

**Self protein-protein interactions are involved in TPPP/p25
mediated microtubule bundling**

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Supplementary Figures Legends

Figure S1 (a) Folding predictions of human TPPP/p25 according to the FOLDINDEX software, which predicts whether a given protein sequence is intrinsically unfolded. The N- and C-terminal domains are estimated to have a high probability of being intrinsically unfolded (red) in contrast to the central core domain (green)³³. **(b)** Charge distribution plot of TPPP/p25 showing that the N-terminus and C-terminus are positively charged. The core domain has alternating charges favoring a proposed “electrostatic zipper” of protein-protein interaction mode.

Figure S2 (a) Coomassie blue staining of full length TPPP/p25 and the supernatant after centrifugation following long term incubation (longer than 24h) with taxol stabilized MTs. **(b)** The proteolytic fragments were analyzed by N-terminal sequencing and the molecular masses were determined by MALDI mass spectrometry. **(c)** The corresponding N-terminal sequences are depicted in red in the amino acid sequence of full length TPPP/p25.

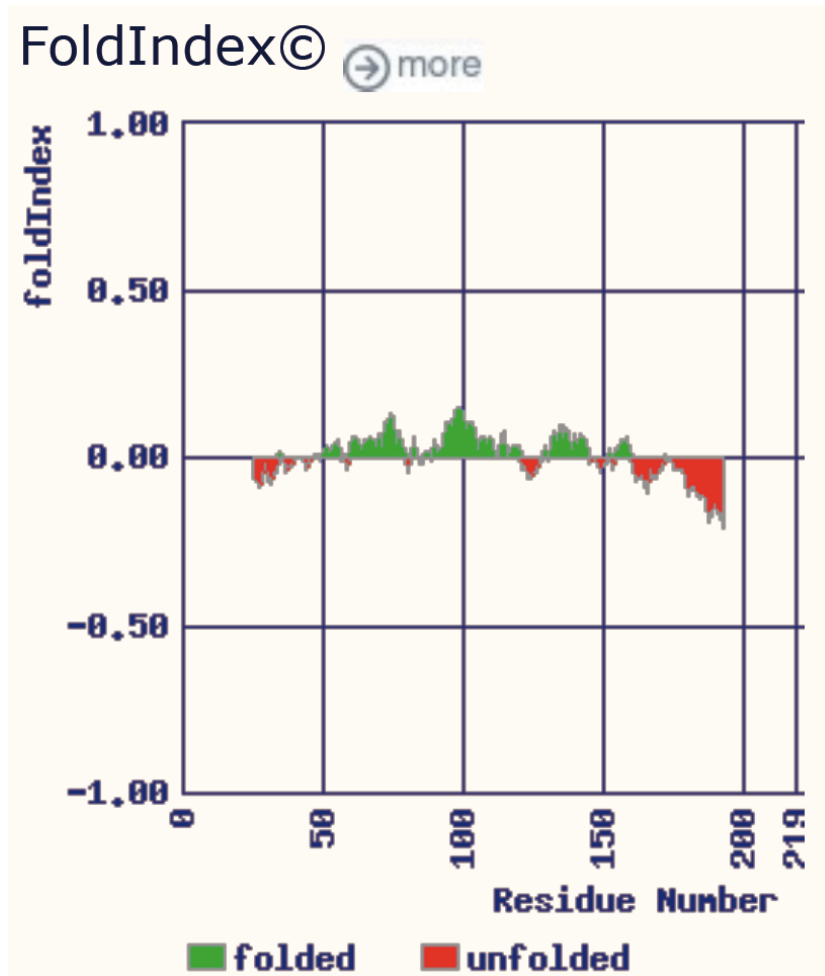
Figure S3 MT binding properties of tag-free TPPP/p25. **(a)** Light scattering assays ($OD_{350\text{ nm}}$) of tubulin solutions ($15\ \mu\text{M}$) in the presence of His-tagged full length (FL) and tag cleaved full length ($\text{FL}^{\text{HISFree}}$) and $\text{core}^{\text{HISFree}}$ domain of TPPP/p25 (ratios of tubulin to TPPP/p25 are shown in parentheses). **(b)** EM images of microtubules assembled from tubulin solutions ($15\ \mu\text{M}$) in the presence of FL and tag-cleaved FL and tag-cleaved core domain of TPPP/p25 (ratios of tubulin to TPPP/p25 are shown in parentheses). Scale bars in the left column of images correspond to $0,5\ \mu\text{m}$; in the right column to $0,2\ \mu\text{m}$). **(c)** Taxol stabilized microtubules ($2\ \mu\text{M}$ tubulin) were incubated in the presence of $12\ \mu\text{M}$ of either tag-cleaved FL or core domain of TPPP/p25 for 15 min

before centrifugation. Coomassie-stained gel of microtubule bound and unbound TPPP/p25 fragments present in the pellets and in the supernatants, respectively. **(d)** EM images of taxol stabilized microtubules incubated with bacterially expressed and purified tag-cleaved TPPP/p25 fragments (ratios of tubulin to TPPP/25 are shown in parentheses) Scale bars correspond to 0,2 μ m.

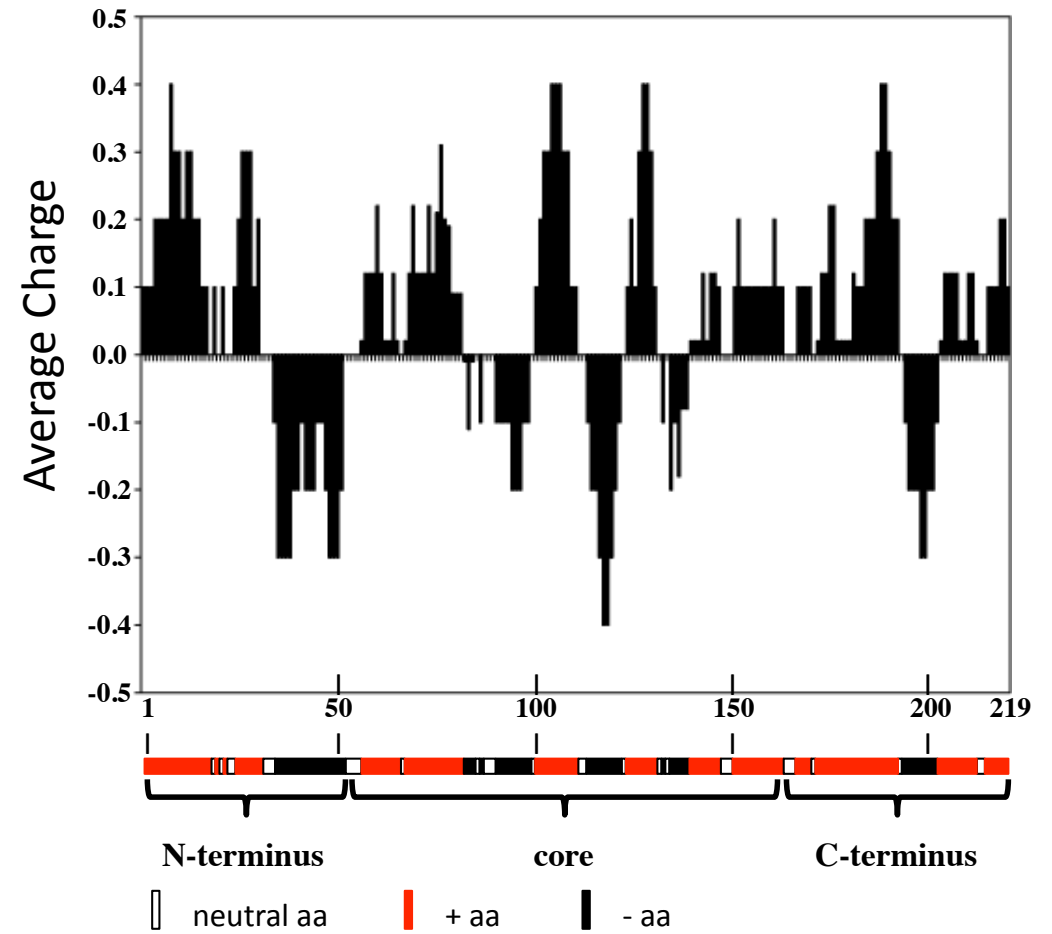
Figure S4 Tag-cleaved TPPP/p25 competes with GFP-tagged TPPP/p25 for MT binding. Taxol stabilized MTs were first co-incubated with 2 μ M of tag-cleaved FL and 2 μ M GFP-FL-TPPP/p25 and then with increasing concentrations of tag-cleaved FL. Each data point represents the mean \pm SD from three independent experiments.

Figure S5 Cells were transfected with either HA-full length **(a)**, Δ C(158) **(b)**, Δ N(49) **(c)** or core -TPPP/p25-VC173 **(d)**. Cells were then fixed and stained for indirect immunofluorescence microscopy using a monoclonal antibody recognizing the HA-tag. Perinuclear bundles are detected in cells transfected with the full length, Δ N(49) and Δ C(158) whereas a diffuse cytoplasmic staining is observed in cells expressing the core.

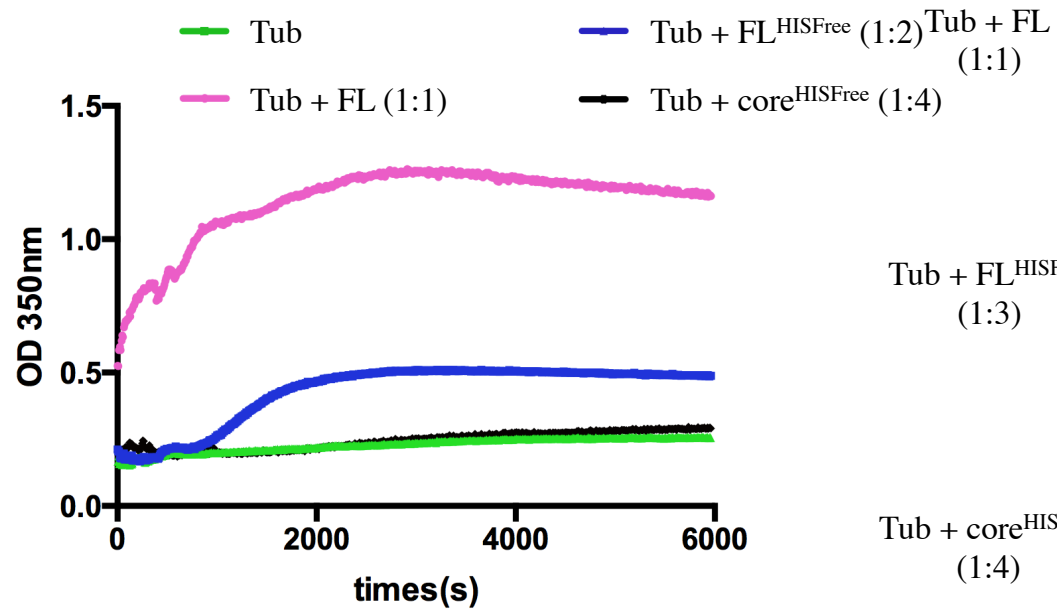
a



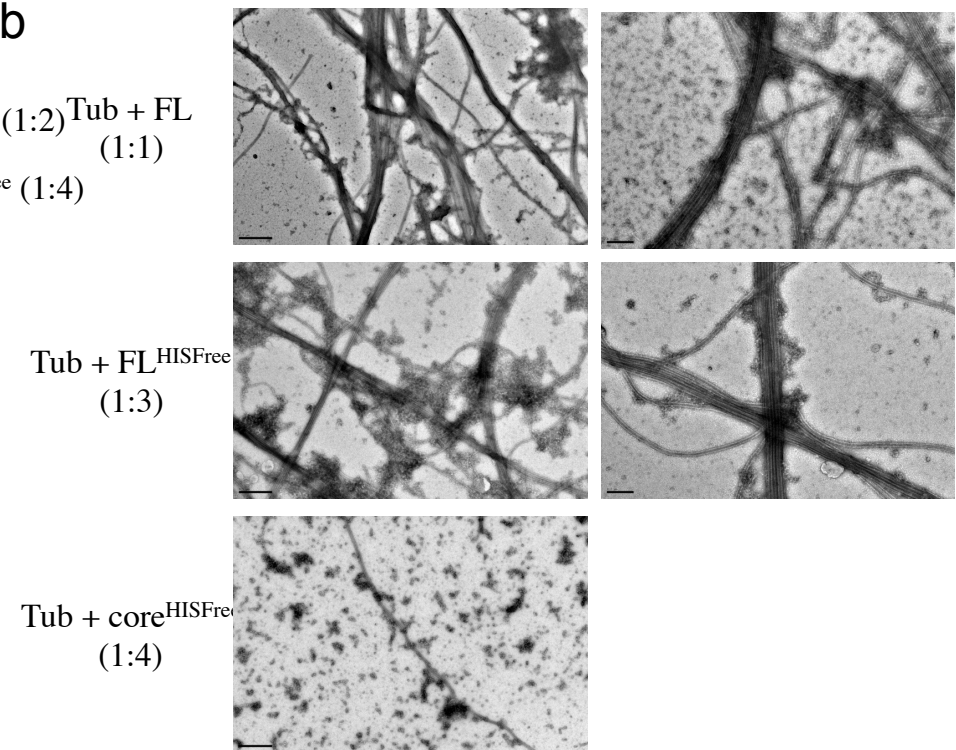
b



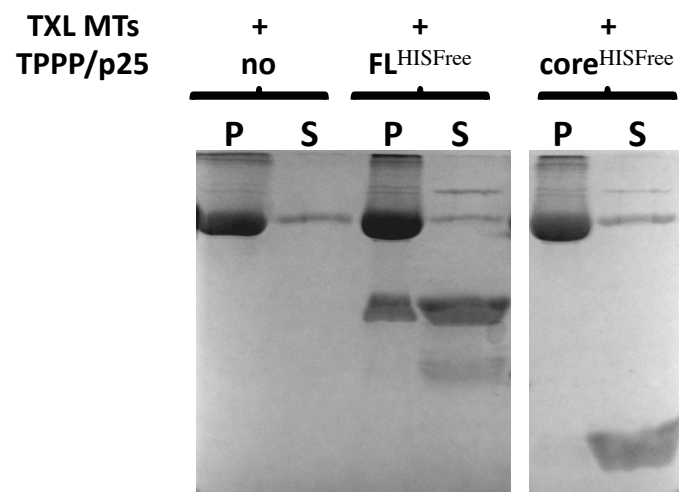
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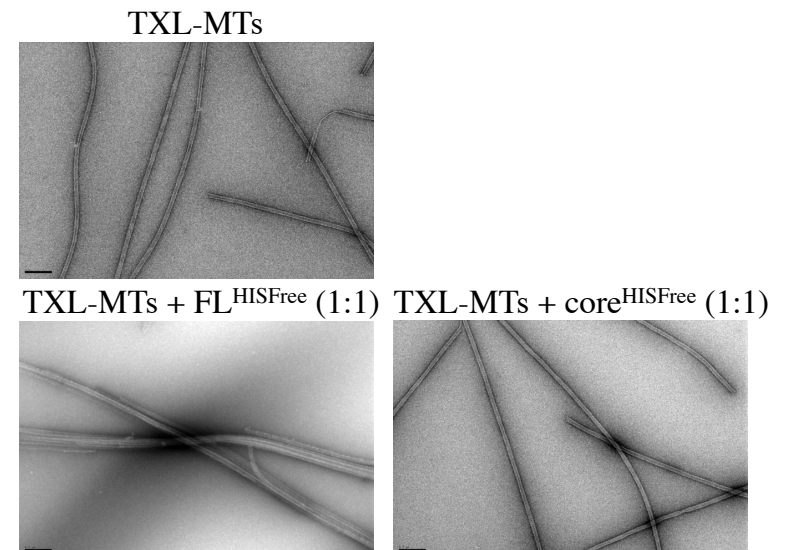
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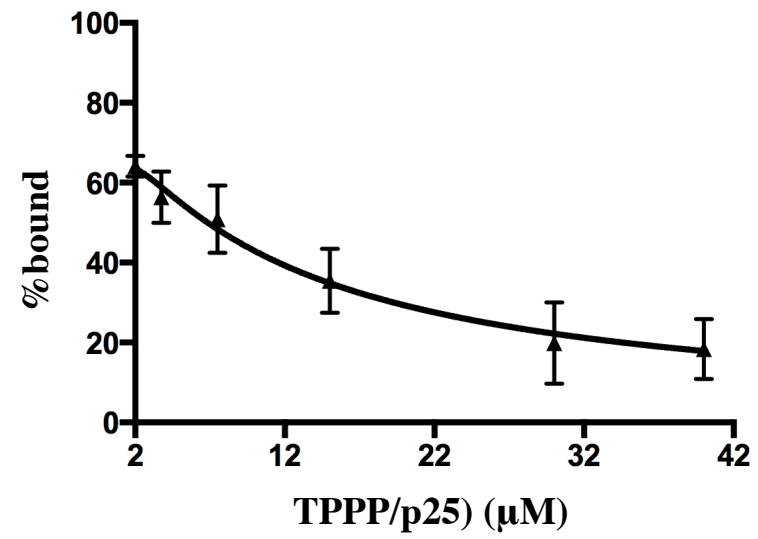


c



d

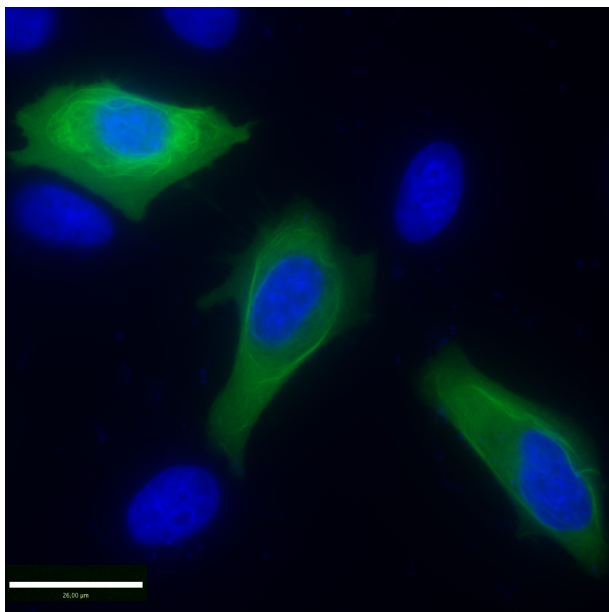




a

HA-TPPP-VC173

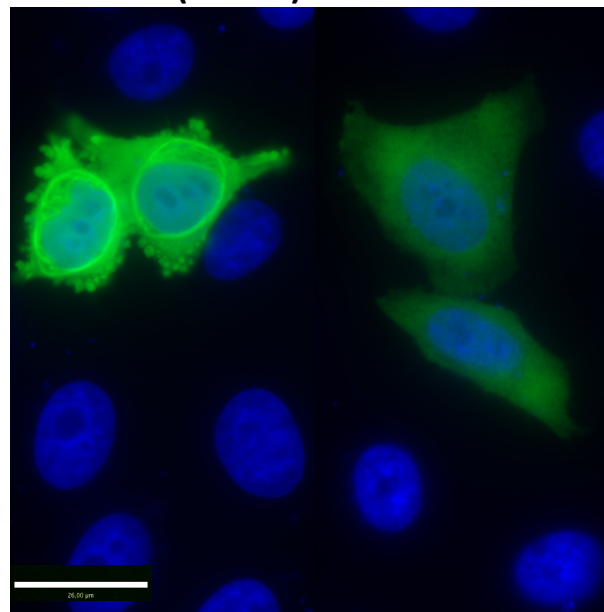
HA / DNA



b

HA-(Δ C158)-TPPP-VC173

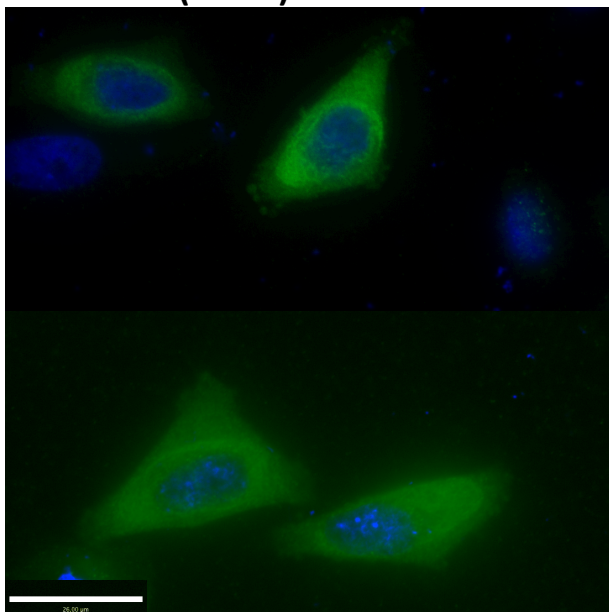
HA / DNA



c

HA-(Δ N49)-TPPP-VC173

HA / DNA



d

HA-(Δ N49- Δ C158)-TPPP-VC173

HA / DNA

