Supplemental Material

Neuroprotective actions of a fatty acid nitroalkene in Parkinson's disease.

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Supplemental Figure 1. Cytotoxicity of 10-NO_2-OA in N27-A dopaminergic cells.

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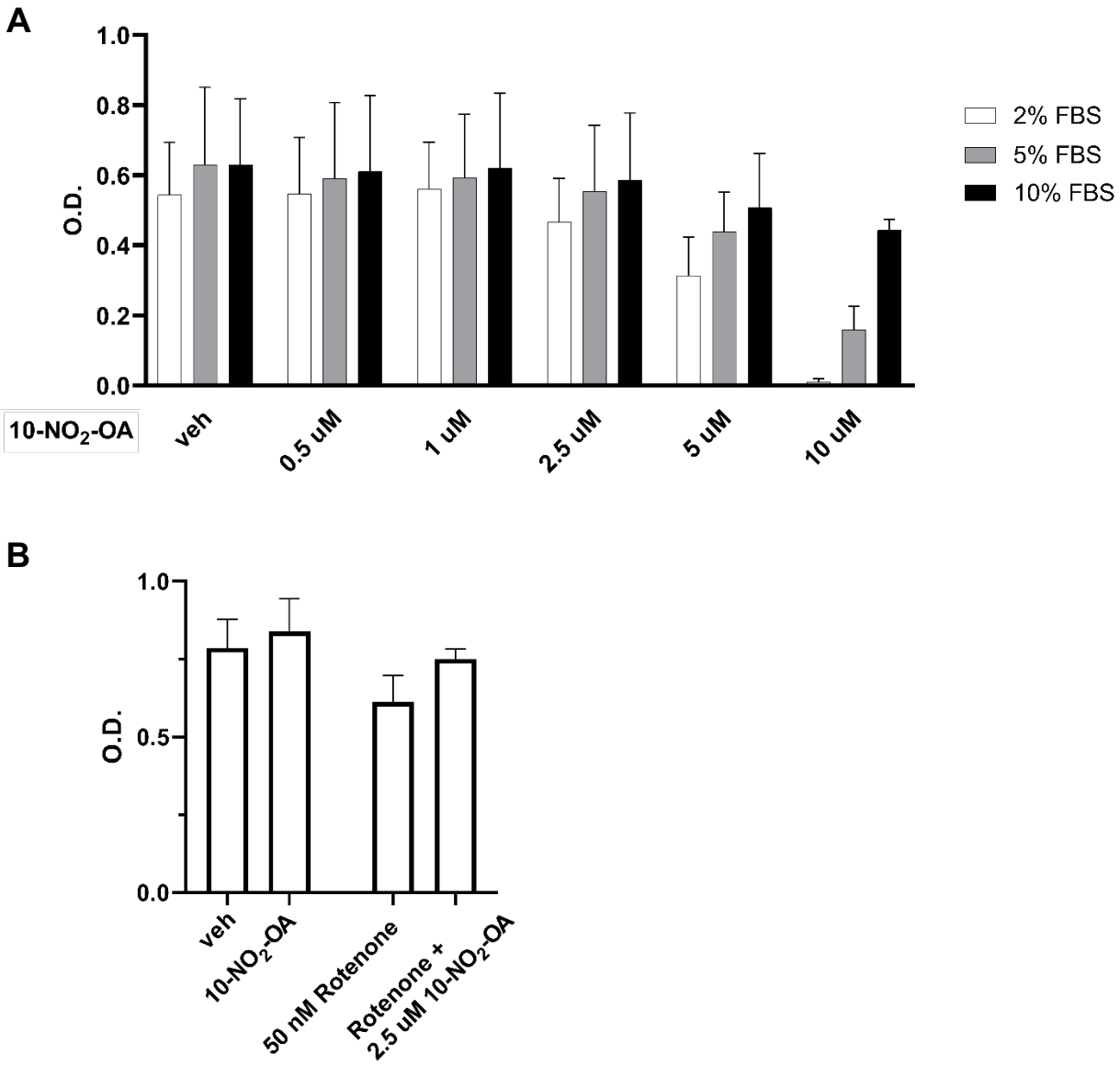
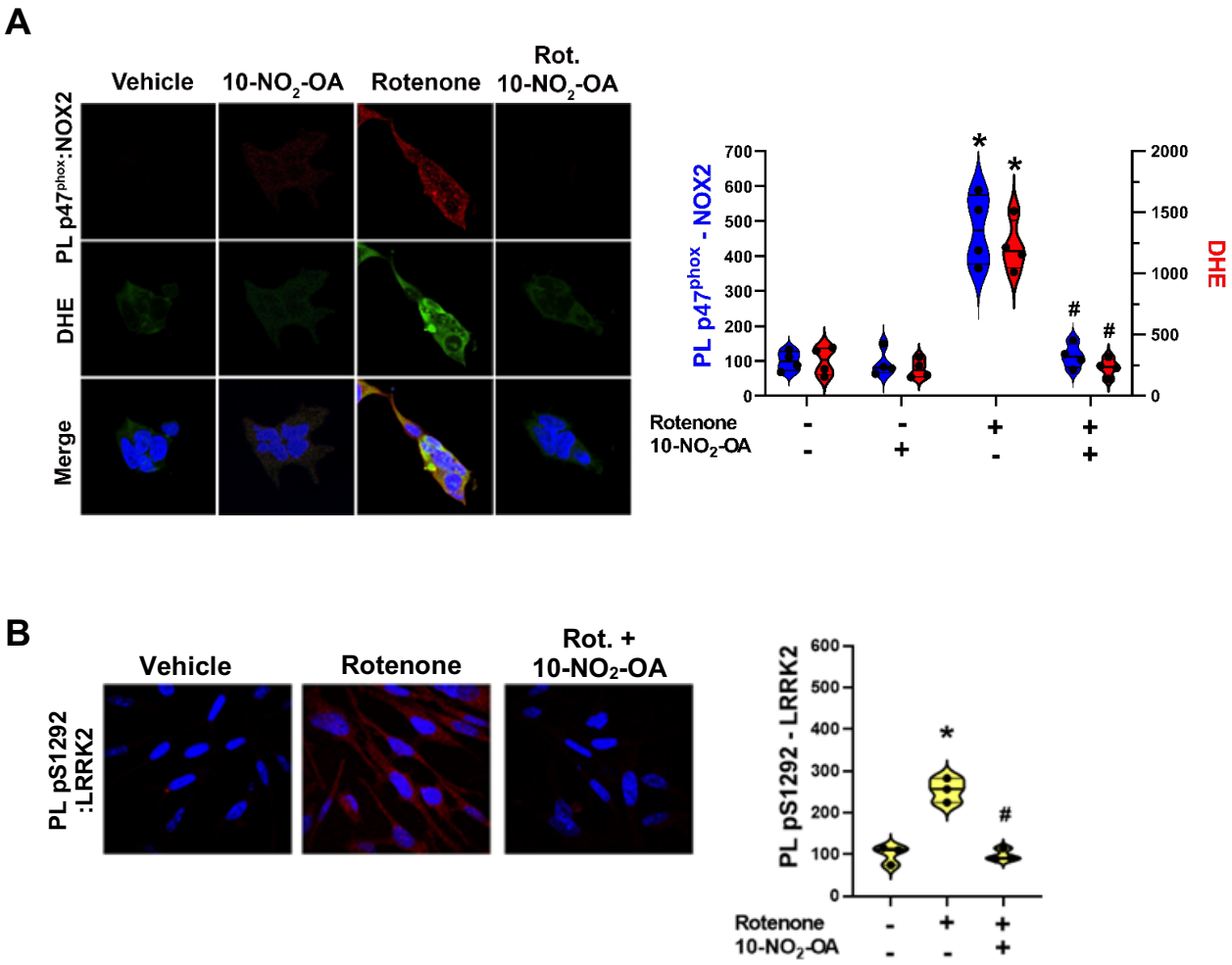
Supplemental Figure 7. 10-NO_2-OA inhibits α-synuclein-mediated mitochondrial import impairment in the subacute PD model of rotenone.

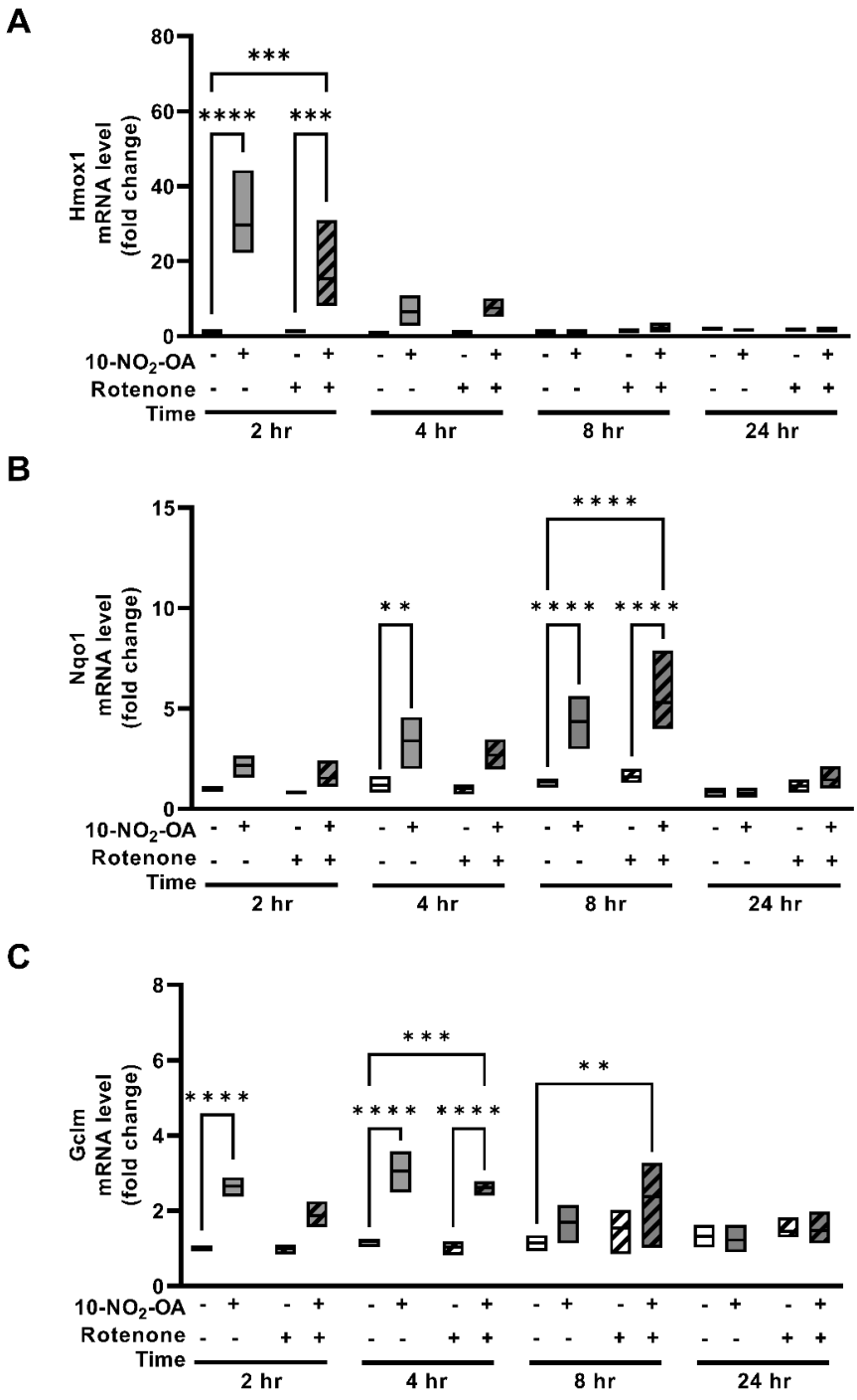
Supplemental Figure 8. Analysis of rotenone in whole rat brain (cohort 2).



Supplemental Figure 1. Cytotoxicity of 10-NO_2-OA in N27-A dopaminergic cells. Cell viability was assessed by MTT assay and recorded by optical density (O.D.). N27A dopaminergic cells were exposed for 24 hours to: **(A)** 2-5-10% FBS, Vehicle and increasing concentrations of 10-NO_2-OA (0.5 μM-10 μM). **(B)** Vehicle, Rotenone (50 nM) and Rotenone (50 nM) + 10-NO_2-OA (2.5 μM) in 2% FBS. All data represents the mean ± SD of at least three independent experiments with n=3.

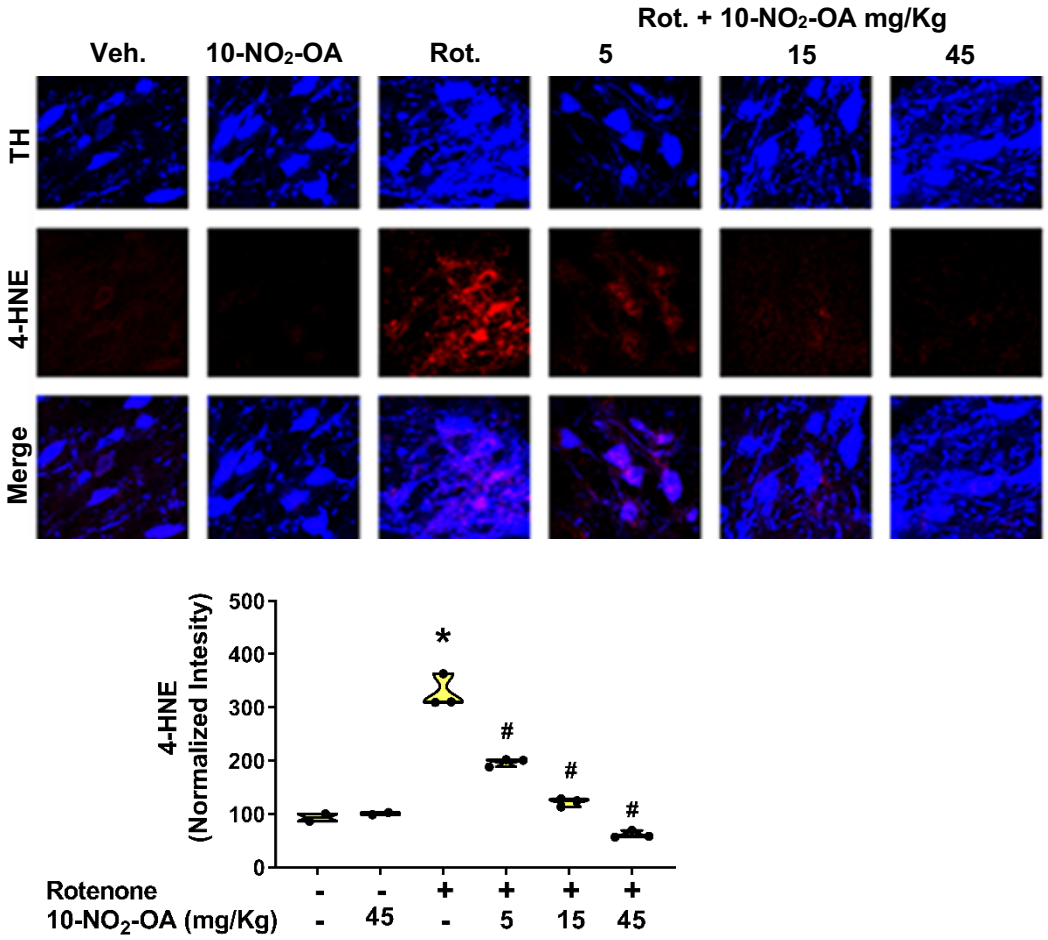


Supplemental Figure 2. 10-NO_2-OA prevents rotenone-induced NOX2 and LRRK2 activation and oxidative stress *in vitro*. N27A dopaminergic cells were exposed for 24 hours to Vehicle, Rotenone (50 nM) and Rotenone (50 nM) + 10-NO_2-OA (2.5 μM). **(A)** Rotenone elicited an increase in NOX2 activation as detected PL $p47^{phox}$:NOX2 (red). This was accompanied by an increase in cytoplasmic superoxide measured by DHE (green). Both NOX2 activation and DHE were prevented by co-treatment with 10-NO_2-OA. On the right, quantitative analysis of PLA-related fluorescence intensity. **(B)** Co-treatment with 10-NO_2-OA prevented rotenone mediated LRRK2 activation (PL pS1292:LRRK2, red). On the right, quantitative analysis of PLA-related fluorescence intensity. Each symbol represents average of normalized signal (with Vehicle set at 100) from a single independent experiment. Statistical testing by ANOVA with post hoc Bonferroni correction (*, $p<0.0001$ compared to Vehicle, #, $p<0.0001$ compared to Rotenone).

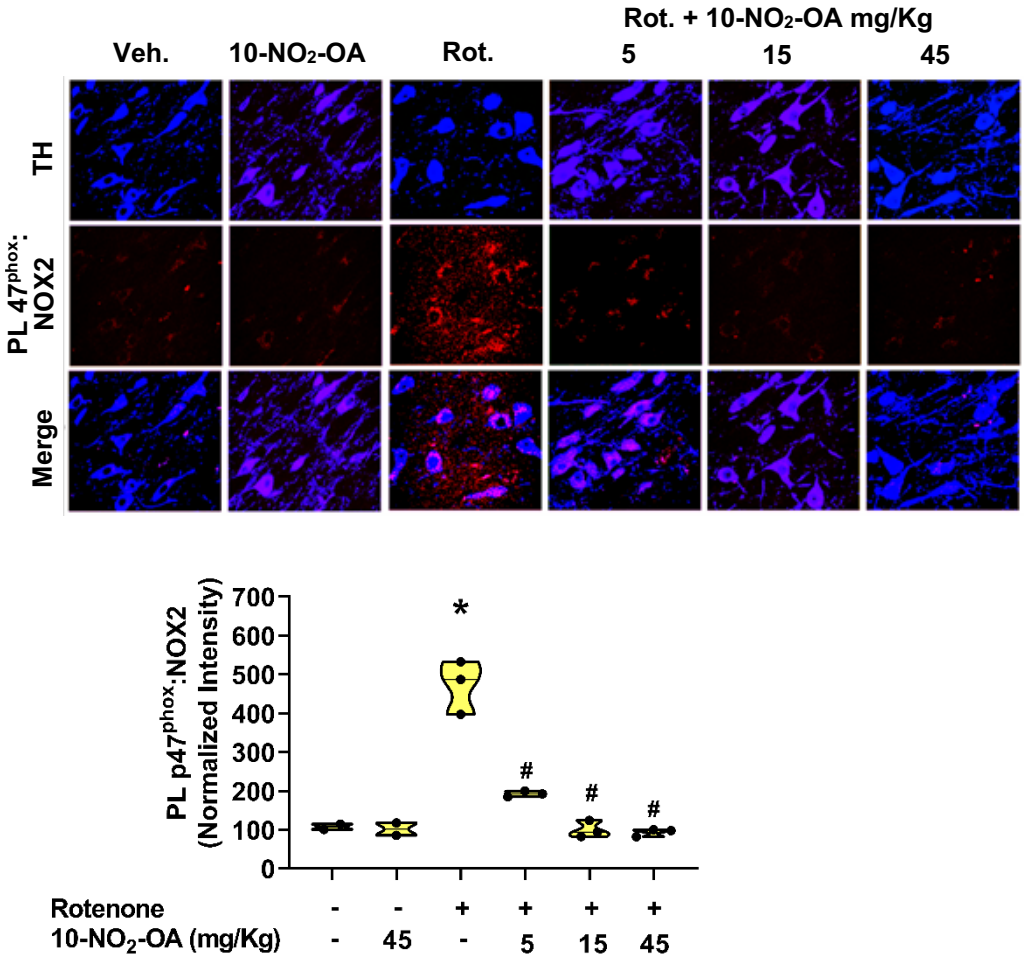


Supplemental Figure 3. 10-NO_2-OA activates Nrf2-dependent gene expression in rotenone treated N27-A dopaminergic cells. Relative mRNA levels of the following Nrf2 target genes: **(A)** heme oxygenase-1 (Hmox1, HO-1), **(B)** NAD(P)H dehydrogenase quinone-1 (Nqo-1), and **(C)** glutamate–cysteine ligase modifier subunit (Gclm), after treatment with 10-NO_2-OA (2.5 μM) and Rotenone (50 nM) for 2, 4, 8 and 24 hours in N27-A rat dopaminergic neural cell line. Values represent the range (minimum-maximum)

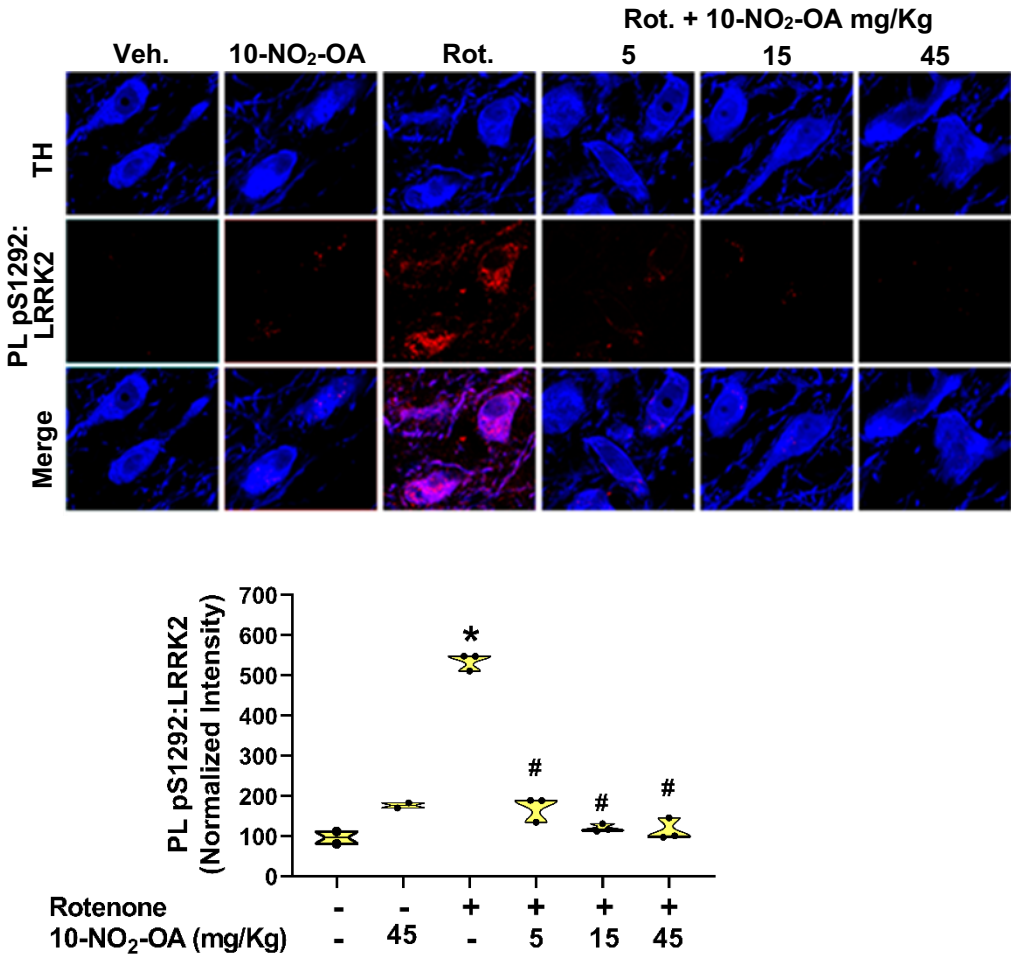
and mean of 4 independent experiments n=1 with statistical significance performed by two-way ANOVA with post hoc Bonferroni correction indicated by **** $p \leq 0.0001$, *** $p \leq 0.001$, and ** $p \leq 0.01$.



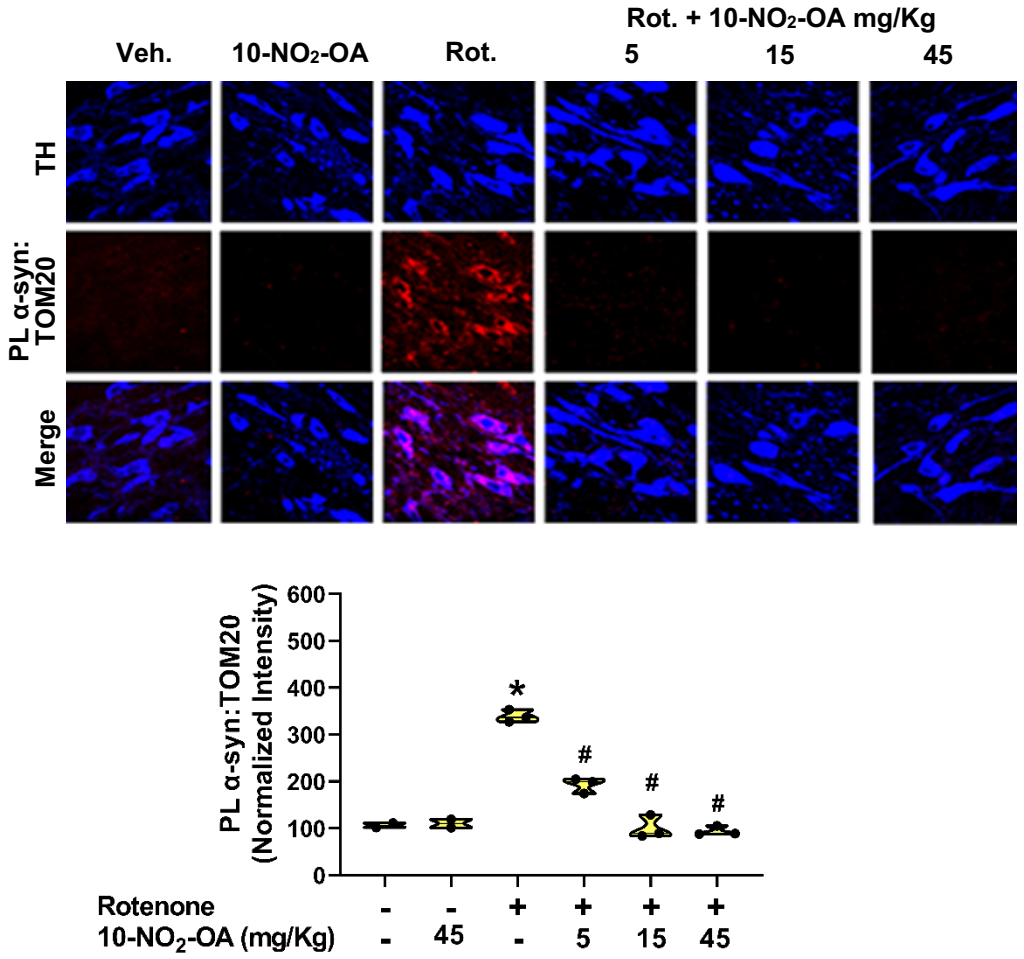
Supplemental Figure 4. 10-NO_2-OA inhibited lipid peroxidation (4-HNE) in the subacute PD model of rotenone. 4-HNE immunostaining (red) in dopaminergic neurons (TH, blue) in the SNpc of rats (cohort 1) with below correspondent quantifications of the fluorescence signal. Symbols represent the normalized means of the intensity (with Vehicle set at 100) from a single animal (6 slices /animal). Statistical analysis was performed by one-way ANOVA with post hoc Bonferroni correction (*, $p<0.0001$ compared to Vehicle, #, $p<0.0001$ compared to Rotenone).



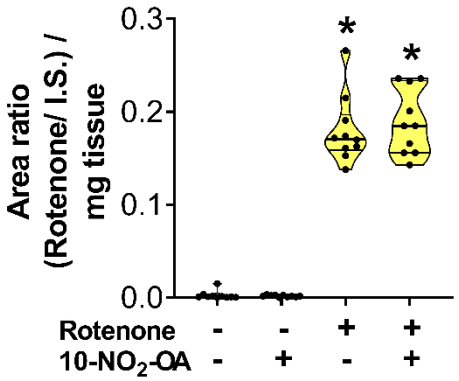
Supplemental Figure 5. 10-NO_2-OA inhibited NOX2 activation in SNpc of rat rotenone model. PL $p47^{phox}$:NOX2 (red) in dopaminergic neurons (TH, blue) in the SNpc of cohort 1 rats with below correspondent quantifications of the fluorescence signal. Symbols represent the normalized means of the intensity (with Vehicle set at 100) from a single animal (6 slices /animal). Statistical analysis was performed by one-way ANOVA with post hoc Bonferroni correction (*, $p<0.0001$ compared to Vehicle, #, $p<0.0001$ compared to Rotenone).



Supplemental Figure 6. 10-NO_2-OA prevented rotenone-induced LRRK2 activation in SNpc. PL pS1292:LRRK2 (red) in dopaminergic neurons (TH, blue) in the SNpc of cohort 1 animals with below correspondent quantifications of the fluorescence signal. Symbols represent the normalized means of the intensity (with Vehicle set at 100) from a single animal (6 slices /animal). Statistical analysis was performed by one-way ANOVA with post hoc Bonferroni correction (*, $p<0.0001$ compared to Vehicle, #, $p<0.0001$ compared to Rotenone).



Supplemental Figure 7. 10-NO_2-OA inhibits α-synuclein-mediated mitochondrial import impairment in the subacute PD model of rotenone. Confocal microscopy of PL α-synuclein:TOM20 (red) in dopaminergic neurons (TH, blue) in the SNpc of rats (cohort 1) with below correspondent quantification of the fluorescence signal. Symbols represent the normalized means of the intensity (with Vehicle set at 100) from a single animal (6 slices /animal). Statistical analysis was performed by one-way ANOVA with post hoc Bonferroni correction (*, $p<0.0001$ compared to Vehicle, #, $p<0.0001$ compared to Rotenone).



Supplemental Figure 8. Analysis of rotenone in whole rat brain (cohort 2). **(A)** HPLC-MS-MS analysis of rotenone levels. Statistical analysis was performed by one-way ANOVA with post hoc Bonferroni correction (*, $p<0.0001$ compared to Vehicle, and 10-NO_2-OA).