

SUPPLEMENTARY MATERIALS AND METHODS

1. Cardiomyocytes derivation from hiPSCs

The iPSC colonies were grown in 6-well dishes on irradiated mouse embryonic fibroblasts (GlobalStem, Rockville, MD) in DMEM/F-12 with 20% KnockOut serum replacement, 1 mM glutamine, 1% non-essential amino acids, 1% penicillin/streptomycin, 0.1 mM Beta-mercaptoethanol (Life Technologies, USA), 20 or 30 ng/mL basic FGF (Stemgent, San Diego, CA) and were passaged manually every 7 or 8 days. When iPSC colonies reached confluency, they were dissociated with Versene (Life Technologies, USA) and were growth on Matrigel-coated plates in mTeSr1 medium (STEM CELLS, Technologies, USA) with 5 μ M ROCK inhibitor (Y27632, Miltenyi Biotec, Germany). Following confluency in the feeder-free condition, iPSC colonies were detached with Accutase (Life Technologies, USA) and then replated on a 12-well Matrigel-coated dish in mTeSr1 medium with 5 μ M ROCK inhibitor (Y27632) (Miltenyi Biotec, Germany) at different cell densities: 0.5, 0.75 or 1 million cells/mL for four days. Induction was performed with 12 μ M CHIR99021 (Selleckchem, USA) in RPMI medium supplemented with B27-insulin (Life Technologies, USA). Twenty-four hours later, the medium was substituted with RPMI with B27-insulin. The following day, the medium was substituted with 2 mL of a medium made up of 1 mL of the medium collected from the well and 1 mL of fresh RPMI with B27-insulin, to which 5 μ M IWP2 (Tocris, Bioscience, USA) was added. This medium was replaced two days later with RPMI with B27-insulin. Two days later, RPMI with B27 was substituted in the wells. First beating areas are expected to form between the 9th and the 12th day from the culture in mTeSr1 medium. Cardiomyocytes with spontaneously beating area were maintained in RPMI/ B27 medium until the 20th day of induction.

SUPPLEMENTARY TABLES

Table S1: phenotypic data of control subject and FRDA patient from which iPSCs were generated by Coriell Institute Repositories.

CODE	Gender	Age (Years)	GAA length repeats	Phenotypic Data
GM23280*A	♀	36		Apparently healthy
GM23404*B	♀	36	330-380	Spinal-cerebral degeneration; myocardiopathy.

Table S2: list of primary and secondary antibodies used in cytofluorimetry (FACS), immunofluorescence (IF) and Western Blot (WB) analysis

Antibody	Species	Dilution
Anti-Hepcidin, Abcam (Ab30760)	Rabbit polyclonal	1:100 for WB 1:100 for IF
Anti-Ferroportin/SLC40A1, Novus biologicals (NBP1-21502)	Rabbit polyclonal	1:500 for WB 1:500 for IF
Cardiac Troponin T, Thermo Fisher Scientific (ms-295-p1/13-11)	Mouse IgG1	1:200 for FACS and IF
MF 20, DSHB	Mouse IgG2b	1:20 for IF
Alexa 488 Goat, Life Technologies (A-21121)	Mouse IgG1	1:1000 for FACS and IF
Alexa 594 Goat, Life Technologies (A-21145)	Mouse IgG2b	1:1000 for IF
Anti-Rabbit IgG-FITC, Sigma (F7512)	Rabbit IgG	1:200 for IF
Anti-Rabbit IgG-HRP, Thermo Fisher Scientific (31460)	Rabbit IgG	1:10000 for WB

Table S3: primer sequences and amplicon length of the genes analysed by real-time PCR in iPSC-derived cardiomyocytes. β -Actin and GAPDH were used as housekeeping genes.

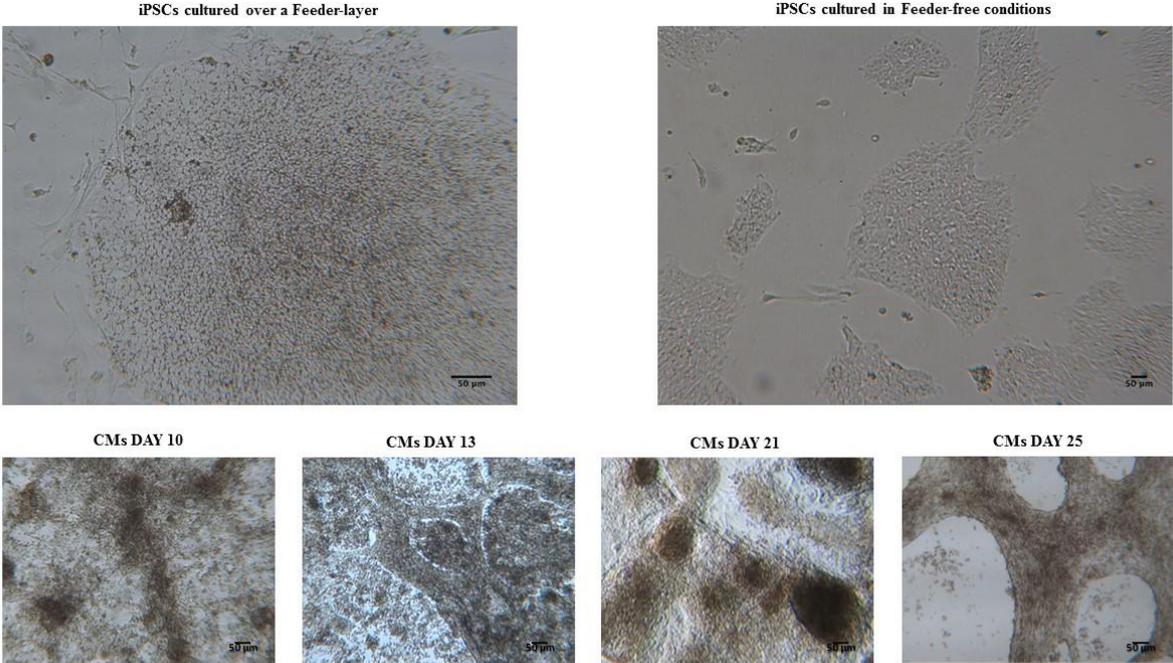
GENE	NCBI RefSeqs*	LEFT PRIMER	RIGHT PRIMER	A.L. (bp)**
β -Actin	NM_0011013	agacctgtacccaacacagt	agtacttgcgctcaggagga	140
GAPDH	NM_002046	cctgacctgccgtctagaaa	tgctgtagccaaattcgttg	233
Frataxin (FXN)	NM_000144	tagcagaggaaacgtggac	gatttctgtttggcgtct	170
Hepcdin (HAMP)	NM_021175.2	gaccagtggctctgtttcc	cacatccacactttgatcg	430
ATOH8	NM_032827	caagaagcgaaggagtgac	gcatcttgagaagaccacga	125
Ferritin (FTH1)	NM_002032.2	ctggcactgacaaaaatga	cagggtgtctgtcaaaaga	168
Ferroportin (FPN)	NM_014585.5	cgtcattgctgctagaatcg	ccaggatgacatgatgaaa	300
Transferrin Receptor (TFRC)	NM_001128148.1	aaaatccggtgtaggcacag	ttaaatgcaggagcgaagg	420
Actinin 2	NM_001103.3	ccgattatgacctggtag	gatgcagtactggcctgat	205
GATA4	NM_001308093.1	tggcctgtcatctcactacg	aagaccaggctgtccaaga	192
SIRPA	NM_001040022.1	agcaaaagccatgacctgaa	cctgggctttcttctgtctg	184
Troponin T2, cardiac type (TNNT2)	NM_000364.3	tccgaacaggatcaacgat	ccattccaacaggagctg	190

*NCBI RefSeqs= NCBI Reference Sequences

**A.L. (bp) =Amplicon length (base pair)

SUPPLEMENTARY FIGURES

Figure S1: timeline of iPSC-derived cardiomyocyte differentiation protocol



SUPPLEMENTARY VIDEO CAPTIONS

SM1: CTR Cardiomyocytes

SM2: FRDA Cardiomyocytes