

## Supplementary materials:

### Title:

Alpha-synuclein Autoimmune Decline in Prodromal Multiple System Atrophy and Parkinson's Disease

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**Supplementary material:** 3 tables and 1 figure.

## Supplementary Tables:

**Table S1: Complete statistics of prodromal(p) Multiple System Atrophy (pMSA), Parkinson's Disease (pPD) and normal controls (NC).**

Model statistics				Multiple comparison test			PD vs MSA	NC vs MSA	NC vs PD
F-statistics	R <sup>2</sup>	p-value		p-value (Group)	p-value (Age)	p-value (Sex)	p-value	p-value	p-value
<b>αSyn Competition</b>									
nM200	F(4,192)=0.49	-0.01	0.746	0.463	0.879	0.912	-	-	-
nM50	F(4,184)=1.09	0.01	0.363	0.379	0.203	0.344	-	-	-
nM12.5	F(4,176)=3.73	0.06	0.006	<b>0.002</b>	0.650	0.256	0.779	<b>0.004</b>	<b>0.020</b>
nM2	F(4,161)=5.90	0.11	0.0002	<b>9.0E-05</b>	0.400	0.484	0.576	<b>0.011</b>	<b>&lt;0.001</b>
<b>Anti-αSyn nAbs subclasses</b>									
IgG-T	F(4,202)=4.64	0.07	0.001	<b>3.0E-04</b>	0.777	0.745	<b>0.001</b>	0.936	<b>0.002</b>
IgG1	F(4,195)=3.32	0.05	0.012	<b>0.006</b>	0.644	0.533	<b>0.018</b>	0.984	<b>0.017</b>
IgG2	F(4,185)=18.0	0.27	1.6E-12	<b>2.7E-13</b>	0.150	0.933	0.609	<b>&lt;0.001</b>	<b>&lt;0.001</b>
IgG3	F(4,200)=11.3	0.17	2.6E-08	<b>2.8E-09</b>	0.551	0.976	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.611
IgG4	F(4,203)=10.3	0.15	1.4E-07	<b>1.2E-06</b>	0.572	<b>0.005</b>	0.999	<b>&lt;0.001</b>	<b>&lt;0.001</b>
IgM	F(4,198)=2.17	0.02	0.073	<b>0.045</b>	<b>0.044</b>	0.349	0.137	0.979	<b>0.054</b>
<b>Total αSyn amount</b>									
αSyn	F(4,157)=5.63	0.10	0.0003	<b>3.0E-04</b>	0.476	0.092	<b>0.004</b>	0.868	<b>&lt;0.001</b>

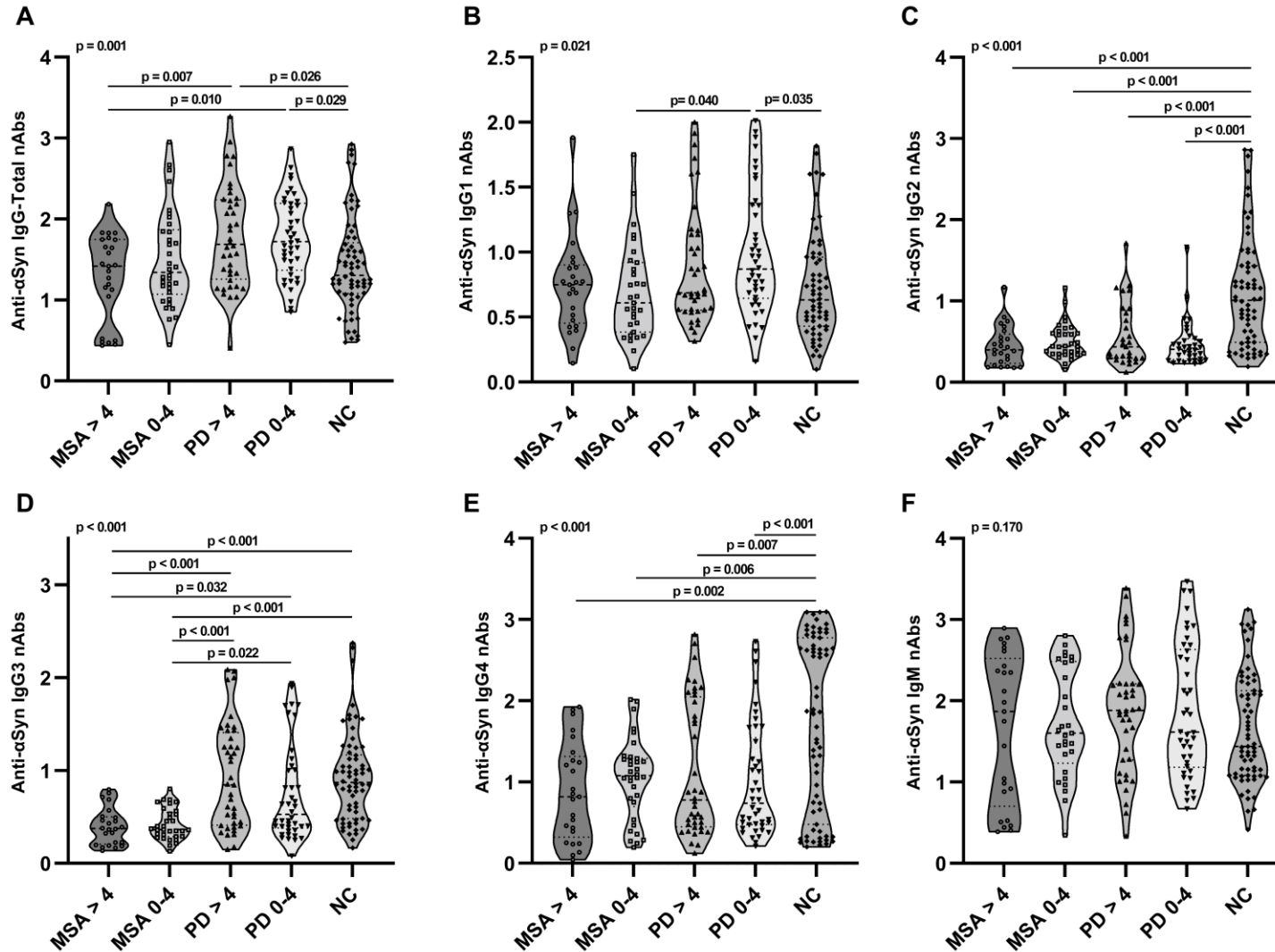
**Table S2: Correlations between prodromal age and measure outcome in prodromal(p) Multiple System Atrophy (pMSA), Parkinson's Disease (pPD) and normal controls (NC).**

MSA					PD			
	<i>r</i>	<i>R</i> <sup>2</sup>	95%CI	p-value	<i>r</i>	<i>R</i> <sup>2</sup>	95%CI	p-value
<b>αSyn Competition</b>								
nM200	0.096	0.009	-0.17 to 0.35	0.472	0.012	0.001	-0.21 to 0.23	0.917
nM50	-0.182	0.033	-0.43 to 0.09	0.189	-0.083	0.007	-0.30 to 0.14	0.472
nM12.5	-0.025	0.001	-0.30 to 0.25	0.858	-0.209	0.044	-0.42 to 0.03	0.080
nM2	<b>-0.367</b>	<b>0.135</b>	<b>-0.08 to -0.37</b>	<b>0.014</b>	0.014	0.001	-0.23 to 0.26	0.914
<b>Anti-αSyn nAbs subclasses</b>								
IgG-T	-0.060	0.004	-0.31 to 0.20	0.651	-0.013	0.001	-0.23 to 0.20	0.906
IgG1	0.090	0.008	-0.18 to 0.35	0.507	-0.148	0.022	-0.35 to 0.07	0.186
IgG2	-0.066	0.004	-0.32 to 0.20	0.624	0.115	0.013	-0.13 to 0.35	0.361
IgG3	0.083	0.006	-0.18 to 0.34	0.541	0.062	0.004	-0.16 to 0.28	0.579
IgG4	-0.042	0.002	-0.30 to 0.22	0.750	0.097	0.009	-0.12 to 0.31	0.388
IgM	0.236	0.055	-0.03 to 0.47	0.086	-0.020	0.001	-0.24 to 0.20	0.858
<b>Total αSyn amount</b>								
αSyn	0.187	0.04	-0.09 to 0.44	0.179	0.239	0.057	-0.01 to 0.46	0.061

Table S3: Complete statistics of prodromal individuals divided into longer period prior to diagnosis (> 4 years) and close to diagnosis (0-4 years) in pMSA, pPD and normal controls (NC).

Model statistics			Multiple comparison test				MSA>4 vs MSA<4	PD <4 vs MSA<4	PD>4 vs MSA <4	NC vs MSA<4	PD<4 vs MSA>4	PD>4 vs MSA>4	NC vs MSA>4	PD>4 vs PD<4	NC vs PD<4	NC vs PD>4
F-statistics	R <sup>2</sup>	p-value	p-value (Group)	p-value (Sex)	p-value (Age)	p-value	p-value	p-value	p-value	p-value	p-value	p-value	p-value	p-value	p-value	p-value
<b>αSyn Competition</b>																
nM200	F(6,190)=0.36	-0.02	0.902	0.776	0.915	0.913	-	-	-	-	-	-	-	-	-	-
nM50	F(6,182)=0.79	0.00	0.576	0.668	0.222	0.358	-	-	-	-	-	-	-	-	-	-
nM12.5	F(6,174)=2.90	0.06	0.010	<b>0.006</b>	0.853	0.237	1.000	0.999	0.701	<b>0.043</b>	0.999	0.828	0.126	0.515	<b>0.017</b>	0.624
nM2	F(6,159)=4.05	0.10	0.0008	<b>7.0E-04</b>	0.463	0.501	0.887	0.992	0.997	<b>0.027</b>	0.699	0.742	0.559	0.999	<b>0.0039</b>	<b>0.0042</b>
<b>Anti-αSyn nAbs subclasses</b>																
IgG-T	F(6,200)=3.46	0.07	0.003	<b>0.001</b>	0.642	0.735	0.572	0.221	0.206	0.990	<b>0.010</b>	<b>0.007</b>	0.734	1.000	<b>0.029</b>	<b>0.026</b>
IgG1	F(6,193)=2.46	0.04	0.026	<b>0.021</b>	0.729	0.497	0.984	<b>0.040</b>	0.316	0.991	0.246	0.746	0.999	0.813	<b>0.035</b>	0.394
IgG2	F(6,183)=12.1	0.26	2E-11	<b>6.2E-12</b>	0.169	0.952	0.973	1.000	0.929	< <b>0.001</b>	0.956	0.682	< <b>0.001</b>	0.961	< <b>0.001</b>	< <b>0.001</b>
IgG3	F(6,198)=8.18	0.17	6.5E-08	<b>1.2E-08</b>	0.740	0.928	0.999	<b>0.022</b>	< <b>0.001</b>	< <b>0.001</b>	<b>0.032</b>	< <b>0.001</b>	< <b>0.001</b>	0.351	0.365	0.999
IgG4	F(6,201)=7.10	0.15	7.2E-07	<b>8.8E-06</b>	0.528	<b>0.005</b>	0.965	0.936	0.999	<b>0.006</b>	0.999	0.912	<b>0.002</b>	0.823	< <b>0.001</b>	<b>0.007</b>
IgM	F(6,196)=1.48	0.01	0.188	0.170	<b>0.040</b>	0.370	-	-	-	-	-	-	-	-	-	-
<b>Total αSyn amount</b>																
αSyn	F(6,155)=4.28	0.11	0.0005	<b>8.0E-04</b>	0.306	0.105	0.596	0.084	<b>0.004</b>	0.999	0.902	0.382	0.675	0.854	0.074	<b>0.003</b>

## Supplementary figures:



**Figure S1.** Serum nAbs affinity-profile towards  $\alpha$ Syn. Binding of anti- $\alpha$ Syn IgG-total, IgG1-4 subclasses and IgM nAbs in individual pMSA ( $n = 59$ ), pPD ( $n = 82$ ) and controls ( $n = 67$ ) for IgG serum fractions to  $\alpha$ Syn in indirect ELISA assay. pMSA and pPD were divided into two prodromal age ranges (0-4 years prior to diagnosis and above 4 years prior to diagnosis) detected for (A) anti- $\alpha$ Syn IgG-total, (B) anti- $\alpha$ Syn IgG1, (C) anti- $\alpha$ Syn IgG2, (D) anti- $\alpha$ Syn IgG3, (E) anti- $\alpha$ Syn IgG4 and (F) anti- $\alpha$ Syn IgM. Differences between groups were tested using multiple linear regression modeling including age and sex as confounding variables. Graphs are represented as truncated violin plots with median and 25% quantiles.