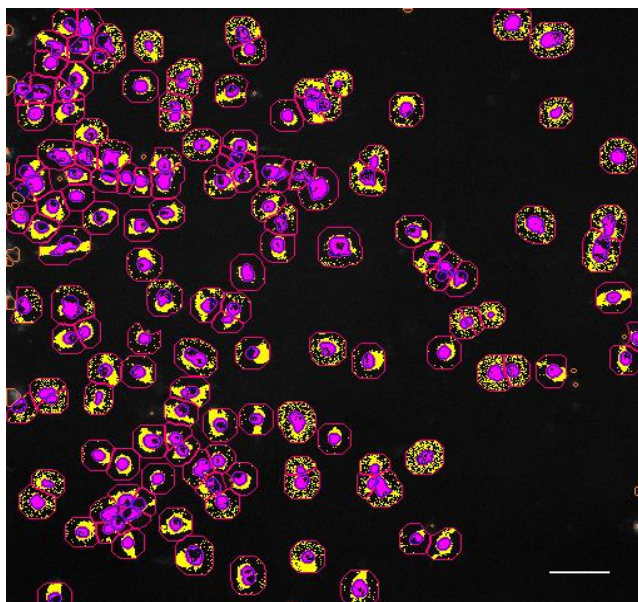| Channel 1, blue (nuclei)	Channel 2, red (TMRM)	Composite

Masking — The software identifies
- o Nuclear regions (purple; selected objects)
- o Cytoplasmic region (red ring; purple nuclear areas excluded)

High-content imaging outcomes — The software calculates
- o Mean fluorescence intensity of TMRM within the cytoplasmic regions

Note: TMRM signals are indicated as yellow

Supplemental Figure 1. Quantitative analysis of high-content imaging of TMRM (Related to Figure 5A-B)**.** Representative images of two channels for nuclei (blue) and cytoplasmic TMRM signals (red) using high-content ArrayScan imaging. The images were analyzed with a mask modifier for Hoechst-stained nuclei and TMRM signals restricted to cytoplasmic area. Scale bar 100 µm.

Supplemental Table 1. Maturation medium

Component	Vendor	Cat#	Stock concentration	Final concentration	Volume for 100 mL
DMEM	Gibco	11995-065	100%	87%	87 ml
Heat-inactivated fetal bovine serum	VWR	SH30396.03HI	100%	10%	10 ml
Glutamax-I	Fisher Scientific	35050079	100 x	1%	1 ml
Non-essential amino acids	Fisher Scientific	11140050	100 x	1%	1 ml
Oleic acid	Sigma Aldrich	O3008-5 ML	400 mM	100 µM	25 µl
Palmitic acid	Sigma Aldrich	P5585-25 G	500 mM	50 µM	10 µl
Penicillin-Streptomycin	Gibco	15140-122	100 x	1%	1 ml

Supplemental Table 2. Primers for qPCR of mtDNA/nDNA ratio

Genes	Description	Sequence	References
DHA	succinate dehydrogenase subunit A	Forward: *TCTCCAGTGGCCAACAGTGTT* Reverse: *GCCCTCTTGTTCCCAT CAAC*	[1]
LPL	lipoprotein lipase	Forward: *CGAGTCGTCTTTCTCCTGAT* Reverse: *TTCTGGATTCCAATGCTTCGA*	[2]
ND	NADH dehydrogenase subunit	F Forward: *CCCTAAAACCCGCCACATCT* Reverse: *GCGATGGTGAGAGCTAAGGT*	[1]
mtCO2	mitochondrial cytochrome oxidase II	Forward: *CGATCCCTCCCTTAC* Reverse: *GAGAGGGGAGAGCAAT*	[3]

References

1   Chong, J. J. *et al.* Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature* **510**, 273-277, doi:10.1038/nature13233 (2014).
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3   Cinsley Gentillon 1, D. L., Meixue Duan 2, Wen-Mei Yu et AL. . Targeting HIF-1α in combination with PPARα activation and postnatal factors promotes the metabolic maturation of human induced pluripotent stem cell-derived cardiomyocytes. *J Mol Cell Cardio*, J Mol Cell Cardio (2019).