

LRRK2 activation as a common mechanism of environmental toxicant-induced Parkinson's disease

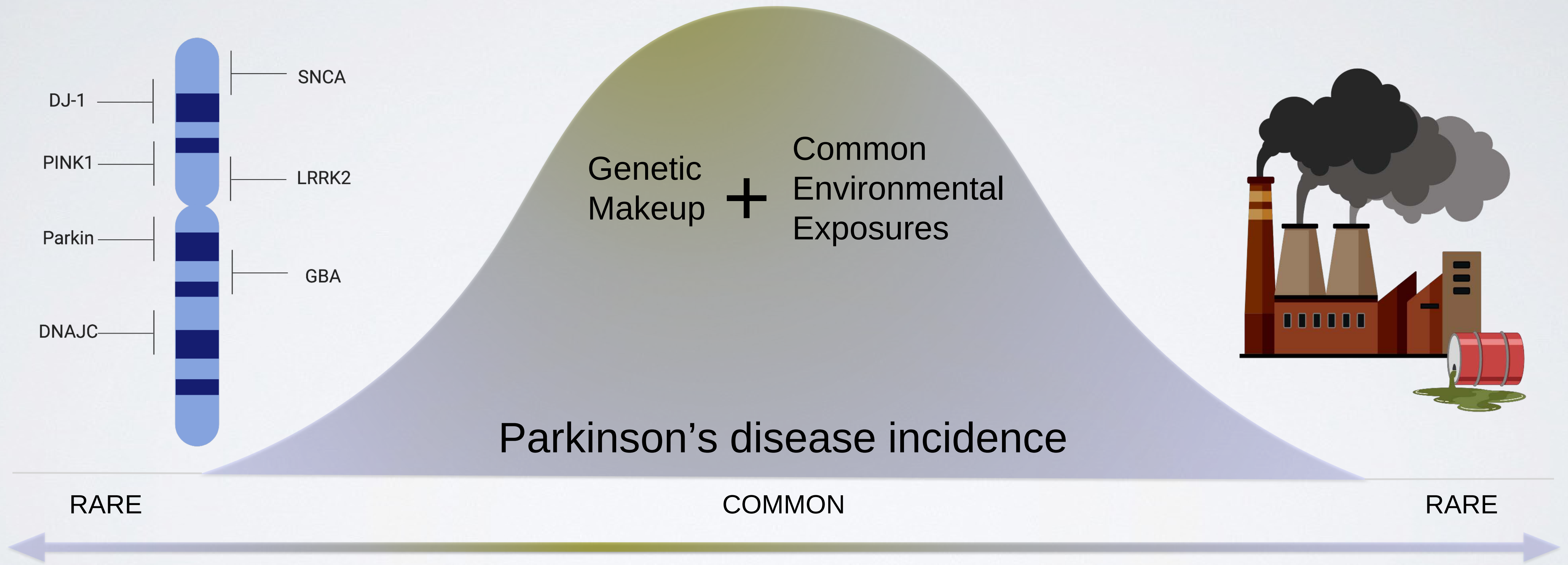


The people who did the work



JTGH Lab 10/2018

GENE x ENVIRONMENT



PARK8: LRRK2

- The most common cause of autosomal dominant PD – May account for 10% of AD PD and ~3% of sporadic PD
- LRRK2 is a kinase - substrates still being defined and verified (Rabs 8 & 10)
- More than 40 point mutations have been identified – At least 6 are clearly pathogenic - generally associated with **increased kinase activity**
- The Gly2019Ser mutation is the most prevalent and is present in 30-40% of PD patients (familial & sporadic) in North African populations & in 13% of Ashkenazi Jews
- Incomplete penetrance
- **Role of LRRK2 in idiopathic PD has been unclear**



How to assess LRRK2 function in iPD?

Assessment of LRRK2 in iPD

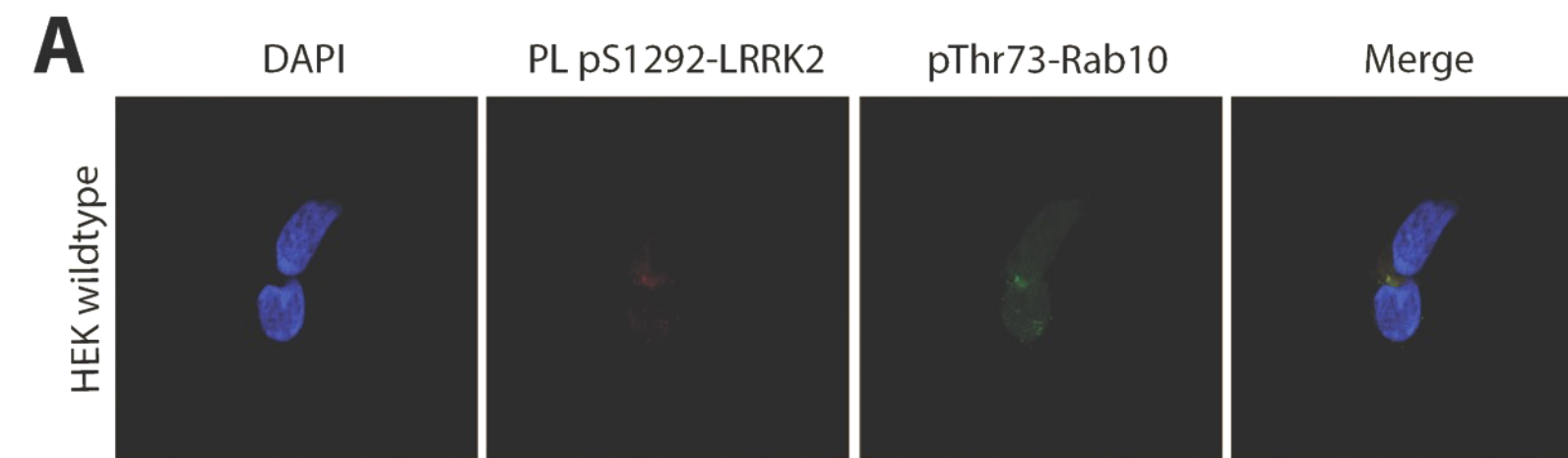
- Growing consensus that S1292 phosphorylation correlates with activity. *"...Ser(P)-1292 LRRK2 may be an effective biomarker surrogate for LRRK2 kinase activity"*
- 14-3-3 may bind to the inactive form of LRRK2 - and 14-3-3 overexpression decreases kinase activity while 14-3-3 dissociation promotes kinase activity
- Can we take advantage of these properties?

Assessment of LRRK2 in iPD

A multiplex PL assay to assess:

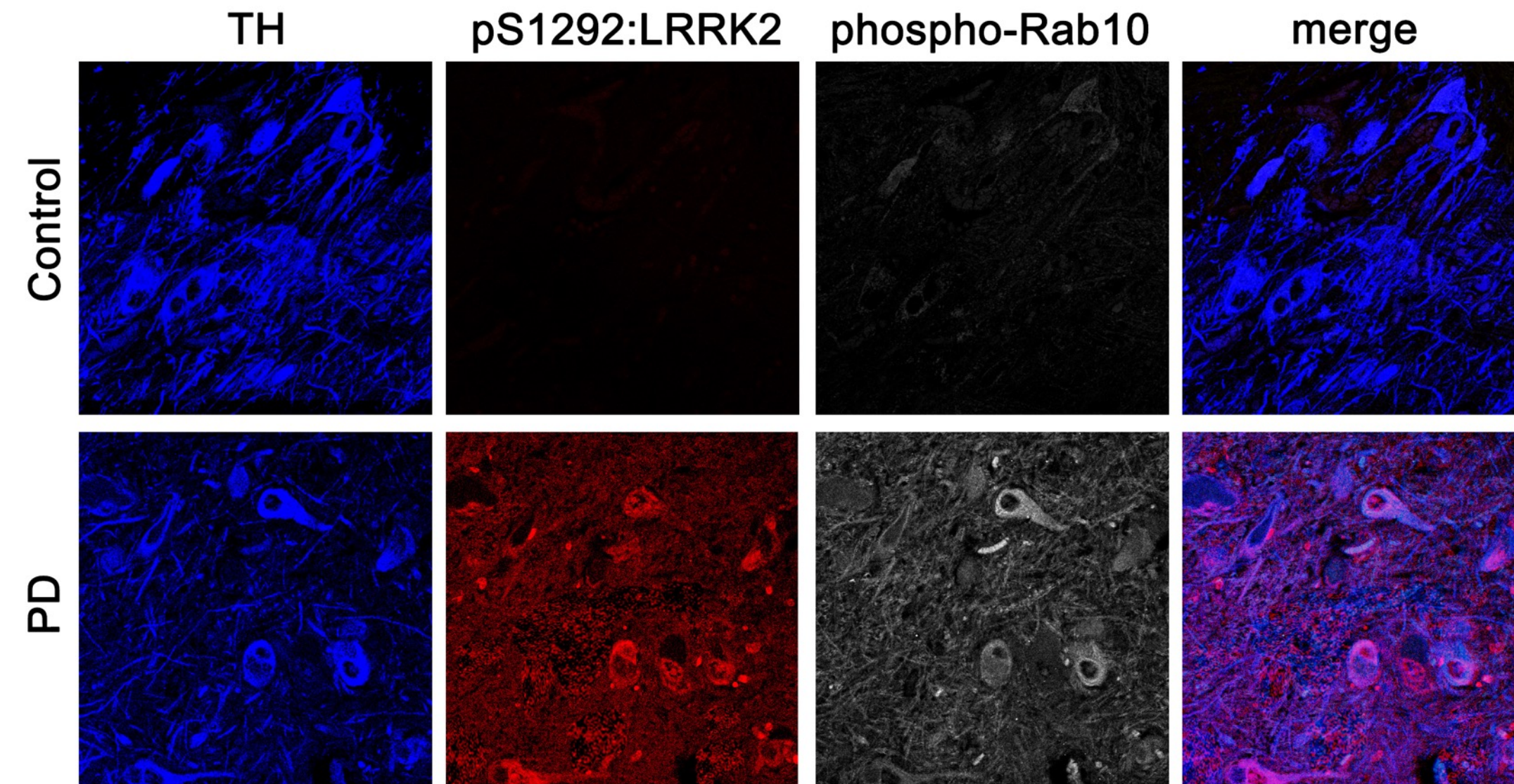
- S1292 phosphorylation state: Amplification allows low Ab concentration (less n.s. binding) and PL eliminates signal from “off-target” Ab binding
- 14-3-3 binding to LRRK2: Allows assessment of endogenous protein:protein interactions *in situ*
- Validation in CRISPR/Cas9-edited LRRK2^{-/-} and LRRK2^{G2019S/G2019S} cells

Assessment of LRRK2 in iPD

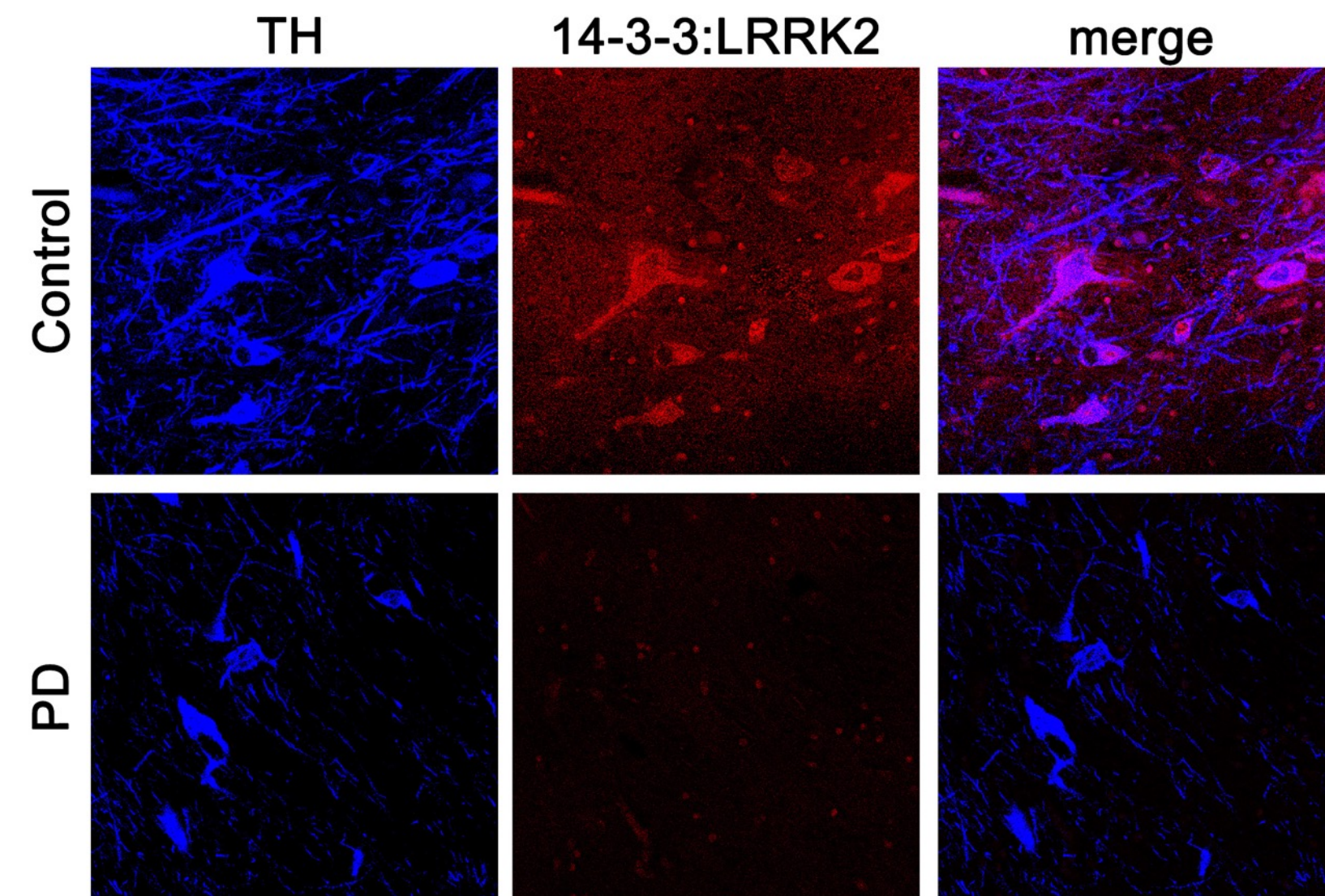
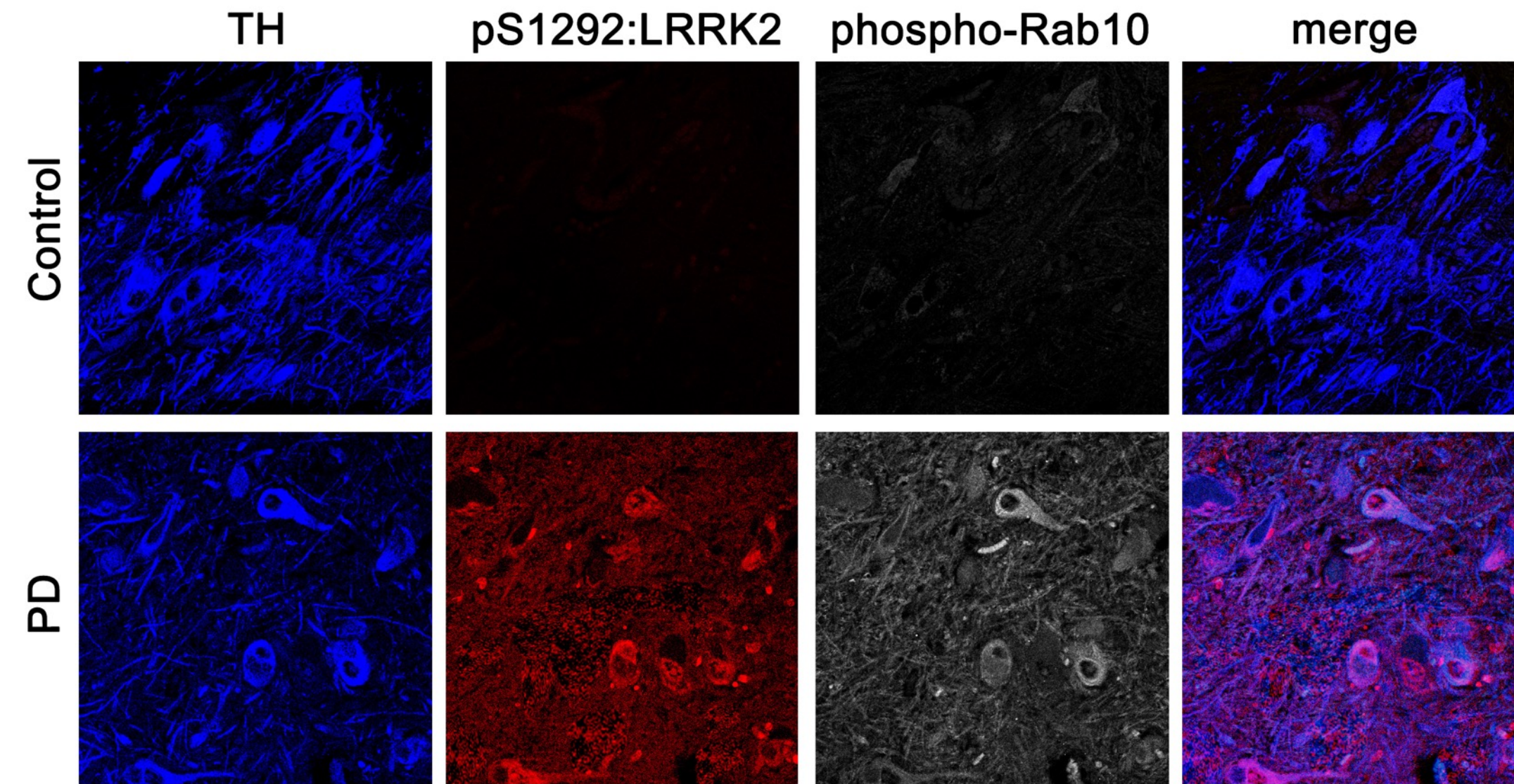


Di Maio et al, *Sci Transl Med*, 2018

Assessment of LRRK2 in iPD

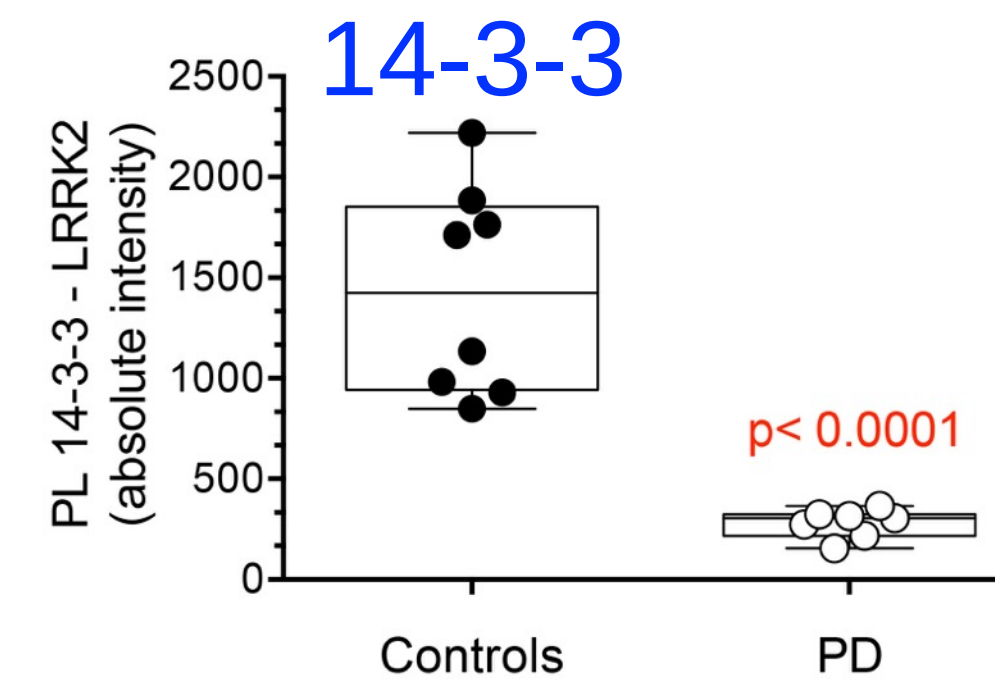
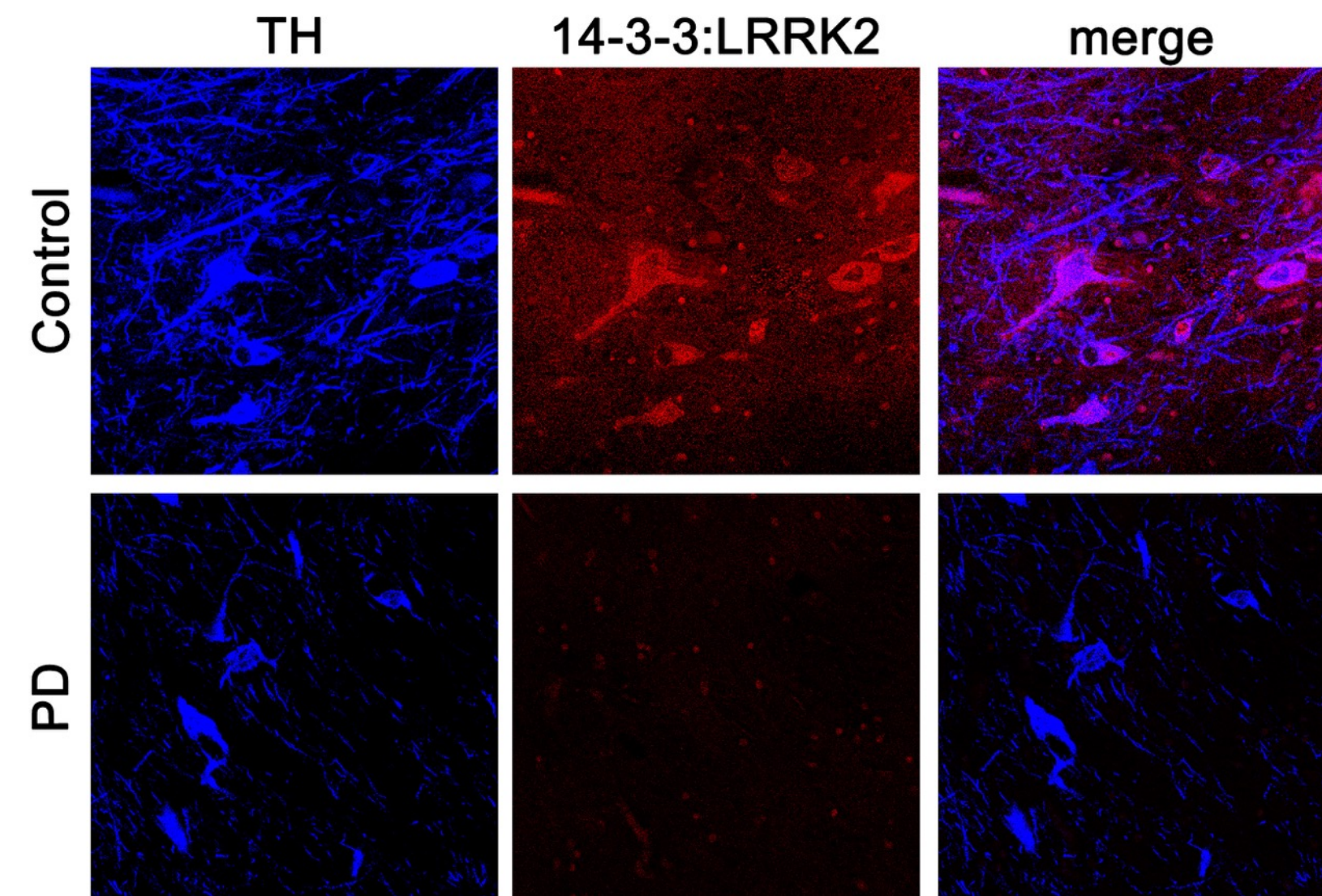
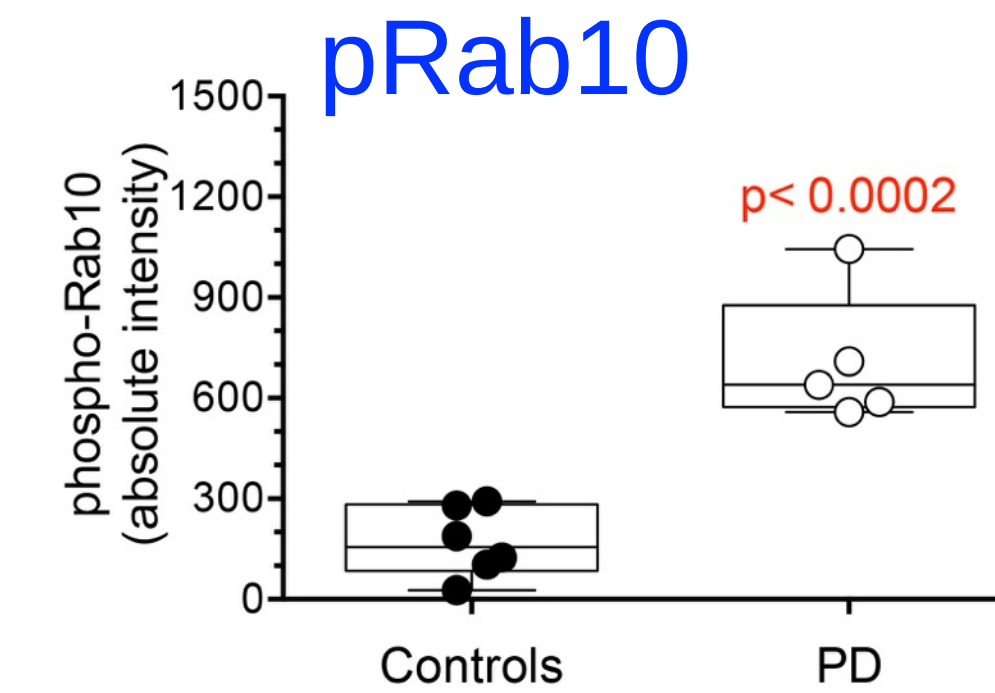
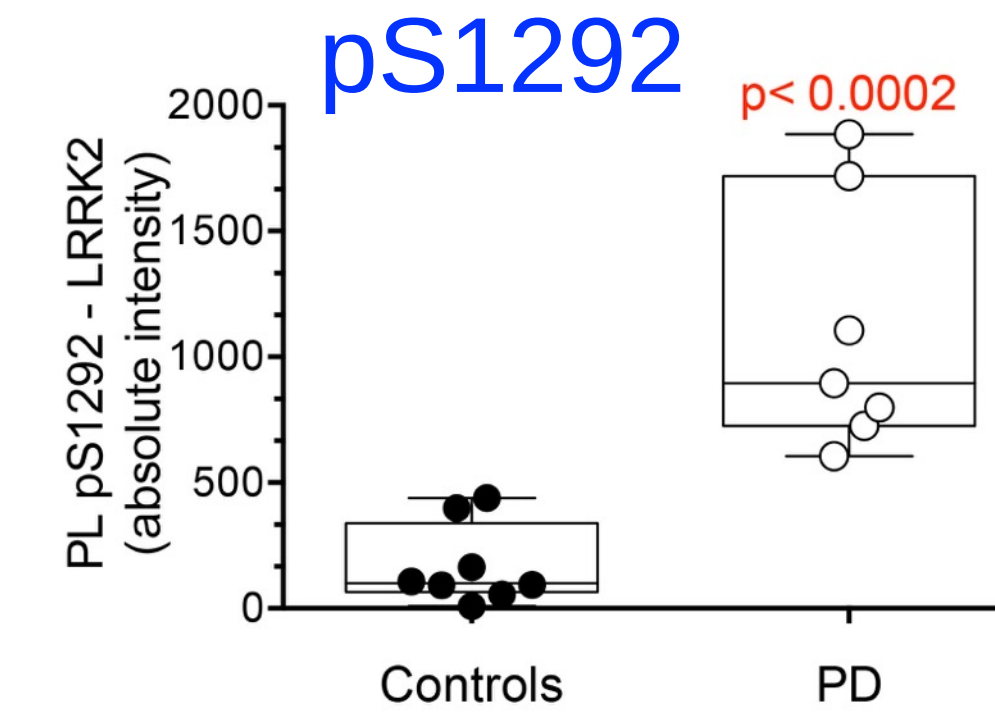
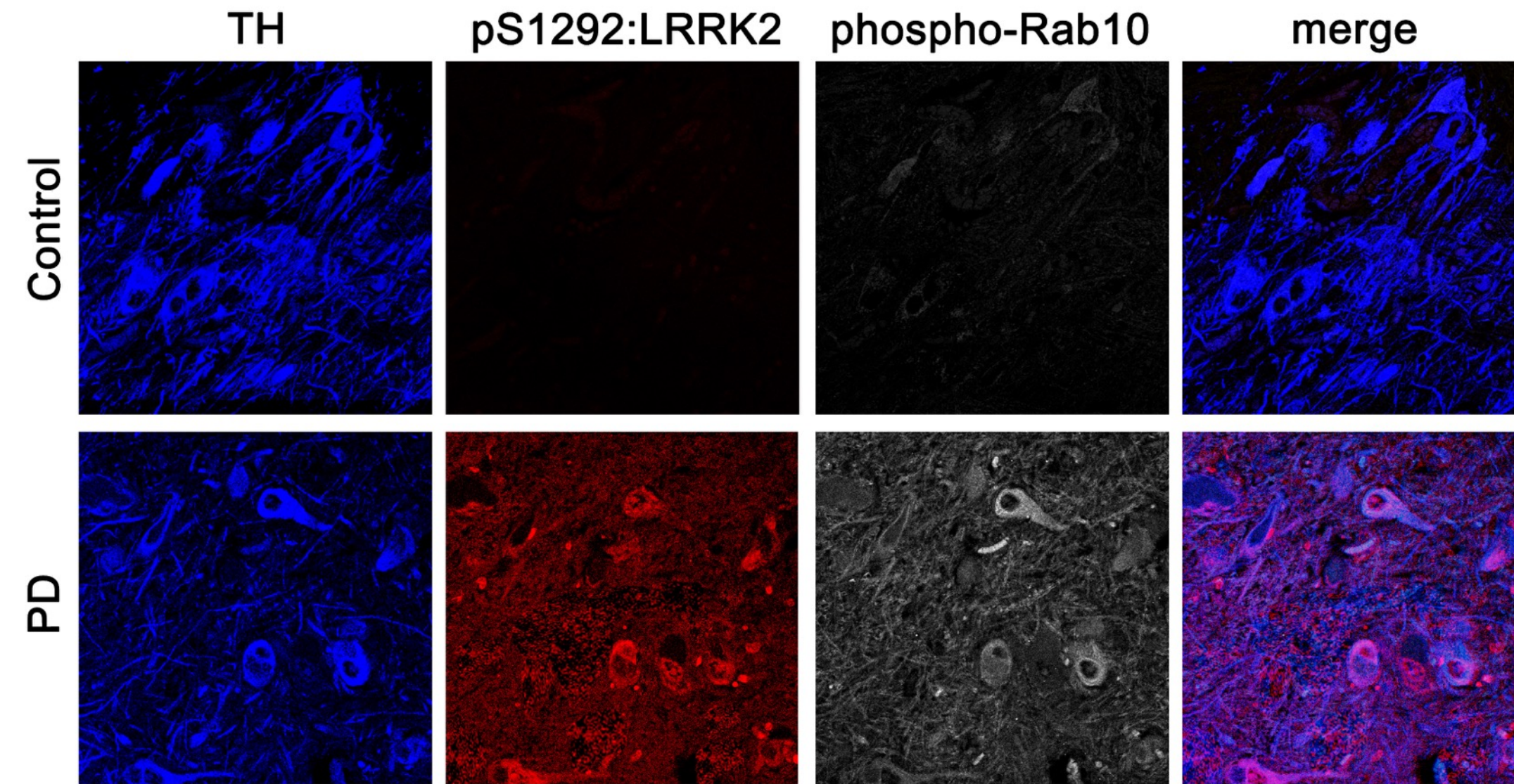


Assessment of LRRK2 in iPD



Human postmortem brain

Assessment of LRRK2 in iPD



Is LRRK2 activation reproduced in animal models of PD?



Rotenone

- Classical high-affinity inhibitor of complex I of the mitochondrial electron transport chain
- A natural product - from several plant species
- Common **pesticide**; the “organic” (natural) alternative to synthetic pesticides
- Used to sample fish populations in reservoirs & kill nuisance fish in lakes
- Highly lipophilic; crosses biological membranes easily & ***independent of transporters***



FAME Study: PD in Agricultural Health Study

Tanner, Kamel et al

Nested, case-control study of 52,000 licensed pesticide applicators and 32,000 spouses

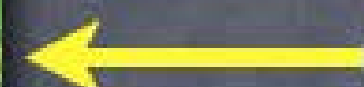
Rotenone → Increased Risk of PD:

All OR = 2.3 (95% C.I.: 1.2, 4.3)
Men OR = 2.8 (95% C.I.: 1.4, 5.8)

Model adjusted for age, gender, state, ever smoking, ever pesticide use



Plant derivative
(worldwide)



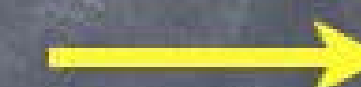
Home gardening

Crop insecticide
(mid-1800's - present)



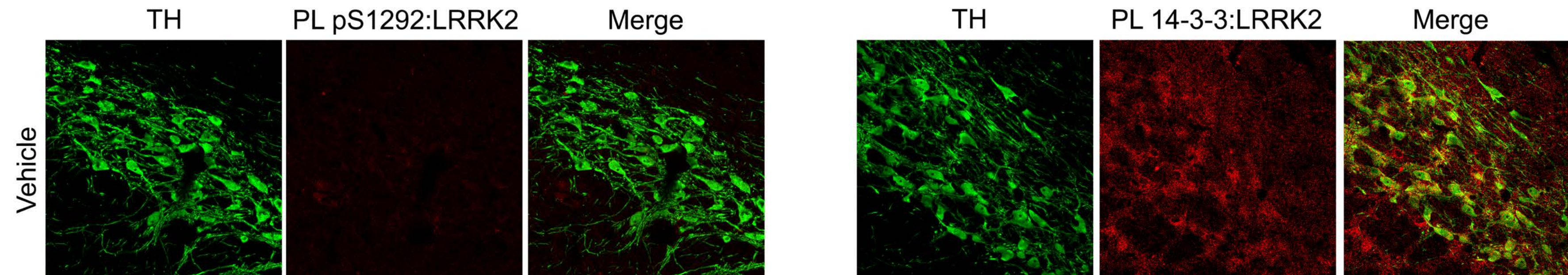
Pest Control

Fish poison
(ancient)



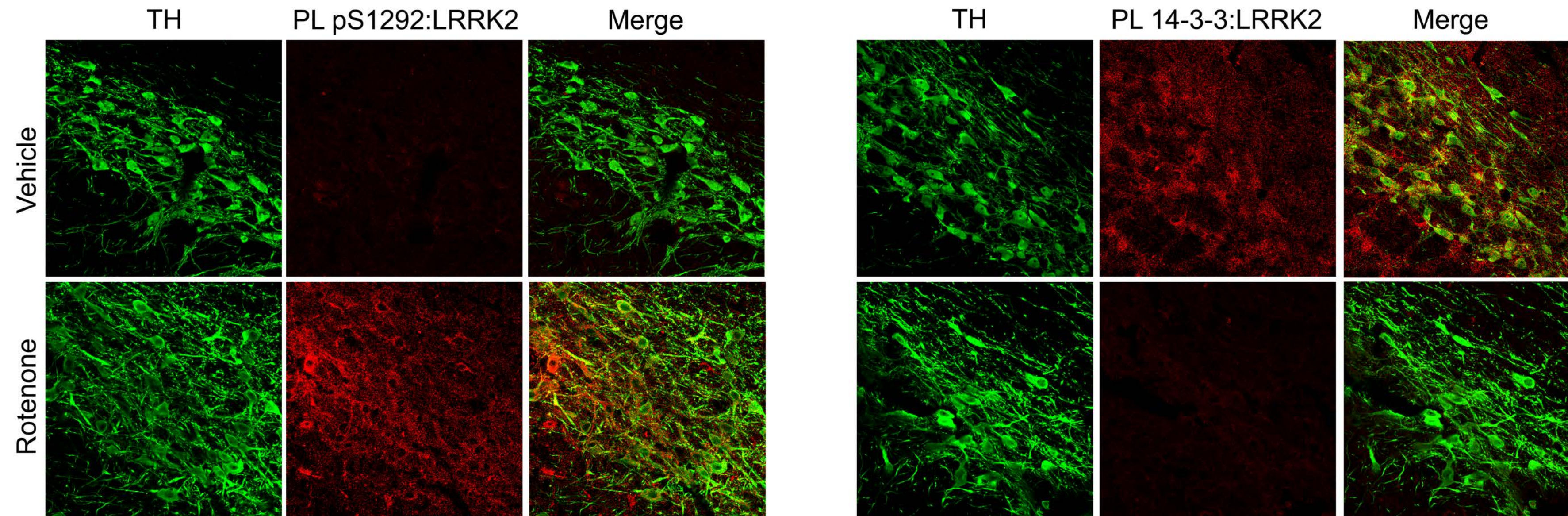
Tanner et al, Environ Health Perspect, 2011

Assessment of LRRK2 in iPD



The rotenone model

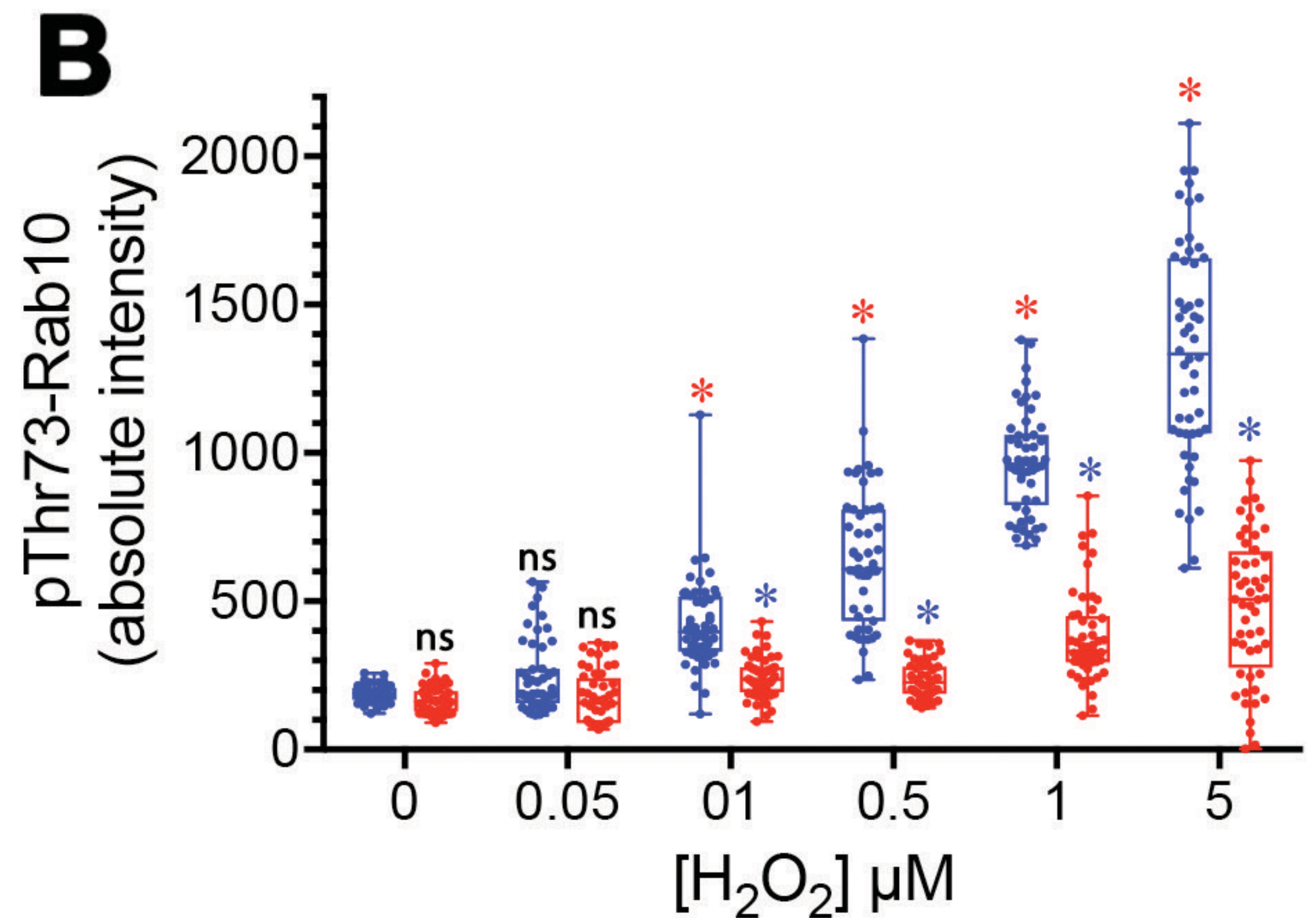
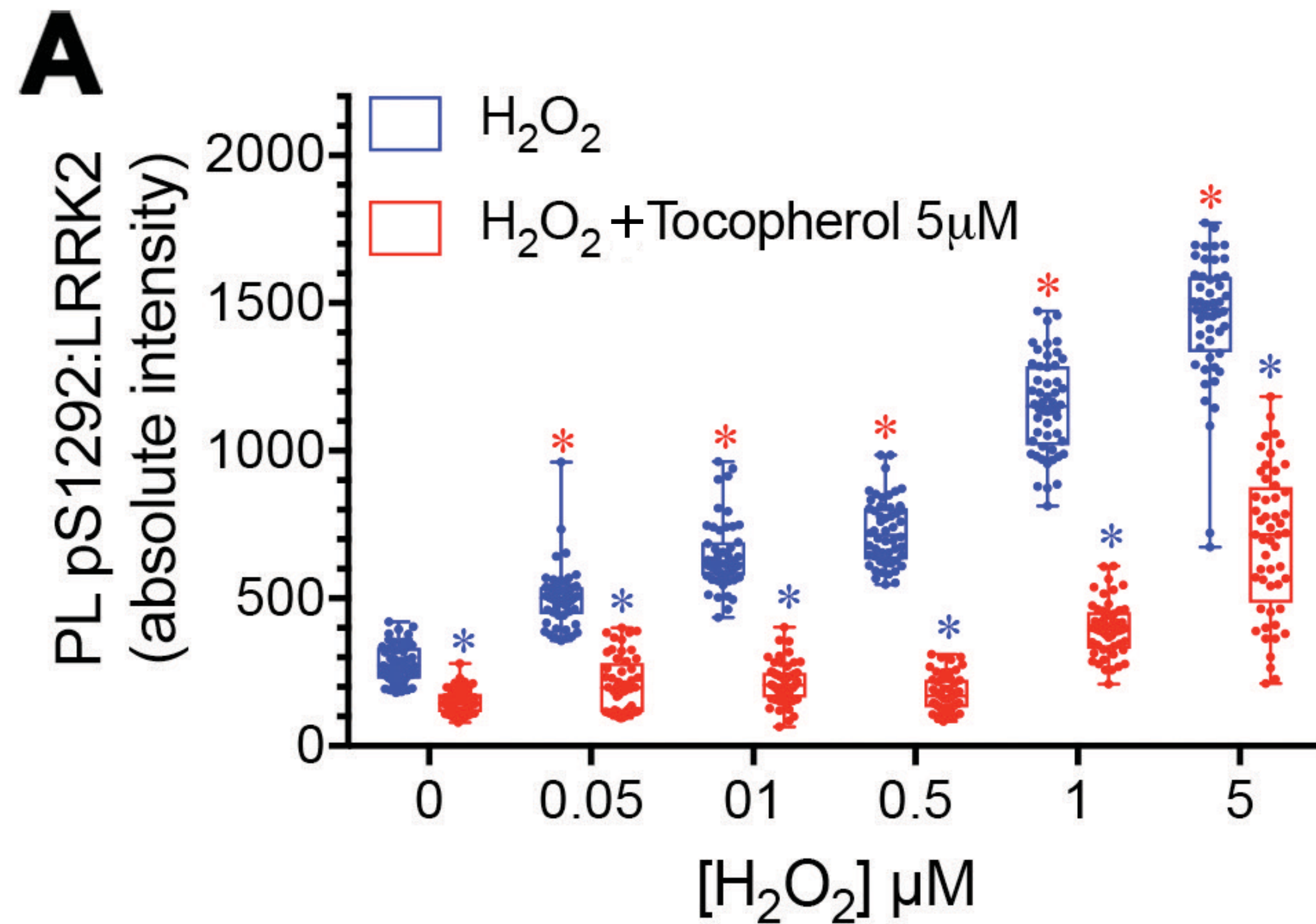
Assessment of LRRK2 in iPD



The rotenone model

Redox activation of LRRK2?

Effects of H₂O₂



Assessment of LRRK2 in iPD

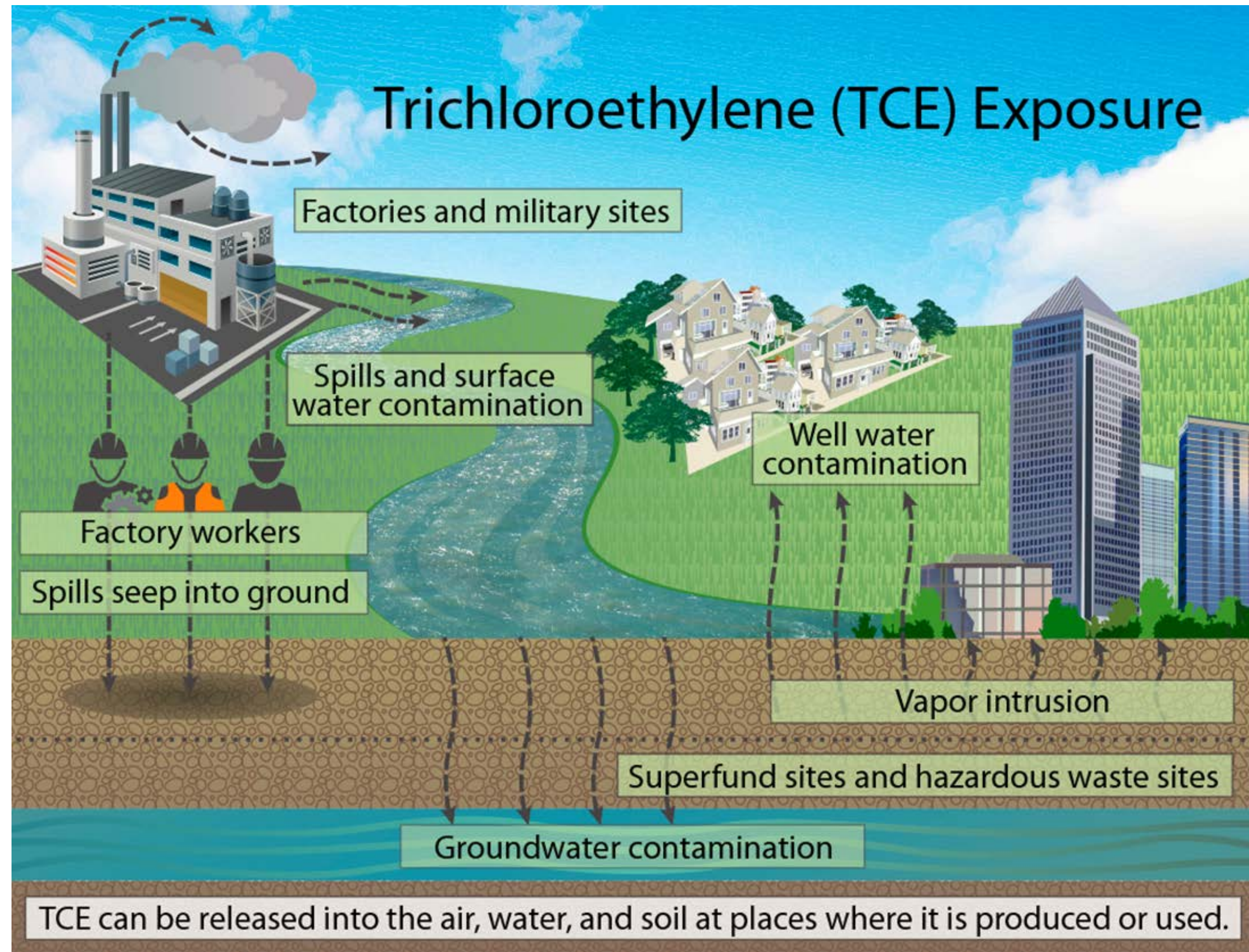
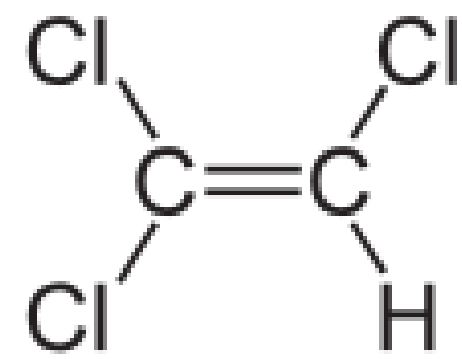
Preliminary conclusions:

- LRRK2 is activated in nigrostriatal neurons in brains of people with iPD
- This is replicated in the rotenone rat model of environmental exposure
- α -Synuclein overexpression also activates LRRK2 in nigrostriatal neurons
- LRRK2 appears to be redox-activated (common to both mitochondrial defects and α -synuclein overexpression)



Is LRRK2 activation a common feature of
PD-associated environmental toxicants?





TCE

TABLE 3: Solvent Exposure Frequencies and Adjusted Pairwise Odds Ratios in PD-Discordant Twins, n = 99 Pairs^a

Solvent	Case ⁻ / Control ⁻	Case ⁺ / Control ⁻	Case ⁻ / Control ⁺	Case ⁺ / Control ⁺	Ever/Never Exposed, OR (95% CI)	<i>p</i>
Toluene	72	11	9	7	1.3 (0.5–3.3)	>0.2
Xylene	88	6	2	3	2.2 (0.4–12)	>0.2
<i>n</i> -Hexane	85	6	7	1	1.3 (0.4–4.1)	>0.2
CCl ₄	74	14	9	2	2.3 (0.9–6.1)	0.088
PERC	93	5	1	0	10.5 (0.97–113)	0.053
TCE	87	9	2	1	6.1 (1.2–33)	0.034
TCE or PERC	85	11	2	1	8.9 (1.7–47)	0.010
Any of 6 solvents	51	19	14	15	1.7 (0.8–3.7)	0.16
Any of 4 excluding TCE and PERC	53	18	15	13	1.5 (0.7–3.1)	>0.2

^aEver exposure, adjusted for respondent type and smoking.

CCl₄ = carbon tetrachloride; CI = confidence interval; OR = odds ratio; PD = Parkinson disease; PERC = perchloroethylene (tetrachloroethylene); TCE = trichloroethylene.



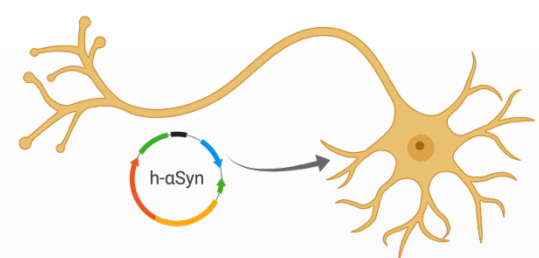
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Goldman et al, Annals Neurol, 2011



Timecourse of toxicant induced ROS in N27-A (h-αSyn) cells

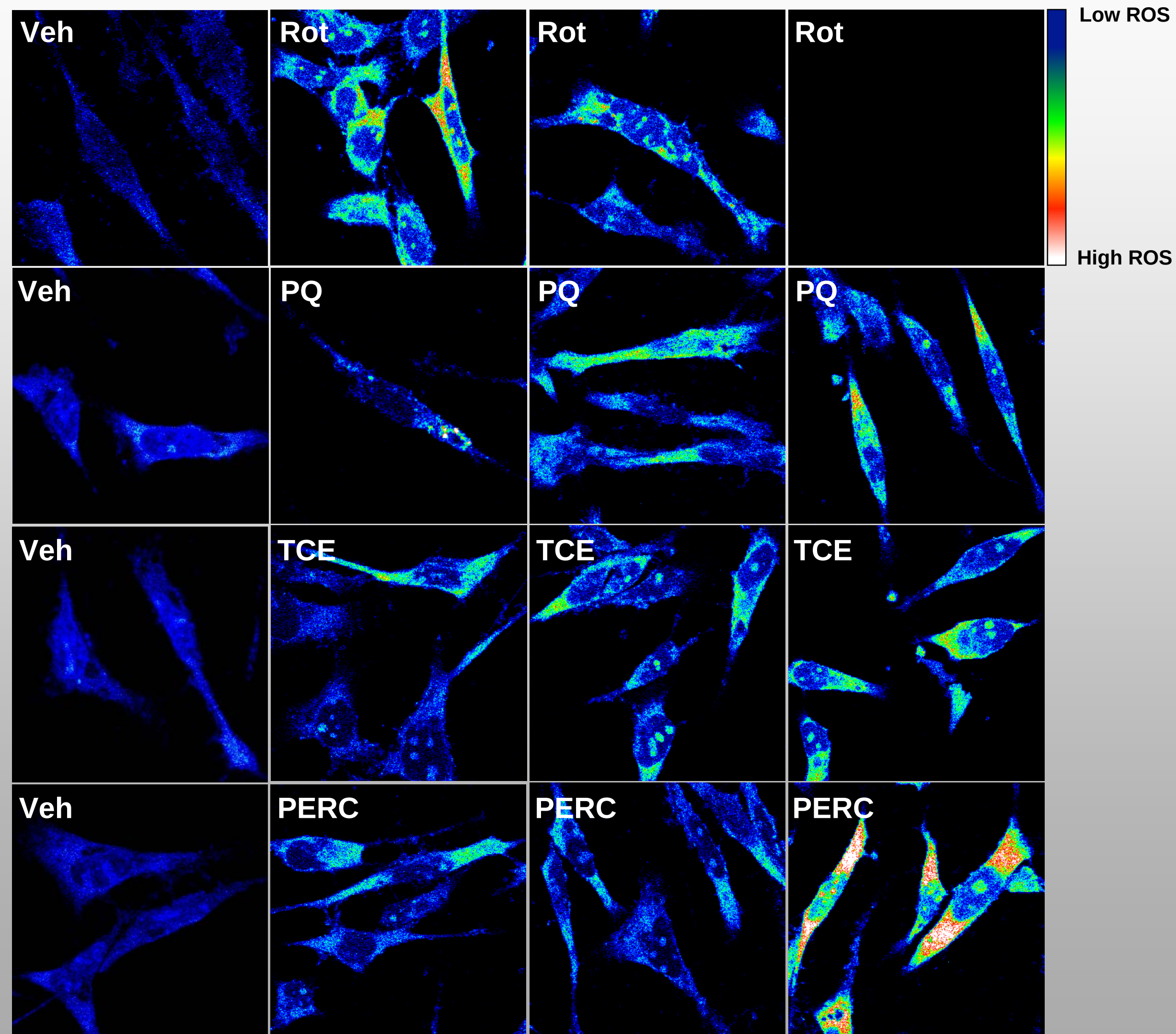


Time post treatment

2 hours

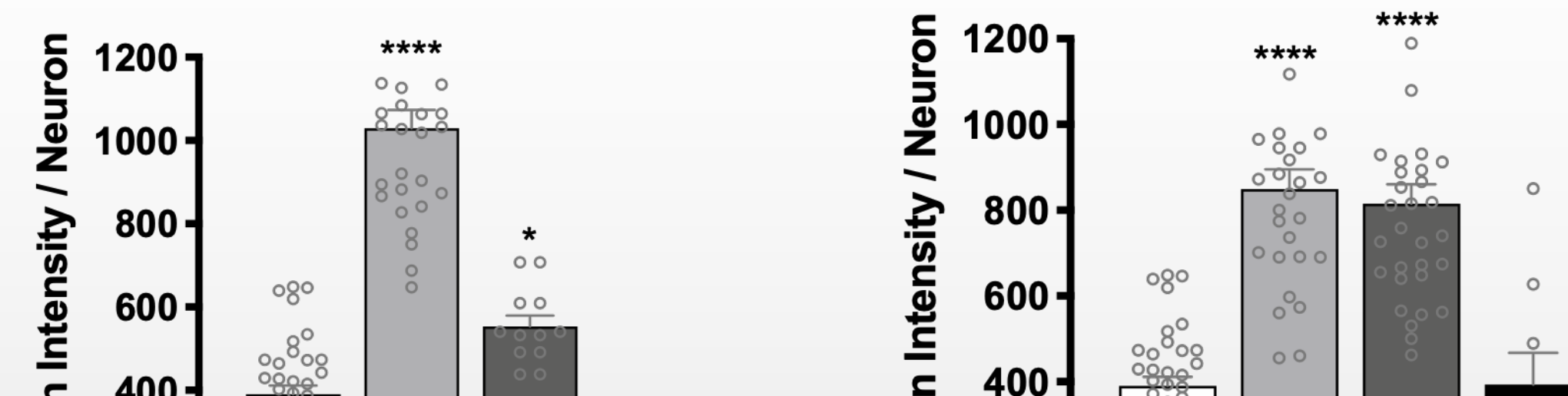
8 hours

24 hours

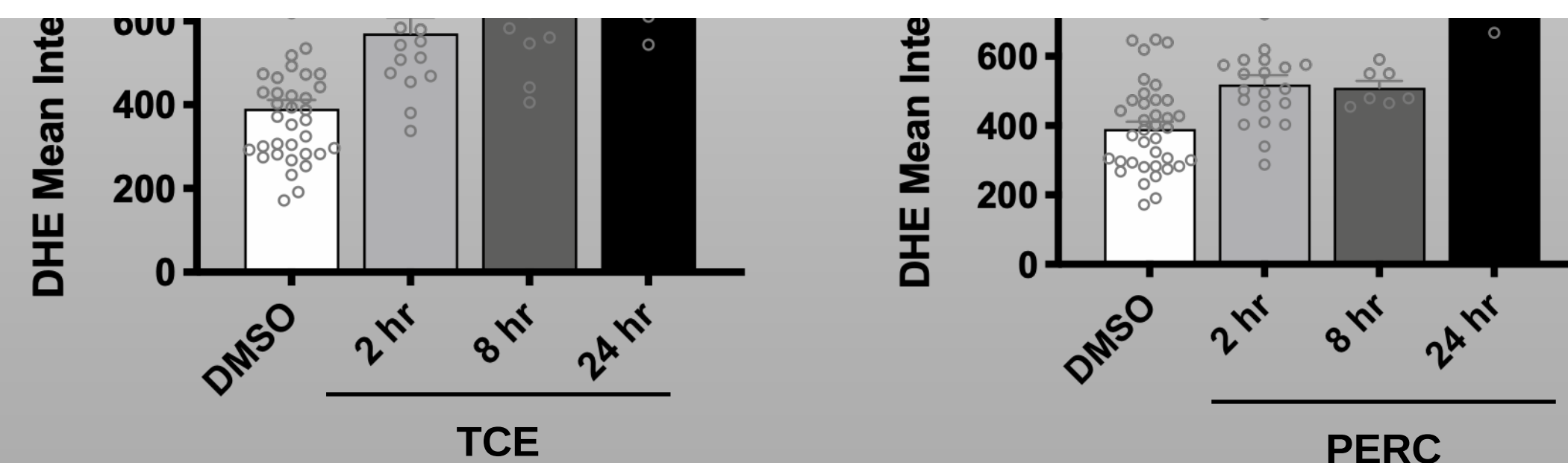
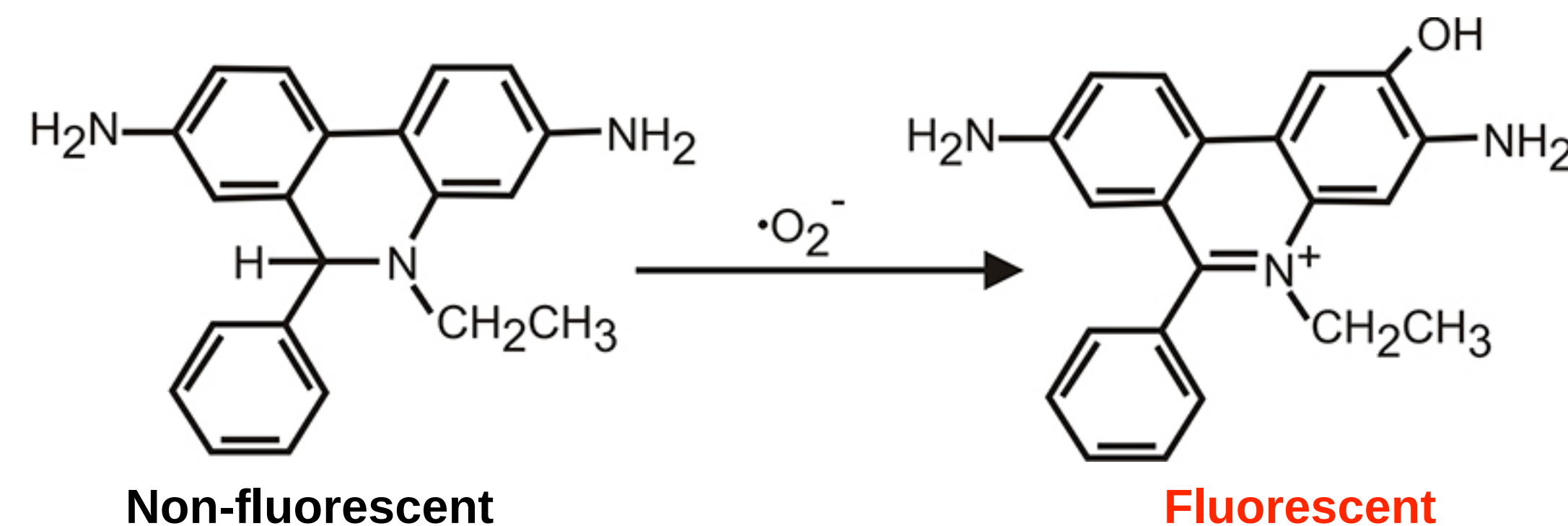


Rotenone (500 nM)

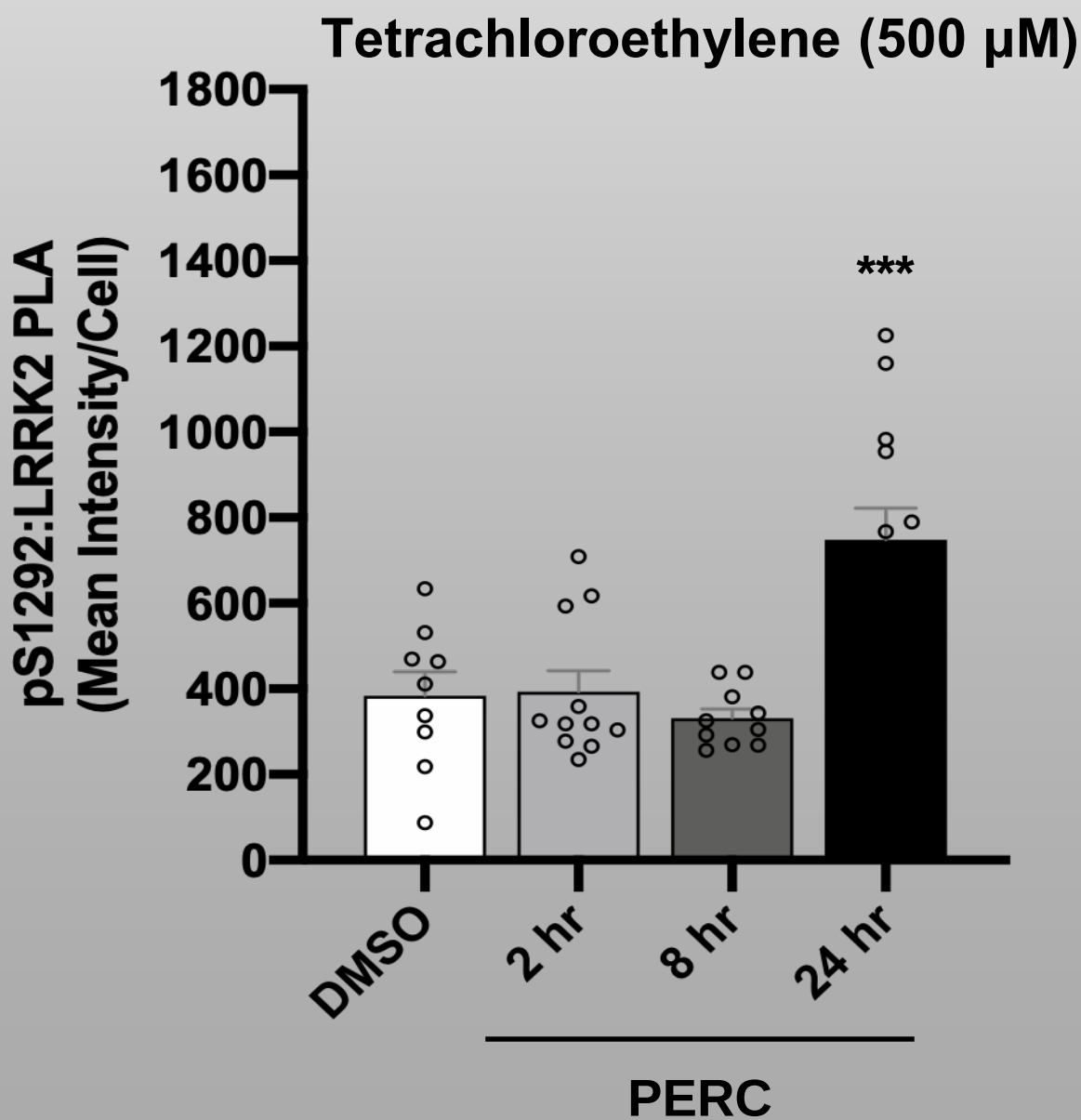
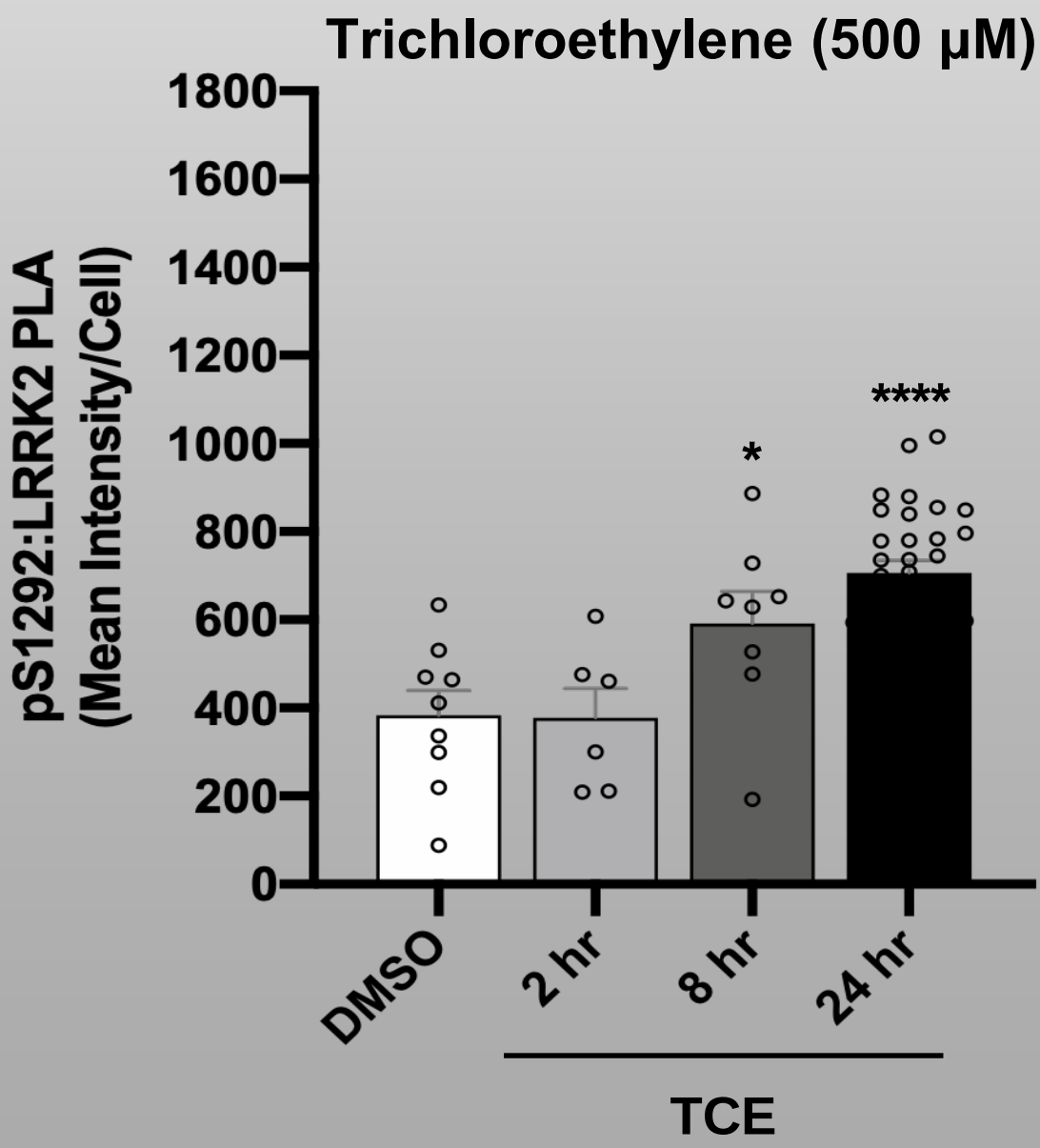
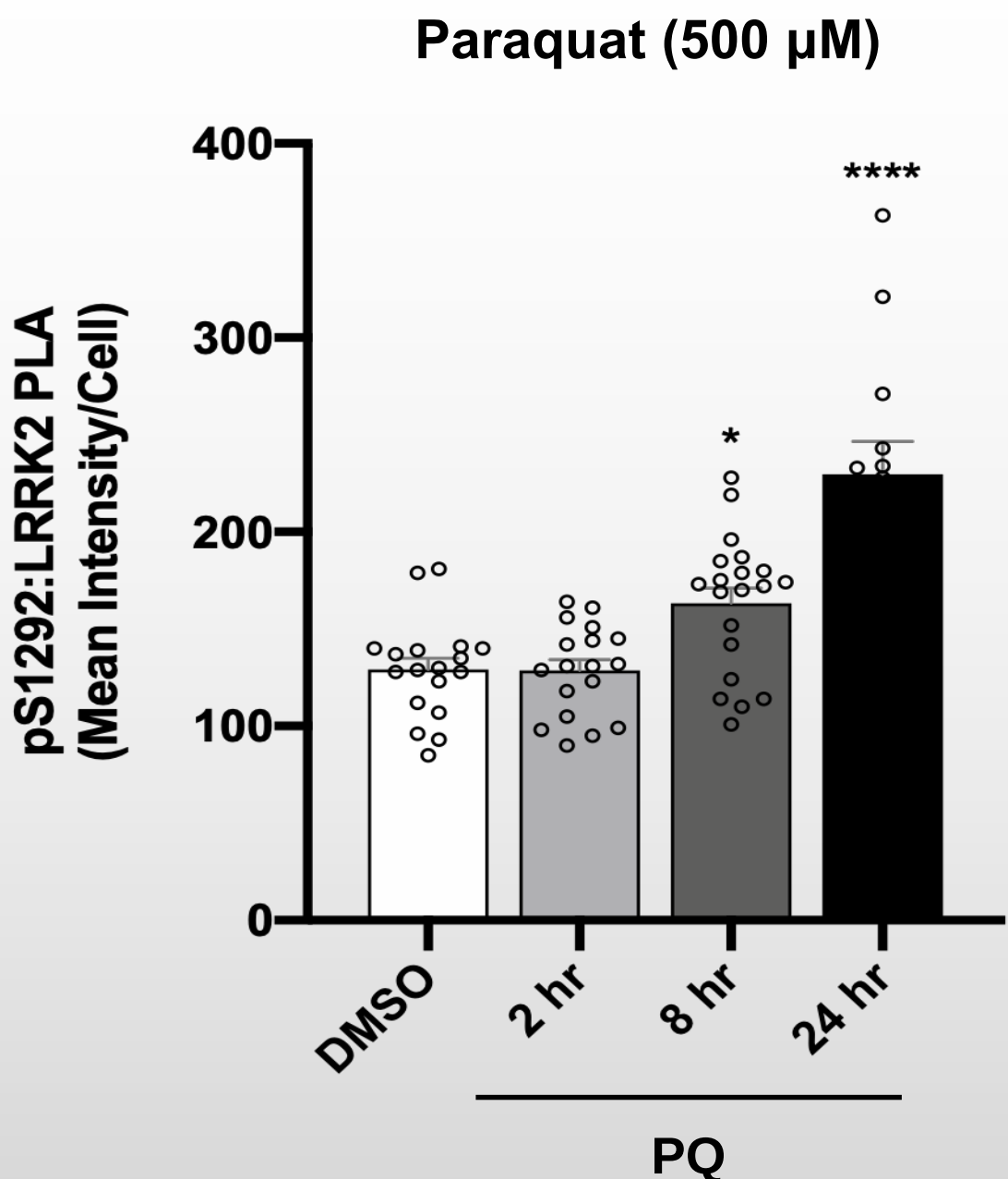
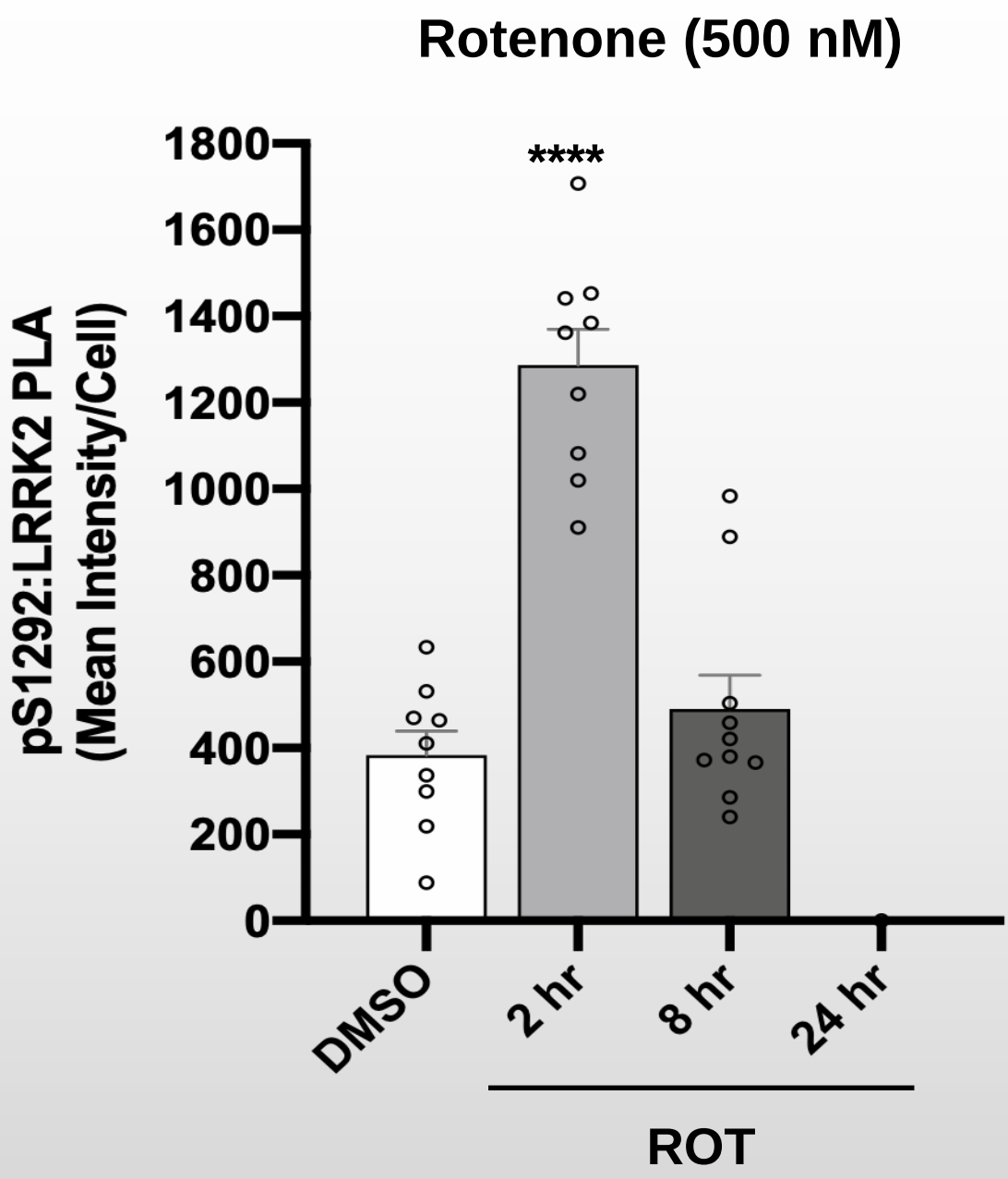
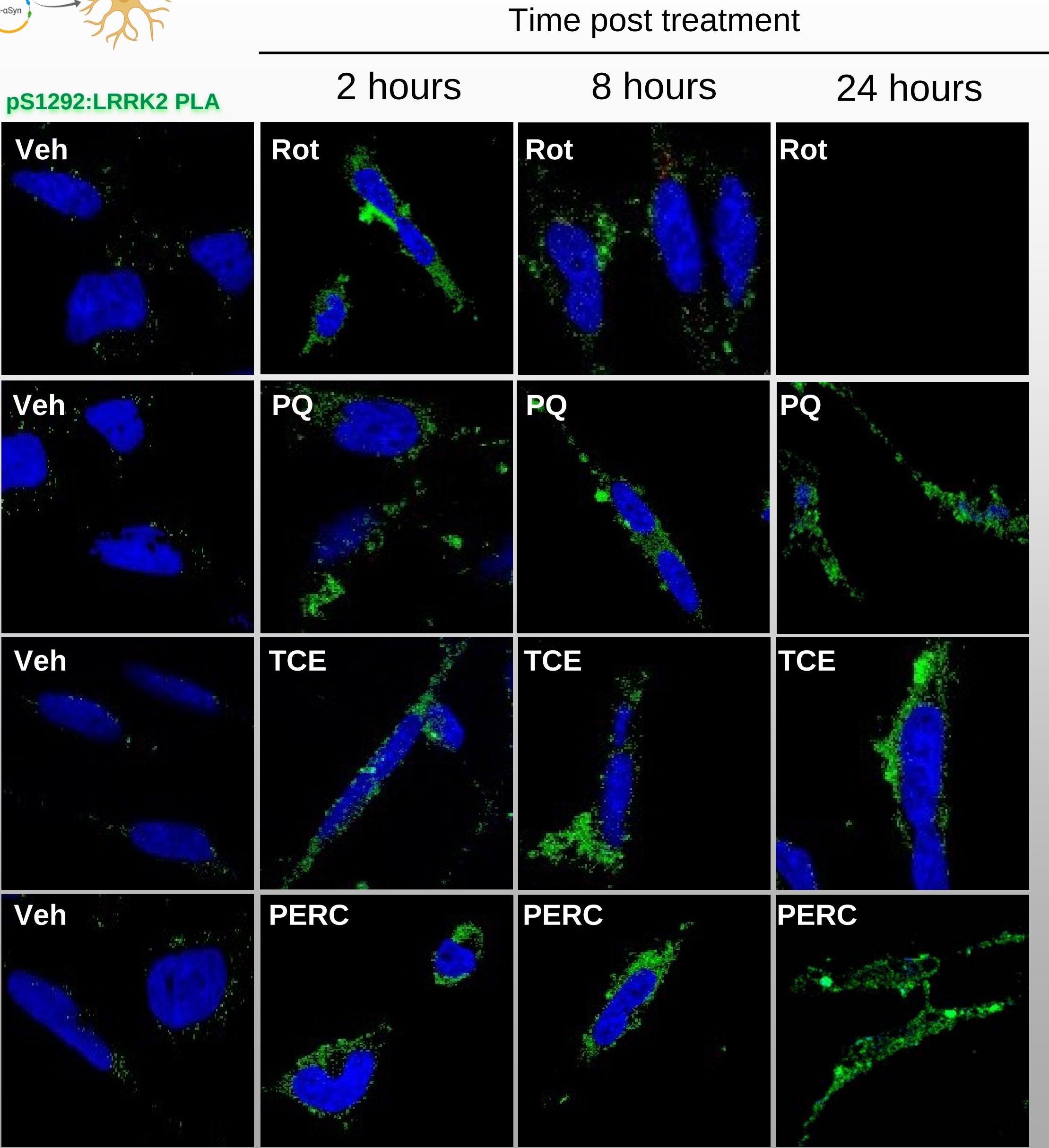
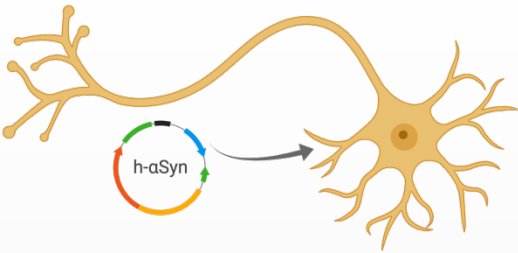
Paraquat (500 μM)



Dihydroethyidium (DHE)



Timecourse of toxicant induced LRRK2 activity in N27-A (h-αSyn) cells



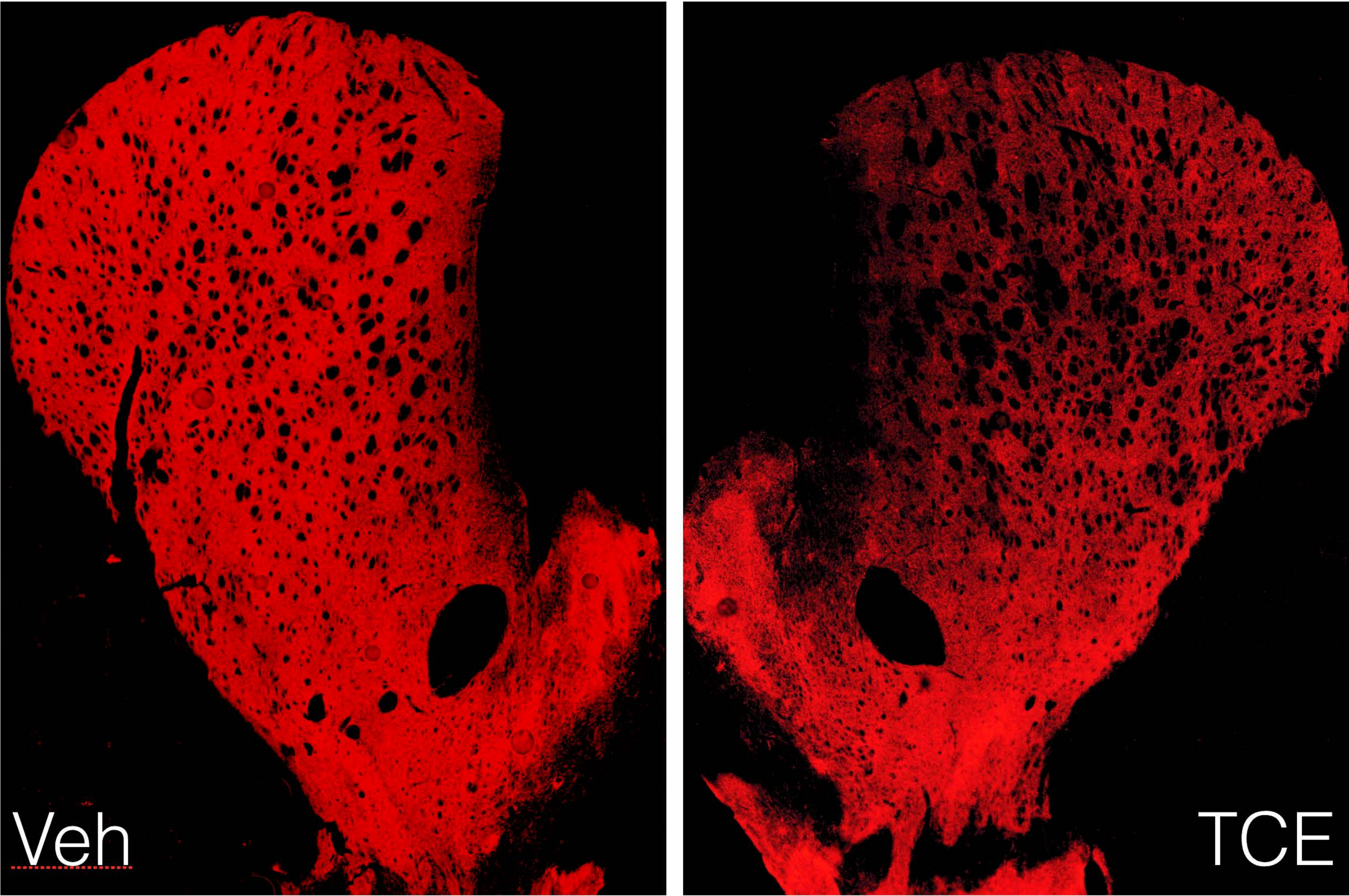
Modeling TCE exposure *in vivo*



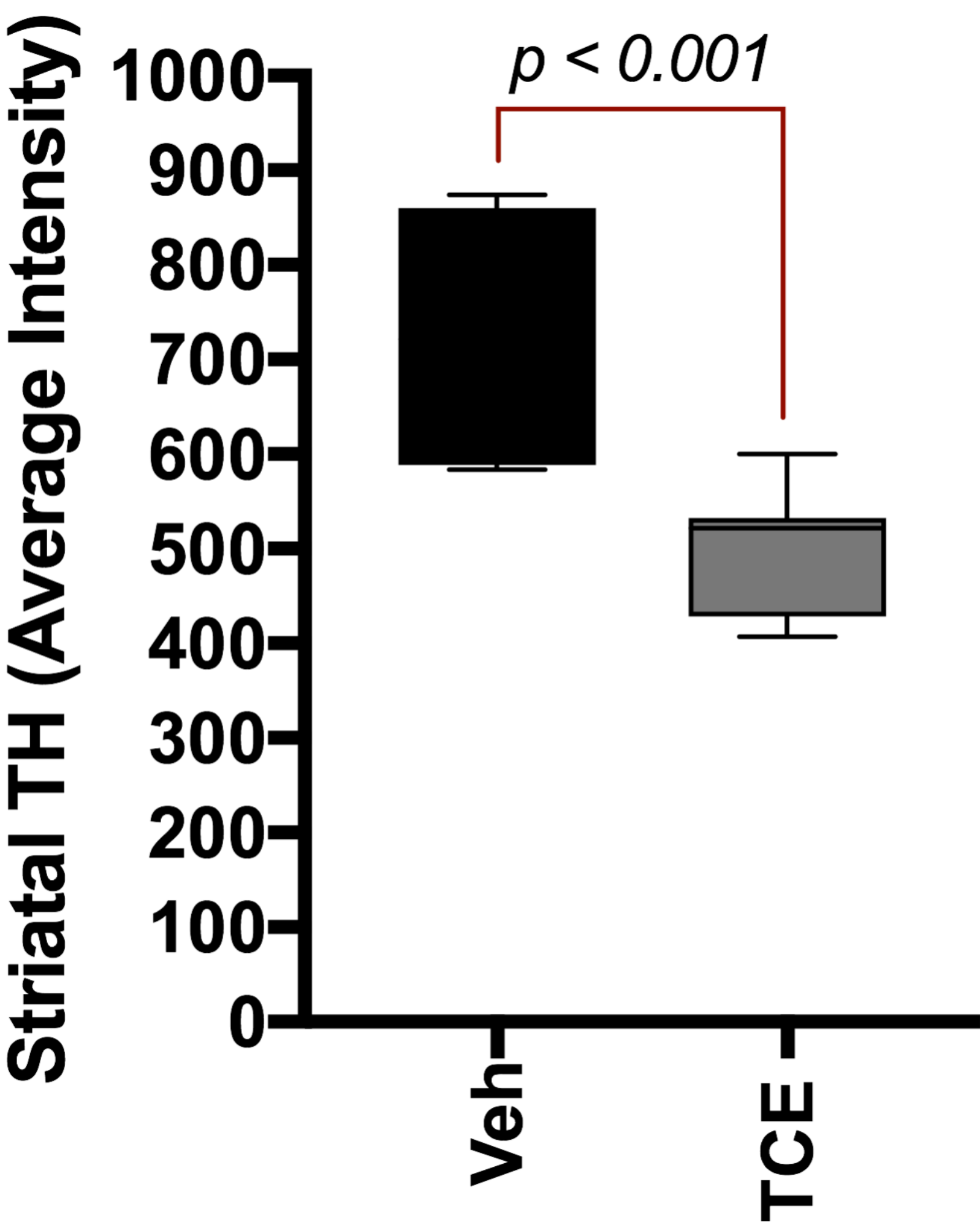
REQUIREMENTS:

1. Produce mitochondrial dysfunction —> oxidative stress
2. Recapitulate Parkinsonian nigrostriatal pathology
3. Limited off-target toxicity
4. Relevant human dose

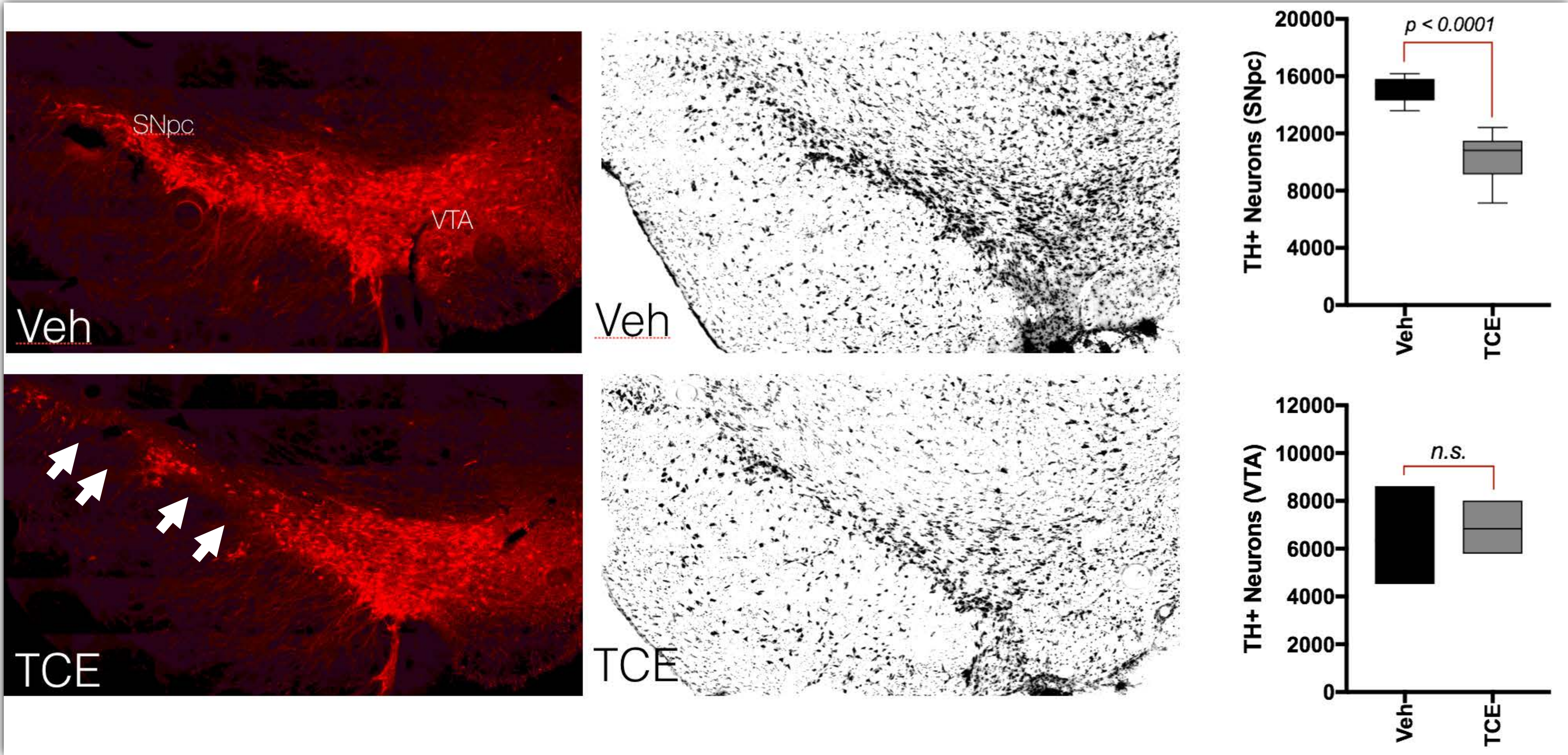
Striatal TH Intensity



*n=6 control, 10 TCE



Stereology

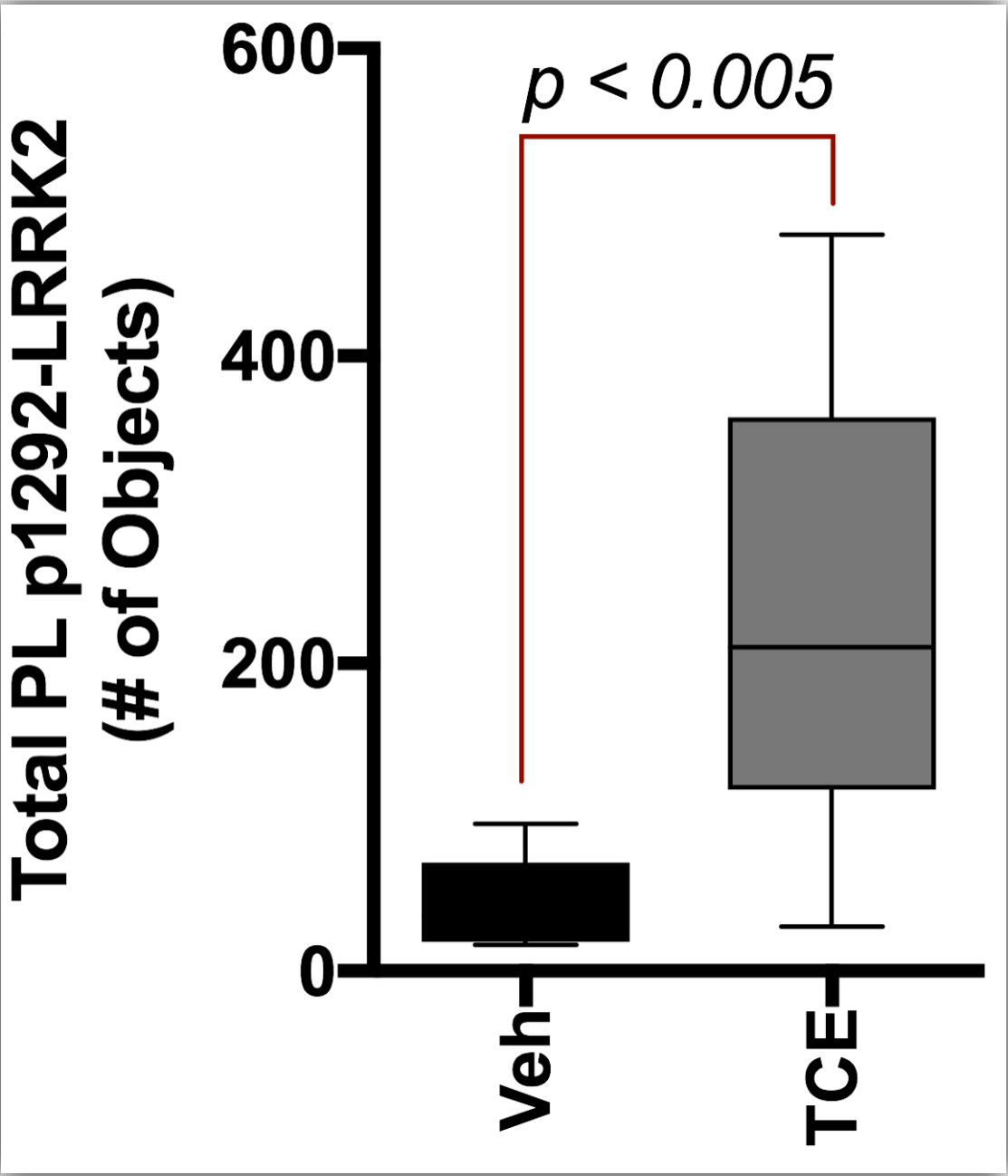
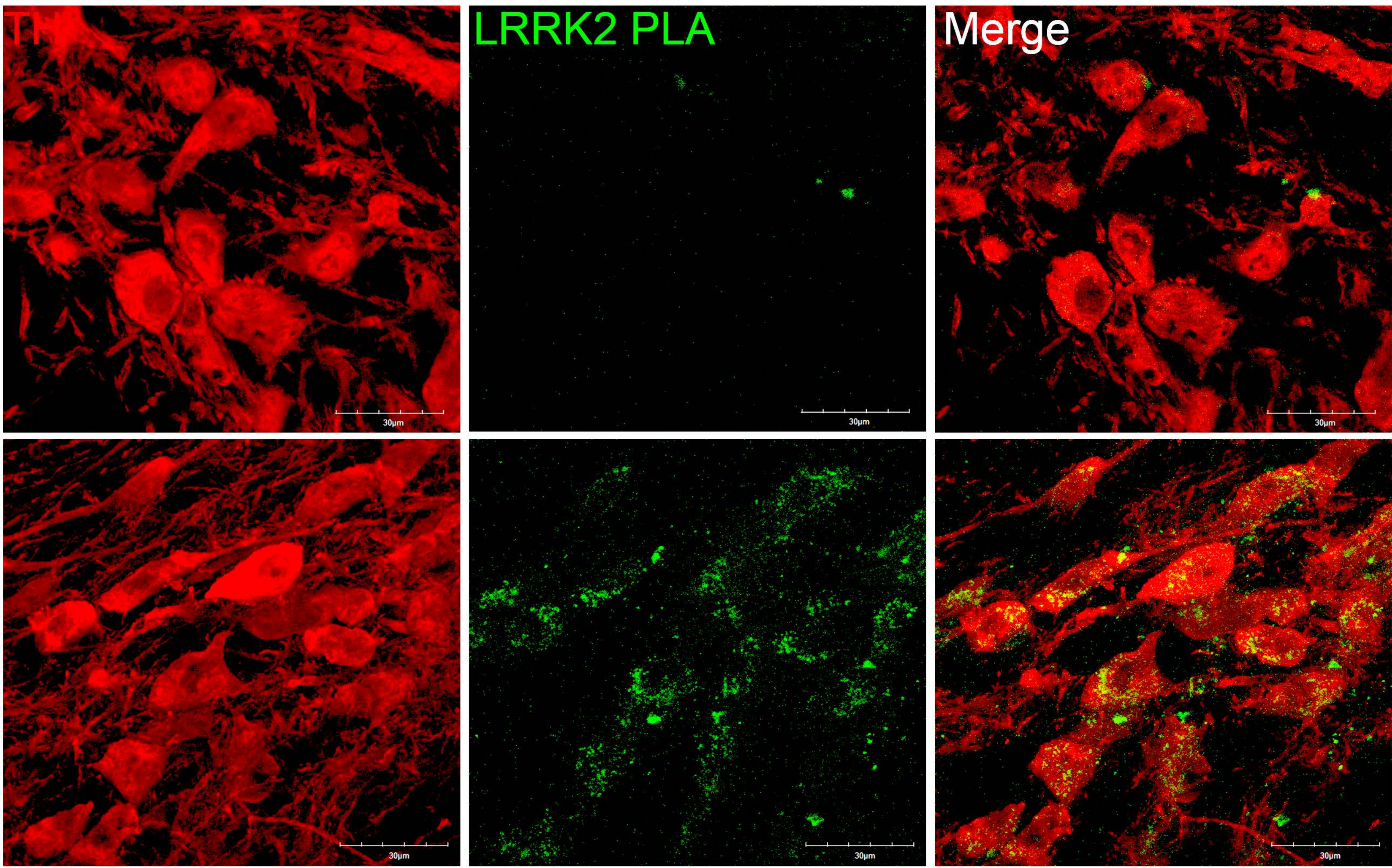


*n=6 control, 10 TCE

TCE exposure induces LRRK2 in dopaminergic neurons

Vehicle

200 mg/kg TCE



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Briana De Miranda et al, unpublished.

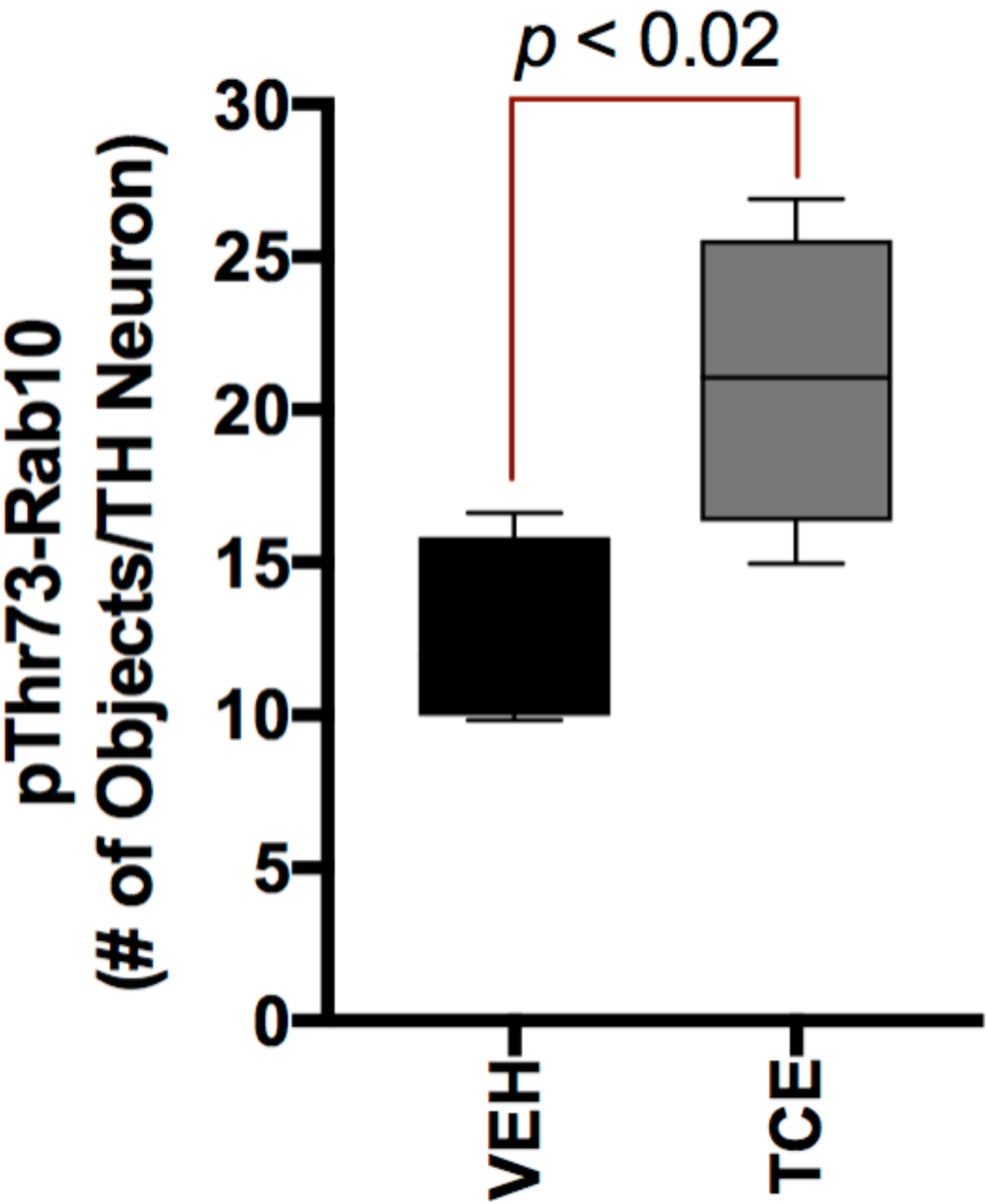
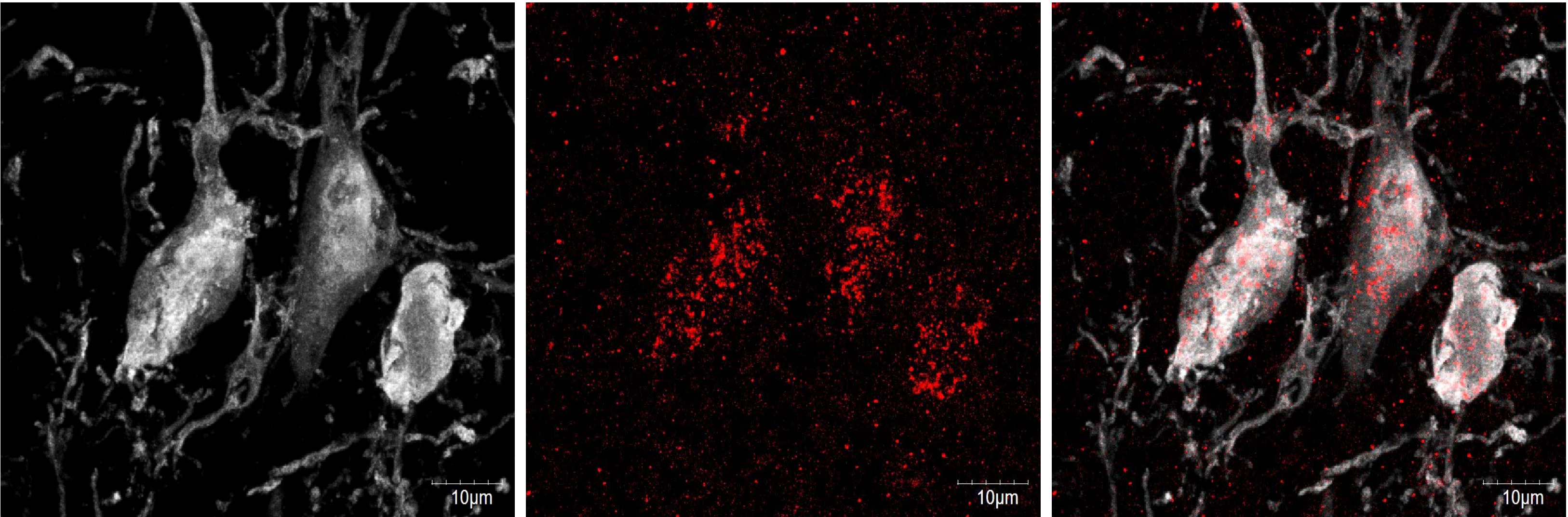


pRab10

Vehicle



200 mg/kg TCE



*n=6 control, 10 TCE



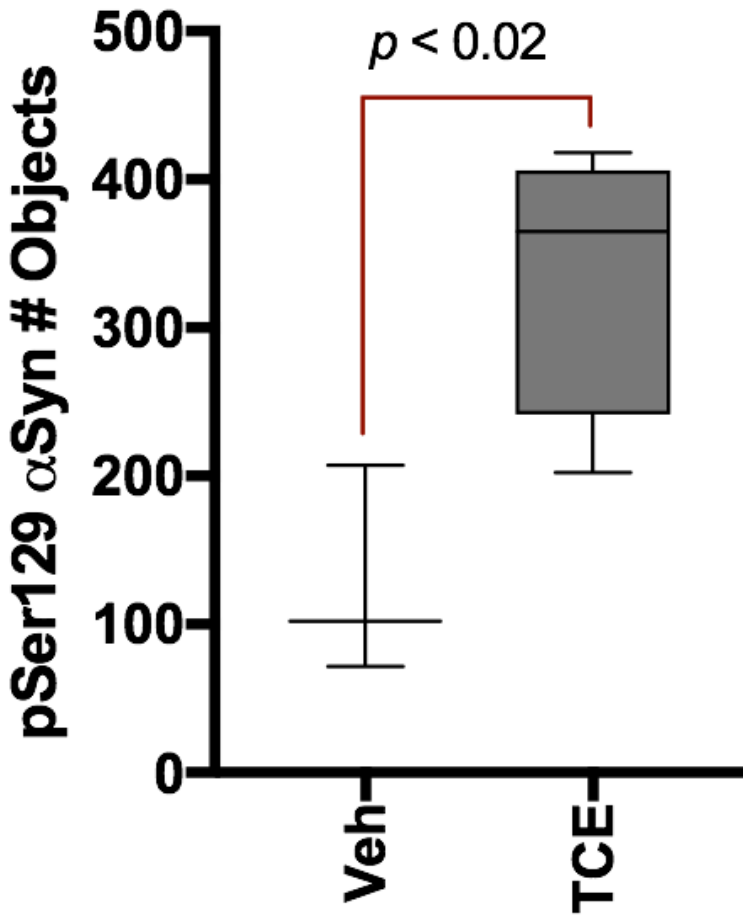
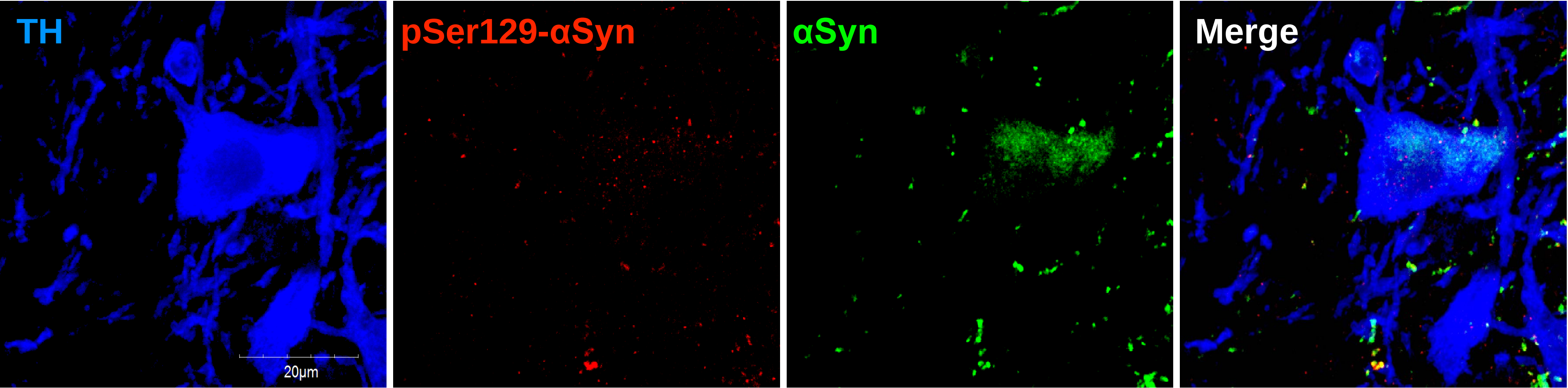
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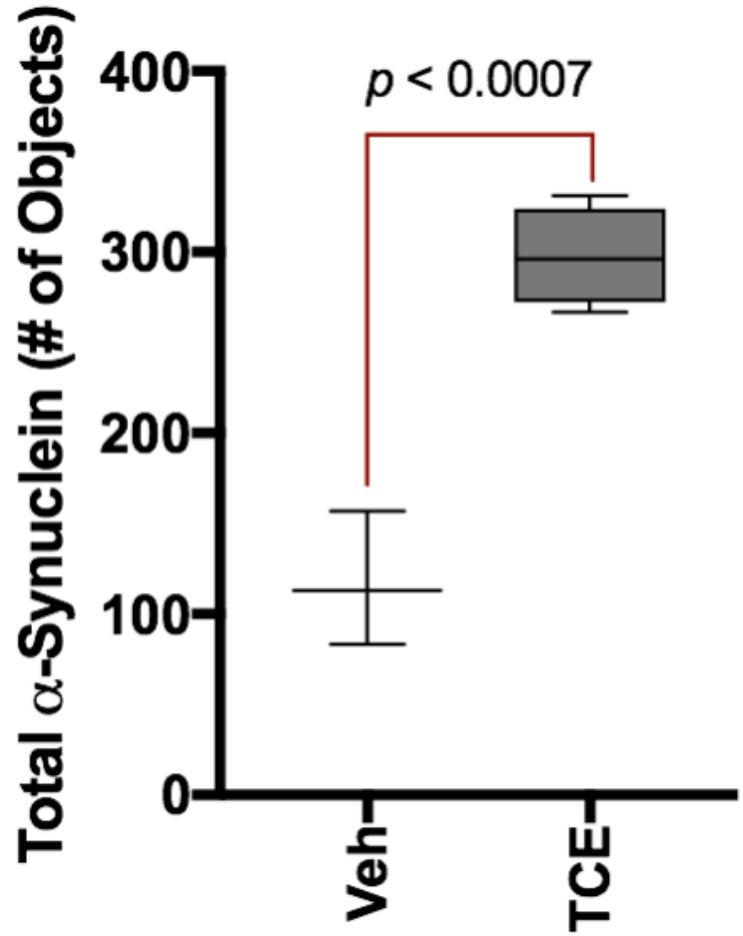
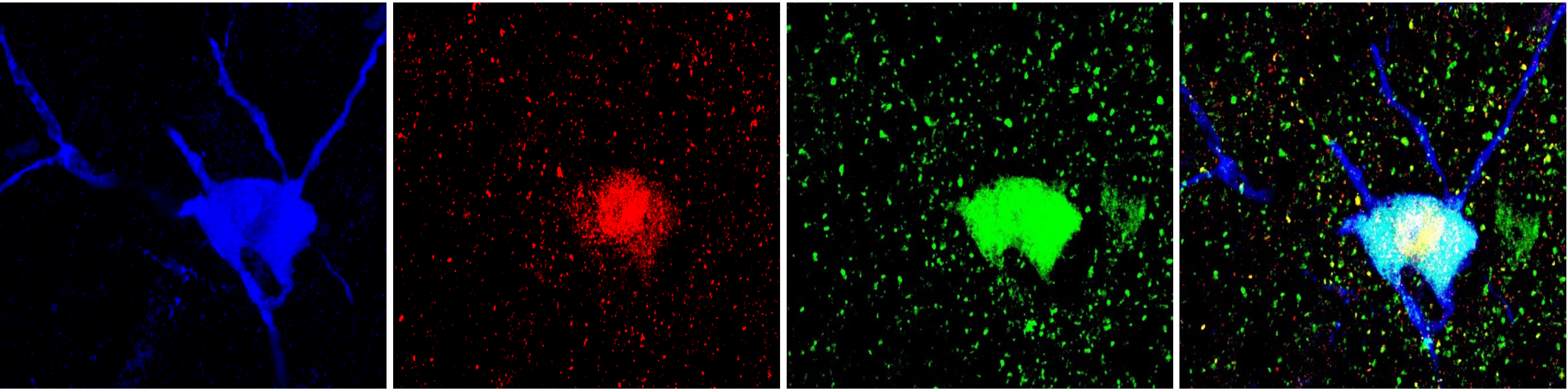
Briana De Miranda et al, unpublished.

pSer129-αSyn

Vehicle



200 mg/kg TCE



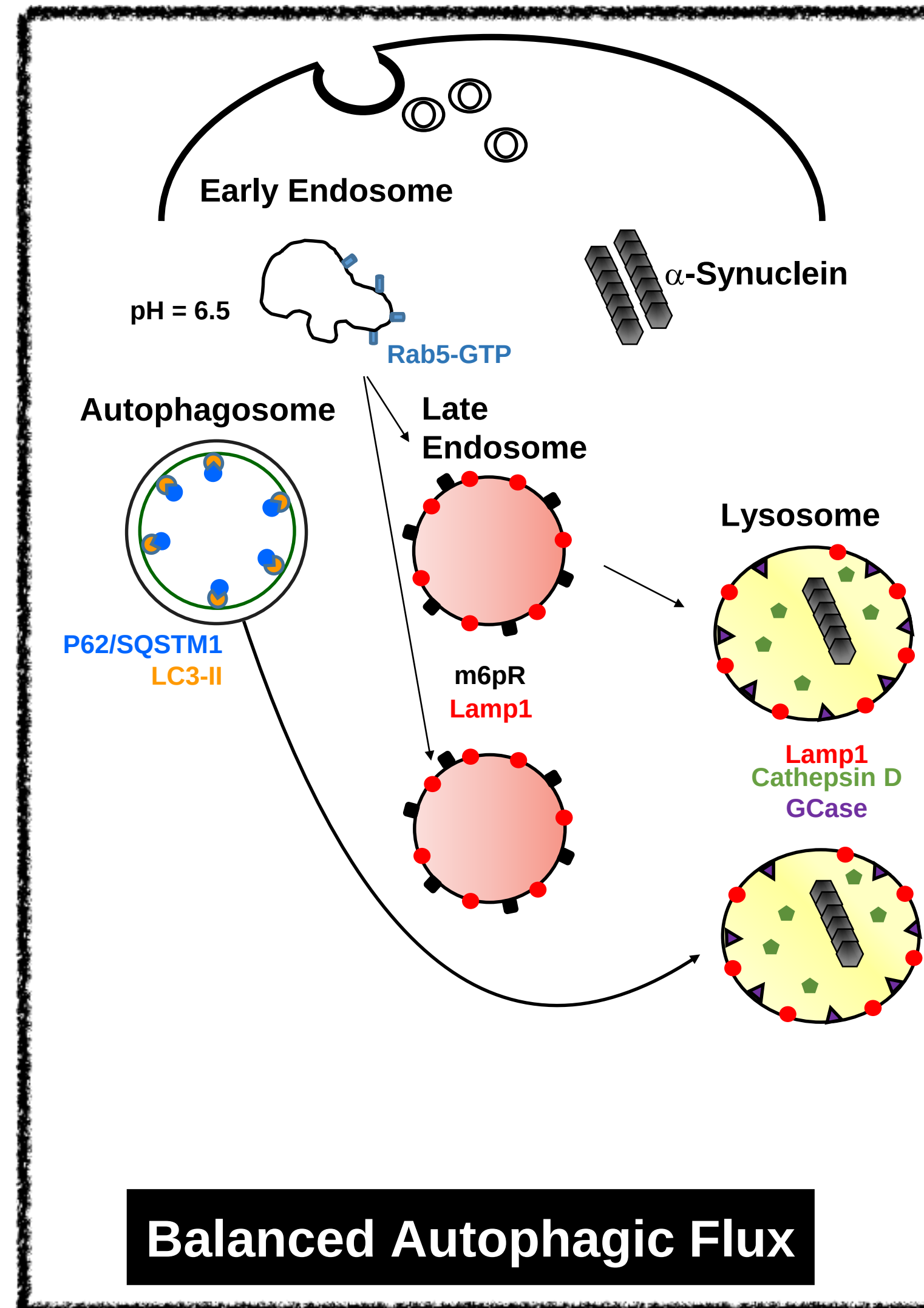
A wide-angle photograph of the Pittsburgh skyline at sunset. The sky is a vibrant mix of orange, yellow, and pink. The city's skyscrapers, including the UPMC Tower, PPG Place, and the two towers of the PNC Tower, are silhouetted against the bright sky. Their lights are reflected in the Allegheny River in the foreground. A bridge is visible on the right side of the river, and some greenery is in the bottom right corner.

Is environmental toxicant-induced LRRK2
activation relevant to idiopathic PD?

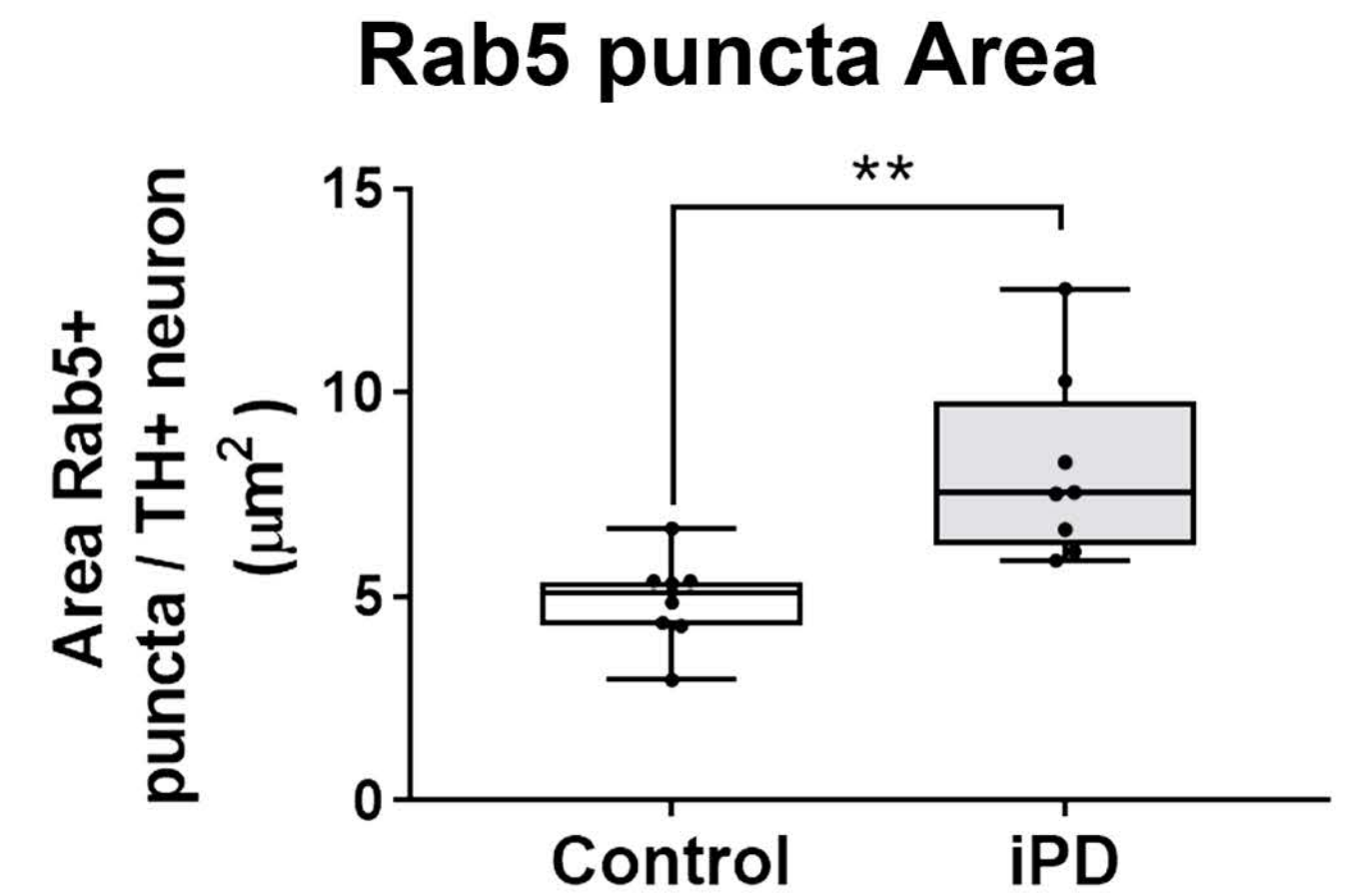
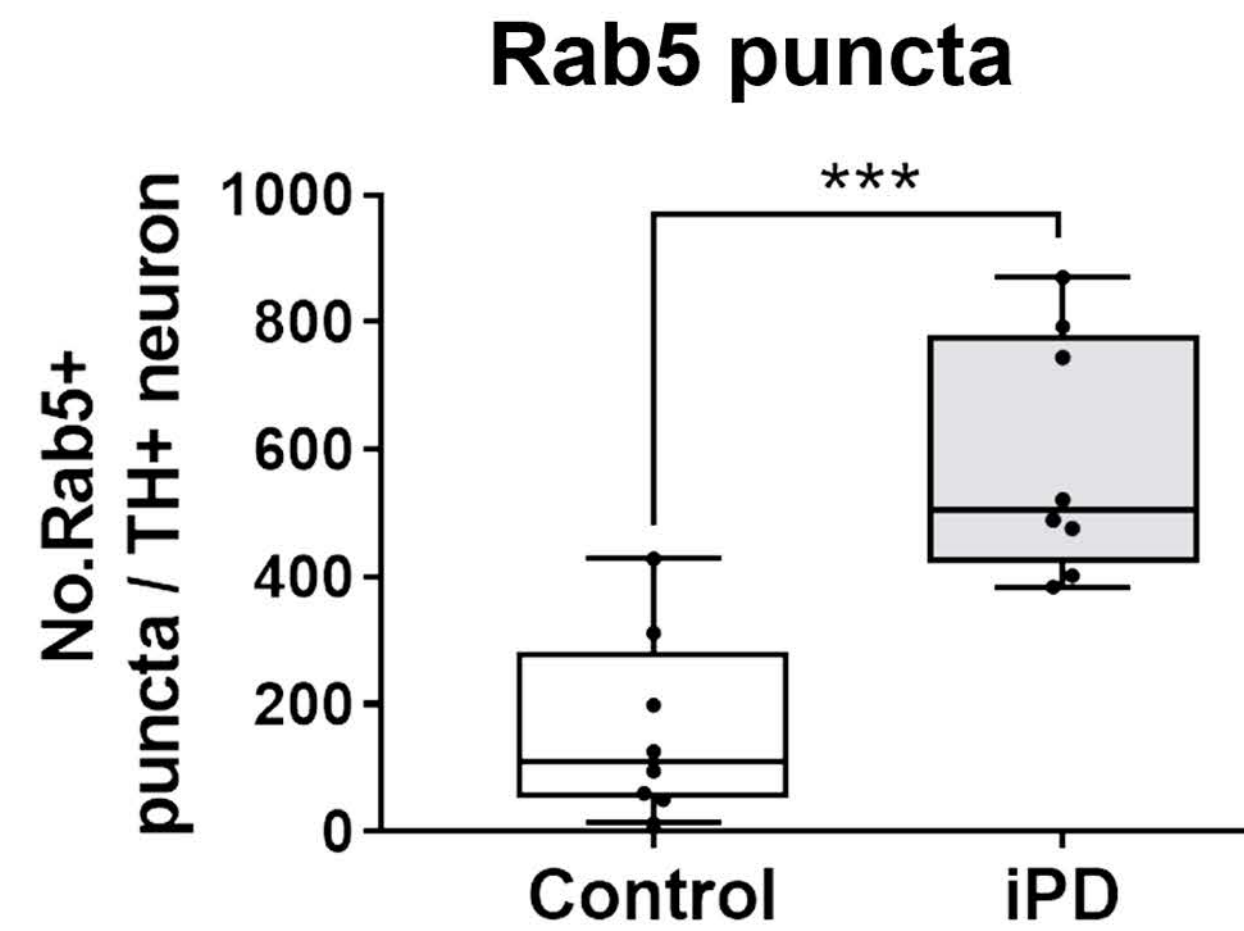
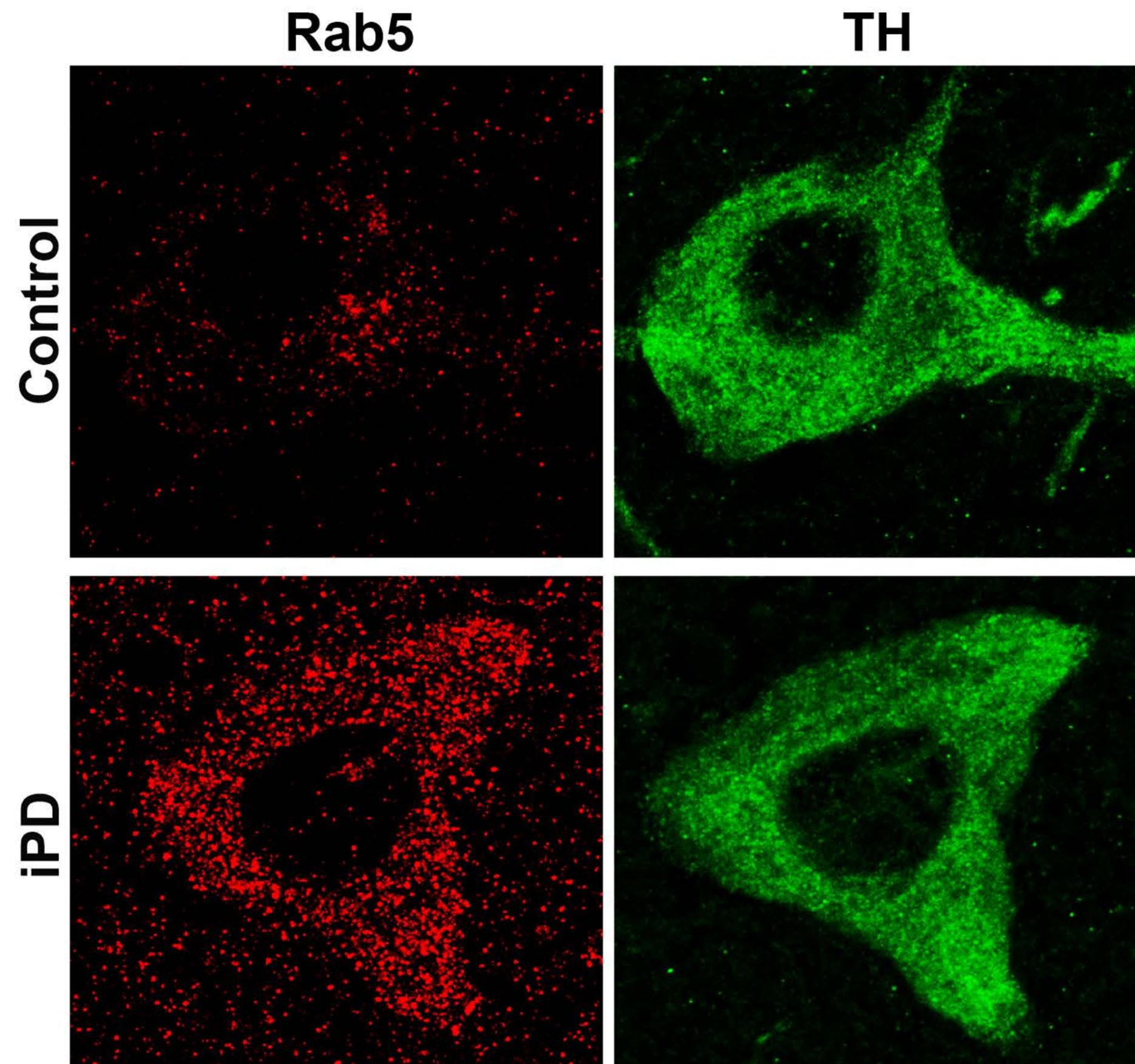
Role of LRRK2 in endolysosomal function in PD

1. Use compartment-selective markers to define status of endolysosomal function in human SNc neurons in iPD
2. Determine the extent to which this is replicated in the rotenone model of PD
3. Assess whether LRRK2 kinase inhibition ameliorates impaired endolysosomal function and protects SNc neurons

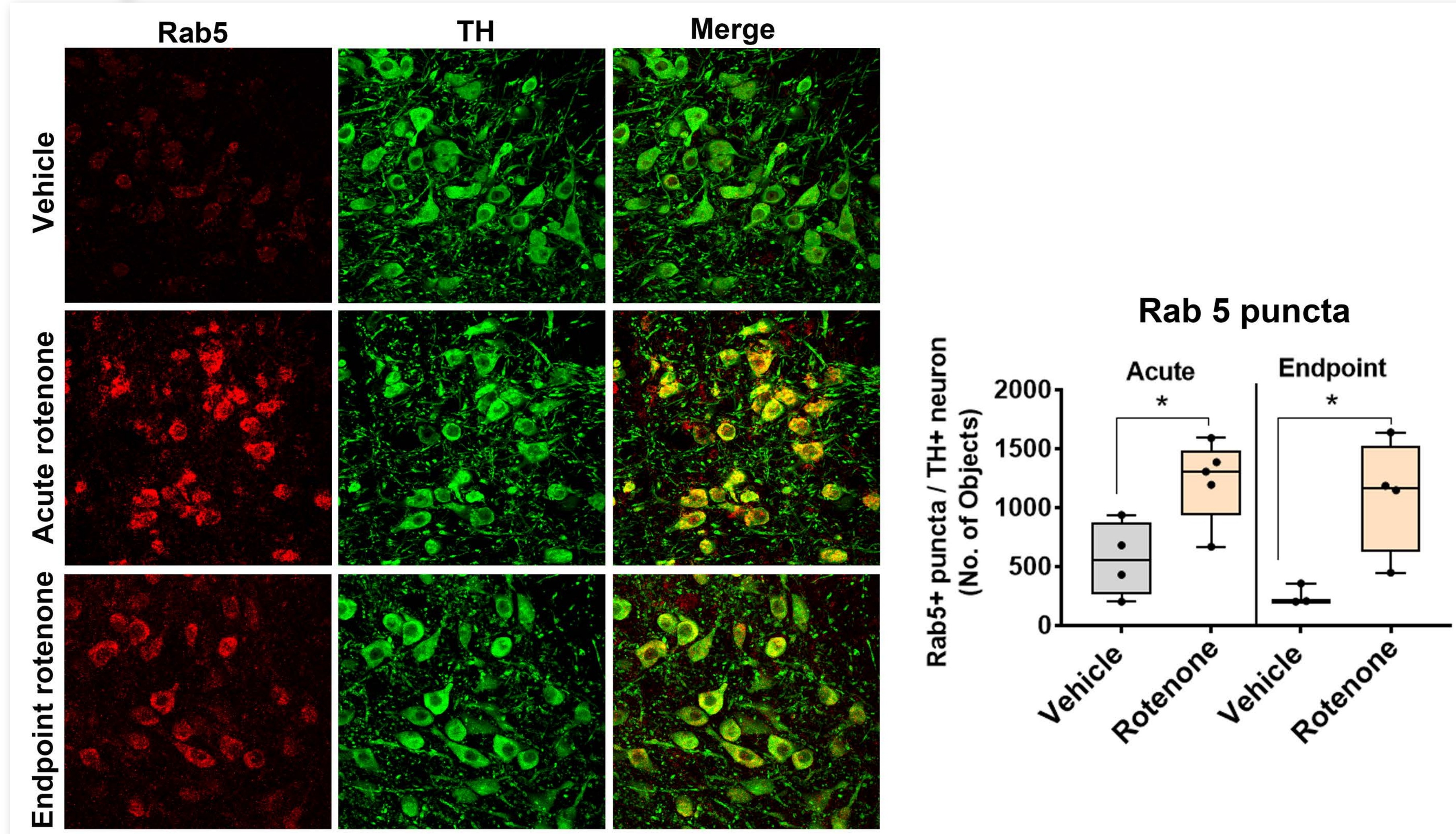
Healthy neuron



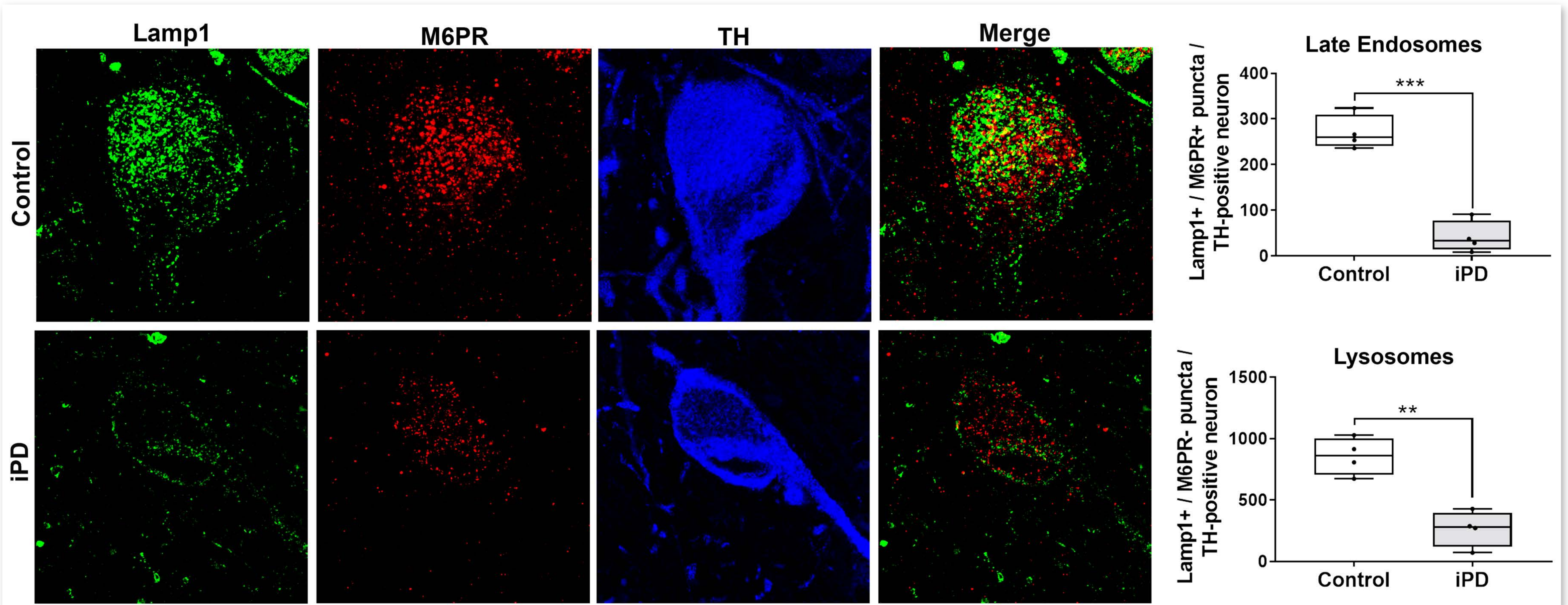
Early Endosomes in iPD



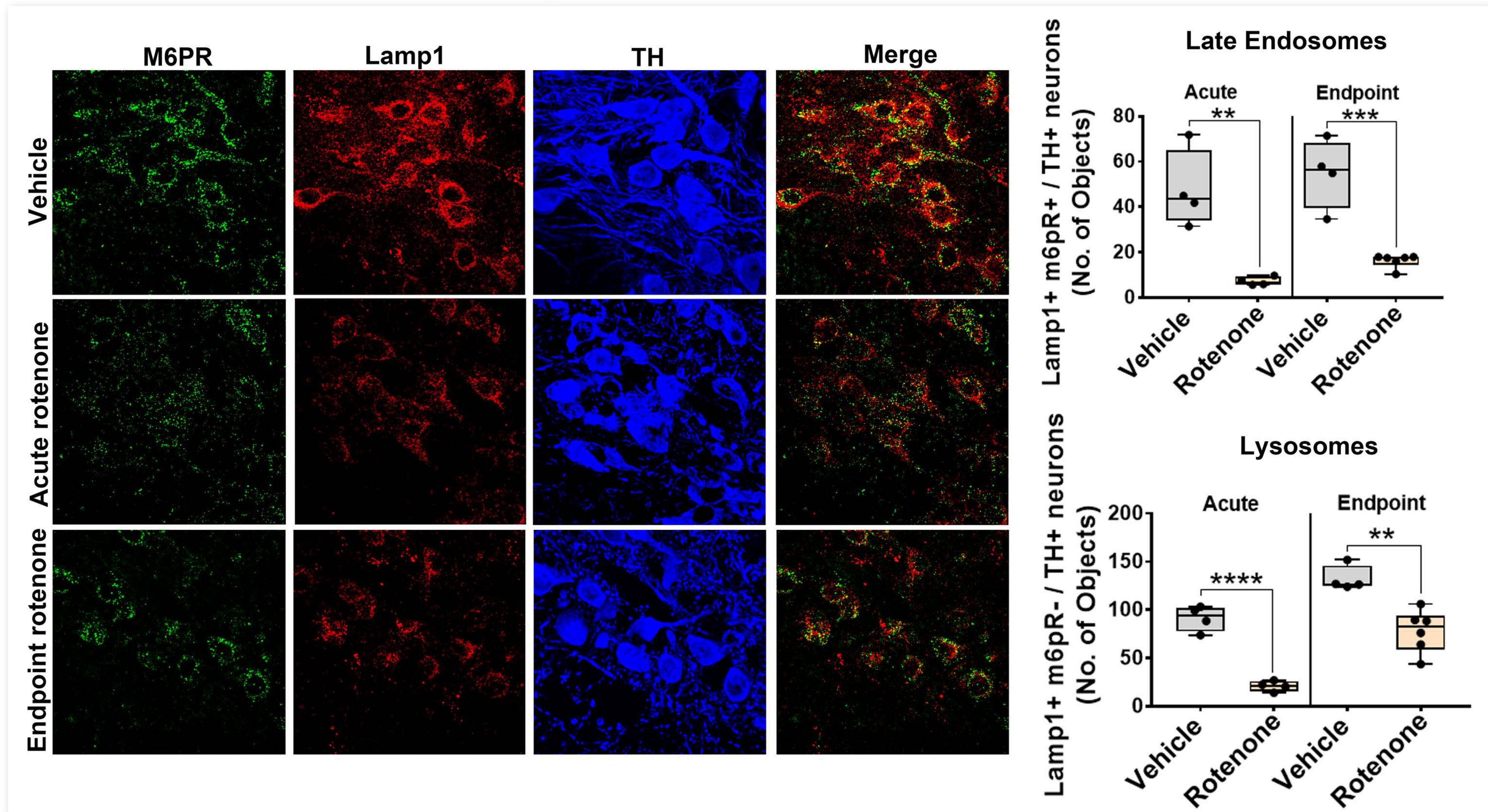
Early Endosomes in the Rotenone Model



Late Endosomes & Lysosomes in iPD



Late Endosomes & Lysosomes in the Rotenone Model



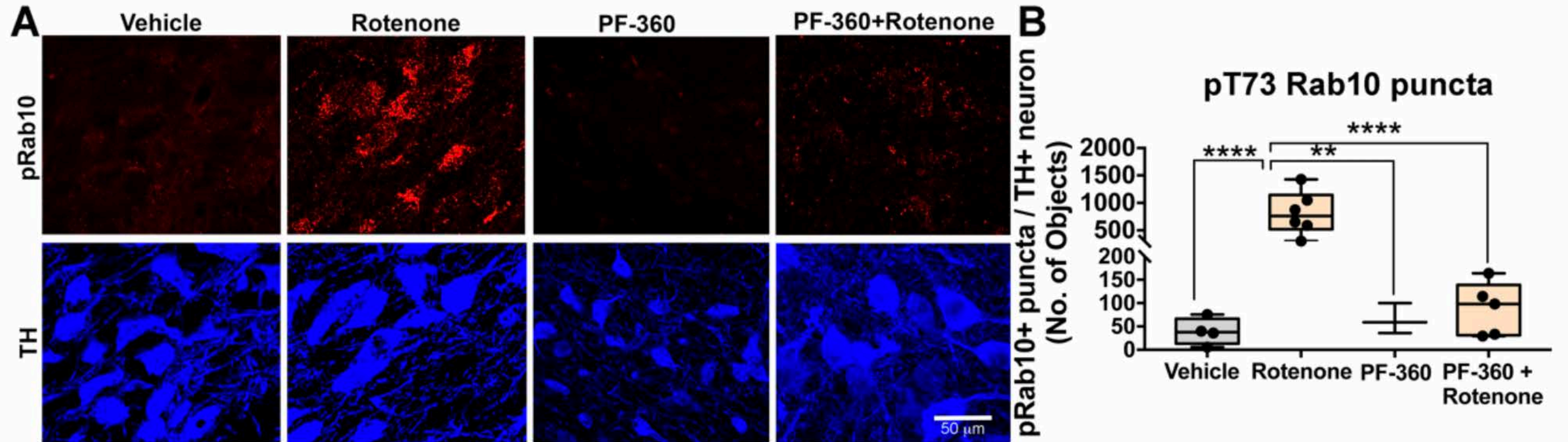
Role of LRRK2 in endolysosomal function in PD

In iPD:

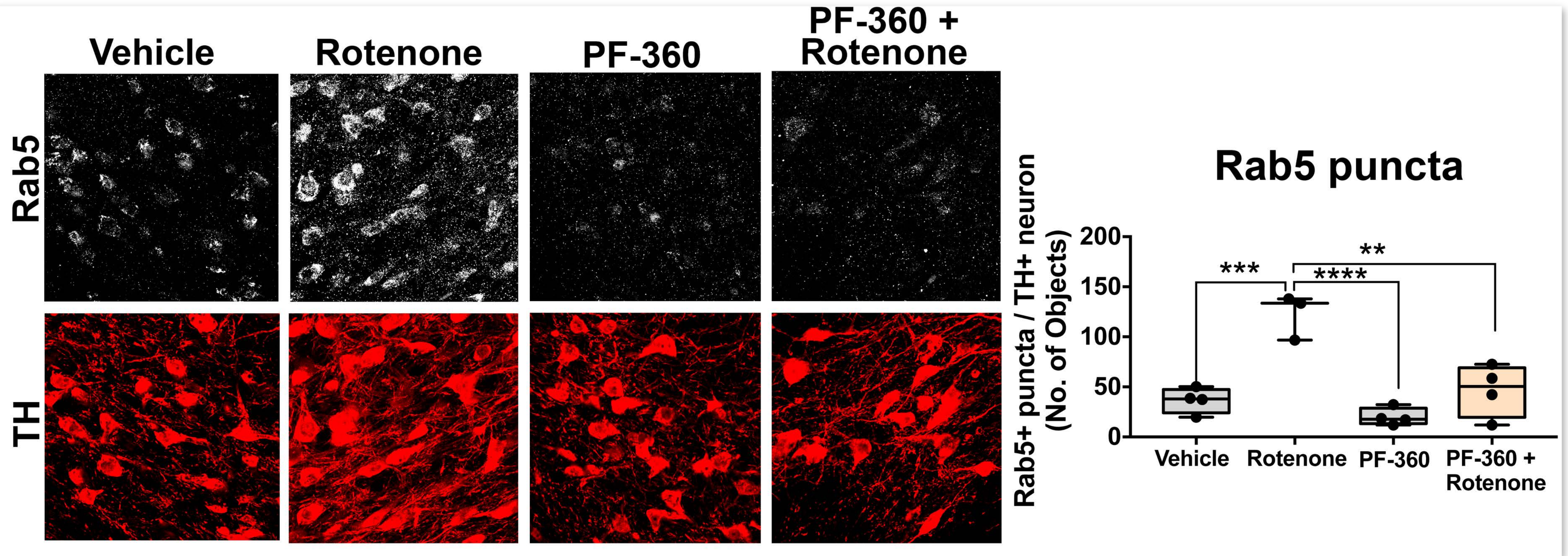
- Accumulation of early endosomes
- Depletion of late endosomes & lysosomes (& Cathepsin D)
- Loss of GCase activity and protein levels
- All of these findings are reproduced in the rotenone model

Does LRRK2 kinase inhibition ameliorate impaired endolysosomal function and/or protect SNc neurons of rats treated to rotenone endpoint?

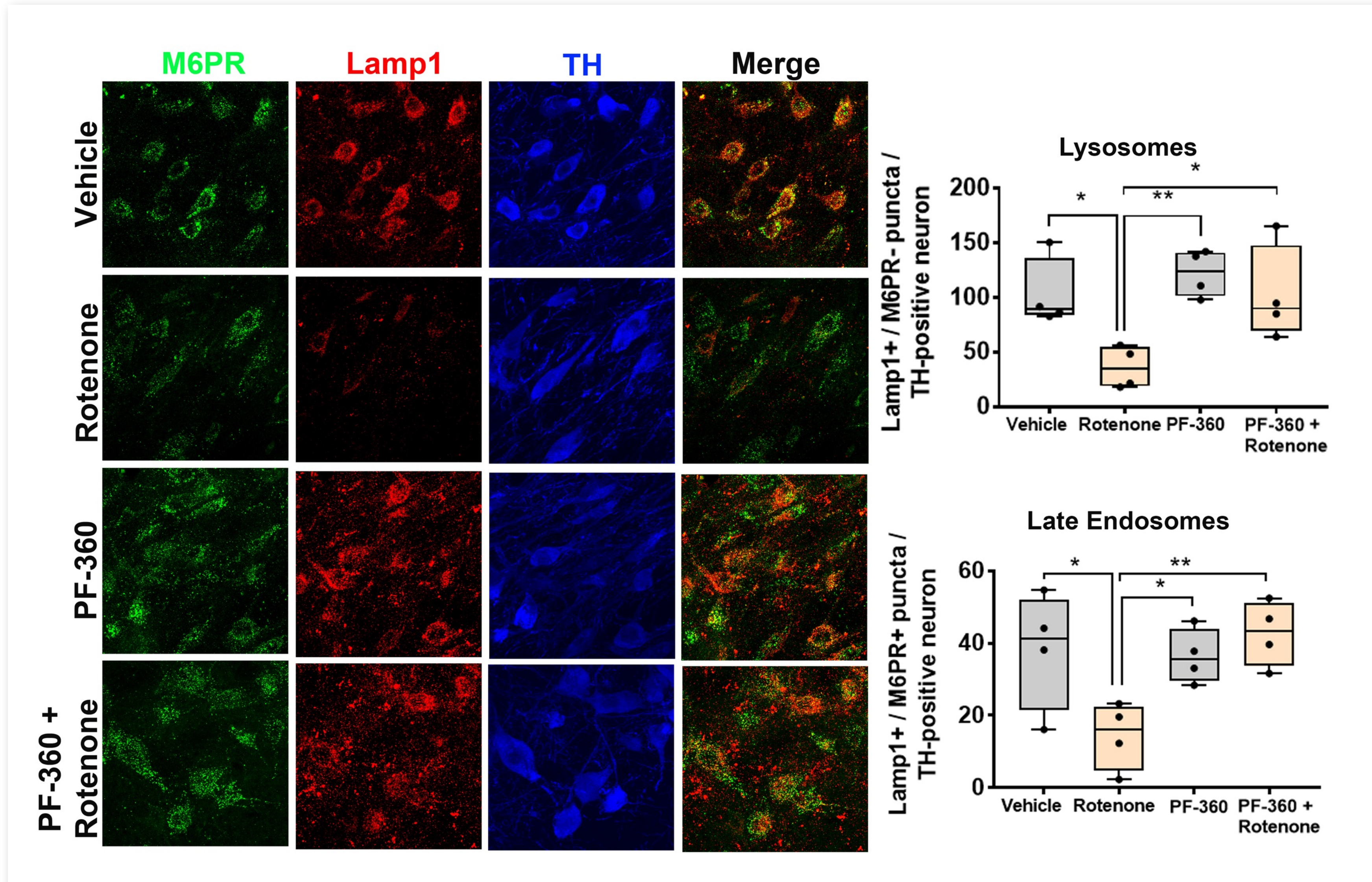
Effects of LRRK2 Inhibition: Target engagement



Early Endosomes: Effects of LRRK2 Inhibition



Late Endosomes & Lysosomes: Effects of LRRK2 Inhibition



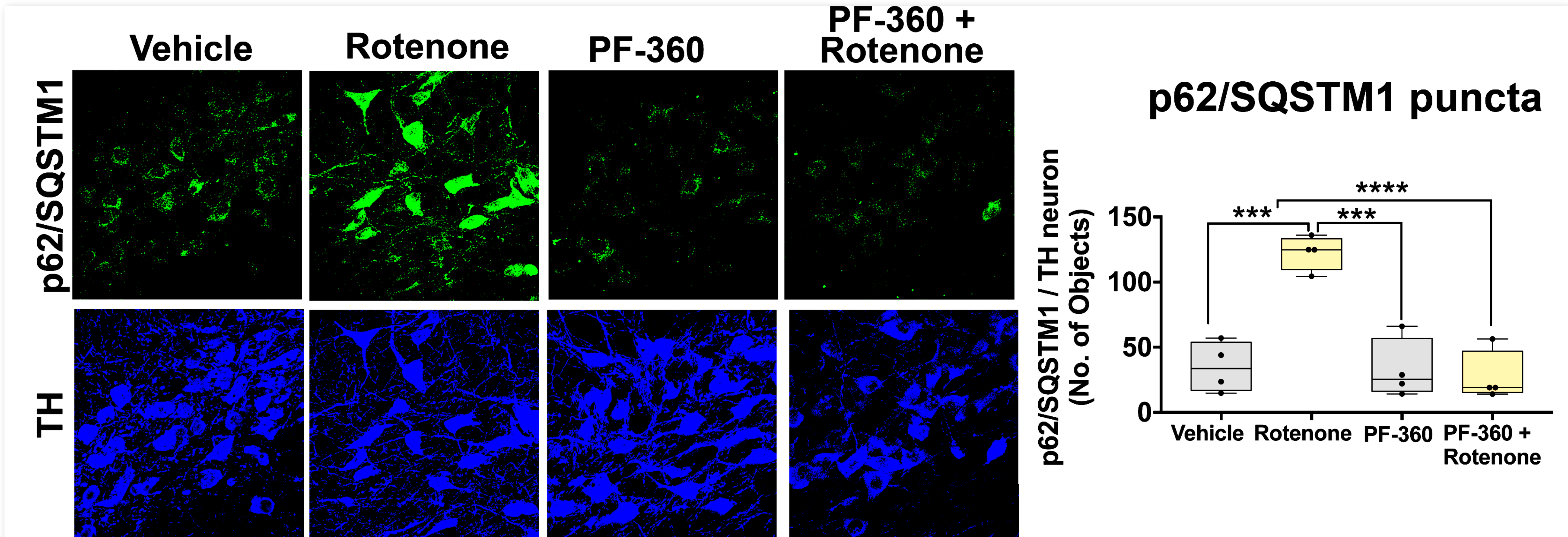
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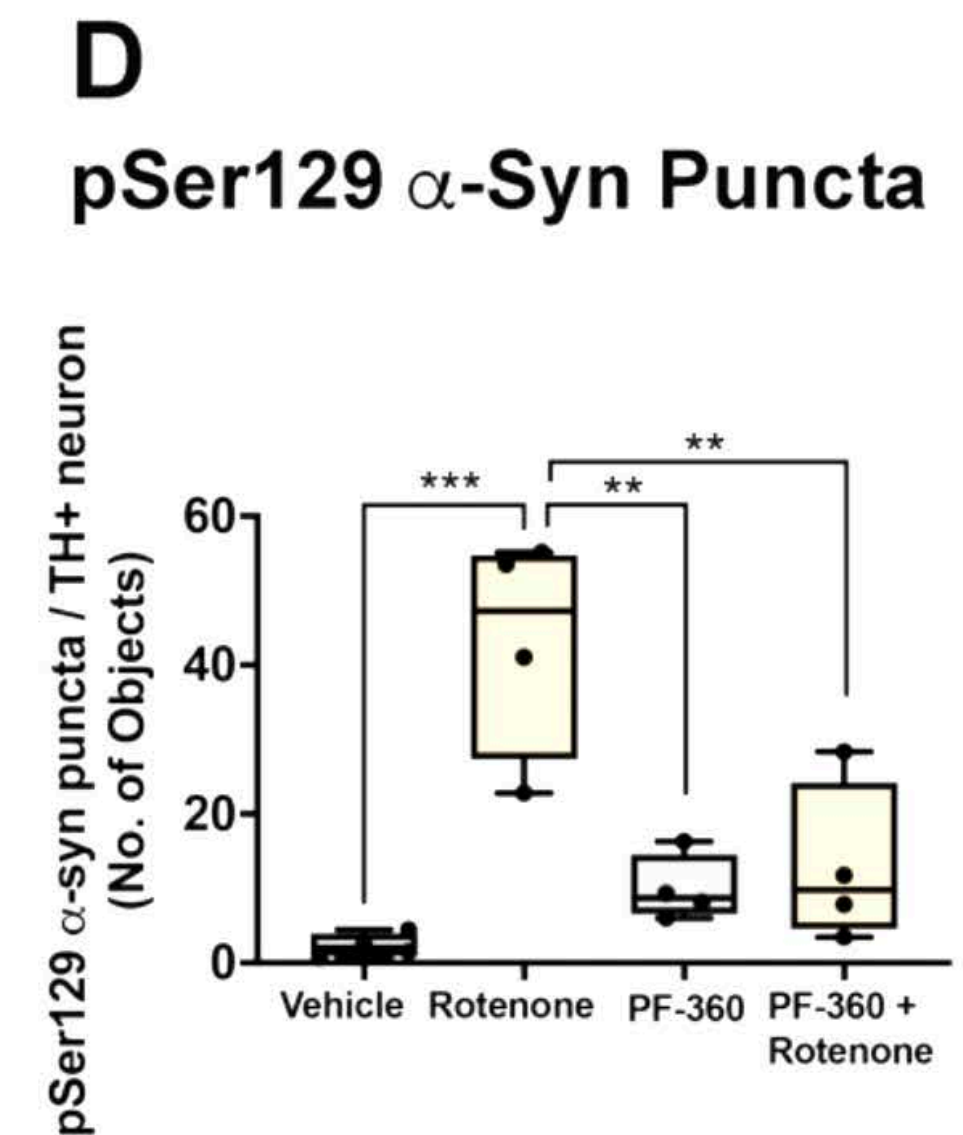
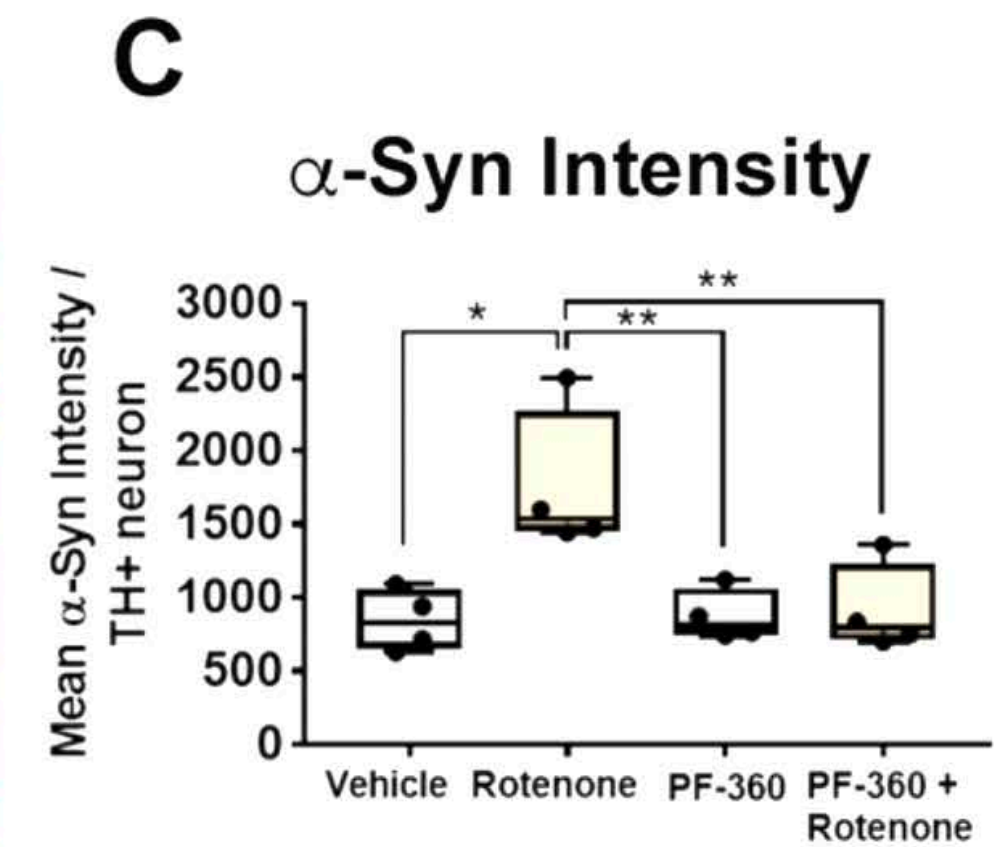
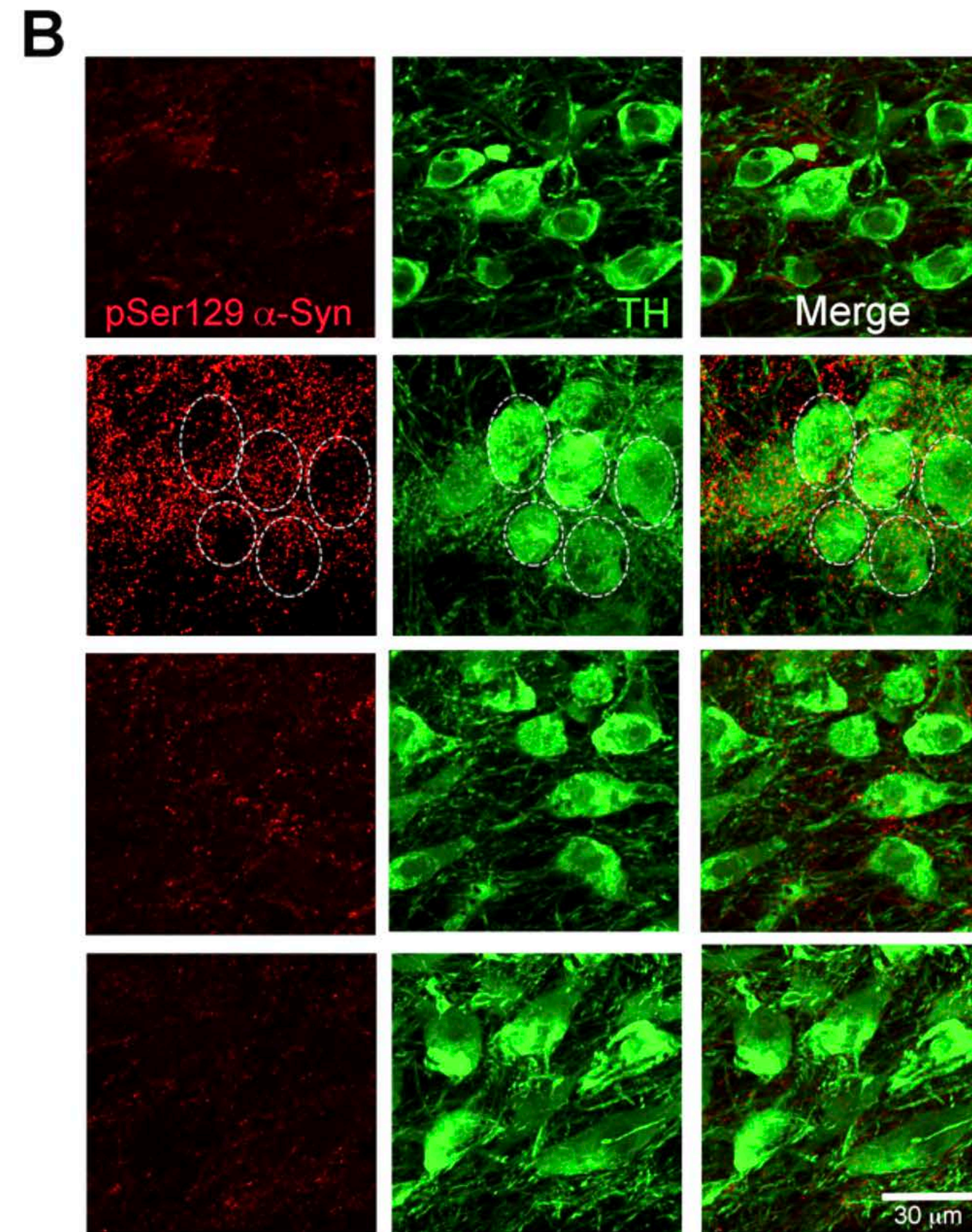
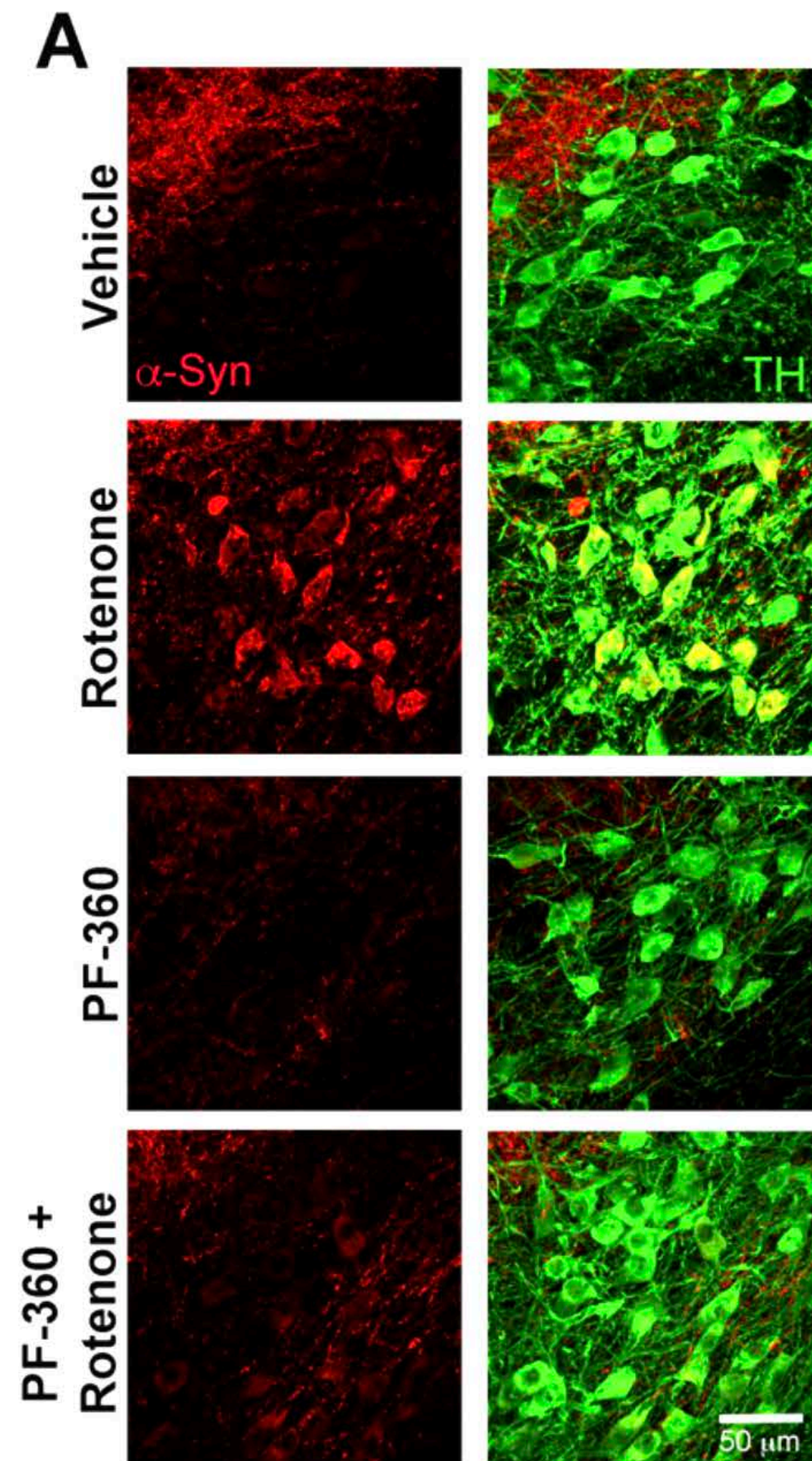
Emily Rocha et al, *unpublished*



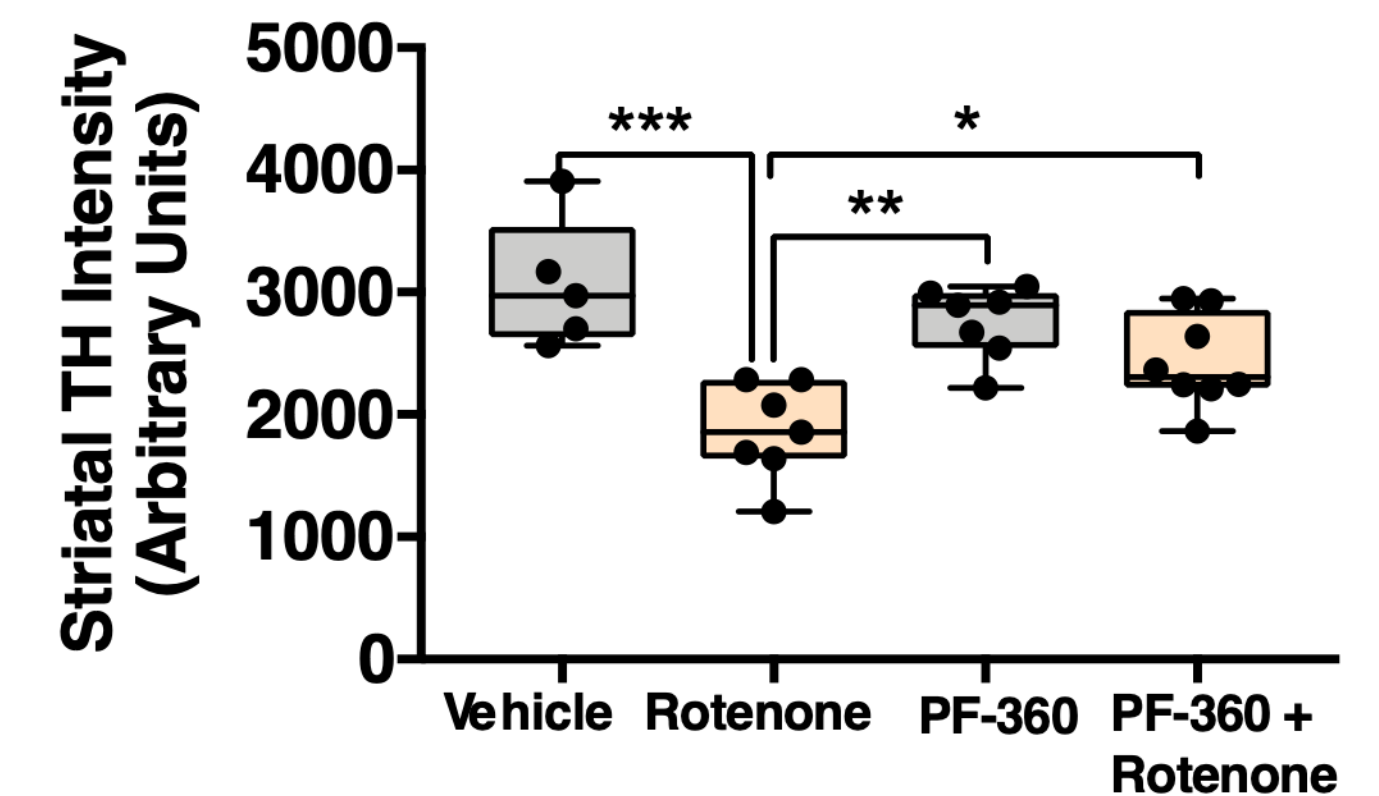
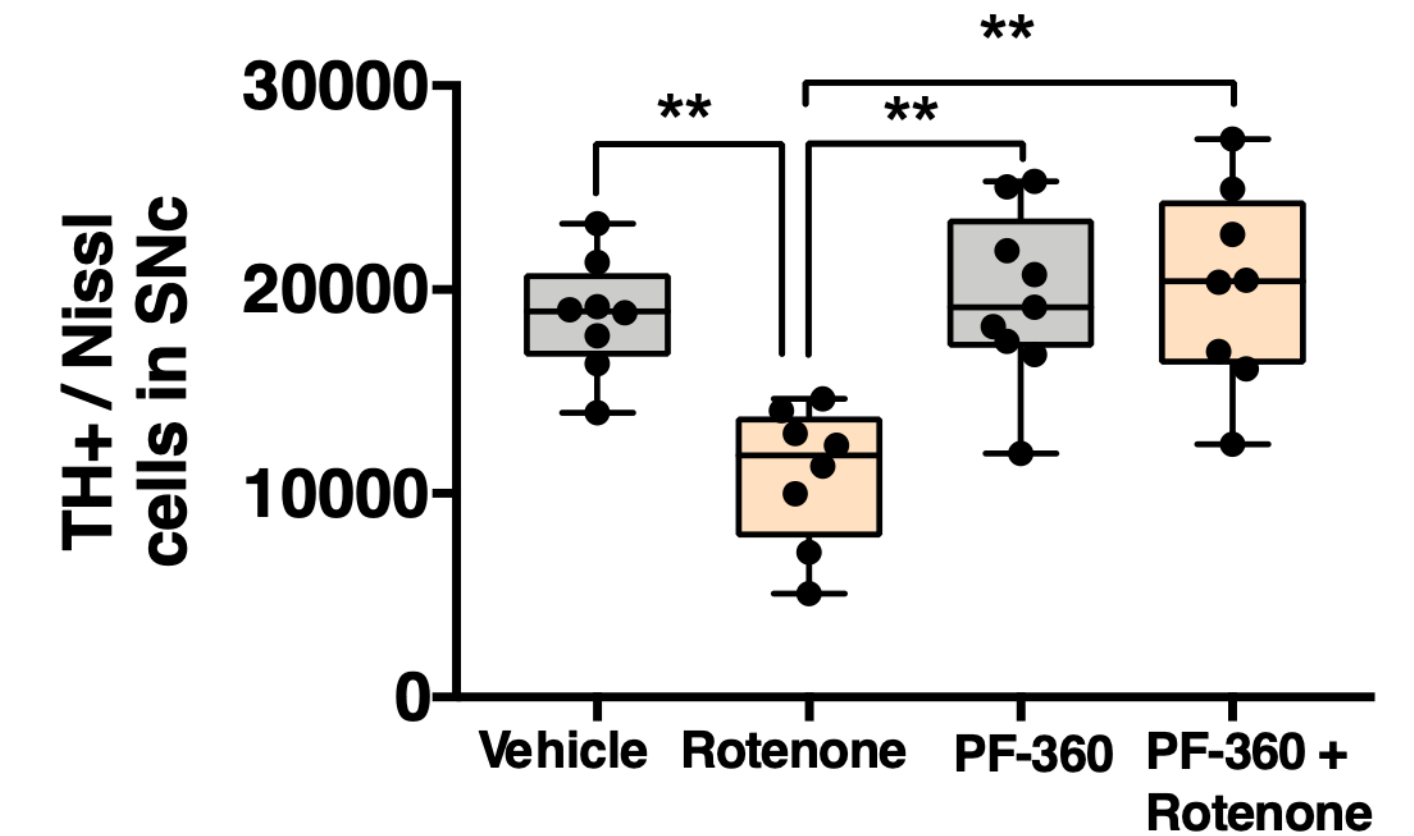
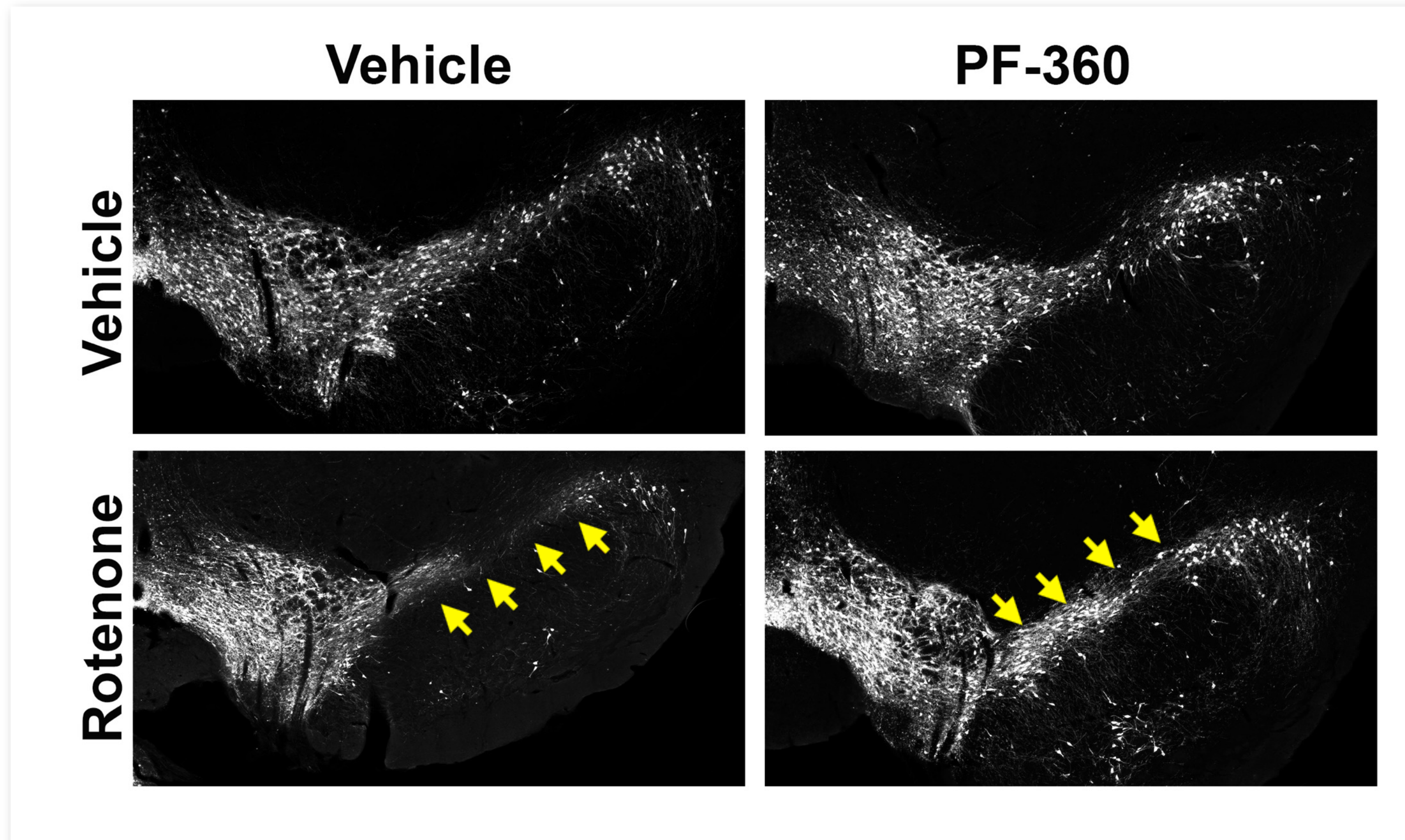
Autophagosomes: Effects of LRRK2 Inhibition

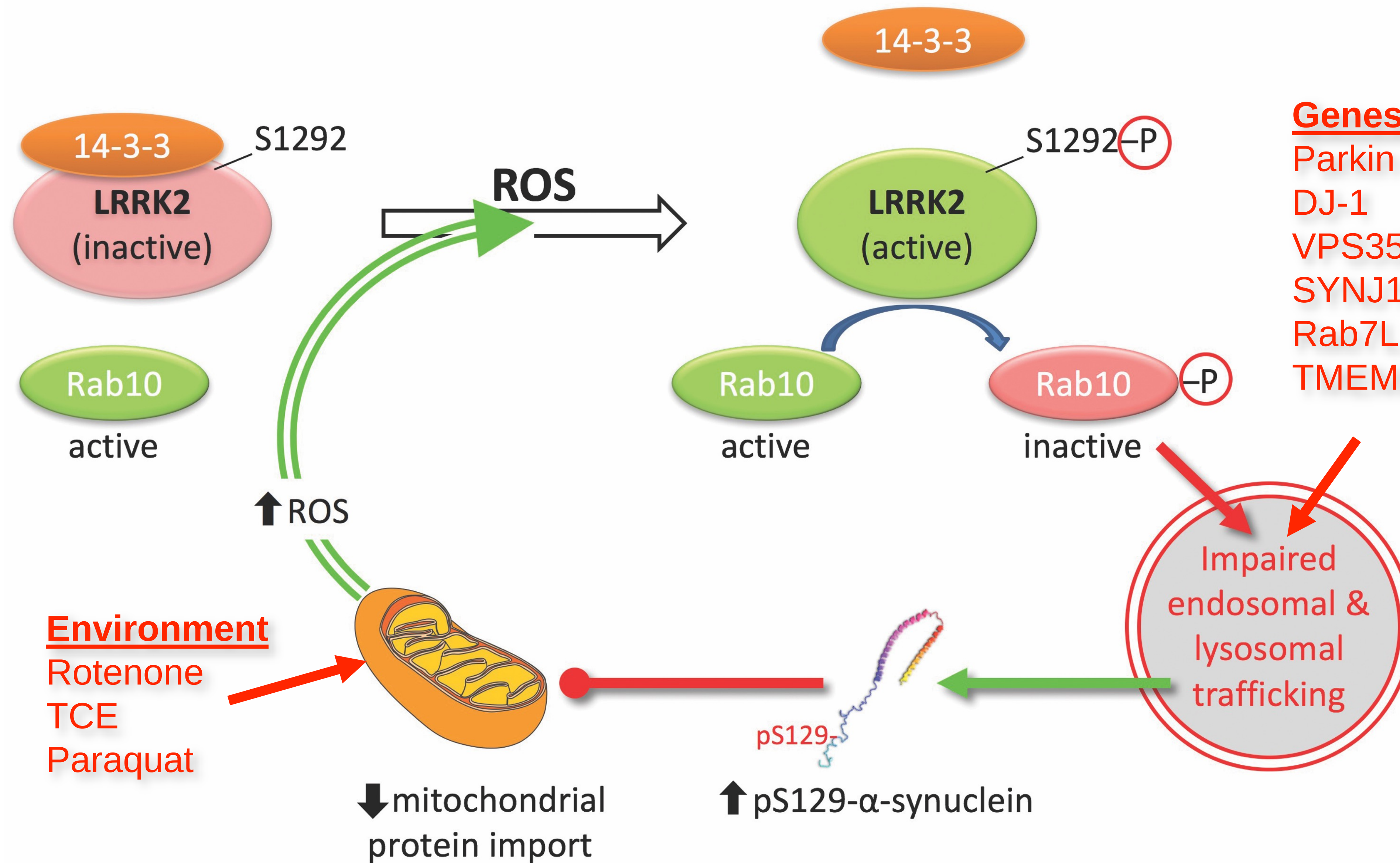


alpha-Synuclein: Effects of LRRK2 Inhibition



SNC cell loss: Effects of LRRK2 Inhibition





Genes

Parkin	PINK1
DJ-1	Fbxo7
VPS35	ATP13A2
SYNJ1	DNAJC6
Rab7L1	GBA
TMEM230	

LRRK2 Therapeutics in iPD

Implications:

- LRRK2 plays a central role in iPD, at least in part, by modulating endolysosomal function
- Rather than targeting only the 2-3% of PD patients with LRRK2 mutations, LRRK2 therapeutics may be useful for individuals with iPD, independent of mutations

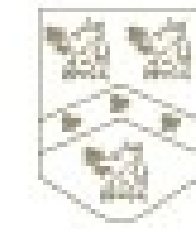
Parkinson's disease:

At the intersection of genes & environment

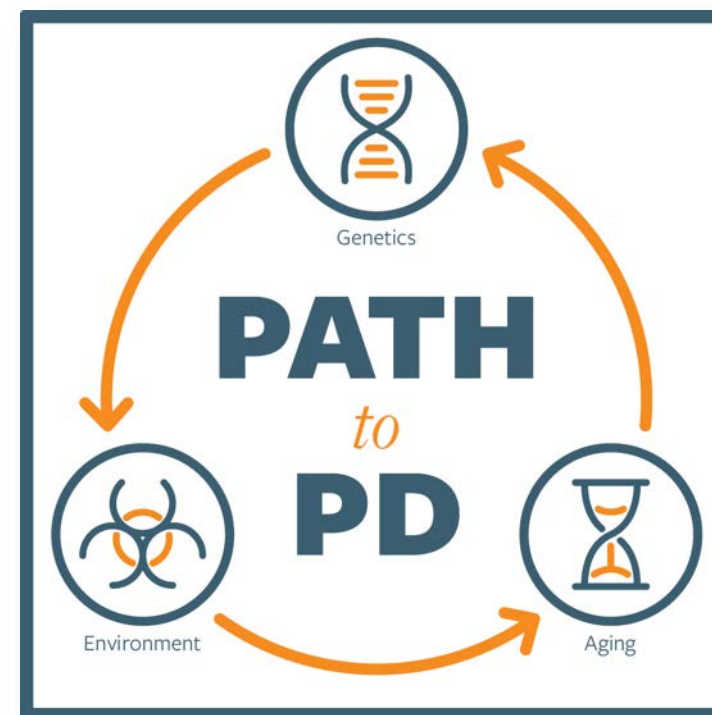
Conclusions:

- Genes implicated in PD (e.g., α -synuclein & LRRK2) strongly influence the neurotoxicity of environmental toxicants (e.g., rotenone, TCE).
- PD-relevant environmental toxicants (e.g., rotenone, TCE) can “activate” gene products implicated in PD (e.g., pS129- α -synuclein and LRRK2).

Funding



DSF Charitable Foundation



BLECHMAN
FOUNDATION
for Parkinson's Research



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