

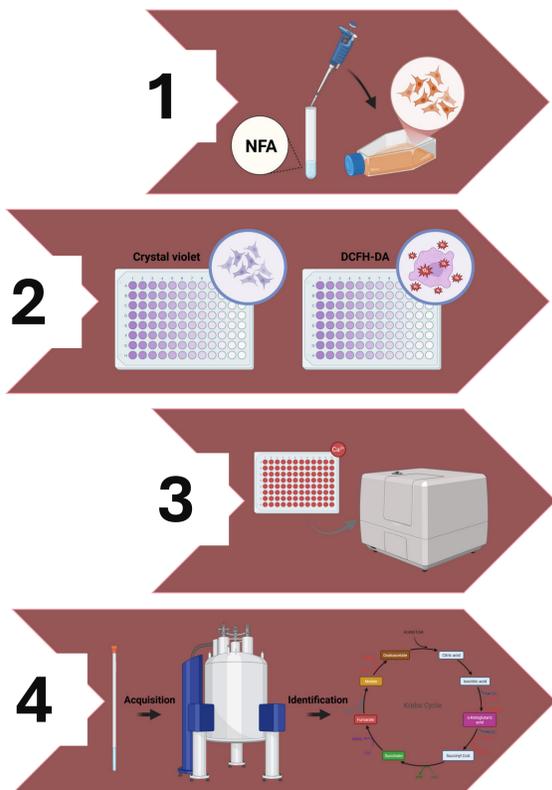
## INTRODUCTION

The small ubiquitin-like modifier 1 (SUMO1) is a key regulator of the sarcoplasmic/endoplasmic reticulum  $Ca^{2+}$  ATPase 2a (SERCA2a), which facilitates the reuptake of  $Ca^{2+}$  in the endoplasmic reticulum after contraction. Co sequentially, any change in SUMO1 could affect SERCA2a's activity and stability, hence cause cardiotoxicity. Succination, an irreversible post-translational modification resulting from the interaction of fumarate with reactive cysteine thiols has been shown in in-house study to target SUMO1. This study aims to understand the impact of SUMO1 succination on SERCA2a SUMOylation, with the hope to better understand heart physiopathology.

## AIMS

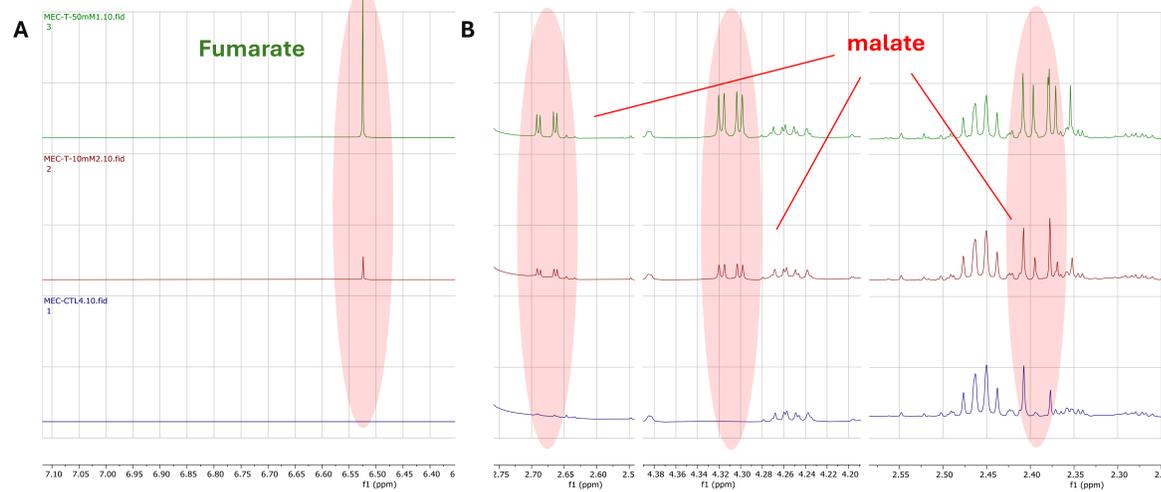
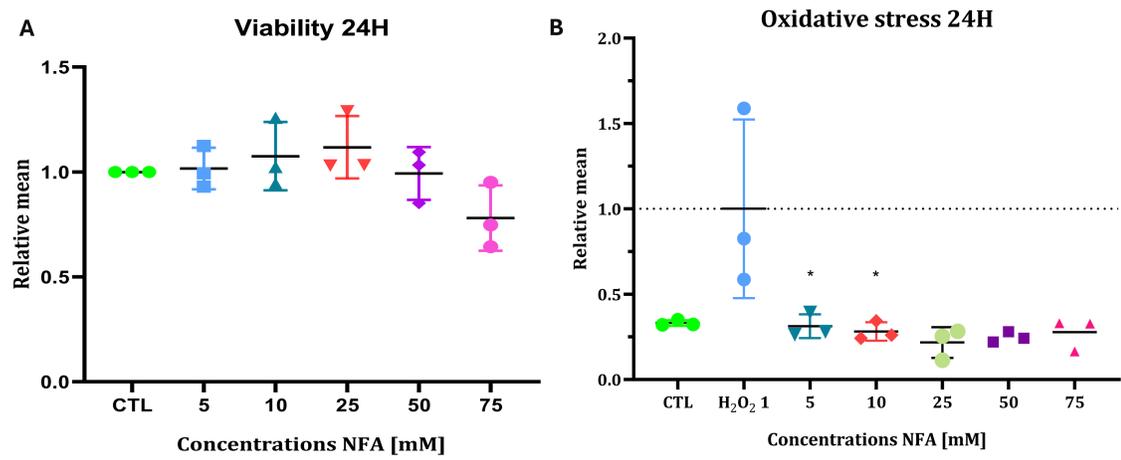
1. To establish a succination model in the AC16 Cardiomyocytes using sodium fumaric acid (NFA).
2. To evaluate the effect of NFA on cell viability and oxidative stress.
3. To assess the metabolic alterations induced by NFA treatment in AC16 cardiomyocytes.
4. To investigate the effect of the NFA on calcium homeostasis in AC16 cells.

## METHODS



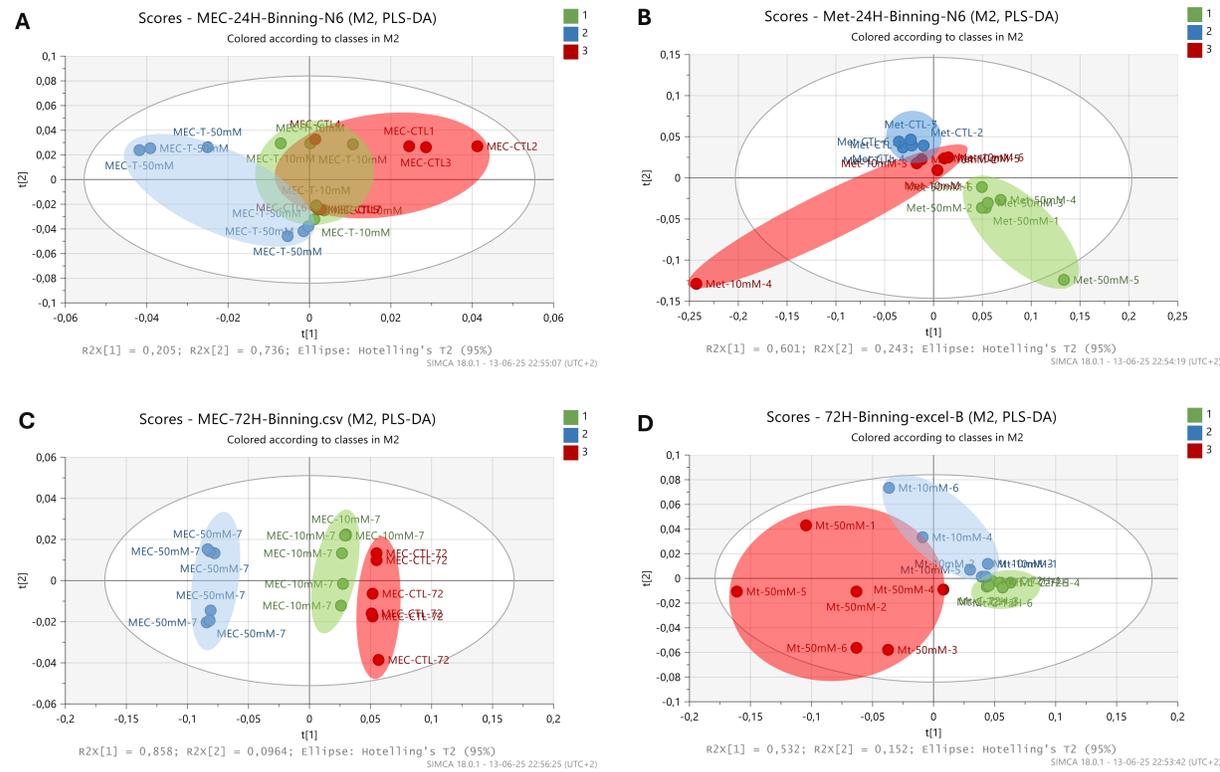
## RESULTS

**Figure 1: Effect of Fumaric Acid (NFA) treatment on cell viability and possible oxidative stress induction in AC16 cardiomyocytes.** A. Relative mean of viability of control cells (negative control) vs NFA enriched cells, using the crystal violet assay. B. Relative mean of oxidative stress in  $H_2O_2$  group (Positive control) vs NFA treated cells. Data are presented as means; each point represents the mean of independent biological replicates  $n=3$ , pipetted 6X,  $\pm$  SD. Control group is represented at 1 (A. CTL; B.  $H_2O_2$ ). Statistical analysis were performed by one-way ANOVA followed by Holmsidak post test. \*  $p \leq 0.05$ .

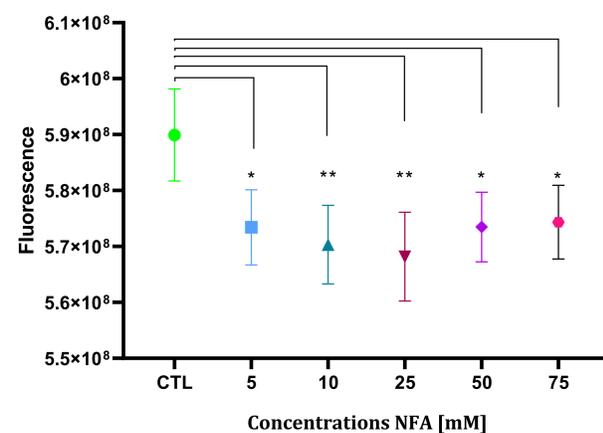


**Figure 2: A.** Extracellular media results showing the elevation in the concentration of Fumarate following the addition of NFA concentrations 10mM and 50mM in comparison to the control group (untreated group) after 24H. **B.** Extracellular media spectra, showing an elevation in malate metabolite in the treated groups (in red) following the addition of 10mM and 50mM of NFA to the media in comparison to the non-treated groups after 24H.

**Figure 3: Score plots of PLS-DA.** A. Results showing the separation between the control group, the 10mM NFA and the 50mM NFA in extracellular media after 24h of treatment. B. Results showing the separation between the control group, the 10mM NFA and the 50mM NFA in polar phase after 24h of enrichment. C. Results showing the separation between the control group, the 10mM NFA and the 50mM NFA in extracellular media after 72h of enrichment. D. Results showing the separation between the control group, the 10mM NFA and the 50mM NFA in polar phase after 72h of enrichment. CV-ANOVA p-value : A. 1, B. 1, C. 0,0025307, D. 0,810605.



## Calcium assay



**Figure 4:** AC16 cells were enriched with 5, 10, 25, 50, and 75mM of NFA for 24 hours before measuring calcium level. The relative intracellular calcium levels were detected (Fluo-4 Direct Calcium Assay Kit; Invitrogen). The results are the average of three independent experiments and are presented as mean  $\pm$  SEM. Statistical analysis were performed by one-way ANOVA followed by Holm Sidak post-test. \*  $p \leq 0.05$ . \*\*  $p \leq 0,01$  compared with cells in the control group.

## CONCLUSION

Preliminary  $^1H$ -NMR results suggest the occurrence of succination in NFA enriched cells starting at a concentration of 10mM of FA. The calcium assay results suggest that the treatment of NFA induces a significant decrease in the intracellular calcium metabolism in the cell, although still not clear how, it would be interesting to investigate whether SERCA2a's function was affected following SUMO1's potential succination. In conclusion, understanding the impact of SUMO1 succination on SERCA2a SUMOylation, could reveal a new regulatory mechanism in cardiac function, and this might allow a better understanding of heart physiopathology.

## REFERENCES

- Gu, Y., Fang, Y., Wu, X. et al. The emerging roles of SUMOylation in the tumor microenvironment and therapeutic implications. *Exp Hematol Oncol* 12, 58 (2023). <https://doi.org/10.1186/s40164-023-00420-3>
- Schmidt C, Sciacovelli M, Frezza C. Fumarate hydratase in cancer: A multifaceted tumour suppressor. *Semin Cell Dev Biol*. 2020 Feb;98:15-25. doi: 10.1016/j.semcdb.2019.05.002. Epub 2019 May 22. PMID: 31085323; PMCID: PMC6974395.
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