Supplementary Information

(-)-Epigallocatechin gallate prevents the aggregation of tau protein into toxic oligomers at substoichiometric ratios

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Supplementary methods

Expression of full length tau protein

The wild type 2N4R (P10636-8) tau expression plasmid (pRK172-Tau 2N4R) was a generous gift from Virginia Lee (University of Pennsylvania). Site-directed mutagenesis to obtain 2N4R C291A/C322A was accomplished using *Pfu* turbo polymerase and transforming into XL 10-Gold ultracompetent cells. The WT as well as mutant plasmid was transformed into BL21DE3-Gold competent cells for protein expression and cells grown in 1L of LB media at 37°C followed by induction with 1 mM IPTG at 37°C when OD 600 was 0.5. The cells were pelleted 2 hours post induction and stored at -80°C. The pelleted cells were re-suspended in ice cold 80 mM PIPES, pH 6.8, 1 mM $MgSO_4$ and 1 mM EGTA and lysed using a French pressure cell press. The cell lysate was then centrifuged at 5,000 rpm for 20 min at 4°C and the supernatant was collected. The supernatant was then boiled at 100°C for 10 min and further centrifuged at 5,000 rpm for 10 min. The supernatant was loaded on a phosphocellulose-packed column equilibrated with lysis buffer and eluted with lysis buffer containing increasing concentrations of NaCl(0.2 - 1.0 M). The column fractions were screened by gel electrophoresis and the peak tau fractions were pooled and dialyzed against buffer containing 10 mM NH_3HCO_3. The dialyzed sample was then aliquoted and lyophilized.

Full length tau aggregation

Lyophilized wt tau monomer was dissolved in water + DTT (25 mM) to a concentration of 40 µM and incubated at room temperature for 1 h. The protein was diluted in aggregation buffer (100 mM HEPES, 100 mM NaCl, 20 µM ThT) and 8 µM heparin (8 µM) were added. Tau DC was prepared under identical conditions, but in the absence of DTT. Protein samples were incubated in a 96 well microplate (Corning #3651), at 37°C in a fluorescence microplate reader (Infinite F200) with intermittent shaking (5 s every 5 min). ThT signals were recorded at 436 nm excitation and 482 nm emission. After 72 h, 10 µl of sample was adsorbed onto mica for 5 min, washed with 4 x 40 µl water and 40 µl ethanol, dried for 2 h and imaged on a NanoMan AFM (Veeco) in tapping mode using a FESP tip.

Supplementary Figures

Supplementary Figure S1

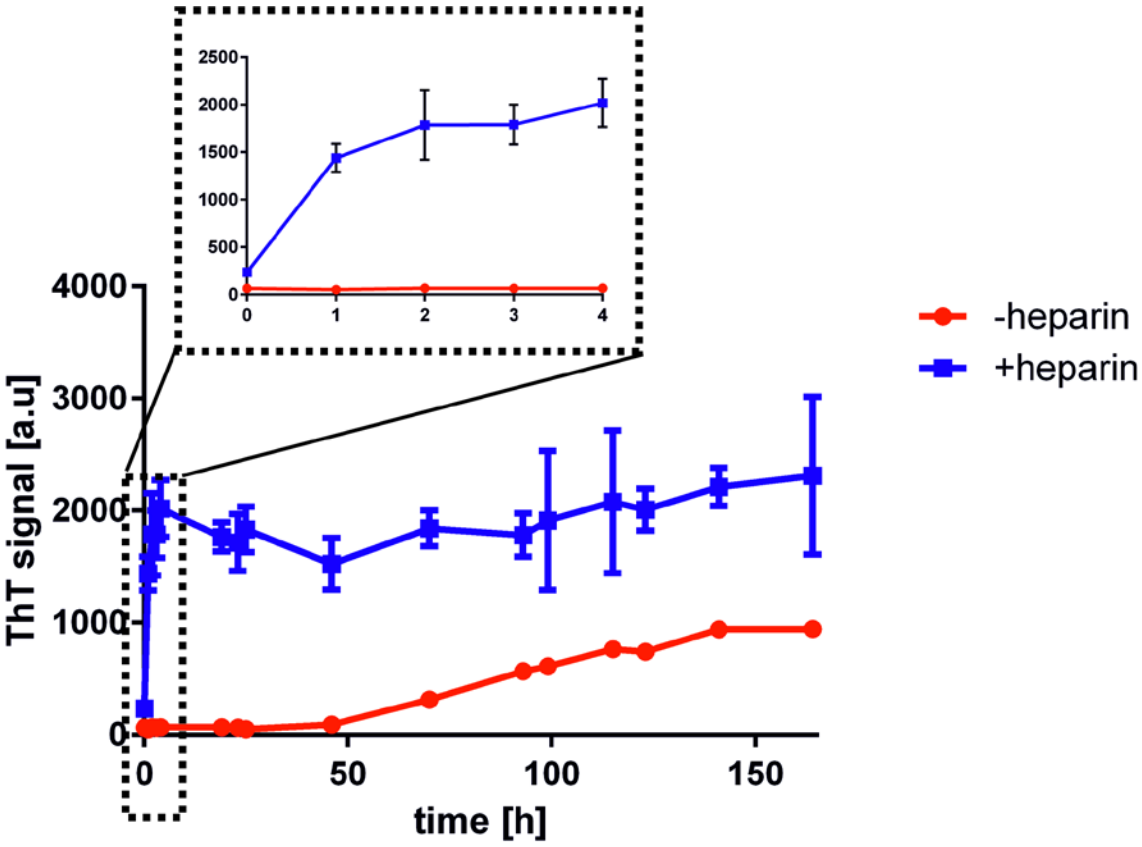

Figure S1 Heparin eliminates the lag phase of the tau aggregation reaction. Aggregation of K18ΔK280 in the presence of heparin (Sigma) as assessed by ThT fluorescence assay (tau protein concentration: 12.5 µM, heparin concentration 5 µM) for 165 h (smaller diagram represents the first 4 hours of aggregation). Results represent mean fluorescence signal ± s.d. (n = 3).

Supplementary Figure S2

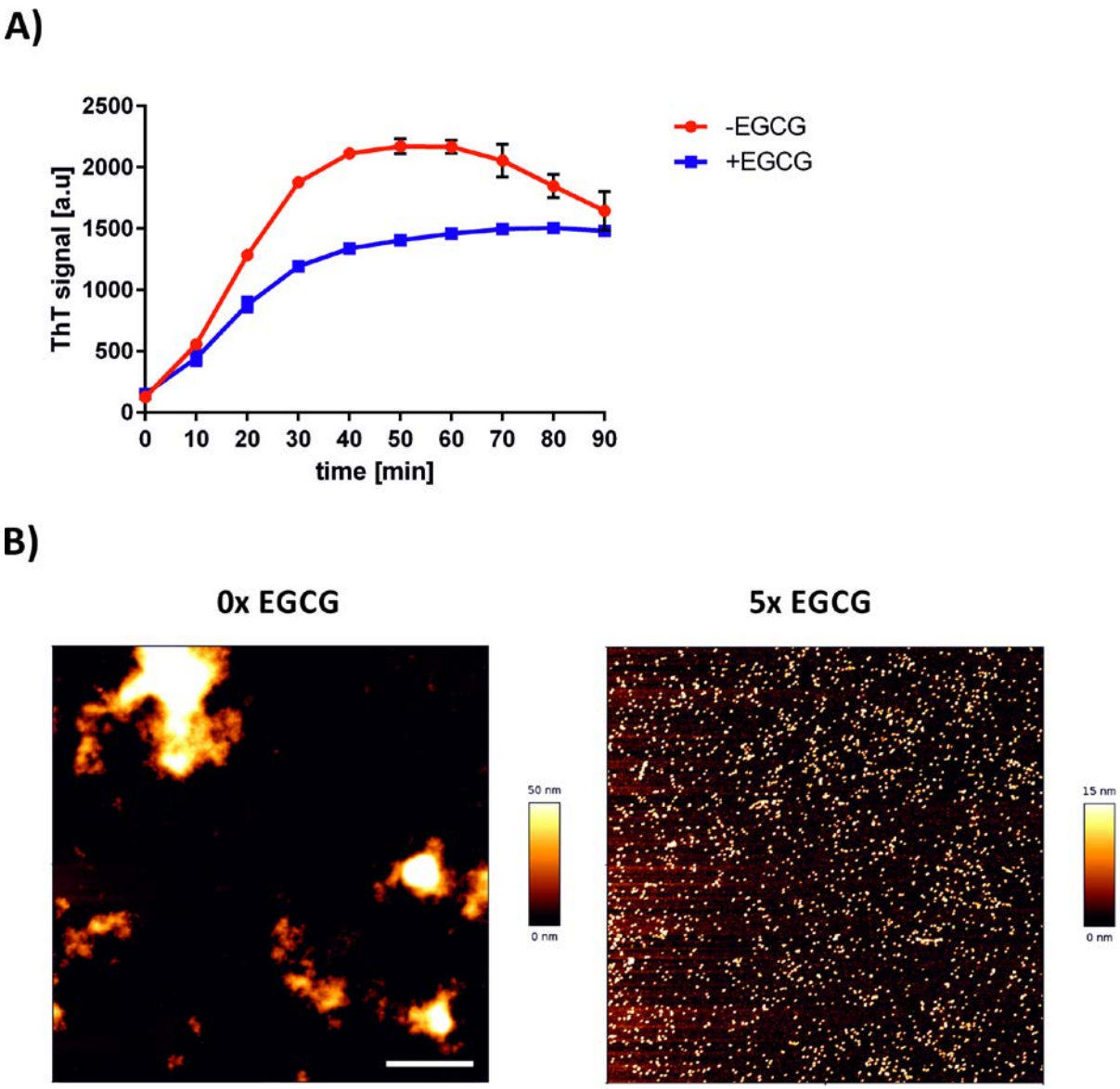

Figure S2 EGCG alters the aggregation kinetics of heparin-induced tau aggregation and alters the aggregate conformation. A) Aggregation of tau (12.5 µM) in the presence of heparin (5 µM) and EGCG (5x molar concentration) as assessed by ThT fluorescence. Results represent mean fluorescence signal ± s.d. (n = 3). B) Analysis of EGCG-treated (5x molar concentration) K18ΔK280 by atomic force microscopy after 3 h incubation. Samples were sonicated for 3 minutes in a water bath. Scale bar = 2µM.

Supplementary Figure S3

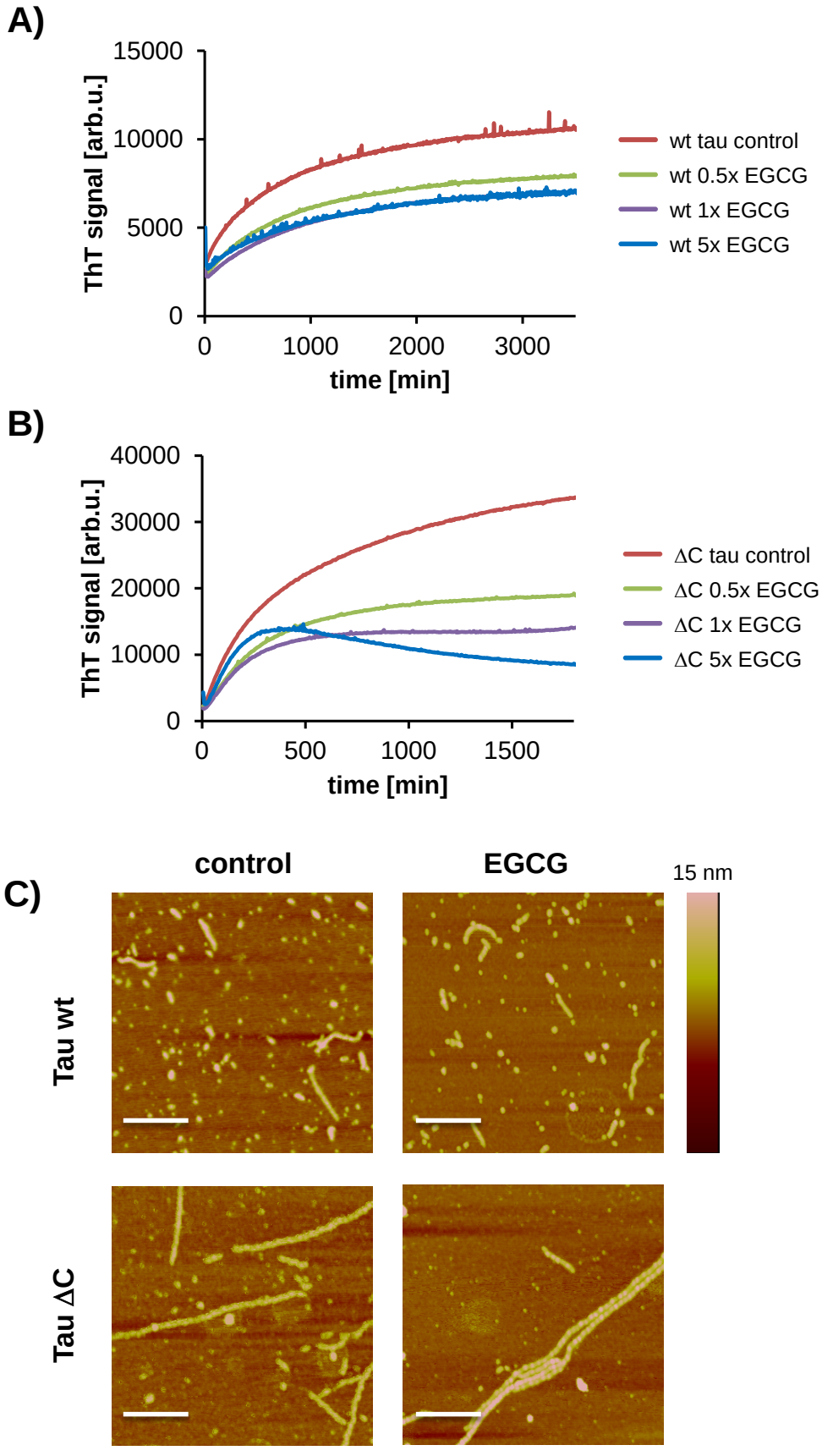

Figure S3 EGCG alters the aggregation kinetics of heparin-induced full length tau aggregation. A) Aggregation of wt full length tau (8 μM) in the presence of heparin (8 μM), DTT (5 mM) and EGCG (4 – 20μM, 0.5x - 5x tau concentration) under reducing conditions as assessed by ThT fluorescence. B) Aggregation of mutant C291A, C322A (ΔC) full length tau (8 μM) in the presence of heparin (8 μM) and EGCG (4 – 20μM, 0.5 - 5x tau concentration) as assessed by ThT fluorescence. ΔC tau aggregates under non-reducing conditions in the presence of heparin. C) Atomic force microscopy images of aggregation end points (72 h) of wt tau and DC tau aggregation assays shown in A), B) in the absence or presence of EGCG (40 μM); scale bar 500 nm.