# Analyzing Differentiation of H9C2 Cells in the Absence of MICU1

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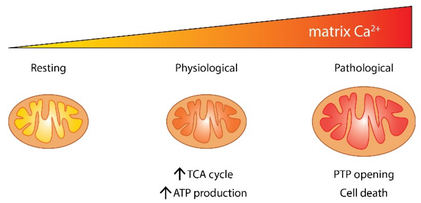
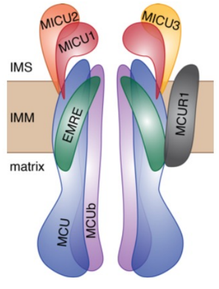
## Introduction

$Ca^{2+}$ entry in the mitochondria is important as it aids in regulating mitochondrial metabolism and creating ATP.[1] However, when excess levels of $Ca^{2+}$ are present in mitochondria, it can potentially lead to cell death because of $Ca^{2+}$ overload.[1]




The Mitochondrial Calcium Uniporter (mtCU) is a transmembrane multi-protein complex that allows $Ca^{2+}$ ions into the mitochondria. One regulatory protein of the mtCU which regulates the uptake of the $Ca^{2+}$ ions is known as the Mitochondrial Calcium Uptake 1 (MICU1).[2] MICU1 serves as a gatekeeper for MCU.




The deletion of MICU1 in different organisms has been found to have certain effects.[1] For example, it leads to myopathic defects in mice and core myopathies in humans as well. It is thought that differentiated H9C2 cells without MICU1 respond differently to the presence of excess calcium compared to control H9C2 cardiac myoblasts.

## Results and Analysis

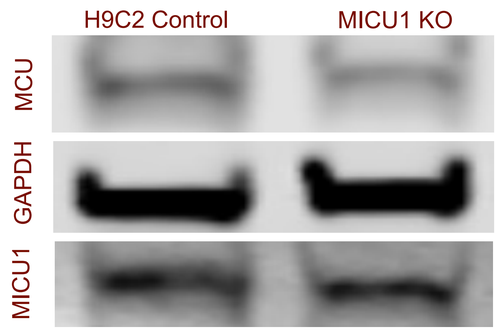



**Figure 1:** When normalized to GAPDH (housekeeping proteins), it was found that around 49% of the MICU1 KO cells were actually deleted of the MICU1 protein. This shows that MICU1 was definitely still present when conducting experiments. MCU was also tested for to compare it to the prevalence of MICU1. MCU expression was 35% knocked out in the MICU1 KO cells.

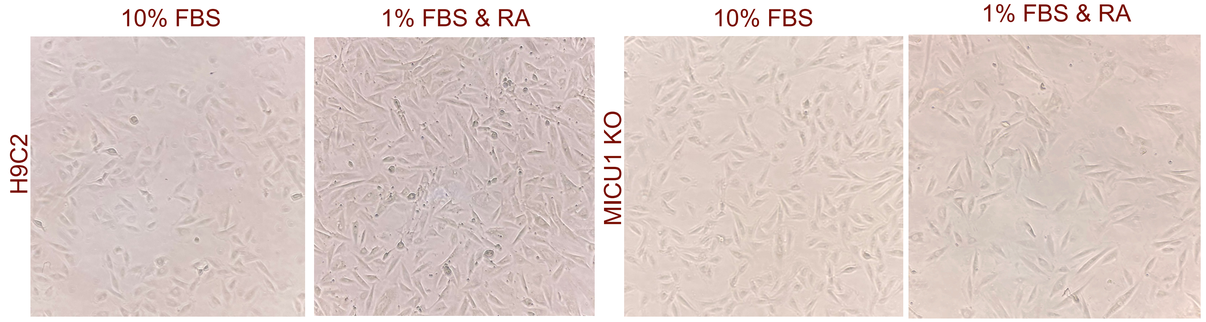



**Figure 2:** The images on the left are the un-differentiated and differentiated H9C2 cells. After differentiation, the cardiac myoblasts turned into "cardiac myocyte-like" cells. Due to mutations that could have occurred, it is hard to tell their exact classification unless further tests are performed. On the right, the differentiated and un-differentiated MICU1 KO cells can be seen. They are also "cardiac myocyte-like" cells but with the deletion of MICU1. In both differentiated cell types, the cells changed shape into a longer form. Previously differentiated H9C2 cells were seen to be longer and more overgrown.[3] This difference could arise from the cells lines being from older passages, wherein the cells could have morphed.

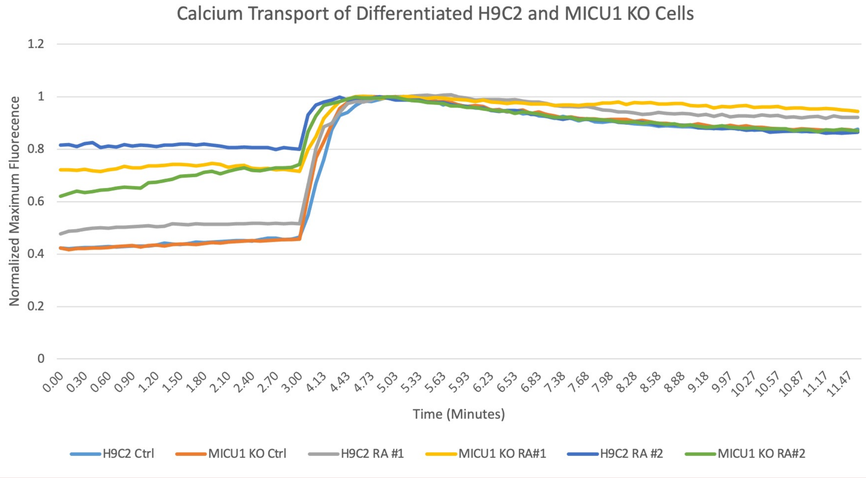



**Figure 3:** The Calcium Transport experiment shows the extra-mitochondrial $Ca^{2+}$ concentration. After the basal is run for 3 minutes, 7uM of $Ca^{2+}$ is added. Due to the different basal fluorescence in each of the cell lines tested, the values were normalized to the maximum fluorescence in each group. As seen in the graph, there is no discrete trend between the non-RA and RA treated cell lines. If the calcium transport experiment had worked as expected, there would be a distinct plateau in the normal MICU1 KO cells and a drop in fluorescence in the H9C2 cells. This is because MICU1 would regulate $Ca^{2+}$ levels and prevent more $Ca^{2+}$ from entering the cell, whereas the H9C2 cells would continue to take in $Ca^{2+}$, decreasing extra-mitochondrial $Ca^{2+}$ levels.

## Conclusion and Future Directions

Due to the results, there is insufficient data to conclude that the MICU1 differentiated cells are different compared to the control H9C2 cells. Further trials can be conducted to obtain a higher percentage of MICU1 knockout cells to fully test the impact of the MICU1 KO. The same project will also be conducted on human myoblast cells (MOO7) as a previous study done on MICU1 deficient mice showed that muscle weakness persisted even after other developmental delays resolved for the most part.[1] This suggests that everyday wear and tear within the muscles were not being repaired properly, potentially due to improper Ca2+ regulation. MICU1 is thus thought to play a large role in muscle cell differentiation.[1]

## Methods

H9C2 MICU1 knockouts were first obtained by designing the desired sgRNA and cloning it into CRISPR/Cas9 plasmids. Bacteria was then used and collected to amplify the clones and DNA concentration was tested by performing a Zymo MiniPrep. Once the sequences were verified, the DNA was transfected into the H9C2 cells via Lipofectamine. Western blot analysis was performed to determine protein expression in both the control H9C2 cells and the MICU1 knockout (KO) cells. To differentiate the cells, they were cultured in 1% FBS DMEM and treated with 10 nM of Retinoic Acid (RA) everyday for 10 days straight.[3] Medium was changed every 2-3 days and the cells were not split. They were then imaged on the 10th day and collected to perform the Calcium Transport experiment. Calcium Transport was finally used as an analysis to test $Ca^{2+}$ regulation function in all of the test cells. 2 cohorts of differentiated cells were used.

## References

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2. Antony A, Paillard M, Moffat C. *et al.* MICU1 regulation of mitochondrial Ca2+ uptake dictates survival and tissue regeneration. *Nat Commun* 7. 2016;10955(2016). doi: 10.1038/ncomms10955
3. Patten V, Chabaesele I, Sishi B, Van Vuuren D. (2017). Cardiomyocyte differentiation: Experience and observations from 2 laboratories. *SA Heart*, 14(2). doi: 10.24170/14-2-2498