5. Supplementary Materials:

I. Gel pictures:

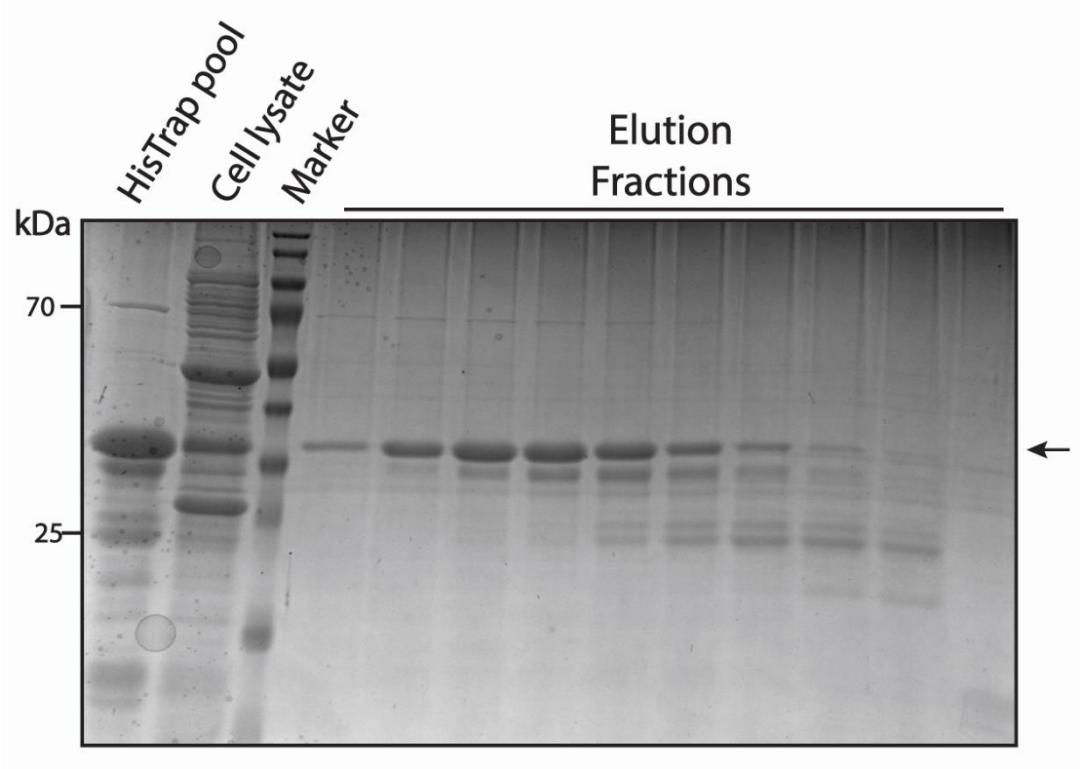

Figure S1.: Purification of pSUMO-AF1(only N-tag). After the Histrap the sample is degraded which cannot be completely separated by SEC using as indicated by the elution fractions. The black arrow indicates full-length AF1 after cleavage of the tag.

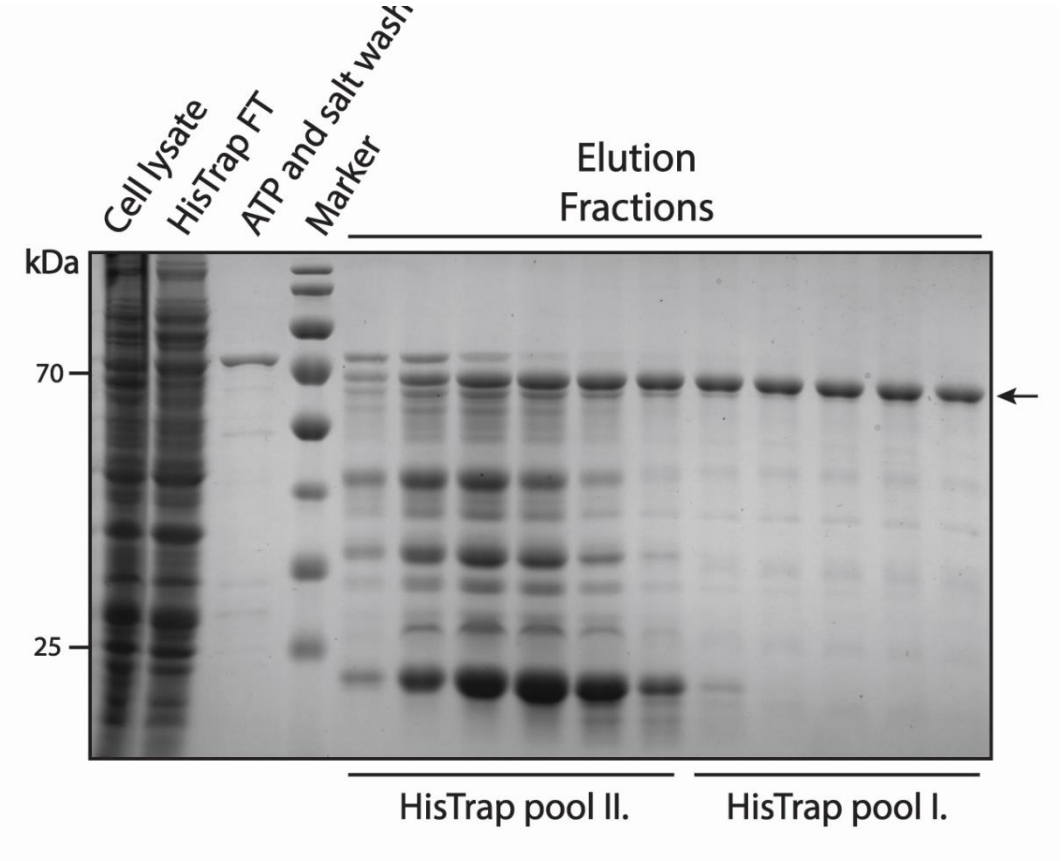

Figure S2.: HisTrap purification of pSUMO-AF1. In the elution fractions there are a clean region of fractions (HisTrap pool I) and a more degraded region (HisTrap pool II). Separately, these were further processed. The black arrow indicates the full-length AF1 before cleaving the tags.

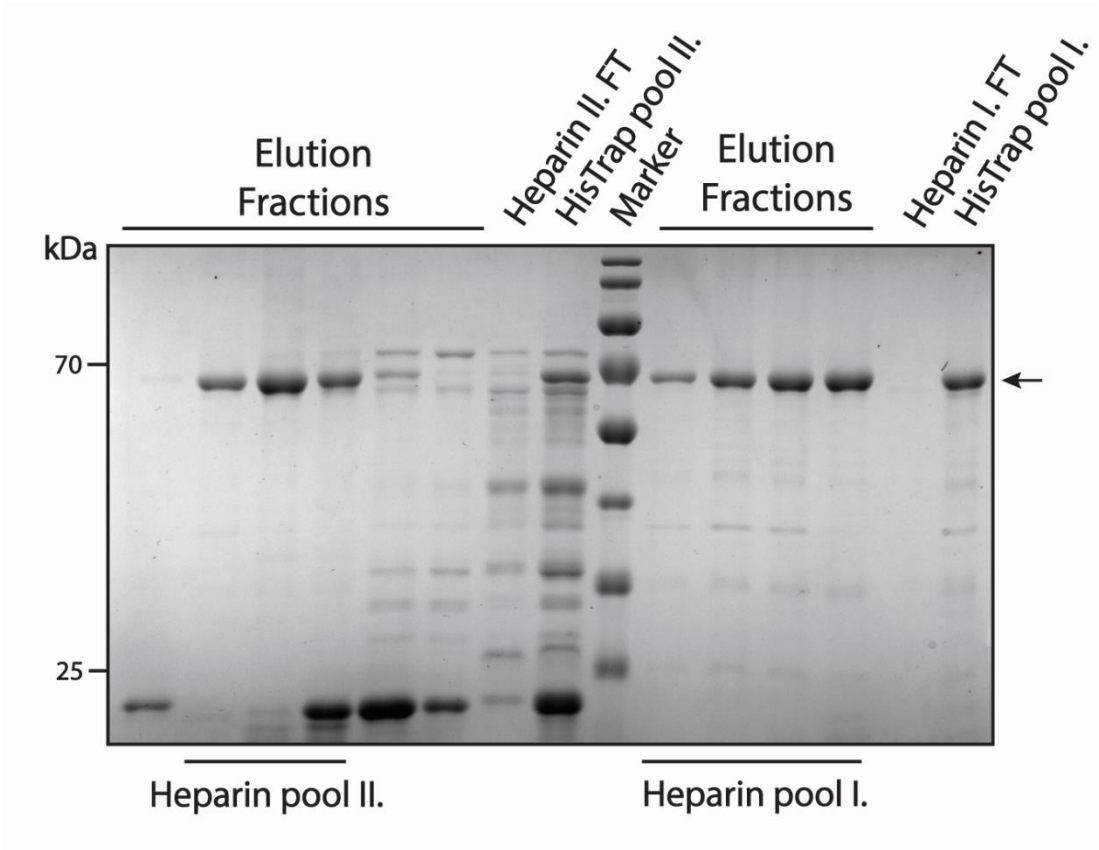

Figure S3.: Heparin purification of AF1. The two different pools from HisTrap (HisTrap pool I and II) were processed separately as Heparin pool I and Heparin pool II. The black arrow indicates the full-length AF1 before cleaving the tags.

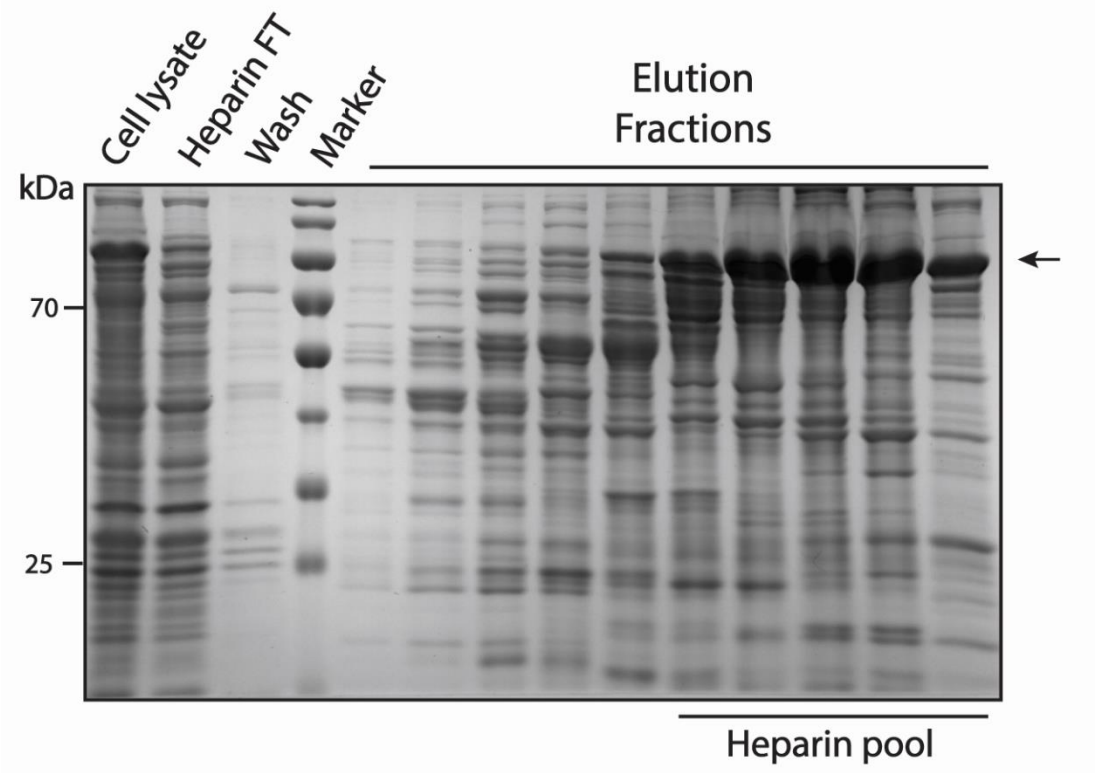

Figure S4.: Heparin purification of pSUMO-Tau-441. The indicated fractions (Heparin pool) were pooled together for the next chromatographic step. The black arrow indicates the full-length Tau-441 before cleaving the tags.

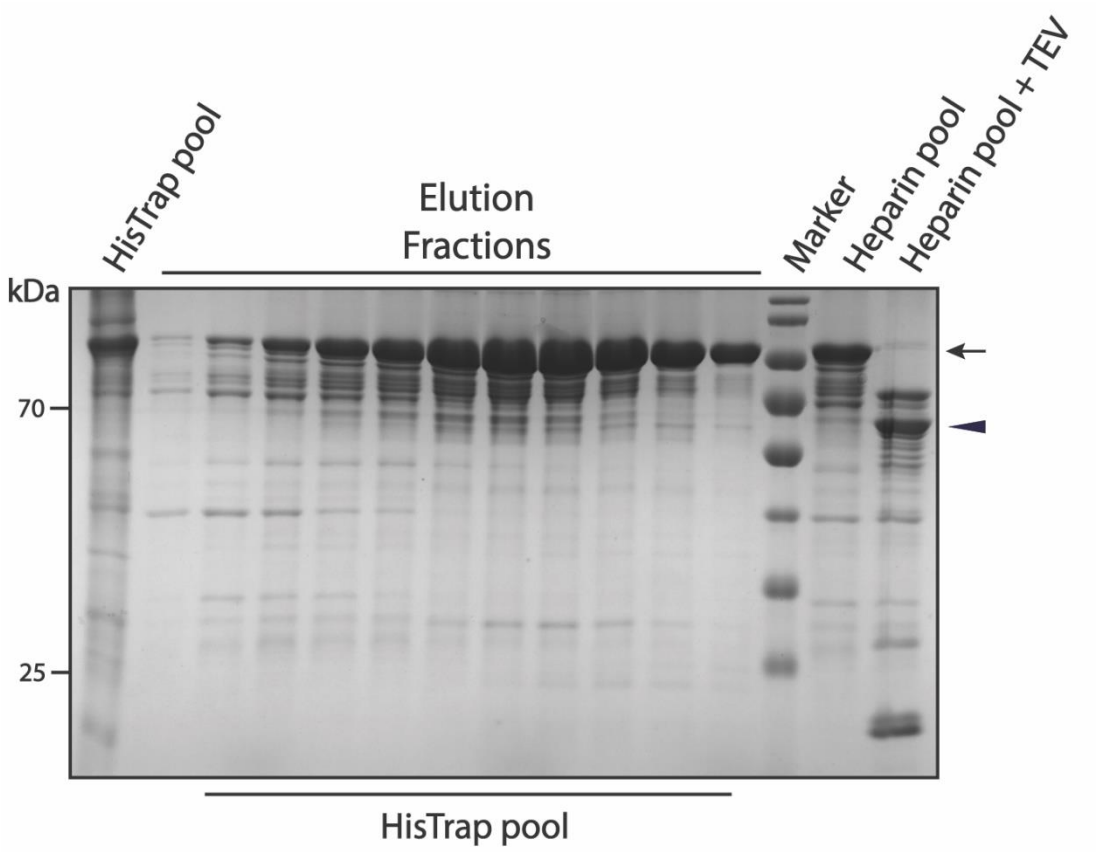

Figure S5.: HisTrap purification of pSUMO-Tau-441. The indicated fractions (HisTrap pool) were pooled for cleavage and subsequent chromatography. The black arrow indicates intact Tau-441 having both affinity tags, while the black triangle indicates the cleaved final product.

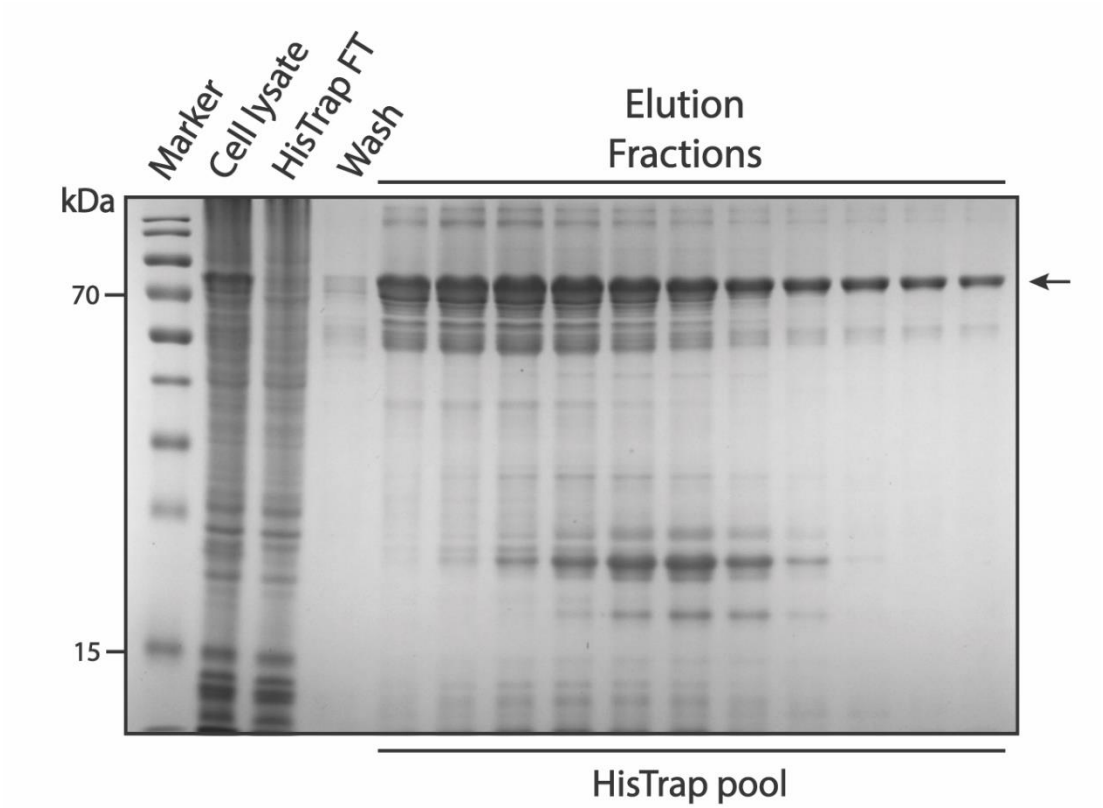

Figure S6.: HisTrap purification of pSUMO-Tau-NTMT. The full-length protein was the most dominant protein species in all elution fractions. Therefore, all elution fractions were pooled together and prepared for Heparin chromatographic separation. The black arrow indicates full-length Tau-NTMT with both affinity tags.

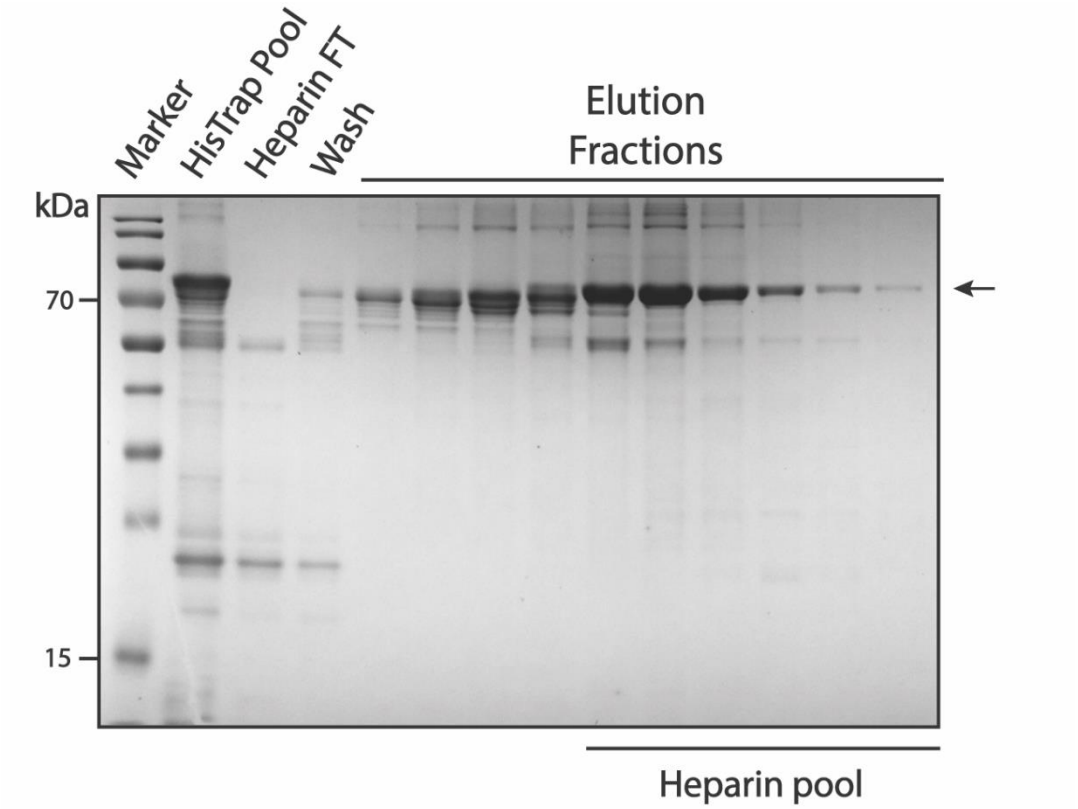

Figure S7.: Heparin purification of pSUMO-Tau-NTMT. Majority of the full-length protein eluted in later fractions. These were pooled together for dialysis and TEV digestion, prior to Reverse HisTrap purification. The black arrow indicates full-length Tau-NTMT with both affinity tags.

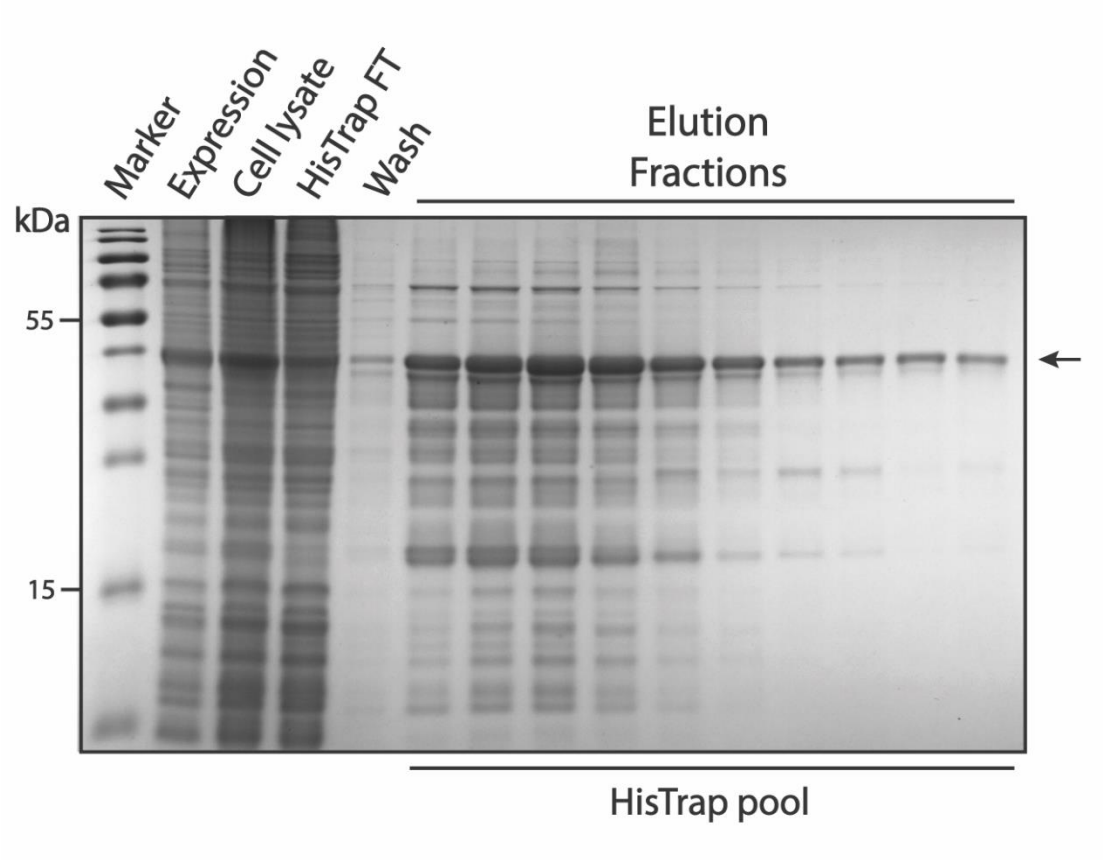

Figure S8.: HisTrap purification of pSUMO-Tau-MTBR. The full-length protein was the most dominant protein species in all elution fractions. Therefore, all elution fractions were pooled together and prepared for Heparin chromatographic separation. The black arrow indicates full-length Tau-MTBR with both affinity tags.

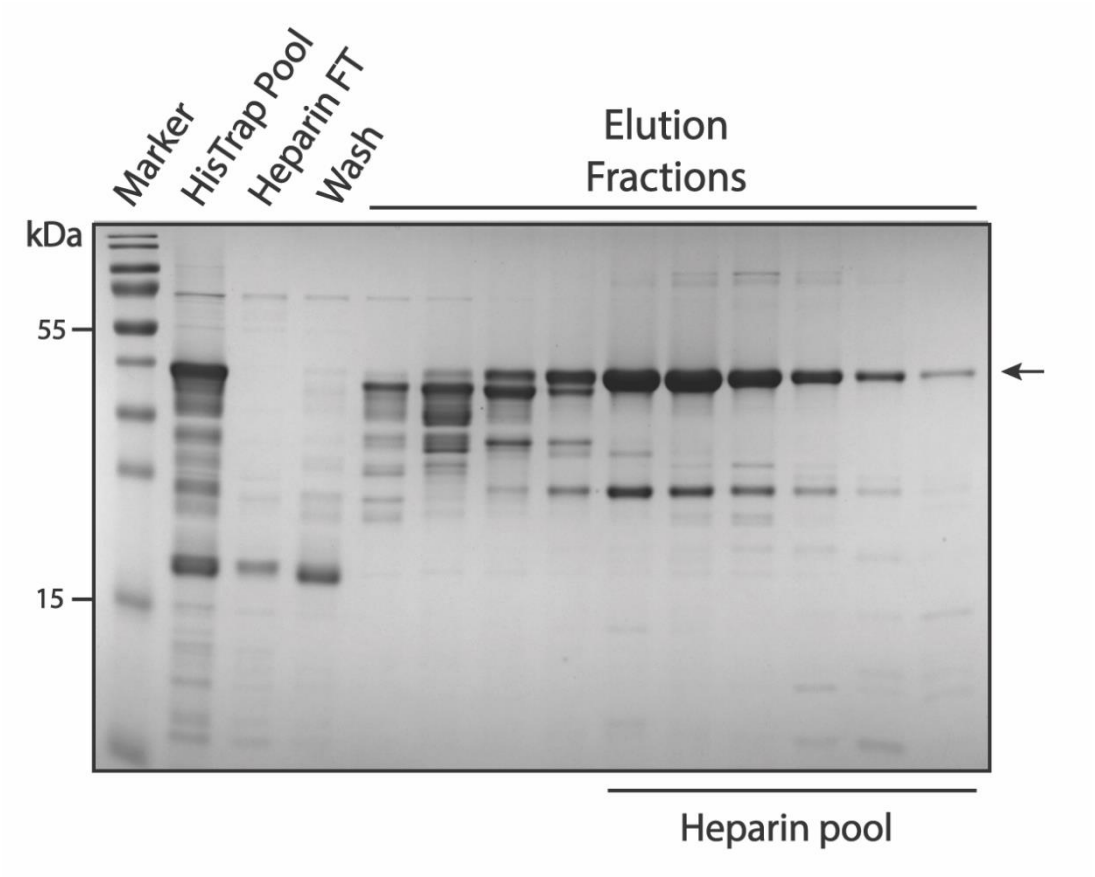

Figure S9.: Heparin purification of pSUMO-Tau-MTBR. Majority of the full-length protein eluted in later fractions. These were pooled together for dialysis and TEV digestion, prior to Reverse HisTrap purification. The black arrow indicates full-length Tau-MTBR with both affinity tags.

II. MS Analysis:

1. MS analysis of p-SUMO-AF1:

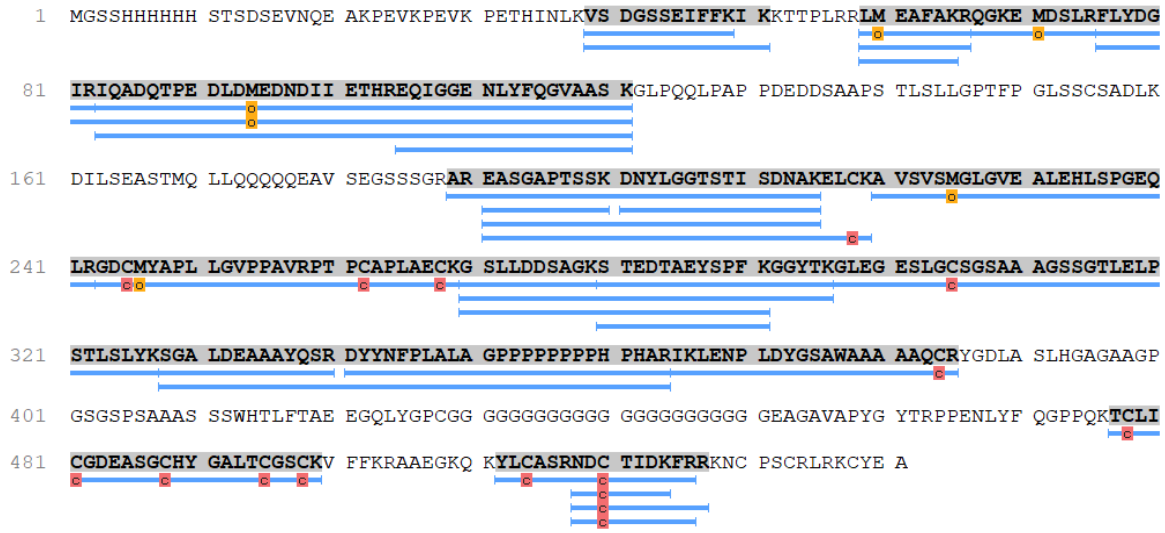

Figure S10.: Alignment of identified peptides from the uncleaved sample (before TEV cleavage) to the sequence uncleaved product (AF1 with both tags), indicating that AF1 is present with both tags. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 466 to residue 541. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

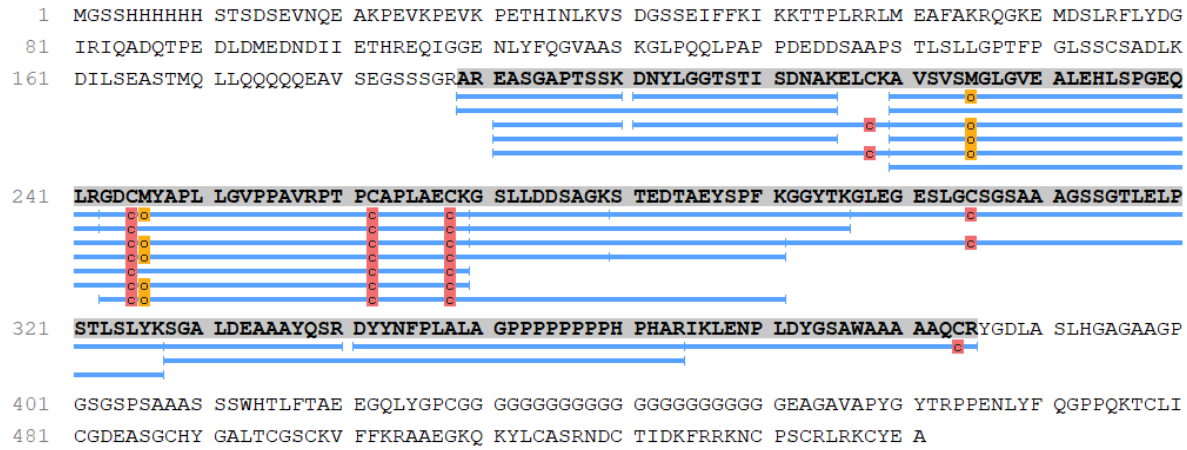

Figure S11.: Alignment of identified peptides from the cleaved sample (final product) to the sequence of uncleaved product (AF1 with both tags), indicating that both tags have been removed. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 466 to residue 541. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

2. MS analysis of p-SUMO-Tau-441:

Figure S12.: Alignment of identified peptides from the uncleaved sample (before TEV cleavage) to the sequence of uncleaved full-length construct (Tau-441 with both tags), indicating that Tau-441 is present with both tags. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 558 to residue 663. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

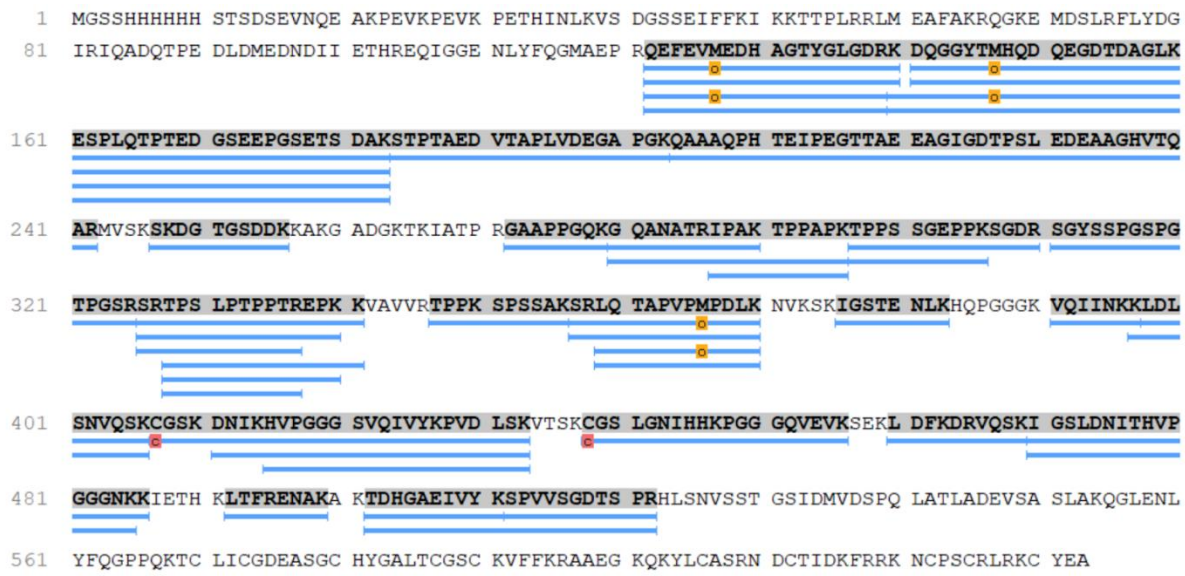

Figure S13.: Alignment of identified peptides from the cleaved sample (final product) to the sequence of uncleaved full-length construct (Tau-441 with both tags), indicating that both tags have been removed. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 558 to residue 663. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

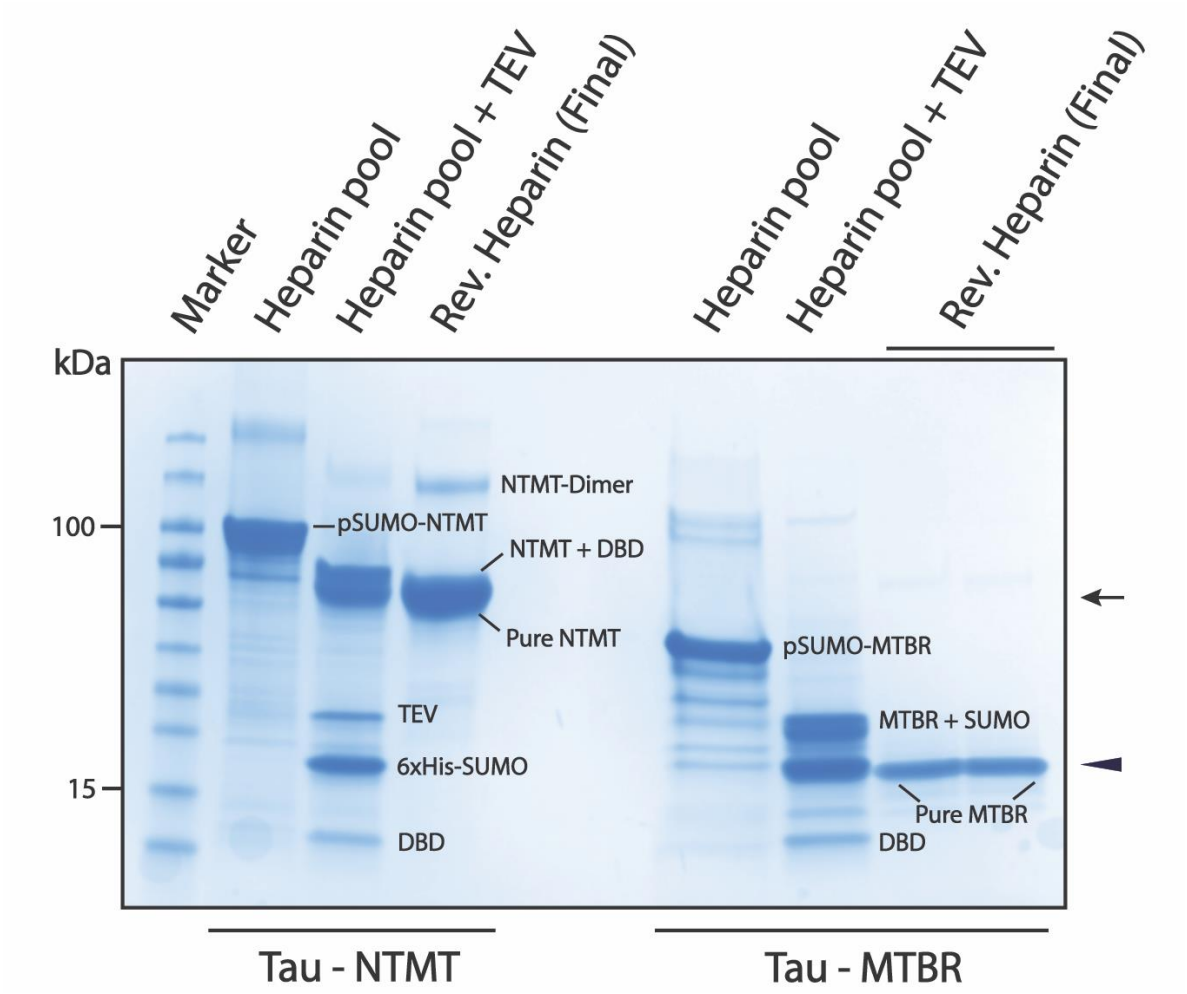

Figure S14.: SDS-PAGE Gel highlighting bands that were extracted, analysed and confirmed by MS. The black arrow indicates purified Tau-NTMT final product, while the black triangle indicates purified Tau-MTBR final product.

3. MS analysis of pSUMO-Tau-NTMT:

pSUMO-NTMT band:

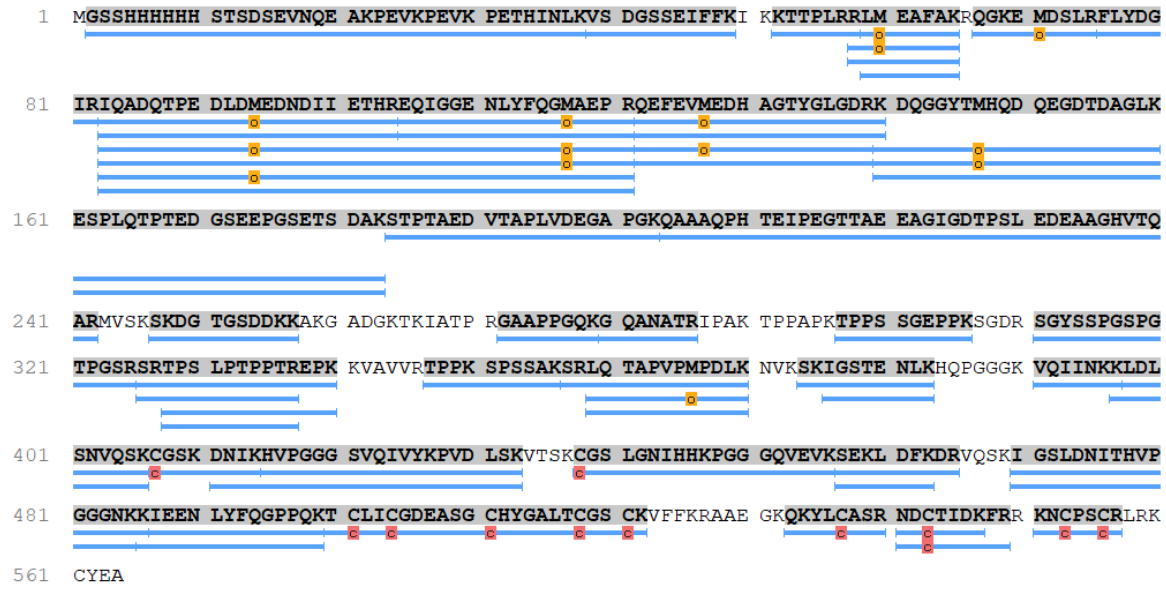

Figure S15.: Alignment of identified peptides from uncleaved sample (before TEV cleavage) to the sequence of uncleaved full-length construct (Tau-NTMT with both tags), indicating that both tags are present before TEV cleavage. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 489 to residue 564. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

NTMT+DBD band:

Figure S16.: Alignment of identified peptides from partially cleaved sample (after TEV cleavage) to the sequence of uncleaved full-length construct (Tau-NTMT with both tags), indicating that only the 6xHis-SUMO-tag has been removed. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 489 to residue 564. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

Pure NTMT band (final product):

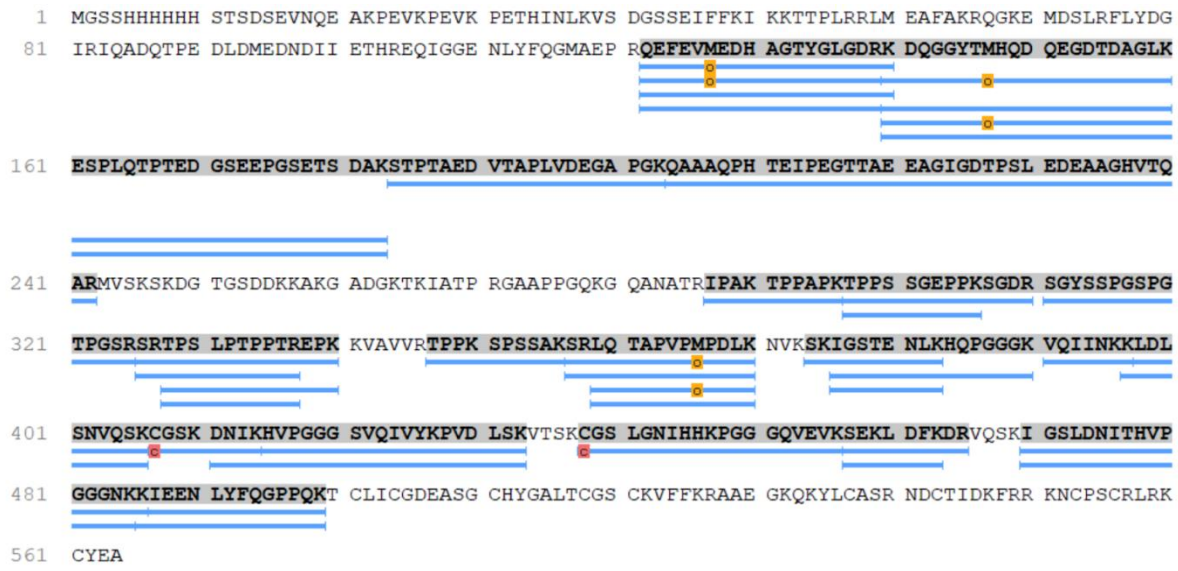

Figure S17.: Alignment of identified peptides from the cleaved sample (final product) to the sequence of uncleaved full-length construct (Tau-NTMT with both tags), indicating that both tags have been removed. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 489 to residue 564. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

NTMT-Dimer band:

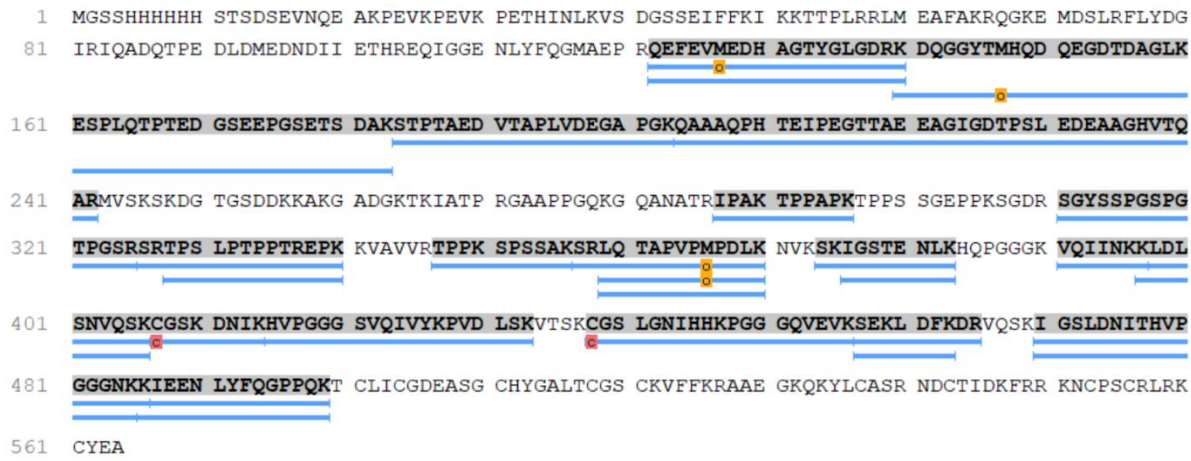

Figure S18.: Alignment of identified peptides from high-molecular-weight (HMW) bands in Tau-NTMT final product (NTMT-Dimer, ~120 kDa band) to the sequence of uncleaved full-length construct (Tau-NTMT with both tags), indicating that the bands were made up of pure Tau-NTMT dimers. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 489 to residue 564. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

4. MS analysis of pSUMO-Tau-MTBR:

pSUMO-MTBR band:

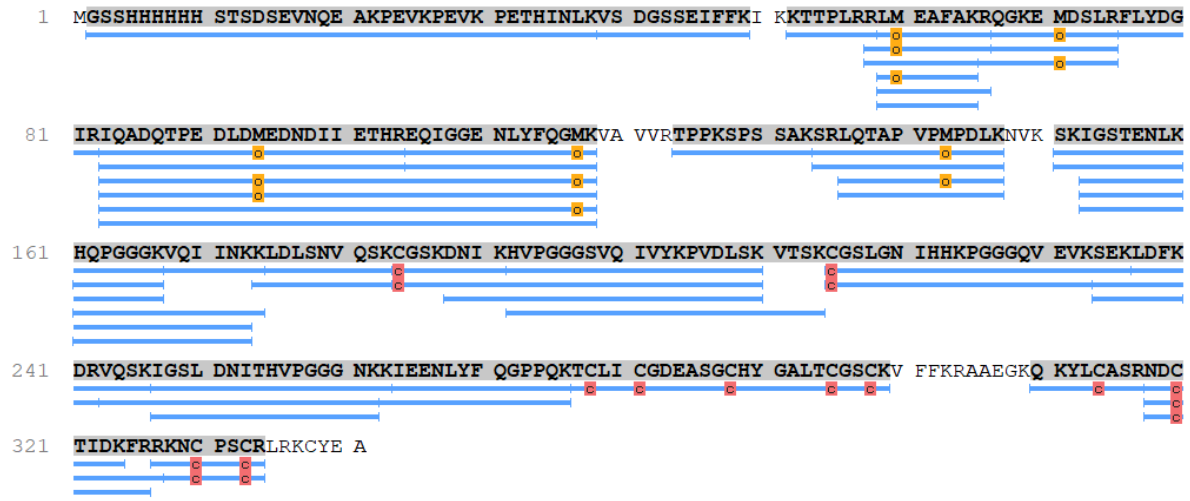

Figure S19.: Alignment of identified peptides from the uncleaved sample (before TEV cleavage) to the sequence of uncleaved full-length construct (Tau-MTBR with both tags), indicating that Tau-441 is present with both tags. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 266 to residue 341. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

MTBR+SUMO band:

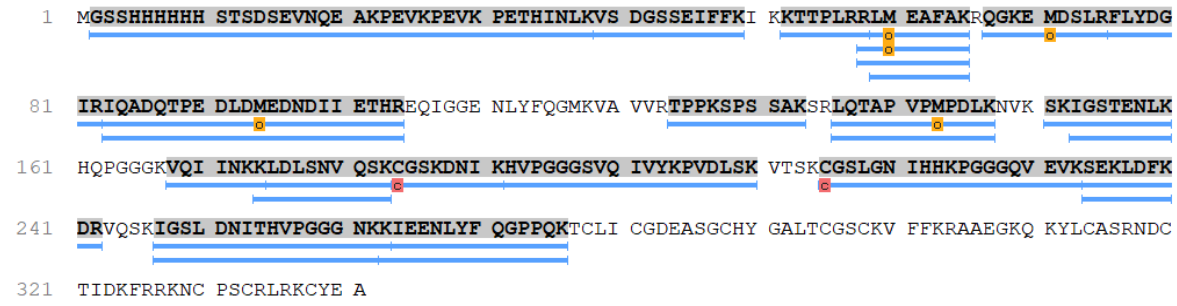

Figure S20.: Alignment of identified peptides from the partially cleaved sample (after TEV cleavage) to the sequence of uncleaved full-length construct (Tau-MTBR with both tags), indicating that Tau-441 still has the N-terminal 6xHis-SUMO-tag. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 266 to residue 341. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.

MTBR band (final product):

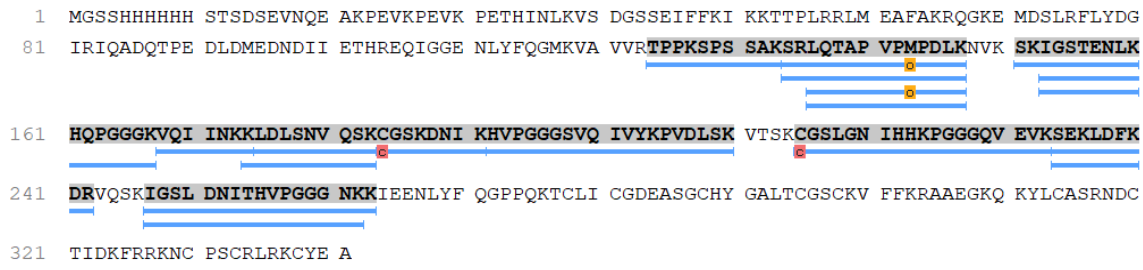

Figure S21.: Alignment of identified peptides from the cleaved sample (final product) to the sequence of uncleaved full-length construct (Tau-MTBR with both tags), indicating that both tags have been removed. The N-terminal 6xHis-SUMO-tag with the TEV cleavage site starts from residue 1 to residue 116, while the C-terminal DBD with the TEV cleavage site starts from residue 266 to residue 341. Oxidised methionines and carbamidomethylated cysteines are peptide modifications highlighted with O-symbol (orange) and C-symbol (red), respectively.