Supplementary Material

Amyloid-β Oligomers Serve as Nucleation Sites for α-Synuclein Aggregation

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Supplementary Figures

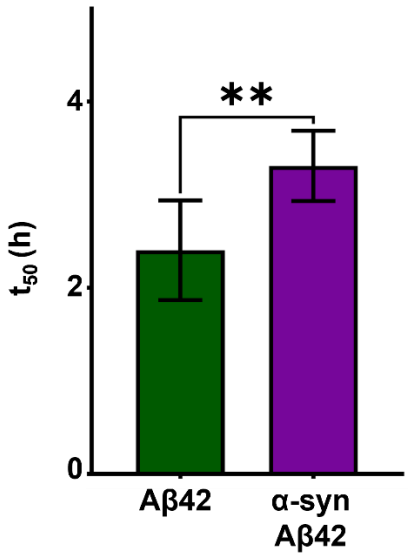

Fig. S1. Aggregation t50 of Aβ42 and α-syn aggregated with Aβ42. The t50 of 3 independent aggregation repeats were averaged. Error bars are shown as SD. Unpaired, non-parametric Mann Whitey's test, where p = ≥0.05 (ns), 0.01-0.05 (*), 0.001-0.01 (**), 0.0001-0.001 (***) and <0.0001 (****).

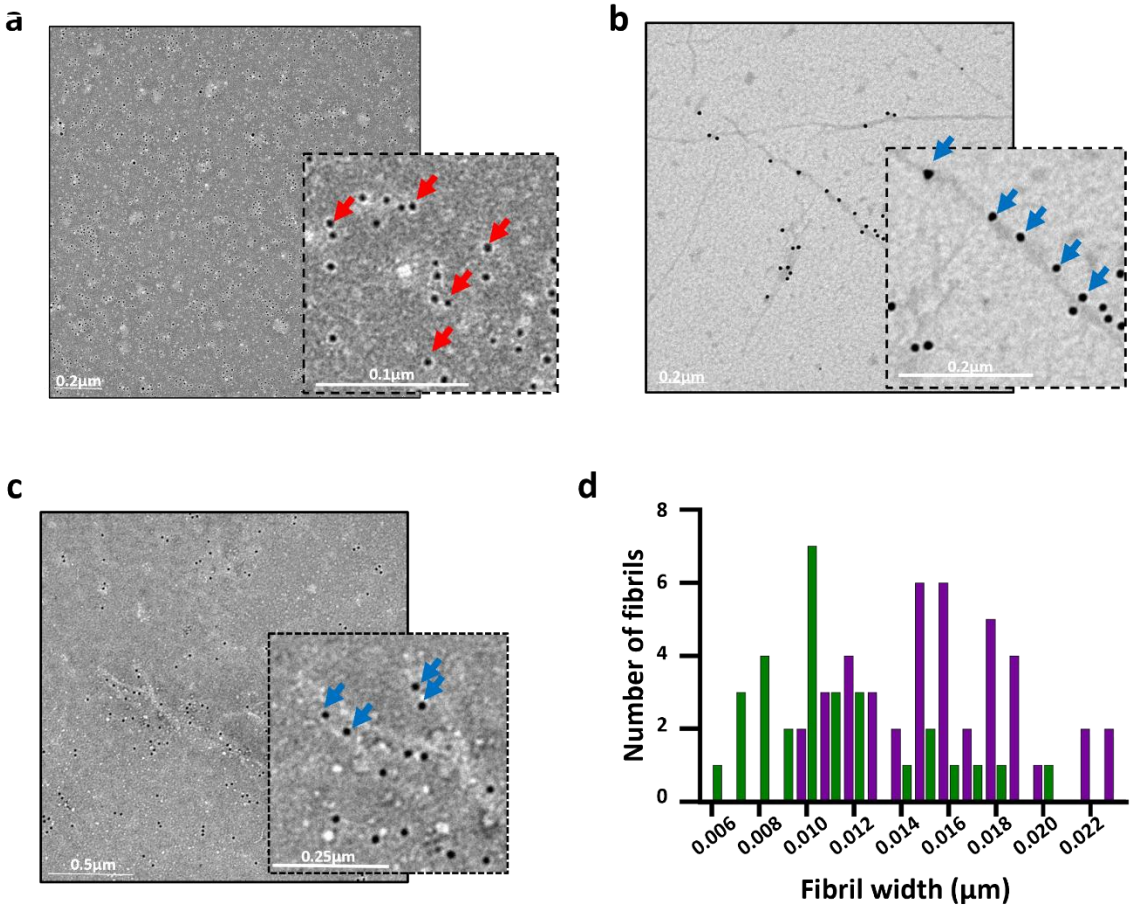

Fig. S2. Immunogold labelling and negative stain TEM of (a) α-syn, (b) Aβ42 monomers and (c) Aβ42 fibrils after aggregation. Red arrows indicate 6nm gold particles which bind to the anti-α-syn primary antibody and blue arrows indicate 10nm gold particles which bind to the 6E10 anti-Aβ antibody. **(d)** Histogram analysis of immuno-labelled fibrils (Fig. 2a and S2b) to show the distribution of fibrils width formed by Aβ42 alone (n=30) and α-syn aggregated with Aβ42 (n=42). Analysis of the width of these fibrils revealed 25th and 75th percentiles of width distributions as 8/12.5 and 123/18 nm for Aβ42 and the co-incubation sample respectively.

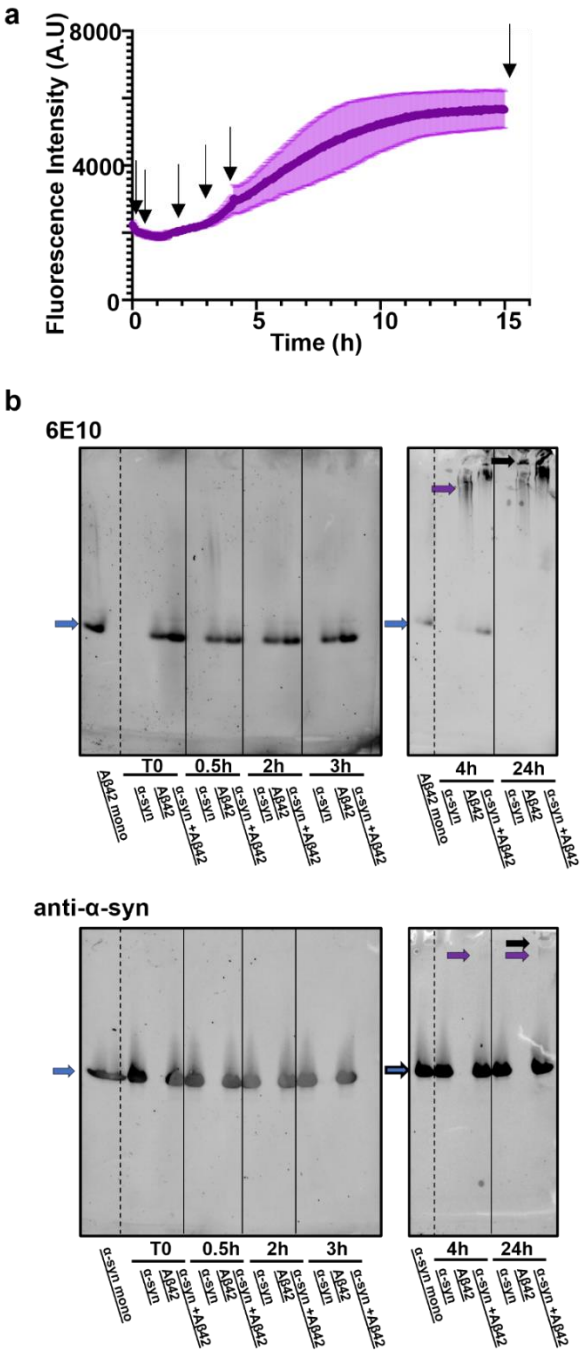

Fig. S3. Time course Native PAGE and western blot analysis of co-incubation samples. (a) Aliquots of α-syn alone, Aβ42 alone and the co-incubation samples (shown) were taken at progressive time points during the aggregation (black arrows). **(b)** Samples were analyzed by Native PAGE and western blotting detected with both the 6E10 (top) and anti-α-syn (bottom) antibodies. Our results show that higher molecular weight assemblies of α-syn (purple and black arrows arrows) are not seen until oligomers of Aβ42 are formed (purple arrow) at 4h, as compared to the monomers of both proteins (blue arrows).

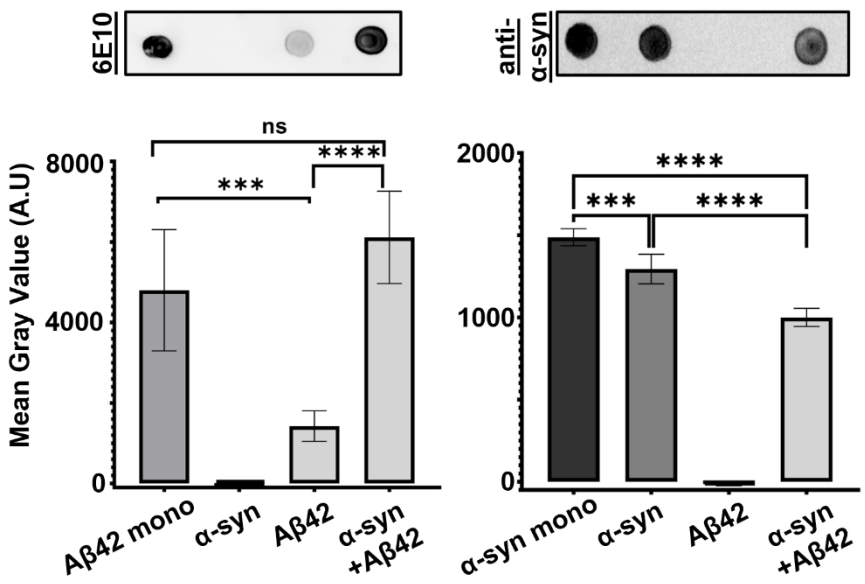

Fig. S4. Solubility of Aβ42 and α-syn after co-incubation. Dot blot analysis and quantifications on the soluble fractions of aggregated samples detected with 6E10 (left) and anti-α-syn (right) primary antibodies. 5 repeats for each sample were quantified. Error bars are shown as SD. Mean grey values were compared with One-way ANOVA, Tukey's multiple comparison test where p = 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***) and <0.0001 (****).