SUPPORTING INFORMATION

Structural disorder in four-repeat Tau fibrils reveals a new mechanism for barriers to cross-seeding of Tau isoforms

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Running title: Cross-seeding barriers in four-repeat Tau fibrils

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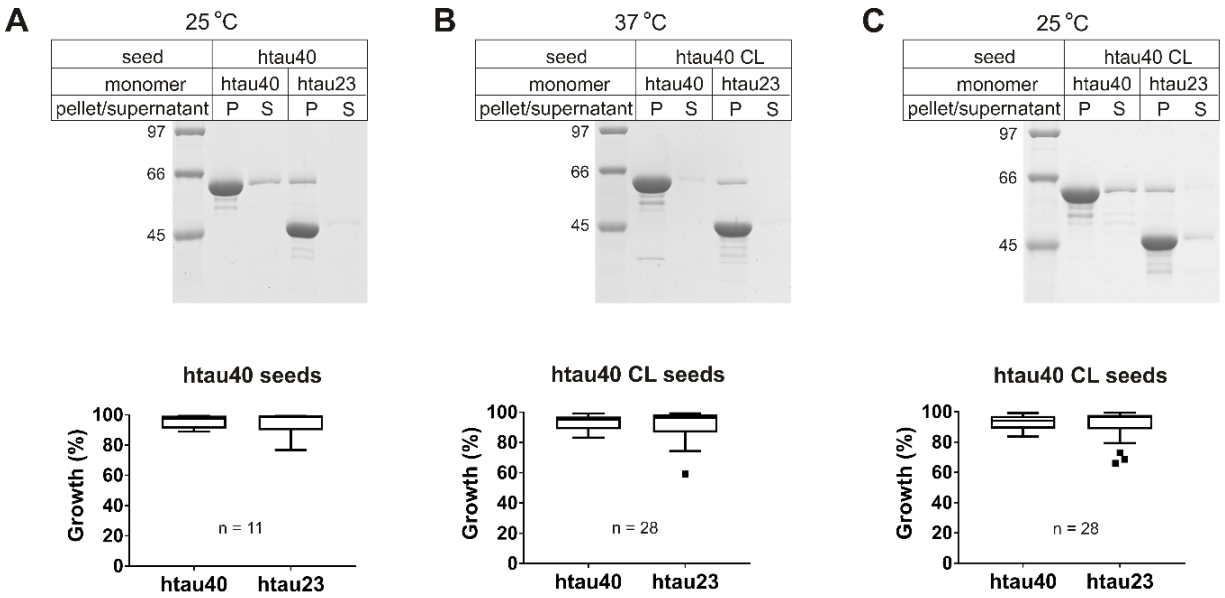

Figure S1. Seeding properties of fibrils composed of htau40 or htau40 cysless formed at varying temperatures. Tau fibrils of htau40 or htau40 cysless (two native cysteines replaced by serines, marked as htau40 CL) were grown at either 25 °C or 37 °C. Fibril seeds were generated by sonication and combined with Tau monomers (10% seeds, 10 μM monomers). Fibril growth was allowed to proceed for 20-24 h at 37 °C. All fibrils were sedimented by ultracentrifugation and analyzed by SDS-PAGE and Coomassie staining. Representative gels (top row) and densitometric quantification of reactions depicted as box-and-whisker plots using the Tukey method (bottom row). (A) htau40-seeded, 25 °C, (B) htau40 CL-seeded, 37 °C, (C) htau40 CL-seeded, 25 °C. P = pellet, S = supernatant, n = number of biological replicates. The data indicate that a replacement of cysteines with serines and a change in reaction temperature from 37 °C to 25 °C during initial aggregation does not affect the cross-seeding properties of htau40 fibrils. In all cases, htau23 is recruited onto the seeds.

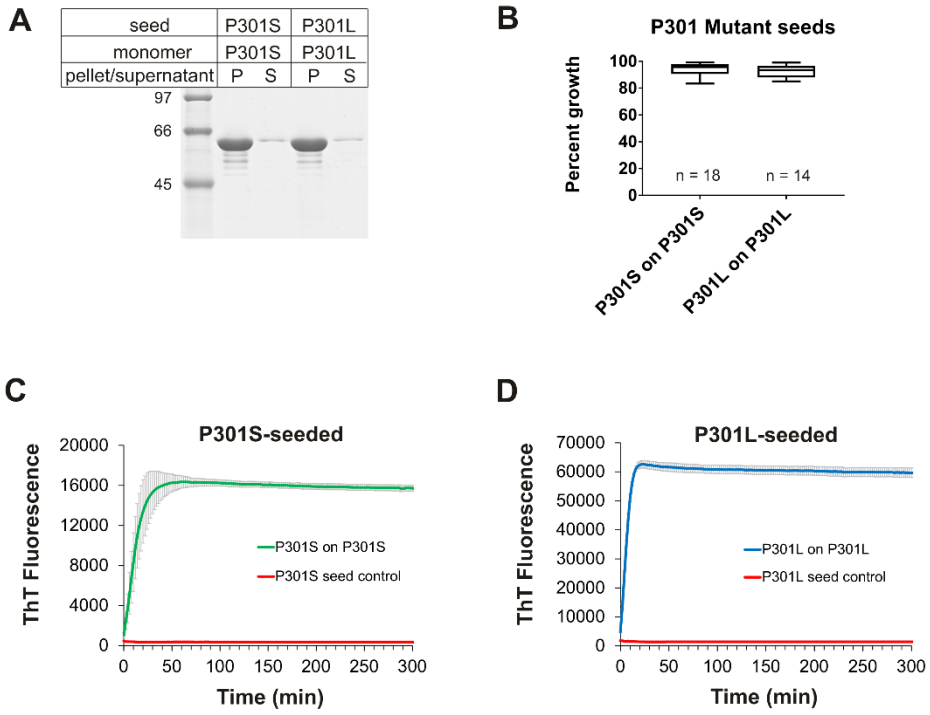

Figure S2. Homotypic seeding of P301S and P301L. Fibril seeds of P301S and P301L were generated by sonication. Ten % seeds were mixed with 10 μM mutant monomers and allowed to grow for 20-24 h at 37 °C. Seeding efficiency was assessed by SDS-PAGE and Coomassie staining after sedimentation. (A) Representative gel. P = pellet, S = supernatant. (B) Densitometric quantification shown as box and whisker plot using the Tukey method. N = number of biological replicates. Elongation kinetics was determined by ThT fluorescence at 37 °C utilizing 5% seeds and 10 μM monomers. (C) P301S-seeded reaction (green trace). (D) P301L-seeded reaction (blue trace). Seed controls with no monomers added (red traces). N = 4. All error bars in C and D represent means ± SD. The results indicate effective recruitment of P301S and P301L monomers onto their respective seeds.

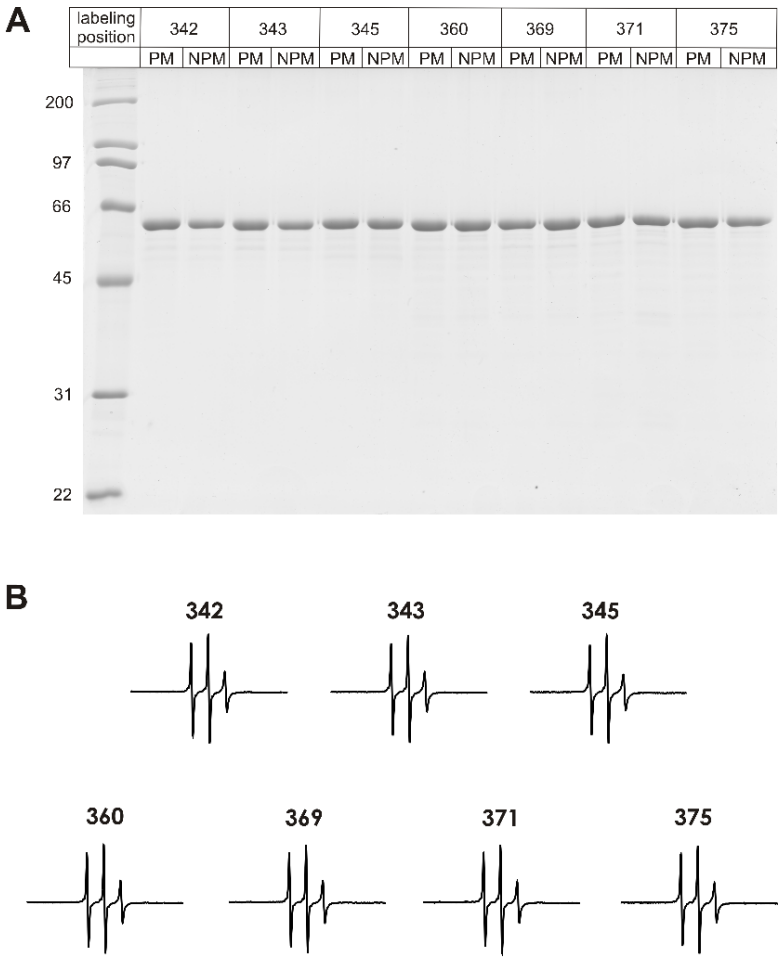

Figure S3. Site-directed spin labeling and EPR analysis of htau40 monomers. (A) Tau monomers labeled at seven single cysteines (342, 343, 345, 360, 369, 371, and 375) were analyzed by SDS-PAGE and Coomassie staining. PM = htau40 monomers labeled with paramagnetic label. NPM = htau40 monomers labeled with nonparamagnetic label. (B) CW EPR spectra collected at 150 G sweep width, 1 G modulation, and 12 mW incident microwave power. The data confirm intrinsic disorder at all sites.

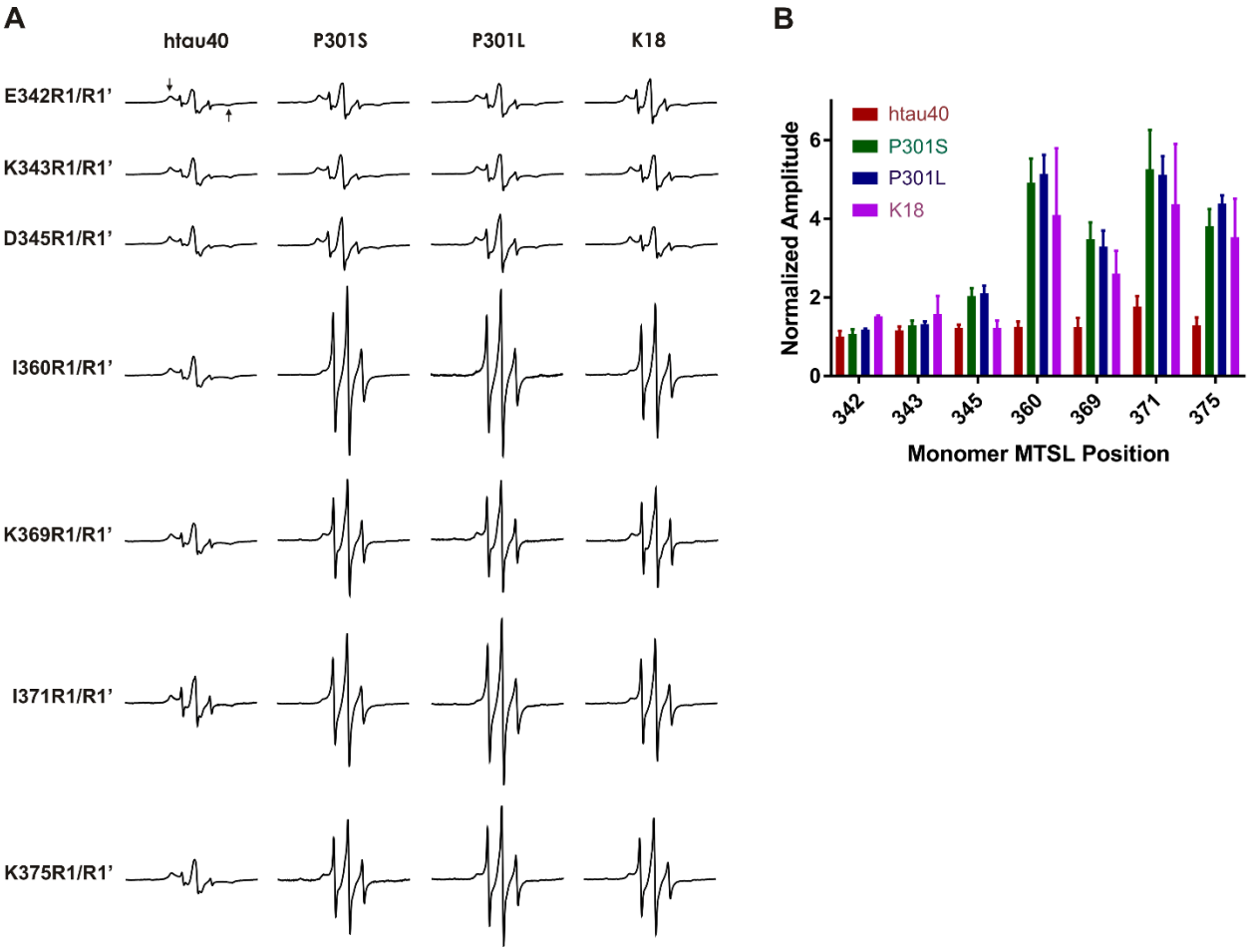

Figure S4. EPR analysis of htau40 fibrils containing a mixture of paramagnetically and nonparamagnetically labeled monomers. Tau seeds (htau40, P301S, P301L, and K18) were combined with a mixture of paramagnetically and nonparamagnetically labeled Tau monomers (molar ratio = 1:4) and allowed to grow for 20-24 h at 37 °C. The fibrils were sedimented and transferred into EPR capillaries. (A) Representative CW EPR spectra collected at X-band. The horizontal row on top signifies the type of seeds that was used. The vertical row on the left identifies the spin labeled htau40 monomer that was grown onto the seed. R1/R1' = cysteines labeled with paramagnetic and nonparamagnetic labels. Arrows indicate outer peaks. All spectra are normalized to the same number of spins. Scan width, 150 G, modulation = 3 G, incident microwave power = 12 mW. (B) Plot of the signal amplitudes. The results are represented as the means ± SD and involve three biological replicates. The data highlight differences in the core structures of the fibrils.