Supplementary Materials

SUPPLEMENTARY TABLES

Supplementary Table 1. Clinical Characteristics of all patients with PD and HC Subjects by αSyn-SAA Status in Serum or Saliva

	Parkinson's disease (PD)						Healthy controls (HC)			
	Serum			Saliva			Serum		Saliva	
	SAA+[a]	All PD	p value	SAA+[a]	All PD	p value	SAA+	All HC	SAA+	All HC
	n=66	n=82	All PD vs HC	n=40 (62[b])	n=49 (83[b])	All PD vs HC	n=4	n=42	n=1 (1[b])	n=26 (48[b])
Age (years), mean (range)	69.35 (44-88)	69.21 (44-88)	0.114	69.29 (49-85)	69.58 (49-87)	**0.003**	69 (59-73)	66.55 (44-81)	66 (66-66)	64.71 (30-81)
Age at diagnosis (years), mean (range)	66.31 (39.00-88.42)	65.84 (39.00-88.42)	0.733	65.15 (40.0-83.5)	65.86 (40.00-83.50)	0.556	NA	63.86 (51.67-74.00)	NA	69.96 (65.92-74.00)
Disease duration, years (range)	4.96 (0-17)	5.05 (0-17)	NA	6.76 (0-31)	6.18 (0-31)	NA	NA	NA	NA	NA
Sex			**0.004**			**0.006**				
Male, number (%)	35 (53.0)	45 (54.9)		31 (50.0)	46 (55.4)		0 (0.0)	11 (26.2)	0 (0.0)	14 (29.2)
Female, number (%)	31 (47.0)	37 (45.1)		31 (50.0)	37 (44.6)		4 (100.0)	31 (73.8)	1 (100.0)	34 (70.8)
mH&Y, mean (SD)	2.05 (0.44)	2.05 (0.48)	NA	2.13 (0.59)	2.09 (0.61)	NA	NA	NA	NA	NA
Schwab & England, mean (SD)	90.23 (7.46)	90.55 (7.11)	**<0.001**	92.24 (6.85)	91.12 (8.12)	**<0.001**	97.5 (5.0)	98.97 (3.07)	100 (0.0)	99.17 (2.82)
MoCA, mean (SD)	26.49 (2.79)	26.69 (2.60)	0.989	26.69 (3.20)	26.74 (3.04)	0.426	27.5 (1.29)	26.70 (2.28)	20 (0.0)	27.24 (2.40)
MDS-UPDRS										
Part 1, mean (SD)	7.69 (5.79)	7.34 (5.82)	NA	7.35 (6.25)	7.58 (6.42)	NA	NA	NA	NA	NA
Part 2, mean (SD)	8.98 (5.65)	8.69 (5.75)	NA	8.26 (6.15)	8.16 (5.91)	NA	NA	NA	NA	NA
Part 3, mean (SD)	31.78 (9.58)	31.46 (10.62)	NA	28.96 (11.32)	29.00 (11.27)	NA	NA	NA	NA	NA
Parts 123, mean (SD)	48.67 (16.75)	47.64 (17.85)	NA	45.64 (17.92)	45.59 (18.07)	NA	NA	NA	NA	NA
Part 4 mean (SD)	1.80 (2.46)	1.81 (2.47)	NA	1.88 (2.57)	1.62 (2.49)	NA	NA	NA	NA	NA
Total score	50.50 (17.54)	49.47 (18.76)	NA	47.58 (18.64)	47.25 (19.03)	NA	NA	NA	NA	NA
PDQ39										
total, mean (SD)	95.99 (84.03)	94.84 (82.14)	**0.001**	79.88 (71.46)	87.17 (82.15)	**0.003**	2.78 (4.81)	35.53 (65.15)	8.3 (0.0)	23.89 (31.10)
mobility, mean (SD)	9.42 (11.81)	9.05 (11.82)	**0.041**	6.76 (10.33)	7.50 (10.98)	0.110	6.25 (12.5)	4.21 (10.64)	0.0 (0.0)	3.21 (7.29)
ADL, mean (SD)	14.79 (13.32)	14.47 (14.15)	**<0.001**	11.76 (11.94)	12.97 (13.60)	**<0.001**	0.0 (0.0)	1.79 (4.77)	0.0 (0.0)	1.39 (4.03)
cognitive impairment, mean (SD)	13.33 (14.98)	12.75 (14.20)	**0.006**	12.32 (14.96)	13.64 (16.52)	0.069	3.12 (6.25)	5.54 (8.13)	0.0 (0.0)	6.55 (8.50)
Patient self-report										
Dementia, number (%)	10 (15.2)	10 (12.2)	0.395	11 (17.7)	12 (14.5)	0.065	1 (25.0)	2 (5.3)	0 (0.0)	1 (2.3)
Hyposmia, number (%)	33 (50.0)	37 (45.1)	**<0.001**	17 (44.7)	21 (42.9)	**0.001**	0 (0.0)	1 (2.6)	0 (0.0)	0 (0.0)
Constipation, number (%)	31 (47.7)	38 (46.9)	**<0.001**	17 (44.7)	24 (49.0)	**0.001**	0 (0.0)	2 (5.3)	0 (0.0)	1 (4.5)
ESS, mean (SD)	5.97 (3.71)	5.95 (3.68)	0.119	6.24 (3.72)	6.66 (3.80)	0.075	3.50 (2.38)	4.87 (2.98)	2.00 (0.0)	5.04 (3.13)
HAM-A, Mean (SD)	7.89 (7.23)	7.70 (7.06)	0.162	7.63 (7.55)	8.00 (7.69)	0.387	8.25 (13.87)	5.85 (5.97)	3.00 (0.0)	6.40 (6.73)
HAM-D, mean (SD)	5.97 (5.64)	5.75 (5.44)	**0.025**	5.64 (5.86)	6.00 (5.83)	0.078	4.25 (7.23)	3.51 (3.98)	2 (0.0)	3.60 (4.31)
RBD, number (%)	29 (48.3)	35 (46.1)	**<0.001**	17 (50.0)	23 (52.3)	**<0.001**	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

[a]Patients with positive αSyn-SAA in the indicated samples. [b]Total number of saliva donors that include a subset of subjects with information on age, age at diagnosis, disease duration, sex, mH&Y, MoCA, and MSD-UPDRS 3 only.

Supplementary Table 2. Clinical Correlation Analysis by αSyn^D Seeding Activities in Serum or Saliva of Patients with PD

	Serum (n = 82)		Saliva (n = 83)	
Pearson's correlation	*r*	*p*	*r*	*p*
Age	-0.12	0.28	-0.10	0.38
Disease duration	0.05	0.63	0.03	0.78
Age at diagnosis	-0.15	0.20	**-0.34**	**0.02**
Schwab & England	-0.14	0.21	0.025	0.87
mH&Y	-0.05	0.69	-0.04	0.70
MoCA	**-0.24**	**0.04**	0.13	0.35
MDS-UPDRS				
Part 1	0.05	0.65	-0.07	0.64
Part 2	0.01	0.93	-0.07	0.66
Part 3	0.02	0.85	-0.05	0.67
Part 123	0.03	0.81	-0.12	0.43
Part 4	0.04	0.73	-0.05	0.76
Total	0.03	0.79	-0.12	0.43
PDQ39				
Total	0.08	0.52	-0.07	0.68
Mobility	0.01	0.93	-0.15	0.34
ADL	<0.01	0.97	-0.17	0.28
Cognitive impairment	**0.21**	**0.07**	-0.07	0.66
ESS	-0.15	0.19	-0.23	0.13
HAM-A	0.13	0.25	0.05	0.76
HAM-D	**0.24**	**0.03**	0.05	0.75
ANOVA	**Higher mean**	*p*	**Higher mean**	*p*
Sex	female	0.95	Female	0.31
RBD	no	0.21	**no**	**0.04**
Orthostatic hypotension (vitals)	yes	0.72	no	0.84
Patient self-report				
Hyposmia	yes	0.11	no	0.63
Orthostatic hypotension	yes	0.97	yes	0.66
Constipation	yes	0.98	Yes	0.50
Dementia	yes	0.16	Yes	0.27

Supplementary Table 3. Correlation Subgroup Analysis by Sex or Age by Serum αSyn^D Seeding Activities of Patients with PD
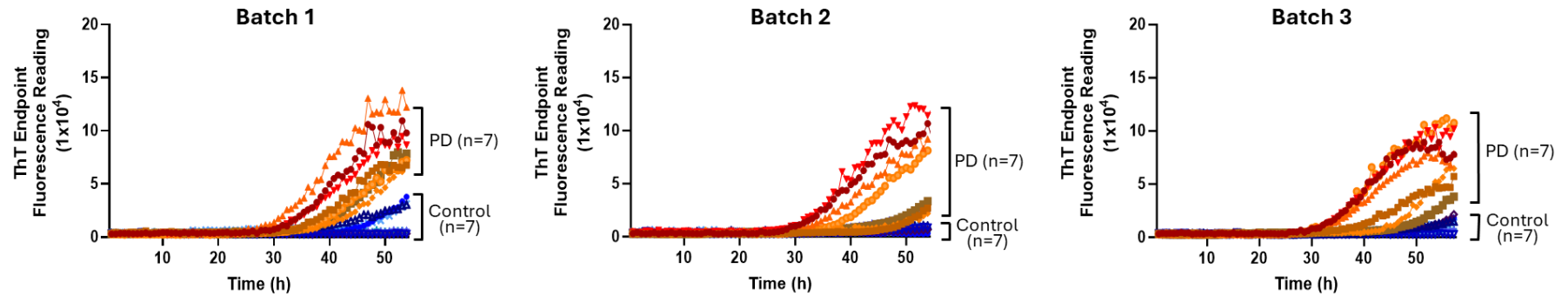
	Sex				Age			
	Female (n = 37)		**Male** (n = 46)		**< 70 years** (n = 39)		**≥ 70 years** (n = 44)	
Pearson's correlation	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age	-0.17	0.33	-0.09	0.57	0.03	0.84	-0.16	0.30
Disease duration	-0.03	0.88	0.12	0.45	0.01	0.95	0.11	0.49
Age at diagnosis	-0.18	0.30	-0.12	0.44	0.01	0.96	-0.14	0.39
Schwab & England	-0.18	0.28	-0.10	0.51	-0.21	0.21	-0.12	0.43
mH&Y	-0.02	0.93	-0.07	0.66	0.05	0.75	-0.08	0.63
MoCA	-0.07	0.70	**-0.38**	**0.01**	-0.25	0.15	**-0.29**	**0.07**
MDS-UPDRS								
Part 1	0.04	0.82	0.07	0.68	0.27	0.12	-0.21	0.19
Part 2	0.09	0.62	-0.02	0.89	0.14	0.44	-0.04	0.78
Part 3	0.25	0.16	-0.15	0.35	0.24	0.16	-0.15	0.35
Part 123	0.19	0.30	-0.07	0.67	0.29	0.09	-0.12	0.45
Part 4	0.09	0.62	<-0.01	0.98	0.08	0.63	0.03	0.85
Total	0.19	0.30	-0.07	0.68	0.29	0.09	-0.14	0.39
PDQ39								
Total	0.10	0.62	0.09	0.62	0.32	0.09	-0.14	0.43
Mobility	0.09	0.63	-0.08	0.64	0.22	0.21	-0.03	0.84
ADL	0.07	0.69	-0.03	0.86	0.17	0.34	-0.10	0.55
Cognitive impairment	**0.32**	**0.07**	0.17	0.27	0.23	0.18	0.16	0.33
ESS	-0.20	0.26	-0.11	0.50	0.02	0.92	**-0.33**	**0.03**
HAM-A	0.21	0.23	0.06	0.69	**0.36**	**0.04**	-0.13	0.40
HAM-D	**0.36**	**0.04**	0.14	0.36	**0.43**	**0.01**	0.02	0.92
ANOVA	**Higher mean**	*p*	**Higher mean**	*p*	**Higher mean**	*p*	**Higher mean**	*p*
Sex	-	-	-	-	male	0.56	female	0.61
RBD	No	0.17	yes	0.68	yes	0.56	**no**	**0.03**
Orthostatic hypotension (vitals)	yes	0.40	no	0.78	**yes**	**0.01**	no	0.31
Patient self-report								
Hyposmia	yes	0.67	yes	0.09	yes	0.09	yes	0.41
Orthostatic hypotension	no	0.48	yes	0.71	yes	0.76	yes	0.83
Constipation	no	0.76	yes	0.78	yes	0.35	no	0.63
Dementia	yes	0.24	yes	0.33	yes	0.26	yes	0.28

Supplementary Table 4. Correlation Subgroup Analysis by Sex or Age by Saliva αSyn^D Seeding Activities of Patients with PD

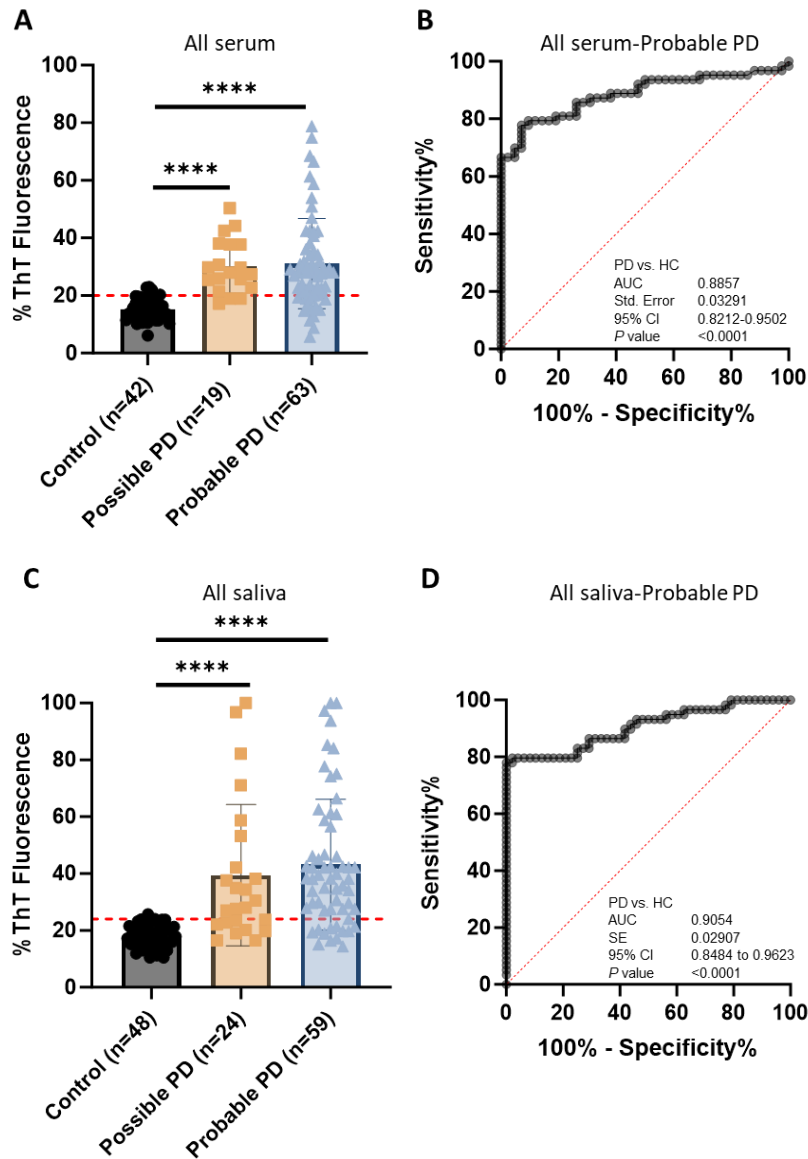|	Sex				Age			
	Female (n = 37)		**Male** (n = 46)		**< 70 years** (n = 39)		**≥ 70 years** (n = 44)	
Pearson's correlation	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age	<0.01	0.99	-0.19	0.21	-0.13	0.44	-0.17	0.28
Disease duration	0.07	0.67	-0.01	0.93	-0.11	0.50	0.12	0.43
Age at diagnosis	-0.33	0.13	**-0.42**	**0.04**	**-0.52**	**0.01**	0.08	0.72
Schwab & England	-0.08	0.70	0.13	0.53	0.33	0.11	**-0.47**	**0.02**
mH&Y	0.25	0.15	-0.17	0.27	-0.10	0.56	<-0.01	0.97
MoCA	0.07	0.77	0.15	0.46	0.18	0.37	0.08	0.72
MDS-UPDRS								
Part 1	0.17	0.45	-0.22	0.31	-0.17	0.45	0.11	0.64
Part 2	0.08	0.73	-0.13	0.56	-0.16	0.46	0.03	0.89
Part 3	0.12	0.50	-0.13	0.39	-0.02	0.89	-0.05	0.75
Part 123	0.16	0.49	-0.32	0.13	-0.18	0.42	-0.02	0.95
Part 4	0.06	0.79	-0.16	0.47	-0.08	0.73	<0.01	0.99
Total	0.16	0.50	-0.33	0.12	-0.18	0.40	-0.01	0.96
PDQ39								
Total	0.33	0.18	-0.28	0.21	-0.22	0.32	0.18	0.47
Mobility	-0.12	0.60	-0.18	0.42	-0.23	0.30	-0.08	0.73
ADL	-0.10	0.68	-0.20	0.35	-0.19	0.40	-0.18	0.50
Cognitive impairment	0.35	0.12	-0.22	0.32	-0.12	0.58	-0.04	0.85
ESS	-0.06	0.78	-0.36	0.09	-0.34	0.11	-0.11	0.64
HAM-A	0.33	0.13	-0.19	0.38	-0.01	0.95	0.08	0.72
HAM-D	0.35	0.13	-0.18	0.43	0.02	0.93	<0.01	0.97
ANOVA	**Higher mean**	*p*	**Higher mean**	*p*	**Higher mean**	*p*	**Higher mean**	*p*
Sex	-	-	-	-	female	0.81	female	0.14
RBD	no	0.13	no	0.34	no	0.13	no	0.15
Orthostatic hypotension (vitals)	no	0.37	yes	0.25	yes	0.28	no	0.62
Patient self-report								
Hyposmia	no	0.61	no	0.92	no	0.09	yes	0.36
Orthostatic hypotension	no	0.84	no	0.70	no	0.37	yes	0.79
Constipation	yes	0.68	yes	0.81	yes	0.67	no	0.69
Dementia	yes	0.98	yes	0.15	yes	0.37	yes	0.51

SUPPLEMENTARY FIGURES

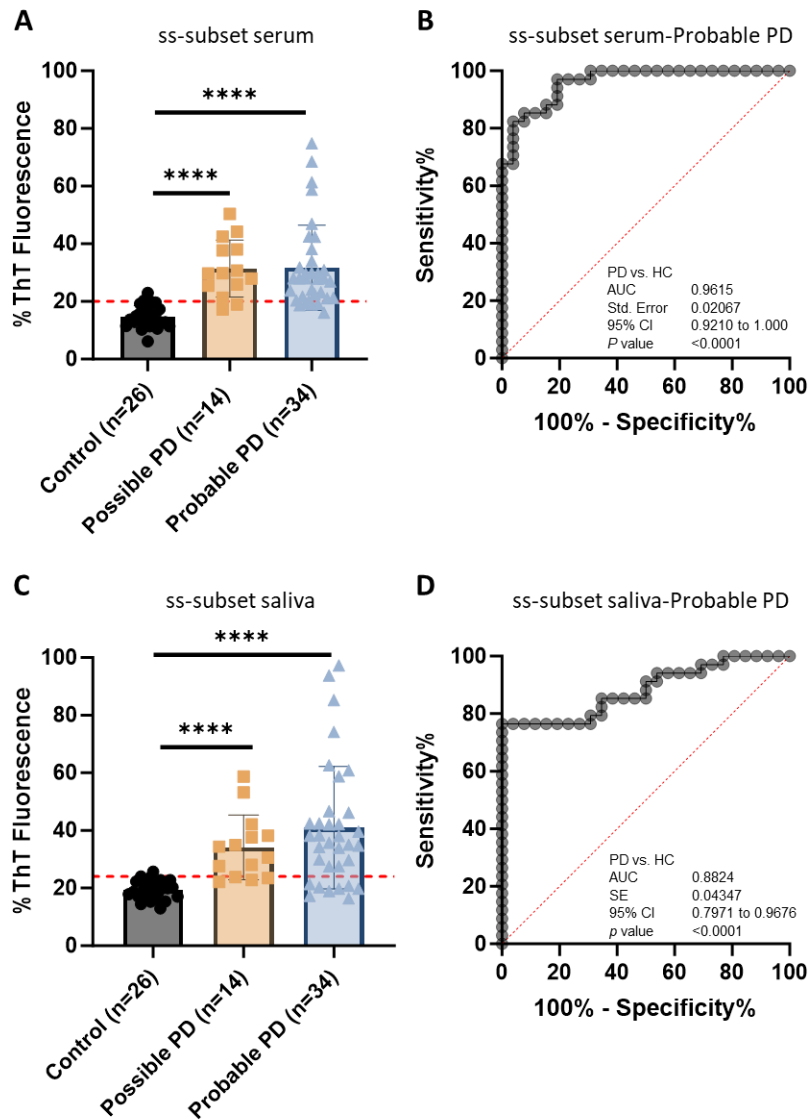**Supplementary Figure 1.** Assessment of αSyn-SAA reproducibility and recombinant αSyn batch variations

Three batches of recombinant αSyn protein were used for αSyn-SAA assays with RT-QuIC on seven biopsy skin samples each from known PD and healthy control subjects (Control). The αSyn-SAA assays of these skin samples were conducted as reported [21]. The ThT fluorescence curves obtained with each of the three batches are presented in the respective panel. Each curve reflects the average of the kinetic curves of quadruplicate wells of a sample. Each PD case is indicated by a distinct color.

Supplementary Figure 2. Comparison of αSyn^D Seeding Activity in All Serum or Saliva Samples from Patients with Probable or Possible PD and Healthy Control (HC) by αSyn-SAA
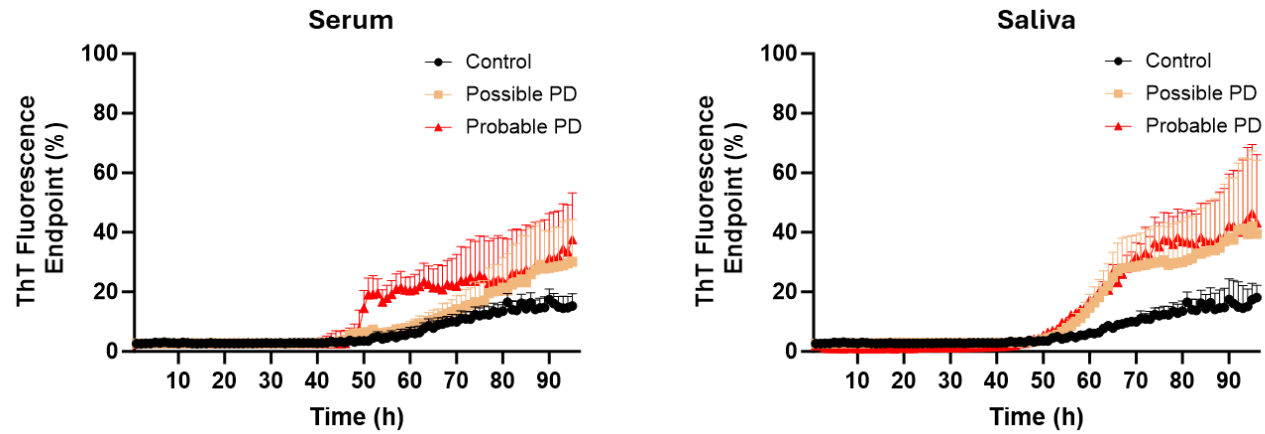

A. Scatter graph of RT-QuIC endpoint ThT fluorescence intensity (αSyn^D seeding activity) of serum samples from all patients with PD and HC subjects. Scatter graph of the average of the endpoint ThT fluorescence in quadruplicate wells as a percentage of the maximum fluorescence (%ThT fluorescence) of serum samples from 19 patients with possible PD, 63 patients with probable PD, and 42 HC subjects. ThT fluorescence cutoff: 52,105. **** $p<0.0001$. **B.** ROC curve and AUC for αSyn^D seeding activity comparisons between serum samples of the patients with probable PD and HC subjects. ROC curve and AUC value for αSyn^D seeding activities in the serum of all patients with probable PD and HC subjects are shown. SE, standard error. 95% CI, 95% confidence interval. **C.** Scatter graph of RT-QuIC endpoint ThT fluorescence intensity (αSyn^D seeding activities) of saliva samples from all patients with PD and HC subjects. Scatter graph of the average of the endpoint ThT fluorescence in quadruplicate wells as a percentage of the maximum possible fluorescence (%ThT fluorescence) of saliva samples from 24 patients with possible PD, 59 patients with probable PD, and 48 HC subjects. ThT fluorescence cutoff: 62,613. **** $p<0.0001$. **D.** ROC curve and AUC for αSyn^D seeding activity comparisons between saliva samples of the 59 probable PD patients and 48 HC subjects. ROC curve and AUC value for αSyn^D seeding activities in the serum of all probable PD patients and HC subjects are shown. SE, standard error. 95% CI, 95% confidence interval.

Supplementary Figure 3. Comparison of αSyn^D Seeding Activity in Serum or Saliva Samples from Patients with Probable or Possible PD and Healthy Control (HC) of the ss-subset by RT-QuIC

A. Scatter graph of RT-QuIC endpoint ThT fluorescence intensity (αSyn^D seeding activity) of serum samples from patients with PD and HC subjects of the ss-subset. Scatter graph of the average of the endpoint ThT fluorescence in quadruplicate wells as a percentage of the maximum possible fluorescence (%ThT fluorescence) of serum samples from 14 patients with possible PD, 34 patients with probable PD, and 26 HC subjects. ThT fluorescence cutoff: 52,105. **** $p<0.0001$. **B.** ROC curve and AUC for αSyn^D seeding activity comparisons between serum samples of patients with probable PD and HC subjects of the ss-subset. ROC curve and AUC value for αSyn^D seeding activities in the serum of patients with probable PD and HC subjects of the ss-subset are shown. SE, standard error. 95% CI, 95% confidence interval. **C.** Scatter graph of RT-QuIC endpoint ThT fluorescence intensity (αSyn^D seeding activity) of saliva samples from patients with PD and HC subjects of the ss-subset. Scatter graph of the average of the endpoint ThT fluorescence in quadruplicate wells as a percentage of the maximum fluorescence (%ThT fluorescence) of saliva samples from 14 patients with possible PD, 34 patients with probable PD, and 26 HC subjects. ThT fluorescence cutoff: 62,613. **** $p<0.0001$. **D.** ROC curve and AUC for αSyn^D seeding activity comparisons between saliva samples of the 34 patients with probable PD and 26 HC subjects. ROC curve and AUC value for αSyn^D seeding activities in the serum of patients with probable PD and HC subjects in the ss-subset are shown. SE, standard error. 95% CI, 95% confidence interval.

Supplementary Figure 4. Cumulative RT-QuIC ThT fluorescence curves for serum and saliva samples of the ss-subset

The cumulative RT-QuIC ThT fluorescence curves for the serum or saliva samples of the 48 patients with PD (34 probable and 14 possible) and 26 HC subjects of the ss-subset shown in Figure 3 are presented. The curves represent the mean and standard deviation of all the serum/saliva samples of the ss-subset over time