Supplementary Tables

Supplementary Table 1. Antibodies used for immunostaining of marker proteins.

Antibody	Vendor	Detection	Concentration
OCT4	Abcam	Nuclear pluripotency markers	1:200
SOX2	Cambridge (UK)		1:250
c-MYC			1:100
KLF-4			1:50
NANOG			1:25
SSEA-4 **(przyżyciowe)**	Abcam	Surface pluripotency markers	1:100
SSEA-4	Cambridge (UK)		1:200
TRA 1-60			1:100
TRA 1-81			1:100
desmin		Myoblast indicator	1:200
AFP	Abcam	Endodermal lineage marker	1:500
SMA	Cambridge (UK)	Mesodermal lineage marker	1:100
TUJ1		Ectodermal lineage marker	1:500
NKX2-5		Cardiac progenitor marker	1:250
TNNT2	Abcam Cambridge (UK)	Mature cardiomyocyte markers	1:200
CNX43			1:200
α-MHC			1:200
α-aktynina	Sigma-Aldrich St. Louis, USA		1:500
Alexa Fluor 488	Abcam Cambridge (UK)	Flurochrome conjugated secondary antibody	1:500
Alexa Fluor 594			1:500
IgG-FITC			1:1000
TexasRed (SAR+TR)			1:1000
Anti-Histon3methylK3		Nuclear marker	1:200

Abbreviations: **OCT4** *octamer-binding transcription factor 4*; **SOX2** sex determining region Y - box 2; **c-MYC** *cellular c-Myc oncogene product*; **Klf4** *Kruppel-like factor 4*; **NANOG** *Nanog homeobox*; **SSEA4** *stage-specific embryonic antigen-4*; **TRA 1-60 / 81** *tissue rejection antigen1-60/1-81*; **AFP** *α-fetoprotein*; **SMA** *smooth muscle actin*; **TUJ-1** *neuron-specific class III beta-tubulin*; **NKX2-5** *NK2 homeobox 5*; **TNNT2** *cardiac troponin T*; **CX43** *connexin-43*; **α-MHC** *myosin heavy chain* α; **IgG – FITC** *anti-human IgG fluorescein isothiocyanate conjugated secondary antibody*; **SAR+TR** *secondary antibody red Texas Red conjugated*.

Supplementary Table 2. Primer sequences for PCR and qRT-PCR.

Gene	Primer	Primer sequence (5'→3')	Product lenght
ACTB	Forward	CTTCCTGGGCATGGAGTCC	192 bp
	Reverse	ATCTTGATCTTCATTGTGCTG	
GAPDH	Forward	GCTCTCTGCTCCTCCTGTTC	112 bp
	Reverse	ACCAAATCCGTTGACTCCGA	
OCT4	Forward	CTCACCCTGGGGGTTCTATT	203 bp
	Reverse	CTGGTTCGCTTTCTCTTTCG	
SOX2	Forward	TGGGTTCGGTGGTCAAGTC	233 bp
	Reverse	CATGTGTGAGAGGGGCAGTG	
NANOG	Forward	TGGTGTGACGCAGAAGGC	233 bp
	Reverse	CACTGGCAGGAGAATTTGGC	
cMYC	Forward	GGTCTTCCCCTACCCTCTCA	296 bp
	Reverse	AGCCTGCCTCTTTTCCACA	
MyoD	Forward	AGCACTACAGCGGCGGACT	242 bp
	Reverse	GCGACTCAGAAGGCACGTC	
Brachyury	Forward	GCCGACTATATGCTGCTCAT	215 bp
	Reverse	TCGTCCAGTAGGTTGTTGGT	
NKX2-5	Forward	AGAGCCGAAAAGAAAGCCTGAA	246 bp
	Reverse	CCGCACAGTAATGGTAAGGGA	
TNNT2	Forward	AGAGCGGAAAAGTGGGAAGA	235 bp
	Reverse	CTGGTTATCGTTGATCCTGT	
MYH6 (α-MHC)	Forward	CAACGCACAAAGTGAGGATG	151 bp
	Reverse	CCTACGCAACTGCCGATACT	
MYH7 (β-MHC)	Forward	GGCAAGACAGTGACCGTGAAG	134 bp
	Reverse	CGTAGCGATCCTTGAGGTTGTA	
CX43	Forward	AGAGGAAGAAGAACTCAAGGTTGCC	182 bp
	Reverse	AGGCCACCTCAAAGATAGACT	
TNNI 3	Forward	TGTGGACAAGGTGGATGAAG	142 bp
	Reverse	AGGCCACCTCAAAGATAGACT	
TNNI 1	Forward	TCCGTGGGAAGTTCAAGCG	238 bp
	Reverse	GACTTGGCGGCATCAAACATC	
KCNJ2	Forward	TTGTCAAGAGCCAAGACACA	171 bp
	Reverse	AGCAACACACATCTGGGAAT	
SERCA2A	Forward	TGGGTGTATGGCAGGAAAGAA	222 bp
	Reverse	ACTGGTCAACTCTTAGTGTGGTA	

Abbreviations: *CX43*—connexin 43; *GAPDH*— glyceraldehyde 3-phosphate dehydrogenase; *OCT4* – octamer-binding transcription factor 4; *SOX2* – SRY (sex determining region Y)-box 2; *MyoD* – myogenin differentiation 1; *NKX2-5* – NK2 homeobox 5; *TNNT2* – cardiac troponin T; *TNNI* – troponin I; *MYH6* (*α-MHC*) – α myosin heavy chain, *MYH7* (*β-MHC*) – β myosin heavy chain, *KCNJ2* – potassium voltage-gated channel subfamily J member 2; *SERCA 2A* – sarcoplasmic/endoplasmic reticulum calcium ATPase; *ACTB*—beta actin.

Supplementary Table 3. qRT-PCR reaction conditions

	qRT-PCR reaction	**PCR reaction**
Predenaturation	95ºC, 1 min	95ºC, 5 min
Denaturation	95ºC, 20 sec	95ºC, 45 sec
Primer annealing	60ºC, 20 sec	60ºC, 45 sec
Elongation	72ºC, 20 sec	72ºC, 1 min
Final elongation (PCR)/melting curve (qRT-PCR)	65ºC-95ºC	72ºC, 10 min
Number of cycles	45	35

Supplementary Table 4. PCR efficiency and correlation coefficients of standard curves

Gene	**qPCR reaction efficiency**	**R2 correlation coefficient**
ACTB	104.80%	0.991
GAPDH	98.90%	1
OCT4	96.90%	0.999
SOX2	97.30%	0.998
cMYC	93.50%	0.998
MyoD	91.90%	0.999
Brachyury	99.20%	0.995
NKX2-5	95.20%	0.997
TNNT2	103.40%	0.991
MYH6 (α-MHC)	101.20%	0.995
MYH7 (β-MHC)	96.70%	0.991
CX43	94%	0.997
TNNI 3	97.30%	0.998
TNNI 1	94.10%	0.997
KCNJ2	96.90%	0.995
SERCA2A	90.20%	0.999

Abbreviations: ***CX43***—connexin 43; ***GAPDH***— glyceraldehyde 3-phosphate dehydrogenase; ***OCT4*** – octamer-binding transcription factor 4; ***SOX2*** – SRY (sex determining region Y)-box 2; ***MyoD*** – myogenin differentiation 1; ***NKX2-5*** – NK2 homeobox 5; ***TNNT2*** – cardiac troponin T; ***TNNI*** – troponin I; ***MYH6* (*α-MHC*)** – α myosin heavy

chain, ***MYH7 (β-MHC)*** – β myosin heavy chain, ***KCNJ2*** – potassium voltage-gated channel subfamily J member 2; ***SERCA 2A*** – sarcoplasmic/endoplasmic reticulum calcium ATPase; ***ACTB***—beta actin.

Supplementary Table 5. Semi-quantitative optimization of cell differentiation protocol with respect to contractility expansion observed in SMiPSC-CMs on day 30 of *in vitro* culture.

option	insulin first 2 days	BMP4	CHIR99021	IWR-1	clone 10	clone 11	clone 13
1	no	25 ng/ml	5 μM	10 μM, medium every other day	+++	+++	+++
2	yes	25 ng/ml	5 μM	10 μM, medium every other day	+	+	++
3	yes	25 ng/ml	5 μM	10 μM, no medium change	++	-	+
4	no	25 ng/ml	5 μM	10 μM, medium every day	+++	+	+++
5	no	25 ng/ml	5 μM	10 μM, medium every other day till beating	+++	++	+++
6	yes	25 ng/ml for48h	5 μM	10 μM, medium every other day	+	+	-
7	yes	25 g/ml	5 μM	10 μM, medium every other day	+	+	++
8	yes	50 ng/ml	5 μM	10 μM, medium every other day	-	+	-
9	yes	25 ng/ml	5 μM	10 μM, medium every other day for 5 days	-	-	++
10	yes	25 ng/ml	5 μM for 72h	10 μM, medium every other day	+	++	-
11	yes	25 ng/ml	10 μM	10 μM, medium every other day	-	+	+
12	yes	25 ng/ml	5 μM	20 μM, medium every other day	-	++	-

(-) no beating, + few beating patches, (++) numerous beating patches,
(+++) synchronous beating throughout the well.

Abbreviation: **BMP4** *bone morphogenetic protein 4*; **CHIR99021** glycogen synthase kinase 3 inhibitor; **IWR-1** 4-(1,3,3a,4,7,7a-hexahydro-1,3-dioxo-4,7-methano-2Hisoindol-2-yl)-N-8-quinolinyl-benzamide.