Supplementary Information

Article title: The chaperone Clusterin participates in α-Synuclein neuropathology in Parkinson's disease brain

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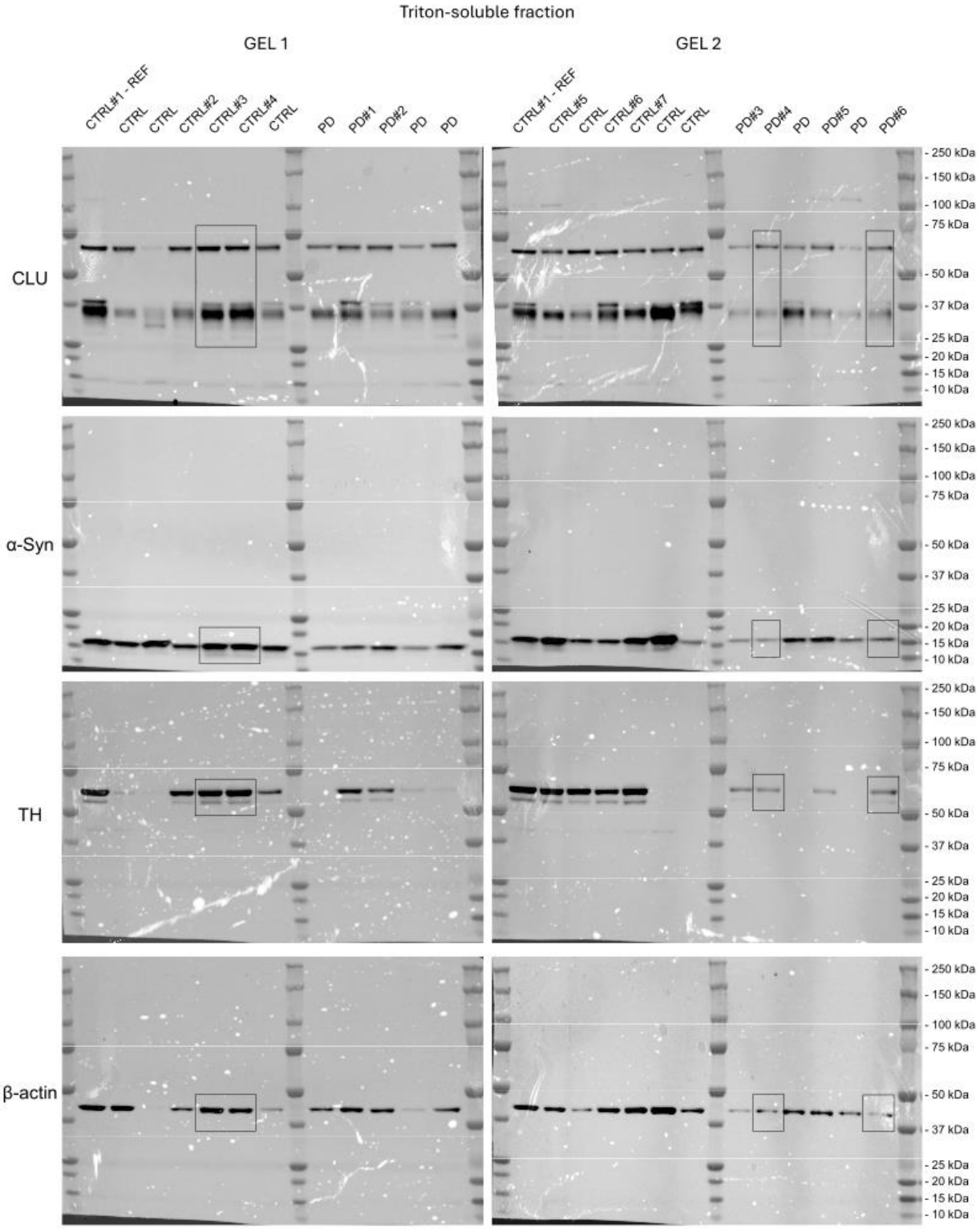
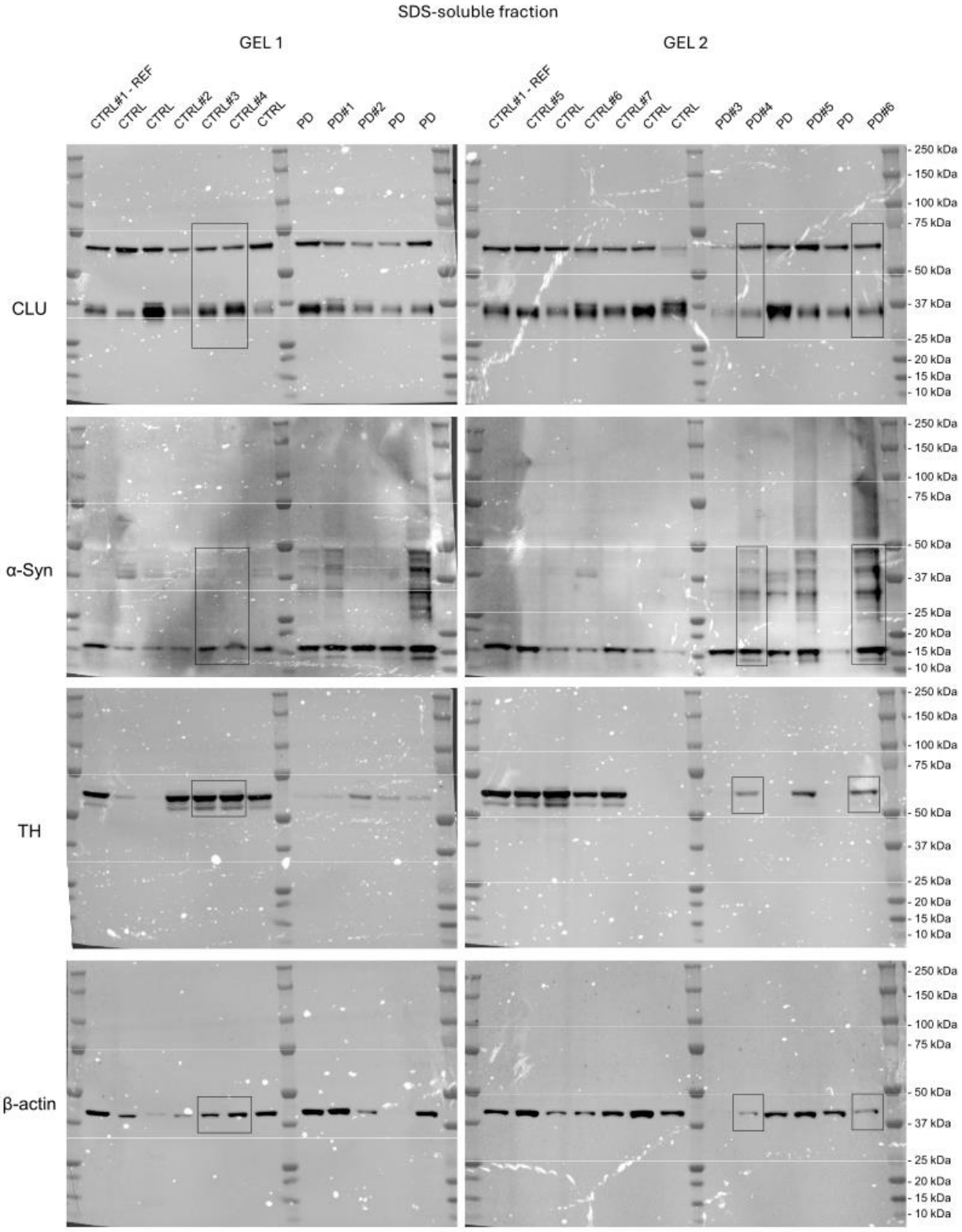
Western blot processing

To accommodate all samples, protein lysates were loaded onto two independent SDS–PAGE gels that were electrophoresed, transferred, and processed in parallel under identical experimental conditions. A common reference sample (REF), indicated in each blot, was included on both gels to allow comparison between membranes. Full-length, uncropped blots, including molecular weight markers, are provided below. Black boxes indicate the representative cropped immunoblots shown in Fig. 1 and Fig. 5.

For each membrane, target proteins were detected sequentially by repeated stripping and re-probing with the indicated primary antibodies. Accordingly, the immunoblots shown for the different target proteins were generated from the same membrane following sequential immunodetection.

More samples were initially loaded than those included in the final study cohort. Samples retained for quantitative analyses are identified by the corresponding numbers reported in Supplementary Table 1. Samples excluded from the analysis showed absent or markedly reduced β-actin and tyrosine hydroxylase (TH) immunoreactivity, precluding reliable normalization and indicating compromised sample integrity, possibly due to protein degradation.


Triton-soluble fraction
GEL 1
GEL 2
CTRL#1 - REF
CTRL
CTRL
CTRL#2
CTRL#3
CTRL#4
CTRL
PD
PD#1
PD#2
PD
PD
CTRL#1 - REF
CTRL#5
CTRL
CTRL#6
CTRL#7
CTRL
CTRL
PD#3
PD#4
PD
PD#5
PD
PD#6
CLU
α-Syn
TH
β-actin
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa


SDS-soluble fraction
GEL 1
GEL 2
CTRL#1 - REF
CTRL
CTRL
CTRL#2
CTRL#3
CTRL#4
CTRL
PD
PD#1
PD#2
PD
PD
CTRL#1 - REF
CTRL#5
CTRL
CTRL#6
CTRL#7
CTRL
CTRL
PD#3
PD#4
PD
PD#5
PD
PD#6
CLU
α-Syn
TH
β-actin
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
10 kDa

Supplementary Table 1 Demographic and clinical characteristics of human brain samples from the Cambridge Brain Bank

Group	Gender	Age at death (years)	AD pathology	LB pathology	PMI (hours)
CTRL#1	F	72	/	/	64.3
CTRL#4	F	69	/	/	54
CTRL#5	M	69	/	/	55
CTRL#6	F	85	/	/	24
CTRL#8	F	61	/	/	71
CTRL#10	M	76	/	/	32.3
CTRL#11	M	76	/	/	41.3
PD#2	M	77	Early AD pathology	Brainstem LBs	7
PD#3	F	79	Severe AD pathology	Sever LB pathology	n.a.
PD#6	F	94	Early AD pathology	Yes	n.a.
PD#7	M	84	Early AD pathology	Yes	n.a.
PD#9	F	85	No	Diffuse LBs	13.3
PD#11	F	73	No	Diffuse LBs	22.3

Supplementary Table 1. AD, Alzheimer's disease; LBs, Lewy bodies; PMI, *post-mortem* Interval; n.a., not assessed**.**

Supplementary Table 2 Demographic and clinical characteristics of human brain samples from the Nervous Tissues Bank in Milan

Group	Gender	Age at death (years)	Disease duration (years)	Hoehn-Yahr stage	Braak stage	Dementia
CTRL#1	F	82	/	/	/	/
CTRL#2	F	93	/	/	/	/
CTRL#3	M	71	/	/	/	/
CTRL#4	M	79	/	/	/	/
CTRL#5	F	91	/	/	/	/
CTRL#6	F	64	/	/	/	/
PD#1	M	87	28	3	6	No
PD#2	F	84	19	n.a.	5-6	n.a.
PD#3	M	75	16	3	6	No
PD#4	M	80	18	4	6	No
PD#5	F	79	20	4	6	No *
PD#6	F	91	38	5	6	Yes
PD#7	F	79	24	4	6	No
PD#8	M	88	28	n.a.	n.a.	n.a.

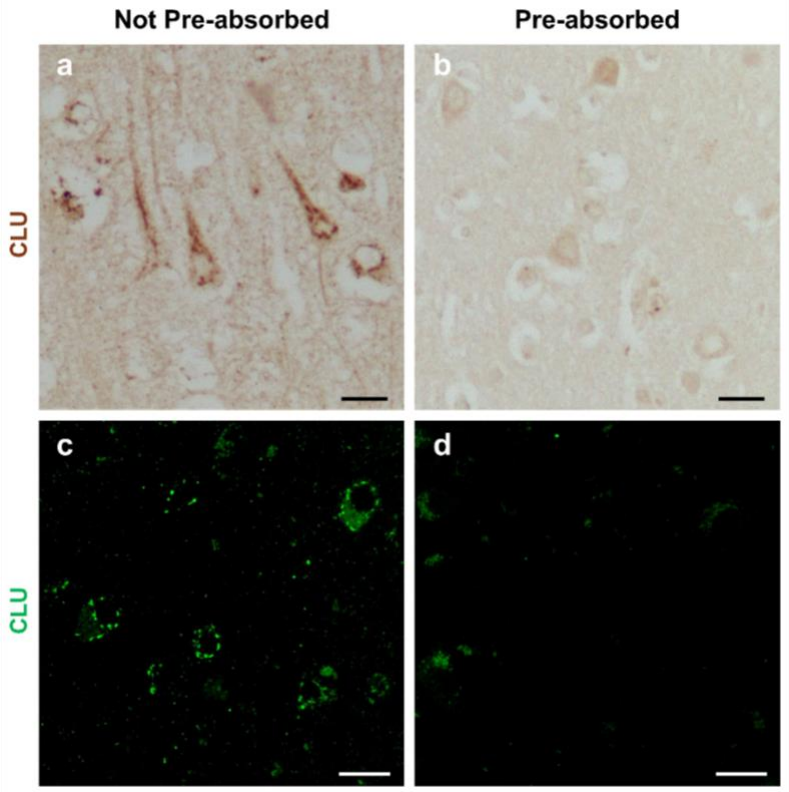
Supplementary Table 2. n.a., not assessed; * Amyloid-β pathology detected.

Table 3 Primary and secondary antibodies used in immunofluorescence

Primary antibodies			
Antigen	**Supplier – Catalog number**	**Host species**	**Dilution factor**
Clusterin	R&D Systems – AF2937	Goat	1:125
Tyrosine hydroxylase	Millipore – 3956930	Rabbit	1:100
Tyrosine hydroxylase	Origene – OT12D4	Mouse	1:50
α-Synuclein	Sigma-Aldrich – S3062	Rabbit	1:2000
S100β	Synaptic Systems – 287006	Chicken	1:300
Iba1	GeneTex – GTX100042	Rabbit	1:500
Iba1	Invitrogen – GT10312	Mouse	1:100
LAMP1	Sigma-Aldrich – L1418	Rabbit	1:100
Acetylated α-tubulin	Sigma-Aldrich – T6793	Mouse	1:300
Secondary antibodies			
Alexa Fluor 488 anti-goat	Jackson ImmunoResearch – 705-545-147	Donkey	1:300
Alexa Fluor 555 anti-rabbit	Abcam – ab150070	Donkey	1:200
Alexa Fluor 647 anti-rabbit	Invitrogen – A32795	Donkey	1:200
CY3 anti-chicken	Jackson ImmunoResearch – 703-165-155	Donkey	1:300
Rhodamine Red X anti-mouse	Jackson ImmunoResearch – 715-295-151	Donkey	1:200
Alexa Fluor 647 anti-mouse	Invitrogen – A32787	Donkey	1:200

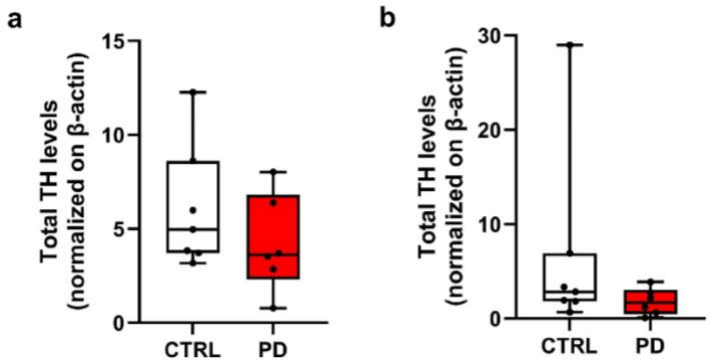
Table 3. List of primary and secondary antibodies used in this study, with product specification and application notes.

Supplementary Fig. 1



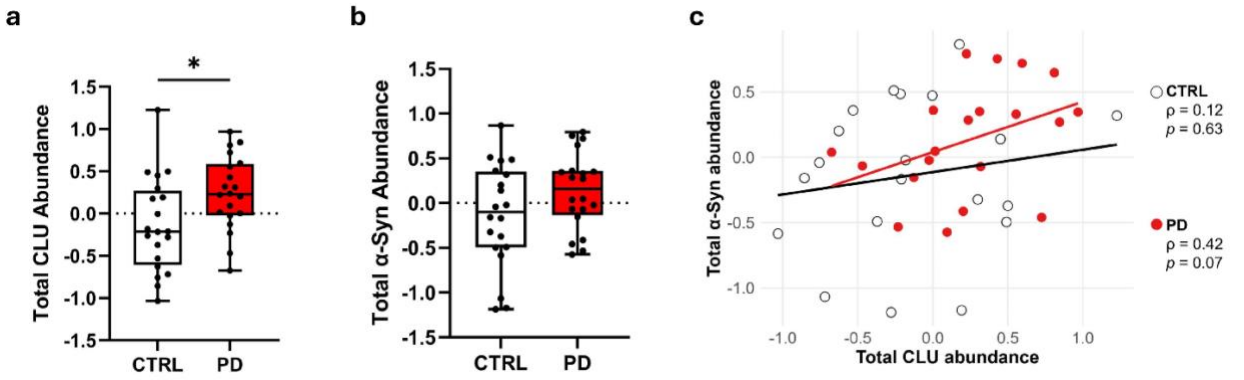
Supplementary Fig. 1 Pre-absorption of Clusterin antibody. a, **b** Representative immunoenzymatic images obtained from *post-mortem* human brains treated with Clusterin (CLU) primary antibody (**a**) and CLU primary antibody pre-incubated with its epitope (recombinant human CLU; **b**). **c**, **d** Representative immunofluorescence images obtained from *post-mortem* human brains treated with CLU primary antibody (**c**) and CLU primary antibody pre-incubated with its epitope (recombinant human CLU; **d**). Scale bar = 25 µm

Supplementary Fig. 2



Supplementary Fig. 2 Tyrosine hydroxylase protein distribution in the *substantia nigra*. a Quantification of tyrosine hydroxylase (TH) protein levels normalized on β-actin in Triton-soluble fraction obtained from controls (CTRL) and Parkinson's disease patients (PD). **b** Quantification of TH protein levels normalized on β-actin in SDS-soluble fraction obtained from CTRL and PD. Box plots represent median, interquartile range and minimum-maximum (CTRL: $n = 7$; PD: $n = 6$). Data were analyzed using the Mann-Whitney test

Supplementary Fig. 3



Supplementary Fig. 3 Proteomics analysis of Clusterin and α-Synuclein in the *substantia nigra*. **a** Quantification of Clusterin (CLU) abundance in controls (CTRL) and Parkinson's disease patients (PD). **b** Quantification of α-Synuclein (α-Syn) abundance in CTRL and PD. Box plots represent median, interquartile range and minimum-maximum (CTRL: $n = 20$; PD: $n = 20$). Data were analyzed using the Mann-Whitney test. * $P < 0.05$. **c** Correlation analysis between CLU and α-Syn abundance in CTRL (white dots) and PD (red dots). Spearman correlation coefficients (ρ) and *P*-values are reported for each group. (CTRL: $n = 20$; PD: $n = 20$)