

Supplementary table 4. Composition of stem cell related culture media

Medium	Components	
Conditioned Medium (CDM)	DMEM/F12 without glutamine 1% (v/v) Non-essential amino acids 1% (v/v) L-glutamine 0.5% (v/v) Penicillin/streptomycin 3.5 µL/500 mL 2-Mercaptoethanol 20% (v/v) Knockout serum replacement 10 ng/mL Basic fibroblast growth factor (bFGF)	After incubation on mitotically inactivated mouse embryonic fibroblasts (strain CF-1) for 24 hours, CDM medium was collected and sterile filtered (0.1 µm filter). Fresh bFGF (30 ng/mL) was supplemented directly before use.
FTDA	DMEM/F-12 without glutamine 2 mM L-glutamine 0.1% (v/v) Lipid mix 5 mg/L Transferrin 5 µg/L Selenium 0.1% (v/v) Human serum albumin	5 µg/mL Insulin 2.5 ng/mL Activin-A 30 ng/mL bFGF 50 nM Dorsomorphin 0.5 ng/mL TGFβ1
Mesoderm induction medium	RPMI 1640 4 mg/mL Polyvinyl alcohol 10 mM HEPES 0.05% (v/v) Human serum albumin 250 µM Phosphoascorbate 5 mg/L Transferrin	5 µg/L Selenium 0.1% (v/v) Lipid mix 10 µM Y-27632 3 ng/mL Activin-A 10 ng/mL BMP4 5 ng/mL bFGF
EB formation medium	FTDA 4 mg/mL Polyvinyl alcohol 10 µM Y-27632	
Cardiac differentiation medium I	RPMI 1640 10 mM HEPES 0.5% (v/v) Penicillin/streptomycin 0.05% (v/v) Human serum albumin 250 µM Phosphoascorbate	5 mg/L Transferrin 5 µg/L Selenium 0.1% (v/v) Lipid mix 1 µM Y-27632 1 µM XAV-939
Cardiac differentiation medium II	RPMI 1640 2% (v/v) B27 plus insulin 10 mM HEPES 0.5% (v/v) Penicillin/streptomycin	500 µM 1-Thioglycerol 1 µM Y-27632 1 µM XAV-939
Cardiac differentiation medium III	RPMI 1640, 2% (v/v) B27 plus insulin 10 mM HEPES	0.5% (v/v) Penicillin/streptomycin 500 µM 1-Thioglycerol 1 µM Y-27632

Supplementary Table 4ff. Composition of stem cell related culture media

Medium	Components	
EHT casting medium	DMEM 1% (v/v) Penicillin/streptomycin	2 mM L-Glutamine 10% (v/v) Horse serum (heat inactivated)
EHT culture medium	DMEM 1% (v/v) Penicillin/streptomycin 10% (v/v) Horse serum	10 µg/mL Insulin 33 µg/mL Aprotinin 0.2 mM Transexamic acid
Fatty acid medium	DMEM (without Glucose) 1% (v/v) Penicillin/streptomycin 10 µg/mL Insulin 33 µg/mL Aprotinin 0.2 mM Transexamic acid	50 ng/mL Hydrocortisone 0.5 ng/mL T ₃ 0.2 mM L-Glutamine 50 µM L-Carnitine hydrochloride 1 mg/mL Linoleic Acid-Oleic Acid-Albumin
Tyrode's solution	120 mM NaCl 5.4 mM KCl 1 mM MgCl ₂ x 6·H ₂ O 0.4 mM NaH ₂ PO ₄	22.6 mM NaHCO ₃ 5 mM Glucose 0.05 mM Na ₂ EDTA 25 mM HEPES (pH 7.4, in PBS)
Mastermix EHT casting	hiPSC-CM EHT casting medium 2x DMEM 0.1% Y-27632 Fibrinogen	1.0x10 ⁶ per EHT 97.68 µL per EHT 6.125 µL per EHT 0.11 µL per EHT 2.78 µL per EHT

Supplementary Table 5. SsODN- and gRNA sequences

Component	Sequence [5'→3']
ssODN Template	CGAGTGGGGGCCCTTCCAGCGCCTCATCT TCTTCCTGCTCAGCGCCAGCATCATTCCCA GTGGCTTCACCGGCCTGTCCTCCGTGTTCC TGATAGCGACCCCGGAGCACCGCTGCCGG GT
OCTN2 (N32S) gRNA	ACAGGCCGGTGAAGCCATTG
OCTN2 (-/-) gRNA 1	TTACATAGGGCGCACGACCA
OCTN2 (-/-) gRNA 2	GGCAGAGGTGATCATCCGCA

Supplementary Table 6: Primer pairs for PCR and Sanger sequencing

Target region	Sequence forward primer [5'→3']	Sequence reverse primer [5'→3']	Product [bp]
OCTN2 (N32S) OT#1	ATCCCAGTAAAAGGCATAAAAGC	TGCTCCATTTTCCCATCAATCT	924
OCTN2 (N32S) OT#2	CAGTTCCACATGCCCTTAGG	CCACCCACTCTTCCTCCTAG	939
OCTN2 (N32S) OT#3	TGTCTGGTGTGCTGGTTTTTC	TTGGTAAGGGTTGGGGAGTG	945
OCTN2 (N32S) OT#4	GCCATGCTGAACTGTGAGTC	TCCCCTCAGCAAAACAGATG	951
OCTN2 (N32S) OT#5	ACAGAGGAGGACAGTGAGTG	GCCAGGAAAGAGCATAGCAC	941
OCTN2 (N32S) OT#6	GTCTTGCATCACACCACCTG	CTTTGTAACCCAGGCTGGAA	1046
OCTN2 (N32S) OT#7	GAGGCACGCTACTCCCTGAG	CAGCAGTGTAGTGGGACGG	910
OCTN2 (N32S) OT#8	TGATTGGCTGCACATGTCAA	TGCTTAGACGGGCTAGAACC	910
OCTN2 (N32S) OT#9	ATGGAACCAGAGCACAGTCT	CCTTCCCAGCCATTCAGAAC	908
OCTN2 (N32S) OT#10	GCGGTAATGCTCACTCATCC	ACCCCTTGTTATGTGAGGA	959
OCTN2 (-/-) gRNA1 OT#1	AAGTCAGGGGGAATGCAGTG	GGGTTGACCAGGCTGCTG	1071
OCTN2 (-/-) gRNA1 OT#2	TCTCACTATGTTGCCCAGGCT	ATTCTAGCAGTGCTGCGTGT	987
OCTN2 (-/-) gRNA1 OT#3	GCCACAGAGCTGGTAATGTATG	GTGGACGATCACGTCCCTTTG	916
OCTN2 (-/-) gRNA1 OT#4	TCTCAATGGTGGGATTCTTCTGTT	AGGGTTAGCCGTCCTCATTG	904
OCTN2 (-/-) gRNA1 OT#5	CCTTTCAAAAGTGTTTCAGCTACA	TCTTTGCAGCTGCTTTTATGGT	1028
OCTN2 (-/-) gRNA1 OT#6	GAGGAATTCGGATCCACCTGA	GTA CT CAGCACTTTGATGGGC	921
OCTN2 (-/-) gRNA1 OT#7	CTTG TAGGTGAGCCCTTGAAT	TGTGGTCAGTGCCTCATTACTTA	980
OCTN2 (-/-) gRNA1 OT#8	CCACGCTGTCCACGATGAT	ATAAGGACCCTGGTCACGCT	938
OCTN2 (-/-) gRNA1 OT#9	ATCGTGCCAGGCCAACTTAT	GAGAGGCAGTGCATTACCA	1000
OCTN2 (-/-) gRNA1 OT#10	ACCTTACTCAGCATAACACCAGA	TGTGTTCTTGGACCTCATGC	960
OCTN2 (-/-) gRNA2 OT#1	TCCTCCAAATTGCCGAGCAT	CACTCAGGCTTGGCTCCATT	932
OCTN2 (-/-) gRNA2 OT#2	ACTCCATGTGAGCAAGAACTATGT	GAAATGAGTGACAGAAACCAGAGC	900
OCTN2 (-/-) gRNA2 OT#3	CAGGCACCGAGTAGAAAGCA	TCATCATCACGCCACCTTCT	934

OCTN2 (-/-) gRNA2 OT#4	GTCCCATAGCCATGGTCAACAC	GGTGTGCCCTCCTTCTCTATTTT	933
OCTN2 (-/-) gRNA2 OT#5	GAAAGCTGCAGTGCCACATATT	CCTCCATCTCTGGGAGCTATT	1099
OCTN2 (-/-) gRNA2 OT#6	AGCAGGGGACGAAGGTTTAC	GAGGGTCTCCGTTCAAGCAAG	931
OCTN2 (-/-) gRNA2 OT#7	GAATCAGTCCTTCTGAGGCAGTT	ATCACTTAGAAGTTGTGAGACCAT	907
OCTN2 (-/-) gRNA2 OT#8	CCCTTTTGTTCCTGCTGTA	ACATGGTTGTGCCTTAGGCTAT	968
OCTN2 (-/-) gRNA2 OT#9	CACAGAAGGAAGAGGCAGCA	CTCCAGGCCATGAGTCCAG	1009
OCTN2 (-/-) gRNA2 OT#10	CAGCAAGACCAAGAGACAAACC	ATCCATTGTCTTCCATCCCTATAA	827
OCTN2 (-/-) gRNA1	ACTTCTCCTTACCTCCGCC	GCTATCAGGAACACGGAGGA	500
OCTN2 (-/-) gRNA2	TCTTGCACCCATCTCCTCAG	GGTGAAAAGAGTGAGGCAGC	659
OCTN2 (-/-) Internal	ATGGGAAAGAAGCCTGCTCT	CTAGTCAACCGGGTCACAGA	530
OCTN2 (-/-) External	TGCCTTCCTAAGCCGAGC	GGTGAAAAGAGTGAGGCAGC	500
SLC22A5 (Exon 1)	GTCTTGGGTGCGCTGCTG	GTCTCCATCGCTAGGGTGTT	652

Supplementary Table 7. Primary antibodies used for flow cytometry/FACS

Antibody	Dilution	Supplier and Cat#
Anti-cardiac troponin T-FITC	1:50	Miltenyi Biotec, 130-119-674
REA Control (I)-FITC	1:50	Miltenyi Biotec, 130-120-709
Rat Anti-Human SSEA-3 Antibody, PE Conjugated	1:50	BD Biosciences, 560237
Rat IgM, κ Isotype Control Antibody, PE Conjugated	1:50	BD Biosciences, 553943

Supplementary Table 8. Primer pairs for qPCR

Target	Sequence forward primer [5'->3']	Sequence reverse primer [5'->3']	Product [bp]
SLC22A5	TTTGGCTACATGGTGCTGCCA	GATGAACCACCAGAGTGCCAC	111
CD36	TCTTTCCTGCAGCCCAATG	AGCCTCTGTTCCAAGTATAGTGA	60
CPT1B	ACATCTCTGCCCAAGCTTCC	ACCATGACTTGAGCACCAGG	175
PDK4	AGAGGTGGAGCATTCTCGC	ATGTTGGCGAGTCTCACAGG	138
HK2	CAAAGTGACAGTGGGTGTGG	GCCAGGTCCTTCACTGTCTC	87
ENO1	GCCGTGAACGAGAAGTCCTG	ACGCCTGAAGAGACTCGGT	79
PKM	ATGTCGAAGCCCCATAGTGAA	TGGGTGGTGAATCAATGTCCA	118
GAPDH	TCGGAGTCAACGGATTTGGT	TCGCCCCACTTGATTTTGA	250
HIF1a	ACCCTAACTAGCCGAGGAAGA	GCACCAAGCAGGTCATAGGT	145
COL1A1	GGCCAGAAGAACTGGTACA	CGCTGTTCTTGCACTGGTAG	199
GUSB	AAACGATTGCAGGGTTTCAC	CTCTCGTCGGTGACTGTTCA	171
βACTIN	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT	250
mtND1	ATGGCCAACCTCCTACTCCTCATT	TTATGGCGTCAGCGAAGGGTTGTA	154
mtND2	CCATCTTTGCAGGCACACTCATCA	ATTATGGATGCGGTTGCTTGCGTG	172
CCN2	TGTGCATTCTCCAGCCATCA	GCCACAAGCTGTCCAGTCTA	131
ACTA2	TGGAAAAGATCTGGCACCCT	GGCATAGAGAGACAGCACCG	188
POSTN	GAGGCTTGGGACAACCTTGA	ACAGTGACAACCCATTAGGA	200
TGFB1	GGAAATTGAGGGCTTTCGCC	CCGGTAGTGAACCCGTTGAT	90
FN1	AATGCACCACAGCCATCTCA	GTCACCTTCTGGTGGCCGTA	188

Supplementary Table 9. Primer pairs for 5'HR probe and 3' HR probe design

Target	Sequence forward primer [5'->3']	Sequence reverse primer [5'->3']	Product [bp]
SLC_5HR1	TGCTTTAGAATCACCTCGCTGCT	CCAAGCCTGCTGGGGACGA	525
SLC_5HR2	TTTTTTTTCCAGTTAGCTTCTGAATAA	CCCGGGCTCGGCTTAGGAAGG	470
SLC_3HR1	TGTGGGTGTGGGTGAGAGGGAC	GAAAATTGACCTGAGCTTCACCCA	1268
SLC_3HR2	AGACTGACCGTGATTTGAGAGCA	GCTTCACCCAGAGTGACTTCCTATG	1232