

Blood-Based Biomarker Development For The Early Diagnosis Of Parkinson's Disease

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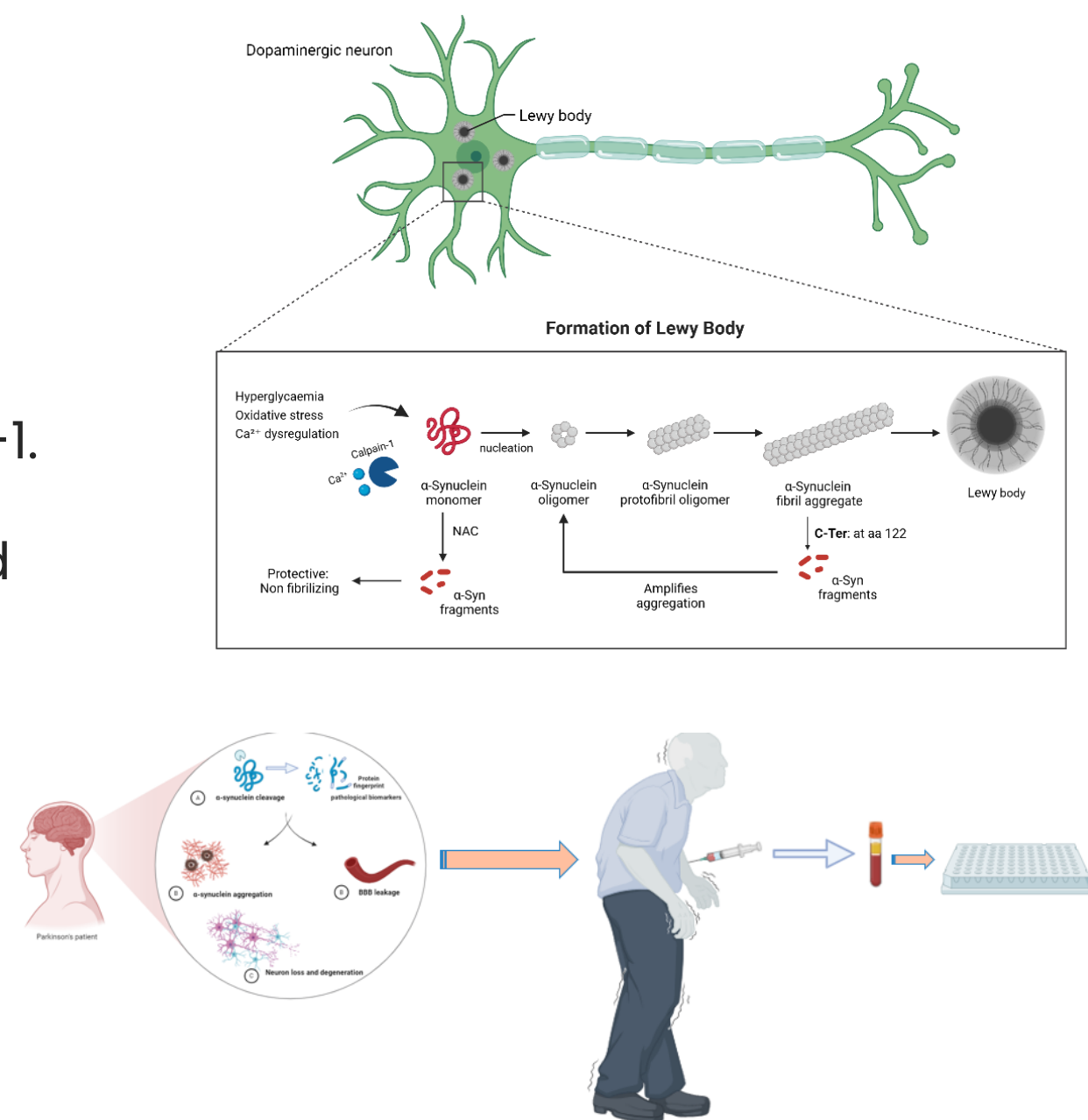
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BACKGROUND

- Roughly 1 in 100 people over 60 years old have PD.
- There isn't a cure, only symptom management.
- PD hallmark: α -synuclein aggregation.
- Early stages: α -synuclein can be cleaved by Calpain-1.
- Biomarkers and advanced diagnostic tools are needed

Hypothesis & Aim

- Developing a sensitive immunoassay that detects α -synuclein fragments for early diagnosis of PD.



METHODS

- The antibody was generated to specifically target α -synuclein fragments cleaved by calpain-1.
- A competitive ELISA was developed to analyze plasma heparin samples from clinical PD cohorts.

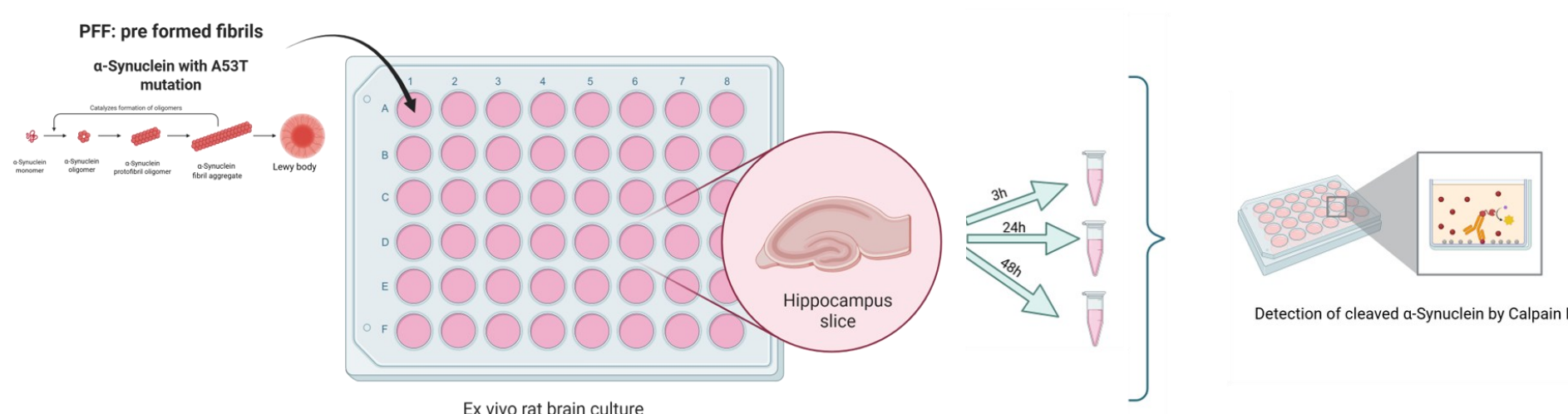
Demographic table for α -SYN-C (NB498) measurement

	Healthy donors (n=40)	Alzheimer's Disease (n=30)	Parkinson's Disease (n=33)	p value
Age, mean (SD)	69 (11.8)	72.3 (10.4)	71.6 (11.3)	0.27
Sex, Male, n (%)	17 (42.5%)	12 (40%)	19 (57.7%)	0.3
Months since diagnosis, mean (SD)	NA	42.6 (17.4)	34 (21.9)	0.15
Stage	NA	Mild, n (%) 17 (56%) Moderate, n (%) 9 (30%) Severe, n (%) 4 (13)	Stage I: 14 (42%) Stage II: 16 (48%) Stage III: 1 (3%) Stage IV: 2 (6%)	
Under treatment n/total (%)	NA	17/19 (89.5%)	6/24 (25%)	

Abbreviation: NA: not applicable. Categorical variables are presented as number (percentage), while continuous variables are reported as mean (standard deviation).

Statistical differences between clinical and patient demographics were assessed using Welch's t-test and Kruskal-Wallis test.

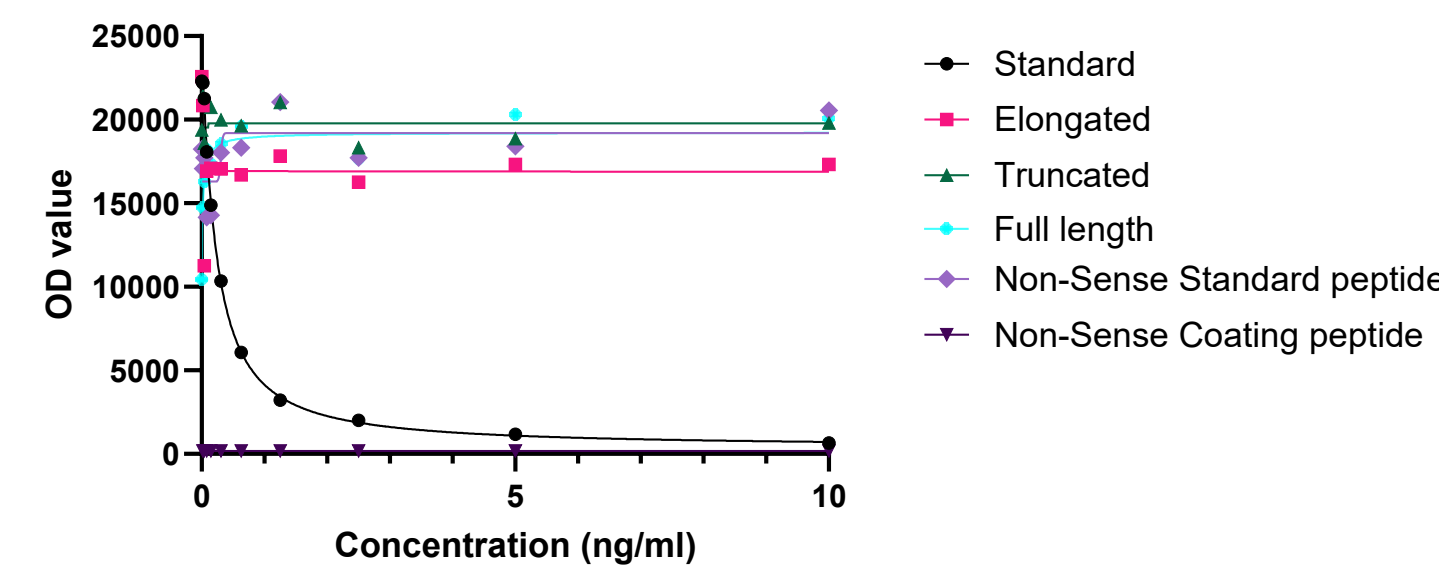
- Rat hippocampal slices were exposed to PFF to mimic Parkinson's disease pathology. The expression of α -synuclein fragments generated by calpain-1 cleavage were then assessed to further validate the immunoassay.



RESULTS

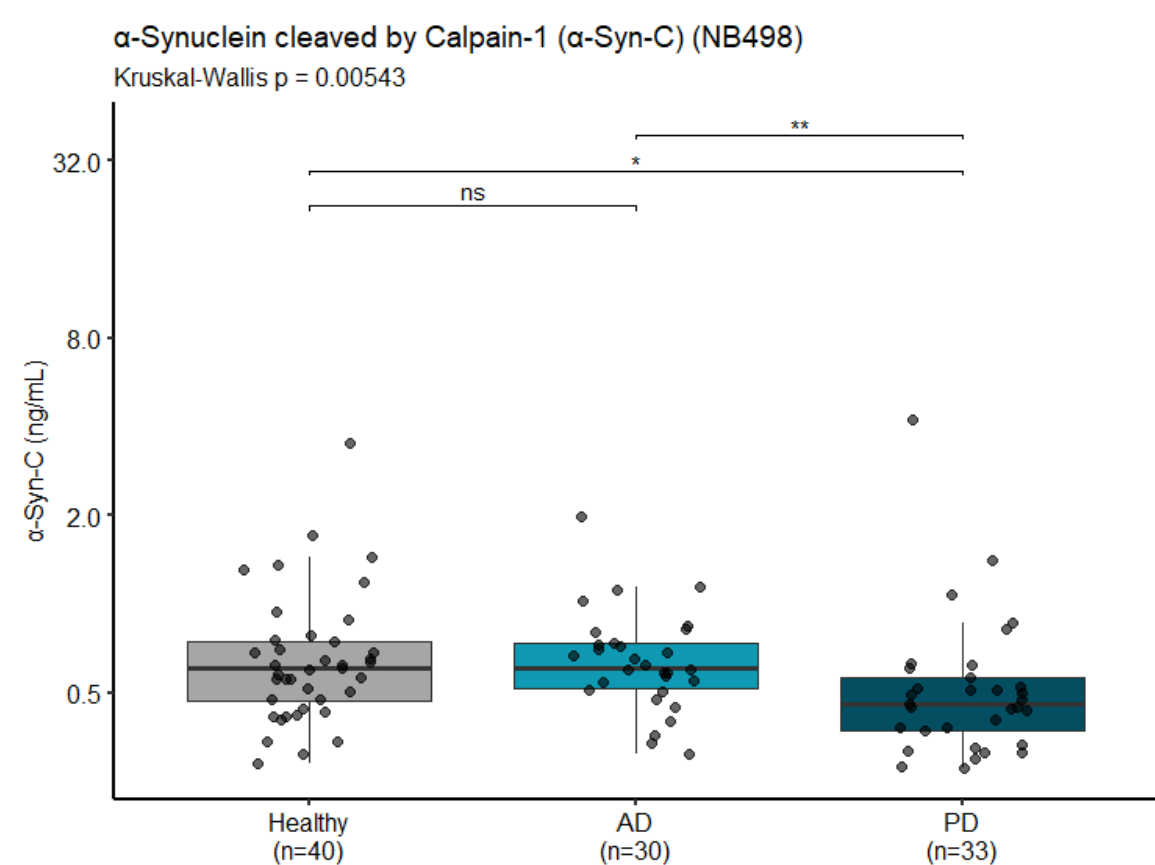
Antibody specificity & immunoassay evaluation

α -Synuclein cleaved by Calpain-1 (α -SYN-C) NB498

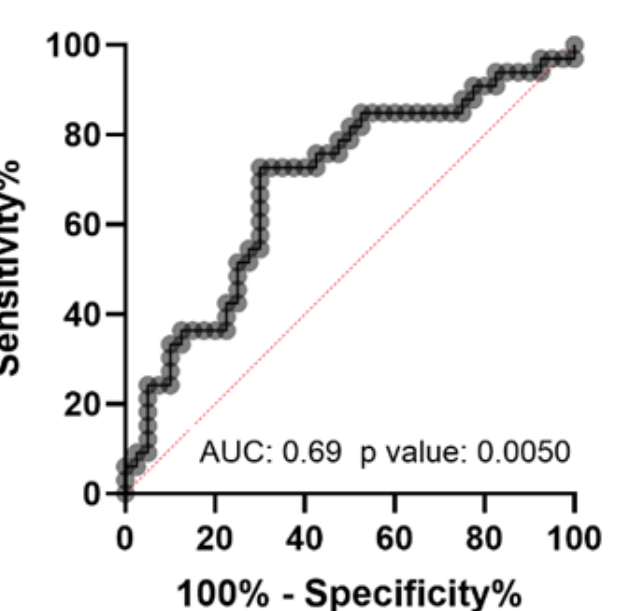


TECHNICAL VALIDATION	
Species possibilities	Human plasma heparin Human Serum Ex vivo Rat brain in DMEM/F-12 media
Standard A	10 ng/mL
Control 1 ($\pm 10\%$)	0.2 ng/mL
Control 2 ($\pm 10\%$)	0.86 ng/mL
Intended matrix (MRD)	1+1
Intra-assay CV%	4.39 % (2.58 – 6.01)
Inter-assay CV%	11.52 % (9.29 – 13.94)
LOB	0.09 ng/mL
Upper Limit of Quantification (ULOQ)	20 ng/mL
Mean slope ($\pm 20\%$)	-1.05 (-1.03) – (-1.09)
Mean IC50 ($\pm 20\%$)	0.22 ng/mL (0.201 – 0.238 ng/mL)
Curve fit model	4-point logistic curve fit

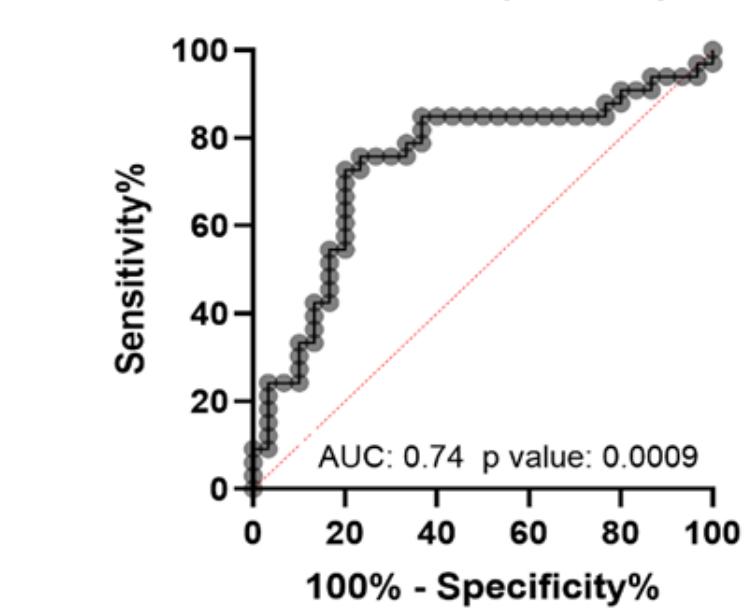
Plasma levels of α -SYN-C are significantly decreased in Parkinson's disease



Healthy vs Parkinson's Disease α -SYN-C (NB498)

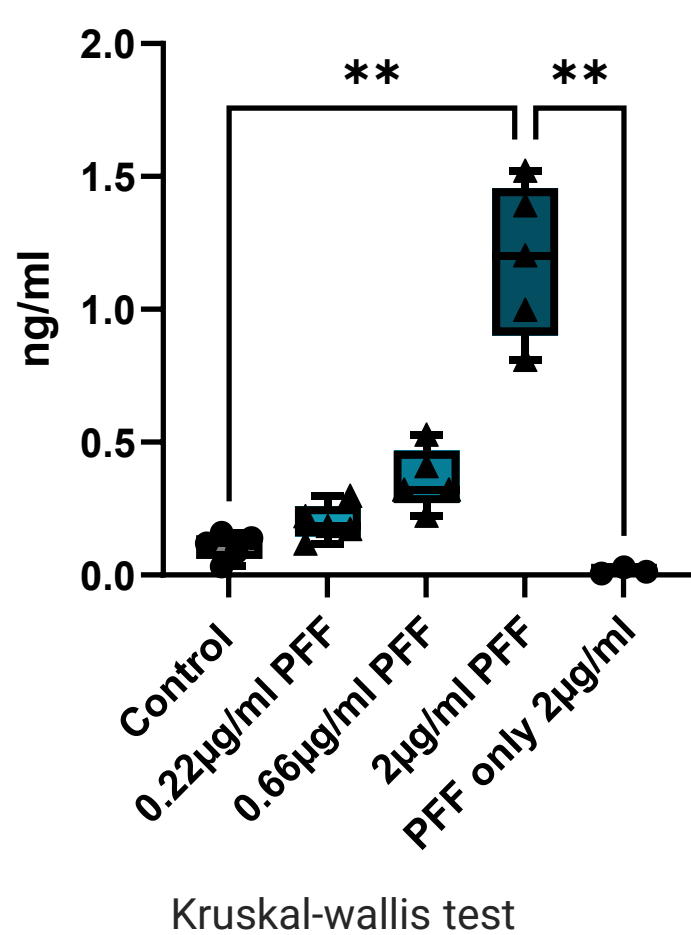


Alzheimer's Disease vs Parkinson's Disease α -SYN-C (NB498)

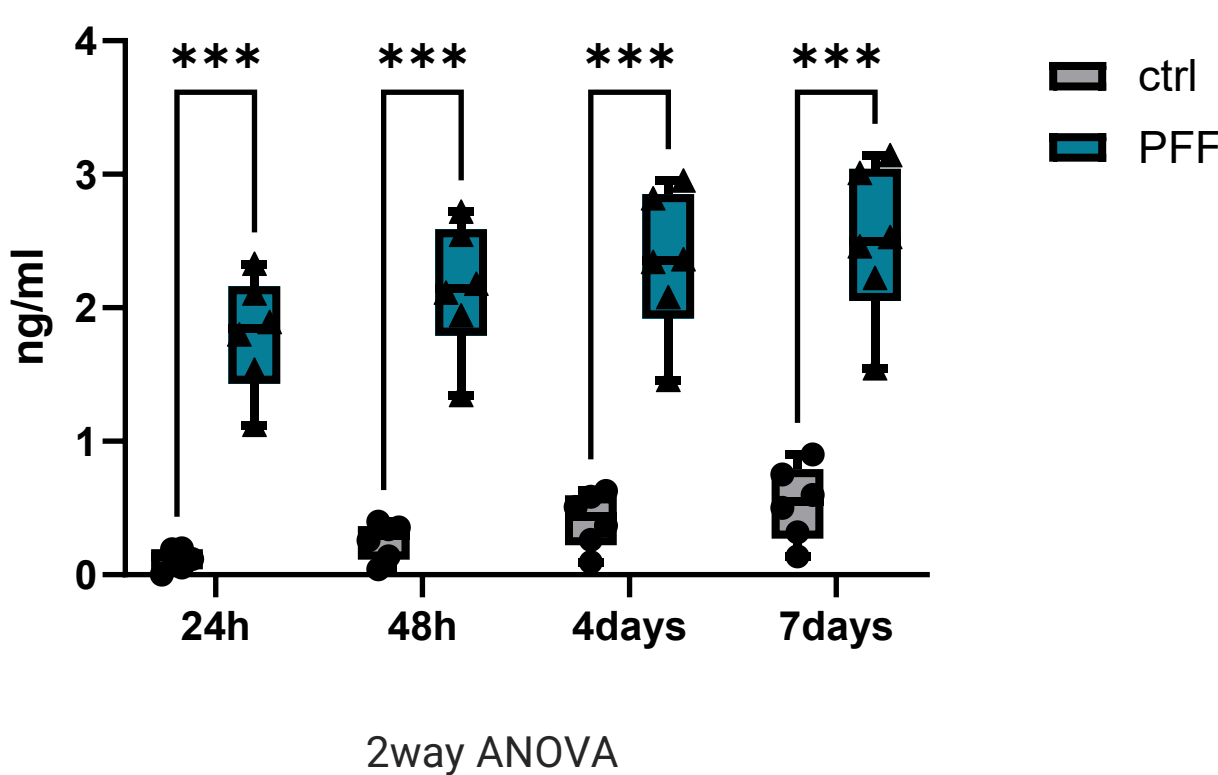


α -SYN-C are elevated in hippocampal organotypic slices of Parkinson's disease model

α -Synuclein cleaved by Calpain1 in rat hippocampus (NB498)



α -Synuclein cleaved by Calpain1 in rat hippocampus (NB498)



* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$



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CONCLUSION

- α -Synuclein fragments cleaved by calpain I represent key early drivers of PD pathology.
- This blood-based biomarker holds promise for early diagnosis and identification of treatment responders.