

Supplementary materials

Wide distribution of alpha-synuclein oligomers in multiple system atrophy brain detected by proximity ligation

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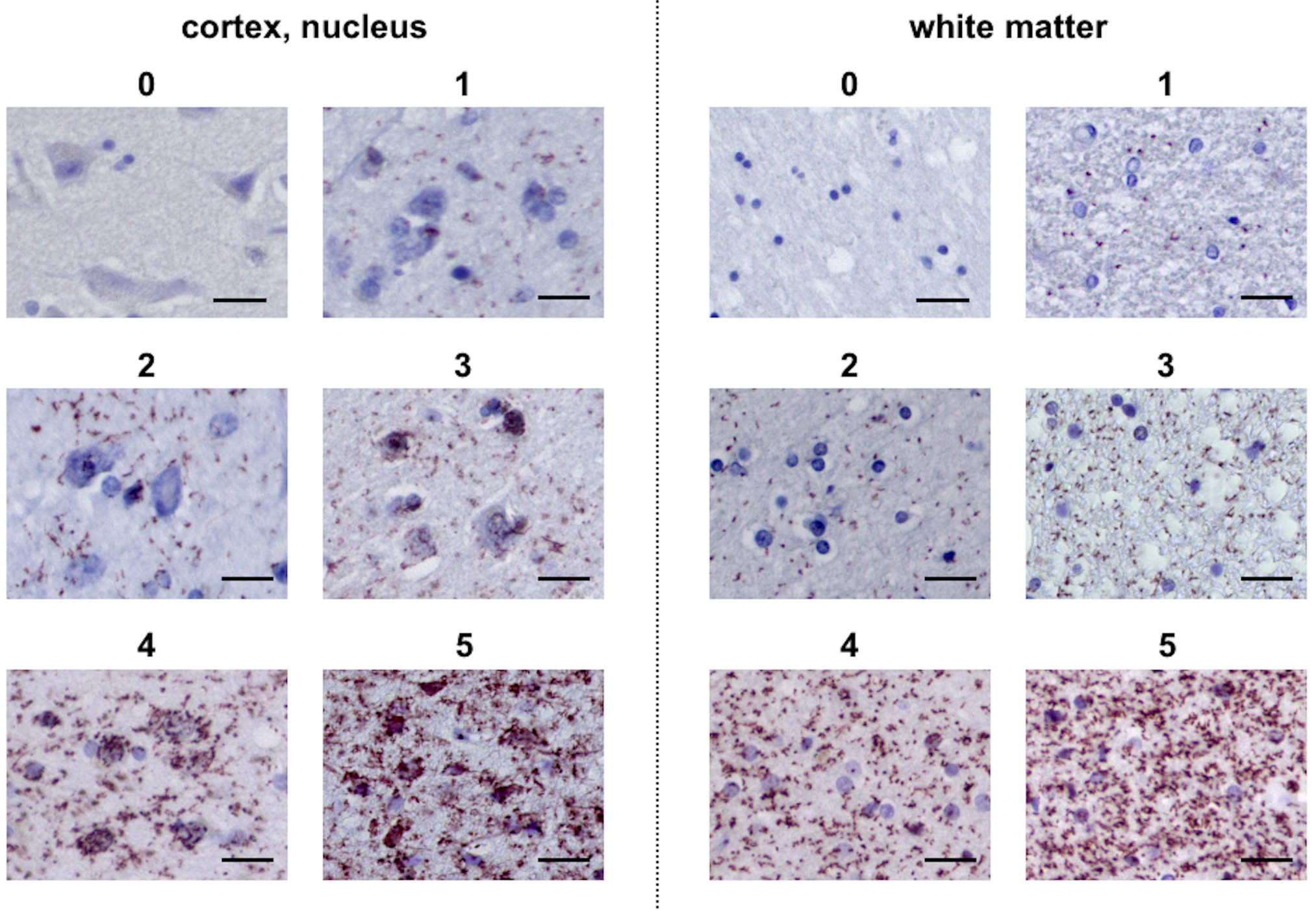
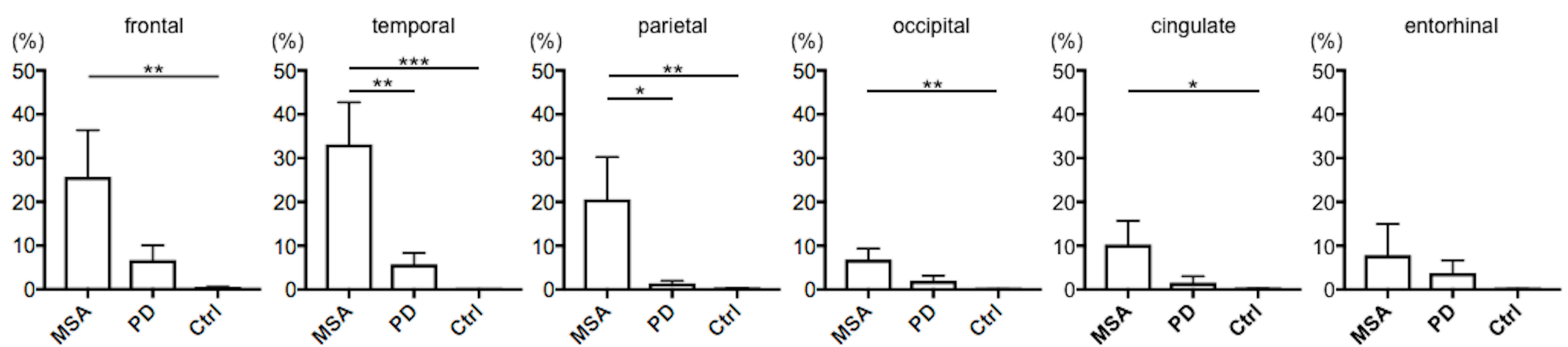


Fig. S1 Pre-made AS-PLA scoring plate

AS-PLA severity in neuropils was scored in representative images at 20 magnification on a scale from 0 (no signal) to 5 (highest signal) by using pre-made scoring plates. Scores of cortices and nuclei were assessed in accordance with left plates, while scores of white matter were assessed in accordance with right plates. *Scale bars* 20 μm.

cortex



white matter

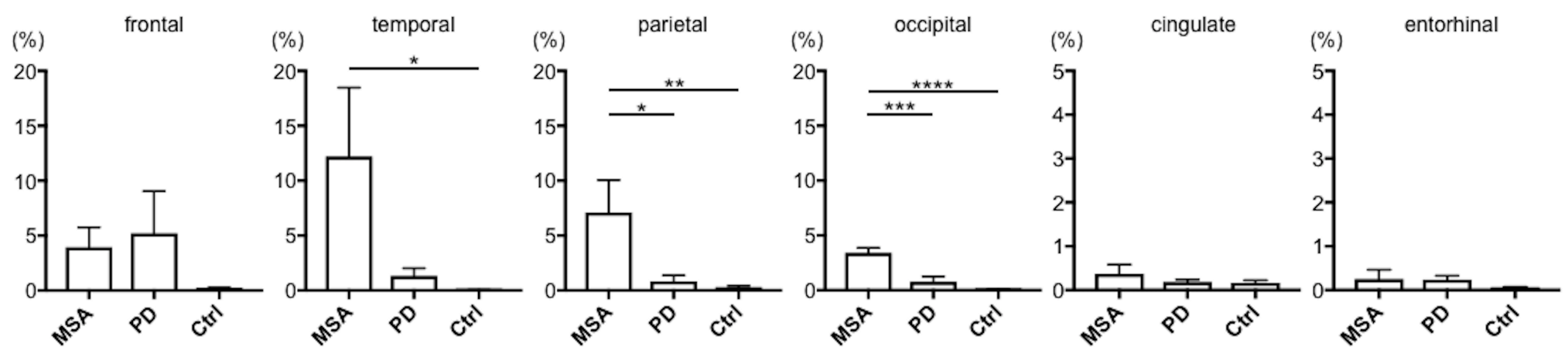


Fig. S2 Areas of AS-PLA staining in each region

The areas of AS-PLA staining in neuropils were measured by using ImageJ software. MSA, multiple system atrophy group; P, parietal cortex; PD, Parkinson's disease group.

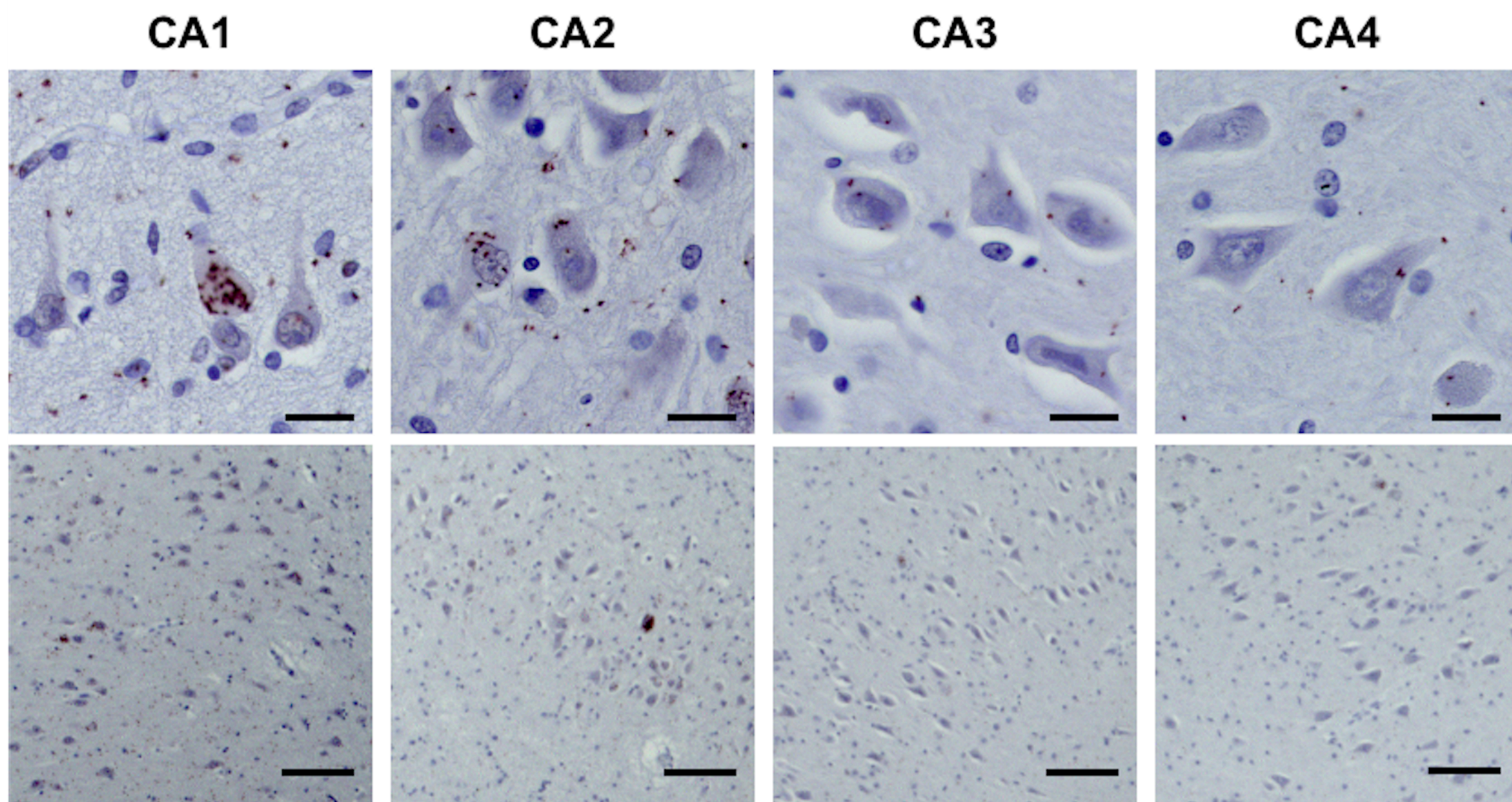


Fig. S3 AS-PLA of the hippocampus in brain sample from patient with multiple system atrophy

The upper panels are high magnification and the lower panels are low magnification. Neurons in CA1 and CA2 exhibit patchy staining and neurons in CA3 and CA4 show punctate staining. *Scale bars 20 μm (upper panels), 100 μm (lower panels).* CA, cornu ammonis.

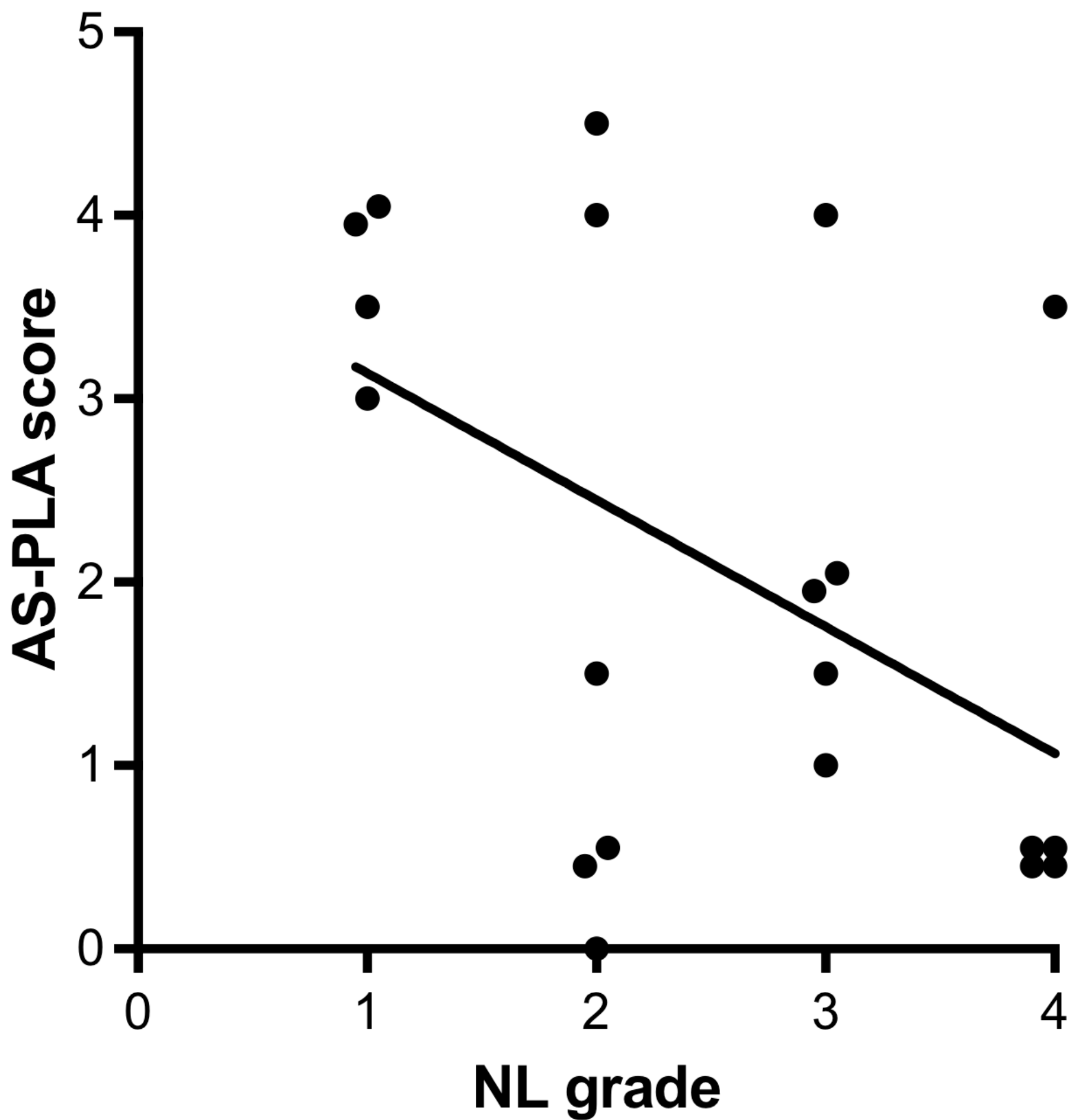
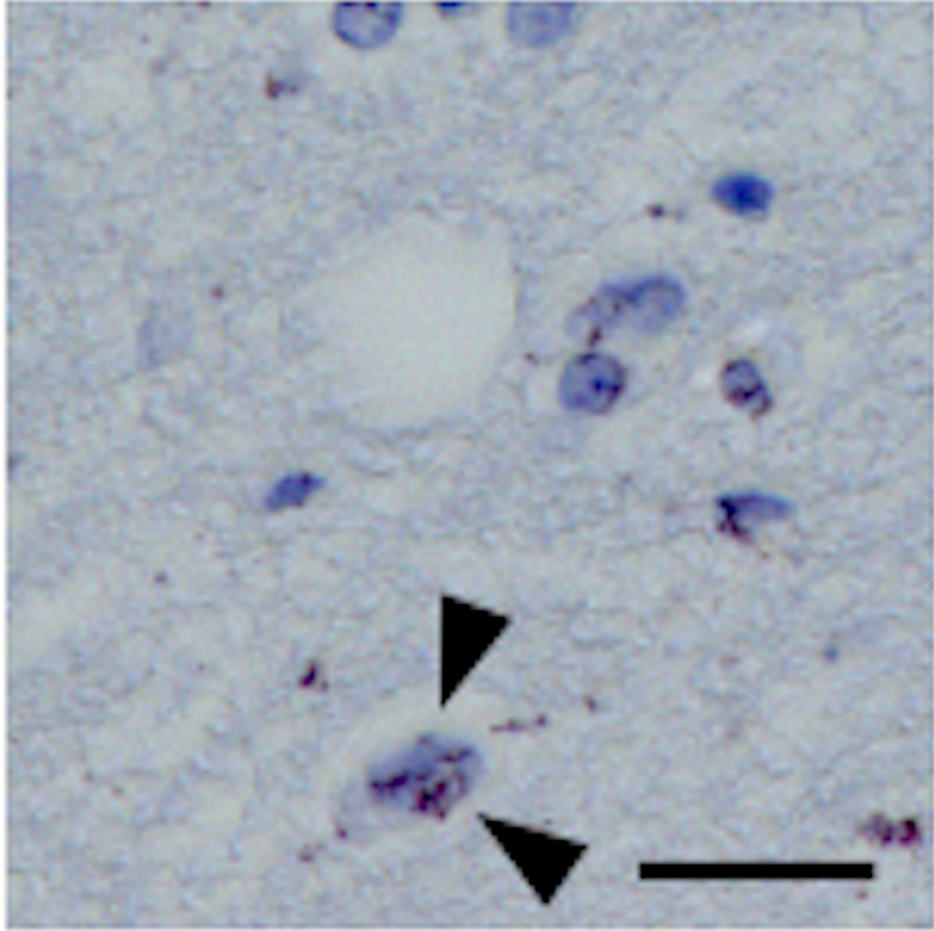


Fig. S4 The correlation of NL and AS-PLA signals in canonical regions of multiple system atrophy

In canonical regions of multiple system atrophy such as putamen, substantia nigra pars compacta, pontine nucleus, and inferior olivary nucleus, AS-PLA signals decreased as NL advanced. There was a significant inverse correlation between AS-PLA and NL. NL, neuronal loss.

AS-PLA



p-AS IHC

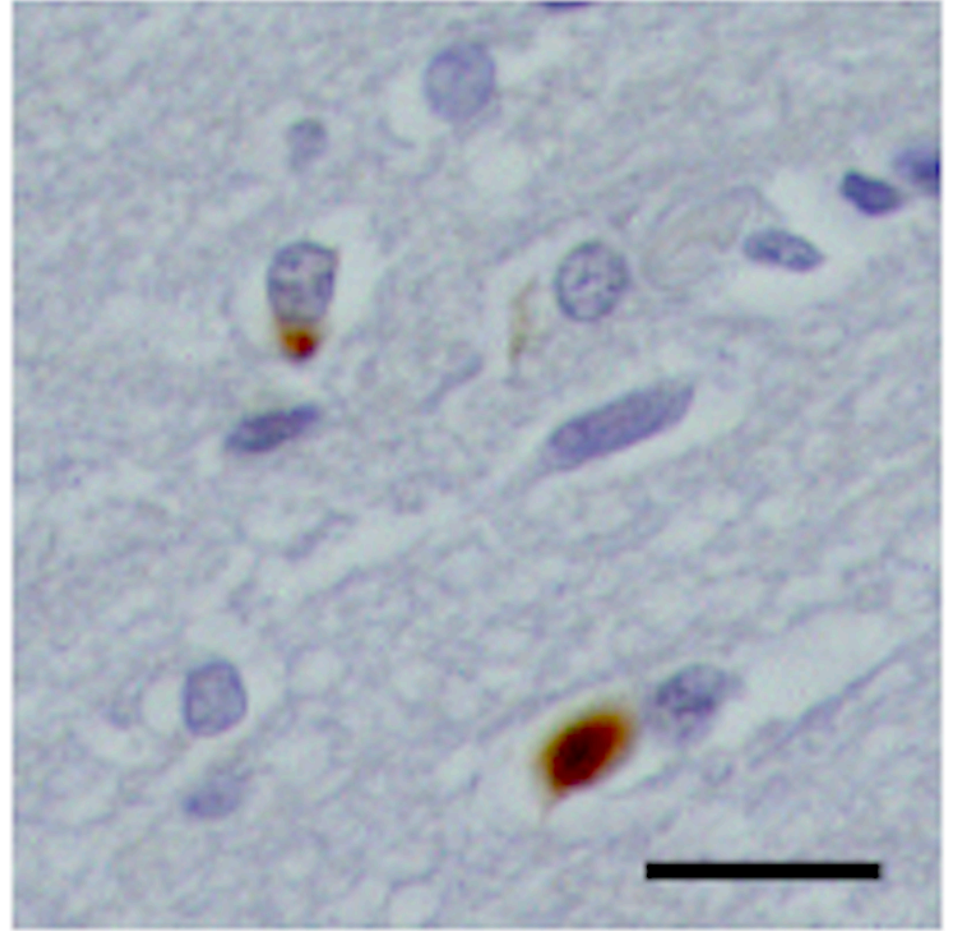


Fig. S5 AS-PLA signals and GCIs

AS-PLA signals were detected in the periphery of GCIs in MSA (arrowhead). GCI, glial cytoplasmic inclusion.

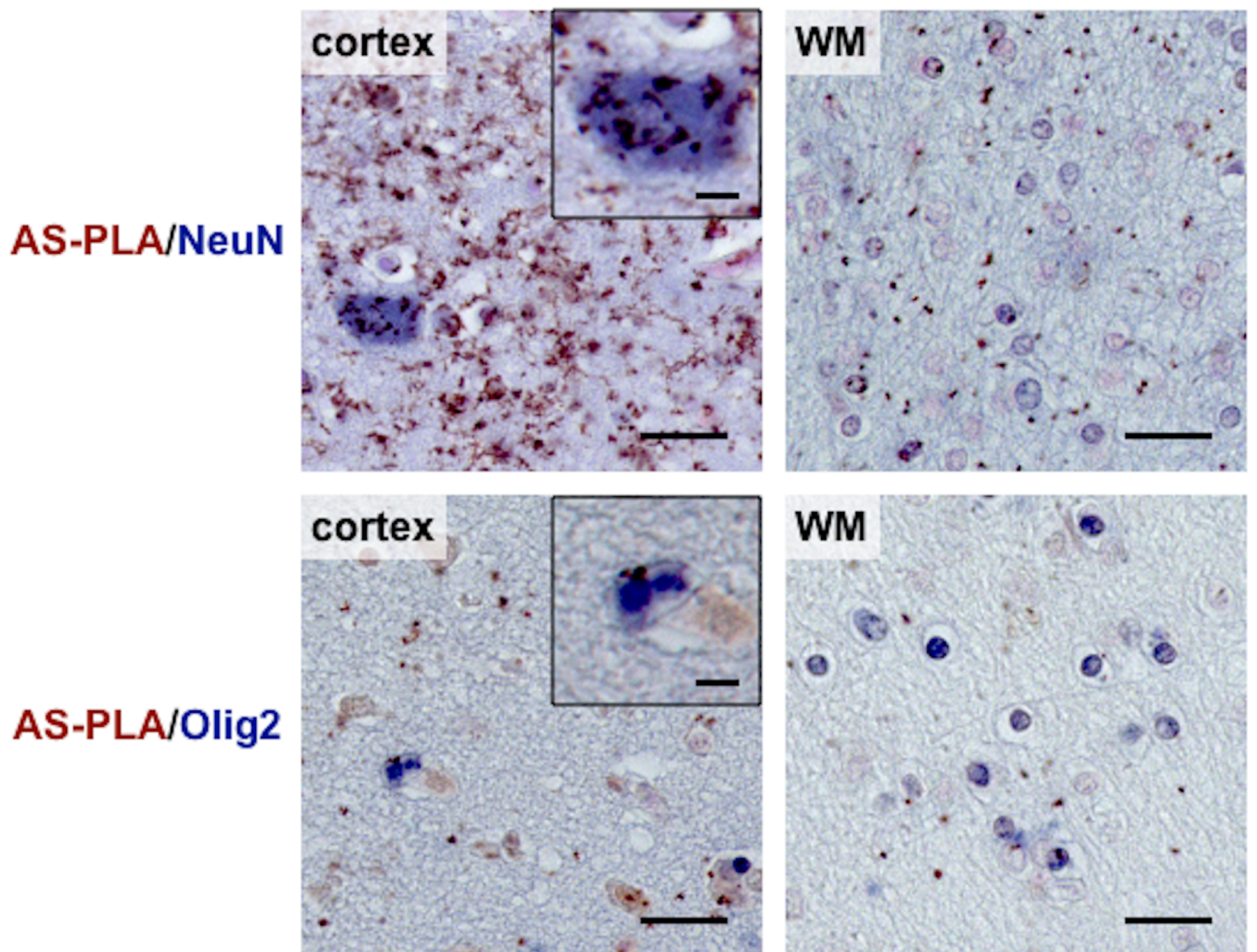


Fig. S6 Double staining of AS-PLA and NeuN or Olig2 immunohistochemistry

AS-PLA signals (brown) and NeuN or Olig2 signals (blue) were colocalized. High-magnification images of the neuron and the oligodendrocyte are shown as insets. AS-PLA signals primarily collocated in neurons of the cortex. Few AS-PLA signals were observed with oligodendrocytes in white matter. *Scale bars* 20 μm . WM, white matter.

Table S1 Correlation of neuronal loss and AS-PLA score in canonical regions of multiple system atrophy

Region	Putamen					SNpc				
ID	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
NL	3	2	3	4	4	2	1	3	3	4
AS-PLA	4	4	1.5	0.5	0.5	4.5	4	2	2	0.5

Region	Pontine nucleus					Inferior olivary nucleus				
ID	M1	M2	M3	M4	M5	M1	M2	M3	M4	M5
NL	4	1	1	2	1	3	2	2	2	4
AS-PLA	3.5	4	3	0.5	3.5	1	1.5	0	0.5	0.5

AS-PLA, alpha-synuclein proximity ligation assay; NL, neuronal loss; SNpc, substantia nigra pars compacta