Supplementary figure 1. Correlations of Neu-N, GFAP and Iba-1 compared to α-synuclein

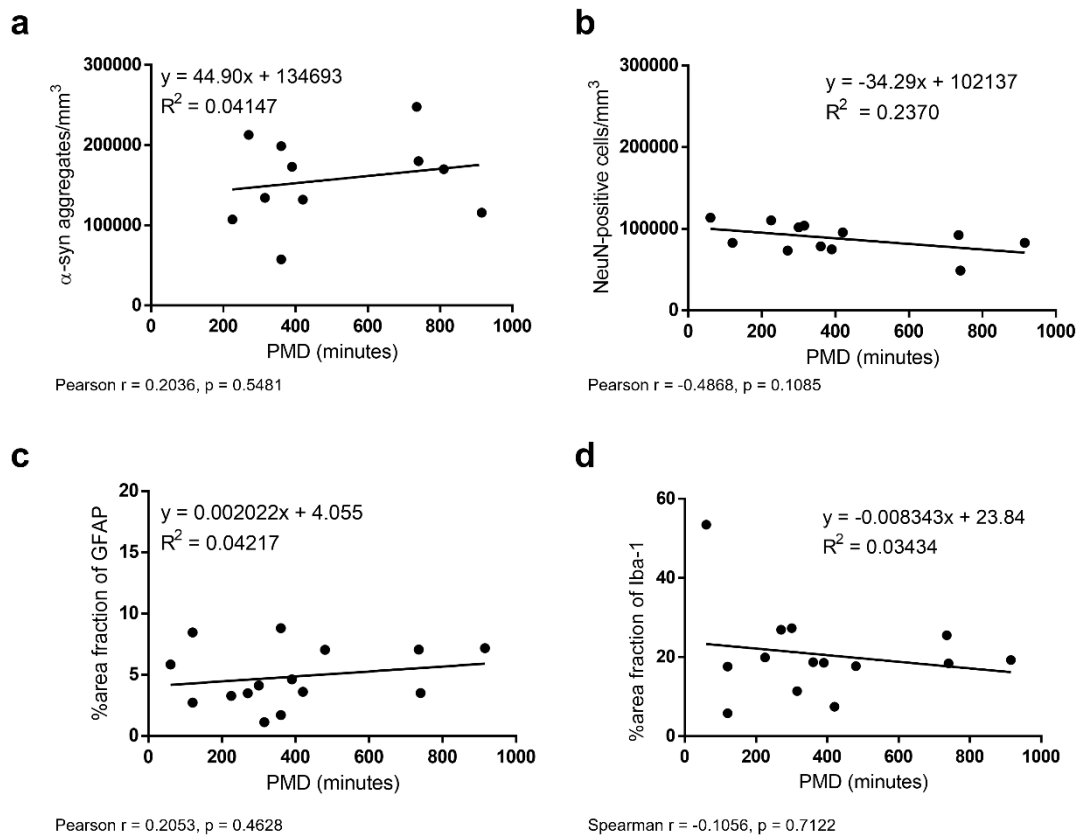**a**

$$y = -0.07102x + 101320$$
$$R^2 = 0.05848$$

NeuN-positive cells/mm^3 (y-axis)
α-syn-aggregates/mm^3 (x-axis)

b

$$y = -0.1266x + 9.182$$
$$R^2 = 0.2011$$

%area fraction of GFAP (y-axis)
%area fraction of α-syn aggregates and fibers (x-axis)

c

$$y = -0.2961x + 30.38$$
$$R^2 = 0.05177$$

%area fraction of Iba-1 (y-axis)
%area fraction of α-syn aggregates and fibers (x-axis)
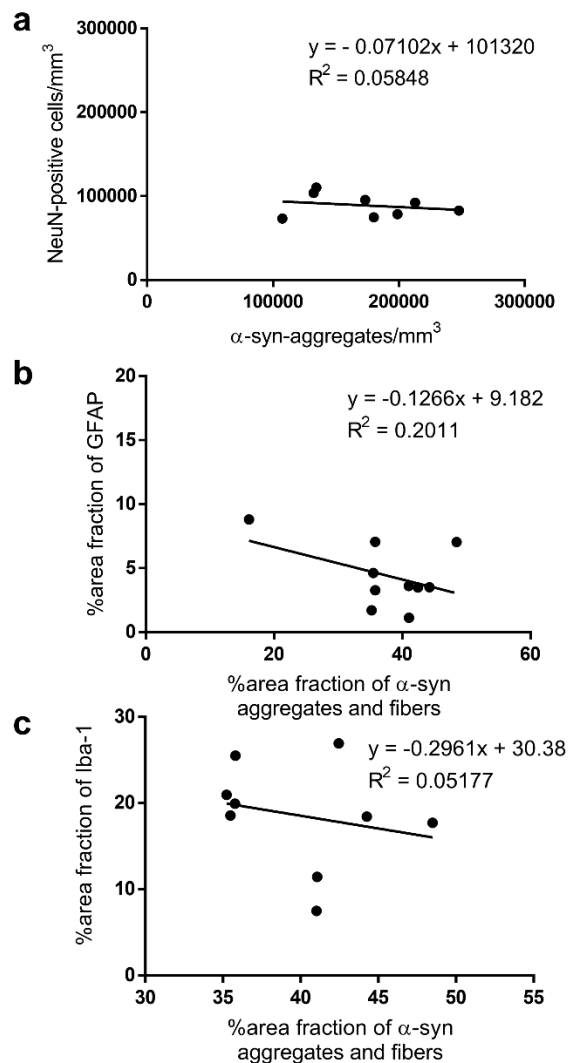
Supplementary Fig. 1. Correlations of Neu-N (cells/mm^3) compared to α-synuclein (aggregates/mm^3) (a), percentage of area fraction of GFAP against percentage of area fraction of α-synuclein (b) and finally, percentage of area fraction of Iba-1 against percentage of area fraction of α-synuclein (c).

Supplementary figure 2. Correlations of α-synuclein, Neu-N, GFAP, Iba-1 compared to disease duration.

a

$y = 4643x + 93994$
$R^2 = 0.1434$

Pearson r = 0.3787, p = 0.2507

b

$y = -1120x + 102844$
$R^2 = 0.1392$

Pearson r = -0.3731, p = 0.1392

c

$y = 0.01209x + 4.820$
$R^2 = 0.0008470$

Pearson r = 0.02910, p = 0.9148

d

$y = -1.622x + 45.07$
$R^2 = 0.3611$

Spearman r = -0.4794, p = 0.0689
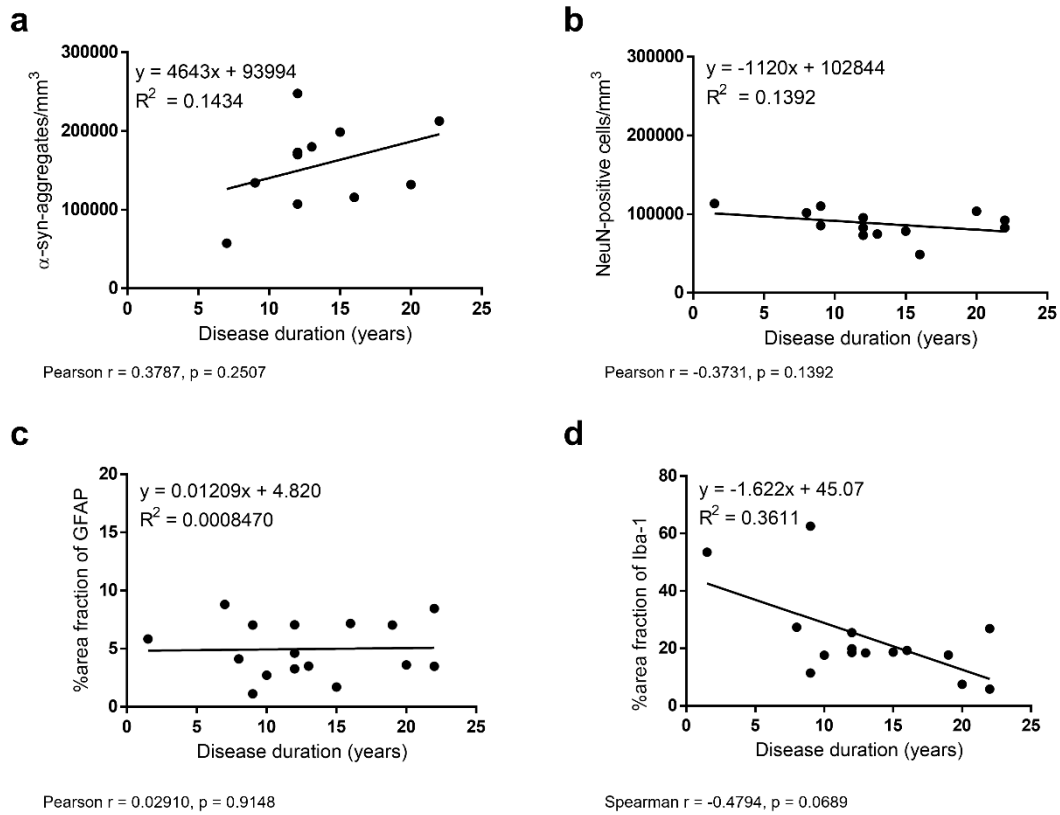
Supplementary Fig. 2. Correlations of α-synuclein (aggregates/mm^3), Neu-N (cells/mm^3), GFAP (percentage of area fraction) and Iba-1 (percentage of area fraction) compared to disease duration, using Pearson and Spearman tests. No substantial correlations were found.

Supplementary figure 3. Correlations of α-synuclein, Neu-N, GFAP, Iba-1 compared to post-mortem interval.

a

$y = 44.90x + 134693$
$R^2 = 0.04147$

Pearson r = 0.2036, p = 0.5481

b

$y = -34.29x + 102137$
$R^2 = 0.2370$

Pearson r = -0.4868, p = 0.1085

c

$y = 0.002022x + 4.055$
$R^2 = 0.04217$

Pearson r = 0.2053, p = 0.4628

d

$y = -0.008343x + 23.84$
$R^2 = 0.03434$

Spearman r = -0.1056, p = 0.7122

Supplementary Fig. 3. Correlations of α-synuclein (aggregates/mm^3), Neu-N (cells/mm^3), GFAP (percentage of area fraction) and Iba-1 (percentage of area fraction) compared to post-mortem interval, using Pearson and Spearman tests. No correlations were detected.

Supplementary Figure 4. Stereological area fraction fractionator

a
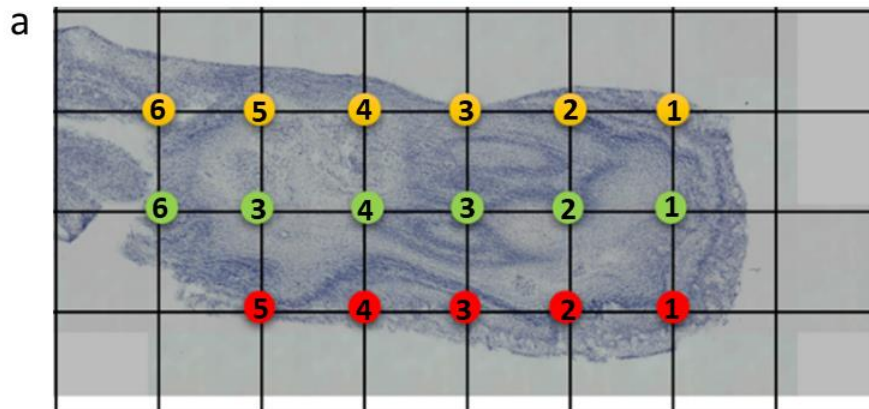
b

Case	Random number	Even/odd number
	Excel f_x=RAND()	
1	0.93	Odd number
2	0.68	Even number
3	0.73	Even number
4	0.77	Odd number
.	.	.
.	.	.
.	.	.

c

d
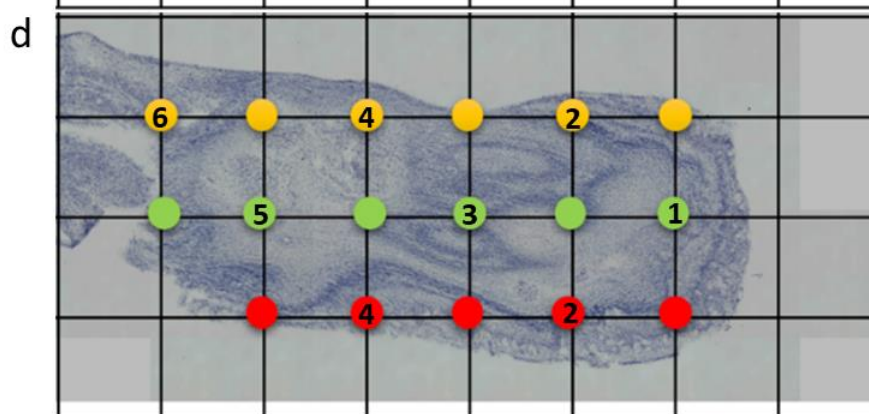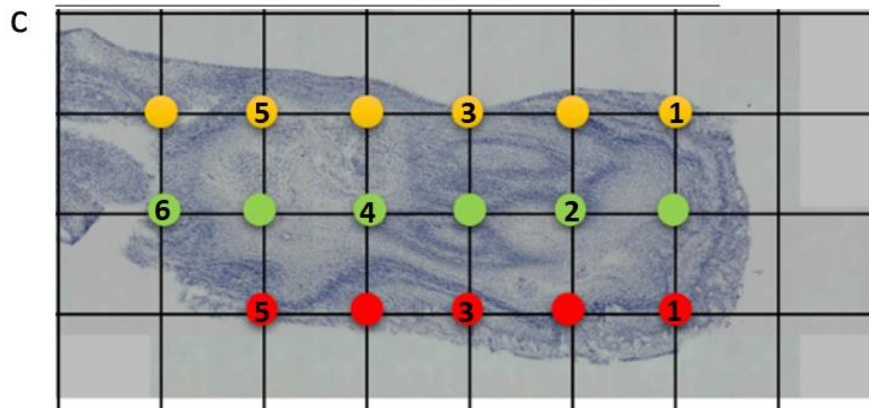
Supplementary Fig. 4. Stereological area fraction fractionator probe to quantify GFAP and Iba-1 staining. Following an unbiased protocol: a millimetric transparent grid simulating an array was randomly overlapped in the slide and crossed matched to the tissue identified (black lines) (a); odd and even numbers were randomly assigned to each human case (b). In odd-numbered cases, the images were taken from the first cross-matching of the tissue following this cross-matching sequence for the first line: 1-3-5-7 (c). In even-numbered cases, the images were captured in the second cross-matching following this sequence: 2-4-6-8 (d). Images were processed with an ImageJ protocol to obtain the percentage of area fraction.