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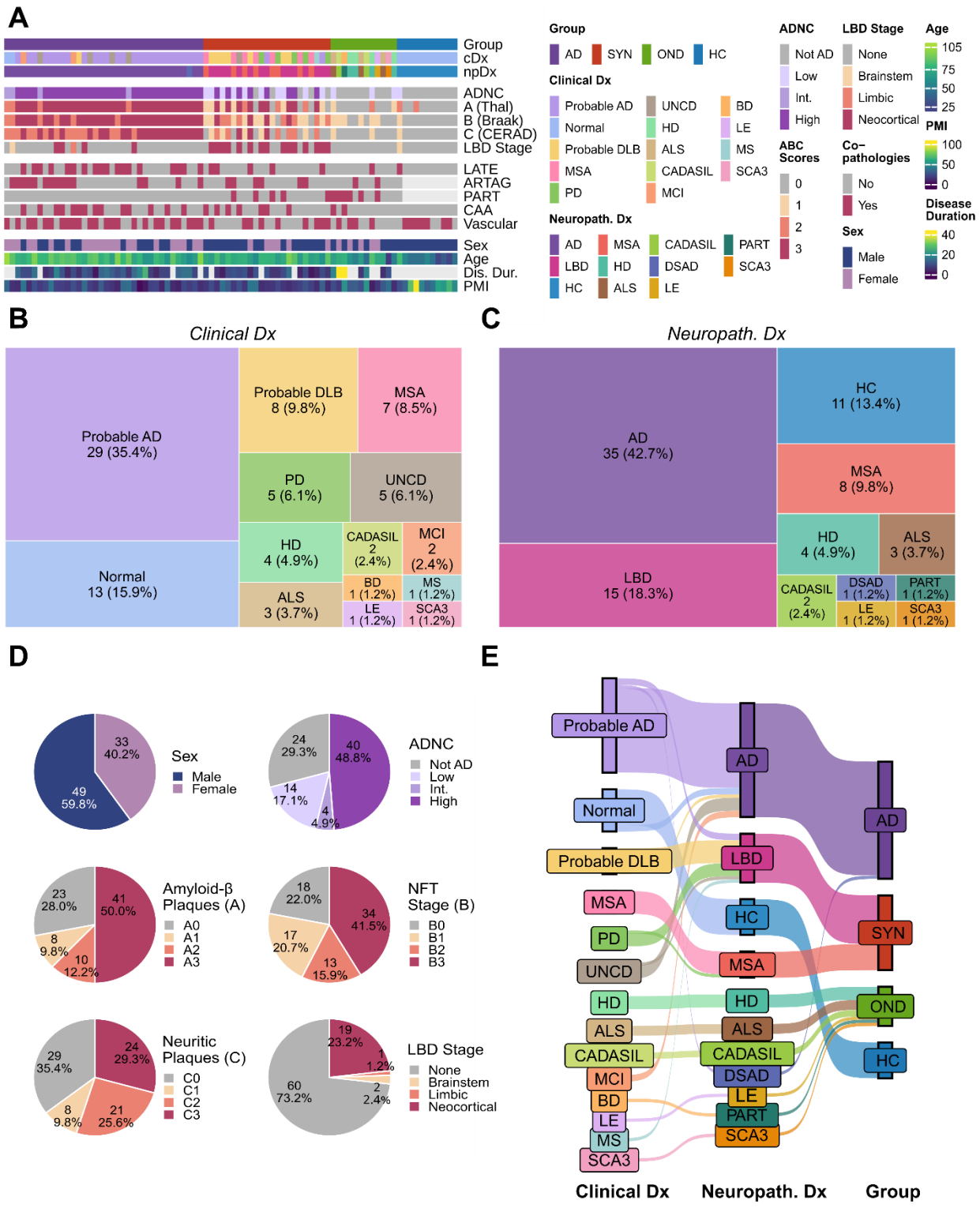
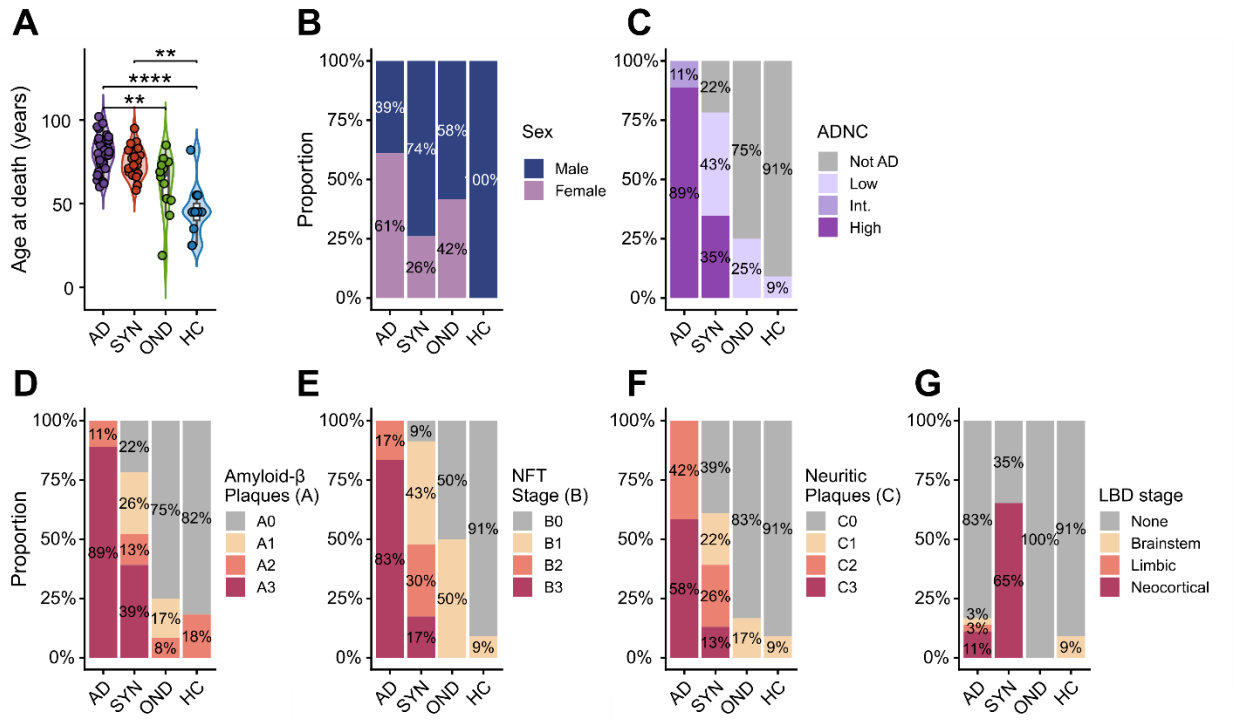
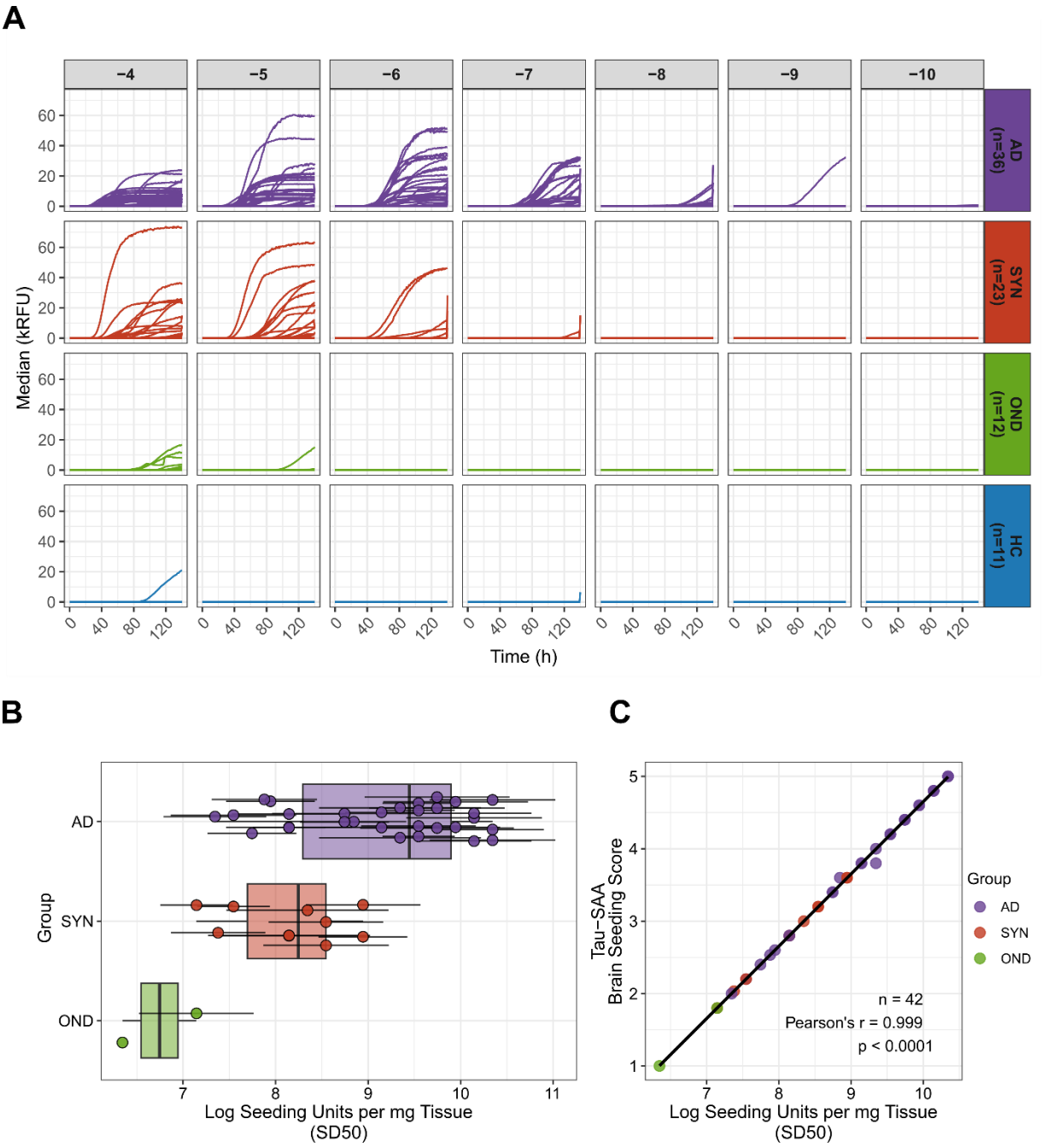


Supplementary Figure 1. Study cohort composition. (A) Annotation heatmap of postmortem brain and CSF cases examined in this study. **(B-C)** Treemap showing distribution of **(B)** Clinical diagnoses (Clinical Dx) and **(C)** neuropathological diagnoses (Neuropath Dx or npDx) across the entire cohort. Number of cases and proportion of all cases indicated as a percent. **(D)** Pie charts

indicating proportions of sex and neuropathological measures including ADNC, Amyloid-β plaques (A), NFT stage (B), Neuritic plaques (C) determined by neuropathological brain examination according to NIA-AA criteria. **(E)** Sankey diagram illustrating the relationship between clinical diagnosis, neuropathological diagnosis, and group assignment for all cases in the cohort. **Abbreviations:** AD, Alzheimer's disease; SYN, synucleinopathy; OND, other neurological disease; HC, healthy control; cDx, clinical diagnosis; npDx, neuropathological diagnosis; ADNC, Alzheimer's disease neuropathological change; LBD, Lewy body disease; MSA, multiple system atrophy; PD, Parkinson's disease; HD, Huntington's disease; ALS, amyotrophic lateral sclerosis; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; MCI, mild cognitive impairment; BD, bipolar disorder; LE, leukoencephalopathy; MS, multiple sclerosis; SCA3, spinocerebellar ataxia type 3; UNCD, unspecified neurocognitive disorder; DSAD, Down syndrome Alzheimer's disease; PART, primary age-related tauopathy; A, amyloid-β plaque score (Thal phase); B, neurofibrillary tangle stage (Braak stage); C, neuritic plaque score (CERAD); LATE, limbic-predominant age-related TDP-43 encephalopathy; ARTAG, aging-related tau astrogliopathy; CAA, cerebral amyloid angiopathy; PMI, postmortem interval; Dis. Dur., disease duration.



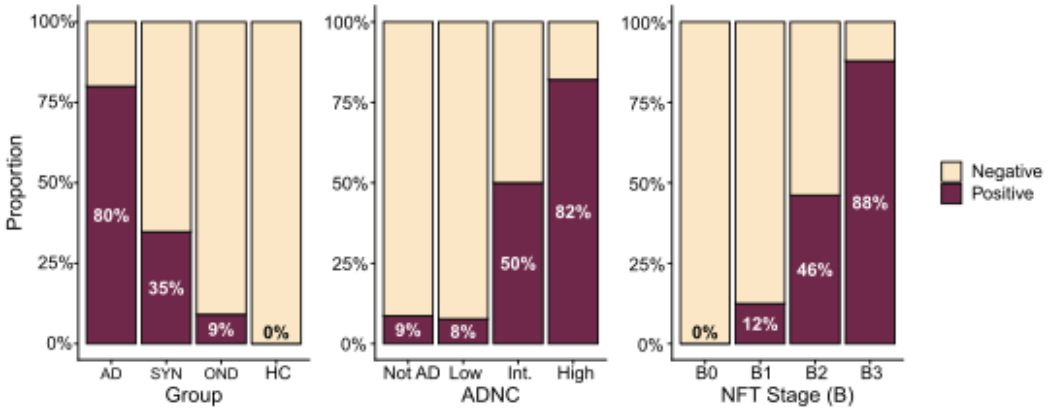
<u>Supplementary Figure 2</u>. Demographics and neuropathological characteristics of experimental groups. (A) Age at death distribution across groups shown as violin plot with overlaid points indicative of individual donors. Significant pairwise differences were determined by Kruskal-Wallis test with Dunn's post-hoc with Benjamini-Hochberg correction and are indicated by brackets. **(B)** Bar plot of sex distribution shown as proportion of male and female. Groups differed in sex distribution (Fisher's exact test, p = 0.002); pairwise comparisons with identified significant differences between AD and HC (*adj p* = 0.002) and AD and SYN (*adj p* = 0.046). **(C-G)** Neuropathological burden across groups show as bar plots representing the proportion of donors at each stage for **(C)** ADNC, **(D)** Aβ/amyloid plaque ("A") score (**E)** NFT stage ("B"), **(F)** neuritic plaque score ("C"), and **(G)** LBD stage.



<u>Supplementary Figure 3.</u> Spearman-Kärber end-point dilution analysis validates Tau-SAA seeding score as a quantitative metric of tau seeding activity in brain tissue. (A) Tau-SAA Thioflavin T (ThT) fluorescence curves from all 82 samples. Brain homogenates (10% w/v) were serially diluted from 10^{-4} to 10^{-10} and used as seeds for Tau-SAA. Traces represent the median ThT (*n* = 5-6 replicates) per sample per dilution. Columns denote the ten-fold serial dilution ($10^{indicated}$) while rows denote experimental group. **(B)** Log-transformed seeding units per mg brain tissue (SD_{50}) estimated using the Spearman- Kärber for 42 samples achieving 100% positivity at one or more dilutions (AD, n = 30, SYN, n = 10, OND, n = 2). Points represent individual donors

while horizontal error bars indicate 95% confidence intervals. **(C)** Correlation analysis between Tau-SAA seeding score and SD_{50} (*n* = 42, Pearson's r = 0.999, p < 0.0001).

A



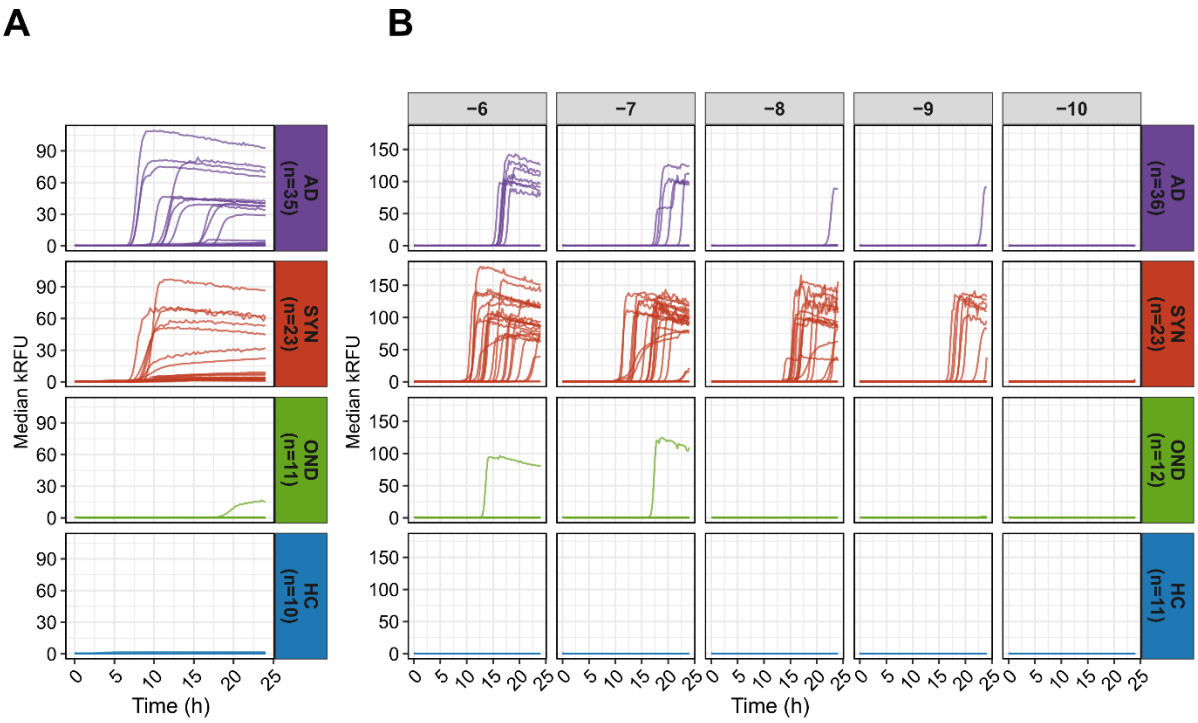
B

CSF Tau-SAA Diagnostic Performance

Comparison	AUC (95% CI)	Optimal Threshold	Sensitivity	Specifcity	PPV	NPV
AD vs HC	0.95 (0.89–0.95)	0.7	0.89	1.00	1.00	0.71
AD vs all others	0.86 (0.77–0.95)	1.7	0.86	0.77	0.75	0.87
High/Int ADNC vs rest	0.92 (0.86–0.99)	1.7	0.90	0.88	0.88	0.90
Braak B2/B3 vs rest	0.92 (0.85–0.98)	1.9	0.80	0.97	0.97	0.78

PPV: Positive Predictive Value; NPV: Negative Predictive Value. Optimal threshold by Youden's index.

<u>Supplementary Figure 4.</u> Tau-SAA CSF neat dilution positivity and diagnostic performance metrics. Related to Figure 2. (A) Proportion of CSF Tau-SAA positive and negative at neat dilution stratified by group (left), ADNC (middle) and NFT stage (right). Samples were deemed positive if at least three of five replicates exceeded a predefined fluorescence threshold of 1000 au. **(B)** Diagnostic performance of CSF Tau-SS as a continuous predictor. Table reports area under the receiver operating characteristic curve (AUC) with 95% confidence interval, optimal threshold determined by Youden's index, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for three binary comparisons: AD versus all other groups, high or intermediate ADNC versus low or absent, and advanced NFT stage (B2/B3) versus early or absent (B0/B1).



Supplementary Figure 5. αSyn-SAA traces for matched brain and CSF samples. (A-B) Thioflavin T (ThT) fluorescence over time traces for **(A)** CSF and **(B)** brain samples. Each trace denotes an individual donor and represents the median of three technical replicates. Rows represent experimental groups with corresponding *n* indicated for each facet. Columns in **(B)** represent ten-fold serial dilutions in $1\times10^{\text{indicated}}$ annotation.

Supplementary Table 1. Cohort demographic and neuropathological characteristics.

Characteristic	Overall N = 82[1]	AD N = 36[1]	SYN N = 23[1]	OND N = 12[1]	HC N = 11[1]	p-value[2]
Age	71.2 (16.8)	79.9 (10.5)	74.5 (8.8)	62.1 (18.0)	45.6 (15.7)	<0.001
Sex						<0.001
Male	49 (60%)	14 (39%)	17 (74%)	7 (58%)	11 (100%)	
Female	33 (40%)	22 (61%)	6 (26%)	5 (42%)	0 (0%)	
Race						0.455
Asian	2 (2.4%)	0 (0%)	1 (4.3%)	1 (8.3%)	0 (0%)	
Black	1 (1.2%)	1 (2.8%)	0 (0%)	0 (0%)	0 (0%)	
Other	1 (1.2%)	1 (2.8%)	0 (0%)	0 (0%)	0 (0%)	
Unknown	2 (2.4%)	0 (0%)	2 (8.7%)	0 (0%)	0 (0%)	
White	76 (93%)	34 (94%)	20 (87%)	11 (92%)	11 (100%)	
PMI	25.4 (16.1)	19.8 (9.6)	20.6 (8.8)	27.0 (14.2)	51.5 (21.6)	<0.001
Disease duration	10.6 (7.3)	10.6 (5.1)	7.5 (3.2)	19.6 (14.9)	NA (NA)	0.026
ADNC						<0.001
Not AD	24 (29%)	0 (0%)	5 (22%)	9 (75%)	10 (91%)	
Low	14 (17%)	0 (0%)	10 (43%)	3 (25%)	1 (9.1%)	
Int.	4 (4.9%)	4 (11%)	0 (0%)	0 (0%)	0 (0%)	
High	40 (49%)	32 (89%)	8 (35%)	0 (0%)	0 (0%)	
A (Thal Phase)						<0.001
A0	23 (28%)	0 (0%)	5 (22%)	9 (75%)	9 (82%)	
A1	8 (9.8%)	0 (0%)	6 (26%)	2 (17%)	0 (0%)	
A2	10 (12%)	4 (11%)	3 (13%)	1 (8.3%)	2 (18%)	
A3	41 (50%)	32 (89%)	9 (39%)	0 (0%)	0 (0%)	
B (Braak Stage)						<0.001
B0	18 (22%)	0 (0%)	2 (8.7%)	6 (50%)	10 (91%)	
B1	17 (21%)	0 (0%)	10 (43%)	6 (50%)	1 (9.1%)	
B2	13 (16%)	6 (17%)	7 (30%)	0 (0%)	0 (0%)	
B3	34 (41%)	30 (83%)	4 (17%)	0 (0%)	0 (0%)	

Characteristic	Overall N = 82[1]	AD N = 36[1]	SYN N = 23[1]	OND N = 12[1]	HC N = 11[1]	p-value[2]
C (CERAD Score)						<0.001
C0	29 (35%)	0 (0%)	9 (39%)	10 (83%)	10 (91%)	
C1	8 (9.8%)	0 (0%)	5 (22%)	2 (17%)	1 (9.1%)	
C2	21 (26%)	15 (42%)	6 (26%)	0 (0%)	0 (0%)	
C3	24 (29%)	21 (58%)	3 (13%)	0 (0%)	0 (0%)	
LBD Stage						<0.001
None	60 (73%)	30 (83%)	8 (35%)	12 (100%)	10 (91%)	
Brainstem	2 (2.4%)	1 (2.8%)	0 (0%)	0 (0%)	1 (9.1%)	
Limbic	1 (1.2%)	1 (2.8%)	0 (0%)	0 (0%)	0 (0%)	
Neocortical	19 (23%)	4 (11%)	15 (65%)	0 (0%)	0 (0%)	
CAA[3]	23 (28%)	14 (39%)	7 (30%)	2 (17%)	0 (0%)	0.054
LATE[3]	25 (30%)	18 (50%)	5 (22%)	1 (8.3%)	1 (9.1%)	0.008
ARTAG[3]	23 (32%)	15 (42%)	6 (26%)	2 (17%)	0 (0%)	0.298
PART[3]	11 (15%)	0 (0%)	5 (22%)	6 (50%)	0 (0%)	<0.001
Cerebrovascular Disease[3]	28 (39%)	20 (56%)	7 (30%)	1 (8.3%)	0 (0%)	0.010
Hippocampal Sclerosis[3]	3 (4.2%)	2 (5.6%)	1 (4.3%)	0 (0%)	0 (0%)	>0.999

[1]Mean (SD); n (%)

[2]Kruskal-Wallis rank sum test (Age, PMI, Disease Duration); Fisher's exact test, Monte Carlo simulated p-value (all categorical variables)

[3]Dichotomized as present or absent.

Supplementary Table 2. Linear mixed model estimates for tau seeding score in AD group donors

Predictor	β	SE	95% CI	t	df	p
Matrix (CSF vs Brain)	**0.679**	**0.282**	**[0.107, 1.252]**	**2.413**	**33**	**0.022**
CSF aSyn-SAA+	**1.076**	**0.411**	**[0.236, 1.916]**	**2.620**	**29**	**0.014**
NFT stage	**2.183**	**0.605**	**[0.946, 3.421]**	**3.608**	**29**	**0.001**
Age at death (scaled)	-0.449	0.247	[-0.954, 0.055]	-1.821	29	0.079
Sex (Female)	0.499	0.453	[-0.428, 1.425]	1.101	29	0.28

Model: tau seeding ~ matrix + CSF aSyn-SAA status + NFT stage + age + sex + (1|donor).

β = unstandardized coefficient; SE = standard error; CI = confidence interval; df = Kenward-Roger degrees of freedom.

Supplementary Table 3. Interaction linear mixed model estimates for tau seeding score.

Predictor	β	SE	95% CI	t	df	p
Matrix (CSF vs Brain)	0.549	0.381	[-0.227, 1.325]	1.442	32.0	0.159
CSF aSyn-SAA+	0.928	0.501	[-0.076, 1.933]	1.853	52.8	0.069
NFT stage	**2.183**	**0.605**	**[0.946, 3.421]**	**3.608**	**29.0**	**0.001**
Age at death (scaled)	-0.449	0.247	[-0.954, 0.055]	-1.821	29.0	0.079
Sex (Female)	0.499	0.453	[-0.428, 1.425]	1.101	29.0	0.28
Matrix × CSF aSyn-SAA+	0.295	0.573	[-0.873, 1.463]	0.515	32.0	0.61
Model comparison (additive vs. interaction)						*0.596*

Model: tau_value ~ matrix × CSF aSyn-SAA status + NFT stage + age + sex + (1|donor).

The interaction term tests whether the effect of CSF aSyn-SAA status on tau seeding differs between brain and CSF matrices. Model comparison p-value derived by likelihood ratio test against the additive model ($\chi^2(1) = 0.281$, $p = 0.596$).

β = unstandardized coefficient; SE = standard error; CI = confidence interval; df = Satterthwaite degrees of freedom.

Supplementary Table 4. Independent linear model estimates for brain and CSF tau seeding scores in AD group donors.

Model	Predictor	β	SE	95% CI	t	df	p
Brain Tau-SAA	**CSF aSyn-SAA+**	**0.837**	**0.339**	**[0.143, 1.531]**	**2.468**	**29**	**0.02**
	NFT stage	**1.822**	**0.500**	**[0.8, 2.844]**	**3.646**	**29**	**0.001**
	Age at death (scaled)	**-0.646**	**0.204**	**[-1.062, -0.229]**	**-3.169**	**29**	**0.004**
	Sex (Female)	-0.015	0.374	[-0.78, 0.75]	-0.041	29	0.967
CSF Tau-SAA	**CSF aSyn-SAA+**	**1.315**	**0.611**	**[0.066, 2.563]**	**2.154**	**29**	**0.04**
	NFT stage	**2.545**	**0.900**	**[0.705, 4.385]**	**2.829**	**29**	**0.008**
	Age at death (scaled)	-0.253	0.367	[-1.003, 0.497]	-0.689	29	0.496
	Sex (Female)	1.013	0.673	[-0.364, 2.39]	1.504	29	0.143

Models were fit separately within each matrix regressing tau seeding score on CSF aSyn-SAA status, NFT stage, age at death, and sex.

β = unstandardized coefficient; SE = standard error; CI = confidence interval; df = residual degrees of freedom.

Supplementary Table 5. Linear model estimates for brain αSyn seeding score in synucleinopathy donors.

Predictor	β	SE	95% CI	t	df	p
Brain Tau-SS	0.092	0.176	[-0.275, 0.46]	0.523	20	0.606
Diagnosis (LBD vs MSA)	**1.484**	**0.420**	**[0.608, 2.36]**	**3.535**	**20**	**0.002**

Model: brain αSyn seeding score ~ brain Tau-SS + neuropathological diagnosis (LBD vs MSA). Age at death and sex were evaluated as additional covariates but were not retained based on AIC comparison.

β = unstandardized coefficient; SE = standard error; CI = confidence interval; df = residual degrees of freedom.