Generation of CRISPR-Cas9 edited human induced pluripotent stem cell line carrying FLNC exon skipping variant

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Lab Resource: Genetically-Modified Multiple Cell Lines

Generation of CRISPR-Cas9 edited human induced pluripotent stem cell line carrying FLNC exon skipping variant

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A B S T R A C T

Loss-of-function (LoF) mutations in FLNC are strongly associated with dilated cardiomyopathy (DCM). Using CRISPR/Cas9 mediated edition in an healthy donor derived iPSC (ICAN-403.3) we subcloned 1 iPSC line harboring LoF mutation in FLNC. All lines are fully pluripotent and isogenic except at edited site where it presents a homozygous (ICAN-FLNC42.1) deletion of splice site leading to skipping of exon 42 traduced into a short filamin form with reduced expression in derived cardiomyocytes. This line would serve for FLNC mutation DCM modeling after differentiation into cardiocytes or beating organoids.

Resource table

Unique stem cell lines identifier	ICANi002-A ICANi002-A-1
Alternative name(s) of stem cell lines	ICAN-403.3 ICAN-FLNC42.1
Institution	ICAN : Institut for Cardiometabolism And Nutrition
Contact information of the reported cell line distributor	Vincent Fontaine (v.fontaine@ican-institute.org) or Eric Villard (eric.villard@sorbonne-universite.fr)
Type of cell lines	iPSCs
Origin	Human
Additional origin info (applicable for human ESC or iPSC)	Age: 30 Sex:M Ethnicity/breed/other genetic background information if known: Caucasian
Cell Source	Skin Fibroblasts
Method of reprogramming	Episomal vectors (Okita et al., 2011)
Clonality	Clonal
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	q-PCR negative for episomal vectors
Cell culture system used	Feeder free
Type of Genetic Modification	Induced mutation by CRISPR Cas9 in FLNC gene
Associated disease	OMIM FLNC-cardimyopathies: 617,047
Gene/locus	FLNC, 7q32.1

(continued on next column)

(continued)

Method of modification/site-specific nuclease used	CRISPR/Cas9 induced non-homologus end-joining (NHEJ)
Site-specific nuclease (SSN) delivery method	Plasmid transfection, (pX459, Addgene #108293)
All genetic material introduced into the cells	- pX459V2.0-SpCas9-HF1 with specific gRNA coding sequence inserted (Addgene #108293) - pCX-cMyc (Addgene #19772) - pCXLE-hOCT3/4 (Addgene #27076) - pCXLE-hSK (Addgene #27078)
Analysis of the nuclease-targeted allele status	PCR and Sanger sequencing, and whole exome sequencing
Method of the off-target nuclease activity surveillance	Targeted PCR and sequencing and whole exome sequencing
Name of transgene	N/A
Eukaryotic selective agent resistance (including inducible/gene expressing cell-specific)	Episomal Puromycin resistance in pX459V2.0-SpCas9-HF1
Inducible/constitutive system details	N/A
Date archived/stock date	August 2021
Cell line repository/bank	https://hpscreg.eu/cell-line/ICANi002-A https://hpscreg.eu/cell-line/ICANi002-A-1
Ethical/GMO work approvals	Human fibroblast purchased from Promocell. See www.promocell.com/ethics

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(*continued*)

Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	pX459V2.0-SpCas9-HF1 was a gift from Yuichiro Miyaoka (Addgene plasmid #108293; http://n2t.net/addgene:108293; RRID: Addgene_108293) pCX-cMyc was a gift from Shinya Yamanaka (Addgene plasmid #19772; http://n2t.net/addgene:19772; RRID: Addgene_19772) pCXLE-hOCT3/4 was a gift from Shinya Yamanaka (Addgene plasmid #27076; http://n2t.net/addgene:27076; RRID: Addgene_27076) pCXLE-hSK was a gift from Shinya Yamanaka (Addgene plasmid #27078; http://n2t.net/addgene:27078; RRID: Addgene_27078)

1. Resource utility

FLNC truncating variants have been mainly associated with DCM. We established a new control human hiPSC line and derived one hiPSC clone with homozygous splicing mutation in *FLNC*. hiPSC-cardiomyocytes derived from this line expressed a shorter form of filamin in reduced quantiy (50%). This line allows cellular and molecular analysis of in-frame *FLNC* exon skipping consequences and it's application to study DCM physiopathology and develop therapy (See Table 1).

2. Resource details

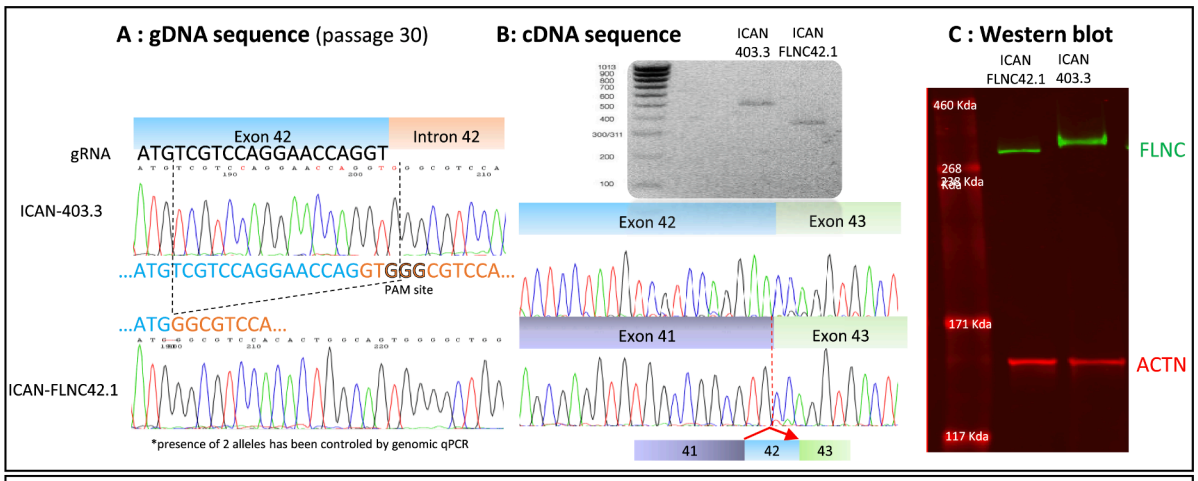
Dilated cardiomyopathy (DCM) is a devastating disease characterized by ventricular enlargement and systolic dysfunction leading to heart failure with little therapeutic options. *FLNC* loss-of-function mutations, including splicing mutations, has been strongly associated with DCM (Ader et al., 2019; Begay et al., 2016). *FLNC* related cardiomyopathies are transmitted on an autosomal dominant mode and mechanisms leading to cardiomyopathies are not fully understood. *FLNC* truncating variants are known causes of DCM likely acting through an haploinsufficiency mechanism. Since cardiomyocytes are not easily accessible, in vitro model are required to improve knowledge on patho-mechanisms. We first generated a control hiPSC line (ICAN-403.3) from a healthy donor and derived one *FLNC* mutated sub-clone using CRISPR/Cas9 induced non-homologous end-joining (NHEJ) leading to in frame exon 42 skipping. Interestingly exons 41 to 43 encode the filamin repeat motif 21 and exon 43 skipping mutation is involved in DCM development in a published family (Begay et al., 2016).

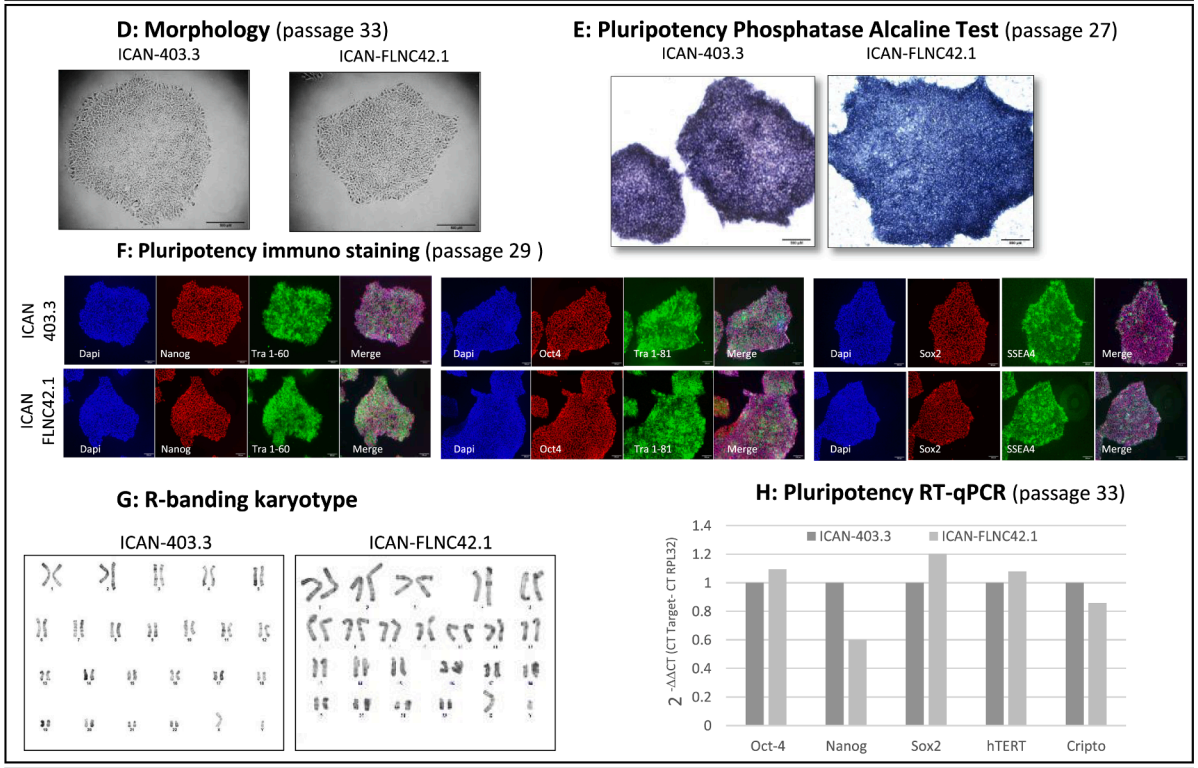
ICAN-403.3 was generated by reprogramming of skin fibroblasts from an healthy donor (PromoCell, Supplementary_S1) using electroporation of episomal vectors (Okita et al., 2011). We genome edit ICAN-403.3 by transfection with pX459V2.0-SpCas9-HF1 and a gRNA targeting FLNC exon 42 3′ boundarie (gRNA-FLNCex42, Table 2) to induce NHEJ (Fig. 1A). One out of 45 puromycin resistant clones was selected after Sanger sequencing. The clone harboured deletion of the donor splice site of intron 42 (ICAN-FLNC42.1 homozygous mutation: Chr7 (GRCh38):g.128854898_128854915del) (Fig. 1A). Presence of the two mutant alleles in ICAN-FLNC42.1 was confirmed by gDNA-qPCR quantitation compare to the ICAN-403.3 DNA (same Ct obtained from identical gDNA input).

Short tandem repeat (STR) indicated matching genetic identities between ICAN-FLNC42.1, the ICAN-403.3 and donor fibroblasts (Supplementary_S2). R and G-banding cytogenetic analysis was performed on the 2 lines, showing a normal karyotype (Fig. 1G). Sanger sequencing demonstrated lack of indels in the *in silico* predicted top-4 off targets

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Typical morphology of hiPSC.	Fig. 1-Panel D
Pluripotency status evidence for the described cell line	Qualitative analysis	1) Positive immune staining for pluripotency markers: Oct4, Nanog, Sox2, Tra1- 60, Tra1-81, SSEA4. 2) Phosphatase alkalin test was positive of all cell lines.	Fig. 1-Panel F Fig. 1-Panel E
	Quantitative analysis	Pluripotency markers Nanog, Sox2, Oct4, hTERT and Cripto are well expressed.	Fig. 1-Panel H
Karyotype	Karyotype (G-banding band and RDG banding)	46XY, resolution 400 bhps.	Fig. 1-Panel G
Genotyping for the desired genomic alteration /allelic status of the gene of interest	PCR across the edited site following by Sanger sequencing has been performed.	One homozygous deletion has been sequenced.	Fig. 1-Panel A Exon sequence is represented in blue and intron sequence in orange
	Transgene-specific PCR	PCR didn't reveal transgene integration in ICAN-FLNC42.1	Supplementary_S6
Verification of the absence of random plasmid integration events	Plasmid specific PCR	PCR didn't reveal Crispr/Cas9 plasmid integration of the edited cell lines	Supplementary Figure_S6
Parental and modified cell line genetic identity evidence	STR analysis, microsatellite PCR (mPCR)	STR Analysis : 16 loci tested and perfect match with the two cell lines.	Supplementary_2, submitted in the archive with journal
Mutagenesis / genetic modification outcome analysis	Sequencing (genomic DNA PCR)	One homozygous deletion has been sequenced.	Fig. 1-Panel A
	PCR-based analyses	*N/A*	N/A
	Western blotting	Western blot using Cterm antibody, has shown a qualitatively reduced transcription of a shorter protein for ICAN- FLNC42.1 homozygous cardiomyocyte.	Fig. 1-Panel C
Off-target nuclease analysis-	1- PCR and Sanger sequencing across top 4/10 predicted top likely off-target sites 2-Exome sequencing	Sanger sequencing for the 4 best scored off target predicted and exome sequencing didn't show unexpected variants in the edited cell lines.	1-Supplementary_S3 2-Analysis in Supplementary_S4. Full exome not show but available with author.
Specific pathogen-free status	Mycoplasma	Mycoplasma absence has been verified for each cell lines by qPCR sequencing.	Supplementary_S5
Multilineage differentiation potential	Directed differentiation	The ability of the cell lines to differentiate into derivatives of all 3 germ layers has been performed by Hpsc Score Card panel after directed differentication in the 3 layers.	Fig. 1-Panel I
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype - additional histocompatibility info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A





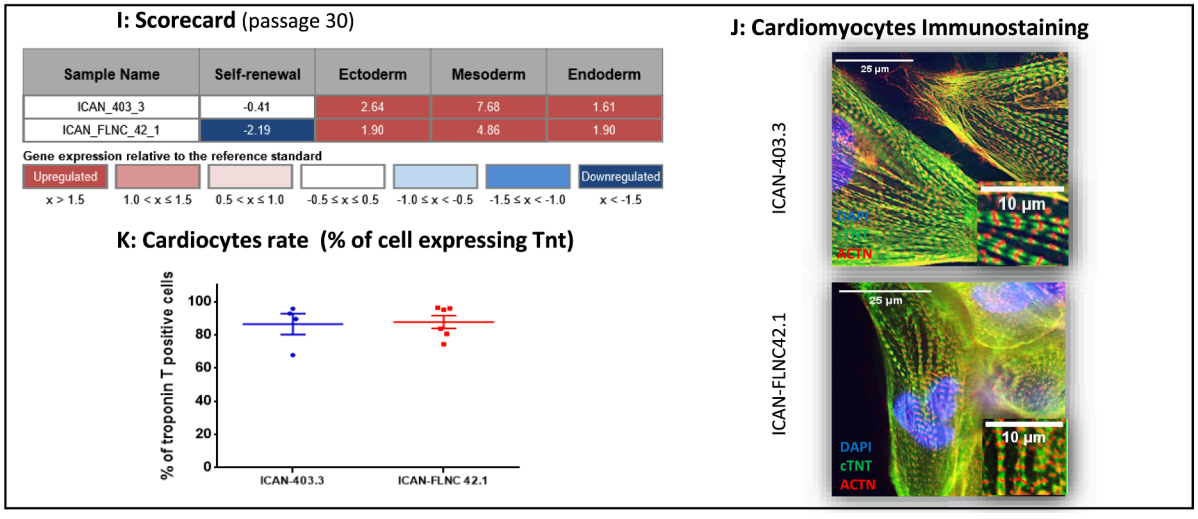


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Fig. 1. A) gDNA sequence of ICAN-403.3 and the edited cell line ICAN-FLNC42.1 showing an homozygous 18bp deletion, in black the sequence of de gRNA used for CRISPR/CAS9 editing ; B) on the top agarose gel migration of cDNA amplification (Primer FLNC40F and 44R) showing a shorter amplicon for ICAN-FLNC42.1 and below the cDNA sequence of the targeted exon junction showing the exon 42 skiping in the ICAN-FLNC42.1 compare to the control ; C) western blot analysis on cardiomyocytes, the green band correspond to the filamin C protein, and the red one a-actinine used as charge marker. The ICAN-FLNC42.1 cell line expressed a shorter protein in reduced among compared the ICAN-403.3 cardiomyocytes ; D) microscopic brightfield view of the two pluripotent cell lines ; E) microscopic brightfield view of the two cell lines after alkalin phosphatase treatment, the purple color observed attest of the pluripotency characteristic of the cell lines ; F) pluripotency immuno staining of ICAN-403.3 and ICAN-FLNC42.1 hiPSc cell lines : immunofluorescence-based detection of human pluripotent specific proteins Oct-4, Sox2, Nanog (red) and surface marker SSEA-4, Tra-1-60 and Tra-1-81 (green). DAPI stained the nucleus (blue) ; G) karyotying analysis of ICAN-403.3 and ICAN-FLNC42.1 hiPSc cell lines shows a normal male karyotype for both cell lines ; H) Confirmation of the mRNA expression of pluripotency-associated genes in ICAN-403.3 and ICAN-FLNC42.1 hiPSc clones (Nanog, Oct-4, Sox2, hTERT, Cripto) ; I) Scorecard results after 4 days of directed differentiation ; H) Percent of Troponin-T positive cells counted by cytometry analysis of independent iPS derived cardiomyocytes differentiation of ICAN-403.3 and ICAN-FLNC42.1cardiomyocytes ; J) a-actinin (green) and troponin-T (red) immunocytochemistry of ICAN-403.3 and ICAN-FLNC42.1 cardiomyocytes at day 21 of differentiation.

sites and exome sequencing confirmed lack of indels at all covered exonic predicted off target of the edited line (Supplementary S3 and S4). All hiPSC lines were mycoplasma-free (Supplementary_S5).

The generated cell lines showed typical human embryonic-stem cell-like morphology (Fig. 1D). Immuno- fluorescence analysis as well as RT-qPCR and alkaline phosphatase labeling demonstrated the expression of pluripotency markers (Fig. 1E-F-H). Episomes used for reprogramation and edition were not found to be integrated using specific PCR (Supplementary_S6). In specific differentiation media each cells lines demonstrate the capacity to differentiate into the three germ lineages. In addition, ScoreCard performed on RNA extracted from cell lines show expression of three germlines genes, and extinction of pluripotent markers (Fig. 1I).

Importantly, all lines were able to differentiate into beating cardiomyocytes using Lian et al protocol (Lian, 2013) showing ACTN2 positive sarcomeres (Fig. 1J). FACS analysis using T-Troponin labelling indicated reproducible and highly efficient production of cardiomyocytes (Fig. 1K). Finally, the molecular effect of splice site deletion in the mutant clone has been characterized by immunolabbeling and revealed a shorter FLNC and less expressed (50%, mimicking haploinsuffisancy) protein compare to FLNC from ICAN-403.3 (Fig. 1C) and the precise deletion of exon 42 verified using sequencing of RT-PCR product from ICAN-FLNC42.1 cardiomyocyte mRNA (Fig. 1B).

3. Materials and methods

3.1. Cell culture

Reprogramming of fibroblasts, isolation of hiPSC clone ICAN-403.3 and hiPSC culture was performed as described (Fontaine et al., 2021).

3.2. CRISPR/Cas9 edition and screening

Sub-confluent hiPSC (3x 10^5 cells/well of 6-well plate) were transfected with 1ug of pX459V2.0-SpCas9-HF1 carying the FLNC specific gRNA by Lipofectamine Stem Transfection Reagent. After puromycin selection, single clones were picked, expanded and screened by Sanger sequencing (Genewiz) of PCR product spanning the target site (Fig. 1A, Table 2).

3.3. hiPSC differentiation

hiPSC were dissociated into single cells and plated at 3.5x10^5 cells/well of 12-well plate with mTeSR1 and 10 μM Y-27632. After 2 days, cells were differentiated into the three layers using RMPI1640 + B27 (ectoderm), with 10 μM CHIR99021 (mesoderm) or with insulin and 100 ng/ml Activine A (endoderm). Cardiomyocytes differentiation was as described (Lian, 2013).

3.4. Pluripotency characterization

hiPSC cultured on Matrigel for 6 days were incubated with alkaline phosphase substrat (20 min, 37 °C) and fixed with ethanol 95% (10 min, 37 °C) (Fig. 1E). For immunostaining and pluripotency qRT-PCR, hiPSC (Fig. 1.F) were processed as described previously (Fontaine et al., 2021). qRT-PCR CT-values were normalized to RPL32 using ΔCT method after having PCR-check absence of amplification from fibroblasts as negative control (Fig. 1H).

3.5. Differentiation capacities characterization

A scorecard kit was used to evaluate the gene expression on 1 μg mRNA from the directed three germ layers differentiations (see 3.3) according to manufacturers instructions (ThermoFisher).

Cardiocytes have been culture for 10 extra days in RPMI + B27 plus Insulin after a 12 days-differentiation period (12 days). Immunolabelling was performed on fixed (PFA 4%, 10 min) and permeabilized (10 min, PBS Triton 0,1%) cells. After blocking (PBS + BSA2%, 30 min) cells were incubated overnight (4 °C) with primary antibodies (Table 2, Fig. 1J). Secondary antibodies were applied for 1 h at RT and DAPI (1/1000) was used to stain nucleus.

3.6. Molecular characterization of the mutation effects

Total mRNA were extracted from cardiocytes and subjected to RT-PCR with MMLV reverse transcriptase, with primer in exons 40 and 44 of filamin. Resulting amplicons were Sanger sequenced (primers in Table 2) (Fig. 1-A). Extracted proteins (10 μg) in standard lysis buffer were migrated into 3–8% Tris Acetate gel (40 mA, 150 V, 4 h) and transfered on nitrocellulose membrane before labelling (Antibodies, Table 2). Revelation was performed on Li-COR Odyssey Infrared Imaging system (Fig. 1.C).

3.7. Genomic integrity controls

Potential episomal integration plasmids was tested by PCR amplification of the vectors backbones on gDNA (primers in Table 2). Cell identity was assessed on gDNA from donor fibroblasts, ICAN-403.3 and ICAN-FLNC42.1 with the 16 STR markers of PowerPlex 16HS kit (Promega) (Supplementary_S2). The 4 best CRISPOR (Broad Institut tool) predicted off-target sites were checked using PCR and Sanger sequencing (Supplementary_S3, primers Table 2). In addition, whole exome sequencing (Integragen, Supplementary_S4) allows for the top 90% CRISPOR predicted score sites checking by visual inspection on aligned reads (Supplementary_S4).

Karyotype of the 2 hiPSC clones was analysed between passages 17 and 39 on more than 10 metaphase spreads (Fig. 1.G) (Supplementary_S7).

3.8. Mycoplasma test

Mycoplasma detection have been performed by standardized qPCR on the culture media (Mycoplasmacheck, Eurofins).

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.

Regeants details				
Assay	Reagent	Company	Reference	Conditions
Cells Culture	mTesR1 complete KIT-GMP	StemCell Technologies	85,850	
	Gentle Cell dissociation	StemCell Technologies	07-174	iPSC dissociation 8–10 min, 37 °C
	Matrigel hESC qualified	Corning	354-277	
	DMEM F-12 W/GLUTAMAX-I	Life technologies	31-765.-027	diluent for Matrigel
	Y-27632	Sigma	Y0503	10 μM
	Triple 10x	Gibco	A121770	Cardiomyocyte dissociation, 2 min, 37 °C
CRISPR/ Cas9 edition	pX459V2.0-SpCas9-HF1	Addgene	108-293	1 μg
	gRNA-FLNCex42	IDT	5′ ATGTCGTCCAGGAACCAGGT 3′	
	Lipofectamine Stem transfection reagent	Life technologies	STEM00001	as manufacturer described
	Puromycin	Sigma	P9620	0.5 μg/μL
PCR	Biotaq DNA polymerase	BIO-Technofix	BIO-21060	as manufacturer described
Sequencing PCR Primers	FLNC exon 42F/43R	IDT	5′ CAGGAGTGGGGATGAAG 3′ 5′ ATGGACCTGCTGTCCTCAAC 3′	touch down Tm60°C 35 cycles (602 bp)
mRNA sequencing	FLNC exon 40F/44R	IDT	5′ AAGTACCGTGGCCAGCAC 3′ 5′ ACGTAGCACTCCTCCACAGC 3′	Tm60°C 35 cycles (477 bp)
Primer sequence for gDNA-qPCR	FLNC q42F/q42R	IDT	5′ AGTTCAGCATCTGGACC 3′ 5′ TGCAATCTCCGCTTTGC 3′	Tm60°C 40 cycles(79 bp)
Primer sequence for edition plasmide integration	AmpR	IDT	5′ GCTATGTGGCGCGGTATTAT 3′ 5′ AAGTTGGCCGCAGTGTTATC 3′	Tm60°C 35 cycles (186 bp)
Primer sequence for reprogrammation plasmide integration	EBNA-1	IDT	5′ ATCAGGGCCAAGACATAGAGATG 3′ 5′ GCCAATGCAACTTGGACGTT 3′	Tm60°C 35 cycles(61 bp)
Pluripotency Characterisation	SigmaFAST BCIP/NBT tablets	Sigma Aldrich	B5655	1 tablets/10 mL water
	D-PBS 1X	Life technologies	14-190-250	
Pluripotency Markers (Immunostaining)	Rabbit anti-Nanog	Cell Signaling	4903	1:200 (in Blocking Buffer1/10 in PBS)
	Rabbit anti-OCT4	Clinisciences	3576–100	1:200 (in Blocking Buffer1/10 in PBS)
	Rabbit anti-Sox2	Millipore	AB5603	1:200(in Blocking Buffer1/10 in PBS)
	Mouse anti-Tra1-60	Millipore	MAB4360	1:100 (in Blocking Buffer1/10 in PBS)
	Mouse anti-Tra1-81	Millipore	MAB4381	1:100 (in Blocking Buffer1/10 in PBS)
	Mouse anti-SSEA4	Santa Cruz	Sc-21704	1:100 (in Blocking Buffer1/10 in PBS)
Secondary antibodies	Goat anti-mouse dylight 594 conjugated	Bethyl	A90-11604	1:500 (in Blocking Buffer1/10 in PBS)
	Goat anti-rabbit Jackson 488 conjugated	Jackson Immuno Research	111-545-144	1:500 (in Blocking Buffer1/10 in PBS)
Mounting Media	Dako Fluoromount	Dako	S3023	
Pluripotency Markers (qPCR)	MMLV reverse transcriptase	Life technologies	28,025,013	as manufacturer described
	Oligo(dT)12–18 Primer	Life technologies	18-418-012	
	Sensifast SYBR NO rox kit	BIO-Technofix	BIO-98005	
Primers sequences for Pluripotency Markers (qPCR)	OCT-4	IDT	5′ CCTCACTTCACTGCACTGTA 3′ 5′ CAGGTTTTCTTTCCCTAGCT 3′	Tm60°C, 40 cycles (164pb)
	Sox2	IDT	5′ CCCAGCAGACTTCACATGT 3′ 5 CCTCCCATTTCCCTCGTTTT 3′	Tm60°C, 40 cycles(151 pb)
	Nanog	IDT	5′ CTCCAACATCCTGAACCTCAGC 3′ 5′ CGTCACACCATTGCTATTCTTCG 3′	Tm60°C, 40 cycles(115 pb)
	hTERT	IDT	5′ CCTGGGTGGCACGGCTTTTGTTC 3′ 5′ CAGCCTTGAAGCCGCGGTTGA 3′	Tm60°C, 40 cycles (167 bp)
	Cripto/TDGF1	IDT	5′ACAGAACCTGCTGCCTGAAT 3′ 5′ATCACAGCCGGGTAGAAATG 3′	Tm60°C, 40 cycles (215 bp)
House-Keeping Genes (qPCR)	RPL32	IDT	5′ AGTTCCTGGTCCACAACGTC 3′ 5′ GTGACTCTGATGGCCAGTTG 3′	Tm60°C,40 cycles (142 pb)
ScoreCard	SuperScript™ III Reverse Transcriptase	Life technologies	18-080-044	as manufacturer described
	Kit taqman hPSC Scorecard Panel, fast 96 well	Life technologies	A15876	
hiPSC differentiation culture media	RPMI 1640 Medium	Life technologies	21-875-091	
	B-27 supplement minus insulin	Life technologies	A1895601	Mixed with RPMI1640
	B-27 supplement with insulin	Life technologies	17-504-044	Mixed with RPMI1640
	Activine A	Peprotech	120-14P	100 ng/mL
	StemMACS CHIR 99,201	Miltenyi	130–103-926	6 μM
	Stem MACS IWP2	Miltenyi	130–105-335	5 μM
FACS immunostaining procedure for hiPSC-CM	Inside Stain Kit	Miltenyi	130–090-477	

(*continued on next page*)

Table 2 (*continued*)

Regeants details				
Assay	Reagent	Company	Reference	Conditions
				As manufacturer inscruction
	Cardiac Troponin T Antibody, anti-human/mouse/rat, REAfinity™	Miltenyi	130–120-403	1:200 (in Blocking Buffer1/10 in PBS)
	REA Control Antibody, human IgG1, APC, REAfinity™	Miltenyi	130–113-446	1:200 (in Blocking Buffer1/10 in PBS)
Primary antibody western blot	Rabbit FLNC -Cterm	Abcam	ab244284	1:500 (in Blocking Buffer1/10 in PBS)
	Mouse anti-α-actinine	Sigma-aldrich	A7811-.2ML	1:1000 (in Blocking Buffer1/10 in PBS)
Western blot secondary antibody	IR dye 680 goat anti mouse	Li-COR Biosciences	92-668-070	1:10 000 (PBS + TWEEN 0,1%+5% milk)
	Ir dye 800 CW donkey anti rabbit	Li-COR biosciences	92-632-213	1:10 000 (PBS + TWEEN 0,1%+5% milk)
	Intergenic KCNK3	IDT	5′ TTTGCTTGGCATGGTGTTAG 3′ 5′ CAGGCTCTGAGCTTCACAGAT 3′	Tm60°C, Touch Down ,35 cycles (363 bp)
	Intergenic LOC	IDT	5′ GTGTTGTGTGGTTTGAGTGC 3′ 5′ CCAAGGCATGCTGTAATCACT 3′	Tm60°C, Touch Down,35 cycles (249 bp)
	Exon MROH5	IDT	5′ GCCTCCCAAAGTGTTGAGAT 3′ 5′ CAAAGCCTCATCATGCAGAA 3′	Tm60°C, Touch Down ,35 cycles (350 bp)
	Intergenic CCNE1	IDT	5′ TGGAAACAGGGAAATCCAAC 3′ 5′ GGAGTCTGCTGACCTGTCTTG 3′	Tm60°C, Touch Down ,35 cycles (358 bp)
Machine used				
Assay	Machine			
PCR	Applied Biosystems™ Thermocycleur SimpliAmp™			
qPCR	LightCycler ® 480 System - Roche Life Science			
ScoreCard qPCR	QuantStudio™ 3 Real-Time PCR System- Applied Biosystems™			
Site-specific nuclease				
Genomic target sequence(s)	Intron 42 FLNC 5′ splicing donor site		Chr7(GRCh38): 128,854,913	
Top off-target mutagenesis predicted site sequencing (for CRISPR/Cas9 and TALENs) primers		Locus name and position	intron MRPS15; chr1:36929402 intergenic CIB4-KCNK3; chr2:26905217 intergenic LOC729987-SNX7; chr1:99103823 exon MROH5; chr8:142484102 intergenic C19orf12-CCNE1; chr19:30227752	

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.2021.102616.

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