Supporting Information

Mitophagy Mediates Metabolic Reprogramming of Induced Pluripotent Stem Cells Undergoing Endothelial Differentiation

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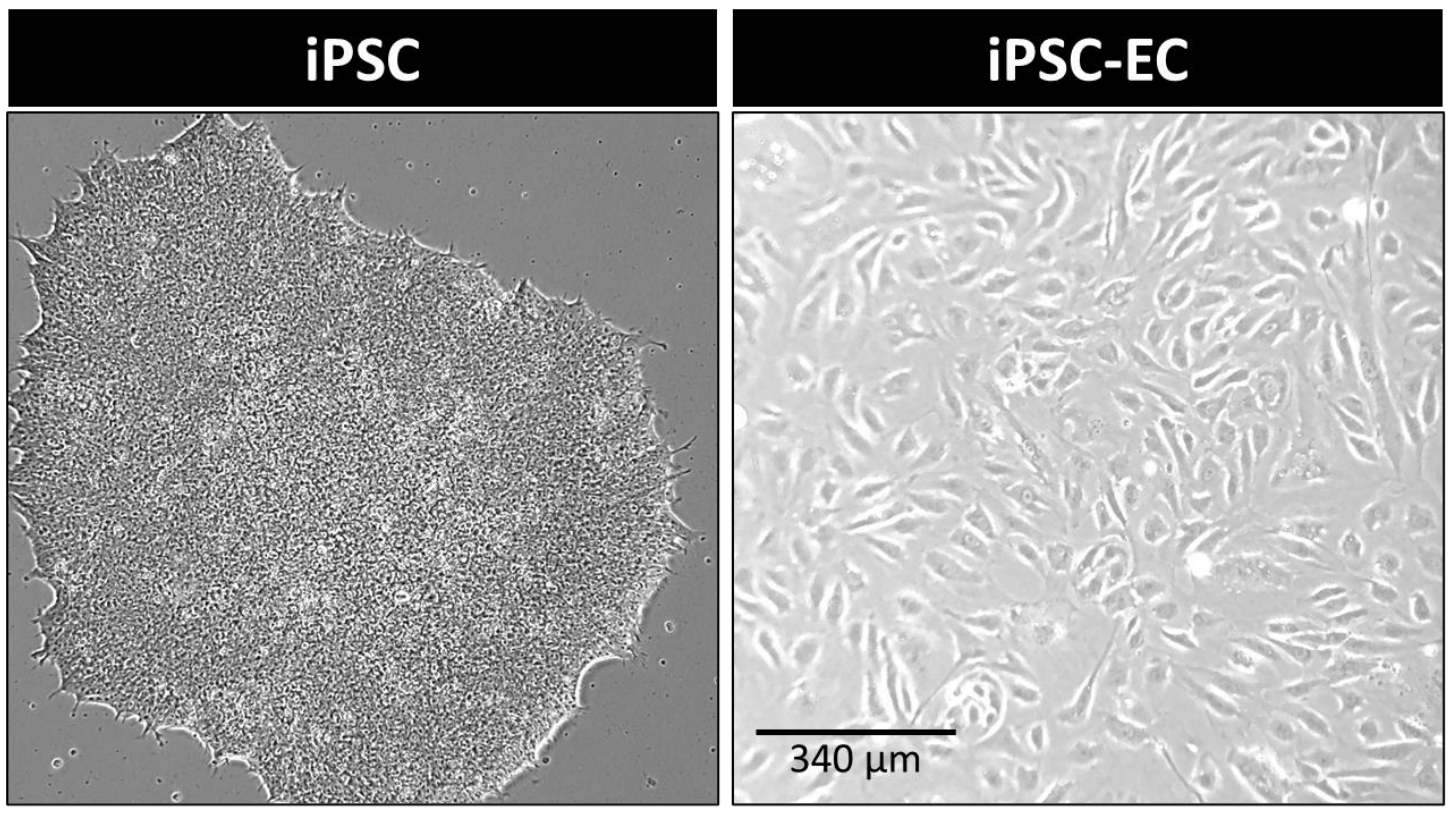
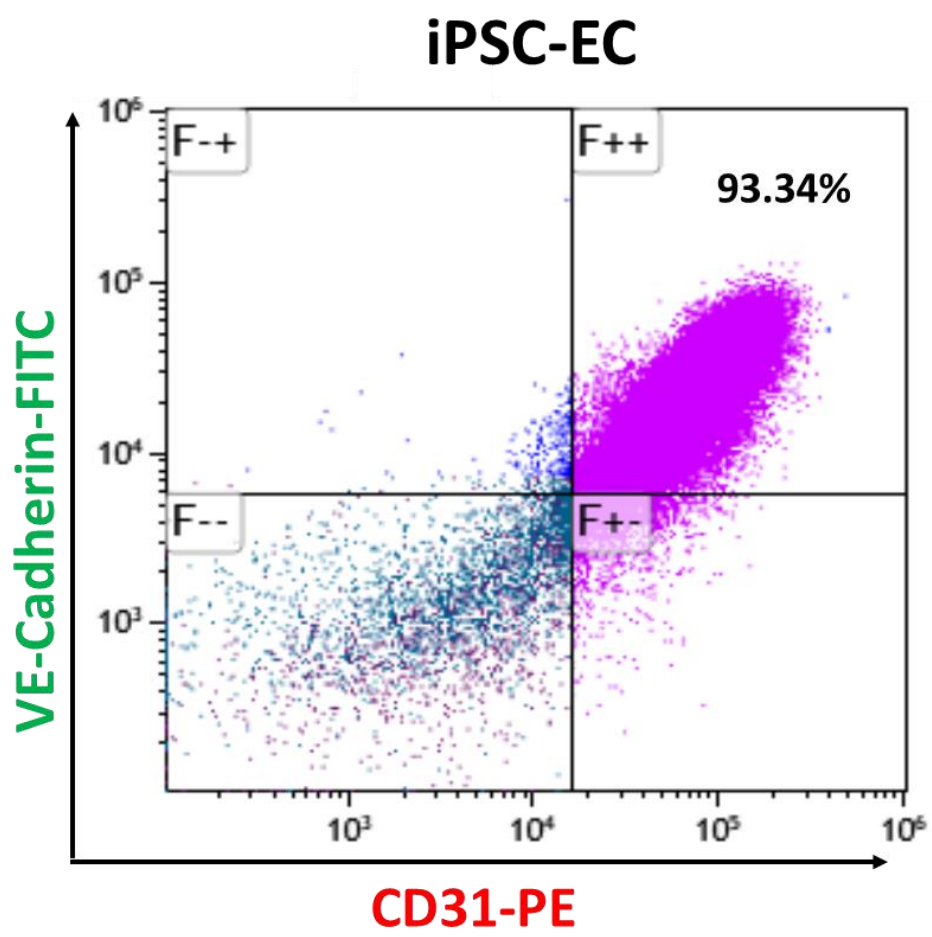
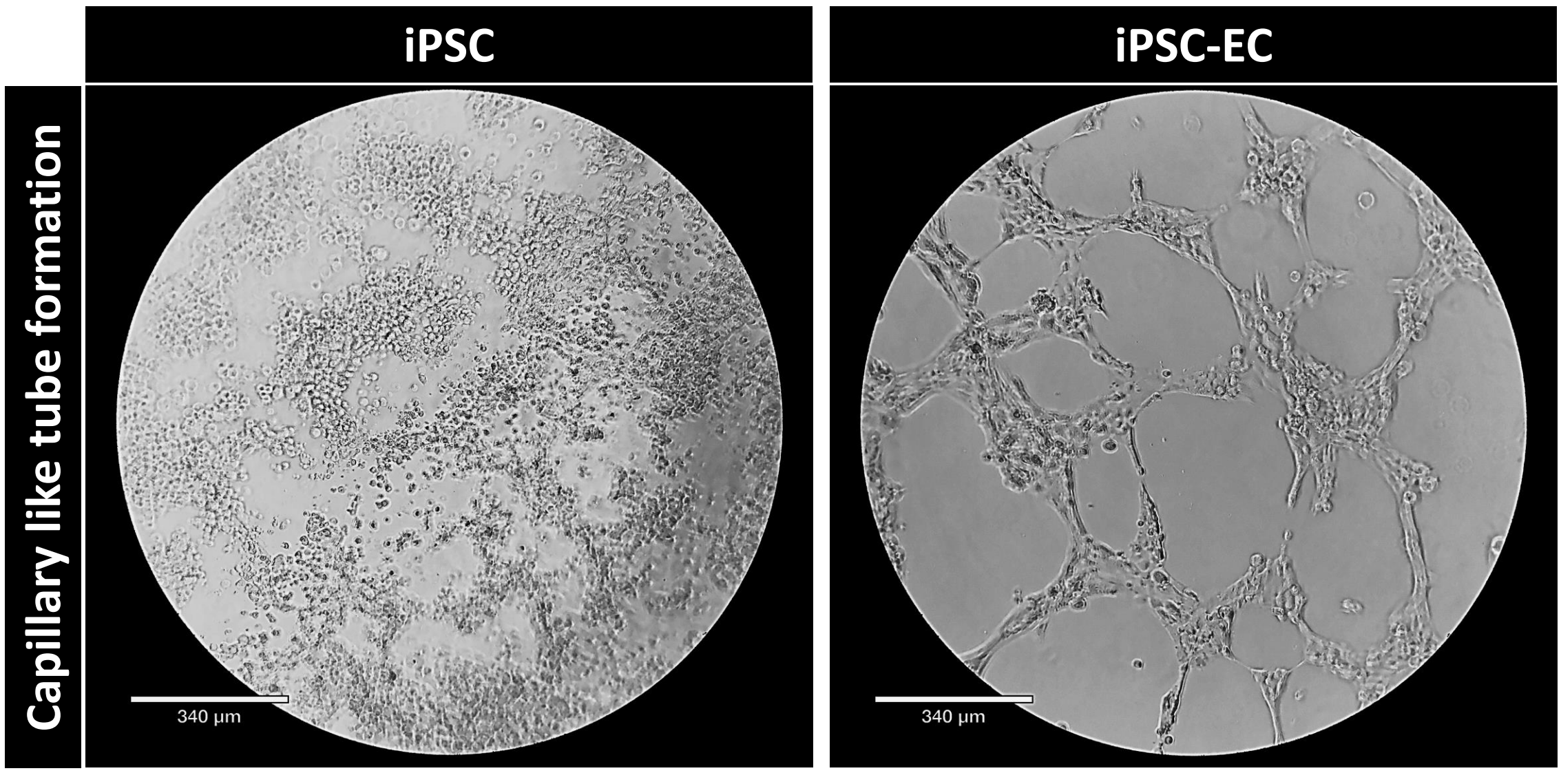
A


iPSC
iPSC-EC
340 µm

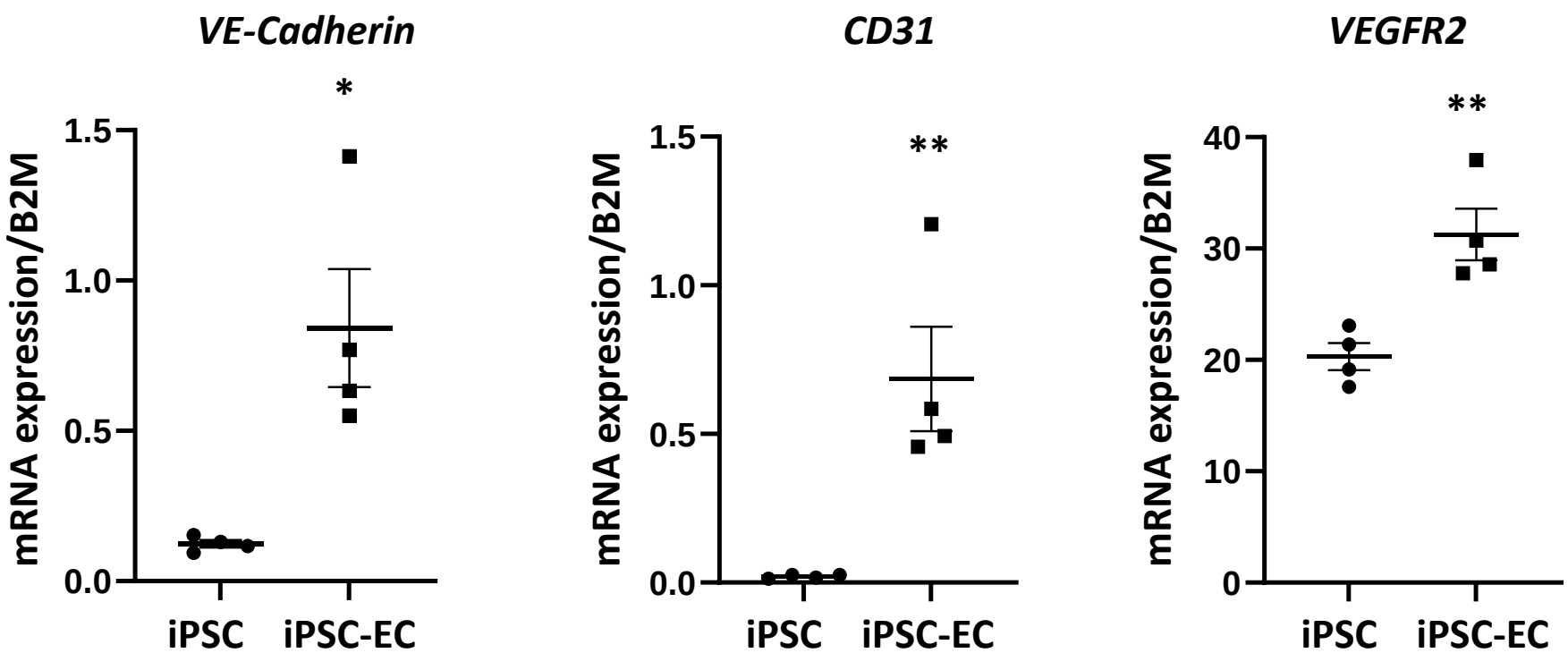
B


iPSC-EC
F-+
F++
93.34%
F--
F+-
VE-Cadherin-FITC
CD31-PE
10⁶
10⁵
10⁴
10³

C

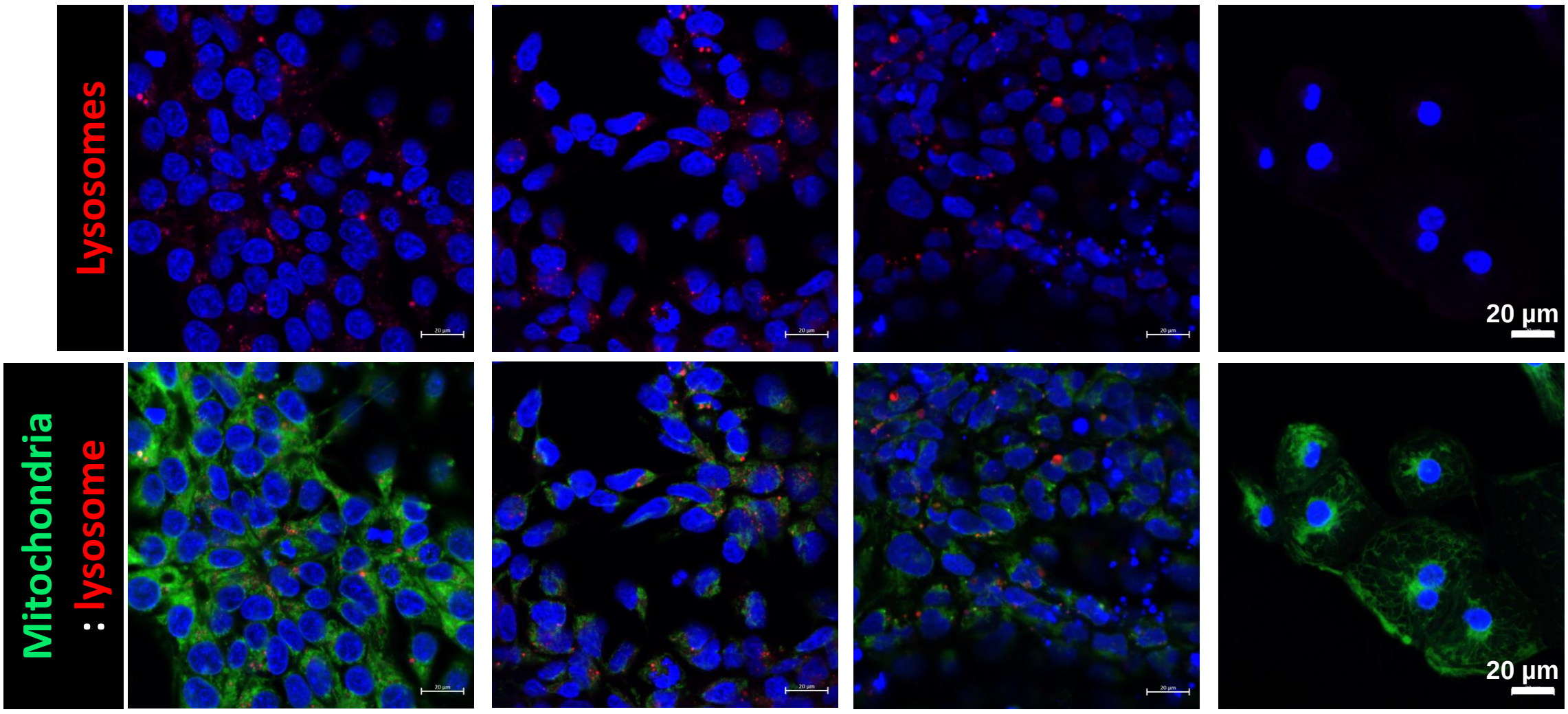

iPSC
iPSC-EC
Capillary like tube formation
340 µm
340 µm

D

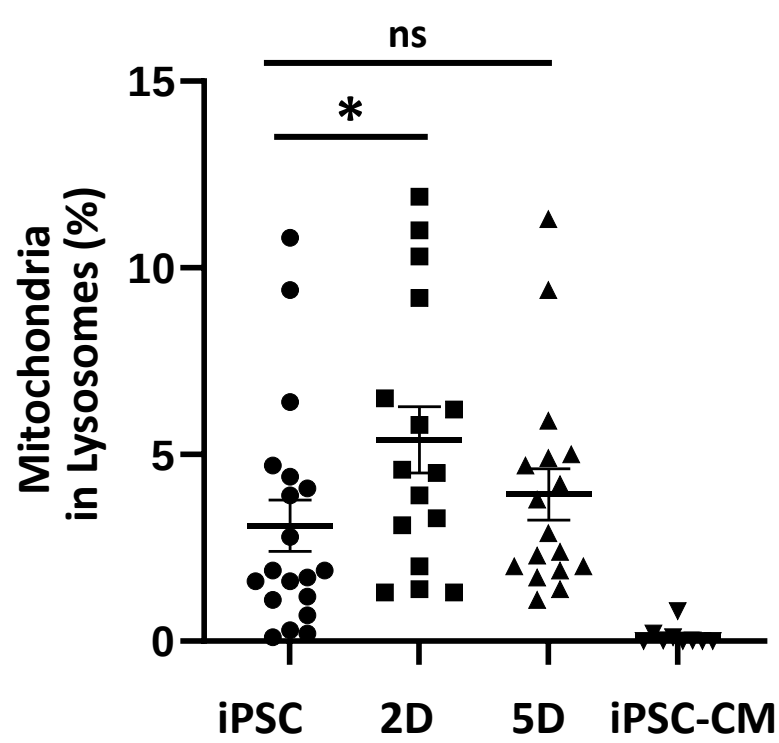

VE-Cadherin
CD31
VEGFR2
mRNA expression/B2M
*
**
**
1.5
1.0
0.5
0.0
40
30
20
10
0
iPSC
iPSC-EC

Supplementary Figure 1. iPSC-ECs exhibit endothelial characteristics. (A) Brightfield images of the cobblestone morphology of iPSC-ECs on 0.2% gelatin. Scale bar = 340 µm. **(B)** Flow cytometry of iPSC-ECs at passage 3 which shows that 93% of iPSC-ECs remain double positive for the endothelial markers CD31 and VE-Cadherin after passaging (passage 3). **(C)** Brightfield images of capillary-like tube formation of iPSCs and iPSC-ECs. Cells were plated on growth factor reduced Matrigel and imaged to visualize tubes after 4 hours. iPSC-ECs show the formation of capillary-like structures indicative of endothelial cells. Scale bar = 340 µm. **(D)** mRNA levels of *VE-Cadherin, CD31, and VEGFR2* were evaluated in iPSCs and VE-Cadherin sorted cells (iPSC-ECs) by RT-qPCR. B2M (β2-Microglobulin) was used as housekeeping gene. iPSC-ECs show an increase in endothelial markers. Mean ± SEM, n=4 with *p<0.05 and **p<0.01.

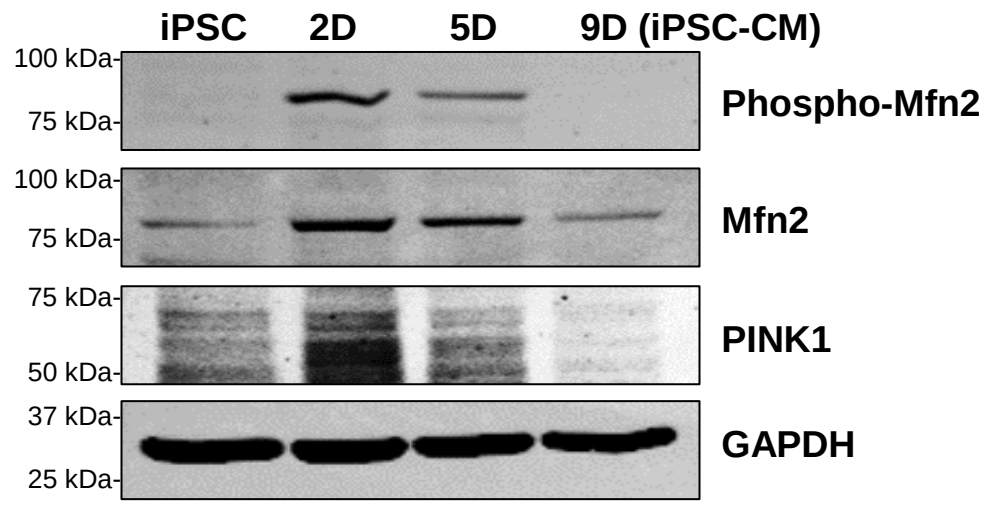
A


iPSC
2D
5D
iPSC-CM
Lysosomes
Mitochondria
: lysosome
20 μm
20 μm

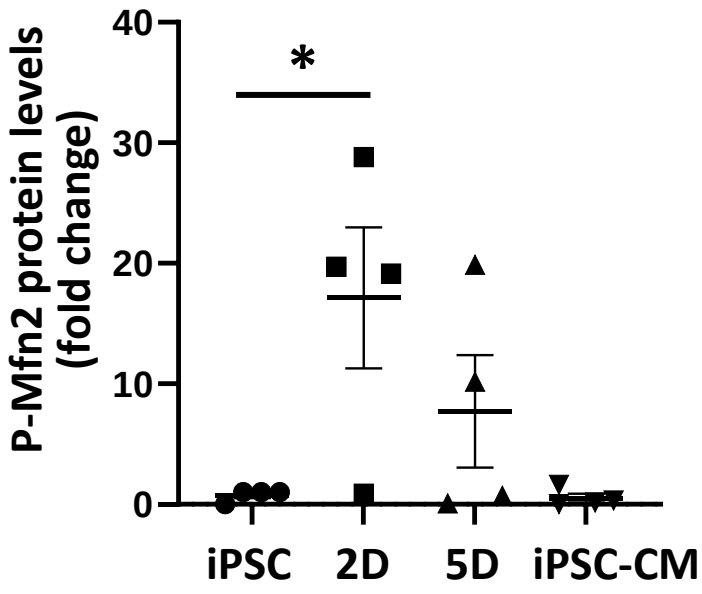
B

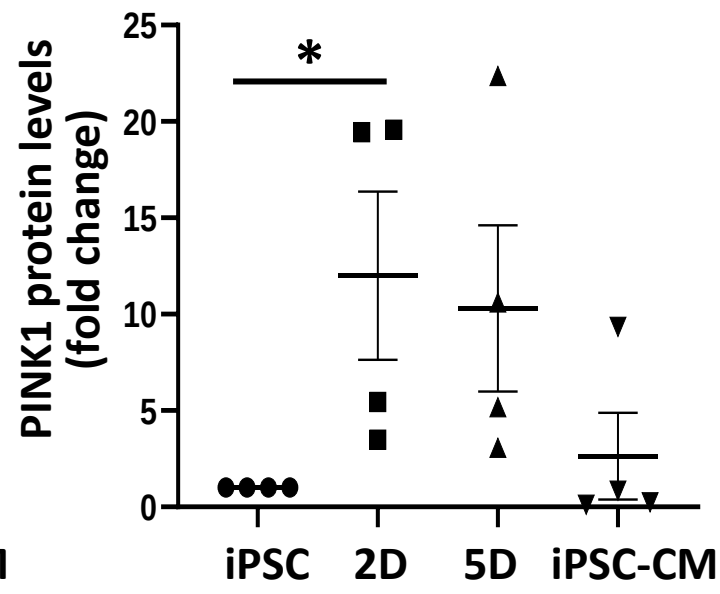

ns
*
Mitochondria in Lysosomes (%)
15
10
5
0
iPSC
2D
5D
iPSC-CM

C

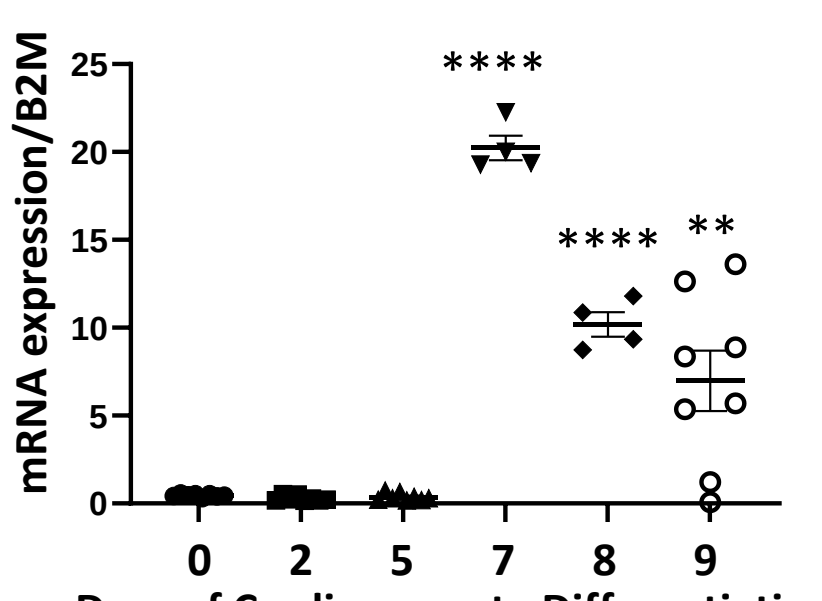

iPSC
2D
5D
9D (iPSC-CM)
100 kDa
75 kDa
Phospho-Mfn2
100 kDa
75 kDa
Mfn2
75 kDa
50 kDa
PINK1
37 kDa
25 kDa
GAPDH

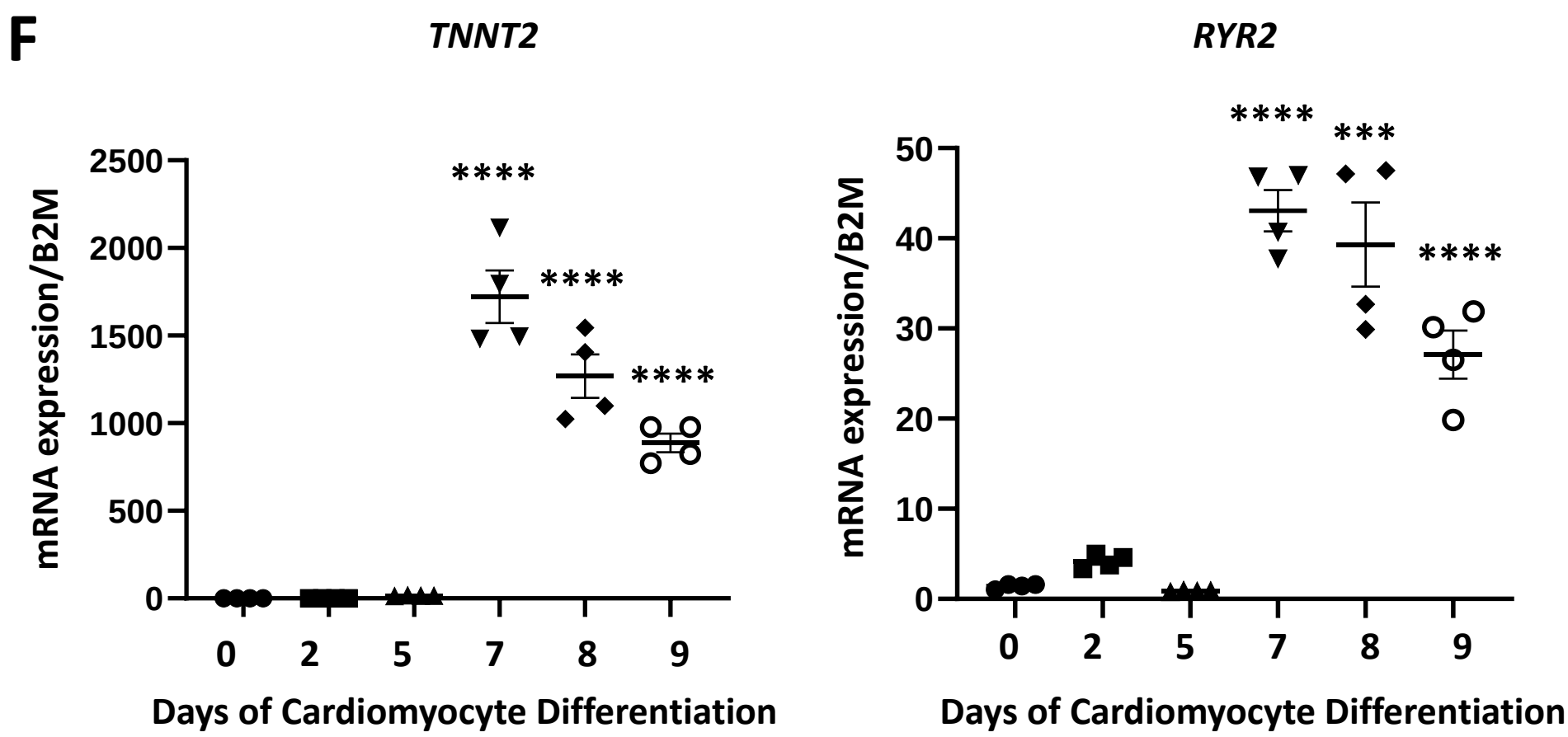
D


Phospho-Mfn2
P-Mfn2 protein levels (fold change)
*
40
30
20
10
0
iPSC
2D
5D
iPSC-CM

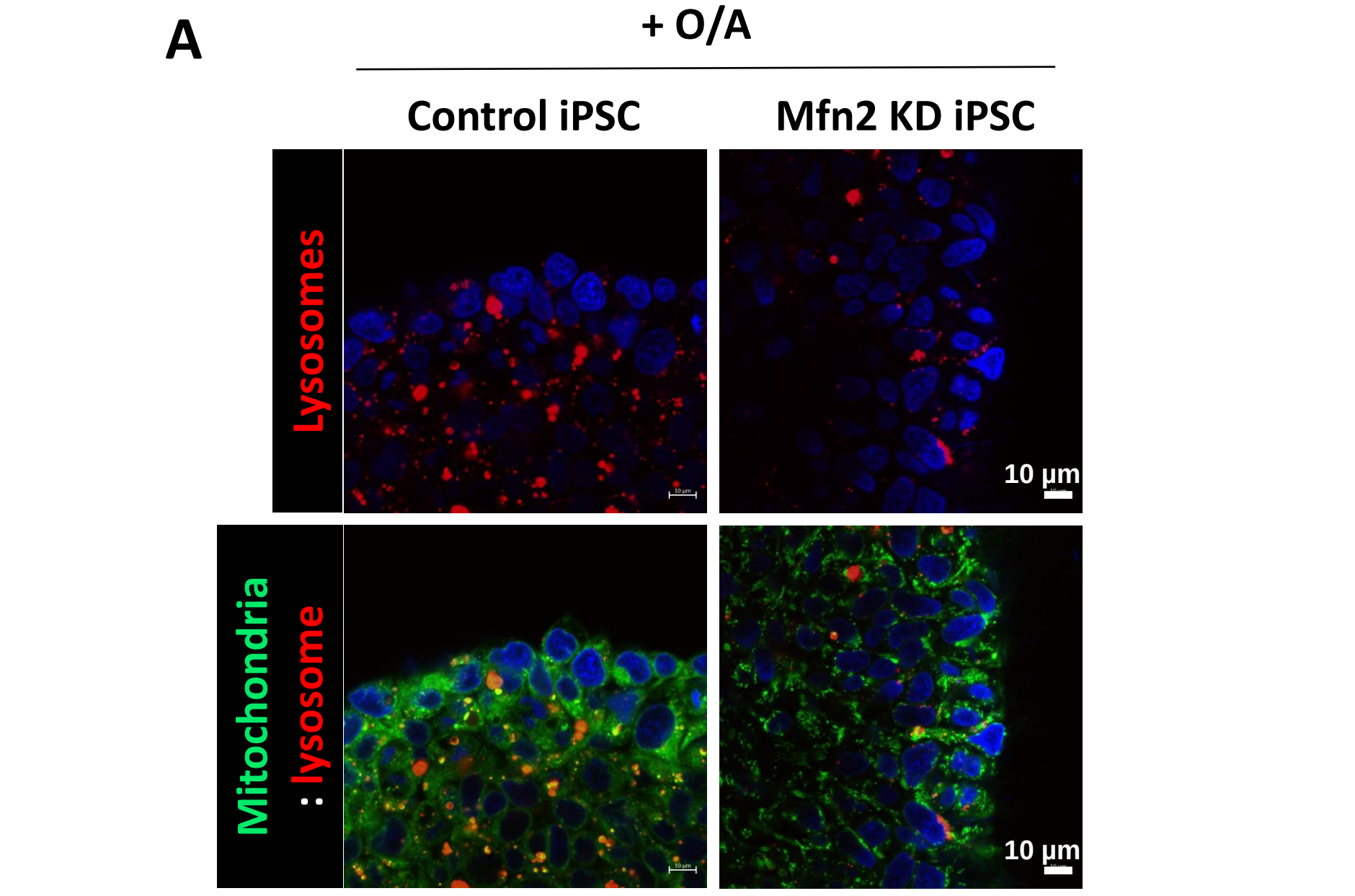

PINK1
PINK1 protein levels (fold change)
*
25
20
15
10
5
0
iPSC
2D
5D
iPSC-CM

E


PGC-1α
mRNA expression/B2M
****
****
**
25
20
15
10
5
0
0
2
5
7
8
9
Days of Cardiomyocyte Differentiation



Supplementary Figure 2. Mitophagy is followed by mitochondrial biogenesis during cardiomyocyte differentiation. **(A)** Live cells were stained with MitoTracker (mitochondria, green), Lysotracker (lysosomes, red), and Hoechst 33342 (nuclei, blue) and imaged at specified timepoints during cardiomyocyte differentiation with confocal microscopy. Scale bar= 20 μm. iPSC-CM indicates 9 day differentiated cardiomyocyte (CM) cells. **(B)** Quantification of (A) indicating the percent of overall mitochondria within lysosomes. Mitophagy is increased on day 2 of cardiomyocyte differentiation. Mean ± SEM with *p<0.05. **(C)** Cells were lysed at different stages of cardiomyocyte differentiation. Western blots show Ser443 Mitofusin 2 phosphorylation, overall Mfn2 levels, PINK1 levels, and GAPDH as loading control. Mitophagy markers are increased on day 2 of cardiomyocyte differentiation. **(D)** Quantification of (C) which shows a higher level of phosphorylated Mfn2 and PINK1 on day 2 of cardiac differentiation. Mean ± SEM, n=4 with *p<0.05. **(E)** mRNA levels of *PGC-1α* were evaluated in differentiating cardiomyocytes by RT-qPCR. The master regulator of mitochondrial biogenesis is increased on days 7, 8, and 9 of differentiation, after mitophagy has occurred. B2M was used as housekeeping control. Mean ± SEM with **p<0.01 and ****p<0.0001. **(F)** mRNA levels of the cardiac markers *TNNT2* and *RYR2* were evaluated in differentiating cardiomyocytes by RT-qPCR. These markers of cardiomyocytes increased in days 7, 8, and 9 indicating that these cells exhibit cardiomyocyte characteristics. B2M (β2-Microglobulin) was used as housekeeping gene. Mean ± SEM, n=4 with ****p<0.0001.



Supplementary Figure 3. Alternate iPSC cell line also shows metabolic reprogramming. (A) Live cells with and without Mitofusin 2 shRNA knockdown were treated with the mitophagy inducers oligomycin (10 µM) and antimycin (5 µM) (O/A) for 3 hours and stained with MitoTracker (mitochondria, green), Lysotracker (lysosomes, red), and Hoechst 33342 (nuclei, blue). Cells were imaged with confocal microscopy. Mitophagy decreases after Mitofusin 2 knockdown. Scale bar = 10 µm. **(B-D)** mRNA levels of **(B)** *K-Glutaminase,* **(C)** *L-Glutaminase,* and **(D)** *Fatty Acid Binding Protein 4 (FABP4)* were evaluated in an alternative cell line of iPSCs (C2 cell line) and iPSC-ECs by RT-qPCR. Results are consistent with 273 cell line from main Figure 3. B2M (β2-Microglobulin) was used as a housekeeping gene. Mean ± SEM, n=4 with *p<0.05, **p<0.01, and ****p<0.0001.