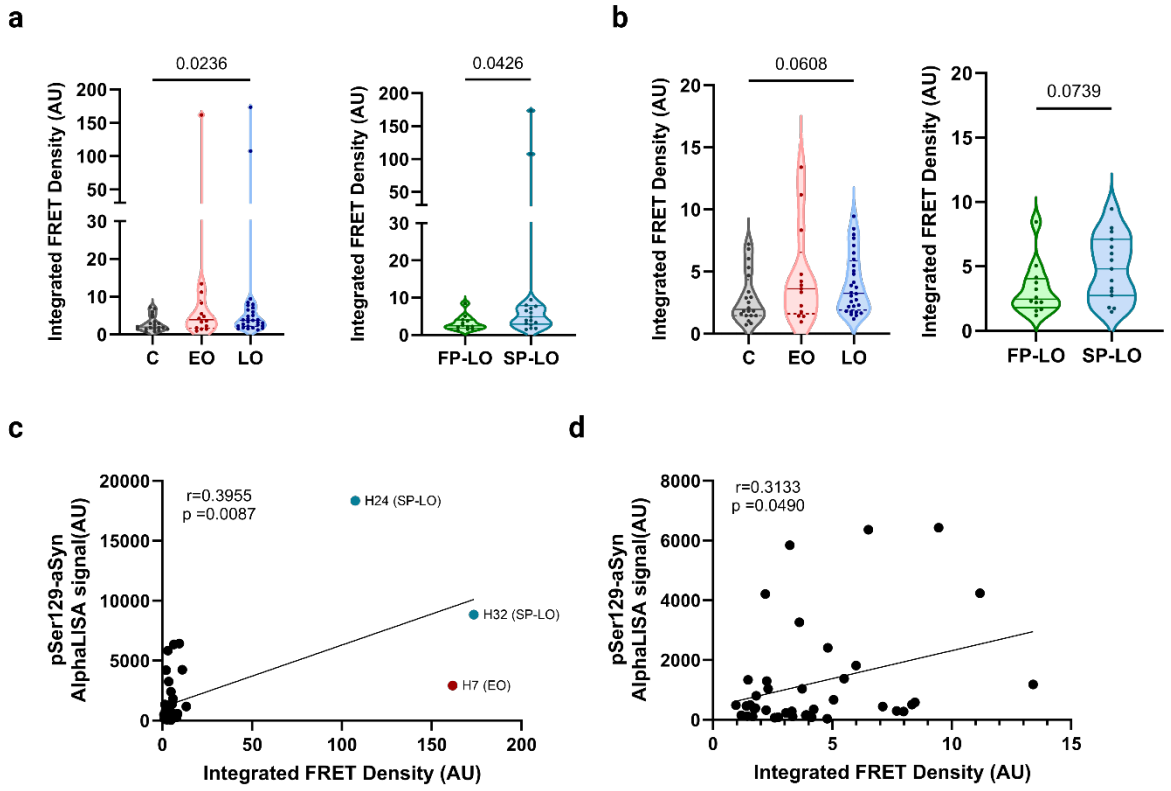
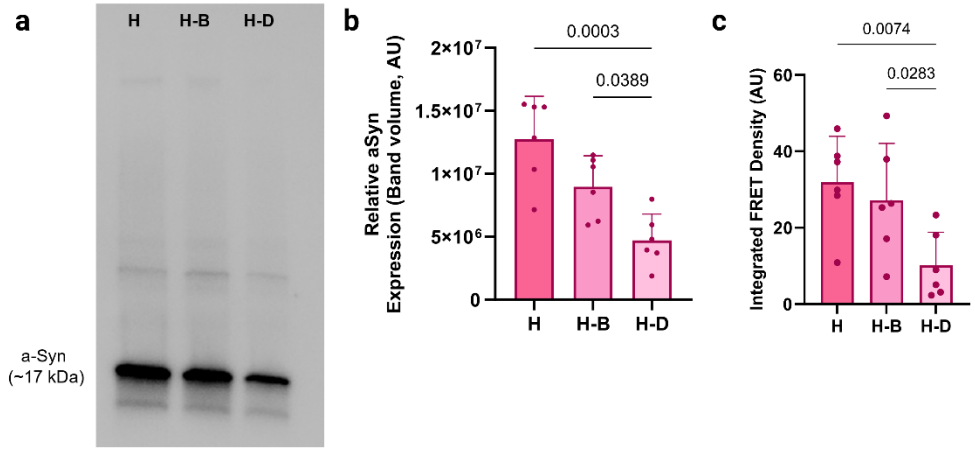
Molecular profiling of alpha-synuclein pathology and seeding activity in Parkinson's disease

Supplementary Figure 1



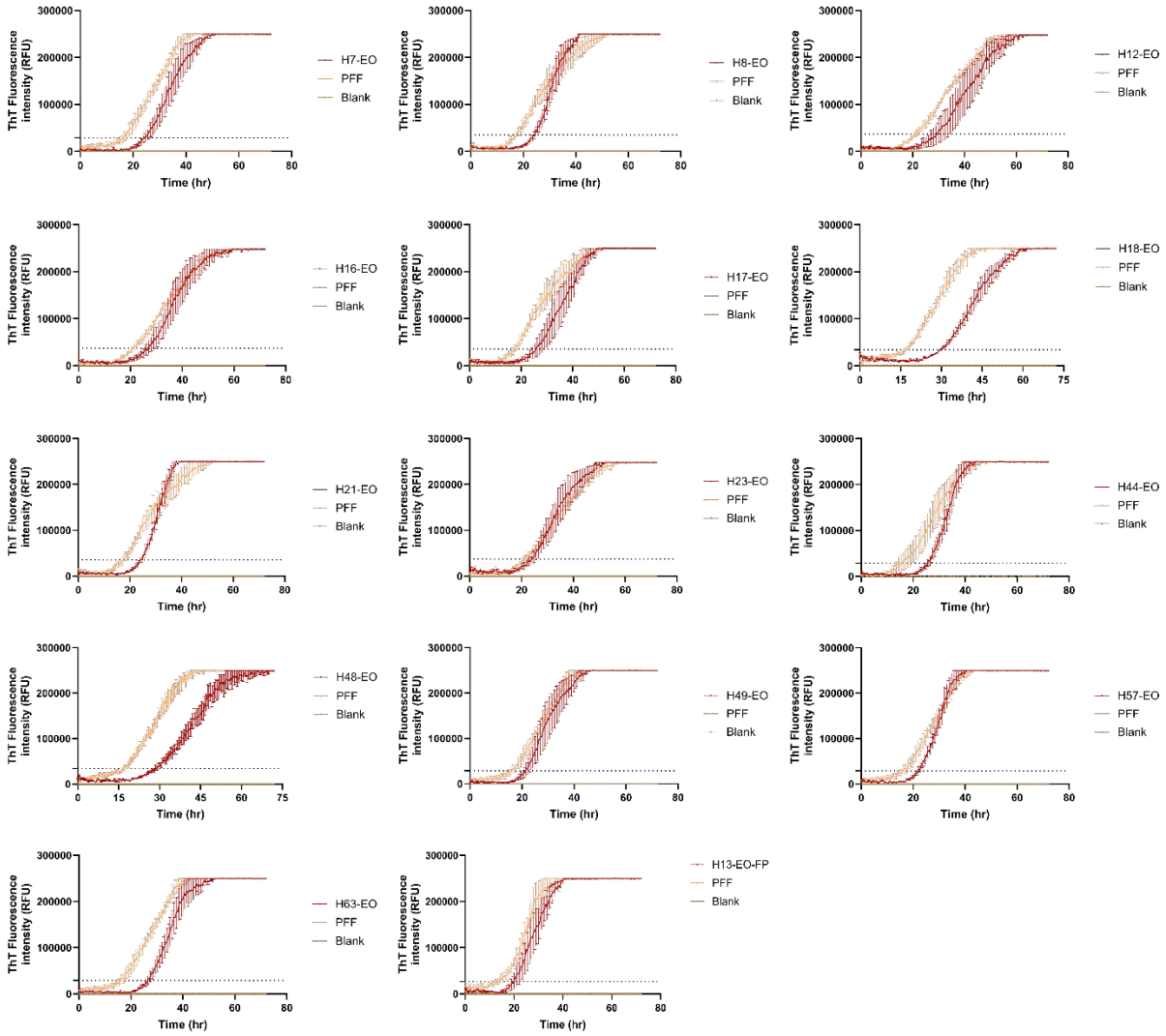
Supplementary Fig. 1 Individual variability in aSyn seeding bioactivity. (**a**) Distribution of FRET-based seeding activity across individual PD cases demonstrates substantial interindividual differences, with several outlier samples contributing to group-level significance. (**b**) When these outliers were excluded, the overall differences between subgroups decreased in significance, although the trend toward elevated seeding activity in LOPD compared to controls (C) remained. (**c**) Spearman correlation analysis revealed a positive association between pSer129-aSyn levels and FRET seeding activity across the cohort. (**d**) Removal of outlier samples reduced the strength of this correlation, suggesting that the relationship is partly driven by individual cases. Together, these analyses highlight the individual variability in seeding activity and emphasize the importance of considering case-specific effects when interpreting subgroup differences. Panels a and c reproduce the analyses shown in Figure 3 and are included here to allow direct comparison with the corresponding analyses performed after exclusion of the three outlier cases.

Supplementary Figure 2



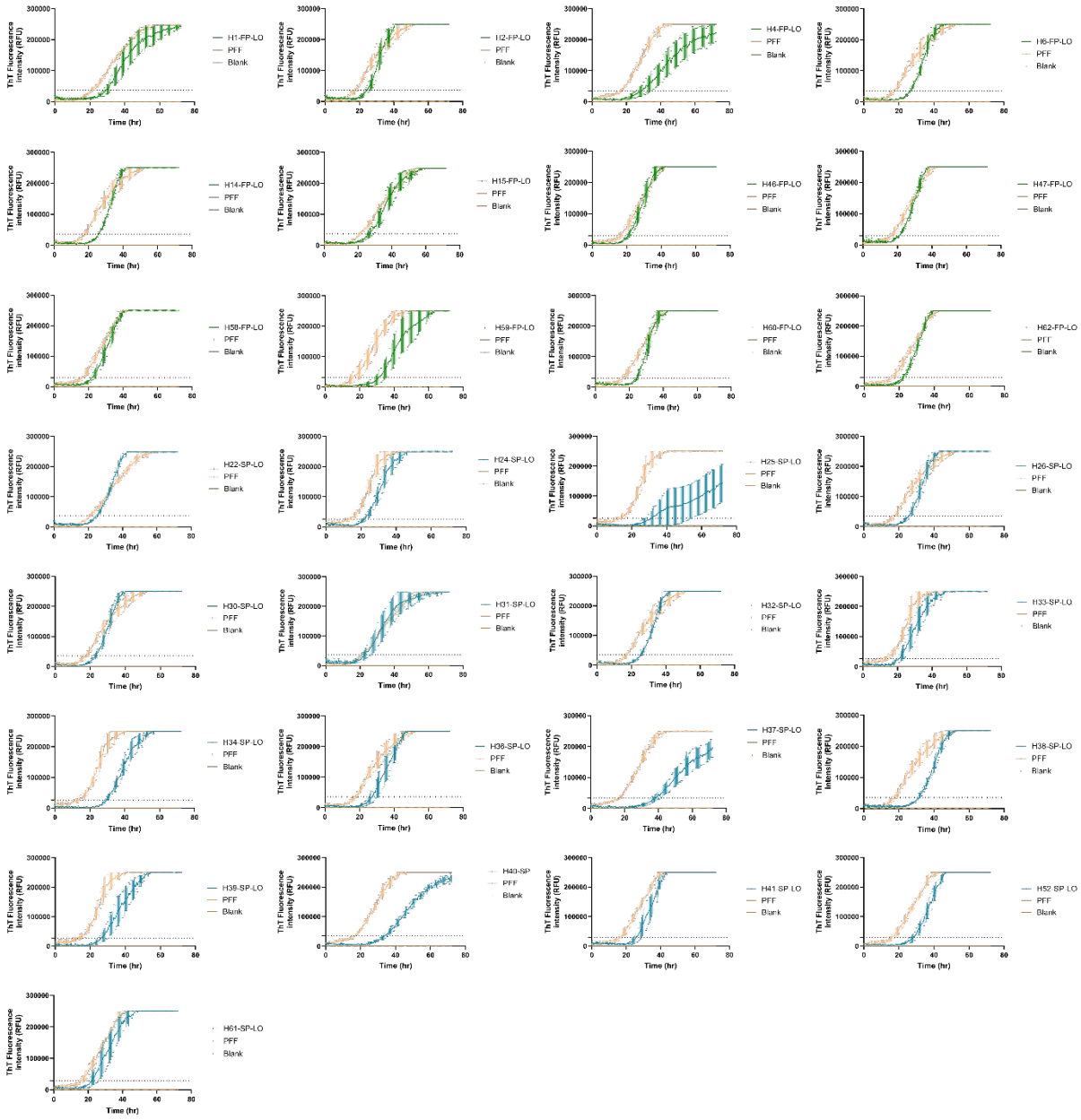
Supplementary Fig. 2 Immunodepletion of aSyn from PD brain homogenates reduces seeding bioactivity. (**a, b**) WB analysis showing reduction of aSyn after immunodepletion with anti-α-synuclein 211 antibody conjugated Protein G Dynabeads (H-D) compared to untreated homogenates (H) and homogenates incubated with unconjugated beads alone (H-B). (**c**) Immunodepleted PD homogenates (H-D) displayed significantly reduced seeding bioactivity in HEK.A53T-C/Y cells compared to untreated homogenates (H) and homogenates incubated with unconjugated beads (H-B). Samples incubated with unconjugated beads (H-B) show a modest reduction consistent with nonspecific protein loss.

Supplementary Figure 3



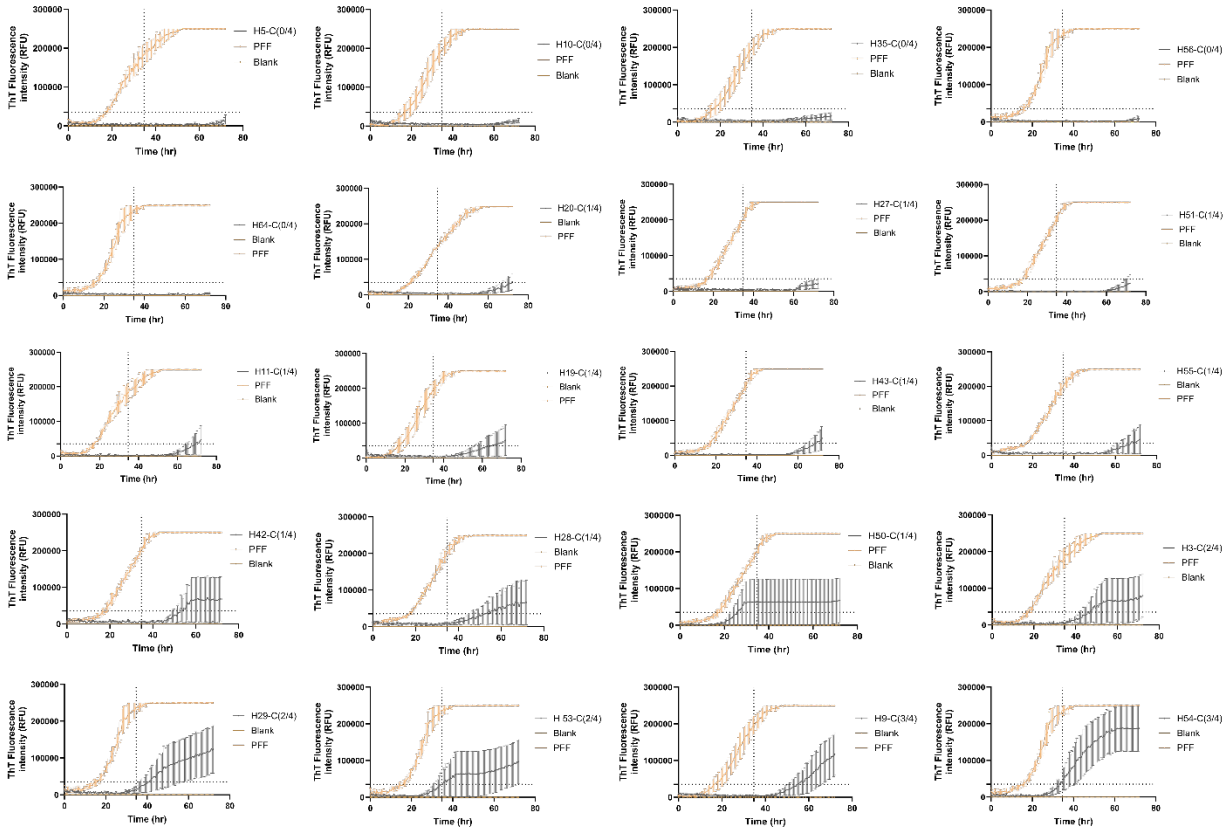
Supplementary Fig. 3 RT-QuIC amplification curves. Individual ThT fluorescence traces for EOPD individual samples. Curves show the mean RFU values of quadruplicate wells for each case. All EOPD samples consistently exhibited rapid and robust amplification (4/4). RFU: Relative fluorescent unit.

Supplementary Figure 4



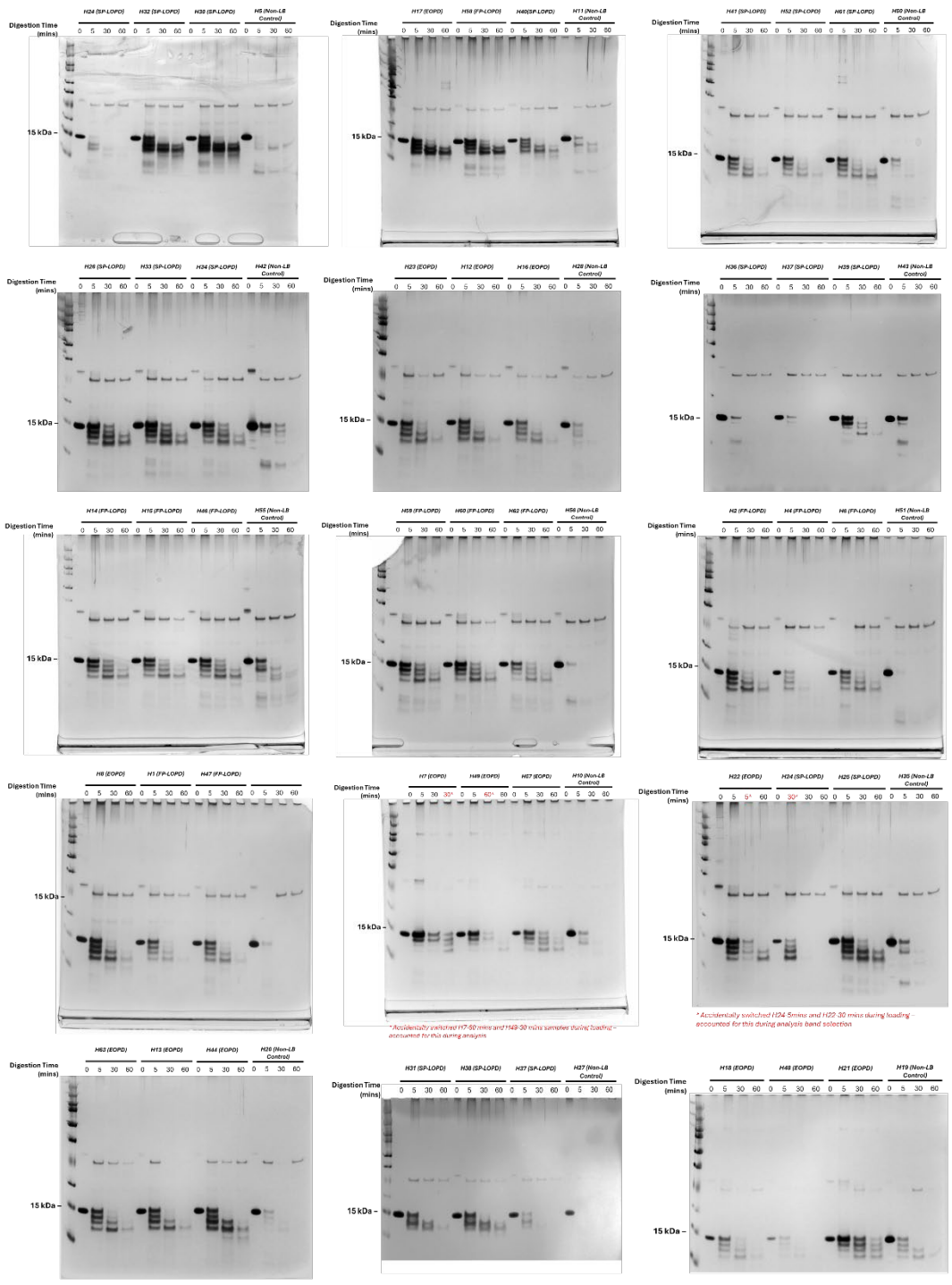
Supplementary Fig. 4 RT-QuIC amplification curves. Individual ThT fluorescence traces for LOPD individual samples. Curves show the mean RFU values of quadruplicate wells for each case. All LOPD samples consistently exhibited rapid and robust amplification (4/4). RFU: Relative fluorescent unit.

Supplementary Figure 5



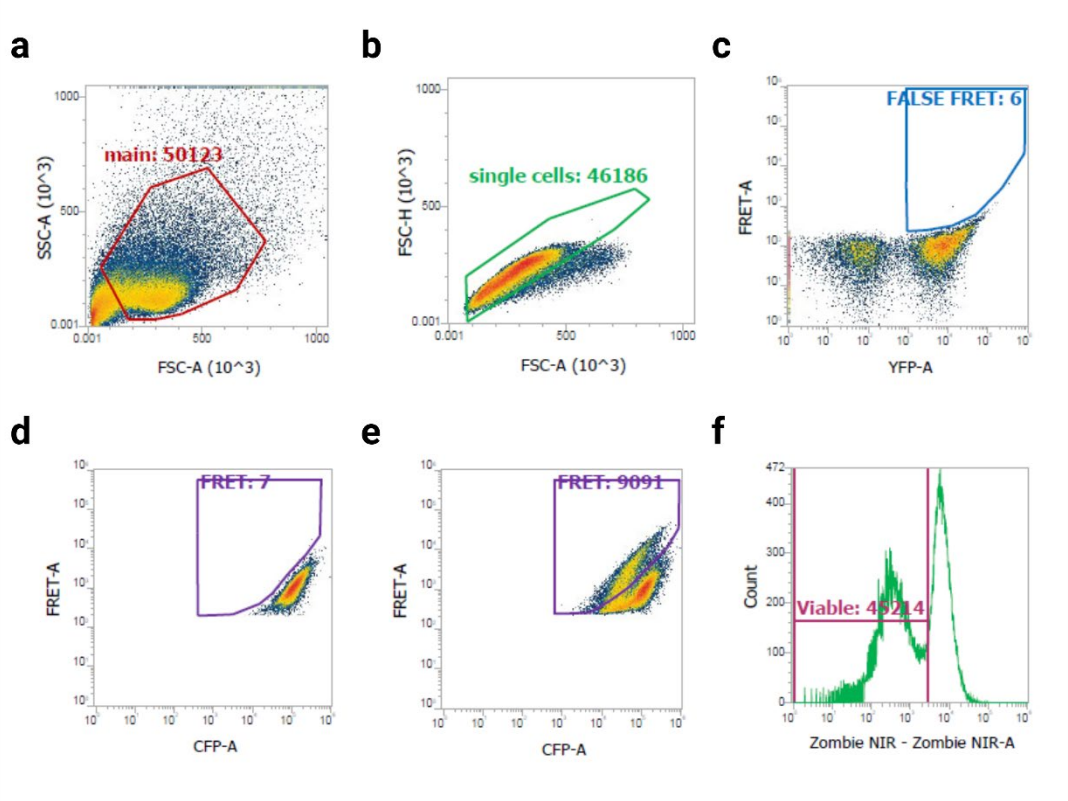
Supplementary Fig. 5 RT-QuIC amplification curves. Individual ThT fluorescence traces for non-LB control individual samples. Curves show the mean RFU values of quadruplicate wells for each case. Among controls, 15 were classified as negative (0 or 1/4), 3 as inconclusive (2/4), and 2 as positive (3/4), reflecting occasional seeding activity in non-diseased samples. Vertical threshold represents lag time cutoff at 34.7 hours. RFU: Relative fluorescent unit.

Supplementary Figure 6



Supplementary Fig. 6 Silver Stain gels used for PK digestion. Note that two sample time points were switched on two gels (*), but these were accounted for during statistical analysis. EOPD: Early-onset PD; SP-LOPD: Slow-progressing late-onset LOPD; FP-LOPD: Fast-progressing late-onset PD; non-LB controls: Non-Lewy Body controls.

Supplementary Figure 7



Supplementary Fig. 7 Gating strategy for FRET-FC. Cell population **(a)** and single cell **(b)** gates are drawn with standard flow cytometry methodology using WT HEK293T cells. **(c)** A false FRET gate is drawn from combination of HEK.A53T-YFP and WT HEK293T cells to eliminate YFP bleed through into the FRET filter. **(d)** A FRET gate is drawn by empty lipofectamine-treated HEK.A53T-C/Y cells. **(e)** A population shift into the FRET gate detected following treatment with seed-positive samples such as PFFs. **(f)** Viability gate is drawn by using WT HEK293T cells and confirmed by empty lipofectamine-treated HEK.A53T-C/Y cells.