

# PHENOTYPIC STUDIES TO DE-RISK PARKINSON'S DISEASE R&D

## INTRODUCTION

To aid in our clients' R&D programs, we specialize in developing *in vitro*, phenotypic screening assays that range from simple monocultures to complex, multi-cell type models. We collaborate with our clients to design custom assays that will answer their most pressing questions.

Here, we outline a few PD-relevant assays that can help inform your go/no-go, R&D decisions.

### Characterize the Neurotoxicity of a Drug Candidate

Understanding how various substances affect cell health better informs PD progression. Furthermore, understanding drug candidates' safety profiles is essential to their development. In this experiment, we measured apoptosis and cell viability in neurons treated with stressors.

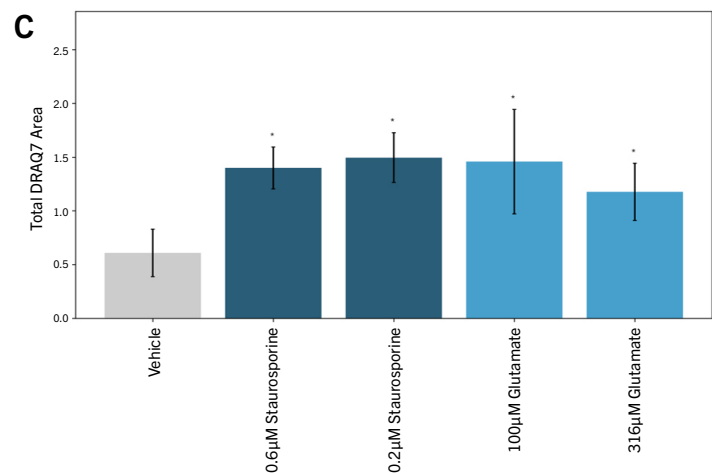
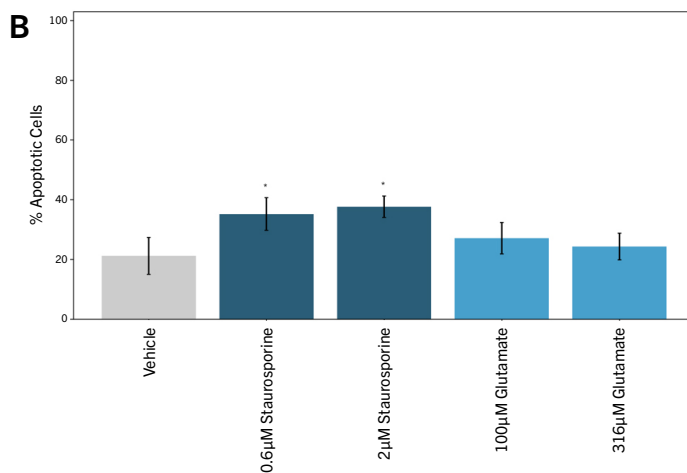
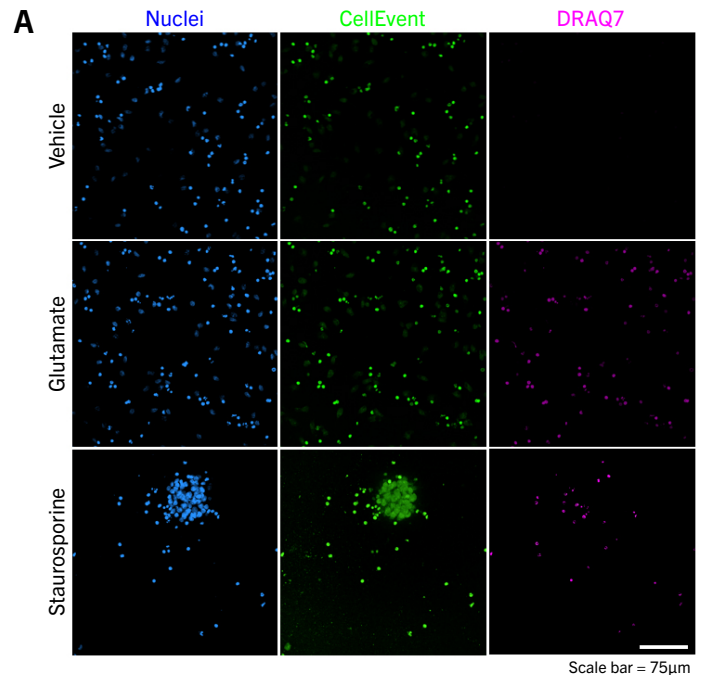
**Cells:** iPSC-derived glutamatergic neurons

**Treatments:** Glutamate, staurosporine

**Markers:** Hoechst (nuclei), CellEvent (apoptosis), DRAQ7 (viability)

**Study Results:** Different drugs or compounds can induce different types of cell death. In this case, staurosporine induced greater levels of apoptosis than glutamate.

**Figure 1.** (A) Representative images of neurons treated with vehicle, glutamate, or staurosporine and stained for nuclei (Hoechst), apoptosis (CellEvent), or death (DRAQ7). Treatment with staurosporine significantly increased percentage of apoptotic cells (B). Treatment with either glutamate or staurosporine significantly increased neuronal death (C). \* *p*-value < 0.05 relative to vehicle.



## Characterize the Effect of a Drug Candidate on Mitochondrial Stress and Dopamine Production

In this experiment, we quantitatively assessed two hallmarks of PD – changes in mitochondrial function and reduced levels of dopamine. To this end, we analyzed differences in mitochondrial membrane polarization and levels of tyrosine hydroxylase (TH) – a key enzyme in dopamine production – after neurons were treated with a reactive oxygen species (ROS).

Cells: Parkin-deficient, dopaminergic neurons

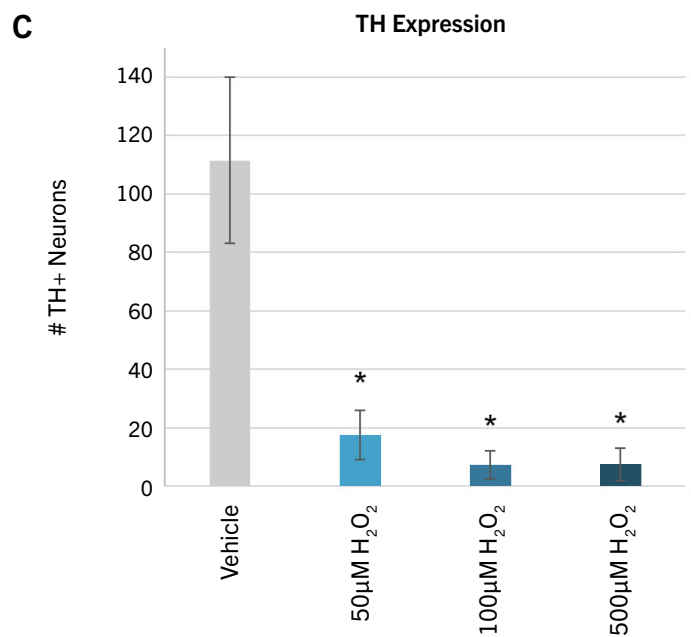
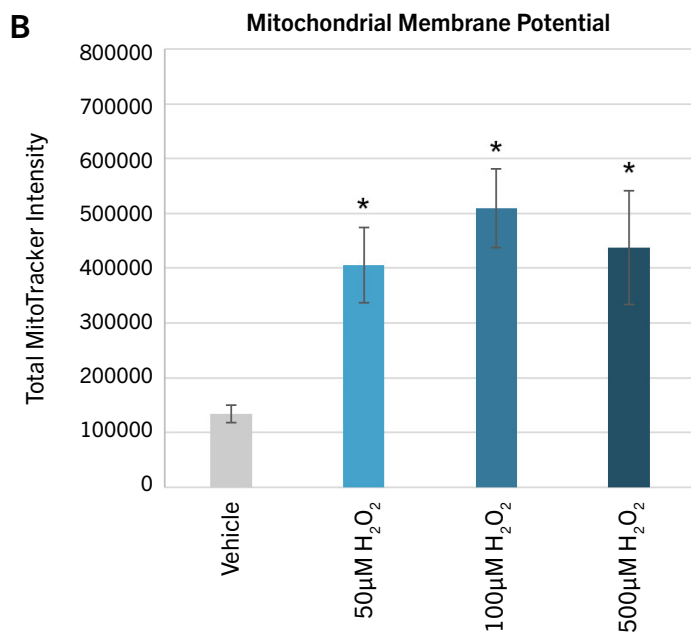
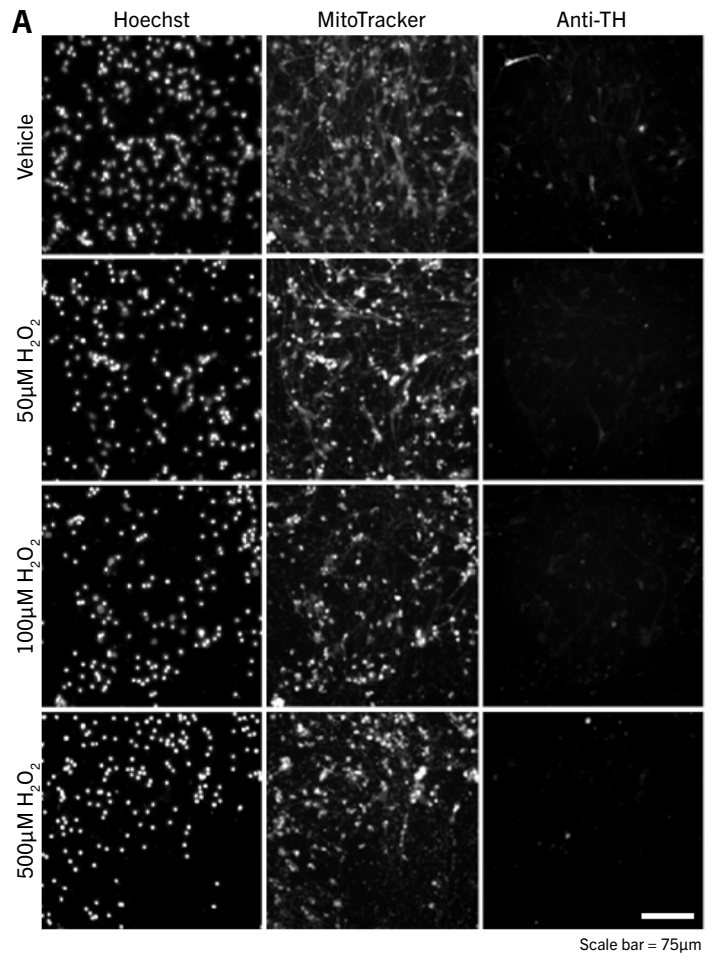
Treatment: Hydrogen peroxide

Markers: Hoechst (nuclei), MitoTracker CMXRos (mitochondrial membrane potential), anti-TH antibody

Study Results:

- The presence of ROS can have significant effects on neuronal function, as measured by alterations in mitochondrial membrane potential.
- The presence of ROS may reduce dopamine production through decreased levels of TH.

**Figure 2.** (A) Representative images of neurons treated with vehicle or hydrogen peroxide and stained for nuclei (Hoechst), mitochondrial membrane potential (MitoTracker), and TH expression (anti-TH). Treatment with hydrogen peroxide significantly increased MitoTracker CMXRos signal (B) and significantly decreased levels of TH expression (C). \* SSMD >2 relative to vehicle.



## Characterize the Effect of a Drug Candidate on Lysosome Function

Lysosome dysfunction is increasingly implicated in PD pathology, as they are indispensable to degradation of substrates/proteins. Protein accumulation and subsequent aggregation is a hallmark of neurodegenerative diseases. In this experiment, we demonstrate the modulation of lysosomal function in iPSC-derived neurons through treatments with various compounds, including autophagy regulators.

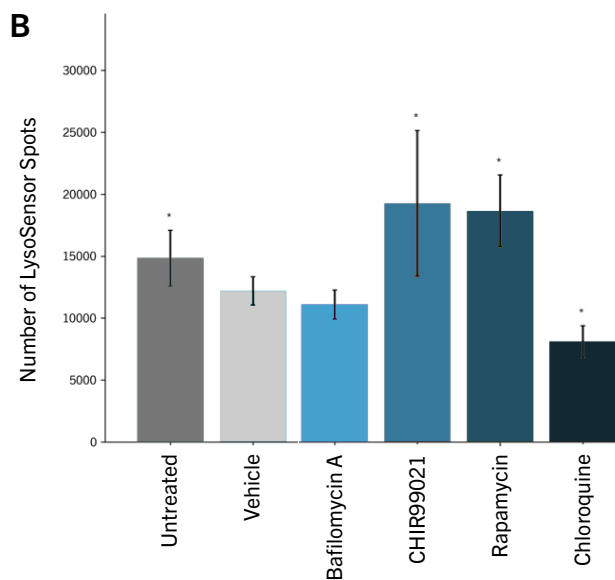
**Cells:** iPSC-derived, glutamatergic neurons

**Treatments:** Bafilomycin A, CHIR99021, rapamycin, chloroquine

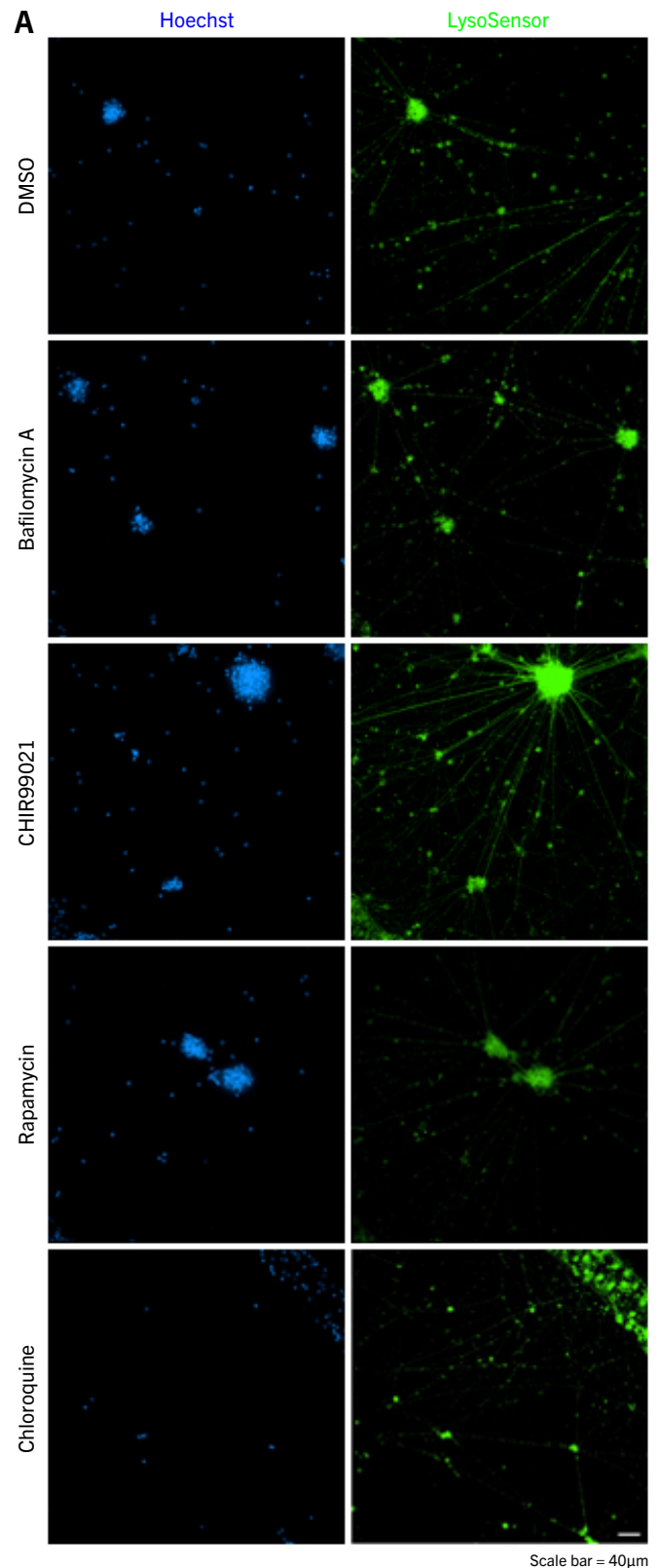
**Markers:** Hoechst (nuclei), LysoSensor Green (acidic lysosomes)

**Study Results:** Lysosomal function can be significantly disrupted or enhanced and quantitatively measured in a neuronal model through treatment with various compounds.

| Drug          | Mechanism of Action                            | Effect on LysoSensor Signal |
|---------------|------------------------------------------------|-----------------------------|
| Bafilomycin A | Inhibits lysosomal pH pump                     | No effect                   |
| CHIR99021     | Inhibits GSK3 → lysosome acidification         | Increase                    |
| Rapamycin     | Induces autophagy                              | Increase                    |
| Chloroquine   | Inhibits fusion of autophagosome with lysosome | Decrease                    |



**Figure 3.** (A) Representative images of neurons treated with vehicle or lysosomal stressors and stained for nuclei (Hoechst) and a lysosomal dye that increases in fluorescence with acidic pH (LysoSensor). (B) Treatment with bafilomycin A had no measurable effect, while treatment with CHIR99021 or rapamycin increased LysoSensor signal, and treatment with chloroquine decreased it. \*  $p$ -value < 0.05 relative to vehicle.



Questions?

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