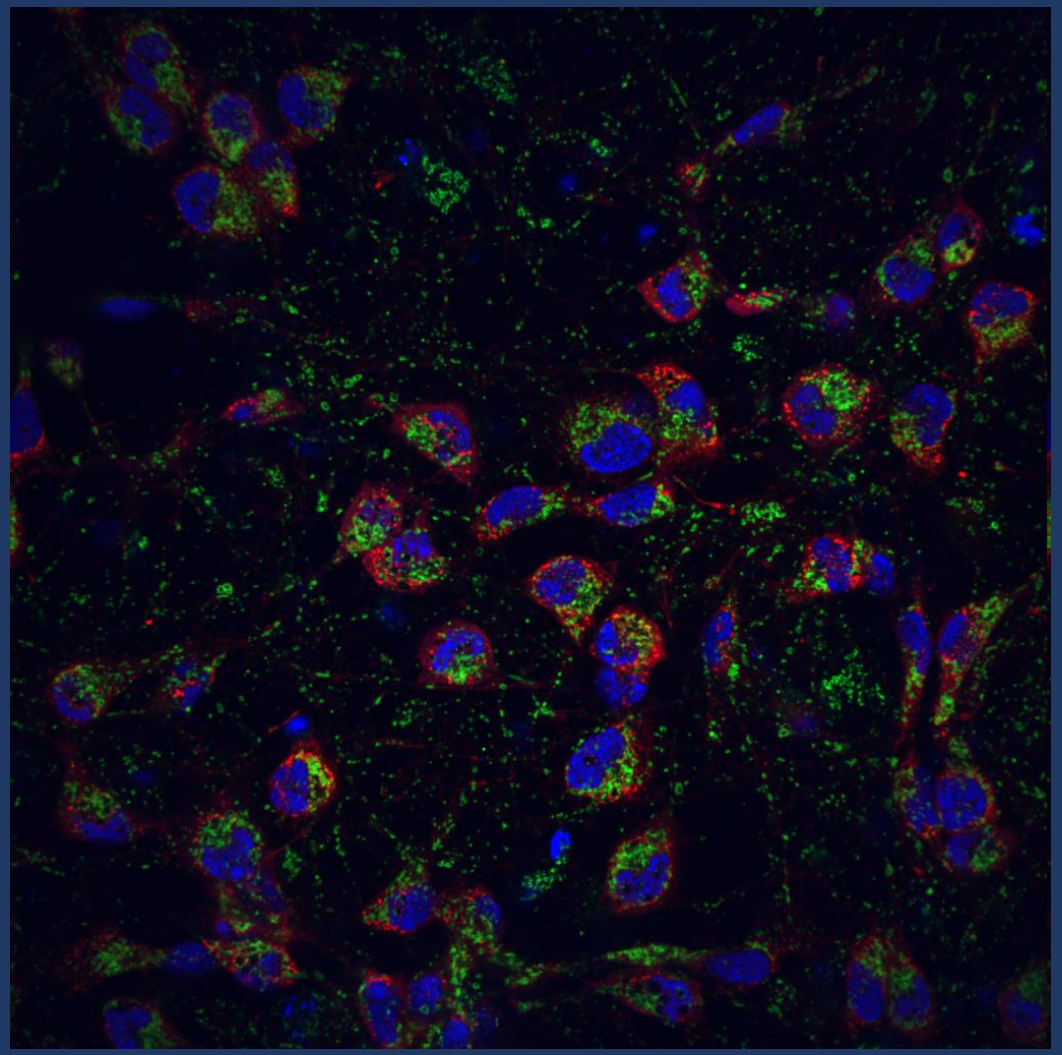
Characterizing the AI-suggested inter-organellar crosstalk between stress granules and mitochondria in human neurons



End of rotation presentation

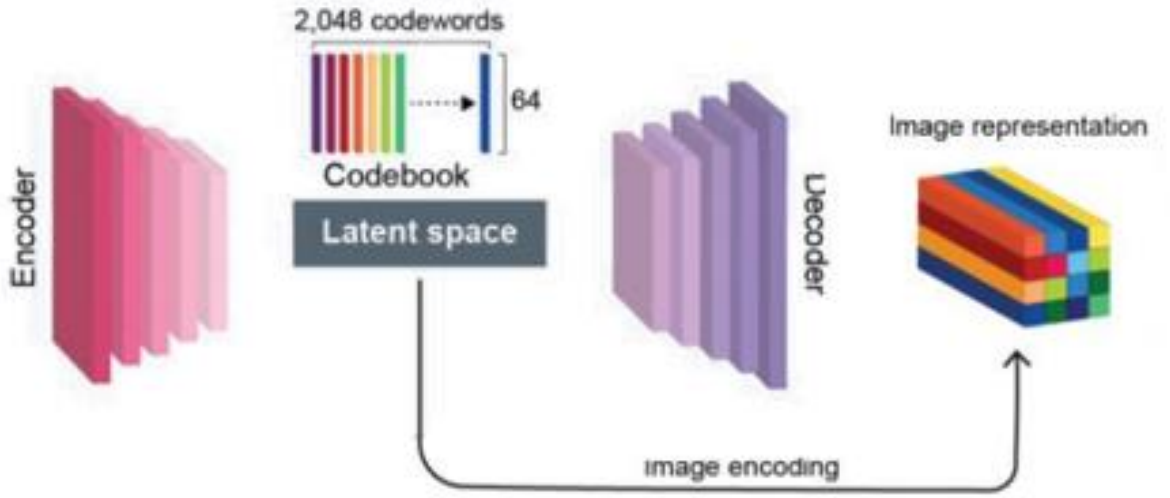
Hornstein's Lab

Lior Lin

16.07.24

A deep learning-Based ‘Organellomics’ Approach

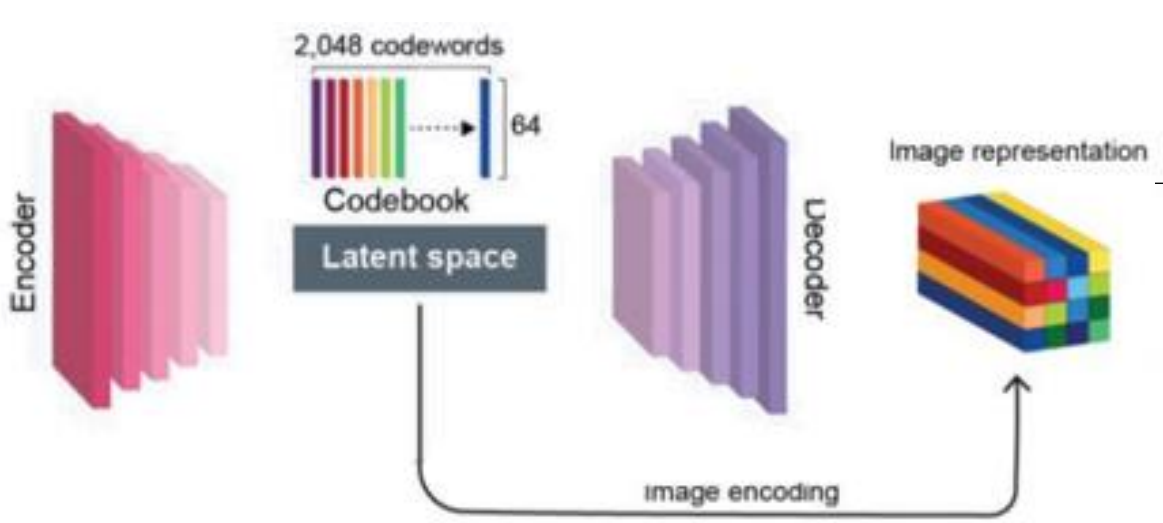
The **NOVA** model
Neuronal Organellomics Vision Atlas

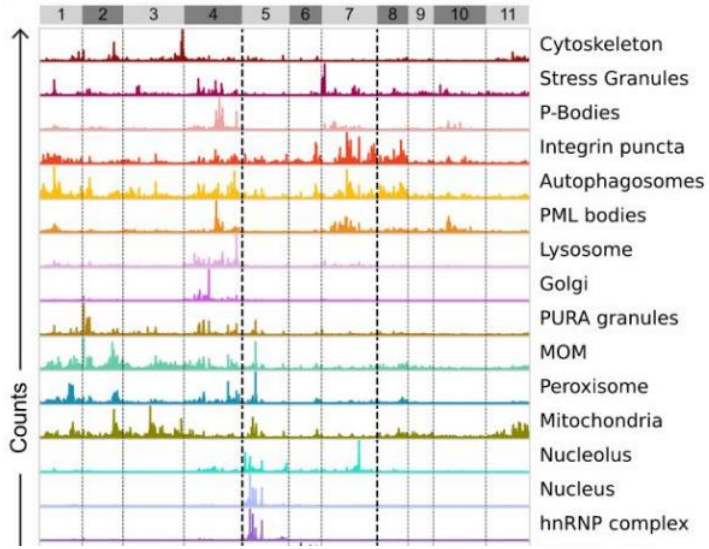


A deep learning-Based ‘Organellomics’ Approach

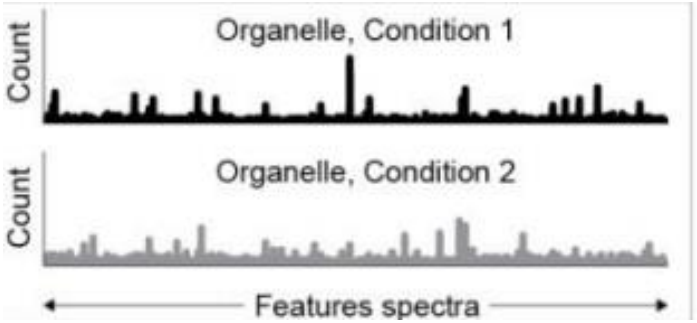
The **NOVA** model
Neuronal Organellomics Vision Atlas

Feature Spectra
Histograms of codeword frequencies





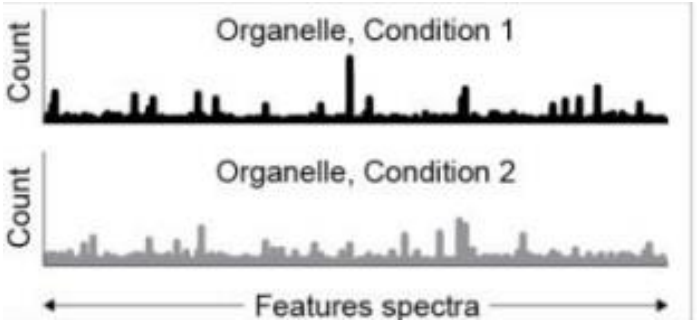
Differential analysis reveals coordination of organellar responses in stressed neurons



Differential analysis reveals coordination of organellar responses in stressed neurons

Sodium Arsenite stress

Untreated



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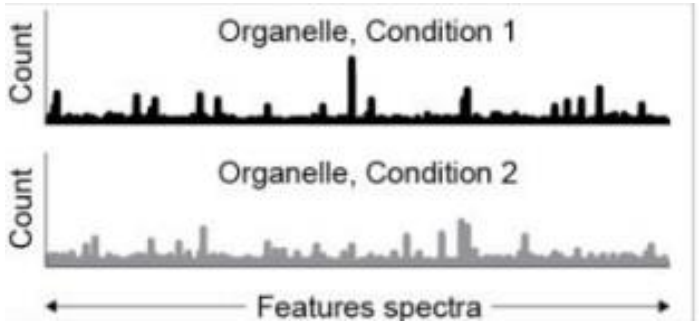

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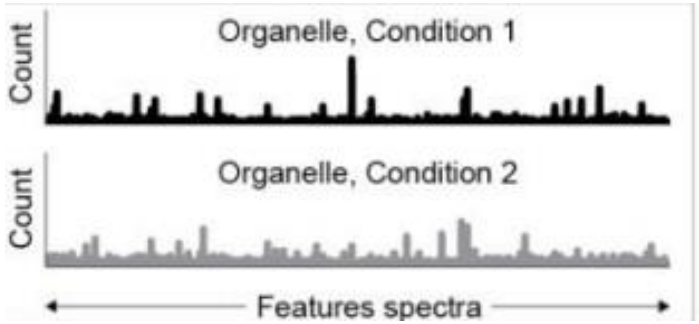
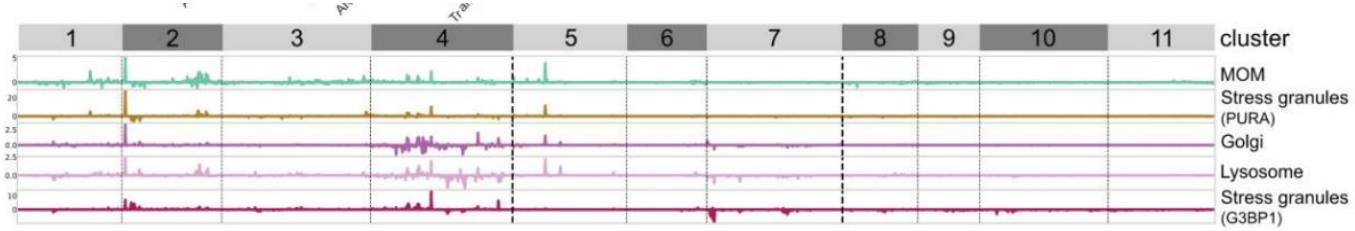


Differential Feature Spectra



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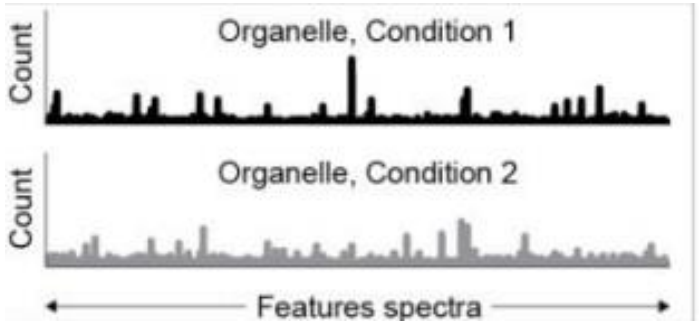

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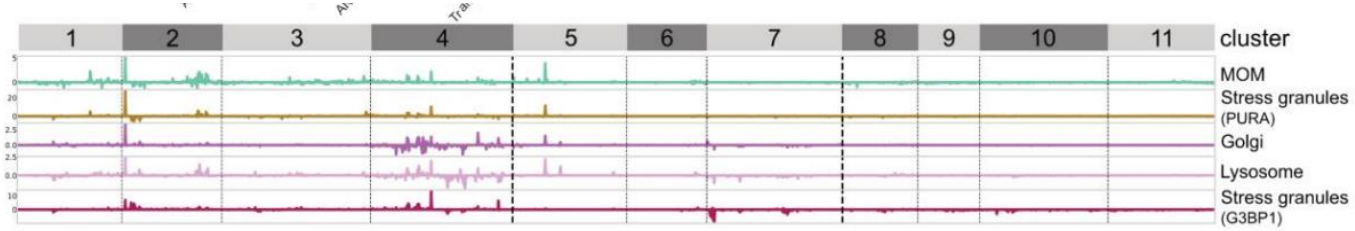
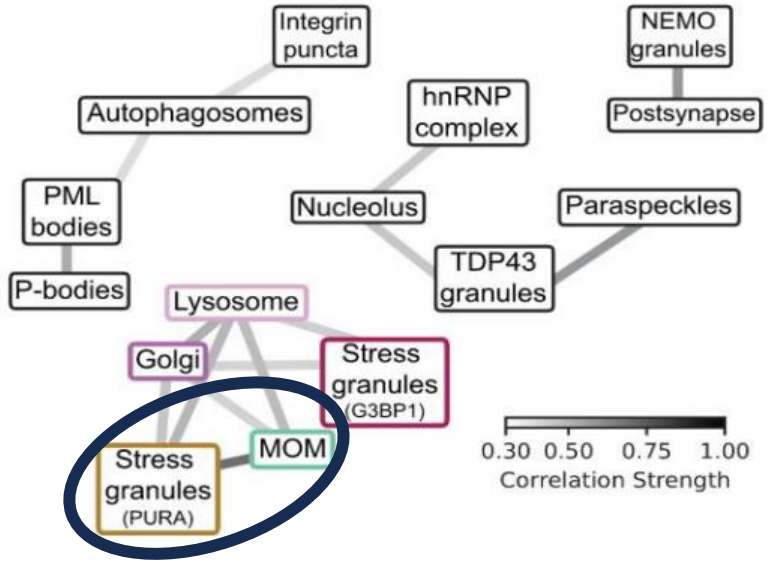
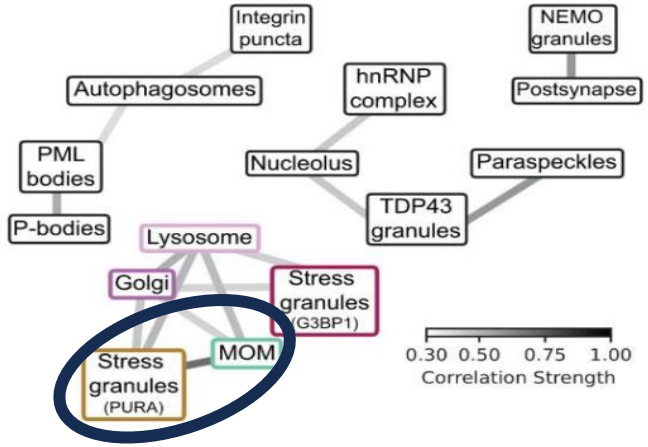


Network Graph of the correlations



Suggested SG-Mitochondrial cross-talk



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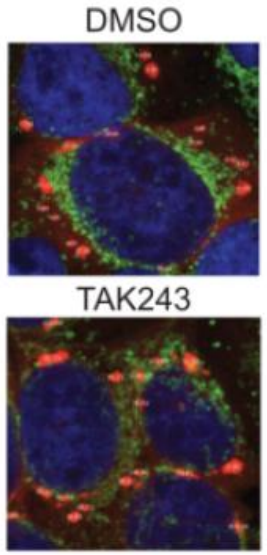
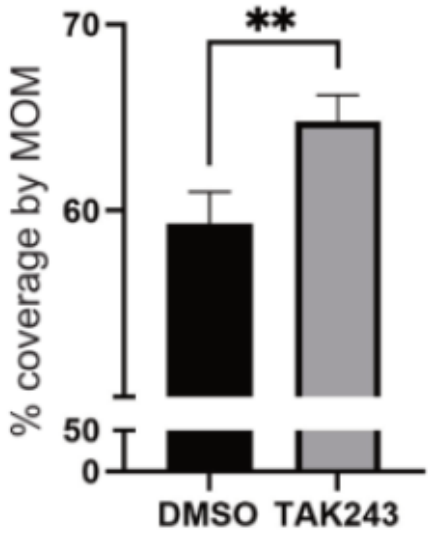
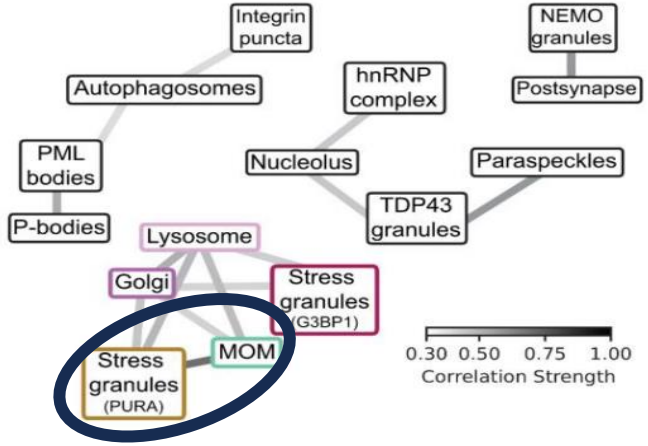




G3BP1
TOMM20
DAPI



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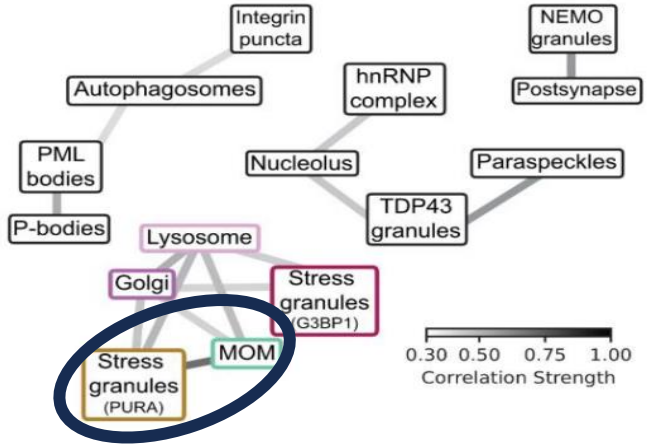

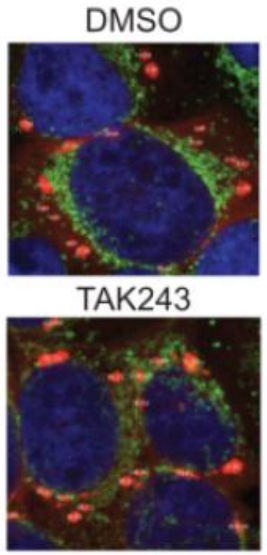

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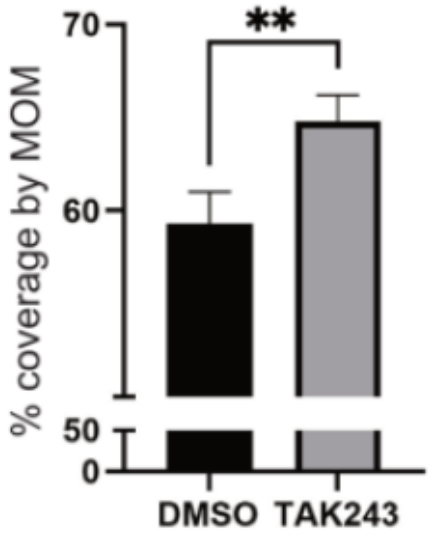
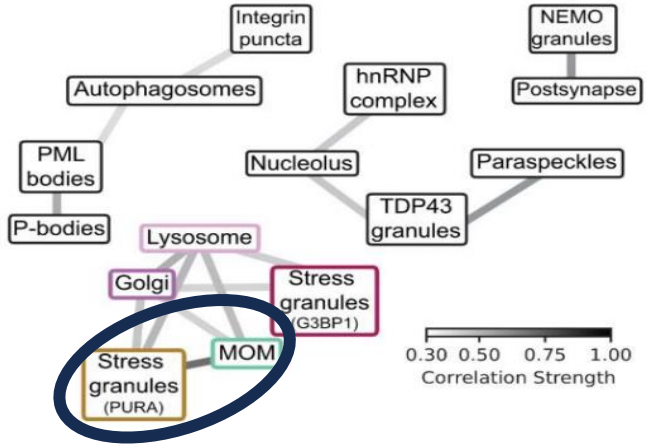
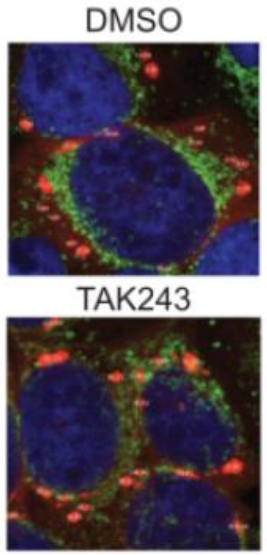


Upon starvation stress of cell, SGs localize to mitochondrial membrane porins blocking fatty acid import and oxidation in the mitochondria.

Amen and Kaganovich 2021

Suggested SG-Mitochondrial cross-talk





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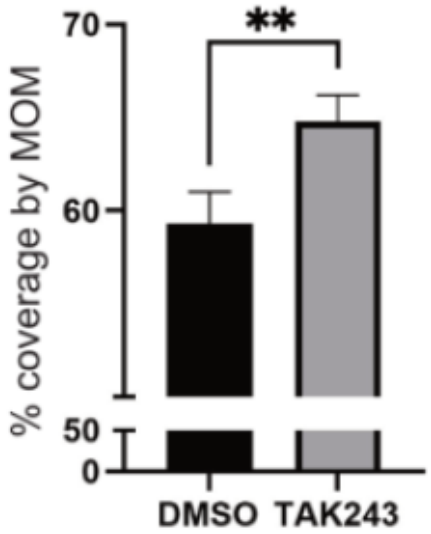
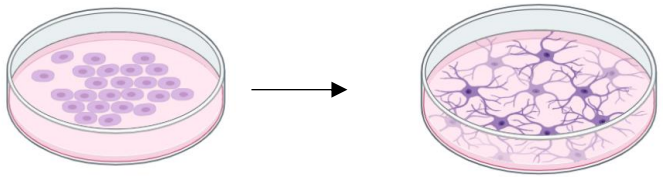


Upon starvation stress of cell, SGs localize to mitochondrial membrane porins, blocking fatty acid import and oxidation in the mitochondria.

Amen and Kaganovich 2021

Hypothesis : There is an inter-organellar cross-talk between SGs and mitochondria in response to cellular stress in human neurons.

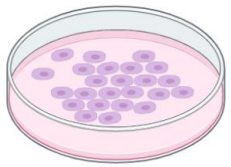
Experimental Procedure



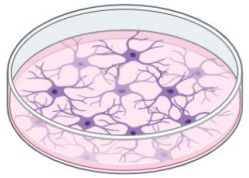
KOLF iPSCs

8 Days
Cortical neurons

Experimental Procedure



KOLF iPSCs



8 Days
Cortical neurons

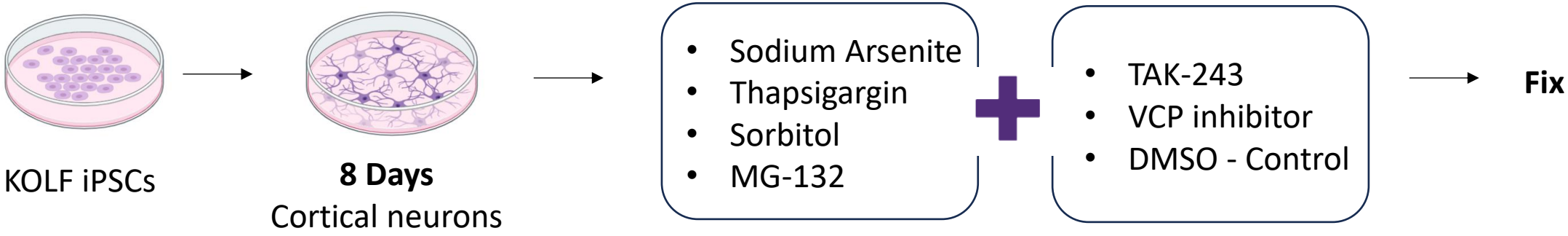
Main stressor

- Sodium Arsenite
- Thapsigargin
- Sorbitol
- MG-132

Additional stressor

- TAK-243
- VCP inhibitor
- DMSO - Control

Experimental Procedure



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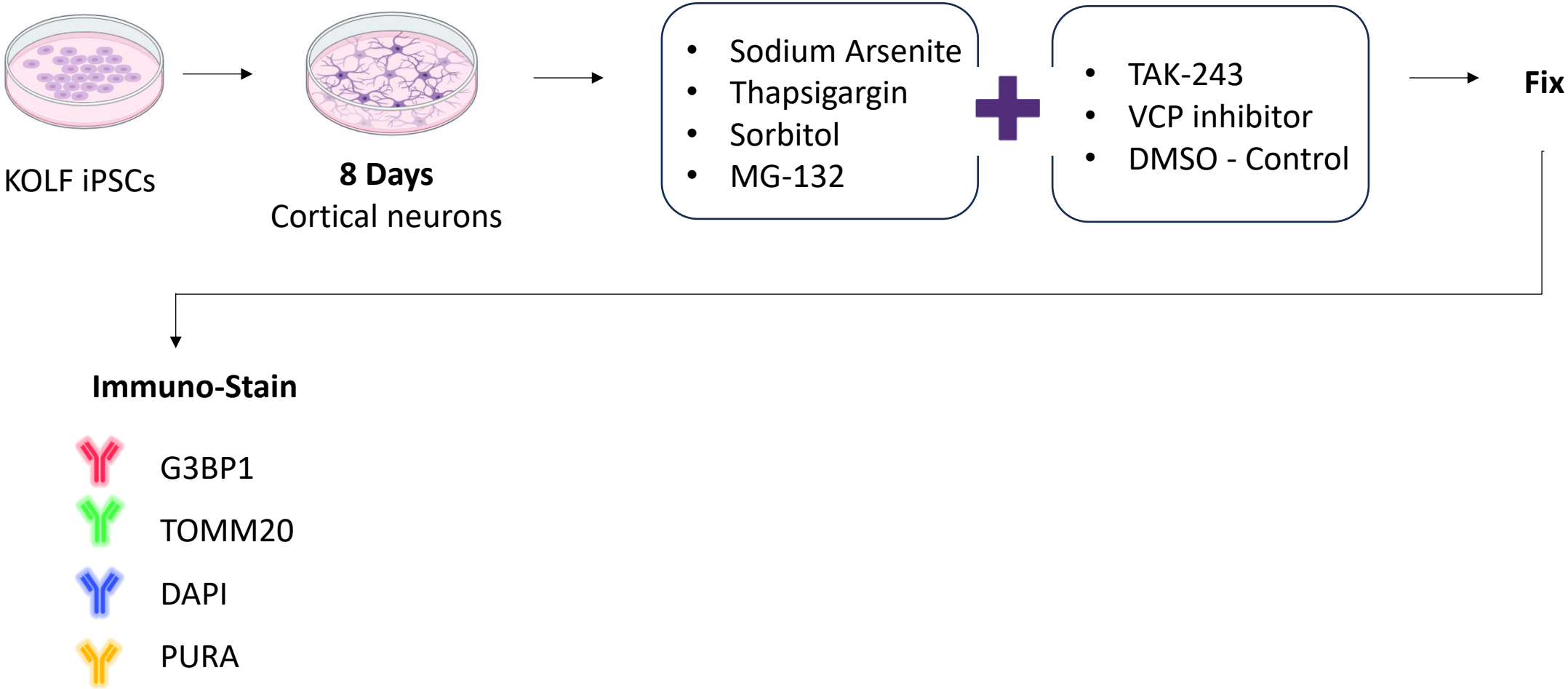

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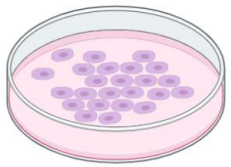

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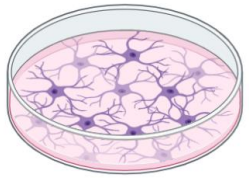

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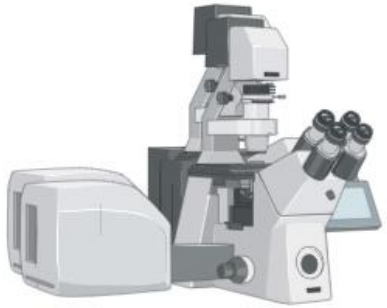
Fix

Immuno-Stain

- G3BP1
- TOMM20
- DAPI
- PURA

Spinning disc confocal microscope



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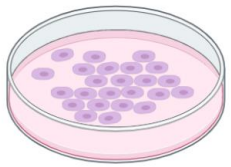

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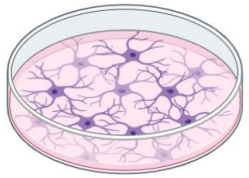

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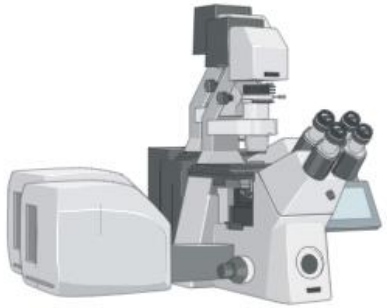
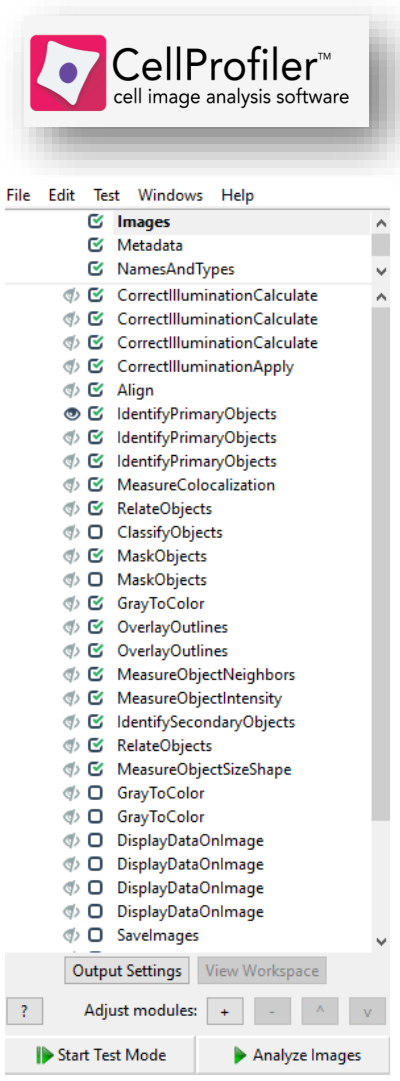


CellProfiler™
cell image analysis software

Object-based image analysis in CellProfiler

Object-based image analysis in CellProfiler



Object-based image analysis in CellProfiler

CellProfiler™
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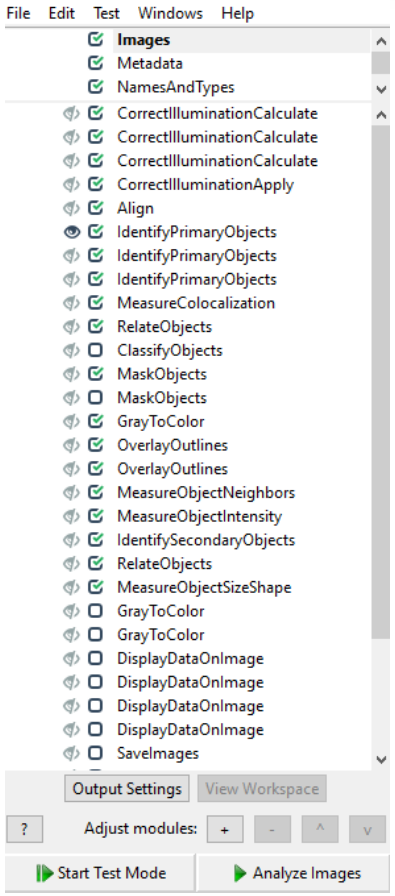

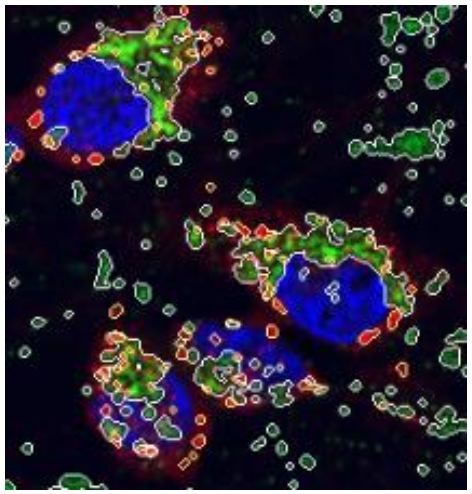

G3BP1
TOMM20
DAPI

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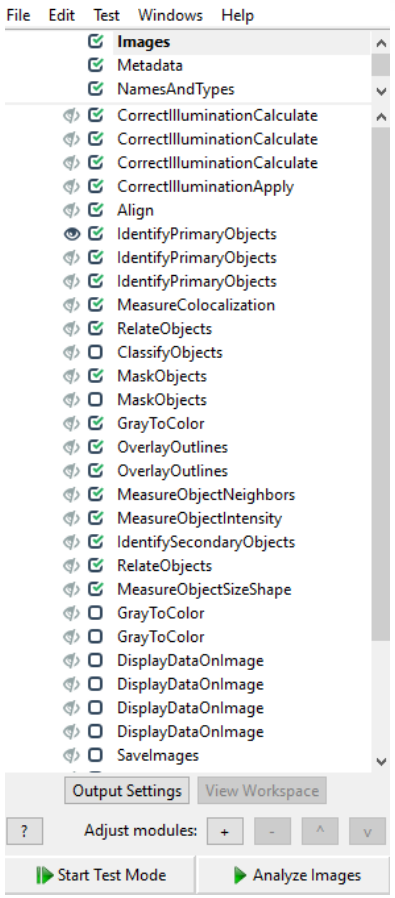
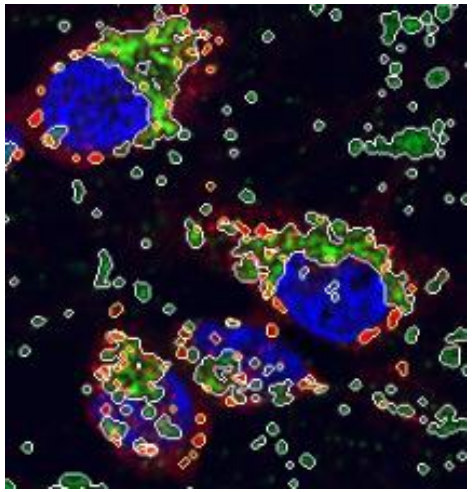




G3BP1
TOMM20
DAPI

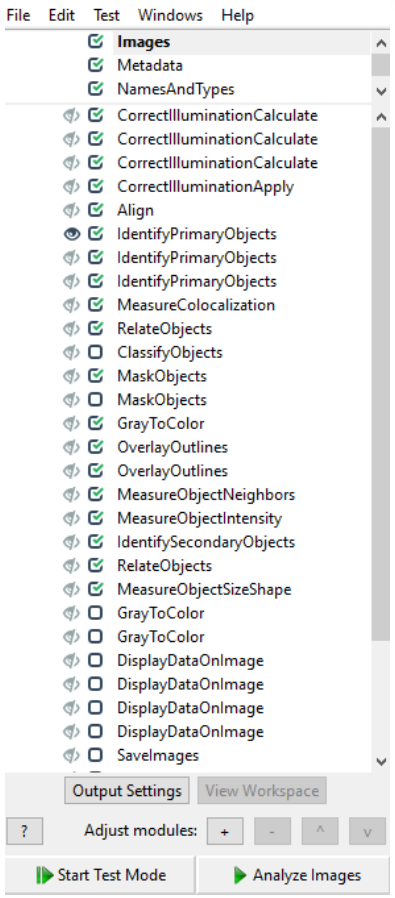
Extract measurements:

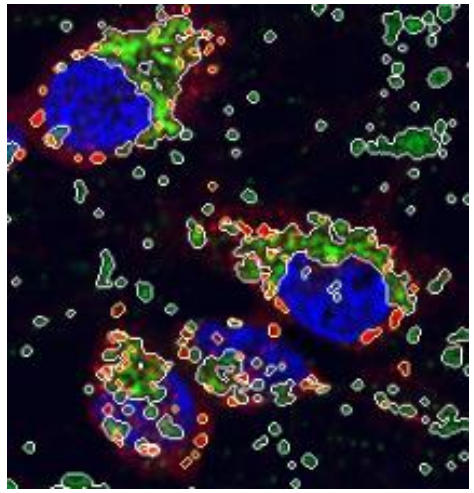
Interaction :
Mean TOMM20 intensity in SGs

Neighborhood :
Percent of SG edge touching TOMM20

Object-based image analysis in CellProfiler

CellProfiler™
cell image analysis software





G3BP1
TOMM20
DAPI

Extract measurements:

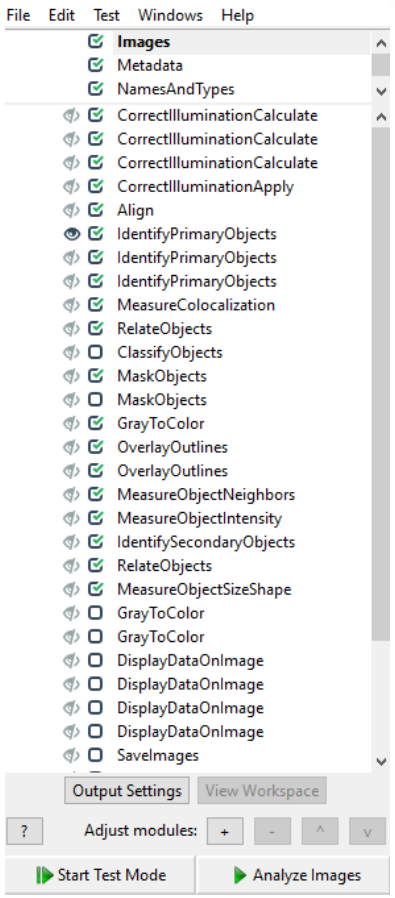
Interaction :
Mean TOMM20 intensity in SGs

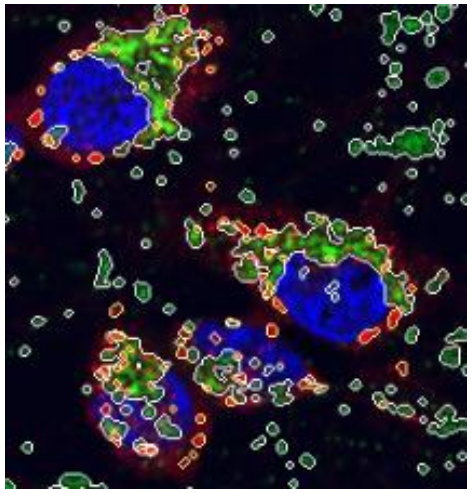
Neighborhood :
Percent of SG edge touching TOMM20

- User-friendly
- Customizable
- Reproducible
- Interpretable results

Object-based image analysis in CellProfiler

CellProfiler™
cell image analysis software





G3BP1
TOMM20
DAPI

Extract measurements:

Interaction :
Mean TOMM20 intensity in SGs

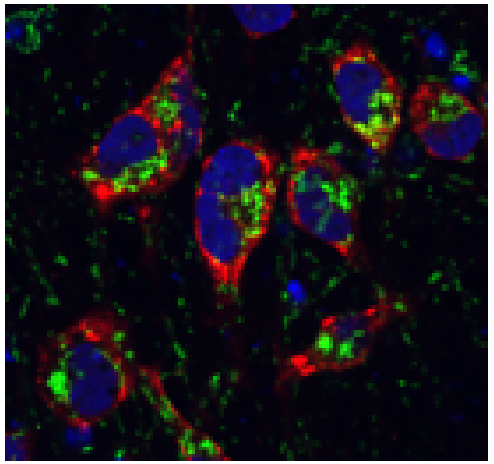
Neighborhood :
Percent of SG edge touching TOMM20

- User-friendly
- Customizable
- Reproducible
- Interpretable results

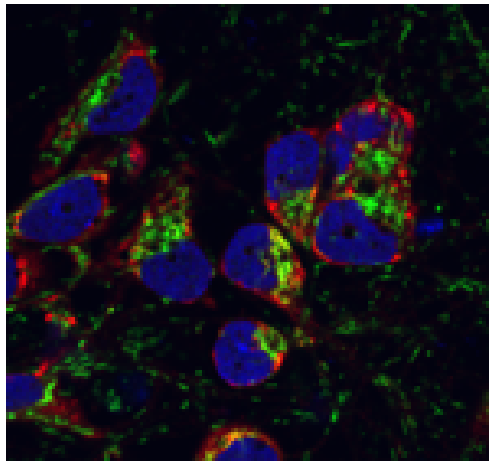
- Biased
- Hypothesis-based
- Object-Based

Only **acute sodium arsenite**, **thapsigargin** and **sorbitol** induced SGs formation

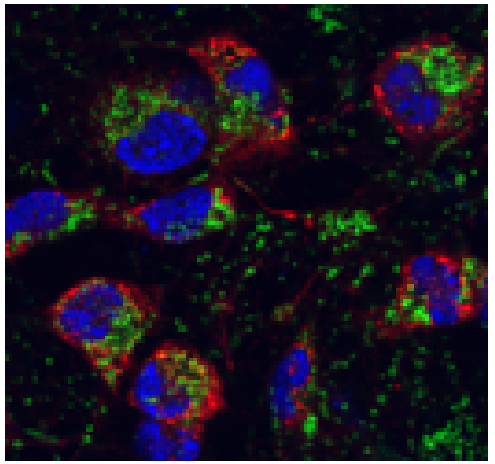
Acute SA



Thapsigargin



Sorbitol



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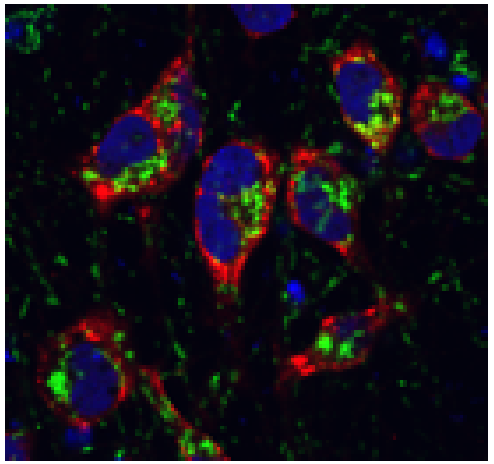
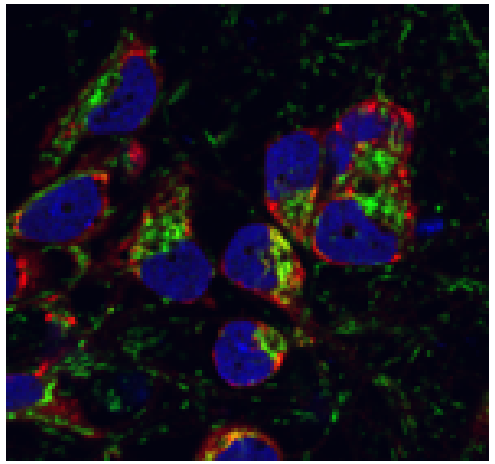


Thapsigargin



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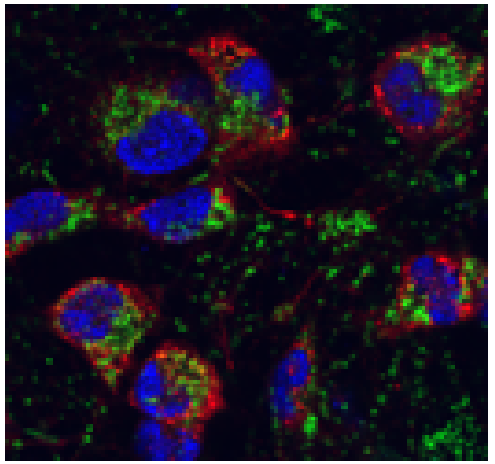

G3BP1
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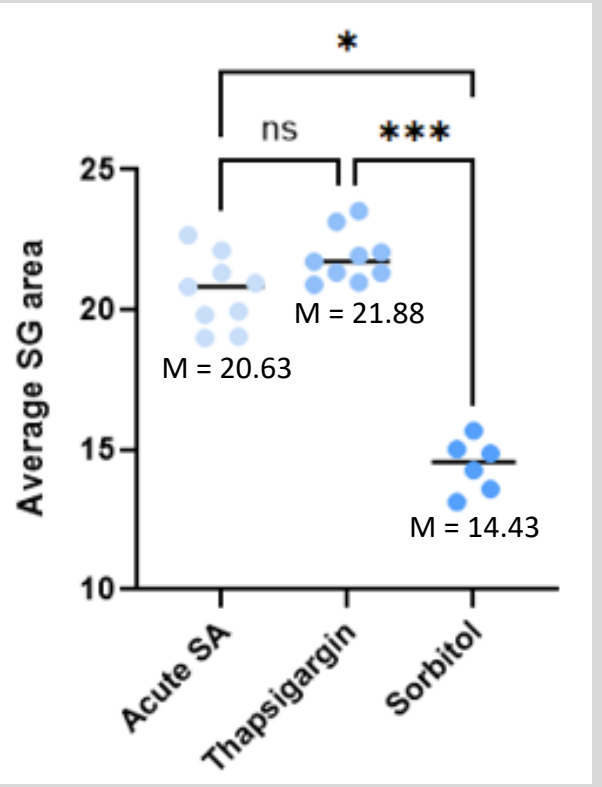
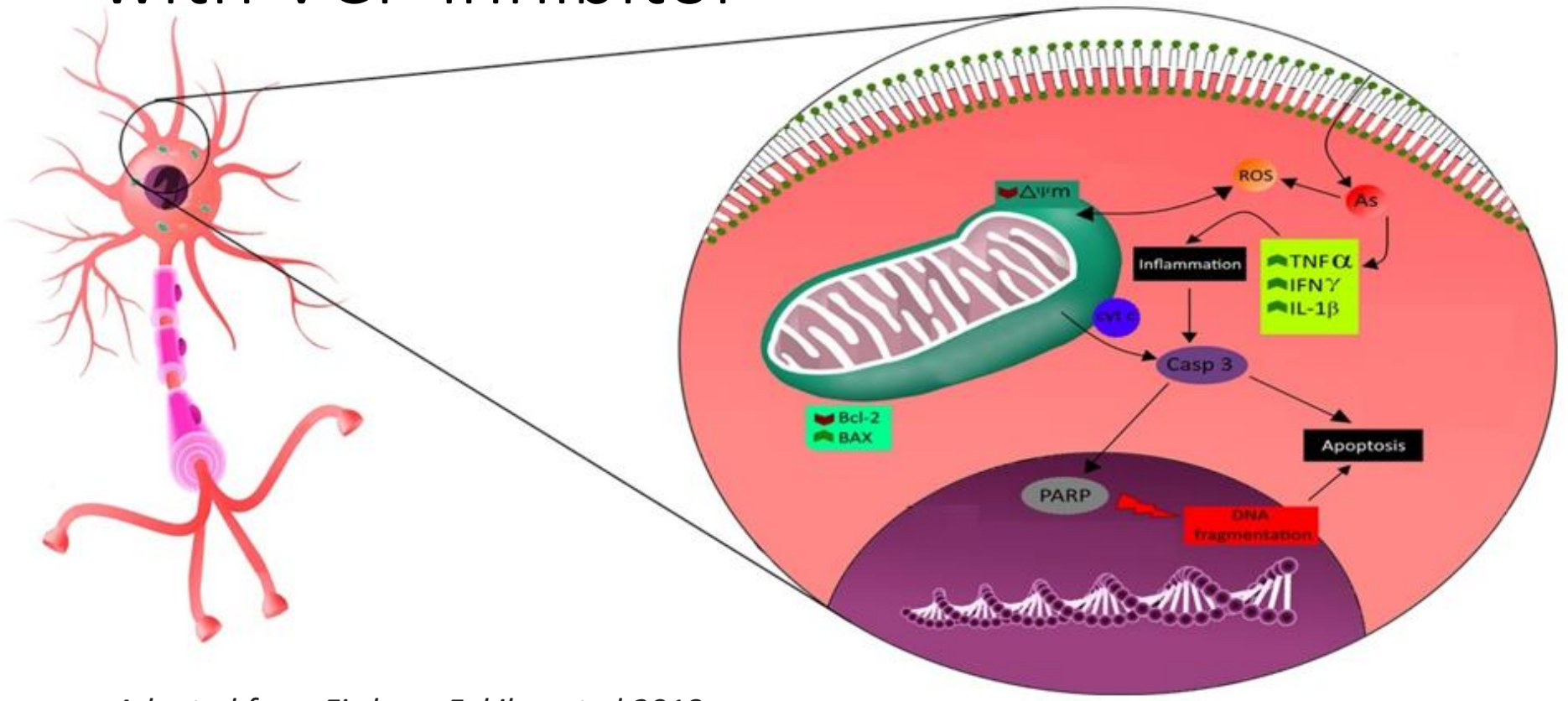
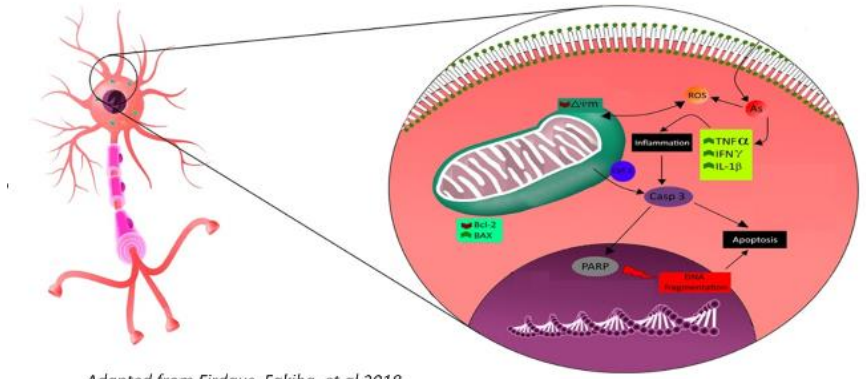


Acute sodium arsenite induces SG-mitochondrial cross-talk with VCP inhibitor



Adapted from Firdaus, Fakiha, et al 2018

Acute sodium arsenite induces SG-mitochondrial cross-talk with VCP inhibitor



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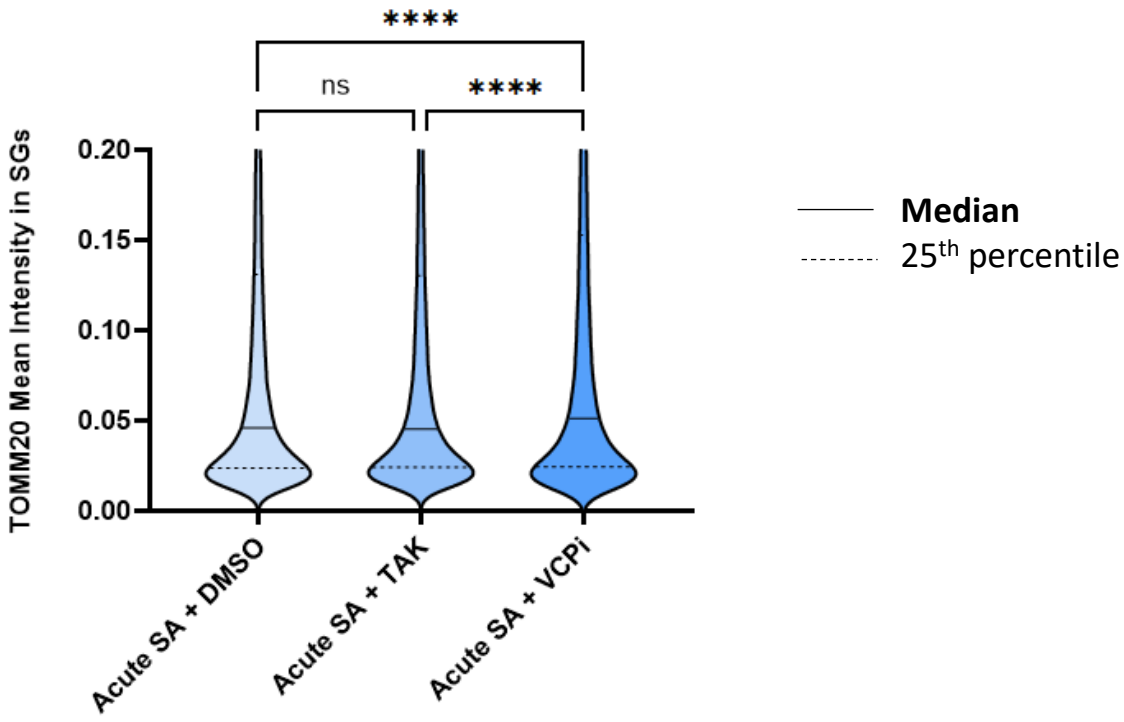
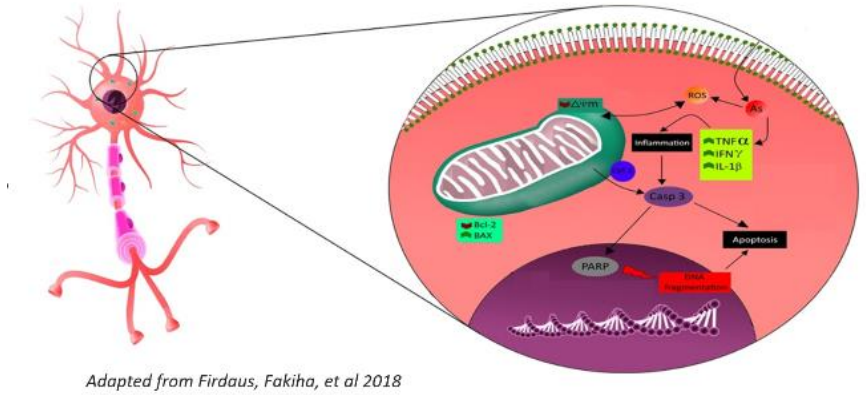
SG-Mitochondria Interaction



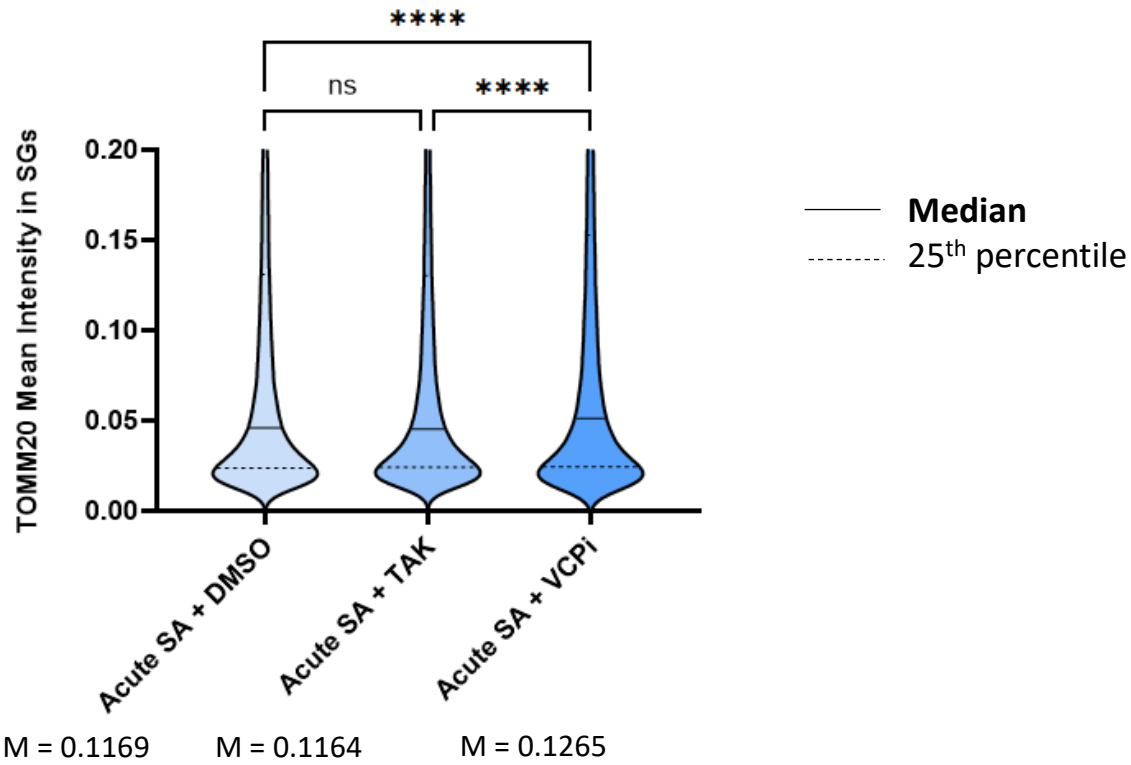
M = 0.1169 M = 0.1164 M = 0.1265

Acute sodium arsenite induces SG-mitochondrial cross-talk with VCP inhibitor

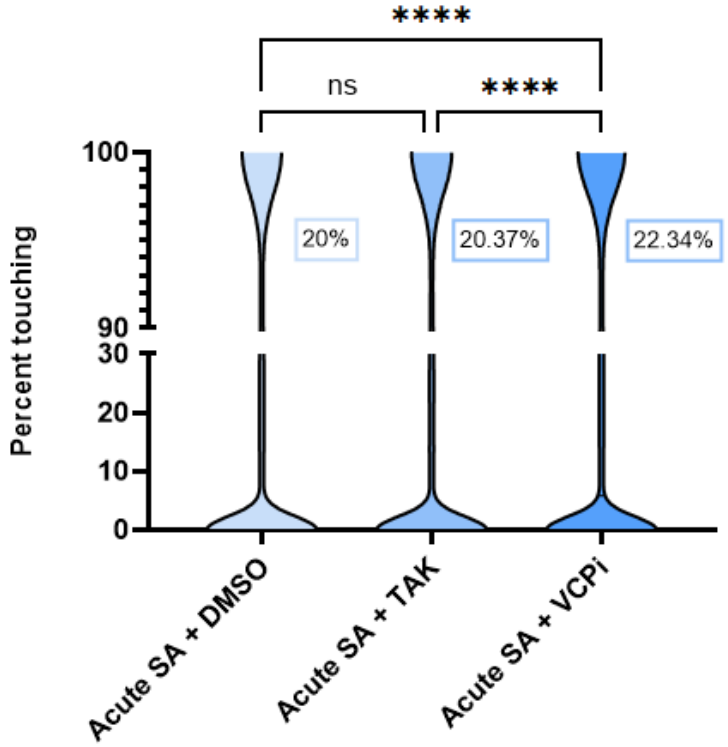


Adapted from Firdaus, Fakiha, et al 2018

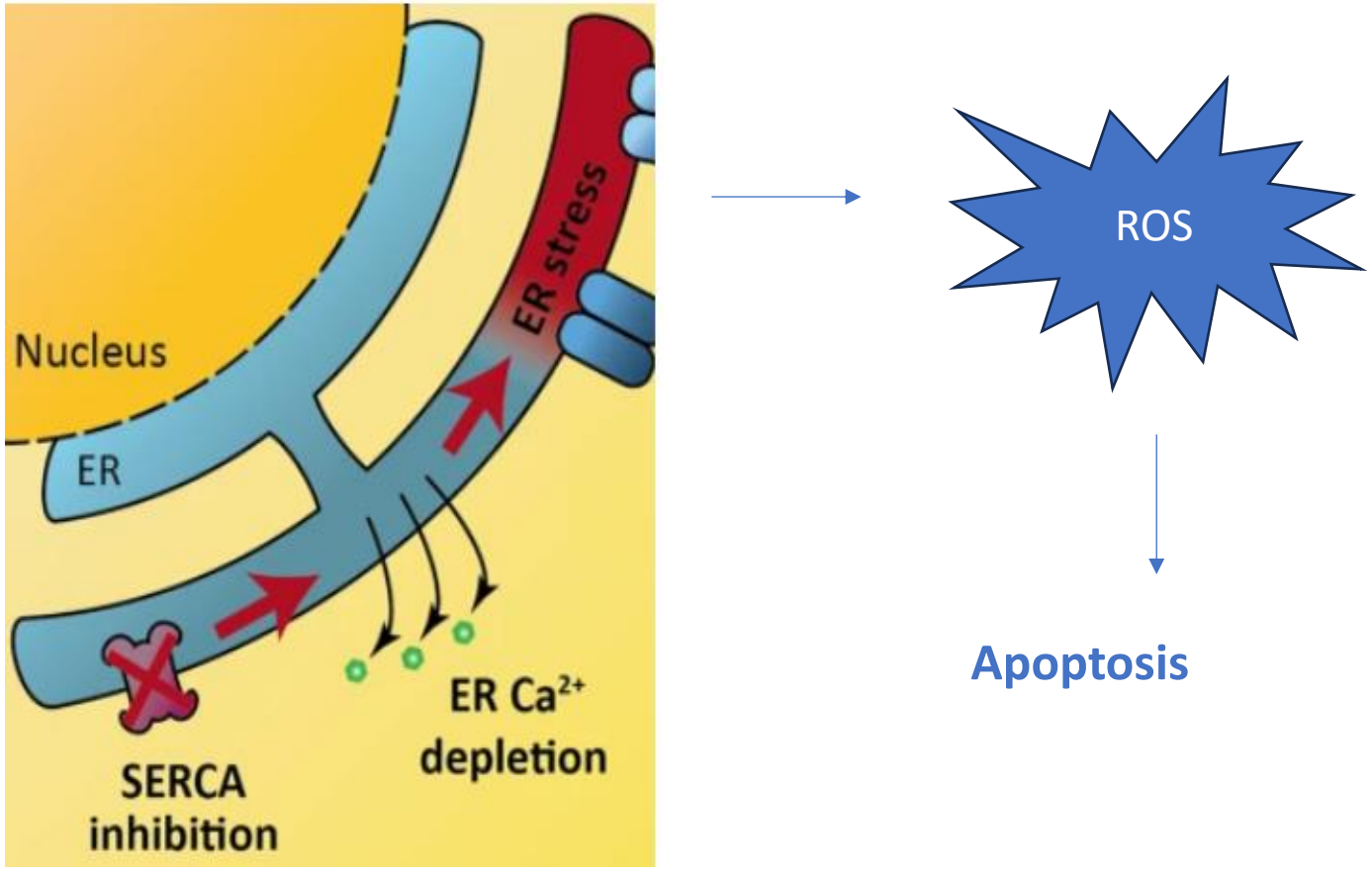
SG-Mitochondria Interaction



SG-Mitochondria Neighborhood

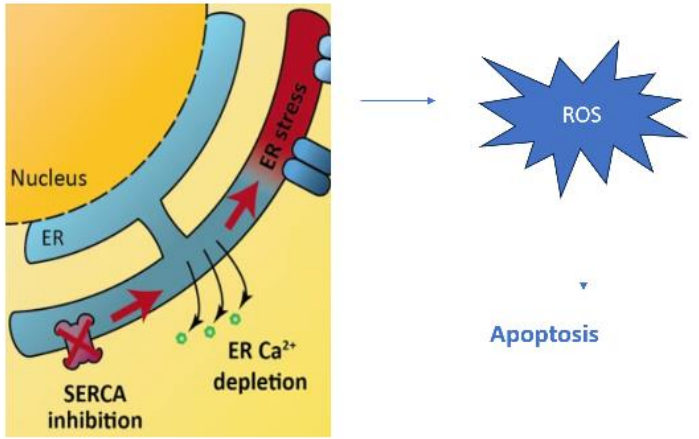


Thapsigargin induces increased cross-talk with both TAK243 and VCP inhibitor



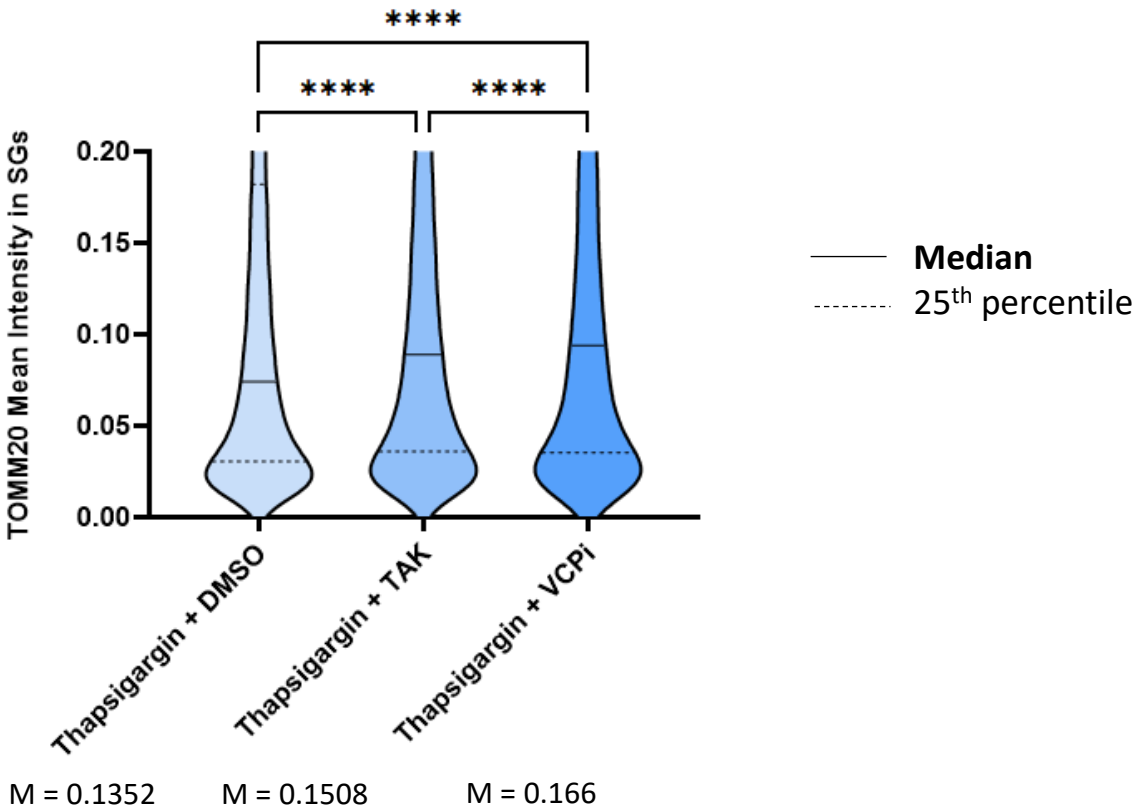
Adapted from Lindner, Paula et al 2020 and Ling,Yating et al 2024

Thapsigargin induces increased cross-talk with both TAK243 and VCP inhibitor

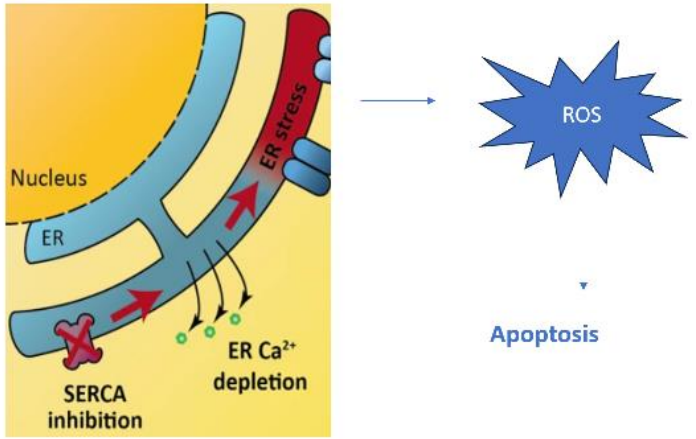


Adapted from Lindner, Paula et al 2020 and Ling,Yating et al 2024

SG-Mitochondria Interaction

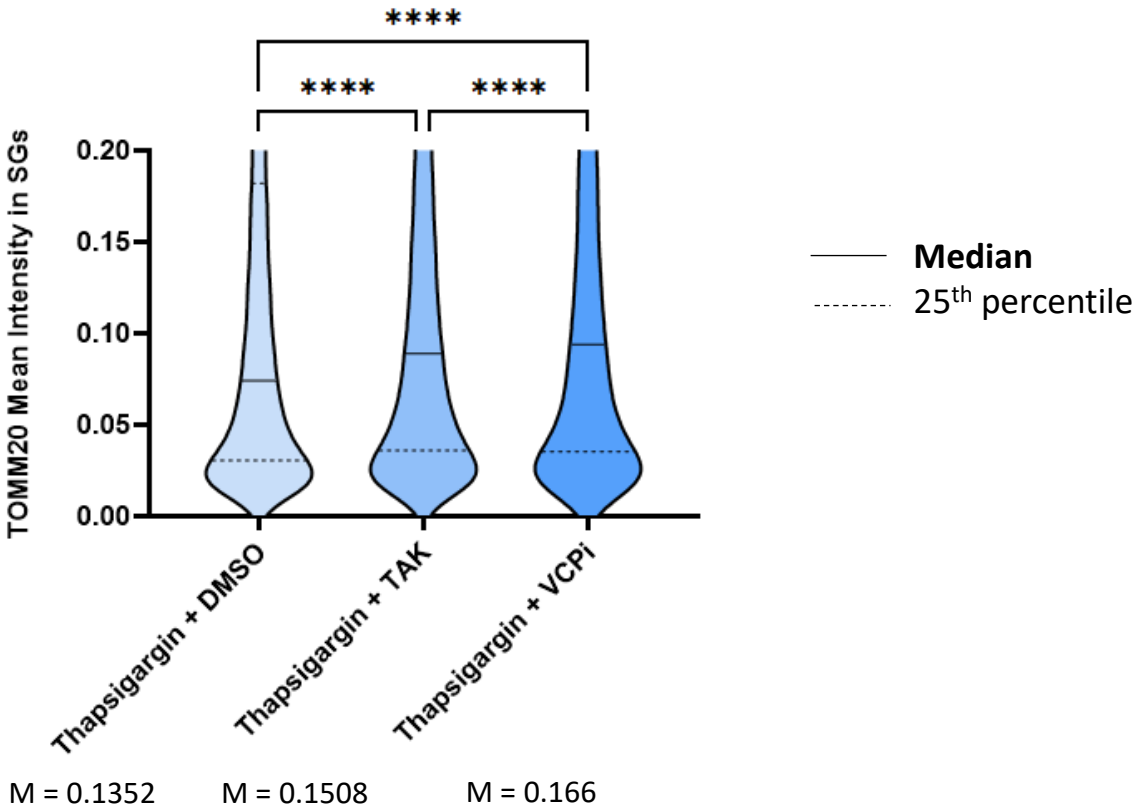


Thapsigargin induces increased cross-talk with both TAK243 and VCP inhibitor

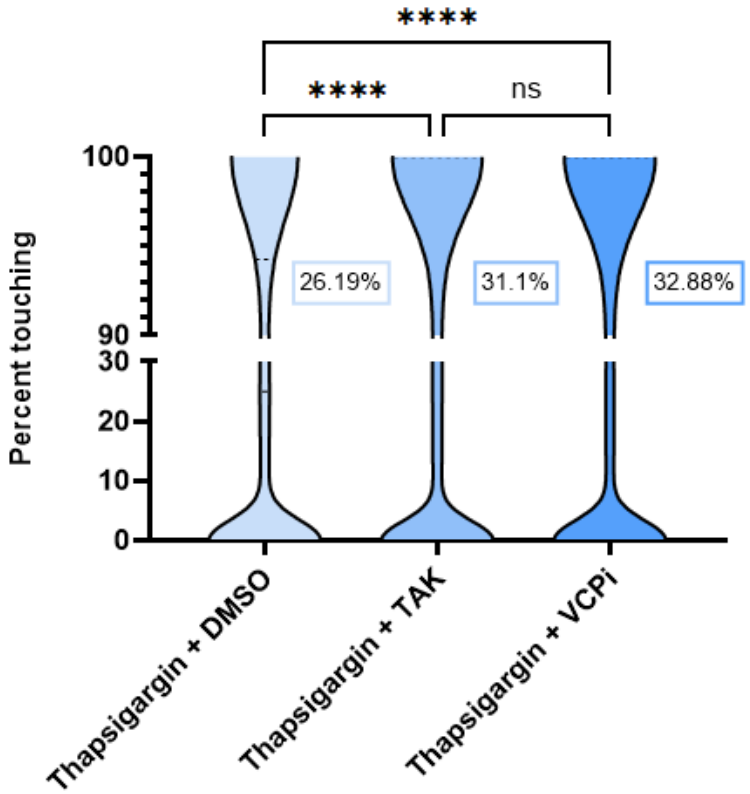


Adapted from Lindner, Paula et al 2020 and Ling,Yating et al 2024

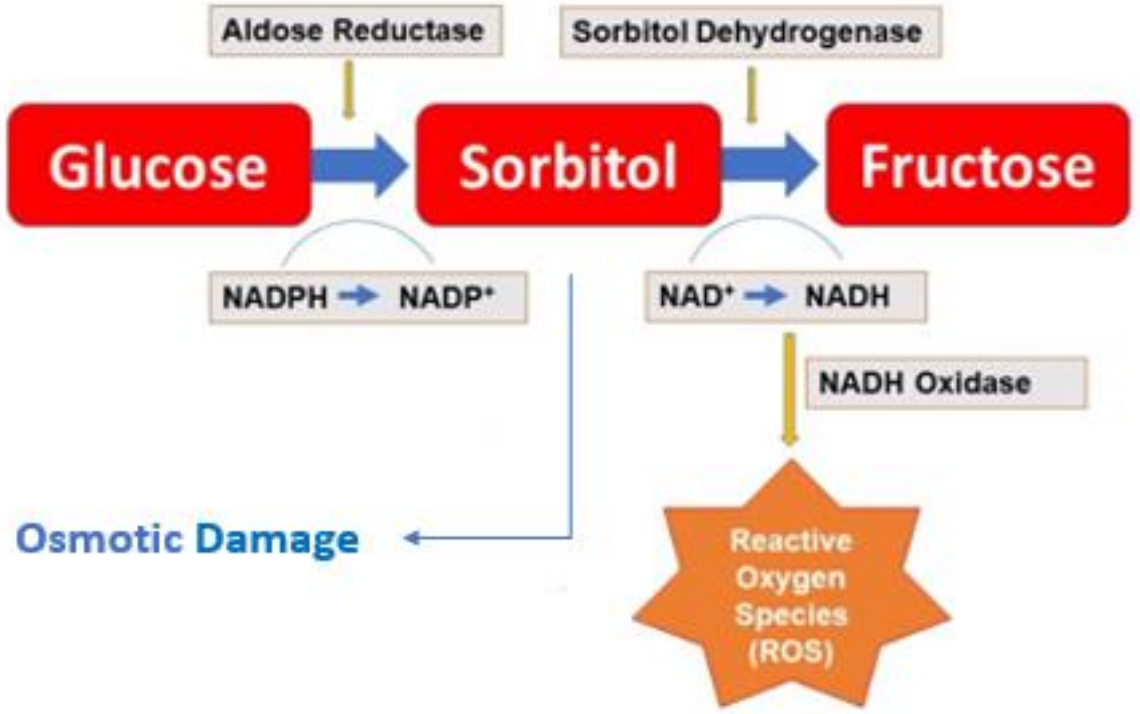
SG-Mitochondria Interaction



SG-Mitochondria Neighborhood

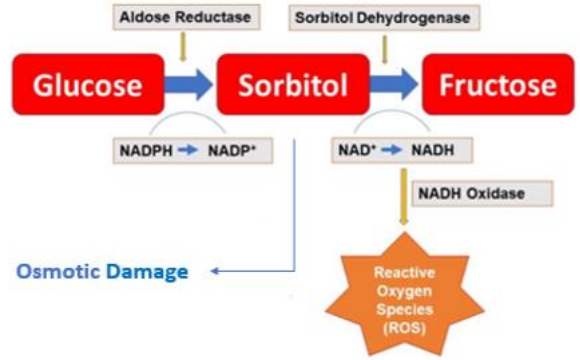


Sorbitol reduced SG-mitochondrial cross-talk with both TAK243 and VCP inhibitor



Adapted from Singh, M., Kapoor, A., & Bhatnagar, A. (2021)

Sorbitol reduced SG-mitochondrial cross-talk with both TAK243 and VCP inhibitor



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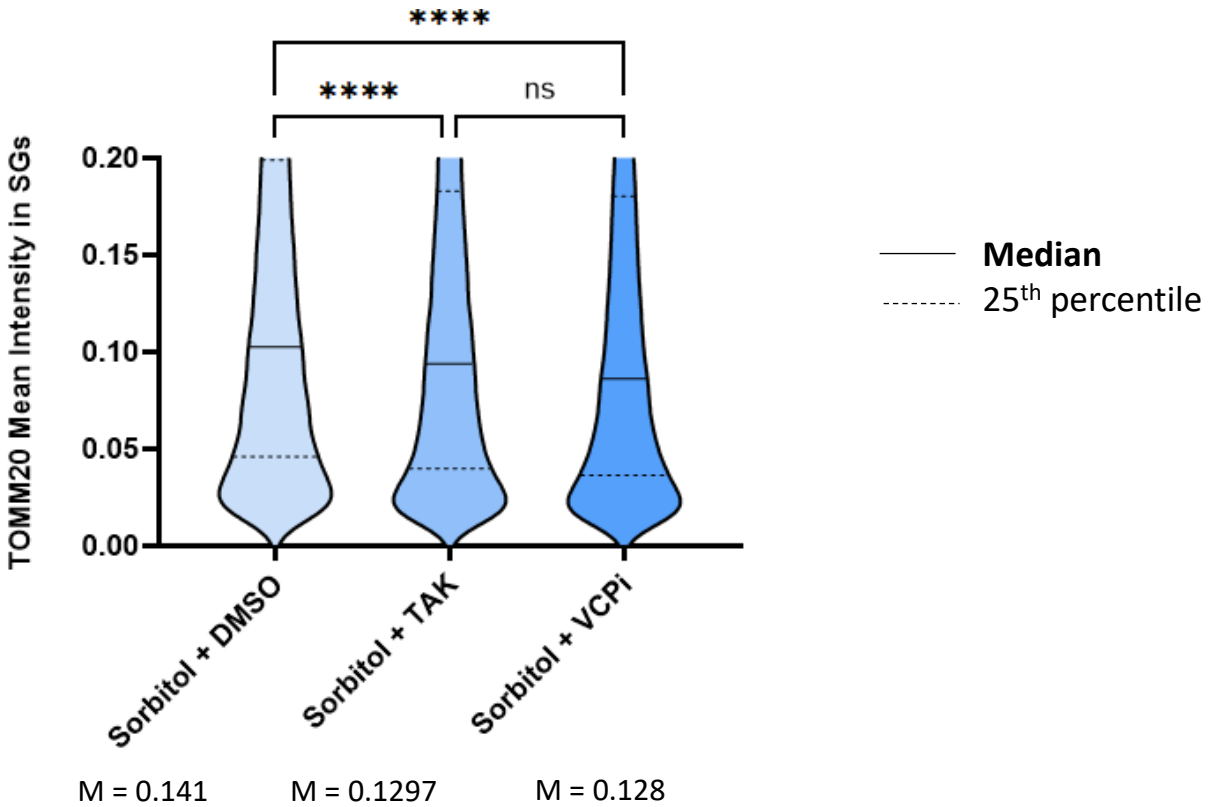
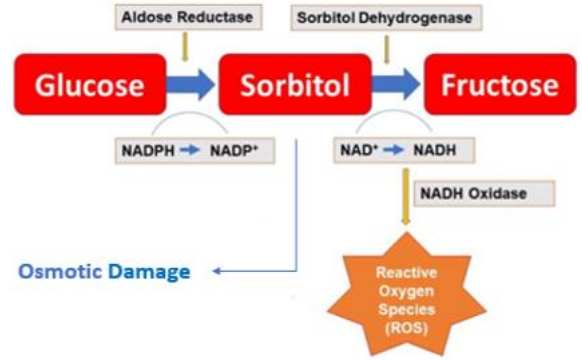
SG-Mitochondria Interaction



Sorbitol reduced SG-mitochondrial cross-talk with both TAK243 and VCP inhibitor



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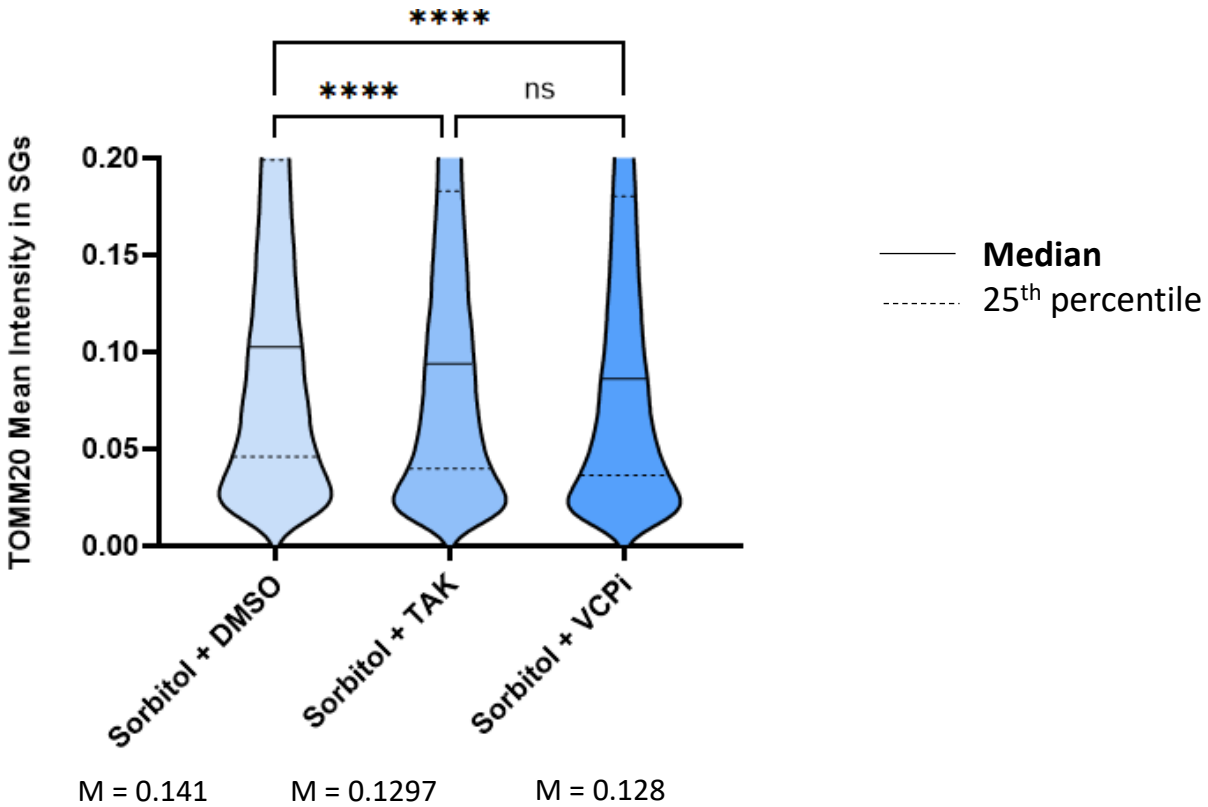
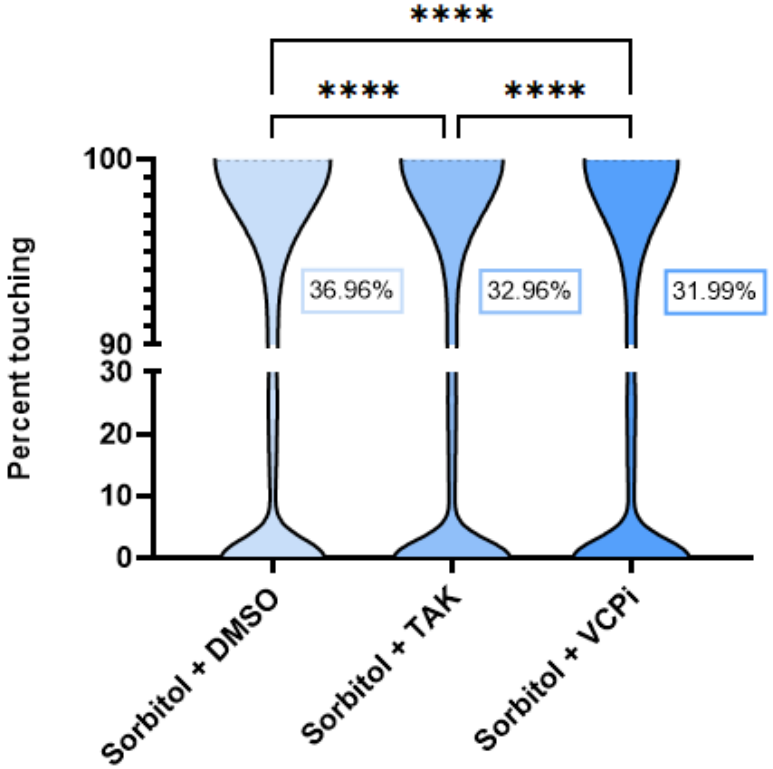
SG-Mitochondria Interaction



SG-Mitochondria Neighborhood

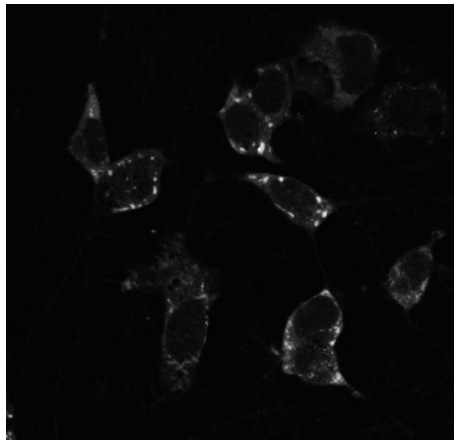


Sorbitol induces the formation of SGs and drives TDP-43 to the cytoplasm

Sorbitol induces the formation of SGs and drives TDP-43 to the cytoplasm

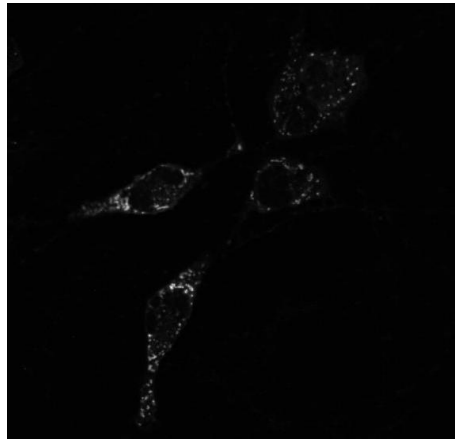
Acute sodium arsenite

G3BP1



Sorbitol

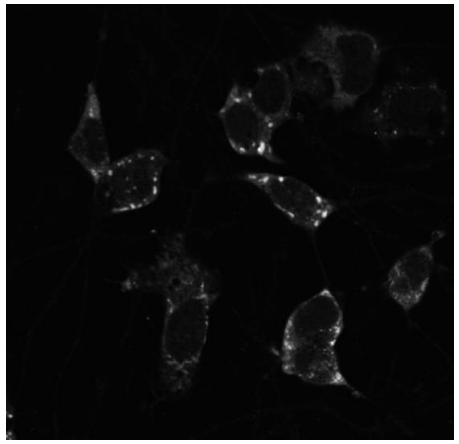
G3BP1



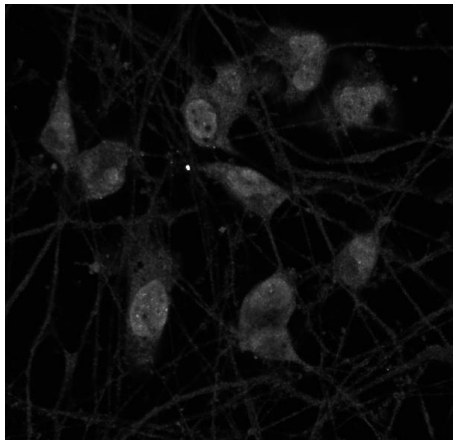
Sorbitol induces the formation of SGs and drives TDP-43 to the cytoplasm

Acute sodium arsenite

G3BP1

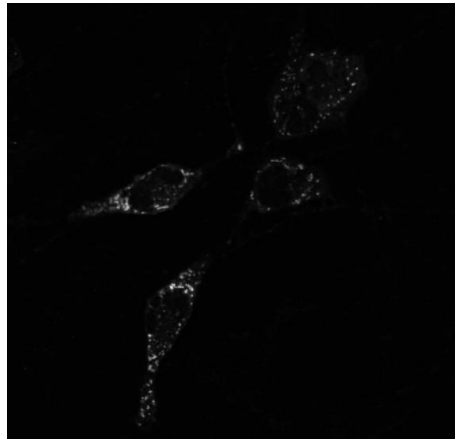


TDP-43

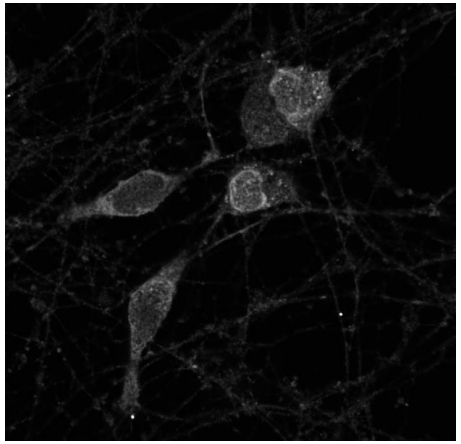


Sorbitol

G3BP1



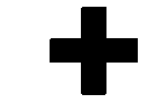
TDP-43



Summary

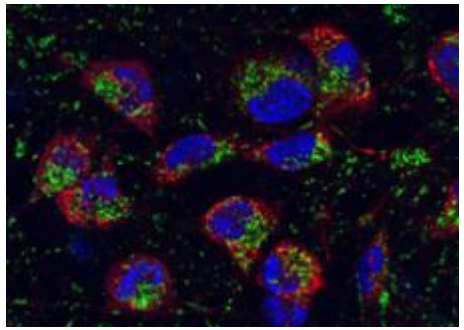
Summary

Acute sodium arsenite
Thapsigargin



Sorbitol

Stress granule formation



Summary

Acute sodium arsenite
Thapsigargin

↓

Larger but less SGs

+

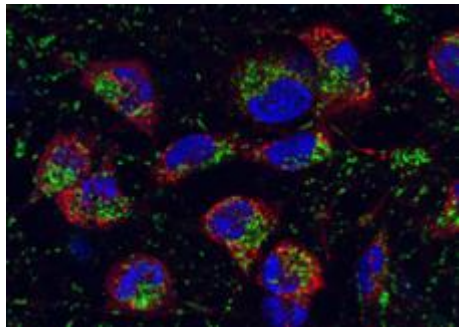
Sorbitol

↓

Smaller but more SGs

→

Stress granule formation



Summary

Acute sodium arsenite
Thapsigargin

↓

Larger but less SGs

Increased SG-mitochondrial interaction with additional stressor

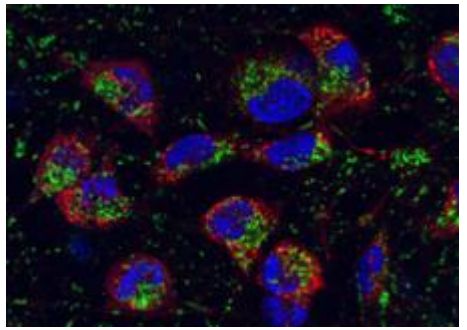
Supports findings from NOVA model with an orthogonal method

+ Sorbitol

↓

Smaller but more SGs

→ Stress granule formation

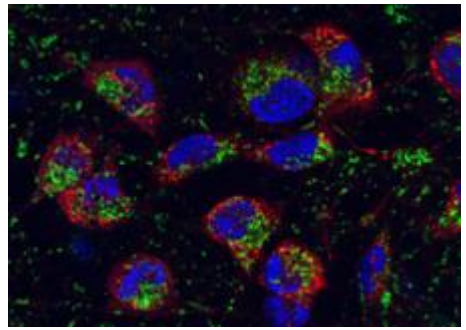


Summary

Acute sodium arsenite
Thapsigargin

Sorbitol

Stress granule formation



Larger but less SGs

Smaller but more SGs

Increased SG-mitochondrial interaction with additional stressor

Decreased SG-mitochondrial interaction with additional stressor

Supports findings from NOVA model with an orthogonal method

Drives TDP-43 to the cytoplasm, mimicking disease phenotype

Thank You !!