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 Tecnologies, 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 Tecnologies, USA) and were growth on Matrigel-coated plates in mTeSr1 medium (STEM CELLS, Tecnologies,USA) with 5µM ROCK inhibitor (Y27632, Miltenyi Biotec, Germany). Following confluency in the feeder-free condition, iPSC colonies were detached with Accutase (Life Tecnologies, USA) and then replated on a 12-well Matrigel-coated dish in mTeSr1 medium with 5µM ROCK inhibitor (Y27632) (Miltenyi Biotec, Germany) at different cell densities: 0.5, 0.75 or 1 milion cells/mL for four days. Induction was performed with 12 µM CHIR99021 (Selleckchem, USA) in RPMI medium supplemented with B27-insulin (Life Tecnologies, 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 µM 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 9^{th} and the 12^{th} day from the culture in mTeSr1 medium. Cardiomyocytes with spontaneously beating area were maintained in RPMI/ B27 medium until the 20^{th} 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	Diluition
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 GADPH were used as housekeeping genes.

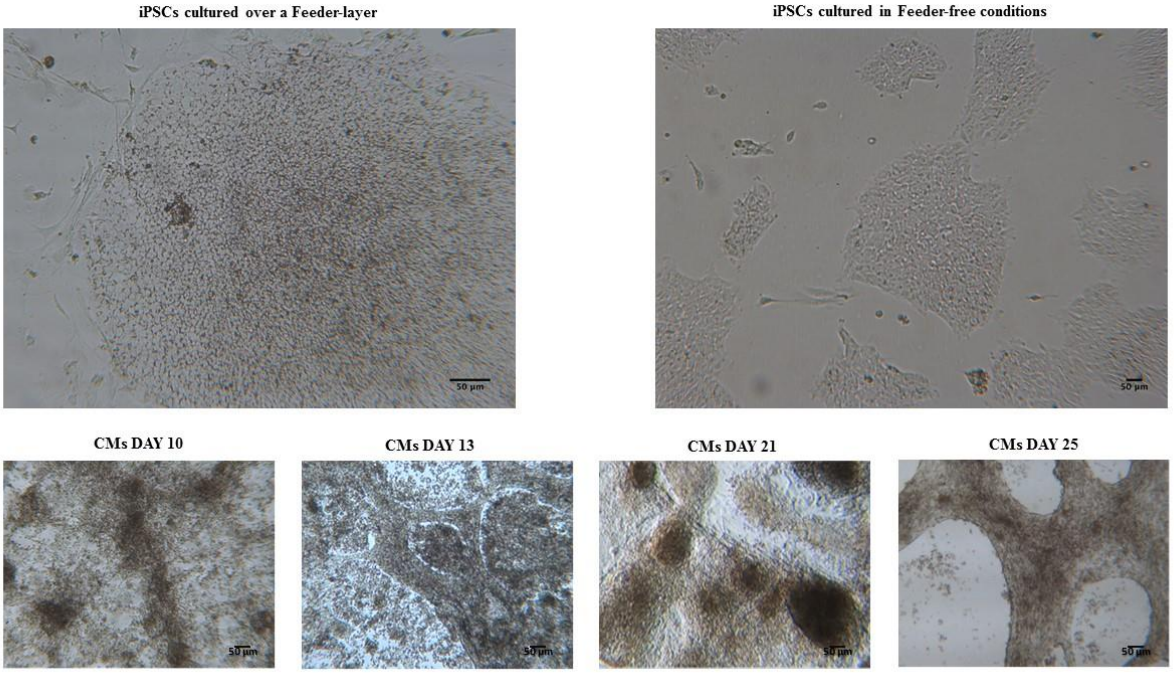
GENE	**NCBI RefSeqs***	***LEFT PRIMER***	***RIGHT PRIMER***	**A.L. (bp)****
β-Actin	NM_0011013	agacctgtacgccaacacagt	agtacttgcgctcaggagga	140
GAPDH	NM_002046	cctgacctgccgtctagaaa	tgctgtagccaaattcgttg	233
Frataxin (FXN)	NM_000144	tagcagaggaaacgctggac	gatttgcttgtttggcgtct	170
Hepcdin (HAMP)	NM_021175.2	gaccagtggctctgttttcc	cacatcccacactttgatcg	430
ATOH8	NM_032827	caagaagcgcaaggagtgac	gcatcttggagaagaccacga	125
Ferritin (FTH1)	NM_002032.2	ctggccactgacaaaaatga	cagggtgtgcttgtcaaaga	168
Ferroportin (FPN)	NM_014585.5	cgtcattgctgctagaatcg	ccaggatgaccatgatgaaa	300
Transferrin Receptor (TFRC)	NM_001128148.1	aaaatccggtgtaggcacag	ttaaatgcagggacgaaagg	420
Actinin 2	NM_001103.3	ccgcattatgaccctggtag	gatgcagtactgggcctgat	205
GATA4	NM_001308093.1	tggcctgtcatctcactacg	aagaccaggctgttccaaga	192
SIRPA	NM_001040022.1	agcaaaagccatgacctgaa	cctgggctttcttctgtctg	184
Troponin T2, cardiac type (TNNT2)	NM_000364.3	tccgaaacaggatcaacgat	ccatttccaaacaggagctg	190

*NCBI RefSqs= NBCI 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