

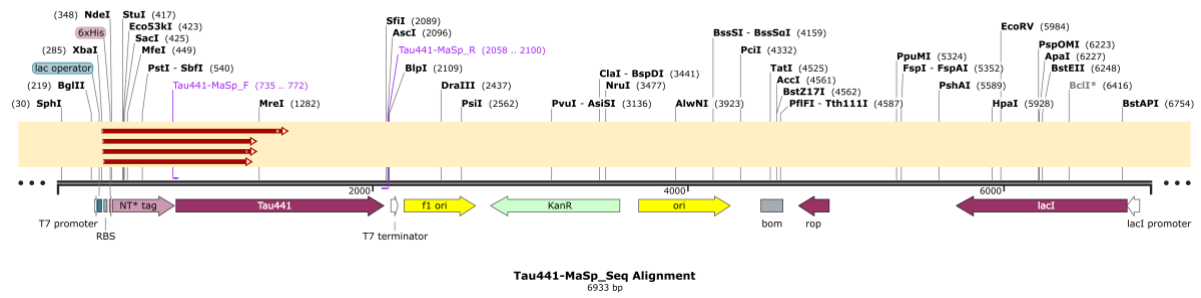
SUPPLEMENTARY MATERIAL

Primer name	Primer Sequence	T _m
Tau441-MaSp_F	tgaatgatgtcagtgctgggATGGCGGAGCCGCGTCAA	74°C
Tau441-MaSp_R	cgcgccggcctgggagggcccttaCAGACCTTGT [*] TCGCCAGGC	78°C
Tau441-TEV-MaSp_F	ttccagagcATGGCGGAGCCGCGTCAA	70°C
Tau441-TEV-MaSp_R	tacaggtttcCCCAGCACTGACATCAT [*] TCATACCTG	67°C
TFT-InsTGGG_F	tgggGAAAACCTGTATT [*] TCCAGAG	58°C
TFT-InsTGGG_R	GCACTGACATCAT [*] TCATAC	58°C
MaSp_P301L_F	CAAGCACGTTctgGGCGGTGGCA	76°C
MaSp_P301L_R	ATGTTATCTTTTGCTACCGCACTTGCTCTG	71°C
MTBR-MaSp2_F	tgaatgatgtcagtgctgggAAAGTGGCGGTGGTTCGTAC	60°C
MTBR-MaSp2_R	cgcgccggcctgggagggcccttaT [*] TCGATT [*] TTCTTGT [*] TGCCACC	56°C
MTBR-MaSp2+TEV_F	ttccagagcAAAGTGGCGGTGGTTCGT	60°C
MTBR-MaSp2+TEV_R	tacaggtttcCCCAGCACTGACATCAT [*] TC	56°C

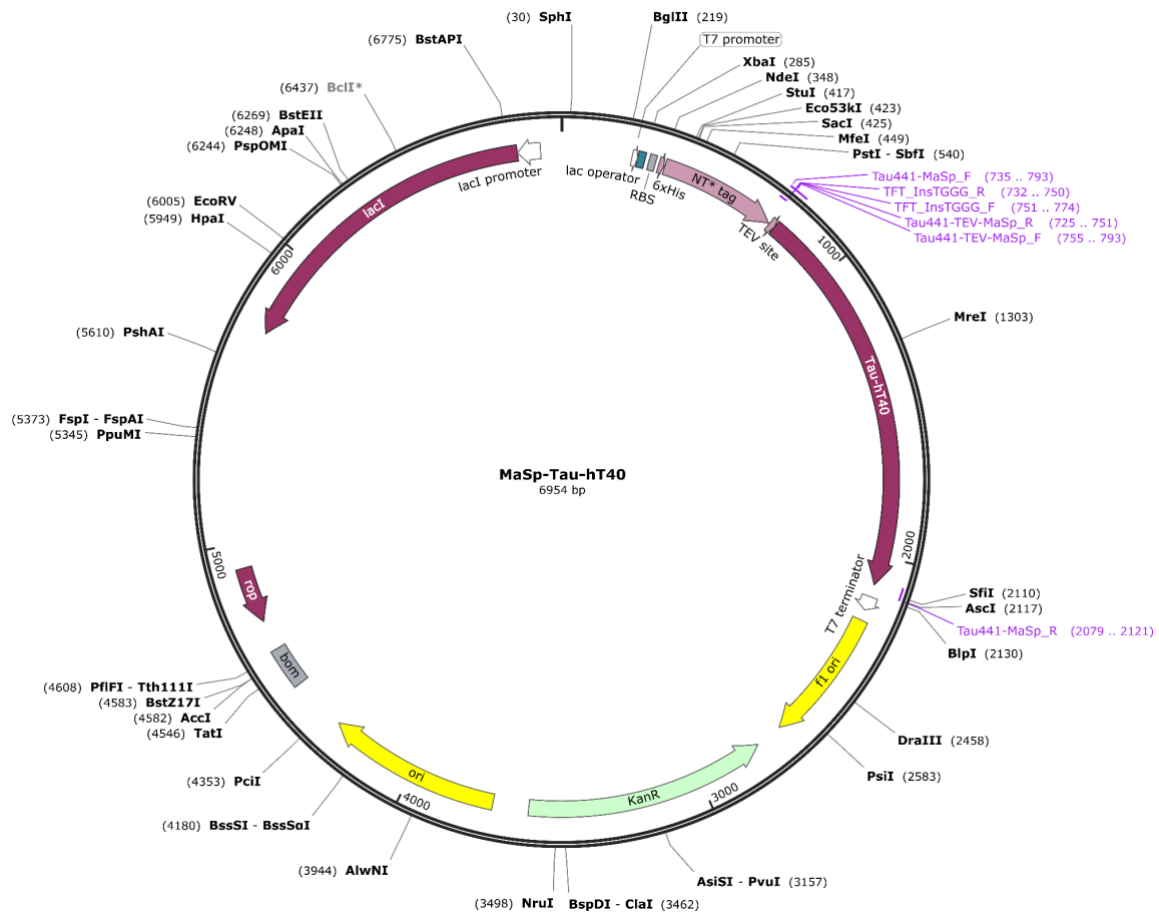
Supplementary Table S1: List of primer sets used in this study for cloning and mutagenesis reactions. All unmodified DNA oligonucleotides used as primers in HiFi DNA Assembly and Q5 site-directed mutagenesis were designed in-house and purchased from Eurofins Genomics (Ebersberg, Germany). Primers were delivered lyophilized, reconstituted in nuclease-free water, and used as 10 μ M stocks in PCR reactions.



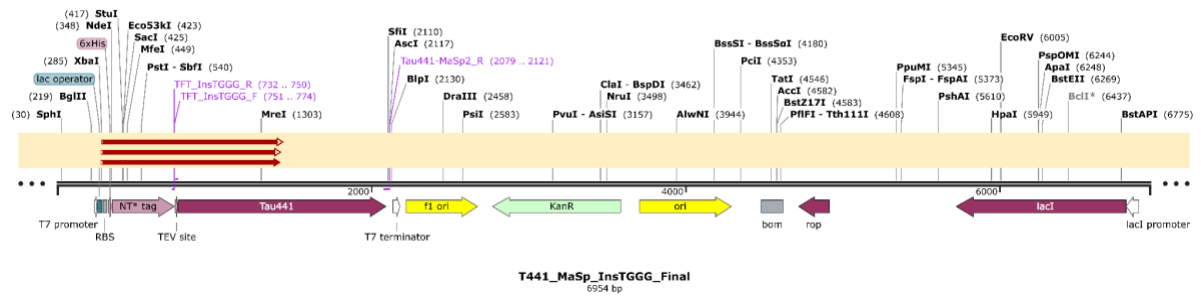
Supplementary Figure S1: Vector map for pT7NT_(MaSp)-Aβ42 expression plasmid used as the primary MaSp construct from which MaSp-Tau-hT40 and MaSp-Tau-MTBR were generated. The plasmid housed T7 promoter elements, a Kanamycin antibiotic-resistance gene, and a 6xHis-NT*tag-Aβ42 coding sequence in its multiple cloning site. The vector map image was exported from SnapGene software (www.snapgene.com).



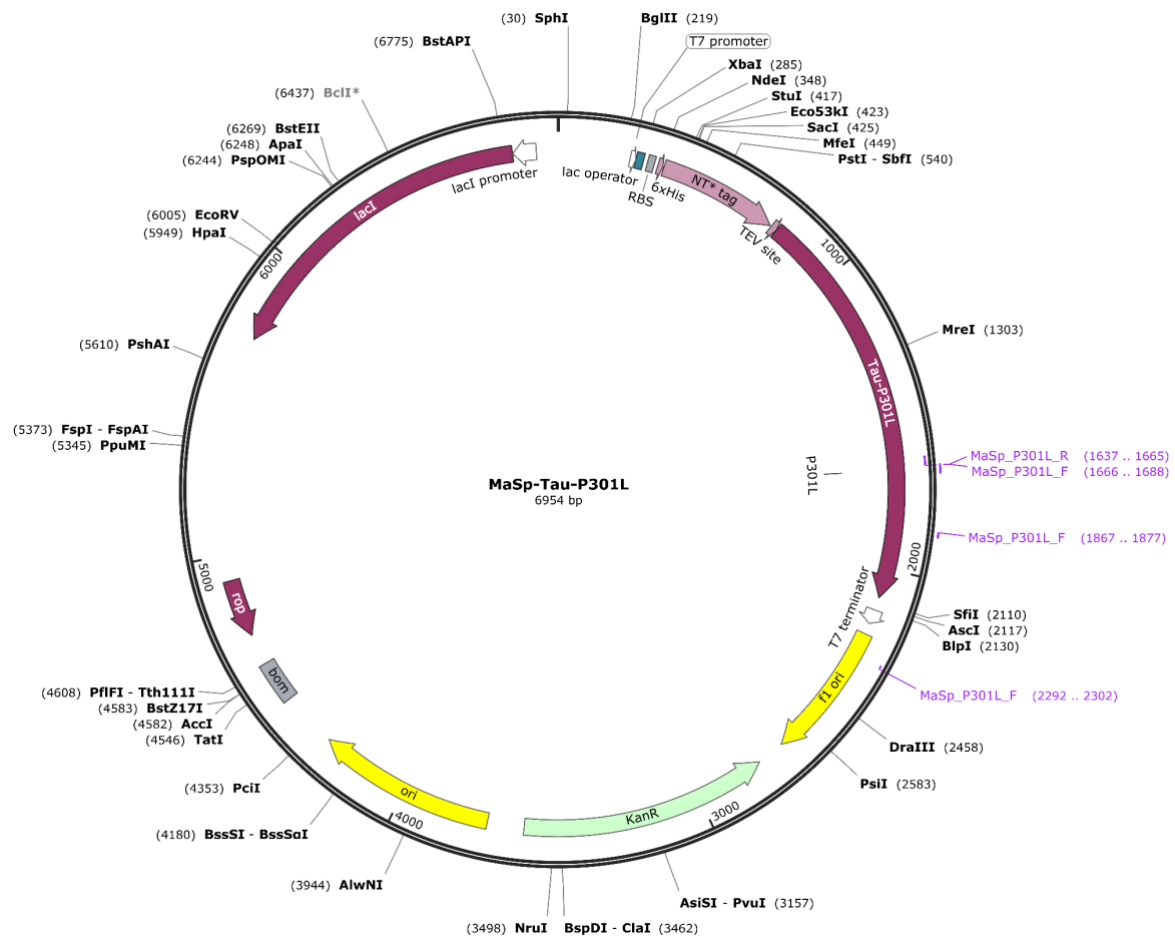
Supplementary Figure S2: Sequencing results to confirm successful insertion of Tau-hT40 coding sequence in the MCS of pT7NT_(MaSp) expression plasmid. Plasmid DNA was extracted using the MN-Nucleospin plasmid isolation kit, and Sanger sequencing was performed using Microsynth® in The Netherlands. The image was exported from SnapGene software (www.snapgene.com).



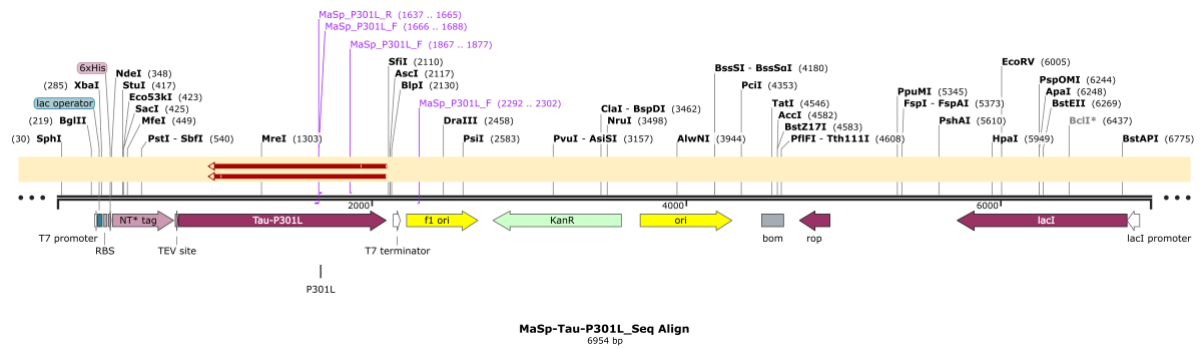
Supplementary Figure S3: Vector map for MaSp-Tau-hT40 plasmid used for bacterial expression of Tau-hT40 recombinant protein. A TEV cleavage site was inserted between the NT* tag solubility tag and the Tau-hT40 coding sequence to facilitate cleavage and separation of purified Tau-hT40 from the cleaved solubility tag during protein purification. The NT*tag housed an N-term 6x His-tag to facilitate its isolation from pure Tau-hT40 during the Reverse HisTrap purification. The vector map image was exported from SnapGene software (www.snapgene.com).



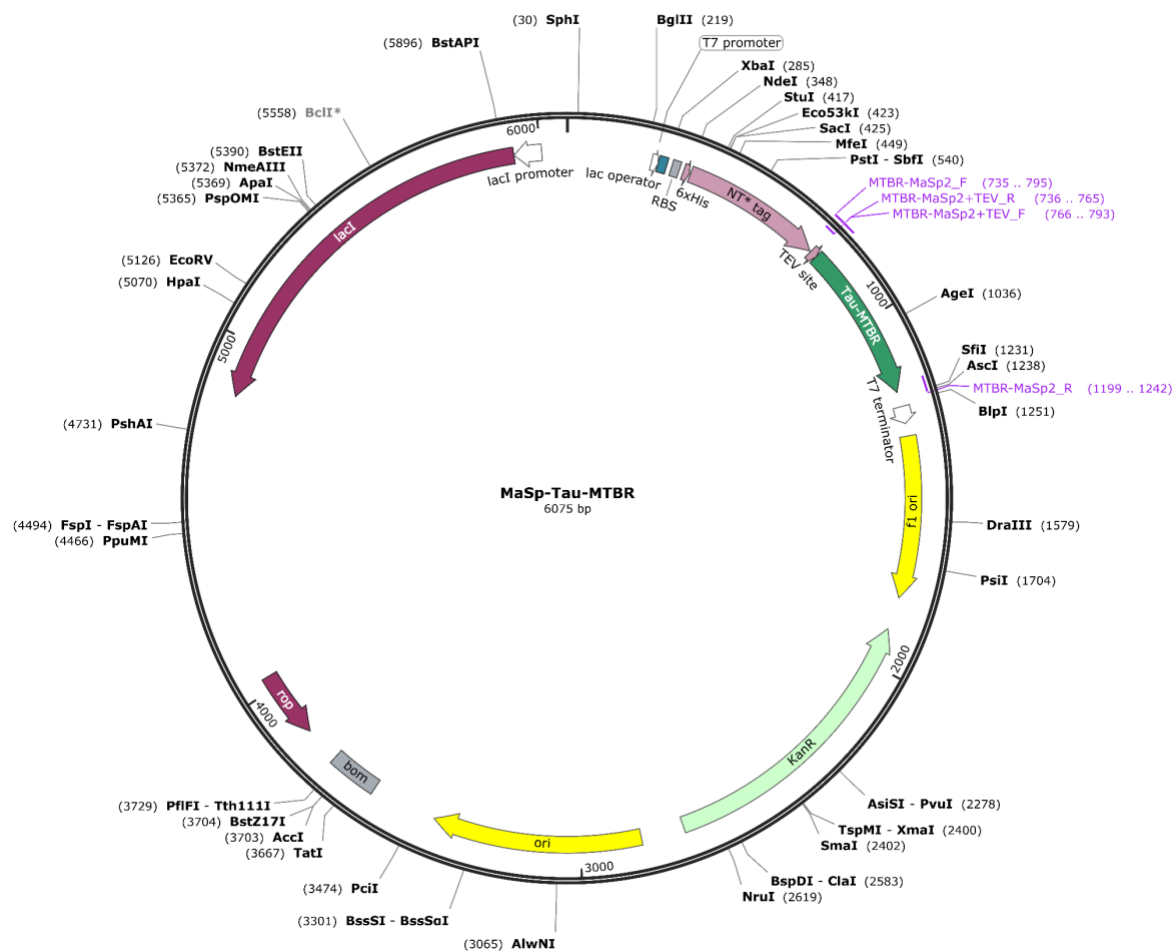
Supplementary Figure S4: Sequencing results to confirm successful insertion of a missing T-nucleotide and an additional three Guanine nucleotides to correct the TEV cleavage site between NT*tag and hT40 coding sequence. Plasmid DNA was extracted using the MN-Nucleospin plasmid isolation kit and Sanger sequencing was performed by Microsynth® in The Netherlands. The image was exported from SnapGene software (www.snapgene.com).



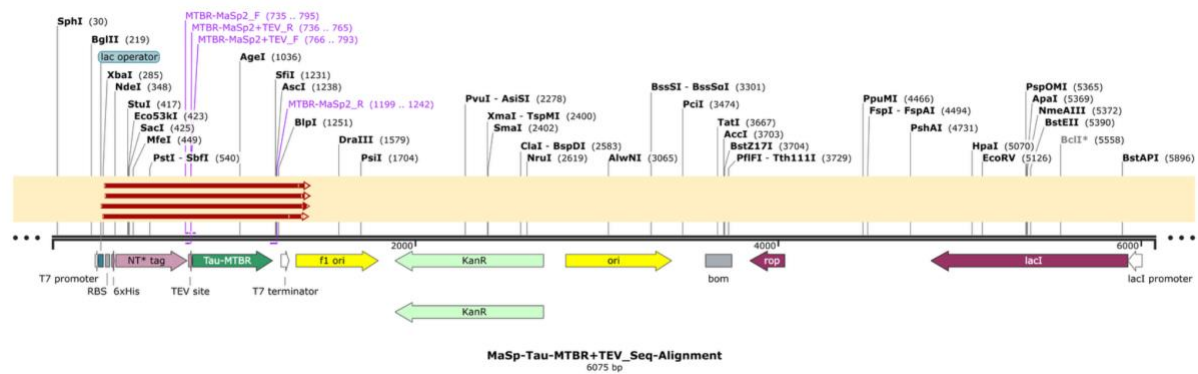
Supplementary Figure S5: Vector map for MaSp-Tau-P301L plasmid used to express recombinant disease-linked P301L Tau mutant protein. Site-directed mutagenesis was done using the NEB Q5 High-Fidelity DNA polymerase kit. The vector map image was exported from SnapGene software (www.snapgene.com).



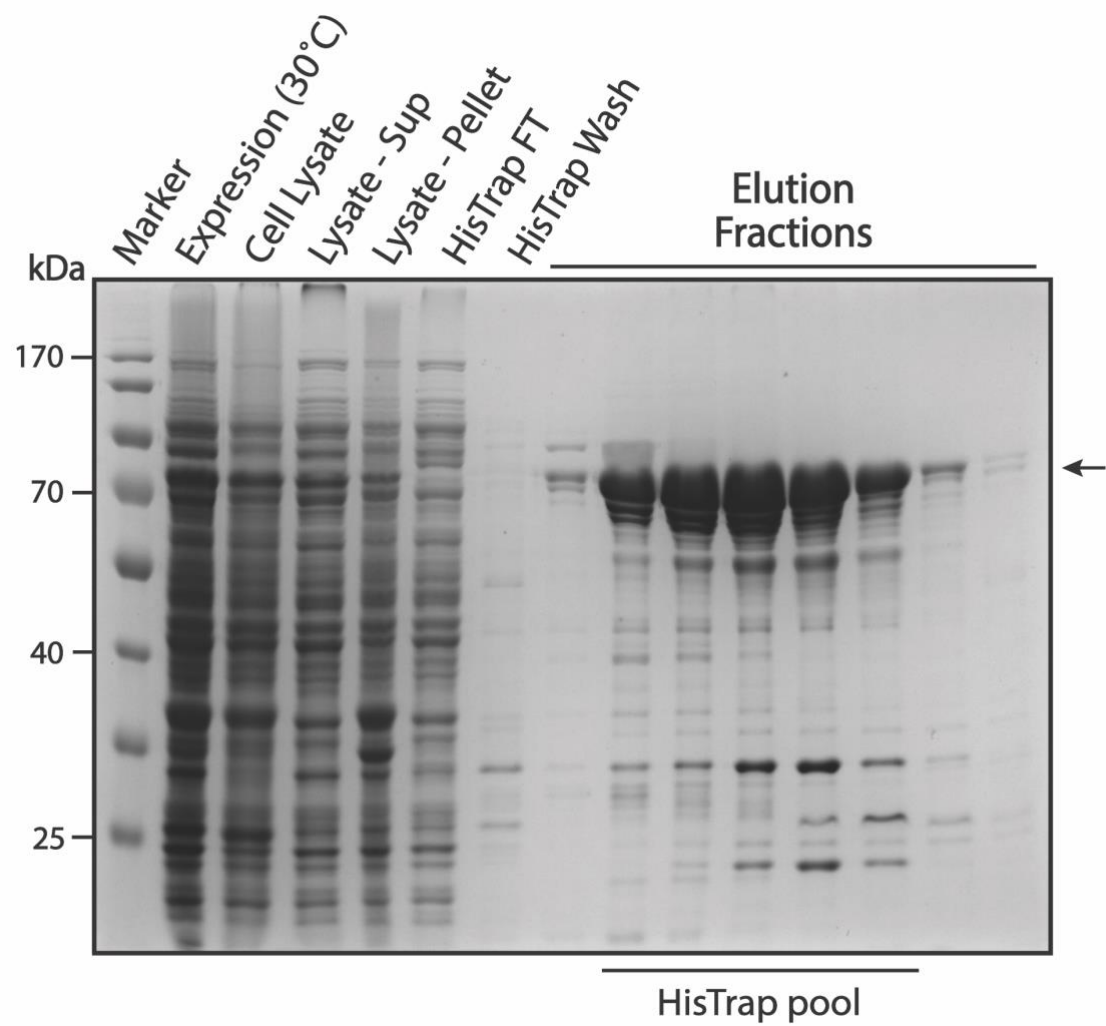
Supplementary Figure S6: Sequencing results to confirm successful mutagenesis of Proline-301 to a Leucine in the Tau-hT40 coding sequence. Plasmid DNA was extracted using the MN-Nucleospin plasmid isolation kit, and Sanger sequencing was performed by Microsynth® in The Netherlands. The image was exported from SnapGene software (www.snapgene.com).



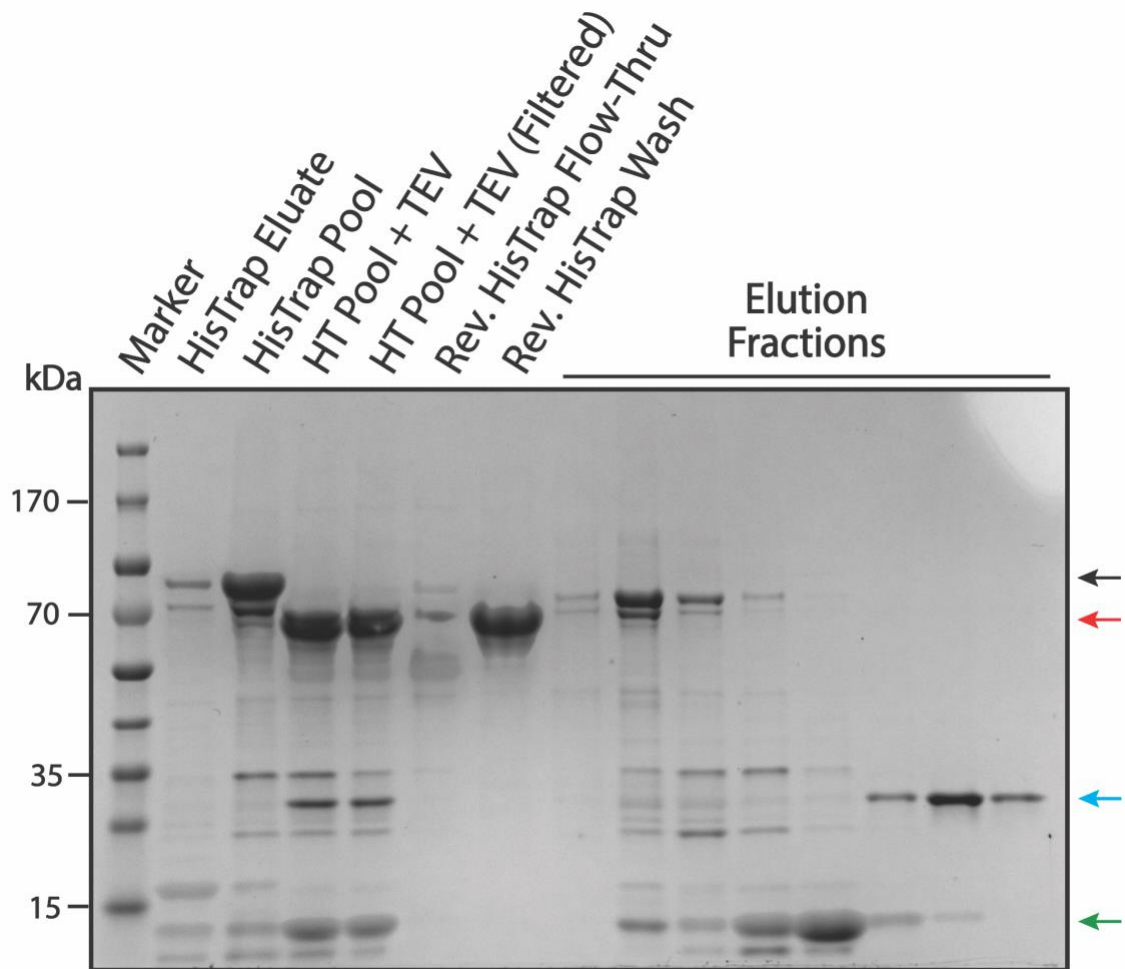
Supplementary Figure S7: Vector map for MaSp-Tau-MTBR expression plasmid used for bacterial expression of Tau's microtubule-binding region. A TEV cleavage site was inserted between the NT* tag solubility tag and the Tau-hT40 coding sequence to facilitate cleavage and separation of purified Tau-MTBR from the cleaved solubility tag during protein purification. The NT*tag housed an N-term 6x His-tag to facilitate its isolation from pure Tau-hT40 during Reverse HisTrap purification. The vector map image was exported from SnapGene software (www.snapgene.com).



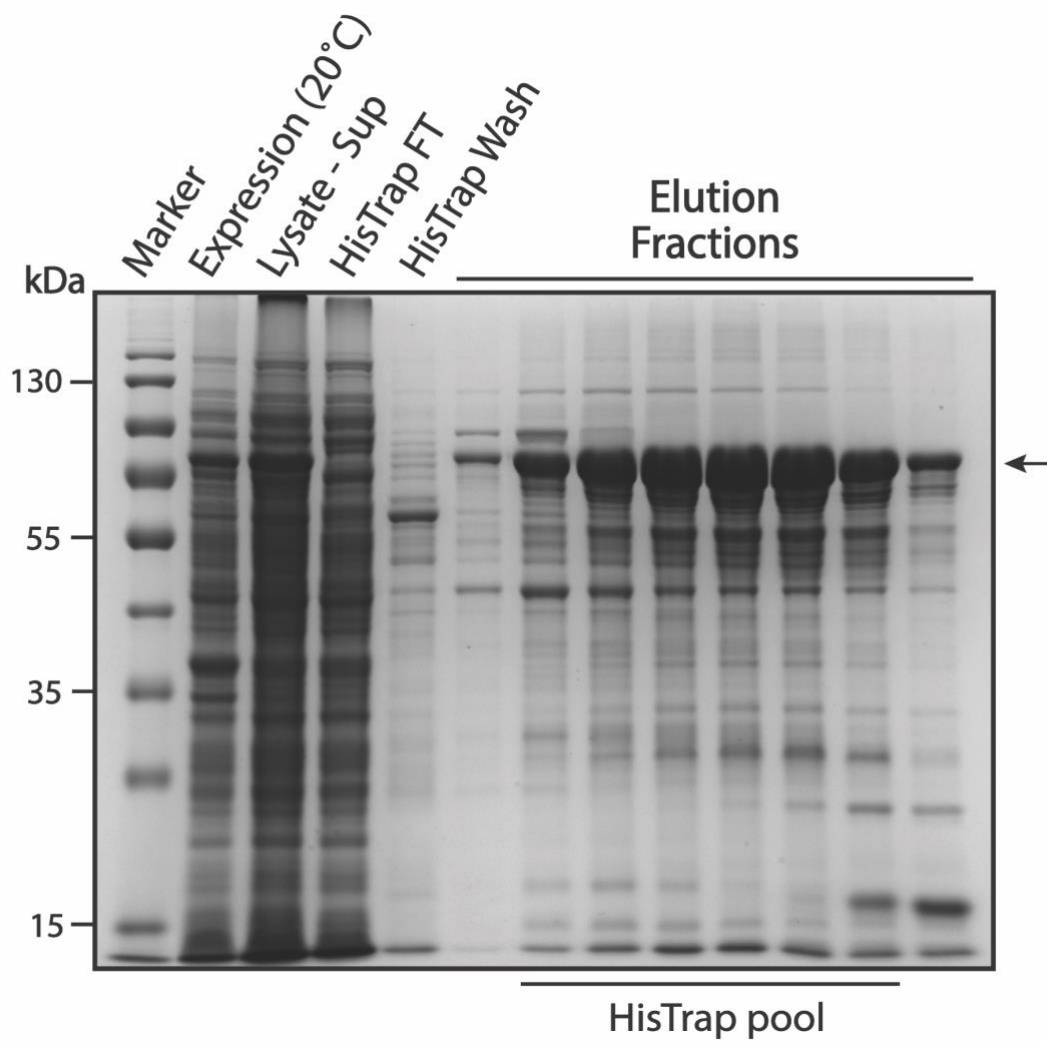
Supplementary Figure S8: Sequencing results to confirm successful cloning of Tau-MTBR coding sequence in the MaSp plasmid and insertion of a TEV cleavage site between the NT*tag and Tau-MTBR coding sequence. Plasmid DNA was extracted using the MN-Nucleospin plasmid isolation kit and Sanger sequencing was performed by Microsynth® in The Netherlands. The image was exported from SnapGene software (www.snapgene.com).



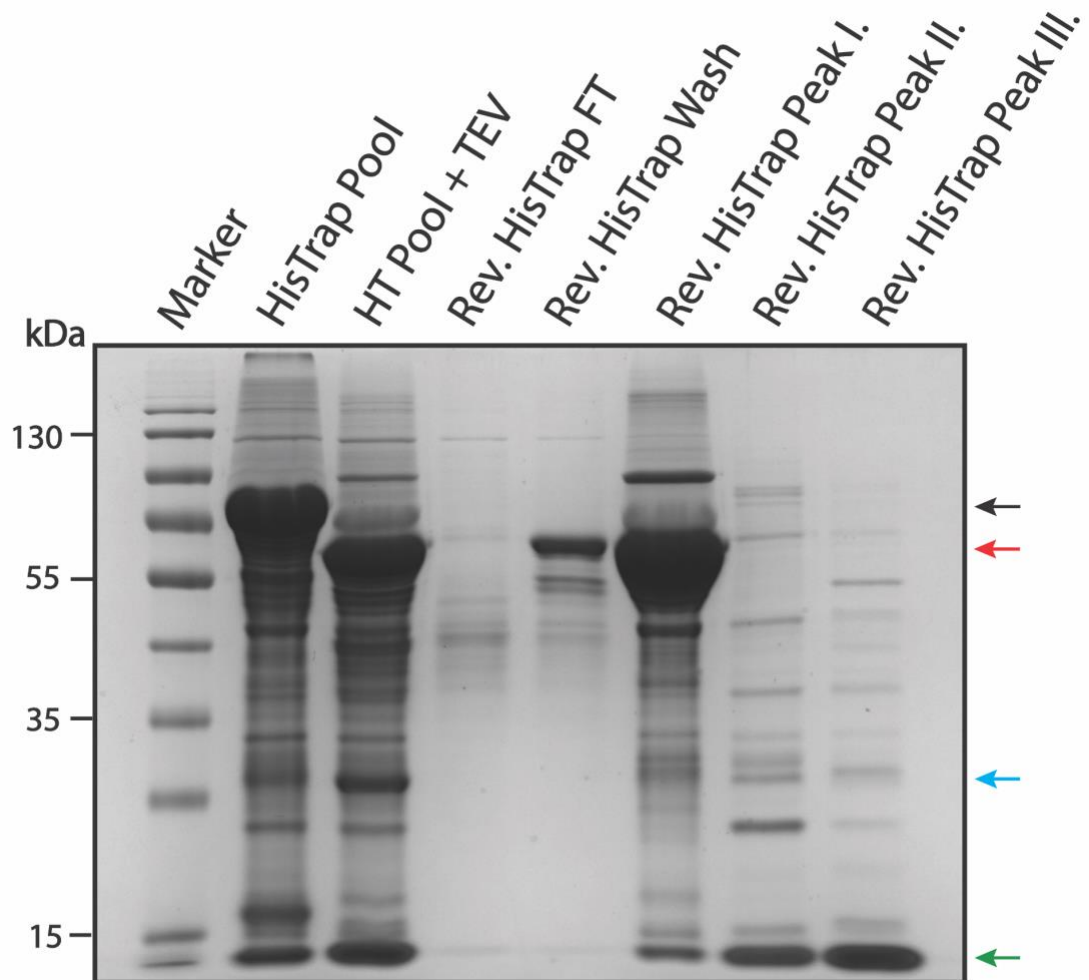
Supplementary Figure S9: HisTrap purification of MaSp-Tau-hT40. SDS-PAGE gel showing the first purification step of MaSp-Tau-hT40 from 1L of TB broth media. The black arrow indicates MaSp-Tau-hT40 fusion protein eluted from two HisTrap-HP-5 mL columns.



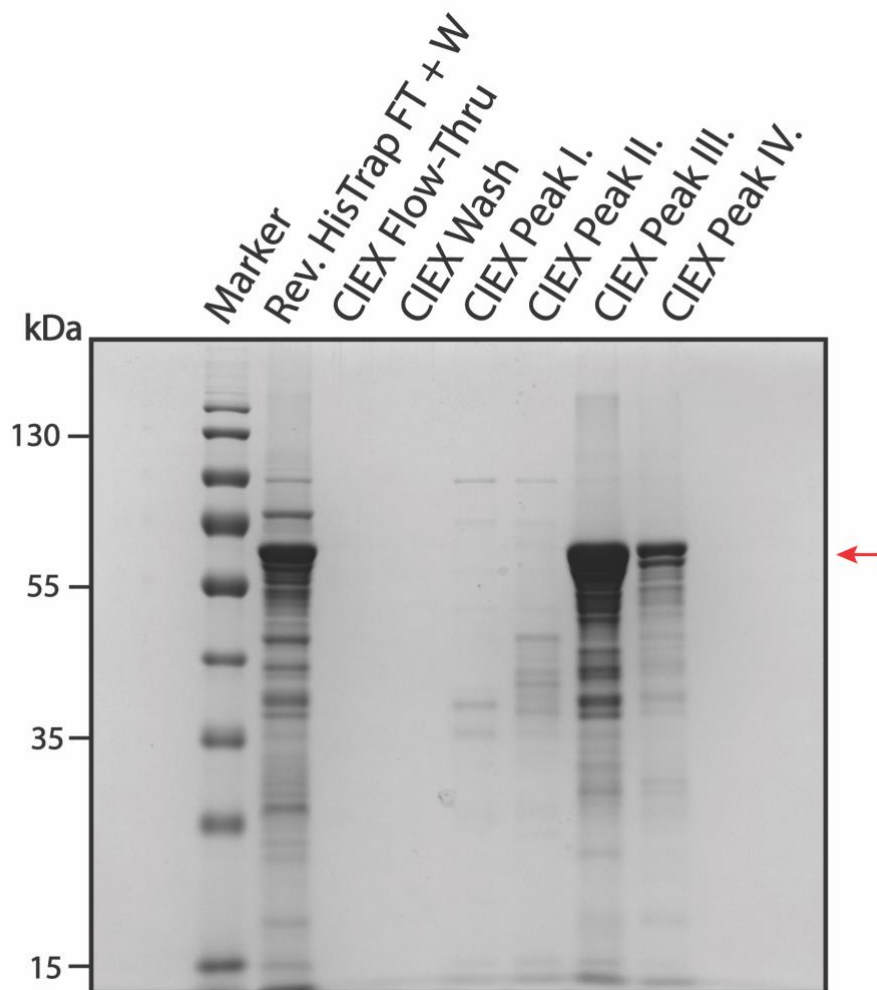
Supplementary Figure S10: Reverse HisTrap purification of MaSp-Tau-hT40. SDS-PAGE gel showing the second purification step of MaSp-Tau-hT40 following TEV digestion to separate Tau-hT40 from cleaved NT* tag and TEV protease enzyme. The black arrow indicates uncleaved MaSp-Tau-hT40, the red arrow indicates free Tau-hT40, the green arrow indicates cleaved NT* tag and the blue arrow indicates the TEV protease enzyme eluted from the HisTrap column.



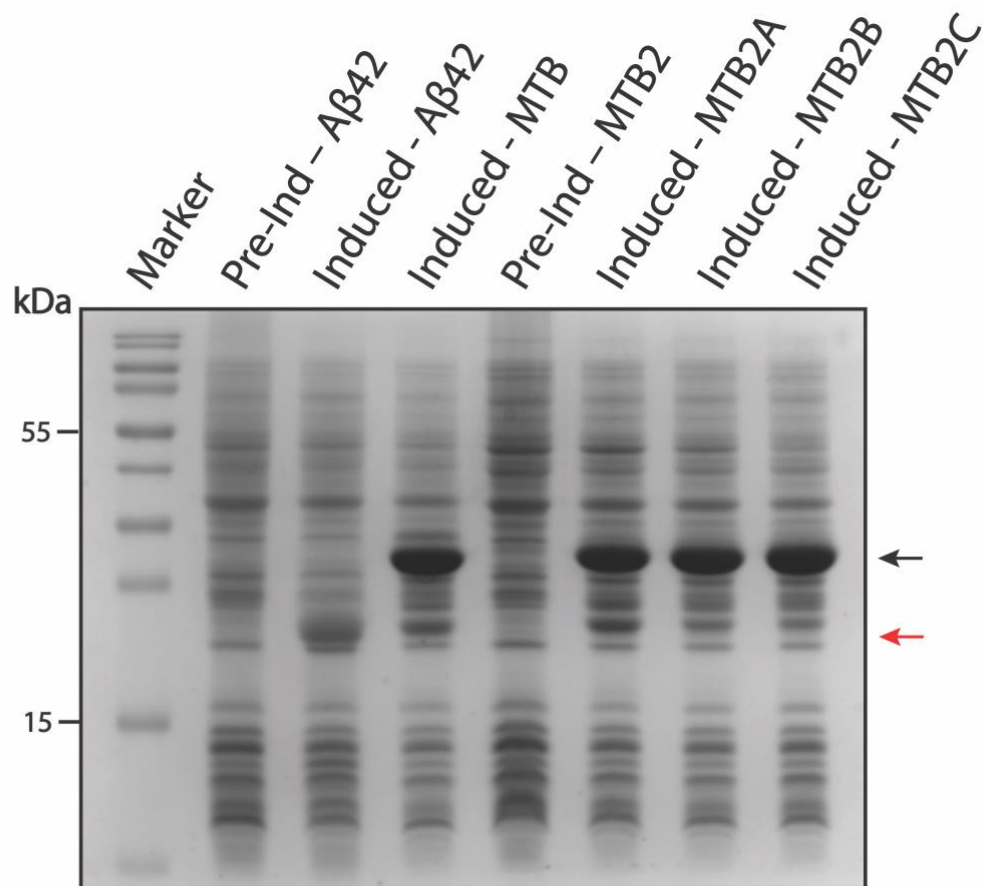
Supplementary Figure S11: HisTrap purification of MaSp-Tau-P301L. SDS-PAGE gel showing the first purification step of MaSp-Tau-P301L from 1L of TB broth media. The black arrow indicates MaSp-Tau-P301L fusion protein eluted from two HisTrap-HP-5 mL columns.



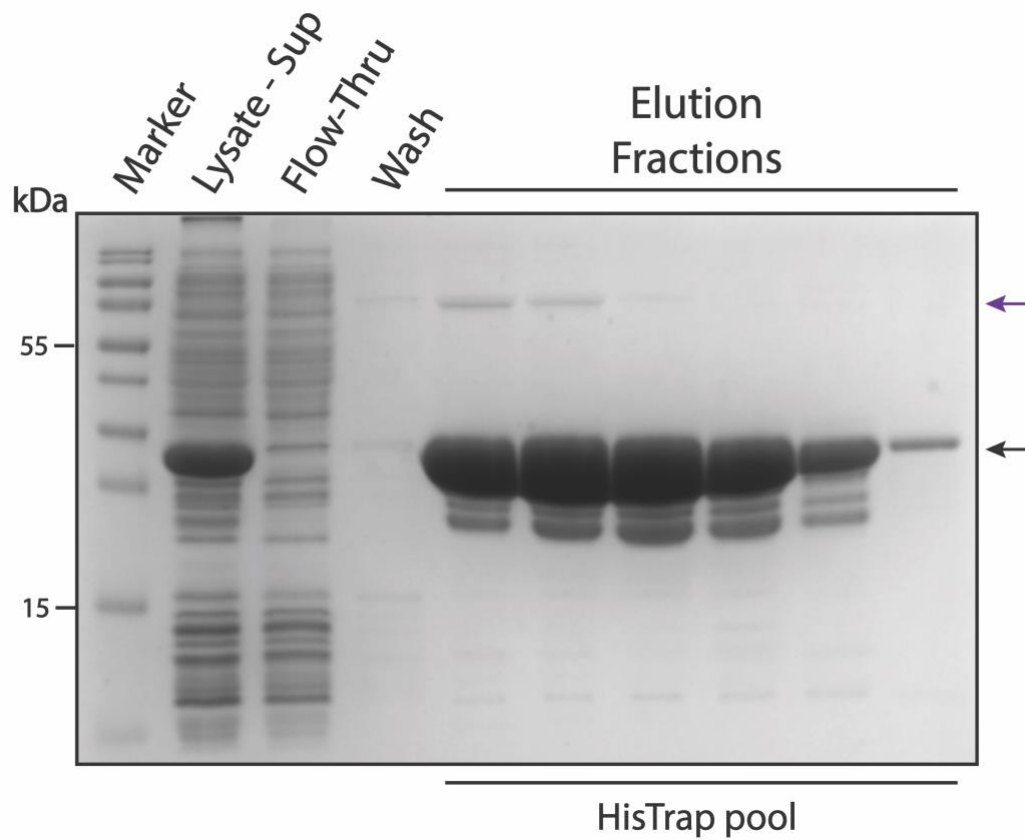
Supplementary Figure S12: Reverse HisTrap purification of MaSp-Tau-P301L. SDS-PAGE gel showing the second purification step of MaSp-Tau-P301L following TEV digestion to separate Tau-hT40 from cleaved NT* tag and TEV protease enzyme. The black arrow indicates uncleaved MaSp-Tau-P301L, the red arrow indicates free Tau-P301L, the green arrow indicates cleaved NT* tag and the blue arrow indicates the TEV protease enzyme eluted from the HisTrap column.



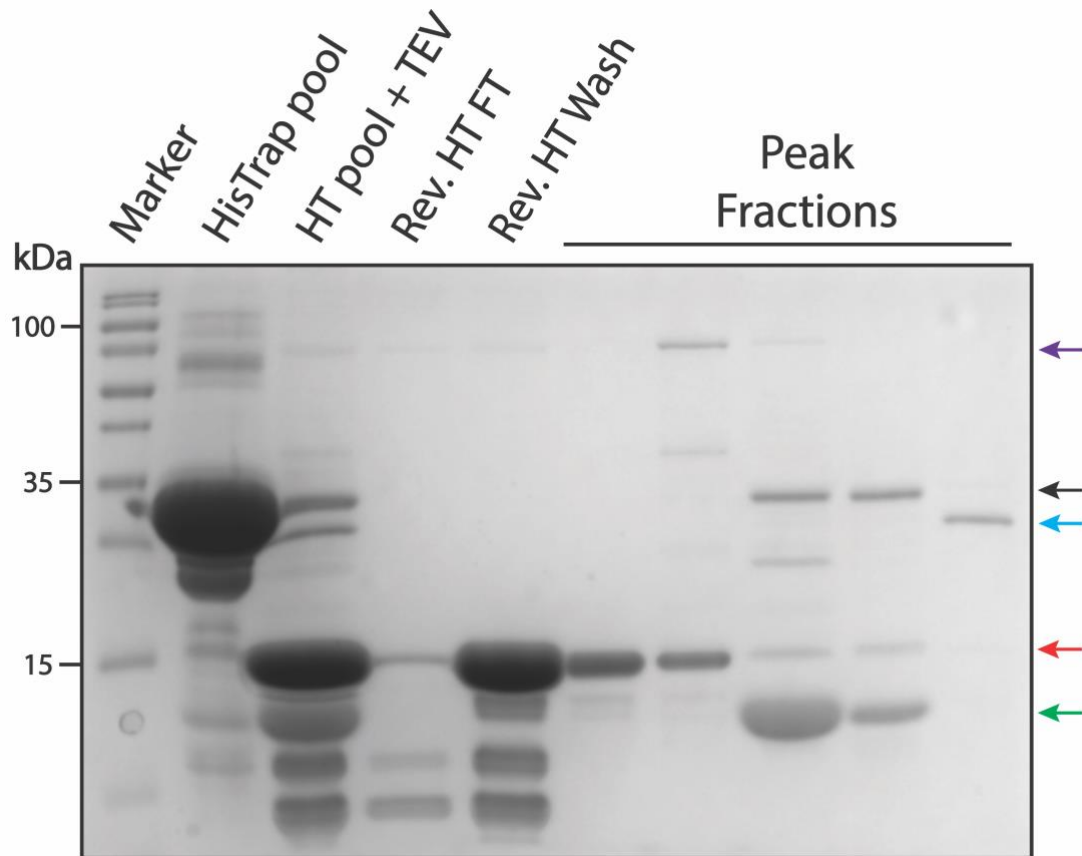
Supplementary Figure S13: Cation exchange (CIEX) chromatography purification of MaSp-Tau-P301L. SDS-PAGE gel showing the final purification step of MaSp-Tau-P301L to get rid of nucleic acid contamination. The red arrow indicates Tau-P301L final preparation eluted from a HiTrap-SP-5 mL column.



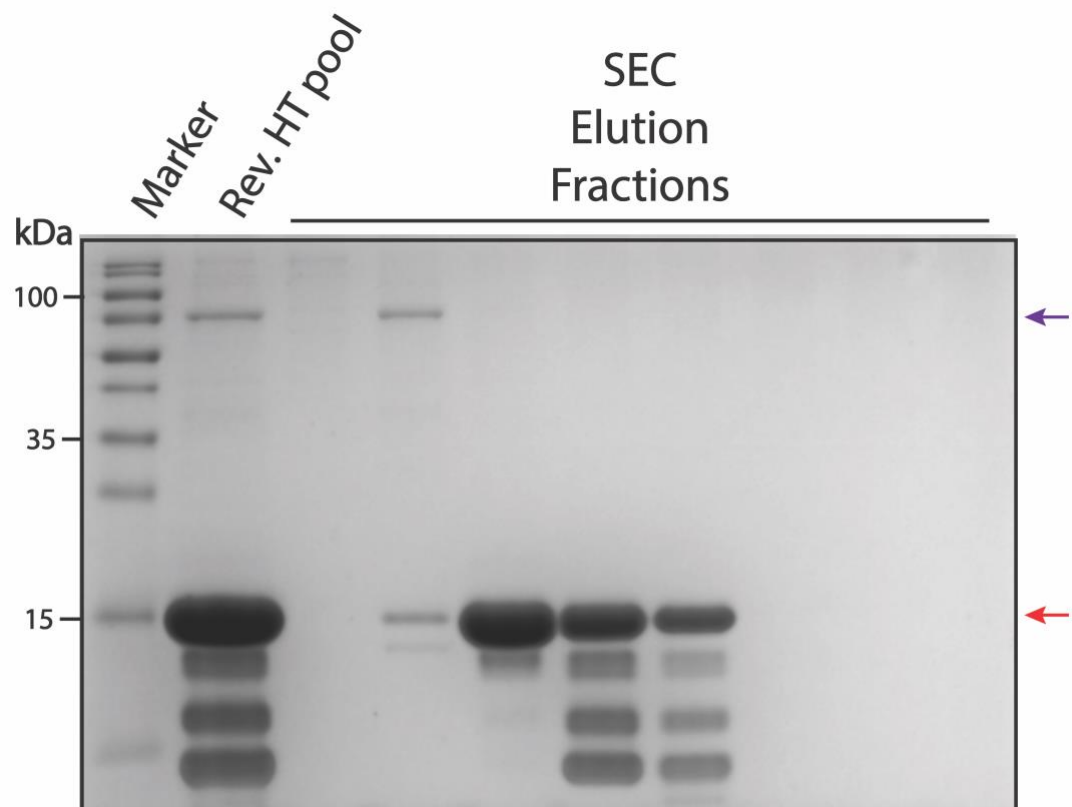
Supplementary Figure S14: Test expression of recombinant MaSp-Tau-MTBR in *E. coli*. SDS-PAGE gel showing the expression of MaSp-Tau-MTBR before (MTB) and after (MTB2) insertion of a TEV cleavage site between the NT* tag and Tau-MTBR coding sequence. The black arrow indicates expressed MaSp-Tau-MTBR recombinant protein in induced samples, while the red arrow indicates expressed amyloid-beta 42 (A β 42) recombinant protein in the post-induction sample.



Supplementary Figure S15: HisTrap purification of MaSp-Tau-MTBR. SDS-PAGE gel showing the first purification step of MaSp-Tau-MTBR from 1L of TB broth media. The black arrow indicates MaSp-Tau-MTBR eluted from two HisTrap-HP-5 mL columns, while the purple arrow indicates DnaK (Hsp70) co-purified with MaSp-Tau-MTBR.



Supplementary Figure S16: Reverse HisTrap purification of MaSp-Tau-MTBR. SDS-PAGE gel showing the second purification step of MaSp-Tau-MTBR following TEV digestion to separate Tau-MTBR from cleaved NT* tag and TEV protease enzyme. The black arrow indicates uncleaved MaSp-Tau-MTBR, the red arrow indicates free Tau-MTBR, the green arrow indicates cleaved NT* tag, the blue arrow indicates the TEV protease enzyme eluted from the HisTrap column, and the purple arrow indicates DnaK (Hsp70) recovered in the elution fractions.



Supplementary Figure S17: Size-exclusion chromatography purification of MaSp-Tau-MTBR. SDS-PAGE gel showing the final polishing step of MaSp-Tau-MTBR. The red arrow indicates purified Tau-MTBR, while the purple arrow indicates residual DnaK (Hsp70) eluted in separate fractions from the Gel Filtration column.