Supplementary Information

Tau stabilizes microtubules by binding at the interface between tubulin heterodimers

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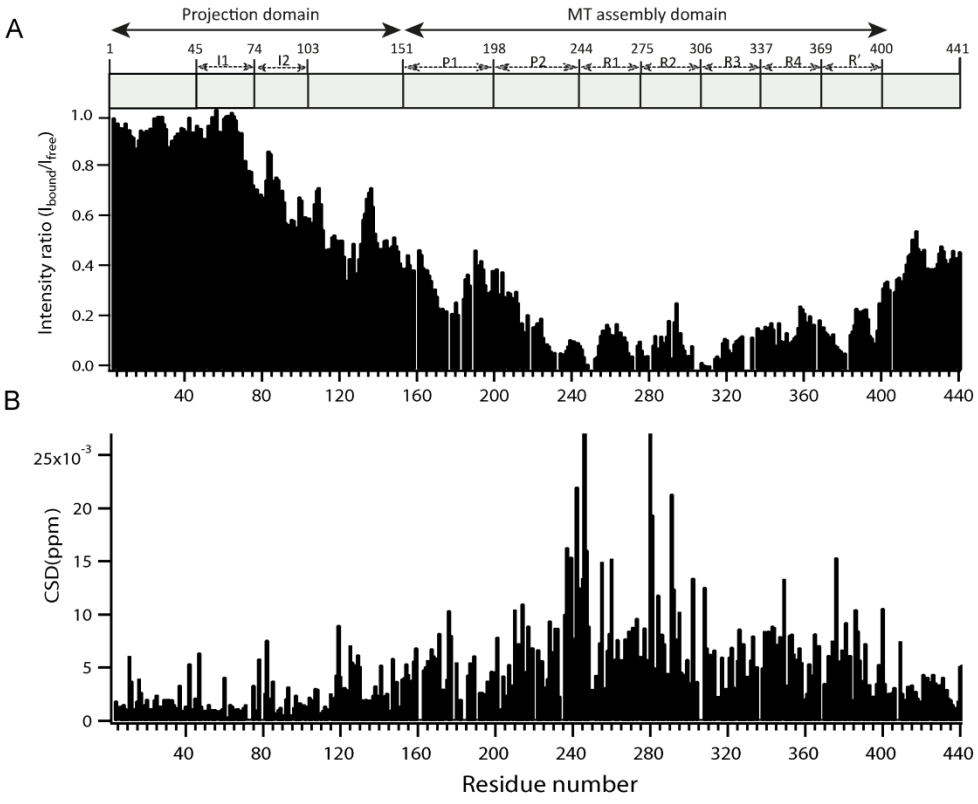
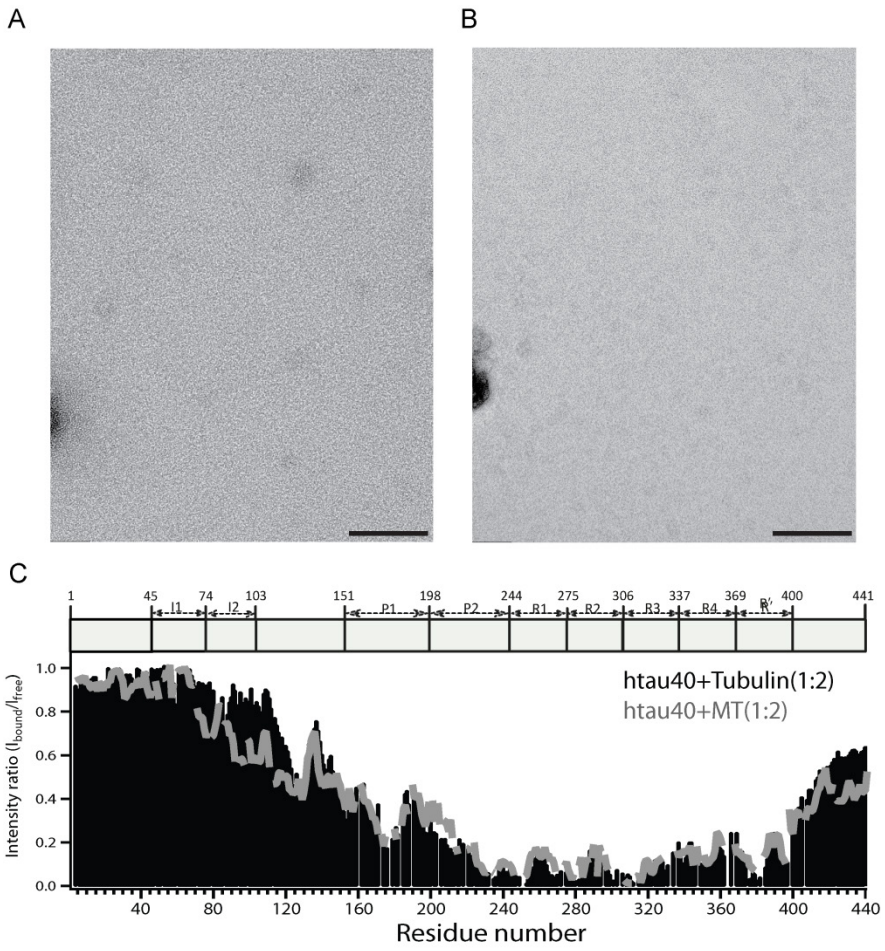
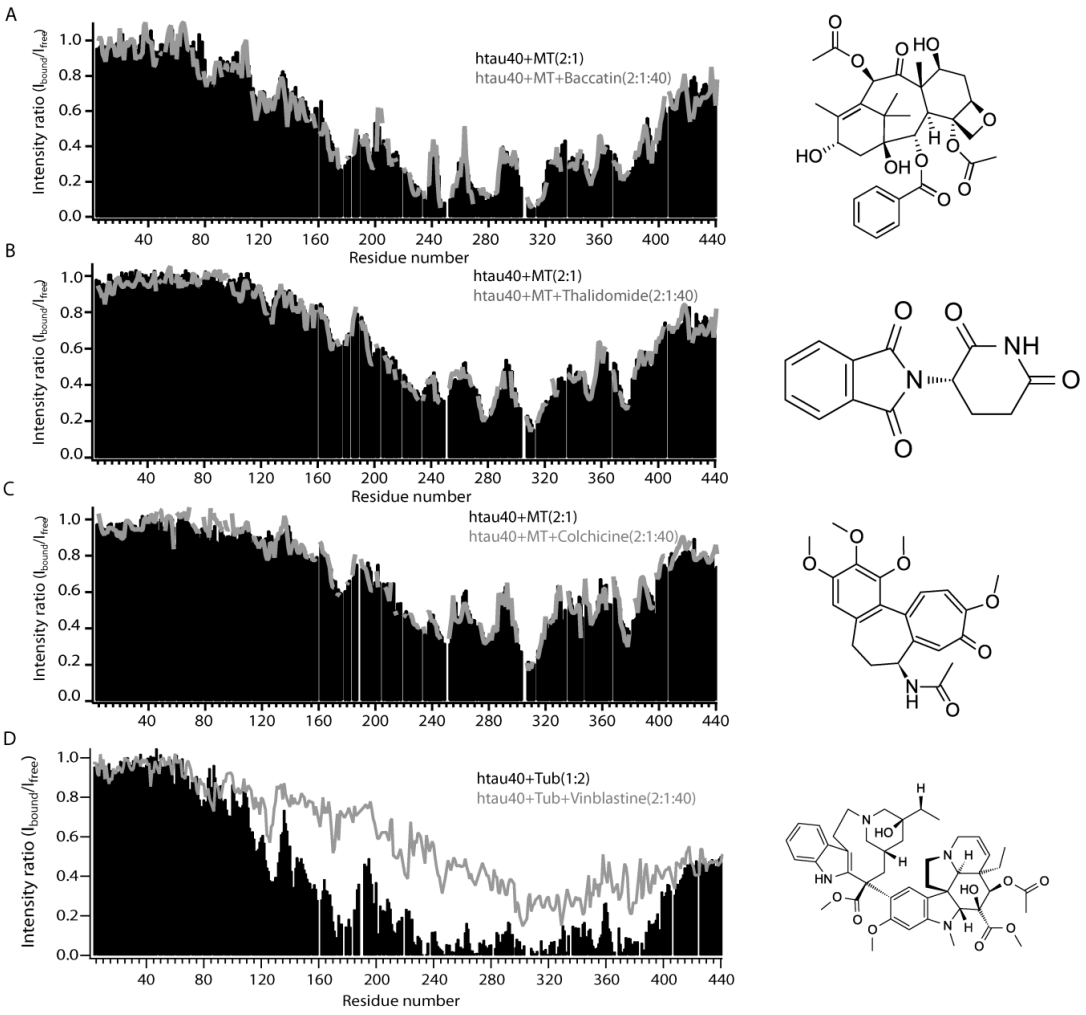


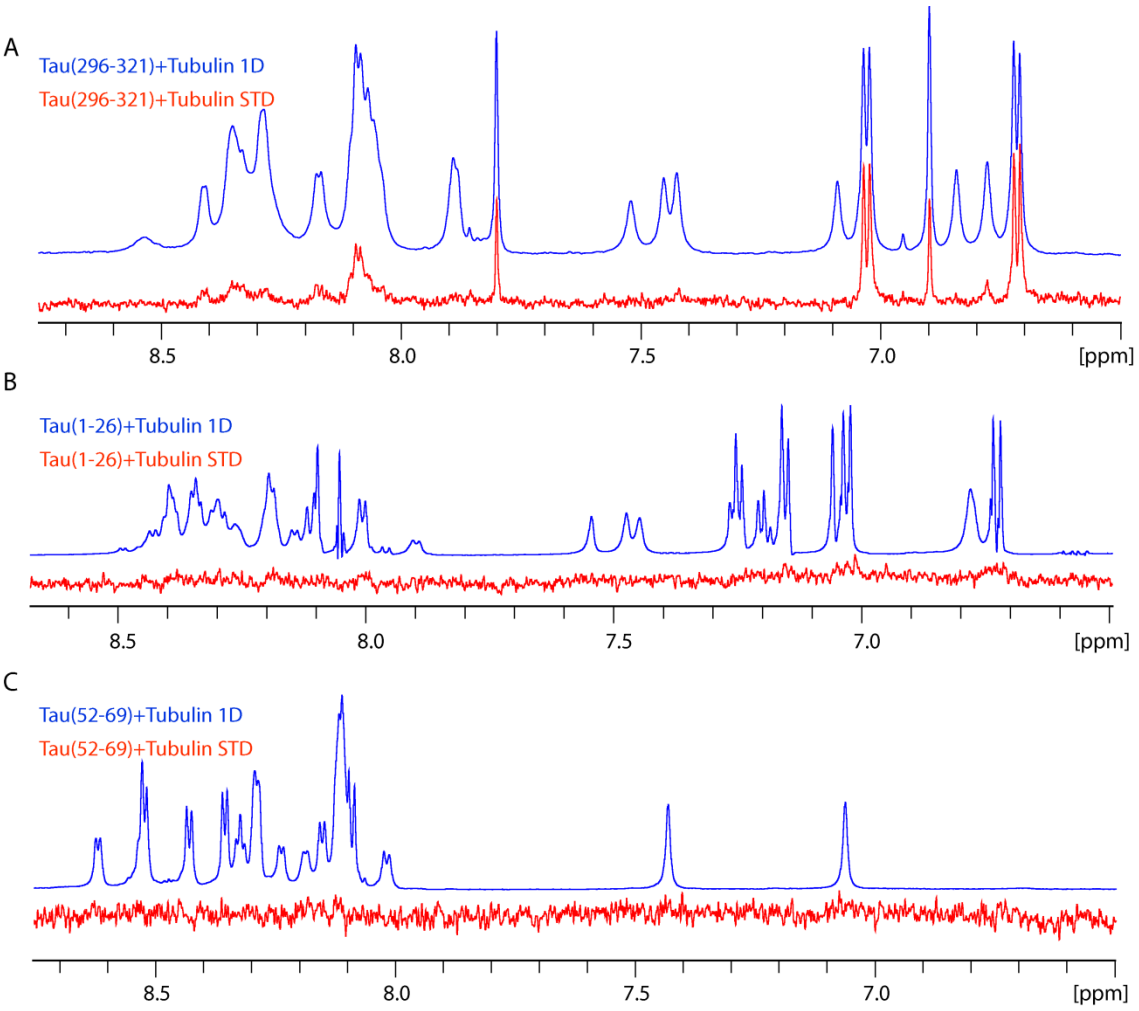
Supplementary Fig. 1. Single-residue analysis of the binding of tau to microtubules and unpolymerized tubulin heterodimers. **(A)** Residue-specific NMR signal intensity ratios of 4-repeat tau (hTau40) obtained from ^{1}H-^{15}N HSQC spectra recorded at 5°C in the presence and absence of microtubules at a htau40:tubulin heterodimer molar ratio of 1:2. **(B)** Changes in NMR signal position in hTau40 upon addition of microtubules.



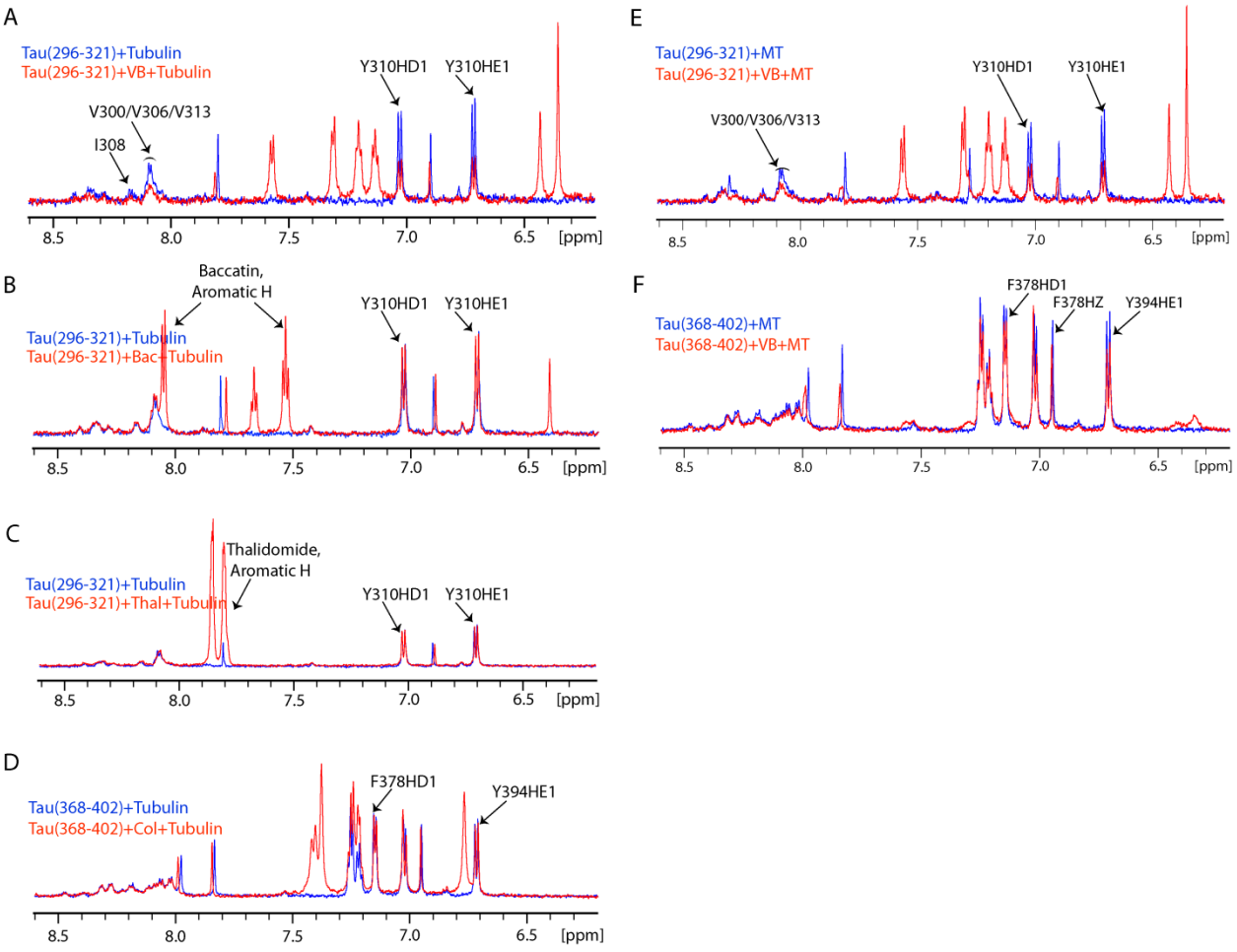
Supplementary Fig. 2. (A,B) EM images of tubulin in the presence of Tau under the conditions of the NMR experiment. 5 µM Tau and 10 µM tubulin (A), 50 µM Tau and 100 µM tubulin (B). No tubulin rings were found in any of the samples**. (C)** Comparison of intensity ratio profiles of hTau40 with microtubules (grey line) and unpolymerized tubulin (black bars), both at a hTau40:tubulin heterodimer ratio of 1:2.



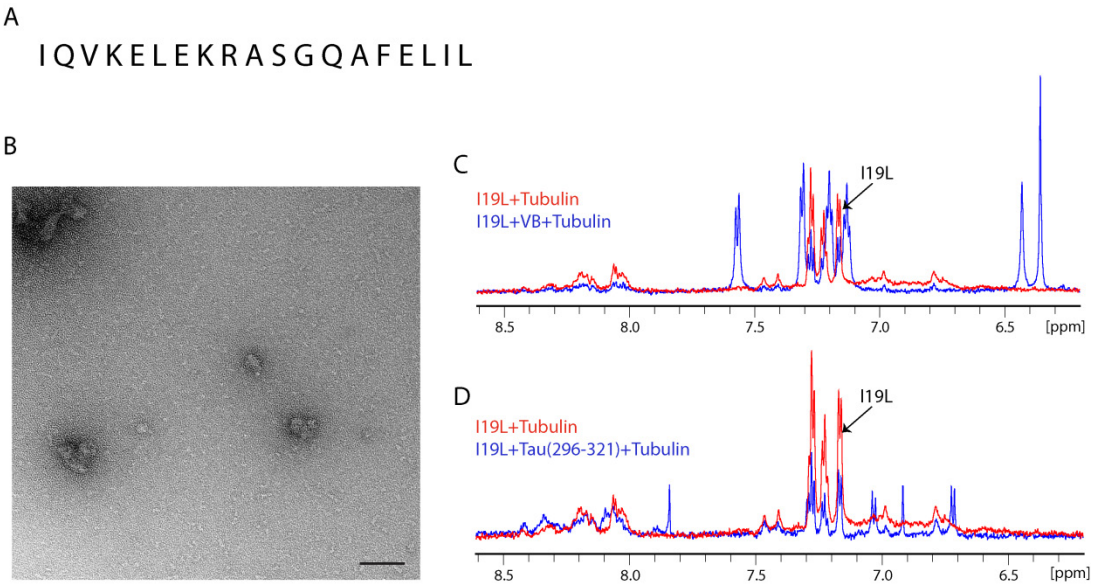
Supplementary Fig. 3. Binding of Tau to tubulin/microtubules in the presence of tubulin drugs. **(A-C)** Competition experiments using two-dimensional ^{1}H-^{15}N HSQC experiments recorded on hTau40. Black bars represent the line broadening of specific residues in hTau40 upon addition of microtubules (molar ratio of 2:1), while grey lines show the corresponding values after addition of a 20-fold excess (with respect to hTau40) of baccatin (**A**), thalidomide (**B**) and colchicine (**C**). **(D)** Vinblastine competes with Tau for binding to tubulin heterodimers at lower concentrations. The concentration of hTau40, tubulin and vinblastine were 5, 10 and 200 μM, respectively. Black bars represent NMR intensity profile of hTau40 in the presence of a 2-fold excess of tubulin heterodimers and grey after the addition of vinblastine. The chemical structure of the respective tubulin drugs are shown against the NMR intensity profiles.



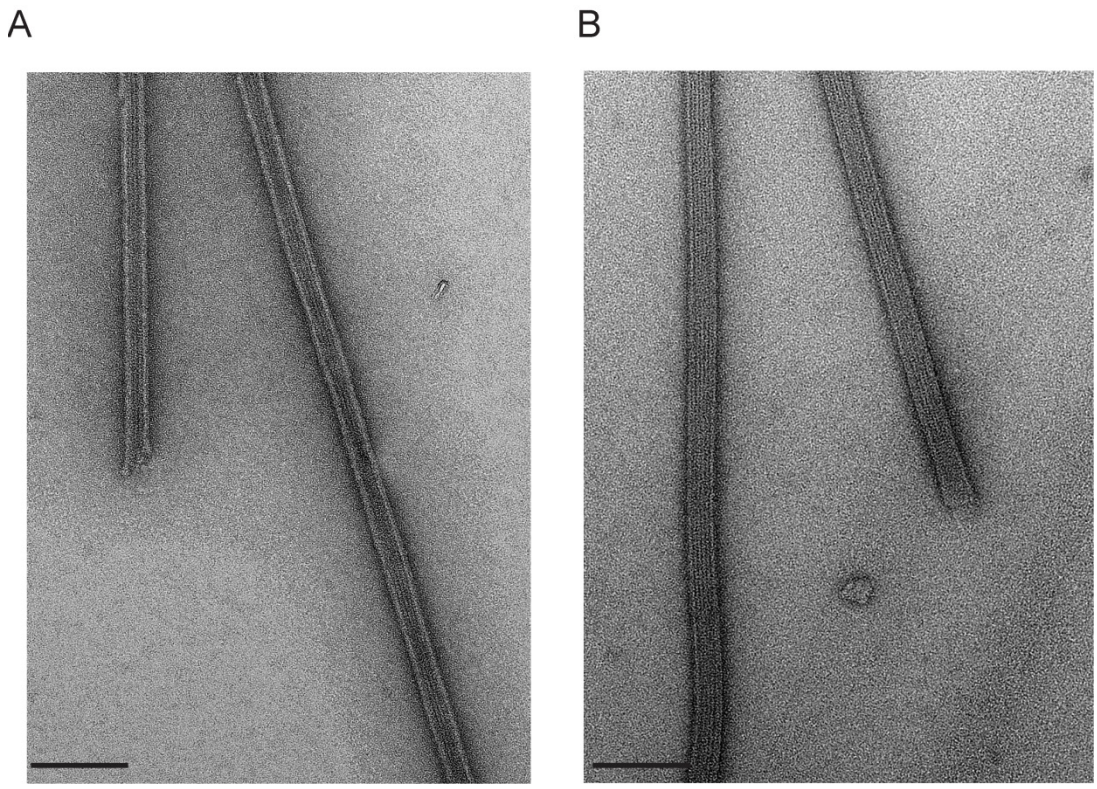
Supplementary Fig. 4. Interaction of Tau peptides with tubulin. **(A-C)** Comparison of one-dimensional ^{1}H spectra (blue) and saturation transfer difference (STD) NMR spectra (red) of Tau(296-321) (**A**), Tau(1-26) (**B**) and Tau(52-69) (**C**) in the presence of tubulin. The peptide concentration was 1 mM, that of tubulin 25 µM. Selective saturation of the protein resonances of tubulin was achieved by irradiation at -0.5 ppm, while the reference spectrum was irradiated at 60 ppm. The signals, which are observed in the STD spectrum of Tau(296-321), demonstrate that Tau(296-321) binds to tubulin. In contrast, no binding was detected under these conditions for Tau(1-26) (**B**) and Tau(52-69) (**C**).



Supplementary Fig. 5. Interaction of Tau peptides with tubulin/microtubules in the presence of tubulin drugs. STD-based competition experiments between Tau(296-321)/Tau(368-402) and tubulin drugs for binding to unpolymerized tubulin. STD experiments were done using 1 mM of peptide and compound and 25 µM of tubulin. Selective saturation of the protein resonances of tubulin was achieved by irradiation at -0.5 ppm, while the reference spectrum was irradiated at 60 ppm. STD spectra in the absence of the tubulin drug are shown in blue, spectra in the presence of vinblastine (**A**) baccatin (**B**), thalidomide (**C**), and colchicine (**D**) are in red. One-dimensional STD NMR spectra of the peptides Tau(296-321) (**E**) and Tau(368-402) (**F**) in the presence of microtubules (blue) and after addition of vinblastine (VB) (red). The concentration of the peptides and vinblastine was 1 mM, that of tubulin 25 µM. Selected Tau protons are labeled. The decrease in STD intensity indicates that the microtubule-binding motifs of Tau and vinblastine compete for the same binding pocket on microtubules.



Supplementary Fig. 6. (**A**) The primary sequence of the stathmin-like peptide I19L. (**B**) EM image of the NMR sample that was used for the STD experiment of the I19L peptide with tubulin heterodimers. The sample contained 10 µM tubulin and 400 µM I19L peptide in 50mM sodium phosphate buffer, pH 6.8. (**C**) STD NMR spectra of I19L and tubulin in the absence (red) and presence (blue) of vinblastine. The decrease in STD intensity of I19L resonances upon addition of vinblastine indicates that I19L and vinblastine compete for the same binding site. (**D**) STD NMR spectra of I19L and tubulin in the absence (red) and presence (blue) of Tau(296-321). The decrease in STD intensity of I19L resonances upon addition of Tau(296-321) indicates that Tau(296-321) and I19L compete for the same binding site.



Supplementary Fig. 7. Electron micrograph of taxol-stabilized MTs, before (A) and after (B) NMR experiment, which shows that the MTs remained stable over the duration of the NMR experiment (8 hours at 5 °C). The black scale bar represents 100nm.

Interprotein cross-links identified between htau40 and tubulin (1:1)

Protein 1	Protein 2	Peptide 1	Peptide 2	Residue 1	Residue 2
α-tubulin	htau40	DVNAAIATIKTK	SPSSAKSR	K336	K240
α-tubulin	htau40	DVNAAIATIKTK	KVAVVR	K336	K225
α-tubulin	htau40	DVNAAIATIKTK	NVKSK	K336	K257
α-tubulin	htau40	TKR	SPSSAKSR	K338	K240
α-tubulin	htau40	DVNAAIATIKTK	ENAKAK	K336	K383

Interprotein cross-links identified between htau40 and microtubules (1:1)

Protein 1	Protein 2	Peptide 1	Peptide 2	Residue 1	Residue 2
α-tubulin	htau40	DVNAAIATIKTK	KVAVVR	K336	K225
α-tubulin	htau40	DVNAAIATIKTK	NVKSK	K336	K257
α-tubulin	htau40	TKR	SPSSAKSR	K338	K240

Interprotein cross-links identified between tau(208-324) and tubulin (1:3)

Protein 1	Protein 2	Peptide 1	Peptide 2	Residue 1	Residue 2
α-tubulin	tau	DVNAAIATIKTK	SPSSAKSR	K336	K240
α-tubulin	tau	DVNAAIATIKTK	KVAVVR	K336	K225
α-tubulin	tau	TKR	HVPGGGSVQIVYKPVDLSK	K338	K311
α-tubulin	tau	TKR	SPSSAKSR	K338	K240

Supplementary Table 1. Interprotein cross-links identified by mass spectrometry in the 4-repeat Tau-tubulin complex, the 4-repeat Tau-microtubules complex and the Tau(208-324)-tubulin complex.