Supplemental Information

In Vivo Maturation of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes in Neonatal and Adult Rat Hearts

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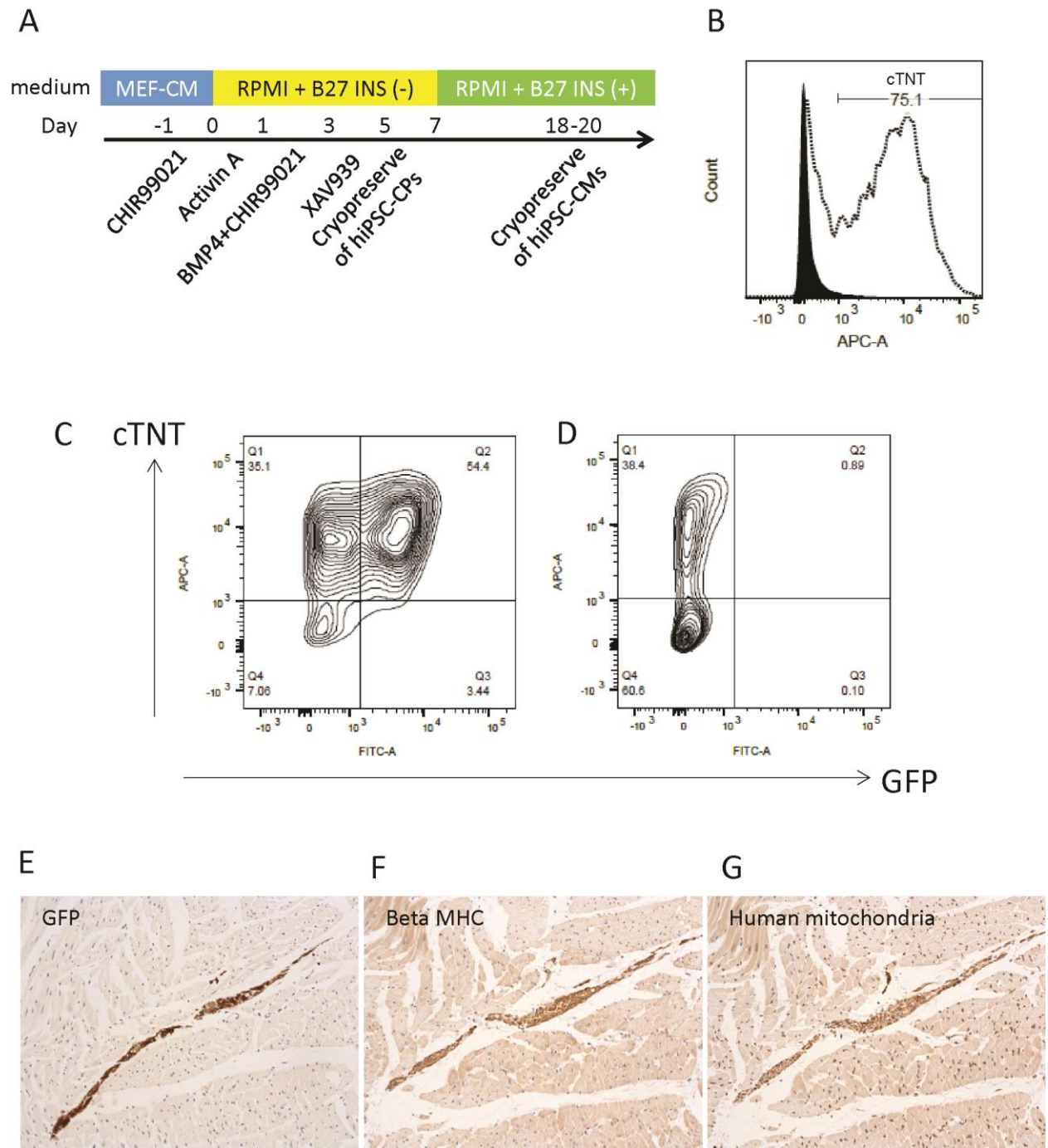
Figure S1. Related to Figure 1

A

| medium | MEF-CM | RPMI + B27 INS (-) | RPMI + B27 INS (+) |

Day: -1 0 1 3 5 7 18-20

CHIR99021
Activin A
BMP4+CHIR99021
XAV939
Cryopreserve of hiPSC-CPs
Cryopreserve of hiPSC-CMs

B

cTNT
75.1

Count

APC-A

C

cTNT

APC-A

FITC-A

Q1 35.1   Q2 54.4
Q4 7.06   Q3 3.44

D

APC-A

FITC-A

Q1 38.4   Q2 0.89
Q4 60.6   Q3 0.10
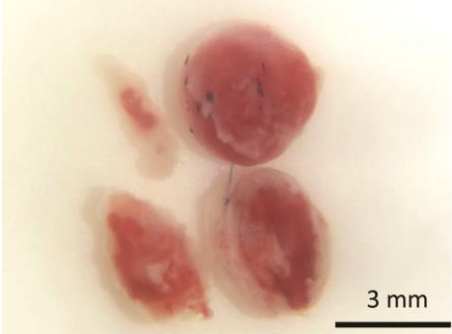
GFP

E

GFP

F

Beta MHC

G

Human mitochondria

Figure S2. Related to Figure 2

Figure S3. Related to Figure 3

A

B

C
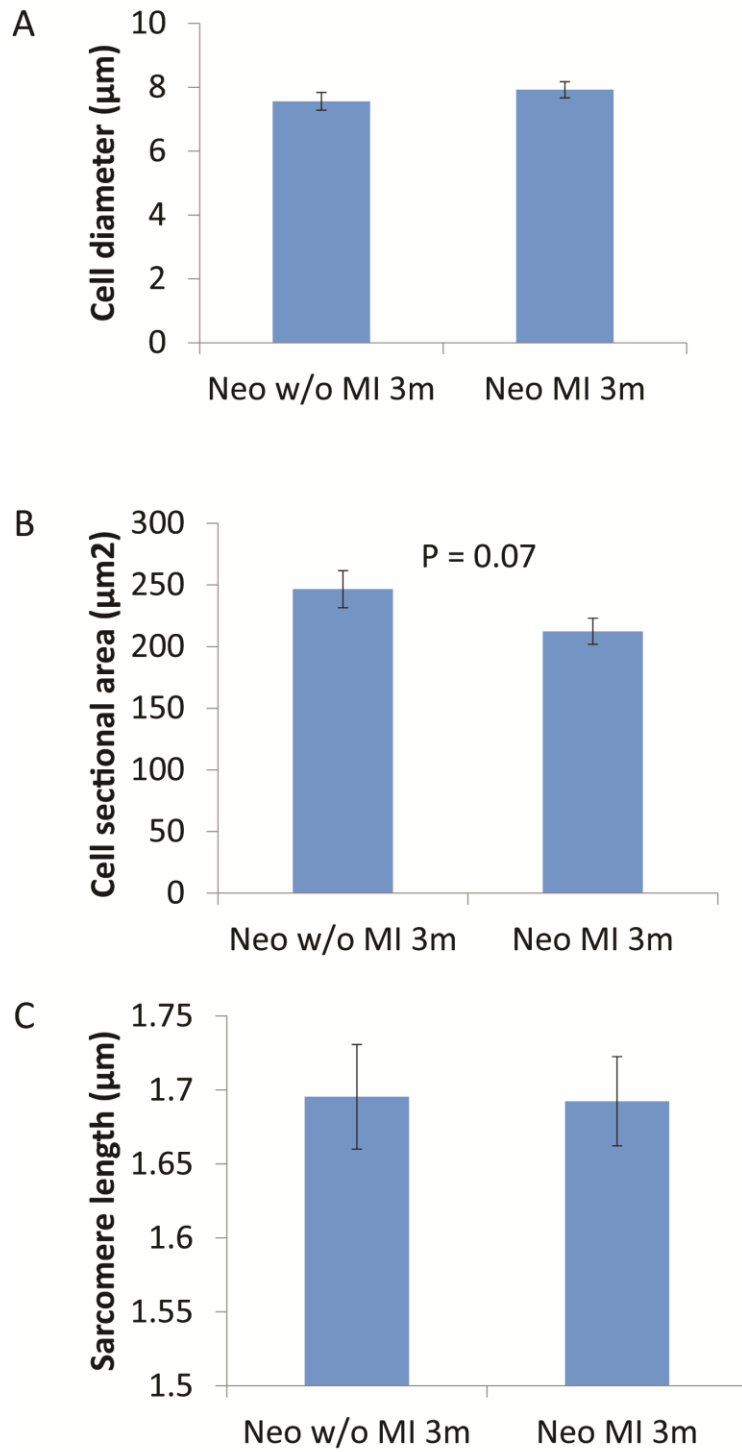
Figure S4. Related to Figure 4

Figure S5. Related to Figure 5

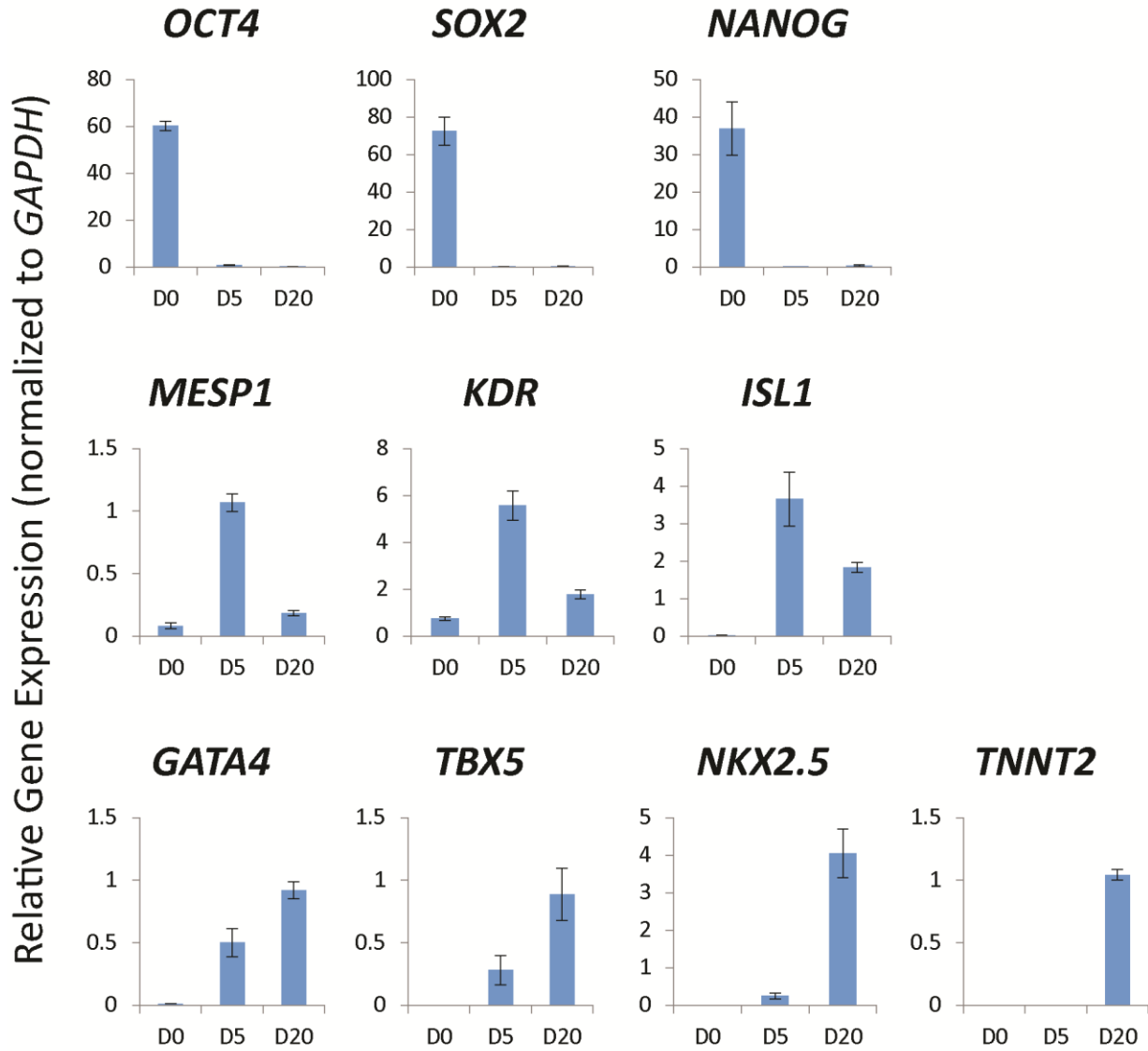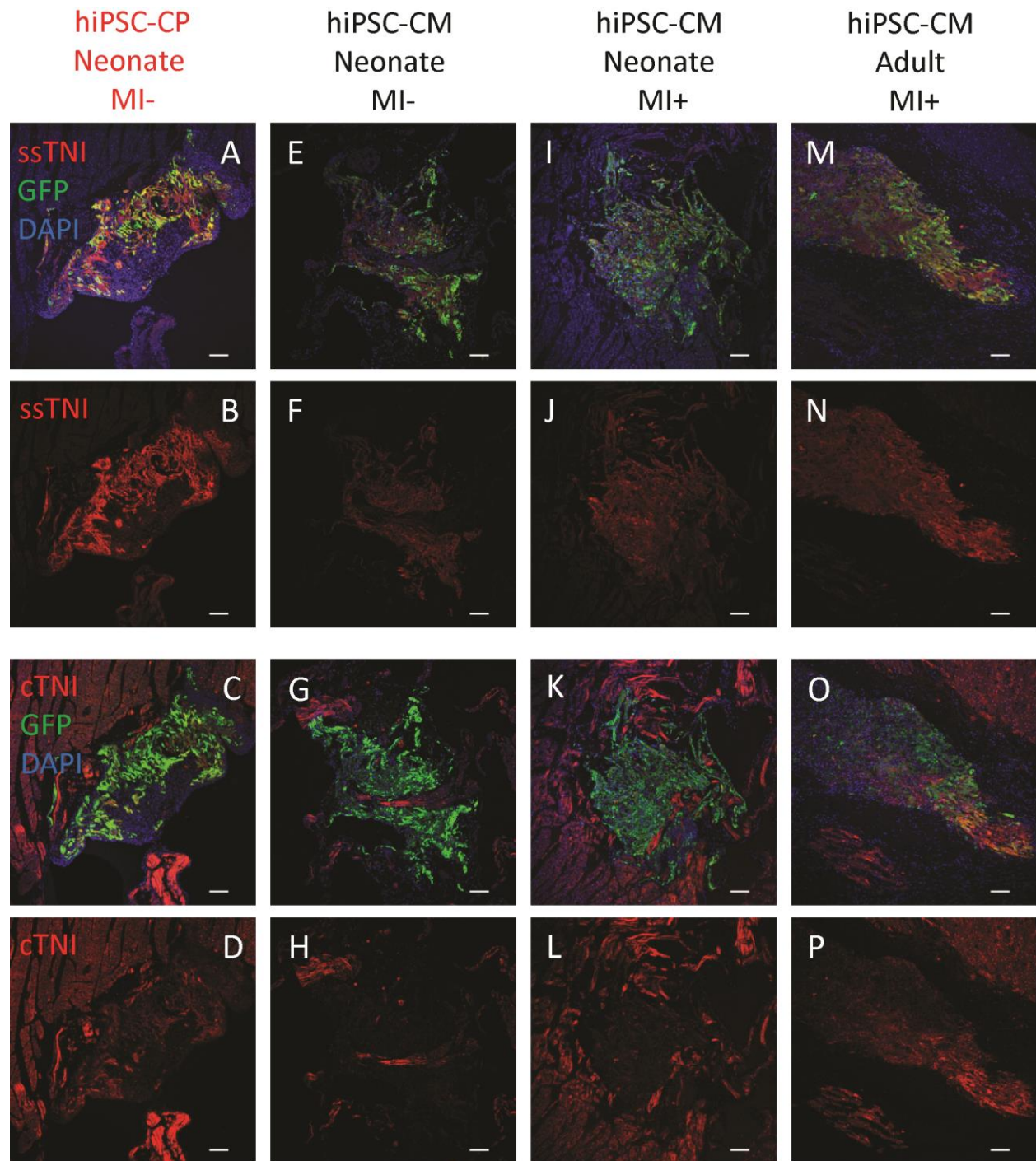| hiPSC-CP Neonate MI- | hiPSC-CM Neonate MI- | hiPSC-CM Neonate MI+ | hiPSC-CM Adult MI+ |

Supplemental figure legends

Figure S1. Differentiation protocol, flow cytometry and immunostaining for detection of hiPSC-CMs.

A: Scheme of monolayer based direct-differentiation protocol. We harvested and cryopreserved hiPSC-derived cardiac progenitors (hiPSC-CPs) and cardiomyocytes (hiPSC-CMs) at day 5 and 18-20 after differentiation, respectively. MEF-CM = mouse embryonic fibroblast-conditioned medium. INS = insulin. **B**: FACS analysis of day 20 hiPSC-derived cells stained cardiac troponin T (cTNT). Black population is negative control which was stained with isotype antibody. **C-D**: Flow cytometry for differentiated cells at day 20 of 253G1-GCaMP3 which were used in this study (**C**) and wild type 253G1 (**D**) stained cTNT. **E-G**: Serial sections of engrafted hPSC-CMs after 84 days of cell injection to uninjured neonatal rat hearts with GFP (**E**), beta-myosin heavy chain (MHC, **F**) and human mitochondria (**G**) staining.

Figure S2. TTC staining.

Representative image of TTC staining of injured heart at 3 days after MI of neonatal rat.

Figure S3. Comparison of hiPSC-CMs in neonatal rat hearts with and without injury.

Comparisons of cell diameter (**A**), cell sectional area (**B**), and sarcomere length (**C**) of engrafted hiPSC-CMs in neonatal rat with and without MI at 3 months after cell injection. N=40 (**A-C**). Data are mean ± SEM.

Figure S4. qRT-PCR.

Horizontal axis shows days after cardiac differentiation. Cardiac mesoderm markers (*MESP1*, *KDR* and *ISL1*) were highly and early cardiac development makers (*GATA4* and T*BX5*) moderately upregulated in day-5 differentiated hiPSC-CPs, although they did neither expressed pluripotent markers (*OCT4*, *SOX2* and *NONOG*), nor cardiac markers (*NKX2.5* and *TNNT2*). N=3 biological replicates. Data are mean ± SEM.

Figure S5. Immunostaining of Troponin I.

Comparison of hiPSC derivatives engrafted in rat hearts after 3 months of cell transplantation with slow skeletal troponin I (ssTNI) and cardiac TnI (cTNI) staining. Representative graft of hiPSC-CP transplantation to neonatal rat without MI (**A-D**), hiPSC-CM transplantation to neonatal rat without MI (**E-H**), neonatal rat with MI (**I-L**), and

adult rat with MI (**M-P**). Scale bar = 100 μm.

Supplemental Tables

Table S1. Antibodies for immunostaining.

Antigen	Antibody type	Company	Catalog number or clone	Concentration	Antigen retrieval
GFP	Rabbit polyclonal	Novus	NB600-308	1:1000	
GFP	Goat polyclonal	Novus	NB100-1770	1:1000	
Beta-Myosin Heavy Chain	Mouse monoclonal	Developmental Studies Hybridoma Bank	A4.951	1:10	Citrate HIER
Human mitochondria	Mouse monoclonal	Millipore	MAB1273	1:100	EDTA HIER
Troponin I type 1 (slow skeletal)	Rabbit polyclonal	Novus	NBP1-56641	1:200	Citrate HIER
Cardiac Troponin I	Rabbit polyclonal	Abcam	ab47003	1:200	Citrate HIER
Alpha-actinin	Mouse monoclonal	Sigma-Aldrich	A7811	1:500	Tris-EDTA HIER
Caveolin 3	Rabbit polyclonal	Abcam	ab2912	1:500	Tris-EDTA HIER
N-cadherin	Mouse monoclonal	Sigma-Aldrich	GC-4	1:200	Citrate HIER
Connexin 43	Rabbit polyclonal	Sigma-Aldrich	C6219	1:200	Citrate HIER
Wheat Germ Agglutinin, Alexa Fluor 594 Conjugate		Thermo Fisher	W11262	1:200	

HIER: Heat-induced epitope retrieval

Table S2. Primers for qRT-PCR.

	Forward primer	**Reverse primer**
OCT4	GGGTTCTATTTGGGAAGGTAT	TTCATTGTTGTCAGCTTCCT
SOX2	GCCGAGTGGAAACTTTTGTCG	GGCAGCGTGTACTTATCCTTCT
NANOG	TTTGTGGGCCTGAAGAAAACT	AGGGCTGTCCTGAATAAGCAG
ISL1	ATTTCCCTATGTGTTGGTTGC	CGTTCTTGCTGAAGCCGATG
KDR	GCACAAAGTGACACGTTGAGAT	AGTGATCGGAAATGACACTGGA
MESP1	TCGAAGTGGTTCCTTGGCAGAC	CCTCCTGCTTGCCTCAAAGTGTC
GATA4	ACACCCCAATCTCGATATGTTTG	GTTGCACAGATAGTGACCCGT
TBX5	GAACCACAAGATCACGCAATTA	ACACCATTCTCACACTGGTAT
NKX2.5	ACCCTGAGTCCCCTGGATTT	TCACTCATTGCACGCTGCAT
TNNT2	TTCACCAAAGATCTGCTCCTCGCT	TTATTACTGGTGTGGAGTGGGTGTGG
GAPDH	ATGGAAATCCCATCACCATCTT	CGCCCCACTTGATTTTGG