Manickam Lab of Biotherapeutics Delivery

https://manickamlab.wordpress.com/
Follow us on Twitter @manickam_lab

DUQUESNE UNIVERSITY

Development of neuron-derived, mitochondria-containing extracellular vesicles (EVs) for ALS treatment

Paromita Paul Pinky, B.Pharm
Pharmaceutical Sciences
Duquesne University

CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

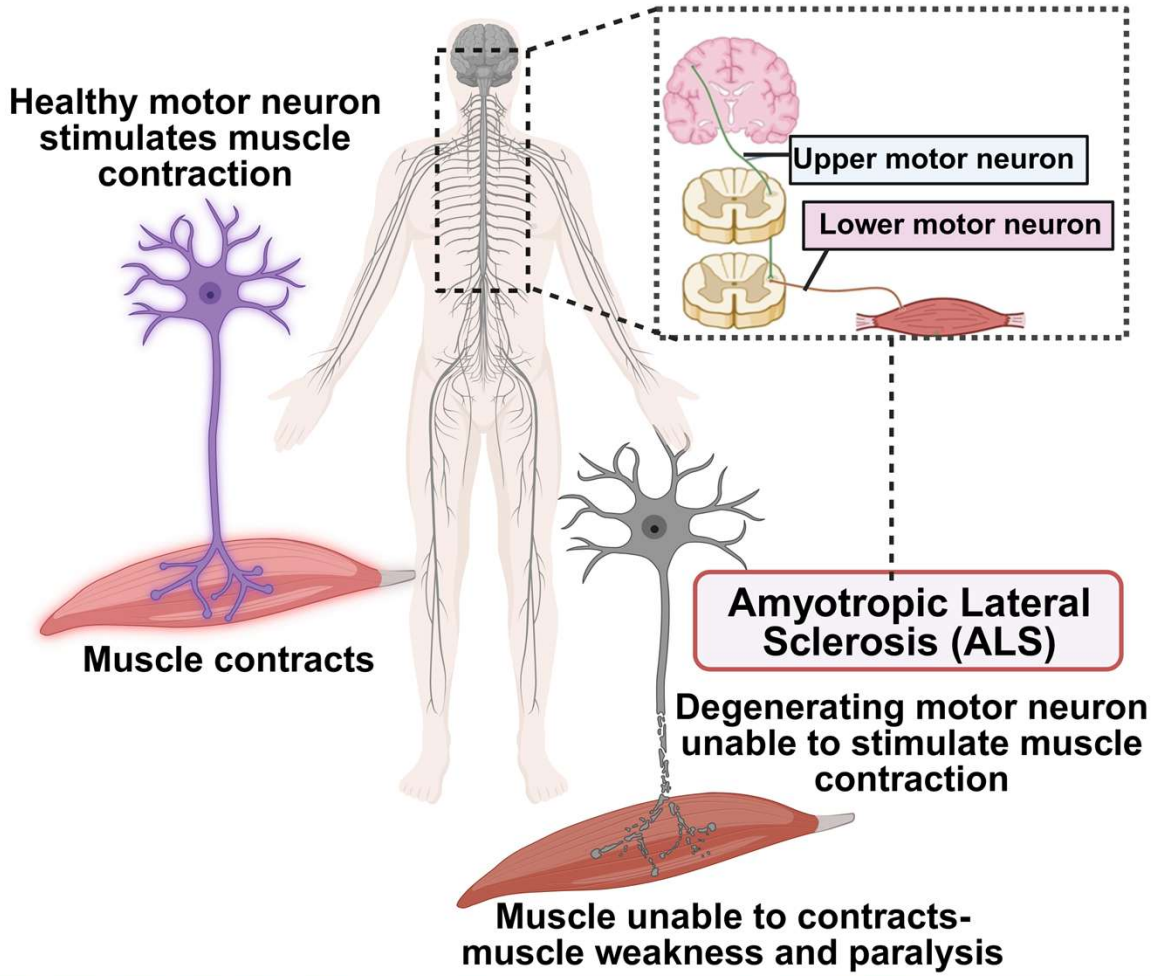
NEXT-GENERATION DELIVERY INNOVATIONS

Outline

- ❑ Introduction of ALS pathophysiology
- ❑ Brief discussion on EVs
- ❑ Hypothesis
- ❑ Result and discussion
- ❑ Future experimental plan
- ❑ Conclusion

Amyotrophic Lateral Sclerosis (ALS)

- Progressive neurodegenerative disease
- Affects upper and lower motor neuron
- Loss of muscle contraction and eventual atrophy paralysis

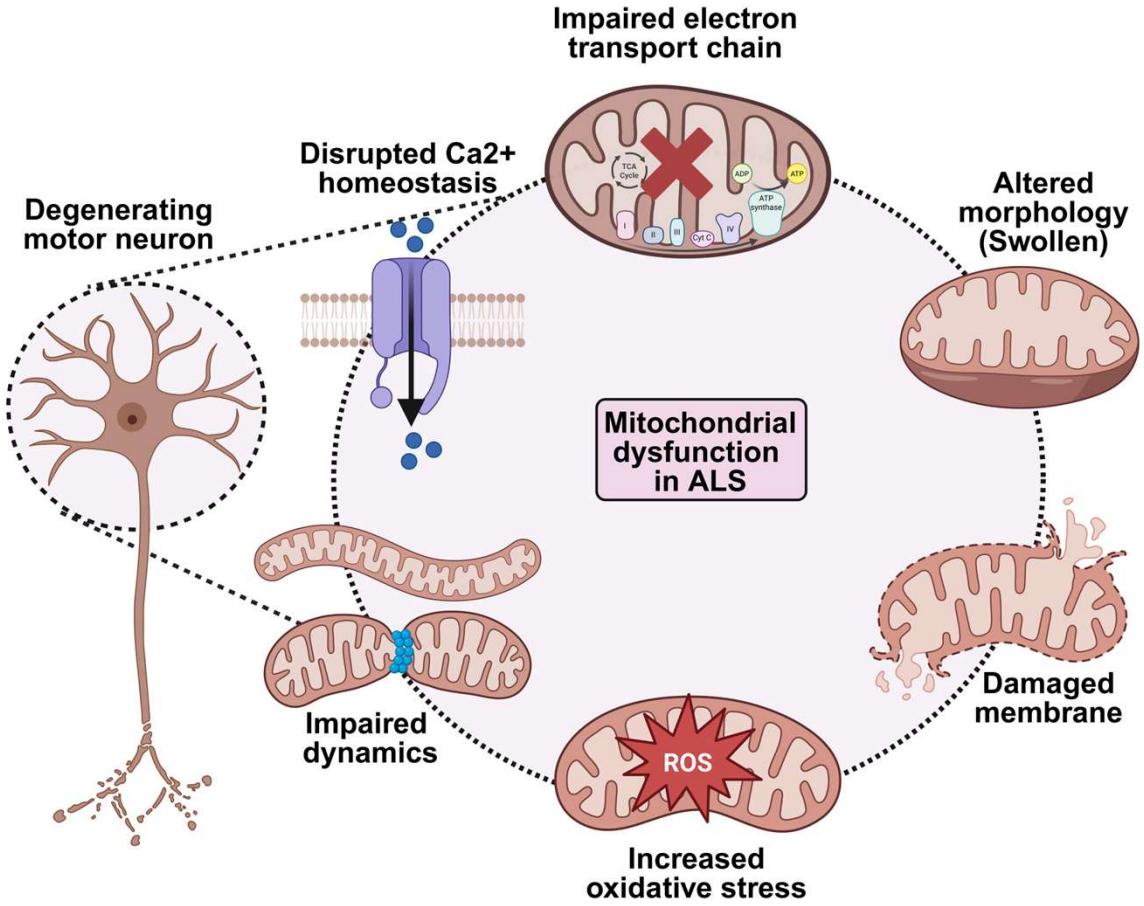


Rizea et al, Understanding Amyotrophic Lateral Sclerosis: Pathophysiology, Diagnosis, and Therapeutic Advances International journal of molecular science, 2024

Image created using Biorender

Mitochondrial dysfunction in ALS

- A key contributor to motor neuron degeneration in ALS



- Kubat et al, Skeletal muscle dysfunction in amyotrophic lateral sclerosis: a mitochondrial perspective and therapeutic approaches, Neurological Sciences ,2024
- Genin et al, Mitochondria, a Key Target in Amyotrophic Lateral Sclerosis Pathogenesis, Genes, 2023

Image created using Biorender

Mitochondria containing extracellular vesicles (EVs)

- Cell-derived, heterogenous nanoparticles
- Mediator of intercellular communication
- Two extensively studied subtypes: lEVs and sEVs
- lEVs carry mitochondria

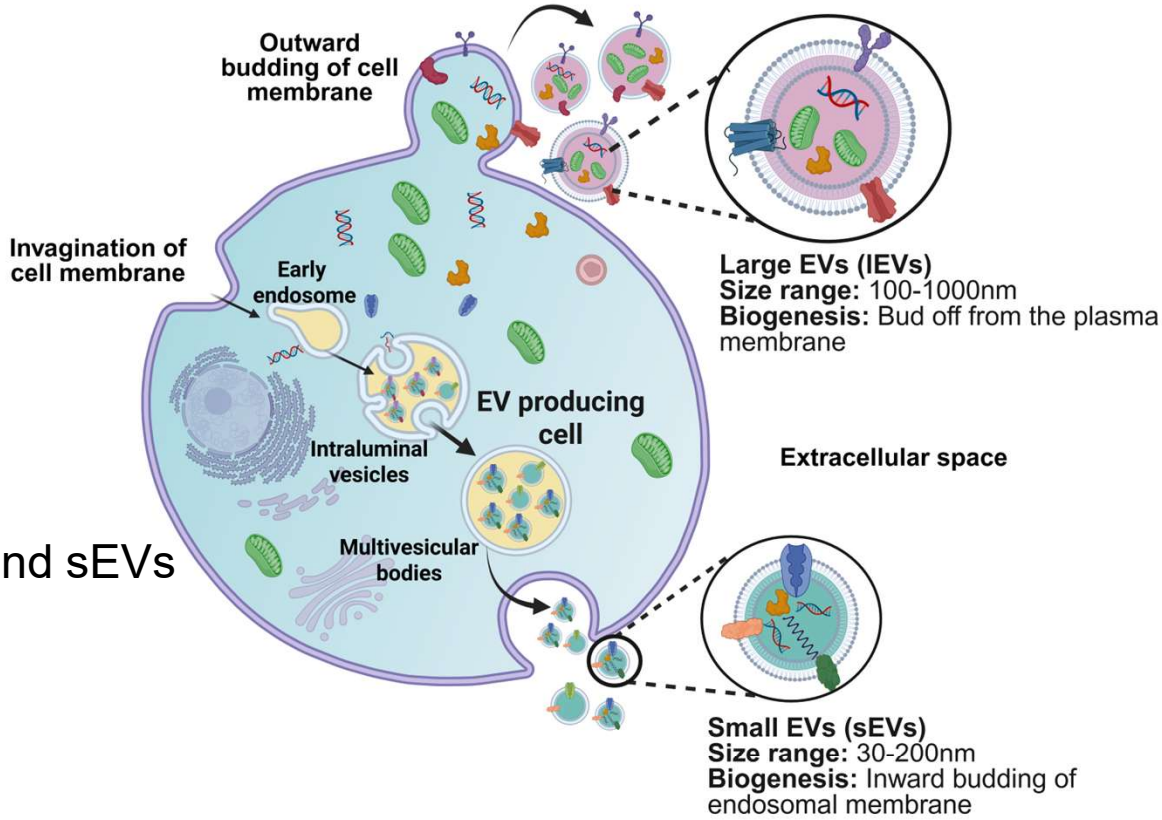
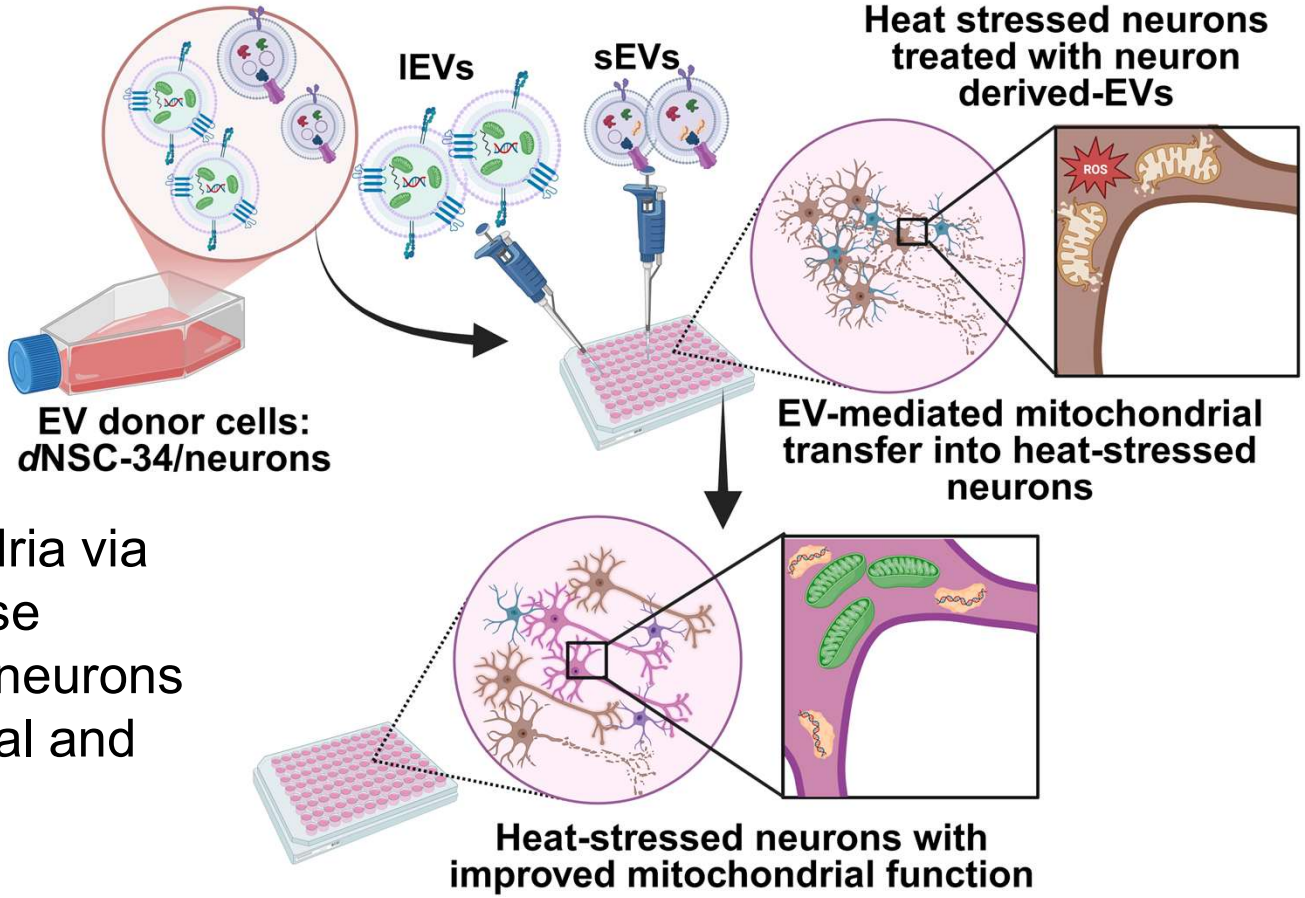


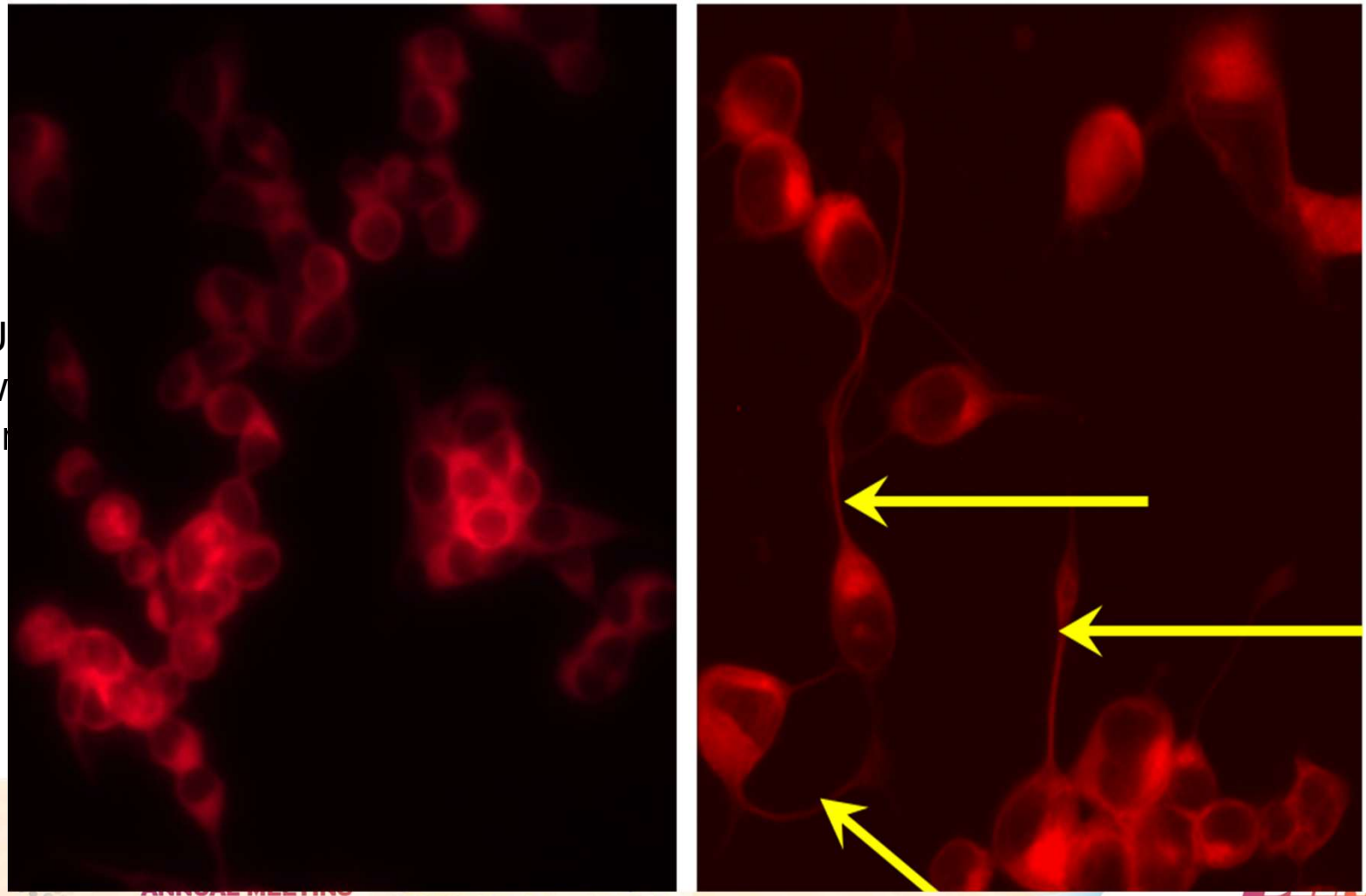
Image taken and modified from: Dave et al, Expert Opinion on Drug Delivery, 2023
Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches, International Society for Extracellular Vesicles, 2024

Hypothesis



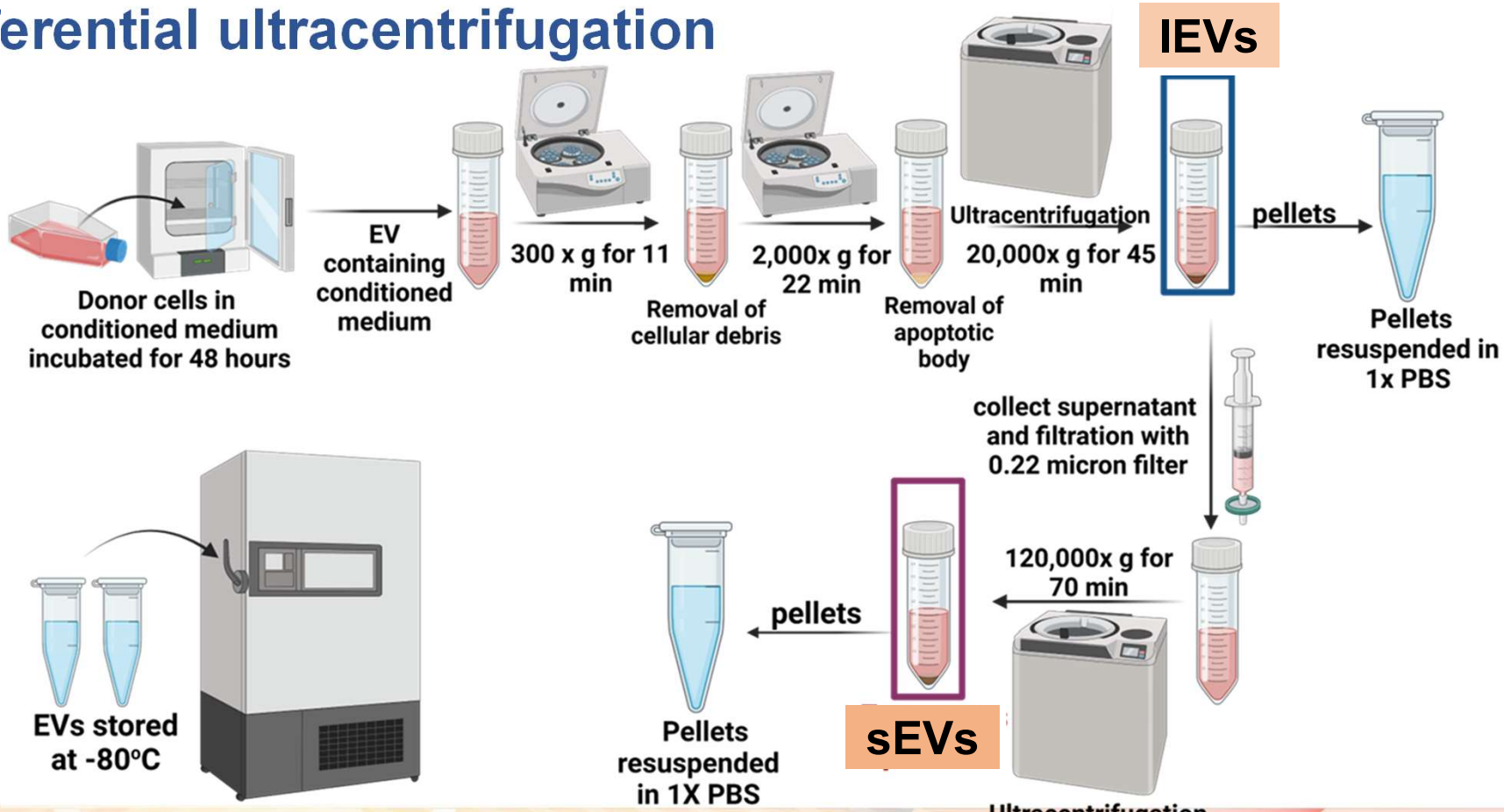
- Delivery of functional mitochondria via neuron-derived lEVs will increase mitochondrial function in motor neurons resulting in improved cell survival and outcomes following ALS.

Prominent neurite outgrowth in *d*NSCs-neuron like



***d*NSC/neuron with prominent neurite outgrowth**

Isolation of neuron derived lEVs and sEVs: Differential ultracentrifugation



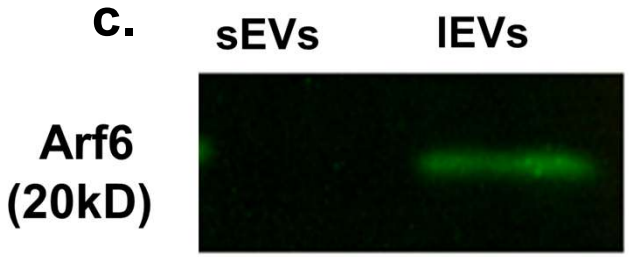
EV particle characteristics and protein marker

a.

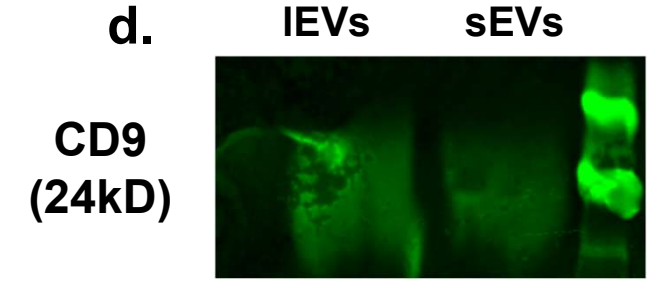
	Neuron-sEVs	Neuron-lEVs
Particle diameter (nm)	153.2 ± 3.4	217± 0.5
Dispersity index	0.26± 0.03	0.28 ± 0.03
Zeta potential (mV)	-22.6 ± 1.3	-37.6 ± 2.0
EV concentration (particles/mL)	$(0.9\pm0.1)\times10^{10}$	$(5.5\pm1.0)\times10^{10}$

Note: Samples were diluted at a 0.1 mg/ml concentration using either PBS or 10mM HEPES at pH 7.4 to measure particle size and zeta potential, respectively. Samples were run in triplicate and data were represented as mean± standard deviation (SD).

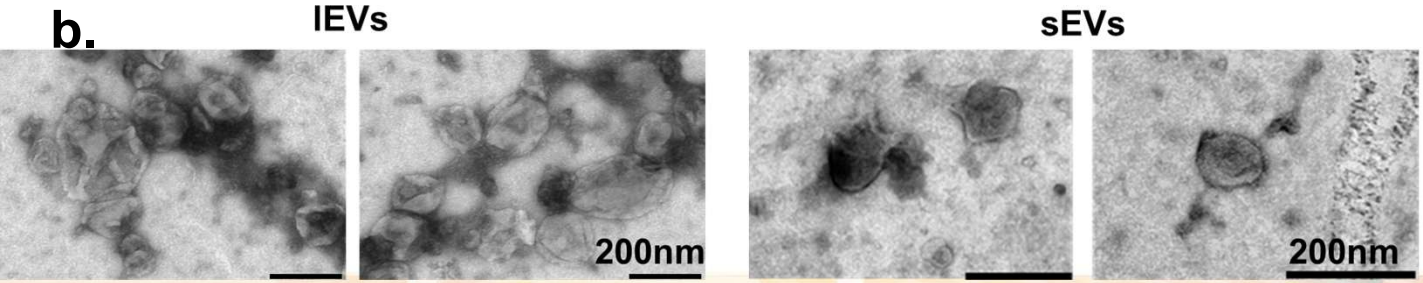
c.

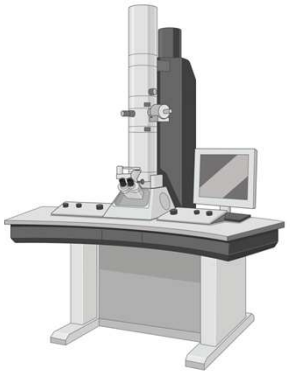


d.



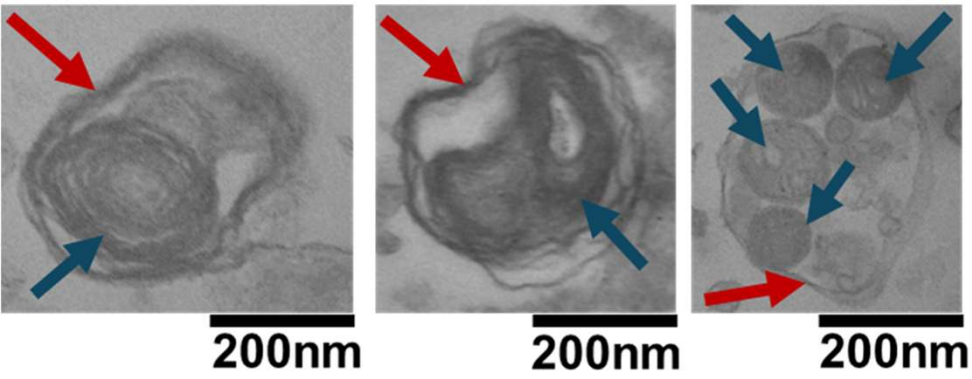
b.





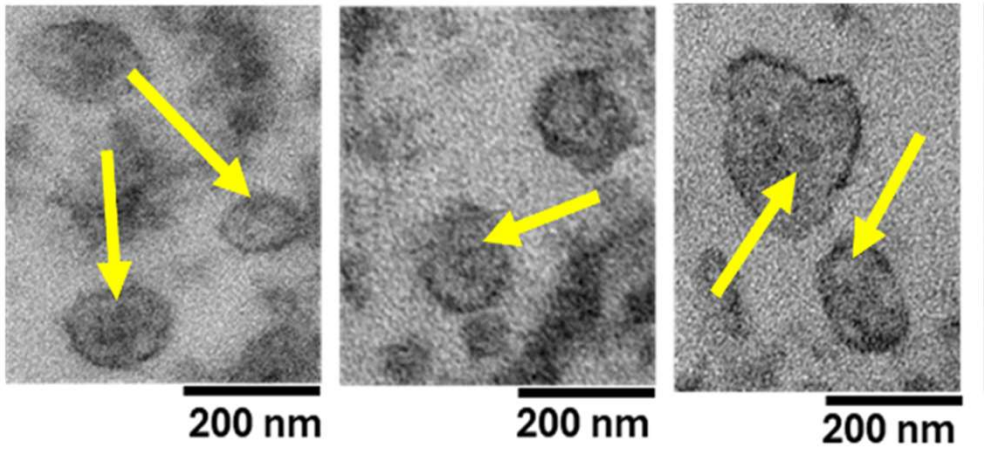
Neuron derived lEVs but not sEVs contain mitochondria

- ❑ TEM images showed presence of mitochondria in lEV lumen
- ❑ sEVs did not show mitochondria



lEVs

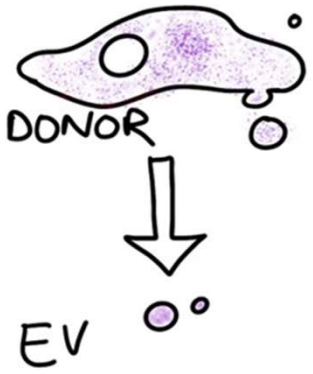
Mitochondria



sEVs

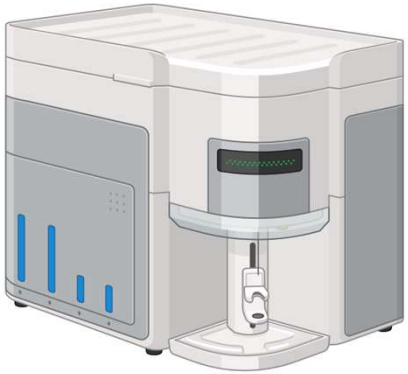
lEVs transferred the mitochondrial load into recipient neurons

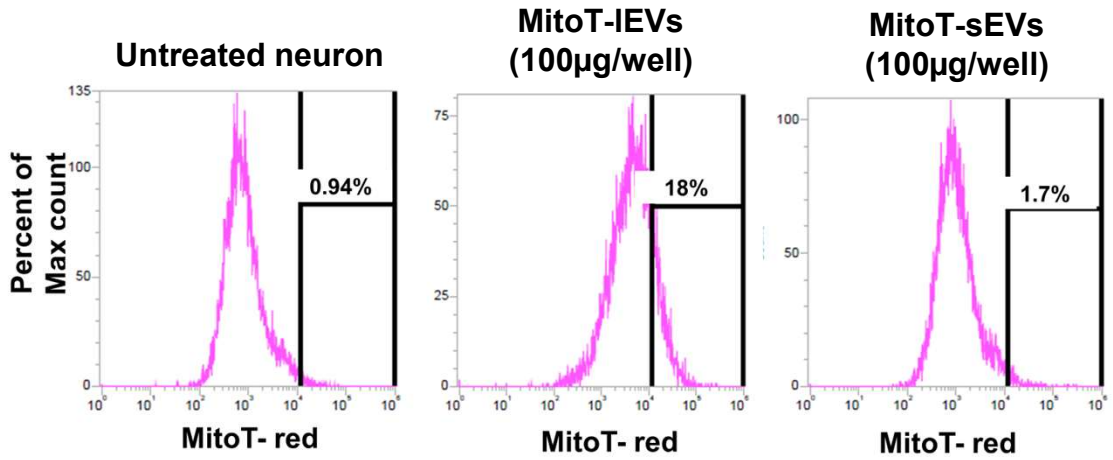
Donor neurons labeled with Mitotracker-Red

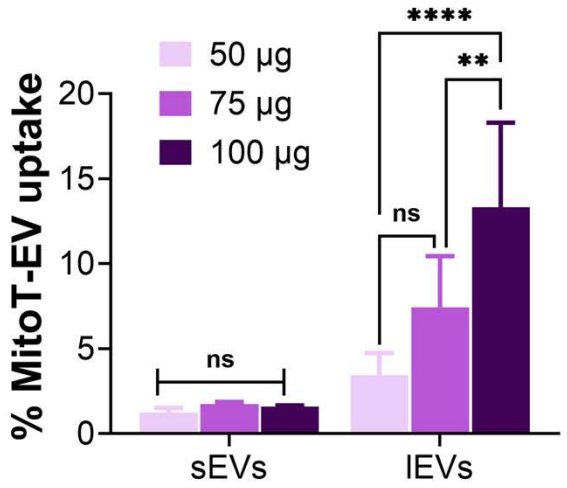


EVs with stained mitochondria

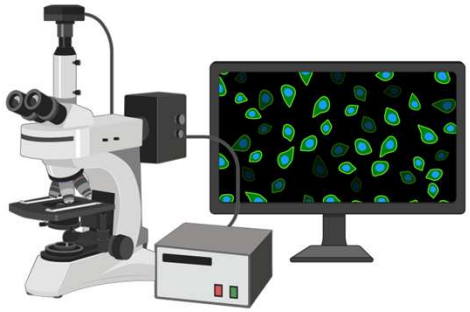
Flow cytometry

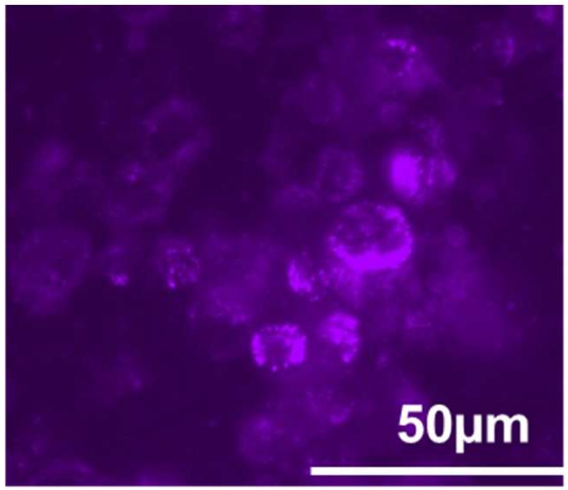




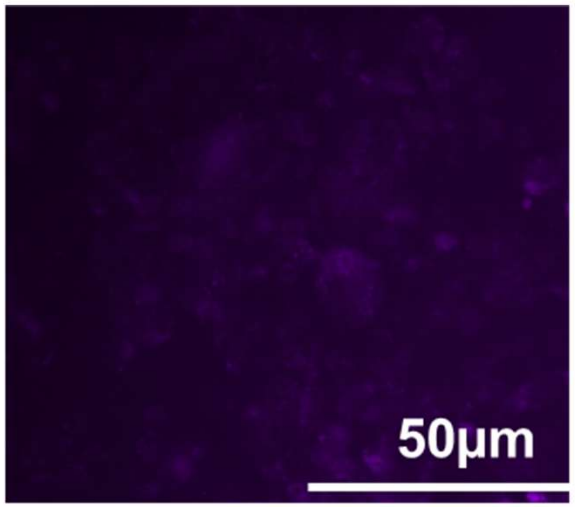


lEVs transferred their mitochondrial load into recipient neurons

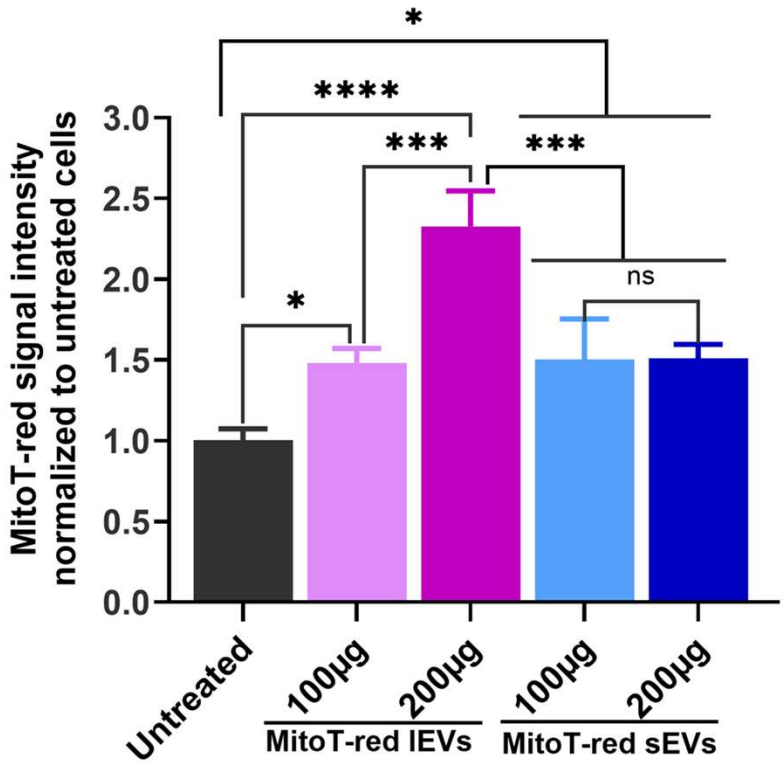




Recipient neurons with MitoT-lEVs (200 µg/well)

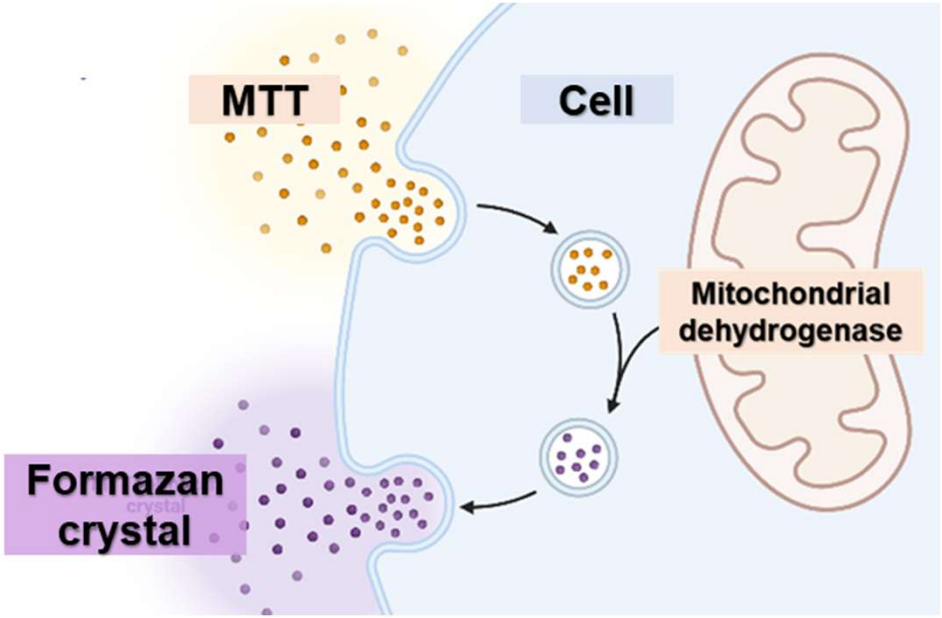


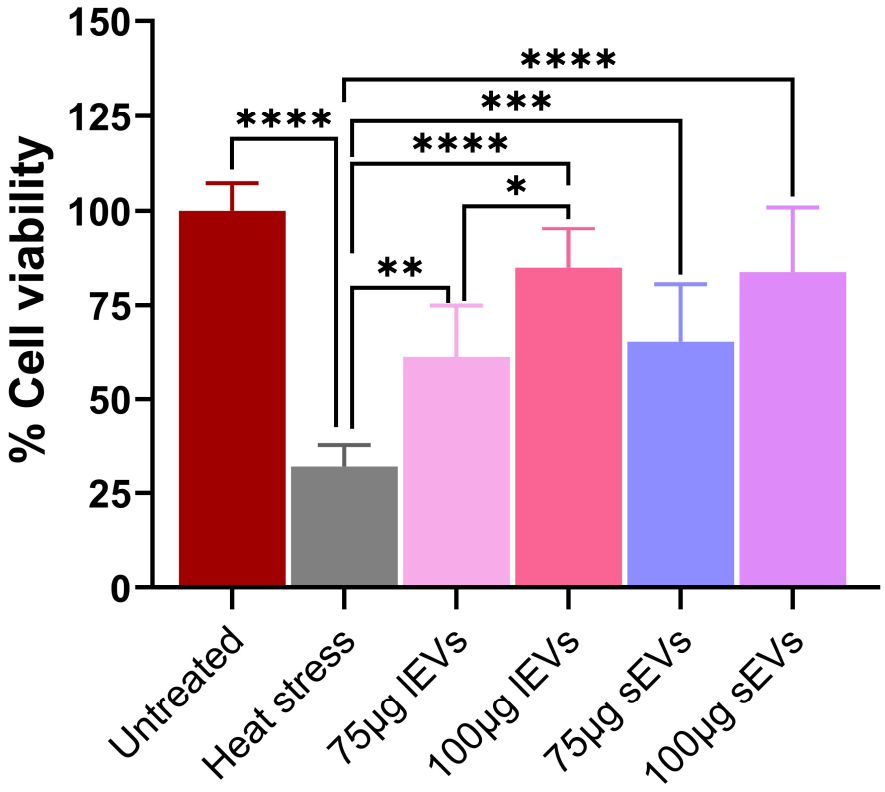
Recipient neurons with MitoT-sEVs (200 µg/well)



Functional effect of neuron derived EVs:

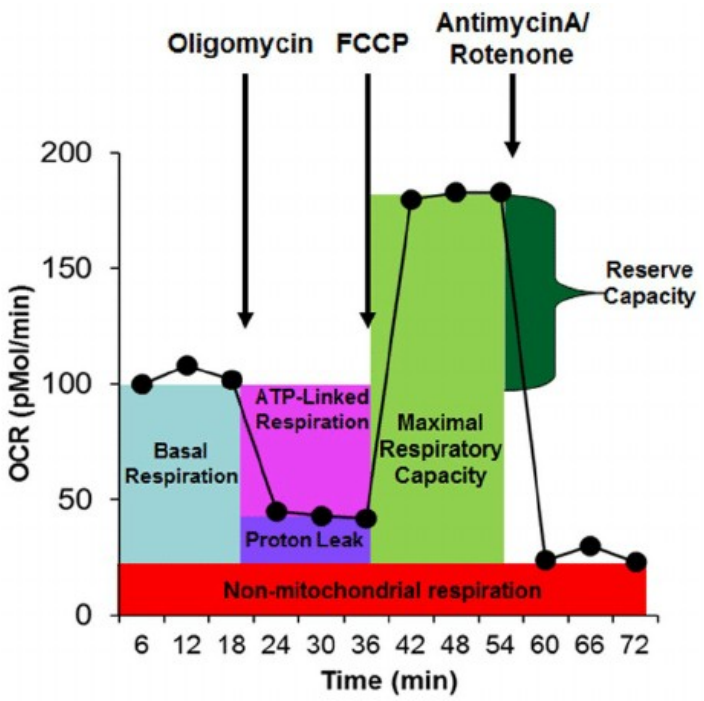
Neuron derived lEVs and sEVs increased cell viability of heat stressed neurons



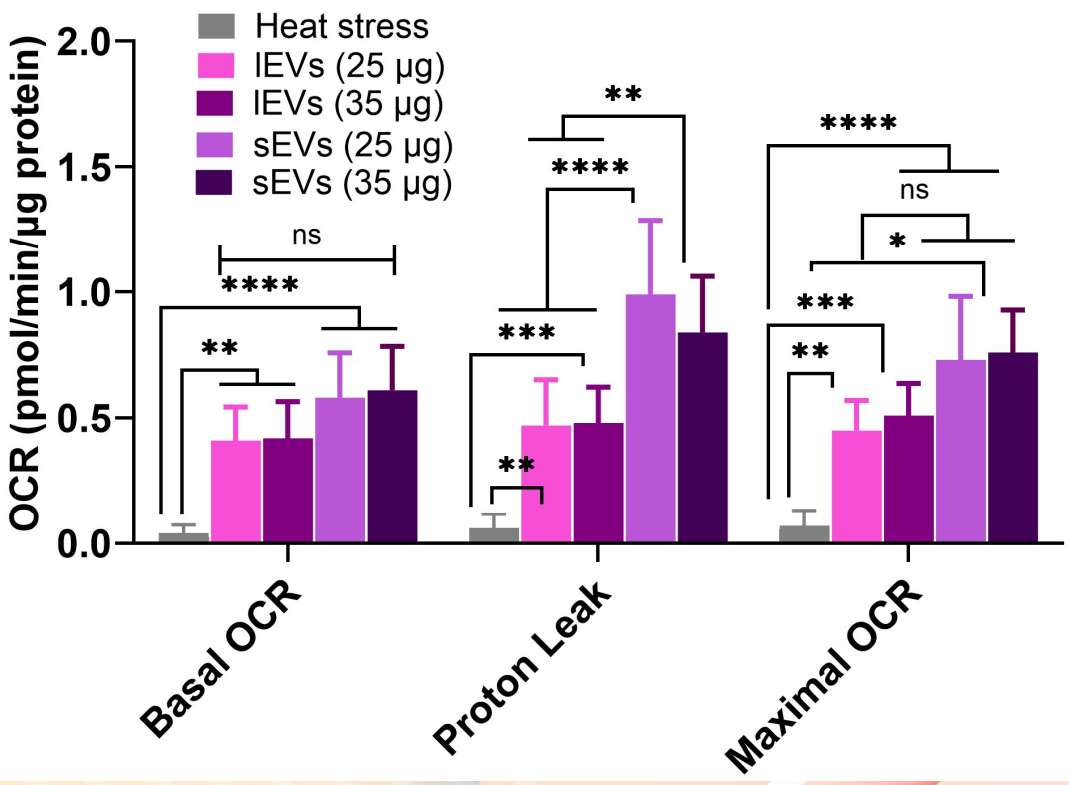


Functional effect of neuron derived EVs:

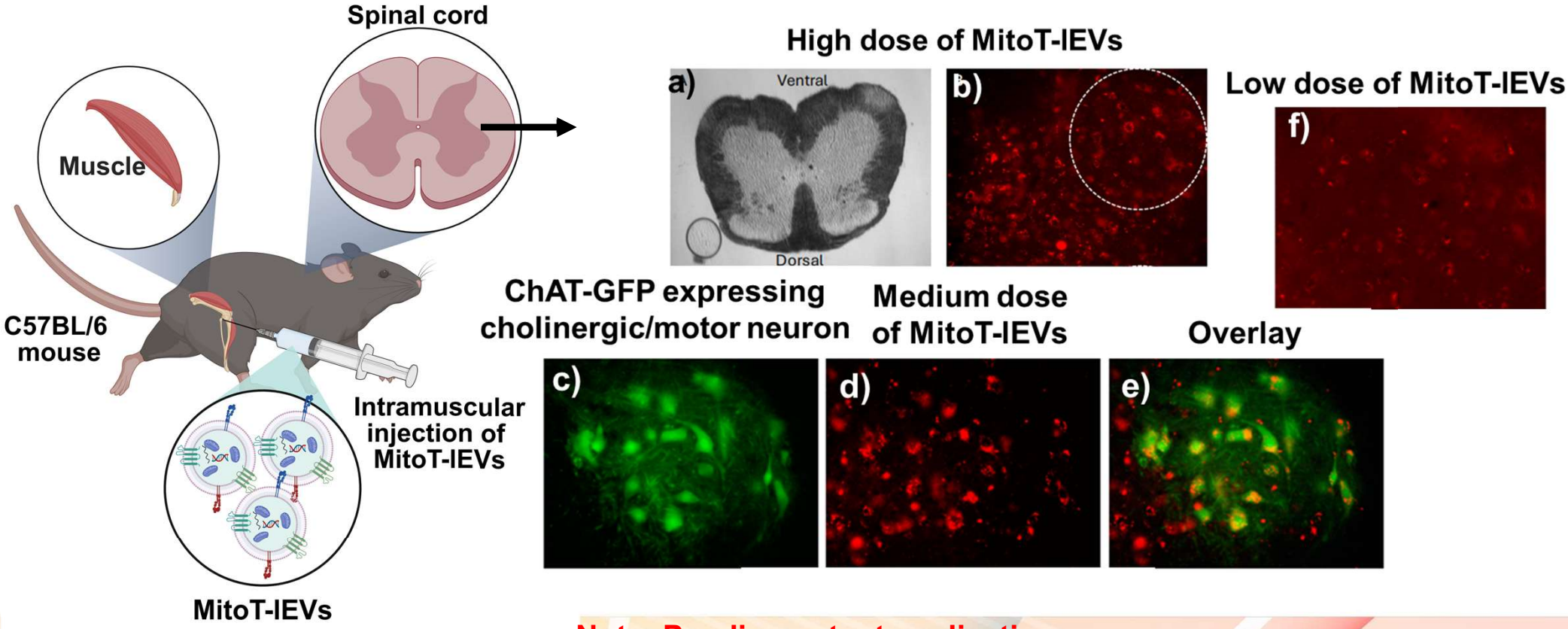
Neuron derived lEVs and sEVs improved mitochondrial function in heat stressed neurons



Mitochondrial respiration



Efficient dose-dependent uptake of MitoT-IEVs by mouse motor neurons *invivo* upon intramuscular injection



Note: Pending patent application
Please do not take photo

Future study

- Quantifying mtDNA in EVs using qPCR
- Comparing the proteomic profiles of sEVs *vs*. lEVs
- Evaluating the therapeutic efficacy of lEV-delivered mitochondria in SOD1^{G93A} transgenic mouse model of ALS

Conclusion

- Developed an innovative therapeutic strategy to treat ALS via lEV-mitochondria.
- Demonstrated increased mitochondrial function in the recipient neurons treated with neuron derived EVs
- Delivery of lEV-mitochondria delivery into mouse motor neurons via intramuscular injection.

Acknowledgement

Advisor

- **Dr. Devika Soundara Manickam**

Former and new lab members

- Kandarp Dave
- Jhanvi Rajan Javeri
- Purva Khare
- Vivek Basudkar
- Balaji Govindaswamy

Collaborators

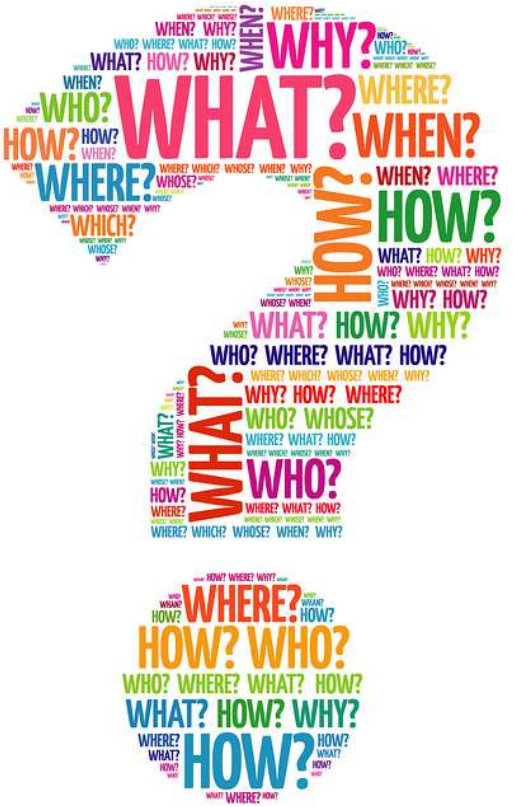
- Dr. Donna B. Stolz
  Center for Biologic Imaging,
  University of Pittsburgh Medical School
- Si-yang Zheng
  Department of Biomedical Engineering,
  Carnegie Mellon University
- Dr. Carol Milligan
  Department of Translational Neuroscience,
  Wake Forest University School of Medicine
- Osvaldo Delbono
  Internal Medicine, Gerontology and Geriatric Medicine
  Wake Forest University School of Medicine
- Sruti S. Shiva
  Pittsburgh Heart Lung Blood Vascular Institute,
  University of Pittsburgh Medical School

Thank you $$$!

Award Number:
HT9425-23-1-0218

DUQUESNE UNIVERSITY


THANK YOU
FOR YOUR
ATTENTION


WHY?
WHAT?
WHERE?
WHEN?
WHO?
HOW?
WHICH?
WHOSE?
WHAT? HOW? WHY?
WHO? WHERE? WHAT? HOW?
WHERE? WHICH? WHOSE? WHEN? WHY?
WHY? HOW? WHERE?
WHO? WHOSE?
WHERE? WHAT? HOW?