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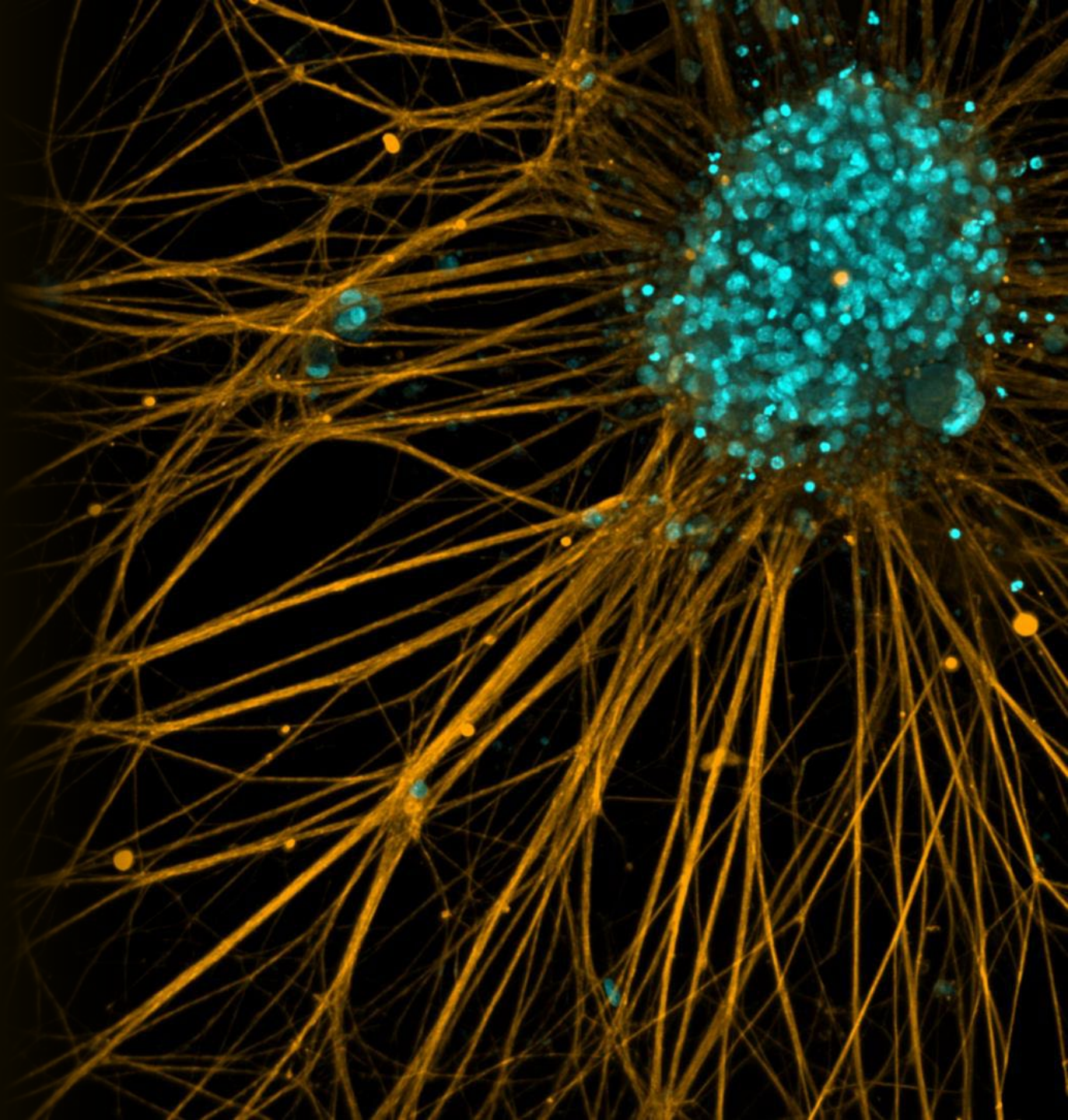
Understanding Complex Biology



CASE STUDY

Evaluating a Therapeutic Candidate's Protective Efficacy in ALS-relevant, Motor-neuron Co-culture Models

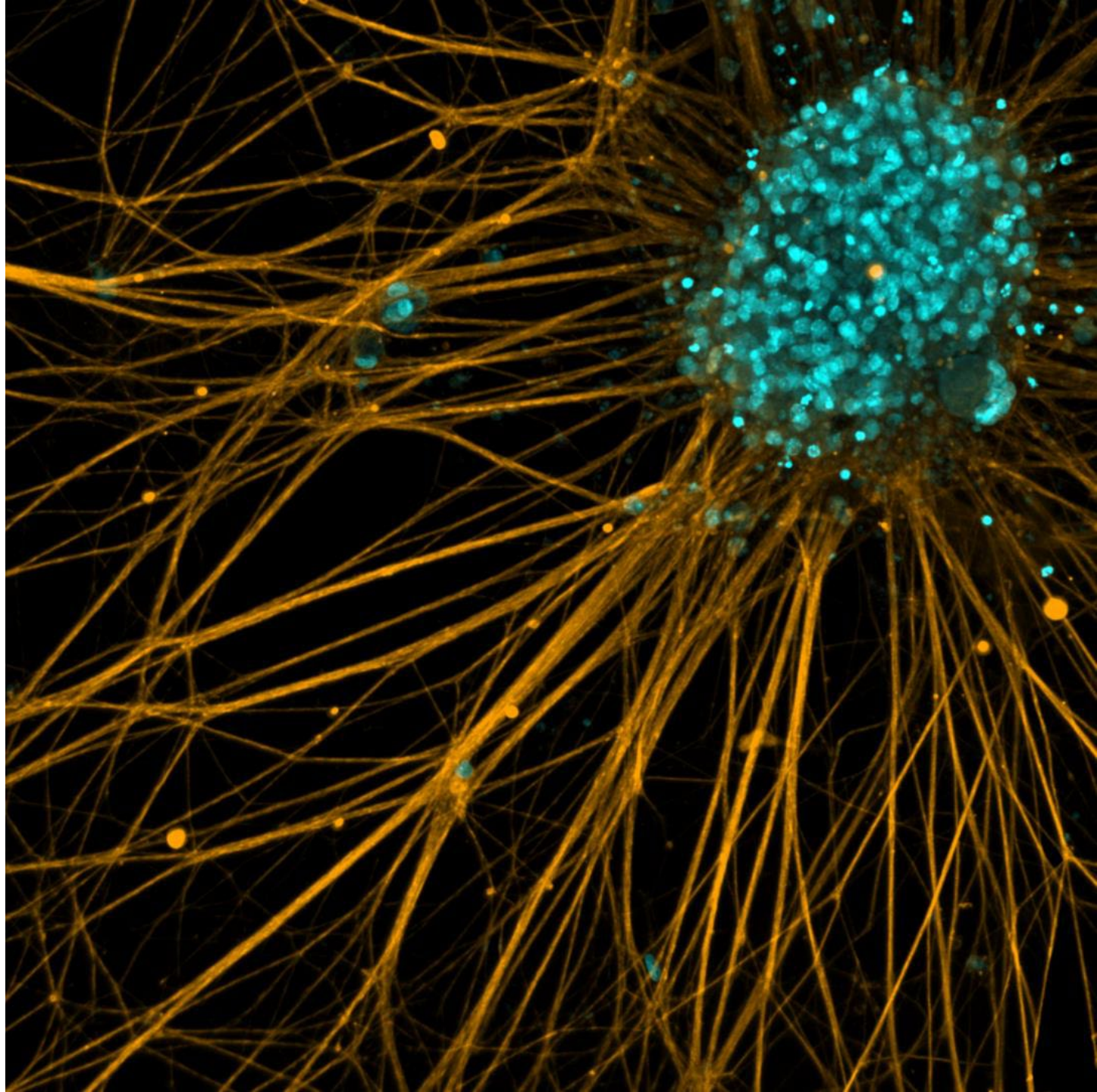
OBJECTIVE

A client requested a custom project evaluating the protective efficacy of their therapeutic candidate in ALS-relevant co-cultures of motor neurons and astrocytes.

Goals

1. Assess the protective efficacy of client's therapeutic candidate against treatment with tunicamycin.

2. Compare results between three, iPSC-derived, motor-neuron lines (wild-type, SOD1A4V, and sporadic ALS) in co-culture with astrocytes.



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EXPERIMENTAL DESIGN

Cell Models – Co-culture

Human, iPSC-derived Motor Neurons
- Wild-type
- SOD1A4V
- Sporadic ALS

Human, iPSC-derived astrocytes

Palette 1

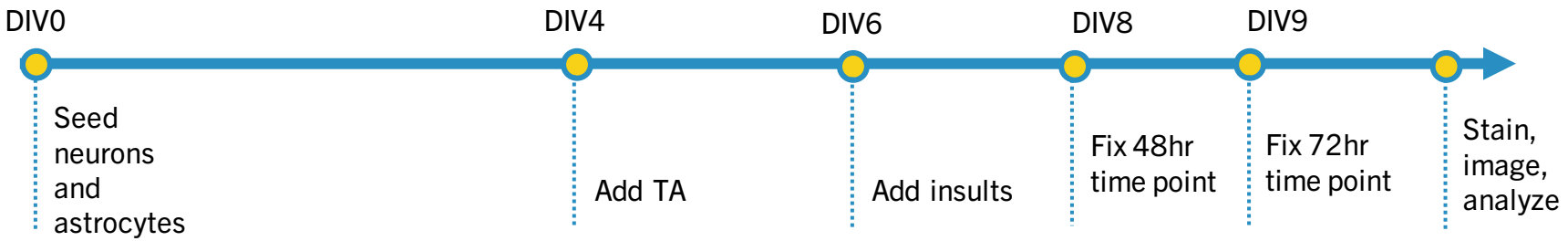
Hoechst (nuclei)
Anti Tuj1 (neurites)
Anti TDP-43 (ALS marker)

Treatments and Timelines

- Culture cells in 384-well, imaging microplates utilizing bespoke culture protocol developed at PhenoVista.
- Pre-treat cultures with test article (TA) plus untreated and vehicle controls.
- 48hrs after treatment with TA, treat with tunicamycin. Include vehicle controls.
- Fix and stain cultures at 48 and 72hrs.

Deliverables

- Quantification may include: Cell count, TDP-43 puncta count in cytoplasm and nucleus, motor neuron network, morphology, and expression level of various markers as well as other metrics as appropriate for the study design.
- Reporting via a presentation-ready report to include detailed methodology, statistical analysis and IC_{50} curve-fits where applicable. Representative images will be provided for controls and for a reasonable selection of test conditions.



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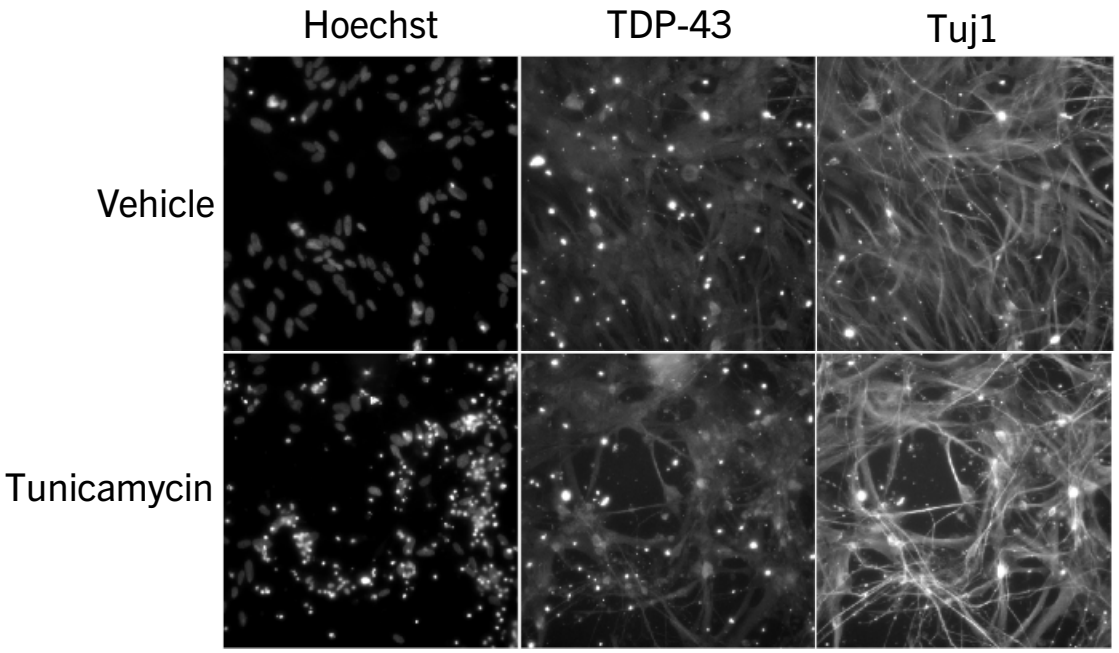
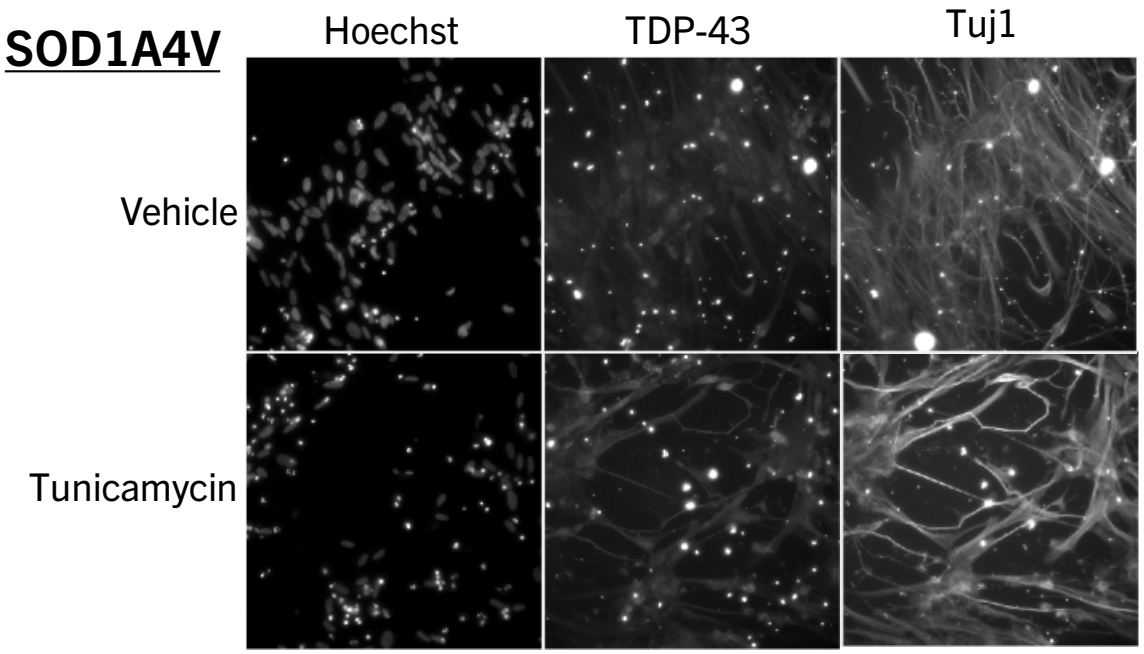
REPRESENTATIVE IMAGES

Co-cultures of motor neurons and astrocytes treated with tunicamycin.

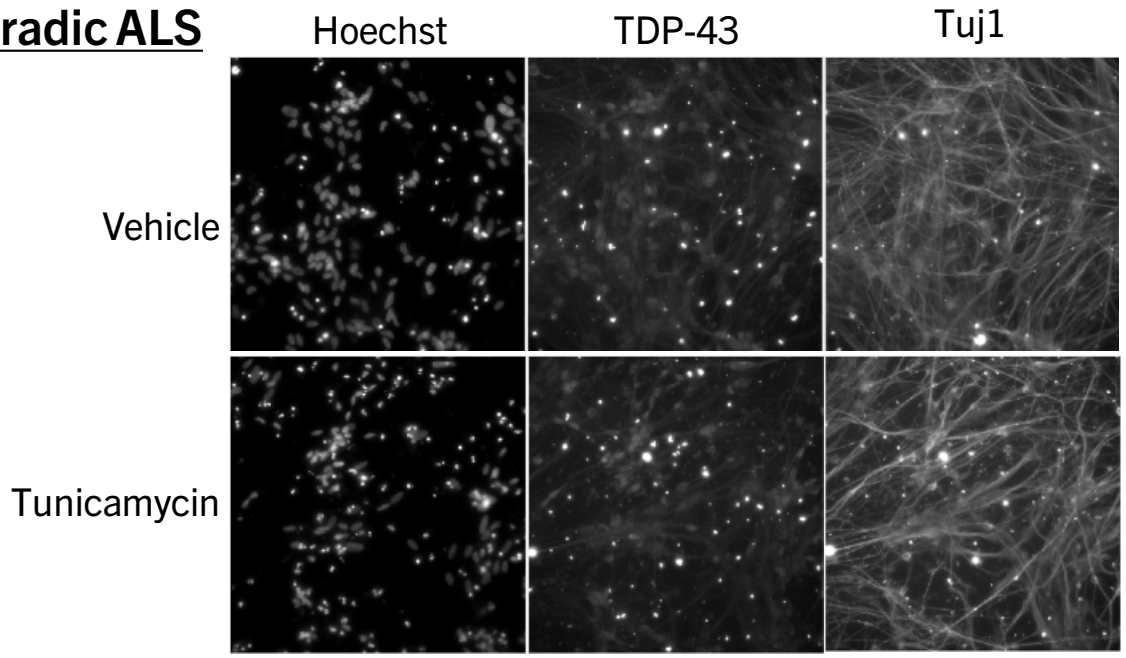
Wild Type



SOD1A4V



Sporadic ALS



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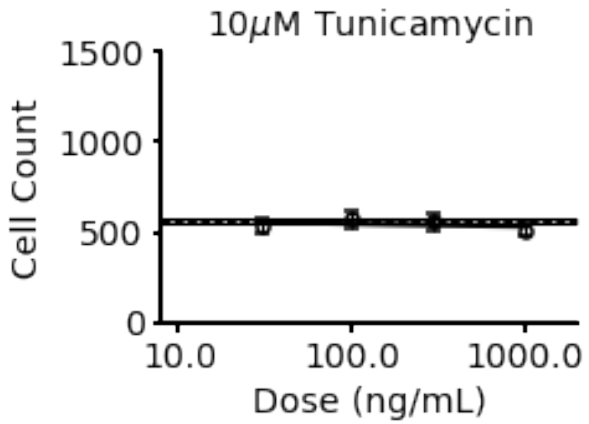
QUANTITATIVE DATA

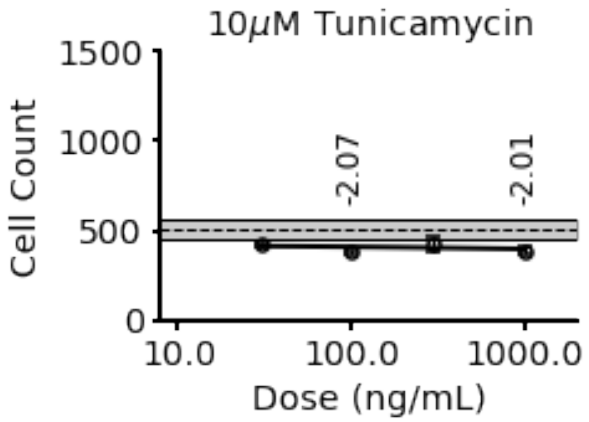
Viable cell count

Viable-cell count was unaffected after treatment with tunicamycin in wild-type and ALS motor neurons. Viable-cell count decreased with tunicamycin treatment in SOD1A4V motor neurons; the TA did not protect against cell death.

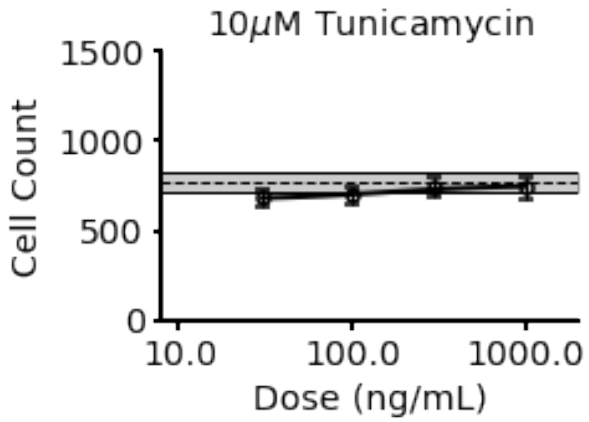
Wild-type



SOD1A4V



ALS

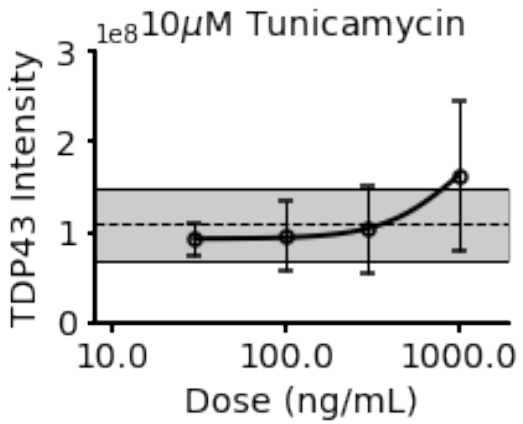


QUANTITATIVE DATA

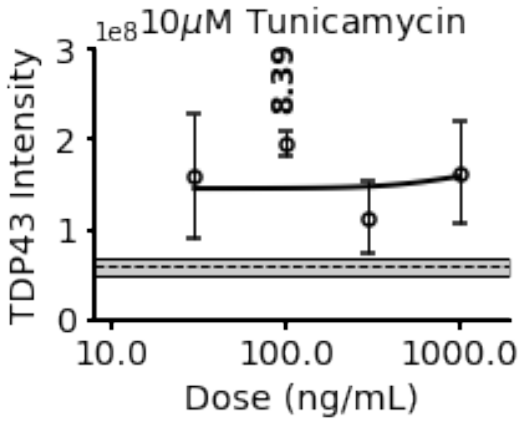
TDP-43 Intensity

TDP-43 intensity was unaffected by tunicamycin treatment in wild-type motor neurons.
The TA did not prevent TDP-43 intensity increases in SOD1A4V motor neurons.
The TA shows a dose-dependent trend toward a protective effect in ALS motor neurons.

Wild-type



SOD1A4V



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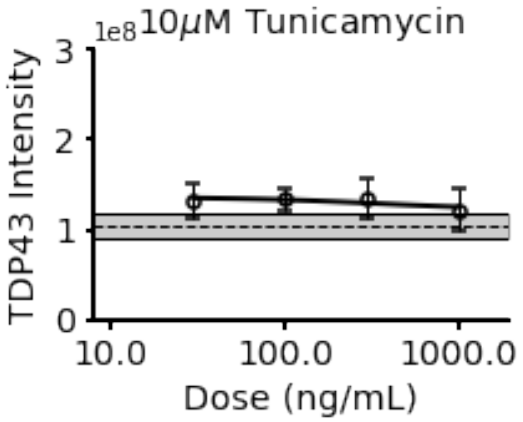
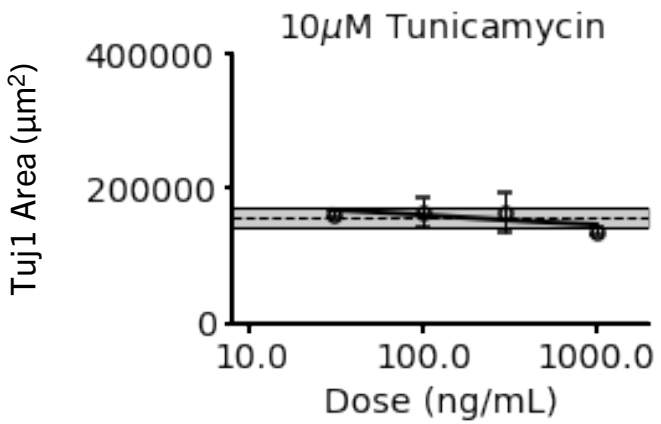


QUANTITATIVE DATA

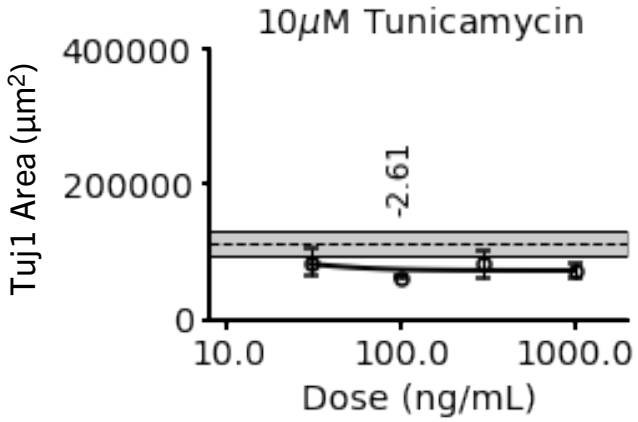
Tuj1 Area

Tuj1 area was unaffected by tunicamycin treatment in wild-type motor neurons.
The TA did not have a protective effect in SOD1A4V.
The TA trends towards a protective effect ALS motor neurons as measured by Tuj1 area.

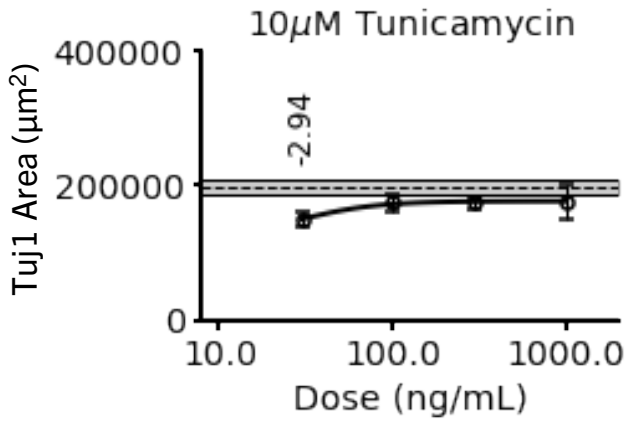
Wild-type



SOD1A4V



ALS

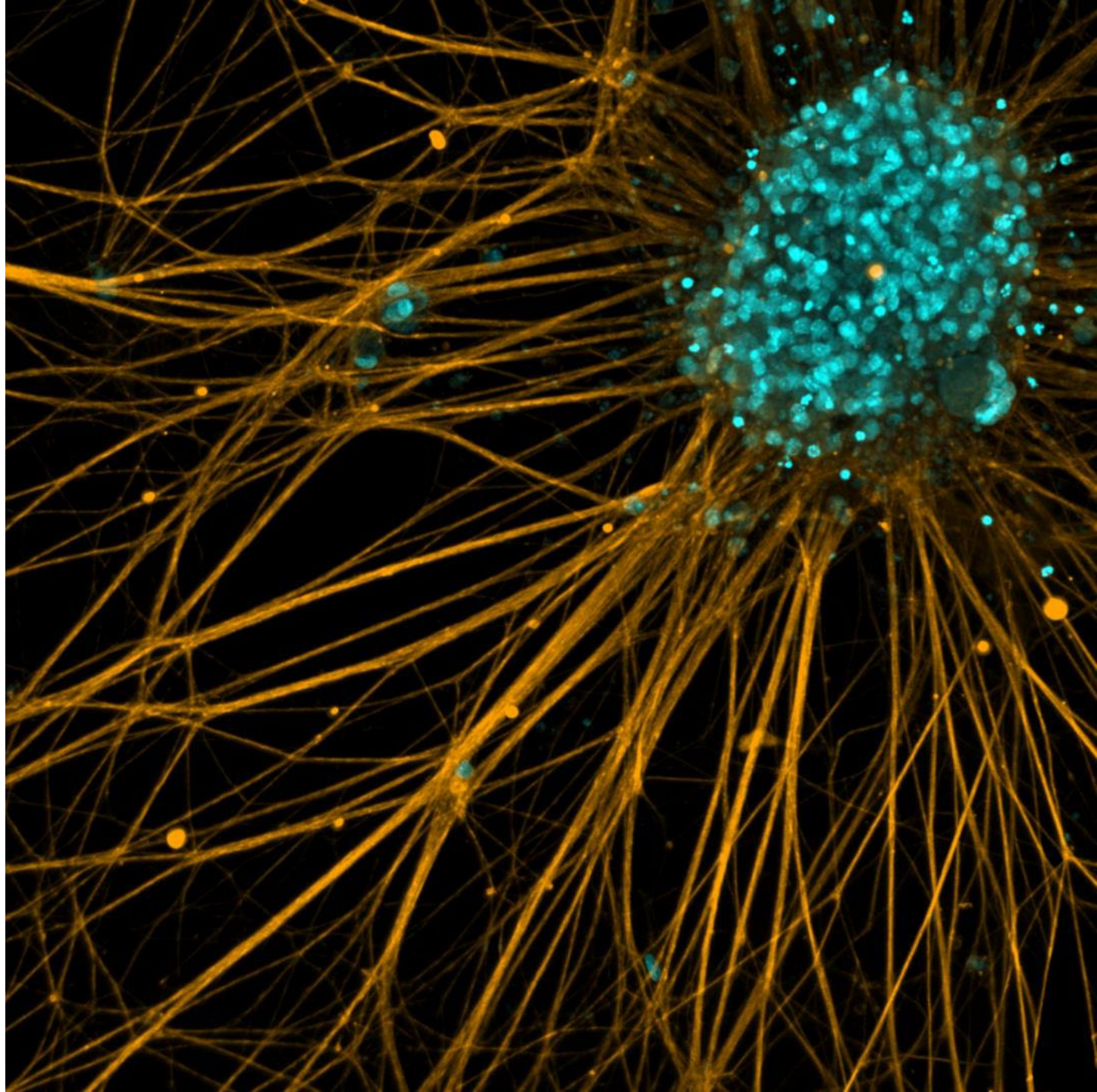


SUMMARY

1. Wild-type motor neurons largely were unaffected by tunicamycin treatment.

2. The TA did not show protective effects in any of the three metrics in SOD1A4V motor neurons.

3. The TA showed potentially protective effects in ALS motor neurons.



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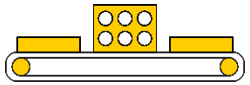
ADDITIONAL RESOURCES

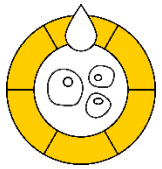
PhenoVista's Services

We develop assays in close collaboration with our clients to ensure that your specific questions will be answered. You can choose from a range of services to select the best fit for your needs. For more information, visit https://phenovista.com/assay-services

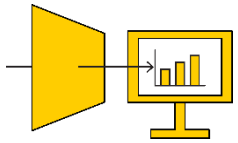
Custom Assay Services
Custom assays to answer your specific, complex biological questions.



Ready-2-Go Assay Services
Defined assay offerings across a range of disease and therapeutic areas.



Cell Painting
Compare your compounds' effects against those of reference compounds.



Imaging & Analysis
Send us plates of fixed & stained cells, and we'll send you data.

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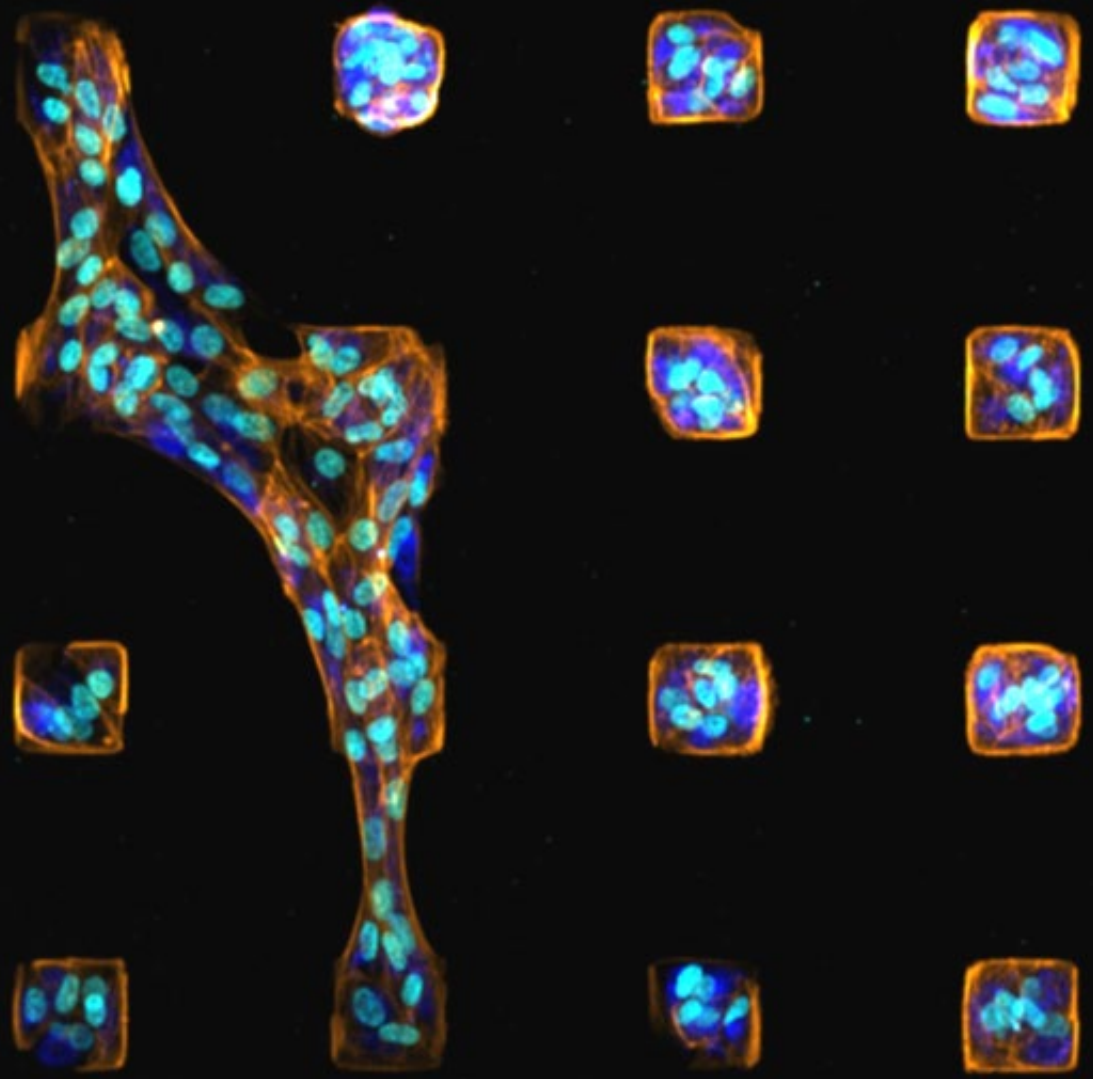

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