

# The Link Between Mitochondrial Dysfunction and Neurodegeneration

## Roles of Parkin, PINK1, and Mitochondria in Parkinson's Disease

### **LifeSensors Inc.**

271 Great Valley Parkway

Malvern PA 19355

Phone: 610-644-8845

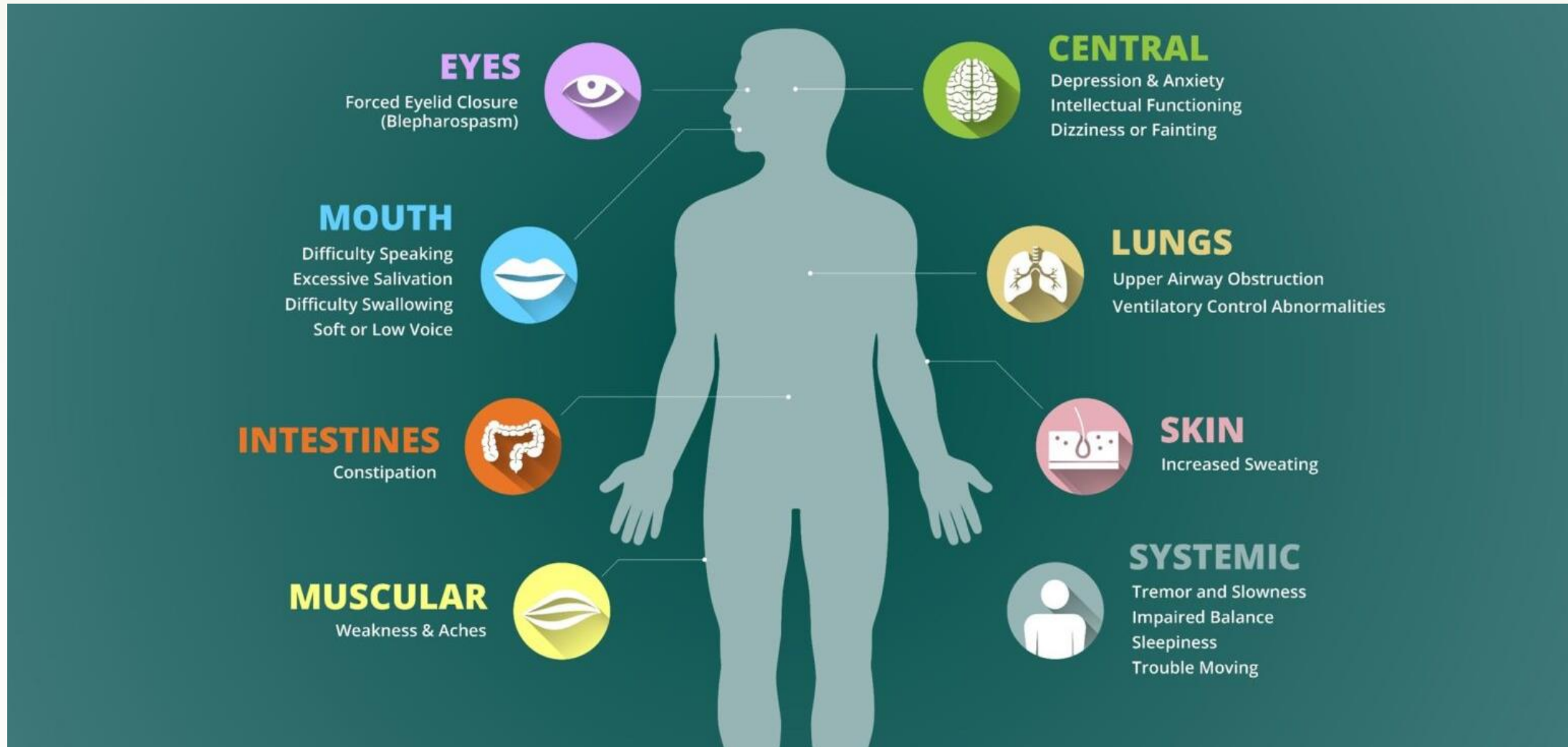
Fax: 610-644-8616

[www.lifesensors.com](http://www.lifesensors.com)

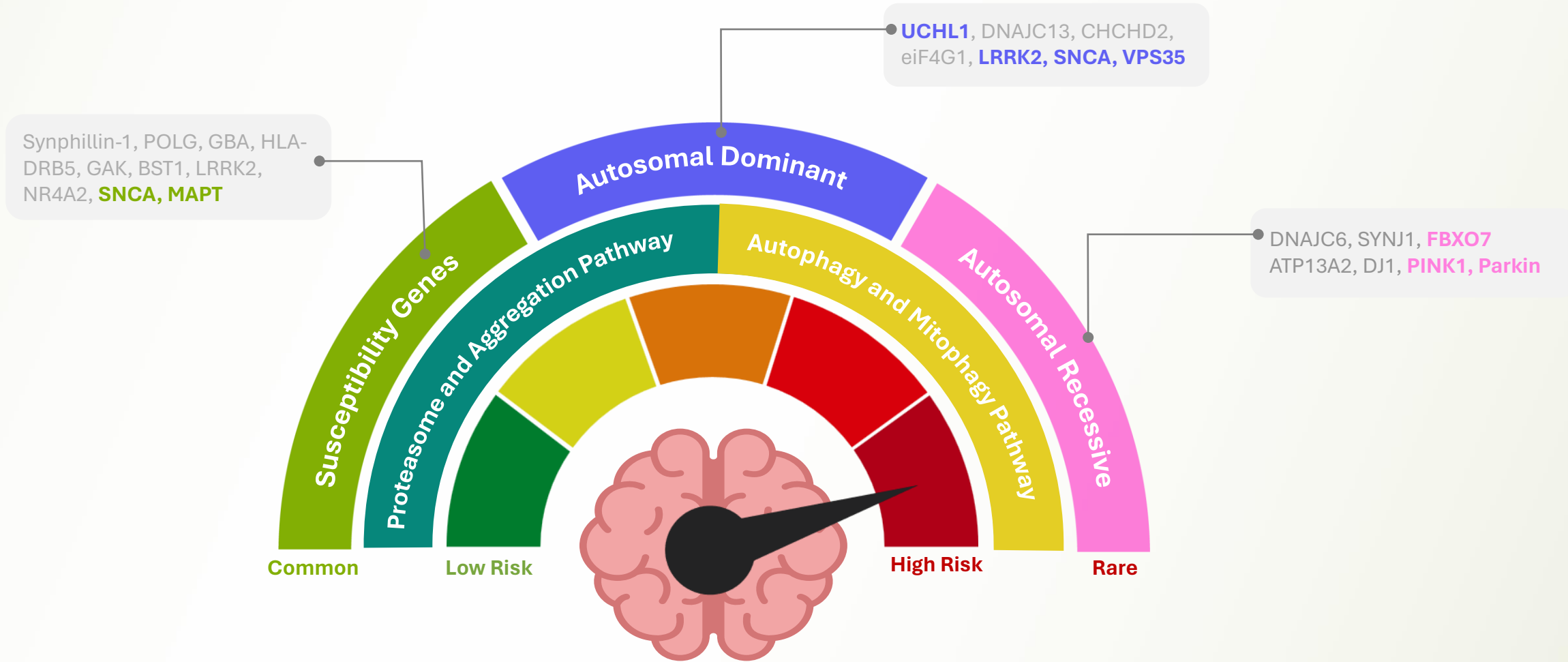
# Neurodegeneration

- Neurodegeneration is defined as slow and progressive loss of neuronal cells, culminating in cell death.
- Parkinson's Disease (PD) and Alzheimer's Disease (AD):
  - Characterized by accumulation of aggregated proteins and defective mitochondria, leading to neuronal cell death.
- The Role of Cellular Pathways:
  - Ubiquitin Proteasome System (UPS) and Autophagy-Lysosomal Pathway (ALP) are crucial in neurodegenerative diseases.
- Therapeutic Potential: Targeted degradation of aggregated proteins and damaged mitochondria holds promise for neuroprotection and transformative therapies in PD and AD.

# Parkinson's Disease: More than a Movement Disorder



# Lessons from Genetics - PD



# E3 Ligase Dependency - Neurodegeneration

## E3 ligases and their roles in neurodevelopmental diseases

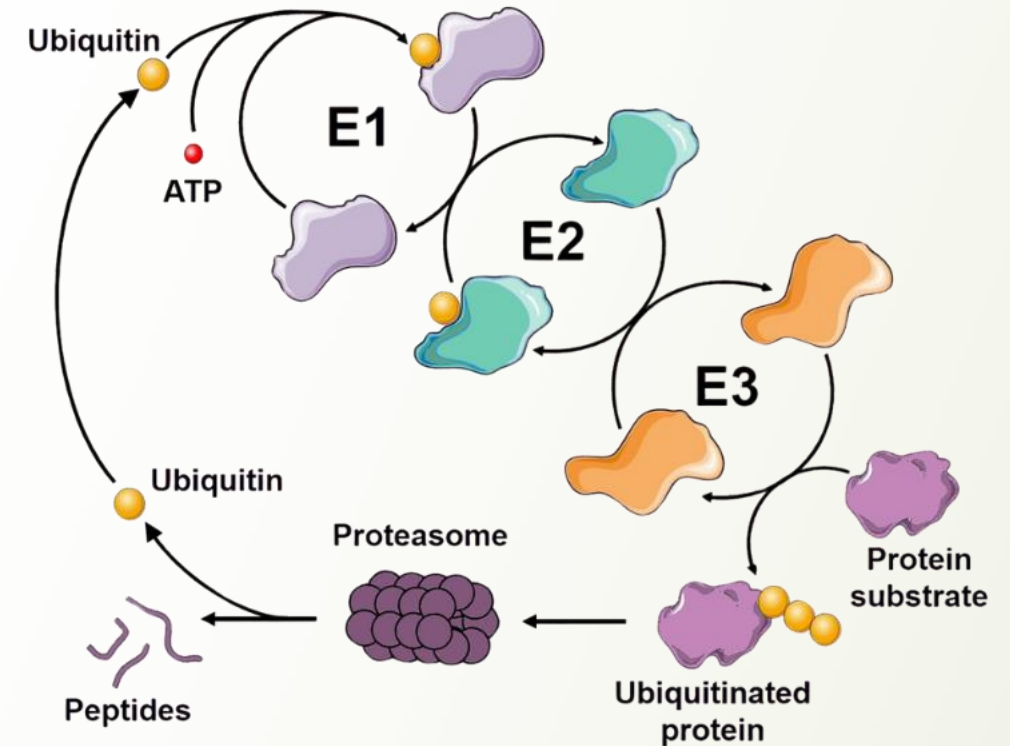


E3 ubiquitin ligases in neurological diseases: focus on gigaxonin and autophagy. *Frontiers in physiology*, 11, 568645.

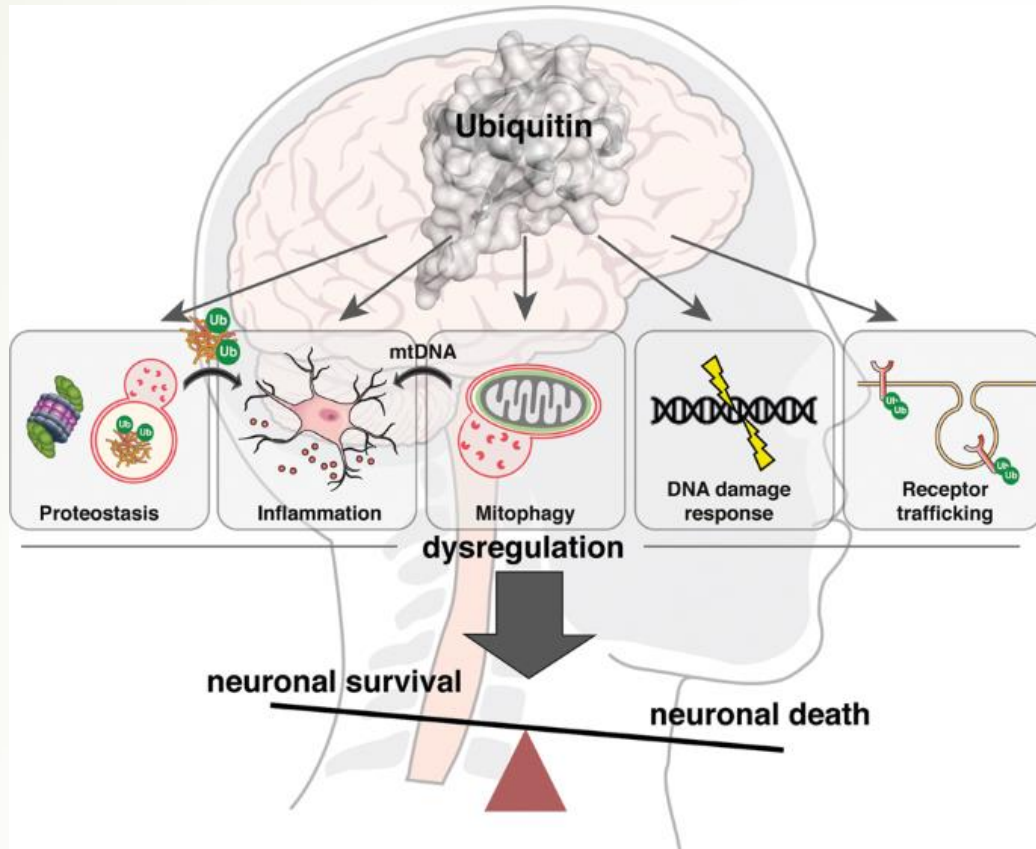
Ubiquitin signalling in neurodegeneration: mechanisms and therapeutic opportunities. *Cell Death & Differentiation*, 28(2), 570-590.

# Ubiquitin Proteasome System – The Guardian of the Cell

- Dysfunction in the Ubiquitin Proteasome System (UPS) leads to aggregation of mis-folded proteins, such as tau and  $\alpha$ -synuclein.
- Protein aggregates disrupt mitochondrial membranes, causing mitochondrial depolarization.
- Dysfunctional mitochondria and loss of ATP is the main cause of neuronal cell death.



# Ubiquitin Proteasome System (UPS) and Parkinson's Disease



Degradation & Non-degradative Ubiquitin Signaling

## Progressive loss of neurons vs UPS

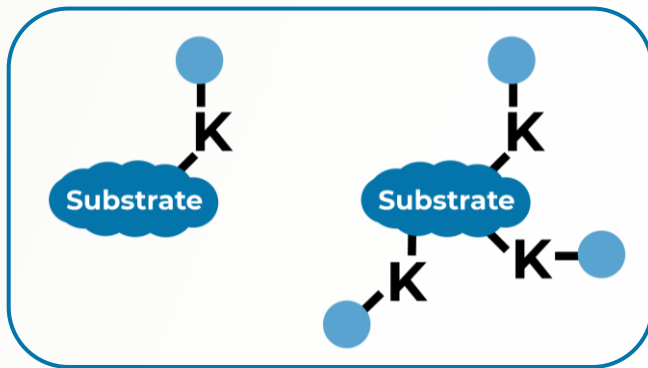
- One of the most overlooked and major component of proteinopathies is **ubiquitin**.
- Ubiquitin here is either an adaptive response to toxic misfolded proteins or evidence of dysregulated UPS driving aggregation.

E3 ubiquitin ligases in neurological diseases: focus on gigaxonin and autophagy. *Frontiers in physiology*, 11, 568645.

Ubiquitin signalling in neurodegeneration: mechanisms and therapeutic opportunities. *Cell Death & Differentiation*, 28(2), 570-590.

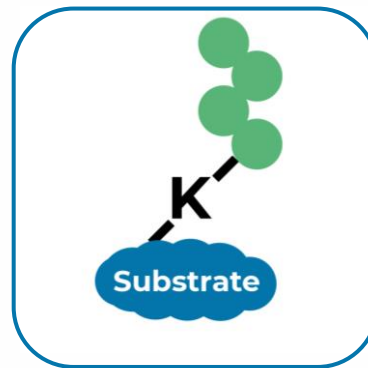
# Ubiquitin Code

Mono & Multi Mono-ubiquitination



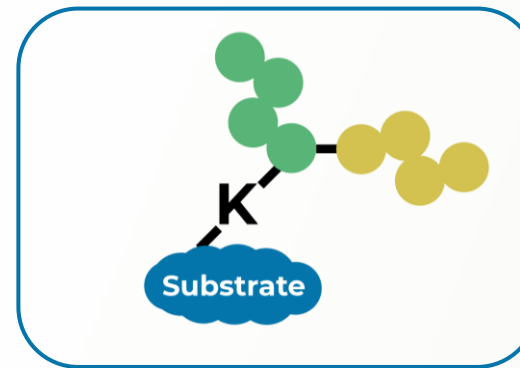
Protein Sorting & Histone modifications

Homotypic Chains



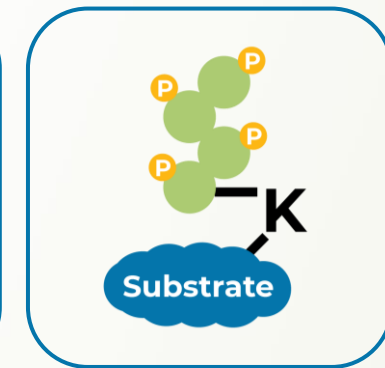
(M1, K6, K11, K27, K29, K33, K48, K63)  
&  
Degradation & Signalling

Heterotypic Chains



Enhanced Degradation & Signalling

Phospho-Ubiquitin



Signalling & Mitophagy

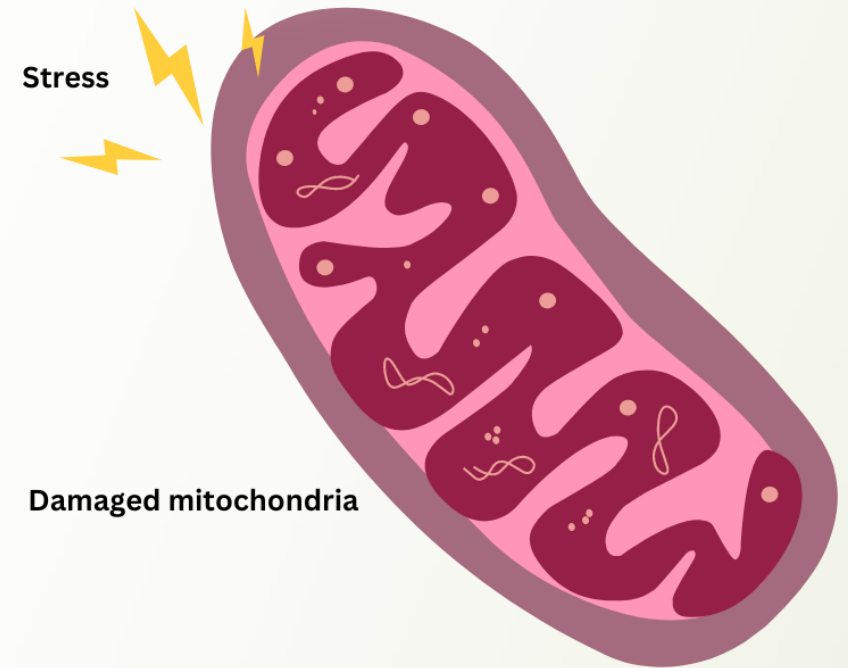


Degradation, Interactions, Localization & Signaling

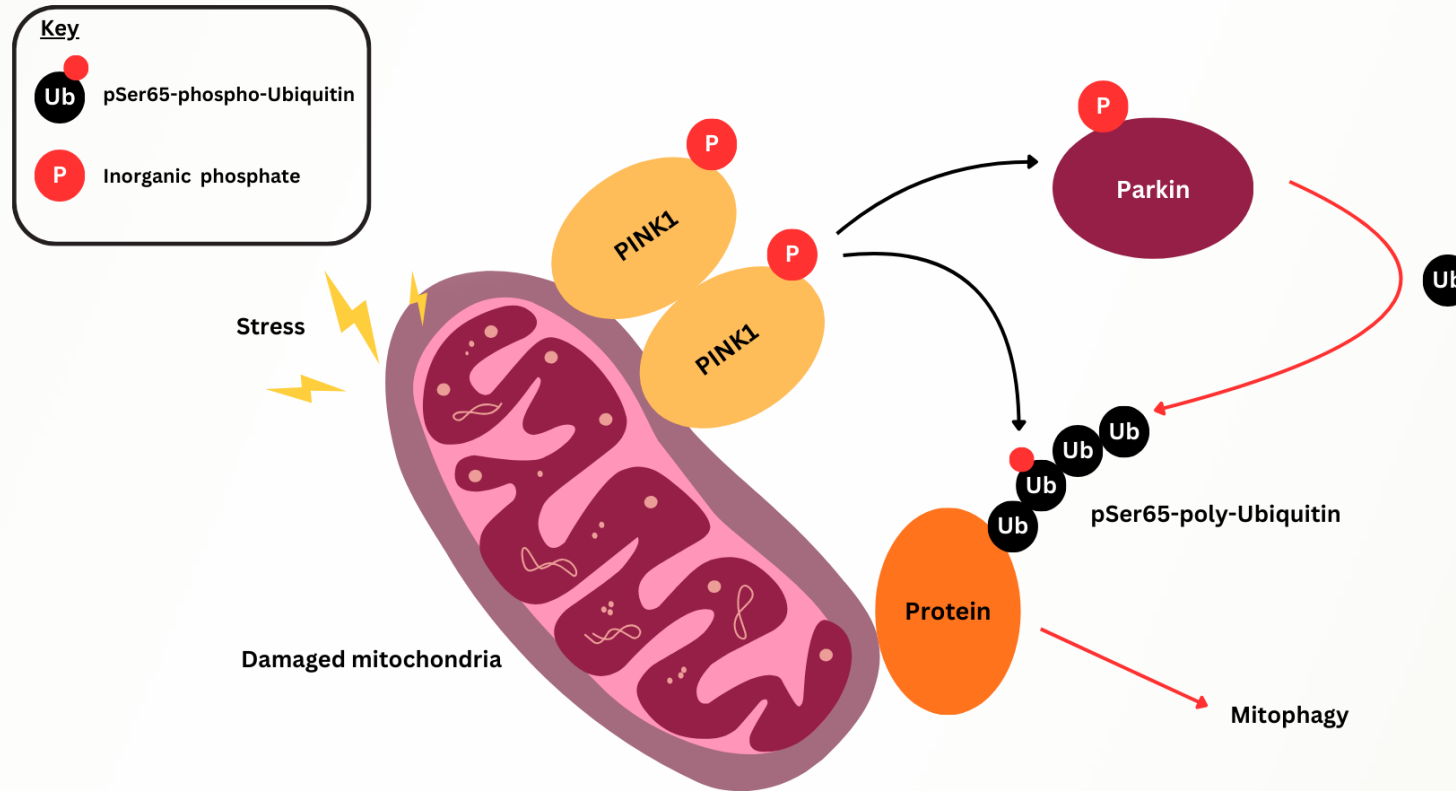


# Mitochondrial Health is Crucial in Neurodegeneration and PD

- Mitochondrial health is pivotal in neurodegeneration and PD, impacting energy supply and oxidative stress.
- [PINK1](#) and [parkin ligase](#) regulates mitochondrial dynamics quality control, initiating mitophagy to remove damaged mitochondria.
- Mutations in PINK1 and parkin genes are linked to familial Parkinson's disease.
- [Phospho-ubiquitin](#) aids in tagging damaged mitochondria for degradation during mitophagy, crucial for preventing neurodegeneration.



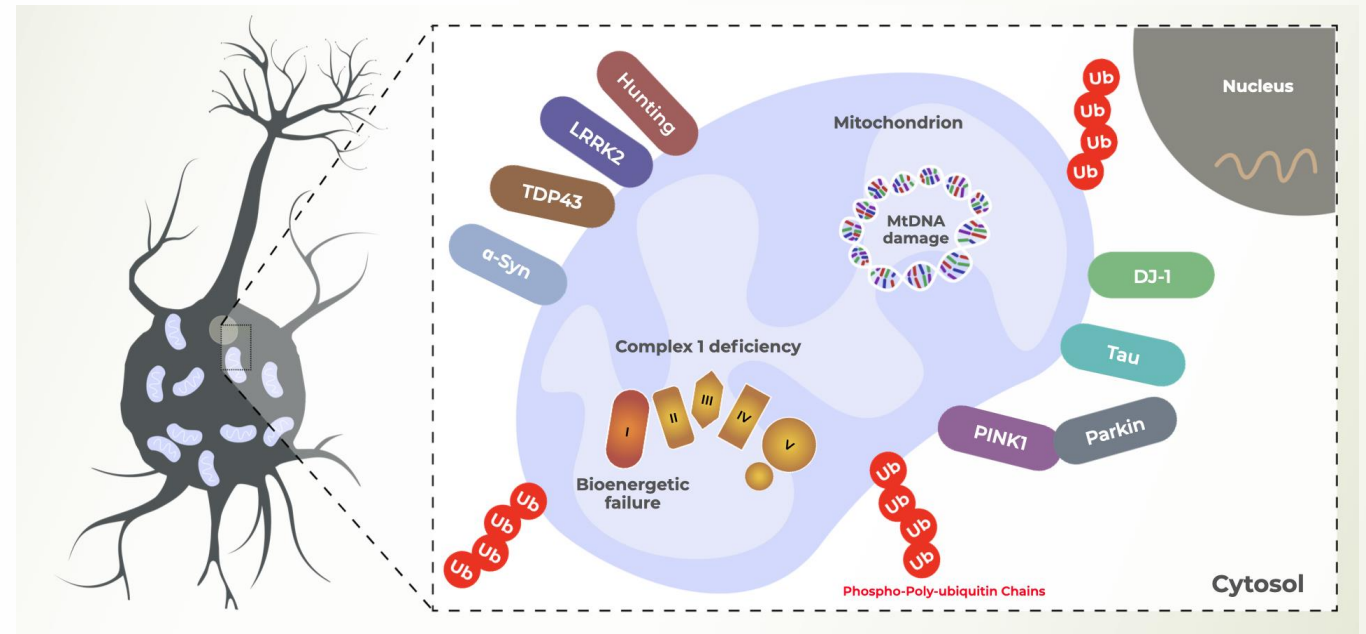
# Phospho-ubiquitin: A Hallmark of Neurodegeneration



- Within the mitochondrial membrane, the **PINK1** kinase becomes activated, triggering the phosphorylation of ubiquitin and parkin ligase.
- **Parkin ligase** then attaches phospho-ubiquitin (pUb) to the outer mitochondrial membranes, initiating mitophagy, the process of removing damaged mitochondria and promoting new mitochondria generation (mitobiogenesis).

# Protein Aggregation and Mitochondrial Damage

- Proteinopathies cause damage to mitochondria
- Many neurodegenerative diseases have protein aggregation problems in neurons
- Increase in [phospho-ubiquitin](#) is an indicator of neurodegeneration
- Development of blood-based phospho-ubiquitin key goal



# Are Tools Available for UPS Biomarker Discovery?

**Ubiquitin antibodies are not suitable for enrichment studies in complex matrices !!!**



---

Ubiquitin is highly conserved across species and is a **poor antigen**

---

Phospho-ubiquitin antibody is monovalent

---

Difficult to develop diagnostic applications

---

Phospho-polyubiquitin chains are heterogenous and antibodies do not detect all epitopes

---

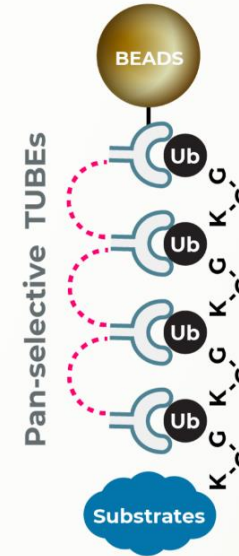
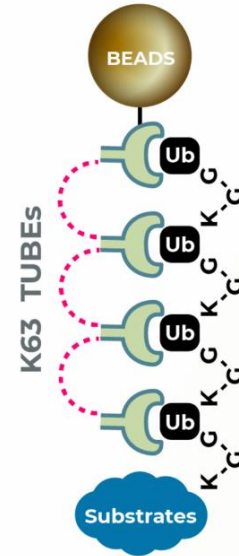
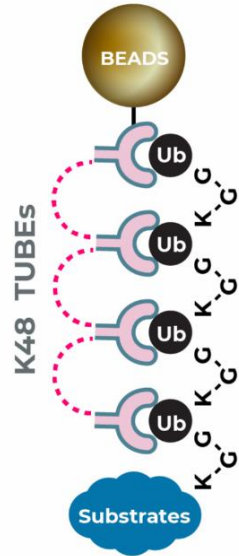
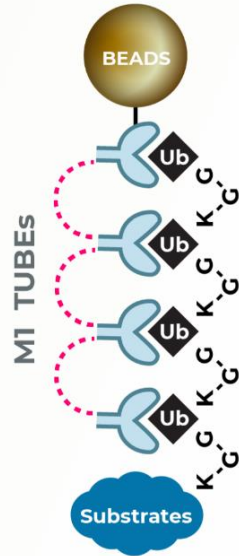
Antibodies **challenged by chain selectivity**, especially in complex media

---

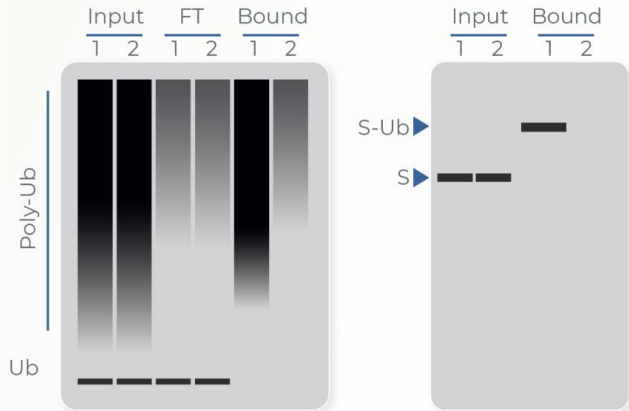
Most importantly, antibodies do not **immuno-precipitate** phospho-polyubiquitinated proteins

# TUBE Technology

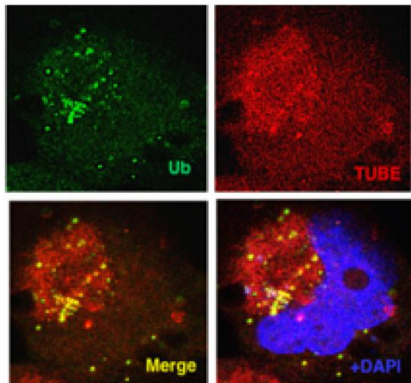
## TUBE (Tandem Ubiquitin Binding Entities)



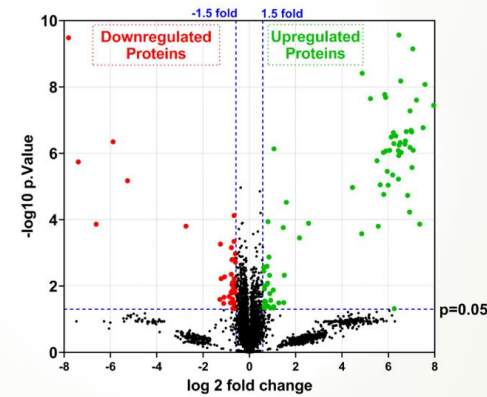
Poly-Ubiquitin Substrate Enrichment & Western Blotting



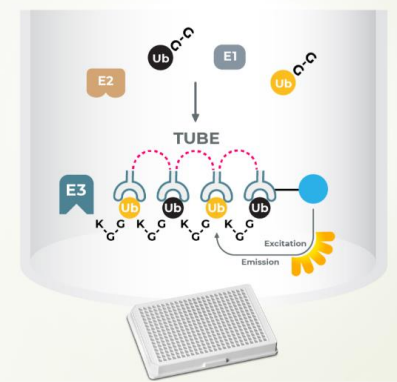
Fluorescent Microscopy



Mass Spectrometry Ubiquitomics



High-Throughput Screening

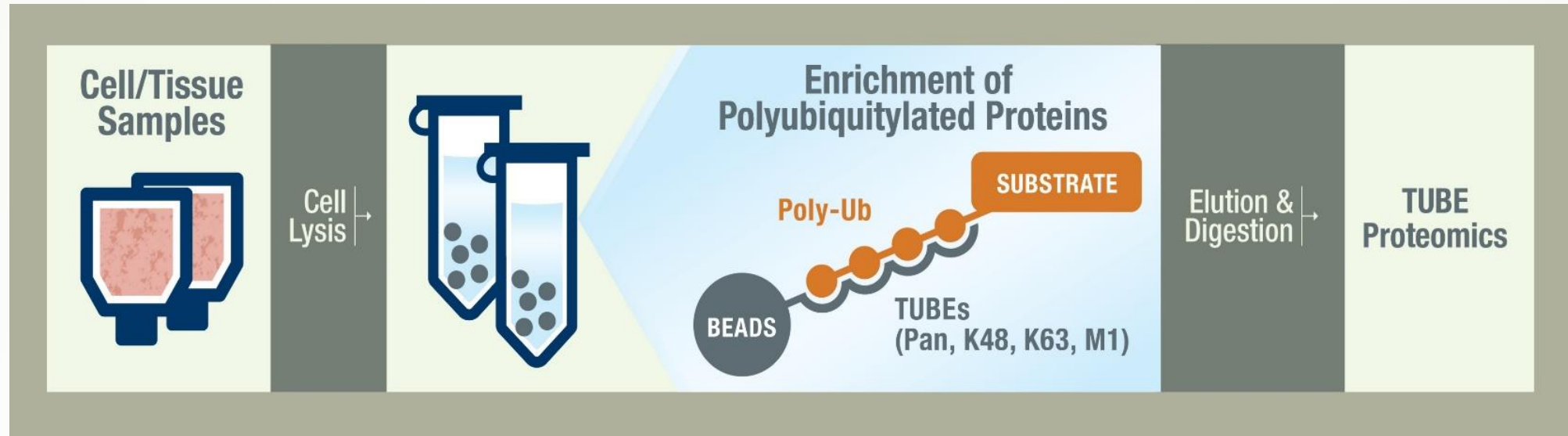


# Phospho-TUBEs: Coming Soon...

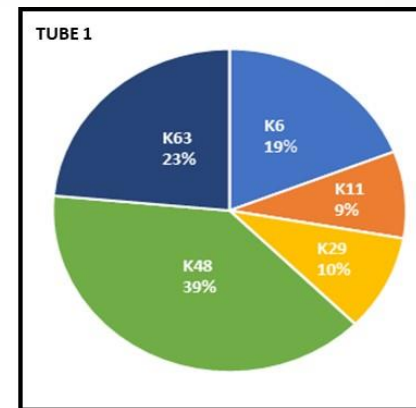
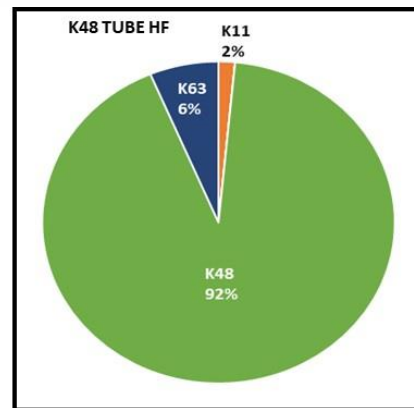
LifeSensors is developing novel phospho-ubiquitin TUBEs and other phospho-ubiquitin tools to unveil Parkinson's disease signature and discover novel PD biomarkers

# TUBE Mass Spec Proteomics

Pan and Chain Selective TUBEs with sub -nM Affinities

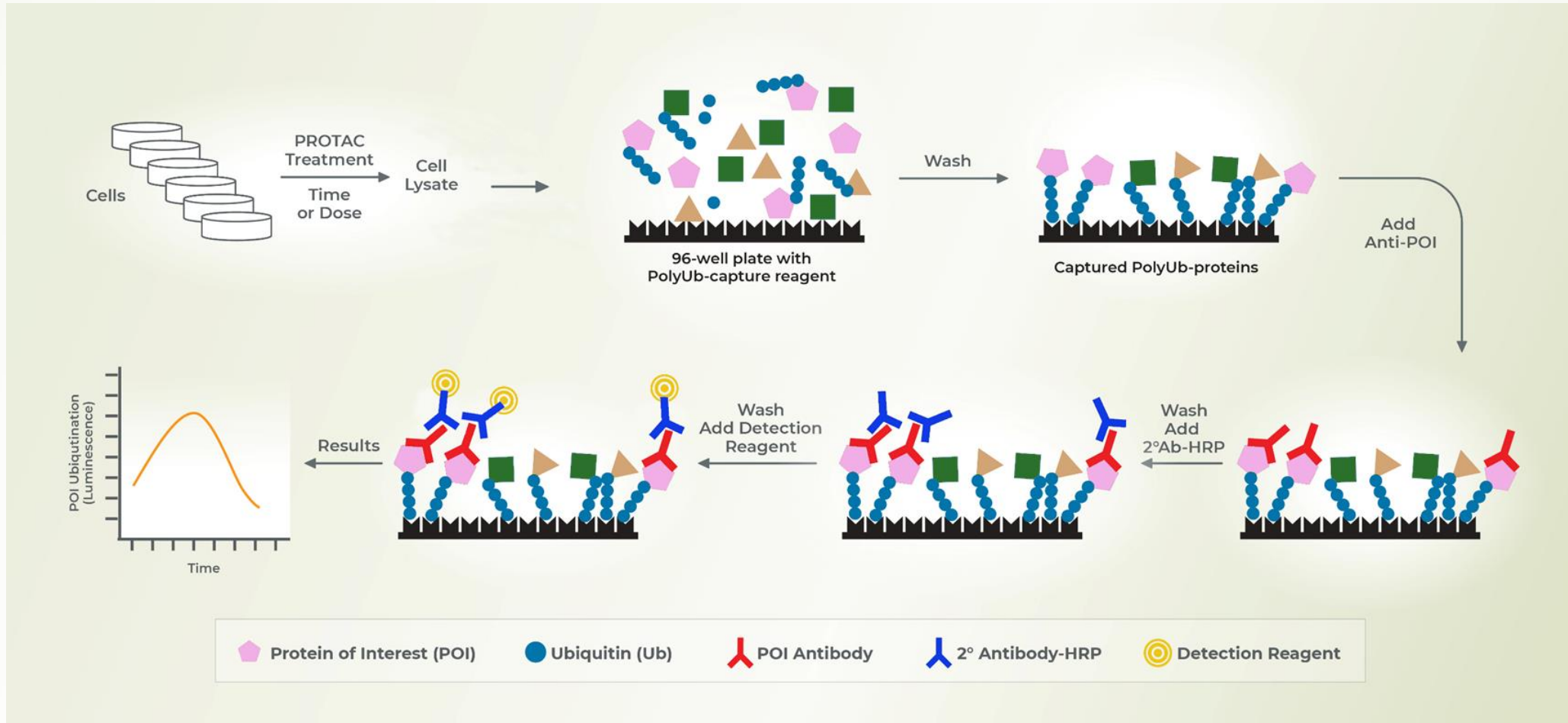


Remarkable selectivity of K48 TUBE HF for K48-linked ubiquitylated substrates in neuronal cells.



# UbiQuant™ ELISA

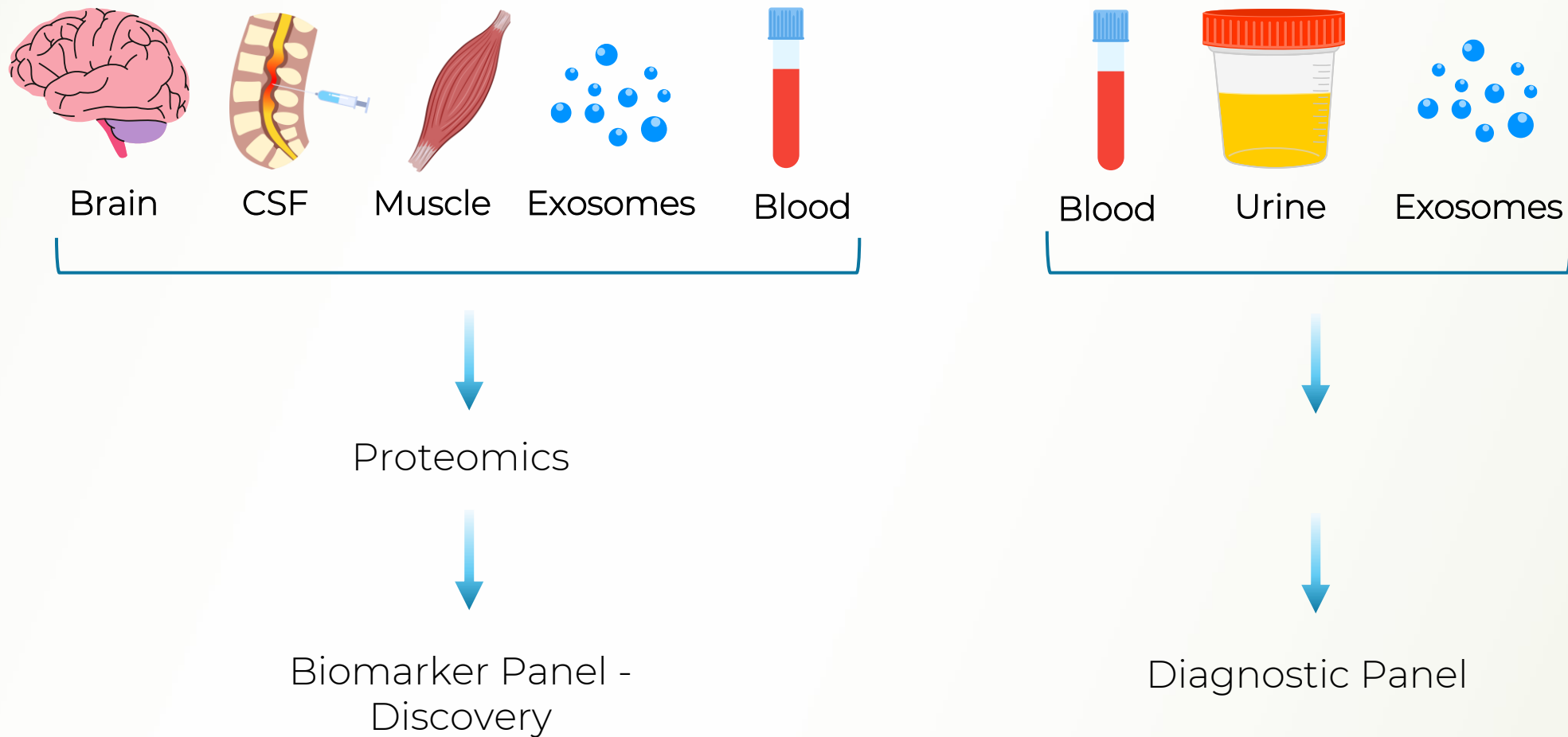
Capturing ubiquitinated proteins on a microtiter plate for absolute quantitation





# Our Vision

## Universal Tools for PD Biomarker Discovery & Diagnostics



# Our Commitment

**A blood-based test to empower Patients, Practitioners & Researchers for better diagnostic solutions and PD Management**

- ✓ Identifying elevated novel UPS biomarkers in Serum.
- ✓ Testing Phospho-S65-Ubiquitin levels in Serum for Familial PD.
- ✓ Harnessing activity data from genetic markers PINK1/Parkin in PBMCs to provide insights into Familial PD.



# LifeSensors' Neurodegeneration Tools

- LifeSensors offers a [suite of tools to support neurodegeneration](#) research and help develop ultra sensitive assays for biomarker development and diagnostic applications:
  - [Phospho-ubiquitin antibodies](#)
  - [Phospho-ubiquitin chains and derivatives](#) (K48, K63, K11, K6, K33, M1)
  - [Neuronal DUBs and Ligases](#)
  - [PINK1 & Parkin pathway proteins](#)
  - [Phospho-polyubiquitinated protein enriched lysates](#)
  - [TUBEs \(Tandem Ubiquitin Binding Entities\)](#)
  - [UbiQuant for monitoring phospho-polyubiquitination](#)

# Thank You

We are your partner in Parkinson's Disease and  
Neurodegeneration Research

## Contact Us!

Research & Product Inquiries R&D

[info@lifesensors.com](mailto:info@lifesensors.com)

610-644-8845 (ext 339)

Custom Service & Assays      BD

[bd@lifesensors.com](mailto:bd@lifesensors.com)

610-644-8845 (ext 310)