SUPPLEMENTAL MATERIAL

Supplemental Methods

Human Pluripotent Stem Cell Culture and Differentiation

hPSCs were cultured with Essential 8 Medium (Thermo Fisher Scientific, MA) in polystyrene plates coated with Matrigel (BD Biosciences). Stem cells were differentiated at 90-100% confluency, typically 4 days after passage. At day 0, RPMI Medium with GlutaMAX plus Gem21 NeuroPlex Serum-Free Supplement without insulin was added to the cells supplemented with 10 μmol/L of CHIR99021 (STEMCELL Technologies, Vancouver, Canada). At day 1, the media was refreshed with RPMI GlutaMAX plus Gem21 without insulin. At day 3, the media was replaced with RPMI Glutamax plus Gem21 without insulin supplemented with 5 μmol/L of IWP-4 (STEMCELL Technologies, Vancouver, Canada). At day 5, the media was refreshed with RPMI GlutaMAX plus Gem21 without insulin. At day 7, the media was refreshed with RPMI GlutaMAX plus Gem21. At day 10 and 13, metabolic purification of cardiomyocytes was performed by replacing the media with RPMI without glucose plus Gem21 supplemented with 5 mmol/L of Sodium L-lactate (Sigma, MO). At day 16 and 19, the media was refreshed with RPMI GlutaMAX pluse Gem21. Between day 20 and 25 of differentiation, spontaneously beating CMs were dissociated with TrypLE Express Enzyme (Thermo Fisher Scientific, MA), centrifuged, resuspended in RPMI GlutaMAX plus Gem21, filtered through 70 μm cell strainer, and re-plated onto Matrigel coated plates for appropriate experiments. hPSC-CMs' purity was quantified and compared between different treatment conditions and cell lines. Highly pure populations of positive cardiomyocytes expressing cardiac troponin T were used in this study (Online Figure II). We also found similar expressions of Myosin regulatory light chain 2, ventricular/cardiac muscle isoform (MLC2v) and Myosin regulatory light chain 2, atrial isoform (MLC2a) together with cardiac troponin T in all three cell lines used in this study (Online Figure III).

Lactate and ATP Measurements

Cellular and tissue lactate levels were measured using Lactate Assay Kit (Sigma, MO). In this assay, lactate is oxidized into pyruvate generating NADH in the process. NADH generated then reacts with a substrate allowing for colorimetric readout, which is proportional to the amount of lactate in the samples. Cellular and tissue ATP levels were measured using CellTiter-Glo Luminescent Assay (Promega, WI). Cell numbers were determined by both PrestoBlue Cell Viability Reagent (Thermo Fisher Scientific, MA) and counting cells using a hemocytometer.

Seahorse Live-cell Metabolic Assay

Live-cell metabolic assays were performed with Seahorse XFe96 Analyzer (Agilent, CA) according to manufacture protocol. hPSC-CMs were plated in 96-well assay plates (Agilent, CA) and cultured with GLM, GFAM and FAM treated with 5 nmol/L Chetomin (CTM, Sigma, MO), 5 μmol/L GSK 2837808A (GSKA, APEXBIO, TX) or controls for 10 days. Sensor cartridges were hydrated with calibration buffer (Agilent, CA) a day prior to each assay. Prior to the measurements, cells were switched to the Seahorse XF Base Medium (Agilent, CA) with added glucose, fatty acids or both plus CTM, GSKA or controls in the appropriate wells. Three baseline measurements were made for each assay before adding oligomycin, Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) and antimycin sequentially into the media. 30,000 CMs were initially seeded onto each well of Seahorse XF96 Cell Culture Microplates (Agilent, CA). Cell numbers were quantified using hemocytometer prior to each experiment for all conditions and all measurements were normalized to the number of cells in FAM control (set to

1). Concentrations of drugs used in Seahorse experiments are as follows: 2 μmol/L oligomycin, 2 μmol/L FCCP and 0.5 μmol/L of antimycin A and rotenone mixture.

Mitochondria Staining and FACS Analysis

MitoTracker Deep Red FM fluorescent dye (Thermo Fisher Scientific, MA) was added at 100nM to hPSC-CMs cultured in GLM, GFAM and FAM treated with CTM, GSKA, or controls. Following multiple washes, CMs were dissociated, centrifuged and resuspended in FACS buffer (PBS with no calcium or magnesium plus 5% FBS and DAPI). CMs were analyzed by BD FACSARIA system (BD Biosciences, CA). Fluorescent intensities at the APC far-red channel (Ex/Em ~633nm/660nm) were recorded. Gating and intensity profile analysis were performed using FlowJo software (FLOWJO, LLC, OR). For all FACS experiments, negative controls were prepared as the experimental samples but using IgG isotype control antibodies.

Immunostaining and Sarcomere Length Measurements

Following multiple washes with cold PBS, cells were fixed for 5 min with 4% paraformaldehyde and washed again with cold PBS. Samples were then incubated for 2 hours at room temperature with a blocking solution consisting of 5% donkey serum and 0.1% Triton-X in PBS. Primary antibodies were diluted in 0.1% Triton-X, 2% donkey serum in PBS and incubated overnight at 4 ˚C. Following multiple washing steps, samples were incubated with secondary antibodies diluted in 0.1% Triton-X and 2% donkey serum for 2 hours in room temperature. Samples were then washed, incubated with DAPI in PBS for 5 minutes and washed again before imaging. The following antibodies were used in this study: anti-HIF-1 alpha (Abcam, Cambridge, UK, ab51608, LOT: GR244245-15), anti-sarcomeric alpha actinin (Sigma, MO, A7811) and Pierce Cardiac Troponin (Thermo Fisher Scientific, MA, MA5-12960, LOT: Q12028791). Secondary antibodies include donkey-anti-mouse 488 (Thermo Fisher Scientific, MA, A21202, LOT: 1423052) and donkey-anti-rabbit 647 (Thermo Fisher Scientific, MA, A-31573, LOT: 1693297). Sarcomere lengths were measured using ImageJ software. Lengths of five sarcomeres from each cell were measured and averaged for each condition. Immunofluorescent stainings were imaged using confocal laser scanning microscope Leica TCS SP8 (Leica, Germany). For all immunofluorescence experiment, secondary antibodies only negative controls were used for adjustment of imaging settings to minimize background signals.

Western Blotting and RT-PCR

Cultured hPSC-CM samples were lysed in RIPA buffer (Boston BioProducts, MA) with protease and phosphatase inhibitors (Thermo Fisher Scientific, MA). Protein concentration was measured by BCA assay (Thermo Fisher Scientific, MA). SDS-PAGE was used to resolve the lysates, which were then transferred onto a PVDF membrane (Merck Millipore ltd. MA) and incubated overnight with primary antibodies. Following multiple washing steps, the membrane was incubated with secondary HRP-linked antibodies (Cell Signaling Technologies, MA) and then treated with SuperSignal™ West Chemiluminescent Substrate (Thermo Fisher Scientific, MA). Chemiluminescent images were acquired using ChemiDoc™ Imaging Systems (Bio-Rad Laboratories, Inc., CA). The antibodies used for western blotting include: anti-HIF-1 alpha (Abcam, Cambridge, UK, ab51608, LOT: GR244245-15), anti-GAPDH (Cell Signaling Technologies, MA, 2118S, LOT: 10), anti-Hexokinase II (Cell Signaling Technologies, MA, C64G5, 2867T, LOT: 5) and anti-rabbit IgG (Cell Signaling Technologies, MA, 7074S, LOT: 27). Signal intensity was quantified by Image Lab software version 5.2.1 (Bio-Rad Laboratories, Inc., CA).

For qPCR experiments, RNA from hPSC-CMs was extracted using RNeasy kit (Qiagen, Germany). Reverse transcriptions were done using iScript kit (Bio-Rad Laboratories, Inc., CA).

qPCR experiments were performed with HotStart-IT SYBR Green kit (Affymetrix, CA) using QuantStudio 3 software (Applied Biosystems, CA).

Confocal Imaging and Single Cell Functional Studies

For single cell imaging, polydimethylsiloxane (PDMS) plates were prepared according to previously established protocol[47]. hPSC-CMs were plated at single cell density on 5kPA PDMS plates with Matrigel coating. Calcium transients were visualized using Fluo-4 AM calcium indicator (Thermo Fisher Scientific, MA). Videos of CMs' calcium transient and contractions were captured using the 488 and DIC channels, respectively, at 50 frames per second. Videos were captured on confocal laser scanning microscope Leica TCS SP8 (Leica, Germany) in resonance scanning mode.

Contractile force generation of single hPSC-CMs were quantified using previously established methods[47]. In brief, movies of single contracting CMs were captured by high-speed confocal microscopy (50 frames per second). Maximum and minimum cell length during contractions as well as cell widths were measured using ImageJ. Maximum contractile forces generated during contractions were then calculated using previously published customized Matlab code[47].

siRNA Transfections

The siRNAs used in this study are MISSION esiRNA human HIF1A (esiRNA1) and MISSION esiRNA targeting EGFP (esiRNA1) (Sigma, MO). siRNAs were transfected to CMs using Lipofectamine 3000 Reagent (Thermo Fisher Scientific, MA). Complexation reaction of RNAs and lipofectamine reagents were performed in Opti-MEM media (Thermo Fisher Scientific, MA). After the incubation period, the mixture was added to the CMs and experiments were performed after 96 hours.

Transmission Electron Microscopy (TEM)

CMs for TEM were fixed with glutaldehyde solution for 2 hours in room temperature and washed with cacodylate rinse buffer. Cells were then scraped from the plates and centrifuged. Following agarose processing and embedding, the samples were then sectioned, and contrast stained for imaging. TEM images were acquired at random locations throughout the samples. JEOL JEM-1011 TEM was used for image acquisitions.

Electrophysiology

hiPSC-CMs were plated on matrigel-coated 12-mm glass coverslips for electrophysiological studies. Action potentials were recorded in current-clamp mode of the whole-cell patch-clamp techniques at 37°C. Signals were low-pass filtered at 5 kHz with an Axopatch 200B amplifier and digitized at 10 kHz with a Digidata 1440A A/D converter, and analyzed with Clampex 10.3 (Molecular Devices Inc.). Borosilicate-glass electrodes had 3 – 5 MΩ tip resistance. Standard Tyrode's solution contains (in mmol/L): NaCl 136, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1.8, NaH_2PO_4 0.33, HEPES 5, Dextrose, 10, pH 7.35 with NaOH. Internal pipette solution contains (in mmol/L): K-aspartate 110, KCl 20, $MgCl_2$ 1, MgATP 5, GTP 0.1, HEPES 10, Na-Phosphocreatine 5, EGTA 0.05, pH 7.3 with KOH. Action potential recordings were analyzed with Clampfit 10.3 (Molecular Devices Inc.).

Online Table I. List of primers used for qPCR.

Sirt1	TGTGTCATAGGTTAGGTGGTGA	AGCCAATTCTTTTTGTGTTCGTG
Sirt3	CCCCAAGCCCTTTTTCACTTT	CGACACTCTCTCAAGCCCA
Sirt6	GATGTCGGTGAATTACGCGG	TGGAACACCACACTGGAAGAC
LDHA	ATGGCAACTCTAAAGGATCAGC	CCAACCCCAACAACTGTAATCT
LDHB	TGGTATGGCGTGTGCTATCAG	TTGGCGGTCACAGAATAATCTTT
PDK1	GAGAGCCACTATGGAACACCA	GGAGGTCTCAACACGAGGT
HK2	TGCCACCAGACTAAACTAGACG	CCCGTGCCCACAATGAGAC
ENO1	GCCGTGAACGAGAAGTCCTG	ACGCCTGAAGAGACTCGGT
VEGFA	AGGGCAGAATCATCACGAAGT	AGGGTCTCGATTGGATGGCA
SDHB	ACAAGGCTGGAGACAAACCT	CCTTCGGGTGCAAGCTAGAG
SDHA	ACTGTTGCAGCACAGCTAGAA	GCTCTGTCCACCAAATGCAC
CA9	GGATCTACCTACTGTTGAGGCT	CATAGCGCCAATGACTCTGGT
DEC1	ATGACAATGAATGTTCTGGAGGC	CATCATGTGTAACACGGCAAGA
PKM	ATGTCGAAGCCCCATAGTGAA	TGGGTGGTGAATCAATGTCCA
PKM1	GGCTGAAGGCAGTGATGTGG	GAGGATAGTCCCCTTTGGCT
PKM2	AAGAACTTGTGCGAGCCTCA	GACGAGCTGTCTGGGGATTC
FoxO1	TGATAACTGGAGTACATTTCGCC	CGGTCATAATGGGTGAGAGTCT
FoxO3	ACGGCTGACTGATATGGCAG	CGTGATGTTATCCAGCAGGTC
PGC-1a	AACAGCAAAAGCCACAAAGACG	GGGGTCAGAGGAAGAGATAAAGTTG
PARP1	TTCAACAAGCAGCAAGTGCC	CCTTTGGGGTTACCCACTCC
PPARA	TTCGCAATCCATCGGCGAG	CCACAGGATAAGTCACCGAGG
P53	TGCTCAAGACTGGCGCTAAA	TTTCAGGAAGTAGTTTCCATAGGT
FGFR1	CCCGTAGCTCCATATTGGACA	TTTGCCATTTTTCAACCAGCG
FOXM1	CGTCGGCCACTGATTCTCAAA	GGCAGGGGATCTCTTAGGTTC
HSF1	GCACATTCCATGCCCAAGTAT	GGCCTCTCGTCTATGCTCC
EGLN1	TCGCAACCCTCATGAAGTACA	TAGCTCGTGCTCTCTCATCTG

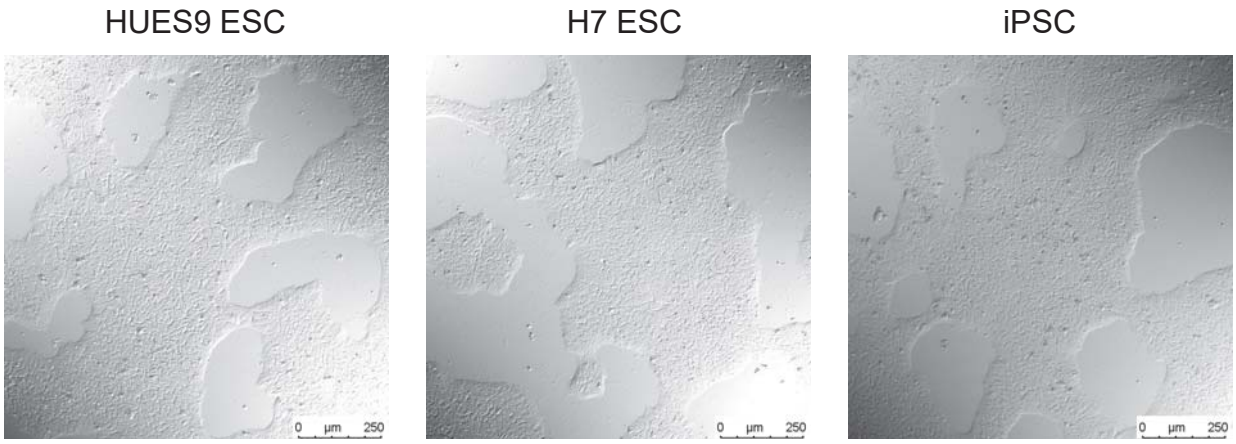
Online Table II: Details on statistical tests and corrections.

Figure	Number of Data Sets	Number of Samples	Passed Normality Test	Statistical Tests and Corrections
1B	3	7,10,9	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
1C	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
1D	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
1E	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
1G	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
1I	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
1J	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
1K	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
2B	6	3	No	Ordinary Two-way ANOVA with Bonferroni's test correction for multiple comparisons
2C	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
2D	6	3	No	Ordinary Two-way ANOVA with Bonferroni's test correction for multiple comparisons
3A	9	3	No	Ordinary Two-way ANOVA with Bonferroni's test correction for multiple comparisons
3B	9	3	No	Ordinary Two-way ANOVA with Bonferroni's test correction for multiple comparisons
3C	9	3	No	Ordinary Two-way ANOVA with Bonferroni's test correction for multiple comparisons
3D	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
4A	4	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
4B	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
4D	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
4E	2	20,27	No	Mann–Whitney test
4F	4	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
5B-F	4	30-50	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons

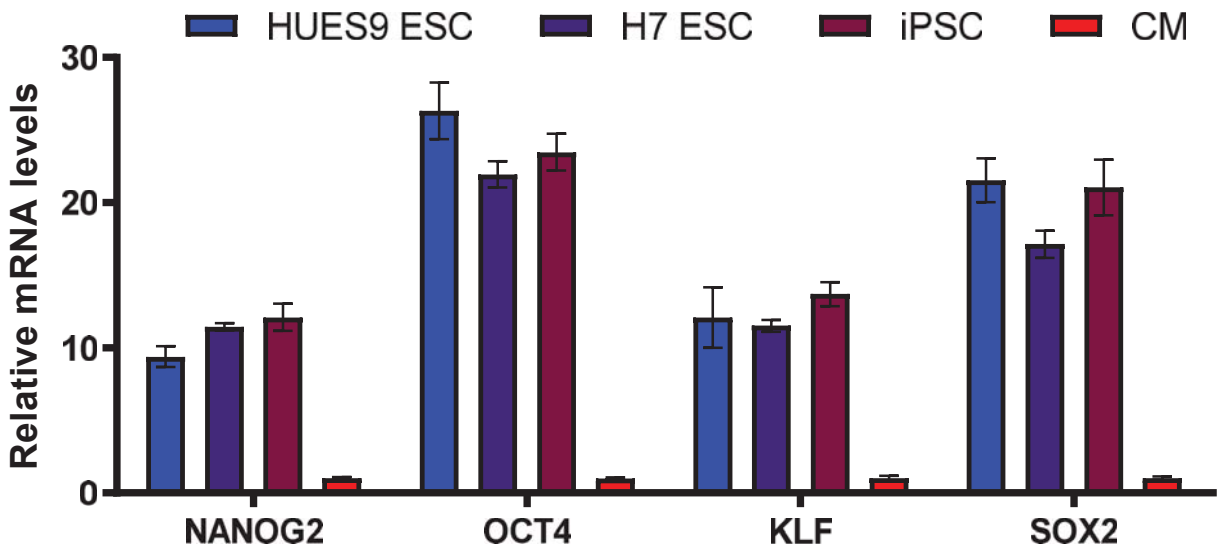
5G	4	10-14	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
5H	4	10-14	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
5I	4	10-14	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
5K	2	6	No	Unpaired T-test
6A	2	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
6B	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
6C	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
6D	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
6E	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S1B	4	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S2A	9	3	No	Ordinary Two-way ANOVA with Bonferroni's test correction for multiple comparisons
S2F	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S4A	3	41,31,34	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S4B	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S4C	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S5	3	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S6A	9	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S6B	9	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S6C	9	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S7A	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S7B	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S7C	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S7D	5	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons

S7E	4	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S8A	4	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S8B	4	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S8C	4	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S8D	4	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons
S8E	4	3	No	Non-parametric One-way ANOVA Kruskal–Wallis test with Dunn's test correction for multiple comparisons

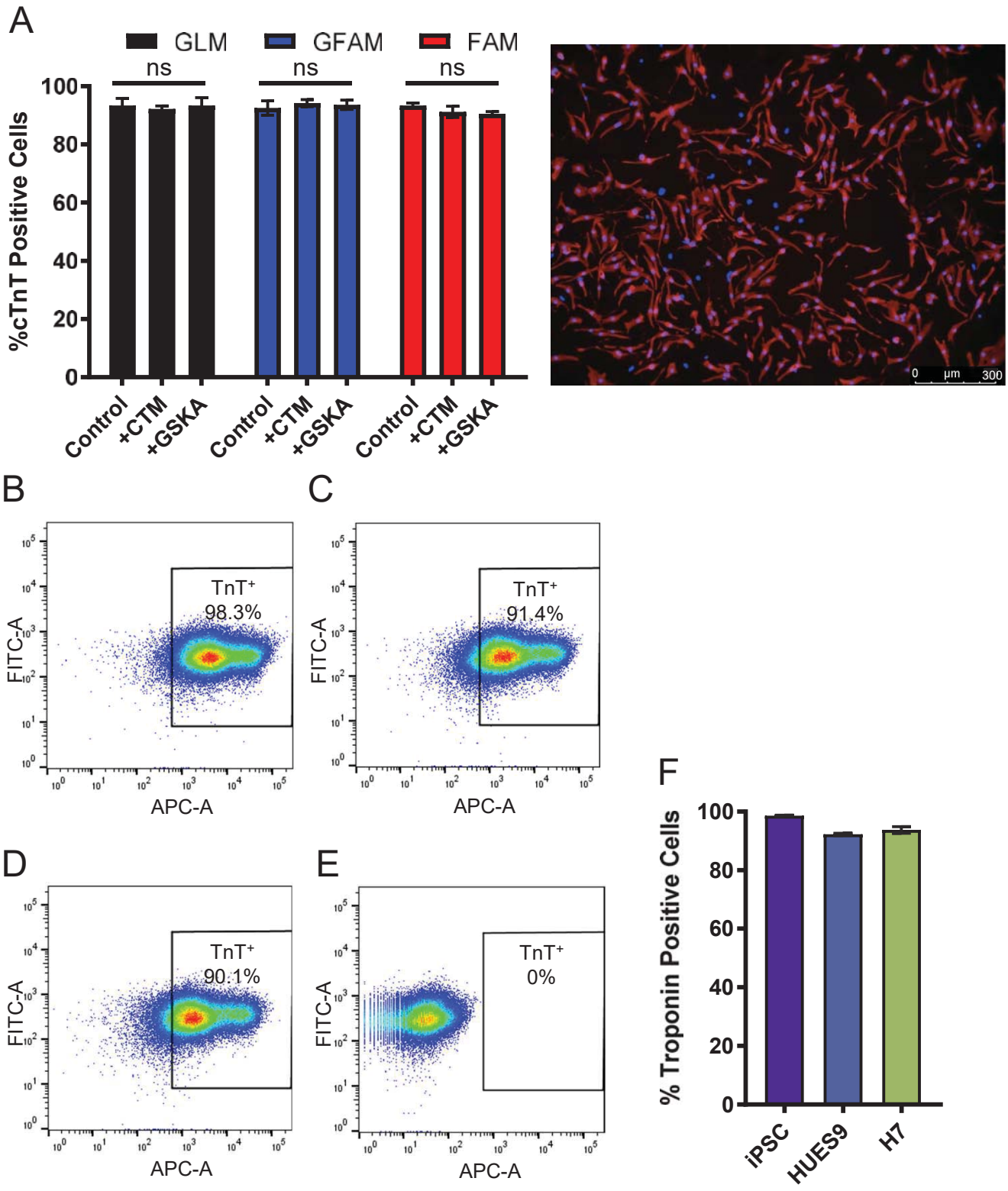
A



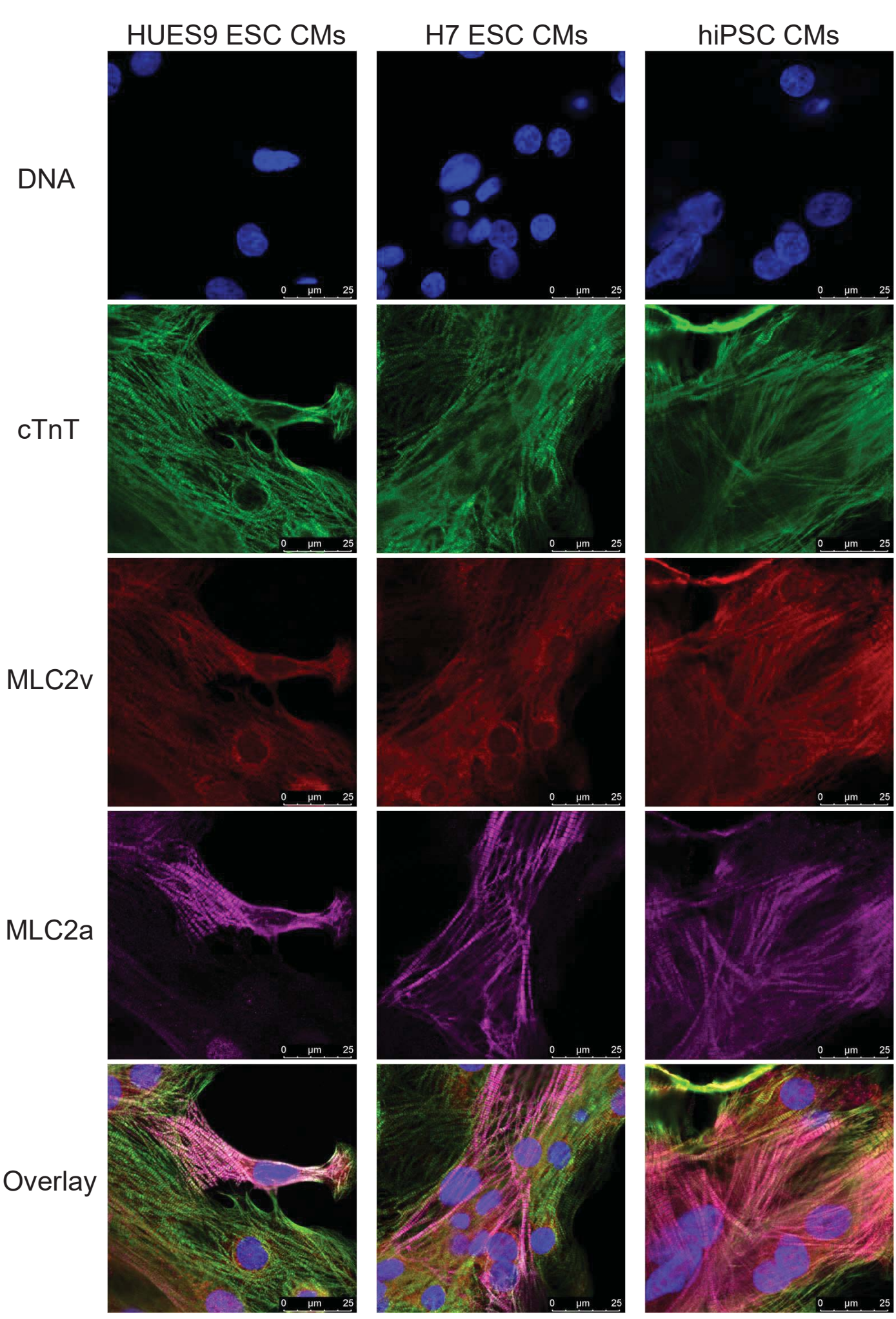
B



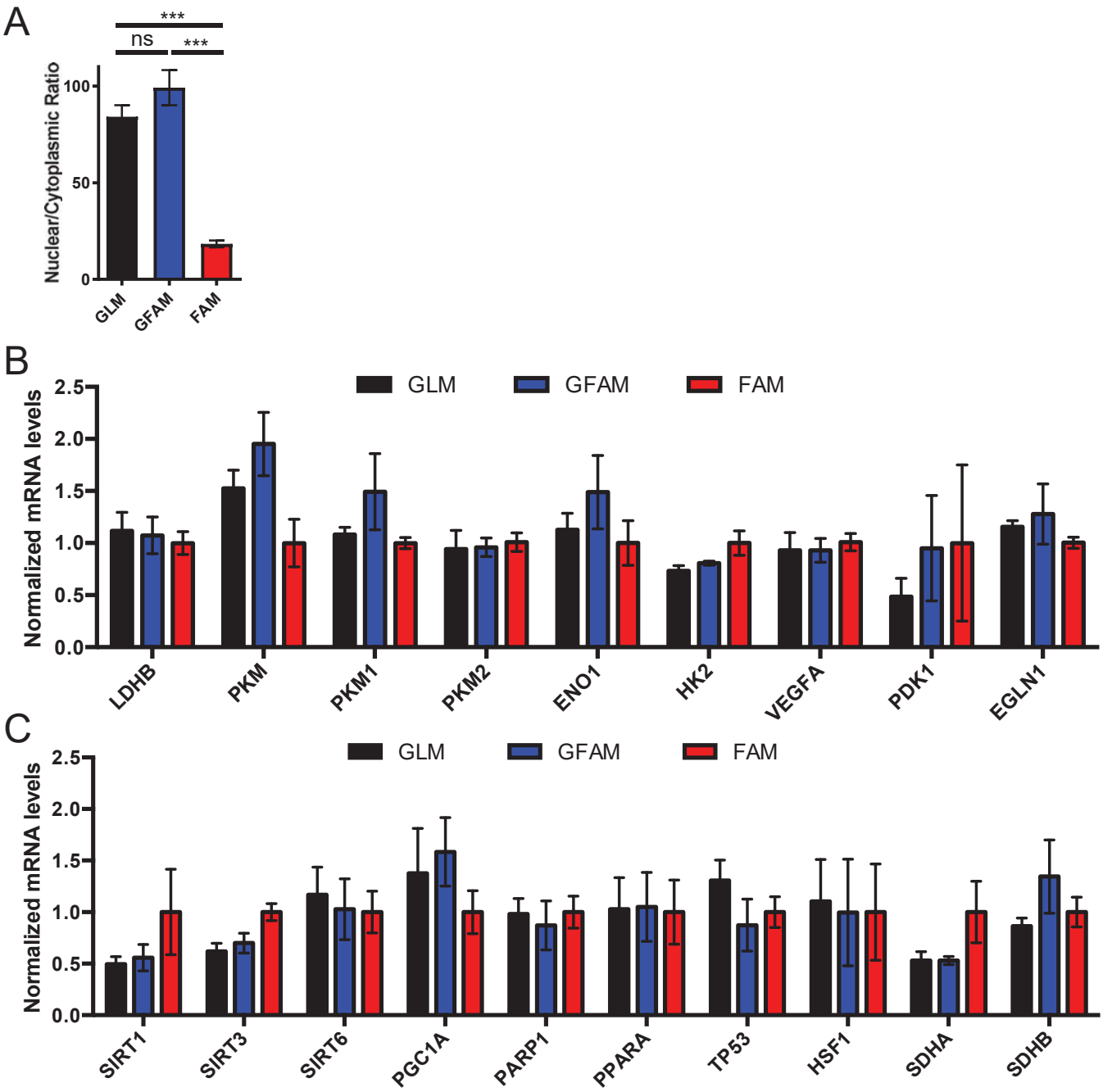
Online Figure I. A) Representative images of in HUES9 ESC, H7 ESC and iPSC, **B)** Relative mRNA levels of pluripotency markers in HUES9 ESC, H7 ESC and iPSC normalized to differentiated CM (n=3 independent experiments)**,** .



Online Figure II. A) Quantification (left, n=3 independent experiments) and a representative image (right) of cardiac Troponin positive cells in HUES9-hESC-CMs cultured in GLM, GFAM and FAM treated with CTM or GSKA. Red is cardiac troponin T and blue is DAPI, **B)** FACS quantifications of troponin labeled cells differentiated from **B)** hiPSC, **C)** HUES9-ESC, **D)** H7-ESC lines, **E)** Negative control sample with isotype control antibody for FACS. **F)** Quantification of % troponin positive cells differentiation from hiPSC, HUES9-ESC, and H7-ESC lines (n=3 independent experiments).

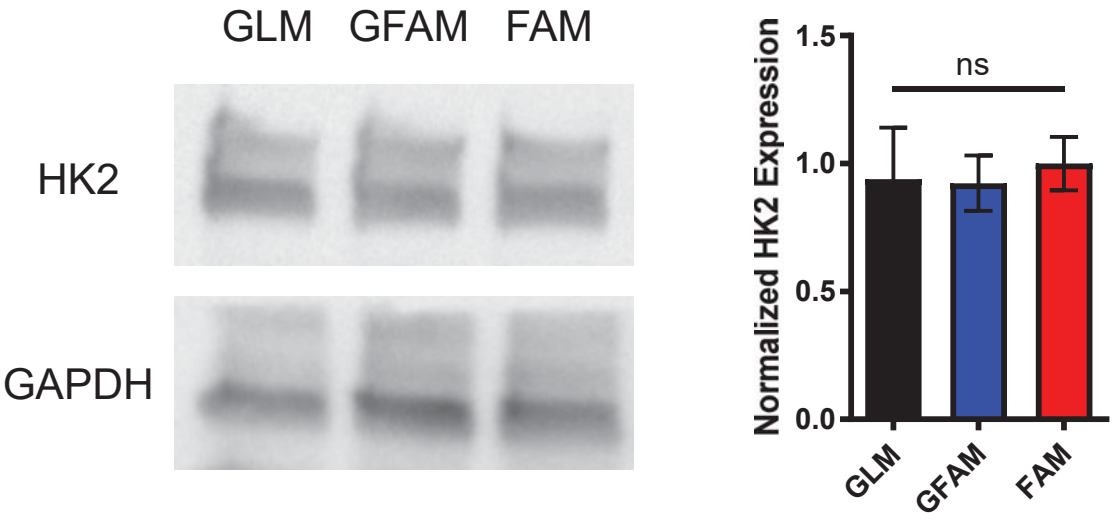

HUES9 ESC CMs
H7 ESC CMs
hiPSC CMs
DNA
cTnT
MLC2v
MLC2a
Overlay
0 µm 25

Online Figure III. Immunofluorescence characterization of HUES9-hPSC-CMs. Immunofluorescent stainings of cardiac troponin T (green), myosin Light Chain 2v (red) and 2a (magenta) together with DAPi labeling DNA in CMs derived from HUES9-ESC, H7-ESC and hiPSC lines at day 20 of cardiac differentiation.



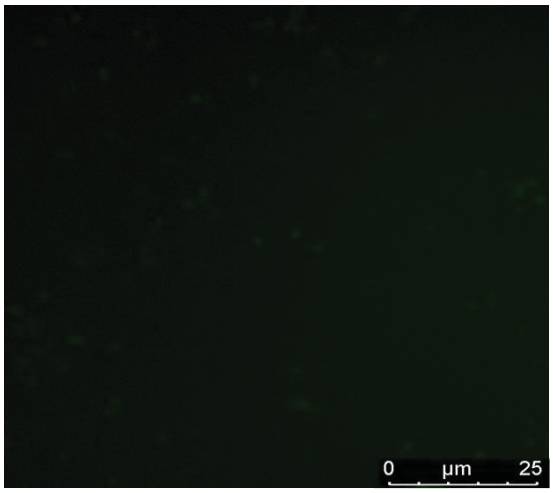
Online Figure IV. A) Quantification of nuclear to cytoplasmic ratios of HIF1α protein expressions in HUES9-hPSC-CMs cultured in GLM, GFAM and FAM, (n=41, 31 and 34 cells from 3 independent experiments), **B)** mRNA levels of select transcripts involved in HIF1α signaling pathways and **C)** mRNA levels of select transcripts in CM glycolytic and oxidative metabolism in HUES9-hPSC-CMs cultured in GLM, GFAM and FAM (n=3 independent experiments). No statistical significance were found among GLM, GFAM and FAM in these transcripts.

A

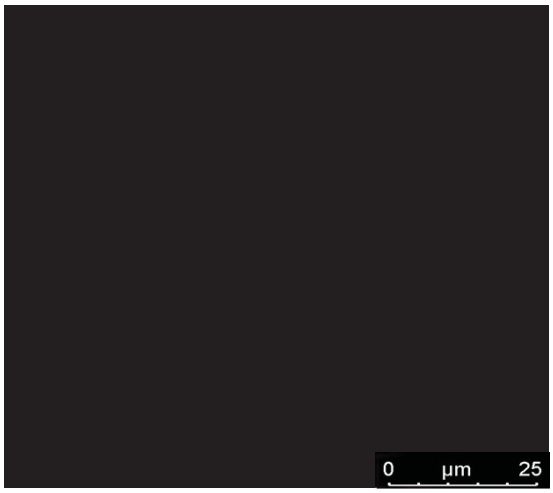


B

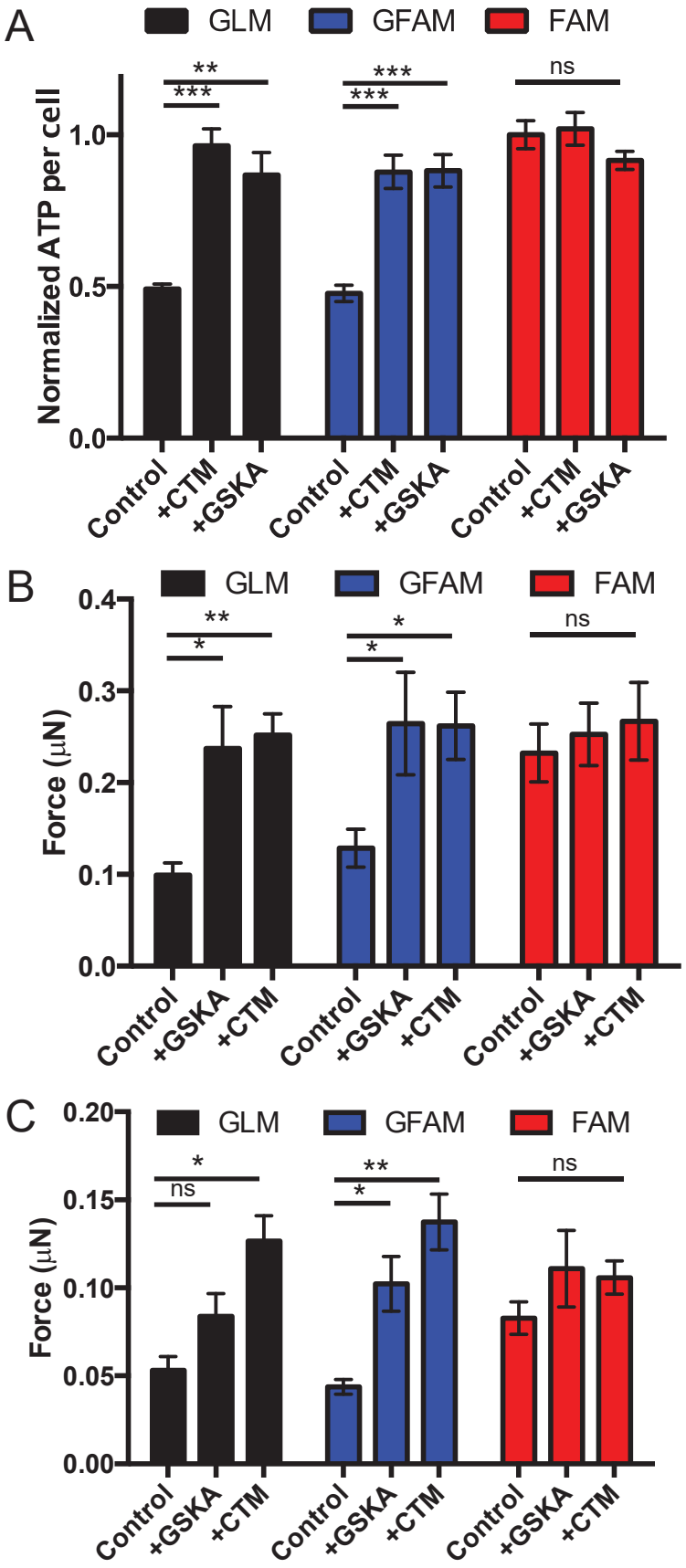
Donkey-anti-mouse 488 (Thermo Fisher Scientific, MA, A21202, LOT: 1423052) only control



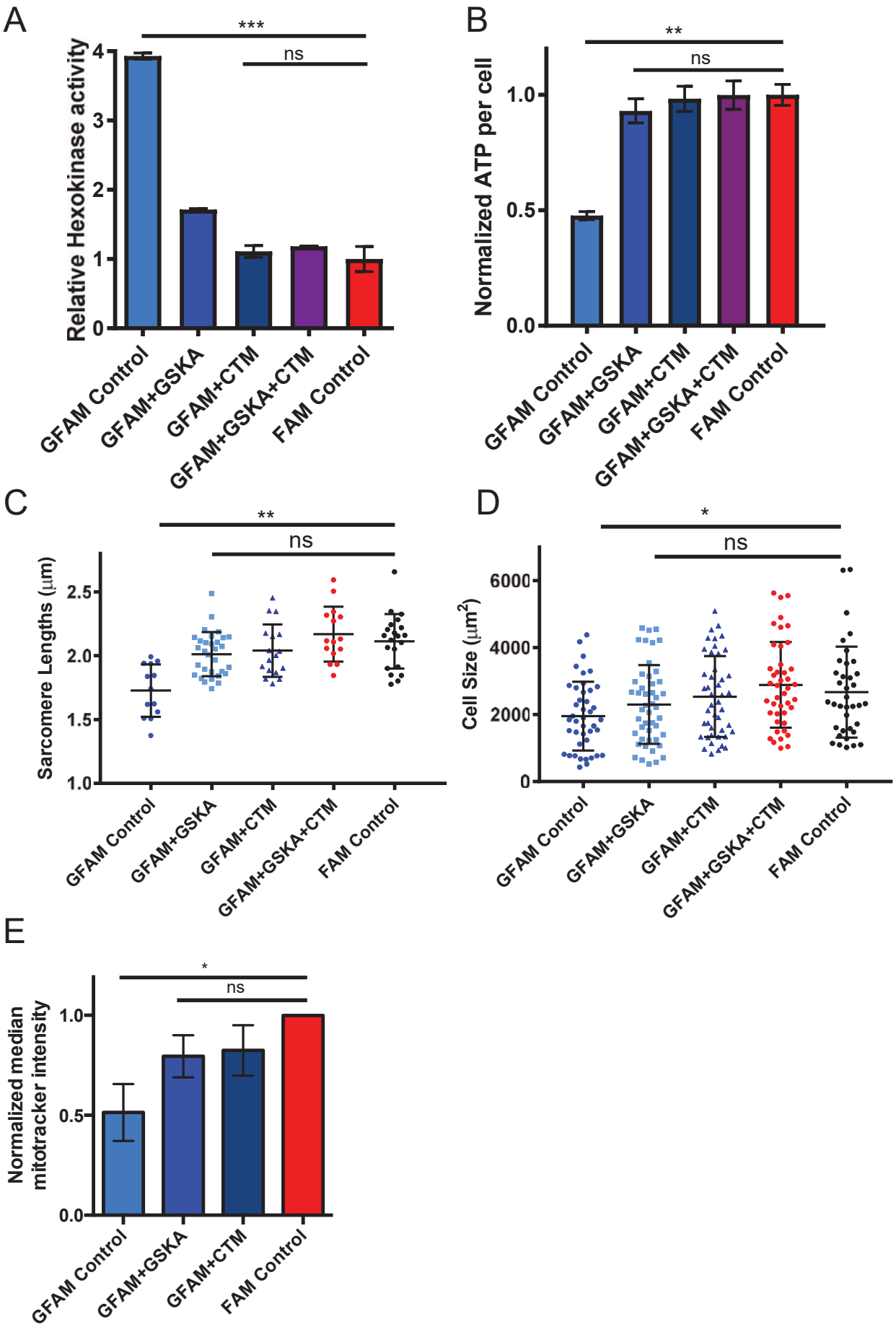
Donkey-anti-rabbit 647 (Thermo Fisher Scientific, MA, A-31573, LOT: 1693297) only control



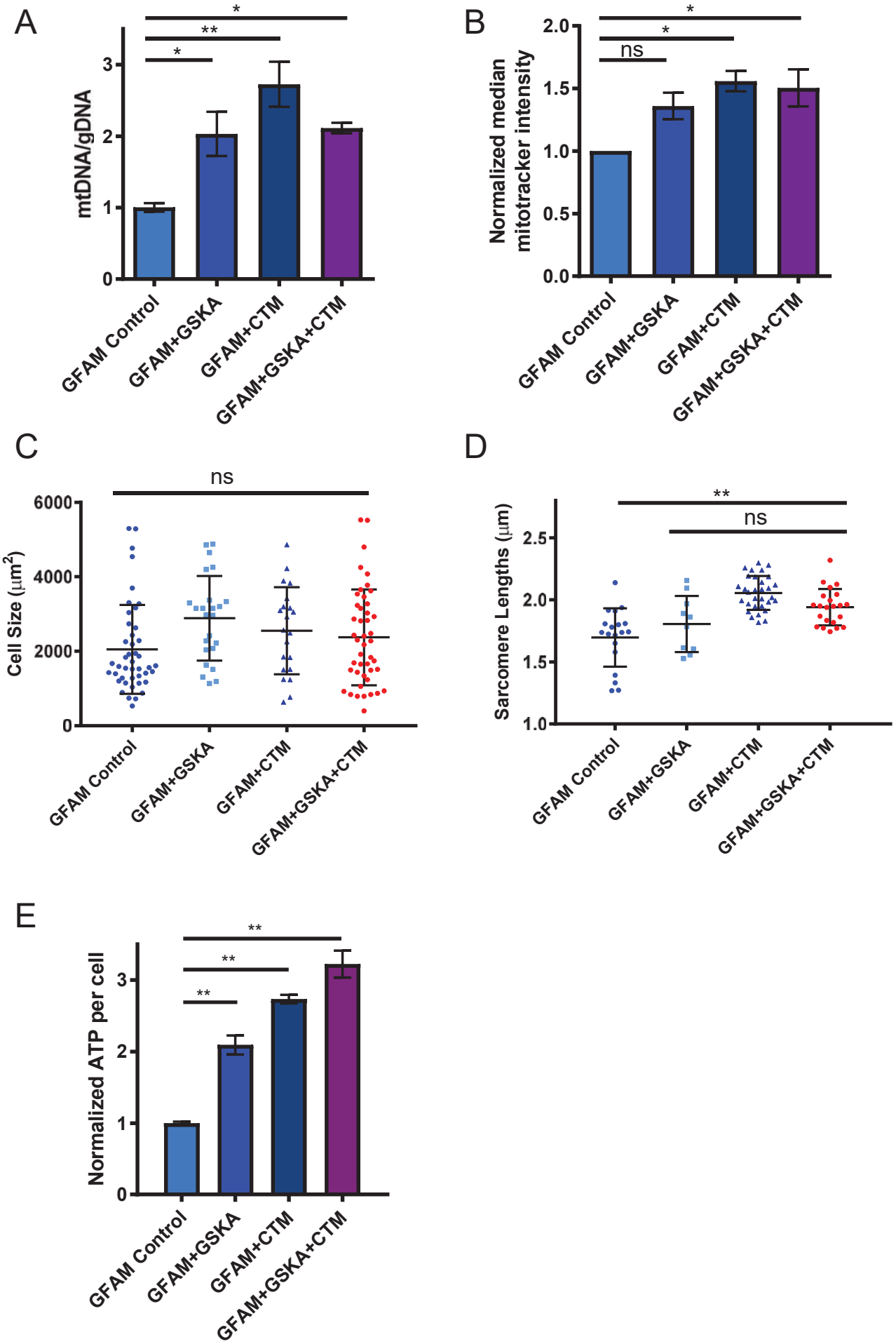
Online Figure V. A) Hexokinase II protein expressions (left panel, western blot and right panel, quantifications) in HUES9-hPSC-CMs cultured in GLM, GFAM and FAM (n=3 independent experiments)**, B) Immunofluorescence Imaging Secondary Antibodies Only Controls.**


A
GLM
GFAM
FAM
Normalized ATP per cell
1.0
0.5
0.0
**
***
***
***
ns
Control
+CTM
+GSKA
B
Force (μN)
0.4
0.3
0.2
0.1
0.0
**
*
*
*
ns
Control
+GSKA
+CTM
C
Force (μN)
0.20
0.15
0.10
0.05
0.00
*
ns
**
*
ns
Control
+GSKA
+CTM

Online Figure VI. Improved ATP and force generation of hPSC-CMs with HIF1α and LDHA inhibitions. A) ATP measurements of hiPSC-CMs cultured in GLM, GFAM and FAM treated with CTM or GSKA (n=3 independent experiments), **B)** Peak contractile force generated by HUES9-hESC-CMs cultured in GLM, GFAM and FAM treated with CTM or GSKA (n=3 independent experiments)**, C)** Peak contractile force generated by H7-hESC-CMs cultured in GLM, GFAM and FAM treated with CTM or GSKA (n=3 independent experiments)**.**

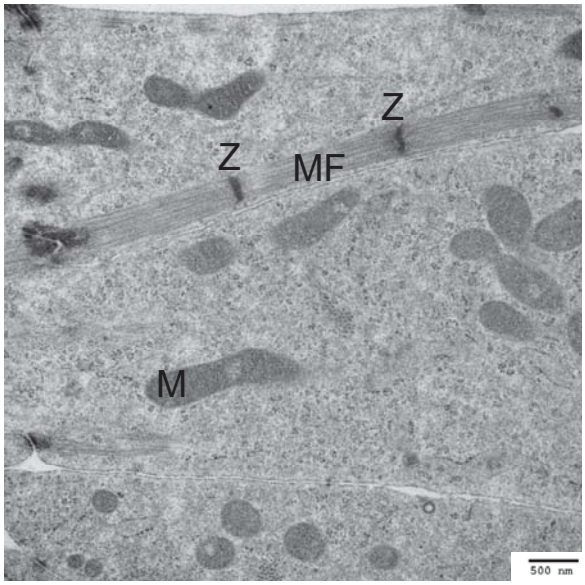

A
***
ns
Relative Hexokinase activity
4
3
2
1
0
GFAM Control
GFAM+GSKA
GFAM+CTM
GFAM+GSKA+CTM
FAM Control
B
**
ns
Normalized ATP per cell
1.0
0.5
0.0
GFAM Control
GFAM+GSKA
GFAM+CTM
GFAM+GSKA+CTM
FAM Control
C
**
ns
Sarcomere Lengths (μm)
2.5
2.0
1.5
1.0
GFAM Control
GFAM+GSKA
GFAM+CTM
GFAM+GSKA+CTM
FAM Control
D
*
ns
Cell Size (μm²)
6000
4000
2000
0
GFAM Control
GFAM+GSKA
GFAM+CTM
GFAM+GSKA+CTM
FAM Control
E
*
ns
Normalized median mitotracker intensity
1.0
0.5
0.0
GFAM Control
GFAM+GSKA
GFAM+CTM
FAM Control

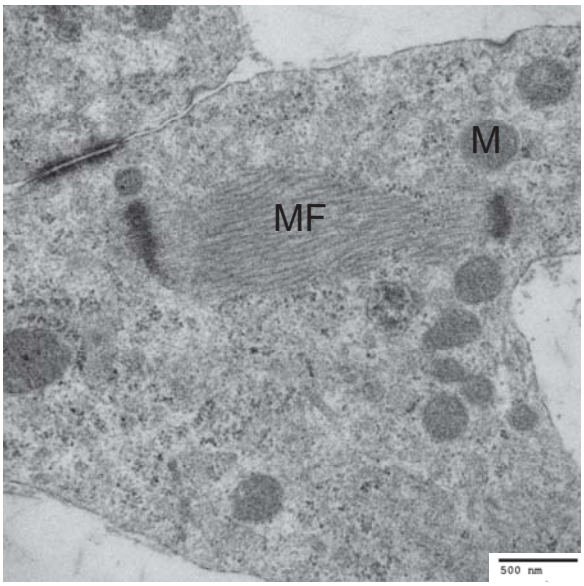
Online Figure VII. Improved maturation of H7-ESC-CMs cultured in GFAM plus HIF1α and LDHA inhibitors. A) Relative hexokinase activity, **B)** ATP per cell, **C)** Sarcomere lengths, and **D)** Cell size of H7-ESC-CMs cultured in GFAM treated with CTM, GSKA, or both and FAM (n=3 independent experiments)**, E)** Quantification of median mitotracker staining intensities of H7-CMs cultured in GFAM treated with CTM or GSKA and FAM (n=3 independent experiments)**.**


A
mtDNA/gDNA
0
1
2
3
*
**
*
GFAM Control
GFAM+GSKA
GFAM+CTM
GFAM+GSKA+CTM
B
Normalized median mitotracker intensity
0.0
0.5
1.0
1.5
2.0
*
*
ns
GFAM Control
GFAM+GSKA
GFAM+CTM
GFAM+GSKA+CTM
C
Cell Size (μm²)
0
2000
4000
6000
ns
GFAM Control
GFAM+GSKA
GFAM+CTM
GFAM+GSKA+CTM
D
Sarcomere Lengths (μm)
1.0
1.5
2.0
2.5
**
ns
GFAM Control
GFAM+GSKA
GFAM+CTM
GFAM+GSKA+CTM
E
Normalized ATP per cell
0
1
2
3
**
**
**
GFAM Control
GFAM+GSKA
GFAM+CTM
GFAM+GSKA+CTM

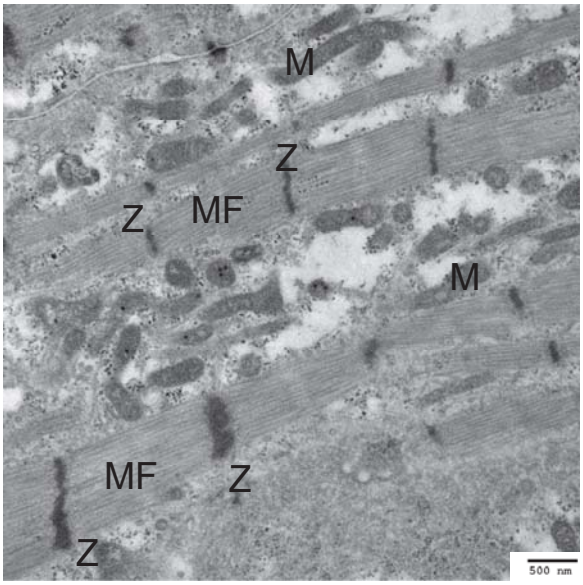
Online Figure VIII. Improved maturation of hiPSC-CMs cultured in GFAM plus HIF1α and LDHA inhibitors for 30 days. A) Ratio of mitochondria DNA to genomic DNA, **B)** Quantification of median mitotracker staining intensitie, **C)** Cell sizes, **D)** Sarcomere lengths, and **E)** ATP per cell of hiPSC-CMs cultured in GFAM treated with CTM, GSKA, or both and FAM (n=3 independent experiments).

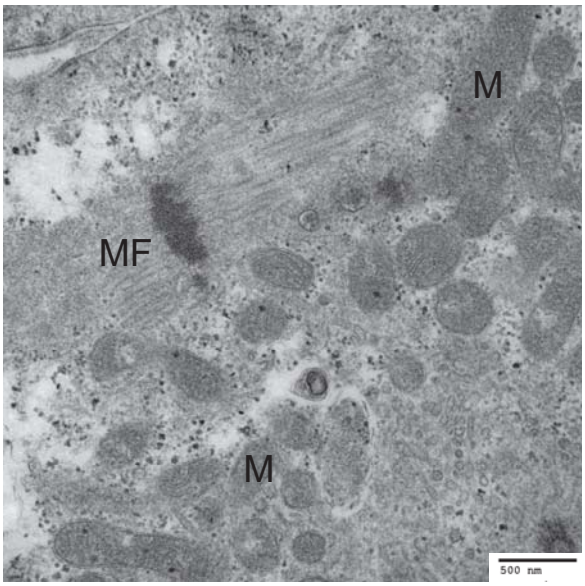
GFAM Control



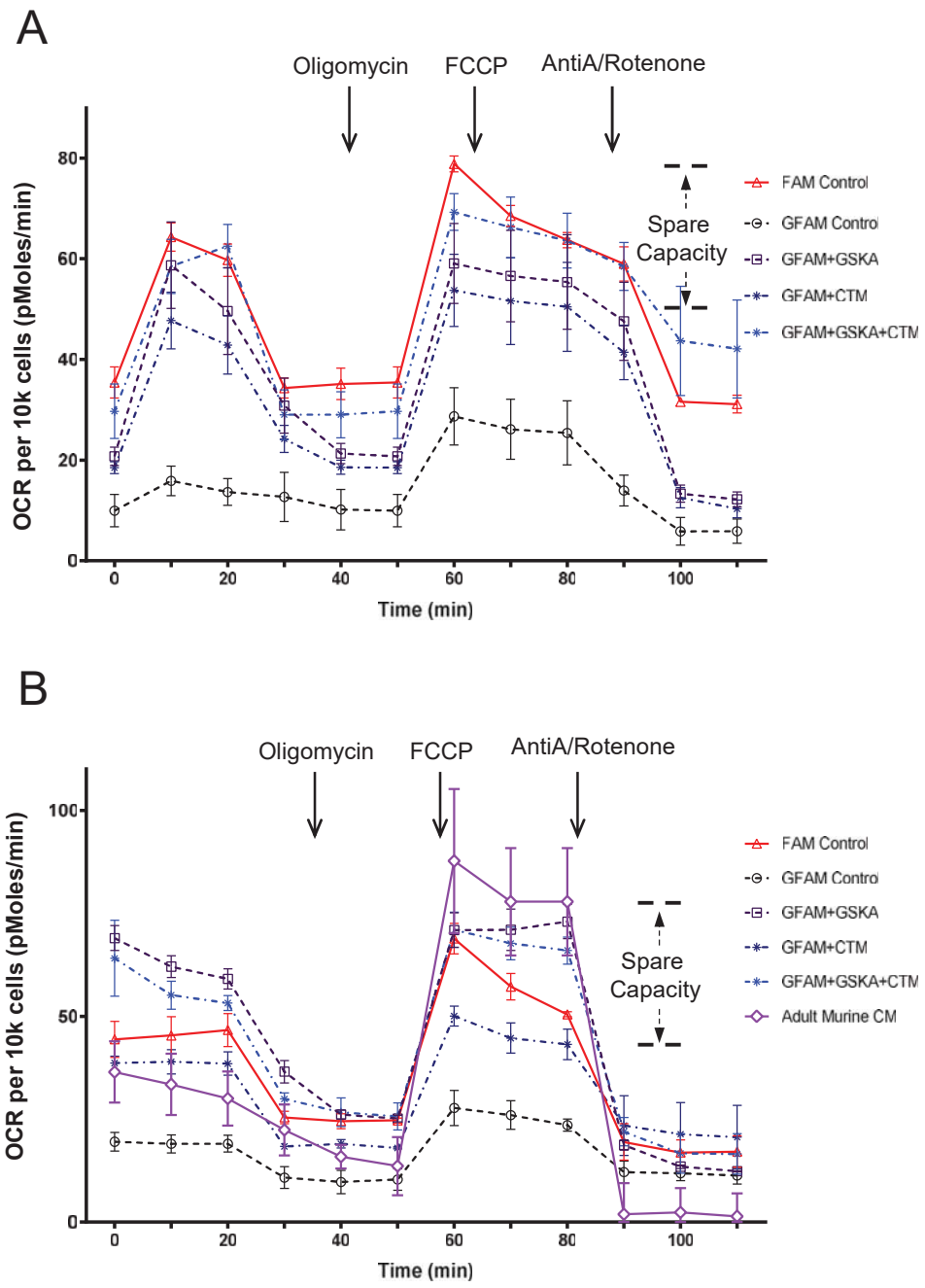


GFAM+CTM





Online Figure IX. Representative transmission electron microscopy images. hiPSC-CMs cultured in GFAM treated with with CTM and control are shown. Z = Z disk, M = Mitochondria, MF = Myofibril



Online Figure X. OCR measurements of CMs with mitochondria stress test. A) H7-CMs.**B).** iPSC-CM treated with inhibitors for 30 days and adult murine CMs. n=3 independent experiments, OCR normalized to 10k cells.