



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Department of Veterinary Medical Sciences

Antonia Cugliari, PhD student

39° Cycle

II year

**Kinetic evidence with evaluation of the
Supramolecular Organization of
Mitochondrial Respiratory Complexes and
mitovesicles production in animal
models of complex human diseases.**

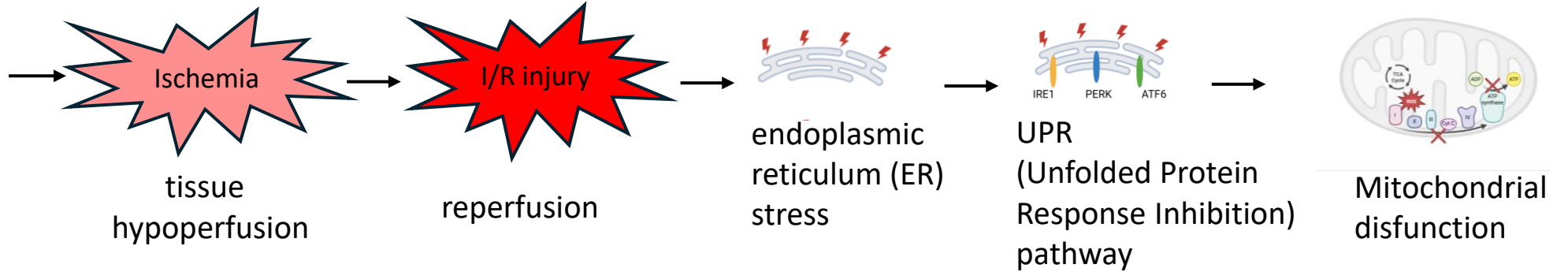
2024/2025

Curriculum: Basic Sciences





ordinary bursary type

Effects of Tunicamycin and Unfolded Protein Response Inhibition on Mitochondrial Supercomplex Organization and Functionality in a Murine Model of Hemorrhagic Shock

Hemorrhagic shock (HS)

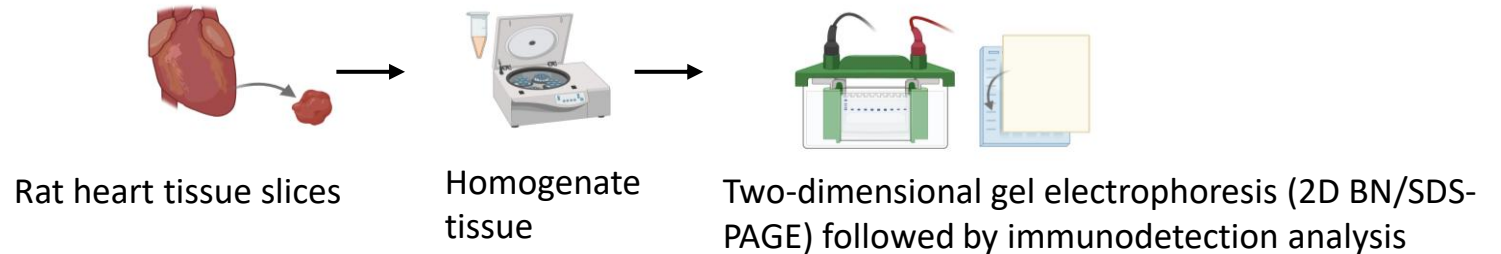


EXPERIMENTAL GROUPS

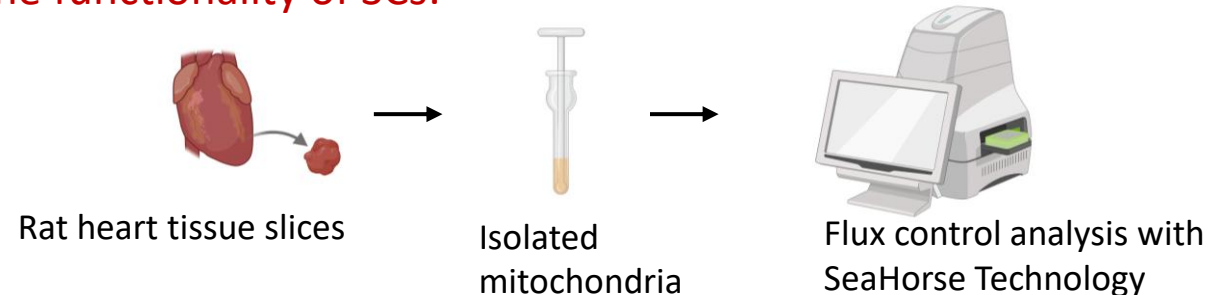
-  Control (**SHAM**)
-  Hemorrhagic shock (**HS**)
-  Hemorrhagic shock+ Tunicamycin (**HS+Tun**)
-  Hemorrhagic shock+ Tunicamycin+ Inhibitors of UPR (**HS+Tun+Inh-UPR**)

METHODS

To study the assembly of SCs:



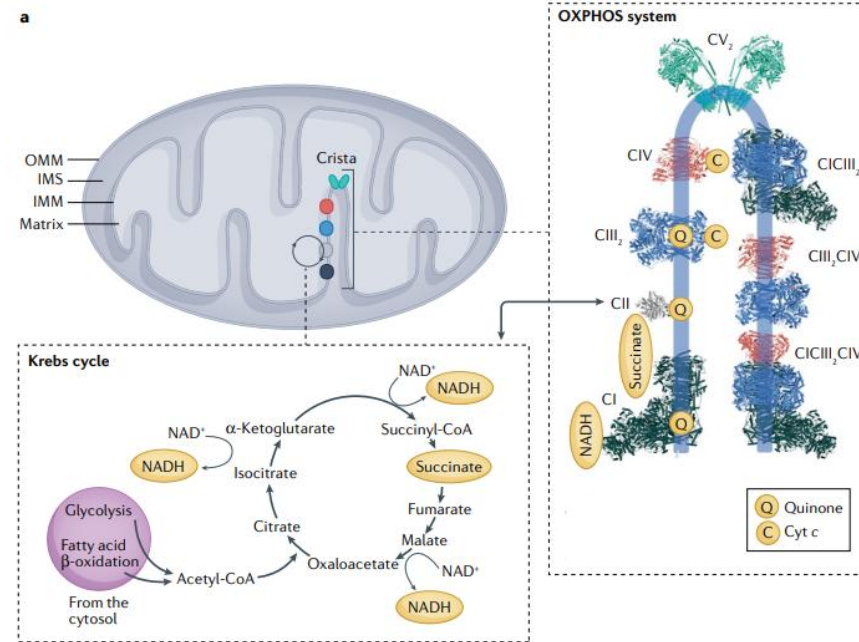
To study the functionality of SCs:



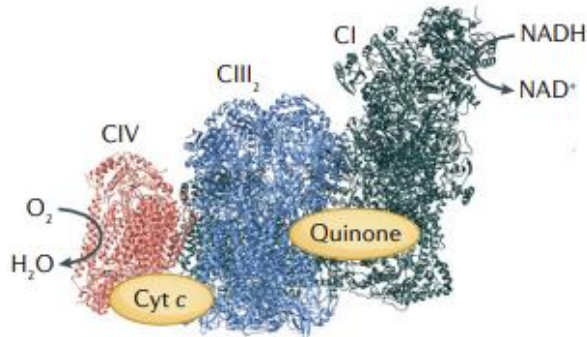
THE ASSEMBLY AND FUNCTION OF MITOCHONDRIAL RESPIRATORY CHAIN COMPLEXES

Advantages of Supercomplex Organization:

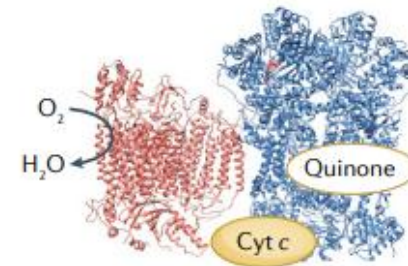
- ↓ **Reactive oxygen species (ROS) production**, limiting oxidative stress and its role in disease and aging.
- ↑ **Catalytic activity** of the respiratory chain components.
- ↑ **Efficiency of electron transfer** through substrate channeling



N-respirasome (CI+CIII₂+CIV_n)

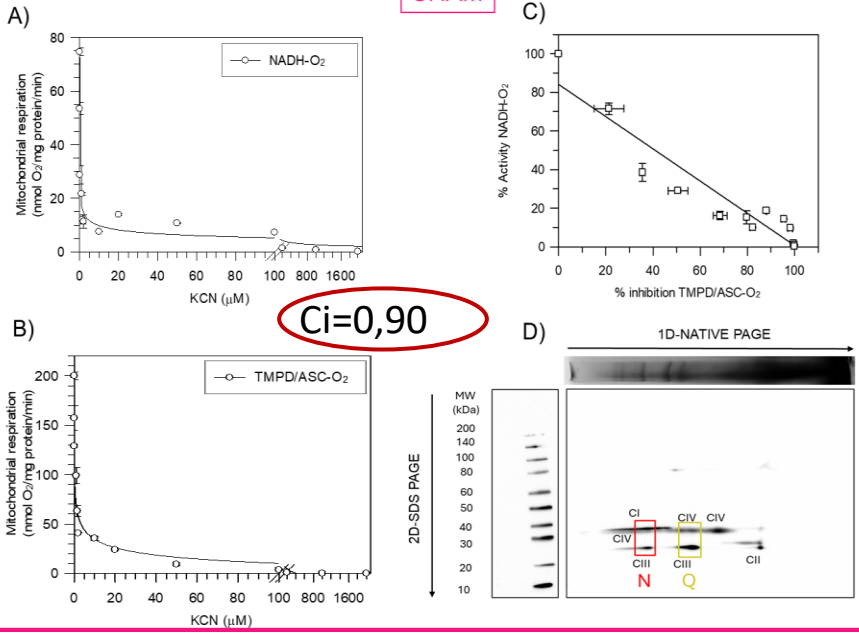


Q-respirasome (CIII₂+CIV_n)

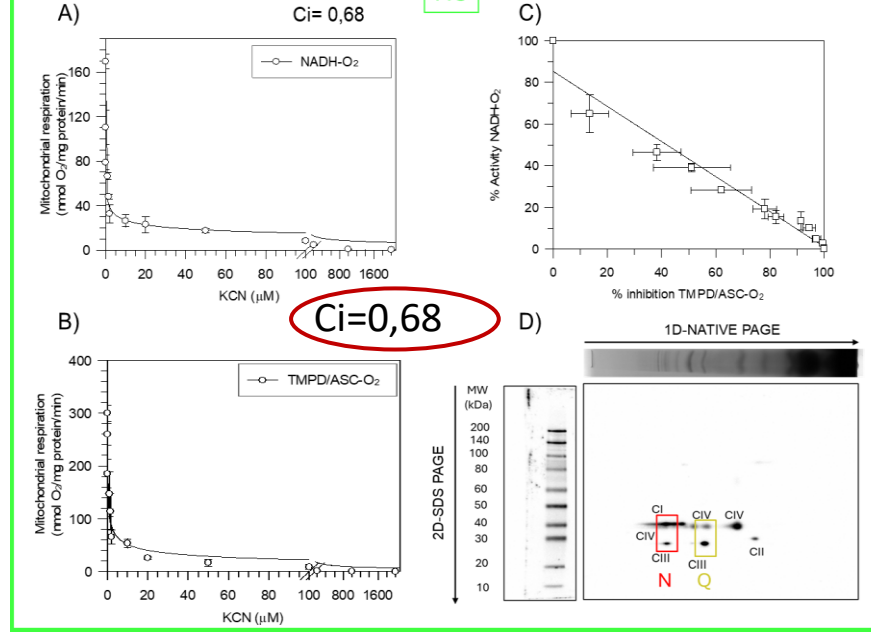


RESULTS

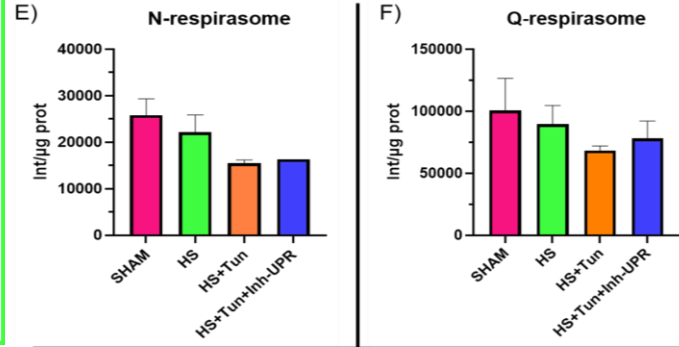
SHAM



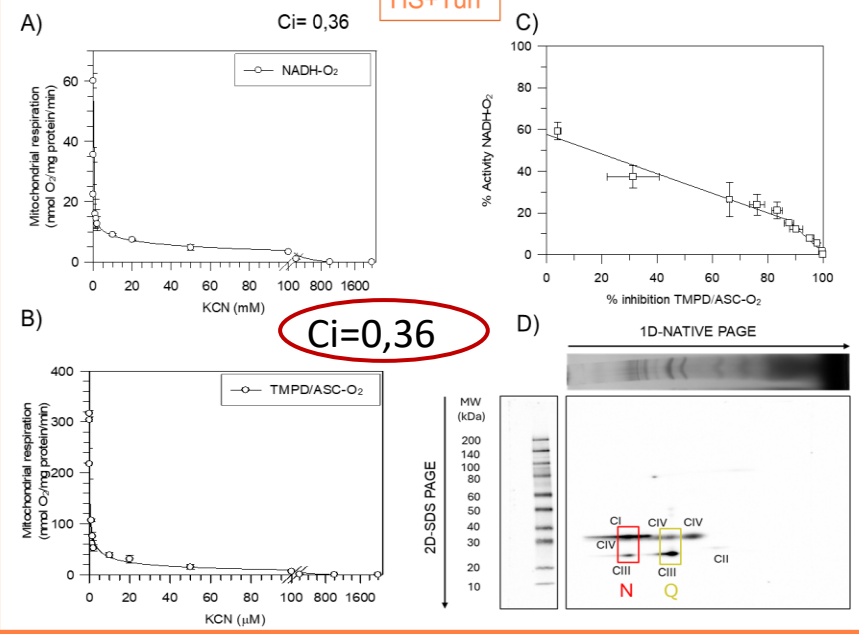
HS



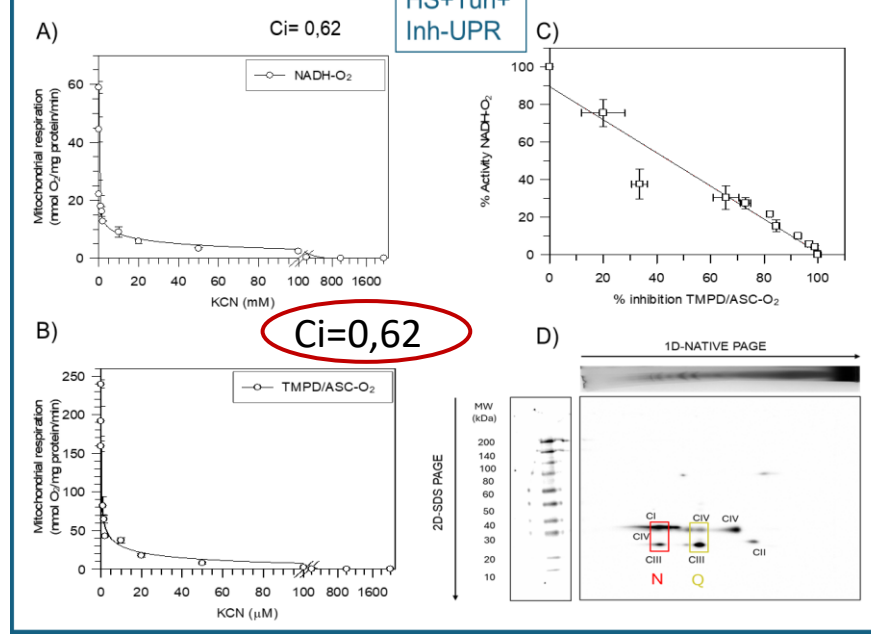
SHAM
HS
HS+Tun
HS+Tun+Inh-UPR



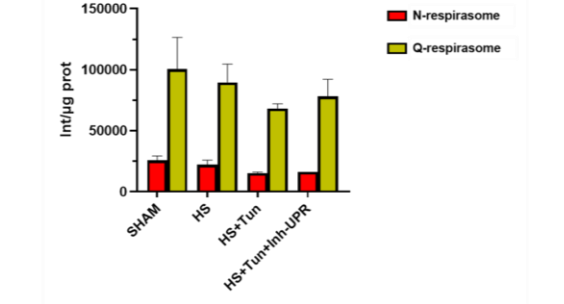
HS+Tun



HS+Tun+Inh-UPR



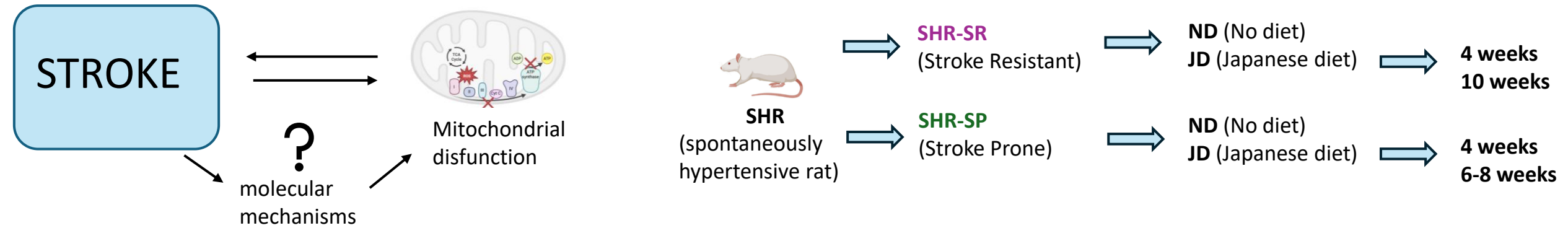
N- and Q-respirasome



CONCLUSIONS #1

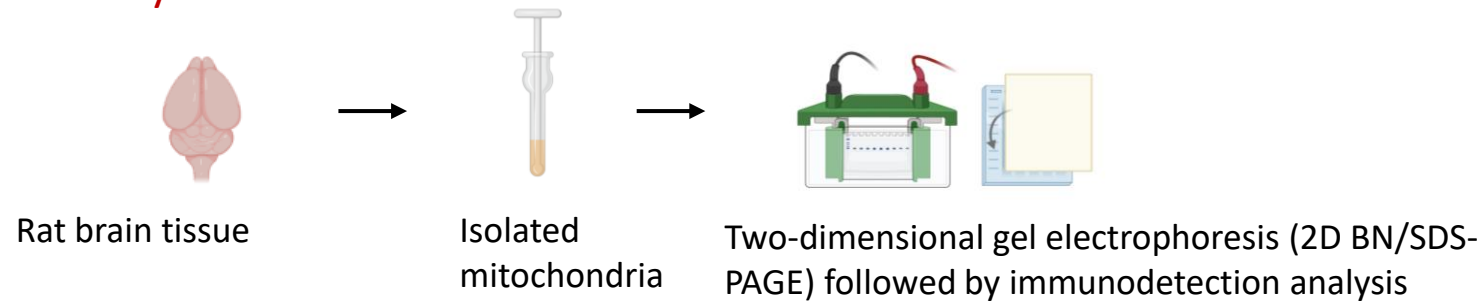
- Sustained UPR-pathway (induced with tunicamycin) causes a reduction in supercomplex (SC) levels and impaired substrate channeling, thereby negatively influences the assembly and functionality of SCs.
- The use of UPR inhibitors counteracts this effect and restores the condition of the untreated HS group, preserving the SCs integrity and increasing electron transfer efficiency.

The function and supramolecular assembly of the mitochondrial respiratory complexes underlie hypertension-related stroke susceptibility in a model of complex human disease

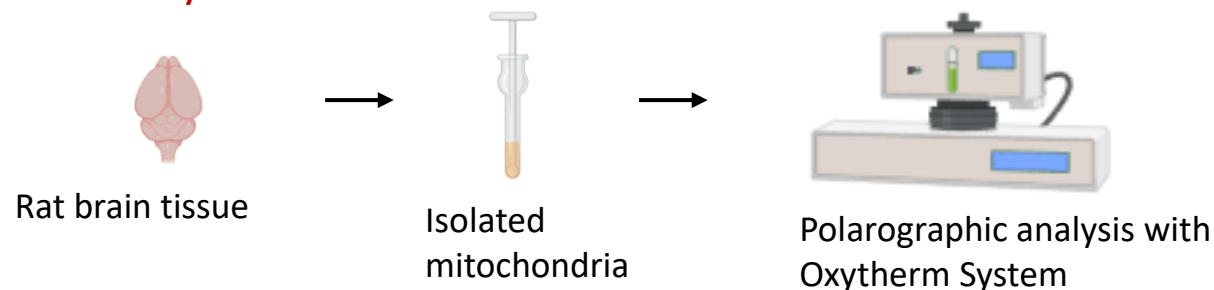


METHODS

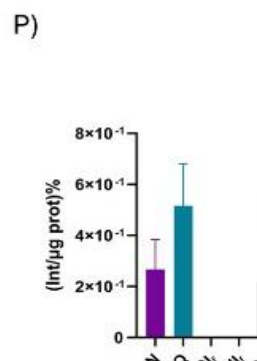
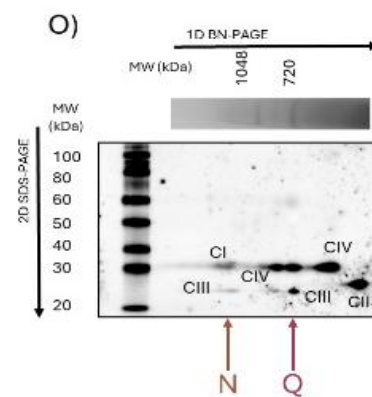
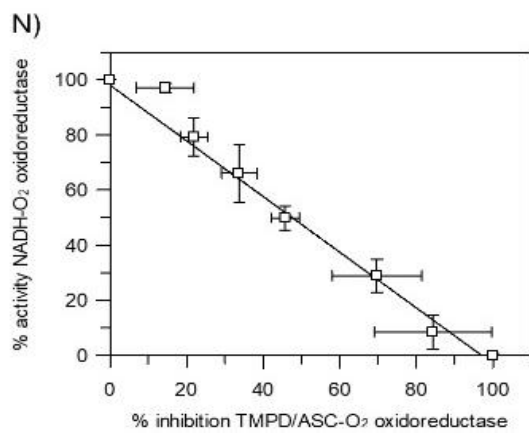
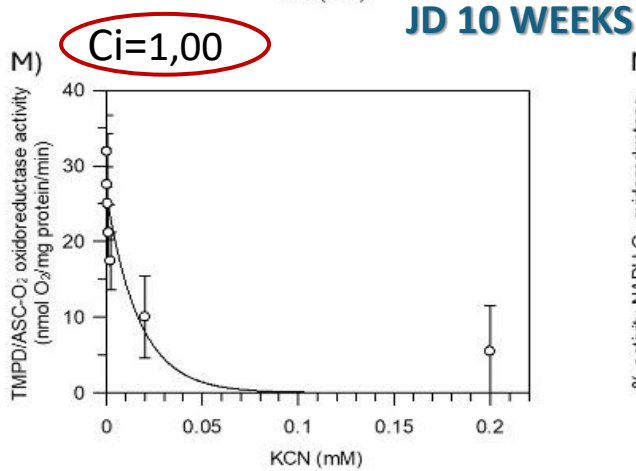
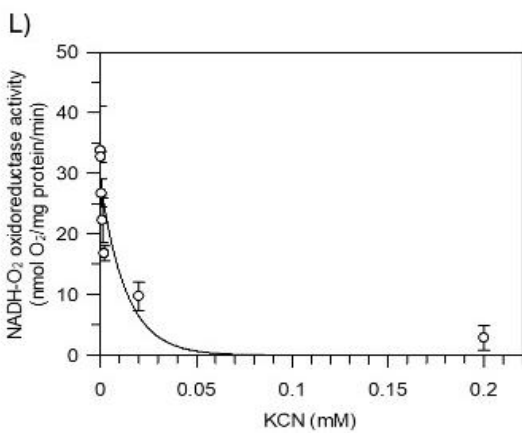
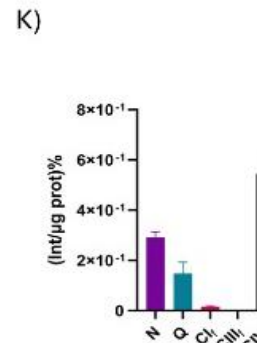
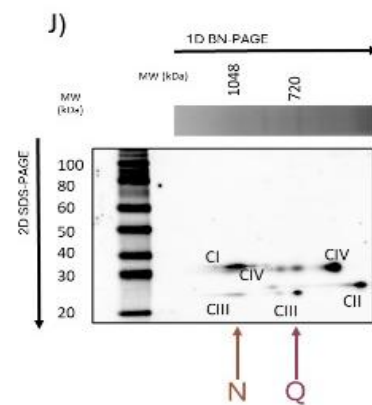
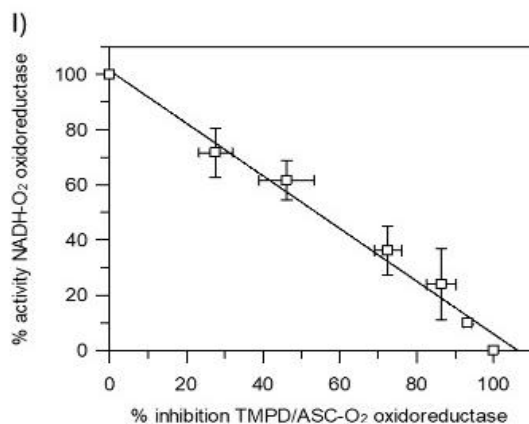
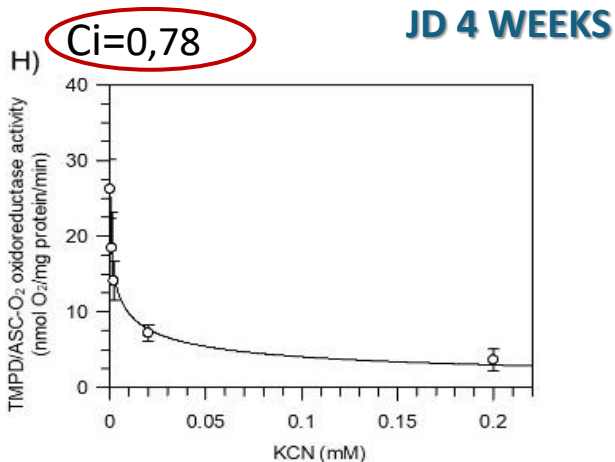
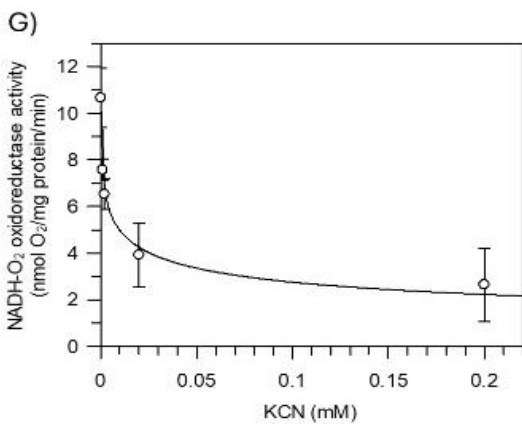
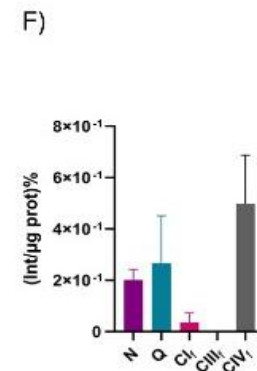
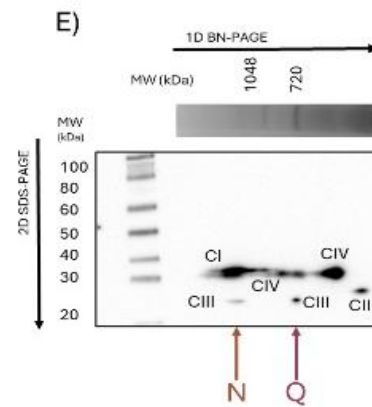
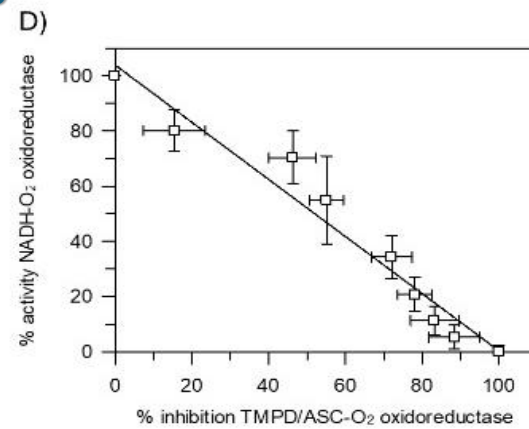
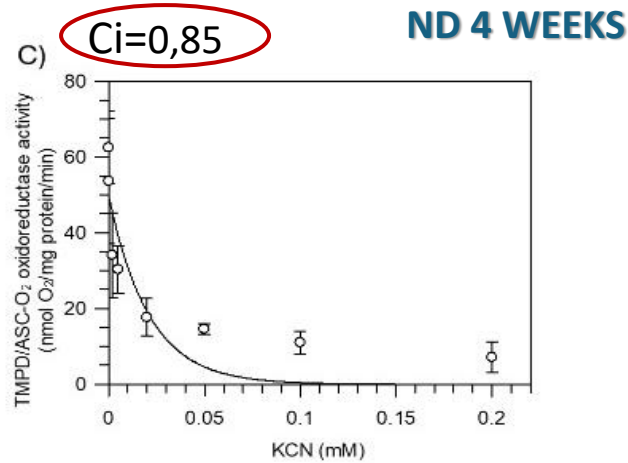
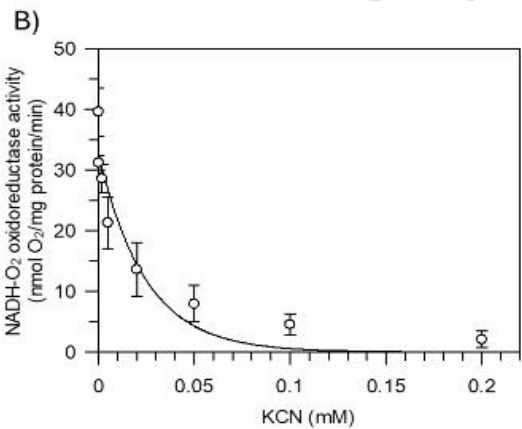
To study the assembly of SCs:



To study the functionality of SCs:

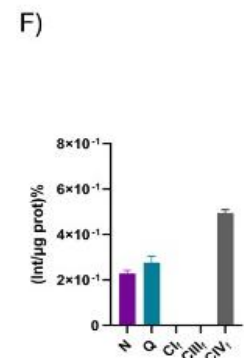
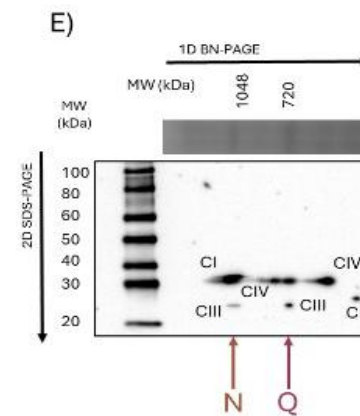
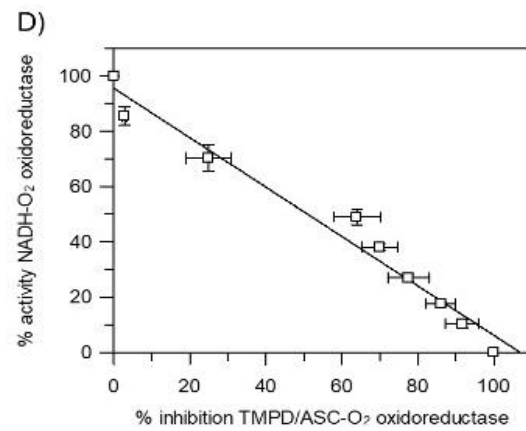
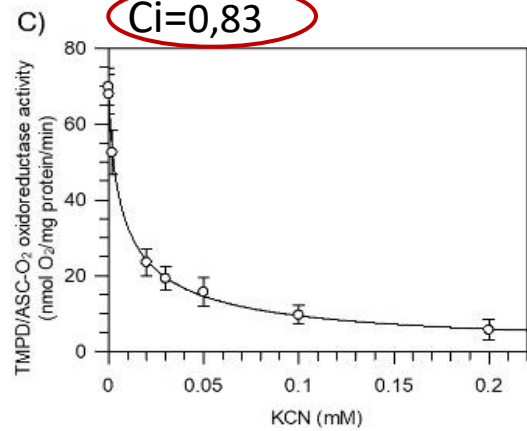
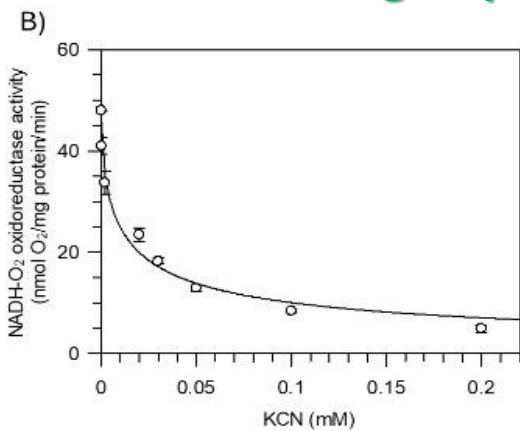


RESULTS SHR-SR group

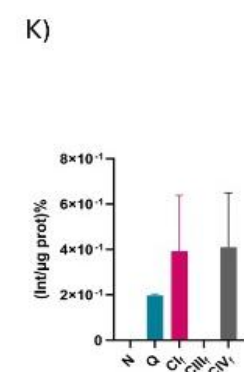
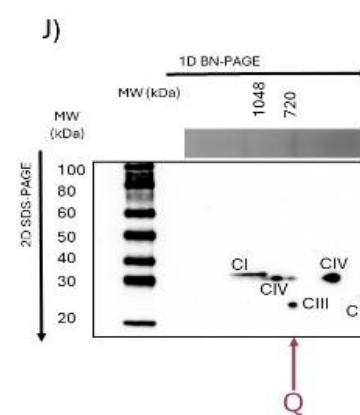
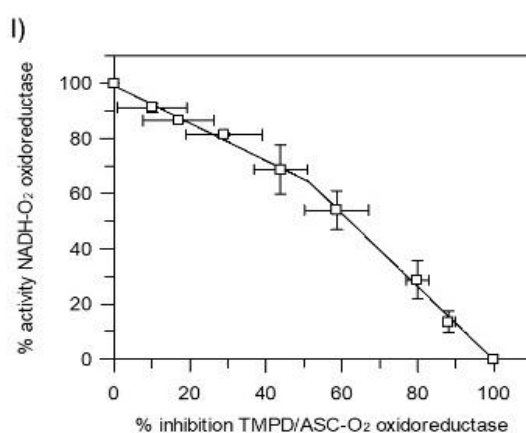
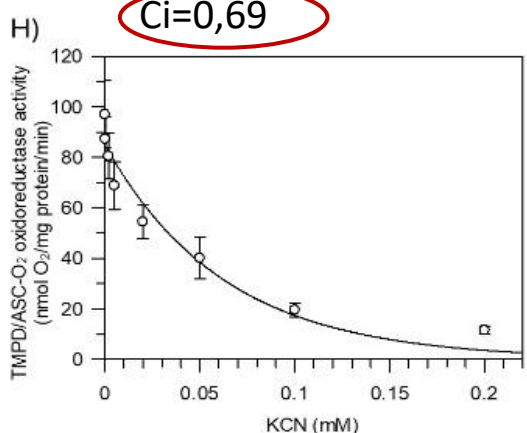
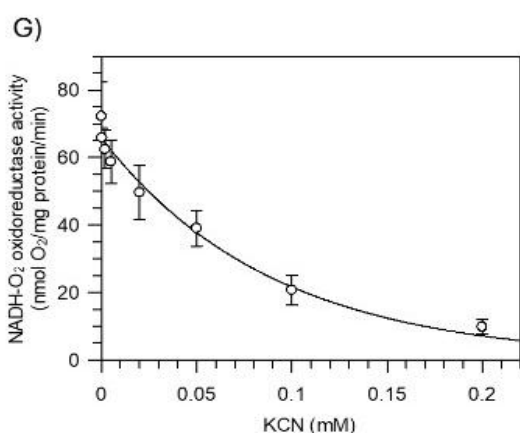


RESULTS SHR-SP group

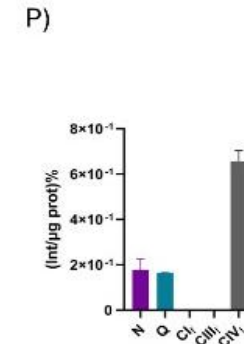
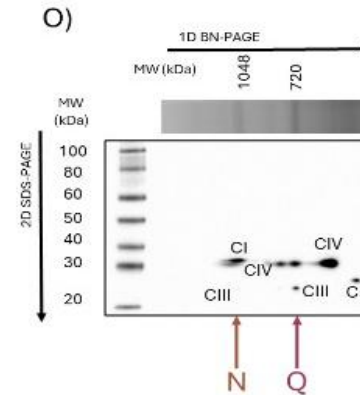
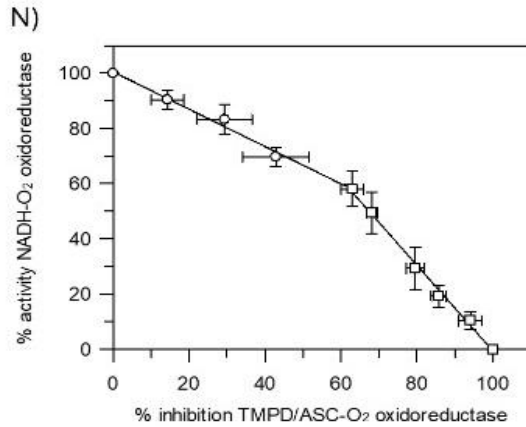
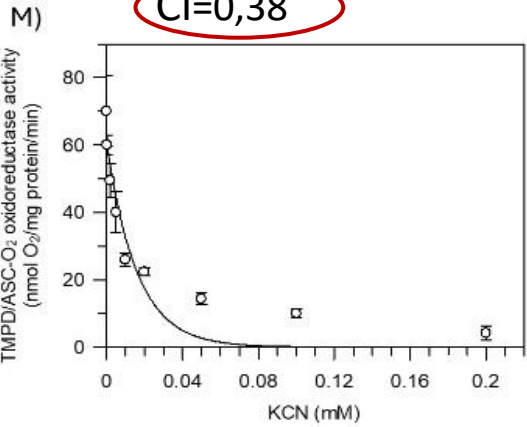
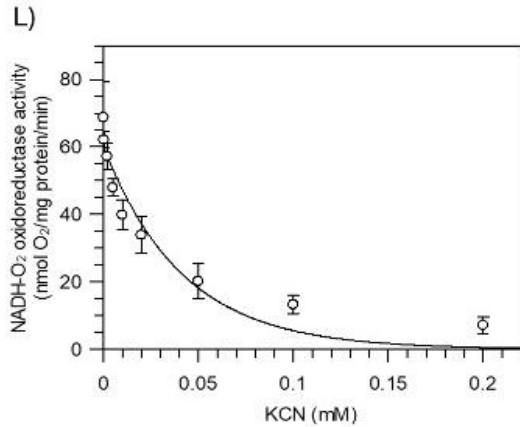
ND 4 WEEKS



JD 4 WEEKS



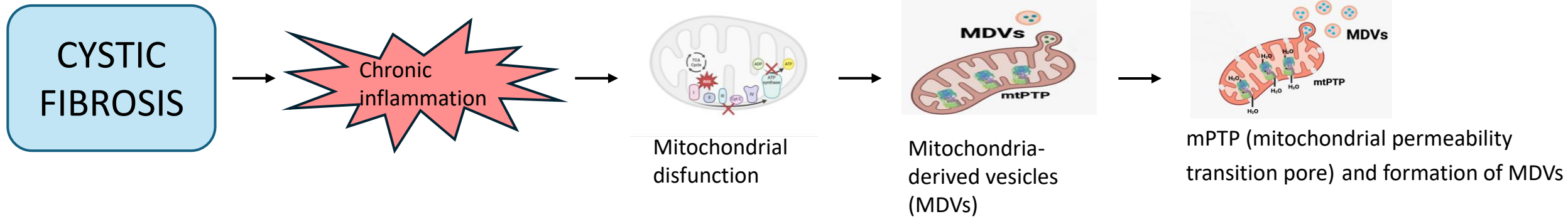
JD 6-8 WEEKS



CONCLUSIONS #2

- The different susceptibility to stroke between SHR-SP and SHR-SR appears to depend on the integrity/loss of mitochondrial SCs organization.
- In SHR-SP, the disassembly of Complex I compromises channeling and reduces the metabolic control by CIV, promoting the mitochondrial dysfunction that accompanies the stroke phenotype.

Isolation and characterization of mitovesicles production from bronchial epithelial cells



METHODS

Analysis of Bioenergetic and Proteomic characteristics of MDVs:

Human bronchial epithelial cells (CF HBE- Δ F508)



-CsA
+CSA



With *Pseudomonas aeruginosa* infection

-CsA
+CSA



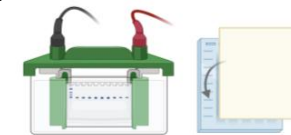
Ultracentrifugation
and sucrose gradient



MDVs

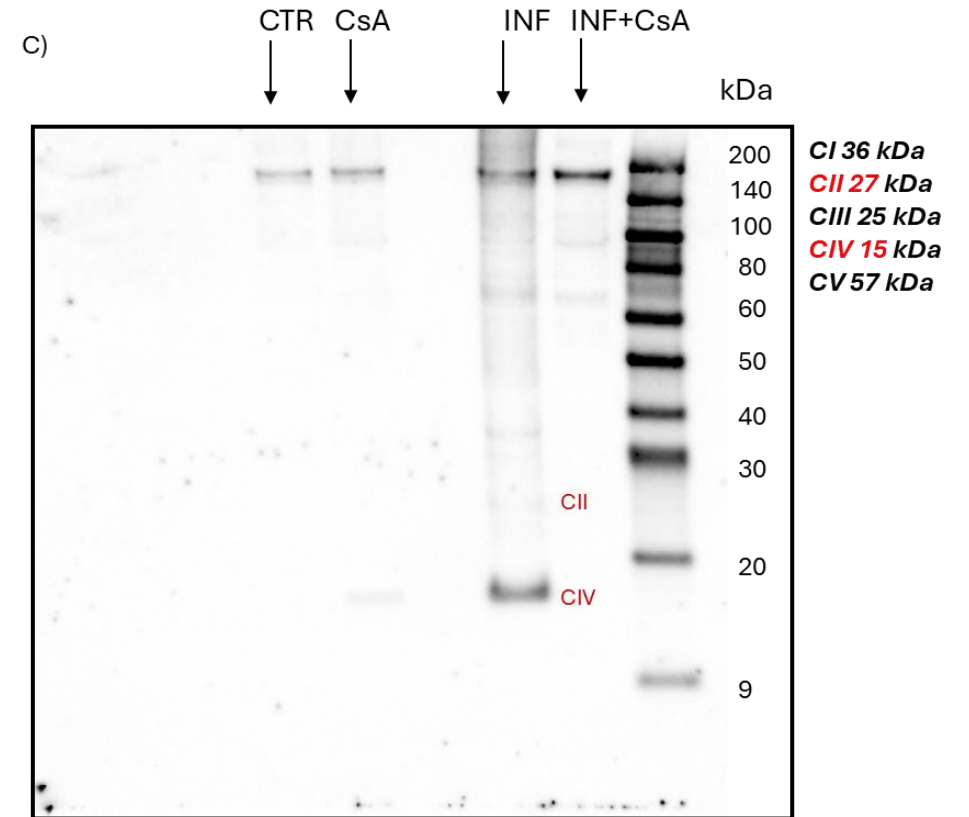
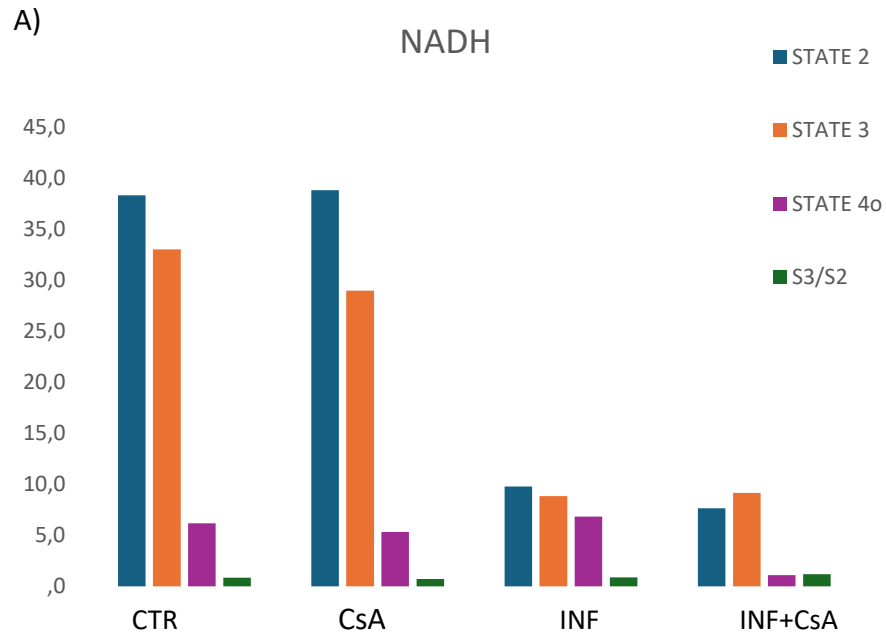


Bioenergetic analysis with
SeaHorse Technology



Proteomic analysis (SDS Page
and Western Blotting)

PRELIMINAR RESULTS



NEXT STEPS

- A Transmission Electron Microscope observation is planned.
- The MDVs isolation protocol will be improved.

PUBLICATIONS (in this year)

- Inside-out submitochondrial particles affect the mitochondrial permeability transition pore opening under conditions of mitochondrial dysfunction. C. Algieri, **A. Cugliari**, P.A. Glogowski, S. Granata, M. Fabbri, F. Trombetti, M.L. Bacci, S. Nesci (2025). *Biochimica et Biophysica Acta – Bioenergetics*, 1866: 149528.
- Melatonin rescues cell respiration impaired by hypoxia/reoxygenation in aortic endothelial cells and affects the mitochondrial bioenergetics targeting the F1FO-ATPase. Algieri, C. Bernardini, **A. Cugliari**, S. Granata, F. Trombetti, P. A. Glogowski, M. Fabbri, G. Morciano, G. Pedriali, P. Pinton, S. Nesci (2025). *Redox Biology*, 82: 103605.
- The acute effect of glyphosate on heart mitochondria does not impair the bioenergetics. **A. Cugliari**, C. Algieri, M. Fabbri F. Trombetti G. Rampazzo, T. Gazzotti, G. Pagliuca, S. Nesci (2025). *Xenobiotica*, 2568631.

CONGRESS (in this year)

- Effects of Tunicamycin and Unfolded Protein Response Inhibition on Mitochondrial Supercomplex Organization in a Murine Model of Hemorrhagic Shock. **Cugliari A.**, Zavadskis S., Algieri C., Granata S., Trombetti F., Fabbri M., Weidinger A., Kozlov A.V., Nesci S. Società Italiana di Biochimica e Biologia Molecolare (SIB) 2025. 10-12 Settembre 2025, Palermo.

THANKS TO...

Mitochondrial Biochemistry Group

Salvatore Nesci, **Group Leader**

Cristina Algieri, **Postdoctoral Researcher**

Patrycja Anna Glogowski, **Research Fellow**

Silvia Granata, **Postdoctoral Researcher**

Fabiana Trombetti, **Assistant Professor**

Micaela Fabbri, **Doctoral**

Collaborations:

Chiara Bernardini, **Associate Professor**

Silvia Piva, **Associate Professor**



Molecular Basis of Organ Failure and Regeneration Group

Adelheid Weidinger, **Group Leader**

Andrey V.Kozlov, **Scientific Advisor**

Annette Vaglio-Garro, **Post doctoral Researcher**

Sergejs Zavadskis, **PhD student**

& Mitochondrial Biology Group

Karin Nowikovsky, **Group Leader**

Clara Abobocioae, **PhD Student**



THANK YOU FOR
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