

Supplementary Material

Growth Factor-Based Manufacturing of Human Pluripotent Stem Cell-Derived Cardiomyocytes Using the Vertical Wheel Bioreactor System

Faisal J. Alibhai^{1*}, Tamilla Valdman Sadikov¹, E. Coulter Montague^{1,2}, Lorena V. Cortes-Medina¹,
Ian Fernandes¹, Gary Sun¹, Juliana Gomez-Garcia^{1,2}, Harriet W. Chung¹, Omar Mourad³,
Beiping Qiang¹, Sara S. Nunes^{2,3}, Gordon Keller^{1,4}, Michael A. Laflamme*^{1,5,6}

¹McEwen Stem Cell Institute, Toronto, ON, Canada, ²Institute of Biomedical Engineering,
University of Toronto, Toronto, ON, Canada, ³Toronto General Hospital Research Institute,
University Health Network, Toronto, ON, Canada, ⁴Medical Biophysics, University of Toronto,
Toronto, ON, Canada, ⁵Peter Munk Cardiac Centre, University Health Network, Toronto, ON,
Canada, ⁶Department of Laboratory Medicine & Pathobiology, University of Toronto, Toronto,
ON, Canada

Supplementary Figure 1. hPSC expansion at different densities within the VWBR. **A)** Flow cytometry histograms and quantification of Oct4 and Sox2 expression in ESI-017 cells expanded in the VWBR with different seeding densities (2×10^4 (n=4), 8×10^4 (n=4), or 15×10^4 (n=5) cells/mL). **B)** Representative immunofluorescent staining for Oct4 and Tra-1-60 (top) and Sox2 and Tra-1-81 (bottom) in hPSC aggregates harvested from VWBRs seeded with 8×10^4 cells/mL, agitation rate set to 40 rpm, and 80% media exchange schedule. **C)** Media glucose and **D)** lactate levels during the 6-day hPSC expansion in VWBRs seeded at different densities. (2×10^4 (n=9), 8×10^4 (n=9), or 15×10^4 (n=5) cells/mL). **E)** Representative aggregate images, and **F)** aggregate diameter distribution during expansion in the VWBR. # $p \leq 0.05$ 2×10^4 cells/mL vs all other groups, ** $p \leq 0.01$ 15×10^4 cells/mL vs all other groups, and $\gamma p \leq 0.05$ 2×10^4 cells/mL vs 15×10^4 cells/mL, analyzed by two-way ANOVA followed by a Tukey post hoc test.

Supplementary Figure 2. Assessing the effect of agitation rate and media exchange on ESI-017 expansion in the 100 mL VWBR. **A)** % viability during expansion at 30, 40 or 60 rpm. **B)** Fold expansion of hPSCs calculated by the cell number at harvest divided by the input cell number at day 0. **C)** Media glucose and **D)** lactate levels during expansion in the VWBR at different agitation rates. † $p \leq 0.05$ 30 rpm vs all other groups, * $p \leq 0.05$ 60 rpm vs all other groups by two-way ANOVA followed by a Tukey post hoc. $\gamma p \leq 0.05$ 40 rpm vs all other groups by one-way ANOVA followed by a Tukey post hoc. n=7 for 30 rpm, n=9 for 40 rpm, and n=6 for 60 rpm. **E)** Media exchange schedules tested for hPSC expansion in the VWBR with a seeding density of 8×10^4 cells/mL and an agitation rate of 40 rpm. **F)** Mean aggregate diameter by day during hPSC expansion in the VWBR. n=9 for 80%, n=3 for 50%, n=3 for 20%. **G)** Flow cytometry histograms and **H)** quantification of Oct4 and Sox2 expression in hPSCs following expansion in the VWBR. n=4 for 80%, n=3 for 50%, n=3 for 20%. Replicates for 40 rpm in the agitation rate experiments are the same as 8×10^4 cells/mL in the density experiments, and 80% in the media exchange experiment.

Supplementary Figure 3. hPSC expansion in StemScale media and testing of hPSC-CM differentiation. **A)** Protocol used for expansion of ESI-017 hPSCs in the 100 mL VWBR with either mTeSR1 or StemScale media. **B-G):** Parameters for hPSCs expanded in each medium, including viable cells/mL (**B**), fold-expansion (**C**), % cell viability (**D**), glucose (**E**), lactate (**F**), and aggregate diameter (**G**). **H)** Representative images of hPSC aggregates in each medium by day, and **I)** aggregate diameter distribution over the expansion period. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ mTeSR1 vs. StemScale analyzed by two-way ANOVA followed by a Tukey post hoc or Student's t-test where applicable. Replicates for mTeSR1 are the same as depicted in Figure 1. **J-M)** ESI-017 hPSCs were expanded in the VWBR in StemScale, as depicted in (**A**), and the cardiomyocyte differentiation potential of StemScale cultures was compared to 2D and 3D cells expanded in mTeSR1. Quantification of **J)** PDGFR α^+ and **K)** PDGFR α^+ /CD56 $^+$ cell frequency on day 3 of differentiation. Quantification of **L)** cardiomyocyte purity (% cTnT $^+$) and **M)** yield on day 16-18 of differentiation. * $p < 0.05$ 3D-StemScale vs. all other groups, and # $p < 0.05$ 2D-mTeSR1 vs. 3D-mTeSR1 by one-way ANOVA followed by a Tukey post hoc. Replicates for 2D-mTeSR1 are the same as 2D in Figure 2, and 3D-mTeSR1 are the same as 8×10^4 cells/mL and 40 rpm in Figure 2.

Supplementary Figure 4. Comparison of RPMI-B27 and SP25 medium for hPSC-CM maintenance and optimization of hPSC-CM differentiation in the VWBR. **A)** Experimental protocol for testing different media for hPSC-CM maintenance. In brief, hPSCs were initially differentiated to hPSC-CMs in 100% StemPro, after which cells were switched to SP25 (25%/75%, v/v; SP/IMDM) on day 6 or maintained on 100% StemPro. On day 12, cells were switched to RPMI-B27 (upper) or maintained on SP25 (lower) until day 20. **B)** Representative cTnT flow cytometry histograms for hPSC-CMs generated by each protocol, as well as quantitation of cardiomyocyte (cTnT) purity (**C**), hPSC-CM yield (**D**), % of cTnT⁺/MLC2v⁺ double-positive cells (**E**), and MLC2v mean fluorescence intensity (MFI) (**F**) on day 20. **G)** Gene expression in day 20 hPSC-CMs generated using the two different protocols. *p<0.05 by two-tailed Student's t-test. **H)** Agitation rate schedule used during the differentiation of 2D (left) and 3D (right) ESI-017 cells in the 100 mL VWBR. **I)** Schematic of the experiment testing the timing of Wnt inhibition (Wnt-i). Cells were removed from the VWBR on days 2, 2.5, and 3, treated with the Wnt inhibitor IWP2, and maintained in 6-well plates until day 14. **J)** hPSC-CM purity assessed by flow cytometry (n=4 per condition). **K)** Flow cytometry plots of c-Kit⁺/CXCR4⁺ populations in the VWBR on day 4 of the differentiation.

Supplementary Figure 5. Expansion of two independent iPSC lines in the VWBR. **A)** Schematic illustrating workflow for expansion of SCTi003-A hPSCs in 100 mL VWBRs. SCTi003-A hPSCs were thawed from a MCB, cultured in 2D for 3 days, passaged once and re-plated for an additional 2 days. hPSCs were then harvested and seeded into VWBRs at a density of 8x10⁴ cells/mL. Undifferentiated cells in suspension culture were maintained in VWBRs operated at 40 rpm for 5 days, with media exchanges as indicated. Number of viable cells/mL (**B**) and % viable cells (**C**) within the VWBR at each day. **D)** hPSC fold expansion over 5 days in the VWBR. **E)** Mean aggregate diameter by day in the VWBR. **F)** Representative aggregate images during expansion in the VWBR. **G)** Media glucose and **H)** lactate levels during the 5-day expansion protocol. n=3 independent runs. **I)** Schematic illustrating workflow for expansion of undifferentiated 585A1-ABo_C12o_To hPSCs in 100 mL VWBRs. 585A1-ABo_C12o_To hPSCs were thawed from a MCB, cultured in 2D for 3 days, passaged once and re-plated for an additional 2 days. hPSCs were then harvested and seeded into VWBRs at a density of 8x10⁴ cells/mL. Undifferentiated cells in suspension culture were maintained in VWBRs operated at 50 rpm for 4 days, with media exchanges as indicated. The effect of 100 µg/mL dextran sulfate (DS) inclusion during VWBR seeding on day 0 was also assessed. Two different media exchange schedules were tested for the +DS condition: 50% on day 3 (+DS-50) or 80% on day 3 (+DS-80). An 80% media exchange on day 3 was performed for the control group, which did not receive dextran sulfate (-DS). Number of viable cells/mL (**J**) and % viable cells (**K**) within the VWBR by day. **L)** hPSC fold expansion over 4 days in the VWBR. **M)** Mean aggregate diameter by day in the VWBR. **N)** Representative aggregate images from -DS and +DS-50 groups during expansion in the VWBR. **O)** Media glucose and **P)** lactate levels during the 4-day expansion protocol. n=3 for -DS, n=4 for +DS-80 and n=9 for +DS-50. *p<0.05 -DS vs all groups, **p<0.01 -DS vs all groups, ***p<0.001 -DS vs all groups, †p<0.05 +DS-50 vs. +DS-80, ‡p<0.05 +DS-50 vs -DS.

Supplementary Figure 6. hPSC-CM differentiation of two independent lines in the VWBR. **A)** Growth factor-based guided differentiation protocol applied to undifferentiated SCTi003-A cells previously expanded in VWBRs. **B-E)** To determine the optimal BMP4 and Activin A concentrations for cardiac differentiation, SCTi003-A hPSCs were first differentiated in 6-well plates and subjected to a range of BMP4 (6-10 ng/mL) and Activin A (4-10 ng/mL) concentrations.

The resulting percentages of PDGFR α ⁺ cells on day 3 (**B**), cTnT⁺ cells (**C**), % MLC2v⁺ cells (**D**), and CM yield (**E**) on day 20 are displayed as heat maps (mean levels for each parameter). n=3 differentiations per condition. Based on these outcomes, 10 ng/mL BMP4 and 10 ng/mL Activin A were selected for subsequent VWBR differentiation runs using SCTi003-A cells. **F-K**) Differentiation of SCTi003-A cells in the 100 mL VWBR. **F**) Using aggregates formed in 6-well plates as a benchmark, we identified a VWBR agitation speed (40 rpm) that formed appropriately sized aggregates on day 1. Scale bar is 100 μ m. **G**) Representative flow cytometry plots for CD56 and PDGFR α on days 2.5 and 3 (left) and quantification on day 3 (right). **H**) Representative flow plot of cTnT and MLC2v on day 20, and corresponding quantification of % cTnT⁺ (**I**), % MLC2v⁺ (**J**), and CM yield (**K**). n=3 differentiation runs. **L**) Growth factor-based guided differentiation protocol applied to undifferentiated 585A1-ABo_C12o_To hPSCs previously expanded in VWBRs. **M-P**) As before, 585A1-ABo_C12o_To hPSCs were first differentiated in 6-well plates and subjected to a range of BMP4 (4-10 ng/mL) and Activin A (4-10 ng/mL) concentrations. The resulting levels of day 3 % PDGFR α ⁺ (**M**) and day 20 % cTnT⁺ (**N**), % MLC2v⁺ (**O**), and CM yield (**P**) are displayed as heat maps of mean levels for each parameter. n=3-6 per condition, X = not tested. 4 ng/mL BMP4 and 4 ng/mL Activin A were selected for subsequent VWBR differentiation runs with this line. **Q-W**) Differentiation of 585A1-ABo_C12o_To cells in the 100 mL VWBR. **Q**) Using the same approach described above, we identified a VWBR agitation speed (30 rpm) that formed appropriately sized aggregates on day 1. Scale bar is 100 μ m. **R**) Representative flow cytometry plots for CD56 and PDGFR α on days 2.5 and 3 (left) and quantification on day 3 (right). **S**) Wnt inhibition (Wnti-i) on day 2.5 and day 3 was compared by treating VWBRs with IWP2 at the two different timepoints. **T**) Representative flow plots of cTnT and MLC2v on day 20, and corresponding quantification of % cTnT⁺ (**U**), % MLC2v⁺ (**V**), and yield (**W**). n=3 differentiations for Wnt-i on day 2.5 and n=4 differentiations for Wnt-i on day 3.

Supplementary Figure 7. Titration of BMP4 and Activin A levels for optimization of ALDH and CD235a expression in day 4 mesoderm populations. **A**) Undifferentiated ESI-017 hPSCs were expanded in the VWBR and differentiated in 6-well plates to define the optimal cytokine concentrations for second heart field progenitors based on ALDH activity and CD235a expression at day 4. BMP4 was varied between 3 and 4 ng/mL, and Activin A varied between 1 and 2 ng/mL. **B**) Representative plots of mesoderm commitment obtained with different BMP4:Activin A concentrations and quantification of **C**) % PDGFR α ⁺ and **D**) % PDGFR α ⁺/CD56⁺. **E**) Representative plots showing aldehyde dehydrogenase (ALDH) activity (Aldefluor) and CD235a⁺ expression on day 4 of differentiation and quantification of **F**) Aldefluor⁺/CD235a⁺ cells and **G**) CD235⁺/Aldefluor⁺ cells. **H-J**) Comparison of day 4 mesodermal markers between our standard ventricular protocol (VLCM-CC, 10 ng/mL BMP4, 6 ng/mL Activin A on days 1-3), optimized ventricular protocol (VLCM+CC, 10 ng/mL BMP4, 6 ng/mL Activin A, and 1 μ M CHIR on days 1-3, and 50 nM Chroman-1 on day 0), and atrial-like cardiomyocyte protocol (ALCM, 4 ng/mL BMP4 and 2 ng/mL Activin A on days 1-3, and 0.5 μ M RA on day 3). **H**) Frequency of CD235a⁺ cells on day 4 of differentiation in VLCM-CC, VLCM+CC, and ALCM conditions. **I**) Frequency of cTnT⁺, MLC2v⁺, and MLC2a⁺ cells and **J**) hPSC-CM yield on day 20 of differentiation for the same conditions. Data presented for VLCM-CC and ALCM are the same as those depicted in Figure 5. n=5 for VLCM-CC, n=4 for VLCM+CC, and n=5 for ALCM. *p<0.05 VLCM-CC vs. VLCM+CC, and **p<0.01 ALCM vs. all other groups by one-way ANOVA followed by a Tukey post hoc.

Supplementary Figure 8. hPSC expansion in 500 mL VWBRs. **A)** Schematic of the protocol used to expand undifferentiated ESI-017 hPSCs in the 500 mL VWBR. Reactors were seeded with 8×10^4 cells/mL and cultured for 5 days at 30 rpm using the depicted medium exchange schedule. **B-D)** Resultant parameters including viable cells/mL and cell viability (**B**), mean aggregate diameter (**C**), and medium glucose and lactate concentrations (**D**) over the 5-day hPSC expansion period. **E)** Representative images of undifferentiated hPSC aggregates during expansion in the 500 mL VWBR and the corresponding **F)** Aggregate diameter distribution. $n=9$ independent VWBR runs. **G)** Representative images of aggregates harvested from 500 mL VWBRs on day 5 of the expansion protocol and immunostained for pluripotency markers.

Supplementary Figure 9. Additional phenotyping of hPSC-CMs generated in 500 mL VWBRs. **A)** hPSC-CM purity (% cTnT⁺) at day 20, 30 and 40 for cells generated in 500 mL reactors and maintained as aggregates in suspension or as replated monolayers. **B)** Representative flow cytometry plots corresponding to Figures 6P and 6Q, showing gating for SIRPα⁺/CD90⁻ and CD90⁺ cells. **C)** Representative flow cytometry plots of SIRPα⁺/CD90⁻/CD151⁺ cells on day 20 and day 34 of differentiation. Plots correspond to Figure 6R and Supplementary Figure 10D. **D)** Quantification of CD151⁺ as a percentage of the SIRPα⁺/CD90⁻ population. $n=5$ independent 500 mL VWBR differentiations. **E)** Relationship between Biowire force generation and MLC2v⁺ cell frequency on day 20. $n=8$ tissues from independent 500 mL VWBR runs. **F)** Viability of 500 mL VWBR produced hPSC-CMs after enzymatic dissociation on day 20 and following cryopreservation and thawing. **G-I)** Assessment of LIN28A expression in hPSC-CM cultures from 500 mL VWBRs using ddPCR. LIN28A copies per 50 μg RNA measured in spike-in standards ($n=3$ independent standard curves ranging from 0.001-10% hPSCs spiked into cardiac fibroblasts) (**G**), the standard curve generated from the spike-in standards (**H**), and the estimated percentage of LIN28A expressing cells in 500 mL VWBR-derived hPSC-CMs ($n=4$ independent differentiation runs) (**I**).

Supplementary Figure 10. Correlation analyses to identify factors impacting hPSC-CM purity, yield, and ventricular specification. Day 4 mesodermal and day 20 parameters were correlated to identify factors that may underlie variability in differentiation outcomes in the VWBR system. Correlation analyses were performed for three different protocols: i) 100 mL VWBR differentiations using our initial ventricular protocol, 10 ng/mL BMP4, 6 ng/mL Activin A, no CHIR or Chroman-1 supplementation (black data points); ii) 100 mL VWBR differentiations using our optimized protocol involving 50 nM Chroman-1 on day 0, and 10 ng/mL BMP4, 6 ng/mL Activin A, and 1 μM CHIR days 1-3 (blue data points); and iii) 500 mL VWBR differentiations using our optimized ventricular protocol involving 50 nM Chroman-1 on day 0, and 10 ng/mL BMP4, 6 ng/mL Activin A, and 1 μM CHIR days 1-3 (red data points). Graphs containing the combined data points from all three conditions are also shown. **A)** Relationship between % cTnT⁺/MLC2v⁺ cells and hPSC-CM yield on day 20. **B)** Relationship between % CD235a⁺/PDGFRα⁺ cells on day 4 and % cTnT⁺/MLC2v⁺ cells on day 20. **C)** Relationship between % c-Kit⁺/PDGFRα^{low} cells on day 4 and % cTnT⁺/MLC2v⁺ cells on day 20. **D)** Relationship between % PDGFRα⁺/c-Kit⁺ cells on day 4 and % cTnT⁺ cells on day 20.

Supplementary Table 1. Aggregation Medium 1A (Day 0-1)

Composition	Supplier	Final Concentration
StemPro-34	Thermo Fisher Scientific	100%
L-Glutamine	Thermo Fisher Scientific	2 mM
L-Ascorbic acid	Sigma	50 µg/mL
Monothioglycerol	Sigma	0.004% (v/v)
Transferrin	Roche	150 µg/mL
BMP4	R&D Systems	1 ng/mL
Y-27632*	Tocris Bioscience	10 µM
Chroman-1*	Tocris Bioscience	50 nM

*Inclusion in media is indicated for each experiment. All ALCM differentiations used Y-27632. All hPSC-CM differentiations in 500 mL VWBRs used Chroman-1.

Supplementary Table 2. Aggregation Medium 1B (Day 0-1)

Composition	Supplier	Final Concentration
StemPro-34	Thermo Fisher Scientific	25% (v/v)
IMDM	Thermo Fisher Scientific	75% (v/v)
L-Ascorbic acid	Sigma	50 µg/mL
Monothioglycerol	Sigma	0.004% (v/v)
Transferrin	Roche	150 µg/mL
ITS-X	Thermo Fisher Scientific	0.75x
BMP4*	R&D Systems	1 ng/mL
Chroman-1	Tocris Bioscience	50 nM

*Included in SCTi003-A differentiations.

Supplementary Table 3. Mesoderm Induction Medium 1A (Day 1-3)

Composition	Supplier	Final Concentration
StemPro-34	Thermo Fisher Scientific	100%
L-Glutamine	Thermo Fisher Scientific	2 mM
L-Ascorbic acid	Sigma	50 µg/mL
Monothioglycerol	Sigma	0.004% (v/v)
Transferrin	Roche	150 µg/mL
BMP4 [#]	R&D Systems	
Activin A [#]	R&D Systems	
bFGF	Peprtech	5 ng/mL
CHIR99021 [*]	STEMCELL Technologies	1 µM

[#]For ventricular CM differentiations with ESI-017 cells, 10 ng/mL BMP4 and 6 ng/mL Activin A were used. For atrial differentiations with ESI-017 cells, 4 ng/mL BMP4 and 2 ng/mL Activin A were used. ^{*}Inclusion in the medium is indicated for each experiment.

Supplementary Table 4. Mesoderm Induction Medium 1B (Day 1-3)

Composition	Supplier	Final Concentration
StemPro-34	Thermo Fisher Scientific	25% (v/v)
IMDM	Thermo Fisher Scientific	75% (v/v)
L-Ascorbic acid	Sigma	50 µg/mL
Monothioglycerol	Sigma	0.004% (v/v)
Transferrin	Roche	150 µg/mL
BMP4 [*]	R&D Systems	
Activin A [*]	R&D Systems	
bFGF	Peprtech	5 ng/mL
CHIR99021	STEMCELL Technologies	1 µM

^{*}For ventricular CM differentiations in 100 mL VWBRs using SCTi003-A cells, 10 ng/mL BMP4, and 10 ng/mL Activin A were used. For ventricular CM differentiations in 100 mL VWBRs using 585A1-ABo_C12o_To cells, 4 ng/mL BMP4, and 4 ng/mL Activin A were used.

Supplementary Table 5. Cardiomyocyte Induction Medium 1A

Composition	Supplier	Final Concentration
StemPro-34	Thermo Fisher Scientific	100%
L-Glutamine	Thermo Fisher Scientific	2 mM
L-Ascorbic acid	Sigma	50 µg/mL
Monothioglycerol	Sigma	0.004% (v/v)
Transferrin	Roche	150 µg/mL
IWP2	R&D Systems	2 µM
VEGF	Peprtech	10 ng/mL
all-trans-retinoic acid*	Sigma	0.5 µM

*Only included in atrial cardiomyocyte differentiations.

Supplementary Table 6. Cardiomyocyte Induction Medium 1B

Composition	Supplier	Final Concentration
StemPro-34	Thermo Fisher Scientific	25% (v/v)
IMDM	Thermo Fisher Scientific	75% (v/v)
L-Ascorbic acid	Sigma	50 µg/mL
Monothioglycerol	Sigma	0.004% (v/v)
Transferrin	Roche	150 µg/mL
IWP2	R&D Systems	2 µM
VEGF	Peprtech	10 ng/mL

Supplementary Table 7. Cardiomyocyte Induction Medium 2 (SP25 Medium).

Composition	Supplier	Final Concentration
StemPro-34	Thermo Fisher Scientific	25% (v/v)
IMDM	Thermo Fisher Scientific	75% (v/v)
L-Ascorbic acid	Sigma	50 µg/mL
Monothioglycerol	Sigma	0.004% (v/v)
Transferrin	Roche	50 µg/mL
VEGF*	Peprtech	5 ng/mL

*VEGF is included until day 10, after which no VEGF is added

Supplementary Table 8. Primary Antibodies

Antibody	Clone	Concentration	Supplier
Oct4 (IF)	C30A3	1:200	Cell Signalling Technology
Sox2 (IF)	D6D9	1:400	Cell Signalling Technology
Tra-1-60 (IF)	Tra-1-60(S)	1:200	Cell Signalling Technology
Tra-1-81 (IF)	Tra-1-81	1:100	Cell Signalling Technology
cTnT (IF)	13-11	1:100	Thermo Fisher Scientific
Oct4-APC	3A2A20	1:200	BioLegend
Sox2-Pacific Blue	14A6A34	1:20	BioLegend
PDGFR α (CD140a)-PE	α R1	1:10	BD Biosciences
NCAM (CD56)-APC	B159	1:100	BD Biosciences
c-kit (CD117)-FITC	104D2	1:100	BD Biosciences
CXCR4-BV421	12G5	1:100	Biolegend
CD235a-APC	GA-R2 (HIR2)	1:100	BD Biosciences
cTnT-APC	REA400	1:100	Miltenyi Biotec
MLC2v-PE	REA401	1:100	Miltenyi Biotec
MLC2a-VioBlue	REA398	1:50	Miltenyi Biotec
α -actinin (IF)	EP2529Y	1:200	Abcam
Ku80 (IF)	C48E7	1:400	Cell Signalling Technology
SIRPa-PeCy7	SE5A5	1:100	Biolegend
CD90-BD Horizon R718	5E10	1:400	BD Biosciences
CD151-PE	14A2.H1	1:200	BD Biosciences
MLC2v (IF) - Polyclonal	Cat#: ab79935	1:400	Abcam
MLC2a (IF)	56F5	1:400	Synaptic Systems

IF – Used for immunofluorescent staining

Supplementary Table 9. Primer List

GENE	Forward 5'-3'	Reverse 5'-3'
ATP2A2	GAAATGTGTAACGCCCTCAAC	AAGATGAGTGGCAAGGGTTC
CASQ2	GCCCAGGTCCTTGAACATAAAG	GCAAACCTCGCCATCAAACCTC
RYR2	AATGGACTGGAGATTGGCTG	CATTGGGACTTGTAGCTTGTG
CACNA1C	TTCGTCATCGTCACCTTTCAG	TGTAAGTGGTGCTGGTTCTTG
TNNI1	GGAGCGATACGACATTGAGG	TCCATCACCTTCAGCTTCAG
TNNI3	GTGGACAAGGTGGATGAAGAG	CGCTTAAACTTGCCTCGAAG
MYL7	TCCAACGTCTTTTCCATGTTTG	CTCTGGGACACTCACCTTC
MYL2	CGGGTCCAATTAACCTTACTGTG	CCGAACGTAATCAGCCTTCAG
MYH7	TGTCCTGCTCTGTGTCTTTC	GACTGCCATCTCCGAATCTC
MYH6	CCCGATGACAAGGAAGAGTTTG	ATCTTGTCGAACTTGGGTGG
HCN4	TCTTCCTCATTGTGGAGACACGCA	TGAGGATCTTCGTGAAGCGGACAA
TBP	GAGAGTTCTGGGATTGTACCG	ATCCTCATGATTACCGCAGC
DDB1	TCAACGGCATGATAGGGCTG	CGCTCGGTGTGAAAGGATCT
TBP	TGAGTTGCTCATACCGTGCTGCTA	CCCTCAAACCAACTTGTCAACAGC
IRX4	TTGGACTCCTGGGAACATGGACAA	ATGCTTCAGGGTATCTGGCCTCTT
TBX5	ACAAAGTGAAGGTGACGGGCCTTA	ATCTGTGATCGTCGGCAGGTACAA
NR2F2	TACCTGTCCGGCTACATCTC	CAGATGTTCTCGATGCCCATAA
MYL2	TGTCCCTACCTTGTCTGTTAGCCA	ATTGGAACATGGCCTCTGGATGGA
KCNJ3	TCATCAAGATGTCCCAGCCCAAGA	CACCCGGAACATAAGCGTGAGTTT
LIN28A	ATCAAAAGGAGACAGGTGCTACAA	GGTGGCAGCTTGCAATTCC
LIN28A- Probe	AGGTCTAGATCATCATGC	