotency Markers (qPCR)	NANOG	CCAAATTCTCCTGCCAGTGAC CACGTGGTTTCCAAACAAGAAA
Pluripotency Markers (qPCR)	SOX2	TCACATGTCCCAGCACTACC CCCATTTCCCTCGTTTTTCT
Pluripotency Markers (qPCR)	TERT	TGGCTGCGTGGTGAACTTG GCGGTTGAAGGTGAGACTGG
Pluripotency Markers (qPCR)	FGF4	CTACAACGCCTACGAGTCCTACA GTTGCACCAGAAAAGTCAGAGTTG
Pluripotency Markers (qPCR)	REX1	CAGATCCTAAACAGCTCGCAGAAT GCGTACGCAAATTAAAGTCCAGA
Pluripotency Markers (qPCR)	GDF3	CTTATGCTACGTAAAGGAGCTGGG GTGCCAACCCAGGTCCCGGAAGTT
Pluripotency Markers (qPCR)	DPPA2	CCGTCCCCGCAATCTCCTTCCATC ATGATGCCAACATGGCTCCCGGTG
House-keeping gene (qPCR)	GAPDH	ATCGTGGAAGGACTCATGACCACA CCCTGTTGCTGTAGCCAAATTCGT
Sendai virus detection	SeV	GGATCACTAGGTGATATCGAGC ACCAGACAAGAGTTTAAGAGATATGTATC
Transgenes detection	KOS	ATGCACCGCTACGACGTGAGCGC ACCTTGACAATCCTGATGTGG
Transgenes detection	c-Myc	TAACTGACTAGCAGGCTTGTCG TCCACATACAGTCCTGGATGATGATG
Transgenes detection	Klf4	TTCCTGCATGCCAGAGGAGCCC AATGTATCGAAGGTGCTCAA

Flow cytometry

hiPSCs were dissociated using Accutase (Innovative Cell Technologies) for 2–4 min at RT, centrifuged at 300 rcf for 5 min and resuspended in PBS + 2% FBS. Anti-human SSEA-4 antibody was added and incubated for 20 min at RT. IgG3, kappa isotype (STEMCELL technologies # 60073PE.1) was used as negative control. The cells were analyzed using a CytoFLEX flow cytometer (Beckman Coulter) and data analyzed by CytExpert 2.0 software (Table 1).

Alkaline phosphatase staining

Alkaline phosphatase staining was carried out using Alkaline Phosphatase Staining Kit II (Stemgent, Cambridge, MA, USA) according to manufacturer's instructions.

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 on https://hpscreg.eu/ as ESi043-A line.

Funding

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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.2018.07.012.

References

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872.

Mycoplasma test by MycoAlert PLUS™ 30/09/2017	
Sample	**Read B/Read A**
Positive control	3,537
Negative control	0,347
ARS-FiPS4F1	0,582