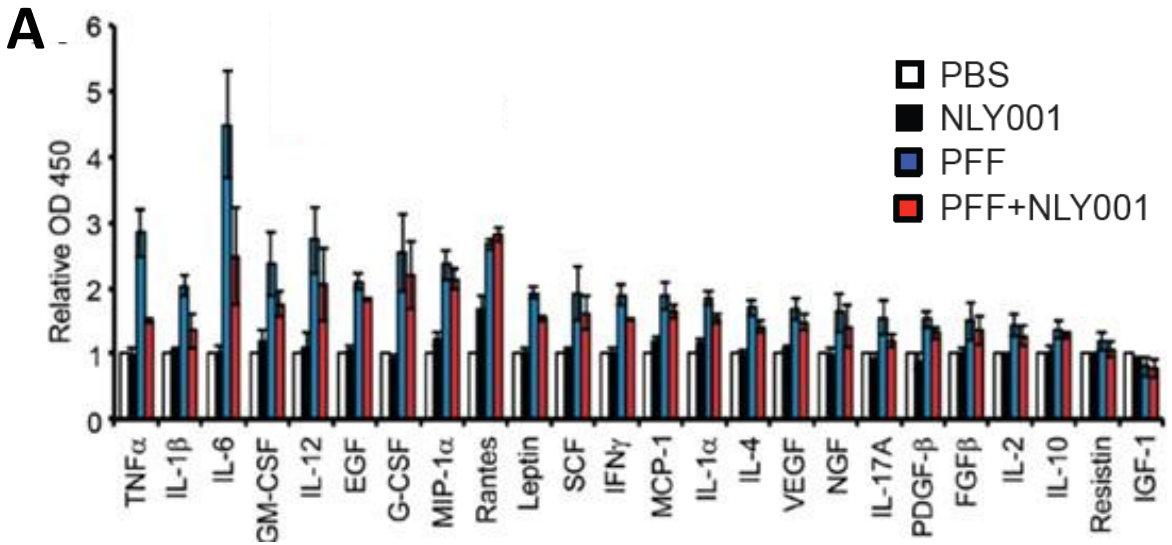
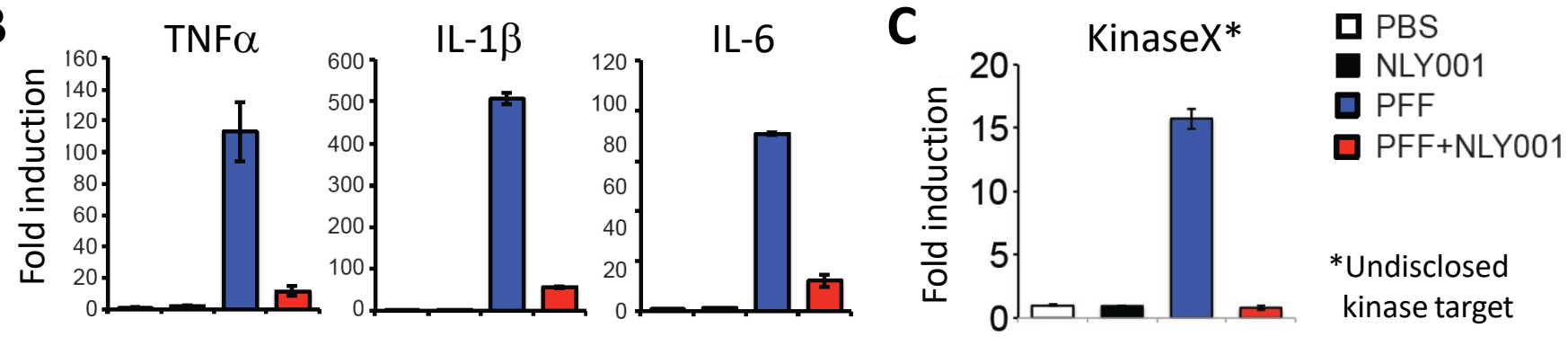
NLY01 Suppresses Cytokines Release from Activated Primary Cultured Microglia

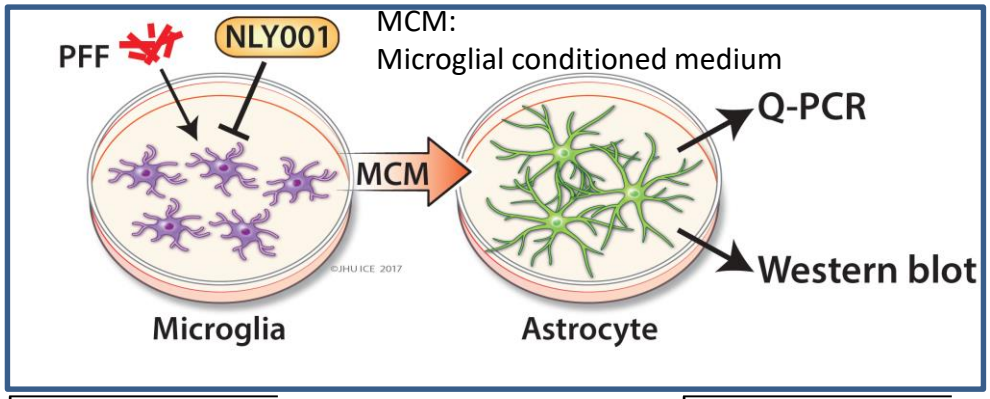
- **NLY01** strongly **suppresses α-syn PFFs-induced neurotoxic cytokines releases** (**TNF-α**, IL-1β, IL-6) and inhibits KinaseX* gene expression in primary microglia



(A) ELISA-based cytokine array for each cytokines.
(B and C) qRT-PCR for the gene expression. Primary microglia was pretreated with PBS or NLY01 (1 μM) for 30 min, and then further incubated with PFFs (1 μg/ml) for 18 h (A) and for 6 h (B and C), respectively.
Bars indicate mean ± s.e.m. (n=3).



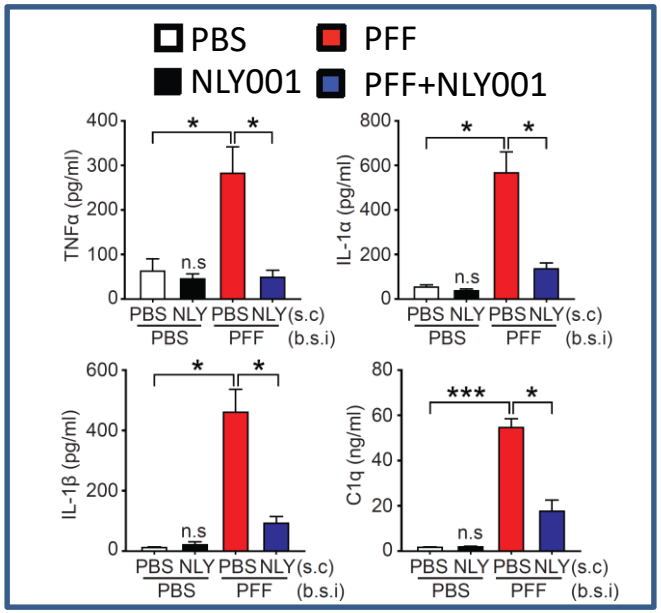
NLY01 Prevents A1 Reactive Astrocyte Formation by Blocking Microglial Activation



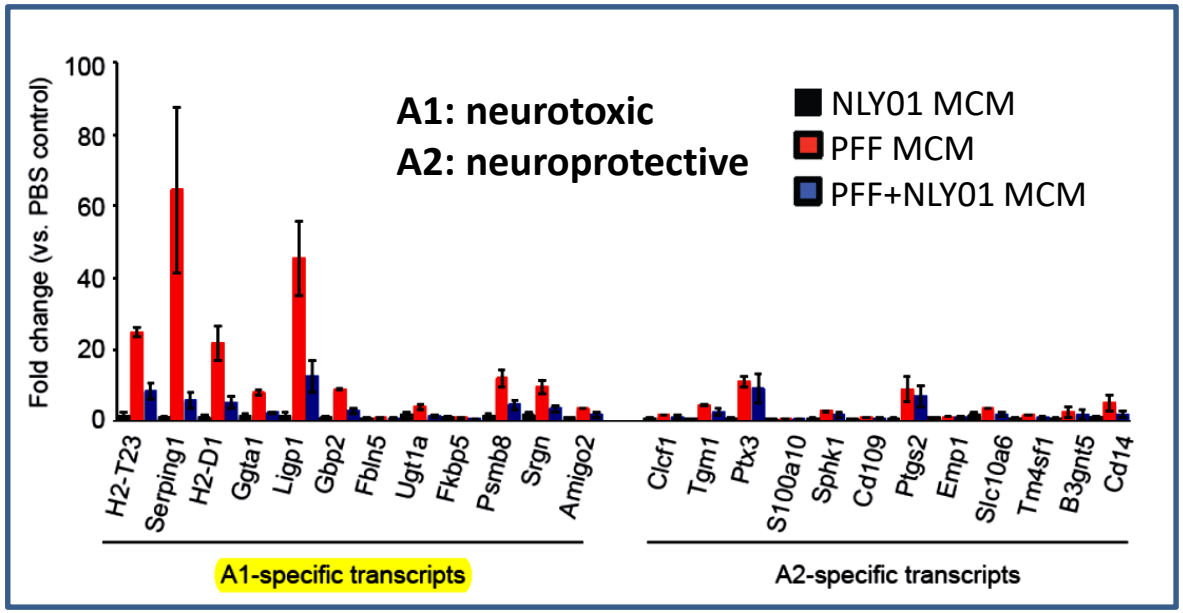
NLY01 inhibits C1q, IL-1α, TNFα (A1 astrocyte inducers) release from PFFs-activated microglia (*lower left*)

Inhibition of microglia activation prevents neurotoxic A1 astrocyte formation, but not anti-inflammatory A2, *in vitro* as evidenced by expression profile of A1/A2 specific markers *(lower right)*

ELISA assay

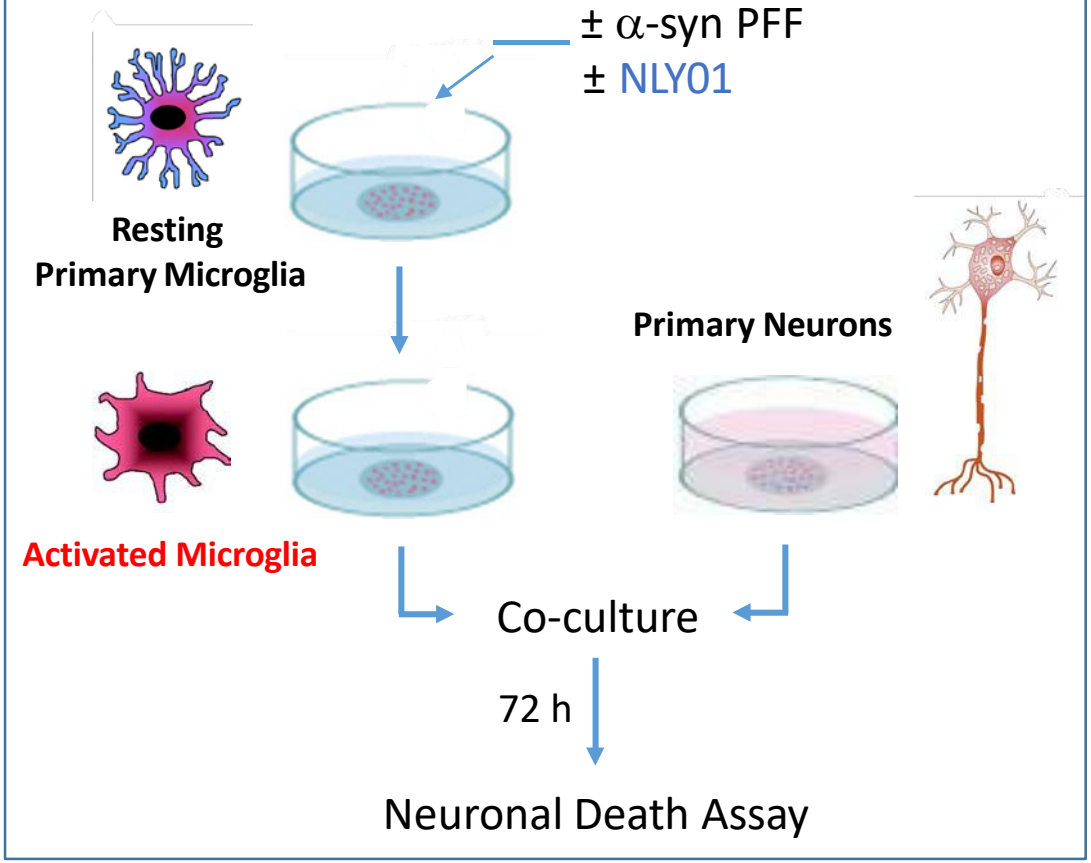


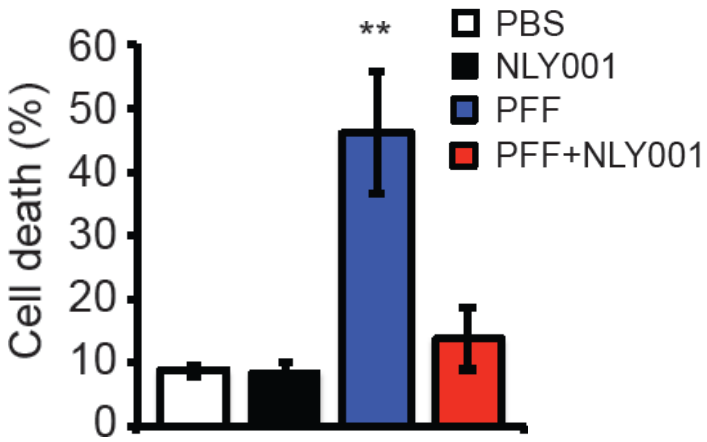
mRNA assay



NLY01 Protects Primary Neuronal Cells from α-synuclein PFFs-activated Microglia

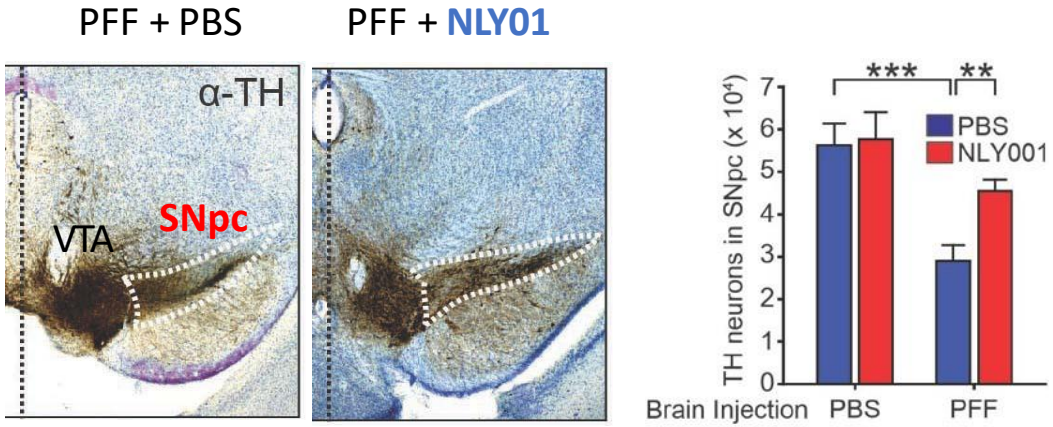
- **NLY01** strongly **protects primary neurons** against α-synuclein PFFs-activated microglia-mediated neuronal cell death.



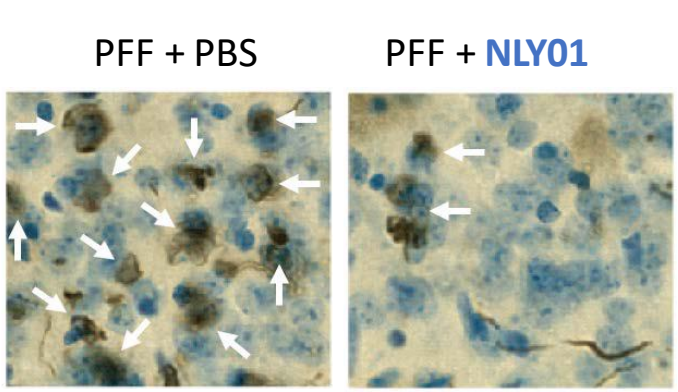


Primary microglia (mouse) was pretreated with or without NLY01 (1 μM) for 30 min, and then further incubated with PFFs (1 μg/ml) for 4 h. The activated microglia was co-cultured with primary cortical neurons (mouse) for 72 h and the neuronal cell death was measured by PI staining. Bars indicate mean ± s.e.m. (n=4-6).

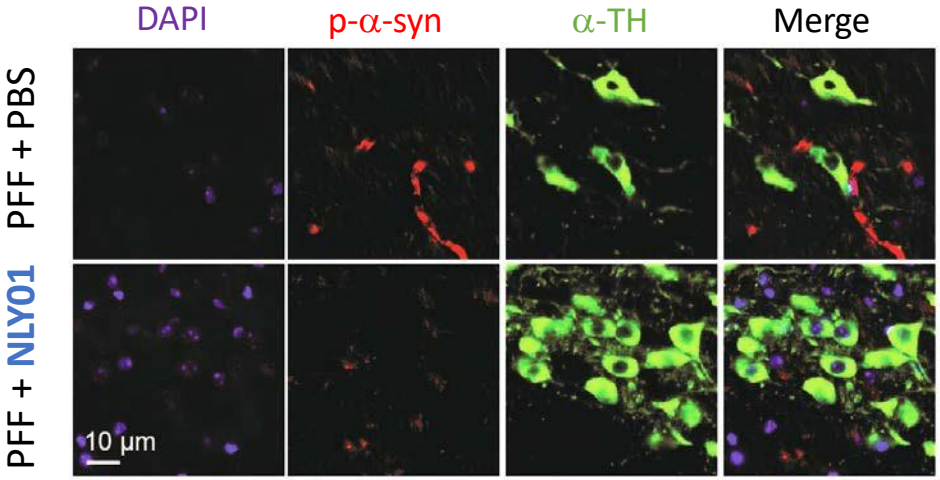
NLY01 Limits Loss of Dopaminergic Neurons and Ameliorates Lewy Body Pathology in α-Syn PFFs induced PD Mice



NLY01 rescues dopaminergic (DA) neurons in the SNpc (substantia nigra, pars compacta) in α-syn PFFs-induced PD mice. Left) Images of IHC for TH in the SNpc. TH (tyrosine hydroxylase, marker of DA neurons). Right) Stereological counting of the number of TH-positive neurons in the SNpc via unbiased stereological analysis (n=6 per each group). ± s.e.m. ***P*<0.01, ****P*<0.001



NLY01 significantly inhibits α-syn accumulation in the brain. Immunostaining with antibody selective for α-syn LB (Brown) in the brain tissues.

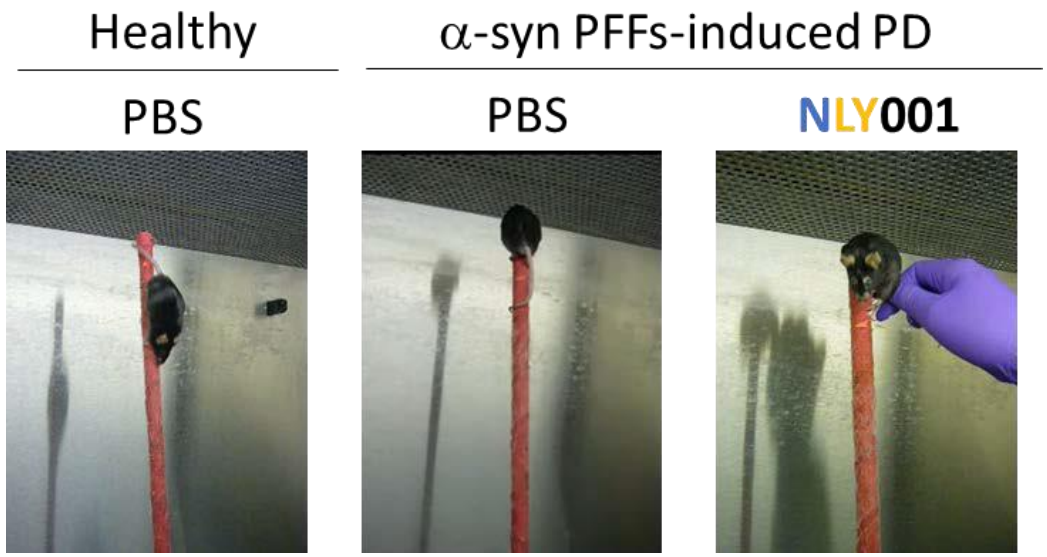


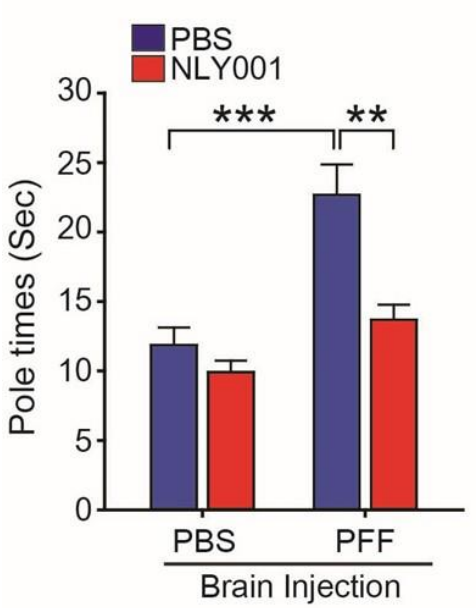
p-α-Syn positive Lewy body pathology induced by α-syn PFFs is reduced by treatment of NLY01 in vivo.

NEURALY

NLY01 Limits Motor Coordination Loss in α-Syn PFF-PD Mouse

- The **Pole Test** provides a very sensitive measure of motor coordination
- α-Syn PFF-induced **PD mice show deficits** in the challenging pole test
- **NLY01 treated PD mice show *no deficits* and *normal behavioral***

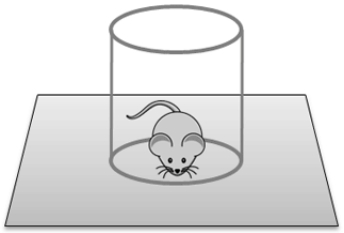


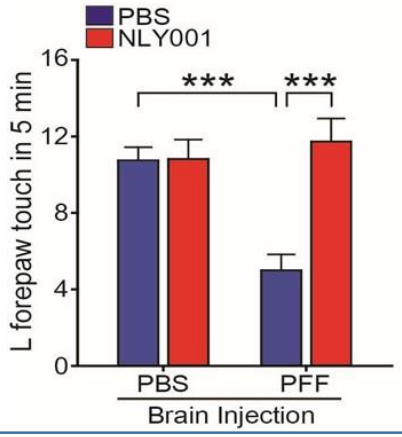


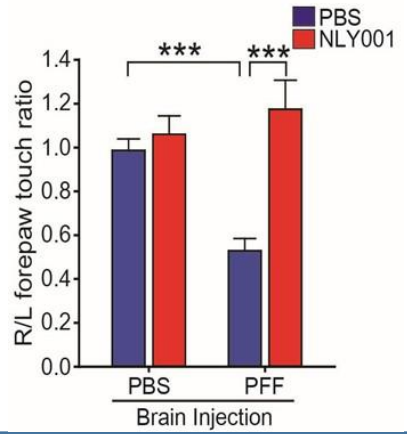
The pole test was conducted in vehicle or **NLY01** treated mice at 6 months after post α-syn PFFs injection. (n=10-12), ± s.e.m. ***P*<0.01, ****P*<0.001. α-Syn PFFs injection led to a significant increase in the time to reach the base of the pole whereas treatment of NLY01 reduced the α-syn PFFs-induced behavioral deficit.

NLY01 Limits Behavioral Deficits in α-Syn PFF-PD Mouse

- The **Cylinder Test** measures asymmetry in spontaneous forelimb use

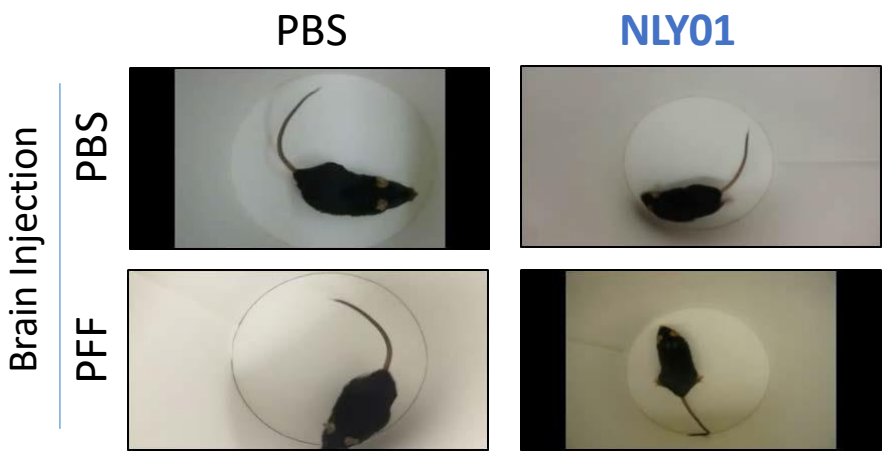


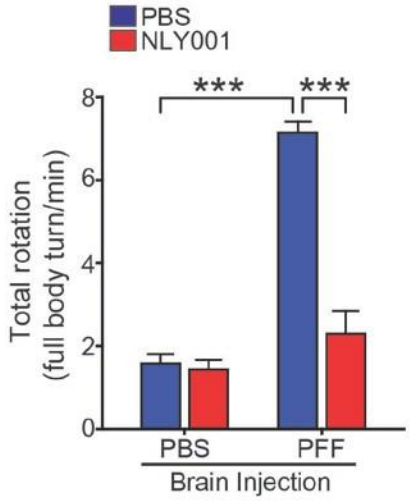




α-Syn PFF-induced PD mice show deficits in forelimb use in the cylinder task while **NLY01 treated PD mice alleviate the motor deficit with balanced use of both forepaws.** (n=10-12), ± s.e.m.***P*<0.01, ****P*<0.001

- The **Amphetamine Rotation Test**



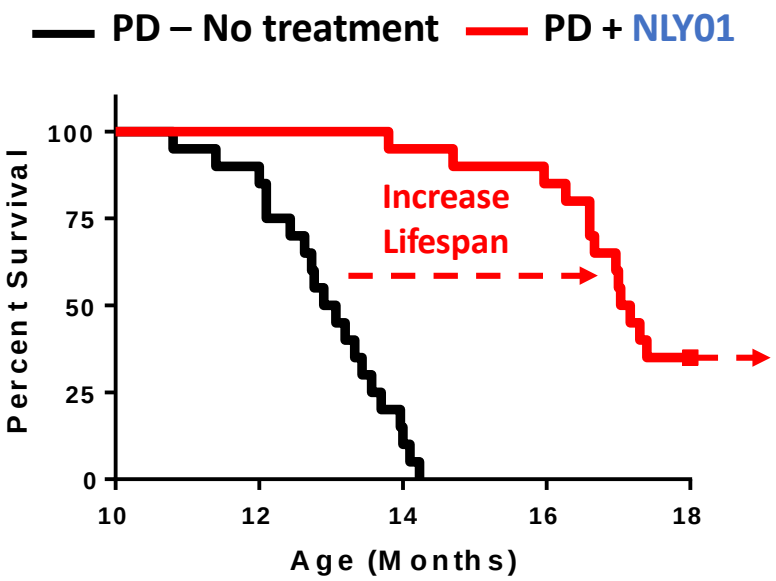


α-Syn PFF injection increased the amphetamine induced rotational behavior by 6-fold indicating loss of dopamine neurons. In contrast, **NLY01 prevents the amphetamine induced rotation indicating that the dopamine neurons are functional** (n=10-12), ± s.e.m. ***P*<0.01, ****P*<0.001

NLY01 effects in lethal constitutive α -synucleinopathy hA53T transgenic mouse model of PD

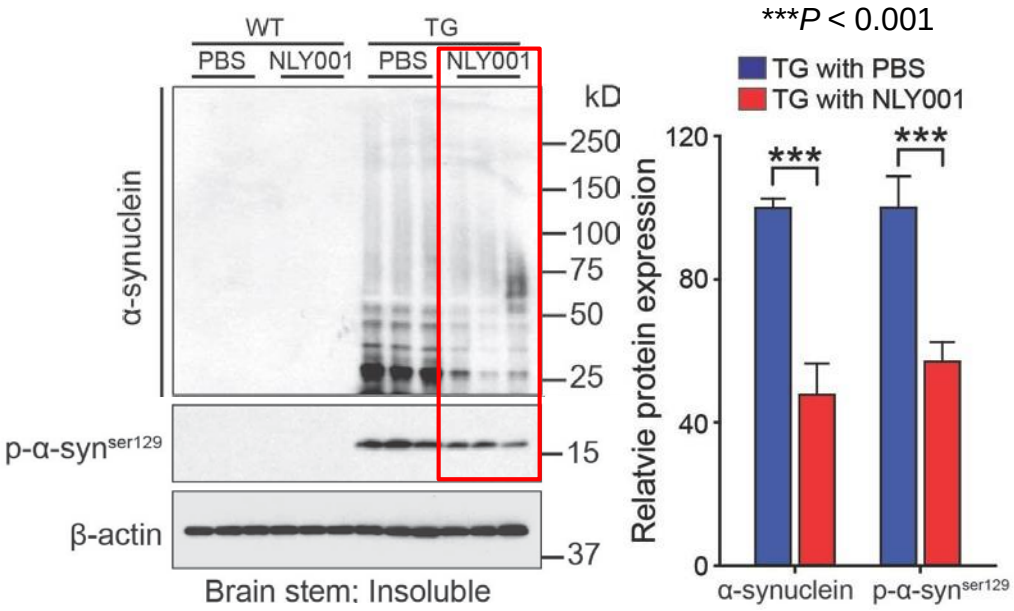
NLY01 clearly 1) increases lifespan and 2) inhibits α-synuclein aggregation and 3) ameliorates LB/LN pathology in A53T Tg PD mice

Survival Proportions (n=20)



Kaplan-Meier survival curve analysis for hA53Tα-syn Tg mice with PBS or NLY01 (treated at 6-month of age, S.C., 50nmol/kg, twice a week)
Median survival (months):
12.9 (A53T), 17.1 (A53T+NLY01)

NLY01 reduces α-synuclein aggregation



Representative immunoblots of α-syn, pS129 α-syn and β-actin in the detergent insoluble fraction of brain stem from 10-month-old hA53Tα-syn Tg mice and age-matched littermate controls with PBS or NLY01.