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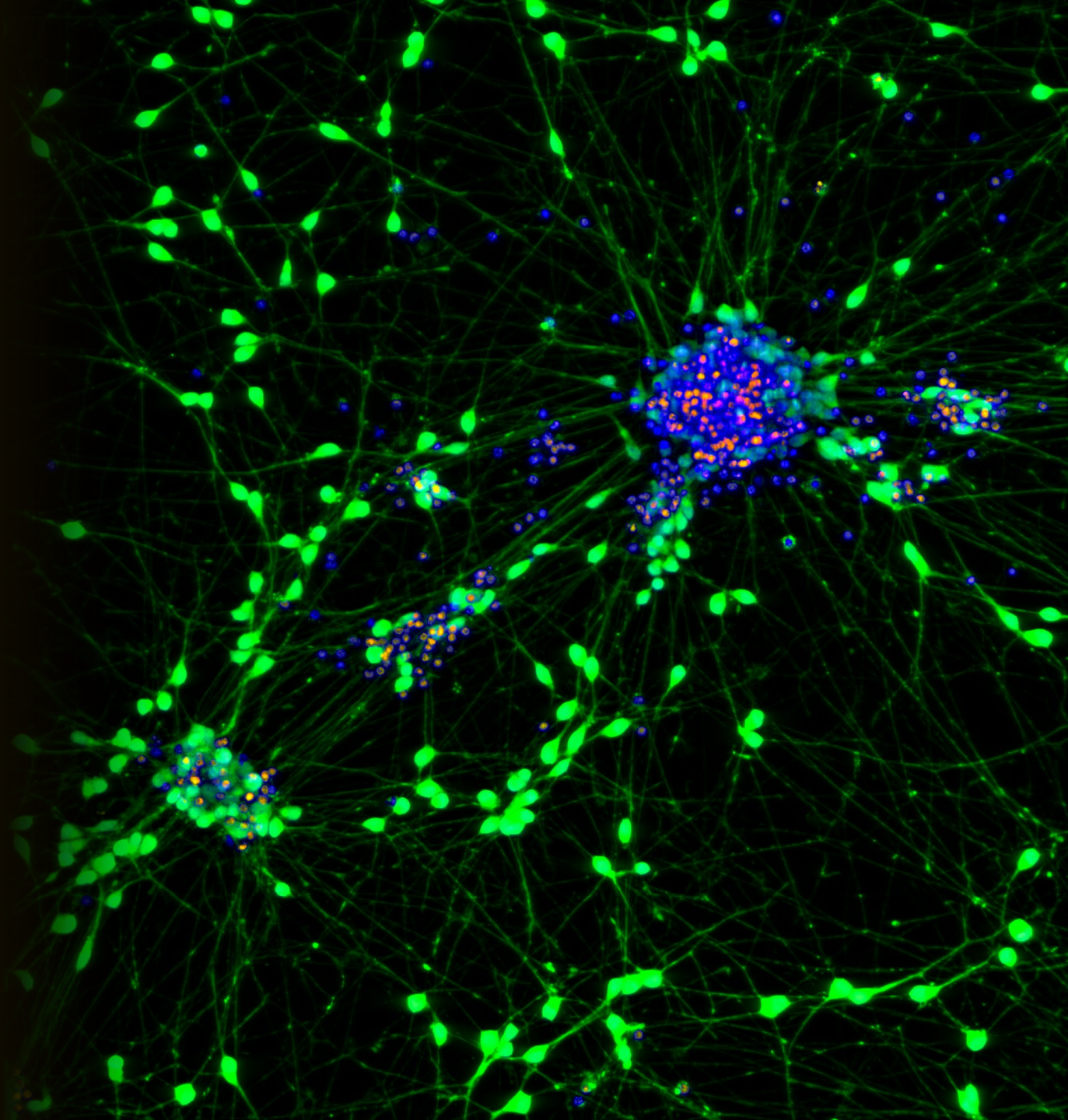
Understanding Complex Biology

CASE STUDY

Evaluating a Therapeutic Agent's Effects on Various Health Metrics in a Neuronal Tri-culture



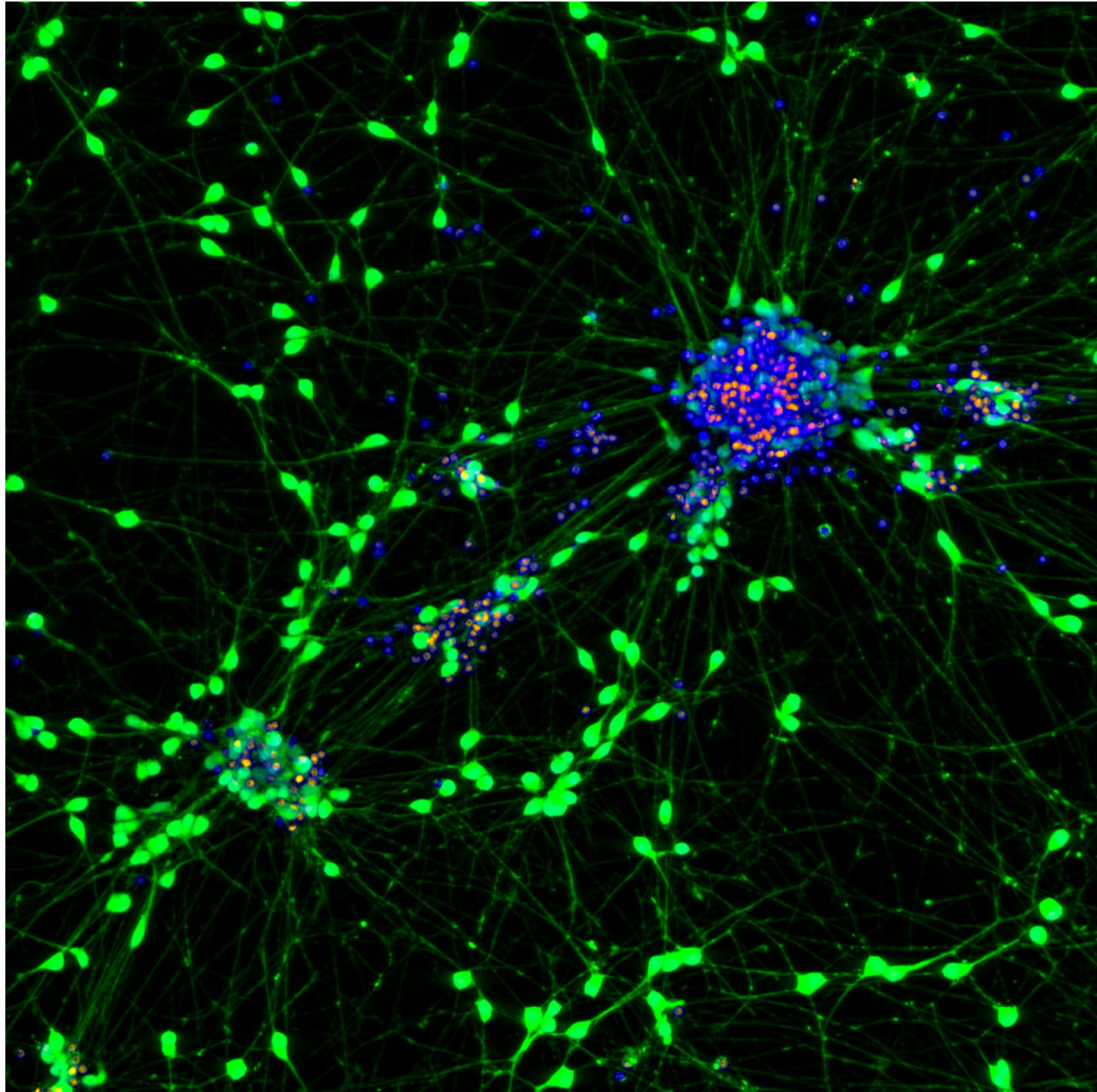
OBJECTIVE

A client requested a custom, neuronal tri-culture model to evaluate their therapeutic agent's effects on various health metrics.

Goals

1. Establish a neuronal tri-culture model using human, iPSC-derived glutamatergic neurons, astrocytes, and microglia.

2. Evaluate the effects of their therapeutic agent on various health metrics, including synapse formation.



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EXPERIMENTAL DESIGN

Cell Models – tri-culture

iPSC-glutamatergic neurons
iPSC-microglia
Primary, human astrocytes

Palette 1

Hoechst (nuclei)
Anti-Tuj1 (neuron/neurite marker)
Anti-GFAP (astrocyte marker)
Anti-Iba-1 (microglia marker)

Palette 2

Hoechst (nuclei)
Anti-Tuj1 (neurons)
Anti-PSD95 (postsynaptic marker)
Anti-synaptophysin (presynaptic marker)

Optimization

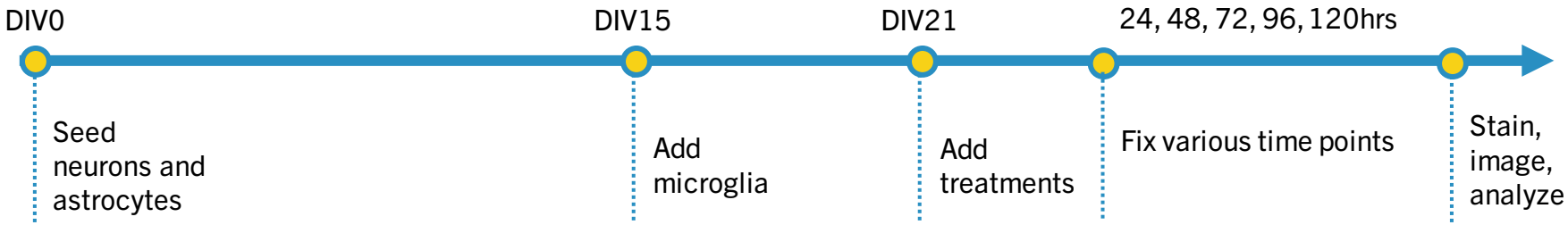
- Establish a neuronal, tri-culture model and measure changes in various health metrics in response to treatment with a therapeutic agent.

Treatments and Timelines

- Culture cells in 384-well, imaging microplates, following custom culture protocol developed at PhenoVista
- Culture neurons and astrocytes until DIV15
- At DIV15, titrate microglia into culture
- At DIV21, treat with client-provided therapeutic agent
- Fix and stain cultures at various time points out to 120 hours post-treatment

Deliverables

- May include: Cell count, neurite-network analysis, neuron morphology, astrocyte morphology, microglia morphology, expression level of various markers, other metrics as appropriate for the study design.
- A presentation-ready report that includes detailed methodology, statistical analysis, and IC_{50} curve-fits where applicable. Representative images will be provided for controls and a reasonable selection of test conditions.



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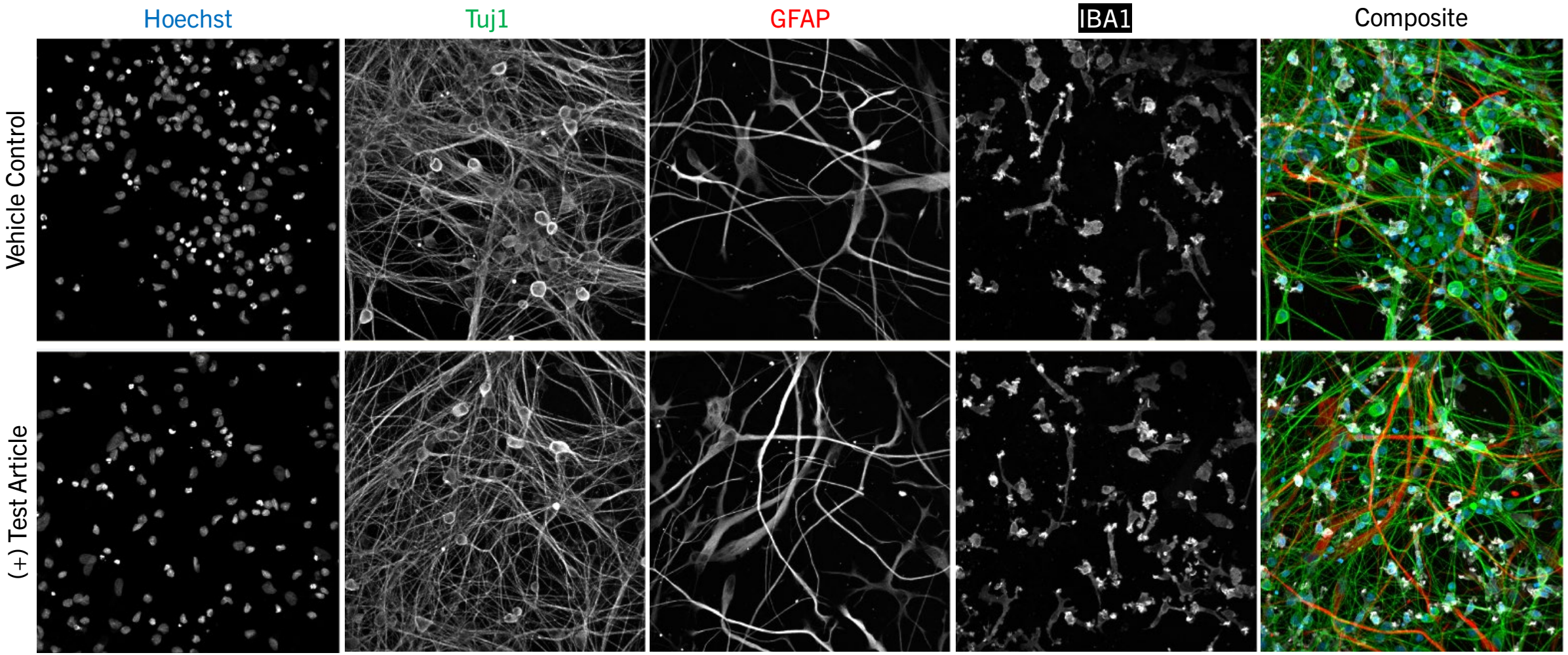
REPRESENTATIVE IMAGES

72hrs post-treatment

Representative images of tri-culture of neurons, astrocytes, and microglia after treatment with client-provided therapeutic agent.



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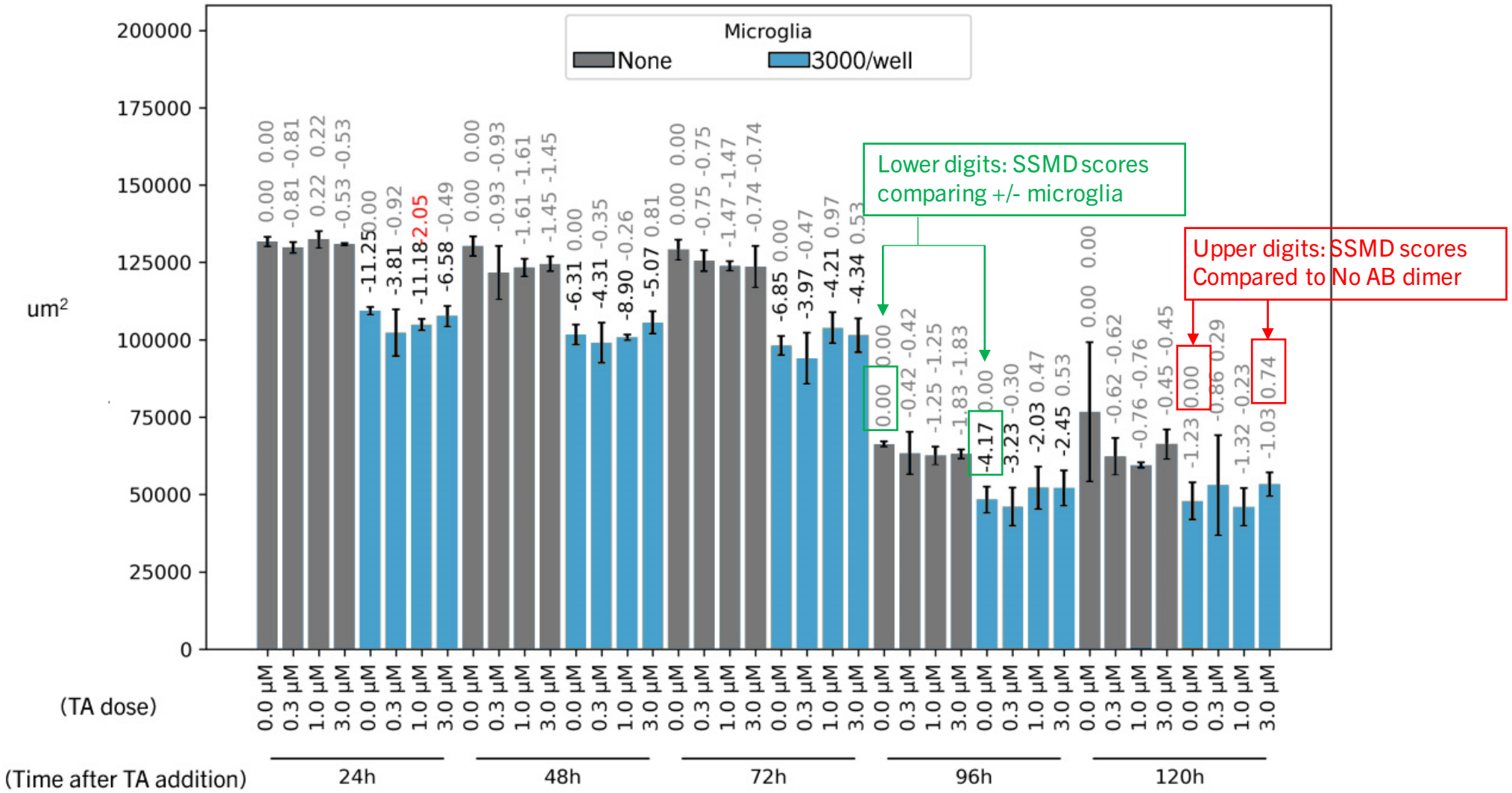
QUANTITATIVE DATA

Neurite Area

Neurite area decreased with presence of microglia compared to no-microglia cultures.

Neurite area decreased in both culture conditions (with and without microglia) over time after addition of therapeutic agent.



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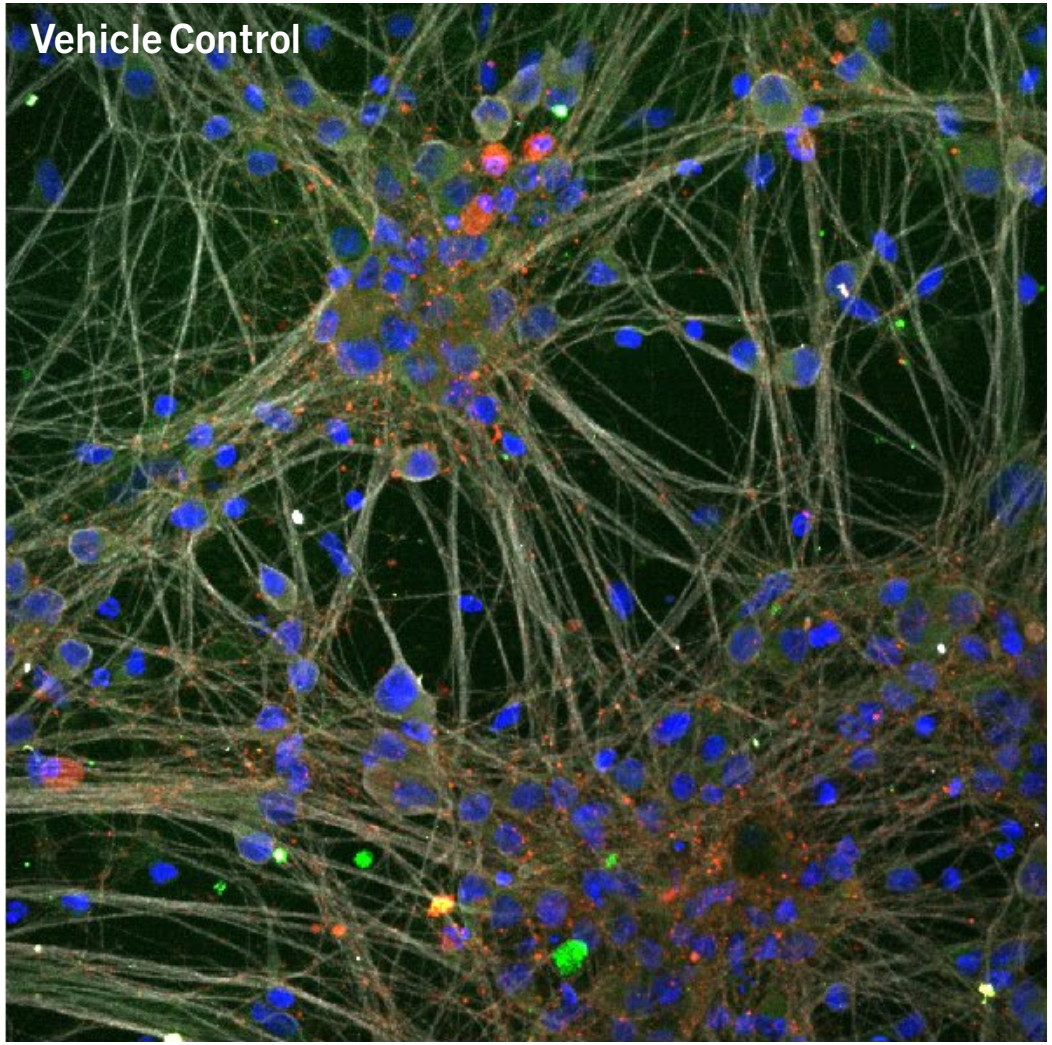
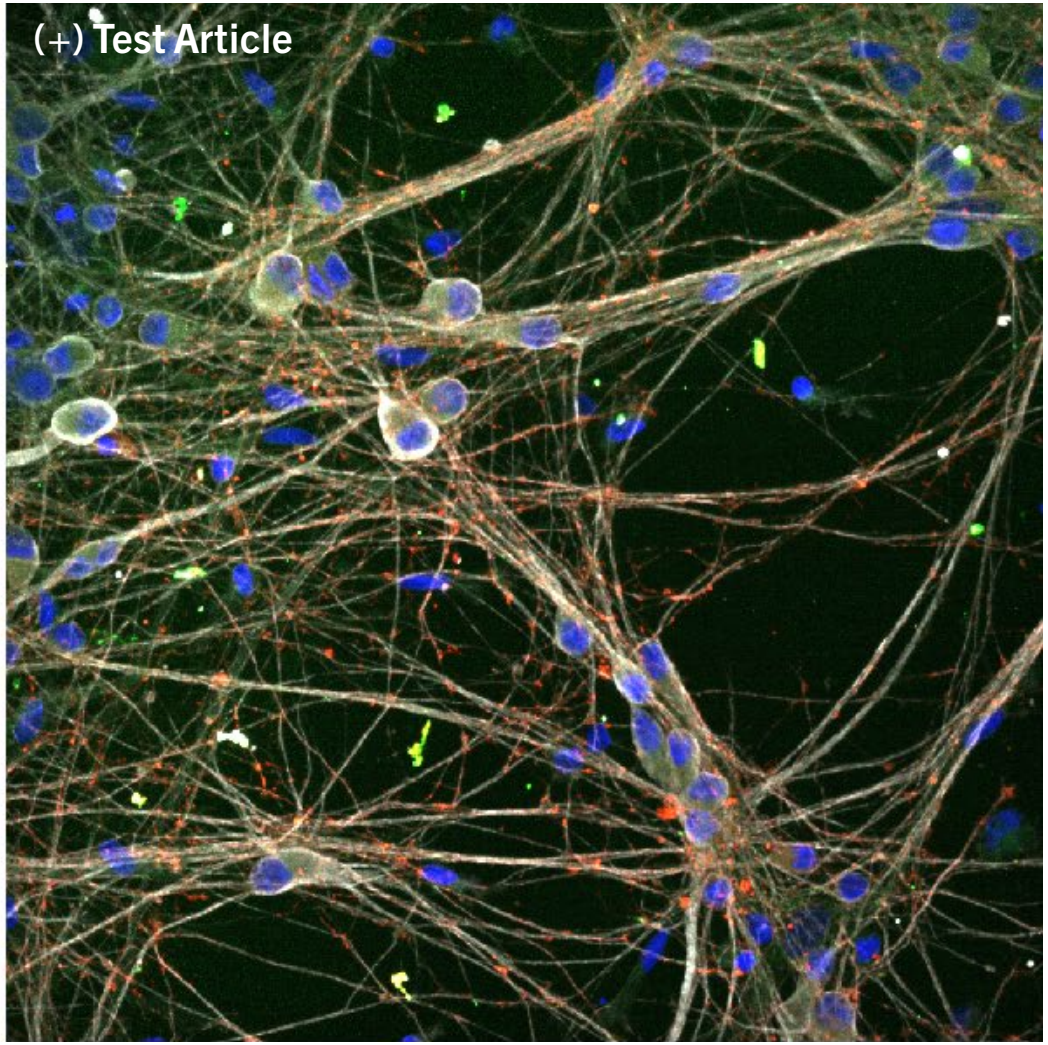
REPRESENTATIVE IMAGES

72hrs post-treatment

Representative images of tri-culture of neurons, astrocytes, and microglia stained for synapses 72hrs after treatment with client's therapeutic agent.





Hoechst

TUJ1

PSD95

Synaptophysin

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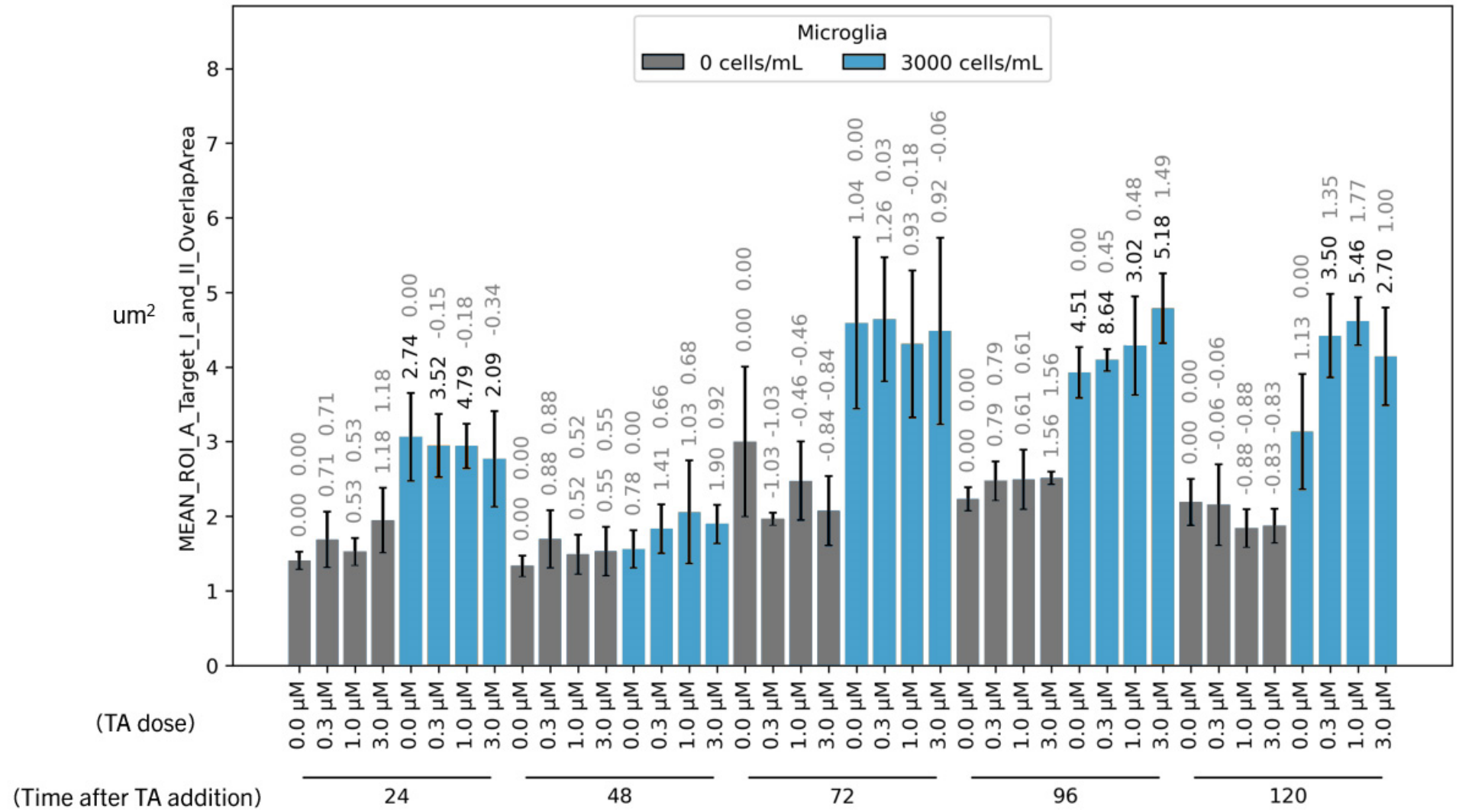
QUANTITATIVE DATA

Synaptogenesis

Synaptogenesis increased with the presence of microglia compared to no-microglia cultures.

Synaptogenesis increased in both culture conditions (with and without microglia) over time after addition of therapeutic agent.



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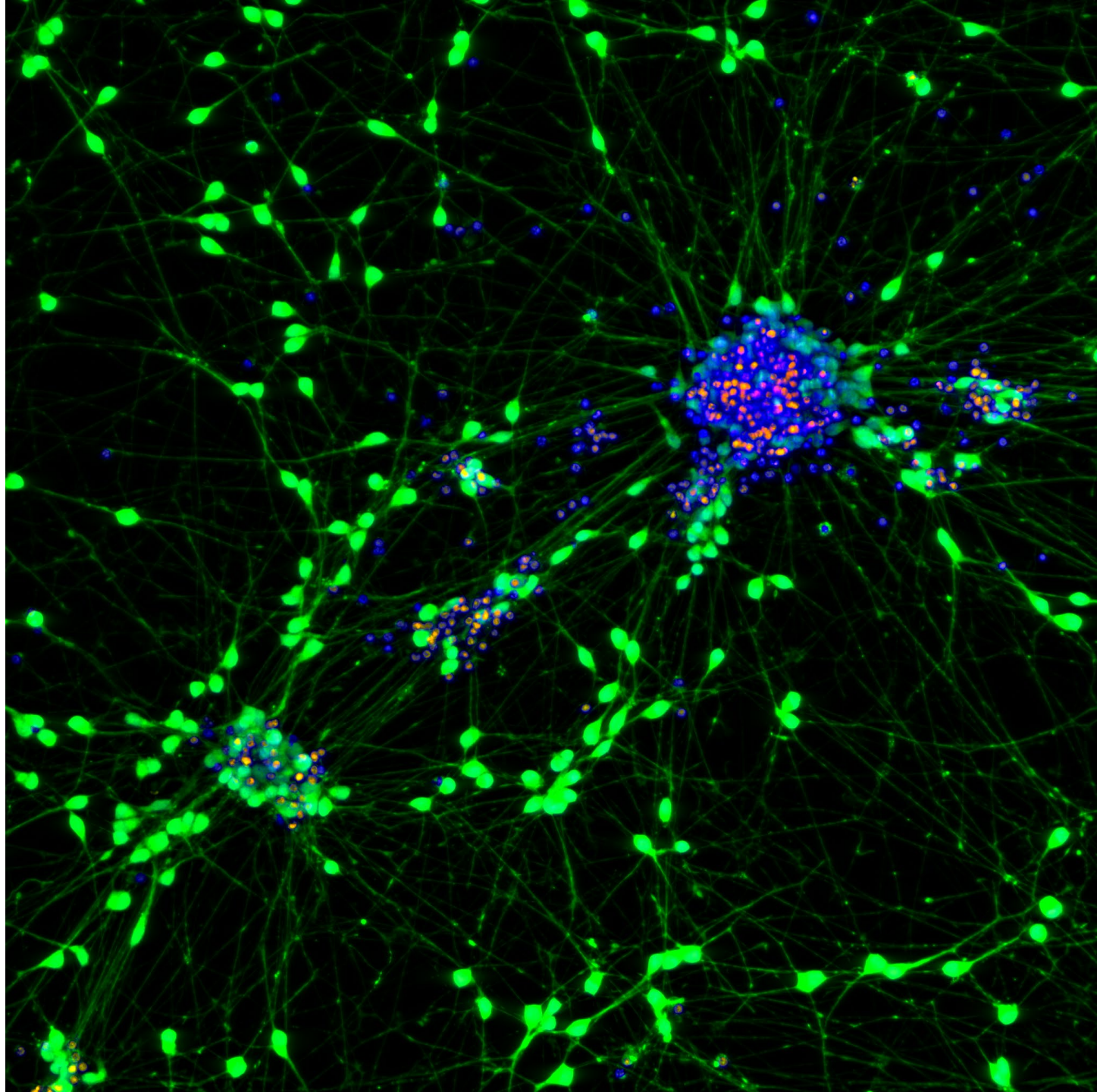
SUMMARY

1. The addition of microglia to neuron-astrocyte co-cultures significantly affected various health metrics.

2. Addition of the therapeutic agent decreased neurite-network area in both culture conditions over time, with overall lower neurite areas in the (+)-microglia culture vs. the (-)-microglia culture.

3. Addition of the therapeutic agent increased synaptogenesis in the tri-culture condition over time.



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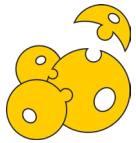
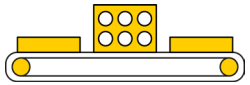
ADDITIONAL RESOURCES

PhenoVista's Services

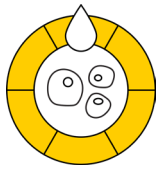
We develop assays in close collaboration with our clients to ensure that your specific questions will be answered. You can choose from a range of services to select the best fit for your needs. For more information, visit https://phenovista.com/assay-services



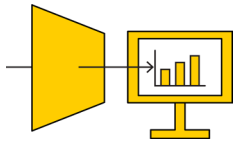
Custom Assay Services
Custom assays to answer your specific, complex biological questions.



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.

Learning Library

Visit https://phenovista.com/resources to browse additional resources such as

- Brochures
- Case studies
- Webinars
- Blog posts

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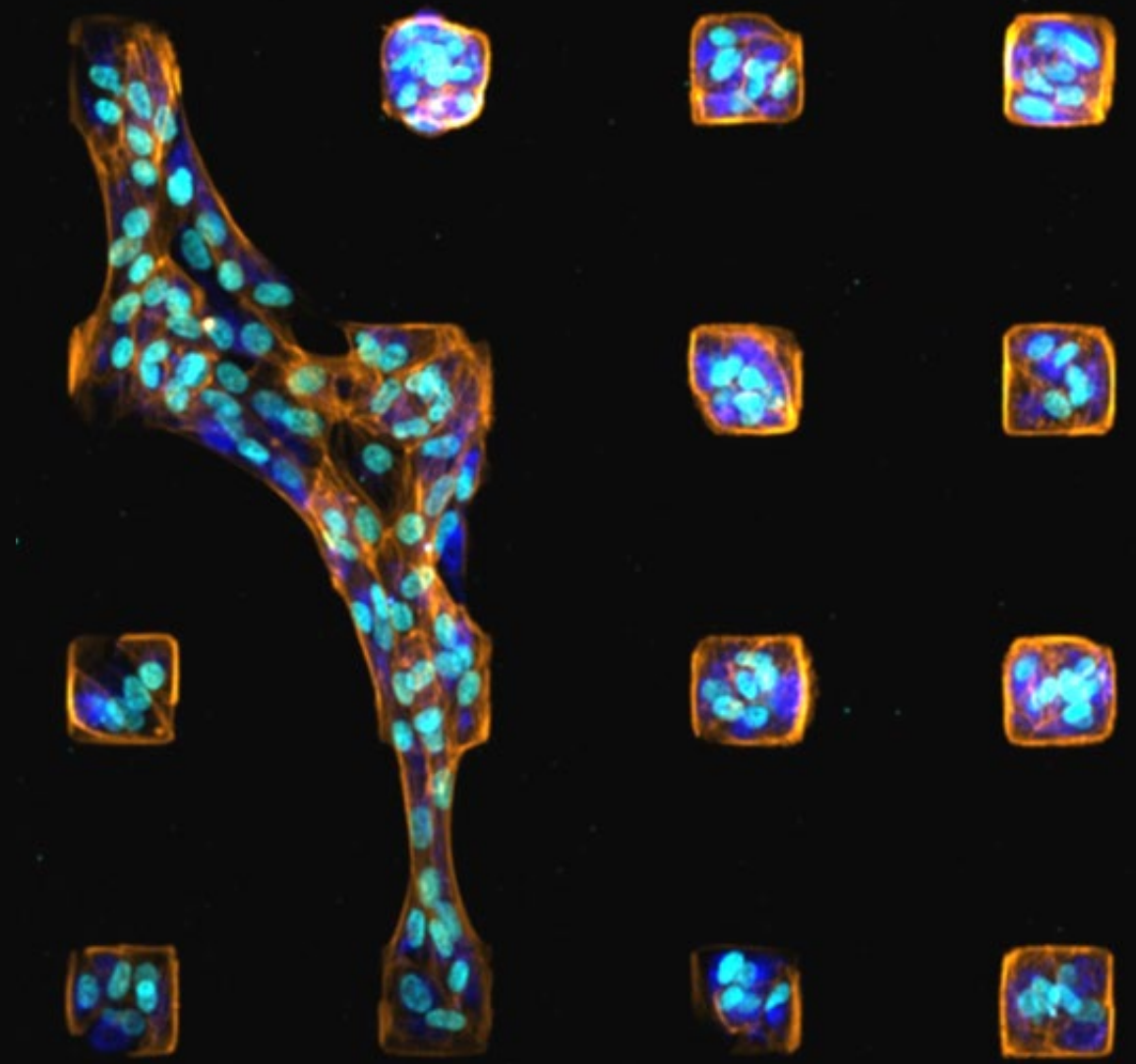

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