Supplemental Materials

Molecular Biology of the Cell

Ramirez-Rios et al.

Tau antagonizes EB tracking at microtubule ends through a phosphorylation-dependent mechanism

Sacnicte Ramirez-Rios, Eric Denarier, Eléa Prezel, Angélique Vinit, Virginie Stoppin-Mellet, François Devred, Pascale Barbier, Vincent Peyrot, Carmen Laura Sayas, Jesus Avila, Leticia Peris, Annie Andrieux, Laurence Serre, Anne Fourest-Lieuvin and Isabelle Arnal

Supplemental Materials:

- Supplemental Figures
- Supplemental Movie Legends
- Supplemental Materials and Methods

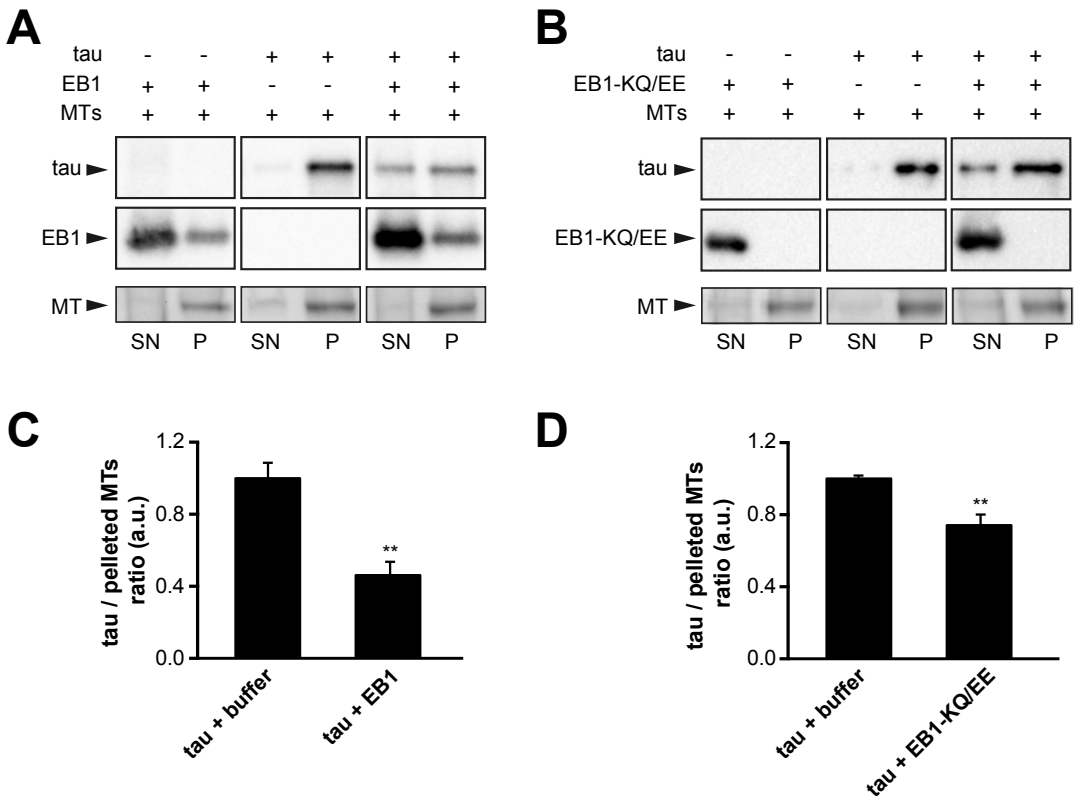

Figure S1. EB1 affects microtubule-binding properties of tau. (A) A two-step co-sedimentation assay was performed to analyse the effect of EB1 on tau-bound microtubules. Taxol-stabilized microtubules (2 µM) were incubated with tau (1µM), centrifuged to remove unbound tau and resuspended in either buffer alone (middle panel) or in the presence of 20 µM EB1 (right panel). Microtubules were then centrifuged again and the relative amounts of tau recovered with pelleted microtubules were quantified. The left panel shows the control experiment without tau. MT, microtubule; P, pellet; SN, supernatant. (B) Similar two-step co-sedimentation assay as in (A) performed with EB1-KQ/EE, a mutated form of EB1 exhibiting impaired microtubule-binding properties. (C) and (D) Quantifications of the ratios of tau to microtubules recovered in the final pellets after incubation with buffer alone (tau + buffer) or with either EB1 (tau + EB1) or EB1-KQ/EE (tau + EB1-KQ/EE). **p<0.01, non-parametric Mann-Whitney U test comparison (n = 5 independent experiments). Errors bars represent SEM.

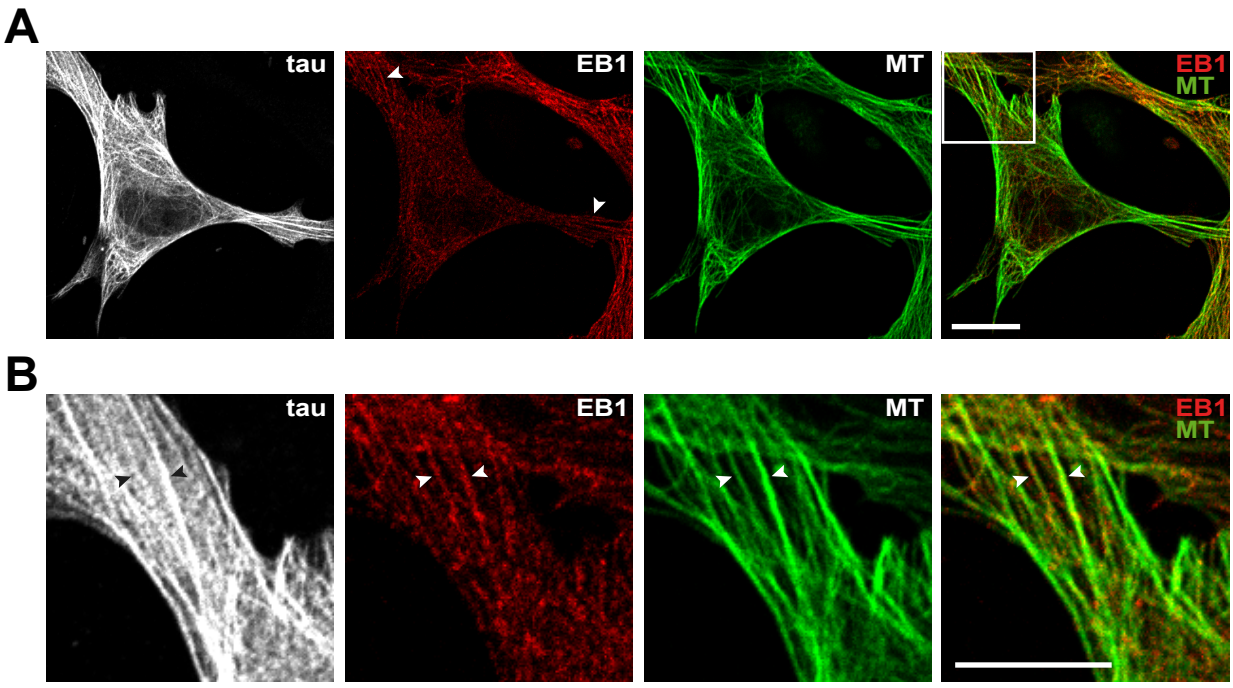

Figure S2. EB1 relocates along tau-induced microtubule bundles in fibroblasts upon high tau expression levels. (A) Mouse embryonic fibroblasts were transfected with pEGFP-tau and stained for EGFP (tau, in gray), EB1 (red) and tubulin (MT, green). A merged image with EB1 and microtubule staining is shown on the right. Images include transfected and non-transfected cells in the same field. Arrowheads point to EB1 relocated along microtubule bundles. Scale bar, 10 μm. (B) Higher magnification of the tau-induced microtubule bundles in the transfected cell. Arrowheads point to microtubules bundles along which EB1 is relocated. Scale bar, 10 μm.

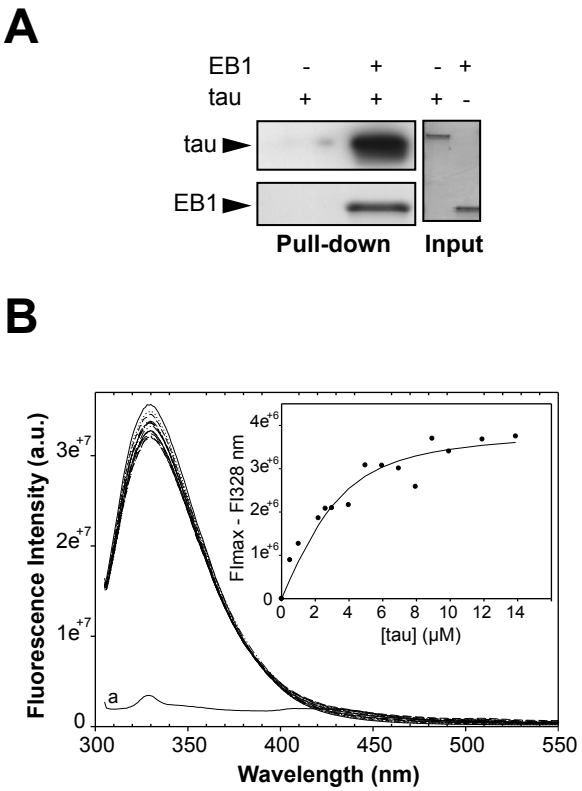

Figure S3. EB1 directly interacts with tau. (A) Pull-down assay of tau and biotinylated-EB1. (B) Representative plot of EB1/tau interaction monitored by fluorescence. EB1 fluorescence emission quenching is followed upon tau addition (λ exc = 295 nm). EB1 (6.3 μM) in BRB80 pH 6.74, 50 mM KCl, was mixed at 25 °C with increasing tau concentrations from 0 (upper spectrum) to 14 μM (lower spectrum). The bottom curve (a) represents the fluorescence spectrum of tau alone (9 μM), which is almost flat due to the absence of tryptophan in the protein. The graph (inset) shows the resulting titration plot as Fmax-F in function of total tau concentration from which we found a value of Kd about 1.7 ± 1.1 μM. Circle symbols indicate the experimental points (collected at 328 nm), and the solid line is the fitting curve obtained as described in Supplemental Materials and Methods).

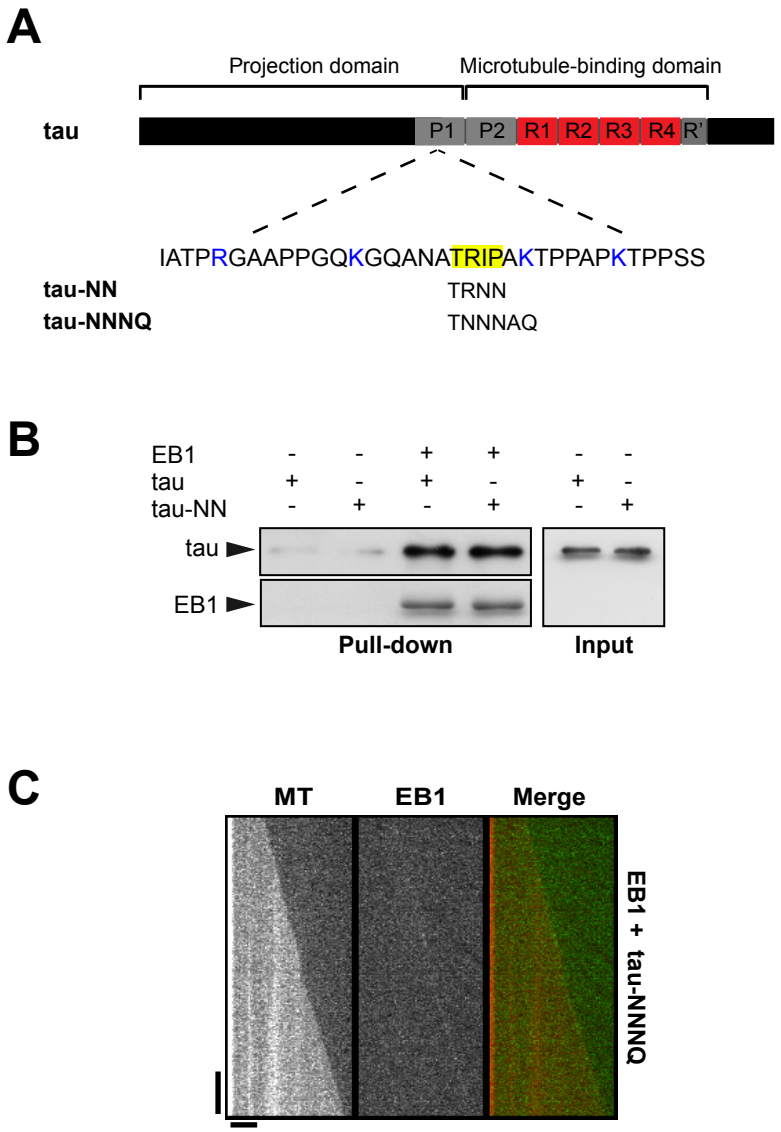

Figure S4. Mutations of the putative SxIP EB1-binding motif of tau does not affect tau/EB1 interaction and does not prevent tau-dependent inhibition of EB1 end-tracking. (A) Tau contains in its proline-rich region P1 a linear Ser-x-Ile-Pro (SxIP) motif (highlighted in yellow) flanking by basic residues (highlighted in blue). This motif shares the characteristic features of EB1-binding sites found on several EBs partners (Honnappa *et al.*, 2009). The replacement of IP or flanking basic residues by Asn (N) or Gln (Q) has been shown to abolish the interaction between EB1 and proteins that contain this motif (Honnappa *et al.*, 2009). (B) Pull-down assay of tau and tau-NN (mutated on IP residues) with biotinylated-EB1. (C) Kymographs of microtubules assembled in the presence of EB1 and tau NNNQ (mutated on xIP and adjacent lysine). Horizontal and vertical scale bars, 5 µm and 60 s.

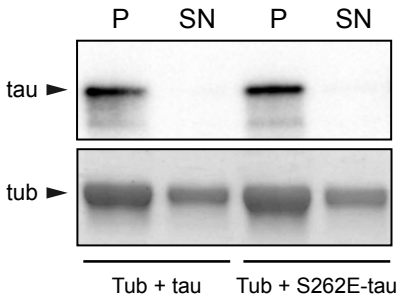

Figure S5. Comparison of the microtubule-binding properties of tau and S262E-tau by co-sedimentation assays. Microtubules were self-assembled in the presence of either tau or S262E-tau under similar tau:tubulin ratio used in TIRF conditions (1:160), before being centrifuged. Quantifications indicate that similar amounts of tau and S262E-tau bind to microtubules: 0.75 ± 0.066 and 0.83 ± 0.12 molecules bound to 100 molecules of polymerized tubulin dimers respectively (three independent experiments, mean ± SD). P, pellet; SN, supernatant; tub, tubulin.

Supplemental Movie Legends

Movie S1. Tau inhibits microtubule end tracking of EB1.

GFP-EB1 (75 nM) tracks growing microtubule ends and promotes catastrophe events (upper panels). Addition of equimolar concentration of tau (75 nM) inhibits the plus-end tracking of GFP-EB1 (lower panels) leading to persistently growing microtubules. Scale bar, 10 μm.

Movie S2. Increasing concentrations of tau reduce GFP-EB3 end tracking.

GFP-EB3 (10 nM) tracks growing ends of microtubules (upper right). Increasing concentration of tau (10 nM middle, and 40 nM lower panel) strongly reduced GFP-EB3 end tracking. Scale bar, 10 μm.

Movie S3. C-terminal deletion of EB3 allows recovering of microtubule end tracking in the presence of tau.

GFP-EB3-NL-LZ (10 nM) tracks growing ends of microtubules in the absence (upper panels) or in the presence of increasing concentrations of tau (10 and 40 nM, middle and lower panels). Scale bar, 10 μm.

Supplemental Materials and Methods

Co-sedimentation assay of taxol-stabilized microtubules, tau and EB1

Taxol-stabilized microtubules were prepared by incubating 70 μM tubulin at 36°C in BRB80 buffer supplemented with 1 mM GTP. Taxol (70 μM final) was then added and microtubules were further incubated for 30 minutes. Microtubules were then centrifuged for 5 minutes at 230,000 g and resuspended in BRB80 buffer.

For co-sedimentation assays, taxol-stabilized microtubules (2 μM) were incubated at room temperature with tau (1 μM) in K-BRB80 and centrifuged for 30 minutes at 230,000 x g to remove unbound tau. Microtubules were resuspended in buffer alone (K-BRB80) or in the presence of 20 μM EB1 or EB1-KQ/EE. After 30 minutes incubation, the mixtures were centrifuged again on a 50 % sucrose cushion for 45 minutes at 230,000 x g. Pellets and supernatants were analysed by SDS-PAGE and immunoblots to quantify the amounts of microtubules and tau, respectively. In the absence of microtubules, neither tau nor EB1 (wild-type and mutated EB1-KQ/EE) was detected in the pellet.

Fluorescence spectroscopy

Fluorescence experiments were done in a Perkin Elmer spectrofluorimeter. When EB1 were excited at 295 nm, the tryptophan residues show fluorescence maximum emission at 328 nm. In presence of different concentrations of tau (that does not have tryptophan residues), this emission is quenched due to the complex formation. EB1 (6 µM) was titrated using a 500 µM Tau solution. We used thermostated cuvettes (1 x 0.2 cm) at 25°C and put the smaller light path toward the excitation direction to give no appreciable inner filter effect (OD < 0.05). The fluorescence intensities (F) obtained at 328 nm for the different concentrations of tau was subtracted from the fluorescence intensity of EB1 alone (Fmax) and plotted versus the total tau concentration. The data were then were fitted to the saturation curve equation by means of nonlinear least-squares regression analysis using:

$$\mathrm{Fmax} - \mathrm{F} = \frac{\mathrm{Fsat} \times [\mathrm{tau}]\mathrm{f}}{\mathrm{Kd} + [\mathrm{tau}]\mathrm{f}}$$

Where Fsat is the fluorescence signal at the saturation, [tau]f is the free tau concentration and Kd is the dissociation equilibrium constant. Concentrations and binding parameters were determined as previously described [36]. Briefly, we used the equation:

$$[\text{tau}]b = \frac{1}{2}\left\{([\text{tau}]_0 + n\,[\text{EB1}]_0 + Kd) - \sqrt{([\text{tau}]_0 + n\,[\text{EB1}]_0 + Kd)^2 - 4n\,[\text{tau}]_0[\text{EB1}]_0}\right\}$$

where $[\text{tau}]_0$ and $[\text{EB1}]_0$ are the total tau and EB1 concentrations, respectively, [tau]b the bound tau concentration, and n the apparent stoichiometry ([tau]/[EB1]) that was fixed to 0.5 to obtain the best fit.