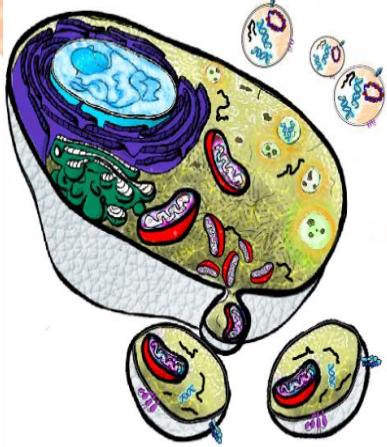


Engineering of mitochondria-enriched brain endothelial cell-derived extracellular vesicles

Manickam Lab of Biotherapeutics Delivery



Follow us on Twitter @manickam_lab

Vivek Basudkar, M.S
First Year Graduate Student
Duquesne University

Advisor: Dr. Devika S. Manickam
UTHealth, Houston



CRS 2025
ANNUAL MEETING & EXPOSITION

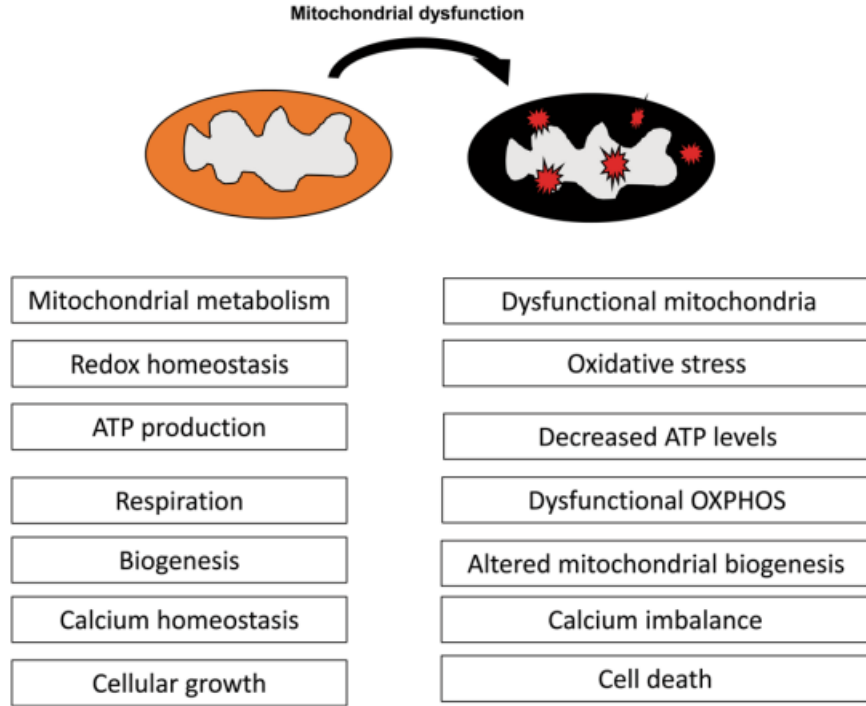
PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION
DELIVERY INNOVATIONS

Content

- Introduction to mitochondrial dysfunction
- Extracellular vesicles
- Hypothesis
- Key findings
- Conclusion
- Future direction

Mitochondrial dysfunction



Ischemic stroke

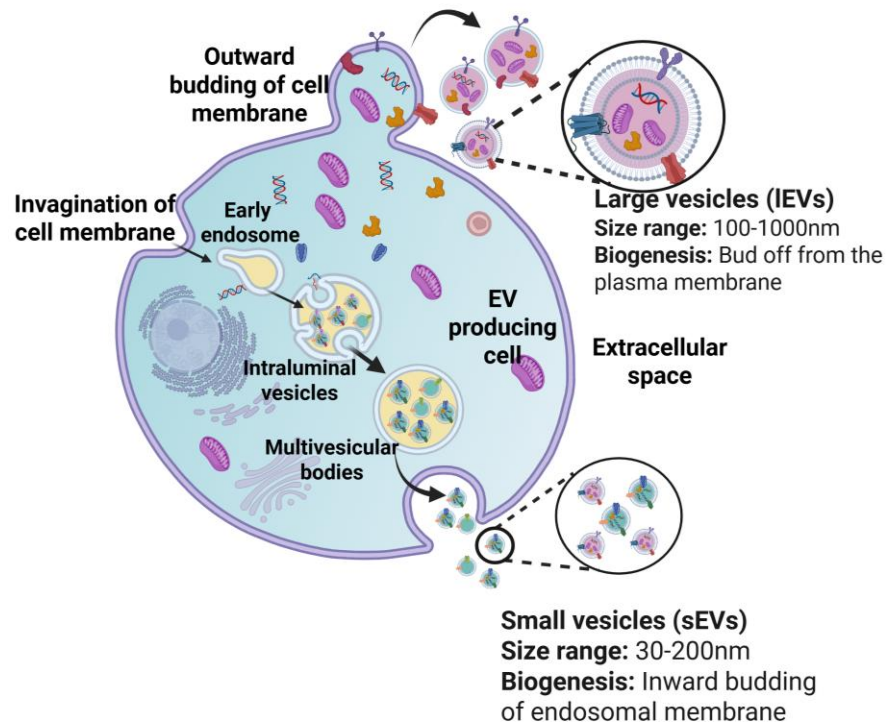
Autism spectrum disorder

Amyotrophic Lateral Sclerosis

Alzheimer's disease

Image adapted from Sreedhar *et al.*, Cell Death and Disease, 2020 and created in Biorender
OXPHOS: Oxidative phosphorylation

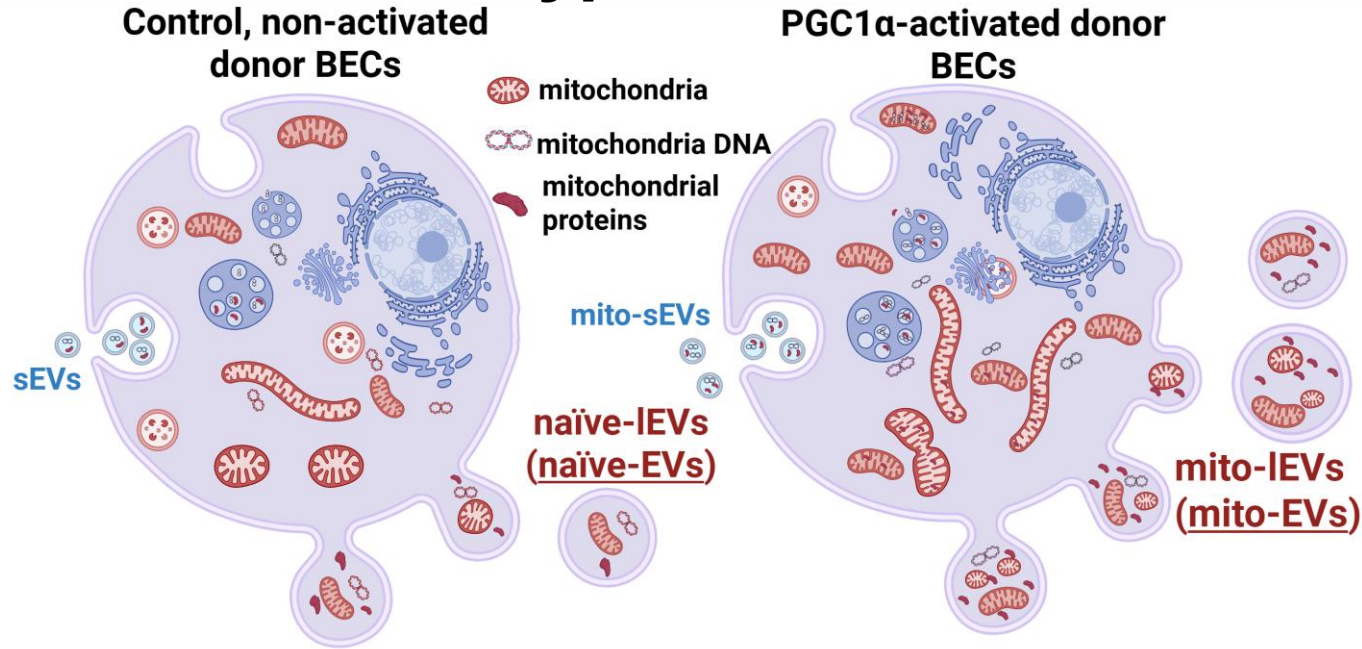
Extracellular vesicles (EVs)



- EVs are enclosed by natural lipid bilayer
- Low immunogenicity and biocompatibility
- EVs can be used as a carrier for mitochondria
- Large vesicles transfer mitochondria to recipient brain endothelial cells (BECs)

Adapted and modified from Dave et al. Expert Opinion on Drug Delivery
Created in Biorender

Hypothesis

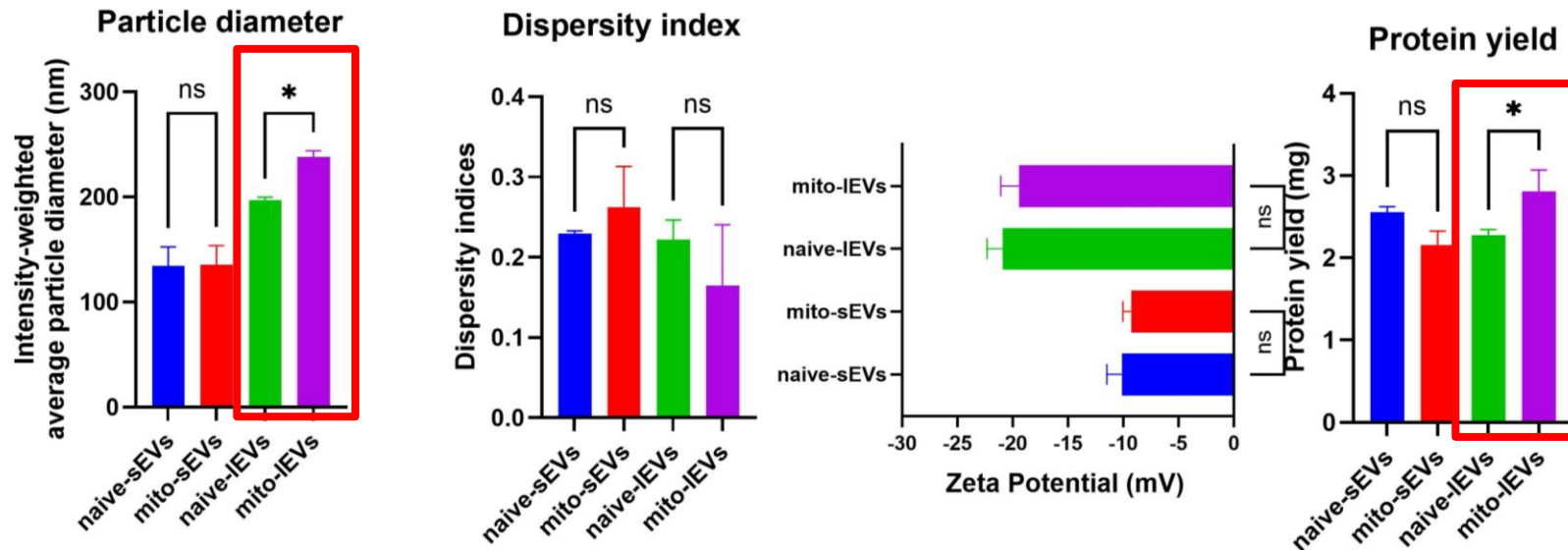


Activating of PGC1 α in donor BECs using resveratrol will increase the quality of mitochondria packed in IEVs

Created in Biorender

Physicochemical characterization

naive-EVs and mito-EVs isolated from b.End3



Deletion of 16p11.2 genetic locus

Autism spectrum disorder is considered a complex genetic disorder with high heritability

16p11.2 deletion syndrome, one of the most common genetic causes of ASD

16p11.2 deletion-induced BEC dysfunction is linked to a bioenergetic failure

In vitro network formation assay to assess angiogenic activity over 24 hours

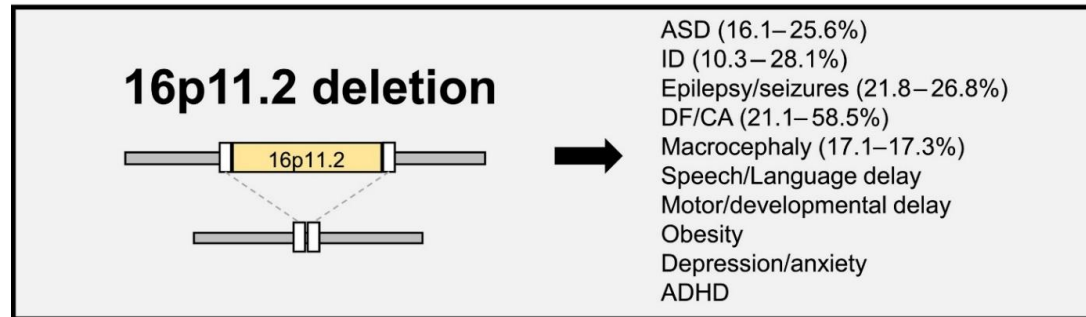


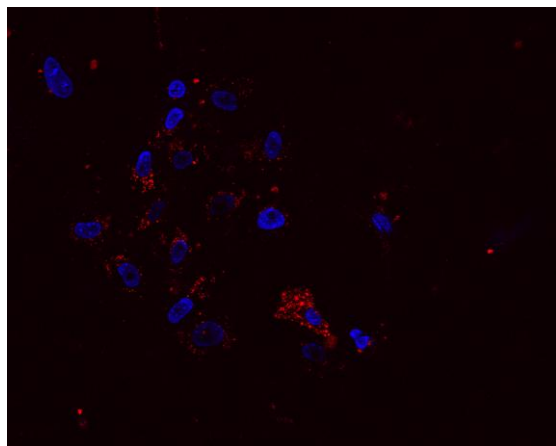
Figure adapted from Sreedhar *et al.* Cell Death and Disease, 2020

ADHD: attention-deficit/hyperactivity disorder

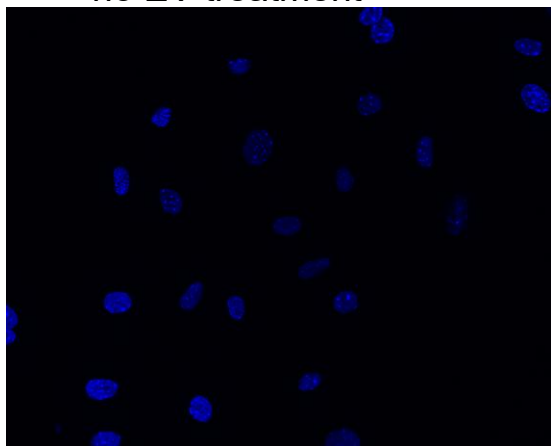
ID intellectual disability

Mito-EV uptake in 16p11.2-deleted BECs

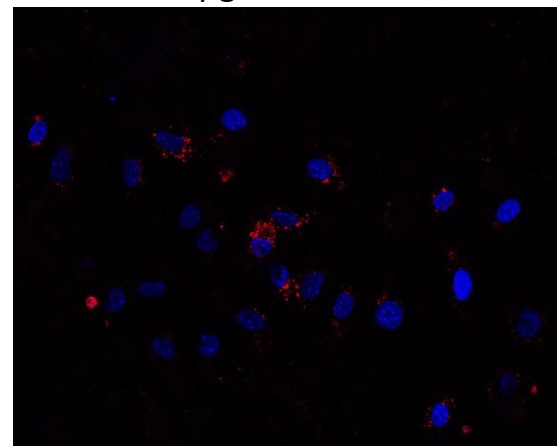
WT 25μg of mito-EVs



16p11.2-deleted BECs
no EV treatment



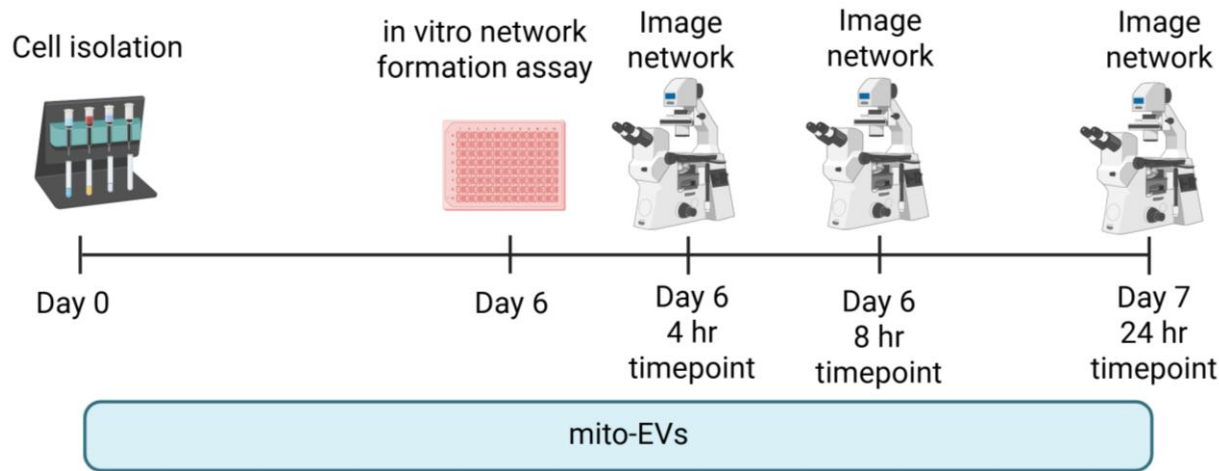
16p11.2-deleted BECs
25μg of mito-EVs



Mito-EVs are internalized by both healthy and ASD 16p11.2-deleted BECs

Data generated in collaboration with Dr. Baptiste Lacoste and Dr. Julie Ouellette

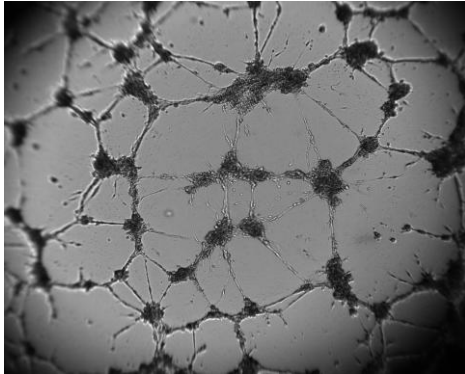
Experimental timeline: *in-vitro* network formation assay



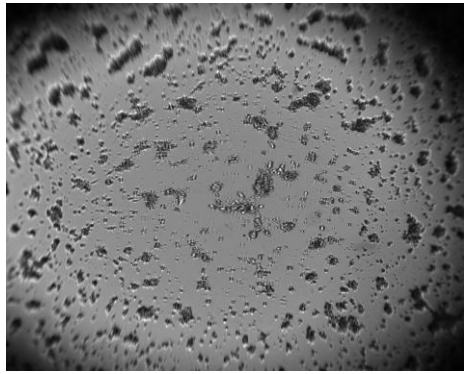
Experimental timeline for treatment of 16p11.2-deleted ECs with mito-EVs following cell isolation and a 24-hour *in-vitro* network formation assay

In-vitro network formation: ASD BECs

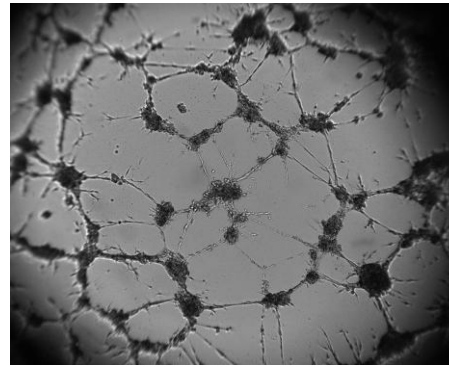
WT-BECs



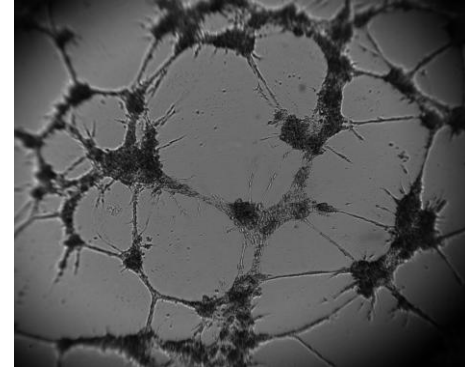
16p11.2-deleted BECs



16p11.2-deleted BECs
25µg mito-IEVs (24 h)



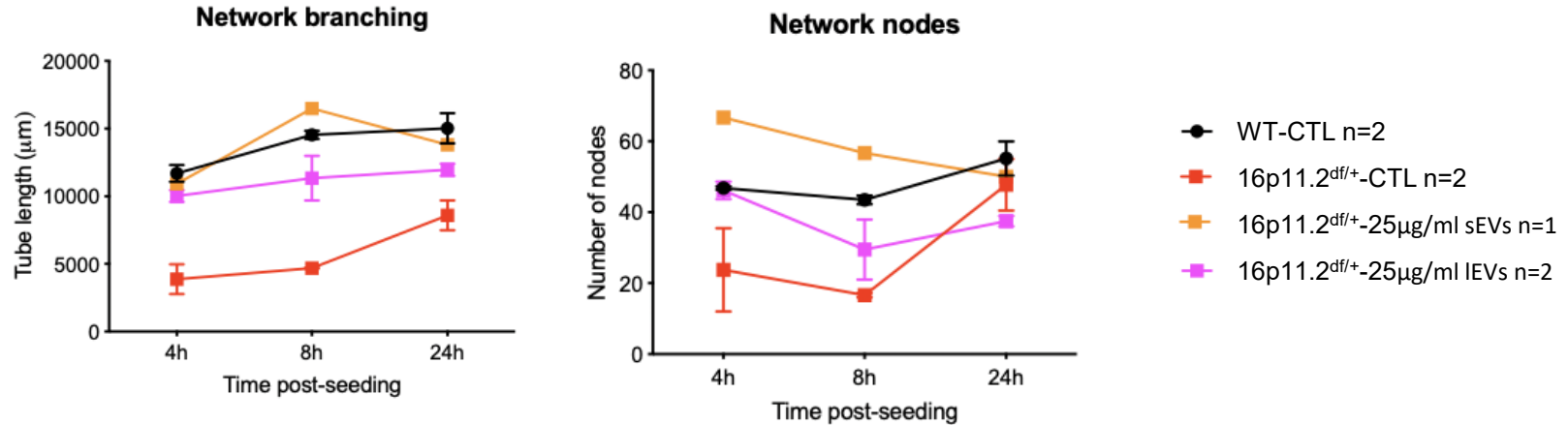
16p11.2-deleted BECs
25µg mito-sEVs (24 h)



Formation of network in 16p11.2 deleted BECs

Data generated in collaboration with Dr. Baptiste Lacoste and Dr. Julie Ouellette

Quantification of network branch and nodes



Mito-EV restores the network branch and branching in 16p11.2 deleted BECs

Data generated in collaboration with Dr. Baptiste Lacoste and Dr. Julie Ouellette

*pilot study

Conclusion

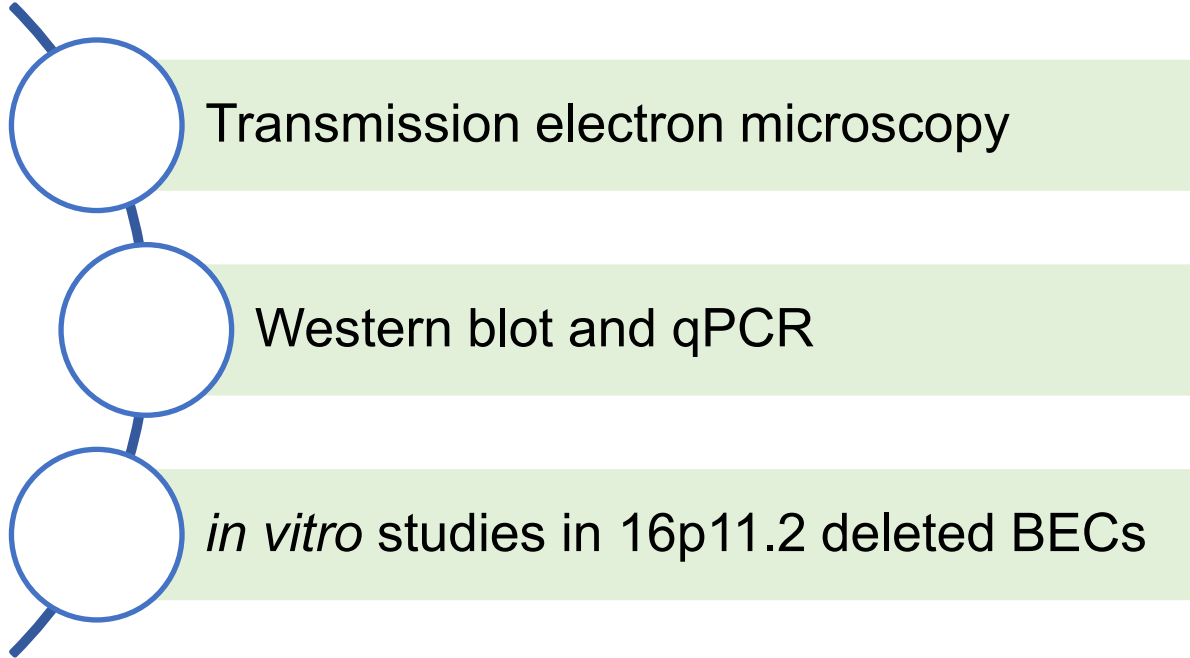
mito-IEVs showed
increase in particle
size and protein
concentration

mito-EVs were
internalized by
16p11.2-deleted
BECs

In-vitro network
formation assay
suggests recovery of
16p11.2-deleted
BECs cells

mito-EVs can be used as a potential treatment for ASD

Future direction



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Lab members

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Balaji Govindaswamy

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Dr. Baptiste Lacoste

Dr. Julie Ouellette



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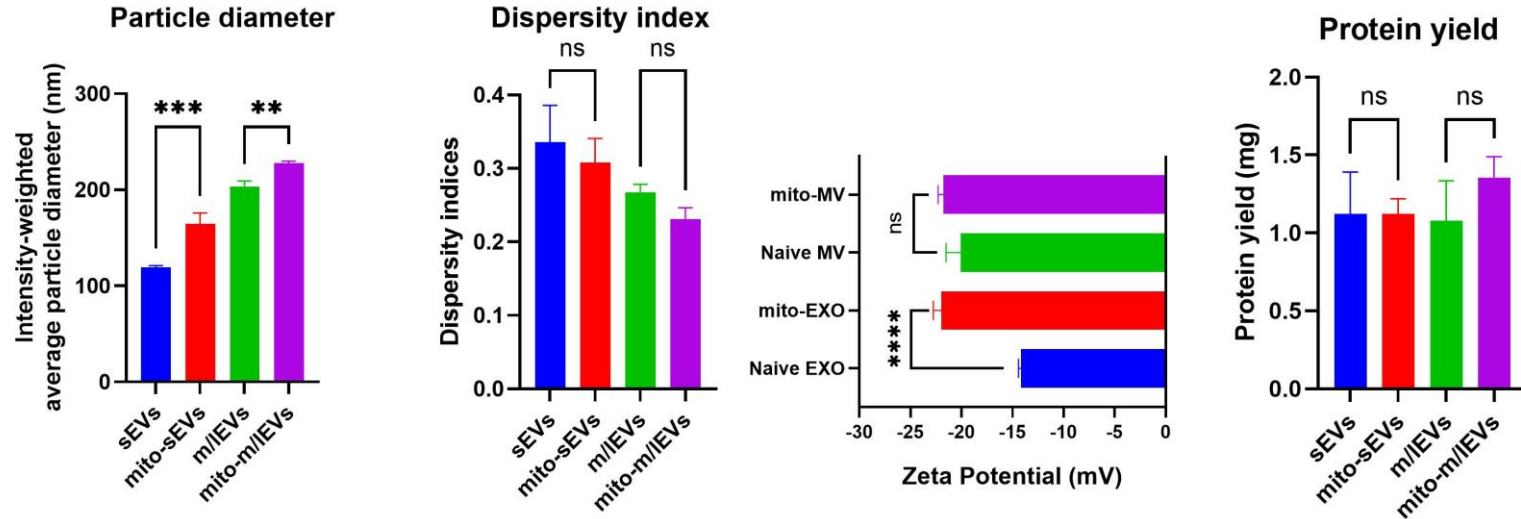


Graduate School of
Pharmaceutical Sciences

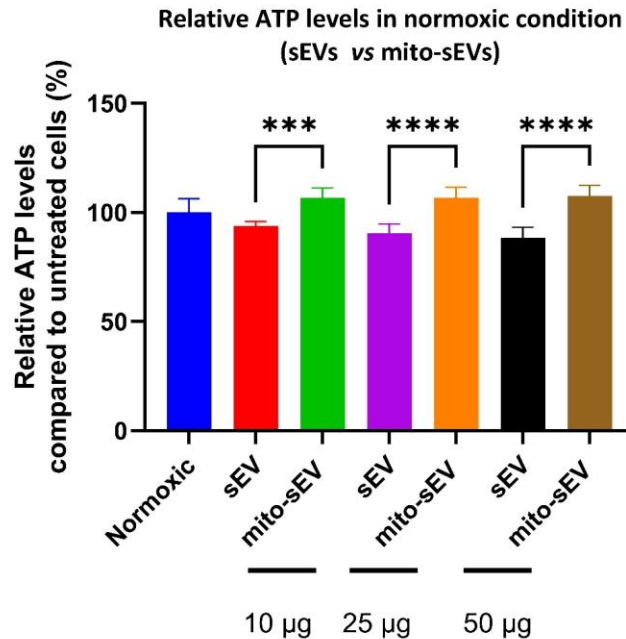
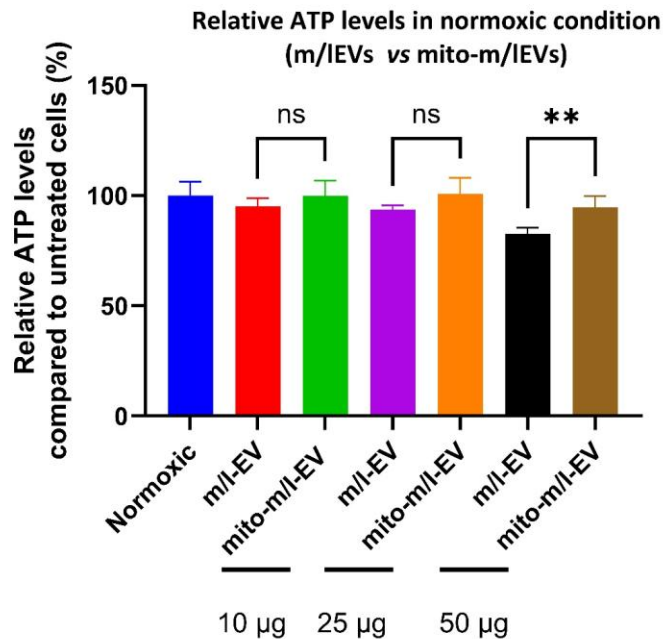
Thank you!

Physicochemical characterization

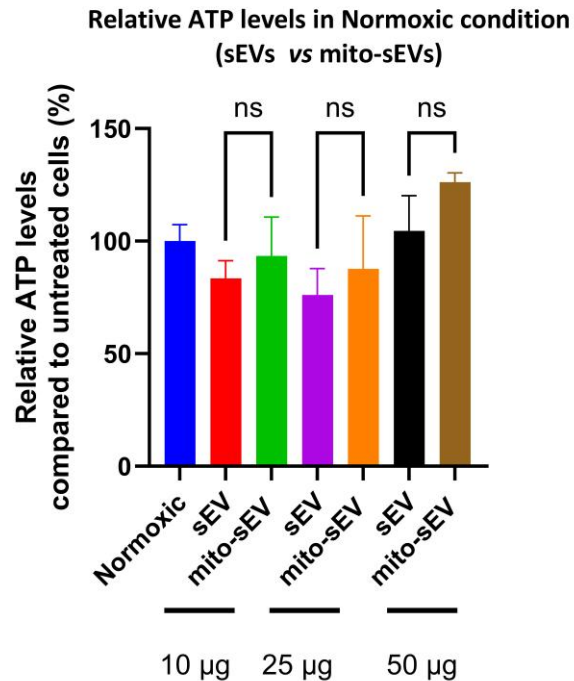
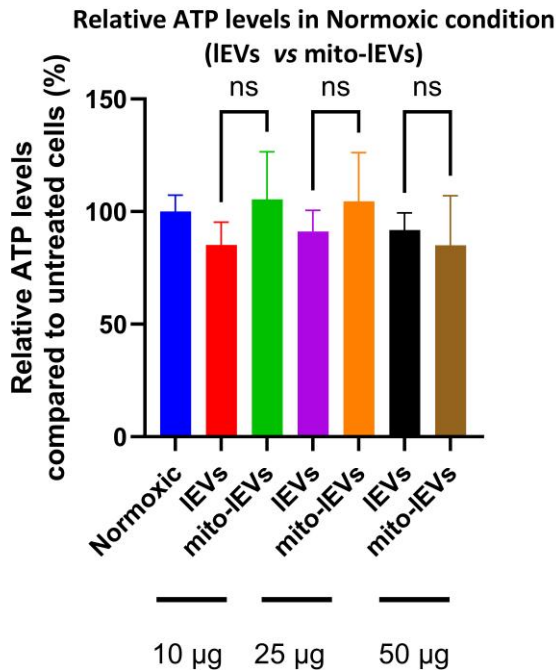
Physicochemical characteristics of EVs and mito-EVs isolated from hCMEC/D3



ATP assay in hCMEC/D3 cell line

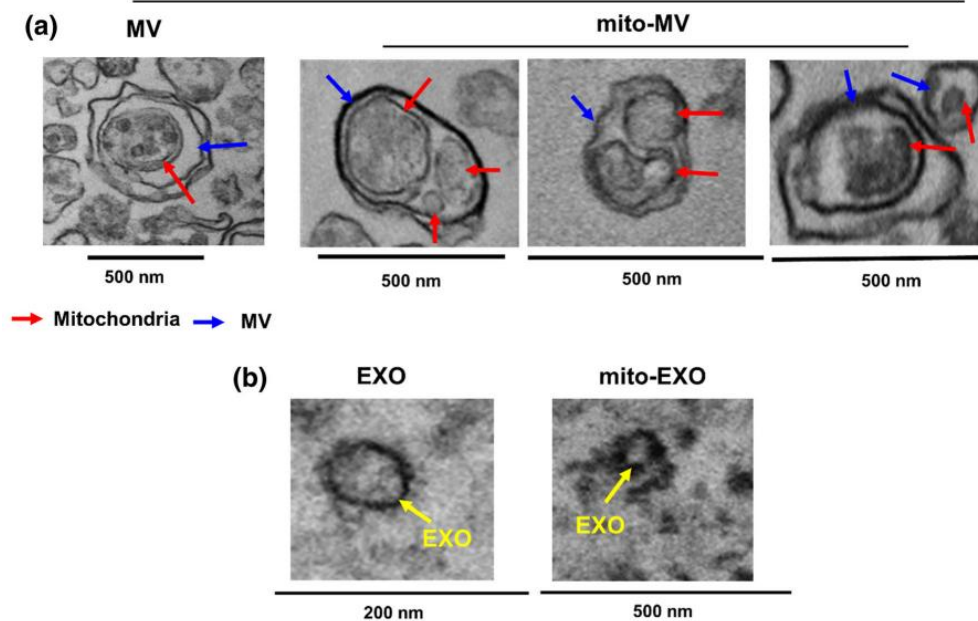


ATP assay in recipient b.End3 cells

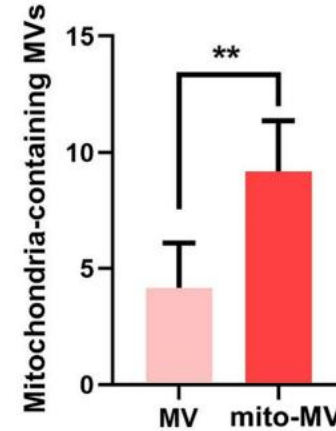


Transmission electron microscopy

TEM of cross-sectioned



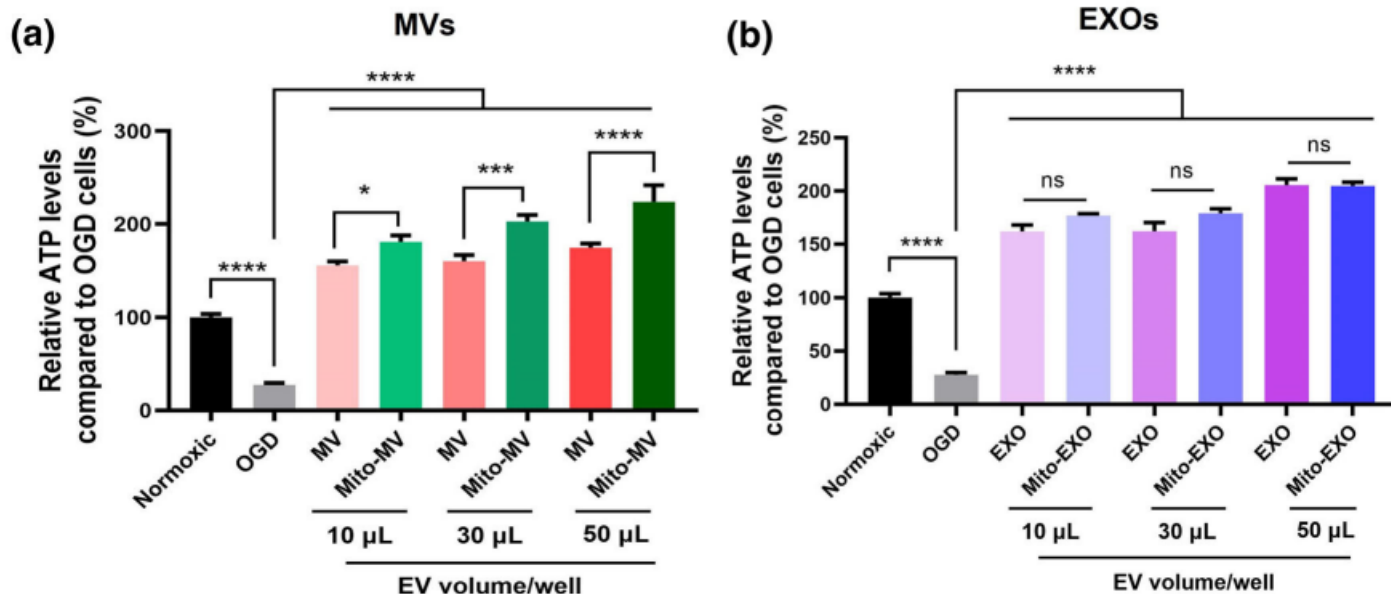
(c) Quantification of mitochondria-containing MVs



Dave *et al.*, Cellular and Molecular Bioengineering 2022

ATP assay

Effect of EVs on relative ATP levels in the recipient BECs



Dave *et al.*, Cellular and Molecular Bioengineering 2022