Supplementary Information

Targeting HIF-1α in Combination with PPARα Activation and Postnatal Factors Promotes the Metabolic Maturation of Human Induced Pluripotent Stem Cell-derived Cardiomyocytes

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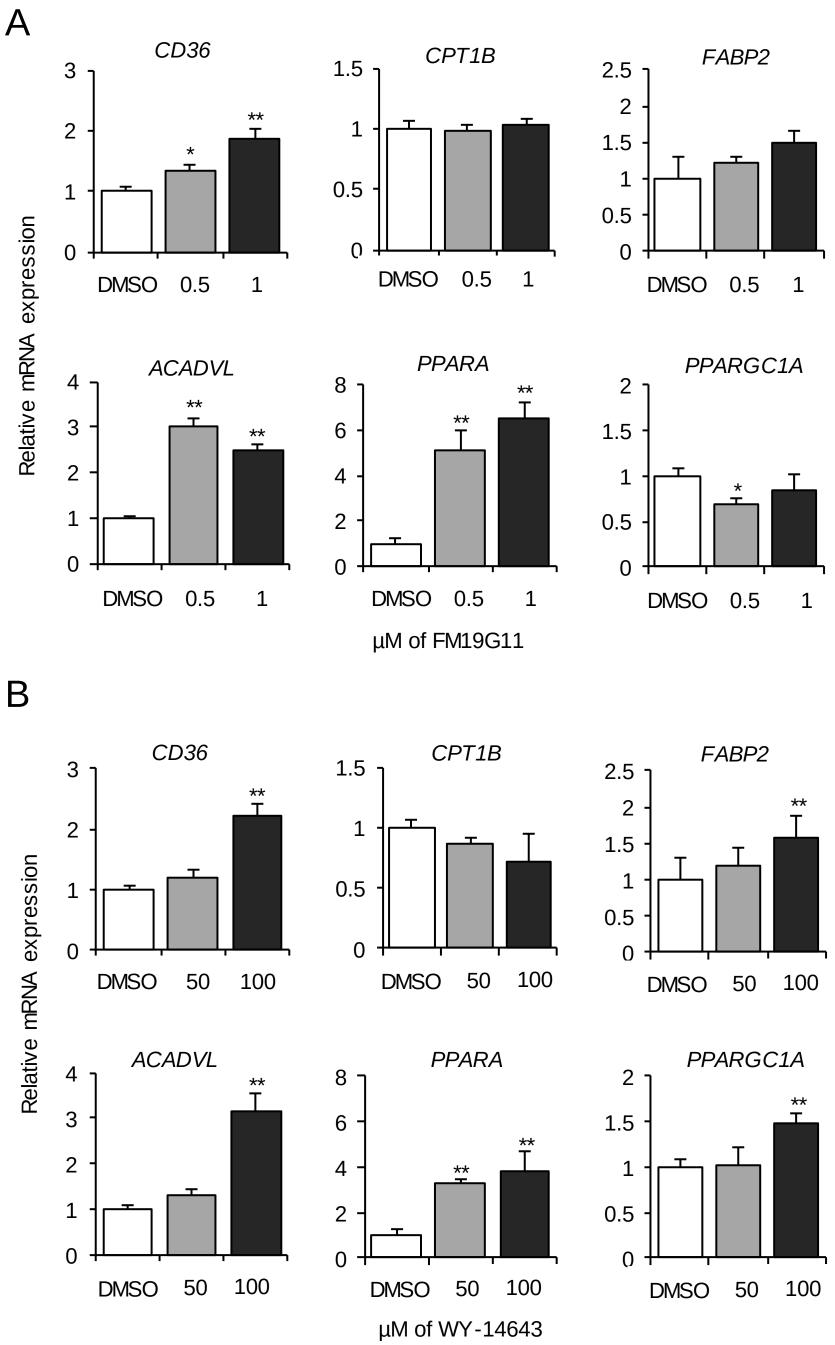
Supplemental Methods

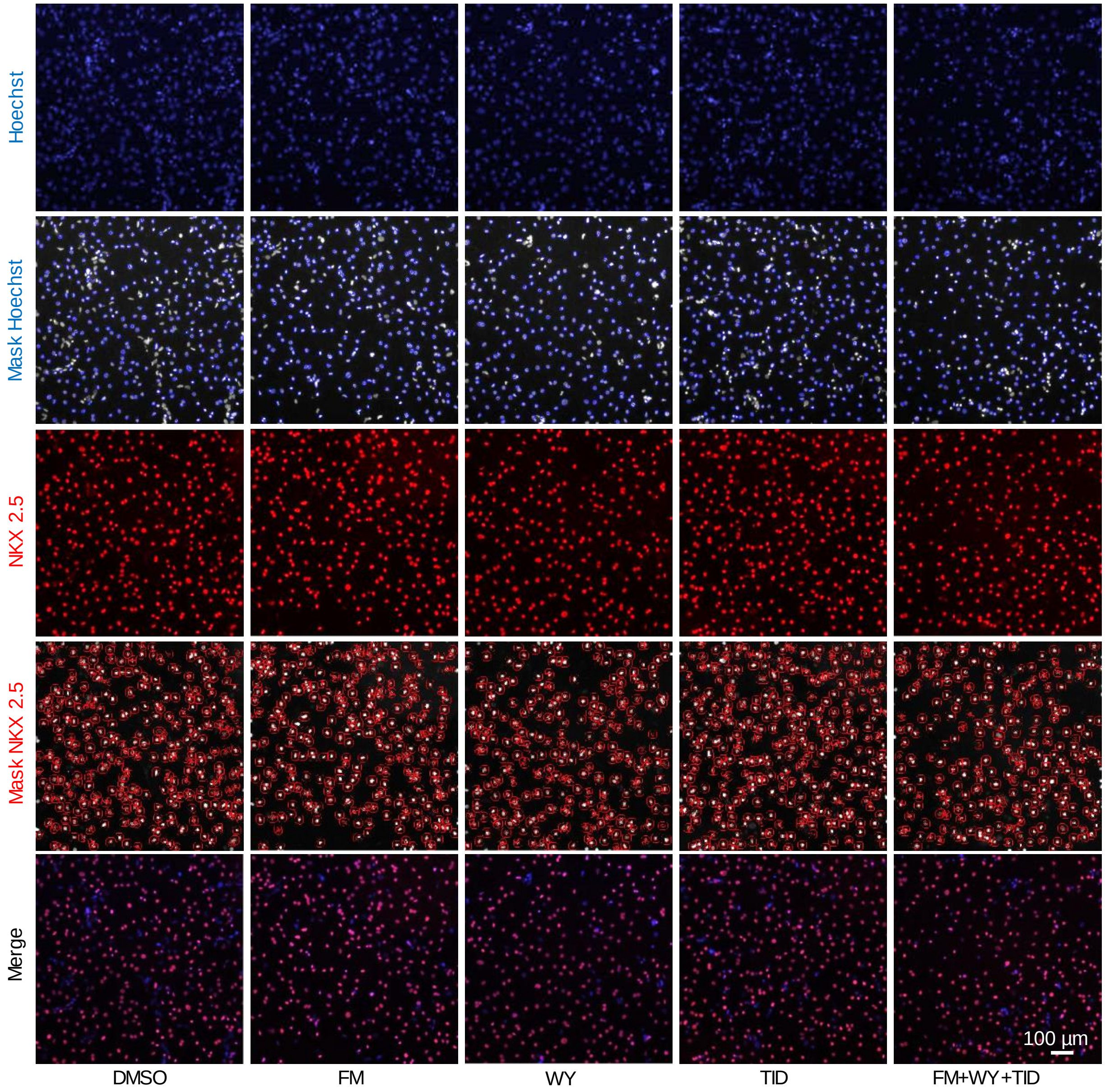
High-content imaging analysis by Arrayscan

Following the maturation treatment, cardiac spheres were dissociated using 0.25% trypsin-EDTA and plated onto a Matrigel-coated 96-well culture plate at a density of 5×10^4 cells/well for the detection of NKX2-5 and 2×10^4 cells/well for the detection of MLC2V and α-actinin. Cells were maintained in the maturation medium for 24 h to allow cells to recover spontaneous beating. The next day the medium was aspirated and cells were washed with PBS, fixed in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO) and permeabilized in ice-cold methanol. The cells were then blocked with 5% normal goat serum (NGS) in PBS at room temperature for 1 h and incubated overnight at 4ºC with the primary antibodies NKX2-5 for the purity assay or α-actinin together with MLC2V for the subtype detection assay. After the incubation with the primary antibodies, the cells were washed twice with PBS and incubated with the corresponding conjugated secondary antibodies followed by two washes with PBS. The nuclei were counterstained with Hoechst in warm buffer and imaged using an ArrayScan™ XTI Live High Content Platform (Thermo Fisher Scientific).

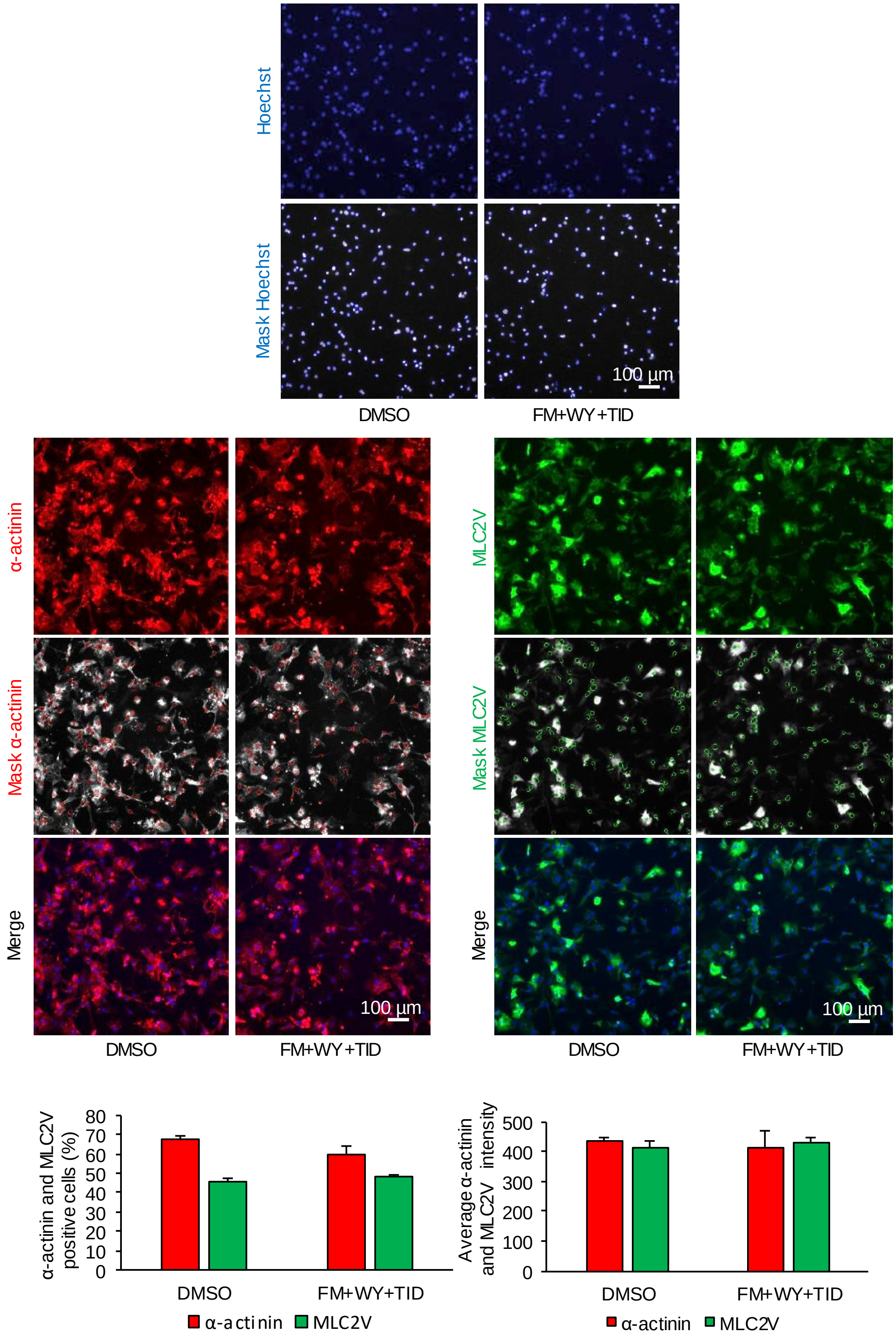
Images of immunocytochemistry were acquired and quantitatively analyzed using ArrayScan™ XTI Live High Content Platform. Twenty fields/well were imaged using a 10x objective. The Acquisition software Cellomics Scan (Thermo Fisher Scientific) was used to capture images and data analysis was performed using Cellomics View Software (Thermo Fisher Scientific). For the NKX2-5 assay, images were analyzed with a mask modifier for Hoechst and NKX2-5-positive cells restricted to the nucleus. The percentage of NKX2-5-positive cells and average of NKX2-5 intensity per well in each treatment were used as readout. In the MLC2V detection assay, images were analyzed with a mask modifier for Hoechst restricted to the nucleus. MLC2V and α-actinin were each quantified with a spot mask that extended 7 units from the nucleus. Spot threshold was set to 10 units and detection limit was set at 25 units. The percentage of MLC2V- and α-actinin-positive cells and average intensity per well were used as readout.



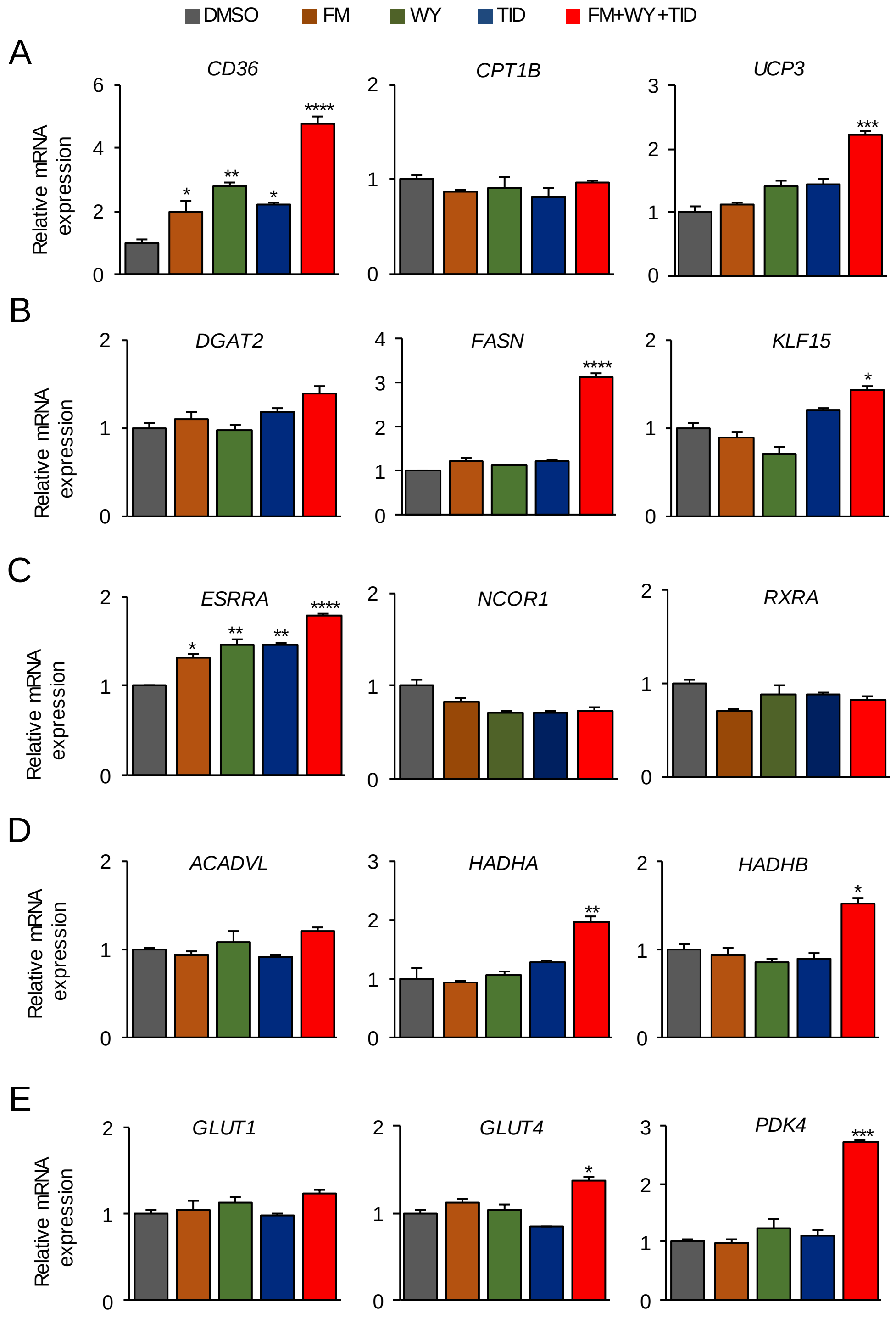
Supplementary Figure 1. Relative gene expression levels of FAO-related genes in hiPSC-CMs treated with (**A**) 0, 0.5 or 1 µM FM19G11 and (**B**) 0, 50 or 100 µM WY-14643



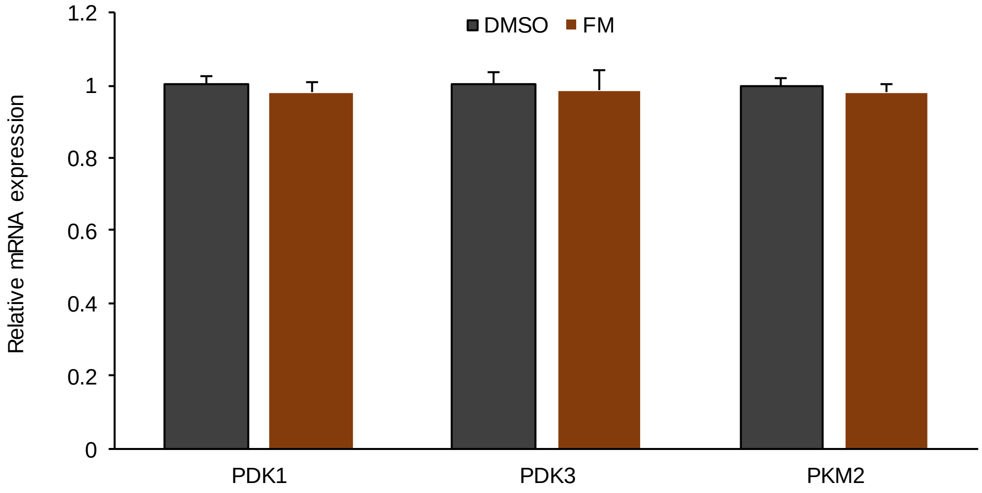
Supplementary Figure 2. Representative images for quantitative analysis of NKX2-5 by high-content imaging using ArrayScan (Related to Fig. 1C). Images were analyzed with a mask modifier for Hoechst and NKX2-5-positive cells restricted to the nucleus. FM, FM19G11; WY, WY-14643; TID, T3 + IGF-1 + dexamethasone.



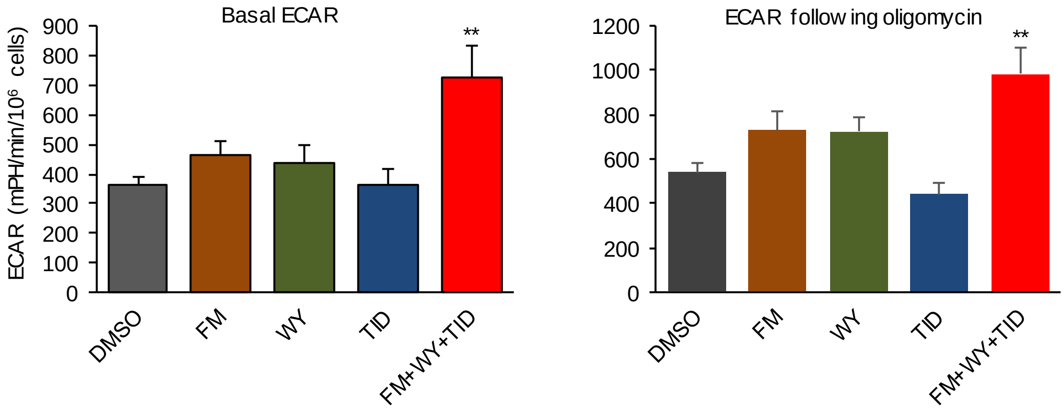
Supplementary Figure 3. Immunocytochemical analysis of MLC2V and α-actinin. Representative images and quantitative analysis of MLC2V and α-actinin by high-content imaging using ArrayScan. Images were analyzed with a mask modifier for Hoechst restricted to the nucleus. MLC2V and α-actinin were each quantified with a spot mask that extended 7 units from the nucleus. FM, FM19G11; WY, WY-14643; TID, T3 + IGF-1 + dexamethasone.



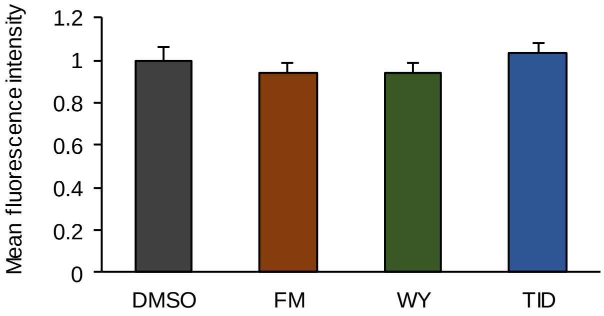
Supplementary Figure 4. Treatments of 3D cardiac spheres with a HIF-1α inhibitor, a PPARα activator and the postnatal factors (T3, IGF-1 and dexamethasone) increase the mRNA expression of metabolic genes. qRT-PCR quantification of the relative expression of genes implicated in (**A**) fatty acid transport, (**B**) lipid storage, (**C**) mitochondrial fatty acid oxidation, (**D**) transcriptional regulation, and (**E**) intracellular glucose uptake. Data are presented as mean ± SEM (n=3). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001; by one-way ANOVA. FM, FM19G11; WY, WY-14643; TID, T3 + IGF-1 + dexamethasone.



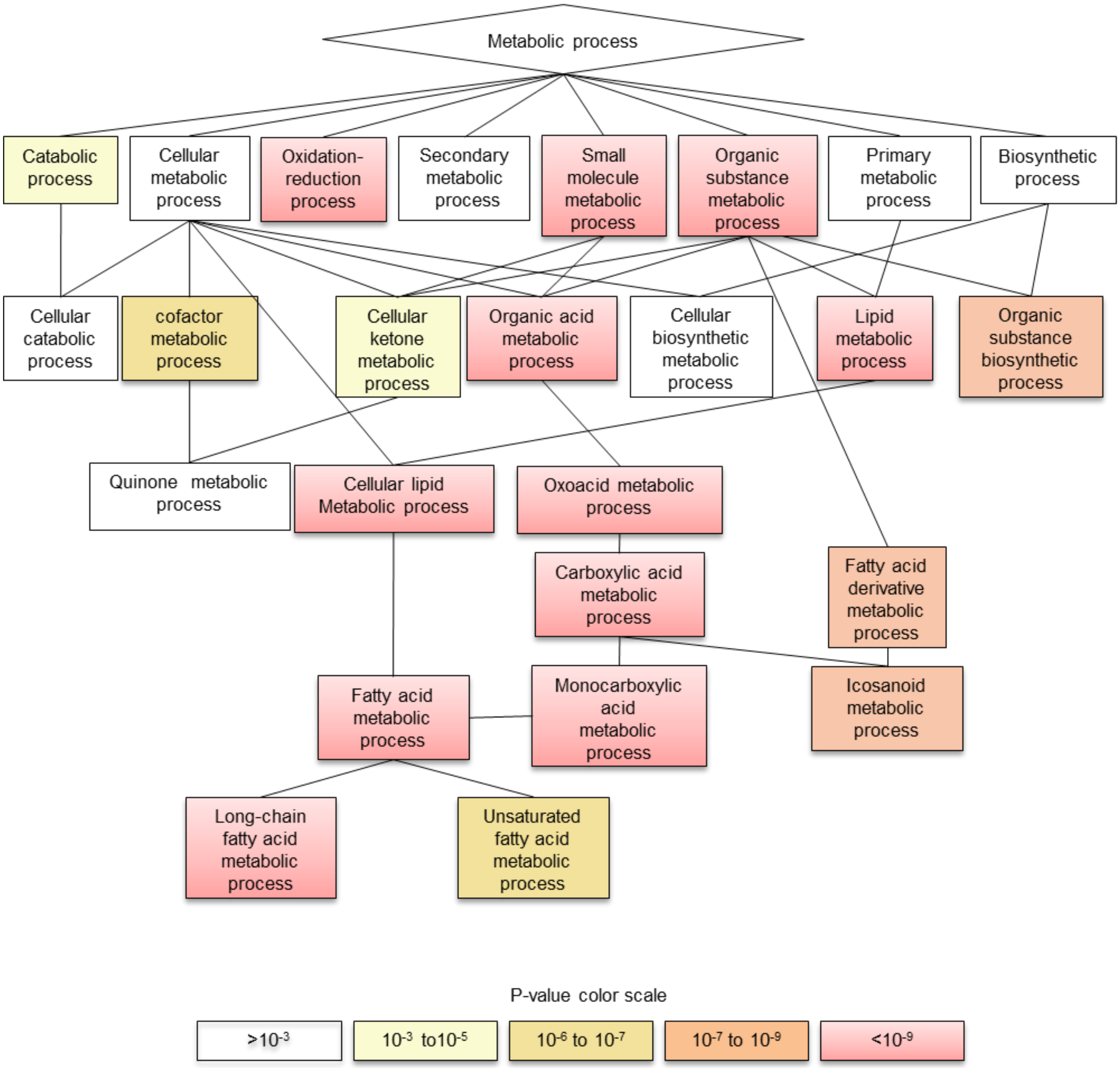
Supplementary Figure 5. qRT-PCR analysis of glucose metabolism-related genes. Data are presented as mean ± SEM (n=3). FM, FM19G11.



Supplementary Figure 6. ECAR of hiPSC-CMs treated with DMSO, FM, WY, TID or FM+WY+TID. ECAR of hiPSC-CMs at basal level and after the addition of oligomycin are presented as mean ± SEM (n=4). FM, FM19G11; WY, WY-14643; TID, T3 + IGF-1 + dexamethasone. Note that ECAR was from the measurement of mitochondrial function using Mito Stress test.



Supplementary Figure 7. Mitochondrial content of hiPSC-CMs. Cardiac spheres were treated with DMSO, FM, WY or TID for 7 days and analyzed for mitochondrial content by flow cytometry. Relative MitoTracker Red fluorescence data are presented as mean ± SEM (n=5). FM, FM19G11; WY, WY-14643; TID, T3 + IGF-1 + dexamethasone.



Supplementary Figure 8. Polyhierarchical graph shows GO terms under the metabolic process ontology. AmiGO visualization was used to determine directionality of hierarchy as shown by the arrows and colors represent p-values of GO terms that are significantly different.

Supplementary Table 1. Antibodies

Type	Antibody target	Origin type	Supplier	Catalog number	Dilution
Primary	α-actinin	Mouse IgG1	Sigma	A7811	1:500
	NKX2-5	Rabbit IgG	Cell Signaling	8792S	1:1600
	Troponin I	Mouse IgG2b	Millipore	MAB4360	1:200
	Troponin T	Mouse IgG1	Thermo Fisher	MS-295-P1	1:200
	MLC2V	Rabbit IgG	Abcam	ab79935	1:200
Secondary	Alexa 488, goat anti-mouse IgG1		Thermo Fisher	A21121	1:1000
	Alexa 594, goat anti-mouse IgG2b		Thermo Fisher	A21145	1:1000
	Alexa 594, goat anti-rabbit		Thermo Fisher	A11012	1:1000
	Alexa 594, donkey anti-mouse IgG		Thermo Fisher	A21203	1:500

Supplementary Table 2. qRT-PCR Primers

Gene	**Full name**	**Accession Code**	**Primer**
ACADVL	Acyl-CoA dehydrogenase, very long chain	NM_000018	ACAGATCAGGTGTTCCCATACC CTTGGCGGGATCGTTCACTT
ACSL5	Acyl-CoA synthetase long-chain family member 5	NM_203379	CTCAACCCGTCTTACCTCTTCT GCAGCAACTTGTTAGGTCATTG
ADRB1	Adrenergic, beta-1-, receptor	NM_000684	ATCGAGACCCTGTGTGTCATT GTAGAAGGAGACTACGGACGAG
ALDH1A1	Aldehyde dehydrogenase 1 family, member A1	NM_000689	CCGTGGCGTACTATGGATGC GCAGCAGACGATCTCTTTCGAT
COQ10A	Coenzyme Q10 homolog A	NM_144576	CTTACCTTCGAGCCGTTCCTT CCATGATTCTACGCTCCGAGTA
CPT1A	Carnitine palmitoyltransferase 1A	NM_001876	ATGCGCTACTCCCTGAAAGTG GTGGCACGACTCATCTTGC
CPT1B	Carnitine palmitoyltransferase 1B	NM_004377	GCGCCCCTTGTTGGATGAT CCACCATGACTTGAGCACCAG
CD36	Collagen type I receptor, thrombospondin receptor	NM_000072	GGCTGTGACCGGAACTGTG AGGTCTCCAACTGGCATTAGAA
DGAT2	Diacylglycerol O-acyltransferase 2	NM_198512	CCTGTAGCTGCTTTTCCACC GAGGGGTCTGGGAGATGG
ESRRA	Estrogen related receptor alpha	NM_004451	CGCTTGGTGATCTCACACTC GCTACCACTATGGTGTGGCA
FABP4	Fatty acid binding protein 4	NM_001442	ACTGGGCCAGGAATTTGACG CTCGTGGAAGTGACGCCTT
FKBP5	FK506 binding protein 5	NM_004117	CTCCCTAAAATTCCCTCGAATGC CCCTCTCCTTTCCGTTTGGTT
FASN	Fatty acid synthase	NM_004104	TCTCCGACTCTGGCAGCTT GCTCCAGCCTCGCTCTC
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_001256799	CTGGGCTACACTGAGCACC AAGTGGTCGTTGAGGGCAATG
GLUT1	Solute carrier family 2 (Facilitated glucose transporter), member 1	NM_006516	ACTCCTCGATCACCTTCTGG ATGGAGCCCAGCAGCAA
GLUT4	Solute carrier family 2 (Facilitated glucose transporter), member 4	NM_001042	AGCACCGCAGAGAACACAG GTCGGGCTTCCAACAGATAG
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	NM_001244949	GATGTAAGCACACAAGTGAGGA TCCGACTCATTAGGCTTTCTTTC
HADHA	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA	NM_000182	CCGTTCCTCTGGAGGTTTTA TGGTAGAAGCATTCGTGCAG

	thiolase/enoyl-CoA hydratase (Trifunctional protein), alpha subunit		
HADHB	Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (Trifunctional protein), beta subunit	NM_000183	GAAAATCTGAGGGCCCATT CTTGCTCCGAGAGGGAGTC
KLF15	Kruppel like factor 15	NM_014079	TCAGAGCGCGAGAACCTC TGTACACCAAAAGCAGCCAC
MAOA	Monoamine oxidase A	NM_000240	GAATCAAGAGAAGGCGAGTATCG GGCAGCAGATAGTCCTGAAATG
MPC2	Mitochondrial pyruvate carrier 2	NM_016098	TTATCAGTGGGCGGATGACAT GCTGTACCTTGTAGGCAAATCTC
NCOR1	Nuclear receptor corepressor 1	NM_006311	CCAGACTGTGAGATCAATGCC CGTCCCACTCCTCAGGC
PCK1	Phosphoenolpyruvate carboxykinase 1	NM_002591	TTGAGAAAGCGTTCAATGCCA CACGTAGGGTGAATCCGTCAG
PDK4	Pyruvate dehydrogenase kinase 4	NM_002612	TGTGAATTGGTTGGTCTGGA TGTGGTAGCAGTGGTCCAAG
PGC1A	Peroxisome proliferator-activated receptor gamma coactivator 1 alpha	NM_013261	GACCAGATGGTGCTGGTTG TGAGTGGAGTGATGGAGCAG
PPARA	Peroxisome proliferator activated receptor alpha	NM_005036	CTGCTAGCAAGTTTGCCTCA AGTGGTGCAGTGACCAATCA
PPARGC1A	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	NM_013261	GACCAGATGGTGCTGGTTG TGAGTGGAGTGATGGAGCAG
RXRA	Retinoid X receptor, alpha	NM_002957	TGTCAATCAGGCAGTCCTTG GAGTGTACAGCTGCGAGGG
UCP3	Uncoupling protein 3	NM_003356	AACGCAAAAAGGAGGGTGTA CTCCAGGCCAGTACTTCAGC
PDK1	Pyruvate dehydrogenase kinase 1	NM_002610	ACTTCGGATCAGTGAATGCTTG ACTCTTGCCGCAGAAACATAAA
PDK3	Pyruvate dehydrogenase kinase 3	NM_005391	CGCTCTCCATCAAACAATTCCT CCACTGAAGGGCGGTTAAGTA
PKM2	Pyruvate kinase M1/2	NM_002654	AAGGACCTGAGATCCGAACTG GCGTTATCCAGCGTGATTTTGA

Supplementary Table 3. Expression of ion channels, solute carriers, calcium transients and contractile/structure-related genes in hiPSC-CMs upon treatment with FM+WY+TID compared with DMSO-treated cells

Symbol	**Gene name**	**Log$_2$ fold change**	**Adjusted p-value**
KCNE3	Potassium voltage-gated channel subfamily E, regulatory subunit 3	0.799	4.48E-02
KCNIP1	Potassium voltage-gated channel interacting protein 1	0.864	3.95E-03
KCNJ16	Potassium voltage-gated channel subfamily J, member 16	1.014	4.09E-05
KCNK5	Potassium two pore domain channel subfamily K, member 5	1.202	1.77E-04
SCNN1A	Sodium channel epithelial 1, alpha subunit	4.887	9.70E-114
SCNN1G	Sodium channel epithelial 1, gamma subunit	1.064	2.37E-04
SLC25A10	Solute carrier family 25, member 10	0.595	2.34E-02
SLC25A5	Solute carrier family 25, member 5	0.54	3.05E-02
SCN5A	sodium voltage-gated channel alpha subunit 5	-0.025	9.76E-01
ATP1A1	ATPase Na+/K+ transporting subunit alpha 1	0.882	1.46E-04
ATP1B1	ATPase Na+/K+ transporting subunit beta 1	0.273	5.34E-01
ATP2A2	ATPase sarcoplasmic/endoplasmic reticulum Ca2+ transporting 2	0.523	1.20E-01
CACNA1C	calcium voltage-gated channel subunit alpha1 C	-0.336	4.12E-06
CACNA1D	calcium voltage-gated channel subunit alpha1 D	0.389	1.72E-01
CALM1	calmodulin 1	-0.142	7.59E-01
FKBP12.6	FKBP prolyl isomerase 1B	0.118	8.18E-01
PLN	phospholamban	-0.455	3.51E-01
RYR2	ryanodine receptor 2	-0.086	9.23E-01
SLC8A1	solute carrier family 8 member A1	-0.091	9.16E-01
ACTC1	actin, alpha, cardiac muscle 1	-0.415	3.60E-01
CDH2	cadherin 2	-0.183	7.31E-01
DES	desmin	-0.689	1.06E-01
GJA1	gap junction protein alpha 1	-0.848	5.19E-02
HCN4	hyperpolarization activated cyclic nucleotide gated potassium channel 4	-0.034	9.61E-01
JPH2	junctophilin 2	-0.18	6.68E-01
MYBPC3	myosin binding protein C, cardiac	-0.224	6.70E-01
MYH6	myosin heavy chain 6	0.577	9.65E-02
MYH7	myosin heavy chain 7	-4.34	3.19E-42
TNNC1	troponin C1, slow skeletal and cardiac type	-0.549	1.91E-01
TNNT2	troponin T2, cardiac type	-0.207	7.34E-01
TTN	titin	-0.31	5.58E-01

Supplementary Table 4. Shared upregulated genes between FM+WY+TID-treated hiPSC-CMs and LV compared to DMSO-treated hiPSC-CMS.

Symbol	**Gene name**	**Log$_2$ ratio FM+WY+TID-treated hiPSC-CMs vs. DMSO-treated hiPSC-CMs**	**Log$_2$ ratio LV vs. DMSO-treated hiPSC-CMs**
ABCG2	ATP binding cassette subfamily G member 2	4.669	2.693
ACSL5	Acyl-CoA synthetase long chain family member 5	2.608	2.18
ADCK3	Aarf domain containing kinase 3	0.871	4.391
ADRB1	Adrenoceptor beta 1	1.119	4.224
AIFM2	Apoptosis inducing factor, mitochondria associated 2	1.096	1.778
AKR1C2	Aldo-keto reductase family 1 member C2	5.816	2.885
ALDH1L1	Aldehyde dehydrogenase 1 family member L1	1.679	6.404
ART3	ADP-ribosyltransferase 3	1.337	4.572
C15orf59	Chromosome 15 open reading frame 59	1.177	3.595
C1orf115	Chromosome 1 open reading frame 115	1.119	2.516
CA4	Carbonic anhydrase 4	3.547	7.937
CADM2	Cell adhesion molecule 2	2.651	3.357
COQ10A	Coenzyme Q10A	1.09	4.1
CRYM	Crystallin mu	1.548	8.523
CTSD	Cathepsin D	0.851	2.782
DUSP13	Dual specificity phosphatase 13	1.791	3.653
EPAS1	Endothelial PAS domain protein 1	1.259	3.737
EPB41L4B	Erythrocyte membrane protein band 4.1 like 4B	1.09	2.732
ETNPPL	Ethanolamine-phosphate phospho-lyase	1.722	3.645
FABP4	Fatty acid binding protein 4	3.459	12.978
FAM155B	Family with sequence similarity 155 member B	1.21	4.562
FAM20A	FAM20A, golgi associated secretory pathway pseudokinase	1.196	2.115
FKBP5	FK506 binding protein 5	1.348	2.262
GALT	Galactose-1-phosphate uridylyltransferase	0.77	1.753
GIPC2	GIPC PDZ domain containing family member 2	1.636	2.623
GPAT3	Glycerol-3-phosphate acyltransferase 3	1.276	2.875
GPNMB	Glycoprotein nmb	1.391	7.626
GPX3	Glutathione peroxidase 3	1.661	5.108
GRB14	Growth factor receptor bound protein 14	1.806	3.153
HLF	HLF, PAR bZIP transcription factor	1.616	6.399
IL15RA	Interleukin 15 receptor subunit alpha	1.467	2.762

IMPA2	Inositol monophosphatase 2	1.267	1.858
IP6K3	Inositol hexakisphosphate kinase 3	2.152	7.356
KLF15	Kruppel like factor 15	1.329	5.537
KLF9	Kruppel like factor 9	1.591	2.935
LOC730102	Quinone oxidoreductase-like protein 2	1.256	4.471
MAOA	Monoamine oxidase A	2.246	5.302
METTL7A	Methyltransferase like 7A	1.201	1.991
MFSD7	Major facilitator superfamily domain containing 7	1.279	3.524
MGAT4A	Mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme A	1.226	1.599
MGLL	Monoglyceride lipase	1.613	4.903
MPC2	Mitochondrial pyruvate carrier 2	0.705	1.999
MRO	Maestro	3.161	5.45
MX1	MX dynamin like GTPase 1	1.283	4.159
NEAT1	Nuclear paraspeckle assembly transcript 1 (non-protein coding)	1.514	2.826
NPR1	Natriuretic peptide receptor 1	1.186	2.768
NR3C2	Nuclear receptor subfamily 3 group C member 2	1.835	4.822
OAS1	2'-5'-oligoadenylate synthetase 1	1.694	2.266
PDK4	Pyruvate dehydrogenase kinase 4	2.033	8.539
PHYH	Phytanoyl-CoA 2-hydroxylase	0.801	3.557
PLA2G16	Phospholipase A2 group XVI	1.282	3.624
PLA2G5	Phospholipase A2 group V	1.622	5.455
PSMB9	Proteasome subunit beta 9	1.447	3.116
PTGER4	Prostaglandin E receptor 4	1.937	2.583
RASSF4	Ras association domain family member 4	0.91	2.012
RGN	Regucalcin	1.187	3.339
RIPK3	Receptor interacting serine/threonine kinase 3	1.324	2.152
S100A9	S100 calcium binding protein A9	3.095	6.292
SERPINB9P1	Serpin family B member 9 pseudogene 1	1.891	3.878
SLC2A12	Solute carrier family 2 member 12	0.806	1.553
SLC2A4	Solute carrier family 2 member 4	0.763	5.144
SQRDL	Sulfide quinone oxidoreductase	0.917	2.036
SULT1A1	Sulfotransferase family 1A member 1	1.131	2.03
THRB	Thyroid hormone receptor beta	1.281	1.574
TINAGL1	Tubulointerstitial nephritis antigen like 1	0.953	4.007
TM7SF2	Transmembrane 7 superfamily member 2	0.887	2.667
TMEM139	Transmembrane protein 139	1.223	2.551
TNFSF10	TNF superfamily member 10	1.427	2.603
VWC2	Von Willebrand factor C domain containing 2	1.708	3.871
WDR62	WD repeat domain 62	0.768	1.38
ZBTB16	Zinc finger and BTB domain containing 16	1.934	3.952
ZNF563	Zinc finger protein 563	1.424	1.959