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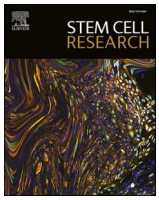

Lab Resource: Genetically-Modified Multiple Cell Lines

Generation of three *TTN* knock-out human induced pluripotent stem cell lines using CRISPR/Cas9 system

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A B S T R A C T

TTN mutations are the common genetic cause for various types of cardiomyopathies (e.g., dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy) and skeletal myopathies. Here, we generated three *TTN* knock-out human induced pluripotent stem cell (iPSC) lines using CRISPR/Cas9 system. These cell lines, which exhibit normal karyotype, typical morphology and pluripotency, could provide useful platform for investigating the role of *TTN* in associated disorders.

Resource Table

Unique stem cell line identifier	YUCMi019-A-3 YUCMi019-A-4 YUCMi019-A-5
Alternative name(s) of stem cell line	hiPSC-TTN_KO_1 hiPSC-TTN_KO_2 hiPSC-TTN_KO_3
Institution	Yonsei University College of Medicine
Contact information of the reported cell line distributor	Boyoung Joung, cby6908@yuhs.ac
Type of cell line	iPSC
Origin	Human
Additional origin info *(applicable for human ESC or iPSC)*	Age: N/A Sex: Male
Cell Source	Cord blood cell
Method of reprogramming	N/A
Clonality	Clonal
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	N/A
Cell culture system used	mTeSR™1 medium on matrigel-coated plate
Type of Genetic Modification	CRISPR/Cas9-mediated knock-out
Associated disease	Dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, various types of skeletal myopathies
Gene/locus	*TTN*, 2q31.2
Method of modification/site-specific nuclease used	CRISPR/Cas9
Site-specific nuclease (SSN) delivery method	Electroporation
All genetic material introduced into the cells	Cas9 protein, single guide RNA (sgRNA)
Analysis of the nuclease-targeted allele status	Sanger sequencing of the targeted allele
Method of the off-target nuclease activity surveillance	N/A
Name of transgene	N/A
Eukaryotic selective agent resistance (including inducible/gene expressing cell-specific)	N/A
Inducible/constitutive system details	N/A
Date archived/stock date	March 2022
Cell line repository/bank	https://hpscreg.eu/cell-line/YUCMi019-A-3 https://hpscreg.eu/cell-line/YUCMi019-A-4 https://hpscreg.eu/cell-line/YUCMi019-A-5
Ethical/GMO work approvals	The hiPSC line, CMC-hiPSC-011 was provided by Korea National Stem Cell Bank originally provided from Catholic University. This study was approved by the Institutional Review Board/Ethics Committee of Severance Hospital, Yonsei

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	University Health System (approval no. 4–2021-1626).
Addgene/public access repository recombinant DNA sources' disclaimers (if applicable)	N/A

1. Resource utility

The generated three *TTN* knock-out iPSC lines could be differentiated into targeted cells, such as ventricular cardiomyocytes, to investigate its roles in various types of cardiomyopathies and skeletal myopathies. Table 1..

2. Resource details

Titin, encoded by *TTN*, is a massive protein that plays important roles in contraction and relaxation of striated muscle, sarcomere organization, force transmission and transduction, and signalling responses (Hinson et al., 2015; Ware and Cook, 2018). In recent years, it has been reported that mutations in different regions of titin seriously disrupt the sarcomere structure and function that are associated with various types of cardiomyopathies (e.g., dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy) and skeletal myopathies (Tskhovrebova and Trinick, 2003; Fomin et al., 2021; Linke and Hamdani, 2014). However, its functional and molecular relevances are not fully understood. In this study, we fortunately generated three *TTN* homozygous knock-out clones by CRISPR/Cas9-based genome editing system (Fig. 1A). These cells maintained normal karyotype without chromosomal abnormalities (Fig. 1B), iPSC-like morphology and alkaline phosphatase activity (Fig. 1C). In addition, these cells expressed pluripotency markers (Sox-2, Oct-3/4, SSEA-4, and TRA-1–81) as shown by immunofluorescence staining (Fig. 1D). Moreover, these cells were positive for OTX2 (ectoderm), Brachyury (mesoderm), and Sox-17 (endoderm), indicating that they possess the differentiation potential into three germ layers (Fig. 1E). Finally, these cells had no mycoplasma contamination (Fig. 1F).

3. Materials and methods

3.1. Cell culture

The hiPSC line, CMC-hiPSC-011 was provided by Korea National Stem Cell Bank originally provided from Catholic University. hiPSCs were cultured in TeSR™-E8™ medium (STEMCELL Technologies) on vitronectin (Gibco)-coated plates at 37 °C in a humidified atmosphere of 5 % CO_2, and subcultured every 4–6 days.

3.2. CRISPR/Cas9-mediated knock-out

A total of 8 X 10^4 hiPSCs were electroporated with preassembled Cas9/sgRNA ribonucleoprotein (RNP) complexes (1.5 μg Cas9 protein and 10 pmoles sgRNA) by Neon™ Transfection System (1200 V, 20 ms, and 2 pulses; Thermo Fisher Scientific). Then, the cells were seeded using the serial dilution method on matrigel (Corning)-coated 96-well plates in mTeSR™1 medium (STEMCELL Technologies) supplemented with 10 μM Y-27632 (Tocris Bioscience). Y-27632 was removed next day and the medium was changed every other day. The single colonies were manually picked up after 12–14 days, and transferred to matrigel-coated 24-well plates for clonal expansion. After isolation of genomic DNA using G-spin™ Total DNA Extraction Mini Kit (iNtRON Biotechnology), the sequence containing knock-out region was confirmed by sanger sequencing at BIONICS Co., ltd (Korea).

3.3. Karyotyping and cell identify

Karyotype analysis was performed at Samkwang Medical Lab (Korea) using standard protocols for GTG banding. Total 20 metaphase chromosome spreads were analyzed at 500–550 band resolution.

For cell line authentication, short tandem repeat (STR) analysis was performed with detection of 18 loci (D5S818, D13S317, D7S820, D16S539, vWA, TH01, TPOX, CSF1PO, AMEL, D3S1358, D21S11, D18S51, D8S1179, FGA, D2S1338, D19S433, Penta D, Penta E) by Cosmogenetech (Korea).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Normal	Fig. 1 panel C
Pluripotency status evidence for the described cell line	Alkaline Phosphatase staining	Positive	Fig. 1 panel C
	Qualitative analysis *(Immunocytochemistry)*	Positive for Sox-2, Oct-3/4, SSEA-4, and TRA-1–81	Fig. 1 panel D
	Quantitative analysis *(RT-qPCR)*	Not performed	N/A
Karyotype	Karyotype (G-banding) and resolution	46XY, Resolution 500–550	Fig. 1 panel B
Genotyping for the desired genomic alteration/ allelic status of the gene of interest	PCR across the edited site or targeted allele-specific PCR	Homozygous knock-out	Fig. 1 panel A
	Transgene-specific PCR	Not performed	N/A
Verification of the absence of random plasmid integration events	PCR/Southern	Not performed	N/A
Parental and modified cell line genetic identity evidence	STR analysis	All sites matched	Submitted in the archive with journal
Mutagenesis / genetic modification outcome analysis	Sequencing (genomic DNA PCR)	Homozygous knock-out	Fig. 1 panel A
	PCR-based analyses	Not performed	N/A
	Southern Blot or WGS; western blotting (for knock-outs, KOs)	Not performed	N/A
Off-target nuclease analysis	PCR across top 5/10 predicted top likely off-target sites, whole genome/exome sequencing	Not performed	N/A
Specific pathogen-free status	Mycoplasma	Mycoplasma testing by RT-PCR, Negative	Fig. 1 panel F
Multilineage differentiation potential	Directed differentiation	Positive for OTX2 (ectoderm), Brachyury (mesoderm), and Sox-17 (endoderm)	Fig. 1 panel E
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

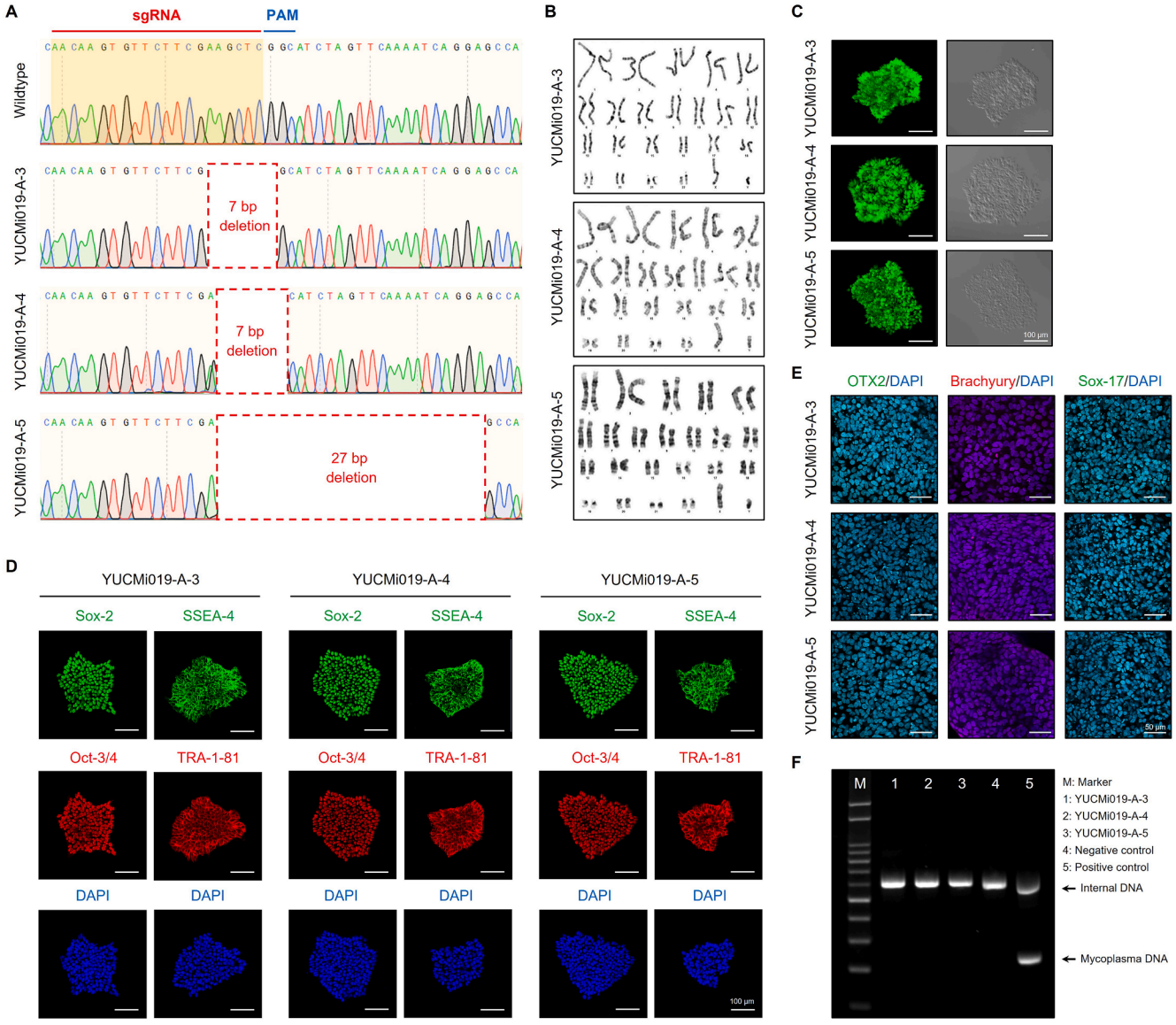

Fig. 1. Generation and characterization of three *TTN* knock-out iPSC lines.

3.4. AP staining

Cells were stained using Alkaline Phosphatase Live stain (Thermo Fisher Scientific) according to the manufacturer's instruction, and visualized by confocal microscopy (Zeiss LSM 710).

3.5. Immunocytochemistry

Cells were fixed with 4 % paraformaldehyde for 15 min and washed three times with PBS. After permeabilization and blocking, cells were incubated with fluorescence-conjugated primary antibodies overnight at 4 °C. Then, cells were washed five times with PBS, stained with Hoechst 33342 (Thermo Fisher Scientific), and observed using confocal microscope (Zeiss LSM 710). All antibody details are listed in Table 2.

3.6. In vitro trilineage differentiation

Cultured cells were differentiated using STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies) according to the manufacturer's instructions. All antibody details are listed in Table 2.

Table 2
Reagents details.

Antibodies and stains used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Sox-2 (E-4) Alexa Fluor® 488	1:200	Santacruz Cat# sc-365823 AF488, RRID: AB_10842165
	Oct-3/4 (C-10) Alexa Fluor® 594	1:200	Santacruz Cat# sc-5279 AF594, RRID: AB_628051
	SSEA-4 Alexa Fluor® 488	1:200	Santacruz Cat # sc-21704 AF488, RRID: AB_628289
	TRA-1–81 Alexa Fluor® 647	1:200	Santacruz Cat# sc-21706 AF647, RRID: AB_628386
Differentiation Markers	OTX2 (D-8) Alexa Fluor® 488	1:200	Santacruz Cat# sc-514195 AF488
	Brachyury (A-4) Alexa Fluor® 594	1:200	Santacruz Cat# sc-374321 AF594, RRID: AB_10990301
	Sox-17 (3.5CH) Alexa Fluor® 488	1:200	Santacruz Cat# sc-130295 AF488, RRID: AB_2286667
Nuclear stain	Hoechst33342	1:2000	Thermo Fisher Scientific Cat # H3570

Site-specific nuclease		
Nuclease information	Cas9	Thermo Fisher Scientific Cat # A36498
Delivery method	Electroporation	Neon transfection system (Thermofisher Scientific)
Selection/enrichment strategy	N/A	N/A

Primers and Oligonucleotides used in this study		
	Target	**Forward/Reverse primer (5′-3′)**
Genotyping	TTN	F: ATCAGTGCCATCAACGATGC R: GCCGGGTTTTCAATGGTCAT
Targeted mutation sequencing	TTN	Sanger sequencing chromatograms in Fig. 1A.
sgRNA	TTN	AACAAGTGTTCTTCGAAGCT

3.7. Mycoplasma detection

Cell culture supernatants were analyzed using SafeDry™ Mycoplasma PCR Detection Kit (CellSafe) according to the manufacturer's instructions.

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.

Acknowledgments

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