Stem Cell Reports, Volume *3*

Supplemental Information

Derivation and Expansion of PAX7-Positive Muscle Progenitors from Human and Mouse Embryonic Stem Cells

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Figure S1

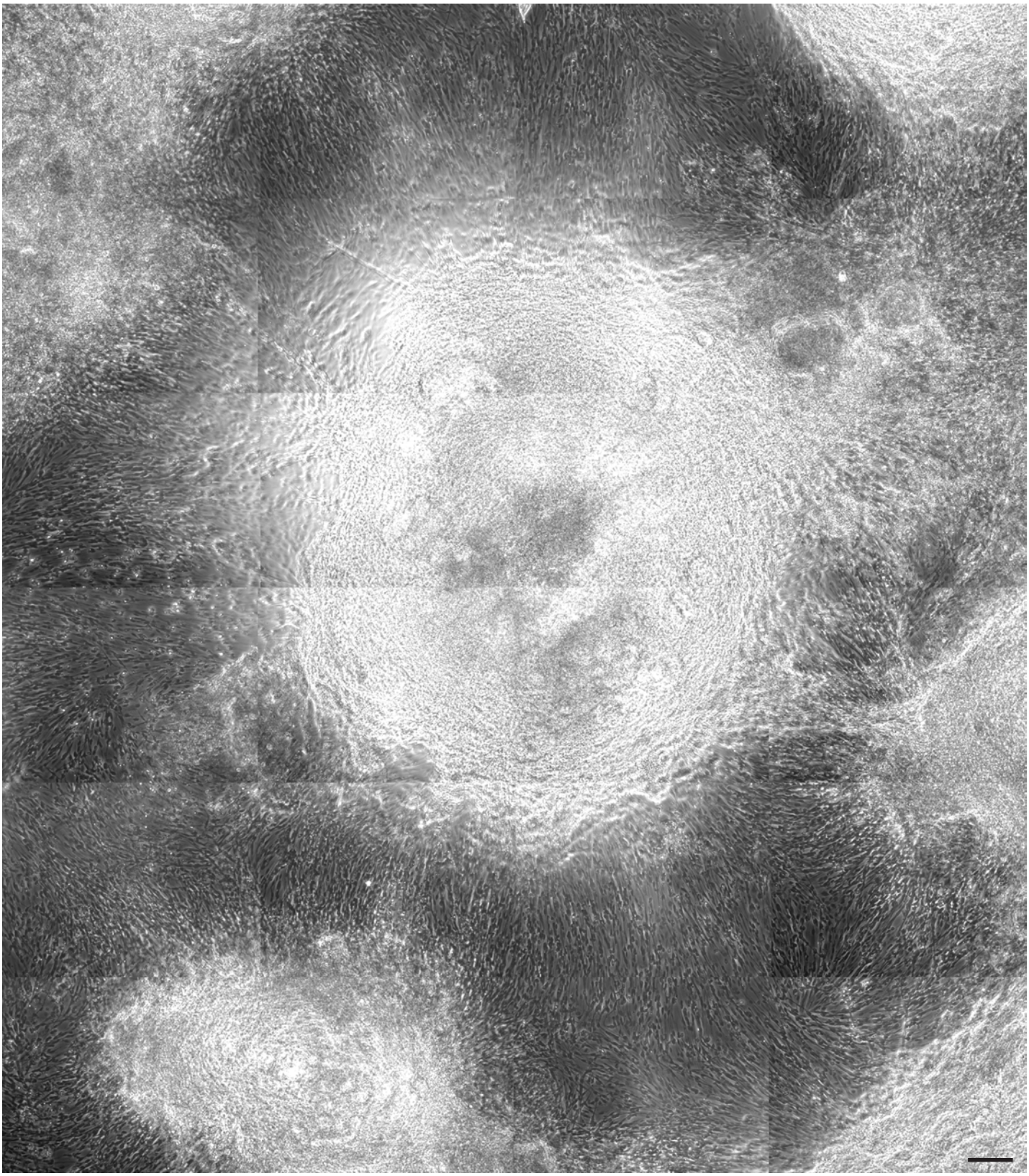

Figure S2

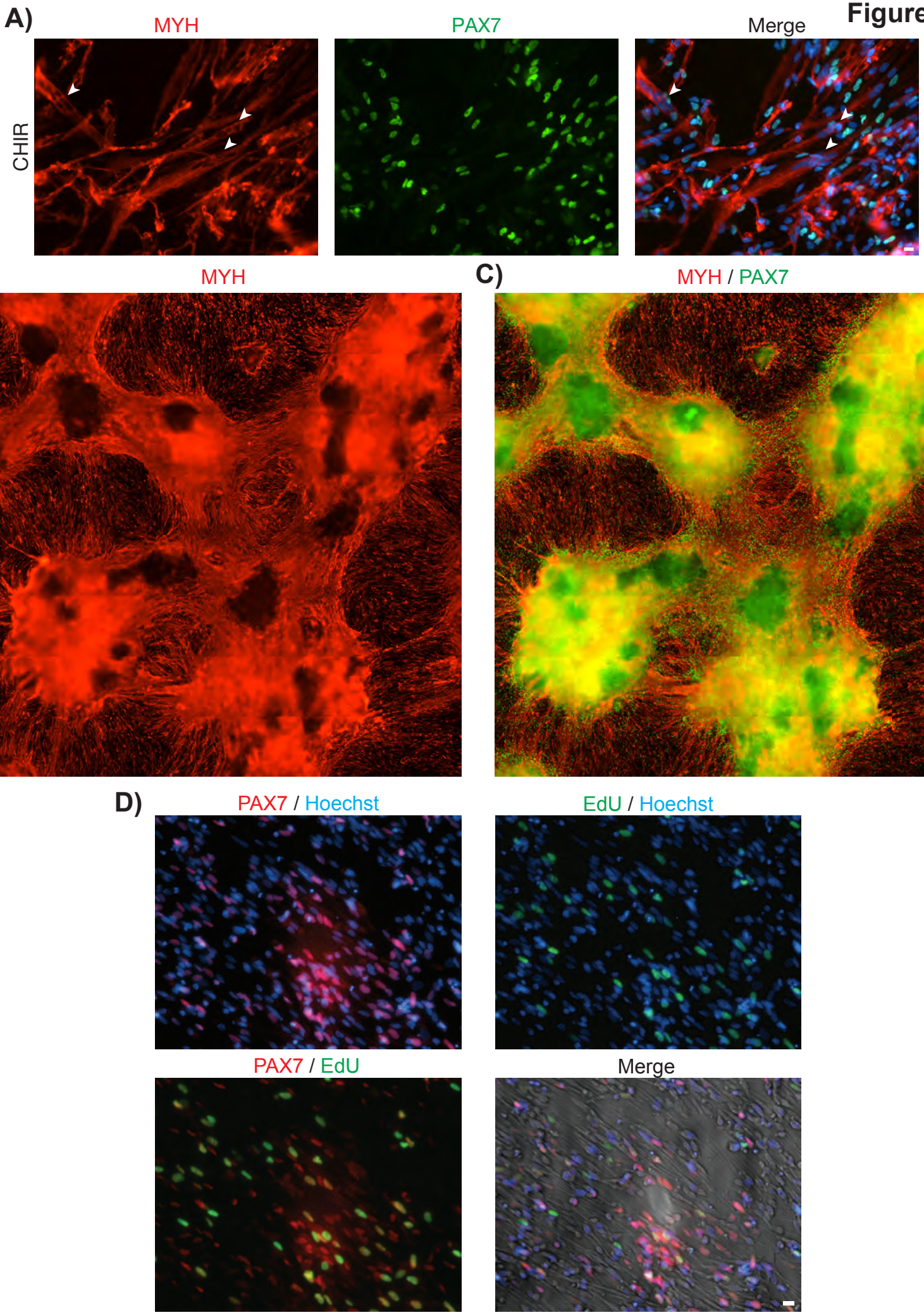
A)
MYH
PAX7
Merge
CHIR
B)
MYH
C)
MYH / PAX7
D)
PAX7 / Hoechst
EdU / Hoechst
PAX7 / EdU
Merge

Figure S3

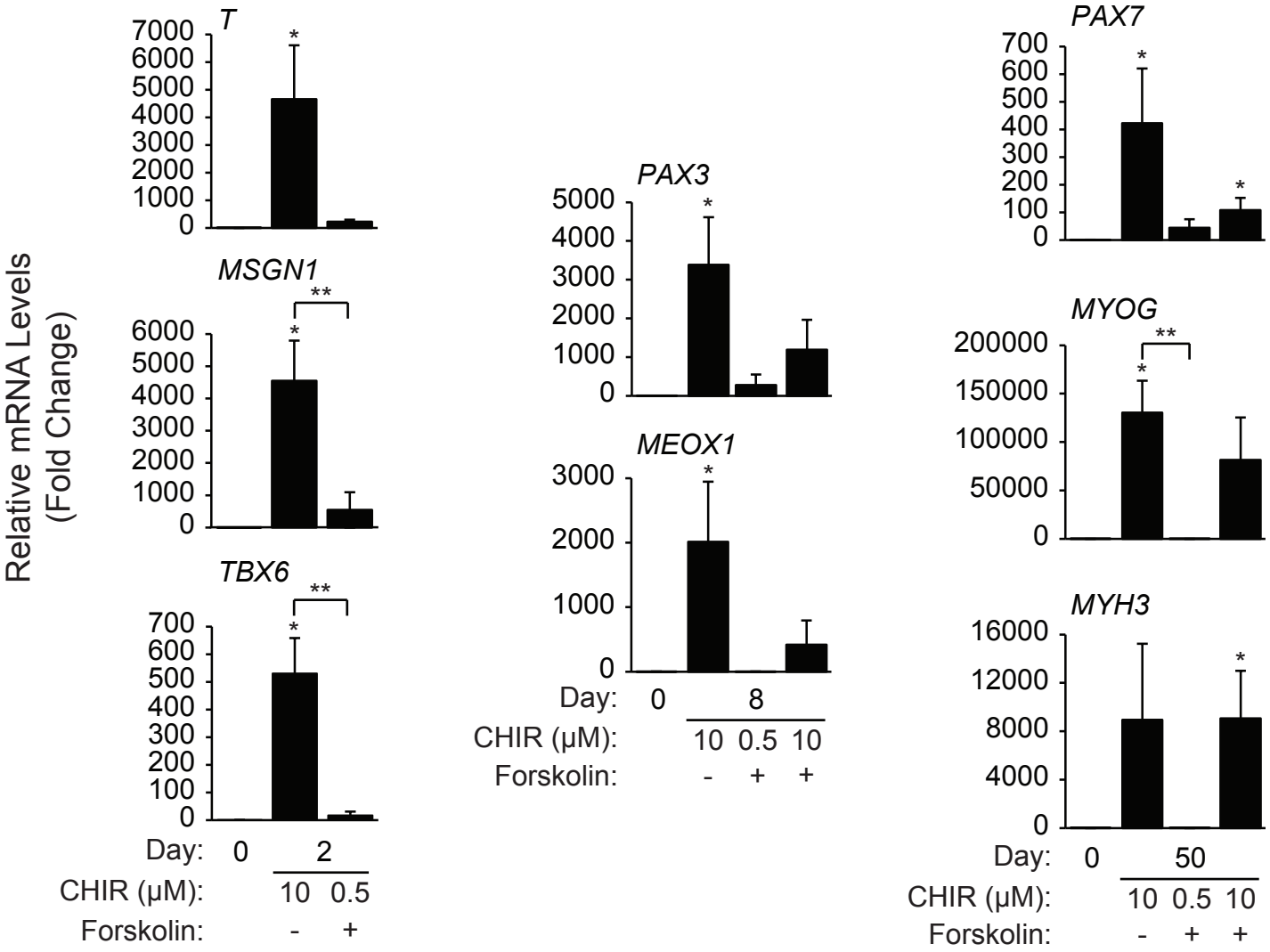
Relative mRNA Levels (Fold Change)
T
MSGN1
TBX6
PAX3
MEOX1
PAX7
MYOG
MYH3
Day: 0 2
CHIR (μM): 10 0.5
Forskolin: - +
Day: 0 8
CHIR (μM): 10 0.5 10
Forskolin: - + +
Day: 0 50
CHIR (μM): 10 0.5 10
Forskolin: - + +

Figure S1. Mature CHIR-differentiated skeletal myocytes display orderly alignment, related to Figure 1. Multiple phase contrast images were captured at day 50 and tiled together using Volocity Software; depicted is an embryoid-body-like cluster of cells (center) and skeletal myocytes aligned radially around its periphery (scale bar = 200 μm).

Figure S2. Fusion competence and high resolution imaging of CHIR-treated cultures, related to Figure 2. A) Immunofluorescence staining with antibodies against myosin heavy chain (MYH) confirms the presence of multinucleated myotubes (white arrowheads) at day 40 (scale bar = 20 μm). **B & C)** Multiple images were captured at day 50 and tiled together using Volocity Software, representing 27.06 mm^2 of culture area. Separate panels are provide for **B)** MYH, and **C)** PAX7 and MYH merged (scale bar = 200 μm). **D)** Day 50 CHIR-treated cells were pulsed with EdU for 4 hours and stained for EdU, PAX7 and with Hoechst dye (scale bar = 20 um).

Figure S3. Forskolin did not enhance the CHIR-driven development of mesoderm or terminal myogenesis, related to Figure 3. The 0.5 μM CHIR, 10 ng/mL FGF2, and 20 μM Forskolin cocktail as performed by Xu et al. was applied for the first 7 days of our differentiation, or FGF2 and Forskolin were applied between days 2 and 7 following our 10 μM CHIR treatment. Including FGF2 and Forskolin during 10 μM CHIR treatment from days 0 to 2 had excessive toxicity towards the cells and was not pursued (data not shown). qPCR for early mesoderm, somite, or terminal myogenic genes showed no significant improvement with the addition of Forskolin in our protocol (n = 3 independent experiments, *$p \leq 0.05$ vs. day 0, **$p \leq 0.05$ Forskolin vs. 10 μM CHIR).

Movie S1. Functional contractions of mature CHIR-differentiated skeletal myocytes, related to Figure 2.

Table S1. Media and components utilized in the growth and differentiation of hESCs, related to Experimental Procedures.

E8/E6 Media	
DMEM/F12	100%
Components:	
$NaHCO_3$	543 µg/mL
Ascorbic Acid	64 µg/mL
Insulin	19.4 µg/mL
Transferrin	10.7 µg/mL
Sodium Selenium	0.014 µg/mL
Gentamicin	50 µg/mL
FGF2 (E8 Only)	100 ng/mL
TGFβ1 (E8 Only)	2 ng/mL

StemPro-34 Media	
StemPro-34 SFM	100%
Components:	
L-Glutamine	2 mM
Monothioglycerol	0.45 mM
Transferrin	10.7 µg/mL
Gentamicin	5 µg/mL
FGF2	5 ng/mL

N2 Media	
DMEM/F12	100%
Components:	
Insulin / Transferrin / Selenium	1%
N2 Supplement	1%
Gentamicin	5 µg/mL

Table S2. Media and components utilized in the growth and differentiation of mESCs, related to Experimental Procedures.

Maintenance Media	
DMEM	85%
Fetal Bovine Serum	15%
Components:	
Non-Essential Amino Acids	1%
Penicillin / Streptomycin	1%
β-Mercaptoethanol	0.1 mM
Leukemia Inhibitory Factor	1000 U/mL

Serum Free Maintenance Media	
Neurobasal	50%
DMEM/F12	50%
Components:	
B27 Supplement	1%
Penicillin / Streptomycin	1%
Bovine Serum Albumin	0.5%
N2 Supplement	0.5%
L-Glutamine	2 mM
Monothioglycerol	0.15 mM
Leukemia Inhibitory Factor	1000 U/mL
BMP4	10 ng/mL

Serum Free Differentiation Media	
IMDM	75%
F12	25%
Components:	
B27 (w/o RA) Supplement	1%
Penicillin / Streptomycin	1%
Bovine Serum Albumin	0.5%
N2 Supplement	0.5%
Monothioglycerol	0.45 mM
Ascorbic Acid	50 µg/mL

StemPro-34 Media	
StemPro-34 SFM	100%
Components:	
Penicillin / Streptomycin	1%
L-Glutamine	2 mM
FGF2	10 ng/mL

N2 Media	
DMEM/F12	100%
Components:	
N2 Supplement	1%
Penicillin / Streptomycin	1%

Table S3. List of qPCR primers, related to Experimental Procedures.

Human Primer Name	Forward Primer (5′ - 3′)	Reverse Primer (5′ - 3′)
T	TTCATAGCGGTGACTGCTTATCA	CACCCCCATTGGGAGTACC
EN1	GAGCGCAGGGCACCAAATA	CGAGTCAGTTTTGACCACGG
FOXA2	TGTGTATTCTGGCTGCAAGG	CCTGCAACCAGACAGGGTAT
GAPDH	TGGTGCTGAGTATGTCGTGGAGT	AGTCTTCTGAGTGGCAGTGATGG
LBX1	CTCGCCAGCAAGACGTTTAAG	CGCTGCCCAAAGATGGTCATA
MEOX1	GCAGGGGGTTCCAAGGAAAT	GTCAGGTAGTTATGATGGGCAAA
MET	AGCGTCAACAGAGGGACCT	GCAGTGAACCTCCGACTGTATG
MSGN1	AACCTGCGCGAGACTTTCC	ACAGCTGGACAGGGAGAAGA
MYF5	AATTTGGGGACGAGTTTGTG	CATGGTGGTGGACTTCCTCT
MYH3	TTGATGCCAAGACGTATTGCT	GGGGGTTCATGGCGTACAC
MYH6	CAACAATCCCTACGACTACGC	ACGTCAAAGGCACTATCGGTG
MYL2	TTGGGCGAGTGAACGTGAAAA	CCGAACGTAATCAGCCTTCAG
MYOD1	TGCACGTCGAGCAATCCAAA	CCGCTGTAGTCCATCATGCC
MYOG	GCTGTATGAGACATCCCCCTA	CGACTTCCTCTTACACACCTTAC
NEUROG1	GCTCTCTGACCCCAGTAGC	GCGTTGTGTGGAGCAAGTC
NKX2-5	GCAGGACCAGACTCTGGAGC	GAGTCCCCTAGGCATGGCTT
NOG	GGCCAGCACTATCTCCACAT	ATGAAGCCTGGGTCGTAGTG
NPPA	TCCTCTGATCGATCTGCCCT	CTCTGGGCTCCAATCCTGTC
PAX3	CTCACCTCAGGTAATGGGACT	CGTGGTGGTAGGTTCCAGAC
PAX7	CCCCCGCACGGGATT	TATCTTGTGGCGGATGTGGTTA
SIM1	TCCATAATCAGACTCACGACCA	TGGGGCTACCACGAAGATGAA
SOX2	TACAGCATGTCCTACTCGCAG	GAGGAAGAGGTAACCACAGGG
SOX10	CCTCACAGATCGCCTACACC	CATATAGGAGAAGGCCGAGTAGA
TBX6	CATCCACGAGAATTGTACCCG	AGCAATCCAGTTTAGGGGTGT

Mouse Primer Name	Forward Primer (5′ - 3′)	Reverse Primer (5′ - 3′)
Actb	AAATCGTGCGTGACATCAAA	AAGGAAGGCTGGAAAAGAGC
T	CTGGACTTCGTGACGGCTG	TGACTTTGCTGAAAGACACAGG
Meox1	TGGCCTATGCAGAATCCATTCC	TGGATCTGAGCTGCGCATGTG
Msgn1	CTTCTGACACCGCTGGTCTG	GTGACTGCCGTAGCCATCG
Myh3	GCATAGCTGCACCTTTCCTC	GGCCATGTCCTCAATCTTGT
Myh6	CAACAACCCATACGACTACGC	ACATCAAAGGGCCACTATCAGTG
Myod1	CCCCGGCGGCAGAATGGCTACG	GGTCTGGGTTCCCTGTTCTGTGT
Myog	GCAATGCACTGGAGTTCG	ACGATGGACGTAAGGGAGTG
Nkx2-5	AAGCAACAGCGGTACCTGTC	GCTGTCGCTTGCACTTGTAG
Pax3	TTTCACCTCAGGTAATGGGACT	GAACGTCCAAGGCTTACTTTGT
Pax7	CTCAGTGAGTTCGATTAGCCG	AGACGGTTCCCTTTGTCGC