Supplemental Information

Major differences between the self-assembly and seeding behavior of heparin-induced- and in-vitro-phosphorylated tau and their modulation by potential inhibitors

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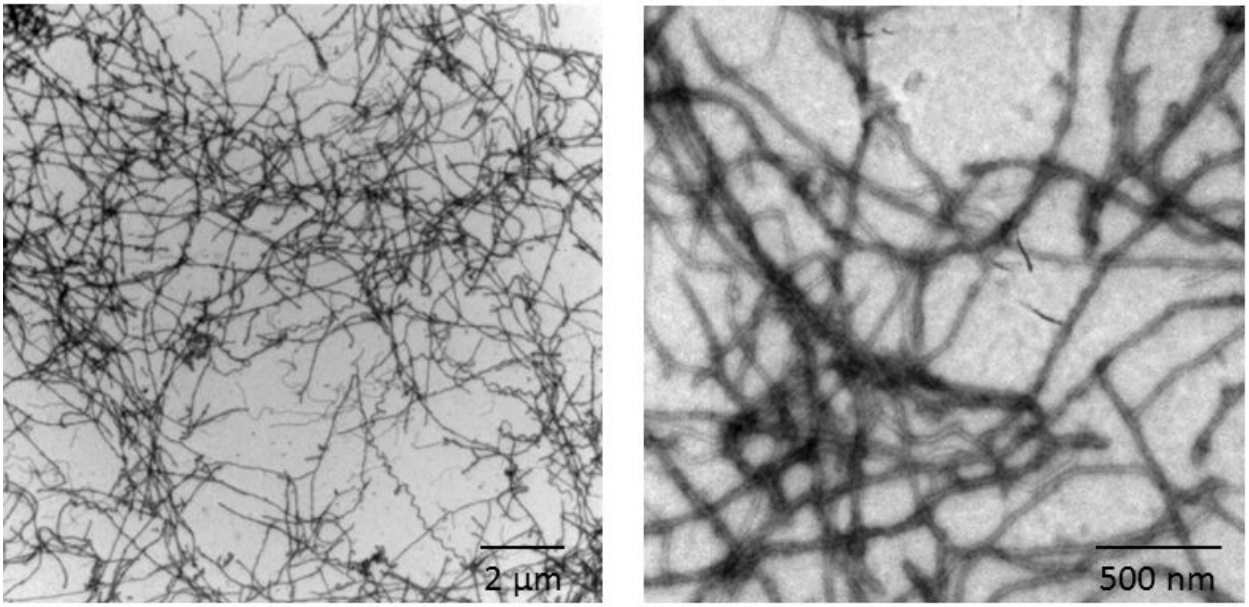
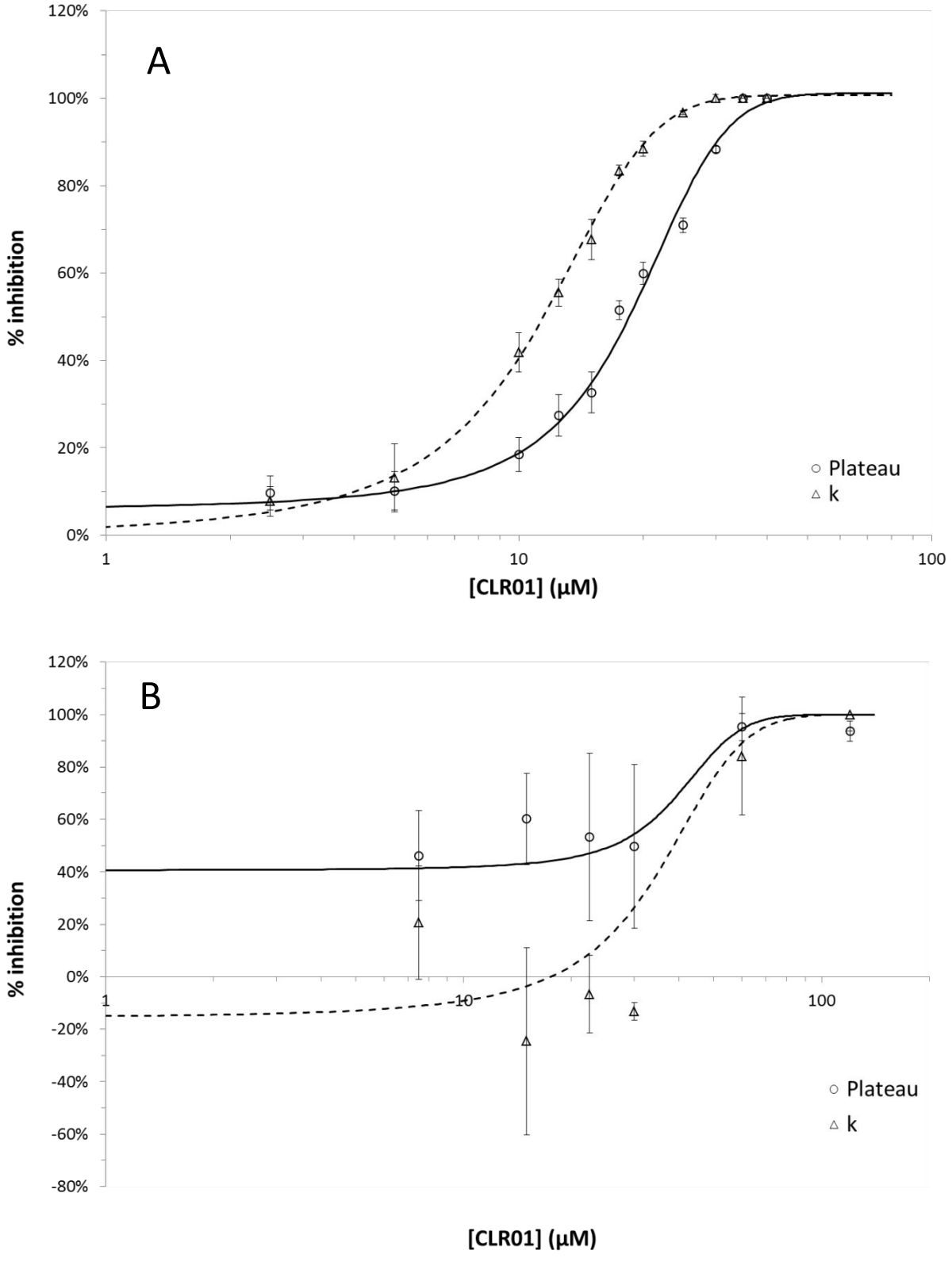
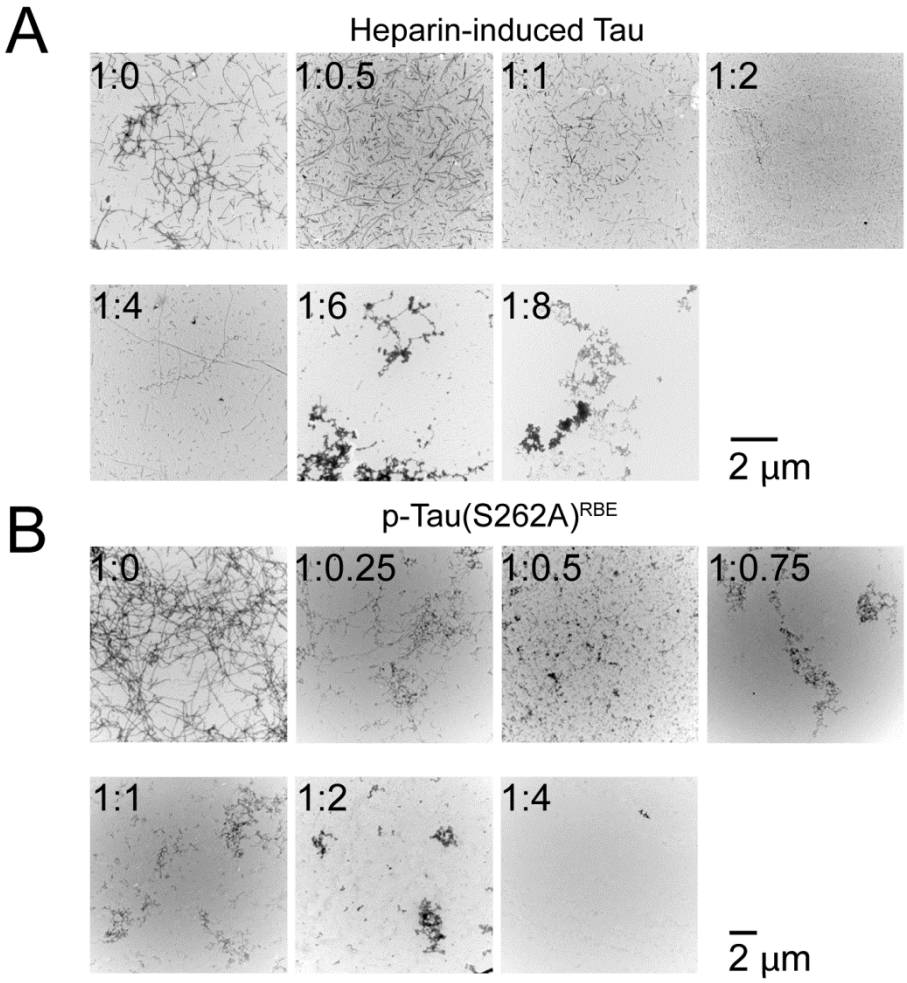


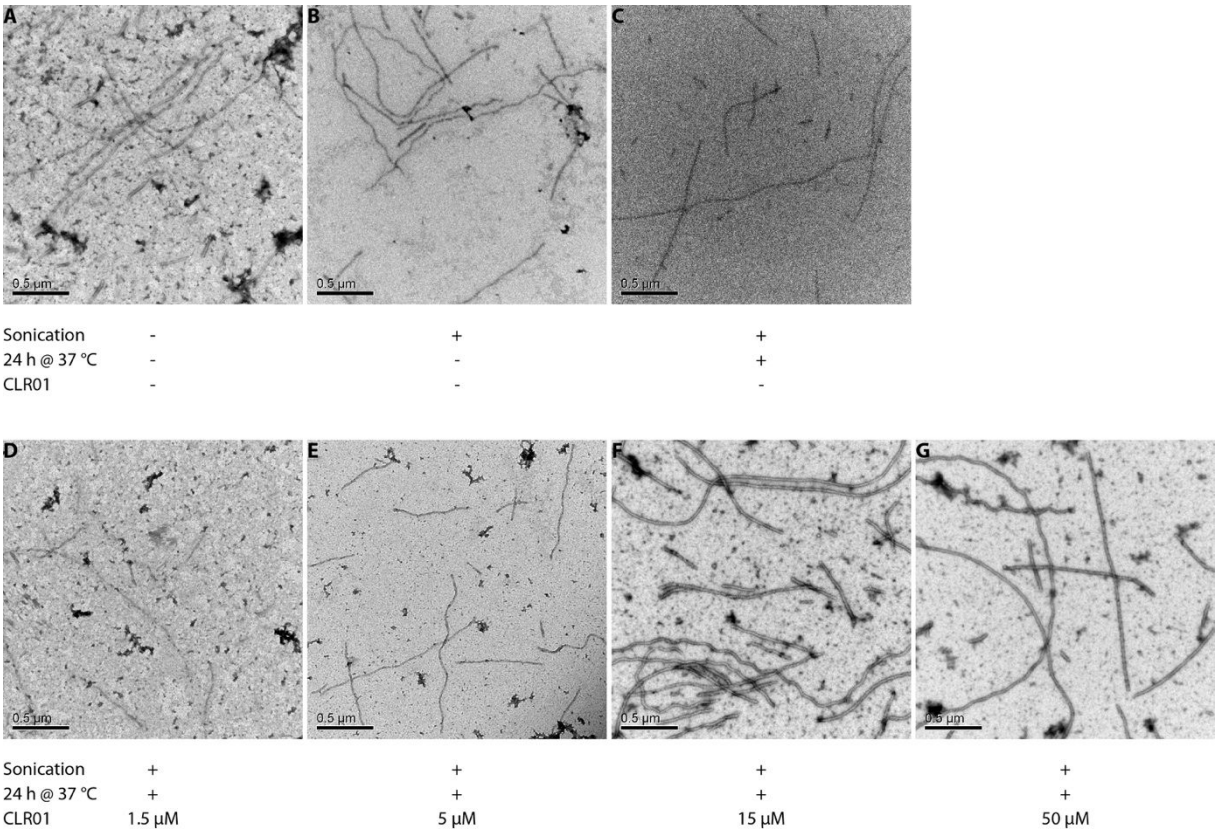
Supplementary Figure S1. Morphological examination by TEM at two different magnifications of p-tau(S262A)^{RBE} fibrils obtained by incubation at 37°C of 30 µM of protein for 100 h.



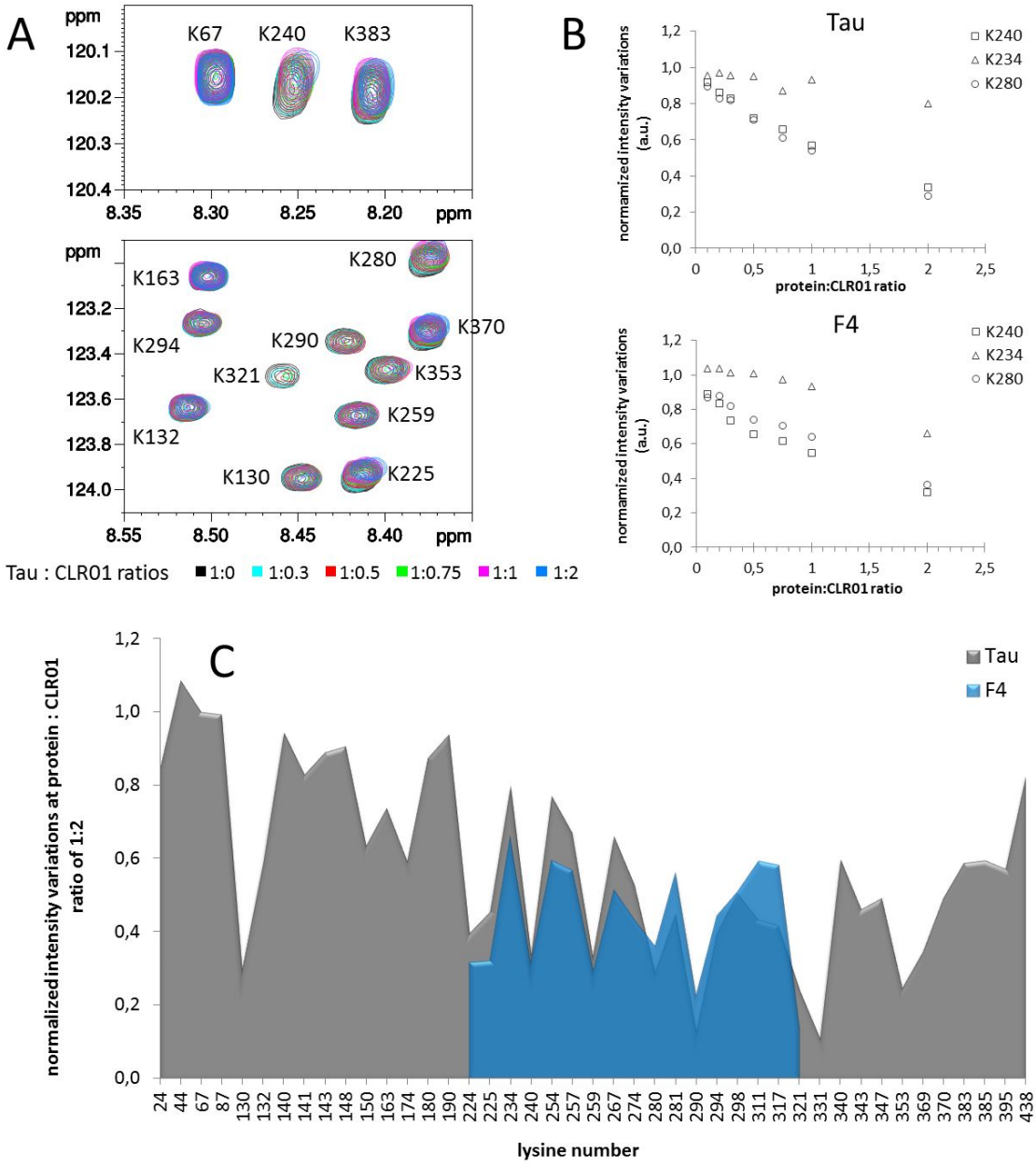
Supplementary Figure S2. Comparison of fitting the plateau vs. the rate constant, k, from aggregation kinetics curves for calculation of half-maximal inhibition by CLR01 of A) aggregation of unphosphorylated tau at 5µM in the presence of 1.25µM heparin, or B) aggregation of p-tau(S262A)^{RBE} at 30µM.



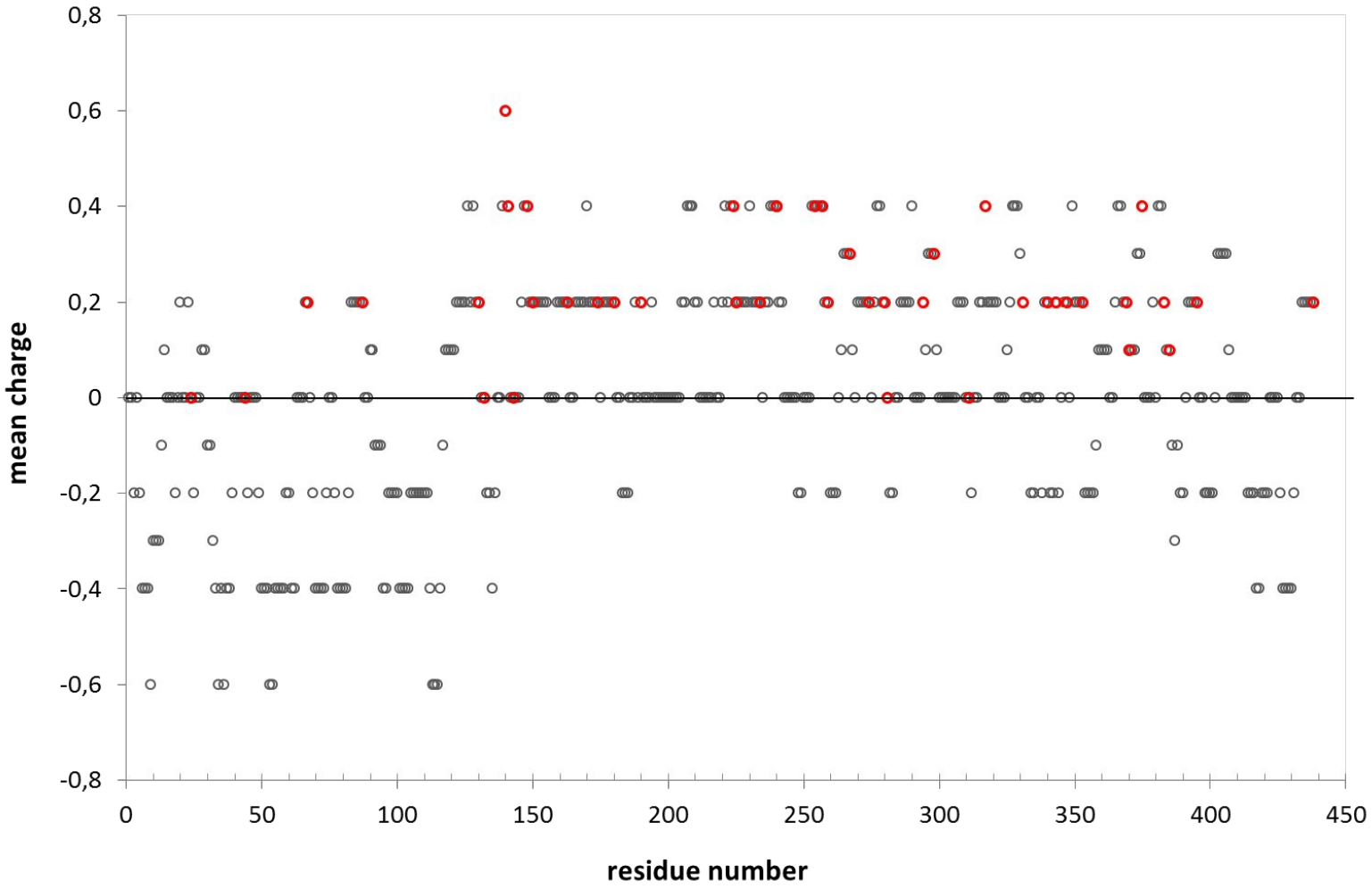
Supplementary Figure S3. Representative TEM images taken at the end of the aggregation reactions shown in Figure 5. A) Images of 5 µM heparin-induced tau in the absence or presence of the indicated tau:CLR01 concentration ratios. B) Images of 30 µM p-tau(S262A)^{RBE} in the absence or presence of the indicated p-tau(S262A)^{RBE}:CLR01 concentration ratios.



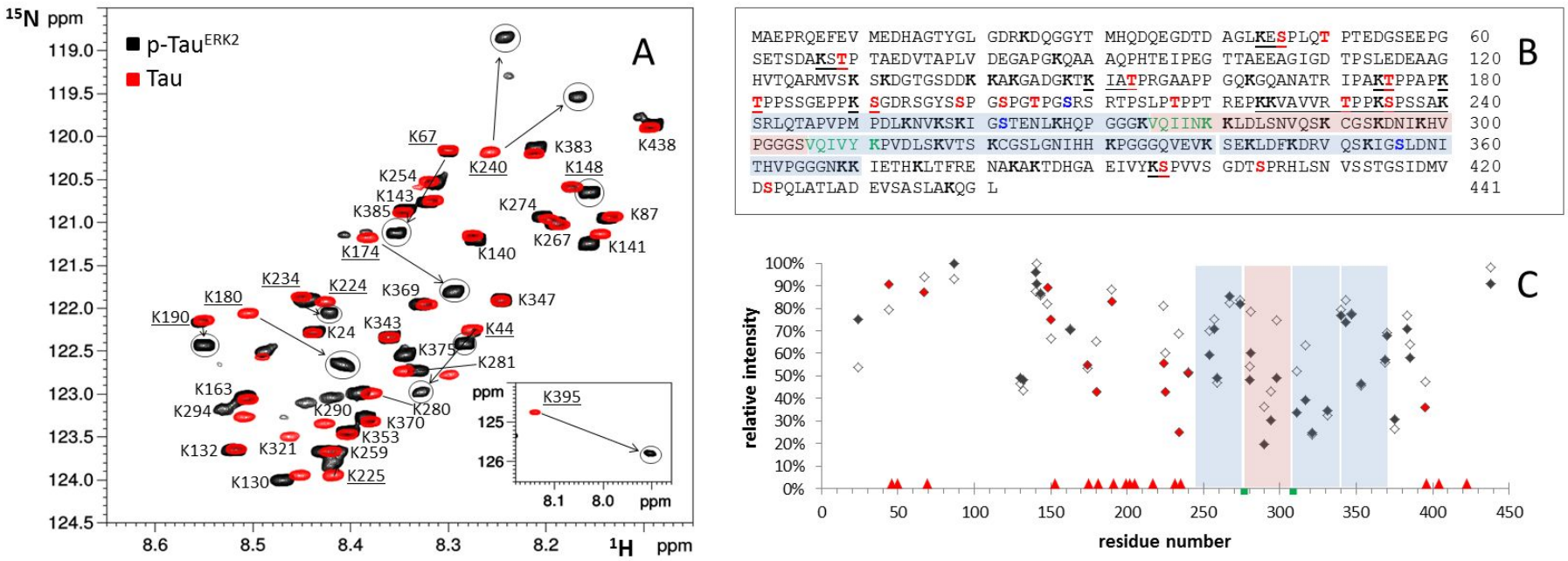
Supplementary Figure S4. Transmission electron microscopy of p-tau(S262A)^{RBE} fibrils in the absence or presence of different concentrations of CLR01. (A-C) p-Tau(S262A)^{RBE} fibrils in the absence of CLR01 before sonication (A), after sonication (B), and after 24-h incubation at 37 °C followed by 10-min sonication (C). (D-G) p-Tau(S262A)^{RBE} incubated for 24 h in the presence of 1.5 µM (D), 5 µM (E), 15 µM (F), or 50 µM (G) of CLR01 followed by 10-min sonication in each case.



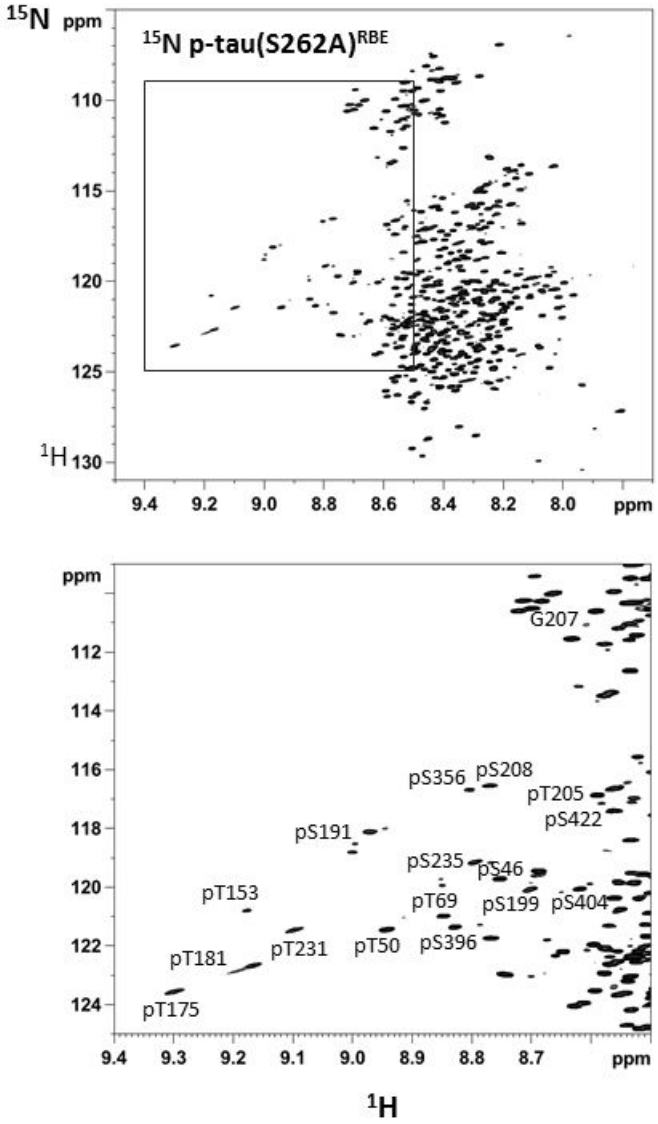
Supplementary Figure S5. Titration of full-length tau and the F4 fragment with CLR01. (A) Superimposition of ^{1}H-^{15}N HSQC spectra of ^{15}N-Lys-tau for different tau:CLR01 concentration ratios. (B) Plots of the normalized resonance intensity variations for three interacting Lys residue K234, K240, K280 in full-length tau and F4. (C) Comparison of signal intensity variations at a two-fold excess of CLR01 for tau (grey) and F4 (blue) for each lysine residue in the tau sequence (longest isoform, 2N4R tau, numbering).



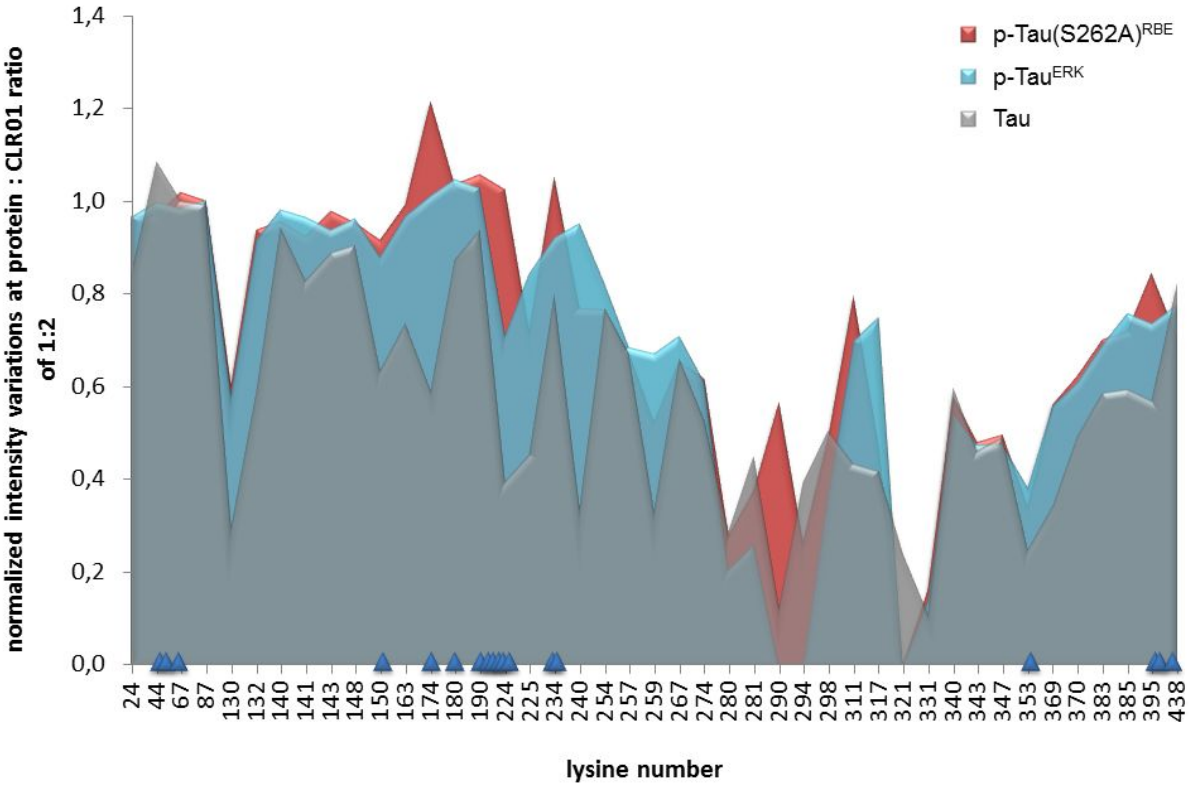
Supplementary Figure S6. Mean charge along the tau sequence calculated using the EMBOSS tool "protein charge" (http://www.bioinformatics.nl/cgi-bin/emboss/charge) with a window of 5 residues. The mean charges of lysine residues are indicated by red dots.



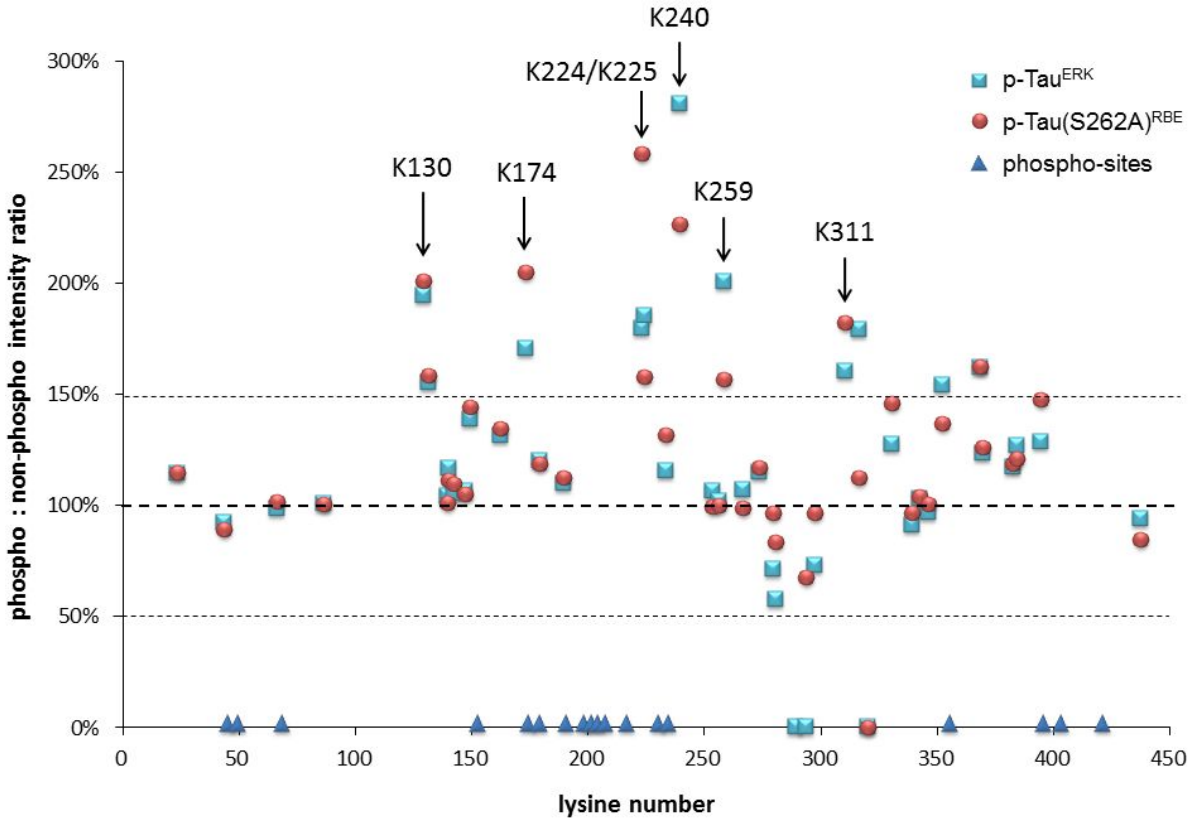
Supplementary Figure S7. Phosphorylation of ^{15}N-lysine,^{13}C-tau by ERK2. (A) Superimposition of ^{1}H-^{15}N HSQC spectra of p-tau^{ERK} (black) and unphosphorylated (red) ^{15}N-lysine,^{13}C-tau showing the effect of phosphorylation of lysine resonances that are in proximity of phosphorylation sites. (B) Sequence of tau in which ERK2 phosphorylation sites are annotated in red, additional RBE phosphorylation sites in blue and PHF6* and PHF6 hexapeptides in green. R1, R3, R4 repeats are underlined in light blue and R2 in light red. Lys residues located in the neighborhood of phosphorylation sites are underlined. (C) Resonance intensity patterns of unphosphorylated tau (open diamonds) and p-tau^{ERK} (filled diamonds). Lys residues close to phospho-sites are depicted by red filled diamonds. In the p-tau^{ERK} sample, the sum of intensities of unphosphorylated and phosphorylated forms is plotted for Lys residues close to phospho-sites. Phosphorylation sites are indicated by red triangles. The hexapeptides PHF6* and PHF6 in the R2 and R3 repeats are indicated by green lines. Regions corresponding to R1-R4 repeats are colored as indicated in (B).



Supplementary Figure S8. ^{1}H-^{15}N HSQC spectra of p-tau(S262A)^{RBE} phosphorylated by a rat brain extract. Lower panels correspond to the box regions in upper panels in which resonances of phosphorylation sites are annotated.



Supplementary Figure S9. Comparison of signal intensity variations in phosphorylated tau, p-tau^{ERK} (cyan) and p-tau(S262A)^{RBE} (red), and non-phosphorylated tau (grey) upon addition of a two-fold excess of CLR01. Intensity variations are plotted against lysine residues number along the tau sequence. Phosphorylation sites of p-tau(S262A)^{RBE} are indicated by blue triangles.



Supplementary Figure S10. Ratio of signal intensity of phosphorylated tau, either p-tau^{ERK} (cyan) or p-tau(S262A)^{RBE}(red), on non-phosphorylated tau upon addition of a two-fold excess of CLR01. Intensity ratios are plotted against lysine residues number along the tau sequence. Phosphorylation sites of p-tau(S262A)^{RBE} are indicated by blue triangles. Ratios higher than 100% indicate stronger interactions with non-phosphorylated tau while ratios lower than 100% indicate stronger interactions with phosphorylated tau. Lysine residues affected in their interaction with CLR01 by phosphorylation are annotated.