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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

Flavie Ader^{a,b,c,*}, Laetitia Duboscq-Bidot^{a,e}, Sibylle Marteau^d, Matthieu Hamlin^d, Pascale Richard^{a,b,d}, Vincent Fontaine^d, Eric Villard^{a,d,e}

^a Sorbonne Université, INSERM, UMR_S 1166, Paris 75013, France

^b Molecular and Cellular Cardiogenetic and Myogenetic Functional Unit, Hôpital Pitié Salpêtrière, DMU BioGem, APHP-Sorbonne Université, Paris, France

^c Département 3, UP de Biochimie, UFR de Pharmacie, Université de PARIS, Paris, France

^d ICAN - Institute for Cardiometabolism and Nutrition, Paris 75013, France

^e APHP, Pitié-Salpêtrière University Hospital, 75013 Paris, France

ABSTRACT

Loss-of-function (LoF) mutations in FLNC are strongly associated with dilated cardiomyopathy (DCM). Using CRISPR/Cas9 mediated edition in a 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 translated 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 | | (continued) | |
|---|--|---|--|
| Unique stem cell lines identifier | ICANi002-A ICANi002-A-1 | Method of modification/site-specific nuclease used | CRISPR/Cas9 induced non-homologous end-joining (NHEJ) |
| Alternative name(s) of stem cell lines | ICAN-403.3 ICAN-FLNC42.1 | Site-specific nuclease (SSN) delivery method | Plasmid transfection, (pX459, Addgene #108293) |
| Institution | ICAN : Institut for Cardiometabolism And Nutrition | 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) |
| Contact information of the reported cell line distributor | Vincent Fontaine (v.fontaine@ican-institute.org) or Eric Villard (eric.villard@sorbonne-universite.fr) | Analysis of the nuclease-targeted allele status | PCR and Sanger sequencing, and whole exome sequencing |
| Type of cell lines | iPSCs | Method of the off-target nuclease activity surveillance | Targeted PCR and sequencing and whole exome sequencing |
| Origin | Human | Name of transgene | N/A |
| Additional origin info (applicable for human ESC or iPSC) | Age: 30 Sex: M Ethnicity/breed/other genetic background information if known: Caucasian | Eukaryotic selective agent resistance (including inducible/gene expressing cell-specific) | Episomal Puromycin resistance in pX459V2.0-SpCas9-HF1 |
| Cell Source | Skin Fibroblasts | Inducible/constitutive system details | N/A |
| Method of reprogramming | Episomal vectors (Okita et al., 2011) | Date archived/stock date | August 2021 |
| Clonality | Clonal | Cell line repository/bank | https://hpscereg.eu/cell-line/ICANi002-A https://hpscereg.eu/cell-line/ICANi002-A-1 |
| Evidence of the reprogramming transgene loss (including genomic copy if applicable) | q-PCR negative for episomal vectors | Ethical/GMO work approvals | Human fibroblast purchased from Promocell. See www.promocell.com/ethics |
| Cell culture system used | Feeder free | | |
| Type of Genetic Modification | Induced mutation by CRISPR Cas9 in FLNC gene | | |
| Associated disease | OMIM FLNC-cardiomyopathies: 617,047 | | |
| Gene/locus | FLNC, 7q32.1 | | |

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* Corresponding author.

E-mail address: flavie.ader@aphp.fr (F. Ader).

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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 quantity (50%). This line allows cellular and molecular analysis of in-frame *FLNC* exon skipping consequences and its application to study DCM physiopathology and develop therapy (See [Table 1](#)).

2. Resource details

Dilated cardiomyopathy (DCM) is a devastating disease

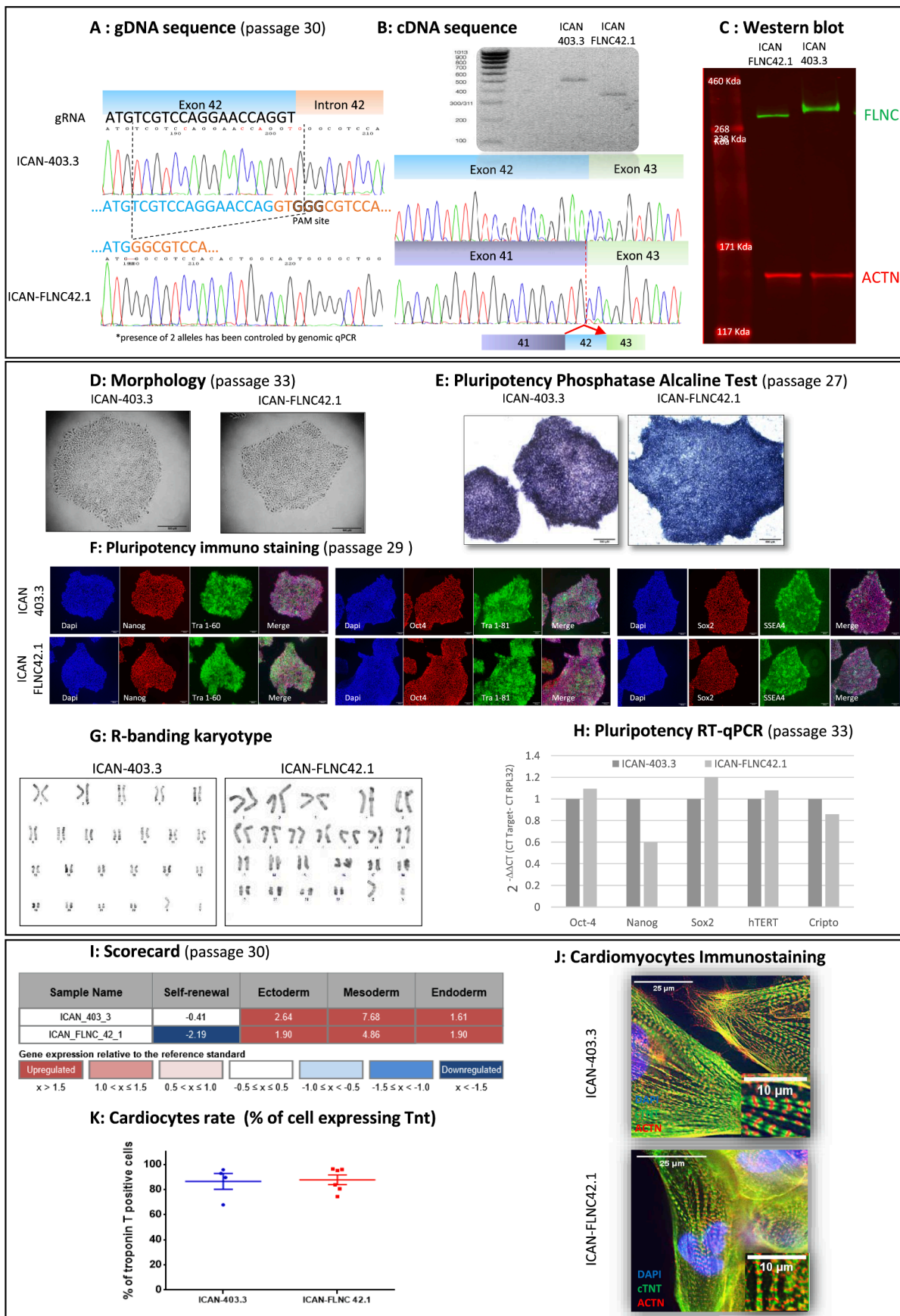
Table 1
Characterization and validation.

| 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 alkaline 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 |
| Verification of the absence of random plasmid integration events | Transgene-specific PCR | PCR didn't reveal transgene integration in ICAN-FLNC42.1 | Supplementary_S6 |
| | 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 |
| <i>Off-target nuclease analysis-</i> | 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 differentiation in the 3 layers. | Fig. 1-Panel I |
| <i>Donor screening (OPTIONAL)</i> <i>Genotype - additional histocompatibility info (OPTIONAL)</i> | HIV 1 + 2 Hepatitis B, Hepatitis C | N/A | N/A |
| | Blood group genotyping | N/A | N/A |
| | HLA tissue typing | N/A | N/A |
| | | | |

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 a 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-*FLNC*ex42, [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-*FLNC*42.1 homozygous mutation: Chr7 (GRCh38):g.128854898_128854915del) ([Fig. 1A](#)). Presence of the two mutant alleles in ICAN-*FLNC*42.1 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-*FLNC*42.1, 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



(caption on next page)

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 skipping 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 α -actinine used as charge marker. The ICAN-FLNC42.1 cell line expressed a shorter protein in reduced amount compared to 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 alkaline 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) karyotyping 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-FLNC42.1 cardiomyocytes ; J) α -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 reprogramming and edition were not found to be integrated using specific PCR (Supplementary S6). In specific differentiation media each cell 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 immunolabeling and revealed a shorter FLNC and less expressed (50%, mimicking haploinsufficiency) 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 (3×10^5 cells/well of 6-well plate) were transfected with 1 μ g of pX459V2.0-SpCas9-HF1 carrying 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 3.5×10^5 cells/well of 12-well plate with mTeSR1 and 10 μ M Y-27632. After 2 days, cells were differentiated into the three layers using RMP11640 + B27 (ectoderm), with 10 μ M 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 phosphatase substrate (20 min, 37 °C) and fixed with ethanol 95% (10 min,

37 °C) (Fig. 1E). For immunostaining and pluripotency qRT-PCR, hiPSC (Fig. 1F) were processed as described previously (Fontaine et al., 2021). qRT-PCR CT-values were normalized to RPL32 using Δ CT 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 μ g 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). Immunolabeling 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 μ g) in standard lysis buffer were migrated into 3–8% Tris Acetate gel (40 mA, 150 V, 4 h) and transferred 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 2Reagents details RRID Requirement for antibodies: use <http://antibodyregistry.org/> to retrieve RRID for antibodies and include ID in the table as shown in examples.

| Reagents 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 Y-27632 Triple 10x | Life technologies Sigma Gibco | 31-765.-027 Y0503 A121770 | diluent for Matrigel 10 µM Cardiomyocyte dissociation, 2 min, 37 °C |
| CRISPR/ Cas9 edition | pX459V2.0-SpCas9-HF1 gRNA-FLNCex42 | Addgene IDT | 108-293 5' ATGTCGTCCAGGAACCAGGT 3' | 1 µg |
| | Lipofectamine Stem transfection reagent | Life technologies | STEM00001 | as manufacturer described |
| PCR Sequencing PCR Primers | Puromycin | Sigma | P9620 | 0.5 µg/µL |
| | Biotaq DNA polymerase FLNC exon 42F/43R | BIO-Technofix IDT | BIO-21060 5' CAGGAGTGGGGATGAAG 3' 5' ATGGACCTGCTGCTCAAC 3' | as manufacturer described 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' GCTATGTGGCCGGTATTAT 3' 5' AAGTTGGCCGAGTGTTATC 3' | Tm60°C 35 cycles (186 bp) |
| Primer sequence for reprogramming plasmide integration | EBNA-1 | IDT | 5' ATCAGGGCCAAGACATAGAGATG 3' 5' GCCAATGCAACTTGGACGTT 3' | Tm60°C 35 cycles(61 bp) |
| Pluripotency Characterisation | SigmaFAST BCIP/NBT tablets D-PBS 1X | Sigma Aldrich Life technologies | B5655 14-190-250 | 1 tablets/10 mL water |
| 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 Sensifast SYBR NO rox kit | Life technologies BIO-Technofix | 18-418-012 BIO-98005 | |
| Primers sequences for Pluripotency Markers (qPCR) | OCT-4 | IDT | 5' CCTCACTTCACTGCCTGTA 3' 5' CAGGTTTCTTTCCCTAGCT 3' 5' CCCAGCAGACTTCACATGT 3' 5' CCTCCATTTCCTCGTTT 3' | Tm60°C, 40 cycles (164pb) |
| | Sox2 | IDT | 5' CTCCAACATCCTGAACCTCAGC 3' 5' CGTCACACCAATTGCTATTCTCG 3' | Tm60°C, 40 cycles(115 pb) |
| | hTERT | IDT | 5' CTGGGTGGCAGCGCTTTTGTT 3' 5' CAGCCTTGAAGCCGCGGTTGA 3' | Tm60°C, 40 cycles (167 bp) |
| | Cripto/TDGF1 | IDT | 5' ACAGAACCTGCTGCCTGAAT 3' 5' ATCACAGCCGGTAGAAATG 3' 5' AGTTCCTGGTCCACACGTC 3' 5' GTGACTCTGATGGCCAGTTG 3' | Tm60°C, 40 cycles (215 bp) |
| | House-Keeping Genes (qPCR) | RPL32 | IDT | 5' AGTTCCTGGTCCACACGTC 3' 5' GTGACTCTGATGGCCAGTTG 3' |
| ScoreCard | SuperScript™ III Reverse Transcriptase Kit taqman hPSC Scorecard Panel, fast 96 well | Life technologies Life technologies | 18-080-044 A15876 | as manufacturer described |
| 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 | Peptotech | 120-14P | 100 ng/mL |
| | StemMACS CHIR 99,201 | Miltenyi | 130–103-926 | 6 µM |
| FACS immunostaining procedure for hiPSC-CM | Stem MACS IWP2 | Miltenyi | 130–105-335 | 5 µM |
| | Inside Stain Kit | Miltenyi | 130–090-477 | |

(continued on next page)

Table 2 (continued)

| Reagents details | | | | |
|---|---|-------------------------|---|--|
| Assay | Reagent | Company | Reference | Conditions |
| Primary antibody western blot | Cardiac Troponin T Antibody, anti-human/mouse/rat, REAfinity™ | Miltenyi | 130–120-403 | As manufacturer instruction |
| | REA Control Antibody, human IgG1, APC, REAfinity™ | Miltenyi | 130–113-446 | 1:200 (in Blocking Buffer1/10 in PBS) |
| | Rabbit FLNC -Cterm | Abcam | ab244284 | 1:200 (in Blocking Buffer1/10 in PBS) |
| | Mouse anti- α -actinin | Sigma-aldrich | A7811-.2ML | 1:500 (in Blocking Buffer1/10 in PBS) |
| Western blot secondary antibody | IR dye 680 goat anti mouse | Li-COR | 92-668-070 | 1:10 000 (PBS + TWEEN 0,1%+5% milk) |
| | Ir dye 800 CW donkey anti rabbit | Li-COR | 92-632-213 | 1:10 000 (PBS + TWEEN 0,1%+5% milk) |
| | Intergenic KCNK3 | IDT | 5' TTTGCTTGGCATGGTGTAG 3' 5' CAGGCTTGAGCTCAGAT 3' | Tm60°C, Touch Down ,35 cycles (363 bp) |
| | Intergenic LOC | IDT | 5' GTGTTGTGGTTGAGTGC 3' 5' CCAAGGCATGCTGTAATCACT 3' | Tm60°C, Touch Down,35 cycles (249 bp) |
| | Exon MROH5 | IDT | 5' GCCTCCAAAGTGTGAGAT 3' 5' CAAAGCCTCATCATGCAGAA 3' | Tm60°C, Touch Down ,35 cycles (350 bp) |
| | Intergenic CCNE1 | IDT | 5' TGAAACAGGAAATCCAAC 3' 5' GGAGTCTGCTGACCTGTCTT 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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