Title: Ultrasonication-based rapid amplification of α-synuclein aggregates in cerebrospinal fluid.

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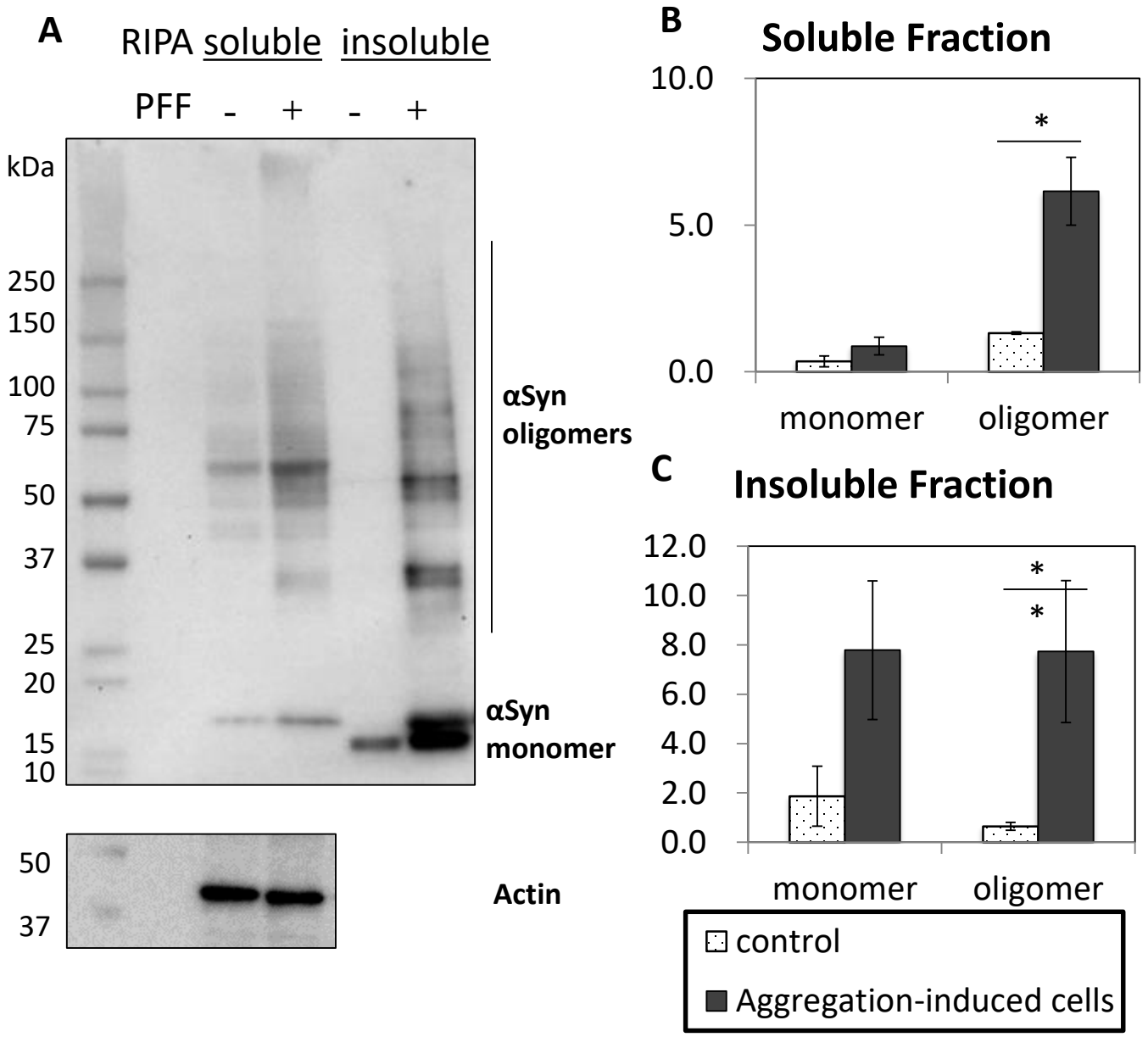


Fig. S1. Intracellular levels of α-synuclein oligomers are elevated in aggregation-induced cells by PFF seeding.

(A) Western blot analysis of the lysates of α-synuclein aggregation-induced cells by PFF seeding and controls. An anti-α-synuclein antibody (Syn 211) was used for detection. (B) Quantitative analysis of the relative amounts of soluble α-synuclein monomers/oligomers adjusted to the levels of actin in the soluble fraction. (C) Quantitative analysis of α-synuclein in the insoluble fraction (n = 4). Aggregation-induced cells had higher levels of α-synuclein oligomers than control cells in the soluble and insoluble fractions ($p < 0.01$ and $p < 0.05$, respectively, unpaired two-tailed t-test).

Fig. S2. A representative full-length blot for α-synuclein of cell lysates with the primary Syn 211 antibody. S1-A. The cropped area indicated by the red box is shown in Fig. S1-A.

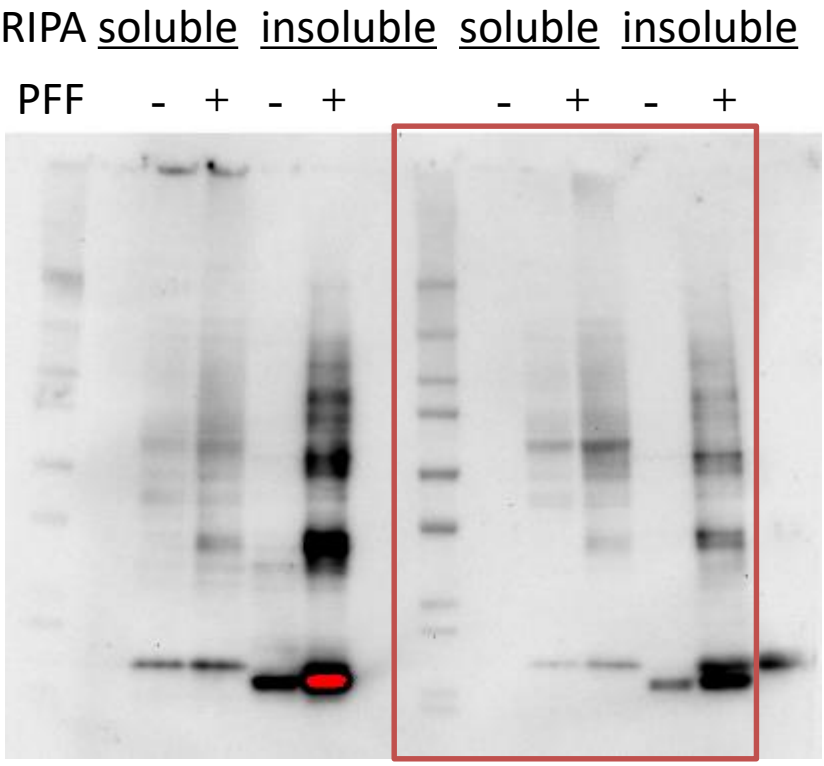
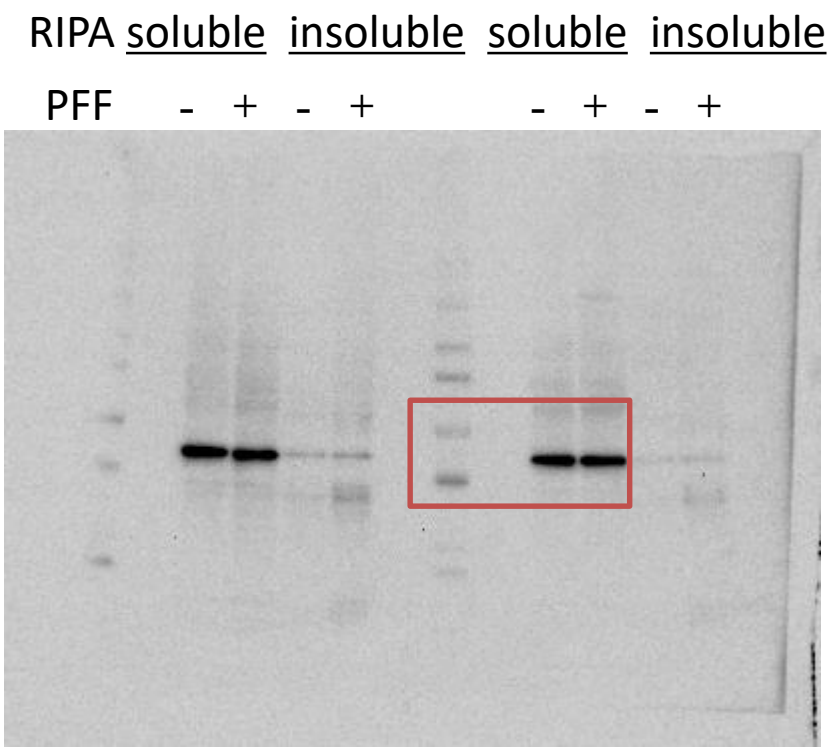


Fig. S3. A representative full-length blot for actin of cell lysates with an anti-actin clone C4 antibody. The cropped area indicated by the red box is shown in Fig. S1-A.



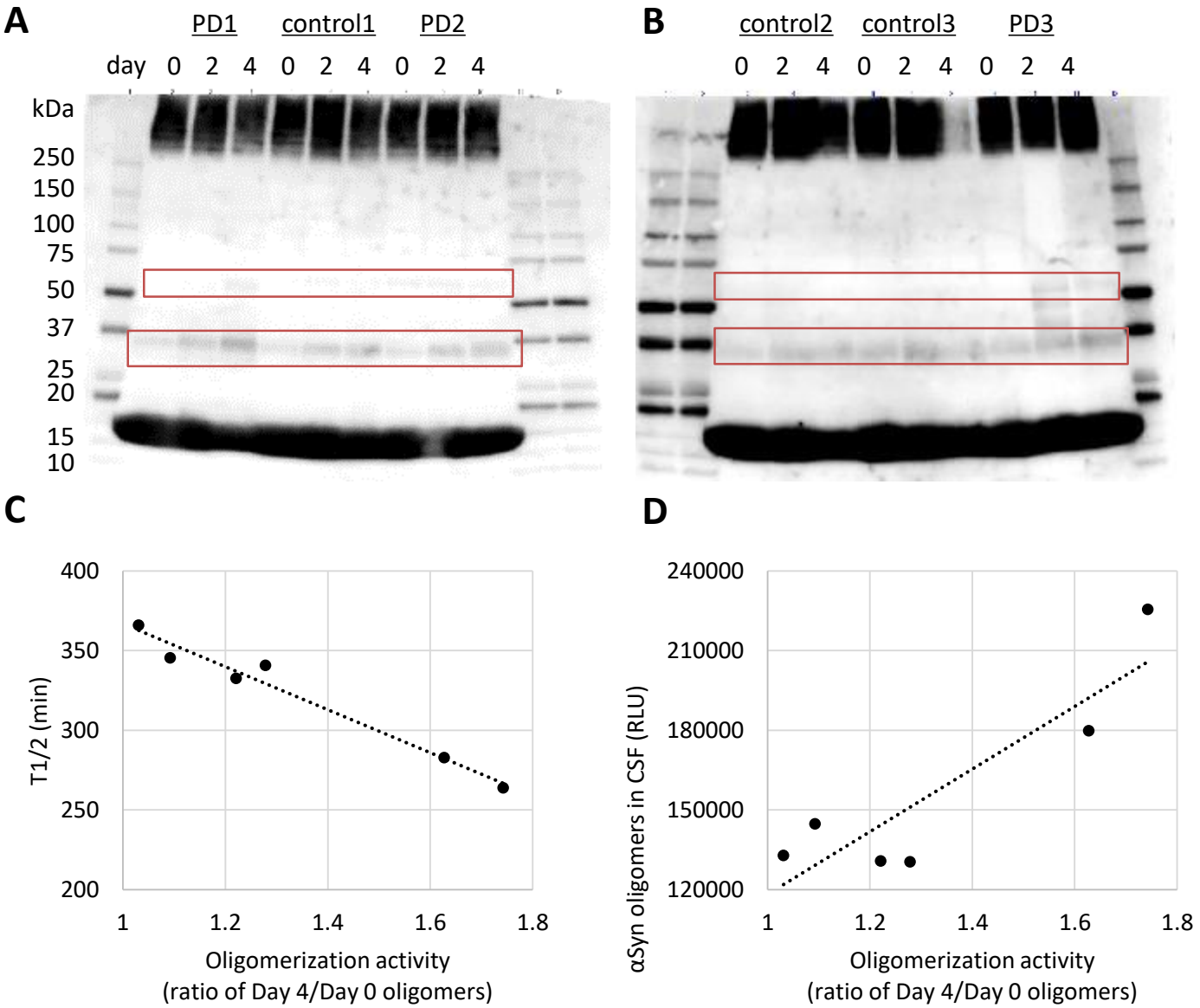


Fig. S4. Oligomeric activity of CSF correlates with the seeding activity of HANABI and α-synuclein oligomers in CSF measured by ELISA.

CSF from six patients (three PD, and three controls) were mixed, respectively, with a pre-filtered α-synuclein monomer solution in 50 mM Tris-HCl and 150 mM NaCl at a v/v ratio of 1:9. Final concentration of α-synuclein was 500 μg/mL. The samples were incubated with continuous shaking (600 rpm) at 37 ° C , and collected at days 0, 2, and 4. The collected samples were centrifuged immediately at 13,500 rpm at 4 ° C for 30 min, and the supernatants were used for the detection of oligomers by western blot analysis. The primary antibody used for western blot was a mouse anti-human α-synuclein oligomer-specific antibody (ASyO5; AS13 2718, Agrisera, Vännäs, Sweden) and the secondary antibody was a sheep anti-mouse peroxidase-linked antibody (NA931; GE Healthcare, Little Chalfont, UK). Oligomerization activity was calculated by the day 4/day 0 ratio of the total intensity of the bands at 33 and 55 kDa.

(A) (B) Western blot analysis of the incubated α-synuclein solution with CSF. The high-molecular band indicated with red boxes was analyzed to calculate the oligomerization activity of each patient. (C) (D) Dot plot of oligomerization of CSF and T1/2 and α-synuclein oligomers in CSF measured by ELISA. Oligomerization activity was significantly correlated with T1/2 (Pearson correlation test, $r = -0.984$, $p < 0.001$) and oligomers in CSF (Pearson correlation test, $r = -0.891$, $p = 0.02$).